SUMMARY

This thesis is concerned with a re-examination of the important processes used to isolate unsaturated fatty acids from suitable seed oils with a view to determining the most economic route.

Attempts were made to find the cost of obtaining acids or their methyl esters of high purity ($\geq$99%) on a moderate scale (1 or 2 Kg. of starting material). The desired acid was separated from other acids by low-temperature cyrstallisation and urea crystallisation with final purification from non-fatty acid material by column chromatography. Column chromatography was also required to separate α-linolenic from linoleic acid because the other technique proved inefficient. Fractions were examined continually by G.L.C., occasionally by von Rudloff oxidation and infrared spectroscopy and finally by T.L.C. Attention was given to the handling of fractions and the storage of final products to prevent or reduce their deterioration.

Initial experiments on a small scale and subsequent experience as the work progressed produced standard methods of applying low-temperature crystallisation, urea crystallisation and column chromatography. A method of costing, which included the materials consumed and the work done, was applied to the isolation of each acid.

Oleic acid ($\geq$99%) was isolated readily and cheaply from olive oil by a route which seemed to be applicable to similar acids from seed oils. However, linoleic acid ($\geq$99%) could not be isolated from safflower oil by this route without using column chromatography to remove α-linolenic from the desired acid. This increased the cost of obtaining linoleic acid above the acceptable level. This problem was overcome by using Evening Primrose oil which yielded both pure linoleic acid and pure γ-linolenic acid by the standard procedures and at a reasonable cost which could be shared between the two acids according to market demand.

Finally, this method of costing was applied to octadec - cis -

"Praying always with all prayer and supplication in the Spirit, and watching thereunto with all perseverance and supplication for all saints; and for me, that utterance may be given unto me, that I may open my mouth boldly, to make known the mystery of the gospel".

EPHESIANS 6: 18,19.

"He asked life of Thee, and Thou gavest it Him, even length of days for ever and ever. His glory is great in Thy salvation: honour and majesty hast Thou laid upon Him. For Thou hast made Him most blessed for ever: Thou hast made Him exceedingly glad with Thy countenance".

PSALM 21: 4,5,6.

To Cynthia and our new-born
son, John Mark.

The isolation and synthesis of long-chain acids and an attempt to cost these processes.

being a thesis

presented by

John McLaughlan

to the

University of St. Andrews

in application for

The Degree of Master of Science

June, 1975.

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 previously been presented in application for a higher degree.

The research was carried out in the Department of Chemistry of the University of St. Andrews, under the supervision of Professor F.D. Gunstone, D.Sc., F.R.I.C.

Certificate

I hereby certify that John McLaughlan has completed five terms of research work under my supervision, has fulfilled the conditions of Resolution of the University Court 1974 No. 2 (St. Andrews) and that he is qualified to submit the accompanying thesis in application for the degree of Master of Science.

Research Supervisor

University Career

After obtaining the necessary passes in the Higher National Certificate examinations I entered the Junior Honours class in chemistry at St. Andrews University in October, 1971 and graduated with B.Sc. (Hons.) in October, 1973.

I was admitted as a research student in the University of St. Andrews in September, 1973 with a grant provided by Professor F.D. Gunstone.

Acknowledgements

I wish to acknowledge the following:

Professor Gunstone not only for his guidance and encouragement in the research and composition of this thesis but also for his personal concern for my career in chemistry.

Dr. C.M. Scrimgeour for his constant advice throughout this research.

Andrew Watson for his help in carrying out part of this research and for his companionship each day in the laboratory.

The Chemistry Department as a whole for providing the means of carrying out this research in pleasant surroundings.

Anne Adamson for the expert typing of the script.

CONTENTS

ABBREVIATIONS

chromatog.	column chromatography.
cryst.	crystals.
DEGS	diethylene glycol succinate.
est.	esterification.
filt.	filtrate or mothor liquor.
G.L.C.	gas-liquid chromatography.
hydrol.	hydrolysis.
LT	low-temperature cyrstallisation.
P	petrol (b.p. 40-60°C).
PE1 (2) etc.	1% (2%) etc. of ether in petrol (by volume).
ppt.	precipitate.
RFE	rotary film evaporator.
sat.	saturated fatty acid or ester.
T.L.C.	thin-layer chromatography.
urea ( )	urea crystallisation of the type indicated (see page 9).

SUMMARY

This thesis is concerned with a re-examination of the important processes used to isolate unsaturated fatty acids from suitable seed oils with a view to determining the most economic route.

Attempts were made to find the cost of obtaining acids or their methyl esters of high purity ($\geq$99%) on a moderate scale (1 or 2 Kg. of starting material). The desired acid was separated from other acids by low-temperature cyrstallisation and urea crystallisation with final purification from non-fatty acid material by column chromatography. Column chromatography was also required to separate α-linolenic from linoleic acid because the other technique proved inefficient. Fractions were examined continually by G.L.C., occasionally by von Rudloff oxidation and infrared spectroscopy and finally by T.L.C. Attention was given to the handling of fractions and the storage of final products to prevent or reduce their deterioration.

Initial experiments on a small scale and subsequent experience as the work progressed produced standard methods of applying low-temperature crystallisation, urea crystallisation and column chromatography. A method of costing, which included the materials consumed and the work done, was applied to the isolation of each acid.

Oleic acid ($\geq$99%) was isolated readily and cheaply from olive oil by a route which seemed to be applicable to similar acids from seed oils. However, linoleic acid ($\geq$99%) could not be isolated from safflower oil by this route without using column chromatography to remove α-linolenic from the desired acid. This increased the cost of obtaining linoleic acid above the acceptable level. This problem was overcome by using Evening Primrose oil which yielded both pure linoleic acid and pure γ-linolenic acid by the standard procedures and at a reasonable cost which

could be shared between the two acids according to market demand.

Finally, this method of costing was applied to octadec - cis - 8 - enoic acid which was prepared by a standard synthetic route. Here, the calculated costs were less significant but allowed a rough comparison with the more accurate values for the "natural" acids.

INTRODUCTION [1].

Increasing interest in the physical, chemical and biological properties of lipids has produced a growing demand for pure fatty acids - both natural and synthetic - and their lipid derivatives. There is little evidence that they are produced in Britain and it is likely that those which are available are isolated at a few sources and marketed - at a high price - by several chemical suppliers. The following table contains a summary of the costs of the more important unsaturated fatty acids (or methyl esters) at the 99% pure level.

This research is concerned with a re-examination of the standard procedures by which pure unsaturated acids are normally isolated from natural sources and with an attempt to discover the cost of producing them. Similar criteria have also been applied to the synthesis of methyl octadec-*cis*-8-enoate required for dietary studies elsewhere.

The natural source of the desired acid must be selected with care. It should be readily available at a reasonable cost, be as rich as possible in the desired acid and contain also only those acids which are readily removed by simple techniques. For this reason seed oils are generally preferred to animal fats since the latter more often contain mixed isomers. Oleic acid, for example, is more likely to be contaminated with its Δ 11 isomer when derived from an animal fat. In addition, most seed oils contain only C18 unsaturated acids so that separation procedures have to take account of differences of unsaturation only and not differences in chain length. The only convenient way of separating homologous acids is by distillation and for polyunsaturated acids, particularly, this is generally undesirable since it may lead to cyclisation, polymerisation, stereomutation or double bond movement.

Cost (£) of pure acids or methyl esters (≥ 99%)

Acid	Wt.(g.)	Britain			Europe		America	
		Sigma (1973)	B.D.H. (1974)	Koch-light (1974)	Fluka[b] (1975)	Merck[c] (1974)	Nu-check[d] (1974)	Serdery[d] (1974)
oleic	10	10[k]	9[e]	-	7[e]	10[e]	6[h]	6[j]
linoleic	10	6[k]	12[e]	6	13[e]	22[e]	6[h]	13[l]
α-linolenic	10	15[k]	19[e]	12	35[f]	22[e]	21[h]	46[m]
γ-linolenic	1	26	-	-	-	-	21	20
eicosatrienoic[a]	1	-	-	-	-	-	170[n]	354[p]
arachidonic	1	23	-	-	-	8[g]	25	33[n]

a Bis-homo-γ-linolenic 20:3 (8,11,14)

b Price quoted in Swiss francs (£1=6.0)

c Price quoted in D.M. (£1=6.1)

d Price quoted in dollars (£1=2.4)

e Based on price for 5 ml.

f Based on price for 1 ml.

g 75% pure

h Based on price for 50 g.

j Based on price for 25 g.

k Based on price for 5 g.

l Based on price for 1 g.

m Based on price for 500 mg.

n Based on price for 100 mg.

p Based on price for 50 mg.

Our general isolation procedure involves steps (i) to (iii). Additional significant points are made in (iv) and (v).

(i) Conversion of the glycerides to acids or methyl esters.

(ii) Upgrading of the crude mixture by low-temperature crystallisation and/or urea crystallisation until high purity material is obtained. Chromatography is employed only when these procedures fail to yield pure material.

(iii) Adsorption chromatography of the purified acids or esters to remove coloured impurities, unsaponifiable material and oxidised products. These are not always revealed by G.L.C. analysis which should therefore be complemented by T.L.C. as a check on their absence.

(iv) The purification process is followed by G.L.C. analysis of methyl esters at each stage. Modified analytical procedures are required for material of high purity in order to get an accurate analysis of minor components in the presence of a major component ($\geq 99\%$). Final samples are checked by T.L.C. analysis, von Rudloff oxidation and infrared spectroscopy.

(v) At all times unsaturated acids must be handled with care to avoid oxidation. This is more serious with polyenoic acids than with monoenes and greater care is needed as the materials are purified and natural antioxidants, originally present, are removed. Attention must also be given to the storage of the purified products.

CHAPTER 1 : Techniques involved in the isolation, analysis and storage of pure acids from natural sources.

Discussion of experimental procedures.

Many purification procedures are available to organic chemists but only those which can be efficiently used on a scale of 100 g. – 1 Kg. were of value for the purpose of this investigation. This effectively ruled out counter-current distribution and some forms of chromatography and, for reasons already explained, distillation was avoided where possible. We found low-temperature crystallisation, urea crystallisation and adsorption chromatography to be most valuable in this connection. It was also necessary to interconvert acids and esters as required. These procedures will now be discussed. Full details of recommended procedures are given in the latter part of this Chapter.

I. Hydrolysis.

Hydrolysis of triglycerides and methyl esters was effected by reaction with hot aqueous ethanolic potassium hydroxide. No more than 1 Kg. of glycerides or esters was hydrolysed in one batch due to the limiting size of the apparatus available (5 l.), but this scale was sufficient to prevent any hold-up in the general procedure. The recovery of hydrolysed acids from glycerides and esters was about 95% (by weight).

II. Esterification.

Small samples (50 mg.) of triglycerides and acids were esterified with acidic methanol to obtain material for G.L.C. analysis. On the large scale, the maximum amount processed in this way was 500 g. due to the same limitation of the size of available apparatus. The esters were recovered in about 98% yield (weight).

III. Low-temperature cyrstallisation[2].

The crystallisation of mixtures of fatty acids at temperatures below zero brings about separation of the acids according to their solubility at the crystallising temperature. In the case of acids present in the seed oils used in these studies relative solubility is given by the sequence 18:3 > 18:2 > 18:1 > 16:0 > 18:0.

We found with mixtures of saturated (mainly 16:0 and 18:0), monoenoic (mainly 18:1) and polyenoic (mainly 18:2 and 18:3(α) or 18:3(γ)) acids or their methyl esters that good separation occurred with 18:2/18:3(γ) mixtures while 18:2/18:3(α) mixtures were difficult to separate especially when 18:3(α) was the minor component (up to 5%). Other mixtures showed moderate separation. When acids were present as impurities at <1% then their concentration dropped when crystallised at any temperature and independently of the nature of the acid.

Low-temperature crystallisation was carried out in acetone solution for low purity samples obtained directly by seed oil hydrolysis or in petrol (b.p. 40-60°C) for samples of higher purity and occasionally on methyl esters in methanolic solution. It is desirable to use solvent in the proportion of 10 ml. per gram of solute but in order to crystallise large quantities of material with the equipment available this was reduced to various levels down to 5 ml. per gram. The efficiency of the crystallisation procedure depends also on the correct choice of crystallising temperature (see Table 1.1) and on thorough and quick washing of the precipitate. This was easier when large crystals were produced by slow cooling of the solution using an acetone-cardice bath at appropriate temperatures. For small flasks (up to 3 l.) we put the refrigerant in a Dewar flask and held the solution at or near the crystallising temperature overnight: for larger

flasks (up to 5 l.) the refrigerant was kept in a plastic container firmly bedded in polystyrene and the temperature was then maintained for 3-5 hours during the working day. The size of the precipitate to be filtered and washed was limited to 600 g. with the apparatus available to us.

Advantages of the low-temperature crystallisation procedure include the very mild treatment to which the unsaturated acids are subjected and the high yield of recovered material (95-100% by weight). A disadvantage is the difficulty of obtaining reproducible results for similar mixtures of acids or esters.

