THE SYNTHESES AND BIODEGRADATION OF MODEL COMPOUNDS RELATED TO HYDROCARBON-TYPE DIELECTRIC FLUIDS

by

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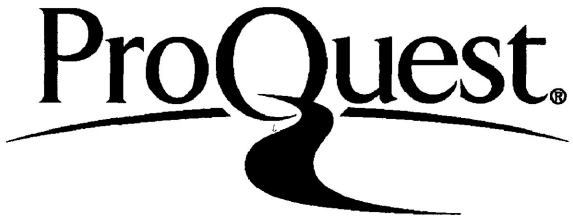

ABSTRACT

Uniroyal PAO-20E, an oligomer of 1-octene, is a dielectric fluid proposed as a replacement for polychlorinated biphenyls. No studies on its biodegradation have been reported in the literature to date.

This thesis contains the results of an investigation of the biodegradability of this commercial product, particularly by micro-organisms. Model compounds, such as 7-methylpentadecane and 7-methyl-9-hexylheptadecane, were employed in the study.

A major portion of the study deals with the synthesis of these model compounds by means of the malonic ester synthesis and with the characterisation of intermediate and final products.

Incubation experiments have been conducted with *Saccharomycopsis lipolytica* using PAO-20E and two synthetic model compounds as substrates. Gas chromatographic analyses of the extracts of such cultures did not result in clear evidence of degradation of these compounds by this organism.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my research supervisor, Dr. W. H. Baarschers, for his constant support and guidance througout the course of this work.

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CHAPTER 1

INTRODUCTION

This thesis describes an investigation of some branched hydrocarbons which have been described (1)[†] and patented (2) for use as dielectric fluids in applications where polychlorinated biphenyls (PCBs) and to a lesser extent, polybrominated biphenyls (PBBs), have been used previously. The justification for the use of such compounds as replacements for PCBs follows from the properties, particularly toxicity and persistence, of these halogen-containing compounds, which may be summarised as follows.

Polychlorinated and polybrominated biphenyls (I[*], X = Cl and X = Br respectively) are multicomponent mixtures of biphenyls in which one or both aromatic rings carry varying numbers of chlorine or bromine atoms as substituents.

Generally, PCBs are prepared on an industrial scale by direct chlorination of biphenyl either with gaseous chlorine (with iron filings as a catalyst) (3) or with a mixture of

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[†] Arabic numerals in parentheses indicate literature references on p. 134.

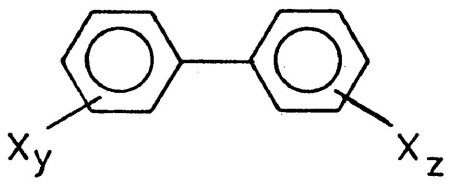
[*] Roman numerals refer to structural formulae (see p. 3)

gaseous and liquid chlorine (using iron(III)chloride as a catalyst) (4,5).

The resulting products (e.g., II: 2,4,6,2',4',6'-hexachlorobiphenyl) which are marketed under trade names such as Clophen, Phenoclor, Kanechlor and Aroclor, generally are liquids with high dielectric constants, thermal stability and are chemically inert. It is these properties that make this group of compounds ideally suited for use as transformer and capacitor fluids, use in hydraulic systems and in the manufacturing of electric cables (6,7).

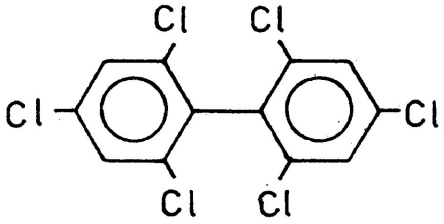
Polybrominated biphenyls (PBBs), the bromine analogs of PCBs are usually solids which have been used as flame retardants, in typewriter ribbons, microfilm processors and in radio (8,9) and other small electrical appliances.

The high chemical and biochemical inertness (7,8) of these compounds are responsible for their environmental persistence. Together with their toxic properties, this has caused a major environmental problem affecting animal and human health (9,10,11,12,13,14).



X = Cl or Br

I



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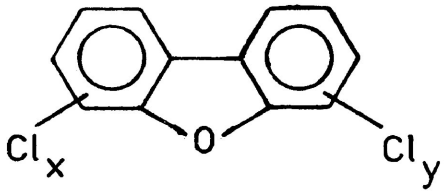

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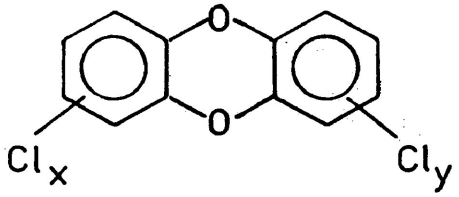

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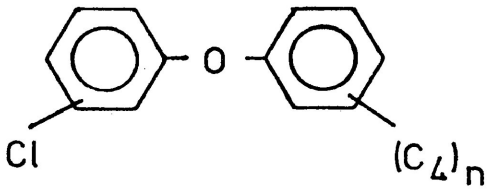

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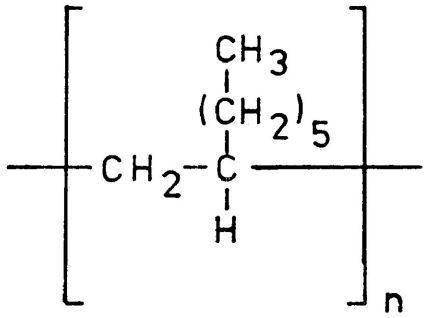


VI

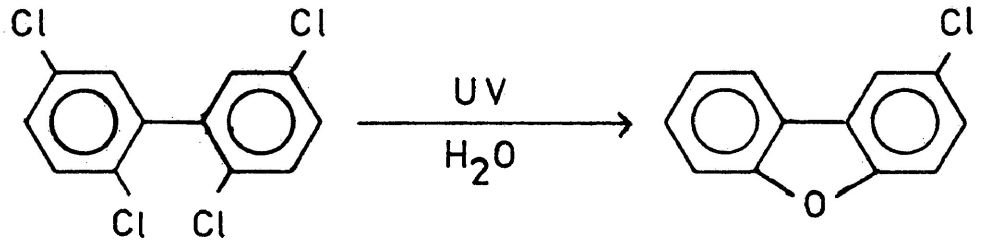
The toxic properties of PCBs and PBBs have been recognised for the past decade. Intake of PCBs has caused lowering of reproductive ability (10,12), including egg production, hatchability and deformities and depressed growth rate of the young in birds, decrease in pup survival rate, fetus abnormalities and even abortion in mammals like mice and rabbits; diseases such as edema in blackbirds (15), anemia, hypoproteinemia, bone marrow hypoplasia in monkeys (10) and even death. Cattle, which had taken in PBBs accidentally, exhibited decrease in milk production, loss in body weight and abnormal hoof growth (9). Both groups of compounds affect similar systems in a similar fashion in mammals, mainly the thyroid systems and the liver (10,14) where metabolism of the compound takes place. Some reactive metabolic intermediates, such as epoxides, may bind tightly with the liver tissues causing severe hepatotoxicity, as suggested by Brodie, *et al.* (16).

As a result of the same properties which make them useful, PCBs and PBBs persist in nature. PCBs have been detected in living organisms, fish (12), birds (12), mammals (12) and even human beings (9). Bioaccumulation is selective, depending on the kind of biospecies. Penta- and hexachlorobiphenyls are most commonly found. PBBs were found to persist in dairy products, to a maximum of 2.8 ppm, from the cattle (14) which had

been accidentally fed with PBBs. PCBs are not readily degraded. The only significant degradation process known is the photochemical progressive reductive dechlorination in hydrocarbon solvents, the reaction conditions of which are not found in the natural environment. On the other hand, although PBBs were expected to be less stable (8), they are not degraded because as solids, they are not easily transported to environmental sites favouring chemical or photo-induced degradation reactions.

An additional complication of the PCBs problem is that not only the compounds themselves are toxic, but also the by-products formed during the manufacture of PCBs have been found to be toxic. One of these byproducts is chlorodibenzofuran (III). This compound was detected in some of the commercial PCBs (17), and was found to cause severe and often lethal liver necrosis in rabbits in a single dose of 0.5-1.0 mg/kg body weight (18). Chlorinated dibenzofurans and dibenzodioxins (another toxic material of current interest) could be generated from PCBs under environmental conditions. The reaction conditions required can be either the ultra-violet components of sunlight (19,20) or heat encountered in wood burning (20). One example is furnished by 2,5,2',5'-tetrachlorobiphenyls in Equation [1] (p. 6)

Eq.[1] D.G. Crosby, *et al.* (20)



Due to their own toxic properties, their persistence in nature, non-degradability and the toxicity of their byproducts, PCBs and PBBs are undersirable. The widespread use of these compounds has led to inevitable leaks and spills and as a result these compounds are found to be widely distributed in the environment. It has thus become evident in recent years that less toxic and less persistent replacements for PCBs are urgently required. This has been recognised by the chemical industry and a number of potential replacements have been proposed.

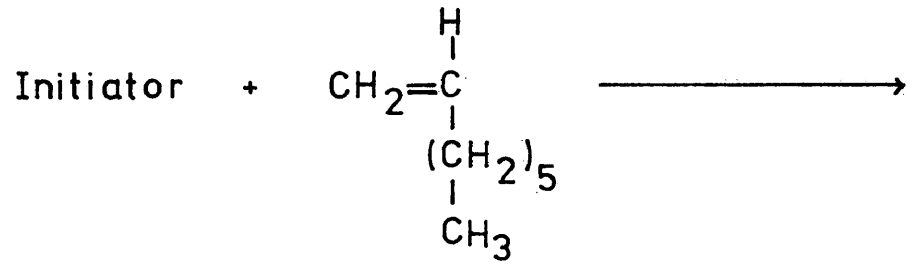
Thus, Monsanto Industrial Chemical Company has prepared hydrocarbon type compounds, MCS 1238 and MC 1588, which do not contain any chlorinated biphenyls. Dow Corning Incorporated has promoted a dimethyl silicone dielectric liquid,

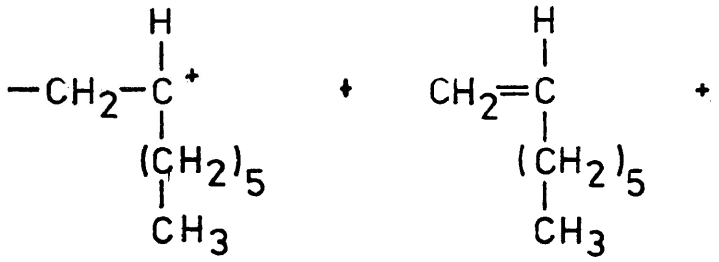
Dow Corning 02-1090, for use in power transformers. It is not biodegradable, but is said not to bioaccumulate. Dow XFS-4169L, a butylated monochlorodiphenyl oxide (V) is manufactured by Dow Chemicals to replace PCBs as capacitor fluid. Prodelec, on the other hand, marketed chloralkylene 12, a mixture of bichlorobiphenyls and their alkylated derivatives which is claimed to be easily biodegradable and non-toxic. In 1974 Drs. A.J. Rutkowski and E.O. Forster at Exxon Research and Engineering Company (21) developed Di-isononyl phthalate (ENJ-2065), an organic ester, as a dielectric liquid in capacitors. Uniroyal Oil Company introduced some new products. They are PAO-20E, a polymerised polyoctene-1, and PAO-13C, PAO-LVC, PAO-40C, PAO-60C and PAO-100C. The last five are all polydecenes with different viscosities.

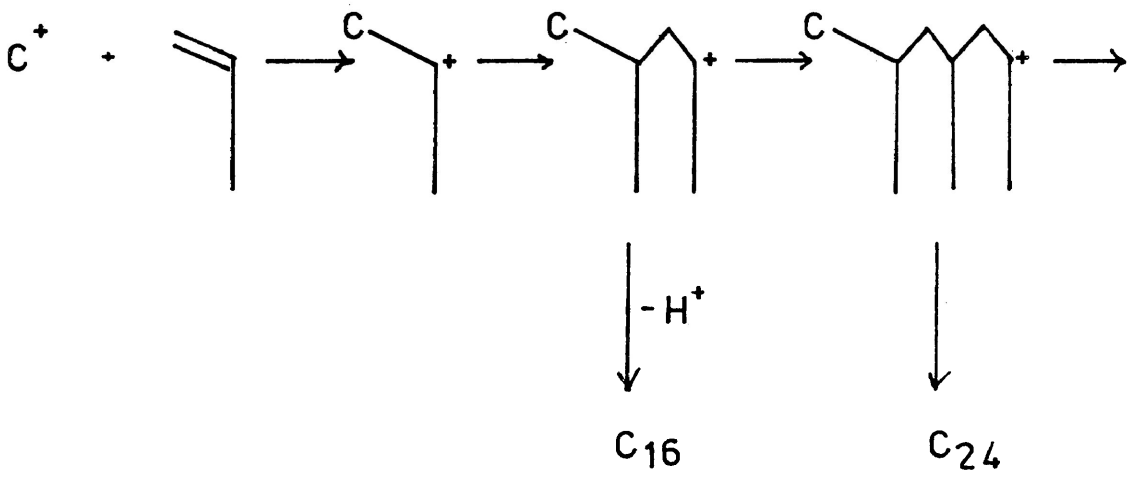
While initial toxicity testing, such as LD_{50}, rabbit skin tests and the like, have been performed on most of these products, a general understanding of their environmental impact is lacking.

As part of a research project on biodegradability, particularly by micro-organisms, presently in progress in this laboratory [e.g., the study of the fungitoxicity of methoxychlor and fenitrothion (22)], the present study deals with the

problems surrounding the persistence and biodegradability of the dielectric fluids produced by Uniroyal Oil Company with specific reference to PAO-20E (VI). This product is a mixture of oligomers of 1-octene with an average molecular weight of 850, which is described in U.S. Patent No. 4,041,098 (2). The polymerisation of 1-octene is initiated by using a coordination complex catalyst system. This system consists of a soluble aluminum alkyl halide (e.g., diethyl aluminum chloride) and an organic halide (a halide with not more than one halogen atom attached to any single carbon atom in the molecule). The chain grows through the double bonds of the monomers and can be terminated at any point by addition of water. The molecular weight distribution and the product yield (yield of oil having molecular weight greater than 114) can be controlled by using the appropriate amount of aluminum alkyl halide. The remaining double bonds are removed by subsequent hydrogenation. Scheme I (23) (p. 9) illustrates the preparation of these compounds.







$$C_8 \xrightarrow{C^+} C_8 + C_{16} + C_{24} + C_{32} + C_{40} + \cdots$$

Scheme I Oligomerisation of 1-octene

The resulting liquid has a high viscosity and is promoted for use as transformer fluid. Other such oligomers produced by the same Company, PAO-13C, PAO-LVC, PAO-40C, PAO-60C and PAO-100C, have similar properties as PAO-20E. These compounds are expected to be similar with respect to their biodegradation characteristics.

Limited toxicity studies have been done on rabbits and rats (23). No research involving micro-organisms has been reported to date. Therefore the microbiological degradability of PAO-20E and the associated synthetic problems have become the subject matter of the present study.

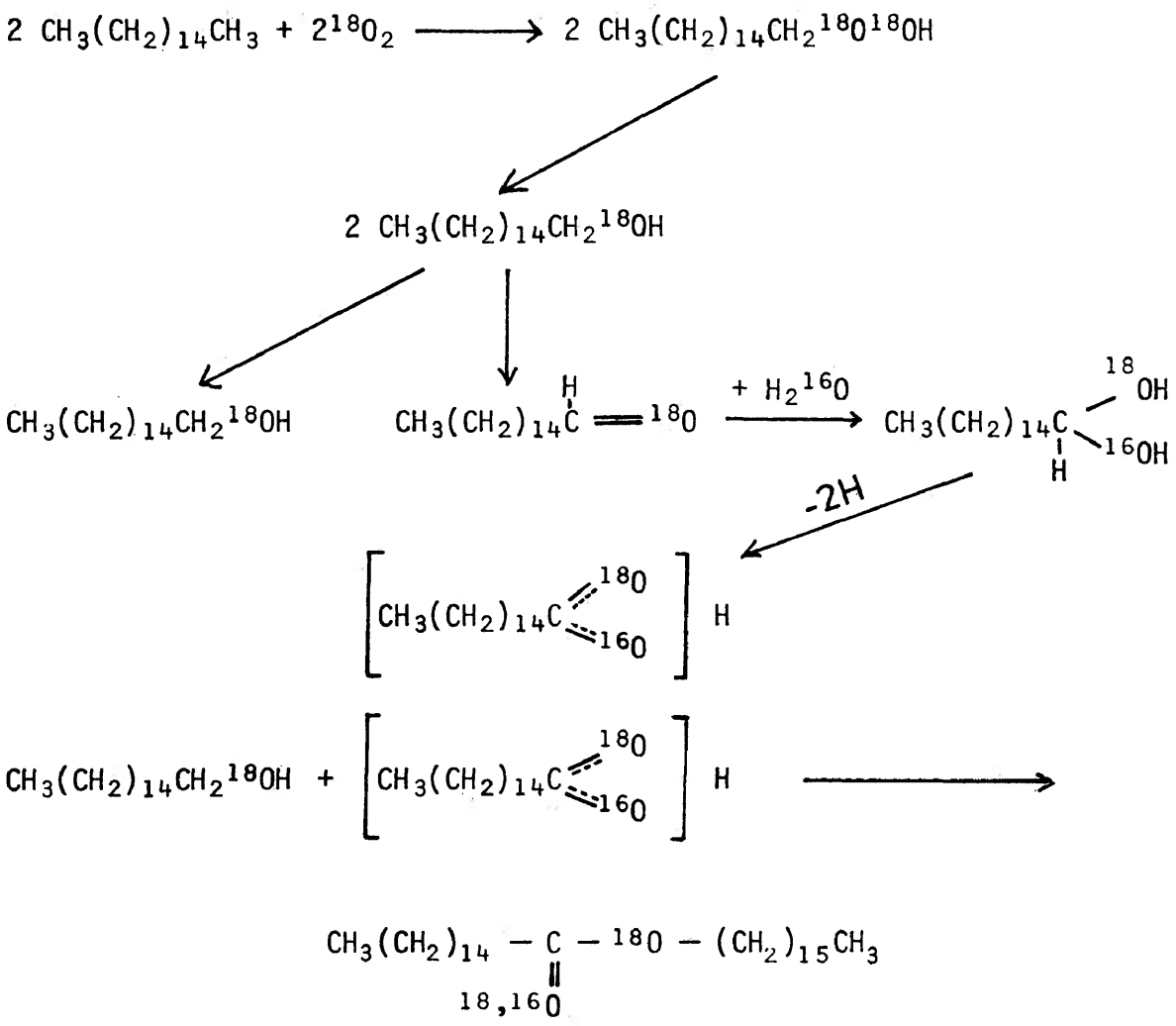
Since the Uniroyal PAO's are branched chain saturated hydrocarbons, a brief summary on the present state of knowledge of the microbiological degradation of such hydrocarbons seems in order. A major portion of this body of knowledge has been obtained from studies on marine organisms, because of the obvious implications of oil spills in marine waters.

Hydrocarbons, whether they be aliphatic (up to C_{40}) (24), olefinic or aromatic, have been found to be attacked by

various micro-organisms, including bacteria, yeasts, molds and fungi. The first relevant observation was reported as early as 1895 by Miyoshi (25). This author found that the bacteria strain *Botrytis cinerea* penetrated paraffin wax which was once thought to be biologically inert. This led to an upsurge of intensive studies with various bacterial species and different hydrocarbons. Some of the most commonly found microbes that oxidize hydrocarbons are the *Pseudomonas* species which grew in kerosene media, *Mycobacterium, Proactinomyces, Actinomyces* (24) and yeast-like organisms such as *Candida lipolytica* (26, 27, 28, 29). These species are found abundantly in soil and in the aquatic environment, especially in oil-polluted areas. The abundance and types of microbes seem to be influenced by the quantities and kinds of hydrocarbon present. Each species metabolizes only a narrow range of hydrocarbon homologues. In many species, the enzymes which catalyse the oxidation of hydrocarbon are adaptive or inducible (30).

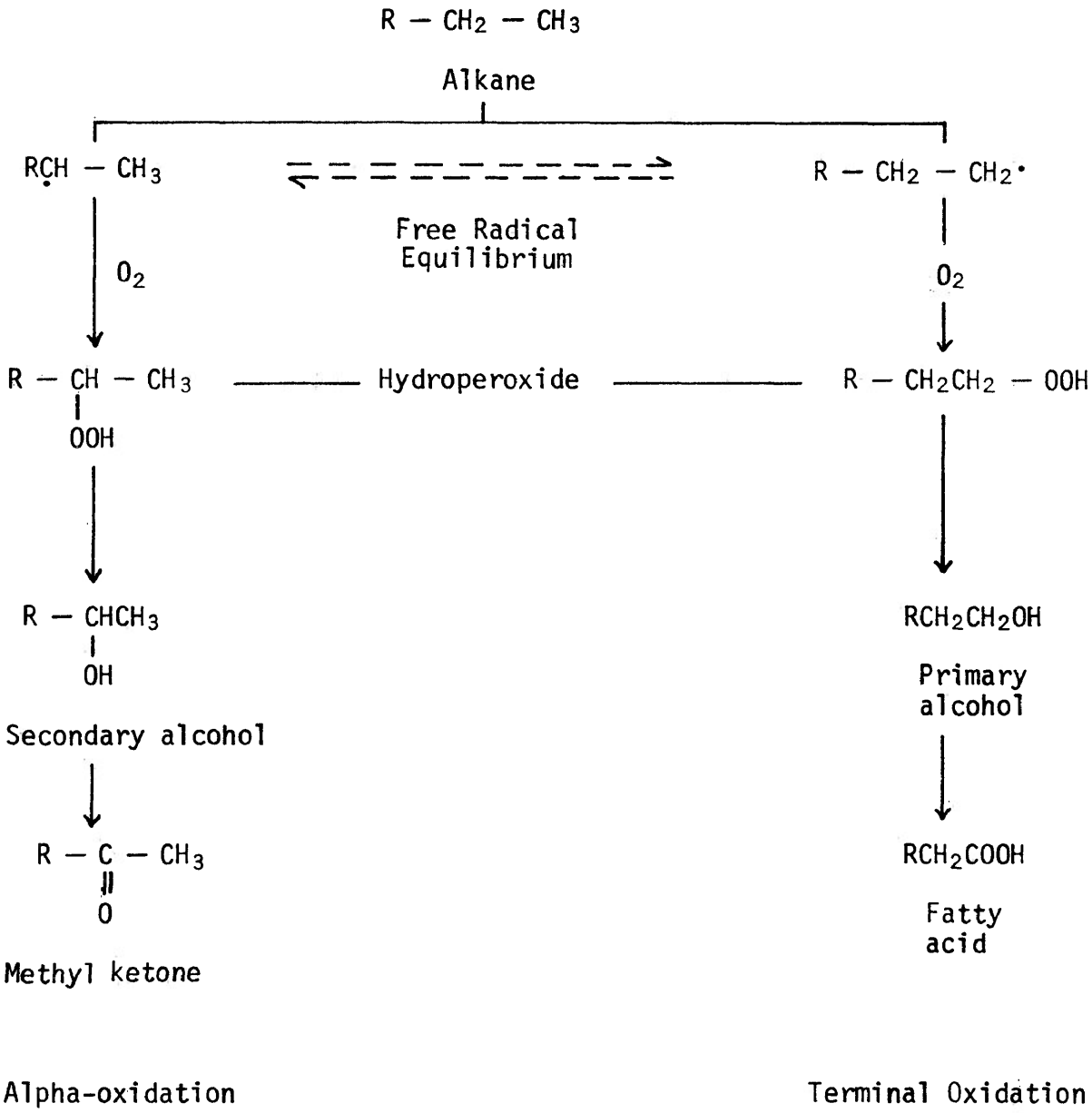
Microbial oxidation of hydrocarbons takes place in very simple media. Wherever there is physiologically balanced mineral salt solution, the presence of nitrogen, phosphorus and free oxygen, the hydrocarbon-oxidiser would be able to utilize hydrocarbons as its only carbon source and assimilate it.

If oxygen is abundant, carbon dioxide is always the final product, but fatty acids, alcohols, unsaturated hydrocarbons and aliphatic esters are frequently found as intermediate products of this metabolic conversion (24,31). Rupture of the hydrocarbon structure occurs in various ways and the metabolic pathways are diverse. Some microbes attack the aliphatic alkanes from one end (i.e., monoterminal oxidation). For long-chain alkanes ($C_{12} - C_{18}$), oxidation takes place via 1-alkyl hydroperoxides and the formation of n-alcohols as the first stable intermediate which in turn are oxidized to *n*-fatty acids (32). Scheme II (33) (p.13) outlines the probable pathway for the bacterial oxidation of *n*-hexadecane. The acid and alcohol condense to form the final product, cetyl palmitate. This was based upon the findings that 75 percent atmospheric oxygen has been incorporated into the ester when the microbe *Micrococcus* was incubated with hexadecane in an $^{18}O_2$ enriched atmosphere (33). The first reaction involves oxidation with molecular oxygen and subsequent reactions the oxygen of water. The fatty acid formed may also be oxidised further via β-oxidation to give fatty acids that are successively two carbons shorter. Certain alkanes of shorter chain length ($C_3 - C_6$) produce methyl ketones (34). Leadbetter and Foster (34) viewed that as a variation of the terminal attack involving a free radical equilibrium

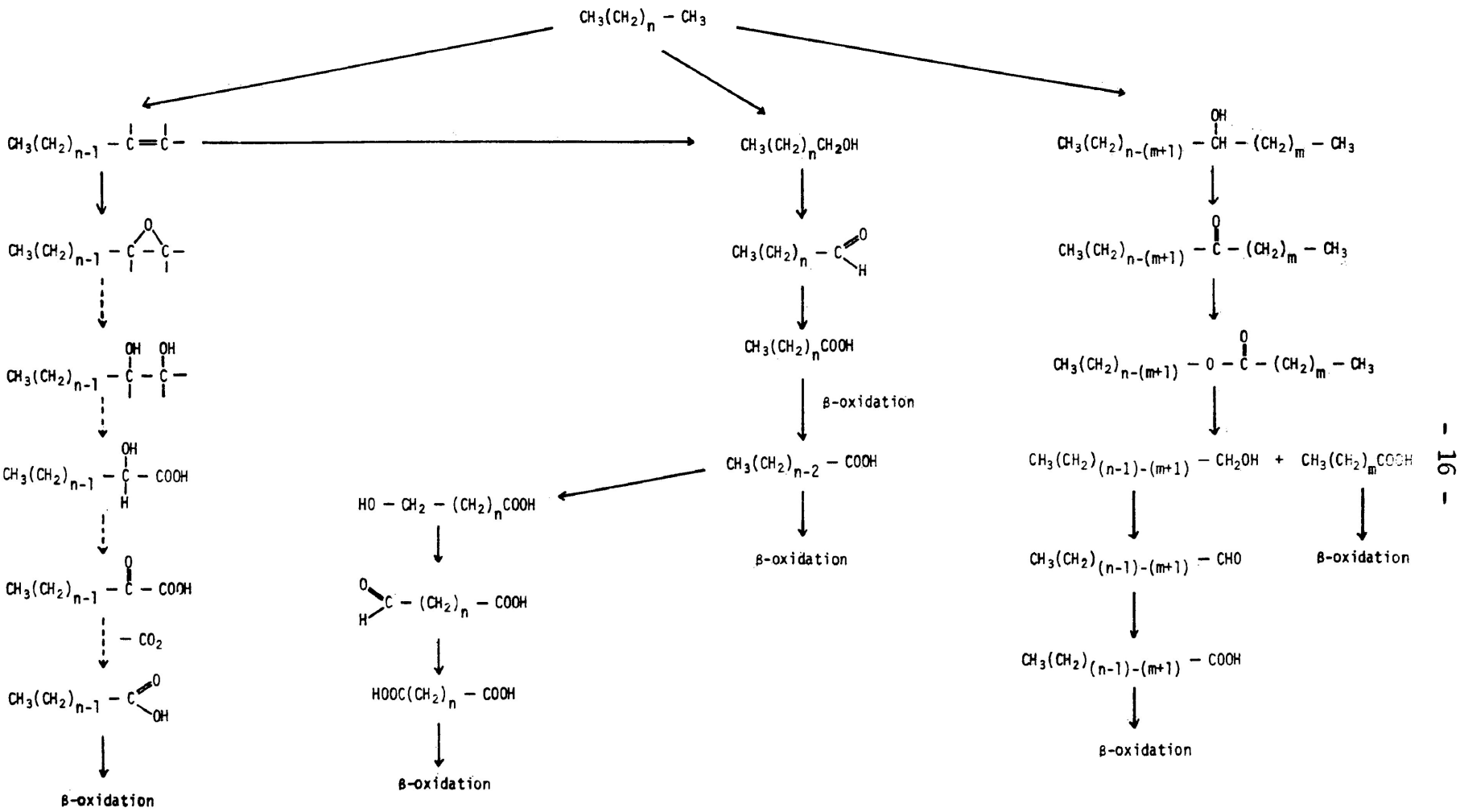


Scheme II. Hypothetical mechanism for bacterial oxidation of *n*-hexadecane and subsequent formation of cetyl palmitate (33).

as illustrated in Scheme III (p.15). Stabilities of alkyl radicals increase in the order $I^{o} < II^{o} < III^{o}$ and also increase with the size of the groups bonded to the carbon atom bearing the unpaired electron. This might explain the difference in the end products for the microbial oxidation of long chain *n*-alkanes and lower molecular weight alkanes. Finally, some intermediate length alkanes ($C_6 - C_{10}$) are dehydrogenated to the corresponding 1-alkenes (32) which are further oxidised to the epoxides or 1-alkanols (35). Considering the metabolic flexibility of micro-organisms, other metabolic pathways might be uncovered. Already discovered are the diterminal oxidation via *w*-oxidation (e.g., by *Corynebacterium* 7EIC (36)) and the subterminal oxidation of the alkane. When the initial attack is on both terminal methyl groups, dicarboxylic acids become the metabolic intermediates. On the other hand, when initial oxidation is subterminal, the intermediates are secondary alcohols, then ketones, followed by ester cleavage. These are outlined in Scheme IV (35,37) (see p.16). However, generally speaking, in the aliphatic series, long-chain hydrocarbons are more readily attacked than compounds having only a few carbon atoms per molecule (24, 36); *n*-alkanes are attacked in preference to branched alkanes, as demonstrated in the oxidation of 2-methylhexane by *Pseudomonas* (38).



