A CHEMICAL INVESTIGATION OF *TULBAGHIA VIOLACEA*

THESIS

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by

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ABSTRACT

Tulbaghia violacea, a member of the family Alliaceae is indigenous to the Eastern Cape and is widely used as a herbal remedy for various febrile and gastro-enteric ailments, particularly in young children. Adverse effects, and even fatalities, have been reported following treatment with the plant extract. The project has involved synthesis of model compounds, chromatographic analysis of flavonoid and other constituents of the plant, and examination of the volatile components.

Some fifteen flavones were synthesised as chromatographic models and in the course of this work, the development of a new method for synthesis of carboxylic anhydrides was completed. Use of the flavone standards permitted identification of the flavonols kaempferol and quercetin in hydrolysed glycosidic plant extracts. In addition, several sugars were identified, *viz.*, D-glucose, D-fructose, L-arabinose and D-galactose as free sugars, and D-glucose, D-galactose, L-rhamnose, D-fucose, D-xylose, L-arabinose and D-fructose as glycosidic sugars, by g.l.c. and g.c. - m.s. analysis of derivatives of isolated sugar mixtures. The presence in the plant extracts of steroidal saponins was also demonstrated.

The sulphur compounds, 2,4,5,7-tetrathiaoctane-2,2-dioxide and 2,4,5,7-tetrathiaoctane were isolated from the plant and characterised spectroscopically. This result, together with analysis of volatiles from the plant, has led to a proposal concerning the nature and origin of sulphur compounds in *Tulbaghia violacea*, showing close correlation with the sulphur compounds in *Allium* species.

Investigation of the biological activity of *Tulbaghia violacea* extracts showed bacteriostatic activity, particularly of extracts which had not been heated, and which had been prepared from mature plants. Treatment of isolated smooth muscle preparations with *Tulbaghia violacea* extracts indicated the presence of a β-adrenergic agonist having an inhibitory effect on normal muscle contraction.

The results of the investigations indicate that while there may be some basis for use of the plant as an antibacterial, or to treat colic, the adverse effects, caused possibly by the sulphur compounds and/or steroidal saponins present, may override the beneficial effects.

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Acknowledgements

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Mr. Neale Bell has been extremely helpful in assisting with obtaining data from the staff of local hospitals and finding information regarding the herbal remedy, and I wish to record my thanks to him. Dr. Santy Daya, Professor Don Hendry, Dr. Lesley Parolis and Mrs. Estelle Brink have generously given of their time and knowledge, to assist with some specialised aspects of my research, for which I thank them. For technical assistance, I record my gratitude to Mr. Aubrey Sonemann, Miss Moira Pogrund, and Mrs. Sally Morley of Rhodes University, Mr. Ivan Antonowitz of the CSIR, and Professor Vincent Brandt of U.O.F.S. Special thanks are due to Mrs. Joan Miles for typing this thesis with such willingness, patience, and efficiency.

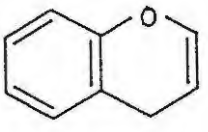
Lastly, but perhaps most importantly, I must express my appreciation of the support and encouragement of my husband, Mike, and daughters, Justine and Leigh, who have sacrificed much during the period of my research.

Note: Nomenclature of Flavones

Although common names are in general use for flavones, it is helpful to clarify the naming and numbering systems found in the literature.

Flavones are ketone derivatives of the 4*H*-benzopyran (1) and hence are phenyl-4*H*-benzopyranones. The systematic name for flavone itself (2) is 2-phenyl-4*H*-1-benzopyran-4-one. Substituted flavones are systematically named using the numbering shown. Compound (3), for example, is named 3,7-dihydroxy-2-(3,4-dihydroxyphenyl)-4H-1-benzopyran-4-one.[1]

A simpler convention treats the flavone molecule as the parent system.[2] Thus, compound (3) would be named 3,3′,4′,7-tetrahydroxyflavone. More often, however, this compound is called by its common name, fisetin, a practice which will be followed in this thesis. When common names are used, substituents are specified using the numbering shown for 3,3′,4′ -tri-*O*-methylfisetin (4). The aromatic rings in the flavone molecule are designated A, B and C respectively, as shown in structure (5).



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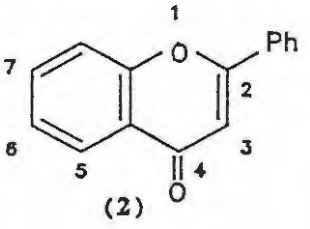

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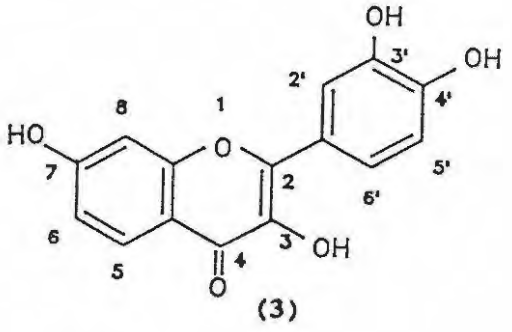

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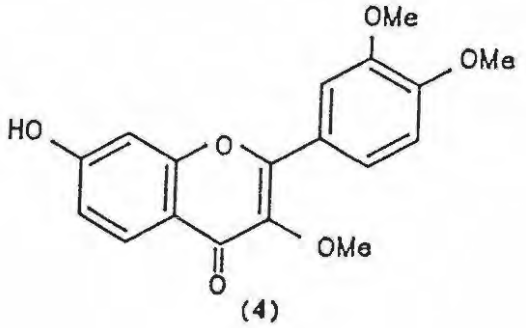

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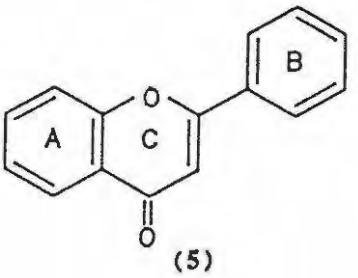


(5)

1. INTRODUCTION

Traditional herbal medicine is an important and widely utilised resource for the indigenous people of South Africa and occasionally it is their only accessible source of medical treatment. The plants used for herbal remedies are drawn from a wide range of taxonomic groups, including many from the superorder Liliiflorae, in which *Tulbaghia violacea* is classified.[3]

The use of *Tulbaghia violacea* (Xhosa name "itswele lomlambo" meaning "evil winds") as a herbal remedy is common in the Eastern Cape, the region to which it is indigenous. An infusion of the plant is prepared by herbalists for use as an enema, in the treatment of conditions including colic, wind, restlessness, headache and fever, largely in young children. Reports, from the King William's Town and East London areas,[4] of adverse effects after such treatment have indicated a variety of symptoms including abdominal pain, gastro-enteritis, acute inflammation and sloughing of the intestinal mucosa, cessation of gastro-intestinal peristalsis, contraction of the pupils, and subdued reactions to stimuli. Some fatalities have been attributed to treatment with the herbal preparation.

The widespread use of the plant as a herbal remedy with apparently toxic, and even fatal, results has prompted this investigation into the chemical constituents of *Tulbaghia violacea*. The aim was to establish the identity of biologically active compounds present, and hence explain the adverse effects of the plant.

1.1 Classification of *Tulbaghia violacea*

A recent classification[3] (Scheme 1.1) separates members of the orders Liliales and Asparagales into several families, and places *Tulbaghia violacea* in the family Alliaceae. Previously *Tulbaghia violacea* was generally regarded as a member of the Liliaceae, and cognisance of this fact must be taken in references to the plant in the older literature. Also, in resolving some irregularities in the genus *Tulbaghia*, Vosa has noted that *Tulbaghia cepacea* Lf. is identical to *Tulbaghia violacea* Harv., and that *Tulbaghia simmerleri* Beauv. is the correct name for *Tulbaghia fragrans*.[5]

Scheme 1.1 Classification of *Tulbaghia violacea* in the Liliiflorae[3]

Superorder : LILIIFLORAE

Order : Asparagales Family : Alliaceae Genus : *Tulbaghia*

Other families relevant to the present study :

Order : Asparagales Family : Amaryllidaceae
Asphodelaceae
Hyacinthaceae
Convallariaceae
Dracaenaceae
Agavaceae

Order : Liliales Family : Iridaceae
Colchicaceae
Liliaceae
Alstroemeriaceae

Order : Dioscorales Family : Dioscoraceae
Trilliaceae

1.2 Medicinal uses of *Tulbaghia violacea* and related plants

In their comprehensive survey of South African poisonous and medicinal plants, Watt and Breyer-Brandwijk[6] give accounts of several species of *Tulbaghia* which are used medicinally. *Tulbaghia violacea* is used for the treatment of infant and mother in *the case* of a depressed fontanelle, and young plants are eaten as a vegetable. Feeding tests for toxicity of this species, on rabbits, were found to be negative.[7] In the Transkei, the root of *Tulbaghia alliacea* Lf. is used for the treatment of rheumatism and paralysis, for reducing temperature, and as a purgative.[6] Early Cape colonists are also reported to have used the bulb for treatment of high temperatures, and other uses of the bulb include treatment of pulmonary tuberculosis and as an antihelmintic. The bulb of *Tulbaghia violacea* (referred to by Watt and Breyer-Brandwijk in this case as *Tulbaghia cepacea*) is also used as a remedy for pulmonary tuberculosis and as an antihelmintic. The green parts of *Tulbaghia violacea* are eaten by the Zulu people as a peppery spinach, and the bulb is used for an emetic love potion. The Zulu people also regard this species as having the property of protecting against snakes.

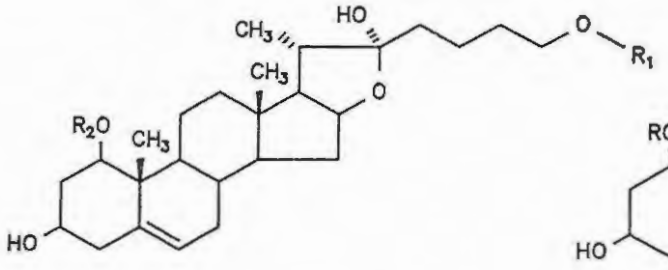
Species of the closely related *Agapanthus* are reported by the same authors to be used as an aphrodisiac, and various *Allium* species, valued in many regions of the world for their marked medicinal properties, are used medicinally for a wide range of purposes.[8] In South Africa, for example, a syrup prepared by steeping onions in sugar is traditionally used to treat whooping cough, the Xhosa people use garlic to treat fever, and *Allium porrum* L. is an old Cape remedy for dropsy.[6] The antibacterial action of some *Allium* species is well established, and *Allium sativum* in particular is used as an antiseptic. Accounts of toxicity to livestock due to various *Allium* species have been reported.[6;9]

1.3 Chemical constituents of plants related to *Tulbaghia violacea*

A wide range of biologically active compounds are present in numerous plants of the Liliiflorae, and *Tulbaghia violacea* is closely related to many of these plants. A survey of the different classes of biologically active compounds found in the plants of the super-order Liliiflorae gave some indication of the active principles which might be found in *Tulbaghia violacea*. The classes of the possible constituents are discussed here under the following headings :- steroidal saponins and cardiac glycosides; alkaloids; flavonoids; anthraquinones; sugars; and sulphur compounds.

1.3.1 Steroidal saponins and cardiac glycosides

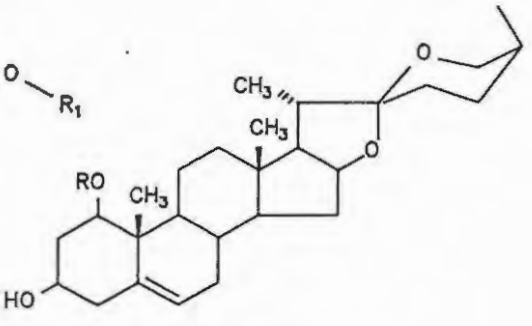
Frequently present in members of the order Asparagales, and to a lesser extent in the order Liliales, are steroidal saponins[3] and their biosynthetic precursors, *viz.*, the sterols.[10;11] The steroidal saponins are glycosidic compounds consisting of steroid skeletons (of the furostanol or spirostanol series[3]) bearing sugar units in varying substitution patterns. Examples from *Allium cepa* include compounds (6) and (7).[12]



R_1 and R_2 = sugar units

(6)

Alliofuroside A



R=disaccharide chain

(7)

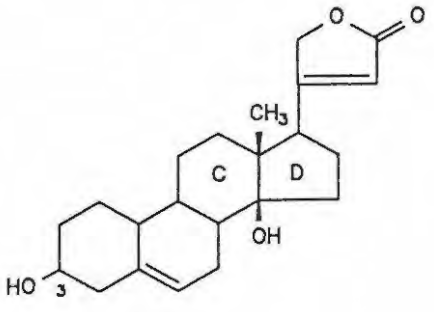
Alliospiroside B

The presence of saponins in plants is evidenced by noticeable foaming of the aqueous extracts (and hence the name relating to soap). The saponin molecules being large, and bearing hydrophobic and hydrophilic portions, tend to form emulsions, colloidal suspensions, and foams.[13] Indeed, some saponin-containing plants such as *Phytolacca dodecandra* and *Sapindus saponaria* are used as soaps for washing clothes.[14] In dilute solutions saponins are poisonous to fish and extracts of saponin-containing plants are used as fish poisons *e.g.* pods of *Swartzia madagascariensis*. Saponins are also effective molluscicides, as illustrated by the example of *Phytolacca dodecandra* berries used in Ethiopia as a means of preventing Schistosomiasis, by interrupting the cycle of the disease at the snail-host stage. Recent research into sources of steroidal saponins (largely in response to recognition of their potential conversion to synthetic steroid hormones[15]) has revealed their presence in widely varying plants including many *Allium* species.[16] Other saponin-containing plants related to *Tulbaghia violacea* include *Agapanthus* species,[6] *Dracaena nitens* (used in several traditional African remedies *e.g.* for haemorrhoids and stomach-ache),[17] *Crinum* species,[6] *Boophone* species,[6] *Albuca* species (used as a purge against venereal disease)[18] and *Eucomis antumnalis* (used as an emetic and an enema, for urinary disease, and during pregnancy).[18]

Saponins as a group of compounds tend to be gastro-intestinal irritants and cause emesis if taken orally.[19] They have a haemolysing effect resulting in symptoms such as hyperaemia of the intestinal mucosa.[18] Fortunately, they are poorly absorbed in the gastro-intestinal tract, since presence of haemolytic saponins in the blood stream causes haemolysis of erythrocytes and subsequent reduction in oxygen-carrying capacity of the blood. The primary action of saponins is one of increasing cell membrane permeability, attributed in some reports to binding of the sapogenin to receptor sites on the membrane, and is thus dependent on initial hydrolysis of the saponin.[20] By the same action, small quantities of saponins can assist in absorption of nutrients and drugs in the small intestine.

Saponins are also proposed to exert an inhibitory effect on tissue respiration and peristaltic action in intestinal muscle.[20]

An unusual group of compounds, the cardiac glycosides, are similar in structure to the steroidal saponins, but are distinguished from them by the presence of an unsaturated lactone ring at C-17, a 14β-hydroxyl group, and *cis*-conformation between the C and D rings as shown for compound (8).[13]



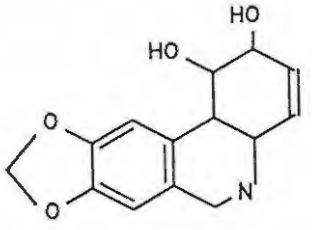
(8)

Cardiac glycosides are often associated with unusual (eg. methylated) sugars which are always linked at C-3. They have a specific cardiotonic effect and some are used as ordeal and arrow poisons,[13] while others *e.g. digitalis* glycosides are used medically in the treatment of cardiac disorders.

In South African herbal remedies, cardiac glycosides are found, for example, in extracts of *Bowiea volubilis* (Hyacinthaceae) (used for treating dropsy, barrenness, headaches, and as an emetic, but has caused at least one fatality) and *Scilla nevosa* (used for treatment of dysentery and rheumatic fever, but toxic).[18] The cardiac glycoside is *Convallaria majalis* (commonly named lily-of-the-valley) is particularly toxic.[21]

1.3.2 Alkaloids

The toxicity of many plants is attributable to the presence in them of alkaloids. The chemical structures of the alkaloids are very diverse, as is their distribution in plants. In the Liliiflorae, members of the Liliales frequently contain alkaloids, and many members of this order are known to be toxic. For example, amongst South African members of the Amarylidaceae [typically containing the Amarylidaceae alkaloids of which compound (9) is an example [22]] *Amaryllis belladona* L. is notoriously poisonous and *Boophone disticha* Herb., while also poisonous, is nevertheless used medicinally in various ways; Watt and Breyer-Brandwijk[6] report many poisonings and fatalities attributable to these plants.



(9)

Lycorine

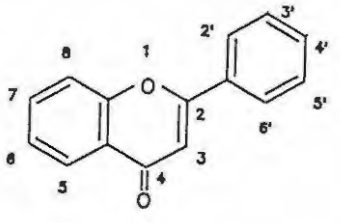
Alkaloids have also been associated with the toxicity of other local herbal remedies *e.g.* colchicine in *Gloriosa superba* (Colchicaceae),[18] cliviine in *Clivia nobilis* (Amarylidaceae),[18] haemanthamine in *Haemanthus natalensis*,[6] and ambelline in *Nerine undulata*,[6] to name but a few. Alkaloids have been reported to be present in *Allium odorum* L.[23] and *Allium ampeloprasum*,[24] but references in the literature to the presence of alkaloids in members of the Alliaceae appear to be rare.

The pharmacological activity of alkaloids is varied, as would be expected from such a diverse group of compounds, but in general, treatment with alkaloid-containing remedies affects the central nervous system.[25] Symptoms of alkaloid toxicity include convulsions, incoordination, pupil dilation and visual disturbances. As specific examples, Lycorine (9) in large doses causes ecchymosis of stomach, intestine, and endocardium; Veratrum alkaloids affect blood pressure and the neuromuscular system;[19] and Solanum alkaloids cause nausea, vomiting, and haemolytic and haemorrhagic damage to the gastro-intestinal tract.[26]

In contrast with these toxic effects, many useful and common drugs are alkaloids (*e.g.* codeine and morphine), and numerous herbal remedies based on the activity of alkaloids are undoubtedly successful when administered appropriately.

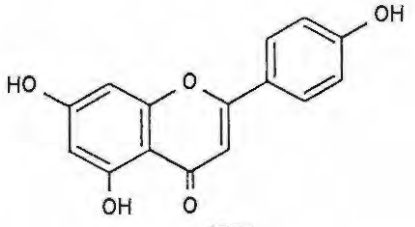
1.3.3 Flavonoids

The flavonoids are a large class of phenolic compounds structurally derived from the flavone skeleton (10).



(10)

Flavones have hydroxyl or, less frequently, methoxyl groups as substituents on the benzene rings, and flavonols have analogous oxygenation patterns but always bear a hydroxyl group at C-3. Naturally occurring flavones and flavonols are found universally in vascular plants, and have substituents most commonly at the 5-, 7-, 3′-, 4′-, and 5′-positions, but rarely at the 2′- (or 6′-) positions.[27] Most common are the flavones luteolin (85) and apigenin (83), and the flavonols kaempferol (87), quercetin (89), myricetin (93) and, to a lesser degree, isorhamnetin (103).[27;28;29]



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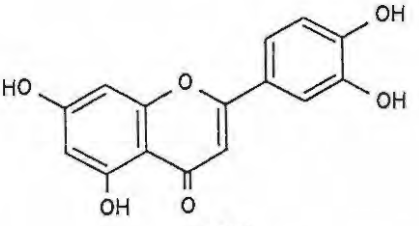

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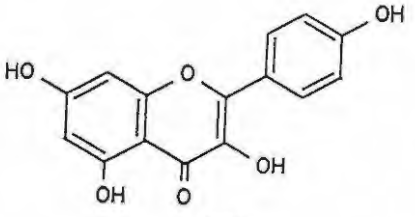

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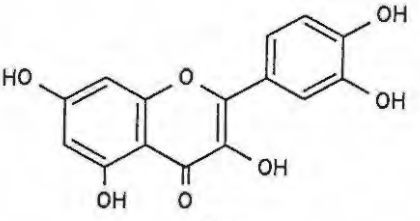

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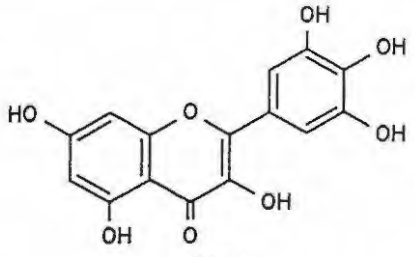

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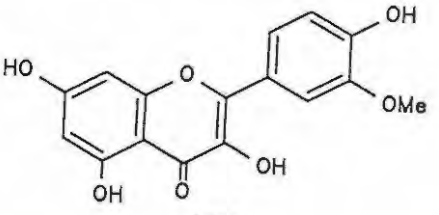


(103)

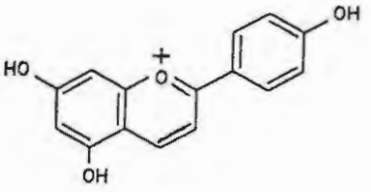
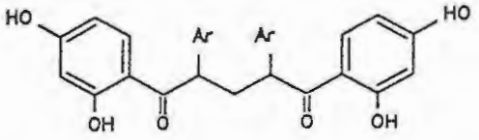
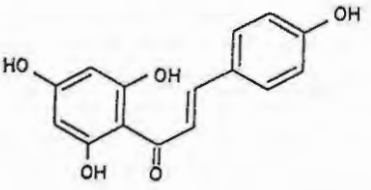
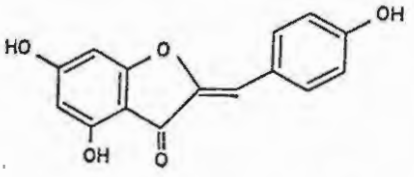
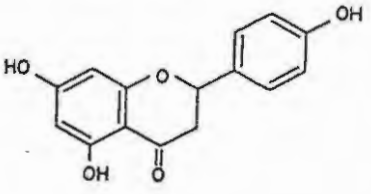
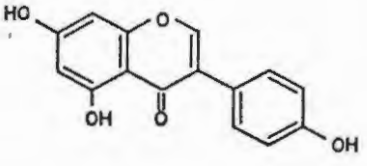
All of these compounds are found in the Liliiflorae,[30] where the distribution of the flavonoids has taxonomic significance. Certain families, for example, contain only flavones (*e.g.* the Wurmbaeoideae) while some contain only flavonols (*e.g.* the Lilioideae) and others contain both (*e.g.* the Scilloideae).[30] Also, isorhamnetin derivatives are known only in the genera *Lilium* and *Convallaria*. In addition to other flavonoids in families of the Liliiflorae, Williams[30] also reports the presence of 5-methylated flavones and 5-deoxyflavones in members of the Liliaceae, thus linking them taxonomically with the families Juncaceae, Cyperaceae, Palmae, and Graminae.

In a comprehensive survey of phenolic constituents in the monocotyledons, Bate Smith[31] reported the presence of kaempferol (87) in *Tulbaghia violacea*.

Other classes of flavonoids which are less widely distributed in the higher plants are shown in Table 1.1.

As in the case with all plant phenolics, flavones and flavonols occur in plants as a wide range of *O*-glycosides, and the positions of the sugar units follow certain trends (see section 1.3.5 for comment on the nature of the sugars). For the flavonols :- 3-*O*-glycosides are most common; 3,7-di-*O*-glycosides are also common; 3,4′-di-*O*-glycosides are unusual; 3′-*O*-glycosides are very rare; and 5-*O*-glycosides do not occur.[27] For the flavones, the sugar is most commonly attached at position 7, and somewhat less often at position 5; 4′-*O*-glycosides of flavones are also found.[27]

Table 1.1 Classes of less common flavonoids found in higher plants[32]

Flavonoid	Typical structure	Distribution
Anthocyanins		Pigments in leaves, petals, fruits
Biflavonols		Gymnosperms
Chalcones		Yellow flower pigments
Aurones		Yellow flower pigments
Flavanones		Leaves and fruit, especially citrus
Isoflavones		Leguminosae

Flavonol glycosides have been isolated and identified from many plant species,[33] and in a few cases attempts have been made to identify all of the glycosides present. The flavonoids found in any one plant species are generally different glycosides of common aglycones. *Allium cepa*, for example, has been found to contain the flavonol quercetin as the 4′-*O*-glucoside, the 7,4′-di-*O*-glucoside and the 3,4′-di-*O*-glucoside.[27]

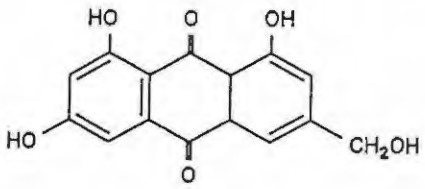
Glycoflavones, or flavone-*C*-glycosides, are found in a small number of families of plants. The sugar units in these flavonoids are linked by carbon-carbon bonds to the flavone skeleton, giving compounds which are characteristically difficult to hydrolyse to separate aglycones and sugars.[2] The C-glycosidic link is always adjacent to a phenolic hydroxyl group, and is usually at position 8. Flavone-*C*-glycosides have been reported in three species of the Liliales *viz.*, *Narthecium ossifragrum* (Melianthioideae), Paradaisia lileastrum (Asphodeleae), and *Urginea maritima* (Scilloideae).[30]

By virtue of their universal presence in higher plants, flavonoids are present in many foods, herbs, and spices, as well as medicinal preparations, and they are generally non-toxic (with the exception of some isoflavones which have been considered responsible for oestrogenic activity resulting in infertility of livestock[34]).

Their nutritive and medicinal value can be attributed partly to their inhibitory action on some enzyme systems, leading to fortification of connective tissue against penetration by invading agents such as cancer cells, viruses, and bacteria, and against the effects of some diseases, *e.g.* the weakening of capillary walls and consequent retinal and peripheral bleeding in diabetes mellitus.[35;36] Other reported beneficial effects of flavonoids include their action on cell membranes which reduces allergic reaction[36] and regulates smooth muscle action.[35]

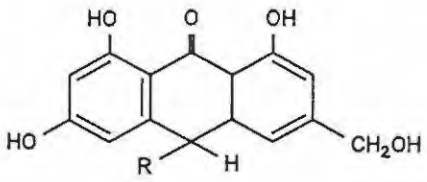
1.3.4 Anthraquinones

A further group of secondary metabolites found in the Liliiflorae are the anthraquinones, naturally occurring quinones of which aloe-emodin (11) is a typical example. Like flavonoids, they generally occur as glycosides.



(11)

Aloe-emodin

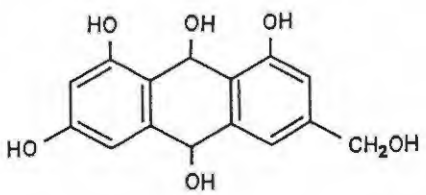


R= Glucose (12)

Barbaloin

In the order Liliales, anthraquinone glycosides are found in the family Asphodeleaceae, members of which are valued, in the practice of traditional medicine, for their marked purgative action. Species of *Aloe*, *Bulbine*, *Kniphofia* and *Haworthia* are members of this family which are used in herbal remedies.[30] The purgative principle in commonly available commercial aloes is a C-glycoside of aloe-emodin (11), named barbaloin (12)[37]. In the order *Agavales*, *Agave americana* is used for its purgative action[6] but is also reported to be toxic.

The physiological activity of plants containing anthraquinones is due to reduction of the anthraquinone glycosides by intestinal bacteria to anthranols [*e.g.* compound (13)] which are cathartics. The reason for storing purgative plants for up to a year before use is to allow slow hydrolysis of the glycosides giving increased anthranol content.[13]



(13)

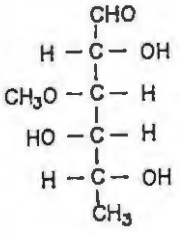
1.3.5 Sugars

The sugars present in glycosides of various secondary metabolites are a large and varied group. In the phenolic glycosides, five monosaccharides are common, these being D-glucose, D-galactose, D-xylose, L-rhamnose, and L-arabinose, while ketohexoses are generally absent.[21] D-glucuronic acid is found attached to some phenolic compounds. The sugars are generally present in the pyranose form (L-arabinose is also found in the furanose form) and are generally β-linked to phenolic hydroxyl groups (with the exception of two known α-arabinosides).

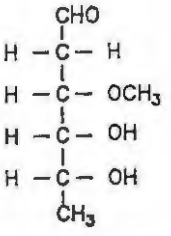
Of the disaccharides found attached to phenolic compounds, the three most common are rutinose (rhamnose α1→ 6 glucose), sophorose (glucose β1→ 2 glucose), and sambubiose (xylose β1→ 2 glucose), and all three are found frequently in association with flavonoids. [A less common but interesting disaccharide is that found in the flavonoid glycosides of *Citrus*, neohesperiodose (rhamnose α1→ 2 glucose), which is largely responsible for the characteristic flavour of *Citrus*]. The presence of trisaccharides combined in phenolic glycosides is less well established, and less common. Characteristically, they contain 1→ 2 or 1→ 6 links, and glucose is frequently present at the reducing end of the trimer.[21]

In flavonol glycosides, glucose and rhamnose are most common, and the di- and trisaccharides present generally contain these sugars. Flavones, on the other hand, are frequently associated with glucose and rutinose, and occasionally with apiosylglucose.[27] The majority of other flavonoids (Table 1.1) are found in combination with glucose, or with glycosidic patterns similar to those of the flavones and flavonols.[27]

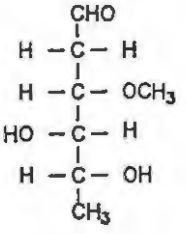
Anthraquinones occur in association with glucose, rhamnose, rutinose, gentiobiose (glucose β1→ 6 glucose), and primeverose (xylose β1→ 6 glucose).[13] In saponins, sugars are often present as oligosaccharides, and differences between saponins are often due to variation of the sugars attached to one sapogenin.[13] The usual sugars associated with cardiac glycosides include D-digitalose (14), D-cymarose (15), and D-sarmentose (16), and acetyl derivatives of these.[13]



(14)



(15)



(16)

Also present in higher plants, are free sugars *e.g.* in *Allium* species :- glucose, fructose, sucrose, maltose, arabinose, rhamnose, xylose, galactose,[38] and polysaccharides, such as glucofructans and fructans which may serve as a means of carbohydrate storage. Pectins, which are complex carbohydrate polymers, are found in association with cellulose in cell membranes.

1.3.6 Sulphur compounds

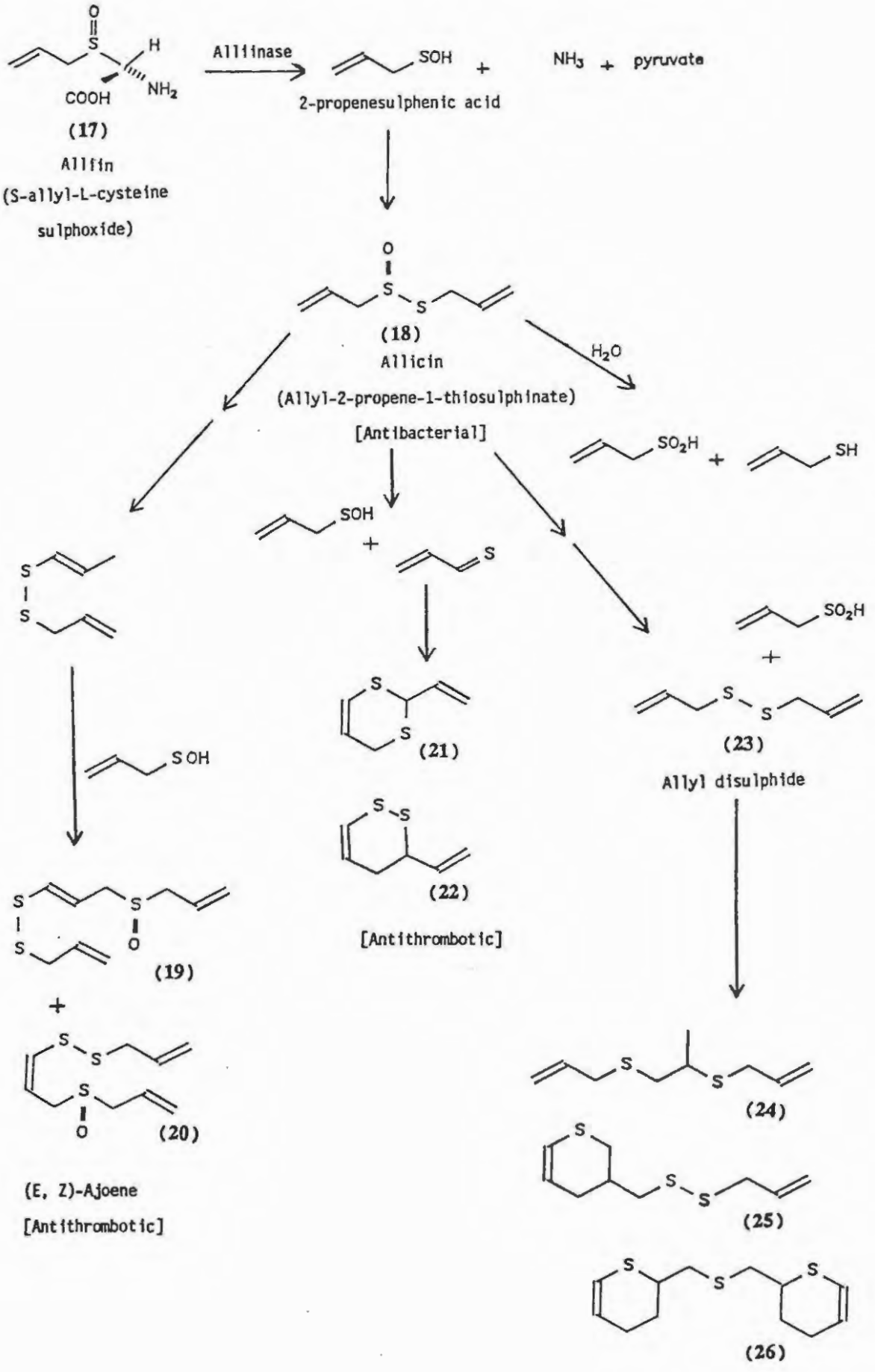
Perhaps the major feature distinguishing the secondary metabolites of the Alliaceae is the presence of low molecular mass sulphur compounds. The *Alliums*, in particular, contain *S*-alk(en)yl-L-cysteine sulphoxides, which give rise to the characteristic odours of the plants by a series of complex and interesting reactions. In the intact cells, these sulphoxides are present in the cytoplasm, and lyase enzymes capable of hydrolysing them are found in the vacuole.[39] Crushing of the plant tissues causes disruption of cells and hence allows the action of the lyase enzyme on the sulphoxide substrates, resulting in the release of volatile sulphur compounds. The sulphoxide precursors are synthesised from glutathione *via* γ-glutamyl peptides, by a pathway involving the chloroplasts and stored in various parts of the plant.[39] The *S*-alkyl-L-cysteine sulphoxide lyase enzymes of *Allium cepa* (onion) and *Allium sativum* (garlic) have been found to differ in physical and kinetic properties[40] although they catalyse analogous reactions, *viz.*, the hydrolysis of *S*-1-propenyl, *S*-propyl, and *S*-methyl cysteine sulphoxides in *Allium cepa* and of *S*-allyl, *S*-propyl, and *S*-methyl cysteine sulphoxides in *Allium sativum*.

In one of the few literature references to *Tulbaghia violacea*, Jacobsen *et al.*[41] report the presence, in this plant, of a carbon-sulphur lyase enzyme whose action is similar to that of the lyases in the various *Allium* species. The presence of three unidentified sulphoxide amino acids is reported by Jacobsen,[42] who also referred to an unsuccessful attempt to analyse the volatiles from *Tulbaghia violacea*. These observations are of significance in the taxonomic relationships of the genera in the Alliaceae, and in fact give support to the modern classification of *Tulbaghia* in the Alliaceae rather than in the Liliaceae as previously.[3]

The biological activity of the *Allium* species, and in particular of *Allium sativum* (garlic) is renowned world-wide, and has been known since as long ago as 1550 B.C. when the ancient Egyptians valued garlic for its therapeutic properties in treating many ailments.[8] *Alliums* have been used in various communities for their antiseptic, antibiotic, antihelmintic, and antithrombotic properties, and a vast amount of research, mostly on garlic, in recent years[43;44] has led to the elucidation of a complex and remarkable set of chemical processes responsible for these properties. Some major results of this work are highlighted in this survey, and thus Scheme 1.2 shows the pathways by which alliin (17) (*S*-alkyl-L-cysteine sulphoxide) in garlic is transformed into various derivatives. Many of these products exhibit notable biological activity. As a point of interest, alliin (17) was the first isolated natural product to have chirality at a sulphur atom.

When garlic is extracted at room temperature, the major product is allicin (18) (alkyl-2-propene-1-thiosulphinate),[45;46] which is formed by enzymic action on alliin (17) in the crushed tissue, and which has marked antibacterial, antiviral, and antifungal activity. Allicin is very unstable and can undergo a variety of transformations[47] which result in products such as the ajoenes (19) and (20).[48] These compounds are strongly antithrombotic due to their ability to inhibit platelet aggregation by binding sulphydryl groups and thus altering the platelet membranes.[49]. The dithiins (21) and (22) are also found to be mildly antithrombotic. Allyl disulphide (23), which is largely responsible for the odour of garlic, can itself be transformed thermally into allyl trisulphide and higher analogues, and into a range of products such as compounds (24), (25), and (26).[50] Compounds such as these three, and the dithiins (21) and (22), are among the components which are responsible for the antioxidant and lyoxygenase inhibitory activity of garlic oils.[50]

Scheme 1.2 Transformations of sulphur compounds from garlic[46-51]



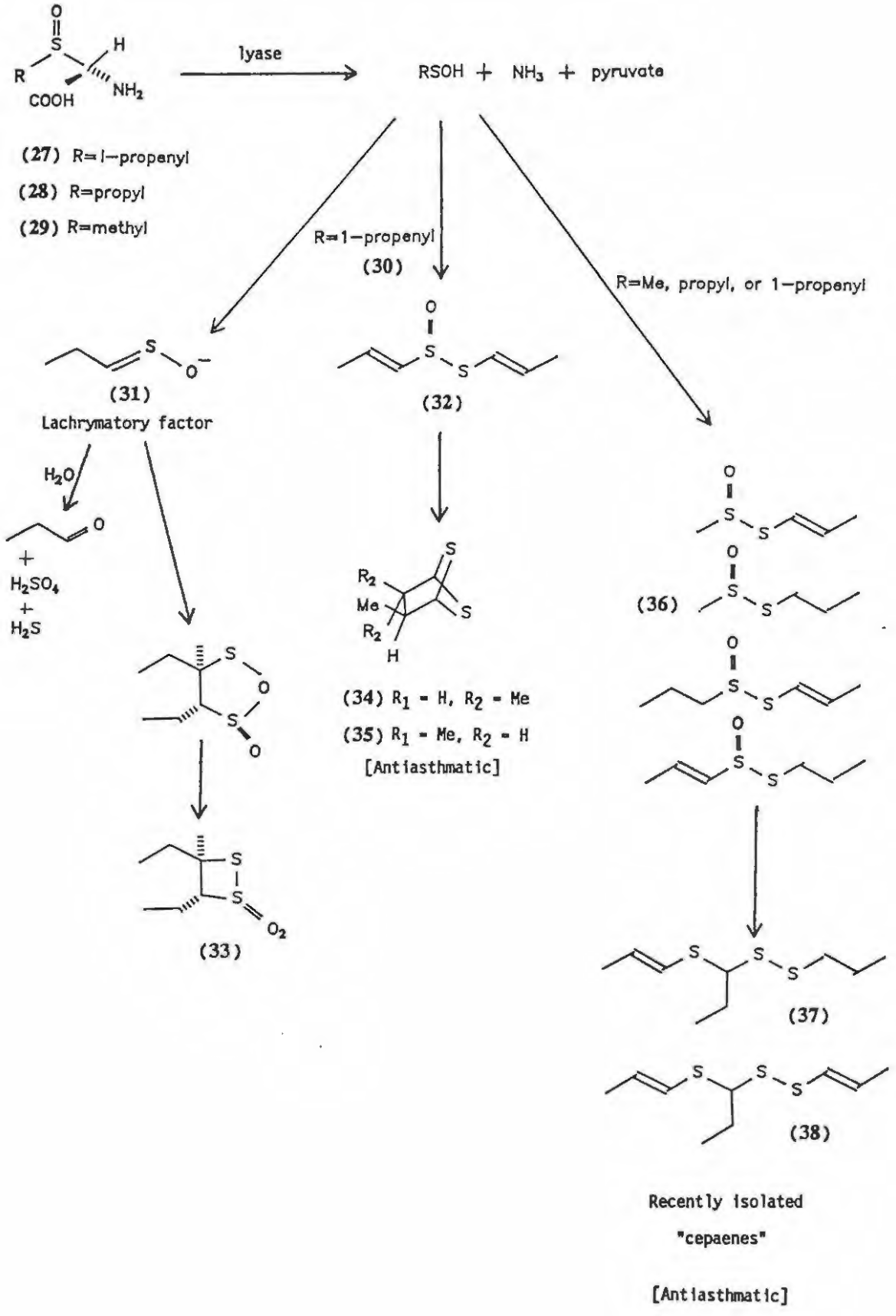
This explains the fact that even aged or heated garlic extracts retain their antithrombotic activity in spite of no longer being antibacterial. These compounds are also responsible for the anti-asthmatic and anti-allergic action reported for garlic oils.[51]

In onion, reactions similar to those of garlic constituents (Scheme 1.3) produce the lachrymatory factor (31) which itself is transformed by self-condensation into the dithietane dioxide (33).[52] The anti-asthmatic compounds (34) and (35), also isolated from onion, are considered to be formed by rearrangement of 1-propenyl-1-propene thiosulphinate (32).[53]

This compound (32) has not been isolated but recently[53] the thiosulphinates (36) have been isolated, together with the anti-asthmatic compounds (37) and (38).[51] Since the alkyl thiosulphinates in the *Alliums* can be transformed into alkyl disulphides and other volatile products, analysis of these volatiles is a useful means of identifying the precursors.[54] Analyses of this type have indicated the presence of propyl, methyl and 1-propenyl thiosulphinate compounds in various *Allium* species *e.g. A. tuberosum*,[55] *A. fistulosum* varieties and *A. chinense*[56] and *A. cepa* and *A. sativum*.[57]

Thus the long-standing medical use of *Allium* species has been substantiated by modern research, and the biological activity of the sulphur compounds in them has proved to be of great value and interest.

Scheme 1.3 Transformations of sulphur compounds from onions[52;53]



1.4 Objectives

The aim of the project was to identify the active principles in *Tulbaghia violacea* and thus elucidate its effects as a herbal remedy. By reference to the chemical literature on the presence of biologically active compounds in plants related to *Tulbaghia violacea*, certain classes of compounds were identified as possible chemical constituents of *Tulbaghia violacea*. In the light of this information, specific objectives were developed as detailed in the following paragraphs.

1. Having regard for the difficulties often encountered in the isolation of workable amounts of natural products, a primary objective was the synthesis of a range of model compounds to be used for chromatographic comparison with constituents extracted from the plant.

2. A second objective was the isolation and/or identification of constituents of *Tulbaghia violacea* using various chromatographic and spectroscopic techniques.

3. In view of the obvious presence of odorous volatile compounds in *Tulbaghia violacea*, the objective was set of analysing these volatiles and monitoring variation over an extended period of time.

4. Examination of the biological activity of *Tulbaghia violacea* extracts was to be carried out for comparison with the reported effects of the herbal remedy.

2.1 SYNTHESIS OF MODEL COMPOUNDS

A range of commonly occurring flavones and flavonols were required for chromatographic comparison with extracts from *Tulbaghia violacea*. Those selected for synthesis are detailed in Table 2.1 (p. 31). The available methods of synthesis were ascertained by survey of the chemical literature.

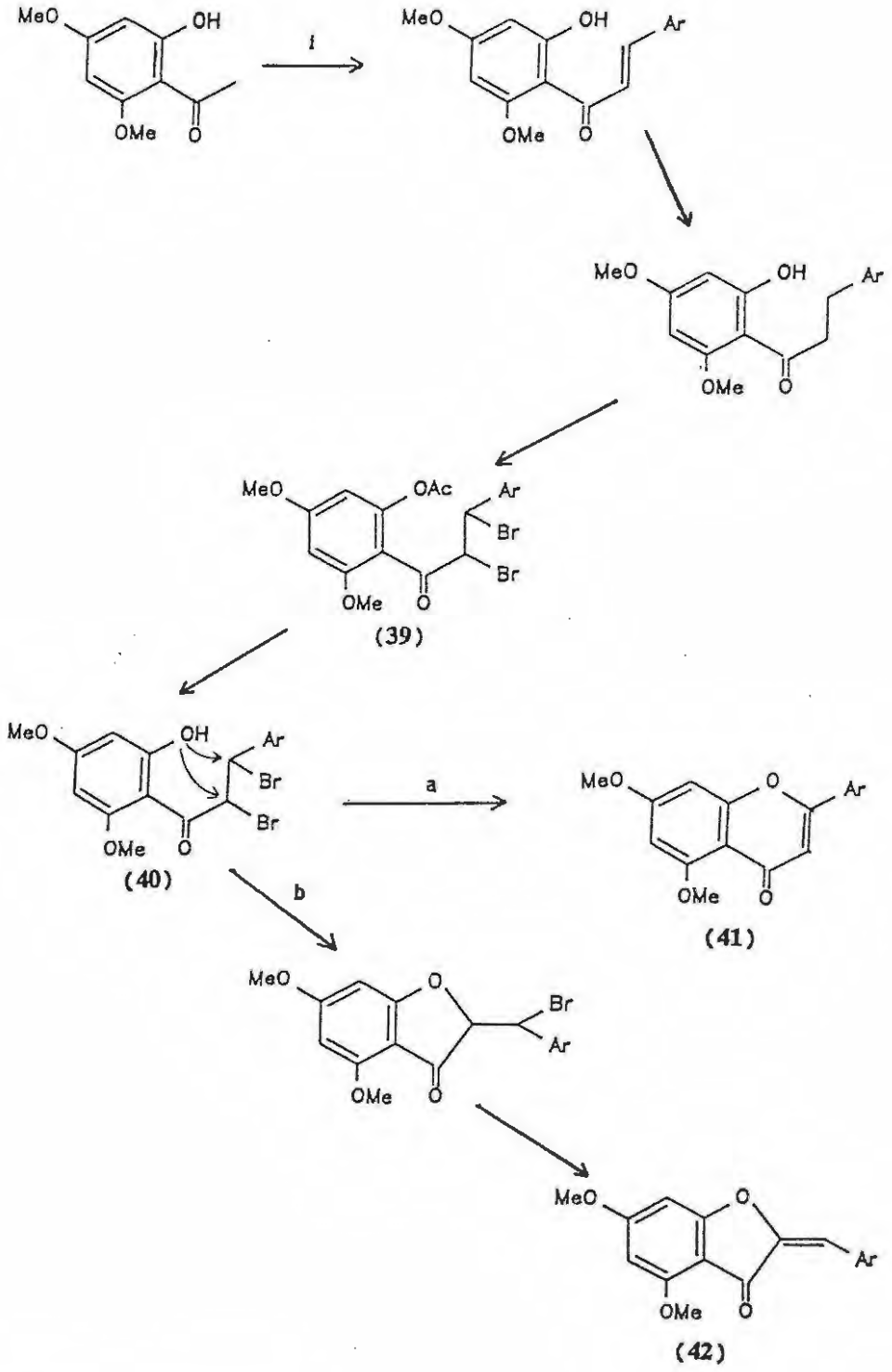
2.1.1 Review of literature methods for flavone synthesis

The development of syntheses for flavones was initiated in the late 1890's.[59] Pioneering work by Kostanecki[60] involved formation of *o*-acetylchalcones from *o*-hydroxyacetophenones and aromatic aldehydes. The chalcones were then brominated, and cyclisation with loss of hydrogen bromide afforded flavones (41) (Scheme 2.1; route a). However, a second product, the 1-benzylidenecoumaran-2-one (42) could also be formed, *via* a different cyclisation (Scheme 1; route b). This is dependent on differences in the reactivity of the electrophilic centres [C-2 and C-3, (40)] arising from the presence of electron-withdrawing substituents in the 3-phenyl group.

Cullinane and Philpott[61] later showed that *o*-hydroxychalcones are converted to benzylidenecoumaranones (42) more readily than to flavones. Hutchins and Wheeler[62] established that *o*-hydroxy and *o*-alkoxy substituents in either aromatic ring of the intermediate (40) favour this conversion. Flavones are formed by heating the *o*-hydroxychalcone dibromides (39) above their melting points, or by adding potassium cyanide. Bhagat and Wheeler[63] recorded successful syntheses of flavones by treating chalcone dibromides (39) with hydrogen bromide in acetic acid.

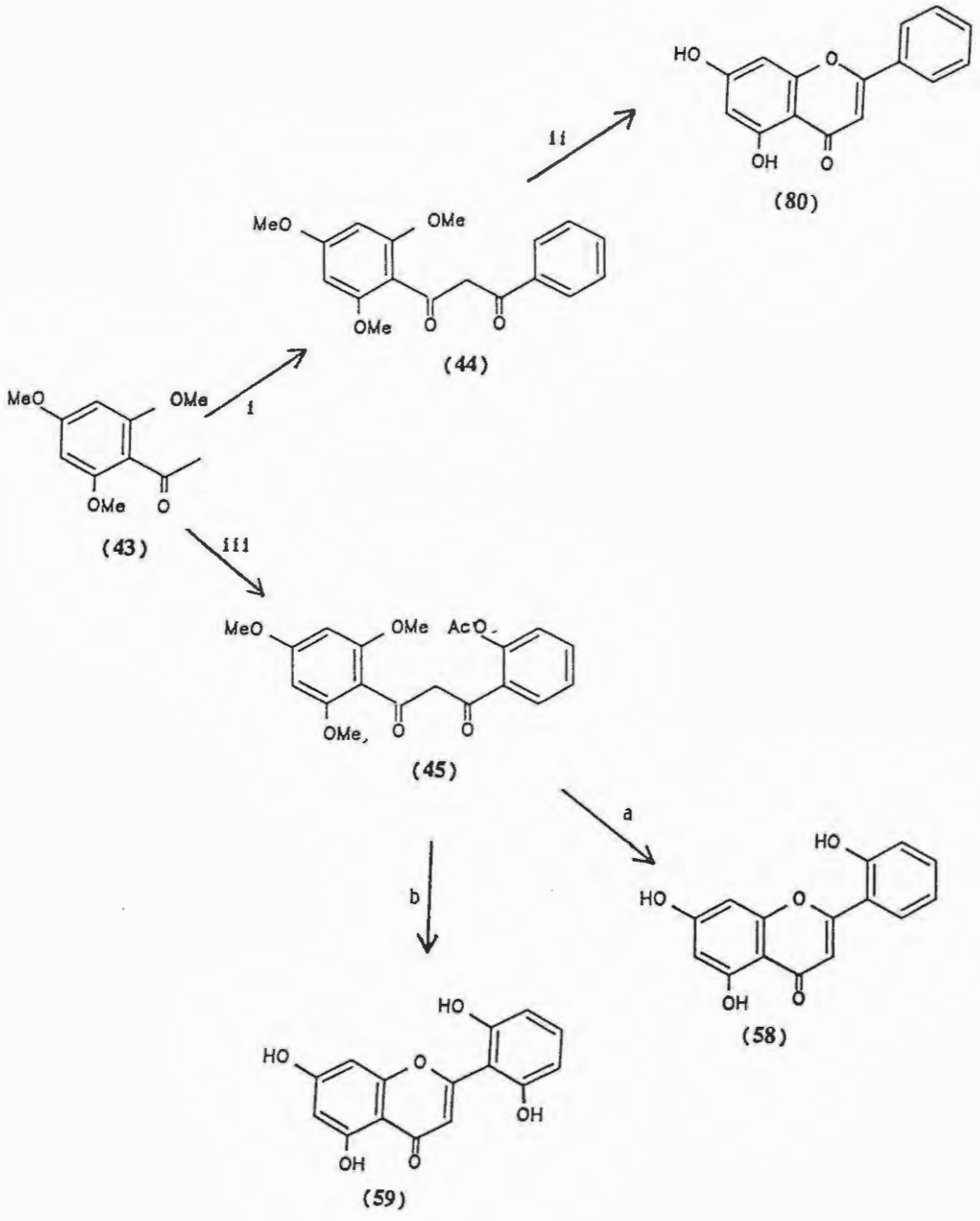
A second approach by Kostanecki and co-workers,[64;65] illustrated in Scheme 2.2, required formation of a β-diketone precursor (44) by Claisen condensation of the acetophenone (43) with an aromatic carboxylate ester (step (i) or iii). Dealkylation and cyclisation were induced by treatment with hydriodic acid. In the case of the intermediate (45), however, nucleophilic attack by either of the two *ortho*-substituent oxygens is feasible, resulting in a mixture of

Scheme 2.1 Flavone synthesis by Kostanecki *et al* (1)



Reagents : 1, Ar-CHO,OH⁻

Scheme 2.2 Flavone synthesis by Kostanecki *et al* (2)



Reagents: i. C_2H_5-O-CO-C_6H_5, Na/EtOH; ii. HI;
iii. C_2H_5-O-CO-C_6H_4($OCOCH_3$), Na/EtOH

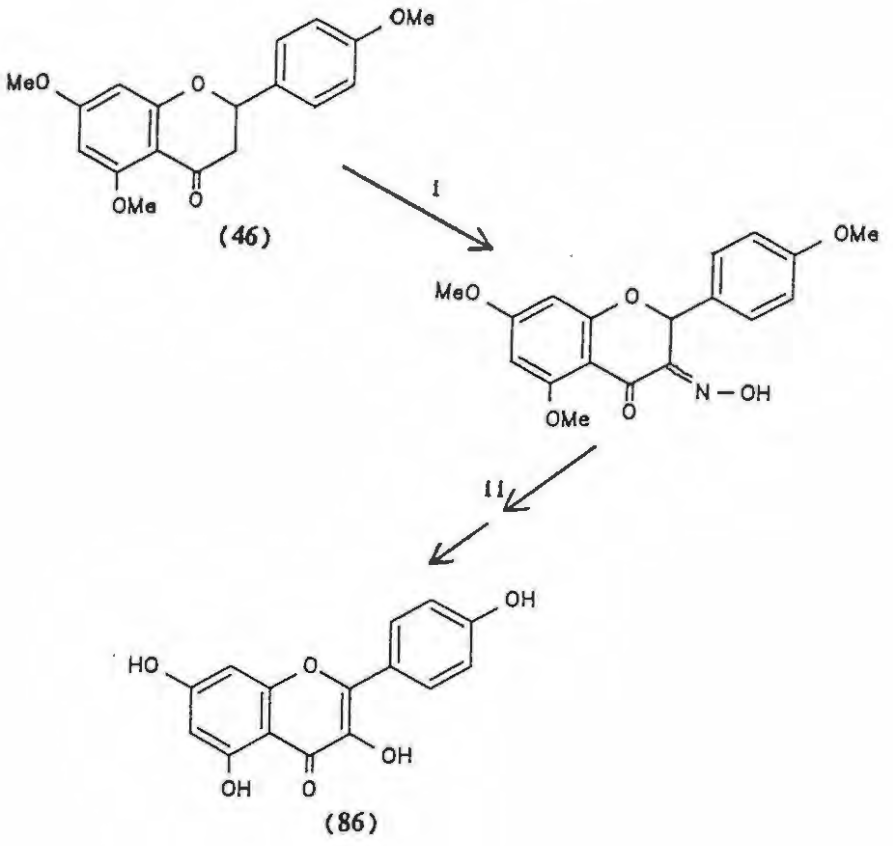
products (Scheme 2.2, route a; b). Kostanecki and Lampe[66] used a nitrosation reaction at position 3 of a methoxyflavanone [(46); Scheme 2.3] to prepare kaempferol (86) *via* its trimethyl ether. The same authors also synthesised chrysin (80) and apigenin (83)[67] by dibromination, and subsequent dehydrobromination, of methoxyflavanones (see Table 2.1, p. 31 for flavone structures).

In attempting to prepare 2,4-dibenzoylresacetophenone, Baker[68] found that *o*-aroylacetophenones (47) rearrange in the presence of base, giving *o*-hydroxy-ω-benzoylacetophenones (48). An intramolecular Claisen condensation mechanism was proposed for this transformation (Scheme 2.4). Ring closure of the β-diketones (48) produced flavones (49). This rearrangement was also described by Venkataraman and is known as the Baker-Venkataraman transformation.[98]

By applying the findings of Baker[68] and Kostanecki,[64] Robinson and co-workers[69] developed a direct and adaptable method for flavone synthesis (Scheme 2.5). An *o*-hydroxyacetophenone (50) is heated with the anhydride and the sodium salt of an aromatic carboxylic acid. Thermal condensation of the intermediate (51) is followed by alkaline hydrolysis to give flavones (53). The reaction may proceed by two possible pathways (routes a and b).[68] Route a is regarded as the most likely, with the sodium salt of the acid catalysing the rearrangement. Intermediate (52) could be further acylated, affording access to flavonols (54), and leading to the possibility of mixtures of flavones (53) and flavonols (54).

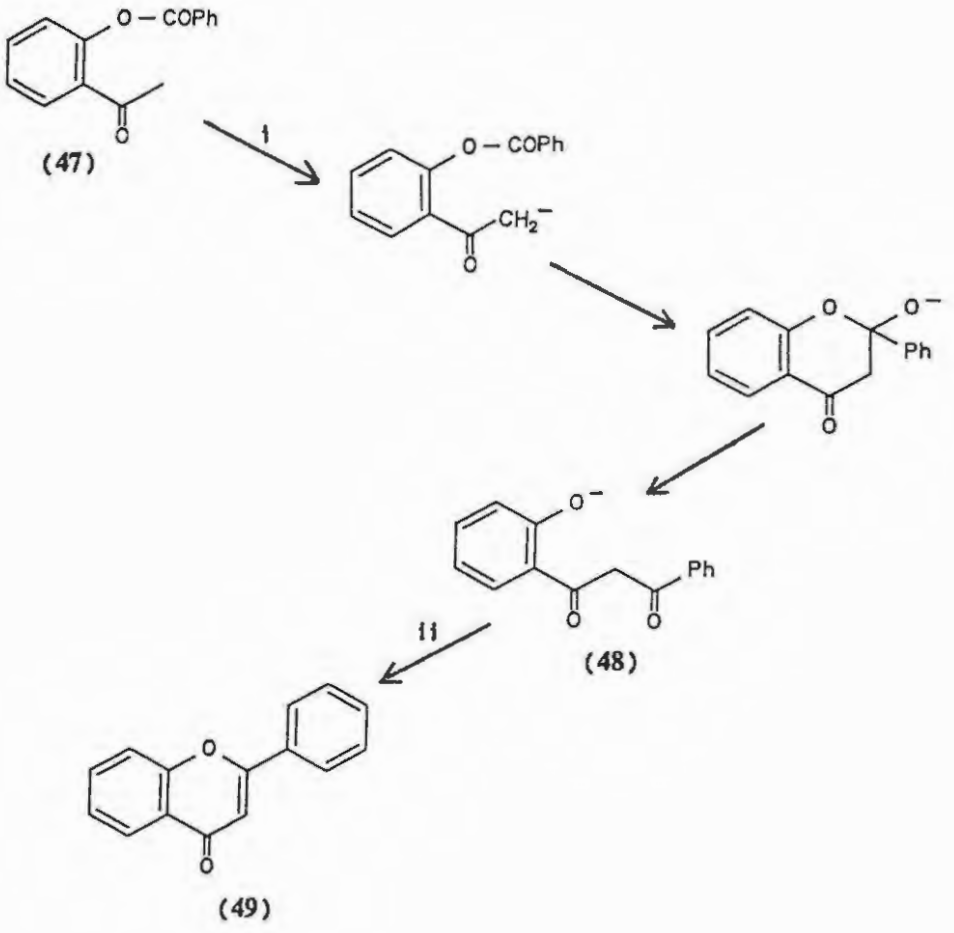
Dunne and co-workers[70] found that certain flavones could be synthesised by heating the *o*-aroylacetophenones (51) in anhydrous glycerol to 250°C. They suggested that the Baker-Venkataraman transformation was initiated by thermally induced loss of the proton α to the carbonyl. More recent work by Teoule and others[71;72] led to the synthesis of flavones by condensation of phenols and benzoylacetates at very high temperatures and reduced pressures. The method can be extended to the synthesis of flavone glycosides by condensation of the *o*-benzylated flavone (56) with acetylated bromoglucose (Scheme 2.6).[73]

Scheme 2.3 Flavone synthesis by Kostaneckl and Lampe



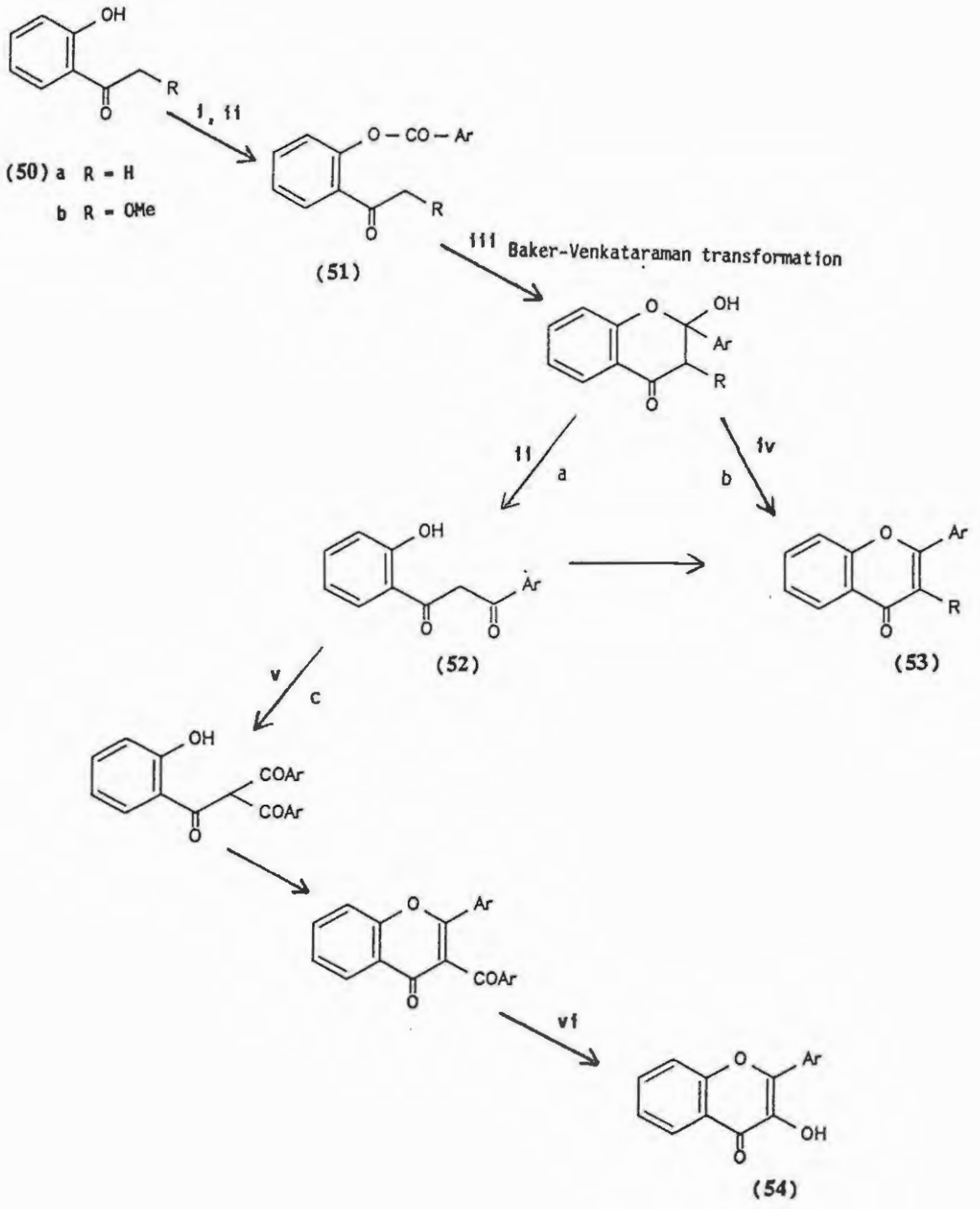
Reagents : i, Amyl nitrite,HCl; ii, AcOH,H_2SO_4

Scheme 2.4 The Baker-Venkataraman rearrangement



Reagents : i, Base; ii, conc. H_2SO_4 or AcOH,NaOAc

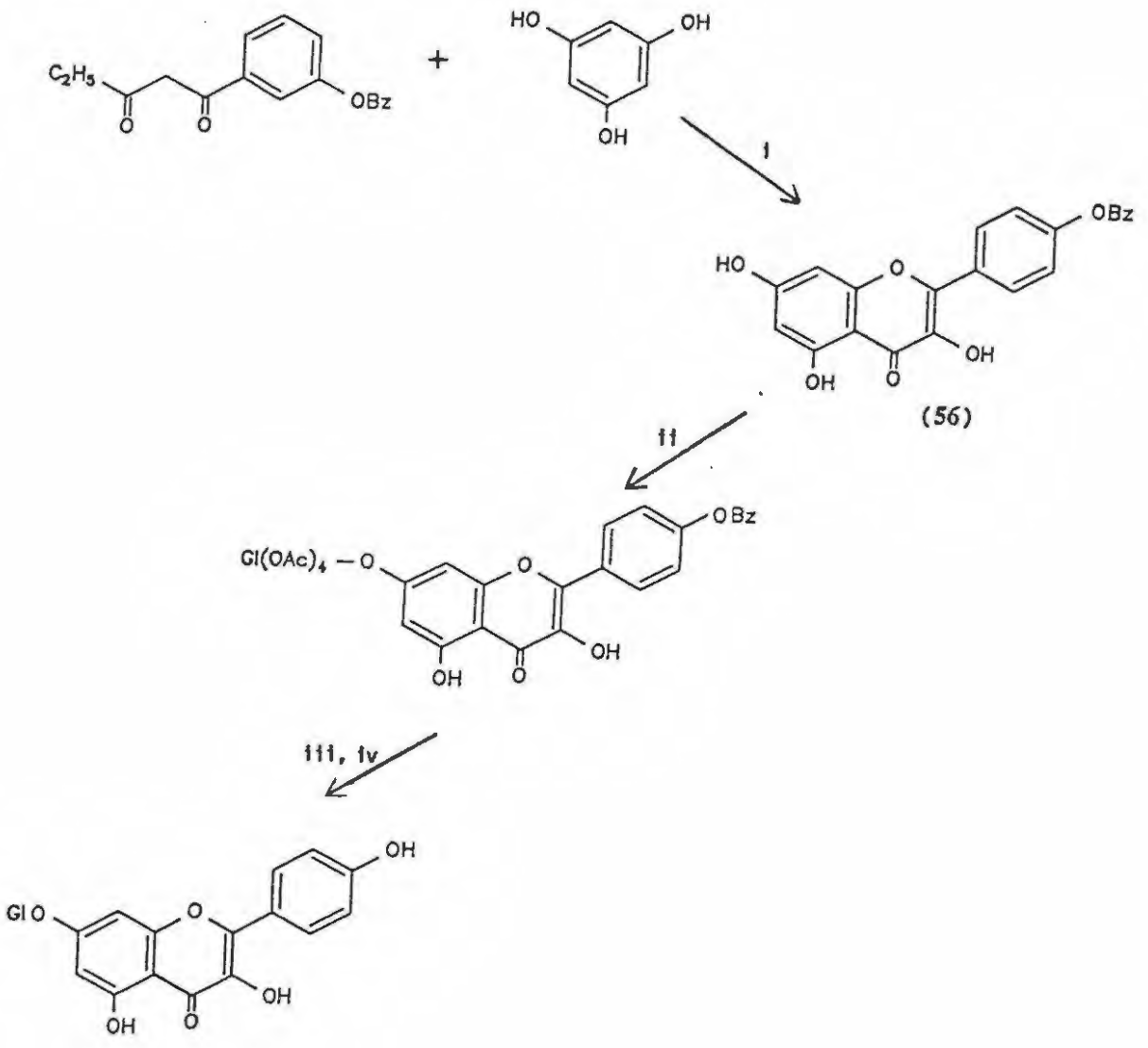
Scheme 2.5 The Kostanecki-Robinson synthesis of flavones



Reagents : i, $(ArCO)_2O$; ii, $Na^+ArCO^-{}_2$; iii, heat (Baker-Venkataraman rearrangement); (55)

iv, base, $-H_2O$; v, $(ArCO)_2O$; vi, base, $-H_2O$

Scheme 2.6 Flavone synthesis by Teoule *et al*



Reagents : i, 230°, 18 mmHg; ii, 2,3,4,6-tetra-*O*-acetyl-1-bromo-D-glucopyranose

iii, H_2-DME ; iv OH^-/H_2O

2.1.2 Synthesis of flavones and flavonols

For most flavones required in the present investigation, the Kostanecki-Robinson synthesis was found to be the most suitable. The method is convenient and the starting materials, carboxylic acid anhydrides and substituted acetophenones, are readily accessible. The general synthetic approach is outlined in Scheme 2.5 (p. 28). Individual starting materials and products are shown in Table 2.1.

2.1.2.1 Synthesis of carboxylic acid anhydrides

Several different aromatic carboxylic acid anhydrides were required, and a method for their preparation was necessary.

In their original work, Robinson and co-workers[74;75] found that boiling the carboxylic acid with acetic anhydride gave the aromatic carboxylic anhydride in acceptable yields. However, this method takes several days, and the possibility of mixed anhydride formation exists. Alternative methods involved use of carboxylic acid chlorides[69] or phosgene.[76]

More recently, numerous anhydride syntheses have been developed, many of which utilise sophisticated reagents and conditions. A few examples are : use of homogenous palladium(ii), in the form of PdOAc, to catalyse carbonylation of aryl halides with carbon monoxide in DMF;[77] treatment of carboxylic acids with thionyl chloride on a 4-vinylpyridine polymer support;[78] reaction of *N,N,N',N'*-tetramethylchloroformamidinium chloride in dichloromethane at -30°C;[79] and utilisation of the phosphorus reagent $PhOP(O)Cl_3$ in $POCl_3$.[80]

A more convenient method for anhydride synthesis was considered desirable. Consequently, the opportunity was taken to extend earlier studies using a supported phosphorus pentoxide reagent.[81;82] Application of the reagent led to the development of a successful general method for the preparation of anhydrides.[83]

Table 2.1 Precursors and products in synthesis of model compounds

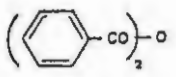
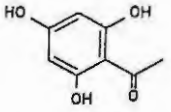
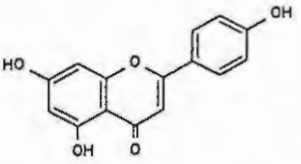
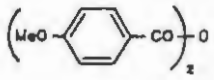
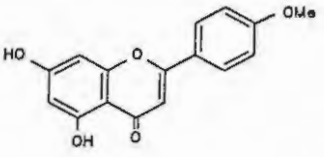
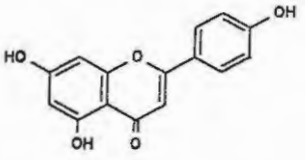
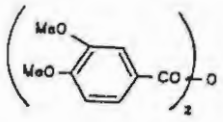
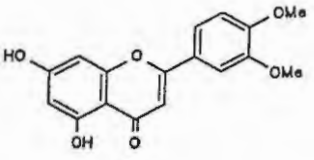
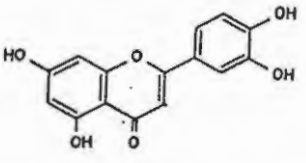
Anhydride precursor	Ketone precursor	Product	Yield (%)	m.p. (°C) observed	m.p. (°C) lit.
 (60)	 (72)	 (80)			
Benzoic anhydride	Phloracetophenone	Chrysin	81	282 - 285	285 - 286[74]
 (61)		 (82)			
Anisic anhydride	Phloracetophenone	4' -*O*-Methylapigenin	35	260 - 263	261[74]
		 (83)			
		Apigenin	35	340 - 344	348 - 350[74]
 (63)		 (84)			
Veratric anhydride	Phloracetophenone	3' ,4' -Di-*O*-methylluteolin	33	256 - 258	258 - 259[84]
		 (85)			
		Luteolin			

Table 2.1 Precursors and products in synthesis of model compounds (contd)

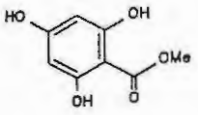
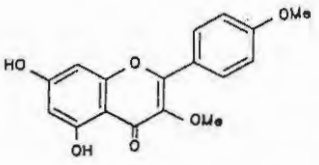
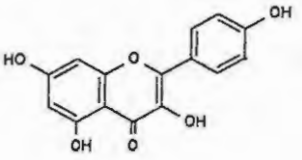
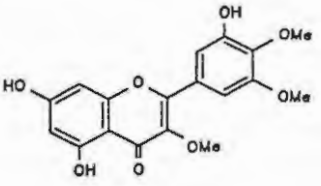
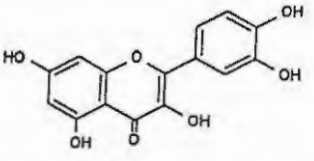
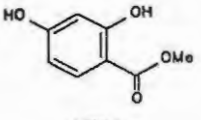
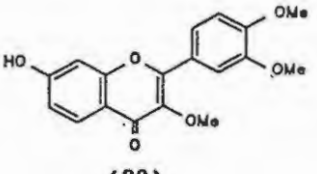
Anhydride precursor	Ketone precursor	Product	Yield (%)	m.p. (°C) observed	m.p. (°C) lit.
	 (74)	 (86)			
Anisic anhydride	ω-Methoxyphlor-acetophenone	3,4′-Di-*O*-methylkaempferol	40	230 - 231	235[98]
		 (87)			
		Kaempferol	90	276 - 278	279 - 280[98]
		 (88)			
Veratric anhydride	ω-Methoxyphlor-acetophenone	3,3′,4′-Tri-*O*-methylquercetin	39	238 - 240	240 - 245[100]
		 (89)			
		Quercetin	86	312 - 314	312 - 316[100]
	 (73)	 (90)			
Veratric anhydride	ω-Methoxyres-acetophenone	3,3′,4′-Tri-*O*-methylfisetin	23	218 - 220	222[100]

Table 2.1 Precursors and products in synthesis of model compounds (contd)

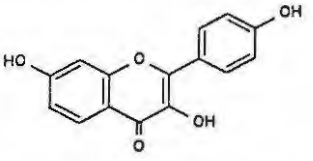
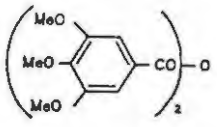
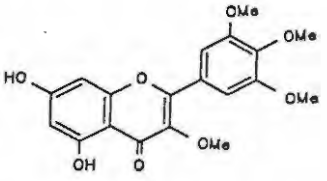
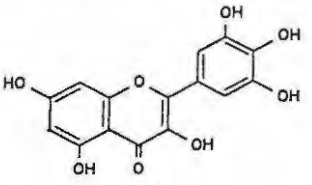
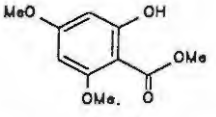
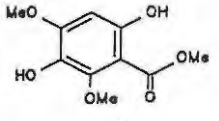
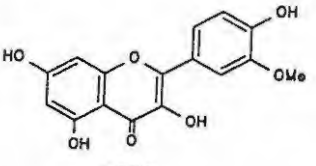
Anhydride precursor	Ketone precursor	Product	Yield (%)	m.p. (°C) observed	m.p. (°C) lit.
		 (91) Fisetin	69	345 - 350	348[98]
 3,4,5-trimethoxy benzoic anhydride	ω-Methoxyphlor-acetophenone	 (92) 3,3′,4′,5′-Tetra-*O*-methylmyricetin	3	268 - 270	276 - 277[101]
		 (93) Myricetin			
		 (77) 2′-Hydroxy-2,4′,6′-trimethoxyacetophenone			
Veratric anhydride		 (79) 2′,5′-Dihydroxy-2,4′,6′-trimethoxyacetophenone			
		 (94) 3,3′,4′,5,7-Penta-*O*-methylquercetagetin			

Table 2.1 Precursors and products in synthesis of model compounds (contd)

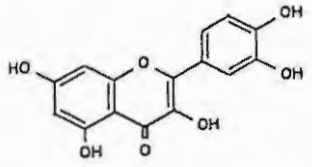
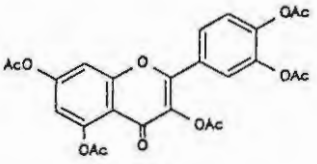
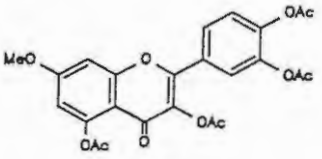
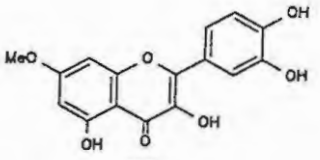
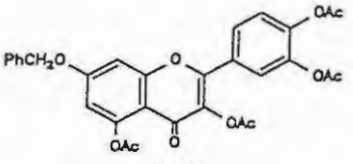
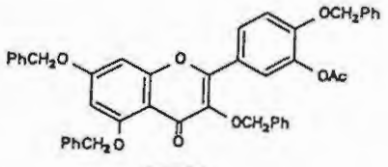
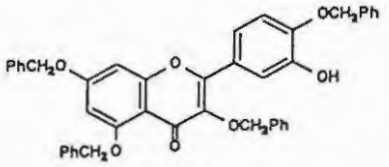
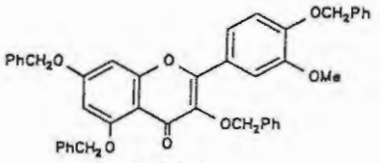
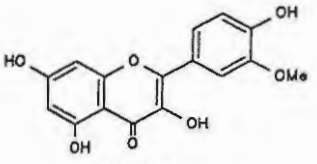
Flavone precursor	Product	Yield (%)	m.p. (°C) observed	m.p. (°C) lit.
	 (96)			
Quercetin	3,3′,4′,5,7-Penta-*O*-acetylquercetin	64	190 - 192	191 - 195[100]
(96)	 (97)			
	3,3′,4′,5-Tetra-*O*-acetylrhamnetin	40	189 - 190	189 - 190[103]
(97)	 (98)			
	Rhamnetin	56	291 - 294	294 - 296[103]
(96)	 (99)			
	3,3′,4′,5-Tetra-*O*-acetyl-7-*O*-benzylquercetin	57	162 - 163	163[103]
(99)	 (100)			
	3′-*O*-Acetyl-3,4′,5,7-tetra-*O*-benzylquercetin	34	175 - 176	176[103]

Table 2.1 Precursors and products in synthesis of model compounds (contd)

Flavone precursor	Product	Yield (%)	m.p. (°C) observed	m.p. (°C) lit.
(100)	 (101) 3,4′,5,7-Tetra-*O*-benzylquercetin	73	165 - 166	166.5 [103]
(101)	 (102) 3,4′,5,7-Tetra-*O*-benzyl-3′-*O*-methylquercetin	37	117 - 120	126 - 127 [103]
(102)	 (103) Isorhamnetin	79	303 - 305	305 - 306 [103]

The procedure involved addition of a supported phosphorus pentoxide reagent (SICAPENT®) to a solution of a carboxylic acid in dry solvent, and heating the stirred mixture for one hour at *ca.* 100°C. Typical experimental details are given in the Experimental section (pp.132-6). In each case, 0.1 moles of the carboxylic acid was dissolved in 60 - 90 ml of solvent, and 15 g of Sicapent® was then added. The general applicability of the method was established by use of various carboxylic acid substrates, as shown in Table 2.2, including aliphatic, unsaturated, and aromatic analogues.

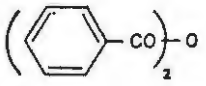
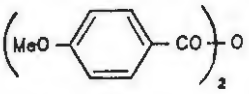
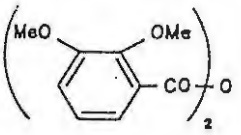
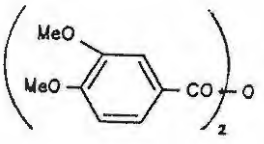
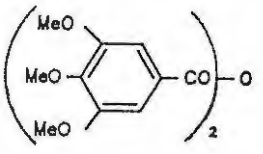
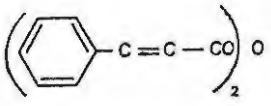
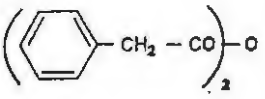
The suitability of three different solvent systems was investigated, *viz.* benzene, 1,2-dimethoxyethane (DME), and toluene. Use of toluene, or preferably benzene, resulted in less charring of the reaction mixtures and afforded cleaner products after filtration and evaporation. However, the toxicity of benzene is a disadvantage, and although toluene is more difficult to remove owing to its higher boiling point, it was the solvent of choice in most cases. DME was only useful in certain cases, due to the insolubility of some carboxylic acids in this solvent.

In each case, after heating, the reaction mixture was filtered and a small amount of anhydrous potassium carbonate was added to the filtrate in an attempt to remove residual carboxylic acid as the insoluble potassium salt. Charcoal was added to decolourise the filtrate. Filtration through celite was followed, in some cases, by further filtration through alumina to remove unreacted carboxylic acid. Products were recovered by evaporation of the solvents under reduced pressure, and recrystallised or distilled to afford the anhydrides.

The conversion of carboxylic acids to anhydrides was followed using ^{1}H n.m.r. spectroscopy, by monitoring the disappearance of carboxylic acid proton signals. In certain cases the ^{1}H n.m.r. spectra were used to calculate the percentage conversion of carboxylic acid to anhydride. [(65), (67); Table 2.2]

Table 2.2 Preparation of Carboxylic Acid Anhydrides

$$2\ RCO_2H \longrightarrow (RCO)_2O + H_2O$$

Product $(RCO)_2O$		Solvent	Yield (%)	m.p. (°C) or b.p. (°C/mmHg) observed	lit.
R =	Compound				
	(60)	benzene	77	40 - 41	42 - 43[85]
		toluene	74		
	(61)	toluene	69	96 - 97	99[85]
		DME	56		
	(62)	toluene	60	66 - 67	97[84]
	(63)	toluene	62	122 - 124	124 - 125[86]
	(64)	toluene	23	158 - 159	159[87]
	(65)	DME	75	135 - 137	138[86]
	(66)	toluene	56	70	71 - 72[85]
		DME			
$[(CH_3(CH_2)_5CO)]_2O$	(67)	benzene	49	118/0.05	186/15[85]
		DME	57		

The identity of the products was established by i.r. and ^{1}H n.m.r. spectroscopy, and by their melting points. The i.r. spectra of carboxylic acid anhydrides show two strong characteristic absorption bands in the region 1870 - 1725 cm^{-1} separated by *ca.* 60 cm^{-1}, with aromatic and α,β-unsaturated carboxylic acid anhydrides absorbing at slightly lower frequencies than saturated analogues. In addition, the lack of a broad absorption band at *ca.* 3000 cm^{-1} confirmed the absence of the carboxylic acid.