Table 1.1

Acids to be crystallised		Crystallising temperature (°C)
saturated	16:0	-15
	18:0	-10
monoenoic	18:1	-35
polyenoic	18:2	-60
	18:3(α)	co-precipitates with 18:2 at -78
	18:3(γ)	remains in mother liquor at -78

IV. Urea crystallisation[3].

Urea forms crystalline inclusion compounds with long-chain acids and esters. Saturated compounds form adducts most readily followed in turn by monoenoic, dienoic and polyenoic compounds.

We used this property to effect the following separations:

saturated acid (16:0 and 18:0) from 18:1 and polyene acids
18:1 from polyene acids
18:2 from 18:3 acid.

Methanol was most commonly employed as solvent and after the inclusion compound had been filtered from the mother liquor the acids or esters were recovered from each fraction. Some preliminary experiments on the separation of oleic and linoleic acids and our subsequent experience with the separation of various acids led us to the following generalisations.

(i) The urea used in the separation complexed between one third and one quarter of its weight of acid/ester.

(ii) Urea crystallisation was most conveniently used to remove relatively small amounts of more easily complexed components from the bulk of the material which then remains in the mother liquor. Examples of this include the removal of saturated acids from olive acids or of saturated and monoenoic acids from safflower acids. This was because the recovery of the acids from the precipitate required a large separating funnel (5 l.) and, for us, this was the limiting factor in urea crystallisation.

(iii) The separation of acids/esters by urea crystallisation was not complete. In separating oleic and linoleic acids, for example, oleic acid concentrated in the complex but was accompanied by some linoleic acid whilst some oleic acid remained in the mother liquor. On the basis of (i) the precipitation of Xg. of acid would ideally require 3X to 4 Xg.of urea but in practice we

found it desirable to use more urea than this especially when the amount of impurity to be removed as a urea complex was small. Table 1.2 shows that for the removal of an impurity at the 1-5% level we recommend the use of 20 portions of urea and at the 30% level we still recommend the use of 10 portions of urea. This limits the maximum amount of material which can be conveniently treated as shown in Table 1.2.

(iv) When it was desired to complex acid/ester present in excess of 60% smaller proportions (3-5x) of urea could be used but the quantity of material which could be conveniently handled was then only 200-400 g. (Table 1.2).

(v) The separations achieved by urea and exemplified later in this thesis were efficient but on this scale of operation, there was some loss of material (5-10%). This we believe to be due to inadequate recovery from aqueous solutions which contain considerable amounts of urea.

Table 1.2

Proportion of material to be complexed(%)		Recommended weight of urea (g.)	Maximum amount of acids (g.) which can be conveniently* handled
(a)	1-5	20a	1200
(b)	6-8	15b	1000
(c)	9-11	12c	900
(d)	12-20	10d	600
(e)	21-30	10e	400
(f)	60-70	5f	340
(g)	71-80	4g	370
(h)	81-98	3h	400

* Acids equivalent to a urea requirement of 1200 g.

We used the urea procedure in several different ways. These are set out in Table 1.3 and the designation in column 1 will be used later in our flow sheets.

Table 1.3

Type	Fractions recovered	Description of process	
A	0	impurity in p.p.t. desired acid in filt.	no acids recovered*
A^1	1	impurity in p.p.t. desired acid in filt.	acids recovered from filt. only
A^{11}	2	impurity in p.p.t. desired acid in filt.	acids recovered from both fractions
B	2	desired acid in p.p.t. impurity in filt.	acids recovered from both fractions
B^1	3	impurity in first p.p.t. and filt. desired acid in second p.p.t.	** acids recovered from all fractions

* The mother liquid is used immediately for further urea crystallisation.

** In this double treatment impurities are first removed as a urea precipitate, more urea is then added to the mother liquor under conditions such that the desired acid is in the second precipitate and other impurities remain in the mother liquor.

V. Column chromatography.

This procedure was used in two ways which are discussed in the following sections.

(a) The removal of coloured impurities, unsaponifiable material and oxidised products.

Each batch of acids/esters which was ≥99% pure by G.L.C. was also examined by T.L.C. Impurities detected in this way include unsaponifiable material and oxidised products which were removed by column chromatography of the acids or esters as appropriate. Quantities up to 60 g. were easily handled on a 30 x 5 cm. column and up to 400 g. on a larger column (90 x 10 cm.) The purified acid/ester was quickly eluted with an appropriate solvent.

(b) The removal of methyl α-linolenate from methyl linoleate[4].

We found it very difficult to remove small amounts of methyl α-linolenate (up to 5%) from methyl linoleate by low-temperature crystallisation or urea crystallisation but chromatography proved to be useful in this connection. Careful elution with non-polar solvents (P, PE1, PE2) was essential for good separation. Using 2 Kg. of silica as adsorbent (the maximum we could handle with the column available) we applied this technique to various mixtures of methyl linoleate and α-linolenate obtained from safflower oil. Table 1.4 shows that the purest eluates from the column yielded pure methyl linoleate in a quantity which depends on the amount of esters put on the column and on the amounts of impurity present in the mixture. For the purification of methyl linoleate by column chromatography we recommend:

(i) Oleate concentration should be reduced to about 0.5% by another method before starting chromatography.

(ii) The concentration of α-linolenate should be less than 4.0% and this can be reduced to this level, when necessary, by running samples through a preliminary column before proceeding to the main column or, less efficiently, by careful low-temperature crystallisation of the esters in methanol (10 volumes) at -78°C.

Table 1.4

Chromatography of methyl linoleate on silica (2 Kg.)

Esters added					Best quality ester (> 98%)					Recovery of 18:2 (%)	Adsorb/ esters
Wt. (g)	G.L.C. (area %)				Wt. (g)	G.L.C. (area %)					
	18:1	18:2	18:3	18:2/18:3		18:1	18:2	18:3	18:2/18:3		
216	0.4	98.9	0.7	141:1	133	0.3	99.6	0.1	996:1	62	9:1
273	0.3	97.2	2.5	39:1	162	0.5	99.2	0.3	331:1	61	7:1
238	0.8	95.0	4.2	23:1	108	0.5	98.9	0.6	165:1	47	8:1
336	0.4	96.0	3.6	27:1	130	0.7	98.7	0.6	165:1	40	6:1
243	0.3	91.9	7.8	12:1	89	0.3	98.1	1.6	61:1	39	8:1

(iii) The ratio of adsorbent to esters should be not less than 7:1 and preferably nearer 10:1.

(iv) A recovery of about 60% of the methyl linoleate in a pure form ($\geqslant$99%) should be possible if (i), (ii) and (iii) are adhered to, but this can be raised by using a higher adsorbent/ester ratio. Additional pure linoleate can be obtained from the less pure fractions by appropriate treatment.

Thus, the limitation to this type of chromatography is effectively the size of the column available. Although total recovery of material from our column was only 85-90% the ester, pure by G.L.C., was also pure by T.L.C. and therefore did not require further treatment to remove unsaponifiable material and oxidised products.

VI. Analytical procedures.

(a) G.L.C. analysis.

Pure acids were analysed as methyl esters by G.L.C. analysis. Relative peak areas were measured from retention times rather than from widths at half-height since impurity peaks were small. As acids approached 99% purity accurate analysis of impurities became difficult. Two additional G.L.C. procedures were therefore examined to discover a more accurate method of estimating impurities in low concentrations.

(i) A known amount of a suitable internal standard (a compound not present in the mixture being examined and well separated from all the components of the mixture) was added to the G.L.C. sample. Two traces were run, one lightly loaded in which the main component was measured

against the standard and the other more heavily loaded in which the impurities were measured against the standard. The impurities were then related to the main component via the standard.

(ii) The impurity peaks were attenuated to measurable proportions by a factor of 10, 15 or 20 as required whilst the main component was kept on scale.

The results (Table 1.5) suggest that the latter method was more consistent than the former and that the major component measured in this way should be accurate to $\pm$ 0.2% However, 100.0% material was required to check this for absolute values and since our interest was to obtain $\geqslant$99% pure material we did not pursue this any further. We used the attenuation procedure to analyse impurities below 1% and, on some occasions, up to about 5%. Final samples were also run neat on G.L.C. to check the absence of impurities coincident with the solvent peak.

Table 1.5

Analysis (area %) of impurities in methyl linoleate ($\geqslant$99%)

Normal trace		Internal reference[1]		Attenuation	
18:1	18:3[2]	18:1	18:3[2]	18:1	18:3[2]
0.4	0.0	0.7	0.1	0.8	0.3
		0.6	0.2	0.7	0.3
		1.0	0.2	0.7	0.2
		0.6	0.3	0.7	0.2
	range:	0.6–1.0	0.1–0.3	0.7–0.8	0.2–0.3

1. methyl stearate (18:0) was used as internal reference
2. methyl γ-linolenate.

(b) Other analyses.

Final products are examined by T.L.C. to check the absence of non-fatty acid impurities and oxidised products. Double bond positions are readily checked by von Rudloff oxidation and their configuration (cis or trans) by infrared spectroscopy. Finally, if necessary, the absence of conjugation can be checked by ultraviolet spectroscopy.

Our samples gave a single spot by T.L.C., the expected double bond positions by von Rudloff oxidation and were free of trans unsaturation by infrared spectroscopy. These clearly showed that our purification techniques did not affect the structure of the acids. On this evidence, it was thought unnecessary to use ultraviolet spectroscopy.

VII. Handling and storage.

Batches of pure material (especially diene and triene) must be stored carefully because final chromatography renders them particularly prone to autoxidation by removing natural antioxidants.

Our material was never allowed to remain at steam bath temperature in air and after removing solvent on a RFE, the sample was cooled under vacuum or in a stream of nitrogen which also helped to remove the last traces of solvent. Final samples were flushed with nitrogen and stored at $-35^{\circ}C$ in tightly stoppered amber bottles. Samples checked after 6 months were still colourless and $\geqslant 99\%$ by G.L.C. but sometimes showed traces of oxidised material by T.L.C. These could be removed by re-chromatography when required.

Costing

We have tried to estimate the cost-to-make of our purified acids/esters by costing each procedure in terms of consumable material used and time employed. The latter is then costed at £1.72 per hour*. We have made no allowance for the cost of equipment nor for any other overhead charges. These estimates, set out in Table 1.6 and in Table 1.7, lead to the following conclusions concerning a choice between the three separation procedures of column chromatography, low-temperature crystallisation and urea crystallisation.

The separation of acids/esters of varying unsaturation by column chromatography is by far the most expensive of the three processes in addition to being limited to a maximum of 300 g. using 2 Kg. of silica in a 90 x 10 cm. column. Even under these conditions and starting with highly concentrated material ($>90\%$) the yield of pure ester ($\geqslant 99\%$) is limited and re-chromatography of some fractions is required.

---

* This figure is based on the salary of a grade 5 technician and on the assumption that he is available for 1740 hours (232 days at 7.5 hours per day).

Grade 5 technician.

	Salary	Company Pension	Graduated Pension	National Insurance	Total
Year 1	2439	244	107	90	2880
2	2529	253	113	90	2985
3	2622	262	119	90	3093

Table 1.6

Procedure	Cost of material (£)[i]	Labour (hours)[i]
hydrolysis of seed oil (1 Kg.)	0.82[ii]	4.5
esterification of acids (500 g.)	0.82	2.0
low-temperature crystallisation[iii] (up to 1 Kg)	0.20x	6 + 0.20x
urea crystallisation[iv] (up to 1.2 Kg)		
type A	0.07y	1 + 0.33y
A^1	0.12y	2 + 0.66y
A^{11} or B	0.20y	3 + 0.66y
B^1	0.20y	4 + 0.66y
column chromatography		
(a) removal of unsaponifiable etc. (200-400 g.)	2.00 + 2.50x	5 + x
(b) ester fractionation (100-300 g.)	2.00 + 5.00x	10 + x

i. x = g of acids $x10^{-2}$, y = g of urea $x10^{-2}$

ii. excluding cost of oil.

iii. maximum quantity which can be handled is 600 g. of precipitate.

iv. for explanation of type of procedure see page 9

Table 1.7

Procedure	ii	Cost (£) of processing different weights of acid (g)[i] 100	200	300	400	500	600	700	800	900	1000
LT		10.9	11.4	11.9	12.5	13.0	13.6	14.1	14.6	15.2	15.7
urea											
(A)	10	2.5	3.3	4.0	4.8	5.6	6.3	7.1	7.9	8.7	9.4
	20	3.0	4.3	5.6	6.8	8.1	9.4	–	–	–	–
	30	3.6	5.6	7.5	9.4	–	–	–	–	–	–
(A^1)	10	5.0	6.5	8.0	9.5	11.0	12.5	14.0	15.5*	17.0*	18.5*
	20	6.0	8.5	11.0	13.5*	16.0*	18.6*	–	–	–	–
	30	7.2	11.0	14.8*	18.6*	–	–	–	–	–	–
(A^{11})	10	6.8	8.4	10.0	11.6	13.2*	14.8*	16.4*	18.0*	19.7*	21.3*
	20	7.8	10.5	13.2*	15.9*	18.6*	21.2*	–	–	–	–
	30	9.2	13.2*	17.2*	21.2*	–	–	–	–	–	–
(B)	95[iii]	9.1	12.9*	16.8*	20.7*	–	–	–	–	–	–
	80[iv]	9.5	13.7*	18.0*	–	–	–	–	–	–	–
(B^1)	95[iii]	10.8	14.7*	18.6*	22.4*	–	–	–	–	–	–
	80[iv]	11.2*	15.5*	19.8*	–	–	–	–	–	–	–
chromatog.											
(a)[v]		–	19.0	23.3	27.5	–	–	–	–	–	–
(b)[v]		32.8	46.4	60.0	–	–	–	–	–	–	–

i. costs are given up to the maximum weight of acid which can be processed in one operation.

ii. proportion (%) of material to be complexed.

iii. 3 g. of urea per g of acids to be complexed.

iv. 4 g. of urea per g of acids to be complexed.

v. see Table 1.6.

