STUDIES ON SEEDS, FATS AND FATTY ACIDS

RESUME

THESIS SUBMITTED FOR THE DEGREE OF
Doctor of Philosophy
IN
CHEMISTRY

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DEPARTMENT OF CHEMISTRY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)
1984
Results of two types of exploratory research are recorded in this thesis: Part-I deals with compositional studies of minor seed oils and Part-II embodies the work related to the synthesis of oxygen/sulfur/nitrogen-containing fatty acid derivatives.

Part - I

In continuation of chemical screening program of wild oil-yielding species to explore the oil seed potential of forest flora, few oils have been examined for their fatty acid profile.

A. Minor Seed Oils

Six wild oil-bearing species Carissa spinarum (Apocynaceae), Vitex trifolia (Verbenaceae), Tithonia diversifolia, Spilanthea acmella (Compositae), Garcinia mangostana (Guttiferae) and Acacia catechu (Leguminosae) have been analysed by using chromatographic and spectroscopic methods. G. mangostana seed oil (60.20% oil content) is characterized by the presence of 96.10% unsaturated acids comprising mainly oleic and linoleic, which have potential use as edible oil. Another important feature of the species
surveyed is the presence of high amount of oleic (51.40%) and docosenoic (31.03%) acids in the seed oils of *C. spinarum* and *V. trifolia* respectively. The remaining three species, *T. diversifolia*, *S. acmella* and *A. catechu* yield oils, have linoleic acid as a major constituent (36.0–57.0%). The seed oil of *A. catechu* contains 12,13-epoxyoleic acid (10.30%).

B. Cyclopropenoid Fatty Acids in Seed Oil of *Gnetum scandens* (Gnetaceae)

The seed oil of *G. scandens*, which responded to Halphen test and showed diagnostic spectral data for cyclopropenoid fatty acids, has been analysed by using GLC of silver nitrate-methanol treated derivatives. The gross fatty acid determination showed the following composition: 14:0, 0.35; 16:0, 15.51; 18:0, 10.20; 18:1, 16:22; 18:2, 15.0; 18:3, 2.85; malvalic, 11.27 and sterculic, 28.57%.

C. A New Hydroxy Acid in *Mirabilis jalapa* (Nyctaginaceae) Seed Oil

The seed oil of *M. jalapa* was found to contain a new hydroxy dienoic acid (4.30%). The structure of hydroxy acid was established as 8-hydroxyoctadeca-cis-11,14-dienoic acid by various spectral and chemical methods.
In this part the results of a variety of reactions on long chain 2-oxo, \(\alpha,\beta\)-unsaturated carbonyl and dioxoene fatty compounds are described. The structures of isolated products have been established by combustion and spectral methods.

A. Preparation of Tetrazole and Amides

An amide (5) was obtained when 2-oxooctadecanoic acid (1) was refluxed with hydrazoic acid (excess) in presence of boron trifluoride (BF\(_3\))-etherate (Scheme-1).

\[
\text{CH}_3(\text{CH}_2)_{15}\text{C-COOH} \xrightarrow{\text{HN}_3\text{(excess)}} \text{BF}_3\text{-etherate, benzene} \rightarrow \text{CH}_3(\text{CH}_2)_{14}\text{-CH}_2\text{-NH-C-COOH} \]

(1) \hspace{2cm} (5)

Methyl 4-oxo-trans-2-octadecenoate (2) on treatment with excess of hydrazoic acid and BF\(_3\)-etherate yielded tetrazole (6) in high yield along with an amide (7) and a cleaved product (8) (Scheme-2).
B. Preparation of Long Chain Thioethers

Branched-chain thioethers (10, 11 and 12) have been prepared from $\alpha,\beta$-unsaturated carbonyl (3) and unsaturated dioxo (4) fatty acids. The reagents involved in these preparations were $\beta$-mercaptoacetic acid and $\beta$-mercaptopropionic acid. The yields of these thioethers are almost quantitative (Schemes 3 and 4).
Scheme-3

\[ R - C - CH = CH - COOCH_3 \]  \( (3) \)

\[ HS-CH_2-COOH, \text{ benzene} \]

\[ H \]

\[ R - C - CH - CH - COOCH_3 \]

\[ S-CH_2-COOH \]

\( (10) \)

\[ R = CH_3(CH_2)_{11} \]

Scheme-4

\[ CH_3(CH_2)_5 - C - CH = CH - C - (CH_2)_7 - COOH \]  \( (4) \)

\[ HSCH_2CH_2COOH, \text{ benzene} \]

\[ H \]

\[ CH_3(CH_2)_5 - C - CH - CH - C - (CH_2)_7 - COOH \]

\[ S-CH_2-CH_2-COOH \]

\( (12) \)
C. Preparation of Oxathiolanes

Methyl 4-oxo-trans-2-hexadecenoate (3) when reacted with β-mercaptoethanol and BF$_3$-etherate in acetic acid afforded methyl 4-oxathiolane-trans-2-hexadecenoate (13), methyl 4-oxathiolane-2(3)-(S-β-mercaptoethylacetate)hexadecanoate (14) and methyl 4-oxathiolane-2(3)-(S-β-mercaptoethanol)hexadecanoate (15) (Scheme-5).

\[
\text{Scheme-5}
\]

\[
\begin{align*}
\text{R-C-CH}=\text{CH-CH}=\text{COOCH}_3 \\
\text{HSCH}_2\text{CH}_2\text{OH, BF}_3\text{-etherate, AcOH} \\
\text{R-C-CH}=\text{CH-CH}=\text{COOCH}_3 + \text{R-C-CH}=\text{CH-CH}=\text{COOCH}_3 + \text{R-C-CH}=\text{CH-CH}=\text{COOCH}_3 \\
(13) \quad (14) \quad (15)
\end{align*}
\]

\(\text{R} = \text{CH}_3(\text{CH}_2)_\text{11}\)

Similar reaction of β-mercaptoethanol with 9,12-dioxo-trans-10-octadecenoic acid (4) gave two products, 9(12)-oxathiolane-12(9)-oxo-10(11)-(S-β-mercaptoethylacetate).
octadecanoic acid (16) in high yield and 9,12-dioxathiolane-10(11)-(S-β-mercaptoethylacetate)octadecanoic acid (17) (Scheme-6).

**Scheme-6**

\[
R - C - CH = CH - C - (CH_2)_7 - COOH
\]

(4)

\[
\text{HSCH}_2\text{CH}_2\text{OH, BF}_3\text{-etherate, AcOH}
\]

\[
R = \text{CH}_3(\text{CH}_2)_5
\]

**D. Preparation of Thiazinones**

An azeotropic reflux of methyl 4-oxo-trans-2-octadecenoate (2) with β-mercaptopropionic acid and \((\text{NH}_4)_2\text{CO}_3\) afforded methyl 4-thiazinone-2(3)-(S-β-mercaptopropionic acid) octadecanoate (18) as a major product along with a minor amount of an ester hydrolysed product (19) of 18 (Scheme-7).
E. Preparation of Thiazolidinedinone and Thiazole Derivatives

Methyl 4-oxo-trans-2-octadecenoate (2) on treatment with thiourea, sodium acetate and dil. HCl afforded the derivatives thiazolidinedinone (20) and thiazole (21) (Scheme-8).
F. Preparation of Enolacetate and α-Acetoxyketone

Methyl 4-oxo-trans-2-octadecenoate (2) was reacted with acetic anhydride and p-toluenesulfonic acid (p-TSA) to give vinyl acetate (22) in high yield. Compound (22) on treatment with lead tetraacetate (LTA) in acetic acid furnished α-acetoxyketone (23) in quantitative yield. LTA oxidation of 2 also afforded 23 in 80.30% of yield (Scheme-9).
G. Preparation of Nitrohydrin and Nitronitrate Derivatives

Nitration of methyl 4-oxo-\textit{trans}-2-octadecenoate (2) with fuming $\text{HNO}_3$ in acetic acid afforded nitrohydrin (24) and nitronitrate (25) (Scheme-10).
Scheme-10

R - C - CH = CH - COOCH₃

(2)

fuming HNO₃, AcOH

R - C - CH

OH

NO₂

CH - COOH

R - C - CH

ONO₂

CH - COOH

NO₂

(24)

(25)

R = CH₃(CH₂)₁₃
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1984
This is to certify that the work embodied in this thesis entitled, 'Studies on Seeds, Fats and Fatty Acids' is the original work of Mr. Jamal Mustafa carried out under my supervision. The thesis is suitable for submission for the award of the degree of Doctor of Philosophy in Chemistry.

( S.M. Osman )
ACKNOWLEDGEMENTS

I have the privilege of carrying out this research work under the able guidance of Professor S.M. Osman. I want to express my deep sense of gratitude to him for his guidance and consistent encouragement.

I am extremely grateful to the Chairman, Department of Chemistry for providing necessary facilities and to Professor M.S. Ahmad for helpful discussions.

I owe Drs. Fasih Ahmad and M. Shamim Ahmad for their suggestions and generous help. In addition I thank Drs. Mushfiq and Rauf along with other colleagues who have rendered assistance in different ways.

Finally, I acknowledge CSIR, New Delhi for financial assistance, the instrumentation services provided by AMU, Aligarh and RSIC, Lucknow.

Jamal Mustafa
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Summary

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surveyed is the presence of high amount of oleic (51.40%) and
docosenoic (31.03%) acids in the seed oils of C. spinarum and
V. trifolia respectively. The remaining three species, I. diversifolia, S. acmella and A. catechu yield oils, have linoleic acid as a major constituent (36.0-57.0%). The seed oil of A. catechu contains 12,13-epoxyoleic acid (10.30%).

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In this part the results of a variety of reactions on long chain 2-oxo, α,β-unsaturated carbonyl and dioxoene fatty compounds are described. The structures of isolated products have been established by combustion and spectral methods.

A. **Preparation of Tetrazole and Amides**

An amide (5) was obtained when 2-oxooctadecanoic acid (1) was refluxed with hydrazoic acid (excess) in presence of boron trifluoride (BF$_3$)-etherate (Scheme-1).

**Scheme-1**

\[
\begin{align*}
\text{CH}_3(\text{CH}_2)_{15}-\text{C}-\text{COOH} & \xrightarrow{\text{HN}_3(\text{excess})}_{\text{BF}_3-\text{etherate, benzene}} \text{CH}_3(\text{CH}_2)_{14}-\text{CH}_2-\text{NH}-\text{C}-\text{COOH} \\
(1) & \quad (5)
\end{align*}
\]

Methyl 4-oxo-trans-2-octadecenoate (2) on treatment with excess of hydrazoic acid and BF$_3$-etherate yielded tetrazole (6) in high yield along with an amide (7) and a cleaved product (8) (Scheme-2).
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Branched-chain thioethers (10, 11 and 12) have been prepared from $\alpha,\beta$-unsaturated carbonyl (3) and unsaturated dioxo (4) fatty acids. The reagents involved in these preparations were $\beta$-mercaptoacetic acid and $\beta$-mercaptopropionic acid. The yields of these thioethers are almost quantitative (Schemes 3 and 4).
\textbf{Scheme-3}

\begin{align*}
  & \begin{tikzpicture}
    \node (R) at (0,0) [text width=3cm, text height=1cm, text depth=0.5cm] {R - \text{C} - \text{CH} = \text{CH} - \text{COOCH}_3};
    \node (O) at (0,0.5) [text width=3cm, text height=1cm, text depth=0.5cm] {O};
    \node (H) at (0,-0.5) [text width=3cm, text height=1cm, text depth=0.5cm] {H};
    \node (S) at (-0.5,-1) [text width=3cm, text height=1cm, text depth=0.5cm] {S-\text{CH}_2\text{-COOH}};
    \node (HS) at (0.5,-1) [text width=3cm, text height=1cm, text depth=0.5cm] {HS-\text{CH}_2\text{-COOH}};
    \node (B) at (1,-1) [text width=3cm, text height=1cm, text depth=0.5cm] {benzene};
    \node (B2) at (2,-1) [text width=3cm, text height=1cm, text depth=0.5cm] {benzene};
    \draw (R) -- (O) -- (H) -- (B);
    \draw (R) -- (S) -- (B2);
    \draw (HS) -- (B);\end{tikzpicture} \\
  & \text{(3)} \\

  \text{R} &= \text{CH}_3(\text{CH}_2)_{11} \\

  & \text{(10)} \quad \text{(11)}
\end{align*}

\textbf{Scheme-4}

\begin{align*}
  & \begin{tikzpicture}
    \node (R) at (0,0) [text width=3cm, text height=1cm, text depth=0.5cm] {\text{CH}_3(\text{CH}_2)_{5} - \text{C} - \text{CH} = \text{CH} - \text{COOCH}_3};
    \node (O) at (0,0.5) [text width=3cm, text height=1cm, text depth=0.5cm] {O};
    \node (H) at (0,-0.5) [text width=3cm, text height=1cm, text depth=0.5cm] {H};
    \node (S) at (-0.5,-1) [text width=3cm, text height=1cm, text depth=0.5cm] {S-\text{CH}_2\text{-CH}_2\text{-COOH}};
    \node (HS) at (0.5,-1) [text width=3cm, text height=1cm, text depth=0.5cm] {HS\text{CH}_2\text{CH}_2\text{COOH}};
    \node (B) at (1,-1) [text width=3cm, text height=1cm, text depth=0.5cm] {benzene};
    \draw (R) -- (O) -- (H) -- (B);
    \draw (R) -- (S) -- (B);
    \draw (HS) -- (B);\end{tikzpicture} \\
  & \text{(4)} \\

  \text{CH}_3(\text{CH}_2)_{5} - \text{C} - \text{CH} = \text{CH} - \text{COOCH}_3 - (\text{CH}_2)_{7} - \text{COOH} \quad \text{(12)}
\end{align*}
C. Preparation of Oxathiolenes

Methyl 4-oxo-trans-2-hexadecenoate \((3)\) when reacted with \(\beta\)-mercaptoethanol and BF\(_3\)-etherate in acetic acid afforded methyl 4-oxathioline-trans-2-hexadecenoate \((13)\), methyl 4-oxathioline-2(3)-(S-\(\beta\)-mercaptoethylacetate)hexadecanoate \((14)\) and methyl 4-oxathioline-2(3)-(S-\(\beta\)-mercaptoethanol)hexadecanoate \((15)\) (Scheme 5).

\[
\text{Scheme 5}
\]

\[
\begin{align*}
\text{R} - \text{C} - \text{CH} &= \text{CH} - \text{COOCH}_3 \\
(3) &
\text{HSCH}_2\text{CH}_2\text{OH}, \text{BF}_3\text{-etherate, AcOH} \\
\text{R-C-CH=CH-COOCH}_3 + \text{R-C-CH-CH-OOCCH}_3 &= \text{R-C-CH-CH-COOCH}_3 + \text{R-C-CH-CH-COOCH}_3 \\
\text{(13)} &
\end{align*}
\]

\[
\begin{align*}
\text{S} &
\end{align*}
\]

\[
\begin{align*}
\text{(14)} &
\text{(15)} \\
&
\text{R} = \text{CH}_3(\text{CH}_2)_\text{11}
\end{align*}
\]

Similar reaction of \(\beta\)-mercaptoethanol with 9,12-dioxo-trans-10-octadecenoic acid \((4)\) gave two products, 9(12)-oxathioline-12(9)-oxo-10(11)-(S-\(\beta\)-mercaptoethylacetate)
octadecanoic acid (16) in high yield and 9,12-dioxathiolane-10(11)-(S-β-mercaptoethylacetate)octadecanoic acid (17) (Scheme-6).

![Scheme-6](image)

R = CH₃(CH₂)₅

D. Preparation of Thiazinones

An azeotropic reflux of methyl 4-oxo-trans-2-octadecenoate (2) with β-mercaptopropionic acid and (NH₄)₂CO₃ afforded methyl 4-thiazinone-2(3)-(S-β-mercaptopropionic acid) octadecanoate (18) as a major product along with a minor amount of an ester hydrolysed product (19) of 18 (Scheme-7).
E. Preparation of Thiazolidinedinone and Thiazole Derivatives

Methyl 4-oxo-trans-2-octadecenoate (2) on treatment with thiourea, sodium acetate and dil. HCl afforded the derivatives thiazolidinedinone (20) and thiazole (21) (Scheme-8).
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Methyl 4-oxo-trans-2-octadecenoate (2) was reacted with acetic anhydride and p-toluenesulfonic acid (p-TSA) to give vinyl acetate (22) in high yield. Compound (22) on treatment with lead tetraacetate (LTA) in acetic acid furnished $\alpha$-acetoxyketone (23) in quantitative yield. LTA oxidation of 2 also afforded 23 in 80.30% of yield (Scheme-9).
G. Preparation of Nitrohydridin and Nitronitrate Derivatives

Nitration of methyl 4-oxo-trans-2-octadecenoate (2) with fuming HNO$_3$ in acetic acid afforded nitrohydridin (24) and nitronitrate (25) (Scheme-10).
Scheme-10

\[ R - C - CH = CH - COOCH_3 \]

(2)

fuming HNO₃, AcOH

\[ \begin{align*}
R & - C - CH - OH \\
& - NO₂ \\
\end{align*} \]

\[ \begin{align*}
R & - C - CH - COOH \\
& - NO₂ \\
\end{align*} \]

(24)

\[ \begin{align*}
R & - C - CH - OH \\
& - NO₂ \\
\end{align*} \]

\[ \begin{align*}
R & - C - CH - COOH \\
& - NO₂ \\
\end{align*} \]

(25)

\[ R = CH₃(CH₂)₁₃ \]
INTRODUCTION

Fats and oils are important in two ways, one in industry and another in human nutrition. Dietary fat plays a significant role in the developing nations as its calorie density is more than twice that of carbohydrates and proteins.

Oils and fats are found increasingly to be physiologically significant and chemically interesting. Renewed activity has arisen from the recognition of fatty acids as essential dietary requirements from their link with the prostaglandins and from their involvement in cell structural membrane. These discoveries followed principally from development of improved chromatographic and spectroscopic techniques for studying these compounds.

In recent years the production and utilization of fatty acids have grown both in size and diversity. In the industrial field there has been a competition between petrochemicals and oleochemicals. A variety of new useful products can be prepared by taking advantage of the inherently present functional groups in fatty acids, some of them are good substitutes for petroleum products. This approach has created an active interest in the study of derivatization of fatty acids.

India, primarily an agricultural country abounds in forest flora. There is a vast potential of agrichemicals derived from the minor oil seeds rich in specific kind of fatty acids.
One of the most exciting properties of fatty acid derivatives is their insecticidal and antimicrobial activity. Basic research in oleochemistry is thus of paramount importance to cope with the ever-increasing demand of fat and their derived products.

In the context of chronic shortage of vegetable oils and fats and the dependence of agrichemicals industry on their availability, the need for chemical screening of oil-yielding plant species is important for a nation like India. It was this objective in view that led to the present investigations on minor seed oils and to synthesize new fatty acid derivatives for possible industrial applications.
Part I

Compositional Studies on Seed Oils
Section I

a. **Fatty Acid Composition of Vegetable Seed Fats**

Recent methods of fatty acid analyses employing chromatographic and spectroscopic techniques have discovered more than eight hundred fatty acids in seed oils. An imposing number of novel and new fatty acids have been discovered possessing structural features quite unusual according to our previous concept. Seed oils having acids containing unusual functional groups like oxo, hydroxy, conjugated unsaturation, cyclopropenoid and epoxy often afford unexpected responses to analytical procedures in frequent use. Oxygenation of unsaturated fatty acids occurs during the ageing of seeds. Small amounts of oxygenated acids such as epoxy and conjugated dienol are widely distributed in seed oils as minor constituents. Spencer et al.¹ have reported that if these acids are present in low concentration then they can be formed after harvest, and may result from the action of lipoxygenase in the seeds. Epoxy acids have been reported in the seed oils from more than 60 species in 12 plant families.

Vernolic acid was the first acid of this class to be discovered.² Kleiman and coworkers³ have discovered a C₂₀ homologue (alchornic acid) of vernolic acid. With respect to the position of epoxide function, there are five 9,10-epoxy acids, three 12,13-epoxy acids and one 15,16-epoxy acid. Vernolic acid has been reported in *Mucuna pruriens*⁴, *Hibiscus cannabinus*⁵ and *Cepharia syriaca*⁶ seed oils. Recently, **cis**-3,4-epoxy-**cis**-11-octadecenoic and **cis**-
12,13-epoxyoctadec-trans-9-enoic acids have been characterized from the author's laboratory in the seed oil of Vernonia roxburghii and Mucuna pruriens respectively. Vernonia volkameriaefolia has been found to be a rich source of vernolic acid. The seed oil of Onopordium acanthium was analysed for the presence of mono(epoxy)octadecenoyl diacyltriacylglyceride which consisted of 63.1\% \alpha- and 36.9\% \beta-epoxytriacylglycerides. Qualitatively the compounds with epoxy group are revealed on the TLC plate by picric acid, whereas the quantitative determination is being made by \(\text{HBr-titration}\) at 3C. The IR, NMR and mass spectral techniques are very useful in determining the structure of epoxy acids. The GLC behaviour of the cis- and trans-epoxy esters is summarized by Gunstone et al.

In the early 1960's India used to be an exporter of oilseeds, but now with the growth in population large imports of edible oils and oilseeds are made for domestic consumption. There is a high potential of minor oilseeds which if properly tapped, can substantially augment the overall supply of vegetable oils and help in bridging the wide gap between supply and demand. The minor oilseeds of trees and herbaceous plants can contribute upto 2 million tonnes of vegetable oils and 12 million tonnes of deoiled meals.

Time to time a number of reviews dealing with natural fatty acids have been published. Pryde published an informative review on natural fatty acids.
methodology for lipid analysis have been noteworthy, particularly over the last decade. The useful and valuable methods in the oil analysis are thin layer chromatography (TLC), complexation chromatography, column chromatography, high performance liquid chromatography (HPLC), counter current distribution, gas liquid chromatography (GLC), chemical methods, urea and thiourea adduct separation and spectroscopic techniques.

Liquid column chromatography (LCC) for analytical uses has drawn a great deal of interest in the past few years. Preparative GLC has successfully been exploited in the isolation of pure fractions from a complex mixture in the recent years. HPLC is the latest milestone in the chain of chromatographic techniques. Fatty acids have been separated as methyl esters, phenyl esters and 2-naphthyl esters employing HPLC.

Likewise, many spectroscopic techniques (high resolution $^1$H NMR, $^{13}$C NMR, LCMS, LCMS and GC-MS) offer satisfactory solutions and sometimes unexpected advantages for the analysis of unknown fatty acids.