Although the procedures had not been optimised, yields for the isolated products varied from moderate to good, as shown in Table 2.2. In the case of 3,4,5-trimethoxybenzoic acid anhydride,(64) the carboxylic acid starting material did not dissolve fully in the toluene initially, and this may account for the low yield obtained.

In preparations where DME was used as the solvent, the crude product was found, in each case, to contain a contaminant which was tentatively identified as the methyl ester of the carboxylic acid. This was evident from an additional peak in the ^{1}H n.m.r. spectra of the crude products, at *ca.* 3.6 - 3.8 ppm (the region typical for methyl ester proton signals). Percentages of these contaminants were estimated from the relative integrals to be : methyl anisate (8%), methyl cinnamate (12%), methyl octanoate (9%), and methyl phenylacetate (*ca.* 30%).

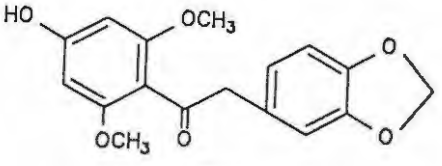
In the preparation of 2,3-dimethoxybenzoic anhydride (62), the product was recrystallised from three different solvent systems, *viz.*, ethyl acetate, ether, and ether-hexane, and in each case the melting point of the recrystallised material was found to be 66 - 67°C. Although this is not in agreement with the literature value, 93°C,[84] the i.r. and ^{1}H n.m.r. spectra of the product confirmed its identity.

2.1.2.2 Synthesis of ω-methoxyacetophenones

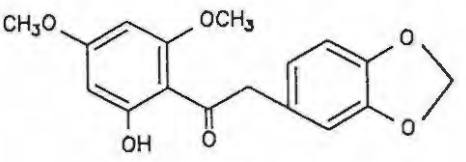
The ketone precursors required in flavonol syntheses, substituted ω-methoxyacetophenones, can be prepared by the Hoesch reaction[87;88] (Scheme 2.7). A nitrile is added to a solution of a phenol such as 1,3,5-trihydroxybenzene (68) in dry ether, and the mixture is then saturated with hydrogen chloride gas. Storage at 0°C enhances the yield of the ketimine hydrochloride salt (70). Hydrolysis in boiling water gives the ketone (71) in good yields. The mechanism proposed by Hoesch involves initial formation on an imino chloride (69), and subsequent electrophilic acylation of the phenol (68), to afford the ketone (71).[89]

ω-Methoxyresacetophenone (73) and ω-methoxyphloracetophenone (74) (Table 2.1, p. 31) were prepared in this investigation by application of the Hoesch reaction, following the method of Slater and Stephen.[88] In both cases the ketimine hydrochloride salt crystallised after several hours of bubbling with hydrogen chloride, at 0°C, in yields of 30% and 56% respectively. Hydrolysis was facilitated by prior removal of as much ether as possible. (Yields could be reduced by loss of material to any ether layer present.) Yields for the hydrolysis were 56% for ω-methoxyresacetophenone (73) and 76% for ω-methoxyphloracetophenone (74).

Yields in the Hoesch synthesis are generally increased by addition of Lewis acid catalysts such as zinc chloride, aluminium chloride or ferric chloride.[89] In certain cases, however, the nature of the product itself is found to depend on the catalyst employed. In the reaction between dimethoxyphenol and piperonylonitrile,[89] both products (75) and (76) are possible. In the presence of ferric chloride both products are formed, but in the presence of zinc chloride, only compound (75) is formed.

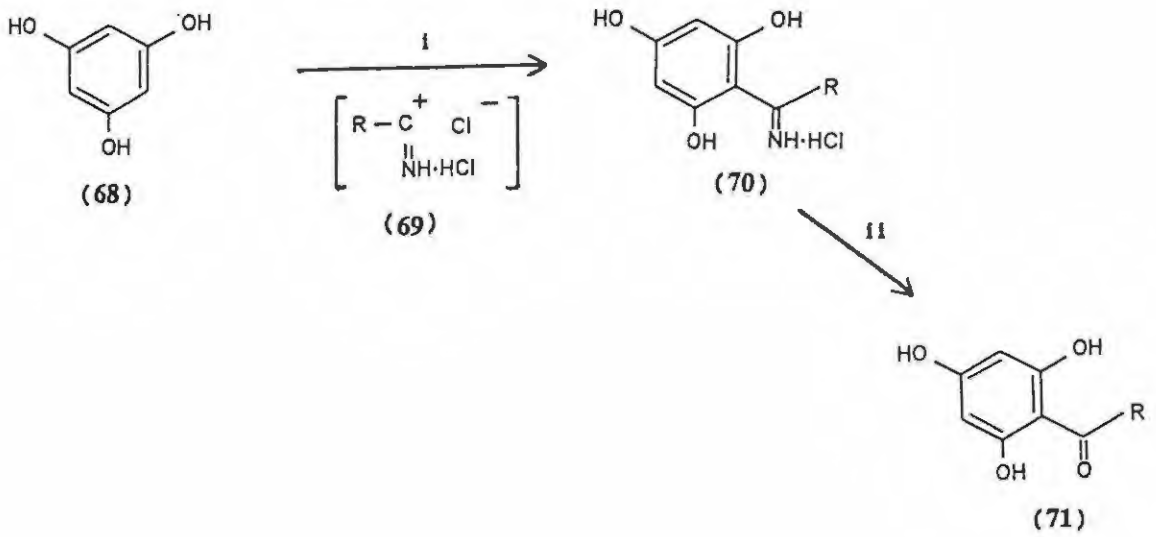


(75)



(76)

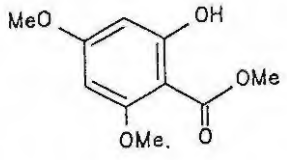
Scheme 2.7 The Hoesch synthesis of ω-methoxyacetophenones



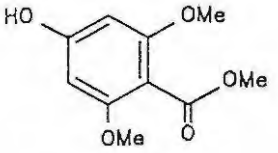
Reagents : i, RCN,HCl, dry ether, $ZnCl_2$; ii, H_2O, reflux

Various factors could be considered to influence this situation, *e.g.* Lewis acid strength, steric factors relating to the size of side chains and the Lewis acid, and electronic effects of hydroxyl and methoxyl groups which may preferentially activate *ortho*- or *para*-positions.

In the reaction between 3,5-dimethoxyphenol and methoxyacetonitrile, the two products (77) and (78) are possible, the former being required for the synthesis of quercetagetin.[90]



(77)



(78)

Contrary to the results of Rao and Seshadri,[90] it was found in this investigation that in this reaction, compound (78) is the major product in the presence of zinc chloride. The ratio of products (77) and (78) was found to be 1 : 5.

2.1.2.3 Synthesis of flavones by the Kostanecki-Robinson method

This method lends itself to the preparation of many different flavone products, since the benzene rings of the precursors [(50), (55); Scheme 2.5, p. 28] may have a variety of substitution patterns. A selection of *O*-methylflavones, flavones and flavonols were prepared, with structures varying in the number and position of hydroxyl or methoxyl substituents [ranging from chrysin, 5,7-dihydroxyflavone (80), to myricetin, 3,3′,4′,5,5′,7-hexahydroxy flavone (93), as shown in Table 2.1, p. 31].

Use of methoxy-substituted aromatic carboxylic acid anhydrides allows preparation of flavones with protected hydroxyl groups. These can be deprotected later by cleavage of the methyl ether linkages. Since polyphenolic compounds are generally susceptible to oxidation, this protection is a most useful aspect of the synthetic method. Products were characterised first as *O*-methylflavones, and later demethylated.

Experimental conditions for the different preparations followed the same general procedure, with variations in duration of heating and in the relative reactant ratios in the reaction mixture. In reactions where the final product is a flavonol, the ketone precursor (50) (Scheme 2.5, p. 28) has an ω-methoxyl group. The inductive effect of this group enhances the acidity of the α-methylene protons, facilitating formation of the anion and subsequent nucleophilic attack at the ester carbonyl function. In cases where this methoxyl group is absent, for example in the preparations of chrysin (80), apigenin (83), and luteolin (85), longer periods of heating and larger amounts of anhydride were called for.[74;75] Yields in such cases can be expected to be lower than those for flavonols.

The synthesis involves thermal condensation at high temperatures (180 - 200°C) and a certain amount of charring was found to be unavoidable, particularly in cases where the reaction mixture thickened to a paste, requiring additional heating to maintain mobility, as in the syntheses of chrysin (80), kaempferol (87), and luteolin (85). Flash chromatography was found useful for separation of products from oxidised or charred material.

2.1.2.4 Demethylation of *O*-methylflavones

As a final step in the synthesis of certain flavones [*eg.* (83), (85) Table 2.1, p. 31] the removal of *O*-methyl groups was necessary. The classic method for cleavage of ethers is hydrolysis with strong acids such as hydriodic or hydrobromic acids, a reaction which has been widely used for several decades.[91] Hydriodic acid was used by Robinson and co-workers to demethylate flavone ethers, with satisfactory results.[74;75]

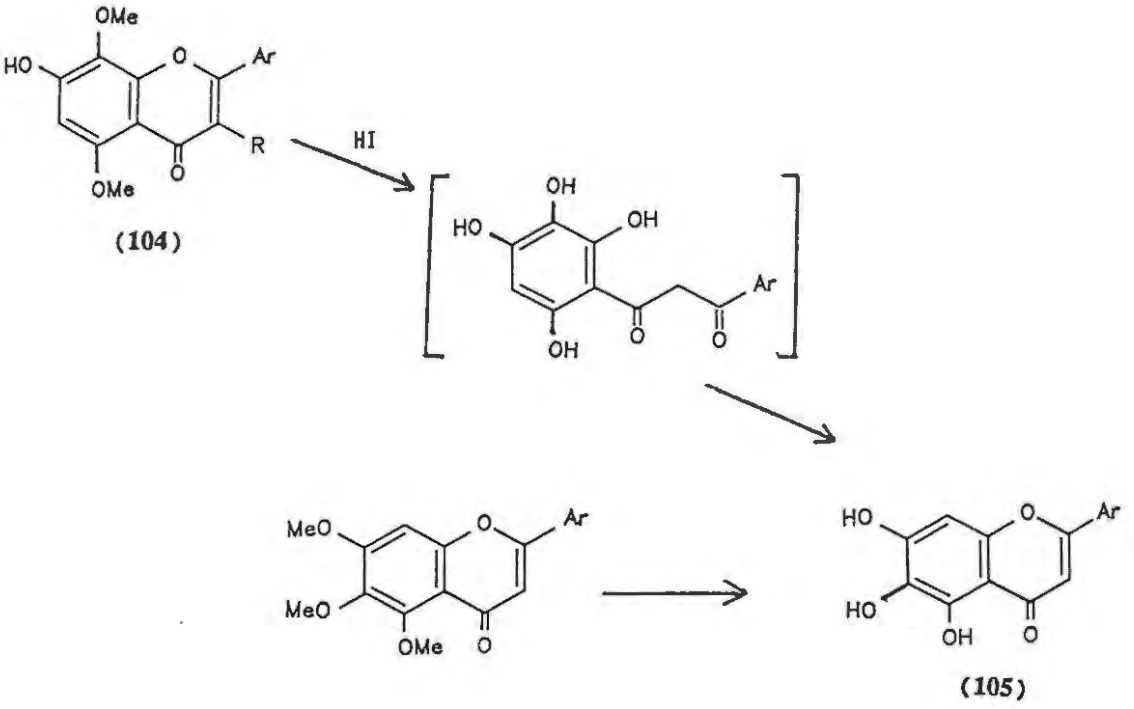
The advantage of using hydriodic acid is that all the methoxyl groups in the flavone ether substrate molecule are hydrolysed, while partial demethylation is common with use of certain other reagents. There is, however, a disadvantage in using hydriodic acid, in some cases. Thus, Wessely and Moser[92] reported a rearrangement of 7-hydroxy-5,8-dimethoxyflavone (104) involving ring opening and cyclisation to yield

5,6,7-trihydroxyflavone [(105); Scheme 2.8], and it is possible for this to occur in other cases where the A-ring has 5- and 8- methoxyl substituents.

Of the various alternative reagents available for cleavage of alkyl aryl ethers, Lewis acids such as boron trifluoride, boron tribromide and aluminium chloride have found wide application. An alternative, albeit expensive, reagent is trimethylsilyl iodide (TMSI) which efficiently cleaves alkyl aryl ethers to yield alkyl halides and aryl trimethylsilyl ethers. An *in situ* method of generating the TMSI from sodium iodide and trimethylsilyl chloride has made the method more generally accessible, and faster.[93] Methyl phenyl ethers have also been cleaved by the sodium salt of *N*-methylaniline in hexamethylphosphorus triamide,[94] by diphenyllithium phosphide,[95] and by sodium cyanide in dimethylsulphoxide.[96]

Since the preparations of some flavones [(80), (83), (85), (87), (89), (91), and (93); Table 2.1] followed the methods of Robinson and co-workers, hydriodic acid was used to demethylate the ethers, using acetic anhydride as co-solvent. In general, the reactions were successful, although yields varied widely, as illustrated by Table 2.1 (p. 31). In each reaction, the quantity of hydriodic acid added was calculated so as to be in *ca.* 50% excess, according to the number of *O*-methyl groups to be hydrolysed. A very large excess was not desirable as (a) this may allow unfavourable side reactions and (b) unused reagent may interfere with isolation of the product.[97] Acetic anhydride was added in a *ca.* 1 : 3 molar ratio with the hydriodic acid.

Scheme 2.8 The Wessely-Moser rearrangement



2.1.2.5 Synthesis of chrysin (74)

Chrysin (80) was prepared directly from phloracetophenone (72) and benzoic anhydride (60), in 81% yield. As confirmation of the structure of the product, a portion was acetylated to give di-*O*-acetylchrysin (81) in 40% yield.

The infra-red spectrum of chrysin (80) shows absorption due to the carbonyl group stretch at 1655 cm^{-1}, which is within the characteristic region (1660 - 1640 cm^{-1}) for flavones.[1] The broad peak centred at *ca.* 3000 cm^{-1} is due to hydroxyl groups which are intramolecularly bonded.

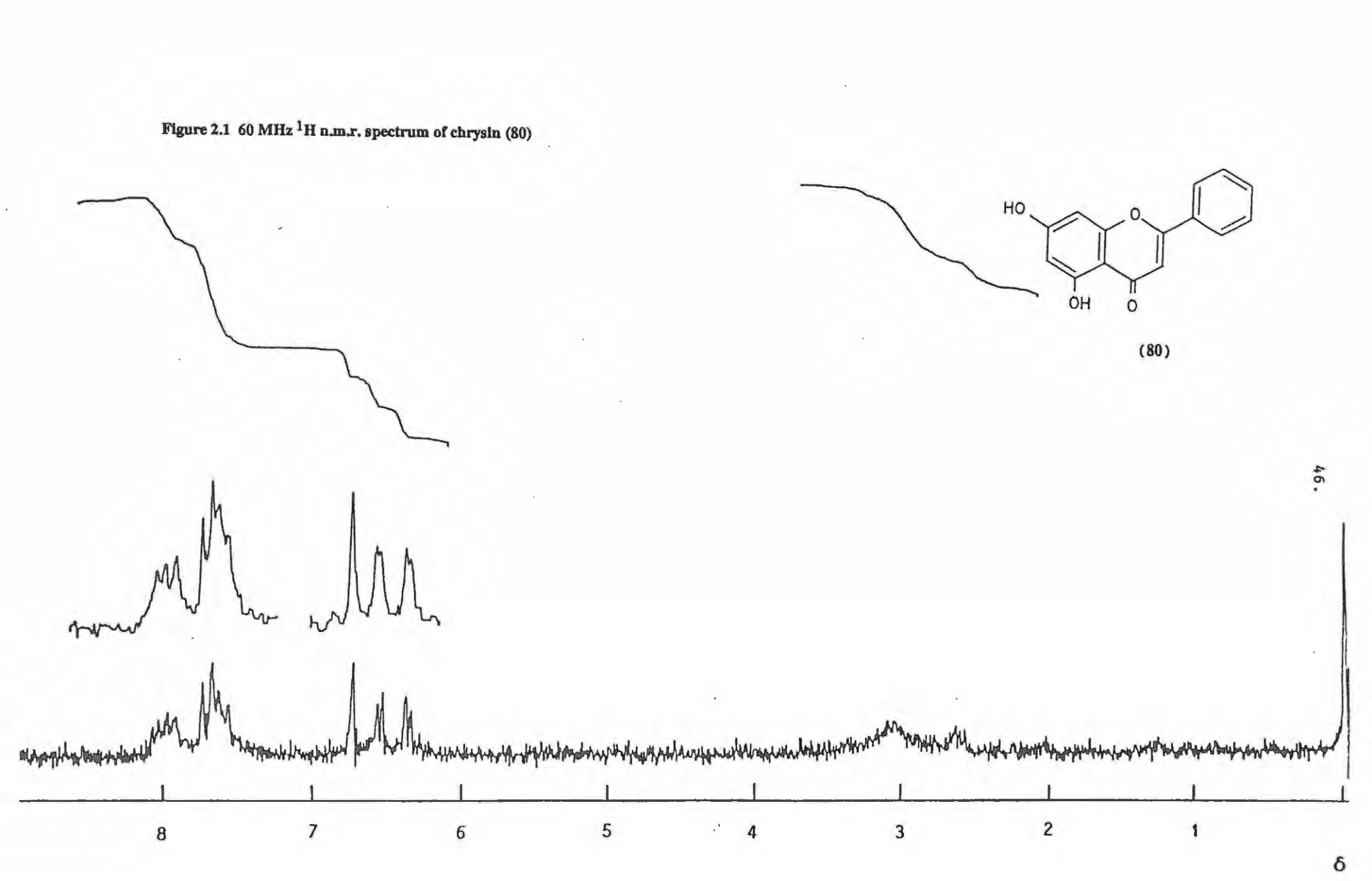
The n.m.r. spectra of flavones are frequently measured in DMSO-d_6 due to lack of solubility in chloroform or methanol. As an alternative, the tri-methylsilyl ethers of flavones can be prepared for n.m.r. spectroscopy.[2] Characteristic features of the ^1H n.m.r. spectra of flavones are : two doublets (J 2.5 Hz) in the range 6 - 6.5 ppm, due to the 6- and 8-H nuclei in 5,7-disubstituted flavones and a singlet at *ca.* 6.3 ppm due to the 3-H nucleus.[2] The B-ring protons generally have signals downfield from the protons of the A-ring, in the range 6.7 - 7.9 ppm. In keeping with these trends, the ^1H n.m.r. spectrum of chrysin (Figure 2.1) shows signals at 6.25 and 6.45 ppm due to the 6- and 8-H nuclei, at 6.72 ppm for the 3-H proton and a multiplet at 7.55 ppm for the B-ring protons.

Synthesis of Apigenin (83)

4′-*O*-Methylapigenin (82), commonly named acacetin, was prepared in 35% yield. Considerable charring of the reaction mixture was apparent after the 6 hour heating period, and flash chromatography and recrystallisation of 1.4 g of the crude product afforded only 0.6 g of the purified product.

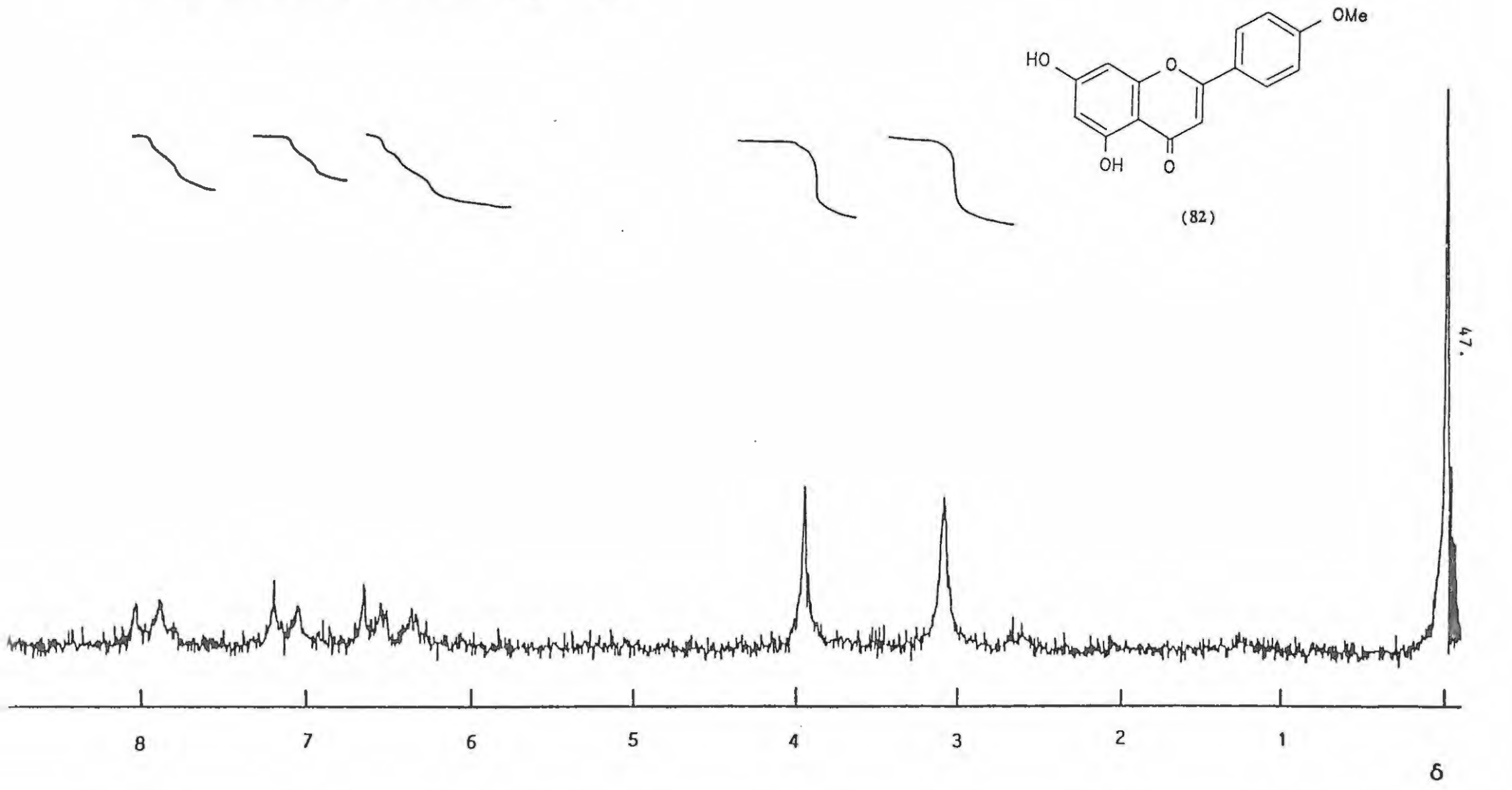
In the ^1H n.m.r. spectrum of 4′-*O*-methylapigenin (82), the B-ring protons constitute an AA′XX′ system. At 60 MHz, however, the signals resemble an AB quartet of two doublets, at δ 7.4 (Figure 2.2). The 3′- and 5′-H nuclei resonate upfield from the 2′- and 6′-H nuclei.[2]

Figure 2.1 60 MHz ^{1}H n.m.r. spectrum of chrysin (80)



(80)

Figure 2.2 60 MHz ^{1}H n.m.r. spectrum of 4′ -*O*-methylapigenin (82)



During hydrolysis of 4′-*O*-methylapigenin (82) with hydriodic acid, difficulty was experienced in obtaining a crystalline product. After heating the aqueous solution on a steam bath, the volume was reduced considerably before crystallisation occurred, and since this

concentration was effected under reduced pressure, some contaminating iodine was removed by sublimation. The product was found to be clean by thin layer chromatography (t.l.c.), and after recrystallisation, gave a melting point of 340 - 344°C (lit.,[98] 348°C).

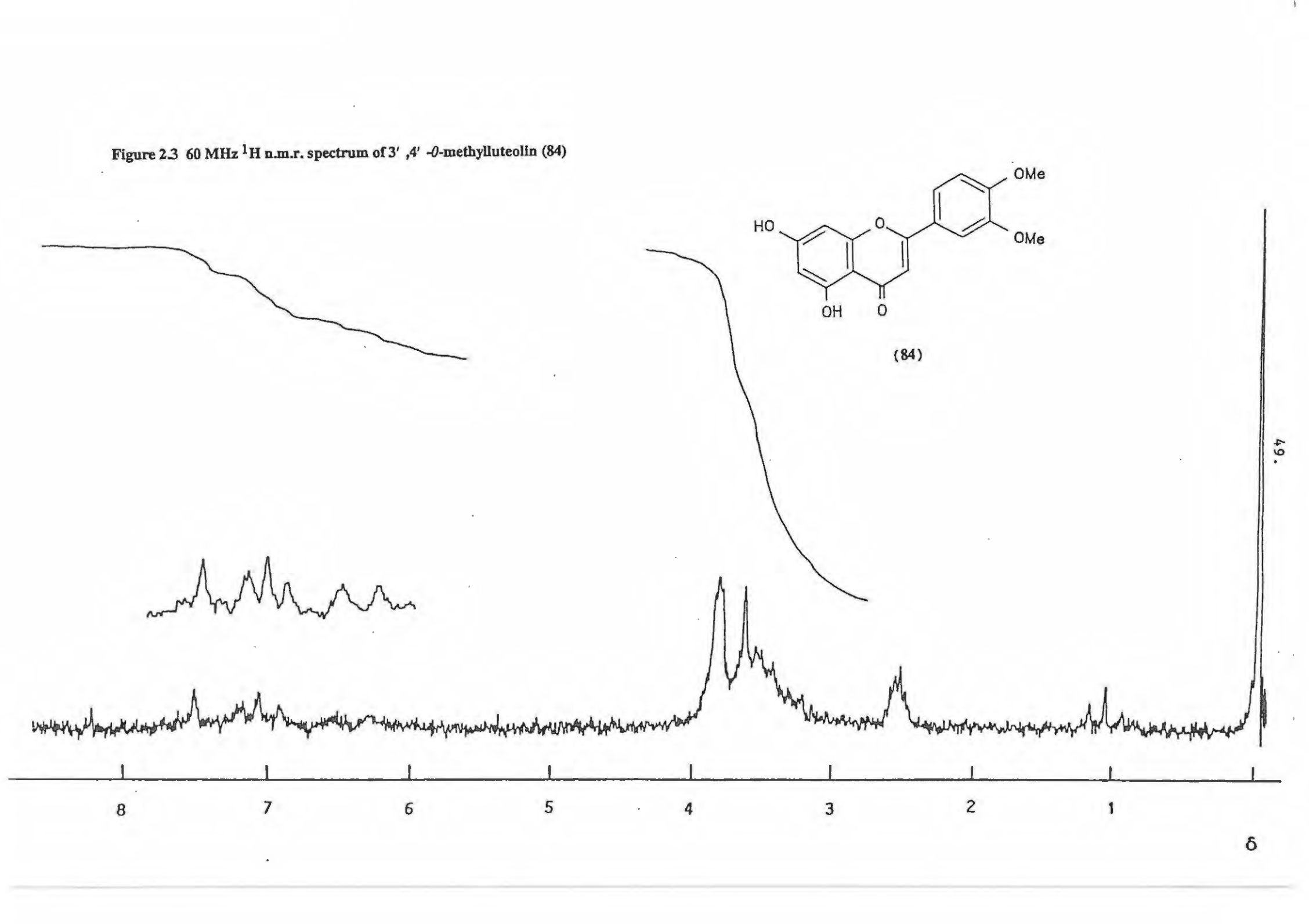
Synthesis of Luteolin (85)

The synthesis of 3′,4′-di-*O*-methylluteolin (84) in 33% yield followed a course similar to that of 4′-*O*-methylapigenin (82). Purification of the crude product required repeated flash chromatography to remove impurities.

Since this product has a 3′,4′-dioxygenation pattern, its 60 MHz ^{1}H n.m.r. spectrum is more complex than that of 4′-*O*-methylapigenin (82). For flavones with this substitution pattern the 5′-H nucleus typically resonates in the range 6.7 - 7.3 ppm as a doublet (*J* 8.5 Hz),[2] and in this case the signal is at 7.3 ppm (Figure 2.3). The 2′- and 6′-H nuclei should given rise to overlapping signals in the range 7.2 - 7.9 ppm, and for compound (84) they are, in fact, found at *ca.* 7.6 ppm.

The demethylation step to prepare luteolin (85) was complicated by the precipitation of a brown non-flavonoid material during the heating of the aqueous solution. This was filtered off, and concentration of the filtrate under reduced pressure gave the crude brown product. Preparative layer chromatography (p.l.c.) resulted in significant loss of product due to oxidation on the plate, and only a small sample of a yellow oil was obtained. Analytical thin layer chromatography (t.l.c.) showed this to contain one major component (yellow in visible light and purple under ultra violet light) which was assumed to be the required product. Further purification was precluded by the low yield of product and its susceptibility to oxidation.

Figure 2.3 60 MHz ^{1}H n.m.r. spectrum of 3′ ,4′ -*O*-methylluteolin (84)



Synthesis of Kaempferol (87)

3,4′-Di-*O*-methylkaempferol (86) was prepared in 40% yield without complication. The presence of the ω-methoxyl group in the ketone precursor, ω-methoxyphloracetophenone (74) enhances the rate of this thermal condensation (p. 42) and consequently a shorter period of heating (3 h) was required.

As in the case of 4′-*O*-methylluteolin (84), the 60 MHz ^{1}H n.m.r. spectrum of this methyl ether (86) shows an apparent AB quartet for the four B-ring protons, the two signals in this case appearing at 6.8 and 7.9 ppm respectively.

Demethylation of compound (86) gave kaempferol (87) in 90% yield. The ^{1}H n.m.r. spectrum of this flavonol (87) (Figure 2.4) shows a broad peak due to four hydroxyl protons at 3.3 ppm in addition to signals for the *meta*-coupled A-ring protons (6- and 8-H) at 6.2 and 6.4 ppm, and the B-ring protons at 6.9 and 8.1 ppm.

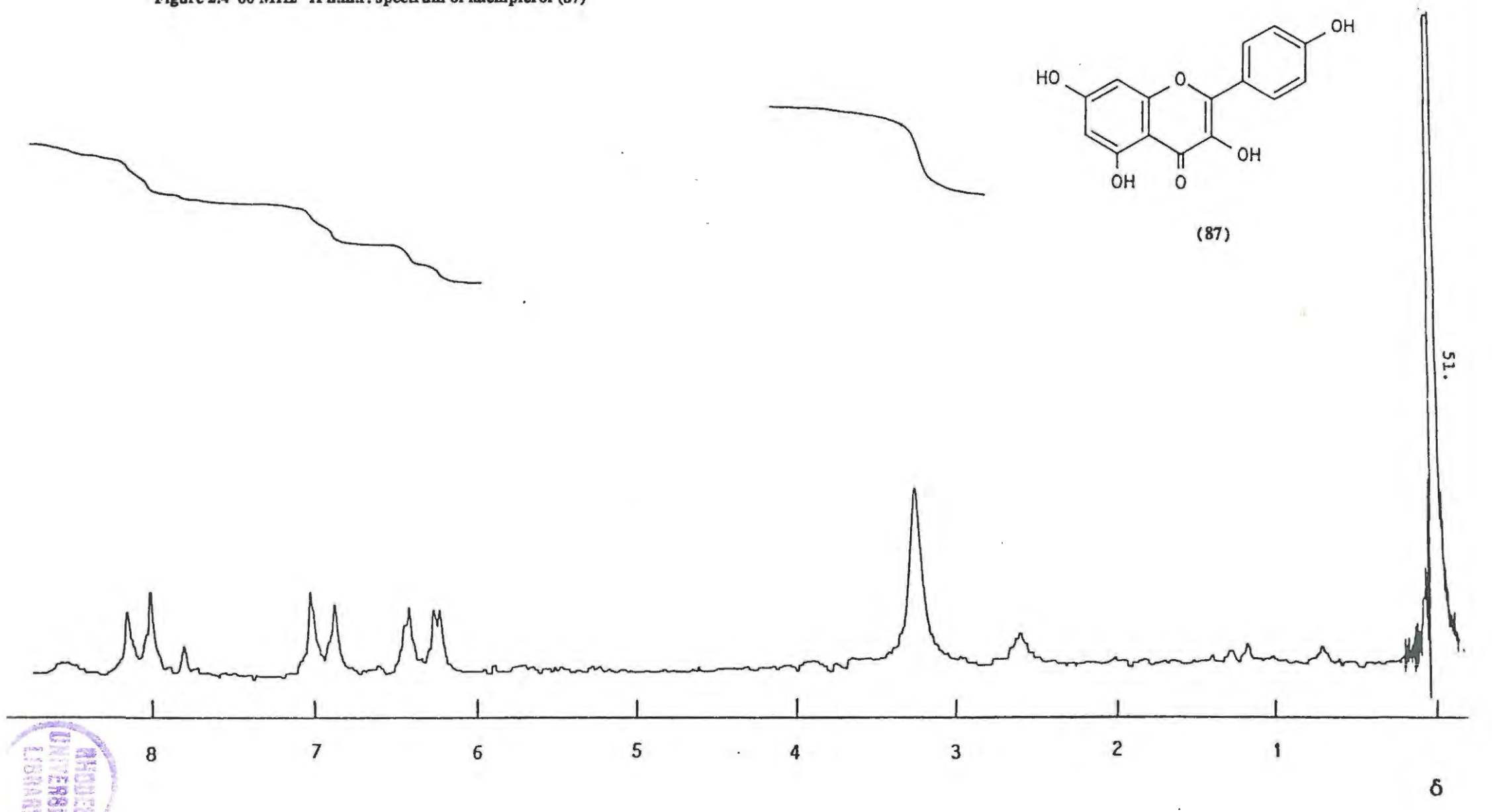
Synthesis of Quercetin (89)

The synthesis of 3,3′-4′-tri-*O*-methylquercetin (88) proceeded smoothly in 39% yield. As in previous examples, purification of the crude product led to considerable reduction in yield as a result of significant charring.

In common with 3′,4′-di-*O*-methylluteolin (84) the ^{1}H n.m.r. spectrum of this methyl ether (88) (Figure 2.5) shows typical signals, *viz.*, two doublets at 6.2 and 6.4 ppm for the A-ring aromatic protons, a doublet at 7.1 ppm due to the 5′-H nucleus and a multiplet at 7.6 ppm due to the 2′- and 6′-H nuclei.

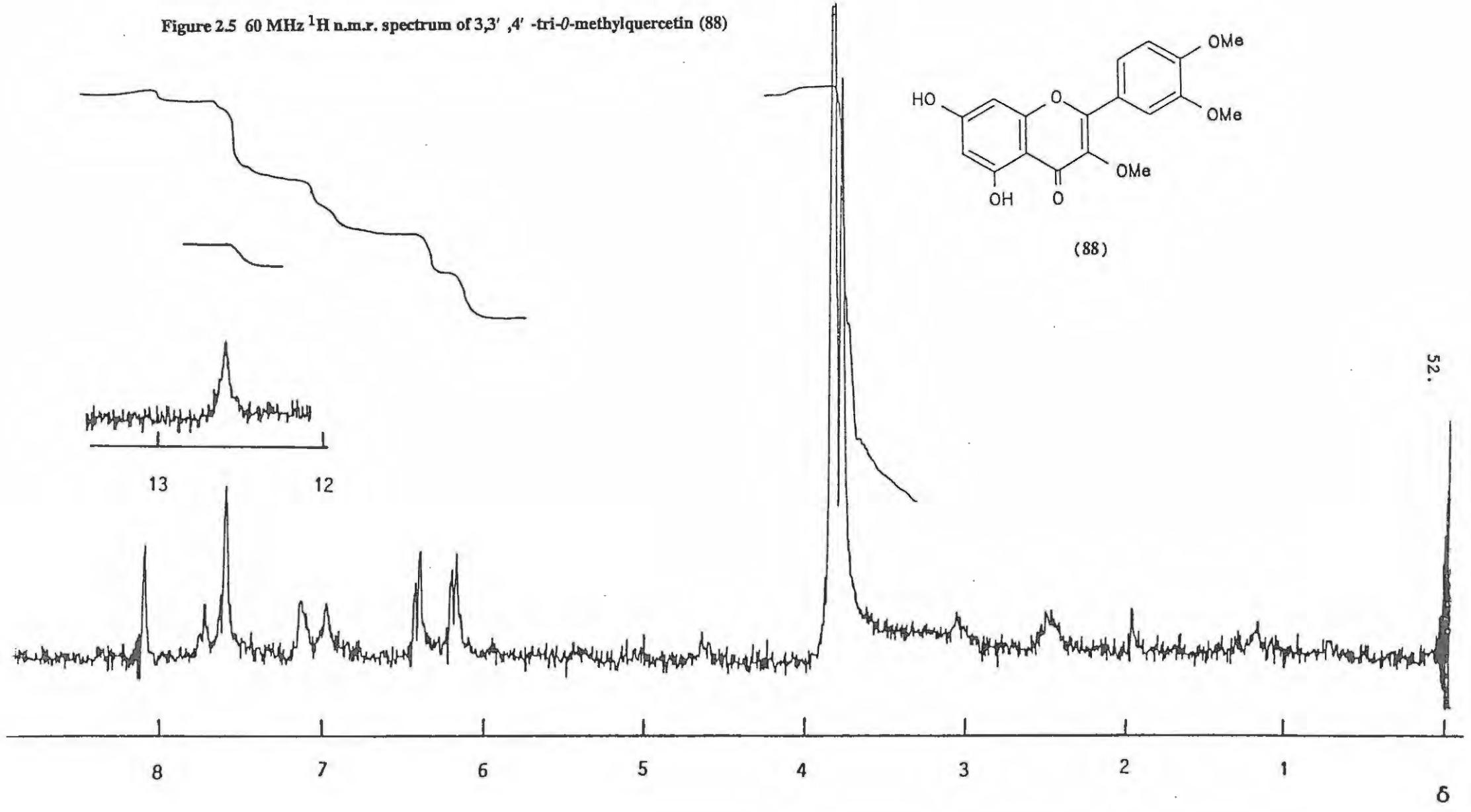
The hydrolysis of the methyl ether (88) afforded quercetin (89) in 86% yield. The ^{1}H n.m.r. spectrum of the flavone (89) (Figure 2.6) shows no methoxyl group protons, and the 5′-H nucleus is shown to be slightly less deshielded than it is in the ether (88). The melting point of quercetin (89) was found to be in closer agreement with that found by Robinson[100] than that reported by Gripenberg.[98] This variation may possibly be attributed to the decomposition of the product near its melting point.

Figure 2.4 60 MHz ^{1}H n.m.r. spectrum of kaempferol (87)



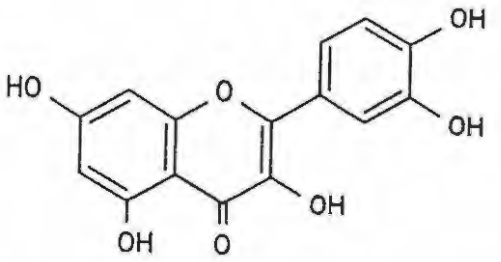
(87)

Figure 2.5 60 MHz ^{1}H n.m.r. spectrum of 3,3' ,4' -tri-*O*-methylquercetin (88)

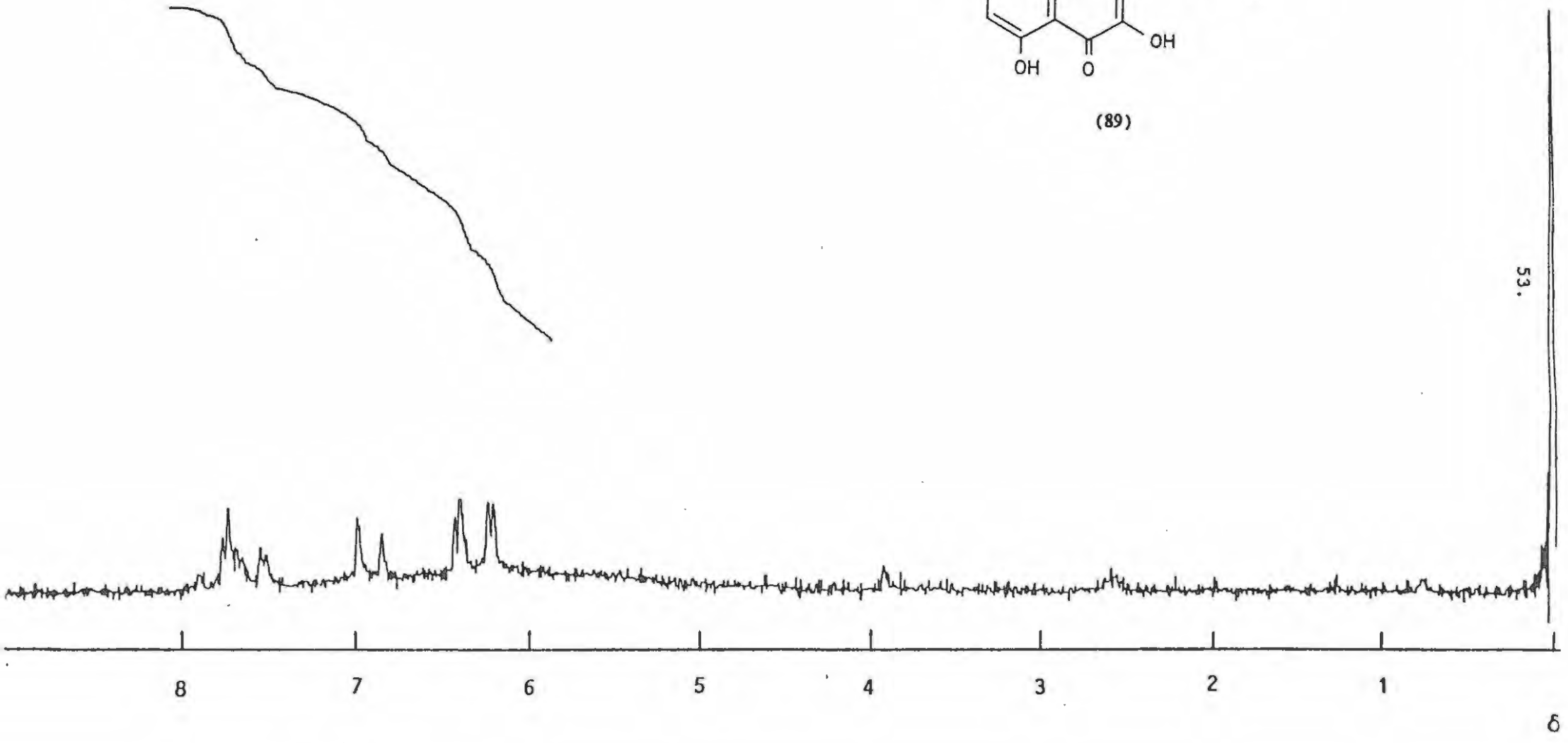


(88)

Figure 2.6 60 MHz ^{1}H n.m.r. spectrum of quercetin (89)



(89)



Synthesis of Fisetin (91)

Fisetin (91) and its trimethyl ether (90) differ from other flavonols in this study, since they lack a 5-hydroxyl group in the A-ring. Their syntheses did not differ in procedure from other flavonol syntheses, but the ketone precursor ω-methoxyresacetophenone [(73); Table 2.1, p. 31] was used in place of ω-methoxyphloracetophenone (74). Since the former precursor has a lower melting point than the latter (74), the reaction was carried out at a slightly lower temperature (175°C).[100] Although the thermal cyclisation reaction afforded the ether product (90) in low yield (23%), the demethylation reaction gave fisetin (91) in 69% yield with no difficulties.

The ^{1}H n.m.r. spectra of products (90) and (91) differ from those of 5,7-dihydroxyflavonols in that a signal for the 5-H nucleus is present. Since this proton is strongly deshielded by the C-4 carbonyl group, the signal is found at *ca.* 8.0 ppm. (Figures 2.7 and 2.8) It is of interest to note that these products (90) and (91), in common with other flavones lacking 5-OH groups,[2] give a characteristic pale fluorescent blue colour under u.v. light, in contrast with 5,7-dihydroxyflavones which give a dark purple colour.

Synthesis of Myricetin (93)

The synthesis of 3,3′,4′,5′-tetra-*O*-methylmyricetin (92) from ω-methoxyphloracetophenone (74) and 3,4,5-trimethoxybenzoic anhydride (64) was carried out in the same way as other flavonol syntheses, following the method of Kalff and Robinson,[101] but flash chromatography of the crude product afforded only a small quantity of amorphous yellow product. T.l.c. of other fractions from the column revealed the presence of starting materials (as well as brown oxidation products) indicating that the shorter period of heating (3 hours) was perhaps insufficient. The product decomposed near its melting point.

The ^{1}H n.m.r. spectrum of product (92) showed methoxyl proton signals, the characteristic 6-H and 8-H proton doublets, and a singlet at 7.4 ppm due to the two B-ring protons, 2′- and 6′-H. (Fig 2.9)

Figure 2.7 60 MHz ^{1}H n.m.r. spectrum of 3,3' ,4' -tri-*O*-methylfisetin (90)

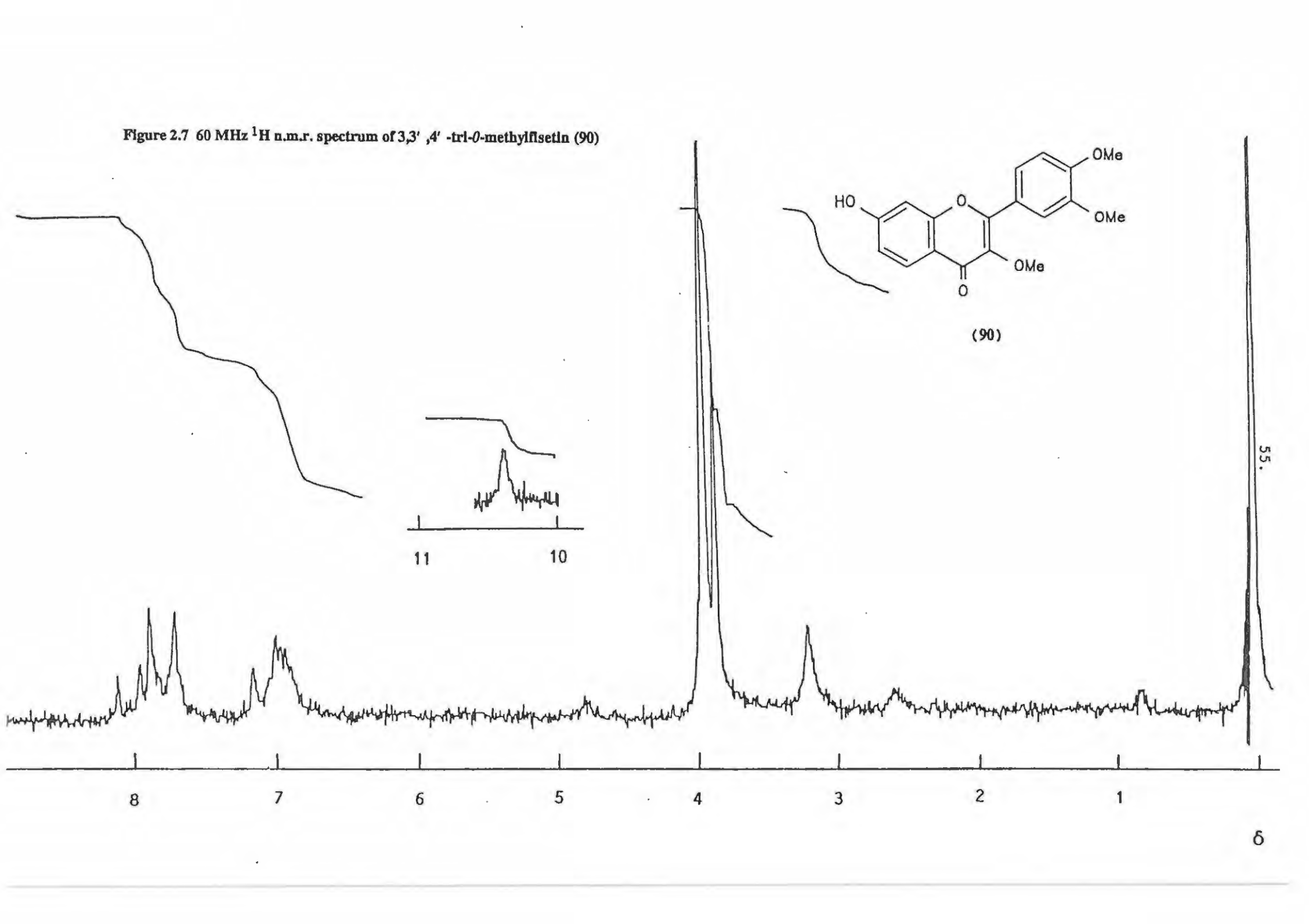
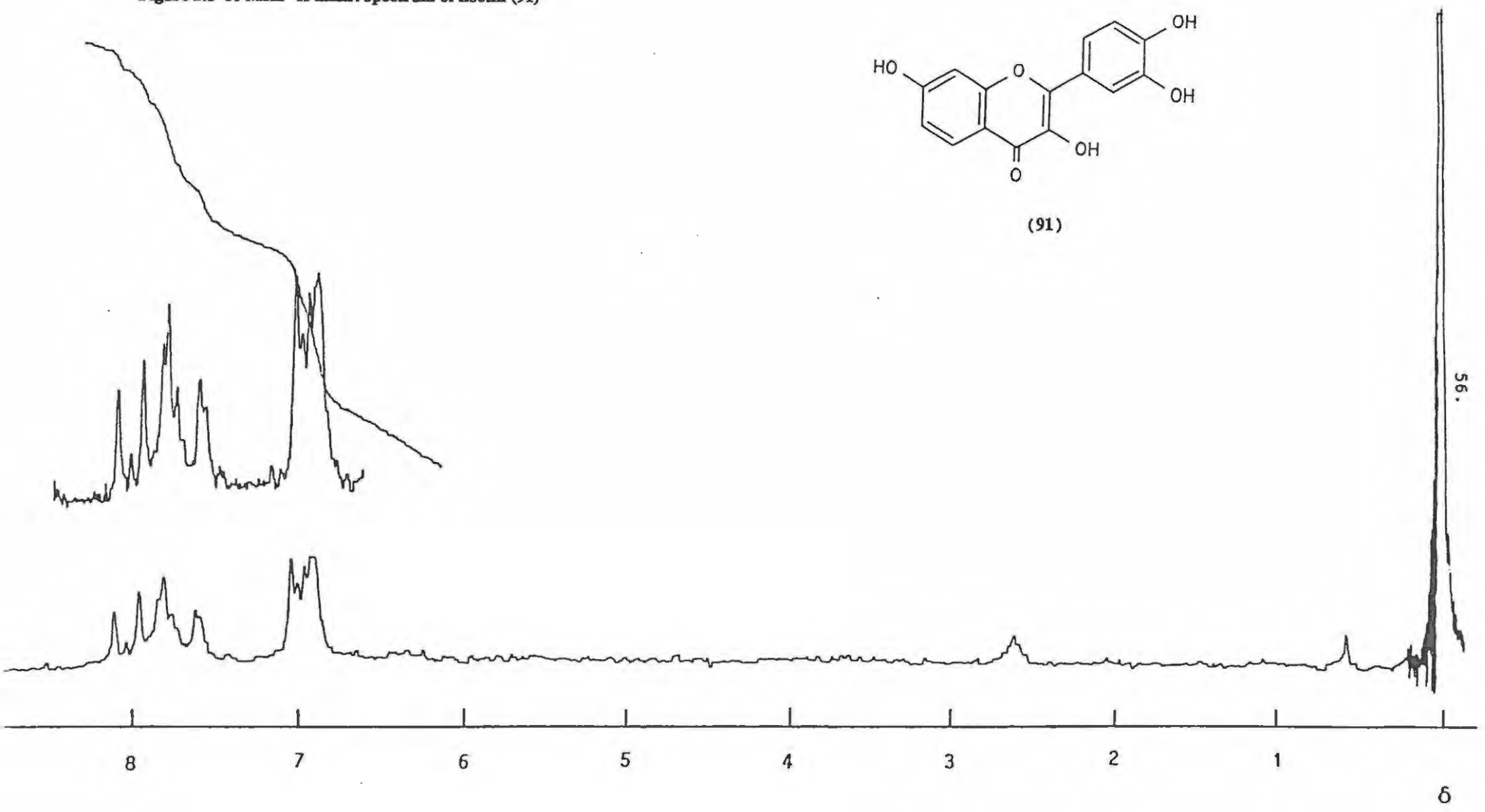
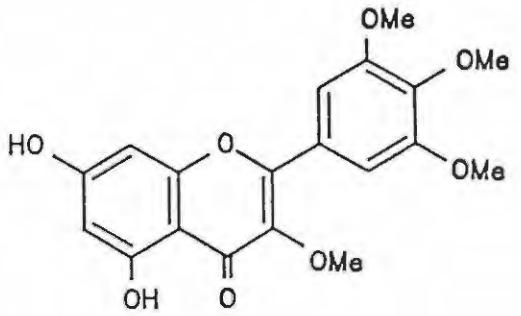


Figure 2.8 60 MHz ^{1}H n.m.r. spectrum of fisetin (91)

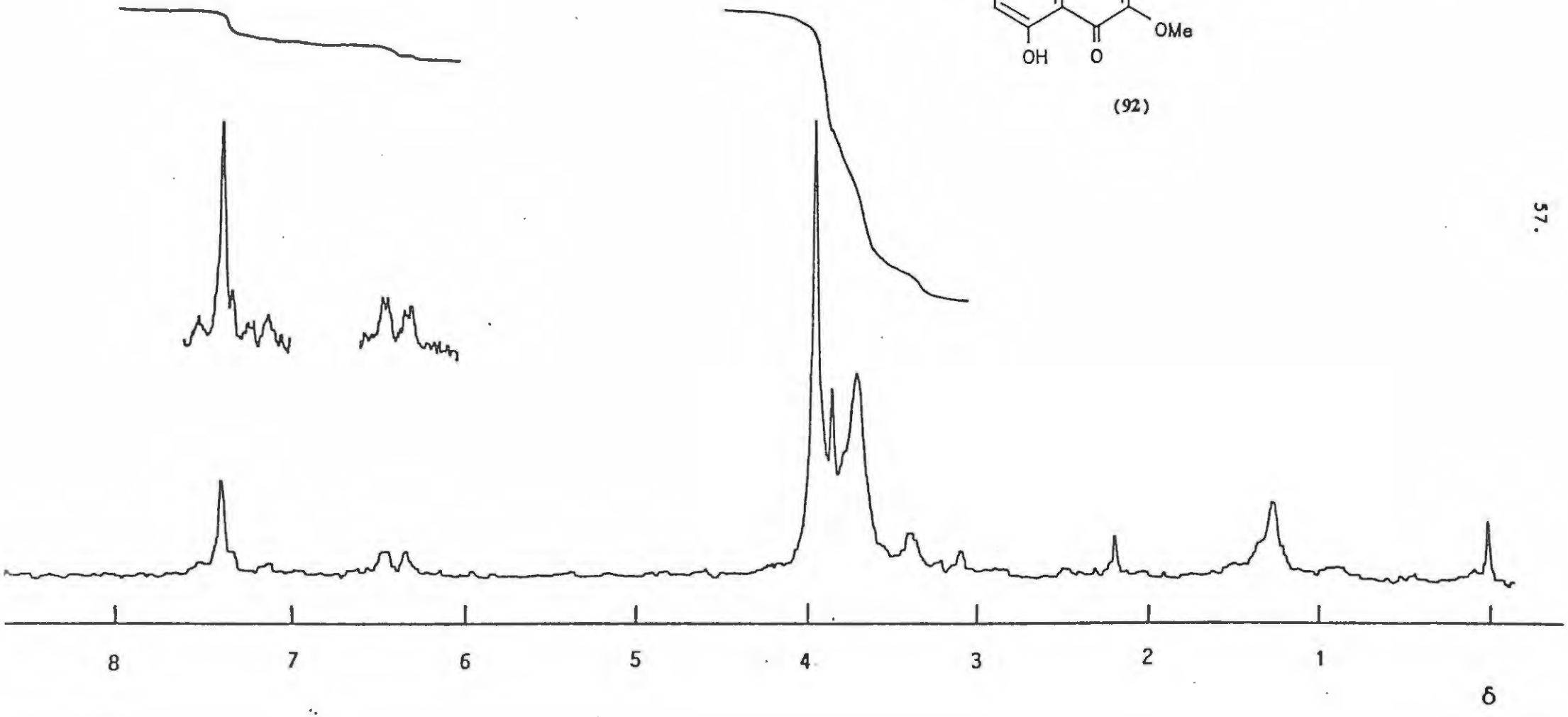


(91)

Figure 2.9 60 MHz ^{1}H n.m.r. spectrum of 3,3' ,4' ,5' -tetra-*O*- methylmyricetin (92)



(92)



Preparation of myricetin (93) by demethylation of the ether (92) afforded a small amount of the crude flavone. In the course of purification by p.l.c. some loss of product occurred because of oxidation on the plate. The recovered sample was shown by t.l.c. to contain one major component (yellow in visible light and purple in ultra violet light) assumed to be myricetin (93). Time did not permit a further attempt to synthesise myricetin.

Synthesis of Quercetagetin (95)

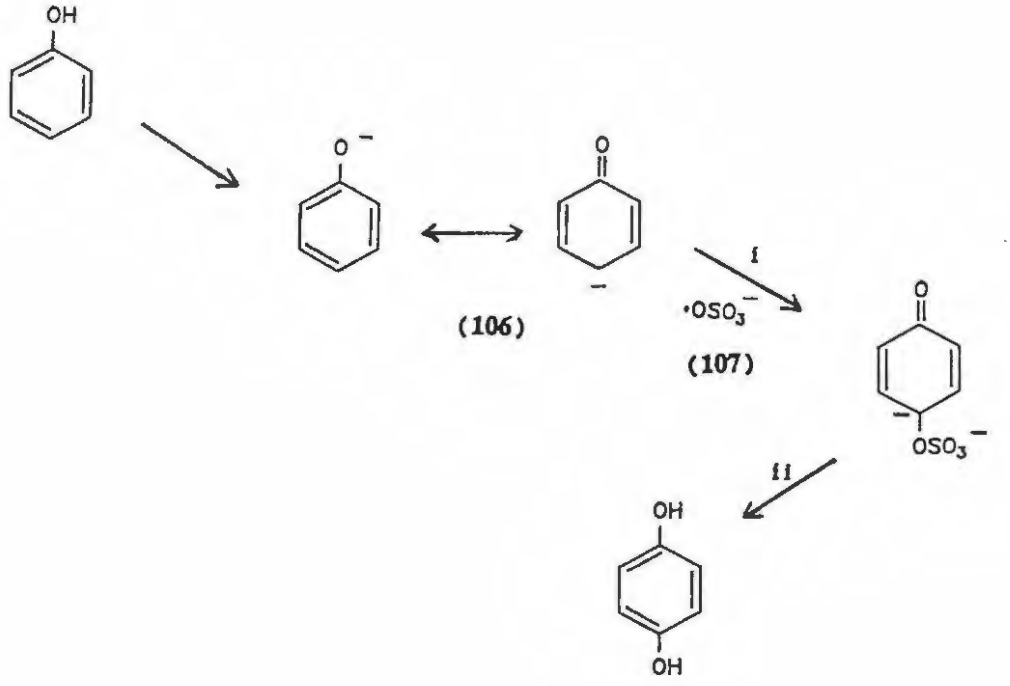
The synthesis of quercetagetin (95) required, as a precursor, 2′,5′-dihydroxy-2,4′,6′-trimethoxyacetophenone (79) which in turn was prepared from 2′-hydroxy-2,4′,6′-trimethoxyacetophenone (77) (see p. 41).

Transformation of compound (77) to the dihydroxy product (79) was effected using the Elbs persulphate oxidation, in which potassium persulphate, $K_2S_2O_8$, is slowly added to an alkaline solution of a phenol under controlled temperature conditions.[102] Yields for the reaction are known to be low (15 - 20%) and in this investigation the reaction gave a 7% yield.

The mechanism for the oxidation has been suggested[102] to involve slow attack by a sulphate ion-radical (107) on the phenoxide ion (106) (Scheme 2.9). *Para*- substitution appears to be favoured but if the *para* position is occupied, *ortho*- substitution is observed to occur in even lower yields.[102] Oxidative coupling of phenyl groups is also possible and may account for the observed darkening of the reaction mixture.

The crude product was used without further purification for the next stage, the synthesis of 3,3′,4′,5,7-penta-*O*-methylquercetagetin (94) The thermal condensation of the ketone precursor (79) with the anhydride (63) was carried out on a small scale and preparative layer chromatography was necessary to isolate the pentamethyl ether product (94). A small amount of product (94) was obtained as an oil, but no attempt was made to demethylate it.

Scheme 2.9 The Elbs persulphate oxidation



Reagents : i, OH^-, $K_2S_2O_8$; ii, H^+

2.1.2.7 Synthesis of rhamnetin and isorhamnetin

Many naturally occurring flavones bear methoxyl groups as aromatic substituents, and their synthesis from existing hydroxyflavones can be performed by selective alkylation reactions.

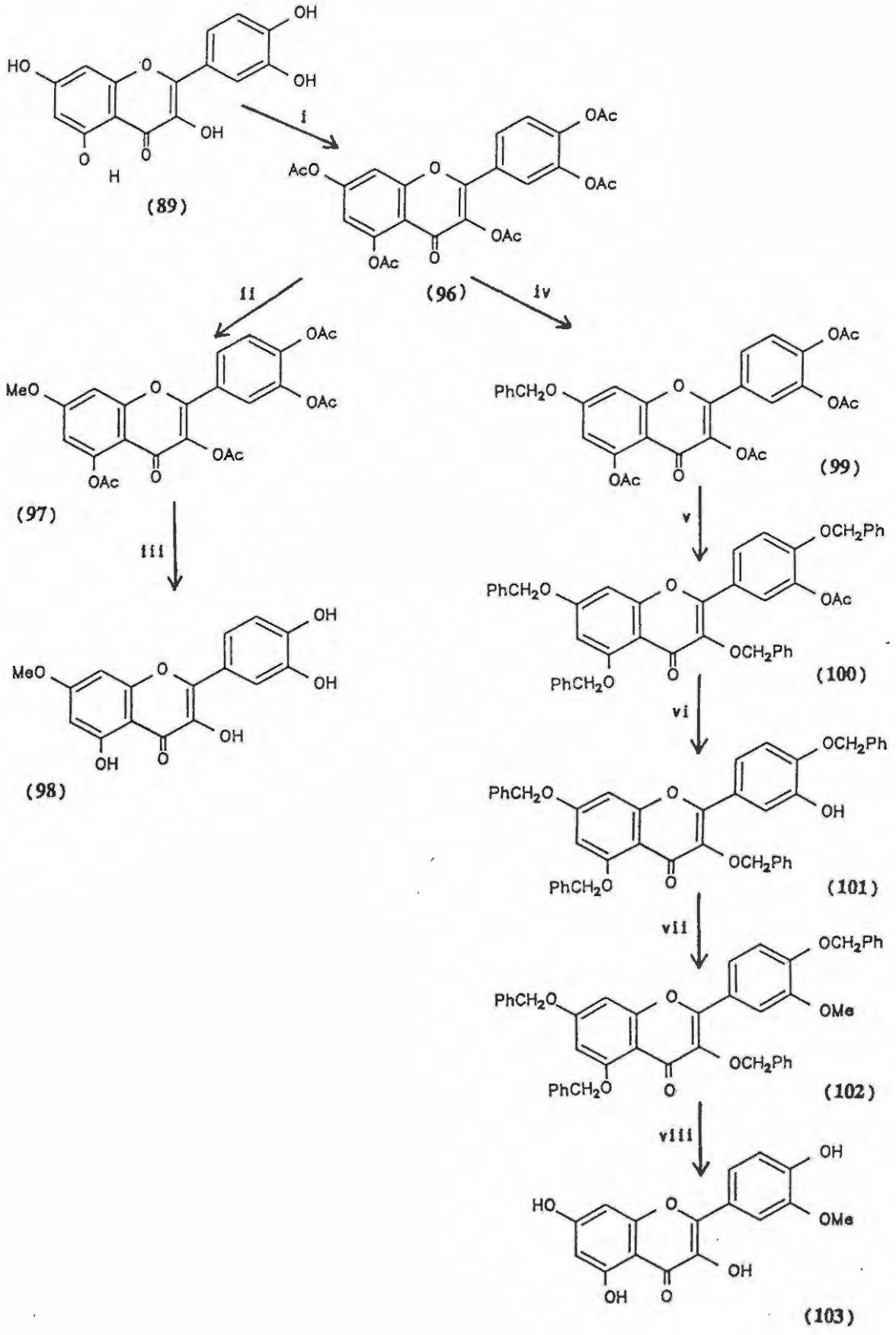
The 5-hydroxyl group is least amenable to alkylation because of intramolecular hydrogen bonding as shown in the flavonol (89) (Scheme 2.10) but complete alkylation of flavones is possible in the presence of a large excess of alkyl halide. Reactions are most successful when carried out in acetone,[1] using potassium carbonate as a base. Dimethyl sulphate is suitable for complete methylation, while diazomethane is used for methylation of all hydroxyl groups other than at the 5- position.[98] Partial methylation can be achieved by protecting certain hydroxyl groups before methylation and by careful control of reaction conditions.[103]

Rhamnetin (98) was successfully synthesised by acetylation of quercetin (89), and subsequent selective hydrolysis and methylation of the penta-acetate (96) at the 7- position only (Scheme 2.10). This was achieved, following the method of Jurd,[103] by carrying out the reaction in refluxing acetone (b.p. 56°C), and using methyl iodide as the methylating agent.

Isorhamnetin (103) was prepared by a similar series of reactions[103] (Scheme 2.10). Use of 2-butanone (b.p. 80°C) as the solvent in step v allowed the reaction to proceed at a slightly higher temperature, leading to replacement of *O*-acetyl groups with *O*-benzyl groups in all but the 3′-position and giving product (100). Hydrolysis of this 3′-*O*-acetyl group to give compound (101), and methylation at this position followed by removal of the protecting benzyl groups by acid hydrolysis, proved to be a very satisfactory route to the desired product, isorhamnetin (103).

Although a multi-step linear synthesis such as this inevitably leads to reduced overall yields, the products at each stage were readily obtained in a pure state as indicated in the following discussion.

Scheme 2.10 Synthesis of rhamnetin and isorhamnetin



Reagents : i, Ac_2O, pyridine; ii, MeI, K_2CO_3, dry acetone;
iii, NaOH, MeOH; iv, $C_6H_5CH_2Cl$, K_2CO_3, dry acetone;
v, $C_6H_5CH_2Cl$, K_2CO_3, dry 2-butanone; vi, NaOH, MeOH; vii, MeI, K_2CO_3, acetone;
viii, AcOH, HCl

Synthesis of 3,3′,4′,5,7-penta-*O*-acetylquercetin (96)

This reaction involved a straightforward acetylation of all the hydroxyl groups of quercetin (89), and the yield after re-crystallisation was 64%.

Presence of the acetyl groups was confirmed by the strong carbonyl group absorbtion in the i.r. spectrum of product (96) at 1775 cm^{-1} (in addition to the flavone carbonyl group absorption at 1650 cm^{-1}) and the lack of a broad hydroxyl absorption band.

The ^1H n.m.r. spectrum of product (96) showed a 15-proton signal at 2.35 ppm due to the five ester methyl groups and otherwise was similar to that of quercetin (89) (Figure 2.6, p. 53).

Synthesis of 3,3′,4′,5-tetra-*O*-acetylrhamnetin (97)

The selective replacement of the 7-*O*-acetyl group by a methyl group was controlled by use of methyl iodide as the methylating agent (this being preferable to use of dimethyl sulphate which could also methylate at the 4′ - position with comparable ease[103]) in a 3 : 1 molar ratio.

The product (97) was obtained in 40% yield and its identity as a methyl ether was confirmed by the ^1H n.m.r. signal at 3.8 ppm, due to the 7-methoxyl protons.

Synthesis of Rhamnetin (98)

Hydrolysis of 3,3′,4′,5-tetra-*O*-acetylrhamnetin (97) was effected in alkaline solution to afford rhamnetin (98) in 56% yield after re-crystallisation. The ^1H n.m.r. spectrum differed from that of quercetin (89) only in the presence of a signal at 3.9 ppm due to the 7-methoxyl substituent.

Synthesis of 3,3',4',5-tetra-*O*-acetyl-7-*O*-benzylquercetin (99)

The synthesis of this compound illustrates the ease of alkylation of the 7-hydroxyl group in flavones. The use of benzyl chloride in a 4 : 1 molar ratio with 3,3',4',5,7-penta-*O*-acetylquercetin (96) in dry acetone resulted in benzylation at the 7- position only. The product (99) was obtained in 57% yield, and the benzyl group was shown to be present by the ^{1}H n.m.r. signals at 5.2 ppm (due to the benzyl methylene protons) and at 7.5 ppm (due to the benzyl aromatic protons).

Synthesis of 3'-*O*-acetyl-3,4',5,7-tetra-*O*-benzylquercetin (100)

Further benzylation of the preceding product (99) was carried out with the higher boiling 2-butanone as solvent, and a higher (10 : 1) reactant ratio, to afford the required product (100) in 34% yield. The additional benzyl groups in the molecule gave rise to ^{1}H n.m.r. signals at 5.2 ppm (benzyl methylene protons) and at 7.4 ppm (benzyl aromatic protons) with integrals in the ratio 8 : 20 respectively, and the one remaining acetyl group gave rise to a 3 proton singlet at 2.3 ppm.

Synthesis of 3,4',5,7-tetra-*O*-benzylquercetin (101)

The hydrolysis of the remaining *O*-acetyl group of compound (100) was effected without difficulty, to afford product (101) in 73% yield. The presence of a broad absorbtion band centred at *ca.* 3300 cm^{-1} in the i.r. spectrum of the flavone (101) confirmed the presence of a hydroxyl group in the molecule.

Synthesis of 3,4',5,7-tetra-*O*-benzyl-3'-*O*-methylquercetin (102)

The 3' -hydroxyl group in compound (101) was methylated as previously, with methyl iodide, in dry acetone. The crude product was a sticky gum, and it was recrystallised with difficulty, to give the crude methylated product (102) in 37% yield.

The i.r. spectrum of product (102) showed no hydroxyl absorption, and its ^{1}H n.m.r. spectrum showed signals for one methoxy group (at 3.7 ppm) and four benzyl groups (at 5.2 and 7.4 ppm), confirming that the methylation had been successful. Hence it was considered unnecessary to purify the product (102) further before using it for the next step of the synthesis.

Synthesis of Isorhamnetin (103)

The final stage in the synthesis of isorhamnetin (103) was the hydrolysis in acid medium of the benzyl ether groups in 3,4′,5,7-tetra-*O*-benzyl-3′-*O*-methylquercetin (102). The reaction proceeded without complication to give the desired product (103) in 79% yield.

2.2 ISOLATION AND IDENTIFICATION OF CHEMICAL CONSTITUENTS OF *TULBAGHIA VIOLACEA*

2.2.1 Methods of Extraction

When *Tulbaghia violacea* is used as a traditional remedy, it is extracted by aqueous infusion of the crushed plant. Since it was an objective of this study to investigate the active principles in the herbal medicine, initial extraction procedures followed the traditional approach (section 2.2.1.1). In addition, the possibility of alkaloids and other lipophilic secondary metabolites being present in the plant necessitated extraction with non-aqueous solvents (section 2.2.1.2). Vacuum distillation techniques were used to obtain samples of volatile constituents in the plant (section 2.2.5).

2.2.1.1 Aqueous extraction

Traditional methods of extracting *Tulbaghia violacea* appear to vary. In some cases, crushed plant material (typically the roots and white aerial parts) is boiled in water for up to half an hour before cooling, while in other cases boiling water is poured on to the crushed plant material and the mixture is then left to cool. Generally, the green parts of the plant are not used, and occasionally only the brown dried outer scales from the base of the plant are extracted.

In this investigation, aqueous infusions were usually obtained by grinding the washed plant, (excluding green leaves) with distilled water, and boiling it briefly (*ca.* 10 min). For comparative biological activity studies (section 2.2.7) infusions were also prepared (a) without boiling the mixture and (b) using green leaves only.

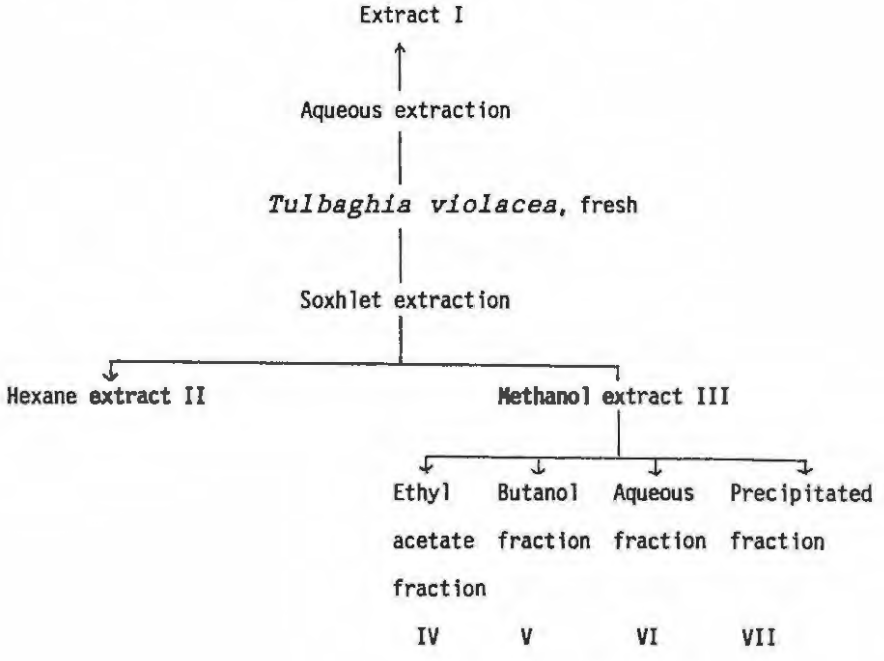
Concentration of the infusion by freeze-drying gave a sticky powder (extract I; Scheme 2.11, p. 66), the colour of which depended on the age of the plant : older plants gave browner extracts due to the accumulation of brown outer scales, while young plants gave colourless extracts.

2.2.1.2 Soxhlet extraction

Successive extraction of chopped fresh plant material (excluding green parts) in a Soxhlet apparatus, using organic solvents of increasing polarity, gave extracts II and III (Scheme 2.11). Partitioning of extract III between water and ethyl acetate and then butanol gave fractions IV, V and VI.

Analysis of the extracts obtained by these methods is described in the following sections :- sugars (2.2.2); aglycones (2.2.3); sulphur compounds (2.2.4); volatiles (2.2.5); and miscellaneous constituents (2.2.6).

Scheme 2.11 Extractions of *Tulbaghia violacea*



2.2.2 ISOLATION AND IDENTIFICATION OF SUGARS IN *TULBAGHIA VIOLACEA*

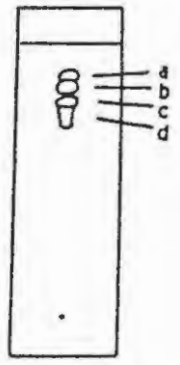
2.2.2.1 Preliminary investigations

The presence of simple sugars in the aqueous plant extract was established initially by t.l.c.[104] (Figure 2.10). When the anisaldehyde - H_2SO_4 spray reagent (1) was employed for visualisation of sugar components, the observed colours provided some information regarding the nature of the sugars present;[104] the colours observed are recorded in Figure 2.12; A.

The presence of glycosidic material in the aqueous extract (I) was established by adapting a most useful technique reported by Heisig and Wichtl,[105] involving hydrolysis of glycosides directly on a t.l.c. plate. The components of extract I were separated by t.l.c. in one direction initially. The plate was then placed in a 'reaction box', the lid of which comprised a p.l.c. plate previously sprayed with dilute hydrochloric acid. The box was heated briefly (*ca.* 10 min) to vapourise the acid and hence effect hydrolysis of the material on the t.l.c. plate. Subsequent elution in the second direction with appropriate solvent systems allowed separation either of aglycones or of sugars. The results of this procedure are shown in Figure 2.11, where chromatogram B represents a control experiment in which no hydrolysis was carried out before the second elution. [Chromatogram C shows the results (obtained later) of hydrolysis following the same procedure, of fraction V from the Soxhlet extraction. (Scheme 2.11, p. 66)].

In order to isolate the aglycones and sugars from these glycosides, a sample of extract I was hydrolysed (see Experimental section, p. 155) giving an aqueous layer containing sugars. This was shown by t.l.c. (Figure 2.12; A) and paper chromatography (p.c.) (Figure 2.12; B) to contain at least three sugar components.

Figure 2.10 T.l.c. of aqueous extract I

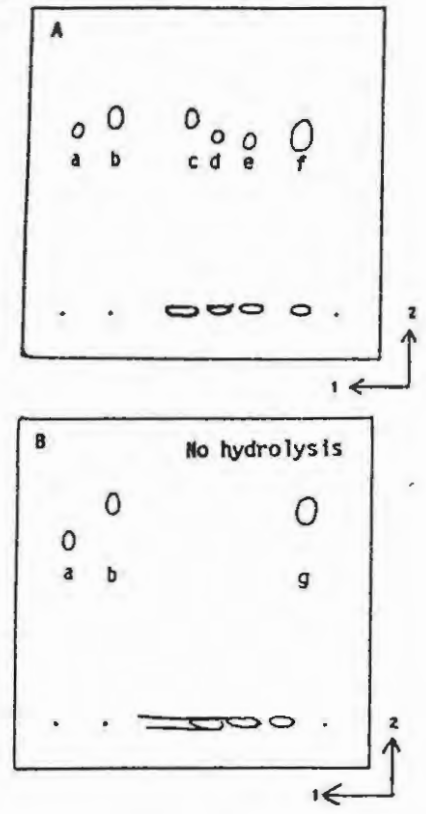


Spot	R_f	Colour
a	0.90	Grey
b	0.84	Violet
c	0.78	Grey/green
d	0.71	Grey/green

Solvent system : EtOH - Prop^{i}OH - H_2O (6:2:1); spray reagent : anisaldehyde - H_2SO_4

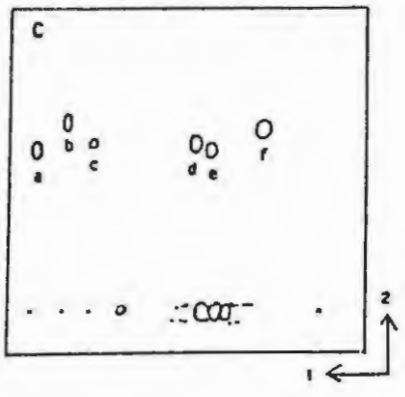
Figure 2.11 2-D t.l.c., with hydrolysis, of extracts I and V

Extract I



Spot	Sample	Colour
a	D-Glucose	Grey/green
b	D-Fructose	Violet/black
c	Sugars from	Dark grey
d	hydrolysed	Grey/green
e	glycosidic	Grey/green
f	material	Dark grey/purple
g		Dark grey/purple

Fraction V (see section 2.2.3.3)

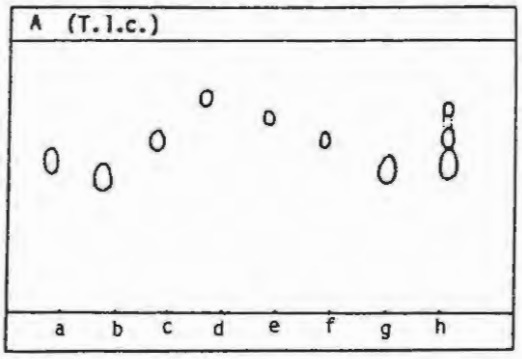


Spot	Sample	Colour
a	D-Glucose	Grey/green
b	D-Fructose	Violet/black
c	D-Xylose	Green
d	Sugars from	Grey/greem
e	hydrolysed	Grey/green
	glycosidic	Dark Grey/purple
	material	
f	Apparently	Dark Grey/purple
	free	
	D-Fructose	

Solvent systems : Bu^tOH - AcOH - H_2O (3:1:1) and BuOH - MeOH - AcOH - H_2O (8:8:1:1);

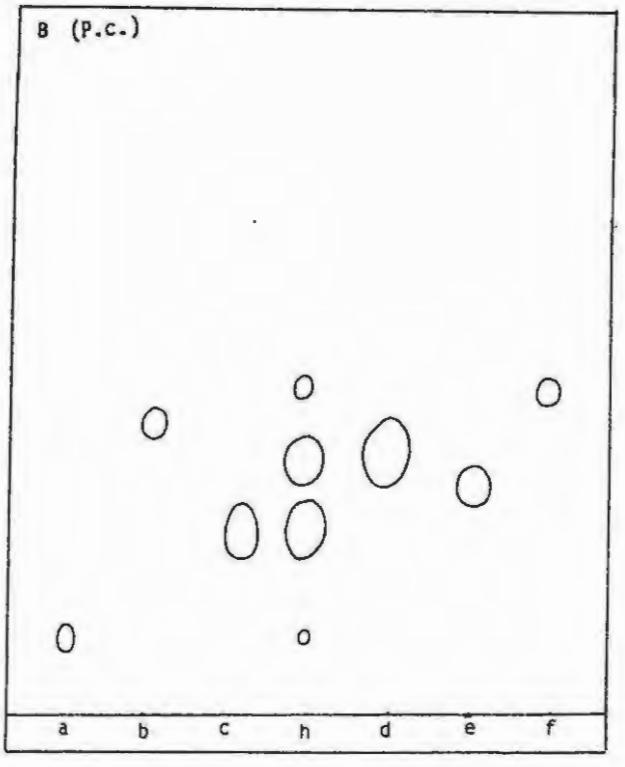
spray reagent : anisaldehyde - H_2SO_4

Figure 2.12 T.l.c. and p.c. of hydrolysed extract I for analysis of sugars



Spot	Sample	R_f	Colour
a	D-Glucose	0.51	Grey/green
b	D-Galactose	0.48	Grey/green
c	D-Mannose	0.61	Green
d	L-Rhamnose	0.75	Green
e	D-Xylose	0.69	Grey/green
f	L-Arabinose	0.61	Yellow/green
g	D-Fructose	0.48	Blue/black
h	Hydrolysed I	0.71	Grey/green
		0.63	Grey/green
		0.52	Blue/black

Solvent system : BuOH - MeOH - AcOH - H_2O (8:8:1:1);
spray reagent : anisaldehyde - H_2SO_4



Spot	Sample	R_f
a	Maltose	0.11
b	L-Arabinose	0.41
c	D-Glucose	0.25
d	D-Fructose	0.37
e	D-Mannose	0.32
f	D-Xylose	0.45
h	Hydrolysed I	0.47
		0.37
		0.26
		0.10

Solvent system : EtOAc - AcOH - HCOOH - H_2O (18:3:1:1);
spray reagents : i, $AgNO_3$ in acetone; ii, NaOH in EtOH;
iii, NaOAc - $Na_2S_2O_3$ - AcOH in H_2O

2.2.2.2 Isolation of free and glycosidic sugars

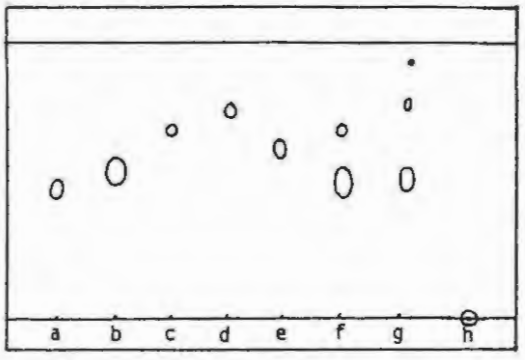
Differentiation between free sugars in *Tulbaghia violacea* and those present as glycosides, and identification of these components, formed an integral part of the analysis of the glycosidic material extracted from the plant. In order to separate glycosidic and free sugars from extract I, the procedure suggested by Mabry, Markham, and Thomas[2] was adapted for use with aqueous solutions. Glycosidic material in the aqueous solution of extract I was adsorbed on to charcoal, leaving the free sugars in solution (FS). The glycosides were recovered by treatment of the filtered charcoal with a hot aqueous phenolic solution. After removal of the phenol by solvent extraction, hydrolysis of the glycosides gave an aqueous solution containing the glycosidic sugars (GS) (see Experimental section). Confirmation of the presence of sugar components in fractions (FS) and (GS), but absence of sugars in the glycosidic material before hydrolysis, was obtained by t.l.c. of these fractions (Figure 2.13).

