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"PHYTOCHEMICAL STUDIES OF SOME BANGLADESHI PLANTS"

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BY

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TO MY FAMILY

ACKNOWLEDGEMENTS

I wish to thank my supervisor Professor J. D. Connolly for his extreme, kindness, constant advice, help and encouragement through the period of his supervision. Without his great understanding and friendship it would have been very difficult for me to achieve the present work. I wish also to express my gratitude to his wife.

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I would like to thank the members of my family for all their help and support over the past few years. I would like to thank my wife for her love and my parents for their affection.

I also thanks to the Association of Commonwealth Universities for the award of a research scholarship.

PHYTOCHEMICAL STUDIES OF SOME BANGLADESHI PLANTS

MD. ENAMUL HAQUE

SUMMARY

Chapter 1 consists of a general description of the plant families Annonaceae, Piperaceae, Meliaceae, Rubiaceae and Euphorbiaceae, in particular in terms of their main secondary metabolites and their biological activity.

Chapter 2 presents a brief account of the biosynthetic pathways leading to the alkaloids, flavonoids and triterpenoids described in later chapters.

Chapter 3 describes the isolation of three novel methylated flavonoids from the bark of *Desmos longiflorus* (Annonaceae). The structures were determined largely by use of HMQC, HMBC and NOE difference techniques. Two other new compounds, a lanostane and a protoberberine alkaloid, were also obtained from the extract together with several known aporphine alkaloids, steroids, benzyl benzoate and the cyclohexane derivative, crotepoxide.

Chapter 4 concerns the structural elucidation, by 2D NMR methods, of a new 2-oxo-11β,16-epoxy-16-epicafestol derivative from *Coffea bengalensis* (Rubiaceae).

Chapter 5 discusses the isolation of several isoquinoline alkaloids and the known sesquiterpenoid spathulenol from the Annonnaceous species *Artabotrys odoratissimus*.

Chapter 6 describes the isolation and characterisation of two new and one known bisdehydroaporphine alkaloids from the bark extract and a known aporphine alkaloid

from the leaf extract of *Polyalthia bullata* (Annonaceae) which is valued in Malaysia as an aphrodisiac (tonquat ali).

Chapter 7 gives an account of the isolation of the cyclohexene derivative zeylenol and two new pseudosugars from another Annonaceae, *Annona roxburghiana.*

In Chapter 8 the isolation of the known compounds, lupeol, lupeone, scopoletin and betulin 3 caffeate from *Antidesma ghaesembilla* (Euphorbiaceae) is discussed.

In Chapter 9 the isolation of one new and six known dammarane triterpenoids and the sesquiterpenoid viridiflorol from *Amoora cucullata* (Meliaceae) is discussed.

The final Chapter 10 gives an account of an investigation of *Piper chaba* (Piperaceae) which produced piperanine, 2,4-decadienoic acid piperidide, pellitorine and the lignan (-)-kusunokinin.

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CHAPTER 1

GENERAL INTRODUCTION

THE ANNONACEAE FAMILY

The Annonaceae is one of the largest of the plant families of the Magnoliales (Annonales, Ranales) which is considered to incorporate the most archaic order of extant dicotyledonous plants[1]. It is a large family comprising 2100 species and 120 genera and consisting of aromatic trees, shrubs or climbers[2]. The family is distributed throughout the tropics, occurring predominantly as trees or lianas in humid, lowland forest regions. The family is also found as trees or shrubs in the Neotropics, growing in drier and more open environments[3].

ANNONACEAE OF ECONOMIC IMPORTANCE

The family Annonaceae contains a number of economically important genera, the most well known being *Annona* which is used as a source of edible fruits; the custard apple (*Annona reticulata*), sweetsop (*A. squamosa*), soursop (*A. muricata*) and cherrimoya (*A. cherimola*)[4]. The fruit pawpaw comes from *Asimina triloba* [pawpaw is also the fruit of *Carica papaya* (Papayaceae) which grows in the tropics] and the genera *Cananga* and *Rollinia* are also grown for their edible fruits[5].

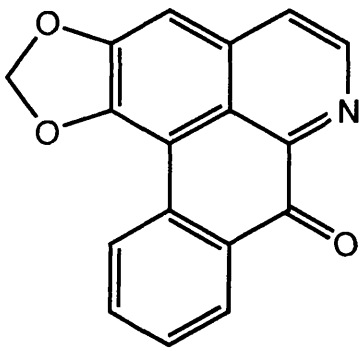
Some of the wood of Annonaceous plants is used for the production of alcohol[6]. The flowers of some of the Central American species are used as spice for flavouring chocolate and in cooking. The fragrant flowers of ylang-ylang (*Cananga odorata*), a native of Indo-China and Malaya, are used as an important raw material for perfumery[7]. The seed oils of some plants are used for the production of soap[8] and edible oils[9]. Strong ropes are made from the bark of certain Brazilian species[3].

ANNONACEAE OF MEDICINAL SIGNIFICANCE

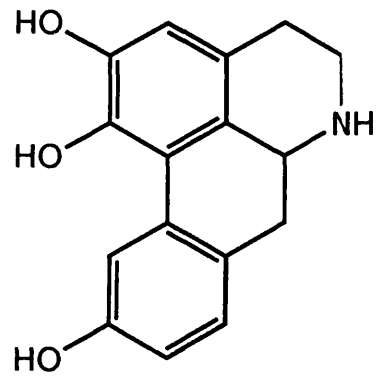
In developing countries herbal remedies are still extensively employed by ethnic groups, despite the development of sophisticated synthetic drugs and medicine in the West. Due to the abundance of Annonaceous plants throughout the tropical world, many of these are documented as herbal remedies. In the Indo-Pak-Bangladesh subcontinent many Annonaceous plants are used medicinally. *Annona reticulata* is used in dysentry, *Cananga odorata* in gout and ophthalmia and *Desmos chinensis* in dysentry and vertigo[10]. In Africa, Annonaceous plants are also used as herbal remedies. *Annona chrysophylla* is used in snake bite, *A. muricata* in coughs, spasms and fevers, *Artabotrys brachypetalus* in gonorrheoea and *Uvaria leptoeladon* in epilepsy[11]. In Borneo the fruit of *Neouvaria merrillii* is used for treating fever and in the Philippines decoctions of all parts of *Goniothalamus* species are taken for fevers[12].

Chemical and pharmacological investigations of Annonaceous plants have intensified in the last two decades. The secondary metabolites of the Annonaceae show a wide variety of interesting biological and pharmacological activities. In most cases the Annonaceae produce alkaloids but a wide range of non-alkaloidal compounds belonging to various phytochemical groups is also found. Many of the alkaloidal and non-alkaloidal constituents are pharmacologically important.

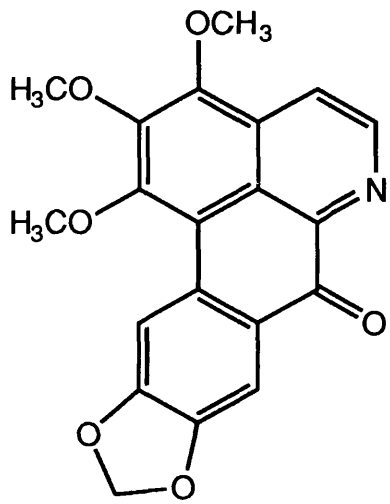
Alkaloids isolated from Annonaceous plants have been found to exhibit cytotoxic, anti-tumour, antimicrobial, antifungal, antiviral and antibacterial activity[13]. Liriodenine (**1**) and other related oxoaporphines from Annonaceous plants show antitumour, antibacterial and antifungal activities[14,15,16]. Higenamine (**2**), from *Annona squamosa*, shows cardiovascular effects[17]. Liriodenine (**1**) and oxophoebine (**3**), from *Xylopia aethiopica* , and 10-methoxyliriodenine (**4**) and 10-hydroxyliriodenine (**5**), from *Meliusa* cf *banacea* , have been found to show DNA topoisomerase inhibitory activity[18].


O
O
N
O

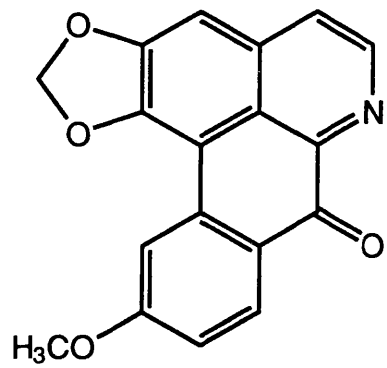
(1)


HO
HO
NH
HO

(2)


OCH3
H3CO
H3CO
N
O
O
O

(3)


O
O
N
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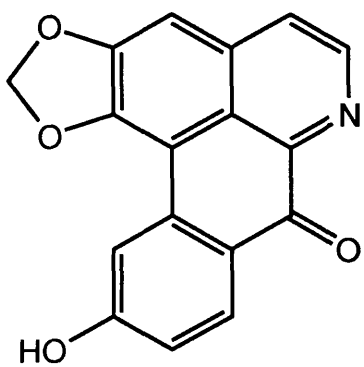
H3CO

(4)


O
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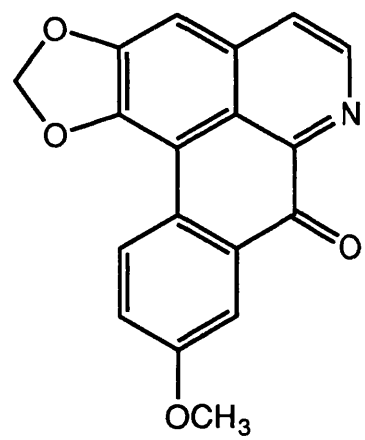
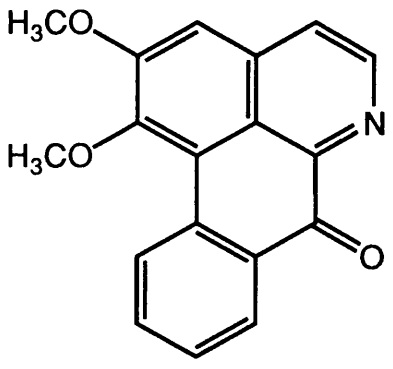
N
O
HO

(5)

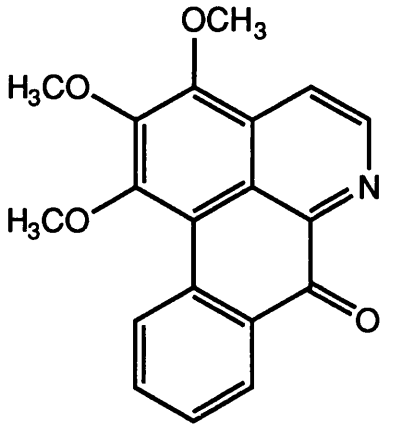

O
O
N
O
OCH3

(6)

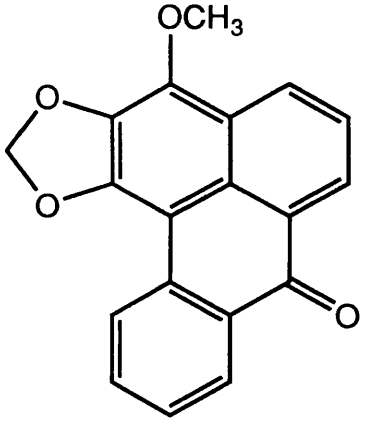
In addition to liriodenine (**1**), lanuginosine (**6**) and lysicamine (**7**), from *Rollinia papilionella*[19], and liridine (**8**) and atherospermidine (**9**), from *Rollinia siricea*[20] have been found to exhibit cytotoxic activity. Asimicilone (**10**) and 6-cis-docosenamide (**11**),



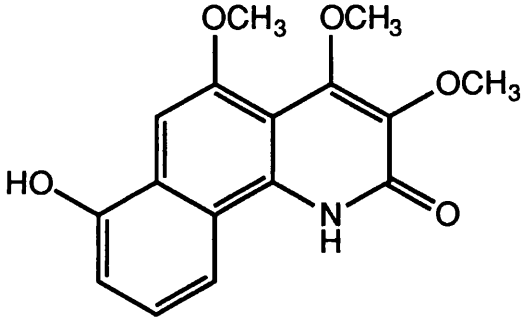
(**7**)



(**8**)



(**9**)



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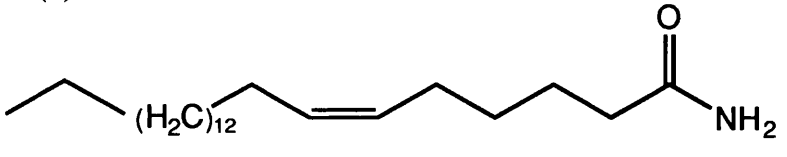
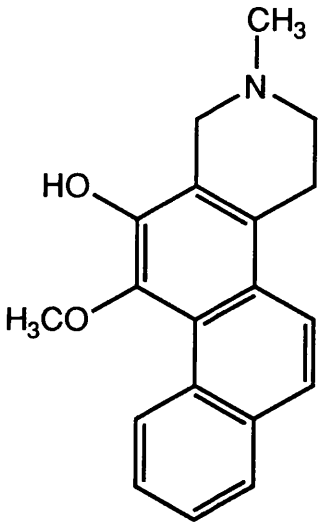


(**11**)

from *Asimina parviflora*[21], and annoretine (**12**) and argentinine (**13**) from *Annona montana*[22], have also been found to exhibit cytotoxic activity. 3-(γ,γ-Dimethylallyl)-indole (**14**), from *Monodora tenuifolia* , shows antifungal and antibacterial activity[23]. Anticandidal activity has been observed for eupolauridine (**15**) and onychine (**16**) from



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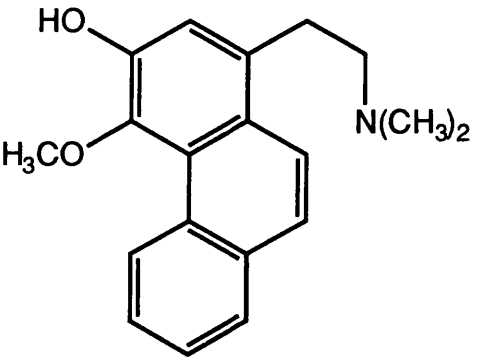

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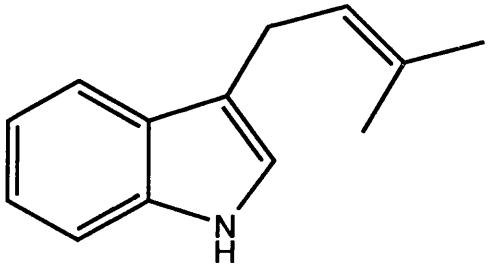

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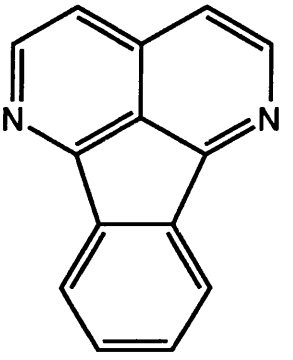

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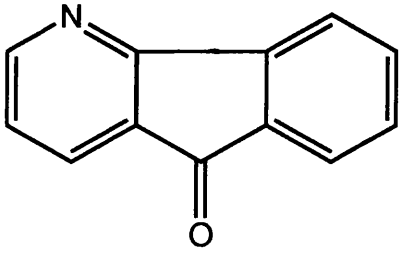
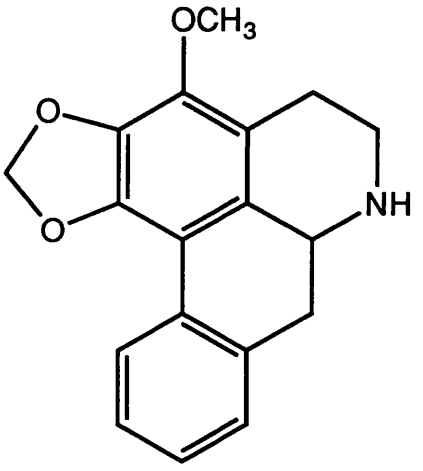


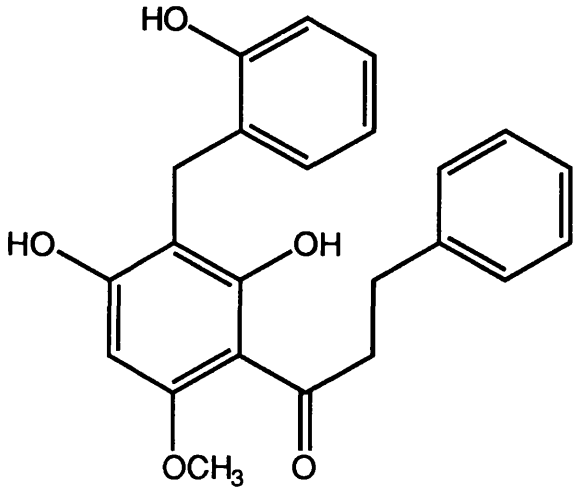
(16)



(17)

Cleistopholis patens[24]. Norstephalagine (**17**) and atherospermidine (**9**), from *Artabotrys maingayi*[25], exhibit muscle relaxation activity.

The C-benzylated flavonoids, eg uvaretin (**18**) and isouvaretin (**19**) from *Uvaria* species, have been found to show cytotoxic and lyphocytic activity[26,27]. Pinocembrin (**20**) and pinostrobin (**21**), also from *Uvaria* species are also cytotoxic[28]. Hufford *et al* have reported the antimicrobial activity for the C-benzylated flavonoids, from *Uvaria chamae*[29,30]. The interesting tribenzylated flavonoid uvarinol (**22**), from the roots of *U. chamae* , has been found to show cytotoxicity and strong antimicrobial activity against various organisms[31]. Uvafzelin (**23**), an unusual methylated aromatic compound from *U. afzelii*, shows strong antimicrobial activity[32].



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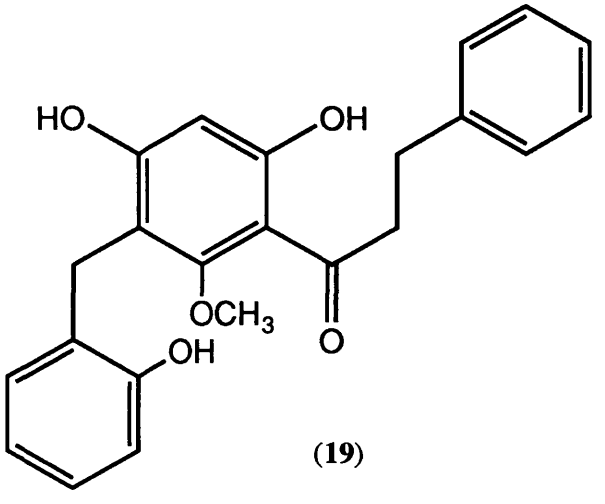

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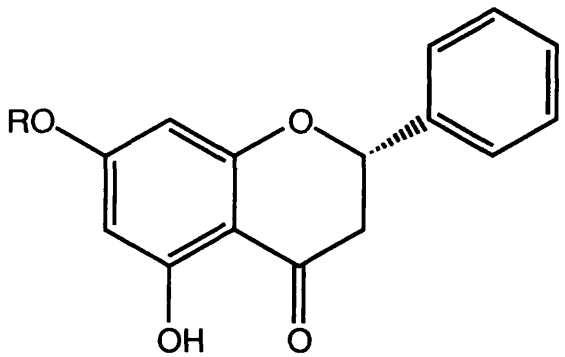
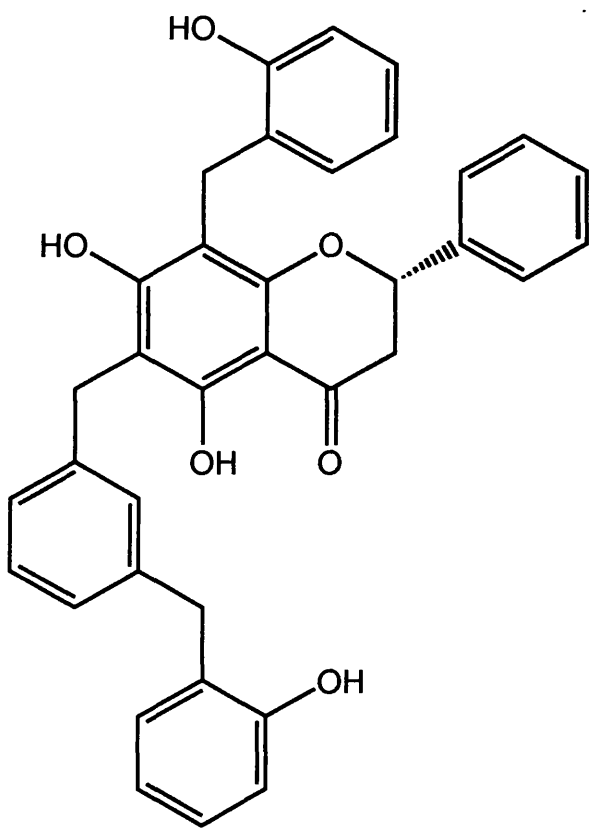
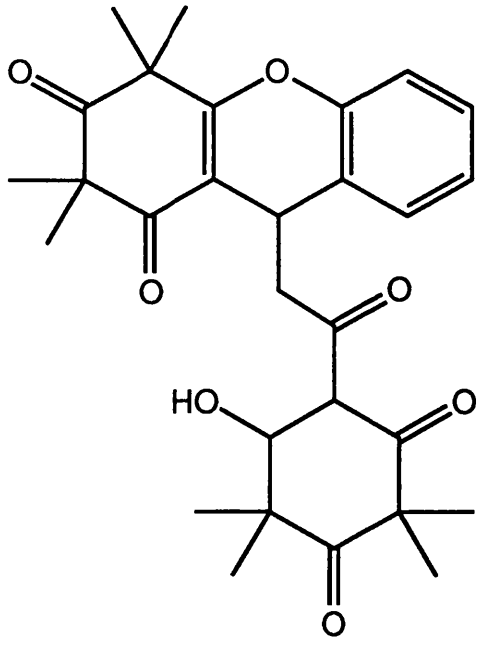


(**20**) R=H

(**21**) R=Me

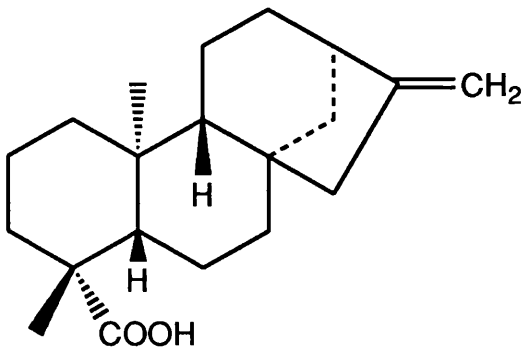


(**22**)



(23)

Terpenoids isolated from Annonaceous plants also exhibit cytotoxic, antibacterial and antimicrobial activity. These include the cytotoxic diterpenoids *ent*-kaur-16-en-19-oic acid (**24**) and *ent*-16β-hydroxy-19-kauranoic acid (**25**)[33,34]. The triterpenoid suberosol (**26**), from *Polyalthia suberosa* , exhibits anti-HIV replication activity[35].



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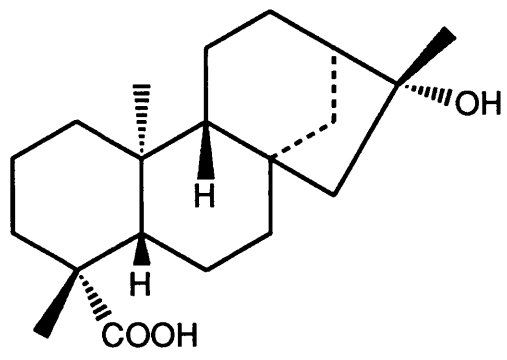

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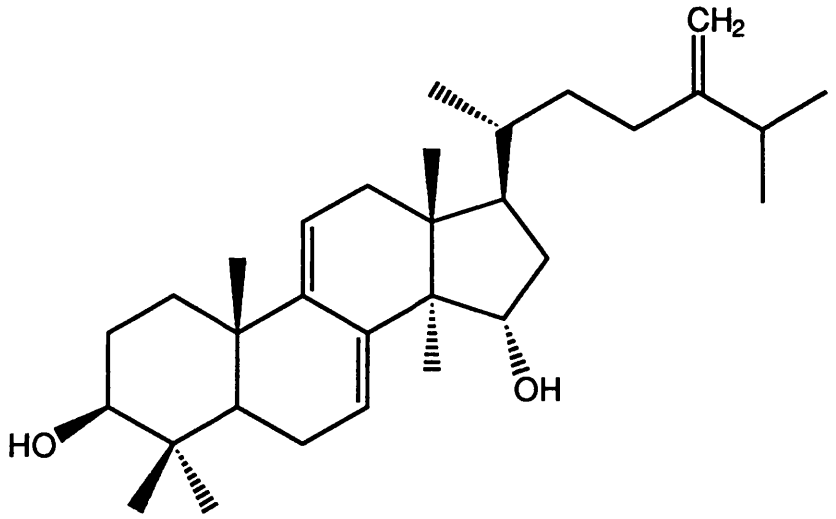
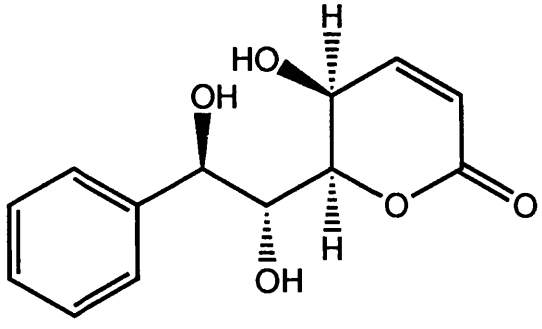


(26)

Goniotriol (**27**), and goniodiol (**28**) , from *Goniothalamus giganteus*[36,37], and goniodiol 7-monoacetate (**29**) and goniodiol 8-monoacetate (**30**), from *G. amuyon*[38,39], have significant cytotoxicity against human tumours cells. The quinone, annoquinone-A (**31**), from *Annona montana* , demonstrated potent antimicrobial activity and cytotoxicity against KB tissue culture[40] while melodorinol (**32**) and acetyl melodorinol (**33**), from *Melodorum fruticosum* , have cytotoxic activity[41,42].



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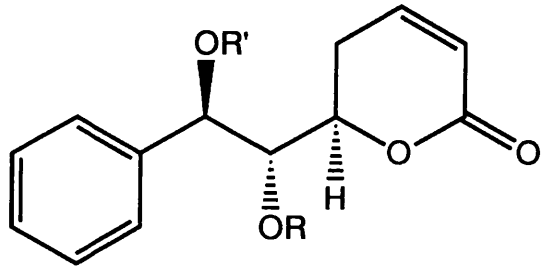
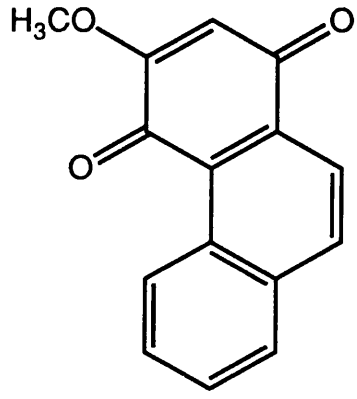


(28) R=H; R'=H

(29) R=Ac; R'=H

(30) R=H; R'=Ac



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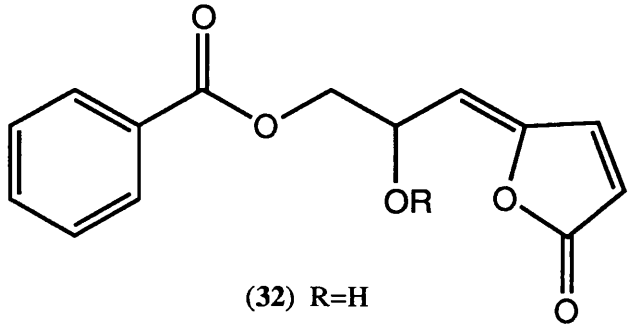
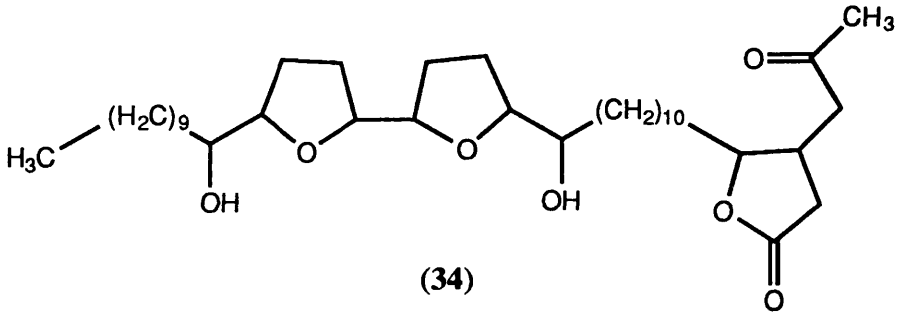


(32) R=H

(33) R=Ac

The most interesting and the most active of the constituents of the Annonaceae are the acetogenins, which have cytotoxic, antitumor, antimalarial, antimicrobial,

immunosuppressent, antifeedant and pesticidal activity[43]. The various structural types are illustrated by rollinone (**34**) from *Rollinia papelionella*[44], bullatacin (**35**) and bullatacinone (**36**), from *Annona bullata* [45], and annomontacin (**37**), annonacinone (**38**) and annonacin (**39**), from *A. montana* [46]. Trilobacin (**40**) , from *Asimina triloba* showed cytotoxic activity against human tumour cells[47]. Bullatacin (**35**) and bullatalicin (**41**), from *A. bullata* , exhibit potent and selective cytotoxic activity[48]. Annomonicin (**42**) and montanacin (**43**), from *A. montana* , have been found to show cytotoxicity against L-1210, P388 and MDA-MB231 cell lines[49]. Several other Annonaceous acetogenins have also been reported to have cytotoxic activity[50,51,52,53].



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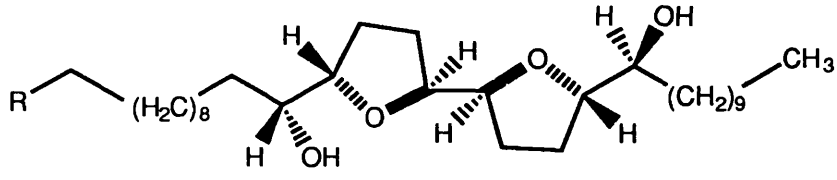
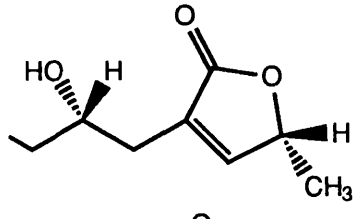
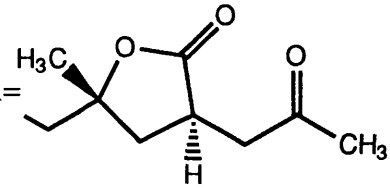
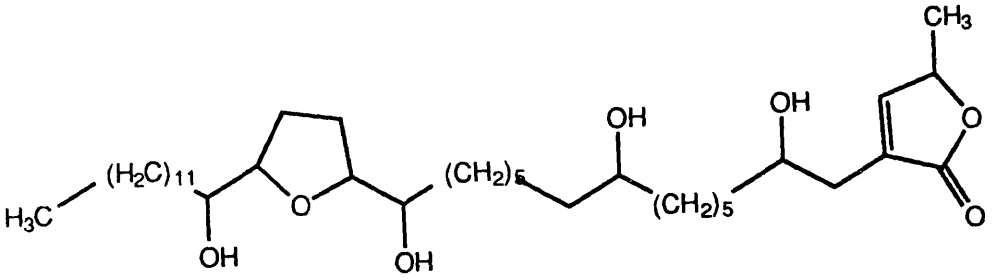


(**35**) R=



(**36**) R=





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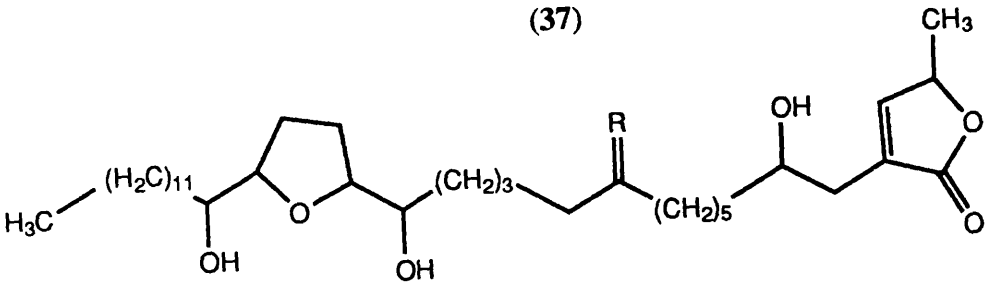
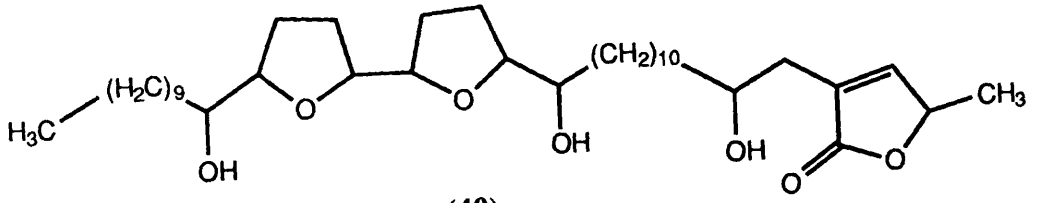
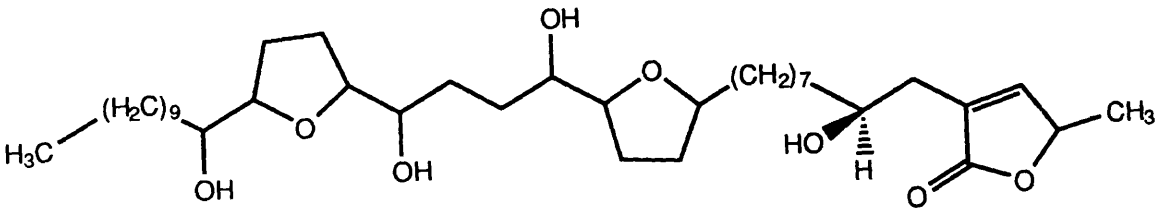


(38) R = O

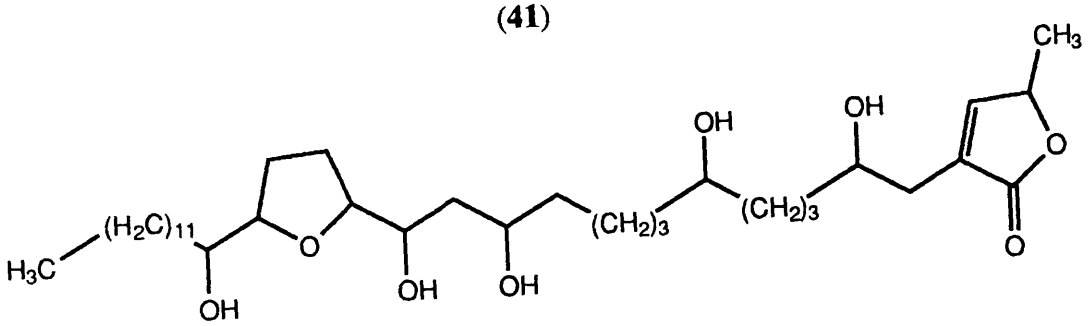
(39) R = H, OH



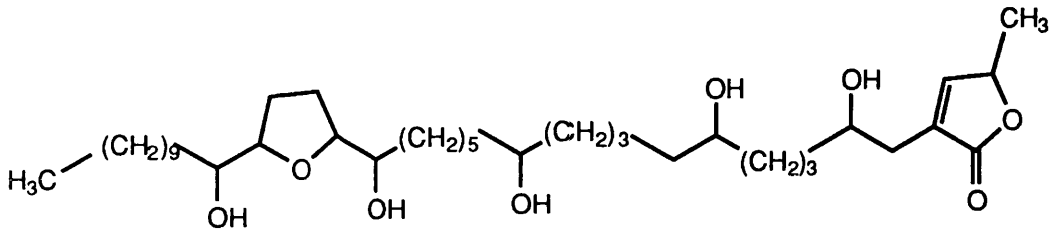
(40)



(41)

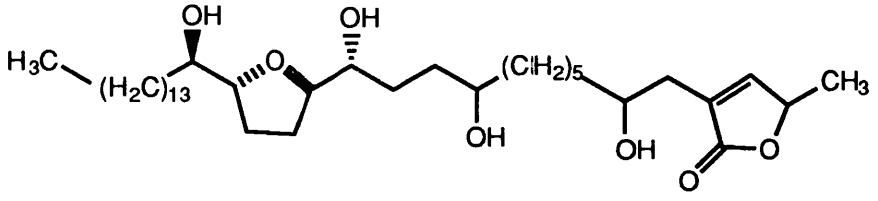


(42)

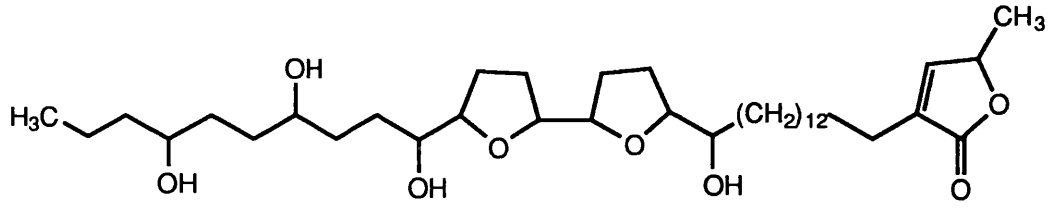


(43)

Goniothalamicin (**44**), from *Goniothalamus giganteus* [54] and bullatacin (**35**), from *Annona bullata*[45] have been found to show insecticidal activity. Cherimoline (**45**) and its derivatives from *Annona cherimolia* [55], have been reported to have antimicrobial activity.



(44)



(45)

THE PIPERACEAE FAMILY

The plants of the family Piperaceae are small trees, shrubs and woody climbers. The family is pantropical in its distribution and inhabits mostly rain forest. The family consists of 5 genera and 2000 species[56].

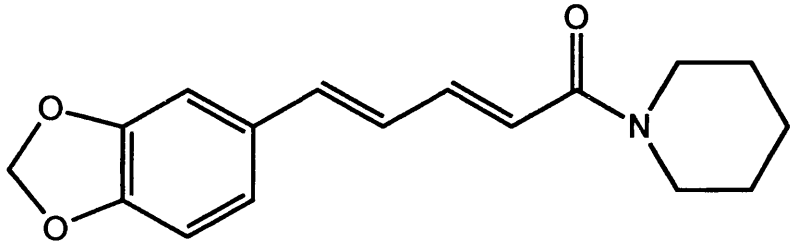
PIPERACEAE OF ECONOMIC IMPORTANCE

The main economic importance of the Piperaceae family is related to pepper. The species *Piper nigrum* is the source of pepper and the Polynesian beverage Kava is made from the roots of *P. methysticum*.. In India and Africa the leaves of *Piper betle* are used as a masticatory[57]. Some species of *Piper* are also grown for their ornamental value.

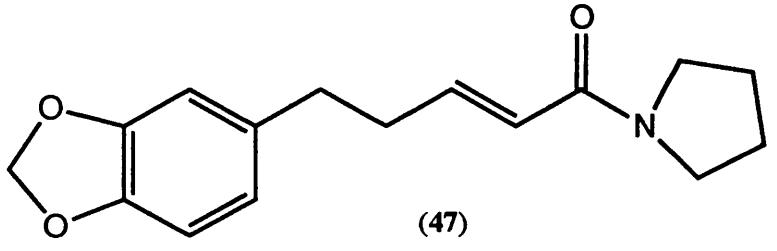
PIPERACEAE OF MEDICINAL SIGNIFICANCE

Medicinally this family has great importance. In the Indian subcontinent, the plants of this family have various medicinal uses. *Piper betle* is used in snake-bite, *P. cubeba* in gonorrhea and cystitis, *P. longum* for colds and *P. nigrum* in cholera, malaria and skin diseases[58]. In Panama the leaves of *Piper alleni* and *P. lucigauden* are used as a snake-bite remedy by the Indians and the leaves of *P. aequale* are used in a decoction to cure rheumatism[59]. In the Philippines the leaves of *P. albidiramum* and *P. penninerve* are applied to ulcers and *P. glabella* is used as a remedy for conjunctivitis in Colombia[59]. The leaves of *P. umbellata* , the fruits of *P. darienense* and the decoction of roots , stem and leaves of *P. alegreanum* are used to relieve toothache[60]. *Piper betle* has been used in the treatment of diphtheria in Africa[61].

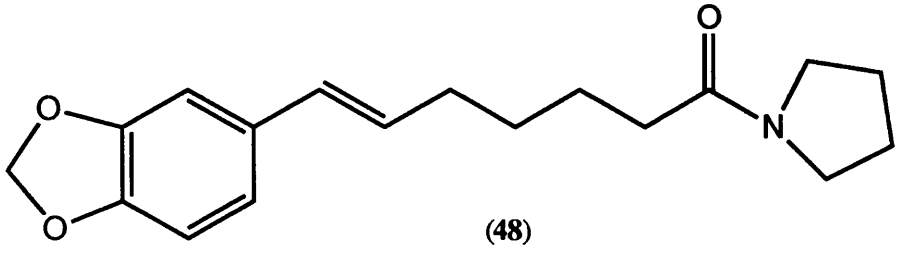
The most common constituents of the Piperaceae are the pepper alkaloids and phenylpropanoid derivatives. The alkaloid piperine (**46**) from *Piper longum*, has a stimulatory action on the central nervous system[62]. Several piperamides from pepper, eg (**47**) and (**48**), showed larvicidal effects against the larvae of *Toxocara canis*[63]. Isobutyl amides, eg pellitorine (see Chapter 10), have important insecticidal properties[63a,63b]. The alkaloids N-(3-methoxy-4,5-methylenedioxydihydrocinnamoyl)-Δ^3-pyridin-2-one (**49**), N-(3-methoxy-4,5-methylenedioxycinnamoyl)-Δ^3-pyridin-2-one (**50**), piplartine (**51**) and piplartine dimer (**52**), isolated from the leaves of *P. aborescens* , have been found to exhibit cytotoxic activity[64].



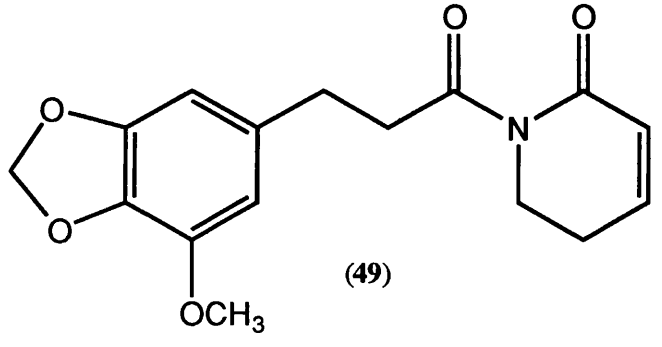
(**46**)



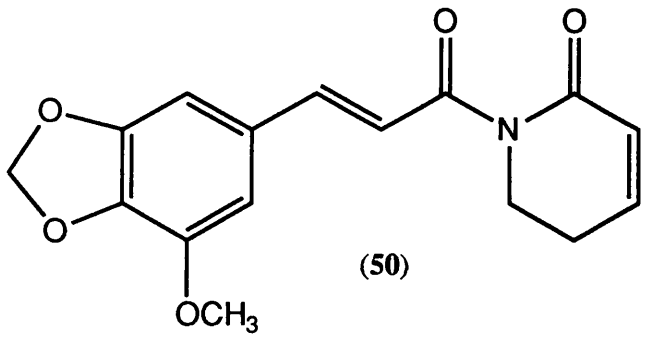
(**47**)



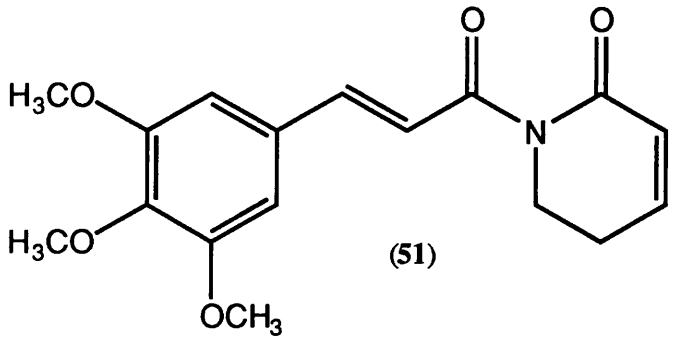
(**48**)



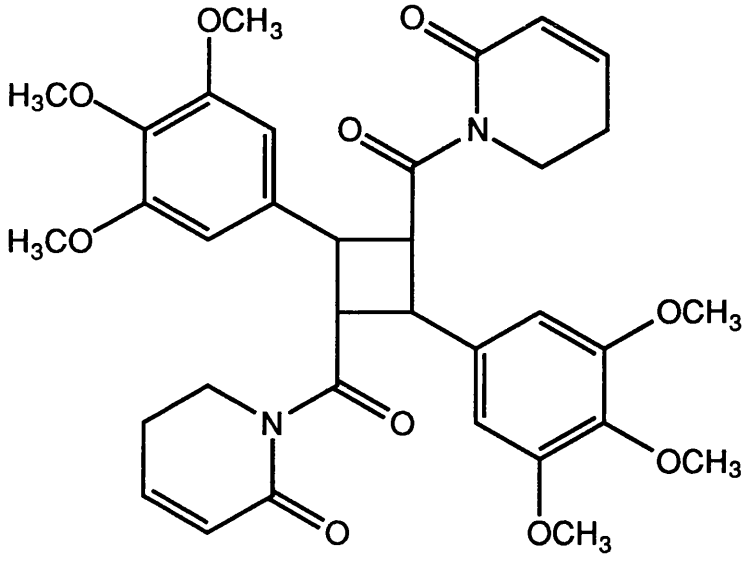
(**49**)



(50)



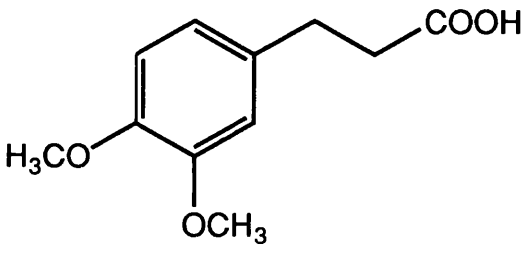
(51)



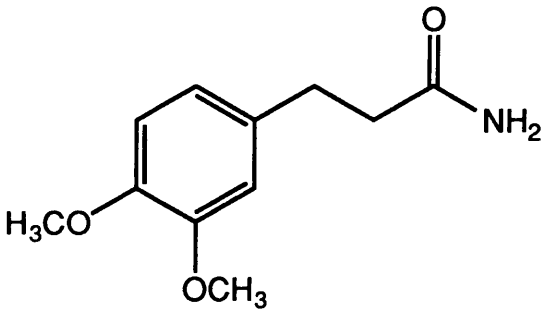
(52)

3-(3,4-Dimethoxyphenyl)-propanoic acid (**53**) and 3-(3,4-dimethoxyphenyl)-propanamide (**54**), from *P. arboricola*, exhibit analgesic effects[65]. Strong antimicrobial activity has been observed for 4,5-dimethoxy-2,3-methylenedioxy-1-allylbenzene (**55**),

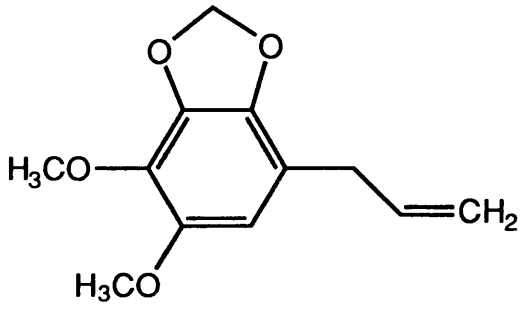
which was isolated from *P. hispidum* and *P. aduncum*[66]. Dihydrochalcones, for example piperaduncin A (**56**), from *Piper aduncum* have cytotoxic and antibacterial activity[67].



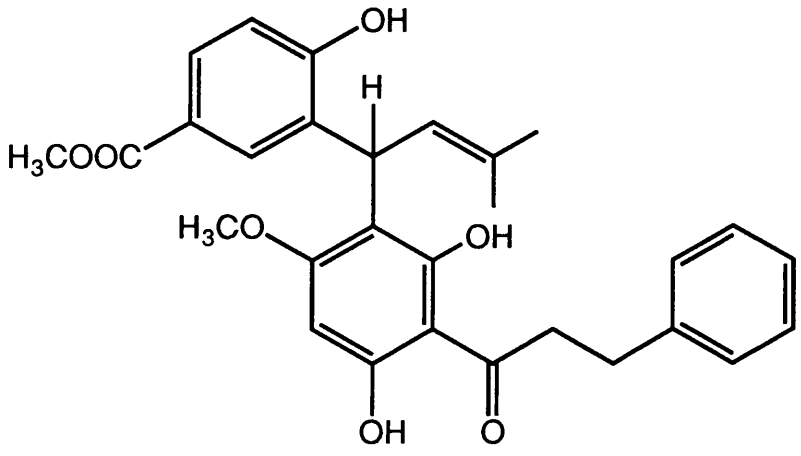
(**53**)



(**54**)



(**55**)



(**56**)

THE MELIACEAE FAMILY

The Meliaceae is mainly a family of trees, restricted to tropical and subtropical regions. The family comprises about 50 genera and 550 species[68]. Most of the species are found in rain forests.

MELIACEAE OF ECONOMIC IMPORTANCE

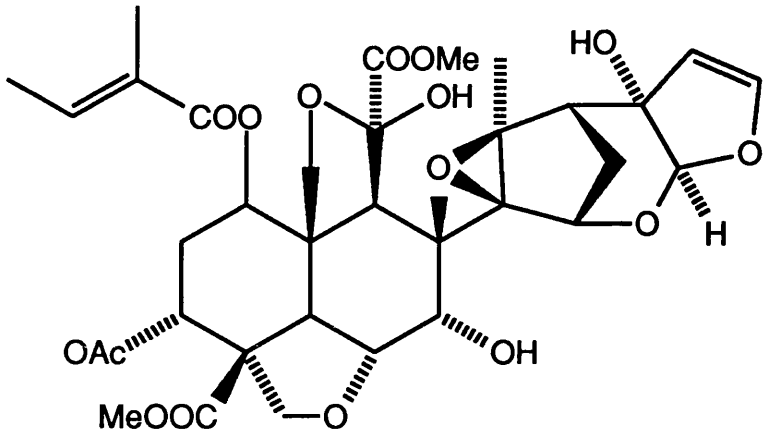
This family has great importance economically. Timber is produced from *Swietenia mahogani*, *Cedrela toona*, *Chukrasia tabularis* and *Chloroxylom swietenia*[69]. The seed oil of *Trichilea emetica* is used for soap making[68]. The fruits of *Aglaia* and *Lansium* species are very important[69]. Some of the genera are used for ornamental purpose.

MELIACEAE OF MEDICINAL SIGNIFICANCE

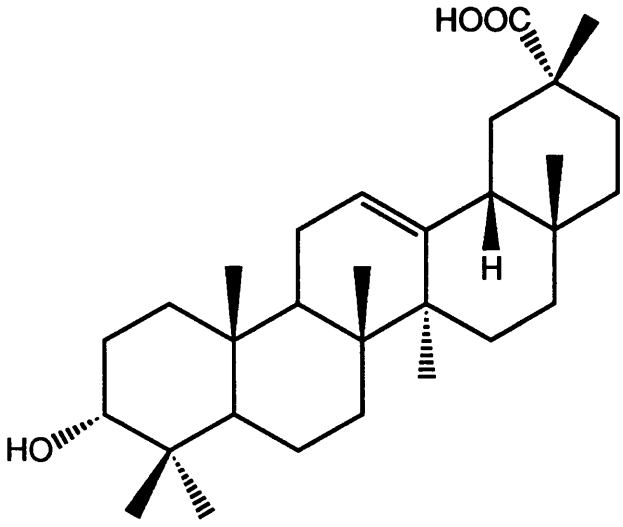
Medicinally this family has some importance. In the Indo-Pak-Bangladesh subcontinent members of the Meliaceae have various medicinal uses. *Cedrela toona* is used in dysentry and as an antiulcer, *Melia azedarach* as an anthelmintic and for nervous headaches, *Carapa granatum* and *Naregamia alata* for dysentry[70]. In the Philippines, the bark of *Vavaea amicorum* and the leaves of *Melia dubia* have been used to relieve internal pain[71]. Insecticides have been isolated from *Azadirachta* and *Melia* species. *Melia azaderach* is used as an insecticide against flies[72]. In Africa the roots of *Ekebergia* species are used as a dysentry remedy while the roots of *Turraea floribunda* are used as an emetic in rheumatism, dropsy and heart disease[73].

The Meliaceae family is a rich source of limonoids (tetranortriterpenoids). The most interesting limonoid is undoubtedly azadirachtin (**57**), from *Azadirachta indica*,

which has potent antifeedent and insecticidal properties[74]. Other limonoids from the Meliaceae family have been found to exhibit antileukemic activity[75]. Limonoids from *Azadirachta indica* and *Dysoxylum roseum* have been found to exhibit cytotoxic activity against human cancer cells[76,77]. The pentacyclic triterpenoid katonic acid (**58**), from *Sandoricum koetjape* (syn *S. indicum*) shows cytotoxicity[78].

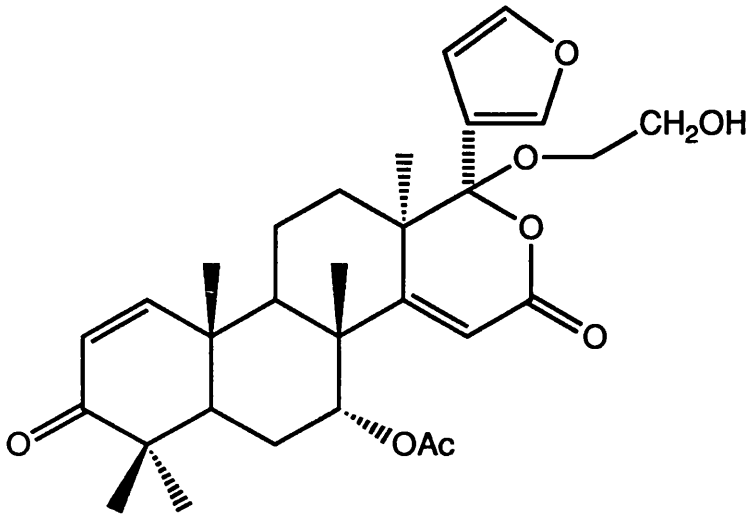


(**57**)

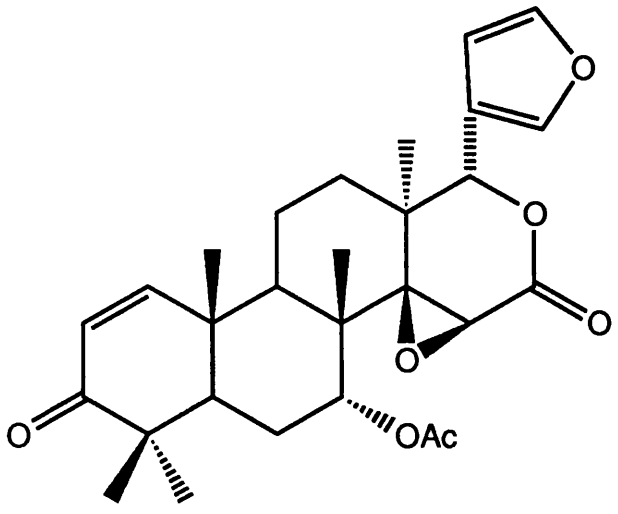


(**58**)

Various triterpenoids from *Melia azedarach* are reported to have antiviral and antibacterial activity[79,80,81]. Antibacterial activity was also observed for mahmoodin (**59**), from *Azadirachta indica*, against various gram-positive and gram-negative organisms[82]. Gedunin (**60**), a metabolite of several Meliaceae species, exhibits antimalarial activity[83] *in vitro* while alkanes of neem leaves are active against mosquito larvae[84].



(59)



(60)

THE RUBIACEAE FAMILY

The Rubiaceae is one of the largest plant families, consisting of 500 genera and about 7000 species. The family is mainly tropical and subtropical in its distribution but some species are also found in temperate and sub-arctic regions[85]. Members of the family found in tropical regions are trees or shrubs. Temperate ones are herbaceous[85].

RUBIACEAE OF ECONOMIC IMPORTANCE

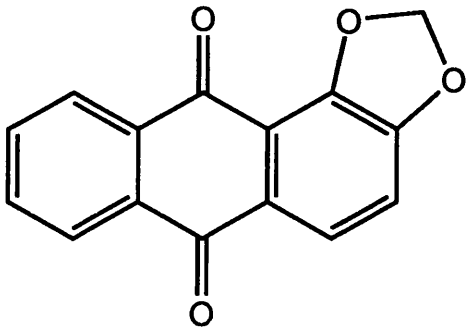
Some of the members of this family are very important in economic terms. The best known product of the Rubiaceae is coffee which comes from *Coffea arabica* , *C. canephora* and other species. Quinine is obtained from various *Cinchona* species[86]. *Cephaëlis* species provides the drug ipecacuanha while the dyes madder and gambier are obtained from *Rubia* and *Uncaria* species[85,87]. Some of the Rubiaceae species are cultivated as ornamental plants and the best known one is *Gardenia jasminoides*.

