ANALYSIS OF SEED LIPIDS AND SYNTHESIS OF FATTY ACID DERIVATIVES

ABSTRACT
THESIS
SUBMITTED FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy
in
Chemistry

BY

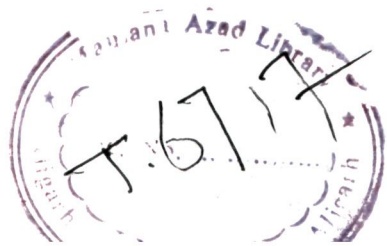## Ms. HUMAIRA PARVEEN

Under the Supervision of
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ALIGARH (INDIA)
2004

This thesis consists of two parts: Part-I deals with the (i) study of *Sesbania aculeata* seed oil and (ii) triacylglycerol-profiles of palm oil and palm-lard admixtures. Part-II illustrates (iii) direct esterification of long-chain fatty acids by using N, N'-dicyclohexylcarbodiimide in the presence of 4-(N,N-dimethylamino)pyridine, (iv) synthesis of α-bromo/5'-methyl tetrazoles from long-chain fatty alkenoates and (v) antimicrobial activity of fatty acid derivatives.

PART-I : ANALYSIS OF SEED LIPIDS

CHAPTER-1

Sesbania aculeata Seed Oil: A Moderate Source of (Z)-12-Hydroxyoctadec-9-enoic Acid

In view of industrial applications, medicinal importance and in search of naturally occurring unusual fatty acids, a study of *Sesbania aculeata* seed oil was undertaken. The most interesting finding was the presence of a hydroxyolefinic fatty acid (9.24%) along with other usual fatty acids such as lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, arachidic and behenic.

On the basis of various analytical techniques, spectral data and chemical methods, the structure of this hydroxy fatty acid was established as (Z)-12-

hydroxyoctadec-9-enoic (ricinoleic) acid, previously unknown in the genus *Sesbania*.

CHAPTER-2

Triacylglycerol-Profiles of Palm Oil and Palm-Lard Admixtures

Palm oil, the second largest edible oil in the world, is a product of great importance due to its nutritional value. To prevent palm oil adulteration in order to protect consumers and food industries, a study of the triacylglycerols (TAG)-profiles of palm oil and palm-lard admixtures by reversed-phase high performance liquid chromatography (RP-HPLC) was undertaken. Lard was mixed with palm oil at the ratio of 0, 5, 10, 15, 20 and 100. In all samples, TAG separation was achieved isocratically in ~15 minutes. The detection limit for lard was observed to be about 5%. The addition of lard in palm oil was detected by observing the composition of molecular species at t_R 7.3 and 8.9.

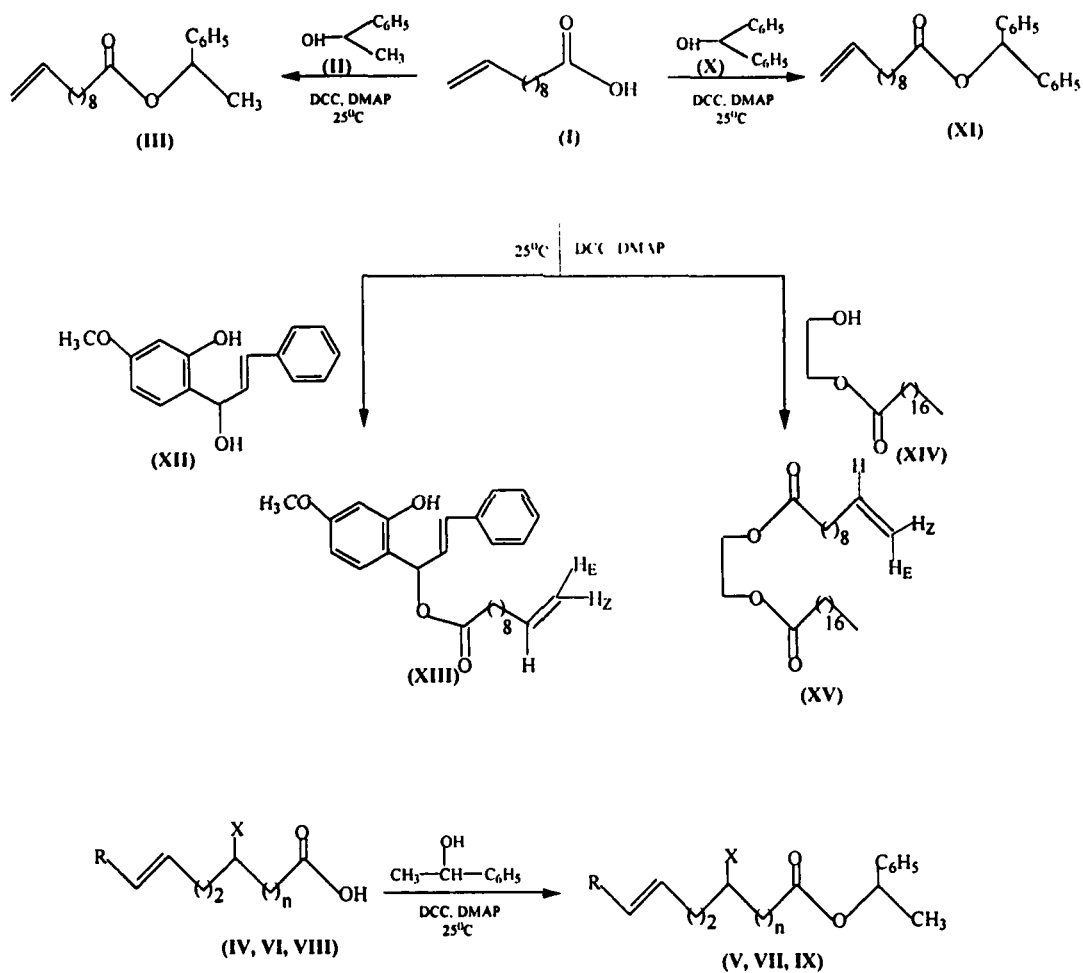## PART-II: SYNTHESIS OF FATTY ACID DERIVATIVES

CHAPTER-3

Direct Esterification of Long-Chain Fatty Acids by using *N,N'*-Dicyclohexylcarbodiimide in the presence of 4-(*N,N*-dimethyl amino)pyridine

Fatty acid derivatives ~~have been found to be~~ *are* associated with various industrial applications and diverse biological activities In the light of these observations and as part of our programme to synthesize biologically ~~acting~~ *active* compounds we have undertaken ~~this problem~~ *the*, "direct esterification of long-

chain fatty acids by using N,N'-Dicyclohexylcarbodiimide (DCC) in the presence of 4-(N,N-dimethyl amino)pyridine (DMAP). It includes the synthesis of 1'-phenylethyl undec-10-enoate (III), 1'-phenylethyl (Z)-octadec-9-enoate (V), 1'- phenylethyl (Z)-12-hydroxyoctadec-9-enoate (VII) and 1'-phenylethyl (Z)-9-hydroxyoctadec-12-enoate (IX) by the reaction of corresponding fatty acid i.e. undec-10-enoic (I), (Z)-octadec-9-enoic (IV), (Z)-12-hydroxyoctadec-9-enoic (VI) and (Z)-9-hydroxyoctadec-12-enoic acid (VIII) with 1-phenylethanol (II) by using DCC in the presence of DMAP at room temperature. Under similar conditions compound (I) was also allowed to reacted with diphenylmethanol (X), 1-(2'-hydroxy-4"-methoxyphenyl)-3-phenyl-prop-2-en-1-ol (XII) and ethylene glycol monostearate (XIV) separately, to yield their corresponding esters, diphenylmethyl undec-10-enoate (XI), 3'-(2"-hydroxy-4"-methoxyphenyl)-1'-phenylpropenyl undec-10-enoate (XIII) and ethylene glycol 1-stearate-2-undecenoate (XV) respectively (Scheme I).

The structure of each product was been established on the basis of elemental analysis and spectral data (IR, ^{1}H-NMR, ^{13}C-NMR EI-MS/ FAB-MS).

DCC: *N,N'*-dicyclohexylcarbodiimide;
DMAP: 4-(*N,N*-dimethylamino) pyridine

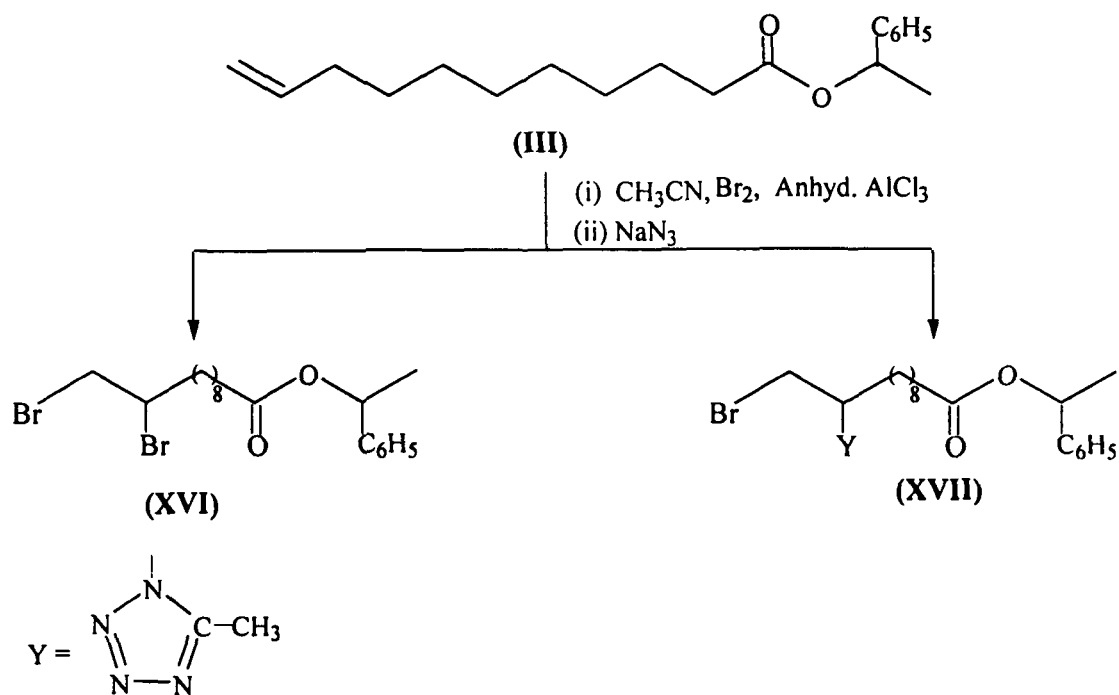| Compound	R	X	n
(IV, V)	$CH_3 (CH_2)_7$	H	4
(VI, VII)	$CH_3(CH_2)_5CH(OH)CH_2$	H	4
(VIII, IX)	$CH_3(CH_2)_4$	OH	7

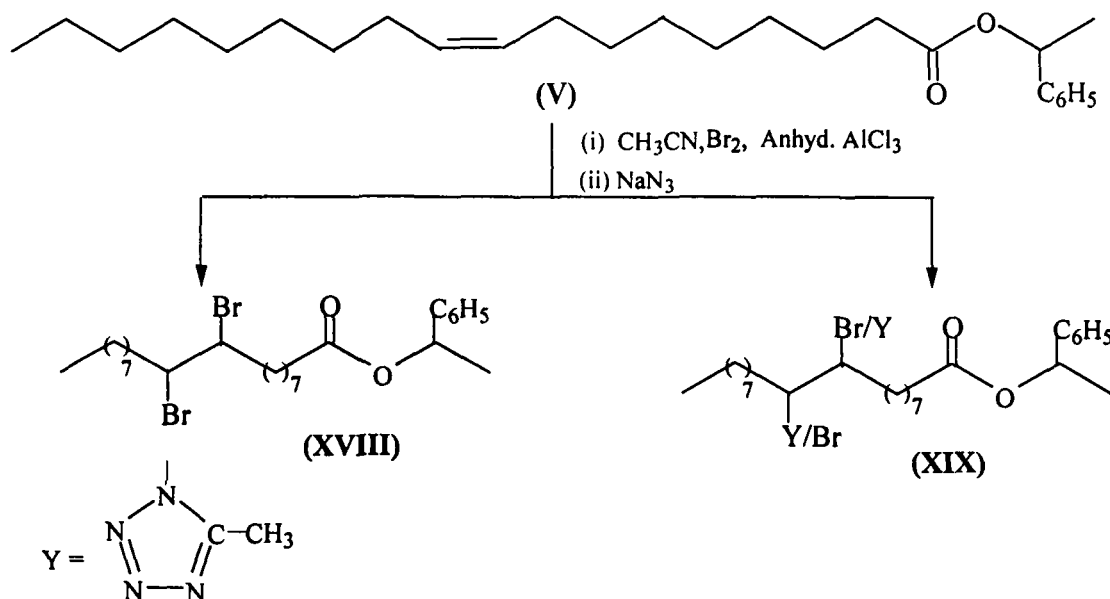**Scheme I:** Preparation of fatty alkenoates by using DCC.

CHAPTER-4

Synthesis of α-Bromo/5'-Methyl Tetrazoles from Long-Chain Fatty Alkenoates

In view of synthetic utility and biological importance of tetrazole derivatives we have carried out the reaction of 1'-phenylethyl undec-10-enoate (III) with bromine, acetonitrile and sodium azide in the presence of anhydrous aluminum chloride to afford the major product 1'-phenylethyl 11-bromo-10-(5'-methyl-1H-tetrazol-1-yl) undecanoate (XVII) along with 1'-phenylethyl 10,11-dibromoundecanoate (XVI). Under the similar reaction conditions 1'-phenylethyl (Z)-octadec-9-enoate (V) and 1'-phenylethyl (Z)-12-hydroxyoctadec-9-enoate (VII) yielded 1'-phenylethyl 9/10-bromo-10/9-(5'-methyl-1H-tetrazol-1-yl) octadecanoate (XIX) and 1'-phenylethyl 12-hydroxy 9/10-bromo-10/9-(5'-methyl-1H-tetrazol-1-yl) octadecanoate (XXI) respectively, in addition to their dibromides, 1'-phenylethyl 9,10-dibromooctadecanoate (XVIII) and 1'-phenylethyl 12-hydroxy-9,10-dibromooctadecanoate (XX) respectively (Scheme II, III and IV).

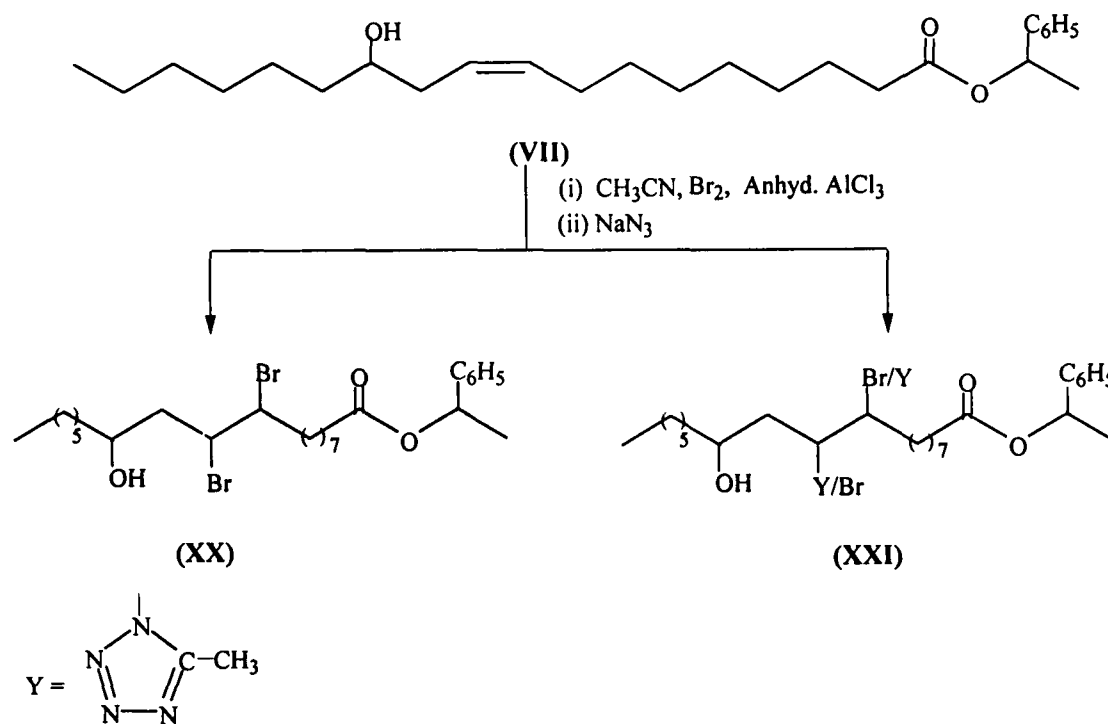The structures of all products were established by elemental and spectral studies.

(III)

(i) CH₃CN, Br₂, Anhyd. AlCl₃
(ii) NaN₃

(XVI)

(XVII)

$$Y = \text{(5'-methyl-1H-tetrazol-1-yl)}$$

Scheme II: Synthesis of 1'-phenylethyl 11-bromo-10-(5'-methyl-1*H*-tetrazol-1-yl) undecanoate (**XVII**).

(V)

(i) CH₃CN, Br₂, Anhyd. AlCl₃
(ii) NaN₃

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Scheme III: Synthesis of 1'-phenylethyl 9/10-bromo-10/9-5'-methyl-1*H*-tetrazol-1-yl) octadecanoate (**XIX**).

Scheme IV: Synthesis of 1'-phenylethyl 12-hydroxy-9/10-bromo-10/9-(5'-methyl-1H-tetrazol-1-yl) octadecanoate (**XXI**).

CHAPTER-5

Antimicrobial Activity of Fatty Acid Derivatives

A total of 10 compounds were tested against Gram +ve and Gram -ve bacteria. The test organisms ~~used in the study~~ included *Staphylococcus aureus* (Gram +ve), *Bacillus subtilis* (Gram +ve) *Escherichia coli* UP 2566 (Gram -ve), ~~*Escherichia*~~ *coli* K-12-J-62 (Gram -ve) and *Pseudomonas aeruginosa* (Gram -ve). ~~The~~ compounds (**III**), (**V**), (**VII**), (**IX**), (**XI**), (**XIII**), (**XV**), (**XVII**), (**XIX**) and (**XXI**) were screened for their antimicrobial activity

in *vitro* by the disc diffusion method. The solvent control (DMSO) showed non-significant inhibition to test organisms.

The compounds **III, XI** and **XIII** demonstrated activity against both Gram +ve and Gram −ve bacteria. Compounds **III, VII, IX, XIII, XV** except **V** showed significant activity against *E. coli* comparable to chloromycetin at the same concentration. Compounds **III, XI** and **XIII** showed moderate activity against *S. aureus* and *P. aeruginosa* whereas the rest of the compounds showed less activity against microorganisms compared to chloromycetin.

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Dated:...27.12.2004 ....

Certificate

This is to certify that the work embodied in this thesis entitled

"*Analysis of Seed Lipids and Synthesis of Fatty Acid Derivatives*" is the original work of **Ms. Humaira Parveen** carried out

under my supervision. The thesis is suitable for submission for the

award of the degree of **Doctor of Philosophy** in Chemistry.

(Dr. Abdul Rauf)

Dedicated

to

My

Dear Ammi

Acknowledgements

I bow my head with all humility and reverence to the Almighty for the completion of this task.

Words fail to express my indebtedness that I owe to my erudite research guide, Dr. Abdul Rauf, who inspired to take up this task, I will cherish throughout my life the valuable suggestions, constructive criticism and unending support that I received from him throughout the course of this study.

I wish to place on record the kind help received from Prof. Shafiullah and Prof. Kabir-ud-Din, who as chairmen of the department provided all the necessary facilities for under taking this research problem.

It is my privilege to have the guidance of Dr. Naseem Ahmed who is always a source of inspiration to me.

I would like to express my profound sense of gratitude and felicitations to Dr. Sayeed Mukhtar for his all out help and manifold cooperation throughout the course of this task.

Heartfelt thanks are due to all my lab colleagues, Dr. Shabana Ahmad, Mrs. Refat Jahan, Mr. Mahavir Singh, Dr. S.P. Singh, Ms. Savitri Verma and Md. Jamal for their cooperation.

There are no words to thank Shahab Ali Asghar Nami for his amicable help and cooperation while preparing this thesis.

I wish to record my debt of gratitude to Dr. Afzal Khan, Dr. Mujeeb-ur-Rahman and Md. Fahimuddin for their kind help and cooperation during the course of this study.

I owe a lot to Dr. Manzoora, Dr. Mandakini and Ms. Musarat for their sisterly and friendly gestures.

I find no words to express my feelings for my generous mother Mrs. Razia Parveen whose blessings and prayers enable me to reach where I am today.

Equally I express my profound sense of gratefulness to my uncles Mr. Altaf Nabi, Mr. Aslam Nabi, my aunt Mrs. Shabana Altaf and my all family members for their extraordinary cooperation and consistent inspiration.

I wish to express my benevolence to Dr. Iqbal Ahmad, Department of Agricultural Microbiology, Faculty of Agricultural Studies, A. M. U., Aligarh, India for his help in the determination of antimicrobial activity of fatty acid derivatives.

Mr. Haroon deserves a word of praise for the excellent typing of the manuscript.

Fine services from Instrumentation Division A. M. U., Aligarh and R.S.I.C., Lucknow are greatfully acknowledged.

(Humaira Parveen)

CONTENTS

PART-II: SYNTHESIS OF FATTY ACID

DERIVATIVES

CHAPTER-3: Direct Esterification of Long-Chain Fatty Acids by Using *N,N'*-Dicyclohexylcarbodiimide in the Presence of 4-(*N,N*-Dimethyl amino) pyridine

CHAPTER-4: Synthesis of *α*-Bromo/5'-Methyl Tetrazoles from Long-Chain Fatty Alkenoates

SUMMARY

This thesis consists of two parts: Part-I deals with the (i) study of Sesbania aculeata seed oil and (ii) triacylglycerol profiles of palm oil and palm-lard admixtures. Part-II illustrates (iii) direct esterification of long-chain fatty acids by using N, N'-dicyclohexylcarbodiimide in the presence of 4-(N,N-dimethylamino)pyridine, (iv) synthesis of α-bromo–5'-methyl tetrazoles from long-chain fatty alkenoates and (v) antimicrobial activity of fatty acid derivatives.

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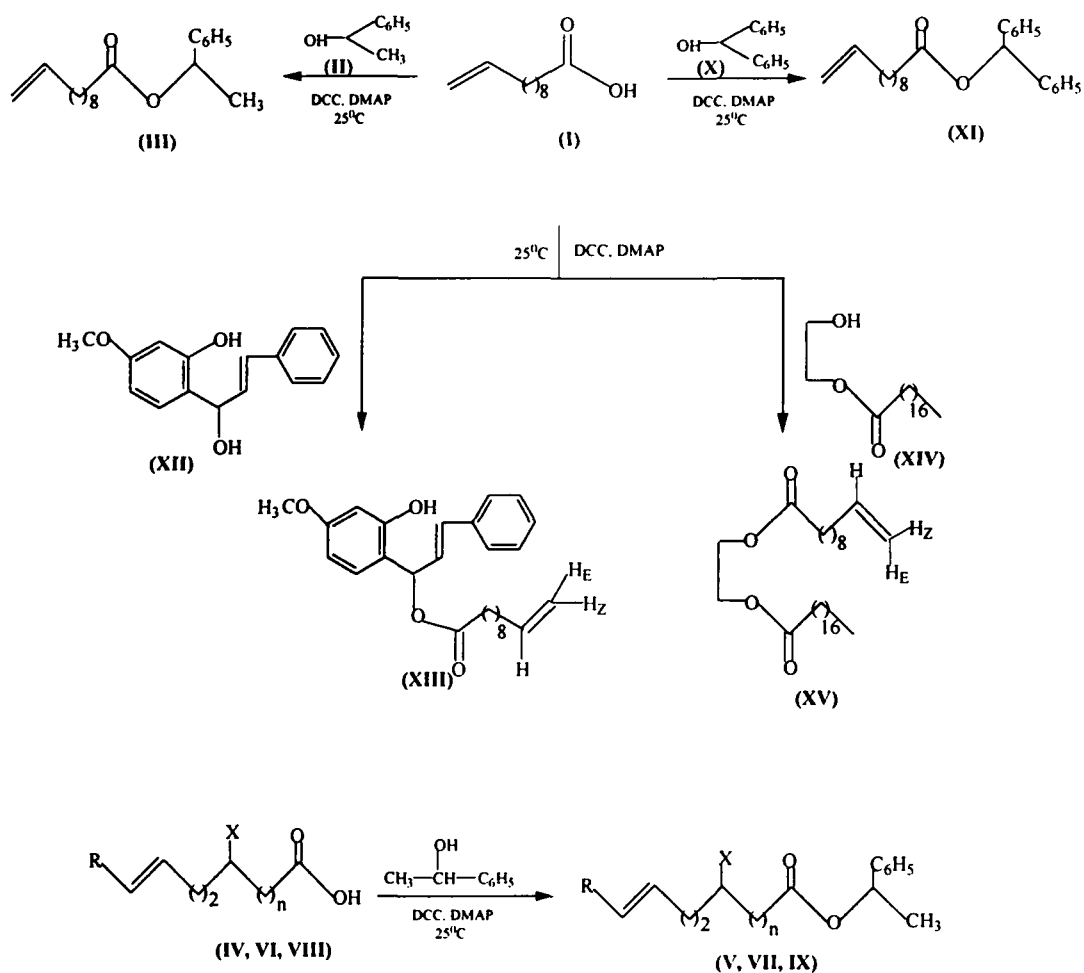
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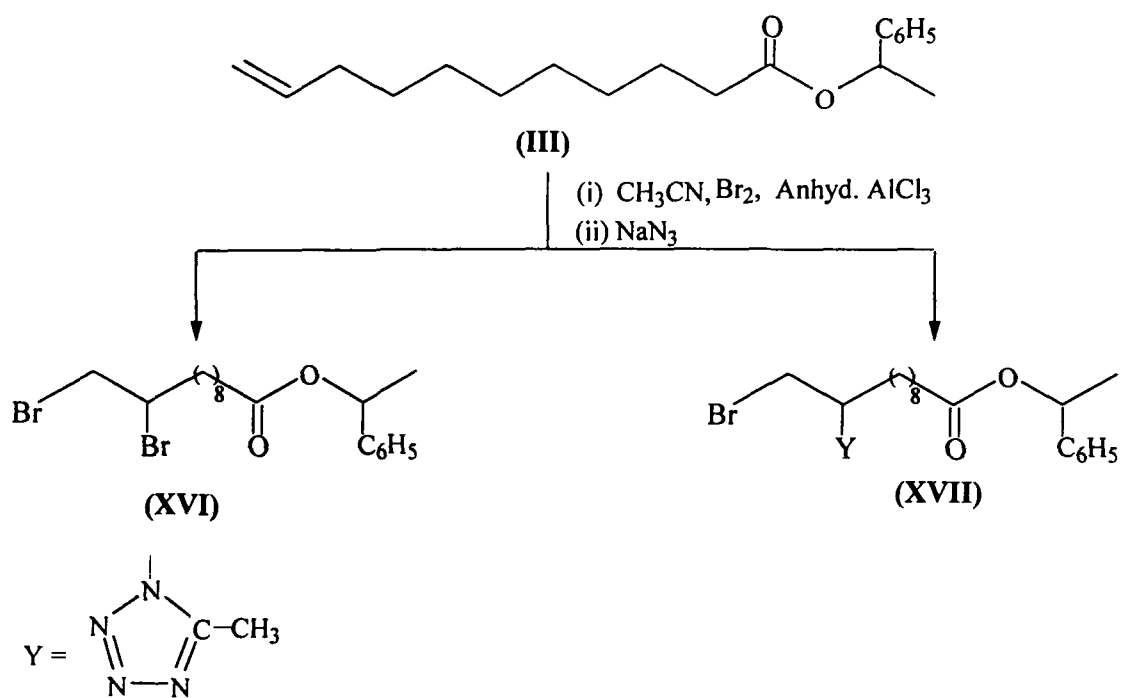| Compound	R	X	*n*
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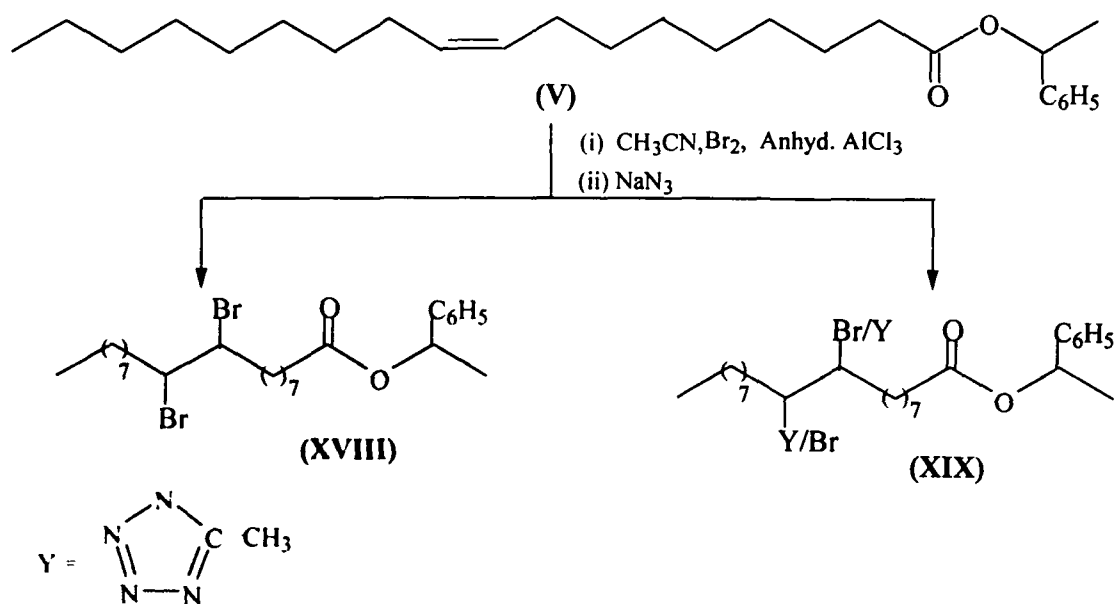**Scheme I:** Preparation of fatty alkenoates by using DCC.

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The structures of all products were established by elemental and spectral studies.

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$$Y = \text{5-methyl-1}H\text{-tetrazol-1-yl}$$

Scheme II: Synthesis of 1'-phenylethyl 11-bromo-10-(5'-methyl-1*H*-tetrazol-1-yl) undecanoate **(XVII)**.

(V)

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Scheme III: Synthesis of 1'-phenylethyl 9/10-bromo-10/9-5'-methyl-1*H*-tetrazol-1-yl) octadecanoate **(XIX)**.

(VII)

(i) CH₃CN, Br₂, Anhyd. AlCl₃
(ii) NaN₃

(XX)

(XXI)

Scheme IV: Synthesis of 1'-phenylethyl 12-hydroxy-9/10-bromo-10/9-(5'-methyl-1H-tetrazol-1-yl) octadecanoate (XXI).

CHAPTER-5

Antimicrobial Activity of Fatty Acid Derivatives

A total of 10 compounds were tested against Gram +ve and Gram -ve bacteria. The test organisms used in the study included *Staphylococcus aureus* (Gram +ve), *Bacillus subtilis* (Gram +ve) *Escherichia coli* UP 2566 (Gram -ve), *Escherichia coli* K-12-J-62 (Gram -ve) and *Pseudomonas* aeruginosa (Gram -ve). The compounds (III), (V), (VII), (IX), (XI), (XIII), (XV), (XVII), (XIX) and (XXI) were screened for their antimicrobial activity

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The compounds (**XVII, XIX** and **XXI**) also demonstrated moderate activity against both Gram +ve and Gram –ve bacteria.

Introduction

Oilseeds and edible oils are two of the most sensitive essential commodities. This is one of the reasons why they deserve worldwide attention. In recent years they have assumed importance due to their increasing use in food and nutrition on one hand and non-food industrial uses as oleochemical intermediates on the other.

Dietary fat is more important since its calorie density is more than twice than that of proteins and carbohydrates. The fatty acids apart from being the constituents of the cell membrane take part in a number of fascinating reactions leading to prostaglandins, thromboxanes and prostacyclines.

In India adulteration is rampant in many foods, but perhaps none as much as edible oil because of chronic shortage and volatility in prices. Oils and fats possess a unique pattern of triacylglycerois (TAG) that can be used to determine origin and to detect adulteration. In order to detect any possible substitution or admixing adulteration, identification of the component TAG of oils and fats becomes an important area of research to many analysts.

The vegetable oil industry in India occupies a pivotal position in the country's development. The growing demand of fatty chemicals as intermediate raw materials of industrial utility has now diverted the attention of oil chemists from analytical aspects of natural fats to the chemistry of unusual fatty acids and their novel derivatization. In recent years new and interesting unusual fatty acids

present in high concentration of certain oils are being exploited for commercial use. Fatty acids with unusual structures are highly important in the production of oleochemicals.