2.2.2.3 Gas-liquid chromatography of isolated sugars

A sensitive method was required for detection of the small quantities of sugars present in the sample solutions and hence, g.c. techniques were employed. To facilitate volatilisation, the sugar samples and selected standard monosaccharides were derivatised, initially using the method of McGinnis[106] in which aldoses were converted to peracetylated aldononitriles (PAANs). This method is suitable for aldoses, and the analysis for keto-sugars was carried out by an alternative method (see section 2.2.2.5).

The preparation of PAANs [Scheme 2.12; (110)] has advantages over the more usual method of derivatisation of sugars by trimethylsilylation, in that the PAANs are stable in the presence of water and may be stored. In addition, while gas chromatograms of TMS-ethers of sugars (111) have at least two peaks per sugar resulting from the different anomeric forms, each sugar forms only one PAAN and hence gives only one peak by g.l.c.

Figure 2.13 T.l.c. of free and glycosidic sugar samples



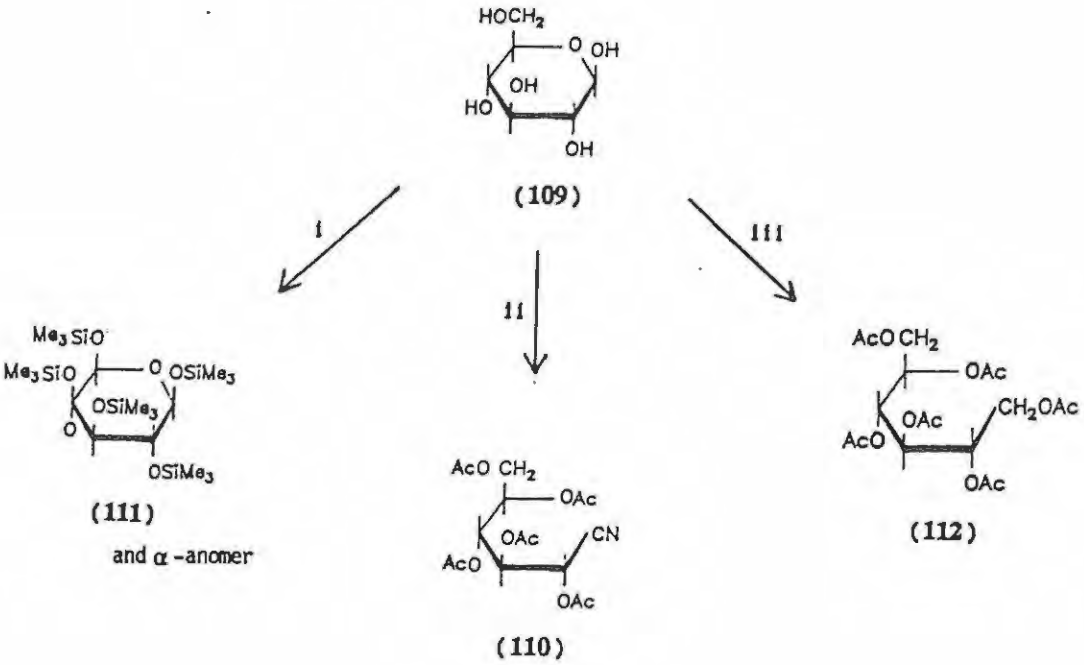
Spot	Sample
a	D-Glucose
b	D-Galactose
c	D-Xylose
d	L-Rhamnose
e	L-Arabinose
f	Fraction FS
g	Fraction GS
h	Isolated glycosides

Solvent system : BuOH - MeOH - AcOH - H_2O (8:8:1:1);

spray reagent : anisaldehyde - H_2SO_4

Scheme 2.12 Derivatisation methods for sugars

[illustrated for D-glucose (109)]



Reagents : i, TMSCl; ii, $NH_2OH.HCl$, N-methylimidazole;
iii, Ac_2O; iv, $NaBH_4$, AcOH; v, Ac_2O, pyridine

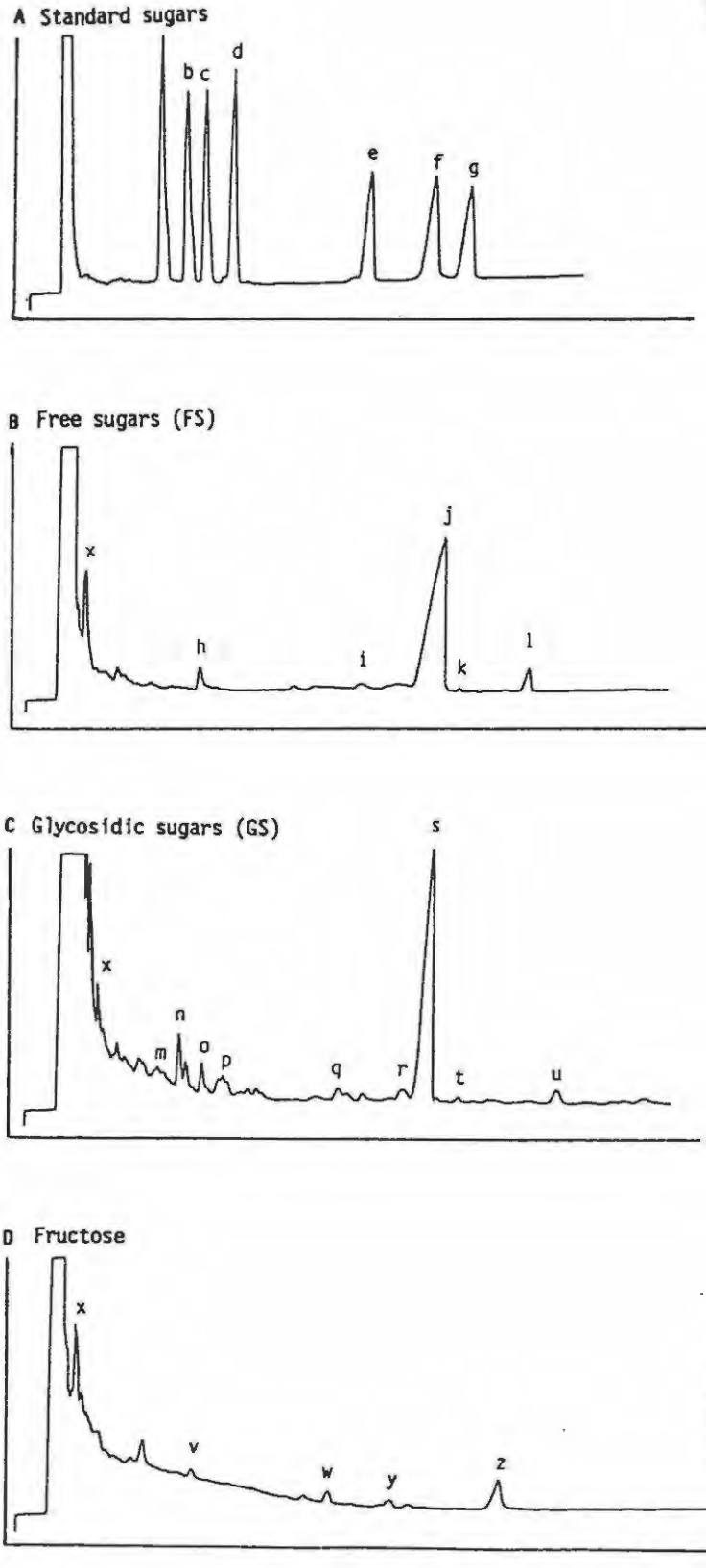
The synthesis of a PAAN is carried out by condensation of the sugar with hydroxylamine hydrochloride in *N*-methylimidazole (which acts as a catalyst and a solvent) to form the oxime. Dehydration and acetylation by acetic anhydride give the PAAN which is isolated by solvent extraction. The reaction is rapid (the rate of condensation depends on the amount of water present but generally the reaction is completed in *ca.* 5 minutes) and it is not affected by the presence of mineral acids or salts. This latter feature makes the method particularly suitable for derivatisation of hydrolysed plant extracts. Isothermal gas chromatography of PAAN derivatives typically results in good separation of components,[107] the dominant factor governing the g.l.c. retention times of the PAANs being the number of acetoxyl groups in the structures. Thus, pentose PAANs are eluted ahead of hexose PAANs.[107]

PAAN derivatives were prepared from the free sugar sample (FS) and the glycosidic sugar sample (GS) obtained as described previously (p. 71). Standard aldoses were derivatised as a mixture and individually, facilitating identification of g.l.c. peaks as shown in Figure 2.14; A. Comparison of the gas chromatogram of the free sugar (FS) PAAN derivatives (Figure 2.14; B)* with that of the standard sugar derivatives showed glucose to be the major component of the free sugar mixture; peak (h) appeared to correspond with arabinose. The gas chromatogram of the glycosidic (GS) PAAN derivatives (Figure 2.14; C) showed components which clearly correlated with the PAAN derivatives of glucose (peak s), rhamnose (peak m), fucose (peak n), and xylose (peak p). In addition, the small peaks (o), (r), and (t) appear to correspond to arabinose, mannose and galactose, in this chromatogram.

Confirmation of these results was obtained by co-elution of the PAAN derivatives of the (FS) and (GS) samples with those of the standard sugars, establishing the identity of some component sugars by the following correlations : (j) - glucose; (k) - galactose; (m) - rhamnose; (n) - fucose; (p) - xylose; (s) - glucose; and (t) galactose.

* The peak (x) on each chromatogram is typical of PAAN preparations, and is due to di-*N*-acetyl-*O*-acetylhydroxylamine.[106]

Figure 2.14 Gas chromatograms of peracetylated aldononitrile derivatives



Peak	Retention time (min)	Sugar
a	7.39	L-Rhamnose
b	8.84	D-Fucose
c	9.43	L-Arabinose
d	11.51	D-Xylose
e	19.16	D-Mannose
f	22.90	D-Glucose
g	24.42	D-Galactose
h	9.82	L-Arabinose
i	17.50	(Fructose)
j	22.69	D-Glucose
k	24.51	D-Galactose
l	28.28	(Fructose)
m	7.27	L-Rhamnose
n	8.46	D-Fucose
o	9.74	L-Arabinose
p	10.92	D-Xylose
q	17.28	(Fructose)
r	18.67	D-Mannose
s	22.37	D-Glucose
t	24.01	D-Galactose
u	29.62	(Fructose)
v	9.61	Fructose
w	17.14	Fructose
y	20.64	Fructose
z	26.84	Fructose

Conditions : DB-225 column; 220°C isothermal

Fructose was treated using the same derivatisation procedure to yield the peracetylated ketoxime (PAKO) (instead of the PAAN as afforded by aldoses), and the gas chromatogram of this derivative (Figure 2.14; D) showed several peaks presumed to be due to partially acetylated products. Comparison of the gas chromatogram of PAANs of samples (FS) and (GS) with that of the fructose PAKO derivative suggested that certain peaks [(h), (i), (l), (o), (q) and (n), Figure 2.14] could be attributed to the presence of fructose in these samples (FS) and (GS).

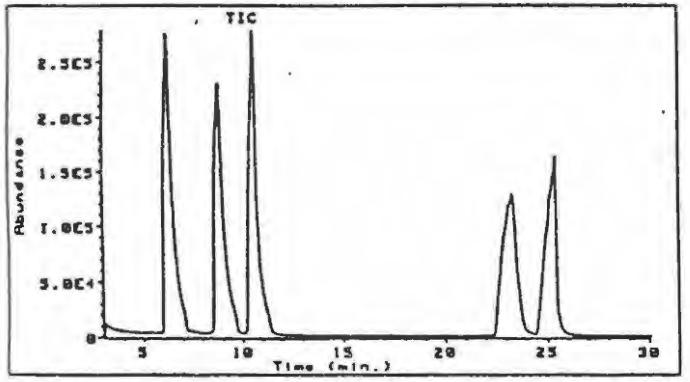
2.2.2.4 G.c. - m.s. of PAAN derivatives

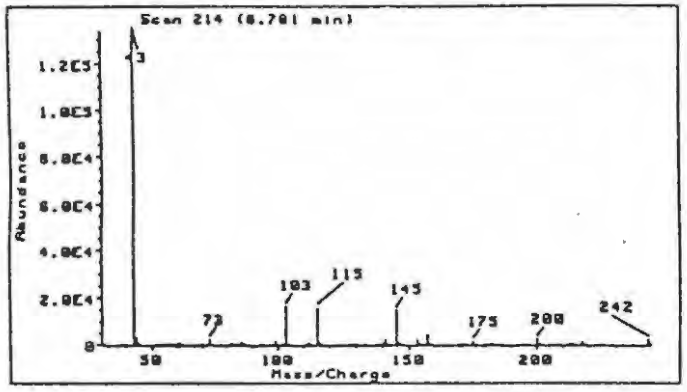
In both the hexose and the pentose series, the mass spectra of diastereomeric PAAN derivatives are very similar, since the compounds have similar fragmentation patterns. However, variation in the proportions of the fragments from particular members of a series results in characteristic mass spectra for each of the diastereomers.

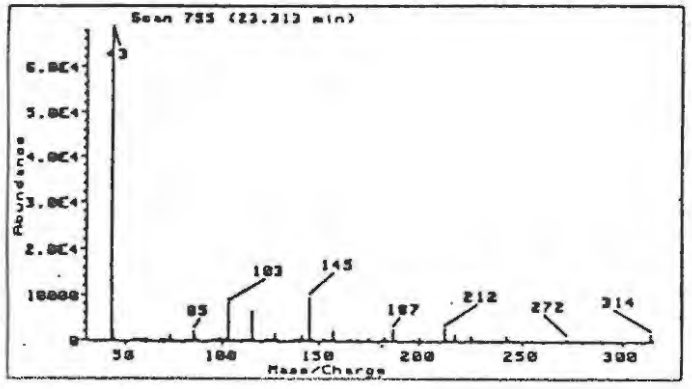
Mass spectra were obtained for the standard sugar PAAN derivatives and for the (GS) and (FS) PAANs, as shown in Figure 2.15. (Full data listings are given in the Appendix). This data was used to supplement the information obtained by g.l.c. analysis, which had been insufficient to fully establish the identity of components giving rise to peaks (h) and (o) in the gas chromatograms (Figure 2.14, p. 75) as being fructose derivatives or arabinose derivatives. The presence of arabinose in the (FS) sample was confirmed by visual comparison of the mass spectrum of the arabinose PAAN in the standard mixture, with that of the component in the (FS) PAAN sample which had the same retention time (Figure 2.15). (This result does not exclude the possibility that the arabinose was present in the free sugar sample owing to hydrolysis of a glycoside containing it, during the aqueous extraction procedure). The component in the glycosidic (GS) PAAN sample which was thought to be the arabinose PAAN, corresponded closely in elution time with the standard arabinose PAAN, but gave a mass spectrum in which there was less convincing correlation.

Figure 2.15 G.c. - m.s. analysis of PAAN derivatives of sugars

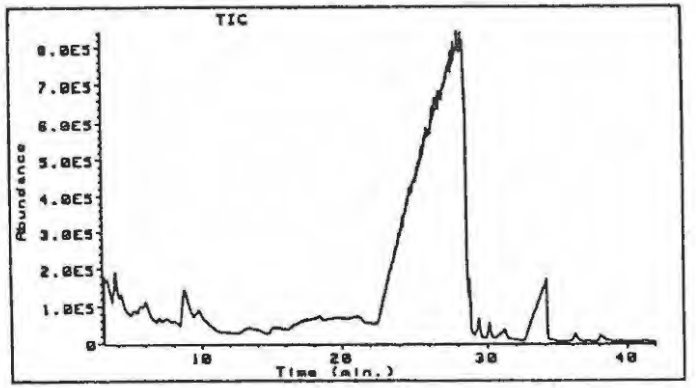
A Standard sugars

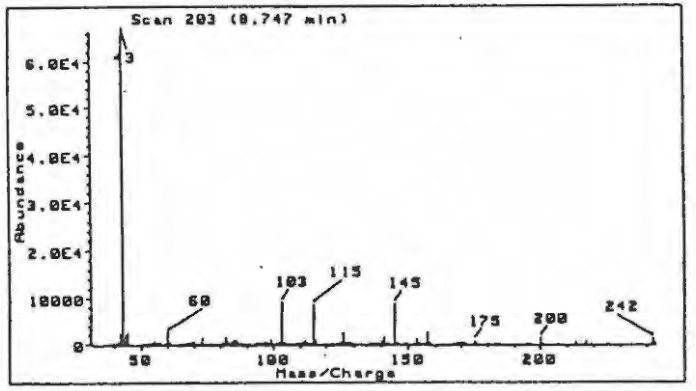


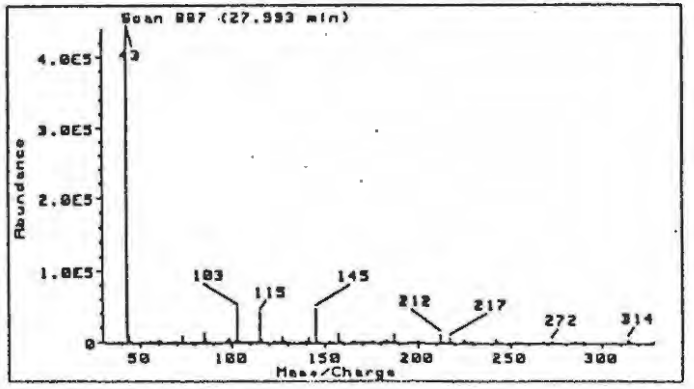




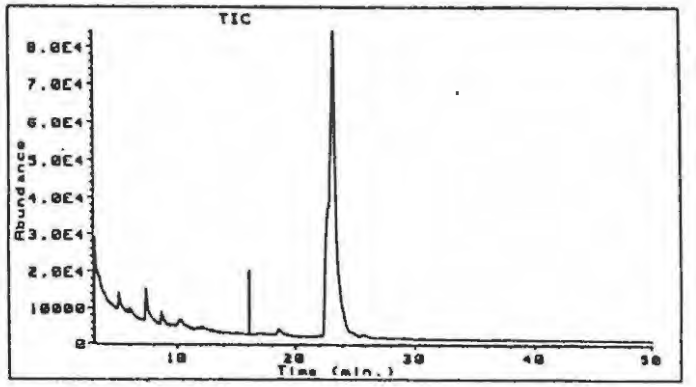
B Free sugars

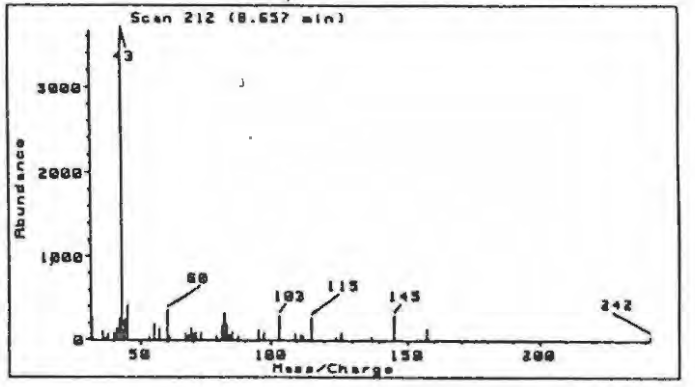






C Glycosidic sugars





Sugar	Retention time (min)
L-Rhamnose	6.170
L-Arabinose	8.781
D-Xylose	10.560
D-Glucose	23.313
D-Galactose	25.398

2.2.2.5 G.c. - m.s. of alditol acetate derivatives of sugars

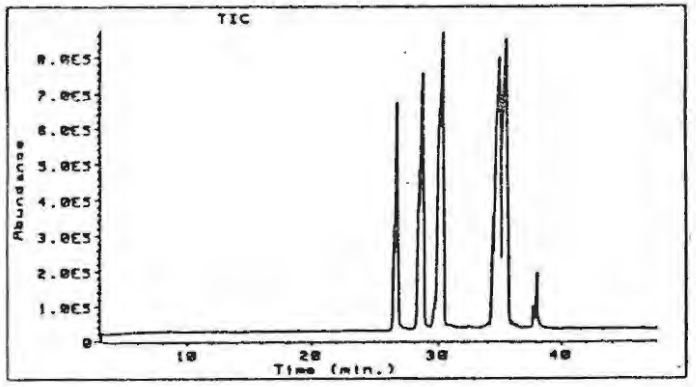
The presence of the ketose, fructose, in extract I as suggested by t.l.c., p.c., and g.l.c., required confirmation. While aldoses are readily identified by analysis of their PAAN derivatives, an alternative method is necessary for analysis of ketoses, since they cannot be converted to PAANs. The preparation of alditol acetates is a useful method for derivatising both aldoses and ketoses [Scheme 2.12, p. 73; (112)]. Reduction of an aldose produces an alditol, but a 2-ketose yields a pair of C-2 epimers, and hence two alditol acetates after acetylation. Fructose, for example, is converted by reduction to glucitol and mannitol. Thus, preparation of the alditol acetate derivatives of a mixture of glucose and fructose would result in a mixture of glucitol and mannitol hexa-acetates, and the relative proportions of the two components would be significant.

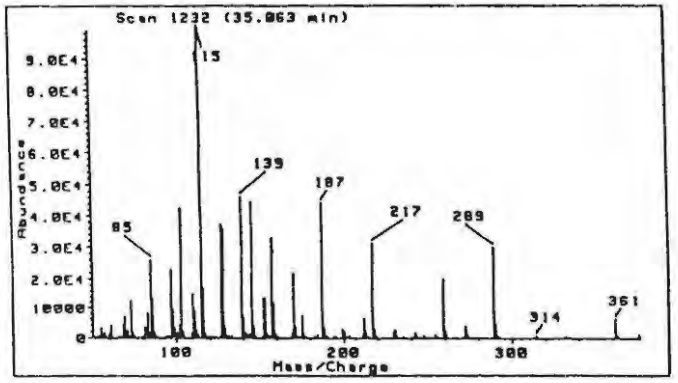
The procedure for derivatisation employs sodium borohydride for the reduction; after removal of unreacted borohydride, acetylation is carried out using acetic anhydride.[108] Additol acetates were prepared from fructose, mannose, and the standard sugars used previously, as well as from the sugar samples (FS) and (GS).

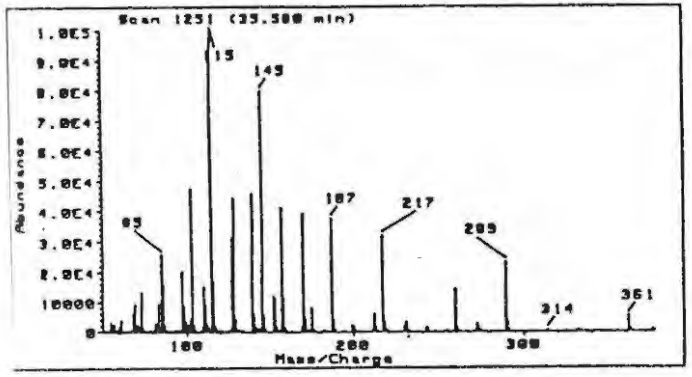
The results of g.c. - m.s. analysis of these alditol acetate derivatives are shown in Figure 2.16. The presence of fructose in both samples (FS) and (GS) is confirmed by the occurrence of components corresponding in retention time to the mannose (and glucose) derivative in both cases, and by the correlation of the mass spectra of these derivatives. Co-elution of the alditol acetates of the (FS) and (GS) samples with that of fructose verified these results.

Figure 2.16 G.c. - m.s. analysis of alditol acetate derivatives of sugars

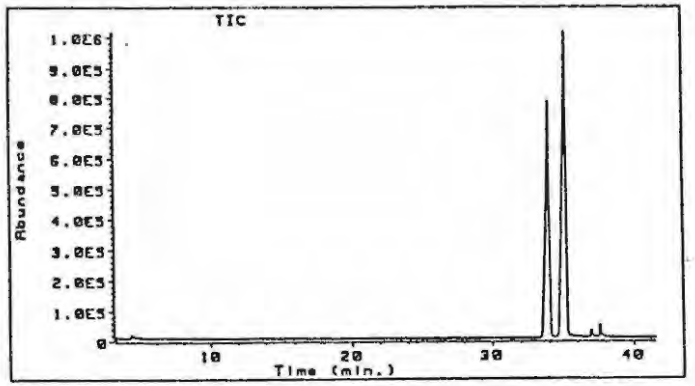
A Standard sugars

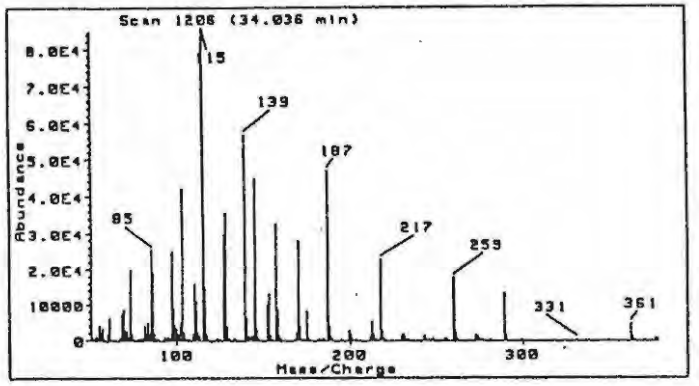


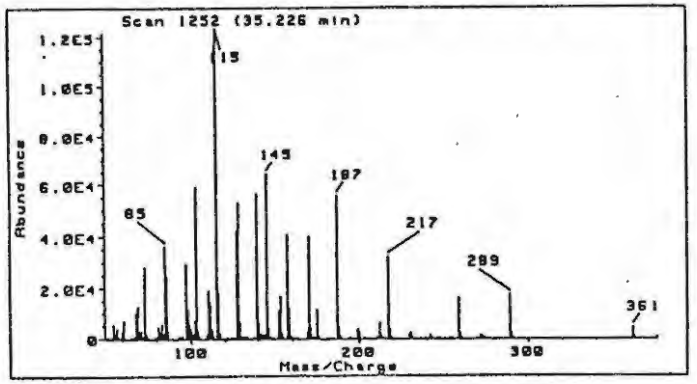




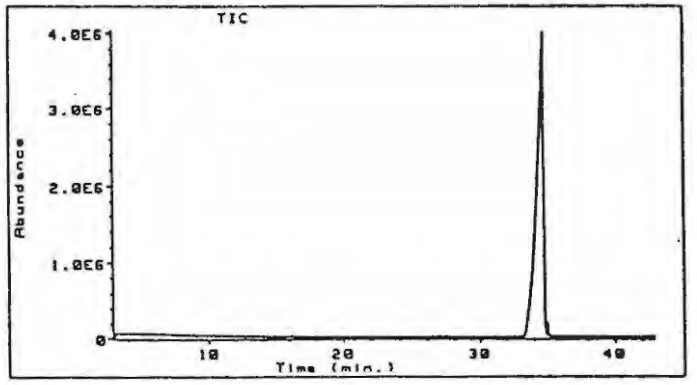
B Fructose

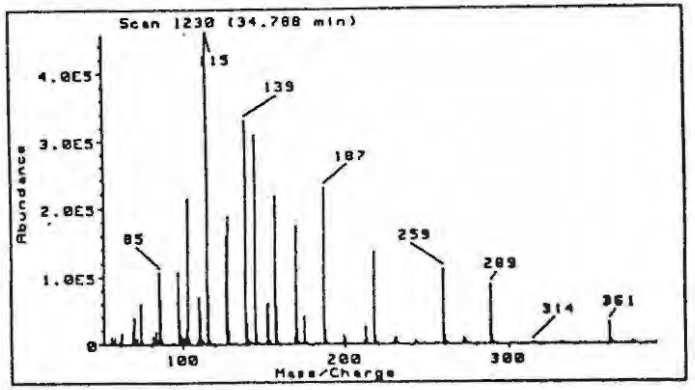






C Mannose

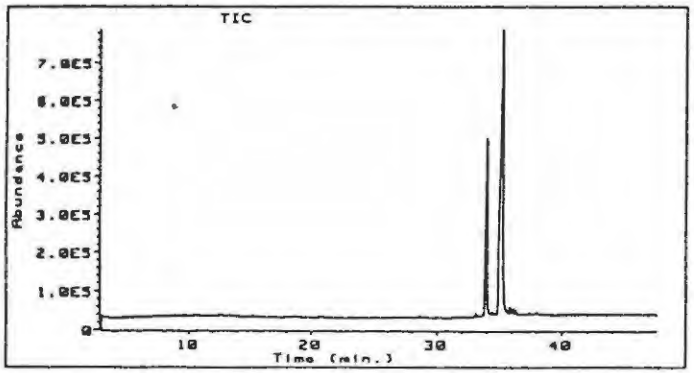


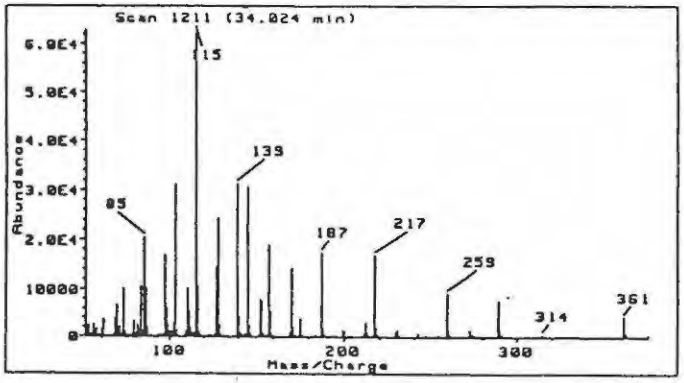


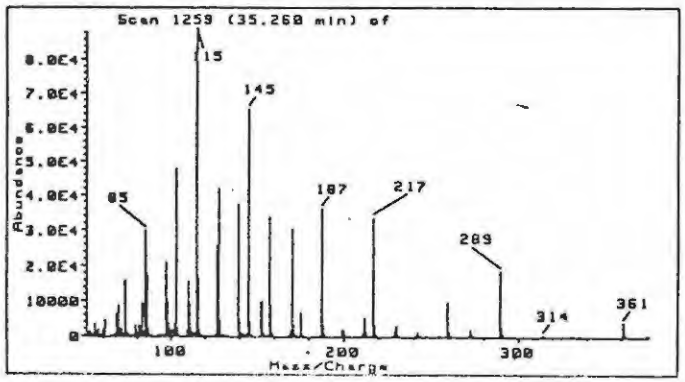
Sugar	Retention time (min)
L-Rhamnose	26.78
L-Arabinose	28.87
D-Xylose	30.47
D-Glucose	35.06
D-Galactose	35.58

Figure 2.16 G.c. - m.s. analysis of alditol acetate derivatives of sugars (contd)

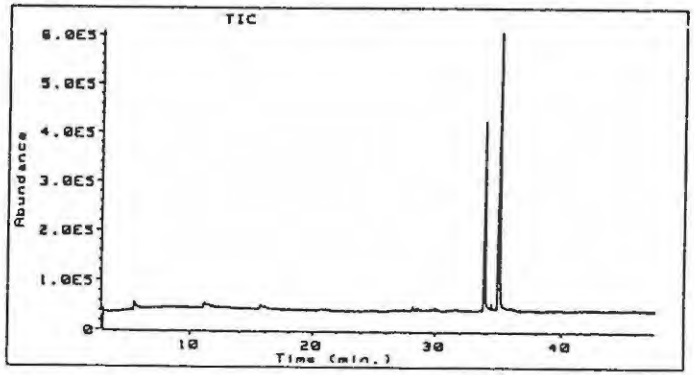
D Free sugars (FS)

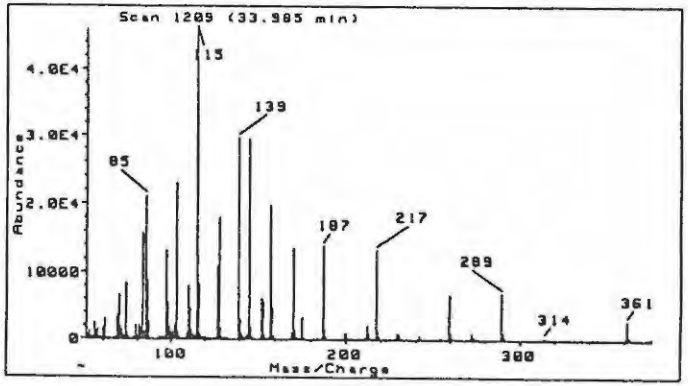


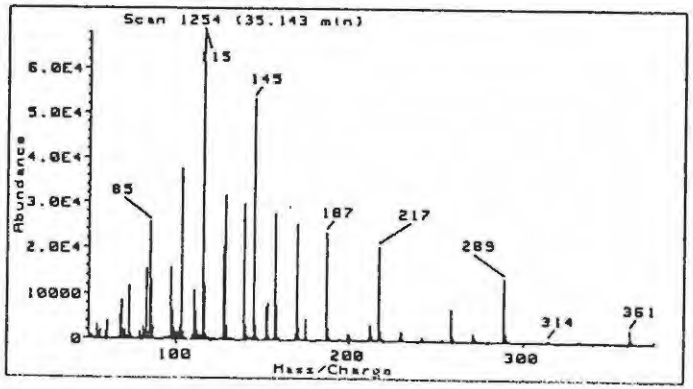




E Glycosidic sugars (GS)







2.2.2.6 A carbohydrate component obtained by methanolic extraction

During the methanolic Soxhlet extraction of *Tulbaghia violacea* and subsequent fractionation, a carbohydrate component was isolated. This solid material precipitated from the methanolic extract III (Scheme 2.11, p. 66). The material, (found to swell in water but to be insoluble) gave a positive Molisch test but did not give a blue colouration with iodine solution. Hydrolysis of the material gave glucose (as revealed by t.l.c.; see Experimental section, p. 161), and the material was presumed to be a polysaccharide.

2.2.2.7 Summary of analysis of sugars in *Tulbaghia violacea*

A range of different chromatographic techniques have thus confirmed the presence of the following sugars in *Tulbaghia violacea* :

	Sugar	Technique used for identification
Free sugars :	D-Glucose	p.c., t.l.c., g.l.c.
	D-Fructose	p.c., t.l.c., g.c. - m.s.
	L-Arabinose	t.l.c., g.l.c., g.c. - m.s.
	D-Galactose	g.l.c.
Glycosidic sugars :	D-Glucose	p.c., t.l.c., g.l.c.
	D-Fructose	p.c., t.l.c., g.c. - m.s.
	L-Rhamnose	g.l.c.
	D-Fucose	g.l.c.
	L-Arabinose	g.l.c., g.c. - m.s.
	D-Xylose	p.c., g.l.c.
	D-Galactose	g.l.c.

2.2.3 ISOLATION AND IDENTIFICATION OF FLAVONE AGLYCONES IN *TULBAGHIA VIOLACEA*

2.2.3.1 Preliminary investigations

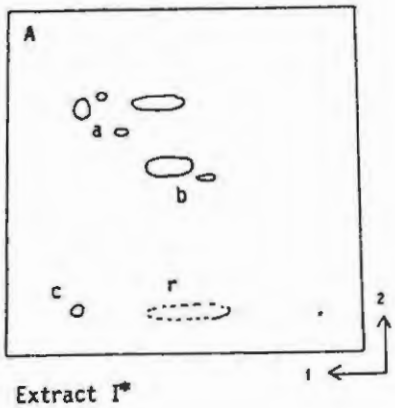
The glycosides in both aqueous and methanolic extracts of *Tulbaghia violacea* were examined by two t.l.c. techniques.

Firstly, two-dimensional t.l.c. (following the method of Mabry, Markham, and Thomas[2] and using aqueous eluants and micro-crystalline cellulose plates) gave the results illustrated in Figure 2.17. A comparison of these chromatograms with those illustrated in the reference suggested the possible presence of 3- and 7-*O*-glycosides of flavonols in the extracts.

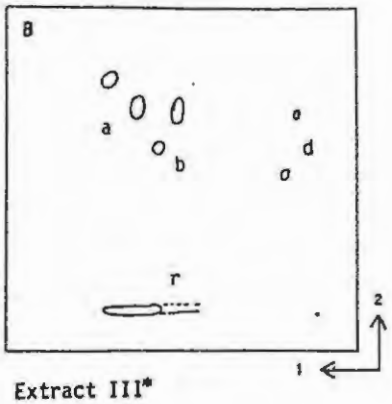
A second two-dimensional technique, involving hydrolysis of material on the t.l.c. plate (described previously in section 2.2.2.1, p. 67) was applied to the aqueous extract I and the butanol-soluble fraction V of the methanolic extract III (Scheme 2.11, p. 66). After hydrolysis, standard flavones were spotted on the plates for comparison with components of the hydrolysed material. A solvent system appropriate for separation of aglycones [*viz.*, ethyl acetate-hexane (1:1)] was used for the second elution. The results of this chromatography are shown in Figure 2.18. These chromatograms also indicated the presence of aglycones; u.v. - fluorescent spots, similar to those of the standard flavone samples in appearance and R_f value, were observed.

Separation of the glycosides was investigated with a view to hydrolysing isolated glycosides and thus obtaining isolated aglycones. Gel filtration (on Sephadex LH-20, using a peristaltic pump system and u.v. detector) was attempted,[109] but poor separation of the 5 detected components, and the small quantities of material in the fractions obtained, made this method unsuitable for larger scale separation of the glycosides. Flash chromatography of the butanol-soluble fraction V (Scheme 2.11, p. 66), using aqueous chloroform-methanol solvent systems of increasing polarity, resulted in the isolation of 6 fractions, but each was found to be contaminated and considerable loss of material occurred due to retention on the column.

Figure 2.17 T.l.c. of extracts I and III on cellulose



Extract I*



Extract III*

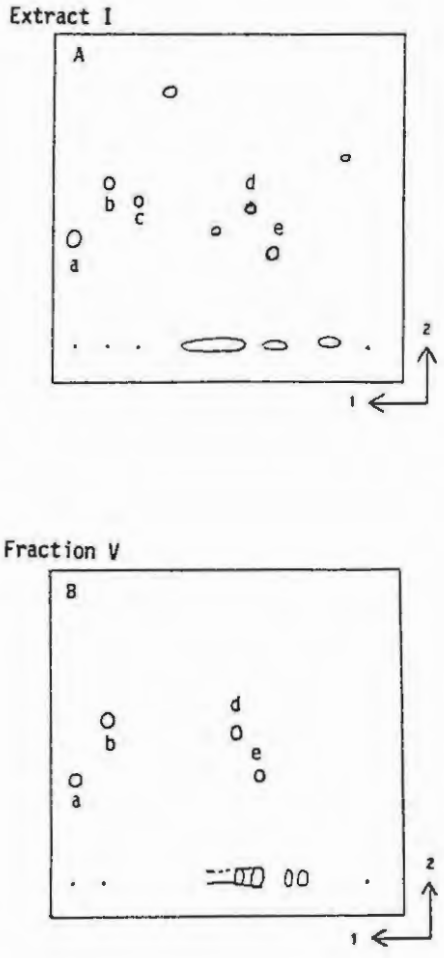
Spot	Possible constituent **
a	Dihydroflavonol-3-*O*-monoglycoside
b	Flavonol-3-*O*-monoglycoside
c	Aglycone
d	Flavonol-3,7-diglycoside
r	Material not mobile in second solvent system

Solvent systems : Bu^tOH - AcOH - H_2O (3:1:1) and 5% AcOH;
visualised with iodine

* See scheme 2.11, p

** Identified by position on the chromatogram.[2]

Figure 2.18 T.l.c., with hydrolysis, of extracts I and V

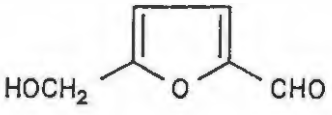


Spot	Constituent
a	Quercetin
b	Kaempferol
c	Isorhamnetin
d	Possible
e	aglycones

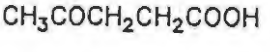
Solvent 1 : Bu^tOH - AcOH - H_2O (3:1:1); hydrolysis with 5M-HCl;

solvent 2 : EtOAc - hexane (1:1).

The most effective method of obtaining aglycones involved extraction of hydrolysed glycosidic mixtures. Hydrolysis of a sample of extract I (Scheme 2.11, p. 66) was carried out (see Experimental section, p. 155), and solvent extraction was expected to separate sugars and aglycones. However, although the aqueous layer was found to contain sugars (see section 2.2.2.1, p. 67), the presence of aglycones in the organic layer was masked by the high concentration of degradation products of free sugars in the original extract. P.l.c. of the concentrated organic layer afforded, as major components, 5-hydroxymethylfurfural (**115**) and 4-oxo-pentanoic acid (**116**) which were identified by ^{1}H and ^{13}C n.m.r. spectroscopy (see Experimental section, p. 156). These products are commonly formed by hexoses under acidic conditions such as those used for the hydrolysis of extract I.[110]



(115)



(116)

In order to obtain a fraction containing the glycosides but no free sugars, the methanolic extract of the plant material (extract III, Scheme 2.11, p. 66) was partitioned between butanol and water.

2.2.3.2 Chromatographic comparison of aglycones with model compounds

Samples of the methanolic extract III and the butanol-soluble fraction V (Scheme 2.11, p. 66) were hydrolysed (see Experimental section, p. 163), and ethyl acetate extracts of the hydrolysates were used to identify the aglycones. The aglycone fraction obtained by hydrolysis of the glycosides isolated after removal of free sugars (see Experimental, p. 157) was also chromatographed [labelled (r) in Figure 2.19]. The standard flavones and methoxyflavones prepared for the purpose (section 2.1) were used as chromatographic standards. T.l.c. using four different solvent systems, as illustrated in Figure 2.19, showed the possible correlations summarised in Table 2.3.

Figure 2.19 T.l.c. comparison of aglycones and model compounds

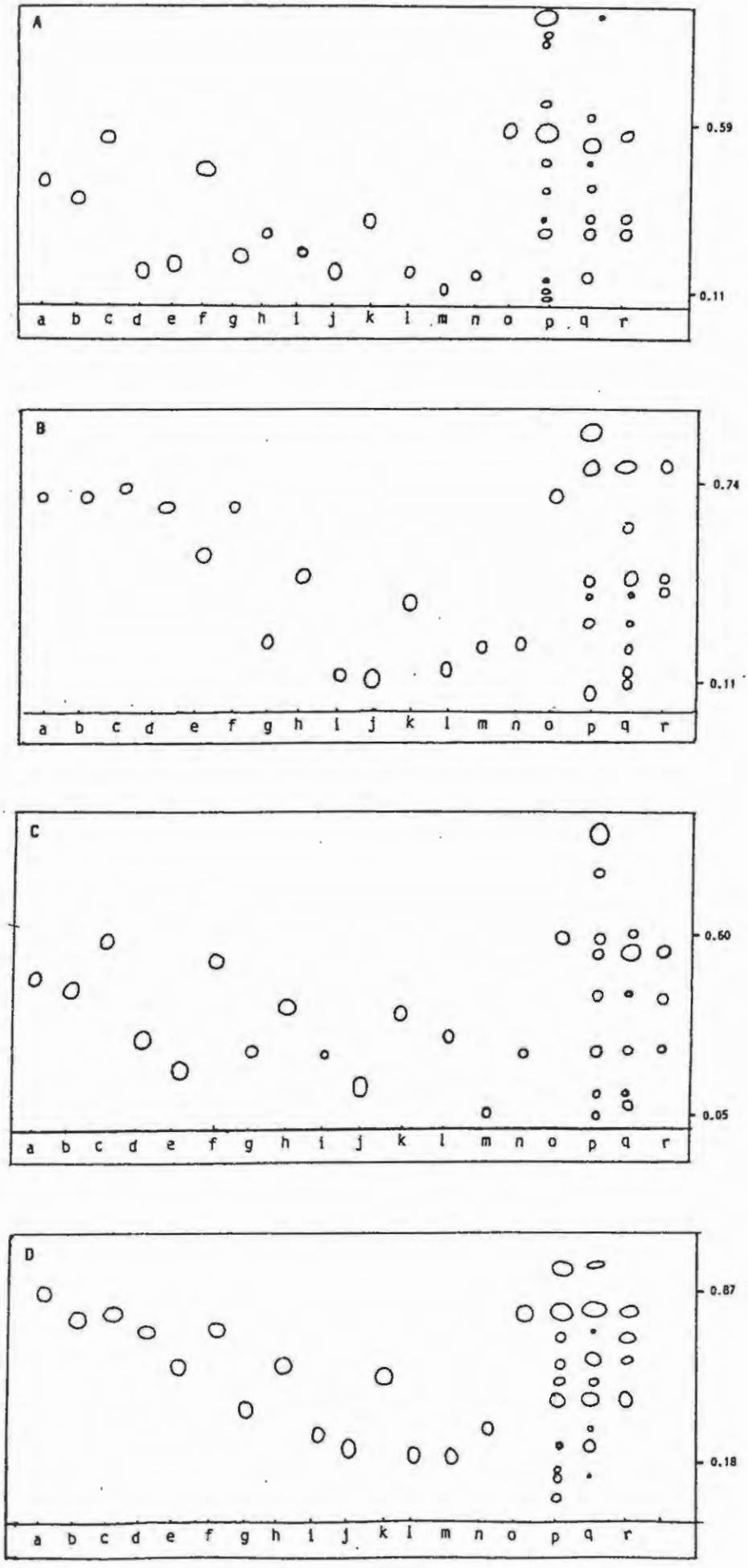


Figure 2.19 T.l.c. comparison of aglycones and model compounds (contd)

Spot	Standard	R_f values in solvent systems (7) - (10) (7)	(8)	(9)	(10)
a	3,3′,4′,5′-Tetra-*O*-methylmyricetin (92)	0.42	0.69	0.47	0.87
b	3,3′,4′-Tri-*O*-methylquercetin (88)	0.38	0.71	0.44	0.77
c	3,4′-Di-*O*-methylkaempferol (86)	0.57	0.74	0.60	0.78
d	3′,4′-Di-*O*-methylluteolin (84)	0.12	0.67	0.28	0.72
e	3,3′,4′-Tri-*O*-methylfisetin (90)	0.15	0.52	0.17	0.59
f	4′-*O*-methylapigenin (82)	0.46	0.65	0.52	0.74
g	Rhamnetin (98)	0.19	0.22	0.24	0.43
h	Isorhamnetin (103)	0.25	0.44	0.37	0.59
i	Myricetin (93)	0.20	0.13	0.24	0.26
j	Quercetin (89)	0.12	0.11	0.13	0.21
k	Kaempferol (87)	0.29	0.36	0.36	0.56
l	Luteolin (85)	0.11	0.14	0.18	0.18
m	Fisetin (91)	0.08	0.32	0.05	0.18
n	Apigenin (83)	0.11	0.33	0.25	0.27
o	Chrysin (80)	0.59	0.69	0.60	0.77

Sample

p	Aglycones from extract III
q	Aglycones from fraction V
r	Aglycones from hydrolysis of glycosides after removal of free sugars (see section 2.2.2.2.), called X

Chromatogram	Solvent system
A	(7) EtOAc - hexane (1:1)
B	(8) $CHCl_3$ - acetone (4:1)
C	(9) EtOAc - toluene (3:2)
D	(10) THF - $CHCl_3$ (1:4)

Table 2.3 Correlation of aglycones with standard flavones

Aglycone extract	Standard component	Observed correlation for solvent systems (7) - (10)*
III	Isorhamnetin	(7) (8)
	Kaempferol	(7)
	3,3',4'-Tri-*O*-Methylquercetin	(7)
	3'-*O*-Methylapigenin	(7) (9)
	Chrysin	(7) (9) (10)
	Quercetin	(7) (8) (9) (10)
	Apigenin	(9)
	Rhamnetin	(10)
V	Isorhamnetin	(7) (8)
	Kaempferol	(7)
	3,3',4'-Tri-*O*-methylquercetin	(7)
	Quercetin	(7) (8) (9) (10)
	3'-*O*-Methylapigenin	(7) (9)
	Luteolin	(8)
	Apigenin	(8) (9)
	Chrysin	(9) (10)
X	Kaempferol	(7) (8) (9)
	Isorhamnetin	(8)
	Apigenin	(9)
	3'-*O*-methylapigenin	(9)
	Chrysin	(9) (10)
	Rhamnetin	(10)
	Quercetin	(7) (8) (9) (10)

* See Figure 2.19

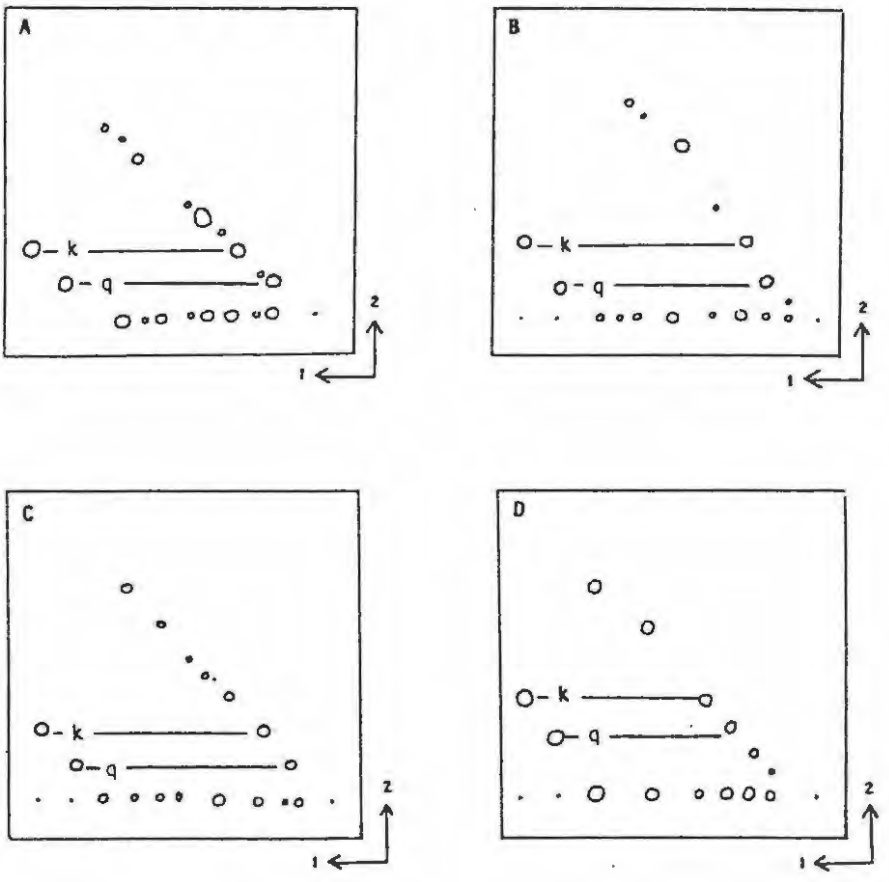
It was evident from these chromatograms that all of the aglycone components (expected in the R_f range 0.11 - 0.87) in the samples (p) and (r) were present in sample (q). Correlation of an aglycone component with a standard in all four solvent systems was taken to indicate the presence of that flavone in the aglycone mixture; this was the case for kaempferol and quercetin. To confirm these observed correlations, and to avoid possible aberrations due to interaction of components on the plates, two-dimensional t.l.c. was used. The aglycone sample (q) from fraction V was chromatographed, using the same 4 solvent systems (for both elutions in each case) to give clearer separation as shown in Figure 2.20.

Thus, on the basis of the foregoing chromatographic evidence, the aglycone mixtures from *Tulbaghia violacea* contain the flavonols, kaempferol and quercetin.

2.2.3.3 Correlation of the sugar and aglycone components of the glycosides

The techniques of hydrolysing glycosides on a t.l.c. plate allowed some correlation of the aglycone and sugar components. Figures 2.11; A and 2.18; A show the results of analysis of extract I, for sugars and aglycones respectively. Correlation of the two chromatograms indicates that the aglycones kaempferol (d) and quercetin (e) in Figure 2.18; A were associated with D-glucose [spots (d) and (e) in Figure 2.11; A]. Similarly for the butanol- soluble fraction V, correlation of Figure 2.11; C with Figure 2.18; B suggested that the aglycones kaempferol and quercetin [(d) and (e) in Figure 2.18; B] were associated with D-glucose [(d) and (c) in Figure 2.11; C]. The presence of xylose was suggested by some green colouration in spot (e). The spot (f) in Figure 2.11; C appeared to correlate with D-fructose in R_f value and colour, and the absence of an associated aglycone, together with its low R_f value in the first elution, suggest the presence of D-fructose as a free sugar.

Figure 2.20 2-D t.l.c. to confirm identity of aglycones in extract V

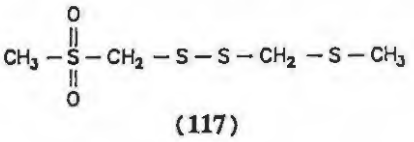


Spot	Flavone
k	Kaempferol
q	Quercetin

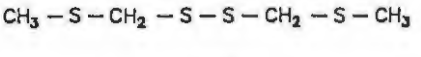
Solvent systems : A, EtOAc - hexane (1:1); B, $CHCl_3$ - acetone (4:1);
C, EtOAc - toluene (3:2); D, THF - $CHCl_3$ (1:4).

2.2.4 ISOLATION AND IDENTIFICATION OF SULPHUR COMPOUNDS FROM *TULBAGHIA VIOLACEA*

In the course of the Soxhlet extraction of *Tulbaghia violacea*, two sulphur compounds were isolated. They were identified, on the basis of n.m.r., i.r., and mass spectroscopic analyses, as 2,4,5,7-tetrathiaoctane-2,2-dioxide (117) and 2,4,5,7-tetrathiaoctane (118).



(117)



(118)

2.2.4.1 Spectroscopic analysis of sulphur compound (117)

Compound (117) was isolated as white crystals from the hexane extract II (Scheme 2.11, p. 66). The 300 MHz ^{1}H n.m.r. spectrum of this compound shows four singlets (Table 2.4 and Figure 2.21) indicating two methyl and two methylene groups, isolated from one another. The 3-proton signal at δ 2.25 is consistent with the presence of a CH_3-S group, and that at δ 3.05 is consistent with the presence of a CH_3-SO_2 group. The chemical shifts of the two methylene signals indicate that they are also adjacent to deshielding groups. The four signals in the ^{13}C n.m.r. spectrum (Table 2.4 and Figure 2.21) correspondingly indicate two non-equivalent methyl groups and two non-equivalent methylene groups. The chemical shifts of these signals are consistent with those cited in the literature for similar compounds.[47;49]

Strong i.r. absorption bands at 1325 and 1140 cm^{-1} indicate the presence of the sulphone group adjacent to straight chain alkyl groups (as opposed to aromatic or branched chain alkyl groups) (Figure 2.22). This type of sulphone group typically shows two absorption bands, in the regions 1330 - 1317 and 1152 - 1136 cm^{-1}. A typical feature of the sulphone absorption in the 1330 - 1317 cm^{-1} region is that the peak is split in the solid state, and this was, indeed, found to be the case in the i.r. spectrum of compound (117) The small peaks at 590 and 550 cm^{-1} are attributed to scissoring and wagging of the SO_2 group.

Figure 2.21 N.m.r. spectra of sulphur compound (117)

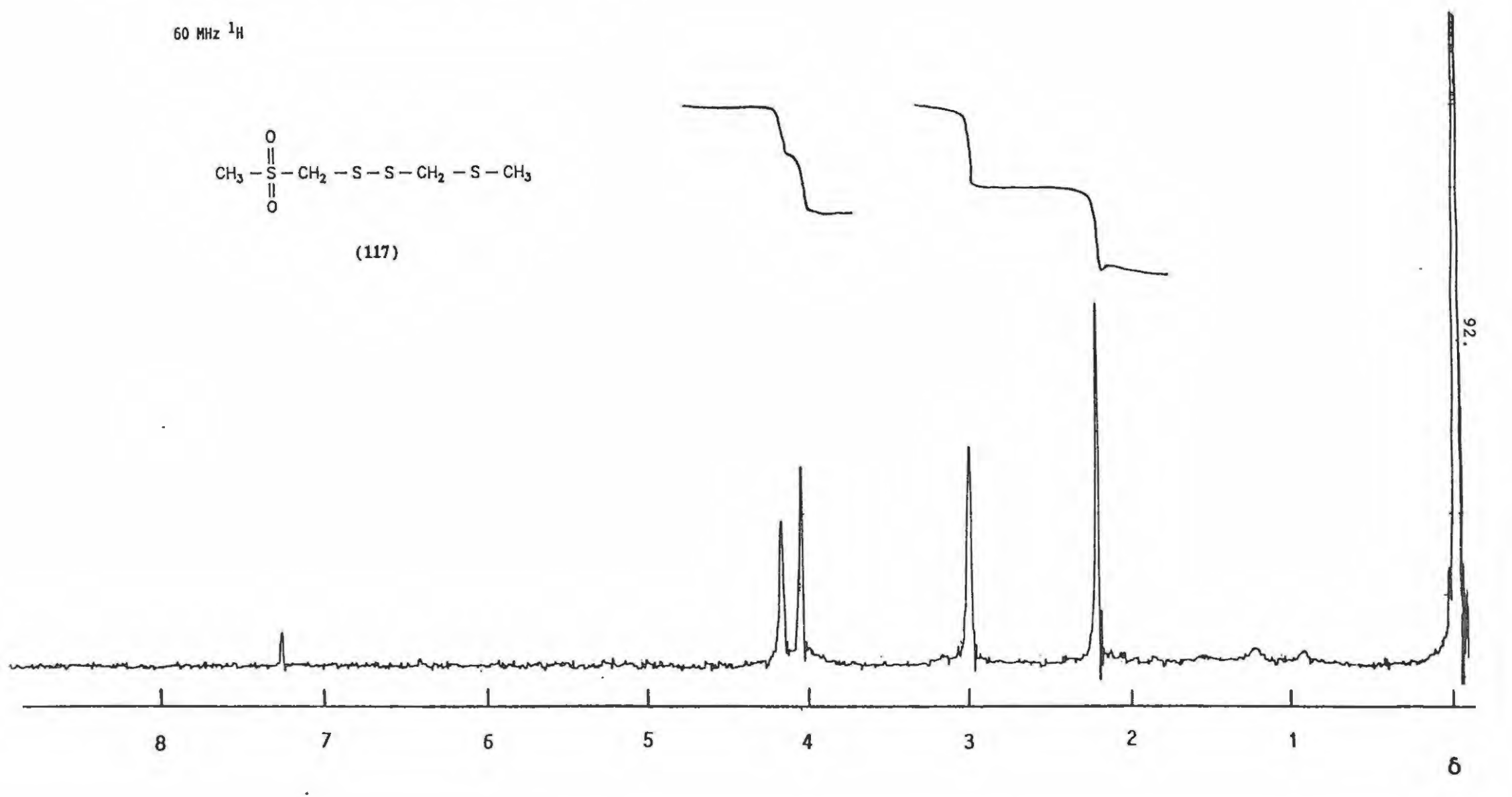
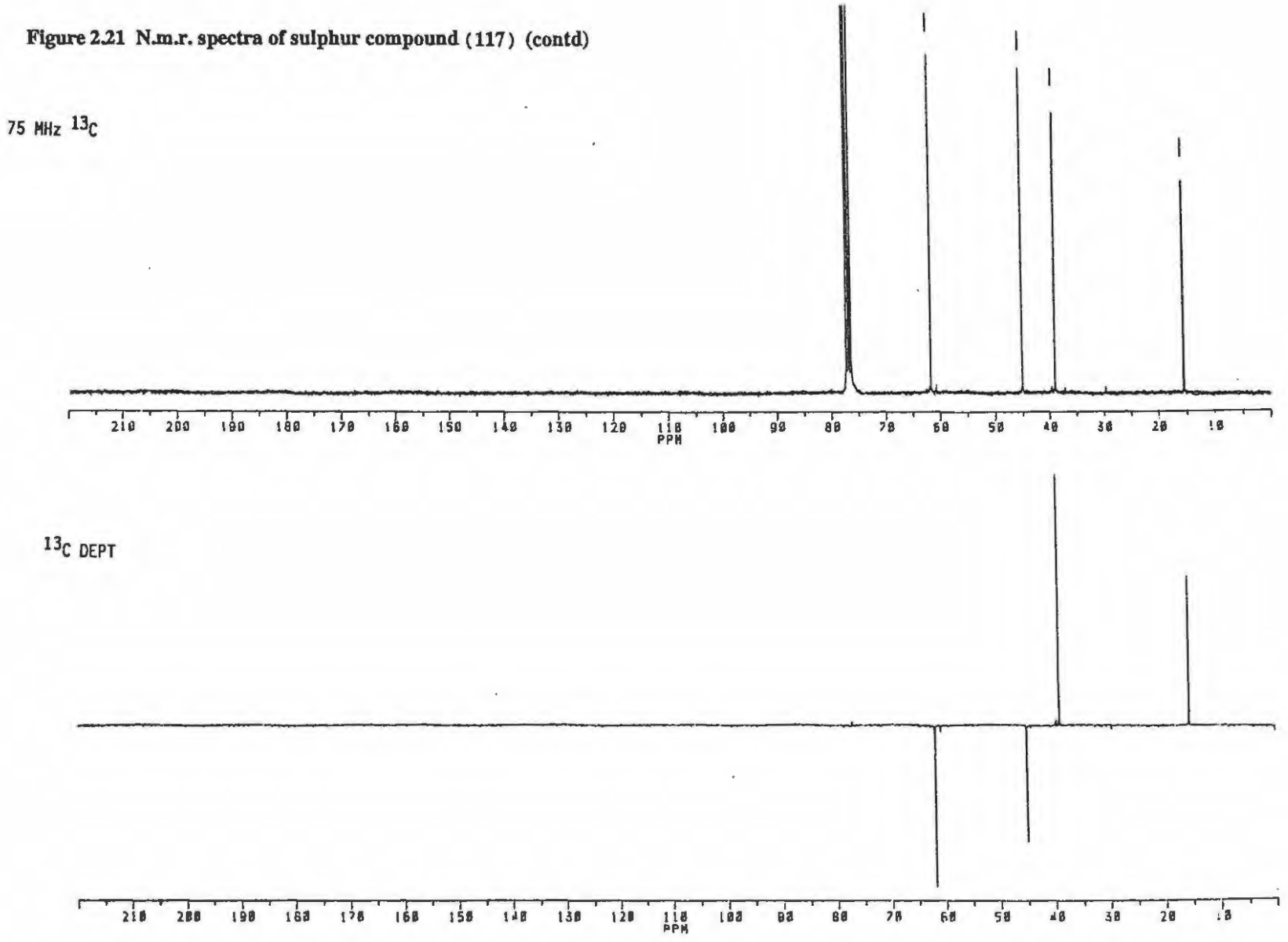


Figure 2.21 N.m.r. spectra of sulphur compound (117) (contd)



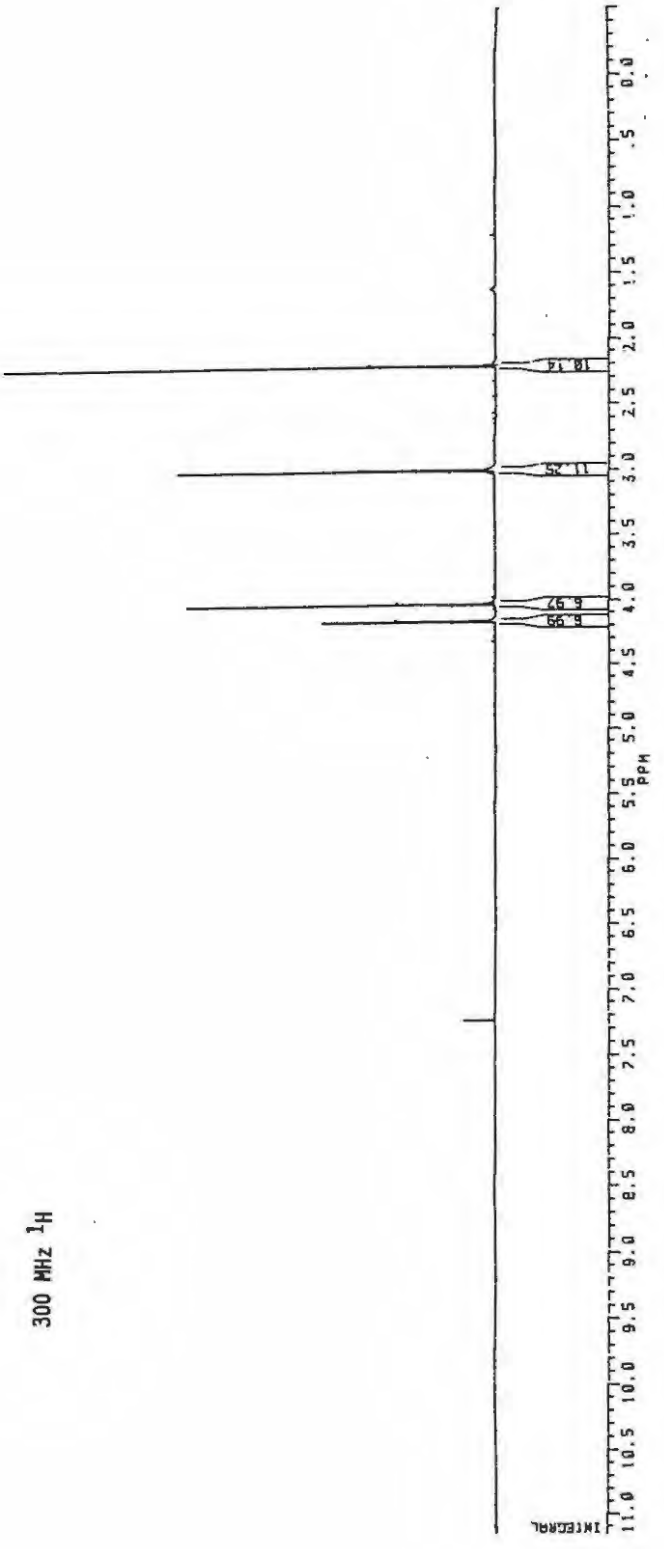
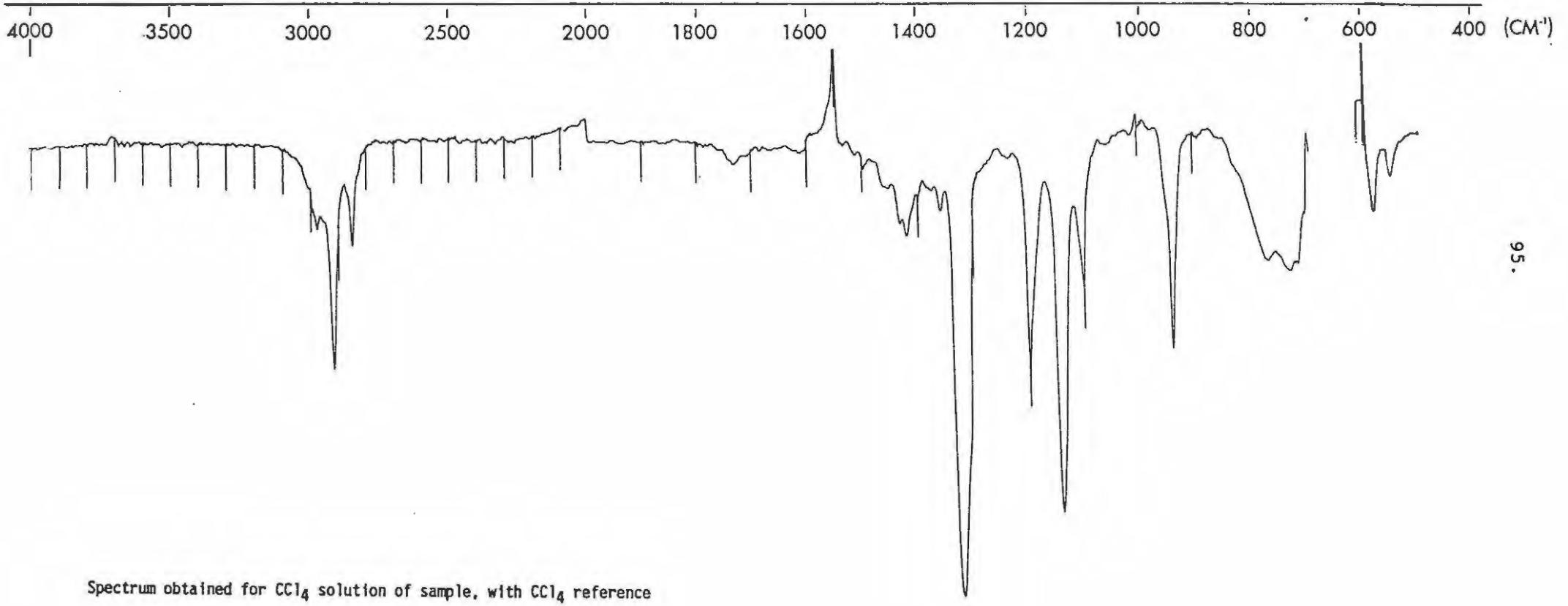


Figure 2.21 N.m.r. spectra of sulphur compound (117) (contd)

Figure 2.22 I.r. spectrum of sulphur compound (117)



Spectrum obtained for CCl_4 solution of sample, with CCl_4 reference

The mass spectrum (Figure 2.26, p. 102) indicates a molecular ion, *m/z* 218.15 with an M : M+2 ratio of 100 : 17.73. This is in agreement with the theoretical relative abundance of the M+2 peak for a compound containing four sulphur atoms per molecule, *viz.*, 100 : 17.76.* (Full data listings are given in the Appendix).

Table 2.4 Spectral data for sulphur compound (117)

^{1}H n.m.r.	δ		^{13}C n.m.r.	δ	
	2.25	(s, 3H)		15.47	(CH_3)
	3.05	(s, 3H)		39.26	(CH_3)
	4.12	(s, 2H)		45.25	(CH_2)
	4.22	(s, 2H)		62.07	(CH_2)

I.r.	ν_{max} (cm^{-1})	M.s.	*m/z*
	1325		218 (M^+, 3%)
	1140		111 (2)
	590		93 (10)
	550		61 (100)
			45 (71)

---

* As calculated from the ratio $\frac{P_{M+2}}{P_M} = y\left(\frac{4.22}{100 - (4.22+0.76)}\right)$

where P_M = abundance of molecules with no heavy isotopes

P_{M+2} = abundance of molecules with y atoms of (M+2) isotopes[112]

2.2.4.2 Spectroscopic analysis of sulphur compound (118)

The compound (118) was isolated as a pale yellow liquid, by p.l.c. of the ethyl acetate fraction IV from the methanolic extraction of *Tulbaghia violacea* (Scheme 2.11, p. 66).

The ^1H n.m.r. spectrum of compound (118) showed only two singlets (δ 2.23 and 3.40) integrating in the ratio 3 : 2 respectively, as shown in Figure 2.23 and Table 2.5. As in the case of compound (117), the lack of coupling and the simplicity of the spectrum indicate the presence of isolated methyl and methylene groups. The chemical shifts of the signals are in keeping with those of CH_3S and $S-CH_2-S$ groups, respectively.[47]

In the i.r. spectrum (Figure 2.24) the absorption band at 680 cm^{-1} is consistent with the presence of C-S bonds, and the 480 cm^{-1} absorption band indicates disulphide S-S stretching. The absorption band at 1190 cm^{-1} is probably attributable to a dithioester degradation product of compound (118), since this band is in the region characteristic of the -S-(CS)- group (1225 - 1190 cm^{-1}).[111] Disulphides bearing α-protons are susceptible to α-elimination reactions, and such a reaction for compound (118), shown by equation (x), would yield compound (119) which is a dithioester.[47]

$$CH_3SCH_2-S-S-\overset{H}{\underset{|}{C}}H-SCH_3 \rightarrow CH_3-S-CH_2S^- + CH_3-S-\overset{S}{\overset{\|}{C}}-H \qquad (x)$$

(119)

G.c. - m.s. analysis of compound (118) (Figure 2.26) indicated a component with an apparent molecular ion, *m/z* 186, which together with the M : M+2 ratio of 100 : 17.56 supports the disulphide structure proposed for the compound. The component giving mass spectrum C (Figure 2.26) is likely to be the trisulphide (120), since disulphides are known to be readily converted to trisulphides when heated on the g.l.c. column (see section 2.2.4.3), as shown in equation (xi).

$$R-S-S-R \longrightarrow R-S-S-S-R + R-S-R \qquad (xi)$$

(120)

(120) R = CH_3SCH_2 *m/z*, M^+ 218

Figure 2.23 ^{1}H **n.m.r. spectrum of sulphur compound (118)**

$CH_3 - S - CH_2 - S - S - CH_2 - S - CH_3$

(118)

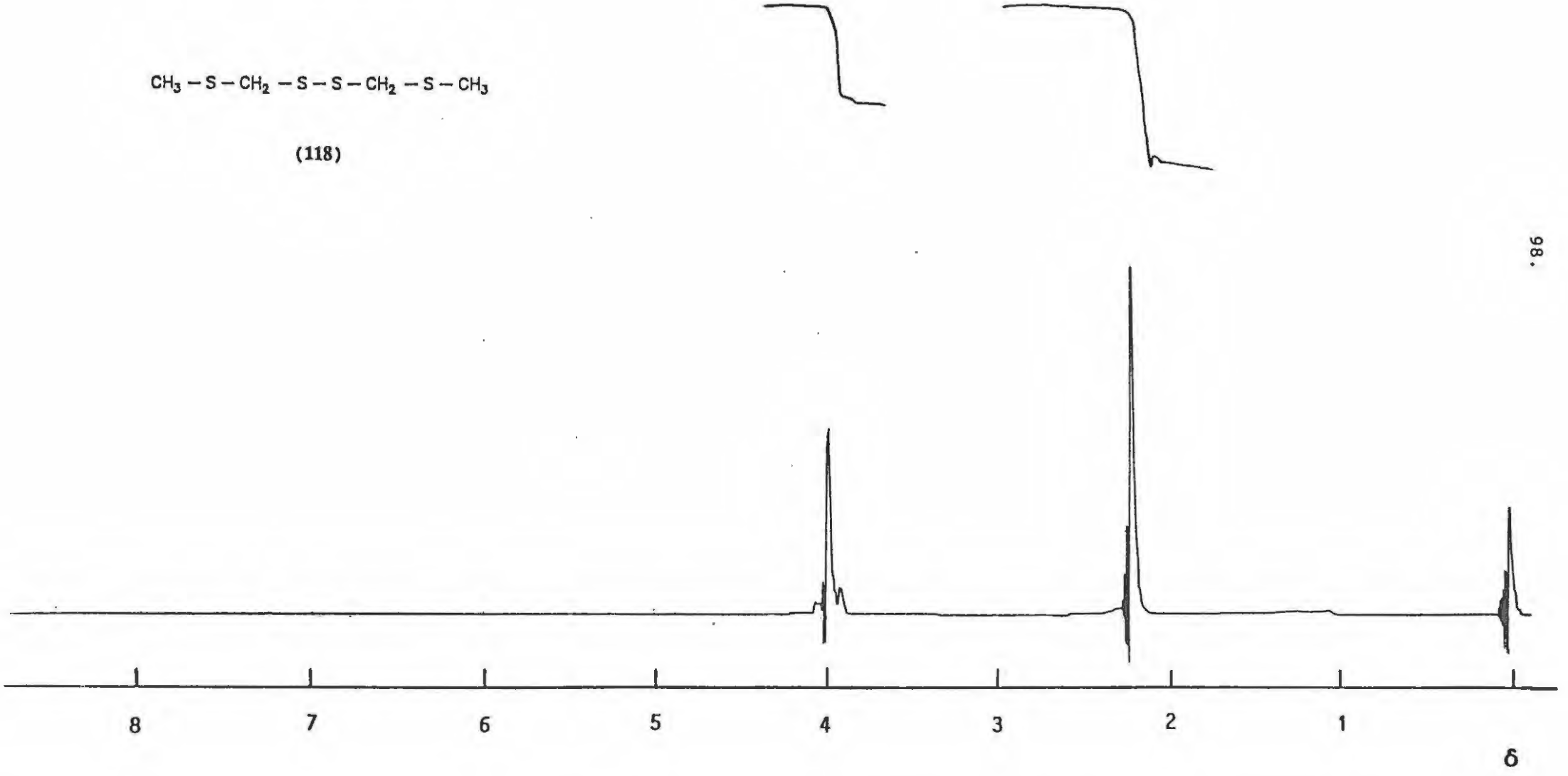
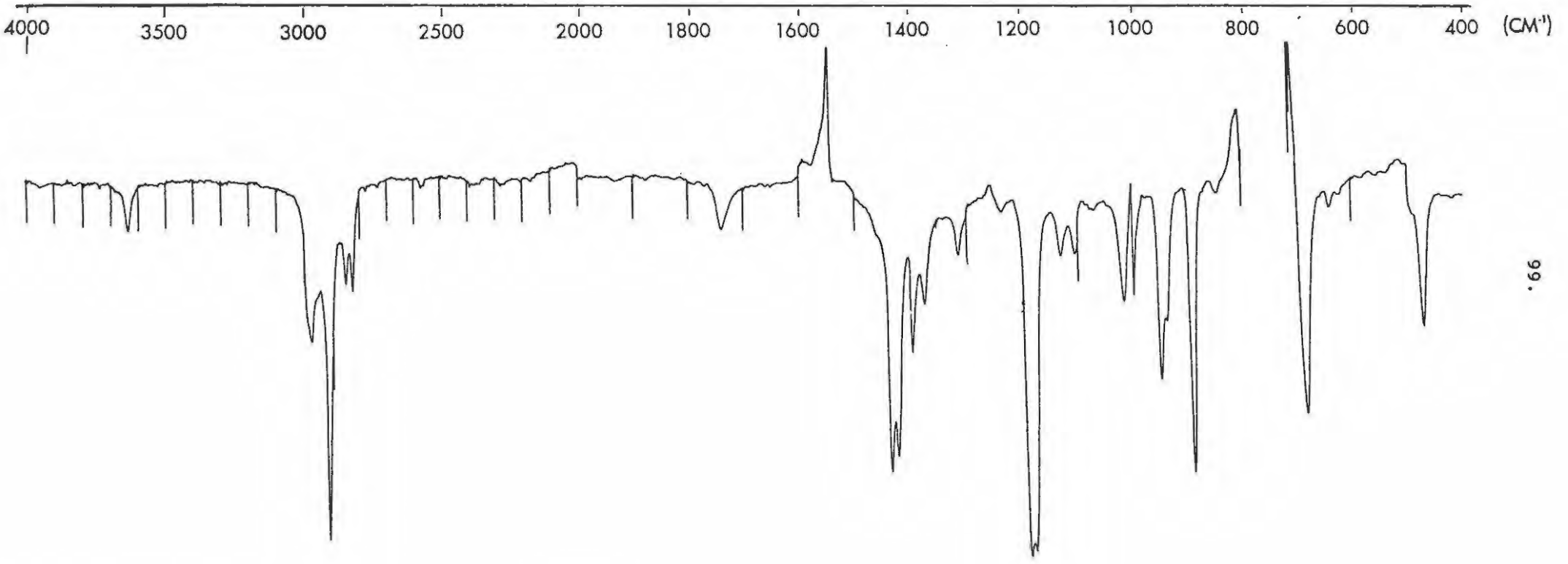


Figure 2.24 I.r. spectrum of sulphur compound (118)



Spectrum obtained for CCl_4 solution of sample, with CCl_4 reference

Table 2.5 Spectral data for sulphur compound (118)

^1H n.m.r.	δ		
	2.23	(3H)	
	3.40	(2H)	
I.r.	ν_{max} (cm^{-1})	M.s.	*m/z*
	1190		186 (M^+, 14%)
	680		93 (2)
	480		61 (100)

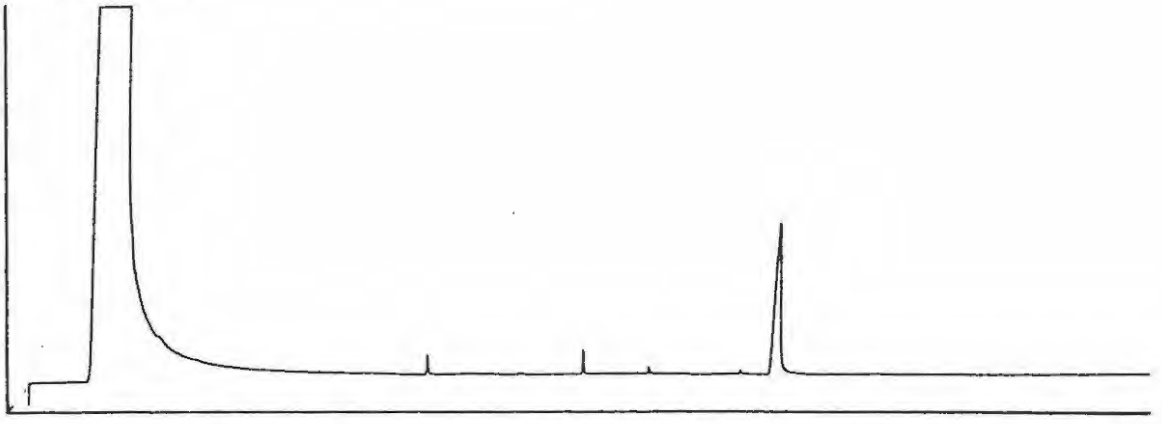
2.2.4.3 G.l.c. and g.c. - m.s. analysis of sulphur compounds (117) and (118)

The disproportionation of alkyl disulphides and related compounds on a g.l.c. column is commonly recognised,[49;50] and the structures of the products depends on the operating conditions. For example, a mixed dialkyl disulphate RSSR′ may disproportionate to give sulphide and trisulphide products such as RSR′ , RS_3R' , RSR and $R'S_3R'$, as well as the disulphides RS_2R and $R'S_2R'$.[47;114] Further disproportionation may lead to the formation of tetrasulphides and sulphides, from trisulphides.

