A REVERSE PHASE HIGH-PRESSURE LIQUID CHROMATOGRAPHIC TECHNIQUE FOR THE ANALYSIS OF SOME COMMON SATURATED AND UNSATURATED FATTY ACIDS AS THEIR p-PHENYLPHENACYL ESTERS

by

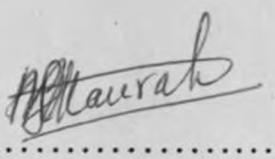
STEPHEN KARIUKI

A thesis submitted in partial fulfilment for the Degree of Master of Science in Chemistry of the University of NAIROBI.

1987

D E C L A R A T I O N

I declare that this thesis is my original work and has not been presented for a degree in any other University. The research was carried out in the Chemistry Department at the University of Nairobi.



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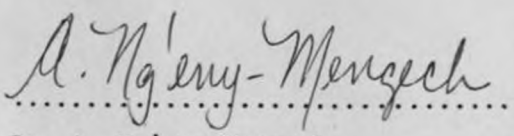

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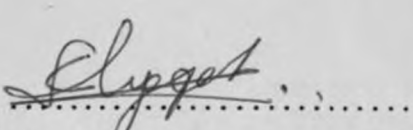

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D E D I C A T I O N

Dedicated to my wife, Mary Mumbi and son, Mwaura (born in November this year) and other members of my family.

A C K N O W L E D G E M E N T S

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A REVERSE PHASE HIGH-PRESSURE LIQUID CHROMATOGRAPHIC TECHNIQUE FOR THE ANALYSIS OF SOME COMMON SATURATED AND UNSATURATED FATTY ACIDS AS THEIR p-PHENYLPHENACYL ESTERS

A Thesis submitted by S. Kariuki for the M.Sc. in Chemistry

ABSTRACT

A technique for the analysis of some common natural fatty acids has been developed by the use of reverse-phase high performance liquid chromatography (HPLC).

The fatty acid standards were derivatised with p-phenylphenacyl bromide via the potassium salt or using tetra-n-butylammonium hydrogen-sulphate as phase-transfer catalyst. The p-phenylphenacyl esters of dodecanoic ($C_{12:0}$), tetradecanoic ($C_{14:0}$), hexadecanoic ($C_{16:0}$), Z-9-hexadecenoic ($C_{16:1;9Z}$), octadecanoic ($C_{18:0}$), Z-9-octadecenoic ($C_{18:1;9Z}$), E-9-octadecenoic ($C_{18:1;9E}$), Z-9, Z-12-octadecadienoic ($C_{18:2;9Z,12Z}$), Z-9, Z-12, Z-15-octadecatrienoic ($C_{18:3;9Z,12Z,15Z}$), 12-hydroxy-Z-9-octadecenoic ($C_{18:1;9Z(12OH)}$) and Z-13-docosenoic ($C_{22:1;13Z}$) acids were synthesized and characterized. The last five esters have not been synthesized previously.

These fatty acid ester derivatives were then chromatographed on a 5μ ultrasphere ODS column using various mobile phases, including absolute methanol, methanol-water, methanol-acetonitrile and methanol-acetonitrile-water mixtures. The best mobile phase was found to be a mixture of methanol, acetonitrile and water, which made it possible to resolve all of the critical pairs (p-phenylphenacyl ester derivatives of hexadecanoic ($C_{16:0}$) & Z-9-octadecenoic ($C_{18:1:9Z}$); tetradecanoic ($C_{14:0}$) & Z-9, Z-12-octadecadienoic ($C_{18:2;9Z;12Z}$); and dodecanoic ($C_{12:0}$) & Z-9, Z-12, Z-15-octadecatrienoic ($C_{18:3;9Z,12Z,15Z}$) fatty acid pairs). The cis-trans isomers of Z-9-octadecenoic ($C_{18:1;9Z}$) and E-9-octadecenoic ($C_{18:1;9E}$) acids were also resolved.

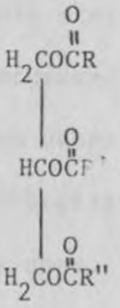
A gradient elution programme was developed when the retention time for the longer-chain fatty acid esters, of octadecanoic ($C_{18:0}$) and Z-13-docosenoic ($C_{22:1;13Z}$) acids was found to be too long under isocratic conditions. The method was applied to the ester mixtures obtained from commercial samples of castor oil and linseed oil.

CHAPTER ONE

INTRODUCTION

1.1 GENERAL PROPERTIES OF FATS AND OILS

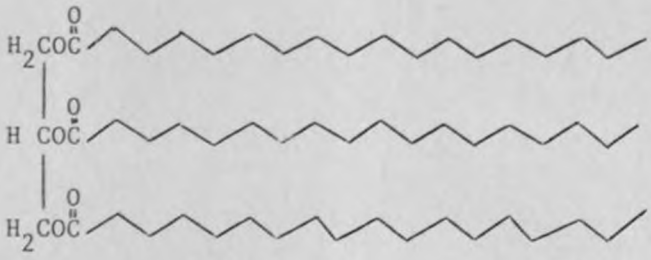
Natural fats and oils are said to be the group of compounds predominantly made up of triglycerides, which are esters of glycerol and fatty acids. Without regard to optical activity, the structure of the triglycerides is generally presented as



where R, R' and R" are the alkyl chains whose structure mainly depends on the source of the fats and oils. Naturally-occuring fats and oils may also contain small amounts of soluble, minor constituents like pigments, sterols, phospholids, lipoproteins, glycolipids, hydrocarbons, vitamin E, vitamin D, waxes, proteins,

carbohydrates, ethers and degradation products of fatty acids. The actual composition depends on the source of the fats and oils.

Thus the chemical structures of fats and oils are very complex owing to the combinations and permutations of fatty acids that can be esterified at any or all of the three hydroxy groups of glycerol. Oils and fats are differentiated from each other mainly by their physical appearance, since oils are liquids while fats are either solids or semi-solids at room temperature (20°C). The alkyl chains of the triglycerides in fats tend to be more saturated than those of oils, as a result of which their stereochemistry is different[1]. The molecules of the saturated alkyl chains tend to pack themselves more closely to each other than those acids with unsaturated alkyl chains and particularly those of the Z-configuration as structures below show.



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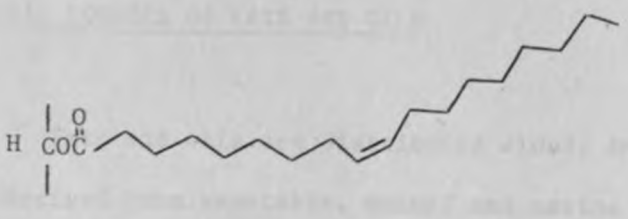


"Z-unsaturated alkyl chain of triglycerides"

The difference in stereochemistry implies that saturated triglycerides have higher melting points due to the relatively closer packing than the unsaturated ones, where very close packing is not possible.

1.2 NATURAL SOURCES OF FATS AND OILS

Fats and oils are distributed widely in nature. They are derived from vegetable, animal and marine sources. They form the main constituents of storage fat cells in these organisms. Table 1 below shows the source of some important fats and oils and from which part of the world it is mainly obtained.

Table 1. Classification and Sources of Significant Fats and Oils.

FAT OR OIL	SOURCE	PRINCIPAL PRODUCTION AREAS (1978)
VEGETABLE:-		
babassu	Orbignya(Attalea) speciosa	Brazil
castor	Ricinus communis	Brazil, India
coconut	Cocos nucifera	Philippines, Indonesia, India
corn	Zea mays	U.S., Europe, Argentina
cottonseed	Gossypium hirsutum, Gossypium harbadense	U.S.S.R, U.S., China, India, Pakistan, others
linseed	Linum usitatissimum	Argentina, Canada, India, U.S., U.S.S.R.
oiticica	Licania rigida	Brazil

Table 1 (Continued)

FAT OR OIL	SOURCE	PRINCIPAL PRODUCTION AREAS (1978)
olive	Olea europaea	Italy, Spain, Greece, North Africa
palm	Elaeis guineensis	Malaysia, Africa, Indonesia
palm kernel	Elaeis guineensis	Malaysia, Africa, Indonesia
peanut(groundnut)	Arachis hypogaea	India, Africa, China, U.S., others
rapeseed	Brassica compestris Brassica napus	Canada, Europe, India, China
safflower	Carthamus tinctorius	U.S., India, others
sesame	Sesamum indicum	India, China, Africa
soybean	Soya max	U.S., China, Brazil, others

Table 1 (Continued)

FAT OR OIL	SOURCE	PRINCIPAL PRODUCTION AREAS (1978)
sunflower	Helianthus annus	U.S.S.R., U.S., Argentina, Europe, others
tung	Aleurites fordii, Aleurites montana	China, Argentina, others
ANIMAL		
butter	Bos gaurus	worldwide
lard	Sus domesticus	worldwide
tallow and grease	Bos taurus, Ouis aries	worldwide
MARINE		
whale	Cetacea	worldwide

Table 1 (continued)

FAT OR OIL	SOURCE	PRINCIPAL PRODUCTION AREAS (1978)
MARINE		
sperm whale	Physeter catodon	worldwide
fish(liver)	Clupea harenqus	Norway, Peru,
	Clupea pilchardus	Japan, U.S.
	Sardinops corrulea	
	Brevoortia tyrannus	

Source: Kirk - Othmer Encylcopedia of Chemical Technology[2].

1.3 USES AND COMMERCIAL IMPORTANCE OF FATS AND OILS

There are many applications of fats and oils and only a few of them will be considered in this section.

Fats and Oils Used As Food

The unsaturated fats and oils tend to be more reactive chemically than the saturated ones. These unsaturated fats and oils cannot remain exposed for a long time without being attacked by oxygen, since the double bonds are reactive sites. In the cooking fats industry, the unsaturated triglycerides are hydrogenated, a process which is known as hardening of oils, and by so doing the triglycerides become quite stable. Heidrich[3] has reported that over 80% of the fats and oils reported to have been isolated and processed are being used as food. In another report of Gander[4], it is estimated that 85.9% of the fats and oils produced in the seasons 1970/71 and 1980/81 respectively were used for edible purposes. These statistics imply that the major usage of fats and oils is for food. Examples of some important fats and oils used for food are coconut, cottonseed, groundnut, maize, neem, palm, olive, perilla, rapeseed, safflower and sunflower oils.

Fats and Oils Used in Surfactants Manufacture

Most of the surfactants produced have alkyl chains in the C_{12} to C_{18} range. Triglycerides like those found in cotton-seed, grapeseed, hempseed, kapok seed, barbados nut and neem oils have been used as good starting materials for the manufacture of surfactants. In the United States, 2,100,100 tons of natural fats and oils were being used in the production of surfactants, as reported by Pryde[5] in 1979. Coconut oil with 230,000 tons was the largest single vegetable oil contributor to these products. Surfactants range from anionic surfactants (sulfonates, sulfates, soaps) to cationic surfactants (quaternary ammonium compounds), non-ionic surfactants (esters, sugar esters, amides) and amphoteric surfactants (carboxylates, sulfo compounds). Among cationic and amphoteric products, fat-derived surfactants predominate. The use of fat-derived surfactants is also preferred in applications where direct human contact is involved, such as in cosmetics. The use of surfactants from natural fats and oils is expected to increase as petroleum becomes more expensive. Soap manufacture requires more fats and oils than any other surfactant. Gander[4] has reported that about 2.6 million tons of fats and oils were used in soap-making industries in the season 1980/81.

Fats and Oils Used in Paints and Varnishes Industry

Paints in the present day are used not only for decoration purposes but also for the protection of a surface. Once a paint is applied, it should dry and hence leave a good coating. Some characteristic triglycerides, for example tung, sunflower, safflower, rapeseed, perilla, oiticica, linseed, hempseed, grapeseed and castor oils, have been used in paints for drying purposes. The specific choice of oil may depend on the prevailing price of the oil. Polymerization of the unsaturated oils brought about by atmospheric oxygen is the major reaction which occurs[6]. The use of fats and oils in the drying oil industries in the United States is said to have been 247 million Kg in 1977, according to a report given from the U.S. Department of Agriculture[7]. Although it has also been reported by Heidrich[3] that the use of these unsaturated triglycerides is decreasing due to the use of synthetics, it remains quite clear that the triglycerides, being renewable, have an advantage over synthetics and even in future could be used to make these synthetics.

Fats and Oils Used in Lubricants Industry

The vegetable oils most commonly used in lubricating systems are castor, rapeseed, tallow and palm oils. Castor

oil derivatives are used for greases, generally as salts of 12-hydroxystearic acid. Rapeseed oil is used as a mold lubricant in the continuous casting of steel[8] ; erucamide, derived from rape, as an antiblocking and slip agent in polyethylene films; pelargonic acid, also derived from rape, in ester lubricants. Palm oil is used as a rolling and drawing oil.

Fats and Oils in Thermochemical Applications

Today's world fuels are obtained principally from non-renewable fossil resources which with time may be depleted. Triglycerides, which are renewable, have a very good potential for fuel-use. As such,interest has been growing in the use of vegetable oils as diesel fuel additives and substitutes. Lipinsky and his colleagues have demonstrated that triglycerides can be used as a fuel to produce steam from water[9]. This steam in turn can be used to generate electricity, if used for turning gas turbines that normally consume No.2 diesel fuel oil. In one experiment, corn oil was burnt in a moving grate burner such that the moving grate could pick up fuel, carry it to the flame zone and return to pick up more fuel.
Melvin Calvin had earlier shown that copaiba balsam

(Copaifera spp) oil[10] could be used to replace diesel directly.

In another experiment[11], eucalyptus oil (specific gravity 0.9166 at 15°C) which had been extracted from the eucalyptus tree was examined in order to find its potential as a fuel. Investigations were done on pure and blended eucalyptus oil. Comparison was done amongst 100% gasoline, 80% gasoline + 20% eucalyptus oil, 60% gasoline + 40% eucalyptus oil, 40% gasoline + 60% eucalyptus oil and 100% eucalyptus oil. It was found that the eucalyptus-blended fuel had lower levels of carbon monoxide emissions than did 100% gasoline. As for the mixing ratios, 40% of eucalyptus oil to 60% gasoline was suitable for use with a regular main jet nozzle (D = 0.650 mm) when tested on a 2-stroke cycle engine. Compared to gasoline, unblended eucalyptus oil was found to have some difficulty in engine starting, although this was said to have been improved by the increase of the mixing ratio of gasoline to eucalyptus oil.

Hall[12] has reported that in the Second World War, China had developed an industrial process for cracking vegetable oils, mostly oil from tung nuts, and thereby turning vegetable

oils into motor fuels. The Chinese also used rapeseed oil and peanut oil. They had previously found that diesel engines could burn vegetable oils, but due to their higher viscosity than conventional diesel fuels, the oils had to be injected into an engine at a higher pressure. Another disadvantage they encountered was that vegetable oils were producing more carbon when they burnt; tung oil in particular formed large molecules which produced gum in the engines. In order to overcome these disadvantages, the Chinese then tested the effectiveness of vegetable oil-gasoline blended fuels.

The National Institute of Technology in Rio de Janeiro in Brazil completed in January, 1980 a series of tests involving running buses on diesel and vegetable oil mixtures[12]. In one trial, the fuel was a mixture of 80% diesel and 20% peanut oil; the other trial used a mixture of 73% diesel, 20% palm oil, and 7% ethanol. In this latter trial, the bus ran about 500 hours for 10,000 km. (it had previously covered 60,000 km over the same time). After the trial the bus reverted to normal running on diesel oil without having any readjustments. The trial showed that with the oil-diesel mixture, the fuel consumption was 3.4% less than with diesel fuel alone.

In one South African study, when the engineers in Pretoria fuelled diesel engines with a high percentage of sunflower oil (actual composition not stated), the injection nozzles became clogged, especially when the engines were not running at full power[12]. They tried to overcome this problem by adding a different fuel filter and by tuning the engines. It was found that contaminants from the fuel - mostly partially burnt oil - found their way into the engine's lubricating oil, but only if the oil was not changed every 200 hours. Further improvements were done by the same group by fuelling diesel engines with a sunflower-oil/ester mixture. This mixture had been obtained by adding either methanol or ethanol to the sunflower oil, after which heating of the mixture was done upto 40°C for a few hours (number of hours have not been specified) resulting in a mixture of fatty acid methyl/ethyl esters, some unreacted oil and alcohol (methanol or ethanol). The viscosity properties of the fuel produced in this way were said to have been very similar to those of ordinary diesel fuel. The fuel obtained was also found to cause less coking and exhaust smoke than diesel fuel; it also had a higher thermal efficiency than diesel fuel, implying that this sunflower-oil/ester mixture had a substantially improved performance.

These results indicate that internal combustion engines are capable of burning non-petroleum fuels if such fuels were available in adequate quantities. Engines can be developed and modified to use alternating fuels.

Other Uses of Fats and Oils

Reports in Chemical and Engineering News[13] state that more than 25 billion pounds per year of ethylene were being sold at approximately $0.26 per pound. In their work, Lipinsky and colleagues[9] have shown that biomass-derived triglycerides can be a good source of olefins. They steam-cracked octadecanoic acid ($C_{18:0}$), Z-9-octadecenoic acid ($C_{18:1}$) and Z-9, Z-12-octadecadienoic acid ($C_{18:2}$) and, depending on temperature, it was found that each acid produced a considerable amount of ethylene which could serve as a feedstock for the polymer industry.

Among the many other uses for fats and oils are polymers of vinyl esters of long chain alcohols used as floor and furniture coatings and in lubricating oils. Vinyl esters of C_{14} - C_{18} acids are used in the manufacture of adhesives[14].

1.4 CONSUMPTION OF FATS AND OILS

By 1984, more than 60 million tons of vegetable and animal fats were being obtained worldwide. Only one-third of the total fat production was being put into the world market by the producing countries (which are U.S.A., Canada, Brazil, Argentina and Malaysia), the rest being consumed internally, according to a report by Gander[4]. Kenya, just like many other countries, has also been producing fats and oils for domestic and industrial use. Table 2 shows the imports and exports of fats and oils in Kenya, as reported in the Statistical Abstracts[15] of Kenya. In Table 3, the value and quantities of fats and oils imported into Kenya from 1979 to 1981 has been shown.

Table 2: Imports and Exports of Kenya's Animal and Vegetable Oils and Fats, Oil Seeds and Oil Nuts From 1975 - 1983.

YEAR / COMMODITY IMPORTED (TONS)	1975	1976	1977	1978	1979	1980	1981	1982	1983
ANIMAL OILS AND FATS	20,574	11,497	8,102	16,638	10,858	19,392	15,019	7,897	4,160
VEGETABLE OILS AND FATS	14,678	39,097	45,786	52,398	47,121	72,381	102,177	99,488	74,001

YEAR / COMMODITY EXPORTED (TONS)	1975	1976	1977	1978	1979	1980	1981	1982	1983
ANIMAL AND VEGETABLE OILS	2,164	2,193	826	600	1,549	1,169	1,593	343	1,185
OIL SEEDS AND OIL NUTS	6,770	5,432	5,692	7,786	13,413	9,579	8,473	5,842	8,492

Table 3: Value$^{\neq}$ and Quantities of Fats and Oils Imported into Kenya.

OIL TYPE	1979		1980		1981	
	TONS	VALUE	TONS	VALUE	TONS	VALUE
Fish/marine oils	6	57	9	208	9	194
Castor oil	112	1,080	17	198	71	495
Crude vegetable oils	47,000	252,200	71,860	361,100	101,360	370,320
Fatty acids	514	3,450	1,270	1,270	1,460	9,910
Tallow	10,850	57,920	19,380	100,500	15,000	92,094
Others	123	1,240	411	4,217	206	2,540
Total	58,627	315,967	92,947	437,505	118,112	475,497

($\neq$ value is the indicated Kshs. times 10^3).

Source: Munavu[16].

As reported by Munavu[16], the fats and oils industry in Kenya is currently based on imported raw materials, mainly crude palm oil and beef tallow. Over 100,000 tonnes of vegetable oils and 15,000 of tallow are imported annually, at a cost of over Kshs.550 million.

The Food and Agricultural Organization (FAO)[17] have revealed that by 1977, the annual per capita consumption of edible and animal oils and fats was far below the recommended level (although the recommended level was not indicated). The details are contained in Table 4.

Table 4: Per Capita Consumption of Oils and Fats for 1972 - 74.

Country/Region	Per Capita Oil Consumption (g/day)		
	Vegetable	Animal	Total
WORLD	26.9	34.1	61.0
AFRICA	29.5	10.9	40.4
Angola	25.1	9.0	34.1
Chad	28.1	8.4	36.6
Congo	22.1	4.6	31.7
Kenya	20.3	11.4	31.7
Mozambique	26.1	5.2	31.3
SOUTH AMERICA	27.0	30.2	57.2
Argentina	43.5	66.2	109.8
Guatemala	22.7	13.7	36.4
Peru	22.8	26.5	49.3
ASIA	19.9	13.4	33.3
Indonesia	15.3	14.5	29.9
India	21.6	7.5	29.0
Phillippines	23.7	2.8	26.5

Table 4 (continued)

Country/region	Per Capita Consumption (g/day)		
	Vegetable	Animal	Total
EUROPE	44.8	86.6	131.4
UNITED STATES	47.3	119.1	166.4
CANADA	28.7	124.5	153.3
UNITED KINGDOM	39.5	105.5	144.6
LESS DEVELOPED COUNTRIES	24.3	12.2	36.4
MORE DEVELOPED COUNTRIES	45.5	88.4	133.9

Source: FAO (1977)[17]

The oil seed species which account for most of the vegetable oils produced in Kenya are sunflower (grown in most farming areas), coconut (grown at the Coast), groundnuts (grown in Western, Coast and Rift Valley Provinces), sesame (grown in Western, Coast, and Rift Valley Provinces), rapeseed (grown in Rift Valley and Central Provinces), cotton (grown in Western, Rift Valley, Central and Eastern provinces), castor (throughout the country)[16].

1.5 FATTY ACIDS FOUND IN TRIGLYCERIDES

It has already been mentioned in section 1.1 that triglycerides are derived from fatty acids and glycerol. The uses of the various triglycerides is largely determined by the nature of the fatty acids they contain. Some of the more common fatty acids obtained from fats and oils are listed in Table 5. As the table shows, there are quite a number of categories of fatty acids; for example, saturated, mono-unsaturated, conjugated polyunsaturated, non-conjugated polyunsaturated, etc. In addition, some branched-chain fatty acids also occur in nature. The table also indicates another important category of fatty acids, those which are epoxylated. The known epoxylated fatty acids are few, though possibly this is because their history is much more recent than that of most of the other categories. The first epoxy acid shown to occur naturally, 12, 13-epoxy-Z-9-octadecenoic acid (XII) was discovered by Gunstone[18] in 1954 in Vernonia anthelmentica seed oil. Since then, others have also been discovered (XIII, XIV, for example).

Table 5: Some Common Fatty Acids Obtained From Natural Sources.

NAME OF ACID	STRUCTURE	ABBREVIATION
Decanoic	$CH_3(CH_2)_8COOH$	$C_{10:0}$
Dodecanoic (I)	$CH_3(CH_2)_{10}COOH$	$C_{12:0}$
Tetradecanoic (II)	$CH_3(CH_2)_{12}COOH$	$C_{14:0}$
Hexadecanoic (III)	$CH_3(CH_2)_{14}COOH$	$C_{16:0}$
Z-9-Hexadecanoic (IV)	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$	$C_{16:1;9Z}$
Octadecanoic (V)	$CH_3(CH_2)_{16}COOH$	$C_{18:0}$
Z-9-Octadecenoic (VI)	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	$C_{18:1;9Z}$
Z-9,Z-12-Octadecadienoic (VII	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$	$C_{18:2;9Z,12Z}$
Z-9,Z-12, Z-15-octadecatrienoic (VIII)	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7COOH$	$C_{18:3;9Z,12Z,15Z}$
12-hydroxy-9-octadecenoic (IX)	$CH_3(CH_2)_5CHOHCH_2CH=CH(CH_2)_7COOH$	$C_{18:1;9Z(12OH)}$
E-9-octadecenoic (X)	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	$C_{18:1;9E}$

Table 5 (continued)

NAME OF ACID	STRUCTURE	ABBREVIATION
Z-13-docosenoic (XI)	$CH_3(CH_2)_7CH=CH(CH_2)_{11}COOH$	$C_{22:1;13Z}$
Eicosanoic	$CH_3(CH_2)_{18}COOH$	$C_{20:0}$
Docosanoic	$CH_3(CH_2)_{20}COOH$	$C_{22:0}$
Tetracosanoic	$CH_3(CH_2)_{22}COOH$	$C_{24:0}$
Hexacosanoic	$CH_3(CH_2)_{24}COOH$	$C_{26:0}$
Octacosanoic	$CH_3(CH_2)_{26}COOH$	$C_{28:0}$
Triacontanoic	$CH_3(CH_2)_{28}COOH$	$C_{30:0}$
Z-9-Decenoic	$CH_2=CH(CH_2)_7COOH$	$C_{10:1;9Z}$
Z-9-Dodecenoic	$CH_3CH_2CH=CH(CH_2)_7COOH$	$C_{12:1;9Z}$
Z-9-Tetradecenoic	$CH_3(CH_2)_3CH=CH(CH_2)_7COOH$	$C_{14:1;9Z}$
Z-9-Eicosenoic	$CH_3(CH_2)_9CH=CH(CH_2)_7COOH$	$C_{20:1;9Z}$
Z-9-Hexacosenoic	$CH_3(CH_2)_{15}CH=CH(CH_2)_7COOH$	$C_{26:1;9Z}$
Z-5,Z-8,Z-11, Z-14-Eicosatetraenoic	$CH_3(CH_2)_4CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_3COOH$	$C_{20:4;5Z,8Z,11Z,14Z}$
Z-9,E-11,E-13-Octadecatrienoic	$CH_3(CH_2)_3CH=CHCH=CHCH=CH(CH_2)_7COOH$	$C_{18:3;9Z,11E,13E}$

Table 5(continued)

NAME OF ACID	STRUCTURE
12:13-Epoxy-Z-9-octadecenoic (XII)	$CH_3(CH_2)_4CH(O)CHCH_2CH=CH(CH_2)_7COOH$
E-9,10-Epoxy-octadecanoic (XIII)	$CH_3(CH_2)_7CH(O)CH(CH_2)_7COOH$
Z,Z-9:10,12:13-Diepoxyoctadecanoic (XIV)	$CH_3(CH_2)_4CH(O)CHCH_2CH(O)CH(CH_2)_7COOH$
Z-9:10-Epoxy-octadecanoic (XV)	$CH_3(CH_2)_7CH(O)CH(CH_2)_7COOH$
9,10-Epoxy-Z-12-octadecenoic	$CH_3(CH_2)_4CH=CHCH_2CH(O)CH(CH_2)_7COOH$
12:13-Epoxy-Z-12-octadecenoic	$CH_3(CH_2)_4C(O)=C(CH_2)_{10}COOH$
15:16-Epoxy-Z-9, Z-12-octadecadienoic	$CH_3CH_2CH(O)CHCH_2CH=CHCH_2CH=CH(CH_2)_7COOH$
C_{14}- Mycosanoic	$CH_3(CH_2)_{17}CHMeCH_2CHMeCOOH$
C_{27}- Phthienoic	$CH_3(CH_2)_{17}CHMeCH_2CHMeCH=CMeCOOH$
C_{32}- Mycocerosic	$CH_3(CH_2)_{19}CHMeCH_2CHMeCH_2CHMeCH_2CHMeCOOH$
Malvalic	$CH_3(CH_2)_7C(=CH_2)C(CH_2)_6COOH$
Sterculic	$CH_3(CH_2)_7C(=CH_2)C(CH_2)_7COOH$

≠ A fatty acid is designated by two numbers separated by a colon; the first number is the total number of carbon atoms in the fatty acid and the

second is the number of unsaturated centres. The positions and stereochemistry of unsaturation is given by additional numbers.

Source: Gunstone[19]

1.6 NATURAL SOURCES OF FATTY ACIDS

The chemical composition of glycerides has been investigated quite extensively. This has been done using chromatographic and non-chromatographic techniques. In chromatographic investigation, the most common technique has been the conversion of the triglycerides into the fatty acid methyl esters to make them ready for gas-liquid chromatography. Gunstone[19] has provided extensive tables showing the fatty acid composition of triglycerides of aquatic origin, some animal fats, milk fats and some vegetable fats, including those containing less common fatty acids. Table 6 shows the fatty acid composition of some seed fats and oils.

Table 6: Major Component Acids (%wt) of Some Vegetable Fats.

SEED	FATTY ACIDS PRESENT : $C_{16:0}$	$C_{18.0}$	$C_{18:1}$	$C_{18:2}$	OTHER ACIDS
Linseed	6	3	17	14	$C_{18:3}$(60%)
Candlenut	7	3	22	37	$C_{18:3}$(31%)
Rubberseed	11	10	25	33	$C_{18:3}$(21%)
Soya	12	4	24	51	$C_{18:3}$(9%)
Safflower	7	3	13	77	
Poppy	8	3	16	73	
Walnut	7	1	16	72	
Tobacco	10	4	14	71	
Niger	7	3	17	68	
Maize	13	2	31	54	
Cottonseed	28	3	18	51	
Sesame	8	4	45	41	
Groundnut	10	4	61	18	C_{20}-C_{24} acids (7%)
Cocoa butter	24	35	38	2	
Rape	2	1	35	18	$C_{18:3}$(10%)
					$C_{20:1}$ (10%)
					$C_{22:1}$(31%)
Palm Kernel	10	4	16	3	$C_{12:0}$(45%)
					$C_{14:0}$(17%)

Table 6 (Continued)

SEED	FATTY ACIDS PRESENT	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	OTHER ACIDS
Coconut		8	4	6	1	$C_{18:0}$(2%)
						$C_{10:0}$(10%)
						$C_{12:0}$(45%)
						$C_{14:0}$(18%)
Tung		5*	--	11	15	Eleostearic (69%)
Vernonia anthelmintica		5*	--	6	17	Vernolic (72%)
Castor		2*	--	3	4	Ricinoleic (89%)

Source: Gunstone[19]

*Total saturated acids.

Gunstone has noted that the composition of an animal fat depends on the type of animal, the kind of food it feeds on, its environmental conditions, age, sex, state of health, stage of life cycle and the part of the body the triglyceride comes from[19]. As for plant sources, he notes that botanically related species often produce similar oils.

Considerable research is currently underway to find new sources of triglycerides, since these compounds are renewable raw materials for industry and fuel use. Along with the triglycerides in plants, there are also other components like hydrocarbons, sterols, fatty alcohols, tannins, etc. All these components are generally known to be constituents of the "whole-plant oil fractions" obtained from plants by extracting the latter with acetone. In their work, Buchanan and Otey[20] of the U.S. Department of Agriculture, when trying to establish the feasibility of developing new multi-use oil-and hydrocarbon-producing crops, have identified about 300 plant species which might be introduced as potential new oil-and hydrocarbon-producing crops onto the U.S. agricultural scene. Carr, Phillips and Bagby[21] have reported the analysis of 96 species from Arizona as multipurpose whole-plant oil-bearing plants of arid or semi-arid environments. Of the species they analyzed, yields ranged from 0.7-6.6% oil

(oils here being considered to be a composition of wax esters, triglycerides, free fatty acids, sterols, fatty alcohols and resins), 1.9 - 29.4% polyphenol, less than 0.1 - 0.9% hydrocarbon and 3.1 - 35.0% protein. Out of the 96 species, 26 were reported to have had at least 3% whole-plant oil; 14 contained at least 4% oil and 12 contained at least 5% on a dry-weight basis. Screening of indigenous Kenya plants for their whole plant oil and hydrocarbon fraction has been carried out by Mengech and Kihumba[22] at the University of Nairobi.

Munavu[16] and colleagues at the University of Nairobi have had a screening programme whereby close to 100 African indigenous seeds have been analyzed for their oil content in attempts of finding new sources of edible or industrial oils. Promising results were obtained for seed oils which had an oil composition greater than 30%. Examples of these seeds are Alerites molucana (54%), Azidirachta indica (56%), Podocarpus gracilor (51%), Macadamia termifolia (70%), Calodendrum capense (60%) and Arecastrum remanzofianum (58%). Investigations on Calodendrum capense seed oil, showed that its fatty acid composition suggests that it can be used for the production of soaps[23]. This screening programme should be able to make Kenya and Africa at large to be less reliant on imported vegetable fats and oils.