RUBIACEAE OF MEDICINAL SIGNIFICANCE

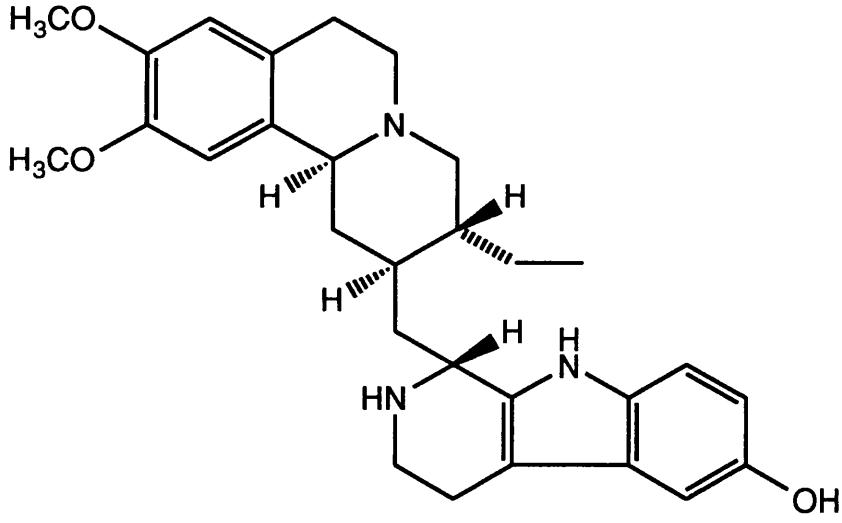
Medicinally this family has great importance. The family Rubiaceae contains some important genera which are used as remedies for various diseases all over the world. In the Indian subcontinent *Anthocephalus indicus* is used in snake-bite, *Cephaëlis ipecacuanha* in amoebic dysentry, *Chassalia chartacea* in pneumonia, *Gardenia jasminoides* in nervous disorder, *Morinda umbellata* in diarrhoea and dysentry and *Ixora coccinea* in dysentry[88]. *Hintonia laliflora* is used in malarial fever in Mexico and *Hedyotis diffusa* is used for stomach ulcers in Singapore while *Contarea hexandra* is used as a remedy for malaria all over the Latin America[89]. *Mussaenda* species are used in small-pox and *Psychotria* species are used in ulcers, T.B. and

internal pains. In Africa *Rubia petiolaris* is used for kidney diseases and *Canthium inerme* is used as a remedy for dysentry and diarrhoea[90].

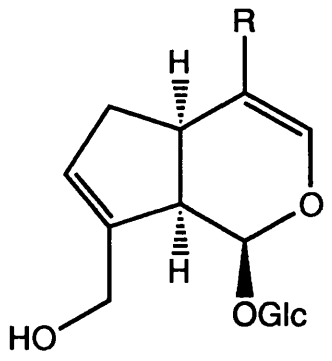
Morindaparvin-A (**61**), from *Morinda parvifolia*[91], tubulosine (**62**), from *Pogonopus speciosus*[92] and tarennoside (**63**), geniposidic acid (**64**), geniposide (**65**), gardenoside (**66**) and genipin (**67**), from *Genipa americana*[93], have all been found to exhibit cytotoxic and antitumour activity.



(**61**)



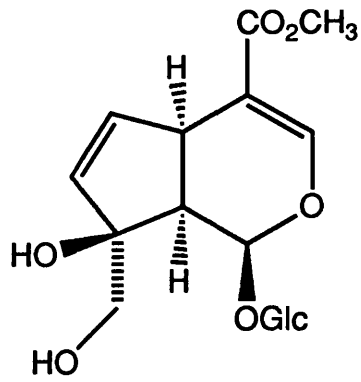
(**62**)



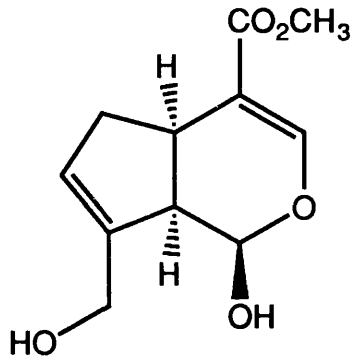
(**63**) R=CHO

(**64**) R=COOH

(**65**) R=$COOCH_3$

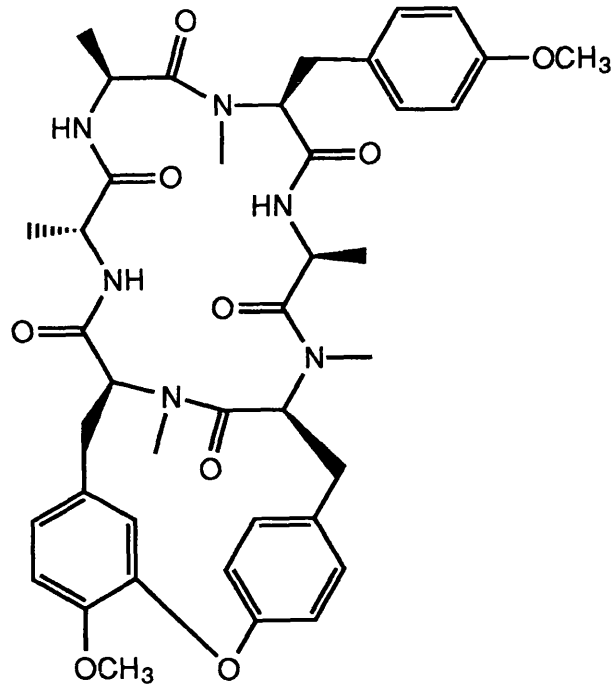


(**66**)

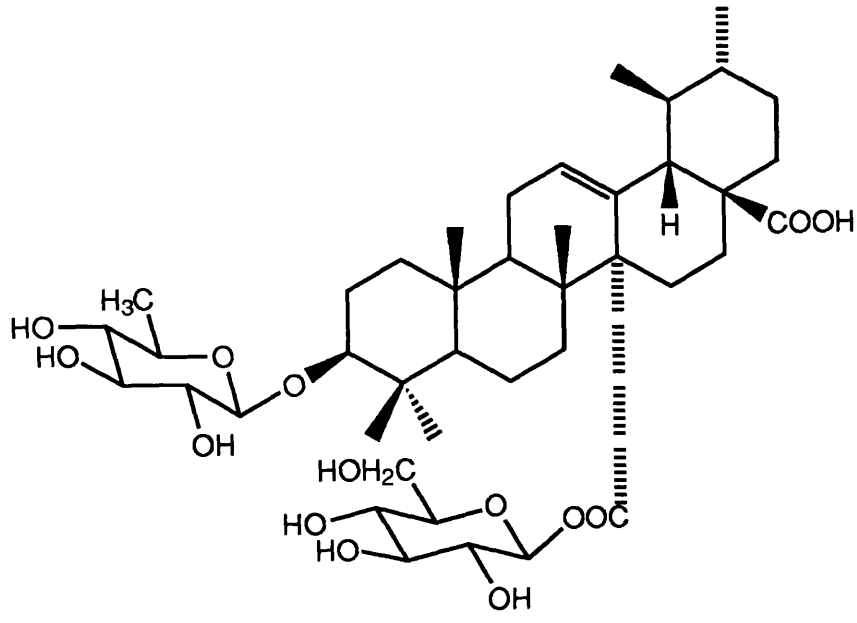


(**67**)

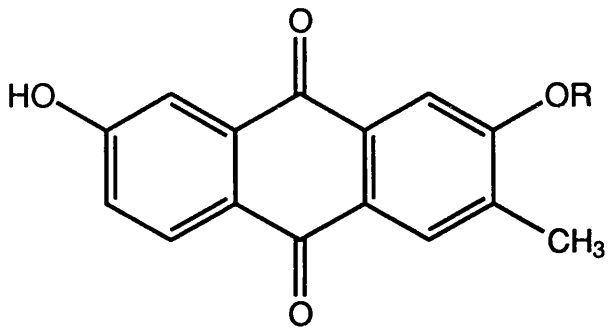
Cyclic hexapeptides (as **68**) from *Rubia cordifolia* and *Rubia akane* have been found to exhibit cytotoxicity and antitumour activity[94,95,96]. Other cyclic hexapeptides and their glycosides from *Rubia* species also exhibit cytotoxic activity[97,98]. Antiviral and antiinflammatory activity has been detected in the quinovic acid glycoside (**69**), from *Uncaria tomentosa* and *Guettarda platypoda*[99,100]. Anthraquinone glycosides, such as (**70**) from *Rubia cordifolia* , have been found to exhibit antibacterial activity[101]. An alkaloid , from *Psychotria oleoides,* has shown somatostatin antagonistic activity[102].


O
OCH3
N
HN
O
HN
O
NH
O
O
N
N
O
O
OCH3
O

(**68**)


H
COOH
H3C
HO
HO
O
O
OH
HOH2C
O
OOC
HO
HO
OH

(**69**)



R= (6'-O-acetyl)-β-D-Glc

(70)

THE EUPHORBIACEAE FAMILY

The Euphorbiaceae is a large plant family consisting of some 300 genera and 5000 species of dicotyledonous herbs, shrubs and trees. The genera *Phyllanthus*, *Euphorbia*, *Croton* and *Acalypha* are very large[103] . The family is predominantly distributed in tropical regions but the genus *Euphorbia* is also found in extratropical regions like U. S. A., the MiddleEast, the Mediterranean basin and South Africa[103]. In South America, particularly in Brazil, about 300 *Croton* species are found.

EUPHORBIACEAE OF ECONOMIC IMPORTANCE

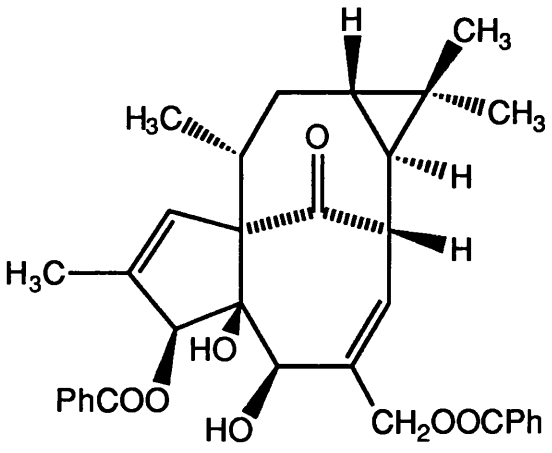
The Euphorbiaceae contains a large number of economically important genera. The most important is *Hevea brasiliensis,* the source of commercial rubber. Rubber is also obtained from the genus *Manihot.* The roots of *Manihot esculentus* (Manioc or Cassava) are used to prepare the food stuff tapioca[104]. Castor oil is obtained from *Ricinus communis*, tung oil from *Aleurites fordii*, croton oil from *Croton tiglium* and candlenut oil from *Aleurites moluccana*[105]. From *Mallotus philippinensis* a red dye is obtained while *Chrozophora tinctoria* produces purple and blue dyes. Some species also produce timber but this is restricted to the genus *Ricinodendron*[106].

EUPHORBIACEAE OF MEDICINAL SIGNIFICANCE

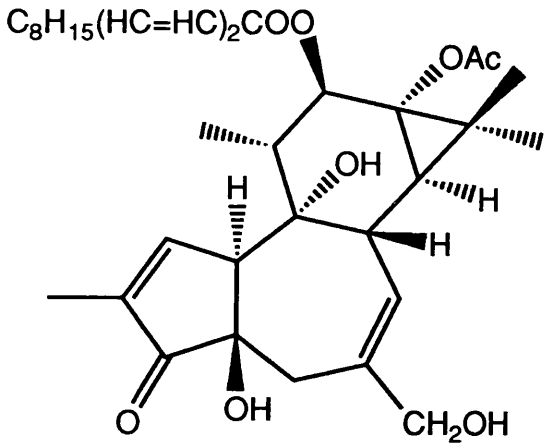
Many of the Euphorbiaceous plants are documented as herbal remedies throughout the tropical world. In the Indo-Pak-Bangladesh subcontinent *Acalypha indica* is used in pneumonia and asthma, *Croton tiglium* in snake bite and fish poison, *Euphorbia* species in rheumatism, asthma, gout and neurological rheumatism, *Homonoia riparia* in gonorrhea and *Jatropha multifida* in scabies[107]. Many Euphorbiaceous genera are used to treat various diseases throughout the world. For

example the leaves of *Breynia* species are used in the Solomon islands to stop toothache and, in the Phillipines, the leaves of *Glochidion* species are applied to ulcers [108]. In Mexico the caustic juice of *Croton cortesianus* is used to treat generalized skin diseases[109].

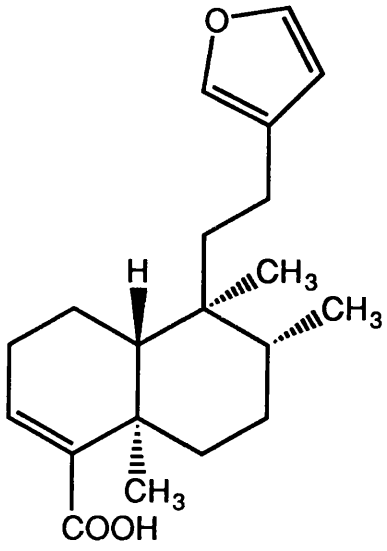
The Euphorbiaceae produce a wide range of diterpenoids, usually as esters. Several ingenol derivatives from various Euphorbiaceous plants have been found to show high antitumour, cytotoxic and antileukemic activity[110,111,112,113]. One of these derivatives is ingenol 3,20-dibenzoate (**71**). Phorbol esters, for example 12-O-undecadienoylphorbol-13-acetate (**72**), from Euphorbiaceous plants have been reported to have cytotoxic activity[114,115,116] but are also widely used in tumour promotion in medical research. Some diterpenoids from Euphorbiaceous plants have been found to have antileukemic activity[117]. Two acidic diterpenoids, (-)-hardwickiic acid (**73**) and 3,4-secotrachylobanoic acid (**74**), from *Croton sonderianus*, have been found to exhibit antimicrobial activity[118].



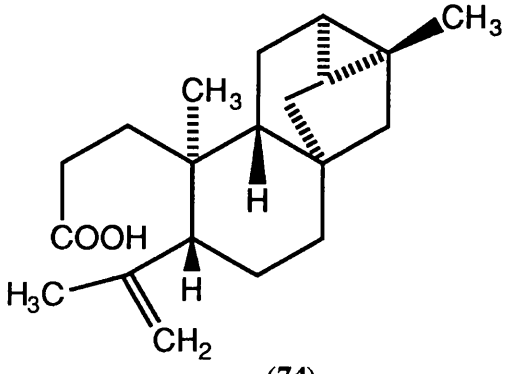
(**71**)


C8H15(HC=HC)2COO
OAc
OH
H
H
H
O
OH
CH2OH

(72)

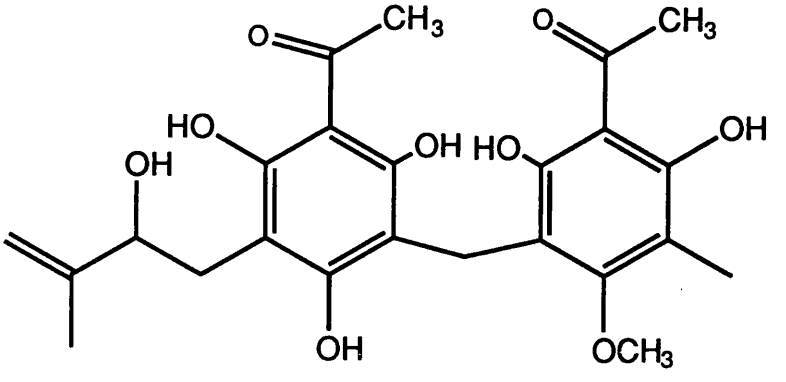

O
H
CH3
CH3
CH3
COOH

(73)

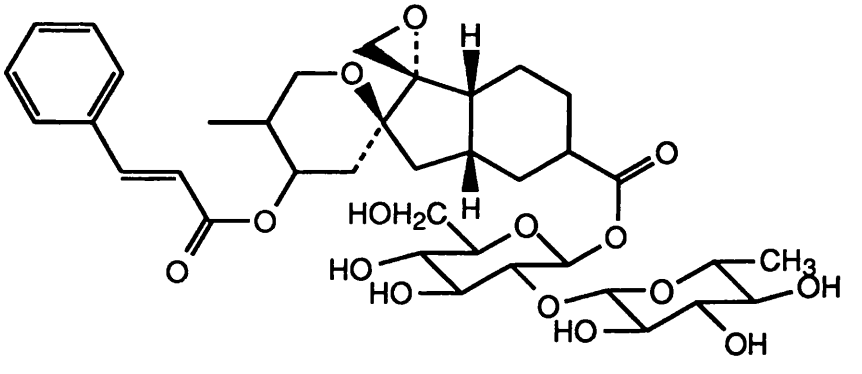

CH3
CH3
H
COOH
H3C
H
CH2

(74)

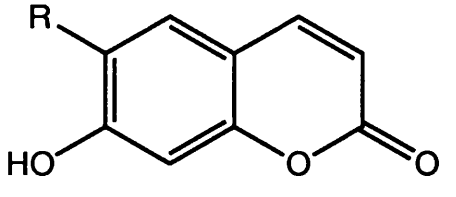
Phloroglucinol derivatives, eg mallotojaponin (**75**) from *Mallotus japonicus* , have been found to exhibit cytotoxic and antitumour activity[119,120,121]. Phyllanthostatin 6 (**76**), an unusual compound from *Phylanthus acuminatus* , has been found to exhibit antineoplastic activity[122]. Some Euphorbiaceous plants also show antiviral activity[123]. Scopoletin (**77**) and umbelliferone (**78**), from *Euphorbia humifusa*, have shown antimicrobial activity[124].



(**75**)



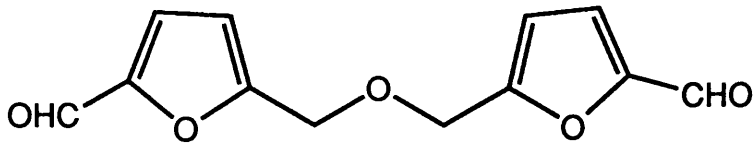
(**76**)



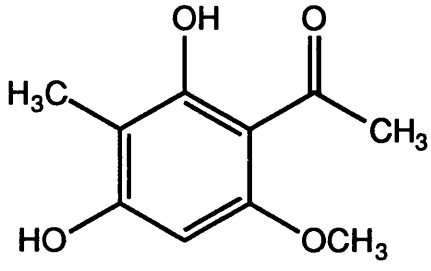
(**77**) R= OMe

(**78**) R= H

The furan derivative (**79**) and 2,4-dihydroxy-6-methoxy-3-methylacetophenone (**80**) from *Euphorbia ebracteolata* have been found to exhibit antituberculosis activity[125].



(79)



(80)

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CHAPTER 2

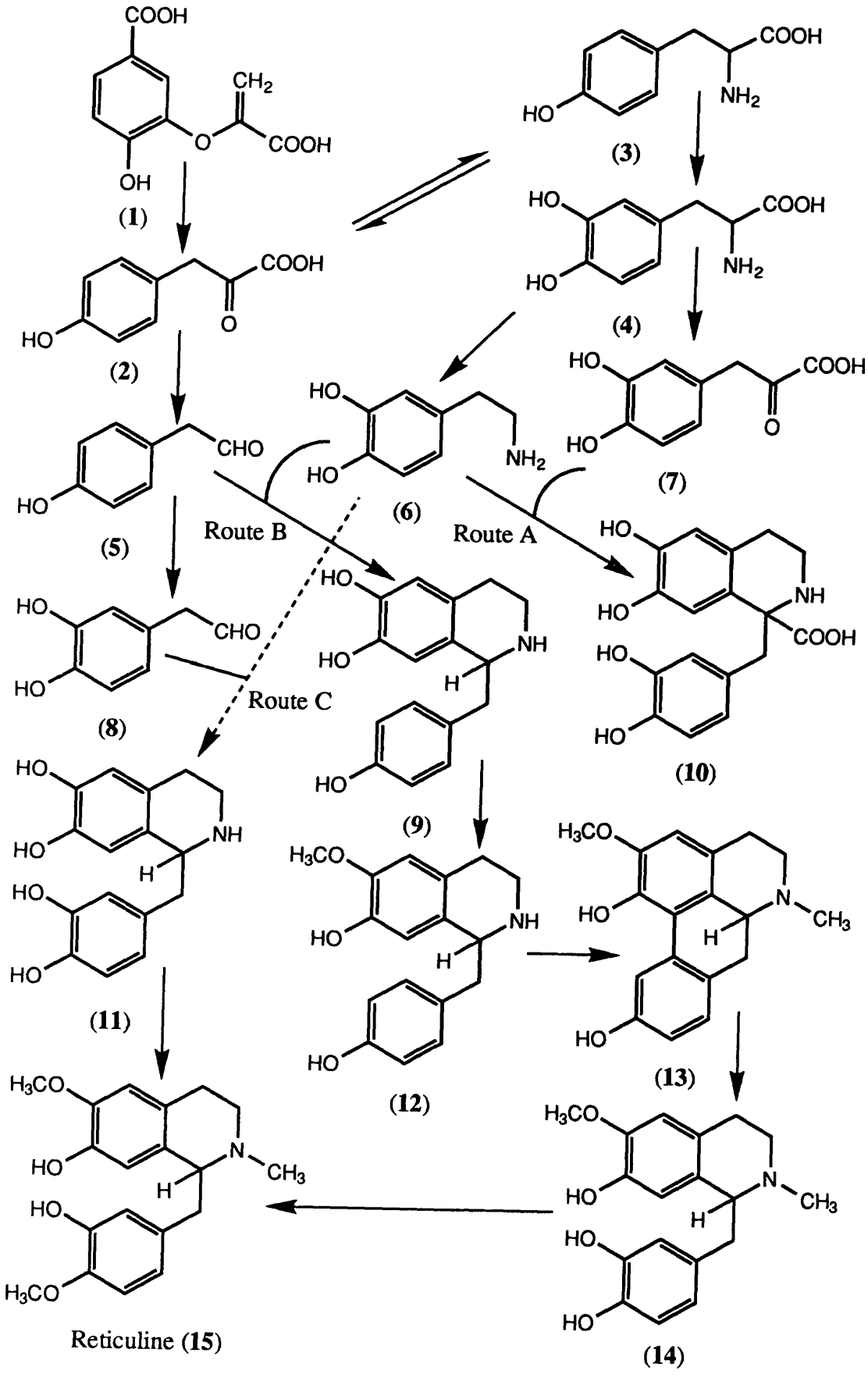
BIOSYNTHESIS OF ALKALOIDS, FLAVONOIDS AND TRITERPENOIDS

BIOSYNTHESIS OF ALKALOIDS

ALKALOIDS DERIVED FROM TYROSINE

THE BENZYLISOQUINOLINE ALKALOIDS

The benzylisoquinoline alkaloids form the largest group of alkaloids of diverse structural types in the plant kingdom[1]. Tyrosine (**3**) is derived from chorismic acid (**1**) via p-hydroxyphenylpyruvic acid (**2**)[2,3]. Two molecules of tyrosine (**3**) form the 1-benzyltetrahydroisoquinoline (1-btiq) skeleton present in reticuline (**15**) and in coclaurine (**12**). At first it was thought that the formation of 1-btiq from tyrosine (**3**) occurred by the condensation of dopamine (**6**) and 3,4-dihydroxyphenylpyruvic acid (**7**) through Route A (Scheme 1). Thus the carboxylated intermediate would be the first 1-btiq (**10**) to be synthesized. Holland *et al*[4] doubted the validity of this route when they observed that labelled DOPA (**4**) was mainly incorporated into the upper portion of 1-btiq molecule but tyrosine (**3**) was equally incorporated into both the upper and lower moieties, an observation that is at variance with the proposed mechanism in Route A. Recently Schumacher *et al*[5] and Zenk *et al*[6] have found that the enzyme (S)-norlaudanosoline synthase, isolated from the family Pavaveraceae, catalyses the condensation of dopamine (**6**) and 3,4-dihydroxyphenylacetaldehyde (**8**) following Route C (Scheme-1) to yield (S)-norlaudanosoline (**11**) as the initial 1-btiq alkaloid. (S)-reticuline (**15**) is formed by the methylation of (S)-norlaudanosoline[6]. However these authors also found that 3,4-dihydroxyphenylpyruvic acid (**7**) is not a substrate for this enzyme and have showed that Route A does not operate in the biosynthesis of isoquinolines. Herbert[7] reported that the enzyme (S)-norlaudanosoline synthase accepted p-hydroxyphenylacetaldehyde (**5**) as an



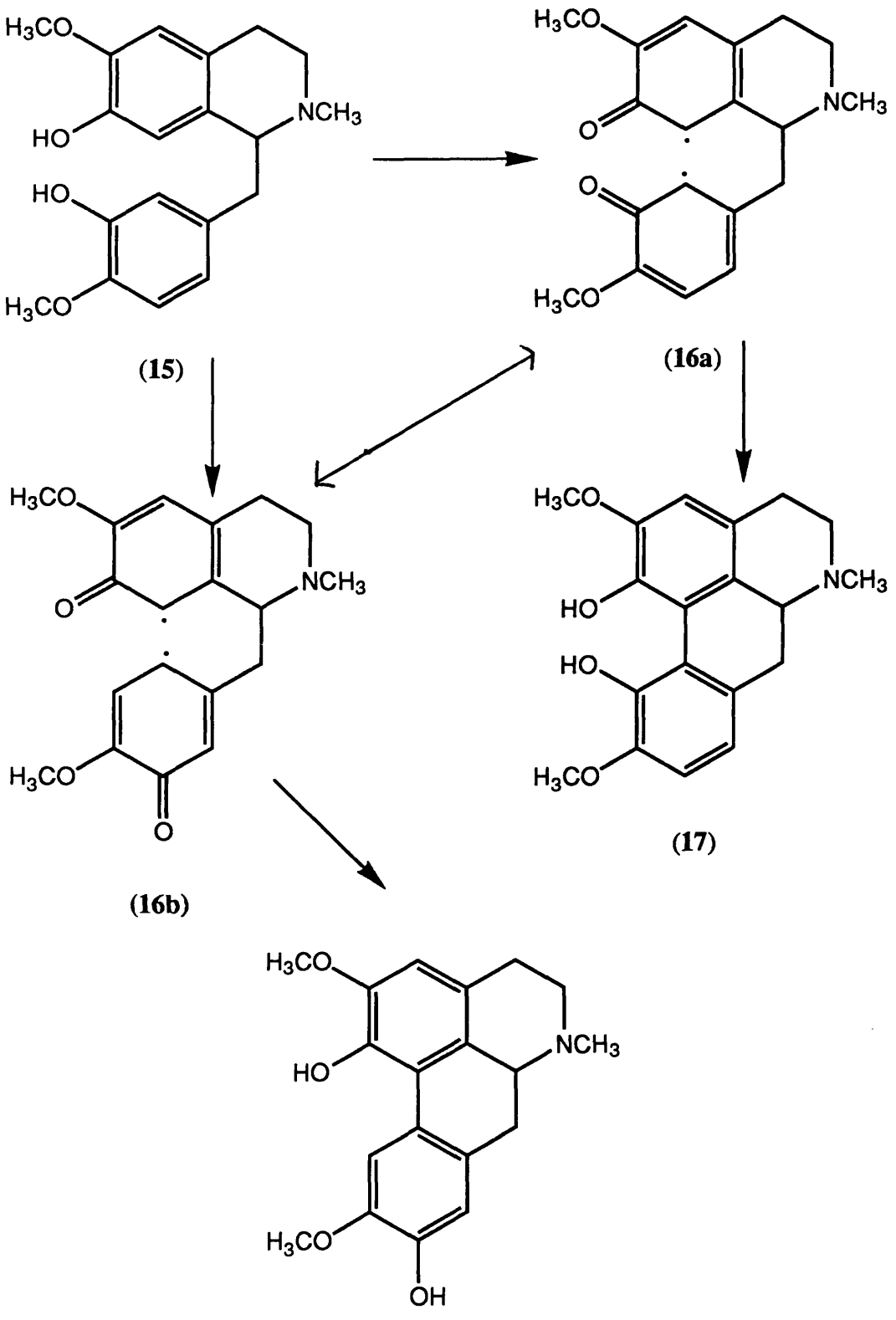
Scheme 1. Biosynthesis of benzylisoquinoline alkaloids.

alternative substrate for condensation with dopamine (**6**) (Route B) to give (S)-norcoclaurine (**9**). This is further supported by Stadler *et al*[8] and Guinaudeae *et al*[9] who have reported that norcoclaurine (**9**) is formed by a stereospecific Pictet-Spengler condensation of dopamine (**6**) and p-hydroxyphenylacetaldehyde (**5**). Norcoclaurine (**9**) has been shown to be building block of many isoquinoline alkaloids[10]. The common building block for the isoquinoline alkaloids, (S)-reticuline (**15**), is obtained from norcoclaurine via a sequence of O- and N-methylation as in coclaurine (**12**), N-methylcoclaurine (**13**) and (S)-3'-hydroxy-N-methylcoclaurine (**14**), strictly governed by the sequential action of stereoselective enzymes. This view has been supported by extensive tracerexperiments[8]. Reticuline (**15**), on metabolism, gives rise to a series of isoquinoline alkaloids (Schemes 1, 2, 3, 4, 5, 6).

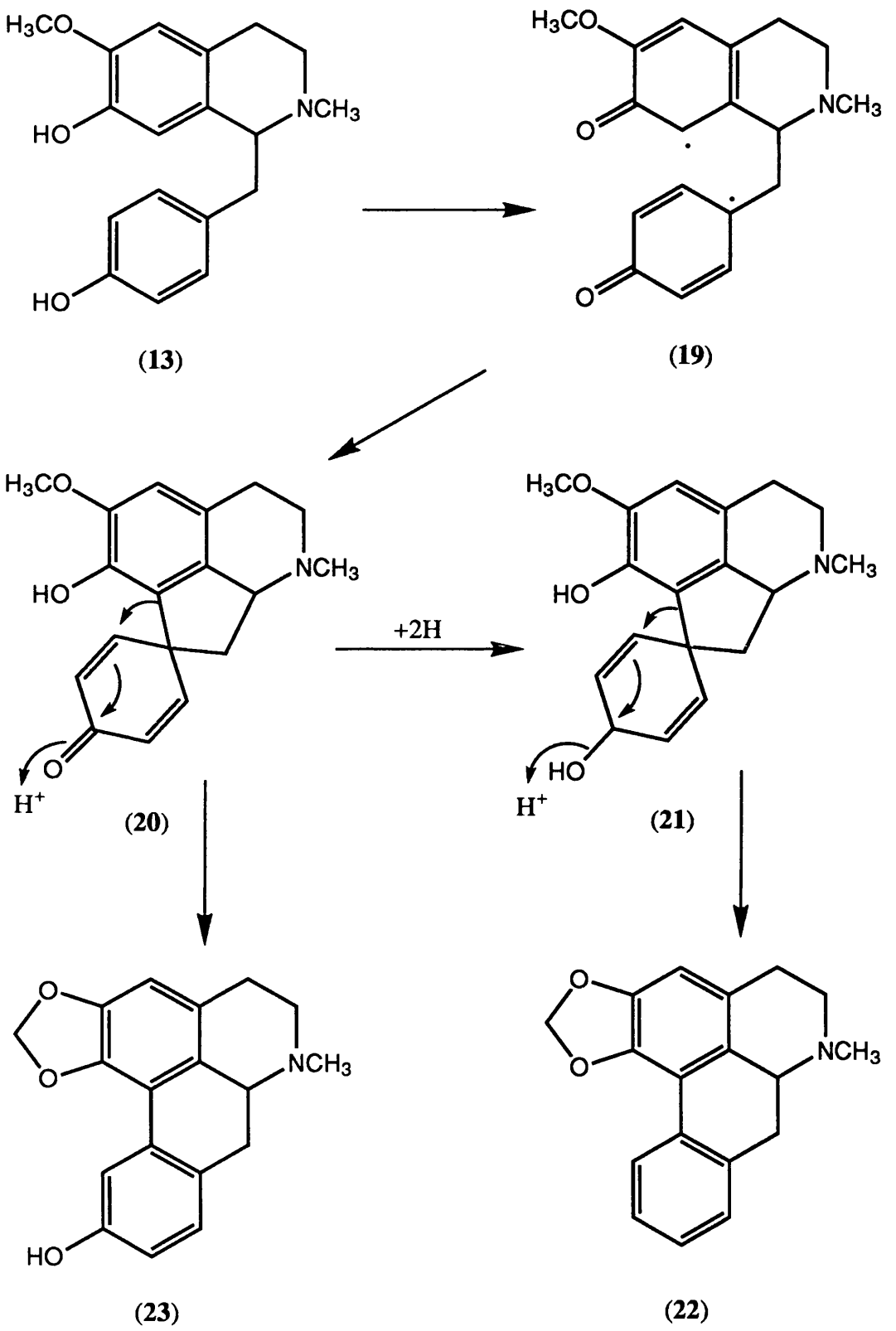
THE APORPHINE ALKALOIDS

That benzyltetrahydroisoquinoline acts as an intermediary in the formation of aporphines is well supported by a number of *in vivo* and *in vitro* studies[11,12]. The aporphines are the direct oxidative coupling products of reticuline (**15**, Scheme 2)[1,11,13,14]. The bis-dienone radicals (**16a**) or (**16b**), derived from reticuline (**15**), are likely to undergo *ortho-ortho* or *ortho-para* coupling giving 1,2,10,11-substituted (**17**) and 1,2,9,10-substituted (**18**) aporphines[11,15].

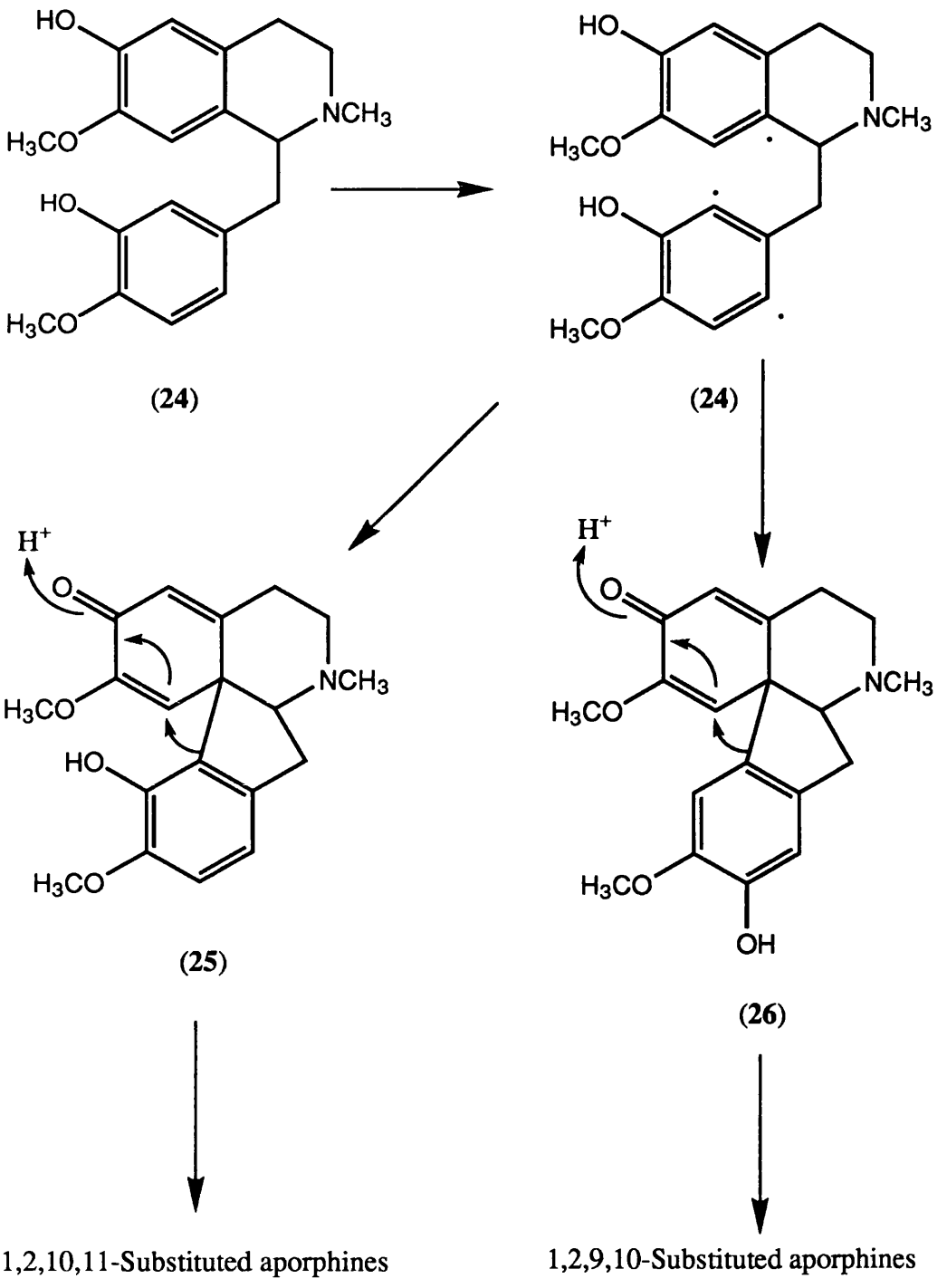
Aporphines can be formed through proaporphine intermediates. Shamma *et al*[11] reported that a trisubstituted 1-btiq, N-methyl coclaurine *ortho-para* diradical (**19**), derived from N-methylcoclaurine, undergoes cyclisation to give a known proaporphine glaziovine (**20**), which subsequently gives rise to the aporphines roemerine (**22**), lacking D-ring oxygenation and mecambroline (**23**), retaining oxygenation in D ring (Scheme 3). One of the intermediates of roemerine formation is the proaporphinol (**21**).



Scheme 2 : Possible biosynthesis of aporphines via a bis-dienone radical.



Scheme 3 : Routes to 1,2- and 1,2,9-substituted aporphines via a proaporphine intermediate.



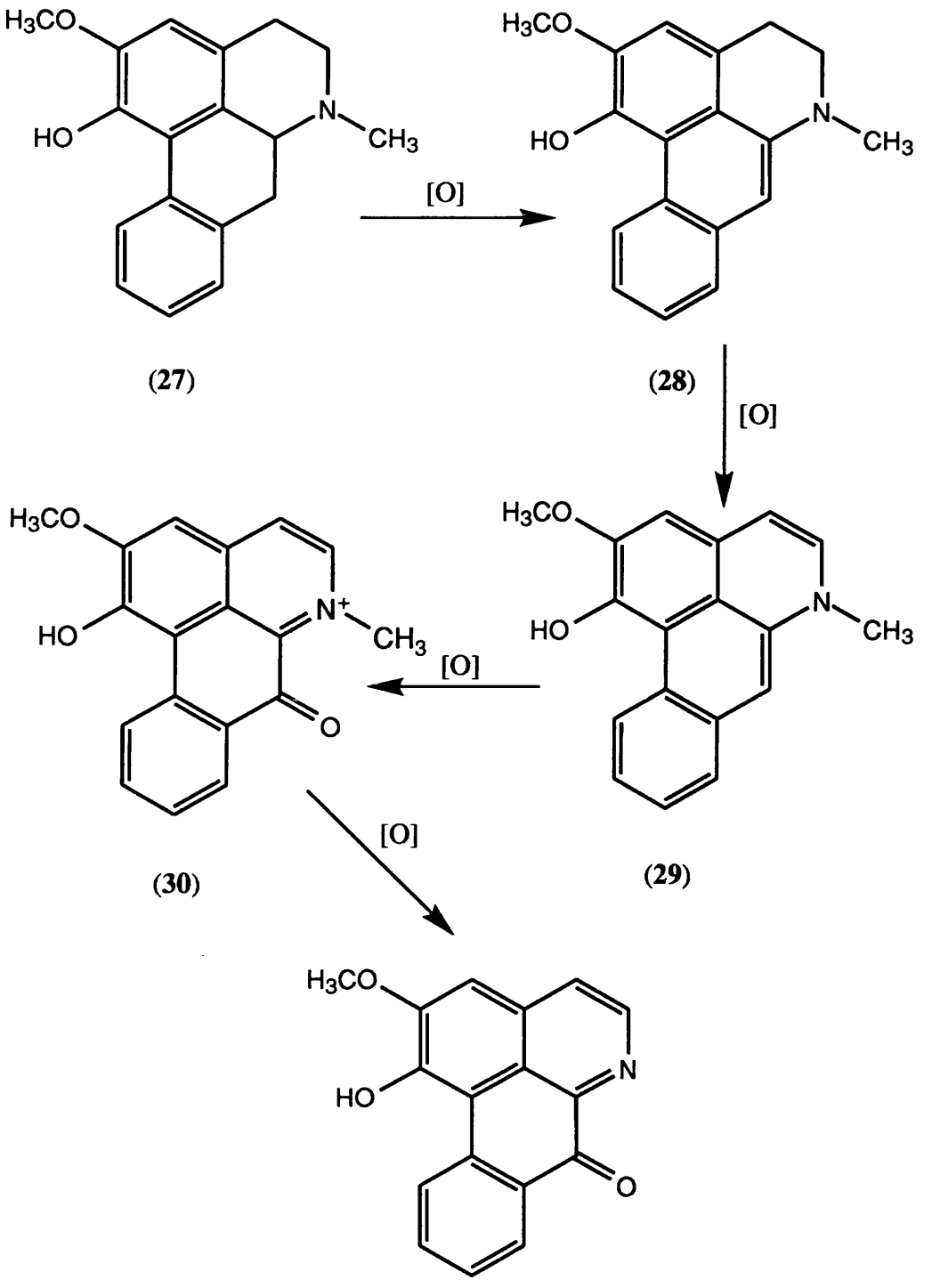
Scheme 4 : Possible route to tetrasubstituted aporphines from norprotosinomenine through neoaporphine intermediates.

Another proposed route[11,16] for the biosynthesis of tetrasubstituted aporphines (Scheme 4) involves the 1-btiq precursor norprotosinomenine (**24**), through the

intermediate neoproaporphines (**25**) and (**26**). It is assumed that neoproaporphine intermediates originate from *para-ortho* and *para-para* coupling between ring A and ring D radicals of norprotosinomenine (**24**). The direct transformation of (**25**) to 1,2,10,11-substituted aporphines and of (**26**) to 1,2,9,10-substituted aporphines has been considered to proceed via protonation and dienone-phenol rearrangement in a manner directly analogous to that for proaporphines. Besides these three proposed biogenic routes, it has been thought that aporphines can also be formed through protoberberineum salts[15] but no hard evidence has been found for this route.

THE DEHYDROAPORPHINE, OXOAPORPHINE AND BISAPORPHINE ALKALOIDS

The biosynthetic studies of dehydroaporphine and oxoaporphines *in vivo* have not yet been carried out so far. Shamma *et al*[11] have suggested a rational sequence of events (Scheme 5) involving the stepwise oxidation of an substituted aporphine (**27**) through the dehydroaporphine (**28**), and then 4,5,6a,7-tetradehydroaporphine (**29**). The 4,5,6a,7-tetradehydroapophine would be susceptible to oxidation at C-7 leading to the generally unstable 7-oxo-N-methylquaternaryoxoaporphinium ion (**30**), which very readily loses the N-methyl group to form the oxoaporphine (**31**). It has been found that aporphines can undergo this process naturally on prolonged exposure to air[17] and it must be considered that there is a possibility of dehydroaporphines and oxoaporphines formation without enzyme mediation. No *in vivo* biogenetic studies of bisaporphines have been carried out. However Shamma *et al*[11] and Cavé[18] have proposed that bisaporphines can be formed from the oxidized monomeric derivatives like dehydroaporphine, tetradehydroaporphine and oxoaporphine. This is further supported by the studies carried out by Guinadeau *et al*[19].

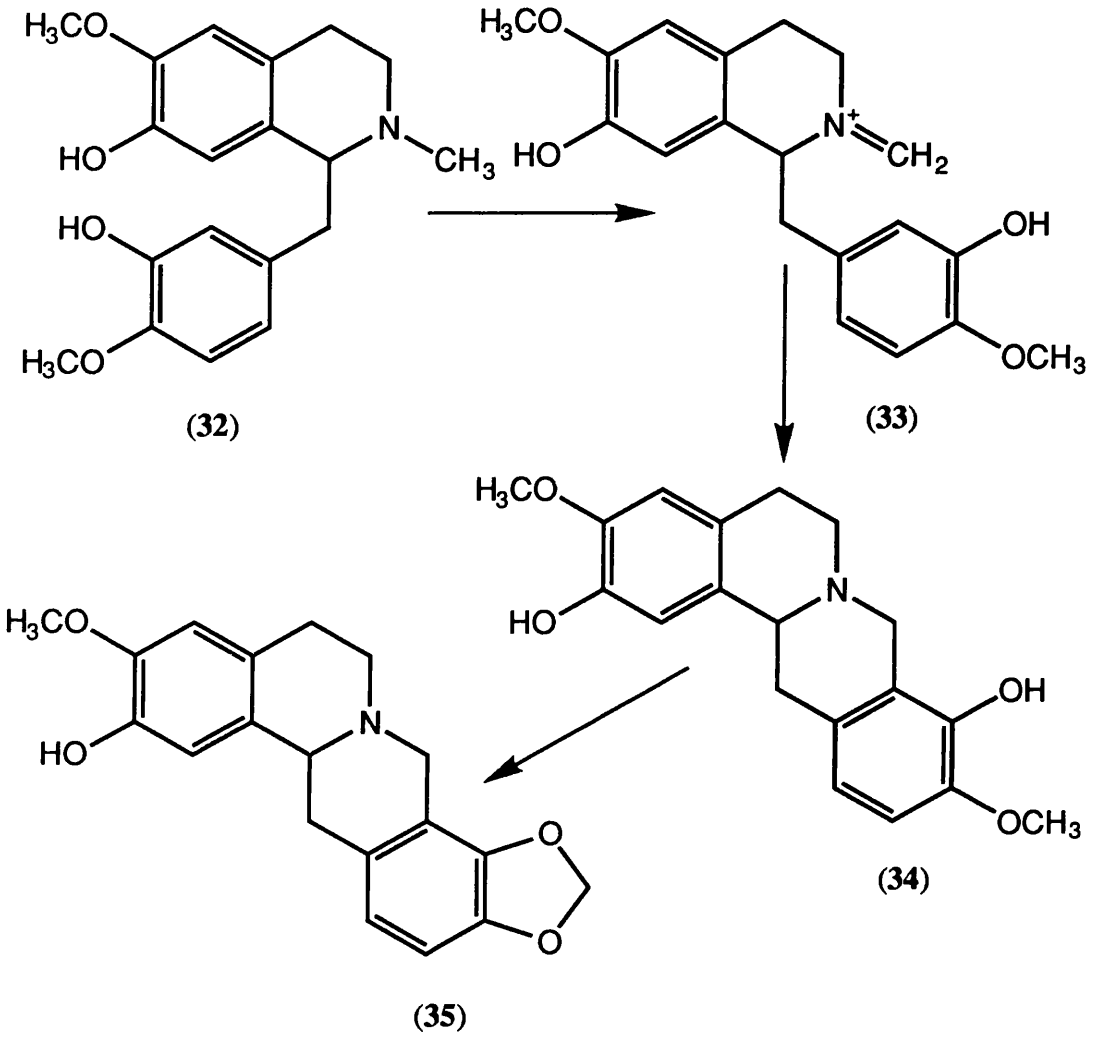


Scheme 5 : Possible biosynthesis of oxoaporphines from aporphines.

THE PROTOBERBERINE ALKALOIDS

The protoberberine skeleton of the type (**34**) (Scheme 6) is the product of an oxidative ring closure of a 1-btiq precursor, eg reticuline (**32**). Tracer experiments established that the so-called "berberine bridge" (C-8) of the berberine group of alkaloids is derived in nature from N-methyl group of 1-benzyltetrahydroisoquinoline precursors[20,21]. The berberine bridge is formed by the oxidative cyclisation of the N-methyl group in a 1-btiq derivative (**32**) in a reaction which is very similar to that of methylenedioxy formation from an O-methyl group[22]. The mechanism involves oxidation of the 1-btiq (**32**) to the corresponding iminium intermediate (**33**), which can cyclise to form the tetrahydroprotoberberine nucleus of (**34**). Cyclisation of the O-methoxyphenol to form a methylenedioxy group can finally yield (+)-sinactine (**35**).

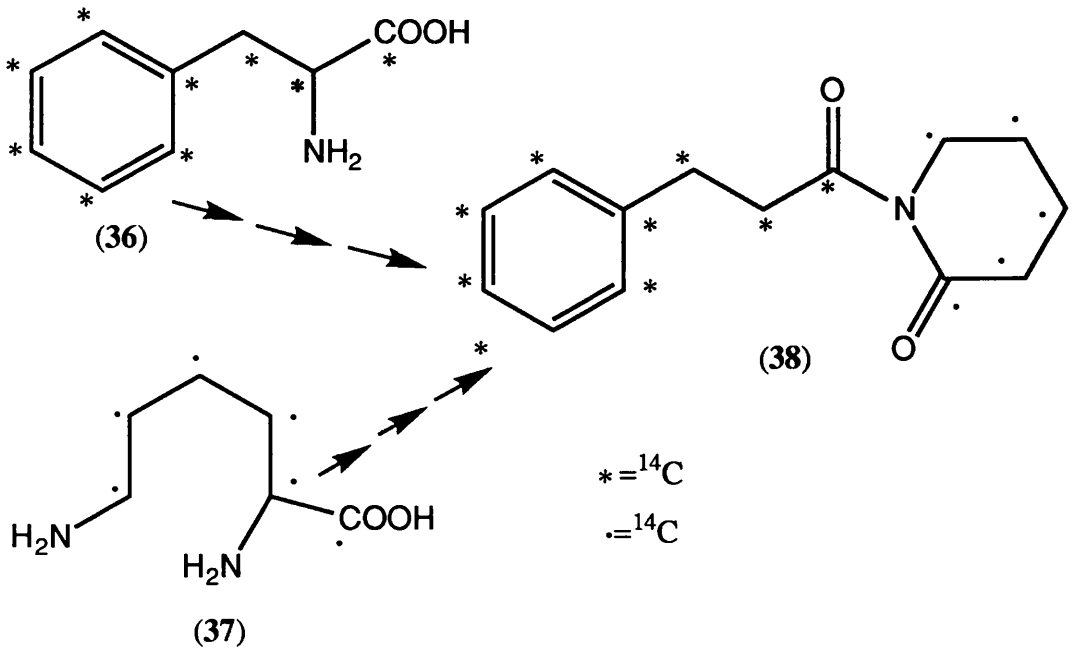
It has been shown that (+)-(S)-reticuline (**32**) is preferred over the (-)-(R)-isomer as a substrate for berberine biosynthesis[23,24]. Bhakuni *et al*[25] reported that when doubly labelled (+)-reticuline was fed to the young cut branches of *Cocculus laurifolius*, labelled (+)-sinactine was isolated. Feeding of (+)-scoulerine (**34**) established its intermediacy in the biosynthesis of (+)-sinactine (**35**)[26]. A similar type of reaction pathway is followed in the biosynthesis of tetrahydropalmitine from (+)-(S)-reticuline[27].



Scheme 6 : Possible biogenesis of protoberberine alkaloids.

BIOSYNTHESIS OF PIPERIDINE AMIDE ALKALOIDS

The alkamides are probably the condensation products of aromatic or long chain aliphatic acids with pyrrolidine, piperidine or isobutylamine moieties. Very little is known about the biosynthesis of alkamides. Leete[28] has reported that the piperidine ring of coniine type alkaloids arises from acetate units. It has been shown, however, using labelled lysine (**36**)[29], that the piperidine nucleus of sedamine and related alkaloids comes from lysine. Prabhu *et al*[30] have studied the possible biogenic pathways for the formation of the piperidine amide alkaloid, piperlongumine (**38**), in *Piper longum.*, using labelled phenylalanine (**37**) and lysine (**36**) (Scheme 7) and have found that the C6-C3 moiety of piperlongumine (**38**) arises from phenylalanine while the piperidone ring is formed from lysine.

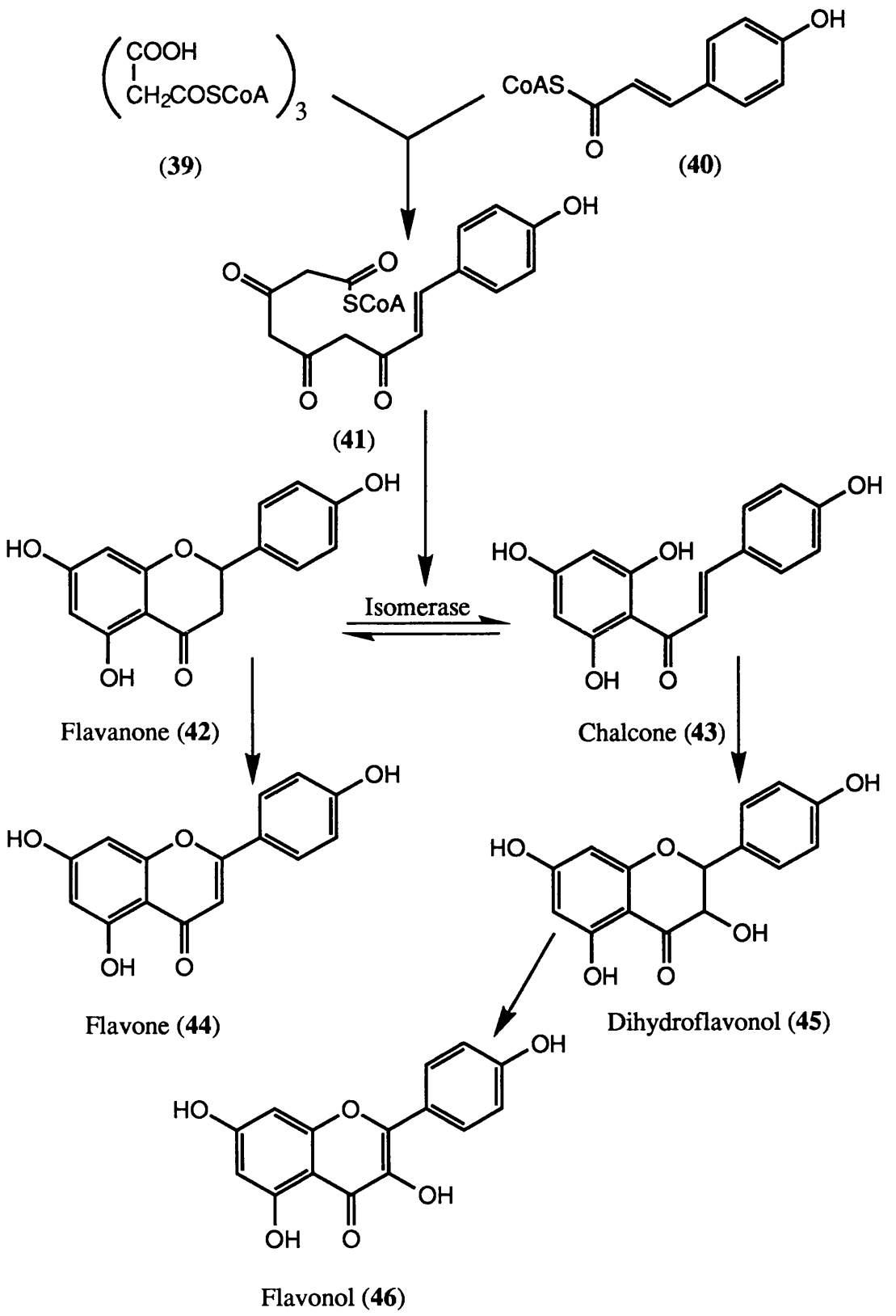


Scheme 7 : Possible biogenesis of piperidine amide alkaloids

BIOSYNTHESIS OF FLAVONOIDS

The flavonoids comprise a large group of secondary metabolites. They are widely founds in higher plants and are responsible for much of the colouring of food and drink of plant origin. They are also responsible for the colour of the flowers and fruits. Many members are physiologically active. By ^{14}C tracer studies it has been found that flavonoids are formed from a common C_{15} precursor, a chalcone[31] (**43**), which is derived from the enzyme mediated condensation of an activated cinnamic acid (**40**) and three molecules of malonyl CoA (**39**). Malonyl CoA (**39**) is derived from acetate pathway and activated cinnamic acid (**40**) from the shikimic acid pathway[32]. Hahlbrock *et al*[33] have also proposed similar route for flavonoid biosynthesis. Subsequently the immediate product of the condensation reaction (**41**), and the enzyme chalcone synthase, responsible for this condensation, were identified[34]. Hahlbrock[35] has pointed out that a central intermediate in the formation of all flavonoids is the chalcone (**43**) or the isomeric flavanone (**42**). The chalcone intermediate is then modified in a number of different ways by ring closure, oxidation or rearrangement to yield a range of flavonoid compounds eg (**44**), (**45**) and (**46**) (Scheme 8).

The nature of the biosynthetic precursors of the flavonoids may be reflected in the patterns of oxygenation in rings A and B. The meta-arrangements of the substituents arise from the malonyl origin of ring A, whereas ring B is derived from cinnamate.