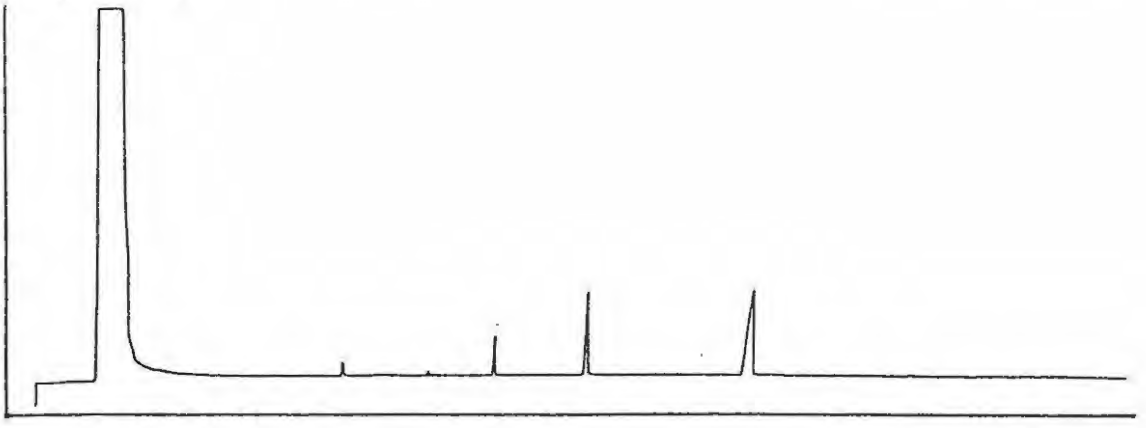
This is apparent in the chromatograms obtained in the g.l.c. analysis of the isolated compounds (117) and (118), and in the chromatogram obtained for a sample of component (117) which had previously been heated, in a sealed tube, to the maximum temperature employed in the gas chromatograph, *viz.*, 200°C (Figure 2.25). M.s. and g.c. - m.s. analyses of these samples (Table 2.6 and Figure 2.26) were used to identify possible components.

Figure 2.25 G.l.c. analysis of sulphur compounds

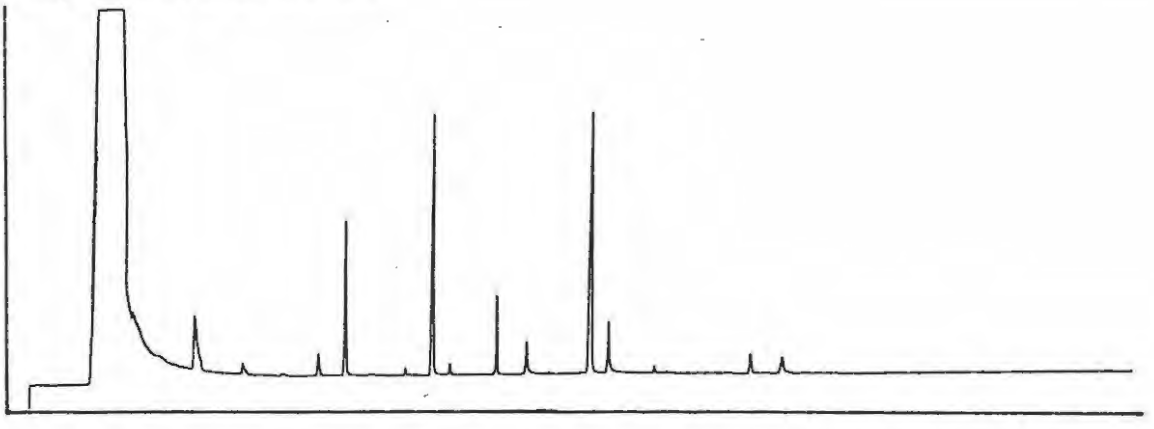
Compound (117)



Compound (118)

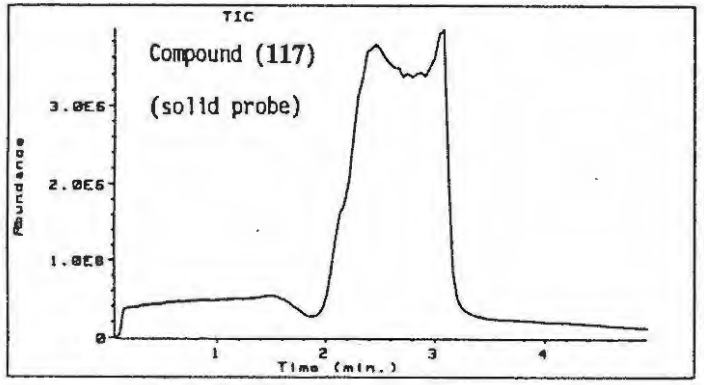


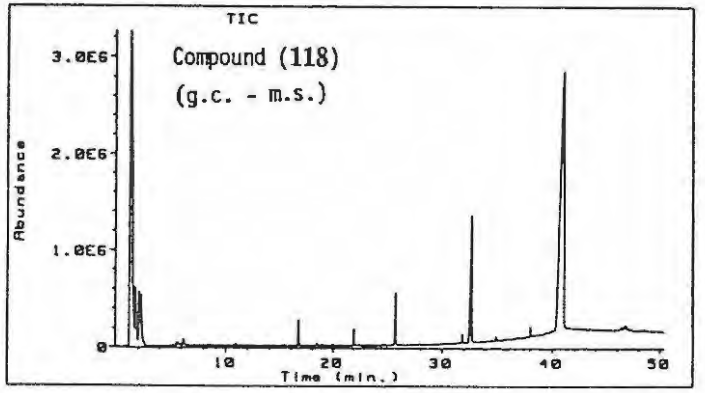
Compound (117) after pyrolysis

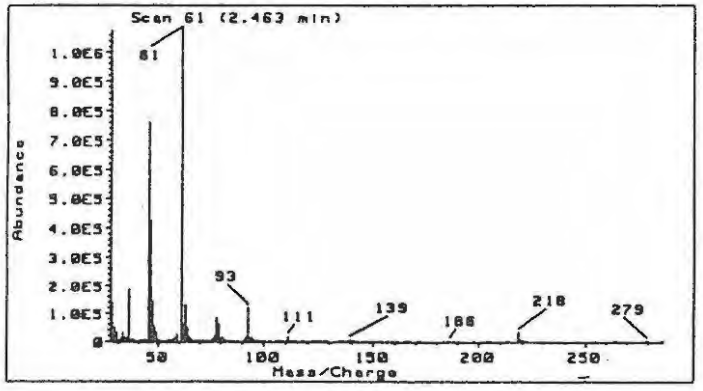


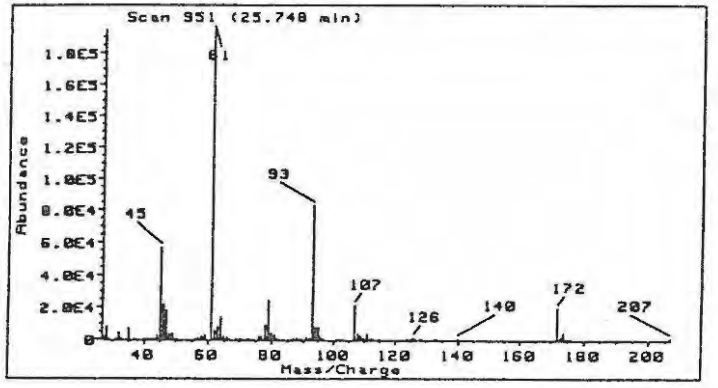
Conditions : HP-1 column; 70 - 200°C, 8°/min.

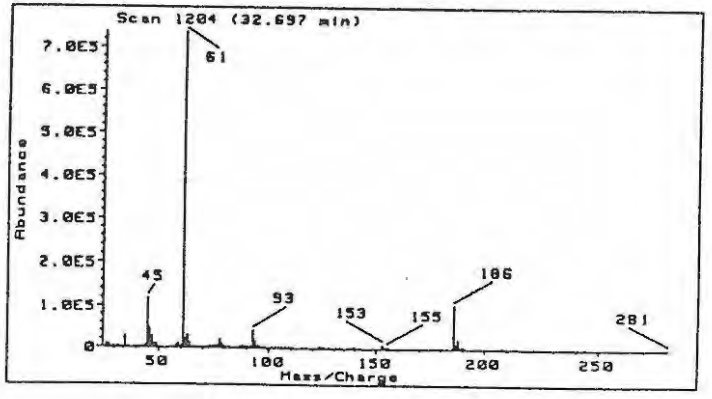
Figure 2.26 M.s. analysis of sulphur compounds (117) and (118)











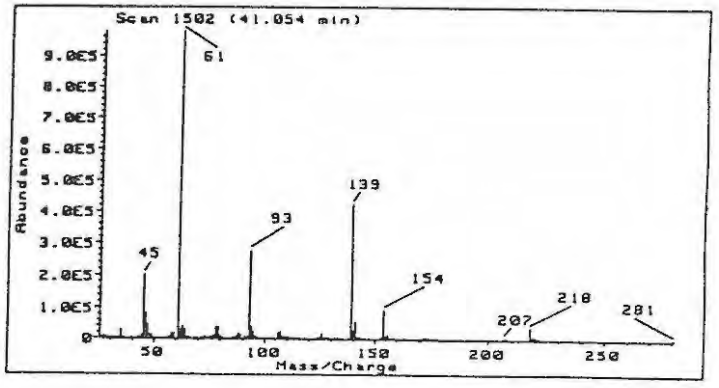
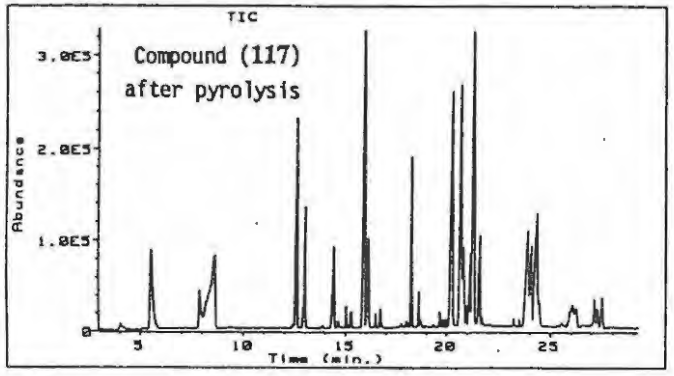
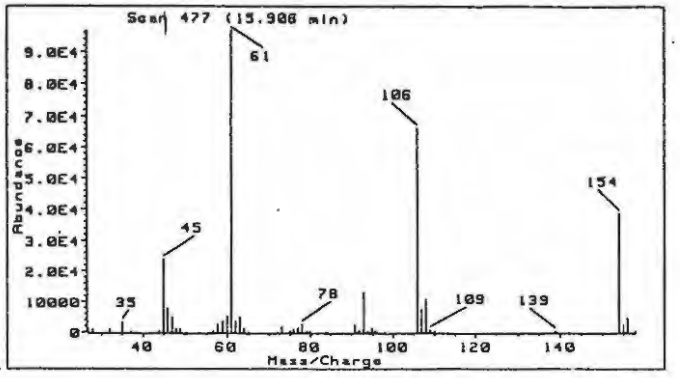
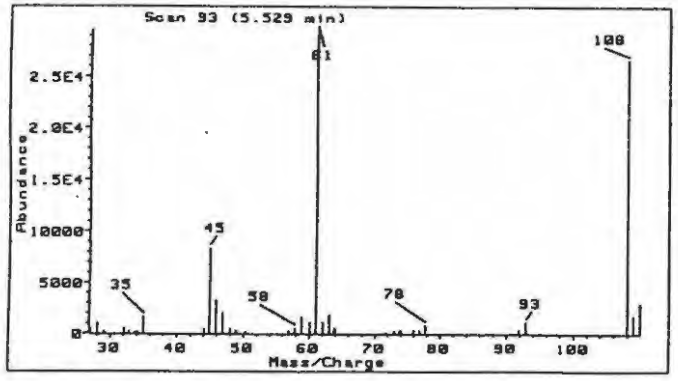
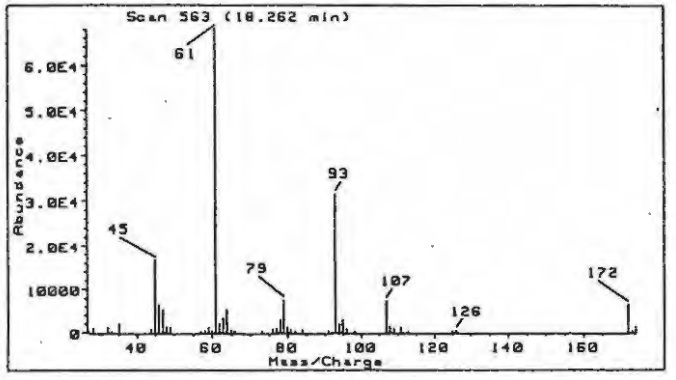


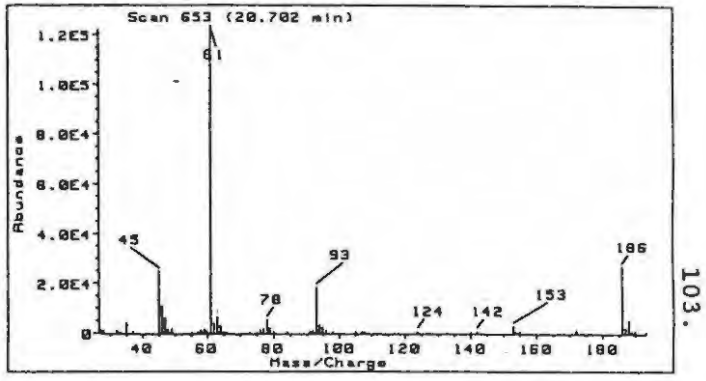
Figure 2.26 M.s. analysis of sulphur compounds (117) and (118) (contd)

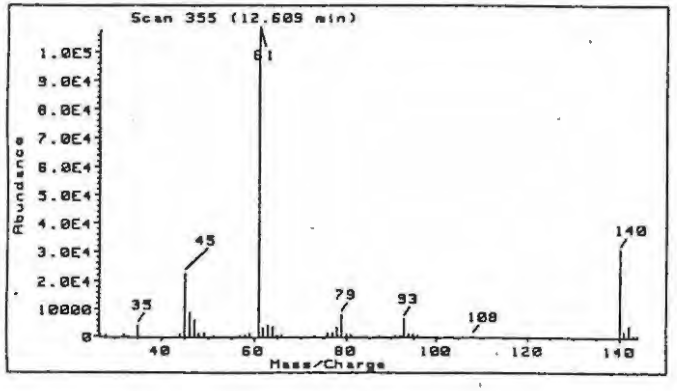


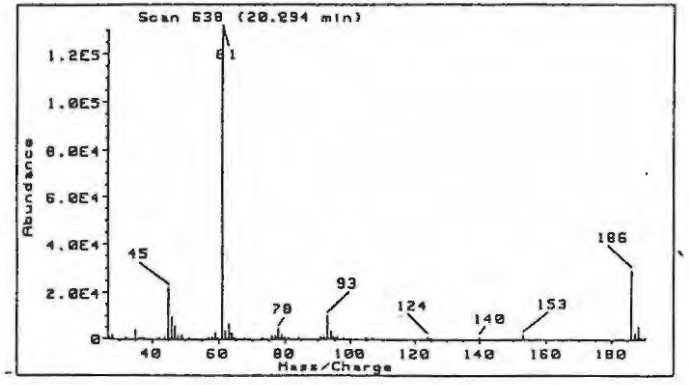












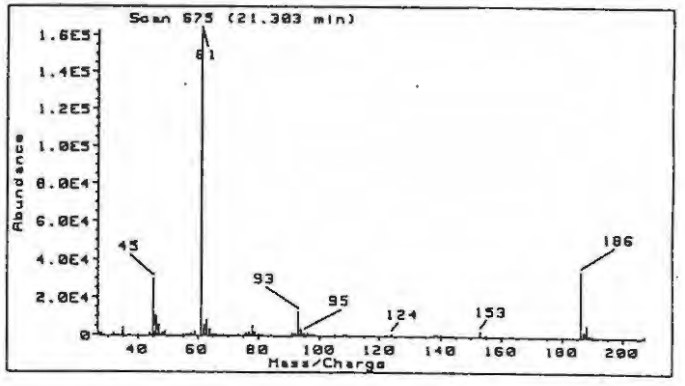


Table 2.6 Components tentatively identified in m.s. analysis of sulphur compounds (117) and (118)

Sample	Conditions	*m/z*, M^+	Tentative structure	No. of S atoms per molecule*
$CH_3SO_2CH_2SSCH_2SCH_3$ (117)	Solid probe	218	$CH_3SO_2CH_2SSCH_2SCH_3$	4
$CH_3SCH_2SSCH_2SCH_3$ (118)	g.c - m.s.	186	$CH_3SCH_2SSCH_2SCH_3$	4
		172	$CH_3SSSCH_2SCH_3$	4
		218	CH_3SCH_2-$SSSCH_2SCH_3$	5
Mixture of products obtained by pyrolysis of compound (117)	g.c. - m.s.	108	$CH_3SCH_2SCH_3$**	2
		140	$CH_3SSCH_2SCH_3$	3
		154	$CH_3SCH_2SCH_2SCH_3$	3
		172	$CH_3SSSCH_2SCH_3$	4
		186	$CH_3SCH_2SSCH_2SCH_3$	4

---

* Calculated from relative abundance of M+2 peak.[112]

** Components postulated to have been formed by disproportionation of the sulphone (117) in a manner analogous with that of thiosulphinates.[49]

Similar fragmentation patterns were observed in the mass spectra of the various components in these samples (Figure 2.26), and some frequently occurring fragments can be tentatively assigned as shown in Table 2.7.

Table 2.7 Assignment of fragments in mass spectra of sulphur compounds (117) and (118)

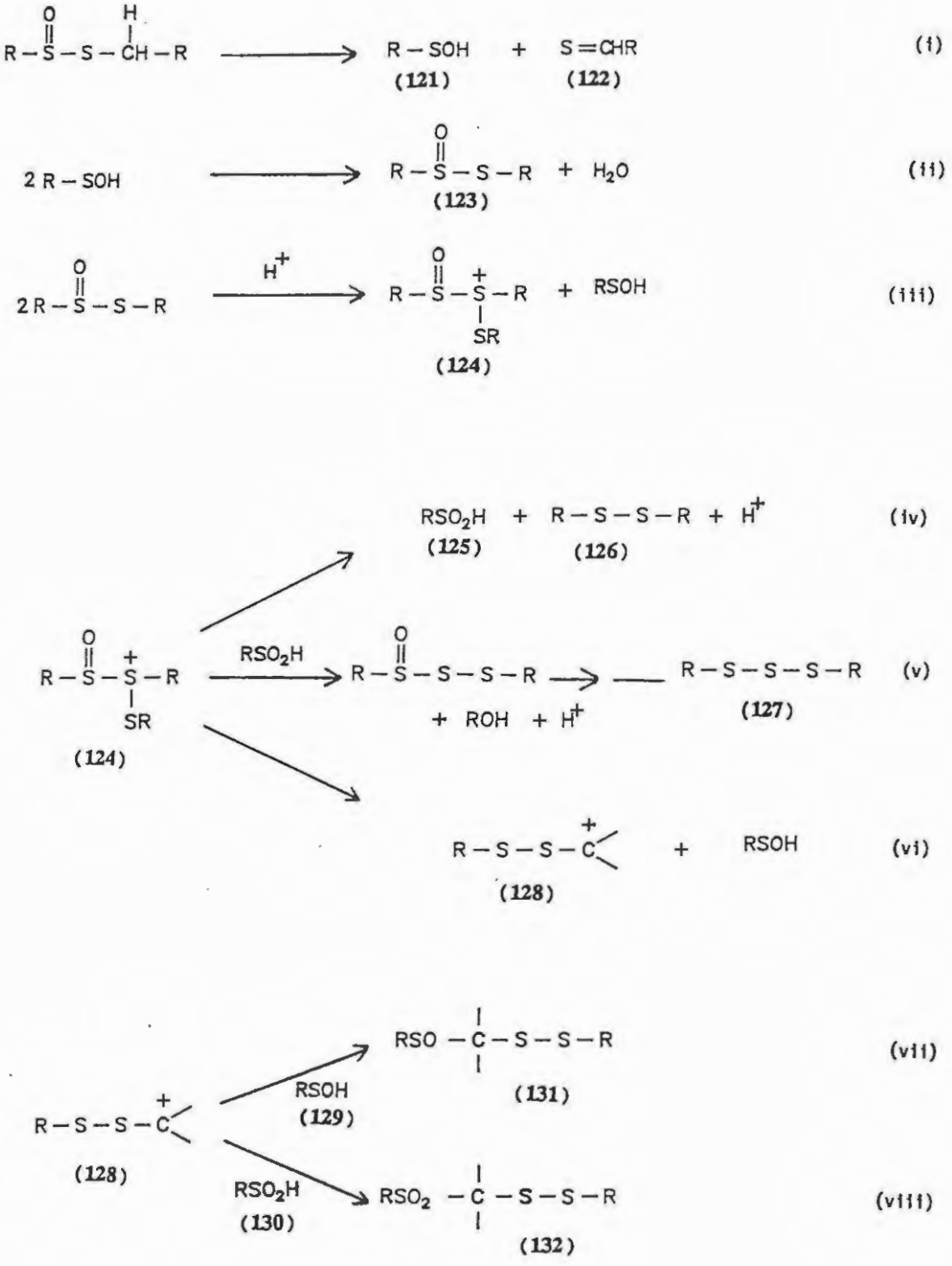
m/z	Fragment
139	$CH_3SCH_2SCH_2S]^+$ or $CH_3SSCH_2SCH_2]^+$
125	$CH_3SCH_2SS]^+$ or $CH_3SSCH_2S]^+$
93	$CH_3SCH_2S]^+$
79	$CH_3SS]^+$
61	$CH_3SCH_2]^+$
47	$CH_3S]^+$
45	$CHS]^+$

2.2.4.4 Discussion of possible origin of sulphur compounds (117) and (118) in *Tulbaghia violacea*

Dialkylthiosulphinates, RS(O)SR', have been shown[47] to undergo a variety of reactions resulting from initial thermal transformation to sulphonic acids, as shown in Scheme 2.13. The initial step (i) involves β-elimination reaction to give an alkanesulphenic acid (121) and a thioaldehyde (122).[49] This is followed by an intermolecular thioalkylation step (iii) which results in formation of a sulphonium ion intermediate (124), and this intermediate can react in various ways. The products may be the alkanesulphinic acid (125) and the disulphide (126), the trisulphide (127), or, by a further β-elimination reaction, the α-dithiocarbocation (128). This intermediate (128) may react with an alkanesulphenic acid (129) to form the α-alkylsulphinyl disulphide (131), or with an alkanesulphinic acid (130) to produce the α-alkylsulphonyl disulphide (132).

In cases where more than one alkylthiosulphinate, or an asymmetrical alkylthiosulphinate, is present, exchange of alkyl groups can lead to a scrambling process, and hence the formation of mixed products.[47]

Scheme 2.13 Thermal reactions of alkylthiosulphinates[46]



For example, the alkylthiosulphinate bearing alkyl groups R and R' could, by disproportionation, give products such as the following :

$R\text{-}SO_2\text{-}S\text{-}R'$

$R\text{-}SO_2\text{-}S\text{-}R$

$R\text{-}S\text{-}S\text{-}R$

$R\text{-}S\text{-}S\text{-}R'$

$R'\text{-}S\text{-}S\text{-}R'$

$R\text{-}SO_2\text{-}S\text{-}S\text{-}R'$

$R\text{-}SO\text{-}S\text{-}R$

$R\text{-}SO_2\text{-}C\text{-}S\text{-}S\text{-}R'$

$R\text{-}SO_2\text{-}C\text{-}S\text{-}S\text{-}R$

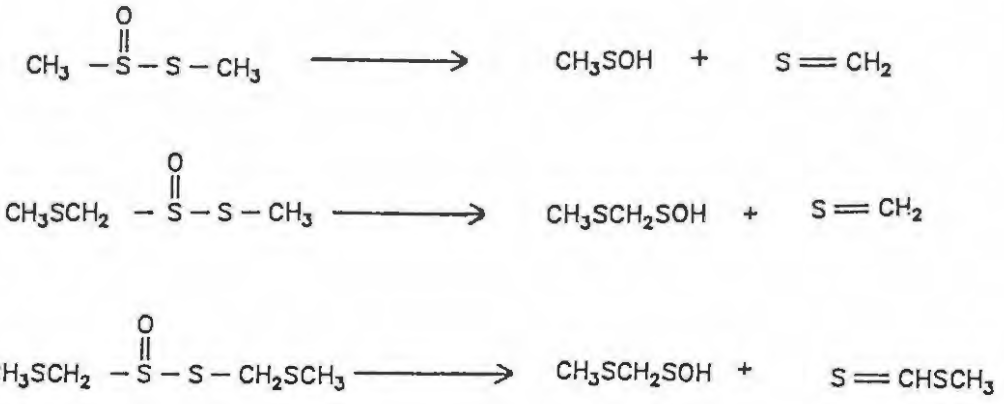
Thiosulphinates are known to be present in *Allium* species as products of enzymic conversion of S-alk(en)yl-L-cysteine sulphoxides.[8] The presence of an alkyl cysteine sulphoxide lyase enzyme and of three amino acid sulphoxides in *Tulbaghia violacea* has been established (see p. 110), and by analogy with *Allium* species, it would seem likely that enzymic conversion of the amino acid sulphoxide to thiosulphinates occurs in *Tulbaghia violacea*. Thus, it is reasonable to expect compounds of the type discussed earlier in this section to be obtained from extracts of *Tulbaghia violacea*.

This is supported by the isolation of compounds **(117)** and **(118)** (see section 2.2.4, p. 91) which are analogous to the products **(132)** and **(126)** respectively, in Scheme 2.13. This suggests that the alkylthiosulphinate precursors in *Tulbaghia violacea* include $RS(O)SR'$ or $R'S(O)SR$ ($R = CH_2SCH_3$; $R' = CH_3$). Scheme 2.14 shows of possible route for the formation of the isolated compounds **(117)** and **(118)** from the postulated precursors.

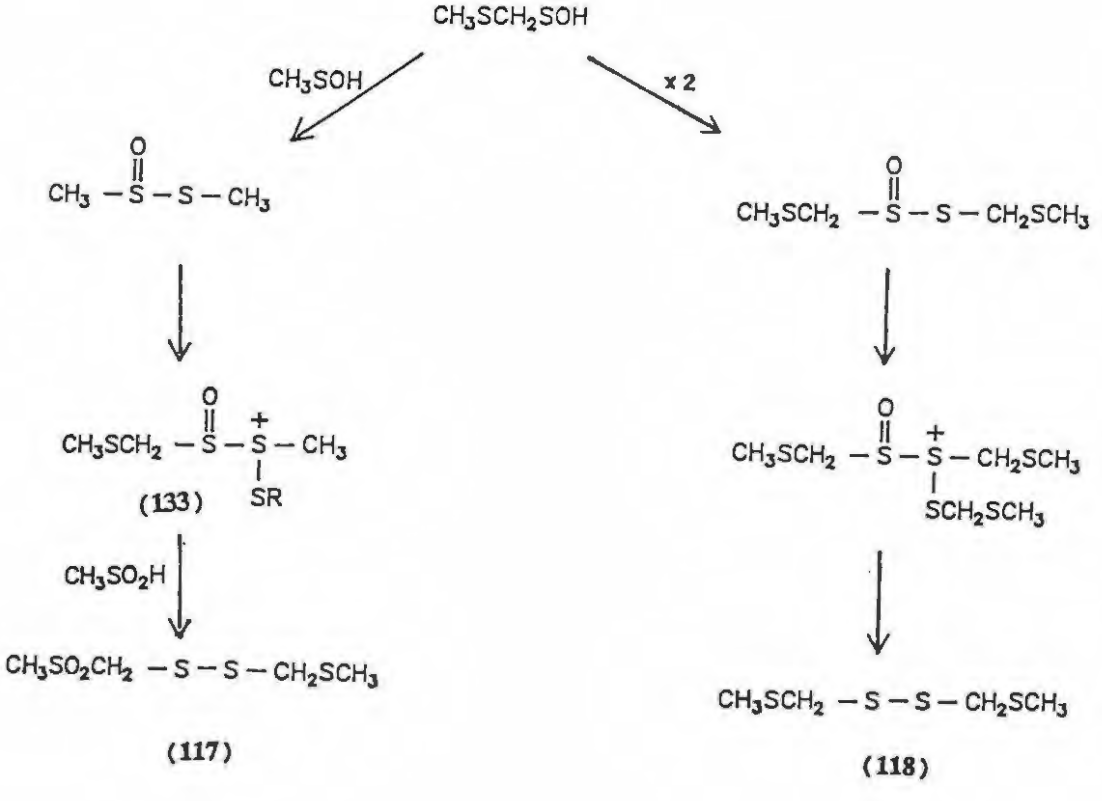
The identification of the group R as CH_3SCH_2 (rather than CH_3CH_2S as was suggested by Jacobsen *et al*[41] in an earlier study on *Tulbaghia violacea*) is confirmed by spectroscopic analyses as discussed previously (section 2.2.4.1, p. 91).

Scheme 2.14 Possible route for formation of sulphur compounds (117) and (118)[47]

Initial reaction for each of three possible alkylthiosulphinate precursors :-



Further possible conversions :-



The fortuitous use of hexane which contained some xylene (see Experimental section, p. 161) could have led to the formation of compound (117), since the thermal reaction producing such sulphinyl disulphides is proved by the presence of aromatic solvents.[47] This has been attributed to the formation of a Π-complex between the aromatic nucleus and the electrophilic sulphonium ion (133) (Scheme 2.14, p. 108).

2.2.5 ANALYSIS OF VOLATILES FROM *TULBAGHIA VIOLACEA*

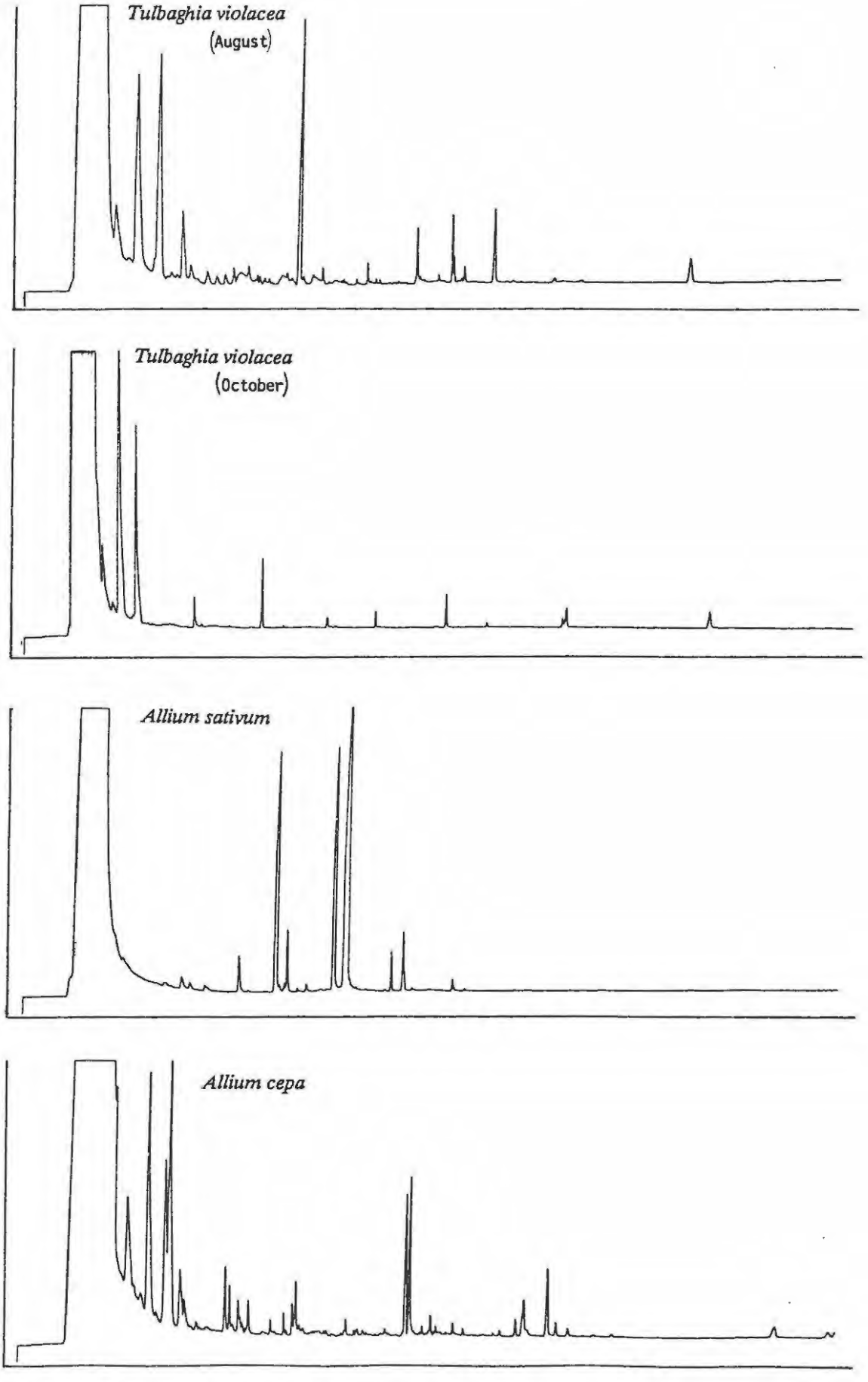
Tulbaghea violacea has been shown to contain a sulphoxide lyase enzyme similar to those found in members of the closely related *Allium* family, and previous investigation of *Tulbaghia violacea* volatiles[41;42] suggested the presence of sulphur compounds which corresponded with those found in *Allium* volatiles. This report postulated the presence of ethyl groups in the alkylthiosulphinate precursors in *Tulbaghia violacea*, which would represent an unusual case, since ethyl groups are not generally found in the sulphur compounds of the *Allium* family.[54]

Extensive research on a wide variety of *Allium* species (into the nature of the volatile components and their formation by enzymic conversion of non-volatile precursors,[53-57] has established precedents for identification of the precursors by analysis of the volatiles.[54] Conditions for extraction procedures and g.l.c. and g.c. - m.s. analyses are well documented,[113;116] and in this study these conditions were reproduced as closely as possible, for the sake of comparison.

2.2.5.1 G.l.c. and g.c. - m.s. analysis of volatiles from *Tulbaghia violacea*

The volatiles were extracted by vacuum distillation of fresh plant material and analysed by g.l.c. on a monthly basis throughout the course of a year (see Experimental section, p. 164). (Improvements in extraction and analysis techniques during the year led to more informative results from later analyses). The results of g.c. - m.s. analysis of the extracted volatiles were compared with the results of g.c. - m.s. analysis of sulphur compounds (117) and (118), and g.c. - m.s. analysis of the volatiles extracted from *Allium cepa* (onion) and *Allium sativum* (garlic) (see Experimental section, p. 164). Comparison showed that the *Tulbaghia violacea* volatile differed considerably from those of the *Allium* species in g.l.c. retention times (see Figure 2.27) and in the mass spectra of components (see Appendix, p. 215), indicating that the sulphur compounds in *Tulbaghia violacea* are not common to the *Alliums*.

Figure 2.27 G.l.c. comparison of volatiles from *Tulbaghia violacea* with those from *Allium cepa* and *Allium sativum*



Column : HP-1; conditions : 70 - 200°, 8°/min.

Two components appeared consistently in the g.c. - m.s. analysis of volatiles extracts obtained at various times of the year, and with columns of varying types (see Experimental section, p. 164). A computer library search indicated that the mass spectrum of 2,4,5-trithiahexane correlated closely with that of the first of these components (Figure 2.28 A); this is in accordance with the explanation given in section 2.2.4.4 for the formation of disulphides from the sulphur containing precursors. The mass spectrum of the second component was observed to correlate closely with that of 2,4,5,7-tetrathiaoctane (118) which was isolated previously (see section 2.2.4.1) (Figure 2.28; B).

It was possible to tentatively identify certain other components shown in the g.c. - m.s. analyses, in the light of the information discussed in section 2.2.4.4 (p. 105), as shown in Table 2.8 and Figure 2.28.*

Table 2.8 Components of volatiles from *Tulbaghia violacea*, identified by g.c. - m.s.

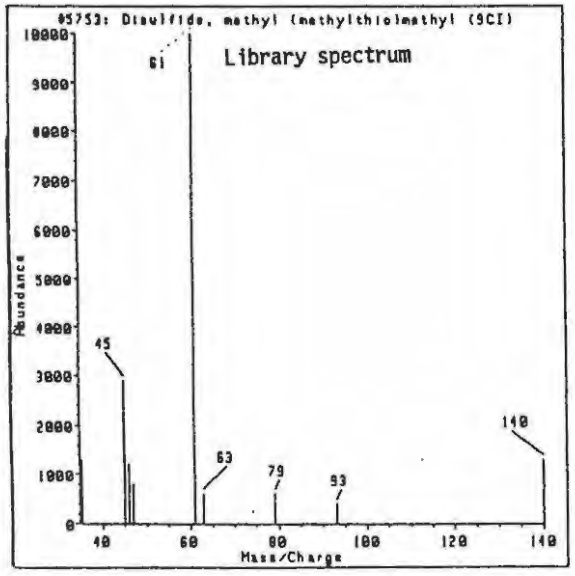
m/z	Possible structure
140	CH_3-S-S-CH_2SCH_3
186	CH_3SCH_2-S-S-CH_2SCH_3
218	CH_3SCH_2-S-S-S-CH_2SCH_3
172	CH_3-S-S-S-CH_2SCH_3
126	CH_3-S-S-S-CH_3

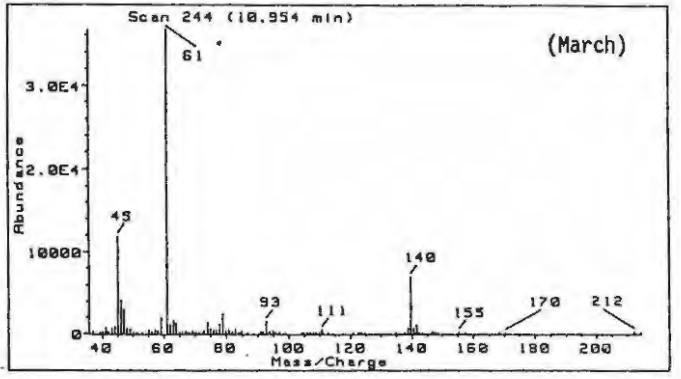
Although components having low retention times were generally found to be inaccesible, the mass spectrum of one component was found to correlate well with 3-hexen-1-ol (89% computer search correlation), and a second component correlated with 4-hexen-1-ol acetate (52% match) (see Figure 2.28; D).

* Complete g.c. - m.s. data listings are given in the Appendix.

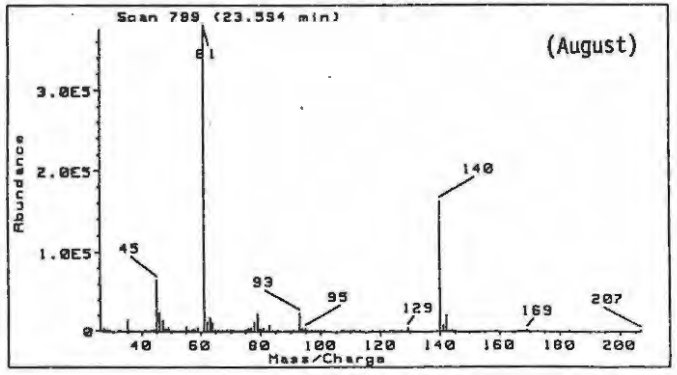
Figure 2.28 Mass spectra of components of volatiles from *Tulbaghia violacea*

A. 2,4,5-trithiahexane





Components of volatiles extracts



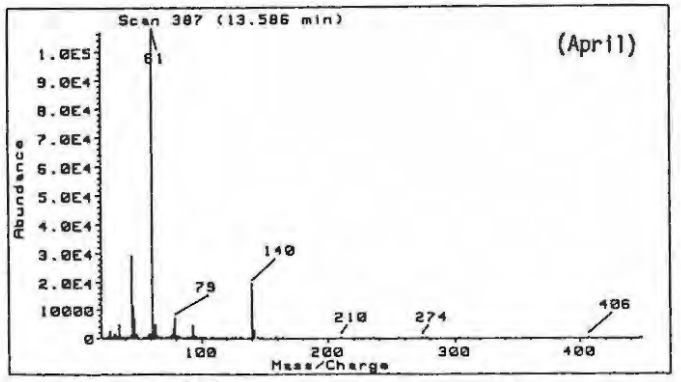
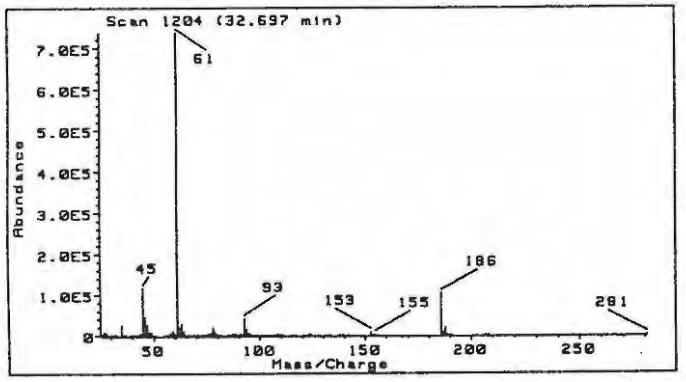
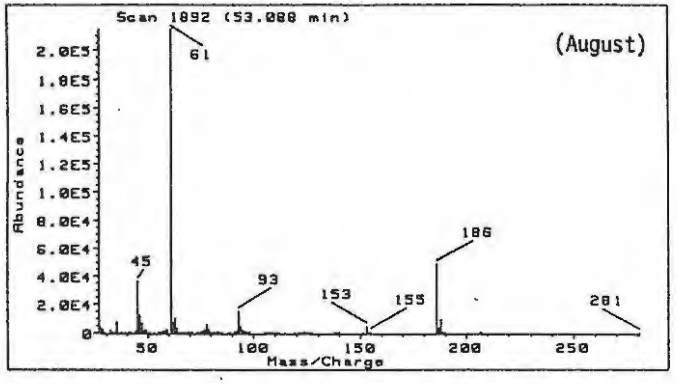


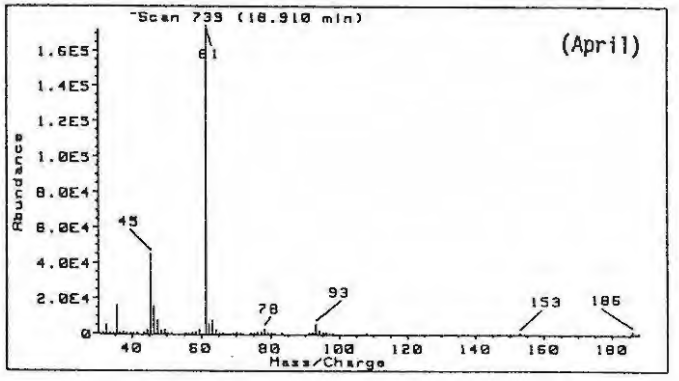
Figure 2.28 Mass spectra of components of volatiles from *Tulbaghia violacea* (contd)

B. 2,4,5,7-tetrathiaoctane [Compound (118)]



Components of volatiles extracts





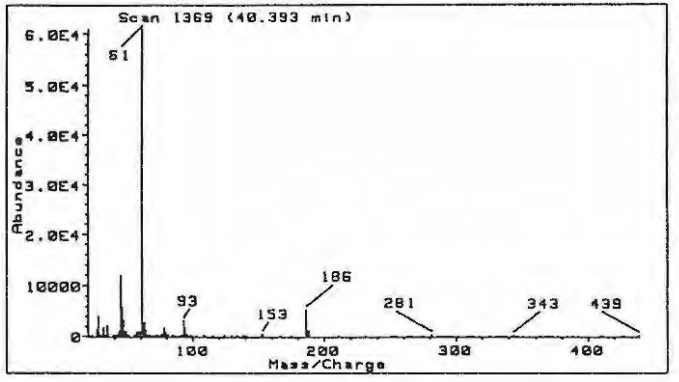
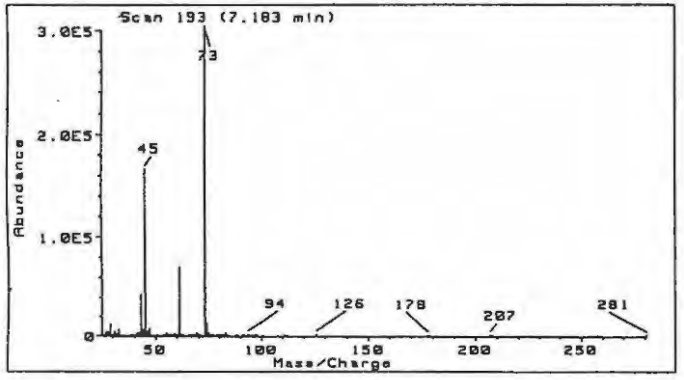
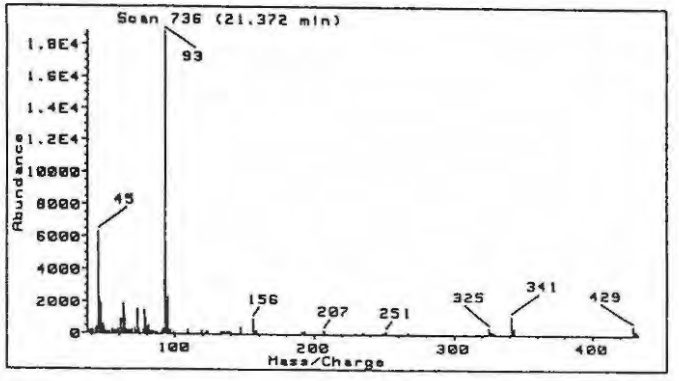
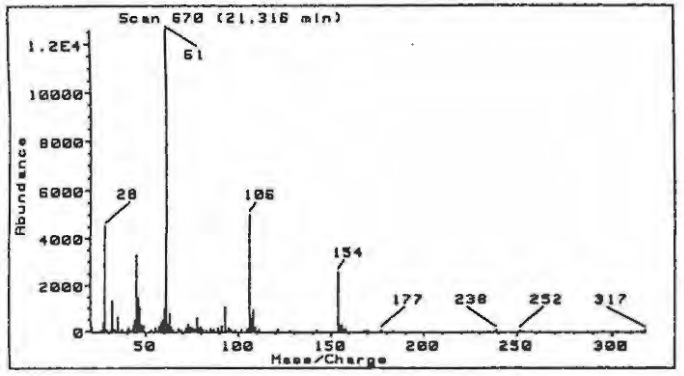


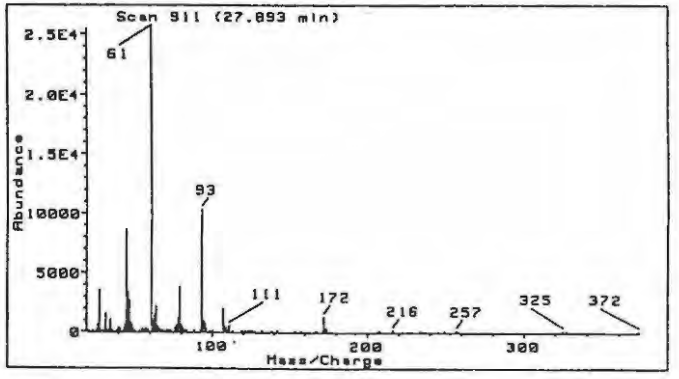
Figure 2.28 Mass spectra of components of volatiles from *Tulbaghia violacea* (contd)

C. Other components of volatiles extracts









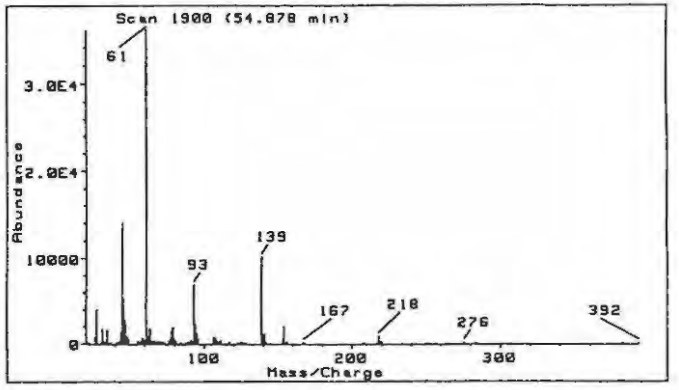
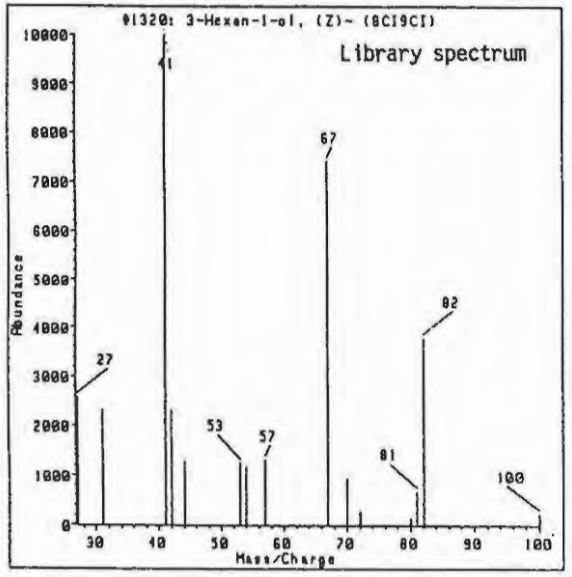
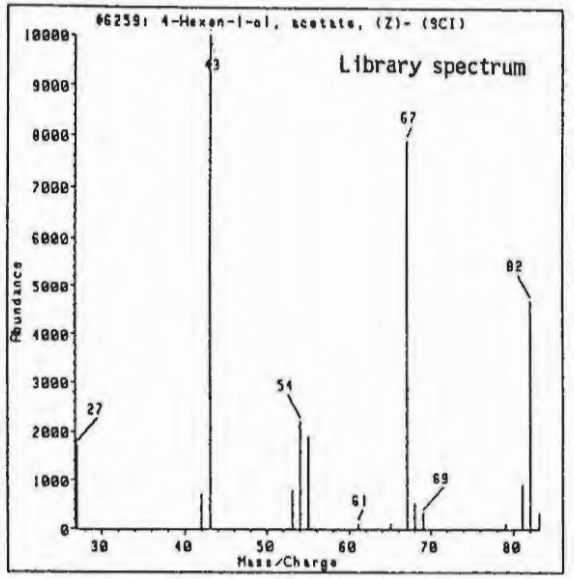


Figure 2.28 Mass spectra of components of volatiles from *Tulbaghia violacea* (contd)

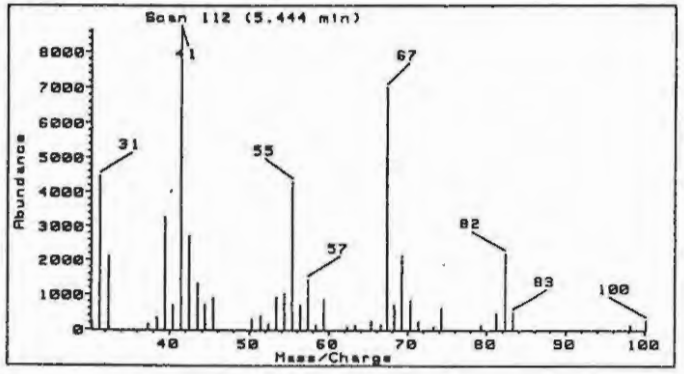
D. Hexen-1-ol

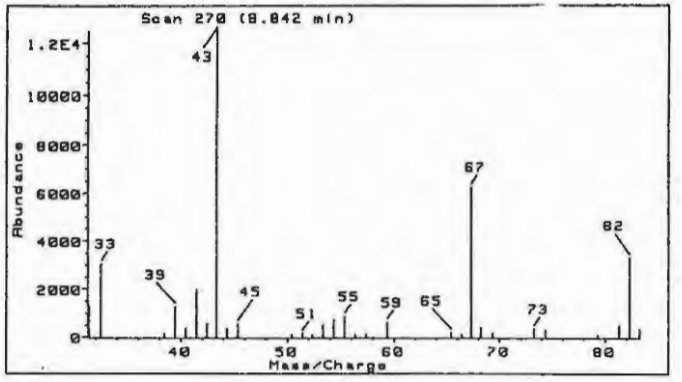


E. Hexen-1-ol acetate



Components of volatiles extracts





2.2.6 MISCELLANEOUS EXTRACTIONS OF *TULBAGHIA VIOLACEA*

The possibility of the presence of secondary metabolites other than flavones and sulphur compounds was investigated by following literature procedures for extraction and analytical chromatography.

2.2.6.1 Examination of *Tulbaghia violacea* for the presence of saponins

Aqueous extracts of *Tulbaghia violacea* were observed to produce a stable foam when shaken, suggesting the presence of saponins. Treatment of aqueous extract I (Scheme 2.11, p. 66) following the method of Hayashi *et al*[117] (see Experimental section, p. 165) afforded a small sample in solution in chloroform.

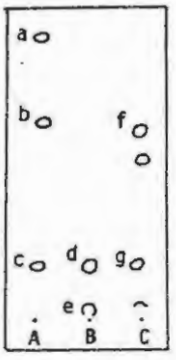
Analysis of this sample by t.l.c., using 3 different sapogenin-specific spray reagents [*viz.*, cinnamaldehyde - sulphuric acid reagent (4),[123] vanillin - phosphoric acid reagent (3),[104] and antimony chloride reagent (5)[104](see Table 3.2, p. 131)] gave positive results as shown in Figure 2.29; A. A similar extraction procedure, following Carle and Reinhard,[15] afforded an extract which gave similar results (see Figure 2.29; B and Experimental section, p. 165). The aglycone sample obtained by hydrolysis of the butanol-soluble fraction V (see Experimental section, p. 163) was chromatographed in the same manner, and also showed positive results (Figure 2.29; C). These results suggested the possible presence of steroidal saponins in the plant but could not be followed up due to time considerations.

2.2.6.2 Examination of *Tulbaghia violacea* for the presence of alkaloids

A small scale extraction to isolate alkaloids[118] was carried out on the aqueous extract I (see Experimental section, p. 165) and the resulting sample was analysed by t.l.c. using alkaloid-specific spray reagents, *viz.*, Drazendorff's reagent (6) and iodoplatinate reagent (7).[104] The results obtained were unconvincing in that the colour reactions observed were not consistent with those expected for alkaloids.

Figure 2.29 T.l.c. analysis of *Tulbaghia violacea* extracts, in examination for steroidal saponins

(1)



Spot	R_f	Colour
a	0.98	Red
b	0.60	Red
c	0.20	Yellow
d	0.20	Blue
e	0.36	Blue
f	0.58	Yellow
g	0.20	Red

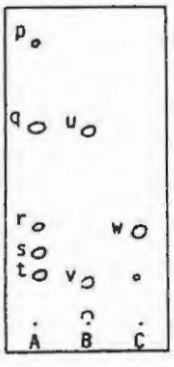
Spray reagent : Cinnamaldehyde - Ac_2O - H_2SO_4 (4)

(2)

h	0.43	Blue
i	0.37	Blue
j	0.94	Purple
k	0.43	Blue
l	0.06	Blue
m	0.62	Blue
n	0.43	Purple
o	0.24	Blue

Spray reagent : Vanillin - H_3PO_4 (3)

(3)



p	0.98	Grey
q	0.67	Purple
r	0.33	Green
s	0.25	Brown
t	0.18	Grey
u	0.67	Grey
v	0.17	Grey

Spray reagent : $SbCl_3$ (5)

Solvent system : Benzene-acetone (4:1) in each case

2.2.6.3 Examination for anthraquinones

A classic test for the presence of anthraquinones, the Borntrager test,[13] was carried out on the aqueous extract I, as well as on an anthraquinone standard, emodin (see Experimental section, p. 166). The red colouration observed for emodin was not observed for the extract of *Tulbaghia violacea*. Analysis by t.l.c. using solvent systems and spray reagents suggested in the literature[104;119] also gave negative results for a hydrolysate of extract I (see Experimental section, p. 166) and it was concluded that no anthraquinones were present in *Tulbaghia violacea*.

2.2.7 BIOLOGICAL ACTIVITY OF *TULBAGHIA VIOLACEA* EXTRACTS

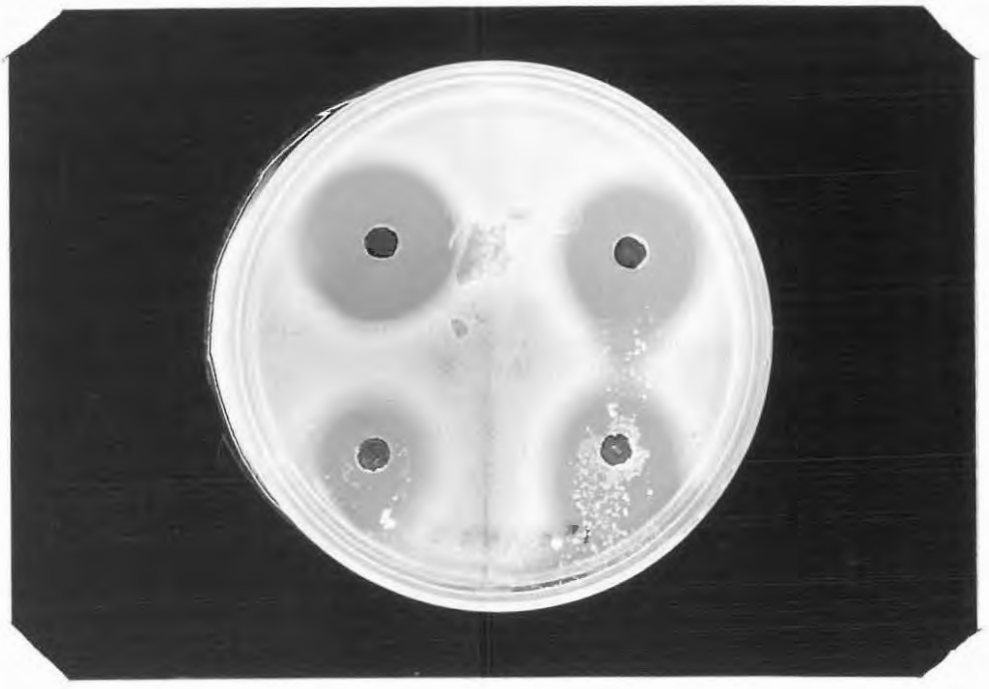
The biological activity of *Tulbaghia violacea* was examined briefly. The effect of the plant extracts on bacterial cultures was investigated, since some anti-bacterial action appeared likely due to the presence of sulphur compounds in the plant; similar compounds in *Allium* species are known to be strongly bacteriocidal.[44;50] In addition, reported impairment of gut contraction in patients treated with *Tulbaghia violacea* extracts (see p. 1) suggested that the extracts had some effect on involuntary muscle, and this was investigated by means of an isolated organ system.

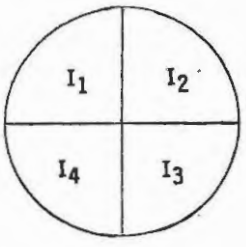
2.2.7.1 Bacteriostatic action

A range of aqueous extracts of *Tulbaghia violacea* (see Experimental section, p. 167) were used to treat four different bacterial strains, cultured by seeding in nutrient agar. Bacteriostatic action was indicated by a circular region of inhibited growth around the well in the agar, into which the extract had been introduced (the region being known as a "halo") (see photograph, Figure 2.30). The results of the initial investigation, using extracts obtained from mature, well-established plants, showed convincing haloes, and dilutions of the extracts caused correspondingly smaller haloes, as summarised in Table 2.9. Repetition of the investigation using extracts from younger plants gave the rather less striking results summarised in Table 2.10, possibly reflecting a lower concentration of secondary metabolites in the younger plants.

Thus, the *Tulbaghia violacea* extracts showed some bacteriostatic activity. The strongest activity was shown by the extract I_1 which had not been boiled (see Experimental section, p. 167), and this may be due to the biologically active compounds in the fresh plant material being transformed by heating into products which have less effect on the bacteria.

Figure 2.30 Photograph : example of bacteriostatic action of *Tulbaghia violacea* extracts





Bacterial culture : *B. subtilis*

White spots on the surface of the plate are due to fungal overgrowth during storage before photographs were taken.

Table 2.9 Bacteriostatic action of *Tulbaghia violacea* extracts (1)

Bacterial species	Extract*	Halo diameter (mm) for varying dilutions			
		1	1:4	1:16	1:64
B. subtilis					
	I_1	29	22	14	12
	I_2	26	19	10	9
	I_3	21	17	9	-
	I_4	17	13	8	-
E. coli					
	I_1	20	15	14	-
	I_2	20	15	14	-
	I_3	17	15	14	-
	I_4	17	15	14	-
S. marcesens					
	I_1	19	17	10	-
	I_2	19	17	10	-
	I_3	19	13	-	-
	I_4	16	13	-	-
S. aureus					
	I_1	29	23	14	-
	I_2	26	17	14	-
	I_3	23	17	14	-
	I_4	21	16	14	-

* See Experimental section, p. 167.

Table 2.10 Bacteriostatic action of *Tulbaghia violacea* extracts (2)

Bacterial species	Extract	Halo diameter (mm) for different dilutions		
		1	1:4	1:16
B. subtilis				
	I_1	14	10	9
	I_2	10	8	-
E. coli				
	I_1	10	10	-
S. aureus				
	I_1	9	-	-
	I_2	9	-	-

2.2.7.2 Pharmacological activity of *Tulbaghia violacea* extract

Drug assessment using isolated involuntary muscle is based on the fact that the tissue will continue to respond normally for several hours after removal from the animal, provided it is maintained in a suitable nutrient solution. In this study, rat intestine was used, and standard procedures[120] were followed (see Experimental section, p. 167).

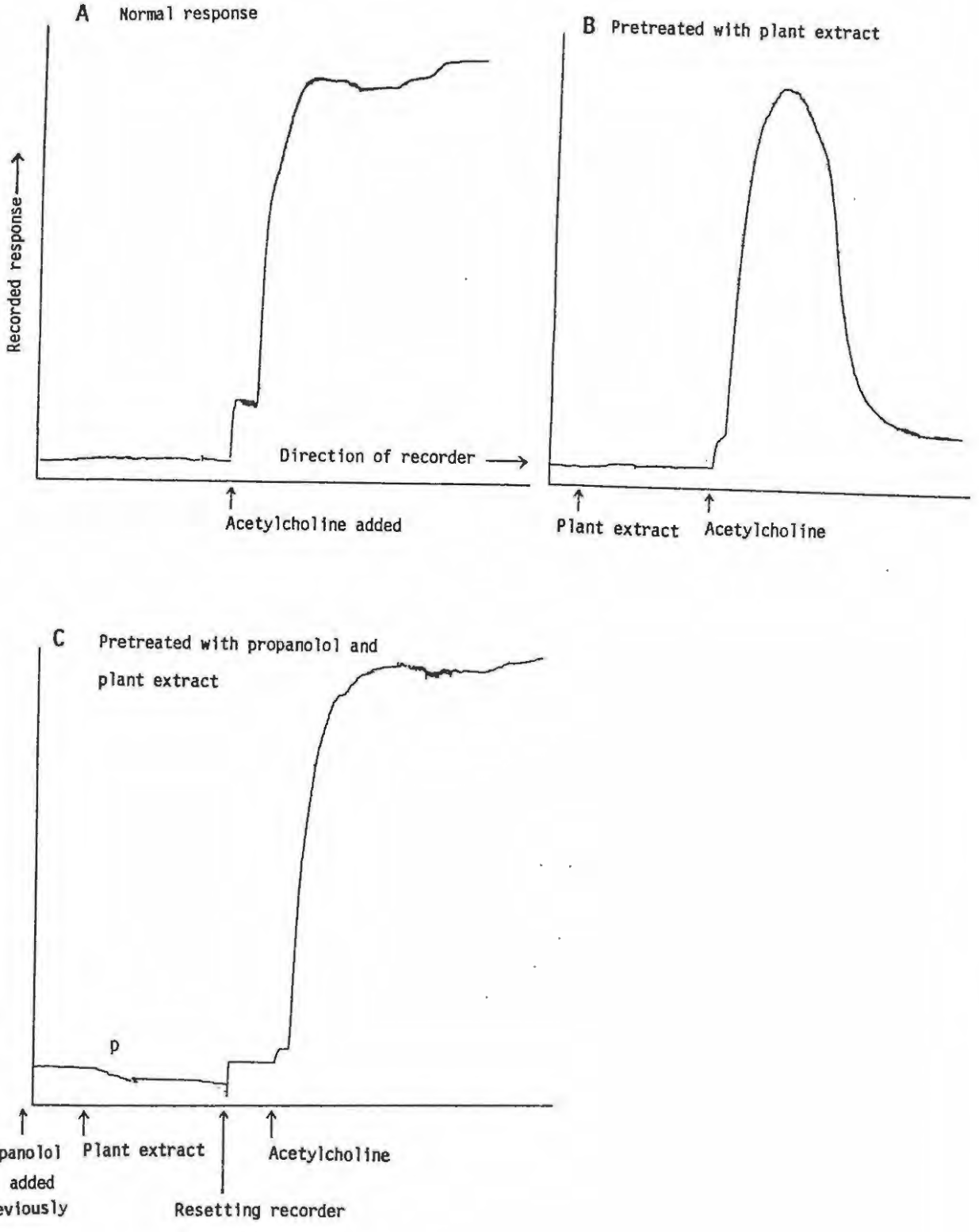
Contraction of the isolated intestinal preparation is achieved by acetylcholine, which initiates contraction by binding to cholinergic muscarinic receptors located on the cell membrane. *In vivo*, endogenous acetylcholine is released and subsequently hydrolysed to prevent continued action. Also present in the intestinal preparation are adrenergic receptors of the β_2 subtype. Stimulation of the receptors by adrenergic agonists such as adrenaline or isoprenaline result in relaxation of the tissue. Thus these β_2-agonists act as functional non-competitive antagonists of acetylcholine and can consequently reduce the size of contractions induced by acetylcholine in an insurmountable manner. The action of β-agonists can in turn be prevented by use of competitive β-adrenergic antagonists, which bind to the β-receptors preferentially.[121]

The effects of the *Tulbaghia violacea* extracts on smooth muscle were determined by two methods : using single dose responses, and dose-response curves.

(a) Single dose responses

The rather dramatic effect of the plant extract (I_2; see Experimental section, p. 167) was shown initially by single doses, as illustrated in Figure 2.31. A single dose of acetylcholine added to the organ-bath caused the muscle preparation to contract, as shown in graph A. The muscle preparation was washed (allowing relaxation) and then pretreated with the plant extract I_2. After 5 minutes of pretreatment, the same dose of acetylcholine was added, producing the response shown in graph B, where contraction is shown to be inhibited. Graph C shows the result of treatment of fresh organ, initially with

Figure 2.31 Single dose responses of isolated smooth muscle with treatment by *Tulbaghia violacea* extracts



propranolol (a β-adrenergic antagonist), followed by the plant extract, and finally the same dose of acetylcholine as before. The dipping of the base line shown in graph B (at p) is indicative of the presence of a β-agonist, causing relaxation.

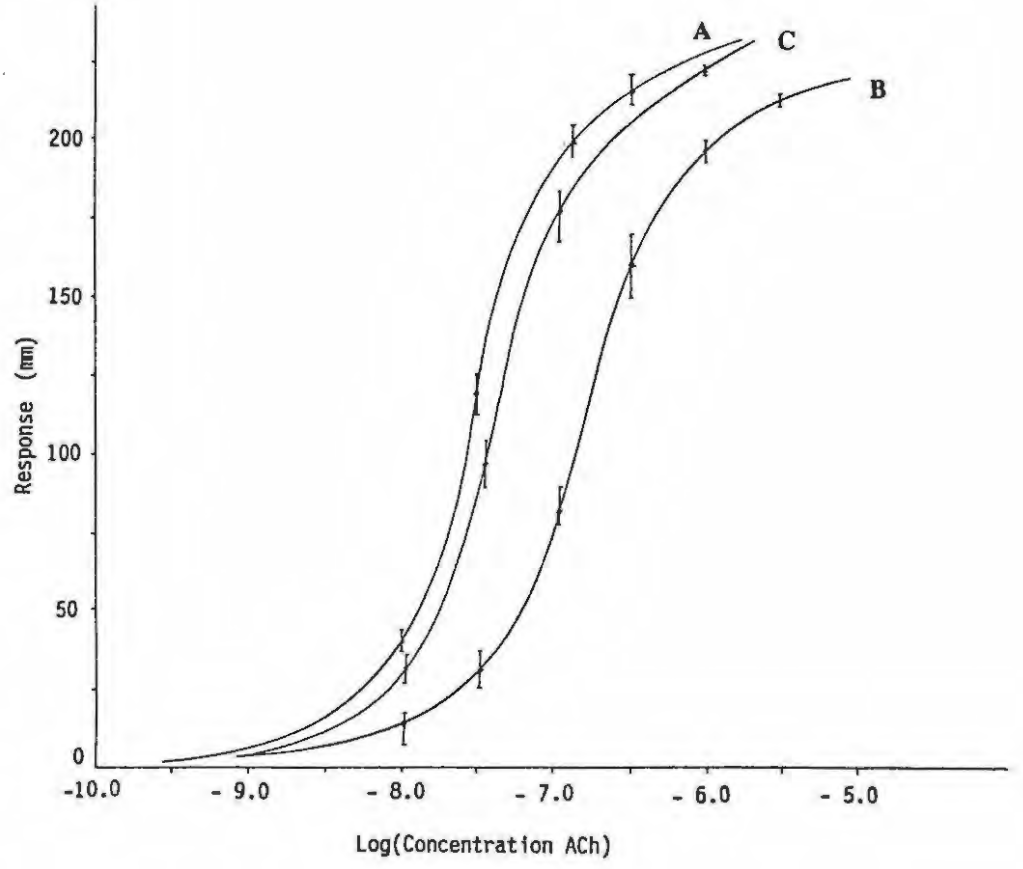
(b) Dose-response curves

A normal dose-response curve was obtained by successively adding increasing amounts of acetylcholine to the organ-bath and recording the size of the corresponding contractions. The organ was then pretreated with plant extract and the dose-response curve was repeated. After thorough washing and resting, the organ was pretreated with propranolol and the plant extract as before, and a third dose-response curve was recorded. The results of these procedures are shown in Figure 2.32, Graphs A, B and C respectively.

The results of this study suggest that the plant extract contains a β-adrenergic agonist, which would oppose the action of acetylcholine on the smooth muscle. When propranolol was used to pretreat the organ, the β-receptors would be blocked, preventing the action of a β-agonist in the plant extract and thus allowing normal contraction in response to stimulation by acetylcholine.

Extension of this hypothesis to the action of the plant extract in patients may explain the reported[4] reduction in the peristaltic action of the gastro-intestinal tract.

Figure 2.32 Dose response curves for isolated smooth muscle, with treatment by *Tulbaghia violacea* extracts



2.3 CONCLUSIONS CONCERNING ACTIVE PRINCIPLES IN *TULBAGHIA VIOLACEA*

From the results of the various investigations carried out, certain conclusions may be drawn regarding the chemical constituents of *Tulbaghia violacea* and their role in the use of this plant as a herbal remedy.

Two sulphur compounds were isolated from the plant, *viz.*, 2,4,5,7-tetrathiaoctane-2,2-dioxide and 2,4,5,7-tetrathiaoctane. These products are postulated to originate from sulphur-containing amino acids, indicating the likely presence in the plant of a system analogous to that giving rise to biologically active sulphur compounds in *Allium* species, particularly garlic. It is possible that the sulphur compounds in *Tulbaghia violacea* are responsible for the gastric inflammation and corrosion reported to be caused by the herbal remedy. These sulphur compounds are also likely to be responsible for the bacteriostatic action of the plant extracts.

However, the irritation of intestinal mucosa could also be attributed to the presence of steroidal saponins in *Tulbaghia violacea*, evidence of which was obtained chromatographically. Steroidal saponins also typically cause inhibition of peristaltic action in smooth muscle, an effect which was clearly demonstrated in the treatment of isolated mammalian intestinal muscle with *Tulbaghia violacea* extracts. The combined effects of sulphur compounds and steroidal saponins may even sufficiently damage the intestinal mucosa (particularly of a young child) so as to allow absorption of steroidal saponins, thus causing inhibition of tissue respiration or haemolysis in the bloodstream, and possibly affecting a patient's responses to stimuli.

The presence in *Tulbaghia violacea* of two flavonols, kaempferol and quercetin, was confirmed chromatographically. It is possible that their presence, as glycosides, in extracts of the plant, has a calming effect on the smooth muscle of the gastro-intestinal tract, and on this basis the herbal remedy may have some efficacy in the treatment of colic conditions.

Several sugars were identified in *Tulbaghia violacea*; the free sugars found were glucose, fructose, galactose and arabinose, and the glycosidic sugars found were glucose, fructose, rhamnose, fucose, arabinose, galactose, and xylose. Since flavones are not often associated with fucose or fructose, it seems likely that these sugars occur in the plant associated with other secondary metabolites. This lends support to the suggested presence of saponins in the extracts.

It would seem from the studies on the biological activity of the *Tulbaghia violacea* extracts that other plants contain higher concentrations of secondary metabolites, which suggests that use of young plants in the preparation of the herbal medicine could result in less adverse effects. Also, since heating could cause hydrolysis of glycosides and transformation of sulphur compounds, an extract which has been boiled might contain less corrosive or less easily absorbed constituents and might therefore be less damaging.

EXPERIMENTAL

3.1 GENERAL

Melting points were measured on a Kofler hot-stage apparatus and are uncorrected. I.r. spectra were recorded on a Perkin-Elmer 180 spectrophotometer, as Nujol mulls, KBr discs, or solutions in CCl_4. ^1H n.m.r. spectra were recorded on the following instruments : Perkin-Elmer R12A, Bruker AM300, and Bruker WM300, using $CDCl_3$ as solvent and TMS (δ = 0) as internal standard, unless otherwise stated. ^{13}C n.m.r. were recorded on a Bruker AM300 instrument. Low resolution mass spectra were obtained on a Hewlett Packard 5988A spectrophotometer.

Solvents were dried as follows : (i) benzene, toluene, and ether, by refluxing over sodium in the presence of benzophenone; (ii) acetone and 2-butanone, by refluxing over anhydrous K_2CO_3; and (iii) DME, by passing through an alumina column. Unless otherwise stated, solutions were dried during work-up with anhydrous $MgSO_4$.

G.l.c. analyses were carried out on a Hewlett Packard 5980A gas chromatograph using N_2 as carrier gas, and flame ionisation detection with hydrogen and synthetic air as detector feeder gases. Conditions are described in the text. For g.c. - m.s. analyses, a Hewlett Packard 5988A gas chromatograph was linked to the Hewlett Packard 5980A mass spectrograph, and He was used as carrier gas, while detection was as for g.l.c. analyses. Columns used for g.l.c. and g.c. - m.s. were as follows : (i) HP-1 (crosslinked methyl silicone gum) 25 m x 0.2 mm x 0.33/μm film thickness; (ii) J + W DB225 (fused silica capillary column) 30 m x 0.25 mm x 0.25 μm film thickness; (iii) HP-20M (carbowax 20 M) 25 x 0.2 mm x 0.2 μm film thickness.

Flash chromatography[122] was carried out using Silica gel 60 [particle size 0.040 - 0.063 mm (230 - 400 mesh ASTM) (Merck)]. T.l.c. analyses were carried out on Silica gel 60 F_{254} precoated plastic plates (Merck) and cellulose precoated plastic plates (Merck). For p.l.c., plates were prepared using Silica gel 60 PF_{254} (Merck).

Solvent systems used for flash chromatography are detailed in the text, while solvent systems and spray reagents used for t.l.c. analyses are listed in Table 3.1 and Table 3.2 respectively.

Table 3.1 Solvent systems used for t.l.c. analyses

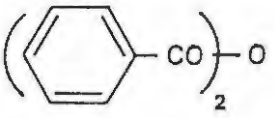
Solvent system	Composition	
(1)	n - BuOH - MeOH - AcOH - H_2O	(8:8:1:1)
(2)	MeCOEt - AcOH - MeOH	(3:1:1)
(3)	EtOAc - Prop^{i}OH - H_2O	(6:2:1)
(4)	AcOH - H_2O	(1:19)
(5)	Bu^{t}OH - AcOH - H_2O	(3:1:1)
(6)	EtOAc - AcOH - HCOOH - H_2O	(18:3:1:1)
(7)	EtOAc - hexane	(1:1)
(8)	$CHCl_3$: acetone	(4:1)
(9)	EtOAc : toluene	(2:3)
(10)	THF : $CHCl_3$	(1:4)
(11)	$CHCl_3$: MeOH : H_2O	(78:20:2)
(12)	$CHCl_3$: MeOH : H_2O	(70:30:10)
(13)	$CHCl_3$: MeOH : H_2O	(65:35:10)

Table 3.2 Spray reagents used in t.l.c. analyses

Spray reagent	Composition
(1) Anisaldehyde - H_2SO_4	EtOH (20 ml), anisaldehyde (1 ml), H_2SO_4 (1 ml) AcOH (0.2 ml)
(2) For p.c. of sugars	(i) Saturated aq. $AgNO_3$ (5 ml) in acetone (20 ml) (ii) 40% aq. NaOH (40 ml) in EtOH (500 ml) (iii) NaOAc (25 g), $Na_2S_2O_3$ (25 g), AcOH (1 ml) in H_2O (500 ml) Paper was dried after spraying with each solution
(3) Vanillin - H_3PO_4	Vanillin (1 g) in H_3PO_4 (50 ml), filtered. Heated 5 min., after spraying, at 100°C
(4) Cinnamaldehyde - H_2SO_4	(i) Cinnamaldehyde (1 g) in EtOH (100 ml) (ii) Ac_2O (12 ml) and H_2SO_4 (1 ml). T.l.c. plate dried after spraying with (i).
(5) $SbCl_3$ - $CHCl_3$	$CHCl_3$ saturated with $SbCl_3$ Heated 5 min. at 100°C
(6) Dragendorff's reagent	See reference (104)
(7) Iodoplatinate	10% aq. Hexachloroplatinic acid (3 ml), H_2O (97 ml) and 6% aq. KI (100 ml) Heated 5 min. at 100°C.
(8) Magnesium acetate	0.5% solution in MeOH
(9) KOH - EtOH	1% solution

3.2 SYNTHESIS OF MODEL COMPOUNDS

Benzoic anhydride (60)



(60)

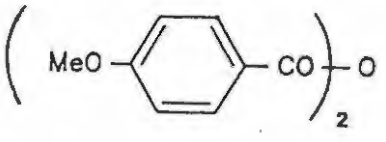
Method 1

Supported P_2O_5 [supplied by E. Merck as "SICAPENT" with indicator (Cat. No. 543)] was added to a solution of benzoic acid (12.2 g, 0.1 mol) in dry toluene, in a flange-flask equipped with an overhead stirrer, reflux condenser, and drying-tube. The stirred mixture was maintained at *ca.* 100 - 110°C (oil-bath temperature) for 1 h, and then filtered. The residue was then washed with dry Et_2O, and the combined filtrate and washings were treated with anhydrous K_2CO_3 (1 g) and charcoal, and boiled under reflux for 15 min before filtering sequentially through celite and alumina. The solvent was evaporated under reduced pressure, and vacuum distillation of the residue afforded benzoic anhydride (60) (8.37 g, 74%), m.p. 39 - 40°C (lit.,[85] 42 - 43°C); ν_{max} (CCl_4) 1795 and 1725 cm^{-1} (anhydride CO); δ_H ($CDCl_3$) 7.55 (6H, m, 3-, 4-, and 5-H) and 8.15 (4H, m, 2- and 6-H).

Method 2

Repetition of this preparation following the above procedure and using benzene as the solvent afforded benzoic anhydride (60) (8.68 g, 77%), m.p. 40 - 41°C.

Anisic anhydride (61) (4-methoxybenzoic anhydride)



(61)

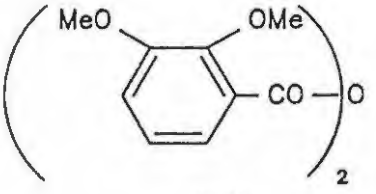
Method 1

Supported P_2O_5 (15 g) was added to a warmed solution of anisic acid (15.2 g, 0.1 mol) in dry DME (60 ml), in a flange-flask equipped with an overhead stirrer, reflux condenser, and drying-tube. The stirred mixture was boiled under reflux for 1 h, and then filtered. The residue was washed with fresh dry DME. The combined filtrate and washings were treated with anhydrous K_2CO_3 and charcoal, boiled under reflux for 15 min., and filtered sequentially through celite and alumina. Evaporation of the solvent gave anisic anhydride (61) (8.0 g, 56%), m.p. 96 - 97°C (from EtOAc) (lit.,[85] 99°C); ν_{max} (CCl_4) 1895 and 1730 cm^{-1} (anhydride CO); δ_H ($CDCl_3$) 3.88 (6H, s, 2 x OCH_3), 6.95 (4H, d, *J* 9Hz, 3- and 5-H), and 8.10 (4H, d, *J* 9Hz, 2- and 6-H).

Method 2

Repetition of the above preparation following the same procedure, and using dry toluene (90 ml) as solvent and dry Et_2O to wash residues, afforded anisic anhydride (61) (9.9 g, 69%), m.p. 96 - 97°C.

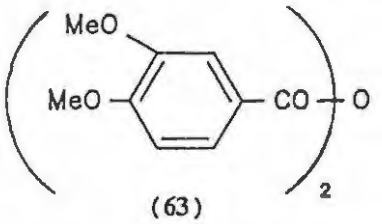
2,3-Dimethoxybenzoic anhydride (62)



(62)

The general procedure described for the synthesis of anisic anhydride (61) (Method 2) was followed, using supported P_2O_5 (15 g), and 2,3-dimethoxybenzoic acid (18.2 g, 0.1 mol) in dry toluene (70 ml). Work-up as before afforded 2,3-dimethoxybenzoic anhydride (62) (10.5 g, 60%), m.p. 66 - 67°C (from Et_2O - hexane) (lit.,[84] 93°C); ν_{max} (CCl_4) 1750 and 1795 cm^{-1} (anhydride CO); δ_H ($CDCl_3$) 3.90 and 3.95 (12H, 2 x s, 2 x OCH_3) and 7.30 (6H, m, Ar-H).