Apart from the above mentioned recent techniques, the purposeful chemical techniques generally used are: partial oxidation, oxidative degradation, hydroxylation, catalytic hydrogenation, partial hydrogenation, hydrogen bromide and Diels-Alder reactions. Recently alkylthiolation reaction has been employed to locate the position of double bond in fatty chain.
b. Analysis of Minor Seed Oils

During recent years the analysis of seed oils from an extensive sampling of the plant kingdom has revealed that a large number of species yield oils of unusual composition. Fatty acids are essential to human beings in a number of ways. Even the impact of low-cost petrochemicals did not reduce their uses. Fats and oils maintained an estimated 50% of surfactant, 40% of paint binder and 15% plasticizer markets, although petrochemicals took greater part of the markets. Several fatty acid uses have been introduced in the past but were not adopted by industry as less expensive petrochemical-based alternatives were available. The growing demand of seed oils for edible purpose and industrial use has stimulated research in the screening of oil-bearing seeds from wild plants. In continuation of 'Minor Seed Oil' screening program carried out in our laboratory, a study of six seed oils belonging to five families was undertaken in the hope of finding some seed oils of possible commercial value.

The petrol extracted oils from the seeds of six species have been analysed for their component acids, mainly by chromatographic and spectroscopic techniques. The UV and IR spectral analyses of seed oils showed no conjugation, trans-unsaturation or any unusual functional group except epoxy function in item 6. Various TLC techniques confirmed the presence of oxygenated acid (epoxy) in item 6. Silver ion TLC of the esters gave clear spots
corresponding to the saturated, monoene, diene and triene parallel to those from authentic linseed ester resolved alongside. The quantitative estimation of fatty acid components on a gas chromatogram was achieved by comparing retention times with those of lipid standards (Sigma, USA). The seed and oil characteristics are given in Table-1 along with the GLC analysis of the methyl esters of the oil.

**Carissa spinarum** (item 1, Vern. Karaunda; family-Apocynaceae) is a forest shrub in dry and rocky situations. Berries are eaten either as a cooked preserve or raw. GLC analysis indicated the total unsaturation (~53.0%), the monoene, oleic acid (51.40%) predominating amongst the unsaturated acids. Item 1 appeared to be an unusual seed oil as it contained common linoleic acid in fraction percentage and contained a higher amount of oleic acid.

**Vitex trifolia** (item 2, Vern. Nichinda) is a tree with 3-foliolate leaves from Verbenaceae family. It is found in Konkan, Deccan and Koromandal. Its leaves are of medicinal importance. This species was reported as a source of essential oil. Fatty acid analysis by GLC showed oleic 21.22, linoleic 23.34, linolenic 5.45 and docosenoic 31.03% acids. The total content of saturated acids was ~19.0%. A noticeable feature of this oil is the higher content of docosenoic acid. Members of this family earlier investigated had roughly equal proportions of oleic and linoleic acids, totalling 70.0-80.0%.
Tithonia diversifolia (item 3, branched glabrous annual) and Spilanthea acmella (item 4, Vern. Akarkara; Annual herb) are the species of family Compositae. Spilanthol obtained from S. acmella flowers showed strong local anaesthetic action. Flowers also produce nonreducing polysaccharide\(^{35}\). Seeds of the same species are chewed to produce salivation when mouth is dry. Crushed plants are used as fish poison\(^{35}\). Seed oils of item 3 and 4 contained the conventional mixture of fatty acids, usually associated with Compositae seed fats, of much linoleic (item 3, 57.0%\(^{18}\); item 4, 50.0%) and less oleic acid (item 3, 13.0%; item 4, 18.17%). A moderate concentration of palmitic acid (22.42%) was present in item 4. Item 3 (I.V. = 116.01) is the rich source of linoleic acid and categorised in the nondrying category along with item 4 (I.V. = 112.38). However, the low oil content restricts its commercial application.

Garcinia mangostana (item 5, Vern. Mangustan, family-Guttiferae) a native of the straits settlements. Cultivated as a garden escape in Madras. Dried fruits are used in dysentery and chronic diarrhoea\(^{36}\). Seeds of item 5 are highly rich in oil content (60.20%). The combined concentration of oleic-linoleic acids is abnormally high (93.35%). This oil somewhat resembles maize oil, which contains oleic and linoleic acids as major components. Further on the basis of iodine value (137.15) the oil may be classified as a semidrying oil and can be used in formulation of synthetic resins.\(^{18}\)
Acacia catechu* (Vern. Khair) is a moderate-sized tree from Leguminosae family. These trees are found in forests throughout N. Oudh, Bundelkhand and Punjab. A. catechu (item 6) seed oil responded to picric acid TLC\(^{11}\) indicating the presence of epoxy group. IR spectrum showed characteristic bands for epoxide at 840 and 826 cm\(^{-1}\). On the basis of IR, UV and TLC the possibility for the occurrence of oxygenated acids other than epoxy acid in the oil was ruled out. The epoxy content of the oil was found to be 10.21\% by HBr-titration\(^{12}\).

GLC of the methyl ester (Prepared by transesterification using 0.5N sodium methoxide) revealed that oleic (28.26\%) and linoleic (36.0\%) acids, amongst the unsaturated acids were found to be the prominent fatty acids. Palmitic acid (21.0\%) is the major saturated acid. GLC also indicated the presence of epoxy acid (10.30\%).

Characterization of epoxy acid was done by direct acetylation of oxygenated triglyceride followed by saponification and acidification. The dihydroxy acid isolated by column chromatography was equivalent to 10.30\% of weight of oil. The methyl ester of the dihydroxy acid derived from A. catechu showed a broad

*After the publication of this result\(^{36a}\), Chowdhury and coworkers\(^{37}\) reported the characteristic of Acacia catechu seeds and its oil. A slight variation in usual fatty acid composition is observed. Only epoxy acid in traces (0.10\%) was reported by these workers in this oil.
IR band at 3450 (hydroxy), 3010 (H-C=C-) and 1740 cm$^{-1}$ ($\text{COOCH}_3$). Its NMR displayed two structure-revealing multiplets at $\delta$ 3.59 (2H, 2xCH$_2$-OH) and 5.42 (2H, -CH=CH-). The dihydroxy acid (m.p. 53-54C) was identified as 12,13-dihydroxyoctadecenoic acid (lit.$^2$ m.p. 54-55C) by comparison with an authentic sample of three 12,13-dihydroxyoctadecenoic acid prepared from the seed oil of *Vernonia anthropimintica*. Confirmation of this assignment was furnished by hydrogenating the original dihydroxy acid. The IR, NMR spectra and the TLC retention characteristics of saturated and unsaturated dihydroxy acids on silica and silica-impregnated with boric acid resemble with those of saturated and unsaturated dihydroxy acids derived from *V. anthropimintica* seed oil.

Permanganate-periodate$^2$ cleavage has been employed to locate the positions of double bond and hydroxy groups in the fatty chain. The original dihydroxy acid (IIa) when oxidatively cleaved produces hexanoic (IV) and azelaic (VI) acids (Scheme-1) as identified by GLC. The saturated dihydroxy acid (III) afforded hexanoic (IV) and dodecanedioic (V) acids (Scheme-1). The cleavage pattern placed the double bond at C$_9$-C$_{10}$ position and the hydroxyls at C$_{12}$ and C$_{13}$ positions. Thus, the original component of the oil from which dihydroxy acid was obtained is *cis*-12,13-epoxyoleic (vernolic) acid.

In general the seed oil compositional data indicate that the oil of *G. mangostana* (item 5; 60.20% oil content) was characterized by the presence of 96.10% unsaturated acids comprising
mainly oleic and linoleic, which have potential for use as edible oil. An agronomic evaluation of this oil-rich species having a fatty acid profile of conventional edible oil may indicate its use as a prospective oil seed crop. Significant features of the other five species surveyed are the presence of oleic 51.40% (item 1), docosenoic 31.03% (item 2) and linoleic 36.0-57.0% (item 3, 4 and 6) acids as dominating components. Low oil content preclude their use as source of minor oil seeds.
### Table 1

Analytical data on seeds and oils

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Species and Family</th>
<th>Seed Analysis</th>
<th>Oil Properties</th>
<th>Methyl Ester Composition(%) by GLC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Oil content</td>
<td>Protein content (Y6.25)</td>
<td>Moisture (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>1.</td>
<td><em>Carissa spinarum</em> (Apocynaceae)</td>
<td>9.30</td>
<td>18.70</td>
<td>13.50</td>
</tr>
<tr>
<td>3.</td>
<td><em>Tithonia diversifolia</em> (Compositae)</td>
<td>19.60</td>
<td>21.00</td>
<td>8.70</td>
</tr>
<tr>
<td>4.</td>
<td><em>Spilanthe acmella</em> (Compositae)</td>
<td>7.70</td>
<td>22.00</td>
<td>3.30</td>
</tr>
<tr>
<td>5.</td>
<td><em>Garcinia mangostana</em> (Guttiferae)</td>
<td>60.20</td>
<td>25.10</td>
<td>12.20</td>
</tr>
<tr>
<td>6.</td>
<td><em>Acacia catechu</em> (Leguminosae)</td>
<td>5.70</td>
<td>27.30</td>
<td>9.50</td>
</tr>
</tbody>
</table>

Others - Item 2: 22:1 (31.03); Item 4: 14:0 (0.42); 16:1 (0.60); Item 6: 16:1 (0.90); 12,13-Epoxycoleic (10.30).

a. Iodine value; b. Saponification value; c. Refractive index.
Scheme 1

\[
\text{CH}_3-(\text{CH}_2)_4-\text{CH}-\text{CH}_2-\text{CH}==\text{CH}-(\text{CH}_2)_7\text{COOR}
\]

(I)

\[
\begin{align*}
1. & \text{CH}_3\text{COOH} \\
2. & \text{KOH} \\
3. & \text{H}^+
\end{align*}
\]

\[
\text{CH}_3-(\text{CH}_2)_4-\text{CH}-\text{CH}_2-\text{CH}==\text{CH}-(\text{CH}_2)_7\text{COOR}
\]

(II)

\[
\begin{align*}
&\text{H}_2 \\
&\text{Pd-C}
\end{align*}
\]

\[
\text{CH}_3-(\text{CH}_2)_4-\text{CH}-\text{(CH}_2)_{10}\text{COOH}
\]

(III)

\[
\begin{align*}
&\text{MnO}_4^-/\text{IO}_4^-
\end{align*}
\]

\[
\text{CH}_3-(\text{CH}_2)_4\text{COOH} + \text{HOOC-(CH}_2)_{10}\text{COOH}
\]

(IV) (V)

\[
\text{CH}_3-(\text{CH}_2)_4\text{COOH} + \text{HOOC-(CH}_2)_7\text{COOH}
\]

(IV) (VI)
EXPERIMENTAL PROCEDURES

Material and Methods

i) Sources of Oilseeds

The seed samples were collected and identified by staff botanists, under contract in various parts of the country or purchase from commercial seed suppliers.

ii) Extraction of Oil

Cleaned and dried samples of seeds were usually ground in a disintegrator. The powdered seeds were extracted exhaustively with petroleum ether (40-60°C) in a Soxhlet apparatus and the extracted oils were dried over anhydrous sodium sulfate (Na₂SO₄). The oils were neutralized by passing it (~1 g) in chloroform solution, through a short column of alumina (10 g). The analytical values of oils and seeds (Table-1) were determined according to the AOCS procedures.¹²

iii) Preparation of Mixed Fatty Acids

Seed oils were refluxed with ethanolic potassium hydroxide. The unsaponifiable material was removed by ether extraction and the free fatty acids were obtained by acidification of aqueous layer.
iv) **Methyl Esters**

Esterification was carried out as follows: Samples were refluxed for one hr in a large excess of absolute methanol containing 1% sulfuric acid (v/v). In each case, resulting mixtures were diluted to the cloud point with water, chilled in ice bath, and then extracted repeatedly with ether. Combined extracts were dried over Na$_2$SO$_4$ and evaporated in vacuo.

v) **Thin Layer Chromatography (TLC)**

Analytical TLC was performed on plates coated with 0.25 mm or 1 mm thick layer of silica gel or 20% silver nitrate-impregnated silica gel with 20 or 30% ether in hexane as developing solvent. The plates were rendered visual by spraying with a 20% aqueous solution of perchloric acid and heating in an oven (120°C) for 10 minutes. The dihydroxy ester was analysed on silica gel impregnated with boric acid and developed with hexane-ether-acetic acid (60:40:1; v/v/v). For reversed-phase TLC the dried coated plates were uniformly impregnated with silicone oil. Acetonitrile-acetic acid-water (70:10:20; v/v/v) were used as developing agent.

vi) **Gas Liquid Chromatography (GLC)**

The quantitative examination of methylesters was carried out by using a Perkin-Elmer Model-154 Vapor Fractometer equipped
with a thermal conductivity detector, using stainless steel packed column (2 m x 3 mm) coated with diethyleneglycol succinate (DEGS), 15% on chromosorb W, 45-60 mesh. The separations were carried out isothermally at 200°C, chart speed 0.76 m/hr with hydrogen flow 70 mL/min.

vii) Infrared (IR)

IR spectra were obtained on Perkin-Elmer Model-621 spectrophotometer as liquid film or as 1% solution in carbon tetrachloride.

viii) Ultraviolet (UV)

UV spectra of the oils were taken on Beckman DK-2A Ultraviolet spectrophotometer in methanol.

ix) Nuclear Magnetic Resonance (NMR)

NMR spectra were recorded on a Varian A60 spectrophotometer in CCl₄. The chemical shifts were reported in ppm (δ) relative to tetramethylsilane (TMS) as an internal standard. The abbreviations 's, t, m and br' denote singlet, triplet, multiplet and broad respectively.
x) **Characterization of Epoxy from A. catechu Seed Oil**

a) **Picric Acid TLC**

Picric acid TLC was carried out on silica gel plates (2.5 x 8.5 cm). The developing system was petroleum ether-ether-acetic acid (75:25:1; v/v/v). The plates, after developing, were sprayed with 0.5M picric acid in 95% ethanol and promptly placed in a jar saturated with the vapors of ether-ethanol (95%) (80:20; v/v). After half an hour the plates were removed and exposed to ammonia fumes for few minutes. The orange spot on a yellow background of the plate showed the presence of epoxy group.

b) **Hydrogen Bromide Titration**

The titration was carried out at 3C as discussed in section II.

c) **Transesterification**

The fatty acid methyl ester of epoxy triglyceride was prepared by sodium methoxide (0.5N) as detailed in section II. The resulted methyl ester was examined by TLC prior to GLC analysis employing *V. anthelmintica* esters as reference standard.

d) **Acetolysis of A. catechu Seed Oil (Scheme-1)**

Seed oil of *A. catechu* (500 mg) was boiled with glacial
acetic acid (10 mL) for 6 hr. The mixture was then saponified, acidified and diluted with water, extracted with ether, dried (Na$_2$SO$_4$). After the evaporation of the solvent, the crude dihydroxy acid (IIa) on crystallization from acetone and petroleum ether (3:1; v/v) gave a low-melting compound, m.p. 53-54°C, and afforded following analysis: Calcd. for dihydroxyoctadecenoic acid, C$_{18}$H$_{34}$O$_4$: C, 68.78; H, 10.82%; Found: C, 68.74; H, 10.79%. The threo-12,13-dihydroxyoctadecenoic acid (derived from _V_._anthelminthica_ seed oil) had m.p., 54-55°C. Cochromatography on TLC plate also produced a single spot. (IIb) IR(CCl$_4$): 3450 (hydroxy), 3010 (H-C=C-) and 1740 cm$^{-1}$ (COOCH$_3$). NMR(CCl$_4$): δ 5.42 m (2H, -CH=CH-), 3.37 s (3H, -COOCH$_3$), 3.59 m (2H, 2xCH-0H), 2.27 m (2H, -CH$_2$-COOCH$_3$), 2.08 m (4H, -CH$_2$-CH=CH-CH$_2$), 1.28 br,s (chain-CH$_2$-), 0.88 t (3H, terminal-CH$_3$), 4.0 br,s (2H, 2xCH-0H, D$_2$O exchangeable).

e) **Hydrogenation of Dihydroxy Acid (IIa) (Scheme-1)**

A 100 mg portion of IIa was hydrogenated in the presence of 10% Pd-C. The usual workup yielded 12,13-dihydroxyoctadecanoic acid (III) and crystallized from ethylacetate (m.p. 96-97°C). No depression of the m.p. was observed on admixture with an authentic sample prepared from vernolic acid. Analysis Calcd. for C$_{18}$H$_{36}$O$_4$: C, 68.35; H, 11.38%; Found: C, 68.32; H, 11.31%.

f) **Permanganate-Periodate Oxidation$^2$ of II and III (Scheme-1)**

A 100 mg portion of II and potassium carbonate (125 mg) were dissolved in t-butyl alcohol (30 mL). To this mixture a solu-
tion of potassium permanganate (1 mL of 0.057M) and sodium metaperiodate (170 mg) in 40 mL of water was added. The stirring of the reaction mixture was allowed at ambient temperature for 24 hr and then reduced with sodium metabisulfite, acidified with HCl, extracted with ether and methylated with ethereal diazomethane prior to GLC. GLC results of these esters gave hexanoic (IV) and azelaic (VI) acids as the cleaved products. Similarly, the cleavage of saturated diol (III) followed by esterification and GLC analysis showed hexanoic (IV) and dodecanedioic (VI) acids.

g) Melting Points

Melting points were taken on a Kofler apparatus and are uncorrected.
Section II

a. **HBr-Reacting Fatty Acids**

A number of naturally occurring fatty acids react quantitatively with hydrogen bromide (HBr) under the conditions prescribed by the American Oil Chemists' Society (AOCS) for the determination of oxirane oxygen. Other than epoxides which absorb approximately stoichiometric amounts of HBr are the \( \alpha \)-hydroxy conjugated diene grouping and a cyclopropene ring containing long chain fatty acids.

Seed lipids of botanical families of the order Malvales (Sterculiaceae, Bombaceae, Malvaceae and Tiliaceae) are known for their cyclopropenoid fatty acid (CPFA) constituents. In 1952 Nunn has reported the first cyclopropenoid acid, sterculic (SA; 9,10-methyleneoctadec-9-enoic) acid from the seed oil of *Sterculia foetida* (Sterculiaceae). Malvalic (MA; 8,9-methyleneheptadec-8-enoic) acid, a homologue of sterculic acid, was first characterized by MacFarlane et al. It is a component of cottonseed oil triglycerides. Usually these acids occur together. Presence of CPFA from six species of *Adansonia* was reported. *Abutilon pannosum* seed oil was also found to contain CPFA. Recently, Berry reported the occurrence of CPFA in *Sterculia monosperma*, *Gnetum gnemon* and *Durio zibethinus* seed oils.

Raju and Reiser reported evidence for CPFA shorter than MA by GLC retention time. Johnson et al. indicated the presence
of \text{C}_{17}\text{-cyclopropene} in certain \text{Malva} species. A \text{C}_{12}\text{-cyclopropenoid} acid was identified in the \text{Euphoria longans}^{51} seed oil. These cyclic acids occur with small amount of their dihydromerivatives in the seed oil of \text{Pachira aquatica}^{52}, \text{P. insignis}^{53} and \text{Bombacopsis glabra}^{53}. Sterculynic (8,9-methyleneoctadec-8-ene-17-ynoic) acid was reported from \text{Sterculia alata}^{54} while Badami \text{et al.}^{55} have highlighted only the presence of MA and SA. In many seed oils CPFA may occur with epoxy acids. Bohannon \text{et al.}^{52} reported the presence of CPFA along with epoxy acid in the \text{Pterygote alata} seed oil.

\text{Pavonia sepium}^{56} seed oil is unusual in being the first member of the Malvaceae family in which SA content was found to be greater than of MA. In the seed oil of \text{Urena lobata} and \text{Sida rhombifolia} of the family Malvaceae, Ahmad \text{et al.}^{4,57} observed similar pattern (SA>MA). \text{Pterospermum acerifolium}^{52} and \text{Eriolaena hookeriana}^{58} seed oils contain more malvalic than sterculic acid.

A fair number of seed oils have been analysed for CPFA in the author's laboratory\textsuperscript{4,57-66}.

SA is reported to have greater physiological activity than \text{MA}\textsuperscript{67}. It is well established that CPFA exert toxic and other adverse effects in a variety of animals\textsuperscript{42,68}. Many physiological disorders in animals, including altered egg production and fertility in chickens, delayed sexual maturity and slow growth in rats are due to cyclopropene ring\textsuperscript{42,69}. CPFA were first reported to cocarcinogenic\textsuperscript{70,71} and then demonstrated to be carcinogenic\textsuperscript{72,73}. 
Recently, it was reported that cyclopropene fatty acids decreased the levels of microsomal cytochrome P-450. Very recently, these effects and their underlying cause have been reviewed.

**Characterization and Quantitation of CPFA**

CPFA quantitation methods have been found to be less than adequate and have resulted in a long search for a technique with acceptable reliability and precision especially at the levels of low range found in materials taken in human food.

It is well known that the CPFA containing seed oils responded to Halphen test. HBr-titration deals a quantitative reaction of CPFA with HBr. Zeringue et al. employed a potentiometric titration for HBr-titration. It was reported that the participation of CPFA in the Tortelli-Jaffe reaction with bromine produced blue color.

There are two basic GLC techniques used in the CPFA quantitation: (a) direct GLC of CPFA and (b) GLC of CPFA derivatives.

Recourt and coworkers used a direct GLC technique for the methyl ester analysis of CPFA containing seed oils and reported that CPFA tend to decompose and isomerize as they travel through GLC column. Recently, Fisher et al. reported a method for the analysis of CPFA with a glass column packed with a methylsilicone substrate on an inner support and concluded that methyl
sterculate and methyl malvalate can be chromatographed without decomposition. GLC of derivatized products of CPFA have been proposed as primary methods. Coleman had concluded that the technique adopted by Raju et al. was unsatisfactory and that the method of Schneider et al. was satisfactory at high level of CPFA. The CPFA contents are suitably modified chemically by hydrogenation or by reaction with mercaptans or silver nitrate in methanol and nonhydroxy solvents (such as acetonitrile or acetone etc.).

The following derivatives formed by treatment of AgNO₃

\[ R''OH \]

\[ R - C = C - R' + AgNO₃ \xrightarrow{R''OH} \]

IR spectra of CPFA illustrated two distinctive prominent bands at 1008-1010 and 1852 cm⁻¹. Measurement of the band at 1008-1010 cm⁻¹ has been suggested as a means of estimating the total content of cyclopropene ring. In NMR cyclopropene methylene protons were observed at δ 0.80 (singlet). NMR is used as a rapid and quantitative method of detecting cyclopropene ring in lipids. Very recently, cyclopropenoid fatty acid methyl esters have been analysed by HPLC.
The silver nitrate derivatives can be successfully used for GC-MS analysis\textsuperscript{45,58}. From the author's laboratory GC-MS of AgNO$_3$-treated methyl esters of \textit{E. hookeriana}\textsuperscript{58} and \textit{Sterculia colorata}\textsuperscript{98} have been reported. The utility of this reaction is that unsaturated components present initially remain unaffected and can be isolated and determined separately.
b. **Cyclopropenoid Fatty Acids in Seed Oil of *Gnetum scandens* (Gnetaceae)**

Recently, cyclopropenoid fatty acids have been a subject of much investigations due to their carcinogenic \(^72,73\), cocarcinogenic \(^70,71\) and biological effects on animals \(^99,100\). As a part of screening program aimed at the search of biologically active cyclopropene acids in minor seed oils, it was found that CPFA is detectable in *G. scandens* (Gnetaceae) seed oil. Gnetaceae seed fats are rarely known for CPFA content.