* urea crystallisations which are more expensive than low-temperature crystallisations for the same amount of material.

We have only used this technique when the other more economical procedures fail and, in particular, for the separation of methyl linoleate from methyl α-linolenate.

In comparing low-temperature crystallisation and urea crystallisation the size limitation must be noted. We were restricted in low-temperature crystallisation to a precipitate of 600 g. and in urea crystallisation to about 1200 g. of urea which can be used to process varying amounts of acid. These limitations are indicated in Table 1.7. Apart from cost considerations account must be taken of the relative efficiency of low-temperature crystallisation against urea crystallisation. It is difficult to generalise because in both cases this will depend on the nature of the acids to be separated and it is a matter of experience to decide how many low-temperature crystallisations or how many urea crystallisations will be required to achieve a desired separation.

From Table 1.7 it is apparent that urea crystallisation type A is always more economical than low-temperature crystallisation, but that type A^1 and A^{11} become more expensive than low-temperature crystallisation when larger amounts are processed. In some of these separations, however, the slightly more expensive urea crystallisations may be preferred to low-temperature crystallisation because the former is usually more efficient.

Urea crystallisation of type B and B^1 are more expensive than low-temperature crystallisation (except with very small amounts) and should therefore only be used when this higher cost is compensated by increased efficiency.

Recommended Experimental Procedures

I. Hydrolysis and esterification

(a) Large scale hydrolysis of glycerides or methyl esters.

Triglycerides or esters (1 Kg.) were added to a solution of potassium hydroxide pellets (230 g.) in ethanol (800 ml.) and water (250 ml.) and the mixture refluxed for one hour. After partial cooling, crushed ice (1 l.) was added, then sulphuric acid (4 M, 1200 ml.) and the mixture transferred to a separating funnel and shaken to ensure good mixing. When the mixture had separated the lower layer was drawn off and extracted with ether (400 ml.). The ether extract was combined with the major organic layer and washed with water (2 x 200 ml.). Solvent was removed (RFE) and the remaining acids were dried by azeotropic distillation with benzene.

(b) Large scale esterification of acids.

Acids (500 g.) were refluxed for one hour with methanol (1 l.) and concentrated sulphuric acid (20 ml.). After cooling, the mixture was added to water (1500 ml.) in a separating funnel. Petrol (b.p. 40-60°C) (1500 ml.) was added and the mixture shaken. The organic extract was washed with water (2 x 100 ml.) and the esters isolated and dried as in (Ia).

(c) Large scale methanolysis of glycerides.

Glycerides (500 g.) were added to a mixture of methanol (1 l.), methylene chloride (500 ml.) and concentrated sulphuric acid (20 ml.) and the mixture was refluxed for two hours. Esters were isolated and dried as in (Ib).

(d) Small scale esterification of acids.

Acids (50 mg.) and methanolic sulphuric acid (0.4 M, 2 ml.) were refluxed for half an hour. The solution was cooled, brine (5%, 5 ml.) and distilled petrol (b.p. 40–60°C) (5 ml.) were added and the mixture shaken. The organic extract was removed by pipette and the aqueous layer was re-extracted with petrol (5 ml.). The combined organic extracts were dried (sodium sulphate) and the solution used directly for G.L.C. analysis.

(e) Small scale esterification of glycerides.

This reaction was done in the same way as in(Id) except that methylene chloride (1 ml.) was added to the mixture which was refluxed for 2 hours.

II. Low-temperature crystallisation.

(a) Linoleic acid (753 g., 84%) from evening primrose oil was dissolved in about 5 volumes (5 ml. per g.) of acetone (3.5 l.) in a conical flask (5 l.). The flask was stoppered and placed in a large acetone-bath along with a small flask containing pure acetone. The bath was cooled to the crystallising temperature (Table 1.1.) over 2 hours and held there for 4 or 5 hours. The crystals were filtered through a Buchner funnel cooled to the crystallising temperature by a cooling jacket containing cardice washed quickly with cooled solvent and sucked free of mother liquor. The precipitate was transferred to a flask by washing with warm petrol (b.p. 40–60°C) and the acids were isolated from both organic solutions by evaporation using a RFE.

(b) Oleic acid (335 g., 89%) from olive oil was dissolved in about 8 volumes of petrol (b.p. 40-60°C) (2.5 l.) in a large stoppered flask (3 l.). The flask was suspended along with a second flask containing pure petrol in a large Dewar flask half full of acetone. The bath was cooled by 10°C and the temperature held constant for an hour. This process was repeated until the bath reached the desired crystallising temperature (-40°C) when it was left overnight. Filtration and recovery of acids was carried out as in (IIa).

III. Urea crystallisation.

Urea (300 g.) was dissolved in a minimum amount of boiling methanol (600 ml.). Acids (702 g.) were added and the mixture reheated until solution was complete. More methanol was added if required. The solution was left overnight at 0°C. The precipitate was filtered, washed quickly with a cold saturated solution of urea in methanol (2 x 200 ml.) and sucked free of mother liquor. The crystals were then dissolved in the minimum amount of hot water (about 1.5 l.) and the solution acidified with dilute hydrochloric acid (3 M). Petrol (b.p. 40-60°C) (300 ml.) was added and the mixture extracted. The aqueous layer was re-extracted with fresh petrol (2 x 300 ml.), the extracts combined, washed with water (2 x 200 ml.) and the acids isolated and dried as before by distillation on a RFE.

The mother liquor was treated similarly after most of the methanol had been removed on a RFE.

IV. Column chromatography.

(a) Removal of unsaponifiable material, coloured impurities and oxidised products.

A glass column (30 x 5 cm.) was packed with a slurry of silica (Sorbsil M60, 500 g.) in distilled solvent (PE2) (1.5 l.) and the acids/esters (50 g.) adsorbed on the top of the column in a narrow band. Material was eluted from the column and the separation was followed by T.L.C. using PE20 for esters and PE30 containing acetic acid (0.2 M) for acids. The eluting solvent was increased in polarity from PE2 by increasing the proportion of ether as required: acids were usually eluted with PE10 and methyl esters with PE5. Fractions showing a single spot by T.L.C. were combined and the solvent removed. Impure fractions were suitably combined and recycled.

(b) Removal of methyl α-linolenate from methyl linoleate.

A glass column (90 x 10 cm.) was packed with a slurry of silica (Sorbsil M60, 2 Kg.) in distilled petrol (b.p. 40-60) (3 l.) and impure methyl linoleate (97%, 273 g.) adsorbed on the top of the column in a narrow band. Material was slowly eluted from the column using petrol initially and then slightly increasing the polarity of the solvent through PE1 to PE2. First traces of esters from the column were detected by T.L.C. (PE20) but, when substantial amounts appeared, the separation was followed by G.L.C. Fractions were combined on the basis of G.L.C. information to give a forerun of impure methyl linoleate containing methyl oleate as major impurity, a middle fraction of pure methyl linoleate ($\geq$ 99%) and a final fraction of methyl linoleate containing an unacceptable proportion of methyl α-linolenate.

V. Analytical procedures.

(a) G.L.C. analysis.

In general, samples of methyl esters in distilled petrol (b.p. 40-60°C) (2 µl., 0.5% solution) were injected into a column (5') of 20% DEGS on chromosorb W at 185°C (flow rate about 60 ml. per minute). Minor components ($\leq$1%) were recorded at an attenuation of 5×10^4 and major components at 50×10^2 after the recorder and amplifier had been set at zero to prevent baseline shift on changing the attenuation.

(b) Other analyses.

Thin-layer chromatography

Methyl esters or acids in distilled petrol (b.p. 40-60°C) were spotted onto microplates (7.5 x 2.5 cm.) obtained by dipping in a silica/chloroform suspension. Esters were run in PE20 and acids in PE30 containing acetic acid (0.2 M). Both were located by spraying with phosphomolybdic acid in ethanol (10% solution) and heating to 120°C.

Von Rudloff oxidation[5].

An oxidising solution (20 ml.) of sodium metaperiodate (2.086 g.) and potassium permanganate (0.0395 g.) in water (100 ml.) and a buffer solution (20 ml.) of potassium carbonate (0.250 g.) in water (100 ml.) were added to the acid/ester (40 mg.) in distilled t-butanol (15 ml.). The mixture was stirred or shaken at room temperature overnight. Concentrated sulphuric acid (1 ml.) was added and sulphur dioxide bubbled through until the solution was colourless. The mixture was extracted with ether (2 x 30 ml.) and the extracts dried over sodium sulphate.

The solvent was removed, the organic residue esterified with methanolic sulphuric acid (0.4 M, 2 ml.) and the resulting esters analysed on G.L.C.

Infrared spectroscopy

Samples of methyl esters (neat) were run as a liquid film on a Perkin Elmer 257 spectrophotometer.

CHAPTER 2: The isolation of methyl oleate from olive oil.

Introduction

Early attempts to isolate oleic acid from vegetable oils or animal fats involved fractional distillation of the oleate concentrate left after saturated esters had been removed by crystallisation at $0^{\circ}C$. When temperatures below zero could be obtained using acetone and cardice methyl oleate was more efficiently isolated by a combination of low-temperature crystallisation and distillation. In a review (1961) on low-temperature crystallisation Schlenk[6] described a typical procedure for the isolation of methyl oleate from olive oil. This employed four crystallisations at $-60^{\circ}C$, $-37^{\circ}C$, $-60^{\circ}C$ and $-60^{\circ}C$ and a final distillation to give methyl oleate (>98% pure) with 50% recovery.

Urea crystallisation was first applied to this problem in 1950 when Schlenk et al.[7] obtained pure methyl oleate (97-98% pure, 40% recovery) from olive oil. Their procedure involved removing saturated esters in two urea precipitates, complexing the oleate in a third precipitate, fractional distillation, and final purification by again complexing the oleate in a urea precipitate. Swern et al.[8] also isolated methyl oleate (95% including 25% of trans isomer) from animal fats and more satisfactorily (98.9% pure) from olive oil. This was achieved on a Kg. scale by (i) urea crystallisation to remove saturated and oleic ester in the precipitate (ii) crystallisation at $-35^{\circ}C$ to remove saturated ester (iii) crystallisation of methyl oleate at $-60^{\circ}C$ and (iv) distillation.

In 1959 Keppler et al.[9] prepared large quantities of pure oleic acid (>99%) for biological investigations. Since it was important that no linoleic acid was present the diene was removed by reaction with an

iodine/maleic anhydride mixture after concentrating oleic acid from olive oil by urea crystallisation and fractional distillation. However, the recovery was only 23% and the product was contaminated with 5% of trans isomer. At the same time large quantities (1.5 Kg.) of pure oleic acid (>99%) were prepared by Rubin et al.[10] using urea crystallisation and a new technique of acid soap crystallisation. This involved the crystallisation of mixtures of fatty acid soaps at low-temperature and, although effective, it seemed to be very involved and does not appear to have been widely employed. The recovery was 43%.

Recent reports on the isolation of oleic acid are concerned with the source of the oleate[11] rather than the development of new or existing separating techniques. For example, Diamond et al.[12] described the isolation of oleic acid from a safflower seed source containing about 80% oleic acid and Kinman[13] suggested that the composition of sunflower seed acids can be varied by changing the environmental conditions to give an oleic acid content of up to 66%.

