A STUDY OF CASHEW NUT-SHELL LIQUID PURIFICATION AND THE SYNTHESIS OF NONIONIC SURFACTANTS FROM THE COMPONENT PHENOLS

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ABSTRACT

The major phenolic lipids from natural and technical Cashew Nut-Shell Liquids were isolated by various techniques including precipitation, chemical purification, distillation, phase separation and chromatography. Cardanol, 3-pentadecyl phenol and cardol were polyethoxylated under base catalysed conditions and the products were characterised by both nmr and HPLC. Their surfactant properties were then analysed by surface tension measurements and their rates and extent of biodegradation were evaluated by means of a modified OECD screening test.

The synthesis of the biosyntetic intermediate 2,4-dihydroxy-6-pentadecyl benzoic acid, by means of a Horner-Emmons modification to the Wittig reaction, is also reported.

Some studies with cavitands are also reported, including the synthesis of some novel macrocycles and some sugar transport studies.

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Science moves, but slowly, slowly

creeping on from point to point.

Alfred, Lord Tennyson

1809 - 1892

To Edel

CHAPTER ONE

REVIEW

THE BIODEGRADATION OF NONIONIC SURFACTANTS

1.1 Introduction

Increasing pressure has been placed on the chemical industry to modify many of its consumer products. Chlorofluorocarbons are gradually being replaced by "ozone friendly" aerosol propellants, non-biodegradable substances are being replaced by chemicals that are "environmentally friendly" and lead is being removed from petrol. The "green revolution" is resulting in consumers becoming more aware of their surroundings and the environmental damage these chemicals can cause.

Biodegradation of organic components in waste waters, in treatment plants and in the ultimate receiving rivers and oceans is primarily the result of bacterial action. In this degradation, the organic molecule passes through many stages before complete conversion to H_2O and CO_2. Thus, biodegradation studies are concerned with both the rate by which this decomposition proceeds and the extent of decomposition. For most organic chemicals, biodegradation in surface water, ground water, soil and sediment is the most important process, usually determining whether a chemical is persistent or degrades relatively rapidly. In addition, biodegradation in waste water treatment plants is a major pollution control process and may determine whether a chemical is released into the environment or not.

The economic importance of nonionic surfactants and raw materials for detergent formulations has grown considerably in recent years[1]. Therefore increasing attention also has to be paid to their environmental compatibility, necessitating, among other things, furnishing proof for their ultimate biodegradability under environmental conditions.

Some fatty alcohol polyethoxylates are considered highly degradable, not only according to test criterion of primary biodegradability as required by German[2] and EEC[3] legislation, but also according to die away test results indicative of ultimate biodegradability[4-9]. Nevertheless, a more detailed knowledge of the environmental fate

of nonionic surfactants is still lacking since very little data has been published on their biodegradability in sewage treatment plants, i.e. the most important means of preventing the pollution of receiving surface waters. This question not only concerns the extent of their ultimate degradation but also the nature and environmental fate of the surfactants, which have not, or have only partially, been degraded in sewage works.

The whole area of biodegradability has been surveyed[10] but little on the biodegradability of nonionic surfactants has appeared in the literature in contrast to the copious literature published on anionic surfactants. As a preliminary to the discussion on biodegradation of nonionic surfactants the most likely metabolic pathways and the more common test methods will be reviewed. Finally, the principal findings of nonionic surfactant biodegradation will be discussed.

1.2 Biochemical Oxidation

Microorganisms are capable of degrading a wide variety of organic compounds and of using them for food and energy requirements. Since surfactants consist of various combinations of similar groups, only a narrow field of biochemical mechanisms is required to characterise surfactant biodegradation.

This review will consider biodegradation of the those principle constituents of nonionic surfactants:-

1) Hydrophobic chain degradation
2) Hydrophilic chain degradation
3) Aromatic ring degradation

1.2.1 Hydrophobic Chain Degradation

Microorganisms, i.e. bacteria, yeasts and moulds can grow on a wide variety of hydrocarbons as sole resources of carbon and energy. They can partially oxidise an

even greater range of such compounds. The list of compounds is extensive and includes straight and branched chain alkanes, alkenes, alicyclic, heterocyclic and aromatic hydrocarbons. There are few compounds that cannot be attacked, at least partially, by some microorganisms, the most recalcitrant molecules probably being the macromolecular polymers such as polystyrene and polyethylene, where there are considerable difficulties for the microorganisms to produce a solubilizing enzyme prior to oxidative degradation. There is no single organism which will utilise all hydrocarbons but, in general, each organism can utilize a range of hydrocarbons as the sole source of carbon and energy.

The most readily assimilated hydrocarbons are the straight chain alkanes from C_{10} to C_{18}. Utilization of long-chain alkanes, eg. plant paraffins of up to C_{35}, is less widespread, but some examples, particularly amongst the bacteria have been reported[11]. *Iso*-alkanes with a single methyl side chain can be utilized for growth and, like the straight chain alkanes, can be incorporated into cell components such as lipids. However, *iso*-alkanes with branched chains at both ends of the molecule tend not to be utilized as readily, and ones with multiple branching, such as pristane-2,6,10,14-tetramethyl pentadecane, are even less readily utilized.

The problems which have to be overcome by an organism utilizing an alkyl chain may be summarised as follows:-

a) **Uptake** - How is the insoluble hydrocarbon taken into the microbial cell?

b) **Attack** - How is the initial oxidation of a hydrocarbon chain accomplished? Is more than one route of attack possible?

c) **Degradation** - Can the compound produced by the initial oxidation step be degraded to provide metabolic intermediates to support subsequent cell growth or can the products be isolated without subsequent degradation?

d) **Energy production** - The energy content of a hydrocarbon is considerable and is greater than the microorganisms require to convert the metabolites into cell

materials. How then does the organism dispose of this energy which is surplus to its requirements?

e) **Assimilation** - Can the alkyl-chain, or its oxidation products be assimilated within the cell? In particular, can the cell take advantage of preformed long-chain alkyl chains to produce various lipids which would be useful to it in the construction of its various membranous organelles?

With regard to this review, points (b) and (c) will be discussed, and point (e) to a lesser extent but a discussion of fatty acid oxidations will also be included as they may produce a variety of chemically useful intermediates.

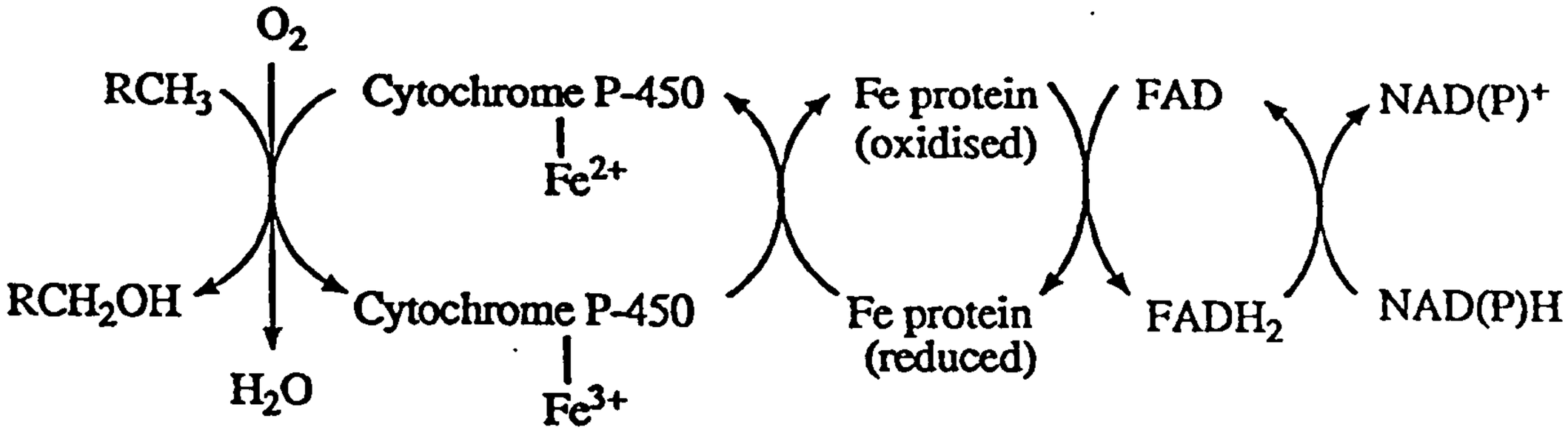
Points (a) and (d) have been discussed in a recent review[12].

Fatty acids have the fundamental advantage over an alkyl chain as far as microbial oxidation is concerned, since the carboxylic acid function confers a specific group on which subsequent degradation can be based. As all microorganisms on their own produce fatty acids it follows that a greater number of microorganisms can utilize a fatty acid than can oxidize an alkane. The need for the specialised oxidative step has gone. All that is required is that the organism should possess a mechanism for taking the fatty acid into its cell. Fatty acids can be used by microorganisms to give selected products which have a higher added value than the starting material.

(a) Primary oxidation of alkanes and alkyl chains

In the majority of organisms, initial oxidation of an alkane or alkyl-chain is at a methyl terminus. The reaction is catalysed by a complex hydroxylase system which is notoriously difficult to stabilize and isolate. There have been no reports of its successful isolation and stabilization for a sufficient length of time to warrant consideration that it may be a commercially useful enzyme in the same way as many hydrolytic and oxidative enzymes which are currently of use in biotechnology.

The alkane hydroxylase (Figure 1) is linked to an electron carrier system, the components of which vary according to the microorganism used. In yeasts and most bacteria (as well as in man), cytochrome P-450 reductase is the electron transfer component. Purification of the first component from yeast *Lodderomyces elongisporus* has recently been accomplished and has given the molecular weight of 79,000 for it[13].

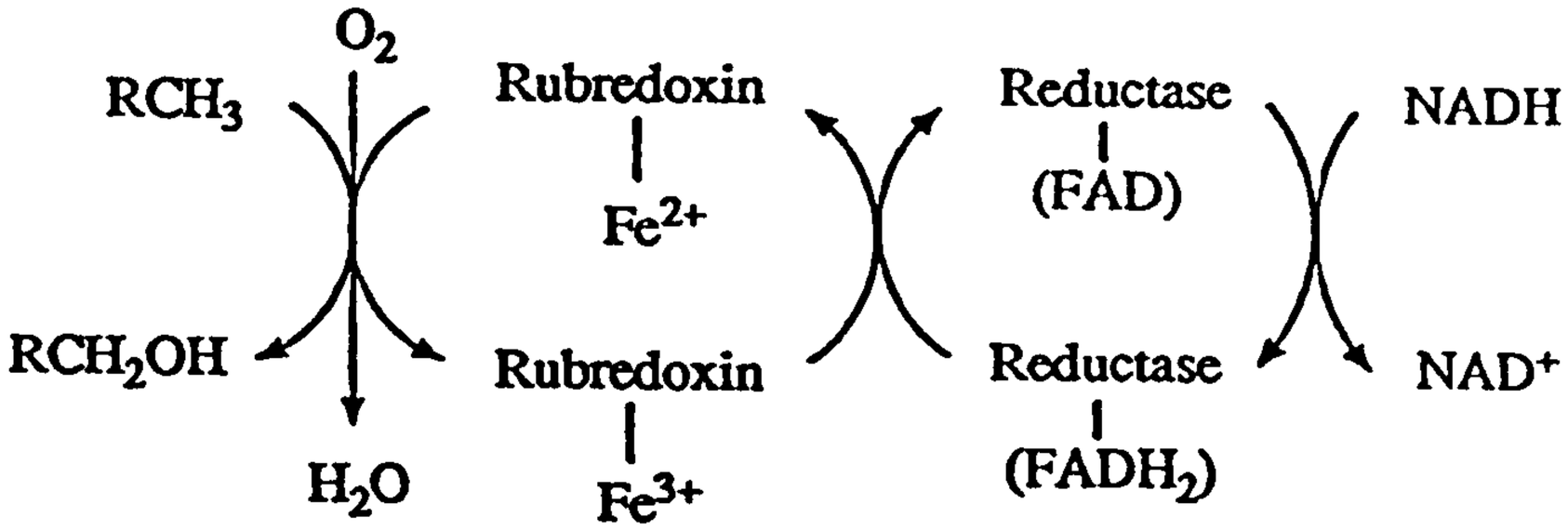


Net reaction - $RCH_3 + NAD(P)H + O_2 \longrightarrow RCH_2OH + NAD(P)^+ + H_2O$

Figure 1. Alkane hydroxylase: the cytochrome P-450 monooxygenase system.