1.7 METHODS OF OBTAINING FATTY ACIDS

Basically there are two sources of fatty acids, namely from synthetic routes and from natural product sources. Synthesis of fatty acids is quite complicated and it may actually be difficult to synthesize certain fatty acids. An example of one synthetic pathway is the procedure of Boughton, Bowman and Ames[24], who synthesized Z-9-tetradecenoic acid. The starting material which they used was 2-hydroxyhexanoic acid, which they converted into 2-acetoxyhexanoyl chloride. They then treated the latter compound with tribenzyl sodio-heptane-1:1:7-tricarboxylate to yield a product which was hydrogenated, decarboxylated and esterified, to give ethyl 10-hydroxy-9-ketotetradecanoate. Reduction of the latter compound over Raney Nickel W7, followed by hydrolysis, yielded mixed hydroxy-acids which were fractionally crystallised from ethanol and then ethanol-ethyl acetate, to give the less soluble erythro- and threo- 9:10-dihydroxytetradecanoic acid. Erythro-9:10-dihydroxytetradecanoic acid was reacted with hydrogen bromide-acetic acid-sulphuric acid to give ethyl threo-9:10-dibromotetradecanoate which was then converted by zinc into ethyl Z-9-tetradecenoate, which on being hydrolysed gave Z-9-tetradecenoic acid.

It remains true that since fatty acids do not exist in appreciable amounts as pure compounds in the natural sources, therefore if any particular fatty acid is wanted in the free state, some separation has to be done since its synthesis cannot be easily done. The techniques of isolation and separation of these fatty acids are generally classified into two, namely the chromatographic and non-chromatographic procedures. Gunstone has in great detail discussed the separation techniques[25]. A few of the non-chromatographic techniques will be outlined below but more details are emphasized on the chromatographic techniques.

1.8 NON-CHROMATOGRAPHIC METHODS OF SEPARATION OF FATTY ACID MIXTURES

Distillation Technique

The technique of distillation depends on the differences in boiling point of the samples under separation. The fact that various fatty acids have different chain lengths made it rather easy for the technique to have been used previously. The chain length contributes more to the differences in boiling point than the degree of saturation does. This technique of separation is among the oldest known by lipid chemists. In 1903, Kraft[26] and Kreiser and Hafner[27] reported that they obtained relatively pure hexadecanoic and octadecanoic acids by the process of fractional distillation. Later in 1906, Haller[28] reported the fractional distillation of the methyl esters derived from coconut oil. Bull, also in 1906, succeeded in isolating 9-eicosenoic acid by a process of fractional distillation of methyl esters prepared from cod-liver oil[29].

Low-Temperature Crystallization Method

It is generally true that the solubility of fatty acids of a homologous series in a polar solvent decreases with increasing chain length and increases with increasing unsaturation. Both the position

of double bonds and their configuration also affect solubility of the fatty acids. This knowledge has been applied to the isolation of fatty acids using the technique of low-temperature crystallization. Back in 1937 Brown and Stoner[30] described the low-temperature crystallization technique for the separation of predominantly saturated and unsaturated acids from a fraction of mixed acids of cottonseed oil. They made a solution of the mixed acids in acetone (1:10) which was cooled overnight at -20°C, after which they washed the crystallized acids with cold acetone. They demonstrated this technique by separating the unsaturated acids from saturated ones. Although not very common, this method for the separation of saturated acids has been used. Hansen and Cooke[31] succeeded in separating decanoic acid by two crystallizations from petroleum naphtha at -70°C and one each from methanol at room temperature and at -60°C, from a distillate fraction of methyl esters of mutton fat. At an earlier date, Brown and Shinowara[32] had succeeded in the preparation of pure Z-9-octadecenoic acid from the acids derived from olive oil. This they had achieved by a process of dissolving the mixed acids in acetone (1g/15.3 ml) and cooling them overnight at -20°C followed by separation of the crystalline fraction and cooling of the filtrate to -60°C. Separation of the crystalline fraction and recrystallization thrice from

acetone at -60°C and once from the same solvent at -35°C was further done. The acetone was then removed from the last filtrate fraction and the residue distilled at 15mm pressure yielding a product of 96% purity.

1.9 CHROMATOGRAPHIC METHODS OF SEPARATING FATTY ACIDS

Partition Chromatography

Partition chromatography is a type of chromatography which involves two immiscible liquids, one of which flows continuously past the second liquid which is held on an inert support. The liquid which flows is known as the mobile phase while the liquid coated on the inert support is referred to as the stationary phase. The components of a mixture being separated are distributed between the two immiscible solvents according to their partition coefficients. Partition chromatography as used in paper chromatography, thin-layer chromatography and gas liquid chromatography will be discussed in this section.

Paper Chromatography

This is a common application of using a reversed phase system (a system whose mobile phase is more polar than the stationary phase), and has been used for the separation of long-chain acids. The reversed phase chromatography use began with the work of Boldingh[33], who showed that by impregnating filter paper strips (Schleicher and Schull No.595)

with dilute vulcanised rubber (30% on a dry weight basis) which is a non-polar substance, the paper could be made to retain its porous nature, thus enabling the movement of the mobile phase by capillary force. The mobile phase used was methanol and a mixture of methanol acetone (1:1). Boldingh demonstrated the method on the separation of ethyl esters of the fatty acids I ($C_{12:0}$), II ($C_{14:0}$), III ($_{16:0}$), V ($C_{18:0}$), VI ($_{18:1}$) and XI ($C_{22:1}$) (Table 5). A solution of Sudan IV in 50% acetone/water was used as a locating agent. Viswanathan and Meera[34] succeeded in separating the fatty acids I, II, III, IV, V, VII and VIII, although the separation of the critical pair of the fatty acids III ($C_{16:0}$) and VI ($C_{18:1}$) was not very good. These scientists found 95% methanol to be a suitable solvent on 10% paraffin-impregnated Whatman No. 3 filter paper, especially when high temperatures of about 55°C were used for the chromatographic separation of fatty acids. Time of developing was found to be 75 minutes and the distance of development was 10 cm. They used a solution of 5-diphenyl carbazide in 95% ethanol as the locating agent. Critical pairs of higher fatty acids which are not easily separable by the usual methods of chromatography have been identified by Kaufmann[35] as hexadecanoic ($C_{16:0}$) and Z-9-octadecenoic ($C_{18:1}$), tetradecanoic ($C_{14:0}$) and Z-9, Z-12-octadecedienoic ($C_{18:2}$); and dodecanoic ($C_{12:0}$) and Z-9, Z-12, Z-15-octadecetrienoic acids ($C_{18:3}$).

The Critical pairs are said to occur when the decrease in chain length by two CH_2 groups is compensated by a double bond. Much work has been done in attempts of isolating the critical pairs, but not much progress has been acquired in paper chromatography.

Schlenk and colleagues[36] tried to separate the critical pairs listed in this section at -5°C, and although the three unsaturated fatty acids were well separated, the three saturated acids, though separated from their unsaturated partners, were not separated from each other. No doubt the temperatures must have affected the separations of the saturated acids from each other.

Thin-Layer Chromatography

The separation technique of thin-layer chromatography has been used since 1956, around the time it was developed by Stahl[37, 38]. It involves separation of mixtures by absorption chromatography on silica gel in the form of a thin layer coated on a glass plate or some other suitable support. The sample to be separated is applied as a solution on one spot on the plate and developed with a suitable solvent capable of differentiating all or most of the polarities of the mixture components. After elution of the chromatogram, the position of the components can be made visible by use of good locating

agents like the ones mentioned in the section on paper chromatography. For the unsaturated fatty acids, quick location can be done by exposure of the developed chromatograms to iodine vapour, a method which was developed by Whitehouse, Breisler and Staple[39]. The procedure can be used either qualitatively or quantitatively. For preparative purposes, 50 - 100 mg of silica gel per plate of 20 x 20 cm is normally used as reported by Gunstone[40].

Lindsay, Holman and Krister[41] had tried a separation of quite a number of unsaturated and epoxy fatty acids and their methyl esters. These fatty acids are IV, VI, IX, X, XII, XIII, XIV, XV, XVI, XVII and XVIII as shown in Table 5. They chromatographed a mixture of the pure, free fatty acids and also their methyl esters, using thin-layer silicic-acid plates. The solvent used for the esters was 10% ethyl ether in petroleum ether (b.p.35-45°) and for the acids, they used the same solvent but added to it 1% ethanoic acid. The chromatogram obtained was developed by heat after spraying with 50% sulphuric acid. The results of the chromatogram show that only those acids with a big difference in Rf values were separated, otherwise a good number of the components which had about the same Rf values showed no appreciable separation. One of the disadvantages of this technique as clearly brought out in this study is that compounds with close Rf values are not separable.

Gas-Liquid Chromatography

Gas-liquid chromatography (GLC) is another form of partition chromatography, differing from the previous techniques in that the mobile phase is a gas which should be stable and inert. The partition coefficients of the components of a mixture are affected both by the volatility of the component and its solubility in the stationary phase and as such, the retention time of each component depends on these two factors. For the analyses of the fatty acids by gas-liquid chromatography (GLC), the methyl derivatives of the acids are preferred to the pure fatty acids since the former are more volatile than the latter. This definitely allows a wider use of stationary phases which can be used at relatively lower temperatures without the stationary phases being volatile.

When a sample is injected, partitioning continues in the column, after which the various components of the sample go to the detector. The detector gives rise to an electrical impulse proportional to the concentration of the organic vapour in the carrier gas at each instant of time. This impulse is fed to the pen of a continuous recorder which draws a curve from which peaks may be analysed on the basis of standards.

Retention time helps identify the components and the area under the peak gives the concentration of the respective component relatively to the mixture injected.

Stationary phases used in gas-liquid chromatography do vary in polarity and as such, choice of the phase has been necessary. Markley[42] has recommended a stationary phase of low viscosity, although there is a limit depending on the sample being analysed. Markley has concluded that Apiezon and silicon grease, both of which are non-polar stationary phases, are not as efficient in the separation of fatty acids as polyester stationary phases which have a higher degree of polarity. A liquid polyester was first introduced by Orr and Callen[43] and since then, polyester stationary phases have been used in lipid chemistry for the chromatography of fatty acids.

Lipsky, Lovelock and Landowne[44] have analysed a known mixture of the methyl esters of saturated and unsaturated fatty acids extending from C_8 to C_{20}. They used a 200-foot stainless capillary column whose internal diameter was 0.010 inch and which was coated with Apiezon 'L'. The column was maintained at 240°C; the inlet pressure of the argon carrier gas was 0.68 atmospheres and the outlet flow rate maintained at 0.5 ml/min.

The sample was introduced into the column by means of a T-shaped glass bypass device maintained at 300°C. In their work, the separation for most of the fatty acid methyl esters was satisfactory except for the Z-9, Z-12-Octadecadienoic ($C_{18:2}$) and Z-9, Z-12, Z-15-Octadecatrienoic ($C_{18:3}$) acid methyl esters which were not separable.

At a later state,Hofstetter, Sen and Holman[45] tried to improve the separation of non-conjugated dienoic methyl or ethyl esters of the same chain length. They derived a table of equivalent chain length (ECL) values for methyl and ethyl esters of various unsaturated fatty acids on four different phases used as standards in the identification of unknown samples by gas-liquid chromatography (GLC). The liquid phases that they used were ethylene glycol succinate (EGS), diethylene glycol succinate (DEGS), β -cyclodextrin acetate (β -CDX-AC) and Apiezon L (approximate 0.84% double bond). The data they obtained has been tabulated by the authors, and it can be observed that the Apiezon L stationary phase was not so good in the separation of the fatty acid methyl and ethyl esters as the other stationary phases. Actually the ECL values of the methyl and ethyl esters of Z-9-octadecenoic:($C_{18:1}$) (VI), E-9-octadecenoic: ($C_{18:1}$) (X) and Z-9-octadecenoic: ($C_{18:1}$) (VI), Z-9, Z-12-octadecadienoic:($C_{18:2}$) (VII) fatty acid pairs obtained when

using the Apiezon L stationary phase implies that each component of each pair is not separable from the other one.

Although methyl esters have been given a lot of emphasis in gas chromatography, other derivatives have also been used so as to enhance detection of the fatty acids being analysed, as will be seen later on in section 1.14 .

Hintze, Roper and Gercken[46] managed to separate the benzyl ester derivatives of tetradecanoic ($C_{14:0}$), hexadecanoic ($C_{16:0}$), octadecanoic ($C_{18:0}$) and eicosanoic ($C_{20:0}$) fatty acids on a stationary phase, EGSS-X on Gas-Chrom Q and with temperature maintained at 210°C. They had synthesized these esters according to a procedure which had been outlined by Klemm, Hintze and Gercken[47]. The procedure involved adding a 1% (weight/volume) solution of the particular fatty acid to phenyldiazomethane in n-pentane at room temperature until the red colour of the phenyldiazomethane persisted. Esterification was observed to occur by the disappearance of the colour and the evolution of nitrogen. Then after 10 - 15 min., the excess of phenyldiazomethane was destroyed by adding a 1% ethereal phosphoric acid solution, and without further purifications, 0.5 - 1.0 µl of the decoloured solution was injected into the

gas-liquid chromatograph.

Separation of the straight-chain C_1-C_8 carboxylic acid anilides has been achieved on a 12-ft. 2.5% sodium dodecylbenzenesulphonate (DBS) on chromosorb G column by Umeh[48].

The experiment was performed on a Perkin Elmer model Fll gas chromatograph equipped with dual flame ionisation detector, and nitrogen was used as the carrier gas. The derivatives had been made by reacting aniline and each of the carboxylic acids together.

Umeh[49] has also done gas chromatogaphic work on the p-bromophenacyl and p-phenylphenacyl ester derivatives of C_2 - C_{10} straight-chain carboxylic acids. Separations on a Perkin-Elmer model Fll gas chromatograph equipped with dual flame ionisation detector with nitrogen serving as the carrier gas, were good. The p-phenylphenacyl and p-bromophenacyl esters were respectively made according to the following equations.

$$p\text{-}C_6H_5.C_6H_4.COCH_2Br + RCOOK \rightarrow p\text{-}C_6H_5.C_6C_4.CO.CH_2.OOCR + KBr$$

$$p\text{-}Br.C_6H_4.CO.CH_2Br + RCOOK \rightarrow pBr.C_6H_4.CO.CH_2.OOCR + KBr$$

The injection in either of the cases into the instrument of clear solution was done after it had been dried. Details on how the clear solution was obtained have not been given.

One of the possible problems which may be encountered when using gas chromatography (GC) may be the analysis of samples whose stability at elevated temperatures may not be guaranteed. Some epoxide fatty acids may possibly have a ring opening and also some unsaturated fatty acids may have a possiblity of re-arrangement at the elevated temperatures.

1.10. TYPES OF HIGH-PRESSURE LIQUID CHROMATOGRAPHY SYSTEMS

The types of high-pressure liquid chromatography (HPLC) discussed in this section differ from one another mainly due to the stationary and mobile phases used in each case.

Normal Phase Chromatography

The name 'normal phase' chromatography is used to denote the use of stationary phases having higher polarity than that of the mobile phases. Non-polar organic solvents are usually employed as mobile phases, although in some cases, chloroform, ethanol or even aqueous acetonitrile have been reported [50] as eluants in normal phase chromatography. The retention time for each component in a mixture depends on the interaction between the polar groups of each component with the stationary phase. This implies that in the normal phase type of chromatography retention increases with the polarity of each component and decreases with increasing mobile phase. The explanation for this is that a polar component interacts for a bigger period with the polar stationary phase than a less polar component would, primarily due to polar interactions. With a stationary phase taken as a constant, a polar solvent would

tend to sweep polar components out of the column faster than would a less polar mobile phase, due to the stronger interactions of the components with the polar mobile phase. The polarity of the stationary phase explains why normal phase chromatography is well suited for applications for the separation of polar groups. Polar bonded phases are made from silica gel by covalent attachment of suitable polar organic groups to the surface of the former[51]. Glyco-phases are examples of weakly polar bonded phases having glycerol type of functional group at the surface. The surface of such stationary phases is covered with an abundance of hydroxyl groups. Bonded phases of intermediate polarity usually have nitrile functions such as cyanoethyl groups covalently bound to the surface. Bonded phases containing alkylamine functions such as γ-aminopropyl groups at the silica surface are examples of highly polar bonded phases, frequently used with non-polar organic solvents or hydro-organic mixtures as the mobile phase.

Reverse Phase Chromatography

Unlike in normal phase chromatography where the stationary phase has a higher polarity than the mobile phase, reverse phase chromatography uses a stationary phase whose polarity is lower than that of the mobile phase. Plain-aqueous, mixed-

aqueous and non-aqueous mobile phases can be employed in this technique, depending on the type of components to be chromatographed. Chromatographic selectivity of the appropriate mobile phase is normally done by exploring a special solvent and observing its effects on the chromatogram. Depending on the results obtained while using one solvent, a mixture of two or three solvents can be tried from which the best composition of the mobile phase can be selected. A predominantly hydro-organic solvent such as water-methanol may also be started with and the concentration of either the organic or inorganic component increased to try and identify the appropriate conditions. Just like in normal phase chromatography, retention time and selectivity in reverse phase chromatography is determined by interaction of the components with the stationary phase, although the nature of the mobile phase also matters. The retention time decreases with the polarity of the component being eluted and increases with increasing mobile phase polarity. The non-polar bonded phases used in reverse phase chromatography contain an alkyl moiety bound to the silica. Octyl- and octadecyl-silica are the most commonly used but stationary phases with shorter chains for instance dimethylsilane are also used. The mobile phases used have mainly been aqueous methanol or acetonitrile or methanol-acetonitrile mixtures. Of late also, a mixture of methanol, water and acetonitrile has been used as a mobile phase.

1.11 DETECTORS FOR HPLC

A liquid chromatographic detector is a device for continuously locating the presence of solutes in a mobile phase coming from a chromatographic column. The electrical signal from the detector is then recorded on a chart recorder. This section describes some types of detectors used in high-pressure liquid chromatography (HPLC).

Ultraviolet (UV) Absorbance Detectors

The theory behind the UV absorbance detectors is that when a species is subjected to radiation of a certain wavelength, it goes to a higher energy electronic level, implying that an electron has been changed from a lower to a higher energy level. The amount of energy a species requires to take it to an excited state depends on its electronic configuration. A bonding electron in a species would be held tightly to the latter implying that a very high energy would be required to excite the species. On the other hand, the non-bonding and π-electrons need less energy for excitation.

UV detectors that are used are either of fixed wavelength type or have variable wavelength. The latter type of detectors

are advantageous in that the wavelength can be monitored so that it coincides with an absorption maximum of the sample of interest.

A deuterium lamp which provides a continuous band covering the range 180 - 400 nm is usually used in the variable wavelength type of detector[52]. If operation in the visible region is required, tungsten lamps of range 400 - 700 nm are used.

The light absorbance of a solution in a cell of fixed length is directly proportional to the concentration of the absorbing species. Absorbance is obtained by use of the Beer-Lambert Law which states that:

$$\text{Absorbance} = \log_{10} \frac{I_o}{I} = abc$$

where a = molar extinction coefficient,

b = path length of the cell,

c = concentration of the absorbing species,

I_o = intensity of the incident light and

I = intensity of the transmitted light.

Fluorescence Detectors

As has been mentioned in the section on UV detectors, many compounds are capable of undergoing electronic excitation when irradiated with sufficient energy of the appropriate wavelength. Once they are excited, the molecules emit a radiation either instantaneously or after some delay. The radiation it emits is said to be of higher energy than the one it is radiated with. This makes it possible to detect the radiation emitted, normally after filtering off the incident radiation.

The light source in many fluorimeters is a xenon arc which provides radiation over the UV range of interest, although other lamps emitting intense emission lines at a single wavelength are also applied. The Grating monochromators are the most commonly used light filters, although cut-off filters are also used. Fluorescence detection is not as common as UV detection, since few compounds display quantum yields of the required magnitude.

Mass Spectrometric Detectors

Unlike in the other types of detectors discussed, mass spectrometic detectors demand a small flow rate of a magnitude

of a few μl min^{-1} as observed by McFadden[53] . For this to be possible, it demands microbore columns which produce flow rate of a few μl min^{-1}. Alternatives to this have been reviewed by McFadden[53] and Arpino and Guichon[54] . The alternative is of developing interfacing devices which permit sample enrichment by evaporation of the solvent before the eluate enters the mass spectrometer. With this alternative the usual HPLC flow rate of about 1 ml min^{-1} is taken care of. One major advantage of using a mass spectrometer as a detector is that it is possible to obtain a spectral identification of a specific compound. Two disadvantages of the interfacing devices is the fact that there are difficulties encountered with polar solvents and the fact that salts must be absent from the mobile phase. The technique of mass spectrometric detection is very expensive and this limits its use to some extent.

Refractive Index Detectors

Refractive index detectors have been in use far longer than any other form of HPLC detectors. They are also said to be the cheapest form of detection. Three types of differential refractometers are available and these are the Fresnel principle type, deflection principle and interferometer principle types,

all of which have been adequately discussed by Simpson[55]. They require thermostatic control during operation, since all of them are temperature-sensitive. In spite of the fact that refractive index is a universal detector, the type of response that can be achieved is very dependent upon the choice of solvent. It should be a type of solvent whose refractive index should be different from any of the components in the mixture being chromatographed, otherwise should it be the same, then no peak would be obtained for that particular component. This principle has been demonstrated by Wheals[52], who has reported that nonane with refractive index 1.410, could not be detected in tetrahydrofuran due to the fact that the tetrahydrofuran has the same refractive index. However if another mobile phase like benzene (refractive index 1.501) had been used, then nonane would have been detected, implying that the proper choice of solvent as the mobile phase is vital.

Electrochemical Detectors

This form of detection is based on the electrochemical reaction of a solute in a cell designed for maximum electrochemical response. As has been reported[52], it is only those compounds which can undergo an oxidation or a reduction reaction at the electrode surface of the cell that are conveniently detectable.

As such, it is expected that the compound of interest be electrochemically active and the eluant should also be capable of conducting a current. The component being detected is made to flow through a cell to which a potential is applied. This potential generates a background current from the eluant and the current increases if the solute undergoes oxidation or reduction as it passes through the detector. The cell design, chemical properties of the solute, the concentration of the solute, the applied potential and the composition of the eluant are the factors which determine the magnitude of the response.

Density Detector

This detector is the most recent one in HPLC field reported by Trathnigg and Jorde[56] . Its principle is based on measurement of change of eluant concentration. The density change d caused by a concentration C_1 of eluted substance is given by:

$$d = (1 - d_o.V_i^*).\ C_i = m_i/V_i.a_i$$

where d_o is the density of eluant,

V_i^* is the apparent specific volume of eluted substance (w/v),

m_i is the mass of eluted substance in the volume V_i and

a_i is the response factor.

It is noted that the more the amount of a component reaching the detector, the higher the density and hence the larger the peak area, implying the density detector is both qualitative and quantitative.

1. 12 HIGH-PRESSURE LIQUID CHROMATOGRAPHY (HPLC) OF UNSATURATED FATTY ACIDS

A mixture of isomers of underivatized C_{20} fatty acids namely 5,8,11,14,17-eicosapentaenoic ($C_{20:5}$); 8,11,14,17-eicosatetraenoic ($C_{20:4}$); 8,11,14-eicosatrienoic ($C_{20:3}$); 11,14,17-eicosatrienoic ($C_{20:3}$); 11,17-eicosadienoic ($C_{20:2}$); and 11-eicosaenoic ($C_{20:1}$) acids have been separated on a high pressure Waters Assoc. liquid chromatograph model 202, equipped with two model 6000 A reciprocating pumps by Batta and his colleagues [57]. Detection was done with a model 450 variable wavelength UV detector set to monitor absorbance at 214 nm. The column used was 100 x 8 mm I.D. Radial - Pak C_{18} cartridge attached to a guard column (49 x 4.6 mm I.D.) dry-packed with C_{18} Corasil reversed-phase material. A methanol-water-acetic acid mixture (89:11:0.2) was used as the mobile phase with a flow rate of 1 ml/min. The separations obtained were adequate except for the 8,11,14-eicosatrienoic acid ($C_{20:3}$) which was not well separated from 11,14,17-eicosatrienoic acid ($C_{20:3}$). The authors, however, managed to separate these positional isomers in a separate chromatography which used a higher percentage of water. Here, the mobile phase was methanol-water-acetic acid mixture (80:20:0.2) at a flow rate of 2 ml/min.

It is observed that although high-pressure liquid chromatography offers a high resolution for the separation of a complicated mixture of fatty acids, their detection is not so easy because they generally show neither strong absorption nor fluorescence in the ultraviolet or visible region, which is widely used as the most sensitive detection system for HPLC. The absorption near 200 nm has been adopted for the detection of non-derivatised fatty acids. However such detection is neither sensitive nor selective. Furthermore, this method is markedly influenced by the properties of the mobile phase. This is a disadvantage for effective application of gradient elution technique to HPLC separation.

1. 13 DERIVATISATION OF FATTY ACIDS FOR CHROMATOGRAPHIC ANALYSIS

The need for derivatising fatty acids arises from the nature of detectors that are used for chromatographic analysis. The fluorescence and absorbance detectors have become popular in the field of lipid chemistry and unlike the refractive index detectors, which do not require chromophores in the samples being analysed, the fluorescence and absorbance detectors do. Since very few naturally-occuring fatty acids possess chromophores which absorb above 220 nm according to a report given by Hitoshi[58] , it is necessary to derivatise the acids so as to increase the ultraviolet (UV) radiation absorption properties of the compounds under investigation and as such enhance the sensitivity of detection. In addition to their function in increasing the sensitivity of detection, derivatising agents may be used to change the chromatographic character of compounds, as a result of which selectivity of homologous compounds is increased, thus implying an improved resolution of the related compounds. Also, derivatisation may be done to enhance the detection of trace amounts at nanogram levels of compounds, due to the fact that the ultraviolet (UV) detectors which are commonly used can detect the presence of a compound whose concentration is much lower than that at which other

detectors like the refractive index can operate.

Derivatives Used in High-Pressure Liquid Chromatography (HPLC)

Quite a number of derivatising agents have been employed in high-pressure liquid chromatography (HPLC) work. When these compounds are reacted with fatty acids, they give derivatives which are easily detected. Some of these derivatives have been listed below in Figure 1.

Figure 1 : Some Fatty Acid Derivatives Employed in High-Pressure Liquid Chromatography (HPLC). R in Each Case is the Alkyl of a Fatty Acid.

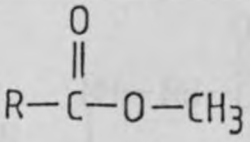
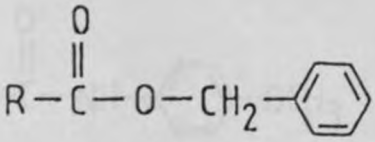
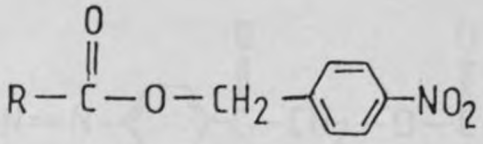
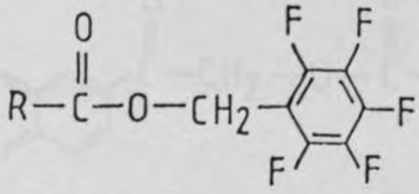
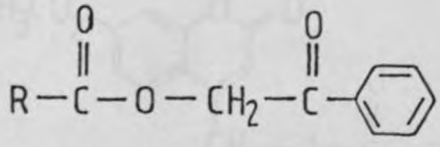
NAME	STRUCTURE AND DESIGNATION
Methyl esters	 (A)
Benzyl esters	 (B)
p-Nitrobenzyl esters	 (C)
Pentafluorobenzyl esters	 (D)
Phenacyl esters	 (E)

Fig. 1 (Continued)

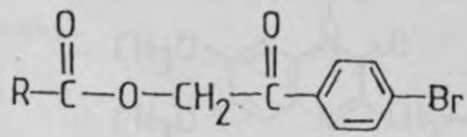
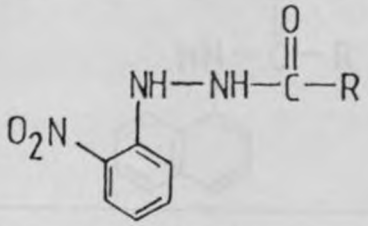
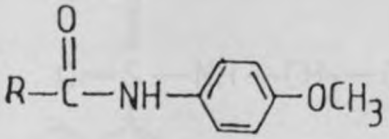
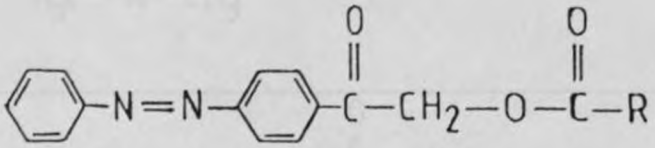
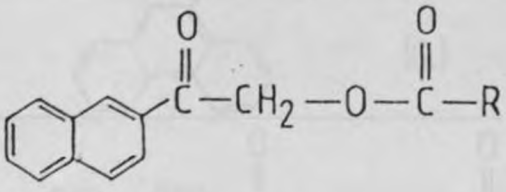
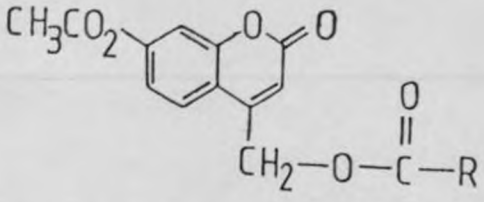
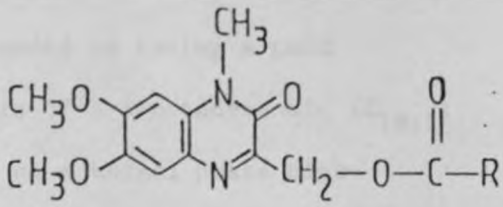
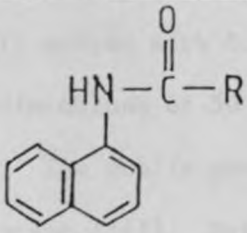
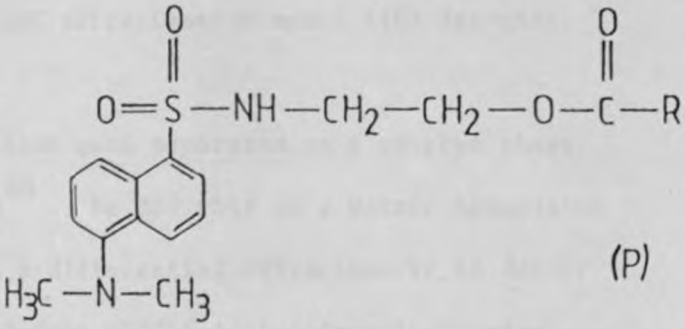
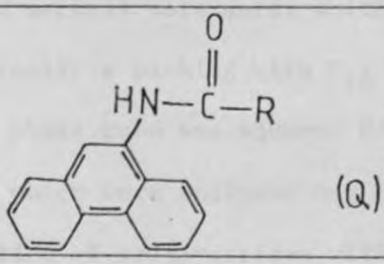
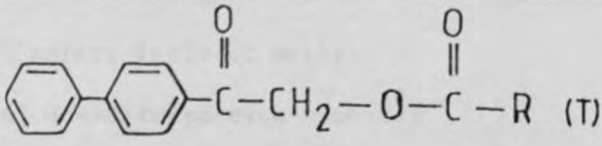
NAME	STRUCTURE AND DESIGNATION
p-Bromophenacyl esters	 (F)
2-Nitrophenylhydrazides	 (G)
p-Methoxyanilides	 (H)
p-Phenylazophenacyl esters	 (J)
2-Naphthacyl esters	 (K)
4-Methyl-7-acetoxycoumaryl esters	 (L)

Fig. 1 (Continued)

NAME	STRUCTURE AND DESIGNATION
3-Methyl-6,7-dimethoxy-1-methyl 2(1H)-quinoxalinone esters	 (M)
1-Naphthylamides	 (N)
5-Dimethylamino-1-naphthalenesulphonyl ethanolamine esters	 (P)
9-Amidophenanthrenes	 (Q)
p-Phenylphenacyl esters	 (T)

Fatty acid methyl ester derivatives (A) have been chromatographed using high-pressure liquid chromatographic equipment. Mikes, Schurig and Gil-av[59], succeeded in having a good separation between the methyl esters of Z-9-octadecenoic ($C_{18:1}$) and E-9-octadecenoic ($C_{18:1}$) acids on a normal phase high-pressure liquid chromatography (HPLC). The column that they used had been packed with Corasil II coated with 0.8% $AgNO_3$-1.75% ethylene glycol and had the dimensions of 50 cm length and 2.1 mm internal diamter (I.D.). The mobile phase used was a mixture of n-hexane and n-heptane (1:1). Detection was done with the help of an LDC refractometer model 1103 detector.