Scheme III Formation of *n*-alcohols and methyl ketones from alkyl free radicals (34).



Scheme IV Degradative pathways of n-alkanes
(n,m are arbitrary number of CH_2 groups)

Bacteria transport the hydrocarbon into the cell and assimilate it within the cell. Söhngen (39) reported that half of the methane assimilated by *Methanomonas methanica* was oxidized to carbon dioxide and the other half converted into bacterial cell material and other metabolic products. A portion of the paraffin oxidised by *Aspergillus flavus* was incorporated into the mycelium (40). This conversion of the hydrocarbons into microbial biomass is considerably less important in the case of fungi. Only 7% of hexadecane, metabolized by *Cladosporium Resinae*, was assimilated by this fungus while 93% was oxidized to carbon dioxide (31).

In general, a large number of the micro-organisms which exist in soil, fresh water and in sea-water have been found to oxidize hydrocarbons. It appears, as mentioned earlier, that many species can be induced to grow in a hydrocarbon environment. However, since nitrogen and phosphate are essential for the growth of micro-organisms, the biodegradation of spilt oil in marine waters is probably limited. Rates of growth are slow when these two essential elements are present in low concentrations in sea water. Kuwait oil had been found to be well degraded in soil and fresh water. But when the oil was put into sea water which was previously inoculated with the same bacteria,

it was degraded very slowly (41). Also, dispersion of hydrocarbons in the soil provides a considerably larger surface area than the thin surface films which may be expected to form from hydrocarbon spills in the aqueous environment. Microbial interaction would therefore seem to be more productive on land than in the aqueous environment.

A study of the biodegradability of PAO-20E would involve incubation of the micro-organisms with the compound at different dose levels, analysis of the unreacted hydrocarbon and determination of possible metabolites.

The commercial product, discussed above, presents three major difficulties which are outlined as follows: (i) The average PAO-20E molecule is made up of 64 carbon atoms. The largest alkane molecule reported found assimilated by micro-organism is C_{40} (24). A question thus arises whether such a large molecule as PAO-20E would be able to penetrate the cell walls of the micro-organisms for biodegradation, and if the answer is negative, whether biodegradation can take place as a result of enzymes released after autolysis of the cells. Such a situation could be simulated by the preparation of cell-free extracts, a method described by Baptist, Gholson and Coon (42).

(ii) An appropriate analytical method has to be selected for analysing the level of residual, non-degraded hydrocarbons. Common spectroscopic methods, such as ultra-violet, infra-red, or proton magnetic resonance would not be suitable where the molecule concerned does not possess any specific functional group.

Its high molecular weight makes the compound not susceptible to standard gas chromatographic analysis. A C-36 alkane molecule took almost fifty minutes to elute from a gas-liquid column (2% OV-1) with temperature programmed to increase from 60°C to 300°C at a rate of 5°C per minute (43). It would take a much longer time for PAO-20E to elute under such conditions. The temperature could not be increased further as most of the column packings decompose above 300°C. This makes gas-liquid chromatography not a feasible analytical tool for PAO-20E.

The only available method would then be high pressure liquid-liquid chromatography which is not as easily accessible and convenient as gas-liquid chromatography.

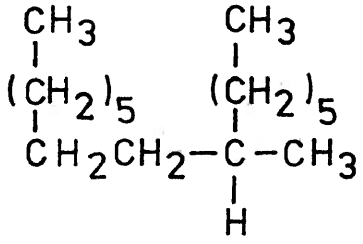
(iii) The last problem encountered is the identification of metabolites. Metabolites from the PAO-20E would be expected to include

carboxylic acids and/or esters (Scheme IV, p. 16). Without the hydrocarbon, the microbes themselves, too, produce esters and carboxylic acids as their natural metabolites (29). To recognise between the two kinds of metabolites would be another difficulty to be overcome.

The use of suitable model compounds could solve some of these problems. The stepwise synthesis of smaller polymeric units of 1-octene, i.e., dimer (VII), trimer (VIII), tetramer (IX), etc. appears to be a suitable approach to this study for a number of reasons.

Firstly, the model compounds suggested are structurally similar but have smaller molecular sizes. They may have the advantage of penetrating the cell-walls of the micro-organisms more easily. As the molecular size is increased gradually (going from dimer (VII) to trimer (VIII) to tetramer (IX) and onwards), it may be possible to establish a limiting size of the macro-molecule that can effectively penetrate the cell walls.

Secondly, since the model compounds have smaller



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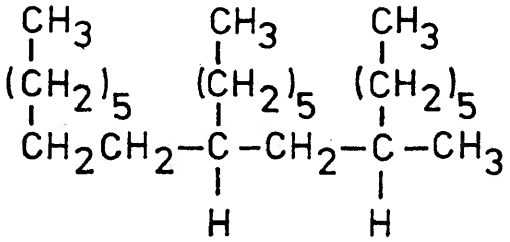
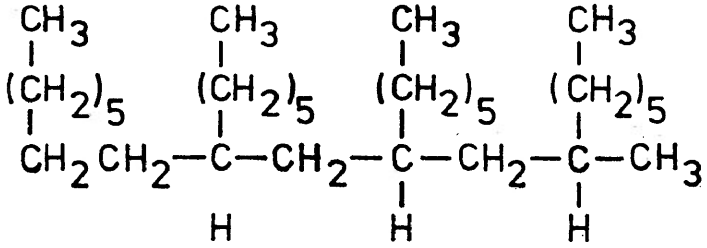


VIII



IX

molecular weights, it would be possible to analyse residual hydrocarbons qualitatively and quantitatively by conventional gas-liquid chromatography, which is readily available in most laboratories.

And thirdly, such model compounds may be labelled with Carbon-14 or Carbon-13, so that the breakdown products may be distinguished from the natural metabolic products of the micro-organisms used in biodegradation studies.

Thus the synthetic approach, using model compounds such as the dimer (VII) and the trimer (VIII) appears to be an attractive method for initial studies on the biodegradability of PAO-20E and its homologues.

Finally, the yeast *Saccharomycopsis lipolytica* (formerly known as *Candida lipolytica*) is chosen as a suitable organism for such initial studies. It is an organism that is found both in the terrestrial and marine environments (27) and it is known to metabolize straight chain saturated hydrocarbons (26,27,28,29) as well as branched alkanes (27). It might be able to oxidize PAO-20E which is also a branched chain hydrocarbon.

In the chapters which follow, the synthesis of the model compounds, the dimer (VII) and the trimer (VIII), and the interaction of these compounds with *S. lipolytica* are reported.

CHAPTER 2

SYNTHESES OF THE DIMER AND TRIMER HYDROCARBON HOMOLOGUES OF PAO-20E AND THE CHARACTERISATION OF INTERMEDIATE PRODUCTS

Possible Synthetic Sequences

Two possible approaches to the preparation of the lower molecular weight homologues of PAO-20E, the dimer (7-methylpentadecane, V*) and the trimer (7-methyl-9-hexylheptadecane, XXV) were considered.

The first utilizes the Reformatsky reaction (44) (Eq. [1]†, p. 25) as a key reaction in a reaction sequence outlined in Scheme I†, p. 26. In a second approach, the malonic ester synthesis (45) (Eq. [2], p. 25) is the key reaction in a sequence as outlined in Scheme II, p. 29. A decision as to which route to the required hydrocarbons would be the most advantageous requires a comparative discussion of both methods.

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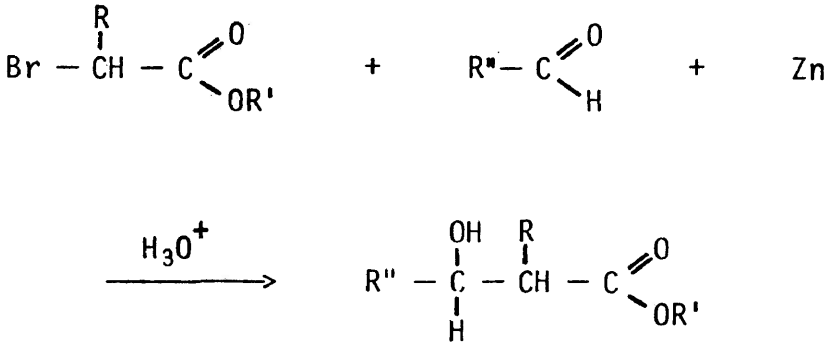
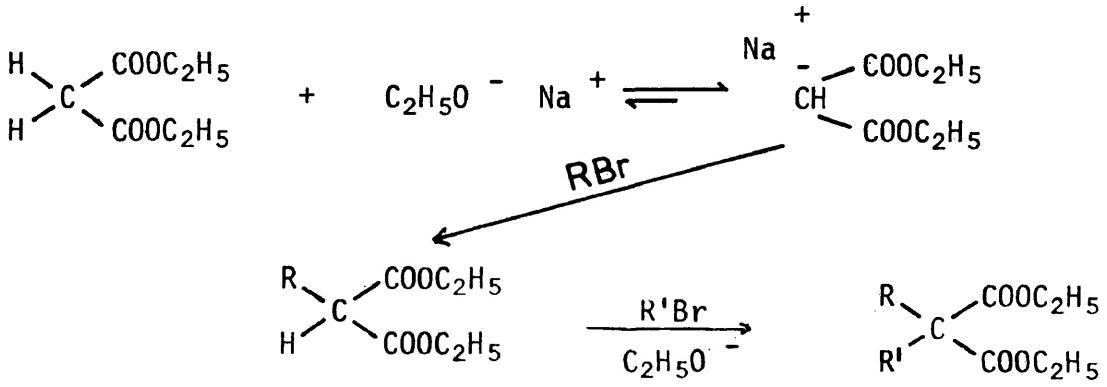
*Structural formulae have been numbered differently from those in Chapter I and the present numbers are retained throughout the remainder of the thesis.

†Equations and schemes are numbered per chapter.

Eq. [1] Reformatsky reaction (44)



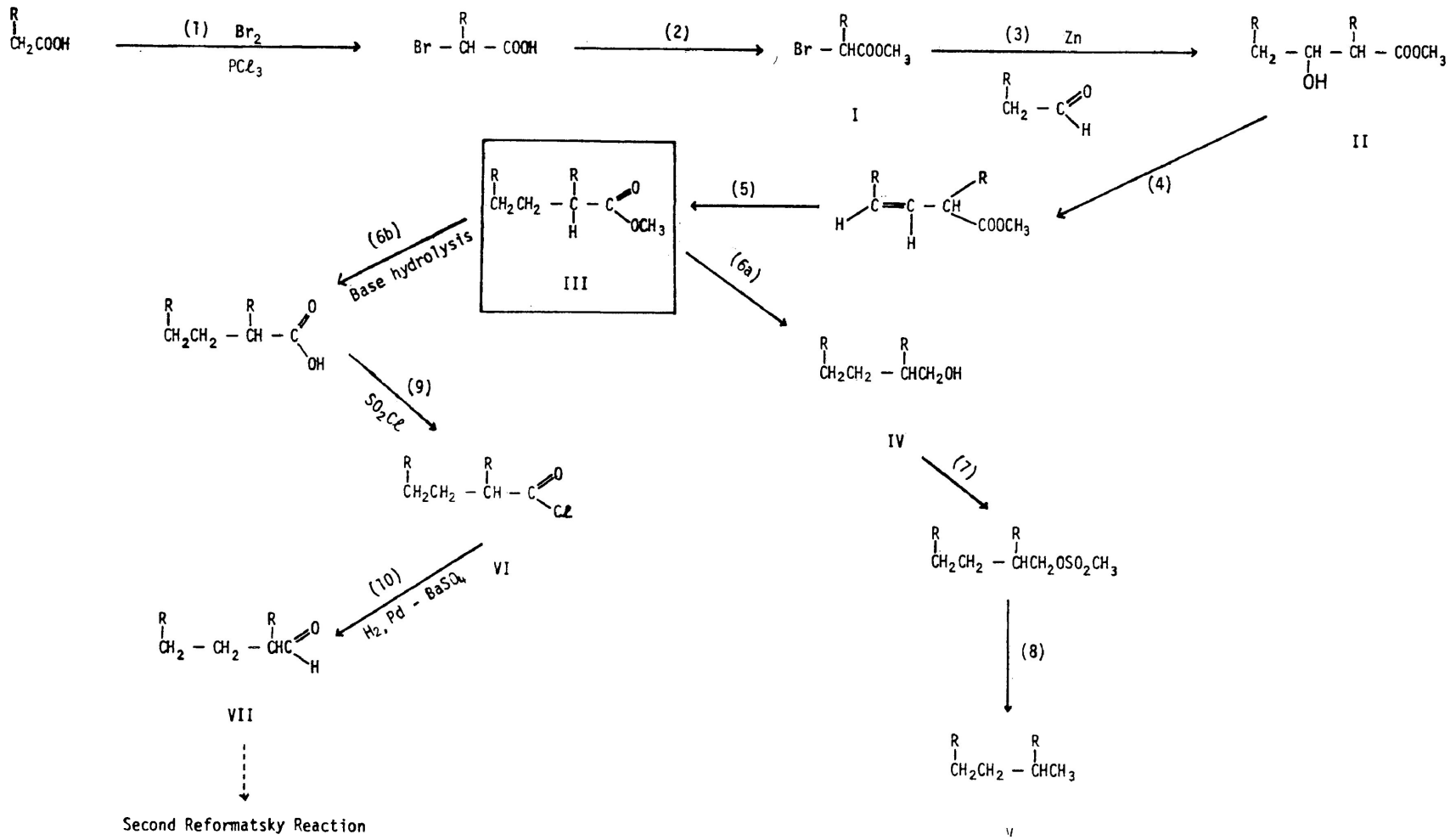
Eq. [2] Malonic ester synthesis (45)



An approach based on the Reformatsky reaction would start with the condensation of a suitable α-bromo ester (e.g., I, Scheme I) with an aldehyde in the presence of zinc powder to form the hydroxy ester (II). The hydroxy ester (II), after dehydration and hydrogenation, would give the branched methyl ester (III). Reduction to the alcohol (IV), mesylation and another reduction would lead to the dimer hydrocarbon (V) in 8 steps. Also, the methyl ester (III) may be converted by base hydrolysis and reduction of the acid chloride

Scheme I Reformatsky reaction approach to the synthesis of lower molecular weight homologues of PGO-2OE
(for legend, see p.27)

$R = CH_3(CH_2)_5$



Scheme I, legend

I	methyl 2-bromo-octanoate	
II	methyl 2-hexyl-3-hydroxy-decanoate	
III	methyl 2-hexyldecanoate	(dimer methyl ester)
IV	2-hexyldecanol	(dimer alcohol)
V	7-methylpentadecane	(dimer hydrocarbon)
VI	2-hexyldecanoyl chloride	
VII	2-hexyldecanal	

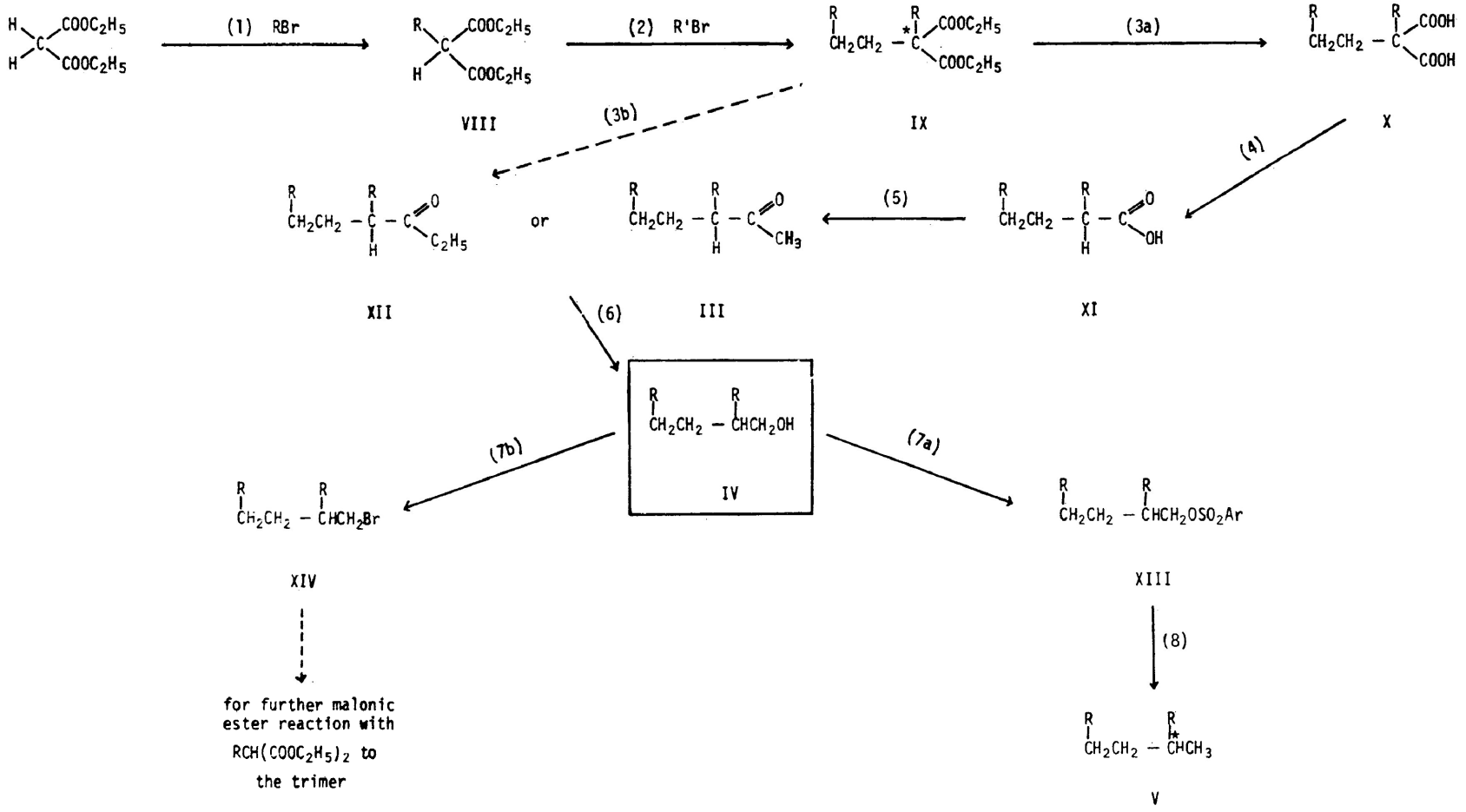
VI to the aldehyde (VII), which would be the starting point for a second cycle, by means of a second Reformatsky reaction with the α-bromo ester (I). This would eventually lead to the trimer hydrocarbon (XXV) in a sequence involving 9 steps.

Alternatively, a synthetic sequence may be based on the malonic ester synthesis (Eq. [2], p.25) and such a sequence is outlined in Scheme II, p. 29 . It requires the preparation of a substituted malonic ester containing two alkyl groups. This dialkylmalonic ester (IX) would be converted to mono-carboxylic acid ester (III or XII) which is then reduced to the alcohol (IV). On one hand, tosylating or mesylating the alcohol and further reduction (Steps 7a, 8, Scheme II) leads to the dimer hydrocarbon (V) in a total of 8 steps (via hydrolysis and decarboxylation of malonic ester) or 6 steps (via decarbalkoxylation of malonic ester, See p. 29). On the other hand, substitution of the hydroxyl group of the alcohol (IV) by a bromine atom (Step 7b, Scheme II) provides an alkyl bromide for a second malonic ester reaction leading eventually to the trimer (XXV) in 8 steps (Scheme V, p. 36).

Thus using either approach, the key reaction is the linking of a second alkyl chain to the parent carbon chain at the C-7 and C-9 positions [as indicated in structural formulae IX,

Scheme II Malonic ester synthesis approach to the preparation of lower molecular weight homologues of PAO-20E
(for legend, see p.30)

R = $CH_3(CH_2)_5$; R' = RCH_2CH_2 ; Ar = CH_3- 



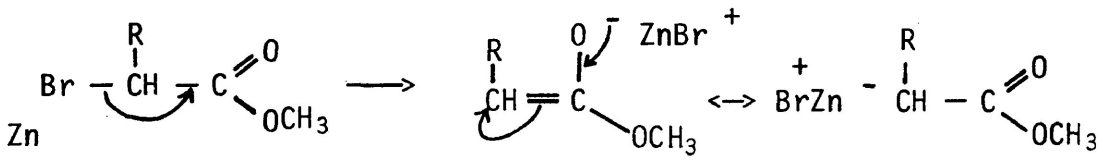
Scheme II, legend

VIII	diethyl hexylmalonate	
IX	diethyl hexyl-octylmalonate	(dimer malonic ester)
X	hexyl-octylmalonic acid	(dimer diacid)
XI	2-hexyldecanoic acid	(dimer monoacid)
XII	ethyl 2-hexyldecanoate	(dimer ethyl ester)
XIII	2-hexyldecyl *p*-toluenesulphonate	(dimer tosylate)
XIV	7-(bromomethyl)pentadecane	(dimer bromide)

Scheme II (p.29), XVII, Scheme V (p.36)]. This occurs at Step 3 in the Reformatsky reaction approach (Scheme I, p. 26) and Steps 1 (Scheme V, p. 36) and 2 (Scheme II, p. 29) in the malonic ester synthesis approach. A more detailed consideration of these key reactions provides a rational choice of the preferred approach to this synthetic problem.

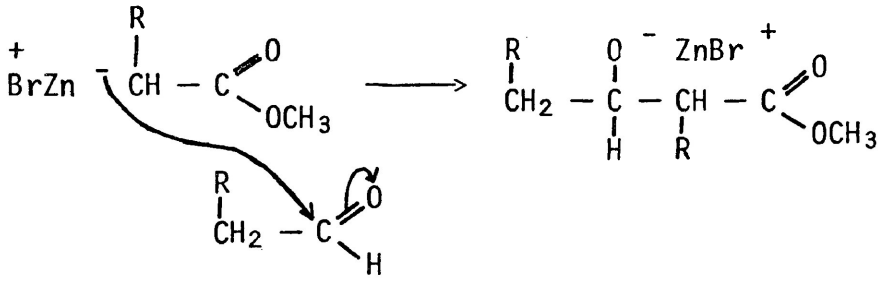
The detailed reaction mechanisms are outlined in Scheme III, p.31-32 and Scheme IV, p. 32.

Step 1.



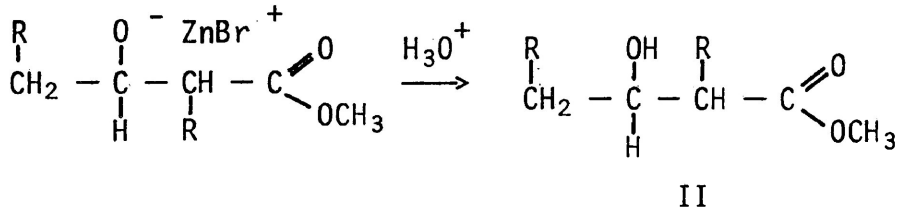
XV

Step 2.



XVI

Step 3.

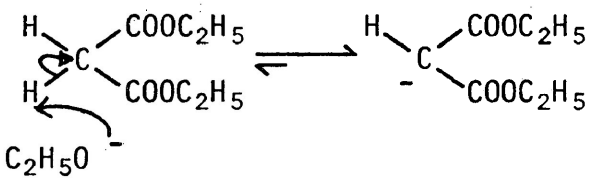


II

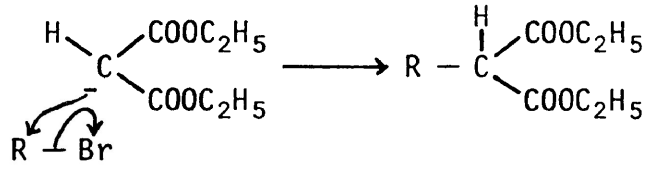
Scheme III Reaction mechanism for the Reformatsky reaction (46)

In the Reformatsky reaction, the reactive intermediate (XV) generated in the first step is the anion of an ester, closely associated with a zinc cation. The succeeding steps involve the nucleophilic addition of this intermediate (XV) to the carbonyl group of the aldehyde (XVI), and the subsequent decomposition of the addition product by dilute acids, leading to the β-hydroxyester (II) as the product.

Step 1.



Step 2.



Scheme IV Reaction mechanism for the malonic ester synthesis reaction

Similarly, the malonic ester, when treated with sodium ethoxide in absolute alcohol, is converted into its salt, sodiomalonic ester. The nucleophilic attack of the resulting carbanion $CH(COOC_2H_5)_2^-$ on the alkyl halide then gives the alkylmalonic ester (VIII). The alkylmalonic ester still contains one enolizable hydrogen, and is therefore capable of further reaction to give the dialkylmalonic ester (IX).

It becomes evident that the two key reactions are mechanistically alike. Thus, other factors have to be considered in deciding which of the two approaches is more suitable for the present synthetic attempt. Three such factors are the number of steps involved in each of the two sequences, the nature of some of the reagents, and the possible future requirement for labelled compounds.

As discussed above, a sequence based on the malonic ester synthesis is clearly the one to be favoured with respect to the total number of steps involved.

Considering the nature of some of the intermediate compounds, it should be noted that in the malonic ester sequence, the esters (III and XII) are easily reduced to the alcohol (IV), which can be converted into the bromide (XIV), the starting point for the second cycle. This is a straightforward reaction which may be

expected to give good yields.

In the Reformatsky reaction, the ester (III) would have to be converted, after hydrolysis, into the acid chloride (VI). The reduction of this acid chloride to the aldehyde (VII) is a reaction which may be expected (47) to be somewhat difficult with at best rather moderate yield. This further lowers the expected yield of the final product, be it the dimer or trimer hydrocarbon.

Thus, based on a comparison of these two reactions which initiate the second synthetic cycle in each case, it is again the malonic ester scheme which is preferred.

Finally, consideration must be given to the possible requirement, at a later stage, to prepare labelled compounds, (positions marked in structural formulae V, p.29 and XXV, p.36). The availability of suitably labelled starting materials is therefore a major consideration. The labelled malonic ester is available commercially at a reasonable price but longer chain carboxylic acids labelled at C-2 are more difficult to obtain. This makes it easier to work with the malonic ester.

Therefore, an attempt was made to use the malonic ester synthesis sequence to prepare the required hydrocarbons. A brief

discussion of the synthesis along this line and the difficulties encountered during the preparations is given in the ensuing paragraphs.