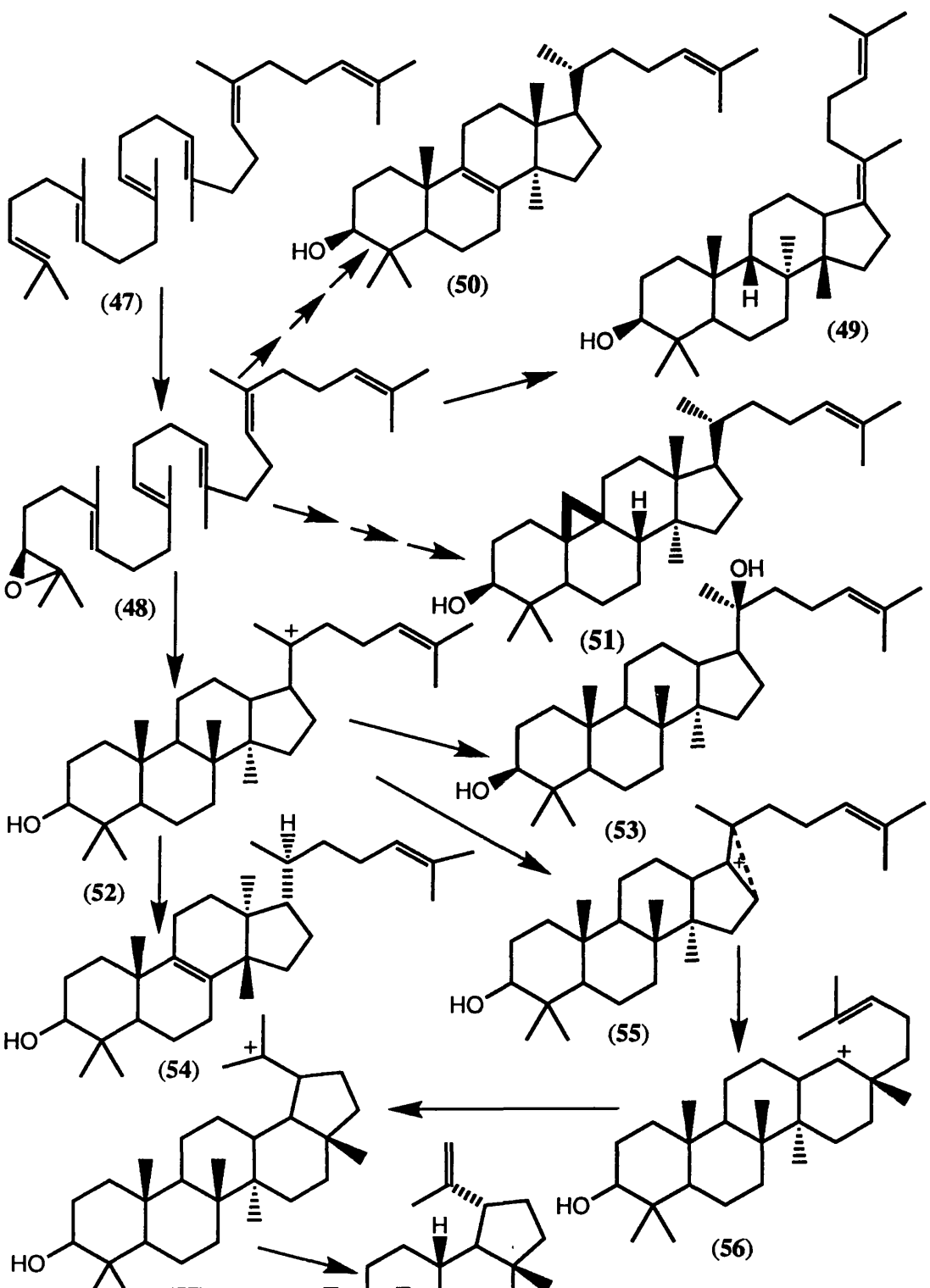


Scheme 8 : Biosynthesis of flavonoids.

BIOSYNTHESIS OF TRITERPENOIDS

The terpenoids represent a large diverse class of secondary metabolites. They are constructed from isoprene (2-methyl butadiene) units. Triterpenoids form a large group of secondary metabolites derived from squalene (**47**). There are more than 4000 triterpenoids isolated so far and more than 40 skeletal types have been identified[36]. Biosynthetically squalene (**47**), or the 3*S* isomer of 2,3-epoxy-2,3-dihydrosqualene (**48**), is the immediate precursor of all triterpenoids[37]. Triterpenoids are formed by the cyclisation of these two precursors followed by rearrangement (Scheme 9). 3(*S*)-2,3-epoxy-2,3-dihydrosqualene (squalene-2,3-epoxide) undergoes cyclisation to give 3β-hydroxytriterpenoids which by oxidation and reduction can be transformed into 3α-hydroxytriterpenoids. In the bacterial cell free systems 3α-hydroxytriterpenoids are formed by the cyclisation of the 3*R* isomer of squalene-2,3-epoxide[38,39,40].

Cyclisation of squalene-2,3-epoxide in a *chair-boat-chair-boat* conformation leads to protosterol (**49**) or, by a subsequent sequence of rearrangements leads to lanosterol (**50**), cycloartenol (**51**) and cucurbitacin I[41]. Cyclisation of squalene-2,3-epoxide in the *chair-chair-chair-boat* conformation leads to the dammarane ring system (**53**). This cyclisation goes through a series of carbonium ion intermediates to a cation (**52**) from which dammaranes, euphanes and tirucallanes are thought to be derived. According to the scheme suggested by Eschenmoser *et al*[42], the transformation of the carbonium ion intermediates into euphol or tirucallol (**54**) occurs either by a concerted process or *via* the appropriate ethylenic intermediates. Triterpenoids containing a fifth ring can be formed by the reaction of the hypothetical cation with the terminal double bond of the side chain[22]. Then a number of hydrogen and methyl migrations occur. Triterpenoids of the lupane series (**58**) are most probably derived through the sequence of cations (**55**), (**56**) and (**57**).



Scheme 9 : Biosynthetic pathways of triterpenoids

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CHAPTER 3

CONSTITUENTS OF THE STEM BARK OF *DESMOS LONGIFLORUS* (ROXB) SAFFORD (ANNONACEAE).

INTRODUCTION

The genus *Desmos* (Annonaceae) comprises 30 species of shrubs and trees, distributed in tropical and subtropical regions[1]. Alkaloids have been isolated from *Desmos tribaghienses*[2], *D. dasymachalus*[3] and *D. dumosus*[4]. Flavanoids have been obtained from *D. dumosus*[4], *D. lawii*[5,6], *D. cochinchinensis*[7] and *D. chinensis*[8,9], aromatic acids and sterols from *D. dasymachalus*[3] and cycloartanes from another *Desmos* species[10].

Desmos longiflorus (Roxb) Safford (Annonaceae) is a shrub which is widely distributed in the hilly areas of Bangladesh. Several isoquinoline alkaloids were isolated from the stem bark[11] and the antibacterial and antifungal activity of these alkaloids was studied[12]. Recently a lanostane derivative has been isolated from this plant[13].

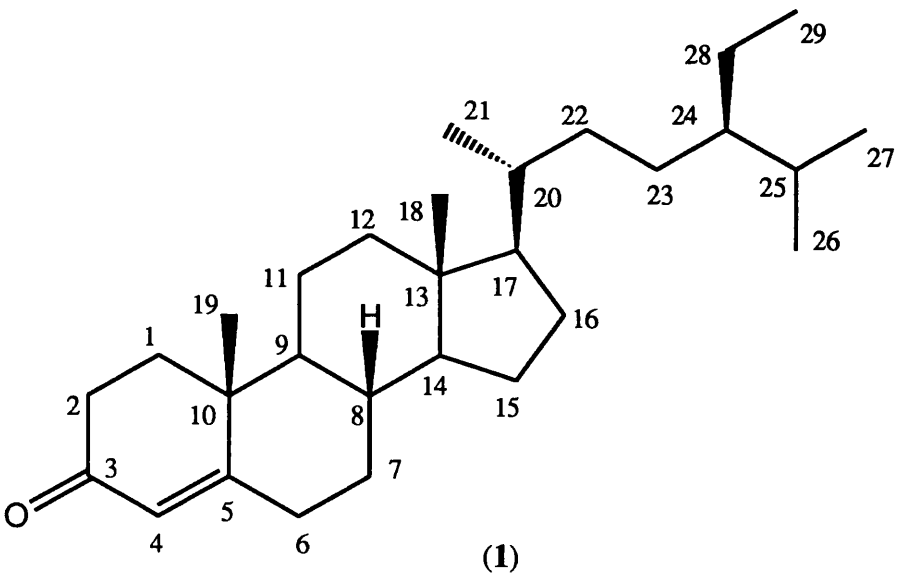
RESULTS AND DISCUSSION

Chromatography over silica gel of the ethyl acetate extract of the stem bark of *D. longiflorus* yielded five known compounds DL-1, DL-2, DL-3, DL-4, DL-5 which were subsequently identified as sitostenone (**1**), 4-stigmastene-3,6-dione (**2**), benzylbenzoate (**3**), crotepoxide (**4**) and N-benzoyl-O-(N'-benzoyl-L-phenylalanyl)-L-phenylalaninol (**5**) and five new compounds. The structures of four of them have been elucidated. These are DL-6, which is 15α-hydroxy-24-methylene-lanosta-7,9 (11)-dien-3-one (**6**), DL-7, DL-8, and DL-9 which are the unusual C-methylated flavanoids (**9**), (**10**) and (**11**). DL-10 has not yet been identified. β-Sitosterol was also isolated from this plant.

Chromatography over silica gel of the methanol extract of the stem bark afforded six isoquinoline alkaloids DL-11, DL-12, DL-13, DL-14, DL-15 and DL-16. Five of

them were identified as the known aporphine alkaloids oxocrebanine (**12**) (DL-11), buxifoline (**15**) (DL-12), oxobuxifoline (**16**) (DL-13), norstephalagine (**17**) (DL-14) and atherospermidine (**18**) (DL-15). The sixth alkaloid DL-16 is a new protoberberine alkaloid, 2-methoxy-3-hydroxy-9,10-methylenedioxytetrahydroprotoberberine (**19**).

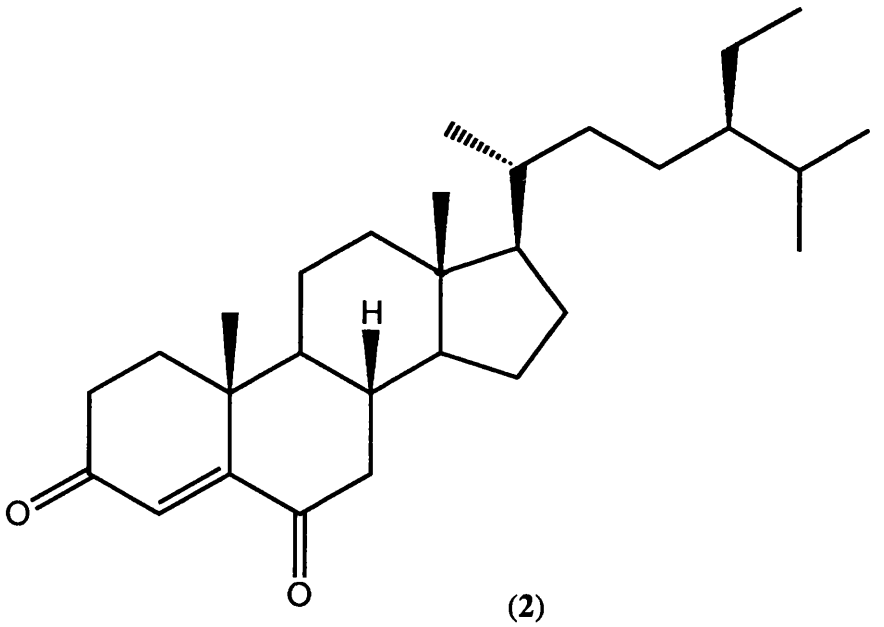
DL-1 (**1**), $C_{29}H_{48}O$, ν_{max} 1680 (ketone), 1620 cm^{-1}, was obtained as needles, mp 95°, $[\alpha]_D$ +81° (c, 1.00 in $CHCl_3$) [lit.[14] sitostenone, mp 95-96.5°. $[\alpha]_D$ +81.3° (c, 2.78 in $CHCl_3$)]. Its mass spectrum [m/z 412 (M^+), 397 (M^+-15), 370 ($C_{27}H_{46}^+$), 327 ($C_{27}H_{39}^+$), 289 ($C_{21}H_{37}^+$), 271 ($C_{19}H_{27}O^+$), 149 ($C_{11}H_{17}^+$), 124 ($C_8H_{12}O^+$, base peak)] indicated its steroidal nature. Its ^1H NMR spectrum shows two tertiary methyls [δ_H 0.67, 1.15], three secondary methyls [δ_H 0.78 (d, J 6.8 Hz), 0.88 (d, J 6.8 Hz) and 1.10 (d, J 6.4 Hz)], a primary methyl [δ_H 0.96, t, J 6.5 Hz)] and an olefinic proton [δ_H 5.67 (sharp s)]. The unresolved protons appear as complex multiplets between δ_H 1.28 and 2.60. These data suggested a steroidal enone with a sitosterol side chain. Comparison with published data[15] led to the identification of DL-1 as sitostenone (**1**).



(**1**)

The ^{13}C NMR spectrum of DL-1 readily supported structure (**1**). Thus it has six methyl groups [δ_C 11.8, 12.2, 17.2, 18.6, 18.9, 19.3] and an αβ-unsaturated ketone [δ_C 199.6] with a trisubstituted double bond [δ_C 123.6 (d), 171.7 (s)]. In addition there are eleven methylenes, seven methines and two quaternary carbons. The ^{13}C data for (**1**) so far have not been reported. The assignments (see Experimental) were made by comparison with 4-androsten-3-one[16] and 24-ethyl-cholest-4-ene-3,6-dione[17]. This is the first report of the isolation of sitostenone from an Annonaceous plant.

DL-2 (**2**), $C_{29}H_{46}O_2$, ν_{max} 1680 (ketone), 1617 cm^{-1}, was obtained as crystals, mp 170°, $[\alpha]_D$ -60.0° (c, 1.00 in $CHCl_3$) [lit.[18] 4-stigmastene-3-6-dione, mp 170-172°, $[\alpha]_D$ -60.5° (c, 0.99 in $CHCl_3$)] and has peaks in its mass spectrum at m/z 426 (M^+), 411 (M^+-15), 285 [M^+-$C_{10}H_{21}$(side chain)], 243 (M^+-$C_{10}H_{21}$-42), 189 ($C_{12}H_{13}O_2^+$), 137 ($C_8H_9O_2^+$) and a base peak at m/z 43 ($C_3H_7^+$). These indicated the steroidal nature of DL-2.

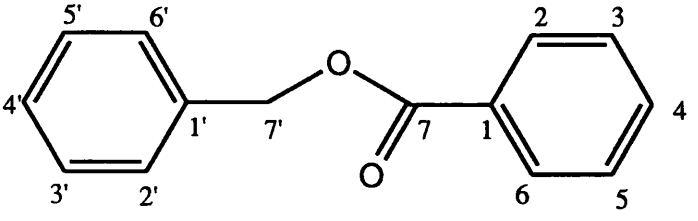


(**2**)

The ^1H NMR spectrum shows two tertiary methyls [δ_H 0.71, 1.15], three secondary methyls [δ_H 0.78 (d, J 6.5 Hz), 0.84 (d, J 6.5 Hz), 0.87 (d, J 6.4 Hz)], a primary methyl [δ_H 0.95, t, J 6.4 Hz] and an olefinic proton [δ_H 6.16 (s)]. These data indicate a steroidal structure and comparison with published data[18,19] readily led to the identification of DL-2 as 4-stigmastene-3,6-dione (**2**).

The ^{13}C NMR spectrum of DL-2 (**2**) is in accord with the proposed structure. Thus it shows six methyl groups [δ_C 11.9, 12.0, 17.5, 18.7, 19.0, 19.8], a trisubstituted double bond [δ_C 125.4 (d), 161.1(s)] and two conjugated ketonic carbonyl [δ_C 197.6, 200.4], in addition to ten methylenes, seven methines and two quaternary carbons. Comparison with published data[17] confirmed the identity of DL-2 as 4-stigmastene-3,6-dione (**2**).

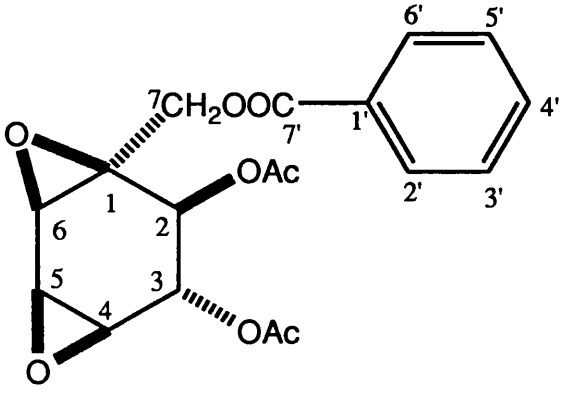
DL-3 (**3**), $C_{14}H_{12}O_2$ was obtained as colourless oil, λ_{max} 228, 235, 260, 273, 280, ν_{max} 3020, 1722, 1603, 1585, 1498 cm^{-1}. Its mass spectrum exhibits a molecular ion (M^+) at m/z 212 corresponding to $C_{14}H_{12}O_2$ and a base peak at m/z 105 ($PhCO^+$), together with fragments at m/z 107 ($C_7H_7^+$) and 77 ($C_6H_5^+$). The ^1H NMR spectrum shows signals for ten aromatic protons [δ_H 8.07 (1H, m), 8.11 (1H, m) and 7.35-7.60 (8H, m)] and a methylene singlet at δ_H 5.38 (Ph-$\underline{CH_2}$-CO). The above data suggest that DL-3 is benzyl benzoate (**3**) and comparison with published data[20] confirmed this..



(**3**)

The ^{13}C NMR spectrum of DL-3 shows the expected features. Thus it has an ester carbonyl [δ_C 166.4], ten aromatic doublets [δ_C 133.0, 129.7 (2), 129.2 (2), 128.6 (2), 128.3, 128.1 (2)], two aromatic singlets [δ_C 136.0, 130.1] and a methylene [δ_C 66.7]. The ^{13}C data for (**3**) so far have not previously been reported. The assignments are given in the Experimental. The shifts of the CH_2OOCPh moiety were assigned by comparison with zeylenol[21] and the shifts of the phenyl group by comparison with alkyl substituted benzenes[22].

DL-4 (**4**), $C_{18}H_{18}O_8$, λ_{max} 281, 273, 223 nm, was obtained as colourless needles, mp 152°, $[\alpha]_D$ +64.4° (c, 1.00 in $CHCl_3$) [lit.[23] crotepoxide, mp 152-153°, $[\alpha]_D$ +64.0° (c, 1.7 in $CHCl_3$)] . It has esters [ν_{max} 1756 and 1732 cm^{-1}], aromatic and C-O absorption in its IR spectrum (see Experimental). Its mass spectrum displays a molecular ion peak (M^+) at m/z 362, a peak at m/z 303 [M^+-CH_3COO], a base peak at m/z 105 ($C_6H_5CO^+$), a peak at m/z 77 ($C_6H_5^+$) and a peak at m/z 43 ($C_2H_3O^+$).



(**4**)

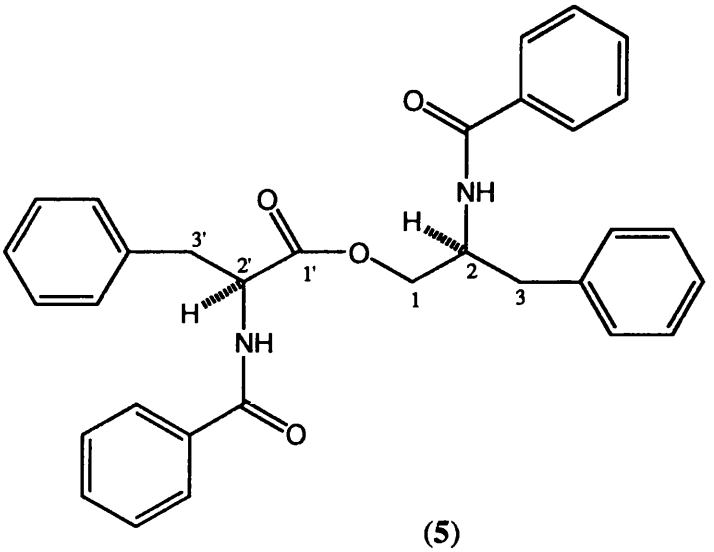
The ^1H NMR spectrum of DL-4 shows signals for five aromatic protons [δ_H 8.01 (2H, m), 7.57 (1H,m), 7.44 (2H, m)] suggesting the presence of a benzoyl group, an AB quartet [δ_H 4.21, 4.55 (J 12.0 Hz)] indicative of an oxygenated methylene group,

five oxygenated methines linked in a continuous chain [δ_H 3.05 (1H, dd, J 4.0, 1.5 Hz, H-4), 3.42 (1H, dd, J 4.0, 2.5 Hz, H-5), 3.65 (1H, d, J 2.5 Hz,H-6), 4.93 (1H, dd, J 10.0, 1.5 Hz, H-3), 5.68 (1H, d, J 10.0 Hz, H-2)] and two acetyl groups [δ_H 2.00 (3H, s), 2.07 (3H, s)]. The chemical shifts of H-2 and H-3 indicate that they are attached to carbons bearing ester groups. The absence of hydroxyl absorption bands in the IR spectrum of DL-4 together with the ^{13}C shifts of the oxygenated carbons (see below) indicated that the compound has two epoxide groups in addition to the three ester groups. Thus the three remaining protons at δ_H 3.05 (H-4), 3.42 (H-5) and 3.65 (H-6) must be associated with the epoxides. In summary DL-4 is a hydroxymethyl cyclohexane derivative bearing two epoxides, two acetates and a benzoate. These features are reminiscent of crotepoxide (**4**) and comparison with published data[23,24,25] confirmed the identification.

The ^{13}C NMR spectrum of DL-4 shows the expected features for DL-4 (**4**). Thus it has an ester carbonyl [δ_C 165.5] and a phenyl ring [δ_C 133.4 (d), 129.7 (2xd), 129.0 (s), 128.5 (2xd)] associated with the benzoate, seven oxygenated carbons [δ_C 70.3 (C-3), 69.4 (C-2), 62.4 (C-7), 59.4 (C-1), 53.7 (C-6), 52.5 (C-4), 48.0 (C-5)], two acetate methyl groups [δ_C 20.6 (2xq)] and two acetate carbonyls [δ_C 170.0, 169.5] associated with two acetyl groups. These data are consistent with the structure of crotepoxide (**4**). Comparison with published data[26] again confirmed the identification of DL-4 as crotepoxide (**4**).

DL-5 (**5**), $C_{32}H_{30}N_2O_4$, was obtained as white crystals, mp 208-210°, $[\alpha]_D$ -75° (c, 0.103 in EtOH) [lit.[27] mp 210°, $[\alpha]_D$ -78.7 (c, 0.14 in EtOH)]. It is UV active and has absorption bands at 215 and 230 nm. Its IR spectrum exhibits strong bands for NH (3340 cm^{-1}), ester carbonyl (1750 cm^{-1}), amide carbonyl (1640 cm^{-1}) and aromatic ring (1600, 1580, 745, 694 cm^{-1}) absorption. Its mass spectrum displays a molecular ion peak (M^+) at m/z 506, corresponding to $C_{32}H_{30}N_2O_4$, and other peaks at m/z 415 (M^+-$PhCH_2$), 385 (M^+-$PhCONH_2$), 294 (M^+-$PhCONH_2$-$PhCH_2$), 252

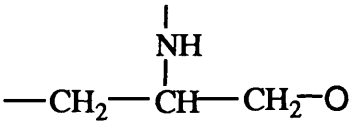
$[PhCH_2CH(NHBz)=C=O]^+$, 224 $(PhCH_2CHNHBz)^+$, 105 $(PhCO^+)$, 91 $(PhCH_2^+)$ and 77 (Ph^+). These data are consistent with the presence of benzyl and benzamide functions. The peaks at m/z 252 and 224 are characteristic of an N-benzoylphenylalaninyl moiety.



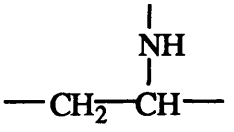
(5)

The ^1H NMR spectrum of DL-5 shows the presence of a total of thirty protons. It reveals the presence of two NH groups as doublets at δ_H 6.62 (J 6.6 Hz) and 6.72 (J 8.2 Hz), twenty aromatic protons, incorporating two benzoyl and two benzyl groups, as multiplets at δ_H 7.71 (4H) and 7.15-7.50 (16H), two benzyl methylenes at δ_H 2.90 (1H, dd, J 13.6, 8.1 Hz) and 3.02 (1H, dd, J 13.6, 6.4 Hz) (2H-3) and at δ_H 3.20 (1H, dd, J 13.8, 7.0 Hz) and 3.32 (1H, dd, J 13.8, 6.6 Hz) (2H-3'), an esterified hydroxymethylene at δ_H 4.05 (1H, dd, J 11.3, 4.2 Hz) and 4.57 (1H, dd, J 11.3, 3.3 Hz) (2H-1) and two methines attached to NH at δ_H 4.62 (1H, m, H-2) and 4.96 (1H, q, J 6.6 Hz, H-2'). These data indicate the presence of an ester grouping linking N-benzoylphenylalanyl and N-benzoylphenylalaninol residues. Comparison with published data[27] confirmed the identity of DL-5 as N-benzoyl-O-(N'-benzoyl-L-phenylalanyl)-L-phenylalaninol (**5**).

The structure was deduced in the following way. The COSY spectrum (Fig. 1) shows two coupled spin systems (a) and (b). System (a) consists of the methylene group at δ_H 2.90 and 3.02 , the methine group at δ_H 4.62, the esterified hydroxymethylene group at δ_H 4.05 and 4.57 and the NH group at δ_H 6.72 . System (b) consists of the methylene group at δ_H 3.20 and 3.32 , the methine group at δ_H 4.96 and the NH group at δ_H 6.62 .



(a)



(b)

The ^{13}C NMR spectrum shows the expected features for DL-5 (**5**). Thus it has two benzyl methylene carbons [δ_C 37.2 and 37.4], two deshielded methines [δ_C 54.5 and 50.2], an ester methylene [δ_C 65.4], twenty aromatic methine carbons [δ_C 126.8 (1C), 127.0 (2C), 127.1 (2C), 127.3 (1C) 128.4 (2C), 128.6 (4C), 128.8 (2C), 129.1 (2C), 129.3 (2C), 131.4 (1C), 132.0 (1C)], four aromatic quaternary carbons [δ_C 133.2, 134.1, 135.7, 137.1], two amide carbonyl carbons [δ_C 167.2, 167.4] and an ester carbonyl carbon [δ_C 171.9].

From the HMQC it was possible to identify all the aliphatic protonated carbons. The carbon at δ_C 37.2 (t) has direct correlations with the methylene protons of 2H-3' [δ_H 2.90 (dd) and 3.02 (dd)] and the carbon at δ_C 37.4 (t) has direct correlations with the methylene protons of 2H-3 [δ_H 2.20 (dd) and 3.32 (dd)]. The ester methylene protons of 2H-1 [δ_H 4.05 (dd) and 4.57 (dd)] correlate with the carbon at δ_C 65.4 (t) while the methine protons H-2 [δ_H 4.62 (m)] and H-2' [δ_H 4.96 (q)] correlate with the carbons at δ_C 50.2 (d) and 54.5 (d) respectively. These correlations assigned the carbons as

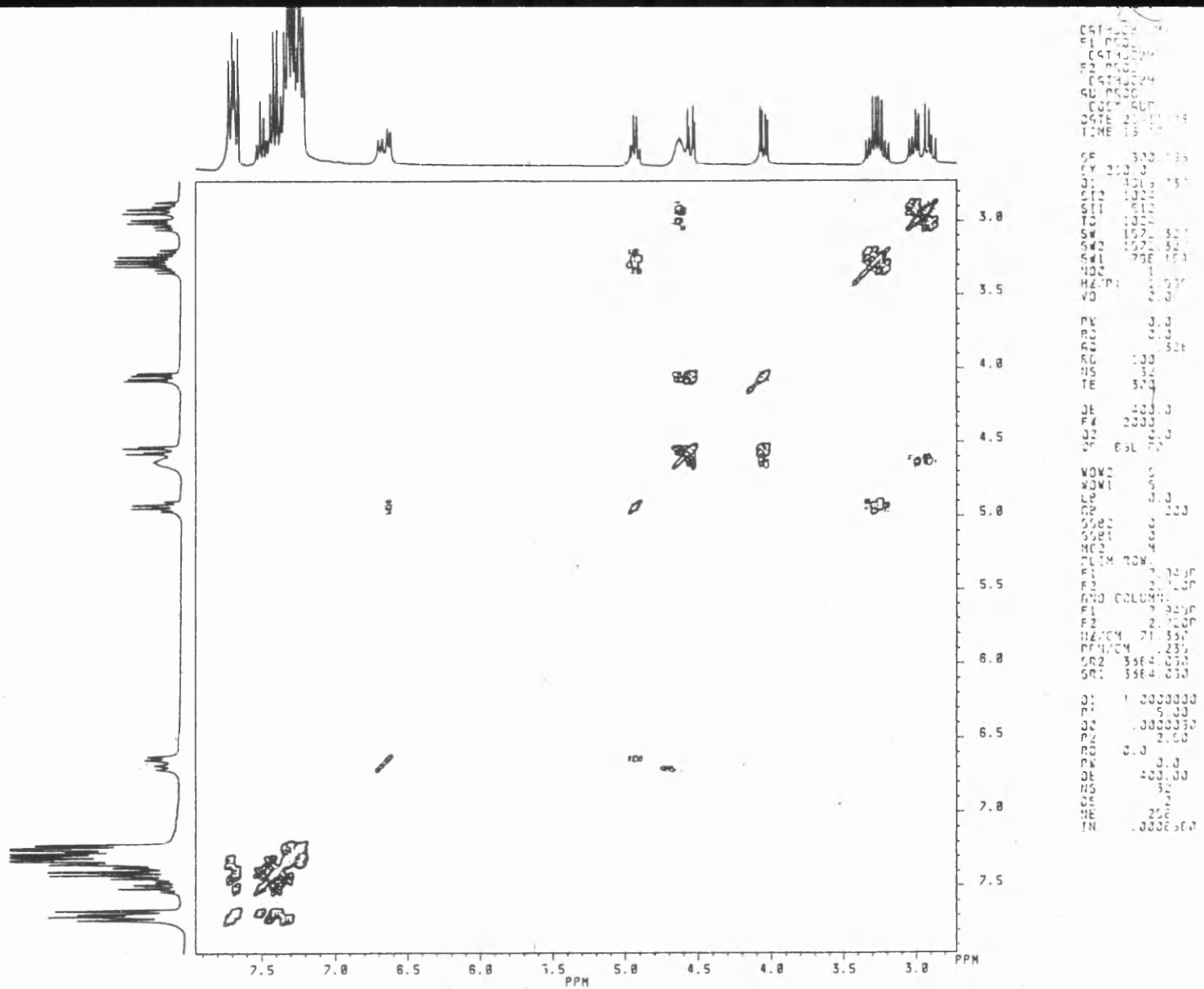
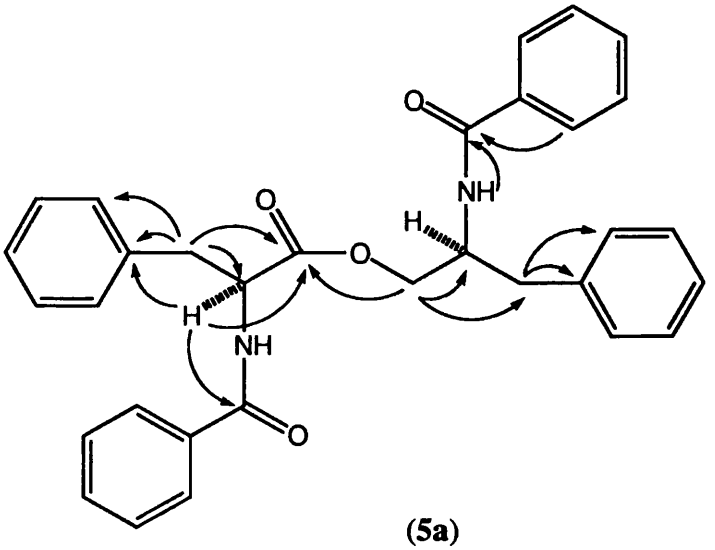


Figure 1. ^{1}H-^{1}H COSY spectrum of DL-5 (**5**).

follows : δ_C 37.2 (C-3'), 37.4 (C-3), 50.2 (C-2'), 54.5 (C-2) and 65.4 (C-1) respectively.



(5a)

From the HMBC spectrum (Fig. 2) it was possible to obtain sufficient connectivity to elucidate the structure. The ester carbonyl carbon [δ_C 171.9 (s)] has correlations with the methylene protons 2H-1 [δ_H 4.05 (dd) and 4.57 (dd)], 2H-3' [δ_H 2.90 (dd) and 3.02 (dd)] and with the methine proton H-2' [δ_H 4.62 (m)], and is therefore C-1'. The amide carbonyl carbon at δ_C 167.4 correlates with the methine proton H-2' [δ_H 4.62 (m)] and the another amide carbonyl carbon δ_C 167.2 correlates with the NH [δ_H 6.62 (d)] and the aromatic protons [δ_H 7.71]. These and the other correlations shown in structure (**5a**) led to structure (**5**) for DL-5. This is the first report of its isolation from an Annonaceous plant.

DL-6 was obtained as colourless needles, mp 156-158°, $[\alpha]_D$ +42.6° (c, 2.00 in $CHCl_3$). The UV spectrum of DL-6 shows characteristic absorptions at λ_{max} 252, 243 and 236 nm, indicating the presence of a conjugated heteroannular diene system. The IR

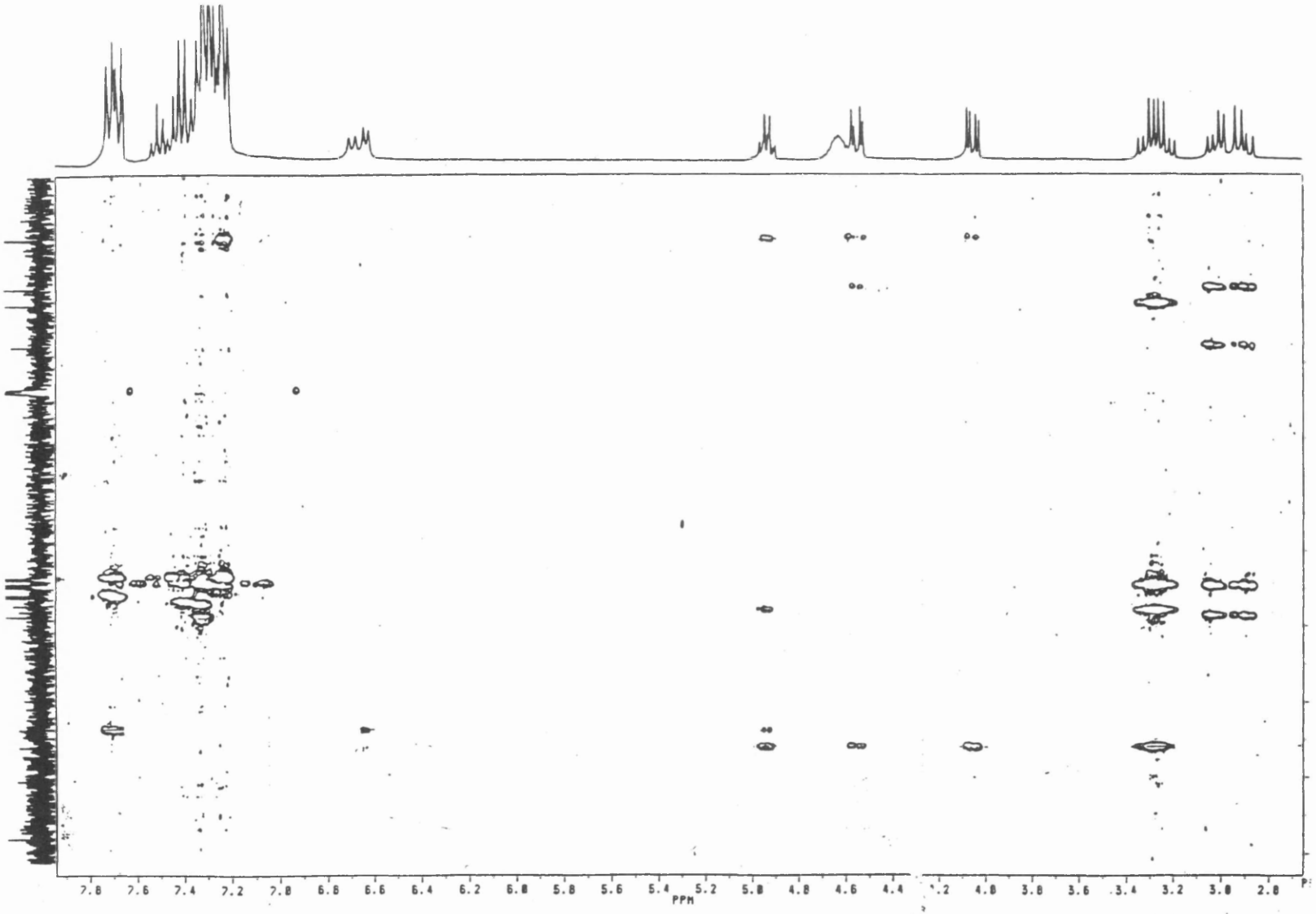
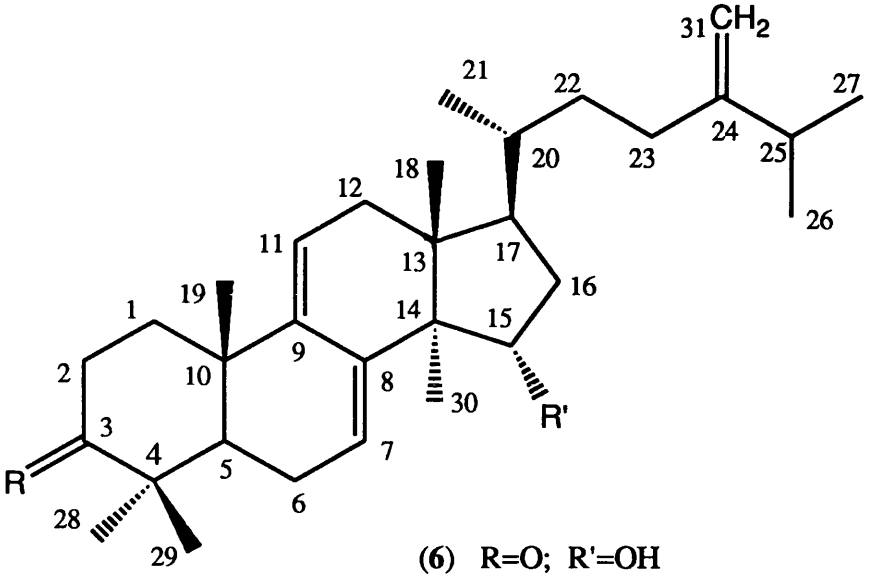


Figure 2. The HMBC spectrum of DL-5 (**5**).

spectrum of DL-6 exhibits absorption bands at 3400 (hydroxyl), 1715 (ketonic C=O) and 1640 (double bond) cm-^{1}. The EIMS shows a molecular ion peak (M^+) at m/z 452 and HREIMS confirmed the molecular formula as $C_{31}H_{48}O_2$. Other mass peaks include m/z 437 (M^+-15), 419 (M^+-15-18), 337 [M^+-C_9H_{17}(side chain)] and 125 ($C_9H_{17}^+$, side chain). These data clearly indicate a triterpenoid with a conjugated diene system and an extra carbon in the side chain.



(**6**) R=O; R'=OH

(**7**) R=H,βOH; R'=H

(**8**) R=H,βOH; R'=OH

The ^{1}H NMR spectrum of DL-6 reveals the presence of three secondary methyl groups [δ_H 0.88 (d, J 6.4 Hz, Me- 21), 1.00 and 1.03 (each d, J 6.8 Hz, Me-26,-27)], five tertiary methyl groups [δ_H 0.62, 0.91, 1.08, 1.11 and 1.18 (Me-18, -30, -29, -28, -19)], two olefinic protons of the conjugated diene system [δ_H 5.37 (d, J 6.0 Hz, H-11) and 5.88(d, J 5.0 Hz, H-7)], an exomethylene group [δ_H 4.64 and 4.71 (each br s, H_Z-31, H_E-31)], an oxygen-bearing methine [δ_H 4.27 (1H, dd, J 9.8, 5.6 Hz, H-15β)] and a deshielded proton [δ_H 2.75 (ddd, J 14.8, 14.5, 6.0 Hz)] suggestive of H-2β in a 3-oxo triterpenoid[28]. These data suggested a tetracyclic triterpenoid of the 24-methylene-

7,9(11)-lanostadien-3-one type with additional secondary hydroxyl group. The attachment of the hydroxyl to C-15 was deduced by comparison of the ^{1}H and ^{13}C (*vide infra*) chemical shift data with those of ganoderiol B[15α,26,27-trihydroxy-7,9(11),24-lanostatrien-3-one][28] and 24-methylene-7,9(11)-lanostadien-3β-ol (**7**)[29].

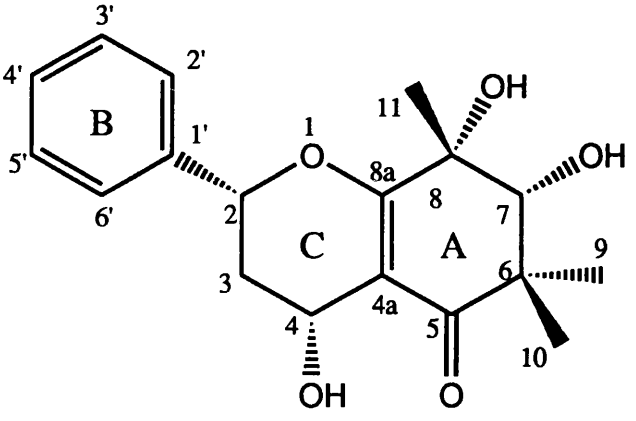
The ^{13}C NMR spectrum contains signals for thirty one carbons including a keto group [δ_C 216.7, (C-3)], two trisubstituted double bonds [δ_C 121.6 (d) (C-7), 141.0 (s) (C-8), 144.7 (s) (C-9), 117.0 (d) (C-11)], an exomethylene [δ_C 156.5 (s) (C-24), 106.1 (t) (C-31)], a secondary alcohol [δ_C 74.6 (d), C-15] and eight methyl groups [δ_C 15.1 (C-18), 22.1 (C-19), 18.4 (C-21), 21.8 (C-26), 22.0 (C-27), 25.4 (C-28), 22.4 (C-29), 17.0 (C-30)], in addition to seven methylene, four methine and four quaternary carbons. The ^{13}C resonances were readily assigned by comparison with those of ganoderiol B and 24-methylene-7,9(11)-lanostadien-3β-ol (**7**) [see **Table 1**] and confirmed the structure of DL-6 as 15α-hydroxy-24-methylene-7,9(11)-lanostadien-3-one (**6**). The coupling constants of H-15 (J 9.8, 5.6 Hz) indicate its configuration as β. This was further confirmed by NOE difference experiments. Irradiation of H-15 (δ_H 4.27) gave a NOE (10%) at Me-18 (δ_H 0.62) while irradiation of Me-18 (δ_H 0.62) gave a NOE (7%) at δ_H 4.27 (H-15).

This is the first report of the isolation of triterpenoid (**6**). The corresponding 3β-hydroxy derivative suberosol (**8**)[30] has recently been isolated from *Polyalthia suberosa* (Annonaceae) and is of particular interest in view of its reported anti-HIV activity. The 15-deoxy analogue (**7**) of suberosol (**8**) has been isolated from *Artabotrys odoratissimus*[29], another member of the Annonaceae. Polycarpol (3β,15α-dihydroxy-7,9(11),24-lanostatriene) has been found in several Annonaceae and is regarded as a useful chemotaxonomic marker[29].

TABLE 1: ^{13}C Chemical Shifts Data of (6), Ganoderiol B and (7).

Carbons	Compounds		
	(6)	Ganoderiol B	(7)
1	36.6	36.9	
2	34.8	35.0	
3	216.7	215.2	
4	47.4	47.5	
5	50.5	51.0	
6	23.6	23.9	
7	121.6	121.6	
8	141.0	142.1	
9	144.7	145.4	
10	37.2	37.6	
11	117.0	117.3	
12	38.4	38.9	
13	44.3	44.6	
14	51.9	52.6	
15	74.6	73.7	
16	40.1	40.5	
17	48.8	49.4	
18	15.1	16.5	
19	22.1	22.2	
20	35.8		36.3
21	18.4		18.5
22	34.8		34.9
23	31.2		31.3
24	156.5		156.8
25	33.8		33.8
26	21.8		21.8
27	22.0		21.9
28	25.4	25.7	
29	22.4	22.9	
30	17.0	18.0	
31	106.1		106.2

DL-7 (**9**) was obtained as gum, $[\alpha]_D$ + 10.7 (c, 0.29 in $CHCl_3$). It is UV active and has an absorption maximum at 250 nm [log ε 3.45] suggesting a conjugated enone system. The IR spectrum exhibits hydroxyl (ν_{max} 3620, 3450 cm^{-1}) and unsaturated carbonyl (ν_{max} 1649 cm^{-1}) absorption. Additional bands at ν_{max} 3020, 1620, 1570, 1522, 1420 cm^{-1}, indicate the presence of an aromatic ring. The EIMS shows a molecular ion peak (M^+) at m/z 318 and HREIMS confirmed the molecular formula as $C_{18}H_{22}O_5$. Other fragments in the mass spectrum include m/z 300 (M^+-H_2O), 282 (M^+-$2H_2O$), 267 (M^+-$2H_2O$-CH_3), 264 (M^+-$3H_2O$), 105 ($C_6H_5CO^+$), 104 ($C_6H_4CO^+$) and 77 ($C_6H_5^+$).

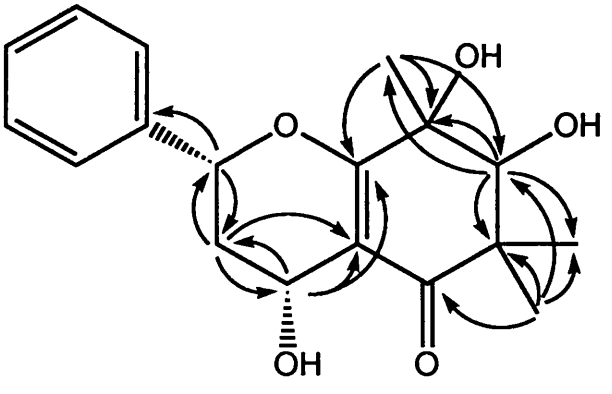


(**9**)

The ^1H NMR spectrum of DL-7 (**9**) shows the five aromatic protons [δ_H 7.30 (m)] of a phenyl substituent, three tertiary methyl groups [δ_H 1.17 (s), 1.33 (s), 1.53 (s)], three oxygenated methines [δ_H 5.10 (1H, dd, J 12.3, 2.2 Hz, H-2), 4.92 (1H, dd, J 10.1, 6.5 Hz, H-4), 3.92 (1H, s, H-7)], a methylene group [δ_H 2.45 (1H, ddd, J 13.7, 6.5, 2.2 Hz, 3-H_a), 2.15 (1H, ddd, J 13.7, 12.3, 10.1 Hz, 3-H_b)] and three hydroxyl groups [δ_H 4.65 (1H, s), 5.70 (1H, s), 2.75 (1H, s)]. Decoupling

experiments readily established the coupled system -CH(O-)-CH_2-CH(O-)- involving the protons at δ_H 5.10 and 4.92 and the methylene group.

The ^{13}C NMR spectrum of DL-7 (**9**) shows eighteen carbons including six aromatic carbons [δ_C 129.0 (d), 128.9 (2xd), 126.4 (2xd) 138.4 (s)], a ketonic carbonyl [δ_C 203.4], a tetrasubstituted enolic double bond [δ_C 111.0 (s), 170.7 (s)], four oxygenated carbons [δ_C 79.5 (d), 77.6 (d), 73.0 (s), 62.6 (d)], three methyl groups [δ_C 24.5, 22.0, 19.6], a methylene [δ_C 36.8] and a quaternary carbon [δ_C 46.2]. The phenyl group, ketonic carbonyl and double bond account for six of the eight double bond equivalents of the molecule which must, therefore, have two additional rings. The lack of connectivity in the ^1H NMR spectrum necessitated recourse to a long range δ_C/δ_H correlation experiment in order to find connectivity. In view of the small amount of material inverse detection was used, ie the HMBC experiment.



(9a)

In the HMBC spectrum (Fig. 3) many correlations were observed and these are summerised in (**9a**) and in **Table 5**. The correlation of the tertiary methyl group protons were particularly helpful. The mutual correlations of the methyls at δ_H 1.17 and 1.33

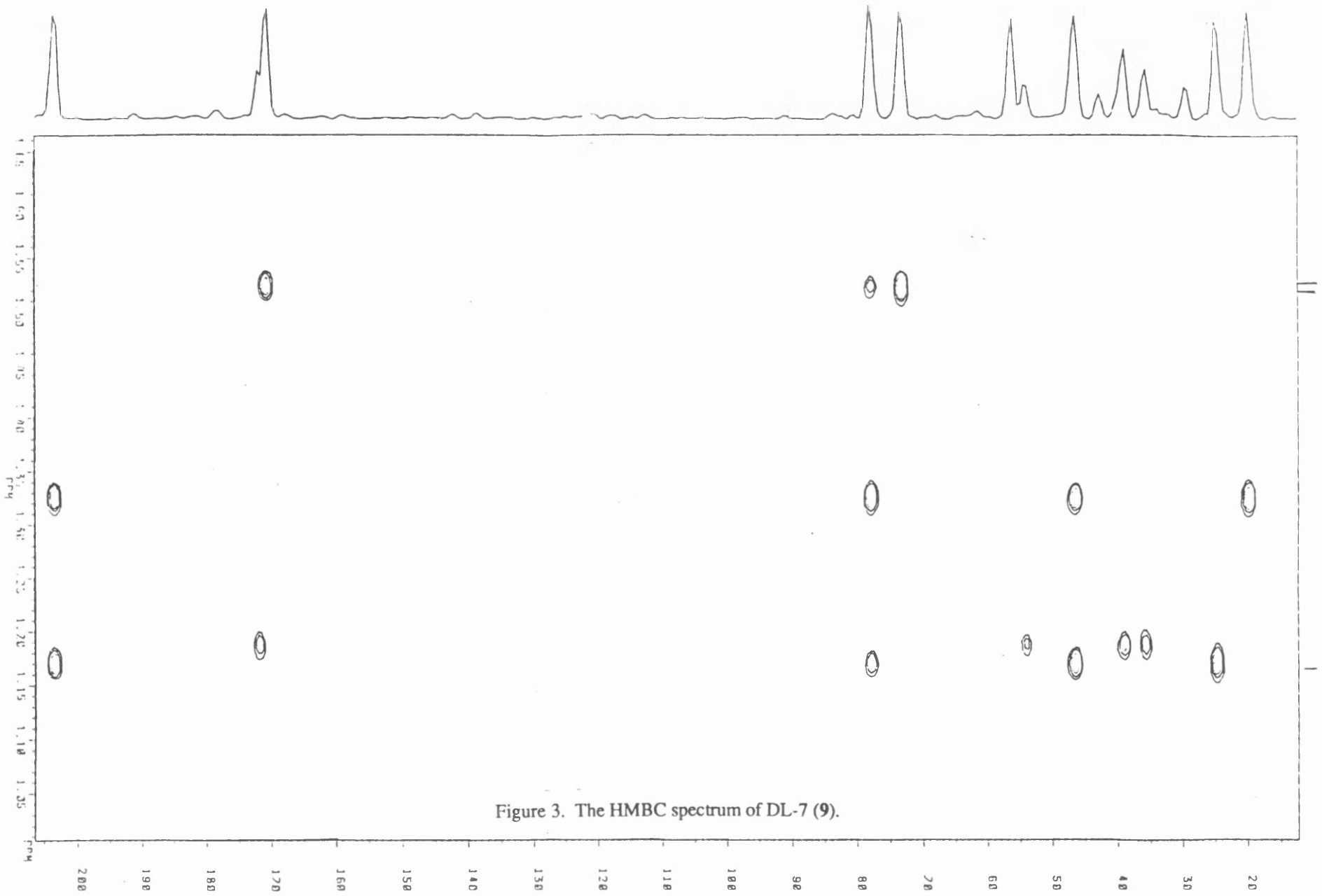
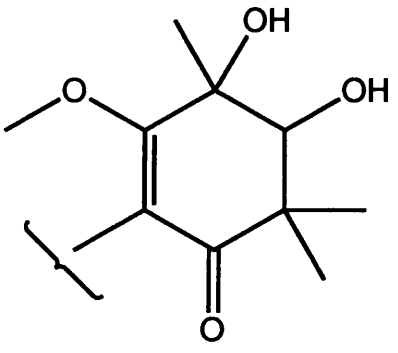


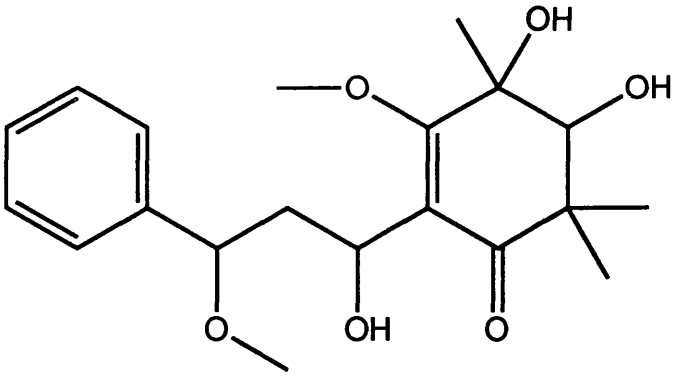
Figure 3. The HMBC spectrum of DL-7 (**9**).

revealed them as a geminal pair attached to the quaternary carbon at δ_C 46.2. In addition both methyls showed $^3J_{CH}$ correlations to the unsaturated ketonic carbonyl group at δ_C 203.4 and to the secondary oxygenated carbon at δ_C 79.5 [associated with the singlet at δ_H 3.92]. The remaining methyl at δ_H 1.53 is attached to a hydroxyl bearing carbon at δ_C 73.0 since it shows correlations to this carbon, in addition to $^3J_{CH}$ correlations to the secondary oxygenated carbon at δ_C 79.5 and to the strongly deshielded (enolic) β carbon of the unsaturated ketone system. These data lead unambiguously to part structure (**9b**) which is also supported by the correlations of the carbinol proton (δ_H 3.92) to all three methyl carbons and the two quaternary carbons at δ_C 46.2 and 73.0.



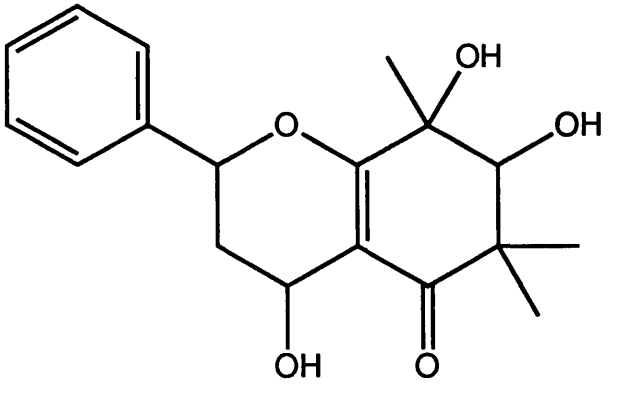
(9b)

Part structure (**9b**) can be extended in the following way. The allylic carbinol proton at δ_H 4.92 correlates to both carbons of the conjugated double bond [δ_C 111.4 and 170.7]. This carbinol proton, which forms part of the four-spin system discussed above, also shows a correlation to its neighbouring methylene group carbon. The lowfield methylene proton [δ_H 2.45] has strong correlation to the vinyl carbon at δ_C 111.4 and to the remaining oxygenated methine at δ_C 62.6. The attachment of the phenyl group to this oxygenated methine is revealed by correlation of its attached proton [δ_H 5.06] to the aromatic carbons at δ_C 126.4 (2d) and 138.4 (s). Thus the part structure can be extended to (**9c**).



(9c)

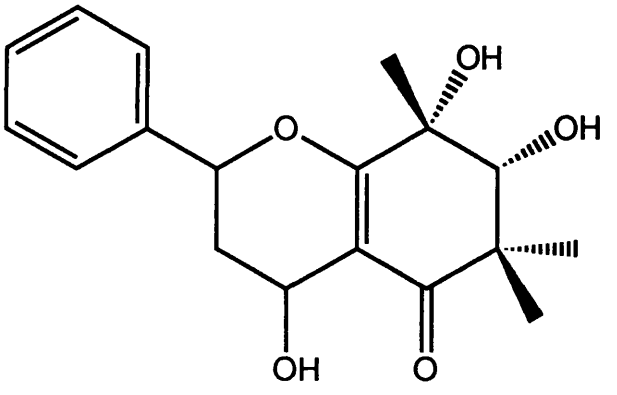
Formation of a pyran ring completes the structure as in (**9d**). The correlation across the oxygen of the pyran from the methine to the lowfield vinyl carbon, absent in this case, is observed in a closely related compound (*vide infra*). Structure (**9d**) is novel and appears to have arisen from a flavonoid precursor by methylation of ring A.



(9d)

The relative stereochemistry of (**9d**) was investigated by NOE difference experiments. The results are given in **Table 4**. The methyl signals at δ_H 1.17 and 1.53

have a 1,3-diaxial relationship since each gives a strong NOE on irradiation of the other. The equatorial methyl at δ_H 1.33 gives a reasonable NOE (4%) to the carbinol proton at δ_H 3.92. In related compounds (*vide infra*) the other two methyls also give NOEs to this carbinol proton which should therefore be equatorial. These results are accommodated by the stereochemistry shown in (**9e**).



(**9e**)

The relative stereochemistry of the pyran ring may be assigned on the basis of the coupling constants of the four spin system. The methine protons [δ_H 5.10 (dd, J 12.3, 2.2 Hz, H-2); 4.92 (dd, J 10.1, 6.5 Hz, H-4)] each have a large coupling and are clearly axial in nature in a flavonoid half-chair conformation of the pyran ring. Irradiation of H-4 affords a NOE at H-3eq [δ_H 2.45 (ddd, J 13.7, 12.2, 6.5 Hz)]. Its geminal neighbour H-3ax [δ_H 2.15 (ddd, J 13.7, 12.3, 10.1 Hz)] is unaffected. The observation of a small NOE from the methyl group at δ_H 1.53 to H-2 reveals the relationship between the rings. The relative stereochemistry is therefore assigned as in (**9**).

TABLE 2. ^1H NMR of Compounds (9), (10) and (11).