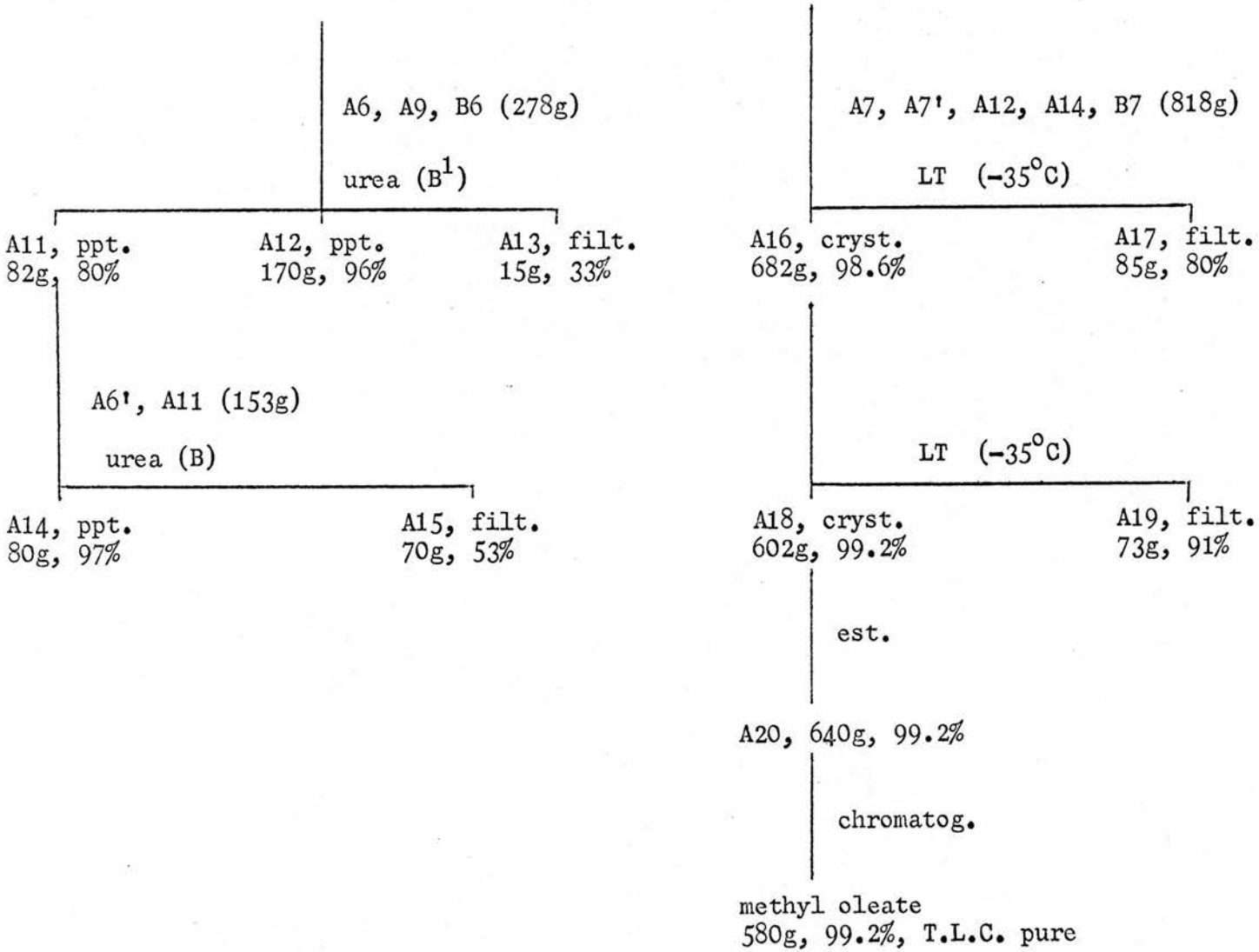
In the earlier work varying levels of purity (95% upwards) were accepted. This arose from the interaction of several factors including (i) failure to recognise the importance of using pure materials particularly in biochemical studies (ii) the increasing difficulty and effort (and therefore cost) of obtaining pure samples and (iii) the difficulty of accurately analysing samples more than 95% pure prior to the wide use of G.L.C.

In the present studies we have tried to find the simplest, most direct and most economical way of isolating oleic acid or methyl oleate from olive oil. We have avoided the use of fractional distillation since (more particularly for the polyene acids/esters) this may produce undesirable changes and we have made a preliminary attempt to determine the cost-to-make of the pure acid/ester.

Scheme 1

The isolation of pure methyl oleate from olive oil (2 Kg.)

For each fraction the weight and proportion of oleic acid are indicated.



* fraction A4 was treated in two batches giving A6, A6' etc.

Analysis of olive fractions

Fraction	Wt. (g.)	G.L.C. (area %) 16:0[a]	18:0	18:1	18:2	18:3 (α)
oil	2000	7.5	2.0	75.0	14.7	0.8
A1	965	7.5	2.0	75.0	14.7	0.8
B1	876	7.5	2.0	75.0	14.7	0.8
A2	44	36.8	18.0	33.8	6.6	4.8
A3	112	27.7	8.4	51.1	11.9	0.9
A4	557	3.5	1.2	91.0	4.3	-
A5	220	1.5	-	51.3	43.7	3.5
B2	89	41.5	14.0	35.5	5.9	3.1
B3	149	15.6	4.8	74.7	4.9	-
B4	296	1.7	0.6	93.1	4.6	-
B5	309	1.7	-	63.9	31.4	3.0
A6	69	9.3	1.9	85.7	2.0	1.1
A6'	71	14.0	3.7	81.6	0.6	-
A7	161	1.0	0.9	95.9	2.2	-
A7'	179	0.7	-	97.6	1.7	-
A8	25	-	-	58.0	40.3	1.7
A8'	18	0.5	-	44.7	51.9	2.9
B6	31	6.3	2.5	87.4	3.8	-
B7	228	0.8	-	97.8	1.4	-
B8	23	0.5	-	61.9	35.9	1.7
A9	178	1.8	-	90.8	7.4	-
A10	336	1.3	-	38.5	56.0	4.2
A11	82	15.2	4.4	80.4	-	-
A12	170	0.8	-	95.6	2.7	0.9
A13	15	0.7	0.2	33.4	62.8	2.9
A14	80	2.1	-	96.6	1.3	-
A15	70	36.0	10.2	52.8	1.0	-
A16	682	0.7	-	98.6	0.5	0.2
A17	85	2.9	0.6	80.0	13.3	3.2
A18	602	0.6	-	99.2	0.2	-
A19	73	3.0	0.3	90.7	4.5	1.5
A20	640	0.6	-	99.2	0.2	-
methyl oleate	580	0.6	-	99.2	0.2	-

a sometimes accompanied by small amounts of 16:1

Summary of products from olive acids (1841 g.)

Fraction	Wt. (g.)	G.L.C. (area %) sat.	18:1	18:2/3
methyl oleate	580	0.6	99.2	0.2
A19	73	3	91	6
A17	85	4	80	16
B3	149	20	75	5
B8	23	1	62	37
A8	25	-	58	42
A15	70	46	53	1
A3	112	36	51	13
A8'	18	1	44	55
A10	336	1	39	60
B2	89	56	35	9
A2	44	55	34	11
A13	15	1	33	66
loss	222			

"Cost"[a] of isolating methyl oleate (580 g.) from olive oil (2 Kg.)

	Material (£)	Labour (£)	Total (£)
hydrolysis (1 Kg., 1 Kg.)	2.64[b]	15.48	18.12
LT A1 (965 g.)[c]	1.93	31.37	33.30
B1 (876 g.)[c]	1.75	30.66	32.41
A5,B5 (529 g.)	1.06	12.14	13.20
urea (B^1) A4 (850 g., 900 g. urea)	3.50	26.75	30.25
B4 (900 g. urea)	1.80	17.10	18.90
A6,A9,B6 (950 g. urea)	1.90	17.66	19.56
(B) A6',A11 (200 g. urea)	0.40	7.43	7.83
LT A7,A7',A12,A14,B7 (818 g.)	1.64	13.14	14.78
A16 (682 g.)	1.36	12.66	14.02
est. A18 (602 g.)	0.82	3.44	4.26
chromatog. A20 (320 g., 320 g.)	20.00	28.20	48.20
	£38.80	£216.03	£254.83
cost per g. (for 580 g.)	6p	37p	43p

a. see Chapter 1, page 15

b. including seed oil.

c. labour is equivalent to 2.3 low-temperature processes; see flow sheet for scheme 1, page 27

Scheme 2

The isolation of pure methyl oleate from olive oil (1 Kg.)

For each fraction the weight and proportion of oleic acid are indicated.

Analysis of olive fractions

Fraction	Wt. (g.)	G.L.C. (area %) 16:0[b]	18:0	18:1	18:2	18:3 (α)
oil	1000	7.5	2.0	75.0	14.7	0.8
A	967	7.5	2.0	75.0	14.7	0.8
B	(702)[a]	2.3	-	78.1	18.4	1.2
C	(208)[a]	17.6	5.9	68.8	5.4	1.1
D	518	0.8	-	74.1	23.4	1.7
E	(142)[a]	8.6	-	87.7	2.4	1.3
F	335	0.4	0.1	89.8	9.1	0.6
G	168	1.3	-	40.7	55.0	3.0
H	256	0.2	-	96.8	3.0	-
J	74	1.3	-	62.7	33.9	2.1
K	236	0.3	-	97.9	1.8	-
L	15	1.5	-	58.4	38.2	1.9
M	213	0.1	-	99.1	0.8	-
N	14	1.1	0.2	80.9	16.9	0.9
P	205	0.1	-	99.1	0.8	-
methyl oleate	197	0.1	-	99.1	0.8	-

a. estimated weights of unrecovered fractions.

b. sometimes accompanied by small amounts of 16:1.

Summary of products from olive acids (967 g.)

Fraction	Wt. (g.)	G.L.C. (area %) sat.	18:1	18:2/3
methyl oleate	197	0.1	99.1	0.8
E	(142)[c]	8	88	4
N	14	1	81	18
C	(208)[c]	24	69	7
J	74	1	63	36
L	15	2	58	40
G	168	1	41	58
loss	149			

c. retained in the form of urea complex, estimated weights of acids.

"Cost"[a] of isolating methyl oleate (197 g.) from olive oil (1 Kg.)

	Material (£)	Labour (£)	Total (£)
hydrolysis (1 Kg.)	1.32[b]	7.74	9.06
urea (A) A (800 g. urea)	0.56	6.26	6.82
(A^1) B (300 g. urea)	0.36	6.85	7.21
LT D (518 g.)	1.04	12.11	13.15
F (335 g.)	0.67	11.47	12.14
H (256 g.)	0.51	11.20	11.71
K (236 g.)	0.47	11.13	11.60
est. M (213 g.)	0.81	3.44	4.26
chromatog. P (205 g.)	7.13	12.11	19.24
	£12.88	£82.31	£95.19
cost per g. (for 197 g.)	7p	42p	49p

a. see Chapter 1, page 15

b. including seed oil.

Discussion

I. Experimental procedures.

Pure methyl oleate ($\geq$ 99%) was isolated from olive oil by two main routes (scheme 1 and scheme 2). Scheme 1 was an attempt to produce the maximum quantity of pure oleate from 2 Kg. of oil without too much regard to the time taken. This is detailed on page 27. Scheme 2 was then devised as a more direct and quicker route using 1 Kg. of oil and was expected to be more economical.

On the basis of our experience we recommend the following procedure taken from scheme 2 for the isolation of pure methyl oleate from olive oil.

(i) Urea crystallisation of olive acids to reduce the content of saturated acids to 1.0% or below. This seemed to require two treatments with urea. In the first, 800 g. of urea reduced the saturated acids from 9.5% to 2.3% in the mother liquor which was then treated directly with a further 300 g. of urea to reduce its saturated acid content to 0.8%. Both urea precipitates were rich in oleic acid and could be employed as a further source of this material.

(ii) Crystallisation from petrol (b.p. 40-60°C) at -40°C until the content of oleic acid exceeds 99.0%. This procedure reduced the polyene acids in four steps (25.1% -> 9.7% -> 3.0% -> 1.8% -> 0.8%) and resulted in a further reduction in the proportion of saturated acid to 0.1%. It is possible that with further experience the removal of polyene acids could be achieved more quickly. The acids in each mother liquor were isolated and all but the first were sufficiently rich in oleic acid to be recycled.

(iii) Finally the pure oleic acid ($\geq$99%) was esterified and chromatographed to remove traces of coloured impurities and oxidised products. The final product (about 96% of that put on the column) was colourless.

It is important not to follow this recipe blindly but to repeat each process until the impurity has been reduced to the value indicated. Of the separation procedures involved urea crystallisation is easily carried out with up to 1.2 Kg. of urea on the 1 Kg. scale and low-temperature crystallisation permits the handling of up to 600 g. of precipitate. Using a 90 x 10 cm. column, chromatography is restricted to about 400 g.

Full details of how to perform each process are given in the appropriate sections of Chapter 1.

II. Costs.

The cost of producing methyl oleate by scheme 1 is 43p per gram and by scheme 2 is 49p per gram. In both cases, material consumed accounts for a small part (13-15%) of the total costs so that, as expected, labour is the major factor in the final cost. In scheme 1, where 2 Kg. of oil is processed and poorer grade fractions are recycled, each major separation procedure is used on, or close to, the optimum weight level. In scheme 2 only 1 Kg. of oil is processed, poorer grade fractions are too small to merit recycling and the later procedures cannot be used at their optimum weight levels. This is most apparent for low-temperature crystallisation.

We prefer scheme 2 to scheme 1 because we believe that this more direct route would be cheaper if carried out on sufficient material to allow the later stages (especially low-temperature crystallisation) to be carried out on the optimum amounts (500–600 g. of precipitate for low-temperature crystallisation). For example, we consider that with 2 Kg. of olive oil the cost would fall to 37p per gram as shown below:

Procedure	Material (£)	Labour (£)	Total (£)
hydrolysis (1 Kg., 1 Kg.)	2.64	15.48	18.12
urea (A) (800 g., 800 g. urea)	1.12	12.52	13.64
(A^1) (300 g., 300 g. urea)	0.72	13.70	14.42
LT (1) (518 g., 518 g.)	2.08	24.22	26.30
(2) (670 g.)	1.34	12.60	13.94
(3) (512 g.)	1.02	12.05	13.07
(4) (472 g.)	0.94	11.94	12.88
est. (426 g.)	0.82	3.44	4.26
chromatog. (410 g.)	12.25	15.65	27.90
	£22.93	£121.60	£144.53
cost per g. (for 394 g.)	6p	31p	37p

In this hypothetical scheme we have assumed that the low-temperature procedures are reproducible up to the limit of 500–600 g. of precipitate. If an additional low-temperature crystallisation were required this would add 3–4p to the cost per gram. If the purification could be effected with one less low-temperature crystallisation this would reduce the cost by a similar amount.