The Malonic Ester Synthesis

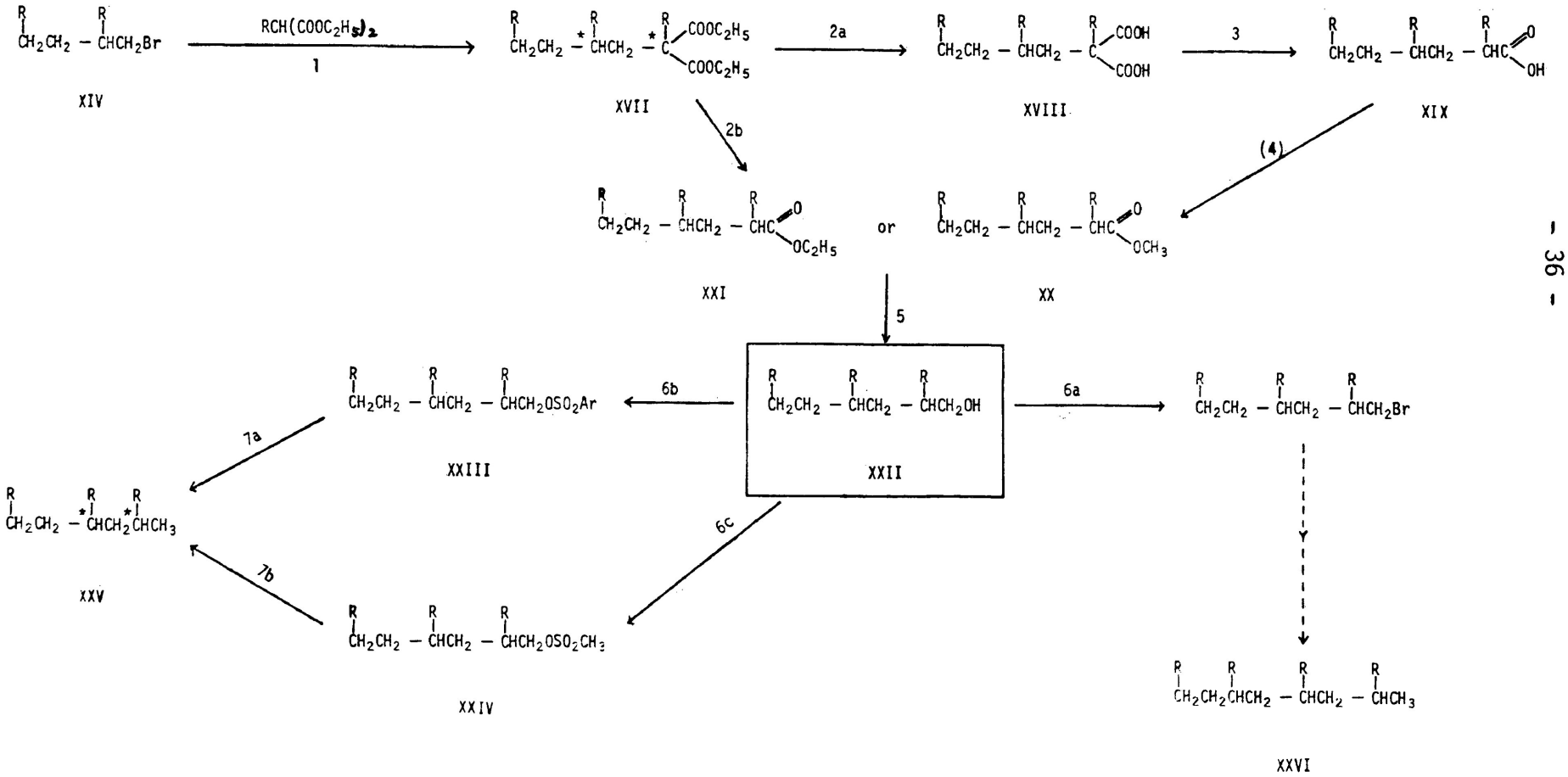
The synthesis begins with the preparation of the alkyl malonic ester, diethyl hexylmalonate (VIII). The reaction follows an S_N2 mechanism which has been discussed earlier in this Chapter (Scheme IV, p.32). A second alkylation with *n*-octyl bromide would lead eventually to the dimer hydrocarbon (V) or alkylation with the dimer bromide $C_8H_{17}CH(C_6H_{13})CH_2Br$ (XIV) will lead to the trimer hydrocarbon (XXV) (Scheme V, p. 36).

As expected, due to steric interaction, the carbanion $C_6H_{13}C(COOC_2H_5)_2^-$ gave a higher yield with *n*-octyl bromide (65%) than with 7-(bromomethyl)pentadecane (dimer bromide, XIV) (59%) within a shorter time (2 1/2 hrs. for the first reaction and 9 hrs. for the latter). In the first reaction with *n*-octyl bromide, the product diethyl hexyl-octylmalonate (dimer malonic ester, IX) was isolated pure by fractional distillation under reduced pressure. The trimer malonic ester (XVII), with a molecular weight higher than the dimer malonic ester (IX) by 112 units, is expected to have a much higher boiling point, probably in the 200° range, under the

Scheme V 2nd cycle of the malonic ester synthesis sequence to the trimer hydrocarbon

(for legend, see p.37)

R = $CH_3(CH_2)_5$; Ar = CH_3-C$_6$H$_4$-



Scheme V, legend

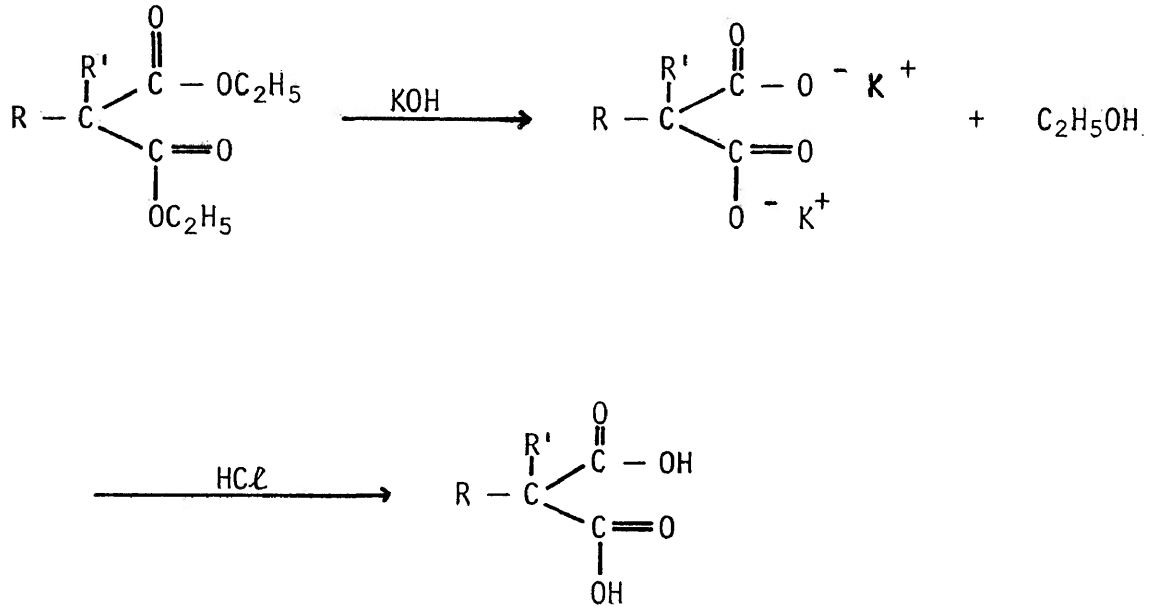
XVII	diethyl hexyl-(2-hexyldecyl)malonate	(trimer malonic ester)
XVIII	hexyl-(2-hexyl-decyl)malonic acid	(trimer diacid)
XIX	2,4-dihexyldodecanoic acid	(trimer monoacid)
XX	methyl 2,4-dihexyldodecanoate	(trimer methyl ester)
XXI	ethyl 2,4-dihexyldodecanoate	(trimer ethyl ester)
XXII	2,4-dihexyldodecanol	(trimer alcohol)
XXIII	2,4-dihexyldodecyl *p*-toluenesulphonate	(trimer tosylate)
XXIV	2,4-dihexyldodecyl methanesulphonate	(trimer mesylate)
XXV	7-methyl-9-hexylheptadecane	(trimer hydrocarbon)
XXVI	7-methyl-9,11-dihexylnonadecane	(tetramer hydrocarbon)

same pressure. This high temperature is difficult to attain and maintain under ordinary laboratory conditions. Therefore the crude product in the second reaction was purified by column chromatography. A considerable amount of trimer ethyl ester (XXI) was isolated as a by-product. Apparently, decarbalkoxylation occurred at the same time as a side-reaction.

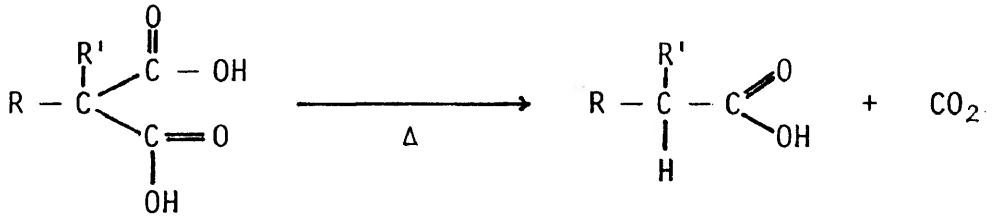
The dialkylmalonic ester has to be converted to an ester with only one carbonyl group for further reduction to the alcohol. Facilitated by the ease of decarboxylation of malonic acid, this can be achieved by hydrolysing the dialkylmalonic ester, decarboxylation and subsequent esterification. Hydrolysis was done with the procedure according to Marvel (48) in the presence of a base. The reaction is represented by Eq. [3], p. 39 and occurs by the so-called nucleophilic addition-elimination mechanism (49) which is illustrated in Scheme VI (p. 40). Treatment with hydrochloric acid yielded the free acids, the hexyl-octylmalonic acid (dimer diacid, X) and hexyl-(2-hexyldecyl)malonic acid (trimer diacid, XVIII).

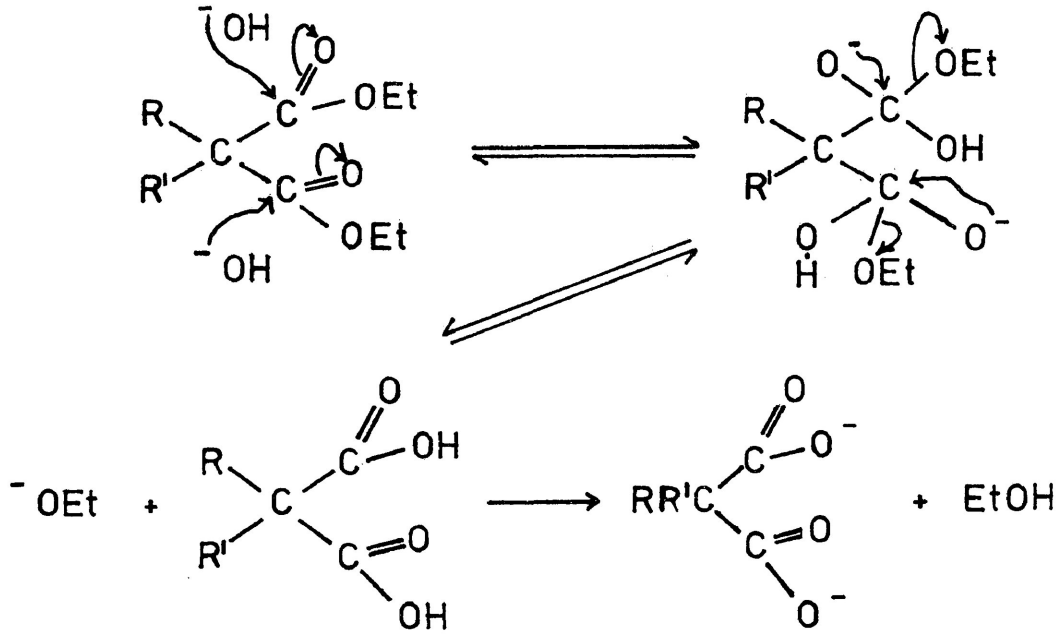
The malonic acids decarboxylate easily by heating, through a cyclic mechanism (Scheme VII, p. 40) proposed by Wesheimer and Jones (50). The 2-hexyldecanoic acid (dimer monoacid, XI) and 2,4-dihexyldodecanoic acid (trimer monoacid, XIX) were prepared from the corresponding dimer diacid (X) and trimer diacid (XVIII),

Eq. [3] Hydrolysis of malonic ester

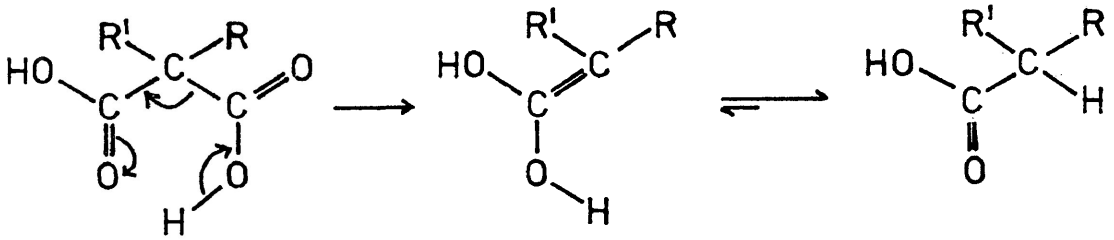


Eq. [4] Decarboxylation of malonic acid





Scheme VI Reaction mechanism for basic hydrolysis of malonic ester



Scheme VII Reaction mechanism for decarboxylation of malonic acid

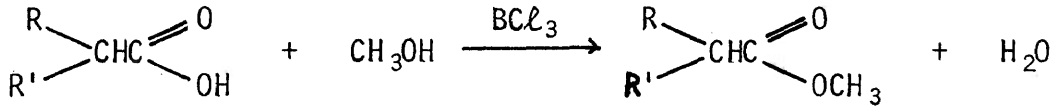
respectively, in this way (Eq. [4], p. 39). To avoid oxidation at such a high temperature (110-145°C), the reaction was carried out in nitrogen. Decarboxylation appeared to proceed more readily in the presence of glass powder.

The next step in the synthesis is the conversion of the monoacids, 2-hexyldecanoic acid (dimer monoacid, XI) and 2,4-dihexyldodecanoic acid (trimer monoacid, XIX) into their methyl esters. The esterification, though a commonplace reaction, often presents difficulties, especially with sterically hindered acids (51). Many various methods have been used to alleviate this difficulty. In the present synthesis, two different agents have been employed. Boron trichloride was used as a catalyst for esterifying the carboxylic acid (XI) with methanol (Eq. [5a], p. 42). The mechanism of this reaction is given in Scheme VIII, p. 43.

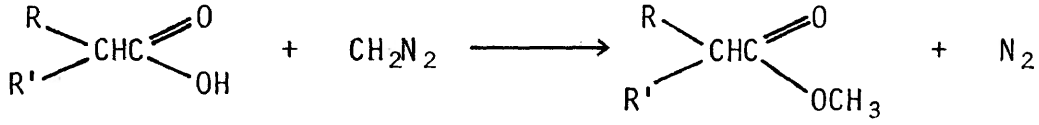
Although the unimolecular S_N1 dissociation of the protonated acid leads to the more sterically favoured acylium ion (XXVII) instead of the usual tetrahedral intermediate (XXVIII, Scheme IX, p. 43), the steps are mostly reversible. The 65% yield for the preparation of the dimer methyl ester (III) may deteriorate to much lower yields when carboxylic acids of higher molecular weight are esterified. Attempts were made using diazomethane (53).

Eq. [5] Methylation of acid

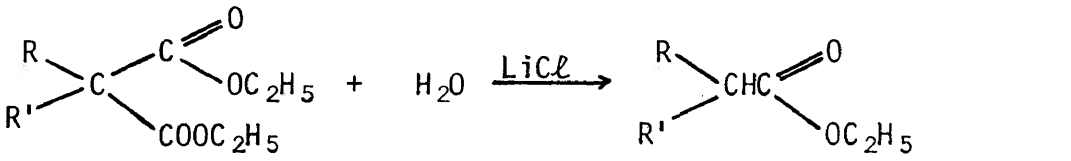
a)



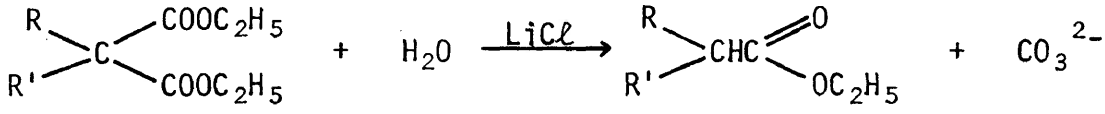
b)

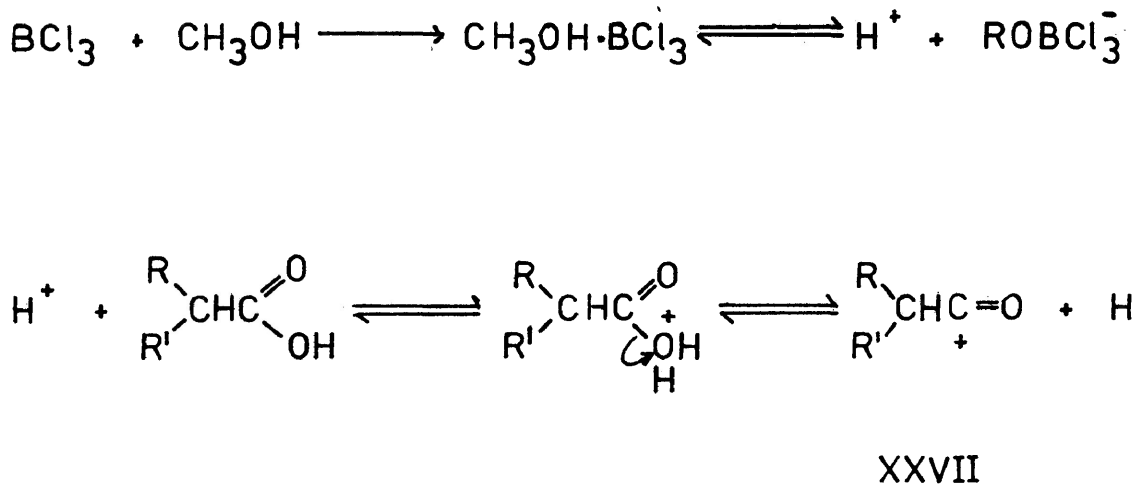


Eq. [6] Decarbethoxylation of malonic ester



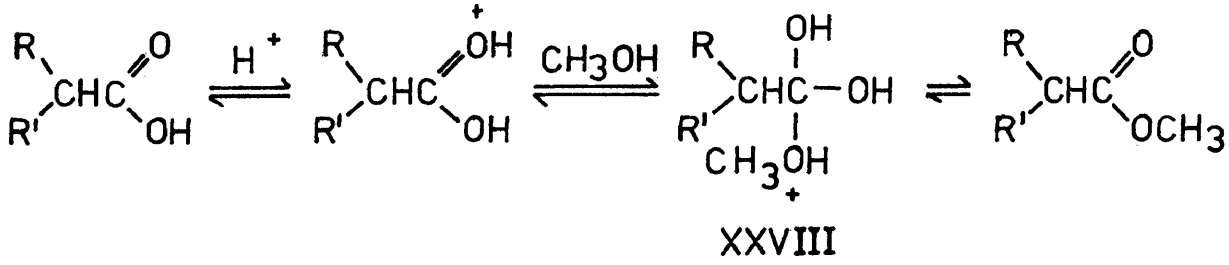
or





XXVII

Scheme VIII Methylation of carboxylic acid with boron trichloride as catalyst (52)



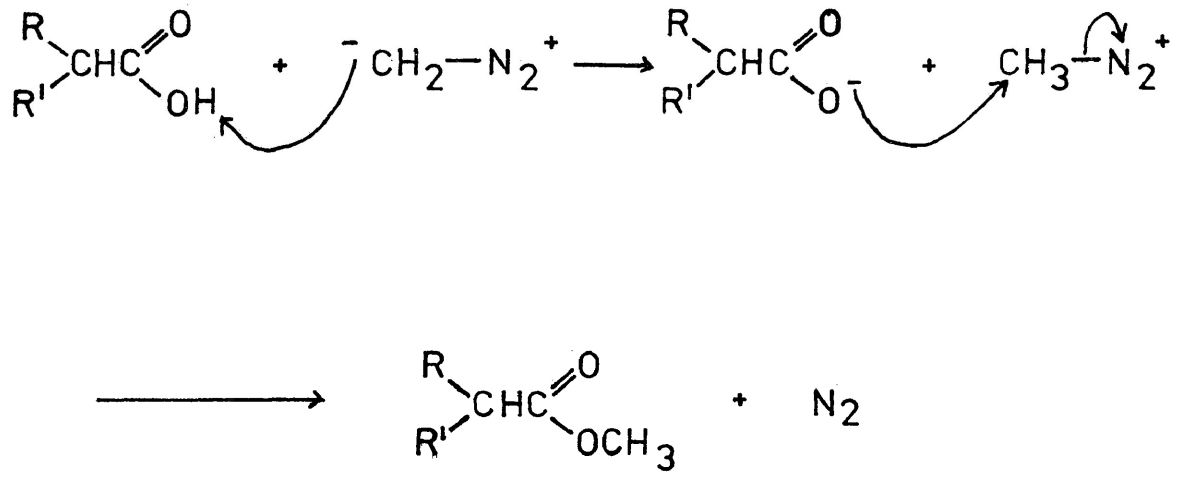
XXVIII

Scheme IX

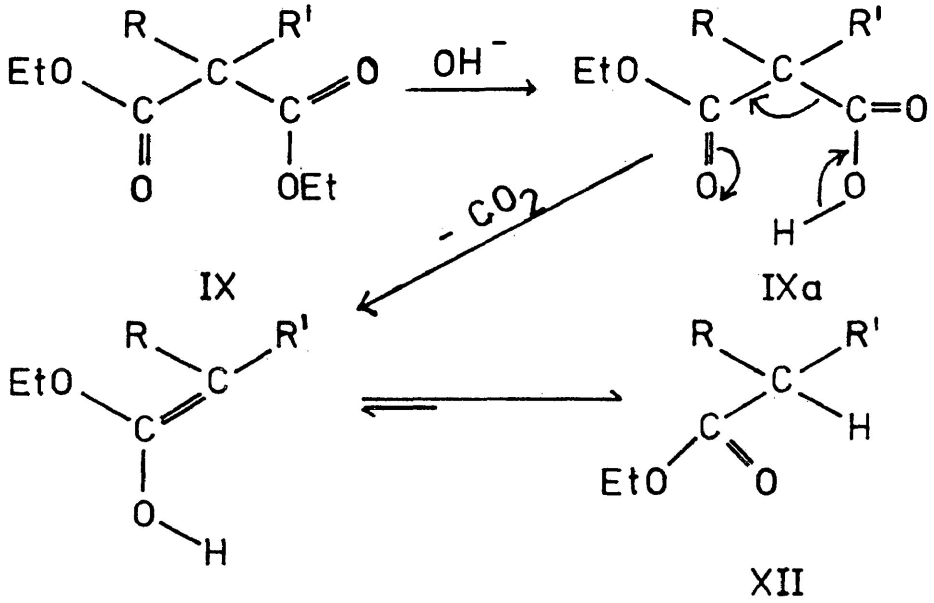
The esterification reaction with diazomethane is outlined by Eq.[5b] (p. 42) and the mechanism described in Scheme X (p. 45, 54). The attack takes place at the more remote oxygen atom of the carbonyl group instead of the acyl carbon atom and therefore suffers from less steric limitations. Esterification using this method proves to be more efficient, giving a yield as high as 99% in preparing the dimer methyl ester (III).

In some preparations of the dimer monoacid (XI), a major impurity was found to be present, which was identified as the dimer ethyl ester (XII). It is assumed that incomplete hydrolysis of the dimer malonic ester (IX) causes the formation of the monoethyl ester acid (IXa), which would decarboxylate to the ethyl ester (XII) by the same mechanism (Scheme XI , p. 45) as the decarboxylation of the diacid (X). Since both the methyl ester (III) and the ethyl ester (XII) lead to the same alcohol (IV) on reduction, there was no need to separate this mixture of monoesters.

In view of the readiness of the malonic esters (IX and XVII) to decarbethoxylate during their preparation (discussed on p.38) or when they are partially hydrolysed and decarboxylated (described above), it is reasonable to speculate that perhaps decarbalkoxylation can take place in one single reaction. In fact, many reactive geminal diesters have been found to decarbalkoxylate



Scheme X Esterification of carboxylic acid with diazomethane (54)



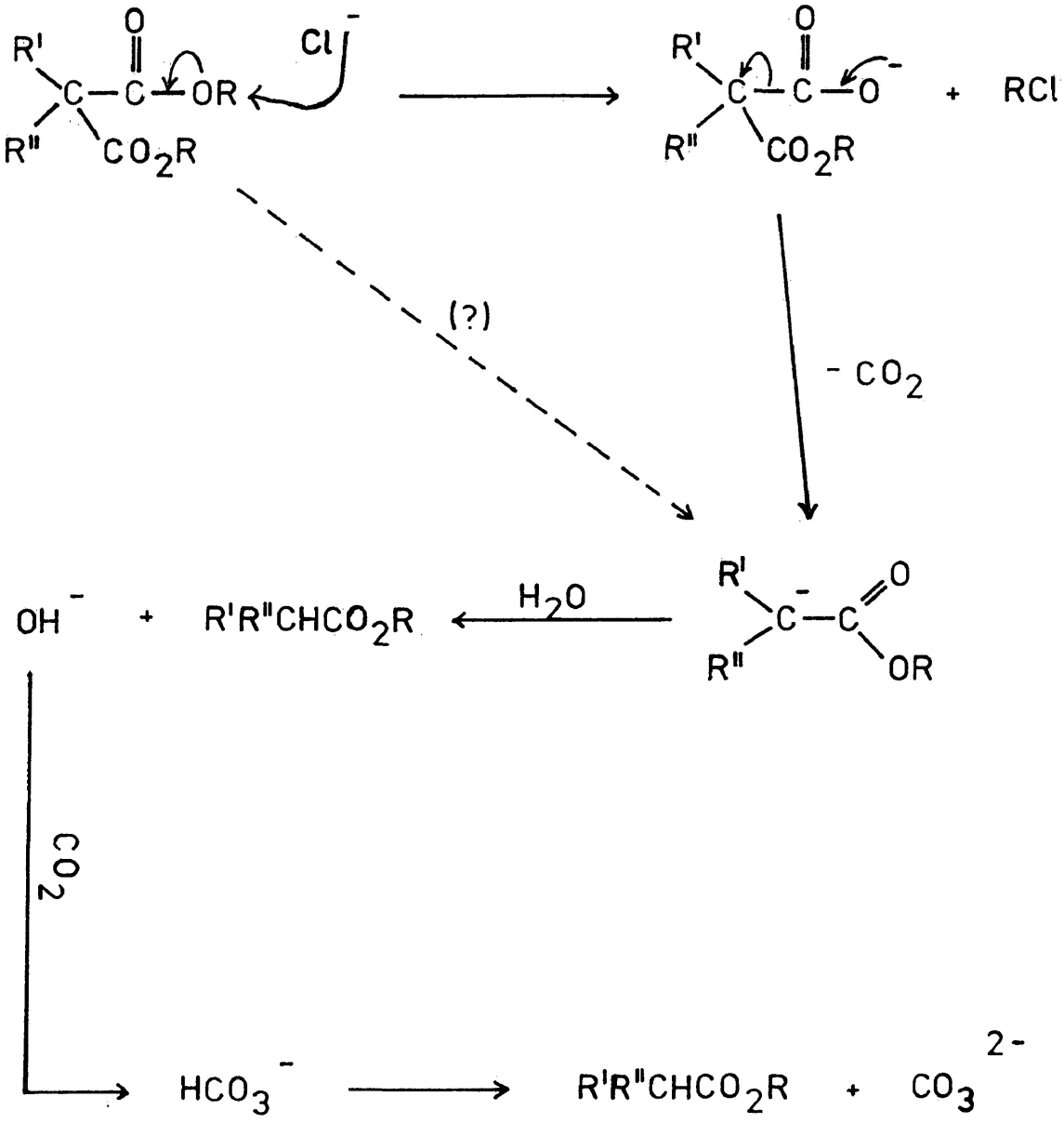
Scheme XI Generation of the ethyl monoester (XII) from the malonic ester (IX) via partial hydrolysis and decarboxylation

$R = CH_3(CH_2)_5$, $R' = CH_3(CH_2)_7$

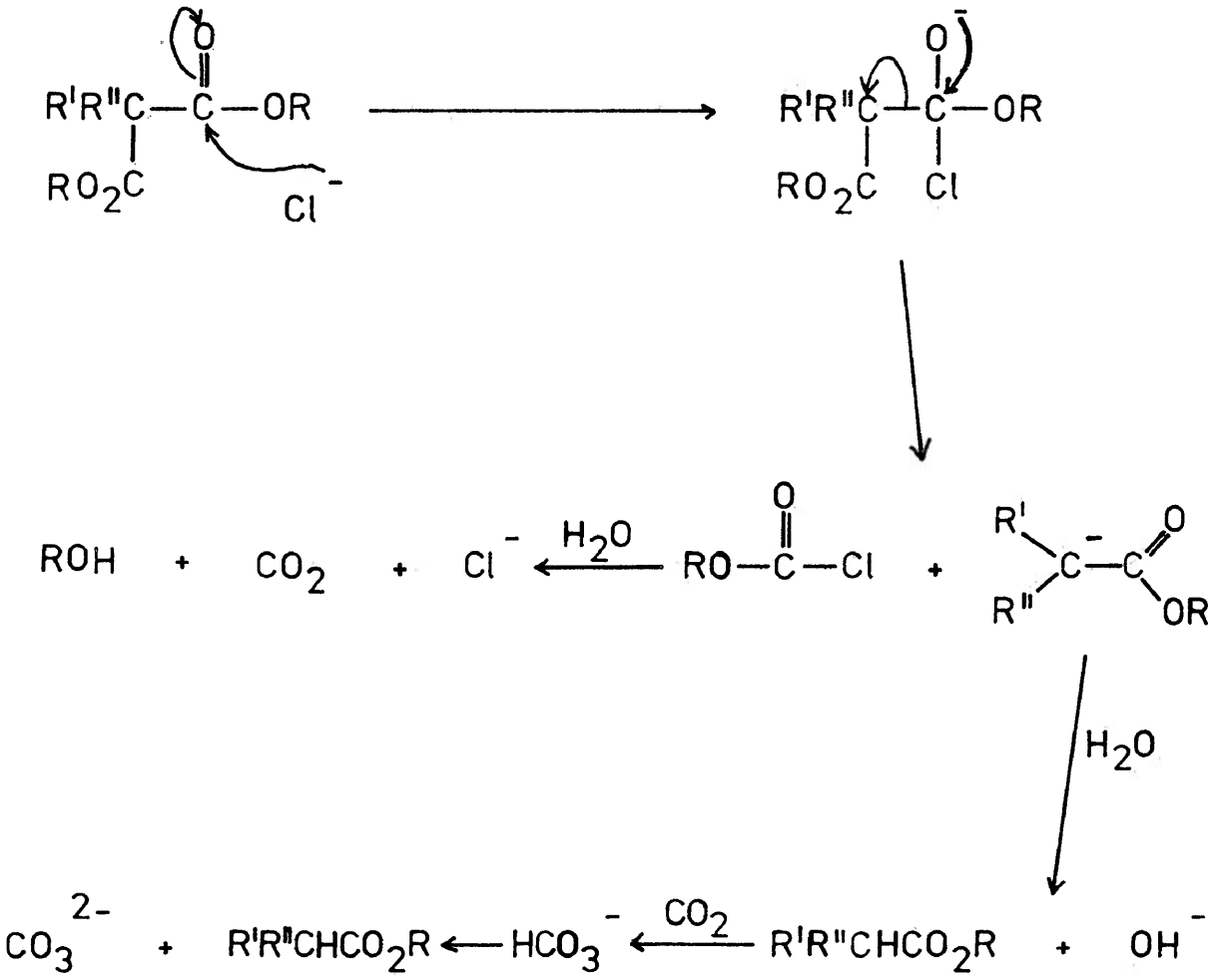
rapidly in wet dimethyl sulfoxide (55, 56). Others, such as disubstituted malonic esters, require the presence of salt (56) such as lithium chloride and potassium cyanide, to bring about the same effect. The reaction can be represented by Eq. [6], p. 42.