In pseudomonal bacteria[14-16], the alkane hydroxylating system contains three protein components: a rubredoxin-like, non-haem iron protein, an NADH-rubredoxin reductase and a ω-hydroxylase (Figure 2). The enzyme system is coded for by DNA contained within a plasmid and thus opportunities exist for transferring the genetic information for hydrocarbon oxidation into organisms that do not possess this activity[17].

Also of importance in the primary oxidation of alkanes is the methane monooxygenase system. This is an enzyme system that can be purified and stabilized from bacteria capable of growing on methane but these bacteria need not be grown on this substrate in order to produce the enzyme. In other words, substrates which are easier to handle than methane can be employed, eg. methanol. The enzyme system is capable of oxidising a wide variety of substrate, from gaseous alkanes to aromatic and alicyclic compounds[18]. It will also react with longer chain alkyl groups, and the oxidation of octane to octanol and octan-2-ol has recently been reported[22].



Net reaction - $RCH_3 + NADH + O_2 \longrightarrow RCH_2OH + NAD^+ + H_2O$

Figure 2. Alkane hydroxylase: the rubredoxin system.

The enzyme is a multicomponent system involving NADH as an essential cofactor. Its mechanism of action is similar to those depicted in Figures 1 and 2. The involvement of NADH precludes the ready large-scale application of the enzyme in an isolated form (and indeed the other alkane hydroxylases) as there is no easy way to accomplish the reduction of NAD^+ back to NADH, which is needed to achieve recycling of this essential cofactor. The intact bacterial cells must therefore be used; their application can be very effective[21].

Although there have been various suggestions that an alk-1-ene may be an intermediate in the reactions, Figures 1 and 2, these claims have not been substantiated[23]. However, Abbot and Casida[24] described isolation of a mixture of internal hexadecenes from hexadecane by *Norcardia salmonicolor* grown on glucose, indicating that under certain circumstances alkenes might be formed.

(b) Subterminal oxidation of alkanes and alkyl chains.

Subterminal oxidation of an alkane represents a radical departure from the usual method of attack. Attack may be seemingly at any carbon atom of the alkane, and the property has been found in several bacteria and moulds[23,25,26]. In a detailed study of the oxidation of tridecane by *Pseudomonas* spp[27-32], the major attack was at the C_2 atom (Figure 3). The key reaction after formation of the secondary alcohol is a Bayer-Villiger type rearrangement[27,28]. The ensuing ester is then hydrolysed by a specific esterase[29,30] to give the primary alcohol undecanol, plus acetic acid.

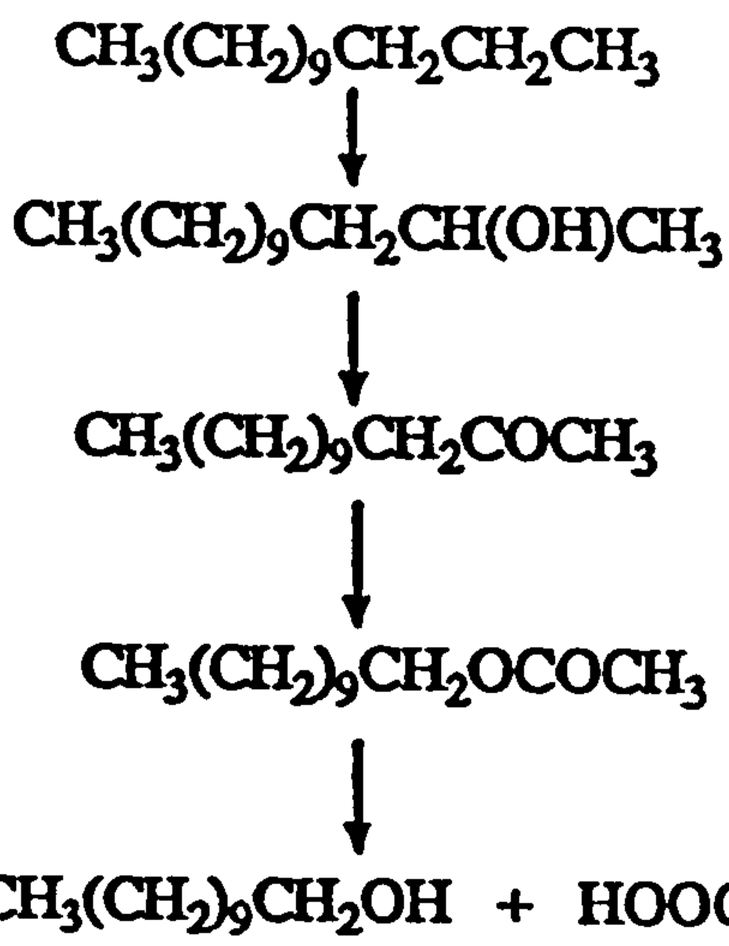


Figure 3. Subterminal oxidation of tridecane by *Pseudomonas aeruginosa* and *Ps. multivorans*[31,32]

There have been no detailed reports on the mechanism of the initial oxidation step of the alkane[33], although the next reaction has been shown to be catalysed by a 2-tridecanone oxygenase which has been purified and characterised[27].

A variation to this scheme has been noted[34] in which a secondary alcohol was attacked by a *Pseudomonas* sp. to give a hydroxy-ketone (Figure 4) that was further oxidised to a diketone, which was then hydrolysed.

In a detailed study of the voracious bacterium *Acineobacter* HO1-N, Finnerty has shown that subterminal oxidative attack occurs on the alkyl chain of various dialkyl ethers, $CH_3(CH_2)_nCH_2OCH_2(CH_2)_nCH_3$, where n = 5 to 8[35]. The resulting products are the alkoxy-acetic acid $CH_3(CH_2)_{n-2}CO_2H$ plus the corresponding dicarboxylic acid $HO_2C(CH_2)_{n-2}CO_2H$

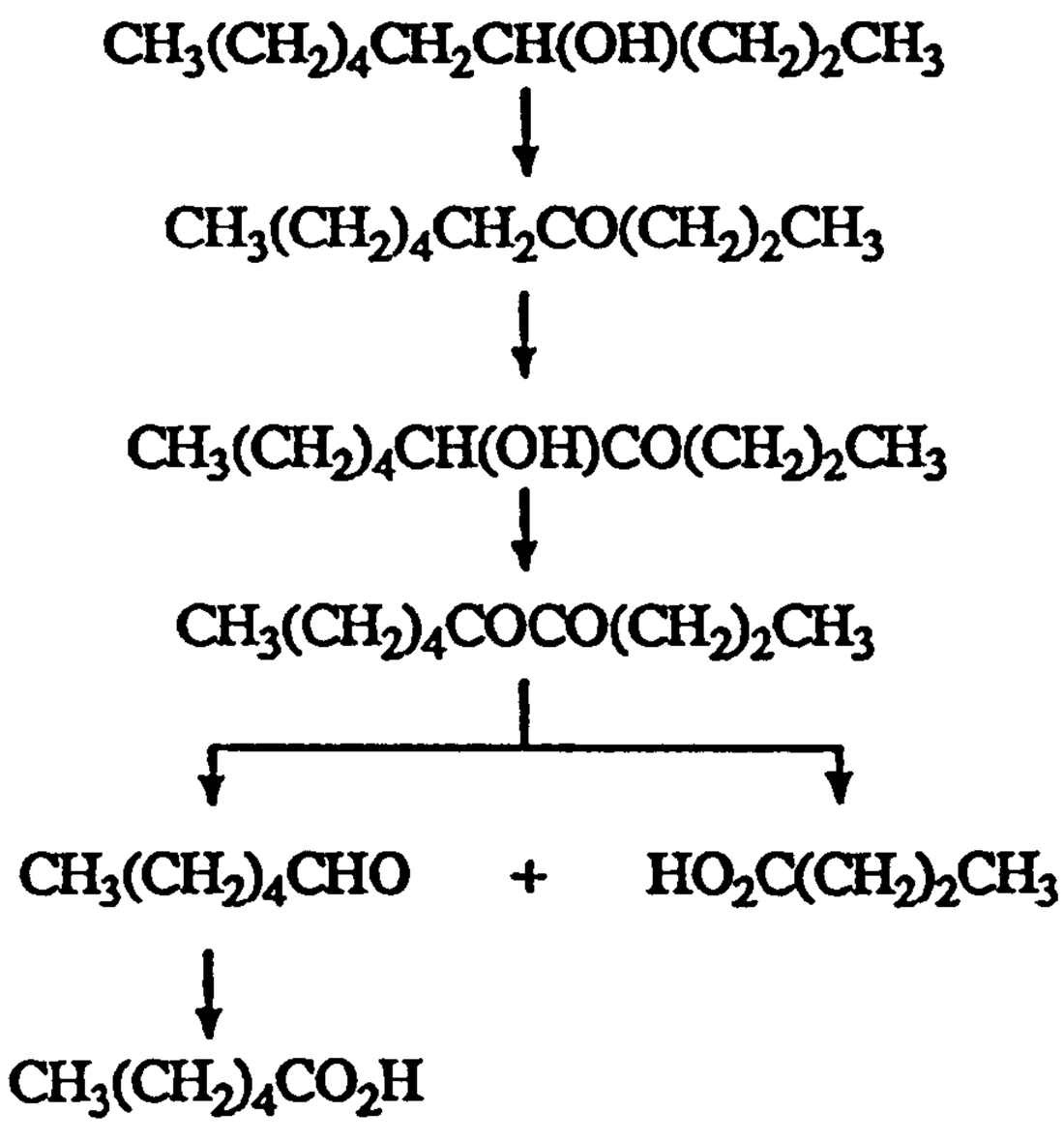


Figure 4. Dissimilation of 4-decanol by *Pseudomonas* sp[34].