Scheme 2 could be further extended to handle 5 Kg. of olive oil but approximate calculations carried out in the same way as for 2 Kg. of oil produce a cost of only 36p per gram. This suggests that with these procedures and their inbuilt limitation of size the cost-to-make is unlikely to fall below a range of 35–40p per gram.

Further economies could only result from procedures where larger amounts could be handled and this would require the following developments.

(i) Instead of filtering through a Buchner funnel, mother liquor would be removed from the vessel containing precipitate and mother liquor by some form of filter stick. This might lead to a less efficient separation of precipitate and mother liquor and make it necessary to repeat the separating process (low-temperature crystallisation or urea crystallisation) more often.

(ii) Separating funnels would have to be replaced by a static vessel with stirring to mix organic and aqueous phases and some means of separating the two layers. Such a vessel (on a 10 or 20 l. scale) might also be adapted for filtration and then used for urea crystallisation and low-temperature crystallisation.

(iii) Column chromatography would also have to be scaled up to handle larger amounts but this should not be too difficult.

CHAPTER 3: The isolation of methyl linoleate from safflower oil and evening primrose oil.

Introduction

Linoleic acid, found in high proportions (40–80%) in many seed oils, was first satisfactorily isolated by debromination of its crystallised tetrabromide. When analytical techniques improved, samples of acids prepared in this way were found to be contaminated with isomers[14]. Low-temperature crystallisation[15], urea crystallisation[16] and combinations of both[17] were later applied with success to the isolation of pure linoleic acid/ester from various seed oils, in particular safflower. Purity levels ranged from 95% to > 99% with average recoveries of about 20%. Initially, analysis was carried out by measuring the degree of unsaturation using iodine values but, as G.L.C. techniques were developed, more accurate data were recorded. Other less successful methods of isolation included solvent partition of mercury complexes[18] and liquid-liquid extraction using a furfural/hexane system[19]. After examination of the latter on a pilot plant scale the liquid-liquid extraction process was expected to operate at a reasonable cost on the industrial scale to produce linoleic acid of 95% purity from safflower oil containing about 75% linoleic acid. Adsorption chromatography on silica[4] has also been used successfully but requires slow elution with large volumes of solvent.

Since low-temperature crystallisation and urea crystallisation were used satisfactorily to isolate pure methyl oleate from olive oil it was expected that the isolation of pure linoleic acid from a suitable source was simply a matter of separating oleic(and saturated acid) from the dienoic acid which would then be pure. The investigation already referred to did not appear to involve the problem of removing trienoic (ω3) acid from linoleic acid because the sources used (mainly safflower oil) did not contain more than 1% of linolenic acid.

We have now isolated methyl linoleate from safflower oil and also from evening primrose oil, which, although not a common commercial oil, is readily available to us. We have also attempted to estimate the cost-to-make on the optimum scale for each source.

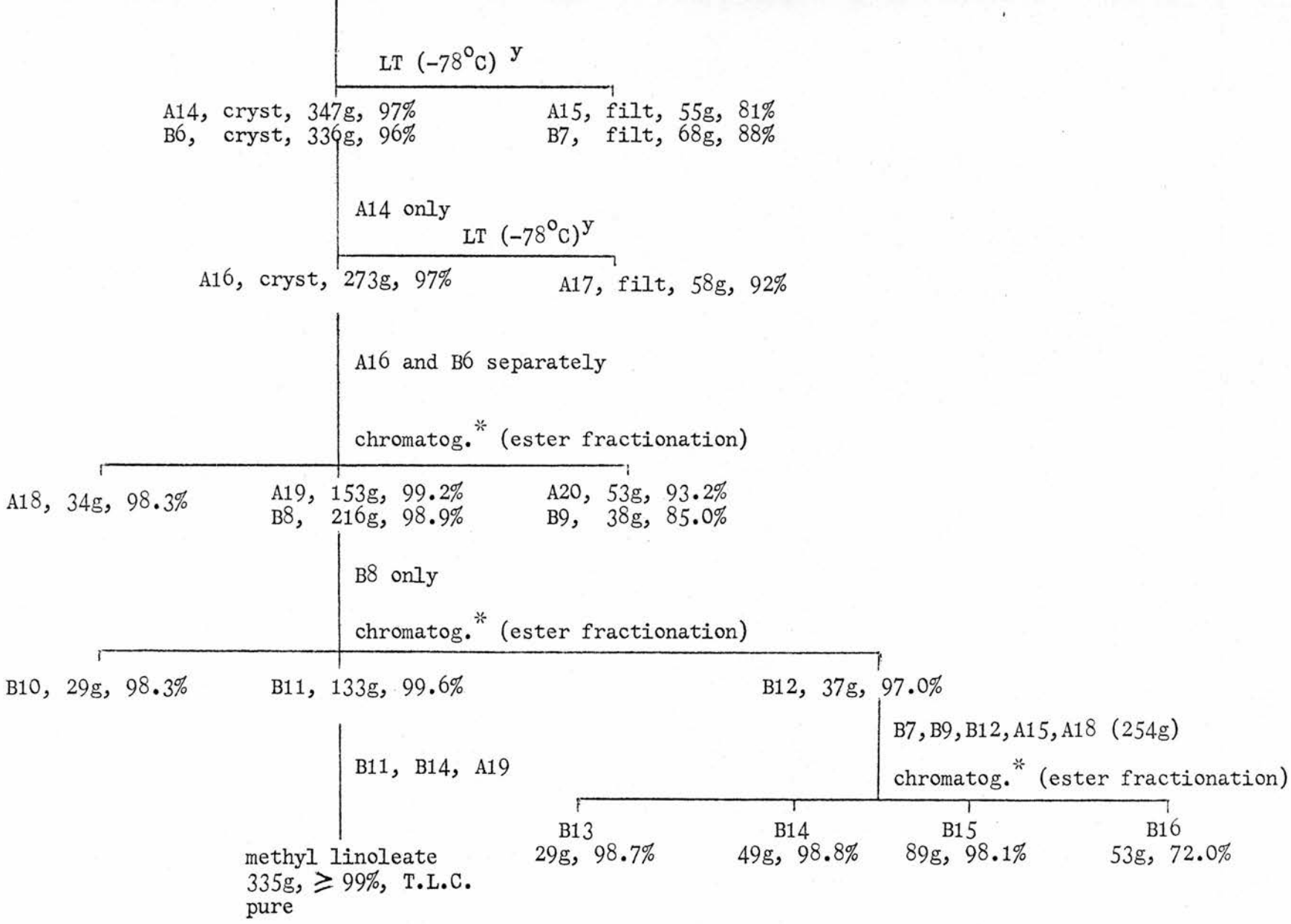
Riley[20] (1949) obtained pure γ-linolenic acid from evening primrose seed oil via the crystallised hexabromide in order to confirm the structure of the isolated acid. Kleiman et al.[21] (1964) and Miller et al.[22] (1968) have recently reported several other seed oils which contain γ-linolenic acid. Although some of these contain more γ-linolenic acid than the evening primrose oil many of them also contain 18:3 (9,12,15) and 18:4 (6,9,12,15) both of which reduce the value of these oils as a source of pure γ-linolenic acid.

In the process of isolating pure methyl linoleate ($\geqslant$99%) from evening primrose oil we have obtained γ-linolenic acid (61%) as a by-product. If desired, however, this trienoic acid could be readily isolated as the more valuable component and we have obtained methyl γ-linolenate of 99% purity by upgrading the above by-product using our standard procedures. Finally, we discuss a hypothetical scheme based on our experiments, which would produce economically combinations of both acids or esters from the evening primrose oil.

Scheme 1

The isolation of pure methyl linoleate from safflower oil (2 Kg.)

For each fraction the weight and proportion of linoleic acid are indicated.



* a methyl α-linolenate rich fraction remained on the column.

x,y see footnotes on page 44

Analysis of safflower fractions

Fraction	Wt. (g.)	G.L.C. (area %) 16:0[b]	18:0	18:1	18:2	18:3 (x)
oil	2000	5.1	2.1	12.6	77.4	2.8
A	967	5.1	2.1	12.6	77.4	2.8
B	944	5.1	2.1	12.6	77.4	2.8
A1	(320)[a]	13.3	6.9	33.9	43.0	2.9
A2	(560)[a]	-	-	2.9	92.0	5.1
B1	(210)[a]	20.2	7.9	35.9	34.2	1.2
B2	(670)[a]	-	-	7.2	88.9	3.9
A3	108	0.4	-	11.3	85.8	2.5
A4	425	-	-	0.7	95.1	4.2
B3	179	-	-	16.4	81.1	2.5
B4	418	-	-	0.8	95.2	4.0
A5	703	-	-	0.3	95.6	4.1
A6	114	-	-	2.4	90.8	6.8
A7	103	0.8	-	30.0	66.8	2.4
A8	245	-	-	0.7	94.0	5.3
A9	49	-	-	7.7	89.3	3.0
A10	877	-	-	0.3	95.8	3.9
A11	13	-	-	1.0	87.1	11.9
A12	13	-	-	0.9	77.8	21.3
A13	439	-	-	0.3	95.8	3.9
B5	438	-	-	0.3	95.8	3.9
A14	347	-	-	0.4	97.0	2.6
A15	55	-	-	1.2	81.1	17.7
B6	336	-	-	0.4	96.2	3.4
B7	68	-	-	0.9	87.5	11.6
A16	273	-	-	0.3	97.2	2.5
A17	58	-	-	0.6	92.3	7.1
A18	34	-	-	1.7	98.3	-
A19	153	-	-	0.5	99.2	0.3
A20	53	-	-	0.2	93.2	6.6
B8	216	-	-	0.4	98.9	0.7
B9	38	-	-	0.1	85.0	14.9
B10	29	-	-	1.6	98.3	0.1
B11	133	-	-	0.3	99.6	0.1
B12	37	-	-	0.1	97.0	2.9
B13	22	-	-	0.9	98.7	0.4
B14	49	-	-	0.5	98.8	0.7
B15	89	-	-	0.3	98.1	1.6
B16	53	-	-	-	72.0	28.0
methyl linoleate	335	-	-	0.4	99.3	0.3

a. estimated weights of unrecovered fractions.

b. sometimes accompanied by small amounts of 16:1.

Products from safflower acids (1911 g.)

Fraction	Wt. (g.)	G.L.C. (area %) sat./18:1	18:2	18:3(ω)
methyl linoleate	335	0.4	99.3	0.3
B13	22	0.9	98.7	0.4
A18	34	1.7	98.3	-
B10	29	1.6	98.3	0.1
B15	89	0.3	98.1	1.6
A9	49	8	89	3
A11	13	1	87	12
A15	55	1	81	18
A12	13	1	78	21
B16	53	-	72	28
A7	103	31	67	2
A1	(320)[a]	54	43	3
B1	(210)[a]	64	34	2
loss[b]	586			

a. retained in the form of urea complexes.

b. high experimental loss resulting from repeated attempts to upgrade A5, A8.

---

x After unsuccessful attempts to upgrade these acids by (i) low-temperature crystallisation of acids in acetone, (ii) low-temperature crystallisation of esters in petrol and in acetone, (iii) urea crystallisation of esters and acids, fractions were recombined to give A10 (part acids, part esters) and A11/A12. After complete esterification the material, as A13 and B5, was crystallised from methanol at -78°C.

y Low-temperature crystallisation of esters in methanol solution at high dilution (10 volumes).

"Cost"[a] of isolating methyl linoleate (335 g.) from safflower oil (2 Kg.)

	Material (£)	Labour (£)	Total (£)
hydrolysis (1 Kg., 1 Kg.)	3.14[b]	15.48	18.62
urea (A) A (1200 g. urea)	0.84	8.53	9.37
B (1000 g. urea)	0.70	7.40	8.10
urea (A^1) A2 (300 g. urea)	0.36	6.85	7.21
B2 (500 g. urea)	0.60	9.12	9.72
urea (B^1) A3, B3, A6 (450 g. urea)	0.90	11.99	12.89
LT A4, B4 (843 g.)	1.67	13.19	14.86
est. A10 (877 g.)	0.82	3.44	4.26
LT A13 (439 g.)	0.88	11.83	12.71
B5 (438 g.)	0.88	11.83	12.71
A14 (347 g.)	0.69	11.51	12.20
chromatog. A16 (273 g.)	15.65	40.68	56.33
B6 (336 g.)	18.80	46.10	64.90
B8 (216 g.)	12.80	35.78	48.58
B7,B9,B12,A15,A18 (254 g.)	14.70	39.04	53.74
	£73.43	£272.77	£346.20
cost per g. (for 335 g.)	£0.22	£0.81	£1.03

a. see Chapter 1 page 15

b. including cost of oil

Scheme 2

The isolation of pure methyl linoleate from safflower oil (1 Kg.)

For each fraction the weight and proportion of linoleic acid are indicated.