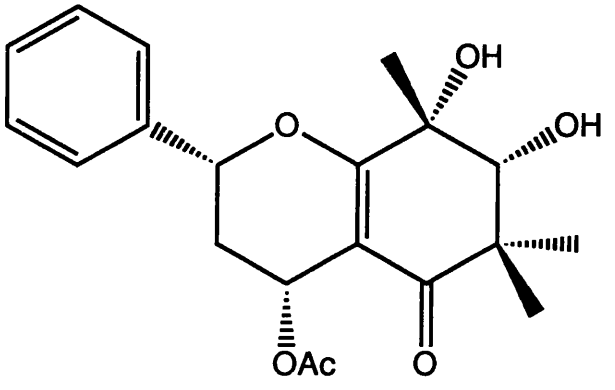
Protons	Compounds		
	(**9**) δ_H, m, J (Hz)	(**10**) δ_H, m, J (Hz)	(**11**) δ_H, m, J (Hz)
Ph	7.40, m	7.32, m	7.40, m
H-2	5.10, dd, 12.3, 2.2	5.40, t, 5.7	5.13, dd, 12.2, 2.5
H-3ax	2.15, ddd, 13.7, 12.3, 10.1	2.40, t, 5.7	1.93, ddd, 14.7, 12.2, 4.0
H-3eq	2.45, ddd, 13.7, 6.5, 2.2	2.40, t, 5.7	2.17, dt, 14.7, 2.3
H-4	4.92, dd, 10.1, 6.5	5.80, t, 5.7	4.65, dd, 4.0, 2.0
H-7	3.92, s	3.98, s	3.82, s
3H-9	1.33, s	1.29, s	1.31, s
3H-10	1.17, s	1.12, s	1.15, s
3H-11	1.53, s	1.55, s	1.53, s
OH	5.70, s	2.70, s	2.45, s
OH	4.65, br s	2.85, s	2.56, s
OH	2.75, s	-	3.10, s
CH_3CO	-	1.61, s	-

TABLE 3. ^{13}C NMR of Compounds (9), (10) and (11).

Carbons	Compounds		
	(9)	**(10)**	**(11)**
C-2	79.5(d)	77.9(d)	76.5(d)
C-3	36.8(t)	33.4(t)	36.4(t)
C-4	62.6(d)	60.9(d)	58.6(d)
C-4a	111.4(s)	108.2(s)	111.3(s)
C-5	203.4(s)	198.6(s)	202.1(s)
C-6	46.2(s)	45.8(s)	45.9(s)
C-7	77.6(d)	77.2(d)	78.0(d)
C-8	73.0(s)	73.2(s)	73.2(s)
C-8a	170.7(s)	171.3(s)	170.3(s)
C-9	24.5(q)	25.1(q)	24.5(q)
C-10	19.6(q)	19.6(q)	19.5(q)
C-11	22.0(q)	20.6(q)	22.2(q)
C-1'	138.4(s)	138.9(s)	139.0(s)
C-2'	126.4(d)	125.2(d)	126.4(d))
C-3'	128.9(d)	128.6(d)	128.8(d
C-4'	129.0(d)	128.0(d)	128.7(d)
C-5'	128.9(d)	128.6(d)	128.8(d)
C-6'	126.4(d)	125.2(d)	126.4(d)
$\underline{C}H_3CO$	-	20.6(q)	-
$CH_3\underline{C}O$	-	170.7(s)	-

The next compound DL-8 was obtained as a gum, $[\alpha]_D$ +5.0° (c, 0.65 in $CHCl_3$), λ_{max} 253 nm (log ε 4.10). Its IR spectrum has bands at 3620, 3547, 1736, 1655 and 1603 cm^{-1}, indicating to presence of hydroxyl, ester and conjugated ketone moieties. The mass spectrum shows a peak at m/z 342, consistent with loss of water

from $C_{20}H_{24}O_6$. Other peaks include m/z 317 [M^+-43(CH_3CO)] , 300 [M^+-60(CH_3COOH)], 283 [M^+-C_6H_5], 282 [M^+-60-H_2O] and a base peak at 43 [CH_3CO] . The spectroscopic properties of DL-8 (see **Tables**) are very similar to those of DL-7 suggesting a closely related structure. The ^1H NMR spectrum shows resonances for a phenyl ring [δ_H 7.32 (5H, m)], three tertiary methyl groups [δ_H 1.12 (s), 1.29 (s) and 1.55 (s)], another methyl at δ_H 1.61 which turns out to be a shielded acetate (*vide infra*), two oxygen-bearing methines [δ_H 5.80 (t, J 5.7 Hz); 5.40 (t, J 5.7 Hz)] which both couple with a methylene group [δ_H 2.40 (2H, t, J 5.7 Hz)] whose protons are accidentally equivalent, a secondary carbinol [δ_H 3.98 (s)] and two hydroxyl protons [δ_H 2.70 and 2.85]. These features differ from those of DL-7 only in the presence of acetate and the accidental equivalence of the methylene protons.

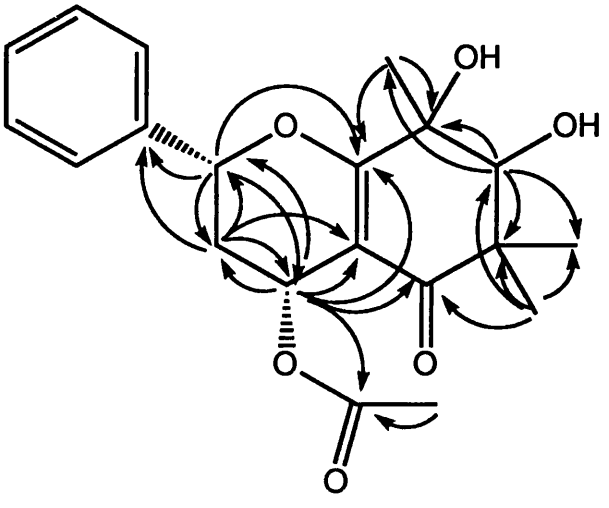


(10)

The ^{13}C NMR spectrum (**Table 3**) confirmed the presence of the above functionality and, in particular, shows the presence of an αβ-unsaturated ketone [δ_C 198.6 (s), 171.3 (s) and 108.2 (s)], a phenyl ring, three oxygenated methines [δ_C 60.9, 77.2, 77.9], an oxygenated quaternary carbon [δ_C 73.2 (s)], a quaternary carbon [δ_C 45.8 (s)], a methylene carbon [δ_C 33.4 (t)], an acetate [δ_C 20.6 (q), 170.7 (s)] and three

tertiary methyls [δ_C 19.6 (q), 20.6 (q), 25.1 (q)]. The protonated carbons were assigned by a 2D δ_C/δ_H direct correlation (HMQC) experiment (Fig. 4).

The structure of DL-8 as (**10**) was deduced largely on the basis of its HMBC spectrum (Fig. 5). The correlations are summarised in (**10a**) and in **Table 5**. The correlations of the methyl groups and the carbinol proton at δ_H 3.98 reveal the same structure of ring A as in DL-7. The benzylic methine H-2 correlates to the phenyl ring as expected but, in addition, shows a correlation across oxygen to the β-carbon of the enone, thus establishing the pyran ring system. The acetate is attached to C-4 of the pyran since H-4 correlates to the acetate carbonyl at δ_C 170.7.



(**10a**)

Two points about DL-8 (**10**) require comment. These concern the shielded nature of the acetate methyl and the accidental equivalence of the methylene protons. The phenyl ring will shield the acetate methyl if both are axial. However irradiation of H-2 affords a NOE at H-4 and *vice versa* suggesting that these protons are axial. Both of these situations can be accommodated if the pyran ring is undergoing rapid conformational

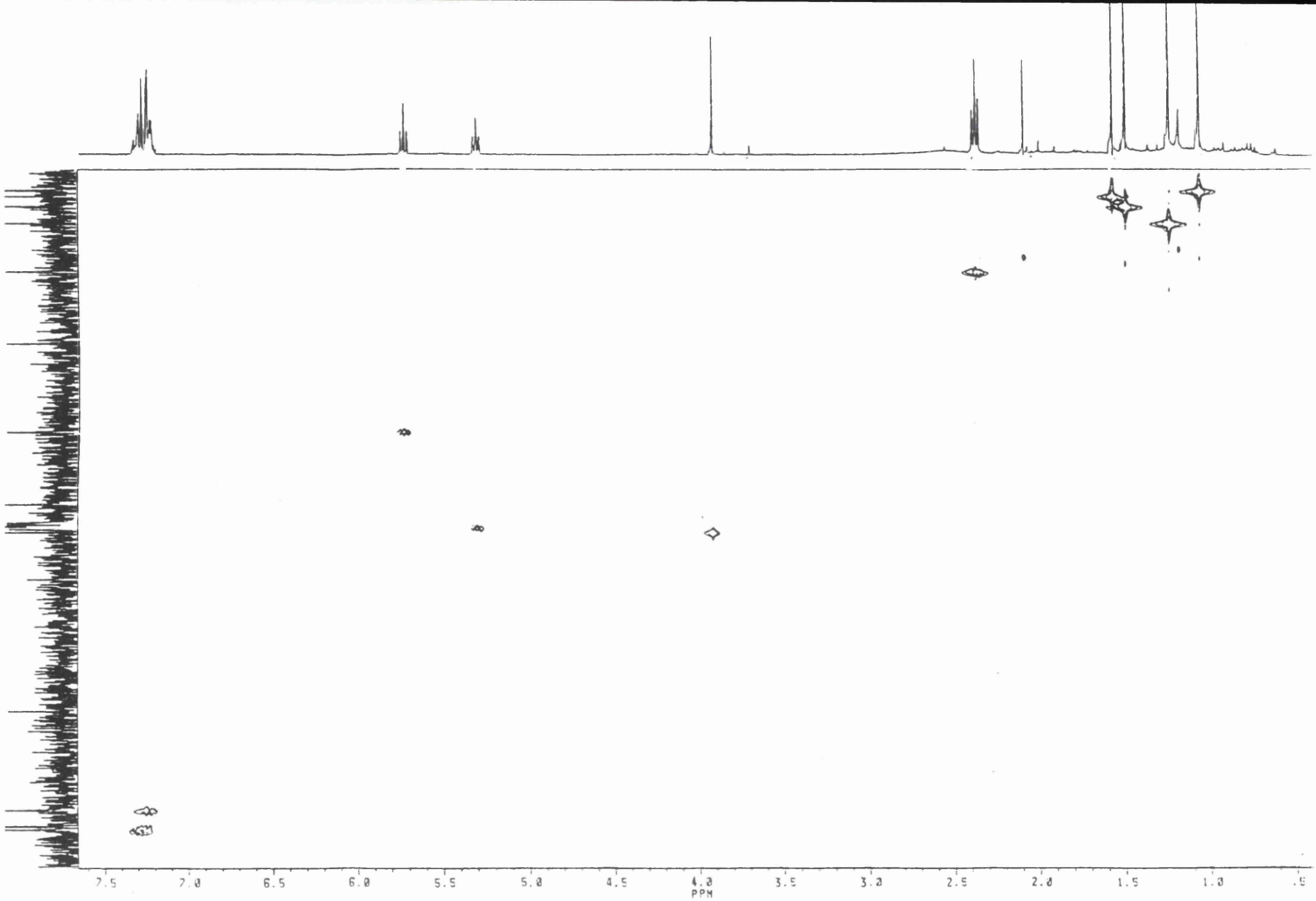


Figure 4. The HMQC spectrum of DL-8 (**10**).

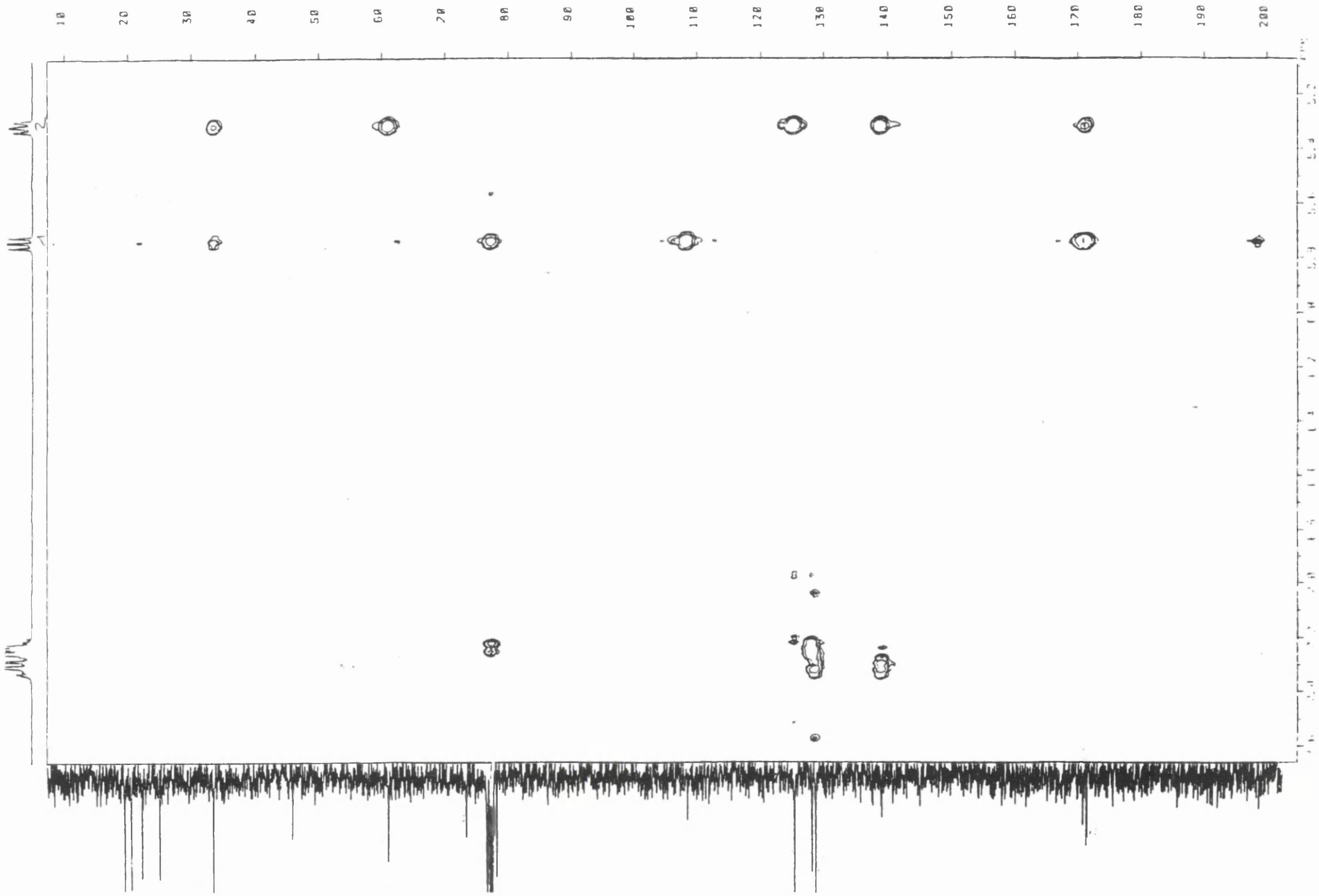


Figure 5. The HMBC spectrum of DL-8 (**10**).

inversion. The accidental equivalence of the diastereotopic methylene protons can also be explained in this way.

TABLE 4. NOEs of Compounds (9), (10) and (11).

Compounds					
(9)		**(10)**		**(11)**	
Irradiation	NOE (%)	Irradiation	NOE (%)	Irradiation	NOE (%)
1.17	1.53 (8)	1.12	1.55 (4.8)	1.15	1.53 (4.5)
1.33	3.92 (4)		1.29 (1.8)	1.31	3.82 (6)
1.53	1.17 (6)		3.98 (1.2)	1.53	1.15 (4.6)
	5.10 (1.4)	1.29	1.12 (1.4)		3.82 (1.5)
-	-		3.98 (6.1)		5.13 (1)
3.92	1.33 (2.5)	1.55	1.12 (4.6)	1.93	4.65 (1.5)
4.65 (OH)	5.10 (2.3)		3.98 (0.9)	2.17	4.65 (1.7)
4.92	2.45 (2)		5.80 (1)		5.13 (2)
5.10	7.40 (1.8)	1.61 (Ac)	7.32 (1.3)	3.82	1.31 (4.5)
7.40	5.10 (3)		3.98 (0.6)	4.65	1.93 (3)
		2.40	5.40 (6.2)		2.17 (1.3)
			5.80 (8.1)	5.13	2.17 (1.3)
			7.32 (4.1)		7.40 (2.7)
		3.98	1.29 (3.4)	7.40	5.13 (4.3)
		5.40	5.8o (1.5)		
			2.40 (3.3)		
			7.32 (4.0)		
		5.80	5.40 (1.3)		
			2.40 (3.5)		

TABLE 5. Long-range Correlations of Compounds (9), (10) and (11).

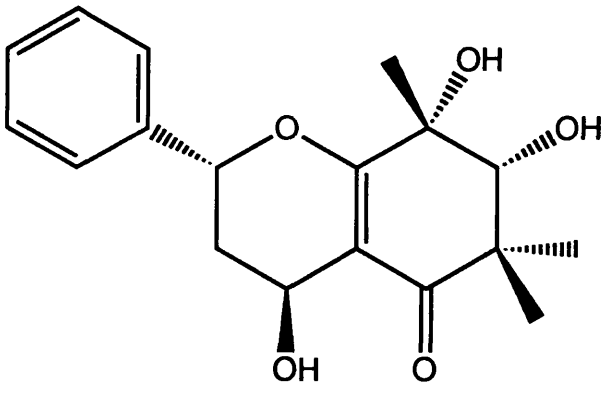
Carbon	Compounds		
	(9)	**(10)**	**(11)**
2	H-3ax, Ar-H	H-4, Ar-H	H-3ax, Ar-H
3	H-4	H-2, H-4	-
4	H-2, H-3ax, H-3eq	H-2, 2H-3	H-3eq
4a	H-3eq, H-4	H-4, 2H-3	H-3eq, H-4
5	Me-9, Me-10	H-4, Me-9, Me-10	H-4, Me-9, Me-10
6	H-7, Me-9, Me-10	H-7, Me-9, Me-10	H-7, Me-9, Me-10
7	Me-9, Me-10, Me-11	2H-3, H-4, Me-9, Me-10, Me-11	H-4, Me-9, Me-10, Me-11
8	H-7, Me-11	H-7, Me-11	H-7, Me-11
8a	H-3ax, H-4, Me-11	H-2, H-4, Me-11	H-4, Me-11
9	H-7, Me-10	H-7, Me-10	H-7, Me-10
10	H-7, Me-9	H-7, Me-9	H-7, Me-9
11	-	H-7	H-7
1'	H-2, H-3ax	H-2, 2H-3	H-2, H-3ax
2'	H-2	H-2	H-2
3'	-	-	-
4'	-	-	-
5'	-	-	-
6'	H-2	H-2	H-2
CH3CO	-	-	-
CH3CO	-	H-4, MeCO	-

The relative stereochemistry of DL-8 as in (**10**) follows from NOE difference experiments (see **Table 4**). All the methyl groups in ring A afford NOEs to the

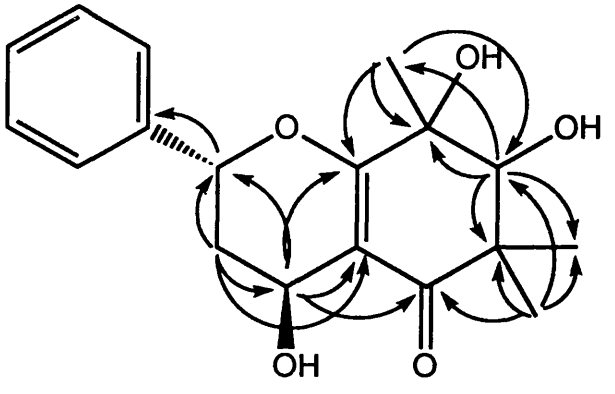
secondary carbinol proton, indicating that both the secondary and tertiary hydroxyl groups must be α. The observation of a NOE from the C-8 methyl at δ_H 1.55 to H-2 confirms the relative stereochemistry of the pyran ring.

The third compound DL-9 was also obtained as a gum, $[\alpha]_D$ +6.5° (c, 0.20 in $CHCl_3$), λ_{max} 253 nm (log ε 3.74), which showed hydroxyl (ν_{max} 3650, 3601, 3554 cm^{-1}) and conjugated ketone (ν_{max} 1645 cm^{-1}) absorption in its IR spectrum. As for DL-7 it also shows an (M^+) peak at m/z 318 and HREIMS also confirmed the molecular formula as $C_{18}H_{22}O_5$. Thus DL-9 is isomeric with DL-7 (**9**). Other mass peaks include m/z 300 [M^+-H_2O], 285 [M^+-H_2O-CH_3], 282 [M^+-$2H_2O$], 267 [M^+-$2H_2O$-CH_3] and 105 [$C_6H_5CO^+$]. The base peak in the mass spectra of both is at m/z 104, which arises from retro Diels Alder cleavage of the pyran ring.

The ^1H and ^{13}C NMR spectroscopic properties of DL-9 are similar to those of DL-7 and DL-8 and the shifts are listed in **Tables 1** and **2**. The ^1H NMR shows the phenyl group [δ_H 7.40 (5H, m)], three tertiary methyls [δ_H 1.15 (s), 1.31 (s), 1.53 (s)], three oxygenated methines [δ_H 4.65 (dd, J 4.0, 2.0 Hz, H-4), 5.13 (dd, J 12.2, 2.5 Hz, H-2), 3.82 (s)], a methylene group [δ_H 2.17 (dt, J 14.7, 2.3 Hz), 1.93 (ddd, J 14.7, 12.2, 4.0 Hz)] which couples to two of the methines, and three hydroxyl groups [δ_H 2.45, 2.56, 3.10 (each bs)]. The ^{13}C NMR spectrum reveals an unsaturated ketone [δ_C 202.1 (s), 170.9 (s), 111.3 (s)], a phenyl ring, three oxygenated methines [δ_C 78.0, 76.4, 58.5], an oxygenated quaternary carbon [δ_C 73.2], a quaternary carbon [δ_C 45.9], a methylene group [δ_C 36.4 (t)] and three methyl groups [δ_C 19.6, 22.6, 24.5]. These data suggest that DL-9 has the same gross structure as DL-7 (**9**) but differs in the stereochemistry (*vide infra*) of at least one centre, in particular at C-4 since H-4 no longer has any large couplings.



(11)



(11a)

The connectivity leading to structure (**11**) for DL-9 was derived from the HMBC spectrum (Fig. 6, 7) as above. The correlations are summerised in (**11a**) and in Table **5**. The substitution pattern of ring A is readily revealed as in (**11**) by the correlations of the methyls and the carbinol proton H-7. The identity of H-4 is clearly established by its correlations to C-2, C-4a, C-5 and C-8a. The couplings of H-4 (J 4.0, 2.0 Hz) suggest that it is equatorial and this is confirmed by NOEs to both H-3ax and H-3eq. Irradiation of the axial H-2 gives, as expected, NOEs to the phenyl group and to H-3eq. The NOEs

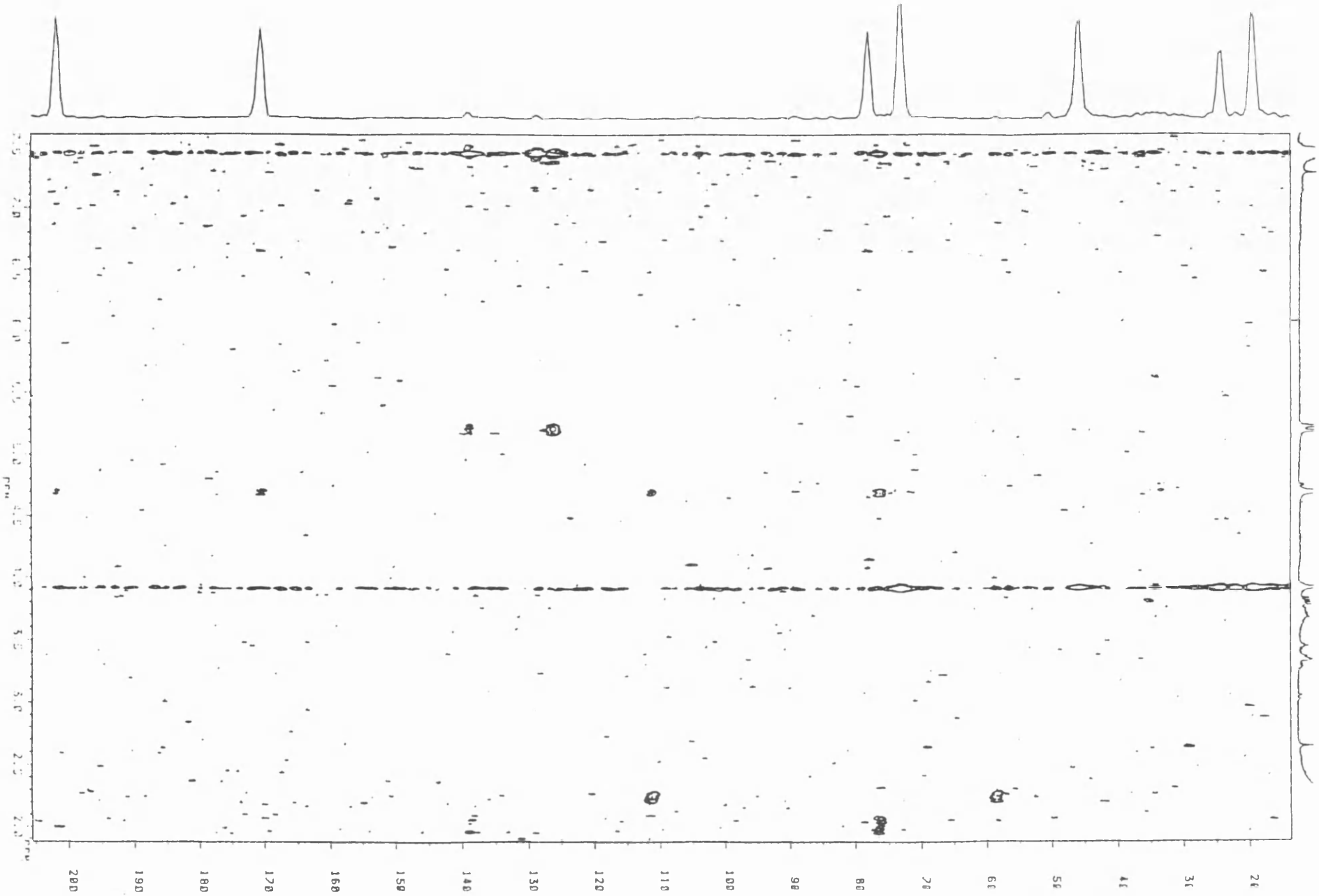


Figure 6. The HMBC spectrum of DL-9 (**11**).

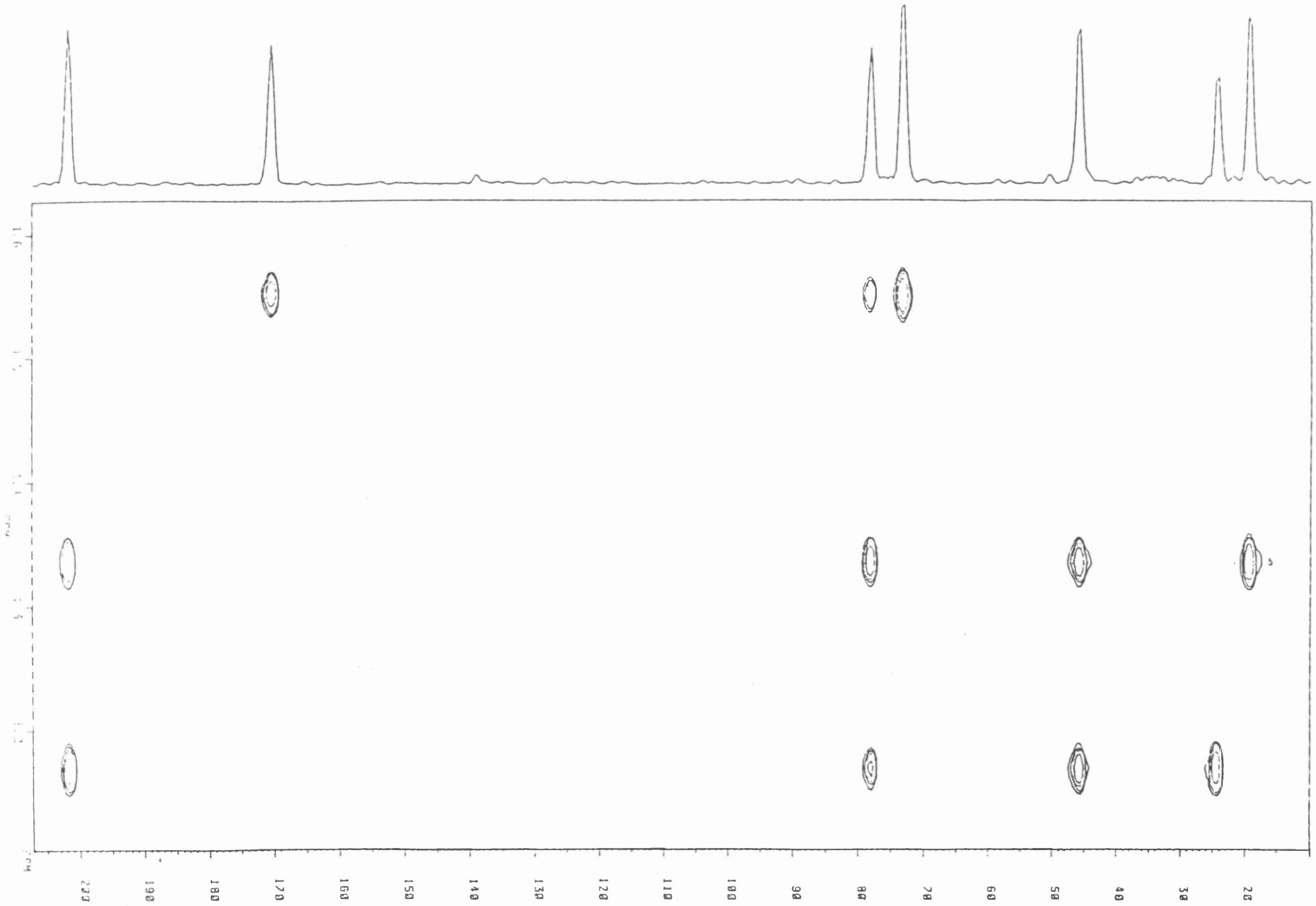
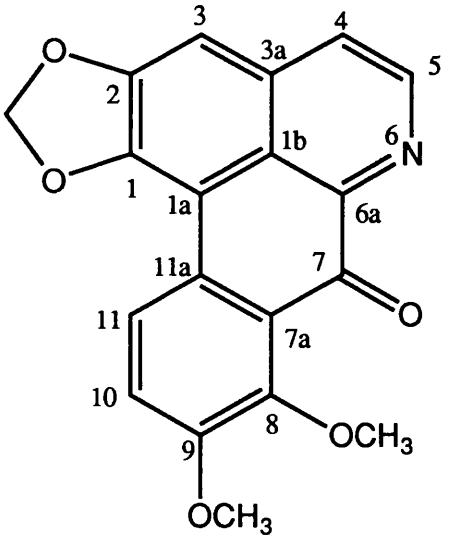


Figure 7. The HMBC spectrum of DL-9(**11**).

obtained for DL-9 are listed in **Table 4** and lead to the relative stereochemistry as in (**11**). Thus DL-9 is the C-4 epimer of DL-7.

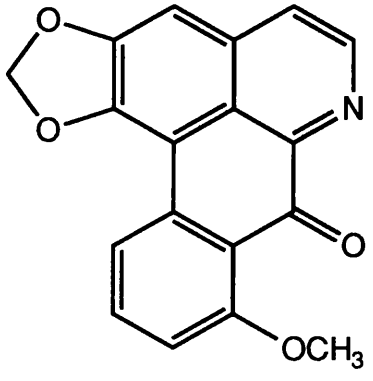
These methylated flavonoids are interesting new compounds. It is unfortunate that they were obtained in only small amounts. Other examples of methylated aromatic compounds have been reported from the Annonaceae (see Introduction).

DL-11 (**12**), $C_{19}H_{13}NO_5$, was obtained as orange-red needles, mp 266-268° [lit.[31] oxocrebanine, mp 265-269°]. It is UV active and shows absorption at λ_{max} 250, 275, 380 nm. It showed a Dragendorff positive spot on tlc, indicating its alkaloidal nature, and its IR spectrum exhibits bands at ν_{max} 1665 (conjugated ketone), 3020, 1603, 1579, 1520, 1468, and 1423 (aromatic) cm^{-1}. Its mass spectrum shows a molecular ion (M^+) at m/z 335 (base peak) together with fragments at m/z 320 (M^+-CH_3), 307 (M^+-CO), 306 (M^+-1-CO), 305 (M^+-OCH_2), 304 (M^+-OCH_3), consistent with an oxoaporphine system.

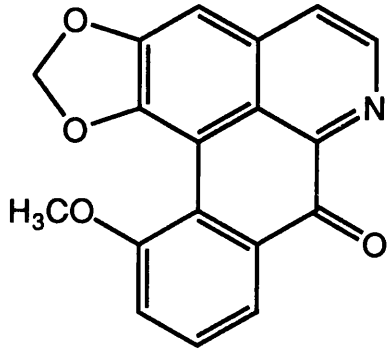


(**12**)

The ^1H NMR spectrum of DL-11 reveals two pyridine protons [δ_H 7.64, 8.78 (ABq, J 5.0 Hz, H-4, H-5)], an aromatic singlet [δ_H 7.03 (1H)], another aromatic AB quartet [δ_H 7.17, 8.31 (each 1H, J 9.0 Hz, H-10, H-11)], a methylenedioxy group [δ_H 6.29 (2H, s)] and two methoxyl groups [δ_H 3.94 (3H, s), 4.00 (3H, s)]. These NMR data are consistent with an oxoaporphine nucleus with methylenedioxy and two methoxyl substituents. Comparison with published data confirmed the identity of DL-11 as oxocrebanine (**12**)[32].



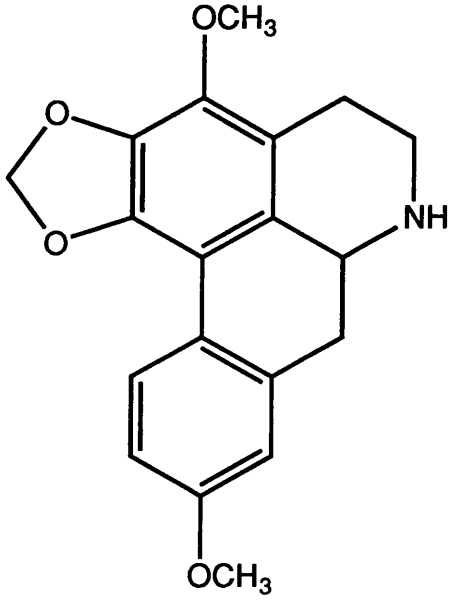
(**13**)



(**14**)

The ^{13}C NMR spectrum is in accord with structure (**12**). It has a carbonyl resonance [δ_C 181.6], five aromatic methines [δ_C 101.9, 117.0, 123.7, 123.8, 144.1], a methylenedioxy group [δ_C 102.3], two methoxyl groups [δ_C 56.0, 61.0] and ten aromatic singlets [see Experimental]. The ^{13}C NMR data for (**12**) have not been reported previously. The assignments (see Experimental) were made by comparison with the ^{13}C NMR data of oxoputerine (**13**) and oxostephanine (**14**)[33]. This is the first report of the presence of oxocrebanine in the genus *Desmos* but it has been isolated previously from other Annonaceae[32].

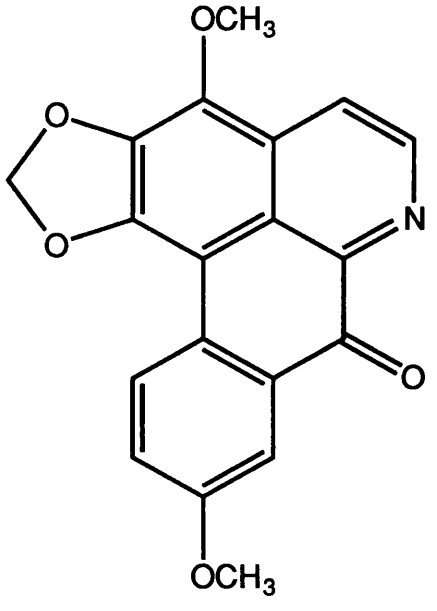
DL-12 (**15**), $C_{19}H_{19}NO_4$ was obtained as crystals, mp >297° [lit.[34] buxifoline, mp >295°]. It is UV active and shows absorption bands at λ_{max} 238, 280 nm. It showed Dragendorff positive spot on tlc, indicating its alkaloidal nature. Its IR spectrum shows bands at ν_{max} 3020, 1610, 1565, 1440 (aromatic) and 1030 (C-O) cm^{-1}. Its mass spectrum shows a parent ion at m/z 325 and other peaks at 324 (M^+-1), 310 (M^+-15), 296 (M^+-OCH), 295 (M^+-OCH_2), 294 (M^+-OCH_3), 279 (M^+-CH_2O_2), 264 (M^+-OCH_3-OCH_2) and 43 (base peak), consistent with an aporphine nucleus for DL-12.



(**15**)

The ^1H NMR of DL-12 (**15**) shows a three spin system at δ_H 7.94 (1H, d, J 8.5 Hz, H-11), 6.85 (1H, dd, J 8.5 Hz, H-10) and 6.80 (1H, d, J 2.5 Hz, H-8) associated with the aromatic ring D, a methylenedioxy group [δ_H 5.91, 6.05 (ABq, J 1.5 Hz)] and two methoxyls [δ_H 3.80 (s), 3.99 (s)]. These data are consistent with the aporphine alkaloid buxifoline (**15**) which has methoxyls at C-3 and C-9 and a methylenedioxy at C-1, C-2. Comparison with published data[34] confirmed that DL-12 is buxifoline (**15**).

DL-13 (**16**), $C_{19}H_{13}NO_5$ was obtained as crystals, mp 270° [lit.[35] oxobuxifoline, mp 268°]. It is UV active and shows absorption at λ_{max} 216, 252, 272, 285 and 329 nm. It gave a positive Dragendorff spot on tlc, indicating its alkaloidal nature. Its IR spectrum has bands at 1650 (C=O), 3020, 1600, 1580, 1460 (aromatic CH), 1220 and 1030 (C-O) cm^{-1} while its mass spectrum shows an $[M^+ + 1]$ peak at m/z 336 and a molecular ion (M^+) at m/z 335 corresponding to $C_{19}H_{13}NO_5$. Other peaks include 334 (M^+-H), 320 (M^+-CH_3), 304 (M^+-OCH_3), 307 (M^+-CO), 306 (M^+-CHO), 305 (M^+-CH_2O), 292 (M^+-CH_3-CO), 290 (M^+-CH_3-OCH_2), 276 (M^+-OCH_3-CO) and a base peak at m/z 28 (CO), consistent with an oxoaporphine system.

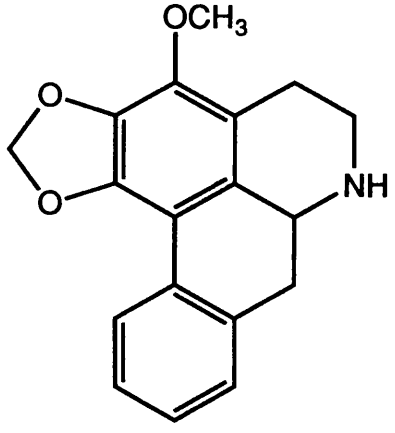


(**16**)

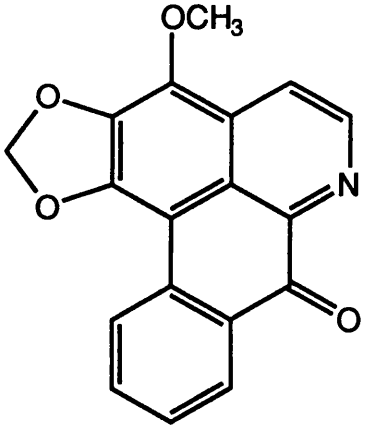
The ^1H NMR of DL-13 reveals the presence of an AB quartet [δ_H 8.78, 8.13 (each 1H, J 5.0 Hz, H-4, H-5)] in a pyridine ring and a three spin aromatic system [δ_H 8.40 (1H, d, J 8.9 Hz, H-11), 7.88 (1H, d, J 2.9 Hz, H-8, 7.22 (1H, dd, J 8.9, 2.9 Hz, H-10)], in addition to a methylenedioxy group [δ_H 6.25 (s)] and two methoxyl groups [δ_H 3.91 (3H, s, MeO-9), 4.21 (3H, s, MeO-3)]. These data are consistent with

an oxoaporphine alkaloid such as oxobuxifoline (**16**). The chemical shifts and coupling constants of H-4 and H-5 confirm the presence of a pyridine ring while the deshielded value of H-8 requires a C-7 carbonyl group. Comparison with published data[35] confirmed the identity of DL-13 as oxobuxifoline (**16**). There are slight differences in chemical shift because of solvent effects, since the original spectra were obtained in TFA[35].

Two further alkaloids DL-14 (**17**) (norstephalagine) and DL-15 (**18**) (atherospermidine) were also isolated. Their physical and spectroscopic properties were identical with those of norstephalagine (**17**) and atherospermidine (**18**), previously isolated from *Artabotrys odoratissimus* (see Chapter 5).



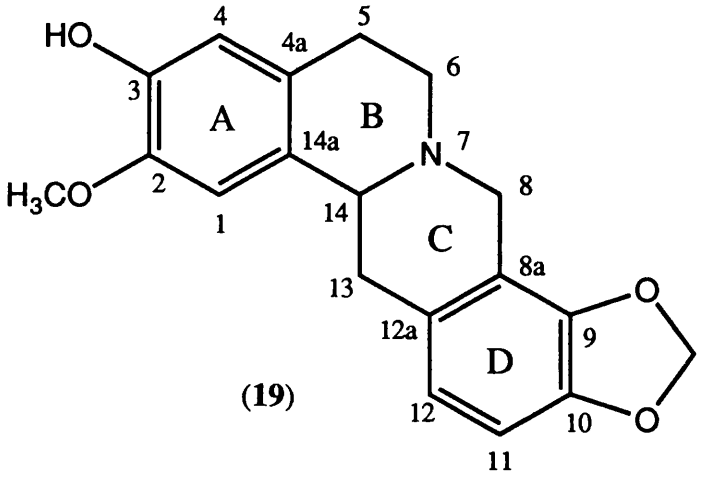
(**17**)



(**18**)

DL-16 (**19**) was obtained as orange-red crystals, mp >315°, $[\alpha]_D$ +34° (c, 0.032 in $CHCl_3$). It is UV active and shows absorption at 240 (log ε 4.37), 277 (log ε 3.85), 287 (log ε 4.08), 347 (log ε 3.73) and 360 (log ε 4.23) nm. Its IR exhibits bands at 3546 (hydroxyl), 3020, 1604, 1520, 1460, 1394, 1356, 1334 (aromatic) and 1030 (C-O) cm^{-1}. Its mass spectrum shows a molecular ion peak at m/z 325 and HREIMS

confirmed its molecular formula as $C_{19}H_{19}NO_4$. Other peaks include m/z 324 (M^+-1), 323 (M^+-1-1), 310 (M^+-15), 295 (M^+-OCH_2), 294 (M^+-OCH_3), 279 (M^+-OCH_3-CH_3), 177 ($C_{10}H_{11}NO_2^+$) and 148 ($C_9H_8O_2^+$). These data are consistent with a protoberberine alkaloid[36]. The appearance of peaks at m/z 177 and 148 suggests a berberine base containing hydroxy and methoxy groups in ring A and a methylenedioxy group in ring D[36].

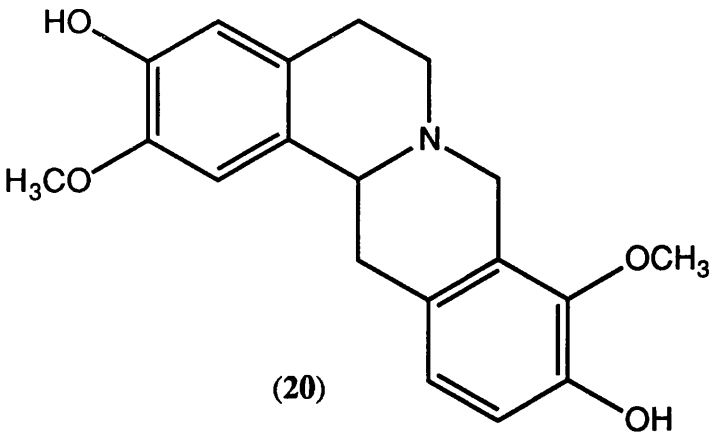


(19)

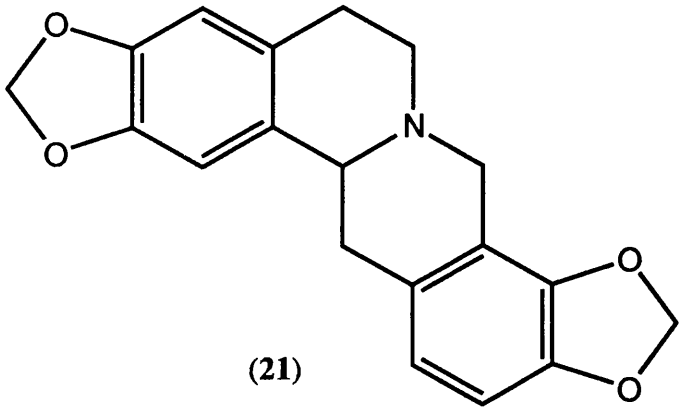
The ^1H NMR of DL-16 shows four aromatic protons [δ_H 6.52-6.74 (4H, m)], a methylenedioxy [δ_H 5.91, 5.96 (ABq, J 1.4 Hz)], a methoxyl [δ_H 3.88 (3H, s)], four methylene groups [δ_H 4.10, 3.52 (ABq, J 15.3 Hz, H_A-8, H_B-8), 3.26 (1H, dd, J 16.0, 4.0 Hz, H_A-13), 2.81 (1H, dd, J 16.0, 11.0 Hz, H_B-13), 2.61 (2H, m, 2H-5) and 3.08 (2H, m, 2H-6)] and a methine proton [δ_H 3.58 (dd, J 11.0, 4.0 Hz, H-14)], confirming the deduction from the mass spectrum that the compound is a tetrahydroprotoberberine alkaloid with methylenedioxy, methoxy and hydroxy substituents. Unfortunately the aromatic protons of DL-16 are not well resolved. To avoid this problem the ^1H NMR spectrum was run in C_6D_6 as solvent. The ^1H NMR in C_6D_6 shows two aromatic singlets [δ_H 6.44 (1H, s, H-1), 6.79 (1H, s, H-4)] and an ABq [δ_H 6.66, 6.53 (each 1H, J 8.0 Hz, H-11, H-12)] in addition to a methylenedioxy [δ_H 5.34, 5.41 (ABq, J 1.4

Hz)], the C-8 methylene [δ_H 4.15, 3.48 (ABq, J 15.2 Hz)], a methoxyl [δ_H 3.20 (3H, s)] and other aliphatic protons [δ_H 3.50 (2H, m, H-14, H_B-5), 3.06 (2H, m, 2H-13), 2.95 (2H, m, 2H-6) and 2.30 (1H, m, H_A-5)]. It seems likely that there are oxygen substituents at C-9/C-10 and C-2/C-3. The mass spectrum suggests that the methylenedioxy group is attached to ring D. To confirm this and to determine the relative positions of the methoxyl and phenolic hydroxy group and the methylenedioxy group we performed some NOE difference experiments. Irradiation of the methoxyl signal (δ_H 3.20) gave a NOE (10%) at H-1(δ_H 6.44) while irradiation at δ_H 6.44 (H-1) gave a NOE (8%) at the methoxyl signal (δ_H 3.20) and additional NOEs at δ_H 3.50 (H-14, 1%) and δ_H 3.06 (2H-13, 2%). Irradiation of δ_H 6.79 (H-4) gave a NOE (3%) at δ_H 2.30 (H-5). These results confirm the position of the methoxyl group at C-2 and the hydroxyl at C-3. Irradiation of H-12 (δ_H 6.53) gave NOEs at δ_H 6.66 (H-11) (5%) and at δ_H 3.06 (2H-13) (2%). These results confirm the attachment of the methylenedioxy to C-9 and C-10.

The ^{13}C NMR spectrum of DL-16 shows the expected features for a tetrahydroprotoberberine alkaloid. Thus it has four aromatic methines [δ_C 114.0 (C-1), 107.7 (C-4), 106.7 (C-11), 120.9 (C-12)], a methylenedioxy [δ_C 101.1], a methoxyl [δ_C 56.0], four methylenes [δ_C 29.0 (C-5), 51.3 (C-6), 53.0 (C-8), 36.5 (C-13)] and a methine [δ_C 59.5 (C-14), in addition to eight aromatic singlets (see Table **6**). Comparison of the ^{13}C chemical shift data with those of descretanine[37] (**20**) for the ring A carbons and stylophine[38] (**21**) for rings B, C and D ring [see **Table 6**] confirms the structural assignments of DL-16 (**19**). There is a difference in the value of C-1 which in our case is δ_C 114.3 but is misassigned (δ_C 123.56) in the literature[37]. DL-16 (**19**) is a new natural product.



(20)



(21)

TABLE 6 : ^{13}C NMR of Compounds (19), (20), (21).

Carbon	Compounds		
	(19)	**(20)**	**(21)**
1	114.2	123.56	
2	144.9*	146.05	
3	145.2*	147.25	
4	107.7	109.28	
4a	129.0	128.36	
5	29.0		29.6
6	51.3		51.2
8	53.0		52.9
8a	116.9		116.9
9	144.0*		146.2
10	143.3*		145.0
11	106.7		106.7
12	120.9		121.0
12a	127.4		128.8
13	36.5		36.5
14	59.5		59.8
14a	128.6	128.18	
CH_2O_2	101.1		100.7
OCH_3	56.0	59.26	

* Assignments may be interchangeable

GENERAL EXPERIMENTAL

Melting points (mp) were determined on a Kofler hot-stage apparatus and are uncorrected. Infra-red (IR) spectra were recorded in $CHCl_3$ solution (unless otherwise stated) on either a Perkin Elmer 580 or Philips 9800 FTIR spectrometer. Ultra-violet (UV) spectra were measured for MeOH solutions (unless otherwise stated) using a Perkin-Elmer lambda 9 UV/Vis/NIR spectrometer. Low resolution mass spectra were determined using a VG updated MS 12 spectrometer while high resolution mass spectra were determined on a modified Kratos MS 9 instrument. Optical rotations were measured on an optical activity AA-100 polarimeter in $CHCl_3$ solution at 20°C.

Unless otherwise stated, nuclear magnetic resonance (NMR) spectra were recorded at 298K and at 4.7T on Bruker WP 200SY and AM 200SY spectrometers (^1H, 200.132 MHz; ^{13}C, 50.32 MHz) . Higher field NMR spectra were recorded using either a Bruker AC 300 instrument (^1H, 300.13 MHz; ^{13}C, 75.47 MHz) at 7.05T or with a Varian VXR600S spectrometer (^1H, 600 MHz; ^{13}C, 150 MHz) at 14.1T. Spectra were recorded for $CDCl_3$ solutions (unless otherwise specified) relative to $CHCl_3$ at δ_H 7.25 and $CDCl_3$ at δ_C 77.00 and chemical shifts are recorded in ppm. Occasionally TMS was used as internal standard at δ 0.0. Tabulated ^1H NMR data have coupling constants (J) in Hz given in parenthesis. Signals indicated as m (multiplet) were unresolved or overlapping. ^1H NMR assignments are based on chemical shifts, correlation with ^{13}C chemical shifts, homonuclear decoupling and NOE difference experiments and also on comparison with published data for similar compounds. ^{13}C assignments are based on chemical shift, correlation with ^1H chemical shifts, multiplicities in DEPT or J. mod. ^{13}C spectra and comparison with published data for similar compounds. ^1H and ^{13}C signals with similar chemical shifts and the same multiplicity are therefore interchangeable unless otherwise confirmed by 2D δ_C/δ_H and δ_H/δ_H correlation experiments.

Homonuclear proton NOE experiments were performed by selectively irradiating a single line of a multiplet for 3s and using a 90° observation pulse to minimise SPT effects. The NOEs reported are given as percentage saturation and are the result of scaling up the observed NOEs in inverse proportion to the degree of saturation in order to obtain values equivalent to the result of complete saturation. The HMQC (^{1}H detected Heteronuclear Multiple Quantum Correlation)[39] and HMBC (Heteronuclear Multiple Bond Correlation)[40] experiments were performed on a Bruker AC 300 spectrometer (^{1}H, 300.13 MHz; 75.47 MHz) and are the inverse of the conventional chemical shift correlation experiments. In these techniques the ^{1}H-^{13}C couplings are observed from the side of the much more sensitive proton which is detected during acquisition rather than the low γ ^{13}C nucleus and this greatly enhances the sensitivity of the NMR experiments. The pulse scheme for HMQC contains a BIRD pulse in order to remove large signals caused by protons not directly attached to ^{13}C. Direct and long-range coupling constants have been chosen as 135.1 Hz and 7.1 Hz respectively for HMQC and HMBC experiments.

The solvents used were either of analytical grade or bulk solvents distilled before use. All plant material was air-dried, ground mechanically and extracted with EtOAc (in some cases by $CHCl_3$ and EtOH) and MeOH. The crude extracts were then analysed by analytical tlc and ^{1}H NMR. Extracts were then fractionated by flash column chromatography (FCC) over silica gel (Merck kieselgel GF_{254}). Eluents for silica gel FCC were in most cases increasing percentages of EtOAc in petroleum ether (in some cases increasing percentage of $CHCl_3$ in petroleum ether) followed by MeOH in EtOAc and finally MeOH. Each of the crude fractions was further purified by preparative thin-layer chromatography (PTLC) over silica gel (Merck kieselgel GF_{254}, 1mm thickness). Tlc plates were developed using various solvent combinations in appropriate concentrations, the most commonly used solvent systems being EtOAc in petroleum ether, MeOH in CH_2Cl_2 and MeOH in $CHCl_3$. After each chromatographic separation the fractions were removed from PTLC plates or collected from columns were checked

by analytical tlc , ^{1}H NMR and sometimes ^{13}C NMR for their compositions. The total amount in each fraction was not always further chromatographed and therefore many of the actual yields are much greater than indicated in the experimental section.

All solvents were removed using a Buchi rotary evaporator and water aspirator. Petroleum ether refers to the fraction boiling between 40^{o} and 60^{o}. All reagents and solvents used in chemical reactions were purified according to literature methods. Analytical tlc was over Merck precoated silica gel 60 F_{254} (0.25 mm thickness). Preparative and analytical plates were visualized under UV light (254 or 366 nm), by Dragendorff's reagent[41], by adsorption of I_2 vapour or by spraying with 25% H_2SO_4 and heating.

Collection and Identification of Plant Material

The plant material collected in Bangladesh was identified by Mr. A. Hasan, Department of Botany, University of Dhaka. Voucher specimens are deposited in the University Herbarium in Dhaka. *Polyalthia bullata* was collected by the botanist of the Forest Research Institute of Malaysia and a voucher specimen is deposited in the Institute Herbarium in Kepong near Kuala Lumpur.

EXPERIMENTAL

Isolation. The plant material of *Desmos longiflorus* was collected from the district of Sylhet, Bangladesh. The dried ground stem bark (900g) was extracted successively with $CHCl_3$ and MeOH. The $CHCl_3$ extract (26g) was concentrated *in vacuo* and a portion (13g) was subjected to flash column chromatography over silica gel. The column was first eluted with petroleum ether, then increasing amounts of EtOAc in petroleum ether and finally MeOH, collecting 100ml fractions. Fractions were monitored by analytical tlc. The early fractions contained mainly fat. The later fractions showed many spots on analytical tlc. Multiple preparative tlc using petroleum ether : EtOAc (85 : 15), (88 : 12) and (97 : 3) of fractions 10, 13 and 7 respectively afforded DL-1 (sitostenone, 100mg), DL-3 (benzyl benzoate, 40mg), DL-6 (15α-hydroxy-24-methylene-lanosta-7,9(11)-dien-3-one, 22mg), DL-2 (4-stigmastene-3,6-dione, 8.0mg) and DL-10 (unidentified, 10 mg). Multiple preparative tlc of fractions 30, 38, 40 using petroleum ether : CH_2Cl_2 : MeOH (14 : 85 : 1) afforded DL-4 (crotepoxide, 60mg), DL-5 [N-benzoyl-O-(N'-benzoyl-L-phenylalanyl)-L-phenylalaninol, 25mg], DL-7 (10mg), DL-8 (22mg) and DL-9 (13mg).

The MeOH extract (10g) was concentrated *in vacuo* and subjected to flash column chromatography over silica gel. The column was eluted with EtOAc, then increasing amounts of MeOH in EtOAc and finally with MeOH. Multiple preparative tlc using CH_2Cl_2 : MeOH (97 : 3), (94 : 6) and (90 : 10) of fractions 13, 15 and 16 afforded DL-12 (buxifoline, 3.0mg), DL-14 (norstephalagine, 10mg), DL-13 (oxobuxifoline, 2.5mg), DL-11 (oxocrebanine, 26mg), DL-15 (atherospermidine, 10mg), and DL-16 (3-hydroxy-2-methoxy -9,10-methylenedioxytetrahydroprotoberberine, 15mg).