*G. scandens* (Vern. Umbli) is a plant used for fish poisoning. Seeds are usually one, oval and brownish hard coated. The plants are distributed throughout Eastern tropical Himalayas and Bihar \(^101\).

In the book 'The Chemical Constitution of Natural Fats', Hilditch \(^102\) reported that *G. scandens* contained only usual fatty acids. The reinvestigation of *G. scandens* seed oil revealed that it contained a high amount of CPFA easily detectable by Halphen test \(^77\). The IR spectrum of the crude oil showed a sharp band at 1010 and weak band at 1852 cm\(^{-1}\). NMR showed cyclopropene methylene protons as a singlet at \(\delta 0.72\) confirmed the cyclopropene ring. UV maxima in the range 228-315 nm (conjugated unsaturation) was not observed. Seed properties and oil characteristics were determined by the standard AOCS procedures \(^12\) and the data are summarized in Table-2. HBr-titration of oil at 55C, indicated the presence of
39.80% of HBr-reacting cyclopropene ring content. Direct TLC revealed the occurrence of only nonoxygenated esters in the seed oil of *G. scandens*. Argentation TLC of methyl esters showed spots of saturated, monoene, diene and triene parallel to those obtained from *S. foetida* esters resolved alongside. An additional spot trailing just behind the spot of saturates was observed which was presumed to be due to the presence of CPFA in the seed oil. Clear spots of usual critical pairs and an additional spot corresponded to cyclopropene esters, as did the *S. foetida* esters, were observed on a siliconised plate.

**Table-2**

Analytical data of *G. scandens* seed oil

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Seeds</strong></td>
<td></td>
</tr>
<tr>
<td>Oil content (%)</td>
<td>15.70</td>
</tr>
<tr>
<td>Protein content Nx6.25 (%)</td>
<td>14.30</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>6.20</td>
</tr>
<tr>
<td><strong>Seed Oil</strong></td>
<td></td>
</tr>
<tr>
<td>Iodine value (Wijs)</td>
<td>85.93</td>
</tr>
<tr>
<td>Saponification value</td>
<td>191.51</td>
</tr>
<tr>
<td>Refractive index, n°</td>
<td>1.4769</td>
</tr>
<tr>
<td>Halphen test</td>
<td>Positive</td>
</tr>
<tr>
<td>HBr-equivalent</td>
<td>39.80</td>
</tr>
</tbody>
</table>
The GLC of methyl esters was done after treatment with silver nitrate-methanol to form stable derivatives of CPFA, following the technique of Schneider et al. The GLC chromatogram clearly established the presence of malvalic and sterculic acids in *G. scandens* seed oil by a comparison of the relative retention times of the derivatives of *S. foetida* esters. The GLC data (Table-3) of the cyclopropenoid acid esters were found close to that obtained by HBr-titration.

Table-3

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>G. scandens</th>
<th>S. foetida</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic (14:0)</td>
<td>0.35</td>
<td>-</td>
</tr>
<tr>
<td>Palmitic (16:0)</td>
<td>15.51</td>
<td>26.00</td>
</tr>
<tr>
<td>Palmitoleic (16:1)</td>
<td>-</td>
<td>1.00</td>
</tr>
<tr>
<td>Stearic (18:0)</td>
<td>10.20</td>
<td>3.40</td>
</tr>
<tr>
<td>Oleic (18:1)</td>
<td>16.22</td>
<td>9.40</td>
</tr>
<tr>
<td>Linoleic (18:2)</td>
<td>15.00</td>
<td>1.30</td>
</tr>
<tr>
<td>Linolenic (18:3)</td>
<td>2.85</td>
<td>0.60</td>
</tr>
<tr>
<td>Malvalic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ether deriv.)</td>
<td>10.36 (+ 11.27)</td>
<td>6.50 (+ 7.10)</td>
</tr>
<tr>
<td>(ketone deriv.)</td>
<td>0.91</td>
<td>0.60</td>
</tr>
<tr>
<td>Sterculic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ether deriv.)</td>
<td>27.25 (+ 28.57)</td>
<td>48.80 (+ 51.20)</td>
</tr>
<tr>
<td>(ketone deriv.)</td>
<td>1.32</td>
<td>2.40</td>
</tr>
</tbody>
</table>

*Standard reference of malvalic and sterculic acids.
Though sterculic acid is always present with malvalic acid, but its very high content is previously reported only in few cases. The physiological activity of SA is reported to be greater than that of MA. Therefore the oil of G. scandens (SA 28.57\%) may be of paramount importance.
EXPERIMENTAL PROCEDURES

i) General Methods

Spectroscopic and chromatographic analyses of oil and esters were carried out in the same way as detailed in the section of analyses of 'Minor Seed Oils'.

ii) Seeds and Oil Characteristics

The analytical values of the oil and seeds were determined according to the procedures recommended by the AOCS\textsuperscript{12} and data are summarised in Table-2.

iii) Halphen Color Reaction\textsuperscript{77}

A solution of sulfur (1\% in CS\textsubscript{2}) was prepared for the Halphen test. Oil (1 mL) was taken in amyl alcohol (1 mL) and mixed with 1 mL of the Halphen reagent. The reaction mixture was then heated on water bath for few minutes till CS\textsubscript{2} had boiled off. On keeping the test tube in an oil bath (110-115\degree C) for 1-2 hr, a red color, characteristic of CPFA, developed.

iv) Hydrogen Bromide Titration\textsuperscript{12,82}

The titration of a weighed amount of oil was carried out with 0.1N hydrogen bromide using crystal violet as an indicator at
55°C to a bluish-green end point, that persists for 30 seconds, permits the quantitation of the cyclopropene. The result was expressed as hydrogen bromide equivalent (HBE). The percentage of CPFA content was calculated by the empirical equation:

\[
\% \text{CPFA} = \frac{29.45 \times N \times V}{\text{weight of the sample}}
\]

where \( N \) = normality; \( V \) = volume of HBr consumed in titration.

v) Preparation of Methyl Ester

The fatty acid methyl esters were prepared by transesterification of triglycerides (1 g) of cyclopropene containing oils in 50 mL of absolute methanol that contained sodium methoxide (0.5N). The reaction was refluxed for 20 minutes, and kept at room temperature for 4 hr. After acidification with acetic acid the methyl esters were extracted with ether as usual and quantitatively examined by TLC prior to GLC analysis, using \( S. \) foetida esters as reference standard.

vi) Characterization of CPFA from \( G. \) scandens Seed Oil

A 200 mg of methyl esters of \( G. \) scandens seed oil were treated with 60 mL of absolute methanol saturated with silver nitrate. The reaction was allowed to proceed with 24 hr shaking at room temperature. The reaction products from cyclopropenes
along with normal esters were recovered from the reaction mixture by adding 100 mL of distilled water and repeated extraction with ether, dried (Na$_2$SO$_4$) and evaporated in a stream of nitrogen.

Methyl esters (freshly prepared) of S. footida oil were also treated with silver nitrate-methanol as detailed above. The total methyl esters containing malvalate and sterculate derivatives were employed as reference standard for GLC analysis. Its GLC data have been compared with that of G. scandens in Table-3.
Section III

a. Hydroxy Fatty Acids

Hydroxy function-bearing fatty acids are widely distributed both in animal and vegetable fats. Downing\textsuperscript{103} and Markley\textsuperscript{104} have reviewed these naturally occurring hydroxy fatty acids. Castor oil of the family Euphorbiaceae is the only hydroxylated vegetable oil available at present as commercial commodity [(D)-(+)\textsuperscript{106,107} hydroxy-cis-9-octadecenoic] acid\textsuperscript{105} (90%). Author's laboratory has reported ricinoleic acid in seed oils of \textit{Phyllanthus niruri} (Euphorbiaceae) and \textit{Hiptage benghalensis} (Malpighiaceae). \textit{Strophanthus} (9-hydroxy-cis-12-octadecenoic) acid, an isomer of ricinoleic acid was first reported by Gunstone\textsuperscript{108} in the seed oil of \textit{Strophanthus sarmentosus} (Apocynaceae). The γ-hydroxy olefinic acid, named as isoricinoleic (Strophanthus) acid was present in high amount in the seed oil of \textit{Holarrhena antidysenterica}\textsuperscript{109}. From author's laboratory it was reported that γ-hydroxy olefinic acid is a dominating component of the fatty acids present in the seed oils of \textit{Wrightia tomentosa}, \textit{W. tinctoria}\textsuperscript{110} and \textit{W. coccinea}\textsuperscript{111} (Apocynaceae).

Smith \textit{et al.}\textsuperscript{112} reported 'Lesquerolic' [(+)-14-hydroxy-cis-11-eicosenoic] acid, a higher homologue of ricinoleic acid from the seed oil of the genus \textit{Lesquerella} (Cruciferae). A new fatty acid, 16-hydroxy-cis-13-docosenoic acid along with lesquerolic acid was found in the seed oil of \textit{Heliophila amplexicaulis} by
Plattner et al.\textsuperscript{113} From author's laboratory isolation and characterization of a new $\beta$-hydroxy olefinic acid, 9-hydroxy-\textit{cis}-11-octadecenoic acid from \textit{Plantago major} seed oil\textsuperscript{114} was reported.

A nonconjugated hydroxy diene, densipolic (12-hydroxy-\textit{cis}-9, \textit{cis}-15-octadecadienoic) acid has been discovered in the species of \textit{Lesquerella densipila}\textsuperscript{115}. Binder et al.\textsuperscript{116} have found a couple of monohydroxy acids from \textit{L. densipila} seed oil.

\textit{L. auriculata} (Cruciferae) seed oil possesses nonconjugated hydroxy diene acid, reported by Kleiman et al.\textsuperscript{117} Smith and Wolff\textsuperscript{118} have discovered a very unusual hydroxy triene acid, $\alpha$-hydroxylinolenic (2-hydroxy-\textit{cis}-9, \textit{cis}-12, \textit{cis}-15-octadecatrienoic) acid in \textit{Thymus vulgaris} (Labiatae) seed oil. Isolation and identification of $\alpha$-hydroxyoleic, $\alpha$-hydroxylinolenic and $\alpha$-hydroxylinoleic acids were carried out by Bohannon and Kleiman\textsuperscript{119} from the \textit{Salvia nilotica} (Labiatae) seed oil. A new nonvicinal diol (9,14-dihydroxyoctadecanoic) acid has been reported in the seed oil of \textit{Peganum harmala} (Rutaceae) from our laboratory\textsuperscript{120}.

A number of acetylenic hydroxy fatty acids were reported from various families i.e. Olacaceae\textsuperscript{121,122}, Compositae\textsuperscript{123}, Santalaceae\textsuperscript{124}. Keogh and Zurita\textsuperscript{125} isolated and characterized a very unusual fatty acid as a $\alpha$-(15-hydroxyhexadecyl) itaconic acid from the seed oil of \textit{Usnea aliphatica}. Recently, Keogh and Duran reported a new hydroxy fatty acid, 3,4-dicarboxy-3-hydroxy-19-oxoeicosanoic acid in \textit{U. meridensis} seed oil. It appears that hydroxy acetylenic fatty acids are nearly as numerous as hydroxy olefinic
acids in seed oils. These acids are logical key intermediates in the biogenetic conversion of linoleic to conjugated triconic acids.

Usual chromatographic and spectral methods were used for the isolation and characterization of hydroxy fatty acids. Eagles et al.\textsuperscript{127} and Siddiqi\textsuperscript{128} successfully studied MS of hydroxy fatty acids using 'remote' group derivatization method for the location of OH group and unsaturation.
Mirabilis jalapa (Nyctaginaceae) is generally found cultivated in gardens over the greater part of India. Dried root is nutrient and is mild purgative. Fresh leaf juice cures wounds. Previous work on M. jalapa seed oil\textsuperscript{129} reported the oil and protein content with HBr-reacting acids (0.30\%). In continuation of our previous work on unusual hydroxy acid-containing seed oils\textsuperscript{106,114,120,130}, it was found that the oil of M. jalapa, not previously examined for fatty acid composition, contained an oxygenated acid, 8-hydroxyoctadeca-cis-11,14-dienoic acid (4.30\%). The UV spectrum of the oil, as well as that of its methyl ester, showed the absence of conjugation. Seed properties and oil characteristics were determined by standard AOCS methods\textsuperscript{12} and the values are given in Table-4. IR spectrum of the oil and its methyl esters

\textbf{Table-4}

Analytical data of M. jalapa seed oil

<table>
<thead>
<tr>
<th>Seeds</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil content (%)</td>
<td>9.80</td>
</tr>
<tr>
<td>Protein content, N\times 6.25 (%)</td>
<td>36.80</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>3.20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Seed Oil</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine value (\text{\text{Wijs}})</td>
<td>98.20</td>
</tr>
<tr>
<td>Saponification value</td>
<td>187.60</td>
</tr>
<tr>
<td>Refractive index, n\textsubscript{D}</td>
<td>1.4679</td>
</tr>
</tbody>
</table>
showed an OH band at 3360 cm\(^{-1}\). TLC of the ester also revealed the presence of a more polar component. The R\(_f\) approximated to that expected for an unsaturated monohydroxy ester. A concentrate of the hydroxy ester was obtained by preparative TLC. This was further purified by column chromatography which yielded a brown viscous oil (VIIb). Since there was no absorption in IR at 967 cm\(^{-1}\), all double bonds must be cis. NMR of its ester gave peaks at \(\delta\) 5.39 m (4H, 2x-CH=CH\(_2\)), 3.61 s (3H, -COOCH\(_3\)), 3.38 m (2H, -CH=OH, D\(_2\)O exchange reduced the peak to its half), 2.72 t (2H, =CH-CH\(_2\)-CH=, J=7 Hz), 2.22 m (6H, allylic to double bond and \(\alpha\) to ester grouping), 1.35 br, s (chain-CH\(_2\)-) and 0.92 t (3H, terminal-CH\(_3\), J=6 Hz). Elemental analysis corresponded to the molecular formula C\(_{19}\)H\(_{34}\)O\(_3\), suggesting a monohydroxy acid with two double bonds. The ester (VIIb) on acetylation yielded a product whose IR spectrum showed strong bands at 1735 br (COOCH\(_3\) and OCOCH\(_3\)), 1230 and 1025 cm\(^{-1}\) (acetate). Its NMR showed no unusual features apart from the expected but significant signals at \(\delta\) 2.02 s (3H, -OCOCH\(_3\)) and 4.80 m (1H, -CH-OCOCH\(_3\)). The signal at \(\delta\) 3.38 was not observed. The appearance of peak at \(\delta\) 0.92 in NMR spectrum as a clear triplet for the terminal methyl protons with a J Value of 6 Hz suggested that one double bond is present at C\(_{14}\). Hopkins\(^{131}\) reported a similar effect in hexadec-cis-12-enoic acid. This was further confirmed by the observation of Frost et al.\(^{132}\), who have reported above NMR data for the methyl cis-11,14-octadecadienoate. Since the two double bonds were methylene interrupted, therefore, the other double bond was located at C\(_{11}\) position. The methine proton
attached to OH function appeared at δ 3.38, thereby showing that the location of OH group was neither at C_{10} nor C_{2} position. If it would have, then the methine proton must be more downfield and would be only a four proton signal at δ 2.22. The structure of hydroxy (VIIa) was further substantiated by the MS of its methyl ester (VIIb). It gave molecular ion peak at m/z 310 (C_{19}H_{34}O_{3}, M^+), along with other salient ion peaks at 311 (M+1, 2), 309 (M-1, 2), 308 (M-2, 3), 281 (a, 1), 279 (M-OCH_{3}, 19), 262 (279-OH, 3), 261 (279-H_{2}O, 4), 187 (f, 11), 185 (187-2H, 14), 167 (g, 55), 155 (f-CH_{3}OH, 22), 149 (g-H_{2}O, 97), 143 (h, 7), 123 (e, 12), 113 (f-C_{3}H_{6}O_{2}, 13), 109 (d, 15), 83 (c, 46), 69 (b, 49) and 55 (principal ion). Some of the assignments were supported by accurate mass measurements.

The peaks at m/z 281 (a), although very weak, is significant as it resulted from allylic cleavage and suggested a \( \triangle^{14} \) double bond. The cleavage between C_{7} and C_{8} produced the mass ion 167 (g) which was the third most intense peak and helped to locate the OH function at C_{8}. The cleavage between C_{8} and C_{9} should give a peak at 173 but it was not observed. Instead of this, it gave a peak at 187 (f) which proved the cleavage between C_{9} and C_{10} and exclusively supported the presence of another \( \triangle^{11} \) double bond.
bond. It is also pertinent to mention here that the absence of peak at 157 and the existence of a peak at 143 (\text{cm}^{-1}) clearly demonstrated the location of the OH group at C_8.

A few reactions were carried out to establish the structure of the hydroxy ester (Scheme-2). Catalytic hydrogenation of VIIb gave a solid compound methyl 8-hydroxystearate (VIIIb) m.p. 55.4°C (lit.\textsuperscript{133} m.p. 55.5°C), which analysed for C_{19}H_{38}O_{3} and had IR band at 3440 cm\textsuperscript{-1} (OH). Jones' oxidation of VIIb furnished methyl 8-oxostearate (IXb), m.p. 44.8°C (lit.\textsuperscript{134} m.p. 44.3-45.1°C). Reductive removal of hydroxy group in VIIb by hydrogen iodide-phosphorus furnished Xb, which was identified as methyloctadecanoate by GLC. This confirmed a normal C_{18} chain length for VIIa. Oxidation of VIIb by permanganate in acetic acid gave a mixture of monobasic (XIa and XIIa) and dibasic (XIIIa and XIVa) acids. The identified fragments were decanoic, undecanoic, heptanedioic and octanedioic esters. Therefore, the hydroxy group was present at C_8 in a normal C_{18} skeleton. Oxidation of the VIIa by permanganate-periodate cleavage\textsuperscript{135} and methylation of the resulting products with CH\textsubscript{2}N\textsubscript{2} gave material with a strong IR absorption at 1775 cm\textsuperscript{-1} characteristic of \(\gamma\)-lactone. GLC analysis at 65°C showed only monobasic, methylbutyrate (XVb). The formation of \(\gamma\)-lactone itself indicated that the double bond and hydroxy group were separated by two methylene groups. The formation of XVb confirmed that the double bond was present between C_{14} and C_{15}. 
GLC analysis of the TMS methyl esters on silicone and polyester columns showed fatty acid composition of the seed oil of *Mirabilis jalapa* (Table-5).

**Table-5**

Fatty acid composition of TMS methyl esters derivative (%) of *M. jalapa* seed oil.

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>% Wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic (14:0)</td>
<td>0.70</td>
</tr>
<tr>
<td>Palmitic (16:0)</td>
<td>22.10</td>
</tr>
<tr>
<td>Stearic (18:0)</td>
<td>0.30</td>
</tr>
<tr>
<td>Oleic (18:1)</td>
<td>54.30</td>
</tr>
<tr>
<td>Linoleic (18:2)</td>
<td>11.10</td>
</tr>
<tr>
<td>Linolenic (18:3)</td>
<td>7.20</td>
</tr>
<tr>
<td>8-Hydroxyoctadeca-cis-11,14-dienoic</td>
<td>4.30</td>
</tr>
</tbody>
</table>
SCHEME 2

\[ \text{CH}_3(\text{CH}_2)_{16} - \text{COOR} \]

(XIIa, b)

\[ \text{CH}_3(\text{CH}_2)_{8} - \text{COOR} + \text{CH}_3(\text{CH}_2)_{9} - \text{COOR} \]

1. HI/P
2. Zn/HCl

\[ \text{K}_{2}\text{MnO}_4 \]

\[ \text{CH}_3(\text{CH}_2)_{9} - \text{CH} - (\text{CH}_2)_{6} - \text{COOR} \]

OH

\[ \text{Jones' Oxidation} \]

\[ \text{CH}_3(\text{CH}_2)_{9} - \text{C} - (\text{CH}_2)_{6} - \text{COOR} \]

(VIIIb)

\[ \text{H}_2 - \text{Pd} \]

\[ \text{ROOC} - (\text{CH}_2)_{5} - \text{COOR} + \text{ROOC} - (\text{CH}_2)_{6} - \text{COOR} \]

(XIIIa, b)

(XIVa, b)

\[ \text{Cl}_3 - (\text{CH}_2)_{2} - \text{CH} = \text{CH} - \text{CH} = \text{CH} - (\text{CH}_2)_{2} - \text{CH} - (\text{CH}_2)_{6} - \text{COOR} \]

(VIIa, b)

\[ \text{K}_{2}\text{MnO}_4 / \text{IO}_4^- \]

\[ \text{CH}_3 - (\text{CH}_2)_{2} - \text{COOR} \]

(XVa, b)

a; \( R = \text{H} \)

b; \( R = \text{CH}_3 \)

\[ + \]

\[ \text{O} \]

\[ \text{C} \]

\[ \text{CH} - (\text{CH}_2)_{6} - \text{COOR} \]

(XVIa, b)
EXPERIMENTAL PROCEDURES

General Methods

Spectroscopic and chromatographic analyses of oil and ester were conducted in the same way as given in the section of analyses of 'Minor Seed Oils'. MS (JEOL JMS-D300) was measured using the direct insertion probe at a source temperature of 140°C and an ionization energy of 75-eV.

Isolation of Hydroxy Ester from Mirabilis jalapa Seed Oil

Petroleum ether (40-60°C) extracted seed oil of M. jalapa seeds was subjected to mild saponification with ethanolic KOH at room temperature for 22 hr. After usual workup, the mixed fatty acids were subjected to methylation (acid-catalysed esterification with MeOH). TLC of the ester gave two spots with petroleum ether-ether (7:3; v/v). The ester was concentrated by preparative TLC (petroleum ether-ether, 4:1; v/v) and the concentrate, further purified by column chromatography using petroleum ether-ether (18:2; v/v), yielded a TLC homogenous product VIIb, as an oil. Analysis—(Found: C, 73.48; H, 10.98%; Calcd. for C_{19}H_{34}O_3: C, 73.50; H, 11.04%). IR(CCl_4): 3360 (OH) and 1738 cm^{-1} (COOCH_3). The compound (VIIb; 40 mg) when heated under reflux with 0.8N KOH in alcohol (∼1 hr), followed by usual workup, afforded the hydroxy acid (VIIa) as an oil (32 mg). IR(neat): 3420–3200 (OH and COOH) and 1710 cm^{-1} (COOH).
Characterization of Hydroxy Ester (VIIb)

Acetylation of VIIb (63 mg) with Ac₂O-pyridine gave a product which showed strong bands at 1735 (COOCH₃, OCOCH₃), 1230 and 1025 cm⁻¹ (acetate). A portion of VIIb (180 mg) was hydrogenated, using 10% Pd-C in EtOAc (2 mL) to give methyl 8-hydroxy-stearate (VIIIb), as a white solid (141 mg) m.p. 55.4°C (lit. 133 m.p. 55.5°C). Analysis—(Found: C, 72.53; H, 12.12%; Calcd. for C₁₉H₃₈O₃: C, 72.56; H, 12.18%). IR(nujol): 3440 (OH), 1735 cm⁻¹ (COOCH₃). Jones' oxidation of VIIib (20 mg) furnished methyl 8-oxo-stearate (IXb), m.p. 44.8°C (lit. 134, m.p. 44.3-45.1°C).