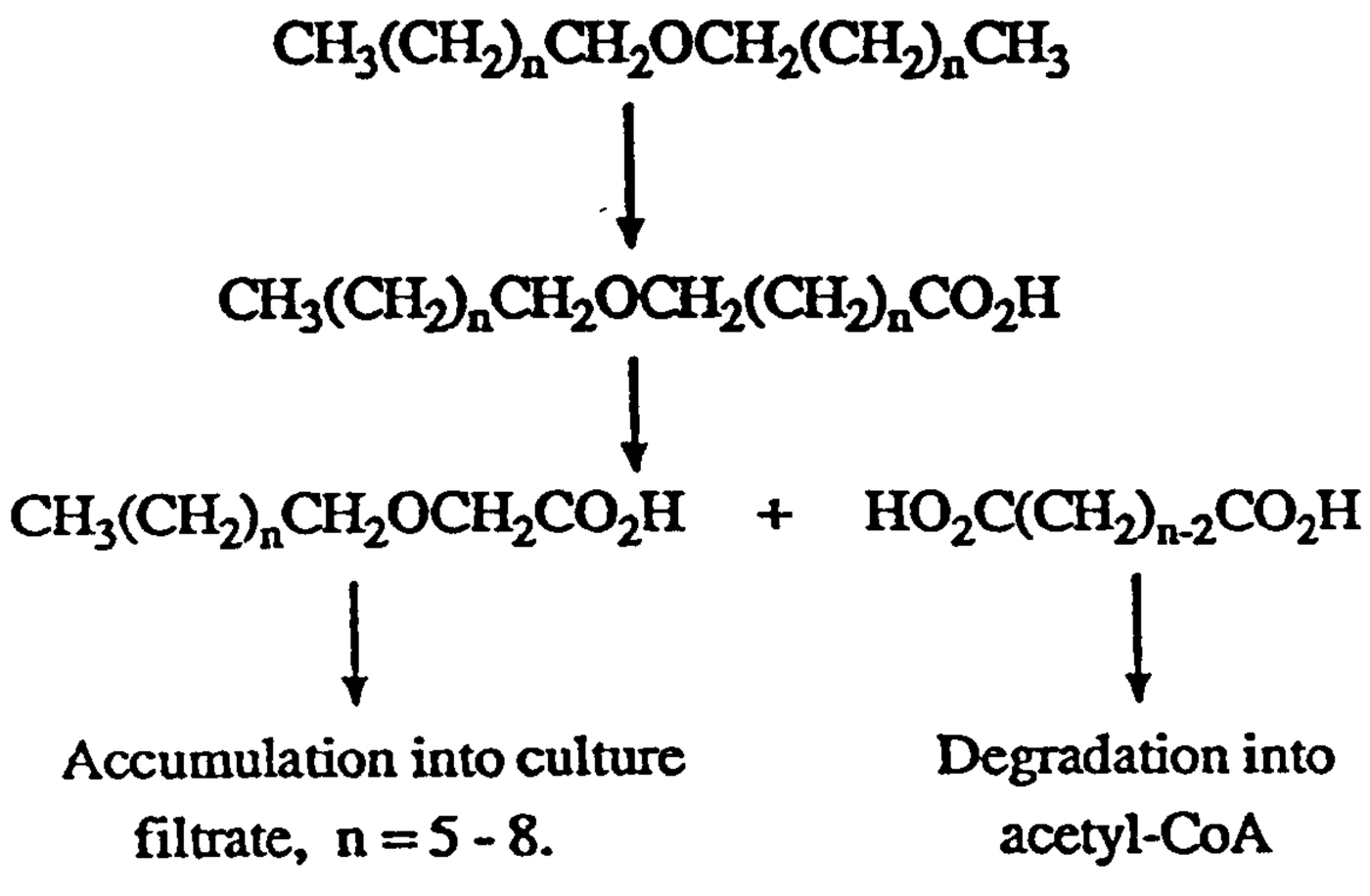


Figure 5. Degradation of alkyl ethers by Acinobacter HO1-N[35,36].

The scheme proposed is given in Figure 5. It is the dicarboxylic acid that is then subsequently degraded to allow the organism to grow. The alkoxy acetic acid accumulates during growth and is apparently not degraded further.

The same bacterium[36] may also attack alkanes and alkyl groups subterminally, although this is probably a minor route. In this way an alkane such as hexadecane is converted to decanedioic acid plus hexane. As other alkanes are also oxidised to the same dicarboxylic acid, the attack is always presumed to be at the C_{10}-C_{11} bond.

(c) Oxidation of alkenes and unsaturated alkyl chains.

Alkenes and unsaturated alkyl chains can be attacked at either their double bonds, wherever they might be in a molecule, or at one of the terminal carbon atoms. Consequently, a variety of products might form (Figure 6).

The majority of work has been with alk-1-enes, as other alkenes are not readily available. The hydroxylating system responsible for attacks at either end of the molecule is usually catalysed by the same enzyme responsible for the initial oxidation of alkanes, i.e. the alkane hydroxylase (Figures 1 and 2).

Epoxide formation has been reported in several instances and may, if the correct conditions are attained, accumulate in some quantity from the oxidation of the alkene[21,37-39]. The epoxide is then oxidised to the corresponding diol which then forms the α-hydroxycarboxylic acid[25]. This is then decarboxylated to the next lower carboxylic acid which then undergoes β-oxidation (see Section 1.2.1 (g)).

(d) Oxidation of primary alcohols (from alkanes and alkyl-chains.

Once the initial oxidation of the alkane or alkyl-chain has been accomplished, the functional group then becomes the focal point for subsequent oxidation. The alcohol is thus converted to the aldehyde and then to the fatty acid. The reactions are catalysed by long-chain alcohol dehydrogenase and aldehyde dehydrogenase (Figure 7).

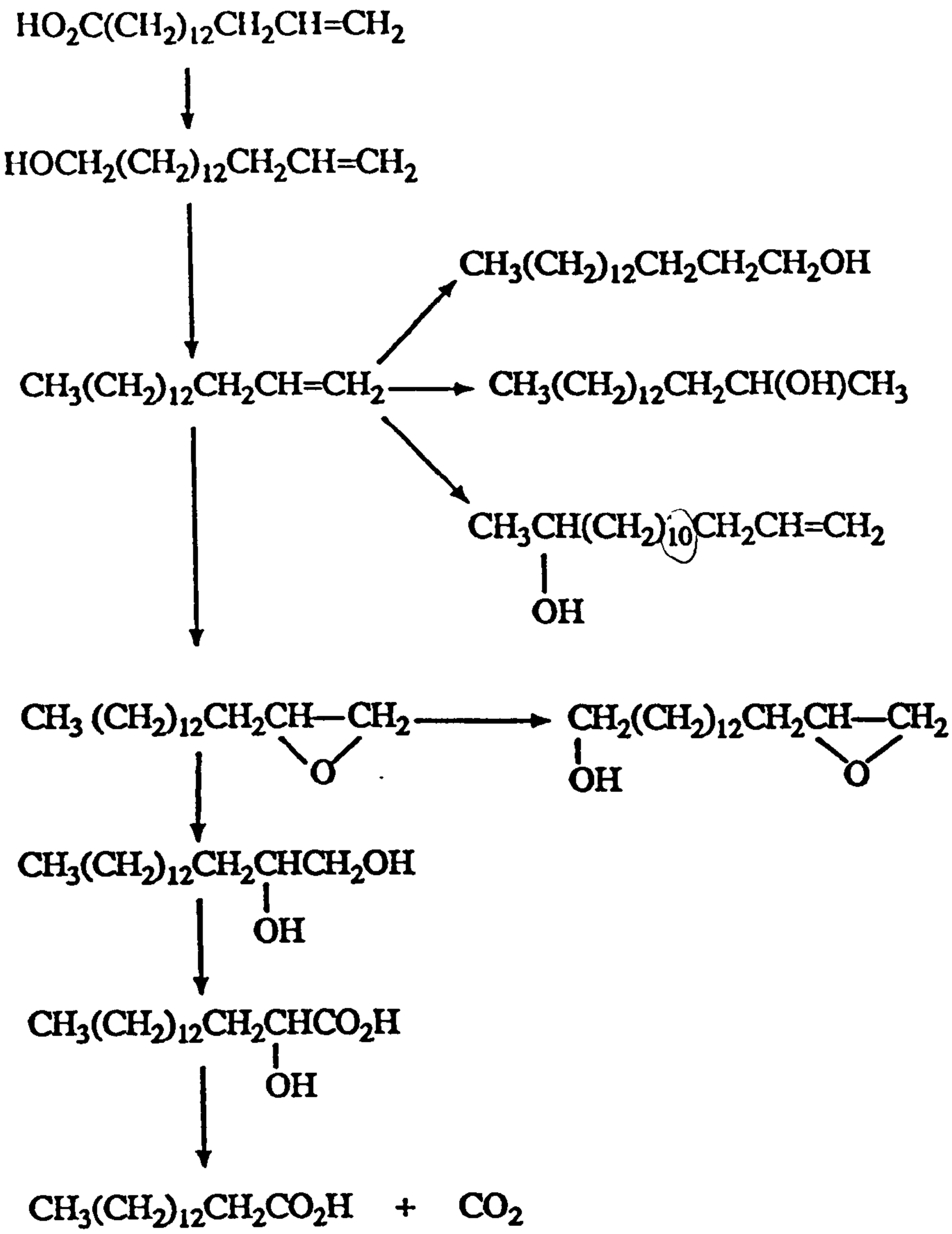


Figure 6. Pathways of alkene dissimilation

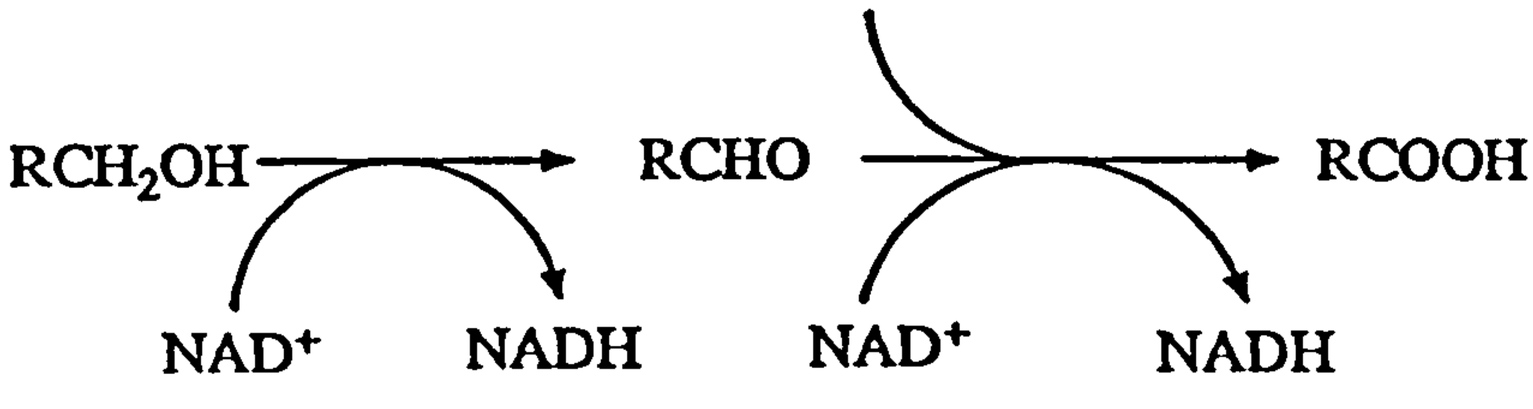


Figure 7. Fatty alcohol and aldehyde dehydrogenases.

The specificity of these enzymes is broad in that substrates from C_{10} to C_{18} can be readily oxidised. There have been suggestions that these two enzymes, plus the alkane hydroxylase, may form a multi-enzyme complex[40] and in certain bacteria all three activities are coded for by DNA of transmissible plasmids[17].

(e) Oxidation of secondary alcohols.