DL-1 (Sitostenone, **1**), needles (MeOH), mp 95°, $[\alpha]_D$ +81.0° (c, 1.00 in $CHCl_3$); IR ν_{max} : 2960, 2850, 1680, 1620, 1410, 1380 cm^{-1}; EIMS m/z (rel. int.) :

412 (36), 397 (5), 370 (16), 327 (5), 289 (19), 271 (21), 269 (11), 230 (11), 229 (42), 187 (11), 161 (13), 149 (26), 148 (16), 147 (30), 135 (27), 134 (13), 133 (20), 131 (13), 125 (14), 124 (100), 123 (25), 121 (27), 119 (20), 109 (27), 107 (30), 105 (28), 97 (20), 95 (39), 93 (30), 91 (30), 83 (22), 81 (37), 79 (30), 69 (33), 67 (27), 57 (27), 55 (64), 53 (66), 43 (40), 28 (46); ^{1}H NMR : δ_H 0.67 (3H, s, Me-18), 1.15 (3H, s, Me-19), 0.78 (3H, d, J 6.8 Hz, Me-26), 0.88 (3H, d, J 6.8 Hz, Me-27), 1.10 (3H, d, J 6.4 Hz, Me-21), 0.96 (3H, t, J 6.5 Hz, Me-29), 5.67 (1H, s, H-4), 1.28-2.60 (30H, m); ^{13}C NMR : δ_C 35.8 (C-1), 33.8 (C-2), 199.6 (C-3), 123.6 (C-4), 171.7 (C-5), 32.8 (C-6), 31.9 (C-7), 34.5 (C-8), 53.7 (C-9), 38.5 (C-10), 21.0 (C-11), 39.4 (C-12), 42.2 (C-13), 55.7 (C-14), 24.1 (C-15), 28.1 (C-16), 55.8 (C-17), 11.8 (C-18), 17.2 (C-19), 36.5 (C-20), 18.9 (C-21), 35.5 (C-22), 21.1 (C-23), 45.6 (C-24), 29.0 (C-25), 18.6 (C-26), 19.3 (C-27), 22.9 (C-28), 12.2 (C-29).

DL-2 (4-Stigmastene-3,6-dione, **2**), crystals (MeOH), mp 170°; $[\alpha]_D$ -60.0° (c, 1.00 in $CHCl_3$); IR ν_{max}: 2960, 2870, 1680, 1617, 1520, 1464, 1380, 1334, 1269, 1215, 930 cm^{-1}; EIMS m/z (rel. int.) : 426 (1), 411 (2), 285 (6), 243 (5), 189 (3), 175 (7), 149 (12), 137 (6), 123 (15), 121 (11), 109 (21), 107 (14), 105 (16), 97 (13), 93 (14), 91 (22), 83 (15), 81 (24), 77 (20), 71 (20), 69 (30), 57 (30), 55 (40), 43 (100); ^{1}H NMR : δ_H 0.71, 1.15 (2x3H, 2xs, Me-18, Me-19), 0.78 (3H, d, J 6.5 Hz, Me-26), 0.84 (3H, d, J 6.5 Hz, Me-27), 0.87 (3H, d, J 6.4 Hz, Me-21), 0.95 (3H, t, J 6.4 Hz, Me-29), 6.16 (1H, s, H-4); ^{13}C NMR : δ_C 34.1 (C-1), 33.8 (C-2), 199.6 (C-3), 125.4 (C-4), 161.1 (C-5), 202.4 (C-6), 46.8 (C-7), 34.2 (C-8), 50.9 (C-9), 39.8 (C-10), 20.8 (C-11), 39.1 (C-12), 42.5 (C-13), 55.8 (C-14), 25.0 (C-15), 28.0 (C-16), 56.7 (C-17), 11.9 (C-18), 17.5 (C-19), 36.0 (C-20), 18.7 (C-21), 34.5 (C-22), 23.0 (C-23), 45.7 (C-24), 29.1 (C-25), 19.0 (C-26), 19.8 (C-27), 24.0 (C-28), 12.0 (C-29).

DL-3 (Benzyl benzoate, **3**), oil, UV λ_{max} (log ε) : 228 (3.63), 235 (3.86), 260 (3.55), 273 (4.13) , 280 (3.96) nm; IR ν_{max} : 1722, 1603, 1585, 1520, 1498, 1450, 1380, 1315, 1277, 1215, 1176, 1115,1072, 1027, 960, 930, 885 cm^{-1}; EIMS m/z (rel.

int.) : 212 (27), 109 (2), 108 (2), 107 (8), 106 (9), 105 (100), 93 (2), 91 (50), 90 (7), 89 (5), 77 (35), 65 (14)), 51 (18), 28 (14); ^{1}H NMR : δ_H 8.07 (1H, m), 8.11 (1H, m), 7.35-7.60 (8H, m), 5.38 (2H, s); ^{13}C NMR : δ_C 136.0 (C-1), 129.7 (C-2'), 128.3 (C-3'), 133.0 (C-4'), 128.3 (C-5'), 129.7 (C-6'), 166.4 (C-7), 130.1 (C-1'), 128.4 (C-2), 128.2 (C-3), 128.0 (C-4), 128.2 (C-5), 128.4 (C-6), 66.6 (C-7').

DL-4 (Crotepoxide, **4**), needles (MeOH), mp 152°; $[\alpha]_D$ +64.4° (c, 1.00 in $CHCl_3$); UV λ_{max} (log ε) : 281 (3.54), 273 (3.43), 223 (4.30) nm; IR ν_{max} : 3020, 2960, 2930, 1756, 1732, 1690, 1602, 1151, 1456, 1372, 1260, 1235, 1210, 1154, 1107, 1070, 1010, 980 cm^{-1}; EIMS m/z (rel. int.) : 362 (1), 303 (1), 249 (1), 231 (2), 227 (8), 207 (3), 185 (2), 163 (9), 157 (30), 138 (6), 125 (4), 115 (8), 106 (8), 105 (100), 97 (100, 77 (23), 71(4); ^{1}H NMR : δ_H 8.01 (2H, m), 7.57 (1H, m), 7.44 (2H, m), 5.68 (1H, d, J 10.0 Hz, H-2), 4.98 (1H, dd, J 10.0, 1.5 Hz, H-3), 3.05 (1H, dd, J 4.0, 1.5 Hz, H-4), 3.42 (1H, dd, J 4.0, 2.5 Hz, H-5), 3.65 (1H, d, J 2.5 Hz, H-6), 4.21, 4.55 (2H, ABq, J 12.0 Hz, Ha-7, Hb-7), 2.00 (3H, s, CH_3-CO-), 2.07 (3H, s, CH_3CO-); ^{13}C NMR : δ_C 59.4 (C-1), 69.4 (C-2), 70.3 (C-3), 52.5 (C-4), 48.0 (C-5), 53.7 (C-6), 62.4 (C-7), 129.0 (C-1'), 129.7 (C-2'), 128.5 (C-3'), 133.5 (C-4'), 128.5 (C-5'), 129.7 (C-6'), 170.0 (2-OO<u>C</u>CH$_3$), 169.5 (3-OO<u>C</u>CH$_3$), 165.5 (CH$_2$O<u>C</u>O), 20.6 (2x<u>Me</u>CO).

DL-5 [N-benzoyl-O-(N'-benzoyl-L-phenylalanyl)-L-phenylalaninol, **5**], white crystals (EtOAc), mp 208-210°; $[\alpha]_D$ -75° (c, 0.103 in EtOH); UV λ_{max} (log ε) : 215 (4.03), 230 (4.35) nm; IR ν_{max} (KBr) : 3340, 3025, 1750, 1640, 1600, 1580, 1530, 1488, 1386, 1350, 1302, 1295, 1275, 1210, 1170, 1160, 1098, 1076, 1025, 1000, 745, 710, 694 cm^{-1}; EIMS m/z (rel. int.) : 506 (M^+) (1), 415 (M^+-$PhCH_2$) (1), 385 (M^+-$PhCONH_2$) (1), 294 (M^+-$PhCONH_2$-$PhCH_2$) (4), 269 (2), 252 [$PhCH_2CH(NHBz)=C=O]^+$ (8), 224 $(PhCH_2CHNHBz)^+$ (9), 223 (2), 213 (2), 149 (9), 148 (10), 147 (10), 146 [252-$PhCHO^+$] (51), 133 (5), 119 (224-$PhCO^+$) (8), 118 (12), 109 (6), 107 (5), 106 (8), 105 ($PhCO^+$) (100), 97 (9), 95 (7), 91 ($PhCH_2^+$) (32),

85 (7), 83 (10), 81 (10), 77 (Ph^+) (38),71 (10), 57 (16), 55 (18), 43 (25), 28 (60); ^1H NMR : δ_H 7.71 (4H, m), 7.15-7.50 (16H, m), 6.72 (1H, d, J 8.2 Hz, NH), 6.62 (1H, dd, J 6.6 Hz, NH), 4.96 (1H, q, J 6.6 Hz, H-2), 4.62 (1H, m, H-2'), 4.57 (1H, dd, J 11.3, 3.3 Hz) and 4.05 (1H, dd, J 11.3, 4.2 Hz) [2H-1], 3.32 (1H, dd, J 13.8, 6.6 Hz) and 3.20 (1H, dd, J 13.8, 7.0 Hz) [2H-3'], 3.02 (1H, dd, J 13.6, 6.4 Hz) and 2.90 (1H, dd, J 13.6, 8.1 Hz) [2H-3]; ^{13}C NMR : δ_C 65.4 (C-1), 54.5 (C-2), 37.2 (C-3), 171.9 (C-1'), 50.2 (C-2'), 37.4 (C-3'), 126.8 (1C, d), 127.0 (2C, d), 127.1 (2C, d), 127.3 (1C, d), 128.4 (2C, d), 128.6 (4C, d), 128.8 (2C, d), 129.1 (2C, d), 129.3 (2C, d), 131.4 (1C, d), 132.0 (1C, d), 133.2 (1C, s), 134.1 (1C, s), 135.7 (1C, s), 137.1 (1C, s), 167.2 (CONH), 167.4 (CONH). HREIMS : Found 506.2223; calculated for $C_{32}H_{30}N_2O_4$ 506.2205.

DL-6 (15α-Hydroxy-24-methylenelanosta-7,9(11)-dien-3-one, **6**), colourless needles (MeOH), mp 156-158°; $[\alpha]_D$ +42.6°(c, 2.00 in $CHCl_3$); UV λ_{max} (logε) : 252 (4.05), 243 (4.11), 236 (3.96) nm; IR ν_{max} : 3400, 1715, 1640, 1462, 1380, 1217, 1065, 1010, 890 cm^{-1}; EIMS m/z (rel. int.) : 452 (M^+, 100), 437 (M^+-CH_3) (13), 419 (M^+-CH_3-H_2O) (11), 337 [M^+-C_9H_{17}(side chain)] (14), 285 (11), 271 (23), 199 (15), 185 (25), 157 (29), 125 (C_9H_{17}, side chain) (26), 123 (34), 119 (39), 105 (32), 95 (38), 83 (47), 69 (76), 55 (91), 43 (82), 28 (59); ^1H NMR : δ_H 0.62 (3H, s, Me-18), 0.88 (3H, d, J 6.8 Hz, Me-21), 0.91 (3H, s, Me-30), 1.00 (3H, d, J 6.8 Hz, Me-26), 1.03 (3H, d, J 6.8 Hz, Me-27), 1.08 (3H, s, Me-29), 1.11 (3H, s, Me-28),1.18 (3H, s, Me-19), 2.75 (1H, ddd, J 14.8, 14.5, 6.0 Hz, Hβ-2), 4.27 (1H, dd, 9.8, 5.6 Hz, Hβ-15), 4.64, 4.71 (2x1H, 2xbr s, H_Z-31, H_E-31), 5.37 (1H, d, J 6.0 Hz, H-11), 5.88 (1H, d, J 5.0 Hz, H-7); ^{13}C NMR (see **Table 1**). HREIMS : Found 452.3662; calculated for $C_{31}H_{48}O_2$ 452.3654.

DL-7 (**9**), a gum , $[\alpha]_D$ +10.7° (c, 0.29 in $CHCl_3$); UV λ_{max} : 250 (log ε 3.45) nm; IR ν_{max} : 3620, 3450, 3020, 2966, 2936, 2872, 1649, 1620, 1570, 1522, 1420, 1217, 1049, 930, 880 cm^{-1}; EIMS m/z (rel. int.) : 318 (M^+) (1), 300 (M^+-H_2O) (1),

289 (8), 282 (M^+-2xH_2O) (5), 271(22), 269 (10), 267 (2), 264 (1), 257 (10), 246 (31), 245 (8), 244 (6), 239 (2), 230 (9), 229 (30), 228 (8), 220 (2), 202 (3), 196 (6), 185 (21), 175 (7), 173 (6), 167 (7), 161 (9), 159 (11), 147 (25), 145 (10), 142 (69), 140 (4), 135 (21), 133 (14), 125 (13), 124 (100), 123 (5), 121 (12), 119 (4), 117 (5), 109 (7), 105 (20), 104 (100), 103 (90), 99 (15), 81 (8), 79 (9), 78 (100), 77 (12), 72 (3), 69 (12), 55 (16), 43 (44), 28 (50); ^{1}H NMR (see **Table 2**); ^{13}C NMR (see **Table 3**). HREIMS : Found 318.1425; calculated for $C_{18}H_{22}O_5$ 318.1467.

DL-8 (**10**), a gum, $[\alpha]_D$ +5.0° (c, 0.65 in $CHCl_3$); UV λ_{max} (log ε 4.10) : 253 nm; IR ν_{max} : 3620, 3547, 3020, 2978, 2860, 1736, 1655, 1603, 1570, 1522, 1475, 1425, 1335, 1211, 1047, 1030, 930, 875, 850 cm^{-1}; EIMS m/z (rel. int.) : 342 (M^+-H_2O) (4), 324 (M^+-2xH_2O) (2), 317 (18), 300 (7), 283 (4), 282 (15), 257 (16), 253 (2), 246 (9), 239 (6), 229 (9), 228 (90), 227 (4), 226 (7), 203 (7), 200 (4), 195 (5), 186 (9), 185 (98), 184 (7), 168 (7), 158 (6), 157 (16), 156 (5), 155 (7), 153 (5), 143 (25), 142 (7), 135 (5), 131 (8), 129 (14), 128 (16), 127 (9), 117 (18), 116 (5), 115 (11), 107 (12), 105 (21), 104 (100), 103 (8), 99 (27), 95 (9), 91 (18), 83 (15), 81 (9), 79 (9), 78 (7), 77 (12), 71 (11), 69 (17), 57 (8), 55 (16), 53 (6), 45 (6), 43 (70), 41 (15), 28 (25); ^{1}H NMR (see **Table 2**); ^{13}C NMR (see **Table 3**). HREIMS : Found [M^+-18] 342.1480; calculated for $C_{20}H_{22}O_5$ 342.1467.

DL-9 (**11**), a gum, $[\alpha]_D$ +6.5° (c, 0.20 in $CHCl_3$); UV λ_{max} : 253 (log ε 3.74) nm; IR ν_{max} : 3650, 3601, 3454, 3020, 2957, 2872, 1647, 1625, 1570, 1560, 1522, 1475, 1420, 1375, 1334, 1220, 1055, 1032.,1008, 964, 930 cm^{-1}; EIMS m/z (rel. int.) : 318 (1), 300 (M^+-H_2O) (3), 285 (M^+-H_2O-CH_3) (1), 282 (M^+-2xH_2O) (3), 267(1), 257 (30), 246 (10), 240 (30, 239 (3), 228 (40), 220 (4), 202 (4), 187 (30, 186 (3), 185 (12), 182 (6), 179 (8), 167 (6), 165 (60, 164 (16), 163 (8), 157 (5), 153 (5), 149 (10), 142 (37), 137 (8), 136 (6), 135 (11), 134 (5), 133 (9), 127 (9), 125 (8), 123 (16), 121 (12), 119 (8), 117 (8), 115 (8), 111 (9), 109 (17), 108 (9), 107 (17), 106 (7), 105 (29), 104 (100), 103 (12), 99 (20), 97 (10), 95 (20), 93 (16), 91 (22), 85 (10), 83 (15), 81

(22), 79 (19), 78 (13), 77 (20), 71 (30), 69 (22), 67 (18), 57 (17), 55 (35), 53 (14), 43 (90), 41 (45); ^{1}H NMR (see **Table 2**); ^{13}C NMR (see **Table 3**). HREIMS : Found 318.1490; calculated for $C_{18}H_{22}O_5$ 318.1467.

DL-11 (Oxocrebanine, **12**), orange-red needles ($CHCl_3$), mp 266-268°; UV λ_{max} (log ε) : 250 (4.32), 275, (4.20) 380 (3.68) nm; IR ν_{max} : 3020, 1665, 1603, 1579, 1520, 1468, 1423, 1335, 1215, 1043, 1022, 974, 927 cm^{-1}; EIMS m/z (rel. int.) : 335 (100), 320 (15), 307 (7), 306 (43), 305 (19), 404 (23), 292 (12), 276 (14), 265 (71), 250 (30), 235 (8), 222 (13), 221 (9), 187 (10), 185 (9), 182 (12), 178 (20), 176 (12), 167 (24), 166 (22), 165 (15), 164 (19), 163 (18), 161 (15), 159 (17), 152 (13), 151 (14), 149 (16), 139 (20), 137 (17), 123 (19), 111 (18), 109 (22), 107 (17), 105 (22), 104 (22), 95 (24), 91 (22), 77 (19); ^{1}H NMR : δ_H 7.64, 8.78 (2H, ABq, J 5.0 Hz, H-4, 5-H), 7.03 (1H, s, H-3), 7.17, 8.31 (2H, ABq, J 9.0 Hz, H-10, H-11), 6.29 (2H, s, CH_2O_2), 3.94 (3H, s, MeO-9), 4.00 (3H, s, MeO-8); ^{13}C NMR : δ_C 145.5 (C-1), 108.0 (C-1a), 121.7 (C-1b), 151.7 (C-2), 101.9 (C-3), 135.4 (C-3a), 123.7 (C-4), 144.1 (C-5), 144.6 (C-6a), 181.6 (C-7), 126.4 (C-7a), 153.3 (C-8)*, 150.8 (C-9)*, 117.0 (C-10), 123.8 (C-11), 128.6 (C-11a), 102.3 (CH_2O_2), 56.0 (OCH_3), 61.0 (OCH_3). HREIMS : Found 335.0793; calculated for $C_{19}H_{13}NO_5$ 335.0794.

* Assignments may be interchangable.

DL-12 (Buxifoline, **15**), crystals (MeOH); mp 292°; UV λ_{max} (log ε) : 238 (4.21), 280 (3.92) nm; IR ν_{max} : 3020, 2960, 2836, 1610, 1565, 1440, 1240, 1030, 980 cm^{-1}; EIMS m/z (rel. int.) : 325 (26), 324 (44), 323 (21), 310 (9), 296 (11), 295 (15), 294 (11), 293 (8), 280 (9), 278 (10), 266 (11), 265 (14), 264 (7), 208 (10), 207 (11), 180 (11), 178 (12), 165 (17), 164 (10), 163 (12), 153 (12), 152 (26), 151 (17), 149 (19), 111 (10), 89 (12), 83 (200), 81 (16), 77 (13), 71 (20), 57 (47), 56 (17), 55 (57), 44 (14), 43 (100), 28 (40); ^{1}H NMR : δ_H 7.94 (1H, d, J 8.5 Hz, H-11), 6.85 (1H, dd, J 8.5, 2.5 Hz, H-10), 6.80 (1H, d, J 2.5 Hz, H-8), 5.91, 6.05 (2H, ABq, J 1.5 Hz, CH_2O_2), 3.80 (3H, s, MeO-9), 3.99 (3H, s, MeO-3).

DL-13 (Oxobuxifoline, **16**) crystals ($CHCl_3$), mp 270°; UV λ_{max} (log ε) : 216 (4.18), 252 (4.33), 272 (4.39), 285 (4.52), 329 (3.60) nm; IR ν_{max} : 3020, 1650, 1600, 1580, 1460, 1220, 1030 cm^{-1}; EIMS m/z (rel. int.) : 335 (43), 334 (3), 320 (12), 307 (10), 306 (30), 305 (5), 304 (15), 292 (16), 291 (8), 290 (10), 276 (12), 264 (12), 263 (18), 234 (15), 206 (17), 205 (11), 178 (14), 177 (14), 176 (16), 164 (17), 163 (24), 162 (13), 151 (15), 150 (15), 149 (14), 111 (6), 110 (10), 99 (9), 98 (10), 88 (13), 77 (9), 75 (15), 69 (15), 57 (20), 55 (30), 43 (33), 41 (26), 32 (19), 29 (26), 28 (100); ^1H NMR : δ_H 8.78, 8.13 (2H, ABq, J 5.0 Hz, H-4, H-5), 8.40 (1H, d, J 8.9 Hz, H-11), 7.22 (1H, dd, J 8.9, 2.9 Hz, H-10), 7.88 (1H, d, J 2.9 Hz, H-8), 6.25 (2H, s, CH_2O_2), 3.91 (3H, s, MeO-9), 4.21 (3H, s, MeO-3).

DL-16 (3-hydroxy-2-Methoxy-9,10-methylenedioxytetrahydroprotoberberine, **19**), orange-red crystals (MeOH), mp >315°, $[\alpha]_D$ +34.4° (c, 0.032 in $CHCl_3$); UV λ_{max} (log ε) : 240 (4.37), 277 (3.85), 287 (4.08), 347 (3.73), 360 (4.23) nm; IR ν_{max} : 3546, 3020, 2934, 2812, 1604, 1520, 1460, 1394, 1356, 1334, 1260, 1215, 1136, 1107, 1055, 1030, 930, 875, 849, 790, 670 cm^{-1}; EIMS m/z (rel. int.) : 325 (36), 324 (20), 323 (16), 322 (21), 310 (3), 295 (2), 294 (2), 279 (4), 177 (20), 176 (16), 149 (23), 148 (100), 147 (10), 133 (6), 105 (6), 92 (7), 91 (22), 89 (13), 78 (6), 77 (8), 69 (6), 57 (8), 43 (16); ^1H NMR ($CDCl_3$) : δ_H 6.52-6.74 (4H, m), 5.91, 5.96 (2H, ABq, J 1.4 Hz, CH_2O_2), 3.88 (3H, s, MeO-2), 4.10, 3.52 (2H, ABq, J 15.3 Hz, H_A-8, H_B-8), 3.26 (1H, dd, J 16.0, 4.0 Hz, H_A-13), 2.81 (1H, dd, J 16.0 , 11.0 Hz, H_B-13), 2.61 (2H, m, 2H-5), 3.08 (2H, m, 2H-6), 3.58 (1H, dd, J 11.0, 4.0 Hz, H-14); ^1H NMR (C_6D_6) : δ_H 6.79 (1H, s, H-4), 6.66 (1H, d, J 8.0 Hz, H-11), 6.53 (1H, d, J 8.0 Hz, H-12), 6.44 (1H, s, H-1), 5.34, 5.41 (2H, ABq, J 1.4 Hz, CH_2O_2), 4.15, 3.48 (2H, ABq, J 15.2 Hz, H_A-8, H_B-8), 3.20 (3H, s, MeO-2), 3.50 (2H, m, H-14, H_B-5), 3.06 (2H, m, 2H-13), 2.95 (2H, m, 2H-6), 2.30 (1H, m, H_A-5); ^{13}C NMR (see **Table 6**). HREIMS : Found 325.1318; calculated for $C_{19}H_{19}NO_4$ 325.1315.

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CHAPTER 4

BENGALENSOL, A NEW 16-EPICAFESTOL DERIVATIVE, FROM THE LEAVES OF *COFFEA BENGALENSIS* ROXB. (RUBIACEAE).

INTRODUCTION

The genus *Coffea* (Rubiaceae) comprises 20 species of shrubs, which are distributed in the tropics[1]. The furanoid diterpenoid cafestol (**1**) is a constituent of coffee (*Coffea arabica*)[2,3] . Two related glycosides, mascaroside (**2**) and mozambioside (**3**) have been isolated from *C. vianneyi*[3] and *C. arabica*[4], and *C. pseudozanguebariae*[5] respectively. *Coffea bengalensis* Roxb. is a shrub with horizontal slender branches which grows widely in Bangladesh, Asam, Nepal and Java island[1]. The fruits of this plant have been previously investigated[6]. We have investigated the leaves of *C. bengalensis* and have isolated[7] a new cafestol type diterpenoid which we have named bengalensol. The evidence for the structure of bengalensol (**4**) is presented below.

RESULTS AND DISCUSSION

On chromatography over silica gel the ethanol extract of the leaves of *C. bengalensis* yielded one compound CB-1, bengalensol, which was subsequently identified as the new diterpenoid *ent*-18(4→19)-abeo-3,18:11α,16α-diepoxy-17-hydroxy-3,18-kauradien-2-one (**4**).

CB-1 (**4**) was isolated as a gum, $C_{20}H_{24}O_4$, $[\alpha]_D$ -286° (c, 0.103 in $CHCl_3$). Its IR spectrum exhibits 3586 (hydroxyl), 3450 (hydroxyl), 1672 (conjugated ketone) and 3020 and 1600 (aromatic) cm^{-1} absorption. Its mass spectrum displays an $[M^+]$ peak at m/z 328, together with fragments at m/z 310 $[M^+-18]$ and 239. The ^1H NMR spectrum (Fig. 8) of CB-1 shows resonances for a tertiary methyl group [δ_H 1.04 (d, J 1.0 Hz)], for protons attached to primary [δ_H 3.80 and 3.64 (ABq, J 11.6 Hz)] and secondary [δ_H 4.43 (t, J 3.4 Hz)] oxygenated carbons, and a disubstituted furan ring [δ_H 6.41 and 7.58 (both d, J 1.8 Hz)]. The ^{13}C NMR spectrum (Fig. 9) of CB-1 (**4**)[5,9] reveals a ketonic

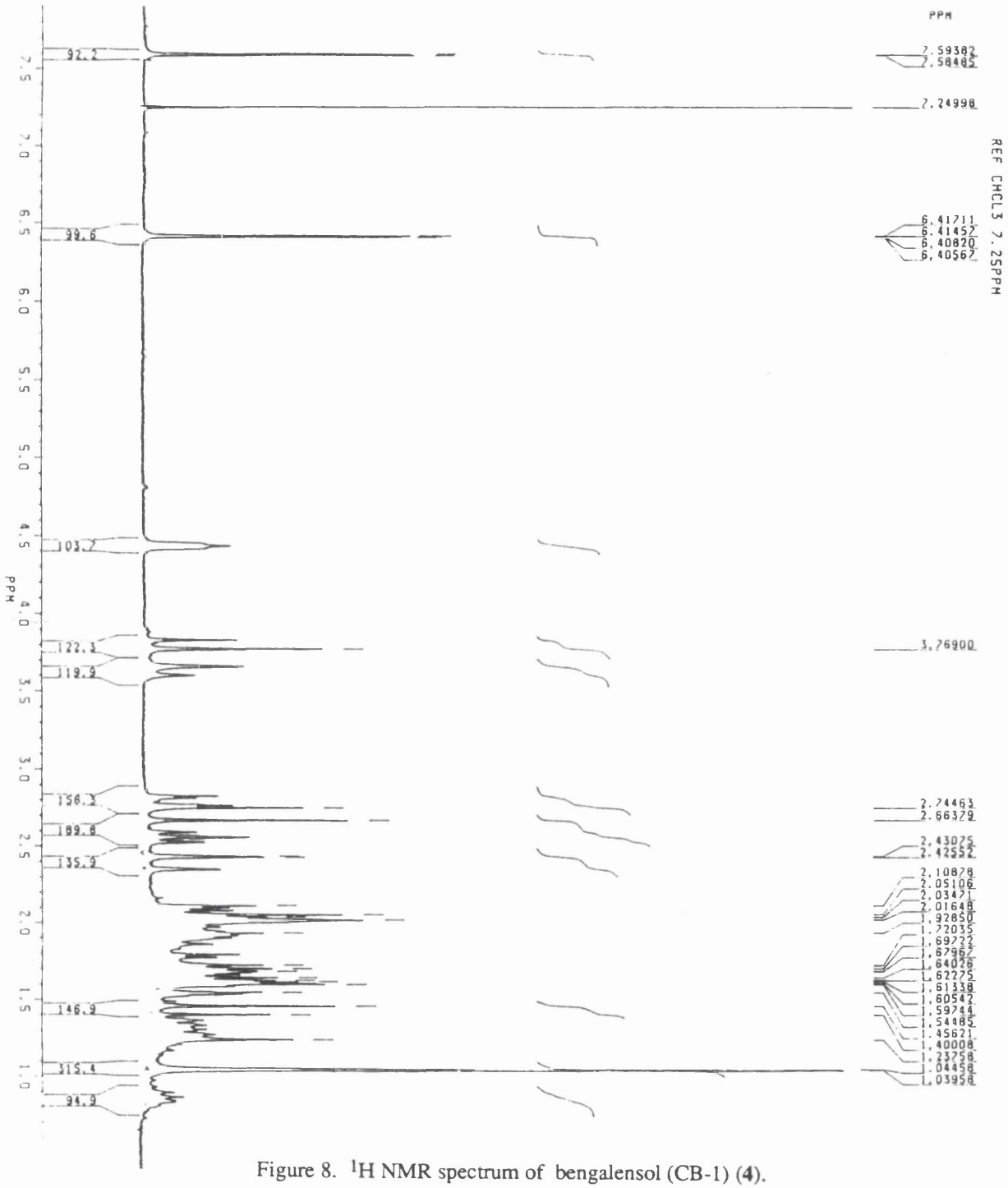
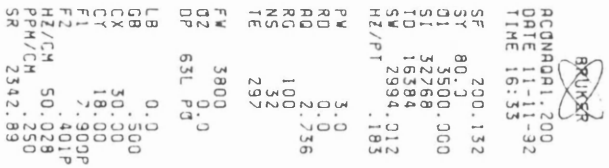


Figure 8. ^{1}H NMR spectrum of bengalensol (CB-1) (**4**).



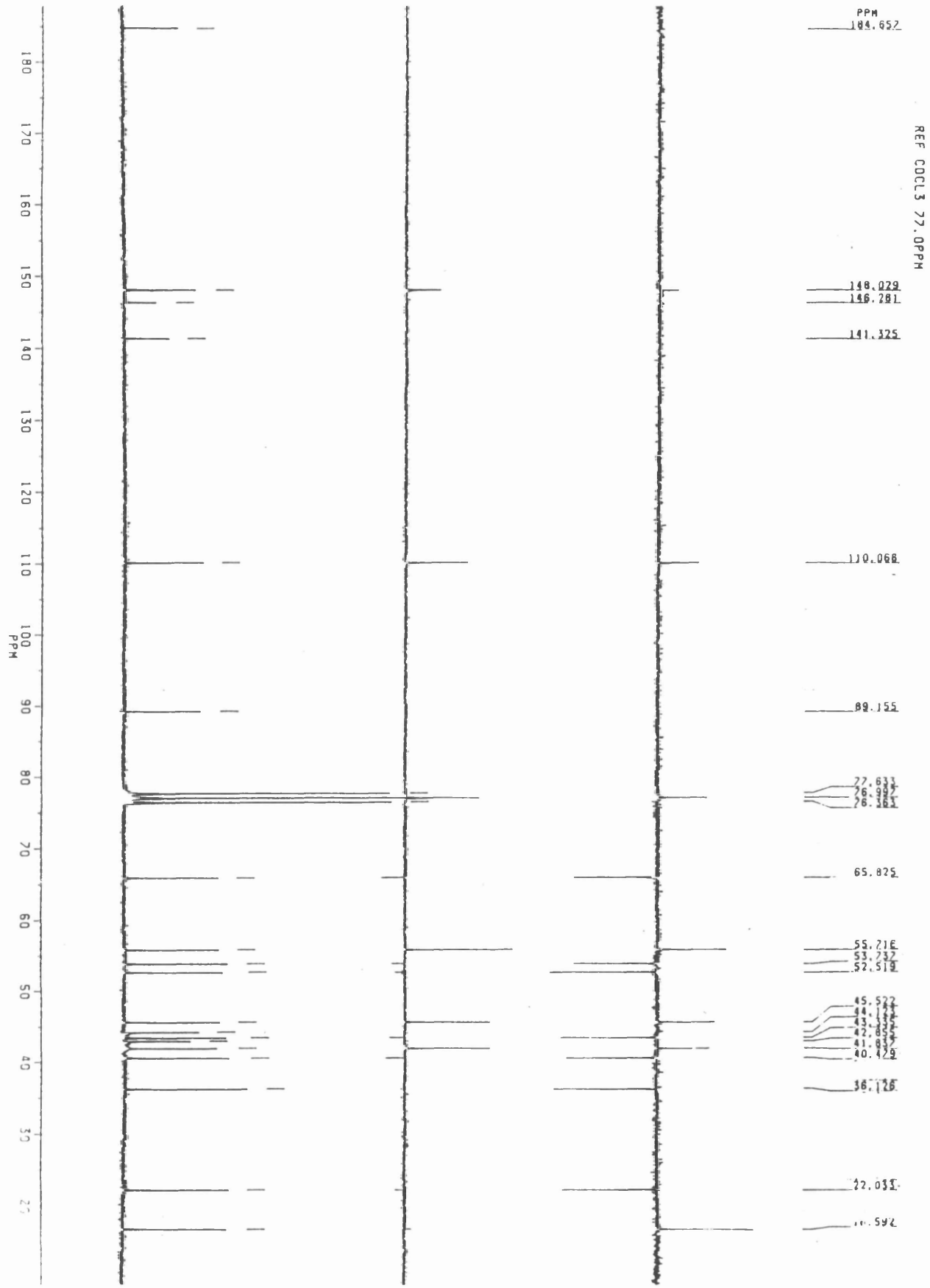
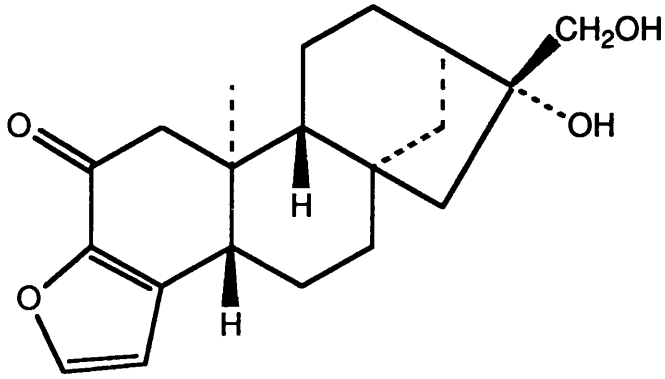
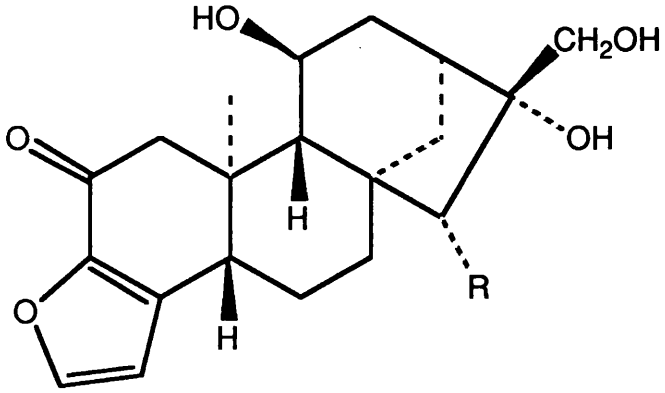


Figure 9. ^{13}C NMR spectrum of bengalensol (CB-1) (**4**).

carbonyl [δ_C 184.7] in conjugation with the disubstituted furan [δ_C 146.3 (s), 141.3 (s), 148.0 (d), 110.1 (d)], primary [δ_C 65.8], secondary [δ_C 77.0]and tertiary [δ_C 89.2] oxygenated carbons and a methyl carbon [δ_C 16.6], in addition to six methylenes [δ_C 53.7, 52.5, 43.3, 40.5, 36.1, 22.0], three methines [δ_C 55.7, 45.5, 41.8] and two quaternary carbons [δ_C 44.1, 42.9]. The molecule is therefore a pentacyclic diterpenoid, in addition to the furan . One of these rings must be a cyclic ether since there are only two oxygens to share among three oxygenated carbons.



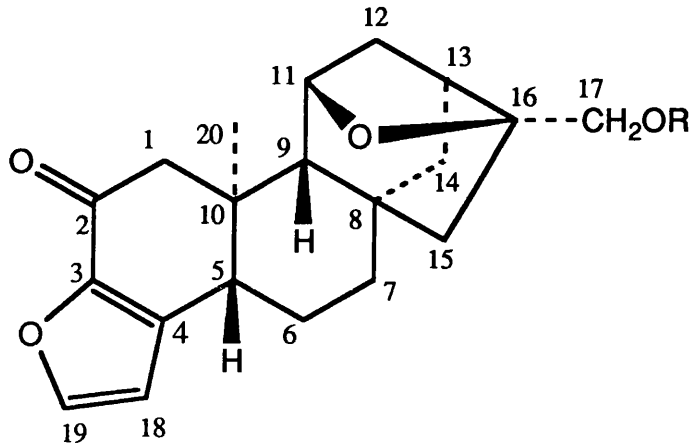
(1)



(2) R=OH; 17-O-β-D-glu

(3) R=H; 11-O-β-D-glu

Acetylation of bengalensol (**4**) afforded a monoacetate (**5**), $C_{22}H_{26}O_5$, [m/z 370.1757], as a gum. The IR spectrum of (**5**) reveals bands for conjugated ketone (ν_{max} 1687 cm-^{1}) and acetate (ν_{max} 1750 cm-^{1}) absorption but lacks hydroxyl absorption. The downfield shift of the AB system (δ_H 4.33 and 4.14, J 11.7 Hz) relative to (**4**) indicates that the acetate is primary. The chemical shift (δ_H 4.45) of the proton attached to the secondary oxygenated carbon remains virtually unchanged.



(**4**) R=H

(**5**) R=Ac

From the COSY (see Fig. 10) and HMQC (see Fig. 11) spectra of CB-1 it was possible to identify all the protons and the protonated carbons (see Experimental). The following spin systems were observed : 2H-1 (Jgem 16.0 Hz) with one of protons showing a long range coupling (J 1.0 Hz) with the methyl group 3H-20; H-5, 2H-6, 2H-7; H-9, H-11, H-12α , H-13, H-14R, H-14S; 2H-15 with the β proton coupled to H-14S. These data are consistent with a cafestol nucleus containing a 2-oxo group, an 11,16 ether and a 17-hydroxyl function and lead to the gross structure (**4**) for CB-1 (bengalensol). Confirmation of this structure and assignment of the non-protonated carbons were obtained from the HMBC spectrum . The principal correlations are listed in **Table 7**.

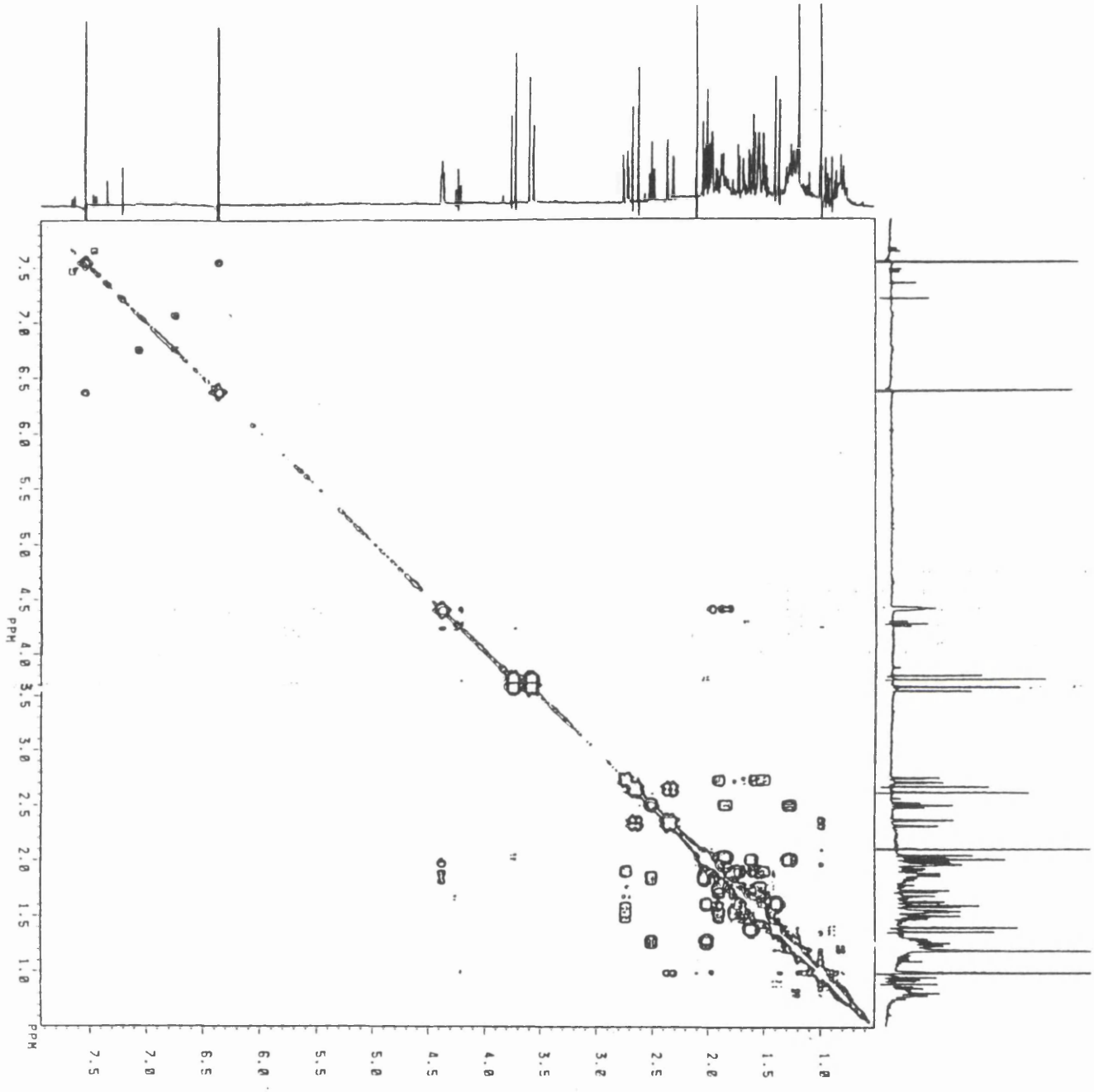


Figure 10. ^{1}H-^{1}H COSY spectrum of bengalensol (CB-1) (**4**).

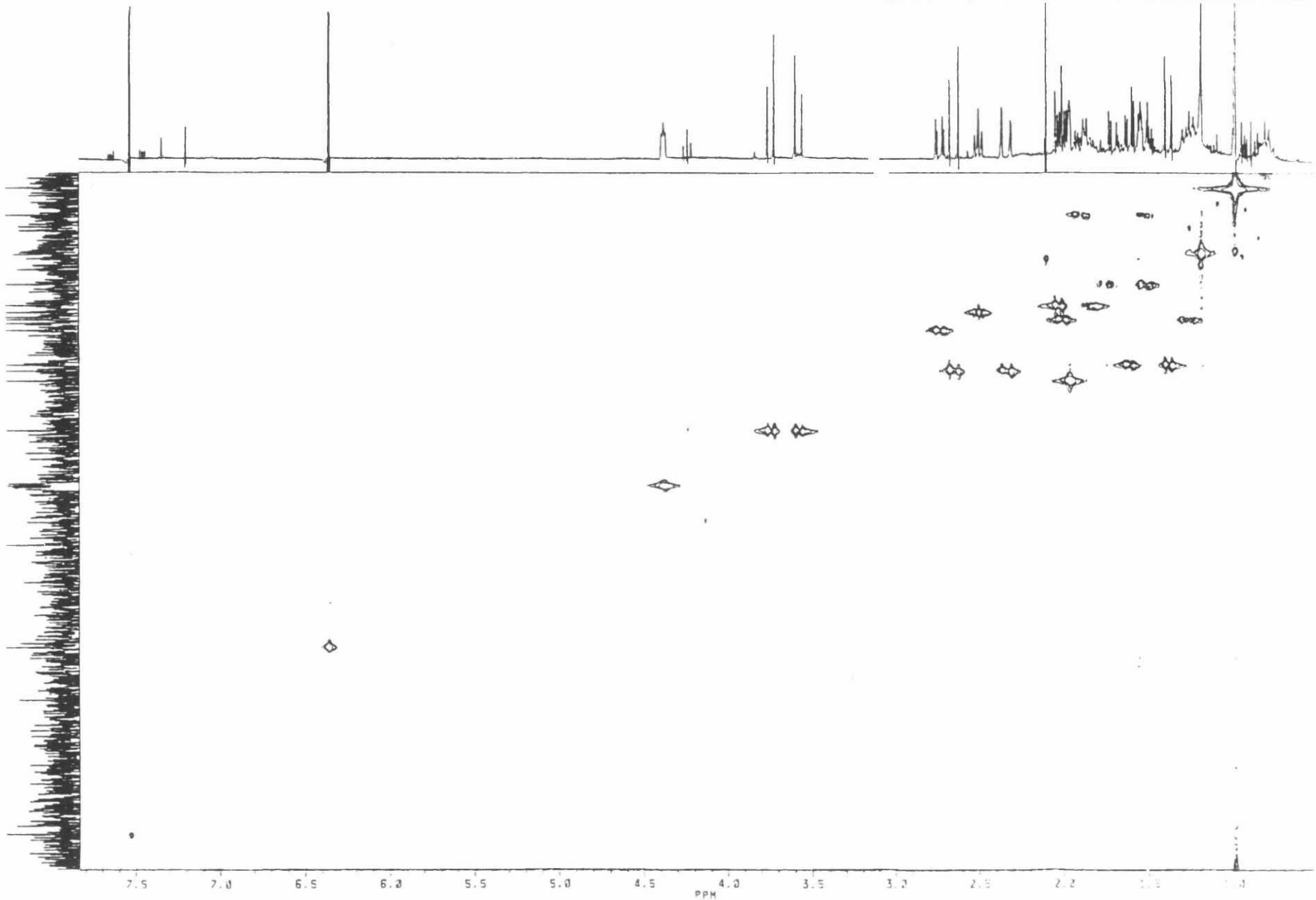


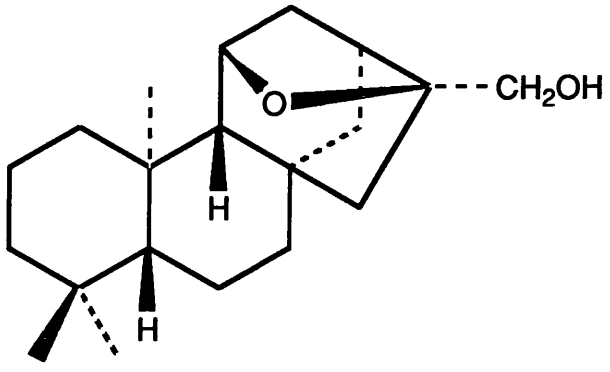
Figure 11. The HMQC spectrum of bengalensol (CB-1) (**4**).

TABLE 7. Long-range Correlations of Bengalensol (4)

Carbon	Long-range correlations
1	3H-20
2	2H-1
3	H-1α, H-18, H-19
4	H-18, H-19
5	H-1α, 3H-20
6	-
7	H-15β
8	H-13, H-15α, H-14R
9	H-1β
10	2H-1, H-9, 3H-20
11	H-13, H-12α, H-9
12	H-9, H-14R
13	2H-17, H-15β, H-14R
14	H-12β
15	H-17 (δ 3.64), H-14S
16	2H-17, H-12β, H-15β
17	H-13, H-15α
18	-
19	H-18
20	2H-1, H-9

Bengalensol has the same oxygenation pattern as the aglycone of mozambioside (**3**), but the presence of the ether requires an inversion of configuration at C-16. A good example of this type of ether is provided by *ent*-11α,16α-epoxy-17-kauranol (**6**), from *Rabdosia liangshanica*[10], whose comparable ^{1}H and ^{13}C shifts [δ_H 4.43 (t, J 3.5 Hz, H-11): δ_C 76.7 (C-11), 89.6 (C-16) and 65.6 (C-17)] are virtually identical with those of (**4**). Confirmation of the configuration at C-16 was obtained by NOE difference experiments. Thus separate irradiation of the primary alcohol 2H-17 protons afforded similar NOEs at H-13 (ca 6%) and at one H-15 (3%) [δ_H 1.38 (d, J 11.2 Hz)] which

must be H-15α since H-15β (δ_H 1.61) is a doublet of doublets (J 11.2, 3.5 Hz) with a W coupling to H-14S. Therefore the C-17 primary alcohol group must also be α ie the configuration at C-16 is the opposite of that observed in the known compounds (**1**), (**2**) and (**3**). The normal kaurane relative stereochemistry of (**4**) is supported by a large



(**6**)

NOE from the C-20 methyl group to H-14S. Irradiation of the methyl group also affords NOEs at H-1α (H-1β has quartet splitting due to the W coupling with the methyl group) and H-11α. The C-11 oxygen function is therefore β (*ent*-α). Irradiation of H-11α results in a large NOE (10%) at H-1α. The conformation of ring C is substantially chair-like since H-9 and H-11α have only a very small vicinal coupling. H-13 appears as a triplet (J 6.5 Hz) and couples only with H-14R and H-12α. The absolute configuration of bengalensol (**4**) has not been rigorously established but its large negative rotation is consistent with *ent* stereochemistry[6].

EXPERIMENTAL

Isolation. The leaves of *Coffea bengalensis* were collected at Savar and Kapashia in the Dhaka district of Bangladesh. Dried, powered leaves (500g) were extracted with EtOH in a Soxhlet extractor. The solvent was removed *in vacuo* and the

residue subjected to flash column chromatography over silica gel using increasing amounts of EtOAc in petroleum ether. Ten fractions (100 ml) were collected and monitored by analytical tlc. Early fractions contained only fat. Preparative tlc of combined fractions 3-5 using acetone : toluene : AcOH (10 : 9 : 0.5) afforded crude bengalensol (**4**) (30mg) which was further purified by preparative tlc using CH_2Cl_2 : MeOH (98 : 2).

The pure compound, *ent*-18(4→19)-abeo-3,18;11α,16α-diepoxy-17-hydroxy-3,18-kauradien-2-one (**4**), was obtained as a gum , $C_{20}H_{24}O_4$, $[\alpha]_D$ -286° (c, 0.103 in $CHCl_3$), IR ν_{max} : 3580, 3450, 3020, 1672, 1600 cm^{-1}; EIMS m/z (rel. int.) : 328 [M^+] (40), 310 [M^+-18] (10), 239 (16), 161 (36), 149 (40), 147 (27), 121 (23), 107 (20), 105 (30), 93 (22), 91 (50), 28 (100); ^1H NMR : δ_H 2.39 (1H, dq, J 16.0, 1.0 Hz, H-1β), 2.69 (1H, d, J 16.0 Hz, H-1α), 2.78 (1H, dd, J 12.2, 2.7 Hz, H-5), 1.90 (1H, m) and 1.50 (1H, m) (2H-6), 1.75 (1H, m) and 1.50 (1H, m) (2H-7), 1.95 (1H, br s, H-9), 4.43 (1H, t, J 3.4 Hz, H-11α), 2.02 (1H, d , J 11.6 Hz, H-12β), 1.83 (1H, m, H-12α), 2.55 (1H, t, J 6.5 Hz, H-13), 2.00 (1H, dd, J 12.0, 3.5 Hz, H-14S), 1.27 (1H, ddt, J 12.0, 6.5, 2.3 Hz, H-14R), 1.38 (1H, d, J11.2 Hz, H-15α), 1.61 (1H, dd, J 11.2, 3.5 Hz, H-15β), 3.80 and 3.64 (both 1H, d, J 11.6 Hz, 2H-17), 6.41 (1H, d, J 1.8 Hz, H-18), 7.58 (1H, d, J 1.8 Hz, H-19), 1.04 (3H, d, J 1.0 Hz, Me-20); ^{13}C NMR : δ_C 53.7 (C-1), 184.7 (C-2), 146.3 (C-3), 141.3 (C-4), 45.5 (C-5), 22.0 (C-6), 36.1 (C-7), 44.1 (C-8), 55.7 (C-9), 42.9 (C-10), 77.0 (C-11), 40.5 (C-12), 41.8 (C-13), 43.3 (C-14), 52.5 (C-15), 89.2 (C-16), 65.8 (C-17), 110.1 (C-18), 148.0 (C-19), 16.6 (C-20).

Acetylation of (**4**) (3mg) with Ac_2O in pyridine at room temperature overnight followed by the usual workup and purification by preparative TLC afforded the acetate (**5**) as a gum, IR (CCl_4) : 1750, 1687 cm^{-1}; EIMS m/z (rel. int.) : 370 (22), 310 (74), 295 (12), 282 (12), 239 (13), 161 (40), 147 (35), 121 (21), 105 (30), 91 (52), 77 (29), 43 (100); ^1H NMR : δ_H 2.38 (dq, J 16.1, 1.0 Hz, H-1β), 2.70 (d, J 16.1 Hz, H-1α),

2.79 (dd, J 12.1, 2.5 Hz, H-5), 4.45 (br t, H-11), 2.48 (t, J 6.5 Hz, H-13), 1.48 (d, J 11.2Hz, H-15α), 1.76 (dd, J 11.2, 3.5 Hz, H-15β), 4.14 and 4.33 (ABq, J 11.7 Hz, 2H-17), 6.41 (d, J 1.7 Hz, H-18), 7.59 (d, J 1.7 Hz, H-19), 1.04 (d, J 1.0 Hz, 3H-20), 2.10 (OAc). HREIMS : Found : m/z 370.1757; $C_{22}H_{26}O_5$ requires m/z 370.1780.

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CHAPTER 5

ALKALOIDS AND TERPENOIDS FROM THE STEM BARK OF *ARTABOTRYS ODORATISSIMUS* R. BR. (ANNONACEAE).

INTRODUCTION

The genus *Artabotrys* (Annonaceae) comprises over a hundred species of climbers and scandent shrubs, distributed in tropical Africa and Asia[1]. Different species of *Artabotrys* have been found to contain benzylisoquinoline, aporphine, oxoaporphine and protoberberine alkaloids. The species are *Artabotrys suaveolens*[2,3,4,5] *A. lastourvellensis*[6,7], *A. venustus*[8], *A. grandifolius*[9] , *A. maingayi*[10] and *A. uncinatus*[11]. *A. pierreanus* contains tannins[12]. Sesquiterpenoids have been isolated from *A. uncinatus*[13,14] and cyanogenic glucosides from *A. suaveolens*[13].

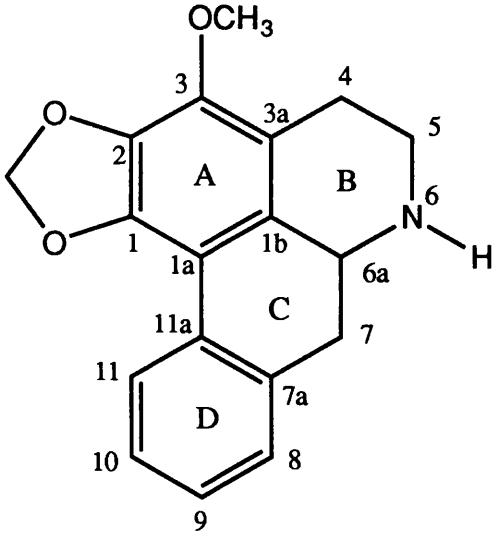
Artabotrys odoratissimus R. Br. is a large climbing ornamental shrub native to Eastern Asia and it is widely distributed in Bangladesh[15]. A decoction of the leaves is given as a remedy for cholera in the Malay penisula[15] and in the Indian subcontinent the flowers are used in Ayurvedic preparations for vomiting, biliousness, diseases of the blood and heart, itchiness, fevers, foul breath, leucoderma, headache, diseases of the bladder and erysipelas[16]. The fruits have been reported to contain fixed and volatile oil, cardiac glycosides and calcium and the extracts were found to exhibit hypotensive and spasmogenic effects[17]. A decoction of the leaves shows antifertility effects in rats[18]. Sterols and sterol glycosides were isolated from the fruit pulp[19] and a lanosterol derivative from the stem bark[20]. Constituents of the stem bark include sterols, aporphine, oxoaporphine and benzylisoquinoline alkaloids and a sesquiterpenoid[21,22].

RESULTS AND DISCUSSION

The ethyl acetate extract of the stem bark of *A. odoratissimus* yielded, on chromatography over silica gel, four compounds AO-1, AO-2, AO-3 and AO-4 which were subsequently identified as the known aporphine alkaloids norstephalagine (**1**),

annonaine (**2**), isopiline (**3**) and oxoaporphine alkaloid liriodenine (**4**). The methanol extract, on concentration and chromatography over silica gel, gave AO-5 and AO-6 which were identified as the known oxoaporphine alkaloid atherospermidine (**5**) and benzylisoquinoline alkaloid N-methylcoclaurine (**6**). The ethyl acetate extract also gave a sesquiterpene AO-7, which was identified as the known compound spathulenol (**7**).

AO-1 was obtained as a yellowish amorphous solid, mp 93-94°, $[\alpha]_D$ -33° (c, 0.90 in $CHCl_3$) [lit.[23] norstephalagine, mp 94-95°, $[\alpha]_D$ -35° (c, 0.98 in EtOH)]. It is UV active and gave a single Dragendorff positive spot on tlc (silica gel), indicating its alkaloidal nature. Its UV spectrum has λ_{max} 243, 278 nm. The IR spectrum exhibits absorption bands at 3020, 1602, 1520, 1420, 1062 and 930 cm^{-1}, indicative of its aromatic skeleton. The mass spectrum shows a molecular ion peak [M^+] at m/z 295 ($C_{18}H_{17}NO_3$) and intense fragment ions at m/z 294 [M^+-1], 280 [M^+-15], 265 [M^+-30] and 264 [M^+-31], consistent with an aporphine nucleus for AO-1.