Reductive Removal of OH Group (VIIib) 136

Methyl 8-hydroxystearate (VIIIb, 40 mg) was refluxed for 17 hr with red P (18 mg) and HI (1.2 mL). Ether extraction of the diluted mixture followed by washing with 5% NaHSO₃ and water gave an oily product (37 mg). This was reduced by refluxing for 4 hr with granular Zn (95 mg), methanol (2.4 mL) and HCl (0.46 mL). Usual workup of reaction mixture produced 14 mg of semisolid ester (Xb). GLC analysis with the authentic sample illustrated the methyl stearate.

Position of the OH Group in VIIib

Oxidative degradation 137 of VIIib (100 mg) with KMnO₄ in acetic acid gave a mixture of monobasic (XΙa and XΙΙa) and dibasic
(XIIIa and XIVa) acids. After methylation with \( \text{CH}_2\text{N}_2 \) these were examined by GLC which indicated the presence of methyl decanoate, methyl undecanoate, methyl heptanedioate and methyl octanedioate.

**Position of Double Bond in VIIa**

VIIa (130 mg), \( \text{K}_2\text{CO}_3 \) (150 mg) and \( \text{t-BuOH} \) (40 mL) were treated with a solution of \( \text{NaIC}_4 \) (450 mg) in 40 mL \( \text{H}_2\text{O} \) and \( \text{KMnO}_4 \) (1.3 mL of 0.057M solution). The mixture was stirred at room temperature for 24 hr reduced with \( \text{NaHSO}_3 \), acidified with HCl and extracted with ether. The ether solution after usual workup gave a semisolid product which was treated with \( \text{CH}_2\text{N}_2\text{-Et}_2\text{O} \) solution and then subjected to GLC. GLC analysis showed one component as methyl butyrate (XVb). The IR of the mixture showed a strong band at 1775 cm\(^{-1}\) which confirmed a \( \gamma \)-lactone (XVIb).
REFERENCES


77. Halphen, G., J. Pharm. 6:390 (1897); J. Chem. Soc. 74:358 (1898).


Part II

Derivatization of Fatty Acids
During the last 30 years, there has been a tremendous resurgence in the interest and research effort directed to the study of the chemistry of fatty acids and their derivatives. Fatty acids usually undergo classical reactions of organic chemistry. Heterocyclic derivatives of fatty acids are few in number but possess biological activity.

Occurrence of heterocyclic compounds in nature as pigments, alkaloids and vitamins is widely known. The current stage in organic chemistry development and closely allied branches of biology is characterized by extensive investigation of physiologically active compounds encountered in the plant and animal world. The search for these active constituents which control the biological process of various systems has acted as a powerful stimulus to the further development of the chemistry of the heterocyclic compounds. Heterocyclic ring-possessing long chain fatty compounds are a nature's rarity.

Keeping in view the importance of heterocyclic compounds, the tetrazole, oxathiolane, thiazinone, thiazolidinedione and thiazole ring-containing fatty acids were prepared. Besides these the preparation of amide, thioethers, vinyl acetate, α-acetate, nitrohydrin and nitronitrate derivatives were also undertaken.
Section-I

Synthesis of Starting Materials

Synthesis of trans-2-Enoic Acids (Va, VIA)

Palmitic (I) and stearic (II) acids afford the corresponding trans-2-enoic acids\(^1,2\) (Scheme-1). The structures of trans-2-enoic acids (Va, VIA) were determined by elemental and spectral analyses of their methyl esters (Vb, VIA). IR spectra of the compounds Vb, VIA showed diagnostic absorption bands at...
1730 (COOCH$_3$), 1645 (–CH=CH–) and 970 cm$^{-1}$ (trans-olefin). The NMR spectra illustrated a doublet of doublet centred at δ 6.89 (J=15 and 5 Hz) ascribable to a proton β to ester carbonyl, a doublet at 6.0 (1H, J=15 Hz with small long range coupling, trans-olefinic proton) attributed to a proton α to ester carbonyl, 2.43 m (2H, –CH$_2$–CH=CH–) and normal signals as observed in fatty acid esters. Double bond configuration is trans as indicated by coupling constant.

**Synthesis of 2-Oxooctadecanoic Acid (Xa)**

Jones' oxidation of 2-hydroxyoctadecanoic acid (X) afforded 2-oxooctadecanoic acid (Xa) (Scheme-2).

**Scheme-2**

CH$_3$–(CH$_2$)$_{15}$–CH–COOH

OH

(X)

↑

Jones' oxidation

CH$_3$–(CH$_2$)$_{15}$–C–COOH

O

(Xa)

The structure of 2-oxooctadecanoic acid (Xa) was established by its elemental analysis (C$_{18}$H$_{34}$O$_3$) and IR, NMR spectro-
scopy. IR spectrum displayed a broad band at 3425 (COOH) and two bands in the carbonyl region at 1710 (COOH) and 1720 cm$^{-1}$ (CO). NMR spectrum showed usual signals along with a multiplet at δ 2.31 for methylene protons α to carbonyl group and a D$_2$O exchangeable broad singlet at δ 8.50 for an acid proton.

Synthesis of Methyl 4-oxo-trans-2-enoates (XIa,XIb)

The allylic oxidation$^3$ of methyl trans-2-hexa- (Vb) and -octadecenoate (VIb) with chromium trioxide in acetic acid and acetic anhydride afforded methyl 4-oxo-trans-2-hexadecenoate (XIa) and methyl 4-oxo-trans-2-octadecenoate (XIb) respectively (Scheme-3).

Scheme-3

```
R-CH$_2$CH=CH-COOC$_3$H$_3$
(Vb,VIb)

CrO$_3$, Ac$_2$O-AcOH

R-C-CH=CH-COOCH$_3$

XIa: R = CH$_3$(CH$_2$)$_{11}$
XIb: R = CH$_3$(CH$_2$)$_{13}$

(XIa,XIb)
```

Compounds XIa and XIb analysed for $C_{17}H_{30}O_3$ and $C_{19}H_{34}O_3$, respectively and gave positive DNP test. Ultraviolet
(UV) spectra (λ_{\text{max}} , 220 nm) suggested these to have S-trans, S-trans-configuration. IR spectra displayed characteristic bands at 1735 (COOCH_3), 1670 (\text{CO-CH=CH-}), 1640 (\text{-C=C-}) and 975 cm^{-1} (\text{trans-unsaturation}). Elemental analysis, IR and UV data suggested that a conjugated dioxoalkenoate moiety was present. The NMR is more informative regarding the conjugated dioxoalkenoate structure. It showed signals at δ 7.06 d (1H, \text{-CH=CH-COOCCH}_3; J=16 Hz), 6.54 d (1H, \text{-CH=CH-COOCCH}_3; J=16 Hz), 2.50 m (2H, \text{CH}_2-C\text{-}). J Value established the configuration of the double bond as trans.

Synthesis of 9,12-Dioxo-trans-10-octadecenoic Acid (XII)

9,12-Dioxo-trans-10-octadecenoic acid (XII) was obtained by vigorous oxidation of castor acids^4. Compound (XII) analysed for C_{18}H_{30}O_4. IR showed characteristic bands at 3425 (COOH), 1710 (COOH), 1680 (\text{COCH=CHCO}), 1645(\text{-C=C-}) and 980 cm^{-1} (\text{trans-unsaturation}). NMR spectrum of the compound displayed two significant signals at δ 6.82 s (2H, \text{-CH=CH}) and 2.80 m (6H, 3\text{CH}_2-C\text{-}).
Section-II

Hydrazoic Acid ———

Preparation of Tetrazole and Amides

Aldehydes and ketones involve in Schmidt reaction. This is the reaction between a carbonyl function and hydrazoic acid in presence of sulfuric acid. Conversion of carboxylic acids and esters into amines can also occur by this reaction. Amides (XIII) are formed when 1 mole equivalent of sodium azide in presence of sulfuric acid is treated.

\[
\begin{align*}
\text{R-C-R'} & \quad \text{NaN}_3/\text{H}_2\text{SO}_4 \\
\text{1 mole} & \quad \rightarrow \\
\text{R-C-NH-R'} & \quad \text{(XIII)}
\end{align*}
\]

Two geometrical isomers are possible, if R and R' are different and anti group is the group which migrates.

When Schmidt reaction is subjected to \(\alpha,\beta\)-unsaturated carboxylic ester (XIV), the enamine is a rearranged product, which hydrolyses to the corresponding ketone (XV).

\[
\begin{align*}
\text{CH} & \quad \text{C-CO}_2\text{CH}_3 \quad \frac{\text{NaN}_3}{\text{H}_2\text{SO}_4} \\
\text{(CH}_2\text{)}_{13} & \quad \rightarrow \\
\text{CH}_2 & \quad \text{C=O} \quad \text{(XV)}
\end{align*}
\]
The five membered doubly unsaturated heterocycle with one carbon and four nitrogen atoms is known as tetrazole. The first tetrazole was recognized in 1885 by Bladin\(^6\) during an investigation of dicyanophenylhydrazine. Treatment of dicyanophenylhydrazine with nitrous acid led to a compound later shown to be 5-cyano-2-phenyltetrazole. Bladin\(^7\) showed the presence of the tetrazole ring system by degrading cyanophenyltetrazole to the parent compound. The considerable stability of the ring with one carbon and four nitrogen atoms is indicated by the ability of these derivatives to withstand the acidic, alkaline, oxidising and reducing agents in the degradation of cyanophenyltetrazole to tetrazole.

An excellent review, touching upon almost every aspect of tetrazole chemistry, is given by Benson\(^8\). Reaction of ketones with 2 moles/excess of sodium azide leads to the formation of the tetrazole (XVI) and urea (XVII) derivatives in major amounts.

\[
\begin{align*}
\text{KH} \quad \text{C} \quad \text{R} & \quad \xrightarrow{\text{NaN}_3/\text{H}_2\text{SO}_4/\text{excess}} \quad \text{R} \quad \text{N} \quad \text{C} \quad \text{R} \quad + \quad \text{R} \quad \text{NH} \quad \text{C} \quad \text{NH} \quad \text{R}' \\
& \text{N} \quad \text{N} \quad \quad \text{N} \quad \text{N} \\
& \text{(XVI)} \\
& \text{(XVII)}
\end{align*}
\]

Schmidt\(^9\) have reported the formation of 80% tetrazole from acetone and diethyl ketone. Smith\(^10\) has subjected the Schmidt reaction of ketones to a critical study and has developed
a more acceptable mechanism involving both amide and tetrazole functions. Following is his proposed mechanism:

\[
\begin{align*}
R-CN &\xrightarrow{H^+} \text{C-OH} \quad \text{(1)} \\
R^+\text{C-OH} + HN_3 &\rightarrow R-C-R \quad \text{H}_2O + R-C-R \quad \text{N-N=NN} \quad \text{(2)} \\
R-N + H_2O &\rightarrow \text{HO-C-R} \quad \text{R-NH-C-R} + H_3O^+ \quad \text{(3)}
\end{align*}
\]

The imidocarbonium ion (XVIII) resulting from step (2) may produce a product other than amide if it reacts a species other than water and is capable of forming a permanent attachment. When it reacts with a second mole of hydrazoic acid a tetrazole (XVI) would be produced.

\[
\begin{align*}
R-N = C-R + HN_3 &\xrightarrow{} R-N \xrightarrow{H^+} C-R + H^+ \\
\text{XVIII} &\quad \text{XVI}
\end{align*}
\]
Tetrazoles have found important biological as well as nonbiological applications. On nonbiological side they are of use in fibre, dyestuff and textile industries and have applications in photography also. On biological side, these are potent stimulants of the central nervous systems and are used clinically to counteract intoxication due to over-dosage of barbiturates. Stimulant, depressant, sedative and analgesic activities are shown by certain tetrazoles. Anticonvulsant, hypotensive and andrenergic blocking actions are also exhibited.

Several papers have appeared describing the synthesis of tetrazoles mainly from steroidal ketones using excess of hydrazoic acid. A survey of the literature revealed that the formation of tetrazoles has not been reported in the field of fatty acids. With the realization of the above-mentioned applications of tetrazoles, efforts were directed towards their synthesis.

Reaction of 2-Oxoocadecanoic Acid (Xa) with Excess of Hydrazoic Acid

Previously, some attempts were made to carry out the reaction of hydrazoic acid at room temperature with methyl 2-oxo-hexadecanoate. These attempts however, failed under the reaction conditions employed. The nonreactivity of 2-oxoester was attributed to peculiar structural feature having an oxo group in proximity of the ester function.
We thought it worthwhile to apply some vigorous reaction conditions and to see their effect on the reactivity of the 2-oxo-octadecanoic acid (Xa) with hydrazoic acid (excess) in the presence of BF₃-etherate as catalyst, refluxed the reaction mixture for 20 hr and successfully isolated the amide (XIX) (Scheme-4).

![Scheme-4](image)

The compound (XIX) was indicated to be a mononitrogenous product by its elemental analysis C₁₈H₃₅O₃N. Its IR spectrum exhibited prominent bands at 3300 for HN (stretching), 1660 and 1625 for the amide (CONH), 1535 and 1520 for NH (bending) and 1710 cm⁻¹ for acid carbonyl. NMR spectrum showed two D₂O exchangeable signals at δ 7.59 and 9.40 integrating for one proton of NH and COOH respectively. A multiplet centred at δ 3.42 for two protons of -CH₂-NH, indicated the presence of NH group in between oxo and alkyl chain. On the basis of these observations the
structure assigned to the compound (XIX) was 3-azanonadeca-2-oxo-noic acid.

Reaction of Hydrazoic Acid (excess) with Methyl 4-oxo-trans-2-octadecenoate (Xlb)

Methyl 4-oxo-trans-2-octadecenoate (Xlb) on treatment with excess of hydrazoic acid at room temperature yielded a solid reaction mixture, which on chromatography separated into three products (Scheme-5).

**Scheme-5**

![Chemical reaction diagram](image)

Characterization of Product (XX)

The compound (XX) was indicated to be a tetranirogenous product by its elemental analysis $C_{19}H_{24}O_2N_4$. The IR spectrum exhibited bands at 3060 w and 1650 cm$^{-1}$ for (-C=C-), 970 cm$^{-1}$ for
trans-olefin. Diagnostic bands for C=N and N=N were observed at 1505, 1460, 1375 cm⁻¹. NMR spectrum of the compound gave conclusive information regarding the structure of tetrazole (XX). It exhibited peaks for vinylic protons at 8 7.81 d (1H, -CH=CH-COCH₂; J=14 Hz) and 6.73 d (1H, -CH=CH-COOCH₃; J=14 Hz). The coupling constant (J) established the configuration of the double bond as trans. The appearance of a triplet at 8 2.98 (J=7 Hz) for two methylene protons α to nitrogen clearly demonstrated the presence of nitrogen in between the alkyl chain and the oxo group. If the nitrogen location is reversed (in between oxo and double bond) then the signals from -CH₂ and β-protons would appear in a higher field. On the basis of these observations the structure assigned to compound (XX) was methyl 5-azanonadec-trans-2-enoate (5,4-d)-tetrazole.

Mass spectrum of XX confirmed the structure by showing molecular ion peak at m/z 350 (Fig. 1) (C₁₉H₃₄O₂N₄). α-Cleavage provided characteristic mass ions at 265 and 153 which established the position of the heterocycle (tetrazole) in the fatty chain (Scheme-6). The other prominent mass fragments were observed at 335 (M-CH₃), 321 (i.e.-C₂H₅), 308 (M-N₃), 307 (321-CH₂), 293 (307-CH₂), 291, 263, 249 (263-N), 237, 235 (249-N), 223 (237-N), 181, 168, 167, 140 (168-N₂), 139 (140-H), 137 (168-OCH₃), 123 (principal ion), 109 (168-COOCH₃), 83, 69 (83-N), 68 and 41 (69-N₂).
Fig. 1 Mass Spectrum of XX.
Scheme 6

\[
\begin{array}{c}
\text{CH}_2-N-C-CH=CH-\text{COOCH}_3 \\
n/z 181
\end{array}
\]

\[
\begin{array}{c}
\text{N}-C-CH=CH-\text{COOCH}_3 \\
n/z 153
\end{array}
\]

\[
\begin{array}{c}
\text{CH}_3(\text{CH}_2)_3-N-C-N=\text{C-CH}=\text{C-CH=CH-} \text{COOCH}_3 \\
M^+ 350
\end{array}
\]

McLafferty rearrangement

\[
\begin{array}{c}
\text{CH}_2-N-C-CH=CH-\text{COOCH}_3 \\
n/z 168
\end{array}
\]

\[
\begin{array}{c}
\text{O}=\text{C-N}=\text{C} \text{N}=\text{C} \text{N}=\text{C} \\
n/z 68
\end{array}
\]

\[
\begin{array}{c}
\text{CH}_3(\text{CH}_2)_3-N+ \text{C-CH}=\text{CH} \\
n/z 265
\end{array}
\]

\[
\begin{array}{c}
\text{CH}_3(\text{CH}_2)_3-N+ \text{C} \text{C} \\
n/z 237
\end{array}
\]

\[
\begin{array}{c}
\text{N} \text{C=CH} \text{CH} \text{N} \text{C=CH} \\
n/z 83
\end{array}
\]
Characterization of Product (XXI)

The compound (XXI) was indicated to be a mononitrogenous by its elemental analysis ($C_{19}H_{35}O_3N$). Its IR spectrum exhibited bands in favour of the structure methyl 5-azanonadec-4-oxo-trans-2-enoate (XXI) at 3310 (NH stretching), 1650, 1620 (CONH), 1540 and 1520 cm$^{-1}$ (NH bending). Bands for double bond were observed at 3060 w, 1640 cm$^{-1}$. A band at 990 cm$^{-1}$ (trans-unsaturation) was also observed. NMR spectrum gave a doublet for trans double bond at $\delta$ 6.94 d ($2H,-CH=CH-COOC_2H_5$; $J=14$ Hz). Two other structure-revealing signals were observed at $\delta$ 6.67m ($1H$, NH, D$_2$O exchangeable) and 3.35 m ($2H,-CH_2-NHCO$). Multiplet at $\delta$ 3.35 confirmed the location of NH group in between oxo and alkyl chain.

Mass spectrum recorded molecular ion peak at m/z 325 (Fig. 2), which was in agreement with its elemental analysis ($C_{19}H_{35}O_3N$). Two $\alpha$-cleavage ions at 240 and 113 confirmed the position of the oxo group. Location of NH group was further confirmed by the mass ions at 212 and 128 (Scheme-7). Other significant ions were observed at 310 ($\delta$-CH$_3$), 294 ($\delta$-OCH$_3$), 266 (294-CO), 265 (266-H), 184 (principal ion), 170, 142, 130, 114, 85 and 69.

Characterization of Product (XXII)

The compound (XXII) was characterized as pentadecamide on the basis of its elemental as well as spectral studies. Compound
(XXII) was analysed for $C_{15}H_{31}ON$. Its IR spectrum showed bands at 3350, 3180 (NH$_2$ stretch.), 1650, 1620 (CONH$_2$), 1410 (C-N) and 1160 cm$^{-1}$ (NH$_2$). The NMR spectrum gave structure-revealing peaks at $\delta$ 5.70 m (2H,-CONH$_2$, disappeared on D$_2$O addition), 2.16 m (2H, CH$_2$-CONH$_2$). Mass spectrum confirmed the product (XXII).

Molecular ion peak was recorded at m/z 241 (Fig. 3) ($C_{15}H_{31}ON$) and other peaks were at 240 (M-H), 212 (N-C$_2$H$_5$), 198 (212-CH$_2$), 184 (198-CH$_2$), 170 (184-CH$_2$), 156 (170-CH$_2$), 142 (156-CH$_2$), 128 (142-CH$_2$), 114, 100, 86, 72, 71, 59 (McLafferty rearrangement), 57, 44 and 43 (principal ion). Different mass fragmentations were consistent (Fig. 3) with the structure given.

Elemental and mass spectral analyses have confirmed that shortening of the chain length had occurred.

Additional evidence for the formation of pentadecamamide (XXII) was arrived at by its conversion to methyl pentadecanoate (XXIII) with MeOH/H$^+$. The methyl ester (liquid) analysed for $C_{16}H_{32}O_2$. Its IR spectrum gave band at 1740 cm$^{-1}$ (COOCH$_3$). The NMR signals at $\delta$ 3.60 s (3H,-COOCH$_3$) and 2.25 m (2H,-CH$_2$-COOCH$_3$) confirmed the structure.
Fig. 3 Mass Spectrum of XXII.
Section-III

Mercaptans

a) **Long Chain Thioethers**

These compounds are generally synthesized by the addition of sulfur or sulfuretted compounds across the double bond of olefinic fats. Such compounds are supposed to be easily assessable intermediates for the synthesis of individual sulfur fatty derivatives.

Alkenic moiety in fatty acids or their esters has reacted with mercaptan (mercaptoacetic acid) to afford thioethers. This mercaptan addition to unsaturation is a widely known process and usually follows a free radical mechanism\(^1\)\(^\text{7-22}\). Mercaptoacetic acid reacts readily with oleic acid (XXIV) to give a thioether (XXV). Terminally unsaturated fatty

\[
\begin{align*}
\text{CH}_3 - (\text{CH}_2)_{17} - \text{CH} &= \text{CH} - (\text{CH}_2)_{7} - \text{COOH} + \text{HSCH}_2 - \text{COOH} \\
\text{(XXIV)} \\
\downarrow \\
\text{CH}_3 - (\text{CH}_2)_{7(8)} - \text{CH} &= (\text{CH}_2)_{8(7)} - \text{COOH} \\
\text{(XXV)} \\
&\text{S-CH}_2 - \text{COOH}
\end{align*}
\]

acid reacts quickly with mercaptoacetic acid\(^1\)\(^\text{6}\). Whereas the preceding products are liquids, the product from 10-undecenoic acid is a crystalline solid of sharp melting point. Depending upon the
way of addition it could be either a branched molecule 10-(carboxymethylthio)undecanoic acid (XXVI) or a linear structure, 11-(carboxymethylthio)undecanoic acid (XXVII).

\[
\begin{align*}
\text{HOOC - CH}_2\text{S} - \text{CH} - (\text{CH}_2)_8 - \text{COOH} & \quad \text{HOOC - CH}_2\text{S}(\text{CH}_2)_{10} - \text{COOH} \\
(\text{XXVI}) & \quad (\text{XXVII})
\end{align*}
\]

X-ray and IR data indicate the existence of linear isomer only. In accordance with a radical mechanism the carboxymethylthio group adds to the terminal carbon of 10-undecenoic acid contrary to Markovnikoff's rule and the rate of reaction can be enhanced by the excess addition of mercaptoacetic acid.

Addition of thiol to 10-undecenoic acid is also capable of producing thioethers. The reaction proceeds rapidly below 100°C of temperature through free radical conditions. Few more types of terminally substituted undecanoic acids are obtained by the reaction of benzenethiol, n-octylthiol and β-mercaptoethanol.