Methyl esters have also been separated on a reverse phase HPLC system by Scholfield[60]. He did this on a Waters Associates ALC - 202 instrument with a differential refractometer to detect the methyl esters. Two 2-foot x 5/16 inch internal diameter stainless steel columns (fixed in series) were used, which were packed with Bondapak C_{18}/Porasil, a packing with C_{18} hydrocarbon bonded to silica. Mobile phase used was aqueous 85% acetonitrile. The methyl esters which were analysed had been obtained through transesterification of triglycerides with sodium methoxide catalyst. The esters included methyl derivatives of the saturated and unsaturated even-numbered fatty acids from C_{12} - C_{18}. The doubly and triply Z-unsaturated

C_{18} fatty acid ester derivatives were also contained in the mixture. Although there was a satisfactory separation between the fatty acid methyl derivatives of the hexadecanoic ($C_{16:0}$)-Z-9-octadecenoic ($C_{18:1}$) and tetradecanoic ($C_{14:0}$)-Z-9-hexadecenoic ($C_{16:1}$) acids, the Z-9 , Z-12-octadecadienoic ($C_{18:2}$)-Z-9-hexadecenoic ($C_{16:1}$) fatty acids were not separated. Also in the same chromatography, the authors were not able to separate the ester derivatives of dodecanoic ($C_{12:0}$), Z-9-tetradecenoic ($C_{14:1}$) and Z-9, Z-12, Z-15-octadecatrienoic ($C_{18:3}$) fatty acids.

The fatty acids found in milk have been analysed as their methyl esters by Christie, Connor and Noble [61] on a column consisting of a 5 cm x 5 mm I.D. guard column and a 25 cm x 5 mm I.D. main column packed with Lichrosorb 10RP 18 in a model 8770 isocratic high-pressure liquid chromatography pump equipped with a Knauer differential refractometer for detection in accordance with a standard solution.

The even-saturated C_4 - C_{18} fatty acids, Z-9-hexadecenoic ($C_{16:1}$) and Z-9-octadecenoic ($C_{18:1}$) fatty acid methyl derivatives were found to have been present in the milk sample that they were analysing. A good separation was obtained except for the Z-9-hexadecenoic ($C_{16:1}$) and tetradecanoic ($C_{14:0}$) fatty acid methyl ester derivatives. An acetonitrile-water mixture

(95:5) was used as the mobile phase at a flow rate of 1 ml/min. The methyl esters had been prepared by firstly dissolving milk fat (20 mg) in acetonitrile (1 ml) with slight warming, then adding to this solution, methyl acetate (25 µl) and 1M potassium methoxide in methanol (50 ml) and then leaving the resulting solution for 5 min before stopping this reaction by the addition of acetic acid (6 µl). The aliquot after being cleaned by centrifugation was ready for injection. No yields were determined.

A mixture of methyl esters of saturated C_{14} - C_{20}; monoenoic C_{16} - C_{20}, C_{24}, C_{26}, and C_{30}; dienoic $C_{18:2}$ and $C_{18:3}$ fatty acids whose stereochemistry of double bonds had not been indicated were chromatographed by Rezanka and Podojil[62] on an HPLC system using SP 8000 apparatus on a 50 cm x 6 mm I.D. column (Separon SIC1, Laboratory Instruments, Czechoslovakia). The mobile phase linear gradient (2 ml/min) ran from a mixture of methanol-water (50:50) into methanol (30 min) and further methanol (60 min). Detection was done with a UV detector set at 210 nm. The methyl esters had been synthesized by means of boron trifluoride-methanol.

Although most of the peaks were well resolved, those of $C_{16:3}$, $C_{15:1}$ were not separated at all. Other methyl ester derivatives which were not resolved well were the sets $C_{17:1}$, $C_{18:2}$, $C_{16:0}$; $C_{18:1}$, $C_{17:0}$; $C_{19:1}$, $C_{20:1}$; and $C_{23:0}$, $C_{24:1}$. The stereochemistry of the double bonds had not been indicated.

The p-Methoxyanilides (H) of fatty acids have also been analysed with high-pressure liquid chromatography by Hoffman and Liao [63]. They prepared these derivatives by converting the fatty acid to an acyl chloride and without isolating it, they heated the chloride solution with p-methoxyaniline to yield the required derivatives. The equation for the reaction is given as:

$$R-\overset{O}{\overset{\|}{C}}-Cl + 2p\text{-}CH_3OC_6H_4NH_2 \rightarrow p\text{-}CH_3OC_6H_4NHCOR + p\text{-}CH_3OC_6H_4NH_3Cl$$

The p-methoxyanilide formed was not separated further, but was injected in the solution in which it was prepared into a model ALC 202 liquid chromatograph with a UV detector which was operated at 254 nm. The column used was of μ-Bondapak C_{18} make. The Chromatograph was operated with a gradient elution

which was started at 100% water and continued to 100% organic solvent (acetonitrile or methanol) within 40 minutes.

One of the methods for making the acyl chloride used triphenylphosphine as shown in the equation below.

$$R\text{-}COOH + (C_6H_5)_3P + CCl_4 \rightarrow RCOCl + (C_6H_5)_3PO + CHCl_3$$

The derivatives chromatographed were of the fatty acids hexanoic ($C_{6:0}$), octanoic ($C_{8:0}$), decanoic ($C_{10:0}$), dodecanoic ($C_{12:0}$), tetradecanoic ($C_{14:0}$) pentadecanoic ($C_{15:0}$), hexadecanoic ($C_{16:0}$), heptadecanoic ($C_{17:0}$), octadecanoic ($C_{18:0}$), eicosanoic ($C_{20:0}$), docosanoic ($C_{22:0}$), tetracosanoic ($C_{24:0}$), Z-9-hexadecenoic ($C_{16:1}$), Z-9-octadecenoic ($C_{18:1}$), Z-9 , Z-12, Z-15-octadecatrienoic ($C_{18:3}$), Z-9 , Z-12-octadecadienoic ($C_{18:2}$), Z-13-docosenoic ($C_{22:1}$), Z-14-tetracosenoic ($C_{24:1}$), 4,7,10,13-eicosetetraenoic ($C_{20:4}$) and 4,7,10,13,16,19-docosehexaenoic ($C_{22:6}$).

It is noted that, in the water-acetonitrile gradient elution, the set of $C_{14:0}$ and $C_{22:6}$ and of $C_{20:4}$ and $C_{16:1}$ appeared as one peak. Resolved poorly in the same gradient elution were the pairs $C_{22:0}$ and $C_{22:1}$; $C_{18:1}$ and $C_{16:0}$; and

$C_{15:0}$ and $C_{18:2}$.

In the water-methanol gradient elution, the fatty acid derivatives in the sets $C_{22:0}$ and $C_{24:1}$; $C_{20:0}$ and $C_{22:1}$; $C_{14:0}$ and $C_{18:3}$; and $C_{15:0}$, $C_{16:1}$ and $C_{20:4}$ appeared as one peak. These results imply that for a given degree of saturation, retention time increases with molecular weight and also for a given alkyl chain length, for instance C_{18}, an increase in unsaturation decreases retention time significantly. As a consequence of this unsaturation/saturation phenomena, some peaks are poorly resolved or not resolved at all. More chromatographic work has continued to determine the appropriate conditions for separation of such a complicated mixture.

Benzyl ester derivatives (B) of fatty acids have also been chromatographed before. Christie, Connor and Noble[61] have already analysed benzyl esters of fatty acids found in milk on a column consisting of a 5 cm x 5 mm I.D. guard column and a 25 cm x 5 mm I.D. main column paked with Li Chrosorb 10RP 18 in a Model 8770 isocratic high-pressure liquid chromatography pump equipped with a Knauer differential refractometer for detection in accordance with a standard solution. The benzyl ester derivatives used were of the fatty acids separated

by these same authors when analysing methyl ester derivatives of milk as discussed earlier in this same section. The derivatives had been synthesized in a similar manner as those of the previously mentioned methyl ester derivatives, except instead of using methyl acetate, benzyl acetate was used. The same column and detector were also used. The flow rate was initially 1 ml/min which was later changed to 2 ml/min.

The resolution obtained was adequate but for the Z-9-hexadecenoic ($C_{16:1}$) and tetradecanoic ($C_{14:0}$) benzyl esters, which were resolved poorly.

A mixture of 2,3,4,5,6-pentafluorobenzyl ester derivatives (D) of octadecanoic ($C_{18:0}$), Z-9-octadecenoic ($C_{18:1}$), Z-9, Z-12-octadecadienoic ($C_{18:2}$) and Z-9, Z-12, Z-15-octadecatrienoic ($C_{18:3}$) fatty acids have been resolved successfully on a normal-phase high pressure liquid chromatograph with a μ Porasil semi-preparative column by Netting and Duffield [64]. The mobile phase used was a mixture of dry dichloromethane and hexane half saturated with water (3:17). The authors used an M440 detector with filters for 254 nm for detection. This was despite the fact that these derivatives have a λ_{max} at 263 nm. Although the separation of the above mentioned derivatives was good, there were about 4 interference peaks in the chromatogram implying

that analysis of a complex mixture of the derivatives may have been interfered with.

On attempts at separating the pentafluorobenzyl esters of saturated C_2, C_3, C_4, C_6, C_8, C_{10}, C_{12}, C_{14} and C_{18} fatty acids, the authors did not get a good resolution, in addition to the fact that the interference peaks were still there. These derivatives that were analysed chromatographically were synthesized using 2,3,4,5,6-pentafluorobenzyl bromide. A fatty acid sample (1 mg) was dissolved in dichloromethane (1 ml) and 1 ml of a solution containing 0.1 mmol of tetrabutylammonium hydrogen-sulphate and 0.2 mmol of sodium hydroxide was added. Pentafluoro-benzyl bromide (20 µl) was then added, and the mixture shaken vigorously at room temperature for 30 min and the dicholoromethane phase was evaporated.

The same authors mentioned above, namely Netting and Duffield managed to separate the pentafluorobenzyl ester derivatives of even saturated C_{14}-C_{22} fatty acids successively obtained from a barley leaf extract at a later date [65]. The authors had synthesized these derivatives according to the procedure described above [64], with the only difference being that samples for injection had been dissolved in isopropanol.

Identification of the esters was done by comparison with standards by gas chromatography (GC) (EGSS-X on Chromosorb W-HP, 100-120 mesh columns:1.5m x 4 mm at 180°C and a flow rate of 20 ml/min; 5.5 m x 4 mm at 200°C and a flow rate of 20 ml/min). The effluent gas was divided: 24 parts to a flame -ionization detector and 1 part to an electron-capture detector (Pye GCV). In some cases these identifications were confirmed by gas chromatography - mass spectrometry (GC-MS). Separations were carried out by reversed phase HPLC on a C_{18} μ Bondapak semi-preparative (300 mm x 7.8 mm, partical size 10 μm) column (Waters Assoc.) with methanol-water mixture (19:1). The detector used had a filter for 254 nm, just like in their previous work.

2-Naphthacyl bromide has also been identified as a good derivatising agent of fatty acids and yields derivatives (K) with a λ_{max} of 247-248 nm. This has been shown by Cooper and Anders[66], when they derivatised among other acids, Z-9-octadecenoic ($C_{18:1;9Z}$), Z-9 , Z-12-octadecadienoic ($C_{18:2;9Z,12Z}$), γ - (6,9,12-) and α - (Z-9, Z-12, Z-15-) octadecatrienoic ($C_{18:3}$) fatty acids and chromatographed them using a 3 ft x 0.07 in internal diameter stainless steel tube packed with Cosasil-C_{18}. Detection was made with the help of a Pharmacia 1205 UV monitor set at 254 nm. The mobile phase used was a mixture of methanol and water at

a proportion of 85:15 and was set at a flow rate of 0.2 ml min^{-1}.

The authors had made each of 2-naphthacyl ester derivatives by first dissolving the acid, 2-naphthacyl bromide and N,N-diisopropylethylamine in dimethylformamide (DMF) according to the right proportions. Secondly, they heated the reaction mixture at 60°C for 10 minutes at which the reaction was said to have been complete. Without any isolation, an aliquot of the reaction mixture was injected into the chromatograph. Percentage yield of reaction was not given.

The chromatogram obtained had a good resolution for the derivatives of Z-9-octadecenoic ($C_{18:1}$) and Z-9, Z-12-octadecadienoic ($C_{18:2}$) acids. However, a separation for the 2-naphthacyl ester derivatives of γ-(6,9,12-) and α-(Z-9, Z-12, Z-15-) octadecatrienoic ($C_{18:3}$) acids was not obtained. The unreacted derivatising agent gave no problems since it elutes earlier than the 2-naphthacyl ester derivatives analysed.

Phenacyl esters (E) have also been used in reverse phase high-pressure liquid chromatography by Engelhardt and Elgass[67] in their work of attempting to separate the even fatty acids between C_6 and C_{22}. They prepared the derivatives by firstly

mixing about 200 mg of the fatty acids, 4 ml of a 5% solution of triethylamine in acetone and 6 ml of a solution of 5g of recrystallized ω -bromoacetophenone in 150 ml of acetone. After a reaction time of at least 30 min, 5µl of this solution were injected into the column whose dimensions were 30 cm x 4.2 mm i.d., packed with a stationary phase SI 100 C_{18} RP.

The phenacyl ester derivatives of the even saturated fatty acids from C_6 - C_{22} were analysed using two gradient elution programs. The first one involved a methanol/water gradient with starting eluant 70% methanol in water, graduating to 100% methanol. The second one involved acetonitrile/water gradient-programmed to start with 70% acetonitrile in water increasing to 100% acetonitrile. The chromatogram showed a good resolution between all the derivatives considered, with acetonitrile/water gradient elution components having relatively lower retention times than methanol/water gradient elution program.

On the same column, the authors tried to isolate the phenacyl ester derivatives of the saturated even-numbered fatty acids from C_6 to C_{18} and of the unsaturated C_{18} fatty acids namely, Z-9-octadecenoic ($C_{18:1}$), E-9-octadecenoic ($C_{18:1}$), Z-9,Z-12-octadecadienoic ($C_{18:2}$) and Z-9 , Z-12, Z-15-octadecatrienoic ($C_{18:3}$) acids with an eluant starting from 50%

acetonitrile in water, increasing to 100% acetonitrile. Resolution was generally satisfactory except for the phenacyl ester derivatives of hexadecanoic ($C_{16:0}$), Z-9-octadecenoic ($C_{18:1}$) and E-9-octadecenoic ($C_{18:1}$) acids; the latter two derivatives did not separate at all.

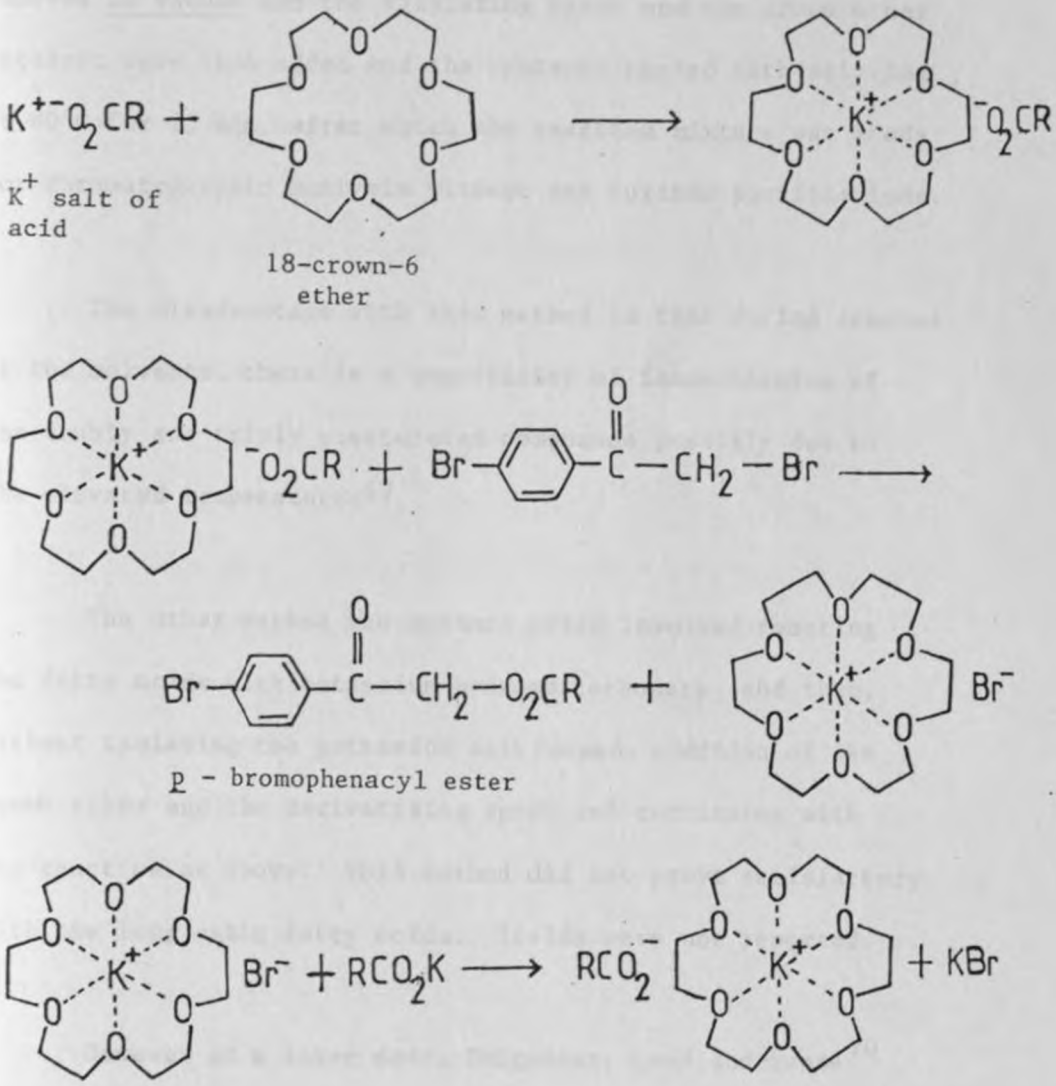
p-Bromophenacyl fatty acid ester derivates (F) have also been used in high-pressure liquid chromatography (HPLC). Durst and co-workers[68] have done the reverse phase high-pressure liquid chromatography of the bromophenacyl ester derivatives of octadecanoic ($C_{18:0}$); Z-9-octadecenoic ($C_{18:1}$); Z-9, Z-12-octadecadienoic ($C_{18:2}$); Z-9, Z-12, Z-15-octadecatrienoic ($C_{18:3}$); dodecanoic ($C_{12:0}$) and eicosanoic ($C_{20:0}$) acids. Detection was provided by an LDC UV detector with an 8 µl cell at 254 nm. The column, 25 cm x 4 mm i.d. stainless steel, had been dry-packed with a reverse-phase packing which consisted of a bonded nonyl group (C_9) to Corasil II. Temperature of the column was maintained at 40°C.

In one of the experimental set-ups, the authors could not accomplish a separation of Z-9, Z-12-octadecadienoic ($C_{18:2}$) acid derivative from Z-9, Z-12, Z-15-octadecatrienoic ($C_{18:3}$) acid derivative; there was also a poor resolution between the p-bromophenacyl ester derivatives of octadecanoic ($C_{18:0}$) and Z-9-octa-

decenoic ($C_{18:1}$) fatty acids. The eluant they used in this particular case was a mixture of methanol (62.5%) and water (37.5%). In this particular case, they had chromatographed only the four fatty acid ester derivatives mentioned.

Another chromatogram they obtained while using a mixture of methanol (75%) and water (25%) as the eluant involved the p-bromophenacyl derivatives of dodecanoic ($C_{12:0}$), octadecanoic ($C_{18:0}$), Z-9-octadecenoic ($C_{18:1}$), Z-9 , Z-12, Z-15-octadecatrienoic ($C_{18:3}$) and eicosanoic ($C_{20:0}$) acids. The chromatogram indicated that the components in this case had been fairly well resolved, implying that the mobile phase had an effect on the chromatography. The drawback in this second chromatography was that they did not include the Z-9, Z-12-octadecadienoic ($C_{18:2}$) p-bromophenacyl ester derivative.

The methods of synthesis used for these derivatives is as given in the equations below[68].



The potassium salt of the fatty acid (RCO_2^- K^+) in each case had been obtained by dissolving the acid in either methanol or water and neutralising it to the phenolphthalein end point with alcoholic KOH solution. The solvent was then

removed in vaccuo and the alkylating agent and the crown ether catalyst were then added and the contents heated with stirring at 80°C for 15 min, after which the reaction mixture was ready for chromatographic analysis without any further purifications.

The disadvantage with this method is that during removal of the solvents, there is a possibility of isomerization of the doubly and triply unsaturated compounds possibly due to the elevated temperatures[69].

The other method the authors tried involved reacting the fatty acids with potassium hydrogencarbonate and then, without isolating the potassium salt formed, addition of the crown ether and the derivatizing agent and continuing with the reaction as above. This method did not prove satisfactory with the long chain fatty acids. Yields were not reported.

However at a later date, Halgunset, Lund and Sunde[70] managed to resolve quite efficiently the p-bromophenacyl esters of Z-9-hexadecenoic ($C_{16:1}$), octadecanoic ($C_{18:0}$), Z-9-octadecenoic ($C_{18:1}$), Z-9, Z-12-octadecadienoic ($C_{18:2}$), Z-9, Z-12, Z-15-octadecatrienoic ($C_{18:3}$) and other fatty acids. They used a mixture of methanol-acetonitrile-water (82:9:9) as the eluant. A good separation was not obtained in the case of the use of methanol-

water or with acetonitrile-water eluants. Column conditions used were: column 49 cm x 4.6 mm guard column dry packed with 40 - μm pellicular reversed phase material (Pelliguard LC - 18) and a 250 x 4.6 mm Supelcosil LC-18 analytical column. Flow rate was maintained at 1.0 ml/min with a pressure of ca 1000 psi. A Consta Metric Spectromonitor - III (LDC) variable-wavelength UV detector set at 254 nm was used for detection.

The synthesis of p-bromophenacyl esters of the fatty acids used in this chromatography was done as described by Jordi[71]. The author did not state the yields.

A mixture of p-bromophenacyl esters of even saturated fatty acids, Z-9-octadecenoic ($C_{18:1}$), Z-9, Z-12-octadecadienoic ($C_{18:2}$) and Z-9, Z-12, Z-15-octadecatrienoic ($C_{18:3}$) fatty acids were well separated as reported by Chaytor[72]. These esters had been obtained by reacting each fatty acid with p-bromophenacyl bromide. The chromatogram obtained was used to analyse the fatty acid composition of samples of butter, melon oil and of shrimp oil. The procedure which was adopted for obtaining these samples is as follows: Fat (50 mg) was first dissolved in 75 ml dichloromethane-methanol mixture (50:25). 0.27M methanolic potassium hydroxide (10 μl) was added to the above solution

(100 µl) and the resulting mixture was shaken and heated for 20 minutes at 80°C. After removing the solvent in a stream of air, 50 µl reagent (0.1 mM p-bromophenacyl bromide and 0.005 mM 18-crown-6/ml in acetonitrile) and 150 µl acetonitrile were added and the mixture heated at 80°C for 30 minutes. 5 microlitres of the aliquot were injected.

The HPLC column used was an Ultrasphere-Octyl column (25 x 0.64 cm). Attempts at using Ultrasphere-ODS column (15 x 0.46 cm) did not work well since the hexadecanoic ($C_{16:0}$) and Z-9-octadecenoic ($C_{18:1}$) p-bromophenacyl esters did not resolve. The detector used was of dual wavelength detection (model 165 detector) with wavelength set at either 214 or 254 nm. The eluant used was a gradient system of an acetonitrile-water mixture programmed as follows: an acetonitrile-water mixture (80:20) flowed at 1 ml/min for 1 minute after which, at the beginning of the 2nd minute, the eluant was changed to 100% acetonitrile over a period of 15 minutes followed by a flow rate change to 2 ml/min over five minutes at the beginning of 21st minute. The flow rate and acetonitrile-water eluant were then changed again to 1 ml/min and 80:20 respectively over a period of 3 minutes at the beginning of the 31st minute.

Miwa and colleagues[73] succeeded in obtaining a good separation of a synthetic mixture of fifteen 2-nitrophenylhydrazides (G) of decanoic ($C_{10:0}$); dodecanoic ($C_{12:0}$); tetradecanoic ($C_{14:0}$); Z-9-tetradecenoic ($C_{14:1}$); hexadecanoic ($C_{16:0}$); Z-9-hexadecenoic ($C_{16:1}$); heptadecanoic ($C_{17:0}$); octadecanoic ($C_{18:0}$); Z-9-octadecenoic ($C_{18:1}$); Z-9, Z-12-octadecadienoic ($C_{18:2}$); Z-9, Z-12, Z-15-octadecatrienoic ($C_{18:3}$); dihomo-γ-linolenic ($C_{20:3}$); 5,8,11,14-eicosatetraenoic ($C_{20:4}$); eicosapentaenoic ($C_{20:5}$) and docosahexanoic ($C_{22:6}$) fatty acid derivatives on a YMC-C8 reversed phase column, 250 x 4.6 mm I.D. maintained at 30°C. This analysis was done using a Shimadzu Model LC-5A liquid chromatograph equipped with a Shimadzu Model SPD-6AV variable-wavelength visible-UV detector, which was set at 400 nm.

The derivatives had been made by adding aqueous 2-nitrophenylhydrazine HCl solution and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride to an ethanolic mixture of the fatty acids and these were heated at 60°C for 20 min. Potassium hydroxide solution in right amount was then added and further heating of the reaction mixture at 60°C for 15 min was done. The mobile phase was an acetonitrile-water mixture (85:15) at a flow rate of 1.2 ml/min. these derivatives had a λ_{max} of 230 nm despite the fact that they were analysed at 400 nm.

This may have had the effect of reducing the lower limit of detection.

The p-phenylazophenacyl ester derivatives (J) of saturated C_{10} - C_{20} acids, excluding the $C_{19:0}$ acid, have been successfully separated with a modular apparatus equipped with an ultraviolet and visible detector of variable wavelength (Biotronic, Model 3030, fixed at 330 nm) by Vioque, Maza and Millan[74]. A C_{18} reversed-phase column containing Bio-Sil ODS, 5 µm (250 x 4 mm, Bio-Rad), Rheodyne injector (20 µl) and a precolumn containing ODS-5S (Bio-Rad) was used. The mobile phase in this analysis was an acetonitrile-water mixture (98:2) flowing at 1ml/min.

In a different chromatography, they also managed to separate Z-9-octadecenoic ($C_{18:1}$), Z-9, Z-12-octadecadienoic ($C_{18:2}$) and Z-9, Z-12, Z-15-octadecatrienoic ($C_{18:3}$) fatty acid derivatives using the same conditions used above. They also separated Z-9-octadecenoic ($C_{18:1}$) from E-9-octadecenoic ($C_{18:1}$) fatty acid derivative in a different chromatography using the same conditions as above.

Derivatisation of the fatty acids was done by reacting 5 - 10 mg fatty acid with 1 ml volume of a 5% triethylamine

solution in acetone and 1.5 ml of acetone containing the equivalent amount of derivatising agent. The mixture was shaken for 30 min. The aliquot on purification and appropriate dilution was injected. No yields were stated.

4-Bromomethyl-7-acetoxycoumarin has been reacted with the C_4 - C_{20} even saturated fatty acids (excluding $C_{14:0}$) to yield ester derivatives (L) which were analysed with reverse-phase high-pressure liquid chromatography on an ODS-6013 (10 μm) column, 250 x 2.1 mm I.D. maintained at 50°C. This work was done by Tsuchiya, Hayashi, Naruse and Takagi[75]. They did the chromatography under gradient elution [aqueous acetonitrile solution 40% (0) - 90% (99)] and detected by a fluorescence spectrofluorimeter model FP - 110 (excitation 365 nm, emission 460 nm). The authors made the derivatives by reacting each fatty acid with 4-bromomethyl-7-acetoxycoumarin in the presence of dibenzo-18-crown, potassium hydrogencarbonate and sodium sulphate for 30 min at 50°C in the dark. After cooling, an aliquot was injected into the column.

At a later date similar derivatives (L) of saturated C_{12} - C_{18} (except $C_{15:0}$), $C_{14:1}$, $C_{16:1}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ and $C_{20:4}$ fatty acids were separated sufficiently by Tsuchiya and colleagues[76] on a Lichrosorb RP-18 column at a column temperature

of 40°C using as the mobile phase firstly a methanol-acetonitrile-water mixture (35:35:30) and secondly a 90% aqueous methanol solution with which a gradient elution was programmed. Flow rate of the eluant was set at 1.2 ml/min and the detection was done by a spectrofluorometer (excitation 365 nm, emission 460 nm). These derivatives had been synthesized in a similar way to the method described here before in the synthesis using 4-bromomethyl-7-acetoxycoumarin.

Yamaguchi and colleagues[77] reacted 3-bromomethyl-6, 7-dimethoxy-1-methyl-2(IH)-quinoxalinone with the even C_4 - C_{20}, C_3, C_5, and C_{17} saturated fatty acids in the presence of 18-crown-6 phase transfer catalyst to give the ester derivatives (M) which they analysed chromatographically on a Hitachi 665 high-performance liquid chromatograph equipped with Hitachi F 1000 flourescence spectrometer equipped with 12 - µl flow cell operating at excitation 370nm and emission of 450nm. A mixture of the fatty acid derivatives when chromatographed gave a good separation. The column which was used was a Radial-Pak C_{18} cartridge (100 x 4 mm I.D.) maintained at a temperature of 20 - 27°C. Gradient elution with 57 - 100% aqueous methanol was carried out using a Hitachi 833A solvent-gradient device, with a flow rate of 2 ml/min. Details of the synthesis of the derivatives and the gradient elution program were not given.

1-Naphthylamide derivatives (N) of tetradecanoic ($C_{14:0}$), hexadecanoic ($C_{16:0}$), Z-9-hexadecenoic ($C_{16:1}$), heptadecanoic ($C_{17:0}$), octadecanoic ($C_{18:0}$), Z-9-octadecenoic ($C_{18:1}$) and Z-9, Z-12-octadecadienoic ($C_{18:2}$) fatty acid mixtures have been chromatographed on a Hitachi high-pressure liquid chromatograph Model 635A equipped with a Hitachi multiwavelength UV monitor set at 280 nm by Ikenda, Shimada and Sakaguchi[78] . They used a μ Bondapak C_{18}(30 x 0.4 cm I.D.) column whose temperature was maintained at 40°C. A methanol-water mixture (81:19) was used as the mobile phase, with a flow rate of 2 ml/min. The resolution of the derivatives was good except for that between $C_{18:1}$ and $C_{16:0}$.

The synthesis of the derivatives involved shaking sufficiently a particular fatty acid (2-1000mmoles dissolved in 0.6 ml of benzene) and 0.6 ml of 2% oxalyl chloride solution in benzene at a temperature maintained at 70°C for 30 min, at the end of which the solvent was removed at a reduced pressure. Into the fatty acid chloride, a 1-naphthylamine solution (made by dissolving the calculated amount of 1-naphthylamine in benzene) and a triethylamine solution in benzene were added and the mixture was reacted at 30°C for 15 min to give the 1-naphthylamide derivatives, whose yields were said to have

been close to 100%.

Ryan and Honeyman [79] have obtained a good separation of 5-dimethylamino-1-naphthalene sulphonylethanolamine derivatives (P) of hexadecanoic ($C_{16:0}$), Z-9-hexadecenoic ($C_{16:1}$), heptadecanoic ($C_{17:0}$), octadecanoic ($C_{18:0}$), Z-9-octadecenoic ($C_{18:1}$), E9-octadecenoic ($C_{18:1}$), Z-9, Z-12, Z-15-octadecatrienoic ($C_{18:3}$) and 5,8,11,14-eicosatetraenoic ($C_{20:4}$) fatty acids done on a Varian Model 5000 high-pressure liquid chromatograph using a Varian fluorochrome for detection. Excitation was at 360 nm and emission was above 420 nm which is at the visible wavelength of light. The analysis was carried out on a 250 x 4.6 nm I.D. Ultrasphere ODS reversed phase column. Acetonitrile-methanol-20 mM aqueous silver nitrate (45:45:10) was used as the mobile phase at a flow rate of 2 ml/min. The synthesis of the derivatives was accomplished by leaving the reaction of the solution resulting from combining 4 mg fatty acid and 3 mg dicyclohexyl carbodiimide with 120 µl 5-dimethylamino-1-naphthalene sulphonylethanolamine solution in chloroform to continue overnight at room temperature with protection from the light. On purification of aliquot, it was ready for chromatographic analysis. No yields were stated.

