# SOME STUDIES OF SEED OILS

being a thesis

presented by

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# CONTENTS

## ABBREVIATIONS

| | |
|---|---|
| ApL | Apiezon L grease |
| b.p. | Boiling Point |
| DEGS | Diethyleneglycolsuccinate polyester |
| ECL | Equivalent Chain Length or Carbon Number |
| GLC | Gas Liquid Chromatography |
| NMR | Nuclear Magnetic Resonance |
| PE 40 | Petrol/ether = 60/40 |
| TLC | Thin Layer Chromatography |
| $Ag^+$/TLC | Silver Ion Thin Layer Chromatography |

## CERTIFICATE

I hereby certify that Mr. Samuel Ronald Steward has spent eleven terms at research work under my supervision, has fulfilled the conditions of Ordnance 51 (St. Andrews), and is qualified to submit the accompanying Thesis for the degree of Master of Science.

Research Supervisor

## DECLARATION

I hereby declare that this Thesis is a record of the results of my own experiments, that it is my own composition and that it has not been presented in application for a higher degree.

ACKNOWLEDGEMENTS

I wish to express my indebtedness to Dr. F. D. Gunstone for the invaluable help he so freely gave during my research studentship.

I would also like to thank Professors Cadogan, Tedder and Wyatt for providing the research facilities and all the members of the teaching and technical for their assistance.

Congratulations are expressed to Miss Fran Christie,M.A. for her success in the preparation of stencils in the face of indecipherable handwriting; the generous help of Mr. T. McQueen in printing this thesis also merits special mention.

Finally, I thank the Tropical Products Institute for the award of a research studentship and Dr. J. A. Cornelius of T.P.I. for the supply of many seed samples.

## 1. Examination of Various Seed Oils

### (a) Introduction

This thesis describes the results obtained during the preliminary examination of a series of seed oils along with a closer examination of the component acids of a few oils which were of special interest. The purpose of this investigation included, in particular, the following objectives:-

(i) The examination of a wide range of seed oils in the hope of discovering some of potential commercial importance.

(ii) The isolation and identification of hitherto unknown acids and a study of their chemical properties.

A similar but more comprehensive study carried out by the U.S. Department of Agriculture over a period of years, mainly at the Northern Regional Research Station, Peoria, Illinois, has yielded valuable information. Several oils of possible commercial value are being studied intensively and many new acids - some with very interesting structures - have been reported.[1] This has followed the routine investigation of more than 2000 species of seeds.

A similar but smaller program has been launched by the Tropical Products Institute under the direction of Dr. J. A. Cornelius and some early results have already been reported.[2] The examination of 83 samples

is reported in this thesis.

(b) Procedure

The procedures used in this study are outlined here and reported in detail in the experimental section.

Seed Oil Extraction

The seeds were separated from the husk and the kernels milled to a fine powder which was extracted with light petrol (b.p. 40-60°) in a Soxhlet apparatus. When this was complete the solvent was evaporated off under vacuum and free fatty acids were removed from the extract by elution through a short alumina column with chloroform. The chloroform was then removed under vacuum to leave neutral triglycerides.

Preparation of methyl esters

The triglycerides were transesterified by refluxing with a methanolic solution of sodium methoxide, acidifying with dilute hydrochloric acid and extracting with ether. After drying over anhydrous sodium sulphate the ether solution was evaporated under vacuum to leave the methyl esters.

Analysis

The mixture of esters analysed by GLC on polar (DEGS, 190°C) and non-polar (APL, 210°C) columns, and TLC (silica and argentation). IR spectroscopy was used to detect unusual features such as trans double-

bonds and UV spectroscopy to estimate conjugated unsaturation.

(c) Results

Full details of the results are given on pp.16-22 and some general points of interest are discussed here.

(i) Yield of Oil

Most of the seeds examined contained a relatively small amount of oil. It is seen from Table I that only 9 seeds gave oils in excess of 40%. Since these are more likely to be of interest their composition is given in Table 2, but it is apparent that the only unusual feature is the large amount of 12:0 (96%) present in C. iners(Lauraceae).

Table 1: Distribution of Yields of Oil

| Oil Yield | Samples | Oil Yield | Samples |
|---|---|---|---|
| 5-9% | 32 | 30-39% | 0 |
| 10-19% | 21 | 40-49% | 2 |
| 20-29% | 21 | 50% upwards | 7 |

(ii) Table 2: Seeds yielding >40% oil

| Family | Species | % Oil | % Composition | | | |
|---|---|---|---|---|---|---|
| | | | 16:0 | 18:0 | 18:1 | 18:2 |
| Lauraceae | Cinnamonum iners | 40 | (contains 12:0 (96%)) | | | |
| Guttiferae | Callophyllum inophyllum | 70 | 17 | 20 | 31 | 32 |
| Anacardiaceae | Spondias pinnata | 53 | 9 | 7 | 37 | 45 |
| Meliaceae | Khaya grandifolia | 59 | 10 | 13 | 58 | 17 |
| " | Khaya ivorensis | 62 | 8 | 5 | 67 | 20 |
| " | Khaya nyasica | 72 | 11 | 12 | 63 | 14 |
| " | Khaya anthotheca | 82 | 15 | 9 | 53 | 22 |
| Combretaceae | Terminalia phallocarpa | 42 | 28 | 4 | 20 | 47 |
| Burseraceae | Canarium vulgare | 63 | 29 | 12 | 49 | 10 |

(iii) Oils rich in polythenoid acids

Attention is drawn in Table 3 to seven oils containing polythenoid acids in excess of 60% since these may be of use as drying oils. Unfortunately none of them come from seeds which are particularly rich in oil, 27% (C. nobile) being the highest yield recorded. Only one of these (L. formosa) had any linolenic acid (16%) and in general the main component was linoleic acid which reached a maximum of 81% in L. indica.

Table 3: Oils rich in polythenoid acids

| Family | Species | % Oil | % Composition | | |
|---|---|---|---|---|---|
| | | | 18:1 | 18:2 | 18:3 |
| Papilionaceae | Lespedeza formosa | 10 | 15 | 55 | 16 |
| Acanthaceae | Ruellia tuberosa | 22 | 11 | 65 | |
| Solanaceae | Capsicum frutescens | 12 | 15 | 64 | |
| Casuarinaceae | Casuarina nobile | 27 | 17 | 71 | |
| Mimosae | Acacia auriculaeformis | 6 | 10 | 67 | * |
| " | Mimosa invisa (var.intermis) | 5 | 13 | 68 | |
| Lythraceae | Lagerstroemia indica | 6 | 6 | 81 | |

* contains 4% of unidentified epoxy acid.

(iv) Oils resembling those already in common use

For an earlier publication[3] attention was drawn to some seed oils which resembled those in common use such as cottonseed oil, groundnut oil, olive oil and cocoa butter. Table 4 lists the characteristic composition of these common oils along with oils similar in composition discovered in the screening program. T. phallocarpa (Combretaceae) is the only seed likely to be of interest since it is similar in nature to cottonseed but yields more oil (42%). All the other seeds listed are not rich enough in oil to provoke commercial interest.

In all 13 oils were found to be similar to cottonseed oil , 1 to cocoa butter, 1 to groundnut oil. There was no oil resembling olive oil.

Table 4: Oils similar in composition (% wt.) to some common oils

| Type or Species (Family) | % Oil | 16:1 | 18:0 | 18:1 | 18:2 | Others |
|---|---|---|---|---|---|---|
| Cottonseed oil | 30 | 28 | 3 | 18 | 51 | |
| Terminalia phaeocarpa (Combretaceae) | 42 | 28 | 4 | 20 | 47 | |
| Bauhinia megalandria (Caesalpiniaceae) | 18 | 23 | 12 | 15 | 48 | |
| Luffa cylindrica (Cucurbitaceae) | 16 | 16 | 13 | 14 | 57 | |
| Leucaena leucocephala (Mimosae) | 11 | 18 | 7 | 16 | 51 | |
| Delonix regia (Caesalpinaceae) | 8 | 18 | 12 | 12 | 56 | |
| Aeschynomene indica (Papilionaceae) | 7 | 19 | 4 | 19 | 51 | 18.3/20.1 4% |
| Pterocarpus indicus (Papilionaceae) | 6 | 22 | 6 | 13 | 49 | 22.0 9% |
| Crotalaria anagyroides (Papilionaceae) | 5 | 18 | 5 | 19 | 52 | 18.3/20.1 4% |
| Caesalpinia pulcherrima (Caesalpiniaceae) | 5 | 17 | 10 | 16 | 53 | |
| C. pulcherrima var. aurea (Caesalpiniaceae) | 5 | 14 | 11 | 15 | 56 | |
| Cassia cobanensis (Caesalpiniaceae) | 5 | 21 | 9 | 21 | 49 | |
| Cassia viscosa (Caesalpiniaceae) | 4 | 24 | 7 | 17 | 47 | |

Table 4 (cont.)

| Type or Species (Family) | % Oil | 16:1 | 18:0 | 18:1 | 18:2 | Others |
|---|---|---|---|---|---|---|
| Cocoa butter | 56 | 24 | 35 | 38 | 2 | |
| Pyrenaria acuminata (Theaceae) | 8 | 10 | 46 | 40 | 3 | |
| Groundnut oil | 50 | 10 | 4 | 61 | 18 | C 20-24 7% |
| Khaya ivorensis (Meliaceae) | 62 | 8 | 5 | 67 | 20 | |

(v) Afzelia and related oils

A source of Afzelia cuanzensis previously examined in this department was shown to be a rich source of crepenynic acid and of dehydrocrepenynic acid.[4] The former is known to be 18:2 (9c12a)[5] and preliminary results suggested the latter was 18:3 (9c12a14c). We have now been able to examine other samples of A. cuanzensis along with oils of some other Afzelia species and to confirm the structure of dehydrocrepenynic acid. The analytical results are summarised in Table 5.

Table 5: Afzelia Oils

| | 16:0 | 18:0 | 18:1 | 18:2 | 20:0 | 20:1/18:3 | 22:0 | 24:0 | 18:2[a] | 18:3[b] |
|---|---|---|---|---|---|---|---|---|---|---|
| A. cuanzensis[c] | 3 | 2 | 6 | 22 | 0.5 | 0.5 | 1 | 3 | 31 | 29 |
| A. cuanzensis[c] | 4 | 3 | 7 | 29 | 0.5 | 0.5 | 1 | 3 | 36 | 16 |
| A. africana | 3 | 3 | 10 | 27 | 1 | 2 | 5 | 8 | 19 | 22 |
| A. bella | 3 | 3 | 13 | 29 | 1 | 1 | 3 | 6 | 17 | 24 |
| A. bipindensis (Pahudia) | 2 | 1 | 6 | 21 | 0.5 | 0.5 | 3 | 10 | 20 | 36 |
| A. rhomboidea | 8 | 7 | 14 | 27 | | | | 6 | 16 | 18 |

a crepenynic acid 18:2(9c12a)

b dehydrocrepenynic acid 18:3(9c12a14c)

c different sources

The seeds of the Afzelia species examined were ovoids ½"-1" in length and jet black with a waxy orange tip which was easily removed . Difficulty was experienced in milling the seeds as they proved to be extremely hard. A sample of methylesters from Afzelia bipendensis was separated by preparative silver ion chromatography into five fractions. By GLC. and where necessary, infra-red and ultra-violet spectroscopy, these were shown to be:- (i) Saturated esters (ii) Methyl oleate (iii) Methyl dehydrocrepenynate (iv) Methyl crepenynate (v) Methyl linoleate.