The increasing cost of petrochemicals has diverted the attention of chemists towards the synthesis of oleochemicals from natural fats and oils and their derivatives. During the past decades increased attention has been directed to explore the utilization of agrochemicals derived from non-edible oils and fats. In recent years the utilization of fatty acids as agrochemicals as a substitute to petrochemicals find their way into a variety of industrial uses and most of them mainly through derivatization. These fatty acid derivatives are becoming essential to a variety of industries such as coatings, surfactants, plasticizers, lubricant additives, cosmetics, perfumes, soaps, detergents, textiles pharmaceuticals, pesticides and polymers. In addition to their various biological properties fatty acid derivatives are also known to be good alternative fuels (biodiesel).

Keeping in view the current researches on oils and fats based on today's needs, the investigations in this thesis covers the two aspects (i) analysis of seed lipids and (ii) synthesis of fatty acid derivatives.

PART-I

Analysis of Seed Lipids

CHAPTER-1

Seed Oil of Sesbania aculeata : A

Moderate Source of

(Z)-12-Hydroxyoctadec-9-enoic Acid

1.1 Theoretical

The naturally occurring unusual fatty acids are widely distributed in plants[1,2]. They include allenic, cyclopropane, cyclopropene, cyclopentane, furan, hydroxy, epoxy, keto groups and double bonds of both the E- and Z-configuration, separated by one or more than one methylene group. Nowadays the number of known fatty acids exceeds thousands[3]. Recently, the interest in fatty acids with unusual structural features in seed oils, that can provide a high concentration of a single fatty acid, has increased as they can be of high value for the chemical and pharmaceutical industries[3-8]. Hydroxyolefinic fatty acids are known to occur in a number of seed oils[9]. Natural long-chain hydroxy acids ranges from C_{16} and C_{22} chain length and may be saturated, unsaturated or contain other functional groups. They are conveniently divided into three categories. One group has the hydroxyl function at or near the carboxyl end of the chain whilst those with mid-chain hydroxyl groups can be subdivided into acids with or without conjugated unsaturation. Badami and Patil[10] have reviewed the structures of these hydroxy fatty acids of plant origin. Hydroxy fatty acids play a vital role in oleochemistry[3]. These are extensively used in protective coatings, plastics, cosmetics, plasticizers, lubricant additives, pharmaceuticals, soaps. detergents, textiles and a variety of synthetic intermediates. The ethoxylated hydroxy fatty acid containing seed oils are used as stabilizers of hydrophobic substances in industries such as perfumes and cosmetics. The polyethoxylated hydroxy fatty acids are non-ionic surfactants and are included in the formulations for cleaning clothes, dishes, hard surfaces,

metals and in textile processing[11]. The derivatized seed oils containing hydroxy fatty acids are useful in lubricating oils and hydraulic oil. The only hydroxylated vegetable oil commercially available is castor (*Ricinus communis*) oil, which contains a high percentage (90%) of (*Z*)-12-hydroxyoctadec-9-enoic (ricinoleic) acid[12] (1).
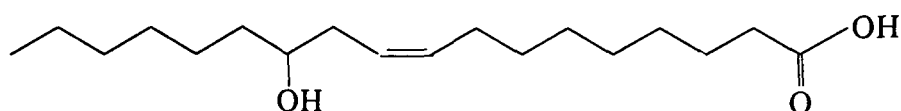

(1)

From our laboratory Siddiqui *et al.*[13] reported 84.3% ricinoleic acid from the seed oil of *Hiptage benghalensis*. The seed oil of *Phyllanthus niruri* (Euphorbiaceae)[14] was analyzed to contain same acid, previously unknown in the genus *Phyllanthus*. Small amount of ricinoleic acid has also been reported in the seed oil of *Nymphaea stellata*[15]. Later Yadav *et al.*[16] discovered a new source of ricinoleic acid in the seed of *Eclipta prostrata*. Recently, Hosamani and co-workers[17-19] have reported the occurrence of ricinoleic acid in the seed oils of *Alternanthera triandra* (Amaranthaceae), *Ochrocarpus africans* (Guttiferae) and *Crotalaria striata* (Leguminoceae). Interest in developing *Lesquerella*[20-22] began when three new hydroxy fatty acids, lesquerolic, densipolic and auricolic acids were discovered in *Lesquerella* species. Other species of the genus *Lesquerella*[23], *Dimorphotheca*[24], *Ostospermum*, *Castalis*[25] and *Coriaria*[26] are known to be rich in hydroxy fatty acids. Plattner *et al.*[27] have reported the presence of (*Z*)-14-hydroxyeicosa-11-enoic (lesquerolic) acid (30%) (2) with a trace of (*Z*)-16-hydroxy-13-docosenoic acid.

Configuration of $\overset{\text{H}}{\underset{\text{OH}}{-C-}}$ *Stereocenters*   5

(2)
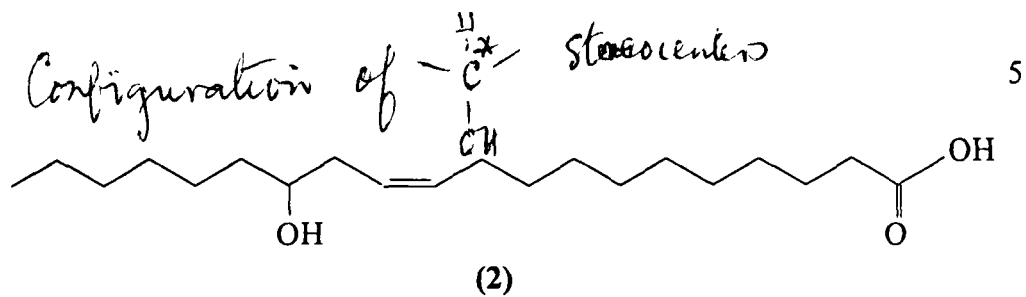
An isomer of ricinoleic acid (isoricinoleic/strophanthus/(Z)-9-hydroxyoctadec-12-enoic acid) (3) was first reported by Gunstone[28] in the seed oil of *Strophanthus sarmentotus*.

(3)
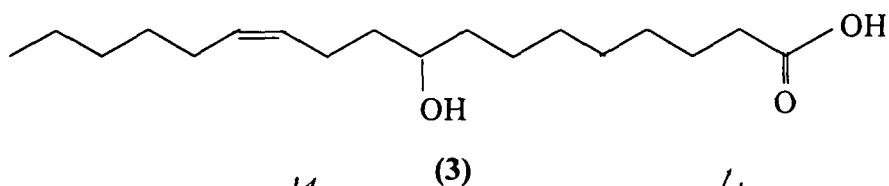
Ansari *et al.*[29] from *the* authors' laboratory reported *the* ~~same~~ acid as a major component of the fatty acids present in the seed oil of *Wrightia tinctoria* and *Wrightia tomentosa* (Apocyanaceae). The seed oil of *Wrightia coccinea*[30] ~~has~~ *is* ~~been found to be~~ the richest known source of isoricinoleic acid. The seed oil and isoricinoleic acid content of the three species of *Wrightia*[29,30] are given below.

Seed	Oil content (%)	Isoricinoleic acid content (%)
W. coccinea	23	76.1
W. tinctoria	30	70.0
W. tomentosa	22	67.0

This hydroxy acid was also found in high amount (73%) in the seed oil of *Holarrhena antidysentrica* (Apocyanaceae)[31].

Gupta *et al.*[32] ~~have~~ reported the occurrence of isoricinoleic acid in the

seed oil of *Solanum hispidium*, a member of family Solanaceae. Farooqi and

co-workers[33] ~~have~~ reported the occurrence of isoricinoleic acid in the seed oil

of *Semicarpus kurzii* (Anacardiaceae). Ansari *et al.*[34] also described the

presence of isoricinoleic acid in the seed oil of *Annona squamosa*

(Annonaceae). Naturally occurring hydroxyolefinic acids have either a β-

hydroxyolefinic or a γ-hydroxyolefinic unsaturation. An isomer of ricinoleic

acid [(Z)-9-hydroxyoctadec-11-enoic acid] (**4**) ~~has been found as~~ a minor

constituent of the seed oil of *Plantago major*[35].

(**4**)
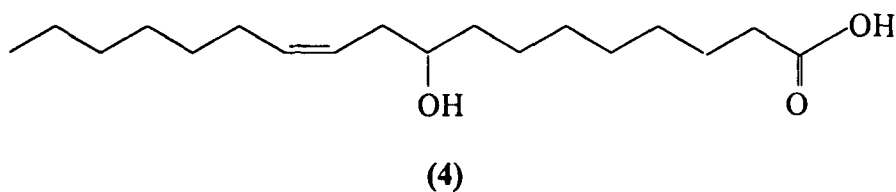
The (Z)-8-hydroxyoctadec-10-enoic acid (**5**) was reported from the seed

oil of *Diospyrous montana* (Ebenaceae)[36].

(**5**)
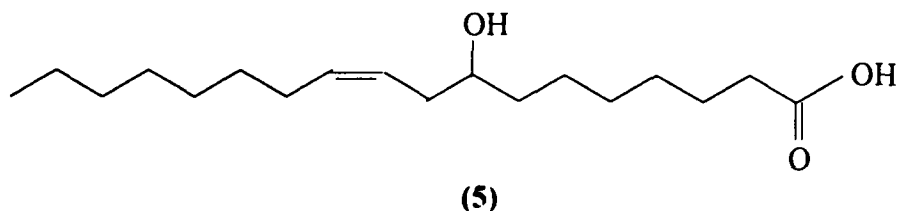
Ahmad and co-workers[17] ~~have~~ reported the presence of (Z,E)-13-

hydroxyoctadec-9,11-dienoic acid (**6**) in the seed oil of *Malva perviflora*

(Malvaceae).

(6)
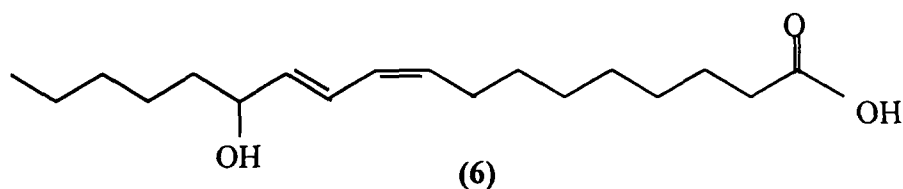
Analysis of *Mirabilis jalapa* (Nyctaginaceae)[38] seed oil ~~has shown~~ *showed* the occurrence of (Z,Z)-8-hydroxyoctadec-11,14-dienoic acid (7).

(7)
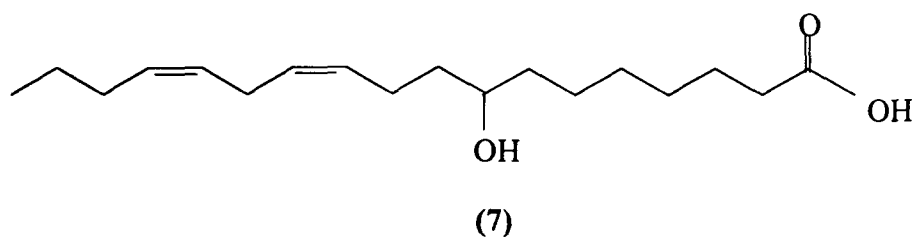
Borch-Jensen and co-workers[39] ~~have~~ reported the occurrence of (E,E)-9-hydroxyoctadec-10,12-dienoic (dimorphecolic) acid (8) in the seed oil of *Dimorphotheca pluvialis*.

(8)
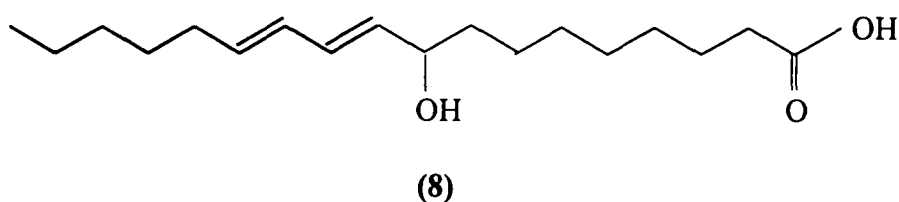
Occurrence of hydroxyolefinic acids with mono-, di- and tri-unsaturation (conjugated or non-conjugated) and without unsaturation has been reported in a number of species of the family Euphorbiaceae[40]. *Baliospermum axillare*[41] seed oil ~~has been shown to~~ contains a new non-vicinol dihydroxy mono-unsaturated acid [(E)-11,13-dihydroxytetracos-9-enoic acid] (8) as a minor constituent of the seed oil glycerides.

(9)
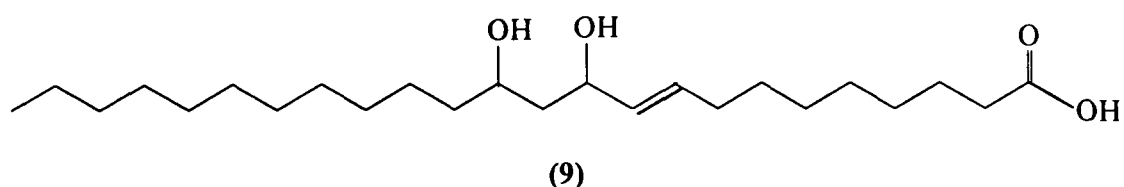
9,14-Dihydroxyoctadec-10, 12-dienoic acid was reported as an artifact

in *Aleurites fordii* seed oil[42]. A first natural non-vicinal diol acid (9, 14-

dihydroxyoctadecanoic acid) (10) ~~have been~~ reported as a minor

triacylglycerols constituent of the seed oil of *Peganum harmala* (Rutaceae)[43].

(10)
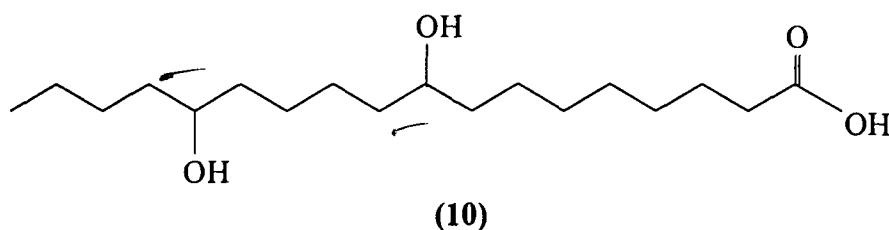
Ahmad *et al.*[44] ~~have~~ isolated 9-hydroxydodecanoic acid (11) from the

seed oil of *Blepharis sindica* (Acanthaceae).

(11)
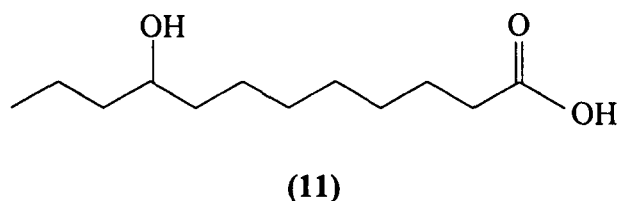
A conjugated ethylenic hydroxy fatty acid, (Z,E,E)-18-hydroxyoctadec-

9,11,13-trienoic acid (12), commonly known as kamlolenic acid was isolated

from the seed oil of *Trewia nudiflora*[45].

(12)
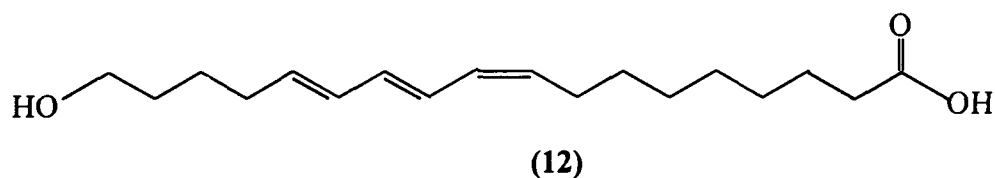
The hydroxyacetylenic fatty acids are also as numerous as hydroxyolefinic fatty acids in seed oils. Miller and co-workers[46] isolated a number of oxygenated acetylenic acids (8-hydroxyoctadec-10,12-diynoic and 8-hydroxy-17-octadecen-10,12-diynoic acid) from the seed oil of *Ongokea gore*.

Conjugated acetylenic hydroxy fatty acid (8-hydroxyximenynic acid) (13) has reported by Spitzer *et al.*[47] in the seed oil of *Iodina rhombofolia*.

(13)
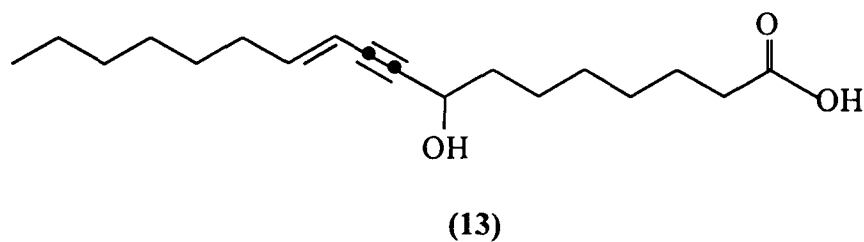
The seed oil of *Pachira aquatia*[48] was found to contain hydroxycyclic fatty (2-hydroxy sterculic) acid (14).

(14)
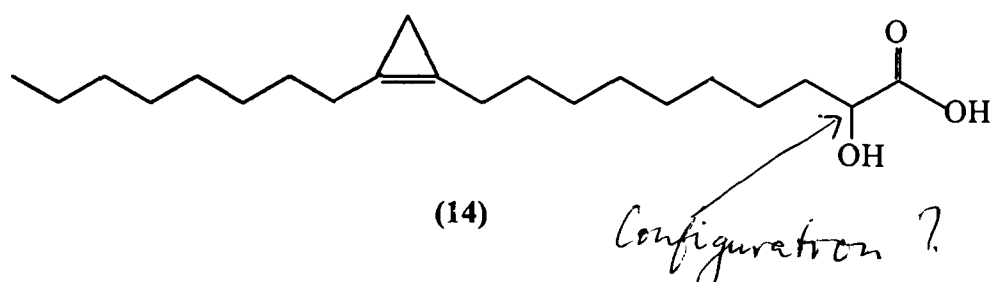
Spitzer *et al.*[49] have reported the occurrence of hydroxyallenic fatty acid

(8-hydroxy-5, 6-octadienoic acid) **(15)** in the seed oil of *Sebastiana*

commersoniana.

HO⌇⌇⌇⌇⌇ ⌇C(=O)OH
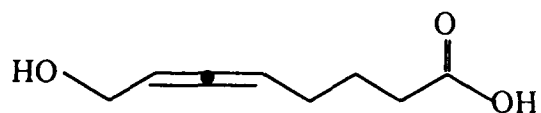
(15)

Keeping in view, the role of hydroxy fatty acids in oleochemistry and in

search of naturally occurring unusual fatty acids, we have analyzed the seed oil

of *Sesbania aculeata*, a member of family Papilionaceae.

1.2 Results and Discussion

Sesbania aculeata, commonly known as Dhaincha is not/food plant except in some instances where leaves and flowers are consumed as vegetables[50]. It is also grown for animal fodder[51] and its wood is useful for fire wood, poles and light construction. The major agricultural use of *Sesbania* species has been as green manure[52-58] to improve production of food crops. Agriculturists have been impressed by their special qualities of vigorous growth, adaptation to varied soil environment, control of soil erosion and the enhancement of soil fertility, where they are grown.

Sesbania seed is a good source of protein (29.33%)[59] and essential fatty acids and may be considered as a potential dietry protein source for monogastrics including fish. However the main constraint to extend their use in animal feeding is the deficiency of essential amino acids and the presence of anti-nutritional factors such as tannins, saponins and trypsin inhibitors[50].

The seed oil of *Sesbania aculeata* has been examined by several groups of workers[60-62]. Ahmad *et al.*[60] have reported the presence of high linoleic acid and poly-unsaturated fatty acid (PUFA) contents, using gas-liquid chromatography of the methyl esters. Wankhede *et al.*[61] studied the isolation, carbohydrate makeup and rheological properties of galactomannan of *S. aculeata* seeds. Afaque and co-workers[62] reported the presence of toxic substances in the defatted seed meal of *S. aculeata*.

The present work was undertaken to determine the fatty acid composition and to confirm the presence or absence of a hydroxyolefinic fatty

acid/ in the seed oil of *S. aculeata*. The most interesting finding was the presence of a hydroxy olefinic fatty acid, which was characterized as (Z)-12-hydroxyoctadec-9-enoic (ricinoleic) acid.

The seed oil was extracted from the finely ground seeds of *S. aculeata* by soxhlet extraction using chloroform and methanol (1:1, v/v) to afford 15.45 % of oil. The seed properties and oil characteristics were determined according to the standard American Oil Chemist's Society (AOCS) procedures[63] and data are summarized in **Table 1**. The seed oil did not respond to the Halphen test[64], picric-acid TLC test[65] and 2,4-dinitrophenylhydrazine (2, 4-DNPH) TLC test[66], thereby indicating the absence of cyclopropenoic, epoxy and keto fatty acids, respectively. The UV and IR spectra of the oil showed no conjugation or *trans*-unsaturation but a strong band at 3433 cm^{-1} was observed in the IR spectrum of the oil as well as methyl ester, indicating the presence of hydroxy function.

Table 1: Analytical values of *Sesbania aculeata* seeds and oil.

Seeds	
Oil content (%)	15.45
Protein content (%)	36
Seed oil	
Iodine value	108.84
Saponification value	218.65
Refractive index at 25°C	1.4887
Halphen test	-Ve
2,4-DNPH TLC test	-Ve
Picric acid TLC test	-Ve

Quantitation of component acids was made by GLC analysis of the

methyl esters as their trimethylsilyl (TMS) derivatives and data are given in

Table 2. The *S. aculeata* seed oil ~~has been found to~~ contain/total saturated fatty

acids (18.91%), total unsaturated fatty acids (71.85%) and hydroxy fatty acid

(9.24%). The seed oil is rich in linoleic acid (45.92%) along with moderate

proportions of palmitic (10.94%) and oleic (17.10%) acid*s*

Table 2: Fatty acid composition (wt.%) of methyl ester of *S. aculeata* seed oil.

Fatty acids	Wt. %
C12:0 (Lauric)	02.51
C14:0 (Myristic)	00.37
C16:0 (Palmitic)	10.94
C16:1 9*(Z)* (Palmitoleic)	03.54
C18:0 (Stearic)	04.02
C18:1 9*(Z)* (Oleic)	17.10
C18:2 9*(Z)*, 12*(Z)* (Linoleic)	45.92
C18:3 9*(Z)*, 12*(Z)*,15*(Z)* (Linolenic)	05.29
C20:0 (Arachidic)	00.03
C22:0 (Behenic)	01.04
C18:1 9*(Z)*,12-OH (Ricinoleic)	09.24

Isolation and characterization of fatty acid

To determine the absolute identity of hydroxy acid, a concentrate of pure hydroxy acid was obtained by Gunstone's partition procedure[71] of freshly prepared mixed fatty acids. The hydroxy acid obtained from methanolic phase was further purified by column chromatography. Methyl ester of hydroxy acid was prepared by acid-catalyzed esterification.

The elemental analysis of the methyl ester corresponded to the molecular formula $C_{19}H_{36}O_3$, suggesting an eighteen carbon fatty acid. The IR spectrum of the pure methyl ester showed a very strong band at 3433 cm^{-1} for hydroxy group.

The ^{1}H-NMR spectrum of the methyl ester of hydroxy fatty acid also gave conclusive information regarding the structure of hydroxy fatty acid. It is in agreement with the presence of a hydroxy function as the chemical shift of hydroxy proton appeared as a broad singlet at δ 3.34 which was disappeared on D$_2$O shake. Other structure revealing signals appeared at δ 3.4 for one methine proton of C-12, a multiplet at δ 5.51-5.36 for two olefinic protons at C-9 and C-10. A singlet appeared at δ 3.6 for ester methyl protons. A multiplet at δ 2.05 and 2.09 was ascribable to the methylene protons at C-11 and C-8 respectively. The signals at δ 2.24 and 1.63 were attributed to the methylene protons at C-2 and C-3 respectively. A broad singlet was appeared at δ 1.27 for chain methylene protons and a distorted triplet at δ 0.88 was assigned to terminal methyl protons. The ^{13}C-NMR spectrum of the methyl ester of hydroxy fatty acid was more informative about the assigned structure. It showed a sharp

singlet at δ 174.4 for carbonyl carbon. Other characteristic signals appeared at

δ 133.8 and 124.5, attributed to 9-C and 10-C respectively, in addition to other

diagnostic signals appeared at δ 27.3 (8-C), δ 31.5 (11-C), 73.8 (12-C), 33.5

(13-C) 51.4 (CH₃).

The structure of hydroxyolefinic fatty acid was further supported by the

mass spectrum of trimethylsilyl (TMSi) derivative[67] of the hydroxy olefinic

ester. The molecular ion observed at m/z 384 indicating C-18 chain, identical to

that of TMSi derivative of methyl ricinoleate, used as reference standard. The

other structure revealing fragment ions were found at m/z 187, 299 and 270,

establishing the position of the hydroxyl group at C-12 and placed the double

bond at C-9.

m/z 187   m/z 299

m/z 270
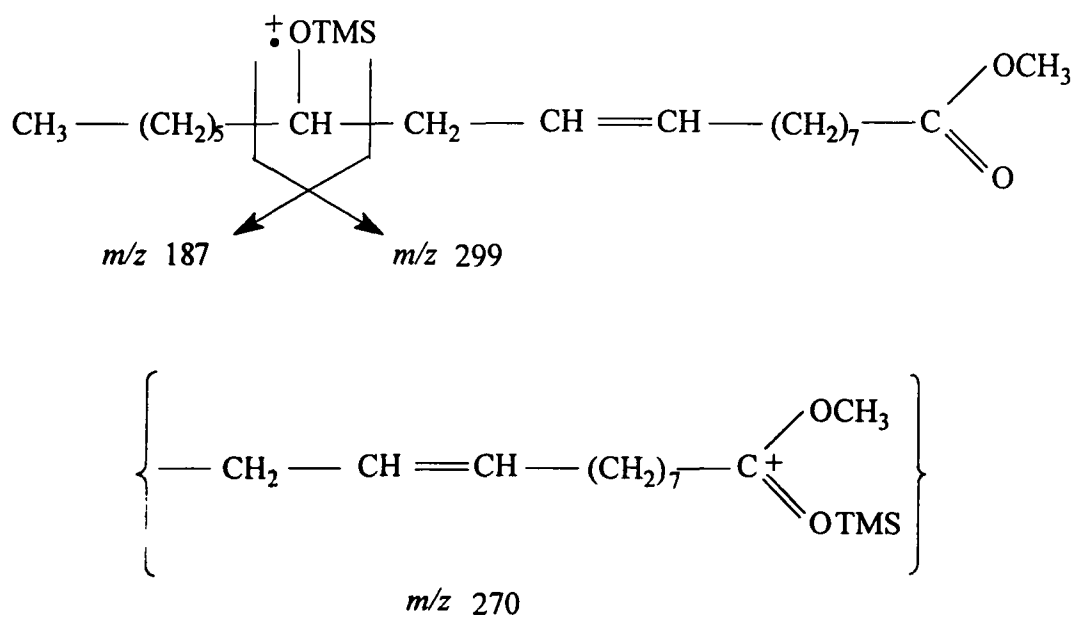
Scheme 1: Mass spectral fragmentation of TMSi derivative of (Z)-12-

hydroxyoctadec-9-enoate.

The acetate derivative of the pure hydroxy ester showed strong band at 1235 cm^{-1} and no hydroxyl absorption at 3433 cm^{-1} in its IR spectrum. The ^{1}H-NMR spectrum (acetate derivative) showed no unusual features apart from two expected but significant signals δ 4.7 as multiplet for one methine proton (CH-OAC) and at 1.9 as singlet for methyl protons (-OCOCH_3). The disappearance of the signal for hydroxyl group confirmed the original acid as hydroxy acid.

Catalytic hydrogenation[68] of hydroxy olefinic ester (Ia) yielded a saturated hydroxy ester (II) (m.p. 54.2-54.3 °C). Reductive deoxygenation[69] of methyl 12-hydroxystearate (II) furnished methyl stearate (III) as identified by GLC and co-TLC. The oxidative cleavage of the original hydroxy fatty acid (Ib) by permanganate/periodate[70] yielded two major fragments, which were identified by GLC after methylation with diazomethane, as azelaic (IV) and 3-hydroxy pelargonic acid (V) (Scheme 2). These results established the double bond at C-9 and hydroxy function at C-12. On the basis of these physical and chemical evidences Ib was characterized as (Z)-12-hydroxyoctadac-9-enoic acid, commercially known as ricinoleic acid.

$$\underset{16}{\wedge\wedge} \overset{O}{\underset{}{\|}} OCH_3$$

(III)

(i) HI/P
(ii) Zn/HCl

$$\underset{5}{\wedge\wedge} \overset{OH}{\underset{}{|}} \underset{10}{\wedge\wedge} \overset{O}{\underset{}{\|}} OCH_3$$

(II)

Pd/C

$$\underset{5}{\wedge\wedge} \overset{OH}{\underset{}{|}} \underset{7}{\wedge\wedge} \overset{O}{\underset{}{\|}} OR$$

(Ia) R = CH₃
(Ib) R = H

KMnO₄ / NaIO₄

$$\underset{5}{\wedge\wedge} \overset{OH}{\underset{}{|}} \overset{O}{\underset{}{\|}} OH$$
(V)

+

$$HO \overset{O}{\underset{}{\|}} \underset{7}{\wedge\wedge} \overset{O}{\underset{}{\|}} OH$$
(IV) .
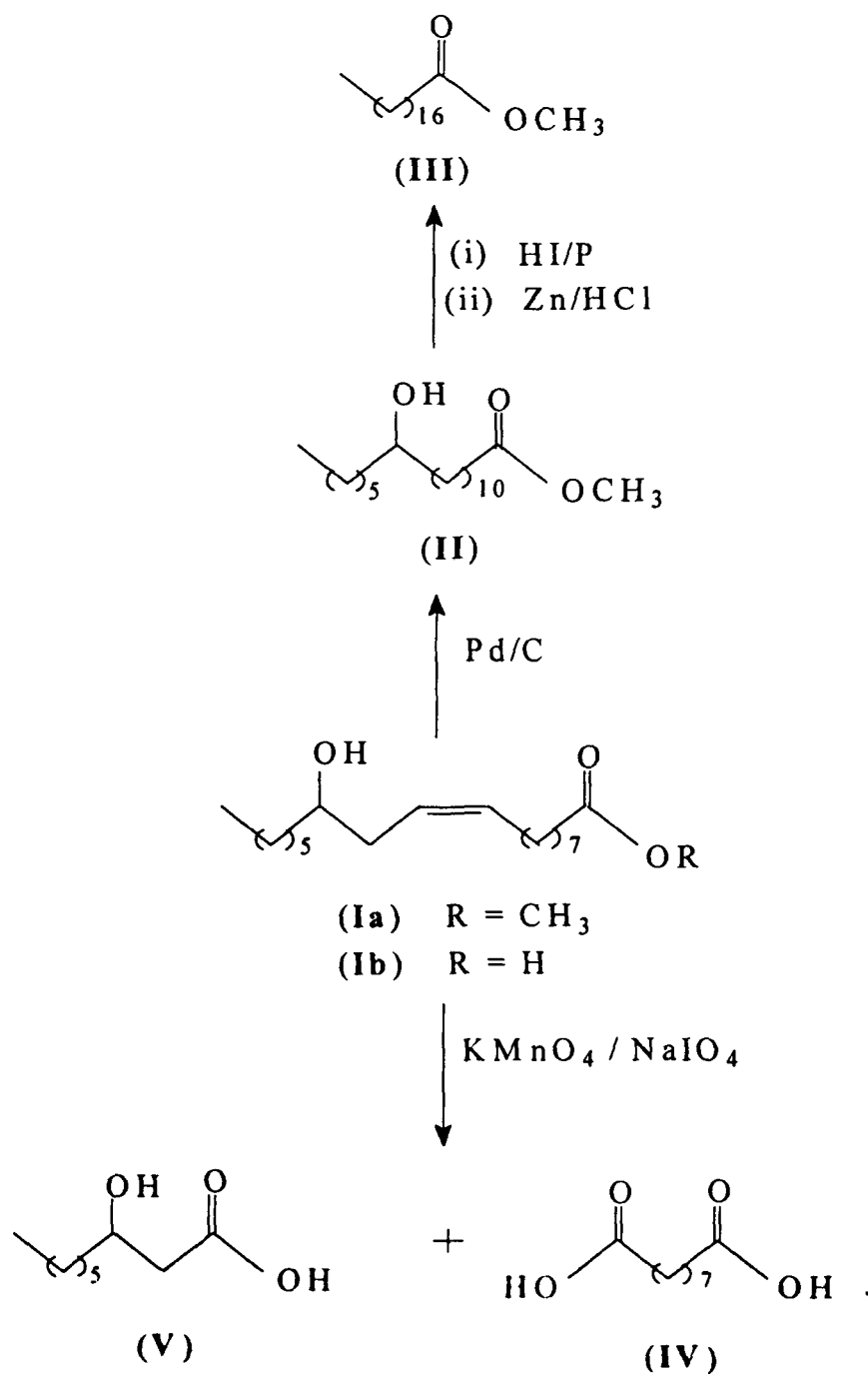
Scheme 2. Chemical transformations of Ia/Ib

1.3 Experimental

Materials and Methods

Source of Seeds

The seeds were purchased from Pratap Nursery, Dehradun (U.P.).

Extraction of Oil

Cleaned and dried seeds were usually ground in a disintegrator. The powdered seeds were extracted with chloroform and methanol (1:1, v/v/) in a soxhlet apparatus. The solvent was removed under reduced pressure. The extracted oil was dried over anhydrous sodium sulphate to yield 15.45% of oil. The seed properties and oil characteristics were determined according to the standard AOCS procedures[63] and data are summarized in **Table 1**.