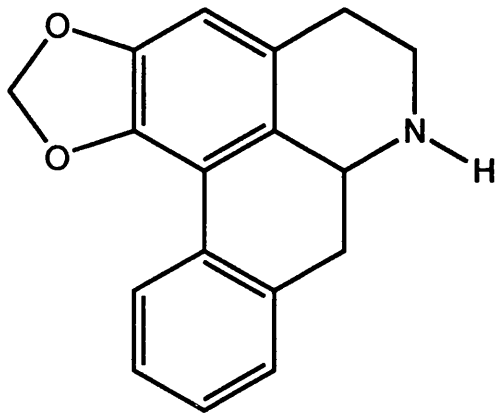
(**1**)

The ^1H NMR spectrum of AO-1 shows a broad one proton doublet at δ_H 8.01 (J 8.5 Hz) suggestive of proton H-11 of an aporphine nucleus, deshielded by an oxygen function at C-1, three other aromatic protons [δ_H 7.16-7.30 (m, H-8, H-9, H-10)], characteristic of the unsubstituted D ring of an aporphine, a methylenedioxy group [δ_H 5.91, 6.06 (ABq, J 1.5 Hz)], and a methoxyl group [δ_H 4.02 (s)], indicating the fully substituted nature of ring A. The ring junction proton H-6a is visible as a double doublet at δ_H 3.87 (J 13.5, 5.0 Hz) whereas the methylene protons are unresolved [δ_H 3.45 (1H, m), 2.70-3.60 (5H, m)]. The ^1H spectral data of AO-1 were compared with those of norstephalagine[23,24,25] (**1**) and were found to be in close agreement.

The ^{13}C NMR spectrum shows four aromatic methines [δ_C 128.0, 127.0, 126.8 and 126.4], a methylenedioxy group [δ_C 100.7 (t)], a methoxyl group [δ_C 59.5] without neighbouring protons, an aliphatic methine [δ_C 43.5 (d)], three methylene groups [δ_C 43.0, 37.0, 23.8] and eight aromatic singlets [δ_C 144.1, 140.4, 135.5, 135.1, 132.0, 129.0, 119.1, 110.7]. Comparison of these ^{13}C spectral data with those of norstephalagine[25] (**1**) confirmed this identity. Norstephalagine has been isolated previously from other Annonaceous plants[23,24,25]. This is the second report of its isolation from an *Artabotrys* species.

AO-2 was obtained as a colourless solid, mp 120-122°, $[\alpha]_D$ -54° (c, 0.20 in $CHCl_3$)[lit.[26] annonaine, mp 123-124°, $[\alpha]_D$ -56° (c, 0.15 in $CHCl_3$)]. It is UV active and gave a single Dragendorff positive spot on tlc (silica gel), indicating its alkaloidal nature. Its UV spectrum has λ_{max} 230, 270, 318 nm. Its IR spectrum exhibits absorption bands at 3020, 1600, 1580, 1550,1460, 940 cm^{-1} indicative of its aromatic skeleton. The EIMS reveals a molecular ion peak [M^+] at m/z 265 ($C_{17}H_{15}NO_2$) and fragment ions at m/z 264 [M^+-1], 250 [M^+-15], 236 [M^+-29] and 235 [M^+-30], consistent with an aporphine nucleus for AO-2.

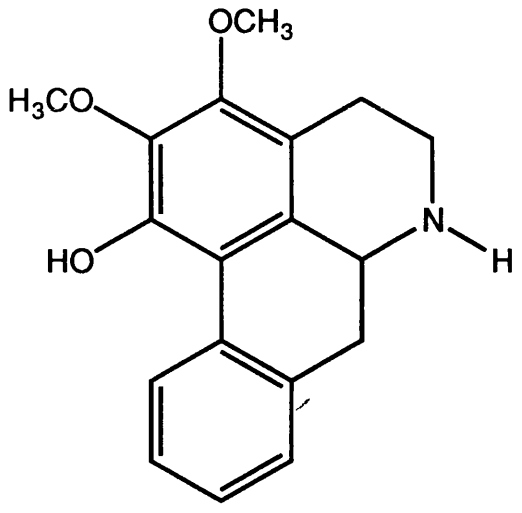
The ^1H NMR spectrum has the characteristic deshielded H-11 signal of an aporphine at δ_H 8.00 (br d, J 8.0 Hz). A sharp aromatic singlet at δ_H 6.56 (H-3), three unresolved aromatic protons at δ_H 7.12-7.30 (m, H-8, H-9, H-10), a methylenedioxy group at δ_H 5.93 and 6.07 (ABq, J 1.5 Hz) and seven unresolved aliphatic protons at δ_H



(2)

2.66-3.88 (m) readily revealed the substitution pattern of the aporphine nucleus. The ^1H spectral data of AO-2 were compared with those of annonaine[26,27,28] and were found to be identical . Thus AO-2 is annonaine (2), previously isolated from other Annonaceae species[27].

AO-3 was isolated as yellowish green solid, mp 150-152°, $[\alpha]_D$ -52.5° (c, 0.30 in $CHCl_3$) [lit.[29] iosopiline, mp 153°, $[\alpha]_D$ -55° (c, 1.06 in $CHCl_3$)]. It is UV active and gave a single Dragendorff positive spot on tlc (silica gel), indicating its alkaloidal nature. Its UV spectrum has λ_{max} 220, 269, 305 nm and its IR spectrum shows phenolic absorption (ν_{max} 3520, 3020, 1600, 1520, 1476, 1230 cm^{-1}). The EIMS reveals a molecular ion [M^+] peak at m/z 297 ($C_{18}H_{19}NO_3$) and intense fragment ions at m/z 296 [M^+-H], 282 [M^+-CH_3], 280 [M^+-OH], 266 [M^+-OCH_3] and 235 [M^+-$C_2H_6O_2$], again pointing to an aporphine nucleus.

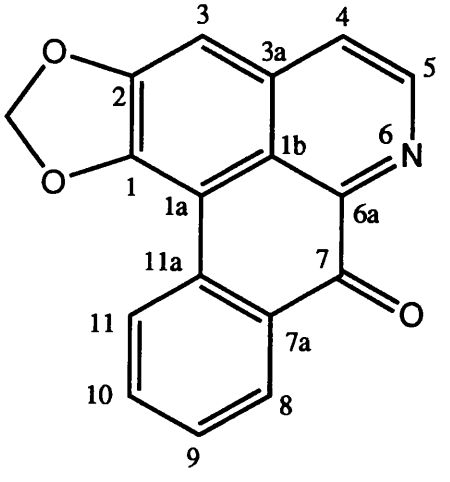


(3)

The ^1H NMR spectrum has a characteristic deshielded broad doublet at δ_H 8.30 (1H, J 8.0 Hz) suggestive of H-11, a three proton multiplet centered at δ_H 7.25 (H-8, H-9, H-10) and two methoxyls [δ_H 3.87 (s) and 3.94 (s)], in addition to the usual unresolved methylene/methine resonances [δ_H 2.65-3.95, 7H, m]. These data suggested structure (**3**), which is isopiline, and comparison with published data[29,30] confirmed the identity. Isopiline has been previously isolated from other Annonaceae species[29,30].

AO-4 was obtained as yellowish needles, mp 281-283° [lit.[31] liriodenine (**4**), mp 280-282°]. It is UV active and gave a single Dragendorff positive spot on tlc (silica gel), indicating its alkaloidal nature. Its UV spectrum has λ_{max} 248, 268, 305 nm and its IR spectrum shows carbonyl (1655 cm^{-1}) and aromatic (3020, 1601, 1525, 1465 cm^{-1}) absorption. The EIMS reveals an [M^++1] peak at m/z 276, a molecular ion peak [M^+] at m/z 275 ($C_{17}H_8NO_3$) and intense fragment ions at m/z 247 [M^+-CO], 219 [M^+-CO-CO] and 217[M^+-CO-CH_2O], consistent with an oxoaporphine system.

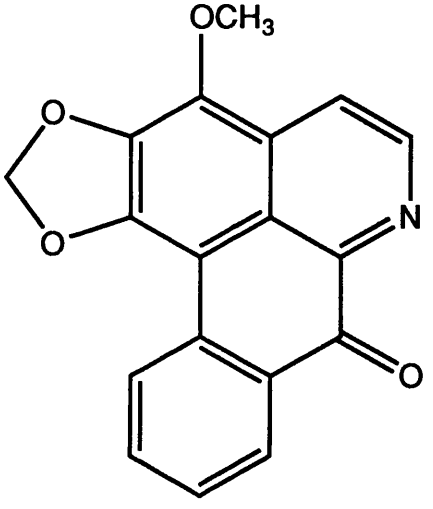
The ^1H NMR spectrum has a characteristic four spin system for the protons of ring D [δ_H 8.56, 8.42 (both dd, J 8.0, 0.8 Hz, H-11, H-8), 7.73 and 7.52 (both, dt, J 8.0, 1.5 Hz, H-9, H-10)], an aromatic singlet [δ_H 7.17 (H-3)], an AB system [δ_H 8.70, 7.80 (both d, J 5.0 Hz, H-4, H-5)] and a methylenedioxy group [δ_H 6.31 (2H, s)]. The chemical shifts and coupling constant of H-4 and H-5 are consistent with the presence of a pyridine ring while the deshielded value of H-8 requires a C-7 carbonyl group. Thus structure (**4**) is assigned to AO-4. This is the structure of liriodenine (**4**) and comparison with published data[31,32] confirmed the identity.



(**4**)

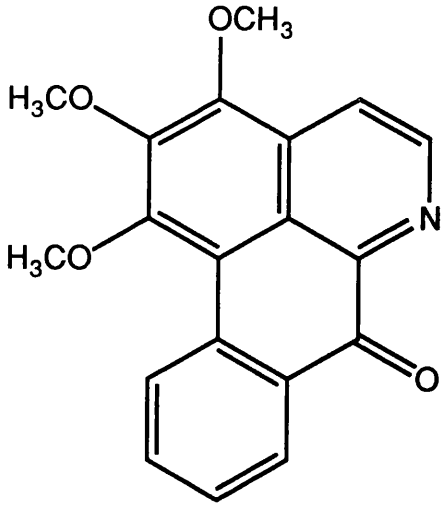
The ^{13}C NMR is in accord with structure (**4**) and has a carbonyl resonance [δ_C 183.3], seven aromatic methines [δ_C 144.5, 135.0, 129.0, 128.8, 128.1, 125.6, 103.6], a methylenedioxy group [δ_C 100.8] and eight aromatic singlets [δ_C 153.0, 151.7, 149.7, 145.0, 136.9, 133.7, 131.2, 123.7]. The assignments are given in the Experimental. Our chemical shift values ($CDCl_3$-CD_3OD) differ slightly from the published data[33] for liriodenine (TFA) because of different solvents.

AO-5 was obtained as a yellowish needles, mp 282-284° [lit.[34] atherospermidine, mp 283-285°]. Again it is UV active and gave a single Dragendorff positive spot on tlc (silica gel), indicating its alkaloidal nature. Its UV spectrum has λ_{max} 247, 279, 310 nm. Its IR spectrum exhibits bands at 1650 (conjugated ketone) and 3025, 1602, 1518 (aromatic) cm^{-1}. The mass spectrum shows an $[M^+ + 1]$ peak at m/z 306, a molecular ion peak at m/z 305 ($C_{18}H_{11}NO_4$) and intense fragment ions at m/z 290 $[M^+ - CH_3]$, 277 $[M^+ - CO]$, 275 $[M^+ - CH_2O]$, 274 $[M^+ - OCH_3]$ and 262 $[M^+ - CH_3 - CO]$, again consistent with an oxoaporphine.



(**5**)

In agreement with this the ^1H NMR reveals pyridine protons [δ_H 8.09 and 8.87 (ABq, J 5.4 Hz, H-4, H-5)], a characteristic ring D four spin system with H-8 and H-11 deshielded [δ_H 8.46 (1H, dd, J 8.0, 0.9 Hz, H-11), 8.52 (1H, dd, J 8.0, 0.9 Hz, H-8), 7.65 (1H, dt, J 7.6, 1.5 Hz, H-9), 7.45 (1H, dt, J 7.6, 1.5 Hz, H-10)], a methylenedioxy group [δ_H 6.28 (s)] and a methoxyl group [δ_H 4.27 (s)]. It is clear from these data that AO-5 is closely related to AO-4 (**4**) but has ring A fully substituted. The ^1H NMR data ($CDCl_3$) are consistent with structure (**5**) which is atherospermidine but differ slightly from published values[35,36] (TFA) because of solvent effects.

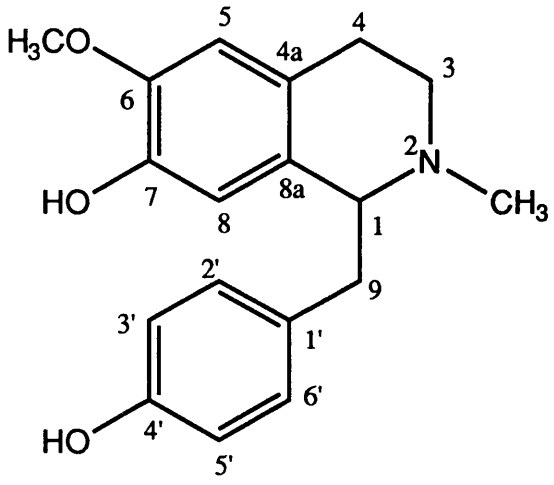


(11)

As expected the ^{13}C NMR is consistent with structure (**5**). Thus it has a conjugated carbonyl [δ_C 182.4], six aromatic methines [δ_C 143.2, 134.0, 128.0, 127.3, 126.3, 119.6], a methylenedioxy [δ_C 102.3], a methoxyl [δ_C 59.7] and nine aromatic singlets [δ_C 150.0, 143.8, 136.5, 135.8, 132.9, 130.5, 129.6, 127.4, 122.3]. The ^{13}C NMR data of atherospermidine have not been reported previously. The assignments (Experimental) are based on the comparison of the ^{13}C NMR data with those of liridine (**11**)[37] (for B, C and D ring carbons) and of norstephalagine (**1**)[25] (for A ring carbons).

AO-6 was obtained as white needles mp 182-184°, $[\alpha]_D$ -93° (c, 1.00 in $CHCl_3$) [lit.[38] N-methylcoclaurine (**6**), mp 181-183°, $[\alpha]_D$ -94.7° (c, 11.8 in $CHCl_3$)]. It is UV active and gave a single Dragendorff positive spot on tlc (silica gel), indicating its alkaloidal nature. Its UV spectrum has λ_{max} 225, 281 nm and the IR spectrum has phenolic absorption [ν_{max} 3680, 3595, 3020, 1600, 1520, 1210 cm^{-1}]. The EIMS shows a molecular ion [M^+] peak at m/z 299 ($C_{18}H_{21}NO_3$), an [M^+-1] peak at m/z 298, a base peak at m/z 192, corresponding to an N-methyl isoquinolium cation with methoxyl

and hydroxyl substituents and arising by the loss of a hydroxybenzyl fragment (C_7H_7O), and a prominent peak at m/z 177 [base peak-15] . The fragmentation pattern is consistent with that of a benzylisoquinoline alkaloid.

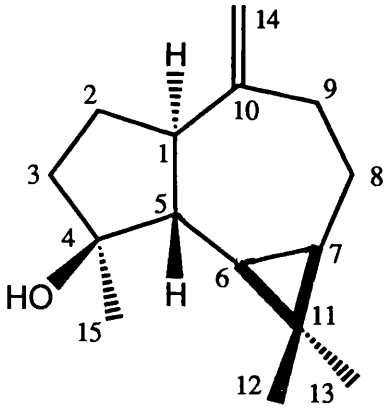


(6)

The ^1H NMR spectrum shows an AA'BB' system [δ_H 6.95 and 6.60 ($J_{AB}+J_{AB'}$ 8.5 Hz), two aromatic singlets [δ_H 6.53, 6.36], a methoxyl [δ_H 3.83 (s)], an N-methyl [δ_H 2.46 (s)], a deshielded methine [δ_H 3.74 (t, J 6.1 Hz)] and six methylene protons [δ_H 2.51-3.35 (m)]. The above data are consistent with a coclaurine (**6**) nucleus. To assign some of the ^1H resonances and to ascertain the substitution pattern NOE difference experiments were carried out. Irradiation of the aromatic singlet at δ_H 6.53 (H-5) affords a strong NOE (10%) at the methoxyl group (δ_H 3.87) while irradiation of the other aromatic singlet at δ_H 6.36 (H-8) affords a NOE (6%) at the H-1 methine at δ_H 3.74. The reverse experiments gave similar results. These results permit the assignment of H-5 (δ_H 6.53) and H-8 (δ_H 6.36) and the position of the methoxyl group at C-6. These data lead to structure (**6**) for AO-6. Comparison with published data for N-methylcoclaurine[38,39] confirmed the identity.

The ^{13}C NMR of AO-6 has resonances for the methines of a p-disubstituted benzene [δ_C 130.5 (C-2', C-6'), 115.2 (C-3', C-5')], two other aromatic methines [δ_C 114.3 (C-5), 111.0 (C-8)], a methoxyl [δ_C 55.6], an N-methyl [δ_C 40.8], a methine [δ_C 65.5 (C-1)] and three methylenes [δ_C 45.4, 39.8 and 23.3 (C-3, C-9, C-4)], in addition to six aromatic singlets [δ_C 155.3, 146.5, 143.7, 128.4, 126.1 and 122.3(C-6, C-7, C-4', C-8a, C-1', C-4a)]. Comparison with the reported ^{13}C NMR data of coclaurine[40] for the coclaurine nucleus and the N-methyl carbon of annonelliptine[41] for the N-methyl group confirmed the identity of AO-6 as N-methylcoclaurine (**6**).

AO-7 was obtained as oil $[\alpha]_D$ +54° (c, 1.00 in $CHCl_3$) [lit.[42] spathulenol, $[\alpha]_D$ +56° in $CHCl_3$]. The IR spectrum shows hydroxyl (ν_{max} 3605 cm^{-1}) and exomethylene (ν_{max} 1635, 895 cm^{-1}) absorption. The EIMS reveals a molecular ion peak [M^+] at m/z 220 ($C_{15}H_{24}O$) and a fragment peak at m/z 177 [M^+-C_3H_7]. Thus AO-7 is a tricyclic sesquiterpenoid alcohol. The ^1H NMR spectrum shows a two proton



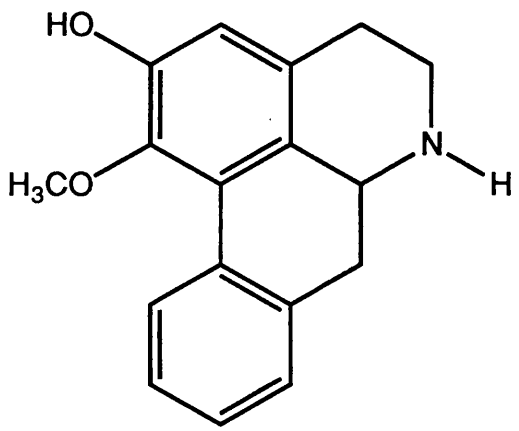
(**7**)

multiplet at δ_H 4.67 indicative of an exomethylene group, three methyl groups [δ_H 1.27 (Me-15), 1.04 and 1.02 (Me-13 and Me-12)], one (Me-15) of which is attached to a carbon bearing a hydroxyl group, and two cyclopropyl protons [δ_H 0.62 (m, H-6, H-7)].

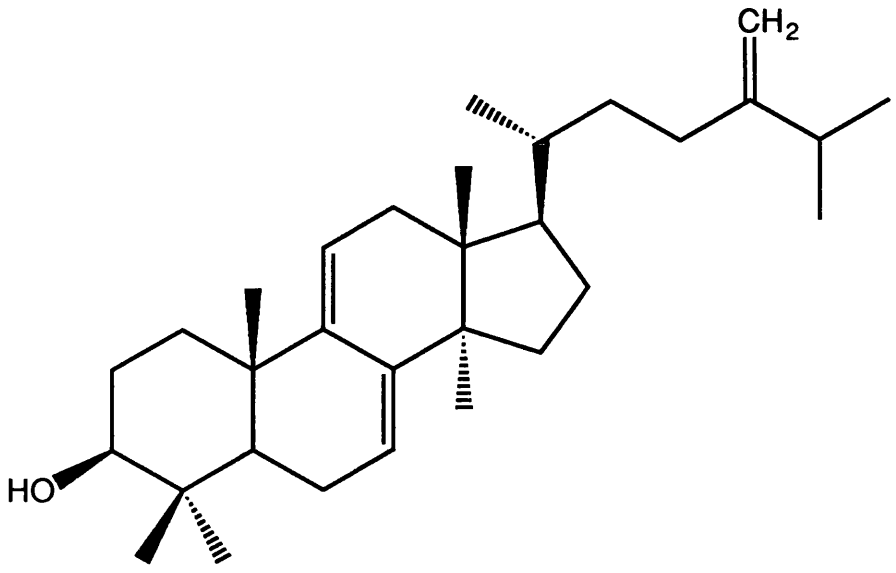
An aromadendrane nucleus seemed a likely possibility for AO-7. Comparison of the ^{1}H NMR data with those of spathulenol (**7**)[42,43,44] established the identity.

The ^{13}C NMR spectrum has the expected resonances for structure (**7**). Thus there are signals for an exomethylene [(δ_C 106.2 (t) (C-14), 153.4 (s) (C-10)], three methyl groups [δ_C 28.7 (C-12), 26.1 (C-15), 16.7 (C-13)], a tertiary carbinol [δ_C 81.0 (C-4)], a cyclopropane ring [δ_C 29.9 (C-6), 27.5 (C-7), 20.3 (C-11)] , four methylenes [δC 41.7, 38.8, 26.7, 24.8] and two methines [δ_C 54.3, 53.4]. The ^{13}C NMR shifts of AO-7 are identical with those published for spathulenol[45] (**7**).

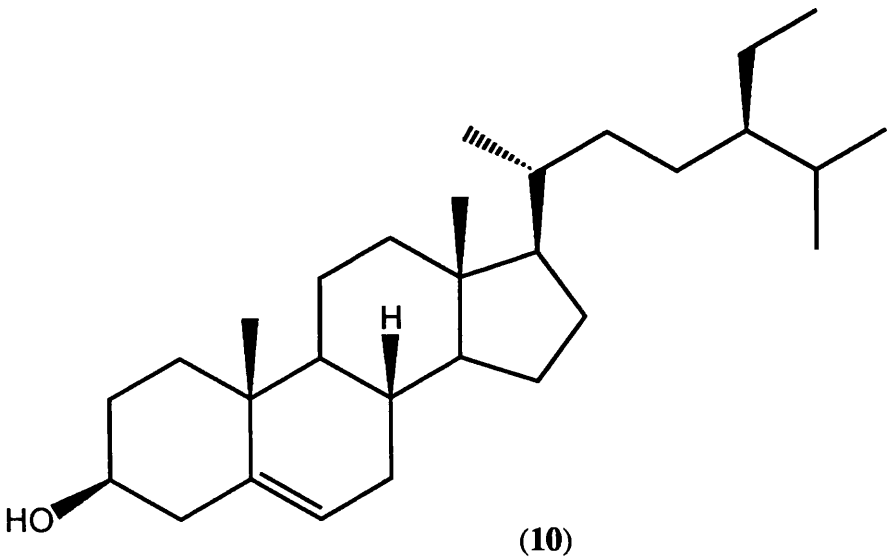
In addition to the above compounds we have also isolated the alkaloid asimilobine (**8**), the triterpenoid 24-methylene lanosta-7,9(11)-dien-3β-ol (**9**) and β-sitosterol (**10**), previously reported from this plant[19,20,21].



(**8**)



(**9**)



(**10**)

EXPERIMENTAL

Isolation. The plant material of *Artabotrys odoratissimus* R. Br. was collected from the Dhaka University campus, Dhaka, Bangladesh. The ground stem bark (800g) was extracted in a Soxhlet apparatus successively with EtOAc and MeOH. The EtOAc extract (6g) was concentrated *in vacuo* and was subjected to flash column chromatography over silica gel. The column was eluted first with petroleum ether and then increasing amounts of EtOAc in petroleum ether and MeOH in EtOAc collecting 50 ml fractions. Fractions were monitored by analytical tlc. The early fractions contained mainly fat. The later fractions showed many spots on analytical tlc. Multiple preparative tlc using CH_2Cl_2 : MeOH (97 : 3), (95 : 5) and (92 : 8) afforded the compounds norstephalagine, AO-1(**1**) (12.0mg), annonaine, AO-2 (**2**) (3.0mg), isopiline, AO-3 (**3**) (3.0mg), liriodenine, AO-4 (**4**) (20mg) and asimilobine (**8**) (40 mg). Preparative tlc of fraction 18 using petroleum ether : EtOAc (92 : 8) afforded AO-7, spathulenol (**7**) (16.4mg) and 24-methylenelanosta-7,9 (11)- dien-3β-ol (**9**) (20mg) and fraction 15 afforded β-sitosterol (**10**) (17mg).

The MeOH extract was concentrated *in vacuo* and subjected to flash column chromatography over silica gel. The column was eluted with EtOAc then increasing amounts of MeOH in EtOAc and finally with MeOH. Multiple preparative tlc using $CHCl_3$: MeOH (90 : 10) and (88 : 12) afforded atherospermidine, AO-5 (**5**) (14.5mg) and N-methylcoclaurine, AO-6 (**6**) (20mg).

AO-1 (Norstephalagine, **1**) , yellow amorphous solid (ether), mp 93-94°; $[\alpha]_D$ -33° (c, 0.90 in $CHCl_3$); UV λ_{max} (log ε) : 243 (4.33), 278 (4.46) nm; IR ν_{max} : 3020, 2401, 1602, 1520, 1420, 1224, 1062, 930, 850, 736, 625 cm^{-1}; EIMS m/z (rel. int.) : 295 (31), 294 (53), 280 (8), 266 (17), 265 (24), 264 (30), 236 (9), 206 (12), 177 (13), 165 (18), 43 (77), 18 (100); ^1H NMR : δ_H 8.01 (1H, br d, J 8.5 Hz, H-11), 7.16-7.30

(3H, m, H-8, H-9, H-10), 5.91, 6.06 (2H, ABq, J 1.5 Hz, CH_2O_2), 4.02 (3H, s, CH_3O-3), 3.87 (1H, m, H-6a), 3.45 (1H, m, H-5_a), 2.70-3.60 (5H, m, 2H-4, 2H-7, H-5_b); ^{13}C NMR : δ_C 144.1 (C-1), 110.7 (C-1a), 129.0 (C-1b), 135.1 (C-2), 140.4 (C-3), 119.1 (C-3a), 23.8 (C-4), 43.0 (C-5), 53.5 (C-6a), 37.0 (C-7), 135.5 (C-7a), 128.0 (C-8), 127.0 (C-9), 126.8 (C-10), 126.4 (C-11), 132.0 (C-11a), 100.7 (CH_2O_2), 59.5 (CH_3O-3).

AO-2 (Annonaine, **2**) , colourless solid (acetone-hexane), mp 120-122°; $[\alpha]_D$ -54° (c, 0.20 in $CHCl_3$); UV λ_{max} (log ε) : 230 (3.89), 270 (4.15), 318 (3.75) nm; IR ν_{max} : 3020, 1600, 1580, 1550, 1460, 1045, 940, 800 cm^{-1}; EIMS m/z (rel. int.) : 265 (11), 264 (14), 250 (8), 236 (12), 235 (7), 132 (5), 77 (9), 28 (100); ^1H NMR : δ_H 8.00 (1H, br d, J 8.0 Hz, H-11), 7.12-7.30 (3H, m, H-8, H-9, H-10), 6.56 (1H, s, H-3), 5.93, 6.07 (2H, ABq, J 1.5 Hz, CH_2O_2), 2.66-3.88 (7H, m, 2H-4, 2H-5, H-6a, 2H-7).

AO-3 (Isopiline, **3**), yellowish green solid (ether), mp 150-151°; $[\alpha]_D$ -52.5° (c, 0.30 in $CHCl_3$); UV λ_{max} (log ε) : 220 (4.20), 269 (4.02), 305 (3.56) nm; IR ν_{max} : 3520, 3020, 2401, 1600, 1520, 1476, 1230, 930 cm^{-1}; EIMS m/z (rel. int.) : 297 (46), 296 (55), 282 (17), 280 (28), 268 (14), 266 (16), 235 (12), 177 (12), 165 (15), 151 (11), 149 (25), 43 (100); ^1H NMR : δ_H 8.30 (1H, br d, J 8.0 Hz, H-11), 7.25 (3H, m, H-8, H-9, H-10), 3.94 (3H, s, CH_3O-3), 3.87 (3H, s, CH_3O-2), 2.65-3.95 (7H, m, 2H-4, 2H-5, H-6a, 2H-7).

AO-4 (Liriodenine, **4**), yellowish needles ($CHCl_3$), mp 281-283°; UV λ_{max} (log ε) : 248 (3.83), 268 (4.12), 305 (3.67) nm; IR ν_{max} : 3020, 2395, 1655, 1601, 1525, 1465, 1050, 965, 615 cm^{-1}; EIMS m/z (rel. int.) : 276 (11), 275 (100), 248 (4), 247 (24), 246 (15), 219 (15), 217 (8), 191 (17), 190 (16), 189 (24), 188 (35), 163 (16), 162 (25); ^1H NMR : δ_H 8.70, 7.80 (each 1H, ABq, J 5.0 Hz, H-4, H-5), 7.73, 7.52 (each 1H, dt, J 8.0, 1.5 Hz, H-9. H-10), 8.56, 8.42 (each 1H, dd, J 8.0, 0.8 Hz, H-8,

H-11), 7.17 (1H, s, H-3), 6.31 (2H, s, CH_2O_2); ^{13}C NMR ($CDCl_3/CD_3OD$) : δ_C 151.7 (C-1), 123.7 (C-1a), 136.9 (C-1b) 153.0 (C-2), 103.6 (C-3), 145.0 (C-3a) 125.6 (C-4), 144.5 (C-5), 149.7 (C-6a), 183.3 (C-7), 131.2 (C-7a), 128.8 (C-8), 129.0 (C-9), 135.0 (C-10), 128.1 (C-11), 133.7 (C-11a), 100.8 (CH_2O_2). HREIMS : Found 275.0545; calculated for $C_{17}H_9NO_3$ 275.0582.

AO-5 (Atherospermidine, **5**) , yellowish needles ($CHCl_3$), mp 282-284°; UV λ_{max} (log ε): 247 (4.43), 279 (4.60), 310 (3.82) nm; IR ν_{max} : 3025, 2380, 1650, 1602, 1518, 1223, 923, 849, 767 cm^{-1}; EIMS m/z (rel. int.) : 306 (23), 305 (86), 290 (25), 277 (10), 276 (26), 275 (100), 274 (23), 266 (16), 262 (13), 234 (12), 206 (11), 204 (9), 177 (5), 176 (17), 175 (5), 149 (22); ^1H NMR : δ_H 8.87, 8.09 (each 1H, ABq, J 5.4 Hz, H-4, H-5), 7.65, 7.45 (each 1H, dt, J 7.6, 1.5 Hz, H-9, H-10), 8.52, 8.46 (each 1H, dd, J 8.0, 0.9 Hz, H-8, H-11), 6.28 (2H, s, CH_2O_2), 4.27 (3H, s, CH_3O-3); ^{13}C NMR ($CDCl_3/CD_3OD$) : δ_C 143.8 (C-1), 122.3 (C-1a), 127.4 (C-1b), 135.8 (C-2), 136.5 (C-3), 129.6 (C-3a), 119.6 (C-4), 143.2 (C-5), 150.0 (C-6a), 182.4 (C-7), 130.5 (C-7a), 127.3 (C-8), 126.3 (C-9), 134.0 (C-10), 128.0 (C-11), 132.9 (C-11a), 102.3 (CH_2O_2), 59.7 (CH_3O-3); HREIMS : Found 305.0672; calculated for $C_{18}H_{11}NO_4$ 305.0688.

AO-6 (N-methylcoclaurine, **6**) , white needles ($CHCl_3$), mp 182-184°; $[\alpha]_D$ -93° (c, 1.00 in $CHCl_3$); UV λ_{max} (log ε) : 225 (4.25), 281 (3.83) nm; IR ν_{max} : 3680, 3595, 3020, 2920, 2850, 2390, 1600, 1520, 1210, 930 cm^{-1}; EIMS m/z (rel. int.) : 299 (1), 298 (1), 192 (100), 178 (8), 177 (34), 144 (9), 107 (8), 91 (4); ^1H NMR : δ_H 6.95, 6.60 (each 2H, AA'BB', $J_{AB}+J_{AB'}$ 8.5 Hz, H-3', H-5', H-2', H-6'), 6.53 (1H, s, H-5), 6.36 (1H, s, H-8), 3.83 (3H, s, CH_3O-6), 2.46 (3H, s, N-CH_3), 3.74 (1H, t, J 6.1 Hz, H-1), 2.51-3.35 (6H, m, 2H-2, 2H-3, 2H-9); ^{13}C NMR : δ_C 65.5 (C-1), 45.4 (C-3), 23.3 (C-4), 122.3 (C-4a), 114.3 (C-5), 155.3 (C-6), 146.5 (C-7), 110.8 (C-8), 128.4 (C-8a) 39.8 (C-9), 126.1 (C-1'), 130.5 (C-2', 6'), 143.7 (C-4'), 115.2 (C-3', 5'), 55.6 (CH_3O-6), 40.8 (N-CH_3).

AO-7 (Spathulenol, **7**) , oil, $[\alpha]_D$ +54° (c, 1.0 in $CHCl_3$); IR ν_{max} : 3605, 3080, 2980-2860, 1635, 1450, 1375, 1095, 915, 895 cm^{-1}; EIMS m/z (rel. int.) : 220 (3), 205 (2), 177 (3), 124 (22), 95 (28), 81 (30), 69 (38), 57 (51), 55 (72), 43 (100), 41 (56); ^1H NMR : δ_H 4.67 (2H, m, 2H-14), 1.27 (3H, s, CH_3-15), 1.04 (3H, s, CH_3-13), 1.02 (3H, s, CH_3-12), 0.62 (2H, m, H-6, H-7), 0.72-2.45 (11H, m, H-1, 2H-2, 2H-3, H-5, 2H-8, 2H-9, OH); ^{13}C NMR : δ_C 53.4 (C-1), 26.7 (C-2), 41.7 (C-3), 81.0 (C-4), 54.3 (C-5), 29.9 (C-6), 27.5 (C-7), 24.8 (C-8), 38.8 (C-9), 153.4 (C-10), 20.3 (C-11), 28.7 (C-12), 16.7 (C-13), 106.2 (C-14), 26.1 (C-15).

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CHAPTER 6

CONSTITUENTS OF THE STEM BARK AND LEAVES OF *POLYALTHIA BULLATA* BL. (ANNONACEAE).

INTRODUCTION

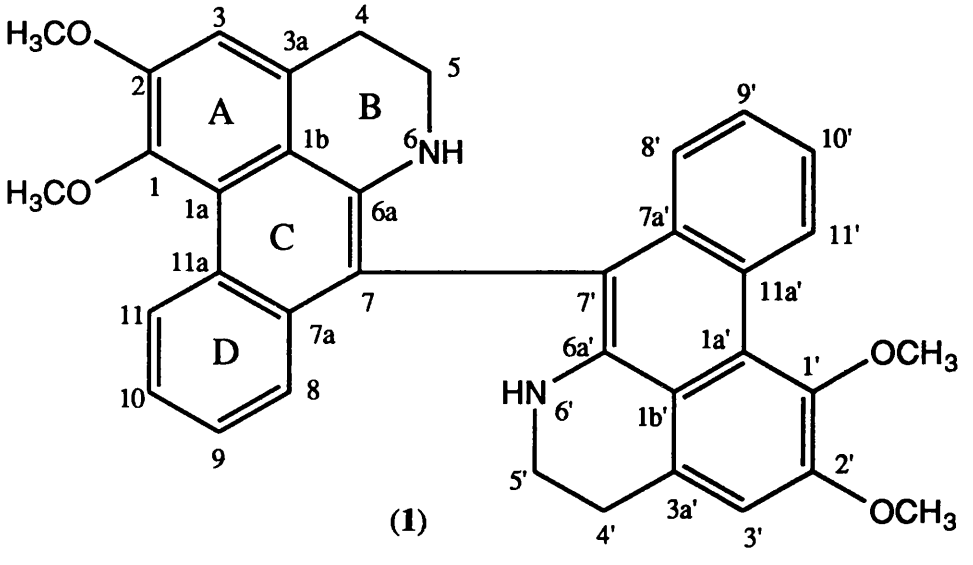
The genus *Polyalthia* (Annonaceae) comprises 120 shrubs and trees which are distributed in tropical and subtropical regions[1]. The chemical work on this genus up to 1981 has been reviewed[2]. There have been many publications on constituents which include alkaloids from *Polyalthia suaveolens*[3,4], *P. beccarii*[5], *P. longifolia*[6,7,8,9], *P. longifolia var pendula*[6], *P. acuminata*[10], *P. cauliflora*[11], *P. macropoda* and *P. stenopetala*[12], *P. nitidissima*[13], and *P. suberosa*[14], diterpenoids from *P. longifolia*[15,16,17], *P. macropoda*[18] and *P. viridis*[19], triterpenoids from *P. suberosa*[20] and *P. longifolia*[21], sesquiterpenoids from *P. suaveolens*[22] , steroids from *P. cerasoides* and *P. suberosa*[23] and *P. longifolia*[24] and flavonoids from *P. longifolia*[25]. *Polyalthia bullata* is a tree which grows widely in the Indian subcontinent and Malay penisula. Thus far no work has been carried out on this plant. It is valued in Malayasia as " tonquat ali", an aphrodisiac.

RESULTS AND DISCUSSION

On chromatography over silica gel the ethyl acetate extract of the stem bark of *P. bullata* yielded PB-1, PB-2 and PB-3 which were subsequently identified as the known bisdehydroaporphine, urabaine (**1**), and two new alkaloids, bis-O-methyldehydroisopiline (**2**) and 7-dehydronornuciferinyl-7'-dehydro-O-methylisopiline (**3**). The ethyl acetate extract of the leaves of *P. bullata* yielded, upon chromatography over silica gel, PB-4, a known oxoaporphine, liridine (**4**).

PB-1 (**1**) was obtained as green crystalline powder, mp >282° [lit.[26] urabaine, mp >280°]. It showed a single spot under UV light and gave a Dragendorff positive spot on tlc (silica gel), indicating its alkaloidal nature. The UV spectrum shows absorption

maxima at 209 [log ε 4.10], 256 [log ε 4.32], 260 [log ε 4.43] and 328 [log ε 4.00] nm, indicating a dehydroaporphine system with substitution at the C-7 position[27]. The IR spectrum has bands at 3404 (NH), 3020, 1600, 1523, 1420, 930 (aromatic CH), 2940, 2840 (aliphatic CH) and 1020 (C-O) cm-^{1}. The mass spectrum of PB-1 exhibits a molecular ion peak $[M]^+$ at m/z 556 corresponding to $C_{36}H_{32}N_2O_4$ and an $[M/2+H]^+$ peak at m/z 279 corresponding to $C_{18}H_{17}NO_2$. The absence of any other significant peak in the region m/z 556-279 suggests a symmetrical dimeric structure for (**1**).

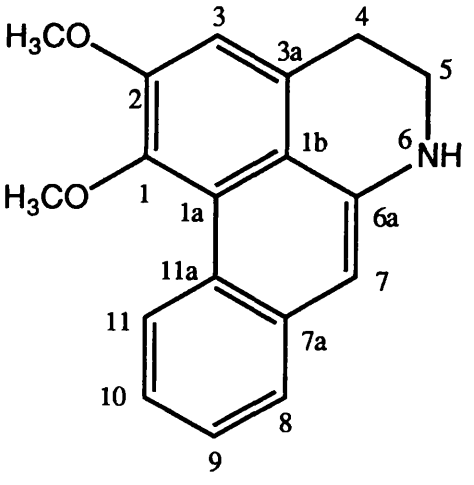


(**1**)

The ^{1}H NMR spectrum of PB-1 is remarkably simple in keeping with its symmetrical nature. Thus it has signals for four (x2) contiguous aromatic protons, one of which is highly deshielded [δ_H 9.65 (br d, J 8.6 Hz, H-11, H-11'), 7.35 (dt, J 8.5, 1.8 Hz, H-10, H-10'), 7.24 (dt, J 8.0, 1.4 Hz, H-9, H-9'), 7.15 (dd, J 8.2, 1.6 Hz, H-8, H-8')], an isolated aromatic proton (x2) [δ_H 7.12 (s, H-3, H-3')], two methoxyl groups (x2) [δ_H 3.99 (s) and 4.06 (s)] and four (x2) aliphatic protons [δ_H 3.15-3.42 (m)]. These data, in conjunction with the mass spectroscopic results, led quickly to a 7,7'-bisdehydroaporphine structure for (**1**). The chemical shift of H-11, H-11' is particularly characteristic of the system with an oxygen at C-1. Comparison with

published data[28] identified PC-1 as urabaine (**1**), which has isolated previously from *Oxandra* cf *major* (Annonaceae)[29].

The ^{13}C NMR spectrum of PB-1 readily accords with structure (**1**). Thus its monomeric unit has five aromatic doublets [δ_C 111.8, 128.0, 127.4, 123.8, 123.1], nine

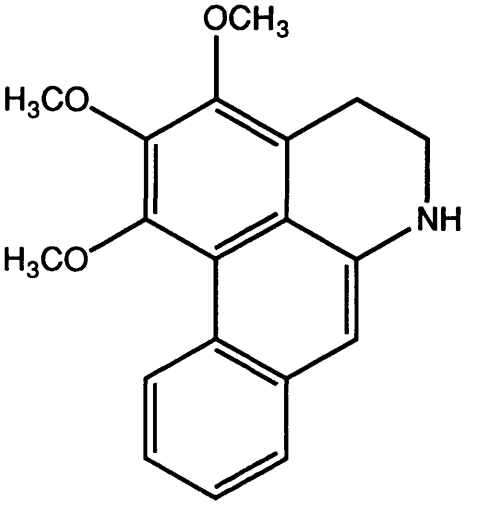


(**5**)

aromatic singlets [δ_C 151.6, 145.7, 139.2, 133.4, 129.9, 125.8, 125.7, 118.1, 118.1], two triplets [δ_C 41.2, 31.9] and two methoxyl carbons [δ_C 59.9, 56.5]. The chemical shifts of the methoxyls show that one of them [δ_C 59.9] has no proton neighbour. The absence of a doublet at δ_C 103.2 [C-7 of the monomer, dehydronornuciferine[30] (**5**)] and appearance of a singlet at δ_C 118.1 are consistent with the dimeric nature of (**1**) (see **Table 8**). Comparison with the published data for urabaine[29] confirmed its identity. This is the first report of urabaine in the genus *Polyalthia*.

PB-2 was obtained as yellowish brown microcrystals, mp 268-270°. It gave a single Dragendorff positive spot on tlc (silica gel), indicating its alkaloidal nature. Its UV spectra has maxima at λ_{max} 213 [log ε 4.53], 265 [log ε 4.62] and 323 [log ε 4.02] nm,

characteristic of a dehydroaporphine system with substitution at C-7 position[27]. The IR spectrum has bands at 3390 (NH), 3020, 1600, 1560, 1420, 930 (aromatic C-H), 2936, 2856 (aliphatic C-H) and 1020 (C-O) cm-[1]. The mass spectrum shows a molecular ion peak $[M]^+$ at m/z 616, corresponding to $C_{38}H_{36}N_2O_6$, and an $[M/2+H]^+$ peak at m/z 309, corresponding to $C_{19}H_{19}NO_3$, equivalent to the $[M+1]^+$ peak for O-methyl dehydroisopiline[31] (**6**). This suggests a symmetrical dimeric structure (**2**) for PB-2 .

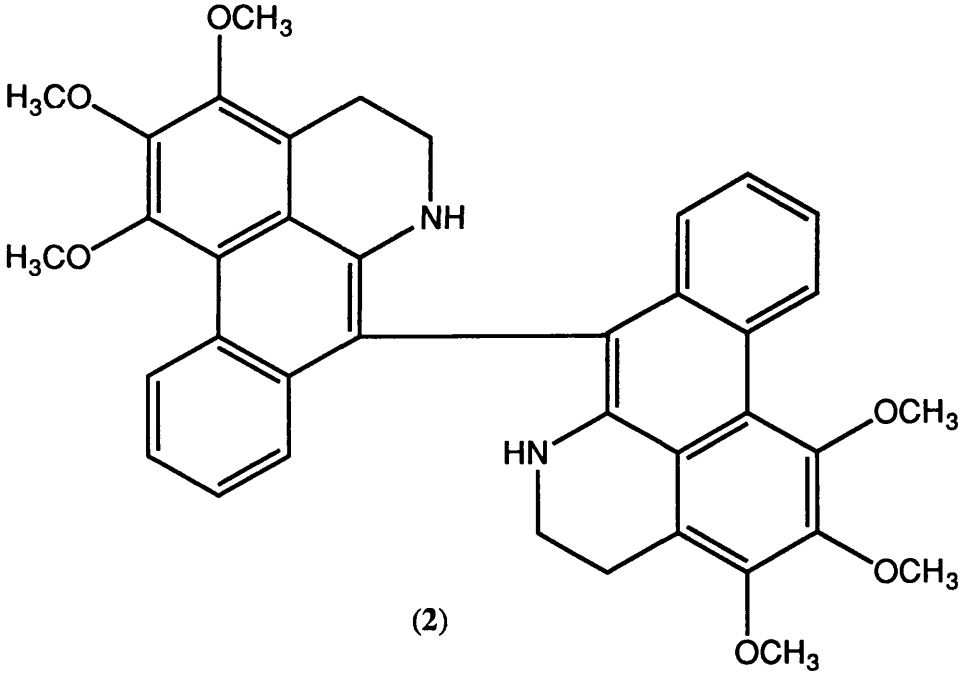


(6)

It was apparent from the ^1H NMR spectrum of PB-2 that it is closely related to urabaine (**1**). Thus its monomeric unit has a strongly deshielded proton [δ_H 9.60 (dd, J 8.6, 0.6 Hz, H-11, H-11'] which forms part of a four spin aromatic system [δ_H 7.15 (dd, J 8.2, 1.5 Hz, H-8, H-8'), 7.23 (dt, J 8.2, 1.4 Hz, H-9, H-9'), 7.37 (dt, J 8.4, 1.8 Hz, H-10, H-10')]. The lack of any further aromatic protons and the appearance of a three methoxyl signals [δ_H 4.14 (s), 4.06 (s), 4.00 (s)] indicates that the second aromatic ring is fully substututed. The aliphatic methylene resonances appear as a complex multiplet between δ_H 3.15 to 3.37. Thus PB-2 is a 7,7'-bisdehydroaporphine with three methoxyls in ring A, ie a dimer (**2**) of O-methyldehydroisopiline (**6**). Comparison with

the published data for urabaine (**1**)[28] and O-methyldehydroisopiline (**6**)[32] support this conclusion.

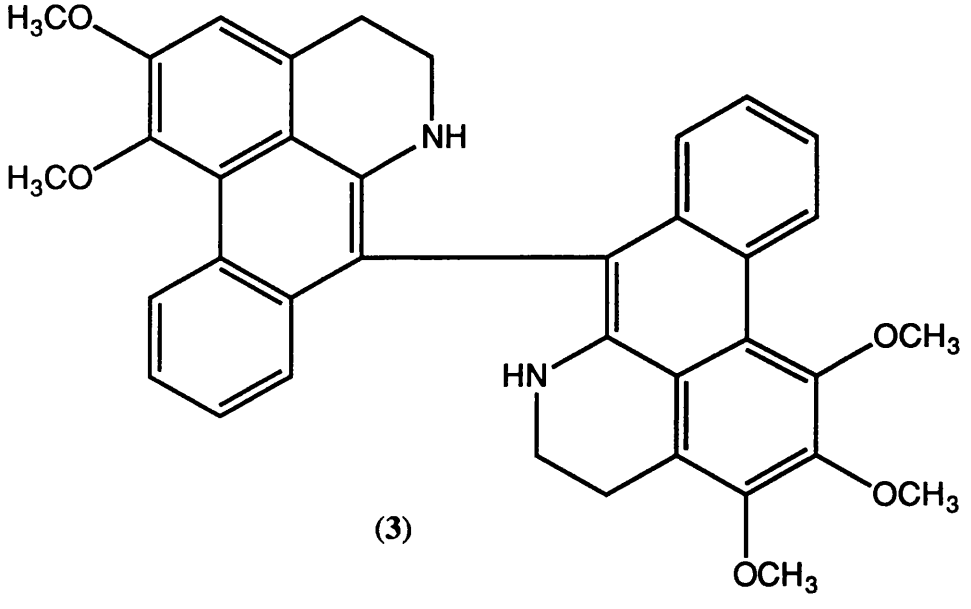
The ^{13}C NMR spectrum of PB-2 shows the expected features of a bisdehydroaporphine with ring A fully substituted. Thus the monomeric unit has four aromatic doublets at [δ_C 127.2, 126.8, 123.8, 123.1], ten quaternary carbons [δ_C 151.1, 148.5, 146.7, 139.5, 132.5, 125.5, 122.5, 121.8, 121.8, 120.1], two methylenes [δ_C 40.5, 23.8] and three methoxyl carbons [δ_C 61.3, 60.9, 60.4]. The chemical shifts of the methoxyl-bearing carbons indicate the lack of neighbouring protons. These data lead to the conclusion that PB-2 (**2**) is a dimer of O-methyldehydroisopiline (**6**). Comparison



(**2**)

with the monomeric unit of urabaine (**1**)[28] for the carbons of C and D rings and with O-methyldehydroisopiline (**6**)[33] for the carbons of rings A and B confirm this conclusion (see **Table 8**). PB-2 (**2**) is a new natural product.

PB-3 was obtained as yellowish brown microcrystals, mp >310°. It gave a single Dragendorff positive spot on tlc (silica gel), indicating its alkaloidal nature. It is UV active with bands at λ_{max} 210 [log ε 3.82], 255 [log ε 4.26], 261 [log ε 4.38] and 327 [log ε 3.76] nm, characteristic of a dehydroaporphine system with substitution at the C-7 position[27]. The IR spectrum exhibits absorption bands at 3391 (N-H), 3020, 1600, 1560, 1522, 1456, 930 (aromatic C-H), 2937, 2853 (aliphatic C-H) and 1020 (C-O) cm^{-1}. The mass spectrum shows a molecular ion $[M]^+$ peak at m/z 586 ($C_{37}H_{34}N_2O_5$), with major fragments at m/z 309 ($C_{19}H_{19}NO_3$) and m/z 279 ($C_{18}H_{17}NO_2$), suggesting that PB-3 has a dimeric structure formed from two different monomers.



(3)

The presence in the ^1H NMR spectrum of PB-3 of two highly deshielded protons at δ_H 9.65 (br d, J 8.5 Hz, H-11) and 9.58 (br d, J 8.1 Hz, H-11'), an aromatic proton singlet at δ_H 7.12 (H-3), six other aromatic protons between δ_H 7.10-7.40 (m), five methyl groups [δ_H 4.13 (s), 4.06 (s), 4.05 (s), 3.99 (x2) (s)] and eight aliphatic protons between δ_H 3.15-3.43 (m) readily led to the conclusion that the two monomeric units are dehydronornuciferine and O-methyldehydroisopiline. Thus PB-3 has the structure (**3**).

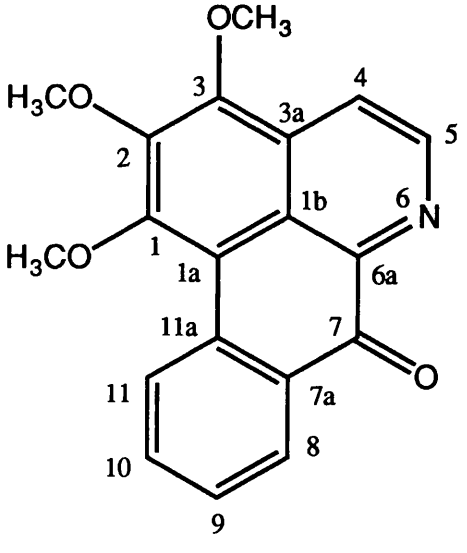
The ^{13}C NMR spectrum is entirely consistent with structure (**3**). The assignments of both ^{1}H and ^{13}C resonances, made by comparison with the appropriate monomers[28,29,32], are listed in the Experimental and in **Table 8** (^{13}C). PB-3 (**3**) is a new natural product.

TABLE 8. ^{13}C NMR Chemical Shifts of Compounds (1), (2) and (3)

Carbon	(1)	(2)	(3)	Carbon	(1)	(2)	(3)
1	145.7(s)	148.5(s)	145.7(s)	7'	118.1(s)	120.1(s)	120.2(s)
1'	145.7(s)	148.5(s)	148.7	7a	133.4(s)	132.5(s)	133.3(s)
1a	125.7(s)	125.6(s)	125.8(s)	7'a	133.4(s)	132.5(s)	132.4(s)
1'a	125.7(s)	125.6(s)	125.6(s)	8	128.0(d)	127.2(d)	128.0(d)
1b	118.1(s)	121.8(s)	118.1(s)	8'	128.0(d)	127.2(d)	127.3(d)
1'b	118.1(s)	121.8(s)	121.7(s)	9	127.4(d)	126.8(d)	127.4(d)
2	151.6(s)	146.7(s)	151.6(s)	9'	127.4(d)	126.8(d)	126.9(d)
2'	151.6(s)	146.7(s)	146.8(s)	10	123.8(d)	123.8(d)	123.6(d)
3	111.8(d)	151.1(s)	111.8(d)	10'	123.8(d)	123.8(d)	124.0(d)
3'	111.8(d)	151.1(s)	151.1(s)	11	123.1(d)	123.1(d)	123.0(d)
3a	129.9(s)	121.8(s)	130.0(s)	11'	123.1(d)	123.1(d)	123.5(d)
3'a	129.9(s)	121.8(s)	121.7(s)	11a	125.8(s)	122.5(s)	125.8(s)
4	31.9(t)	23.8(t)	30.7(t)	11'a	125.8(s)	122.5(s)	122.4(s)
4'	31.9(t)	23.8(t)	23.6(t)	MeO-1	59.9(q)	60.4(q)	59.9(q)
5	41.2(t)	40.5(t)	41.1(t)	MeO-1'	59.9(q)	60.4(q)	60.4(q)
5'	41.2(t)	40.5(t)	40.7(t)	MeO-2	56.5(q)	60.9(q)	56.5(q)
6a	139.2(s)	139.5(s)	139.3(s)	MeO-2'	56.5(q)	60.9(q)	60.9(q)
6'a	139.2(s)	139.5(s)	140.0(s)	MeO-3	-	61.3(q)	-
7	118.1(s)	120.1(s)	118.1(s)	MeO-3'	-	61.3(q)	61.3(q)

PB-4 was obtained as yellow crystals mp 186° [lit.[34] liridine, mp 188°]. It is UV active and has absorption maxima at λ_{max} 234 (log ε 4.25) and 271 (log ε 4.36) nm. The IR spectrum exhibits absorption bands 1702 (C=O), 3020, 1620, 1572, 1480, 1324, 930 (aromatic) and 1020 (C-O) cm^{-1}. The mass spectrum displays an $[M^+]$ peak at m/z 321 ($C_{19}H_{15}NO_4$) together with fragments at m/z 306 [M^+-15], 291 [M^+-15-15], 278 [M^+-15-28] and 263 [M^+-15-15-28], consistent with an oxoaporphine alkaloid.

The ^1H NMR of PB-4 shows a double doublet at δ_H 9.02 (1H, J 8.4, 0.6 Hz, H-11) which is part of an aromatic spin spin system with three other protons resonating at δ_H 7.68 (dt, J 7.5, 2.0 Hz, H-10), 7.46 (dt, J 7.5, 1.9 Hz, H-9) and 8.48 (dd, J 7.6, 1.6 Hz, H-8), an AB system [δ_H 8.87 and 8.14 (each d, J 5.4 Hz, H-4, H-5)] and three methoxyl signals [δ_H 4.06 (s), 4.09 (s), 4.17 (s)]. Comparison with published data[34] readily led to the identification of PB-4 as liridine (**4**).



(**4**)

The ^{13}C NMR of PB-4 is in accord with the structure (**4**) and has a carbonyl resonance [δ_C 182.5], six aromatic doublets [δ_C 144.6, 134.4, 129.0, 128.2, 127.6, 119.2], nine aromatic singlets [δ_C 156.5, 148.5, 147.2, 145.5, 134.5, 131.4, 131.1, 122.8, 115.6] and three methoxyl carbons [δ_C 61.8, 61.5, 61.0]. The assignments are given in the Experimental. Comparison with published data[35] confirmed the identity of PB-4 as liridine (**4**).

EXPERIMENTAL

Isolation. The plant material of *Polyalthia bullata* was collected in Malayasia. The dried stem bark (400g) and leaves (350g) were extracted in a Soxhlet apparatus with EtOAc. The EtOAc extracts of the stem bark (3.6g) and leaves (3.0g) were concentrated *in vacuo* and fractionated by flash column chromatography over silica gel, eluting with petroleum ether, increasing amounts of EtOAc in petroleum ether and finally with MeOH (50 ml fractions). The fractions were monitored by analytical tlc. Early fractions contained only fat. Multiple preparative tlc, using petroleum ether : EtOAc [(80 : 20), (88 : 12) and (85 : 15)] of the stem bark fractions gave the following results :- fraction 15 gave PB-1 (17mg), fraction 8 PB-2 (55mg) and fraction 10 PB-3 (21mg). Similarly preparative tlc, using CH_2Cl_2 : MeOH (98 : 2), of the fraction 19 of the leaf extract afforded PB-4 (10mg).

PB-1 (**1**), green crystalline powder (MeOH), mp > 282°; UV λ_{max} : 209 (log ε 4.10), 256 (log ε 4.32), 260 (log ε 4.43), 328 (log ε 4.00) nm; IR ν_{max} : 3404 (NH), 3020, 2940, 2840, 1523, 1420, 1020, 930 cm^{-1}; EIMS m/z (rel. int.) : 556 $[M]^+$ (100), 279 $[M/2+H]^+$ (44), 264 $[279-CH_3]^+$ (14), 248 $[279-OCH_3]^+$ (7), 233 (9), 217 (8); ^1H NMR : δ_H 9.65 (br d, J 8.6 Hz, H-11, H-11'), 7.35 (dt, J 8.5, 1.8 Hz, H-10, H-10'), 7.24 (dt, J 8.0, 1.4 Hz, H-9, H-9'), 7.15 (dd, J 8.2 , 1.6 Hz, H-8, H-8'), 7.12 (s, H-3, H-3'), 3.15-3.42 (m, 2H-4, 2H-4', 2H-5, 2H-5'), 3.99 (s, CH_3O-1, CH_3O-1'), 4.06

(s, CH_3O-2, CH_3O-2') ; ^{13}C NMR (see **Table 8**); HREIMS : Found $[M]^+$ $C_{36}H_{32}N_2O_4$ 556.2349; requires 556.2362; found $[M/2+H]^+$ $C_{19}H_{17}NO_2$ 279.1248; requires 279.1259.