Recently, fatty acid derivatives containing thioether linkage were successfully prepared at author's laboratory. Terminal, internal and α,β-unsaturated fatty acid esters were allowed to react with 3-mercaptopropan-1,2-diol to produce addition products.

α,β-Unsaturated ketones were also subjected for the addition of β-mercaptoethanol in piperidine by Romo et al.
addition of β-mercaptoethanol is similar to benzyl mercaptan in its capacity to add to α,β-unsaturated ketone.

Sulfur-bearing fatty materials are expected to attract both industrial and academic interests. Mercaptans of higher molecular weight have industrial applications as anticorrosive agents for lubricating oils and starting materials for manufacturing chemicals. Recently, some sulfides (thioethers) have been tested for fungicidal, neurotropic, bactericidal, antiinflammatory, hypolipemic and anticholesteremic activities. Few thioethers have been positively tested for tranquilizers.

The reaction of mercaptans with α,β-unsaturated fatty acids has been quite well known. The reaction, however, took a much longer time to reach completion than that for a terminal olefinic fatty acid. We made an attempt to modify this reaction so as to get the desired product in a shorter period of time. For this we have taken α,β-unsaturated carbonyl and dioxoene fatty acids rather than the α,β-unsaturated olefinic fatty esters and successfully obtained the addition products in one hour under reflux conditions.

Reaction of β-Mercaptoacetic Acid with Methyl 4-oxo-trans-2-hexadecenoate (XIa)

Methyl 4-oxo-trans-2-hexadecenoate (XIa) was refluxed with β-mercaptoacetic acid in benzene. The reaction mixture showed a single spot on TLC plate. The reaction mixture on workup yielded
a solid product, which on crystallization from petroleum ether-ether at low temperature gave a crystalline product (XXVIII) (Scheme-8).

Scheme-8

\[
\begin{align*}
\text{CH}_3-(\text{CH}_2)_{11}-&-\text{C}-\text{CH}=&\text{CH}-\text{COOCH}_3 \\
\text{(XIa)}
\end{align*}
\]

\[
\begin{align*}
\text{HS}-\text{CH}^-\text{COOH}, \\
\text{benzene(Reflux)}
\end{align*}
\]

\[
\begin{align*}
\text{CH}_3-(\text{CH}_2)_{11}-&-\text{C}-\text{CH}=&\text{CH}-\text{COOCH}_3 \\
&-\text{S}-\text{CH}_2^-\text{COOH}
\end{align*}
\]

(Product (XXVIII) analysed for C_{19}H_{34}O_5S. IR spectrum illustrated bands at 3400 cm\(^{-1}\) (COOH) and three carbonyl bands at 1735 (COOCH\(_3\)), 1720 (-CO-) and 1710 cm\(^{-1}\) (COOH). 4-Oxo grouping remained unaffected as indicated by IR. A couple of IR absorptions for sulfur were also present at 1440 (S-CH\(_2\) deformation) and 1260 cm\(^{-1}\) (S-CH\(_2\) wagging). NMR spectrum showed signals at \(\delta\) 3.23 m (3H, -CO-CH-S-CH\(_2\)-COOH), 2.40 m (4H, protons \(\alpha\) to carbonyl functions) and 9.60 br.s (1H, -COOH, disappeared on D\(_2\)O addition). On the basis of above informations the product (XXVIII) was formulated as methyl 4-oxo-2(3)-(S-\(\beta\)-mercaptoacetic acid)hexadecanoate.
The MS ($M^+$ 374, Fig. 4) of thioether (XXVIII) revealed its composition as indicated by elemental analysis ($C_{19}H_{34}O_5S$). Emergence of fragment ions, $m/z$ 163 and 177 clearly demonstrated the attachment of sulfur on $\alpha$- and $\beta$-carbons. $\alpha$-Cleavage ions at 197 and 113 and McLafferty ion at 128 (principal ion) fixed the position of an oxo group at C$_4$ (Scheme-9). Other significant peaks observed were at 343 ($M^+\text{-CH}_3$), 342 ($M^+\text{-CH}_2\text{OH}$), 324 (342-H$_2$O), 315, 298 (343-COOH), 296 (324-CO), 284 ($M^+\text{C}_2\text{H}_5\text{O}_2S$), 283, 282, 256, 255, 251 (282-OCH$_3$), 223 (255-S and/or 282-COOCH$_3$), 159 (177-H$_2$O), 145 (177-S and/or 163-H$_2$O), 144 (177-HS), 143 (177-H$_2$S), 131 (163-S), 130 (163-HS) and 129 (163-H$_2$S). Thus the structure of XXVIII was methyl 4-oxo-2(3)-($S$-$\beta$-mercaptoacetic acid)hexadecanoate.

Reaction of $\beta$-Mercaptopropionic Acid with Methyl 4-oxo-trans-2-hexadecenoate (XIa)

A solution of methyl 4-oxo-trans-2-hexadecenoate (XIa) in benzene when refluxed with $\beta$-mercaptopropionic acid yielded a single product which on crystallization with petroleum ether-ether gave XXIX (Scheme-10).

The elemental analysis of the product (XXIX) corresponded to $C_{20}H_{36}O_5S$ as suggested from microanalysis. The characteristic IR bands appeared at 3400 (COOH), 1740 (COOCH$_3$), 1720 (CO), 1715 (COOH), 1435 and 1260 cm$^{-1}$ (S-CH$_2$). The NMR peaks observed were at $\delta$ 3.22 m (1H, -COCH-S-), 2.75 m (2H, -S-CH$_2$-CH$_2$-), 2.38 m (6H,
Fig. 4 Mass Spectrum of XXVIII.
3xCH$_2$-CO-), 8.50 br.s (1H,-COOH, disappeared on D$_2$O shake). The elemental and spectral analyses established the structure (XXIX) as methyl 4-oxo-2(3)-(S-$\beta$-mercaptopropionic acid)hexadecanoate.

![Scheme-10](image)

Its mass spectrum was quite consistent with the structure of XXIX and showed molecular ion peak at m/z 388 (C$_{20}$H$_{36}$O$_5$S) (Fig. 5). Mass ions at 191 and 177 successfully indicated the isomeric nature of the product (XXIX). Fragmentations at 197, 113 ($\alpha$-cleavage) and 128 (McLafferty rearrangement) confirmed the presence of an oxo group at C$_4$ (Scheme-11). Other important peaks were at 357 (M-OCH$_3$), 356 (M-CH$_3$OH), 338 (356-H$_2$O), 329, 315, 310 (338-CO), 284 (M-C$_3$H$_4$O$_2$S), 283, 282, 256, 255, 251 (282-OCH$_3$), 223 (255-S or/and 282-COOCH$_3$), 173 (191-H$_2$O), 159 (191-S and/or 177-H$_2$O), 157 (191-H$_2$S), 145 (177-CH$_3$OH), 127 (159-CH$_3$OH) and 111 (145-H$_2$S).
Scheme 11

\[
\begin{align*}
CH_3(CH_2)_2 C - \text{CH} \rightleftharpoons & \text{COOCH}_3 \\
\text{m/z 283} & \\
\end{align*}
\]

\[
\begin{align*}
CH_3(CH_2)_2 C = \text{C} \equiv O^+ & \\
\text{m/z 197} & \\
\end{align*}
\]

\[
\begin{align*}
CH_3(CH_2)_2 C - \text{CH} - \text{COOCH}_3 & \\
\text{m/z 177} & \\
\end{align*}
\]

\[
\begin{align*}
CH_3(CH_2)_2 C - \text{CH} - \text{COOCH}_3 & \\
\text{m/z 315} & \\
\end{align*}
\]

\[
\begin{align*}
CH_3(CH_2)_2 C - C - \text{CH} - \text{COOCH}_3 & \\
\text{m/z 255} & \\
\end{align*}
\]

\[
\begin{align*}
CH_3(CH_2)_2 C - \text{CH} = \text{CH} - \text{COOCH}_3 & \\
\text{m/z 282} & \\
\end{align*}
\]

McLafferty rearrangement

\[
\begin{align*}
\text{HOT} & \\
\text{m/z 128} & \\
\end{align*}
\]

\[
\begin{align*}
\text{CH}_2 = \text{C} - \text{CH} = \text{CH} - \text{COOCH}_3 & \\
\text{m/z 113} & \\
\end{align*}
\]

\[
\begin{align*}
\text{M}^+ 388 & \\
\text{m/z 191} & \\
\end{align*}
\]

\[
\begin{align*}
\text{CH}_2 = \text{CH} - \text{COOCH}_3 & \\
\text{S-CH}_2 - \text{CH}_2 - \text{COOH} & \\
\text{m/z 329} & \\
\end{align*}
\]

\[
\begin{align*}
\text{CH}_3(CH_2)_2 C - \text{CH} - \text{COOCH}_3 & \\
\text{S-CH}_2 - \text{CH}_2 - \text{COOH} & \\
\text{m/z 256} & \\
\end{align*}
\]
Reaction of β-Mercaptopropionic Acid with 9,12-Dioxo-trans-10-octadecenoic acid (XII)

A similar treatment of β-mercaptopropionic acid with 9,12-dioxo-trans-10-octadecenoic acid (XII) gave a semisolid product, 9,12-dioxo-10(11)-(β-β-mercaptopropionic acid)octadecanoic acid (XXX) (Scheme-12).

**Scheme-12**

\[
\text{CH}_3 - (\text{CH}_2)_5 - \text{C} - \text{CH} = \text{CH} - \text{C} - (\text{CH}_2)_7 - \text{COOH} \\
\text{HSCH}_2\text{CH}_2\text{COOH}, \\
\text{benzene (Reflux)} \\
\downarrow \\
\text{CH}_3\text{(CH}_2)_5 - \text{C} - \text{CH} - \text{C} - (\text{CH}_2)_7 - \text{COOH} \\
\text{S-CH}_2\text{CH}_2\text{COOH} \\
(\text{XXX})
\]

Elemental analysis of the product (XXX) corresponded to the formula C_{21}H_{36}O_{6}S. IR spectrum of the compound (XXX) displayed bands at 3400 (OH), 1715 (free carbonyl), 1710 (acid carbonyl) and 1440, 1265 cm^{-1} (S-CH$_2$). NMR spectrum illustrated important signals at δ 3.26 m (1H, CO-CH$_2$-), 2.70 m (2H, -S-CH$_2$-CH$_2$), 2.30 m (1OH, 5xCH$_2$-CO-) and 9.30 br,s (2H, 2xCOOH, D$_2$O exchangeable). All
these evidences led to the conclusion that the compound (XXX) was 9,12-dioxo-10(ll)-(S-β-mercaptopropionic acid)octadecanoic acid.

The structure of (XXX) was further substantiated by its mass spectrum. It showed molecular ion peak at m/z 416 (C_{21}H_{36}O_{6}S) (Fig. 6). Mass ions at 289 and 303 were observed due to the isomeric type of mercaptan addition to olefinic carbons. Presence of oxo group was fixed by the fragment ions at 113, 225 and 240 (C_{12}) and 171, 167 and 182 (C_{9}) (Scheme-13). The other significant peaks observed were at 384 (M−S), 383 (M−SH), 382 (M−H_{2}S), 381 (M−H_{2}O+OH), 380 (M−2xH_{2}O), 346, 310, 288, 271 (303−S), 270 (303−SH), 269 (303−H_{2}S), 268 (303−H_{2}O+OH), 256 (289−SH), 253 (289−2xH_{2}O), 223 (240−OH), 164 (182−H_{2}O) and 153 (171−H_{2}O).
Fig. 6 Mass Spectrum of XXX.
b) Fatty Oxathiolanes

In previous studies quite a number of steroidal as well as nonsteroidal ketones have been reported to condense promptly with β-mercaptoethanol to produce oxathiolane (cyclic hemithio-ketals)\textsuperscript{32}. Condensation of ketones with β-mercaptoethanol or ethanediethiol are influenced by the use of sodium sulfate and zinc chloride\textsuperscript{24,33}, hydrogen chloride in ether\textsuperscript{34,35}, p-toluenesulfonic acid (p-TSA) in benzene under azeotropic distillation employing a water separator\textsuperscript{36} and an exchange method\textsuperscript{32}.

\[
\begin{array}{c}
R_1 \quad C=O \\
R_2
\end{array} + \text{OH} \quad \rightarrow \quad \begin{array}{c}
R_1 \quad C \\
R_2 \quad S
\end{array}
\]

Initial attack on the carbonyl carbon proceeds through the SH rather than OH function of the reagent. This fact also seems to be a substantial evidence why only one isomer is furnished in the reaction of 3- and 17-oxosteroids with β-mercaptoethanol and γ-mercaptopropanol inspite of the fact that two isomers are possible. Sulfur attacks from the unhindered rear side (α) so that all the hemithioketals have the C-S (α) and C-O (β) configurations\textsuperscript{32}.

Fieser\textsuperscript{37} synthesized thioketals, using boron trifluoride-etherate as a condensing agent by a more simpler method that appear to be of comparable applicability.
Condensation of \( \beta \)-mercaptoethanol with the acetylenic ketones have also been capable of forming thioketal as hemithio-ketal derivatives. Nakhmanovitch \textit{et al.}\textsuperscript{38} have reported the synthesis of oxathiolane in fairly good yield by the reaction of \( \beta \)-mercaptoethanol with \( \alpha \)-bromoacetylene. They also conducted cyclocondensation of acetylenic ketone with \( \beta \)-mercaptoethanol to produce oxathiolane\textsuperscript{39}.

Oxathiolanes are converted to parent ketones on acid hydrolysis\textsuperscript{24}, but they are stable to base and lithium aluminium hydride (LAH).

Earlier ethylene hemithioketals were somewhat confined to short chain and cyclic ketones\textsuperscript{24,32,33}. Recently, the work in the author's laboratory was involved in preparing nonsubstituted\textsuperscript{40} and substituted\textsuperscript{41} cyclic hemithioketals carrying fatty acid chains.

The oxoolefinic acids are capable of undergoing the reaction typical of the oxo and olefinic functions, because of their bifunctionality. Keeping in view the point, the reactions of \( \alpha,\beta \)-unsaturated carbonyl and dioxoene fatty acids with \( \beta \)-mercaptoethanol were carried out in the present study and the results of these reactions are discussed.
Condensation of Methyl 4-oxo-trans-2-hexadecenoate (XIa) with β-Mercaptoethanol

Methyl 4-oxo-trans-2-hexadecenoate (XIa) when reacted with β-mercaptoethanol and BF$_3$-etherate in acetic acid afforded an oily product. Fractionation of the reaction mixture over silica gel column gave three products (Scheme-14).

**Scheme-14**

\[
\text{(XIa)} \xrightarrow{\text{HSCH}_2\text{CH}_2\text{OH}, \text{BF}_3\text{-etherate, AcOH}} \text{(XXXI)} + \text{(XXXII)} + \text{(XXXIII)}
\]

\[R = \text{CH}_3(\text{CH}_2)_{11}\]

**Characterization of Product (XXXI)**

Elemental analysis of the compound (XXXI) corresponded to the formula C$_{19}$H$_{34}$O$_3$S. IR bands at 3030, 1650 and 980 cm$^{-1}$ proved the presence of trans-double bond. Some diagnostic bands at 1440 (S-CH$_2$ deformation), 1200, 1220, 1280 (S-CH$_2$ wagging),
1020 and 1040 cm$^{-1}$ (oxathiolane ring) supported the formation of oxathiolane (cyclic hemithioketal) grouping at the site of 4-oxo. Conclusive support in favour of methyl 4-oxathiolane-trans-2-hexadecenoate (XXXI) had come from its NMR spectrum. It gave signals for trans-unsaturation at $\delta$ 6.82 d (1H, -CH=CH-COOCH$_3$; J=14 Hz) and 5.82 d (1H, -CH=CH-COOC$_2$H$_5$; J=14 Hz). Downfield appearance of two broad multiplets at $\delta$ 4.30-3.90 and 2.98-2.78 integrating for two protons each were ascribable to the protons $\alpha$ to oxygen and sulfur in oxathiolane ring respectively. A multiplet appeared at $\delta$ 1.80 for protons $\alpha$ to oxathiolane ring.

The MS of XXXI corroborated the suggested structure by showing characteristic fragmentations (Scheme-15). Molecular ion at m/z 342 (Fig. 7). Characteristic mass ions at 257 and 173 (principal ion) fragmenting from $\alpha$-cleavage confirmed the location of the oxathiolane ring at C$_4$ (Scheme-15). Some other significant peaks observed were at m/z 311 (M-OCH$_3$), 310 (M-CH$_3$OH), 283, 251 (283-S), 250 (283-SH), 225 (257-S), 224 (257-SH), 223 (257-H$_2$S), 199 (257-C$_2$H$_2$S), 197, 174 (173+H), 141 (173-S), 140 (173-SH), 139 (173-H$_2$S), 129, 113, 97 (129-S).

**Characterization of Product (XXXII)**

Compound (XXXII) was found to have a composition C$_{23}$H$_{42}$O$_5$S$_2$ consistent with methyl 4-oxathiolane-2(3)-(S, S'-mercaptoethylacetate)hexadecanoate. IR spectrum showed characteristic bands at 1740 (OCOOCH$_3$, COOCH$_3$) and 1270 cm$^{-1}$ for acetate moiety
Fig. 7 Mass Spectrum of XXXI.

CH₃-(CH₂)₅-C=CH=CH-COOCH₃
along with bands, characteristic for oxathiolane (1020 cm\(^{-1}\)) and S-CH\(_2\) (1435, 1250 cm\(^{-1}\)) grouping. Elimination of IR bands at 3030, 1650 and 980 cm\(^{-1}\) and vinylic protons in NMR spectrum indicated the consumption of the double bond and formation of thioether. Emergence of a five proton multiplet at \(\delta\) 3.18-2.52 (protons \(\alpha\) to sulfur in ring, -CH-S-CH\(_2\)) indicated that the attack of \(\beta\)-mercaptoethanol is through sulfur, not through oxygen. Otherwise its integration would have been one proton less and integration of signal at \(\delta\) 4.42-3.91 (protons \(\alpha\) to oxygen in ring and mercaptan side chain) would have shown five protons instead of four. Illustration of two sharp singlets of equal intensities at \(\delta\) 3.65 and 3.62 for ester protons showed the formation of an isomeric mixture of thioethers on \(\alpha\)- as well as \(\beta\)-carbons. A sharp singlet of three protons at \(\delta\) 2.02 supported the acetylation of hydroxy group in the side chain. Protons \(\alpha\) to ring were observed at \(\delta\) 1.80 \(\alpha\) \(\alpha\) a multiplet.

Parent ion at m/z 462 (Fig. 8) corresponded to molecular weight and confirmed the elemental composition \((\text{C}_{23}\text{H}_{42}\text{O}_{5}\text{S}_{2})\). The diagnostic mass ions at 257 (principal ion) and 293 fragmenting from \(\alpha\)-cleavage established the position of the ring at \(\text{C}_4\). Two characteristic fragment ions at 329 and 131 confirmed the isomeric nature of the compound as well as the attachment of mercaptan side chain through sulfur at \(\beta\)- and \(\alpha\)-carbons respectively (Scheme-16). Other salient peaks were at 431, 430, 403, 402, 385, 375, 343, 342, 328, 326, 321, 310, 307, 271, 258, 248, 225 (257-S), 224 (257-SH), 197, 173, 145 and 101.
Characterization of Product (XXXIII)

Compound (XXXIII) analysed for $C_{21}H_{40}O_4S_2$. IR and NMR spectra of the compound methyl 4-oxathiolane-2(3)\((\text{S}-\beta\text{-mercaptoethanol})\)hexadecanoate showed almost identical values as observed in the previous case (XXXII), except bands for acetate grouping. A band for hydroxy function at 3400 cm$^{-1}$ in IR, indicated non-acetylation of hydroxy function in the side chain. A noticeable feature of its NMR spectrum was nonappearance of an acetoxy singlet in the region of $\delta$ 2.0 which further supported the presence of hydroxy function. Methylene protons $\alpha$ to hydroxy in the side chain appeared in a broad multiplet at $\delta$ 4.32-3.92 along with the protons $\alpha$ to oxygen in oxathiolane ring. A $D_2O$ exchangeable broad singlet at $\delta$ 3.90 was assigned to a hydroxy proton. Mass spectrum of compound (XXXIII) showed highest ion peak at m/z 420 ($M^+$, Fig. 9). Other peaks were observed at 402, 389, 388, 375, 360, 343, 342, 329, 328, 310, 279, 271, 265, 258, 257 (principal ion), 251, 225 (257-S), 224 (257-SH), 223 (257-H$_2$S), 206, 197, 173, 145 and 131. Mode of fragmentations (Scheme-17) was same as observed in the previous case (XXXII).

Condensation of 9,12-Dioxo-trans-10-octadecenoic Acid (XII) with $\beta$-Mercaptoethanol

Similar condensation of 9,12-dioxo-trans-10-octadecenoic acid (XII) with $\beta$-mercaptopropanol gave an oily mass which afforded two products by column chromatography (Scheme-18).
Fig. 9 Mass Spectrum of XXXIII.
Characterization of Product (XXXIV)

The microanalysis of (XXXIV) corresponded to formula $C_{24}H_{42}O_{6}S_2$. The IR spectrum had prominent bands at 1440, 1240 ($S-\text{CH}_2$), 1740 ($\text{COOCH}_3$), 1720 ($\text{-CO-}$), 1710 ($\text{COOH}$), 1270 (acetate) and 1030 cm$^{-1}$ (oxathiolane ring). NMR spectrum of the compound
(XXXIV) showed a four proton broad multiplet at $\delta$ 4.40-3.95 attributable to the protons $\alpha$ to oxygen in the ring and mercaptan side chain. Another broad multiplet assigned to the protons (5H) $\alpha$ to sulfur in ring and $\text{CH}_2\text{S-CH}_2$ was observed at $\delta$ 3.20-2.60. A singlet at $\delta$ 1.99 supported the acetylation of hydroxy group in the side chain. Other significant NMR signals were at $\delta$ 2.30 m (protons $\alpha$ to CO-and-COOH) and 1.79 m (protons $\alpha$ to oxathiolane ring). On the basis of the above data the product (XXXIV) was identified as 9(12)-oxathiolane-12(9)-oxo-10(11)-(S-$\beta$-mercaptoethylacetate)octadecanoic acid.