Chromatographic techniques

Thin layer chromatography (TLC) was done on glass plates (20 × 5 cm) with a layer of silica gel G (Merck, Mumbai, India, 0.5mm thickness). Mixture of petroleum ether-diethyl ether-acetic acid (80:20:1, v/v) was used as developing solvent. The column chromatography was carried out with silica gel (Merck, 60-12 mesh).

Instrumentation

IR spectra were recorded on a Shimadzu 8201 PC (Kyoto, Japan) spectrophotometer. ^{1}H-NMR spectra were recorded on a Bruker DRX 300 spectrometer (Fallenden, Switzerland) in $CDCl_3$ using TMS as internal standard. Chemical shifts (δ) were quoted in ppm. ^{13}C-NMR spectra were recorded on Bruker DRX 300 spectrometer in $CDCl_3$ with $CDCl_3$ (δ = 77.00

ppm). GLC was carried out by using a Varian Vista 6000 instrument equipped with FID (290°C) detector using a stainless steel column (2M × 2mm I.d.) packed with 15% of OV-275 on chromosorb-W (80-100 mesh). Separations were carried out at a programmed temperature of 140-200°C ($10°C$ min^{-1}). The UV spectra were recorded on Cintra 5 UV-visible spectrometer (Dandenong, Australia) in methanol.

Isolation of hydroxy fatty acid

Saponification of the seed oil was carried out by refluxing it with 0.5 N alcohlic KOH. The unsaponifiable material was removed by ether extraction. The mixed fatty acids were separated into oxygenated and non-oxygenated fractions by Gunstone's partition method[71] between petroleum ether (40-60°C) and 80% methanol. The oxygenated fraction was chromatographed over a column of silica gel. Elution with petroleum ether-ethyl acetate (70:30, v/v) gave pure hydroxy fatty acid (Ib).

Preparation of methyl ester and trimethylsilyl ether derivatives

The methyl ester of hydroxy acid was prepared by refluxing Ib for 2 hours in a large excess of absolute methanol containing 1% sulphuric acid (v/v). The resulting mixture was diluted with water and then extracted with diethyl ether. The extract was dried over anhydrous sodium sulphate and solvent evaporated in vacuo to yield pure ester (Ia) which was converted into trimethylsilyl ether derivative by following method.

To the hydroxy fatty acid (10 mg) pyridine (1 mL), hexamethyl-disilazane (0.3 mL) and trimethylchlorosilane (0.1 mL) were added. After

shaking the mixture for 30 seconds, it was allowed to stand for 5 minutes. Then

Hexane (5 mL) and water (5 mL) were added followed by shaking. The hexane

layers were dried over anhydrous sodium sulphate and solvent was removed in

a stream of nitrogen to yield pure derivative. The derivative was subjected to

GLC analysis and MS.

Acetylation of hydroxy methyl ester

The hydroxy methyl ester (50 mg) was dissolved in acetic anhydride in

pyridine (2 mL, 5:1, v/v) and left at room temperature overnight. The solvent

was removed in a stream of nitrogen with gentle warming and the acetylated

ester was purified by TLC on silica gel layers.

Position of double bond

Catalytic hydrogenation[68] of **Ia** with 10% Pd/C in ethanol (5 mL) gave

saturated hydroxy ester melting at 54.2-54.3 °C which was identified as methyl

12-hydroxystearate **(II)** by comparing mp. (54.2-54.3 °C) with an authentic

sample.

Reductive deoxygenation[69] of **II** by hydrogen iodide-phosphorous/carried out

as follows.

Compound **(II)** (40 mg) was refluxed for 17 hours with red phosphorus

(18 mg) and hydriodic acid (1.2 mL). Diethyl ether extraction of the diluted

mixture followed by washing with 5% sodium metasulphite gave an oily

product, which was reduced by refluxing for 4 hours with granular zinc (100

mg). Workup with diethyl ether of the mixture afforded methyl stearate **(III)** as

identified by GLC and co-TLC, confirming a normal C-18 skeleton for

hydroxy acid.

Position of hydroxy function

The oxidative degradation[69] of the original hydroxy acid was performed as follows.

One mL of stock oxidant solution (prepared by mixing 20.86g of sodium metaperiodate and 0.39g of potassium permanganate in 1 L water) and potassium carbonate solution (1 mL) was added to **Ia** (1 mg) in *tert*-butanol (1mL). The reaction mixture was slowly stirred at room temperature for 1 hour. After that, the solution was acidified by 1 drop of concentrated sulphuric acid, extracted with diethyl ether and dried over anhydrous sodium sulphate. The solvent was evaporated in a stream of nitrogen to yield two major products identified by GLC after methylation with diazomethane as azelaic (**IV**) and 3-hydroxypelargonic acid (**V**). The formation of these products placed the hydroxyl group at C-12. The identity of the cleavage products (**IV**) and (**V**) was made by comparing their retention times with those of authentic samples prepared by the oxidation of ricinoleic acid obtaind from castor oil.

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CHAPTER-2

Triacylglycerols Profiles of Palm Oil and Palm-Lard Admixtures

2.1 Theoretical

Oils and fats are extremely important food products due to their relatively high calorie value and accordingly much attention has been given to their analysis. Adulteration has been a problem in the oils and fats for a long time[1]. It is sometimes deliberate and sometimes accidental[2].

In general, adulteration of edible oil refers blending cheaper oil with premium oil. Higher the price of premium oil greater is the propensity to adulterate it with low priced oil. For ages, raw groundnut oil, the premium-cooking medium had been mixed with castor oil to the extent of 5-10 % since it used to be the cheapest vegetable oil in the country and groundnut oil the most expensive. After refined palmolein started to come in 25 years ago, it became a ready adulterant as it blended well with groundnut oil. In the last two years, there have been reports of high-priced refined sunflower oil mixed with cheaper oils. There were deaths due to dropsy following suspected adulteration of mustard oil. Also there were reports concerning contamination of groundnut oil with sunflower oil and adulteration of both groundnut and sunflower oils with cheaper oil such as soy or rape[2].

Two general approaches can be taken in the adulteration of food stuffs[3]. One is identification of a major constituent and its property and the other is identification of the adulterant. Oils and fats possess a unique pattern of triacylglycerols (TAG). In order to detect any possible substitution or admixing adulterations, identification of the component TAG of oils and fats has become an important area of research to many analysts[4,5]. The separation of

TAG in edible vegetable oils has been studied by a wide range of techniques such as gas chromatography (GC)[3,6,7], liquid chromatography (LC)[8,9], super and sub-critical fluid chromatography (SFC and Sub FC)[10,11] and capillary electro chromatography (CEC)[12]. The most common approach to the analysis of TAG is to release free fatty acids (FFA) and perform GC or GC/mass spectrometry after derivatization. Grob[13] has discussed methods for the detection of adulterated edible oils and fats (e.g. olive, rapeseed, and specialty oils), including control methods based on fatty acid composition, sterol analysis, detection of raffination and detection of solvent extraction oil. Rossell[14] described the purity criteria of palm and other oils, which is based on the composition of fatty acid at the triacylglycerol 2-position, triacylglycerol compositions by high temperature gas liquid chromatography and tocol analysis. Jee[15] has reviewed adulteration and authentication of oils and fats. Yamazaki et al.[16] have discussed the detection of soybean oil in adulterated sesame oil by fatty acid profiling analysis. Adhikari and co-worker[17] have reported semi-quantitative detection of rape-mustard oil in rice bran oil. Shukla et al.[18] have reported a highly economical qualitative technique for the identification of physically refined rice bran oil and its simple detection in other oils. They have shown that a small quantity of alkaline solution of suspected oil sample when treated with small quantity of benzenediazonium chloride solution at 0-5 °C followed by shaking of the mixture, a brilliant orange-red colour of 5-phenylazo-γ-oryzanol or 5-phenylazoferulic acid (a dye material) develops within the next few seconds indicating the presence of rice

bran oil adulteration in the test sample. Mottram *et al.*[19] described high resolution detection of adulteration of maize oil using multi-component compound specific δ ^{13}C values of major and minor vegetable oil components and discriminant analysis. The current status and recent trends in improving methodologies for detecting adulteration of olive oil have been reviewed by Li-Chan[20]. For olive oil the criterion of purity is based on the trilinolein and presence of atypical fatty acids. Among the established methods for the control of the authenticity of olive oil, the determination of fatty acids and triacylglycerols seem to be very useful. Zabaras *et al.*[21] have reported an improved method for the detection of pressed hazelnut oil in admixtures with virgin olive oil by the analysis of polar components which was based on the isolation of the polar fraction followed by reversed-phase high performance liquid chromatography (RP-HPLC) analysis with UV detection. Christopoulou *et al.*[22] investigated the effectiveness of the determination of fatty acids and triacylglycerols for the detection of adulteration of olive oil with vegetable oils.

Palm oil, the second largest edible oil in the world, is a product of great importance because its nutritional value has been acknowledge internationally. So it is thus important to safeguard it from adulteration in order to protect consumers and food industries. In the past, fraudulent addition of cottonseed oil with palm oil has been reported.[23] Similarly, palm oil was reported to have been adulterated with relatively cheaper palm stearin[2,23]. It is also a problem when animal fats such as lard are mixed with palm oil, either for the purpose of adulteration or new product development, where the consumption of lard is

restricted by the Islamic and Orthodox Jewish religions[24]. Both the genuine and randomized lard are found to possess high amounts of palmitic and oleic acids that are also the predominant fatty acids in refined-bleached-deodorized (RBD) palm oil. After adulteration, RBD palm oil may become inferior in quality due to the difference in the triacylglycerols composition of lard. Also, lard is associated with the risk of cancer[25]. The detection of lard as adulterant has received much attention and a number of detection methods have been reported by several workers. Lambelet and Ganguli[26] have studied the differential scanning calorimetry (DSC) to detect adulteration of ghee (a popular dairy product in India) with lard. Farag *et al.*[27] have demonstrated a method based on fractional crystallization followed by gas chromatographic (GC) analysis of fatty acid methyl esters to detect butter fat with lard. Saeed *et al.*[28] have used HPLC analysis of derivatized TAG to detect pork in adulterated sample of mutton and beef. Rashood *et al.*[24] have shown that the HPLC analysis of TAG could be used as a method to identify genuine/randomized lard from other animal fats. Che Man and Mirghani[29] developed a method for detecting lard in mixtures of body fats such as chicken, lamb and cow based on fourier transform infrared (FTIR) spectroscopy. Recently, differential scanning calorimetry thermal profiles of oils and fats have been reported for the detection of lard and randomized lard as adulterant in RBD palm oil[30,31]. RP-HPLC is a good separation technique and has application to analysis of animal and vegetable oils and fats[4,5,32].

The objective of this study was to examine and compare the TAG profiles of palm, lard and their admixtures by RP-HPLC. Such profiling characterization is of value for the genuinely testing and checking of adulteration of lard in palm oil.

2.2 Results and discussion

The fatty acid composition generally used to evaluate the stability and nutritional quality of oils and fats but not always their functional properties. It is the TAG composition that ultimately determines the final physical and functional properties of the fats and oils. **Figure 1** shows the TAG-separation of genuine lard and palm oil by RP-HPLC. The complete elution of the TAG molecular species of the samples took 15 minutes. The reproducibility of the retention times of all the separated peaks was very good for standard and other samples. Molecular species of lard and palm oil were characterized by comparing retention times of the sample to that of the standard sample. Pig fat (lard) contains a number of fatty acids but common are six (72% w/w)[34]. If three stereospecific positions on glycerol molecule were assumed to be equivalent and isomers are not separated, then for six fatty acids, the total possible TAG species were calculated to be 56 by using the formula $(n^3 + 3n^2 + 2n)/6$ (where n = total number of fatty acids)[35]. However, the practically observed value for TAG is always less than the calculated number[36]. TAG composition of lard, palm oil and their admixtures are given in **Table 3**.

Figure 2 shows the TAG profile of binary laboratory admixtures of the palm oil containing 5 and 10% lard (w/w). Two characteristic peaks with retention times (t_R-values) of 7.3 and 8.9 minutes curene selected for testing of lard in palm. Palm contains 31.38% SPL molecular species at t_R 7.3 where as lard have 13.70%. Addition of lard in palm oil shows gradual decrease in

concentration of SPL TAG. TAG (SPO) composition at t_R 8.9 increases on addition of lard in palm **(Table 3)**. From the **Table 3**, it is clear that addition of lard in palm oil is easily detectable by observing the composition of molecular species at t_R 7.3 and 8.9.

The TAG-profiling method is simple, rapid and needs no derivatization. The TAG-profiling of palm oil, lard and their admixtures is of great value for the detection of lard in palm oil. The detection limit for pig fat was about 5%.
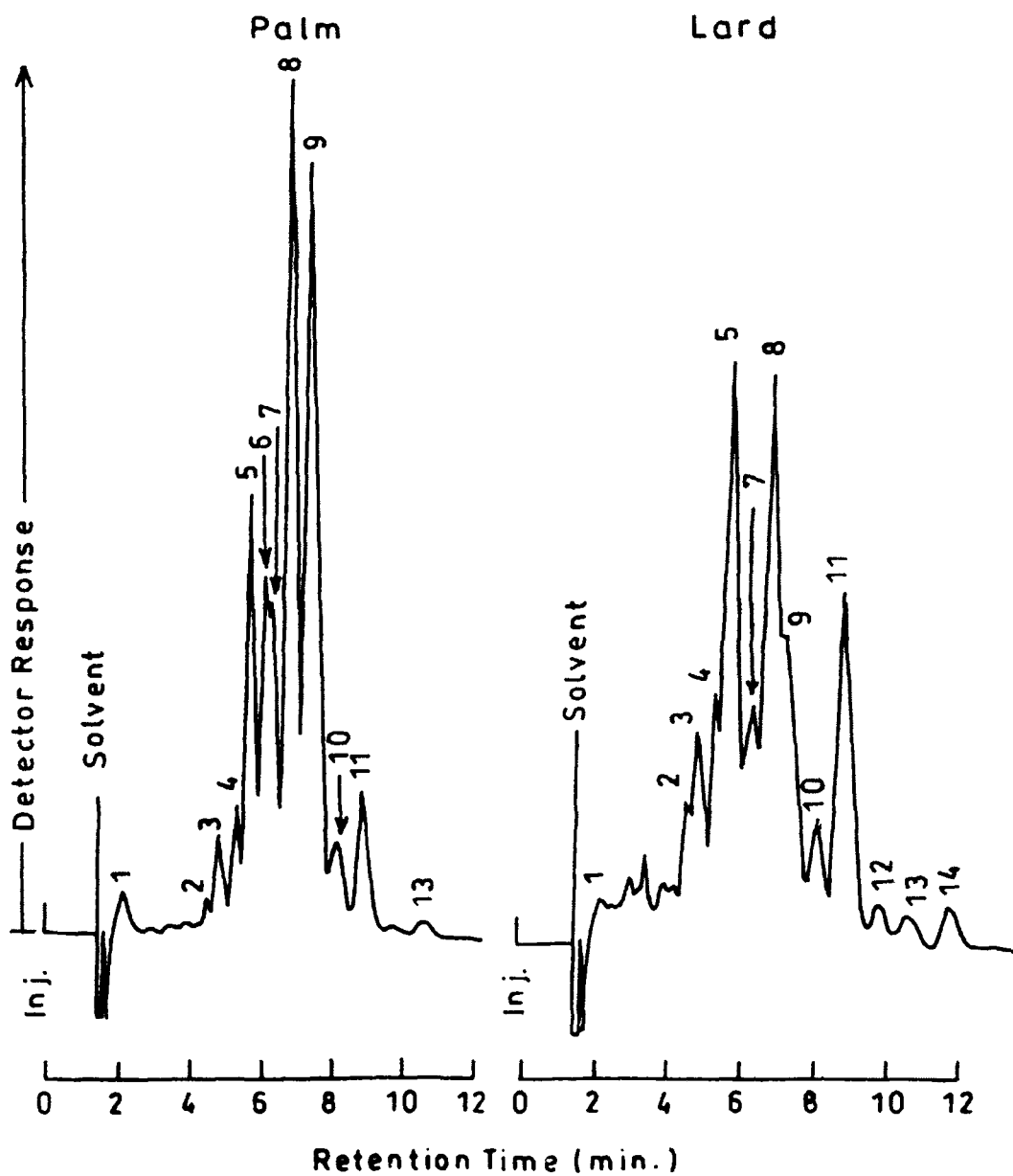
Figure 1: HPLC chromatogram of triacylglycerols of palm oil (P) and lard (L). Tentative identification of peaks 1,2,3.... are given in **Table 3**.
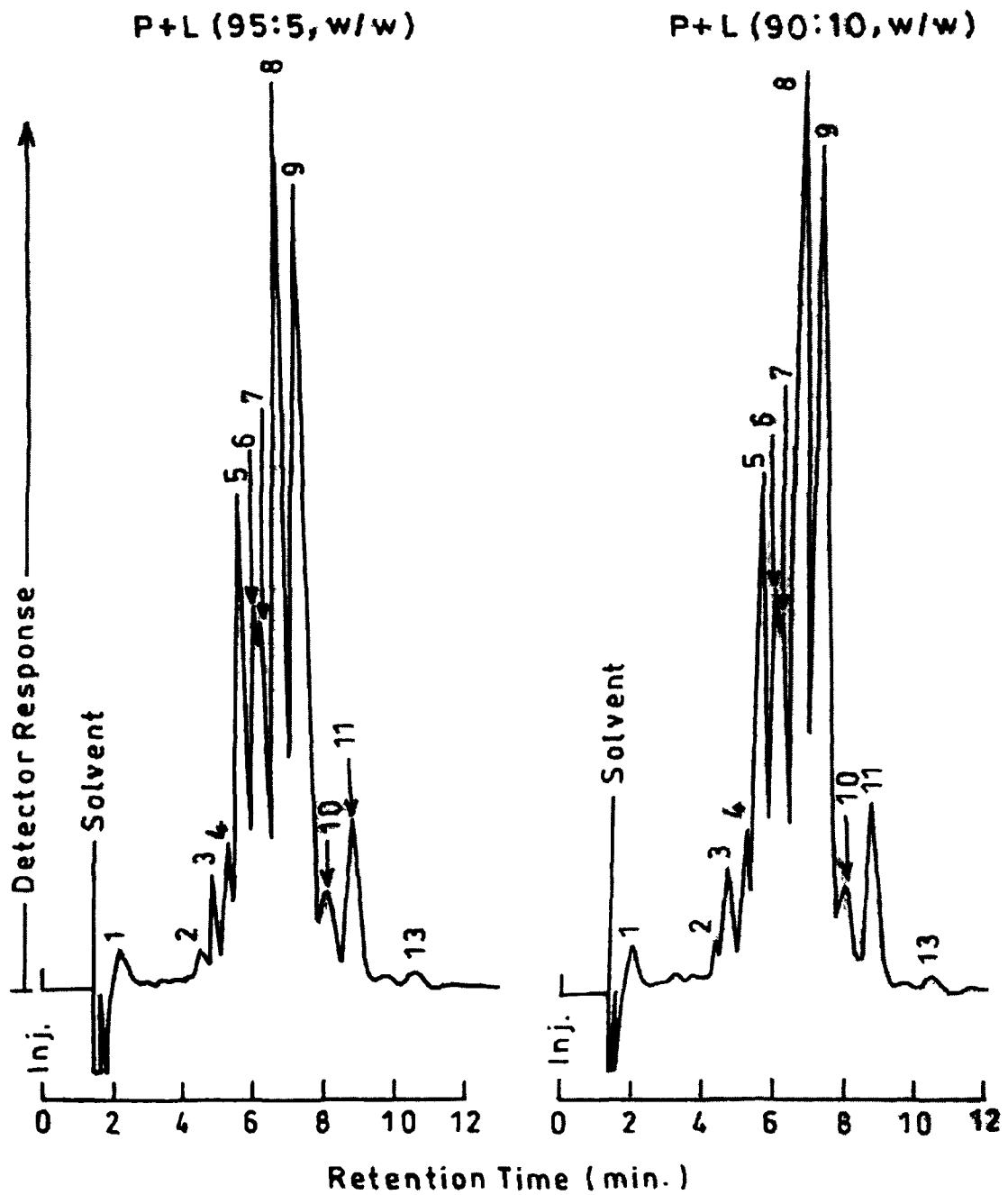
Table 3: TAG composition of lard, palm and lard-palm admixtures by high performance liquid chromatography.

Peak No.[#]	Retention time (min.)	TAG composition (%)[*]					
		Palm	**LP-5**	**LP-10**	**LP-15**	**LP-20**	**Lard[+]**
1.	2.2	1.10	1.17	0.99	1.05	0.96	0.42
2.	4.6	0.45	0.54	0.63	0.68	0.79	2.26
3.	4.9	2.48	2.65	2.87	2.99	3.24	5.71
4.	5.4	2.58	2.67	2.81	2.92	3.08	4.69
5.	5.7	10.92	11.31	11.73	11.83	12.47	19.75
6.	6.2	7.56	7.55	7.09	6.90	6.97	-
7.	6.4	7.48	7.11	7.35	7.36	7.20	7.94
8.	6.8	26.75	26.47	26.33	26. 13	25.93	21.08
9.	7.3	31.38	30.50	29.73	28.97	28.10	13.70
10.	8.2	3.35	3.32	3.44	3.49	3.33	4.07
11.	8.9	5.44	5.98	6.47	6.72	7.18	14.43
12.	9.9	-	-	-	0.33	0.14	0.97
13.	10.7	0.51	0.73	0.56	0.63	0.61	1.09

[+]Other TAG, RT (%): 3.0 (0.62, ?) 3.2 (0.16, ?), 3.4 (0.94, LLLe), 3.9 (0.20, LLL), 11.9 (1.97, S_3)

[#]Tentative identification of peaks: 2,3,4,5,6,7,8,9,10,11,12 and 13 are L_3O, OML + L_2P, O_2L, LPO, P_2L + O_2S, O_2P, SOL+P_2O, SPL, O_2S, SPO, P_2S, S_2O respectively. Other TAG are not identified. Each TAG represents all possible isomers of constituent fatty acids, where Le = 18:3, L=18:2, O=18:1, S=18:0, P=16:0, M=14:0

[*]Average of three determinations.

P+L (95:5,w/w)

P+L (90:10,w/w)

Detector Response

Solvent

Solvent

Inj.

Inj.

8

9

5 6 7

3 4

2

1

10

11

13

8

9

5 6 7

3 4

2

1

10

11

13

0 2 4 6 8 10 12

0 2 4 6 8 10 12

Retention Time (min.)

Figure 2: Triacylglycerol-profiling of palm oil (P) – lard (L) admixture. Tentative identification of peaks 1,2,3 ..... are given in **Table 3**.

2.3 Experimental

Materials

Pork was obtained from the local market. Fat was extracted from the minced samples by a modified Folch *et al.*[33] procedure with $CHCl_3$ / CH_3OH (2:1, v/v) as solvent.

A. Palm oil sample was obtained from the local market. All chemicals used in this experiment were of analytical or HPLC-grade. For HPLC-analysis HPLC-grade solvents were filtered through a 0.22 μm Millipore filter before use.

Blend Proportions

Adulterated palm oil (P) samples (**Table 4**) were prepared by mixing lard (L) in the following proportions:

Table 4: Adulterated palm oil samples prepared by mixing lard (w/w).

Sample	Palm (P)	Lard (L)
LP-5	95	5
LP-10	90	10
LP-15	85	15
LP-20	80	20

Isolation of neutral triacylglycerols

Triacylglycerols (TAG) were isolated from fat/oil and mixed samples by column chromatography. A small chromatographic column (16 cm x 2 cm, i.d.) was carefully packed with slurry of 5 g silica gel (60-120 mesh) in n-hexane. After settle down of the silica, 0.5 g of the dried fat/oil in 0.5 ml of n-hexane was introduced into the column and sample was allowed to adsorb on the surface of silica. The column was eluted with 25 ml of n-hexane followed by 5% diethyl ether in n-hexane until the TAG were completely eluted. Fractions (20 ml, each) were collected and solvent was removed. The purity of the TAG fractions was assessed on thin-layer (0.25 mm thickness) chromatography plate (TLC) using n-hexane/diethyl ether/acetic acid (75:25:1, v/v) as the developing system. The spots were visualized by charring after spraying with a 20% aqueous solution of perchloric acid.

Reversed-phase high performance liquid chromatography (RP-HPLC)

The separation of TAG was performed on LiChrospher 100 RP-18 (5 μm column, 12.5 cm x 4 mm i.d., E. Merck, Darmstadt, Germany). The analysis was carried out isocratically with a mobile phase consisting of acetonitrile/dichloromethane (58:42, v/v).

TAG samples (5%) were dissolved in HPLC-grade mobile phase and 20 μl aliquots were injected onto the column (column embedded into a CTO-10A column oven, solvent delivery module, LC-10AD, Shimadzu, Japan) and eluted at a flow rate of 1 ml/min at 25°C. The effluent was monitored with a Waters 410 RI detector and chromatogram recorded (chart speed 5 mm/min) with a C-R4A chromatopac multifunctional data processor. TAG were tentatively identified by comparing retention times to pure standards.

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PART-II

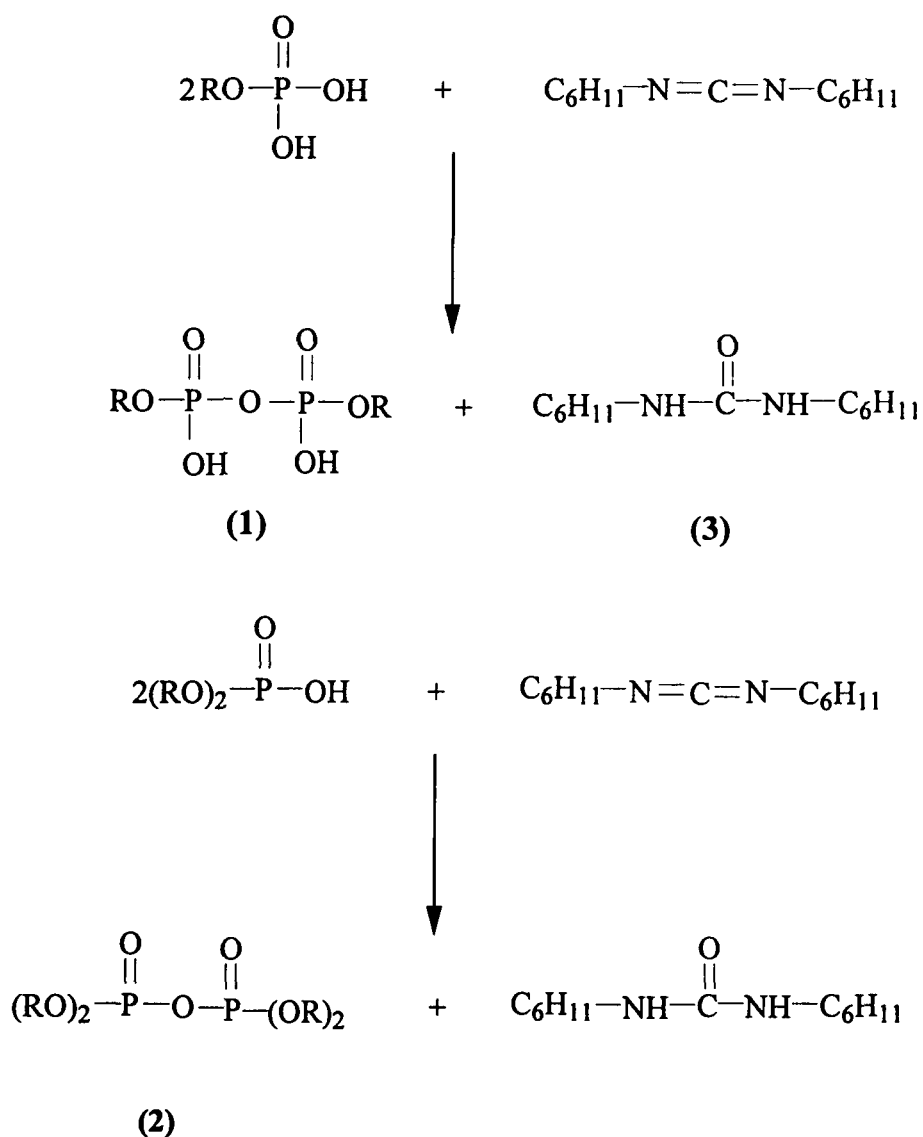## Synthesis of Fatty Acid Derivatives

CHAPTER-3

Direct Esterification of Long-Chain

Fatty Acids by using

N,N'-Dicyclohexylcarbodiimide

in the presence of

4-(N,N-Dimethylamino)pyridine

3.1 Theoretical

Fatty acid derivatives have been the subject of extensive study in the recent past. Numerous reports in the form of reviews[1,2] have appeared ~~in the literature~~ which highlight the chemistry and uses, especially their synthesis. Fatty acid derivatives have been found to be associated with diverse biological activities such as herbicidal[3], antifeedant[4], neuroprotective[5], bactericidal[6], fungicidal[7], antioxidant[8], antimicrobial[7-9,11], antifogging[12], antiparasitic[13], and anti-inflammatory.[14] Some fatty acid esters are found very effective for the treatment of cardiovascular, hepatic and renal diseases.[15] Similarly fatty acid esters are useful for plasticizer in biodegradable plastic materials[16] and are known to be good alternative fuels (biodiesel).[17] Also, fatty acid monoesters are widely used in industry due to their lubricating and softening properties.[18] Fatty acid esters constitute an important group of fatty acid derivatives, especially when we consider the large number of representatives of this type, which have been synthesized and studied. These synthetic esters include the simple alkyl esters, the esters of aromatic alcohols and the more complex esters such as those of cellulose, starch and allied compound.
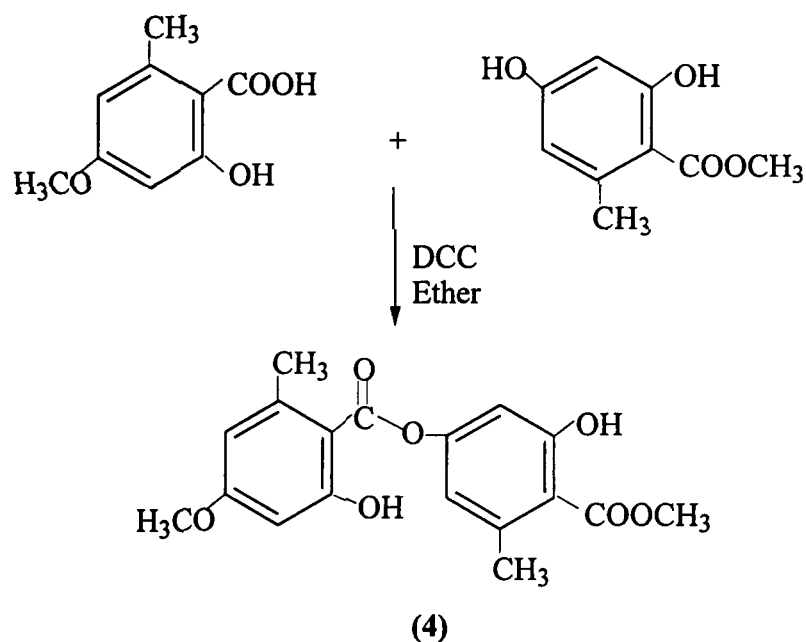
A number of methods[19] have been reported to esterify fatty acids but most of them require either the acidic or basic medium and application of heat. The utilization of N,N'-dicylohexylcarbodiimide (DCC) as an activating agent in the direct room temperature esterification has drawn chemist's attention. Khorana et al.[20] observed that DCC reacts with mono- and di- esters of

phosphoric acid at room temperature to yield corresponding di- (1) and tetra- (2)

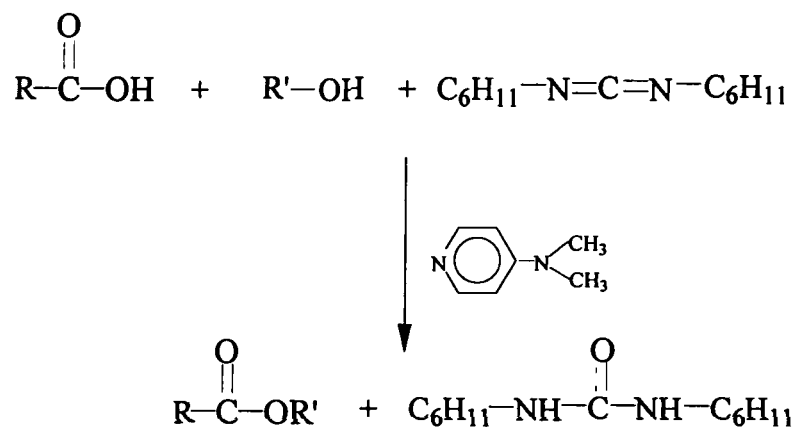esters of pyrophosphoric acid, with the precipitation of dicyclohexylurea (3).

$$2\,RO-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle OH}{|}}{P}}-OH \quad + \quad C_6H_{11}-N=C=N-C_6H_{11}$$

$$RO-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle OH}{|}}{P}}-O-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle OH}{|}}{P}}-OR \quad + \quad C_6H_{11}-NH-\overset{\overset{\displaystyle O}{\|}}{C}-NH-C_6H_{11}$$

(1)   (3)

$$2\,(RO)_2-\overset{\overset{\displaystyle O}{\|}}{P}-OH \quad + \quad C_6H_{11}-N=C=N-C_6H_{11}$$

$$(RO)_2-\overset{\overset{\displaystyle O}{\|}}{P}-O-\overset{\overset{\displaystyle O}{\|}}{P}-(OR)_2 \quad + \quad C_6H_{11}-NH-\overset{\overset{\displaystyle O}{\|}}{C}-NH-C_6H_{11}$$

(2)

In the earlier studies[21], it was observed that being an activating agent

DCC promotes esterification of primary and secondary alcohols under very

mild conditions while tertiary alcohols results in very low yield. Pless and

co-workers[22] prepared esters by the condensation of N-protected amino acid

and phenol with DCC. Neelakantan *et al.*[23] used DCC successfully for the synthesis of Lichen depsides e.g. 3-methyl evernate (4).

$$CH_3$$
$$COOH$$

$$H_3CO \qquad OH$$

$$+$$

$$HO \qquad OH$$
$$COOCH_3$$
$$CH_3$$

$$\downarrow \quad \begin{array}{l} DCC \\ Ether \end{array}$$

$$CH_3 \quad O$$
$$\overset{\parallel}{C} - O \qquad OH$$

$$H_3CO \qquad OH \qquad COOCH_3$$
$$CH_3$$

(4)

Later, Hassner *et al.*[24] ~~have~~ introduced a modification of the DCC-mediated esterification, which consists in the addition of a catalytic amount of 4-dialkylamino pyridine (DMAP) to a mixture of acid, alcohol and carbodiimide.
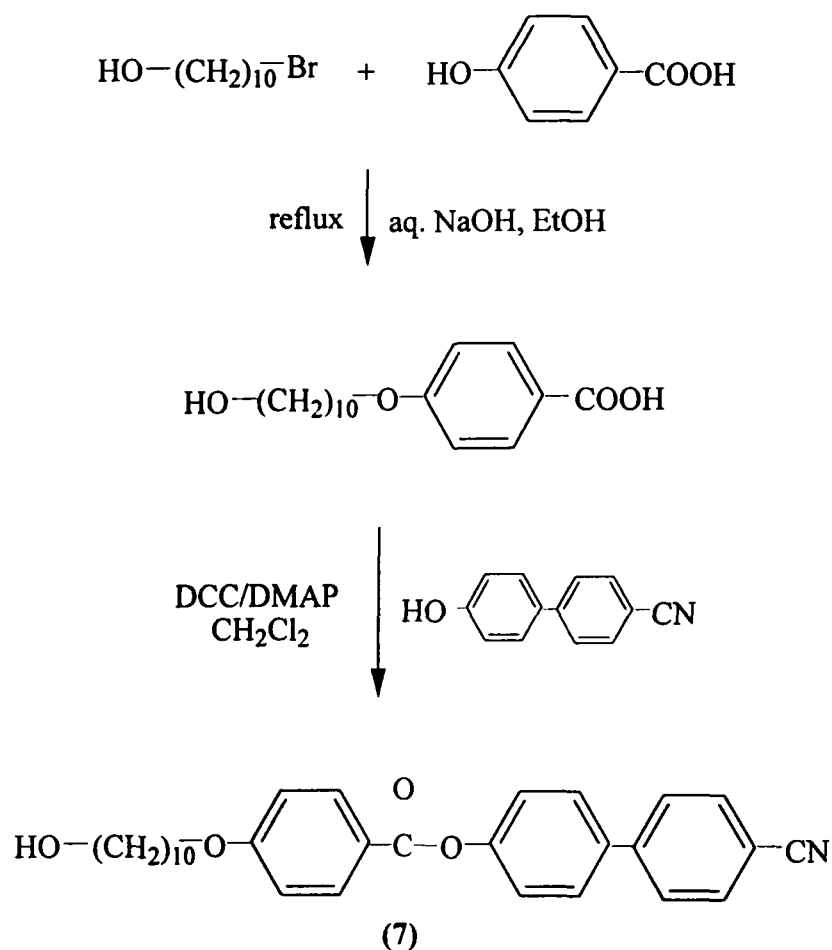
$$\overset{O}{\overset{\parallel}{R-C-OH}} \quad + \quad R'-OH \quad + \quad C_6H_{11}-N=C=N-C_6H_{11}$$

$$\downarrow$$

$$N \overset{}{\bigcirc} - N \overset{CH_3}{\underset{CH_3}{\diagup}}$$

$$\downarrow$$

$$\overset{O}{\overset{\parallel}{R-C-OR'}} \quad + \quad C_6H_{11}-NH-\overset{O}{\overset{\parallel}{C}}-NH-C_6H_{11}$$

Neises *et al.*[25] ~~have~~ synthesized tert-butylethyl fumarate (5) by reacting

italics

~~the~~ monoester of (*E*)-2-butenedioic acid with tert-butyl alcohol, using DCC-

mediated esterification.