The mechanism proposed is a blend of the $B_{AL}2$ (Scheme XII, p. 47) and the $B_{AC}2$ (Scheme XIII, p. 48) pathways in competition (56b). The anion of the added salt (e.g., $C\ell^-$) is involved in the initial nucleophilic attack on the acyl carbon ($B_{AC}2$ mechanism) or on the alkoxyl carbon ($B_{AL}2$ mechanism). It was suggested (56b) that simple reactive esters decarbalkoxylate predominantly via the $B_{AC}2$ mechanism. However, in hindered esters, such as disubstituted malonic esters, the $B_{AL}2$ route appears to be the main pathway.

The trimer malonic ester (XVII) decarbethoxylated readily in $LiC\ell$-H_2O-DMSO to the trimer ethyl ester (XXI) with an average yield of 72.9%. This does not only prove to be a feasible alternate route to the three-step sequence of hydrolysis, decarboxylation and methylation, but could be more preferable. This is indeed the case when other factors, such as yield and ease of the preparation, are considered. The hydrolysis and decarboxylation reactions do not present much difficulties. But methylation, however, becomes more and more difficult as the carboxylic acid becomes more sterically hindered. For example, with diazomethane, the dimer methyl ester



Scheme XII Reaction mechanism for decarbalkoxylation via $B_{AL}2$ pathway (56b)



Scheme XIII Reaction mechanism for decarbalkoxylation via $B_{AC}2$ pathway (56b)

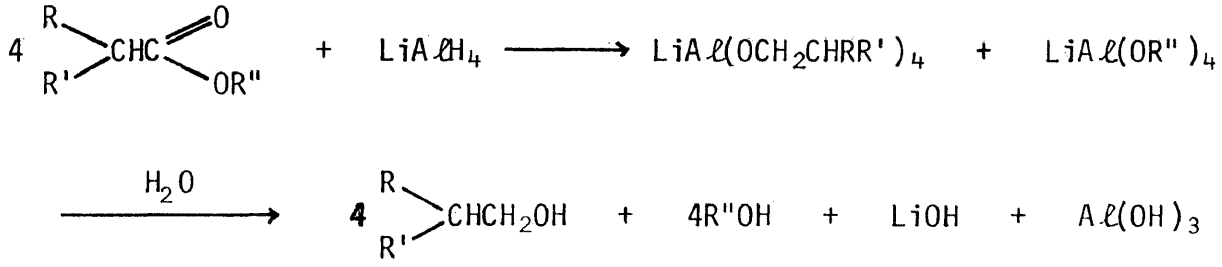
(III), was generated with an average yield of only 64.8%. When the α-carbon atom is substituted with a more bulky 2-hexyldecyl group (trimer monoacid, XIX) instead of a second hexyl group (dimer monoacid, XI), the yield of the diazomethane reaction decreased further to only ~50%. Bulky substitutions on the malonic ester may not affect the decarbalkoxylation reaction as much, since it is basically an elimination process and can proceed by the $B_{AL}2$ pathway (Scheme XII, p. 47). Thus the synthetic route via decarbalkoxylation is shorter and more efficient and is therefore preferred to the other alternate route.

The methyl (III, XX) or ethyl ester (XII, XXI) obtained can be reduced to the respective alcohols (IV, XXII) by lithium aluminium hydride (57). The reaction is represented by Eq. [7], p. 50.

Once the alcohol is prepared, the corresponding alkane can be easily obtained via the reduction of the corresponding sulphonate esters. Using Tipson's procedure (58), the tosylate of the dimer, the 2-hexyldecyl *p*-toluenesulphonate (XIII) was prepared successfully. The reaction is represented by Eq. [8], p. 50 and mechanistically is the nucleophilic substitution of the alcohol to the sulfonyl group (Scheme XIV, p. 51). Again, when the α-carbon of the alcohol becomes more heavily substituted (e.g., the trimer

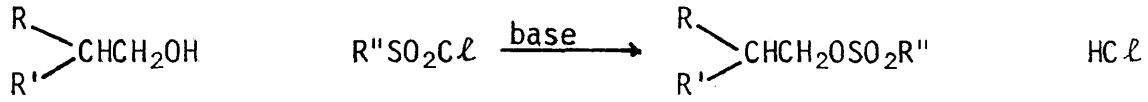
Eq. [7] Reduction of the ester

(R" = CH_3 or C_2H_5)

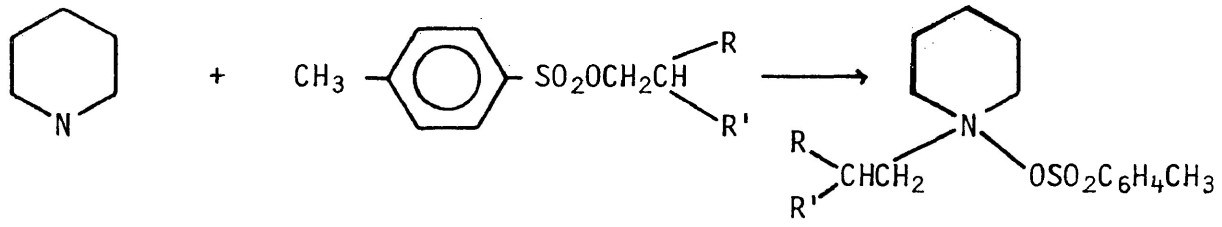


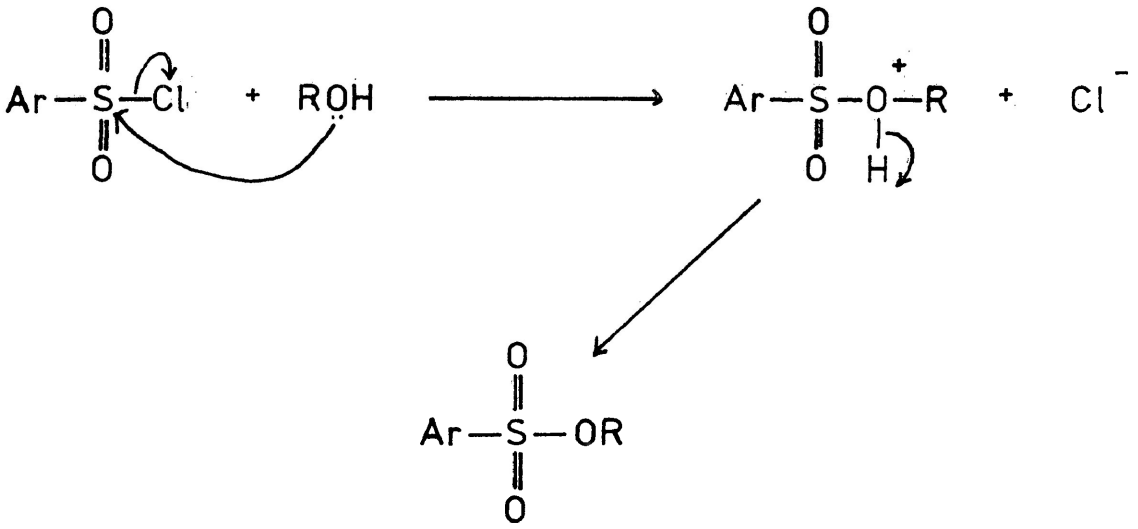
Eq. [8] Conversion of the alcohol to the sulphonate

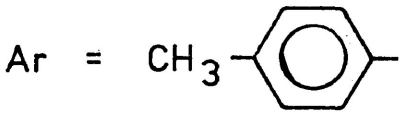
(R" = CH_3 or CH_3-C$_6$H$_4$-)



Eq. [9] Alkylation of pyridine by the tosylate







Scheme XIV Reaction mechanism of formation of *p*-toluenesulphonate from the alcohol

alcohol, XXII), Tipson's procedure does not seem to work. There is little reaction at 0° (the recommended temperature) and tosylation has to be carried out at room temperature. Though 2,4-dihexyldodecyl *p*-toluenesulphonate (trimer tosylate, XXIII) was successfully synthesized with an 84.2% yield in a first attempt, the result was not reproducible. The high temperature might have enhanced the undesirable side-reaction (Eq. [9], p. 50) in which the solvent, pyridine, was alkylated by the product (59). This and the long reaction time (~17 hrs.) limit the applicability of this method.

Although tosylate may be prepared by the reaction of the corresponding alkyl iodide with silver tosylate (60), the procedure requires two synthetic steps from the corresponding alcohol. There are other limitations to other existing methods (58).

Because of this difficulty in preparing some of these tosylate esters, other alternatives were considered. Methanesulphonate esters are also useful synthetic intermediates and can be easily displaced by nucleophiles such as hydride ion (61).

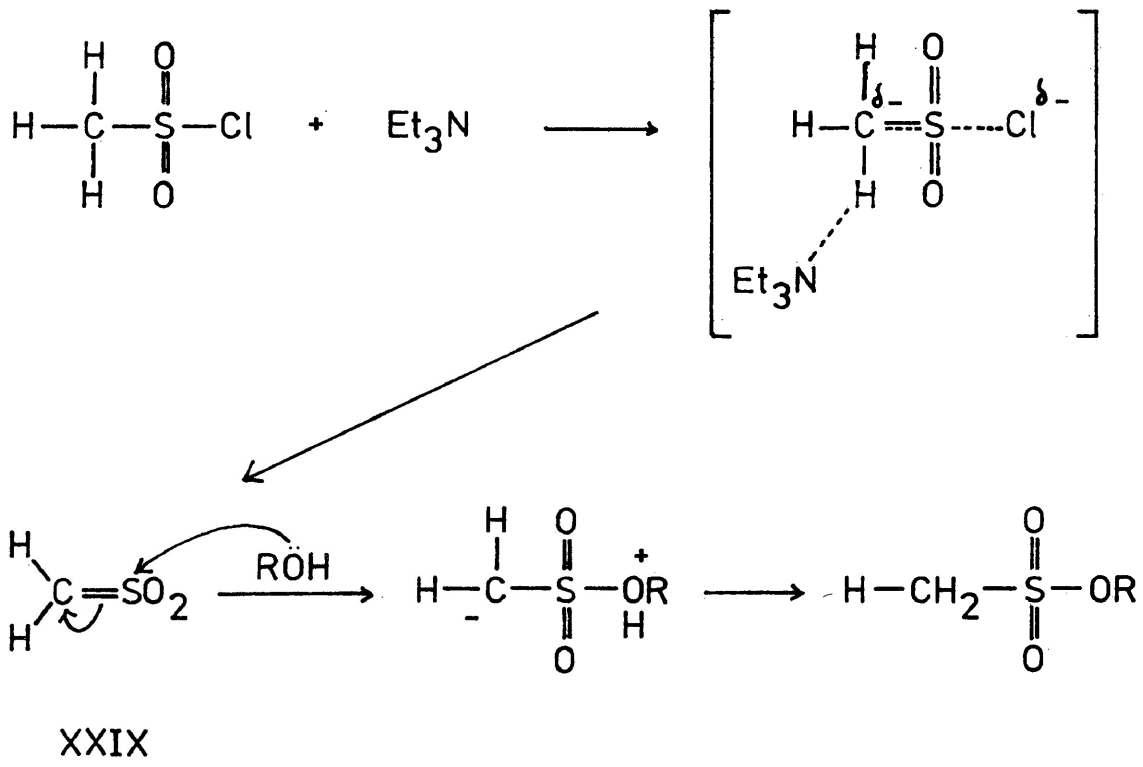
An attempt was made to prepare methanesulphonate using the procedure suggested by Crossland, *et al.* (62). This method differs from Tipson's procedure by using triethylamine as the base and methylene chloride as the solvent. Mechanistically, it is proposed

(62) to be the nucleophilic addition of the alcohol to the sulfene (XXIX) derived by E2 elimination of hydrogen chloride from the mesyl chloride (63), as is illustrated in Scheme XV, p. 54. Also, the mesylates are much less reactive towards solvolysis than the corresponding tosylates (64). It was found that the preparation of the required mesylate esters took place quite readily with an average yield of 87%. This makes it more convenient to take the synthetic route via mesylate as the synthetic intermediate.

The sulphonates can be readily reduced by metal hydrides (Eq. [10], p. 55) such as lithium aluminium hydride (61), or sodium borohydride in DMSO or sulfolane (65). Thus, the alcohols (IV and XXII) lead readily to the dimer (V) and trimer (XXV) hydrocarbons respectively via the tosylate or mesylate esters.

On the other hand, the alcohol (IV) can also be converted into the bromide (XIV) so as to serve as the alkylating agent for the alkylation of a malonic ester in the next synthetic cycle to the higher hydrocarbon homologue as illustrated in Scheme III (see p. 31). The bromination reaction probably involves a S_N2 mechanism (Scheme XVI, p. 56).

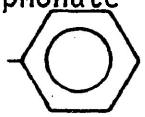
A diffculty was encountered in a first attempt to synthesize the dimer bromide [7-(bromomethyl)pentadecane, XIV] from the dimer

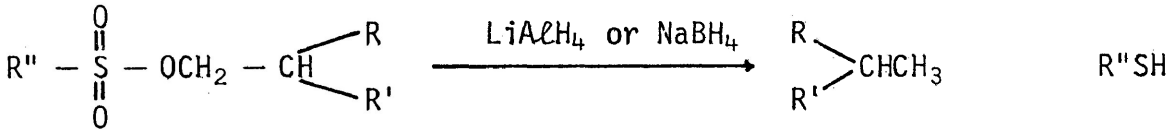


XXIX

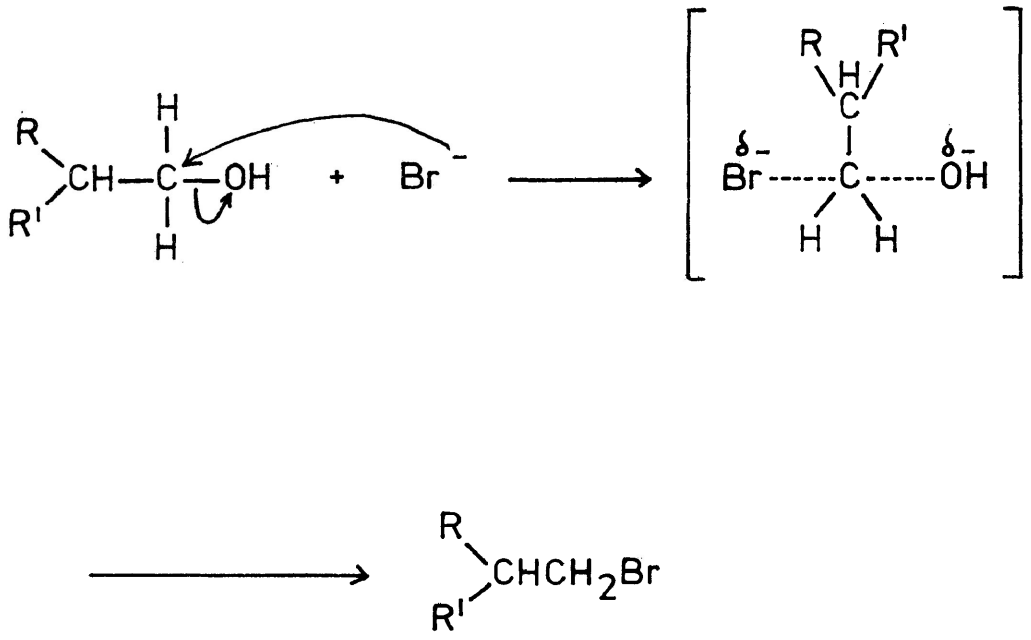
Scheme XV Reaction mechanism for the formation of methanesulphonate using triethylamine as the base

Eq. [10] Reduction of sulphonate

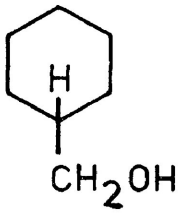
($R'' = CH_3$ or )



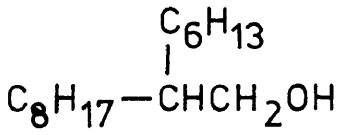
Eq. [11] Conversion of alcohol to bromide



Scheme XVI Reaction mechanism for the conversion of alcohol to the bromide



XXX



IV

alcohol (IV). Using phosphorus tribromide as the brominating agent gave a yield of only 13.5%. Therefore an alternate procedure was designed. Using cyclohexylmethanol (XXX) as a model compound because of its structural similarity to the dimer alcohol (IV), it was found that refluxing the alcohol XXX (and also alcohol IV) with concentrated aqueous hydrobromic acid and passing gaseous hydrogen bromide through the reaction mixture was a more successful method.

The resulting bromide was then used for the alkylation of diethyl hexylmalonic ester (VIII) so as to initiate a second cycle of the synthetic pathway. This led to the trimer hydrocarbon (XXV) without further difficulties.

The Feasibility of Labelling

As discussed earlier (p. 22), a study of the metabolism of these branched chain hydrocarbons may require the use of labelled compounds. Two main factors ought to be considered.

Yield

Table 1, p.58, outlines the average yields of all the reactions in the synthetic sequence which leads to the trimer hydrocarbon (XXV). Thus, starting with 5g of labelled malonic ester, and

Table 1 Intermediate reactions of the malonic ester synthesis sequence to the dimer and trimer hydrocarbons

	Reaction	Average yield (%)	
		R = n-C_6H_{13} , R'= n-C_8H_{17}	R = n-C_6H_{13} , R'= n-$C_8H_{17}CRHCH_3$
1.	$CH(COOC_2H_5)_2 \xrightarrow{RBr} RCH(COOC_2H_5)_2$	70.3	70.3
2.	$RCH(COOC_2H_5)_2 \xrightarrow{R'Br} R'RC(COOC_2H_5)_2$	65.0	59.3
3.	$R'RC(COOC_2H_5)_2 \longrightarrow R'RC(COOH)_2$	80.3	90.2
4.	$R'RC(COOH)_2 \longrightarrow R'RCHCOOH$	89.8	75.6
5.	$R'RCHCOOH \longrightarrow R'RCHCOOCH_3$	64.8	49.8
6.	$R'RC(COOC_2H_5)_2 \longrightarrow R'RCHCOOC_2H_5$		72.9
7a.	$R'RCHCOOC_2H_5 \longrightarrow R'RCHCH_2OH$		92.5
b.	$R'RCHCOOCH_3 \longrightarrow R'RCHCH_2OH$	81.5	87.0
8a.	$R'RCHCH_2OH \longrightarrow R'RCHCH_2OSO_2C_6H_4CH_3$	85.4	-
b.	$R'RCHCH_2OH \longrightarrow R'RCHCH_2OSO_2CH_3$	-	87.5
9a.	$R'RCHCH_2OSO_2C_6H_4CH_3 \longrightarrow R'RCHCH_3$	67.0	49.9
b.	$R'RCHCH_2OSO_2CH_3 \longrightarrow R'RCHCH_3$	-	59.9
10.	$R'RCHCH_2OH \longrightarrow R'RCHCH_2Br$	86.3	

using the shortest and most efficient synthetic route, a yield of 0.965g of labelled dimer hydrocarbon (V) or 0.456g of labelled trimer hydrocarbon (XXV) could be expected. With suitable dilution, this quantity of material is sufficient for conducting biodegradation studies with micro-organisms at concentration levels high enough to be detected with liquid scintillation counting (for C-14 labelled compounds) and to cause significant enhancement in ^{13}C NMR spectroscopy. Thus this synthetic route appears to be feasible for the preparation of labelled hydrocarbons for such metabolic studies.

While it may be assumed that carbon-14 labelling and a subsequent search for radioactive metabolites is directly applicable to the present project, the use of the non-radioactive carbon-13 label may not be quite as straightforward. Nevertheless, the use of carbon-13 labelling does have some advantages, one of which is the avoidance of the health hazards associated with radioactive materials. It is therefore useful to briefly consider the feasibility of this method.

Carbon-13 labelling

In the C-13 NMR spectrum, the strength of the signal due to a given carbon atom is, of course, proportional to the relative abundance of the C-13 isotope with respect to that carbon atom.

Thus, a moderate enrichment with C-13 in a given position will enhance the signal resulting from that carbon atom.

The use of C-13 labelling techniques has solved many of the problems in biosynthesis (66) and elucidating reaction mechanisms (67). It may be expected that, by suitably labelling the substrate before feeding to a micro-organism culture, it would be possible to trace the origin of the labelled carbon atoms in the C-13 NMR spectrum of the resulting metabolites. The only prerequisite to the use of this technique is an assigned spectrum of the compound(s) in question.

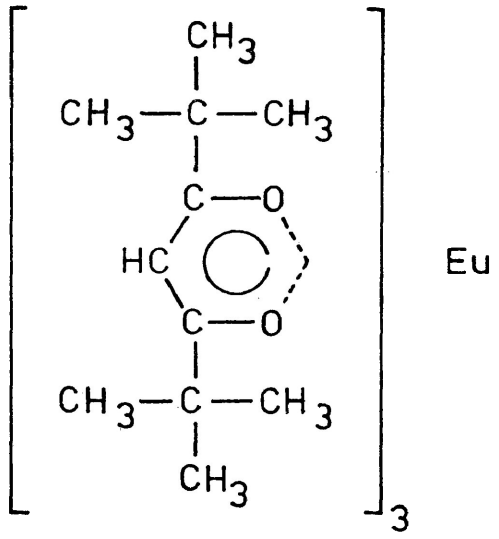
The substrates of the present biodegradation studies would be the PAO-20E itself, and its lower molecular weight homologues, the dimer hydrocarbon (V) and the trimer hydrocarbon (XXV). As a suitable example, the Carbon-13 NMR spectrum of the dimer hydrocarbon (V), Fig. 8 (p. 83) may be considered. The signals are well defined and well separated. Based on all the available spectral information, such as chemical shifts and signal multiplicities, it is possible to assign the spectral lines to all the carbon atoms in the molecule (see pp. 82-85). Of principal importance is the recognition of the signal due to the C-7 atom in the spectrum, since this is the probable position to be labelled. Although the doublet in the off-resonance ^{1}H decoupling spectrum is not well resolved due to the

overlap with the adjacent triplet, it is reasonable to predict that enhancement in its intensity after labelling may make its multiplicity more discernible.

Though C-13 NMR spectrum of the trimer hydrocarbon (XXV) has not been obtained, an attempt has been made to obtain a comprehensible spectrum for its mono-methyl ester (XX), the hydrocarbon backbone of which is identical to the trimer hydrocarbon (XXV). Because of the close resemblance of the carbon atoms on the long hydrocarbon chain, their chemical shifts differ very little. Using the off-resonance ^{1}H decoupling technique alone is insufficient for the full interpretation of the spectrum.

Some rare-earth complexes are known to be able to induce shifts in both the proton (68a) and ^{13}C NMR (68b). This moves some crowded signals far away from one another. By comparing the spectra of the uncomplexed and complexed samples, it is possible to make assignments to carbon atoms which cannot be identified otherwise.

$Eu(thd)_3$ [thd = 2,2,6,6-tetramethyl-3,5-heptanedione, also named as dipivalomethanato(DPM)] was added to the trimer mono-acid (XIX) for this purpose. Some of the signals were shifted downfield, but the spectrum is not distinctive enough to be interpreted. Time did not allow further investigation to exhaust the application of this chemical shift dispersion agent in this problem. Further



$Eu(thd)_3$ or $Eu(DPM)_3$

investigation can be done by varying the [Eu(thd)$_3$]: [substrate] ratio. An investigation into the applicability of other techniques such as chemical shift correlation (69) (comparison of chemical shifts in molecular framework with those found in suitable model compounds), selective decoupling (70), labelling (other than ^{13}C) (70) to make C-atom assignment can also be carried out.

Once the spectra can be assigned, ^{13}C enriched compounds, [the dimer (V) and perhaps the trimer (XXV)] could be used in biodegradation studies.

Characterisation and Identification of Intermediate and Final Products

In most cases, the intermediate and final products in a synthetic sequence are characterized by spectroscopic methods, combustion analysis and melting and/or boiling points. Such methods not only allow identification of functional groups, but also serve as criteria for the purity of a given compound.

The nature of the compounds encountered in the present synthetic sequence does not allow for these conventional methods to be used.

Firstly, nearly all compounds, except the dimer diacid (X),

are oily liquids, which are often difficult to purify to a purity sufficient for combustion analysis. Small quantities of solvent from isolation procedures are often trapped in the sample, and are difficult to remove from compounds which are themselves relatively volatile, particularly when quantities are too small for purification by distillation.

Secondly, nearly all compounds contain a single functional group in a molecule that has a strong hydrocarbon character. This severely limits the use of spectroscopic methods for characterisation purposes since spectra are almost entirely dominated by the hydrocarbon character of the compounds. The following paragraphs will show that although some use could be made of spectroscopic methods, alternative methods have to be found for the proper characterization of some of the compounds.

Characterisation By ^{1}H NMR Spectroscopy

The proton NMR spectra were found to be particularly poor for the purpose of characterizing some of the compounds. A comparison of the spectra of the dimer alcohol (IV, Fig. 1, p. 65), the trimer alcohol (XXII, Fig. 2, p. 66), and the trimer malonic ester (XVII, Fig. 3, p. 67) will illustrate this quite clearly.