Ikenda, Shimada, Sakaguchi and Matsumoto[80] have already achieved a fair separation of 9-amidophenanthrene derivatives (Q) of a tetradecanoic ($C_{14:0}$), hexadecanoic ($C_{16:0}$), Z-9-hexadecenoic ($C_{16:1}$), heptadecanoic ($C_{17:0}$), octadecanoic ($C_{18:0}$), Z-9-octadecenoic ($C_{18:1}$), Z-9, Z-12-octadecadienoic ($C_{18:2}$) and 5,8,11,14-eicosatetraenoic ($C_{20:4}$) fatty acid mixture on a Hitachi high-pressure liquid chromatograph Model 635 A equipped with a JASCO FP-110 spectrofluorometer and a Hitachi multiwavelength UV monitor. The column used was a μBondpak C_{18}(30 x 0.4 cm I.D.), and fluorescence detection was done with the excitation wavelength set at 303 nm and the emission wavelength at 376 nm. Methanol-acetonitrile-water mixture (53:27:20) was used as the mobile phase, with a flow rate of 2.0 ml/min. However, the sets of peaks $C_{14:0}$, $C_{16:1}$, $C_{18:2}$ and $C_{20:4}$ did not resolve very well. The procedure used in the synthesis of these derivatives was similar to the procedure employed in the synthesis of 1-naphthylamide derivatives, a technique which has already been discussed[78]. The last step is the only one which was unique since in this case, the mixture of triethylamine, 9-aminophenanthrene and the fatty acid chloride solution was allowed to react at 70°C for 45 min. Also the benzene-removing step was done under a stream of nitrogen gas.

1.14 THE USE OF p-PHENYLPHENACYL ESTERS AS DERIVATIVES FOR FATTY ACID ANALYSIS BY HPLC

P-Phenyphenacyl esters have been analysed chromatographically, both in gas chromatography and in liquid chromatography. In gas chromatography, Umeh[49] chromatographed the p-phenylphenacyl ester derivatives of C_2 - C_{10} straight-chain carboxylic acids. He used a Perkin-Elmer model F11 gas chromatograph equipped with dual flame ionisation detector with nitrogen serving as the carrier gas. The separation for each derivative was satisfactory. The author prepared each derivative by first saponifying the fatty acid with KOH after which p-phenylphenacyl bromide (the derivatising agent) was added and the reaction carried out. The reaction can be represented by the equation:-

$$p\text{-}C_6H_5C_6H_4CO.CH_2Br + RCOOK \rightarrow P\text{-}C_6H_5C_6H_4.CO.CH_2.OOCR + KBr$$

A clear solution of the reaction mixture was injected into the chromatograph without further purifications. No yields were reported.

The p-phenylphenacyl ester derivatives of the saturated even C_{12} - C_{22} fatty acids have been separated by Mengech and

Kihumba[81] . This was done by reverse phase high-pressure liquid chromatography (HPLC) on a Beckman chromatograph equipped with two model 110A pumps, an Altex model 420 system controller programmer and a Beckman model 210 sample injector valve. Detection was done by the use of Hitachi model 100-40 UV-vis spectrophotometer with an UV-Vis variable wavelength detector, operated at a wavelength of 286 nm. The eluant used was 100% methanol, at a flow rate of 2 ml/min through a 25 cm x 4.6 mm i.d. Altex Ultrasphere - ODS column. The chart speed was 0.5 cm per min using a Kipp and Zonen BD 41 recorder. A good resolution was obtained.

These p-phenylphenacyl ester derivatives of the saturated even C_{12} - C_{22} acids were synthesized by the method of Umeh[49] which has already been discussed in this section. A weighed amount (0.00125 moles) of fatty acid was dissolved in absolute ethanol (10 ml) to which water (5 ml) was added. Into the solution, a drop of phenolphthalein indicator was added and then neutralised with aqueous 5 M potassium hydroxide. The solution which was then made slightly acidic to phenolphthalein, was added to 0.00125 moles of p-phenylphenacyl bromide and then refluxed for a maximum of 2 hrs with temperature maintained at 60°C. Unlike Umeh[49] who did not isolate the derivatives

from the aliquot, the author did. He then determined the percentage yields which averaged 78.66%.

Attempts to extend this method of separation to the separation of the unsaturated p-phenylphenacyl ester derivatives of C_{18} acids failed in that the Z-9-octadecenoic ($C_{18:1}$) and Z-9, Z-12-octadecadienoic ($C_{18:2}$) p-phenylphenacyl derivatives were not resolved.

This method for the synthesis of p-phenylphenacyl ester derivatives avoided the problem mentioned by Durst [68], since isomerization may be possible in the latter case in the solvent removal step as discussed in section 1.13.

The p-phenylphenacyl esters showed a λ_{max} at 286 nm and therefore the variable wavelength detector was set at 286 nm [81]. Of the derivatives discussed in section 1.13, the p-phenylphenacyl ones have among the higher λ_{max} values (greater than 254 nm).

The melting point of the p-phenylphenacyl fatty acid ester derivatives are relatively higher than those of related derivatives reported above. A comparison of three of these derivatives has been done for three fatty acids as shown in Table 7.

Table 7. Melting Points of a Few p-phenylphenacyl Esters Compared with those of Related Esters.

Acid	Phenacyl ester m.p. (°C)	p-bromophenacyl ester m.p. (°C)	p-phenyl-phenacyl ester m.p. (°C)
dodecanoic ($C_{12:0}$)	48.9	76.0	84
octadecanoic ($C_{18:0}$)	69	90	91
Z-9-octadecenoic ($C_{18:1,9Z}$)	oil	oil	60.5

The original objectives of this study were threefold, namely

(i) to devise an efficient method for the synthesis in high yield of the p-phenylphenacyl esters of the common saturated and unsaturated fatty acids found in nature,

(ii) to determine the optimum conditions for the separation of these derivatives by HPLC using UV-detection, with special attention to the critical pairs, and

(iii) to apply this method in the determination of the fatty acid composition of samples of natural fats and oils. By so doing, the application of the HPLC technique to the analysis of natural products will be extended, and some of the inherent disadvantages of GC analysis, such as possible isomerisation at the site of the double bonds and difficulties in post-column recovery of volatile derivatives, will be overcome.

The present research work has included the synthesis of the p-phenylphenacyl esters of I($C_{12:0}$), II($C_{14:0}$), III($C_{16:0}$), IV($C_{16:1;9Z}$), V($C_{18:0}$), VI($C_{18:1;9Z}$), X($C_{18:1;9E}$), IX($C_{18:1;9Z,12OH}$), VII($C_{18:2;9Z,12Z}$), VIII($C_{18:3;9Z,12Z,15Z}$) and XI($C_{22:1;13Z}$) fatty acids. The p-phenylphenacyl esters of I($C_{12:0}$), II($C_{14:0}$), III($C_{16:0}$) and V($C_{18:0}$) fatty acids had been prepared by Mengech and Kihumba[81] using a method that has already been discussed in this section.

The p-phenylphenacyl ester derivative of IV($C_{16:1;9Z}$) has been synthesized before from smaller fragments by Boughton, Bowman and Ames[24]. The synthesis was carried out by first dibrominating the erythro-9, 10-dihydroxyhexadecanoic acid to yield a dibromo-acid, which was esterified, debrominated and distilled giving Z-9-hexadecenoic acid ($C_{16:1}$) which formed a p-phenylphenacyl ester with melting point 56.5-57° from acetone. Further details of this reaction have not been given. The yield was not stated, furthermore.

The p-phenylphenacyl ester derivative of VI($C_{18:1;9Z}$) has been synthesized by Drake and Bronitsky[82]. This was done by reacting the sodium salt of VI($C_{18:1;9Z}$) with p-phenylphenacyl bromide as shown by the equation below.

$$RCO_2Na + C_6H_5C_6H_5C_6H_4COCH_2Br \rightarrow C_6H_5C_6H_4COCH_2OCOR + NaBr$$

The p-phenylphenacyl derivatives of X ($C_{18:1,9E}$), IX($C_{18:1,9Z,12OH}$), VII ($C_{18:2;9Z,12Z}$), VIII ($C_{18:3;9Z,12Z,15Z}$) and XI ($C_{22:1;13Z}$) have not been reported prepared and as such they are new compounds.

These compounds (I - XI) have been analysed by reverse phase high-pressure liquid chromatography and the relationship of retention time with respect to increasing gram molecular mass among the saturated derivatives and increase in degree of unsaturation among the C_{18} acids determined. This has been done in a separation under isocratic elution conditions. Gradient elution of the mixture has also been done through compositions of methanol, acetonitrile and water.

The method has been applied to samples of castor and linseed oils.

CHAPTER TWO

EXPERIMENTAL

PART A: SYNTHESIS OF THE p-PHENYLPHENACYL ESTERS OF SOME COMMON FATTY ACIDS.

2A - 1. REAGENTS AND CHEMICALS

All pure samples of dodecanoic acid (lauric acid) (I), tetradecanoic acid (myristic acid) (II), hexadecanoic acid (palmitic acid) (III), Z-9-hexadecenoic acid (palmitoleic acid) (IV), octadecanoic acid (stearic acid) (V), Z-9, Z-12-octadecadienoic acid (linoleic acid) (VII), Z-9, Z-12, Z-15-octadecatrienoic acid (linolenic acid) (VIII), 12-hydroxy-Z-9-octadecenoic acid (ricinoleic acid) (IX), E-9-octadecenoic acid (elaidic acid) (X) and Z-13-docosenoic acid (erucic acid) (XI) were obtained commercially from Sigma Chemical Company Limited. They were of known structure and of purity better than 99%. Z-9-octadecenoic acid (VI) was a laboratory-grade reagent. The esterifying reagent,

p-phenylphenacyl bromide, was a laboratory grade reagent, and was recrystallized from absolute ethanol to a constant melting point (125-126°C)[83] before use. The tetra-n-butylammonium hydrogensulphate (crystalline) was obtained from Sigma Chemical Company, and was of purity above 99%. Ethyl ethanoate (ethyl acetate), dichloromethane, absolute ethanol and absolute methanol used were of analytical grade and were distilled before use. Hydrochloric 5M and 1M aqueous solutions were made from the concentrated acid and standardized with potassium hydroxide, using phenolphthalein indicator. Castor oil and linseed oil were purchased locally from the shops in Nairobi. The castor oil had been labelled as "pure", while the linseed oil as "raw".

2A - 2. INSTRUMENTATION

The mass spectrometry was performed by electron impact on a VG 12-250 Analytical spectrometer, at a scan time of 1 sec. at 70eV; detection was done over the mass range of 0-500.

All nuclear magnetic resonance spectroscopy was performed on a Perkin Elmer 90 MHz model except for derivatives of $C_{18:2;9Z,12Z}$ and $C_{18:3;9Z,12Z,15Z}$ which was done on a Perkin Elmer 60 MHz instrument. A 2-6 % solution of each ester was made in deuterated chloroform, using tetramethylsilane as the internal standard. Infrared spectra were run on a Perkin Elmer 598 double-beam spectrometer calibrated with styrene. Potassium bromide discs were prepared by mixing the p-phenylphenacyl ester with analytical-grade KBr in the ratio of 1:6 - 1:8 by weight. The ultraviolet (UV) spectra were run on a Perkin Elmer UV/Vis Lambda 3 instrument in dichloromethane solution.

2A - 3. GENERAL DERIVATISATION PROCEDURES

Two different methods were used, as described below, for the preparation of the p-phenylphenacyl esters of the fatty acids.

2A - 3.1. Method A - Preparation Using Phase-Transfer Catalysts

A weighed amount (0.00125 moles) of the fatty

acid was dissolved in 35 ml of dichloromethane in a 100 ml round-bottomed flask. To this solution 5.4×10^{-3} moles (0.1834g) of tetrabutylammonium hydrogensulphate was added, followed by 0.00179 moles (0.1004g) of potassium hydroxide dissolved in 6 ml of distilled water, after which two immiscible layers formed.

A weighed amount of p-phenylphenacyl bromide, 0.00125 moles (0.3439g) was then added to the flask, and the mixture stirred for a maximum of one and a half hours at room temperature (20°C). The course of the reaction was followed by thin layer chromatography on Kieselgel 60G brand of silica gel using cyclohexane: acetone (8:2) as eluant. In most cases, the reaction was complete within 30 minutes. The organic layer was then separated from the aqueous layer and the latter extracted with a total of 30 ml of dichloromethane in 6 ml portions. The organic extracts were in turn combined with the original dichloromethane layer from the mixture, and then dried over anhydrous magnesium sulphate. The dichloromethane was removed in vacuuo at 30°C to yield the crude product. Two recrystallizations of the crude

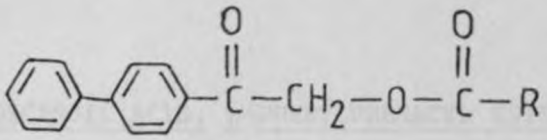
esters yielded derivatives of constant melting point. Recrystallization of the p-phenylphenacyl esters was done from either methanol or absolute ethanol, depending on the polarity of the ester being prepared. Two recrystallizations were required before the purity of each derivative was confirmed by thin layer chromatography on ultraviolet active silica gel and elution with cyclohexane-acetone (8:2). The structures of the p-phenylphenacyl esters prepared by this method are found in Figure 2 except for the ester derivatives of compounds VIII and XI, which were prepared only by method B, described below.

2A - 3.2 Method B - Preparation Using Simple Displacement

Just as in method A, 0.00125 moles of the fatty acid were weighed and dissolved in absolute ethanol (15 ml). Phenolphthalein (3 drops) was added, followed by addition of aqueous 5 M potassium hydroxide solution until the solution appeared pink. The excess potassium hydroxide was then neutralised with aqueous 1M hydrochloric acid until the pink colour was just discharged.

Into the resulting solution 0.00125 moles (0.3439g) p-phenylphenacyl bromide was added and then the flask stirred at 60°C for a period of at least two hours or until TLC showed the reaction to have been complete. The p-phenylphenacyl esters were extracted from the reaction mixture with ethyl acetate (60 mls) in 5x12 ml portions, and the organic layer dried over anhydrous magnesium sulphate. Reduction of the solvent *in vacuuo* at 50°C followed until about 5 ml remained, after which shiny white crystals formed on cooling in an ice-salt mixture. The resulting crude p-phenylphenacyl esters were recrystallized three times from either absolute ethanol or from methanol, depending on the polarity of the ester, to yield a constant melting-point product. This method of analysis resembles the one that Kihumba[81] had used for his synthesis of p-phenylphenacyl esters, as mentioned in the discussion. Each of the resulting p-phenylphenacyl esters was subjected to mass spectrometry, ultraviolet and infrared spectroscopy and to nuclear magnetic resonance spectrometry. The esters prepared by this method are shown in Figure 2.

Figure 2. The Synthesized p-Phenylphenacyl Esters of Some Common Fatty Acids.



(T)

R =	Compound Number	Trivial Name of Parent Acid
$CH_3(CH_2)_{10}-$	XIX	Lauric
$CH_3(CH_2)_{12}-$	XX	Myristic
$CH_3(CH_2)_{14}-$	XXI	Palmitic
$CH_3(CH_2)_5\overset{Z}{(CH)}_2(CH_2)_7-$	XXII	Palmitoleic
$CH_3(CH_2)_{16}-$	XXIII	Stearic
$CH_3(CH_2)_7\overset{Z}{(CH)}_2(CH_2)_7-$	XXIV	Oleic
$CH_3(CH_2)_7\overset{E}{(CH)}_2(CH_2)_7-$	XXV	Elaidic
$CH_3(CH_2)_4\overset{Z}{(CH)}_2CH_2\overset{Z}{(CH)}_2(CH_2)_7-$	XXVI	Linoleic
$CH_3CH_2\overset{Z}{(CH)}_2CH_2\overset{Z}{(CH)}_2CH_2\overset{Z}{(CH)}_2(CH_2)_7-$	XXVII	Linolenic
$CH_3(CH_2)_5CHOHCH_2\overset{Z}{(CH)}_2(CH_2)_7-$	XXVIII	Ricinoleic
$CH_3(CH_2)_7\overset{Z}{(CH)}_2(CH_2)_{11}-$	XXIX	Erucic

2A - 4. DODECANOIC ACID, p-PHENYLPHENACYL ESTER (XIX)

The title compound was prepared using both of the general methods outlined above. The product obtained from method A was recrystallized twice and from method B thrice, each from absolute ethanol, to yield from method A 0.42g (85.27%) of ester and from method B, 0.39g (79.18%). Plate-like white crystals were obtained with m.p. 83.0 - 85.0°C (lit. m.p. 84°)[84]; λ_{max} 286nm; ν_{max} 3040, 1740, 1690, 1595 cm^{-1}; τ ca 1.95 - 2.72 (9H, m, aromatic), 4.65 (2H, s, $-COCH_2O-$), 7.5 (2H, t, $-CH_2-CO-$), 8.7 (18H, m, $-(CH_2)_9-$) and 9.1 (3H, $-CH_3$); M^+ at 394.2965 (1%). A table of significant fragments obtained by mass spectroscopy is below (Table 8).

Table 8. Significant MS Fragments of p-phenylphenacyl Ester of Dodecanoic Acid. (XIX).

POSTULATED FRAGMENT	OBSERVED FRAGMENT (m/e)	CALCULATED ≠ (m/e)	RELATIVE ABUNDANCE
$C_6H_5C_6H_4CO^+$	181.2171	181.0654	100%
$C_6H_5C_6H_4^+$	153.2519	153.0705	11.2%
$C_6H_5C_6H_4COCH_3^+$	196.1654	196.0889	3.1%
$CH_3(CH_2)_4^+$	71.2404	71.0861	3.3%
$CH_3(CH_2)_5^+$	85.1672	85.1018	1.6%
$CH_3(CH_2)_{10}CO^+$	183.2569	183.1750	1.7%
$CH_3(CH_2)_{10}COOH_2^+$	213.1328	213.1856	1.6%
M^+	394.2965	394.2509	1.0%

≠ C = 12.00000, H = 1.00783 and O = 15.9949

2A - 5. TETRADECANOIC ACID, p-PHENYLPHENACYL ESTER (XX)

Compound XX was prepared using both of the methods outlined above. Two recrystallizations of the product from method A and three from method B gave plate-like white crystals whose yield was 0.4679 (88.70%) and 0.4232 (80.22%) respectively. The ester had m.p. 89 - 90.5°C (lit. m.p. 90°C)[84]; λ_{max} 286 nm; ν_{max} 3040, 1740, 1690, 1595 cm^{-1}; τ_{ca} 1.95 - 2.75 (9H, m, aromatic), 4.65 (2H, s, $-COCH_2O-$), 7.5 (2H, t, $-CH_2-CO-$), 8.72 (22H, m, $-(CH_2)_{11}-$) and 9.2 (3H, t, $-CH_3$); $M^+ + 1$ at 423.3349 (0.2%). A table of significant fragments obtained by mass spectroscopy is below (Table 9).

Table 9. Significant MS Fragments of p-Phenylphenacyl Ester of Tetradecanoic Acid. (XX).

POSTULATED FRAGMENT	OBSERVED FRAGMENT (m/e)	CALCULATED ≠ (m/e)	RELATIVE ABUNDANCE
$C_6H_5C_6H_4CO^+$	181.2619	181.0654	100%
$C_6H_5C_6H_4^+$	153.2190	153.0705	10.2%
$CH_3(CH_2)_4^+$	71.1807	71.0861	5.2%
$CH_3(CH_2)_5^+$	85.1012	85.1018	2.2%
$CH_3(CH_2)_8^+$	127.1758	127.1488	1.5%
$CH_3(CH_2)_{12}^+$	183.1483	183.2114	1.1%
$CH_3(CH_2)_{12}CO^+$	211.2401	211.2063	1.3%
$M^+ + 1$	423.3349	423.2822	0.2%

≠ C = 12.00000, H = 1.00783 and O = 15.9949

2A - 6. HEXADECANOIC ACID, p-PHENYLPHENACYL ESTER (XXI).

This ester was prepared using both of the methods described above. Method A yielded 0.5018g (89.21%) and method B gave 0.4708 (83.70%) after recrystallization as described in section 2A - 3. White, plate-like crystals resulted of m.p. 91 - 92°C (lit. m.p. 91°C)[84]; λ_{max} 286 nm; ν_{max} 3040, 1740, 1690, 1595 cm^{-1}; τ_{ca} 1.96 - 2.6 (9H, m, aromatic), 4.66 (2H, s, $-COCH_2O-$), 7.52 (2H,t,$-CH_2CO-$), 8.74 (26H, m, $-(CH_2)_{13}-$ and 9.3 (3H, t, $-CH_3$); M^+ at 450.3819 (0.7%). A table of significant fragments obtained by mass spectroscopy is below (Table 10).

Table 10. Significant MS Fragments of p-Phenylphenacyl Ester of Hexadecanoic Acid (XXI).

POSTULATED FRAGMENT	OBSERVED FRAGMENT (m/e)	CALCULATED ≠ (m/e)	RELATIVE ABUNDANCE
$C_6H_5C_6H_4CO^+$	181.2829	181.0654	100%
$C_6H_5C_6H_4^+$	153.2626	153.0705	17.9%
$CH_3(CH_2)_3^+$	57.1104	57.0705	9.0%
$CH_3(CH_2)_4^+$	71.1440	71.0861	2.8%
$CH_3(CH_2)_5^+$	85.1641	85.1018	1.7%
$CH_3(CH_2)_8^+$	127.1300	127.1488	2.5%
$CH_3(CH_2)_{12}^+$	183.1667	183.2114	1.2%
M^+	450.3819	450.3135	0.7%

≠ C = 12.00000, H = 1.00783 and O = 15.9949

2A - 7. Z-9-HEXADECENOIC ACID, p-PHENYLPHENACYL ESTER (XXII)

The above compound was prepared by each of the two methods described above, with method A yielding 0.4771g (85.19%) and method B 0.4631g (82.70%). The white shiny plate-like crystals obtained had m.p. 56.5 - 58.0° (lit. m.p. 56.5 - 57°C)[24]; λ_{max} 286 nm; ν_{max} 3040, 1740, 1690, 1595 cm^{-1}; τ ca 1.96 - 2.63 (9H, m, aromatic), 4.61 - 4.72 (4H, m, - CH = CH - and -CO-CH_2-O-), 7.52 (2H, t, -CH_2CO-), 8.0 (4H, m, -CH_2-C = C-CH_2-), 8.68 (18H, m, -$(CH_2)_9$-) and 9.13 (3H, t, -CH_3); M^+ at 448.3902 (2%). A table of significant fragments obtained by mass spectroscopy is below (Table 11).

Table 11. Significant MS Fragments of p-Phenylphenacyl Ester of Z-9-Hexadecenoic Acid (XXII).

POSTULATED FRAGMENT	OBSERVED FRAGMENT (m/e)	CALCULATED ≠ (m/e)	RELATIVE ABUNDANCE
$C_6H_5C_6H_4CO^+$	181.2829	181.0654	100%
$C_6H_5C_6H_4^+$	153.2626	153.0705	17.9%
$CH_3(CH_2)_4^+$	71.1440	71.0861	2.8%
$CH_3(CH_2)_5^+$	85.1641	85.1018	1.7%
$CH_3(CH_2)_8^+$	127.1300	127.1488	2.5%
$CH_3(CH_2)_5(CH)_4^+$	137.1713	137.1331	3.9%
$CH_3(CH_2)_{12}(CH)_2^+$	197.1822	197.2271	3.2%
M^+	448.3902	448.2978	2.0%

≠ C = 12.00000, H = 1.00783 and O = 15.9949

2A - 8. OCTADECANOIC ACID, p-PHENYLPHENACYL ESTER (XXIII)

This ester was prepared by the above two described methods to yield 0.5033g (84.25%) from method A and 0.4762g (79.70%) from method B. The white plate-like crystals had m.p. 93.5 - 95.0°C (lit. m.p. 94°C)[85]; λ_{max} 286 nm; ν_{max} 3040, 1740, 1690, 1595 cm^{-1}; τ ca 1.97 - 2.77 (9H, m, aromatic), 4.67 (2H, s, $-CO-CH_2-O-$), 7.51 (2H, t, $-CH_2-CO-$), 8.72 (30H, m, $-(CH_2)_{15}-$)and 9.13 (3H, t, $-CH_3$); M^+ at 478.4446 (0.5%). A table of significant fragments obtained by mass spectroscopy is below (Table 12).

Table 12. Significant MS Fragments of p-Phenylphenacyl Ester of Octadecanoic Acid (XXIII).

POSTULATED FRAGMENT	OBSERVED FRAGMENT (m/e)	CALCULATED ≠ (m/e)	RELATIVE ABUNDANCE
$C_6H_5C_6H_4CO^+$	181.2171	181.0654	100%
$C_6H_5C_6H_4^+$	153.2656	153.0705	9.0%
$CH_3(CH_2)_3^+$	57.2147	57.0705	24.4%
$CH_3(CH_2)_4^+$	71.1623	71.0861	7.5%
$CH_3(CH_2)_5^+$	85.1840	85.1018	3.2%
$CH_3(CH_2)_8^+$	127.1437	127.1488	1.3%
$CH_3(CH_2)_{12}^+$	183.2171	183.2114	1.2%
M^+	478.4446	478.3448	0.5%

≠ C = 12.00000, H = 1.00783 and O = 15.9949

2A - 9. Z-9-OCTADECENOIC ACID, p-PHENYLPHENACYL ESTER (XXIV)

This ester was synthesized by use of the above two methods. Method A yielded 0.4984g (83.76%) and method B gave 0.4641g (78.00%) of white rounded crystals with m.p. 60.5 - 61.0°C (lit. m.p. 60°C)[82]; λ_{max} 286 nm, ν_{max} 3040, 1740, 1690, 1595 cm^{-1}; τca 1.99 - 2.78 (9H, m, aromatic), 4.68 (4H, m, -CH = CH - and -CO-CH_2-O-), 7.52 (2H, t, -CH_2-CO), 7.99 (4H, m, -CH_2-C = C - CH_2), 8.71 (22H, m, -$(CH_2)_{11}$-) and 9.12 (3H, t, -CH_3); M^+ at 476.4385 (0.1%). A table of significant fragments obtained by mass spectroscopy is below (Table 13).

Table 13. Significant MS Fragments of p-Phenylphenacyl Ester of Z-9-Octadecenoic Acid (XXIV).

POSTULATED FRAGMENT	OBSERVED FRAGMENT (m/e)	CALCULATED ≠ (m/e)	RELATIVE ABUNDANCE
$C_6H_5C_6H_4CO^+$	181.2936	181.0654	100%
$C_6H_5C_6H_4^+$	153.2611	153.0705	11.2%
$CH_3(CH_2)_3^+$	57.2117	57.0705	15.7%
$CH_3(CH_2)_4^+$	71.1424	71.0861	4.2%
$CH_3(CH_2)_5(CH)_2^+$	111.1891	111.1175	1.7%
$CH_3(CH_2)_9(CH)_2^+$	167.1453	167.1801	4.0%
$CH_3(CH_2)_{12}(CH)_2^+$	209.2489	209.2271	0.1%
M^+	476.4385	476.3291	0.1%

≠ C = 12.00000, H = 1.00783 and O = 15.9949

2A - 10. E-9-OCTADECENOIC ACID, p-PHENYLPHENACYL ESTER(XXV)

The title compound was synthesized by use of methods A and B above which yielded 0.5216g (87.66%) and 0.5216g (80.66%) white rounded crystals respectively with m.p. 73.0 - 74.0°C; λ_{max} 286; ν_{max} 3040, 1740, 1690, 1595, 960 cm^{-1}; τca 2.12 - 2.9 (9H, m , aromatic), 4.77 (4H, -CH = CH- and $-OCH_2-CO-$), 7.56 (2H, t, $-CO-CH_2-$), 8.08 (4H, m, $-CH_2-C = C - CH_2$), 8.75 (22H, m, $-(CH_2)_{11}-$) and 9.13 (3H, t, $-CH_3$); M^+ at 476.4209 (0.6%). A table of significant fragments obtained by mass spectroscopy is below (Table 14).

Table 14. Significant MS Fragments of p-Phenylphenacyl Ester of E-9-Octadecenoic Acid (XXV).

POSTULATED FRAGMENT	OBSERVED FRAGMENT (m/e)	CALCULATED ≠ (m/e)	RELATIVE ABUNDANCE
$C_6H_5C_6H_4CO^+$	181.2144	181.0654	100%
$C_6H_5C_6H_4^+$	153.2113	153.0705	15.4%
$CH_3(CH_2)_3^+$	57.0461	57.0705	14.8%
$CH_3(CH_2)_4^+$	71.0858	71.0861	4.0%
$CH_3(CH_2)_5(CH)_2^+$	111.2364	111.1175	3.8%
$CH_3(CH_2)_9(CH)_2^+$	167.2061	167.1801	6.1%
$CH_3(CH_2)_{12}(CH)_2^+$	209.2125	209.2271	0.2%
M^+	476.4209	476.3291	0.6%

≠ C = 12.00000, H = 1.00783 and O = 15.9949

2A - 11. Z-9, Z-12-OCTADECADIENOIC ACID, p-PHENYLPHENACYL ESTER (XXVI)

This ester was synthesized through the two methods outlined above. Method A yielded 0.4902g (82.73%) and method B gave 0.4462g (75.30%) of white rounded crystals of m.p. 35 - 36°C; λ_{max} 286 nm; ν_{max} 3038, 1740, 1695, 1595 cm^{-1}; τ ca 1.80 - 2.72 (9H, m, aromatic), 4.68 (6H, m, $-O-C\underline{H}_2-CO$ and $(-C\underline{H} = C\underline{H}-)_2$), 7.1 - 8.87 (24H, m, $-(CH_2)_{12}-$), 9.13 (3H, t, $-CH_3$); M^+ at 474.4255 (0.3%). A table of significant fragments obtained by mass spectroscopy is below (Table 15).

Table 15. Significant MS Fragments of p-Phenylphenacyl Ester of Z-9, Z-12-Octadecadienoic Acid (XXVI).

POSTULATED FRAGMENT	OBSERVED FRAGMENT (m/e)	CALCULATED ≠ (m/e)	RELATIVE ABUNDANCE
$C_6H_5C_6H_4CO^+$	181.2370	181.0654	100%
$C_6H_5C_6H_4^+$	153.2385	153.0705	17.9%
$CH_3(CH_2)_3^+$	57.1326	57.0705	10.3%
$CH_3(CH_2)_4^+$	71.0998	71.0861	4.7%
$CH_3(CH_2)_4(CH)_2^+$	97.1468	97.1018	4.0%
$CH_3(CH_2)_5(CH)_2^+$	111.1694	111.1175	4.0%
$CH_3(CH_2)_{12}(CH)_4^+$	235.2691	235.2427	0.2%
M^+	474.4255	474.3136	0.3%

≠ C = 12.00000, H = 1.00783 and O = 15.9949

2A-12. Z-9, Z-12, Z-15-OCTADECATRIENOIC ACID, p-PHENYLPHENACYL ESTER (XXVII).

Compound XXVII was synthesized by method B only, yielding 0.4529g (76.76%) of white rounded crystals of m.p. 33.0 - 34.0°C; λ_{max} 286 nm; ν_{max} 3040, 1740, 1690, 1595 cm^{-1}; τca 1.98 - 2.76 (9H, m, aromatic), 4.65 (8H, m, $-O-CH_2-CO-$ and $(-CH = CH-)_3$), 7.05 - 8.85 (20H, m, $-(CH_2)_{10}-$), 9.0 (3H, t, $-CH_3$); M^+ at 472.4700 (0.3%). A table of significant fragments obtained by mass spectroscopy is below (Table 16).

Table 16. Significant MS Fragments of p-Phenylphenacyl Ester of Z-9, Z-12, Z-15-Octadecatrienoic Acid (XXVII).

POSTULATED FRAGMENT	OBSERVED FRAGMENT (m/e)	CALCULATED ≠ (m/e)	RELATIVE ABUNDANCE
$C_6H_5C_6H_4CO^+$	181.1608	181.0654	100%
$C_6H_5C_6H_4^+$	153.1608	153.0705	14.5%
$CH_3(CH_2)_3^+$	57.0169	57.0705	16.5%
$CH_3(CH_2)_4^+$	71.1577	71.0861	3.9%
$CH_3(CH_2)_4(CH)_2^+$	97.1162	97.1018	3.6%
$CH_3(CH_2)_5(CH)_2^+$	111.1364	111.1175	1.7%
$CH_3(CH_2)_{12}(CH)_2^+$	209.1942	209.2271	1.1%
M^+	472.4700	472.2979	0.3%

≠ C = 12.00000, H = 1.00783 and O = 15.9959

2A - 13. 12-HYDROXY-Z-9-OCTADECENOIC ACID, p-PHENYLPHENACYL ESTER (XXVIII)

The compound XXVIII was synthesized according to the two procedures given above. Method A yielded 0.5326g (86.6%) and method B 0.4797g (78%) of white rounded crystals with m.p. 73.5 - 74°C, λ_{max} 286 nm; ν_{max} 3320, 3040, 1740, 1690, 1595 cm^{-1}; τ ca 1.93 - 2.72 (9H, m, aromatic), 4.64 (4H, m, -CH = CH- and -O-CH_2-CO), 6.39 (1H, m, CH-OH), 7.5 (2H, t, -CH_2-CO-), 7.76 - 9.04 (24H, m, -$(CH_2)_{12}$-) and 9.22 (3H, t, -CH_3); M^+ - 18 at 474.4969 (0.2%). A table of significant fragments obtained by mass spectroscopy is below (Table 17).