(5)
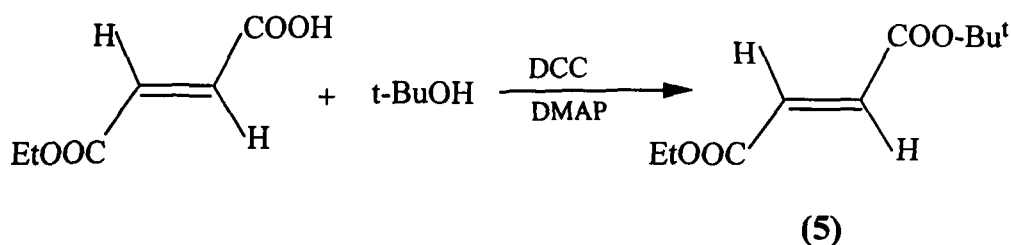
Perrone *et al.*[26] took advantage of this procedure to develop ~~an~~ improved

method for the esterification of sugar acetals with DCC in the presence of

N,N-(dimethylamino)pyridine. Wen *et al.*[27] ~~have~~ reported ~~a~~ synthesis of

fluorinated aromatic acid sterol esters by reacting cholesterol or cholestanol

with ~~a~~ *n* F$_n$-phenyl-A (n = 2-3 and A = a single bond or HC=CH) carboxylic acid

in the presence of DCC and DMAP at 10-50°C. Sonpatki *et al.*[28] ~~have~~

synthesized v-keto ester (6) by using this method.

(6)
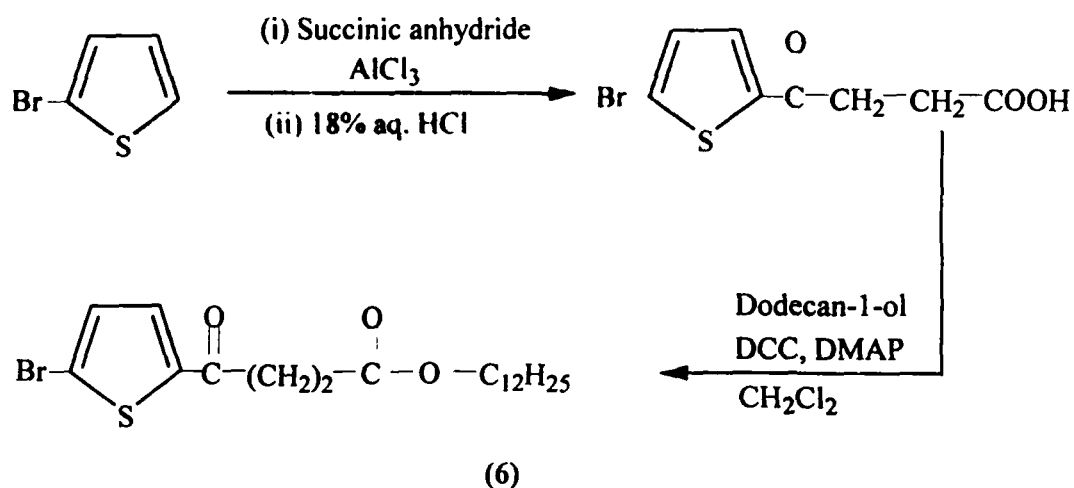
By employing this route, Dardel *et al.*[29] ~~have~~ synthesized liquid crystalline promotor (7).

$$HO-(CH_2)_{\overline{10}}Br \quad + \quad HO-\langle\bigcirc\rangle-COOH$$

reflux | aq. NaOH, EtOH

$$HO-(CH_2)_{\overline{10}}O-\langle\bigcirc\rangle-COOH$$

DCC/DMAP
CH_2Cl_2 | $HO-\langle\bigcirc\rangle\langle\bigcirc\rangle-CN$

$$HO-(CH_2)_{\overline{10}}O-\langle\bigcirc\rangle-\overset{\overset{\displaystyle O}{\|}}{C}-O-\langle\bigcirc\rangle\langle\bigcirc\rangle-CN$$

(7)

Recently Greetings and co-workers[15] used DCC-mediated esterification in the syntheses of natural compounds and their derivatives including tyrosol and hydroxy tyrosol esters represented in formula (8). They ~~have~~ showed that the reaction of 2-[3,4-dihydroxyphenyl] ethanol (i.e. hydroxytyrosol) with 3 equivalent of stearic acid and DCC in the presence of DMAP gave the triester (9).
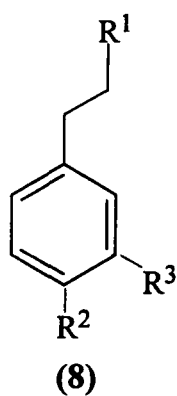
R¹ & R² = OH, OCO-alkyl or OCO-alkenyl

R³ = H, OH, OCO-alkyl or OCO-alkenyl

(8)

$+ \; 3CH_3\!-\!(CH_2)_{16}\!-\!COOH \xrightarrow[\text{rt}]{\text{DCC/DMAP}}$

(9)
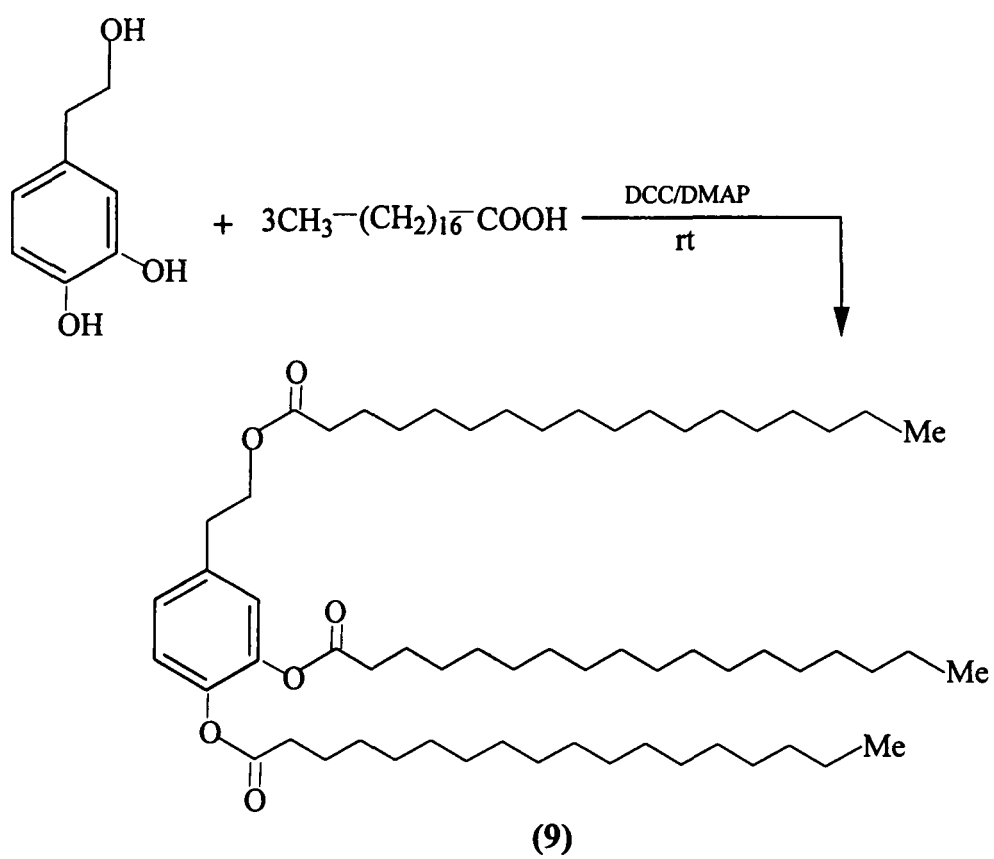
This invention relates to the use of **8** for prevention or treatment of cardiovascular, hepatic and renal diseases and for cosmetic applications.

To our knowledge the analogous reaction had not been extensively applied to esterify long-chain fatty acids. Prompted by these observations and as part of our aim in the syntheses of fatty acid derivatives we ~~have~~ *undertook* ~~under taken~~ *(the* ~~this problem~~ "Direct room temperature esterification of long-chain fatty acids *))* by using DCC in the presence of DMAP. It includes the synthesis of 1'-phenylethyl undec-10-enoate (III), 1'-phenylethyl (Z)-octadec-9-enoate (V), 1'- phenylethyl (Z)-12-hydroxyoctadec-9-enoate (VII) and 1'-phenylethyl (Z)-9-hydroxyoctadec-12-enoate (IX) by the reaction of corresponding fatty acid i.e. undec-10-enoic (I), (Z)-octadec-9-enoic (IV), (Z)-12-hydroxyoctadec-9-enoic (VI) and (Z)-9-hydroxyoctadec-12-enoic acid (VIII) with 1-phenylethanol (II) by using DCC in the presence of DMAP at room temperature. Under similar *ool* conditions compound (I) ~~was~~ also ~~allowed to~~ react *with* diphenylmethanol (X), 1-(2'-hydroxy-4''-methoxyphenyl)-3-phenyl-prop-2-en-1-ol (XII) and ethylene glycol monostearate (XIV) separately, to yield their corresponding esters, diphenylmethyl undec-10-enaote (XI), 3'-(2''-hydroxy-4''-methoxyphenyl)-1'-phenylpropenyl undec-10-enoate (XIII) and ethylene glycol 1-stearate-2-undecenoate (XV) respectively.

3.2 Results and Discussion

Preparation of 1'-phenylethyl undec-10-enoate (III)

The reaction of undec-10-enoic acid (I) with 1-phenylethanol (II) (Scheme 1) was carried out using the procedure of Hassner et al.[24]. The progress of reaction was monitored by TLC (silica gel G, petroleum ether-diethyl ether, 8:2, v/v). The completion of the reaction was finally checked by TLC, which revealed the presence of a single spot, labelled as III. On workup with diethyl ether the crude product was obtained which was chromatographed over a column of silica gel (60-120 mesh). Elution with petroleum ether-diethyl ether (95:5, v/v) gave the product (III) in 95% yield.
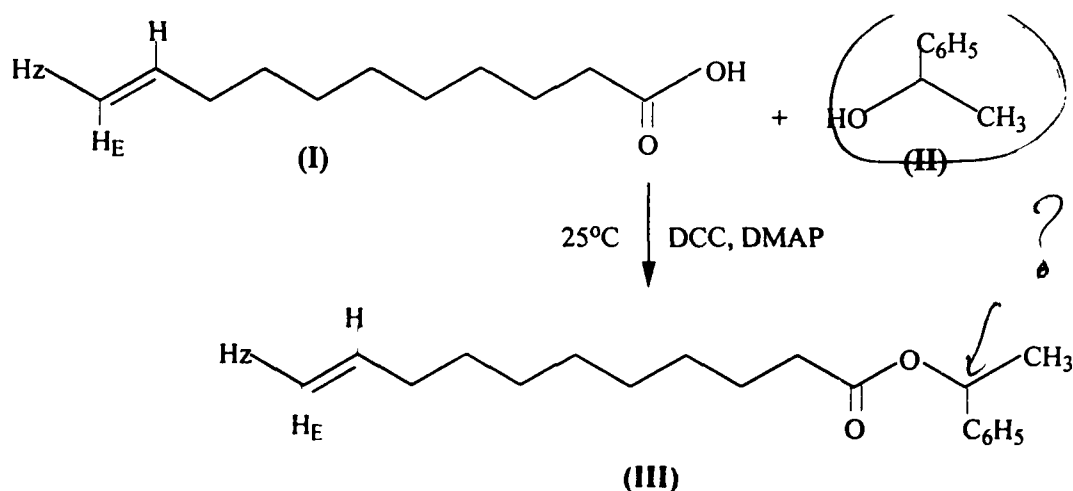
Scheme 1: Preparation of 1'-phenylethyl undec-10-enoate (III).

Structure elucidation of the product (III)

It was a colourless liquid. The structure of III has been confirmed by IR, EI-MS, ^{1}H- and ^{13}C-NMR. The IR spectrum (KBr) of III displayed characteristic band at 1735 cm^{-1} confirming the presence of an ester carbonyl group. The mass spectrum of III derived by utilizing electron impact ionization showed a molecular ion peak at m/z 288 which is consistent with its molecular formula $C_{19}H_{28}O_2$. Also, it gave a distinctively McLafferty rearrangement fragmentation ion at m/z 164 that clearly established the newly introduced functionality to reside at C_1 of the alkyl chain. The base peak observed at m/z 105 was formed by the cleavage of the O-C bond of the ester group. The fragmentation pattern is shown in **Chart 1**. The assignments of all ^{1}H- and ^{13}C-NMR signals to individual H- and C-atoms were performed on the patterns basis of typical chemical shift values, splitting constants, multiplicity and relative integrations. The characteristic signals for aromatic protons were observed at δ 7.34 and δ 7.28. The signal at δ 5.88 (J = 6.6 Hz) was attributed to one methine proton. A doublet for the methyl protons appeared at δ 1.54, a distorted triplet at δ 2.32 and a multiplet at δ 1.61 was attributed to α- and β-methylenic protons with respect to the carbonyl group and a multiplet at δ 2.03 for two allylic protons. Two doublet of doublet at 4.91 and 5.01 were assigned to two terminal olefinic protons and a doublet of doublet at δ 5.80 for one olefinic proton. A broad singlet at δ 1.34 was assigned to the protons of the aliphatic chain. The ^{13}C-NMR spectrum was more informative about the assigned
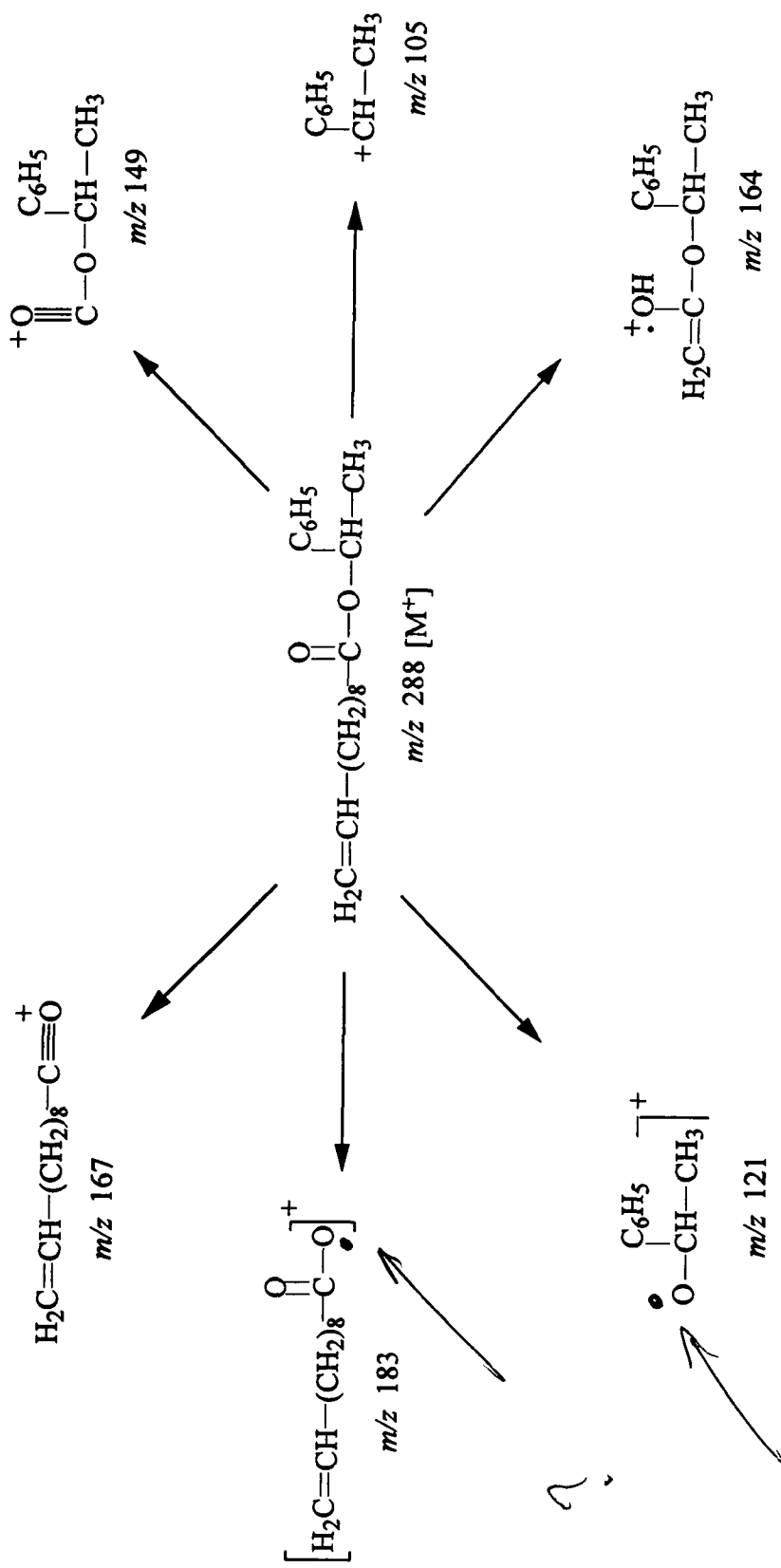
**Chart 1:** Mass fragmentation pattern of III.

structure which showed a peak at δ 173.0 ascribable to carbonyl carbon and a

able

peak at δ 72.0 ~~was~~ assigned for one methine proton bearing carbon at ester

the *please rephrase*

side. The characteristic peaks for aromatic ~~ring~~ carbons were observed at

δ 141.8, 128.4, 127.7 and 126.0.

On the basis of above facts the product (III) was characterized as

1'-phenylethyl undec-10-enoate.

Preparation of 1'-phenylethyl (Z)-octadec-9-enoate (V)

The reaction of (Z)-octadec-9-enoic acid (IV) with 1-phenylethanol (II)

(**Scheme 2**) was carried out as given above. The examination of the final

reaction product revealed one distinctive spot, indicating complete conversion

gave

of acid into ester. Purification over a column of silica gel, ~~pure product~~ (V) ~~was~~

~~obtained~~ in 95% yield.

racemate ?
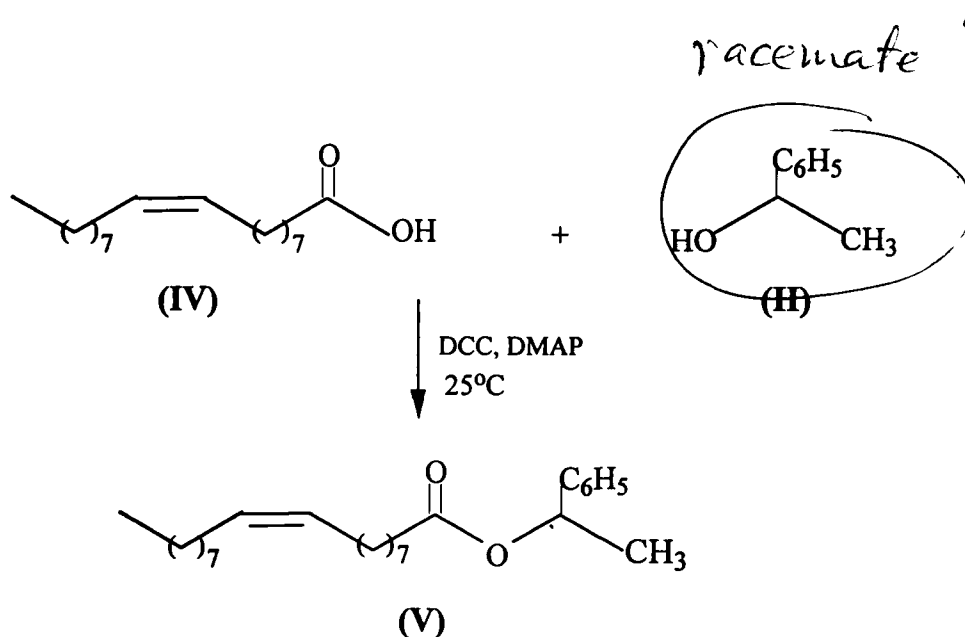
Scheme 2: Preparation of 1'-phenylethyl (Z)-octadec-9-enoate (V).

Structure elucidation of the product (V)

obtained as

Ester (V) ~~it~~ was a colourless oily liquid. Elemental analysis of the pure product

(V) corresponded to formula $C_{26}H_{42}O_2$. Its IR spectrum displayed characteristic

band at 1730 cm^{-1} indicating the presence of an ester carbonyl group. The

EI-MS spectrum showed *a* molecular ion peak at m/z 386. The other diagnostic

peaks were observed at m/z 265 (M-OCH(C_6H_5)CH$_3$), 237 (M-COOCH

(C_6H_5)CH$_3$), 149 (M-CH$_3$(CH$_2$)$_7$CH=CH(CH$_2$)$_7$), 139 (M-(CH$_2$)$_7$COOCH

(C_6H_5)CH$_3$). The base peak at m/z 105 ~~was arised~~ *formed* by the cleavage of O-C bond

~~at the side of ester~~. Another characteristic peak arised at m/z 164 by McLafferty

rearrangement (Chart 2). The ^{1}H-NMR spectrum of V showed signals at

δ 7.35 and 7.27 for aromatic protons. A quartet at δ 5.88 was attributed to one

methine proton and a doublet at δ 1.53 for methyl protons at ester side. Three

multiplets at δ 5.35, δ 2.30 and δ 1.61 were assigned for two olefinic protons,

α- and β-methylenic protons (with respect to carbonyl group) respectively. A

multiplet at δ 2.01 was attributed to four methylenic protons at C_8 and C_{11}. The

structure of V was further supported by its ^{13}C-NMR spectrum, which showed

an ester

a sharp singlet at δ 173.0 ascribable to carbonyl carbon and a peak observed at

δ 72.0 for methine carbon ~~at ester side~~. The diagnostic peaks for aromatic

carbons were observed at δ 141.8, 128.4, 127.7 and 126.0. On the basis of

spectral data compound V was characterized as *1'-phenylethyl (Z)-octadec-9-*
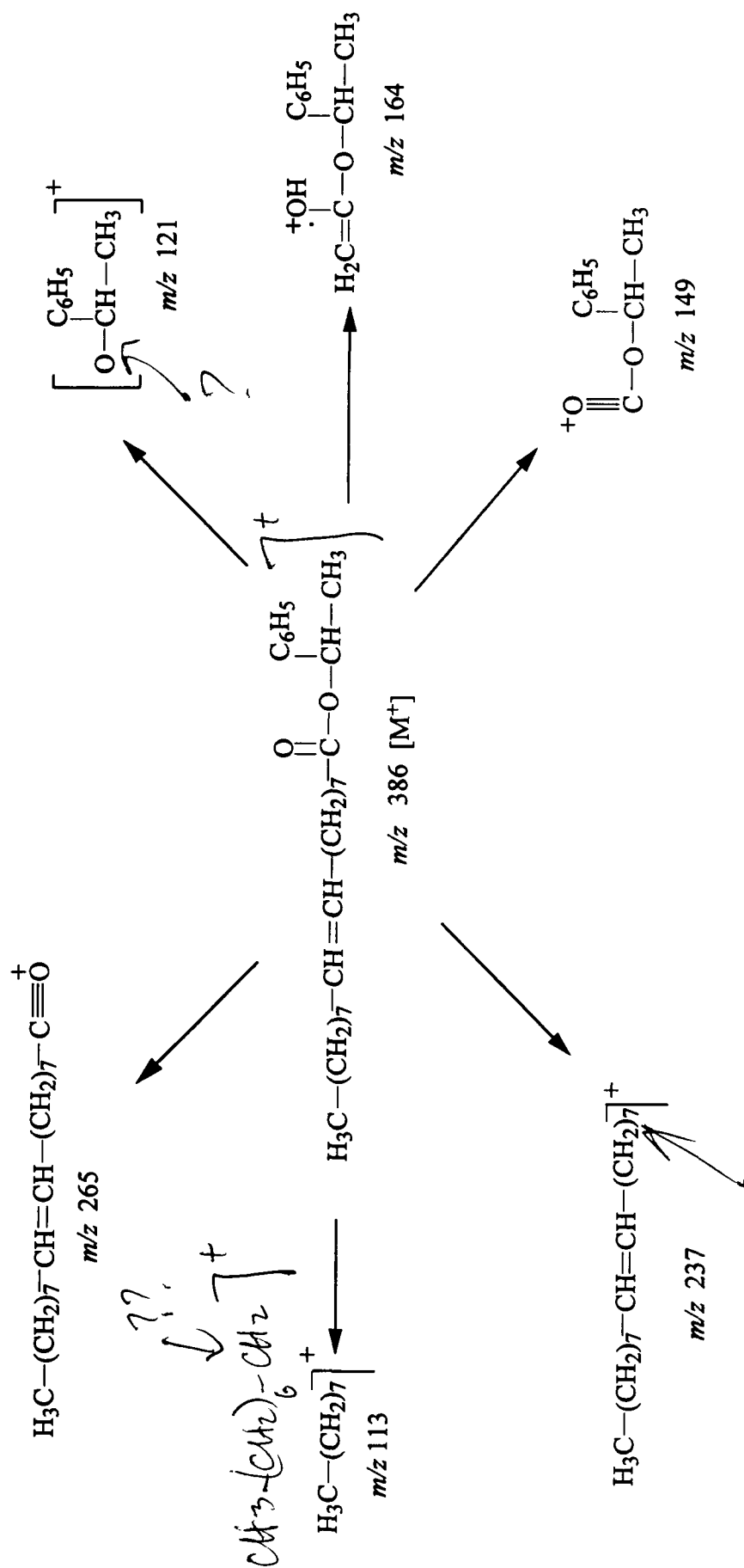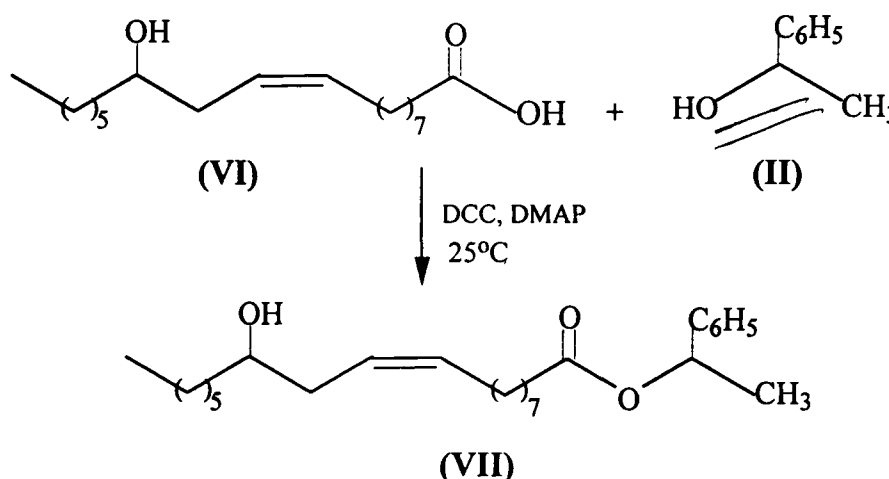
enoate. *racemic ?*

Chart 2: Mass fragmentation pattern of V.

Preparation of 1'-phenylethyl (Z)-12-hydroxyoctadec-9-enaote (VII)

Reaction of (Z)-12-hydroxyoctadec-9-enoic acid (VI) with 1-phenylethanol (II) by using DCC and DMAP in dichloromethane resulted in the formation of VII, as an oily liquid (Scheme 3). On workup with diethyl ether pure product (VII) was obtained in 90% yield.

Scheme3 : Preparation of 1'-phenylethyl (Z)-12-hydroxyoctadec-9-enoate (VII).

Structure elucidation of the product (VII)

It was a colourless liquid. Its structure has been established by IR, FAB-MS, ^{1}H- and ^{13}C-NMR spectra. IR spectrum of VII suggested the presence of ester carbonyl group by exhibiting absorption band at 1735 cm^{-1}. The FAB-MS spectrum of VII showed a peak at m/z 403, corresponding with [M+H]$^+$, which confirmed its molecular weight (402) (C$_{26}$H$_{42}$O$_3$). The base peak observed at m/z 105 by the cleavage of O-C bond at the side of ester. The other diagnostic

peaks resulted from bond cleavage next to C=O, were observed at *m/z* 281, 253, 149 and 121. The mode of fragmentation is shown in **Chart 3**. The ^{1}H-NMR spectrum of **VII** showed (in addition to normal signals of fatty acid ester) diagnostic signals at δ 7.34 and at δ 7.28 for aromatic protons. A multiplet at δ 5.87 attributed to one methine proton and a doublet at δ 1.51 assigned to methyl protons at the side of ester. A multiplet was observed at δ 5.55-5.37 for two olefinic protons. A multiplet assigned for one methine proton at C_{12}, was observed at δ 3.59 and a multiplet at δ 2.19 and 2.03 attributed to methylenic protons at C_{11} and C_8. The signal for hydroxy proton appeared at δ 2.30, which was disappeared on D_2O shake. The ^{13}C-NMR spectrum of **VII** also gave good agreement with the proposed structure. It gave a chemical shift at δ 173.0 corresponding to carbonyl carbon and at δ 72.0 for methine carbon at ester side. The chemical shifts of aromatic carbons were observed at δ 141.9, 128.4, 127.7 and 126.0.