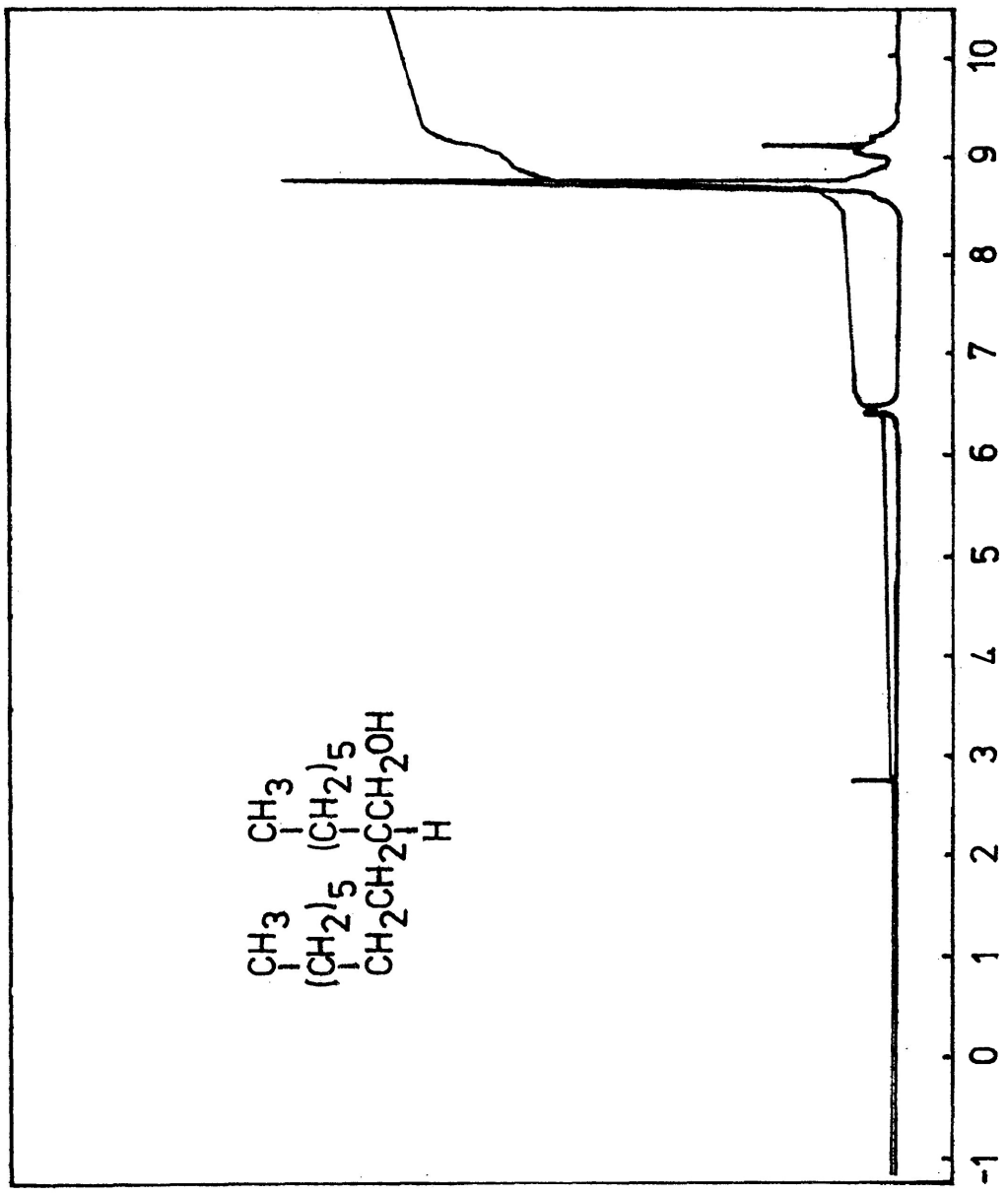


Fig. 1 ^{1}H NMR spectrum of 2-hexyldecanol (dimer alcohol, IV) in deuterochloroform

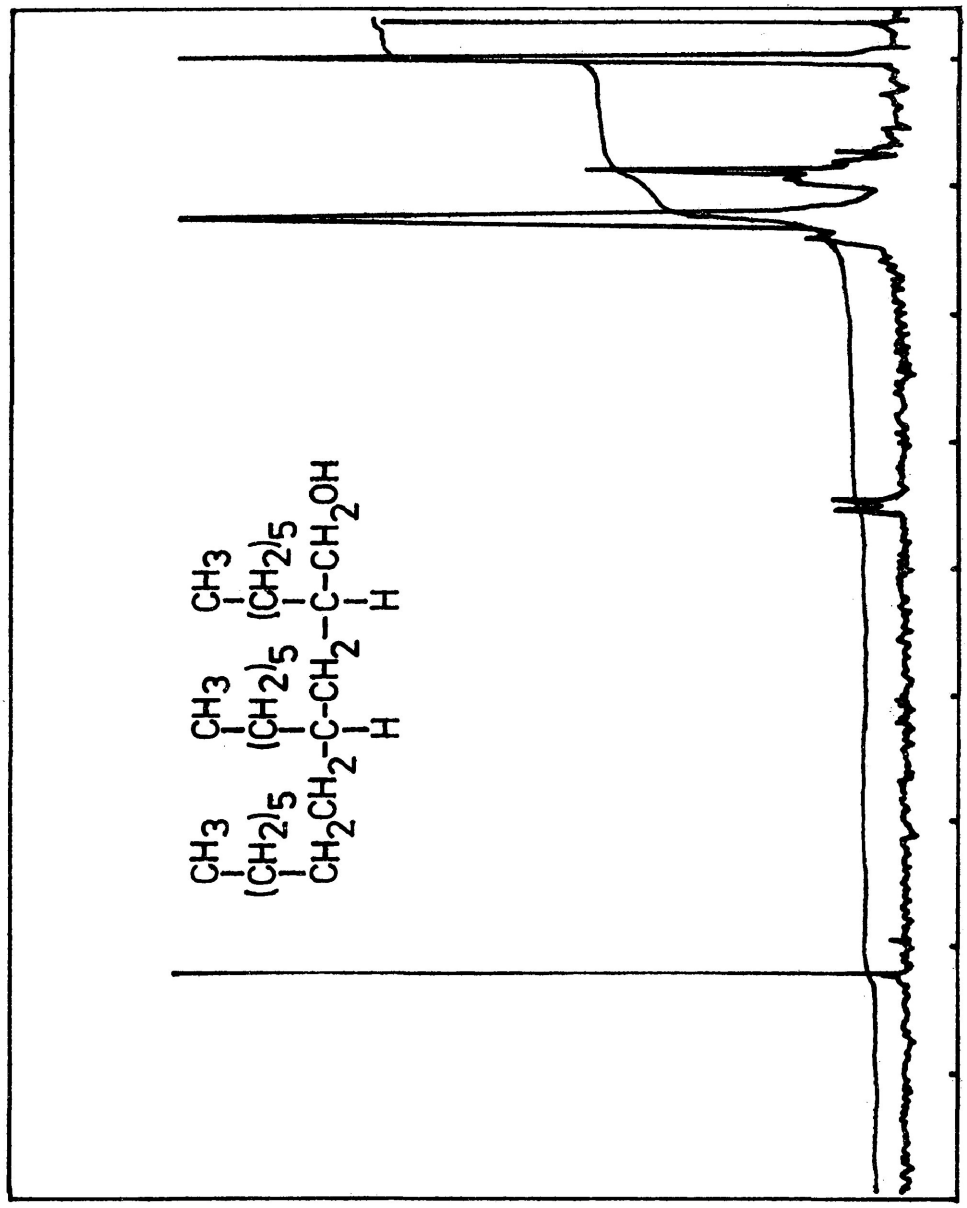


Fig. 2 ^{1}H NMR spectrum of 2,4-dihexyldodecanol (trimer alcohol, XXII) in deuterochloroform

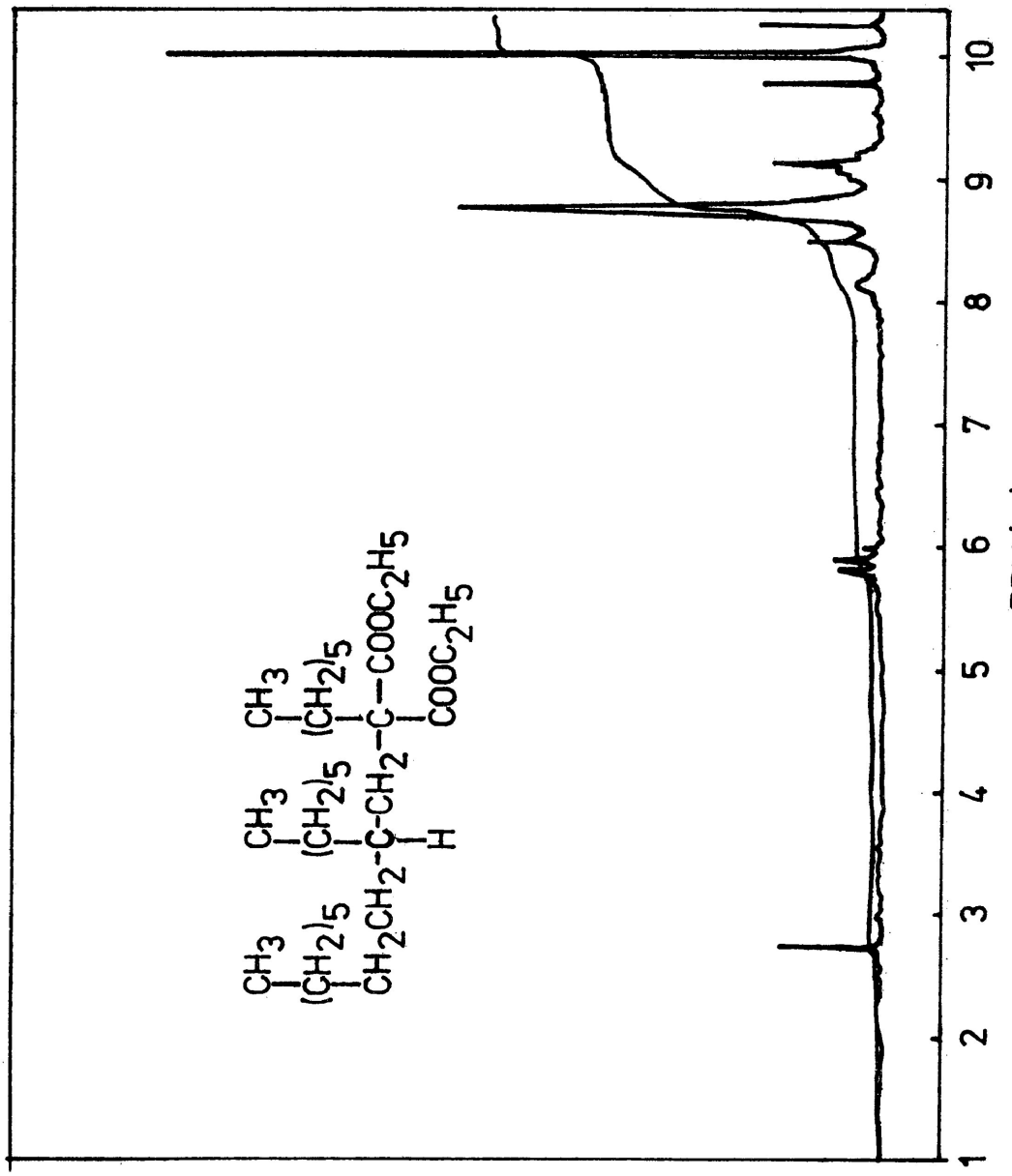
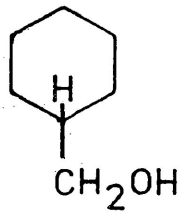


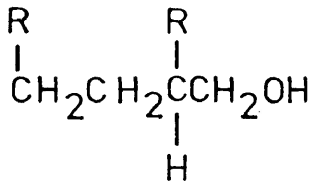
Fig. 3 ^{1}H NMR of diethyl hexyl-(2-hexyldecyl)malonate (trimer malonic ester, XVII) in deuterochloroform

The protons on the main hydrocarbon chain are so much alike in their chemical environment that the signals all merge at τ 9.13 ppm (methyl protons) and τ 8.75 ppm (methylene protons). Varying the nature of the functional group ($-OH$ to $-\overset{O}{\overset{\|}{C}}-OC_2H_5$) and the hydrocarbon chain (dimer to trimer) does not result in any significant change in this part of the spectra. Particularly, the spectra of the dimer alcohol (IV) and the trimer alcohol (XXII) are almost identical. The spectra do not provide enough information such that the various protons on the hydrocarbon chain, especially the single proton on C-2, can be identified.

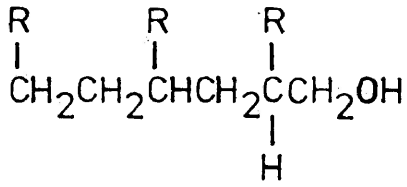
The only feature that allows for some distinction is the signal due to the protons on C-1 of compounds bearing $-OH$, $-Br$ and $-OTs$ as functional groups. The effect of the substituent on the chemical shifts of these protons may shed some light on the nature of the substituent. Some readily available model compounds were used to confirm this observation. Cyclohexylmethanol (XXX), which is commercially available, its tosylate (XXXa) and the corresponding bromide (XXXb) show a structural similarity to the dimer and trimer derivatives containing the same functional groups. It was found that the chemical shift of the protons attached to C-1 correlates well with the nature of the functional group. In all cases, these protons appear as a doublet due to spin-spin coupling with the single proton attached to the tertiary carbon atom C-2. The chemical shifts are tabulated in



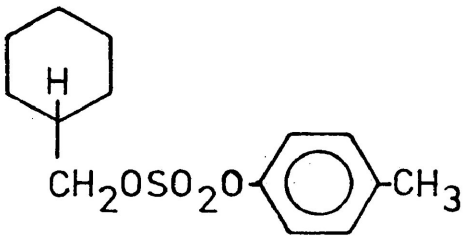
XXX



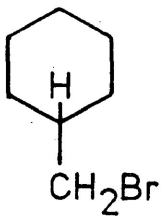
IV



XXII



XXXa



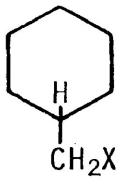
XXXb

Table 2, p. 71. Replacement of the hydroxyl group by a tosyl group causes a downfield shift of 0.4 - 0.8 ppm and replacement by bromide results in a shift of ~0.15 ppm upfield. This information does not only help to establish the presence of hydroxyl group on C-1 of the compounds IV and XXII, it also offers a method to establish the successful conversion of these compounds into their tosylates and bromides. This is especially important because the mass spectra of alcohols and tosylates do not give sufficient information for identification purposes.

Characterisation By Mass Spectroscopy

The molecular ions of the alcohols and the *p*-toluenesulphonates do not appear in their mass spectra (m.s.). This can be seen in the spectra drawn for the dimer alcohol (IV, Fig. 4, p. 72) and dimer tosylate (XIII, Fig. 5, p.73). The largest fragment at $^{m}/e$ 224 corresponds to the ion (i) (Scheme XVII, p. 74) which could be derived from the molecular ion by elimination of the element of water (from the alcohol) or of *p*-toluenesulphonic acid (from the tosylate). The hydrocarbon fragment (i) then undergoes fragmentation, the pattern of which is characteristic of hydrocarbons and is the same for both compounds. This, therefore, cannot provide sufficient evidence for characterizing the two compounds.

Table 2 Chemical shifts of the C-1 protons of $R'RCHCH_2X$ in ^1H NMR spectra

Compound	τ values (ppm)		
	X = OH	X = OTs	X = Br
	6.53	5.72	6.68
$CH_2CH_2 - CHCH_2X$ (R" = $CH_3(CH_2)_5$) a)	6.45	6.04	6.61
$CH_2CH_2 - CHCH_2 - CHCH_2X$ (R" = $CH_3(CH_2)_5$) a)	6.50	6.12	

a) R" = $CH_3(CH_2)_5$

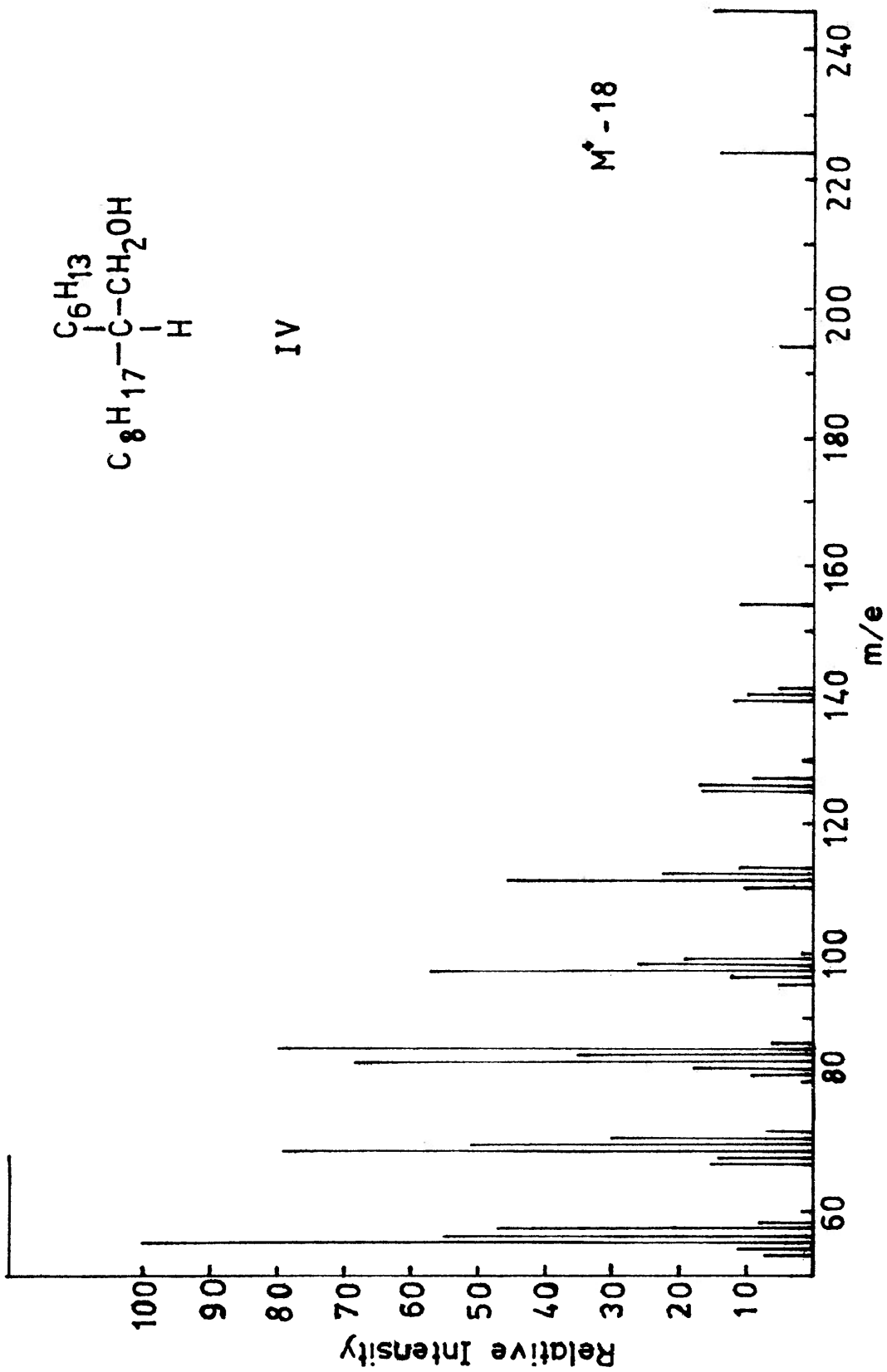


Fig. 4 Mass spectrum of 2-hexyldecanol (dimer alcohol, IV)

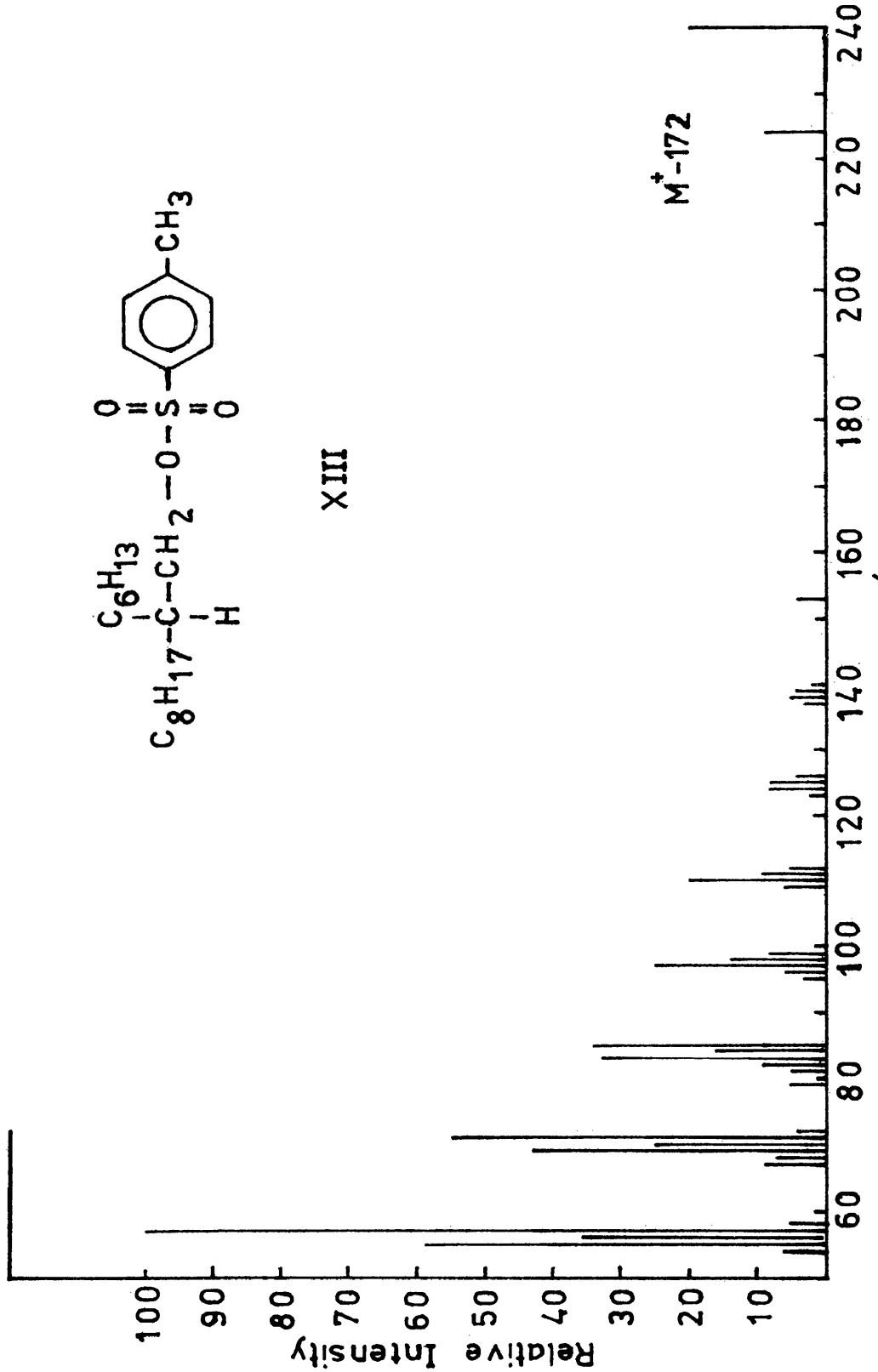
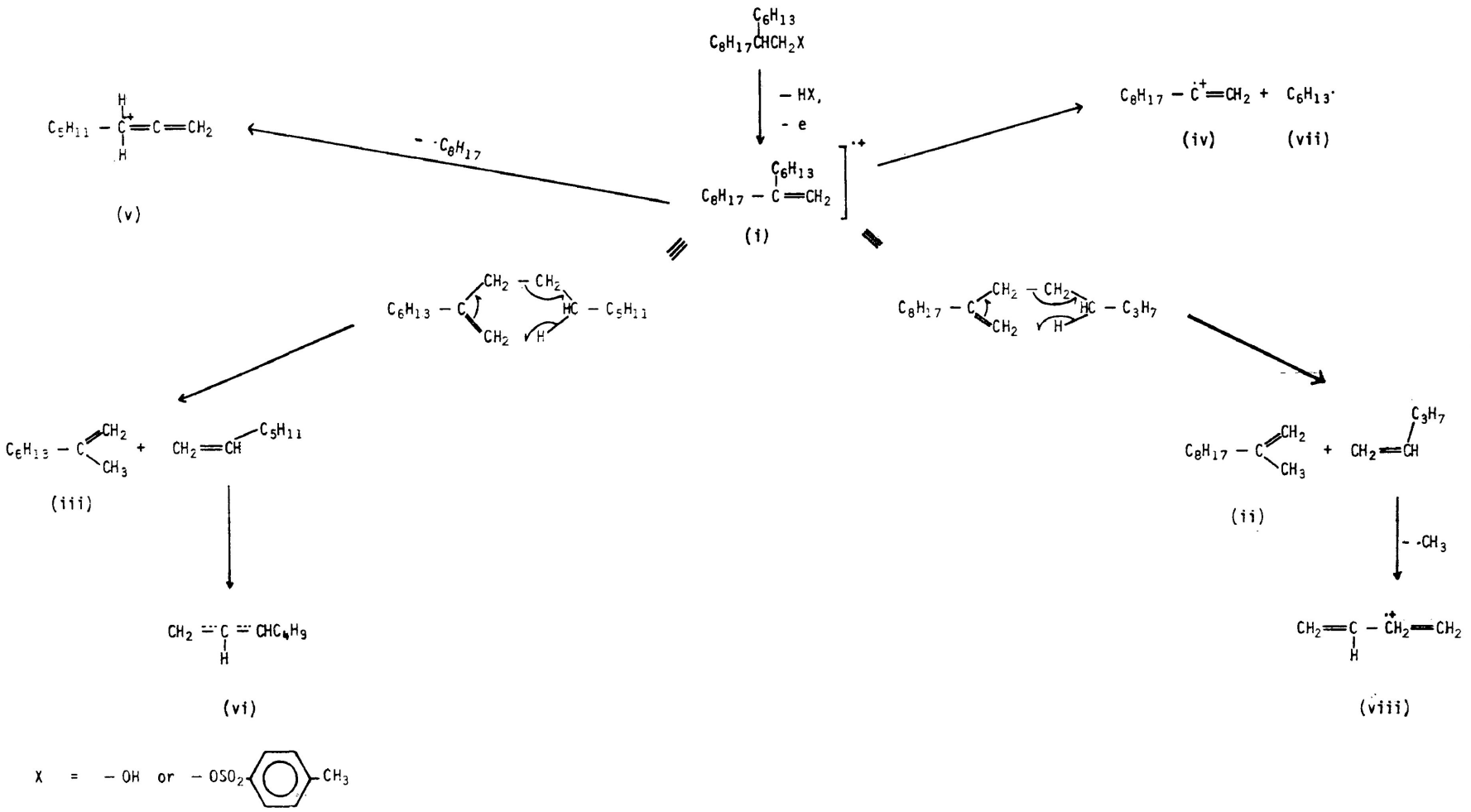


Fig. 5 Mass spectrum of 2-hexyldecyl p-toluenesulphonate (dimer tosylate, XIII)

Scheme XVII Mass spectral fragmentation pattern proposed for the dimer alcohol IV and dimer tosylate XIII



X = – OH or – OSO_2–C6H4–CH_3

(i) m/e 224, (ii) m/e 154, (iii) m/e 126, (iv) m/e 139, (v) m/e 111, (vi) m/e 97, (vii) m/e 85, (viii) m/e 55.

Similar observations may be made for several other compounds in this series.

Thus, it is clear that there are considerable difficulties in the use of both NMR and mass spectroscopy for the characterization of these compounds. It was found, however, that these methods could be successfully complemented by the use of gas-liquid chromatography.

Characterization By Gas-liquid Chromatography

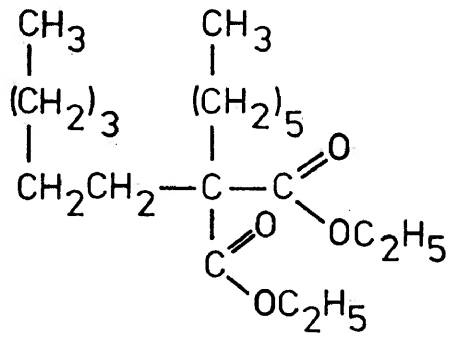
All the compounds prepared in the present project were found to be susceptible to gas-liquid chromatography (GLC) analysis without the need for derivatization, with the exception of the mono- and dicarboxylic acids which had to be esterfied.

James and Martin (71) found that there was a linear relationship between the chain length of a homologous series of hydrocarbons and the log of their respective retention volumes. The retention volume is defined as the volume of carrier gas passing through the column before the centre of the peak emerges. The retention volume is constant for a given substance on a given column provided the temperature and the carrier gas flow rate remain constant. This enabled these authors to assign the correct structural formula to an unknown aliphatic hydrocarbon on the basis of its retention volume.

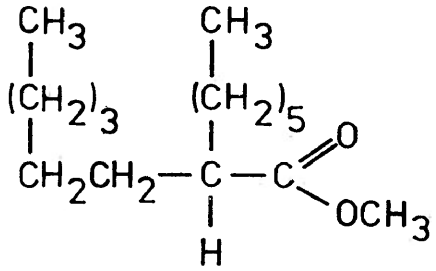
It was found that this method could be used for confirming the identity of some of the compounds in the present series.