Table 17. Significant MS Fragments of p-Phenylphenacyl Ester of 12-Hydroxy-Z-9-Octadecenoic Acid (XXVIII).

POSTULATED FRAGMENT	OBSERVED FRAGMENT (m/e)	CALCULATED ≠ (m/e)	RELATIVE ABUNDANCE
$C_6H_5C_6H_4CO^+$	181.2619	181.0654	100%
$C_6H_5C_6H_4^+$	153.2603	153.0705	14.3%
$CH_3(CH_2)_4^+$	71.0934	71.0861	1.1%
$CH_3(CH_2)_5^+$	85.1242	85.1018	2.4%
$CH_3(CH_2)_8^+$	127.1284	127.1488	2.0%
$CH_3(CH_2)_5(CH)_2^+$	111.1606	111.1175	4.4%
$CH_3(CH_2)_9(CH)_2^+$	167.2718	167.1801	11.0%
$M^+ - 18$	474.4969	474.3136	0.2%

≠ C = 12.00000, H = 1.00783 and O = 15.9949

2A - 14. Z-13-DOCOSENOIC ACID, p-PHENYLPHENACYL ESTER (XXIX)

The ester above was prepared by method B only giving 0.5309g (79.83%) of white round crystals. The product obtained had m.p. 70 - 72°C; λ_{max} 286 nm; ν_{max} 3040, 1740, 1690, 1595 cm^{-1}; τca 2.07 - 2.84 (9H, m, aromatic), 4.72 (4H, m, -CH = CH- and -O-CH_2-CO), 7.53 (2H, t, -CO-CH_2-), 7.75 - 8.95 (34H, m, -$(CH_2)_{17}$-) 8.95 (34H, m, -$(CH_2)_{17}$-) and 9.12 (3H, t, -CH_3); M^+ at 532.5738 (0.2%). A table of significant fragments obtained by mass spectroscopy is below (Table 18).

Table 18. Significant MS Fragments of p-Phenylphenacyl Ester of Z-13-Docosenoic Acid (XXIX).

POSTULATED FRAGMENT	OBSERVED FRAGMENT (m/e)	CALCULATED ≠ (m/e)	RELATIVE ABUNDANCE
$C_6H_5C_6H_4CO^+$	181.2401	181.0654	100%
$C_6H_5C_6H_4^+$	153.2523	153.0705	17.3%
$CH_3(CH_2)_3^+$	57.1509	57.0705	19.4%
$CH_3(CH_2)_8^+$	127.2244	127.1488	1.9%
$CH_3(CH_2)_9(CH)_2^+$	167.2458	167.1801	6.3%
$CH_3(CH_2)_{13}^+$	197.2599	197.2271	2.3%
$CH_3(CH_2)_{16}(CH)_2^+$	265.3333	265.2897	0.0%
M^+	532.5738	532.3918	0.2%

≠ C = 12.00000, H = 1.00783 and O = 15.9949

2A - 15. PREPARATION OF FREE FATTY ACIDS FROM NATURAL OILS

The component fatty acids present in linseed and castor oils were liberated using the procedure of Miloslav and Hokl[86] who liberated the fatty acids present in castor oil.

Castor oil (10g) was mixed with a 50% aqueous solution of KOH (10 ml) in a porcelain dish until the resulting emulsion was thick and would not separate into layers. Heating of the mixture was then done for two hours on a water bath to complete the emulsification. The emulsion was then dissolved in 200 ml of hot distilled water, and then a thin stream of a 1:1 solution of HCl (13.5 ml) added, and the mixture boiled for 10 min. Before the dilution, there was a homogeneous solution, but by the end of the dilution an oily layer containing the free fatty acids separated on the top. This was decanted and washed five times with water (deionized) until the washings contained no trace of inorganic acids as detected by methyl orange indicator. The fatty acids obtained were dried and weighed.

Anhydrous magnesium sulphate was used to dry these fatty acids. This procedure also applied to the preparation of free fatty acids from linseed oil (10g).

2A - 16. DERIVATIZATION OF FATTY ACIDS ISOLATED FROM NATURAL OILS

The castor oil fatty acids prepared as described in the previous section 2A - 15 (0.559g) were weighed in a 100 ml flask, and absolute ethanol (20 ml) and water (5 ml) added to the flask; a homogeneous solution formed. Phenolphthalein indicator (3 drops) was then added and 5 M aqueous KOH was added to the solution until a pink colour was obtained. The resulting solution was then neutralised with 1 M HCl. p-Phenylphenacyl bromide (0.57g) was then added and the contents of the flask were stirred in an oil bath at 60°C for 3½ hours. The progress of the reaction was followed by TLC until completion when p-phenylphenacyl bromide was observed to remain unreacted. The unreacted p-phenylphenacyl bromide was removed by means of preparative TLC using cyclohexane:acetone(8:2)as eluant. Recrystallization was done on the mixed esters so obtained from absolute ethanol which yielded 0.7910g of white crystalline material.

The same procedure was used for the derivatization of linseed oil fatty acids. The linseed oil fatty acid mixture (0.559g) yielded 0.7497g of mixed esters after recrystallization.

2B ANALYSIS OF THE FATTY ACID p-PHENYLPHENACYL ESTERS BY REVERSE - PHASE HPLC.

2B - 1. INSTRUMENTATION

Throughout this work, chromatograms were run on a Beckman model 110 high performance liquid chromatograph equipped with two model 110A pumps; a Beckman model 210 sample injector valve; a Hitachi model 115,100-40 UV-VIS spectrometer with an UV-Vis variable wavelength detector; and a Kipp and Zonen BD 41 recorder. The column used was a 4.6 mm i.d. x 25 cm C_{18} - Ultrasphere - ODS type. The λ_{max} was predetermined and set at 286 nm and the sensitivity range of the detector set at 0.05 throughout unless otherwise stated.

All solvents used for elution in this analysis were purified by distillation and de-aerated by boiling. Methanol and acetonitrile used in this analysis were analytical grades. Water had been double distilled. Before the beginning of the chromatography, the column was prepared by flushing with pure methanol and then with the eluant in use. For the gradient elution, a mixture of methanol-acetonitrile-water (87:5:8) was flushed after flushing the column with methanol.

2B - 2. PREPARATION OF THE FATTY ACID STANDARD SOLUTIONS

A stock solution of each fatty acid p-phenylphenacyl ester derivative synthesized in part 2A of this thesis was made by dissolving 1.00×10^{-2} g in 50 ml of either Analar dichloromethane or Analar benzene, producing a solution equivalent to 200 µg/ml which was diluted to 10 µg/ml according to appropriate dilution. A mixed standard solution of the fatty acid ester derivatives synthesized in part 2A of this thesis was prepared by mixing 5 ml of the 100 µg/ml solution of each of these esters and diluting them together with dichloromethane to make a solution containing 10 µg/ml of each ester.

Mixed standards were also prepared containing p-phenylphenacyl ester derivatives of the following saturated acids: dodecanoic acid ($C_{12:0}$), tetradecanoic acid ($C_{14:0}$) and hexadecanoic acid ($C_{16:0}$). The mixed standards for the ester derivatives of C_{18} fatty acids were similarly made. These acids were Z-9-octadecenoic acid ($C_{18:1;9Z}$); Z-9, Z-12-octadecadienoic acid ($C_{18:2;\ 9Z,\ 12Z}$) and Z-9, Z-12, Z-15-octadecatrienoic acid ($C_{18:3;\ 9Z,\ 12Z,\ 15Z}$) p-phenylphenacyl esters.

2B - 3. IDENTIFICATION OF THE APPROPRIATE MOBILE PHASE

Resolution of the standard mixture of saturated and unsaturated fatty acid esters was first started by use of 100% methanol as the mobile phase at a flow rate of 1.0 ml/min and chart speed of 0.5 cm/min. Pressure which developed was 2200 psi. As found earlier by Mengech and Kihumba[81], it was noted that the solvent did not produce good resolution between the saturated and unsaturated fatty acid ester derivatives.

Methanol mixed with distilled water in various proportions (e.g. 80:20, 90:10, 95:5) was then used to enhance the separation, but a good resolution was not obtained with the above mixtures. For 95:5 methanol to water proportion, the flow rate of the methanol-water mobile phase was 0.75 ml/min and the chart speed was 0.19 cm/min. The pump pressure which developed was 3500 psi.

Methanol and acetonitrile mixtures were made and tested as the mobile phase (e.g. 80:20, 90:10, 95:5) but none was found to bring very outstanding results

in resolution. For methanol to acetonitrile proportion of 95:5, flow rate and chart speed were 1 ml/min and 0.5 cm/min respectively. The operating pressure was 2000 psi.

The next mobile phase tested was a mixture of methanol, acetonitrile and water in varied proportions of methanol to acetonitrile to water (e.g. 90:5:5, 80:10:10, 85:5:10, 87:5:8). It was found that a mixture of methanol, acetonitrile and water in the ratio of 87:5:8 brought about efficient separation. The flow rate was 0.75 ml/min and chart speed was 0.1 cm/min at an operating pressure of 3400 psi.

2B - 4. QUALITATIVE ANALYSIS OF THE FATTY ACID DERIVATIVES UNDER ISOCRATIC CONDITIONS

In order to determine the retention time for each fatty acid derivative synthesized, three samples containing 10 μg/ml were introduced into the HPLC and the retention time of each noted, from which the average retention time (in minutes) was calculated. The mobile phase used was a mixture of methanol, acetonitrile and water (87:5:8); the flow rate was set at 0.75 ml/min while the chart speed was 0.1 cm/min. The pump pressure registered at 3400 psi. A mixture containing all the fatty acid ester derivatives listed in Fig.2 was then injected and chromatographed. The retention times obtained when injecting the individual derivatives were used in identifying the components of the mixture.

2B - 5. QUALITATIVE ANALYSIS OF THE FATTY ACID ESTER STANDARDS BY GRADIENT ELUTION

Gradient elution was introduced when chromatography under isocratic conditions gave very long retention times for some of the esters studied, notably those of octadecanoic acid and Z-13-docosenoic acids. One pump (A) was programmed to pump 100% of the mixture of solvents used under isocratic conditions (methanol: acetonitrile:water - 87:5:8) from zero to 50 minutes; from 50 to 70 minutes, the percentage of this ternary solvent was gradually reduced from 100% to 0%. The second pump (B) was programmed to begin pumping absolute methanol from the 50th minute, increasing its mobile phase composition up to 100% over the next 20 minutes.

The solutions of varying concentrations of the individual standard fatty acid esters were injected as described earlier in section 2B - 1 and then the average retention time for each in minutes was determined. The mixed standard containing all the fatty acid ester derivatives was then injected, and analysed by the

foregoing gradient elution programme. The flow rate and chart speed were maintained at 0.75 ml/min and 0.1 cm/min respectively throughout the gradient elution programme.

2B - 6. QUALITATIVE IDENTIFICATION OF THE p-PHENYLPHENACYL ESTERS OF THE FATTY ACIDS FROM CASTOR OIL AND LINSEED OIL

Qualitative identification of the castor oil and linseed oil fatty acid ester derivatives was done under isocratic conditions. A stock solution was made of the castor oil fatty acid p-phenylphenacyl esters by dissolving 8.54 mg of the ester mixture as prepared in section 2A -16 in 50 ml Analar benzene. This solution was further diluted to make solutions containing 25.62 µg/ml. Each of the foregoing solutions was then chromatographed at a flow rate of 0.75 ml/min and chart speed of 0.1 cm/min, using methanol-acetonitrile-water (87:5:8) as the mobile phase. The retention time of each peak was measured and compared to the known retention times for the fatty acid ester standards as determined in section 2B - 4.

The linseed oil fatty acid ester derivatives stock solution was made by dissolving 10 mg of the prepared esters in 50 ml benzene. This solution was then diluted to 75 µg/ml and the chromatographic procedure above was repeated, as in the case of the castor oil fatty acid ester derivatives.

2B - 7. QUANTITATIVE ANALYSES OF CASTOR OIL AND LINSEED OIL FATTY ACID ESTER DERIVATIVES

The solutions used here were the same as those used in the qualitative analyses of the fatty acid ester derivatives, as described in section 2B - 6. For linseed oil fatty acid ester derivatives, 18.75 ml of a benzene solution containing 10 mg/50 ml was diluted to 50 ml with benzene, resulting in a solution containing 75 µg/ml. This was chromatographed three times under isocratic conditions using methanol, acetonitrile, water (87:5:8). The solutions of castor oil fatty acid ester derivatives that were used were of concentrations of 25.62 µg/ml in benzene.

Each of the samples had its components identified by comparing their retention times with those of the standards. Concentration of each of the components was estimated by determining the area of each peak by the tangent method and then expressing it as a percentage of the total areas of all the components of the sample.

CHAPTER THREE

RESULTS AND DISCUSSION

3A PART A - SYNTHESIS OF FATTY ACID p-PHENYLPHENACYL ESTERS

3A-1 GENERAL DERIVATIZATION PROCEDURES FOR THE PREPARATION OF FATTY ACID p-PHENYLPHENACYL ESTERS

Preparation Using Phase-transfer Catalysis

Fatty acid p-phenylphenacyl esters were prepared as described in section 2A-3. When aqueous KOH was added to the solution of the fatty acid in dichloromethane, two immiscible layers formed, implying that reaction between any two reactants in the different phases would not have occured easily had it not been for the presence of tetrabutylammonium hydrogensulphate which was added as a phase-transfer catalyst. The derivatizing agent was also dissolved in the organic layer.

The tetrabutylammonium hydrogensulphate facilitated the reaction in that it was capable of transporting the hydroxide ions from the aqueous layer into the organic layer. In return, the phase transfer catalyst transported bromide ions into the aqueous layer

at the time of which the tetrabutylammonium hydrogensulphate was regenerated in the aqueous phase after the tetrabutylammonium ions reacted with hydrogensulphate ions as shown in the scheme of equations below, as suggested by Durst and colleagues[53].

In the aqueous layer:

1. $(n\text{-}Bu)_4N^+HSO_4^- + K^+OH^- \rightleftharpoons (n\text{-}Bu)_4N^+OH^- + K^+HSO_4^-$

In the organic layer:

2. $(n\text{-}Bu)_4N^+OH^- + RCOOH \rightleftharpoons (n\text{-}Bu)_4N^+\bar{O}_2CR + H_2O$

3. $(n\text{-}Bu)_4N^+\bar{O}_2CR + C_6H_5C_6H_4COCH_2Br \rightarrow (n\text{-}Bu)_4N^+Br^- + C_6H_5C_6H_4COCH_2O_2CR$

In the aqueous phase:

4. $(n\text{-}Bu)_4N^+Br^- + K^+HSO_4^- \longrightarrow (n\text{-}Bu)_4N^+HSO_4^- + K^+Br^-$

This scheme serves to explain why only a small amount of the phase-transfer catalyst need be used, since it functions as a transport agent which is being regenerated in the course of the reaction. The reaction represented by equation 3 above most likely proceeded through an SN_2 reaction mechanism. This is

possible due to the fact that the bromide is a good leaving group and the carboxylate anion ($RCOO^-$) is a relatively good nucleophile. This method of derivatization is quite a good one, in that it can be done at low temperatures (about 20°C) thereby preventing the isomerization of the double bond which has been observed to occur at higher temperatures by Herbert [69]. The reaction time was about 30 minutes which is a short time and the reaction yields were quite high, averaging to 85.93%. Possibly the yields here could have been improved by extracting the aqueous layer with 5-ml portions of dichloromethane about 10 times instead of five times which were done (section 2A-3.1)

Preparation via the Potassium Salt of the Acid

Although much weaker than the strong mineral acids, the fatty acids are capable of forming salts when treated with aqueous hydroxides. This was the first step done in the derivatization procedure as reported by Kihumba [81].

$$RCOOH + KOH \rightarrow RCOO^-K^+ + H_2O$$

The reaction proceeded instantaneously and no heating was required. Neutralisation of any excess potassium hydroxide solution using

dilute hydrochloric acid was done so as to avoid any alkalinic conditions which might cause decomposition of the p-phenylphenacyl bromide as observed by Kihumba[81] . Again the potassium salts of the fatty acids reacted with the p-phenylphenacyl bromide, the derivatizing agent, most likely through an SN_2 reaction mechanism, as explained in the previous procedure. This reaction occured as represented by the equations:

$$RCOOH + K^+OH^- \rightarrow RCOO^-K^+ + H_2O$$

$$RCOO^-K^+ + C_6H_5C_6H_4COCH_2Br \rightarrow RCOOCH_2COC_6H_4C_6H_5 + KBr$$

Just as in the method of derivatization using phase-transfer catalysis, the reaction yields by direct displacement were quite high, although slightly lower than the former method, averaging to 79.46%. A table comparing the reaction yields of the products from both methods is shown below (Table 19).

Table 19. Percentage yields of the synthesized p-phenylphenacyl esters.

p-PHENYLPHENACYL ESTER DERIVATIVE OF FATTY ACID	% YIELD	
	METHOD A	METHOD B
Dodecanoic ($C_{12:0}$)(XIX)	85.27%	79.18%
Tetradecanoic ($C_{14:0}$)(XX)	88.70%	80.22%
Hexadecanoic ($C_{16:0}$)(XXI)	89.21%	83.70%
Z-9-Hexadecenoic ($C_{16:1}$)(XXII)	85.19%	82.70%
Octadecanoic ($C_{18:0}$)(XXIII)	84.25%	79.70%
Z-9-Octadecenoic ($C_{18:1}$)(XXIV)	83.76%	78.00%
E-9-Octadecenoic ($C_{18:1}$)(XXV)	87.66%	80.66%
Z-9,Z-12-Octadecadienoic ($C_{18:2}$)(XXVI)	82.73%	75.30%
Z-9,Z-12,Z-15-Octadecatrienoic ($C_{18:3}$) (XXVII)	--	76.76%
12-Hydroxy - Z-9-Octadecenoic (XXVIII)	86.60%	78%
Z-13-Docosenoic ($C_{22:1}$) (XXIX)	--	79.83%

The p-phenylphenacyl esters of E-9-octadecenoic ($C_{18:1}$) (XXV), Z-9, Z-12-Octadecadienoic ($C_{18:2}$) (XXVI), Z-9,Z-12,Z-15-Octadecatrienoic ($C_{18:3}$) (XXVII), Z-13-docosenoic ($C_{22:1}$) (XXIX) and 12-hydroxy-Z-9-octadecenoic ($C_{18:1;9Z(12OH)}$) (XXVIII), have not been reported in the available literature and as such they constitute new compounds.

3A-2 INFRARED SPECTROMETRY RESULTS FOR THE SYNTHESIZED p-PHENYLPHENACYL ESTERS

The results of the IR spectroscopy obtained from the following p-phenylphenacyl ester derivatives of the following fatty acids is tabulated in Table 20 below: dodecanoic ($C_{12:0}$) (XIX), tetradecanoic ($C_{14:0}$) (XX), hexadecanoic ($C_{16:0}$) (XXI), octadecanoic ($C_{18:0}$) (XXIII), Z-9-hexadecenoic ($C_{16:1}$) (XXII), Z-9-octadecenoic ($C_{18:1}$) (XXIV), E-9-octadecenoic ($C_{18:1}$) (XXV), Z-9,Z-12-octadecadienoic ($C_{18:2}$) (XXVI), Z-9,Z-12,Z-15-octadecatrienoic ($C_{18:3}$) (XXVII), 12-hydroxy-Z-9-octadecenoic ($C_{18:1;9Z(12OH)}$) (XXVIII) and Z-13-docosenoic ($C_{22:1}$) (XXIX) acids. Of the very many peaks observed in each case, only the ones considered to be diagnostic to the structures mentioned have been shown in the table. The IR spectra of compounds XXV, XXVI, XXVII, XXVIII and XXIX are shown in Figures 3 to 7.

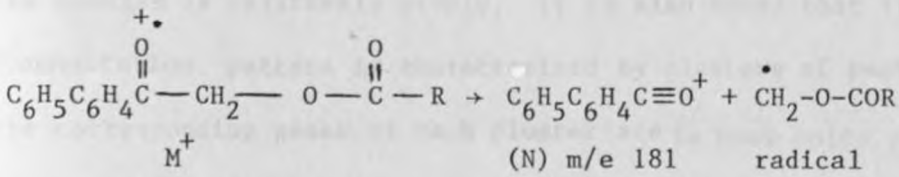
Table 20 : Major IR Absorptions for the p-phenylphenacyl Esters of Some Common Fatty Acids.

MAJOR FREQUENCY IR ABSORPTIONS (ν , cm^{-1})	ASSIGNMENT	p-PHENYLPHENACYL ESTERS SHOWING THIS ABSORPTION
3030-3050 (weak)	C-H aromatic stretch	All the esters XIX-XXIX
3140-3540 (broad)	O-H stretch	$C_{18:1;9Z(12OH)}$ (XXVII)
2780-2900 (strong)	C-H stretch	All the esters XIX-XXIX
1740 (strong)	ester carbonyl stretch, OCO	All the esters XIX-XXIX
1690 (strong)	free carbonyl (C=O) stretch	All the esters XIX-XXIX
1595 (strong)	C=C aromatic stretch	All the esters XIX-XXIX
960 (strong)	Trans RHC=CHR H-bending	$C_{18:1;9E}$ (XXV)
834 (strong)	Ar-H bends of two adjacent H's showing p-substitution	All the esters XIX-XXIX

From the results tabulated in Table 20 , it is observed that all the p-phenylphenacyl esters (XIX - XXIX) have a strong ester stretch at 1740 cm^{-1}. This verifies the reaction between the p-phenylphenacyl bromide and the individual fatty acids, since neither of the starting materials contains an ester group. The presence of an OH group in the p-phenylphenacyl ester (XXVIII) of 12-hydroxy - Z-9-octadecenoic acid has been confirmed by the broad absorption from 3140 - 3540 cm^{-1}. The presence of the trans- H-bending (RHC = CHR) in E-9-octadecenoic p-phenylphenacyl ester (XXV) at 960 cm^{-1} differentiates this ester from that of Z-9-octadecenoic acid ester (XXIV) which did not have an absorption in this region. Lack of this absorption at 960 cm^{-1} in the other unsaturated p-phenylphenacyl esters synthesized from the cis- fatty acid starting materials confirms that no cis-trans isomerization took place during either of synthetic procedures outlined in sections 2A-3. No absorption associated with a free C=C stretch was observed for any of the unsaturated ester derivatives. This implies that this particular absorption was masked by the aromatic C=C absorption.

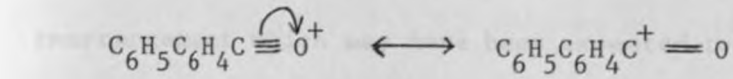
3A-3 MASS SPECTROSCOPY RESULTS FOR THE SYNTHESIZED p-PHENYLPHENACYL ESTERS

Mass spectrometry data for the individual p-phenylphenacyl ester derivatives of dodecanoic ($C_{12:0}$) (XIX); tetradecanoic ($C_{14:0}$) (XX); hexadecanoic ($C_{16:0}$) (XXI); octadecanoic ($C_{18:0}$)(XXIII); Z-9-octadecenoic ($C_{18:1}$) (XIV); E-9-octadecenoic ($C_{18:1}$) (XXV); Z-9, Z-12-octadecadienoic ($C_{18:2}$) (XXVI); Z-9, Z-12, Z-15-octadecatrienoic ($C_{18:3}$) (XXVII); 12-hydroxy-Z-9-octadecenoic ($C_{18:1;9Z(12OH)}$) (XXVIII) and Z-13-docosenoic ($C_{22:1}$) (XXIX) fatty acids has been tabulated in sections 2A-4 - 2A-14. It is noted that all the p-phenylphenacyl ester derivatives have a common peak of the highest abundance whose m/e value is at 181. The reason is well understood in that the fragment with m/e value of 181 is a very stable one in relation to all the other fragments. The mechanism of its formation is postulated below:

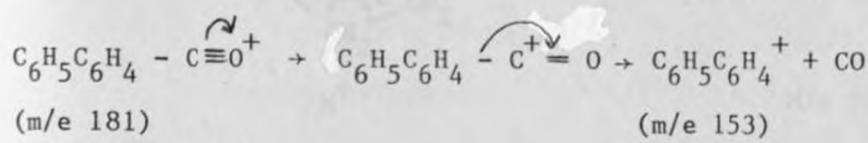


As implied from the equation, the fragment (m/e = 181) has an even number of electrons and hence a high stability, since a

species with even electrons is more stable in comparison to one which has an odd number of electrons[87]. In addition to this, the species N has further stability due to resonance, as shown below:

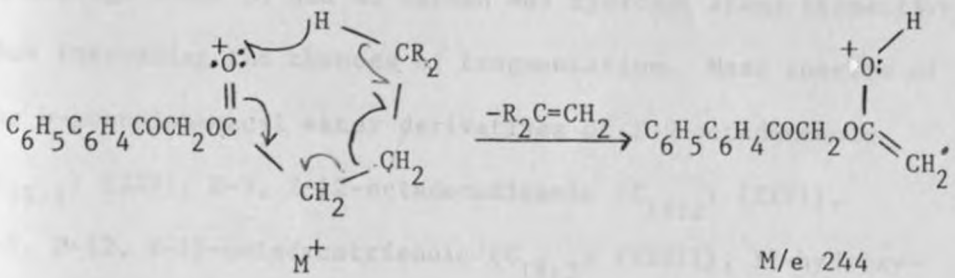


Another fragment which is quite abundant (relative abundance averaging about 14.3%) and common to all the p-phenylphenacyl esters is the one with m/e at about 153. Its postulated mechanism of formation is shown below:



Again due to its even number of electrons and high degree of resonance, the species is relatively stable. It is also noted that the fragmentation pattern is characterized by clusters of peaks, and the corresponding peaks of each cluster are 14 mass units apart, due to loss of a $-CH_2-$ unit. The fragmentation pattern of the saturated and unsaturated looks quite similar. However, in the unsaturated esters, most of the latter have the C_nH_{2n-1} peak as the most intense in each cluster while as for the saturated esters this trend has not been kept and the C_nH_{2n} or C_nH_{2n+1} peak is the

most intense. In addition to this, all the saturated esters have shown the peak with m/e 43 as being more intense than the peak with m/e 55. The unsaturated esters show the opposite since nearly all of them have shown the peak with m/e 55 as being more intense than m/e 43 peak. It has also been noted that the McLafferty rearrangement which may have been expected to result in a peak of m/e 244 (via the mechanism shown below) for all these esters was not favoured.



M^+ M/e 244

All of the p-phenylphenacyl ester derivatives except two (those of $C_{14:0}$ (XX) and $C_{18:1;9Z(12OH)}$ (XXVIII) showed molecular ion peaks. The p-phenylphenacyl ester of tetradecanoic ($C_{14:0}$) (XX) fatty acid showed an $(M+1)^+$ ion and not M^+ peak. This may have been due to either the gaining of a hydrogen atom from a neutral molecule onto M^+ or isotopic effect of hydrogen during the mass spectrometry [87].

The p-phenylphenacyl ester derivative of 12-hydroxyoctadecenoic acid ($C_{18:1;9Z,(12OH)}$) (XXVIII) gave an $(M-18)^+$ fragment

of relative abundance of 0.2%. This can be explained due to the well-known dehydration of alcohols that occurs during mass spectrometry[87].

It can be noted that the discussed M^+, $(M+1)^+$ and $(M-18)^+$ fragments for the ester derivatives have a very low intensity averaging about 0.55%. This is explainable due to the long chain of the derivatives containing at least two oxygen atoms and having on average about 31 and 42 carbon and hydrogen atoms respectively, thus increasing the chances of fragmentation. Mass spectra of the p-phenylphenacyl ester derivatives of E-9-octadecenoic ($C_{18:1}$) (XXV), Z-9, Z-12-octadecadienoic ($C_{18:2}$) (XXVI), Z-9, Z-12, Z-15-octadecatrienoic ($C_{18:3}$) (XXVII), 12-hydroxy-Z-9-octadecenoic ($C_{18:1;9Z,(12OH)}$) (XXVIII) and Z-13-docosenoic ($C_{22:1}$) (XXIX) acids have been shown in Figures 8 and 12 respectively. The mentioned facts stand as a confirmation that postulated ester products had indeed been synthesized and had the structures as assigned in this work.

The IR spectra for compounds $C_{18:1;9E}$ (XXV), $C_{18:2;9Z,12Z}$ (XXVI), $C_{18:3;9Z,12Z,15Z}$ (XXVII), $C_{18:1;9Z(12OH)}$ (XVIII) and $C_{22:1;13Z}$ (XXIX) is shown in Figures 3 - 7.

3A-4 NUCLEAR MAGNETIC RESONANCE RESULTS FOR THE SYNTHESIZED p-PHENYLPHENACYL ESTERS

The nuclear magnetic resonance results for each of the p-phenylphenacyl ester derivatives of dodecanoic ($C_{12:0}$) (XIX), tetradecanoic ($C_{14:0}$) (XX), hexadecanoic ($C_{16:0}$) (XXI), octadecanoic ($C_{18:0}$) XXIII, Z-9-hexadecenoic ($C_{16:1}$) (XXII), Z-9-octadecenoic ($C_{18:1}$) (XXIV), E-9-octadecenoic ($C_{18:1}$) (XXV), Z-9, Z-12-octadecadienoic ($C_{18:2}$) (XXVI), Z-9, Z-12, Z-15-octadecatrienoic ($C_{18:3}$) XXVII), 12-hydroxy - Z-9-octadecenoic ($C_{18:1;9Z(12OH)}$) (XXVIII) and Z-13-docosenoic ($C_{22:1}$) (XXIX) acids has been shown in section 2A-4 through 2A-14. NMR spectra for five of these esters namely p-phenylphenacyl esters of $C_{18:2;9Z,12Z}$, $C_{18:1;9Z(12OH)}$, $C_{18:3;9Z,12Z,15Z}$, $C_{18:1;9E}$ and $C_{22:1;13Z}$ have been shown in Figures 13 to 17.

The presence of the two aromatic rings in each of the esters is confirmed since all of the esters have a 9- proton multiplet in the region of τ1.92-2.75. The group O-$\underline{CH}_2$-CO is shown to be present in all the esters by the presence of a 2- proton singlet at around τ4.6. For the p-phenylphenacyl esters which are unsaturated, the vinyl protons are not resolved from these O-$\underline{CH}_2$-CO protons. However, in the case of the p-phenylphenacyl ester of

12-hydroxy-Z-9-octadecenoic acid ($C_{18:1;9Z,(12OH)}$) (XXVIII), the vinyl protons were shifted downfield (τ 4.52) compared to the other esters whose vinyl protons were at about τ 4.65, although the resolution from the vinyl protons is not complete. This implies that the hydroxy group has an effect on the electronic environment of the -O-$\underline{CH}_2$-CO protons. Evidence for the presence of the OH also comes from the fact that the proton -$\underline{CH}$-OH was observed as a broad multiplet at τ 6.39 as can be observed in Figure 16.

Due to the fact that vinyl protons overlap with the methylene (-O-$\underline{CH}_2$-CO-) ones, the NMR spectroscopy cannot be used to differentiate cis from trans (Z from E) isomers at this level of resolution using the 60 MHz or 90 MHz instruments.

All the unsaturated esters show the presence of allylic protons (-$\underline{CH}_2$-C=C-$\underline{CH}_2$-) in the region of τ 7.65 - 8.1. The presence of the methylene protons (-CH_2-$\underline{CH}_2$-CO-) in all the esters has been marked by the presence of a triplet around τ 7.34 - 7.6. Each ester has also shown the rest of the methylene and the methyl protons of the alkyl chain at around τ 8.7 and 9.15 respectively. The integration of the spectra for each of the ester derivatives was according to the expectations for each of the compounds. The NMR spectroscopy has therefore served to make a distinction between

the number of carbon-carbon double bonds (C=C) in the compounds being discussed. For the monounsaturated esters ($C_{18:1;9Z}$, $C_{18:1;9E}$, $C_{18:1;9Z(12OH)}$, $C_{16:1;9Z}$ and $C_{22:1;13Z}$) there were 4 protons (vinyl and $-O-C\underline{H}_2-CO$) in the τ ca 4.6 region already discussed. For the diunsaturated ester ($C_{18:2;9Z,12Z}$) there were 6 protons and in the triunsaturated ester ($C_{18:3;9Z,12Z,15Z}$) there were 8 protons attributed to vicinal vinyl protons of the type -CH=CH- overlapping with the $-O-C\underline{H}_2-CO-$ signal. Therefore, the NMR spectroscopy also confirms the postulated structures of the compounds which were synthesized in this work.