The GLC peak (DEGS) of ECL 24 which appeared in all of the Afzelia oils was present in the first band and is therefore likely to be a saturated ester rather than a more unsaturated derivative of dehydrocrepenynic acid. The latter has been reported to occur in Tricholoma grammopodium[6] mould and is of interest in the biosynthesis of some highly unsaturated compounds from linoleic acid via crepenynic acid. The Afzelia seeds are the best known source of dehydrocrepenynic acid.

| Order and Family | Species | Origin (see footnotes) | Oil % | 16:0 | 18:0 | 18:1 | 18:2 | Others % |
|---|---|---|---|---|---|---|---|---|
| Iridaceae | 1. Trimeza martinicensis | S | 4 | 16 | 17 | 17 | 29 | 14:0 18 |
| Cyperaceae | 2. Gahnia tristis | S | 20 | 6 | 2 | 48 | 44 | |
| Palmae | 3. Oreodoxa regia | S | 21 | 10 | 2 | 26 | 11 | 12:0 35<br>14:0 15 |
| Palmae | 4. Ptychosperma macarthuri | S | 8 | 56 | 10 | 28 | 5 | |
| Palmae | 5. Oncosparma tigillarium | S | 5 | 9 | 2 | 10 | 4 | 12:0 44<br>14:0 30 |
| Palmae | 6. Astrocaryum vulgare | S | 16 | 6 | 4 | 12 | 3 | 12:0 47<br>14:0 26 |
| Cycianthaceae | 7. Carludovica palmata | S | 24 | 13 | 16 | 45 | 22 | |
| Casuarinales Casuarinaceae | 8. Casuarina nobile | S | 27 | 7 | 5 | 17 | 71 | |
| Santales Caryophyllaceae | 9. Honckenya ficifolia | S | 8 | 14 | 7 | 24 | 53 | |

| Order and Family | Species | Origin | Oil % | 16:0 | 18:0 | 18:1 | 18:2 | Others |
|---|---|---|---|---|---|---|---|---|
| Ranales | | | | | | | | |
| Anonaceae | 10. Canangium odoratum | S | 8 | 12 | 4 | 44 | 39 | |
| Anonaceae | 11. Anaxagorea javaneca | S | 19 | 17 | 15 | 26 | 40 | |
| Lauraceae | 12. Cinnamonum iners | S | 40 | - | - | - | - | 12:0 96% |
| Rosales | | | | | | | | |
| Mimosae | 13. Parkia roxburghii | S | 20 | 9 | 14 | 16 | 44 | 20:0 4%<br>22:0 9% |
| Mimosae | 14. Acacia auriculaeformis | S | 6 | 10 | 2 | 10 | 67 | 22:0 4%, epoxy 18:1 4% |
| Mimosae | 15. Leucaena leucocephala | M | 11 | 18 | 7 | 16 | 51 | |
| Mimosae | 16. Mimosa invisa (var. intermis) | M | 5 | 16 | 2 | 13 | 68 | |
| Caesalpinaceae | 17. Cassia surattensis | S | 11 | 19 | 7 | 26 | 43 | |
| Caesalpinaceae | 18. Sindora wallichii | S | 5 | 15 | 5 | 13 | 51 | 24:0 12% |
| Caesalpinaceae | 19. Bauhinia acuminata | S | 12 | 12 | 12 | 11 | 63 | |
| Caesalpinaceae | 20. Bauhinia megalandra | S | 18 | 23 | 12 | 15 | 48 | |

| Order and Family | Species | Origin | Oil % | 16:0 | 18:0 | 18:1 | 18:2 | Others |
|---|---|---|---|---|---|---|---|---|
| Caesalpinaceae | 21. Cassia cobanensis | M | 5 | 21 | 9 | 21 | 49 | |
| " | 22. Cassia fruticosa | S | 5 | 27 | 7 | 32 | 29 | |
| " | 23. Cassia viscosa | M | 4 | 24 | 7 | 17 | 47 | 20:0 5% |
| " | 24. Caesalpinia pulcherrima | S | 5 | 17 | 10 | 16 | 53 | |
| " | 25. Caesalpinia pulcherrima (var. aurea) | S | 5 | 14 | 11 | 15 | 56 | |
| " | 26. Pahudia romboidea | S | 6 | 8 | 7 | 14 | 27 | (note 1) |
| " | 27. Erythrophleum guineensis | S | 29 | 9 | 11 | 34 | 35 | 16:1 8% |
| " | 28. Trachylobium verrucosum | S | 4 | 8 | 4 | 23 | 49 | 22:0 7% 24:0 9% |
| " | 29. Delonix regia | S | 8 | 18 | 12 | 12 | 56 | |
| " | 30. Gleditsia fera | HK | 5 | 12 | 2 | 11 | 74 | |
| Papilionaceae | 31. Myroxylon toluiferum | S | 29 | 11 | 7 | 59 | 8 | 22:0 9% |
| " | 32. Calopogonium caeruleum | M | 11 | 25 | 7 | 23 | 42 | |
| " | 33. Calopogonium mucunoides | M | 7 | 23 | 8 | 20 | 40 | 22:0 4% |
| " | 34. Pterocarpus indicus | S | 6 | 22 | 6 | 13 | 49 | 22:0 9% |
| " | 35. Clitoria rubiginosa | T | 19 | 8 | 4 | 55 | 4 | 20:1 12%<br>22:0 8%<br>24:0 5% |
| " | 36. Psophocarpus palustris | M | 16 | 11 | 4 | 27 | 40 | 22:0 9% |
| " | 37. Crotolaria anagyroides | M | 5 | 18 | 5 | 19 | 52 | 18:3/20:1 4% |
| " | 38. Ormosia semicastrata | HK | 6 | 14 | 5 | 32 | 46 | |
| " | 39. Lespedeza formosa | HK | 10 | 10 | 2 | 15 | 55 | 18:3 16% |
| " | 40. Aeschynomene indica | HK | 7 | 19 | 4 | 19 | 51 | 18:3/20:1 4% |

| Order and Family | Species | Origin | Oil % | 16:0 | 18:0 | 18:1 | 18:2 | Others |
|---|---|---|---|---|---|---|---|---|
| Papilionaceae | 41. Clitoria ternatea | T | 7 | 15 | 12 | 40 | 25 | |
| " | 42. Clitoria rubiginosa | T | 10 | 7 | 2 | 56 | 2 | 18:3 9%<br>20:1 7%<br>22:0 9%<br>24:0 5% |
| " | 43. Millettia bussei | T | 28 | 7 | 4 | 42 | 12 | 18:3 12%<br>22:0 14%<br>24:0 5% |
| " | 44. Psophocarpus palustris | T | 8 | 16 | 5 | 18 | 36 | 18:3 14%<br>22:0 9% |
| " | 45. Stizolobium atterrimum | T | 5 | 26 | 9 | 13 | 46 | |
| " | 46. Tephresia noctiflora | T | 9 | 10 | 3 | 25 | 39 | 20:1/18:3 4%<br>22:0 13%<br>24:0 4% |
| " | 47. Canavalia cathartica | T | 6 | 18 | 2 | 50 | 11 | 18:3 10%<br>20:1 10% |
| Thymeliaceae | 48. Wikstroemia viridiflora | S | 28 | 13 | 6 | 45 | 35 | |
| Crentionales Burseraceae | 49. Canarium vulgare | S | 63 | 29 | 12 | 49 | 10 | |
| Apocynaceae | 50(Lochnera)Vinca rosea (var. alba) | S | 25 | 16 | 9 | 63 | 11 | |
| " | 51. Holarrhena wulfsbergii | N | | 5 | 7 | 13 | 23 | 18:3 52% |
| " | 52. Thevetia peruviana | S | 7 | 20 | 7 | 48 | 23 | |

| Order and Family | Species | Origin | Oil % | 16:0 | 18:0 | 18:1 | 18:2 | Others |
|---|---|---|---|---|---|---|---|---|
| Simarubaceae | 53. Quassia amata | S | 27 | 15 | 23 | 54 | 6 | |
| Dilleniaceae | 54. Dillenia indica | S | 23 | 10 | 2 | 21 | 17 | 12:0 8%, 14:0 42% |
| Acanthacae | 55. Ruellia tuberosa | S | 22 | 20 | 4 | 11 | 65 | |
| Acanthacae | 56. Asystasia coromandeliana | S | 16 | 3 | 3 | 47 | 14 | 20:0 10% 20:1 9% 22:0 5% 24:0 9% |
| Malpighiaceae | 57. Tristellateia australasica | S | 19 | 9 | 12 | 26 | 23 | 20:0 12% 20:1 14% |
| Meliaceae | 58. Khaya ivorensis | N | 62 | 8 | 5 | 67 | 20 | |
| Meliaceae | 59. Khaya grandifoliola | N | 59 | 10 | 13 | 58 | 17 | |
| Meliaceae | 60. Khaya nyasica | R | 72 | 11 | 12 | 63 | 14 | |
| Meliaceae | 61. Khaya anthotheca | U | 82 | 15 | 9 | 53 | 22 | |
| Euphorbiaceae | 62. Baccaurea motleyana | S | 16 | 13 | 7 | 22 | 19 | 18:3(9c12c15c) 39% |

| Order and Family | Species | Origin | Oil % | 16:0 | 18:0 | 18:1 | 18:2 | Others |
|---|---|---|---|---|---|---|---|---|
| Sapindales Conorbiataceae | 63. Ipomoea digitata | S | 7 | 27 | 11 | 18 | 33 | |
| Anacardiaceae | 64. Spondias pinnata | S | 53 | 9 | 7 | 37 | 45 | |
| Celastrales Aquifoliaceae | 65. Ilex pubescens | HK | 19 | 10 | 6 | 28 | 55 | |
| Rhamnales Rhamnaceae | 66. Paliurus ramosissimus | H | 16 | 9 | 3 | 45 | 37 | |
| Malvales Malvaceae | 67. Hibiscus cannabinus | M | 15 | 24 | 4 | 32 | 34 | |
| " | 68. Hibiscus mutabilis | HK | 9 | 29 | 2 | 13 | 46 | |
| Sterculiaceae | 69. Reevesia thrysoidea | S | 26 | 9 | 3 | 38 | 50 | |
| Guttiferae | 70. Calophyllum inophyllum | S | 70 | 17 | 20 | 31 | 32 | |
| Theaceae | 71. Pyrenaria acuminata | S | 8 | 10 | 46 | 40 | 3 | |
| Myrtales Lythraceae | 72. Lagerstroemia indica | HK | 6 | 8 | 2 | 6 | 81 | |
| Combretaceae | 73. Combretum grandiflorum | S | 23 | 19 | 2 | 32 | 16 | 14:0 31% |
| " | 74. Terminalia phallocarpa | S | 42 | 28 | 4 | 20 | 47 | |

| Order and Family | Species | Origin | Oil (%) | 16:0 | 18:0 | 18:1 | 18:2 | Others* |
|---|---|---|---|---|---|---|---|---|
| Crentionales Asclepiadaceae | 75. Calotropis procera | N | 23 | 16 | 12 | 37 | 33 | |
| Polemoniales Solanaceae | 76. Capsicum frutescens | HK | 12 | 17 | 4 | 15 | 64 | |
| Cucurbitales Cucurbitaceae | 77. Luffa cylindrica | Z | 16 | 16 | 13 | 14 | 57 | |
| Cucurbitaceae | 78. Cucumis africanus | Z | 13 | 9 | 8 | 9 | 74 | |

Origin:

HK= Hong Kong
M = Malaya
N = Nigeria
R = Rhodesia
S = Singapore
U = Uganda
T = Tanzania
Z = Zambia



*Acids present at the 3% level or below have been recorded but are not included here.

Note (1) This oil contains crepenynic acid (16%), dehydrocrepenynic acid (18%), and an unidentified acid (6%).

## (vii) Common Names

10. Ylang-Ylang, Perfume tree.
11. Twin seed.
12. Wild cinnamon.
14. Acacia tree, Australian wattle.
15. Wild mimosa.
17. Glaucous cassia.
19. Wild bauhinia.
20. West Indian bauhinia.
22. Drooping cassia.
24. Pride of Barbados.
27. Ordeal tree, Red Water tree.
28. Gum copal tree.
29. Flamboyant, Flame of the Forest.
31. Balsam of Tolu.
34. Malay Paduak, Amboyna.
41. Blue pea, Blue vine.
45. Bengal bean.
50. West Indian periwinkle, Blue periwinkle.
51. False rubber tree.
53. Bitterwood.
54. Elephant apple.
55. Monkey gun, Minnie roots.
58. Ivory Coast mahogany.
59. Big leaf mahogany.

60. Red mahogany.

61. White mahogany.

62. Rambai mahogany.

64. Common hog plum.

67. Kenaf, Stock rose, Deccan hemp.

68. Changeable rose.

70. Alexandrian laurel, Beauty leaf.

71. Bat's apple.

72. King of flowers, Cape myrtle, Crepe tree, Indian lilac, Pride of India.

74. Pigs mango.

75. Swallow wort, Giant milkweed, Sodom apple, Dead Sea fruit.

76. Bird pepper, Cayenne pepper, Chilli bean, Red pepper.

77. Smooth loofah.

78. Bitter apple, Wild cucumber.

79,80. Lucky bean, Pod mahogany.

81. Mahogany bean.

(d) Experimental

(i) Extraction of Triglycerides from seeds

The seeds (about 5g, shelled if possible) were ground as finely as possible in an electrically driven seed mill, then reduced to a fine powder using a mortar and pestle and a little petrol. The meal was extracted with petrol (b.p. 40° - 60° C) for 3 hours in a Soxhlet apparatus, then reground and extracted for a further 3 hours. At the end of this period the solvent was removed on a rotary film evaporator, leaving behind the extracted triglycerides and some free fatty acids. The latter were removed by passing the extracted oil (1g) through a column (5" x 1", 10g) of activated alumina (Type H) using chloroform (600ml) as eluting solvent. This treatment gives the neutralised oil after removal of solvent under reduced pressure. The chromatographic procedure should not be prolonged otherwise the alumina may effect partial hydrolysis of triglyceride.

(ii) Transesterification

The methyl esters of the component acids in the neutralised triglycerides were then prepared by transesterification with sodium and methanol. This involved refluxing the triglycerides (1g) under anhydrous conditions for three minutes with solium methoxide in dry methanol[7] (Sodium, 0.2N, 15ml). The mixture was then cooled with distilled water, acidified with dilute sulphuric acid, and extracted (3 x 20ml) with solvent ether. The ether extract was washed with saturated sodium bicarbonate solution (1ml) and with distilled water (1ml) before being left to dry over anhydrous sodium sulphate. After a few hours the drying agent was filtered off and the ether solution evaporated to leave the methyl esters, which were then weighed and stored at 0° C under nitrogen.