On the basis of above facts, the structure of **VII** was formulated as *1'-phenylethyl (Z)-12-hydroxyoctadec-9-enoate*.

Preparation of 1'-phenylethyl (Z)-9-hydroxyoctadec-12-enoate (IX)

The reaction of (Z)-9-hydroxyoctadec-12-enoic acid (**VIII**) with 1-phenylethanol (**II**) (Scheme 4) carried out as given above. Final TLC examination of the reaction mixture showed one distinctive spot. On workup with diethyl ether an oily product was obtained, which was chromatographed

$H_3C-(CH_2)_5-\underset{\underset{\textstyle OH}{|}}{CH}-CH_2-CH=CH-(CH_2)_7-C\equiv \overset{+}{O}$

m/z 281

$H_3C-(CH_2)_5-CH$
$\overset{+}{O}H$

m/z 115

$H_3C-(CH_2)_5-\underset{\underset{\textstyle OH}{|}}{CH}-CH_2-CH=CH-(CH_2)_7\Big]^+$

m/z 253

$\left[\begin{array}{c} C_6H_5 \\ | \\ O-CH-CH_3 \end{array}\right]^+$

m/z 121

$H_3C-(CH_2)_5-\underset{\underset{\textstyle OH}{|}}{CH}-CH_2-CH=CH-(CH_2)_7-\overset{\overset{\textstyle O}{||}}{C}-O-\underset{\underset{\textstyle C_6H_5}{|}}{CH}-CH_3$

m/z 403 [M+H]$^+$

$\overset{+}{O}=C-O-\underset{\underset{\textstyle C_6H_5}{|}}{CH}-CH_3$

m/z 149

$\left[\begin{array}{c} C_6H_5 \\ | \\ H_3C-(CH_2)_5-CH-CH_2 \\ | \\ OH \end{array}\right]^+$

m/z 129

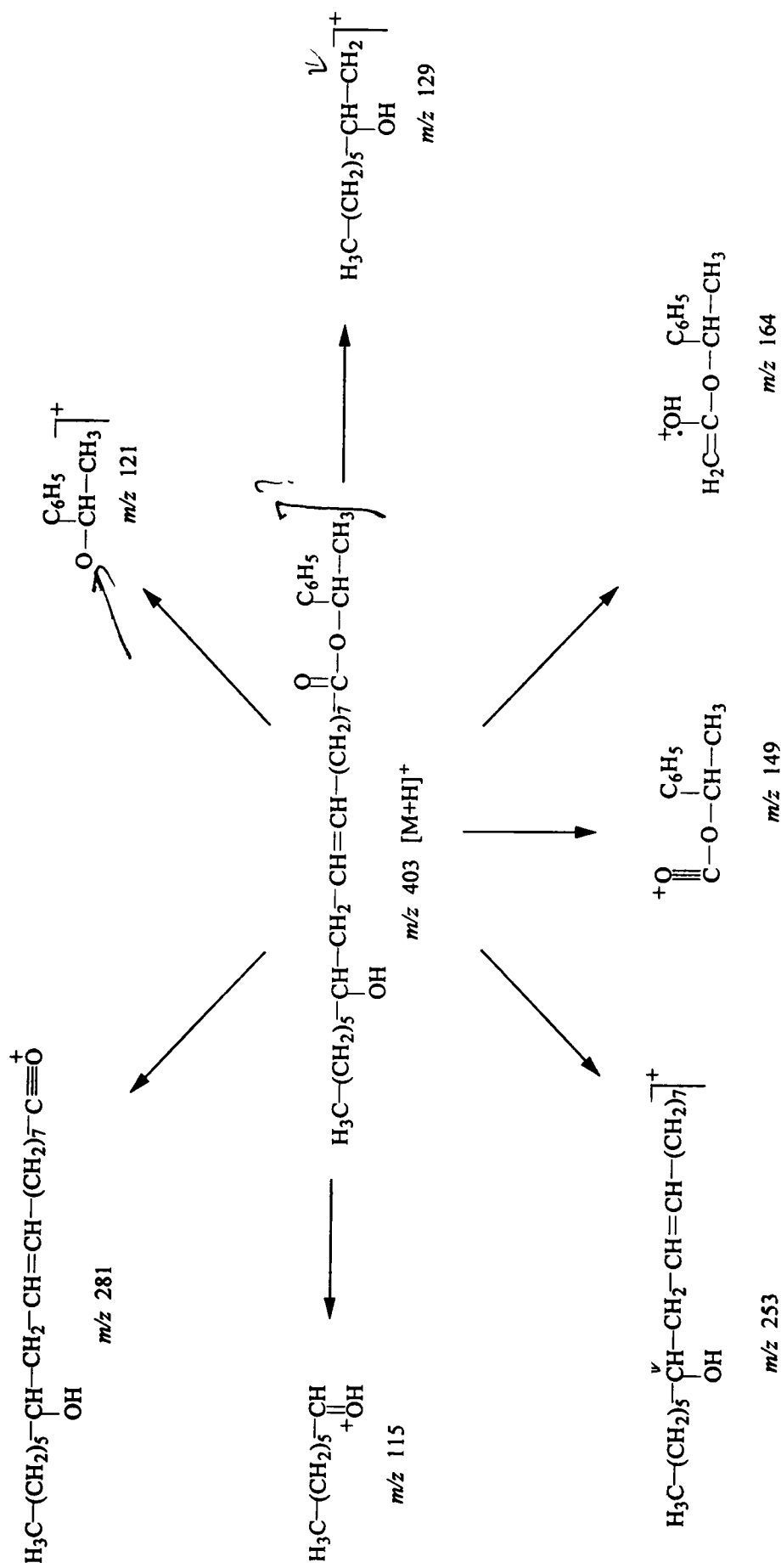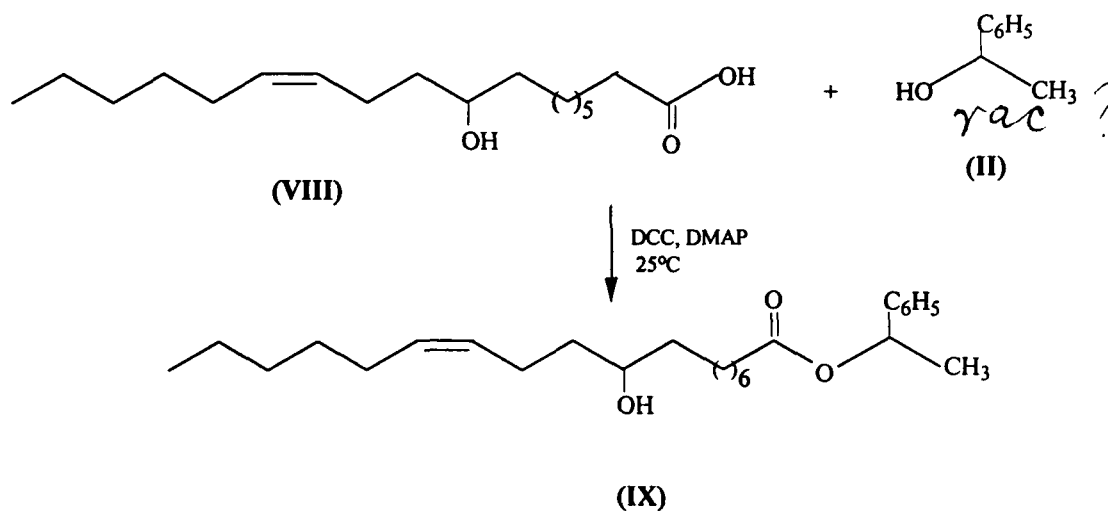$\overset{+}{:}OH \quad C_6H_5$
$H_2C=C-O-CH-CH_3$
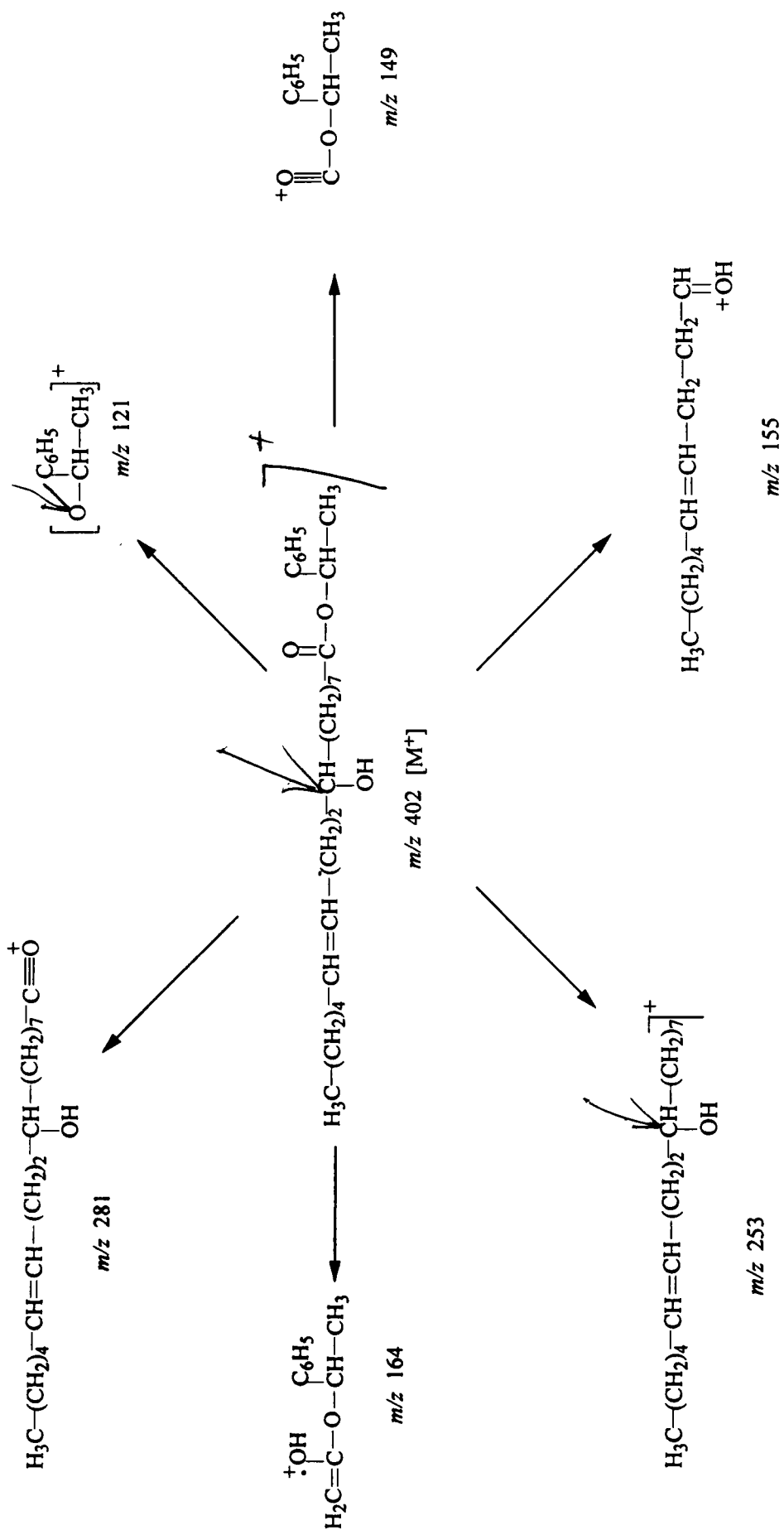
m/z 164

Chart 3: Mass fragmentation pattern of VII

over a column of silica gel. Elution with petroleum ether-diethyl ether (95:5, v/v) gave pure product (IX) in 90% yield.

Scheme 4: Preparation of 1'-phenylethyl (Z)-9-hydroxyoctadec-12-enoate (IX).

Structure elucidation of the product (IX)

It was a colourless oily liquid. The structure of IX has been confirmed by IR, EI-MS and ^{1}H-NMR spectra. IR spectrum of IX displayed characteristic band at 1740 cm^{-1} suggesting the presence of an ester carbonyl group. The EI-MS spectrum showed no molecular ion peak due to loss of water molecule from the parent molecule. The other characteristic peaks resulted from bond cleavage next to C=O, were observed at m/z 281, 121, 253 and 149. The distinctive peak for McLafferty rearrangement fragmentation ion was appeared at 164. The mode of fragmentation pattern is shown in Chart 4. The ^{1}H-NMR spectrum of IX showed structure revealing diagnostic signals at δ 7.34 and 7.25

C_6H_5
$+O\!\equiv\!C\!-\!O\!-\!CH\!-\!CH_3$ *m/z* 149

$\left[O\!-\!CH\!-\!CH_3\right]^+$ with C_6H_5 *m/z* 121

$H_3C\!-\!(CH_2)_4\!-\!CH\!=\!CH\!-\!CH_2\!-\!CH_2\!-\!CH\!=\!+OH$ *m/z* 155

$H_3C\!-\!(CH_2)_4\!-\!CH\!=\!CH\!-\!{}^7(CH_2)_2\!-\!CH\!-\!(CH_2)_7\!-\!C\!-\!O\!-\!CH\!-\!CH_3$ with $\overset{O}{\|}$ and C_6H_5, OH

m/z 402 [M$^+$]

$H_3C\!-\!(CH_2)_4\!-\!CH\!=\!CH\!-\!(CH_2)_2\!-\!CH\!-\!(CH_2)_7\!-\!C\!\equiv\!\overset{+}{O}$ OH

m/z 281

$+\!:OH \quad C_6H_5$
$H_2C\!=\!C\!-\!O\!-\!CH\!-\!CH_3$

m/z 164

$\left[H_3C\!-\!(CH_2)_4\!-\!CH\!=\!CH\!-\!(CH_2)_2\!-\!CH\!-\!(CH_2)_7\right]^+$ OH

m/z 253

Chart 4: Mass fragmentation pattern of IX

for aromatic protons. The characteristic signals assigned for one methine

proton and three methylenic protons at ester side, were observed at δ 5.87 and

1.51 respectively. The normal signals of a fatty acid ester were also observed as

in case of compound **VII**. On the basis of spectral data the product **IX** was

characterized as *1'-phenylethyl (Z)-9-hydroxyoctadec-12-enoate*.

Preparation of diphenylmethyl undec-10-enoate (XI)

Undec-10-enoic acid (**I**) was allowed to react with diphenylmethanol

(**X**) at room temperature (**Scheme 5**), as described earlier. The final TLC

monitoring of the reaction mixture showed one spot. Purification by column

chromatography of the reaction mixture yielded pure product (**XI**) in 95%

yield.
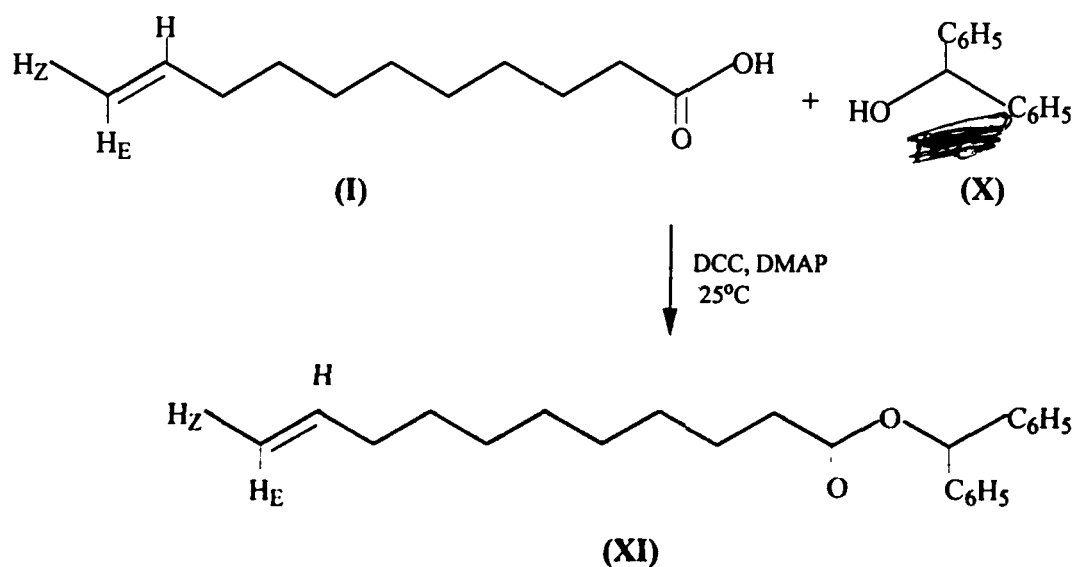
Scheme 5: Preparation of diphenylmethyl undec-10-enoate (XI).

Structure elucidation of the product (XI)

This colourless liquid ~~was~~ analyzed for $C_{24}H_{30}O_2$. Its IR spectrum suggested the presence of an ester carbonyl group by exhibiting/absorption band at 1730 cm^{-1}. The EI-MS spectrum of XI showed /molecular ion peak at m/z 350 along with other salient peaks at m/z 167, 183 and 139 resulted/from bond cleavage next to ester carbonyl group. The base peak ~~observed~~ at m/z 105 ~~was arised~~ by cleavage of/O-C bond of the ester group. The distinctive peak for McLafferty rearrangement ~~fragmentation ion was~~ appeared at m/z 164 suggesting the incorporation of/diphenylmethyl moiety in the chain. The mode ~~of~~ fragmentation pattern is shown in Chart 5. The ^{1}H-NMR spectrum of XI further supported the assigned structure. It showed characteristic signals at δ 7.32 and 7.26 for aromatic protons. The signal at δ 5.84 was attributed to one methine proton at ester side. Other diagnostic signals were observed at δ 5.77 corresponding to one olefinic proton and at δ 4.91 and 5.01 for two C-11 methylene protons, along with a broad singlet at δ 1.33 for methylenic protons of/alkyl chain. In addition, other structure revealing signals were observed at δ 2.41 and at 1.65, attributed to α- and β- methylenic protons with respect to carbonyl group. On the basis of these data the structure of XI was formulated as *diphenylmethyl undec-10-enoate*.
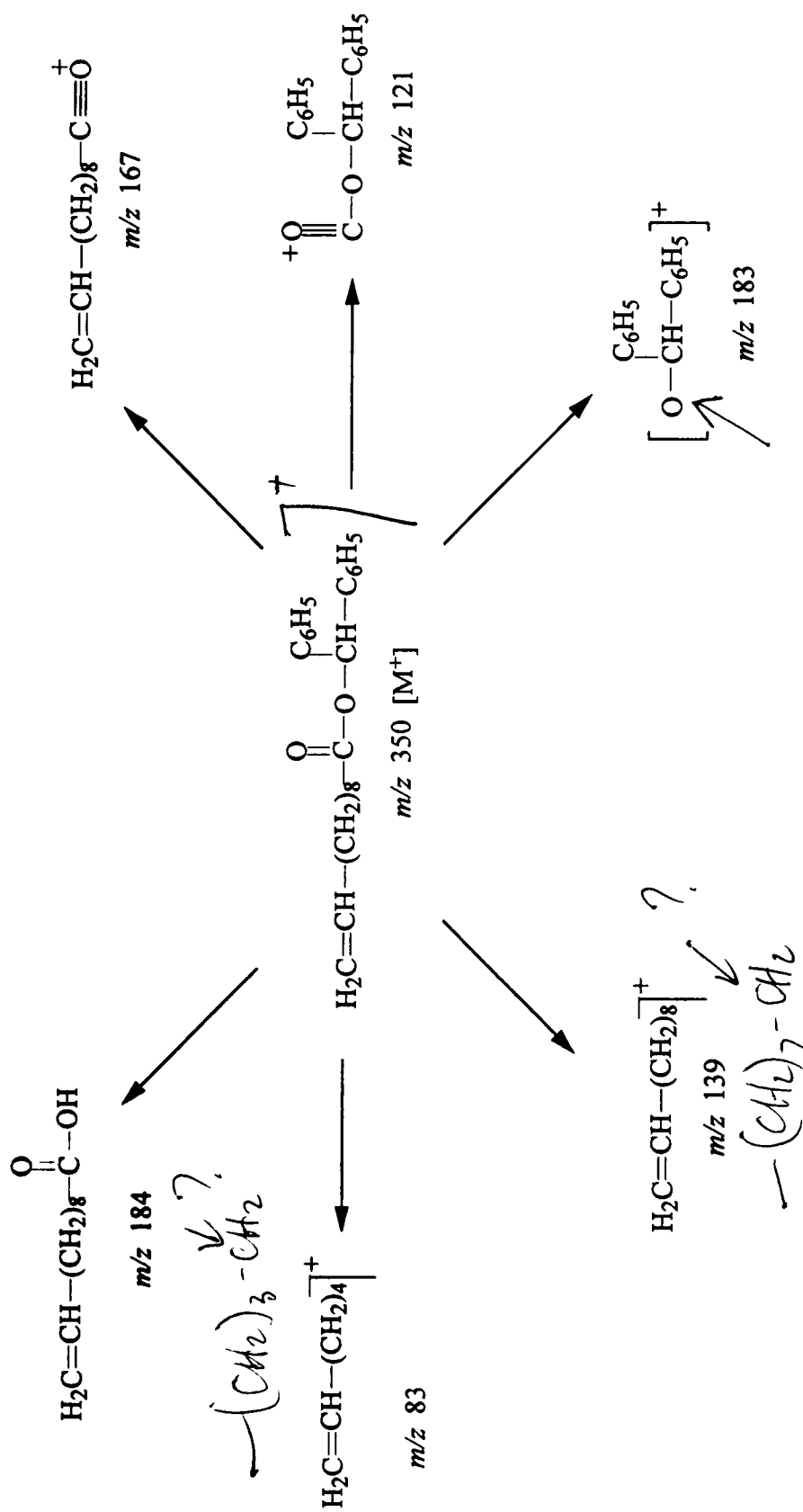
$H_2C{=}CH{-}(CH_2)_8{-}C{\equiv}\overset{+}{O}$

m/z 167

$\underset{\substack{|\\ C_6H_5}}{\overset{+}{O}{\equiv}C{-}O{-}CH{-}C_6H_5}$

m/z 121

$H_2C{=}CH{-}(CH_2)_8{-}\underset{\substack{\|\\O}}{C}{-}O{-}\underset{\substack{|\\C_6H_5}}{CH}{-}C_6H_5$

m/z 350 [M$^+$]

$\left[O{-}\underset{\substack{|\\C_6H_5}}{CH}{-}C_6H_5 \right]^+$

m/z 183

$H_2C{=}CH{-}(CH_2)_8{-}\underset{\substack{\|\\O}}{C}{-}OH$

m/z 184

$H_2C{=}CH{-}(CH_2)_4$]$^+$

m/z 83

$H_2C{=}CH{-}(CH_2)_8$]$^+$

m/z 139

Chart 5: Mass fragmentation pattern of **XI**

Preparation of 3'-(2''-hydroxy-4''-methoxyphenyl)-1'-phenylpropenyl undec-10-enoate (XIII)

The reaction of undec-10-enoic acid (I) with 1-(2'-hydroxy-4''-methoxyphenyl)-3-phenylprop-2-en-1-ol (XII) and DCC in the presence of DMAP was carried out as described previously. Final TLC examination of the reaction mixture showed a distinctive spot along with unreacted acid. The reaction mixture was purified over a column of silica gel to give pure product (XIII) in 80% yield (Scheme 6).
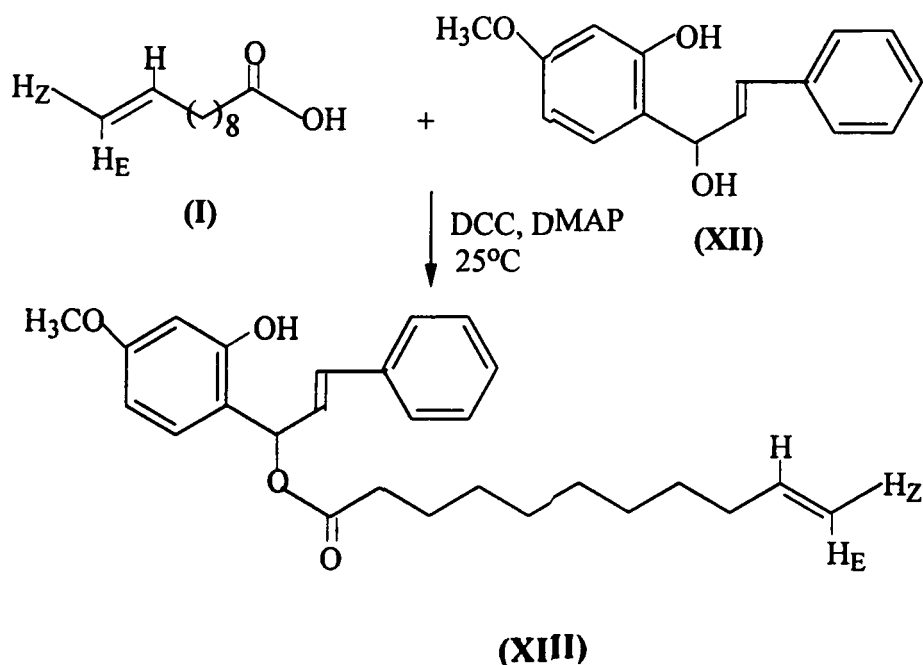
(XIII)

Scheme 6: Preparation of 3'-(2''-hydroxy-4''-methoxyphenyl)-1'-phenylpropenyl undec-10-enoate (XIII).

Structure elucidation of the product (XIII)

Ester (XIII) was obtained as a ~~by MS~~

~~It was a~~ yellowish liquid *and* analyzed for $C_{27}H_{15}O_4$. The IR spectrum of

XIII gave distinctive bands at 1749 and 3427 cm⁻¹ corresponding to ester

carbonyl and hydroxyl group respectively. The band observed at 1140 cm⁻¹ is

indicative of the ~~depicted~~ for C-O-C group. The EI-MS spectrum of XIII showed *a* molecular ion

~~peak~~ at m/z 422. A characteristic peak at m/z 167 ~~was arised~~ *originated* by the bond

cleavage next to ester carbonyl group. The other prominent peaks at m/z 390,

298, 239, 183, 123 and 83 (base peak), were also observed. The mode of

fragmentation pattern is shown in **Chart 6**.

The ¹H-NMR spectrum of **XIII** was more informative about the

assigned structure. It displayed characteristic signals at δ 7.15-7.35 ascribable

to aromatic protons. A multiplet at δ 6.23 was attributed to one methine proton,

a doublet at δ 6.61 for two olefinic protons, two multiplets at δ 2.54 and at δ

1.71 were attributed to α- and β- methylenic protons with respect to *the* carbonyl

group. A multiplet at δ 2.02 was assigned to two allylic protons, a doublet at δ

3.37 (*J* = 6.6 Hz) for hydroxy proton and a singlet at δ 3.78 was assigned *to*

methoxy protons. Two doublet of doublet *s* at δ 4.90 and 5.01 were assigned *for*

two terminal olefinic protons, a multiplet at δ 5.79 *for* one olefinic proton and a

broad singlet at δ 1.54-1.25 was assigned to the protons of *the* aliphatic fatty chain.

On the basis of above data compound **(XIII)** was characterized as

*3'-(2''-hydroxy-4''-methoxyphenyl)-1'-phenylpropenyl-undec-10-enoate.*
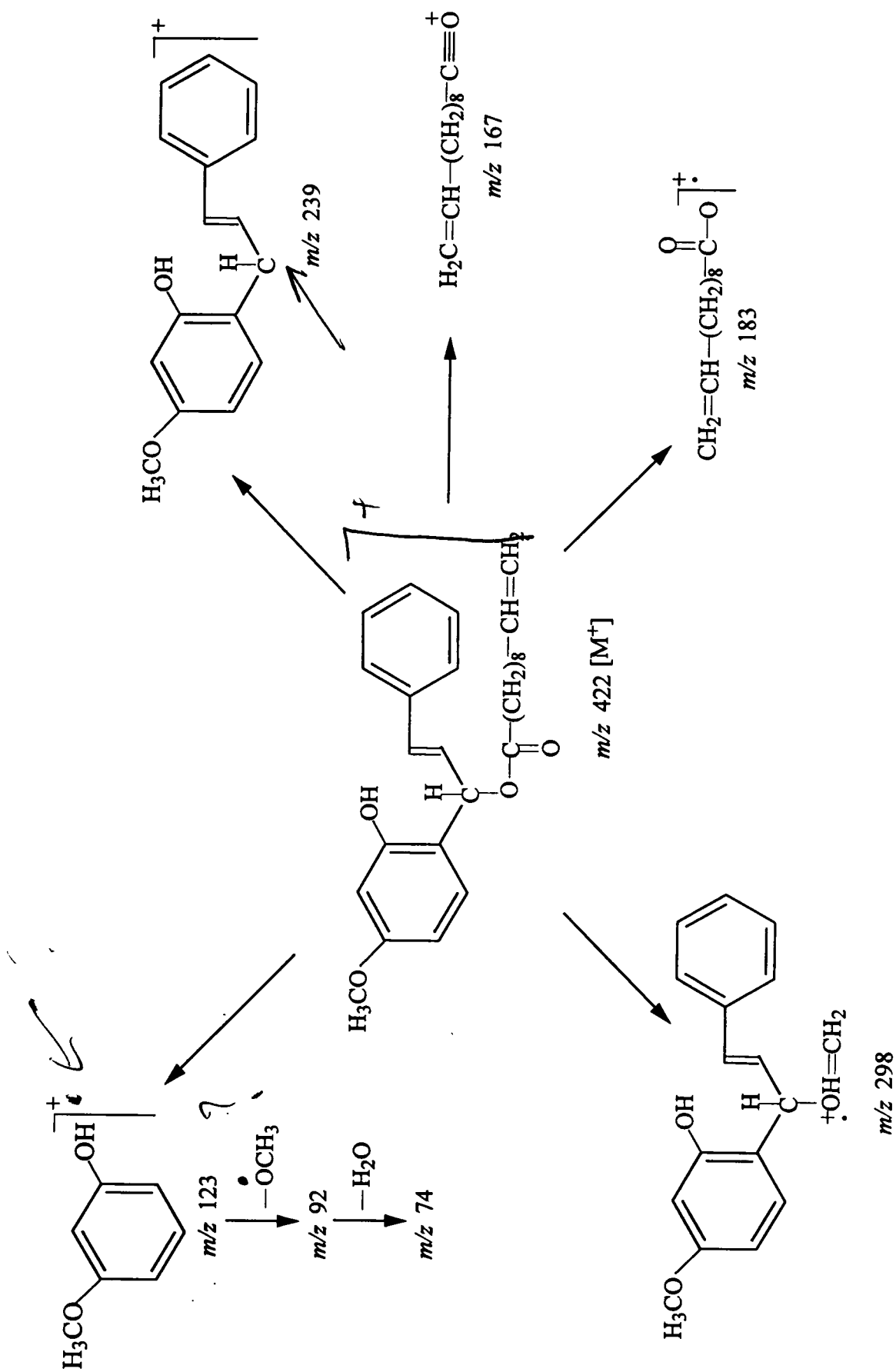
Chart 6: Mass fragmentation pattern of **XIII**

## Preparation of ethylene glycol 1-stearate-2-undecenoate (XV)

The satisfactory results obtained from the reaction of undec-10-enoic acid (I) with different hydroxy compounds by using DCC prompted us to carry out the same reaction with ethylene glycol monostearate (XIV) under similar conditions as described earlier. The reaction was monitored by TLC. The ~~final~~ reaction mixture was chromatographed over a column of silica gel to give pure product (XV) in 90% yield.
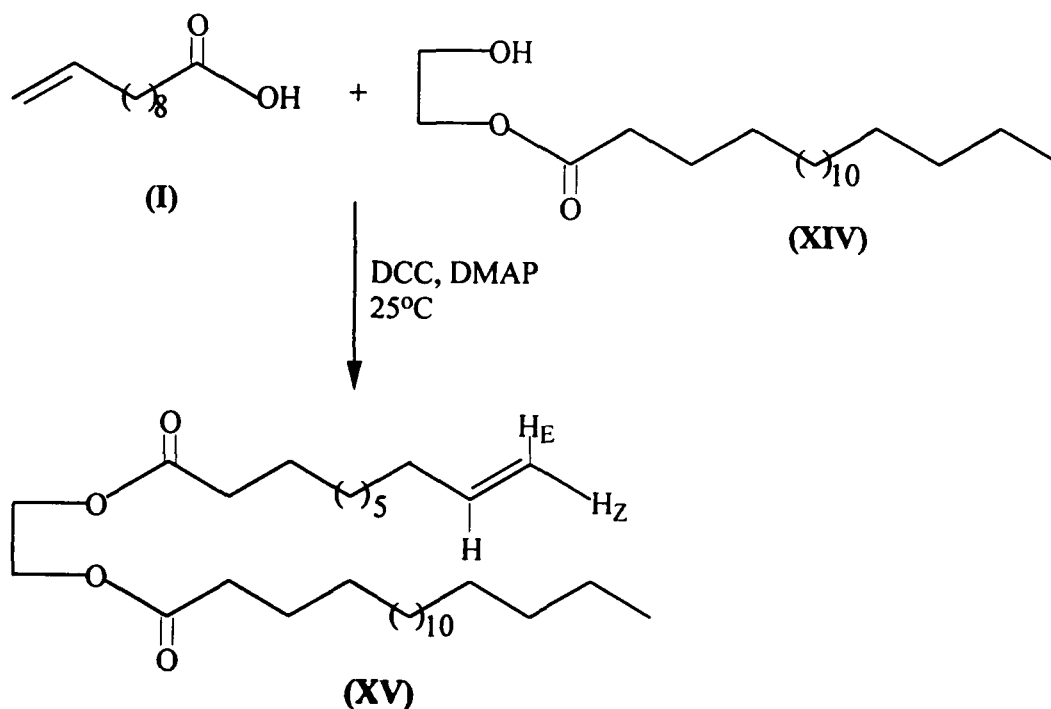
Scheme 7: Preparation of ethylene glycol 1-stearate-2-undecenoate (XV).