PART B HIGH PRESSURE LIQUID CHROMATOGRAPHY RESULTS OF RESOLUTION OF A MIXTURE OF FATTY ACID p-PHENYLPHENACYL ESTER DERIVATIVES

3B-1 CHROMATOGRAPHY USING ABSOLUTE METHANOL

The p-phenylphenacyl ester derivatives of the fatty acids $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{16:1;9Z}$, $C_{18:0}$, $C_{18:1;9Z}$, $C_{18:1;9E}$, $C_{18:1;9Z(12OH)}$, $C_{18:2;9Z,12Z}$, $C_{18:3;9Z,12Z,15Z}$ and $C_{22:1;13Z}$ were chromatographed on a 4.6 mm I.D. x 25 cm Altex Ultrasphere-ODS column using absolute methanol as the mobile phase at a flow rate of 1 ml min^{-1} and chart speed of 0.5 cm min^{-1}. The chromatograph obtained is shown in Figure 18. From the chromatogram, it can be noted that the resolution of the p-phenylphenacyl esters of the fatty acids $C_{14:0}$, $C_{16:1;9Z}$, $C_{18:2;9Z,12Z}$ and $C_{18:3;9Z,12Z,15Z}$ (peaks 3,4 and 5) is not particularly good, since even the ester derivatives of $C_{14:0}$ and $C_{16:1;9Z}$ fatty acids appear as one peak (peak 4). Another set of ester derivatives with a poor resolution are those of the $C_{16:0}$, $C_{18:1;9Z}$ and $C_{18:1;9E}$ fatty acids, all of which appear as one peak (peak 6). However the p-phenylphenacyl esters of the fatty acids $C_{18:1;9Z(12OH)}$ (peak 1), $C_{12:0}$ (peak 2), $C_{18:0}$ (peak 7) and $C_{22:1;13Z}$ (peak 8) were properly resolved. Analysis time was 26.4 min. The results here agree with the ones obtained by Kihumba[81], who found that absolute methanol

used as a mobile phase was not capable of resolving a mixture of saturated and unsaturated p-phenylphenacyl ester derivatives when using the same column as used in this work. Kihumba, however, had found that absolute methanol was good in separating the saturated p-phenylphenacyl esters of the even C_{12} - C_{24} fatty acids.

3B-2. CHROMATOGRAPHY USING METHANOL:WATER (95:5) AS THE MOBILE PHASE

The same mixture of fatty acid ester derivatives chromatographed above were chromatographed here while using a methanol: water mixture (95:5) as the mobile phase at a flow rate of 0.75 ml min^{-1} and chart speed of 0.19 cm min^{-1}. A pump pressure of 3500 psi developed. The chromatogram is shown in Fig. 19. From the chromatogram, it is noted that inspite of the increased time for analysis (about 99 min), there is a better resolution than in the chromatography obtained while using methanol (100%) as the mobile phase. The p-phenylphenacyl derivatives of $C_{18:1;9Z(12OH)}$ (peak 1), $C_{12:0}$ (peak 2), $C_{18:2;9Z,12Z}$ (peak 5), $C_{18:0}$ (peak 9) and $C_{22:1;13Z}$ (peak 10) were well resolved. However, there is poor resolution between the set of p-phenylphenacyl ester derivatives of $C_{16:0}$ (peak 6), $C_{18:1;9Z}$ (peak 7) and $C_{18:1;9E}$ (peak 8). There was also a poor resolution between tetradecanoic ($C_{14:0}$), Z-9, Z-12, Z-15-octadecatrienoic ($C_{18:3}$) and Z-9-hexadecenoic ($C_{16:1}$) fatty acid ester derivatives.

3B-3 CHROMATOGRAPHY USING A MIXTURE OF METHANOL:ACETONITRILE (95:5) AS THE MOBILE PHASE.

The methanol:acetonitrile mixture (95:5) as the mobile phase also gave results which look comparatively different from the others described in the previous two sections. The chromatogram had been done at a flow rate of 0.5 ml/min and chart speed of 0.5 cm/min. It is noted from the chromatogram (Figure 20) that resolution of octadecanoic ($C_{18:0}$) and Z-13-docosenoic ($C_{22:1}$) was satisfactory like in the other chromatograms discussed. However, it is also noted that the p-phenylphenacyl ester derivatives of tetradecanoic ($C_{14:0}$), Z-9-hexadecenoic ($C_{16:1}$), Z-9, Z-12, Z-15-octadecatrienoic ($C_{18:3}$) and Z-9, Z-12-octadecadienoic ($C_{18:2}$) acids (peaks 3 and 4) have not resolved well although peak 4 is appearing as a shoulder. As compared with the chromatography using methanol (100%) as the mobile phase, this solvent shows some advantage in the resolution of p-phenylphenacyl esters of hexadecanoic ($C_{16:0}$), Z-9-octadecenoic ($C_{18:1}$) fatty acids. The pump pressure was also more satisfactory (2000 psi) than in the cases already described. Analysis time (42 min.) was shorter than that of the chromatogram obtained using a mixture of methanol and water (95:5) as the mobile phase (99 min.).

3B-4. CHROMATOGRAPHY USING A MIXTURE OF METHANOL-WATER-ACETONITRILE (87:8:5) AS THE MOBILE PHASE

The composition of methanol:water:acetonitrile (87:8:5) was tried after failure of getting a good resolution using varied proportions of either water and methanol or methanol and acetonitrile. The flow rate was set at 0.75 ml min^{-1} and chart speed at 0.1 cm min^{-1}. The pump pressure which developed was 3400 psi. As can be seen from the chromatogram in Fig. 21 , the resolution obtained by use of the above-mentioned mobile phase is satisfactory. This mixed solvent has the power of separating the E and Z isomers (denoted by the peaks 9 and 8) of p-phenylphenacyl ester derivatives of $C_{18:1;9E}$ and $C_{18:1;9Z}$ respectively. The whole separation required about 80 minutes, which is quite a long period. This period might have been made shorter by raising the flow rate, but this looked inadvisable due to the high pump pressures that might have developed (beyond 3400 psi). As can be noted, the ester derivatives of the fatty acid $C_{18:0}$ and $C_{22:1;13Z}$ have not been included in the mixture of esters that were being separated in this solvent system, because these derivatives have a very long retention time (greater than 80 minutes) using this solvent mixture, and as such this becomes a drawback of chromatography of these

derivatives under isocratic conditions. However, it can be observed that p-phenylphenacyl derivatives of the fatty acids $C_{18:1;9Z(12OH)}$, $C_{12:0}$, $C_{18:3;9Z,12Z,15Z}$, $C_{14:0}$, $C_{16:1,9Z}$, $C_{18:2;9Z,12Z}$, $C_{16:0}$, $C_{18:1,9Z}$ and $C_{18:1,9E}$ (peaks 1 - 9 respectively) were resolved properly, unlike in the case of other eluants mentioned above. In addition to the fact that members of a homologous series differing by a $-(CH_2)_2-$ can be separated, two members with same chain length but differing in degree of unsaturation can also be separated. The system can also distinguish between the geometrical isomers $C_{18:1,9Z}$ and $C_{18:1,9E}$. It can be noted that this solvent system enables a good separation of the critical pairs, namely the p-phenylphenacyl esters of $C_{18:3;9Z,12Z,15Z}$ and $C_{14:0}$ (peaks 3 and 4); $C_{18:2;9Z,12Z}$ and $C_{16:1;9Z}$ (peaks 5 and 6); and $C_{16:0}$ and $C_{18:1}$ (peaks 7 and 8) fatty acids.

3B-5. QUALITATIVE ANALYSIS OF FATTY ACIDS USING A MIXTURE OF METHANOL: WATER:ACETONITRILE (87:8:5) AS THE ELUANT

It can be noted from Fig. 21 that the polarity of a particular fatty acid derivative is important in determining the relative retention time. The more polar the derivative, the lower the retention time of the fatty acid ester derivative in this reverse-phase system. Of all the samples considered, the ester derivative of the fatty acid $C_{18:1;9Z(12OH)}$ with a retention time of 15.8 minutes is the most polar. Its increased polarity is due to the presence of the OH group which forms the basis of H-bonding in the compound. The hydrogen bonding has been confirmed by the IR spectrum. The increase in retention times of the saturated fatty acid ester derivatives as increasing from $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, ... can be noted. The reason attributed to this is the fact that addition of an ethyl group reduces the polarity of the molecule. For the ester derivatives of unsaturated fatty acids, their retention times increase in the order $C_{18:3;9Z,12Z,15Z}$ (35.2 minutes), $C_{18:2;9Z,12Z}$ (48.6 minutes) $C_{18:1;9Z}$ (72.6 minutes) and $C_{18:0}$ (greater than 80 minutes) due to the fact that each additional double bond increases the polarity of a derivative. Therefore, with a very slight difference in polarities between two components,

a resolution can occur between them. Using this same mobile phase it is possible to achieve a separation of the natural fatty acids as their p-phenylphenacyl ester derivatives. It is on this basis of using these standards that it becomes possible to identify an unknown mixture of fatty acids.

The retention times of the fatty acid p-phenylphenacyl ester derivatives chromatogramed when using the methanol:water: acetonitrile mixture (87:8:5) are tabulated below in Table 21.

Table 21 : Retention Times of Some Common Fatty Acid Esters Using Methanol:Water:Acetonitrile (87:8:5) As Eluant Under Isocratic Conditions. Column: 4.6mm I.D. x 25 cm Altex Ultrasphere - ODS; Flow Rate: 0.75 ml; Chart Speed: 0.25 cm min^{-1}.

p-PHENYLPHENACYL ESTER DERIVATIVE OF THE ACID	RETENTION TIME (MINUTES)
$C_{18:1;9Z(12OH)}$	15.8
$C_{12:0}$	22.4
$C_{18:3;9Z,12Z,15Z}$	35.2
$C_{14:0}$	38.4
$C_{16:1;9Z}$	41.4
$C_{18:2;9Z,12Z}$	48.6
$C_{16.0}$	68.4
$C_{18:1;9Z}$	72.6
$C_{18:1;9E}$	78.4

3B-6. GRADIENT ELUTION IN THE ANALYSIS OF p-PHENYLPHENACYL ESTER DERIVATIVES OF FATTY ACIDS

A mixture of the p-phenylphenacyl ester derivatives of $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{16:1;9Z}$, $C_{18:0}$, $C_{18:1;9Z}$, $C_{18:1;9E}$, $C_{18:1;9Z(12OH)}$, $C_{18:2;9Z,12Z}$, $C_{18:3;9Z,12Z,15Z}$ and $C_{22:1;13Z}$ fatty acids was also chromatogramed using gradient elution. This was done in order to cause the elution of the $C_{18:0}$ and $C_{22:1;13Z}$ ester derivatives and to lower the elution time for the derivatives. The gradient elution was done by pumping a mixture of methanol:water:acetonitrile (87:8:5) from one pump for the first 50 minutes, after which absolute methanol was pumped from a second pump from the 51st minute to the 70th minute (over a period of 20 minutes) increasing the relative methanol concentration from 87% to 100%. Elution was continued with 100% methanol until all of the components injected were eluted. The flow rate was maintained at 0.75 ml min^{-1} and the chart speed at 0.1 cm min^{-1}. The resolution was found to be satisfactory as can be seen from Fig. 22 . In addition to good resolution, the retention times for the longer-chain esters that were previously taking too long to elute were considerably shortened, with the last component ($C_{22:1;13Z}$) eluting with a retention time of 88 minutes. This gradient elution technique therefore has the advantage of making it

possible for one to analyse a given sample containing unsaturated acids of less than 24 carbon atoms in less than 90 minutes.

The retention times of the components have been tabulated below in Table 22 .

Table 22: Retention Time of Some Common Fatty Acid Esters Using Gradient Elution: A Methanol:Water:Acetonitrile Mixture (87:8:5) For the First 50 Min And Then Over 20 Min Methanol Raised From 87% to 100%. Column: 4.6 mm I.D. x 25 cm Altex Ultrasphere-ODS; Flow rate: 0.75 ml min^{-1}; Chart Speed: 0.1 cm min^{-1}.

p-PHENYLPHENACYL ESTER DERIVATIVE OF THE ACID	RETENTION TIME (MINUTES)
$C_{18:1;9Z(12OH)}$	15.2
$C_{12:0}$	22.0
$C_{18:3;9Z,12Z,15Z}$	34.8
$C_{14:0}$	37.4
$C_{16:1;9Z}$	40.2
$C_{18:2;9Z,12Z}$	46.8
$C_{16:0}$	62.8
$C_{18:1;9Z}$	65.8
$C_{18:1;9E}$	68.2
$C_{18:0}$	78.0
$C_{22:1;13Z}$	87.9

3B-7. HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC) RESULTS FOR CASTOR AND LINSEED OILS UNDER ISOCRATIC CONDITIONS

The fatty acids of the commercial samples of castor and linseed oils were derivatized according to the procedure explained in section 2A-16. In the case of castor oil, a concentration of 25.62 μ_c/ml in benzene of the mixed fatty acid ester derivatives were chromatographed as already discussed using a mixture of methanol, acetonitrile and water at a proportion of 87:5:8 respectively with a chart speed of 0.1cm min^{-1}. The spectrum obtained is shown in Figure 23. Similarly, a mixture of the fatty acid ester derivatives of linseed oil was also chromatographed. The resulting spectrum has been shown in Figure 24 . For each component of the p-phenylphenacyl ester mixtures from the castor oil and linseed oil, qualitative and quantitative results have been obtained. For the qualitative analysis, a comparison of the retention time of the peaks obtained here was done with those of the standards obtained when using a chart speed of 0.25 cm min^{-1}. For quantitative purposes, the total areas were obtained by using the tangent approximation method of measuring the areas under each major peak. The concentration of each component was obtained by expressing the area of the latter as a percentage of the total

area under all the peaks on each spectrum.

The castor oil fatty acid ester mixture was found to contain mainly 12-hydroxy -Z-9-octadecenoic ($C_{18:1;9Z(12OH)}$ derivative (R.T. = 15.4 min; relative concentration = 94.8%), which is denoted as peak 1 in Figure 23 . The other components in the castor oil ester mixture were very minor. One of the minor components is the p-phenylphenacyl ester of Z-9,Z-12-octadecadienoic ($C_{18:2}$) acid (R.T. = 47.5 min; concentration = 0.7%), denoted by peak 2 in Figure 23 . The other minor component identified is the p-phenylphenacyl ester of the Z-9-octadecenoic ($C_{18:1}$) acid which appears in peak 3 in Figure 23 (R.T. = 65.2 min; concentration = 1.1%). Peaks 4 and 5 (R.T. of 69.5 min and 115 min) were not identified. Their total composition is jointly very minimal. Besides p-phenylphenacyl ester of the 12-hydroxy - Z-9-octadecenoic acid (peak 1), there seems to be another unidentified peak not very clearly resolved as that appearing as a shoulder off peak 1.

Four of the most abundant components of p-phenylphenacyl ester derivatives of the linseed oil fatty acids have been identified. Peak 3 (R.T. = 34.5 min; concentration = 24.8%)

represents the ester derivative of Z-9,Z-12,Z-15-octadecatrienoic acid ($C_{18:3}$); peak 4 (R.T. = 46.5 min; concentration = 8.3%) is the ester derivative of Z-9,Z-12-octadecadienoic acid ($C_{18:2}$); peak 5 (R.T. = 63 min; concentration = 21.3%) is the ester derivative of hexadecanoic acid $C_{16:0}$, and peak 6 (R.T. = 66.9 min, concentration (30.4%) is the p-phenylphenacyl ester of Z-9-octadecenoic acid ($C_{18:1}$). Peak 7, when analysed by a procedure explained in the next section (3B-8), can be predicted to be the p-phenylphenacyl ester of octadecanoic acid ($C_{18:0}$). Its retention time and concentration were found to be 109.3 min. and 9.3% respectively. The retention time agrees well with that for $C_{18:0}$ (111 min) deduced from Figure 25. The peaks 1 and 2 whose total concentration is 5.9% were not identified. However, with greater availability of the required standards, all of these fatty acids could be identified without ambiguity.

The levels of 12-hydroxy-Z-9-octadecenoic (95%), Z-9-octadecenoic (1.1%) and Z-9,Z-12-octadecadienoic (0.7%) ester derivatives found in our castor oil vary from those reported in the literature as shown in Table 6. This may have been due to incomplete esterification of the oil and favouring derivatisation of 12-hydroxy-Z-9-octadecenoic acid, hence reducing the relative concentration of the other fatty acid derivatives during analysis. Alternatively this Kenyan species of castor may be a superior producer of 12-hydroxy-Z-9-octadecenoic acid than others that have been assessed before; further work might clarify this matter.

Also as can be observed, the results obtained from the linseed oil analysis vary with reported values. Our oil sample showed less Z-9, Z-12, Z-15-octadecatrienoic acid ester derivative (24.8%) and more of the fatty acid derivatives (30.3%) were detected in our oil, compared to the values reported in Table 6. The linseed oil we analysed and had been labelled 'raw' and hence possibly the great variation in the results might be due to the presence of impurities or adulteration. Further investigations might reveal the cause for these discrepancies.

3B-8. RELATIONSHIP OF THE RETENTION TIMES OF THE p-PHENYLPHENACYL ESTERS WITH MOLECULAR WEIGHT AND DEGREE OF UNSATURATION

The p-phenylphenacyl esters synthesized in this work were found to show a linear relationship between gram molecular weight and the logarithm of the respective retention times. A graph has been plotted (Figure 25) using the data from Table 23 .

Table 23 : Gram Molecular Weight, Retention Time and Logarithm of Retention Time For p-Phenylphenacyl Esters.

p-PHENYLPHENACYL ESTER DERIVATIVES OF THE FATTY ACID	GRAM MOLECULAR WEIGHT (GMW)	RETENTION TIME (R.T) (MIN)	LOG_{10} R.T
$C_{12:0}$	394	22.4	1.35
$C_{14:0}$	422	38.4	1.58
$C_{16:0}$	450	68.4	1.84
$C_{16:1;9Z}$	448	41.4	1.62
$C_{18:1;9Z}$	476	72.6	1.86
$C_{18:2;9Z,12Z}$	474	48.6	1.69
$C_{18:3;9Z,12Z,15Z}$	472	34.8	1.54

Conditions: Mobile phase used was a mixture of methanol, water and aceteonitrile (87:8:5 respectively) at a flow rate of 0.75 ml/min.

From the plot, three observations can be made. Firstly, the fact that in the saturated series, the larger the GMW, the longer is the retention time. This is contributed to by the fact that every $-(CH_2)_2-$ group added to a saturated series adds to the latter some non-polar character and hence causes it to have a longer retention time in this reverse-phase system. It is noticed that each additional $-(CH_2)_2-$ group produces the same increase between every saturated fatty acid ester derivative. This makes it possible to predict, for example, the retention times of p-phenylphenacyl ester derivatives of $C_{18:0}$ (111.0 min). This implies that an unknown saturated fatty acid p-phenylphenacyl ester derivative can be identified by use of the plot shown in Figure 25 . Secondly, although only two mono-unsaturated members of the homologous series has been plotted, an increase in chain length by a $-(CH_2)_2-$ group seems to have the same effect on retention time as in the case of the saturated series. However, prediction of the retention time of a member of the homologous series of the Z-9 unsaturated acid esters is only tentative, since the plot was determined using only two points, which strictly speaking are not sufficient.

Thirdly, it can be observed that on the "C_{18} degree of saturation" curve, the greater the number of double bonds in a fatty acid of the same chain length, the shorter the retention time. This is explainable by the fact that each additional double bond increases the polarity of the fatty acid and hence the latter is eluted faster.

In conclusion, it can be said that the mixture of methanol, water and acetonitrile (87:8:5) used as a mobile phase is adequate for the separation and estimation of common saturated and unsaturated fatty acids of chain length C_{10} - C_{22} in an isocratic elution system.

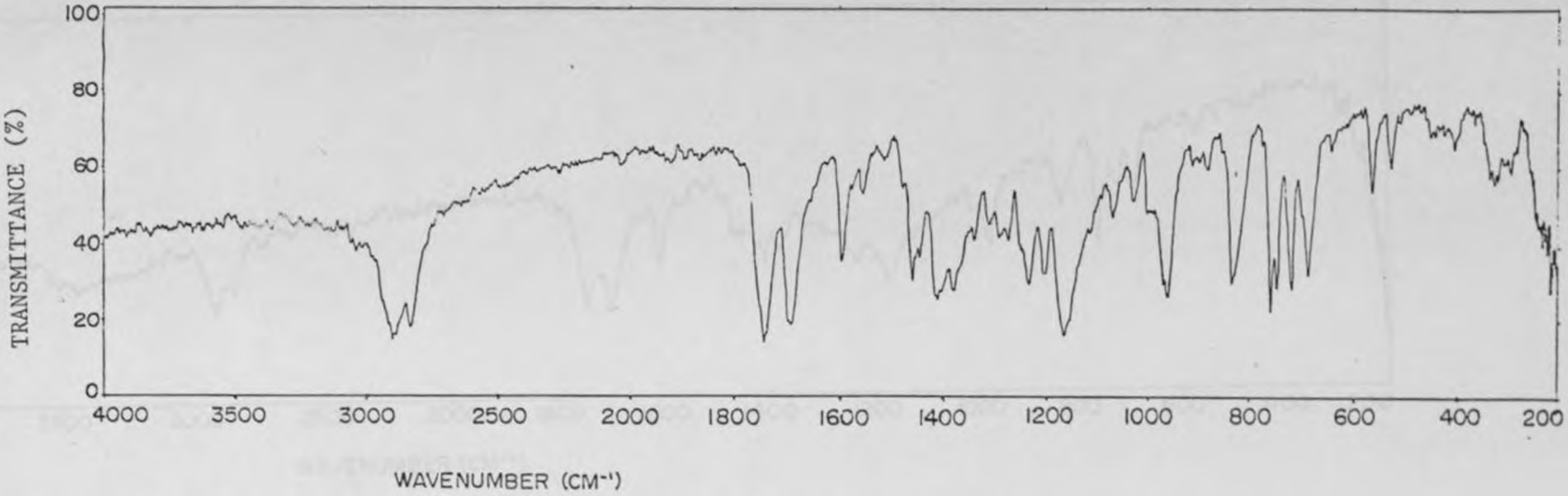


Figure 3: IR Spectrum of E-9-Octadecenoic acid, p-phenylphenacyl ester.

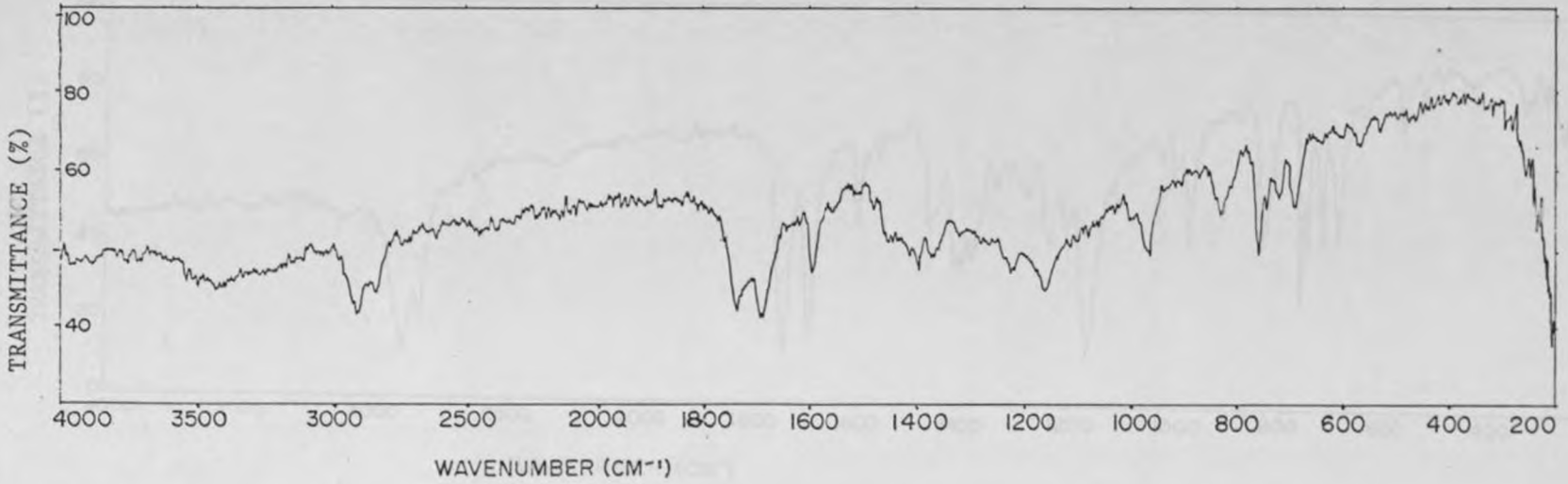


Figure 4: IR Spectrum of Z-9,Z-12-Octadecadienoic acid, p-phenylphenacyl ester.

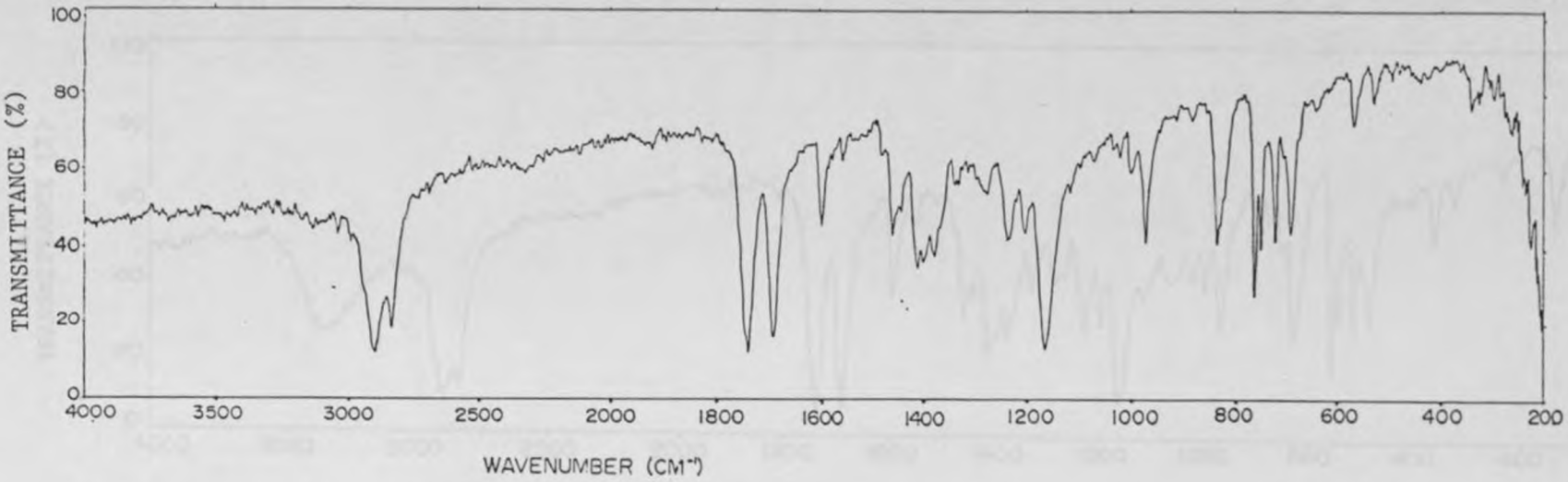


Figure 5: IR Spectrum of Z-9,Z-12,Z-15-Octadecatrienoic acid, p-phenylphenacyl ester.

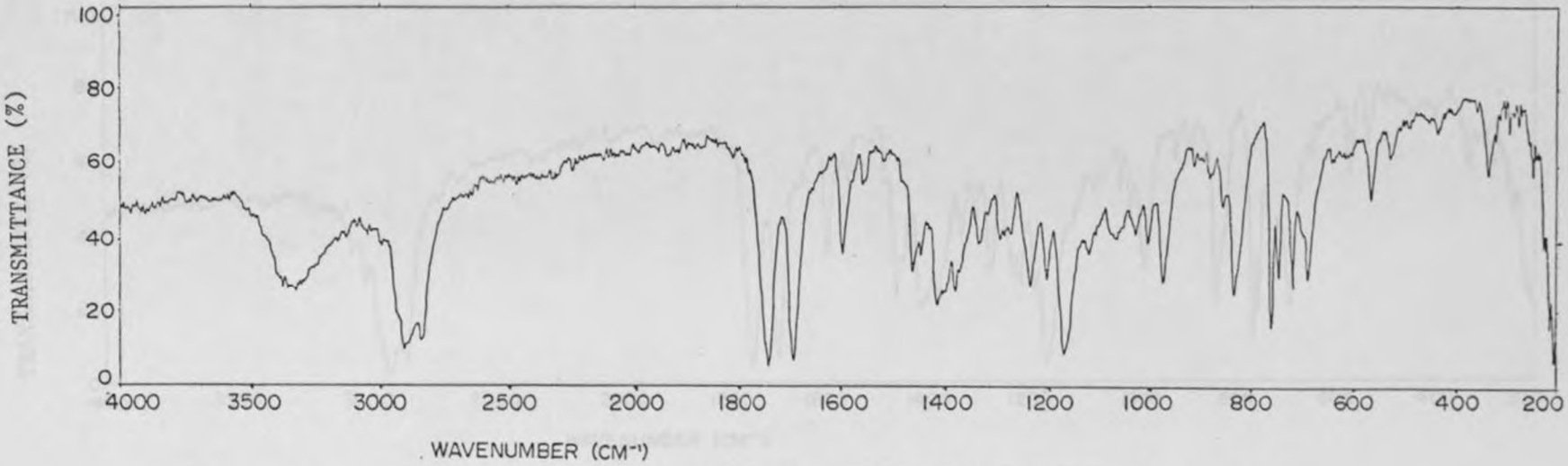


Figure 6: IR Spectrum of 12-hydroxy-Z-9-Octadecenoic, p-phenylphenacyl ester.

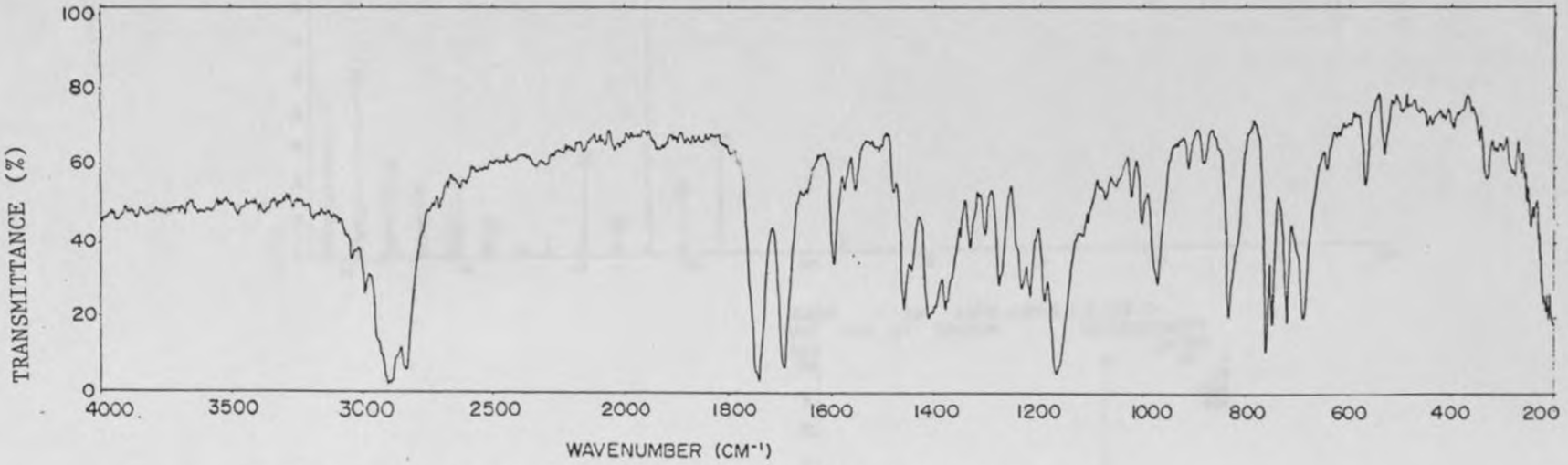


Fig. 7: IR Spectrum of Z-13-docosenoic acid, p-phenylphenacyl ester.