Before pursuing any analytical procedures it was necessary to check that transesterification was complete. This was tested by comparing the TLC behaviour of adjacent spots of neutralised oils and transesterified product on a silica plate (0.25mm thick) with PE 30. The separated material

was rendered visual by spraying with an ethanolic solution of phosphomolybdic acid (10%) and heating at 100°C for 30 minutes or by exposing to iodine vapour. If the transesterification was complete the product appeared as one spot with a fairly high Rf value, corresponding to the methyl esters of all the non-oxygenated acids present in most seed oils. Below this spot minor spots due to epoxy-, oxo- or hydroxy- methyl appeared, whereas unchanged triglycerides ran slightly higher than the methyl esters and monoglycerides and diglycerides much lower.

Having been assured that the oil was completely in ester form, the mixed methyl esters were examined by chromatographic and spectrophotometric procedures.

## (iii) Chromatographic Techniques

### Gas Liquid Chromatography (GLC)

GLC was employed extensively in the identification of component acids. All the mixtures to be analysed were run twice on a 5' polar column, using 20% DEGS (Diethyleneglycolsuccinate) as stationary liquid phase, and once on a 5' non-polar column using 5% ApL (Apiezon L grease). The usual operating temperatures were 190°(DEGS) and 210°(ApL). The machine used was a Pye 104 Chromatograph with a flame-ionisation detector (FID). From a study of the chromatograms obtained from both columns a tentative identification was possible. Assuming that the detector response to each component was the same the area % of each component could be found from the area under its peak. The peaks were taken to be gaussian and the areas were calculated as (height)x(width at half height). In dealing with retention data the notion of "equivalent chain length" (ECL) or "carbon number" is usual, since to a large degree these are specific for each component depending only on the particular column used and being almost invariant of other factors (e.g. column temperature, carrier gas flow-rate etc.)

For non-saturated esters the ECL is taken to be the chain length: for other esters the ECL is usually non-integral and is found from the straight-line plot of ECL v. log (Retention time). As already mentioned, ECLs differ from column to column, and so comparing the results of runs on a polar column such as DEGS (on which saturated esters preceed unsaturated ones of the same chain length) with those on a non-polar one such as ApL (on which the order is reversed) can give valuable information on the composition of the mixture. For example, both methyl eicosenoate and methyl linolenate give the same ECL (20.5) on DEGS but since they differ greatly on ApL (19.6 for methyl eicosenoate, 17.4 for methyl linolenate) they can be individually estimated.

Thin Layer Chromatography (TLC)

In TLC the mobile phase is a liquid (e.g. ether) and the stationary phase a thin layer (0.25 - 100mm.) of silica (Silica Gel G nach Stahl) or of silica impregnated with silver nitrate (~10%) coated on to a glass plate. Silica TLC will separate esters according to their polarity whereas argentation chromatography will separate esters of different unsaturation. For analytical work layers 0.25 - 0.30mm. thick are used whereas for preparative purposes it is best to use 1mm layers.

TLC was usually employed to give qualitative support to the GLC results, although in cases of doubt saturated, monoethenoid and polyethenoid esters were separated by preparative thin layer argentation and the resulting components re-examined by GLC.

## Ultraviolet (UV) Spectroscopy

Absorption in the ultraviolet region indicated the presence of conjugated chromophores (conjugated diene has a $\lambda MAX$ = 234mμ and $E^{1\%}_{1cm} \approx 1000$; conjugated enyne exhibits a peak at 229mμ with an inflection at 240mμ and $E^{1\%}_{1cm} \approx 500$; conjugated triene absorbs at 261mμ, 271mμ, 281mμ and $E^{1\%}_{1cm} \approx 2000$).

A Pye Unicam SP800 Spectrophotometer was used and the spectra measured on methanolic solutions of the esters in quartz cells (1cm pathlength)

## Infrared Spectra

The infrared (IR) spectra of the mixed esters will provide evidence for the presence of hydroxyl- groups (2.9μ, 3448cm$^{-1}$) or oxo- groups (5.8μ, 1724cm$^{-1}$) in addition to indicating the presence of trans- double bonds (10.3μ, 975 cm$^{-1}$).

The spectrum was measured on a Perkin Elmer 257 Grating Spectrophotometer on a thin film of the liquid esters.

## The partial reduction of some acetylenic acids by hydrazine

### (a) Introduction

#### (i) Objectives

Three C18 acids containing an acetylenic group [crepenynic acid (9c12a), xymenynic acid (9allt) and dehydrocrepenynic acid (thought to be 9c12a14c)] have been submitted to partial reduction by hydrazine for the following reasons:

(1) To study the relative rates of reduction by hydrazine of olifinic and acetylenic groups in differing environments. In these three acids the olifinic centre is sometimes cis- and sometimes trans- and is sometimes conjugated and sometimes non-conjugated.

$$CH_3(CH_2)_4 - C \equiv C - CH_2 - CH^{c} = CH - (CH_2)_7 - CO_2H$$

Crepenynic acid

$$CH_3 - (CH_2)_5 - CH \overset{t}{=} CH - C \equiv C - (CH_2)_7 - CO_2H$$

Xymenynic acid

$CH_3(CH_2)_2 - CH \overset{c}{=} CH - C \equiv C - CH_2 - CH \overset{c}{=} CH - (CH_2)_7CO_2H$

Dehydrocrepenynic acid

(2) To confirm the structure of dehydrocrepenynic acid by isolating and identifying some of the products of partial reduction.

(ii) Hydrazine reduction of unsaturated acids

One of the most reliable methods of determining the position and configuration of unsaturated centres in polyethenoid acids is based on their partial reduction with hydrazine. Hydrazine hydrate in alcoholic solution reduces polyenoic acids in a stepwise manner ultimately to saturated acids. Reduction occurs without double-bond migration or stereomutation[8,9] and the conditions can generally be arranged to give monoenes in good yield. The monoenes can then be isolated by $Ag^+$/TLC and identified by GLC examination of the products of ozonolysis or Von Rudloff oxidation.[10] For example, the linoleic type acid (18:2, 9c12t) gives a mixture of monoenes (9c and 12t) on partial reduction with hydrazine. Since trans-monoenes run higher than cis-monoenes on $Ag^+$/TLC the two monoenes can be separated and subjected to Von Rudloff oxidation. This yields a mixture of C9 monobasic and C9 dibasic acids (with 18:1,9c) and C6 monobasic and C12 dibasic acids in the case of 18:1,12t. These are then identified by GLC and unequivocally prove the structure of the original acid.

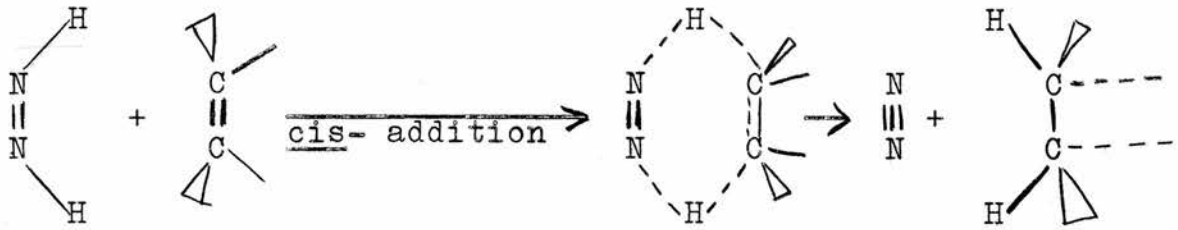
Catalytic hydrogenation is unsuitable for this purpose since partial reduction occurs with double bond migration and stereomutation.

Unique solutions are also possible using a variety of partial oxidation techniques. Oxidation of a polyenoic acid with insufficient performic acid[11] or osmium tetroxide[12] leads to partially hydroxylated products. These can then be hydrogenated (Pd/charcoal) and double bonds then cleaved by oxidative fission to give characteristic fragments. Another method described uses partial ozonolysis.[13]

Aylward has made a prominent contribution to the understanding of the hydrazine reaction.[14] He found that oxygen was necessary for the reaction, that vigorous stirring was essential and that carboxylic acids catalytically accelerated the reaction (provided the solution itself was alkaline). Aylward then postulated the presence of di-imide as a reactive intermediate and suggested the following mechanism for the reaction:-

$$N_2H_4 \xrightarrow[\text{from } CH_3CO_2H]{H^+} N_2H_5^+ \xrightarrow[-H_2O]{\frac{1}{2}O_2} N_2H_3^+ \xrightarrow{N_2H_4} N_2H_2 + N_2H_5^+$$

di-imide
syn:anti = 1:1




syn- form only reacts.

It was also found[15] that cupric ions catalytically accelerated the reduction and were regenerated by atmospheric oxygen from cuprous ions. Aylward explained this by suggesting that di-imide was being formed from hydrazine in two univalent stages via the hydrazyl radical.

(iii) Crepenynic and Dehydrocrepenynic Acids

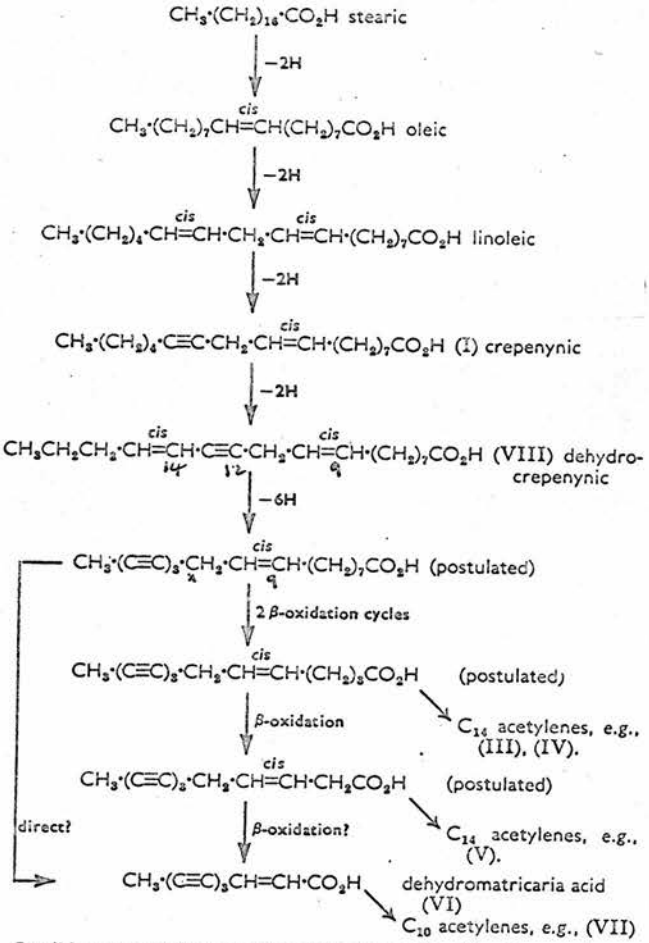
Crepenynic acid [18:2(9c12a)] was first discovered in the seed oils of the Crepis genus (Compositae)[16] and later found in Afzelia (Caesalpiniaceae) oils. Interest in this unusual acid was increased following the suggestion that it is a key intermediate in the biosynthetic route to short- and long-chain polyenynes[17] which are known to occur in the leaves and seeds of many plants. The scheme (1) proposed by Bu'Lock and Smith[18] involves the stepwise desaturation of stearic acid via oleic, linoleic and crepenynic acids and can account for several of the acetylenic compounds found in the higher plants of the Compositae, Araliacea and Umbelliferae. These two workers provided some experimental support for this hypothesis by isolating [10-$^{14}$C] - labelled linoleic and crepenynic acids from the polyacetylene producing fungus Tricholoma grammopodium grown in a medium containing [10-$^{14}$C] oleic acid. By degradative methods they demonstrated that the C18 chain of both acids was derived intact from the oleic acid.

They also discovered trace amounts of dehydrocrepenynic acid from the same incubations.

They believed this to be the next product in the chain of desaturation processes. The Afzelia oil examined by Gunstone et al[19] and the larger series of Afzelia oils now examined proved to contain large amounts of both crepenynic and dehydrocrepenynic acids and preparative $Ag^+$/TLC of two members of this genus; A. cuanzensis and A. bipendensis, provided the starting materials for the reactions described later.

Some preliminary investigations of the partial reactions with hydrazine of both crepenynic and dehydrocrepenynic esters have previously been reported by Gunstone et al.[20] They found the olefinic group of methyl crepenynate to be reduced more quickly than the acetylenic group; methyl dehydrocrepenynate gave a complex mixture of products containing monoacetylenic ester, conjugated enoate and several unidentified esters.

BIOSYNTHESIS OF NATURALLY-OCCURRING ACETYLENES




Possible sequence from stearic acid to the various $C_9$—$C_{14}$ acetylenes, showing the role of crepenynic and dehydrocrepenynic acids. Presumably, as in the first step, special derivatives rather than the free acids are the actual intermediates.