In two recent patents[21], it has been claimed that various methane-utilising yeasts each have a secondary alcohol-specific dehydrogenase (linked to NAD^+) which is capable of converting such secondary alcohols as propan-2-ol, butan-2-ol, pentan-2-ol and hexan-2-ol to the corresponding 2-ketone. The range of compounds which these organisms attack tend to be of shorter chain lengths, but a wider range of substrate other than alcohols can be oxidised by these organisms. The secondary alcohols used in these reactions could be produced by the oxidation of the corresponding alkane, which is a reaction the methane bacteria can readily accomplish.

(f) Fatty-acid degradation.

Fatty-acids may form by oxidation of alkanes or alkenes. Fatty-acids, however, may be presented directly to the cells, either in their own right or in the form of triacylglycerols. A wide variety of microorganisms are capable of hydrolysing oils and fats, enabling them to utilize and thus grow on the glycerol and fatty-acids, in the form of detergents or soaps, and has ecological importance. Microorganisms can utilize a range of fatty-acids, usually from C_{10} to C_{18}, although there are reports of fatty-acids of both longer and shorter chain lengths being used.

The first reaction for a fatty-acid to undergo is the formation of a thiol-ester, with Coenzyme A (Figure 8). This reaction proceeds rapidly and serves to decrease the concentration of free fatty acids within the cell. The reaction is catalysed by long-chain fatty-acyl Co-A synthetase. Such enzymes have been studied in detail in the yeast *Yarrowia* (= *Saccharomycopsis* = *Candida*) *lipolytica*.

Figure 8. Formation of fatty-acyl Co-A from a free fatty acid and Coenzyme A

In this yeast, and possibly in others too, there are two distinct fatty acyl-CoA synthetases which have different roles according to whether the yeast is growing on a carbohydrate or alkyl chain. One enzyme (Synthetase 1) is involved in the direct transfer of fatty acyl-CoA esters into lipids: the other enzyme links the fatty acid arising from the alkyl chain oxidation to the β-oxidation used for subsequent degradation.

(g) β-Oxidation

The β-oxidation system of fatty acid degradation follows the 'classical' textbook pathway elucidated with animal and plant cells (Figure 9). There are four enzymes required to decrease the chain length of the fatty acyl-CoA ester by two carbon atoms: (a) a dehydrogenase, (b) an enoyl-CoA hydrase. In yeasts, the process occurs in the peroxisome organelle and is not linked to the production of metabolic energy[12, 42]. No intermediates of this degradative sequence are ever released as all are tightly bound to the enzyme complex

(h) α-Oxidation

Evidence for the α-oxidation of fatty acids is very fragmentary, although the system does occur in plants. In this scheme, a fatty acyl-CoA is converted to the next lower homologue with the loss of CO_2. Reports of the occurrence of this system in the bacteria *Orthrobacter simplex* and in a yeast, *Candida utilis*, have been given[12]. This system of oxidation is usually invoked to explain the occurrence of fatty acyl groups (in the cell lipids) with even number of carbon atoms arising from all alkyl chains with an odd number of carbons. There are, however, alternative explanations for these occurrences[12]. It is possible that intermediates might be isolated from this process as α-

hydroxypalmitic acid was identified as a product from palmitate degradation by the above bacterium. The pathway, however, has not been extensively studied.

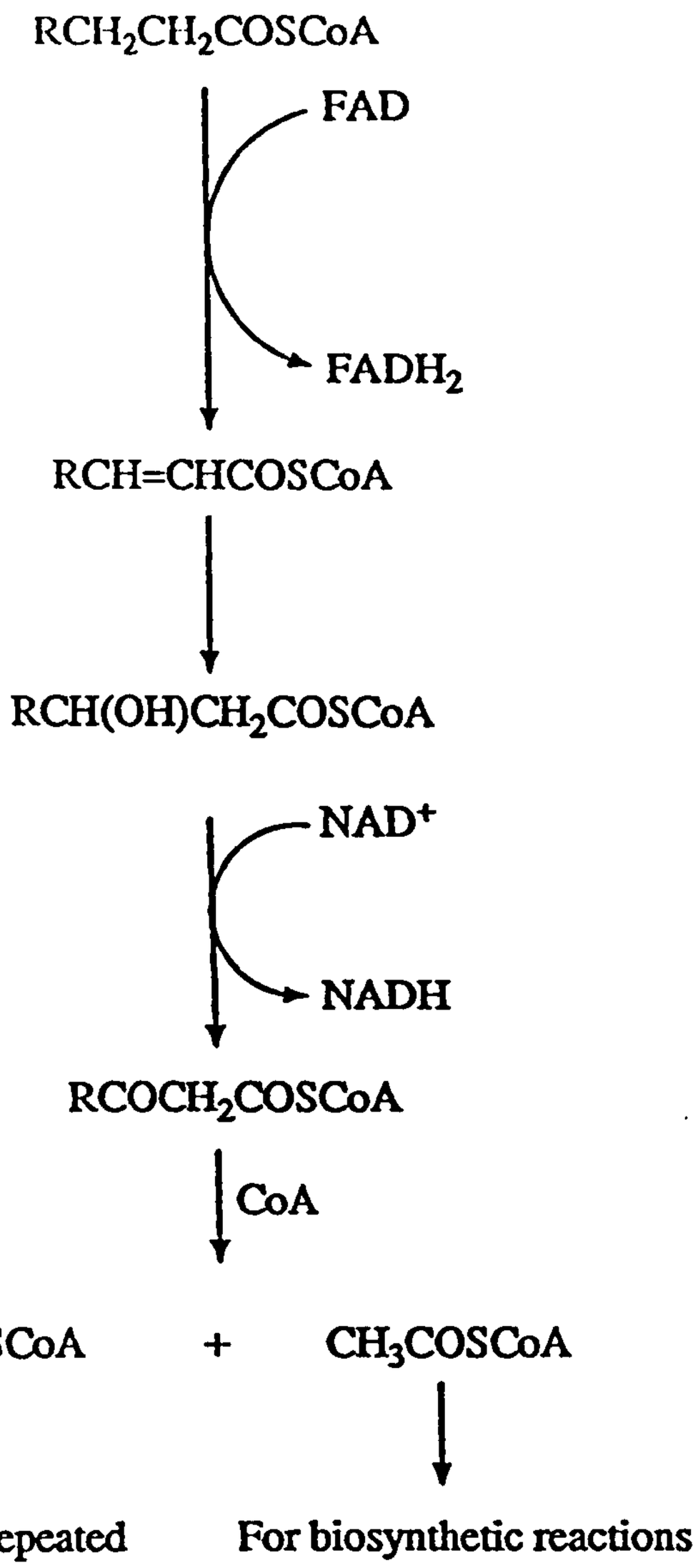


Figure 9 β-Oxidation cycle for the oxidation of fatty acyl CoA esters.

(i) ω-Oxidation (Diterminal Oxidation)

In this mode of oxidation, end-products can be and have been isolated in some abundance. The oxidation, the scheme for which is shown in Figure 10, occurs in both

bacterial and yeasts. Both the α, ω-dioic acids and the ω-hydroxyfatty acids have been isolated from culture filtrates of various organisms; many patents covering various aspects of this process have appeared[12] and the subject has been extensively covered in two recent reviews[43, 44]. Using mutants of *Candida Doacae*, conversions of alkyl chain to dioic acid have been as high as 70% with the process being carried out on a 300l scale.

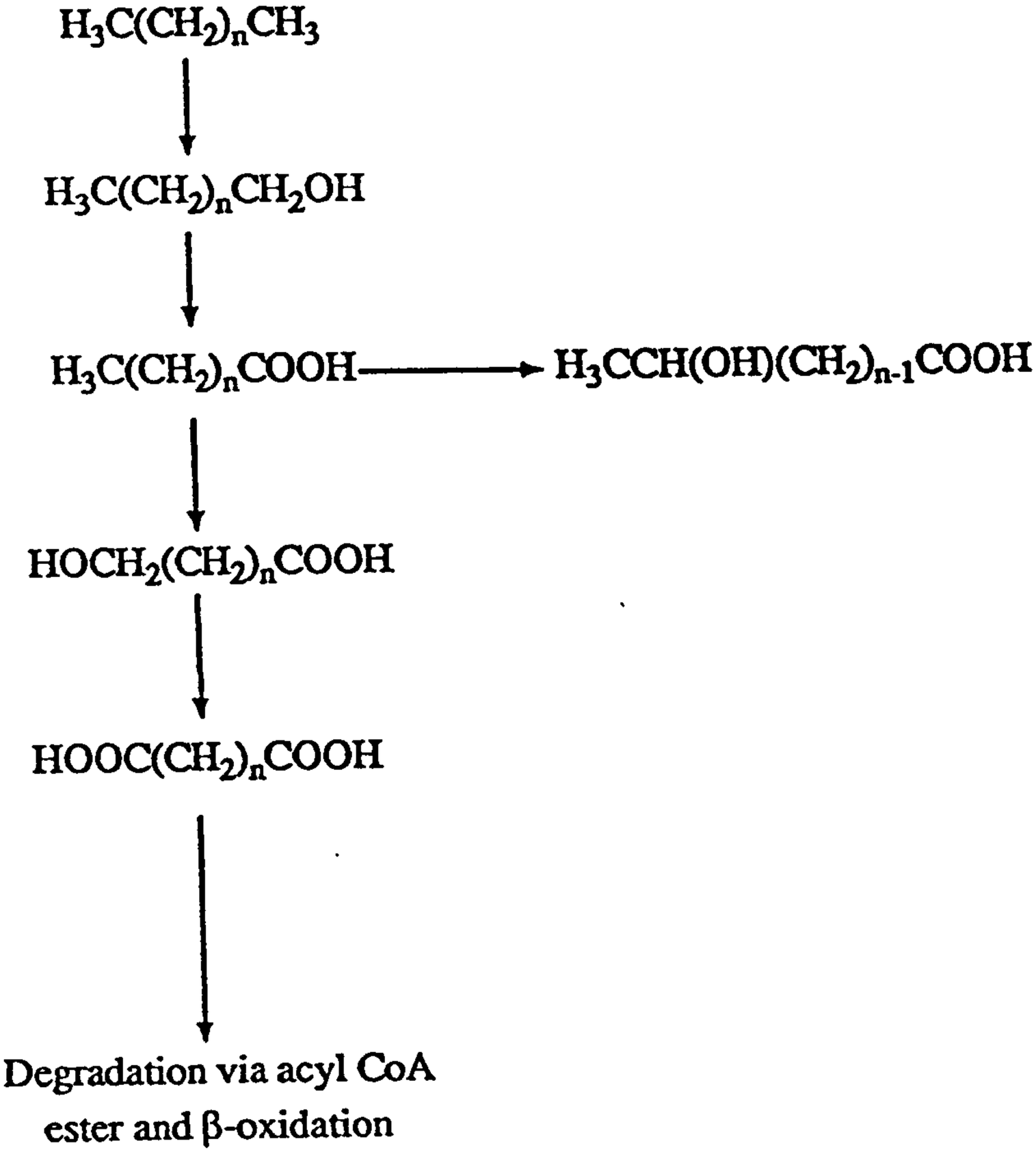


Figure 10 Diterminal oxidation of alkanes.