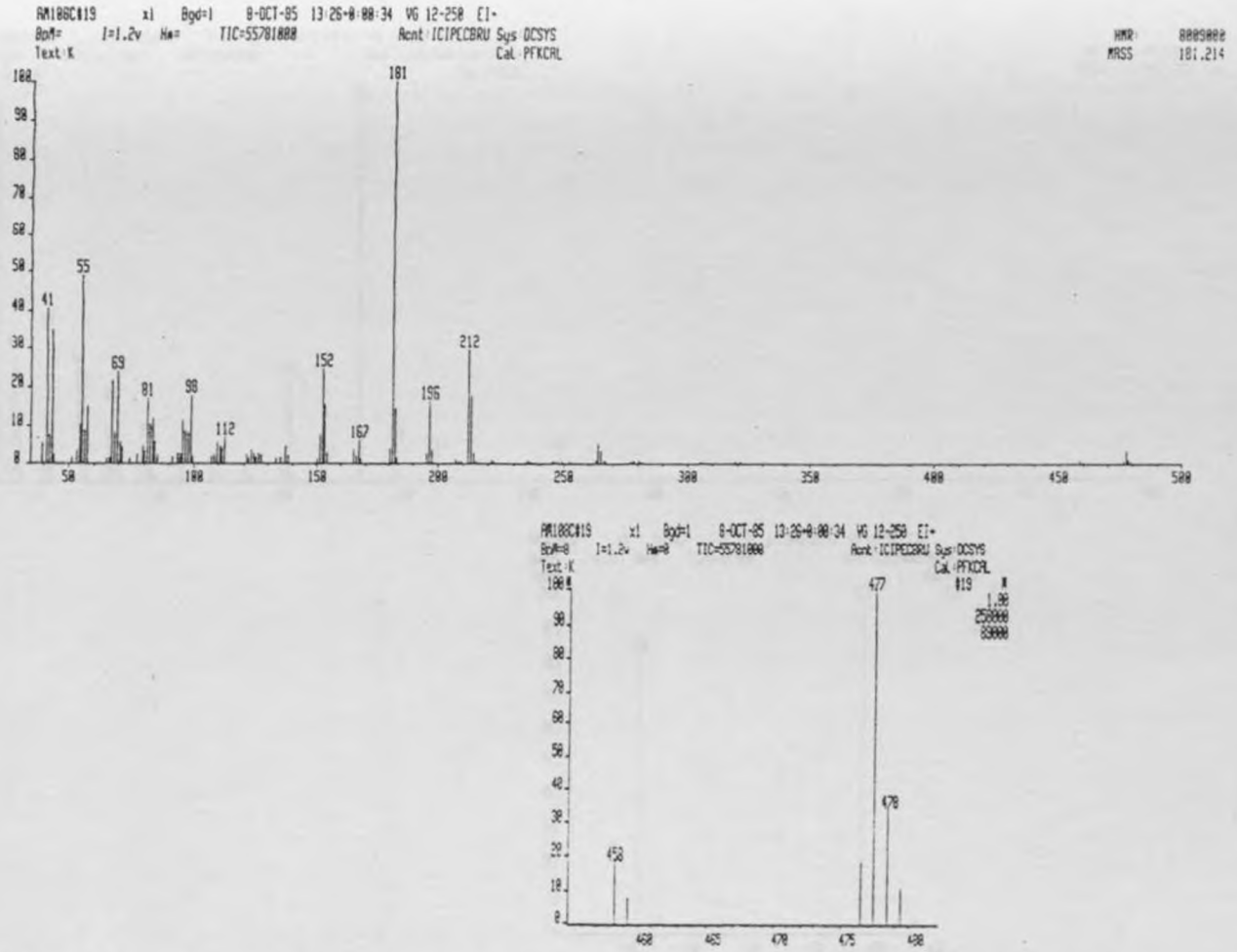


Figure 8. Mass Spectrum of p-phenylphenacyl ester of E-9-octadecenoic acid.

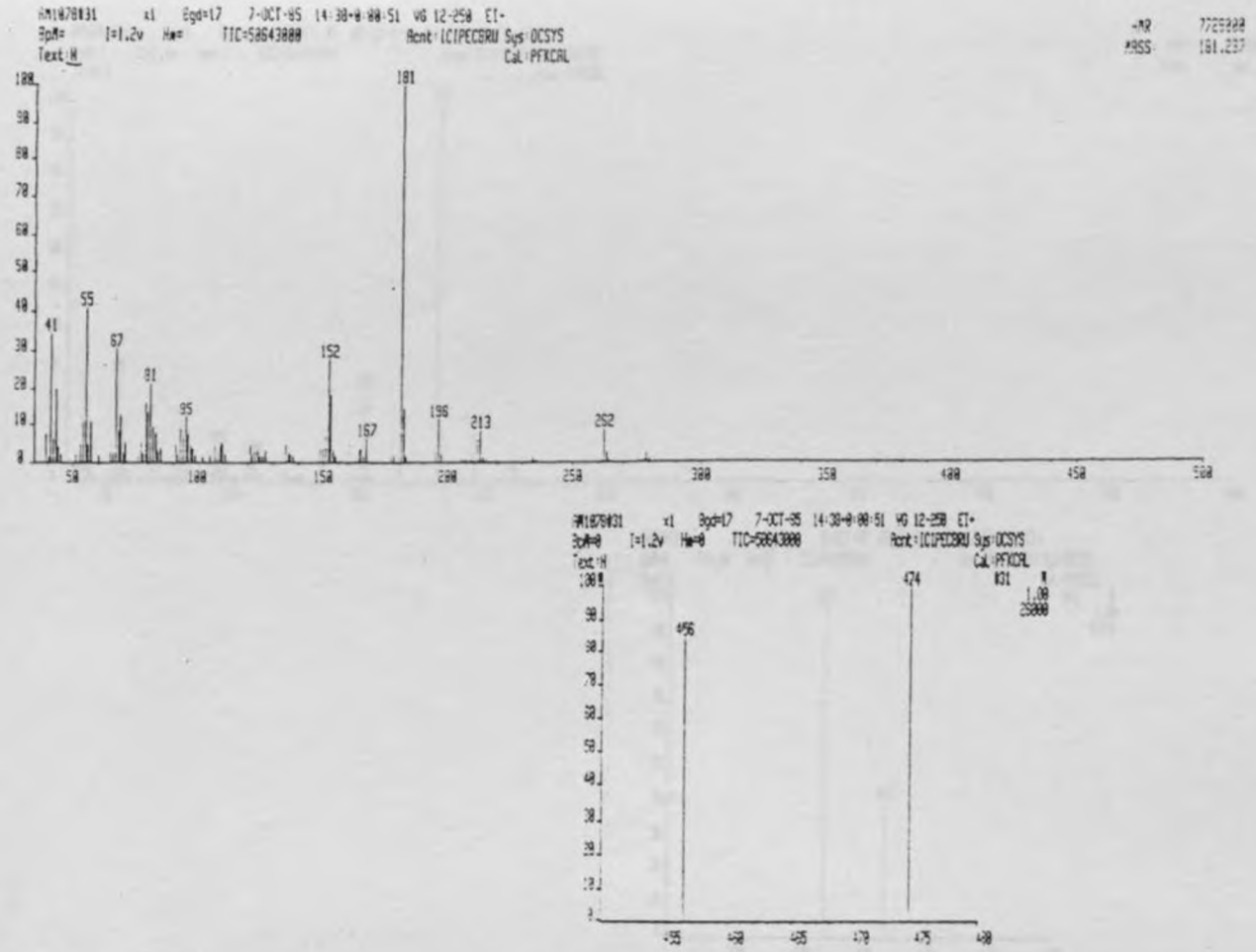


Figure 9. Mass Spectrum of p-phenylphenacyl ester of Z-9, Z-12-octadecadienoic acid.

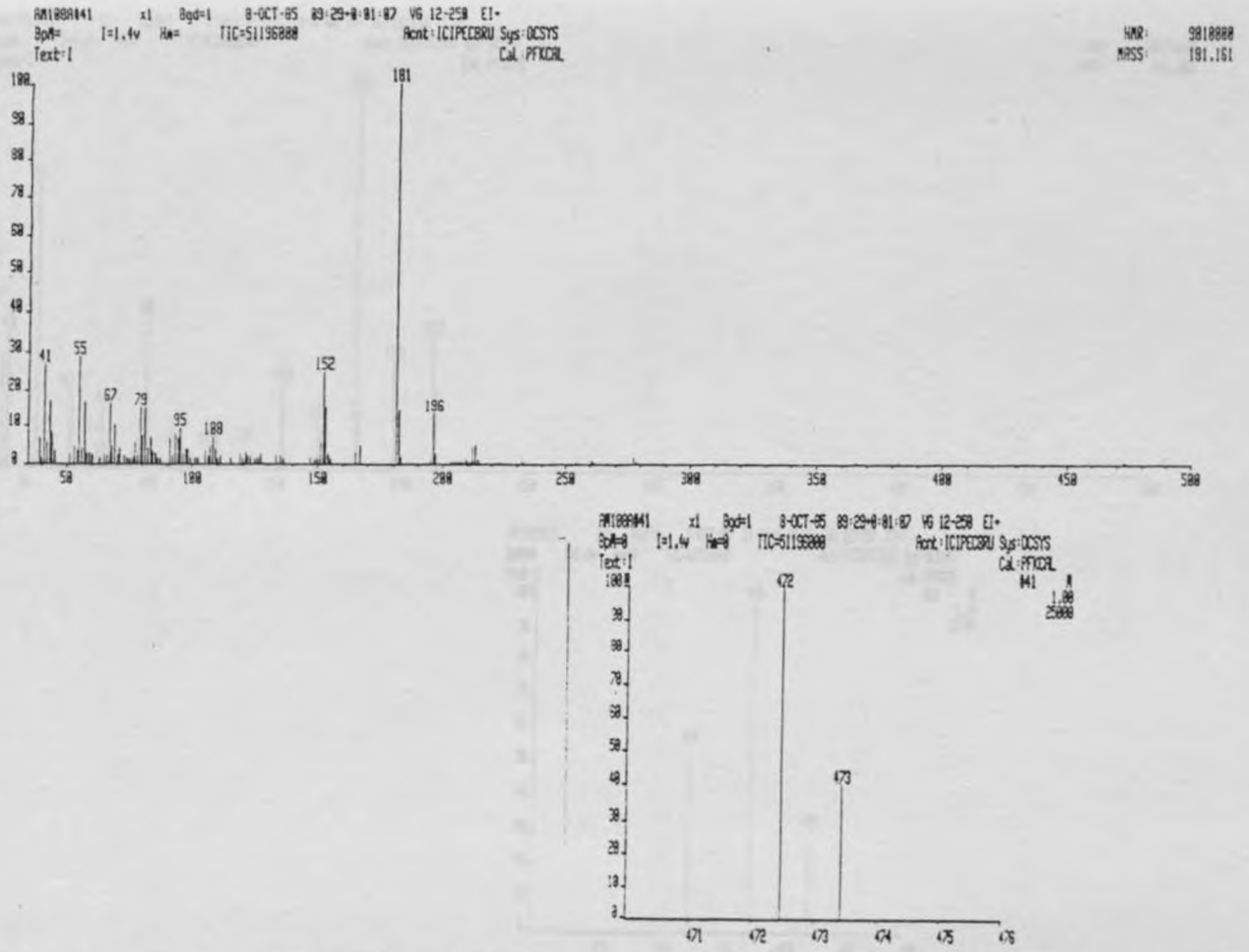


Figure 10. Mass Spectrum of p-phenylphenacyl ester of Z-9, Z-12, Z-15-octadecatrienoic acid.

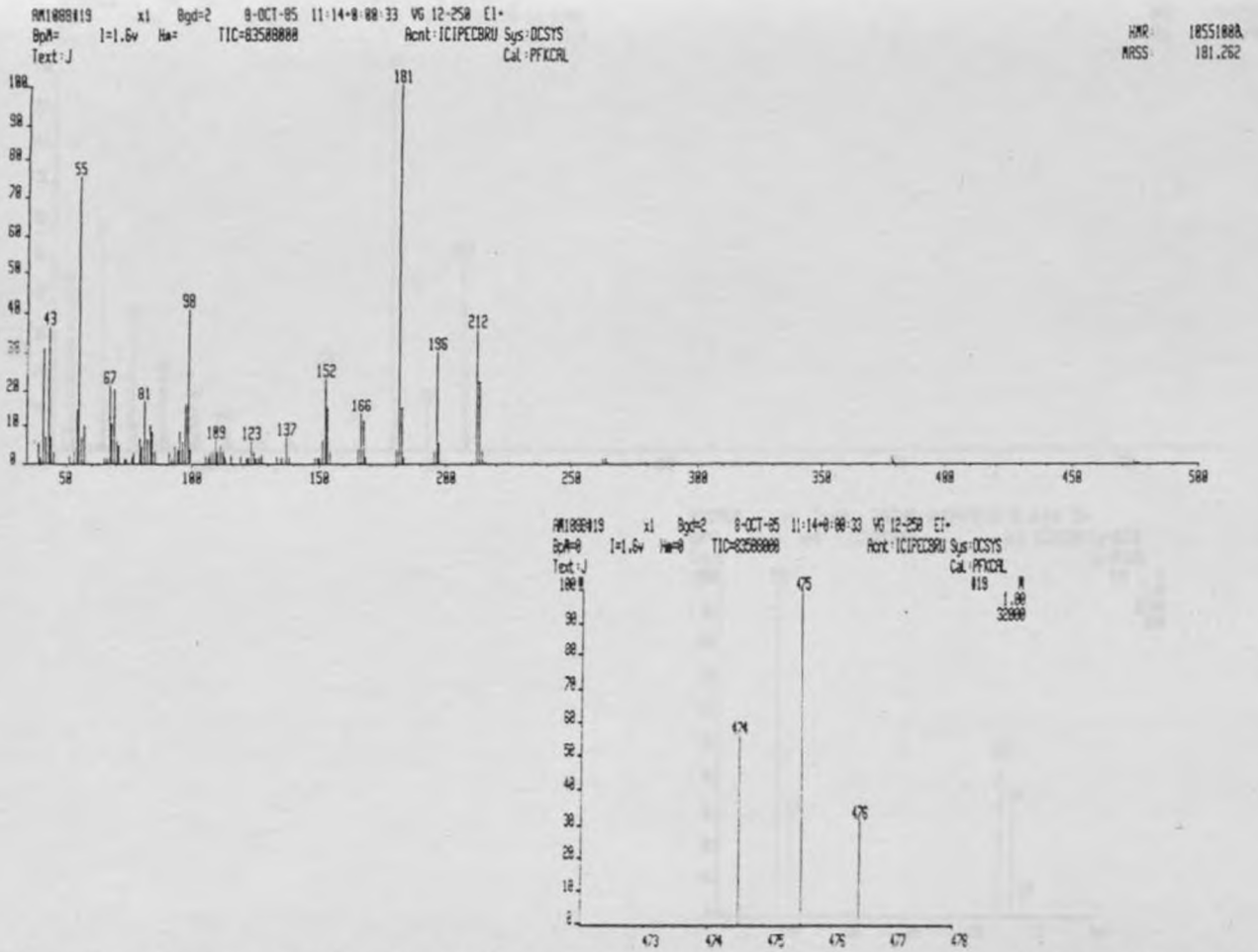


Figure 11. Mass Spectrum of p-phenylphenacyl ester of 12-hydroxy-Z-9-octadecenoic acid.

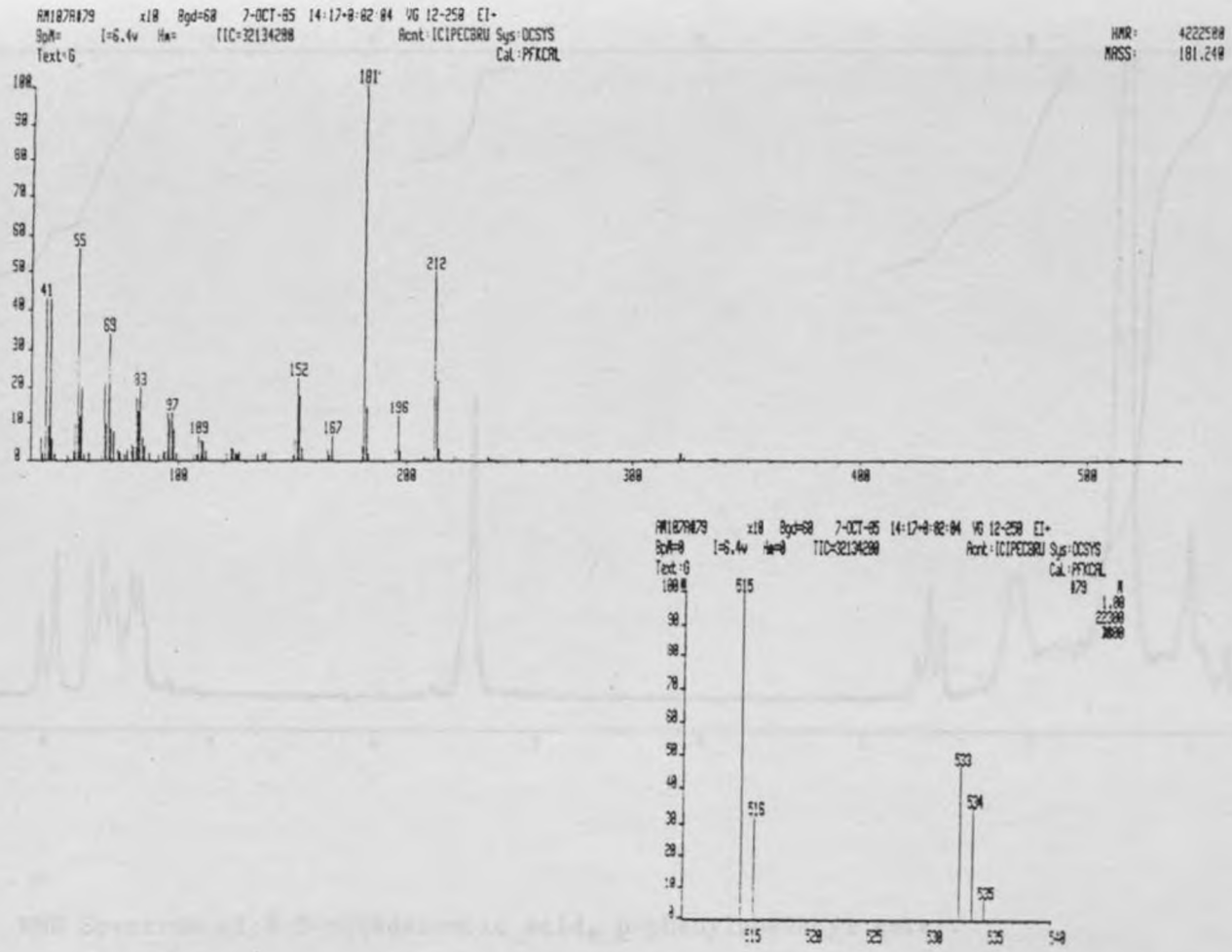


Figure 12. Mass Spectrum of p-phenylphenacyl ester of Z-13-docosenoic acid.

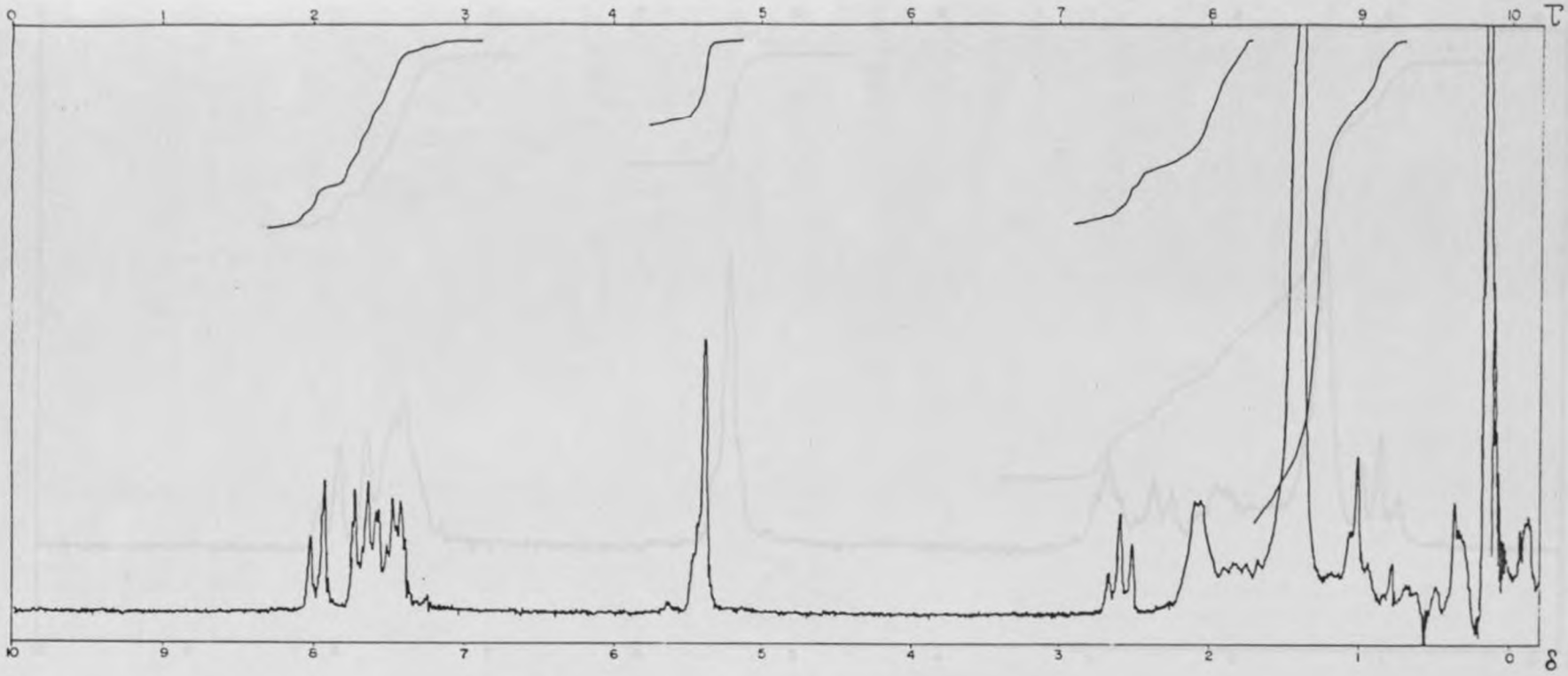


Figure 13. NMR Spectrum of E-9-octadecenoic acid, p-phenylphenacyl ester.

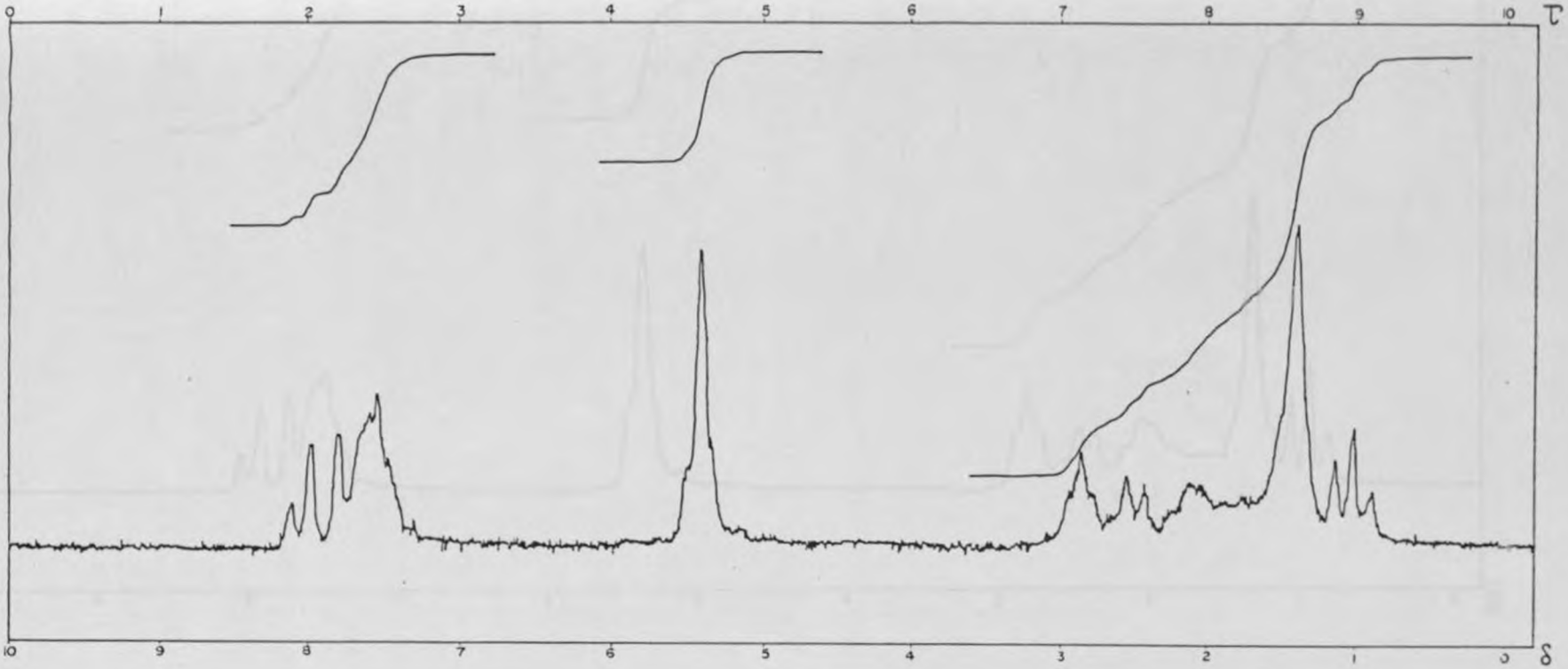


Figure 14. NMR Spectrum of Z-9, Z-12-octadecadienoic acid, p-phenylphenacyl ester.

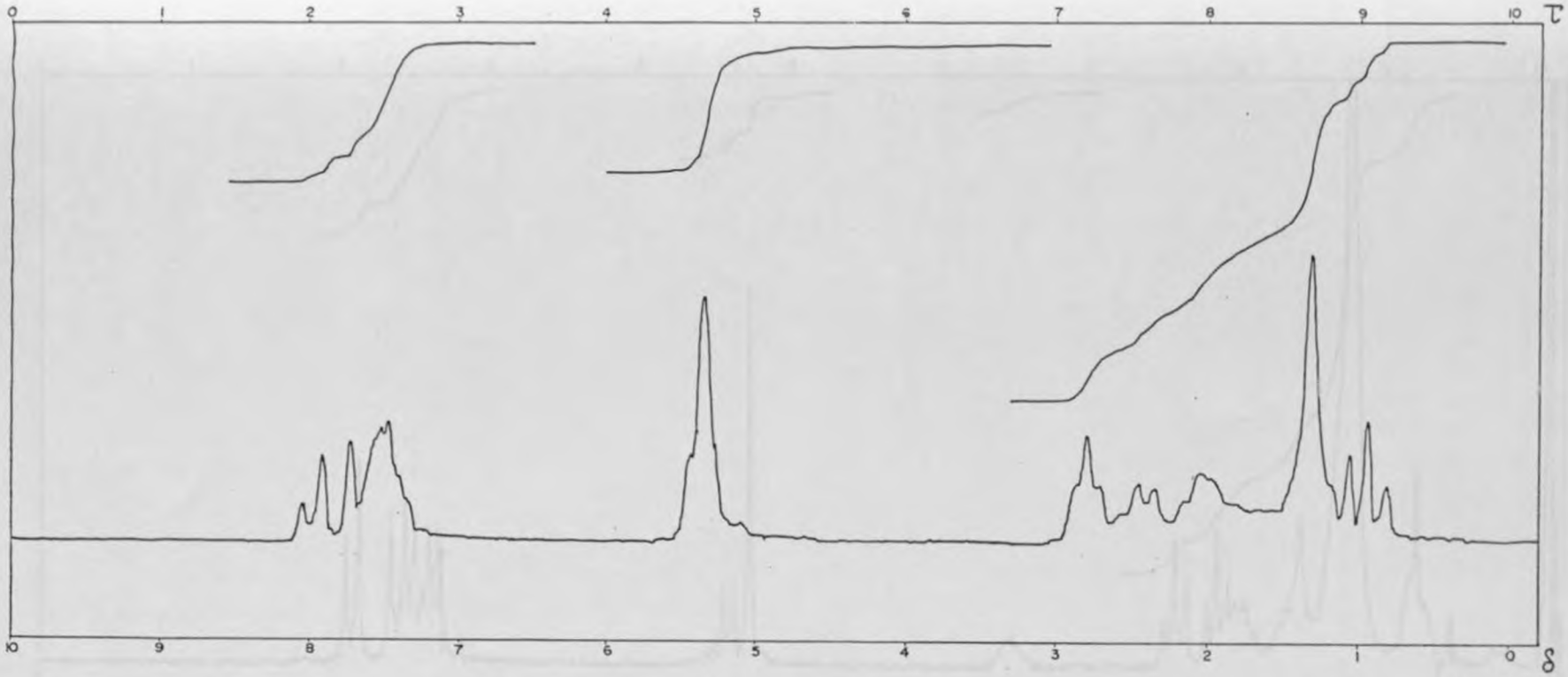


Figure 15. NMR Spectrum of Z-9, Z-12, Z-15-octadecatrienoic acid, p-phenylphenacyl ester.

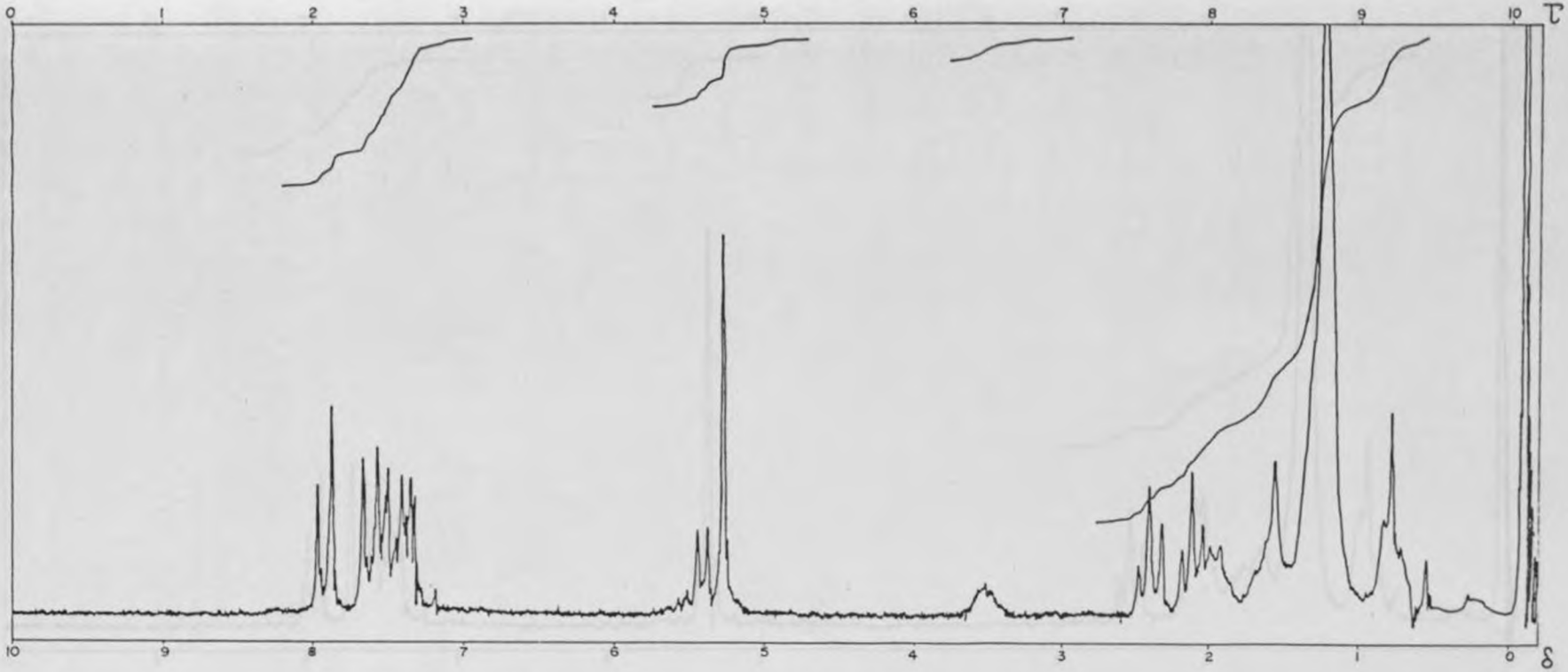


Figure 16. NMR Spectrum of 12-hydroxy - Z-9-octadecenoic acid, p-phenylphenacyl ester.