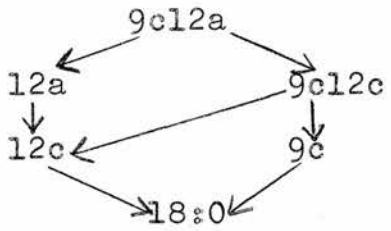
AS PROPOSED BY

J.D.Bu'Lock and G.N.Smith, J.Chem.Soc. 1967(C).332–6

(b) Results

(i) The partial reduction of crepenynic acid

The possible processes are outlined below:-




(1) The reaction was first examined using methyl crepenynate in methanol solution at 45-50°C in the presence of hydrazine hydrate and a little acetic acid. Throughout the reaction the mixture was stirred vigorously and air continuously bubbled through. Aliquots were removed at intervals, the esters recovered and examined by GLC. Under these conditions the reaction was very slow, the progress of reaction did not seem to be reproducible and there was evidence that some of the esters were converted to hydrazides:-

$$RCO_2Me + N_2H_4 \longrightarrow RCONHNH_2 + MeOH$$

(2) The reaction was repeated with the free acid, which would be expected to form the hydrazide less readily and the products were subsequently methylated by reaction with methanolic $BF_3$ (3 minutes) prior to GLC examination. The reaction was still slow although carried out at 45-50°C.

(3) It was not possible to use methanol at a higher temperature than this whilst maintaining a vigorous air flow and the conditions finally employed used ethanol as solvent, at 55-60°C, and without a flow of air through the solution. Reaction then proceeded at a reasonable rate for investigation.

By GLC examination of the reaction products at various time intervals it was possible to determine the amount of each reduction product except that the two monoene esters (9c and 12c) were not separated. Attempts to plot reaction composition v. time showed that reaction rates varied from experiment to experiment. This difficulty was overcome by plotting composition v. the extent of reduction. The latter was measured in double bond equivalents and was easily calculated from the composition of the mixture as determined by GLC. The results of one experiment are given in Table 6 and the results plotted on a graph (Fig. 2).

Table 6

| Component | ECL (DEGS) | Composition (%) of each aliquot | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0.0hr | 1.5hr | 3.8hr | 7.8hr | 12.7hr | 24.5hr |
| 18:2(9c12a) | 21.6 | >95 | 55.1 | 29.8 | 10.9 | 1.9 | 0.0 |
| 18:1(12a) | 20.4‡ | 0.0 | 26.4 | 37.0 | 40.8 | 30.7 | 12.9 |
| 18:2(9c12c) | 19.3 | 0.0 | 9.5 | 9.7 | 5.4 | 1.5 | 0.0 |
| 18:1(9c) } — 18:1(12c) } | 18.5 | 0.0 | 8.4 | 17.8 | 25.1 | 21.3 | 10.6 |
| 18:0 | 18.0 | 0.0 | 0.5 | 3.9 | 17.1 | 43.6 | 76.3 |
| DBE* | | 2.85 | 2.46 | 2.01 | 1.50 | 0.91 | 0.26 |

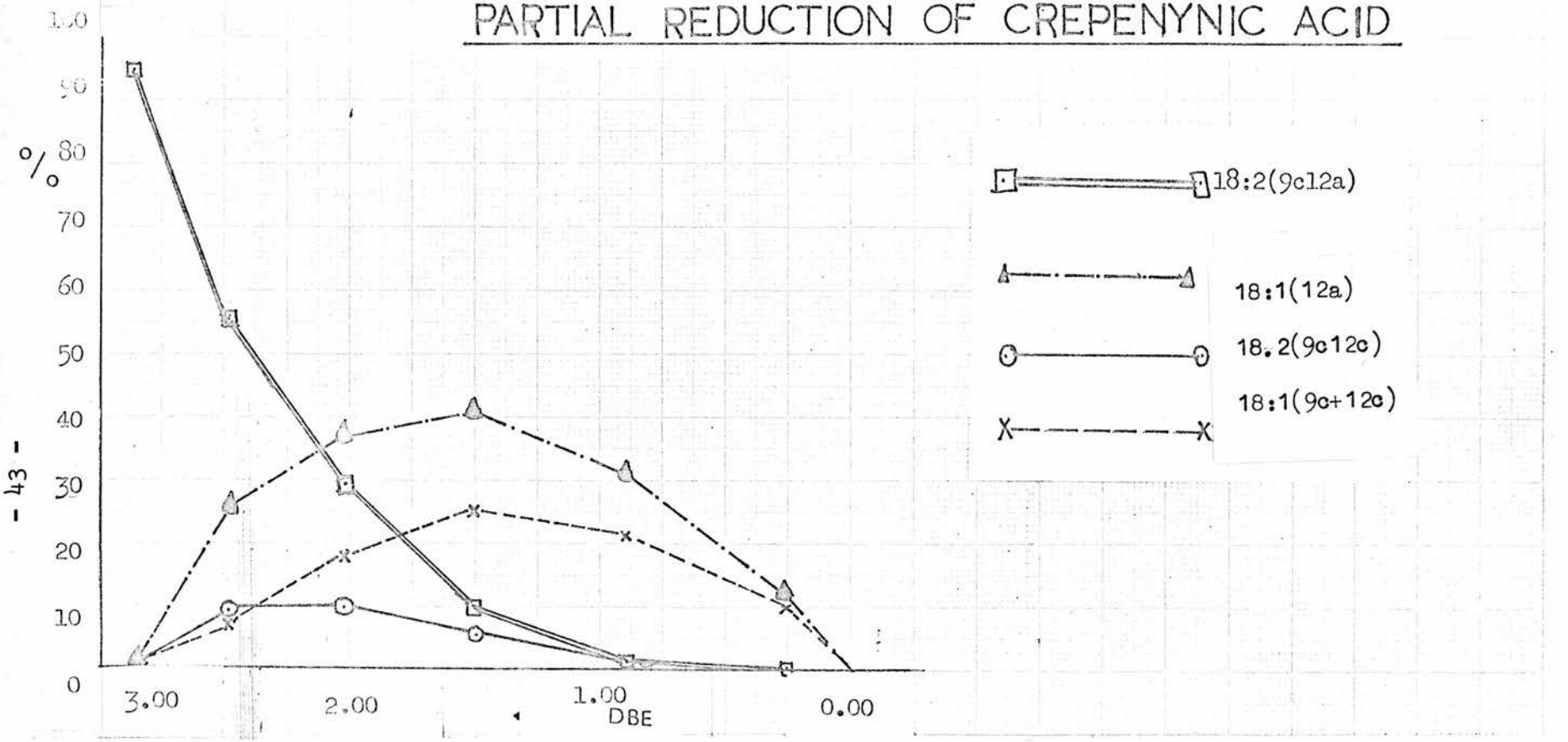


* Double bond equivalent

‡ Small shoulder ~20.7

Fig2

# PARTIAL REDUCTION OF CREPENYNIC ACID



From these results the following conclusions may be drawn:-

(i) There is some selectivity of reduction but this is not very marked. Crepenynic acid is still present after reaction is one third complete (~30%) but almost completely reduced when the reaction is two thirds complete.

(ii) The major unsaturated product at all stages of the reaction is octadecynoic acid and this attains a maximum value of ~40% half way through the reaction. The other product of the first hydrogenation step - linoleic acid - never exceeds ~10%.

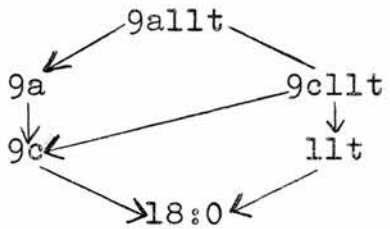
(iii) The two monoene acids are formed quickly but do not exceed ~25%.

These results are consistent with the conclusion that cis-olifinic centres are reduced by $N_2H_4$ more readily than acetylenic groups. In the partial reduction of a long-chain acid with both olifinic (cis) and acetylenic groups the latter will be more resistant to reaction and products in which

these are present could be readily isolated and identified. This could be valuable in the structural identification of such compounds.

(ii) The partial reduction of xymenynic acid

The possible reaction pathways are:-




A partially reduced sample of xymenynic acid (18:2, 9allt) gives five peaks arising from $C_{18}$ acids on a DEGS column. Those of ECL 18:0 (stearate), 18.5 (octadecenoate isomers, 9c and 11t), and 22.9 (xymenynate) are readily assigned to the esters shown. The other peaks have ECL's of 20.4 and 21.1. The first of these represents two esters (9a and 9cllt) and the second is thought to be the 9tllt dienoate which may result from isomerisation of the 9cllt dienoate either during the reaction or on the GLC column. The results are set out in Table 7.

Table 7

| Component | ECL (DEGS) | Composition (%) of each aliquot | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0.0hr | 1.0hr | 4.0hr | 8.8hr | 12.0hr | 28.0hr |
| 18:2(9allt) | 22.9 | 97.7 | 65.0 | 22.5 | 7.3 | 2.1 | 0.0 |
| 18:2(9tllt) | 21.1 | 0.8 | 1.7 | 3.0 | 2.5 | 1.3 | 0.0 |
| 18:1(9a) / 18:2(9cllt) | 20.4 | 0.8 | 29.1 | 43.0 | 26.3 | 21.1 | 2.8 |
| 18:1(9c) / 18:1(11t) | 18.5 | 0.4 | 3.4 | 17.4 | 20.0 | 16.1 | 3.4 |
| 18:0 | 18.0 | 0.3 | 0.7 | 12.2 | 43.9 | 58.5 | 93.5 |
| 16:0* | 16.0 | 0.1 | 0.1 | 0.6 | 0.0 | 0.2 | 0.3 |
| DBE | | 2.97 | 2.60 | 1.77 | 1.00 | 0.77 | 0.09 |

* Impurity in starting material

It is particularly unfortunate that the peak at ECL 20.4 should arise from the two primary products of reduction. These two esters are separated on a 50m ApL capillary column but then the 18:0 and 9cl1t esters are incompletely resolved (see Table 8 ).

Table 8

ECL's of Components of reduction of Xymenynic acid

| Component | 20% DEGS 190° | ApL 210°* |
|---|---|---|
| 18:0 | 18.0 | 18.00 |
| 18:1 | 18.5 | 17.63 9c<br>17.75 11t |
| 18:1, 9a | 20.4 | 17.75 |
| 18:2, 9c11t | 20.4 | 18.09 |
| 18:2, 9a11t | 22.9 | 18.73 |

* 50m capillary column

Quantitative UV spectroscopy was used in an attempt to overcome this difficulty. The complex maximum around 230 mμ is caused by three esters - 9allt, 9cllt and 9tllt* and it is possible to determine the amount of these by a combination of GLC (which gives the content of 9allt and 9tllt) and UV spectroscopy (which gives the content of 9cllt by difference) as shown in Tables 10 and 11.

* See Table 9.

Table 9

UV absorption of 9cllt, 9tllt and 9allt

| Component | $E^{1\%}_{1cm}$ of Acid | $E^{1\%}_{1cm}$ of methyl ester* | $\lambda$ Max. mμ |
|---|---|---|---|
| 9allt | 596 | 568 | 229 |
| 9cllt | 878 | 836 | 233 |
| 9tllt | 1170 | 1115 | 233 |

* $E^{1\%}_{1cm}$ for methyl ester = $E^{1\%}_{1cm}$ for acid x $\frac{\text{Molecular Wt. of Acid}}{\text{Wt. of Ester}}$