In order to obtain a sufficient number of points on the straight line plot of retention time (which is related to retention volume) versus the number of carbon atoms, an additional series of compounds in which one of the alkyl side chains of the substituted malonic ester was two carbon atoms shorter (i.e., the C_4-C_6 series XXXI – XXXV, pp. 77, 78) was synthesized. The malonic ester synthetic sequence (discussed on p. 35) was followed and the synthesis was initiated by alkylating the diethyl hexylmalonate (VIII) with *n*-hexyl bromide instead of with *n*-octyl bromide as in the C_6-C_6 dimer series.

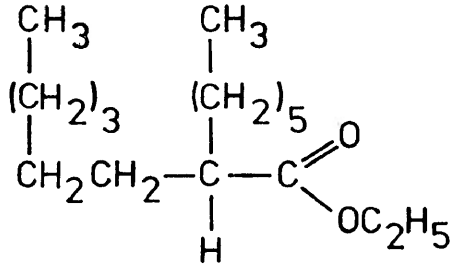
Provided the conditions (flow rate, column temperature, etc.) remain constant in each series, a linear relationship between the log of the retention time and the number of carbon atoms was indeed found. This finding is illustrated in Fig. 6, p. 80 (for the malonic esters VIII, IX, XVII and XXXI; the mono- methyl esters III, XX and XXXII; and the mono ethyl esters XII, XXI and XXXIII) and Fig. 7, p.81 (for the alcohols IV, XXII and XXXIV; and the hydrocarbons V, XXV and XXXV). Although these linear relationships would not be sufficient by themselves for the characterisation of a given compound, these data, together with the limited information obtained from NMR



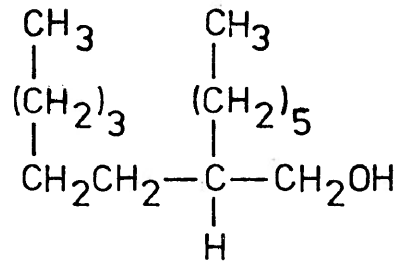
XXXI diethyl dihexylmalonate



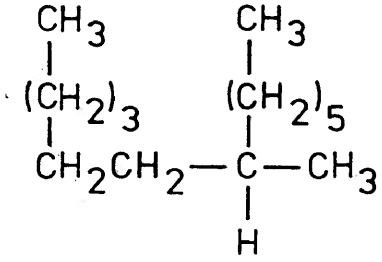
XXXII methyl 2-hexyloctanoate



XXXIII ethyl 2-hexyloctanoate



XXXIV 2-hexyloctanol



XXXV 7-methyltridecane

Table 3 Retention time of the malonic esters, esters, alcohols and hydrocarbons on GLC

Compound	Retention time[c] (min)			
	R' = n-C_6H_{13}			
	R = H	R = n-C_6H_{13}	R = n-C_8H_{17}	R = n-$C_8H_{17}CH(n\text{-}C_6H_{13})CH_2$
1) $RR'C(COOC_2H_5)_2$ [a]	1.30	4.55	8.45	54.2
2) $RR'CHCOOC_2H_5$ [a]		2.18	4.08	31.36
3) $RR'CHCOOCH_3$ [a]		2.12	4.24	38.30
4) $RR'CHCH_2OH$ [b]		3.50	6.40	30.70
5) $RR'CHCH_3$ [a]		1.10	2.02	19.40

a) The stationary phase is SE-30 (5%).

b) The stationary phase is EGSS-X (10%).

c) The retention time is measured at the same temperature for each homologous series: 1) $RR'C(COOC_2H_5)_2$, 210°; 2) $RR'CHCOOC_2H_5$, 200°; 3) $RR'CHCOOCH_3$, 190°; 4) $RR'CHCH_2OH$, 170°; 5) $RR'CHCH_3$, 190°.

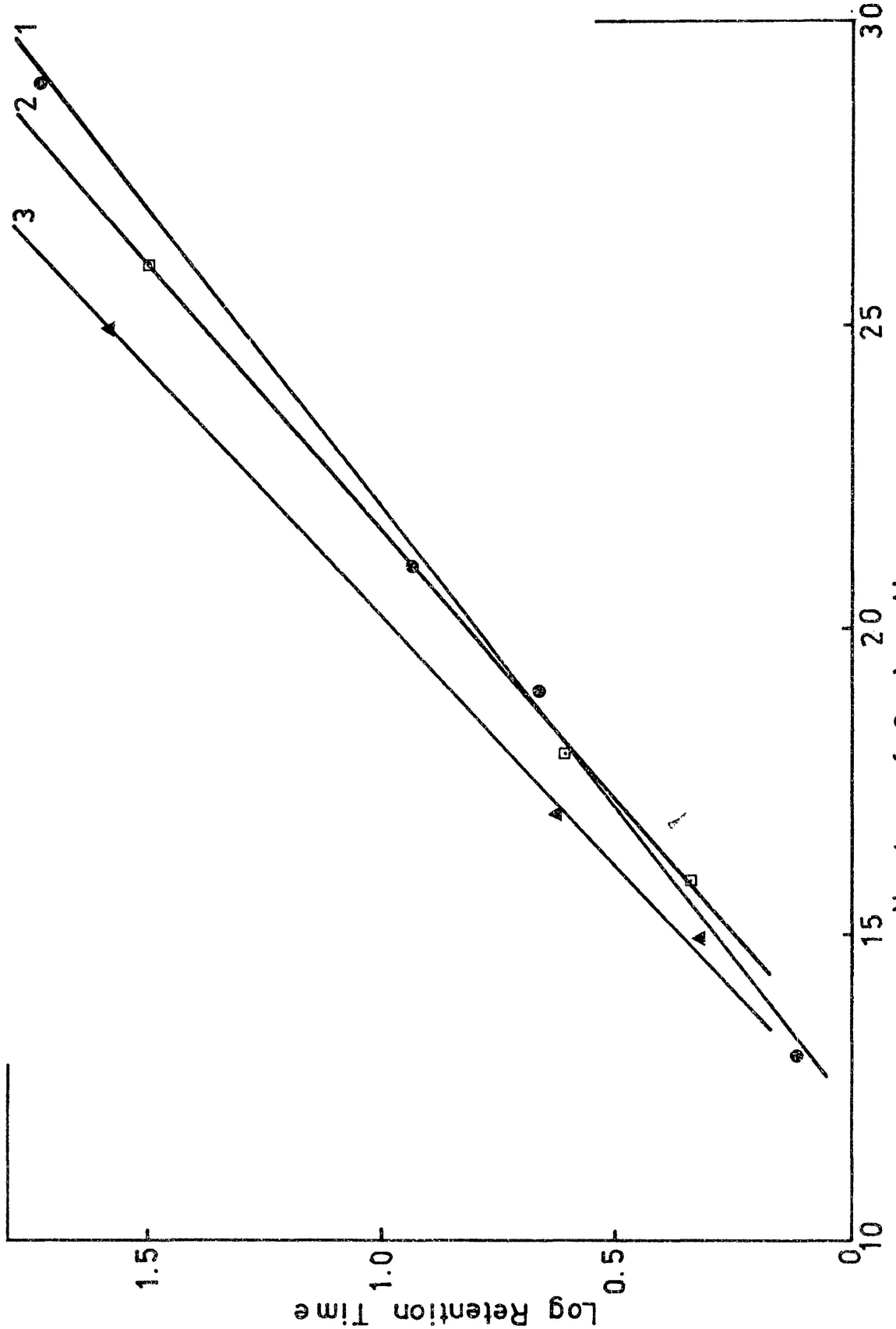


Fig. 6 Relation of log retention time and number of carbon atoms in the molecule
1: $RR'C(COOC_2H_5)_2$; 2: $RR'CHCOOC_2H_5$; 3: $RR'CHCOOCH_3$

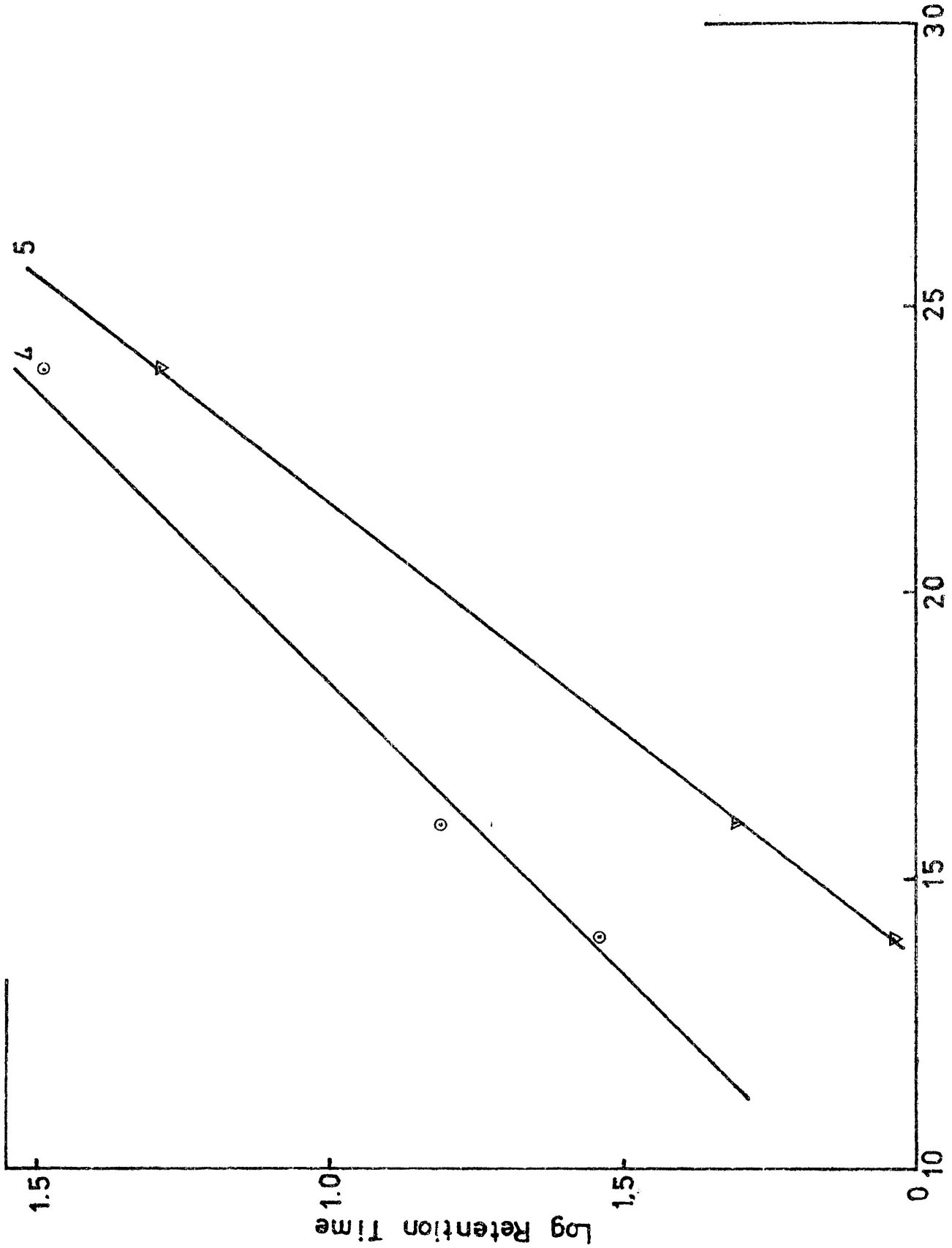


Fig. 7 Relation of log retention time and number of carbon atoms in the molecule
4: $RR'CHCH_2OH$; 5: $RR'CHCH_3$

and m.s. provides sufficient information to establish unambiguously the identity of any compound in the synthetic series.

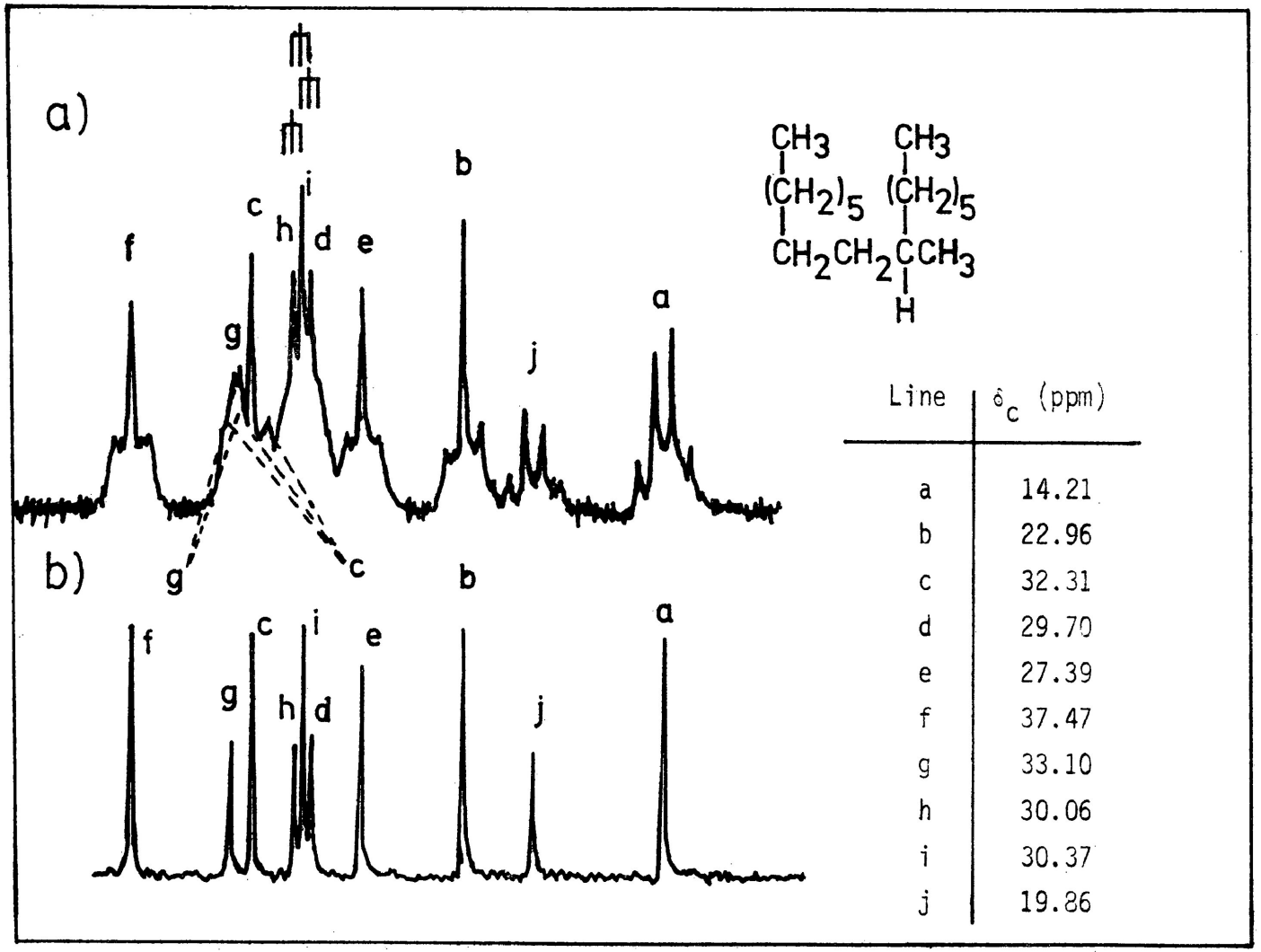
Similar ralationships could not be established for the tosylates and the free carboxylic acids, since in both these series the retention times of the higher members are unacceptably long.

It is, of course, self-evident that for all compounds which are amenable to gas-liquid chromatographic analysis, the gas chromatograms also provide a good criterion for the purity of a given compound.

Characterization by ^{13}C NMR Spectroscopy

Unlike proton NMR, ^{13}C NMR spectra provide information about the molecular framework of molecules.

^{13}C NMR spectra of only a few of the synthesized compounds were studied. The dimer hydrocarbon (V) gave a well defined spectrum (Fig. 8) on p. 83. The broad band 'H decoupled spectrum showed the presence of ten different types of carbon atoms in the molecule with distinctive resonance frequency. The signal multiplicities of most of the peaks observed in the off-resonance 'H decoupled spectrum suggested the immediate environment ('H) of the atom, e.g., the quartets at lines



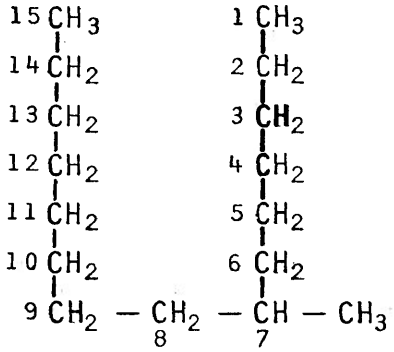
Line	δ_C (ppm)
a	14.21
b	22.96
c	32.31
d	29.70
e	27.39
f	37.47
g	33.10
h	30.06
i	30.37
j	19.86

Fig. 8 20.1 MHz ^{13}C NMR spectrum of 7-methylpentadecane (dimer hydrocarbon,V) in deuterochloroform a) Off-resonance ^{1}H decoupled , b) Broad band ^{1}H decoupled

Table 4 ^{13}C NMR spectral data of the dimer hydrocarbon V[a)]

Peak	Carbon atom	Chemical shift (ppm)	
		δ_C (calc.)	δ_C (obs.)
a	1/15	13.86	14.21
b	2/14	22.65	22.96
c	3/13	32.4	32.31
d	4/12	29.71	29.70
e	5/9	27.27	27.39
f	6/8	36.92	37.47
g	7	32.52	33.10
h	10	30.21	30.06
i	11	30.21	30.37
j	CH_3	19.63	19.86

a) The structure of the dimer hydrocarbon V with assigned carbon atoms is shown below



(a) and (b) corresponding to the terminal methyl groups and the triplets to the methylene group. Though there is some overlapping, (e.g., triplets of d,i,h), the signals can still be distinguished as illustrated in Fig. 8. Making use of this information and comparing the observed chemical shifts with the expected values [calculated using 'Lindeman-Adams rule' (72)], all carbon atoms were readily assigned to the spectral lines (see Table 4, p. 84). The only ambiguity is in the lines at δ 30.06 and 30.37 ppm. These signals most likely belong to C-10 and C-11 which were expected to resonate at the same frequency (Table 4 peaks h and i). The presence of a methyl group at the γ carbon atom is expected to cause an upfield shift (70) for C-10. This enables the assignment of the high field methylene signal to C-10 and the line at 30.37 ppm to C-11.

The spectrum of the trimer methyl ester (XX) was well defined. The compound contains 26 carbon atoms. With the exception of the methoxy carbon atom, all the others have similar chemical environment. This accounts for the close proximity of the signals. The coupling patterns by the adjacent protons overlap each other to such an extent that attempts to distinguish between individual signal are unsuccessful.

An attempt was made to obtain further information on the assignment of the various signals by the use of the shift reagent

$Eu(thd)_3$, but was unsuccessful. Clearly, further work on this method of characterization is required (see Recommendations, p. 63).

CHAPTER 3

INTERACTION OF PAO-20E AND ITS SYNTHETIC HOMOLOGUES WITH *SACCHAROMYCOPSIS LIPOLYTICA*

PAO-20E, being a dielectric oil, will inevitably enter into natural environment through leaks and/or spills during transportation and handling. Because of its low solubility in water, it may be anticipated that the oil would be adsorbed on soil and/or suspended matter as it finds its way into the aquatic environment. The fate of the oil will be determined by factors such as photodegradation, chemical decomposition and biodegradation. The latter is the subject of interest at present, particularly with respect to micro-organisms.

Microbial degradation of hydrocarbons, both aliphatic and aromatic, has been reported in the literature (see Chapter 1). Most of the work done on the aliphatic series, however, was on straight chain hydrocarbons. *Pseudomonas, Micrococcus* and *Candida lipolytica* are some of the species used in such studies.

It was decided to use *Saccharomycopsis lipolytica* (formerly *Candida lipolytica*) as a test organism to carry out initial experiments on the biodegradability of the PAO-type hydrocarbons. Studies were carried out in pure cultures.

Before interaction studies between PAO-20E and its homologs and *S. lipolytica* can be carried out, some preliminary experiments were conducted so as to select a suitable growth medium for the micro-organism. Since *C. lipolytica* was known to grow in a mineral-salt medium with glucose or hydrocarbon as the carbon source (Medium A*, 29a), cultures were prepared using that culture medium. However, normal growth, measured in terms of the dry weight of the filtered mycelium (73), was unsatisfactory in this medium (see Table† 1, p. 89). Other culture media B* and C* were tried out. Medium B contains casamino acid and yeast extract as the nitrogen source and in Medium C, these two compounds were replaced by the same amount of yeast nitrogen base (Difco). A comparison of the dry weights of filtered mycelia of the yeast growing in the different growth media (see Table 1) suggests that the yeast seems to grow best in Medium B containing glucose (0.5%). Therefore, this nutrient medium has been chosen for the present work.

As mentioned earlier (Chapter 1), hydrocarbon oxidizing enzymes may be either adaptative or constitutive in nature. More specifically, it has been shown (74) that in *Candida lipolytica* these enzymes are almost certainly adaptative. Therefore, the hydrocarbons to be tested were added at the time of inoculation,

*See Appendix for recipes of growth media

†Tables are numbered per Chapter

Table 1 Growth rate of *S. lipolytica* in different culture media (represented by the dry weights of filtered mycelium)

Medium	Carbon Source (%)	Duration of Incubation (days)	Dry weight of filtered mycelium [a] (g)
A	–	8	0.004
B	–	10	0.036
A	glucose (1)	8	0.030
B	glucose (0.5)	8	0.085
B	glucose (0.5)	10	0.135
C	glucose (0.5)	10	0.080

[a] Average of triplicate dry weights of filtered mycelium

immediately after autoclaving of the medium, so that the yeast would grow in the presence of the hydrocarbon substrate. Growth periods of 10-12 days (see Table 2, p. 91) were employed for most of the experiments.

The following experiments were carried out.

(i) Incubation with commercial PAO-20E

The yeast was grown in culture Medium B containing PAO-20E for 10 days, after which time its mycelium was filtered. The dry weights of the filtered mycelia were compared with those obtained from control cultures grown without PAO-20E. The results are listed in Table 3, p. 92 .

It has to be emphasized that there was no other carbon source in (i) except amino acids present in a very small quantity. Therefore, it appears that the addition of a small amount of PAO-20E enhances the growth of the yeast somewhat.

To test for the probable oxidation of the oil by the yeast, the cultures of (ii) were extracted with diethyl ether.

As primary alcohols and monocarboxylic acids had been

Table 2 Rate of growth of *S. lipolytica* in culture Medium B (containing 0.5% glucose)

Duration of Incubation (days)	Dry weight of filtered mycelium[a] (g)
8	0.085
10	0.135
12	0.149
14	0.135

[a] Average of triplicate dry weights of filtered mycelium

Table 3 The growth of *S. lipolytica* in culture Medium B after 10 days of incubation

Experiment	Carbon Source/Substrate (%)	Dry weight of filtered mycelium[a] (g)
(i)	—	0.036
	PAO-20E (0.5)	0.071
(ii)	glucose (0.5)	0.135
	glucose (0.5) + PAO-20E (0.005)	0.145

[a] Average of triplicate dry weights of filtered mycelium

identified (29a) in cell-fluids of this species grown on glucose, infra-red analysis were done on the present extracts. The spectra indicated the presence of alcohols and carboxylic acids in the extracts grown on both PAO-20E and glucose alone. Gas-liquid chromatography (on SE-30 5%) of the whole extracts of these cultures gave very similar results for both test cultures and controls. When the extracts were washed with 2N sodium hydroxide solution, a change could be observed in the gas chromatograms (Fig. 1*, p. 94). The peaks (2) and (3) almost disappeared on saponification. This suggests that carboxylic acids and/or esters were removed from the extract by the NaOH treatment. However, these changes were the same for both control and test cultures. Thin layer chromatography showed that several new compounds were present in the test cultures as compared to the controls. But no attempt has been made to isolate and identify these compounds.

(ii) Recovery efficiency of PAO-20E

In order to test if the hydrocarbon is being assimilated, it is best to measure its recovery rate quantitatively. This was done using gas-liquid chromatography. However, PAO-20E, being a hydrocarbon with a high molecular weight, would not readily elute from the column

*Figures are numbered per Chapter

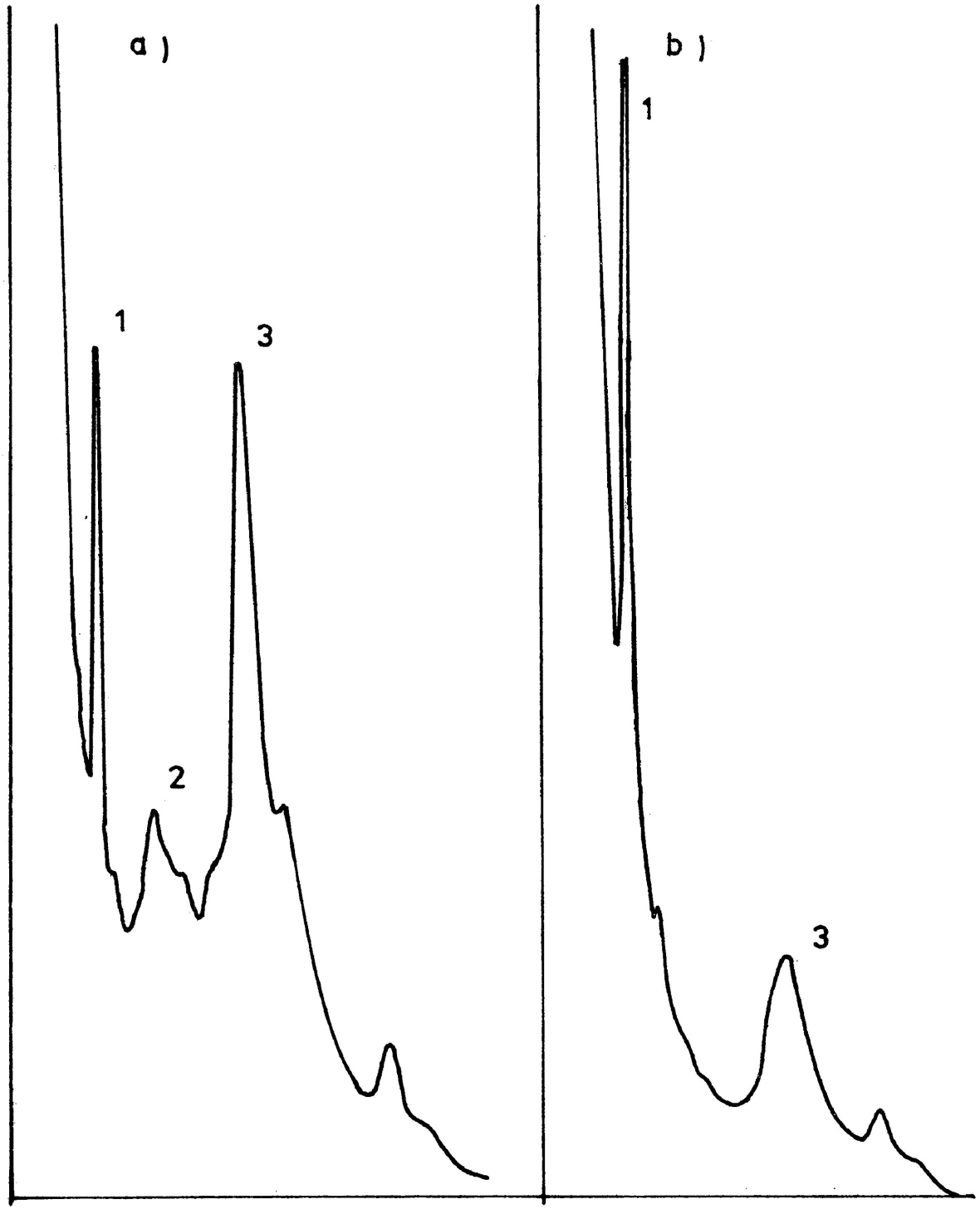


Fig. 1 Gas-chromatograms of extracts of *S. lipolytica* incubated with glucose on the SE-30 (5%) column a) before being washed with 2N NaOH and b) after

(see p. 19). In view of this result, further biodegradation studies have been limited to 7-methylpentadecane (dimer hydrocarbon, V) and 7-methyl-9-hexylheptadecane (trimer hydrocarbon, XXV), synthesized for this purpose. Since only a small quantity of the synthesized compounds was available, it was not possible, at the present stage, to use the hydrocarbon substrate as the only carbon source. Glucose (0.5%) was added (unless otherwise stated) for all studies done.