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Structure elucidation of the product (XV)

Product (XV), a colourless liquid, on microanalysis gave the composition $C_{31}H_{58}O_4$. Its IR spectrum suggested the presence of ester carbonyl group by exhibiting absorption band at 1741 cm^{-1}. Its mass spectral data showed molecular ion peak at m/z 494. The other distinctive peaks (Chart 7) resulted by the bond cleavage next to ester carbonyl group, were observed at m/z 255, 239 and 267, along with McLafferty rearrangement fragmentation ion at 254, that clearly established the newly introduced functionality to reside at C_1 of the alkyl chain of compound (I). The structure of XV was further supported by its ^{1}H-NMR spectrum, which displayed characteristic signal at δ 5.80 for one olefinic proton. The signals at δ 4.96 and 5.00 were assigned for two terminal olefinic protons. A singlet appeared at δ 4.27 for methylenic protons of glycol unit along with other diagnostic signals (given in experimental section). On the basis of above the structure of compound (XV) was assigned as *ethylene glycol 1-stearate-2-undecenoate*.
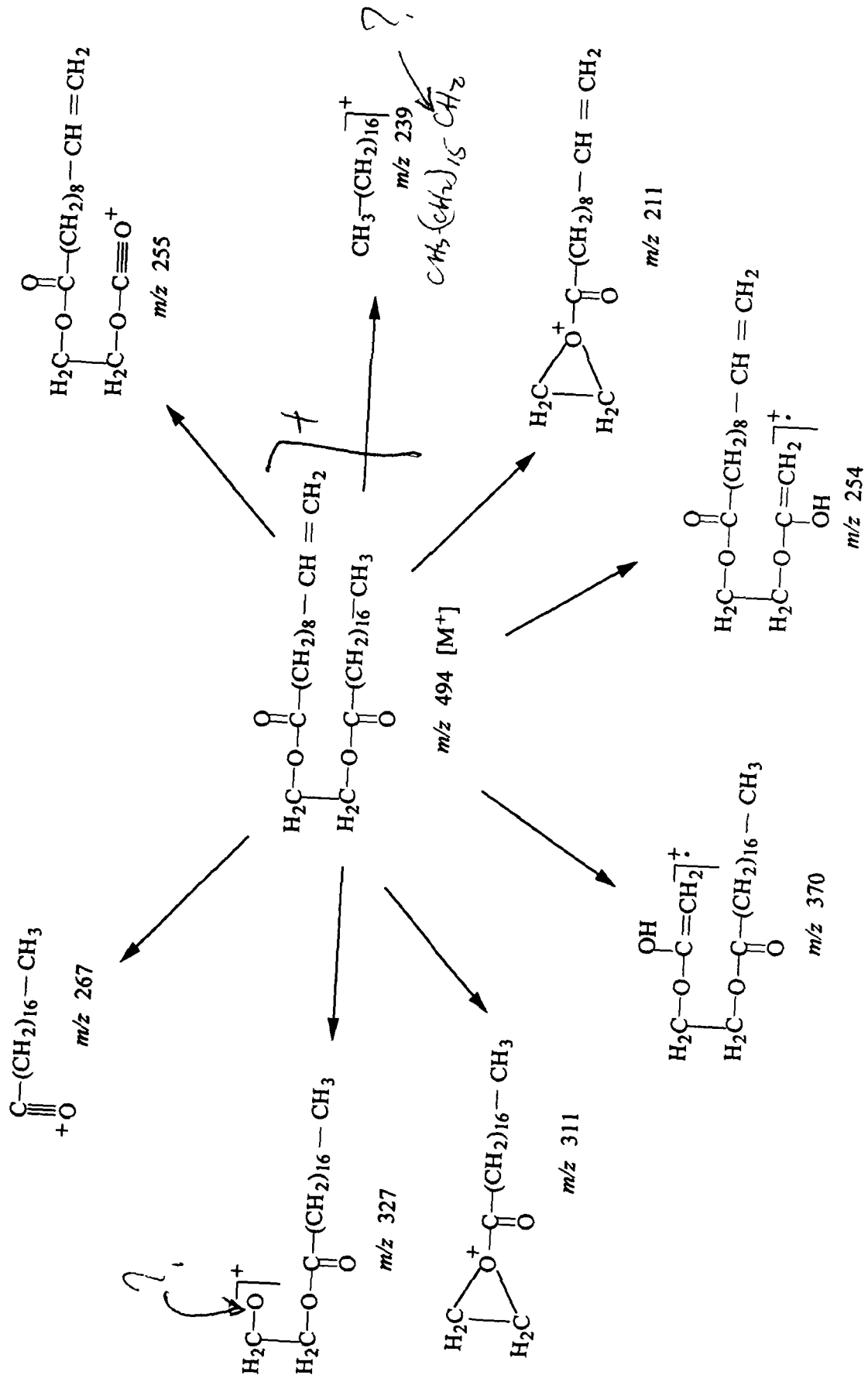
Chart 7: Mass fragmentation pattern of **XV**

3.3 Experimental

Reagents were of commercial grade and were used without further purification. IR spectra were recorded on Shimadzu 8201-PC (Kyoto, Japan) spectrophotometer in KBr-pellets or Nujol or in solvent and wave number (v_{max}) was recorded in cm^{-1}. ^{1}H-NMR spectra were recorded with a Bruker DRX-300 spectrometer (Fallanden, Switzerland) in $CDCl_3$ using TMS as internal standard. Chemical shifts (δ) are quoted in ppm and coupling constants (J) are given in Hz. The abbreviations s, d, t, q, m, dd denoted singlet, doublet, triplet, quartet, multiplet and double doublet. ^{13}C-NMR spectra were recorded on a Bruker DRX-300 spectrometer in $CDCl_3$ with $CDCl_3$ ($\delta = 77.00$ ppm). The EI-MS measurements were recorded on JEOL-D-300 (EI) spectrometer at 70 eV ionization voltage. The FAB mass spectra were recorded on a JEOL-SX 102/DA-6000 (Tokyo, Japan) mass spectrometer. Thin layer chromatography (TLC) was carried out on glass plates (20 × 5 cm) with a layer of silica gel G (Merck, Mumbai, India, 0.5 mm thickness). Spots were visualized on exposure to iodine vapors using iodine chamber. The column chromatography was performed with silica gel [Merck, (60-120) mesh] using 25-30 g per g of material to be separated and purified. The organic solvents used were of L.R. grade and were distilled before use. Undec-10-enoic (purity, 98%) and (Z)-octadec-9-enoic (97%) acids, DCC (99%) and 4-(N,N-dimethylamino) pyridine (DMAP, 98%) were purchased from Fluka chemicals (Bucks, Switzerland). Ethylene glycol monostearate was purchased from Pioneer

Chemicals (New Delhi, India). (Z)-12-hydroxyoctadec-9-enoic (ricinoleic acid,

98%) and (Z)-9-hydroxyoctadec-12-enoic acids (isoricinoleic acid, 98%) were

isolated from *Ricinus communis* and *Wrightia tinctoria* seed oils, respectively,

following Gunstone's partition procedure.[30]

1-Phenylethanol (II)

1-phenylethanol was prepared following a published procedure[31] by

reducing acetophenone with sodium borohydride in the presence of ethanol. A

solution of acetophenone (6.0g, 50 mmol) in ethanol (30 mL) was stirred

magnetically. Then a solution of sodium borohydride [prepared by dissolving

1.97g, 52 mmol of $NaBH_4$ in cold water (10 mL)] was added drop by drop to

stirred ethanolic solution at room temperature. After adding all the sodium

borohydride solution the mixture was stirred for a further 40 minutes. The

progress of the reaction was monitored by TLC. After completion, the reaction

mixture was pour in cold water, extracted with diethyl ether and washed with

water several times, then dried over anhydrous sodium sulphate. The solvent

was evaporated under reduced pressure to give pure compound (II).

IR (KBr) v_{max}: 3380 (OH), 2990 (C-H) 1610 (C=C phenyl).

^{1}H-NMR (CDCl$_3$) δ_H: 7.2 (m, Ar-*H*), 4.81 (q, 1H, C*H*OH), 1.9 (s, 1H,

CHO*H*).

Diphenylmethanol (X)

Diphenylmethanol (X) was prepared as described above by reducing

benzophenone with sodium borohydride. To the magnetically stirred solution

of benzophenone (1.8g, 10 mmol) in 10 mL of ethanol, sodium borohydride

solution (0.45g, 12 mmol) dissolved in 10 mL of cold water was added ~as~

~mentioned~~above~ and the mixture was further stirred for 40 minutes.

Then the mixture was poured into a 250 mL beaker containing a mixture

of cold water (100 mL) and concentrated hydrochloric acid (10 mL). After a

few minutes the precipitated product was collected by suction filtration and

washed with water (2 × 50 mL). The crude product was dried by suction at the

filter pump for 10 minutes and then recrystallized ~by~ from petroleum.

IR (KBr) v_{max}: 3385 (OH), 2980 (C-H), 1610, 1590 (C=C, phenyl).

^{1}H-NMR (CDCl$_3$) δ_H: 7.1-7.64 (m, Ar-H), 5.4 (s, 1H, CHOH), 1.87 (s, 1H,

CHOH).

1-(2'-hydroxy-4''-methoxyphenyl)-3-phenyl-prop-2-en-1-ol (XII)

1-(2'-hydroxy-4''-methoxyphenyl)-3-phenyl-prop-2-en-1-ol (**XII**) was

prepared as described above by reducing 2'-hydroxy-4'-methoxychalcone with

sodium borohydride. A solution of 2'-hydroxy-4'-methoxy chalcone (0.20g,

0.8 mmol) in ethanol (5 mL), ~was~ stirred magnetically. To this solution, sodium

borohydride (0.03g, 0.8 mmol) dissolved in cold water (1.5 mL) was added as

described above. The reaction mixture was further stirred and the progress of

the reaction was monitored by TLC. After completion, the reaction mixture

was worked up as described above furnishing a pale yellow liquid which was

chromatographed over a column of silica gel using petroleum ether-diethyl ether (8:2, v/v) as eluent to obtain pure compound (XII).

IR (KBr) v_{max}: 3440 (OH), 2836 (C-H), 1116 (C-O-C), 1565, 1510 (Phenyl).

ABX- system ?

^{1}H-NMR (300 MHz, CDCl$_3$) δ_H : 7.14-7.35 (m, 3Ar-H), 7.16 (d, 1H, CH=CH,

J = 16.20), 6.55 (dd, 1H, CH=CH, J = 16.20 and 7.20), 4.47 (s, 1H, CHOH),

4.14 (d, 1H, CHOH, J = 7.20), 11.88 (s, 1H, Ar-OH).

1'-Phenylethyl undec-10-enoate (III)

A solution of undec-10-enoic acid (I) (0.92 g, 5 mmol), *1-phenylethanol*

(0.61g, 5 mmol), DCC (1.13g, 5 mmol) and DMAP (0.16g, 5 mmol) in

dichloromethane (50 mL) was slowly stirred at 25°C for 6 hours. The *N,N*-

dicyclohexylurea was filtered off and *the* filtrate was washed with water (3 × 50

mL), 5% acetic acid solution (3 × 50 mL), again with water (3 × 50 mL) and

then dried over anhydrous sodium sulphate. The solvent *was* evaporated under

reduced pressure to give an oily colourless liquid, which was chromatographed

over a column of silica gel. Elution with petroleum ether-diethyl ether (95:5,

v/v) gave the pure product, 1.4g (95%). Calculated for C$_{19}$H$_{28}$O$_2$: C, 79.2; H,

9.7%. Found : C, 79.1; H, 9.6%.

IR (KBr) v_{max}: 1735 (COO), 3074, 2980, 2927 (C-H), 1641 (C=C), 1110

(C-O-C).

EI-MS: *m/z*: 228 [(M$^+$, 6.6)], 183 (9.2), 167 (7.7), 164 (3.8), 149 (18.5), 121

(42.4), 111 (8.5), 105 (100.0), 97 (10.1), 77 (72.3).

^{1}**H-NMR (400MHz, CDCl₃)** δ_H : 7.34 (m, 3 Ar-H), 7.28 (m, 2 Ar-H). 5.88

(q, 1H, OCH, J = 6.6), 5.80 (tdd, 1H, $J_{H-^2CH_2}$ = 6.6, J_{H-H_E} = 10.2, J_{H-H_z} = 17.1,

CH₂ =CH), 4.91 (dd, 1H, J_{H_E-H} = 10.2, $J_{H_E-H_z}$ = 2.2, HC=CH_E), 5.01 (dd, 1H,

J_{H_z-H} = 17.1, $J_{H_z-H_E}$ = 2.2, HC=CH_z), 2.32 (dist.t, 2H, CH_2COO, J = 7.48), 2.03

(m, 2H, H₂C=CH-CH_2), 1.61 (m, 2H, CH_2CH₂COO), 1.54 (d, 3H, CH-CH_3,

J = 4.95), 1.34 (br s, 10H, alkyl chain).

^{13}**C-NMR (CDCl3)** δ_c: 173.0 (C-1), 34.6 (C-2), 24.9 (C-3), 29.0 (C-4), 29.2

(C-5-7), 30.4 (C-8) 28.9 (C-9) 139.1 (C-10) 130.8 (C-11) 72.0 (-OCH), 22.0

(CH-CH_3) 141.8 (C-Ar-1), 128.4 (C-Ar-2) 127.7 (C-Ar-3), 126.0 (C-Ar-4).

1'-phenylethyl (Z)-octadec-9-enoate (V)

(Z)-octadec-9-enoic acid (IV) (1.41g, 5 mmol), 1-phenylethanol (II)

(0.61g, 5 mmol), DCC (1.13g, 5.5 mmol) and DMAP (0.61g, 5 mmol) in

dichloromethane (50 mL) were stirred mechanically for 5 hours at room

temperature. After completion of the reaction the reaction mixture was worked

up as described earlier to yield crude product, which was resolved by column

chromatography over silica gel. Elution with petroleum ether-diethyl ether

(95:2, v/v) gave colourless liquid. 1.83g (95%). Calculated for C₂₆H₄₂O₂:

C, 80.8; H, 10.8%. Found: C, 80.5; H, 10.6%.

IR (KBr) v_{max} : 1730 (COO), 1635 (C=C), 2850 (C-H) 1130 (C-O-C).

EI-MS: m/z: 386 [(M$^+$, 0.7)], 265 (2.3), 237 (1.6), 211 (3.1) 164 (4.6), 149

(16.2), 139 (8.4), 122 (100.0), 121 (36.9), 113 (3.8), 105 (93.9).

¹H-NMR (400MHz, CDCl₃) δ_H: 7.35 (m, 3 Ar-*H*), 7.27 (m, 2 Ar-*H*), 5.88

(q, 1H, OC*H*, *J* = 6.6), 5.35 (m, 2H, C*H*=C*H*), 2.30 (m, 2H, C*H₂*COO), 2.01

(m, 4H, C*H₂*-CH=CH-C*H₂*), 1.61 (m, 2H, C*H₂*CH₂COO), 1.53 (d, 3H,

CH-C*H₃*, *J* = 4.95), 1.27 (br s, 16H, alkyl chain) 0.88 (dist. t, 3H, C*H₃*).

¹³C-NMR (CDCl₃) δ_c: 173.0 (C-1), 34.5 (C-2), 25.0 (C-3), 29.1 (C-4), 29.6

(C-5-7), 27.2 (C-8), 130.0 (C-9), 130.2 (C-10), 27.2 (C-11), 29.6 (C-12), 29.6

(C-13), 29.7 (C-14), 31.5 (C-15), 31.9 (C-16), 22.7 (C-17), 14.0 (C-18), 72.0

(-OC*H*), 22.2 (CH-C*H₃*), 141.8 (C-Ar-1), 128 (C-Ar-2), 127.7 (C-Ar-3), 126

(C-Ar-4).

1'-phenylethyl (Z)-12-hydroxyoctadec-9-enoate (VII)

The reaction of (Z)-12-hydroxyoctadec-9-enoic acid (**VI**) (1.50g,

5 mmol), 1-phenylethanol (**II**) (0.61g, 5 mmol), DCC (1.13g, 5.5 mmol) and

DMAP (0.61g, 5 mmol) in dichloromethane (50 mL) was carried out under

similar conditions as described above. The crude product was chromatographed

over a column of silica gel. Elution with petroleum ether-diethyl ether (96:4,

v/v) gave the pure product (**VII**) as an oily liquid, 1.82g, (90%). Calculated for

C₂₆H₄₂O₃: C, 77.6; H, 10.4%. Found: C, 77.4; H, 10.2%.

IR (KBr) v_max: 1735 (COO), 3350 (OH), 2900(C-H) 1625 (C=C, phenyl).

1135 (C-O-C).

FAB-MS: *m/z*: 403 [(M⁺+1)], 385 (20.8), 325 (20.8), 297 (16.6), 281 (87.4),

253 (4.2), 245 (24.9), 211 (12.5), 164 (4.2), 155 (12.5), 149 (10.4), 129 (12.5),

121 (22.8), 115 (18.7), 105 (100.0).

^{1}H-NMR (CDCl$_3$, 400MHz) δ_H: 7.34 (m, 3 Ar-*H*), 7.28 (m, 2 Ar-*H*), 5.87

(q, 1H, OC*H*, *J* = 4.95), 5.55-5.37 (m, 2H, C*H*=C*H*), 3.59 (m, 1H, C*H*-OH),

2.30 (br s, 1H, CHO*H*), 2.19 and 2.03 (m, each for 2H, C*H$_2$*-CH=CH-C*H$_2$*),

1.62 (m, 2H, C*H$_2$*COO), 1.51 (d, 3H, CH-C*H$_3$*, *J* = 4.95) 1.27 (br s, 18H, alkyl

chain), 0.87 (distorted t, 3H, C*H$_3$*).

^{13}C-NMR (CDCl$_3$) δ_c: 173.0 (C-1), 34.5 (C-2), 24.9 (C-3), 29.0 (C-4), 29.5

(C-5-7), 27.3 (C-8), 133.2 (C-9), 133.2 (C-10), 27.3 (C-11), 71.4 (C-12), 29.5

(C-13), 29.5 (C-14), 29.3 (C-15), 31.8 (C-16), 22.6 (C-17), 14.0 (C-18), 72.0

(OC*H*), 22.2 (CH-C*H$_3$*), 141.9 (C-Ar-1), 128.4 (C-Ar-2), 127.7 (C-Ar-3), 126.0

(C-Ar-4).

1′-phenylethyl (Z)-9-hydroxyoctadec-12-enoate (IX)

A mixture of (Z)-9-hydroxyoctadec-12-enoic acid (VIII) (1.50g,

5 mmol), (±)-1-phenylethanol (II) (0.61g, 5 mmol), DCC (1.13g, 5.5 mmol) and

DMAP (0.61g, 5 mmol) in dichloromethane (50 mL) was stirred and worked

up as described earlier to yield an oily colourless liquid. Silica gel column

chromatography using petroleum ether-diethyl ether (96:4, v/v) as eluent, gave

pure product (IX), 1.83g (90%). Calculated for C$_{26}$H$_{42}$O$_3$: C, 77.6; H, 10.9%.

Found: C, 77.4; H, 10.1%.

IR (KBr) v_{max} : 1740 (COO), 3345 (OH), 1625 (C=C), 2850 (C-H), 1130

(C-O-C).

EI-MS: *m/z*: 402 [(M⁺, 3.8)], 385 (1.5), 297 (30.8), 279 (100.0) 281 (10.0)

253 (0.8), 239 (1.6) 164 (3.3), 155 (91.6), 149 (5.4), 121 (24.7).

¹H-NMR (400MHz, CDCl₃) δ_H: 7.34 (m, 3 Ar-*H*), 7.25 (m, 2 Ar-*H*), 5.87 (q, 1H, OC*H*, *J* = 4.95), 5.38 (m, 2H, C*H*=C*H*), 3.59 (m, 1H, C*H*-OH), 2.31 (m, 2H, C*H₂*COO), 2.29 (br s, 1H, CH-O*H*), 2.15 and 2.03 (m, each for 2H, C*H₂*-CH=CHC*H₂*), 1.60 (m, 2H, C*H₂*CH₂COO), 1.51 (d, 3H, CH-C*H₃*, *J* = 4.95), 1.28 (br s, 18H, alkyl chain), 0.83 (dist. t, 3H, C*H₃*).

Diphenylmethyl undec-10-enoate (XI)

The reaction of undec-10-enoic acid (**I**) (0.92g, 5 mmol) with diphenylmethanol (**X**) (0.92g, 5 mmol), DCC (1.13g, 5.5 mmol), and DMAP (0.61g, 5 mmol) in dichloromethane (50 mL) was carried out as described above. The final workup afforded a colourless liquid, which was chromatographed over a column of silica gel using petroleum ether-diethyl ether (95:5, v/v) to yield pure product (**XI**), 1.6g, (95%). Calculated for $C_{24}H_{30}O_2$: C, 82.3; H, 8.6%. Found: C, 82.1; H, 8.5%.

IR (KBr) v_{max}: 1730 (COO), 1640 (C=C), 2900 (C-H), 1110 (C-O-C).

EI-MS: *m/z*: 350 [(M+, 6.9)] 184 (100.0) 183 (69.3), 167 (98.5), 164 (1.5), 139 (3.1), 83 (4.6).

¹H-NMR (400 MHz, CDCl₃) δ_H : 7.32 (m, 2 × 3Ar-*H*), 7.26 (m, 2 × 2Ar-*H*), 5.84 (q, 1H, OC*H*, *J* = 6.9), 5.77 (tdd, 1H, $J_{H-'CH_2}$ = 6.9, J_{H-H_E} = 10.2, J_{H-H_z} = 17.2, CH₂ =C*H*), 4.91 (dd, 1H, J_{H_E-H} = 10.2, $J_{H_E-H_z}$ = 2.1, HC=C*HE*),

5.01 (dd, 1H, J_{H_Z-H} = 17.2, $J_{H_L-H_E}$ = 2.1, HC=CH_Z), 2.41 (dist. t, 2H, CH_2COO), 2.01 (m, 2H, H$_2$C=CH-CH_2), 1.65 (m, 2H, CH_2CH$_2$COO), 1.33 (br s, 10H, alkyl chain).

3'-(2''-hydroxy-4''-methoxyphenyl)-1'-phenylpropenyl undec-10-enoate

(XIII)

3'-(2''-hydroxy-4''-methoxyphenyl)-1'-phenyl-propenyl   undec-10-enoate (XIII) was prepared following the procedure as describe earlier by stirring a solution of undec-10-enoic acid (I) (1.84g, 10 mmol), 1-(2'-hydroxy-4'-methoxyphenyl)-3-phenyl-prop-2-en-1-ol (XII) (2.56g, 10 mmol) DCC (3.61g, 11 mmol) and DMAP (1.22g, 10 mmol) in dichloromethane (50 mL) at room temperature. The reaction mixture on usual workup yielded a yellowish liquid, which was fractioned over a column of silica gel to give pure product (XIII) 3.30g (80%). Calculated for $C_{27}H_{15}O_3$: C, 77.7; H, 8.06. Found: C, 77.6; H, 8.1%.

IR (KBr) v_{max} : 1749 (COO), 3427 (OH), 2832 (C-H), 1115 (C-O-C), 1572, 1511 (phenyl), 1472 (C-H bend).

EI-MS: *m/z*: 422 [(M+, 3.8)], 298 (1.6), 283 (1.5), 256 (60.1), 104 (59.3), 92 (11.5), 83 (100.0), 74 (8.5).

^{1}H-NMR (300 MHz, CDCl$_3$) δ_H : 7.15-7.34 (m, Ar-*H*), 6.75 (d d, 1H, C*H*=CH, J = 16.20) 6.61 (d, 1H, CH=C*H*-Ar, J = 16.20 and 7.20), 6.23 (m, 1H, C*H*-O), 5.79 (tdd, 1H, $J_{H-^5CH_2}$ = 6.9, J_{H-H_E} = 10.2, J_{H-H_Z} = 17.1,

$CH_2=CH$), 4.90 (dd, 1H, $J_{H_E-H}= 10.2$, $J_{H_E-H_z} = 2.1$, HC=CH_E), 5.01 (dd, 1H,

$J_{H_z-H} = 17.1$, $J_{H_z-H_E} = 2.1$, HC=CH_Z), 3.78 (s, 3H, OCH_3), 3.37 (d, 1H, OH,

$J = 6.6$), 2.54 (m, 2H, CH_2COO), 2.02 (m, 2H, CH_2-CH=CH-CH₂), 1.71

(m, 2H, CH_2CH₂COO), 1.54-1.25 (br s, 10H, CH₂ chain).

Ethylene glycol 1-stearate-2-undecenoate (XV)

As a general method a solution of undec-10-enoic acid **(I)** (1.84g, 10

mmol), ethylene glycol monostearate (3.28g, 10 mmol), DCC (3.61g, 11 mmol)

and DMAP (1.22g, 10 mmol) in dichloromethane (50 mL) was stirred at room

temperature. Final workup afforded a colourless liquid, which was

chromatographed over a column of silica gel using petroleum ether-diethyl

ether (8:2, v/v) to yield pure compound **(XV)**, 4.44g (90%). Calculated for

$C_{31}H_{58}O_4$: C, 75.30; O, 12.96%. Found : C, 75.29; O, 12.94%.

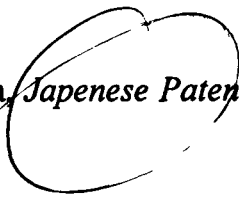**IR (KBr)** v_{max} : 1741 (COO), 3075, 2926, 2854 (C-H), 1640 (C=C), 1130

(C-O-C).

EI-MS: *m/z*: 494 [(M⁺, 2.4)], 439 (3.6), 370 (2.4), 355 (5.9), 329 (41.6), 327

(57.1), 325 (88.1), 311(35.7), 298 (23.8), 267 (7.7), 255(38.1), 254 (26.2), 211

(11.9), 196 (53.5), 183 (100.0).

¹H-NMR (300 MHz, CDCl₃) δ_{11}: 5.80 (tdd, 1H, $J_{H-^3CH_2} = 6.9$, $J_{H-H_E} = 10.26$,

$J_{H-H_z} = 17.2$, CH₂=CH), 4.96 (dd, 1H, $J_{H_E-H} = 10.26$, $J_{H_E-H_z} = 2.1$,

HC=CH_E), 5.00 (dd, 1H, $J_{H_z-H} = 17.2$, $J_{H_z-H_E} = 2.1$, HC=CH_Z), 4.27

(s, 4H, OCH_2-CH_2O), 2.31 (t, 2H, CH_2COO, $J = 4.4$), 2.03 (m, 2H, CH₂=CH-

CH_2), 1.60 (m, 2H, CH_2CH₂COO), 1.29 (br s, alkyl chain) 0.88 (dist. t, 3H,

CH_3).

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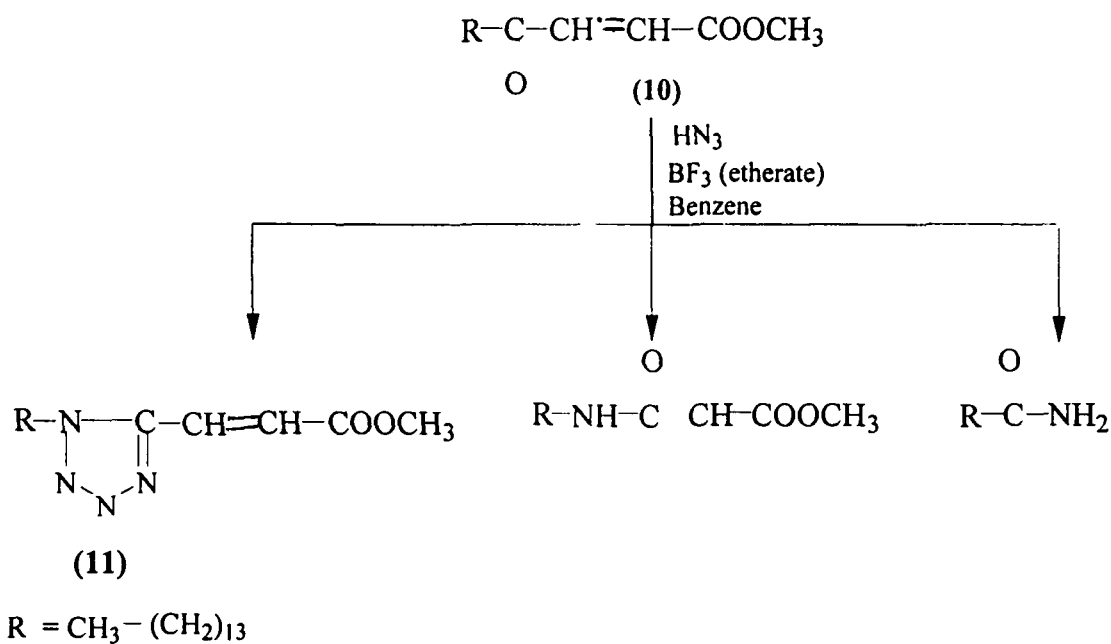
CHAPTER-4

Synthesis of α-Bromo 5'-Methyltetrazoles from Long-Chain Fatty Alkenoates

4.1 Theoretical

Tetrazoles are an increasingly popular functionality[1] with wide ranging applications. They are regarded as a biological equivalent for carboxylic acid group and extensive work have been carried out in the field of medicinal chemistry[2]. Thus a retained pharmacological and more favourable pharmacokinetic profile are often achieved by the replacement of a carboxyl group with a metabolically stable tetrazole. They play an important role in co-ordination chemistry, as useful ligands and in various material science applications, including specialty explosives[3]. Similarly they are also found as a precursor of carbenes in flash vacuum pyrolysis[5]. The discovery of the pharmacological and biochemical properties of tetrazole derivatives has resulted in enormous development over the past few years[6]. Tetrazole derivatives are reported to exhibit a variety of biological activities[7] such as antiallergic, antihypertensive, antipyretic, analgesic, anti-inflammatory and antibacterial properties. Some tetrazoles are used as antiulcer[8] and antihypercholesterolemic agents.[9] Similarly 1,5-disubstituted 1H-tetrazoles have long been known for their pharmaceutical activity[10] as stimulants or depressants on the central nervous system and are reported to show oral antidiabetic,[11] antithrombotic[12] and antimicrobial properties[13-15]. Recently Ishmetova[16] have reported antituberculous and radioprotector activity of disubstituted tetrazoles. In addition to their various biological properties,

tetrazoles also serve as precursors for the synthesis of ~~further interesting~~ heterocycles, such as 1,3,4-oxadiazoles[17].

A number of methods for the synthesis of 1,5-disubstituted tetrazoles have been reported[10]. The conventional synthesis of 1,5-disubstituted tetrazoles involves the thermal addition of organic azides to electron deficient nitriles, thermolysis, photolysis, cyclization of *in situ* generated imidoylazides, azidolysis of azalactones, cyclodehydration of acetyl tetrazoles, alkylation of 5-substituted tetrazoles and reaction of acetylhydrazines with diazonium compounds. Mustafa *et al.*[18] have synthesized fatty tetrazoles (**11**) along with other products by the reaction of methyl octadec-4-oxo-*trans*-2-enoate (**10**) with excess of hydrazoic acid at room temperature.

$$R-C-CH^{\cdot}=CH-COOCH_3$$
$$\underset{O}{} \qquad (10)$$

HN$_3$
BF$_3$ (etherate)
Benzene

$$R-\underset{\underset{N}{N\diagdown\diagup N}}{\overset{}{N}}-\overset{\overset{}{C}}{\underset{\parallel}{C}}-CH=CH-COOCH_3 \qquad \underset{O}{\overset{}{R-NH-C}} \ CH-COOCH_3 \qquad \underset{O}{\overset{}{R-C-NH_2}}$$
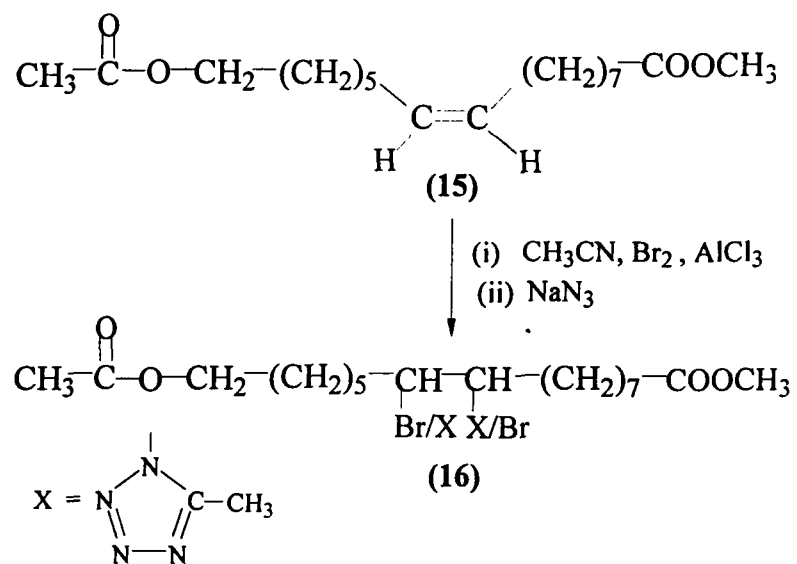
(**11**)

R $= CH_3-(CH_2)_{13}$

An effective and straight forward route to the synthesis of tetrazole

derivatives ~~is found by~~ *involves* the cycloaddition reaction of halogen to olefins,

proceeding via a cyclic 3-membered halonium ion intermediate. ~~The main goal~~

~~To~~ Introduction of nitrogen functions[19,20] into organic compounds led to

~~development of~~ ~~envisage~~ *development of* a pathway whereby a neutral molecule i.e. a nitrile, ~~might~~ act *s* as a

nucleophile in the opening of a halonium ion intermediate **(12)**, thus leading to

a nitrilium ion **(13)**. At this stage, the addition of sodium azide to the solution

results in the formation of tetrazole **(14)**.