Table 10
Partial reduction of xymenynic acid

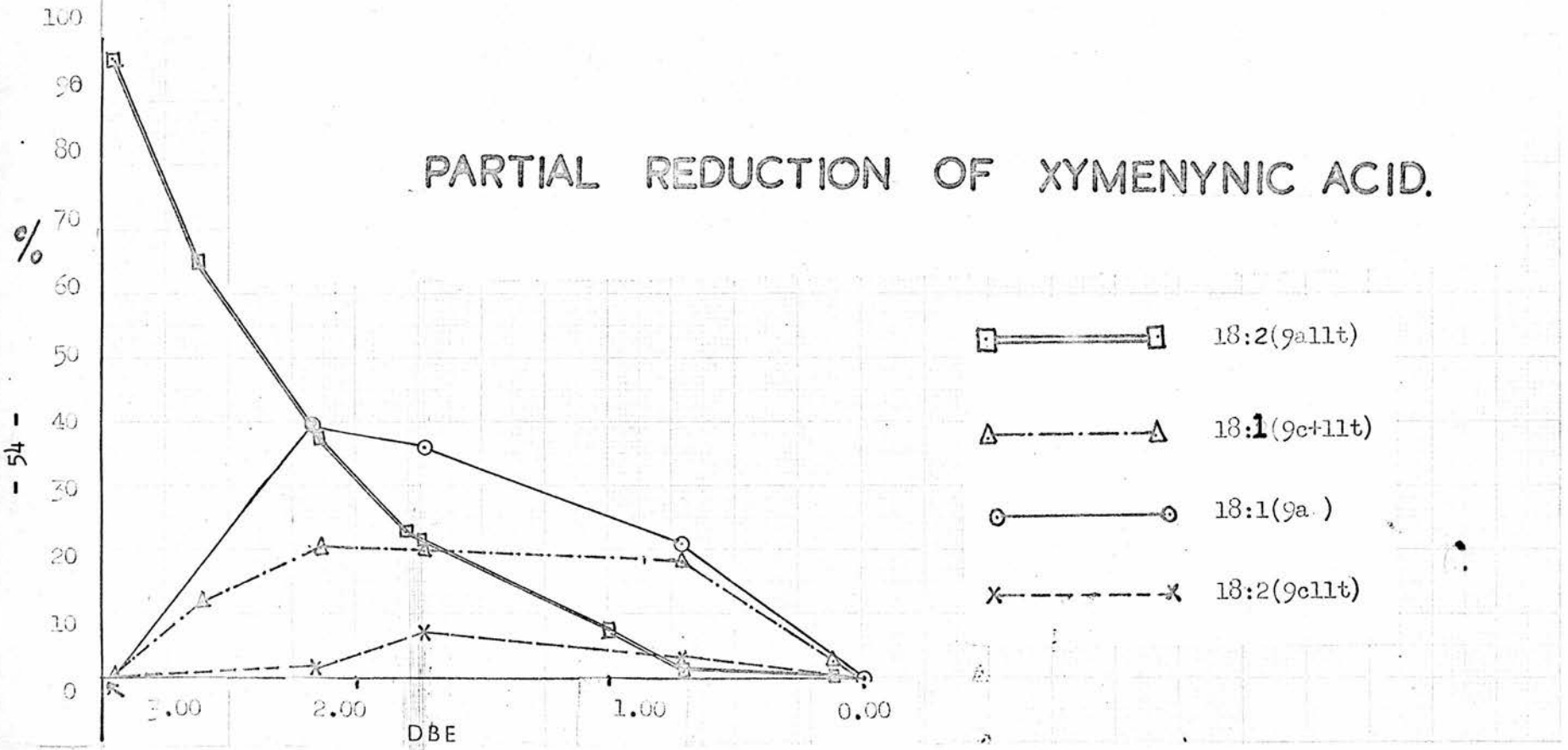
| Reaction Time(hrs) | $E^{1\%}_{1cm}$ solution | % 9allt | $\dot{E}^{1\%}_{1cm}$ for 9allt | % 9tllt | $\ddot{E}^{1\%}_{1cm}$ for 9tllt | $E^{1\%}_{1cm} - (\dot{E}^{1\%}_{1cm} + \ddot{E}^{1\%}_{1cm}) \equiv$ | % 9cllt |
|---|---|---|---|---|---|---|---|
| 0.0 | - | 97.7 | - | 0.8 | - | - | - |
| 2.0 | 291.3 | 37.2 | 212.0 | 6.9 | 77.0 | 2.3 | 0.3 |
| 4.0 | 201.5 | 20.1 | 114.0 | 2.6 | 29.0 | 58.5 | 7.0 |
| 8.5 | 44.2 | 1.7 | 9.7 | 0.9 | 10.0 | 24.5 | 2.9 |

Thus the composition (% wt.) of the reaction aliquots can be set out as follows:-

Table 11

| Reaction Time(hrs) | DBE | 16:0 | 18:0 | 18:1 | 9allt | 9tllt | 9cllt | 9a |
|---|---|---|---|---|---|---|---|---|
| 0.0 | 2.95 | 0.1 | 0.3 | 0.4 | 97.7 | 0.8 | ? | 0.0 |
| 2.0 | 2.13 | 0.3 | 5.6 | 12.0 | 37.2 | 6.9 | 0.3 | 37.6 |
| 4.0 | 1.72 | 0.3 | 14.1 | 19.5 | 20.1 | 2.6 | 7.0 | 36.5 |
| 8.5 | 0.71 | 0.4 | 55.8 | 18.1 | 1.7 | 0.9 | 2.9 | 20.3 |

Fig3


PARTIAL REDUCTION OF XYMENYNIC ACID.
%
100
90
80
70
60
50
40
30
20
10
0
3.00
2.00
1.00
0.00
DBE
18:2(9allt)
18:1(9c+11t)
18:1(9a )
18:2(9c11t)

The results of the experiments detailed in Tables 7,10&11 are represented graphically in Fig. 3. These results suggest that in this conjugated enyne system (9allt), as in the non-conjugated enyne system previously examined (9c12a), the olifinic bond is more readily reduced than the acetylenic bond and that the major partial reduction product is again the octadecynoic acid which reaches almost 40%. The conjugated diene acid and the two octadecenoic acids are never present in excess of ~20%.

(iii) The partial reduction of dehydrocrepenynic acid

The possible reaction steps with this more complex acid are set out in Table 12. The results of a preliminary experiment indicated that there would be some difficulty in obtaining detailed results similar to those described for crepenynic and xymenynic acids. Because of the large number of peaks present in the GLC of partially reduced dehydrocrepenynic acid there was some difficulty in identifying all these. Also some of the peaks contained more than one component (Table 13). Fig. 4 expresses the variation of those components which can be estimated with certainty.

In a second experiment conducted for 4 hours the product was separated by $Ag^+$/TLC into five fractions. Each of these was examined by GLC and shown to contain the following methyl esters:

(i) stearate

(ii) 18:1 (9c + 12c + 14c) + 18:1 (12a) + 2 unidentified components

(iii) dehydrocrepenynate

(iv) crepenynate

(v) crepenynate + 18:2 (9c12c + 9c14c)

Table 12

Partial reduction of dehydrocrepenynic acid

Possible schemes:-

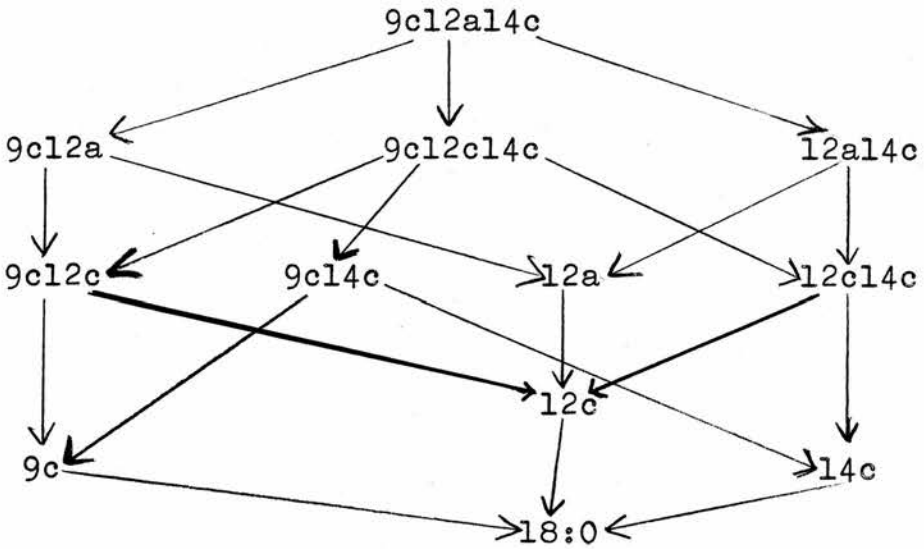


Table 13

| Component | ECL (DEGS) | Composition (%) of each aliquot | | | |
|---|---|---|---|---|---|
| | | 0.0hr | 0.8hr | 4.3hr | 24.8hr |
| 18:3(9c12a14c) | 22.3 | >98 | 45.0 | 11.6 | 0.0 |
| 18:2(9c12a)+ ? | 21.6** | 0.0 | 13.9 | 26.9 | 8.9 |
| ? | 20.6 | 0.0 | 1.9 | 4.1 | 3.7 |
| 18:1(12a) | 20.3 | 0.0 | 1.9 | 20.4 | 24.3 |
| 18:2(9c12c+9c14c) | 19.2 | 0.0 | 3.1 | 5.2 | 3.5 |
| 18:1(9c+12c+14c) | 18.5 | 0.0 | 3.2 | 9.0 | 19.7 |
| 18:0 | 18.0 | 0.0 | 0.8 | 4.0 | 26.5 |
| * | 23.1 | 0.0 | 24.4 | 13.4 | 12.6 |
| * | 24 | 0.0 | 5.8 | 1.5 | 1.8 |
| DBE | | 3.92 | 2.35 | 1.87 | 1.01 |



* Absent from repeat reductions

** Unsymmetrical peak

The major fraction (ii) was still a complex mixture. In an attempt to identify the acetylenic ester present this fraction was treated with mercuric acetate in methanol as described elsewhere in this thesis. The product, further separated by $Ag^{+}$/TLC, was shown to be a mixture of methyl octadecenoates, oxostearates, and possibly of a conjugated oxo-octadecenoate. The MS of the methyl oxostearates showed these to be a mixture of 12- and 13- oxoesters. It is considered that fraction (ii) is a mixture of:

18:1 (9c, 12c and 14c)

18:1 (12a) $\xrightarrow{Hg(OOCCH_3)_2}$ 12- and 13-oxo 18:0

18:2 (12a14c) $\xrightarrow{Hg(OOCCH_3)_2}$ 13-oxo 18:1 (14c)

The following conclusions can be drawn from the above results:-

(1) The acetylenic site in dehydrocrepenynic acid lies in the 12,13- position.

(2) The acetylenic bond is more resistant to

hydrogenation than either the conjugated or non-conjugated olefinic bond.

(1) and (2) illustrate the value of partial reduction with hydrazine followed by hydration with mercuric acetate as a means of pinpointing acetylenic sites in polyunsaturated acids.

## Experimental

### (i) Starting materials

Methyl crepenynate and dehydrocrepenynate were isolated by preparative $Ag^+$/TLC of the mixed esters of Afzelia bipendensis or A. cuanzensis. The neat esters ( 100mg) were manually streaked on to a silver nitrate/silica plate (1mm thick; ~15% $AgNO_3$) and double developed in PE 20. The plates were sprayed with a methanolic solution of 2', 7'-dichlorofluorescein (~0.5%) and rendered visual under ultraviolet light. The following five bands separated:-

1. Methyl palmitate, stearate, lignocerate(24:0)
2. Methyl oleate
3. Methyl dehydrocrepenynate
4. Methyl crepenynate
5. Methyl linoleate

Bands 3 and 4 were extracted (4x20ml) with solvent ether, dried over anhydrous sodium sulphate and the ether was evaporated off in vacuo. The esters were dissolved in petrol (b.p. 40 - 60°) and stored at 0° C

under nitrogen since both crepenynate and dehydro-crepenynate have been reported to be prone to autoxidation.

(ii) Hydrolysis of methyl esters

The esters (100mg) were hydrolysed by shaking with ethanolic potassium hydroxide (5ml, ~0.5N) for 2.5 hours in the dark and under nitrogen. The mixture was then acidified with dilute hydrochloric acid (2N), extracted (4x20ml) with solvent ether and dried over anhydrous sodium sulphate. The ether was removed in vacuo and the resulting acids (98mg) stored in petrol over nitrogen at 0°.

Xymenynic (Santalbic) acid was prepared by treating the oil (10g) of Santalum album (Santalaceae) with boiling alcoholic potassium hydroxide for 1 hour. The gum which separated was discarded and the mixture acidified and ether extracted (4x50ml) to give the mixed acids. Recrystallisation of these acids from light petrol (b.p. 40-60°) gave crude xymenynic acid (~5g; m.p. 34-39°) and further recrystallisation yielded the pure acid (~3g; m.p. 38-39.5°). This was stored in petrol at 0° under nitrogen.

Esterification with $BF_3$/methanol

The acid (100mg) was refluxed for 3 minutes with a methanolic solution of boron trifluoride (6ml; ~2% $BF_3$) before the addition of saturated brine (~1ml). The methyl ester was extracted (4x20ml) with ether, washed with sodium bicarbonate solution (~1ml; 5%) then distilled water (1ml) and dried over anhydrous sodium sulphate. After removal of the solvent in vacuo the pure ester (99mg) remained.

Partial reduction of crepenynic acid with hydrazine

Crepenynic acid (83mg) was stirred vigorously in an open flask at 56-60° with hydrazine hydrate (3.6g) and glacial acetic acid (0.5ml) in ethanol (200ml). Aliquots (25ml) were withdrawn at intervals of 0.0, 1.5, 3.8, 7.8, 12.7 and 24.5 hours, acidified with dilute sulphuric acid (2N), salted and extracted with light petrol (b.p. 40-60°, 4x20ml). The solvent was removed under reduced pressure (~40°) and the resulting acids converted into methyl esters by treatment with a methanolic solution of boron trifluoride as described earlier. The mixture of esters was then analysed by GLC (20% DEGS, 190°C) and the results appear in Table 6.

Partial reduction of xymenynic acid with hydrazine

Xymenynic acid (100mg) was partially reduced by stirring with hydrazine hydrate (4.5g) in ethanol (100ml) in the presence of acetic acid (0.5ml) at 55-60°. After 0.0, 1.0, 4.0, 8.8, 12.0 and 28.0 hours aliquots (10ml) were removed and treated as before to yield the mixed methyl esters. Table 7 gives the GLC analysis (DEGS, 190°C) of these.

The UV spectra of the aliquots were run in methanol and the results appear in Table 10. The first three mixtures were run on an analytical $Ag^+$/TLC plate (0.3mm thick; ~15% $AgNO_3$) in PE 20 along with the following methyl esters:-

(i) Methyl stearolate (18:1, 9a)
(ii) 18:2, 9c11t + 18:2, 10t12c + 18:2, 9c12c

The spots were rendered visual by spraying the plate with an ethanolic solution (10%) of phosphomolybdic acid and heating at 100° for 30 minutes. The results indicated that methyl xymenynate and the conjugated dienoate mixture had almost identical Rf values and methyl stearolate ran only slightly

lower. Different petrol/ether solvent systems were tried but as no distinct separation could be effected no conclusions were drawn.