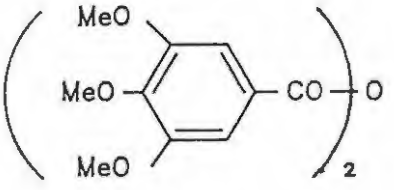
Veratric anhydride (63) (3,4-dimethoxybenzoic anhydride)



(63)

The general procedure described for the synthesis of anisic anhydride (61) (Method 2) was followed using supported P_2O_5 (15 g), and veratric acid (18.2 g, 0.1 mol) in dry toluene (60 ml). Work-up as described previously, but omitting the final filtration through alumina, afforded veratric anhydride (63) (10.7 g, 62%), m.p. 122 - 124°C (from EtOAc) (lit.,[86] 124 - 125°C); ν_{max} (CCl_4) 1790 and 1745 cm^{-1} (anhydride CO); δ_H ($CDCl_3$) 3.95 and 3.98 (2 x 6H, 2 x s, 4 x OCH_3), 6.95 (2H, d, *J* 8Hz, 2-H) and 7.85 (4H, m, 5- and 6-H).

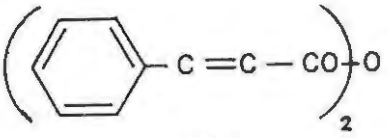
3,4,5-trimethoxybenzoic anhydride (64)



(64)

The general procedure described for the synthesis of veratric anhydride (63) was followed, using supported P_2O_5 (12 g), and 3,4,5-trimethoxybenzoic acid (12 g, 0.06 mol) in dry toluene (70 ml), and using dry toluene to wash residues. Work-up as before afforded 3,4,5-trimethoxybenzoic anhydride (64) (5.19 g, 23%), m.p. 158 - 159°C (from EtOAc) (lit.,[87] 159°C); ν_{max} (CCl_4) 1780 and 1730 cm^{-1} (anhydride CO); δ_H ($CDCl_3$) 3.98 and 4.00 (18H, 2 x s, 6 x OCH_3) and 7.49 (4H, s, 2- and 6-H).

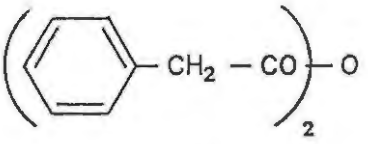
Cinnamic anhydride (65) (3-phenyl-2-propenoic anhydride)



(65)

The general procedure described for the synthesis of veratric anhydride (63) was followed, using supported P_2O_5 (15 g), and cinnamic acid (14.8 g, 0.1 mol) in dry DME (60 ml). After boiling for 1 h, additional Sicapent® (5 g) and DME (10 ml) were added and boiling was continued for 15 min. Work-up as before afforded cinnamic anhydride (65) (10.5 g, 75%), m.p. 135 - 137°C (from EtOAc) (lit.,[86] 138°C); ν_{max} (CCl_4) 1725 and 1790 cm^{-1} (anhydride CO); δ_H ($CDCl_3$) 6.5 (4H, 2 x s, CH=CH) and 7.6 (10H, m, Ar-H).

Phenylacetic anhydride (66) (2-phenylethanoic anhydride)



(66)

Method 1

The general procedure described for the synthesis of anisic anhydride (61) (Method 1) was followed, using supported P_2O_5 (15 g), and phenylacetic acid (13.6 g, 0.1 mol) in dry DME (60 ml). The crude product was obtained as a brown oil, distillation of which gave three fractions. Each of these fractions was shown by ^1H n.m.r. to contain varying proportions of a contaminant [tentatively identified as methyl phenylacetate on the basis of the ^1H n.m.r. signal at δ 3.25 (CO_2CH_3)]. The overall yield of the required product was estimated by ^1H n.m.r. spectroscopy to be 3.7 g (30%).

Method 2

Repetition of the above preparation, following the same procedure, and using toluene (70 ml) as the solvent, afforded, after evaporation of the solvent, crystalline phenylacetic anhydride (66) (7.06 g, 56%), m.p. 70°C (from Et_2O) (lit.,[85] 71 - 72°C); ν_{max} (CCl_4) 1745 and 1810 cm^{-1} (anhydride CO), δ_H ($CDCl_3$) 3.70 (4H, s, CH_2) and 7.30 (10H, s, Ar-H).

Octanoic anhydride (67)

$$[(CH_3(CH_2)_5CO)]_2O$$

(67)

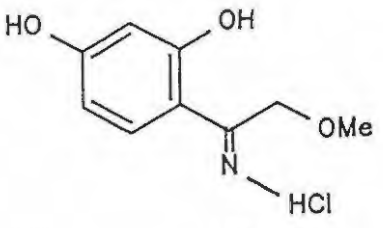
Method 1

The general procedure described for the synthesis of anisic anhydride (61) (Method 1) was followed, using supported P_2O_5 (15 g) and octanoic acid (14.4 g, 0.1 mol) in dry DME (60 ml). Work-up as before afforded octanoic anhydride (67) (8.1 g, 57%), b.p. 140°/0.2mmHg (lit.,[85] 186°/15mmHg); ν_{max} (CCl_4) 1760 and 1820 cm^{-1} (anhydride CO); δ_H ($CDCl_3$) 0.95 (6H, s, CH_3), 1.30 (20H, br signal, CH_2), and 2.35 (4H, m, CH_2CO).

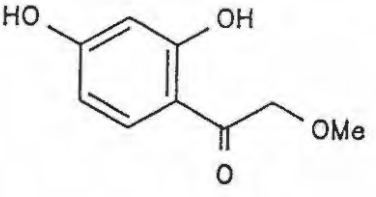
Method 2

Repetition of the above preparation following the same procedure and using benzene as the solvent, afforded octanoic anhydride (67) (6.6 g, 49%), b.p. 118°/0.05mmHg.

ω-Methoxyresacetophenone (73) (2',4'-dihydroxy-2-methoxyacetophenone)[88]



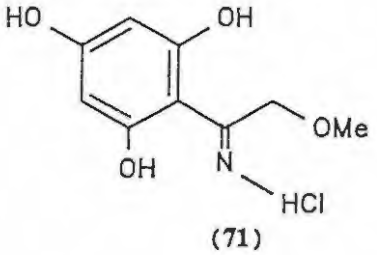
(70)



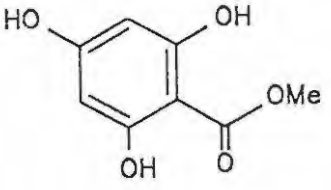
(73)

Methoxyacetonitrile (1.2g, 0.017 mol) was added to a solution of resorcinol (1.6 g, 0.015 mol) in dry Et_2O (50 ml). Dry HCl gas was bubbled through the stirred solution for 2 h at 0°C (during which time a cream solid formed) and the resulting mixture was stored at -4°C for 2 d. The solvent was decanted and the residual solid was rinsed with fresh dry Et_2O before recrystallisation from MeOH, to give the ketimine hydrochloride salt (70) (1.02 g, 30%), m.p. 201 - 202°C (dec.) [lit.,[88] 205 - 207°C (dec.)]. The hydrochloride salt (70) was dissolved in water and the solution was warmed to 80°C for 0.5 h to effect hydrolysis. Cooling afforded ω-methoxy-resacetophenone (73) as colourless crystals (0.48 g, 56%) m.p. 134 - 136°C (dec.) [lit.,[88] 136°C (dec.)]; ν_{max} (KBr) 3360 (OH) and 1750 cm^{-1} (CO); δ_H ($CDCl_3$) 3.55 (3H, s, OCH_3), 4.65 (2H, br s, 2 x OH), 4.75 (2H, s, CH_2), 6.50 (2H, m, 5′- and 6′-H), and 7.70 (1H, s, 3′-H).

ω-Methoxyphloracetophenone (74) (2′,4′,6′-trihydroxy-2-methoxyphloracetophenone)[88]



(71)

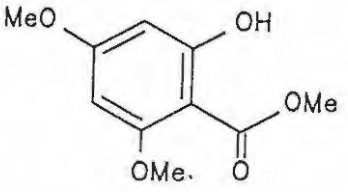


(74)

Methoxyacetonitrile (5 g, 0.08 mol) was added to a solution of phloroglucinol (9 g, 0.08 mol) in dry Et_2O (50 ml). Dry HCl gas was bubbled through the stirred solution for 2 h at 0°C (during which time a pale yellow solid formed) and the resulting mixture was stored at -4°C for 3 d. The solvent was decanted and the residual solid was rinsed with fresh dry Et_2O. Recrystallisation from MeOH gave the ketimine hydrochloride salt (70) (10.5 g, 56%), m.p. 236 - 240° (dec.) (lit., 238 - 241°C). The hydrochloride salt (70) was dissolved in water (50 ml) and boiled to effect hydrolysis. On

cooling, pale yellow needles formed which were recrystallised from water, giving ω-methoxyphloracetophenone (74) (6.8 g, 76%), m.p. 191 - 192°C (dec.) [lit.,[88] 190 - 192°C (dec.)]; ν_{max} (KBr) 3270br (OH) and 1640 cm^{-1} (CO), δ_H (CD_3OD) 3.50 (3H, s, OCH_3), 4.71 (2H, s, CH_2), 4.90 (3H, br s, 3 x OH), and 5.90 (2H, s, 2 x Ar-H).

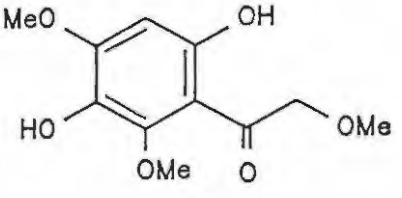
2'-Hydroxy-2,4',6'-trimethoxyacetophenone (77)[90]



(77)

Methoxyacetonitrile (10 g, 0.14 mol) was added to a solution of 3,5-dimethoxyphenol (10 g, 0.065 mol) and anhydrous $ZnCl_2$ (2 g, 0.015 mol) in dry Et_2O. Dry HCl gas was bubbled through the stirred solution for 2.5 h at 0°C (during which time a pink solid crystallised) and the resulting mixture was stored at -4°C for 3 d. The supernatant liquid was decanted and the residual solid was washed with fresh dry Et_2O. A solution of the solid in H_2O (150 ml) was heated on a steam-bath for 1 h, cooled, and then extracted with ether (5 x 30 ml). The dried ether fraction was concentrated under reduced pressure to afford a yellow oil which was recrystallised from EtOH, giving 2'-hydroxy-2,4',6'-dimethoxyacetophenone (77) (3.08 g, 21%), m.p. 102 - 104°C (lit.,[90] 103 - 104°C); δ_H ($CDCl_3$) 3.50 (3H, s, 2-OCH_3), 3.88 (6H, 2xs, 4'- and 6'-OCH_3), 4.58 (2H, s, CH_2), 5.9 (1H, d, *J* 2Hz, 3'-H), 6.15 (1H, d, *J* 2Hz, 5'-H), and 13.7 (1H, s, OH).

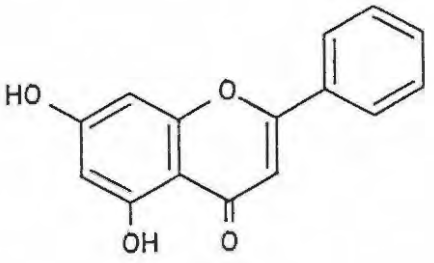
2',5'-Dihydroxy-2,4',6'-trimethoxyacetophenone (79)[90]



(79)

A stirred solution of 2'-hydroxy-2,4',6'-trimethoxyacetophenone (77) (3.0 g, 0.014 mol) in 5% NaOH solution (50 ml) was maintained at 15°C and a solution of $K_2S_2O_8$ (4 g, 0.015 mol) was added during the course of 1 h. Stirring was continued for 3 h and the mixture was then left to stand for 18 h. Conc. HCl (2 ml) was added to acidify the solution which was then filtered. Further conc. HCl (15 ml) was added, and the aqueous layer was extracted with Et_2O (6 x 30 ml). Evaporation of the dried ether layer gave a dark brown oil which was purified by chromatography [flash chromatography on silica; elution with EtOAc - hexane (8 : 2)] to yield, as a yellow oil, 2',5'-dihydroxy-2,4',6'-tri-methoxyacetophenone (79) (0.20 g, 7%), δ_H ($CDCl_3$) 3.5 and 3.7 (9H, 2 x s, 3 x OCH_3), 3.85 (2H, br s, 2 x OH), 4.42 (2H, s, CH_2) and 6.15 (1H, s, Ar-H).

Chrysin (80) (5,7-dihydroxy-2-phenyl-4H-1-benzopyran-4-one)[74]

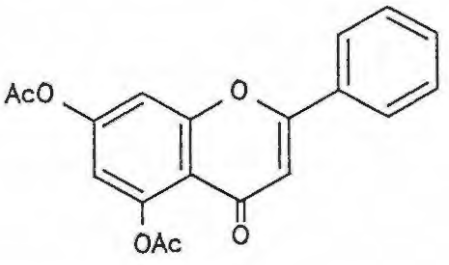


(80)

Phloracetophenone (1g, 0.006 mol), benzoic anhydride (10 g, 0.044 mol), and sodium benzoate (1.2 g, 0.008 mol) were placed in a three-necked 100 ml round-bottomed flask fitted with thermometer and condenser. The stirred mixture was maintained at 180 - 185°C for 6 h. After cooling, the resulting mixture was dissolved in EtOH (60 ml) by boiling under reflux for 0.5 h. A solution of KOH (6.4 g, 0.12 mol) in H_2O (4 ml) was slowly added to the solution and boiling was continued for 0.5 h. After removal of the EtOH under reduced pressure, the residue was dissolved in H_2O (50 ml). Solid CO_2 was added to saturate the aqueous solution, precipitating crude product

which was purified by chromatography [flash chromatography on silica, elution with EtOAc - hexane (55 : 45) affording chrysin (80)] (0.75 g, 81%), m.p. 282 - 285°C (from MeOH) (lit.,[74] 285 - 286°C); ν_{max} (KBr) 3180 - 2620br (OH) and 1655 cm^{-1} (CO); δ_H (DMSO-d_6) 6.25 (1H, d, *J* 2Hz, 6-H), 6.45 (1H, d, *J* 2Hz, 8-H), 6.72 (1H, s, 3-H), 7.85 (5H, m, Ar-H) and 11.50 (2H, br s, 2 x OH).

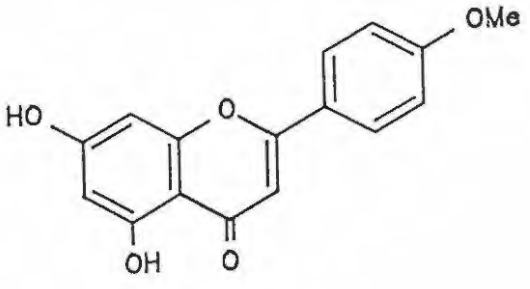
5,7-Di-O-acetylchrysin (81) (5,7-diacetoxy-2-phenyl-4H-1-benzopyran-4-one)[74]



(81)

Chrysin (80) (0.4 g, 0.0016 mol) was placed in a round-bottomed 25 ml flask with Ac_2O (2.5 ml, 0.03 mol) and pyridine (0.5 ml, 0.006 mol). The mixture was boiled under reflux for 2 h before pouring on to crushed ice (30 g) and extracting with Et_2O. The Et_2O extract was dried (anhydrous $MgSO_4$), evaporated to dryness, and the solid product recrystallised from MeOH, to yield 5,7-di-*O*-acetylchrysin (81) (220 mg, 39%), m.p. 185 - 186°C (lit.,[74] 185°C); ν_{max} 1775s (CO) and 1630s (CO); δ_H ($CDCl_3$) 2.37 and 2.47 (2x3H, 2 x s, 2 x CH_3CO), 6.74 (1H, s, 6-H), 6.96 (1H, d, *J* 2Hz, 8-H) and 7.60 (5H, m, Ar-H).

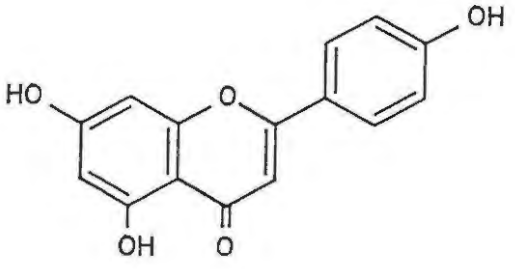
4'-O-Methylapigenin (82) [5,7-dihydroxy-2-(4-methoxyphenyl) -4H-1-benzopyran-4-one][74]



(82)

A stirred mixture of phloracetophenone (1 g, 0.006 mol), anisic anhydride (61) (10 g, 0.05 mol), and potassium anisate [prepared by neutralisation of anisic acid (4 g, 0.025 mol) in MeOH with KOH (1.4 g, 0.025 mol), and subsequent evaporation of the solvent] (1.5 g, 0.008 mol) was maintained at 180 - 185°C for 6 h, then cooled and dissolved in EtOH (60 ml). A solution of KOH (6.4 g, 0.1 mol) in H_2O (4 ml) was added slowly and the resulting solution was boiled under reflux for 0.5 h. The EtOH was removed by distillation and the residue was dissolved in H_2O (100 ml). Saturation of the aqueous solution with CO_2 precipitated the crude product which was purified by chromatography [flash chromatography cn silica; elution with EtOAc - hexane (55 : 45)] and recrystallisation from EtOH to yield yellow crystals of 4'-*O*-methylapigenin (82) (0.6 g, 35.2%), m.p. 260 - 263°C (lit.,[98] 261°C); ν_{max} (KBr) 3140br (OH) and 1655 cm^{-1} (CO); δ_H (DMSO-d_6) 2.96 (3H, s, OCH_3), 3.93 (2H, s, OH), 6.30 (1H, d, *J* 3Hz, 6-H), 6.50 (1H, d, *J* 3Hz, 8-H), 6.62 (1H, s, 3-H), 7.1 (2H, d, *J* 8Hz, 3'- and 5'-H) and 7.93 (2H, d, *J* 8Hz, 2'- and 6'-H).

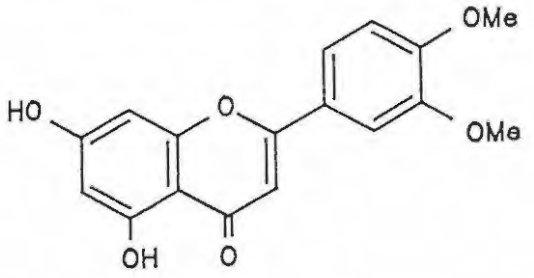
Apigenin (83) [5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one][74]



(83)

A mixture of 4'-*O*-methylapigenin (82) (0.06 g, 0.2 mmol), Ac_2O (0.3 ml, 3 mmol) and HI (d = 1.7 g/ml; 1 ml, 5 mmol) boiled under reflux for 2 h. H_2O (30 ml) was added and the aqueous solution was heated on a steam-bath for 0.25 h. The solution was cooled and after standing at room temperature for 2 d, some yellow product crystallised. Reduction of the volume and cooling in ice afforded more yellow crystals of apigenin (83) (0.02 g, 35%), m.p. 340 - 344°C (from MeOH) (lit.,[98] 348 - 350°); ν_{max} (KBr) 3200br (OH) and 1650 cm^{-1} (CO).

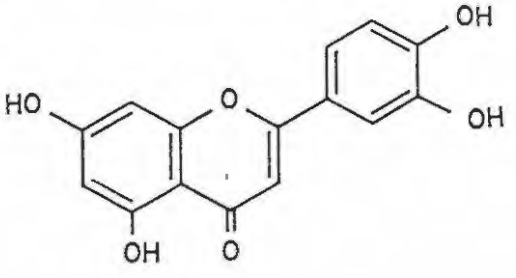
3',4'-Di-O-methylluteolin (84) [2-(3,4-dimethoxyphenyl) -5,7-dihydroxy-4H-1-benzopyran-4-one][75]



(84)

Phloracetophenone (0.5 g, 0.003 mol) was added to veratric anhydride (63) (5.0 g, 0.01 mol) and potassium veratrate [prepared by neutralisation of a solution of veratric acid (8.3 g, 0.046 mol) in MeOH with KOH (2.5 g, 0.045 mol), followed by evaporation of the solvent] (2.0 g, 0.01 mol). The stirred mixture was maintained at 180 - 185°C for 5 h, then cooled and dissolved in EtOH (60 ml) by boiling under reflux. A solution of KOH (6.4 g, 0.1 mol) in H_2O (4 ml) was slowly added and boiling under reflux was continued for 0.5 h. The EtOH was removed under reduced pressure leaving a brown residue which was dissolved in water (30 ml). Saturation of the aqueous solution with CO_2 precipitated brown crude product which was purified by chromatography [flash chromatography on silica; elution with EtOAc - hexane (7 : 3) and subsequently benzene - Et_2O (1 : 1)] to afford 3',4'-di-*O*-methylluteolin (84) (0.31 g, 33%), m.p. 256 - 258°C (lit.,[75] 258 - 259°C); ν_{max} (KBr) 3300br (OH) and 1645 cm^{-1} (CO); δ_H (DMSO-d_6) 3.55 (2H, br s, OH), 3.85 (6H, s, OCH_3), 6.82 (1H, d, *J* 2Hz, 6-H), 6.95 (1H, s, 3-H), 7.10 (1H, d, *J* 2Hz, 8-H), 7.30 (1H, m, 5'-H), 7.52 (1H, s, 2'-H), and 7.65 (1H, m, 6'-H).

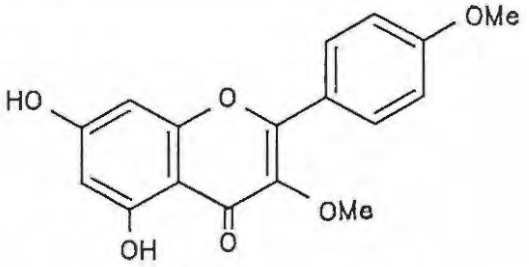
Luteolin (85) [2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4H-1-benzopyran-4-one][75]



(85)

A mixture of 3',4'-di-*O*-methylluteolin (84) (0.06 g, 0.2 mmol), Ac_2O (0.3 ml, 3 mmol), and HI (d = 1.7 g/ml; 1.3 ml, 6 mmol) was boiled under reflux for 2 h. H_2O (30 ml) was added and the aqueous solution was heated on a steam-bath for 15 min. After cooling and filtering, the solution was reduced to a very small volume. MeOH (1 ml) was added, and separation by chromatography [p.l.c. on silica; elution with EtOAc - hexane (1 : 1)] afforded the crude product as a yellow oil (20 mg, 36%). Chromatography [t.l.c. on silica; elution with EtOAc - hexane (1 : 1)] of the crude product showed the product to contain one major yellow (fluorescent purple in u.v. light) component, assumed to be the required product, and some brown material. The product was found to be very susceptible to oxidative decomposition.

3,4'-Di-O-methylkaempferol (86) [5,7-dihydroxy-3-methoxy-2-(4-methoxyphenyl)-4H-1-benzopyran-4-one][99]

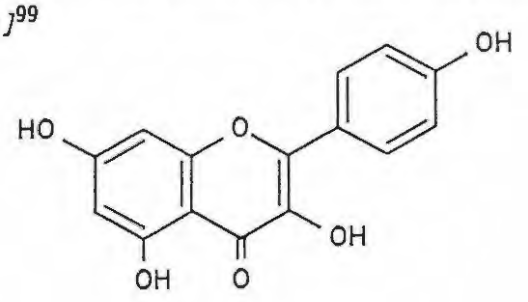


(86)

ω-Methoxyphloracetophenone (74) (1.9 g, 0.01 mol) was added to anisic anhydride (61) (7.6 g, 0.03 mol) and potassium anisate (4.7 g, 0.025 mol), and the stirred mixture was maintained at 180 - 185°C for 3 h. After cooling, the mixture was dissolved in EtOH (100 ml) by boiling under reflux. A solution of KOH (8.5 g, 0.15 mol) in H_2O (6 ml) was added slowly, and boiling under reflux was continued for 0.5 h. Removal of the EtOH by distillation left a dark residue which was dissolved in H_2O (100 ml). Saturation of the aqueous solution with CO_2 precipitated the yellow product which was purified by

chromatography [flash chromatography on silica; elution with EtOAc - hexane (45 : 55)] and recrystallisation from aqueous AcOH to yield 3,4′-di-*O*-methyl-kaempferol (86) (1.2 g, 40%), m.p. 230 - 231°C (lit.,[98] 234°C); ν_{max} (KBr) 3100br (OH) and 1655 cm^{-1} (CO); δ_H (CD_3OD) 3.60 and 3.72 (6H, 2 x s, 2 x OCH_3), 4.50 (3H, br s, OH), 6.05 (1H, d, *J* 2Hz, 6-H), 6.30 (1H, d, *J* 2Hz, 8-H), 6.88 (2H, d, *J* 8Hz, 3′ - and 5′-H), and 7.92 (2H, d, *J* 8Hz, 2′ - and 6′-H).

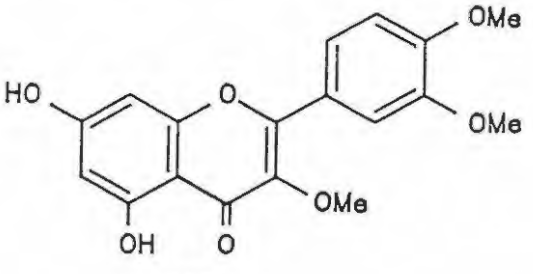
Kaempferol (87) [3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one][99]



(87)

A solution of 3,4′-di-*O*-methylkaempferol (86) (0.2 g, 0.6 mmol) in freshly distilled HI (d = 1.7 g/ml; 2 ml, 0.03 mol) was heated to 140°C for 1 h before pouring into H_2O (40 ml). The aqueous solution was heated on a steam-bath for 15 min. The precipitated yellow product was filtered, dried under reduced pressure, and recrystallised from EtOH, yielding kaempferol (87) (0.15 g, 90%), m.p. 276 - 278°C (lit.,[98] 279 - 280°C); ν_{max} (KBr) 3100br (OH) and 1655 cm^{-1} (CO); δ_H (DMSO-d_6) 3.20 (4H, br s, OH), 6.20 (1H, d, *J* 2Hz, 6-H), 6.40 (1H, d, *J* 2Hz, 8-H), 6.90 (2H, d, *J* 9Hz, 3′ - and 5′-H), and 8.05 (2H, d, *J* 9Hz, 2′ - and 6′-H).

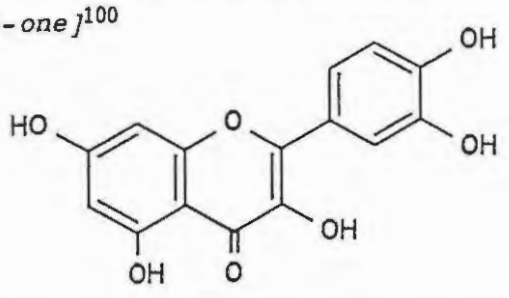
3,3′,4′-Tri-O-methylquercetin (88) [2-(3,4-dimethoxyphenyl)-5,7-dihydroxy-3-methoxy-4H-1-benzopyran-4-one][100]



(88)

A stirred mixture of ω-methoxyphloracetophenone (74) (0.6 g, 0.003 mol), veratric anhydride (63) (5.0 g, 0.014 mol) and potassium veratrate (2.0 g, 0.01 mol) was maintained at 175 - 180°C for 4.5 h. After cooling, the mixture was dissolved in EtOH (30 ml) by boiling under reflux. A solution of KOH (1.8g, 0.03 mol) in H_2O (5 ml) was added, and boiling was continued for 0.5 h. The EtOH was removed by distillation and the residue was dissolved in water. Saturation of the aqueous solution with CO_2 resulted in precipitation of the crude product which was purified by chromatography [flash chromatography on silica ; elution with EtOAc - hexane (7 : 3)] and recrystallisation from EtOAc, to yield 3,3′,4′-tri-*O*-methylquercetin (88) (0.43 g, 39%), m.p. 238 - 240°C (lit.,[100] 240 - 245°C); ν_{max} (KBr) 3130br (OH) and 1650 cm^{-1} (CO); δ_H (DMSO-d_6) 3.86 and 3.95 (9H, 2 x s, 3 x OCH_3), 6.36 (1H, d, *J* 3Hz, 6-H), 6.48 (1H, d, *J* 3Hz, 8-H), 7.15 (1H, m, 5′-H), 7.80 (2H, m, 2′- and 6′-H), and 12.60 (2H, s, 2 x OH).

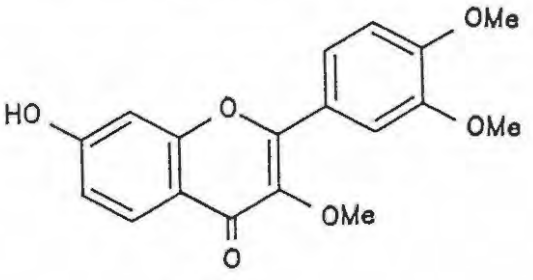
Quercetin (89) [2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy -4H-1-benzopyran-4-one][100]



(89)

A mixture of 3,3′,4′-tri-*O*-methylquercetin (88), (0.2 g, 0.7 mmol), Ac_2O (0.5 ml, 5 mmol), and HI (d = 1.7 g/ml; 1.5 ml, 0.02 mol), was maintained at 140°C for 2 h. After cooling, H_2O (50 ml) was added to the contents of the flask, and the resulting mixture was heated on a steam-bath for 20 min. The mixture was again cooled and the solid product was filtered off, dried *in vacuo*, and recrystallised from MeOH to afford quercetin (89) (0.15 g, 86%), m.p. 312 - 314°C (lit.,[100] 312 - 316°C; ν_{max} (KBr) 3200br (OH) and 1620 cm^{-1} (CO); δ_H ($CDCl_3$) 6.20 (1H, d, *J* 2Hz, 6-H), 6.39 (1H, d, *J* 2Hz, 8-H), 6.92 (H, d, *J* 9Hz, 5′-H), and 7.60 (2H, m, 2′- and 6′-H).

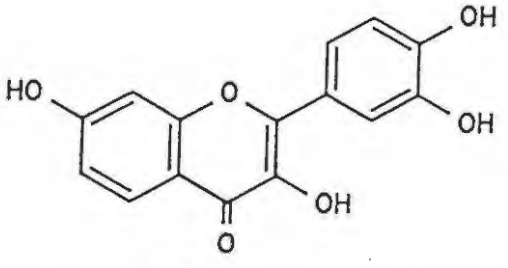
3,3',4'-Tri-O-methylfisetin (90) 2-(3,4-dimethoxyphenyl) -7-hydroxy-3-methoxy-4H-1-benzopyran-4-one][100]



(90)

A stirred mixture of ω-methoxyresacetophenone (73) (0.5 g, 0.003 mol), veratric anhydride (63) (5.0 g, 0.015 mol) and potassium veratrate (2 g, 0.01 mol), was maintained at 175°C for 4.5 h. After cooling, the mixture was dissolved in EtOH (30 ml) by boiling under reflux for 0.5 h. A solution of KOH (1.6 g, 0.03 mol) in H_2O (5 ml) was added slowly, and boiling was continued for 0.5 h. The EtOH was removed by distillation and the residue was dissolved in H_2O (30 ml). Saturation of the aqueous solution with CO_2 precipated the crude product which was purified by chromatography [flash chromatography on silica; elution with EtOAc - hexane (7 : 3)] and recrystallisation from EtOAc, affording 3,3',4'-tri-*O*-methylfisetin (90) (0.205 g, 23%), m.p. 218 - 220°C (lit.,[100] 220°C); ν_{max} (KBr) 3210br (OH) and 1610 cm^{-1} (CO); δ_H (DMSO-d_6) 3.85 and 3.90 (9H, 2 x s, 3 x OCH_3), 6.90 (2H, m, 5- and 6-H), 7.10 (1H, s, 8-H), 7.7 - 7.9 (3H, br m, 2'-, 3'- and 5'-H), and 10.30 (1H, s, OH).

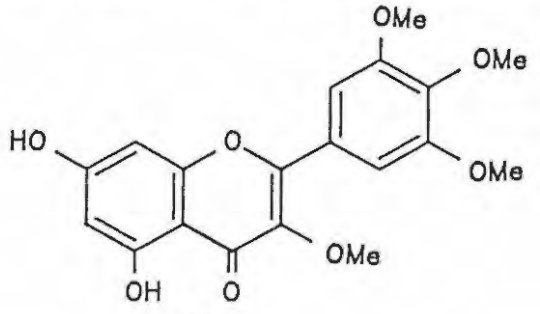
Fisetin (91) [2-(3,4-dihydroxyphenyl)-3,7-dihydroxy-4H-1-benzopyran-4-one][100]



(91)

A mixture of 3,3′,4′-tri-*O*-methylfisetin (91) (0.2 g, 0.6 mmol), Ac_2O (0.2 ml, 2 mmol), and HI (d = 1.7 g/ml; 1.0 ml, 0.01 mol) was maintained at 140°C for 2 h. The solution was cooled, and H_2O (30 ml) was added. The aqueous solution was heated on a steam-bath for 0.3 h. After cooling, the crude product, which precipitated, was filtered off, washed with H_2O, and recrystallised from MeOH to give fisetin (91) (0.12g, 69%), m.p. 345 - 350°C (lit.,[98] 348°C); ν_{max} (KBr) 3300br (OH) and 1620 cm^{-1} (CO); δ_H (DMSO-d_6) 6.90 (2H, m, 6- and 8-H), 7.75 (3H, m, 2′-, 5′-, and 6′-H), and 7.96 (1H, d, *J* 9Hz, 5-H).

3,3′,4′,5′-Tetra-O-methylmyricetin (92) [5,7-dihydroxy-3-methoxy-2-(3,4,5-trimethoxyphenyl)-4H-1-benzopyran-4-one][101]

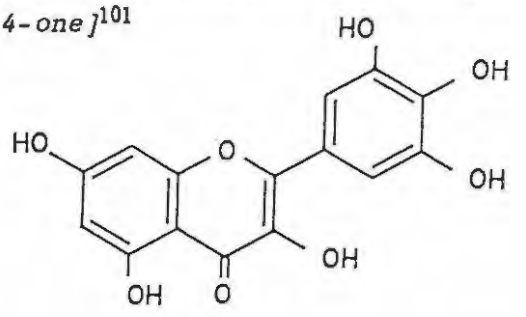


(92)

ω-Methoxyphloracetophenone (74) (1.0 g, 0.005 mol) was added to 3,4,5-trimethoxybenzoic anhydride (64) (5.0 g, 0.01 mol) and sodium 3,4,5-trimethoxybenzoate [prepared by neutralisation of 3,4,5-trimethoxybenzoic acid (9.1 g, 0.045 mol) in MeOH with NaOH (1.68 g, 0.042 mol), and subsequent evaporation of the solvent] (2.2 g, 0.01 mol). The stirred mixture was maintained at 175°C for 3 h, and then cooled. The residual mixture was dissolved in EtOH (30 ml) by boiling under reflux. A solution of KOH (1.7 g, 0.03 mol) in H_2O (4 ml) was added and boiling was continued for 0.5 h. The EtOH was removed under reduced pressure leaving a residue which was dissolved in H_2O (30 ml). Saturation of the aqueous solution with CO_2 gave a

brown precipitate which was purified by chromatography [flash chromatography on silica; elution with EtOAc - hexane (65 : 35)] to give 3,3',4',5'-tetra-*O*-methylmyricetin (92) (0.06 g, 3%), m.p. 268 - 273°C (dec.) (lit.,[101] 276 - 277°C), ν_{max} (KBr) 3400br (OH) and 1650 cm^{-1} (CO); δ_H (CD_3OD) 3.70 (2H, br s, OH), 3.84 and 3.95 (12H, 2 x s, 4 x OCH_3), 6.31 (1H, d, *J* 2Hz, 6-H), 6.45 (1H, d, *J* 2Hz, 8-H), and 7.40 (2H, s, 2'- and 6'-H).

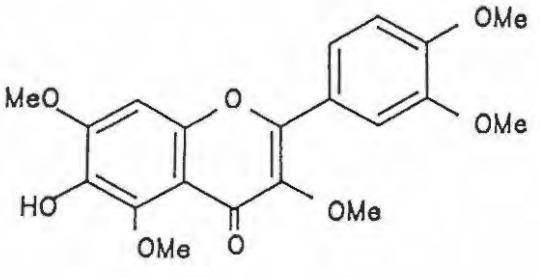
Myricetin (93) [3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl) -4H-1-benzopyran-4-one][101]



(93)

A mixture of 3,3',4',5'-tetra-*O*-methylmyricetin (92) (0.05 g, 0.13 mmol), Ac_2O (0.1 ml, 1 mmol) and HI (d = 1.7 g/ml; 0.3 ml, 1.4 mmol) was maintained at 140°C for 1.5 h. H_2O (10 ml) was added, and the aqueous solution was heated on a steam-bath for 15 min. The solution was then concentrated under reduced pressure diluted with MeOH, and chromatographed [p.l.c. on silica; elution with EtOAc - hexane (1 : 1)]. A yellow (fluorescent purple in u.v.light) major band was isolated and used, without further purification, as a chromatographic standard. The susceptibility of this material to oxidative decomposition was evident from its tendency to turn brown while on a t.l.c. plate.

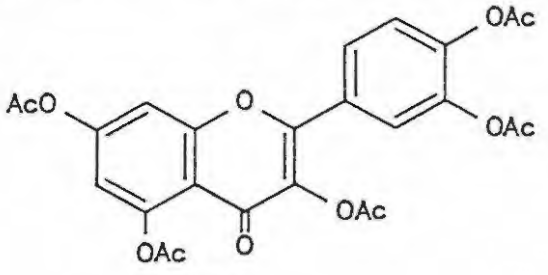
*3,3',4',5,7-Penta-O-methylquercetagetin (94) [2-(3,4-*dimethoxyphenyl*) -6-hydroxy-3,5,7-trimethoxy-4H-1-benzopyran-4-one]*[90]



(94)

A stirred mixture of 2′,5′-dihydroxy-2,4′,6′-trimethoxyacetophenone (79) (0.2 g, 1 mmol), veratric anhydride (63) (2.1 g, 6 mmol), and potassium veratrate (0.6 g, 3 mmol), was maintained at *ca.* 180°C for 4 h. After cooling, the residual mixture was dissolved in EtOH (30 ml) by boiling under reflux. A 10% aqueous solution of KOH (14 ml) was added slowly and boiling was maintained for 0.5 h. The EtOH was removed under reduced pressure and the residue was dissolved in H_2O. Saturation of the aqueous solution with CO_2 precipitated a small amount of crude product which was filtered off. Extraction of the filtrate with Et_2O afforded further crude product. Chromatography of the combined material [p.l.c. on silica; elution with EtOAc - hexane (1 : 1)] gave 3 fractions, one of which (30 mg; Rf 0.46) was shown by ^1H n.m.r. spectroscopy to be crude 3,3′,4′,5,7-penta-*O*-methyl-quercetagetin (94) δ_H (CD_3OD) 3.76 and 3.96 (15H, 2 x s, 5 x OCH_3), 6.15 (1H, s, 8-H), and 7.33 (3H, m, 2′-, 5′-, 6′-H).

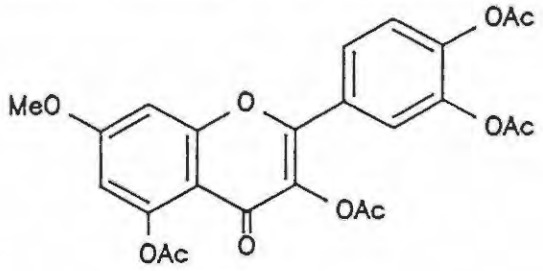
3,3′,4′,5,7-Penta-O-acetylquercetin (96) [3,5,7-triacetoxy-2-(3,4-diacetoxyphenyl)-4H-1-benzopyran-4-one][100]



(96)

Quercetin (89) (15 g, 0.045 mol) was dissolved in Ac_2O (75 ml, 0.7 mol) and pyridine (10 ml). The solution was boiled under reflux for 2 h before pouring on to crushed ice (300 g), whereupon the crude solid separated as a brown solid. Recrystallisation of the dried solid from EtOH - $CHCl_3$ afforded needles of 3,3′,4′,5,7-penta-*O*-acetylquercetin (96) (15.6 g, 64%), m.p. 190 - 192°C (lit.,[100] 191 - 195°C); ν_{max} (KBr) 1775 (CO) and 1650 cm^{-1} (CO); δ_H ($CDCl_3$) 2.32 and 2.4 (15H, 2 x s, s x $COCH_3$), 6.85 (1H, d, *J* 2Hz, 6-H), 7.30 (1H, d, *J* 2Hz, 8-H), 7.40 (1H, m, 5′-H), and 7.65 (2H, m, 2′- and 6′-H).

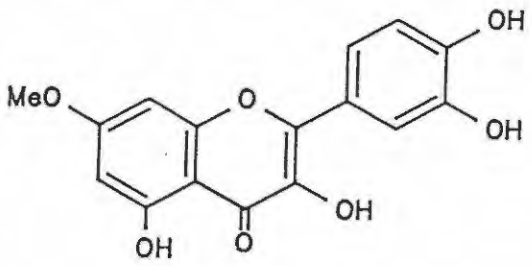
3,3′,4′,5-Tetra-O-acetylrhamnetin (97) [3,5-diacetoxy-2-(3,4-diacetoxyphenyl)-7-methoxy-4H-1-benzopyran-4-one][103]



(97)

Methyl iodide (10 ml, 0.03 mol) was added to a mixture of 3,3′,4′,5,7-penta-*O*-acetylquercetin (96) (5 g, 0.009 mol) and anhydrous K_2CO_3 (13 g, 0.1 mol) in dry acetone (75 ml). The mixture was boiled under reflux for 20 h. Undissolved solids were then filtered off and washed with fresh dry acetone. The combined filtrate and washings were concentrated to a gum which was dissolved in warm benzene (30 ml). The solution was filtered, diluted with hexane (30 ml), and concentrated to *ca.* 10 ml; on cooling, the crude product crystallised out (4.31 g, 91%). Recrystallisation from acetone - MeOH afforded colourless needles of 3,3′,4′,5-tetra-*O*-acetylrhamnetin (97) (1.8 g, 40%), m.p. 189 - 190°C (lit.,[103] 189 - 190°C); ν_{max} (KBr) 1770 (CO) and 1635 (CO); δ_H ($CDCl_3$) 2.29 and 2.49 (12H, 2 x s, 4 x $COCH_3$), 3.85 (3H, s, 7-OCH_3), 6.48 (1H, d, *J* 2Hz, 6-H), 6.78 (1H, d, *J* 2Hz, 8-H), 7.20 (1H, s, 5′-H), and 7.61 (2H, m, 2′- and 6′-H).

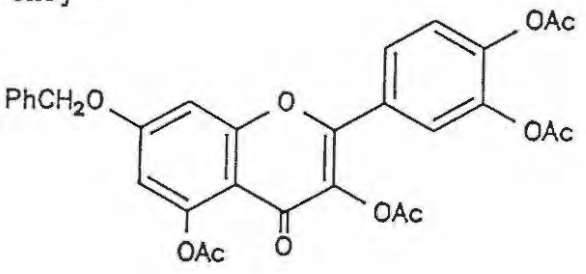
Rhamnetin (98) [2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-7-methoxy-4H-1-benzopyran-4-one][103]



(98)

A solution of 3,3′,4′,5-tetra-*O*-acetylrhamnetin (97) (1.0 g, 0.002 mol) in 10% aqueous NaOH solution (1.1 ml, 0.004 mol) was warmed on a steam-bath for 3 min. H_2O (3 ml) was added, and after a further 5 min., concentrated HCl (1 ml) was added. Heating on the steam-bath was continued for 0.75 h, during which time the crude yellow product precipitated. This was filtered off and recrystallised from acetone - MeOH to yield rhamnetin (98) (0.38 g, 56%), m.p. 291 - 294°C (lit.,[103] 294 - 296°C); ν_{max} (KBr) 3150br (OH) and 1660 cm^{-1} (CO); δ_H (DMSO-d_6) 3.96 (3H, s, OCH_3), 6.45 (1H, d, *J* 3Hz, 6-H), 6.75 (1H, d, *J* 3Hz, 8-H), 7.05 (1H, d, *J* 8Hz, 5′-H), 7.94 (2H, m, 2′- and 6′-H).

3,3′,4′,5-Tetra-O-acetyl-7-O-benzylquercetin (99)
[3,5-diacetoxy-7-benzyloxy-2-(3,4-diacetoxyphenyl)-4H-1-benzopyran-4-one][103]

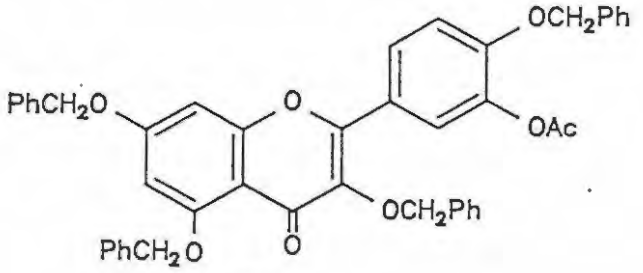


(99)

3,3′,4′,5,7-Penta-*O*-acetylquercetin (96) (10.0 g, 0.02 mol), KI (1.0 g, 0.001 mol), anhydrous K_2CO_3 (25 g, 0.2 mol), and freshly distilled benzyl chloride (10.0 ml, 0.08% mol) were added to dry acetone (250 ml). The mixture was boiled under reflux for 21 h. Undissolved solids were then filtered off and washed with fresh dry acetone. The combined filtrate and washings were concentrated under reduced pressure to give an oil which was dissolved in warm benzene (100 ml), and hexane (50 ml) was added. On cooling the solution, the crude colourless product crystallised. Recrystallisation of the

crude product from acetone - MeOH gave 3,3′,4′,5-tetra-*O*-acetyl-7-*O*-benzyl-quercetin (99) (6.18 g, 57%), m.p. 162 - 163°C (lit.,[103] 163°C); ν_{max} (KBr) 1775 (CO) and 1640 cm^{-1} (CO); δ_H ($CDCl_3$) 2.25 and 2.35 (12H, 2 x s, 4 x $COCH_3$), 5.22 (2H, s, $PhCH_2$), 6.82 (1H, d, *J* 2Hz, 6-H), 6.92 (1H, d, *J* 2Hz, 8-H), 7.44 (1H, s, 5′-H), 7.52 (5H, s, C_6H_5), and 7.70 (2H, m, 2′ and 6′-H).

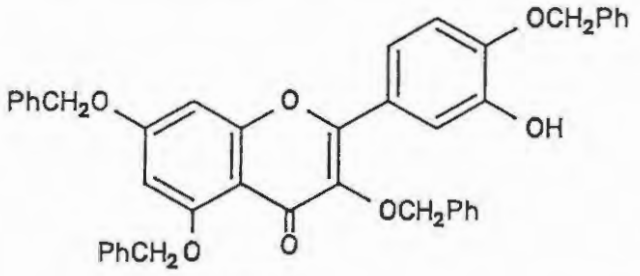
3′-O-Acetyl-3,4′,5,7-tetra-O-benzylquercetin (100) [2-(3-acetoxy-4-benzyloxyphenyl)-3,5,7-tribenzyloxy-4H-1-benzopyran-4-one][103]



(100)

3,3′,4′,5-Tetra-*O*-acetyl-7-*O*-benzylquercetin (99) (2.5 g, 0.004 mol), KI (0.25 g, 0.002 mol), anhydrous K_2CO_3 (10 g, 0.07 mol), and freshly distilled benzyl chloride (5 ml, 0.04 mol) were added to dry 2-butanone (50 ml). The mixture was boiled under reflux for 20 h. Undissolved solids were then filtered off and washed with fresh dry 2-butanone. The combined filtrate and washings were concentrated to an oil which was dissolved in benzene (20 ml). Hexane was added to the cooled solution until crystallisation of the crude product commenced. Recrystallisation of the crude product from acetone - MeOH afforded 3′-*O*-acetyl-3,4′,5,7-tetra-*O*-benzylquercetin (100) (0.95 g, 33.8%), m.p. 175 - 176°C (lit.,[103] 176°); ν_{max} (KBr) 1770 (CO) and 1640 cm^{-1} (CO); δ_H ($CDCl_3$) 2.25 (3H, s, $COCH_3$), 5.26 (8H, m, 4 x $PhCH_2$), 6.50 (2H, 2 x s, 6- and 8-H), 7.38 (21H, m, 4 x C_6H_5 and 5′-H), and 7.78 (2H, s, 2′- and 6′-H).

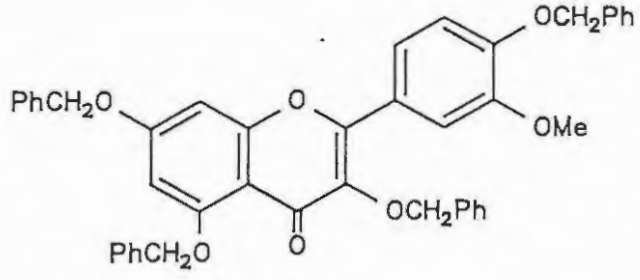
3,4′,5,7-Tetra-O-benzylquercetin (101) [3,5,7-tribenzyloxy -2-(4-benzyloxy-3-hydroxyphenyl)-4H-1-benzopyran-4-one][103]



(101)

3′-*O*-Acetyl-3,4′,5,7-tetra-*O*-benzylquercetin (100) (0.8 g, 1.4 mmol) was dissolved in acetone (5 ml), and MeOH (10 ml) and 10% aqueous NaOH (2 ml, 8 mmol) were added. The solution was heated on a steam-bath for 10 min, and then diluted with H_2O (30 ml) and acidified with conc. HCl. The yellow product which precipitated was filtered off and recrystallised from acetone - MeOH to afford 3,4′,5,7-tetra-*O*-benzyl-quercetin (101) (0.68 g, 73.3%), m.p. 165 - 166°C (lit.,[103] 166.5°C); ν_{max} (KBr) 3300br (OH) and 1630 cm^{-1} (CO); δ_H ($CDCl_3$) 5.15 (8H, m, 4 x $PhCH_2$), 6.55 (2H, m, 6- and 8-H), 7.00 (1H, s, 5′-H), and 7.45 (22H, m, 4 x C_6H_5, 2′- and 6′-H).

3,4′,5,7-Tetra-O-benzyl-3-O-methylquercetin (102) [3,5,7-tribenzyloxy -2-(4-benzyloxy-3-methoxyphenyl)-4H-1-benzopyran-4-one][103]

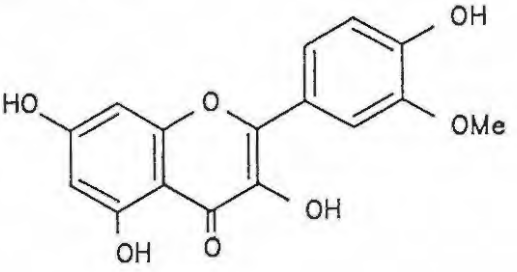


(102)

3,4′,5,7-Tetra-*O*-benzylquercetin (101) (0.4 g, 0.6 mmol), anhydrous K_2CO_3 (1.5 g, 0.01 mol), and methyl iodide (1 ml, 0.015 mol) were added to dry acetone (10 ml), and the mixture was boiled under reflux for 20 h. Undissolved solids were then filtered off and washed with fresh dry acetone, and the combined filtrate and washings were

concentrated to a gum. Water (10 ml) was added, precipitating crude product which was recrystallised from benzene - hexane to yield 3,4′,5,7-tetra-*O*-benzyl-3′-*O*-methylquercetin (**102**) (0.15 g, 36.7%), m.p. 119 - 120°C (lit.,[103] 126 - 127°C); ν_{max} (KBr) 1630 cm^{-1} (CO); δ_H ($CDCl_3$) 3.16 (3H, s, OCH_3), 5.25 (8H, m, 4 x $PhCH_2$), 6.50 (1H, d, *J* 2Hz, 6-H), 6.60 (1H, d, *J* 2Hz, 8-H), 6.85 (1H, s, 5′-H), and 7.42 (22H, m, 4 x C_6H_5, 2′- and 6′-H).

Isorhamnetin (103) [3,5,7-trihydroxy-2-(4-hydroxy-3-methoxyphenyl)-4H-1-benzopyran-4-one][103]



(**103**)

3,4′,5,7-Tetra-*O*-benzyl-4′-*O*-methylquercetin (**102**) (0.14 g, 0.2 mmol) was dissolved in glacial AcOH (5 ml, 0.08 mol), and conc. HCl (2.5 ml) was added. The solution was heated on a steam-bath for 1.5 h, and then cooled and diluted with H_2O (10 ml). The precipitated yellow product was filtered off and recrystallised from acetone - MeOH, affording isorhamnetin (**103**) (0.05 g, 79%), m.p. 303 - 305°C (lit.,[103] 305 - 306°C); ν_{max} (KBr) 3100br (OH) and 1655 cm^{-1} (CO); δ_H (DMSO-d_6) 3.91 (3H, s, OCH_3), 6.23 1H, d, *J* 3Hz, 6-H), 6.42 (1H, d, *J* 3Hz, 8-H), 6.98 (1H, d, *J* 9Hz, 5′-H), 7.10 (4H, br s, OH), and 7.72 (2H, m, 2′- and 6′-H).

3.3 ISOLATION AND CHROMATOGRAPHIC ANALYSIS OF CONSTITUENTS OF *TULBAGHIA VIOLACEA*

The plant material was identified as *Tulbaghia violacea* Harv., by Mrs. E. Brink of the Albany Museum Herbarium, Grahamstown, where voucher specimens are deposited (Vouchers A7418 and A7419).

3.3.1 Aqueous extraction of *Tulbaghia violacea* (Extract I)

Fresh plant material, including green parts, was washed and crushed in a domestic blender with distilled water (100 ml per 30 g plant). The stirred slurry was boiled for 10 min, and then filtered while hot, through celite, to give a light brown filtrate which was freeze-dried. The residual product, extract I, was obtained as a sticky hygroscopic powder, and was chromatographed using various techniques as follows.

(i) T.l.c. [on silica; elution with EtOAc - prop^{i}OH - H_2O (6:2:1), visualised with anisaldehyde - H_2SO_4 spray (see Figure 2.10, p. 68)].

(ii) Two-dimensional (2-d)-t.l.c. [on cellulose; elution with Bu^{t}OH - AcOH - H_2O (3:1:1) and 5% AcOH consecutively; visualised with iodine (see Figure 2.11, p. 69)].

(iii) Gel filtration [with Sephadex LH-20; elution with EtOH - H_2O (3:2), using a peristaltic pump, flow rate 0.2 ml/min; detection by u.v. absorption (see section 2.2.3.1, p. 82)].

The results of this chromatography indicated the presence of at least 3 sugars, and the presence of several glycosidic components.

3.3.2 Hydrolysis of Extract I

Extract I (9.3 g) was added to 10% HCl (170 ml) and the mixture was heated on a steam-bath for 0.5 h, cooled, and then extracted with Et_2O (3 x 80 ml) and EtOAc (3 x 80 ml). The combined organic extracts were dried, concentrated under reduced pressure, and

chromatographed [p.l.c. on silica; elution with EtOAc - hexane (3:2)] to give as major components the hexose degradation products :- 5-hydroxymethylfurfural (**115**), δ_H (300 MHz, $CDCl_3$) 2.7 (1H, br s, OH), 4.6 2H, s, CH_2OH), 6.5 (1H, s, CH), 7.2 (1H, s, CH), and 9.5 (1H, s, CHO); δ_C (75 MHz, $CDCl_3$) 55.5 (t, CH_2OH), 109.5 (d, CH), 120.5 (d, CH), and 176.0 (d, CHO); and 4-oxopentanoic acid (**116**) δ_H (300 MHz, $CDCl_3$) 2.15 (3H, s, CH_3CO), 2.53 (2H, t, *J* 5 Hz, CH_2), and 2.70 (2H, t, *J* 5 Hz, CH_2). δ_C (75 MHz, $CDCl_3$) 27.5 (t, CH_2), 29.8 (q, CH_3), 37.5 (t, CH_2), 177.5 (s, COOH) and 208.1 (s, CO).

The aqueous layer remaining after solvent extraction was neutralised with Ag_2CO_3, filtered, and chromatographed using

(i) thin layer chromatography [co-chromatography with standard sugars on silica; elution with BuOH - MeOH - AcOH - H_2O (8:8:1:1) visualised with anisaldehyde - H_2SO_4 spray (see Figure 2.12; A, p. 70)] and

(ii) paper chromatography [Whatman 3 M paper; downward elution overnight with EtOAc - AcOH - HCOOH - H_2O (18:3:1:1); visualised with a), $AgNO_3$ in acetone; b), NaOH in EtOH, c), NaOAc, $Na_2S_2O_3$, AcOH in H_2O (see Table 3.2) (Figure 2.12; B, p. 70)].

The results of this chromatography indicated the presence of glucose, fructose and xylose in the extract I.

3.3.3.1 Hydrolysis of extract I on a t.l.c. plate[105]

An aqueous solution of extract I was spotted in one corner of each of two 65 x 65 mm t.l.c. plates. After the first elution with BuOH - AcOH - H_2O (3:1:1), the plates were dried, examined under u.v. light, and placed in the reaction box[105], face up. A p.l.c. plate, sprayed with 5-M HCl (5 ml), was placed face down over the t.l.c. plates and supported by the rim of the box. The whole box was placed in an oven at *ca.* 100°C for 10 min, after which the box was opened and the t.l.c. plates removed and dried in the oven. Standard compounds were spotted in line with sample spots, above the solvent front, and a

second elution was carried out perpendicular to the direction of the first elution, using BuOH - MeOH - AcOH - H_2O (8:8:1:1) for one plate, and EtOAc - hexane (1:1) for the other. The first plate was visualised with anisaldehyde - H_2SO_4 spray to reveal sugar components (see Figure 2.11, p. 69) and the second plate was examined under u.v. light to detect aglycones (see Figure 2.18, p. 84). The results of this chromatography suggested the presence of the sugars glucose and fructose, and aglycones which correlated with flavone standards.

3.3.3.2 Hydrolysis of fraction V of methanolic extract III on a t.l.c. plate

The procedure for hydrolysis of extract I was followed using the butanol soluble fraction V (Scheme 2.11, p. 66). The resulting chromatograms showed aglycones corresponding to the flavone standards kaempferol and quercetin (see Figure 2.18, p. 84) and sugars corresponding to glucose and fructose (see Figure 2.11, p. 69).

3.3.4 Isolation of free and glycosidic sugars from extract I[2]

Extract I (0.4 g) was dissolved in H_2O (30 ml), and powdered charcoal (1 g) was added. The mixture was stirred for 0.5 h, and then filtered, and the residue was washed with fresh water. The combined filtrate and washings were evaporated to dryness under reduced pressure to give a fraction (FS) (170 mg) containing free sugars. The charcoal residue was added to a hot solution of phenol (3.5 g) in H_2O (47 ml), the stirred mixture was heated to *ca.* 80°C for 10 min before filtering, and the residue was washed with fresh water. The combined filtrate and washings were concentrated under reduced pressure to *ca.* 20 ml and extracted with Et_2O (4 x 20 ml) to remove the phenol. The aqueous layer and fraction (FS) were chromatographed [t.l.c. on silica; elution with BuOH - MeOH - AcOH - H_2O (8:8:1:1); visualisation with anisaldehyde - H_2SO_4 spray; (see Figure 2.13, p. 72)] to confirm the presence of free sugars in fraction (FS), and the absence of free sugars in the aqueous solution. The aqueous layer

was concentrated further, to *ca.* 5 ml, added to 10% HCl (7 ml), and the mixture was then heated on a steam-bath for 0.5 h, cooled, and extracted with EtOAc (4 x 20 ml). The aqueous layer was neutralised with 2-M NH_3 solution and concentrated to *ca.* 5 ml, giving a fraction (GS) containing glycosidic sugars. T.l.c. [as for fraction (FS)] indicated the presence of sugars in the fraction (GS) (Figure 2.13, p. 72). Fractions (FS) and (GS) were used for derivatisation procedures. The EtOAc layer was dried, concentrated, and chromatographed [t.l.c. on silica; elution with solvents (7) - (10) (see Figure 2.19, p. 86)] to show the presence of flavone components.

3.3.5 Preparation of peracetylated aldononitriles (PAANs) of sugars[106]

3.3.5.1 Standard mixture of sugar PAANs

A stock solution was prepared containing 50 mg of each of the following sugars : D-glucose, D-mannose, D-galactose, D-xylose, L-arabinose, L-rhamnose and D-fucose, in H_2O (0.5 ml). Hydroxylamine hydrochloride (0.5 g) was dissolved in *N*-methylimidazole (20 ml), and this solution was stored at -4°C. A mixture of the stock sugar solution (0.2 ml) and the hydroxylamine hydrochloride solution (0.4 ml) was heated in a closed tube at 80°C for 10 min, and then cooled. Ac_2O (5 ml) was added while the solution was cooled in ice. After 5 min, $CHCl_3$ (1 ml) and then H_2O (1 ml) were added. The tube was shaken and allowed to stand; the aqueous (upper) layer was discarded and the $CHCl_3$ layer was dried. This $CHCl_3$ solution was used as a standard mixture for g.l.c. analysis of the isolated sugar sample (FS).

3.3.5.2 Individual standard sugar PAANs

The general procedure described for the preparation of the standard mixture of sugar PAANs was followed using, in each case, a standard sugar (from those listed above and including D-fructose) (4 mg) in H_2O (0.2 ml). G.l.c. analysis of each of these samples was used to identify the peaks in the gas chromatogram of the standard mixture of derivatised sugars (Figure 2.14; A, p. 75).

3.3.5.3 Free sugar (FS) PAANs

The general procedure described for the preparation of the standard mixture of sugar PAANs was followed, using a solution of sample (FS) (60 mg) in water (0.2 ml) to give a solution of (FS) PAANs which was analysed by g.l.c. and g.c. - m.s., using the standard sugar PAANs for comparison.

3.3.5.4 Glycosidic sugar (GS) PAANs

The general procedure described for the preparation of the standard mixture of sugar PAANs was followed, using the solution (GS) (1 ml) as the sugar solution, to give a solution of (GS) PAANs which was analysed by g.l.c. and g.c. - m.s., using the standard sugar PAANs for comparison.

3.3.5.5 G.l.c. and g.c. - m.s. conditions for analysis of PAANs

G.l.c. analysis : DB225 column; N_2 carrier gas, flow rate 25 ml/min; isothermal 220°C; inlet purge time 0.5 min; inlet and detector temperatures 230°C; column head pressure 100 kPa.
G.c. - m.s. analysis : DB225 column; He carrier gas, flow rate 1.5 ml/min through column; isothermal 230°C; inlet purge time 1 min; inlet and detector temperatures 240°C.

3.3.6 Preparation of alditol acetate derivatives of sugars[108]

3.3.6.1 Standard mixture of sugar alditol acetates

$NaBH_4$ (0.5 g) was added to a solution containing 40 mg of each of the following sugars : D-glucose, D-galactose, L-arabinose, D-xylose and L-rhamnose, in water (0.4 ml). The mixture was allowed to stand at room temperature for 1 h, and then acidified with 50% AcOH before being evaporated to dryness. MeOH (5 ml) was added, and the mixture was again evaporated to dryness. Treatment with MeOH was repeated twice to remove boron residues. Pyridine (1 ml) and Ac_2O (1 ml) were

added to the residue, and the mixture was maintained at 100°C for 1 h before being poured on to crushed ice (*ca.* 10 g). When the ice had melted, the solution was extracted with $CHCl_3$ (3 x 10 ml). The $CHCl_3$ layer was washed (consecutively with 10% H_2SO_4 solution, saturated $NaHCO_3$ solution, and H_2O), dried, and concentrated to *ca.* 0.2 ml. This solution was used as the standard mixture of sugar alditol acetates (AAs) for g.c. - m.s. analysis of isolated sugar samples (FS) and (GS).

3.3.6.2 Fructose and Mannose alditol acetates

The general procedure described for the preparation of the standard mixture of alditol acetates was followed, using a solution of fructose (40 mg) in H_2O (0.4 ml), and a solution of mannose (40 mg) in H_2O (0.4 ml). The resulting derivatives were used as g.c. - m.s. standards to confirm the presence of fructose in the isolated sugar samples (FS) and (GS) (see section 2.2.2.5, p. 78).

3.3.6.3 Free sugars

The general procedure described for the preparation of the standard mixture of alditol acetates was followed, using sample (FS) (20 mg) in water (0.4 ml). The derivatised mixture was analysed by g.c. - m.s. using the standard mixture of alditol acetates for comparison (see section 2.2.2.5, p. 78).

3.3.6.4 Glycosidic sugars

The general procedure described for the preparation of the standard mixture of alditol acetates was followed, using the sample (GS) (0.4 ml) as the sugar solution. The derivatised mixture was analysed by g.c. - m.s. using the standard mixture of alditol acetates for comparison (see section 2.2.2.5, p. 78).

3.3.6.5 G.l.c. and g.c. - m.s. conditions for analysis of alditol acetates

G.l.c. analysis : DB225 column; N_2 carrier gas, flow rate 25 ml/min; isothermal 230°C; inlet purge time 0.5 min, inlet and detector temperatures 240°C; column head pressure 100 kPa.
G.c. - m.s. analysis : DB225 column; He carrier gas, flow rate 1.5 ml/min through the column; isothermal 230°C; inlet purge time 1 min; inlet and detector temperatures 240°C.

3.3.7 Soxhlet extraction of *Tulbaghia violacea* (Extracts II - VI) and isolation of sulphur compounds (117) and (118)

Fresh, hand-chopped plant material (excluding green parts) (1.5 kg) was extracted in a Soxhlet apparatus, with hexane (containing 30% xylene and 30% toluene) (5 l) for 2 d (see Scheme 2.11, p. 66). The extract II was concentrated to a small volume (100 ml) and stored at -4°C for 5 d, during which time a white material crystallised out. This solid (0.26 g) was filtered off, dissolved in $CHCl_3$ (1 ml) and chromatographed [p.l.c. on silica; EtOAc - hexane (60:40) as eluant] to give white crystalline *2,4,5,7-tetrathiaoctane-2,2-dioxide* (117) (0.13 g), m.p. 59 - 60°C, (Found C, 22.98; H, 4.60. $C_4H_{10}S_4O_2$ requires C, 22.00; H, 4.62%); ν_{max} (CCl_4) 1325 and 1140 (SO_2), 685 (CH_3-S), 590, and 550 cm^{-1} (SO_2); δ_H ($CDCl_3$) 2.25 (3H, s), 3.05 (3H, s), 4.12 (2H, s), and 4.22 (2H, s); δ_C ($CDCl_3$) 15.47 (CH_3), 39.26 (CH_3), 45.25 (CH_2), and 62.07 (CH_2); *m/z* 218 (M^+, 3%), 93 (10), 61 (100), and 45 (71). (See section 2.4.1).

The residual plant material in the Soxhlet apparatus was then extracted with MeOH (5 l) for 2 d. A colourless precipitate (VII) was filtered from the MeOH extract (III) and air dried. This material gave a positive Molisch test and was found to char and effervesce when treated with conc. H_2SO_4, indicating a carbohydrate. Hydrolysis of the solid (0.1 g) was effected in dilute HCl (3 ml) by heating on a steam-bath for 15 minutes. Neutralisation of the solution with 2M-aq.NH_3 and subsequent concentration to small volume, afforded glucose, identified by chromatography [t.l.c. on silica; elution with BuOH - MeOH - AcOH - H_2O (8:8:1:1); visualised with anisaldehyde - H_2SO_4 spray reagent] using glucose, fructose, rhamnose and xylose as standards.

The filtered MeOH extract (III) was concentrated under reduced pressure to a syrup (300 ml) which was then dissolved in water (300 ml). A small portion (30 ml) of this aqueous solution was hydrolysed (see next section, p.163), and the remainder was extracted with EtOAc, and then n-BuOH, giving fractions IV and V respectively.

The dried EtOAc extract (IV) was concentrated under reduced pressure to small volume (2 ml) and chromatographed [p.l.c. on silica, elution with $CHCl_3$] to yield 2,4,5,7-tetrathiaoctane (118) as a pale yellow liquid (100 mg), ν_{max} (CCl_4) 680 (CH_3-S) and 480 cm^{-1} (S-S); δ^H ($CDCl_3$) 2.23 (3H, s) and 3.40 (2H, s); *m/z* 186 (M^+, 14%), 93 (2) and 61 (100) (see section 2.4.1).

The BuOH-soluble fraction was evaporated to dryness, giving a sticky yellow powder, fraction V (1.7 g). Chromatography of fraction V was carried out as follows :

(i) Flash chromatography [on silica; sequential elution with $CHCl_3$ - MeOH - H_2O (78:20:2), (70:30:10), (65:35:10)] giving 6 crude fractions as shown in Table 3.3 below; (see section 2.2.3.1, p. 82).

(ii) T.l.c. [on cellulose; elution with Bu^tOH - AcOH - H_2O (3:1:1) and 5% AcOH; visualisation with I_2] (see section 2.2.3.1, p. 82).

(iii) 2-D-t.l.c. [on silica with hydrolysis after first elution with Bu^tOH - AcOH - H_2O (3:1:1); second elution with BuOH - MeOH - AcOH - H_2O (8:8:1:1) for sugars, and EtOAc - hexane (1:1) for aglycones; visualisation with anisaldehyde - H_2SO_4 spray for sugars] (see sections 2.2.2.1 and 2.2.3.1, pp. 67 and 82).

The results of this chromatography showed the presence of glycosidic material in fraction V, comprising aglycones corresponding to the flavone standards, kaempferol and quercetin, and sugars corresponding to glucose and possibly xylose. Fructose appeared to be present as a free sugar (Figure 2.11; C) (see section 2.2.3.3, p. 89).

Table 3.3 Fractions from flash chromatography of extract V

R_f*	Mass of fraction (mg)
0.59	20
0.38	18
0.28	13
0.22	24
0.15	40
0.08	13

* in solvent system : $CHCl_3$ - MeOH - H_2O (78:20:2)

3.3.8 Hydrolysis of methanolic extract III

A portion of the aqueous solution of methanolic extract III (see Scheme 2.11, p. 66) (30 ml) was added to a 10% methanolic solution of HCl (30 ml), and the mixture was heated on a steam-bath for 0.5 h. The solution was concentrated to a small volume (20 ml) and extracted with EtOAc (3 x 20 ml). Concentration of the dried EtOAc layer gave a mixture which was chromatographed using t.l.c. [co-chromatography on silicon with standard flavones; elution with solvents (7) - (10) (see Figure 2.19, p. 86)], to show components correlating with flavones (see section 2.2.3.2).

3.3.9 Hydrolysis of BuOH extract V

A solution of extract V (Scheme 2.11, p. 66) (60 mg) in MeOH (3 ml) and 5M - HCl (2 ml) was heated on a steam-bath for 2 h. The solution was evaporated to dryness, and H_2O (2 ml) was added. The aqueous solution was extracted with $CHCl_3$. Concentration of the dried $CHCl_3$ extract gave a mixture which was chromatographed using

(i) t.l.c. [co-chromatography on silica with flavone standards; solvent systems (7) - (10) (see Figure 2.19, p. 86)].

(ii) 2-d t.l.c. [on silica; elution with each of solvents (7) - (10) in both directions (see Figure 2.20, p. 90)].

(iii) t.l.c. [on silica; elution with EtOAc - hexane (7:3); visualised with vanillin - H_3PO_4 and cinnamaldehyde - Ac_2O - H_2SO_4 reagent (see Table 3.2) (section 2.2.6.1, p. 117)].

The results of the t.l.c. analyses (i) and (ii) showed the presence of the flavonols, kaempferol and quercetin, as aglycones, and the analysis (iii) indicated the possible presence of steroidal sapogenins in the aglycone mixture (see section 2.2.6.1, p. 117).

3.3.10 Extraction of volatiles from *Tulbaghia violacea*

Fresh plant material (aerial parts) was rapidly frozen using liquid N_2, ground in a pestle and mortar while frozen, then allowed to return to room temperature, and extracted by vaccum distillation at 10mm Hg and *ca.* 40°C for 2 h. The distillate, collected in a cold trap, was extracted with Et_2O; the Et_2O extract was dried and concentrated under reduced pressure at *ca.* 10°C, to small volume. The resulting sample was analysed by g.l.c. [on HP-1 column; carrier gas N_2 total flow rate 15 ml/min; temperature programme 70 - 200°C at 8°/min; inlet purge time 1 min; inlet and detector temperatures 210°C] (see section 2.2.5.1) and g.c. - m.s. [(i) on HP-1 column; carrier gas He, flow rate through column 1.5 ml/min; column head pressure 62 kPa; temperature programme 60 - 180°C at 6°/min; inlet purge time 3 min; inlet and detector temperatures 200°C; (ii) on HP-20 M (Carbowax 20 M) column; carrier gas He, flow rate through column 1 ml/min; column head pressure 62 kPa; temperature programme 65 - 210°C at 2°/min, inlet and detector temperatures 200°C] (see section 2.2.5.1).

3.3.11 Extraction of volatiles from *Allium cepa* and *Allium sativum*

The procedure for extraction of volatiles from *Tulbaghia violacea* was followed, using fresh onion bulbs and fresh garlic bulbs (both obtained locally). The extracts were analysed by g.l.c. and g.c. - m.s. as described previously.

3.3.12 Miscellaneous constituents

3.3.12.1 Examination of *Tulbaghia violacea* for presence of saponins

Method 1[117]

A solution of extract I (scheme 2.11, p. 66) (1 g) in MeOH (20 ml) and 0.1 M-H_2SO_4 (5 ml) was heated on a steam-bath for 1 h. H_2O (20 ml) was added, and the solution was concentrated under reduced pressure before extracting with $CHCl_3$. The organic layer was washed consecutively with saturated $NaHCO_3$ solution and saturated NaCl solution, dried, and evaporated to dryness. The resulting sample was analysed by t.l.c. [on silica; elution with benzene - acetone (4:1); visualised with spray reagents :- (i) cinnamaldehyde - Ac_2O - H_2SO_4 reagent (4); (ii) vanillin - H_3PO_4 reagents (3); and (iii) $SbCl_3$ reagent (5) (See Table 3.2)].

Method 2[15]

A solution of extract I (200 mg) in 2M-methanolic HCl (35 ml) was boiled under reflux for 2 h and then filtered. The solid residue was dissolved in 10% aqueous NH_3 and the solution was evaporated to dryness, at 60°C. Extraction with $CHCl_3$ gave a solution which was dried, concentrated, and chromatographed following the procedures in method 1.

Method 3

The sample of aglycones obtained by hydrolysis of the butanol-soluble fraction V (section 3.2.9, p. 163) was chromatographed as described in Method 1.

The results of this analysis (shown in Figure 2.29, p. 118) indicate the presence of saponins in *Tulbaghia violacea*.

3.3.12.2 Examination of *Tulbaghia violacea* for alkaloids[118]

A mixture of extract I (Scheme 2.11, p. 66) (2 g) in EtOH (30 ml) was boiled under reflux for 2 h, filtered, and the filtrate was evaporated to dryness. The remaining material was dissolved in 5%

AcOH and the solution was washed with Et_2O before being basified with 10% Na_2CO_3 solution. Extraction of the alkaline solution with $CHCl_3$ gave a solution which was dried, concentrated, and chromatographed [t.l.c. on silica; elution with $CHCl_3$ - MeOH (9:1); visualised with :- (i) Dragendorff's reagent (6); (ii) iodoplatinate reagent (7) (see Table 2.3)] but the results were inconclusive (see section 2.6.2, p. 117).

3.3.12.3 Examination of *Tulbaghia violacea* for anthraquinones

(a) Borntrager test[13]

A solution of extract I (Scheme 2.11, p. 66) (100 mg) in 5% aqueous KOH solution was boiled for 5 min, cooled, acidified with 5M-HCl, and extracted with benzene (5 ml). The benzene layer was shaken with 2M-NaOH solution. The lack of red colouration in the alkaline phase indicated the absence of anthraquinones. [A control test was carried out using emodin (1 mg), and the alkaline phase was observed to turn red in this case.]

(b) T.l.c. analysis

The hydrolysed sample prepared for analysis of saponins (see section 3.2.12.1, p. 165) was chromatographed [on silica; elution with benzene - ethyl formate - formic acid (75:24:1); visualised with :- (i) magnesium acetate reagent (8)[104]; and (ii) KOH - EtOH reagent (9) (see Table 3.2)] using emodin as a standard. No components in the sample showed red or purple spots (while emodin gave a red spot in both cases) and these results were taken to be negative.

3.4 BIOLOGICAL ACTIVITY OF *TULBAGHIA VIOLACEA* EXTRACTS

3.4.1 Examination of effects of *Tulbaghia violacea* extracts on bacterial cultures

Aqueous extractions of *Tulbaghia violacea* were carried out (using 50 g fresh plant material and 250 ml H_2O in each case) as follows :-

Extract I_1 : Roots and white aerial parts were ground in a blender with water, allowed to stand for 1 h, and then filtered.

Extract I_2 : Roots and white aerial parts were ground in a blender with water, the mixture was boiled for 10 min, cooled and filtered.

Extract I_3 : Green leaves were ground in a blender with water, the mixture was allowed to stand for 1 h, and then filtered.

Extract I_4 : A sample of extract I_1 was stored at -4°C for *ca.* 3 weeks.

Dilutions of 1:4, 1:16, and 1:64 were made for each extract. Nutrient agar solution (30 ml per petri dish) was seeded with cultures of *B. subtilis*, *E. coli*, *S. aureus*, and *S. marcesens* and wells were punched in each quarter of the agar plates. The plant extracts (and diluted samples) were added to the wells as indicated in Table 2.9 (p. 122) and the cultures were maintained at 37°C for 24 h. Bacteriostatic action was assessed by measurement of the diameter of the circular regions of inhibited growth in the agar plates (see photograph, p. 121). The results of this investigation (listed in Tables 2.9 and 2.10) show definite bacteriostatic activity, particularly in the case of fresh extracts of mature plants.

3.4.2. Isolated organ study on effect of *Tulbaghia violacea* extracts

A portion of cleaned rat small intestine, *ca.* 2 cm, was mounted in a 50 ml organ-bath, (see Figure 3.1) in Tyrode's solution (see Table 3.4) and maintained at 37°C. Contractions were recorded *via* a thread tying the muscle preparation to a transducer connected to a recorder.

Figure 3.1 Organ-bath as used for isolated organ study

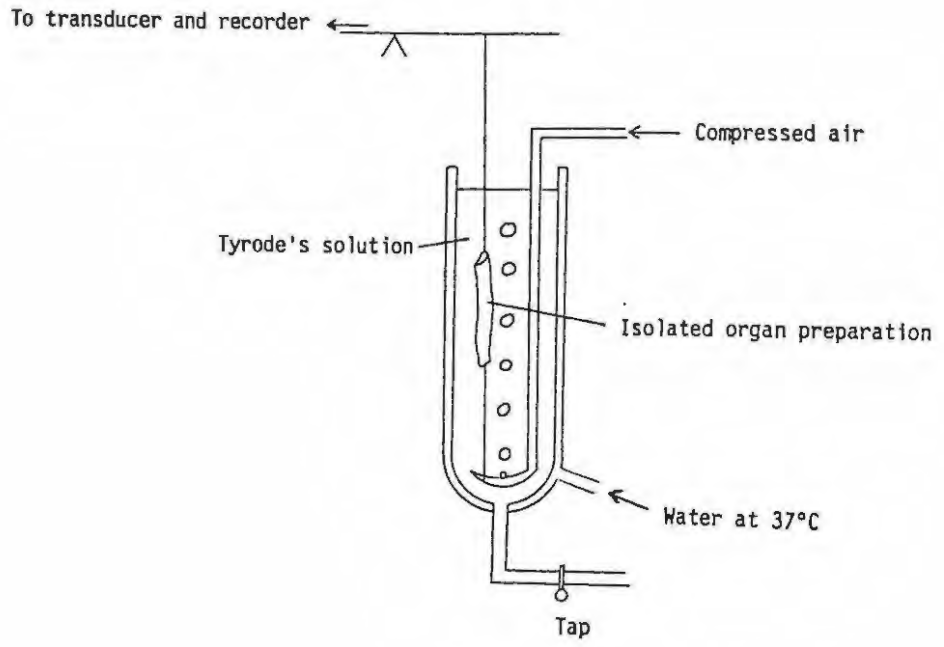


Table 3.4 Tyrode's solution[120]

Constituent	Mass required per litre of solution (g)
NaCl	8.00
KCl	0.20
$MgSO_4.7H_2O$	0.26
$NaH_2PO_4.2H_2O$	0.26
$NaHCO_3$	1.00
$CaCl_2$	0.36
Glucose	1.00

Single doses were recorded [using 10^{-4} M-acetylcholine (ACh) (0.1 ml) and the pretreatment solutions (i) extracts I_2 (see section 3.2.13.1) (0.5 ml) and (ii) 10^{-3} M-propranolol (0.5 ml)] as shown in Figure 2.31 (p. 124). The final concentrations obtained in the organ-bath are indicated in Table 3.5. Dose-response curves were obtained by adding increasing amounts of ACh to the organ-bath in the order indicated, to give concentrations in the organ-bath as shown, in Table 3.5.

Table 3.5 Doses of ACh for dose-response curves

Concentration of ACh solution ($mol.dm^{-3}$)	Volume ACh solution added (ml)	Final concentration in organ-bath ($mol.dm^{-3}$)	Log (concentration)
1×10^{-7}	0.1	3×10^{-10}	-9.5
1×10^{-7}	0.5	1×10^{-9}	-9.0
1×10^{-6}	0.1	3×10^{-9}	-8.5
1×10^{-6}	0.5	1×10^{-8}	-8.0
	etc		

Graphs plotted of response *vs* log (concentration ACh) are shown in Figure 2.32 (p. 126). Dose-response curves were obtained using

(i) ACh only to give the normal dose-response curve;

(ii) pretreatment with plant extract I_2 (0.5 ml), 5 min pre-treatment time, and additions of ACh as before; and

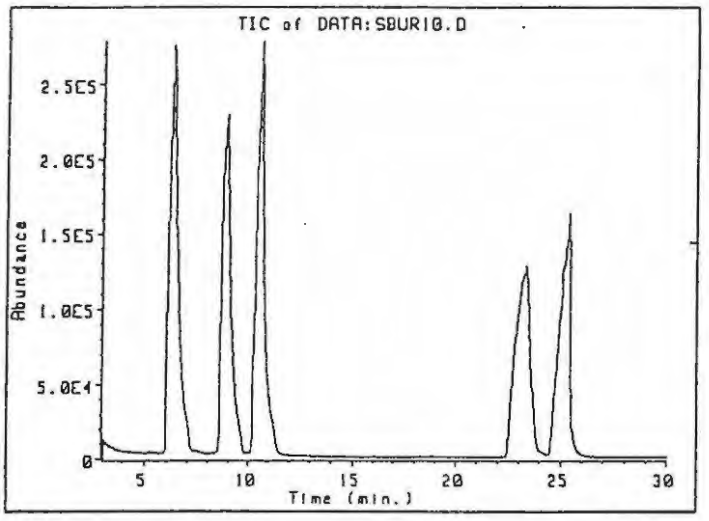
(iii) pretreatment with 10^{-3} M-propranolol solution (0.5 ml), 5 minutes pretreatment, treatment with extract I_2 (0.5 ml), 5 minutes further pretreatment, and additions of ACh as before.