(12)   **(13)**

NaN$_3$

(14)
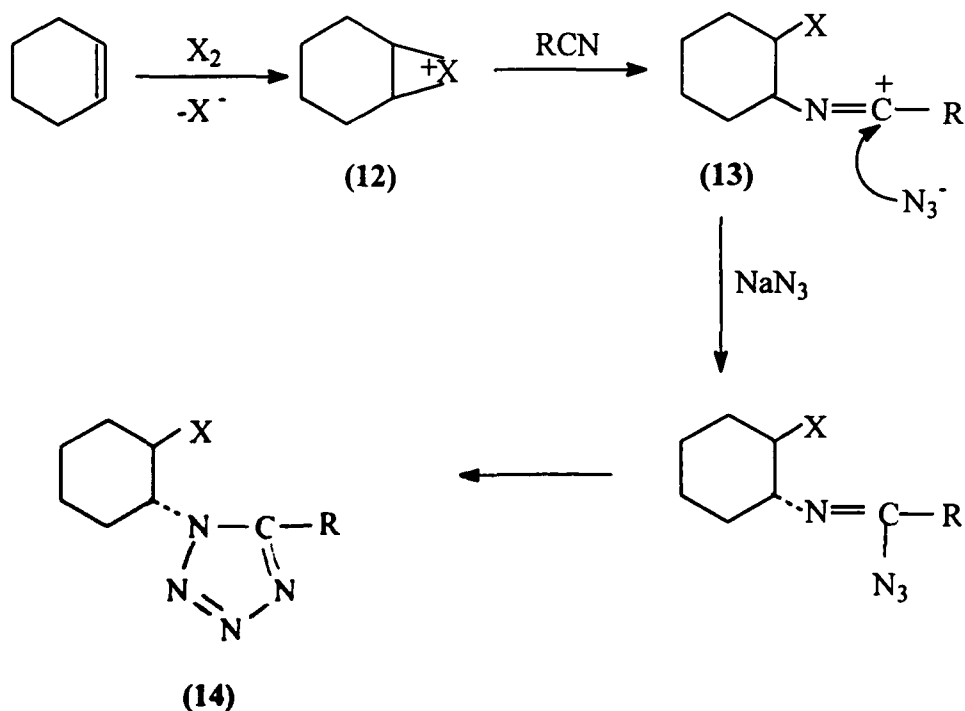
Later, Agarwal and co-workers[21] first reported the synthesis of 5-methyltetrazoles from long-chain fatty acids by this route.
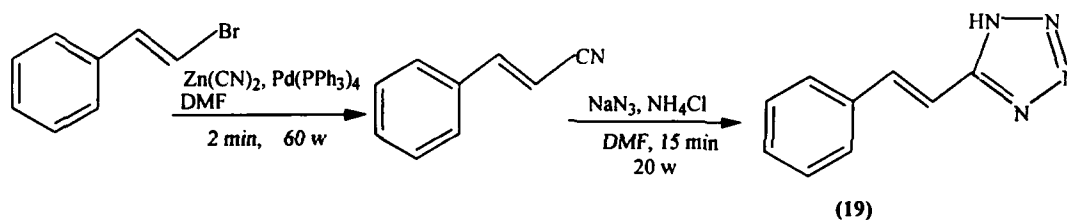
Sarkar *et al.*[22] took advantage of this route to synthesize 5-methyltetrazoles (16) from olefinic ester derivatives (15) of aleuritic acid.

$$CH_3-\overset{\overset{\displaystyle O}{\|}}{C}-O-CH_2-(CH_2)_5$$

(CH₂)₇—COOCH₃ ... C=C ... H, H

(15)

(i) CH₃CN, Br₂, AlCl₃
(ii) NaN₃

$$CH_3-\overset{\overset{\displaystyle O}{\|}}{C}-O-CH_2-(CH_2)_5-CH-CH-(CH_2)_7-COOCH_3$$

Br/X X/Br

(16)

X =

N
N   C—CH₃
N—N

Synthesis of (±)-*erythro*-methyl 16- acetoxy-9/10-bromo-10/9-5'-methyl-1-*H*-tetrazol-1-yl) hexadecanoate (17), starting from (±) *threo*-aleuritic acid, has also been reported[23].

$$CH_3COOCH_2(CH_2)_5 \quad H \diagdown \underset{C=C}{} \diagup (CH_2)_7COOCH_3 \diagup \diagdown H$$

(i) CH₃CN, Br₂, AlCl₃

(ii) NaN₃

$$CH_3COOCH_2(CH_2)_5 \quad Q \diagdown H—C—C \diagup (CH_2)_7COOCH_3 \diagdown P$$

(17a,b)

17a: P = Br, Q = ;   17b: P =   Q = Br

Recently, synthesis of tetrazoles from cyanides has received much

attention and new preparation methods have appeared in literature²⁴.

Alterman *et al.*²⁵ have synthesized aryl and vinyl tetrazoles (18,19) by

flash heating of cyano compounds, prepared from the corresponding bromides

using palladium-catalyzed reaction with microwave irradiation employed as the
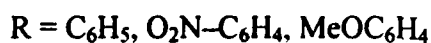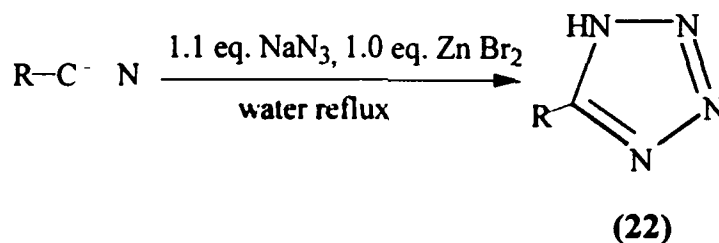
energy source.

(18)

(19)

Takeuchi[26] ~~have~~ prepared 1-(3,4-dihydroxyphenyl)-1*H*-tetrazole derivative (21) by the reaction of 3,4-diacetoxy benzonitrile (20) with sodium azide in the presence of ammonium chloride.
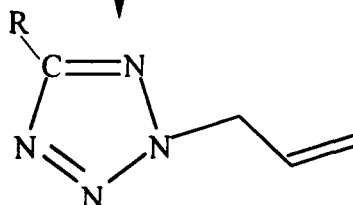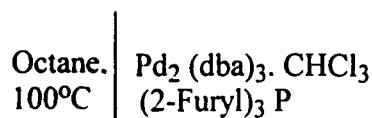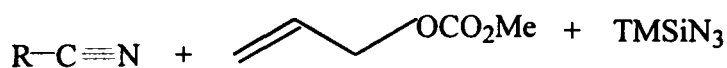

(20)   (21)
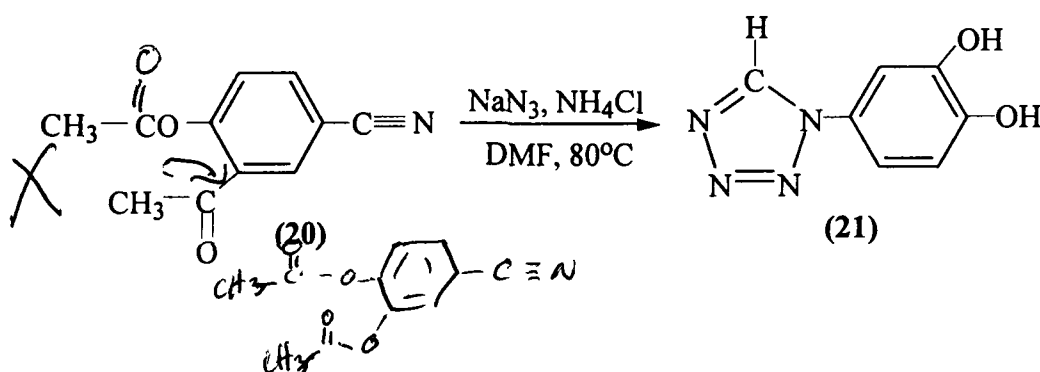
Demko and co-workers[27] have recently carried out the addition reaction of nitrile compounds with sodium azide in water to yield their corresponding tetrazoles (22).


$$R-C \equiv N \xrightarrow[\text{water reflux}]{1.1 \text{ eq. NaN}_3, 1.0 \text{ eq. Zn Br}_2}$$

(22)

R = C$_6$H$_5$, O$_2$N-C$_6$H$_4$, MeOC$_6$H$_4$

number of 2-allyltetrazoles (23) in good to excellent yield ~~have been~~

~~reported~~ by the palladium-catalyzed three component coupling (TCC)

reaction of the corresponding allylmethyl carbonate and trimethylsilyl azide

(TMSiN₃) under a catalytic amount of Pd₂ (dba)₃.CHCl₃ (2.5 mol %) and tri (2-

furyl) phosphine (10 mol %) *was reported.²⁸*

$$R-C \equiv N \quad + \quad \diagup\diagdown\diagup^{OCO_2Me} \quad + \quad TMSiN_3$$

Octane. | Pd₂ (dba)₃. CHCl₃
100°C | (2-Furyl)₃ P

$$\begin{matrix} R \\ \diagdown \\ C = N \\ / \quad \diagdown \\ N \quad\quad N-\diagup\diagup \\ \diagdown\diagdown \quad \diagup \\ N \end{matrix}$$

(23)

R= Me₂N, OC₄H₈N, O₂NC₆H₄, (NO₂)₂ C₆H₃, PhO, Ts

Shie *et al.*²⁹ ~~have~~ reported *the* direct conversion of aldehydes to tetrazoles in

aqueous media by one-pot tandem reaction. They have shown that a variety of

aldehydes reacted with iodine in ammonia water at room temperature to give

nitrile intermediates (24) which were trapped by the addition of sodium azide

to produce corresponding tetrazoles (25).

R—C⟨=O / —H → (I₂, aq. NH₃, r.t.) → R—C≡N → (NaN₃, ZnBr₂) → (25)

[R—C⟨=N—I / —H :NH₃] (24)

What does the arrow mean
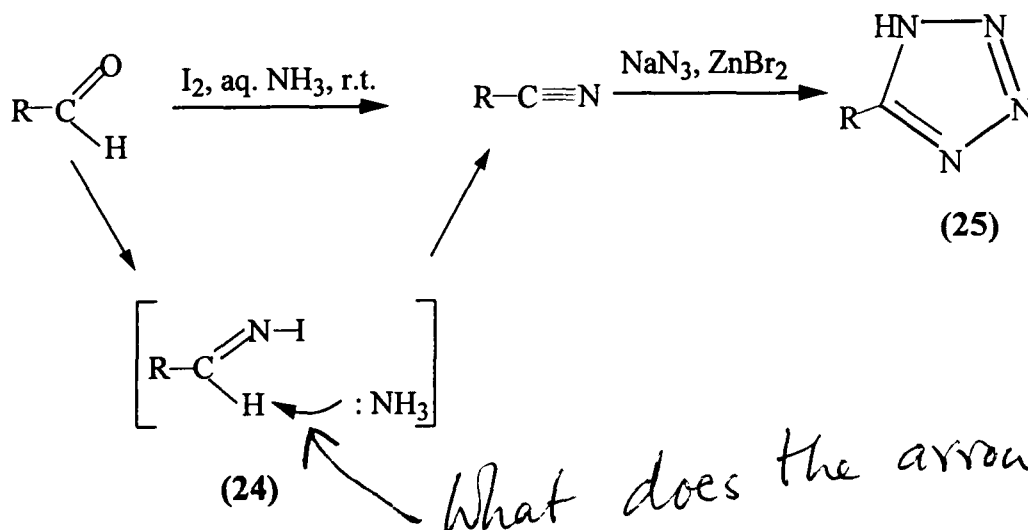
R = C₆H₅, 4-MeOC₆H₄, 4-O₂NC₆H₄, 2-NCC₆H₄, 4-OHCC₆H₄, CH₃(CH₂)₃, Me₃C,

C₆H₅CH=CH

Recently, Furmeier *et al.*[30] have synthesized 5-alkyl-1*H*-tetrazoles (26)

from fatty nitriles by using 3 equivalent of sodium azide and triethylamine

hydrochloride in dry toluene.

R—C≡N → (NaN₃, NEt₃⁻ HCl / Toluene) → (26)
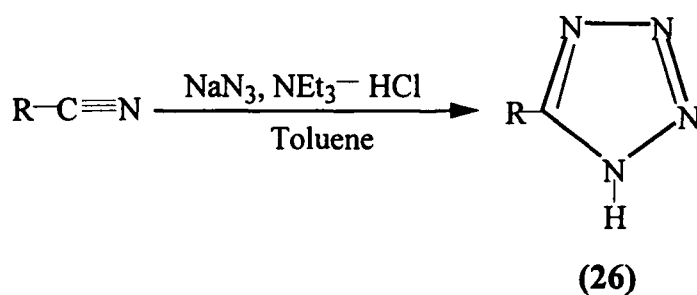
R = CH₃(CH₂)₁₀, CH₃(CH₂)₁₆, CH₃(CH₂)₇CH=CH(CH₂)₇,

CH₃(CH₂)₁₂, CH₂=CH(CH₂)₈, NC(CH₂)₁₀⁻

seek

Prompted by these observations and as part of our aim to ~~achieve~~ access

to pharmacologically interesting compounds based on renewable raw materials

that serve as important feedstocks for the chemical industry[31], we ~~have~~

embarked

~~undertaken this problem~~ on the syntheses of α-bromo 5'-methyltetrazoles from

please rephrase

long-chain fatty alkenoates having aromatic ring at the side of ester. It includes

the reaction of 1'-phenylethyl undec-10-enoate (III) with bromine, acetonitrile

and sodium azide in the presence of anhydrous aluminum chloride to afford the

major product 1'-phenylethyl 11-bromo-10-(5'-methyl-1H-tetrazol-1-yl)

undecanoate (XVII) along with 1'-phenylethyl 10,11-dibromoundecanoate

(XVI). Under ~~the~~ similar ~~reaction~~ conditions 1'-phenylethyl (Z)-octadec-9-

enoate (V) and 1'-phenylethyl (Z)-12-hydroxyoctadec-9-enoate (VII) yielded

1'-phenylethyl 9/10-bromo-10/9-(5'-methyl-1H-tetrazol-1-yl) octadecanoate

(XIX) and 1'-phenylethyl 12-hydroxy 9/10-bromo-10/9-(5'-methyl-1H-

tetrazol-1-yl) octadecanoate (XXI) respectively, in addition to their dibromides,

1'-phenylethyl 9,10-dibromooctadecanoate (XVIII) and 1'-phenylethyl 12-

hydroxy-9,10-dibromooctadecanoate (XX) respectively.

4.2 Results and Discussion

Reaction of 1'-phenylethyl undec-10-enoate (III) with bromine, sodium azide and acetonitrile.

Reaction of compound (III) with bromine and sodium azide in acetonitrile afforded a mixture of two components, one minor (XVI) and other major (XVII) (Scheme 8). These products were separated and purified by column chromatography over a column of silica gel using petroleum ether-diethyl ether as eluent.
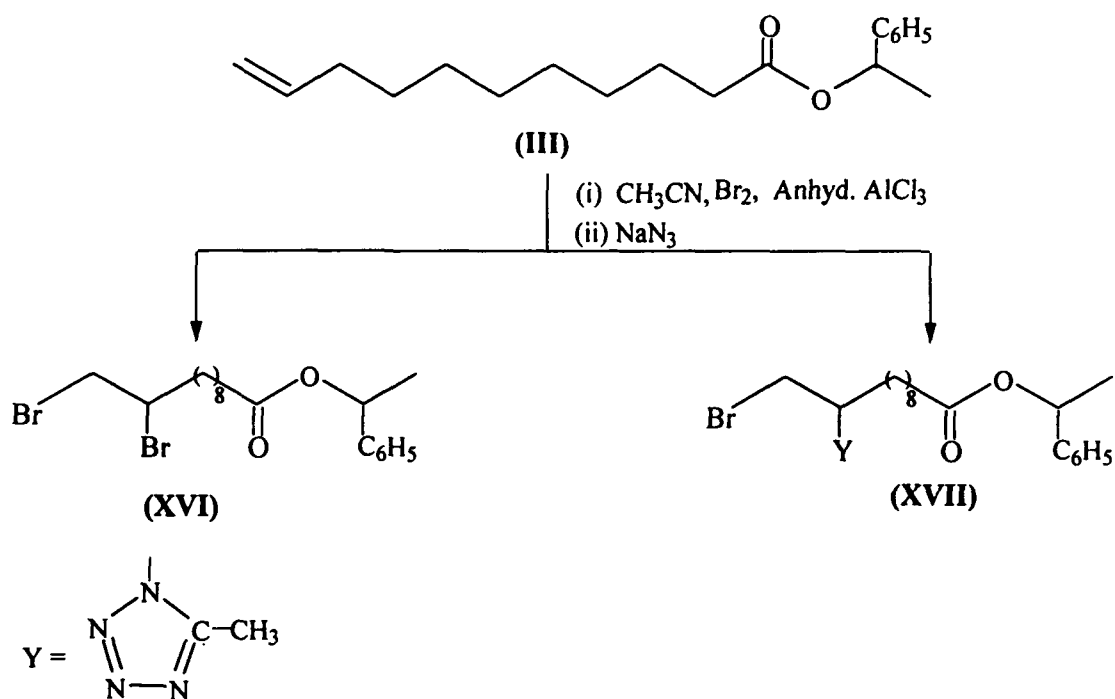
Scheme 8: Synthesis of 1'-phenylethyl 11-bromo-10-(5'-methyl-1H-tetrazol-1-yl) undecanoate (XVII).

Structure elucidation of the product (XVI)

Rephrase as before

It was a brownish liquid. It gave positive Beilstein test for halogen. Its

IR spectrum exhibited characteristic bands at 1735 (COO) and 670 cm^{-1} (C-Br).

The ^{1}H-NMR spectrum of **XVI** gave diagnostic signals at δ 3.59-4.4 (m, 3H,

resonance

CH$_2$-Br and CH-Br). The ~~chemical shift values~~ at δ 5.87 as a quartet (J = 6.6

resonances

Hz) was attributed to one methine proton at ester side. The ~~chemical shifts~~ at δ

s

7.35 and 7.25 both as multiplet were assigned to five aromatic protons. The

normal signals of a fatty acid ester were also observed (details in experimental

section). On the basis of these spectral data compound **XVI** was characterized

as *1'-phenylethyl-10,11-dibromoundecanoate*.

Structure elucidation of the product (XVII)

Rephrase

It was also a brownish liquid. It ~~was~~ appeared to be a tetranitrogenous

gave a positive

compound by its elemental analysis (C$_{21}$H$_{31}$O$_2$N$_4$Br) and ~~responded to~~ Beilstein

for halogen

test. The IR spectrum of **XVII** exhibited characteristic absorption bands at

a *C-Br*

1244 and 984 cm^{-1} for ~~CN$_4$~~tetrazole ring and at 668 cm^{-1} for ~~bromo~~ function.

The absorption bands for N=N, C=N and COO were observed at 1520, 1368

a

and 1735 cm^{-1} respectively. The EI-MS spectrum of **XVII** showed molecular

ion at m/z 450/452 and an intense peak at m/z 422/424 due to loss of one

m/z

nitrogen molecule from the tetrazole ring. The diagnostic mass ion at 357 and

189/191 arising from α- cleavage to the ring, established the position of the

the

tetrazole ring at C-10. Fragment ion at m/z 93/95 showed the presence of

bromine atom at C-11. Other salient peaks were observed at m/z 370 (M-HBr),

prominent ?

345/347 (M-CH(C₆H₅)CH₃) and 261 (M-CH₂BrCHCN₄CH₃). The mode of fragmentation is presented in **Chart 8**.

The ¹H-NMR spectrum of the compound **(XVII)** gave conclusive information regarding the assigned structure. It is in agreement with the formation of a tetrazole ring as the chemical shift of C-10 proton has shifted upfield from δ 5.80 to δ 4.65 due to the ring formation. Two methylene protons (H$_a$, H$_b$) attached to bromine are magnetically non-equivalent by virtue of their different stereochemistry, therefore they have different chemical shift in their ¹H-NMR spectrum and appeared at δ 3.9 (s, 1H, H$_a$-C-Br) and 3.8 (dist. d, 1H, H$_b$-C-Br J = 3.0) respectively. In addition to normal signals of fatty acid ester, other characteristic signal was observed at δ 2.61 for 5′-methyl protons of the tetrazole ring. Based on the above evidences, compound **XVII** was characterized as *1′-phenylethyl 11-bromo-10-(5′-methyl-1H-tetrazol-1-yl) undecanoate*.

Reaction of 1′-phenylethyl (Z) octadec-9-enoate (V) with bromine, sodium azide and acetonitrile.

A 6 hours stirring of 1′-phenylethyl (Z)-octadec-9-enoate (V) in acetonitrile at 0°C in the presence of bromine and sodium azide followed by column chromatographic separation on silica gel afforded two oily products, a minor **XVIII** and a major **XIX** (Scheme 9).
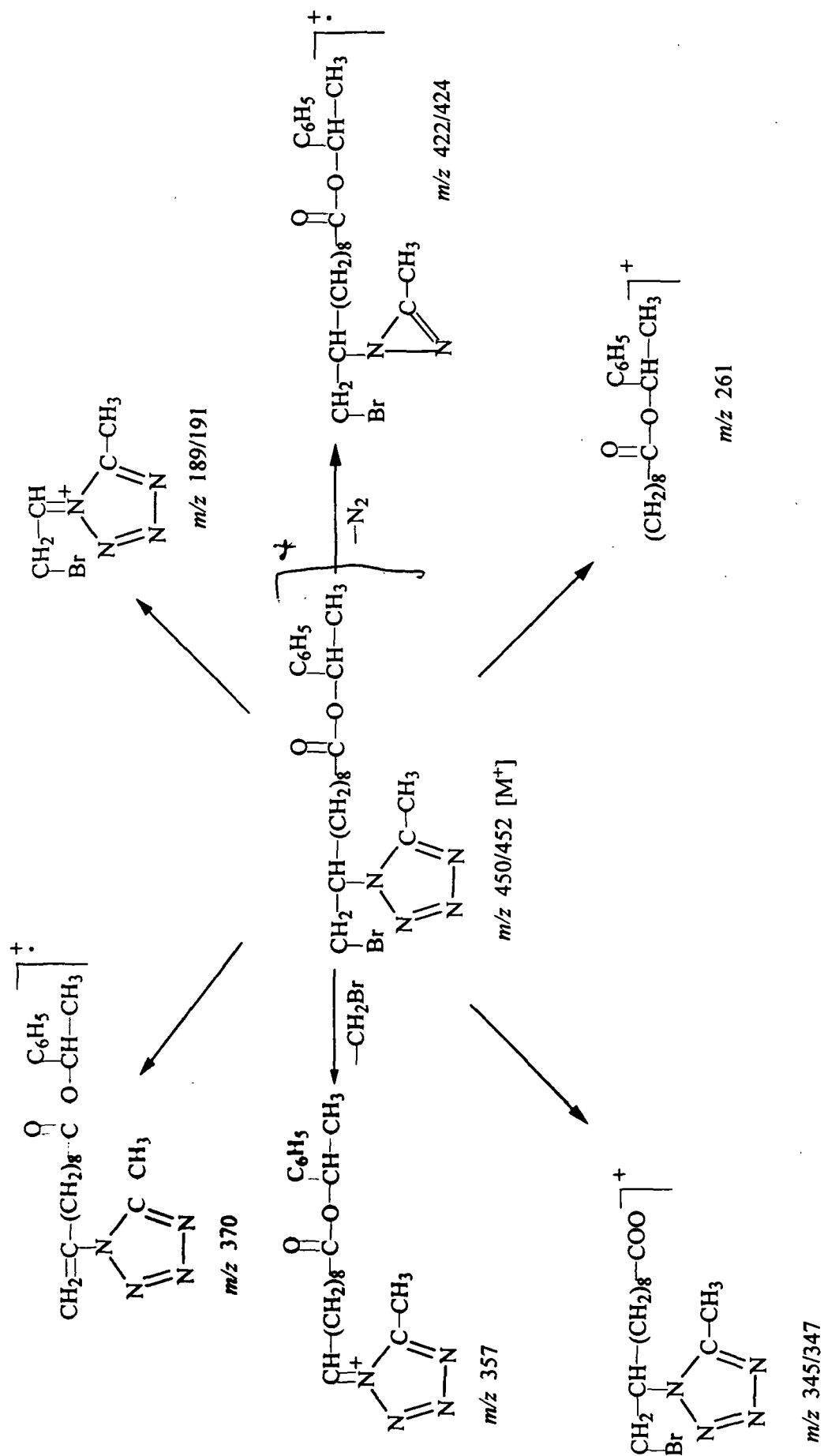
Chart 8: Mass fragmentation pattern of **XVII**

(V)

(i) CH₃CN,Br₂, Anhyd. AlCl₃
(ii) NaN₃

(XVIII)

(XIX)

$$Y = $$

C–CH₃
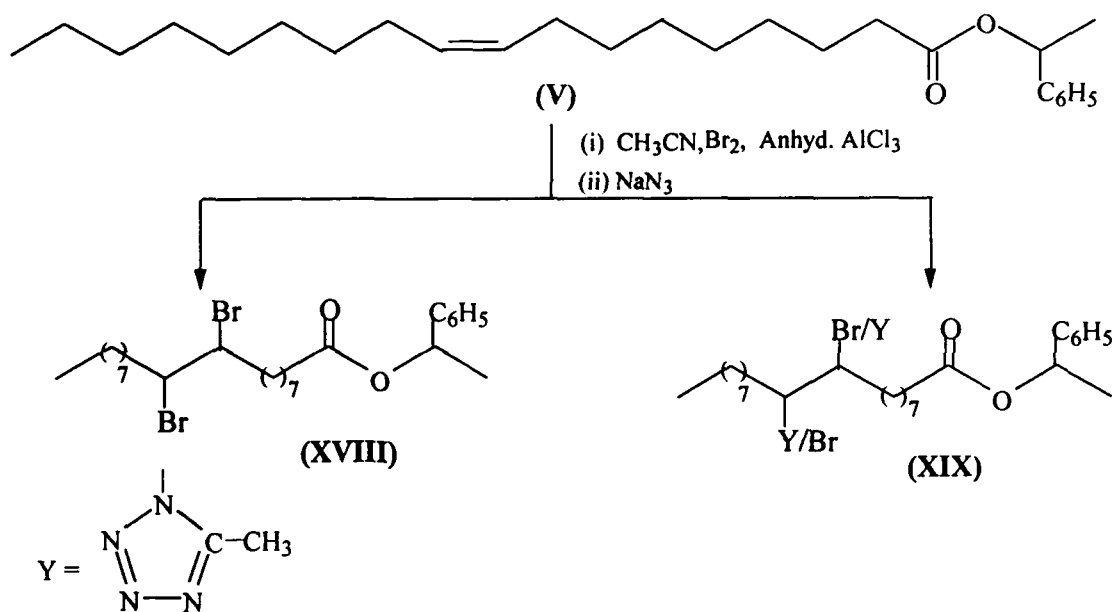
Scheme 9: Synthesis of 1'-phenylethyl 9/10-bromo-10/9-5'-methyl-1*H*-tetrazol-1-yl) octadecanoate **(XIX)**

Structure elucidation of the product (XVIII)

Compound (XVIII) obtained as

It was a brownish liquid and found to be halogenous by responding

showed

positively to Beilstein test. Its IR spectrum illustrated characteristic bands at

1735 (COO) and 665 cm⁻¹ (C-Br). Its ¹H-NMR spectrum showed the structure-

revealing signal at δ 4.21 as a multiplet (4H, 2 × C*H*-Br). Other characteristic

signals were observed at δ 7.33 for three aromatic protons, 7.27 for two

aromatic protons, 5.89 for methine proton at ester side, 2.32 and 1.61 for α-

and β-methylene protons with respect to carbonyl group, 1.53 for methyl

protons at ester side and at 0.87 for terminal methyl protons. On the basis of

these data compound **XVIII** was formulated as *1'-phenylethyl 9,10-dibromooctadecanoate*.

Structure elucidation of the product (XIX)

It was a light brown liquid. Its structure has been established by IR, EI-MS and ¹H-NMR spectra. The elemental analysis of the compound **XIX** corresponded to $C_{28}H_{45}O_2N_4Br$ and showed positive Beilstein test for halogen. The IR spectrum showed characteristic bands at 1507, 1357 cm⁻¹ for N=N, C=N, 1268, 1037 for CH₄ ring and at 669 cm⁻¹ for C-Br. The EI-MS spectrum of **XIX** showed molecular ion peak at *m/z* 548/550. An intense peak appeared at *m/z* 520/522, due to the loss of one nitrogen molecule from the tetrazole nucleus. Mass fragments at *m/z* 343, 339/341, 209, and 205/207 confirmed that product **XIX** is a mixture of two isomers **(Chart 9)**. The ¹H-NMR spectrum of the product **XIX** showed a signal at δ 4.68 as a multiplet for the C-9 and C-10 methine protons to which bromine and tetrazole ring are attached. As expected, the signal at δ 2.59 as a singlet was observed and integrated for three protons which further confirmed the presence of 5'-methyl protons in the tetrazole ring. Spectral data coupled with mechanistic consideration indicated the structure of compound **XIX** as *1'-phenylethyl threo-9/10-bromo-10/9-(5'-methyl-1H-tetrazol-1-yl)octadecanoate*. *Threo* confirmation of **XIX** was designated on the basis of literature report and mechanistic approach[33].

C_6H_5
$CH-CH_3$
O
$CH-(CH_2)_7-C-O$
CH_3
C
N
N—N

m/z 343

C_6H_5
$CH-CH_3$
O
$CH_3-(CH_2)_7-CH-CH-(CH_2)_7-C-O$
Br
CH_3
C
N
N—N

m/z 548/550 [M⁺]

C_6H_5
$CH-CH_3$
O
$CH_3-(CH_2)_7-CH-CH-(CH_2)_7-C-O$
Br
CH_3
C
N

m/z 520/522

—N₂

C_6H_5
$CH-CH_3$
O
$CH-(CH_2)_7-C$
Br⁺

m/z 339/341

$CH_3-(CH_2)_7-CH$
Br⁺
CH

m/z 205/207

C_6H_5
$CH-CH_3$
O
$CH_3-(CH_2)_7-CH-CH-(CH_2)_7-C-O$
Br
CH_3
C
N

m/z 520/522

—N₂

C_6H_5
$CH-CH_3$
O
$CH_3-(CH_2)_7-CH-CH-(CH_2)_7-C-O$
Br
CH_3
C
N
N—N

m/z 548/550 [M⁺]

CH_3
C
N
N—N
$CH_3-(CH_2)_7-CH$

m/z 209
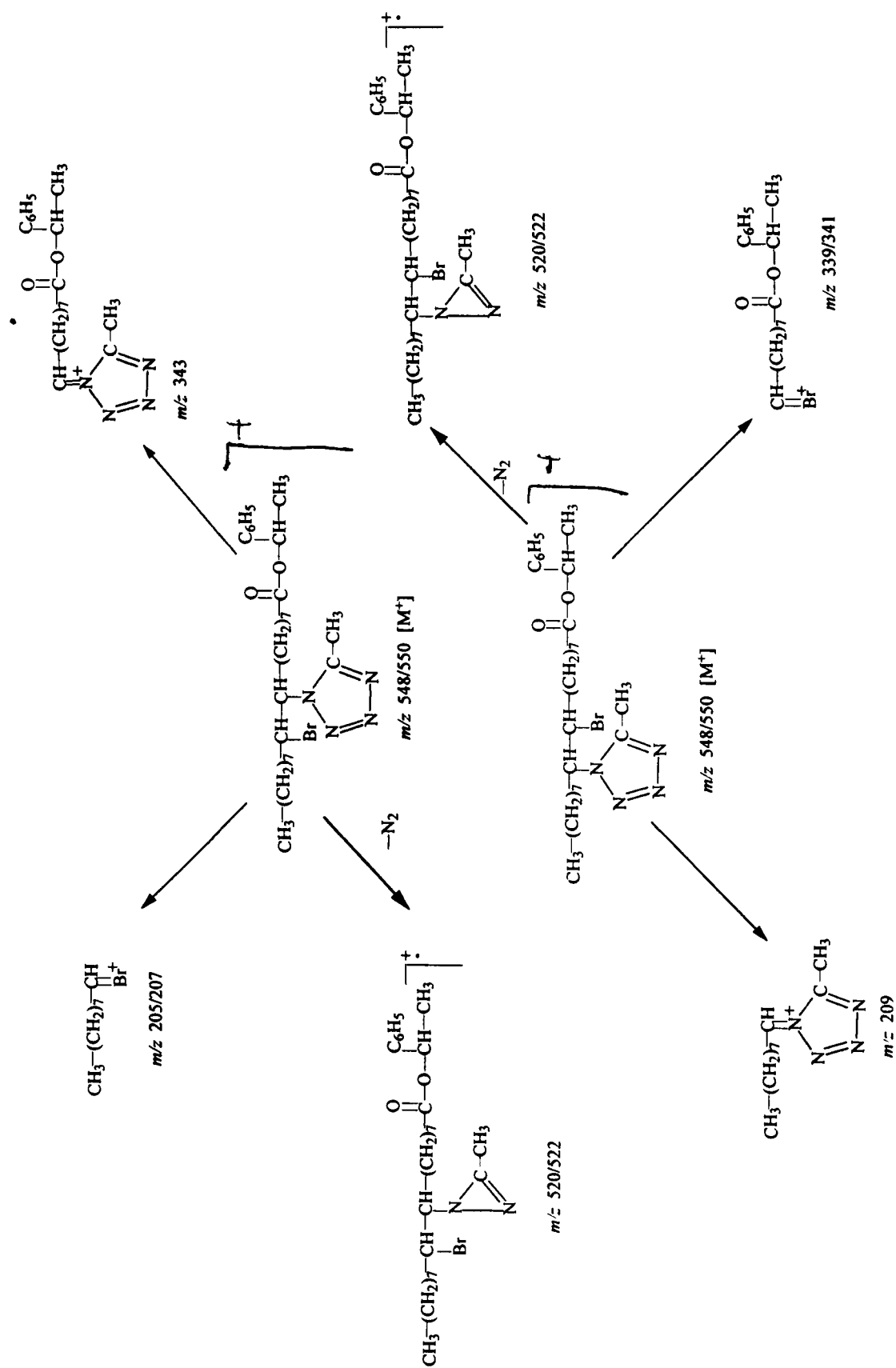
Chart 9: Mass fragmentation pattern of XIX

Reaction of 1'-phenylethyl (Z)-12-hydroxyoctadec-9-enoate (VII) with bromine, sodium azide and acetonitrile.