PB-2 (**2**), yellowish brown microcrystals (MeOH), mp 268-270°; UV λ_{max} : 213 (log ε 4.53), 265 (log ε 4.62), 323 (log ε 4.02) nm; IR ν_{max} : 3390 (NH), 3020, 2936, 2856, 1600, 1560, 1420, 1020, 930 cm^{-1}; EIMS m/z (rel. int.) : 616 $[M]^+$ (100), 309 $[M/2+H]^+$ (35), 294 $[309-CH_3]^+$ (9), 278 $[309-CH_3O]^+$ (10), 263 (13); ^1H NMR : δ_H 9.60 (dd, J 8.6, 0.6 Hz, H-11, H-11'), 7.37 (dt, J 8.4, 1.8 Hz, H-10, H-10'), 7.23 (dt, J 8.2, 1.4 Hz, H-9, H-9'), 7.15 (dd, J 8.2, 1.5 Hz, H-8, H-8'), 3.15-3.37 (m, 2H-4, 2H-4', 2H-5, 2H-5'), 4.00 (s, CH_3O-1, CH_3O-1'), 4.06 (s, CH_3O-2, CH_3O-2') 4.14 (s, CH_3O-3, CH_3O-3'); ^{13}C NMR (see **Table 8**); HREIMS : Found $[M]^+$ $C_{38}H_{36}N_2O_6$ 616.2510; requires 616.2502; found $[M/2+H]^+$ $C_{19}H_{19}NO_3$ 309.1345; requires 309.1330.

PB-3 (**3**), yellowish brown microcrystals (MeOH), mp >310°; UV λ_{max} : 210 (log ε 3.82), 255 (log ε 4.26), 261 (log ε 4.38), 327 (log ε 3.76) nm; IR ν_{max} : 3391 (NH), 3020, 2937, 2853, 1600, 1560, 1522, 1456, 1020, 930 cm^{-1}; EIMS m/z (rel. int.) : 586 $[M]^+$ (100), 309 $[M-278+H]^+$ (22), 279 $[M-308+H]^+$ (30); ^1H NMR : δ_H 9.65 (br d, J 8.5 Hz, H-11), 9.58 (br d, J 8.1 Hz, H-11'), 7.12 (s, H-3), 7.10-7.40 (m, H-8, H-8', H-9, H-9', H-10, H-10', H-11, H-11'), 3.15-3.43 (m, 2H-4, 2H-4', 2H-5, 2H-5'), 3.99 (s, CH_3O-1, CH_3O-1'), 4.06 (s, CH_3O-2), 4.05 (s, CH_3O-2'), 4.13 (s, CH_3O-3'); ^{13}C NMR (see **Table 8**); HREIMS : Found $[M]^+$ $C_{37}H_{34}N_2O_5$ 586.2468; requires 586.2474.

PB-4 (**4**), yellow crystals (MeOH), mp 186°; UV λ_{max} : 234 (log ε 4.25), 271 (log ε 4.36) nm; IR ν_{max} : 3020, 1701 (C=O), 1620, 1572, 1480, 1324, 1020, 930 cm^{-1}; EIMS m/z (rel. int.) : 321 $[M]^+$ (100), 306 $[M^+-15]$ (61), 291 $[M^+-15-15]$ (22), 278 $[M^+-15-28]$ (44), 263 $[M^+-15-15-28]$ (53), 248 (26), 235 (30), 220 (54), 192 (23), 164

(40), 137 (28); ^{1}H NMR : δ_H 9.02 (dd, J 8.4, 0.6 Hz, H-11), 8.87, 8.14 (each, d, J 5.4 Hz, H-4, H-5), 8.48 (dd, J 7.6, 1.6 Hz, H-8), 7.68 (dt, J 7.5, 2.0 Hz, H-10), 7.46 (dt, J 7.5, 1.9 Hz, H-9), 4.17 (s, CH_3O-2), 4.09 (s, CH_3O-3), 4.06 (s, CH_3O-1); ^{13}C NMR : δ_C 145.5 (C-1), 115.6 (C-1a), 122.8 (C-1b), 147.2 (C-2), 148.5 (C-3), 131.1 (C-3a), 119.2 (C-4), 144.6 (C-5), 156.5 (C-6a), 182.5 (C-7), 131.4 (C-7a), 128.2 (C-8), 127.6 (C-9), 134.4 (C-10), 129.0 (C-11), 134.5 (C-11a), 61.8 (CH_3O-1), 61.5 (CH_3O-2), 61.0 (CH_3O-3).

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CHAPTER 7

ZEYLENOL AND TWO NEW PSEUDOSUGARS FROM THE STEM BARK OF *ANNONA ROXBURGHIANA* L. (ANNONACEAE).

INTRODUCTION

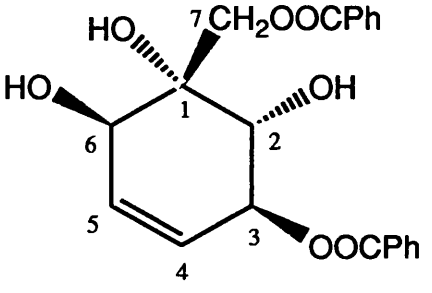
The genus *Annona* (Annonaceae) consists of about 120 species of trees, shrubs and herbs. They are widely distributed in the tropics and the neotropics and to a lesser extent in Africa[1]. *Annona roxburghiana* is a shrub of 2-4 ft in height and is distributed through out Bangladesh. So far no work has been carried out on this plant. The chemical work on this genus up to 1981 has been reviewed[2]. Recently alkaloids have been isolated from *A. montona*[3,4,5], *A. crassiflora*[6], *A. glabra*[7], *A. elliptica*[8], *A. hayesii*[9], *A. paludosa*[10], *A. spraguei*[11] and *A. salzmanii*[12]. The existence of acetogenins in this genus has been reviewed[13]. Further reports of acetogenins from *A. montana*[14], *A. densicoma*[15], *A. muricata* and *A. cherimolia*[16] have appeared. Monoterpenoids and sesquiterpenoids have been isolated from *A. atemoya*[17,18] and diterpenoids from *A. bullata*[19].

RESULTS AND DISCUSSION

On chromatography over silica gel , the chloroform extract of the stem bark of *A. roxburghiana* yielded AR-1, subsequently identified as the known cyclohexene derivative, zeylenol (**1**). The sticky methanol extract was acetylated and chromatographed over silica gel to yield AR-2 and AR-3 which were identified as two new acetylated isomeric cyclitol (pseudosugar) derivatives with the gross structure (**2**). The stereochemistry of AR-2 is tentatively assigned as in (**4**).

AR-1 was obtained as colourless needles, mp 132-134°, $[\alpha]_D$ +113°(c, 0.50 in $CHCl_3$) [lit.[20] zeylenol, mp 132-133°, $[\alpha]_D$ +113.5° (c, 0.60 in $CHCl_3$)]. It was UV

active [λ_{max} 230 (log ε 4.53) nm] and gave a single spot . Its IR spectrum showed hydroxyl (3450 cm-^{1}), ester (1718, 1275 cm-^{1}), alkene and aromatic (3020, 1600, 1585, 1490, 710 cm-^{1}) absorption. The mass spectrum of AR-1 displayed an [M^+] ion at m/z 384, an [M^+-OH] peak at m/z 367 and intense fragments ions at m/z 262 [M^+-PhCOOH], 245 [M^+-PhCOOH-OH] and 244 [M^+-PhCOOH-H_2O]. The base peak at m/z 105 is suggestive of the $PhCO^+$ ion which loses 28 mass unit (CO) to give m/z 77.

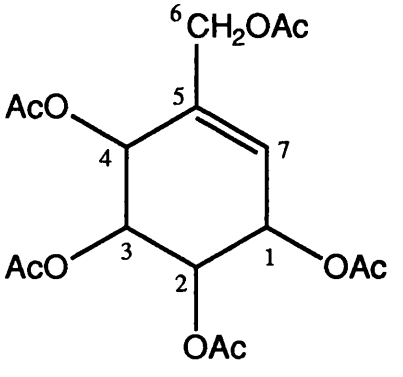


(**1**)

The ^{1}H NMR spectrum of (**1**) shows signals for ten aromatic protons [δ_H 8.02-7.95 (4H, m), 7.54 (2H, m) and 7.38 (4H, m)] suggestive of the presence of two slightly different benzoyl groups, an AB quartet [δ_H 4.89 and 4.73 (J 12.3 Hz)] indicative of a benzoyloxymethylene group, two vinyl protons on a *cis* disubstituted double bond [δ_H 6.00 (ddd, J 10.1, 3.7, 1.8 Hz, H-5); 5.85 (ddd, J 10.1, 2.5, 1.0 Hz, H-4)] associated with two allylic oxygenated methines [δ_H 4.32 (dd, J 3.7, 1.0 Hz, H-6); 5.69 (dddd, J 6.1, 2.5,1.8,1.2 Hz, H-3)]. H-3 has vicinal coupling to H-4 and H-2 [δ_H 4.25 (d, J 6.1 Hz)], an allylic coupling (1.8 Hz) with H-5 and a homoallylic coupling (1.0 Hz) with H-6. The attachment of the second benzoate group to C-3 is revealed by the downfield shift of H-3. These data are consistent with structure (**1**) which is zeylenol, previously isolated from *Uvaria zeylanica* (Annonaceae)[21]. Comparison with published data[20,21] confirmed the assignment.

The ^{13}C NMR spectrum of (**1**) showed the expected features. Thus it has two ester carbonyls (δ_C 167.9, 167.2) and two phenyl rings [δ_C 133.5, 133.4, 129.8, 128.5, 128.4 (all d), 129.4 (s)], associated with the two benzoates. The olefinic carbons appear at δ_C 126.9 (d) and 129.5 (d), while the oxygenated carbons resonate at δ_C 68.9, 70.9, 74.3 (all d), 66.8 (t) and 76.0 (s). These values compare well with the published data for zeylenol[21]. This is the second report of the isolation of zeylenol from the Annonaceae and the first from the genus *Annona*.

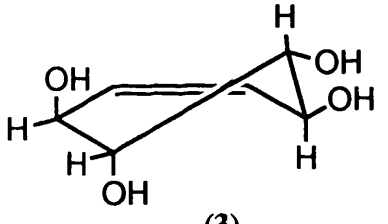
AR-2 was obtained as a gum. Its IR spectrum exhibits ester carbonyl (1745, 1700 cm^{-1}), double bond (3020, 1500, 1425, 1370, 1225 cm^{-1}) and C-O (1025 cm^{-1}) absorption. The ^{13}C DEPT spectrum indicated the molecular formula $C_{12}H_{22}O_{10}$. Thus it has five acetate groups [δ_C 20.9 (2), 20.8 (2), 20.7 (CH_3), 170.3, 170.2, 170.1, 170.0, 169.9 (C=O)], a trisubstituted double bond [δ_C 133.7 (s), 126.3 (d)], four oxygenated methines [δ_C 69.4, 69.3, 67.8, 67.2] and an oxygenated methylene [δ_C 63.3] . Thus the molecule is monocarbocyclic. Its mass spectrum shows an M^+-60 peak at m/z 326 and other peaks at m/z 266 (M^+-60-60), 224 (M^+-60-60-42), 207 (M^+-60-60-59), 182 (M^+-60-60-42-42), 140 (182-42) and a base peak at 122 (140-18).



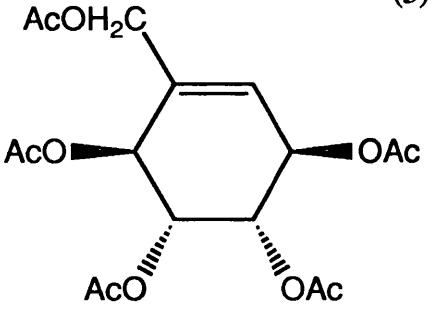
(**2**)

The ^1H NMR of AR-2 shows an AB system at δ_H 4.45 (d, J 13.6 Hz) and 4.63 (d, 13.6 Hz) and five deshielded methines [δ_H 5.29 (dd, J 6.7, 2.7 Hz, H-2) , 5.35 (dd, J 4.9, 2.7 Hz, H-3), 5.48 (dd, J 6.3, 1.7 Hz, H-1) , 5.51(br d, J 4.9 Hz, H-4) and 5.92 (br d, J 3.0 Hz, H-7)], the last associated with the vinyl proton H-7, in addition to the expected resonances for five acetates [δ_H 2.05, 2.06, 2.07, 2.09, 2.10]. These data indicate that AR-2 is the pentaacetate of a hydroxymethylcyclohexene tetrol ie a cyclitol or pseudosugar as in (**2**).

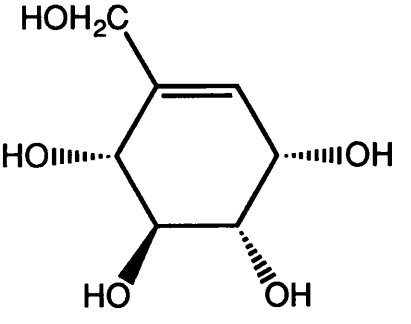
From the ^1H-^1H COSY (Fig. 12) it was possible to establish the proton-proton connectivity. The primary acetate protons 2H-6 are allylic and show allylic coupling to the vinyl proton H-7 [δ_H 5.92]. The sequence H-6→H-1[δ_H 5.48]→H-2 [δ_H 5.29]→H-3 [δ_H 5.35]→H-4 [δ_H 5.51] is readily apparent, confirming the assignments of the protons in (**2**). There are also some indications of homoallylic coupling of H-1 and the methylene protons 2H-6.



(**3**)



(**4**)



(**5**)

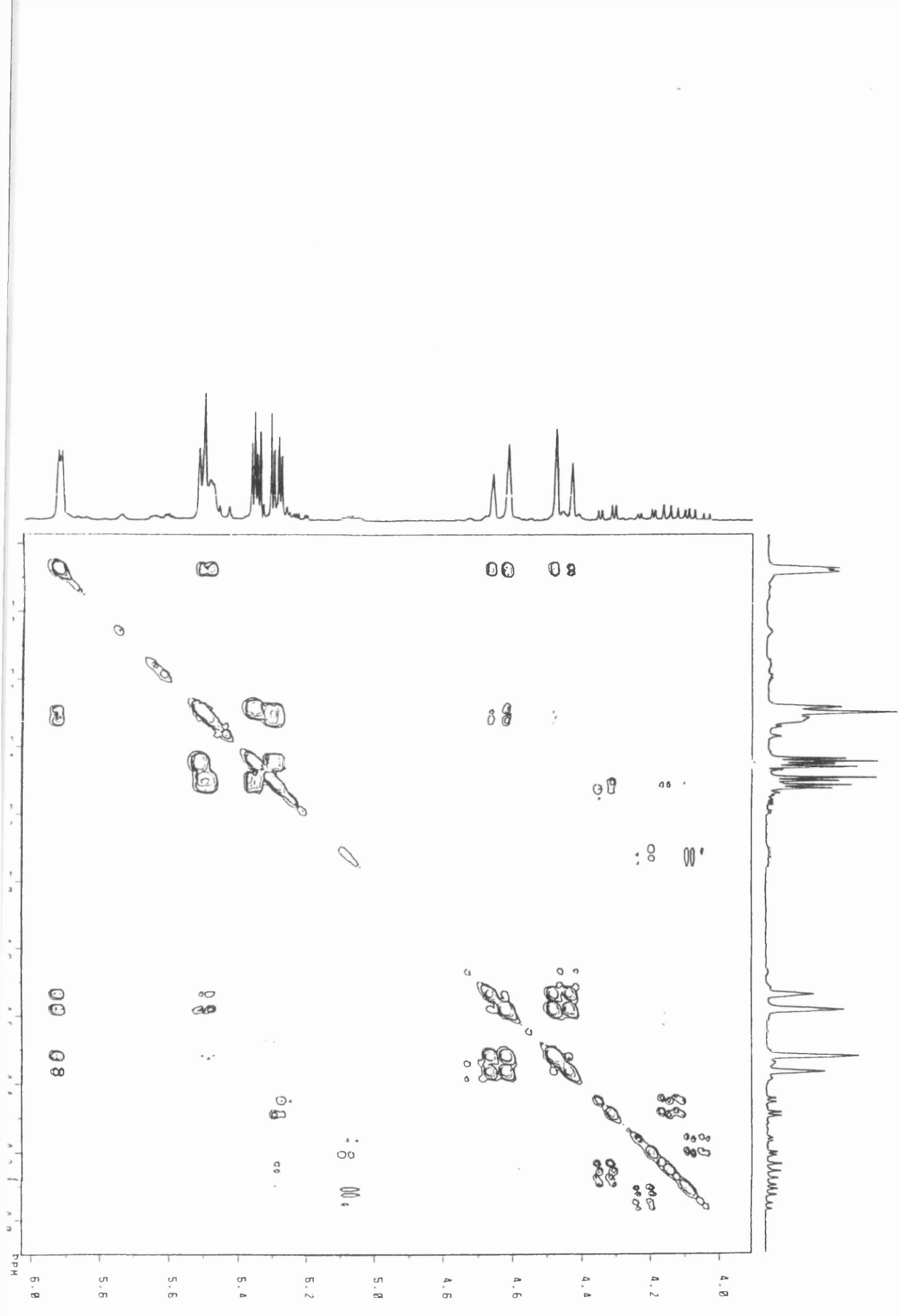


Figure 12. ^{1}H-^{1}H COSY spectrum of AR-2 (**4**).

In the absence of independent evidence, eg NOEs, for the conformation of the cyclohexene ring it is difficult to assign the relative stereochemistry. A tentative stereochemistry may be suggested on the basis of a half chair conformation (**3**) as follows. The allylic proton H-1 is pseudoaxial and couples with the axial H-2 (6.7 Hz). All other couplings are small and must involve equatorial protons . H-4 shows no allylic coupling to the vinyl proton and should be more or less in the plane of the double bond. Thus AR-2 has the relative stereochemistry as in (**4**).

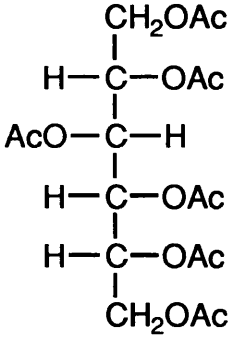
AR-2 appears to be a new cyclitol. The only free cyclitol to have been reported is streptol (**5**)[22] from *Streptomyces* sp. The structure and stereochemistry of streptol have been confirmed by synthesis[23]. Cyclitols often contain amino functions and form part of larger antibiotic molecules.

The next compound AR-3 was also obtained as a gum, ν_{max} 3020, 1745, 1700, 1500, 1045 cm^{-1} . It is clear from its ^{13}C NMR spectrum that it is another cyclitol derivative. It has five acetates [δ_C 20.9 (2), 20.7 (2), 20.4, 170.3, 170.1, 169.9, 169.8, 169.7], a trisubstituted double bond [δ_C 133.6 (C-5), 125.5 (C-7)], four oxygenated methines [δ_C 70.7, 69.2, 68.4, 67.0] and an acetoxymethylene [δ_C 62.7 (C-6)]. Thus it has the same gross structure (**2**) as AR-2.

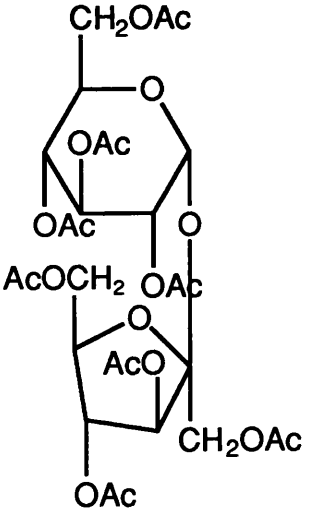
Its mass spectrum also shows an (M^+-60) peak at m/z 326 and other major peaks at m/z 266 (M^+-60-60), 224 (M^+-60-60-42), 206 (M^+-60-60-60), 182 (224-42), 140 (182-42) and a base peak at m/z 122 (140-18). Its ^1H NMR shows an AB system at δ_H 4.43 (1H, d, J 13.5 Hz) and 4.70 (1H, d, J 13.5 Hz), three one proton double doublets at δ_H 5.21 (1H, dd, J 8.0, 2.1 Hz), 5.62 (1H, dd, J 3.6, 1.9 Hz) and 5.63 (1H, dd, J 3.8, 1.5 Hz), a broad doublet at 5.74 (1H, br d, J 1.3 Hz) and another broad doublet at 5.85 (1H, br d, J 8.0 Hz)]. The five acetate methyl groups appear at δ_H 2.02, 2.04, 2.06, 2.07 and 2.14 .

The ^1H-^1H COSY, though limited in quality because of the small sample provided some connectivity. The narrow doublet at δ_H 5.74 shows coupling to the acetoxymethylene protons and apparently to two other protons [δ_H 5.85 and 5.60]. The lowest field signal [δ_H 5.85] has a large coupling (J 8.0 Hz) to the proton at δ_H 5.21 which in turn couples to the proton at δ_H 5.60. This proton appears to couple to both the narrow doublet at δ_H 5.74 and to the doublet of doublets at δ_H 5.63 . Not all the couplings are visible in the COSY spectrum since the proton at δ_H 5.63 shows no further correlations. Since it is difficult to decide which proton is the vinyl proton the assembly of a rational coupled system is not possible. Until the more material is available the detailed structure of AR-3 must remain undetermined. There is no doubt , however, that it is a new cyclitol.

This is the first report of the isolation of cyclitols from the Annonaceae. In addition, sorbitol acetate (**6**) and sucrose acetate (**7**) were also isolated.



(**6**)



(**7**)

EXPERIMENTAL

The plant material of *Annona roxburghiana* was collected from the district of Comilla, Bangladesh. The ground stem bark (175g) was extracted successively, in a Soxhlet apparatus, with $CHCl_3$ and MeOH. Following concentration *in vacuo,* the $CHCl_3$ extract (450mg) was fractionated by flash column chromatography over silica gel, eluting with petroleum ether, petroleum ether and increasing amounts of $CHCl_3$ and finally MeOH (50 ml fractions). Multiple preparative tlc of fraction 6 [$CHCl_3$: MeOH (98 : 2) afforded AR-1 (12mg) (**1**).

AR-1 (zeylenol, **1**)) was obtained as colourless needles (MeOH), mp 132-134°, $[\alpha]_D$ +113° (c, 0.50 in $CHCl_3$) ; UV λ_{max} (MeOH) : 230 (log ε 4.53) nm ; IR ν_{max} : 3450 3020, 1715, 1600, 1585, 1490, 1450, 1372, 1278, 1175, 1115, 1070, 1010, 970, 710 cm^{-1}; EIMS m/z (rel. int.) : 384 [M^+] (1), 367 [M^+-OH] (1), 262 [M^+-PhCOOH] (1), 245 (3), 244 (4), 231 (2), 215 (3), 203 (4), 190 (2), 163 (3), 123 (8), 122 (20), 110 (5), 106 (12), 105 (100), 99 (10), 77 (52); ^1H NMR : δ_H 8.02-7.95 (4H, m, H-2', H-2"), 7.54 (2H, m, H-4', H-4"), 7.38 (4H, m, H-3', H-3"), 6.00 (1H, ddd, J 10.1, 3.7, 1.8, H-5), 5.85 (1H, ddd, J 10.1, 2.5, 1.0 Hz, H-4), 5.69 (1H, dddd, J 6.1, 2.5, 1.8, 1.2 Hz, H-3), 4.89, 4.73 (2H, ABq, J 12.3Hz, 2H-7), 4.32 (1H, dd, J 3.7, 1.0 Hz, H-6), 4.25 (1H, d, J 6.1 Hz, H-2); ^{13}C NMR δ_C: 76.0 (C-1), 68.7 (C-2), 74.3 (C-3), 126.9 (C-4), 129.5 (C-5), 70.0 (C-6), 66.7 (C-7), 128.5 (2x C-3'), 128.4 (2x C-3"), 129.8 (2xC-2', 2xC-2"), 133.5 (C-4'), 133.4 (C-4"), 129.4 (C-1', C-1"), 167.9 (C-7'), 167.2 (C-7").

The methanol extract was concentrated *in vacuo* to give a sticky mass (1.2g) which was acetylated under standard conditions with acetic anhydride and pyridine overnight. The acetylated extract was fractionated by flash column chromatography using $CHCl_3$ and increasing amounts of MeOH (50 ml fractions). Multiple preparative

tlc [petroleum ether : EtOAc (65: 35)] of fractions 6 and 7 afforded AR-2 (**4**) (5.0mg) and AR-3 (**2**) (4.5mg). In addition fraction 7 afforded sorbitol acetate (**6**) (5.0mg) and fraction 8 sucrose acetate (**7**) (12.0mg).

AR-2 (**4**), gum, IR ν_{max} : 3020, 1745, 1700, 1500, 1425, 1370, 1225, 1025 cm^{-1}; EIMS m/z (rel. int.) : 326 [M^+-60] (1), 266 [M^+-60-60] (1), 224 [M^+-60-60-42] (19), 223 (5), 206 [M^+-60-60-60] (2), 183 (10), 182 [M^+-60-60-42-42] (94), 164 (17), 153 (21), 145 (8), 140 (182-42) (80), 139 (12), 124 (9), 123 (30), 122 [140-18] (100), 115 (14), 111 (26), 103 (14), 98 (22), 95 (15), 94 (40), 81 (9); ^1H NMR : δ_H 4.45 , 4.63 (2H, ABq, J 13.6 Hz, 2H-6), 5.29 (1H, dd, J 6.7, 2.7 Hz, H-2), 5.35 (1H, dd, J 4.9, 2.7 Hz, H-3), 5.48 (1H, dd, J 6.3, 1.7 Hz, H-1), 5.51 (1H, br d, J 4.9 Hz, H-4), 5.92 (1H, d, J 3.0 Hz, H-7); ^{13}C NMR : δ_C 20.9 (2) , 20.8 (2) , 20.7 (all q), 170.3, 170.2, 170.1, 170.0, 169.9 (all s), 133.7 (s), 126.3 (d), 69.4 (d), 69.3 (d), 67.8 (d), 67.2 (d), 63.3 (t).

AR-3 (**2**), gum, IR ν_{max} : 3020, 1745, 1700, 1500, 1430, 1370, 1220, 1045 cm^{-1}; EIMS m/z (rel. int.) : 326 [M^+-60] (1), 266 [M^+-60-60] (1), 224 [M^+-60-60-42] (8), 223 (3), 206 [M^+-60-60-60] (3), 183 (3), 182 [224-42] (28), 164 (9), 153 (9), 145 (2), 141 (8), 140 [182-42] (20),139 (4), 124 (5), 123 (19) 122 [140-18] (100), 115 (4), 111 (9), 103 (10), 98 (7), 95 (13), 94 (11), 81 (7); ^1H NMR : δ_H 4.43, 4.70 (2H, ABq, J 13.5 Hz, 2H-6), 5.21 (1H, dd, J 8.0, 2.1 Hz), 5.60 (1H, dd, J 3.6, 1.9 Hz), 5.63 (1H, dd, J 3.8, 1.5 Hz), 5.74 (1H, br d, J 1.3 Hz), 5.85 (1H, br d , J 8.0 Hz); ^{13}C NMR : δ_C 20.9 (2), 20.7 (2), 20.4 [all q], 170.3, 170.1, 169.9, 169.8, 169.7 (all s), 133.6 (s), 125.5 (d), 70.7 (d), 69.2 (d), 68.4 (d), 67.0 (d), 62.7 (t).

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CHAPTER 8

THE CONSTITUENTS OF THE STEM BARK OF *ANTIDESMA GHAESEMBILLA* GAERTN (EUPHORBIACEAE).

INTRODUCTION

The genus *Antidesma* (Euphorbiaceae) comprises 60 species of trees or shrubs which are distributed throughout tropical Asia, Africa, Australia and the Pacific[1]. Triterpenoids have been isolated from several species including *Antidesma bunius*[2], *A. pentandrum*[3] and *A. menasu*[4,5]. *A. ghaesembilla* is a tree which is widely distributed in Bangladesh. The bark of this plant is considered an astringent and tonic in Indochina[6]. Thus far no phytochemical work has been done on this plant.

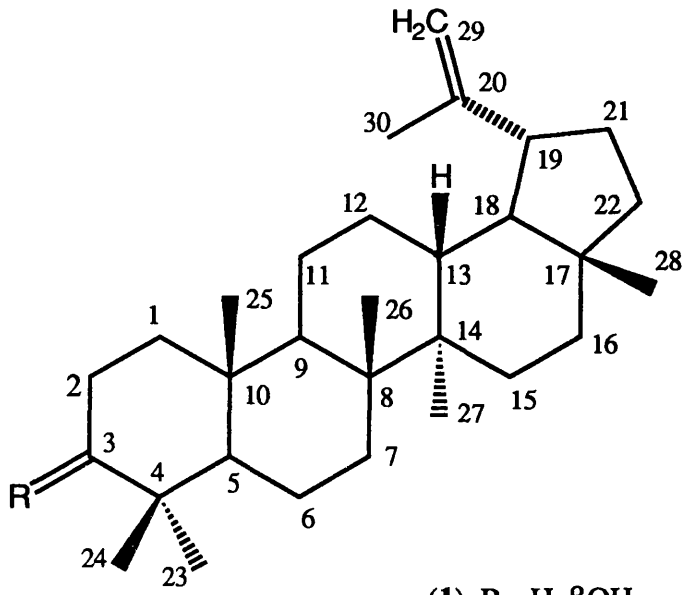
RESULTS AND DISCUSSION

On chromatography over silica gel the EtOAc extract of the stem bark of *A. ghaesembilla* yielded four compounds AG-1, AG-2, AG-3 and AG-4 which were subsequently identified as the triterpenoids lupeol (**1**) and lupeone (**2**), the coumarin scopoletin (**3**) and the ester betulin 3-caffeate (**4**).

AG-1 (**1**) was isolated as white crystals, mp 210-212°, $[\alpha]_D$ +30.4° (c, 0.58 in $CHCl_3$) [lit[7] lupeol, mp 212°, $[\alpha]_D$ +32° (c, 0.50 in $CHCl_3$)]. Its IR spectrum exhibits hydroxyl [ν_{max} 3615, 1030 cm^{-1}] and exomethylene [ν_{max} 3070, 1638, 887 cm^{-1}] absorption. Its mass spectrum displays an [M^+] peak at m/z 426 ($C_{30}H_{50}O$) together with fragments at m/z 411 [M^+-15] and 408 [M^+-18] and a base peak at m/z 43 [$(C_3H_7)^+$].

The ^1H NMR spectrum reveals signals for six tertiary methyls [δ_H 0.74, 0.77, 0.81, 0.93, 0.94 and 1.01], a vinyl methyl [δ_H 1.66 (br d, J 0.5 Hz)], a secondary carbinol [δ_H 3.20 (dd, J 9.6, 6.2 Hz)] and an exomethylene group [δ_H 4.58 (1H, d, J 0.4 Hz) and 4.65 (1H, dq, J 0.4, 0.5 Hz)]. These data indicated a pentacyclic

triterpenoid of the lupeol type and comparison with published data[8,9] confirmed the identity of AG-1 as lupeol (**1**).



(**1**) R= H, βOH

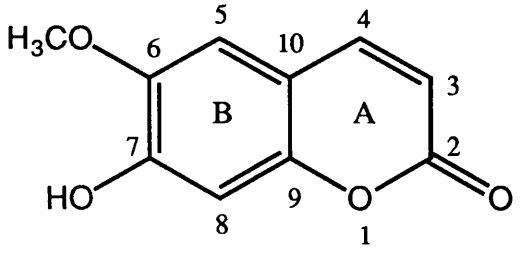
(**2**) R= O

The ^{13}C NMR spectrum of AG-1 (**1**) shows seven methyl groups [δ_C 28.0, 19.3, 18.0, 16.1, 15.9, 15.4, 14.5], an exomethylene group [δ_C 150.8 (C-20), 109.3 (C-29)] and a secondary hydroxyl bearing carbon [δ_C 78.9 (C-3)], in addition to ten methylene, five methine and five quaternary carbons. These data (see Experimental) are identical to those of lupeol (**1**)[10,11].

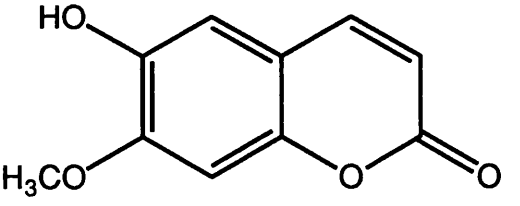
AG-2 (**2**) was obtained as crystals mp 166-168°, $[\alpha]_D$ +60° (c, 0.52 in $CHCl_3$) [lit.[12] lupeone, mp 166-168°, $[\alpha]_D$ +60.6° (c, 0.50 in $CHCl_3$)]. Its IR spectrum exhibits ketonic carbonyl [ν_{max} 1701 cm^{-1}] and an exomethylene group [ν_{max} 3070, 1640, 887 cm^{-1}]. Its mass spectrum suggests it is a pentacyclic triterpenoid [m/z 424 (M^+, $C_{30}H_{48}O$); 409 (M^+-15); 394 (M^+-15-15); 381 (M^+-15-28); 43 ($C_3H_7^+$, base peak)]] and this was confirmed by the ^1H and ^{13}C NMR spectra (see Experimental)

which show *inter alia* six tertiary methyls [δ_H 0.73, 0.86, 0.89, 0.96, 1.00 (6H, s) , δ_C 26.6, 21.0, 18.0, 15.9, 15.7, 14.4], a vinyl methyl [δ_H 1.61 (3H, br d, J 0.5 Hz, Me-30) , δ_C 19.3 (C-30)], a ketonic carbonyl group [δ_C 218.3 (C-3)] and an exomethylene group [δ_H 4.51 (1H, d, 0.4 Hz) , 4.62 (1H, dq, J 0.4, 0.5 Hz) , δ_C 150.9 (C-20), 109.4 (C-29)]. Comparison with published data[12,10] readily led to the identification of AG-2 as lupeone (**2**).

Compound AG-3 (**3**) was obtained as needles, mp 204° [lit.[13] scopoletin, mp 202-203°]. On tlc it showed as a single blue fluorescent spot under UV light and has characteristic hydroxy-coumarin absorption in the UV (λ_{max} 342, 297, 260, 251 and 225 nm) and IR [ν_{max} 3530 (OH), 1720 (C=O), 3020, 1604, 1583 (aromatic) cm^{-1}]. The mass spectrum indicates a molecular formula $C_{10}H_8O_4$ [m/z 192] with fragments at m/z 177 [M^+-15] and 161 [M^+-31]. The coumarin nature of AG-3 was readily deduced from its ^1H NMR spectrum which shows a characteristic ring A AB quartet [δ_H 6.28 and 7.59 (each d , J 9.5 Hz, H-3 and H-4)], two aromatic singlets [δ_H 6.91 and 6.84 (both s, H-5, H-8)] and a methoxyl group [δ_H 3.95, s]. These data can be accommodated by



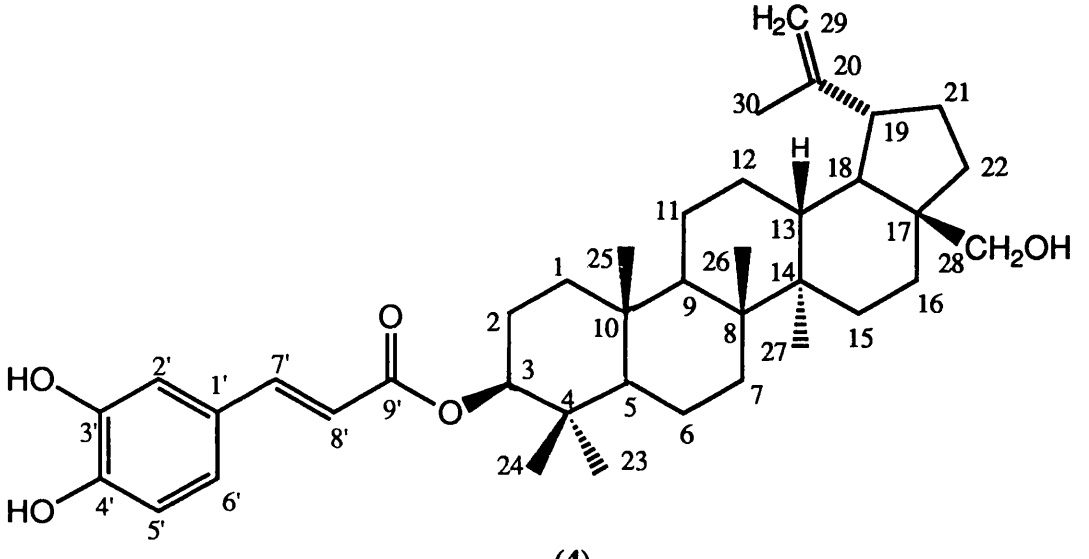
(**3**)



(**5**)

structures (**3**), scopoletin, or (**5**), isoscopoletin. The identity of AG-3 as scopoletin (**3**) was confirmed by comparison with published data[13,14].

AG-4 (**4**) was isolated as an amorphous solid, $[\alpha]_D$ +33.2° (c, 0.50 in $CHCl_3$) [lit.[15] betulin 3-caffeate, $[\alpha]_D$ +33.6° (in $CHCl_3$)]. It is UV active and shows absorption at λ_{max} 221, 234, 246, 295 and 350 nm. The IR spectrum has bands at 3550 (OH), 1710 (conjugated ester), 1637, 1604, 1522 and 929 (aromatic and vinyl) cm-[1]. The mass spectrum has an [M^+] ion at m/z 604, with additional fragments at m/z 589 [M^+-15], 434 [M^+-170], 163 [$C_9H_7O_3^+$ (base peak)] and 161 [$C_9H_5O_3^+$].



(**4**)

The ^1H NMR spectrum shows signals for an isopropenyl group [δ_H 4.66 and 4.56 (1H each, br s) and (1.66, 3H, s)], five tertiary methyl groups [δ_H 0.99, 0.96, 0.89, 0.86, 0.84], a secondary ester function (δ_H 4.56, 1H, m, H-3), a primary alcohol [δ_H 3.80 and 3.36 (ABq, J 11.0 Hz, 2H-28)] and an allylic proton [δ_H 2.36 (1H, m, H-19)]. These data are consistent with the presence of a lupeol type triterpenoid skeleton with a hydroxyl at C-28. The appearance of signals at δ_H 6.24 (1H, d, J 15.8 Hz, H-8'), 6.85 (1H, d, J 7.8, H-5'), 6.96 (1H, br d, J 8.3 Hz, H-6'), 7.08 (1H, br s, H-2') and 7.53 (1H, d, J 15.8 Hz, H-7') indicates the presence of a 3',4'-dihydroxycinnamoyloxy moiety. Thus AG-4 is most likely to be betulin 3-caffeate (**4**) and comparison with published data[15] confirmed this conclusion.

The ^{13}C NMR spectrum of AG-4 is also in accord with this proposal. Thus it has five methyls [δ_C 28.1, 16.6, 16.2, 16.1, 14.6], an isopropenyl group [δ_C 150.3 (s), 109.7 (t), 19.0 (q)], a primary alcohol [δ_C 60.5], a secondary ester [δ_C 81.2] and ten other methylenes, five methines and five quaternary carbons (see Experimental). The appearance of three aromatic methines [δ_C 122.1, 115.3, 114.1], two olefinic methines [δ_C 144.8, 115.7], three aromatic singlets [δ_C 146.8, 144.3, 127.2] and an ester carbonyl [δ_C 167.8] confirms the 3',4'-dihydroxycinnamoyloxy moiety. Comparison with published data[15] again confirmed the structure of AG-4 as betulin 3-caffeate (**4**).

EXPERIMENTAL

Isolation. The plant material of *A. ghaesembilla* was collected from the Khulna district of Bangladesh. The stem bark (500g) was extracted in a Soxhlet apparatus with EtOAc. The EtOAc extract (8.0g) was concentrated *in vacuo* and a portion (4.0g) was fractionated by flash column chromatography over silica gel, eluting with petroleum ether, increasing amounts of EtOAc in petroleum ether and finally with MeOH (100ml fractions). The fractions were monitored by analytical tlc . The early fractions contained only fat. Fraction 7 gave AG-1 (100mg) and fraction 5 gave AG-2 (25mg) upon multiple preparative tlc using petroleum ether : EtOAc [(90 : 10) and (96 : 4)]. Similarly , preparative tlc of fraction 15 [petroleum ether : EtOAc (75 : 25)] afforded AG-4 (20mg) and of fraction 18 [CH_2Cl_2 : MeOH (98 : 2)] gave AG-3 (8.0mg).

AG-1 (**1**), lupeol, white crystals (MeOH), mp 210-212°; $[\alpha]_D$ +30.4° (c, 0.58 in $CHCl_3$); IR ν_{max} : 3615, 3070, 3020, 1638, 1520, 1380, 1217, 1030, 887 cm^1; EIMS m/z (rel. int.) : 426 [M^+] (2), 411 [M^+-CH_3] (3), 408 [M^+-H_2O] (3), 218 (5), 207 (6), 189 (58), 163 (80), 135 (57), 107 (68), 105 (55), 79 (54), 43 (100); ^1H NMR : δ_H 0.74, 0.77, 0.81, 0.93, 0.94, 1.01 (Me-28, Me-23, Me-24, Me-25, Me-26, Me-27), 1.66 (3H, br d, J 0.5 Hz, Me-30), 3.20 (1H, dd, J 9.6, 6.2 Hz, Hα-3), 4.58 (1H,

d, J 0.4 Hz, H_a-29), 4.65 (1H, dq, J 0.4, 0.5 Hz, H_b-29) ; ^{13}C NMR : δ_C 38.7 (C-1), 27.4 (C-2), 78.9 (C-3), 38.8 (C-4), 55.3 (C-5), 18.3 (C-6), 34.2 (C-7), 40.8 (C-8), 50 4 (C-9), 37.1 (C-10), 20.9 (C-11), 25.1 (C-12), 38.0 (C-13), 42.8 (C-14), 27.4 (C-15), 35.5 (C-16), 42.9 (C-17), 48.2 (C-18), 47.9 (C-19), 150.8 (C-20), 27.8 (C-21), 40.0 (C-22), 28.0 (C-23), 15.4 (C-24), 16.1 (C-25), 15.9 (C-26), 14.5 (C-27), 18.0 (C-28), 109.3 (C-29), 19.3 (C-30).

AG-2 (**2**) , lupeone, crystals (MeOH), mp 166-168°; $[\alpha]_D$ +60° (c, 0.52 in $CHCl_3$); IR ν_{max} : 3070, 3018, 1701 (C=O), 1640, 1460, 1350, 887 cm^{-1}; EIMS m/z (rel. int.) : 424 [M^+] (8), 409 [M^+-CH_3] (9), 394 (10), 381 (13), 313 (12), 218 (22), 205 (52), 189 (28), 149 (28), 135 (35), 121 (57), 107 (68), 105 (44), 95 (82), 81 (88), 79 (56), 55 (72), 43 (100); ^1H NMR : δ_H 0.73, 0.86, 0.89, 0.96 (4xMe) , 1.00 (6H, s, 2xMe), 1.61 (3H, br d, J 0.5 Hz, Me-30), 4.51 (1H, d, J 0.4 Hz, H_a-29) , 4.62 (1H, dq, J 0.4, 0.5 Hz, H_b-29) ; ^{13}C NMR : δ_C 39.5 (C-1), 34.1 (C-2), 218.3 (C-3), 47.3 (C-4), 54.8 (C-5), 19.6 (C-6), 33.5 (C-7), 40.7 (C-8), 49.7 (C-9), 36.8 (C-10), 21.4 (C-11), 25.1 (C-12), 38.1 (C-13), 42.8 (C-14), 27.4 (C-15), 35.5 (C-16), 42.9 (C-17), 48.2 (C-18), 47.9 (C-19), 150.9 (C-20), 29.8 (C-21), 39.9 (C-22), 26.6 (C-23), 21.0 (C-24), 15.7 (C-25), 15.9 (C-26), 14.4 (C-27), 18.0 (C-28), 109.4 (C-29), 19.3 (C-30).

AG-3 (**3**), scopoletin, needles (EtOAc-petroleum ether), mp 204°; UV λ_{max} (log ε) : 342 (3.62) 297 (4.05), 260 (3.75), 251 (3.82), 225 (3.90) nm; IR ν_{max} : 3530, 2932, 2856, 1720, 1604,1583, 1560, 1518, 1422, 1388, 1262, 1218, 1140, 1017, 930, 850 cm^{-1}; EIMS m/z (rel. int.) : 192 [M^+] (27), 177 (26), 163 (10), 161 (30), 149 (36), 121 (33), 109 (22), 107 (25), 105 (21), 95 (32), 81 (41), 79 (36), 69 (65), 53 (63), 43 (100); ^1H NMR : δ_H 6.28 and 7.59 (ABq, J 9.5 Hz, H-3, H-4), 6.91 (1H, s, H-5), 6.84 (1H, s, H-8), 3.95 (3H, s, CH_3O-6).

AG-4 (**4**), betulin 3-caffeate, amorphous solid, $[\alpha]_D$ +33.2° (c, 0.50 in $CHCl_3$); UV λ_{max} (log ε) : 221 (4.21), 234 (4.03), 246 (3.86), 295 (3.69), 350 (3.95) nm; IR ν_{max} : 3550 (OH), 1710 (C=O), 1637, 1604, 1522, 929 (C=CH_2) cm^{-1}; EIMS m/z (rel. int.) : 604 [M^+] (1), 589 [M^+-CH_3] (2), 434 [M^+-170] (4), 189 (13), 180 (18), 164 (11), 163 ($C_9H_7O_3^+$) (100), 161 ($C_9H_5O_3^+$) (10), 147 (13), 145 (12), 135 (30), 134 (20), 133 (16), 129 (13), 123 (14), 121 (27), 119 (15), 112 (16), 109 (15), 107 (34), 105 (29), 95 (21), 93 (39), 91 (25), 89 (13), 84 (19), 81 (19), 79 (15), 77 (21), 69 (28), 55 (40), 43 (22); ^1H NMR : δ_H 7.53 and 6.24 (ABq, J 15.8 Hz, H-7', H-8'), 7.08 (1H, br s, H-2'), 6.96 (1H, br d, J 8.3 Hz, H-6'), 6.85 (1H, d, J 7.8 Hz, H-2'), 4.66, 4.56 (each 1H, br s, H_a-29, H_b-29), 1.66 (3H, s, Me-30), 0.99 (3H, s, Me-26), 0.96 (3H, s, Me-27), 0.89 (3H, s, Me-24), 0.86 (3H, s, Me-23), 0.84 (3H, s, Me-25), 4.56 (1H, m, H-3), 3.80 and 3.36 (ABq, J 11.0 Hz, H-28), 2.36 (1H, m, H-19); ^{13}C NMR : δ_C 38.4 (C-1), 23.8 (C-2), 81.2 (C-3), 38.0 (C-4), 55.4 (C-5), 18.2 (C-6), 33.9 (C-7), 40.6 (C-8), 50.3 (C-9), 37.1 (C-10), 20.8 (C-11), 25.3 (C-12), 37.0 (C-13), 42.4 (C-14), 26.9 (C-15), 29.5 (C-16), 46.9 (C-17), 50.3 (C-18), 49.2 (C-19), 150.3 (C-20), 29.6 (C-21), 33.9 (C-22), 28.1 (C-23), 16.6 (C-24), 16.2 (C-25), 16.1 (C-26), 14.6 (C-27), 60.5 (C-28), 109.7 (C-29), 19.0 (C-30), 127.2 (C-1'), 115.3 (C-2'), 144.3 (C-3'), 146.8 (C-4'), 114.1 (C-5'), 122.1 (C-6'), 144.8 (C-7'), 115.7 (C-8'), 167.8 (C-9').

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CHAPTER 9

CONSTITUENTS OF THE STEM BARK OF *AMOORA CUCULLATA* ROXB. (MELIACEAE).

INTRODUCTION

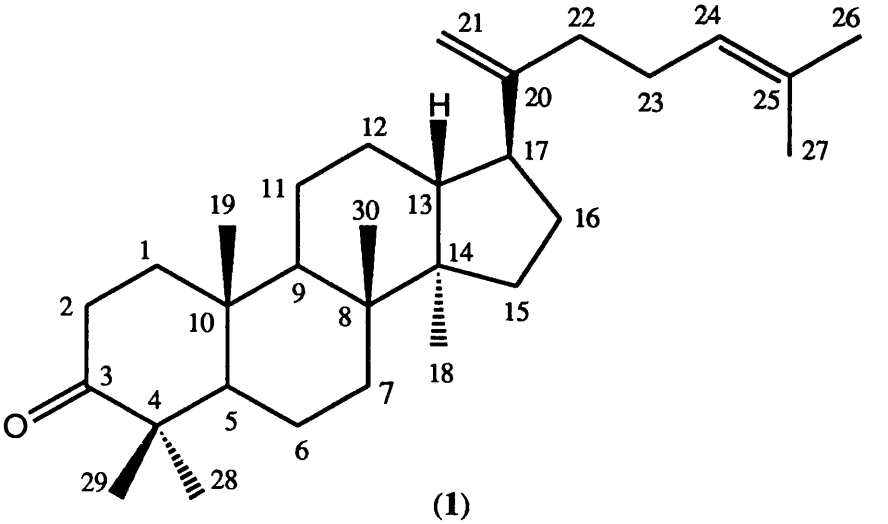
The genus *Amoora* (Meliaceae) comprises 16 species of trees which are distributed in the Indian subcontinent, Malay penisula and Australia[1]. Limonoids have been isolated from the species *Amoora grandifolia*[2,3,4] and *A. rohituka*[5,6,7,8,9,10], sesquiterpenes from *A. grandifolia*[11] and sterols from *A. rohituka*[12,13] and *A. wallichii*[14]. *Amoora cucullata* Roxb. is a tree which grows widely in the coastal forest of Bangladesh. It has been reported that bruised leaves are applied to remove inflamation[15]. Thus far no phytochemical work has been carried out on this plant.

RESULTS AND DISCUSSION

The EtOAc extract of the stem bark of *A. cucullata* yielded, upon chromatography over silica gel, seven known compounds AC-1, AC-2, AC-3, AC-4, AC-5, AC-6 and AC-7 which were subsequently identified as dammara-20,24-dien-3-one (**1**), 20(S)-hydroxydammar-24-en-3-one (**2**), cabraleone (**3**), cabraleadiol (**4**), cabralealactone (**5**), viridiflorol (**6**) and isofouquierone (**7**) and a new compound AC-8 was identified as 20(S), 24- dihydroxydammaran-3-one (**8**).

AC-1 ,$C_{30}H_{48}O$, was obtained as crystals, mp 76°, $[\alpha]_D$ +91.2° (c, 1.0 in $CHCl_3$) [lit.[16] dammara-20,24-dien-3-one, mp 75° , $[\alpha]_D$ +91.6 (c, 1.02 in $CHCl_3$)], ν_{max} 3050,1701 (ketone), 1630, 980, 880 cm^{-1}, whose mass spectrum [m/z 424 [M^+], 409 (M^+-15), 315 [M^+-C_8H_{13} (side chain)], 109 [base peak, C_8H_{13} (side chain cleavage)] indicates its triterpenoid nature. The ^1H NMR of AC-1 shows five quaternary methyls [δ_H 0.85, 0.94, 0.99, 1.02 and 1.06], two vinyl methyls [δ_H 1.60 and 1.66], an

exomethylene [δ_H 4.70 (2H, m)] and a vinyl proton [δ_H 5.12 (m)]. These data revealed a tetracyclic triterpenoid structure. Comparison with published data[16] led to the identification of AC-1 as dammara-20,24-dien-3-one (**1**).

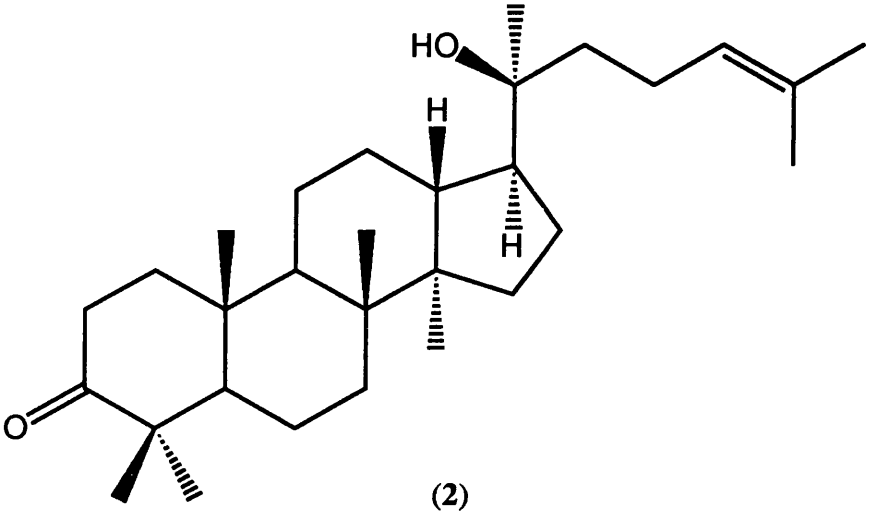


(**1**)

The ^{13}C NMR spectrum of AC-1 readily agreed with structure (**1**). Thus it has seven methyl signals [δ_C 15.3, 15.7, 16.1, 17.7, 21.0, 25.7 and 27.0], a trisubstituted double bond [δ_C 124.4 (d) and 131.4 (s)], an exomethylene [δ_C 107.5 (t) and 152.5 (s)] and a ketonic carbonyl group [δ_C 218.1], in addition to ten methylene, four methine and four quaternary carbons. The ^{13}C NMR data for (**1**) have not previously been reported. The assignments (see Experimental) were made by comparison with 20(S)-hydroxydammar-24-en-3-one[17] for the 3-oxodammarane nucleus and with dammara-20,24-dien-3β–ol[18] for the side chain.

AC-2, $C_{30}H_{50}O_2$, was obtained as white crystals, mp 134-136°, $[\alpha]_D$ +66° (c, 1.0 in $CHCl_3$) [lit.[19] 20(S)-hydroxydammar-24-en-3-one, mp 135-136°, $[\alpha]_D$ +65° (in $CHCl_3$)], ν_{max} 3600 (hydroxyl), 1698 (ketone) cm^{-1}, whose mass spectrum [m/z 427

(M^+-15), 424 (M^+-18) and 315 (M^+-sidechain)] again indicated a triterpenoid, probably a dammarane. The ^1H NMR spectrum of AC-2 shows eight tertiary methyls [δ_H 0.87, 0.93, 0.98, 1.02, 1.08, 1.13, 1.61 and 1.68] and a vinyl proton [δ_H 5.11 (m)]. These spectral data suggested a 3-oxodammarane nucleus with an uncyclized side chain containing a hydroxyl function at C-20 and a $\Delta^{24,25}$double bond. Comparison with the published data[20] for 20-hydroxydammar-24-en-3-one confirmed the identity of AC-2 as (**2**).

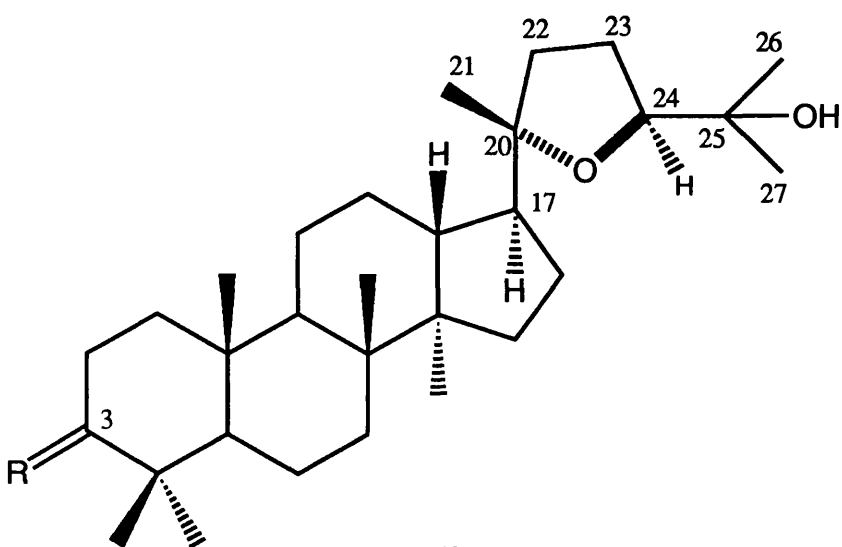


(**2**)

The ^{13}C NMR spectrum is in agreement with this structural proposal and has signals for eight methyls [δ_C 15.2, 16.0, 16.3, 17.7, 21.0, 25.4, 25.7.and 26.6], a tertiary carbinol [δ_C 75.3], a trisubstituted double bond [δ_C 124.6 (d) and 131.6 (s)] and a ketonic carbonyl [δ_C 218.1], in addition to ten methylene, four methine and four quaternary carbons. The ^{13}C data of AC-2 were identical with published values[17] for 20(S)- hydroxydammar-24-en-3-one (**2**), thus confirming its identity. The ^{13}C NMR chemical shifts of selected carbons provide a method for distinguishing between the R^{21}

and S[17] configurations at C-20. The chemical shifts of the two series (20R : 20S) are as follows : C-16 (δ_C 23.5, 24.8), C-17 (δ_C 49.4, 49.7), C-21 (δ_C 25.2, 25.4), C-22 (δ_C 41.8, 40.4).