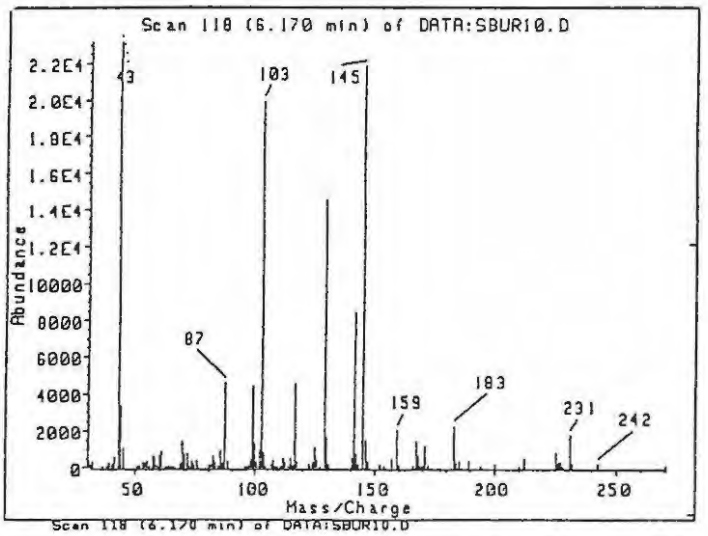
The experiment was repeated on four occasions using fresh muscle preparations, and the graphs represent the mean of the four sets of results. The study shows that the plant extract reduces the effect of ACh on smooth muscle, and that this action is prevented by propranolol.

APPENDIX

(1) G.C. - m.s. data - Sugars

(i) Standard sugar PAANs

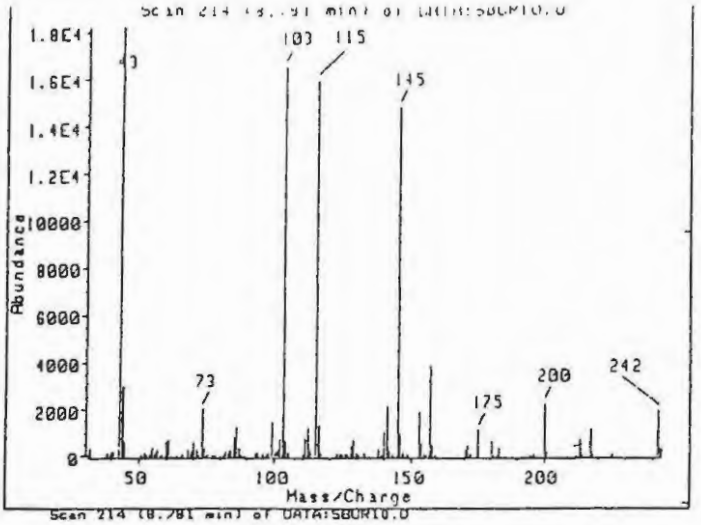




L-Rhamnose PAAN derivative

Scan 118 (6.170 min) of DATA:SBUR10.D

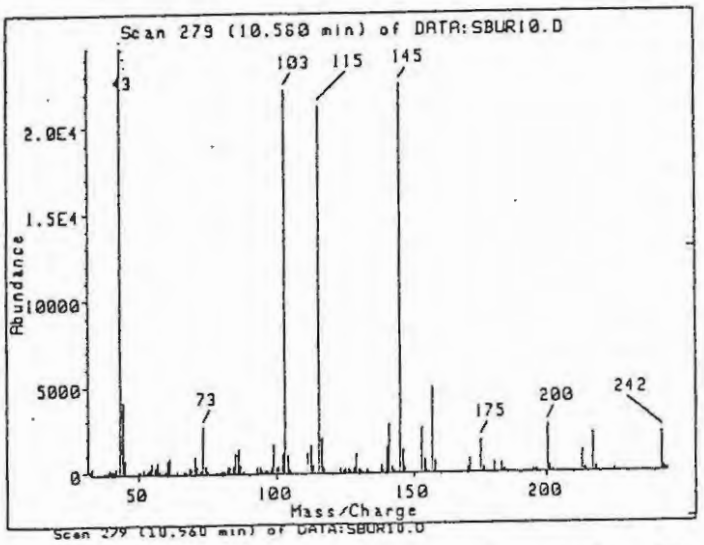
m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
31.00	125	70.00	799	109.00	68	154.00	66
32.10	339	71.10	796	110.00	56	157.10	480
35.95	52	72.10	107	111.10	78	159.10	2010
38.05	86	73.00	343	112.00	502	160.10	161
38.95	278	74.00	176	113.10	299	166.10	133
40.05	193	75.10	389	114.10	75	167.10	1449
41.05	595	79.00	50	115.10	541	168.05	643
42.95	151360	79.95	208	116.10	160	169.05	54
43.95	3359	80.95	220	117.10	4550	170.15	124
45.05	1078	82.05	645	118.00	285	171.05	1161
51.05	64	83.05	318	123.00	205	172.15	131
52.05	156	84.05	146	124.05	301	183.15	2224
53.05	292	84.95	921	125.05	1117	184.15	260
54.05	266	86.05	232	126.05	404	185.15	365
55.05	359	86.95	4600	127.15	76	189.15	366
56.05	140	88.05	348	129.05	14574	194.10	99
57.05	631	96.05	72	130.05	1605	200.10	223
58.00	613	97.05	96	131.05	185	210.20	95
59.10	126	98.15	413	139.35	41	212.05	498
60.00	682	99.05	4473	140.15	529	225.15	841
61.00	878	99.95	1358	141.05	8418	226.15	252
62.10	48	101.05	298	142.05	787	227.15	292
63.00	57	102.00	925	143.05	185	228.15	56
64.10	72	103.00	19944	145.05	21872	231.15	1724
65.10	102	104.00	842	146.00	1513	232.15	178
66.00	95	105.00	158	147.10	289	242.20	177
67.90	249	107.00	198	152.10	215	270.15	97
69.00	1480	108.00	443				



L-Arabinose PAAN derivative

Scan 214 (8.781 min) of DATA:SBUR10.D

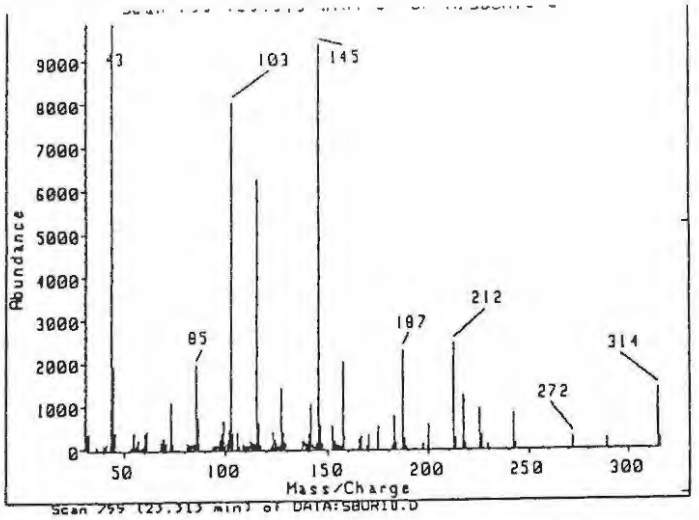
m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
31.20	82	73.00	2058	110.10	62	153.10	1901
32.00	342	74.00	319	111.00	793	154.10	560
32.95	83	75.10	59	112.00	1236	155.10	94
38.95	169	77.10	53	113.10	248	156.10	69
40.05	207	78.10	51	115.10	15932	157.10	3860
40.95	212	79.95	25	116.10	1328	158.10	501
42.95	133568	80.95	77	117.10	174	159.20	72
44.05	2984	82.05	262	123.10	132	170.15	308
45.05	646	83.05	249	124.05	99	171.15	489
51.05	83	84.05	309	124.95	109	172.15	92
52.05	172	89.05	831	126.05	96	175.05	1134
53.05	72	85.95	1255	127.05	117	180.05	691
54.05	105	87.05	313	128.05	450	181.05	63
55.05	399	87.95	64	129.05	690	182.05	36
56.05	142	89.05	40	130.05	147	183.15	362
57.05	292	93.05	157	131.05	84	195.10	56
58.00	58	93.95	172	133.05	147	196.20	101
59.00	49	96.05	92	138.05	346	200.10	2285
60.00	654	97.05	79	139.25	55	201.10	183
61.00	681	98.05	174	140.05	1047	212.15	71
62.10	31	99.05	1479	141.05	2152	213.15	727
64.10	36	100.05	220	142.05	254	214.15	80
66.00	103	101.05	306	143.05	83	217.15	1201
68.00	286	102.00	745	145.15	14821	218.05	126
69.00	230	103.00	16552	146.10	1010	225.05	120
70.00	587	104.00	708	147.10	151	242.10	1982
71.00	273	105.00	153	149.00	73	243.10	325
72.10	79						



D-Xylose PAAN derivative

Scan 279 (10.560 min) of DATA:SBUR10.D

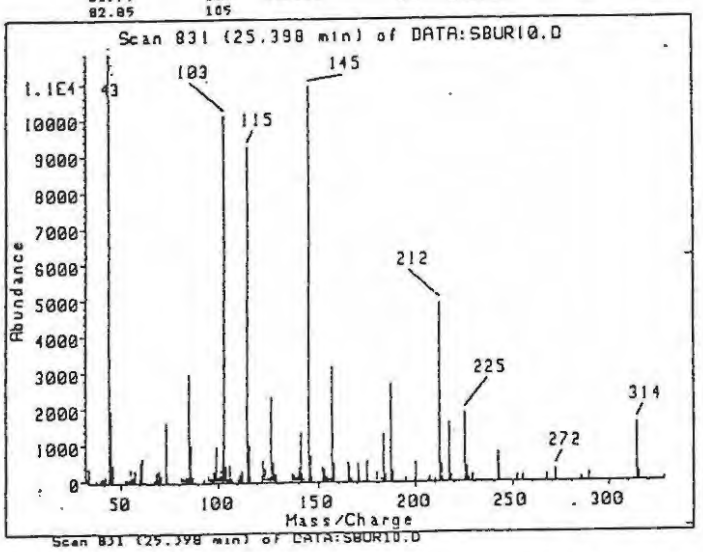
m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
31.10	120	70.00	858	102.00	1065	146.00	1249
32.00	295	71.10	313	103.00	22176	147.10	225
32.05	34	72.00	81	104.00	949	153.10	2513
32.95	79	73.00	2656	105.00	174	154.10	695
38.95	182	74.00	301	111.10	1095	155.10	114
39.95	152	75.00	89	112.00	1526	157.10	4885
41.15	258	77.10	36	113.00	400	158.10	570
43.05	153008	79.20	32	115.10	21264	170.05	260
43.95	4059	79.30	38	116.10	1936	171.05	710
44.95	713	79.95	69	117.10	220	175.15	1816
50.05	70	80.95	96	123.10	197	176.05	102
50.95	103	82.05	317	124.05	88	180.05	540
51.95	185	82.95	299	125.05	191	183.05	483
53.15	85	84.05	395	126.05	196	184.25	56
54.05	180	85.05	1085	127.05	116	195.10	65
55.05	495	86.05	1305	128.05	241	196.10	102
56.05	267	87.05	401	129.05	985	200.10	2643
57.05	587	87.95	89	130.05	162	201.10	258
57.80	57	93.05	246	131.05	76	202.10	41
59.10	68	93.95	248	133.05	157	213.15	1124
60.00	706	94.95	78	138.05	386	214.15	197
61.00	806	95.95	70	139.15	63	217.15	2191
63.90	56	97.05	83	140.05	1396	218.15	214
66.00	111	98.05	274	141.15	2704	225.15	117
67.00	73	99.05	1550	142.15	292	242.20	2194
68.00	273	99.95	259	143.15	81	243.20	236
69.00	205	101.05	335	145.05	22504	244.20	56



D-Glucose PAAN derivative

Scan 799 (23.313 min) of DATA:SBUR10.D

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
31.10	84	84.05	144	122.00	101	165.10	237
32.00	344	85.05	1936	123.10	373	166.20	280
36.05	54	86.05	687	124.05	198	168.95	93
39.15	84	87.05	125	124.95	96	170.05	297
39.95	90	93.95	48	126.05	111	175.05	502
41.05	143	95.05	95	127.05	1406	182.05	95
43.05	68208	96.05	76	128.05	384	183.05	240
43.95	1930	97.05	242	129.05	134	184.15	121
44.95	367	98.05	349	132.05	187	187.15	2274
53.15	39	99.05	647	138.05	180	188.15	227
53.95	54	100.15	108	139.05	124	189.15	40
55.05	368	101.05	192	140.15	343	197.10	117
55.95	92	102.00	426	141.15	1024	200.10	539
56.95	200	103.00	7993	142.15	109	212.15	2431
58.00	42	104.00	349	143.15	62	213.15	242
59.10	43	106.00	363	144.05	125	217.15	1218
60.00	361	107.00	59	145.05	9375	218.15	111
61.00	392	109.00	99	146.10	549	225.15	923
68.10	157	110.00	75	147.10	102	226.15	297
69.00	265	111.10	89	152.10	514	229.15	85
70.00	242	112.00	196	153.10	187	242.20	794
71.00	128	112.90	166	154.10	101	243.10	110
73.00	1065	114.10	137	155.10	79	272.15	245
74.10	115	115.10	6242	156.10	62	289.20	216
80.95	121	116.10	592	157.10	2010	314.15	1357
81.95	120	117.10	77	158.10	294	315.25	209
82.85	105						

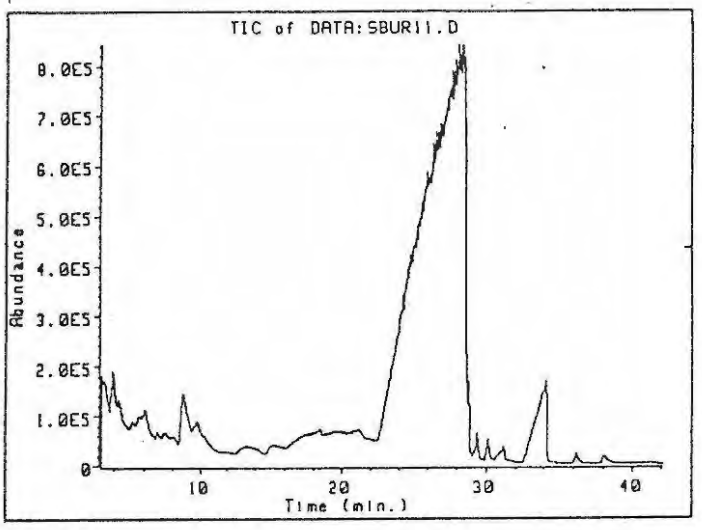


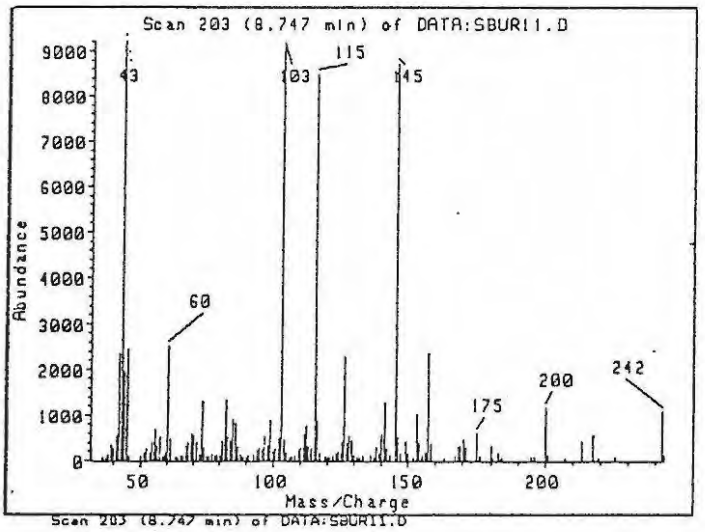
D-Galactose PAAN derivative

Scan 831 (25.398 min) of DATA:SBUR10.D

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
31.10	51	85.95	1020	128.05	512	183.15	1310
32.10	364	87.05	132	129.05	194	184.15	188
35.95	49	92.95	67	129.85	30	187.15	2695
37.95	40	95.05	163	137.05	214	188.15	278
39.05	108	96.15	111	138.05	195	195.10	76
41.05	172	97.05	233	139.05	122	199.10	43
42.95	81848	97.95	277	140.15	361	200.10	504
44.05	1963	98.95	945	141.05	1324	207.20	90
45.05	462	100.05	140	142.05	202	210.20	38
52.05	68	101.05	323	143.05	80	212.05	4904
53.05	80	102.00	516	145.15	10922	213.15	449
54.05	50	103.00	10102	146.10	677	214.05	82
55.05	340	104.00	429	147.00	93	217.15	1604
55.95	140	104.90	77	152.10	382	218.05	171
57.05	300	106.00	443	153.10	334	225.15	1873
60.00	520	107.10	59	154.10	125	226.15	385
61.00	640	110.00	60	155.00	80	227.15	77
67.10	41	111.00	177	156.10	89	229.15	162
68.00	255	112.00	306	156.30	92	242.10	766
69.00	276	113.00	211	157.10	3152	243.10	118
70.00	301	115.10	9215	158.10	468	252.20	127
71.00	198	116.10	998	165.10	499	255.20	142
73.00	1639	117.00	108	166.10	389	267.15	145
74.00	169	122.10	169	167.10	65	272.15	304
81.05	127	123.00	584	170.05	491	285.10	96
82.05	110	124.05	327	171.15	78	289.20	175
83.05	138	125.05	128	175.05	930	314.15	1566
84.05	156	126.05	97	180.05	244	315.25	229
85.05	2969	127.05	2344	182.15	88	328.20	49

(ii) Free sugar PAANs

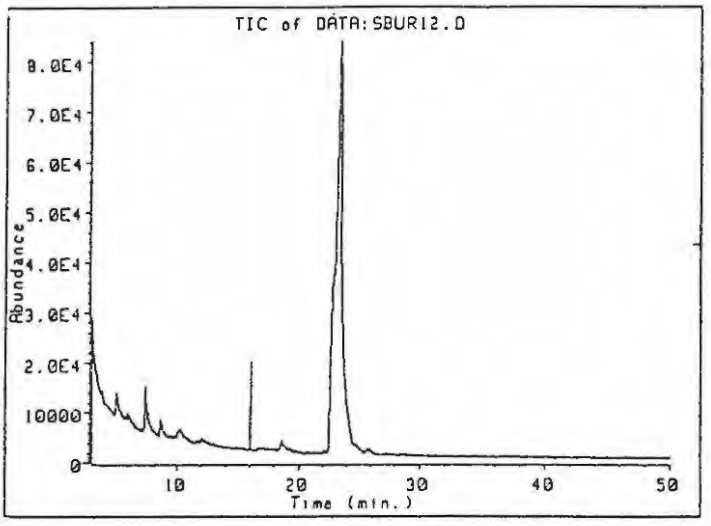


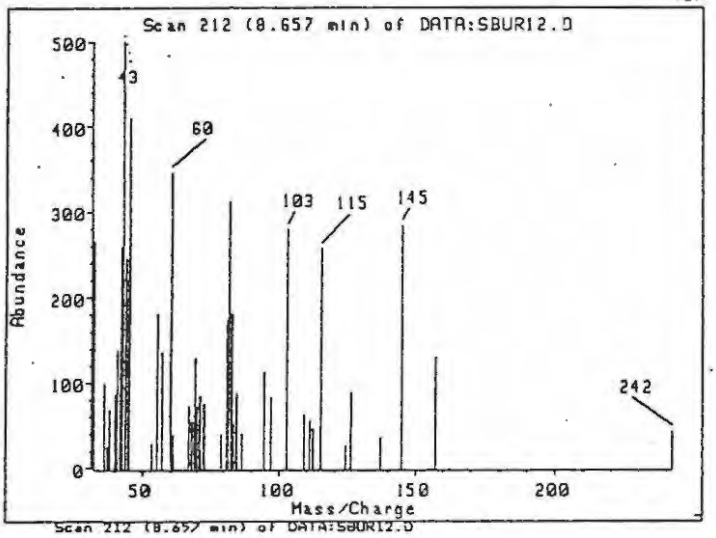


Scan 203 (8.747 min) of DATA:SBUR11.D

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
32.00	224	73.00	1296	108.10	101	146.10	517
35.95	71	74.00	269	109.10	234	147.10	136
37.15	38	75.00	83	110.10	308	149.00	419
37.95	132	76.00	77	111.10	586	150.10	147
39.05	339	77.00	141	112.00	758	152.00	72
40.05	282	78.10	104	113.00	323	153.10	1016
41.05	549	79.05	104	114.10	276	154.10	399
42.05	2340	80.05	115	115.10	8490	155.00	102
43.05	66408	80.95	431	116.10	889	156.20	189
44.05	1950	82.05	1329	117.20	150	157.10	2337
44.95	2432	82.95	498	119.10	76	158.10	364
46.05	77	84.05	435	120.00	74	163.10	43
49.55	30	85.05	907	121.00	55	167.05	113
50.05	84	86.05	818	122.05	105	168.05	311
50.95	146	87.05	308	123.05	151	169.05	313
52.05	242	88.05	101	124.05	208	170.05	465
53.05	159	89.05	72	125.05	407	171.15	279
54.05	383	90.05	68	126.05	2272	175.15	588
55.05	669	91.05	108	127.05	397	176.05	88
56.05	312	93.05	145	128.05	525	180.05	325
57.00	502	94.05	227	129.05	425	181.05	32
58.10	91	95.05	265	130.05	120	183.15	150
59.10	172	96.05	255	131.05	57	184.25	39
60.00	2501	97.05	534	132.15	52	195.00	67
61.10	461	98.05	345	133.05	79	196.10	58
63.00	86	99.05	879	136.05	112	200.10	1161
64.00	53	100.05	208	137.15	72	201.10	118
65.10	101	101.00	272	138.05	290	207.10	40
66.00	119	102.00	474	139.05	155	213.15	408
67.00	313	103.00	9066	140.15	584	217.15	564
68.00	406	104.10	497	141.15	1281	219.05	29
69.00	580	105.00	175	142.15	248	225.15	63
70.00	563	106.10	66	143.15	121	242.20	1062
71.10	384	107.00	84	145.00	8715	243.10	118
72.10	145						

(iii) Glycosidic sugar PAANs

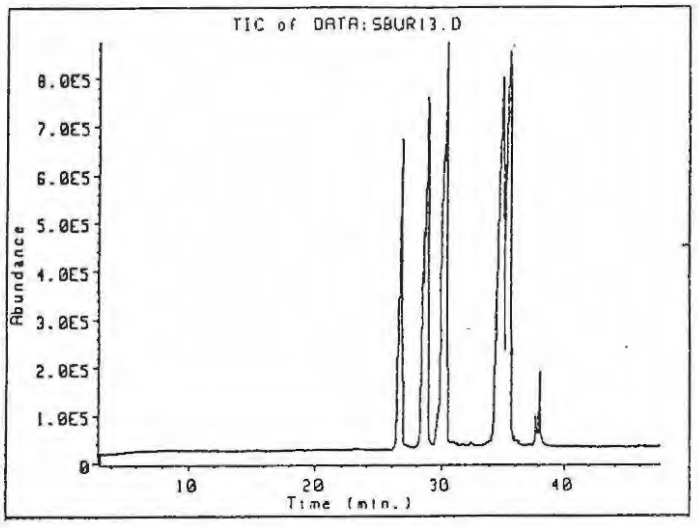


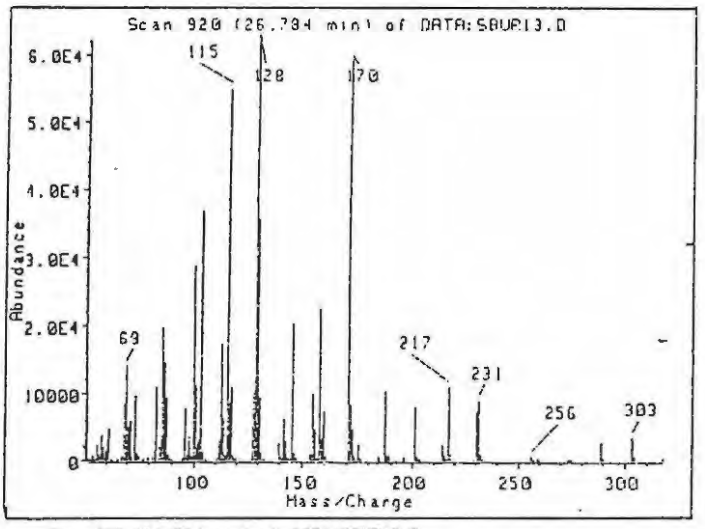


Scan 212 (8.657 min) of DATA:SBUR12.D

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
32.00	265	55.05	180	79.15	40	109.10	63
35.95	99	57.00	136	81.05	174	111.10	55
37.25	25	60.00	346	82.05	312	112.10	46
37.95	69	60.90	39	83.05	180	115.10	257
40.05	87	67.00	73	84.05	53	124.15	27
41.05	138	68.10	55	85.05	88	126.05	89
42.05	259	69.10	130	87.15	40	137.15	35
42.95	3662	70.20	73	95.05	112	145.10	284
43.95	246	71.10	85	97.15	82	157.10	130
45.05	410	73.00	75	103.00	280	242.10	43
53.15	29						

(iv) Standard sugar alditol acetates (AAs)

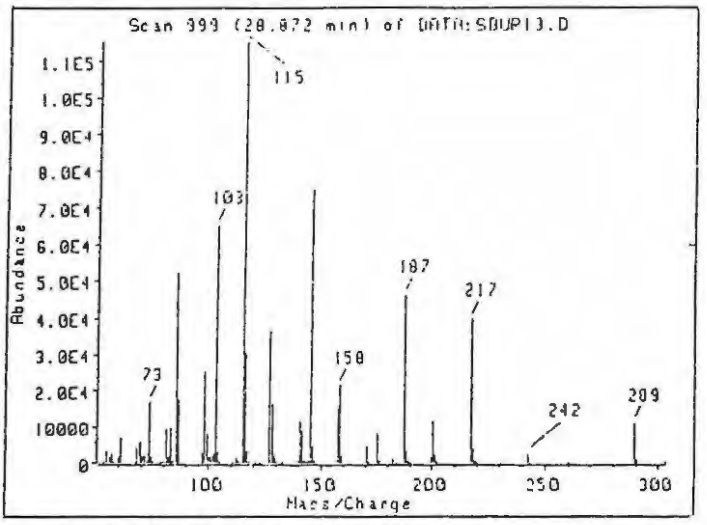




L-Rhamnose AA derivative

Scan 920 (26.784 min) of DATA:SBUR13.D

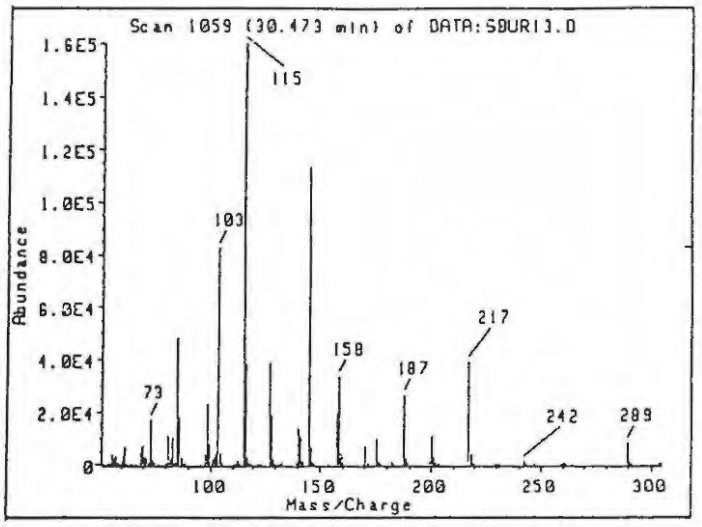
m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
51.05	196	85.05	19784	126.15	289	185.15	108
52.05	259	86.05	14953	127.05	10133	187.15	10304
53.05	832	87.05	9398	128.05	62064	188.20	880
54.05	270	88.15	865	129.05	35616	189.20	709
55.05	2379	89.05	172	130.15	9388	196.10	602
56.05	1043	90.05	58	131.15	1379	201.20	8078
57.10	3774	94.05	476	132.15	236	202.20	719
58.10	2168	95.15	7828	133.15	163	203.20	185
59.10	369	96.15	1051	139.15	2783	213.25	176
60.00	1527	97.05	3700	140.15	671	214.15	2423
61.00	4795	98.05	878	141.15	6350	215.25	781
62.10	189	99.05	28752	142.15	2985	216.15	169
63.20	31	100.15	11268	143.15	546	217.15	10947
65.10	223	101.10	1843	144.10	500	218.15	980
66.00	164	102.00	2823	145.10	20272	219.25	134
67.10	758	103.00	36824	146.10	1180	229.05	154
68.10	8552	104.00	1528	147.10	322	230.15	7341
69.10	14114	105.10	199	153.10	1172	231.20	8999
70.10	5238	109.10	67	154.10	10039	232.20	958
71.10	5969	110.10	586	155.10	4691	233.20	191
72.80	695	111.10	2953	156.20	580	256.15	626
73.10	9472	112.10	17320	157.20	22552	257.25	211
74.10	1172	113.10	6306	158.10	3385	259.15	347
75.10	698	114.20	1028	159.10	7449	260.15	78
76.20	47	115.10	55024	160.10	707	273.15	228
77.10	147	116.10	8577	170.15	99144	274.25	103
78.10	92	117.10	11054	171.15	8294	275.20	146
79.05	484	118.10	858	172.15	4681	289.20	2696
80.15	77	119.10	177	173.15	659	290.20	323
81.05	854	120.00	242	174.15	86	303.15	3566
82.05	1658	122.00	105	175.15	2393	304.15	627
82.95	11033	123.05	34	176.15	220	305.15	104
84.05	2205	125.15	140	184.15	744	317.15	302



L-Arabinose AA derivative

Scan 999 (28.872 min) of DATA:SBUR13.D

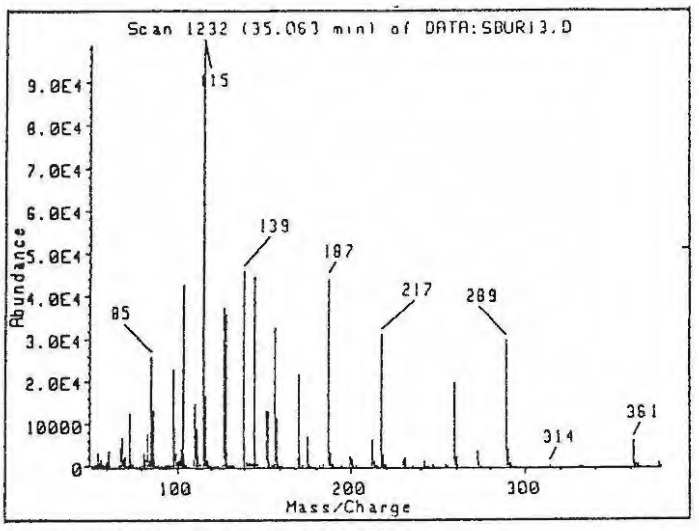
m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
51.15	164	86.05	17568	129.05	2126	183.15	180
52.15	243	86.95	2359	130.05	389	184.15	90
53.15	521	88.05	177	131.05	156	187.15	46120
54.05	522	89.05	152	132.05	162	188.10	3395
55.05	3304	95.15	177	133.15	464	189.10	729
56.05	1829	96.15	119	139.15	901	190.10	57
57.10	2781	97.05	3409	140.15	11612	199.10	1821
58.10	348	98.05	25240	141.15	9002	200.10	11724
59.00	196	99.05	8177	142.15	1019	201.20	2372
60.00	1948	100.15	1770	143.05	344	202.20	308
61.00	7040	101.10	2003	144.10	727	203.10	164
62.10	200	102.10	2823	145.10	74928	207.10	86
63.10	71	103.10	65160	146.10	4806	217.15	39792
67.20	131	104.00	3309	147.10	683	218.15	3904
68.00	4998	109.00	460	148.10	85	219.15	615
69.10	5801	110.10	126	157.10	15351	220.15	33
70.10	2111	111.10	387	158.20	21576	229.15	249
71.10	2162	112.10	1574	159.20	2017	230.15	134
72.10	483	113.10	591	161.10	107	242.20	2437
73.00	16848	115.10	119432	163.10	41	243.20	340
74.10	1845	116.10	30632	169.15	89	244.20	77
75.10	348	117.10	3408	170.15	4763	247.20	114
77.10	78	118.10	504	171.15	545	257.35	39
78.10	111	119.00	121	172.15	67	259.15	260
79.05	411	120.10	199	173.15	81	260.15	440
80.05	631	121.10	98	174.15	44	261.15	257
81.05	9743	122.05	138	175.15	8152	289.20	11224
82.05	1792	123.05	101	176.15	735	290.20	1393
82.95	9869	124.05	89	177.15	150	291.20	222
84.05	1108	127.05	36392	182.15	1229	303.15	363
85.05	92128	128.05	16359				



D-Xylose AA derivative

Scan 1059 (30.473 min) of DATA:SBUR13.D

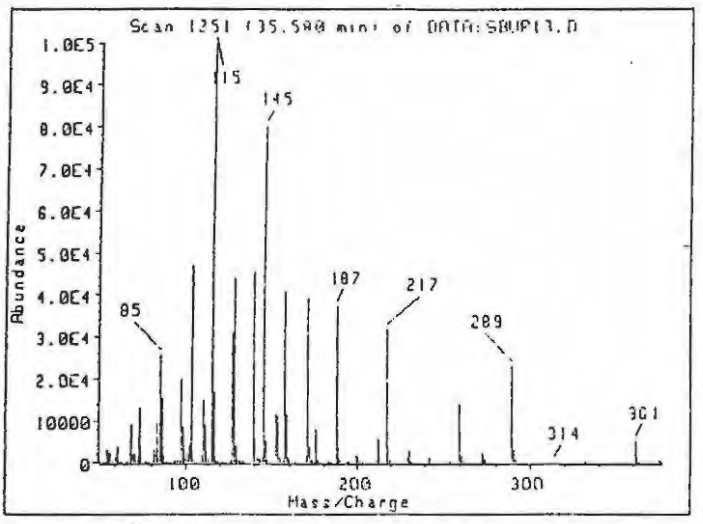
m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
51.05	316	84.05	990	122.05	171	177.15	169
52.05	255	85.05	47936	123.05	86	182.15	1115
53.05	537	86.05	18000	125.05	58	183.15	217
54.15	520	87.05	2384	127.05	38720	187.15	26488
55.05	3647	88.05	214	128.05	18728	188.10	2453
56.05	2242	89.05	166	129.15	2043	189.10	489
57.00	3193	94.15	70	130.15	264	199.10	1300
58.00	572	95.05	130	131.05	127	200.10	10849
59.00	149	96.05	74	132.05	236	201.10	2814
60.10	1837	97.05	4026	133.05	758	202.10	378
61.10	6461	98.15	23296	139.05	1120	203.10	159
62.10	200	99.05	7696	140.15	13936	217.15	39320
64.00	81	100.15	1912	141.15	10431	218.25	3935
65.10	82	101.00	2585	142.15	1313	219.15	686
66.10	47	102.00	4028	143.15	281	219.95	58
67.00	133	103.00	82656	145.10	113192	229.25	222
68.10	4643	104.10	4034	146.10	6652	230.25	145
69.10	6854	105.10	693	147.10	997	231.20	72
70.10	2866	109.10	52	148.10	75	242.20	1345
71.10	2527	110.10	235	153.10	94	243.20	231
72.10	524	111.10	425	156.10	75	247.20	90
73.10	17040	112.10	1980	157.10	19376	259.15	261
74.10	1748	113.10	592	158.10	33720	260.25	387
75.10	390	115.10	160128	159.10	2678	261.15	222
77.10	94	116.10	38464	160.20	457	289.20	8162
79.05	274	117.10	2937	169.15	122	290.20	1147
80.05	611	118.10	488	170.05	6853	291.20	223
81.05	11158	119.00	152	171.05	692	303.15	451
82.05	2030	120.00	262	179.15	9590	304.25	90
82.95	10123	121.10	87	176.05	821		



D-Glucose AA derivative

Scan 1232 (35.063 min) of DATA:SBUR13.D

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
51.05	114	96.05	455	143.15	792	211.15	890
52.05	130	97.05	22944	144.10	910	212.15	6235
53.05	544	98.05	9217	145.10	44576	213.15	1299
54.15	303	99.05	3185	146.10	3378	214.15	195
55.05	3049	100.15	1430	147.10	560	215.15	98
56.05	924	101.00	1714	151.10	126	217.15	31144
57.10	1545	102.00	4179	152.10	13347	218.15	2791
58.00	262	103.00	42584	153.10	13071	219.25	438
59.10	124	104.10	1882	154.10	1175	229.15	1519
60.00	1154	105.10	291	155.10	231	230.25	2160
61.10	3619	107.10	71	156.20	317	231.20	458
62.00	105	109.10	1621	157.10	32760	235.10	44
65.00	177	110.10	14793	158.10	11542	241.10	51
66.10	126	111.10	8682	159.10	1144	242.20	1224
67.00	285	112.10	2469	160.20	125	243.20	193
68.10	4220	113.10	857	161.20	73	244.20	33
69.10	6791	115.10	98832	168.15	93	247.10	293
70.10	1918	116.10	16840	169.15	1781	254.15	588
71.00	2276	117.10	1515	170.15	21680	255.15	60
72.00	395	118.10	234	171.05	3186	259.15	19680
73.10	12427	119.10	122	172.15	327	260.15	2255
74.10	1285	120.00	183	173.15	144	261.15	349
75.10	389	121.10	99	174.15	68	271.25	91
77.10	122	122.00	115	175.15	7102	272.15	3979
79.05	202	123.15	104	176.15	912	273.15	1086
80.05	458	124.15	103	177.15	130	274.25	223
81.05	3374	126.15	414	182.15	291	289.20	29664
82.05	1731	127.05	37456	184.15	680	290.20	4179
82.95	8010	128.05	35864	187.15	44152	291.10	842
84.05	1435	129.05	3264	188.10	3335	292.20	88
85.05	25824	130.15	383	189.10	691	314.25	287
86.05	13359	131.15	250	190.10	64	331.20	100
87.05	1693	132.15	157	194.20	189	332.20	93
88.05	145	133.05	410	195.10	115	361.25	6031
89.05	394	139.15	46072	199.10	2376	362.20	903
91.05	91	140.05	6576	200.10	1910	363.20	256
93.05	290	141.15	1408	201.20	239	379.20	768
94.15	316	142.15	863	203.10	206	376.20	98
95.15	416						

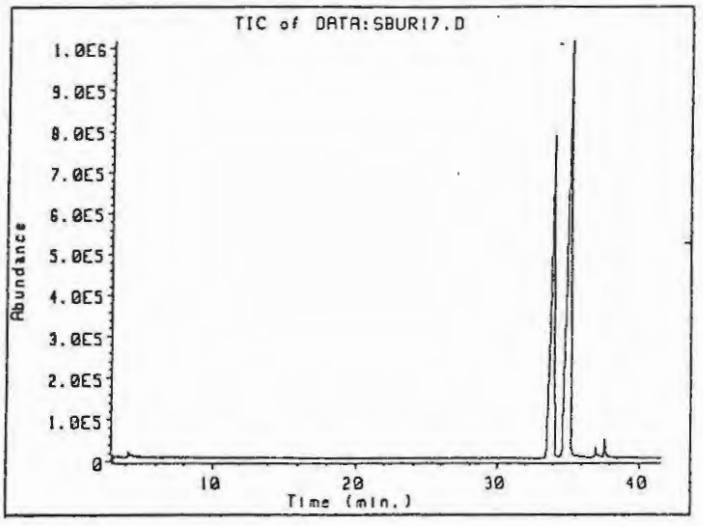


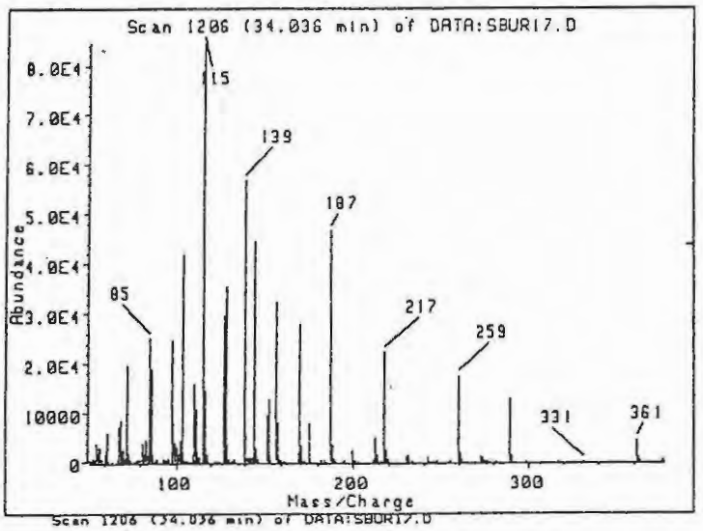
D-Galactose AA derivative

Scan 1251 (35.580 min) of DATA:SBUR13.D

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
51.05	112	89.95	41	139.05	45592	203.00	163
52.05	124	91.15	104	140.05	5587	207.10	61
53.05	394	93.05	456	141.15	1255	211.15	707
54.15	228	94.05	317	142.15	997	212.15	5678
55.05	2994	95.05	501	143.15	586	213.15	1390
56.05	1040	96.15	388	144.10	1021	214.15	163
57.00	2396	97.05	20064	145.00	79928	215.05	63
58.00	273	98.05	8556	146.10	5340	217.15	31680
59.00	198	99.05	3481	147.10	631	218.15	3087
60.10	1439	100.05	2233	152.10	11607	219.15	654
61.10	3798	101.00	2209	153.10	11081	220.25	48
62.10	121	102.00	4076	154.10	1251	229.15	1419
63.00	76	103.00	47208	155.10	132	230.25	2983
64.00	30	104.10	2140	156.10	345	231.20	570
65.00	141	105.10	317	157.10	40864	232.10	109
66.10	163	108.00	59	158.10	11661	242.20	1241
67.10	298	109.10	1504	159.10	1058	243.20	175
68.00	5682	110.10	14976	160.20	150	247.10	123
69.00	9079	111.10	8969	163.10	62	254.15	282
70.00	2191	112.10	2315	169.15	1566	259.15	14012
71.00	2071	113.10	1160	170.05	38992	260.15	1504
72.10	918	115.10	100000	171.15	4072	261.25	290
73.10	13154	116.10	16944	172.15	530	271.25	73
74.10	1167	117.10	1560	173.15	184	272.15	2309
75.10	234	118.00	305	174.15	108	273.25	979
76.30	33	119.00	147	175.15	7792	274.25	147
77.10	83	120.00	198	176.15	674	279.20	41
78.10	84	121.00	157	177.05	138	289.20	22968
79.05	228	122.00	96	182.15	292	290.20	3134
80.05	367	124.05	126	183.15	35	291.20	488
81.05	3100	125.15	146	184.15	222	314.25	215
81.95	1775	126.05	960	187.15	37272	331.20	118
82.95	9418	127.05	30984	188.10	3588	332.20	136
84.05	1389	128.05	43920	189.10	604	333.10	61
85.05	25704	129.05	4097	194.10	100	361.25	5127
86.05	15484	130.15	507	195.10	75	362.20	832
87.05	2216	131.15	366	199.10	1911	363.20	147
88.05	226	132.05	228	200.10	1557	375.20	523
89.05	260	133.15	330	201.10	334	376.20	81

(v) Fructose alditol acetate (AA) derivative

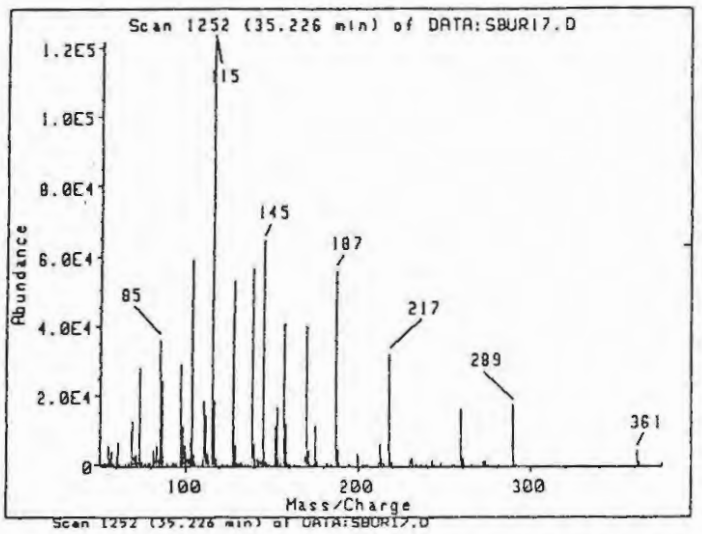




Mannitol AA derivative

Scan 1206 (34.036 min) of DATA:SBUR17.D

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
51.05	388	95.05	867	144.15	1183	213.15	1475
52.05	365	95.95	304	145.10	44584	214.15	119
53.15	1043	97.05	24848	146.10	2932	215.15	83
54.15	485	98.05	8681	147.10	508	215.75	63
55.05	3940	99.05	4153	152.10	9697	217.15	22424
56.05	1619	100.05	3232	153.10	12819	218.15	2413
57.05	3147	101.00	1598	154.10	1222	219.15	421
58.00	469	102.00	4576	154.90	173	229.15	1166
60.00	1528	103.00	41872	157.10	32416	230.15	1459
61.00	6822	104.10	1973	158.20	8267	231.15	518
62.00	271	105.00	363	159.20	829	233.10	26
63.90	168	106.20	47	160.20	246	242.20	873
67.00	486	107.20	168	162.00	41	243.30	146
68.00	7303	109.10	1602	168.05	116	245.20	88
69.10	8577	110.10	15759	168.25	115	247.20	309
70.10	2607	111.10	10853	169.05	2022	254.10	322
71.10	2588	112.00	2125	170.15	27872	255.15	84
72.10	788	113.10	1142	171.15	3531	259.15	17416
73.00	19600	115.10	84656	172.15	135	260.15	2078
74.00	1719	116.10	14625	173.25	127	261.25	438
75.00	257	117.10	1510	175.15	8011	272.15	1200
77.10	155	118.20	148	176.15	460	273.25	993
78.20	133	122.20	71	182.25	318	274.05	173
79.05	543	123.05	118	184.25	330	275.25	71
80.15	519	123.85	161	185.05	110	276.35	53
81.05	3871	125.15	122	187.15	46672	281.10	69
82.05	1668	127.05	29664	188.15	3652	289.20	12903
83.05	4771	128.05	35456	189.20	731	290.20	1355
84.05	1683	129.15	3645	190.20	67	298.85	53
85.05	25280	130.05	556	194.20	105	317.15	69
86.05	10948	132.15	104	195.10	195	331.10	100
86.95	1870	133.15	413	199.10	2524	361.25	4396
87.75	77	139.15	56824	200.10	1591	362.15	922
88.05	139	140.15	5955	201.10	247	363.15	188
89.05	459	141.05	899	203.10	149	375.20	454
93.05	695	142.15	838	211.15	697	376.10	87
94.05	472	143.05	900	212.15	4988	376.40	60

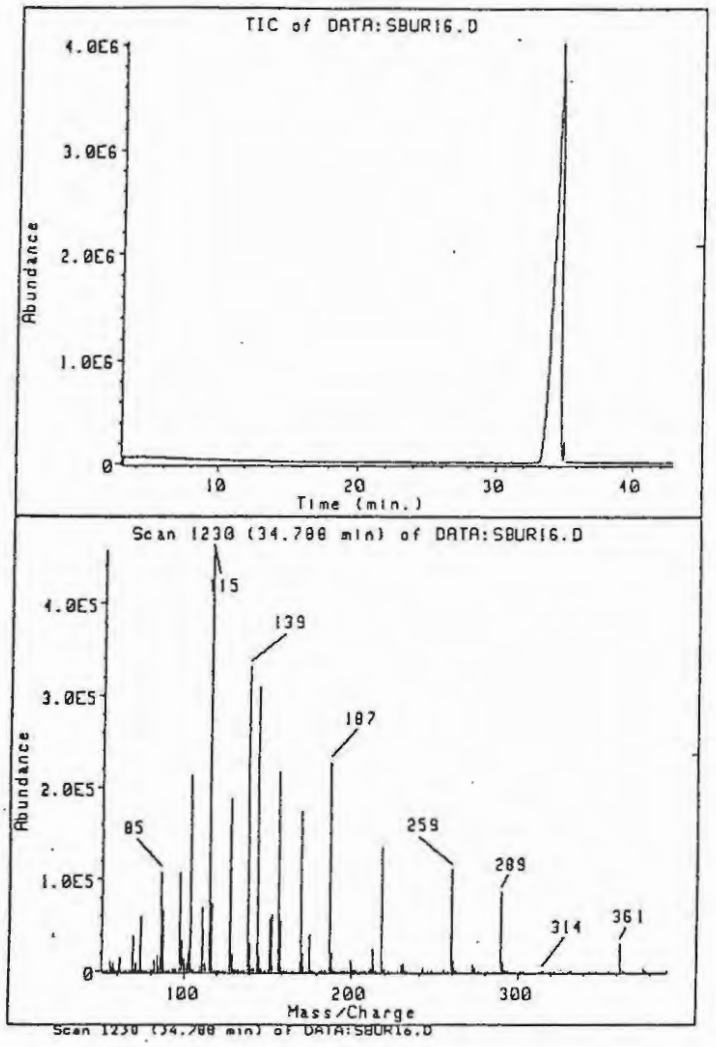


Glucitol AA derivative

Scan 1252 (35.226 min) of DATA:SBUR17.D

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
51.05	551	94.05	623	142.15	1540	203.30	119
52.05	344	95.05	875	143.05	1328	205.20	76
53.05	703	97.05	29024	144.15	1419	211.15	722
54.05	611	98.05	11257	145.10	64304	212.15	5937
55.05	5539	99.05	5685	146.10	3873	213.15	1621
55.95	1706	100.05	3482	147.10	666	214.25	293
57.10	3748	101.00	1858	148.00	118	215.15	105
58.10	573	102.10	6926	149.00	143	217.15	31968
58.90	192	103.00	58792	151.20	123	218.15	3611
60.00	2642	104.00	3072	152.10	11016	219.15	755
61.00	6670	104.90	543	153.10	16616	229.25	1371
61.90	128	108.30	81	154.10	1850	230.15	2071
62.20	139	109.10	1446	155.10	295	231.15	608
65.10	361	110.10	18600	157.10	40568	233.20	81
66.10	269	111.10	14495	158.20	11705	236.30	40
67.10	1068	112.10	3481	159.20	867	239.20	96
68.00	9908	113.00	1479	160.20	95	242.10	1212
69.10	12624	115.10	121840	161.00	83	243.20	151
70.10	2764	116.10	18384	163.10	45	247.20	372
71.10	3173	117.10	1968	169.15	2649	254.20	218
72.10	703	118.00	160	170.15	39760	255.15	44
73.00	27944	121.20	127	171.15	4468	259.15	15975
74.00	1765	122.00	121	172.15	458	260.15	1968
75.10	586	123.95	201	173.05	252	261.15	310
77.10	293	125.15	247	174.35	239	265.25	36
79.05	723	127.05	42072	175.15	11213	272.15	1139
80.15	594	128.05	52728	176.15	1008	273.15	1018
81.05	4323	129.05	6070	182.15	364	274.15	245
82.05	2217	130.05	728	184.25	167	275.25	66
83.05	5729	131.05	527	187.15	55664	281.20	67
84.15	1779	132.05	379	188.15	4492	289.20	17184
85.05	36088	133.15	627	189.10	794	290.20	2208
86.05	24368	135.15	37	194.10	207	291.20	262
86.95	2743	137.35	167	195.20	129	361.25	4062
89.05	288	139.15	56384	199.10	3240	362.15	678
92.35	128	140.15	5969	200.10	1951	375.20	531
93.05	706	141.15	1744	202.50	50		

(vi) Mannose alditol acetate

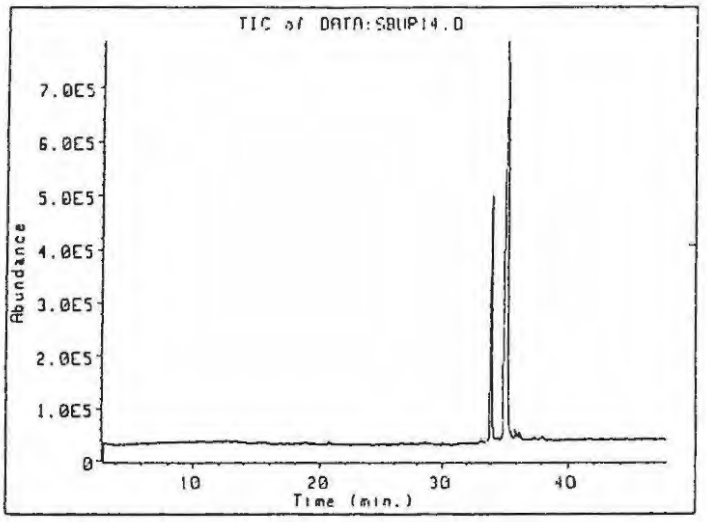


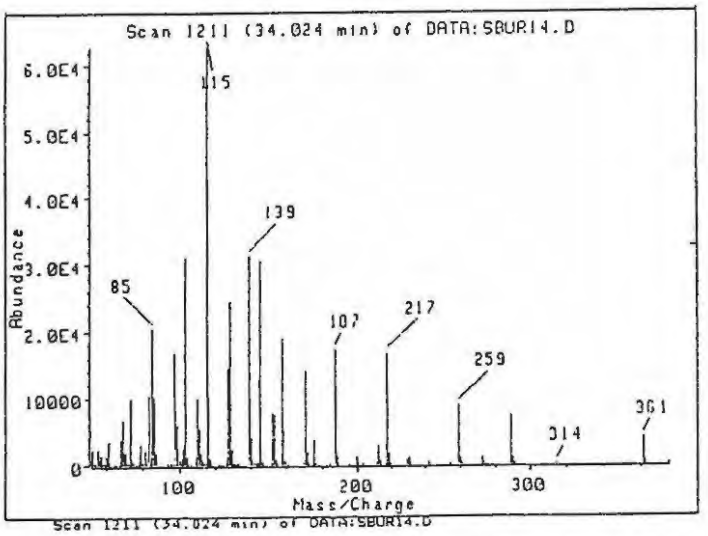
Scan 1230 (34.700 min) of DATA:SBUR16.D

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
50.95	422	90.15	291	136.25	160	189.25	532
52.05	417	91.05	390	137.15	325	187.15	222200
53.05	1814	93.05	2437	139.05	331072	188.15	20768
54.05	981	94.05	1647	140.15	29824	189.10	4319
55.05	10608	95.05	2408	141.15	6819	190.20	301
56.05	3673	97.05	107632	142.15	3568	191.00	111
57.05	8452	98.05	34208	143.05	3706	194.10	618
58.10	1257	99.05	13725	145.00	309504	195.10	687
59.00	440	100.05	9880	146.10	16316	195.80	72
59.20	425	101.00	8173	147.10	2885	197.40	67
60.10	9867	102.00	20608	148.10	114	198.10	164
61.10	15268	103.00	212864	149.20	173	199.10	12080
62.00	528	104.10	8428	149.90	52	200.10	8766
63.20	66	105.10	1632	151.10	835	201.10	1075
64.00	74	106.10	225	152.10	58280	202.30	107
64.30	90	108.10	339	153.10	60808	203.10	753
65.00	692	109.10	6413	154.10	6302	205.10	284
66.10	681	110.10	69824	155.10	949	207.20	176
67.10	1321	111.10	45888	157.10	216064	208.70	70
68.00	20184	112.10	9572	158.10	54320	210.20	78
69.00	37976	113.10	5073	159.10	4561	210.50	100
70.00	7016	115.10	456896	160.20	412	211.15	3931
71.00	9449	116.10	74248	161.20	544	212.15	24368
72.10	1686	117.10	5987	162.30	54	213.15	7998
73.10	59400	118.10	998	165.10	79	214.15	1004
74.10	4325	119.10	299	166.50	39	215.05	385
75.10	725	120.00	299	169.15	9510	217.15	135040
76.50	55	121.00	249	170.05	173888	218.15	11858
77.10	437	122.10	195	171.15	19640	219.25	2240
79.05	753	123.15	366	172.15	2102	220.05	266
79.95	1819	124.15	617	173.05	841	222.15	62
81.05	12252	125.05	524	174.15	517	227.35	90
82.05	7176	127.05	159296	175.15	39632	229.25	6938
82.95	17934	128.05	187712	176.15	3921	230.25	8434
84.05	9961	129.09	18560	177.15	801	231.15	2204
85.05	107608	130.15	2015	178.15	91	232.25	422
86.05	65920	131.05	1540	181.45	127	241.20	432
87.05	7278	132.05	989	182.15	1853	242.20	4358
87.95	817	133.15	2181	183.15	383	243.20	729
89.05	832	135.05	248	184.15	1345	245.10	288

m/z	abund.
247.20	934
248.20	113
254.20	1195
255.15	117
257.15	104
259.15	110528
260.25	11628
261.25	2212
262.25	270
271.25	237
272.25	7727
273.25	5100
274.25	848
275.75	35
281.10	76
285.50	59
289.10	86848
290.20	10430
291.20	1547
292.20	200
293.30	55
301.15	147
303.05	187
314.15	679
315.25	291
317.15	97
319.15	241
322.20	37
331.20	487
332.10	370
333.10	183
343.25	55
346.95	38
359.35	29
361.25	30032
362.25	4485
363.25	1029
364.30	171
375.20	3858
376.20	580
377.20	138
389.35	37

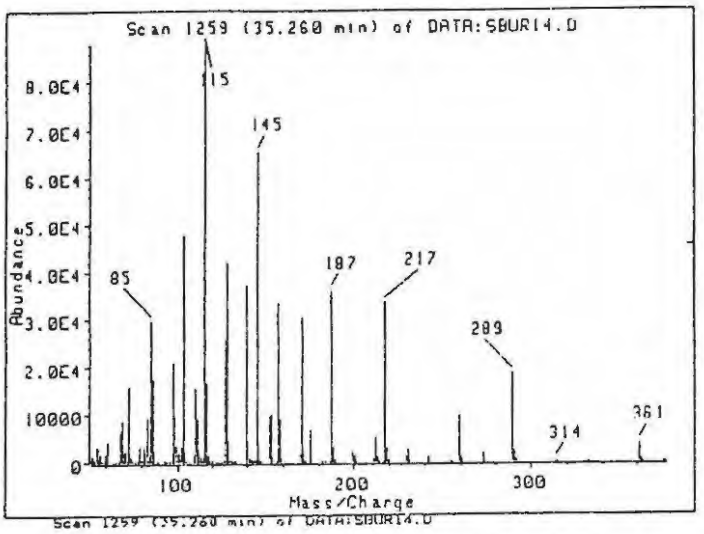
(vii) Free sugar alditol acetate (AA) derivatives





Scan 1211 (34.024 min) of DATA:SBUR14.D

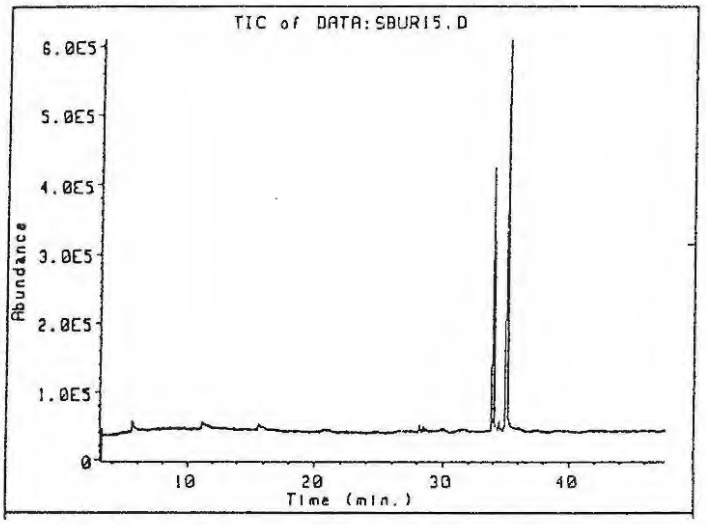
m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
51.05	1055	89.05	141	130.05	228	189.10	181
52.05	2279	91.05	104	131.05	188	194.10	87
53.05	583	93.05	352	133.05	211	195.10	85
54.05	206	94.05	296	137.15	32	199.10	1277
55.05	2464	95.05	348	139.05	31120	200.10	955
56.05	898	96.05	289	140.05	4148	203.10	145
57.00	1514	97.05	16800	141.05	825	211.15	540
58.00	310	98.05	6070	142.15	394	212.15	2848
59.00	157	99.05	2321	143.15	459	213.15	731
60.00	1377	100.15	1050	144.10	525	214.15	98
61.00	3512	101.00	1864	145.00	30584	216.15	71
62.10	107	102.00	2656	146.00	2233	217.15	16696
64.10	29	103.00	31024	147.00	464	218.15	1769
65.00	167	104.00	1250	151.10	66	219.15	278
66.00	115	105.10	220	152.10	7031	229.15	787
67.00	282	107.00	38	153.00	7455	230.15	1167
68.00	3849	109.00	1115	154.10	282	231.20	219
69.10	6677	110.00	10074	155.10	100	242.10	904
70.10	1725	111.10	5393	156.10	202	247.10	73
71.00	2034	112.10	1750	157.10	18880	254.05	245
72.10	302	113.10	218	158.10	5419	259.15	8875
73.00	10011	114.10	615	159.10	656	260.15	1231
74.10	1007	115.00	62816	160.10	82	261.15	164
75.10	307	116.10	10538	161.20	109	272.15	1232
76.00	79	117.10	936	169.05	885	273.15	462
77.10	123	118.00	329	170.05	14093	274.15	65
78.10	570	119.00	129	171.05	1934	275.20	57
78.95	3142	120.00	132	172.05	263	289.10	7372
80.05	568	122.00	129	173.15	119	290.10	1057
81.05	2349	123.05	57	175.15	3710	291.20	229
82.05	1189	124.15	70	176.15	257	314.15	160
82.95	10449	125.15	23	177.15	44	361.15	4005
83.95	1173	126.05	308	182.15	91	362.10	665
84.95	20368	127.05	14380	184.05	186	363.10	97
86.05	10190	128.05	24320	187.15	17256	375.20	167
87.05	1793	129.05	2365	188.10	1550		

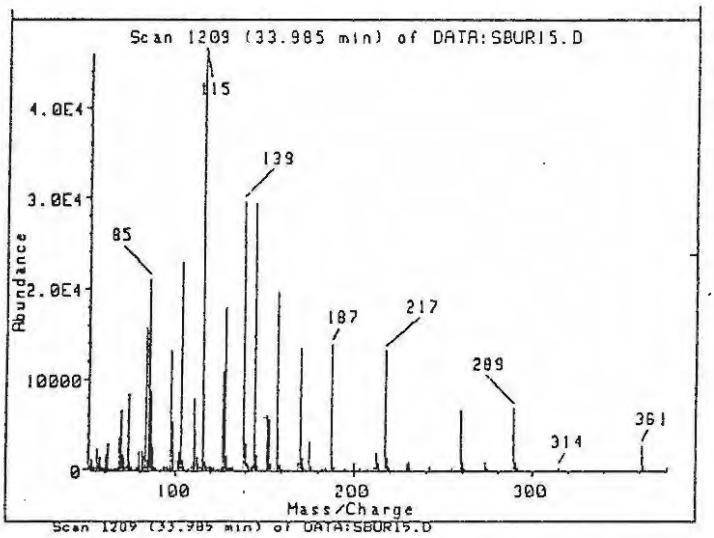


Scan 1259 (35.260 min) of DATA:SBUR14.D

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
51.05	700	94.05	363	139.15	32360	203.20	157
52.05	1352	95.05	377	140.05	4431	207.20	34
53.05	661	96.05	312	141.15	1066	211.05	877
54.05	325	97.05	20888	142.05	860	212.15	5284
55.05	3266	98.05	9736	143.05	577	213.15	1123
56.15	1063	99.05	3604	144.10	764	214.15	207
57.00	1805	100.05	2070	145.10	65368	216.15	71
58.00	344	101.00	1843	146.10	4018	217.15	33464
59.00	131	102.00	3496	147.10	604	218.15	3066
60.00	1806	103.00	47784	148.10	63	219.15	504
61.00	4400	104.00	2437	152.10	9570	229.15	1484
64.10	59	105.00	354	153.10	10004	230.15	2753
65.00	175	107.10	46	154.10	1111	231.10	456
66.00	203	109.10	1406	155.00	196	241.20	67
67.10	420	110.00	15557	156.10	303	242.10	1093
68.00	6188	111.00	9112	157.10	33640	243.10	167
69.10	8739	112.10	2254	158.10	8863	247.10	141
70.10	2011	113.10	1106	159.10	886	254.15	285
71.00	2208	114.20	985	161.10	64	255.05	60
72.10	454	115.10	88152	168.05	67	259.15	9611
73.00	15725	116.10	16720	169.05	1578	260.15	1257
74.10	1287	117.00	1521	170.15	30376	261.15	269
75.00	371	118.00	286	171.15	3238	272.15	1275
76.10	72	119.00	146	172.15	373	273.15	782
77.10	88	120.00	205	173.05	153	275.10	47
78.10	433	121.20	70	175.15	6744	289.20	18464
78.95	3102	121.90	85	176.15	473	290.20	2398
79.95	651	123.05	108	177.15	142	291.20	450
81.05	3100	124.05	186	182.15	248	314.25	144
82.05	1913	125.05	75	184.15	233	315.25	63
82.95	9386	126.05	385	186.15	144	331.10	59
84.05	1363	127.05	25688	187.15	30072	332.10	61
84.95	29800	128.05	41984	188.10	3181	361.15	3956
86.05	17464	129.05	4523	189.10	586	362.10	756
87.05	2244	130.05	477	195.10	85	363.10	126
87.95	143	131.05	337	199.10	2000	375.10	314
89.05	222	132.15	150	200.10	1356	376.20	61
92.95	480	133.15	339	201.10	236		

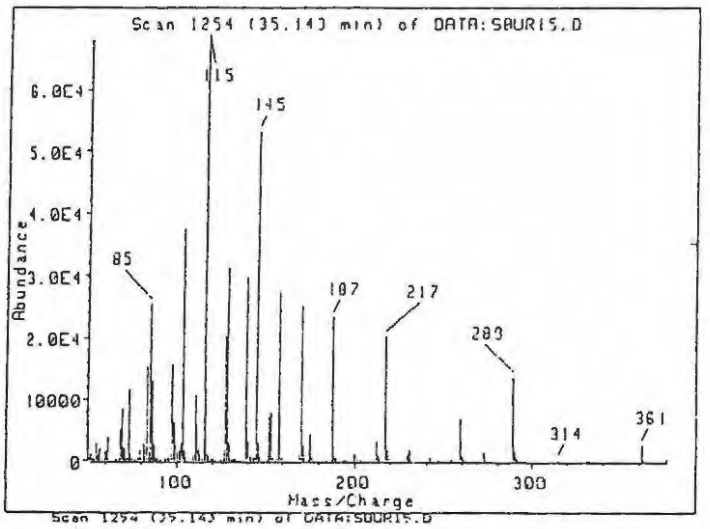
(viii) Glycosidic sugar alditol acetate (AA) derivatives





Scan 1209 (33.985 min) of DATA:SBUR15.D

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
51.05	558	88.05	135	128.05	17920	189.00	301
52.05	1161	89.05	145	129.05	1582	195.10	84
53.15	328	92.15	40	130.05	142	199.10	788
54.05	204	93.05	368	131.05	166	200.10	794
55.05	2311	94.05	234	132.05	75	201.10	99
56.05	996	95.05	316	133.05	170	202.10	28
57.00	1423	95.95	196	139.05	29536	203.10	120
58.00	259	97.05	13187	140.15	2895	207.10	52
59.00	108	98.05	5310	141.15	761	211.15	330
60.00	1633	99.05	1819	142.15	385	212.15	1928
61.00	2924	100.05	1101	143.05	323	213.05	727
62.00	102	101.00	1014	144.00	564	214.15	89
63.00	80	102.00	1996	145.00	29384	217.15	13214
63.80	41	103.00	22912	146.10	1693	218.15	1280
65.10	142	104.00	1096	147.10	347	219.15	243
66.10	91	105.10	241	152.10	6054	229.15	673
67.00	269	107.10	42	153.10	5577	230.15	819
68.00	3601	108.10	37	154.10	606	231.10	175
69.00	6587	109.10	910	156.10	180	242.20	415
70.00	1724	110.10	7929	157.10	19632	254.15	193
71.00	1387	111.10	4460	158.10	4658	259.15	6625
72.00	349	112.10	1403	159.10	479	260.15	821
73.10	8346	113.00	685	160.10	63	261.15	81
74.00	894	114.10	573	169.05	897	272.15	821
75.10	279	115.10	45904	170.05	13428	273.25	324
77.20	77	116.10	8327	171.15	1294	274.25	61
78.00	298	117.10	901	172.15	180	289.10	6846
79.05	1925	118.00	316	173.15	74	290.20	872
80.05	390	118.90	198	175.05	3156	291.20	140
80.95	2155	119.90	356	176.05	234	314.15	107
81.95	1600	121.00	62	182.05	88	315.15	39
82.95	15742	122.00	153	184.15	201	361.25	2707
83.95	1123	124.05	71	186.25	85	362.10	416
85.05	21024	125.05	83	187.15	13847	363.10	90
86.85	8724	126.05	224	188.00	1370	375.20	127
86.95	2621	127.05	10932				

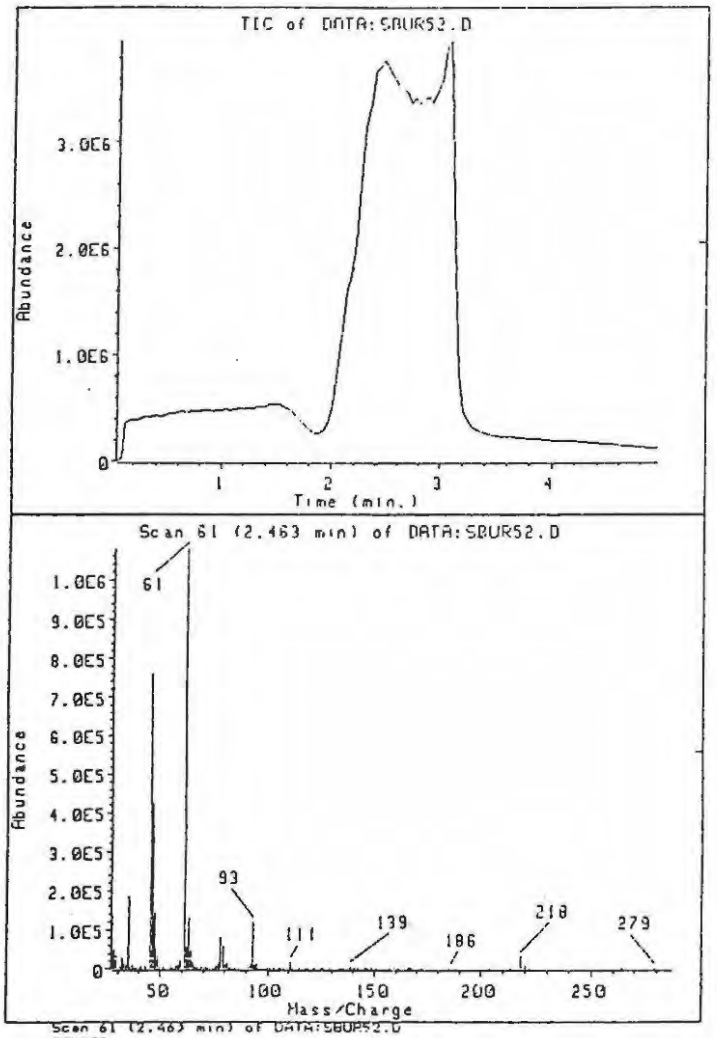


Scan 1254 (35.143 min) of DATA:SBURIS.D

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
51.05	669	88.05	161	129.15	2921	194.10	71
52.05	1061	88.95	213	130.15	295	195.10	91
53.05	447	91.05	71	131.05	183	199.10	1085
54.05	226	93.05	416	131.95	184	200.10	1110
55.05	2890	94.05	322	133.05	267	201.10	164
56.05	974	95.05	413	139.05	29616	203.20	122
57.00	1926	96.05	342	140.15	3074	211.15	486
58.00	253	97.05	15578	141.15	827	212.15	3108
59.20	135	98.05	6442	142.15	616	213.15	986
60.00	1554	99.05	2476	143.05	416	214.15	88
61.00	3883	100.05	1582	144.10	796	217.15	20272
62.10	89	101.00	1590	145.00	53152	218.15	1833
64.00	59	102.00	2727	146.10	3063	219.15	418
65.10	164	103.00	37472	147.10	531	229.25	909
66.00	95	104.10	1581	151.10	71	230.15	1727
67.10	343	105.10	285	152.10	7202	231.10	237
68.00	5248	109.00	1005	153.10	7975	242.20	596
69.00	8543	110.10	10615	154.10	729	243.20	113
70.00	2041	111.10	5986	156.10	203	247.10	57
71.00	1839	112.10	1875	157.10	27432	254.15	234
72.10	327	113.10	929	158.10	7466	259.15	6861
73.10	11671	114.10	791	159.20	620	260.15	803
74.10	1036	115.10	68128	160.20	98	261.15	150
75.10	322	116.10	11702	169.05	1111	272.15	1386
76.00	98	117.10	1137	170.05	25176	273.25	605
77.10	83	118.00	396	171.05	2679	274.15	73
78.00	328	119.90	230	172.15	281	289.10	13656
79.05	1810	120.00	300	174.05	52	290.20	1619
80.05	467	121.00	127	175.15	4433	291.20	271
81.05	2834	122.00	144	176.05	420	314.15	110
81.95	1805	124.15	108	182.05	166	332.20	62
82.95	15366	125.05	87	184.15	152	361.25	2631
84.05	1332	126.05	354	187.15	23312	362.10	491
85.05	25448	127.05	20256	188.00	2287	363.10	77
86.05	13029	128.05	31488	189.10	373	375.20	218
86.95	2657						

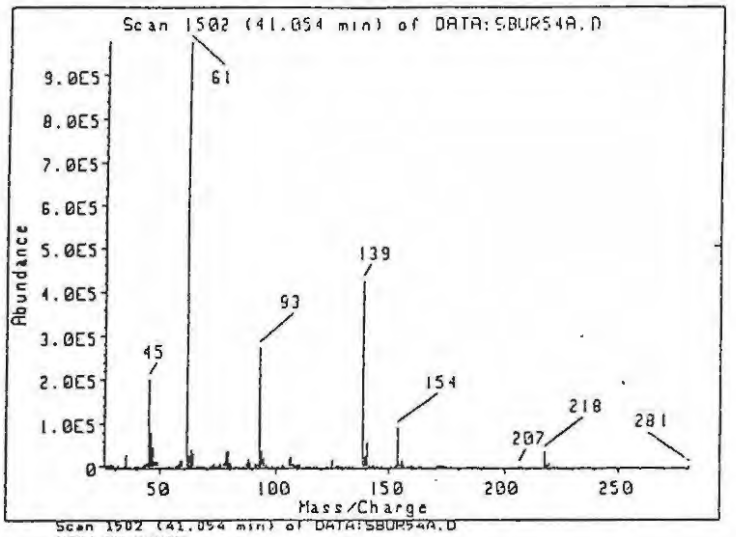
(2) G.c. - m.s. data - Sulphur compounds and Volatiles

(1) 2,4,5,7-tetrathiaoctane-2,2-dioxide - Compound (117)



Scan 61 (2.463 min) of DATA:SBUR52.D
SBUR52

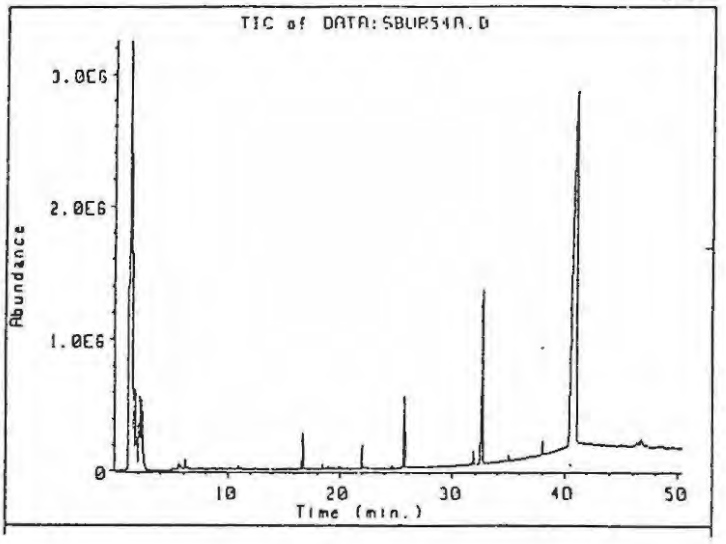
m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
27.05	136768	63.95	47696	101.00	272	145.20	98
28.05	49248	64.95	16213	102.70	104	146.10	122
29.15	32400	65.95	4906	103.20	258	147.10	182
30.05	1872	67.05	2527	105.10	597	148.20	206
31.15	8787	67.95	897	106.10	1168	149.10	551
32.05	28384	68.15	881	107.10	1208	150.40	153
33.05	12990	69.15	3431	108.05	2048	151.20	168
34.05	11403	70.05	917	109.05	1726	153.20	262
35.05	196112	71.05	3110	110.05	1714	154.05	349
36.05	2494	72.05	396	110.95	16104	157.15	380
37.05	8395	73.15	1081	112.05	1156	159.15	136
37.90	204	74.05	411	112.95	2198	161.25	160
38.10	197	75.05	478	114.05	191	165.55	69
39.00	1548	75.95	8170	115.05	481	166.35	90
40.00	1084	76.95	19328	117.15	118	166.65	64
41.10	6319	77.95	80584	117.65	190	167.15	235
42.10	1307	79.05	58952	118.15	111	171.05	1010
43.10	7485	80.05	10665	119.15	616	172.05	414
45.00	259744	81.05	14067	121.05	360	183.40	201
46.00	422976	82.15	1715	122.15	183	185.20	425
47.00	113296	83.05	1807	123.05	1119	186.10	500
48.00	70640	89.10	748	130.15	195	172.05	524
49.00	34424	91.00	8947	131.20	585	173.05	848
50.00	4135	92.00	14209	132.20	240	174.05	320
51.00	2693	93.00	107312	133.20	847	175.25	297
52.00	335	94.00	9952	134.20	441	176.25	75
53.10	2572	95.10	14506	135.10	1632	177.15	162
54.00	2081	96.10	3480	136.10	812	178.10	130
55.10	17824	97.10	4456	137.10	1373	180.20	173
56.10	4128	98.10	1640	138.30	727	182.30	74
57.10	20712	99.10	1687	139.10	4498	183.90	81
58.10	13615	100.10	251	140.10	652	185.10	3405
59.10	25360	101.10	292	141.00	3051	186.00	1563
61.05	1072128	102.10	111	142.00	672	187.10	752
62.05	50896	103.10	386	143.10	790	188.00	188
63.05	140608	104.10	289	144.10	222	189.30	606
63.95	50176	105.10	2055	145.20	639	190.20	150
64.95	17600	106.20	1330	146.20	164	191.30	293
65.95	6105	107.20	3488	147.20	247	194.20	122

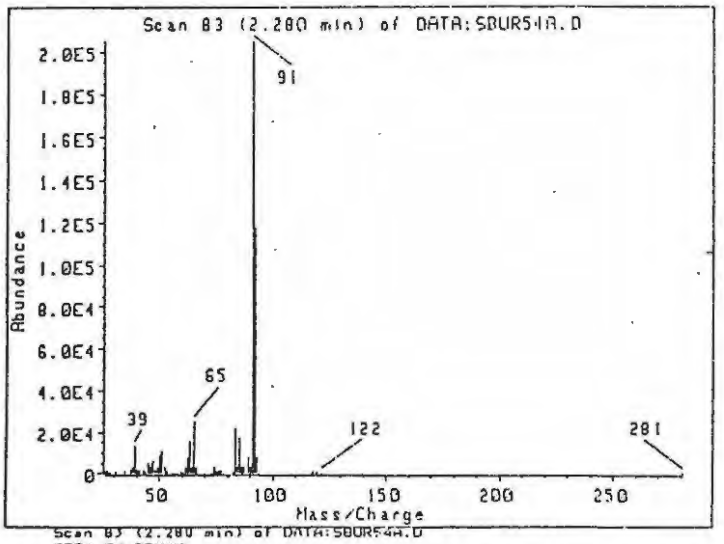


Scan 1502 (41.054 min) of DATA:SBUR54A.D
SB54 ON OBWAX

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
26.05	543	62.95	40264	98.80	501	140.75	54544
27.05	6811	63.95	30768	99.90	406	141.75	3184
28.05	6809	64.85	3050	100.90	1893	142.85	2855
29.05	2594	65.80	3720	102.00	1113	143.75	272
29.85	1184	66.80	570	102.90	2505	144.85	332
31.05	2013	68.00	432	104.00	401	146.85	397
31.95	3703	68.80	848	105.80	17528	150.80	167
33.15	1039	70.00	1025	106.90	23200	152.80	4657
33.95	241	71.00	1691	107.85	8990	153.80	90688
34.95	26984	72.00	2271	108.85	4025	154.80	6921
35.95	476	72.90	8059	109.85	4852	155.80	12786
36.95	1447	73.80	855	110.85	6570	156.70	2358
39.10	325	74.90	3130	111.75	1232	157.70	1157
39.90	311	75.90	8636	112.75	1206	158.80	476
41.00	1174	76.90	9367	113.85	463	159.60	353
42.00	1108	77.90	36304	114.95	703	160.90	206
42.90	5794	78.90	37640	115.95	312	162.90	344
43.90	11760	79.85	9454	116.95	1034	163.95	153
45.00	200960	80.75	4167	118.05	205	170.95	359
46.00	79544	81.85	662	118.75	930	171.75	2245
47.00	44112	82.75	609	120.95	477	172.95	327
48.00	12269	83.95	371	122.90	462	173.65	634
48.90	11704	84.95	654	123.60	1645	174.75	433
50.00	634	85.85	1111	124.80	5411	176.95	391
50.90	719	86.85	7781	125.70	16236	185.80	983
52.05	260	87.85	17448	126.90	1405	187.70	214
53.15	284	88.95	11824	127.70	2248	191.00	141
53.95	197	90.85	8735	128.80	400	206.80	1061
55.05	1203	91.95	4726	129.70	220	207.80	266
55.95	735	92.05	275008	130.80	945	217.70	35752
56.95	5371	93.80	37616	132.00	322	218.60	2716
57.95	16720	94.80	21504	132.90	2024	219.65	7176
58.95	16346	95.80	6515	134.10	254	220.65	706
60.95	977600	96.90	1547	138.75	424640	221.55	625
61.85	27576	97.90	1215	139.85	25176	280.75	241