The ω-hydroxylation of fatty acids (Figure 10) is carried out by the same alkane hydroxylase enzyme used in the initial attack of an alkyl chain (Figure 1). Alkenes may also be attacked by the same enzyme (Figure 6). However, a fatty acid hydroxylase has been isolated from *Bacillus megaterium* which is an organism unable to grow an alkanes. This hydroxylase does not oxidise alkanes but produces 13-, 14- and 15-hydroxypalmitic acids when presented with palmitic acid[45] and epoxy acids from

unsaturated acids. Degradation of α, ω-dioic acids is probably by β-oxidation (Figure 9) beginning at one of the termini. Shorter chain dicarboxylic acids, C_2 to C_9, have been recovered in certain instances although in most cases, degradation would appear to continue until succinic acid is reached, which can then be oxidised via the tricarboxcylic acid cycle.

1.2.2 Hydrophilic Chain Degradation

When discussing the degradation of the hydrophilic chain very few mechanistic pathways need to be discussed due to the fact that a polyethylene oxide chain only degrades by one or two mechanisms. Pearce and Heydeman[48] carried out a study on pure culture classes of bacteria isolated from differing sources that would readily degrade polyethylene oxide chains of differing lengths. It was found from their results (Table 1, n = number of EO groups) that low molecular weight chains could be metabolized by *Acineobacter*, while low (n = 1) to medium molecular weight chains (n = 5) could be metabolized by *Aeromonas* grown from river water extracts. High molecular weight polyethylene oxide chains (n = 5-9) were utilized by *Pseudomonas* grown from sewage sludge extracts.

It has been found recently[49] that *Flavobacterium* sp. grows well on a dialysis culture containing a glycol with a very high molecular weight (~6000) unlike *Pseudomonas* sp. The reason given was that the three glycol utilizing enzymes, PEG dehydrogenase, PEG-aldehyde dehydrogenase and PEG-carboxylate dehydrogenase were present in *Flavobacterium* but the first two were absent in *Pseudomonas*. Thus in order to utilise the high molecular weight glycols, all three enzymes must be present.

It has long since been established that polyethylene oxide chains break down by the loss of one C_2 fragment at a time[50], mainly as ethanol, but traces of acetic acid and acetaldehyde have been reported.

Source n =	1	2	2.5	5	7.5	12.5	19	65
Soil (*Acinobacter*)	-	+	+	-	-	-	-	-
River water	+	+	+	+	-	-	-	-
Soil (*Pseudomonas*)	-	-	+	+	-	-	-	-
Sewage extracts	-	-	+	+	+	+	-	-
Sewage extracts	-	-	-	+	+	+	+	-

Table 1 Bacteria were isolated as described[48] and tested for growth on various polyethoxylated chains (0.1%, w/v) on mineral base E agar.

A mechanism for formation of acetaldehyde was proposed which is initiated by dehydration of the glycol followed by hydrolysis and loss of a C_2 moiety (Figure 11).

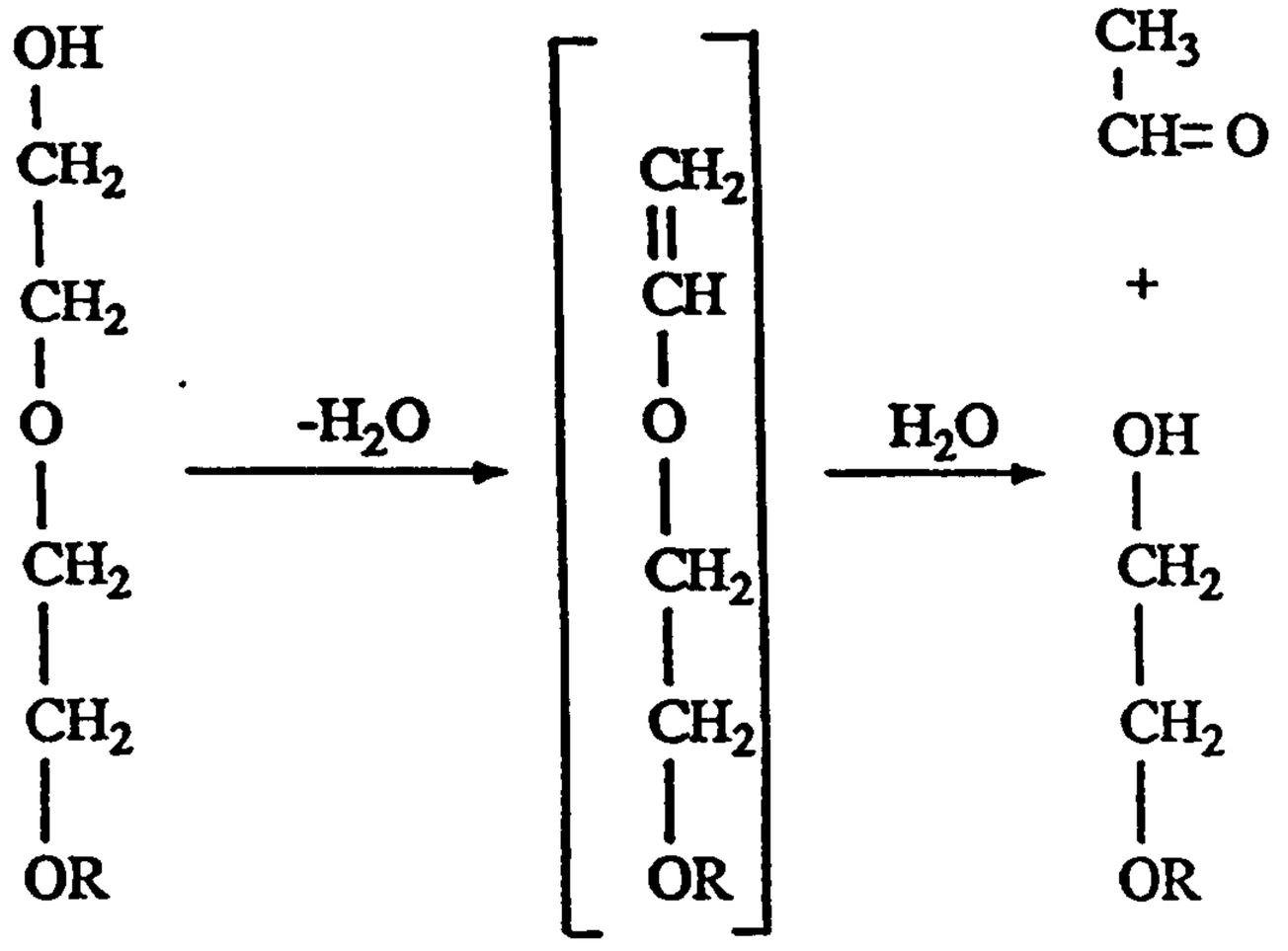


Figure 11 Suggested mechanism for the reduction of a glycol by a C_2 unit to give acetaldehyde.

For alcohol ethoxylates the first degradation step is fission into alkyl and polyethylene oxide chains followed by rapid oxidation of the alkyl chain and a somewhat slower oxidation of the polyethylene oxide chain. This was proved by Kravetz[51] who labelled the alkyl chain with ^3H and the ethylene oxide chain with ^{14}C, and showed the rapid appearance of ^3H_2O in the early stages of biodegradation ('shake flask') accompanied by very little CO_2 evolution (Figure 12).

1.2.3 Biodegradation of Aromatic Rings

Small changes in chemical structure can appreciably alter its susceptibility to microbial degradation. As described earlier, β-oxidation is an important pathway for degrading alkyl-chains and highly branched compounds degrade very slowly.

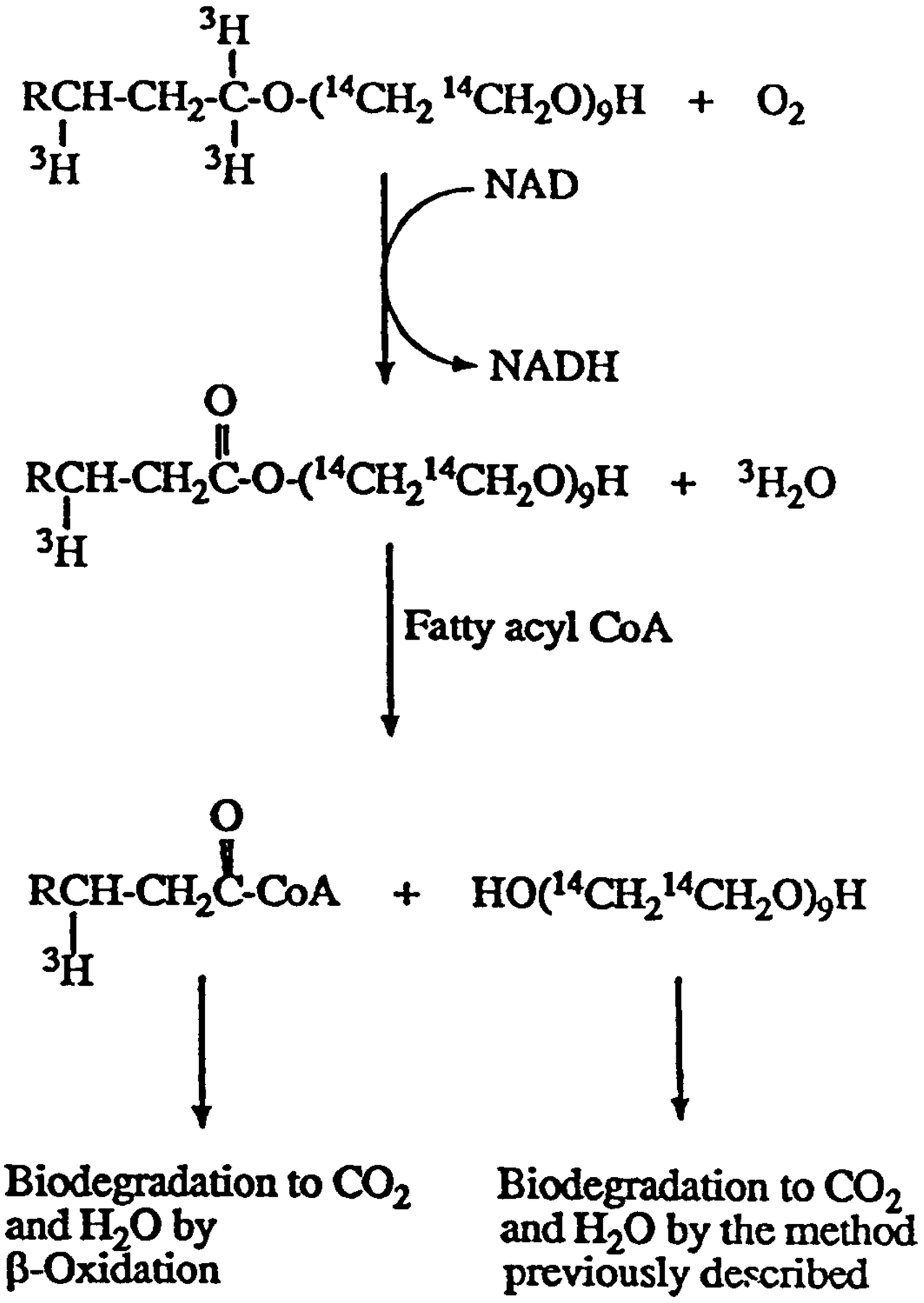


Figure 12 Proposed degradation mechanism for alcohol polyethoxylates.

β-Oxidation is also a very important pathway for the degradation of substituted aromatic rings[52].