AC-3, $C_{30}H_{50}O_3$, was isolated as needles, mp 163°, $[\alpha]_D$ +60° (c, 1.10 in $CHCl_3$) [lit.[22] cabraleone, mp 163-166.5°, $[\alpha]_D$ +60° (c, 1.20 in $CHCl_3$)], ν_{max} 3446 (OH), 1697 (ketone) cm^{-1}, whose mass spectrum did not show a molecular ion. The highest detectable peak is at m/z 443, evidently resulting from the loss of 15 mass units (Me-group) from m/z 458, the presumed molecular ion $C_{30}H_{50}O_3$. Other major fragments include peaks at 428 [M^+-15-15], 399 [M^+-59], 315 [M^+-$C_8H_{15}O_2^+$ (side chain)], 143 [$C_8H_{15}O_2^+$ (side chain)], 125 [$C_8H_{15}O_2^+$-18] and 59 [$(CH_3)_2COH]^+$. The ^1H NMR spectrum of AC-3 shows the presence of eight tertiary methyls [δ_H 0.87, 0.93, 1.00, 1.03, 1.07, 1.10, 1.14 and 1.18] and an oxygenated methine [δ_H 3.65 (dd, J 7.0, 6.2 Hz, H-24)]. Comparison with published data[22,23,24] confirmed the identity of AC-3 as cabraleone (**3**).



(**3**) R=O

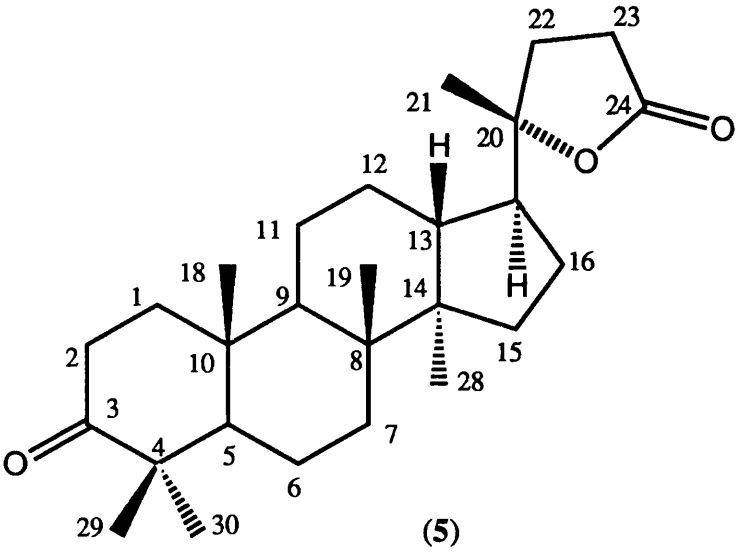
(**4**) R=H, α OH

The ^{13}C NMR spectrum reveals the presence of a ketonic carbonyl [δ_C 218.1], three oxygenated carbons [δ_C 70.1(s), 86.3(d), 86.2(s)] and eight methyls [15.0, 16.0, 16.1, 20.8, 23.9, 26.6, 27.0, 27.6], in addition to ten methylenes, four methines and four quaternary carbons. AC-3 is therefore a tetracarbocyclic triterpenoid ketone with a side chain containing a cyclic ether and a tertiary hydroxyl. These data readily led to structure (**3**) for AC-3 which is cabraleone. Comparison with published data[24,25] confirmed its identity.

AC-4, $C_{30}H_{52}O_3$, was obtained as needles, mp 175° $[\alpha]_D$ +18.2° (c, 1.00 in $CHCl_3$) [lit.[23] cabraleadiol, mp 175-176°, $[\alpha]_D$ +18° (c, 1.00 in $CHCl_3$)] , ν_{max} 3618 (hydroxyl), 3586 (hydroxyl), 1460, 1060 cm^{-1} and has peaks in its mass spectrum at m/z 460 [M^+], 445 (M^+-15), 427 (M^+-18-15), 143 [base peak, $C_8H_{15}O_2^+$ (side chain)] and 125 (side chain-18). The ^1H NMR spectrum shows eight methyl singlets [δ_H 0.84, 0.86, 0.89, 0.94, 0.97, 1.11, 1.15 and 1.19] and two protons attached to oxygenated carbons [δ_H 3.40 (t, J 3.6 Hz, H-3) and 3.65 (dd, J 7.2, 5.8 Hz, H-24)]. The above data suggested a 3α-hydroxydammarane with a side chain containing a cyclic ether and a tertiary hydroxyl group. Comparison with published data[22,23,24] revealed its identity as cabraleadiol (**4**).

The ^{13}C NMR spectrum confirmed this deduction, showing signals for four oxygenated carbons [δ_C 76.2 (d, C-3), 70.2 (s, C-25), 86.6 (s, C-20) and 86.2 (d, C-24)], in addition to the expected eight methyl signals [δ_C 15.5, 16.0, 16.5, 22.1, 24.0, 27.1, 27.8 and 28.3], ten methylene, four methine and four quaternary carbons (see Experimental). Comparison with published data[24] again confirmed the identity .of AC-4 as cabraleadiol (**4**).

AC-5 was obtained as crystals, mp 182-184°, $[\alpha]_D$ +70.4° (c, 1.00 in $CHCl_3$) [lit.[23] cabralealactone, mp 181-183°, $[\alpha]_D$ +70° (c, 0.57 in $CHCl_3$)], ν_{max} 1701 (ketone), 1759 (γ-lactone) cm^{-1}. The mass spectrum exhibits a molecular ion [M^+] peak at m/z 414 corresponding to $C_{27}H_{42}O_3$ and peaks at m/z 399 (M^+-15), 384 (M^+-15-15) and 205 (base peak). The ^1H NMR spectrum of AC-5 shows six tertiary methyl signals [δ_H 0.88, 0.92, 0.98, 1.02, 1.06 and 1.37] including one (Me-21) which is deshielded with respect to (**4**). No downfield signals are observed. Comparison with published data[22,23] led to the identification of AC-5 as cabralealactone (**5**).

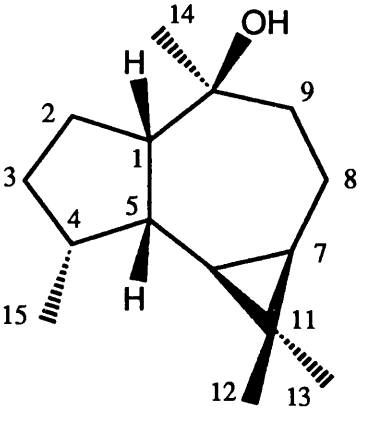


(**5**)

The ^{13}C NMR spectrum of AC-5 reveals a ketonic carbonyl [δ_C 218.1], a lactone carbonyl [δ_C 176.8], a quaternary oxygenated carbon [δ_C 90.1], six methyl groups [δ_C 15.1, 16.0, 16.1, 21.0, 25.4, 26.6], in addition to ten methylene, four methine and four quaternary carbons. The loss of three carbons , including two methyl groups , relative to a normal triterpenoid suggested oxidative cleavage of the end of the side chain with

formation of a γ-lactone. This reasoning led to the cabralealactone structure (**5**) for AC-5. Comparison with published data[26] confirmed its identity with cabralealactone.

AC-6 was obtained as crystals, mp 74°, $[\alpha]_D$ +3.2° (c, 0.10 in $CHCl_3$) [lit.[27] viridiflorol, mp 74° , $[\alpha]_D$ +2.0° (in $CHCl_3$)], ν_{max} 3603, 3450 cm^{-1}. The mass spectrum shows a molecular ion peak at m/z 222, corresponding to $C_{15}H_{26}O$. AC-6 is therefore a sesquiterpenoid. Its ^1H NMR spectrum reveals the presence of a cyclopropane ring [δ_H 0.11 (1H, dd, J 9.1 Hz) and 0.61 (1H, m)], in addition to three tertiary methyl signals [δ_H 0.99, 1.01 and 1.14] and a secondary methyl [δ_H 0.93 (d, J 6.6 Hz)]. Comparison with published data[27] led to the identification of AC-6 as viridiflorol (**6**).

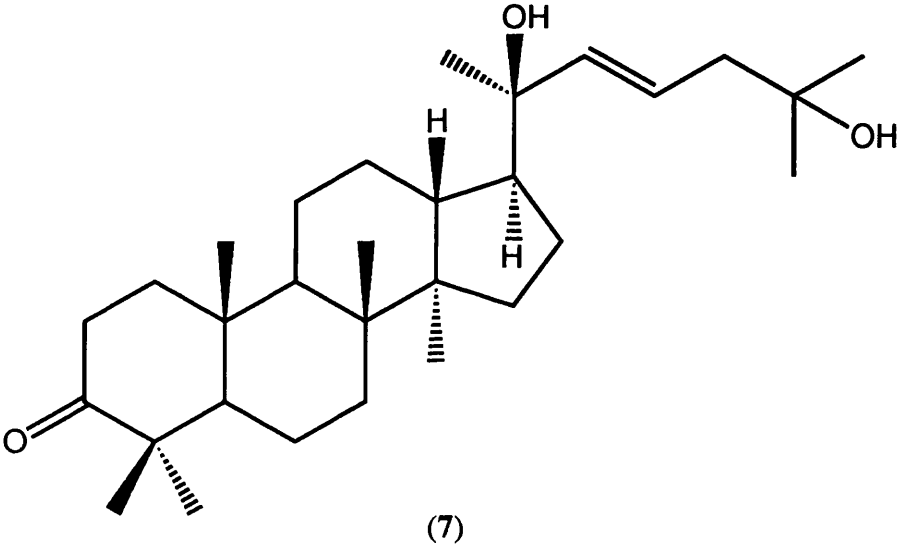


(**6**)

The ^{13}C NMR spectrum of AC-6 exhibits four methyls [δ_C 16.1, 16.3, 28.7 and 32.1] and a quaternary oxygenated carbon [δ_C 74.6] and confirms the presence of the cyclopropane [δ_C 18.4 (s), 22.2 (d), 28.5 (d)]. AC-6 is therefore a tricyclic cyclopropane-containing sesquiterpenoid. The most obious conclusion is that it has an

aromadendrane nucleus. The remaining carbon resonances, four methylenes [δ_C 18.8, 25.8, 29.0 and 37.7] and three methines [δ_C 38.4, 39.7 and 58.2], are consistent with this suggestion. Comparison with published data[27] confirmed to the identification of AC-6 as viridiflorol (**6**).

AC-7, $C_{30}H_{50}O_3$, was isolated as gum, $[\alpha]_D$ +33.0° (c, 1.00 in $CHCl_3$) [lit.[24] isofouquierone, $[\alpha]_D$ +34° (c, 0.10 in $CHCl_3$)], ν_{max} 3607 (hydroxyl), 3453 (hydroxyl), 1710 (ketone) cm^{-1} and is clearly a triterpenoid. The mass spectrum displays peaks at m/z 459 (M^++1), 443 (M^+-15), 425 (M^+-15-18), 359 (M^+-$C_6H_{11}O$) and 82 [base peak, $(C_6H_{10})^+$]. The ^1H NMR spectrum shows eight methyl singlets [δ_H 0.85, 0.92, 0.97, 1.01, 1.05, 1.12, 1.32 (2)], two olefinic protons [δ_H 5.66 (m)] and two deshielded methylenes [δ_H 2.18 and 2.45 (both 2H, m)]. Consideration of the ^{13}C NMR data (below) and comparison with published[24] data reveals the identity of AC-7 as isofouquierone (**7**).

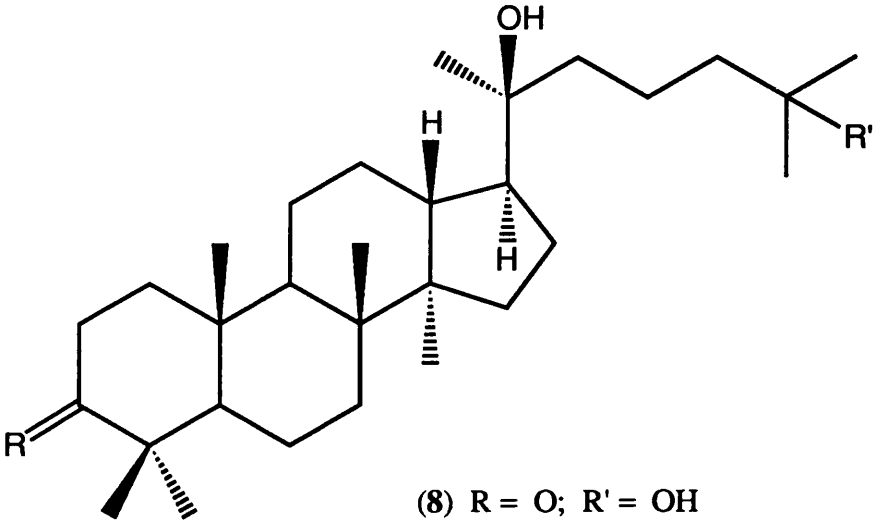


(**7**)

The ^{13}C NMR spectrum revealed a ketonic carbon [δ_C 218.3], two tertiary hydroxyl bearing carbons [δ_C 70.7 and 75.0], disubstituted olefinic carbons [δ_C 122.2 (d) and 142.0 (d)] and eight tertiary methyl groups [δ_C 15.1, 16.0, 16.3, 21.0, 25.6, 26.6, 29.8 and 29.9], in addition to nine methylene, four methine and four quaternary carbons. These data are consistent with a 3-ketodammarane structure (**7**), containing an unsaturated dihydroxy side chain. This structure belongs to the known compound isofouquierone and comparison with published data[24] confirmed its identity with AC-7. There is a difference in chemical shift of one methylene signal [δ_C 24.8 for AC-7, 27.4 in ref.(24)] which we assume is due to an error in the published value. This is the second report of the isolation of isofouquierone from a plant source.

AC-8 was also obtained as gum, $[\alpha]_D$ +17.8° (c, 0.59 in $CHCl_3$), ν_{max} 3570 (hydroxyl), 3452 (hydroxyl), 1698 (ketone) cm^{-1}. The mass spectrum indicates that AC-8 is a triterpenoid, showing *inter alia* peaks at m/z 442 (M^+-18), 427 (M^+-18-15), 424 (M^+-2x18) and 412 (M^+-18-2x15). Other peaks associated with the side chain are observed at m/z 315 (M^+-$C_8H_{17}O_2$), 145 [$C_8H_{17}O_2^+$ (side chain)], 127 [base peak, $C_8H_{15}O^+$ (side chain-18)] and 109 ($C_8H_{13}^+$). The ^1H NMR shows eight methyl singlets [δ_H 0.85, 0.91, 0.96, 1.00, 1.04, 1.12, 1.21 (2)] and a deshielded methylene [δ_H 2.45 (m)] and suggested a close similarity to isofouquierone (**7**) but with a saturated dihydroxy side chain. Comparison of the ^1H chemical shifts of AC-8 with isofouquierone[24] for the 3-oxodammarane nucleus protons and with dammarane-20(S),25-diol[28,29] for side chain protons suggests that AC-8 is 20S,25-dihydroxydammarane-3-one (**8**).

The ^{13}C NMR spectrum of AC-8 confirmed the presence of a ketone [δ_C 218.3] and two tertiary alcohols [δ_C 70.9, 75.3] and shows, in addition, eight methyls [δ_C 15.1, 16.0, 16.3, 20.9, 25.4, 26.6, 29.2, 29.3], eleven methylenes, four



(**8**) R = O; R' = OH

(**9**) R = O; R' = H

(**10**) R = H,H; R' = OH

methines and four fully substituted carbons. Comparison of the ^{13}C chemical shifts of AC-8 with those of 20S-hydroxydammarane-3-one[17] (**9**) (for the carbon skeleton) and 20*S*, 25- dammaranediol[17] (**10**) (for the side chain) are shown in **Table 9**. It is apparent that AC-8 is 20*S*, 25-dihydroxydammaran-3-one (**8**), which is a new compound.

TABLE 9 : ^{13}C NMR of compounds (8), (9) and (10)

Carbons	Compounds		
	(8)	(9)	(10)
C-1	39.8	39.5	
C-2	34.0	33.8	
C-3	218.3	217.6	
C-4	47.3	47.1	
C-5	55.3	55.1	
C-6	19.5	19.4	
C-7	34.4	34.3	
C-8	40.2	40.0	
C-9	49.9	49.8	
C-10	36.7	36.5	
C-11	21.9	21.8	
C-12	27.4	27.3	
C-13	42.3	42.1	
C-14	50.2	50.0	
C-15	31.1	31.0	
C-16	24.7	24.5	
C-17	49.7	49.3	
C-18	16.0	15.8	
C-19	15.1	15.0	
C-20	75.3		75.4
C-21	25.4		25.6
C-22	40.9		41.1
C-23	18.3		18.5
C-24	44.3		44.6
C-25	70.9		71.1
C-26	29.2*		29.3*
C-27	29.3*		29.5*
C-28	26.6	26.5	
C-29	20.9	20.8	
C-30	16.3	16.2	

* may be interchangeable

EXPERIMENTAL

Isolation. The plant material of *Amoora cucullata* was collected from the coastal forest , Sundarban, Khulna district, Bangladesh. The ground stem bark (840g) was extracted in a Soxhlet apparatus with EtOAc. The EtOAc extract (19g) was concentrated *in vacuo* and a portion (12g) was fractionated by flash chromatography over silica gel. The column was first eluted with petroleum ether and increasing amounts of EtOAc in petroleum ether and finally with MeOH (100ml fraction). Fractions were monitored by analytical tlc. The early fractions contained mainly fat while the later fractions showed many spots on analytical tlc. Multiple preparative tlc of fraction 5 using petroleum ether : EtOAc (94 : 6) gave AC-1 (90mg). Fraction 6 and 7 afforded AC-2 (3.0g) as crystals. Multiple preparative tlc of fractions 6, 8, 9 using petroleum ether : EtOAc (85 : 15) afforded AC-3 (100mg), AC-4 (10mg), AC-5 (16mg) and AC-6 (13mg). Preparative tlc of fraction 17 using CH_2Cl_2 : MeOH (98 : 2) afforded AC-7 (36mg) and AC-8 (50mg).

AC-1 (dammara-20,24-dien-3-one, **1**), colourless crystals (MeOH), mp 76°, $[\alpha]_D$ +91.2° (c, 1.00 in $CHCl_3$); IR ν_{max} : 3050,1701,1630, 1222,1150, 1112, 1076, 980, 895, 880 cm^{-1}; EIMS m/z (rel. int.): 424 (M^+) (42), 409 (M^+-15) (6), 315 (M^+-C_8H_{13}) (9), 300 (8), 205 (54), 190 (10), 175 (13), 147 (28), 109 ($C_8H_{13}^+$) (100), 81 (50), 43 (70); ^1H NMR : δ_H 0.85, 0.94, 0.99, 1.02, 1.06 (Me-18, Me-19, Me-28, Me-29, Me-30), 1.60, 1.66 (Me-26, Me-27), 4.70 (2H, m, 2H-21), 5.12 (1H, m, H-24); ^{13}C NMR : δ_C 40.0 (C-1), 34.0 (C-2), 218.1 (C-3), 47.3 (C-4), 55.2 (C-5), 19.6 (C-6), 34.0 (C-7), 40.3 (C-8), 50.2 (C-9), 36.8 (C-10), 21.8 (C-11), 27.0 (C-12), 45.3 (C-13), 49.3 (C-14), 31.3 (C-15), 28.8 (C-16), 47.7 (C-17), 15.7 (C-18), 15.3 (C-19), 152.5 (C-20), 107.5 (C-21), 39.9 (C-22), 24.9 (C-23), 124.4 (C-24), 131.4 (C-25),

25.7 (C-26), 17.7 (C-27), 27.0 (C-28), 21.0 (C-29), 16.1 (C-30). HREIMS : Found 424.3700; calculated for $C_{30}H_{48}O$ 424.3705.

AC-2 (20*S*-hydroxy -dammar-24-en-3-one, **2**), white crystals (ether), mp 134-136°; $[\alpha]_D$ +66° (c, 1.00 in $CHCl_3$); IR ν_{max} : 3600, 3020, 1698, 1520, 1425, 928 cm^{-1}; EIMS m/z (rel. int.) : 427 [M^+-CH_3] (2), 424 [M^+-H_2O] (20), 315 (5), 205 (13), 147 (9), 127 (15), 109 (100), 105 (12), 95 (54), 82 (20), 67 (43), 43 (94); ^1H NMR : δ_H 0.87, 0.93, 0.98, 1.02, 1.08 (Me-18, Me-19, Me-28, Me-29, Me-30), 1.13,1.61, 1.68 (Me-21, Me-26, Me-27), 5.11 (1H, m, H-24); ^{13}C NMR : δ_C 39.8 (C-1), 34.1 (C-2), 218.1 (C-3), 47.8 (C-4), 55.3 (C-5), 19.6 (C-6), 34.5 (C-7), 40.2 (C-8), 49.9 (C-9), 36.8 (C-10), 22.0 (C-11), 27.5 (C-12), 42.3 (C-13), 50.2 (C-14), 31.1 (C-15), 24.8 (C-16), 49.7 (C-17), 16.0 (C-18), 15.2 (C-19), 75.3 (C-20), 25.4 (C-21), 40.4 (C-22), 22.5 (C-23), 124.6 (C-24), 131.6 (C-25), 25.7 (C-26), 17.7 (C-27), 26.6 (C-28), 21.0 (C-29), 16.3 (C-30).

AC-3 (cabraleone, **3**), needles (MeOH), mp 163°; $[\alpha]_D$ +60° (c, 1.10 in $CHCl_3$); IR ν_{max} : 3440, 1697, 1460, 1222, 1070 cm^{-1}; EIMS m/z (rel. int.): 443 [M^+-CH_3] (10), 428 [M^+-CH_3-CH_3] (4), 399 [M^+-C_3H_7O] (4), 381 (12), 315 [M^+-$C_8H_{15}O_2$ (side chain)], 143 [$C_8H_{15}O_2^+$ (side chain)] (35), 125 ($C_8H_{15}O_2^+$-18) (100), 59 (70), 43 (30); ^1H NMR: δ_H 0.87, 0.93, 1.00, 1.03, 1.07 (Me-18, Me-19, Me-28, Me-29, Me-30), 1.10, 1.14, 1.18 (Me-21, Me-26, Me-27), 3.65 (1H, dd, J 7.0, 6.2 Hz, H-24); ^{13}C NMR: δ_C 40.1 (C-1), 33.9 (C-2), 218.1 (C-3), 47.2 (C-4), 55.1 (C-5), 19.3 (C-6), 34.4 (C-7), 39.7 (C-8), 50.0 (C-9), 36.7 (C-10), 22.1 (C-11), 25.7 (C-12), 42.8 (C-13), 49.8 (C-14), 31.3 (C-15), 26.8 (C-16), 49.6 (C-17), 16.0 (C-18), 15.0 (C-19), 86.2 (C-20), 23.9 (C-21), 34.5 (C-22), 26.2 (C-23), 86.3 (C-24), 70.1 (C-25), 26.6 (C-26), 27.0 (C-27), 27.6 (C-28), 20.8 (C-29), 16.1 (C-30).

AC-4 (cabraleadiol, **4**), needles (MeOH), mp 175°; $[\alpha]_D$ +18.2° (c, 1.00 in $CHCl_3$); IR ν_{max}: 3618, 3586, 2965, 1460, 1060 cm^{-1}; EIMS m/z (rel. int.) : 460 [M^+] (1), 445 [M^+-CH_3] (1), 427 [M^+-CH_3-H_2O] (1), 401 (2), 383 (8), 191 (5), 143 [$C_8H_{15}O_2^+$ (side chain)] (100), 125 ($C_8H_{13}O^+$) (10), 59 (78); ^1H NMR : δ_H 0.84, 0.86, 0.89, 0.94, 0.97 (Me-18, Me-19, Me-28, Me-29, Me-30), 1.11, 1.15, 1.19 (Me-21, Me-26, Me-27), 3.40 (1H, t, J 3.6 Hz, Hβ–3), 3.65 (1H, dd, J 7.2, 5.8 Hz, H-24); ^{13}C NMR : δ_C 33.6 (C-1), 25.8 (C-2), 76.2 (C-3), 37.6 (C-4), 50.6 (C-5), 18.2 (C-6), 34.7 (C-7), 40.5 (C-8), 49.8 (C-9), 37.2 (C-10), 21.6 (C-11), 25.3 (C-12), 42.7 (C-13), 50.1 (C-14), 31.4 (C-15), 26.9 (C-16), 49.5 (C-17), 16.0 (C-18), 15.5 (C-19), 86.6 (C-20), 24.0 (C-21), 35.1 (C-22), 26.3 (C-23), 86.2 (C-24), 70.2 (C-25), 27.1 (C-26), 28.3 (C-27), 27.8 (C-28), 22.1 (C-29), 16.5 (C-30).

AC-5 (cabralealactone, **5**), colourless crystals (MeOH), mp 182-184°; $[\alpha]_D$ +70.4° (c, 1.00 in $CHCl_3$); IR ν_{max} : 3020, 1759, 1701, 1560, 1223, 930, 850 cm^{-1}; EIMS m/z (rel. int.): 414 [M^+] (65), 399 [M^+-CH_3] (9), 396 (7), 329 (11), 328 (9), 316 (23), 315 (35), 205 (100), 195 (13), 135 (32), 121 (35), 109 (38), 107 (46), 99 (56), 95 (70), 81 (64), 79 (36), 69 (38), 67 (49), 55 (63), 43 (30); ^1H NMR : δ_H 0.88, 0.92, 0.98, 1.02, 1.06, 1.37 (Me-18, Me-19, Me-28, Me-29, Me-30, Me-21); ^{13}C NMR : δ_C 39.8 (C-1), 34.0 (C-2), 218.1 (C-3), 47.4 (C-4), 55.2 (C-5), 19.6 (C-6), 34.4 (C-7), 40.2 (C-8), 49.8 (C-9), 36.8 (C-10), 21.9 (C-11), 26.8 (C-12), 43.2 (C-13), 50.1 (C-14), 31.1 (C-15), 29.2 (C-16), 49.2 (C-17), 16.0 (C-18), 15.1 (C-19), 90.1 (C-20), 25.4 (C-21), 25.0 (C-22), 31.0 (C-23), 176.8 (C-24), 21.0 (C-29), 16.1 (C-30); HREIMS : Found 414.3137; calculated for $C_{27}H_{42}O_3$ 414.3133.

AC-6 (viridiflorol, **6**), crystals (MeOH), mp 74°, $[\alpha]_D$ +3.2° (c, 0.10 in $CHCl_3$); ν_{max} : 3603, 3450, 3020, 2400, 1522, 1420, 1375, 1120, 1020, 985, 930 cm^{-1}; EIMS m/z (rel. int.) : 222 [M^+] (1), 204 [M^+-H_2O] (10), 189 (13), 164 (9), 105 (40), 93 (48),

91 (41), 83 (44), 81 (62), 69 (57), 55 (73), 43 (100); ^{1}H NMR : δ_H 0.11 (dd, J 9.1 Hz, H-6), 0.61 (m, H-7), 0.99, 1.01, 1.14 (Me-12, Me-13, Me-14), 0.93 (d, J 6.6 Hz, Me-15); ^{13}C NMR : δ_C 58.2 (C-1), 37.7 (C-2), 25.8 (C-3), 38.4 (C-4), 39.7 (C-5), 28.5 (C-6), 22.2 (C-7), 18.8 (C-8), 29.0 (C-9), 74.6 (C-10), 18.3 (C-11), 16.3 (C-12), 28.7 (C-13), 32.1 (C-14), 16.1 (C-15).

AC-7 (isofouquierone, **7**), gum, $[\alpha]_D$ +33.0° (c, 1.00 in $CHCl_3$); IR ν_{max} : 3607, 3453, 2964, 1710, 1522, 1460, 1030, 930 cm^{-1}; EIMS m/z (rel. int.) : 459 [M^++1] (1), 443 [M^+-CH_3] (1), 425 [M^+-15-18], 359 [M^+-$C_6H_{11}O$)] (26), 315 [M^+-$C_8H_{15}O_2$] (6), 205 (14), 159 (22), 143 (15), 135 (23), 127 (31), 125 (40), 95 (55), 82 (100); ^{1}H NMR : δ_H 0.85, 0.92, 0.97, 1.01, 1.05 (Me-18, Me-19, Me-28, Me-29, Me-30), 1.12 (Me-21), 1.32 (Me-26, Me-27), 2.18 (m, 2H-22), 2.45 (m, 2H-2), 5.66 (m, H-23, H-24); ^{13}C NMR : δ_C 39.8 (C-1), 34.1 (C-2), 218.3 (C-3), 47.4 (C-4), 55.2 (C-5), 19.6 (C-6), 34.4 (C-7), 40.2 (C-8), 49.9 (C-9), 36.7 (C-10), 21.9 (C-11), 24.8 (C-12), 42.4 (C-13), 50.2 (C-14), 31.1 (C-15), 27.4 (C-16), 49.7 (C-17), 16.0 (C-18), 15.1 (C-19), 75.0 (C-20), 25.6 (C-21), 43.3 (C-22), 122.2 (C-23), 142.0 (C-24), 70.7 (C-25), 29.9 (C-26), 29.8 (C-27), 26.6 (C-28), 21.0 (C-29), 16.3 (C-30).

AC-8 (20*S*, 25-dihydroxydammaran-3-one, **8**), gum, $[\alpha]_D$ +17.8° (c, 0.59 in $CHCl_3$); IR ν_{max} : 3570 (OH), 3452 (OH), 2960, 1698 (C=O), 1522, 1460, 1030, 925 cm^{-1}; EIMS m/z (rel. int.) : 442 [M^+-H_2O] (1), 427 [M^+-H_2O-CH_3] (5), 424 [M^+-2xH_2O] (3), 412 [M^+-H_2O- 2xCH_3] (1), 315 [M^+-$C_8H_{17}O_2$ (side chain)] (5), 205 (9), 163 (11), 145 [$C_8H_{17}O_2^+$ (side chain)] (15), 135 (11), 127 [$C_8H_{15}O^+$] (100), 109 [$C_8H_{13}^+$] (60), 95 (36), 82 (44), 43 (40); ^{1}H NMR δ_H 0.85 , 0.91, 0.96, 1.00, 1.04 (Me-18, Me-19, Me-28, Me-29, Me-30), 1.12 (s, Me-21), 1.20 (s, Me-26, Me-27), 2.45 (m, 2H-2); ^{13}C NMR (see **Table 9**). HREIMS : Found [M^+-H_2O-Me] 427.3595; calculated for $C_{29}H_{47}O_2$ 427.3575.

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CHAPTER 10

CONSTITUENTS OF THE STEM BARK OF *PIPER CHABA* HUNTER (PIPERACEAE).

INTRODUCTION

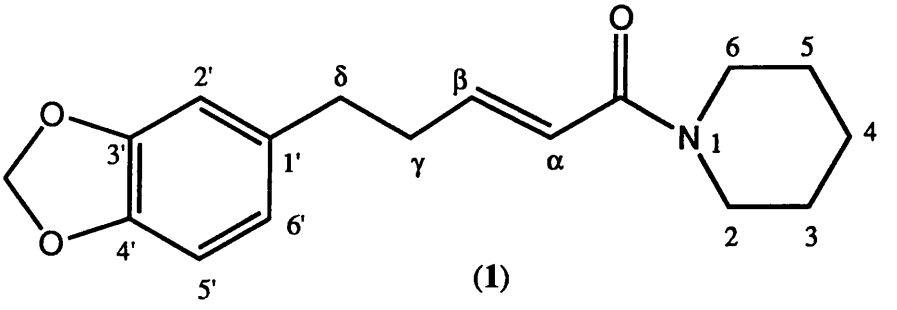
The genus *Piper* (Piperaceae) comprises 500 species which are distributed in tropical and subtropical regions. They are mostly shrubs and rarely herbs or trees[1]. *Piper chaba* Hunter is a much branched, rambling shrub which is cultivated in Bangladesh, India and Malay[2]. The fruit is used for treatment of coughs and common cold[3] and the stem bark for asthma, cough, cold, indigestion, loss of appetite and piles[4]. The antitubercular properties of this plant have also been reported[5]. Preliminary pharmacological examination revealed a hypotensive and smooth muscle relaxant action[6]. Earlier phytochemical work on this plant resulted in the isolation of alkaloids[7,8,9,10], amino acids and monosaccharides[11] and sterols[8,9]. Recently alkamides and a lignan have been isolated[12].

RESULTS AND DISCUSSION

On chromatography over silica gel the ethyl acetate extract of the stem bark of *P. chaba* yielded four known compounds PC-1, PC-2, PC-3 and PC-4 which were subsequently identified as the known piperidine amide alkaloids piperanine (**1**) and 2,4-decadienoic acid piperidide (**2**), the alkamide alkaloid pellitorine (**3**) and the dibenzyl butyrolactone lignan , (-)-kusunokinin (**4**).

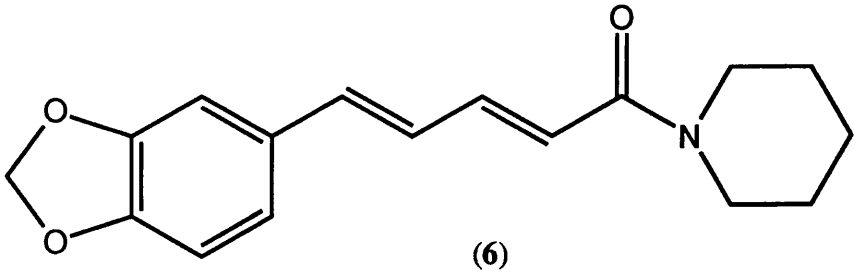
PC-1 (**1**), $C_{17}H_{21}NO_3$, was obtained as white crystals, mp 80° [lit.[13] piperanine, mp 79-80°]. It is UV active [λ_{max} 234, 290, 345 nm] and gave a single Dragendorff positive spot on tlc (silica gel), indicating its alkaloidal nature. It has bands in the IR spectrum arising from amide [ν_{max} 1670 cm^{-1}] and aromatic and double bond [ν_{max} 3020, 1624, 1600, 1580, 1446, 1022 cm^{-1}] absorption. The mass spectrum displays a molecular ion [M^+] at m/z 287 [$C_{17}H_{21}NO_3$] and other fragments at m/z 286 [M^+-1],

203 [M^+-84 (piperidine ring)], 201[M^+-$C_5H_{12}N$], 175 [M^+-$C_6H_{10}NO$], 135 [$C_8H_7O_2^+$ (base peak)], 112 [$C_6H_{10}NO^+$] and 84 [$C_5H_{10}N^+$ (piperidine ring)], indicating its piperidine amide nature.



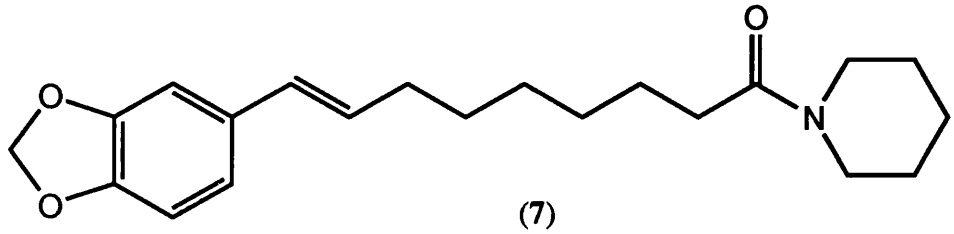
(1)

The ^1H NMR spectrum of PC-1 (**1**) shows signals at δ_H 1.66 [6H, m, 2H-3, 2H-4, 2H-5], 2.40-2.75 [4H, m, 2H-γ, 2H-δ], 3.55 [4H, br, 2H-2, 2H-6], 5.95 [2H, s, CH_2O_2], 6.35 [1H, d, J 14.6 Hz, H-α] and 6.60-6.90 [4H, m, H-β, H-2', H-5', H-6']. These data revealed a piperidine amide alkaloid with α,β-unsaturation and a methylene dioxy group in the aromatic ring. Comparison with published data[13] led to the identification of PC-1 as piperanine (**1**).



(6)

The ^{13}C NMR spectrum of PC-1 (**1**) is in accord with the proposed structure. Thus it has seven methylene signals [δ_C 46.9, 43.2, 34.5, 34.4, 26.7, 25.6, 24.9], five methines [δ_C 142.5, 122.5, 120.0, 108.8, 105.6], a methylenedioxy group [δ_C 101.7], an amide carbonyl [δ_C 165.4] and three aromatic quaternary carbons [δ_C 148.2, 148.1, 131.1]. The ^{13}C data for (**1**) have not previously been reported. The assignments (see Experimental) were made by comparison with piperine (**6**)[14] and pipernonaline (**7**)[15]. This alkaloid has been previously isolated from other *Piper* species[16].

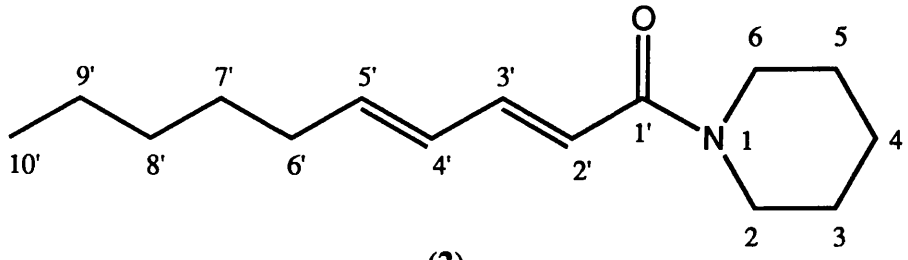


(**7**)

PC-2 (**2**), $C_{15}H_{25}NO$, was obtained as gum. It is UV active (λ_{max} 250 nm) and gave a Dragendorff positive spot on tlc (silica gel), indicating its alkaloidal nature. It has bands in its IR spectrum at 1655 (amide C=O) and 1637, 1603, 1550 and 1016 (CH=CH) cm-^{1}. Its mass spectrum displays a molecular ion peak [M^+] at m/z 235 ($C_{15}H_{25}NO$) together with fragments corresponding to the loss of various alkyl groups at m/z 206 [M^+-C_2H_5], 193 [M^+-C_3H_6], 192 [M^+-C_3H_7], 178 [M^+-C_4H_9], 164 [M^+-C_5H_{11}, base peak], 151 [M^+-$C_5H_{10}N$ (piperidine ring)] and 138 [M^+-C_7H_{13}]. A peak at m/z 84 [$C_5H_{10}N^+$ (piperidine ring)] indicates the piperidine amide nature of the compound.

The ^1H NMR of PC-2 (**2**) has signals at δ_H 6.13 (1H, d, J 14.8 Hz, H-2'), 7.22 (1H, dd, J 14.8, 10.0 Hz, H-3'), 6.00-6.30 (2H, m, H-4', H-5'), 3.58 (4H, br, 2H-2, 2H-6), 1.58 (6H, br, 2H-3, 2H-4, 2H-5), 2.13 (2H, dt, J 7.0, 6.0 Hz, 2H-6'), 1.27 (6H, m, 2H-7', 2H-8', 2H-9') and 0.87 (3H, t, J 7.0 Hz, Me-10'). These data revealed

a piperidine amide alkaloid with an aliphatic chain having double bonds at 2' and 4' position. Comparison with published data[17] led to the identification of PC-2 as 2,4-decadienoic acid piperidide (**2**).

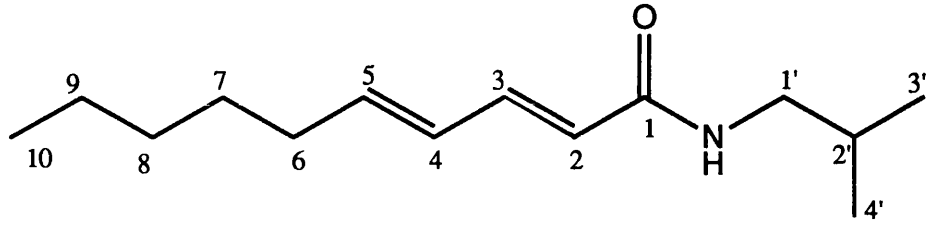


(**2**)

The ^{13}C NMR of PC-2 (**2**) readily accorded with the proposed structure. Thus it has five methylene signals for the piperidine ring [δ_C 43.2 (C-2), 26.6 (C-3), 24.7 (C-4), 25.6 (C-5), 46.9 (C-6)], an amide carbonyl [δ_C 165.7 (C-1'), four olefinic methines [δ_C 118.5 (C-2'), 142.7 (C-3'), 128.8 (C-4'), 142.9 (C-5')], another four methylene of the aliphatic chain [δ_C 32.9 (C-6'), 28.5 (C-7'), 31.4 (C-8'), 22.5 (C-9')] and a methyl carbon [δ_C 14.0 (C-10')]. The ^{13}C data for (**2**) have not previously been reported. The assignments were made by comparison with piperine (**5**)[14] and pellitorine (**3**)[18] (see Experimental). This is the first report of the occurrence of this alkaloid in *Piper* species.

PC-3 (**3**), $C_{14}H_{25}NO$, was obtained as colourless needles, mp 89° [lit.[19] pellitorine, mp 89-90°]. It is UV active [λ_{max} 260 nm] and showed a single Dragendorff positive spot on tlc (silica gel), indicating its alkaloidal nature. Its IR spectrum exhibits NH (ν_{max} 3400 cm^{-1}), amide (ν_{max} 1665 cm^{-1}) and double bond (ν_{max} 1620,1560, 1522, 1480, 1388, 1018 cm^{-1}) absorption. The mass shows a parent ion [M^+] at m/z 223 and fragments at m/z 222 [M^+-1], 208 [M^+-15], 180 [M^+-15-28], 167, 152 [M^+-C_4H_9N], 151 [M^+-$C_4H_{10}N$ (base peak)] and 72 [$C_4H_{10}N^+$, isobutylamine], suggesting an isobutylamide alkaloid.

The ^1H NMR of PC-3 (**3**) reveals a methyl [δ_H 0.89 (t, J 7.0 Hz, Me-10)], three methylenes [δ_H 1.30 (m, 2H-7, 2H-8, 2H- 9)], an allylic methylene [δ_H 2.14 (dt, J 7.0, 6.0 Hz, 2H-6)], four olefinic protons [δ_H 5.80 (1H, d, J 15.0 Hz); 5.95-6.20 (2H, complex m); 7.20 (1H, ddd, J 15.0, 8.8, 1.4 Hz)] and signals associated with an isobutylamide grouping [δ_H 0.92 (6H, d, J 6.7 Hz, Me-3', Me-4'); 1.77 (1H, m, H-2'), 3.15 (2H, t, J 6.7 Hz, 2H-1'); 5.88 (1H, br t, J 6.0 Hz, NH)]. These data are consistent with the presence of an isobutyryl alkamide alkaloid containing aliphatic acid chain (C_{10}) with double bonds at the 2 and 4 positions. Comparison with published data[18,19] led to the identification of PC-3 as pellitorine (**3**).

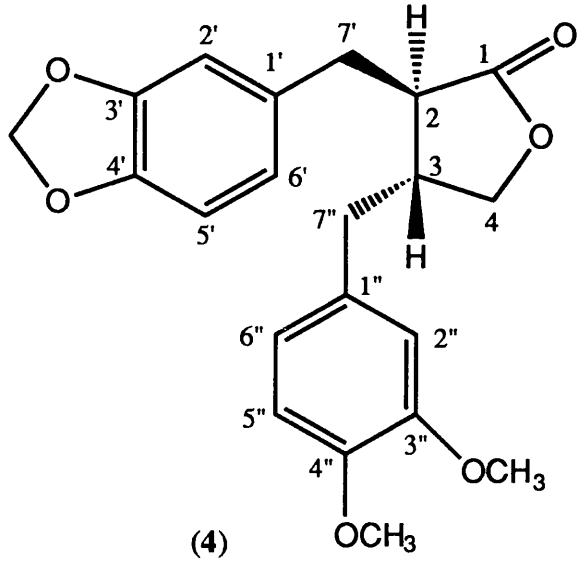


(**3**)

The ^{13}C NMR spectrum of PC-3 (**3**) shows an amide carbonyl [δ_C 166.5 (C-1)], four olefinic methines [δ_C 121.8 (C-2), 141.1 (C-3), 128.1 (C-4), 143.0 (C-5)], five methylenes [δ_C 32.8 (C-6), 28.4 (C-7), 31.3 (C-8), 22.4 (C-9), 46.8 (C-1')], one methine [δ_C 28.5 (C-2')] and three methyl carbons [δ_C 13.9 (C-10), 20.0 (C-3'), 20.0 (C-4')]. These data are in agreement with published data[18] and confirm the structure of pellitorine (**3**), which has been previously isolated from several *Piper* species[16,20].

PC-4 (**4**), $C_{21}H_{22}O_6$, was obtained as gum, $[\alpha]_D$ -34.2 (c, 0.50 in $CHCl_3$) [lit.[21] (-)-kusunokinin, $[\alpha]_D$ -34.5 (c, 0.52 in $CHCl_3$)]. It is UV active and has absorption bands at λ_{max} 230, 282, 285 nm. Its IR spectrum shows bands at 1763 (γ-lactone), 3020, 1601, 1577, 1560, 1504, 1442, 1018 (aromatic) and 1250 (C-O

streching) cm-[1]. Its mass spectrum has a molecular ion [M^+] at m/z 370, together with fragments at m/z 235 [M^+-135($C_8H_7O_2$)], 219 ($C_{12}H_{11}O_4^+$), 218 ($C_{12}H_{10}O_4^+$), 192 ($C_{10}H_8O_4^+$), 151 ($C_9H_{11}O_2^+$) and a base peak at m/z 135 ($C_8H_7O_2^+$), consistent with a lignan type structure.

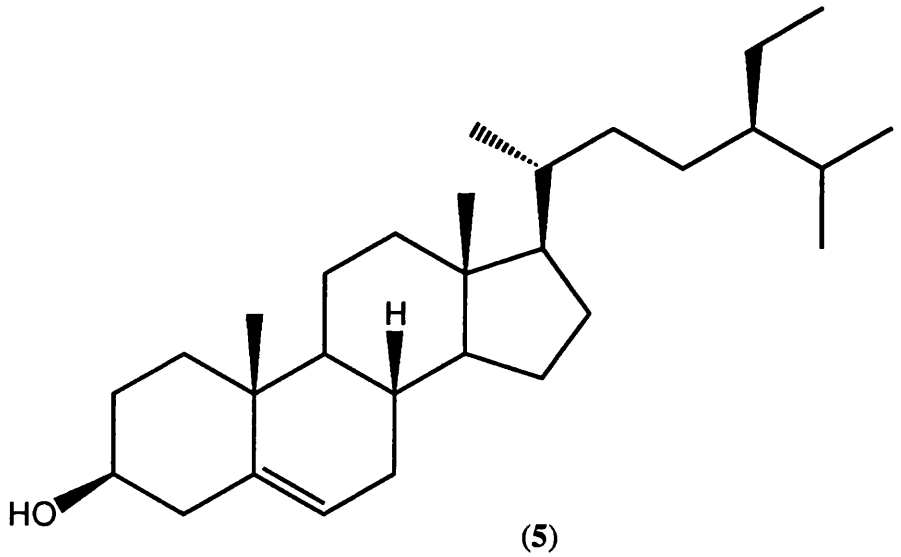


(4)

The ^1H NMR of PC-4 (**4**) shows signals for two 3,4-disubstituted benzyl rings and a butyrolactone ring system . It has signals for the first benzyl ring at δ_H [6.60 (br s, H-2'), 6.68 (m, H-5'), 6.58 (dd, J 8.2, 2.0 Hz, H-6'), 5.92, 5.94 (ABq, J 1.5 Hz, 3',4'- CH_2O_2), 2.83 (dd, J 14.0, 6.5 Hz, H_a-7') and 2.96 (dd, J 14.0, 5.0 Hz, H_b-7')] and for the second benzyl ring at δ_H [6.42 (d, 2.0 Hz, H-2"), 6.76 (d, J 8.2 Hz, H-5"), 6.56 (dd, J 8.2, 2.0 Hz, H-6"), 3.83 (s, MeO), 3.85 (s, MeO) and 2.50 (m, 2H-7")]. For the butyrolactone ring it has signals at δ_H [2.50 (m, H-2, H-3), 3.88 (m, H_a-4) and 4.14 (dd, J 9.3, 6.9 Hz, H_b-4)]. Comparison with published data[21] confirmed the identity of PC-4 as (-)-kusunokinin (**4**).

The ^{13}C NMR of PC-4 shows the expected features of a 3,4-disubstituted dibenzylbutyrolactone. Thus it has six aromatic methines [δ_C 109.4 (C-2'), 111.5 (C-2"), 108.1 (C-5'), 111.2 (C-5"), 122.2 (C-6'), 120.5 (C-6")], six aromatic quaternary carbons [δ_C 131.3 (C-1'), 130.3 (C-1"), 147.8 (C-3'), 149.0 (C-3"), 146.4 (C-4'), 147.8 (C-4")], two methylenes [δ_C 34.7 (C-7'), 38.2 (C-7")], a methylenedioxy [δ_C 101.0] and two methoxyl groups [δ_C 55.8, 55.7]. There are also signals [δ_C 178.5 (s), (C-1), 46.4 (d), (C-2), 41.1 (d), (C-3), 71.2 (t), (C-4)] arising from a butyrolactone ring substituted at the 2 and 3 positions. These data (see Experimental) are in agreement with the published data[22] for (-)-kusunokinin (**4**) . This is the first report of the isolation of (**4**) in Piperaceae.

In addition β-sitosterol (**5**) and the alkaloid piperine (**6**), previously reported constituents[8,9], were also isolated from this plant.



(**5**)

EXPERIMENTAL

Isolation. The plant material of *Piper chaba* was collected from the district of Khulna, Bangladesh. The ground stem bark (450g) was extracted in a Soxhlet apparatus with EtOAc. The ethyl acetate extract (3g) was concentrated *in vacuo* and fractionated by flash column chromatography over silica gel. The column was first eluted with petroleum ether and then increasing amounts of EtOAc in petroleum ether and finally MeOH. Each of the fractions was monitored by analytical tlc. The early fractions contained only fat. Multiple preparative tlc of the later fractions gave the following results: fraction 16 [CH_2Cl_2 : MeOH (99 : 1)] yielded PC-1 (**1**) (25mg); fraction 15 [petroleum ether : EtOAc (65 : 35)] afforded PC-4 (**4**) (20.5mg); fraction 14 [petroleum ether : EtOAc (75 : 25)] afforded PC-2 (**2**) (17mg) and β-sitosterol (**5**) (6.3mg); fraction 12 [petroleum ether : EtOAc (85 : 15)] yielded PC-3 (**3**) (92 mg); fraction 18 [CH_2Cl_2 : MeOH (99 : 1)] afforded piperine (**6**) (12.8mg).

PC-1 (**1**), white crystals (EtOAc-hexane), mp 80°; UV λ_{max} (log ε) : 234 (4.35), 290 (3.76), 345 (3.18) nm; IR ν_{max} : 3020, 1670, 1624, 1600, 1580, 1446, 1040, 1022, 929, 852 cm^{-1} ; EIMS m/z (rel. int.): 287 (M^+) (10), 286 (M^+-1) (3), 285 (10), 203 [M^+-84 (piperidine ring)] (3), 201 (M^+-86) (16), 176 (10), 175 (8), 174 (13), 138 (6), 136 (11), 135 (100), 115 (25), 112 (10), 105 (8), 86 (31), 84 (79), 77 (17), 57 (20); ^1H NMR : δ_H 6.60-6.90 (4H, m, H-β, H-2', H-5', H-6'), 6.35 (1H, d, J 14.6 Hz, H-α), 5.95 (2H, s, CH_2O_2), 3.55 [4H, br, 2H-2, 2H-6), 1.66 (6H, m, 2H-3, 2H-4, 2H-5), 2.40-2.75 (4H, m, 2H-γ, 2H-δ); ^{13}C NMR : δ_C 131.1 (C-1'), 105.6 (C-2'), 148.1 (C-3'), 148.2 (C-4'), 108.8 (C-5'), 122.5 (C-6'), 101.7 (CH_2O_2), 120.0 (C-α), 142.5 (C-β), 34.4 (C-γ), 34.5 (C-δ), 165.4 (C=O), 43.2 (C-2), 26.7 (C-3), 24.9 (C-4), 25.6 (C-5), 46.9 (C-6). HREIMS : Found 287.1527; calculated for $C_{17}H_{21}NO_3$ 287.1521.

PC-2 (**2**), gum, UV λ_{max} : 250 (log ε 3.52) nm; IR ν_{max}: 2940, 1655, 1637, 1603, 1550, 1016, 952 cm^{-1}; EIMS m/z (rel. int.) : 235 (M^+) (54), 206 (M^+-C_2H_5) (15), 193 (M^+-C_3H_6) (18), 192 (M^+-C_3H_7) (68), 178 (M^+-C_4H_9) (15), 164 (M^+-C_5H_{11}) (58), 151 (M^+-$C_5H_{10}N$) (42), 138 (M^+-C_7H_{13}) (40), 112 (33), 95 (30), 84 ($C_5H_{10}N^+$) (100); ^{1}H NMR : δ_H 7.22 (1H, dd, J 14.8 , 10.0 Hz, H-3'), 6.13 (1H, d, J 14.8 Hz, H-2'), 6.00-6.30 (2H, m. H-4', H-5'), 3.58 (4H, br, 2H-2, 2H-6), 1.58 (6H, br, 2H-3, 2H-4, 2H-5), 2.13 (2H, dt, J 7.0, 6.0 Hz, 2H-6'), 1.27 (6H, m, 2H-7', 2H-8', 2H-9'), 0.87 (3H, t, J 7.0 Hz, Me-10'); ^{13}C NMR : δ_C 165.7 (C-1'), 118.5 (C-2'), 142.7 (C-3'), 128.8 (C-4'), 142.9 (C-5'), 32.9 (C-6'), 28.5 (C-7'), 31.4 (C-8'), 22.5 (C-9'), 14.0 (C-10'), 43.2 (C-2), 26.6 (C-3), 24.7 (C-4), 25.6 (C-5), 46.9 (C-6). HREIMS : Found 235.1931; calculated for $C_{15}H_{25}NO$ 235.1936.

PC-3 (**3**), colourless needles (pentane), mp 89°; UV λ_{max} : 260 (log ε 4.54) nm; IR ν_{max} : 3400, 3020, 1665, 1620, 1560, 1522, 1480, 1388, 1018, 929, cm^{-1}; EIMS m/z (rel. int.) : 223 (M^+) (25), 222 (M^+-1) (26), 208 (M^+-15) (10), 180 (M^+-15-28) (5), 167 (10), 152 (M^+-$C_4H_{10}N$) (30), 151 (M^+-$C_4H_{10}N$) (100), 150 (M^+-$C_4H_{11}N$) (99), 137 (20), 135 (8), 113 (5), 112 (24), 111 (22), 109 (22), 96 (48), 81 (47), 78 (30), 72 ($C_4H_{10}N$) (18), 57 (60), 43 (70); ^{1}H NMR : δ_H 7.20 (1H, ddd, J 15.0, 8.8, 1.4 Hz), 5.95-6.20 (2H, complex m), 5.80 (1H, d, J 15.0), 2.14 (2H, dt, J 7.0, 6.0, 2H-6), 1.30 (6H, m, 2H-7, 2H-8, 2H -9), 0.89 (3H, t, J 7.0 Hz, Me-10), 3.15 (2H, t, J 6.7 Hz, 2H-1'), 1.77 (1H, m, H-2'), 0.92 (6H, d, J 6.7 Hz, Me-3', Me-4'), 5.88 (1H, br t, J 6.0 Hz, NH); ^{13}C NMR : δ_C 166.5 (C-1), 121.8 (C-2), 141.1 (C-3), 128.1 (C-4), 143.0 (C-5), 32.8 (C-6), 28.4 (C-7), 31.3 (C-8), 22.4 (C-9), 13.9 (C-10), 46.8 (C-1'), 28.5 (C-2'), 20.0 (C-3'), 20.0 (C-4'). HREIMS : Found 223.1938; calculated for $C_{14}H_{25}NO$ 223.1936.

PC-4 (**4**), gum, $[\alpha]_D$ -34.2° (c, 0.50 in $CHCl_3$); UV λ_{max} (log ε) : 230 (3.68), 282 (3.82), 285 (3.42) nm; IR ν_{max}: 3020, 1763, 1601, 1577, 1560, 1504, 1442, 1250, 1018, 930, 850 cm^{-1}; EIMS m/z (rel. int.) : 370 (M^+) (60), 235 (M^+-135) (5),

219 ($C_{12}H_{11}O_4^+$) (2), 218 ($C_{12}H_{10}O_4^+$) (2), 192 ($C_{10}H_8O_4^+$) (5), 177 (16), 151 ($C_9H_{11}O_2^+$) (85), 135 ($C_8H_7O_2^+$) (100), 107 (16), 91 (11), 77 (35); ^{1}H NMR : δ_H 6.60 (1H, br s, H-2'), 6.68 (1H, m, H-5'), 6.58 (1H, dd, J 8.2, 2.0 Hz, H-6'), 6.42 (1H, d, J 2.0 Hz, H-2"), 6.76 (1H, d, J 8.2 Hz, H-5"), 6.56 (1H, dd, J 8.2, 2.0 Hz, H-6"), 5.92, 5.94 (2H, ABq, J 1.5 Hz, CH_2O_2), 3.83 and 3.85 (both s, 2xCH_3O), 2.83 (1H, dd, J 14.0, 6.5 Hz, H_a-7'), 2.96 (1H,dd, J 14.0, 5.0 Hz, H_b-7'), 2.50 (4H, H-3, H-4, 2H-7"), 4.14 (1H, dd, J 9.3, 6.9 Hz, H_b-4), 3.88 (1H, m, H_a-4); ^{13}C NMR : δ_C 178.5 (C-1), 46.4 (C-2), 41.1 (C-3), 71.2 (C-4), 131.3 (C-1'), 109.4 (C-2'), 147.8 (C-3'), 146.4 (C-4'), 108.1 (C-5'), 122.2 (C-6'), 34.7 (C-7'), 130.3 (C-1"), 111.5 (C-2"), 149.0 (C-3"), 147.8 (C-4"), 111.2 (C-5"), 120.5 (C-6"), 38.2 (C-7"), 101.0 (CH_2O_2), 55.8 (CH_3O), 55.7 (CH_3O). HREIMS : Found 370.1415; calculated for $C_{21}H_{22}O_6$ 370.1416.

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