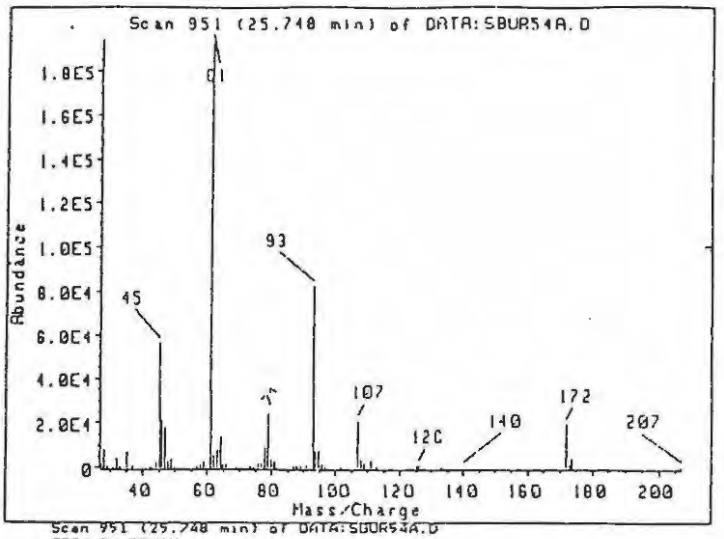
(11) 2,4,5,7-tetrathiaoctane - Compound (118)





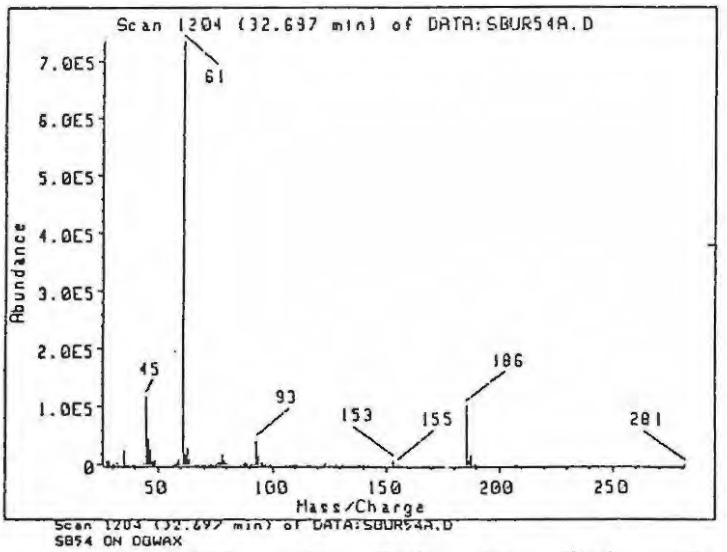
Scan 83 (2.280 min) of DATA:SBUR54A.D
SB54 ON DBWAX

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
26.15	635	45.90	3185	63.95	3330	86.85	4036
27.15	1341	47.00	5818	65.05	25368	87.85	417
27.75	315	48.00	2220	65.90	2910	88.95	7944
28.25	323	49.00	3092	69.80	163	90.05	3413
30.95	851	50.00	8984	72.00	354	90.95	209248
34.95	1164	51.00	11137	72.90	800	91.95	117432
36.05	409	52.05	3226	74.00	3095	92.95	8112
36.95	2223	53.05	1366	74.80	1485	93.70	231
38.00	3527	55.95	180	75.80	1202	116.85	231
39.00	13182	56.65	103	76.90	7004	117.85	682
40.00	1600	58.05	116	81.05	955	118.95	344
41.00	1955	59.05	648	82.85	22016	119.75	523
42.10	372	60.05	592	83.05	3079	121.80	180
43.10	1589	60.95	3524	84.85	17208	139.85	111
43.90	693	61.95	8193	85.85	3482	280.55	205
45.00	4915	62.95	15755				



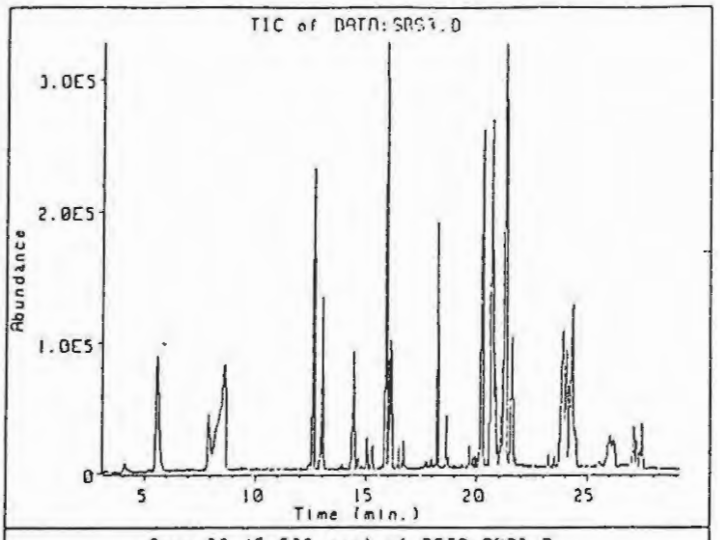
Scan 951 (25.748 min) of DATA:SBUR54A.D
SB54 ON DBWAX

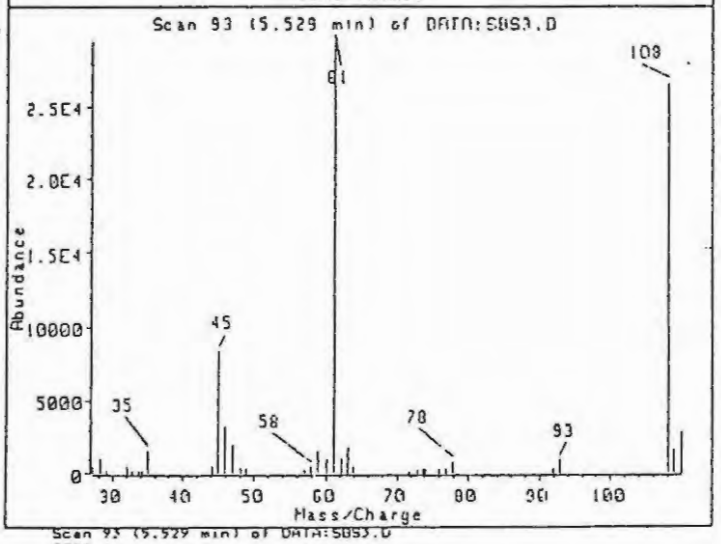
m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
27.05	2195	56.85	873	78.90	24128	107.95	3427
28.05	7900	57.95	1877	79.85	3601	108.85	2085
28.95	465	58.95	2812	80.85	2682	109.75	567
31.05	399	60.95	194432	81.75	374	110.85	3264
31.95	4088	61.95	9455	86.75	967	111.95	256
32.95	530	62.95	7947	87.85	825	112.75	498
35.05	6832	63.95	13375	89.15	739	114.85	172
36.95	455	64.95	1737	90.85	1276	123.90	188
39.90	414	65.90	1437	91.85	901	124.90	560
41.00	320	66.70	305	92.05	82272	125.70	976
42.00	172	68.90	228	93.80	7562	127.80	241
42.90	821	69.90	158	94.90	2345	133.00	180
44.00	2604	70.70	203	95.70	1881	139.75	294
45.00	56464	72.00	256	96.70	486	171.75	19240
46.00	21344	73.00	502	100.90	149	172.85	1507
46.90	17584	74.40	139	101.70	154	173.75	3960
47.90	3263	75.90	2129	102.00	159	174.85	178
48.90	3455	76.80	1912	103.10	113	175.95	220
50.00	187	77.90	9005	106.80	20936	206.60	386
55.95	171						



Scan 1204 (32.697 min) of DATA:SBUR54A.D
SB54 ON DBWAX

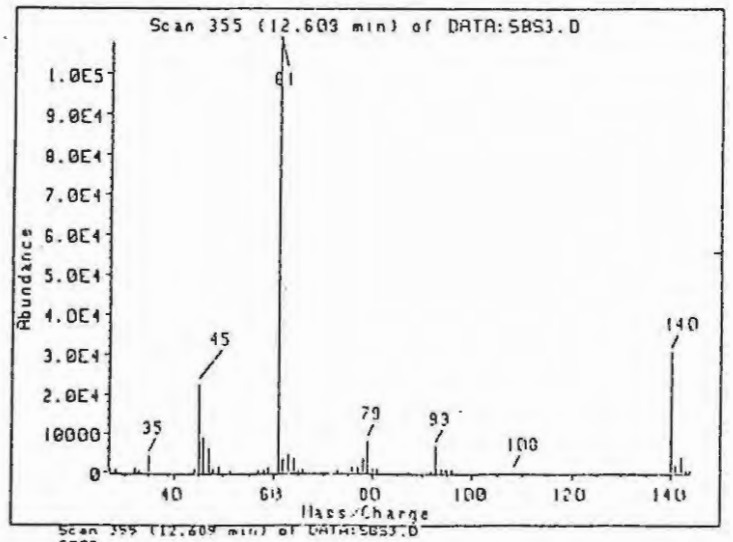
m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
25.95	244	58.95	9564	87.05	1787	116.85	293
27.05	8058	59.95	2917	87.95	4413	120.05	249
28.05	7547	60.95	735936	88.95	2822	122.70	160
29.05	1221	61.95	18392	90.05	355	122.90	165
30.15	316	62.95	28304	90.85	2706	123.70	2030
30.85	850	63.85	10355	91.85	2937	124.70	435
32.05	3671	64.95	969	92.85	40560	125.70	610
32.95	678	65.90	960	93.80	14648	127.80	230
35.05	25128	66.80	228	94.90	4169	131.00	249
36.05	377	68.00	213	95.80	3153	132.90	561
36.95	1434	69.00	507	96.80	423	137.65	1204
39.10	201	70.10	318	97.80	730	138.85	535
39.90	536	70.80	643	98.70	206	139.85	1295
41.00	946	72.00	501	99.10	197	141.05	215
42.00	499	73.00	3199	100.80	465	152.80	2748
42.90	2320	74.00	292	101.80	398	153.80	565
43.90	5062	74.90	1769	102.90	833	154.80	1131
44.90	117064	75.90	4080	103.90	191	155.90	192
45.90	45424	76.90	5410	104.90	555	185.70	102864
47.00	26304	77.90	18120	105.90	443	186.70	7776
47.90	7174	78.90	8054	106.90	614	187.70	10064
48.90	2897	79.75	2329	107.95	1043	188.80	1190
49.90	500	80.05	962	108.85	739	189.80	1287
50.90	353	81.95	270	109.75	215	190.80	328
54.95	592	83.05	355	109.95	212	206.80	480
55.85	301	85.15	180	110.65	510	208.00	202
56.95	1887	85.95	332	115.05	343	280.75	205
57.95	6553						





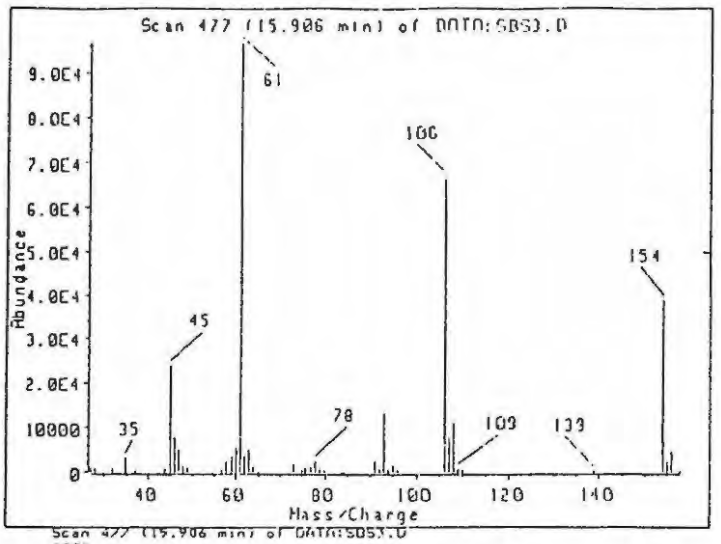
Scan 93 (5.529 min) of DATA:SBS3.D
SBS3

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
26.95	503	44.90	8321	60.05	1029	76.00	334
28.05	971	45.90	3236	60.95	29536	76.90	291
29.05	189	46.90	1992	62.05	1098	77.80	792
32.05	467	48.00	395	63.05	1781	91.85	306
32.85	201	48.90	298	63.85	905	92.85	1012
33.75	147	50.30	124	71.70	109	108.00	26640
34.15	170	56.95	240	73.00	250	108.90	1677
34.95	1592	57.85	470	73.80	325	109.90	2885
44.10	456	58.85	1561	74.10	301		



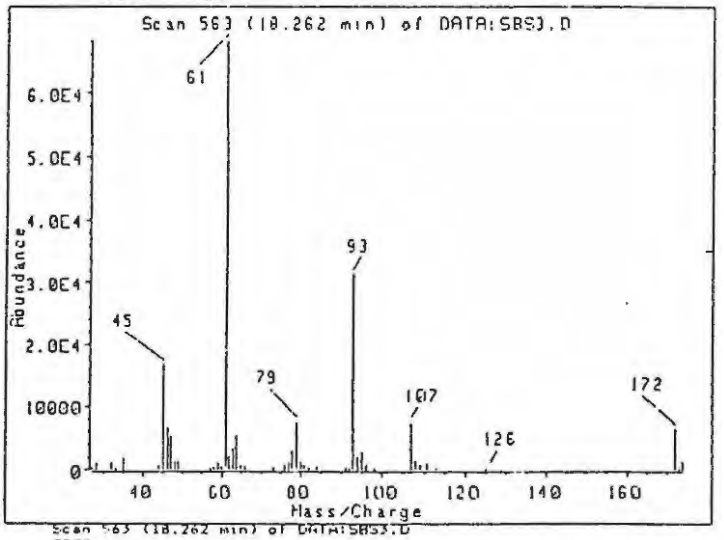
Scan 355 (12.609 min) of DATA:SBS3.D
SBS3

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
26.95	970	51.20	253	75.80	1266	92.85	6562
28.05	621	56.05	339	76.90	1307	93.85	918
31.95	815	57.95	611	77.90	3566	94.85	798
32.95	347	58.95	1282	78.90	8028	95.95	525
34.95	4186	60.95	107800	80.00	869	108.10	164
44.00	940	61.05	3383	80.90	833	140.00	30728
45.00	22424	62.95	4435	84.10	161	140.90	1929
46.00	8753	63.95	3734	87.25	176	142.00	4038
47.00	5997	64.95	440	90.35	471	142.90	392
47.90	1054	65.95	630	91.95	332	143.80	287
49.00	1331	72.90	266				



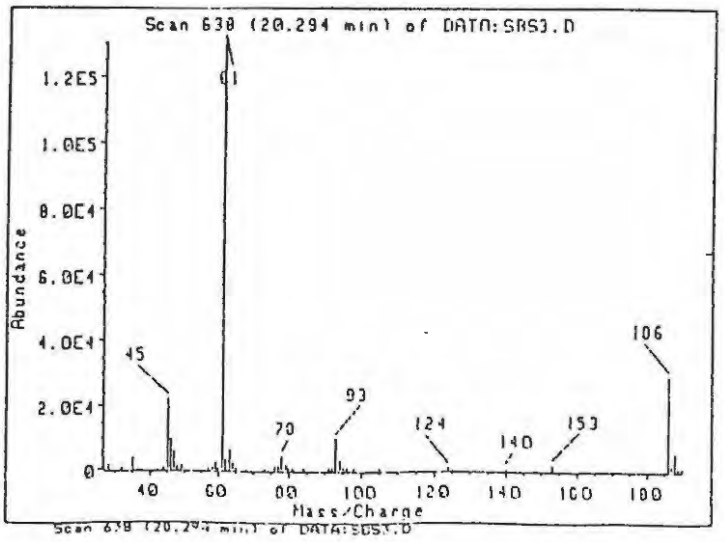
Scan 477 (15.906 min) of DATA:SBS3.D
SBS3

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
26.85	1160	58.95	3695	77.90	2567	105.90	66464
27.95	862	59.95	5360	79.00	708	106.90	7282
31.95	903	60.95	96600	79.80	608	107.90	11033
34.95	3240	61.95	3634	83.90	144	108.80	721
37.05	335	62.95	5047	84.20	150	109.00	734
43.80	851	63.95	1270	90.05	2486	110.00	550
44.90	23824	65.05	207	91.85	829	138.80	351
45.90	7872	72.00	194	92.95	13167	139.00	330
47.00	4850	73.00	1791	93.95	629	153.95	38704
47.90	1328	75.00	464	94.85	1439	154.95	2513
48.90	1082	75.80	1077	95.75	517	155.95	4734
56.75	524	76.90	1328	98.05	212	157.85	318
57.75	2617						



Scan 563 (18.262 min) of DATA:SBS3.D
SBS3

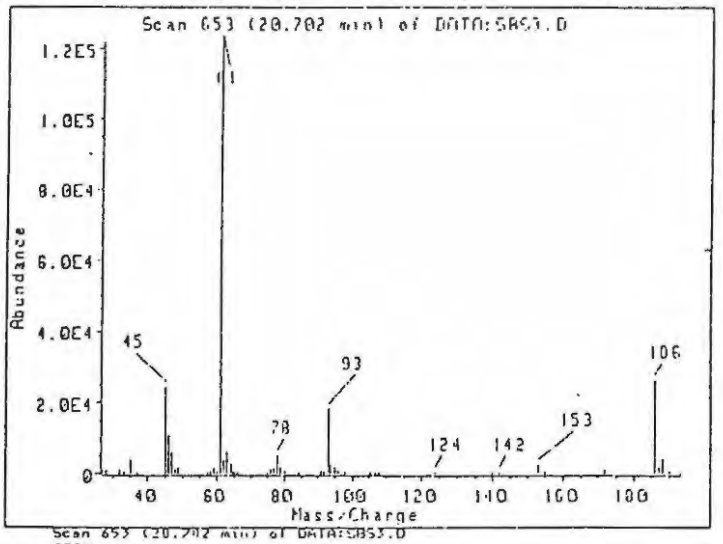
m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
26.85	494	58.05	474	77.90	2935	98.05	287
28.05	1027	59.05	1121	78.90	7492	106.90	7308
31.95	1112	59.95	522	79.90	1276	107.90	1413
33.05	219	60.95	68136	80.80	748	109.00	795
35.05	1849	61.85	2170	82.00	322	110.80	1095
43.80	813	62.85	3344	84.00	611	112.80	295
44.90	16608	63.85	5364	90.95	449	124.95	263
45.90	6498	64.95	671	91.85	262	126.05	319
47.00	5231	65.85	502	92.85	31440	171.90	6375
47.90	1351	73.00	410	93.85	1884	173.00	313
48.90	1195	75.90	803	94.85	2882	173.90	1204
56.95	271	76.90	1013	95.95	815		



Scan 638 (20.294 min) of DATA:SBS3.D

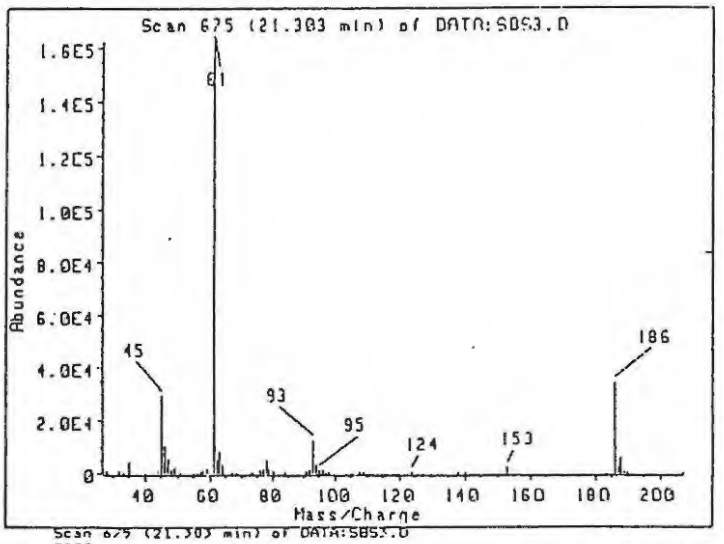
SBS3

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
26.95	706	49.00	1300	78.80	1053	97.85	342
27.95	1289	51.10	163	76.90	1322	105.00	361
31.95	568	56.85	495	77.90	4204	106.90	263
34.95	3748	57.95	790	78.90	1709	123.85	691
36.15	194	58.95	2313	79.80	684	124.95	129
36.85	216	59.25	574	81.00	353	139.70	290
37.45	173	60.95	130440	84.10	488	140.00	216
42.10	172	61.95	3314	90.85	751	152.95	1541
43.00	706	63.05	6441	91.75	819	105.85	29280
44.90	21952	63.95	2152	92.85	9990	186.95	2191
45.90	9542	64.85	528	93.95	3039	187.95	5363
46.90	5594	67.65	158	94.85	832	188.75	324
47.90	1183	73.10	253	95.85	801	189.95	329



Scan 653 (20.702 min) of DATA:SBS3.D
SBS3

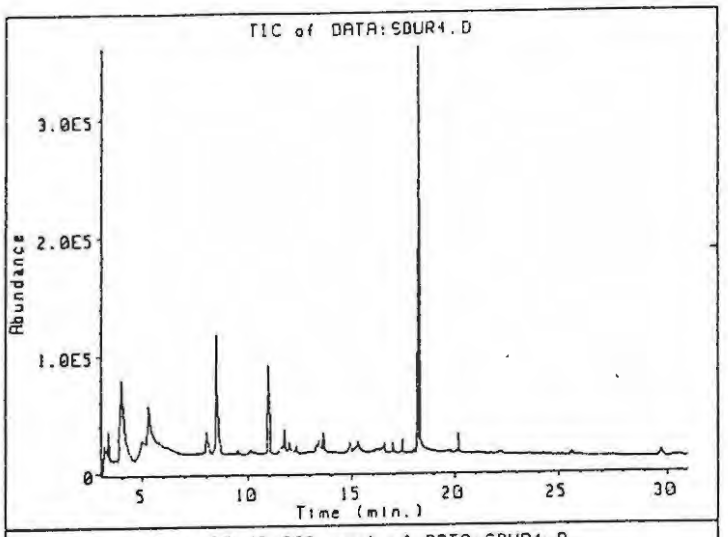
m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
26.95	1365	58.95	1585	78.80	1912	123.75	383
27.95	942	59.85	666	80.00	607	139.90	323
32.05	944	60.95	122032	84.10	460	141.90	441
33.05	343	61.95	3820	90.95	597	152.85	2429
34.95	3859	62.95	6264	91.85	875	153.75	165
36.95	288	63.95	2755	92.95	18192	154.85	369
44.90	24504	64.75	349	93.85	2953	171.90	951
45.90	10784	64.95	323	94.85	2117	185.95	26320
46.90	5914	65.95	333	95.85	914	186.95	1929
47.80	1323	72.80	277	97.95	413	187.95	4556
48.90	1588	74.90	290	105.10	322	188.85	392
55.15	147	75.90	1329	106.90	307	189.85	620
57.05	495	76.90	1694	107.80	499	193.10	168
57.95	821	77.90	5157				

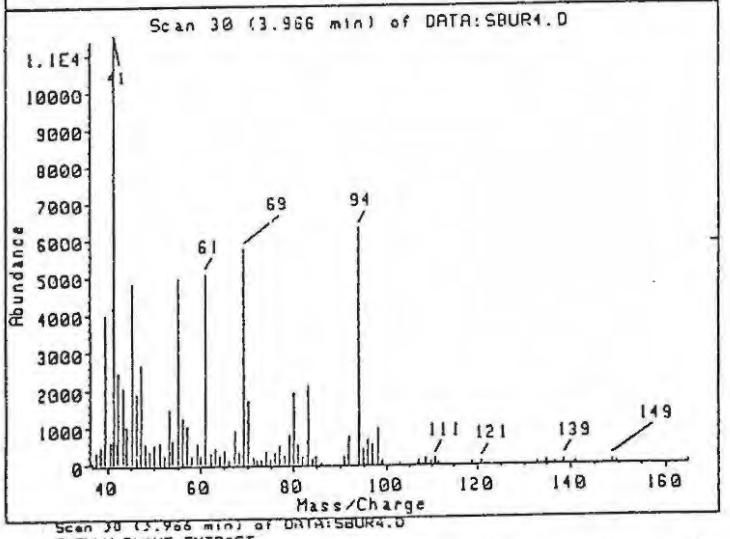


Scan 675 (21.303 min) of DATA:SBS3.D
SBS3

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
27.05	1157	56.95	585	76.90	1050	107.80	498
28.05	799	57.75	1064	77.90	4923	108.80	249
31.85	945	58.95	1990	78.00	1782	109.10	265
33.15	188	60.95	162368	79.90	804	123.85	681
34.95	4106	61.95	5412	80.90	307	137.90	393
37.15	226	62.85	8200	84.00	511	138.70	321
40.00	120	63.95	3324	90.85	1106	140.10	413
43.80	1172	64.85	337	91.85	1374	152.95	2520
44.90	20552	65.85	208	92.85	12540	154.85	393
45.90	10538	66.85	115	93.85	3426	185.95	34528
46.90	5464	68.45	122	94.75	1245	186.95	2585
48.00	1376	73.00	332	95.95	1189	187.85	6449
48.90	1794	73.50	163	97.05	264	180.85	555
50.70	212	75.10	220	97.95	523	189.85	527
54.90	148	75.90	1275	105.10	338	206.95	317
55.95	228						

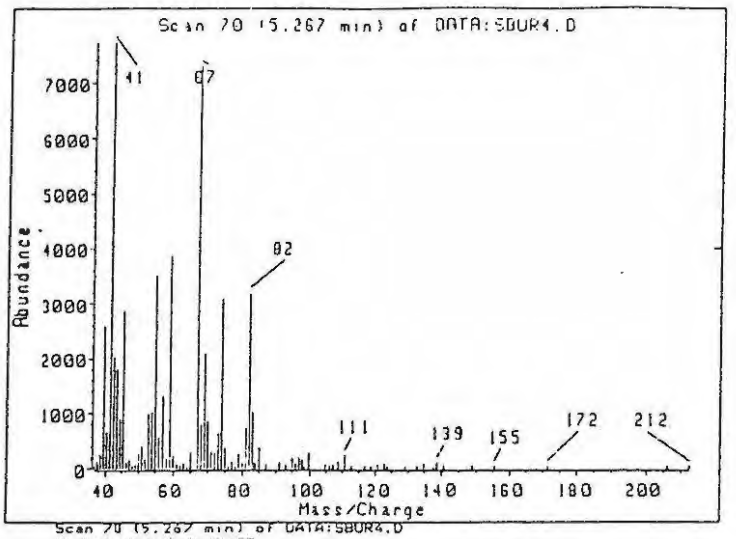
(iv) Volatiles - March





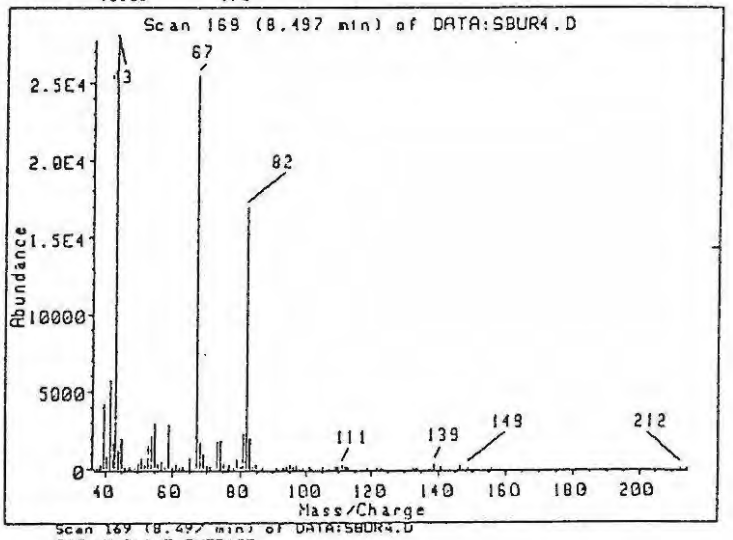
Scan 30 (3.966 min) of DATA:SBUR4.D
SMELLY PLANT EXTRACT

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
36.20	57	55.10	4960	73.90	391	97.90	926
37.20	369	56.10	1266	74.90	123	98.90	88
38.20	486	57.10	1041	75.90	325	102.75	34
39.20	3997	58.10	240	76.90	535	106.85	86
40.30	630	59.10	581	77.90	235	108.70	146
41.20	11373	60.00	228	78.90	802	109.80	57
42.20	2480	61.00	5084	80.00	1920	110.70	149
43.20	2082	62.00	329	80.85	549	111.50	49
44.05	1033	62.95	451	81.95	218	118.75	48
45.15	4810	63.95	253	82.95	2139	120.75	55
46.15	1939	65.05	390	83.95	159	132.70	50
47.15	2676	65.95	100	84.85	210	134.70	63
48.05	523	67.05	933	85.95	38	136.55	32
49.05	328	68.05	384	90.90	200	138.55	105
50.15	555	69.05	5753	91.80	758	140.85	36
51.15	621	70.05	1730	93.80	6288	148.60	81
52.15	200	71.15	196	94.80	430	149.50	32
53.10	1488	72.00	105	95.80	665	164.50	32
54.00	670	73.00	138	96.90	548		



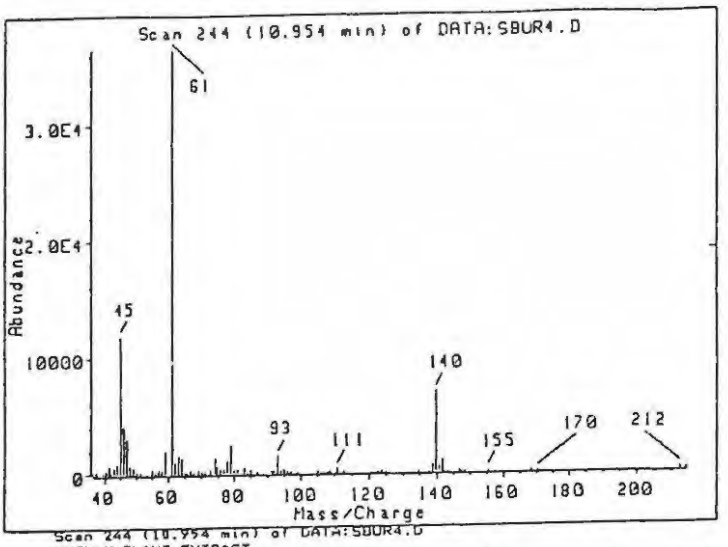
Scan 70 (5.267 min) of DATA:SBUR4.D
SMELLY PLANT EXTRACT

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
36.10	50	57.10	1316	78.00	53	106.95	58
37.20	135	58.10	176	79.00	222	108.80	131
38.20	262	59.10	3867	79.90	133	110.70	237
39.20	2591	60.10	251	80.95	754	112.80	42
40.20	671	61.00	79	81.95	3187	116.70	54
41.20	7745	62.00	65	82.95	1016	118.75	53
42.20	2018	63.05	112	83.75	101	120.75	60
43.20	1822	64.05	44	83.95	103	122.75	29
44.15	902	64.95	301	84.85	322	123.75	35
45.15	2863	66.95	7313	86.85	95	128.80	36
46.25	134	67.95	806	90.80	102	132.60	49
47.05	165	69.05	2092	92.90	62	134.70	29
48.05	57	70.05	855	95.00	192	137.45	32
49.15	29	71.15	298	95.90	108	138.65	115
50.15	286	72.00	274	96.90	202	140.55	65
51.15	418	73.00	653	97.80	167	148.70	48
52.05	178	74.00	3093	98.50	37	155.45	50
53.10	1001	75.00	386	99.85	266	171.50	49
54.00	1045	75.90	56	104.85	64	206.20	39
55.10	3516	77.00	148	105.95	42	212.35	44
56.10	570						



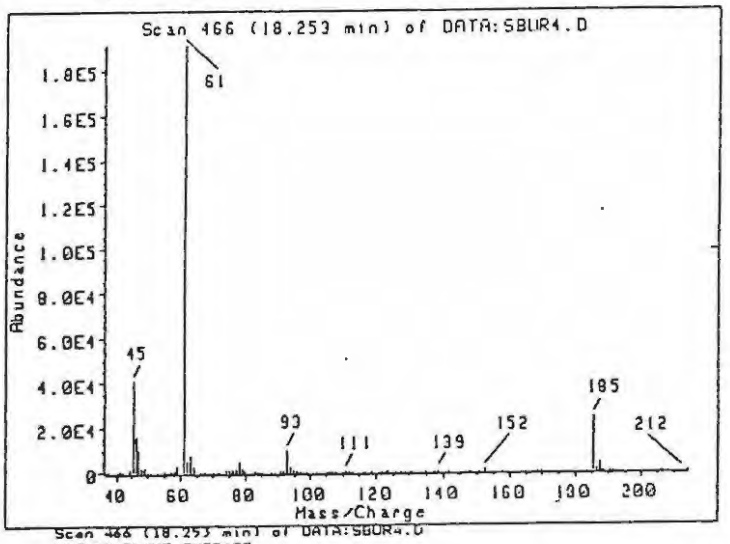
Scan 169 (8.497 min) of DATA:SBUR4.D
SMELLY PLANT EXTRACT

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
36.20	46	58.10	189	80.95	2300	109.70	66
37.20	121	59.10	2897	81.95	16926	110.80	237
38.20	335	60.20	138	82.85	1994	111.80	134
39.20	4184	61.10	311	83.85	166	112.70	85
40.20	899	62.00	64	84.75	307	118.75	77
41.20	5765	62.95	184	86.85	71	119.65	54
42.20	1670	63.95	63	90.80	114	120.75	48
43.20	27752	64.95	708	91.80	57	121.65	32
44.15	1291	67.05	25512	92.90	113	122.85	47
45.15	2016	68.05	1705	93.80	171	132.70	59
46.15	121	69.05	993	94.80	209	133.80	36
47.25	188	69.95	258	95.80	118	138.55	341
48.15	62	71.05	162	96.90	247	140.55	140
49.05	80	73.00	1759	98.00	43	146.50	246
50.15	373	74.00	1825	98.90	83	148.60	126
51.15	709	75.00	404	100.75	161	155.55	85
52.15	298	76.10	104	101.65	55	157.45	52
53.10	1532	76.90	275	104.75	74	158.45	30
54.10	2190	78.00	89	106.85	63	169.40	62
55.10	2942	79.00	681	107.75	59	212.35	97
56.10	375	80.10	141	108.80	179	214.15	58
57.10	511						



Scan 244 (10.954 min) of DATA:SBUR4.D
SMELLY PLANT EXTRACT

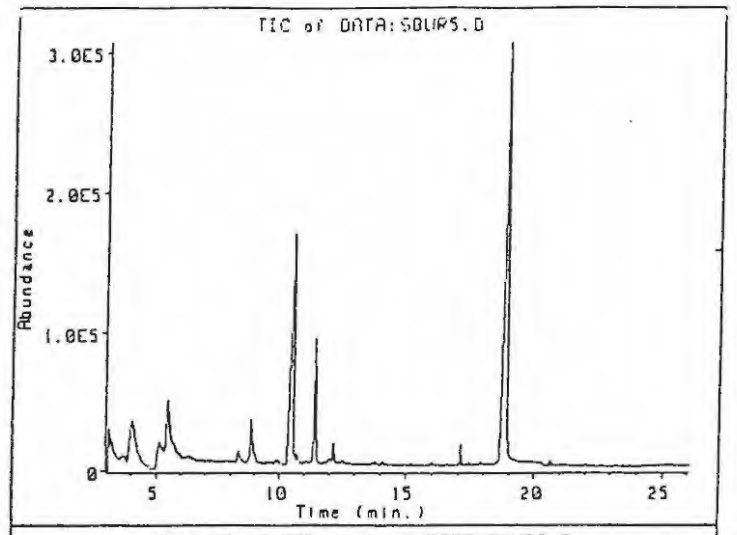
m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
36.20	98	62.00	1078	83.75	50	122.45	48
37.20	247	63.05	1506	84.05	59	122.75	46
39.30	143	63.95	1284	84.85	288	123.05	49
40.20	270	64.95	127	86.85	84	123.65	106
41.20	687	65.85	154	90.90	216	124.85	34
42.10	173	66.95	282	91.70	187	134.80	101
43.20	634	67.95	87	92.80	1479	137.65	33
44.15	908	69.15	269	93.80	239	138.65	635
45.15	11828	70.15	114	94.90	324	139.55	6921
46.15	4080	71.15	112	95.80	127	140.55	621
47.05	3031	72.10	50	96.90	169	141.55	1015
48.05	676	72.90	378	98.80	51	142.55	64
49.05	508	74.00	1349	104.15	29	143.55	48
50.15	127	74.90	595	104.85	88	146.60	193
51.25	96	75.90	398	105.95	45	147.50	73
53.05	73	76.80	450	106.85	83	148.50	85
55.10	395	77.90	1118	107.85	54	155.45	163
56.10	155	78.90	2435	108.70	133	157.45	43
57.10	418	79.80	282	109.80	95	168.40	136
58.00	280	80.85	411	110.70	424	170.40	48
59.10	1931	81.95	151	112.70	103	212.15	198
61.00	36560	82.85	459	120.75	53	214.25	119

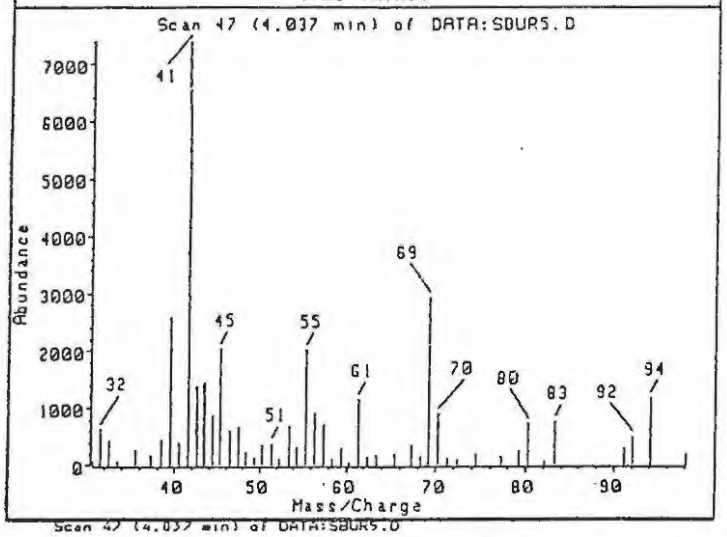


Scan 466 (18.253 min) of DATA:SBUR4.D
SMELLY PLANT EXTRACT

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
36.20	153	66.95	219	97.70	170	137.55	310
37.20	587	67.95	91	98.80	66	138.55	842
39.20	138	69.05	292	101.85	45	139.55	138
40.20	281	70.05	143	104.85	101	140.55	233
41.20	661	71.05	162	105.75	130	141.55	61
42.20	181	72.00	71	106.85	227	146.50	282
43.20	515	72.90	384	107.75	239	147.60	82
44.15	1860	73.90	922	108.70	295	148.50	76
45.15	40912	75.00	921	109.80	149	150.70	87
46.15	15939	75.90	1112	110.70	604	152.50	1413
47.05	10276	76.90	1673	111.90	112	153.50	144
48.05	2234	77.90	4984	112.80	167	154.35	204
49.15	2223	78.90	1790	114.80	39	155.45	290
50.15	253	79.80	561	116.70	70	157.55	88
51.15	198	80.95	345	118.75	73	164.60	58
53.05	84	81.95	139	120.75	85	168.50	100
54.20	70	82.85	480	121.75	53	169.50	90
55.00	462	83.85	124	122.75	106	185.30	24928
56.10	233	84.85	393	123.55	523	186.30	1761
57.10	733	85.95	58	124.65	137	187.30	3850
58.10	1157	88.95	47	125.65	95	188.30	295
59.00	3153	90.70	766	127.60	54	189.30	301
61.00	191616	91.00	1009	128.70	90	190.40	115
62.10	5296	92.80	10251	129.90	25	191.45	40
63.05	8071	93.80	2794	130.60	50	206.20	37
63.95	2751	94.80	1098	134.70	113	212.15	159
64.85	360	95.80	584	136.65	74	214.15	60
65.95	375	96.90	216				

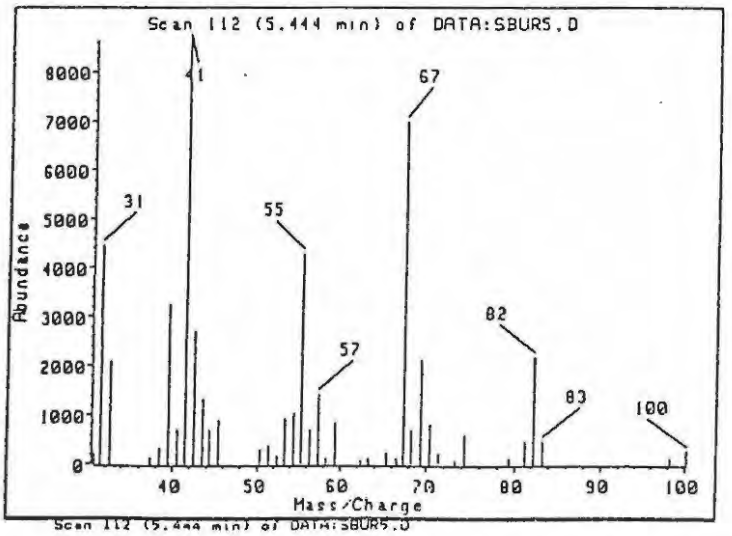
(v) Volatiles - April





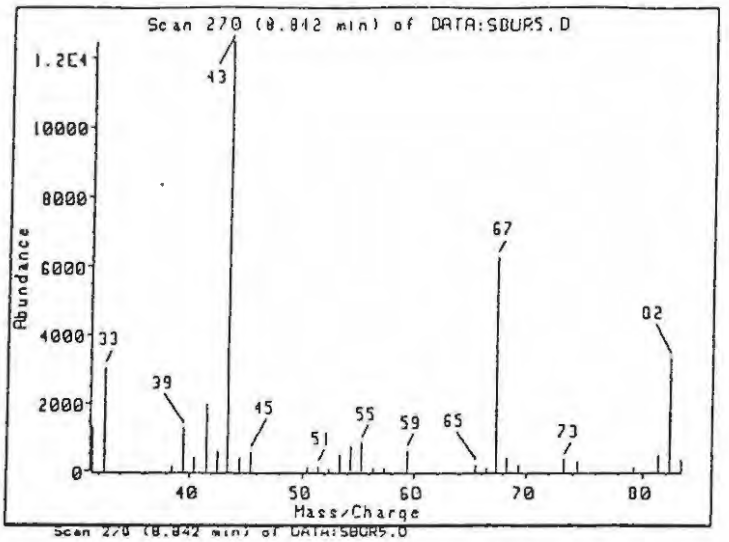
Scan 47 (4.037 min) of DATA:SBUR5.D

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
30.50	68	45.30	2038	57.35	724	71.25	115
31.50	639	46.40	621	58.35	123	72.35	109
32.40	432	47.40	676	59.45	309	74.35	195
33.50	57	48.40	227	60.30	48	77.20	160
35.50	264	49.40	140	61.30	1166	79.20	258
37.35	179	50.30	360	62.30	143	80.30	734
38.45	457	51.40	389	63.30	181	82.20	80
39.45	2575	52.35	125	65.40	187	83.25	765
40.45	407	53.45	700	67.30	361	91.20	314
41.45	7424	54.35	326	68.35	162	92.10	500
42.45	1391	55.35	2026	69.25	2938	94.10	1179
43.45	1443	56.35	917	70.25	903	98.15	202
44.35	877						



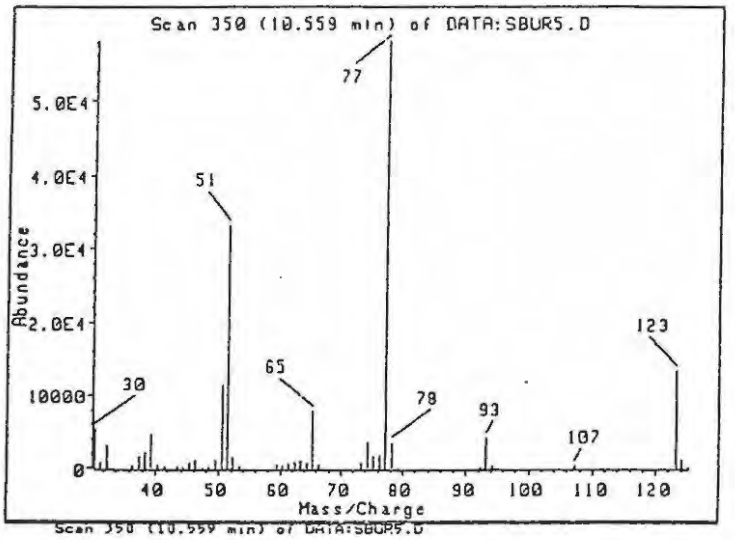
Scan 112 (5.444 min) of DATA:SBUR5.D

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
30.50	220	44.35	709	58.35	127	71.35	227
31.40	4449	45.40	891	59.35	841	73.25	68
32.50	2090	50.30	294	62.30	68	74.25	605
33.35	150	51.40	370	63.30	112	79.30	117
38.45	338	52.35	162	65.30	236	81.20	460
39.45	3258	53.35	915	66.50	124	82.30	2174
40.45	697	54.35	1027	67.30	6992	83.25	469
41.45	8650	55.35	4206	68.25	697	98.15	111
42.45	2701	56.35	704	69.25	2118	100.15	259
43.45	1327	57.35	1425	70.35	824		



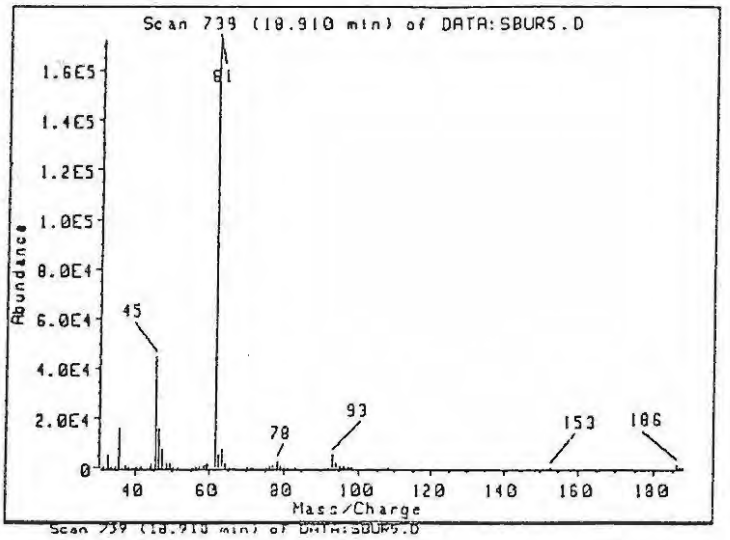
Scan 270 (8.842 min) of DATA:SBUR5.D

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
31.40	1305	44.35	329	56.35	102	69.35	165
32.50	3043	45.40	557	57.35	120	73.25	345
38.45	142	50.40	92	59.35	609	74.35	292
39.45	1269	51.40	149	65.40	183	79.30	103
40.45	382	52.35	84	66.40	105	81.30	468
41.45	1953	53.35	499	67.30	6251	82.30	3315
42.45	567	54.35	732	68.25	405	83.25	327
43.35	12492	55.35	856				



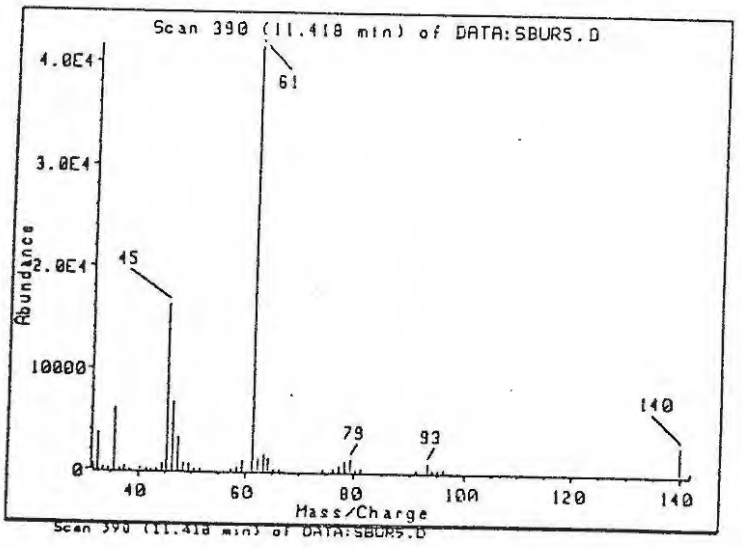
Scan 350 (10.559 min) of DATA:SBUR5.D

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
30.50	5356	49.30	835	60.30	294	77.20	58128
31.50	833	46.30	1080	61.30	580	78.20	3472
32.50	3132	47.40	67	62.30	718	79.20	127
36.50	293	48.40	137	63.30	1050	92.20	125
37.45	1659	49.40	1205	64.30	787	93.20	4178
38.45	2289	50.40	11459	65.30	7798	94.20	317
39.35	4579	51.40	33200	66.30	429	107.10	325
40.35	449	52.25	1581	73.25	827	123.10	13359
41.45	278	53.35	146	74.25	3512	124.00	1011
43.45	204	55.45	105	75.20	1670	125.10	6
44.35	162	59.35	482	76.30	1854		



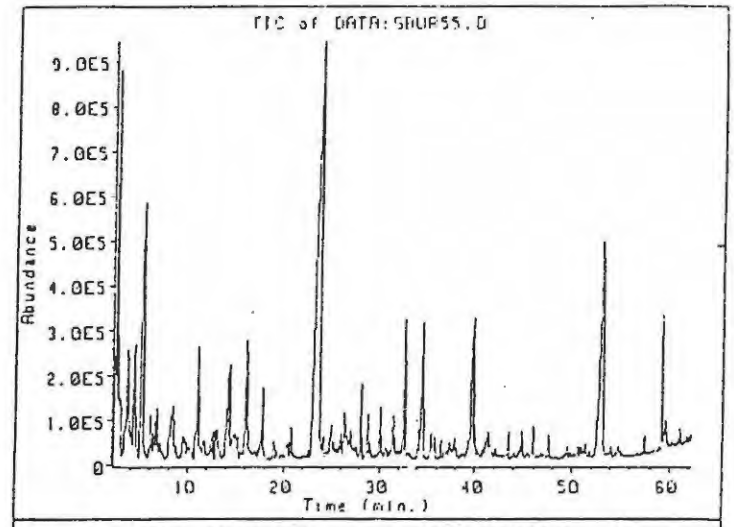
Scan 739 (10.910 min) of DATA:SBUR5.D

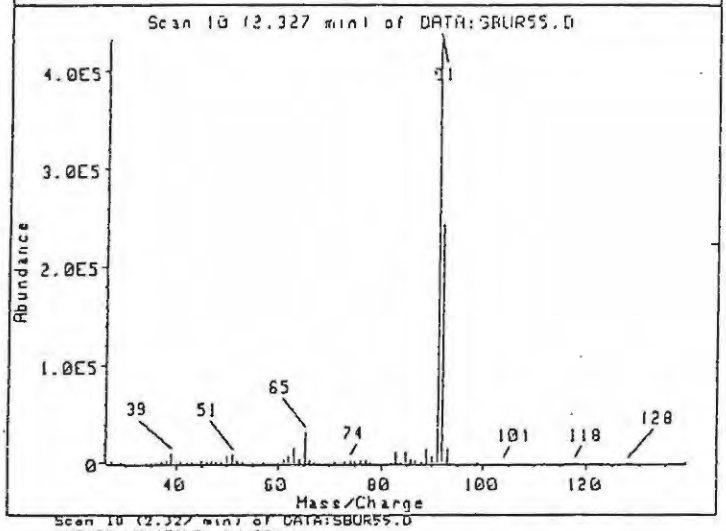
m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
30.50	128	46.30	15633	64.20	1937	83.25	64
31.40	450	47.40	7915	65.30	248	91.10	307
32.40	4810	48.40	1830	66.30	189	92.10	336
33.40	360	49.40	2054	69.25	87	93.10	5196
34.50	448	50.30	174	70.25	76	94.10	1741
35.50	15908	51.40	240	71.35	85	95.10	571
36.40	299	55.35	152	74.25	146	96.10	740
37.35	937	56.35	115	75.20	245	97.20	52
38.35	46	57.35	622	76.10	688	98.05	226
39.45	144	58.35	883	77.20	889	108.10	182
40.35	259	59.35	2079	78.20	2667	152.90	197
41.45	481	61.30	172288	79.20	701	185.80	1378
43.45	331	62.20	5336	80.20	337	186.70	120
44.35	1977	63.20	7549	81.20	157	187.70	375
45.30	44752						



Scan 390 (11.418 min) of DATA:SBUR5.D

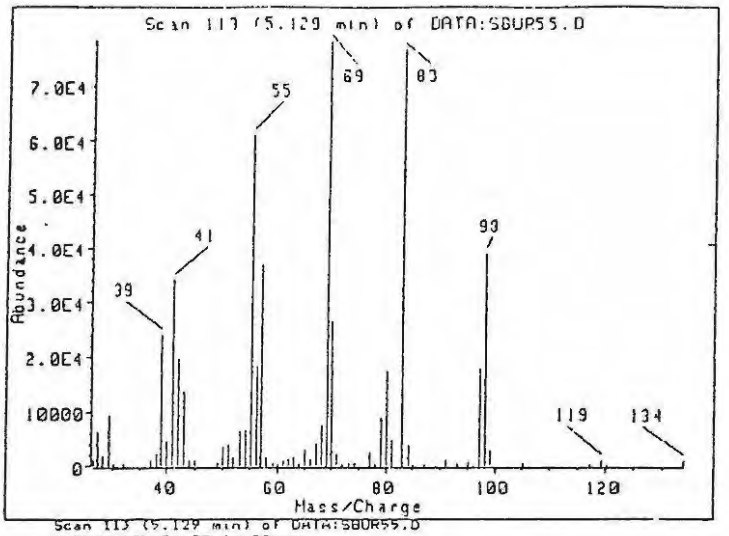
m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
31.50	675	43.45	109	59.35	955	79.20	1233
32.40	3578	44.45	720	61.30	41600	80.20	194
33.40	238	45.30	16300	62.30	1234	81.20	200
34.50	214	46.30	6768	63.30	1758	91.10	101
35.50	6156	47.30	3262	64.20	1324	93.10	910
36.50	132	48.40	738	65.20	170	94.10	159
37.45	348	49.40	678	66.20	134	95.10	115
38.45	48	50.40	101	74.35	194	96.10	201
40.35	286	51.40	194	76.10	243	139.90	2798
41.45	108	57.35	169	77.20	592	141.90	246
42.45	37	58.25	367	78.20	993		





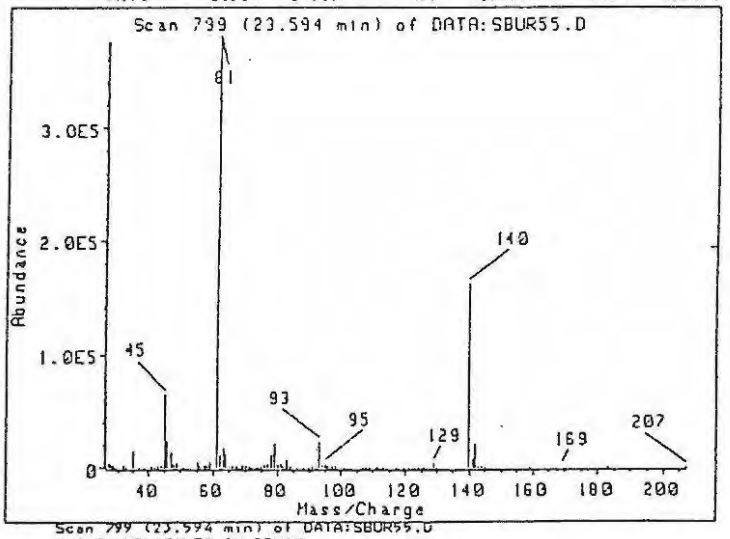
Scan 10 (2.327 min) of DATA:SBUR55.D
AUGUST VOLATILES ON DBWAX

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
26.05	745	47.80	993	69.10	534	90.05	6756
27.05	1574	49.00	1436	70.00	659	91.05	433536
27.95	256	50.00	6710	70.90	1017	91.95	243136
28.25	235	51.10	9280	72.90	1588	93.05	14462
28.95	719	51.95	2330	74.00	2899	94.00	345
35.05	552	53.05	1135	75.00	1658	95.80	145
35.85	140	55.05	925	76.00	2719	97.10	201
36.95	1281	57.05	660	77.00	2812	99.00	212
38.10	2434	58.95	506	78.00	372	104.90	290
39.00	9951	60.05	696	81.05	245	116.85	359
40.10	1313	60.95	3380	81.95	569	117.05	481
41.00	1651	61.95	7173	82.95	11108	118.75	207
42.10	353	63.05	16304	83.95	1776	118.95	206
43.00	1992	64.05	3446	84.95	10953	119.85	400
44.10	470	65.05	32184	85.85	3663	120.65	160
45.00	3012	66.00	3125	86.05	2826	127.90	335
46.10	1121	66.90	332	88.05	1026	138.95	114
47.00	2042	67.90	232	89.05	14961		



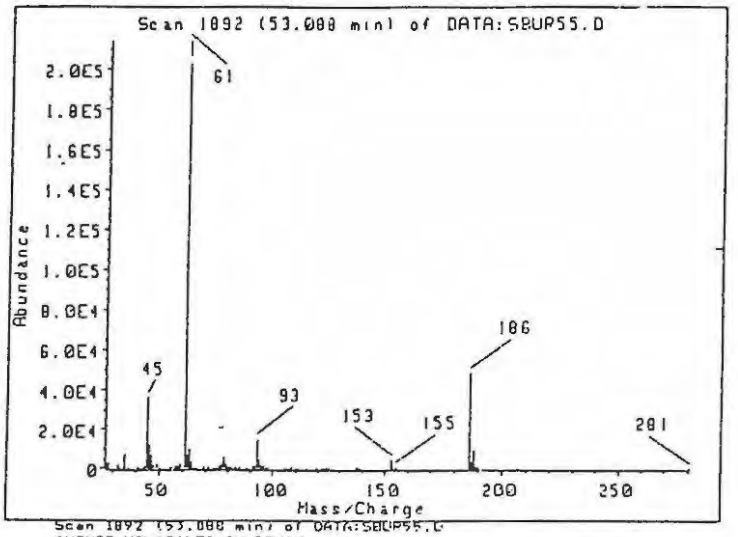
Scan 113 (5.129 min) of DATA:SBUR55.D
AUGUST VOLATILES ON DBWAX

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
26.05	1216	49.10	716	64.95	3015	84.05	3914
27.05	6456	50.00	3363	66.00	1173	84.95	230
28.05	1869	51.00	4008	67.00	4173	87.15	241
29.15	9401	51.85	1585	68.00	7583	89.25	145
30.05	244	53.05	6489	69.00	78504	91.05	1035
30.25	227	54.05	6609	70.00	26536	92.95	414
31.95	562	55.05	60984	70.90	2218	95.00	238
32.05	1076	56.05	18512	72.00	219	96.10	117
38.00	2261	56.95	36744	73.10	404	97.00	17848
39.00	24048	57.85	1567	74.10	422	98.00	39024
39.90	4626	59.15	385	77.00	2367	99.00	2841
41.00	33952	60.05	287	78.00	359	105.10	444
42.00	19608	60.95	982	79.10	8912	117.15	208
43.00	13767	62.05	1271	80.05	17504	119.05	1158
44.00	1155	63.05	1752	81.05	4863	120.15	266
44.90	1032	64.05	479	82.95	76904	133.90	916



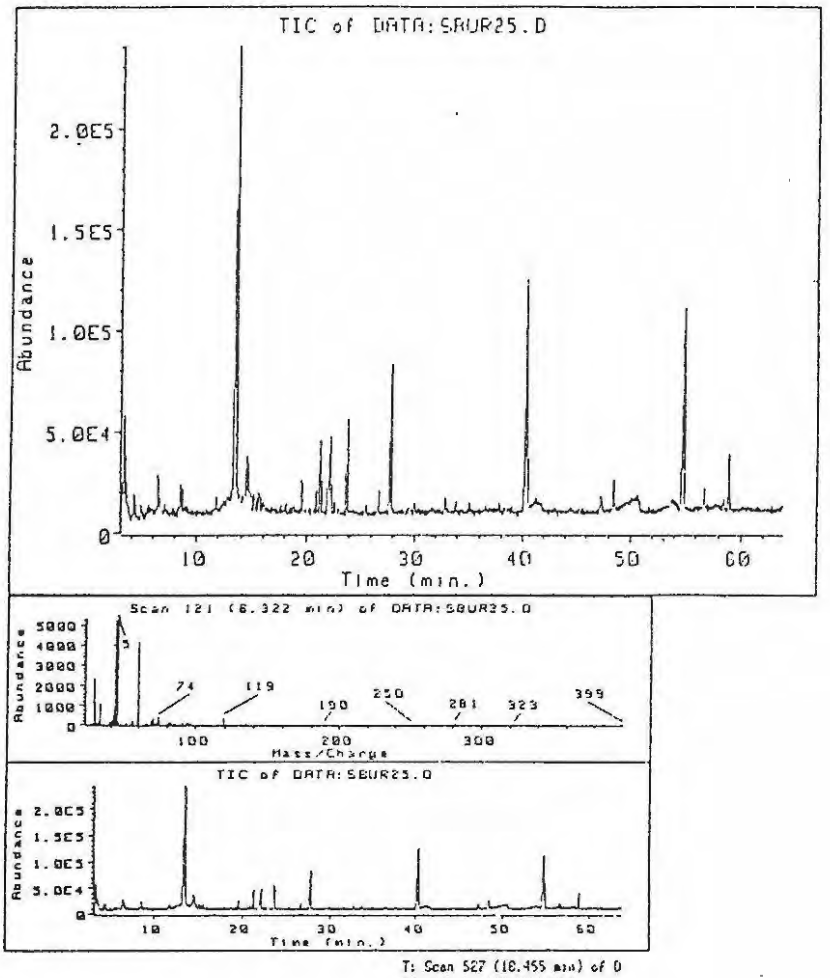
Scan 799 (23.594 min) of DATA:SBUR55.D
AUGUST VOLATILES ON DBWAX

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
26.15	164	57.05	2266	81.95	1081	110.95	596
26.95	3705	57.85	2233	82.95	7315	111.15	597
28.05	1903	58.95	4644	84.05	1041	113.15	263
29.15	582	61.05	376320	85.15	592	117.15	172
31.95	1885	62.05	11957	86.05	299	119.25	144
32.95	414	63.05	17272	88.05	164	121.15	521
35.05	14544	63.95	11318	89.05	202	123.20	242
36.05	220	64.85	1377	89.75	141	124.00	250
37.05	791	65.90	1097	89.95	141	125.10	501
39.00	651	67.10	1021	90.95	1749	126.00	219
41.00	1443	68.00	593	91.95	1274	127.00	995
42.00	484	68.90	1896	92.95	22456	129.10	4077
43.00	1722	70.00	2093	93.90	3219	130.10	487
43.90	2039	71.00	1162	94.90	3208	138.85	289
44.90	64592	71.90	245	95.80	1066	139.85	162176
45.90	23672	72.10	257	97.00	1709	140.95	8009
47.00	13327	73.10	350	98.00	1504	141.95	20920
48.00	3109	73.90	312	99.00	542	142.85	1079
49.00	3888	75.00	1400	100.00	231	143.95	1318
50.10	571	75.90	3146	101.00	807	144.95	306
51.00	418	76.80	4110	106.90	525	159.20	535
52.95	441	77.90	11619	107.85	609	169.05	610
53.95	275	79.00	21624	108.95	650	183.10	399
55.05	5098	79.95	3326	109.95	586	206.90	183
55.95	1247	80.95	3217				



Scan 1892 (53.088 min) of DATA:SBUP55.D
AUGUST VOLATILES ON DBWAX

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
27.05	2202	57.95	1377	81.95	404	114.85	121
28.05	2705	58.95	2482	83.05	760	119.05	238
29.05	220	61.05	214400	84.05	209	121.05	416
32.05	1827	61.95	6681	85.05	496	122.00	164
32.95	189	62.95	10074	87.95	133	122.90	286
34.15	270	63.85	3366	88.65	204	123.80	882
35.05	7311	64.75	408	89.05	276	124.90	474
37.15	326	65.05	336	90.95	1172	125.80	276
39.00	187	66.00	405	91.95	1197	126.90	134
41.00	787	67.00	506	92.95	14919	137.75	644
41.20	178	68.00	185	93.90	4719	138.75	454
43.20	397	69.10	849	94.80	1856	139.85	473
43.90	1271	70.00	253	95.80	1304	150.90	197
44.90	36192	71.00	728	96.80	457	152.80	4477
45.90	12136	73.10	309	98.00	530	153.90	281
47.00	6960	73.90	245	105.10	269	154.80	548
48.00	1774	74.90	478	106.00	244	185.80	48616
49.00	2189	75.80	1543	106.90	414	186.80	3469
50.10	183	76.90	2153	107.85	615	187.80	9225
50.80	260	78.00	5609	108.85	451	188.80	532
52.95	130	78.90	2473	110.05	204	189.80	680
55.05	257	79.95	903	111.05	463	207.00	161
55.95	314	80.95	897	112.05	171	260.15	157

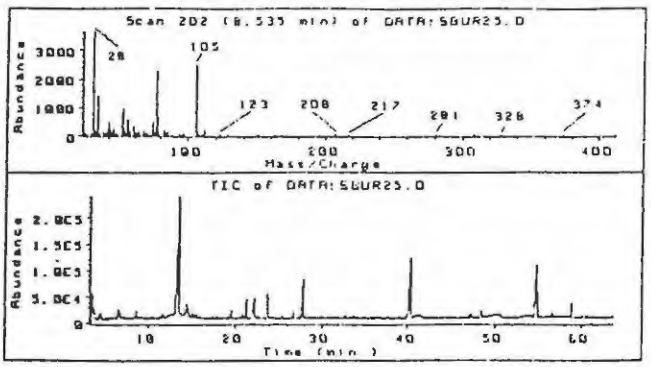


T: Scan 527 (18.455 min) of D
Z: TIC of DATA:SBUR25.D
Y: Scan 22 (3.619 min) of DAT
X: Scan 121 (6.322 min) of DA

DRAW done
[DE]

Scan 121 (6.322 min) of DATA:SBUR25.D
AUGUST VOLATILES

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
22.30	28	44.15	372	75.10	145	115.30	58
24.40	106	44.95	5282	77.40	41	119.10	359
26.40	106	45.85	150	79.00	116	120.80	22
26.60	182	47.05	142	81.10	172	121.10	38
28.10	2318	54.75	83	82.95	160	130.95	50
29.20	720	55.25	231	84.25	60	134.25	86
30.10	70	56.15	165	84.45	72	140.25	35
30.30	77	59.10	284	85.25	36	140.45	35
31.10	199	60.10	4128	86.15	55	146.15	56
32.10	1058	64.30	40	88.85	78	181.05	17
34.10	36	65.00	65	90.95	140	189.55	39
35.00	31	66.10	56	93.15	122	196.20	17
35.30	75	69.00	212	94.05	144	250.10	22
38.85	223	70.10	322	95.15	114	281.25	85
39.15	144	71.10	63	97.15	106	321.95	22
39.95	233	72.20	49	103.45	43	323.35	27
41.15	486	73.10	144	104.70	28	332.80	17
42.05	836	74.10	380	105.30	24	399.40	23
43.05	5230	74.80	112	109.10	40		

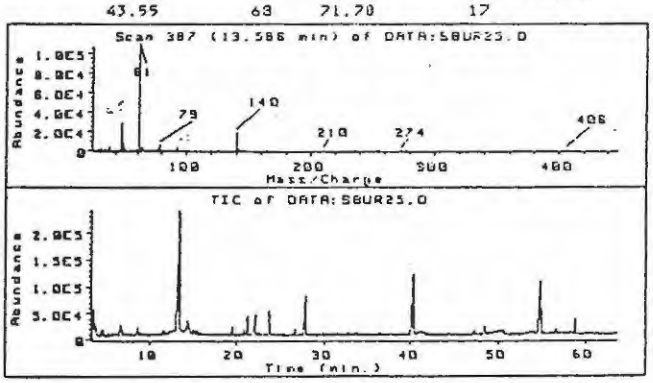


T: Scan 22 (3.619 min) of DAT
Z: TIC of DATA:SBUR25.D
Y: Scan 121 (6.322 min) of DA
X: Scan 202 (8.535 min) of DA

DRAW done
(MS1)

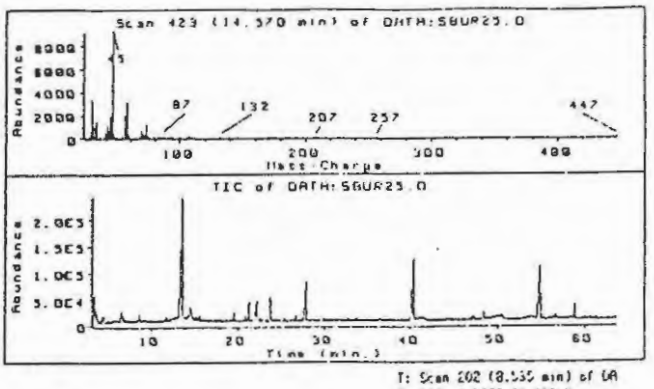
Scan 202 (8.535 min) of DATA:SBUR25.D
AUGUST VOLATILES

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
20.50	86	44.15	264	73.00	208	116.60	19
21.30	55	45.45	96	74.10	523	116.90	26
22.00	23	46.05	75	75.20	156	121.60	39
22.90	59	46.95	21	76.20	117	122.90	69
23.10	64	50.05	710	77.10	2281	127.95	49
26.20	139	51.05	940	78.10	674	148.95	19
27.00	46	52.05	282	79.20	125	150.90	56
28.00	3653	52.95	154	82.15	214	162.80	15
29.20	171	53.35	57	83.15	130	167.90	22
31.20	138	55.05	579	85.15	131	195.25	20
31.40	152	56.25	190	89.05	23	207.50	62
32.10	1433	57.05	123	93.25	80	217.20	19
33.10	50	59.10	367	94.45	50	233.15	15
36.00	174	60.00	106	94.75	67	280.95	32
37.55	53	61.00	95	95.95	109	281.25	60
38.25	149	62.10	86	97.15	57	283.65	15
38.95	251	62.90	155	105.00	2490	308.20	54
40.05	496	64.00	23	106.10	2469	308.75	28
40.75	262	67.10	174	107.00	210	328.35	18
41.15	185	68.20	86	108.10	26	374.25	37
42.15	126	69.00	89	110.10	51	389.20	20
42.75	132	69.50	46	111.40	27	399.40	17
43.35	78	71.00	35	112.00	237	411.85	23
43.55	68	71.70	17				



Scan 387 (13.586 min) of DATA:SBUR25.D
AUGUST VOLATILES

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
22.10	99	53.95	54	79.00	6973	115.10	91
22.40	95	54.95	86	80.00	838	117.10	68
24.20	93	55.25	124	81.00	600	119.30	31
24.50	121	55.85	142	81.95	176	120.20	47
24.80	166	56.25	148	83.05	167	124.10	113
26.00	214	57.15	660	83.95	35	126.30	65
26.30	182	58.05	1002	84.55	28	126.50	64
27.10	1811	59.00	1578	84.85	44	127.15	26
28.10	2612	60.00	571	86.15	64	130.35	31
29.00	563	61.00	106640	87.15	76	130.85	123
30.20	166	62.00	3025	88.05	119	132.15	30
31.10	1543	63.00	4887	90.95	631	137.45	21
32.10	1450	63.90	4425	92.05	239	139.25	60
33.00	271	65.00	590	93.05	4482	140.05	19472
34.10	129	65.90	341	94.05	726	141.05	1063
35.10	4885	67.00	78	94.85	635	142.05	2653
37.05	436	67.80	62	96.05	284	144.15	178
37.65	40	68.10	200	98.15	27	145.35	27
39.05	149	69.00	279	99.05	107	148.05	38
39.95	159	70.00	74	99.95	80	158.30	53
41.05	778	70.80	128	100.45	39	162.10	169
42.15	138	71.80	38	100.95	44	162.10	162
42.95	484	72.10	80	101.05	108	209.80	26
44.05	1450	73.10	141	102.05	43	235.35	20
44.95	29192	74.00	91	102.95	44	255.10	11
45.95	11080	74.30	112	103.35	29	273.55	26
47.05	6644	75.10	118	107.10	100	277.35	12
48.05	1444	75.90	1026	108.10	168	406.25	34
49.05	1088	76.90	1360	109.00	106	448.35	11
50.95	156	78.00	3697	112.10	41		

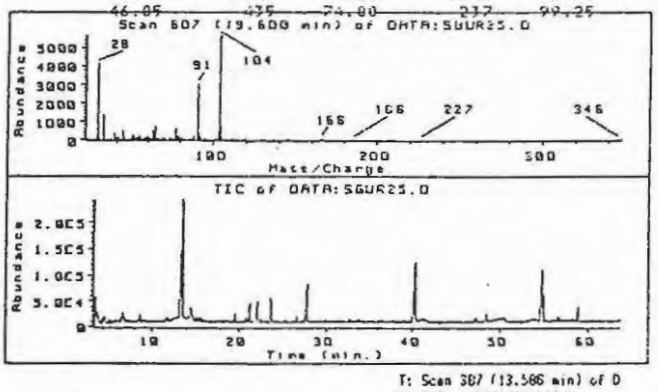


T: Scan 202 (8.535 min) of DA
Z: TIC of DATA:SOUR25.D
Y: Scan 387 (13.586 min) of D
X: Scan 423 (14.570 min) of D

DRAW done
[MSI]

Scan 423 (14.520 min) of DATA:SOUR25.D
AUGUST VOLATILES

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
21.50	128	46.85	184	74.20	215	100.15	39
21.70	126	48.15	35	74.50	91	100.85	37
22.00	138	48.45	52	75.70	10	102.05	100
25.30	63	50.95	139	76.00	33	105.10	22
26.20	181	52.95	210	79.10	125	106.40	113
27.10	624	55.05	2060	80.10	133	107.20	42
28.00	3391	56.15	357	83.15	158	108.10	37
29.10	999	57.05	3154	83.75	69	108.40	53
30.00	132	58.15	296	84.15	121	109.30	37
31.00	986	59.00	287	85.15	92	109.90	11
32.00	1470	61.90	41	86.15	71	112.00	13
34.90	110	63.10	77	87.05	199	132.25	82
37.45	42	64.00	53	87.45	108	139.25	30
37.95	137	64.60	43	87.75	85	145.15	37
39.05	792	67.10	241	89.95	27	169.40	23
39.95	350	68.20	124	91.15	79	190.85	29
41.05	1236	69.10	672	91.55	20	192.90	22
41.95	569	69.90	328	93.15	134	207.30	46
43.05	1956	70.90	341	94.15	104	256.70	68
43.95	1255	72.10	214	97.95	95	432.10	14
44.95	9117	73.20	1242	98.75	22	446.85	14
46.85	435	74.00	237	99.25	48		

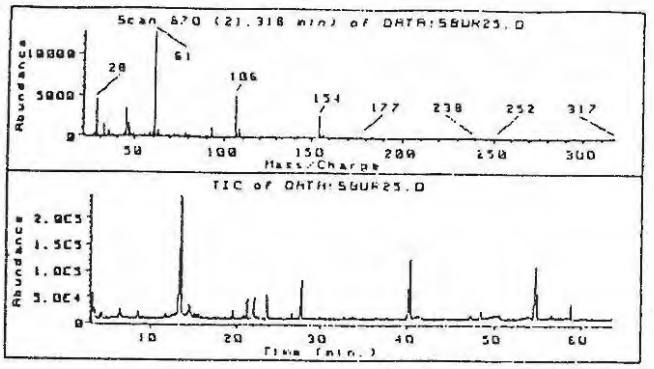


T: Scan 387 (13.586 min) of D
Z: TIC of DATA:SOUR25.D
Y: Scan 423 (14.570 min) of D
X: Scan 607 (19.600 min) of D

DRAW done
[MSI]

Scan 607 (19.600 min) of DATA:SOUR25.D
AUGUST VOLATILES

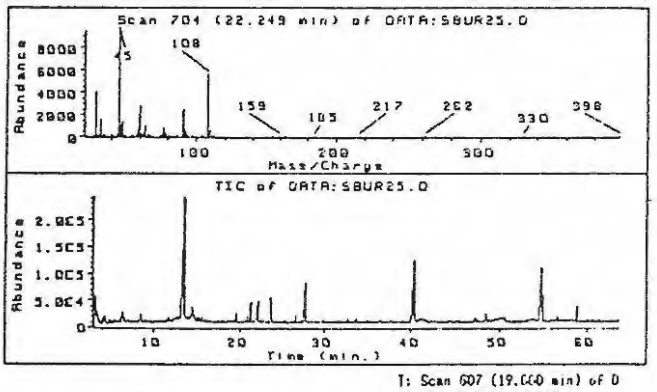
m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
20.80	40	55.05	223	78.80	136	106.00	71
22.80	41	55.85	90	79.20	257	107.20	35
25.50	57	57.25	104	79.60	140	108.50	31
25.90	43	58.05	54	79.80	138	111.80	68
27.00	171	58.85	136	81.30	150	114.50	43
28.00	4133	60.10	218	82.15	104	118.00	32
29.10	170	60.80	102	83.15	36	119.40	71
32.10	1385	61.60	39	85.85	40	129.45	25
34.10	55	62.20	72	87.25	94	132.15	18
36.45	107	63.00	467	87.65	31	136.25	16
38.95	363	64.10	169	89.05	227	145.55	45
40.05	182	65.10	749	91.05	3032	164.60	14
41.25	162	66.20	86	92.05	375	166.30	68
42.05	113	67.10	74	92.95	136	184.05	11
44.05	518	69.30	119	95.05	99	186.15	47
45.15	181	70.30	64	97.05	26	201.30	39
47.25	30	71.00	43	101.35	42	207.40	26
49.25	93	74.20	150	102.15	101	227.25	15
50.05	220	75.10	56	103.05	497	319.65	35
51.15	294	75.80	93	104.05	5656	345.70	35
51.85	71	77.10	466	105.00	709	348.10	19
53.15	103	78.00	610				



T: Scan 423 (14.570 min) of D
Z: TIC of DATA:SBUR25.D
Y: Scan 607 (19.000 min) of D
X: Scan 670 (21.318 min) of D

DRAW done
[MS1]

20.90	189	40.95	226	75.20	136	106.00	4907
25.60	54	52.35	41	76.00	98	107.10	527
27.00	344	52.95	39	77.00	75	108.00	918
28.00	4515	55.15	143	78.00	560	109.00	158
29.10	50	57.05	216	79.20	148	111.00	72
31.20	51	58.15	407	80.00	173	121.20	77
32.10	1321	59.00	488	81.20	99	128.05	16
33.30	85	60.00	983	82.95	53	142.05	38
35.10	612	61.00	12584	85.25	97	154.00	2538
37.15	66	61.90	296	86.85	52	155.00	206
37.35	57	63.00	762	89.15	116	156.10	266
37.75	39	63.00	170	91.05	218	157.00	41
40.15	108	64.80	39	93.05	1027	158.20	99
40.75	72	67.80	84	94.85	107	168.40	40
41.15	128	69.10	48	95.15	101	170.20	26
42.15	16	71.40	78	96.15	45	177.15	31
43.15	251	71.90	140	98.35	35	183.25	12
44.05	504	72.80	271	101.55	69	238.45	25
45.05	3269	73.10	254	101.75	69	239.05	23
46.05	1481	74.10	165	104.35	112	251.60	17
46.95	1043	75.00	134	105.20	81	317.35	18
47.95	260						

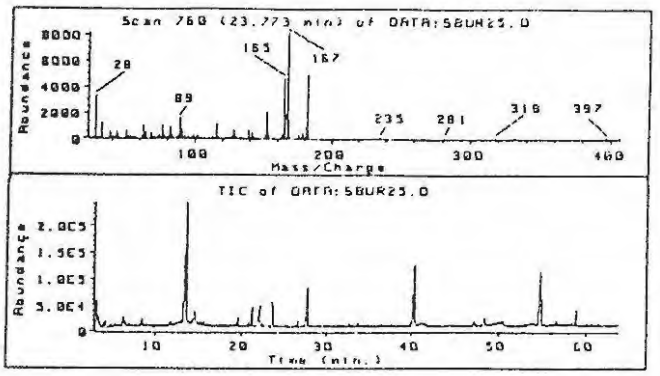


T: Scan 607 (19.000 min) of D
Z: TIC of DATA:SBUR25.D
Y: Scan 670 (21.318 min) of D
X: Scan 704 (22.249 min) of D

DRAW done
[MS1]

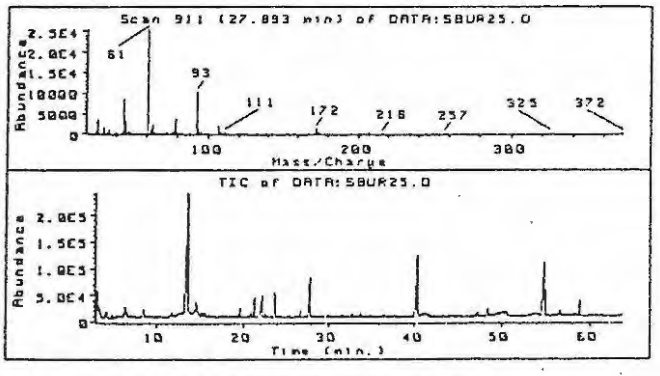
Scan 704 (22.249 min) of DATA:SBUR25.D
AUGUST VOLATILES