Functional groups can either increase or decrease the biodegradability of an aromatic ring. Hydroxyl and carboxyl groups on benzene rings usually increase the biodegradability of the ring whereas halogen, nitro and sulphonate groups have the opposite effect[53]. As we are dealing, in this review, with the biodegradation of nonionic surfactants, the only residues on the aromatic ring we need to consider are hydroxyl, carboxyl and alkyl as these are the most likely metabolites from the degradation of the hydrophobic and hydrophilic chain degradations. Masunaga *et al*[54] analysed the metabolic intermediates of *o*-cresol and 3-methylcatechol by phenol acclimated sludge, one of the first groups to look at phenolic degradation by a mixed bacterial culture. They measured the decrease in *o*-cresol by UV spectrophotometry and GC-MS. From these results they were able to assign up to 24 metabolite peaks and thus proposed biodegradation pathways for both compounds (Figures 13 and 14).

They concluded that the major pathway of *o*-cresol degradation was via 3-methyl-catechol where metabolites can be further degraded to CO_2 and H_2O by the mechanisms previously described.

Hanibabu[57] *et al* describes how a *Micrococus* sp. utilised a number of substituted benzoic acids, tested, as the sole source of carbon and energy. The organism degraded benzoic acid and anthranilic acid through the intermediate formation of catechol. While salicylate was metabolized through gentisic acid and, β-hydroxy benzoic acid was degraded through protocatechuic acid (Figure 15). Catechol and protocatechuate were further metabolized through the *ortho*-cleavage pathway described above.

The β-ketoadipate formed is a well known intermediate in the catabolism of lysine to 2-acetyl CoA, while maleyl pyruvate is an intermediate in the catabolism of certain amino acids.

Several investigations have dealt with the effect of position of attachment of these various functional groups, the first of which was carried out by Blankenship and

Piccolini[56], who studied variations in the rate of degradation with octylphenols, finding that *meta*- and *para*- substitution of groups degrade somewhat faster than *ortho*-substitution. This relationship has been reported more recently by several workers who have studied substituted chlorophenols[57], nitrophenols and amino phenols, and the whole subject of structure/biodegradability relationships has recently been reviewed by Howard *et al*[60].

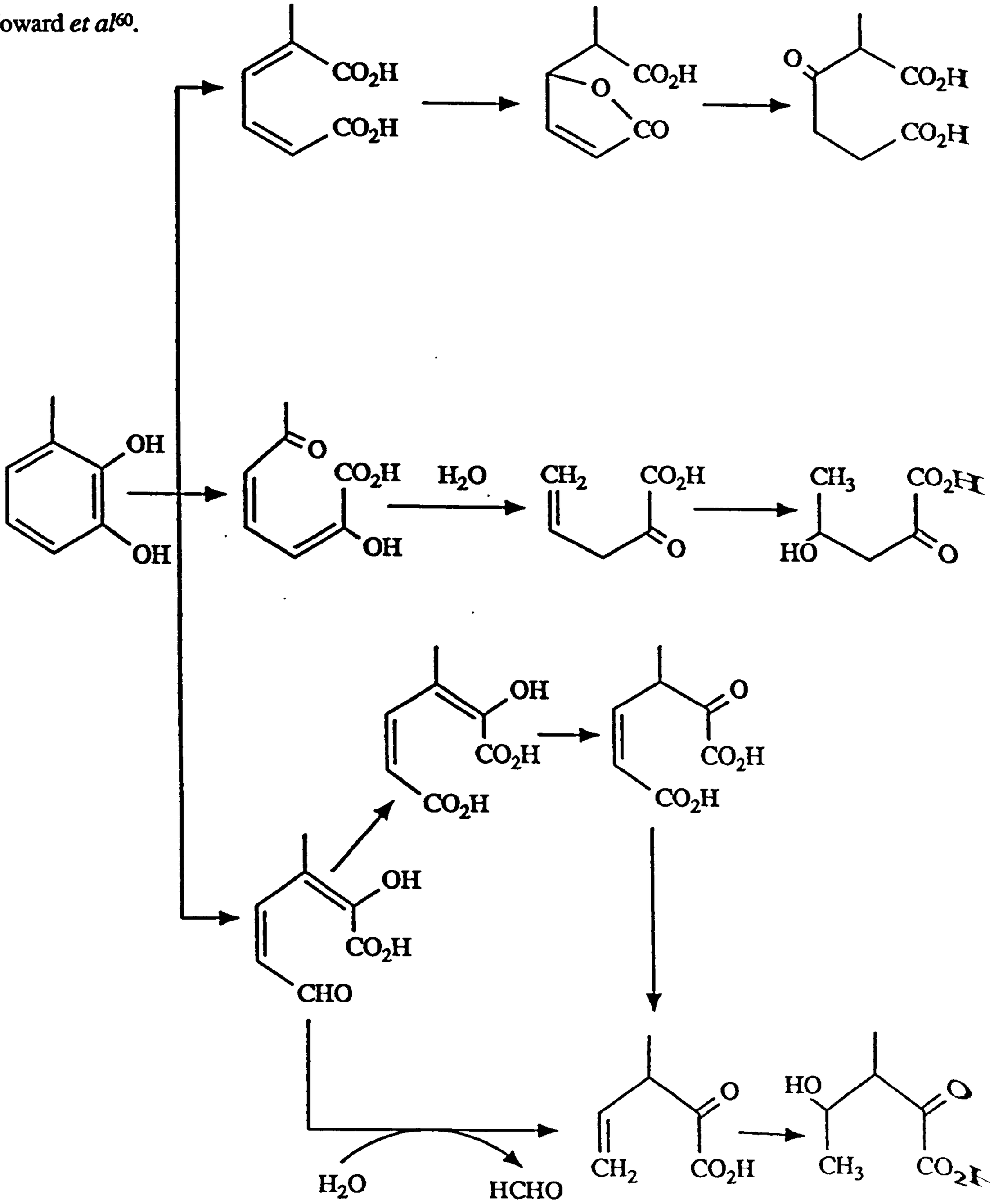


Figure 13. All the probable biodegradation pathways of 3-methyl catechol.

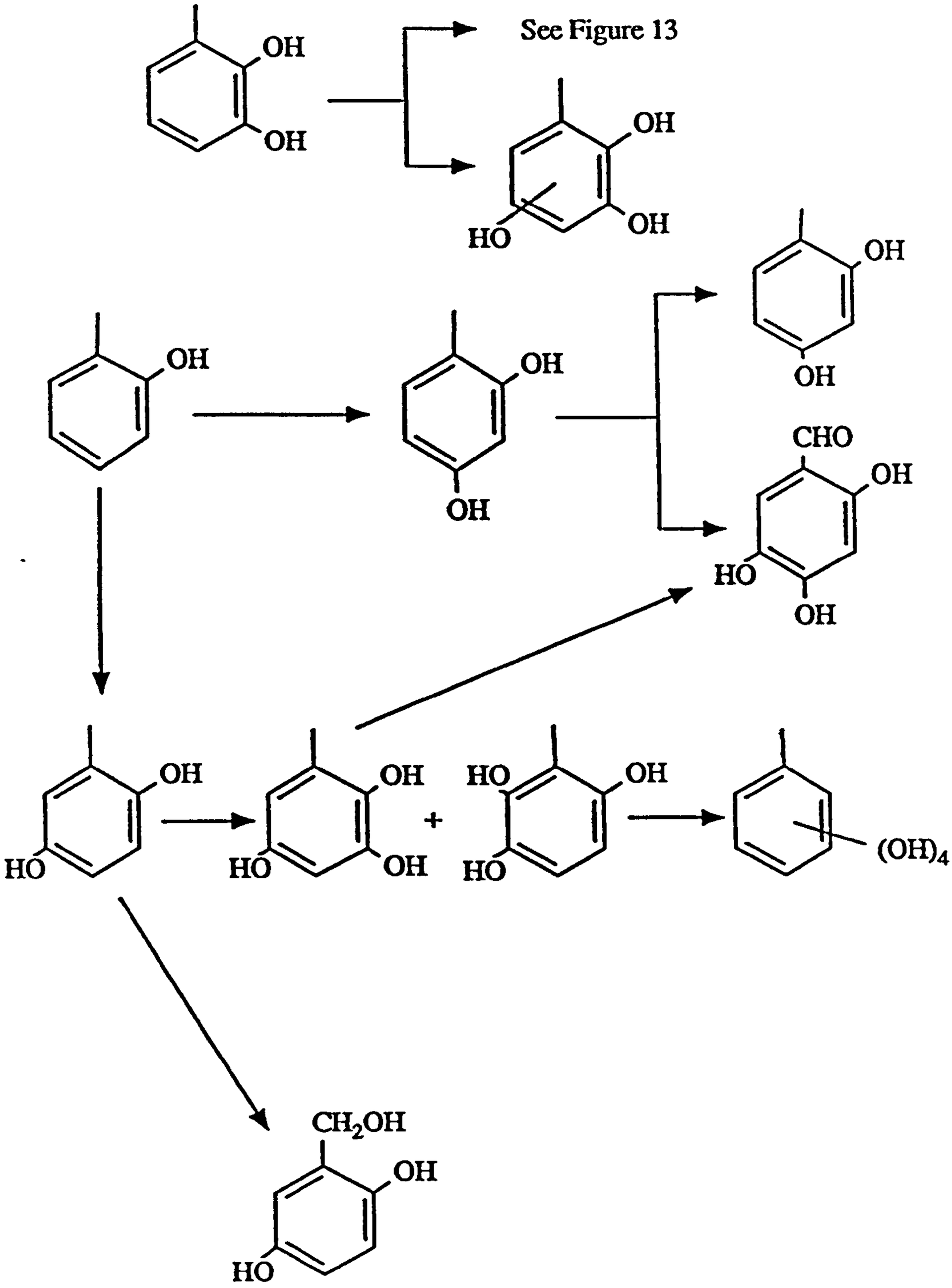


Figure 14. Biodegradation pathways of *o*-cresol by phenol acclimated sludge.