Partial reduction of dehydrocrepenynic acid

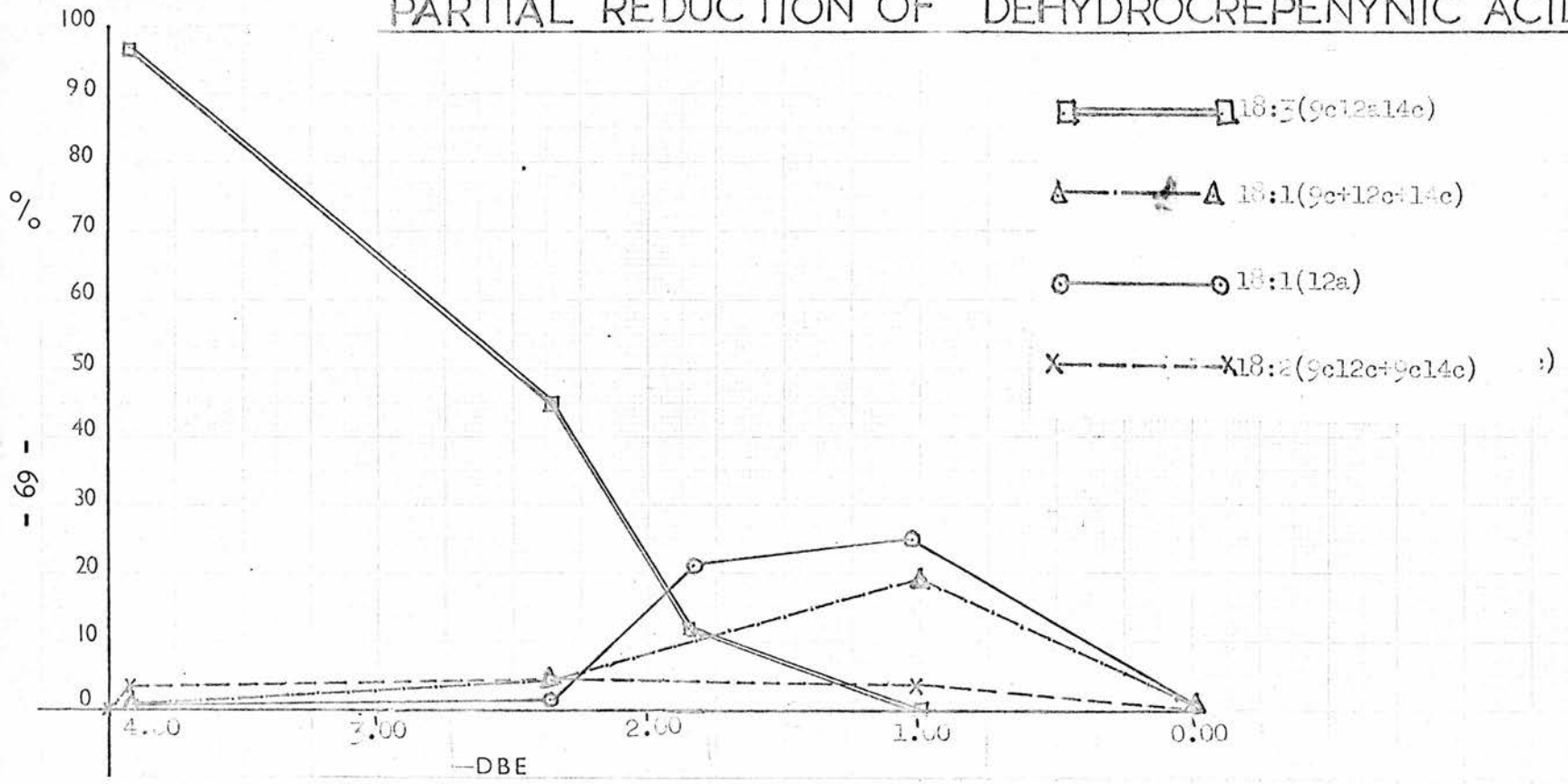
Dehydrocrepenynic acid (26mg) was reduced with hydrazine hydrate (1.5g) as before and aliquots were removed at 0.0, 0.8, 4.3 and 24.8 hours. These were worked up to yield the methyl esters which were examined on GLC (DEGS). (See Table 13 )

In a second experiment (see scheme ) dehydrocrepenynic acid (85mg) was reduced for 4.0 hours with hydrazine hydrate (3.5g). The product (after spillage, 41mg) showed the following composition:-

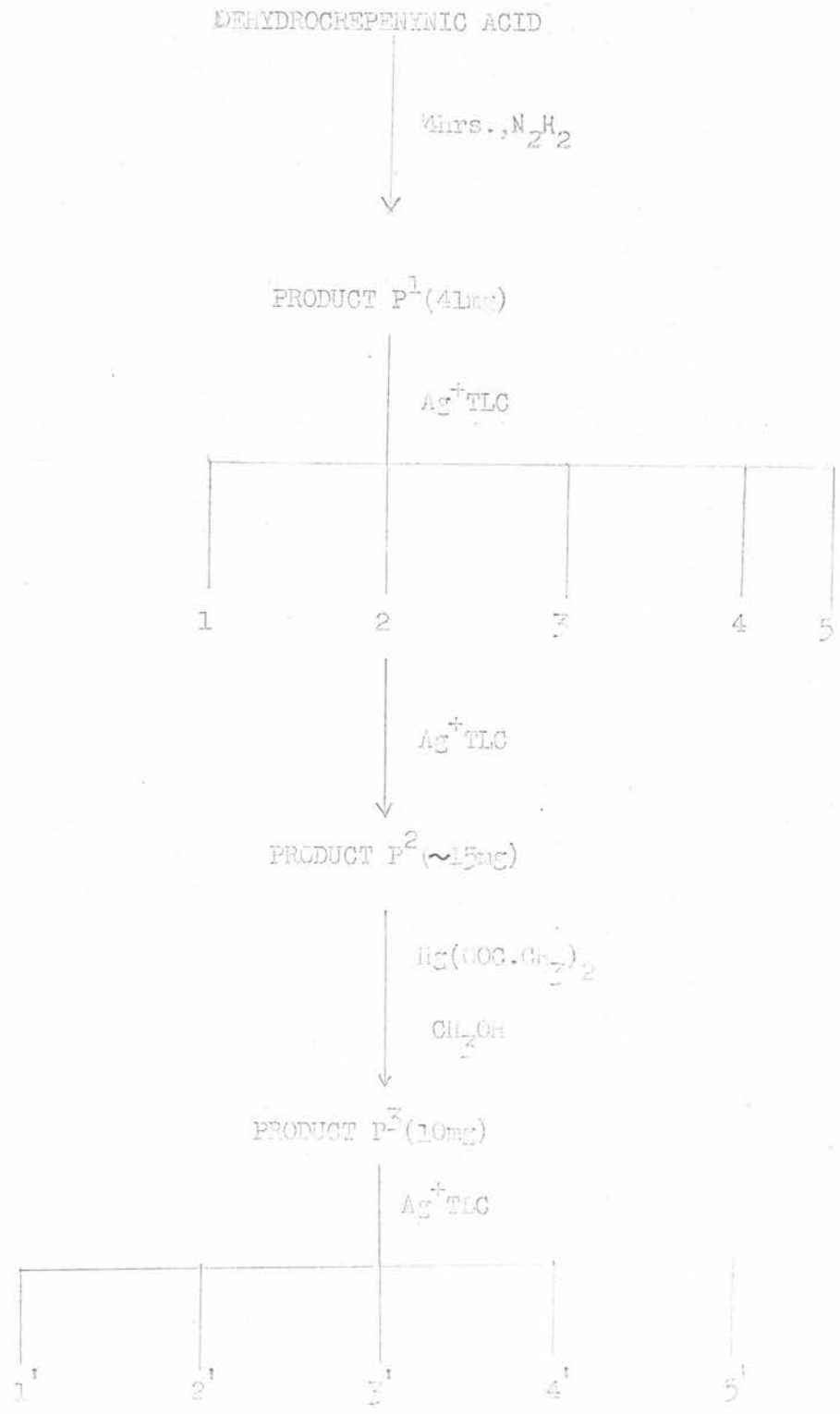
| ECL | 18.0 | 18.6 | 19.2 | 20.3 | 20.5 | 21.6 | 22.4 | ~23.2 |
|---|---|---|---|---|---|---|---|---|
| % | 7.5 | 13.7 | 5.7 | 20.5 | 5.7 | 34.6 | 11.3 | 1.0 |

Fig4

# PARTIAL REDUCTION OF DEHYDROCREPENYNIC ACID



SCHEME 3


DEHYDROCREPENYNIC ACID
4hrs., $N_2H_2$
PRODUCT $P^1$(41mg)
$Ag^+$TLC
1
2
3
4
5
$Ag^+$TLC
PRODUCT $P^2$(~15mg)
$Hg(OOC.CH_3)_2$
$CH_3OH$
PRODUCT $P^3$(10mg)
$Ag^+$TLC
1'
2'
3'
4'
5'

Preparative $Ag^+$/TLC of product

The total product (41mg) was streaked on to a preparative silver nitrate/silica plate (1mm thick; 10% $Ag^+$), developed in PE 20 and sprayed with a methanolic solution of 2', 7'-dichlorofluorescein before viewing under ultraviolet. The mixture separated into five distinct bands which were extracted with ether (4x20ml) and the extract run on GLC(DEGS, 190°) to give the analysis below. (Tables 14(a) and (b))

Table 14

(a) DEGS, 190°C

| Component | ECL | ~Composition(%) of bands | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 18:3(9c12a14c) | 22.4 | - | - | 80+ | - | - |
| 18:2(9c12a)+? | 21.6 | - | 40 | - | 80+ | 45 |
| ? | 20.5 | - | 10 | - | - | - |
| 18:1(12c) | 20.3 | - | 30 | - | - | - |
| 18:2(9c12c+9c14c) | 19.2 | - | - | - | - | 45 |
| 18:1(9c+12c+14c) | 18.6 | - | 20 | - | - | - |
| 18:0 | 18.0 | 80+ | - | - | - | - |

Table 14

(b) ApL 210°C

| Component | ECL | ~Composition(%) of bands | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5' |
| ? | 16.0 | - | - | - | 10 | - |
| 18:2(9c12c+9c14c) | 17.5 | - | - | - | - | 50 |
| 18:1(9c+12c+14c) | ~17.7 | - | 15 | - | - | - |
| 18:3(9c12c14c) + ? | 17.9 | - | 70 | 90+ | 65 | - |
| 18:0+18:2(9c12a) | 18.0 | 80+ | - | - | - | 30 |
| ? | 18.2 | - | - | - | 5 | - |
| ? | 18.4 | - | 15 | - | - | - |
| ? | 19.4 | - | - | - | 20 | - |

Band 1 contained methyl stearate. Band 3, with an $E^{1\%}_{1cm} \sim 400$ and ECLs of 22.3 (DEGS) and 18.0 (ApL) contained unreacted methyl dehydrocrepenynate.

The major component of Band 4 had ECLs of 21.6 (DEGS) and 18.0 (ApL) and an $E^{1\%}_{1cm} \sim 370$. When subjected to von Rudloff oxidation it gave azelaic acid and no other dibasic acids - no monobasic acids were observed. This component was thus thought to be methyl crepenynate.

Band 5 was considered to contain a mixture of methyl crepenynate and linoleate on the basis of GLC evidence.

Band 2, the most complex of the fractions, was again subjected to preparative $Ag^+$/TLC (20% $Ag^+$; PE 25) in the hope of further separation but only a mixture $P^2$ of slightly different composition could be obtained, viz.:-

| ECL(DEGS) | 18.6 | 20.3* | 21.5 |
|---|---|---|---|
| % | 61 | 27 | 12 |

* small but distinct shoulder ~ 20.7

$P^2$ was then treated with mercuric acetate (120mg) in methanol to give a product $P^3$ (15mg) of composition:-

| ECL(DEGS) | 18.7 | 20.7 | 25.1 | 26.9 |
|---|---|---|---|---|
| % | 62 | 7 | 21 | 10 |

Preparative $Ag^+$/TLC (~ 15% $Ag^+$; 1mm thick, PE 25) separated this mixture into five bands (see Table 15 below).

Table 15

| Component | ECL (DEGS) | Composition(%) of bands | | | | |
|---|---|---|---|---|---|---|
| | | 1' | 2' | 3' | 4' | 5' |
| 18:1(9c+12c+14c) | 18.7 | - | 95 | - | - | - |
| 12-oxostearate<br>13-oxostearate | 25.2 | - | - | 95 | 5 | - |
| | 26.9 | - | - | - | 75 | - |

Bands 1' and 5' showed no definite peaks on GLC; Band 2' contained unchanged methyl oleate.