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
20.80	45	53.15	98	77.90	331	109.20	193
22.40	96	54.15	95	79.10	263	109.90	588
26.20	114	55.15	143	80.20	72	111.40	118
26.40	107	55.85	93	80.80	119	112.10	69
28.10	4066	57.15	326	82.05	51	112.40	53
29.10	188	58.05	686	82.95	66	119.40	59
29.70	139	59.00	2003	84.95	79	134.95	40
31.10	99	60.00	2782	85.15	59	149.25	17
32.10	1622	60.80	251	87.05	182	154.10	15
33.10	229	62.00	180	89.95	2424	159.40	66
34.10	149	63.00	622	90.95	2459	163.70	16
35.80	152	64.00	1091	91.95	685	184.55	36
36.75	19	65.00	327	92.95	468	185.15	28
37.05	30	66.10	123	93.95	152	217.10	64
39.95	280	66.90	94	95.05	101	221.35	11
41.05	167	67.20	106	96.05	49	248.20	10
42.85	165	68.10	96	97.25	39	258.90	23
43.95	827	69.00	89	99.35	46	260.70	22
45.05	9493	73.90	137	100.15	60	262.40	34
45.95	795	75.10	158	103.25	27	330.15	22
47.05	1118	76.10	481	104.15	141	397.30	14
47.95	1399	77.00	870	100.00	5590	397.70	24
49.05	127						



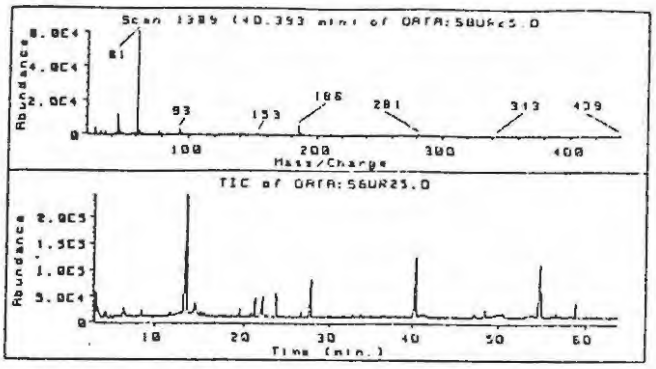
Scan 760 (23.773 min) of DATA:SBUR25.D

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
23.00	25	62.00	261	93.05	156	143.15	95
24.10	45	63.00	993	94.15	35	145.45	24
25.10	17	64.00	198	94.35	34	150.10	226
25.40	21	65.10	550	95.05	104	151.10	327
26.10	52	67.20	51	97.15	85	152.10	2019
27.00	108	68.00	97	98.05	178	153.10	654
28.10	3306	68.80	432	99.15	204	154.10	191
29.20	148	69.40	199	101.25	115	157.30	38
30.20	24	70.30	35	102.05	263	162.30	74
30.50	46	71.30	142	103.05	124	163.10	347
30.80	41	73.20	132	104.80	63	164.20	367
31.10	66	74.10	259	105.90	23	165.20	4644
32.10	1228	75.10	374	110.10	51	166.20	1936
32.95	80	76.10	1032	113.00	95	167.20	8185
39.05	502	77.00	344	115.10	1098	168.20	1337
39.95	300	78.10	272	116.20	123	169.30	159
41.05	238	79.20	157	116.90	63	176.05	314
41.25	209	80.20	76	119.10	18	177.05	106
42.85	192	81.20	340	119.30	20	178.15	441
44.05	520	82.15	882	122.20	12	179.15	285
44.95	230	82.65	855	125.20	90	181.15	862
46.95	71	82.85	798	126.20	119	182.15	4941
50.05	225	83.55	213	127.10	152	183.15	718
51.05	639	83.75	207	128.05	624	192.45	10
51.95	141	84.45	49	129.15	174	200.70	41
53.15	136	85.05	95	129.95	20	235.05	21
54.15	101	86.15	174	132.15	35	235.35	34
55.25	158	86.95	255	133.25	58	281.05	27
55.75	65	88.15	333	133.95	58	318.45	21



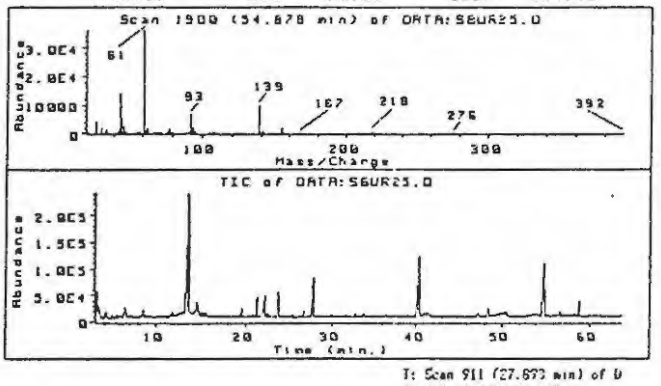
Scan 911 (27.893 min) of DATA:SBUR25.D
AUGUST VOLATILES

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
20.80	17	50.25	97	81.00	374	124.30	42
21.90	44	53.25	41	82.15	74	125.20	87
23.90	67	55.05	208	82.75	146	127.00	27
24.20	56	55.85	90	83.05	70	132.25	67
25.80	57	57.15	263	83.35	54	138.35	24
26.00	62	58.05	184	88.15	87	142.25	105
27.00	625	59.00	170	91.95	135	153.30	18
28.10	3545	61.00	25432	92.95	10243	157.20	29
29.10	98	62.00	896	94.05	943	167.00	25
31.00	109	63.00	1423	95.15	796	167.30	19
32.10	1525	64.00	2145	96.05	255	167.60	37
33.30	95	65.00	426	98.15	38	171.20	45
34.10	83	66.00	248	98.55	57	172.10	1244
35.00	1030	66.90	102	100.45	73	173.15	177
36.20	102	67.70	79	107.00	1941	174.05	290
39.05	74	69.10	128	108.10	433	207.10	32
39.95	340	71.10	161	109.00	296	207.90	49
41.05	268	72.60	68	110.10	77	216.20	86
43.15	181	73.20	65	110.90	495	234.15	21
44.05	633	73.60	49	113.30	22	249.20	19
45.05	8552	76.10	315	119.10	61	256.90	22
46.05	3368	77.00	549	121.30	45	257.90	11
47.05	2694	78.10	1099	123.00	79	324.95	27
48.05	769	79.00	3765	123.30	43	327.25	20
48.95	525	80.00	545	123.60	35	372.35	19



Scan 1369 (40.393 min) of DATA:SBUR25.D
AUGUST VOLATILES

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
21.50	23	48.95	972	79.10	822	129.25	20
22.20	56	49.85	83	80.10	208	133.05	38
23.90	92	50.95	103	81.10	321	133.35	26
27.10	1354	55.05	316	83.35	88	134.25	31
28.10	3929	56.05	138	84.15	31	138.05	95
29.00	291	57.05	868	85.05	172	139.45	80
30.30	49	58.05	418	85.95	96	141.05	29
31.20	129	59.10	987	86.35	73	152.30	47
32.00	1636	60.00	400	86.55	79	152.50	35
33.10	66	61.00	60872	89.15	64	153.10	340
34.00	49	62.00	1991	90.95	321	166.60	36
35.10	2084	63.00	2691	92.05	221	175.05	44
35.90	120	64.00	1169	93.05	3008	186.05	5262
39.05	191	64.90	135	93.95	1504	187.05	427
39.95	182	66.00	201	95.05	359	187.95	1062
41.15	180	67.00	233	95.95	250	188.95	49
41.75	142	69.10	192	97.95	72	210.70	24
42.25	92	71.10	130	98.95	116	211.10	22
42.95	502	72.20	154	100.35	50	231.05	37
43.95	1003	73.20	69	102.45	24	281.25	167
44.95	11820	74.90	165	108.00	166	343.30	28
46.05	5725	75.90	400	109.10	100	410.25	12
47.05	3141	77.00	441	115.20	89	438.90	32
48.05	900	78.00	1617	124.00	177		

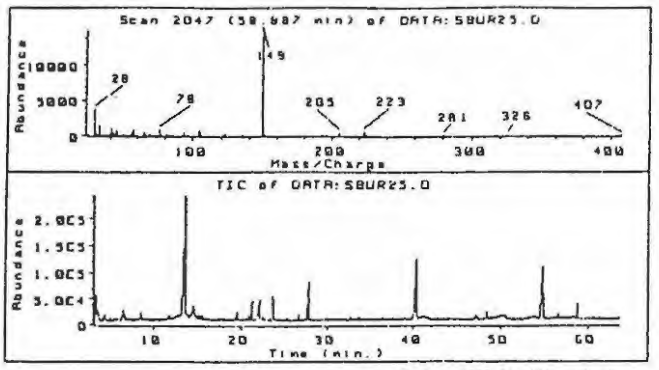


1: Scan 911 (27.693 min) of D
2: TIC of DATA:SBUR25.D
Y: Scan 1369 (40.393 min) of
X: Scan 1900 (54.878 min) of

DRAW done
(MS1)

Scan 1900 (54.878 min) of DATA:SBUR25.D
AUGUST VOLATILES

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
21.80	25	57.05	189	78.00	1424	122.70	18
22.70	45	58.05	508	79.00	1836	123.00	30
27.00	754	58.95	605	80.00	505	125.00	140
28.10	4002	60.10	369	81.10	198	126.10	96
30.90	99	61.10	35992	84.15	64	128.15	14
32.00	1668	62.00	761	87.45	49	138.95	10043
33.10	150	63.00	1542	88.95	110	139.95	468
34.00	137	63.90	1673	88.95	143	140.85	1101
35.10	1499	64.90	177	90.95	388	142.05	102
37.15	82	65.90	269	92.05	139	153.20	195
41.05	128	67.10	233	92.95	6835	154.00	1918
41.35	58	68.10	134	94.05	2208	156.10	150
42.45	85	69.00	249	94.95	1107	167.40	24
43.05	249	69.90	42	95.95	442	207.90	26
43.95	1340	70.40	80	105.90	565	218.05	272
44.95	13963	70.90	146	107.00	691	219.25	61
45.95	5247	71.10	153	108.00	429	219.95	167
47.05	2682	72.30	117	109.20	134	275.55	33
48.05	813	73.20	50	109.90	106	283.55	31
49.05	585	76.00	307	111.00	275	381.40	13
55.05	243	77.10	496	117.10	75	392.10	24
56.05	145						

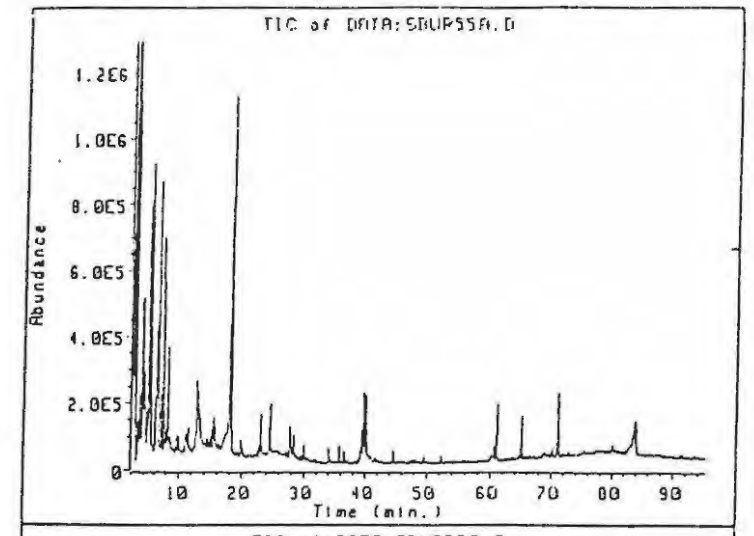


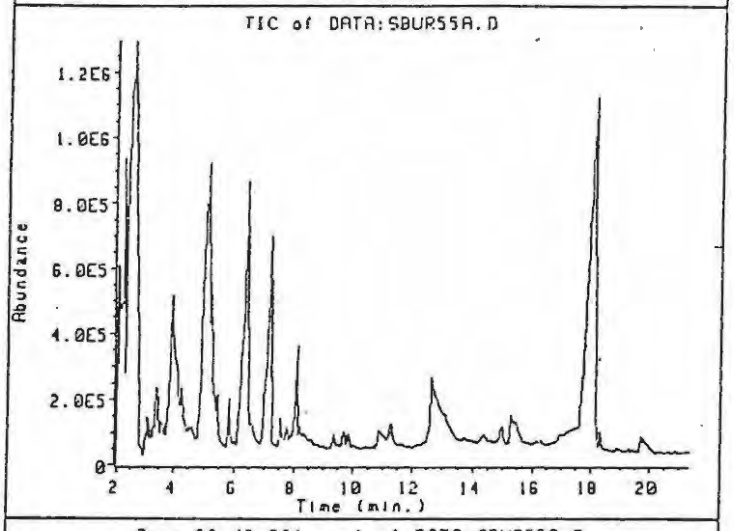
T: Scan 1387 (40.393 min) of
Z: TIC of DATA:SBUR25.D
Y: Scan 1900 (54.878 min) of
X: Scan 2047 (58.887 min) of

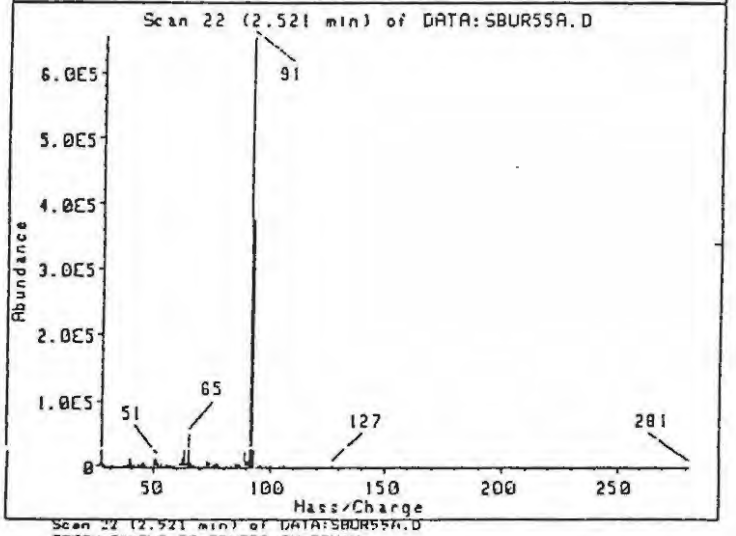
DRAW done
(MSI)

Scan 2047 (58.887 min) of DATA:SBUR25.D
AUGUST VOLATILES

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
22.40	56	52.45	53	81.20	203	122.10	270
24.00	43	53.25	72	81.95	31	123.20	210
28.00	3774	55.05	427	82.95	111	129.05	22
29.00	632	56.05	651	84.95	105	131.95	87
30.90	29	57.15	886	86.15	101	135.05	103
32.00	1432	58.05	144	88.35	36	135.95	32
34.00	56	58.95	84	90.05	37	149.05	14988
34.30	73	61.10	61	91.15	156	150.00	1342
34.60	71	64.10	140	91.95	55	151.00	106
36.20	89	65.00	612	93.05	581	175.25	15
36.85	87	65.80	83	94.05	106	175.95	13
37.05	90	68.10	34	95.25	120	193.15	19
39.05	238	69.10	197	95.45	86	195.15	37
39.95	320	70.00	92	96.25	34	205.10	391
40.95	1207	70.90	79	97.05	77	217.90	27
41.85	210	71.70	195	99.15	18	223.15	684
43.05	484	72.90	135	99.95	12	224.15	93
44.05	854	73.20	142	100.25	14	225.15	13
45.05	266	73.90	137	101.15	26	280.85	20
47.05	60	74.10	136	104.05	775	325.65	67
48.25	66	76.20	950	105.00	751	355.05	19
49.05	62	77.10	180	105.90	49	359.05	38
49.95	316	78.80	61	110.20	34	398.00	15
50.75	158	79.20	136	115.30	30	398.40	30
51.25	100	80.50	11	121.10	287	407.35	31

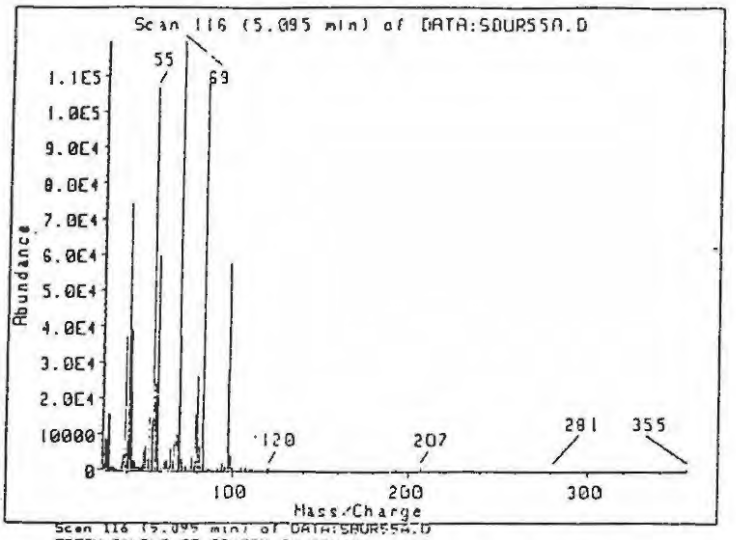






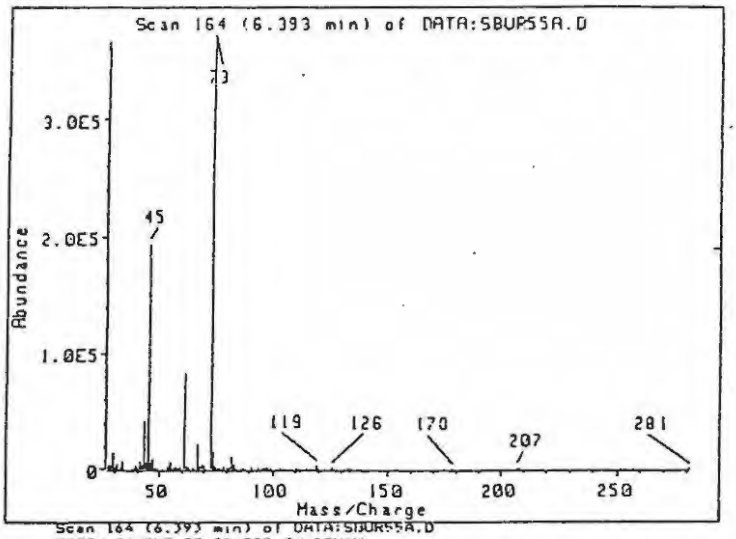
Scan 22 (2.521 min) of DATA:SBUR55A.D
FRESH SAMPLE OF SBUR55 ON DBWAX

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
26.05	933	49.00	1495	67.00	514	86.95	2617
27.05	1932	49.90	9507	67.90	206	87.95	763
28.15	228	51.00	12281	68.90	504	89.05	20304
29.15	644	51.95	3312	69.90	590	90.05	9334
30.95	352	53.05	1768	71.10	870	91.05	654400
35.95	162	55.05	1559	72.00	482	92.05	374336
37.05	1408	56.05	379	73.00	2426	92.95	24288
38.10	2321	57.05	670	73.90	5473	93.90	951
39.00	11331	58.05	321	75.00	2782	95.10	217
40.00	1149	58.85	523	76.10	4368	96.40	142
41.00	1821	60.05	960	77.00	3841	98.20	199
42.00	602	60.95	6100	78.10	552	100.00	464
42.90	2619	61.95	11674	80.95	641	103.90	221
44.10	666	63.05	24296	83.05	446	106.00	470
45.10	5960	63.95	4886	83.95	1044	126.90	347
46.10	2300	65.05	49800	84.95	3247	127.10	355
47.00	1130	66.00	5365	85.95	4701	280.95	152
47.80	507						



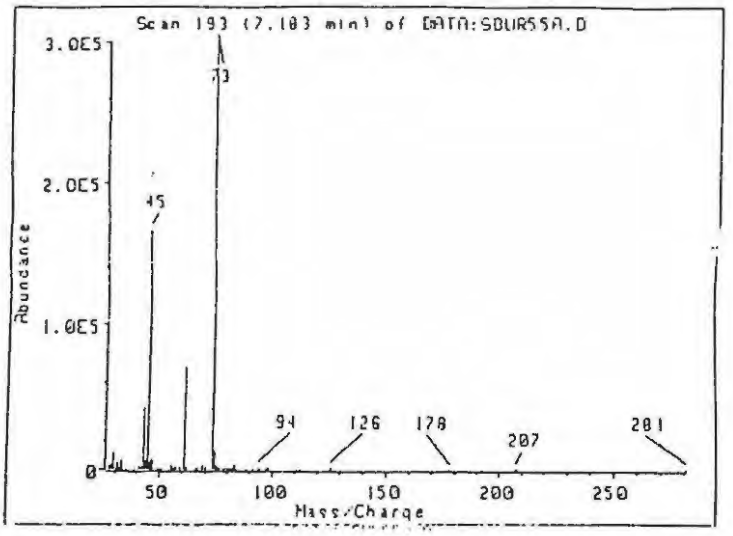
Scan 116 (5.095 min) of DATA:SBURSSA.D
FRESH SAMPLE OF SBURSS ON DBWAX

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
26.05	1402	50.00	5246	69.00	119592	91.85	238
27.05	8855	51.10	6825	70.00	37224	92.95	354
28.05	3031	52.05	2787	71.00	3258	94.00	1904
29.05	15357	52.95	14569	71.90	388	95.00	1169
29.85	362	53.95	13402	72.80	1070	96.00	602
30.95	726	54.95	106408	73.90	686	97.00	26520
32.15	726	56.05	23728	75.90	1022	97.90	57400
33.95	121	57.05	59800	77.00	3863	99.00	4127
37.15	1497	58.05	2794	78.10	781	100.10	280
38.00	4346	59.05	815	79.90	15578	105.00	473
39.00	32544	59.85	469	79.95	26008	107.10	189
40.00	2845	60.95	2374	80.95	6642	107.95	585
41.00	74056	61.95	2331	81.95	1149	109.85	131
42.00	39344	63.05	2761	82.95	109624	110.85	159
43.00	21064	63.95	498	84.05	5615	118.95	271
43.90	2394	64.15	547	84.85	513	120.15	280
45.00	1994	65.05	5703	86.05	170	192.75	106
46.00	598	65.90	2107	87.05	175	206.80	532
47.10	864	66.90	7951	88.15	203	280.85	264
48.00	437	68.00	10107	91.05	505	354.70	219
49.10	950						

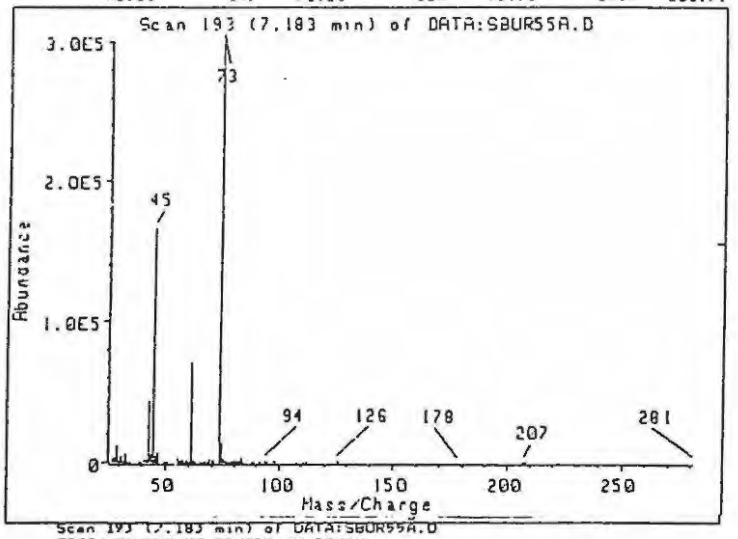


Scan 164 (6.393 min) of DATA:SBURSSA.D
FRESH SAMPLE OF SBURSS ON DBWAX

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
26.15	549	50.00	442	72.90	367232	95.00	729
27.05	4159	51.10	825	74.00	14943	95.80	272
27.95	3691	52.15	366	75.00	2462	96.80	1025
28.95	14448	53.05	2194	76.00	738	97.90	1087
30.05	853	53.95	2042	76.90	805	99.00	167
30.95	5060	54.95	5873	77.70	190	102.90	179
32.05	1010	56.05	1276	78.10	227	105.00	308
33.05	6988	57.05	1933	78.90	2147	108.05	176
37.40	111	58.05	476	79.95	1241	109.85	180
38.10	311	58.95	1002	80.95	2586	110.95	584
39.00	3271	60.95	82184	81.95	10074	114.85	217
40.00	768	61.95	1977	83.05	4069	116.85	418
40.90	7050	62.95	943	83.95	265	119.05	2742
41.90	3908	64.05	394	84.85	309	120.05	326
43.00	42080	65.05	1339	87.05	173	126.10	1368
44.00	6131	65.80	524	87.95	517	133.10	271
45.10	193664	66.90	21968	88.05	496	134.00	601
46.00	5901	68.00	1867	88.95	290	178.10	222
47.00	8463	69.00	4114	90.85	996	206.90	740
48.10	426	70.00	2904	91.05	540	207.90	164
48.80	143	71.20	624	93.90	1404	280.75	427

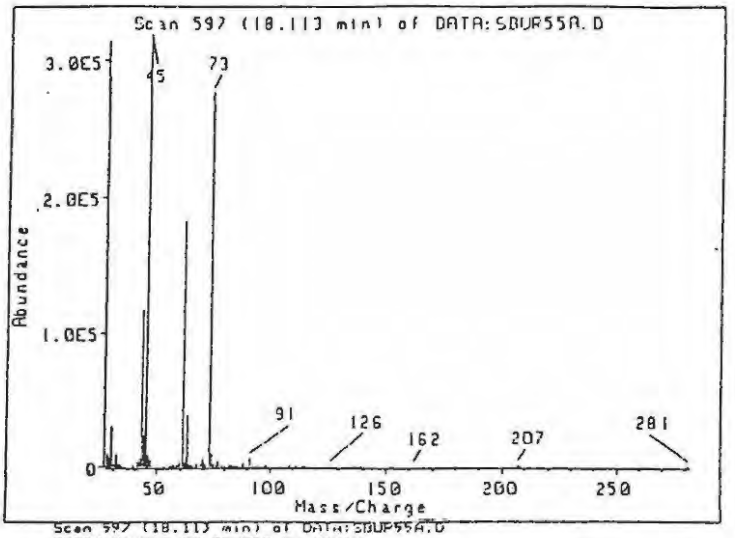


28.95	14438	53.05	2154	76.00	778	97.90	1087
30.05	863	53.95	2042	76.90	805	99.00	167
30.95	9060	54.95	5873	77.70	190	102.90	179
32.05	1010	56.05	1276	78.10	227	105.00	308
33.05	6988	57.05	1933	78.90	2147	108.05	126
33.40	111	58.05	476	79.95	1241	109.85	188
38.10	311	58.95	1002	80.95	2586	110.95	584
39.00	3271	60.95	82184	81.95	10074	114.85	217
40.00	768	61.95	1977	83.05	4069	116.85	418
40.90	7050	62.95	943	83.95	245	119.05	2742
41.90	3908	64.05	394	84.85	309	120.05	326
43.00	42080	65.05	1339	87.05	173	126.10	1368
44.00	6131	65.80	524	87.85	517	133.10	271
45.10	193664	66.90	21968	88.05	496	134.00	601
46.00	5901	68.00	1867	88.95	790	178.10	222
47.00	8463	69.00	4114	90.05	996	206.90	740
48.10	426	70.00	2904	91.85	540	207.90	164
48.80	143	71.20	624	93.90	1404	280.75	427



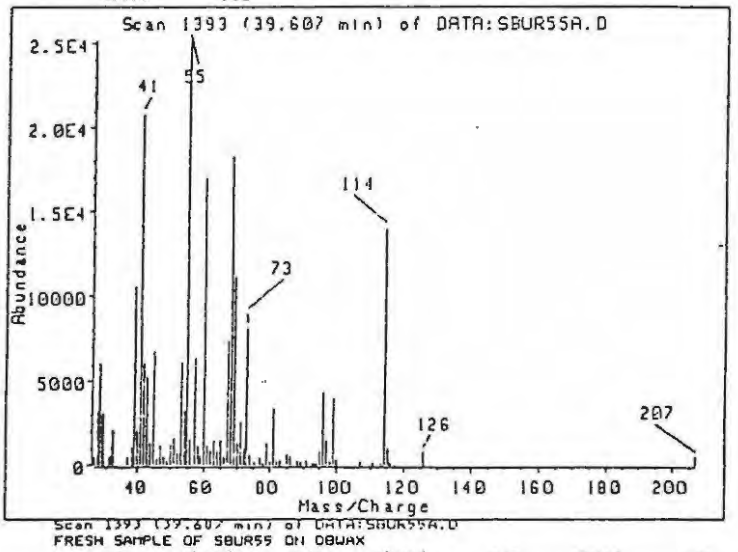
Scan 193 (7.183 min) of DATA:SBUR55A.D
FRESH SAMPLE OF SBUP55 ON DBWAX

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
25.95	363	47.90	333	65.80	202	88.05	657
27.05	3422	49.20	210	66.10	225	88.85	614
28.05	3990	49.90	210	67.00	1261	91.05	345
29.05	11872	50.10	201	68.00	519	91.85	656
30.15	856	50.40	179	69.00	2746	93.80	1413
31.05	4554	50.70	131	70.00	1906	94.90	332
31.95	1422	51.10	287	71.00	623	96.00	523
33.05	6715	53.05	650	73.00	300864	97.10	741
34.15	221	54.05	472	74.00	13239	98.00	939
35.25	183	54.95	3072	74.90	2365	100.80	168
38.10	216	55.75	931	76.00	900	107.05	139
38.90	1311	55.95	919	77.00	471	110.75	484
40.00	416	57.05	1674	77.90	185	112.05	178
41.00	2883	58.05	464	79.00	1149	125.90	1384
42.00	2946	59.05	1946	79.95	810	177.90	218
43.00	43064	60.05	591	80.85	974	206.70	652
44.00	6215	61.05	70376	81.85	721	206.90	656
45.00	166272	61.95	1886	82.95	3147	207.90	165
46.00	4934	63.05	621	83.95	373	280.85	490
47.00	7325	65.05	415				



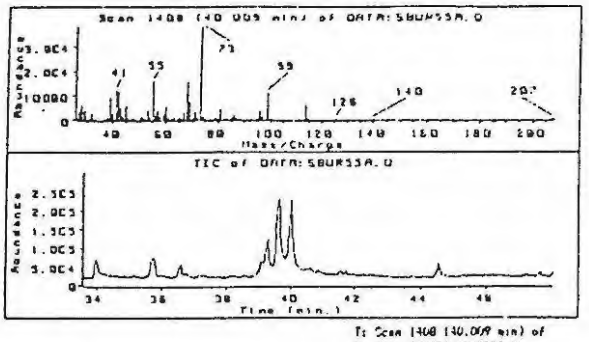
Scan 597 (18.113 min) of DATA:SBUR55A.D
FRESH SAMPLE OF SBUR55 ON DBWAX

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
26.05	1951	51.30	217	74.00	10679	96.00	422
27.05	9474	53.05	821	75.00	2929	96.90	669
27.95	6734	53.85	493	76.00	767	97.90	903
28.95	31328	55.05	1458	77.00	4147	103.80	248
30.05	1644	56.05	902	77.90	405	105.10	516
31.05	10045	57.05	2000	78.90	1195	106.10	184
32.05	1710	58.05	1194	79.85	670	108.05	297
33.05	1746	58.95	1221	80.95	879	108.95	259
34.05	416	59.95	4162	81.95	763	111.05	396
34.95	244	60.95	181952	82.95	1212	114.05	159
39.00	1065	61.95	7842	84.05	261	115.05	355
40.00	700	63.05	39320	84.95	522	125.90	466
40.90	4076	63.95	1646	85.85	277	136.95	154
42.00	7629	65.05	971	86.95	443	139.15	169
43.00	117528	65.80	434	88.05	2489	151.90	291
44.00	24056	66.90	2401	88.95	474	162.00	236
45.00	314752	68.00	1437	90.95	6468	180.00	163
46.10	8946	69.10	3124	91.95	700	202.00	858
47.00	4766	70.00	6408	93.05	336	208.00	199
47.70	473	71.10	1517	93.90	1023	280.85	185
47.90	491	73.00	272248	95.00	797	281.95	124
49.90	312						



Scan 1393 (39.607 min) of DATA:SBUR55A.D
FRESH SAMPLE OF SBUR55 ON DBWAX

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
26.15	518	50.00	1230	69.00	11131	90.95	302
27.15	3175	51.00	1588	70.00	1327	92.95	163
28.05	6026	51.95	729	71.00	2650	93.70	164
29.05	3044	52.95	6080	72.10	1052	94.00	148
30.95	588	54.05	3241	72.90	8944	95.00	849
32.05	2118	54.95	25176	73.90	683	95.90	4316
36.95	531	55.95	1570	75.10	197	97.00	1452
38.10	1074	57.05	6333	77.00	464	98.00	208
39.00	10524	58.15	1195	78.00	142	98.90	3940
39.90	2101	58.95	685	79.00	1326	99.80	323
41.00	20816	60.05	17008	79.95	372	107.10	234
41.90	6063	60.95	1140	80.05	359	110.85	173
43.00	5196	62.05	886	80.95	3405	113.15	167
44.00	1366	63.05	1475	82.05	274	114.05	13966
45.00	6716	63.85	939	83.05	337	115.05	992
46.00	384	65.05	1452	84.95	637	115.85	130
47.00	1148	65.90	614	85.95	514	125.90	784
47.90	520	66.90	7377	87.95	309	206.80	902
49.00	253	67.90	18248	88.95	189		



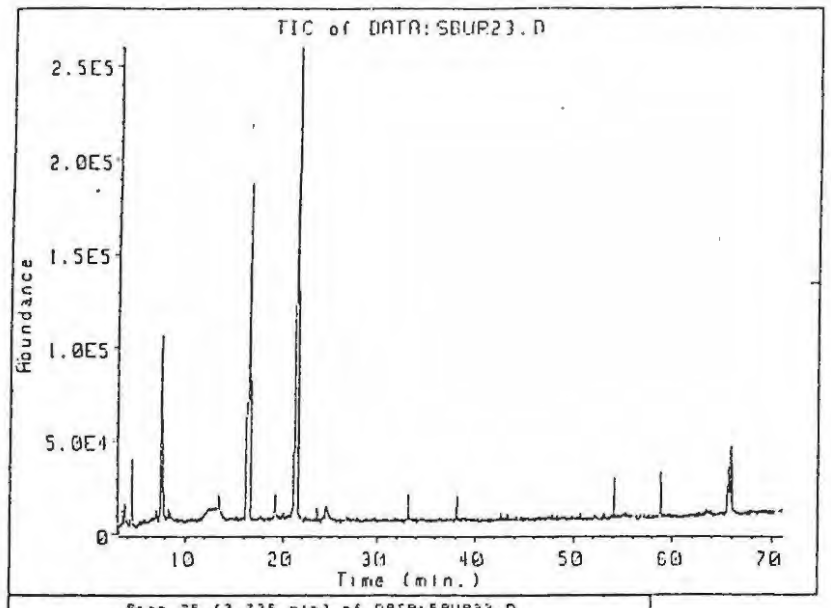
T: Scan 1408 (40.009 min) of
Z: TIC of DATA:SBUR55A.D
Y: Scan 1373 (39.007 min) of
X: Scan 1409 (40.009 min) of

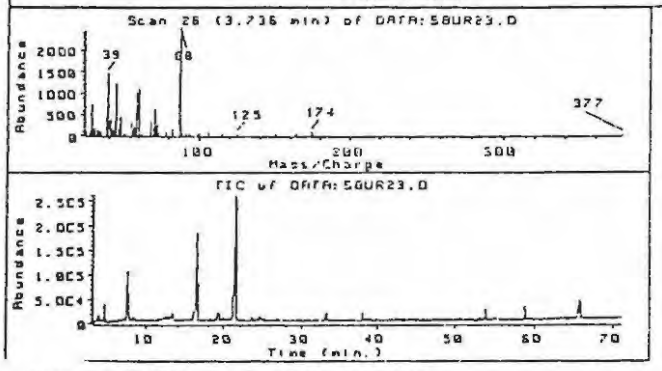
DRAW done
(DE)

Scan 1408 (40.009 min) of DATA:SBUR55A.D
FRESH SAMPLE OF SBUR55 ON DBWAX

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
26.05	450	47.90	537	68.90	15631	91.05	374
26.95	3270	49.00	202	69.90	7352	92.95	358
28.05	5816	50.00	1057	70.10	739	94.00	245
29.05	3653	50.90	1714	71.00	3458	94.90	1189
31.05	707	51.95	716	72.00	712	95.90	3859
31.95	2613	52.95	4307	73.00	38272	96.80	1630
33.15	164	54.05	1606	74.00	1368	97.90	230
35.95	144	55.05	15693	75.00	345	98.90	11068
36.95	459	56.05	3402	76.80	431	100.90	425
39.90	1222	58.95	3982	79.80	178	101.10	169
38.10	1207	57.95	1374	78.90	909	111.05	156
39.00	9077	59.05	2423	80.05	193	112.75	187
40.00	2882	60.05	5603	80.95	4267	113.05	238
41.10	12379	60.95	1041	82.05	424	113.95	6109
42.00	11574	61.95	651	82.95	491	114.85	441
43.00	5215	63.05	991	84.05	241	125.80	450
43.90	1536	64.05	825	84.95	1273	130.90	126
44.90	5625	65.05	1294	85.95	2131	139.85	135
45.90	431	65.90	488	86.95	540	206.80	558
47.00	564	67.00	3524	88.05	193	207.90	143

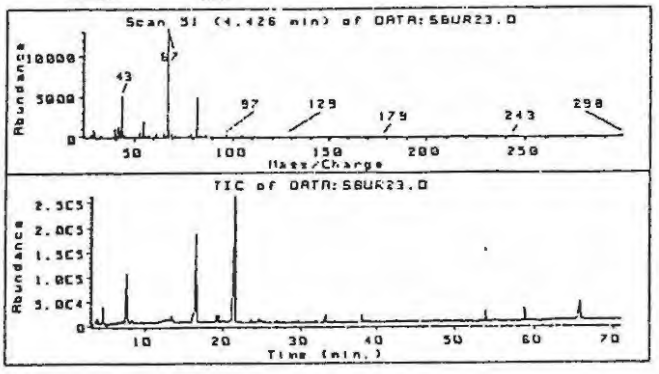
(ix) Volatiles - garlic





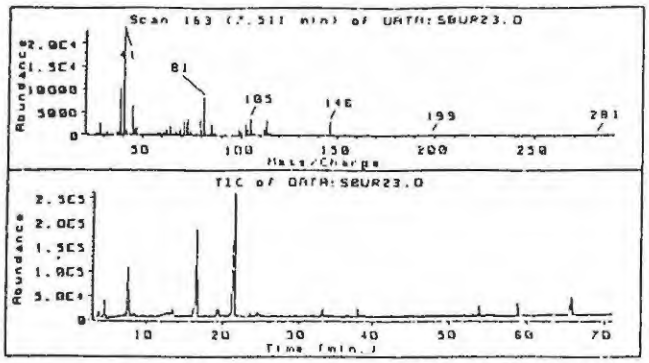
Scan 26 (3.736 min) of DATA:SBUR23.D
GARLIC EXTRACT

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
23.60	105	44.05	348	59.10	1009	87.95	2451
26.10	40	45.05	1207	60.10	1090	88.95	236
27.30	113	46.05	171	62.30	33	91.25	74
28.20	129	49.95	436	69.00	339	93.85	87
29.30	189	50.25	34	70.10	184	100.05	62
31.90	125	51.35	40	71.10	616	106.10	87
34.20	109	55.15	209	72.10	150	114.90	33
37.95	436	55.65	127	73.10	229	125.40	55
39.05	1460	55.95	133	74.10	79	150.00	50
40.15	384	56.15	137	79.10	95	163.30	28
40.95	148	57.05	230	83.15	152	174.45	99
42.05	154	58.05	176	87.05	1445	377.00	25
43.15	120						



Scan 51 (4.426 min) of DATA:SBUR23.D
GARLIC EXTRACT

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
22.90	55	43.05	5131	65.10	492	89.05	119
25.40	98	44.15	533	65.90	167	95.05	80
26.20	40	45.25	221	67.00	13011	97.05	249
27.10	441	49.45	120	68.10	999	99.15	96
28.10	771	51.45	96	69.20	565	105.00	196
29.00	450	53.05	662	71.30	172	111.10	72
32.00	146	54.05	1366	73.20	111	112.90	60
36.30	65	55.15	1847	77.30	71	116.20	51
37.95	90	55.95	159	79.00	438	128.95	126
39.05	909	56.15	165	81.20	938	178.65	43
39.75	192	57.15	282	82.05	4714	242.90	53
40.15	200	58.15	126	83.15	281	279.15	28
41.15	1350	60.10	100	84.65	64	298.40	64
42.15	466	61.10	304	86.85	214		

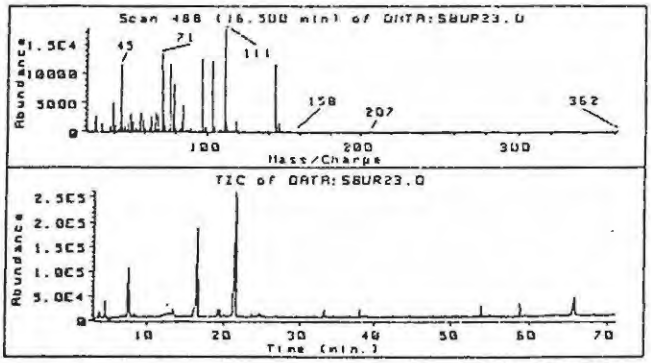


T: Scan 26 (3.750 min) of DAT
Z: TIC of DATA:SBUR23.D
Y: Scan 51 (4.420 min) of DAT
X: Scan 163 (7.511 min) of DA

DRAW done
(MSI)

Scan 163 (7.511 min) of DATA:SBUR23.D
GARLIC EXTRACT

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
21.00	34	46.05	1087	67.10	558	86.95	313
22.00	78	47.05	1540	67.00	140	92.05	112
25.40	104	47.95	111	68.00	156	99.05	1273
26.00	60	48.95	85	68.20	155	99.95	690
27.20	647	50.35	30	69.00	1303	102.95	2254
28.10	2641	50.75	39	70.00	230	104.05	1163
29.20	176	51.15	88	71.00	2662	105.00	3625
30.10	29	53.05	403	72.10	2288	106.10	570
31.20	103	54.05	267	73.00	3635	106.90	467
32.10	743	55.05	454	74.00	994	112.10	1126
33.00	80	56.25	129	75.00	236	113.10	2969
34.20	57	57.05	243	77.00	286	114.10	396
37.05	601	58.05	743	78.00	352	115.10	1[illegible]

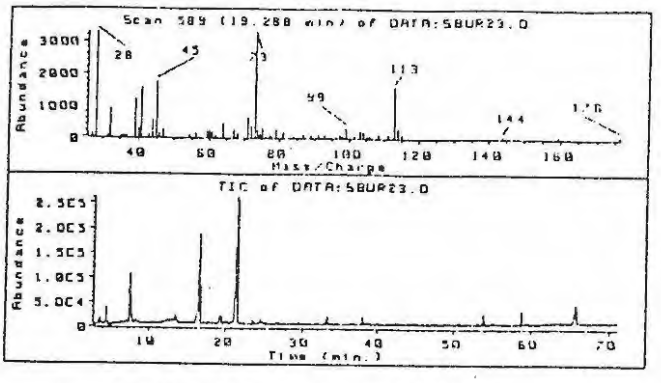


T: Scan 51 (4.426 min) of DAT
Z: TIC of DATA:SBUR23.D
Y: Scan 163 (7.511 min) of DA
X: Scan 488 (16.500 min) of D

DRAW done
(MSI)

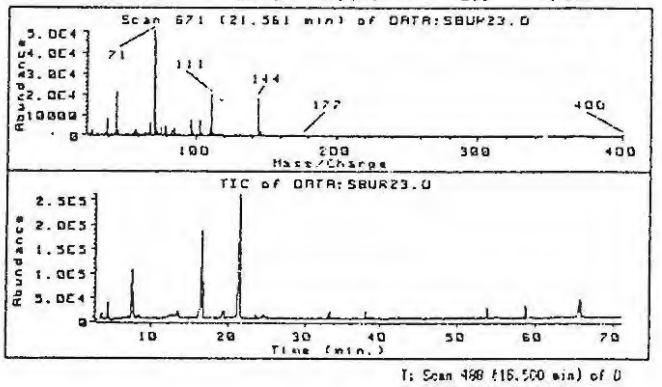
Scan 488 (16.500 min) of DATA:SBUR23.D
GARLIC EXTRACT

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
22.30	111	53.15	1677	78.10	2978	108.00	153
24.80	63	54.15	720	79.10	8114	109.00	742
25.10	74	55.15	316	80.10	986	110.10	1278
26.10	334	55.85	183	81.10	327	111.10	17656
27.00	1732	57.05	1836	82.05	416	112.10	1577
28.00	2646	58.05	3179	82.95	471	113.10	902
28.80	108	59.10	1956	83.95	1984	113.90	144
32.00	1379	60.10	429	85.05	4348	114.90	128
33.10	134	61.10	585	86.05	444	116.10	159
37.05	447	62.20	346	86.95	353	117.00	327
38.05	831	63.00	749	87.95	177	118.00	1702
39.05	4813	63.90	2536	88.95	139	119.10	132
39.95	504	65.00	1933	89.95	434	120.10	298
41.15	1091	66.10	1424	91.75	126	123.00	45
42.15	247	67.10	3265	95.15	332	133.05	105
43.15	253	68.00	234	97.05	12542	138.75	43
44.05	725	69.00	2691	98.05	693	144.05	11462
44.95	11469	70.00	525	99.05	829	145.05	998
46.05	803	71.00	13330	102.05	87	146.05	1266
47.05	1012	72.10	10416	103.05	11989	147.25	62
47.85	108	73.00	2227	104.05	670	152.90	55
49.05	183	74.00	927	105.00	963	206.90	58
50.05	1613	75.10	89	107.10	57	324.75	37
51.05	3082	76.00	281	107.30	61	362.05	77
52.05	1020	77.10	11403				



Scan 589 (19.208 min) of DATA:SBUR23.D
GARLIC EXTRACT

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
25.90	117	45.95	125	74.50	114	99.05	307
27.10	121	46.95	248	75.10	288	101.45	44
28.10	3303	54.45	78	77.30	59	103.05	227
31.30	109	56.05	130	77.70	62	104.05	157
32.10	896	59.10	211	79.00	262	105.10	57
34.80	59	59.90	196	80.80	82	106.10	67
35.50	98	60.70	153	81.20	153	108.50	77
36.00	50	61.90	44	84.15	47	111.20	79
36.75	68	63.90	431	86.95	87	113.10	1603
39.05	1198	67.00	252	89.15	82	114.10	302
40.05	288	68.20	130	91.05	105	115.10	101
41.05	1553	71.10	635	92.95	78	143.75	55
43.05	147	72.10	370	96.05	48	159.40	47
43.95	540	73.00	3198	97.15	93	176.45	71
45.05	1742	73.90	253	97.95	58		

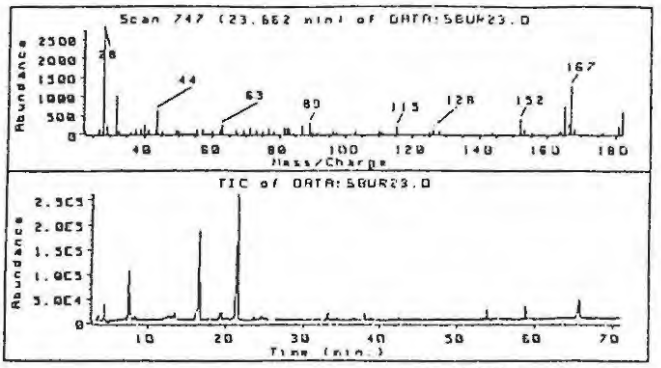


T: Scan 488 (16.500 min) of D
Z: TIC of DATA:SBUR23.D
Y: Scan 589 (19.288 min) of D
X: Scan 671 (21.561 min) of D

DRAW done
[MS1]

Scan 671 (21.561 min) of DATA:SBUR23.D
GARLIC EXTRACT

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
25.20	171	54.05	158	77.00	3662	104.05	773
25.50	207	55.15	391	78.00	1016	105.00	644
26.10	452	56.15	185	79.10	4486	109.00	371
27.10	1111	57.05	1715	80.20	2269	110.00	2345
28.00	2766	57.95	2397	81.10	221	111.10	19848
32.00	1032	59.00	1805	82.85	204	112.10	1468
32.90	165	60.10	675	83.05	216	113.00	1051
34.10	93	61.10	238	83.95	2167	114.00	116
35.20	82	62.10	107	85.05	3207	115.30	84
37.05	1113	63.10	237	86.05	340	116.10	268
38.05	1528	64.00	291	87.05	304	117.00	593
39.05	8081	65.00	1352	88.15	138	119.00	137
39.85	401	66.10	509	89.05	120	128.95	352
41.05	712	67.10	1234	90.05	329	131.25	44
43.25	154	68.10	698	94.85	60	138.95	32
44.05	1180	69.00	6248	95.35	49	144.15	18288
44.95	20880	70.00	1444	97.05	7307	145.05	1683
45.95	2316	71.00	50664	98.05	1864	145.95	2126
46.95	1917	72.00	47888	99.05	897	148.15	141
48.05	249	73.10	6120	100.15	277	168.70	52
48.85	136	74.10	2196	101.05	277	176.75	60
51.15	411	75.10	310	102.05	123	382.30	26
52.05	217	75.90	338	103.05	6671	400.25	31
53.05	717						

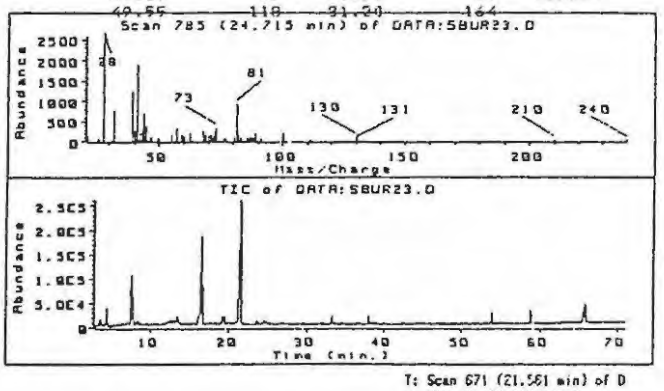


T: Scan 569 (19.268 min) of D
Z: TIC of DATA:SBUR23.D
Y: Scan 671 (21.561 min) of D
X: Scan 747 (23.662 min) of D

DRAW done
(MS1)

Scan 747 (23.662 min) of DATA:SBUR23.D
GARLIC EXTRACT

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
22.70	72	50.15	78	81.75	167	125.10	66
23.00	92	55.25	97	82.55	190	126.10	113
26.70	76	56.05	110	82.75	164	127.95	83
27.10	131	57.35	98	83.05	151	143.85	37
28.10	2743	59.80	79	84.35	29	152.10	406
29.10	204	60.10	94	87.05	226	153.20	105
32.00	1013	62.00	124	89.05	302	155.40	43
32.70	64	67.60	131	89.85	74	163.30	89
36.00	40	63.10	240	91.25	62	164.10	112
37.55	149	67.20	105	95.25	30	165.20	716
38.95	137	69.10	104	96.25	80	166.20	266
39.95	226	71.30	160	97.25	53	167.20	1276
41.15	104	73.00	122	102.95	88	168.30	145
43.25	189	74.90	76	110.20	69	176.15	42
43.85	626	76.90	152	111.00	60	181.05	193
45.15	93	78.30	67	115.20	207	182.15	601
49.55	118	81.20	164				

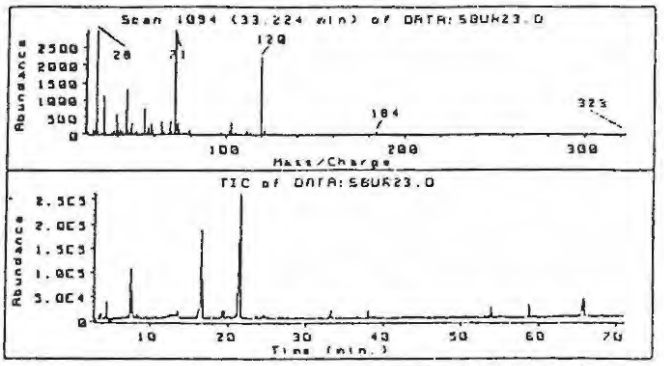


T: Scan 671 (21.561 min) of D
Z: TIC of DATA:SBUR23.D
Y: Scan 747 (23.662 min) of D
X: Scan 785 (24.715 min) of D

DRAW done
(MS1)

Scan 785 (24.715 min) of DATA:SBUR23.D
GARLIC EXTRACT

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
21.00	120	46.15	46	69.80	145	85.75	84
26.00	82	47.05	93	71.10	157	87.15	114
28.10	2587	49.55	44	72.10	124	87.85	103
30.90	55	50.05	81	73.00	327	88.95	183
32.10	762	55.15	173	76.40	36	91.15	83
39.05	1252	56.95	323	76.90	78	99.85	188
39.95	289	59.20	175	77.10	70	100.05	193
41.05	1882	60.10	124	80.10	147	111.10	46
42.15	218	63.00	199	81.10	933	130.05	103
43.05	325	68.00	259	81.95	161	130.55	26
43.95	678	68.60	09	82.75	98	210.20	29
45.05	382	69.10	171	85.15	59	239.65	36

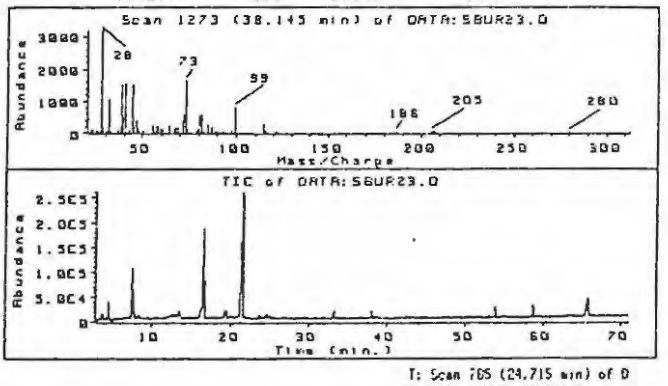


T: Scan 747 (23.062 min) of D
Z: TIC of DATA:SBUR23.D
Y: Scan 765 (24.715 min) of D
X: Scan 1094 (33.224 min) of

DRAW done
(MS1)

Scan 1094 (33.224 min) of DATA:SBUR23.D
GARLIC EXTRACT

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
22.90	87	42.95	161	59.00	162	94.95	30
26.20	156	44.05	733	63.90	349	97.05	66
26.80	83	45.05	1302	65.50	84	101.25	125
27.10	99	45.75	141	68.00	167	103.05	334
28.00	2990	46.05	143	69.00	387	105.10	38
29.10	126	47.25	158	70.00	97	111.00	92
30.70	49	47.95	331	71.00	2869	113.20	60
32.10	1139	48.95	63	72.00	2859	120.00	2222
33.20	82	50.65	100	73.00	337	121.30	56
36.75	86	53.55	61	74.10	134	121.90	138
38.15	148	55.15	756	76.00	67	127.00	29
39.05	559	56.05	103	77.10	43	183.95	61
39.95	342	57.15	224	79.30	126	323.05	26
41.25	166	58.15	325				

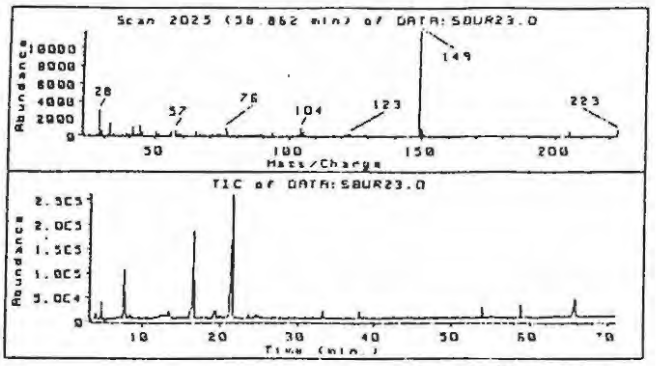


T: Scan 765 (24.715 min) of D
Z: TIC of DATA:SBUR23.D
Y: Scan 1094 (33.224 min) of
X: Scan 1273 (38.145 min) of

DRAW done
(MS1)

Scan 1273 (38.145 min) of DATA:SBUR23.D
GARLIC EXTRACT

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
20.90	67	39.95	491	59.50	104	84.95	269
22.30	80	41.05	1531	59.90	101	87.15	195
23.20	134	42.25	62	61.00	145	89.05	56
25.00	77	43.05	150	64.10	255	89.45	45
25.80	58	44.15	1080	67.20	180	91.15	53
27.10	106	45.05	1507	68.10	160	93.05	44
28.10	3177	45.95	203	69.20	191	97.05	84
28.90	164	47.05	380	71.00	354	99.15	804
29.80	73	47.65	102	72.00	557	114.10	290
30.10	62	47.95	123	73.00	1651	115.10	59
30.80	29	48.45	49	74.00	129	116.10	100
31.40	99	50.45	102	75.10	70	121.20	51
32.10	1075	55.25	229	78.90	109	186.15	41
32.80	53	56.25	93	79.50	94	205.30	66
36.65	62	57.15	104	80.00	520	207.20	60
37.05	140	58.05	236	81.20	590	279.95	29
38.05	220	59.20	165	83.05	51	312.75	26
39.05	1499						

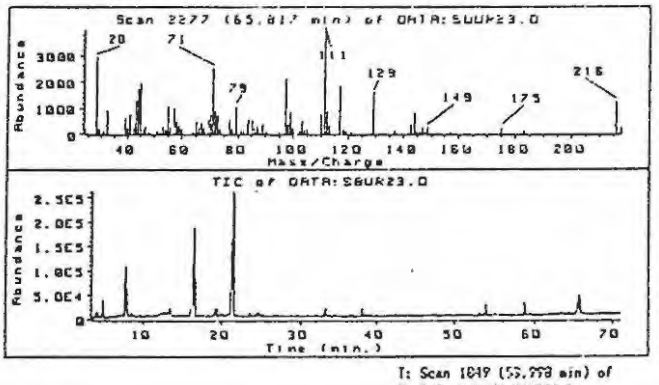


T: Scan 1273 (36.145 min) of
Z: TIC of DATA:SBUR23.D
Y: Scan 1849 (53.998 min) of
X: Scan 2025 (58.862 min) of

DRAW done
[MS1]

Scan 2025 (58.862 min) of DATA:SBUR23.D
GARLIC EXTRACT

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
21.70	88	42.05	35	74.90	115	102.25	109
22.00	63	50.15	429	75.20	93	103.05	163
23.60	68	51.15	194	76.10	850	104.15	863
25.00	70	54.95	236	77.00	486	105.10	439
27.20	294	55.05	2981	79.00	90	106.10	131
28.00	2934	56.15	513	81.20	61	116.40	25
29.00	577	57.15	675	84.05	106	121.10	243
31.30	141	58.15	149	85.65	41	122.00	259
32.10	1471	59.10	166	87.05	92	123.20	164
33.80	167	65.10	460	88.15	128	129.05	45
36.95	42	66.20	199	89.15	216	135.15	110
39.05	341	67.20	97	90.05	107	145.95	77
40.05	220	68.20	44	93.15	404	149.15	11832
41.85	1043	70.20	119	96.95	104	150.10	890
42.15	138	71.10	267	97.15	105	151.10	161
43.15	373	73.20	221	99.05	97	205.10	532
44.05	1164	74.00	200	101.05	44	223.15	645
45.05	421						



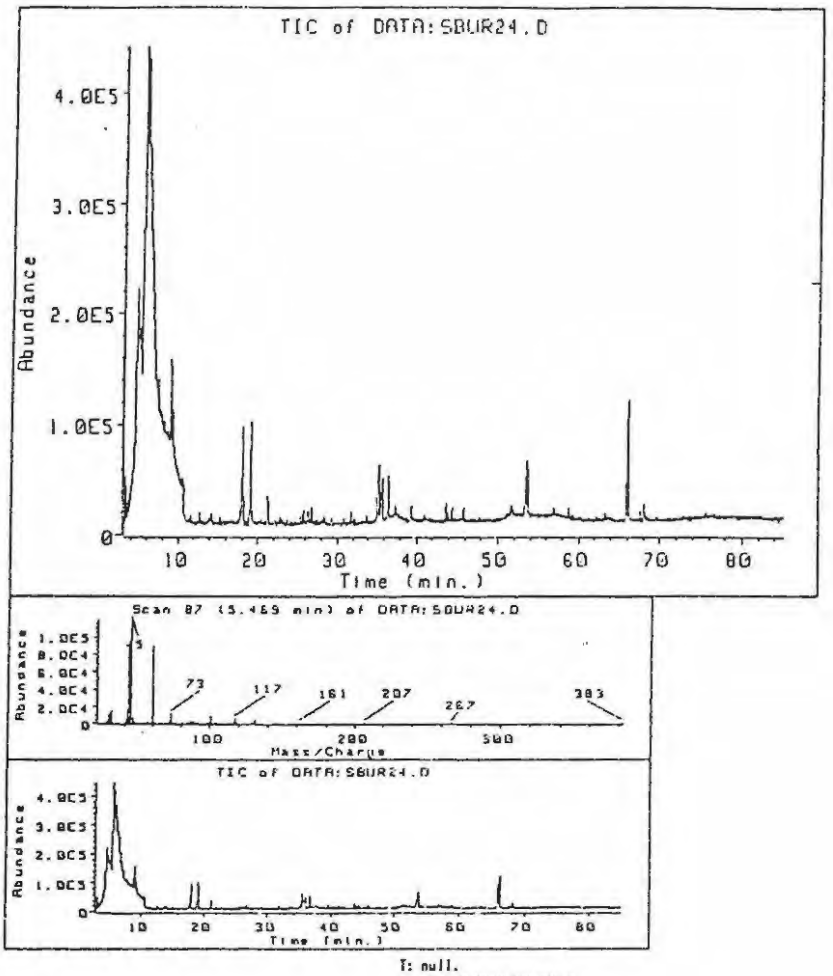
T: Scan 1849 (53.998 min) of
Z: TIC of DATA:SBUR23.D
Y: Scan 2025 (58.862 min) of
X: Scan 2277 (65.817 min) of

DRAW done
[MS1]

Scan 2277 (65.817 min) of DATA:SBUR23.D
GARLIC EXTRACT

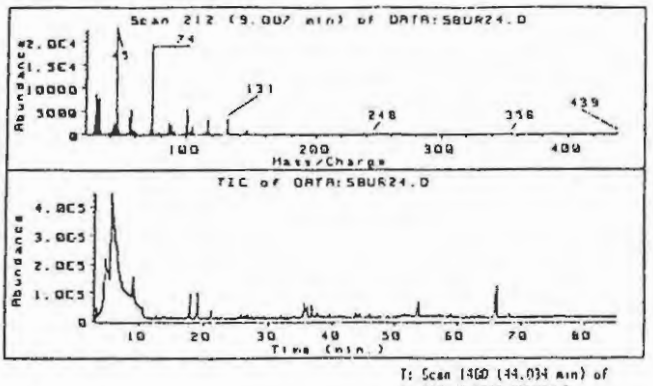
m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
23.30	182	55.05	1064	82.05	171	112.10	876
24.50	33	56.15	259	83.25	403	113.10	311
28.10	2953	57.15	1007	84.05	567	117.00	1841
29.10	199	58.15	474	85.05	533	118.00	159
31.00	92	59.00	341	86.05	192	119.00	135
32.00	893	60.10	200	87.15	312	120.90	49
38.95	630	63.80	82	88.15	193	129.05	1611
39.95	256	64.00	79	89.05	398	137.05	114
41.05	742	65.20	478	90.05	118	141.95	54
42.05	297	66.10	251	90.25	118	143.05	409
43.05	1319	67.00	435	95.05	123	144.05	813
43.95	1740	67.90	266	96.25	36	145.15	45
44.95	1924	69.10	562	97.05	2100	146.15	131
46.15	144	70.20	766	98.05	327	147.15	258
46.95	318	71.10	2538	99.05	876	148.15	76
48.05	58	72.10	911	100.05	232	149.05	263
49.15	41	73.10	736	102.05	141	152.40	50
50.95	114	74.00	216	103.05	497	157.30	51
51.15	108	77.00	562	104.15	171	175.05	196
52.15	49	78.10	266	104.90	152	183.15	103
53.15	282	79.10	1058	110.10	730	216.20	1241
54.05	186	81.10	159	111.10	3964	218.05	234

(x) Volatiles - onion



Scan 87 (5.469 min) of DATA:SBUR24.D
ONION EXTRACT

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
22.50	115	58.05	1396	90.05	478	131.15	4471
25.10	542	59.10	2539	91.15	929	132.25	324
26.10	1120	60.00	89728	91.95	125	144.25	248
27.10	2513	61.10	2236	93.95	98	145.25	903
28.10	2013	62.60	279	98.15	212	148.25	1199
29.10	11460	65.10	109	99.15	210	147.15	136
30.10	1093	66.90	71	100.15	2284	157.40	98
31.10	14878	69.20	166	101.15	8727	159.30	88
32.10	819	70.10	489	102.05	499	160.10	55
35.40	145	71.10	988	103.05	1181	161.20	103
36.35	41	72.00	3242	103.95	378	165.40	35
39.25	163	73.00	11530	105.10	625	174.65	40
39.95	1127	74.10	415	107.20	129	179.15	44
41.05	5592	75.10	456	109.10	44	184.35	46
42.05	15682	77.00	222	109.40	64	186.65	66
43.05	86248	78.90	265	112.20	51	191.25	77
44.15	6443	79.20	258	113.20	108	193.05	32
45.05	118920	81.20	114	114.00	439	197.60	26
46.05	7542	82.15	115	115.10	1161	207.20	219
47.05	838	83.15	219	116.10	1715	214.50	31
48.15	35	84.05	195	117.10	6044	241.80	61
50.05	80	85.05	415	118.10	530	267.15	132
54.25	50	86.05	1174	119.20	185	281.05	67
54.95	228	87.05	4028	128.25	55	352.15	30
56.15	331	88.05	992	129.05	301	382.90	30
57.05	1150	89.05	2826	130.15	1442		

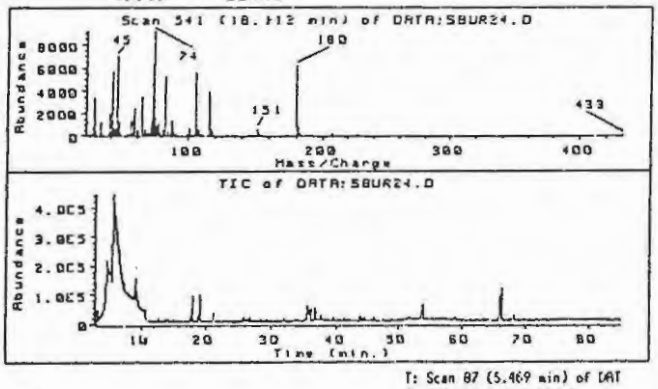


T: Scan 1460 (44.034 min) of
Z: TIC of DATA:SBUR24.D
Y: Scan 87 (5.469 min) of DAT
X: Scan 212 (9.007 min) of DA

DRAW done
(MSI)

Scan 212 (9.007 min) of DATA:SBUR24.D
ONION EXTRACT

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
21.20	88	46.05	4496	75.00	1006	103.15	739
23.90	203	47.15	1195	76.10	170	104.15	357
25.20	401	47.85	141	77.00	255	105.00	1600
26.10	1911	48.65	70	78.00	127	106.10	179
27.10	4457	48.85	69	79.10	644	114.20	225
28.10	8801	50.35	91	81.00	130	115.10	260
29.10	7739	52.05	72	81.40	40	116.10	729
30.10	3611	53.15	441	82.15	129	117.10	3117
31.10	3550	54.15	150	82.85	159	118.80	380
32.00	1054	55.05	3739	85.15	143	119.10	210
33.10	44	56.15	2821	85.95	784	130.15	674
33.40	77	57.05	5493	87.05	2420	131.05	3337
36.10	86	58.15	1050	88.05	660	132.05	156
36.35	65	59.00	872	89.15	1826	133.15	72
38.05	60	60.10	525	90.15	253	144.25	127
38.25	66	61.00	425	91.15	627	145.15	462
39.05	251	67.10	38	92.05	128	146.15	635
39.95	300	70.00	130	98.35	60	238.75	28
41.15	582	71.10	880	99.05	97	246.40	42
42.05	1810	72.10	2229	100.05	1689	248.90	29
43.05	2810	73.10	19400	101.15	5273	355.55	32
43.95	2212	74.00	18184	102.15	489	438.70	32
45.05	22440						

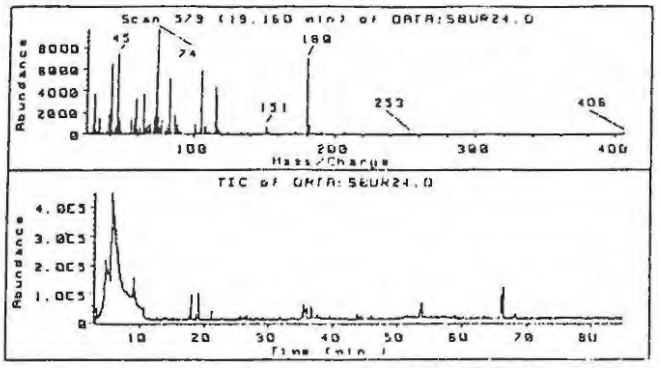


T: Scan 87 (5.469 min) of DAT
Z: TIC of DATA:SBUR24.D
Y: Scan 212 (9.007 min) of DA
X: Scan 541 (18.112 min) of D

DRAW done
(MSI)

Scan 541 (18.112 min) of DATA:SBUR24.D
ONION EXTRACT

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
22.50	49	54.05	80	77.00	883	107.00	599
23.60	42	54.25	85	77.90	1140	108.00	543
25.00	86	55.15	1312	79.00	326	109.10	447
26.10	146	56.25	195	81.10	472	110.90	173
26.90	785	57.15	613	82.05	430	115.10	3973
28.00	3399	57.95	1572	83.05	5237	116.10	3495
29.00	458	59.00	2445	84.15	327	117.20	504
40.05	116	75.00	1704	106.00	5495	433.00	37
53.15	197	76.00	751				

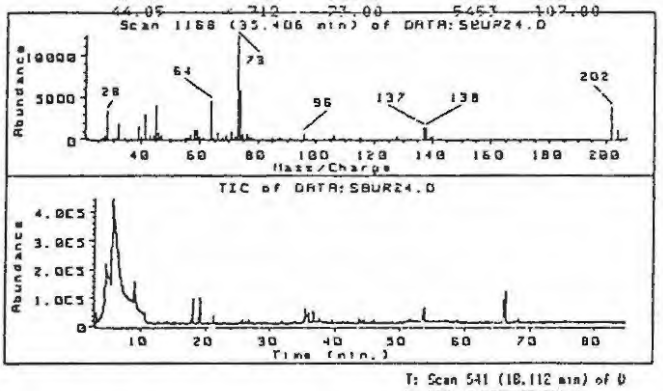


T: Scan 212 (9.807 min) of D
Z: TIC of (DATA:SBUR24.D
Y: Scan 541 (18.112 min) of D
X: Scan 579 (19.160 min) of D

DRAW done
(MS1)

Scan 579 (19.160 min) of DATA:SBUR24.D
ONION EXTRACT

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
23.40	102	45.05	7402	74.10	9671	108.10	562
24.20	41	45.95	1334	75.10	1560	109.00	585
24.50	61	46.85	1170	76.10	543	111.00	162
25.50	31	47.95	221	77.00	617	112.80	50
26.10	207	51.15	33	78.00	1245	114.20	92
27.10	789	53.25	83	79.10	416	115.10	4281
28.00	3662	55.15	1337	80.10	116	116.10	3599
29.00	360	57.05	710	81.10	722	117.00	478
30.30	60	57.95	1430	82.05	272	118.00	312
30.90	155	59.00	3238	83.05	4981	119.00	104
32.00	1441	59.90	495	84.15	294	119.90	33
33.20	71	61.10	619	86.35	143	124.40	111
34.40	133	63.00	91	87.05	1635	133.25	64
34.60	143	64.00	3625	88.05	833	139.35	53
36.00	58	65.00	382	89.05	278	151.10	657
37.05	260	65.80	433	89.85	57	153.10	141
37.75	115	66.00	441	90.15	55	172.10	117
38.05	112	67.10	685	90.05	144	180.05	6960
39.05	1849	68.10	213	101.05	849	181.15	586
39.85	535	69.00	907	102.15	53	182.15	853
41.05	6451	70.00	231	102.95	138	191.05	51
42.05	420	71.00	1397	105.10	386	207.20	122
43.15	462	72.00	1792	106.00	9818	252.80	31
44.05	712	73.00	5453	107.00	447	406.35	30

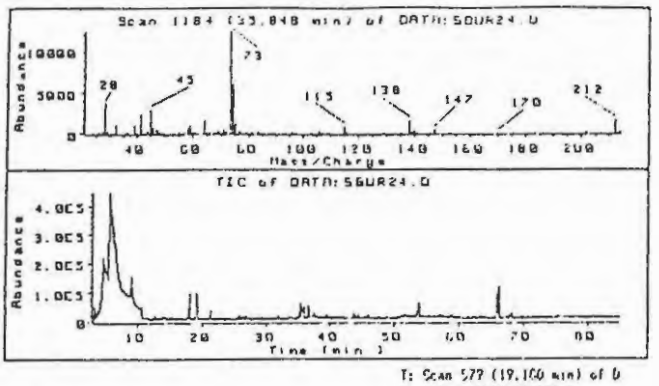


T: Scan 541 (18.112 min) of D
Z: TIC of (DATA:SBUR24.D
Y: Scan 579 (19.160 min) of D
X: Scan 1168 (35.406 min) of

DRAW done
(MS1)

Scan 1168 (35.406 min) of DATA:SBUR24.D
ONION EXTRACT

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
20.80	212	45.95	725	73.00	12356	106.10	505
21.40	94	46.85	543	74.10	5838	108.10	115
26.00	168	49.95	65	75.00	658	108.90	165
26.90	493	55.15	166	76.10	678	109.10	185
28.00	3386	56.15	165	77.00	366	109.90	46

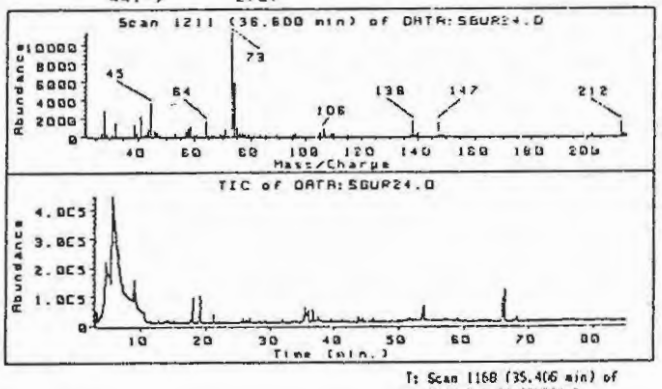


T: Scan 577 (19.100 min) of D
Z: TIC of DATA:SBUR24.D
Y: Scan 1168 (35.406 min) of
X: Scan 1184 (35.848 min) of

DATA done
(MSI)

Scan 1184 (35.848 min) of DATA:SBUR24.D
ONION EXTRACT

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
20.70	87	45.85	721	70.00	86	97.05	130
24.00	109	47.05	509	71.00	590	103.15	263
25.40	54	48.05	175	72.00	357	106.00	298
25.90	225	49.05	112	73.10	12443	106.90	85
27.00	316	52.95	96	74.10	6044	108.90	117
28.10	3741	53.15	83	75.00	1160	109.90	36
29.00	324	55.15	213	76.10	380	115.10	971
30.20	71	56.95	334	77.00	272	116.10	185
31.10	78	58.05	835	78.00	97	123.30	48
31.30	79	59.00	1080	78.90	114	129.05	26
32.00	1049	59.90	166	79.10	102	135.65	69
36.00	152	60.50	124	80.90	187	138.05	1578
36.95	155	60.90	115	83.15	191	139.15	266
38.95	1076	61.10	103	84.05	83	140.05	393
39.75	217	64.00	1678	85.05	137	145.55	87
40.15	267	64.80	231	88.05	96	147.05	525
41.15	2353	66.00	269	89.15	135	170.20	57
42.05	127	67.20	137	92.95	115	201.90	219
43.15	246	68.00	140	95.95	240	212.10	1596
44.05	514	69.20	285	96.55	61	214.20	270
44.95	2987						

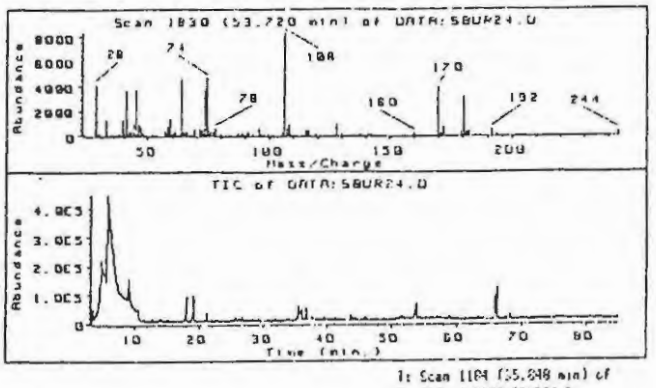


T: Scan 1168 (35.406 min) of
Z: TIC of DATA:SBUR24.D
Y: Scan 1184 (35.848 min) of
X: Scan 1211 (36.600 min) of

DATA done
(MSI)

Scan 1211 (36.600 min) of DATA:SBUR24.D
ONION EXTRACT

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
21.50	66	47.05	408	73.10	11391	105.00	428
22.90	77	47.95	163	74.10	5932	106.10	923
26.90	460	50.15	113	75.00	972	107.00	370
28.00	2916	53.15	270	76.10	431	108.00	86
29.10	385	55.15	90	77.00	370	109.10	336
30.90	182	55.35	75	78.00	162	110.10	264
32.00	1491	56.15	99	79.10	143	115.10	223
34.00	40	57.05	623	81.10	207	116.20	61
34.50	95	58.05	834	81.95	107	124.30	40
35.90	52	59.00	1082	83.05	179	137.05	201
37.15	52	60.00	144	85.15	163	138.05	1637
38.15	92	61.10	191	85.95	34	139.05	144
38.95	1415	64.00	1677	89.05	168	139.95	417
39.95	450	64.90	214	95.15	183	147.05	1590
41.05	2217	65.90	136	95.95	264	148.15	130
42.05	235	66.90	129	97.05	120	149.05	249
42.55	88	69.10	260	97.95	53	202.00	384
43.15	475	70.10	134	100.75	70	212.10	1495
44.05	894	71.00	796	101.05	72	213.20	262
44.95	3615	72.10	226	104.35	46	214.20	180
45.95	610						

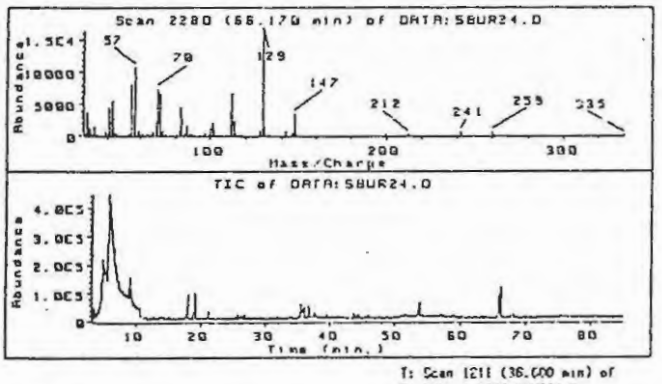


1: Scan 1184 (35.848 min) of
2: TIC of DATA:SBUR24.D
Y: Scan 1211 (36.600 min) of
X: Scan 1830 (53.720 min) of

DRAW done
(MS1)

Scan 1830 (53.720 min) of DATA:SBUR24.D
ONION EXTRACT

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
21.90	159	52.95	70	83.05	156	116.10	409
22.70	30	53.15	72	84.45	64	119.10	94
27.10	453	57.05	465	86.95	250	123.60	46
28.90	4166	59.05	1813	88.15	226	127.15	854
29.10	282	59.80	1408	89.65	145	129.85	891
30.00	41	60.00	201	90.15	53	129.95	126
32.10	1343	61.00	457	90.85	293	134.15	60
34.20	86	62.30	32	91.45	83	135.15	56
34.80	72	63.10	40	91.65	66	137.15	54
35.00	55	64.00	4646	92.95	101	138.05	165
36.95	134	64.90	293	93.15	93	141.05	96
37.95	125	65.90	377	95.85	591	141.95	127
38.25	158	68.00	135	96.85	140	159.90	222
39.05	1295	69.20	523	97.95	125	170.00	3927
39.95	344	71.10	662	99.15	175	171.00	317
40.95	3617	72.10	518	101.05	71	172.10	627
42.05	352	73.00	3760	105.10	451	179.05	481
42.95	382	74.10	4679	106.00	8314	180.05	3132
44.05	1054	75.00	660	107.00	614	181.15	317
45.05	3245	76.10	455	108.10	960	182.15	389
45.95	903	77.10	379	109.00	106	191.95	516
46.95	762	78.10	640	110.10	77	194.05	93
48.15	200	80.10	142	111.10	42	207.20	61
49.05	48	82.15	81	115.10	475	244.10	315
52.05	126						



1: Scan 1211 (36.600 min) of
2: TIC of DATA:SBUR24.D
Y: Scan 1830 (53.720 min) of
X: Scan 2280 (66.170 min) of

DRAW done
(MS1)

Scan 2280 (66.170 min) of DATA:SBUR24.D
ONION EXTRACT

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
26.30	33	59.10	850	84.05	2852	116.20	79
27.20	351	60.10	180	85.15	565	117.00	69
28.10	3675	61.00	185	85.95	345	122.15	183
29.10	1306	63.40	46	86.95	1623	128.05	974
31.20	236	64.00	108	87.95	198	129.05	16328
32.10	1462	65.10	102	88.95	247	130.05	1405
33.50	52	66.00	66	89.75	61	131.05	219
39.05	648	66.20	64	90.05	87	133.15	129
39.95	320	67.10	622	95.25	171	142.15	580
41.05	4429	68.00	533	97.15	475	145.35	106
42.05	1400	69.10	2224	99.05	232	146.25	424
43.05	5501	70.10	7435	100.05	1217	147.15	3494
44.05	1332	71.10	6536	101.15	1923	148.15	286
45.05	475	72.00	482	102.15	870	149.25	95
45.75	62	73.00	727	103.15	180	157.20	133
48.35	63	74.00	184	104.90	68	199.30	203
53.05	401	75.20	39	106.10	71	212.30	230
54.25	329	77.20	87	109.10	67	241.30	678
55.05	8123	79.00	139	111.00	3711	242.20	167
56.15	3068	81.00	266	112.10	6641	289.40	601
57.15	10035	82.05	763	113.20	2309	260.40	121

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