Molecular ion peak at m/z 490 (Fig. 10) was consistent with the molecular weight. $\alpha$-Cleavage provided mass ions at 173/113 and 405 (oxathiolane ring/oxo), 171/231 and 347 (oxo/oxathiolane ring) and two McLafferty rearranged ions at 300, 242 from mass ion 370, established the presence of oxathiolane ring and oxo functions in isomeric form at C$_9$ and C$_{12}$. Further, fragment ions at 303/243 and 245/185 confirmed the attachment of mercaptan side chain at C$_{10}$ and C$_{11}$ respectively (Scheme-19). In addition, other significant peaks present were at 430, 412 (430-H$_2$O), 352 (370-H$_2$O), 293 (352-C$_2$H$_3$S), 292 (352-C$_2$H$_4$S), 267 (300-SH), 266 (300-H$_2$S), 210 (242-S), 209 (242-SH), 208 (242-H$_2$S), 199 (231-S), 198 (231-SH), 197 (231-H$_2$S), 187, 153 (171-H$_2$O), 141 (173-S), 139 (173-H$_2$S) and 127. Thus the structure of XXXIV was 9(12)-oxathiolane-12(9)-oxo-10(11)-(S-$\beta$-mercaptoethylacetate)octadecanoic acid.
Fig. 10 Mass Spectrum of XXXIV.
Characterization of Product (XXXV)

The compound (XXXV) was analysed for $\text{C}_{26}\text{H}_{46}\text{O}_6\text{S}_3$. IR spectrum showed bands at 1740, 1710, 1420, 1260, 1220, 1030 cm$^{-1}$. No IR band for isolated ketone, indicated the formation of oxathiolane ring at both $\text{C}_9$ and $\text{C}_{12}$ carbonyls. NMR spectrum displayed significant signals at $\delta$ 4.39-3.90 br,m (6H, protons $\alpha$ to oxygen in both rings, mercaptan side chain), 3.0-2.50 br,m (7H, protons $\alpha$- sulfur in both rings and -CH-S-CH$_2$-CH$_2$-OCOCH$_3$), 2.0 s (3H,-OCOCH$_3$) and 1.78 m (6H, protons $\alpha$ to both rings).

Mass spectrum was more informative to confirm the structure of XXXV. Molecular ion peak at m/z 550 (Fig. 11). $\alpha$-Cleavage produced mass ions at 173, 465 and 231, 407 which confirmed the location of two oxathiolane rings, one each at $\text{C}_9$ and $\text{C}_{12}$. Mass ions at 303 and 245 confirmed the presence of thioether linkage on both the olefinic carbons (Scheme-20). The other prominent peaks observed were at 490, 472 (490-H$_2$O), 430, 412 (430-H$_2$O), 352 (412-C$_2$H$_4$S), 292 (352-C$_2$H$_4$S), 199 (231-S), 198 (231-SH), 197 (231-H$_2$S), 187, 171, 141 (173-S), 140 (173-HS), 139 (173-H$_2$S) and 113. All these spectroscopic data established the structure of XXXV as 9,12-dioxathiolane-10(ll)-(S-p-mercaptoethylacetate)octadecanoic acid.

There are certain parameters which control the formation of cyclic hemithioketal and addition of mercaptan. Their rate of formation is generally enhanced by increase in temperature...
Fig. 11 Mass Spectrum of XXXV.
Scheme-20

\[ \text{CH}_3(\text{CH}_2)_5\text{C}^+ \quad m/z 113 \]

\[ \text{CH}_3(\text{CH}_2)_5\text{C}^+ \text{CH=CH CH=CH(C(CH_2)_7\text{COOH})}^+ \quad m/z 430 \]

\[ \text{CH}_3(\text{CH}_2)_5\text{C}^+ \quad m/z 173 \]

\[ \text{CH}_3(\text{CH}_2)_5\text{C}^+ \text{CH=CH CH=CH(C(CH_2)_7\text{COOH})}^+ \quad m/z 407 \]

\[ \text{CH}_3(\text{CH}_2)_5\text{C}^+ \quad m/z 231 \]

\[ \text{CH}_3(\text{CH}_2)_5\text{C}^+ \text{CH=CH CH=CH(C(CH_2)_7\text{COOH})}^+ \quad m/z 465 \]

\[ \text{CH}_3(\text{CH}_2)_5\text{C}^+ \quad m/z 187 \]

\[ \text{CH}_3(\text{CH}_2)_5\text{C}^+ \text{CH=CH CH=CH(C(CH_2)_7\text{COOH})}^+ \quad m/z 245 \]

\[ \text{CH}_3(\text{CH}_2)_5\text{C}^+ \quad m/z 303 \]

\[ \text{CH}_3(\text{CH}_2)_5\text{C}^+ \text{CH=CH CH=CH(C(CH_2)_7\text{COOH})}^+ \quad m/z 490 \]

\[ \text{CH}_3(\text{CH}_2)_5\text{C}^+ \quad m/z 245 \]
and reaction time. Addition of mercaptan in excess is also capable of increasing the rate of the reaction. Dioxathiolane (XXXV) was produced in a comparative high yield (45%), when dioxoene (XII) with β-mercaptoethanol was heated under reflux. When compound (XXXIV) was allowed to react with excess of β-mercaptoethanol for a long time at room temperature again XXXV was obtained in 100% yield. These results drew the conclusion that increase in temperature, time and mercaptan concentration enhanced the yield of XXXV.
c. **Long Chain Thiazinones**

Thiazinones are six-membered heterocyclic compounds containing one sulfur and one nitrogen atom. Thiazinones may be known as derivatives of thiazines. Smolanka et al. synthesized 5,6-dihydro-1,3-thiazinone-4. They observed that SC(NH\textsubscript{2})\textsubscript{2} and Br(CH\textsubscript{2})\textsubscript{2}COOC\textsubscript{2}H\textsubscript{5} when heated on a steam bath form Br(CH\textsubscript{2})\textsubscript{2}COSC(NH)-NH\textsubscript{2}. This does not undergo cyclization to form thiazine when refluxed in organic solvent. On the other hand Br(CH\textsubscript{2})\textsubscript{2}COCl and NH\textsubscript{4}CNS on heating in toluene form Br(CH\textsubscript{2})\textsubscript{2}CONCS which with NH\textsubscript{3} form Br(CH\textsubscript{2})\textsubscript{2}-CONHCSNH\textsubscript{2}. This cyclises to produce 2-amino(imino)-4-oxo-5,6-dihydro-1,3-thiazinium bromide.

A combine method for the synthesis of thiazolidinones and thiazinones was reported by Natsukawa et al. Heindel and coworker prepared benzothiazinones from C-mercaptobenzamides. They reported that the condensation of C-mercaptobenzamides with methyl acetylenedicarboxylate or propiolate takes place with trans addition of the SH function to the alkyne linkage. The resulting vinyl sulfide adducts can be cyclised to 1,3-benzothiazin-4-ones in good yield.

As six-membered heterocyclic fatty derivatives are comparatively rare or little known, an attempt has been made to prepare thiazinones from methyl 4-oxo-trans-2-octadecenoate (XIIb) with β-mercaptopropionic acid and (NH\textsubscript{4})\textsubscript{2}CO\textsubscript{3}. 

Reaction of β-Mercaptopyruvic Acid, $(NH_2)_2CO_2$ with Methyl 4-oxo-trans-2-octadecenoate (XIIb)

An azeotropic reflux of methyl 4-oxo-trans-2-octadecenoate with β-mercaptopyruvic acid and $(NH_2)_2CO_2$ followed by column separation afforded methyl 4-thiazinone-2(3)-(S-β-mercaptopyruvic acid)octadecanoate (XXXVI) and 4-thiazinone-2(3)-(S-β-mercaptopyruvic acid)octadecanoic acid (XXXVII) (Scheme-21).

Scheme-21

\[
\begin{align*}
R - C - CH = CH - COOCH_3 \\
(XIIb) \\
\text{HS-CH}_2-\text{CH}_2\text{COOH,} \\
(NH_2)_2\text{CO}_2, \text{benzene}
\end{align*}
\]

\[
\begin{align*}
R-C-CH-C(O)CH_2\text{COOCH}_3 \\
S\quad S-\text{CH}_2-\text{CH}_2-\text{COOH} \\
\text{CH}_2\text{C}=\text{O} \\
\text{CH}_2
\end{align*}
\]

(XXXVI)

\[
\begin{align*}
R-C-CH-C(O)\text{COOH} \\
S\quad S-\text{CH}_2-\text{CH}_2-\text{COOH} \\
\text{CH}_2\text{C}=\text{O} \\
\text{CH}_2
\end{align*}
\]

(XXXVII)

\[
R = \text{CH}_3(\text{CH}_2)_{13}
\]
Characterization of Product (XXXVI)

The liquid major product (XXXVI) exhibited the element composition (C, H, N) corresponding to the formula \( \text{C}_{25}\text{H}_{30}\text{O}_{5}\text{S}_{2}\text{N} \). Its IR spectrum showed characteristic absorptions at 3425 (COOH), 3165 (-NH stretch.), 1735 (\( \text{C}O\text{OCH}_{3} \)), 1710 (COOH), 1670 (Lactam carbonyl, -CONH), 1440 (S-CH\(_2\)), 1410 (C-N stretch.), 1240 (S-CH\(_2\)) and 780 cm\(^{-1}\) (-NH wag.). These data are in agreement with the structure (XXXVI). NMR spectrum showed the structure-revealing signals at \( \delta \) 8.50 br,s (1H, -COOH, D\(_2\)O exchangeable), 4.30 br,s (1H, -NH, disappeared after D\(_2\)O shake), 3.20-2.80 br,m (5H, two protons \( \alpha \) to sulfur in ring and-CH-S-CH\(_2\)-CH\(_2\)-COOH), 2.60 unresolved triplet (2H, -CH\(_2\)-CONH-), 2.30 br,m (protons \( \alpha \) to ester carbonyl, -CH\(_2\)-COOH), 1.70 m (protons \( \alpha \) to ring). Appearance of two sharp singlets at \( \delta \) 3.68 and 3.70 for ester protons indicated the attachment of mercaptan side chain both on \( \alpha \)- and \( \beta \)-carbons. On the basis of these values the compound (XXXVI) was formulated as methyl 4-thiazinone-2(3)-(S-\( \beta \)-mercaptopropionic acid)octadecanoate.

The MS of XXXVI was deprived of molecular ion peak at m/z 503 (Fig. 12). However, diagnostic mass ions were observed at 431 (\( \text{C}_{3}\text{H}_{4}\text{O}_{2} \)), 416 (431-NH), 384 (416-S), 366 (384-H\(_2\)O), 356 (416-C\(_2\)H\(_4\)S), 338, 311 (312-H), 310 (311-H), 284 (312-\( \text{CO} \)), 283 (312-COH), 280 (312-S), 279 (312-SH), 278 (312-H\(_2\)S), 252 (312-C\(_2\)H\(_4\)S), 224 (252-\( \text{CO} \)), 223, 173 (191-H\(_2\)O), 159 (177-H\(_2\)O), 129, 128 (129-H), 118 (191-C\(_3\)H\(_5\)O\(_2\)), 115, 113, 104 (177-C\(_3\)H\(_5\)O\(_2\)). Fragment
Fig. 14 Mass Spectrum of XXXIX.
ions at 312 and 200 established the position of thiazinone ring at C₄. Mass ions at 191 and 177 confirmed the isomeric attachment of mercaptan side chain both on β- and δ-carbons (Scheme-22).

The compound (XXXVII) is an ester hydrolysed product of XXXVI as confirmed by its elemental (C₂₄H₄₄O₅S₂N) IR, NMR and Mass analyses. Nonappearance of a singlet for three ester protons in NMR spectrum further showed the structure (XXXVII) as 4-thiazinone-2(3)-(S-β-mercaptopropionic acid) octadecanoic acid.

It is interesting to observe two chemical shifts for ester protons in NMR spectra of only those compounds (XXXII, XXXIII, XXXVI) in which C₄ is occupied by a ring whereas no such observation was made in the absence of a ring at C₄ (XXVIII, XXIX).
Section IV

Thiourea ———

Thiazolidinediones and Thiazole Derivatives

Thiazolidinediones are the derivatives of thiazolidinones and these in turn are the derivatives of thiazolidine which belong to a significant group of heterocyclic compounds. Thiazolidinediones with a carbonyl group at position 2, 4 or 5 have been a topic of extensive study in the recent past. Numerous reports on their use and chemistry have appeared in the literature. Thiazolidinediones and their derivatives have been found to be associated with diverse biological activities.

2-Imino-4-oxo-5-thiazolidineacetic acid can be obtained by refluxing equimolar amounts of substituted or nonsubstituted thiourea and maleic anhydride. Thiourea reacts in enol form with maleic or fumaric acid to afford the 2-imino-4-oxo-5-thiazolidineacetic acid. Nagasaka et al. reported the formation of ethyl ester of 2-imino-4-oxo-5-thiazolidine acid from diethylmaleate and thiourea. This reaction followed the following mechanism:

\[
\begin{align*}
\text{HOOC} & \quad \text{COOH} \\
\text{HC=CH} & \quad \text{HOOC-C=NH} \\
\text{HOOC} & \quad \text{HOOC-CII2} \\
\text{H}_2\text{N} & \quad \text{S} \\
\text{C} & \quad \text{C} \\
\text{NH}_2 & \quad \text{NH}_2 \\
\text{O} & \quad \text{OH} \\
\text{C} & \quad \text{C} \\
\text{=NH} & \quad \text{=NH} \\
\end{align*}
\]
The 4-thiazolidinones can be synthesized from various N-substituted acetylthioacetamide on treatment with monochloroacetic acid in the presence of AcONa in refluxing with AcOH\textsuperscript{61}. Substituted 2-imino-4-thiazolidinones are obtained in good yields by the reaction of symmetrical and unsymmetrical thioureas with various substituted and nonsubstituted α-haloalkanoic acids and their esters\textsuperscript{62}. The synthesis of 2-imino-4-thiazolidinones-4-\textsuperscript{14}C has been reported by using thiourea and sodium salt of the labeled monochloroacetic acid\textsuperscript{63}. Mixed anhydride reacts with substituted and nonsubstituted thioureas to give various 2-imino-4-thiazolidinones\textsuperscript{64,65}.

Various 2-(substituted-imino)-4-thiazolidinones undergo hydrolysis at position 2 on treatment with dilute hydrochloric acid or sulfuric acid to give 2,4-thiazolidinedinones and amines\textsuperscript{66-70}.

Thiazole may be synthesized by a number of methods. Cyclocondensation of α-bromoketone with substituted thiourea gave 2-aminothiazoles\textsuperscript{71}. Yang et al.\textsuperscript{72} reported the formation of substituted 2-aminothiazoles. Durguya et al.\textsuperscript{73} reported the formation of 2-amino-5-mercaptomethyl thiazole by the reaction of thiourea and potassium thiocyanate with epoxide. Recently, the synthesis of nonsubstituted fatty thiazoles were reported from the author's Laboratory\textsuperscript{41}. The thiazoles and their derivatives\textsuperscript{45} have shown biological and nonbiological activities.
The importance of nitrogen and sulfur possessing heterocyclic ring compounds led to the preparation of fatty thiazolidinedione and thiazole.

**Reaction of Thiourea with Methyl 4-oxo-trans-2-octadecenoate (XIIb)**

A solution of methyl 4-oxo-trans-2-octadecenoate (XIIb) in alcohol was refluxed with thiourea, CH₃COONa and dil. HCl. Reaction product on fractionation finally yielded two products (Scheme-23).

**Scheme-23**

\[
R - C - CH = CH - COOCH₃
\]

(XIIb)

\[
\text{NH₂CSNH₂, CH₃COONa, alcohol, dil. HCl (Reflux)}
\]

\[
R - C - CH₂ - CH = CH - COOH
\]

(XXXVIII)

\[
R - C = C - CH₂ - COOH
\]

(XXXIX)

\[
R = CH₃(CH₂)₁₃
\]
Characterization of Product (XXXVIII)

The microanalysis of the compound (XXXVIII) corresponded to the molecular formula $\text{C}_{19}\text{H}_{33}\text{O}_3\text{SN}$. The IR spectrum displayed characteristic bands for NH grouping at 3230 and 3150 cm$^{-1}$. It also showed three bands in carbonyl region at 1710 (CO), 1755, 1690 cm$^{-1}$ (CONHCO). NMR spectrum exhibited diagnostic peaks at $\delta$ 3.21 ill defined triplet (1H, CH$_2$), 2.50 m (4H, CH$_2$-CO-CH$_2$) and 4.20 br, s (1H, -NH, disappeared on D$_2$O addition). On the basis of these data the compound (XXXVIII) was formulated as 5-(2-oxohexadecyl) 2,4-thiazolidinedinone.

MS was further useful in supporting the assigned structure. Highest ion peak at m/z 355 ($M^+$, Fig. 13). Mass ions at 116 and 117 fragmenting from $\alpha$-cleavage of the ring established the location of the ring. The diagnostic mass ions at 225 and 158 were supposed to arise from the cleavage $\alpha$ to oxo function (Scheme-24). Other significant peaks were recorded, 341 ($\equiv$-N), 340 ($\equiv$-NH), 313, 312, 294, 281, 280 (281-H), 253, 239, 157 (158-H), 130, 129, 98 (158-SCO) and 83 (98-NH).

Characterization of Product (XXXIX)

Compound (XXXIX) analysed for $\text{C}_{19}\text{H}_{34}\text{O}_2\text{N}_2\text{S}$. In its IR spectrum, absorption bands were appeared at 3480, 3245 (-NH$_2$), 3400 (COOH), 1710 (COOH), 1630 (-C=O-), 1570, 1370 cm$^{-1}$ (C=N, C-N). The NMR spectrum of the compound showed structure -revealing
Fig. 13 Mass Spectrum of XXXVIII.
multiplets at δ 2.22 (2H, -CH₂-C=C-) and 3.0 (2H, -CH₂-COOH). Signal at δ 3.0 appeared as a multiplet, due to long range coupling. Two D₂O exchangeable signals were also appeared, one at δ 4.0 br.s (2H, -NH₂) and another at δ 8.90 br.s (1H, -COOH). These data are in agreement with structure of the compound (XXXIX) as 2-amino-5-tetradecyl-4-methylenecarboxy-thiazole.

The structure of XXXIX was further substantiated by its mass spectrum. It gave molecular ion peak at m/z 354 (Fig. 14). MS afforded prominent β-cleavage ions at 309 and 171 which located the position of the thiazole ring. Fragment ions at 157, and 295 resulted from α-cleavage of the ring (Scheme-25). Other significant ions were present at 338 (M-NH₂), 337 (M-OH), 336 (M-H₂O), 307 (336-COH), 291 (307-NH₂), 279 (307-CNH₂), 264 (279-NH), 263, 262 (295-SH), 261 (295-H₂S), 153 (171-H₂O), 126, 125 (157-S), 124 (157-SH), 121 (153-S), 120 (153-SH), 115, 112 (157-COOH), 109 (125-NH₂), 83 and 74.
Section V

Lead Tetraacetate (LTA)

Preparation of Enolacetate and α-Acetoxyketone

Enolate ion occupies a central place in the chemistry of oxo compounds. The composition of the enolate mixture may be governed by thermodynamic and kinetic factors. Hancu has synthesized enolacetates by heating diethyl ketones and diphenyl ketones with acetic anhydride and sodium acetate. Enolacetates of cyclic ketones were obtained with greater ease. Paul has reported a general method for the synthesis of enolacetates, by using a mixture of ketone, acetic anhydride and p-TSA.

In the author's laboratory various long chain oxo fatty acids were reacted with p-TSA and acetic anhydride to afford the enolacetates. When enolacetate has been treated with lead tetraacetate (LTA), it has yielded oxoacetate and the location of the acetoxy function has depended upon the position of double bond in the parent enolacetate.

Lead tetraacetate reacts with ketones to give α-acetoxyketones in high yields. BF₃-etherate can be used to catalyse these oxidations. It is presumed to function by catalysis of enolization and it is assumed that the enol is the reactive species. Evidence to confirm the intermediacy of the enol in the reaction comes from many sources. Moon and Bohm reported a number of oxoacetates.
Recently, in the author's laboratory, long chain oxo fatty acids were reacted with LTA in presence of acetic acid to produce α-acetoxyketones.

Enolacetate derivatives have applications in oil based industries. The carbonyl groups are especially important in producing such type of functional groups. If enolates are allowed to react with acetic anhydride, a quick formation of enolacetate occurs. In the present study long chain enolacetate has been prepared from methyl 4-oxo-trans-2-octadecenoate (XIIb). Further, enolacetate derivative was converted to the corresponding α-acetate with LTA.

Synthesis of Enolacetate with Methyl 4-oxo-trans-2-octadecenoate (XIIb)

Methyl 4-oxo-trans-2-octadecenoate (XIIb) was heated under reflux for 1 hr with acetic anhydride and p-TSA and the reaction mixture on column chromatographic purification yielded XL (Scheme-26).

Compound (XL) analysed for C_{21}H_{36}O_{4}. The IR spectrum of the product (XL) exhibited the band at 1750 cm\(^{-1}\) for vinyl acetate carbonyl and an absorption at 1730 cm\(^{-1}\) for ester carbonyl. Two intense bands at 1650 and 970 cm\(^{-1}\) were attributed to unsaturation and trans unsaturation respectively. Other significant bands were also observed at 1220 (acetate) and 1170 cm\(^{-1}\) (C-O). The values
were in agreement with the enolacetate structure of product (XL).

Scheme-26

The NMR spectrum furnished conclusive support in favour of XL. It showed two doublets at $\delta$ 7.20 ($J=14$ Hz) and 6.96($J=14$ Hz) each for one proton of C- and a-carbon respectively. $J$ Value confirmed trans-olefin. A triplet and a multiplet appeared at $\delta$ 5.70 ($J=10$ Hz) and 2.30 which were assigned to one vinylic and two allylic protons. A sharp singlet at $\delta$ 2.21 was due to the three
acetoxy protons. Thus on the basis of these results the structure of compound (XL) was established as methyl 4-acetoxy-trans-2-4-octadecadienoate.

Enolacetates on reaction with LTA in acetic acid were reported to yield oxoacetates in the light of this report LTA oxidation of XL was conducted in acetic acid. Reaction mixture showed the formation of one product. Column chromatographic separation gave XLI (Scheme-26).

The microanalysis of XLI corresponded to formula $C_{21}H_{36}O_5$. IR spectrum had bands at 1740, 1730 and 1680 cm$^{-1}$ for acetate, ester carbonyl and conjugated oxo groups respectively. Other characteristic bands were observed at 1640 ($\delta$-C=C), 1730 (acetate) and 970 (trans-unsaturation), 1060 and 1015 cm$^{-1}$ (C=O). The NMR spectrum displayed two doublets at $\delta$ 7.32 (J=14 Hz) and 6.59 (J=14 Hz) for $\beta$- and $\alpha$-unsaturated protons. J Value confirmed the configuration of the double bond as trans. A triplet for a methine proton $\alpha$ to acetoxy and oxo function appeared at $\delta$ 5.10 (J=6 Hz). Three acetoxy protons appeared in a singlet at $\delta$ 2.11. These data suggested the structure of XLI as methyl 5-acetoxy-4-oxo-trans-2-octadecenoate.

Formation of the product (XLI) was substantiated by its $^1$H NMR study (m/z 1, 369; Fig. 15). Structure-revealing mass ions are given in Scheme-27. The peaks were observed at 337 ($m/z$ $\text{C}_2\text{H}_4\text{O}_3$), 326 ($m/z$ $\text{C}_2\text{H}_2\text{O}$), 310 ($m/z$ $\text{COCCH}_2$), 309, 295 (326-$\text{OCH}_3$), 267 (310-$\text{COCH}_3$),
251 (309-OCOCH₂), 186, 156 (155+H), 155, 144, 129 (128+H), 128, 127 (186-OCOCH₃), 114 and 43 (principal peak).