Analysis of safflower fractions

Fraction	Wt. (g.)	G.L.C. (area %) 16:0[b]	18:0	18:1	18:2	18:3(α)
oil	1000	5.1	2.1	12.6	77.4	2.8
A	957	5.1	2.1	12.6	77.4	2.8
B	(650)[a]	-	-	6.6	89.4	4.0
C	(263)[a]	25.2	10.2	32.7	29.6	2.3
D	530	-	-	1.6	93.8	4.6
E	(90)[a]	0.6	-	26.6	70.7	2.1
F	530	-	-	1.6	93.8	4.6
G	100	-	-	4.6	95.2	0.2
H	96	-	-	1.9	97.9	0.2
J	142	-	-	0.1	94.7	5.2
methyl linoleate	148	-	-	0.6	99.0	0.4

a. estimated weights of unrecovered fractions.

b. sometimes accompanied by small amounts of 16:1.

Summary of products from safflower acids (957 g.)

Fraction	Wt. (g.)	G.L.C. (area %) sat./18:1	18:2	18:3 (α)
methyl linoleate	148	0.6	99.0	0.4
H	96	2	98	-
G	100	5	95	-
J	142	-	95	5
E	90	27	71	2
C	263	68	30	2
loss	118			

"Cost"[a] of isolating methyl linoleate (148 g.) from safflower oil (1 Kg.)

	Material (£)	Labour (£)	Total (£)
hydrolysis (1 Kg.)	1.57[b]	7.74	9.31
urea (A) A (800 g. urea)	0.56	6.26	6.82
urea (A^1) B (500 g. urea)	0.60	9.12	9.72
est. D (530 g.)	0.82	3.44	4.26
chromatog. F (265 g., 265 g.)	30.50	79.98	110.48
	£34.05	£106.54	£140.59
cost per g. (for 148 g.)	23p	72p	95p

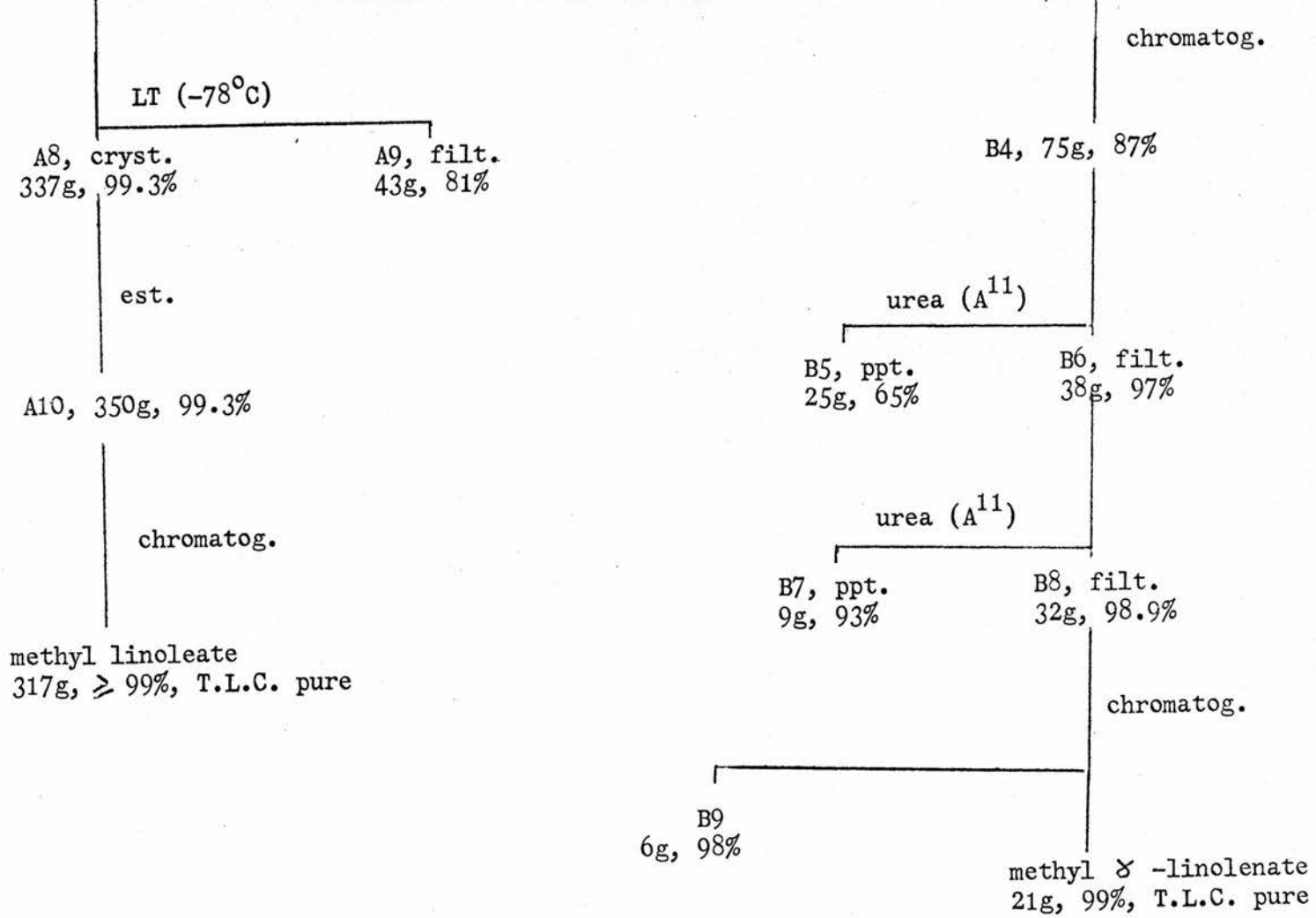
a. see Chapter 1, page 15

b. including cost of oil.

Scheme 3

The isolation of pure methyl linoleate and pure methyl γ-linolenate from evening primrose oil (1 Kg.)

For each fraction the weight and proportion of linoleic acid or γ-linolenic acid are indicated.



* Fractions designated A are enriched in 18:3 to the level shown and fractions designated B are enriched in 18:2 (ϒ) to the level shown.

Analysis of evening primrose fractions

Fraction	Wt. (g.)	G.L.C. (area %)					
		16:0[b]	18:0	18:1	18:2	18:3 (γ)	comp. x[c]
oil	1000	5.1	1.4	9.1	76.4	8.0	-
A	930	5.1	1.4	9.1	76.4	8.0	-
A1	(120)[a]	31.5	7.0	27.9	32.8	0.8	-
A2	786	-	-	5.1	87.0	7.9	-
A3	573	0.2	-	5.8	92.8	1.2	-
B	144	-	-	4.1	35.1	60.8	-
A4	(160)[a]	0.7	-	20.3	79.0	-	-
A5	403	-	-	1.5	96.9	1.6	-
B1	(44)[a]	-	-	12.9	78.5	8.6	-
B2	95	0.1	-	-	12.5	87.0	0.4
A6	(15)[a]	-	-	7.2	92.8	-	-
A7	382	-	-	0.7	97.4	1.9	-
B3	96	0.1	-	-	12.5	87.0	0.4
B4	75	0.1	-	-	12.5	87.0	0.4
A8	337	-	-	0.4	99.3	0.2	0.1
A9	43	-	-	1.9	80.7	17.4	-
B5	25	-	-	0.5	33.7	65.4	0.4
B6	38	0.1	-	-	2.1	97.1	0.7
A10	340	-	-	0.4	99.3	0.2	0.1
B7	9	0.1	-	0.2	6.6	92.6	0.5
B8	32	-	-	0.1	0.2	98.8	0.9
B9	6	-	-	0.3	1.1	97.7	0.9
methyl linoleate	317	-	-	0.4	99.3	0.2	0.1
methyl γ-linolenate	21	-	-	0.1	0.2	98.8	0.9

a. estimated weights of unrecovered fractions

b. sometimes accompanied by small amounts of 16:1

c. an unknown component

Summary of products from evening primrose acids (930 g.)

Fraction	Wt. (g.)	G.L.C. (area %) sat./18:1	18:2	18:3 (γ)	comp. x [b]
methyl linoleate	317	0.4	99.3	0.2	0.1
A6	(15)[a]	7	93	-	-
A9	43	2	81	17	-
A4	(160)[a]	21	79	-	-
A1	(120)[a]	66	33	1	-
methyl γ-linolenate	21	0.1	0.2	99.0	0.7
B9	6	0.1	0.2	99.0	0.7
B7	9	-	7	93	-
B5	25	1	33	65	-
B1	(44)[a]	13	78	9	-
loss	169				

a. retained in the form of urea complexes

b. an unknown compound.

"Cost"[a] of isolating methyl linoleate (317 g.) and methyl γ-linolenate (21 g.) from evening primrose oil (1 Kg.)

methyl linoleate

	Material (£)	Labour (£)	Total (£)
hydrolysis (1 Kg.)	7.82[b]	7.74	15.56
urea (A^1) A (600 g. urea)	0.72	10.25	10.97
LT A2 (786 g.)	1.57	13.02	14.59
urea (A^1) A3 (500 g. urea)	0.60	9.12	9.72
A5 (120 g. urea)	0.14	4.80	4.94
LT A7 (382 g.)	0.76	11.63	12.39
est. A8 (337 g.)	0.82	3.44	4.26
chromatog. A10 (350 g.)	10.75	14.62	25.37
	£23.18	£74.62	£97.80
cost per g. (for 317 g.)	7p	24p	31p

methyl γ-linolenate

	Material (£)	Labour (£)	Total (£)
hydrolysis (1 Kg.)	7.82[b]	7.74	15.56
urea (A^1) A (600 g. urea)	0.72	10.25	10.97
LT A2 (786 g.)	1.57	13.02	14.59
urea (A^1) B (150 g. urea)	0.18	5.14	5.32
est. B2 (95 g.)	0.82	3.44	4.26
chromatog. B3 (96 g.)	4.40	10.25	14.65
urea (A^{11}) B4 (100 g. urea)	0.20	6.30	6.50
B6 (20 g. urea)	0.04	5.38	5.42
chromatog. B8 (32 g.)	2.80	9.15	11.95
	£18.55	£70.67	£89.22
cost per g. (for 21 g.)	£0.88	£3.37	£4.25

a. see Chapter 1, page 15

b. including cost of oil.

Hypothetical cost of isolating methyl linoleate from evening primrose oil (2 Kg.)

	Material (£)	Labour (£)	Total (£)
hydrolysis (1 Kg., 1 Kg.)	15.64[a]	15.48	31.12
urea (A^1) (600 g., 600 g. urea)	1.44	20.50	21.94
LT (780 g., 780 g.)	3.12	26.04	29.16
urea (A^1) (1) (1 Kg. urea)	1.20	14.79	15.99
(2) (240 g. urea)	0.29	6.16	6.45
LT (760 g.)	1.52	12.93	14.45
est. (660 g.)	0.82	3.44	4.26
chromatog. (330 g., 330 g.)	20.50	28.55	49.05
	£44.53	£127.84	£172.44
cost per g. (for 630 g.)	7p	20p	27p

a. including cost of oil.

Hypothetical cost of isolating methyl linoleate and methyl γ-linolenate from evening primrose oil (5 Kg.)

methyl linoleate

	Material (£)	Labour (£)	Total (£)
hydrolysis (5 x 1 Kg.)	39.10[a]	38.70	77.80
urea (A^1) (3 x 1 Kg. urea)	3.60	44.38	47.98
LT (5 x 780 g.)	7.80	65.02	72.82
urea (A^1) (1) (2 x 1.2 Kg. urea)	2.88	34.13	37.01
(2) (600 g. urea)	0.72	10.25	10.97
LT (3 x 640 g.)	3.84	37.57	41.41
est. (3 x 560 g.)	2.46	10.32	12.78
chromatog. (5 x 350 g.)	53.75	73.10	126.85
	£114.15	£313.47	£427.62
cost per g. (for 1580 g.)	7p	20p	27p

methyl γ-linolenate

	Material (£)	Labour (£)	Total (£)
hydrolysis (5 x 1 Kg.)	39.10[a]	38.70	77.80
urea (A^1) (3 x 1 Kg. urea)	3.60	44.38	47.98
LT (5 x 780 g.)	7.80	65.02	77.82
urea (A^1) (750 g. urea)	0.90	11.95	12.85
est. (475 g.)	0.82	3.44	4.26
chromatog. (480 g.)	14.00	16.86	30.86
urea (A^{11}) (1) (500 g. urea)	1.00	10.80	11.80
(2) (100 g. urea)	0.20	6.30	6.50
chromatog. (160 g.)	6.00	11.35	17.35
	£73.42	£208.80	£282.22
cost per g. (for 105 g.)	£0.70	£1.99	£2.69

a. including cost of oil.

Discussion

1. Methyl linoleate from safflower oil.

The general recommendations described in Chapter 2 for the isolation of pure methyl oleate from olive oil failed to produce 99% methyl linoleate when applied to safflower oil. Hydrolysis, removal of saturated and monoenoic acids by urea crystallisation, low-temperature crystallisation, upgrading to the 99% level and final purification of the ester by chromatography, furnished methyl linoleate at the excessively high cost of £1.03 per gram (Scheme 1). In a slightly modified procedure this was reduced only to £0.95 per gram (Scheme 2).