Band 3' showed IR absorption characteristic of a non-conjugated oxo-ester (1710, 1735cm$^{-1}$) and its mass spectrum was identical with that expected of a mixture of 12- and 13-oxostearates (see Table 16 p. 82)

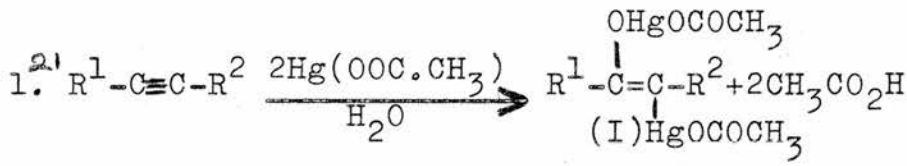
The major component (~75%) of band 4' had an ECL(DEGS) of 26.9, identical with that of a conjugated oxoester, but gave an anomalous IR spectrum with unexpectedly low oxo-absorption (1700-1800cm$^{-1}$) and a small multiplet (~ 930cm$^{-1}$) in the trans-absorption region. After hydrogenation (Pd/charcoal) band 4' on GLC(DEGS) showed a peak only at ECL 25.1, this replacing 26.9, suggesting saturation of a conjugated oxo-ester to give the non-conjugated oxoester appearing in band 3'. The mass spectrum of this hydrogenated product showed the molecular ion 312 and major fragments at mass numbers 128, 185 and 242, consistent with fragments $\left[CH_2 = \underset{\underset{OH}{\|}}{C} - (CH_2)_5CH_3\right]^+$,

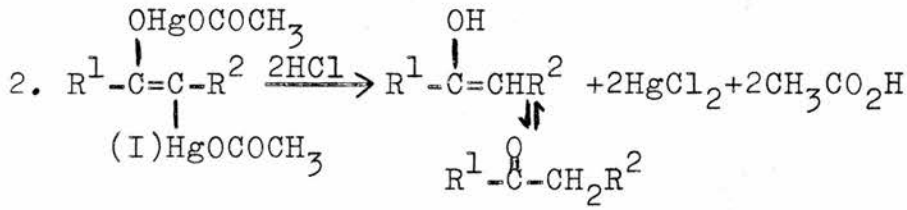
$\left[CH_3 - O - \underset{\underset{O}{\|}}{C} - (CH_2)_9\right]^+$ and $\left[CH_3-O-\underset{\underset{O}{\|}}{C}-(CH_2)_{10}-\underset{\underset{OH}{\|}}{C}=CH_2\right]^+$ .

## The mercuric acetate catalysed hydration of acetylenes

### (a) Introduction

Water can be addedto the triple bond of acetylenes to give the corresponding ketones often by treatment with hot acid alone, but a more efficient method involves the catalytic use of mercuric sulphate in the presence of sulphuric acid or reaction with mercuric acetate. In the latter case the reaction conditions are quite mild and in a homogeneous medium (e.g. methanol, acetone) good yields of a mercury complex can be obtained. This forms the corresponding ketone on acidification with dilute mineral acid or treatment with hydrogen sulphide:-





This method has been employed as a quantitative method of estimating acetylenes[22] since one mole of the complex (I) yields two moles of acetic acid, which can be titrated by standard methods. Alternative mercuric salts which have been used are the chloride,

acetamide or toluene-p-sulphonamide - the last two are particularly useful in that acid-labile acetylenes can be hydrated under sufficiently neutral conditions.

Although in the above reaction scheme only one ketone is shown, in fact two isomers are possible, depending on the direction of addition of the nucleophile to the acetylene, and this is governed by the electrophilic or nucleophilic nature of the groups $R^1$ and $R^2$ which flank the double bond in the complex (I). If $R^1$ is more electron-withdrawing than $R^2$ the anion ($OHgOCOCH_3$) is attached to the carbon atom next to $R^1$ leading to the ketone shown above; but if $R^1$ and $R^2$ are similar in nature a mixture of ketones is obtained.

In the ensuing experiments three acetylenic esters:-

(a) methyl stearolate 18:2 (9a)

(b) methyl crepenynate 18:2 (9c12a)

(c) methyl xymenynate 18:2 (9allt)

were converted into mercury complexes by shaking for 24 hours at room temperature with an excess of mercuric acetate in methanol. Under these conditions the mercuric salt also added to any olefinic centres but in the subsequent acidification step the double bonds were regenerated unchanged whereas an oxo-group appeared at what was originally the acetylenic site, usually at both possible positions. It was hoped

by means of this reaction to pinpoint acetylenic sites in molecules in the presence of additional olefinic unsaturation, conjugated or not.

(b) Results

(i) Methyl stearolate (18:1, 9a)

When shaken with mercuric acetate in methanol for 24 hours methyl stearolate gave a major product amounting to (97%) and this was isolated by silica/TLC and gave IR absorptions characteristic of a saturated oxoester. The MS, presented as a graph of mass number vs. relative intensity (taking mass 156 as 100%), was identical with that recorded[23] for a mixture (50:50) of 9- and 10-oxostearates (see figs. 5 and 6). Thus when reacted with mercuric acetate as described, methyl stearolate gives an almost quantitative yield of 9- and 10-oxo-stearates in approximately equal proportions.

out of place



Table 17

Hydration of methyl xymenynate

Mass spectrum of major product

| m/e | % | m/e | % |
|---|---|---|---|
| 97 | 55 | 185 | 13 |
| 111 | 15 | 193(d-32) | 10 |
| 125 | 15 | 195 | 5 |
| 139(b) | 100 | 199(a) | 5 |
| 140 | 12 | 206 | 8 |
| 149 | 53 | 211 | 5 |
| 154(c+1) | 63 | 224 | 7 |
| 155 | 21 | 225(d) | 35 |
| 167(a-32) | 12 | 279 | 13 |
| | | 310 | 5 |

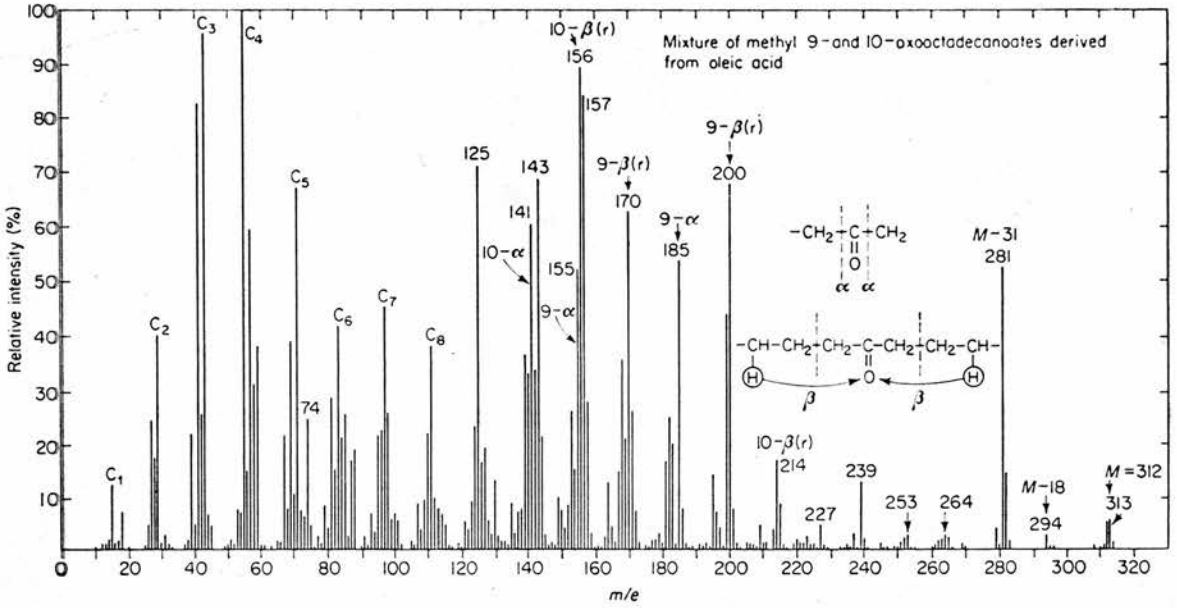



**Fig. 22.** Mass spectrum of equimolecular mixture of methyl 9- and 10-oxooctadecanoates derived from oleic acid. (Kenner and Stenhagen, unpublished.)

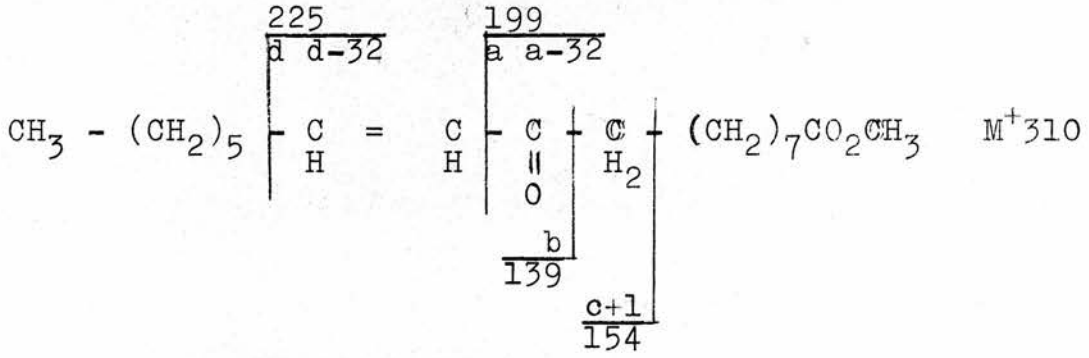
(ii) Hydration of methyl xymenynate (18:2,9allt)

Methyl xymenynate underwent hydration by shaking with mercuric acetate using the conditions described before. The IR spectrum and GLC analysis of the product indicated that the major component (~50%) was a conjugated oxoester and this component was isolated silica/TLC. Its MS (see table 17) was consistent with that predicted for methyl 10-oxo-octadec-trans-11-enoate*, and IR spectrum indicated the presence of a trans-unsaturation. After hydrogenation this component was replaced by one showing the GLC behaviour of a saturated oxoester and its MS was very similar to that observed$^{R}$ for methyl 10-oxostearate - impurities could account for the minor variations. It is believed that the above reaction converts methyl xymenynate predominantly into methyl 10-oxo-octadec-trans-11-enoate, the triple bond undergoing hydration whereas the conjugated double bond remains unchanged in position and configuration. This being so, this method may prove to be valuable in the pinpointing of acetylenic sites in polyunsaturated esters.

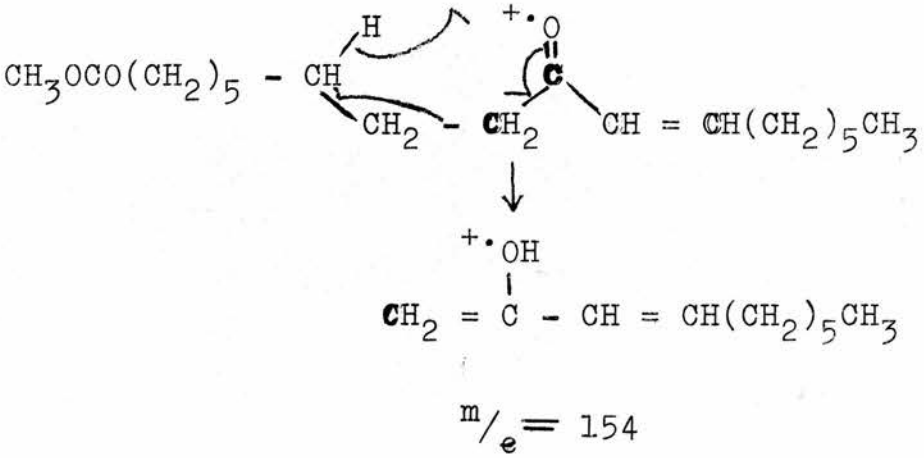
* The MS of unsaturated esters are not always unique[24].

Table 17

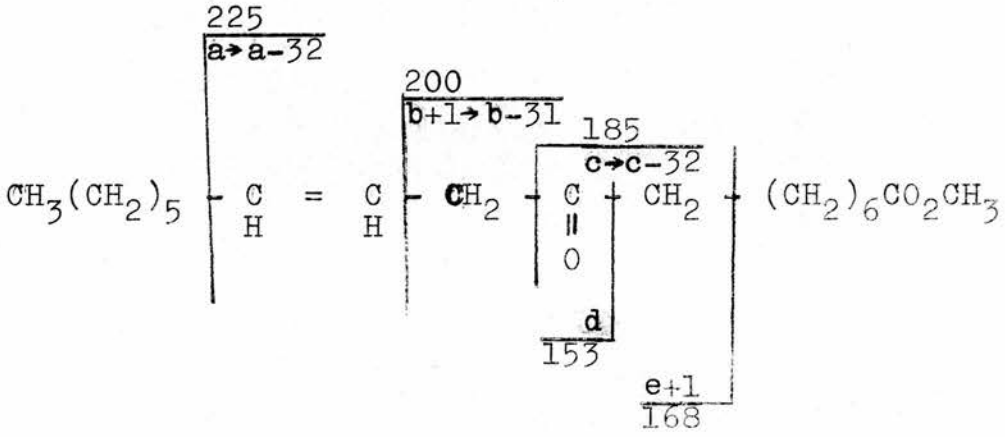
Expected cracking pattern of methyl 10-oxo-octadec-trans-11-enoate




c + 1 is the result of the following McLafferty Rearrangement.