**LTA Oxidation of Methyl 4-oxo-trans-2-octadecenoate (XIIb)**

Methyl 4-oxo-trans-2-octadecenoate (XIIb) was refluxed with LTA in acetic acid for 2 hr. Reaction mixture on column chromatographic separation yielded a product (Scheme-26) which possessed identical cochromatographic and spectral behaviour similar to those of XLI.
Section VI

Fuming Nitric Acid

Preparation of Nitrohydrin and Nitronitrate Derivatives

Nitric acid is usually employed for the introduction of nitro group. Nitration under drastic conditions affords cleavage products. Unsaturated aliphatic compounds undergo nitration readily than saturated hydrocarbons. A well known method for nitration is the use of a mixture of nitric acid and glacial acetic acid. The use of this nitrating mixture usually produced mononitro derivatives. The reaction mechanism of nitration is a topic of much discussion. It has been shown that for nitration with mixed acids, the nitrating species is the nitronium cation.

Chung-gi-Shin et al.\textsuperscript{83} reported the nitration of $\alpha,\beta$-unsaturated carboxylic ester with fuming nitric acid. Two products, $\alpha,\beta$-unsaturated $\alpha$-nitrocarboxylic ester and $\alpha$-hydroxy-$\beta$-nitrocarboxylic ester were formed. There appeared few reports in literature about the preparation of long-chain nitro derivatives. Therefore it was considered desirable to use fuming nitric acid in the synthesis of nitrogen-containing fatty derivatives. An $\alpha,\beta$-unsaturated oxo fatty acid ester has been selected for the present study.
Nitration of Methyl 4-oxo-trans-2-octadecenoate (XIIb)

A solution of methyl 4-oxo-trans-2-octadecenoate (XIIb) in acetic acid was nitrated with fuming HNO₃. Examination of the final reaction product by TLC showed two spots. Silica gel chromatographic separation gave XII and XIII (Scheme-28).

Scheme-28

\[
\begin{align*}
R & - C - CH = CH - COOCH₃ \\
& \xrightarrow{\text{Fuming } HNO₃, AcOH} \\
R - C - CH & \text{-COOH} \quad \text{ONO}_2 \quad \text{CH-CCH}_2 \\
& \quad \text{(XII)} \quad \text{(XIII)} \\
R & = \text{CH}_3 - (\text{CH}_2)_{13}
\end{align*}
\]

Characterization of Product(XII)

Elemental analysis corresponded to the formula C₁₈ᴴ₃₃O₆N. IR spectrum showed bands at 3400 br (COOH, OH), 1715 (CO), 1710 cm⁻¹ (COOH). The nitro group had two identical NO bonds which vibrate asymmetrically, showed strong absorption at 1560 cm⁻¹ and symmetrically showed somewhat weaker absorption at 1360 cm⁻¹. NMR spectrum gave conclusive support in favour of the structure.
of product (XLII). It exhibited resonances at $\delta$ 8.45 br,s ($1H$, COOH, $D_2O$ exchangeable), 4.30 d ($1H$, $-CH-NO_2$), 3.90 br,m (2H, $-CH-OH$, signal reduced to its half after $D_2O$ shake) and 2.30 m (2H, $-CH_2-CO$). On the basis of the above data the compound (XLII) was formulated as 4-oxo-2(3)-nitro-3(2)-hydroxyoctadecanoic acid.

**Characterization of Product (XLIII)**

Microanalysis gave the composition $C_{18}H_{32}O_8N_2$ for compound (LXIII). Its IR spectrum displayed bands for nitrate grouping at 1640 (asymmetrical stretching), 1250 (symmetrical stretching), 860 (N-O stretch.) and 750 cm$^{-1}$ (out of plane deformation). Nitro grouping absorptions were observed at 1565 and 1350 cm$^{-1}$. NMR spectrum showed a structure-confirming broad multiplet at $\delta$ 4.71-4.30 for two methine protons, one each for nitrate and nitro groupings. The protons $\alpha$ to free carbonyl appeared in a multiplet at $\delta$ 2.25 and an acid proton at $\delta$ 8.80 ($D_2O$ exchangeable). Elemental and spectral analyses elucidated the compound (XLIII) as 4-oxo-2(3)-nitro-3(2)-nitratooctadecanoic acid.

The nitrohydrin (XLII) was probably formed as a result of the addition of HONO$_2$ across the double bond and the simultaneous esterification of hydroxy group to the nitrate ester led to the formation of XLIII.
EXPERIMENTAL

All melting points were observed on a Kofler apparatus and are uncorrected. Infrared (IR) spectra were obtained with a Perkin-Elmer 621 spectrophotometer. IR values are given in cm⁻¹. Ultra-violet (UV) spectra were determined on Perkin-Elmer 202 Ultra-violet visible and Backman DK-2A spectrophotometers. Nuclear magnetic resonance (NMR) spectra were run on a Varian A60 instrument with tetramethylsilane (TMS) as internal standard. NMR values are given in ppm (δ). The abbreviations, 'w, s, d, m, br and t' denote 'weak, singlet, doublet, multiplet, broad and triplet', respectively. Mass spectra (MS) were measured with a AEI-Ms–902 at 70-eV. In the absence of 'ACCURATE' and 'DEUTERATED' mass spectra all the fragmentation schemes are to be considered tentative.

Thin layer chromatographic (TLC) plates were coated with silica gel and impregnated with a 20% aqueous solution of perchloric acid. TLC plates were charred at 110°C for 10 minutes. Column chromatography was carried out with silica gel (60-120 mesh) using 25-30 g per g of material to be separated. Elution was usually effected with petroleum ether (bp.40-60°C) or benzene containing increasing proportions of ether.

Preparation of trans-2-Enoic Acids (Va, Via)

Syntheses of trans-2-hexa-(Va) and octadecenoic (Via) acids were carried out from palmitic (I) and stearic (II) acids
following the method of Palameta and Prostenik as adopted in the author's laboratory.

**General Procedure**

Dry bromine (50 mL) was added drop by drop at 90°C in a period of 7 hr to a well-stirred mixture of saturated acid (100 g) and red phosphorous (4.6 g). The mixture was vigorously stirred during the addition of bromine by using a mercury sealed stirrer. Heating was continued for 24 hr and the cooled solution was poured into cold water and left overnight. The solid product was filtered, taken up in ether, washed successively with 10% aqueous solution of sodium sulfite and distilled water and dried over sodium sulfate (Na₂SO₄). The 2-bromoacid obtained after evaporation of the solvent was heated under reflux with powdered potassium iodide (96 g) in 95% ethanol (700 mL) for 6 hr. To the cooled solution potassium hydroxide (64 g) was added and the contents were refluxed for another 4 hr. Most of the alcohol was evaporated in vacuo and the residue diluted with water, acidified with hydrochloric acid (dilute) and extracted with ether. The combined ether extracts were washed with water and dried over anhydrous Na₂SO₄. After evaporation of the solvent, a mixture of α,β-unsaturated, 2-hydroxy and 2-ethoxy acids was obtained.

The 2-hydroxy acids (IX, X) were separated from α,β-unsaturated acids as copper chelate by treatment with cupric
acetate in acetic acid and ethanol. The remaining two fractions obtained after removal of 2-hydroxy acids were separated by silica gel column chromatography to furnish the individual components. Elution with petroleum ether-ether (94:6; v/v) gave pure \( \alpha, \beta \)-unsaturated acids as colorless products, yield \( \approx 52.0\% \). Crystallization-petroleum ether-ethanol (75:25; v/v). The 2-enoic acids on acid catalysed esterification yielded their corresponding esters.

**trans-2-Hexadecenoic acid** (V, m.p. 54°, lit. 84, m.p. 53.5°). Analysis— (Found: C, 75.46; H, 11.84%; Calcd. for \( C_{16}H_{30}O_2 \): C, 75.53; H, 11.89%). IR(CCl\(_4\)): 1730 (COOCH\(_3\)), 1645 (\(-CH=CH\)-) and 970 cm\(^{-1}\) (trans-olefin). NMR(CCl\(_4\)): \( \delta \) 6.89 d,d (1H, \(-CH=CH-COOCH\(_3\); J=15 and 5 Hz), 6.0 d (1H, \(-CH=CH-COOCH\(_3\); J=15 Hz), 3.71 s (3H, \(-COOCH\(_3\)), 2.43 m (2H, \(-CH_2=CH-CH=CH\)), 1.30 br.s (chain-\(CH_2\)) and 0.90 t (3H, terminal-\(CH_3\)).

**trans-2-Octadecenoic acid** (V\(_I\), m.p. 58-59°, lit. 84, m.p. 58.5°). Analysis— (Found: C, 76.51; H, 12.10%; Calcd. for \( C_{18}H_{34}O_2 \): C, 76.54; H, 12.13%). The spectral values (IR, NMR) of V\(_I\)b are identical as of V\(_b\).

**Preparation of 2-Oxooctadecanoic Acid** (Xa)

Jones' oxidation of 2-hydroxyoctadecanoic acid (X) afforded 2-oxooctadecanoic acid (Xa, m.p. 75°, lit. 85, m.p. 74.5°). Analysis— (Found: C, 72.30; H, 11.40%; Calcd. for \( C_{18}H_{34}O_3 \):
C, 72.43; H, 11.48%. IR(CCl₄): 3425 (COOH), 1720 (−CO−), and 1710 cm⁻¹ (COOH). NMR(CCl₄): δ 2.31 m (2H, −CH₂−CO−), 1.30 br, s (chain−CH₂−) and 0.88 t (3H, terminal−CH₃), 8.50 br, s (1H, −COOH, D₂O exchangeable).

Preparation of Methyl 4-oxo-trans-2-hexa-(XIa) and octadecenoate (XIIb)

A solution of chromium trioxide was prepared by addition of chromium trioxide (5 g, 0.05 mol) in small portions to a mixture of glacial acetic acid (25 mL) and acetic anhydride (12.5 mL)³. The solution was diluted with benzene (25 mL) under cold conditions. Compound Vb (2.68 g, 0.01 mol) or VIb (2.96 g, 0.01 mol) in benzene (5 mL) was added drop by drop with constant stirring in the solution of above reagent. The reaction mixture was kept below 15°C. The compound Vb or VIb was consumed within two hr as monitored by TLC. The reaction mixture was diluted with water, neutralized with an aqueous solution of sodium hydroxide, extracted with ether and dried. After evaporation of solvent and crystallization from hexane, white crystals were obtained, XIa (ϕ 84.0%, m.p. 64-65°C, lit. 86, m.p. 65-66°C); XIIb (ϕ 83.50%, m.p. 68°C). Analysis (XIa)-(Found: C, 72.22; H, 10.64%; Calcd. for C₁₇H₃₀O₃: C, 72.29; H, 10.71%); (XIb) (Found: C, 73.34; H, 9.97%; Calcd. for C₁₉H₃₄O₃: C, 73.50; H, 10.03%). UV(λmax): 220 nm. IR(KBr): 1735 (COOCH₃), 1670(=CO−CH=CH), 1640 (−C=C−) and 975 (trans-olefin), 1260, 1210, 1180 cm⁻¹(C−O stretching). NMR(CDCl₃): δ 7.06 d (1H, −CH=CH−COOCH₃;
J=16 Hz), 6.54 d (1H, -CH=CH-COOCH₃; J=16 Hz), 2.50 m(2H, -CH₂-CO-), 1.30 br, s (chain-CH₂) and 0.90 t (terminal-CH₃).

Preparation of 9,12-Dioxo-trans-10-octadecenoic Acid (XII)

A vigorously stirred solution of castor acids (100 g) (saponified oil of Ricinus communis seeds) in acetic acid (1100 mL) was oxidised by the addition of a solution of sodium bichromate dihydrate (73 g, 0.24 mol) in water (90 mL), acetic acid (650 mL) and concentrated H₂SO₄ (40 mL). More oxidising agent [sodium bichromate dihydrate (82 g, 0.27 mol), water (400 mL), acetic acid (200 mL) and conc. H₂SO₄ (40 mL)] was added, when the temperature had fallen from 54 to 47°C. The reaction content was stirred for one hr at 40-45°C. The desired product (XII) was precipitated with ice cold water and recrystallised from 90% ethanol. Yield is 22.0%, m.p.111°C (lit. ⁴, m.p.111-112°C). Analysis—(Found: C, 69.63; H, 9.71%; Calcd. for C₁₈H₃₀O₄: C, 69.64; H, 9.73%). UV(λmax.): 228 nm. 1R(KBr): 3425 (COOH), 1710 (COOH), 1680 (CO-CH=CH-CO), 1645 (C=C), 1210, 1180 (C=O), 980 cm⁻¹ (trans-olefin). ¹H NMR(CDC₁₃): δ 6.82 s (2H, -CH=CH-), 2.80 m (6H, 3xCH₂-CO), 1.32 br, s (chain-CH₂) and 0.88 t (3H, terminal-CH₃), 8.80 br, s (1H, -COOH, D₂O exchangeable).

Reaction of Excess Hydrazoic Acid with 2-Oxoocadecanoic Acid (Xa)

The method of Moural and Syhora¹² was used to prepare hydrazoic acid solution. 2-Oxoocadecanoic acid (Xa, 2 g; 0.006 mol)
in benzene (10 mL) was added drop by drop over a period of 4 hr to a cooled solution of hydrazoic acid and boron trifluoride (BF₃)-etherate (1.5 mL, freshly distilled). The mixture was refluxed for 20 hr. Solvent was removed _in vacuo_. The residue was dissolved in ether, washed with water, sodium bicarbonate (5%), water and dried over anhydrous Na₂SO₄. After evaporation of solvent, a semisolid was obtained that was chromatographed over silica gel to give XIX (semisolid) in ~100% yield. Analysis- (Found: C, 68.81; H, 11.30; N, 4.50%; Calcd. for C₁₈H₃₅O₃N: C, 68.90; H, 11.25; N, 4.46%). IR(nujol): 3400 (COOH), 3300 (NH stretching), 1710 (COOH), 1660, 1625 (CONH), 1535, 1520 (NH bending), 1170,1050 cm⁻¹ (C-O). _¹H NMR(CCl₄):_ 7.59 br,s (1H,-COOH, D₂O exchangeable), 3.42 m (2H,-CH₂-NH), 1.30 br,s (chain-CH₂), 0.90 t (terminal-CH₃), 9.40 br,s (1H,-COOH, D₂O exchangeable).

**Reaction of Methyl 4-oxo-trans-2-octadecenoate (XIIb) with Excess of Hydrazoic Acid**

Methyl 4-oxo-trans-2-octadecenoate (XIIb, 2 g; 0.006 mol) in benzene (10 mL) was added drop by drop over a period of 4 hr to a cooled solution of hydrazoic acid and BF₃-etherate (1.5 mL, freshly distilled). The mixture was stirred for 72 hr at room temperature and the solvent was removed _in vacuo_. The residue was dissolved in ether, washed with water, sodium bicarbonate (5%), water and dried over anhydrous Na₂SO₄. When ether was evaporated, a solid was obtained that was chromatographed over silica gel to
give three products. Elution with benzene-ether (96:4; v/v), gave XX. Crystallization at 10°C from petroleum ether (60-80°C) gave a yield of 66.0%, m.p. 52°C. Analysis— (Found: C, 65.01; H, 9.60; N, 15.40%; Calcd. for C_{19}H_{34}O_{2}N_{4}: C, 65.10; H, 9.70; N, 15.90%).

IR(nujol): 3060 w, 1720 (\text{\text{-COOCH}_{3}}), 1650 (C=C), 1505, 1460, 1375 (C=N, N=N), 1070, 1030, 1005 (C-O), 970 cm\(^{-1}\) (trans-olefin).

NMR(CDCl\(_3\)): \(\delta\) 7.81 d (1H, -\text{\text{-CH=CH-COOCH}_{3}}; J=14 Hz), 6.73 d (1H, -\text{\text{-CH=CH-COOCH}_{3}}; J=14 Hz), 3.82 s (3H, -\text{\text{-COOCH}_{3}}), 2.98 t (2H, -H\_2\mathbf{N-}) \text{\text{-C-}}, J=7 Hz), 1.30 br, s (chain-\text{\text{-CH}}\_2\mathbf{)} and 0.90 t (3H, terminal-\text{\text{-CH}}\_3\mathbf{)}.

Mass: \(M^+\) 350.

Further elution with benzene-ether (85:15; v/v) gave XXI. The yield is 10.0%, m.p. 96°C. Analysis— (Found: C, 69.60; H, 10.50; N, 4.0%; Calcd. for C_{19}H_{35}O_{3}N: C, 70.10; H, 10.80; N, 4.30%). IR(nujol): 3310 (NH stretch.), 3060 w (C=C), 1735 (\text{\text{-COOC}_{3}}), 1650, 1620 (CONH), 1640 (C=C), 1540, 1520 (NH bend.), 1160, 1030 (C-O) and 990 cm\(^{-1}\) (trans-olefin). NMR(CDCl\(_3\)): \(\delta\) 6.94 d (2H, -\text{\text{-CH=CH-COOCH}_{3}}; J=14 Hz), 6.67 m (1H, \text{\text{-NH}}, J=20 exchangeable), 3.82 s (3H, -\text{\text{-COOCH}_{3}}), 3.35 m (2H, -\text{\text{-CH}_{2}-NHCOC}) \text{\text{-H}}, 1.35 br, s (chain-\text{\text{-CH}}\_2\mathbf{)} and 0.88 t (3H, terminal-\text{\text{-CH}}\_3\mathbf{)}.

Mass: \(M^+\) 325.

Final elution with benzene-ether (80:20; v/v) gave XXII. The yield is 7.0%, m.p. 100°C. Analysis— (Found: C, 74.40; H, 12.70; N, 5.60%; Calcd. for C_{15}H_{31}N\(_2\mathbf{)}\): C, 74.60; H, 12.90; N, 5.80%).
IR (nujol): 3350, 3180 (NH\textsubscript{2} stretch.), 1650, 1620 (\textsuperscript{CONH\textsubscript{2}}), 1410 (C=\textsuperscript{N}) and 1160 cm\textsuperscript{-1} (NH\textsubscript{2}). N\textsubscript{MR} (CDCl\textsubscript{3}): \textsuperscript{5} 5.70 m (2H, \textsuperscript{CONH\textsubscript{2}}, disappeared on D\textsubscript{2}O addition), 2.16 m (2H, \textsuperscript{CH\textsubscript{2}CONH\textsubscript{2}}), 1.30 br, s (chain-CH\textsubscript{2}-) and 0.90 t (3H, terminal-CH\textsubscript{3}). Mass: M\textsuperscript{+} 241.

A solution of XXII in MeOH and catalytic amount of conc. H\textsubscript{2}SO\textsubscript{4} was refluxed for 6 hr to produce XXIII in 100\% yield. Analysis- (Found: C, 75.0; H, 12.60\%; Calcd. for C\textsubscript{16}H\textsubscript{32}O\textsubscript{2}: C, 74.90; H, 12.50\%). IR (neat): 1740 cm\textsuperscript{-1} (\textsuperscript{COOCH\textsubscript{3}}). N\textsubscript{MR} (CDCl\textsubscript{3}): \textsuperscript{3} 3.60 s (3H, \textsuperscript{COOCH\textsubscript{3}}), 2.25 m (2H, \textsuperscript{CH\textsubscript{2}-COOCH\textsubscript{3}}), 1.25 br, s (chain-CH\textsubscript{2}-) and 0.90 t (terminal-CH\textsubscript{3}).

Reaction of \textbeta-Mercaptoacetic Acid with Methyl 4-oxo-trans-2-hexadecenoate (XI\textalpha)

A solution of \textbeta-mercaptoacetic acid (1.7 g; 0.018 mol) and methyl 4-oxo-trans-2-hexadecenoate (XI\textalpha, 2g; 0.007 mol) in benzene (15 mL) was refluxed for 1 hr. Reaction mixture on workup gave a solid product which on crystallization in petroleum ether-ether (4:1; v/v) gave XXVIII in 98.0\% yield, m.p. 73\textdegree C. Analysis- (Found: C, 60.88; H, 9.10\%; Calcd. for C\textsubscript{19}H\textsubscript{34}O\textsubscript{5}S: C, 60.93; H, 9.15\%). IR (nujol): 3400 (\textsuperscript{COOH}), 1735 (\textsuperscript{COOCH\textsubscript{3}}), 1720 (\textsuperscript{CO}), 1710 (\textsuperscript{COOH}), 1440 (S-CH\textsubscript{2} deformation), 1260 (S-CH\textsubscript{2} wagging), 1170, 1060 cm\textsuperscript{-1} (\textsuperscript{C-O}). N\textsubscript{MR} (CDCl\textsubscript{3}): \textsuperscript{3} 3.70 s (3H, \textsuperscript{-COOH}), 3.23 m (3H, \textsuperscript{-CO-CH-S-CH\textsubscript{2}-COOH}), 2.40 m (4H, 2\times \textsuperscript{CH\textsubscript{2}-CO}), 1.30 br, s (chain-CH\textsubscript{2}-), 0.90 t (terminal-CH\textsubscript{3}) and 9.60 br, s (1H, \textsuperscript{-COOH}, disappeared on D\textsubscript{2}O shake). Mass: M\textsuperscript{+} 374.
Reaction of β-Mercaptopropionic Acid with Methyl 4-oxo-trans-2-hexadecenoate (XIa)

To a solution of methyl 4-oxo-trans-2-hexadecenoate (XIa, 2 g; 0.007 mol) in benzene (15 mL), β-mercaptopropionic acid (1.7 g; 0.016 mol) was added. The contents were refluxed for 1 hr. The reaction mixture on usual workup yielded a product, which crystallized in petroleum ether-ether (4:1; v/v) yielded (98.0%) a solid (XXIX) melting at 76°C. Analysis— (Found: C, 61.80; H, 9.20%; Calcd. for C_{20}H_{36}O_{5}S: C, 61.82; H, 9.32%). In(nujol): 3400 (COOH), 1740 (COOCH$_3$), 1720 (CO), 1715 (COOH), 1435 and 1260 (S-CH$_2$), 1160, 1120 cm$^{-1}$ (C-O). IR(CDC$_3$): δ 3.87 s (3H, -COOCH$_3$), 3.22 m (1H, -COCH$_2$-), 2.75 m (2H, -S-CH$_2$-CH$_2$-), 2.38 m (6H, 3xCH$_2$-CO-), 1.30 br,s (chain-CH$_2$-), 0.90 t (terminal-CH$_3$), 8.50 br,s (1H, -COOH, disappeared on D$_2$O addition). Mass: M$^+$ 388.