This situation is related to the presence of about 3% of α-linolenic acid in our safflower oil and the difficulty of separating linoleic and α-linolenic acids. Attempts to separate these two acids by low-temperature crystallisation of both acids and esters from acetone, urea crystallisation of the acids or esters and low-temperature crystallisation of the methyl esters from methanol at $-78^{\circ}C$ all failed though the final method showed slight promise. We preferred, however, to purify the linoleate by column chromatography. This is a very expensive process especially when the yield of pure linoleate is low and the separation has to be undertaken several times. Chromatography accounted for 65% and 79% of the total cost of Scheme 1 and 2 respectively.

It is obvious that linoleic acid should only be isolated from oils which contain little or no α-linolenic acid. Some possible sources are listed in Table 3.1 from which it is apparent that we were unfortunate in using a safflower oil which contained more than the usual proportion of α-linolenic acid.

Table 3.1

oil	G.L.C. (area %) sat.	18:1	18:2	18:3 (α)	Others
sesame	15.9	41.9	42.0	-	0.2
kapok	28.5	21.8	35.7	1.4	12.6
cottonseed	27.9	19.3	51.9	-	0.9
niger	19.5	7.0	72.6	0.9	-
sunflower	13.4	23.7	60.2	0.8	1.9
safflower	9.6	13.5	76.6	0.3	-
corn	13.7	28.6	57.0	0.7	-

Other work in this department had shown that evening primrose oil which contains linoleic acid (76%) and γ-linolenic acid (8%) but no α-linolenic acid is a good source of both the diene and the triene ω6 acids which are easily separated from each other. We have therefore made a preliminary investigation of the cost of isolating both these acids from 1 Kg. of the oil.

2. Methyl linoleate from evening primrose oil.

(i) Experimental procedures.

Pure methyl linoleate (≥99%) was readily isolated from evening primrose oil by Scheme 3, based on the recommendations in Chapter 2 for the isolation of pure methyl oleate from olive oil. After hydrolysis of the oil (1 Kg.), evening primrose acids were treated with 600 g. of urea which reduced the proportion of saturated and oleic acids from 15.6% to 5.1%. Further separation using urea was not achieved until the exceptionally high level of non-saponifiable material (about 8%) had been reduced. This was done by low-temperature crystallisation at -78°C in acetone. The precipitate gave linoleic acid (93%) and the mother liquor

contained most of the non-saponifiable material, which had previously interfered with the urea separation, along with a concentrate of γ-linolenic acid (61%). The saturated and oleic acids in the linoleic acid concentrate were then further reduced to 1.5% and to 0.7% with two batches of urea (500 g. and 120 g. respectively). A second low-temperature crystallisation then gave linoleic acid (99.3%) and chromatography of the esters gave methyl linoleate which was pure by T.L.C.

The side-fractions (A4, A6, A9) could be used as a source of linoleic acid or, in the case of A9, of the more valuable γ-linolenic acid. As in the case of the purification of methyl oleate (Chapter 2) Scheme 3 will only be reproducible qualitatively if each procedure is repeated until the impurity has been reduced to the level indicated.

(ii) Costs.

The cost of producing methyl linoleate by Scheme 3 is 31p per gram (see page 53) which is below the range obtained for methyl oleate in Chapter 2 (35-40p per gram). Labour is again the major factor contributing to overall costs but the most expensive part of this sequence is chromatography (26% of total cost). By using each procedure on the optimum amount of material the cost-to-make could be lowered. For example, with 2 Kg. of oil the cost would be 27p (see page 54). It is noticeable that chromatography now accounts for 29% of the total cost and it would be desirable to economise on the use of this technique. For example, 660 g. of esters could perhaps be handled in one batch on a larger column thereby reducing labour costs. We have also calculated a hypothetical cost for methyl linoleate starting from 5 Kg. of oil (page 55). In this case, the cost-to-make remains at 27p per gram and there is no advantage

over starting with 2 Kg. The cost might be further reduced by using procedures where larger amounts of material could be handled. Examples are given in Chapter 2 (page 38).

It is obvious that evening primrose oil is a useful, economical source of methyl linoleate regardless of its high price (£7 per Kg.) but a suitable safflower oil (containing $\angle$ 1% α-linolenic acid) may also provide cheap methyl linoleate using Scheme 3. Safflower oil of suitable quality is to be preferred to evening primrose oil because it is more readily available and at a cheaper price unless it is desired to produce both linoleic and γ-linolenic acids (see page 61).

3. Methyl γ-linolenate from evening primrose oil.

(i) Experimental procedures.

In recognition of the importance of pure methyl γ-linolenate in biological studies and because of the lack of reports on its isolation and purification, we have developed a method of isolating this ester from evening primrose oil.

The original scheme developed in this department for the isolation of methyl linoleate from evening primrose oil was extended to give Scheme 3 which provides the best route to both 18:2 and 18:3ω6 acids/esters from 1 Kg. of oil. After the first low temperature crystallisation the mother liquor contained a high concentration of non-saponifiable material and γ-linolenic acid (61%). The organic residue from this liquor was treated with urea (150 g.) which reduced the more saturated components from 39.2% to 12.6%. The fraction in the urea complex was rich enough in γ-linolenic acid (9%) to be recycled. Further treatment of the mother liquor with urea was not profitable until

the non-saponifiable material had been removed. Since work already done in this department had shown that γ-linolenic acid could not be upgraded beyond about 90% with urea unless the esters were used, the acids were esterified and the level of non-saponifiable material greatly reduced by column chromatography of the esters. A recovery of 78% by weight was obtained from the column. The esters were then treated with two batches of urea (100 g. and 20 g. respectively) which reduced the more saturated components from 12.5% to 2.1% to 0.3%. Chromatography also removed the last traces of non-saponifiable material and caused slight ester fractionation to give methyl γ-linolenate (98.9%, pure by T.L.C.) and a less pure fraction of methyl γ-linolenate (97.7%). The three fractions, B5 (65%), B7 (93%) and B9 (98%) contained valuable quantities of methyl γ-linolenate which could be recovered by recycling.

(ii) Costs.

The cost of producing methyl γ-linolenate only by Scheme 3 is £4.25 per gram. This high figure is related in part to the high cost of evening primrose oil (£7 per Kg.) but more to the low content of this acid in the oil (8%). The major proportion of the cost can be attributed to labour with the exception of hydrolysis where material costs equal labour costs because of the high cost of starting oil. This cost-to-make could be reduced by using each procedure on the optimum amount of material. For example by using 5 Kg. of oil (page 55) the cost is reduced from £4.25 to £2.69 per gram. The use of a larger scale than this would provide little saving and we calculated that the cost would still be £2.57 per gram using 10 Kg. of oil. However, these figures made no allowance for the valuable intermediate fractions (B5, B7, B9) which could furnish additional pure methyl γ-linolenate at lower cost. For example, on the 1 Kg. scale, the cost of £4.25 per gram could be reduced to £2.99

per gram if allowance is made for these fractions thus:

B5*	25 g. at	29p per g.	£7.25
B7*	9 g. at	87p per g.	£7.83
B9*	6 g. at	£1.89p per g.	£11.34
			£26.42
	revised cost (for 21 g.)		£62.80
		cost per g.	£2.99

By allowing for similar intermediate fractions on a hypothetical 5 Kg. scale the cost-to-make is reduced to £1.67 per gram.

4. Methyl linoleate and methyl γ-linolenate from the same batch of evening primrose oil.

The cost of isolating methyl linoleate from evening primrose oil has been discussed independently of the cost of isolating methyl γ-linolenate. Using 5 Kg. of oil and only the appropriate section of Scheme 3 it has been shown that this would furnish 1580 g. of methyl linoleate at 27p per gram and 105 g. of methyl γ-linolenate at £2.69 per gram. Some of the operations are common to both these procedures. It is of interest to consider the effect on cost of isolating both these esters from the same 5 Kg. batch of evening primrose oil.

After allowing for the appropriate operations common to the isolation of each ester (but with no allowance for intermediate fractions) it is considered that 5 Kg. of evening primrose oil would give 1580 g. of methyl linoleate and 105 g. of methyl γ-linolenate at a total cost of £501.

---

* B5, B7, B9 were valued according to Scheme 3 by equating these fractions with the cost to produce B (144 g., 61%), B4 (75 g., 87%) and B6 (38 g., 97%) respectively.

The cost of each ester is then dependent on the way in which this sum is distributed between the two products. Given an adequate market for both esters these costs can be distributed in various ways as shown:

cost of 18:2 (p per g.)	5	10	12	15	18	20	22	25	30
cost of 18:3 (£ per g.)	4.00	3.25	3.00	2.50	2.00	1.75	1.50	1.00	0.25

Since there is good market for linoleate at a cost-to-make of 15-25p per gram it is possible to produce γ-linolenate at a price between £2.50 and £1.00 per gram. Since no allowance has been made for intermediate fractions it should be possible to reduce this somewhat.

CHAPTER 4: The synthesis of methyl octadec - cis - 8 - enoate.

Introduction

A large quantity of methyl octadec - cis - 8 - enoate free from saturated and trans isomers was required for dietary studies elsewhere. Since this ester does not occur in a natural source suitable for isolation it is best obtained by synthesis.

Various methods have been devised for preparing acids/esters of varying chain lengths and degrees of unsaturation. These have been reviewed by Kunau[23] who discusses, in particular, the most recently described routes to methylene interrupted polyunsaturated acids. The Δ 2 to Δ 17 18:1 acetylenic and trans ethylenic acids have been prepared by Barve[24] and Ismail[25] has prepared all the 18:1 cis ethylenic acids by stereospecific catalytic reduction of the corresponding acetylenic acids.

We prepared pure methyl octadec - cis - 8 - enoate (70 g.) using the method of Barve to obtain octadec - 8 - ynoic acid which was then converted to the corresponding cis acid. We chose this method because of previous experience of working with acetylenic intermediates in this department and because this method reduces the possibility of the formation of unwanted isomers. Full details of the synthetic sequence are given in the next sections.

Scheme 4

Preparation of methyl octadec - cis - 8 - enoate

Chemicals required (including solvents etc.)

1. Thionyl chloride, pyridine, ether.
2. Sodium iodide, acetone, ether.
3. Sodium, liquid ammonia, acetylene, ether.
4. Compound C, sodium, liquid ammonia, ether.
5. Potassium cyanide, dimethyl sulphoxide, hydrogen chloride, methanol.
6. Potassium hydroxide, ethanol, sulphuric acid (concentrated) ether, petrol (b.p. 40-60°C).
7. Lindlar's catalyst, quinoline, hydrogen, methanol, silica, ether, petrol (b.p. 40-60°C).

Purity of products (by G.L.C.)

B, E	100%
C	92%
F	80%. The impurity was the C17 acetylenic iodide which reacted along with the chloride in the next stage.
G	89%. The impurity was not removed by column chromatography.
H	99%. After repeated crystallisation from petrol (b.p. 40-60°C).
J	99%
K	99% (G.L.C. and T.L.C.)

"Cost" of preparing pure methyl octadec - cis - 8 - enoate (70g) from 1,6 - hexanediol (590g) and 1 - bromononane (470g)

	Material (£)	Labour (£)[a]	Total (£)
Cost of hexanediol (590g)	1.37	-	1.37
Cost of bromononane (470g)	14.10	-	14.10
Preparation of: B (586g)	5.37	20.64	26.01
C (351g)	7.97	41.28	49.25
E (145g)	8.46	61.92	70.38
F (130g)	3.43	41.28	44.71
G (110g)	3.49	22.36	25.85
H[b] (75g)	1.48	6.88	8.36
J (75g)	2.62	6.88	9.50
K[c] (70g)	4.70	12.04	16.74
	£52.99	£213.28	£266.27
cost per g. (for 70g)	£0.76	£3.04	£3.80

a. man-hours costed at £1.72 per hour (see page 15).

b. including repeated crystallisation of product.

c. including column chromatography of final product.

Discussion

The cost of making pure methyl octadec - cis - 8 - enoate (70g) from 1,6 - hexanediol (590g) and 1 - bromononane (470g) by Scheme 4 is £3.80 per gram. Material consumed amounts to about 20% of the total cost so that labour is the major factor contributing to cost.

At each stage the cost is dependent on the time taken to carry out the reaction and purify the product. The first four stages are particularly expensive because these have to be carried out on a large scale and involve a time-consuming fractional distillation. The preparation of the C18 ester would have been more economic if each step in the synthesis had been carried out in a single operation. On this basis the cost could have been reduced to £2.42 per gram as follows.

	Material (£)	Labour (£)	Total (£)
Cost of hexanediol (590g)	1.37	-	1.37
Cost of bromononane (470g)	14.10	-	14.10
Preparation of: B (586g)	5.37	10.32	15.69
C (351g)	7.97	20.64	28.61
E (145g)	8.46	30.96	39.42
F (130g)	3.43	20.64	24.07
G (110g)	3.49	11.18	14.67
H (75g)	1.48	6.88	8.36
J (75g)	2.62	3.44	6.06
K (70g)	4.70	12.04	16.74
	£52.99	£116.10	£169.09
cost per g. (for 70g)	£0.76	£1.66	£2.42

The overall cost of synthesising this "unnatural" acid is still high compared to that of isolating the natural $\Delta 9$ isomer, oleic acid, which was obtained from olive oil at a "cost" of 49p per gram (Scheme 2, Chapter 2). This large difference is due to the different nature of the processes involved. It requires much less effort to isolate pure methyl oleate from a suitable natural source than to prepare the synthetic isomer. Nevertheless it is recognised that the cost of the synthetic acid might be further reduced by improvements in yields and techniques since this synthesis was carried out only once in comparison to the techniques employed to isolate the natural acids. These were used repeatedly and some success achieved in discovering the most economic conditions for each procedure.