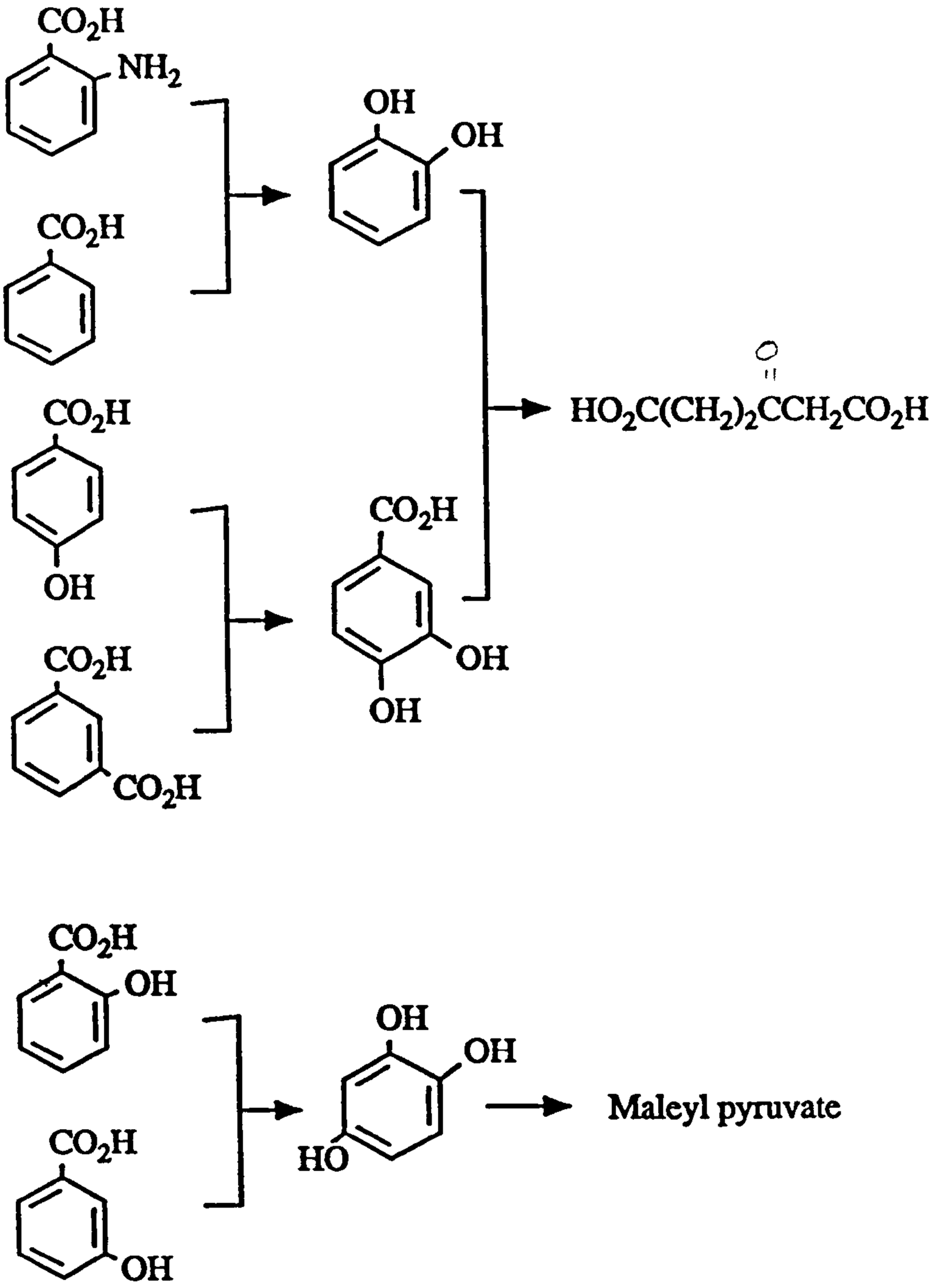


Figure 15. Pathways for the biodegradation of benzoic acids.

Pure culture microbiology is a powerful tool for identifying possible pathways for degradation but the organisms isolated by enrichment are not necessarily active in the environment. Thus, even if a pure culture is isolated that will degrade a chemical, it does not mean that the chemical will necessarily degrade in the environment. Moreover, since mixed populations generally have higher catabolic versatility then pure cultures, failure to isolate a pure culture that can degrade a chemical does not necessarily imply that the chemical will not degrade in the environment. Therefore, mixed microbial cultures from natural samples should be used for determining biodegradation in the environment.

1.3. Definition of General Terms

Primary biodegradation - means biodegradation of a substrate to an extent sufficient to remove a characteristic property of the original intact molecule. For surfactants this has been measured by loss of foaming capacity or ability to reduce surface tension. Primary biodegradation can leave high levels of organic residues altered in form from the original material.

Ultimate biodegradation - is biodegradation which proceeds through a sequence of enzymatic attacks to ultimately produce the simplest structures possible in the biodegradation media. In aerobic biodegradation, such as that which consumes oxygen in the aeration solutions of sewage treatment plants, CO_2, H_2O and mineral salts of other elements present are generated. In anaerobic septic tank systems in which microbial attack occurs with little oxygen present, methane is generated in addition to the products already mentioned.

1.4. Physical Tests for Biodegradability

1.4.1 Foaming.

The capability of aqueous solutions of surfactants to foam is greatly reduced when they are subjected to microbial attack. A decrease in foam height is frequently used to measured the primary biodegradability of surfactants. However, foam measurements can be misleading criteria if surfactants biodegrade to chemical intermediates which are resistant to further biodegradation yet foam readily. This has been reported for alkylphenol polyethoxylates in which the polyoxyethylene chain was considerably shortened to produce products which still foamed[61]. For surfactants like alcohol polyethoxylates, where biodegradation is more rapid, foam data provide a better measurement of primary biodegradability than for alkylphenol polyethoxylates. However, even rapidly biodegradable surfactants like alcohol polyethoxylates do not

provide very accurate primary biodegradability data when foaming is the criterion because of the interferences of low foaming biodegradation intermediates produced from organic materials present in sewage streams.

1.4.2 Surface tension.

Reduction of surface tension in aqueous media by surfactants can be used to follow their primary biodegradation. As the surfactants degrade, surface tension tends to rise. The limitations mentioned for foaming also apply to surface tension measurements.

1.5. Analytical Tests for Biodegradability

1.5.1 Cobalt thiocyanate and Bismuth iodide.

These widely used primary biodegradation test methods take advantage of the formation of metal complexes with the oxygen atoms in the polyoxyethylene structure of nonionic polyethoxylates. Cobalt thiocyanate forms a blue coloured complex with nonionics which may be extracted with a chlorocarbon solvent and determined spectrophotometrically. Bismuth iodide forms a precipitate with nonionics which is dissolved and titrated for bismuth. Biodegradation reduces the complexing capabilities of the surfactants. The advantage of these techniques is that they can be used in laboratory-scale biodegradation tests as well as in sewage treatment plants and receiving waters where other organic materials are present. However, nonionic polyethoxylates with very short (generally less than five) or very long (generally greater than 20) polyoxyethylene units do not complex with the cobalt and bismuth reagents. Hence, nonionics of this type may go undetected by these analytical methods. Accuracy is also limited by interferences from other products found in environmental waters, particularly in sewage effluents and receiving waters where the surfactant concentrations are low. The most serious limitation is the inability of the cobalt and bismuth methods to differentiate between different sufactants containing a polyoxyethylene chain.

1.5.2 Instrumental and Chromatographic analysis.

Ultraviolet and infrared spectroscopy[62] have been used to determine nonionic polyethoxylates and the rates at which they degrade. These primary biodegradability approaches are limited by interferences from other materials present in environmental samples and by their inability to identify the many varying chain length structures of alcohol polyethoxylates and alkylphenol polyethoxylates. Thin layer chromatography (TLC) has had considerable success in measuring the effect of nonionic structure on degradation rates[63]. In recent years, a number of workers have used a method in which nonionic polyethoxylates and their reaction intermediates are extracted from environmental samples and treated with hydrobromic acid. The resulting chain scission products - ethylene bromide from the polyoxyethylene chain of alcohol polyethoxylates and alkylphenol polyethoxylate and alkyl bromides from alcohol polyethoxylates - are identified and quantified by gas chromatography. It is likely that such approaches as high performance liquid chromatography and gas chromatography, interfaced with mass spectrometry, will find increasing use in identifying nonionic polyethoxylates and their biodegradation intermediates in environmental samples.

1.6 Ultimate Biodegradability

The ultimate biodegradation of alcohol polyethoxylates and alkylphenol polyethoxylates under aerobic conditions may be represented by the following stoichiometry:

(a) $C_{13}H_{27}O(CH_2CH_2O)_9H + 42\,O_2 \rightarrow 31\,CO_2 + 32\,H_2O$

for alcohol polyethoxylates having 13 carbon atoms in the alkyl chain and on average of 9 ethylene oxide units/mole.

(b) $C_{15}H_{23}O(CH_2CH_2O)_9H + 43\,O_2 \rightarrow 33\,CO_2 + 30\,H_2O$

for alkylphenol polyethoxylates having 9 carbon atoms in the alkyl chain and an average of 9 ethylene oxide units/mole.

The alcohol polyethoxylates and the alkylphenol polyethoxylates are highly simplistic average structures. Commercial samples of these surfactants are actually complex mixtures of single chemical entities.

It should also be noted that the ultimate biodegradation sequences (a) and (b) are theoretical. Even the most biodegradable compounds, like glucose, do not oxidise completely to CO_2 and water since a portion of the organic matter is used by biodegrading bacteria to form new bacterial cells.

Theoretical equations (a) and (b), however, are useful in determining the extent of ultimate biodegradation of the respective substrates. If analytical methods are available, determination of oxygen uptake, disappearance of organic carbon, CO_2 evolution and the formation of water from a known substrate will measure the extent to which that substrate biodegrades to CO_2 and water. The following analytical methods are used to determine ultimate biodegradability.

1.6.1 Biochemical Oxygen Demand (BOD).

This is one of the oldest methods used to measure oxygen uptake. Substrate, bacterial innoculum and oxygen are generally placed in a glass vessel and O_2 uptake determined by chemical analysis, manometrically or by an oxygen electrode. However, BOD tests to determine the ultimate biodegradability of a single substrate do not simulate realistic sewage plant conditions where organic materials other than the substrate to be tested are present. Other limitations include use of unacclimated inocula, possible interference from inorganics like sulphur compounds, which can also consume oxygen and form materials which can destroy bacteria and give false negative values on the biodegradability of a substrate.

1.6.2 Total Organic Carbon (TOC) and Chemical Oxygen Demand (COD) analysis.

These methods determine residual organic carbon in biodegradation media. In TOC the organics in an aqueous sample are pyrolysed to CO_2 in the presence of a catalyst. CO_2

levels are then determined in an IR spectrophotometer interfaced with the combustion unit. In the COD method the sample is oxidised by a mixture of potassium dichromate and sulphuric acid. The quantity of dichromate used is calculated as oxygen equivalents.

TOC and COD are useful in dilute bacterial laboratory media where a test substance is the major or only organic present and in environmental samples where the cumulative TOC contribution from all organics present might be desired. A major limitation is the inability of these methods to determine organic carbon from a specific substrate in environmental samples.

1.6.3 CO_2 Evolution.

In this method the CO_2 evolving from a closed biodegradation system is trapped in a basic medium. The carbonate produced are titrated with acid to determine CO_2 evolved. This method is finding increasing use in laboratory experiments having a single surfactant present as the major substrate[67, 68]. It suffers from the same limitations as the TOC and COD methods since it cannot be used to differentiate between specific substrates simultaneously present in environmental samples.

1.6.4 Radiotracers.

Use of radiolabelled compounds[67] provides an extremely sensitive method of determining the presence of two levels of substrate. When coupled with organic carbon and/or CO_2 evolution tests it becomes a most powerful technique to determine ultimate biodegradation and the presence of intermediate biodegradation products. The use of radiolabelled compounds eliminates the major limitations of the organic carbon and CO_2 evolution tests already discussed since the radiolabelled substrate and its biodegradation products can be followed accurately in the presence of much higher levels of other organics. Limitations to the use of radiolabelled substrates include the high costs and complexity of synthesis, the difficulty of interpreting results without undertaking a detailed study requiring additional sophisticated analytical techniques and

the virtual impossibility of using radiolabelled substrates in large scale plant studies because of the large quantities required. For these reasons radiotracers cannot be considered for use in routine biodegradability testing.