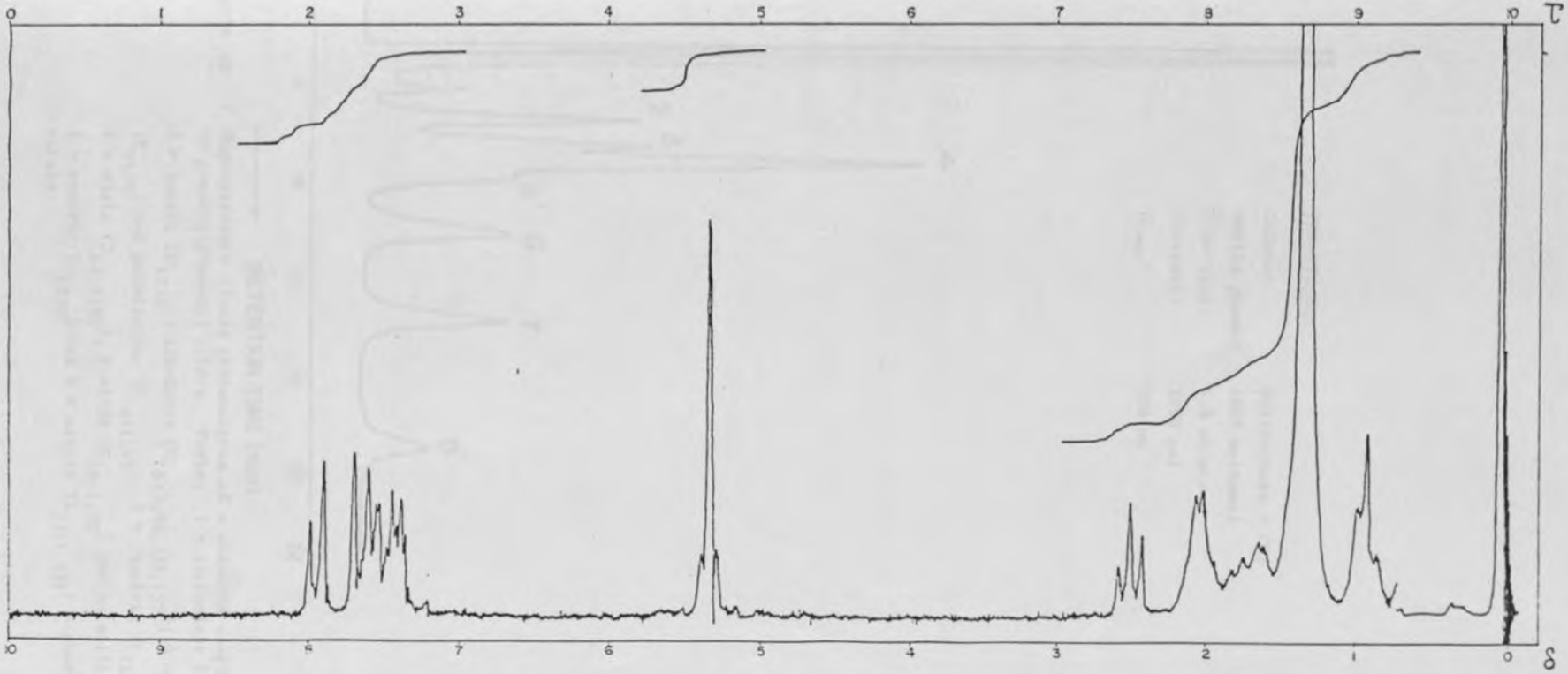


Figure 17. NMR Spectrum of Z-13-docosenoic acid, p-phenylphenacyl ester.

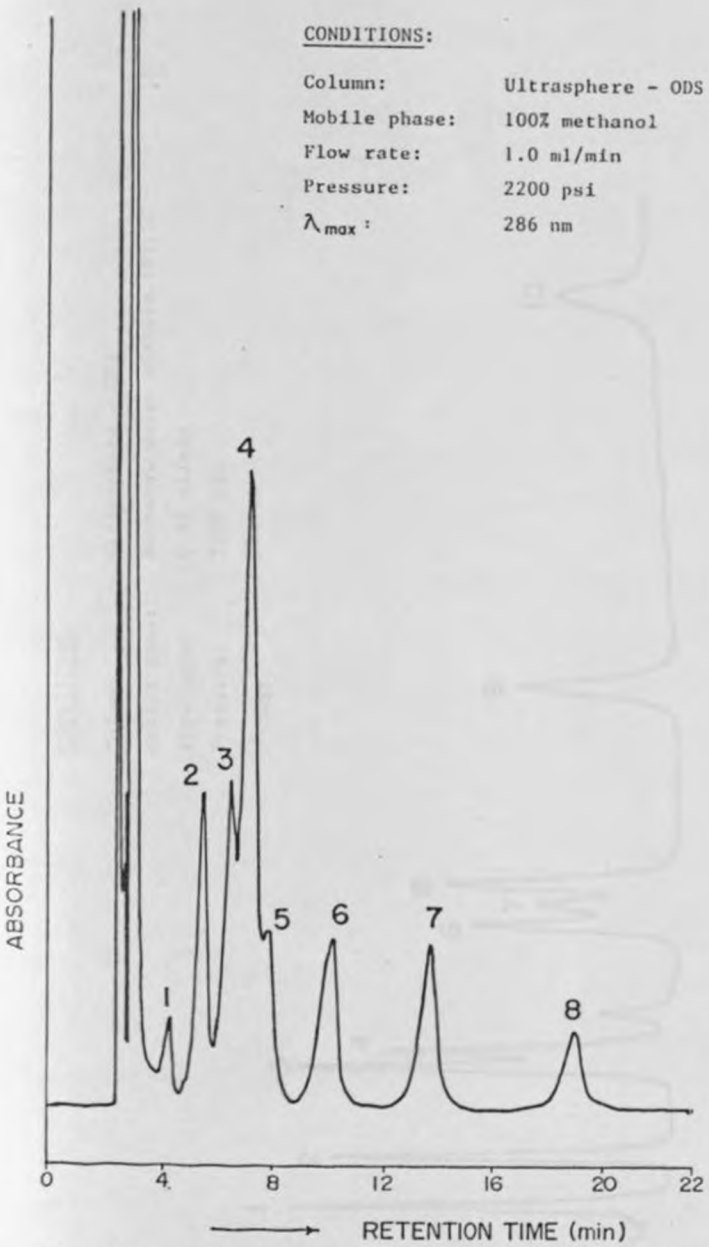


Figure 18 : High-pressure liquid chromatogram of a standard sample mixture of p-phenylphenacyl esters. Peaks: 1 = ricinoleic ($C_{18:1;9Z,12OH}$) 2 = lauric ($C_{12:0}$); linolenic ($C_{18:3;9Z,12Z,15Z}$); 4 = myristic ($C_{14:0}$) and palmitoleic ($C_{16:1;9Z}$), 5 = linoleic ($C_{18:2;9Z,12Z}$); 6 = oleic ($C_{18:1;9Z}$), elaidic ($C_{18:1;9E}$) and palmitic ($C_{16:0}$); 7 = stearic ($C_{18:0}$) and 8 = erucic ($C_{22:1;13Z}$) p-phenylphenacyl esters.

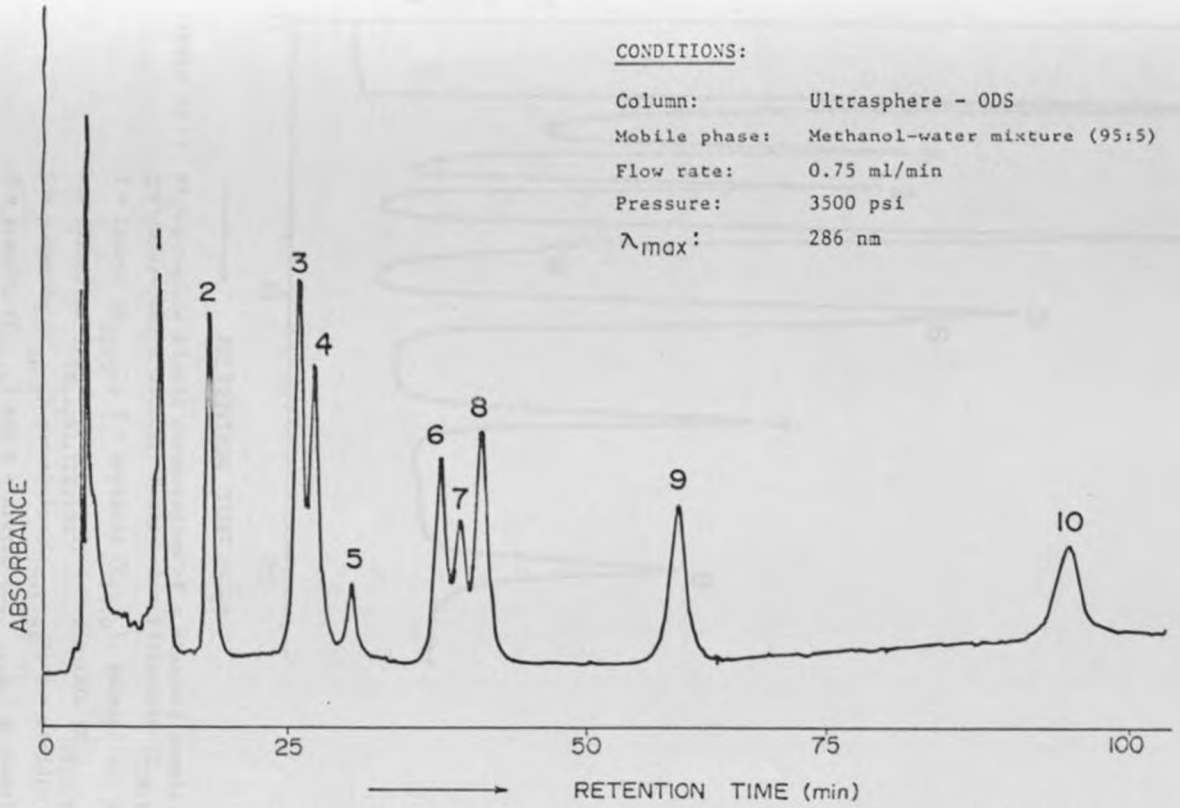


Figure 19 : High-pressure liquid chromatogram of a standard sample mixture of p-phenylphenacyl esters. Peaks: 1 = ricinoleic ($C_{18:1;9Z,12OH}$); 2 = lauric ($C_{12:0}$); 3 = myristic ($C_{14:0}$) and linolenic ($C_{18:3;9Z,12Z,15Z}$); 4 = palmitoleic ($C_{16:1;9Z}$); 5 = linoleic ($C_{18:2;9Z,12Z}$); 6 = palmitic ($C_{16:0}$); 7 = oleic ($C_{18:1;9Z}$); 8 = elaidic ($C_{18:1;9E}$); 9 = stearic ($C_{18:0}$) and 10 = erucic ($C_{22:1}$) p-phenylphenacyl esters.

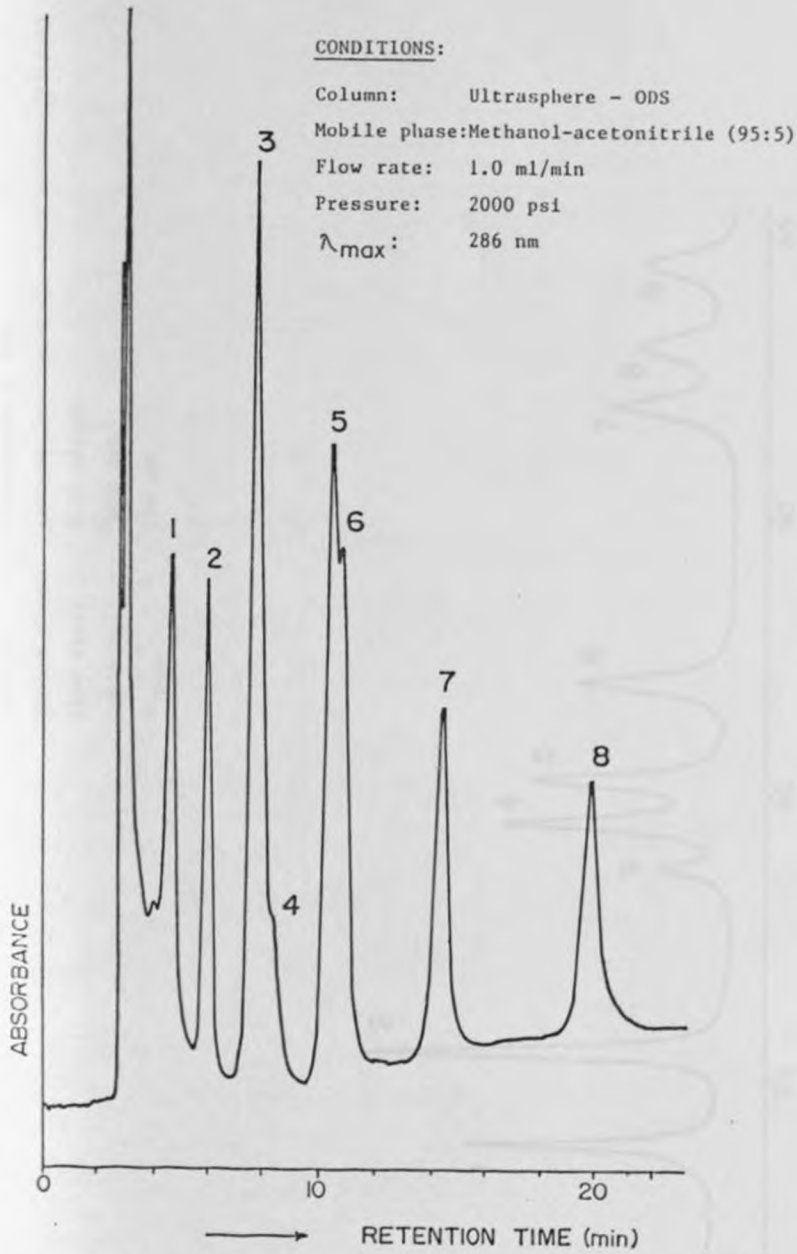


Figure 20 : High-pressure liquid chromatogram of a standard sample mixture of p-phenylphenacyl esters. Peaks: 1 = ricinoleic ($C_{18:1;9Z,12OH}$); 2 = lauric ($C_{12:0}$); 3 = myristic ($C_{14:0}$), palmitoleic ($C_{16:1,9Z}$) and linolenic ($C_{18:3;9Z,12Z,15Z}$); 4 = linoleic ($C_{18:2;9Z,12Z}$), 5 = palmitic ($C_{16:0}$) and oleic ($C_{18:1;9Z}$); 6 = elaidic ($C_{18:1,9E}$) 7 = stearic ($C_{18:0}$) and 8 = erucic ($C_{22:1;13Z}$) p-phenylphenacyl esters.

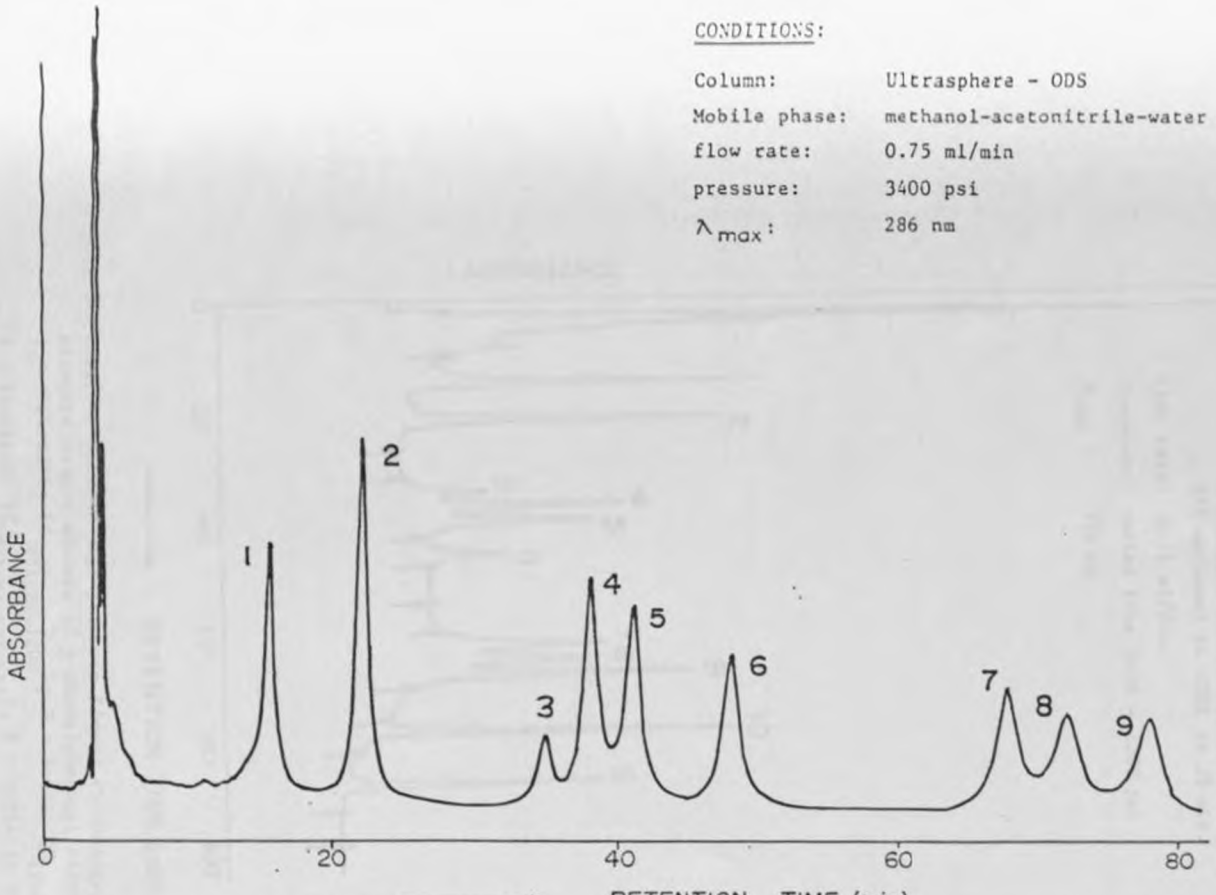


Figure 21. High-pressure liquid chromatogram of a standard sample mixture of p-phenylphenacyl esters. Peaks: 1= ricinoleic ($C_{18:1;9Z,12OH}$); 2 = lauric = ($C_{12:0}$); 3 = linolenic($C_{18:3;9Z,12Z,15Z}$); 4 = myristic ($C_{14:0}$); 5 = palmitoleic ($C_{16:1;9Z}$); 6 = linoleic ($C_{18:2;9Z,12Z}$); 7 = palmitic ($C_{16:0}$); 8 = oleic ($C_{18:1;9Z}$) and 9 = elaidic ($C_{18:1;9E}$) p-phenylphenacyl esters.

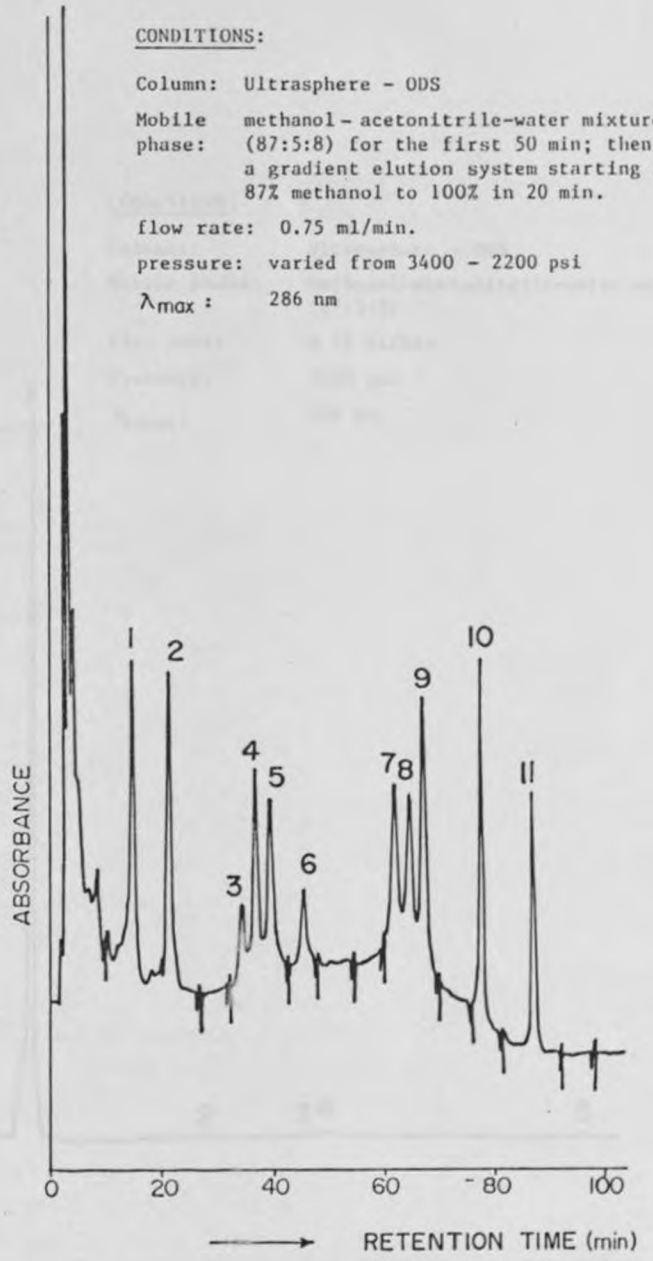


Figure 22: Gradient elution high-pressure liquid chromatogram of a standard sample mixture of p-phenylphenacyl esters. Peaks: 1 = ricinoleic ($C_{18:1;9Z,12OH}$); 2 = lauric ($C_{12:0}$); 3 = linolenic ($C_{18:3;9Z,12Z,15Z}$); 4 = myristic ($C_{14:0}$); 5 = palmitoleic ($C_{16:1;9Z}$); 6 = linoleic ($C_{18:2;9Z,12Z}$); 7 = palmitic ($C_{16:0}$); 8 = oleic ($C_{18:1;9Z}$); 9 = elaidic ($C_{18:1;9E}$) 10 = stearic ($C_{18:0}$) and 11 = erucic ($C_{22:1;13Z}$) p-phenylphenacyl esters.

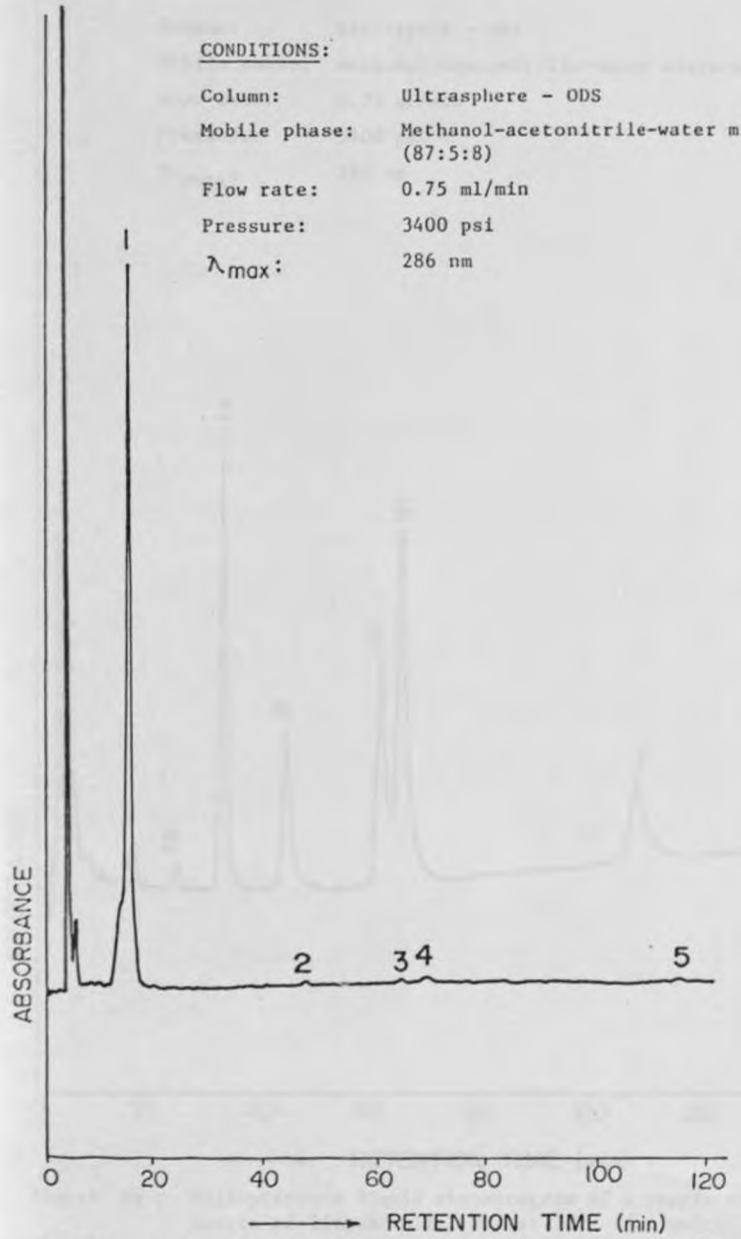


Figure 23 : High-pressure liquid chromatogram of a sample of p-phenylphenacyl esters of castor oil. Peaks: 1 = ricinoleic ($C_{18:1;9Z,12OH}$); 2 = linoleic ($C_{18:2,9Z,12Z}$) and 3 = oleic ($C_{18:1;9Z}$) p-phenylphenacyl esters. Peaks 4 and 5 have not been identified.

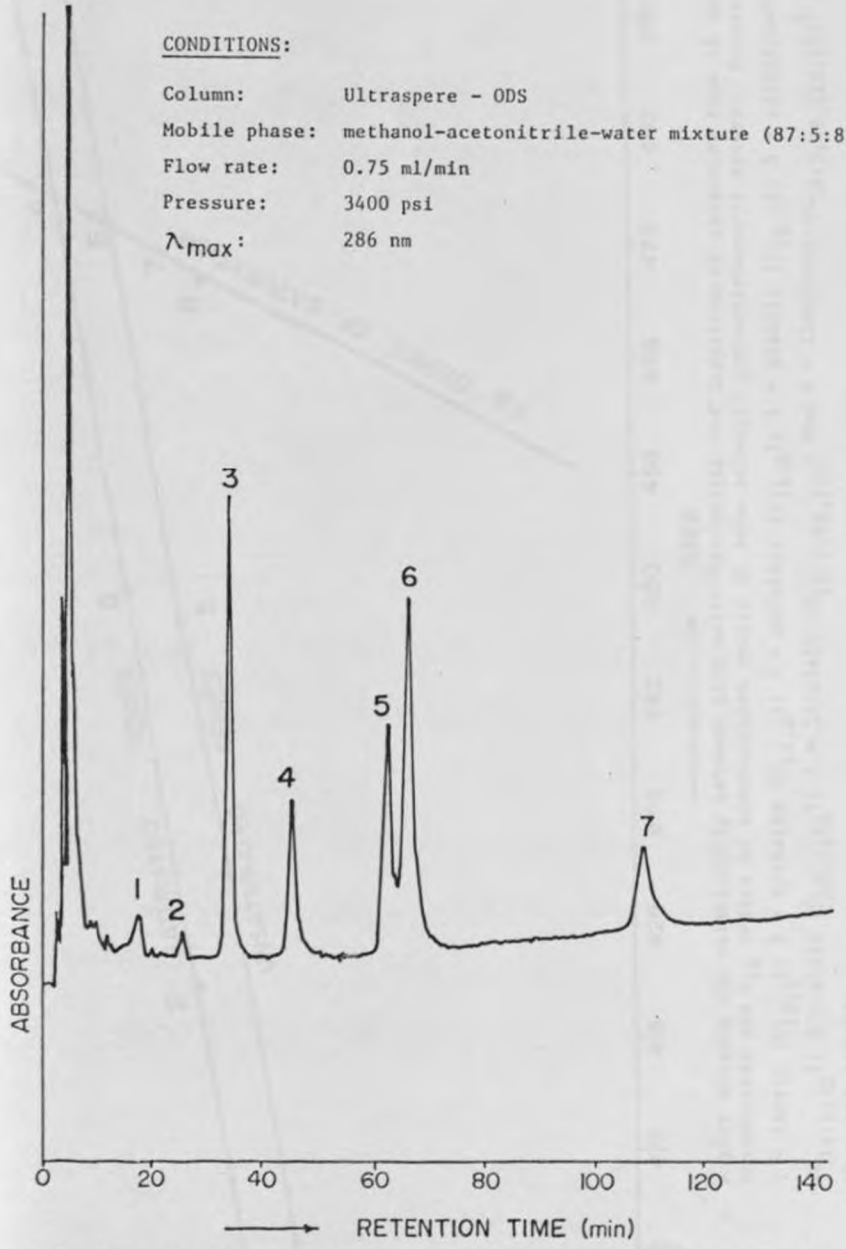


Figure 24 : High-pressure liquid chromatogram of a sample of p-phenylphenacyl esters of linseed oil. Peaks: 3 = linolenic ($C_{18:3;9Z,12Z,15Z}$); 4 = linoleic ($C_{18:2;9Z,12Z}$); 5 = palmitic ($C_{16:0}$) and 6 = oleic ($C_{18:1;9Z}$) p-phenylphenacyl esters. The peaks 1,2 and 7 have not been identified.

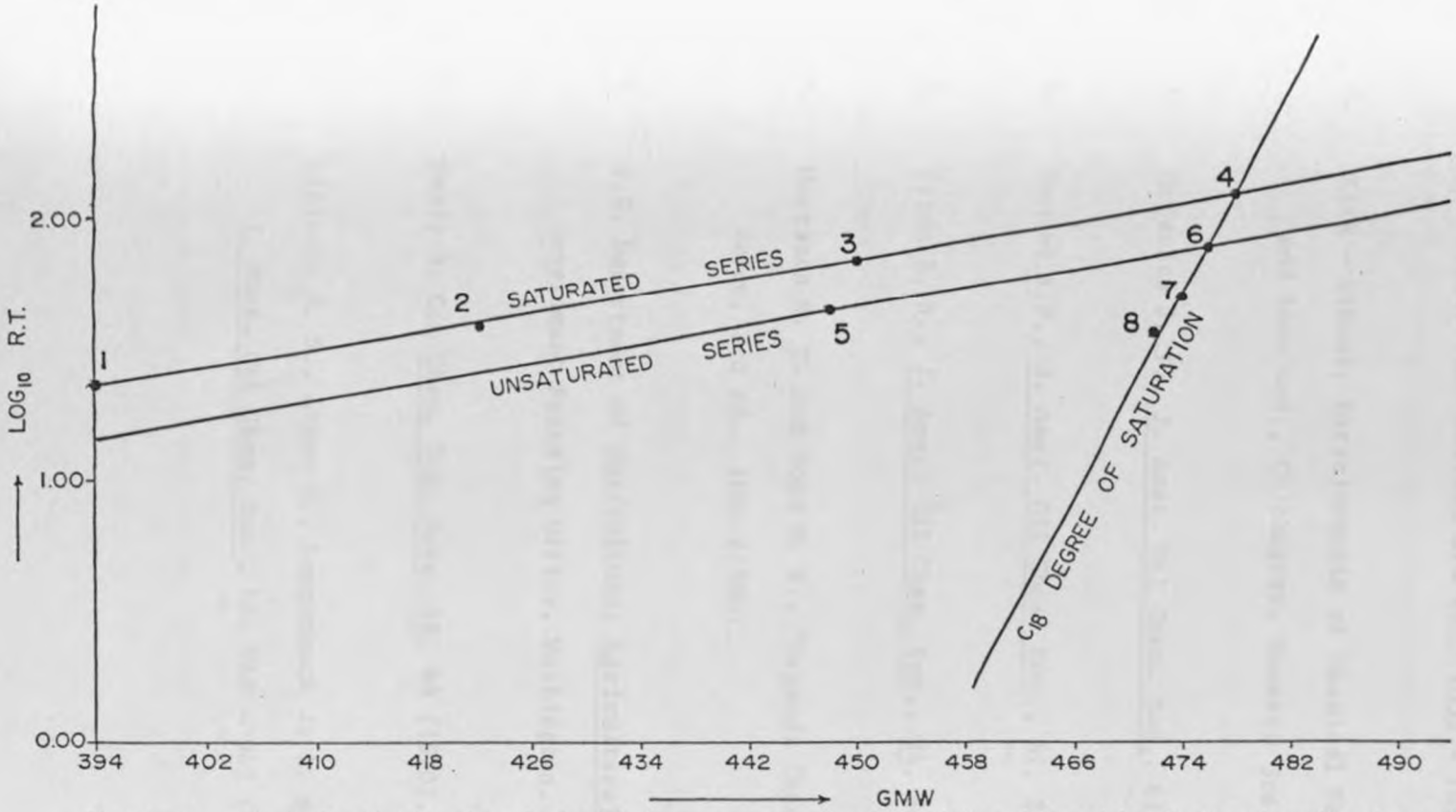


Figure 25 : A plot showing the relationship between gram molecular weight and logarithm of retention time of saturated, unsaturated and C_{18} degree of unsaturation series of some selected p-phenylphenacyl esters. Points: 1 = lauric ($C_{12:0}$); 2 = myristic ($C_{14:0}$); 3 = palmitic ($C_{16:0}$); 4 = stearic ($C_{18:0}$); 5 = palmitoleic ($C_{16:1;9Z}$); 6 = oleic ($C_{18:1;9Z}$); 7 = linoleic ($C_{18:2;9Z,12Z}$) and 8 = linolenic ($C_{18:3;9Z,12Z,15Z}$) p-phenylphenacyl esters.

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