(iii) Incubations with the dimer hydrocarbon (V)

After incubation of the yeast with the dimer hydrocarbon, it was found that no hydrocarbon could be recovered from the test cultures. Subsequent experiments showed that the hydrocarbon was also lost from a set of control flasks containing only the medium, but no inoculum. Also, flasks containing water (100 ml) and hydrocarbon in the same amounts as used for the cultures were found to contain very little hydrocarbon after 16 days on the shaker.

It is assumed that the dimer hydrocarbon which has a relatively low molecular weight ($C_{16}H_{34}$) is lost by evaporation. This compound would therefore not be suitable for incubation in aerobic cultures. For further experiments with *S. lipolytica*, only the trimer hydrocarbon (XXV) was used, since a similar set of

experiments showed that its volatility would not be a problem.

The effect of the volatility of these compounds on recovery efficiency are summarised in Table 4, p. 97, and the analytical details of these experiments are reported in Chapter 4 (see p.129).

(iv) Incubations with the trimer hydrocarbon (XXV)

The yeast was incubated with 20 ppm of trimer hydrocarbon (XXV) for ten days. A set of control cultures were grown under the same conditions and the same quantity of the compound was added to these at the end of the growth period. The cultures were extracted with diethyl ether and the extracts were analysed by gas-liquid chromatography as described (see p.101). About 91% of the trimer hydrocarbon was recovered from the test cultures. This suggests that some biodegradation took place and a portion of the hydrocarbon had been consumed by the yeast.

This first experiment was repeated under the same conditions for different growth periods, but no biodegradation was evident.

Several other incubations were also carried out with 10 ppm trimer hydrocarbon (XXV) and approximately 100% of the hydrocarbon

Table 4 Recovery efficiency of the dimer (V) and trimer (XXV) hydrocarbons from water and/or culture Medium B (containing 0.5% glucose).

Time on the shaker (days)	Recovery efficiency of addend (%)[a]		
	Dimer hydrocarbon (V)		Trimer hydrocarbon (XXV)
	b	c	c
0	73.5	67.3	97.6
1	70.0		
2	72.5		
3	-	-	94.7
4	71.1	25.6	-
6	65.2	-	94.5
8	38.1	22.1	-
9	-	-	98.4
16	15.6	13.7	-

a) Average of triplicate analysis of recovery efficiency of addend

b) Analysis of recovery efficiency of addend from water

c) Analysis of recovery efficiency of addend from culture Medium B (0.5% glucose added)

Table 5 Quantitative analysis of extracts of *S. lipolytica* incubated with trimer hydrocarbon (XXV).

Experiment	Duration of Incubation (days)	Amount of trimer hydrocarbon added (mg)	Average recovery[a] (corrected) of addend (%)
1	10	2.026	91
2	13	2.0	100
3	15	2.006	100
4	10	1.003	100
5	15	1.003	100
6	10	1.0	100
7[b]	13	10.0	100

[a] Average of triplicate *S. lipolytica* culture extract analysis

[b] The glucose content was reduced by half, i.e., 0.25% glucose content.

was recovered in all cases.

The results of the various experiments are summarized in Table 5, p. 98.

Since glucose was also present in the medium, the organism might selectively grow on it instead of utilize the added hydrocarbon. Thus, in Experiment 7, the amount of glucose was decreased by half and more hydrocarbon (i.e., 100 ppm.) was added. But the recovery rate stayed the same (100%), indicating that the hydrocarbon was not degraded.

Since most studies reported in the literature were carried out in nutrient media with the hydrocarbon in question as the sole carbon source, it would be too early to say that the species *Saccharomycopsis lipolytica* does not degrade the branched hydrocarbon, 7-methyl-9-hexylheptadecane (XXV).

Clearly, further experimentation is required in order to show conclusively whether or not *Saccharomycopsis lipolytica* is able to biodegrade this type of hydrocarbon.

CHAPTER 4

EXPERIMENTAL METHODS

A) Analytical Methods

Nuclear magnetic resonance details of instrumentation: ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Wp-80 spectrometer, employing deuterochloroform with tetramethylsilane as an internal reference.

Infra-red spectral details of instrumentation: Infra-red (IR) spectra were recorded on a Beckman IR 12 infra-red spectrophotometer. Samples were prepared in solutions using carbon tetrachloride or chloroform as solvent. Infra-red cavity cells with path length 0.1 mm were used.

Mass spectral details of instrumentation: A double focusing mass spectrometer (Hitachi-Perkin-Elmer RMU-7), operating at an ionizing voltage of 80 eV was used.

Thin layer chromatography (TLC) method: Unless stated otherwise, benzene was used as the eluent and iodine as developer for the silica gel plates.

Gas-liquid chromatography (GLC) method: A Perkin Elmer Model 3920 B gas chromatograph, equipped with a flame ionization detector (FID) was used. Dual glass columns (6 ft., 2 mm i.d.) packed with Gaschrom Q (80 - 100 mesh) coated with SE-30 silicone rubber gum (5%), dexsil 300 (3%) or EGSS-X (10%) were used. The column temperatures were 120-240° and nitrogen was used as the carrier gas (25 ml/min.).

Quantitative analysis was made by the use of internal standards [diethyl azelate, p.129, or di-*n*-butyl phthalate, p.130 (75)]. Standard solutions were prepared by dissolving 0.25, 0.4, 0.8, 1.0, 1.25, 1.5, 1.75, 2.0 and 2.25 mg of the hydrocarbon each in 1 ml of the appropriate internal standard solution. The peak height ratios obtained from their chromatograms were plotted against the corresponding weight ratios to yield a calibration curve. However, the trimer hydrocarbon (XXV) concentration in the extracts from incubation experiment 7 (p. 99) did not fall within the above concentration range. Thus, another set of standard solutions was required. This was obtained by dissolving 0.9, 1.8, 4.2, 6.0, 7.8, 10.2 and 12 mg. of trimer hydrocarbon (XXV) each in 2 ml. internal standard solution. A new calibration curve was prepared immediately prior to the actual analysis for each set of samples.

B) Synthetic Chemistry

All starting materials were commercially available, solvents were reagent grade and were used without further purification.

All the synthetic products were dried with anhydrous sodium sulphate. Melting points were determined with an electro-thermal melting point apparatus and were uncorrected. The purity of each compound, after distillation or column chromatography, was determined by thin layer and gas-liquid chromatography.

diethyl hexylmalonate (VIII)

The preparation was carried out according to Adams and Kamm (76). Dry ethanol (50 ml, ethanol was refluxed with one-twentieth its weight of sodium and distilled immediately prior to use) was placed in a three-necked round-bottomed flask (300 ml), which was fitted with a reflux condenser, a dropping funnel and a magnetic stirrer. Sodium (2.29 g) was added in small pieces. When the sodium had dissolved, the temperature was adjusted to 50°C, and diethyl malonate (16.11 g) was added. To the resulting clear solution *n*-hexyl bromide (16.31 g) was added dropwise.

The reaction mixture was refluxed until it was neutral to

pH paper (2 1/2 hr). A major portion of the solvent was removed by distillation and the remaining residue was dispersed in water (40 ml). The aqueous mixture was extracted with methylene chloride (25 ml), the extract washed with water and dried. Removal of the solvent yielded the crude product (23.64 g), which was distilled at 15.9 mm Hg. A low boiling impurity was discarded, and diethyl hexylmalonate (20.12 g, 81.9%), isolated as a fraction with boiling point 132-140°, was pure as determined by thin layer chromatography (TLC) and gas-liquid chromatography (GLC). The infra-red (IR) spectrum contained absorption maximum at 1765 cm^{-1} ($-\overset{O}{\overset{\|}{C}}-OC_2H_5$). The NMR spectrum contained a complex signal at τ 8.62 - 8.84 (19H), a triplet at τ 6.56 - 6.78(1H, C-2 H) and a quartet at τ 5.65 - 5.94 (4H, 2 $-OCH_2-$) ppm. The mass spectrum (MS) contained the molecular ion M^+ at $^m/e$ 244 (3%, $C_{13}H_{24}O_4^+$) and major fragments at $^m/e$ 199 (21%, $C_{11}H_{19}O_3^+$), $^m/e$ 173 (29%, $C_9H_{17}O_3^+$), $^m/e$ 160 (100%, $C_7H_{12}O_4^+$) $^m/e$ 133 (25%, $C_6H_{12}O_3^+$) and $^m/e$ 88 (21%, $C_4H_7O_2^+$).

diethyl hexyl-octylmalonate (dimer malonic ester, IX, 76)

Dry ethanol (60 ml) was placed in a three-necked bottomed flask (300 ml), which was fitted with a reflux condenser, a dropping funnel and a magnetic stirrer. Sodium (2.93 g) was added in small pieces. When the sodium had dissolved, diethyl hexylmalonate (VIII, 17.9 g) was added. To the resulting clear solution, *n*-octyl bromide

(12.90 g) was then added.

The reaction mixture was refluxed until it was nearly neutral to pH paper (2 1/2 hr). Most of the ethanol was removed by distillation. The remaining residue was dispersed in water (2 x 25 ml). The aqueous mixture was extracted with methylene chloride (20 ml). The extract was washed with water and dried. Removal of the solvent yielded a crude product which was then distilled at 12.4 mmHg. A low boiling impurity was discarded, and diethyl hexyl-octylmalonate (19.17g, 80.6%), isolated as a fraction with boiling point 180-190°, was found pure on TLC and GLC. The NMR contained a complex signal at τ 8.0 - 9.22 and a quartet at τ 5.66 - 5.97 (with a ratio of 9:1) ppm. The mass spectrum contained the molecular ion at $^{m}/e$ 356 (2%, $C_{21}H_{40}O_4^+$) and major fragments at $^{m}/e$ 310 (13%, $C_{19}H_{34}O_3^+$), $^{m}/e$ 282 (26%, $C_{18}H_{34}O_2^+$), $^{m}/e$ 271 (44%, $C_{15}H_{28}O_4^+$), $^{m}/e$ 243 (62%, $C_{13}H_{24}O_4^+$), $^{m}/e$ 226 (11%, $C_{14}H_{25}O_2^+$), $^{m}/e$ 173 (100%, $C_8H_{14}O_4^+$) and $^{m}/e$ 170 (21%, $C_{10}H_{18}O_2^+$).

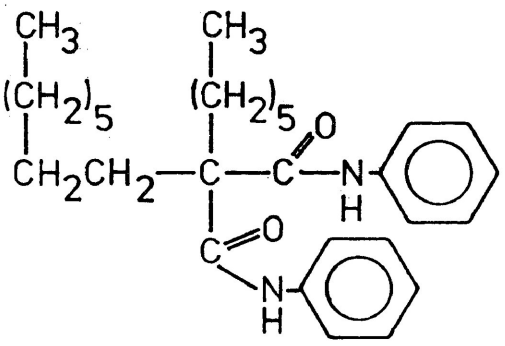
hexyl-octylmalonic acid (dimer diacid, X)

Diethyl hexyl-octylmalonate (IX) was hydrolysed according to Marvel (48). Potassium hydroxide (11.64 g) was dissolved in water (9 ml), and to the resulting aqueous solution diethyl hexyl-octylmalonate (IX, 19.17 g) was added dropwise with continuous stirring.

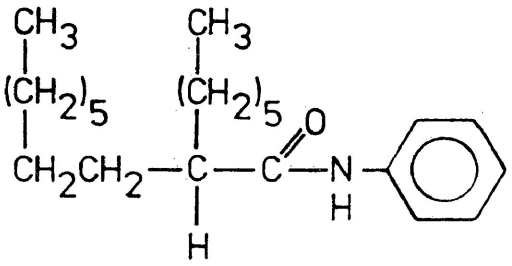
Stirring was continued and the mixture was heated (5 hr). As the reaction progressed, ethanol produced was removed under vacuum and water was added occasionally to prevent solidification of the reacting mixture.

The resulting mixture was cooled in an ice-bath, acidified with hydrochloric acid to pH 1-2, and extracted with diethyl ether (3 x 100 ml). The combined ethereal extracts were washed with water, dried and the solvent removed to give white crystals of hexyl-octyl-malonic acid (X, 14.33 g, 88.4%). The product was pure as determined by thin layer chromatography. The IR spectrum contained absorption maxima at 1730 cm^{-1} ($-\overset{O}{\overset{\|}{C}}-$) and at 3000 cm^{-1} (— OH). The NMR spectrum contained a complex signal at τ 7.91 - 9.25 (30 H) and a broad singlet centred at τ 1.86 ($-\overset{O}{\overset{\|}{C}}-OH$) ppm. The product was further characterized by conversion into hexyl-octylmalonanilide (Xa, see p.106 for structural formula) which was prepared as follows.

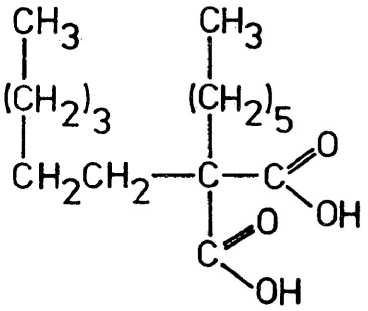
To the hexyl-octylmalonic acid (X, 0.61 g) was added thionyl chloride (3 ml). This mixture was refluxed (30 min) under anhydrous condition. Excess thionyl chloride was distilled off under vacuum leaving hexyl-octylmalonyl dichloride as an oily residue to which benzene (5 ml) was added. Aniline (2.2 g) in benzene (20 ml) was added dropwise, and the resulting mixture shaken with excess dilute hydrochloric acid. The organic layer was collected, washed



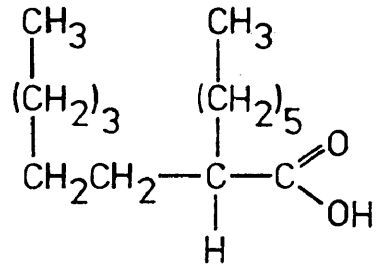
Xa



XIa



XXXIa



XXXIb

with water and dried. Removal of the solvent yielded the crude product (1.03 g) which was recrystallized from methanol and the pure product had m.p. 119.5-120.0°. The IR spectrum contained absorption maxima at 1700 cm^{-1} ($-\overset{O}{\overset{\|}{C}}-$), 1540 cm^{-1} ($-N-H$ bending) and 3495 cm^{-1} ($-N-H$). The mass spectrum contained the molecular ion at $^m/e$ 450 (4%, $C_{29}H_{42}N_2O_2^+$), and major fragments at $^m/e$ 366 (6%, $C_{23}H_{30}N_2O_2^+$), $^m/e$ 358 (5%, $C_{23}H_{36}NO_2^+$, $^m/e$ 338 (9%, $C_{21}H_{26}N_2O_2^+$), $^m/e$ 330 (12%, $C_{22}H_{36}NO^+$), $^m/e$ 268 (9%, $C_{16}H_{15}N_2O_2^+$), $^m/e$ 246 (7%, $C_{15}H_{20}NO_2^+$ and $C_{16}H_{24}NO^+$), $^m/e$ 232 (10%, $C_{16}H_{26}N^+$), $^m/e$ 120 (22%, $C_7H_6NO^+$) and $^m/e$ 93 (100%, $C_6H_7N^+$).

Analysis: Calcd. for $C_{29}H_{42}N_2O_2$: C, 77.29; H, 9.39; N, 6.22; Found: C, 77.19; H, 9.00; N, 5.85%.

2-hexyldecanoic acid (dimer monoacid, XI)

A mixture of the dicarboxylic acid (X, 2.01 g) and fine glass powder (1.72 g) was heated at 140-150° in a nitrogen atmosphere for three and one-half hours.

Ether (25 ml) was added and the mixture was filtered through a plug of silica gel and the solvent was removed to give crude 2-hexyldecanoic acid (XI, 1.62 g) as a colourless oil. Thin layer chromatography (solvent: Benzene/chloroform 1:1) indicated

that the expected product contained traces of the dicarboxylic acid (X). The product was characterized by conversion into the 2-hexyldecananilide (XIa) which was prepared as follows.

To the crude 2-hexyldecanoic acid (0.60 g), thionyl chloride (3 ml) was added and the mixture was refluxed under anhydrous condition for 30 min. Excess thionyl chloride was distilled off under vacuum, leaving 2-hexyldecanoyl chloride as an oily residue which was then dissolved in benzene (5 ml). To this solution aniline (2.2 g) in benzene (20 ml) was added dropwise. The resulting mixture was shaken with excess dilute hydrochloric acid and the organic layer was collected, washed with water and dried. Removal of the solvent yielded the crude product (1.22 g), which was recrystallized from methanol (m.p. 78-78.5°). The infra-red spectrum contained absorption maxima at 1700 cm^{-1} ($-\overset{O}{\overset{\|}{C}}-$), 1540 cm^{-1} ($-N-H$ bending) and 3495 cm^{-1} ($-N-H$). The mass spectrum contained molecular ion at $^{m}/e$ 331 (22%, $C_{22}H_{37}NO^{+}$), with major fragments at $^{m}/e$ 302 (3%, $C_{21}H_{36}N^{+}$), $^{m}/e$ 260 (5%, $C_{17}H_{26}NO^{+}$), $^{m}/e$ 240 (3%, $C_{16}H_{31}O^{+}$), $^{m}/e$ 232 (4%, $C_{15}H_{22}NO^{+}$), $^{m}/e$ 219 (7%, $C_{14}H_{21}NO^{+}$), $^{m}/e$ 210 (5%, $C_{15}H_{30}^{+}$), $^{m}/e$ 154 (5%, $C_{11}H_{22}^{+}$ and $C_{10}H_{17}O^{+}$), $^{m}/e$ 148 (12%, $C_{9}H_{10}NO^{+}$), $^{m}/e$ 120 (19%, $C_{7}H_{6}NO^{+}$) and $^{m}/e$ 93 (100% $C_{6}H_{7}N^{+}$).

Analysis: Calcd. for $C_{22}H_{37}NO$: C, 79.70; H, 11.25; N, 4.22; Found: C, 79.69; H, 11.19; N, 4.11%.

Methyl 2-hexyldecanoate (dimer methyl ester, III)

Method I:

2-hexyldecanoic acid (XI, 3.12 g) was refluxed with methanol-boron trichloride (10% boron trichloride, 5 ml) on a water bath (30 min.) under anhydrous conditions. Excess methanol was removed under vacuum. Diethyl ether (25 ml) was added and the mixture was poured into water (75 ml). The ethereal layer was separated, dried and filtered. The solvent was removed to yield crude methyl 2-hexyldecanoate (III, 2.93 g) as a colourless liquid. Thin layer chromatography indicated the presence of some impurities which were removed by column chromatography on silica gel. Elution with toluene gave the pure product (2.12 g, 64.4%).

Method II (53):

Diazomethane was generated by adding finely powdered nitrosomethylurea to a mixture of ether (100 ml) and 50% potassium hydroxide solution (30 ml), and blown into 2-hexyldecanoic acid (XI, 2.70 g) in ether, with nitrogen. The reaction vessel was immersed in ice-water so as to retain as much diazomethane in the reaction mixture as possible. The solvent was removed to give methyl 2-hexyldecanoate (III, 2.83 g, 99.1%).

The ester, obtained from both reactions, was pure on TLC and GLC. The IR spectrum contained an absorption band at 1750 cm^{-1} ($\_ \overset{O}{\overset{\|}{C}} \_$). The mass spectrum showed the molecular ion at $^m/e$ 270 (6%, $C_{17}H_{34}O_2^+$), major fragments at $^m/e$ 239 (3%, $C_{16}H_{31}O^+$), $^m/e$ 227 (2%, $C_{16}H_{34}O^+$), $^m/e$ 186 (44%, $C_{11}H_{22}O_2^+$), $^m/e$ 158 (67%, $C_9H_{18}O_2^+$), $^m/e$ 157 (11%, $C_{10}H_{21}O^+$), $^m/e$ 101 (21%, $C_7H_{16}^+$) with the base peak at $^m/e$ 87 (100%, $C_4H_7O_2^+$).

2-hexyldecanol (dimer alcohol, IV)

Lithium aluminium hydride (0.68 g) was suspended in dry tetrahydrofuran (15 ml) in a round-bottomed flask (100 ml), fitted with a reflux condenser equipped with a drying tube. The ester (III, 5.72 g) was dissolved in dry tetrahydrofuran (10 ml) and the resulting solution was added slowly with continuous stirring. The reaction mixture was refluxed for 3 hr. With external cooling (ethanol and dry ice), a saturated solution of sodium sulphate was added dropwise. When effervescence had ceased, more anhydrous sodium sulphate was added and the residue extracted with ether. The mixture was then filtered. Removal of the solvent gave 2-hexyldecanol (IV, 5.07 g, 98.8%) as a clear, colourless liquid. The product was pure on TLC and GLC. The IR spectrum contained an absorption peak at 3650 cm^{-1} (— OH). The NMR spectrum contained a triplet at τ 9.06 - 9.19 (6H, 2 — CH_3), a cluster of peaks at τ 8.72 (25H) and a

doublet at τ 6.45 (2 C-1 H) ppm. The mass spectrum (Fig. 4 , p.72) contained major fragments at $^{m}/e$ 224 (14%, $C_{16}H_{32}^{+}$), $^{m}/e$ 154 (11%, $C_{11}H_{22}^{+}$), $^{m}/e$ 140 (10%, $C_{10}H_{30}^{+}$), $^{m}/e$ 139 (12%, $C_{10}H_{19}^{+}$), $^{m}/e$ 126 (17%, $C_9H_{18}^{+}$), $^{m}/e$ 111 (46%, $C_8H_{15}^{+}$), $^{m}/e$ 97 (57%, $C_7H_{13}^{+}$), $^{m}/e$ 85 (80%, $C_6H_{13}^{+}$) and the base peak at $^{m}/e$ 55 (100%, $C_4H_7^{+}$).

2-hexyldecyl *p*-toluenesulphonate (dimer tosylate, XIII, 58)

The alcohol (1.06 g) was dissolved in dry pyridine (10 ml) and the solution cooled to -5°. To the cold solution was added *p*-toluenesulphonyl chloride (1.02 g) in one portion. The suspension was swirled, with cooling in an ice-salt bath, until all the tosyl chloride had dissolved. After the mixture was kept at 0° for a further 2 hrs., water (10 ml) was added in portions (1 + 1 + 1 + 2 + 5 ml) at intervals of five minutes, with swirling and cooling so that the temperature did not rise above +5°. The solution was then diluted with water (100 ml). The aqueous pyridine solution was extracted with chloroform (3 x 25 ml) and the combined chloroform extracts washed successively with ice-cold dilute sulphuric acid, water and sodium bicarbonate solution, and then dried. Removal of solvent gave the *p*-toluenesulphonate (XIII, 1.53 g, 88.5%) as a colourless liquid. The compound was pure on TLC and GLC. The infra-red spectrum contained strong signals at 1190 - 1200 cm^{-1} (doublet, sulphonate $-O-SO_2-$). The mass spectrum (Fig. 5 , p. 73) contained the principal fragment

at $^{m}/e$ 224 (9%, $C_{16}H_{32}^{+}$) and the base peak at $^{m}/e$ 57 (100%, $C_4H_9^{+}$). The NMR spectrum contained signals at τ 8.28 - 9.1 (33H), τ 7.56 (3H, $-C_6H_4\underline{CH_3}$), τ 6.0 - 6.09 (2H, doublet, C-1 H), and τ 2.09 - 2.75 (4H, aromatic H) ppm.

Analysis: Calcd. for $C_{23}H_{40}SO_3$: C, 6.97; H, 10.1; Found: C, 70.07; H, 9.79%.

7-methylpentadecane (dimer hydrocarbon, V, 65)

A solution of the tosylate (XIII, 5.04 g) in dimethyl sulphoxide, DMSO (2 ml, dried and distilled over calcium hydride at reduced pressure) was added to a solution of sodium borohydride (1.65 g) in DMSO (20 ml). The reaction mixture was stirred and refluxed for 2 hours, then diluted with water and extracted with ether (3 x 25 ml). The combined extracts were dried and removal of the solvent gave a colourless liquid (2.91 g). TLC and GLC indicated the presence of some impurities which could be removed by column chromatography on silica gel (90 g; column i.d., 3.5 cm). Pure 7-methylpentadecane (V, 1.96, 68.1%) was eluted, first with petroleum ether (30-60°), then with chloroform. The IR spectrum contained only C-H absorption signals at 1380 cm^{-1}, 1470 cm^{-1} and 2860 - 2970 cm^{-1}. The mass spectrum contained the molecular ion at $^{m}/e$ 226 (3%, $C_{16}H_{34}^{+}$) and other major fragments at $^{m}/e$ 211 (2%, $C_{15}H_{31}^{+}$), $^{m}/e$ 140

(15%, $C_{10}H_{20}^+$), ^{m}/e 113 (9%, $C_8H_{17}^+$), ^{m}/e 112 (24%, $C_8H_{16}^+$), ^{m}/e 85 (31%, $C_6H_{13}^+$), ^{m}/e 71 (75%, $C_5H_{11}^+$) and ^{m}/e 57 (100%, $C_4H_9^+$).

Analysis: Calcd. for $C_{16}H_{34}$: C, 84.96; H, 15.04; Found: C, 84.48; H, 14.60%.

7-(bromomethyl)pentadecane (dimer bromide, XIV)

The alcohol (IV, 5.07 g) and 48% hydrobromic acid (3.42 g) were refluxed (4 hr.) while gaseous hydrogen bromide was bubbled through the reaction mixture. The mixture was extracted with benzene (25 ml) and the extract was dried. Removal of the solvent yielded 7-(bromomethyl)pentadecane (XIV, 6.11 g, 95.7%) as a brown liquid. The product was found pure by TLC and GLC. The NMR spectrum contained signals at τ 8.94 - 9.34 (6H, triplet, terminal methyl H), τ 8.5 - 8.87 (25H), and τ 6.6 - 6.63 (2H, doublet, C-1 H). The mass spectrum contained the molecular ion at ^{m}/e 304, 305 (1%, $C_{16}H_{33}Br^+$). Other major fragments were at ^{m}/e 246, 248 (2.5%, $C_{12}H_{24}Br^+$), ^{m}/e 224 (33%, $C_{16}H_{32}^+$), ^{m}/e 218, 220 (4%, $C_{10}H_{20}Br^+$), ^{m}/e 210 (6%, $C_{15}H_{29}^+$), ^{m}/e 168 (5%, $C_{12}H_{22}^+$), ^{m}/e 154 (9%, $C_{11}H_{22}^+$), ^{m}/e 113 (15%, $C_8H_{17}^+$), ^{m}/e 85 (44%, $C_6H_{13}^+$), ^{m}/e 71 (65%, $C_5H_{11}^+$) and ^{m}/e 57 (100%, $C_4H_9^+$).

diethyl hexyl-(2-hexyldecyl)malonate (trimer malonic ester, XVII)

Diethyl hexyl-(2-hexyldecyl)malonate (XVII) was prepared by the same method (76) as used for diethyl hexylmalonate (VIII, see p.102). Pieces of sodium (0.51 g) were dissolved in dry ethanol (11.3 ml) in a three-necked round-bottomed flask (100 ml), equipped with a dropping funnel and a reflux condenser. Diethyl hexylmalonate (VIII, 5.49 g) and 7-(bromomethyl)pentadecane (XIV, 6.67 g) were added dropwise and the mixture was refluxed for 9 hours, when the pH was about 8. Most of the solvent was removed and the non-volatile residue was dispersed in water (40 ml). The aqueous mixture was extracted with methylene chloride (2 x 25 ml), the combined extracts were dried and the solvent was removed, giving a dark-brown oily liquid (10.09 g). Thin layer chromatography indicated the presence of a number of byproducts from which the product could be isolated by column chromatography on silica gel (150 g, column i.d., 3.5 cm.). Elution with a mixture of cyclohexane and chloroform, beginning with a 1:1 ratio and increasing the proportion of chloroform to one hundred percent, gave the product (6.75 g, 65.9%). The IR spectrum showed a peak at 1740 cm^{-1} ($-\overset{O}{\overset{\|}{C}}-$). The mass spectrum contained M^+ at $^m/e$ 468 (0.8%, $C_{29}H_{56}O_4^+$) and major fragments at $^m/e$ 423 (2.5%, $C_{28}H_{55}O_2^+$), $^m/e$ 394 (8.1%, $C_{26}H_{50}O_2^+$), $^m/e$ 244 (31%, $C_{13}H_{24}O_4^+$), $^m/e$ 225 (7%, $C_{16}H_{33}^+$) and $^m/e$ 173 (100%, $C_8H_{13}O_4^+$).

hexyl-(2-hexyldecyl)malonic acid (trimer diacid, XVIII)

Potassium hydroxide (6.75 g) was dissolved in a mixture of ethanol (47 ml) and water (13ml). To the resulting solution, the trimer malonic ester (XVII, 6.75 g) was added slowly with continuous stirring. The reaction mixture was refluxed (5 hr.), acidified with hydrochloric acid, and extracted with ether (2 x 100 ml). The ethereal extracts were dried and the solvent was removed to yield hexyl-(2-hexyldecyl)malonic acid (XVIII, 5.67 g, 95.4%), as a brown waxy solid, which was found (TLC, solvent: chloroform) to contain a trace amount of a less polar compound. The IR spectrum contained a sharp band at 1750 cm^{-1} () and a broad band at 3470 cm^{-1} (– OH). The mass spectrum contained the fragment $M^+ - CO_2$ at $^m/e$ 368 (11%, $C_{24}H_{48}O_2^+$), and other major fragments at $^m/e$ 283 (11%, $C_{18}H_{35}O_2^+$), $^m/e$ 255 (24%, $C_{17}H_{35}O^+$), $^m/e$ 224 (67%, $C_{16}H_{32}^+$), $^m/e$ 157 (100%, $C_9H_{17}O_2^+$) and $^m/e$ 144 (47%, $C_8H_{16}O_2^+$).