Compound **(VII)** was stirred with bromine, sodium azide and anhydrous aluminum chloride in acetonitrile at 0°C for 7 hours. Examination of the~~reaction~~ reaction mixture by TLC showed two spots. Separation by column chromatography over silica gel yielded two products **XX** and **XXI** in minor and major amounts **(Scheme 10)**.
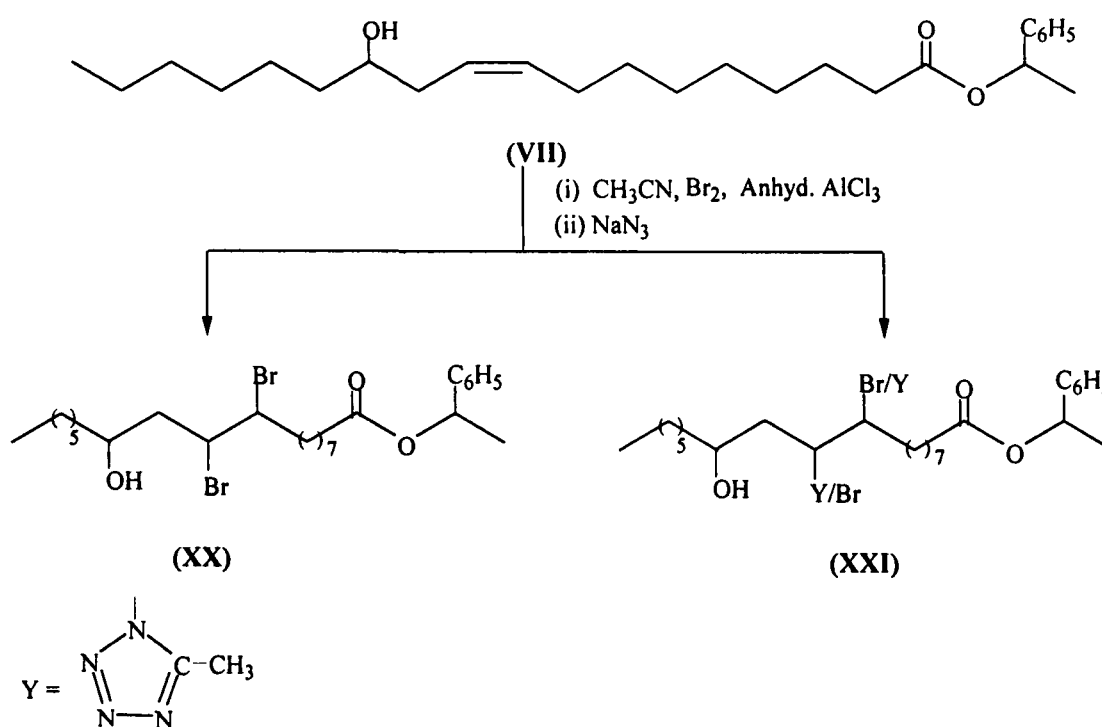

Scheme 10: Synthesis of 1'-phenylethyl 12-hydroxy-9/10-bromo-10/9-(5'-methyl-1*H*-tetrazol-1-yl) octadecanoate **(XXI)**.

Structure elucidation of the product (XX)

It was an oily liquid. It gave positive Beilstein test for halogen. Its IR spectrum displayed characteristic bands at 1736 for ester carbonyl group and at 669 cm^{-1} for bromo function. Its ^{1}H-NMR spectrum exhibited characteristic signal at δ 5.29 as a multiplet for two methine protons at C-9 and C-10. A multiplet appeared at δ 3.59 for methine proton at C-12 and a broad singlet at δ 2.31 for hydroxyl proton. The chemical shift value for three aromatic protons observed at 7.34 as a multiplet and for two aromatic protons at δ 7.25 also as a multiplet. On the basis of the above facts compound XX was characterized as *1´-phenylethyl 12-hydroxy-9,10-dibromooctadecanoate.*

Structure elucidation of the product (XXI)

The structure of XXI was established by IR, EI-MS and ^{1}H-NMR spectra. It appeared to be a tetranitrogenous compound by its elemental analysis (C$_{28}$H$_{45}$O$_3$N$_4$Br) and it gave a positive Beilstein test. The IR spectrum of XXI exhibited characteristic bands at 1211 and 1095 cm^{-1} for CN tetrazole ring, at 665 cm^{-1} for bromo function. The absorption bands for N=N, C=N and COO were observed at 1530, 1375 and 1735 cm^{-1} respectively. The EI-MS spectrum of XXI showed a molecular ion peak at m/z 564/566. The base peak appeared at m/z 536/538 due to loss of one nitrogen molecule from the tetrazole nucleus. Mass fragment ions at m/z 343, 339/341, 225, and 221/223 confirmed that product XXI is a mixture of two isomers. Other prominent peaks arising due to loss of OH and HBr appeared at 547/549 and 483 respectively (**Chart 10**). The ^{1}H-NMR spectrum gave a characteristic signal at 5.38 as a
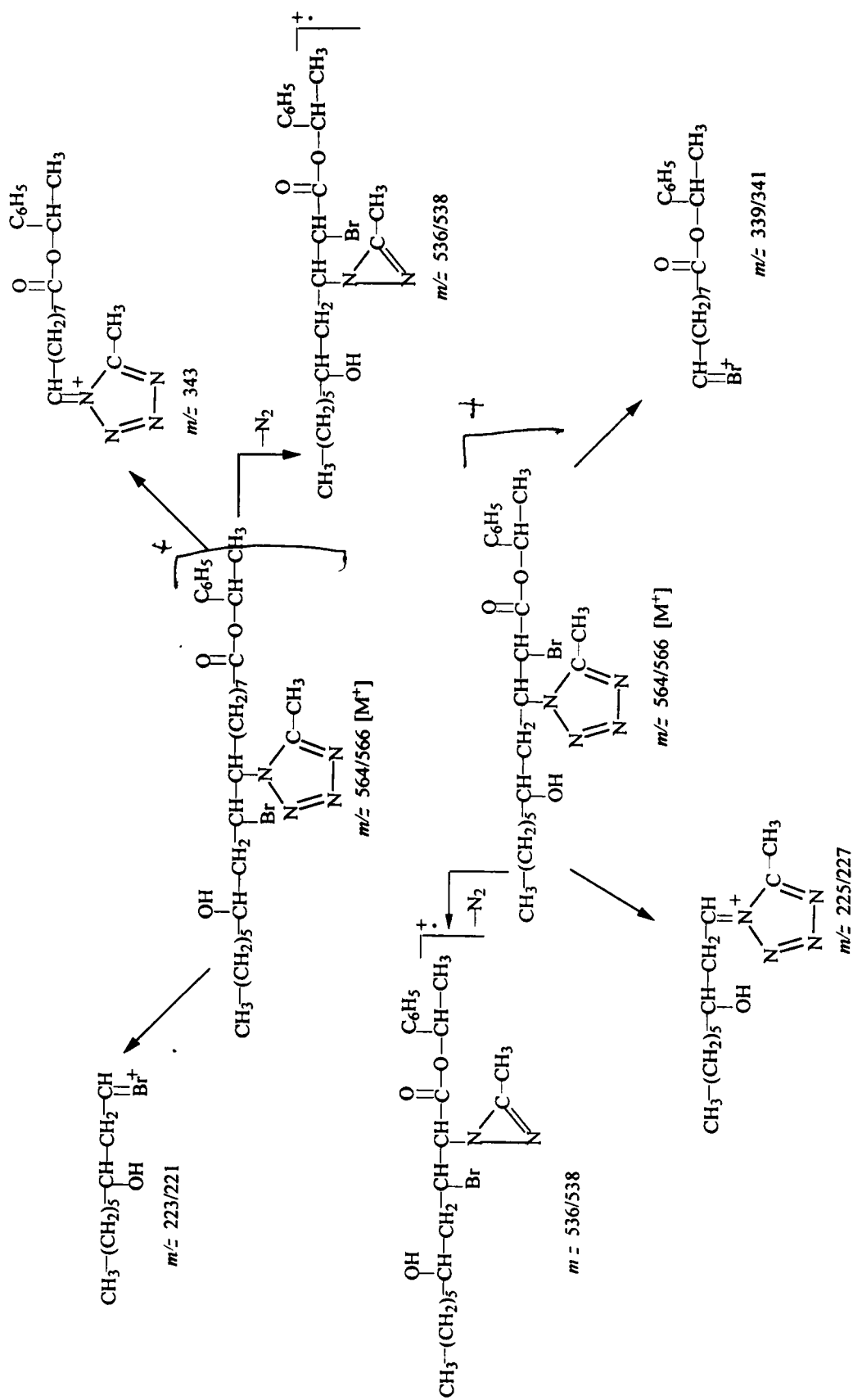
Chart 9: Mass fragmentation pattern of **XIX**

multiplet for C-9 and C-10 methine proton to which bromine and tetrazole ring

were attached. The signal for methyl protons in the tetrazole ring was observed

as a singlet at δ 2.56 along with other usual signals associated with fatty acid

ester. On the basis of spectral data coupled with mechanistic consideration, the

structure of **XXI** was assigned as *1'-phenylethyl threo-12-hydroxy-9/10-bromo-*

10/9-(5'-methyl-1H-tetrazol-1-yl)octadecanoate.

The mechanism of bromo tetrazole (**XVII, XIX** and **XXI**) formation has *is*
demonstrated
been shown in **Scheme 11**, which is similar to that of reported by Hassner

et al.[19,20] In this case nitrile, present in excess, acts as a nucleophile in the

opening of bromonium ion intermediate (**A**), leading to the formation of

nitrilium ion (**B**). The addition of azide ion to the (**B**), resulted *then results* in the formation

of bromo tetrazole (**C**).

$$RHC = CH - R' \xrightarrow[-Br^-]{Br_2}$$

$$\bar{N} = \overset{+}{C} - CH_3 \longleftrightarrow N \equiv C - CH_3$$

(A)

$$CH_3CN \xrightarrow{\quad}$$

$$\begin{array}{c} RHC-CH-R' \\ | \quad | \\ Br \quad N = \overset{+}{C} - CH_3 \end{array} \xrightarrow{NaN_3}$$

(B)

$$\begin{array}{c} RHC-CH-R' \\ | \quad | \\ Br \quad N \\ \quad \overset{\diagup}{\diagdown} C - CH_3 \\ N \quad N \\ \overset{-}{N} = \overset{+}{N} \end{array}$$

Intramolecular cyclization

$$\begin{array}{c} RHC-CH-R' \\ | \quad | \\ Br \quad N \\ N \overset{\diagup}{\diagdown} C - CH_3 \\ N - N \end{array}$$

(C)
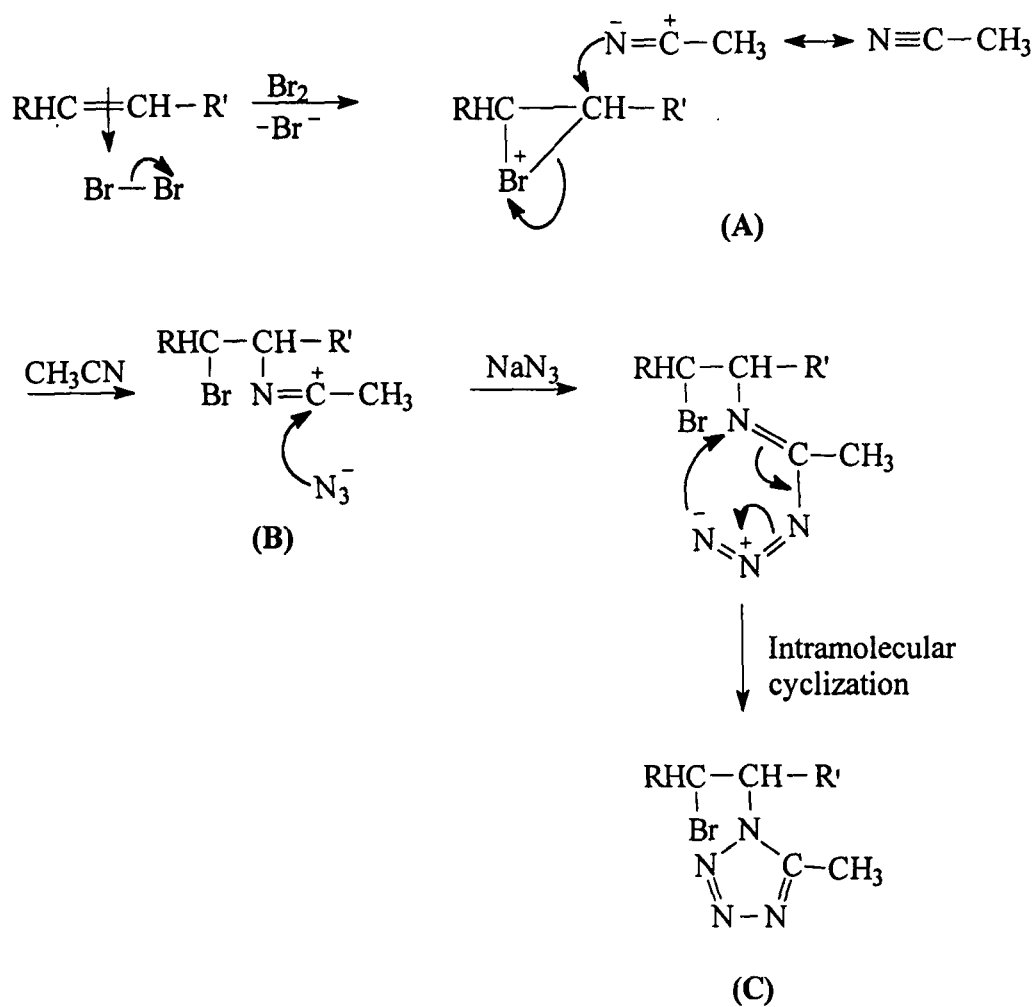
(XVII): R = H, R' = $(CH_2)_8$ – COO – CH (C_6H_5) – CH_3

(XIX) : R = CH_3 – $(CH_2)_7$, R' = $(CH_2)_7$ – COO – CH (C_6H_5) – CH_3

(XXI): R = CH_3 – $(CH_2)_5$– CH(OH) – CH_2, R' = $(CH_2)_7$ – COO – CH (C_6H_5) – CH_3

Scheme 11: Mechanism of the formation of α-bromo-5'-methyl tetrazole

4.3 Experimental

Reaction of 1'-phenylethyl undec-10-enoate (III) with bromine, sodium azide and acetonitrile.

Bromine (0.41g, 3 mmol) was added to a well stirred and cooled (0°C) solution of 1'-phenylethyl undec-10-enoate **(III)** (0.86g, 3 mmol) and anhydrous aluminum chloride (0.35g, 3 mmol) in acetonitrile (10 mL). Within a few minutes sodium azide (0.16g, 3 mmol) was added in portions. The reaction mixture was allowed to attain room temperature and stirred for 5 hours and filtered. Filtrate was diluted with water and extracted with dichloromethane (100 mL × 4). The extract was washed with water and then dried over anhydrous sodium sulphate. Solvent was evaporated under reduced pressure. The crude mixture was resolved by silica gel column chromatography. Elution with petroleum ether-diethyl ether (95:5, v/v) gave **XVI** (0.21g, 16%).

IR (KBr) v_{max}: 1735 (COO), 1610 (C=C, phenyl), 670 (C-Br).

^{1}H-NMR (CDCl$_3$) δ_H: 7.35 (m, 3H, 3Ar-H), 7.25 (m, 2H, 2Ar-H), 5.87 (q, 1H, O-CH, J = 6.6), 3.59-4.4 (m, 3H, CH_2-Br and CH-Br), 2.32 (dist. t, 2H, CH_2COO, J = 7.4), 2.00 (m, 2H, Br-CH$_2$-CH-Br-CH_2), 1.34 (br s, chain CH_2).

Final elution with petroleum ether-diethyl ether (80:20, v/v) yielded a brownish liquid **(XVII)**, (1.07g, 80%). Calculated for C$_{21}$H$_{31}$O$_2$N$_4$Br: C, 55.8; H, 6.9; N, 12.4%. Found: C, 56.2; H, 6.7; N, 12.2%.

IR (KBr) v_{max} : 1735 (COO), 1520, 1368 (N=N, C=N), 1244, 984 (CN$_4$ ring),

668 (C-Br).

EI-MS: *m/z* 450/452 [(M$^+$, 35.3)], 422/424 (100), 370 (23.5), 357 (29.4),

345/347 (41.1), 261 (29.4), 189/191 (58.8), 93/95 (17.2).

^{1}H-NMR (CDCl$_3$) δ_H: 7.34 (m, 3H, 3Ar-*H*), 7.25 (m, 2H, 2Ar-*H*), 5.89 (q, 1H,

OC*H*, *J* = 6.6), 4.65 (m, 1H, N-C*H*), 3.9 (s, 1H, H_a-C-Br) 3.8 (s, 1H, H_b-C-Br,

J = 3.0) 2.61 (s, 3H, 5'-C*H$_3$*), 2.31 (dist.t, 2H, C*H$_2$*COO), 1.61 (m, 2H,

C*H$_2$*CH$_2$COO), 1.54 (d, 3H, CHC*H$_3$*, *J* = 6.6), 1.26 (br s, 14H, chain C*H$_2$*).

Reaction of 1'-phenylethyl (Z) octadec-9-enoate (V) with bromine, sodium

azide and acetonitrile.

To the ice cooled solution of 1'-phenylethyl (Z)-octadec-9-enoate (V)

(1.16g, 3 mmol) in acetonitrile (10 mL), anhydrous aluminum chloride (0.35g,

3 mmol) was added. Then bromine (0.13 mL, 3 mmol) was added to the above

cooled (0°C) and well stirred mixture, followed by the addition of sodium azide

(0.16g, 3 mmol) in portions and stirred for 5 hours at room temperature. The

reaction mixture was worked up as described above and the crude product

chromatographed over a column of silica gel. The first elution with petroleum

ether-diethyl ether yielded a brownish liquid compound (XVIII), (0.24g, 15%).

IR (KBr) v_{max}: 1735 (COO), 1611 (C=C, phenyl), 665 (C-Br).

¹H-NMR (CDCl₃) δ_H: 7.33 (m, 3H, 3Ar-*H*), 7.27 (m, 2H, 2Ar-*H*), 5.89 (q, 1H,

O-C*H*, *J* = 6.6), 4.21 (m, 4H, 2×C*H*-Br), 2.32 (dist. t, 2H, C*H₂*COO), 1.61 (m, 2H,

C*H₂*CH₂COO), 1.53 (d, 3H, CHC*H₃*, *J* = 6.6), 0.83 (dist. t, 3H, terminal C*H₃*).

Final elution with petroleum ether-diethyl ether (60:40, v/v) afforded the

major product **XIX** (1.15g, 70%). Calculated for $C_{28}H_{45}O_2N_4Br$: C, 61.1; H, 10.2;

N, 10.2%. Found: C, 60.0; H, 10.2; N, 10.1%.

IR (KBr) v_{max}: 1734 (COO), 1611 (C=C, phenyl), 1507, 1375 (N=N, C=N), 1268,

1037 (CN₄ ring), 669 (C-Br).

EI-MS: *m/z* 548/550 [(M⁺, 13)], 520/522 (77), 506/508 (77), 468 (5), 435 (100),

339/341 (13), 343 (26), 301 (18), 209 (10), 205/207 (18).

¹H-NMR(CDCl₃) δ_H: 7.34 (m, 3H, 3Ar-*H*), 7.25 (m, 2H, 2Ar-*H*), 5.89 (q, 1H,

OC*H*, *J* = 6.6), 4.68 (m, 2H, C*H*=C*H*), 2.59 (s, 3H, 5'-C*H₃*), 2.31 (t, 2H,

C*H₂*COO), 2.03 (m, 4H, C*H₂*-CH=CH-C*H₂*), 1.64 (m, 2H, C*H₂*CH₂COO) 1.53

(d, 3H, CH-C*H₃*, *J* = 4.95), 0.87 (dist. t, 3H, terminal C*H₃*), 1.24 (br s, 24H, chain

C*H₂*).

Reaction of 1'-phenylethyl (Z)-12-hydroxyoctadec-9-enoate (VII) with

bromine, sodium azide and acetonitrile.

In a similar manner using the conditions as described above, a mixture of

1'-phenylethyl (Z)-12-hydroxyoctadec-9-enoate (**VII**) (1.21g, 3 mmol), anhydrous

aluminum chloride (0.35g, 3 mmol), bromine (0.13 mL, 3 mmol) and sodium azide (0.16g, 3 mmol) in acetonitrile (10 mL) was stirred for 6 hours. After workup with dichloromethane (100 mL × 4) the extract was washed with water, dried over anhydrous sodium sulphate and the solvent evaporated under reduced pressure. The residue was chromatographed over a column of silica gel. Elution with petroleum ether-diethyl ether yielded an oily liquid (XX), (0.34g, 20%).

IR (KBr) v_{max}: 3352 (OH), 1736 (COO), 1610 (C=C, phenyl), 669 (C-Br).

^{1}H-NMR (CDCl$_3$) δ_H: 7.34 (m, 3H, 3Ar-H), 7.25 (m, 2H, 2Ar-H), 5.87 (q, 1H, OCH, J = 4.95), 5.29 (m, 2H, CH=CH), 3.59 (m, 1H, CHOH), 2.32 (m, 2H, CH_2COO), 2.31(br s,1H, OH merged in part with the signal at δ 2.32) 2.18 and 2.03 (m, each for 2H, CH_2-CH=CH-CH_2), 1.62 (m, 2H, CH_2CH$_2$COO), 1.52 (d, 3H, CHCH_3, J = 4.95), 1.28 (br s, chain CH_2), 0.88 (dist. t, 3H, terminal CH_3).

Final elution with petroleum ether-diethyl ether (70:30, v/v) gave brownish liquid (XXI), (1.27g, 75%). Calculated for C$_{28}$H$_{45}$O$_3$N$_4$Br: C, 59.50; H, 7.9; N, 9.9 %. Found: C, 58.6; H, 8.5; N, 9.4%.

IR (KBr) v_{max}: 3350 (OH), 2911 (C-H), 1735 (COO), 1622 (C=C, phenyl), 1530, 1375 (N=N, C=N), 1211, 1095 (CN$_4$ ring), 665 (C-Br).

EI-MS: m/z 564/566 [(M$^+$, 28)], 547/549 (23), 535/537 (100), 522/524 (7.1), 483 (6.6) 437, (27.4) 358 (2.4), 343 (3.6), 339/341 (11.2) 316/318 (11.0), 247 (6.6), 225/227 (9.5), 221/223 (7.1).

¹H-NMR (CDCl₃) δ_H: 7.34 (m, 3 Ar-*H*), 7.28 (m, 2Ar-*H*), 5.87 (q, 1H, OC*H*,

J = 4.95), 5.38 (m, 2H, C*H*=C*H*)), 3.59 (m, 1H, C*H*OH), 2.56 (s, 3H, 5'-C*H₃*),

2.32 (m, 2H, C*H₂*COO), 2.30 (br s, 1H, O*H*), 2.19 and 2.03 (m, each for 2H, C*H₂*-

CH=CH-C*H₂*), 1.60 (m, 2H, C*H₂*CH₂COO), 1.51 (d, 3H, CHC*H₃*, *J* = 4.95), 1.27

(br s, 18H, chain C*H₂*), 0.87 (dist. t, 3H, terminal C*H₃*).

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CHAPTER-5

Antimicrobial Activity of Fatty Acid Derivatives

5.1 Theoretical

are

Fatty acid derivatives ~~have been found to be~~ associated with diverse antimicrobial activities[1-5]. Mod *et al.*[6] reported that sulphur derivatives of N,N-disubstituted amides of long-chain fatty acids are active against various microorganisms. A number of nitrated heterocycles, particularly heterocycles with five membered rings, ~~have been shown~~ *possess* potential antimicrobial and antiparasitic properties[7].

In view of biological importance of fatty acid derivatives we have carried out antimicrobial screening of some fatty acid derivatives (reported in Chapter 3 *5* and 4).

5.2 Antimicrobial activity of long-chain fatty alkenoates

The test organisms ~~used in the study~~ included *Escherichia coli* UP 2566 (Central Drug Research Institute, Lucknow, India), clinical isolates of *Staphylococcus aureus* (IOA-106) and *Pseudomonas aeruginosa* (IOA-110) provided by the Department of Microbiology, J. N. Medical College, A. M. U., Aligarh, India.

Culture media and inoculum

Nutrient broth and agar were obtained from Hi-Media Pvt. Ltd. (Mumbai, India). Freshly grown microbial cultures at 37°C were appropriately diluted in sterile normal saline solution to obtain a cell suspension of 10^6 *CFU/mL.

*CFU = Colony forming unit

Antimicrobial assay

The compounds (III), (V), (VII), (IX), (XI), (XIII) and (XV) were screened for their antimicrobial activity in *vitro* by the disc diffusion method[8]. The test organisms were grown in agar media and spread over prepoured petriplates. The test samples were loaded on 6 mm sterilized filter paper and placed on petriplates. The test compounds were dissolved in dimethylformamide (DMF). Incubation was carried out at 37°C for 24 hours. Chloromycetin was used as standard drug. The diameters of zone of inhibition were measured in mm for 10μg/mL concentration.

Results and Discussion

Seven compounds were tested against one gram +ve bacteria (*Staphylococcus aureus*) and two Gram –ve bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The results for the antimicrobial study of the tested compounds are depicted in **Table 5**. Solvent control (DMSO) showed non-significant inhibition to test microorganisms. The compounds **III, XI** and **XIII** demonstrated activity against both Gram +ve and Gram –ve bacteria. Compounds **III, VII, IX, XIII, XV** except **V** showed significant activity against *E. coli* comparable to chloromycetin at the same concentration. Compounds **III, XI** and **XIII** showed moderate activity against *S. aureus* and *P. aeruginosa* whereas rest of the compounds showed less activity against microorganisms compared to chloromycetin.

Table 5: Antimicrobial activity of long-chain fatty alkenoates

Test organism	Inhibition zone size in mm							
	III (10 µg/disc)	V (10 µg/disc)	VII (10 µg/disc)	IX (10 µg/disc)	XI (10 µg/disc)	XIII (10 µg/disc)	XV (10 µg/disc)	Antibiotic Control (10 µg/disc)
Staphylococcus aureus (Gram +ve)	13.50	12.00	11.50	10.00	14.50	15.00	11.00	19.00
Escherichia coli (Gram –ve)	14.00	8.50	14.50	13.00	13.50	14.50	13.00	20.00
Pseudomonas aeruginosa (Gram –ve)	14.00	10.00	9.00	11.50	13.50	14.00	12.50	18.00

5.3 Antimicrobial activity of fatty tetrazoles

The test microorganisms used in antimicrobial screening included two

Gram +ve bacteria (*Staphylococcus aureus* IOA-106, *Bacillus subtilis*

MTCC-121) and two Gram –ve bacteria (*Escherichia coli* K-12-J-62,

Pseudomonas aeruginosa).

Culture media and inoculum

The bacteria were maintained in nutrient agar. Freshly grown microbial

cultures at 37°C were diluted in sterile normal saline solution to obtain a cell

suspension of 10^5 CFU/mL. *You need to be consisten*

In the Chemistry section you

Antimicrobial assay *abbreviated milliliter by mL !!!*

The disc diffusion method[9] with little modification was used. Briefly 0.1

mL of diluted inoculum (10^5 CFU/mL) of test organism was spread on nutrient

agar (NA) plates. Sterile paper disc impregnated with 50 μg of compound and a

disc without compound was used as a negative control. The plates were incubated

for 5-6 days at 28 °C. The antimicrobial activity was evaluated by measuring zone

of inhibition against test organisms. Antibiotic control chloramphenicol (Hi-media

Pvt. Ltd., Mumbai, India) was used in the test system as positive control.

Results and Discussion

A total of three compounds (**XVII**), (**XIX**) and (**XXI**) were tested against

two Gram +ve bacteria (*S. aureus, B. subtilis*) and two Gram –ve bacteria (*E. coli,*

P. aeruginosa).

The results for the antimicrobial study of the tested compounds (**XVII, XIX** and **XXI**) are given in **Table 6**. All compounds (**XVII, XIX** and **XXI**) demonstrated moderate activity against both Gram +ve and Gram –ve bacteria.

Table 6: Antimicrobial activity of fatty tetrazoles

| Test organism | Inhibition zone size in mm | | | |
	XVII (50 µg/disc)	**XIX** (50 µg/disc)	**XXI** (50 µg/disc)	Antibiotic Control (30 µg/disc)
Staphylococcus aureus (Gram +ve)	10.50	11.00	8.00	33.00
Bacillus subtilis (Gram +ve)	11.00	11.50	8.50	38.00
Escherichia coli (Gram –ve)	10.00	8.00	10.50	33.00
Pseudomonas aeruginosa (Gram –ve)	11.00	10.00	8.50	26.00

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LIST OF PUBLICATIONS AND PRESENTATIONS

1. Direct Esterification of Fatty Acids with Phenyl Alkanols by using Dicyclohexylcarbodiimide. *Eur. J. Lipid Sci. Technol.*, 106, **2004**, 97-100.

2. Synthesis and Characterization of α-Bromo-5-Methyl-Tetrazoles from Long Chain Fatty Alkenoates. *J. Oleo Sci.*, 53, **2004**, 279-283.

3. Triacylglycerols-Profiling by High Performance Liquid Chromatography for Detection of Lard in Palm Oil. *J. Oil Tech. Assn. India*, 35, **2003**, 45-48.

4. Triacylglycerols-Analysis of three Medicinal Seed Oils using Pancreatic Lipase. *J. Oleo Sci.*, 53, **2004**, 531-536.

5. Preparation, Characterization and Antimicrobial Activity of Fatty Alkenoates. *Indian J. Chem.* **(B)**, (in press).

6. Use of Dicyclohexylcarbodiimide in the Direct Esterification of Fatty Acid with Alcohol. 57th Annual Convention & International Seminar, September 21-22, 2002, Harcourt Butler Technological Institute, Kanpur 208002, India.

7. Chemical Composition and Physiologically Active Substances in Ricebran Oil. 57th Annual Convention & International Seminar, September 21-22, 2002, Harcourt Butler Technological Institute Kanpur 208002, India..

8. Synthesis and Characterization of α-Bromoalkyltetrazoles From Long-Chain Fatty Alkenoates. 58th Annual Convention and International Seminar, November 21-23, 2003, Park Hotel, Kolkata, India.

9. Preparation, Characterization and Antimicrobial Activity of Esters of 10-Undecenoic Acid. 59th Annual Convention and National Seminar, December 4-5, 2004, Hotel Le Meridien, New Delhi 110 001, India.

10. Seed Oil of *Sesbania aculeata*: A Moderate Source of (*Z*)-12-Hydroxyocta-dec-9-enoic Acid. 59th Annual Convention and National Seminar, December 4-5, 2004, Hotel Le Meridien, New Delhi 110 001, India.