A possible impurity is the 9-oxo-11t isomer which would probably give the following splittings:




e+1 and d might be expected to lead to major fragments by considering similar molecules. This would lead to masses of 168 and 153; these however are missing from the spectrum and it would appear that this impurity is not present.

p 84 follows p 80

(iii) Methyl crepenynate (18:2, 9c12a)

The product of the hydration of methyl crepenynate could be a mixture of methyl-12-oxo-octadec-cis-9-enoate and the 13-oxo isomer. However, methylene interrupted oxo-octadecenoates are known to alter on $Ag^+$/TLC to give conjugated oxoesters and various autoxidation products[25], and in the presence of mercuric acetate it was considered that a similar process might occur with the 12-oxoester. Thus the reaction product would be complex, containing the unchanged 13-oxoester and derivatives of the 12-oxoester.

When it was reacted with mercuric acetate methyl crepenynate gave rise to mainly two products of ECLs (DEGS) 25.0(50%) and 27.7(40%) which were separated by silica/TLC. The upper band yielded the component of ECL 25.0 which showed the IR absorption of a cis non-conjugated oxoester. After hydrogenation an ECL of 24.5 was obtained. The MS of this compound showed a molecular ion 312 and the major fragments of masses 114, 241 and 256 consistent with

$$\left[CH_3(CH_2)_4\overset{OH}{\overset{|}{C}} = CH_2\right]^+, \quad \left[\overset{O}{\overset{\|}{C}}(CH_2)_{11}CO_2CH_3\right]^+, \quad \left[CH_2 = \overset{OH}{\overset{|}{C}} - \underset{CO_2CH_3}{(CH_2)_{11}}\right]^+$$

The upper band was therefore considered to contain

methyl 13-oxo-octadec-9-cis-enoate.

The lower band contained the component of ECL 27.7, the IR spectrum of which showed insignificant absorption in the 1730 - 1680 $cm^{-1}$ (carbonyl) or ~3600 $cm^{-1}$ (hydroxyl) regions. It remained unchanged after attempted hydrogenation (Pd/charcoal) and the MS (table) showed a molecular ion 340 and large fragments at masses 113, 149, 183 and 227. It seems possible that this is an autoxidation derivative of the 12-oxo-octadec-cis-9-enoate but no real substantiation for this has been found and no further investigations into its structure were carried out due to lack of time.

(c) Experimental

Mercuric acetate catalysed hydration of acetylenic compounds

(i) Methyl stearolate (18:1, 9a)

Methyl stearolate (100mg; $2 \times 10^{-4}$ moles) was stored for 24 hours in the dark with mercuric acetate (217mg; $4 \times 10^{-4}$ moles) in dry methanol (20ml).[26] The solvent was then removed in vacuo, the residue taken up in chloroform (50ml) and the filtered extract evaporated in vacuo to leave a white solid. This was treated with dilute hydrochloric acid (2N, 2ml) extracted (4x20ml) with solvent ether and dried over anhydrous sodium sulphate. The solution was filtered and solvent evaporated to leave a solid (90mg) having the following composition:-

| ECL(DEGS) | 24.8 | 20.4* | 19.2 |
|---|---|---|---|
| % | 97 | 2 | 1 |

* unreacted methyl stearolate

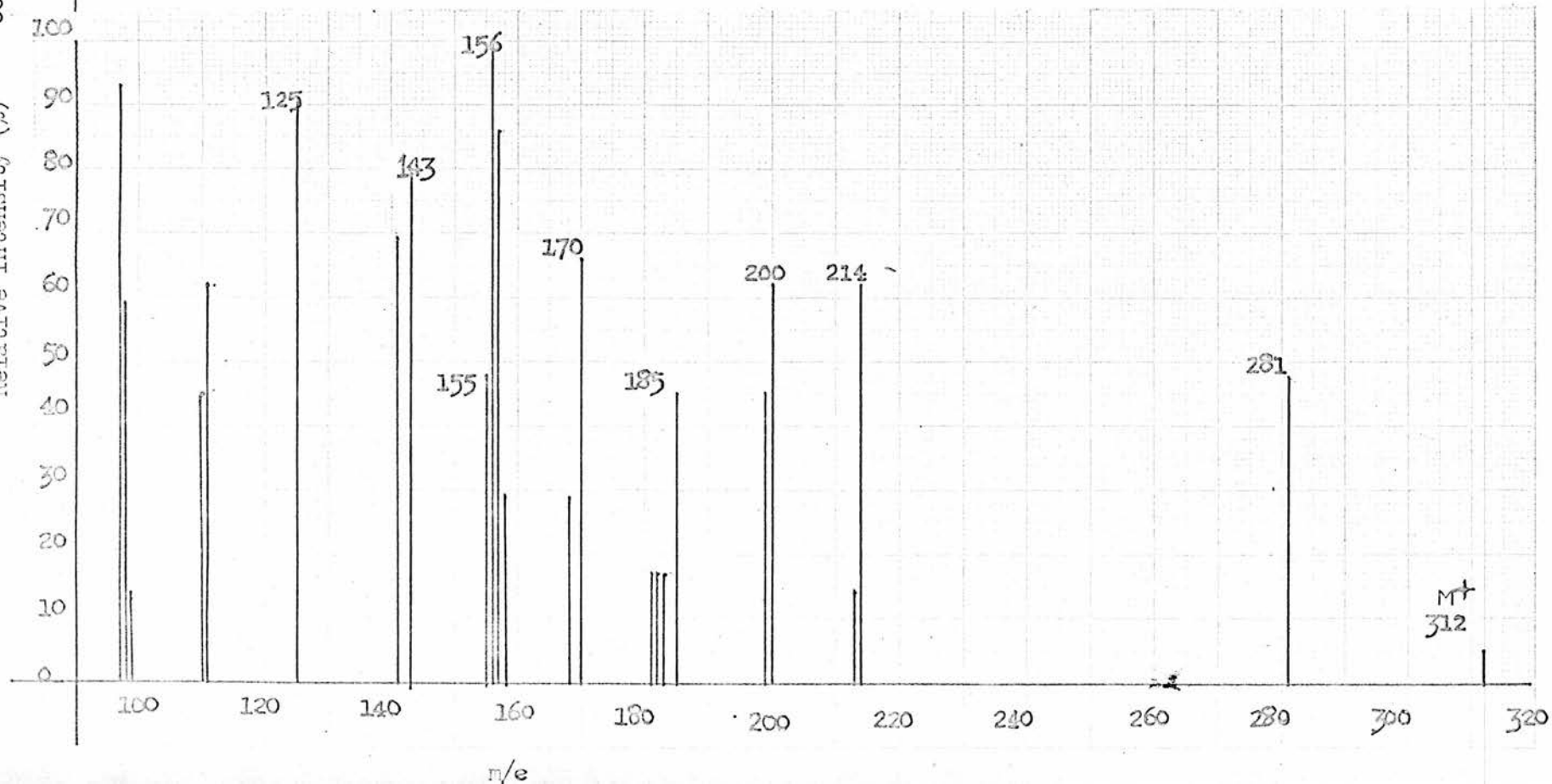
The IR spectrum of the product indicated the presence of non-conjugated oxo-($1710cm^{-1}$) and ester ($1740cm^{-1}$) functions. There was no methoxyl ($1100cm^{-1}$) or hydroxyl ($\sim 3600cm^{-1}$) absorption.

The product was compared on an analytical TLC plate (0.3mm thick) with a mixture of 9- and 10-oxostearates. This was developed in PE 20 and then immersed in a tank of iodine vapour, this causing brown spots to appear. The reaction product gave one major spot identical in Rf with the authentic oxostearates.

After preparative $Ag^+$/TLC (1mm thick; 15% $Ag^+$; PE 20) the major product of the reaction was isolated (over 99% pure). Its mass spectrum was found to be identical to that recorded by Stenhagen and Kenner[26] for a mixture (50:50) of methyl 9- and 10-oxostearates (see figs. 5 and 6).

Fig.

# MASS SPECTRUM OF HYDRATION OF METHYL STEAROLATE — MAJOR PRODUCT



(ii) Methyl xymenynate (18:2, 9allt)

Methyl xymenynate(100mg;$2x10^{-4}$moles) was hydrated with mercuric acetate(450mg;$1x10^{-4}$moles) as described above. The product (90mg) showed the following composition on GLC(DEGS):

| ECL(DEGS) | 17.4 | 21.6 | 22.9 | 24.4 | 24.9 | 25.5 | 26.7 |
|---|---|---|---|---|---|---|---|
| % | 5 | 15 | 5 | 15 | 5 | 5 | 50 |

The product was separated on a silica plate (1mm thick, PE 20) into three bands the lowest of which was the largest. On GLC(DEGS) this had an ECL of 26.7 (~98%) and its IR spectrum indicated conjugate oxo- ($1675cm^{-1}$, $1690cm^{-1}$), trans-olefinic ($980cm^{-1}$) and ester ($1740cm^{-1}$) functions.

This product gave an ECL(DEGS) of 24.5 when hydrogenated (Pd/charcoal) and the trans-, and conjugated oxo absorptions in the infrared were replaced by non-conjugated oxo absorption at $1710cm^{-1}$. The MS of this compound showed a molecular ion 312 and major fragments at 141, 156 and 281 consistent with

$\left[CH_3(CH_2)_7 - \overset{O}{\overset{\|}{C}}\right]^+$, $\left[CH_3(CH_2)_7 - \overset{OH}{\overset{|}{C}} = CH_2\right]^+$ and M - 32

from methyl 10-oxostearate.

(iii) Methyl crepenynate

Methyl crepenynate (65mg; $1.4 \times 10^{-4}$ moles) was hydrated with mercuric acetate (300mg; $4.2 \times 10^{-4}$ moles) as before. The product (50mg) showed the following composition on GLC(DEGS):

| ECL(DEGS) | 24.5 | 25.0 | 26.4 | 27.7 |
|---|---|---|---|---|
| % | 5 | 50 | 5 | 40 |

The product was separated by silica/TLC (PE 20) into 2 major bands. The lower band yielded the component of ECL 25.0 which absorbed in the infrared at 1735cm$^{-1}$ (ester) and 1720cm$^{-1}$ (non-conjugated oxo).

The lower band contained the component of ECL 27.7. The IR spectrum of this showed an ester function (1735cm$^{-1}$) and also very slight absorption at 1680, 1690 and 1720cm$^{-1}$ - probably due to trace impurities. It remained unchanged after hydrogenation, ruling out the presence of double bonds. The MS of this component is set out in Table 18.

**Table 18**

| m/e | % | m/e | % |
|---|---|---|---|
| 109 | 44 | 201 | 55 |
| 111 | 53 | 212 | 58 |
| 113 | 83 | 227 | 66 |
| 123 | 42 | 238 | 31 |
| 139 | 47 | 309 | 19 |
| 149 | 100 | 340 | 14 |
| 183 | 71 | | |

## REFERENCES


1. e.g. Mikolajczak, K.L. et al. J. Org.Chem. 29, 318, 1964.
2. Cornelius, J.A. et al. J.Sci.Fd. Agric. 21, 1970.
3. Gunstone, F.D. et al. Chem. Phys. Lipids 1, 349, 1967.
4. Gunstone, F.D. et al. Chem. Comm. 295, 1967.
5. Mikolajczak, K.L. et al. J. Org. Chem., 29, 318, 1964.
6. Bu'lock, J.D. and Smith, G.N. J. Chem. Soc. 332-6, 1967 (C).
7. Gauglitz, E.J. and Lehmann, L.W. J.A.O.C.S., 40, 197, 1963.
8. Dutton, J.H. et al. J.A.O.C.S., 40, 175, 1963.
9. Aylward, F. and Sawistowska, M. Chem. and Ind., 433, 1961.
10. Lemieux and von Rudloff, E. Can. J. Chem. 33, 1701-9, 1955.
11. Isii, Y. J. Chem. Soc. Ind. Japan, 43 255-6, 1940.
12. Gunstone, F.D. "An Introduction to the Chemistry and Biochemistry of Fatty Acids and their Glycerides", p.98, (Chapman and Hall, 1967).
13. Ibid. p.104.
14. Dutton, H.J. et al. J.A.O.C.S., 40, 175, 1963.
15. Aylward, F. and Sawistowska,M. Chem. and Ind., 433, 1961.
16. as for reference 1.
17. Bu'lock, J.D. and Smith, G.N. J. Chem. Soc., 332, 1967(C).
18. Bu'lock, J.D. and Smith, G.N. J. Chem. Soc., 333, 1967(C).
19. As for reference 4.
20. Gunstone, F.D. and Kilcast,D. Unpublished observations.
21. Raphael "Acetylenic Compounds in Organic Synthesis", p.40.

22. Koulkes, M. Bull. Soc. Chim. 402, 1953.

23. Boekenoogen "Analysis and Characterization of Oils, Fats and Fat Products" Vol.2. p.25, (Wiley and Sons, 1968).

24. Ibid. p.233.

25. Raphael "Acetylenic Compounds in Organic Synthesis", p.203, (Butterworths, 1955)

26. As for reference 23.