Experimental

Purification of solvents.

Acetone was stored over anhydrous sodium sulphate for two hours, decanted and distilled from anhydrous calcium sulphate.

Ether was stored over anhydrous calcium chloride for two days, decanted, distilled and stored over sodium wire.

Methanol was refluxed with magnesium methoxide for two hours and distilled[26].

Pyridine was stored over potassium hydroxide pellets for two days, decanted, distilled and stored over potassium hydroxide pellets.

Dimethyl sulphoxide, t-butanol and quinoline were distilled under vacuum before use.

Analytical techniques.

Infrared spectroscopy, von Rudloff oxidation, column chromatography, thin-layer chromatography and gas-liquid chromatography were carried out as already described (Chapter 1).

Nuclear magnetic resonance spectroscopy was carried out on a 100 MHz spectrometer (Varian) with samples in carbon tetrachloride using tetramethylsilane as internal standard.

Preparation of methyl octadec - cis - 8 - enoate.

In each of the following reactions, organic extracts were washed with water, dried using anhydrous sodium sulphate and the solvent removed using a rotary film evaporator.

1,6 - Dichlorohexane (B).

Hexane-1,6-diol (295g, 2.50 moles) was stirred in a three-necked flask fitted with a condenser and drying tube (silica gel). After addition of dry pyridine (30 ml.) the flask was cooled in ice and thionyl chloride (700 ml., 10.09 moles) added slowly from a dropping funnel. The mixture was then stirred for three hours at room temperature and refluxed for a further hour. After cooling, iced water was carefully added to destroy excess thionyl chloride. More water was added until two distinct layers were seen. The organic layer was collected and the aqueous layer extracted with ether (2 x 400 ml.). The combined organic layers, washed with sodium bicarbonate solution (10%) and water until neutral, gave the crude dichloride. The reaction was repeated using the same amount of diol and the combined crude product distilled to give 1,6 - dichlorohexane [586g, 76%, b.p. 94°/20 mm. (literature[25] 86°/15 mm)].

1,6 - Chloro-iodohexane (C).

A solution of 1,6 - dichlorohexane (310g, 2.00 moles) in dry acetone (500 ml.) was placed in a three-necked flask fitted with a mechanical stirrer, condenser with drying tube (silica gel) and dropping funnel. The mixture was stirred and brought to reflux. A solution of sodium iodide (300 g, 2.00 moles) in dry acetone (1500 ml.) was added at a rate which allowed the mixture to continue refluxing. The mixture was refluxed for a further three hours and most of the acetone (1.5 l.) was then distilled off. The residue was cooled, diluted with water (1 l.) and the dihalide mixture was extracted with ether (2 x 400 ml.). The reaction was repeated with more dichloride (276g) and the combined dihalides were fractionally distilled through a Fenske column. Fractions were combined on the basis of G.L.C. and boiling point data to give 1,6 - chloro-iodohexane [351g, 38% yield, 92% pure, b.p. 101-102°/5 mm. (literature[25] 76-78°/0.7 mm)].

Undec - 1 - yne (E).

Liquid ammonia (1 l.) was poured from a cylinder into a three-necked flask fitted with a cardice-acetone condenser, dropping funnel and magnetic stirrer. Ferric nitrate (1g) was added and the stirrer started. Sodium (13g) was added in small pieces (0.25g each), each piece being added when the previous piece had reacted (a blue colour, formed initially, disappeared as the white solid, sodamide, formed). When about half the sodium had been added, dry acetylene (pretreated to remove acetone) was passed briskly through the mixture while the rest of the sodium was being added. The acetylene was passed through the mixture (now a black colour) for a total of three hours. 1 - Bromononane (110g, 0.53 moles) in dry ether (400 ml.) was then added dropwise. The reaction mixture was allowed to reflux for a further two hours before the condenser was removed and the ammonia allowed to evaporate overnight at room temperature. Next morning, cold water (1 l.) was carefully added to destroy excess sodium acetylide and sodamide. The mixture was extracted with ether (3 x 300 ml.) and the combined ether extracts gave crude hydrocarbon. The reaction was repeated with further quantities of 1 - bromononane (3 x 120g) and the combined crude products were fractionally distilled through a Fenske column to give undec - 1 - yne [145g, 42%, b.p. 71-72°/8 mm. (literature[24] 84-86°/15 mm)] which was 100% pure by G.L.C.

1 - Chloroheptadec - 7 - yne (F).

Sodamide was prepared as described previously from sodium (8g) and liquid ammonia (1 l.). To this stirred suspension was added dropwise undec - 1 - yne (45g, 0.30 moles) in dry ether (200 ml.) and after two

hours a solution of 1,6 - chloro-iodohexane (95g, 0.37 moles) in dry ether (200 ml.). Stirring was continued for a further three hours. Excess ammonia was allowed to evaporate overnight and the crude chloride isolated. The reaction was repeated with more undec - 1 - yne (2 x 50g) and distillation of the combined crude product gave 1 - chloroheptadec - 7 - yne which contained 1 - iodoheptadec - 7 - yne* (20% by G.L.C.) [130g, 62%, b.p. 150-165°/2 mm. (literature[24] 151°/2 mm.)]. This mixture was used in the next stage of the reaction where both halides reacted in the same way.

Methyl octadec - 8 - ynoate (G)

Potassium cyanide (17g, 0.26 moles) in dimethyl sulphoxide (250 ml.) was placed in a round-bottomed flask fitted with a condenser and magnetic stirrer. A solution of the 1 - chloroheptadec - 7 - yne/ 1 - iodoheptadec - 7 - yne mixture (65g, 0.29 moles) in dimethyl sulphoxide (60 ml.) was added while stirring. The reaction flask was then maintained at a temperature of 120-130°C using an oil bath for 3.5 hours. The mixture was cooled, diluted with brine (5%, 1 l.) and extracted thoroughly with ether (4 x 250 ml.). The combined ether extracts were washed with brine (10%) and gave crude heptadec - 8 - ynyl cyanide (53g, 0.20 moles) which was 98% pure by G.L.C. The crude cyanide was dissolved in a solution of hydrogen chloride (60g) and methanol (180 ml.) and stirred at room temperature overnight. The mixture was diluted with brine (10%, 600 ml.) and extracted with ether (3 x 200 ml.) to give the crude

---

* The acetylenic iodide was identified by G.L.C. and by the nuclear magnetic resonance spectrum (100 MHz) which was similar to that of the chloride except that it showed a triplet at δ 3.14 ($-CH_2I$) instead of δ 3.46 ($-CH_2Cl$).

ester. The reaction was repeated with the same amount of starting material and the products combined to give methyl octadec - 8 - ynoate (110g, 65%). The ester contained an impurity (11% by G.L.C.) which was not detected by T.L.C. (PE20) nor removed by column chromatography. It was therefore necessary to crystallise the free acid to obtain pure material for the hydrogenation reaction.

Octadec - 8 - ynoic acid (H).

Methyl octadec - 8 - ynoate (110g, 0.37 moles) was refluxed for one hour with a solution of potassium hydroxide pellets (23g) in ethanol (80 ml.) and water (25 ml.). After partial cooling, crushed ice (100 ml.) was added followed by sulphuric acid (4 M, 120 ml.) and the mixture was transferred to a separating funnel and shaken to ensure good mixing. When the mixture had separated the lower layer was drawn off and extracted with ether (50 ml.). The ether extract was combined with the major organic layer and washed with water (2 x 20 ml.). Solvent was removed using a RFE and the residue was dried by azeotropic distillation with benzene before being crystallised from petrol (b.p. 40-60°C, 10 ml. per g.) to give octadec - 8 - ynoic acid [75g, 71%, m.p. 45-46° (literature[24] 47°)] which was 99% pure by G.L.C.

Octadec - cis - 8 - enoic acid (J).

Octadec - 8 - ynoic acid (35g, 0.13 moles) was dissolved in methanol (200 ml.) in a long-necked round-bottomed flask (1 l.). Lindlar's catalyst (2.0g) and freshly distilled quinoline (1 ml.) were added and the flask connected to a hydrogenation apparatus which operated at atmospheric pressure. Hydrogenation occurred quickly and the reaction product was checked by G.L.C. whenever the uptake of hydrogen ceased.

The reaction was repeated with more octadec - 8 - ynoic acid (40g). The combined reaction mixtures were filtered to remove the catalyst and the solution used in the next stage.

Methyl octadec - cis - 8 - enoate (K).

The solution of octadec - cis - 8 - enoic acid (75g, 0.27 moles) and quinoline (2 ml.) in methanol (430 ml.) from the previous stage was evaporated to a volume of about 200 ml. Sulphuric acid (2.5g) in methanol (25 ml.) was added carefully and the mixture refluxed for one hour. After cooling, the mixture was added to water (300 ml.) in a separating funnel. Petrol (b.p. 40-60°C, 300 ml.) was added and the mixture shaken. The organic extract was washed with water (2 x 20 ml.) and the ester isolated by evaporation (RFE) and dried with benzene. The crude product (99% pure by G.L.C.) was purified by column chromatography on silica (500g) using PE5 as eluting solvent to give methyl octadec - cis - 8 - enoate (70g, 89%) which gave a single spot on T.L.C.

In this final product, the double bond was shown to be in the Δ 8 position by von Rudloff oxidation which gave the C8 diacid and the C10 monoacid only and its infrared spectrum, corresponding to that of pure methyl octadec - cis - 8 - enoate, showed no trace of trans unsaturation at 963 $cm.^{-1}$.

REFERENCES

1. O.S. Privett, Prog. in the Chem. of Fats and other lipids (ed. R.T. Holman), 9, 407, Pergamon, Oxford (1966).

2. J.B. Brown and D.K. Konb, Prog. in the Chem. of Fats and other lipids (ed. R.T. Holman), 3, 57, Pergamon, Oxford (1955).

3. H. Schlenk, Prog. in the Chem. of Fats and other lipids (ed. R.T. Holman), 2, 243, Pergamon, Oxford (1954).

4. R.W. Riemenschneider, S.F. Herb and P.L. Nichols Jr., J. Amer. Oil Chemists' Soc., 26, 371, (1949).

5. W.W. Christie, 'lipid analysis', 137, Pergamon, Oxford (1973).

6. H. Schlenk and R.T. Holman, J. Amer. Oil Chemists' Soc., 38, 728, (1961).

7. H. Schlenk and R.T. Holman, J. Amer. Oil Chemists' Soc., 72, 5001, (1950).

8. D. Swern and W.E. Parker, J. Amer. Oil Chemists' Soc., 29, 431 and 614, (1952).

9. J.G. Keppler, S. Sparreboom, J.B.A. Stroink and J.P. Von Mikursch, J. Amer. Oil Chemists' Soc., 36, 308, (1959).

10. L.J. Rubin and W. Paisley, J. Amer. Oil Chemists' Soc., 37, 300, (1960).

11. T.H. Applewhite, J. Amer. Oil Chemists' Soc., 43, 406, (1966).

12. M.J. Diamond and G. Fuller, J. Amer. Oil Chemists' Soc., 47, 362, (1970).

13. M.L. Kinman, J. Amer. Oil Chemists' Soc., 49, 36, (1972).

14. N.L. Matthews, W.R. Brode and J.B. Brown, J. Amer. Oil Chemists' Soc., 63, 1064, (1941).

15. B. Sreenivasan, J.B. Brown, E.P. Jones, V.L. Davidson and J. Nowakawska, J. Amer. Oil Chemists' Soc., 39, 255, (1962).

16. A.R. Johnson and G.M. Ali, J. Amer. Oil Chemists' Soc., 38, 453, (1961).

17. D. Swern and W.E. Parker, J. Amer. Oil Chemists' Soc., 30, 5, (1953).

18. E.M. Stearns Jr., H.B. White Jr. and F.W. Quackenbush, J. Amer. Oil Chemists' Soc., 30, 61, (1962).

19. R.E. Beal and O.L. Brekke, J. Amer. Oil Chemists' Soc., 36, 397, (1959).

20. J.P. Riley, J. Chem. Soc., 2728, (1949).

21. R. Kleiman, F.R. Earle, I.A. Wolff and Q. Jones, J. Amer. Oil Chemists' Soc., 41, 459, (1964).

22. R.W. Miller, F.R. Earle, I.A. Wolff and A.S. Barclay, Lipids, 3, 43, (1968).

23. W.H. Kunau, Chem. and Phys. of Lipids, 11, 254, (1973).

24. J.A. Barve, PhD. Thesis, University of St. Andrews, (1970).

25. I.A. Ismail, PhD. Thesis, University of St. Andrews, (1966).

26. A.I. Vogel,'Practical Organic Chemistry', 168, Longmans (1948).