2,4-dihexyldodecanoic acid (trimer monoacid, XIX)

The diacid (XVIII, 1.35 g) and glass powder (0.23 g) were placed in a round-bottomed flask (100 ml) fitted with a cold finger condenser. The mixture was heated, in nitrogen, in an oil bath at 110-145° for 2 1/4 hr. The product was worked up as described for the dimer monoacid (XI, p.107) to give 2,4-dihexyldodecanoic acid (XIX, 1.13 g, 94.1%). The product was pure on TLC. The mass spectrum

showed the molecular ion at $^{m}/e$ 368 (17%, $C_{24}H_{48}O_2^+$) and major fragments at $^{m}/e$ 283 (10%, $C_{18}H_{35}O_2^+$), $^{m}/e$ 255 (25%, $C_{16}H_{31}O_2^+$), $^{m}/e$ 225 (89%, $C_{16}H_{33}^+$), $^{m}/e$ 157 (100%, $C_9H_{17}O_2^+$), and $^{m}/e$ 144 (41%, $C_8H_{16}O_2^+$).

methyl 2,4-dihexyldodecanoate (trimer methyl ester, XX)

2,4-dihexyldodecanoic acid (XIX, 5.75 g) was esterified with diazomethane (53) as on p. 109. The reaction mixture was dried. The crude product (3.32 g) was chromatographed on a column of silica gel (50 g, column i.d. 2.5 cm). Elution with a mixture of cyclohexane and chloroform (4:1) gave the pure ester (XX, 2.97 g, 49.8%) as a colourless oil. The IR spectrum contained the carbonyl absorption at 1745 cm^{-1}. The mass spectrum contained the molecular ion at $^{m}/e$ 382 (5%, $C_{25}H_{50}O_2^+$) and major fragments at $^{m}/e$ 295 (7%, $C_{19}H_{37}O_2^+$), $^{m}/e$ 268 (11%, $C_{18}H_{36}O^+$), $^{m}/e$ 225 (8%, $C_{16}H_{33}^+$), $^{m}/e$ 170 (73%, $C_{10}H_{18}O_2^+$), $^{m}/e$ 157 (100%, $C_9H_{17}O_2^+$) and $^{m}/e$ 86 (82%, $C_6H_{14}^+$).

ethyl 2,4-dihexyldodecanoate (trimer ethyl ester, XXI, 56b)

The trimer diethyl malonic ester (XVII, 1.03 g), lithium chloride (0.18g), dimethyl sulphoxide (3.6 ml, dried and distilled over calcium hydride) and water (0.04 ml) were placed in a round-bottomed flask (100 ml). The mixture was refluxed for 4 hr. A pale brownish yellow colour developed and a light precipitate (Li_2CO_3)

was formed. The reaction mixture was poured into ice-water (15 ml) and the aqueous layer saturated with salt (NaCℓ). The ester was extracted with hexane (8 x 25 ml). The extracts were dried and the solvent removed to yield a brownish liquid (0.74 g) which was chromatographed on silica gel (15 g, column i.d., 2 cm). Elution with a mixture of cyclohexane and chloroform (1:1) gave pure ethyl 2,4-dihexyldodecanoate (XXI, 0.72 g, 82.8%). Its IR spectrum contained absorption peak at 1740 cm^{-1} (_ $\overset{O}{\overset{\|}{C}}$ _). The mass spectrum contained molecular ion at $^{m}/e$ 396 (5.5%, $C_{26}H_{52}O_2^+$) and major fragments at $^{m}/e$ 351 (2%, $C_{24}H_{47}O^+$), $^{m}/e$ 311 (10%, $C_{26}H_{39}O_2^+$), $^{m}/e$ 283 (8%, $C_{18}H_{35}O_2^+$), $^{m}/e$ 185 (56%, $C_{11}H_{21}O_2^+$) and $^{m}/e$ 173 (100%, $C_{10}H_{21}O_2^+$).

2,4-dihexyldodecanol (trimer alcohol, XXII)

Lithium aluminium hydride (0.13 g) was weighed into dry tetrahydrofuran (10 ml) in a round-bottomed flask (100 ml) equipped with a reflux condenser and a drying tube. The trimer ethyl ester (XXI, 0.61 g) was added dropwise and the mixture was refluxed with stirring (4 hr.), and then worked up as for 2-hexyldecanol (IV, p.110) to give a colourless liquid (0.53 g, 96.3%). The product, which was pure on TLC and GLC, contained a band at 3650 cm^{-1} (— OH) in the IR spectrum. The mass spectrum contained the fragment M^+-18 at $^{m}/e$ 336 (4%, $C_{24}H_{48}^+$) and other major fragments at $^{m}/e$ 251 (9%, $C_{18}H_{35}^+$),

$^{m}/e$ 224 (12%, $C_{16}H_{32}^{+}$), $^{m}/e$ 210 (11% $C_{15}H_{30}^{+}$), $^{m}/e$ 153 (9%, $C_{11}H_{21}^{+}$), $^{m}/e$ 126 (25%, $C_{9}H_{18}^{+}$), $^{m}/e$ 112 (14%, $C_{8}H_{15}^{+}$), $^{m}/e$ 97 (61%, $C_{7}H_{15}^{+}$), $^{m}/e$ 71 (93%, $C_{5}H_{11}^{+}$) and $^{m}/e$ 69 (100%, $C_{5}H_{9}^{+}$).

2,4-dihexyldodecyl methanesulphonate (trimer mesylate, XXIV, 62)

To an approximately 0.2 M solution of 2,4-dihexyldodecanol (XXII, 0.50 g) in methylene chloride (7 ml) containing a 50% molar excess of triethylamine (0.21 g; 0.29 ml; refluxed over phthalic anhydride, distilled, and then redistilled from potassium hydroxide pellets), a 10% excess of methanesulphonyl chloride (0.18 g; 0.12 ml) was added over a period of 5-10 min. at -10°. Stirring was continued for an additional 10-15 min., and the mixture was extracted successively with water, cold 10% hydrochloric acid, saturated sodium hydrogencarbonate solution and brine. Removal of the solvent, after drying, gave 2,4-dihexyldodecyl methanesulphonate (XXIV, 0.55 g, 90.3%). The mesylate was pure on TLC [solvent: cyclohexane/chloroform (3:2)]. The IR spectrum contained sharp peaks at 1180 and 1350 cm^{-1} (S = O in sulphonate ester). The product was not further characterized, but its identity was established from its reduction to the hydrocarbon (p.120).

2,4-dihexyldodecyl *p*-toluenesulphonate (trimer tosylate, XXIII, 58)

The trimer alcohol (XXII, 2.70 g) and *p*-toluenesulphonyl chloride (1.61 g) were dissolved in dry pyridine (27 ml) and the mixture was allowed to stand at room temperature for 22 hr. Water (100 ml) was added and the aqueous solution was extracted with chloroform (3 x 25 ml). The combined chloroform extracts were washed with ice-cold dilute sulphuric acid, water and sodium bicarbonate solution respectively. Removal of solvent, after drying, gave the crude product (2.97 g) which was then purified on a column of silica gel (40 g; column i.d., 2.5 cm). Elution with a cyclohexane-chloroform mixture (starting with a 2:1 ratio and increasing the concentration of chloroform to 100%) yielded the pure *p*-toluenesulphonate (XXIII, 1.45 g, 38.8%). Its IR spectrum contained a doublet at 1200 cm^{-1} (S = O in tosylate). No further attempts were made to characterize the compound but its identity could be established from its reduction to the hydrocarbon (see p.120).

7-methyl-9-hexylheptadecane (trimer hydrocarbon, XXV)

Method I (65):

The tosylate (XXIII, 0.44 g) in dry DMSO (2 ml) was added to a mixture of sodium borohydride (0.15 g) and DMSO (10 ml) with continuous stirring. The reaction mixture was refluxed (2 hr.), water was then added, and the organic portion was extracted with ether (3 x 25 ml). The extracts were dried, and the solvent removed to give a crude product (1.63 g), which was purified by column chromatography (50 g silica gel; columni.d., 2.5 cm). Elution with petroleum ether yielded the trimer hydrocarbon (XXV, 0.16 g, 54.9%) as a colourless oil.

Method II (61):

The trimer hydrocarbon (XXV) was prepared from 2,4-dihexyldodecyl methanesulphonate (XXIV) as follows.

The methanesulphonate (XXIV, 0.47 g) was added slowly to a mixture of lithium aluminium hydride (0.23 g) and dry tetrahydrofuran (10.5 ml) with constant stirring. The mixture was refluxed for two hours under anhydrous conditions. Saturated sodium sulphate solution was added dropwise, with the flask immersed in a dry ice-

ethanol cooling bath (-70°). More anhydrous sodium sulphate was added, and the organic phase isolated by filtration. Removal of the solvent yielded 7-methyl-9-hexylheptadecane (XXV, 0.24 g, 64.4%) as a colourless liquid. The product was pure on TLC and GLC.

The IR spectrum contained only peaks assigned to hydrocarbon vibrations at 1380, 1470, and 2860 - 2970 cm^{-1}. The mass spectrum contained the molecular ion at $^{m}/e$ 338 (0.2%, $C_{24}H_{50}^{+}$), and other fragments at $^{m}/e$ 253 (14%, $C_{18}H_{37}^{+}$), $^{m}/e$ 225 (15%, $C_{16}H_{33}^{+}$), $^{m}/e$ 210 (7%, $C_{15}H_{30}^{+}$), $^{m}/e$ 127 (10%, $C_{9}H_{19}^{+}$), $^{m}/e$ 113 (13%, $C_{8}H_{17}^{+}$), $^{m}/e$ 85 (53%, $C_{6}H_{13}^{+}$), $^{m}/e$ 71 (85%, $C_{5}H_{11}^{+}$) and $^{m}/e$ 57 (100%, $C_{4}H_{9}^{+}$).

Cyclohexylmethanol (XXX)

Cyclohexylmethanol was obtained from Aldrich Chemical Co., Inc. and was used without further purification. Its NMR spectrum contained a cluster of peaks at τ 8.0 - 9.22 (11H) and a doublet at τ 6.38 - 6.69 (2H, C-1 H) ppm. The mass spectrum contained molecular ion at $^{m}/e$ 114 (1%, $C_{7}H_{14}O^{+}$), the base peak at $^{m}/e$ 55 (100%, $C_{4}H_{7}^{+}$), and other major fragments at $^{m}/e$ 96 (32%, $C_{7}H_{12}^{+}$), $^{m}/e$ 83 (69%, $C_{6}H_{11}^{+}$), $^{m}/e$ 68 (15%, $C_{5}H_{8}^{+}$), and $^{m}/e$ 67 (40%, $C_{5}H_{7}^{+}$).

Cyclohexylmethyl *p*-toluenesulphonate (XXXa)

Cyclohexylmethanol (1.0 g) was tosylated (58) with

p-toluenesulphonyl chloride (2.05 g) in the same way as 2-hexyldecanol (IV) described on p.111. Cyclohexylmethyl *p*-toluenesulphonate (1.83 g, 78.0%), pure on TLC, was obtained. Its IR spectrum contained strong signals at 1190 - 1200 cm^{-1} (doublet, sulphonate S = O). The NMR spectrum contained a cluster of signals at τ 7.87 - 9.37 (11H), a singlet at τ 7.53 (3H, CH_3), a doublet of doublet at τ 5.72 (2 C-1 H) and a doublet of doublet at τ 2.09 - 2.75 (4 aromatic H) ppm. The mass spectrum contained the molecular ion at $^m/e$ 268 (1%, $C_{14}H_{20}SO_3^+$), and major fragments at $^m/e$ 172 (5%, $C_7H_8SO_3^+$) and $^m/e$ 96 (100%, $C_7H_{12}^+$).

Cyclohexylmethyl bromide (XXXb)

Cyclohexylmethanol (0.51 g) and 48% aqueous hydrobromic acid (1.13 g) were refluxed (1 hr.) while gaseous hydrogen bromide was passed into the reaction mixture. The mixture was extracted with benzene (10 ml). Removal of the solvent, after drying, gave a crude product (0.49 g). Thin layer chromatography indicated the presence of unreacted alcohol, which was removed by column chromatography on silica gel. Elution with toluene gave the pure cyclohexylmethyl bromide (XXXb, 0.36 g, 46.2%). The NMR spectrum contained a doublet at τ 6.68 (2 C-1 H), and a cluster of peaks at τ 8.0 - 9.25 (11 H) ppm. The mass spectrum contained the molecular ion at $^m/e$ 176, 177 (3%, $C_7H_{13}Br^+$), the base peak at $^m/e$ 97 (100%, $C_7H_{13}^+$) and a major

fragment at $^{m}/e$ 98 (9%, $C_7H_{13}^+$).

diethyl dihexylmalonate (C_4-C_6 malonic ester, XXXI, 76)

Small pieces of sodium (0.30 g) were dissolved in dry ethanol (6.5 ml). Diethyl hexylmalonate (VIII, 3.19 g) and *n*-hexyl bromide (2.13 g) were added, dropwise, successively, and the reaction mixture was refluxed until it was neutral (about 1 1/2 hr.). Most of the ethanol was removed by distillation, and the residue was washed with water (2 x 20 ml), and extracted with methylene chloride (25 ml). The organic extract was dried, and the solvent removed to give a crude product (3.72 g) which was purified by distillation. The pure diethyl dihexylmalonate (XXXI, 4.24 g, 50.1%) was collected at 184-192° at 27.5 mm Hg. The mass spectrum contained molecular ion at $^{m}/e$ 328 (0.02%, $C_{19}H_{36}O_4^+$), and major fragments at $^{m}/e$ 284 (6.2%, $C_{18}H_{36}O_2^+$), $^{m}/e$ 256 (8.6%, $C_{16}H_{32}O_2^+$), $^{m}/e$ 245 (38%, $C_{13}H_{25}O_4^+$), $^{m}/e$ 174 (11%, $C_8H_{14}O_4^+$), $^{m}/e$ 173 (100%, $C_{10}H_{21}O_2^+$) and $^{m}/e$ 160 (26%, $C_7H_{12}O_4^+$).

ethyl 2-hexyloctanoate (C_4-C_6 ethyl ester, XXXIII, 56b)

A mixture of diethyl dihexylmalonate (XXXI, 2.58 g), dry dimethyl sulphoxide (13 ml), lithium chloride (0.65 g) and water (0.13 ml) was refluxed for 4 hours. The mixture was poured into water (52.3 ml)

and the aqueous layer saturated with common salt. Extraction with hexane (4 x 30 ml) and removal of the solvent, after drying, gave a crude product which was contaminated with a trace of dimethyl sulphoxide. The crude was purified on a short column of silica gel. Elution with benzene gave the pure ethyl 2-hexyloctanoate (0.69 g, 34.6%). The mass spectrum contained the molecular ion at $^{m}/e$ 256 (3%, $C_{16}H_{32}O_2^+$), and other major fragments at $^{m}/e$ 227 (3%, $C_{14}H_{27}O_2^+$), $^{m}/e$ 185 (14%, $C_{11}H_{21}O_2^+$), $^{m}/e$ 172 (100%, $C_{10}H_{20}O_2^+$), $^{m}/e$ 157 (8%, $C_9H_{17}O_2^+$ or $C_{10}H_{21}O^+$), $^{m}/e$ 143 (17%, $C_9H_{19}O^+$ or $C_8H_{15}O_2^+$), $^{m}/e$ 115 (11%, $C_7H_{15}O^+$) and $^{m}/e$ 101 (90%, $C_5H_9O_2^+$).

dihexylmalonic acid (C_4-C_6 malonic acid, XXXIa, 48)

Diethyl dihexylmalonate (XXXI, 2.08 g) was added dropwise to an aqueous solution of potassium hydroxide (1.33 g KOH/1.05 ml H_2O) with constant stirring. The mixture was refluxed for 5 hours. As the reaction progressed, ethanol was removed continuously by vacuum and water was added occasionally to prevent solidification. The mixture was acidified with concentrated hydrochloric acid with external cooling, and extracted with diethyl ether (3 x 100 ml). The combined extracts were dried and the solvent was removed to yield dihexylmalonic acid (XXXIa, 1.75 g) as a white solid. The IR spectrum contained absorption peaks at 1720 cm^{-1} ($-\overset{O}{\overset{\|}{C}}-$) and 3000 cm^{-1} (broad, $-OH$ for carboxylic acid). The product was not further purified and

characterized but its identity was established by methylating its decarboxylated product.

2-hexyloctanoic acid (C_4-C_6 mono acid, XXXIb)

A mixture of dihexylmalonic acid (XXXIa, 1.54 g) and glass powder (0.27 g) was heated at 105-140°, in nitrogen, for 5 hours. Diethyl ether (25 ml) was added and the mixture was filtered through a plug of silica gel. The solvent was removed to give 2-hexyloctanoic acid (XXXIb, 1.25 g, 96.9%) as a colourless liquid. The product was pure on TLC. The mass spectrum contained the molecular ion at $^m/e$ 228 (2%, $C_{14}H_{28}O_2^+$), $^m/e$ 172 (12%, $C_{10}H_{20}O_2^+$), $^m/e$ 144 (10%, $C_8H_{16}O_2^+$), $^m/e$ 115 (14%, $C_6H_{11}O_2^+$), $^m/e$ 101 (38%, $C_5H_9O_2^+$), $^m/e$ 84 (26%, $C_6H_{12}^+$), $^m/e$ 73 (78%, $C_3H_5O_2^+$) and $^m/e$ 60 (100%, $C_2H_4O_2^+$).

methyl 2-hexyloctanoate (C_4-C_6 methyl ester, XXXII).

2-hexyloctanoic acid (XXXIb, 1.25 g) was esterified with diazomethane (53) as described on p.109. The crude product (1.42 g) was purified by column chromatography (30 g silica gel; column i.d., 2 cm). Elution with cyclohexane-chloroform mixture (from 4:1 ratio to 100% chloroform) yielded pure methyl 2-hexyloctanoate (XXXII, 0.75 g, 56.9%). The compound absorbed at 1745 cm^{-1} ($-\overset{O}{\overset{\|}{C}}-$) in the IR spectrum. The mass spectrum contained the molecular ion at $^m/e$ 242

(3%, $C_{15}H_{30}O_2^+$) and major fragments at $^m/e$ 210 (3.5%, $C_{14}H_{26}O^+$), $^m/e$ 184 (4%, $C_{13}H_{28}^+$), $^m/e$ 170 (18%, $C_{10}H_{18}O_2^+$), $^m/e$ 157 (88%, $C_9H_{17}O_2^+$), $^m/e$ 129 (17%, $C_8H_{17}O^+$), $^m/e$ 114 (33%, $C_8H_{18}^+$), $^m/e$ 100 (16%, $C_7H_{15}^+$) and $^m/e$ 86 (100%, $C_6H_{14}^+$).

2-hexyloctanol (C_4-C_6 alcohol, XXXIV)

Methyl 2-hexyloctanoate (XXXII, 0.75 g) and ethyl 2-hexyloctanoate (XXXIII, 0.69 g) were added dropwise to a mixture of dry tetrahydrofuran (21.5 ml) and lithium aluminium hydride (0.33 g) under anhydrous conditions. The resulting mixture was refluxed (5 1/2 hr.) and worked up as described on p.110. The product, 2-hexyloctanol (XXXIV, 0.98 g) was pure on TLC and GLC. The IR spectrum had a sharp absorption peak at 3640 cm^{-1} (— OH). The mass spectrum contained the fragment M^+-18 at $^m/e$ 196 (9%, $C_{14}H_{28}^+$) and other major fragments at $^m/e$ 126 (10%, $C_9H_{18}^+$), $^m/e$ 112 (13%, $C_8H_{16}^+$), $^m/e$ 111 (25%, $C_8H_{25}^+$), $^m/e$ 85 (39%, $C_6H_{13}^+$), $^m/e$ 71 (64%, $C_5H_{11}^+$), and $^m/e$ 57 (100%, $C_4H_9^+$).

2-hexyloctyl *p*-toluenesulphonate (C_4-C_6 tosylate, XXXIVa, 58)

2-hexyloctanol (XXXIV, 0.71 g) was dissolved in dry pyridine (10 ml) and the solution was cooled to -5°. To the cold solution

p-toluenesulphonyl chloride (2.12 g) was added and the mixture allowed to stand at 0° for two hours. The product was worked up, using the method on p.111, to give a brownish yellow liquid (XXXIVa, 0.87 g, 71.1%). The IR spectrum contained a doublet at 1180-1190 cm^{-1}(S = O for tosylate). The mass spectrum contained the molecular ion at $^{m}/e$ 368 (0.1%, $C_{21}H_{36}SO_3^+$), and major fragments at $^{m}/e$ 196 (6%, $C_{14}H_{28}^+$), $^{m}/e$ 126 (9%, $C_9H_{18}^+$), $^{m}/e$ 111 (24%, $C_8H_{15}^+$), $^{m}/e$ 70 (28 %, $C_5H_{10}^+$), $^{m}/e$ 69 (62%, $C_5H_9^+$), $^{m}/e$ 56 (100%, $C_4H_8^+$) and $^{m}/e$ 55 (68%, $C_4H_7^+$).

7-methyltridecane (C_4-C_6 hydrocarbon, XXXV)

A mixture of 2-hexyloctyl *p*-toluenesulphonate (XXXIVa, 0.87 g) sodium borohydride (0.36 g) and dry dimethyl sulphoxide (17 ml) was refluxed for 3 hours. On dilution with water, the reaction mixture was extracted with diethyl ether (3 x 25 ml). The combined extracts were dried and the solvent removed to give 7-methyltridecane (XXXV, 0.30 g, 64.0%) as a colourless liquid. The product was pure on TLC and GLC. Its IR spectrum contained absorption bands of hydrocarbon at 1380, 1470, and 2870 - 2980 cm^{-1}. The mass spectrum contained the molecular ion at $^{m}/e$ 198 (20%, $C_{14}H_{30}^+$), and other fragments at $^{m}/e$ 183 (1%, $C_{13}H_{27}^+$), $^{m}/e$ 127 (1%, $C_9H_{19}^+$), $^{m}/e$ 112 (22%, $C_8H_{18}^+$), $^{m}/e$ 71 (72%, $C_5H_{11}^+$) and $^{m}/e$ 57 (100%, $C_4H_9^+$).

C) Biodegradation Studies

(i) Growth media for and its incubation with PAO-20E for S. lipolytica

Saccharomycopsis lipolytica, ATCC No. 16617 (formerly *Candida lipolytica*) was grown in aerobic liquid cultures (100 ml) on a rotatory table shaker (stroke, 12 cm., 90 r.p.m.) at room temperature for 8-14 days. The following growth media were used: (i) Medium A, the mineral medium described by Klug and Markovetz (29a) (ii) Medium B and (iii) Medium C (see Appendix I for recipes of the nutrient media). All media were supplemented with/without glucose (0.5%) and/or PAO-20E (0.005 or 0.5%).

Incubation procedures were performed by adding hydrocarbon PAO-20E in ether (1 ml) immediately after autoclaving (15 min. at 15 psi.). This method was employed so that the ether would evaporate from the hot medium. The cooled medium was inoculated with inoculum (5 x 5 mm^2) cut from the active growing front of the previous plate cultures (Medium B with 0.6% glucose and 1.5% agar). At the end of the growth period, the mycelia were filtered, and washed with ether (5 ml). Mycelial growth was estimated by determining the dry weight of the culture (73).

(ii) Volatility studies on the dimer (V) and trimer (XXV) hydrocarbons

Hydrocarbon (dimer V or trimer XXV) in ether (1 ml) was added immediately after autoclaving, at two dose levels (15 and 20 ppm), to the hot water (100 ml) or hot Medium B (100 ml, supplemented with 0.5% glucose). The flasks were put on the rotatory table shaker (stroke 12 cm., 90 rpm) for periods of time varying from 0 to 16 days. Triplicate sets were made for each concentration. At the end of each period, the content of each flask was extracted with diethyl ether (2 x 25 ml). The combined extracts were dried (Na_2SO_4), and the solvent removed. The dried extract was dissolved in ethyl acetate (1 ml) containing an appropriate internal standard for quantitative gas-liquid chromatography (see p.101). The dimer hydrocarbon (V) was analysed with a SE-30 (5%) column using diethyl azelate (5.0 mg/ml) as the internal standard at a column temperature of 150°. Di-*n*-butyl phthalate (2.0 mg/ml) was the internal standard when trimer hydrocarbon (XXV) was analysed on the SE-30 (5%) column at 240°.

(iii) Incubation with trimer hydrocarbon (XXV)

Liquid cultures (100 ml) prepared in nutrient Medium B (supplemented with 0.25% or 0.5% glucose) and inoculated as before (p.128) were grown in triplicate after the addition of trimer

hydrocarbon (XXV, 10-100 ppm) in ether (1 ml) for periods of time varying from 10 to 15 days on a rotatory table shaker (stroke 12 cm., 90 rpm). For each of these experiments, a triplicate set of control cultures to which only ether (1 ml) was added, was grown under identical conditions. At the end of the incubation period, the same quantity of trimer hydrocarbon (XXV) was added to the control culture. All cultures were extracted with ether (3 x 25 ml). The combined extracts were washed with aqueous sodium hydroxide (2N, 20 ml), water (20 ml), and dried (Na_2SO_4). The dried extracts were evaporated to dryness, and dissolved in ethyl acetate (1 or 2 ml) containing di-*n*-butyl phthalate (2.0 mg/ml or 2.25 mg/ml) as the internal standard. The gas-liquid columns used were SE-30 (5%) at 240° and dexsil 300 (3%) at 200°. Analysis of the control cultures established the recovery efficiency for the hydrocarbon.

CONCLUSIONS AND RECOMMENDATIONS

The lower molecular weight homologues of PAO-20E, 7-methyl-pentadecane (dimer hydrocarbon V) and 7-methyl-9-hexylheptadecane (trimer hydrocarbon, XXV) have been synthesized by the malonic ester synthetic sequence. *Saccharomycopsis lipolytica* have been incubated with the commercial and synthesized compounds and the cultures analysed. No evidence has been found for the biodegradation of the PAO-20E type hydrocarbons by this yeast.

Further incubation experiments should be done, using initially lower concentrations of the hydrocarbon and longer periods of incubation, and increasing the amounts of hydrocarbon in subsequent cultures, so that true adaptation of the organism may be achieved. Such investigations may lead to more conclusive results concerning the probable biodegradation of the PAO-20E type hydrocarbons by the yeast.

Other hydrocarbon-utilizing species, such as *Pseudomonas*, can be examined for their ability to assimilate the hydrocarbons studied.

Appendix I Recipe for growth media

1) Medium A

KH_2PO_4	7g
Na_2HPO_4	1.2g
$MgSO_4 \cdot 7H_2O$	200mg
NH_4SO_4	3g
$CaC\ell_2 \cdot 2H_2O$	50mg
$NaC\ell$	50mg
$FeC\ell_3 \cdot 6H_2O$	1mg
Solution A	1ml
Distilled water	1000ml

Solution A:-

$CuSO_4 \cdot 5H_2O$	80mg
KI	200mg
$MnSO_4 \cdot H_2O$	27.1mg
$Na_2MoO_4 \cdot 2H_2O$	10.8mg
$ZnSO_4 \cdot 7H_2O$	142.4mg
Boric acid	500mg
Distilled water	1000ml

2) Medium B

Casamino acid	1g
Yeast extract	0.5g
KH_2PO_4	1g
$MgSO_4 \cdot 7H_2O$	0.5g
*Vitamin Stock	0.2ml
†Mineral Stock	2ml
Distilled water	1000ml

3) Medium C

Yeast nitrogen base	1g
KH_2PO_4	1g
$MgSO_4 \cdot 7H_2O$	0.5g
*Vitamin Stock	0.2ml
Mineral Stock	2ml
†Distilled water	1000ml

*Vitamin Stock solution

Biotin	25µg
thiamine	500µg
pyridoxine	500µg
Inositol	25µg

Ethyl alcohol (40%) to 100 ml

†Mineral Stock solution

$FeC\ell_3 \cdot 6H_2O$	98mg
$CuSO_4 \cdot 5H_2O$	78.5mg
$MnSO_4 \cdot 4H_2O$	40.5mg
$ZnSO_4 \cdot 7H_2O$	88mg

Distilled water to 250 ml

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