Reaction of β-Mercaptopropionic Acid with 9,12-Dioxo-trans-10-octadecenoic Acid (XII)

A similar reaction of β-mercaptopropionic acid (1.7 g; 0.016 mol) with 9,12-dioxo-trans-10-octadecenoic acid (XII, 2 g; 0.006 mol) in benzene (15 mL) was carried out for 1 hr. Usual workup yielded a semisolid (XXX) which was purified by column chromatography (80:20, v/v; petroleum ether-ether). Yield is 92.0%. Analysis— (Found: C, 60.50; H, 8.60%. Calcd. for C$_{21}$H$_{36}$O$_6$S: C, 60.55; H, 8.70%). IR(CCl$_4$): 3400 (COOH), 1715 (CO),
1710 (COOH), 1440, 1265 (S-CH\textsubscript{2}), 1160, 1060 cm\textsuperscript{-1} (C-O). N\textsubscript{MRCCl}\textsubscript{4}: δ 3.26 m (1H, CO-CH-S-), 2.70 m (2H, -S-CH\textsubscript{2}-), 2.30 m (10H, 5xCH\textsubscript{2}-CO), 1.30 br,s (chain-CH\textsubscript{2}-), 0.90 t (terminal-CH\textsubscript{3}), 9.30 br,s (2H, 2xCOOH, D\textsubscript{2}O exchangeable). Mass: +416.

Reaction of Methyl 4-oxo-trans-2-hexadecenoate (XIa) with Excess of β-Mercaptoethanol

A solution of methyl 4-oxo-trans-2-hexadecenoate (XIa, 2 g; 0.007 mol) in acetic acid (30 mL), β-mercaptoethanol (8 mL) and BF\textsubscript{3}-etherate (16 mL) was stirred at room temperature for 50 minutes. Progress of the reaction was monitored by TLC. At the end of the reaction, solvent was removed in vacuo. The residue was extracted with ether, washed with 5% solution of sodium bicarbonate and dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}. After evaporation of solvent, an oil was obtained which was chromatographed over silica gel column and afforded three products. Elution with petroleum ether-ether (94:6; v/v) gave XXXI; yield is 10.40%. Analysis—(Found: C, 60.90; H, 9.12%; Calcd. for C\textsubscript{19}H\textsubscript{34}O\textsubscript{3}S: C, 60.92; H, 9.14%). λ\textsubscript{max}(neat): 3030 (HC=CH), 1730 (COOC\textsubscript{2}H\textsubscript{5}), 1650 (-C=O-), 1440 (S-CH\textsubscript{2} deformation), 1200, 1220, 1280 (S-CH\textsubscript{2} wagging), 1120 (C-O), 1020, 1040 (oxathiolane or cyclic hemithioketal grouping), 980 cm\textsuperscript{-1} (trans-olefin). N\textsubscript{MRCCl}\textsubscript{4}: δ 6.82 d (1H, -CH=CH-CCOCl\textsubscript{3}; J=14 Hz), 5.82 d (1H,-CH=CH-COOCH\textsubscript{3}; J=14 Hz), 4.30-3.90 br,m (2H, protons α to oxygen in oxathiolane ring), 3.75 s (3H,-COOH), 2.98-2.78 br,m (2H, protons α to sulfur in oxathiolane ring),
Further elution with petroleum ether-ether (90:10; v/v) gave XXXII; yield is 48.0%. Analysis—(Found: C, 59.60; H, 9.14%; Calcd. for C_{23}H_{42}O_{5}S_{2}: C, 59.70; H, 9.14%). IR(nujol): 1740 br (OCOCH_{3}, COOCH_{3}), 1435 (S-CH\_2 def.), 1270 (acetate), 1250 (S-CH\_2 wag.), 1110 (C-O), 1020 cm\^{\text{-1}} (oxathiolane ring). NMR(CCl\_4): 
\delta 4.42-3.91 br,m (4H, two protons α to oxygen in oxathiolane ring, -CH\_2-O-OCOCH\_3), 3.65,3.62 s (3H,-COOCH\_3), 3.18-2.52 br,m (5H, two protons α to sulfur in oxathiolane ring,-CH-S-CH\_2), 2.46 m (protons α to ester), 2.02 s (3H,-OCOCH\_3), 1.80 m (protons α to oxathiolane ring), 1.50 br,s (chain-CH\_2-), 0.85 t (3H, terminal-CH\_3). Mass: M^+ 462.

Final elution with petroleum ether-ether (85:15; v/v) gave XXXIII; yield is 38.10%. Analysis—(Found: C, 59.89; H, 9.59%; Calcd. for C_{21}H_{40}O_{4}S_{2}: C, 59.95; H, 9.58%). IR(nujol): 3400 (OH), 1740 (OCOCH\_3), 1410 (S-CH\_2 def.), 1250 (S-CH\_2 wag.), 1125 (C-O) and 1020 cm\^{\text{-1}} (oxathiolane ring). NMR(CCl\_4): \delta 4.32-3.92 br,m (4H, 2 protons α to oxygen in oxathiolane ring,-CH\_2-OH), 3.90 br,s (1H, -CH\_2-OH, D\_2O exchangeable), 3.70,3.68 s (3H,-COOCH\_3), 3.45-2.72 br,m (5H, two protons α to sulfur in oxathiolane ring,-CH-S-CH\_2), 2.43 m (protons α to ester carbonyl), 1.72 m (protons α to oxathiolane ring), 1.25 br,s (chain-CH\_2-), 0.82 t (3H, terminal-CH\_3). Mass: M^+ 420.
Reaction of 9,12-Dioxo-trans-10-octadecenoic Acid (XII) with Excess of β-Mercaptoethanol

A solution of 9,12-dioxo-trans-10-octadecenoic acid (XII, 2 g; 0.006 mol), β-mercaptoethanol (10 mL) and BF$_3$-etherate (10 mL) in acetic acid (32 mL) was stirred at room temperature. Reaction was completed in 80 minutes as monitored by TLC. After usual workup and evaporation of solvent, a liquid reaction mixture was obtained which on column fractionation produced two products. Elution with benzene-ether (98:2; v/v) gave XXXIV; yield is 70.0%. Analysis—(Found: C, 58.73; H, 8.61%; Calcd. for C$_{24}$H$_{42}$O$_6$S$_2$: C, 58.74; H, 8.62%). IR(nujol): 3400 (COOH), 1740 (OCOCH$_3$), 1720 (CO), 1710 (COOH), 1440 (S-CH$_2$ def.), 1270 (acetate), 1240 (S-CH$_2$ wag.), 1175 (C=O), 1030 cm$^{-1}$ (oxathiolane ring). NMR(CCl$_4$): $\delta$ 4.40-3.95 br,m (4H, two protons α to oxygen in oxathiolane ring, -CH$_2$-O-CO), 3.20-2.60 br,m (5H, two protons α to sulfur in oxathiolane ring, -CH-S-CH$_2$), 2.30 m (protons α to CO and COOH), 1.99 s (3H, -OCOCH$_3$), 1.79 m (protons α to oxathiolane ring), 1.30 br,s (chain-CH$_2$-), 0.90 t (3H, terminal-CH$_3$), 8.70 br,s (1H, -COOH, D$_2$O exchangeable). m/z 490.

Second elution with benzene-ether (90:10; v/v) gave XXXV; yield is 28.0%. Analysis—(Found: C, 56.66; H, 8.40%; Calcd. for C$_{26}$H$_{46}$O$_6$S$_3$: C, 56.69; H, 8.41%). IR(nujol): 3400 (COOH), 1740 (OCO-CH$_3$), 1710 (COOH), 1420 (S-CH$_2$ def.), 1260 (acetate), 1220 (S-CH$_2$ wag.), 1140 (C=O), 1030 cm$^{-1}$ (oxathiolane
ring). NMR(CCl₄): δ 4.39-3.90 br,m (6H, four protons α to oxygen in both rings and S-CH₂-CH₂-OCOCH₃), 3.0-2.50 br,m (7H, four protons α to sulfur in both rings, -CH-S-CH₂), 2.70 m (2H,-CH₂-COOH), 2.0 s (3H,-OCOCH₃), 1.78 m (6H, protons α to both rings), 1.30 br,s (chain-CH₂-), 0.91 t (3H, terminal-CH₃), 8.50 br,s (1H, -COOH, D₂O exchangeable). Mass: M⁺ 550.

9,12-Dioxo-trans-10-octadecenoic acid(XII, 2 g; 0.006 mol), AcOH (32 mL), p-mercaptoethanol (10 mL) and BF₃-etherate (10 mL) were heated under reflux for one hour. Reaction products showed two distinct spots corresponding to XXXIV and XXXV in their Rf values. After usual workup the chromatographic separation gave compound (XXIV) 50.0% and compound (XXXV) 45.0% which showed identical composition and spectral behaviour as mentioned above.

A solution of XXXIV (0.5 g; 0.001 mol) in acetic acid (10 mL), p-mercaptoethanol (5 mL) and BF₃-etherate (6 mL) was stirred at room temperature. The reaction was completed in about 10 hr and gave one product as evidenced by TLC. This compound showed similar cochromatographic, elemental and spectral properties as of XXXV.

Reaction of β-Mercaptopropionic Acid and (NH₄)₂CO₃ with Methyl 4-oxo-trans-2-octadecenoate (XIIb) (Azeotropic Condition)

A solution of methyl 4-oxo-trans-2-octadecenoate (XIIb, 2 g; 0.006 mol) and β-mercaptopropionic acid (2 g; 0.002 mol) in
dry benzene (30 mL) was refluxed in a water collector for 30 hr following the procedure of Paryzek and coworker in the presence of \((\text{NH}_4)_2\text{CO}_3\). Reaction mixture showed two distinct spots on TLC. After usual workup the reaction products were purified by column chromatography using silica gel and a mixture of benzene-ether (80:20, v/v) gave XXXVI in 86.0% of yield. Analysis—(Found: C, 59.50; H, 9.3; N, 2.72%). Calcd. for \(C_{25}H_{45}O_5S_2N\): C, 59.60; H, 9.0; N, 2.78%. IR(\text{nujol}): 3425 (COOH), 3165 (NH stretch.), 1735 (\text{COOCH}_3), 1710 (COOH), 1670 (Lactam carbonyl, \text{CONH}-), 1440 (S-CH2 def.), 1410 (C-N), 1240 (S-CH2 wag.), 1120, 1070 (C-O) and 780 cm\(^{-1}\) (NH wag.). \text{NMR}(\text{CCl}_4): \delta 4.30 br,s (1H, -NH; D\(_2\)O exchangeable), 3.68, 3.70 s (3H,-\text{COOCH}_2), 3.20–2.80 br,m (5H, two protons \(\alpha\) to sulfur in ring, -CH-S-CH2-CH2-), 2.60 unresolved triplet (2H, -CH2CONH), 2.30 br,m (protons \(\alpha\) to ester carbonyl,-CH2-COOH), 1.70 m(protons \(\alpha\) to ring), 1.30 br,s (chain-CH2-), 0.90 t (terminal -CH3) and 8.50 br,s (1H, -COOH, disappeared on D\(_2\)O addition). Mass: \text{M}^+ 503 (absent), 431 (M-\text{C}_3\text{H}_4\text{O}_2).

Subsequent elution with benzene-ether (60:40; v/v), followed by crystallization from petroleum ether-ether gave XXXVII in 13.10% yield, m.p. 82°C. Analysis—(Found: C, 58.80; H, 8.80; N, 2.83%; Calcd. for \(C_{24}H_{43}O_5S_2N\): C, 58.86; H, 8.85; N, 2.86%). IR(\text{nujol}): 3430 (COOH), 3160 (NH stretch.), 1715 br (COOH), 1665 (Lactam carbonyl, \text{CONH}), 1435 (S-CH2 def.), 1410 (C-N), 1240 (S-CH2 wag.), 1170, 1070 (C-O) and 775 cm\(^{-1}\) (NH wag.). \text{NMR}(\text{CCl}_4): \delta 4.29 br,m (1H, -NH; D\(_2\)O exchangeable), 3.20–2.81 br,m (5H, protons...
α to sulfur in ring, -CH=S-CH₂-CH₂-COOH), 2.59 unresolved triplet (2H, -CH₂-CO-NH-), 2.30 br, m (protons α to acid carbonyls), 1.70 m (protons α to ring), 1.30 br, s (chain-CH₂-), 0.90 t (terminal-CH₃), 9.10 br, s (2H, 2xCOOH, disappeared on D₂O shake). Mass: M⁺ 489(8), m/z 471 (M-H₂O; 12), 453 (471-H₂O; 13), 312 (17) and 292 (8) [α-cleavage of the ring at C₄], 177 (8) and 163 (10) [for isomeric attachment of mercaptan side chain both at β- and ς-carbons] and 45 (principal ion).

Reaction of Thiourea with Methyl 4-oxo-trans-2-octadecenoate (XIIb)

To a solution of methyl 4-oxo-trans-2-octadecenoate (XIIb, 3 g; 0.009 mol) in ethanol (30 mL), sodium acetate (anhydrous) (1.1 g; 0.01 mol) and thiourea (0.72 g; 0.009 mol) were added and the mixture was refluxed on water bath for 2 hr. After adding dil. HCl the contents were refluxed for another hr. After evaporation of alcohol, reaction mixture was extracted with ether. After evaporation of solvent, a solid reaction mixture was obtained. The reaction mixture was fractionated by column chromatography to give two products. First elution with petroleum ether-ether(93:7; v/v) produced XXXVIII; yield is 71.30%, m.p. 55°C. Analysis- (Found: C, 64.20; H, 9.40; N, 3.90%; Calcd. for C₁₉H₃₃O₃SN: C, 64.18; H, 9.35; N, 3.93%). IR(nujol): 3230, 3150 (>NH), 1710 (>C=O), 1755, 1690 (CONHCO), 1460 (C-N), 1180 (C-O), 780 cm⁻¹ (NH). NMR(CCl₄): δ 4.20 br, s (1H, -NH-, D₂O exchangeable), 3.21 ill defined triplet (1H, CH=S), 2.50 m (4H, -CH₂-CO-CH₂), 1.30 br, s (chain-CH₂-), 0.90 t (3H, terminal-CH₃). Mass: M⁺ 355.
Second elution with petroleum ether–ether (89:11; v/v) gave XXXIX; yield is 13.0%, m.p. 61°C. Analysis—(Found: C, 64.30; H, 9.60; N, 7.90%; Calcd. for C_{19}H_{34}O_{2}N_{2}: C, 64.36; H, 9.66; N, 7.90%). IR(nujol): 3480, 3245 (−NH_{2}), 3400 (COOH), 1710 (COOH), 1630 (>C=C<), 1570, 1370 (C=N, C=N), 1160 cm\(^{-1}\) (C=O). \(\mathrm{^1}H\) NMR(CCl\(_4\)): δ 4.0 br, s (2H, −NH\(_2\), disappeared on D\(_2\)O shake), 3.0 m (2H, CH\(_2\)-COOH), 2.22 m (2H, −CH\(_2\)-C=C), 1.30 br, s (chain−CH\(_2\)-), 0.90 t (3H, terminal −CH\(_3\)), 8.90 br, s (1H, −COOH, D\(_2\)O exchangeable). M\(^+\) 354.

Synthesis of Enolacetate from Methyl 4-oxo-trans-2-octadecenoate (XIIb)

Methyl 4-oxo-trans-2-octadecenoate (XIIb; 2 g; 0.006 mol) was added to a mixture of acetic anhydride (3.7 mL) and p-TSA (0.5 g; 0.003 mol) and refluxed for 1 hr following the method of Paul.\(^76\) The temperature was kept below 125°C. The reaction mixture was poured into water and taken up in ether. The solvent layer was washed with water and sodium bicarbonate (5%) and then dried. Evaporation of the solvent yielded a liquid product which was chromatographed over silica gel. Elution with petroleum ether–ether (96:4; v/v) gave XL in 85.0% yield. Analysis—(Found: C, 71.56; H, 10.30%; Calcd. for C_{21}H_{36}O_{4}: C, 71.55; H, 10.29%). IR(neat): 3030 (CH=CH), 1750 (OCOC\(_3\)), 1730 (COOC\(_3\)), 1650 (C=C−, C=C), 1220 (acetate), 1170, 1080 cm\(^{-1}\) (C=O) and 970 (trans-olefin). \(\mathrm{^1}H\) NMR(CCl\(_4\)): δ 7.20 d (1H, −CH=CH−COOCH\(_3\); J=14 Hz), 6.96 d (1H, −CH=CH−COOCH\(_3\); J=14 Hz), 5.70 t (1H, −CH=C−; J=10 Hz), 3.67 s (3H, OAc).
-COOCH_3), 2.21 s (3H, -OCOCH_3), 2.30 m (2H, -CH_2-CH=CH_2), 1.30 br, s (chain-CH_2) and 0.88 t (3H, terminal-CH_3). Subsequent elution with petroleum ether-ether (86:14; v/v) afforded starting 4-oxo (XIIb) in 14.50% of yield.

**Preparation of LTA**

\[
Pb_3O_4 + 8C_2H_3O_2 \rightarrow Pb(C_2H_3O_2)_4 + 2Pb(C_2H_3O_2)_2 + 4H_2O
\]

A mixture of 10 g of acetic anhydride and glacial acetic acid 45 g is placed in a three-necked flask of one litre capacity equipped with a thermometer and a mercury sealed stirrer. The mixture was heated to 55-60°C, stirred vigorously and 25 g of lead powder (dry) was added in portions of 2-3 g. A fresh addition was done only after the color due to the preceding portion had largely vanished. The temperature of the content was not permitted to rise above 65°C. In order to complete the reaction it was necessary to heat the reaction mixture above 80°C towards the end. The thick and somewhat dark solution was cooled at the end of the reaction and LTA (precipitated) was filtered off and washed with glacial acetic acid. The crude product was dissolved in hot glacial acetic acid, containing a small amount of acetic anhydride. The solution was treated with a little decolorising carbon and filtered through a warm water funnel and cooled. 12 g of colorless crystalline product was filtered off and dried in vacuum desiccator over KOH pellets.
LTA Oxidation of Methyl 4-acetoxy-trans-2,4-octadecadienoate (XL)

Methyl 4-acetoxy-trans-2,4-octadecadienoate (XL, 0.5 g) was refluxed for 2 hr with equimolar amount of LTA (0.62 g) in 45 mL of acetic acid following the method of Noon et al. Acetic acid was removed in vacuo and the reaction mixture was extracted with ether, washed with 5% aq. sodium bicarbonate, water and dried. Column chromatography purification yielded XLI in 100% yield.

Analysis—(Found: C, 68.39; H, 9.70%; Calcd. for C_{21}H_{38}O_{5}: C, 68.44; H, 9.80%). IR(CCl₄): 3010 (CH=CH), 1740 (OCOCH₃), 1730 (OCOCOCH₃), 1680 (OC=CH=CH), 1640 (-C=O), 1230 (acetate), 1060 and 1015 (C-O), 970 cm⁻¹ (trans-unsaturation). NMR(CCl₄): δ 7.32 d (1H, -CH=CH-COOCH₃; J=14 Hz), 6.59 d (1H, -CH=CH-COOCH₃; J=14 Hz), 5.10 t (1H, -CH–O–COOCH₃, J=6 Hz), 3.85 s (3H, -COOCH₃), 2.11 s (3H, -OCOCH₃), 1.30 br,s (chain-CH₂), 0.90 t (terminal-CH₃). Mass:M+1 369.

LTA Oxidation of Methyl 4-oxo-trans-2-octadecenoate (XIIb)

Methyl 4-oxo-trans-2-octadecenoate (XIIb, 2 g) was refluxed for 2 hr with equimolar amount of LTA (2.88 g) in 45 mL of acetic acid by the method adopted in the previous case (XL). Acetic acid was removed in vacuo and the reaction mixture was extracted with ether, washed with 5% aq. sodium bicarbonate, water and dried. Reaction mixture was passed over a column of silica gel. Elution with petroleum ether-ether (90:10, v/v) gave the parent oxo (XIIb); yield is 17.0%. Subsequent elution with
petroleum ether-ether (80:20; v/v) afforded the product (XLJ), the yield is 80.30%.

Nitration of Methyl 4-oxo-trans-2-octadecenoate (Xlb)

Methyl 4-oxo-trans-2-octadecenoate (Xlb, 2g; 0.006 mol) in glacial acetic acid (20 mL) was vigorously stirred at room temperature and treated with fuming nitric acid (6 mL, d=1.5, 0.14 mol) over a period of one hour. Reaction mixture was extracted with ether, washed successively with water, sodium bicarbonate (2%), water and dried (Na2SO4). On evaporation of solvent a semi-solid product was obtained which when chromatographed over silica gel column produced two products. Elution with petroleum ether-ether (95:5; v/v) produced XLII in 75.10% yield. Analysis-(Found: C, 60.11; H, 9.23; N, 3.88%; Calcd. for C18H33O6N: C, 60.14; H, 9.25; N, 3.89%). IR(neat): 3400 br (COOH, OH), 1715 (-CO-), 1710 (COOH), 1560 and 1360 (C-NO2), 1160 cm⁻¹ (C-O). NMR(CCl4): δ 4.30 d (1H, -CH-NO2), 3.90 br, m (2H, -CH2-OH after D2O shake signal reduced to its half), 2.30 m (2H, -CH2-C=O), 1.30 br, s (chain-CH2-), and 0.90 t (3H, terminal-CH3), 8.45 br, s (1H, -COOH, D2O exchange-able).

Subsequent elution with petroleum ether-ether (70:30; v/v) gave XLIII in 22.50% yield. Analysis-(Found: C, 53.32; H, 7.99; N, 6.88%; Calcd. for C18H32O6N2: C, 53.45; H, 7.97; N, 6.93%). IR(neat): 3425 (COOH), 1720 (-CO-), 1710 (COOH), 1640 (C-CNO2), 1565 and 1350 (C-NO2), 1250 (C-ONO2), 1160 (C-O), 860,
750 cm$^{-1}$ (C-ONO$_2$). NMR(CCl$_4$): $\delta$ 4.71-4.30 br, m (2H, -CH$_2$-ONO$_2$, -CH$_2$-NO$_2$), 2.25 m (2H, CH$_2$-CO-), 1.30 br, s (chain-CH$_2$) and 0.88 t (3H, terminal-CH$_3$), 8.80 br, s (1H, -COOH, D$_2$O exchangeable).
REFERENCES


75. Mannich and Hancu, Ibid. 41:564 (1908).
Publications and Presentations

1. Acacia catechu Seed Oil—Characterization of H\textsubscript{2}R-Reactive Acid.

2. Synthesis of Tetrazole from $\alpha,\beta$-Unsaturated Carbonyl Fatty Acid.

3. Synthesis of Sulfur Heterocycles from $\alpha,\beta$-Unsaturated Carbonyl Fatty Acid Ester.
   Ibid. (Published in May, 1984).

4. An 8-Hydroxy Dienoic Acid from Mirabilis jalapa Seed Oil.
   Phytochem. (in press).

5. Studies on Minor Seed Oils—XV.
   Fette, Seifen, Anstrichm. (in press).

6. Synthesis of N-Heterocyclic Derivatives from $\alpha,\beta$-Unsaturated Carbonyl Fatty Acid.
   In 37th Annual Convention of OTAI, held on Feb. 13-14, 1982 at Kanpur, India.

7. Synthesis of S-Heterocyclic Derivatives from $\alpha,\beta$-Unsaturated Oxo Fatty Acid.
   In 2nd Annual Conference of Indian Council of Chemists', held on Nov. 6-8, 1982 at Srinagar, India.