1.7. Biodegradation Test Methods

Swisher[61] and Gilbert and Watson[62] have reviewed many test methods which have been used for determining the biodegradability of surfactants in aqueous media. Those used most frequently are summarised here:-

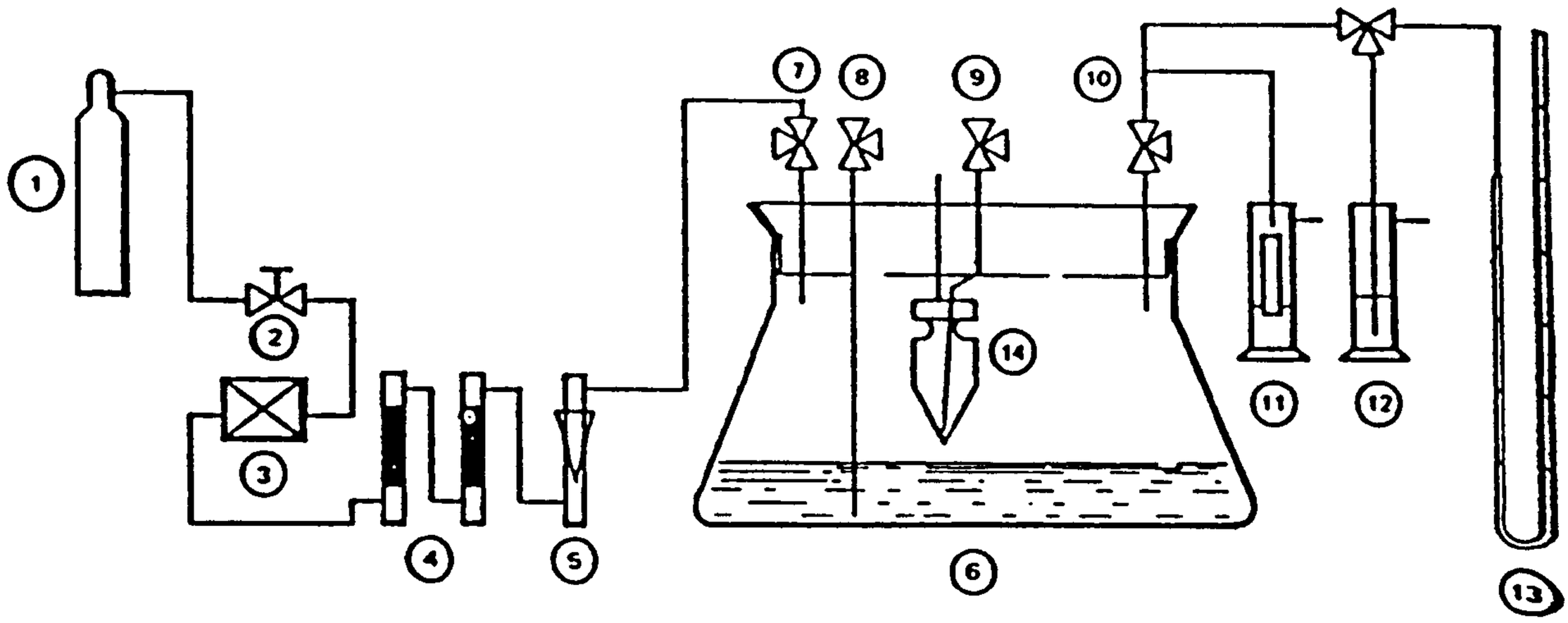
1.7.1 Shake Flask.

Substrate, dilute bacterial innoculum, usually obtained from a sewage treatment plant, and inorganic supplements are added to Erlenmeyer-type flasks. The flask is mounted, open mouthed, on a reciprocating or oscillating shaker to permit air to enter the medium. Samples are withdrawn at intervals and analysed for the presence of surfactant by the techniques already discussed. Shake flash systems vary from Erlenmeyer flasks, when only primary biodegradability is to be measured, to very complicated equipment (Figure 16) when the ultimate biodegradation data is required and radiolabelled surfactants are used. The dilute bacterial media present in shake flask systems do not simulate sewage treatment plant conditions. However, the system is excellent for screening a variety of substrates.

1.7.2 Activated Sludge.

These systems are concentrated, biological solids obtained in aeration units of sewage treatment plants. Laboratory-scale activated sludge reactor systems with forced air introduction simulate sewage treatment plant conditions more closely than shake flask systems. Generally, they are used to obtain primary biodegradability data but may be modified to obtain CO_2 evaluation data. Activated sludge tests for surfactants are practiced in semi-continuous units, mostly in the U.S. and in continuous units in Europe. Limitations of laboratory activated sludge systems include the frequent

absence of several realistic sewage conditions such as influent parameters and sludge wasting.



1. Gas Cylinder, 70% O_2/30% N_2, CO_2 Free Biodegradation Atmosphere
2. Shut Off Valve
3. Pressure Relief Valve
4. Ascarite Scrubbers to Remove CO_2
5. Purge Rotameter – 1 for Each Unit
6. 4 Liter Erlenmeyer Flask with No. 12 Rubber Stopper, Covered with Black Polyethylene Tape and Aluminum Foil
7. O_2/N_2 Purge Gas Inlet Valve
8. Test Media Sample Valve and Line
9. Base Sample/Charge Line
10. O_2/N_2 Purge Gas Outlet Valve
11. Bubbler to Monitor Purge Gas Flow During Sampling
12. LiOH Scrubber Used While Sampling ^{14}C Containing Units
13. Manometer to Monitor Internal Pressure on 1000 mg Units
14. Base (LiOH) Reservoir

Figure 16. Apparatus for measuring the ultimate biodegradation of a radiolabelled surfactant

1.7.3 River Die-away.

This is an attempt to simulate the action of a receiving water on a substrate. It is similar to a 'shake flask' system except that it is under static conditions. Its limitations are the same as those of the shake flash method - somewhat unrealistic in accurately simulating a river die-away situation. In addition, the use of a single substrate in static river die-away tests does not simulate the dynamic flow-through system of an environmental stream where bacteria feed on many substrates which change in type and concentration with time.

1.7.4 Sewage Treatment Plant Study.

This is a highly realistic test in which the influent of a sewage treatment plant is dosed with surfactant at a specific feed rate at levels considerably above background. Various sections of the plant are then analyzed for the presence of surfactant. The method is limited by the fact that it can be used to obtain primary biodegradability data only, it requires a plant to operate without upsets during the course of the study and depends on co-operative attitudes of plant personnel.

1.7.5 Monitoring Study.

This is the most realistic study possible since data are obtained under normal operating conditions. The substrate to be studied must be present in the influent through normal conditions of home use. A monitoring study is limited by the availability of specific and sensitive analytical methods for determining the substrate in its course through the plant and in the receiving waters, and has been reported for nonionic surfactants primarily because specific analytical methods for differentiating between alcohol polyethoxylates and alkylphenol polyethoxylates have been available.

1.8 Results from Biodegradation Experiments

Many workers have carried out primary and ultimate biodegradation tests on alkylphenol and alcohol ethoxylates of different origins and degrees of ethoxylation. Here, we shall discuss some of the more important findings from these tests and attempt to put into context the results and inferences made by these researchers.

1.8.1 Primary Biodegradation.

The primary biodegradability of alcohol polyethoxylates and alkylphenol polyethoxylates has been extensively investigated, generally in separate studies, for these two nonionic classes. In those studies where both alcohol polyethoxylates (AE) and alkylphenol polyethoxylates (APE) have been compared directly, AE with predominantly linear alkyl chains biodegraded considerably faster than APE[63,70-72]. An

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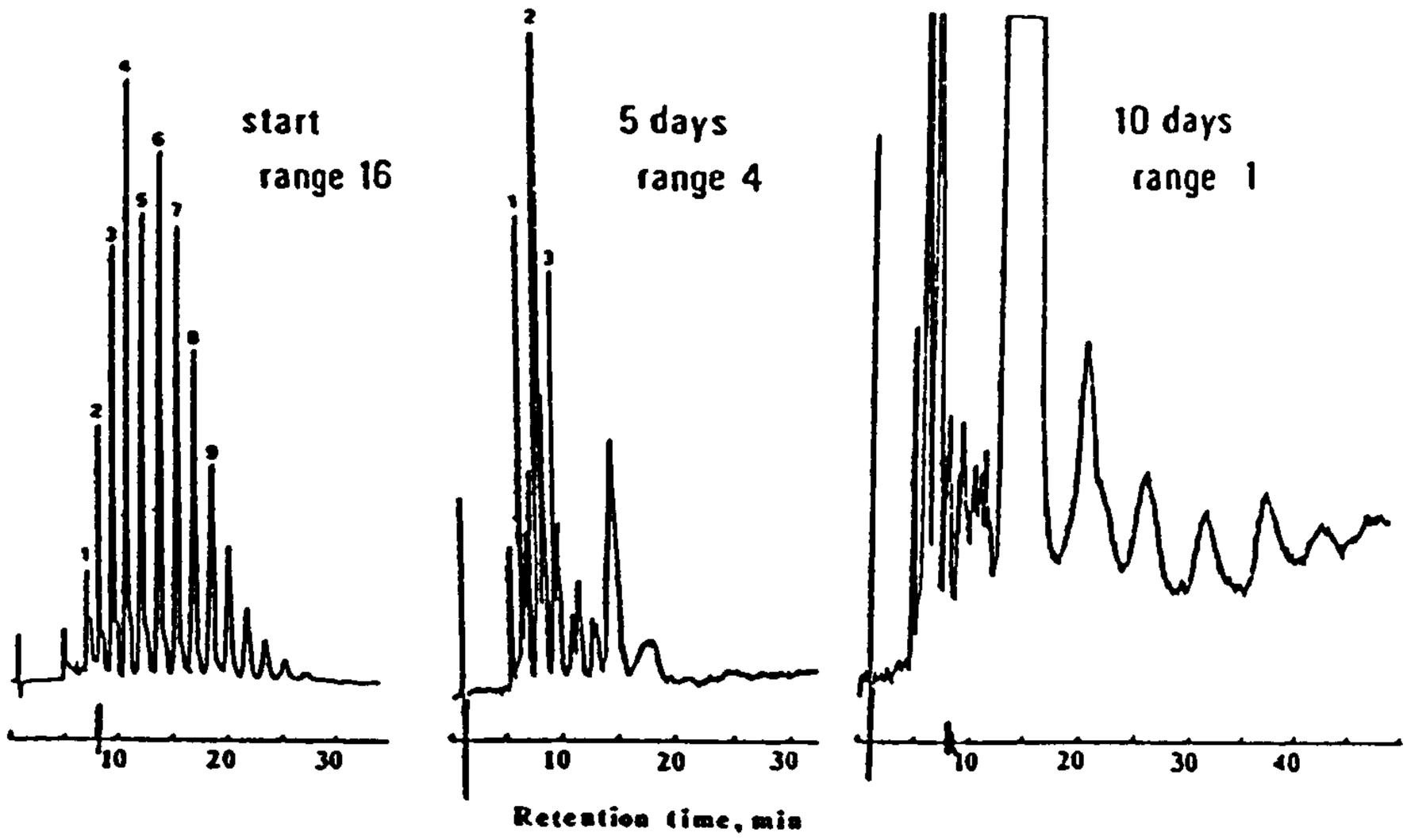

Figure 18. HPLC analysis following the biodegradation of C_9APE_9.

Bruschweiler *et al*[74] used the bismuth iodide test to follow primary biodegradation but they found that nonionics with less than about 4EO groups could not be detected by this method. This test was used in conjunction with DOC (ultimate) and UV (ring) analyses to follow the degradation of C_9APE_{11} and C_9APE_{23} by an activated sludge test method (Figure 19).

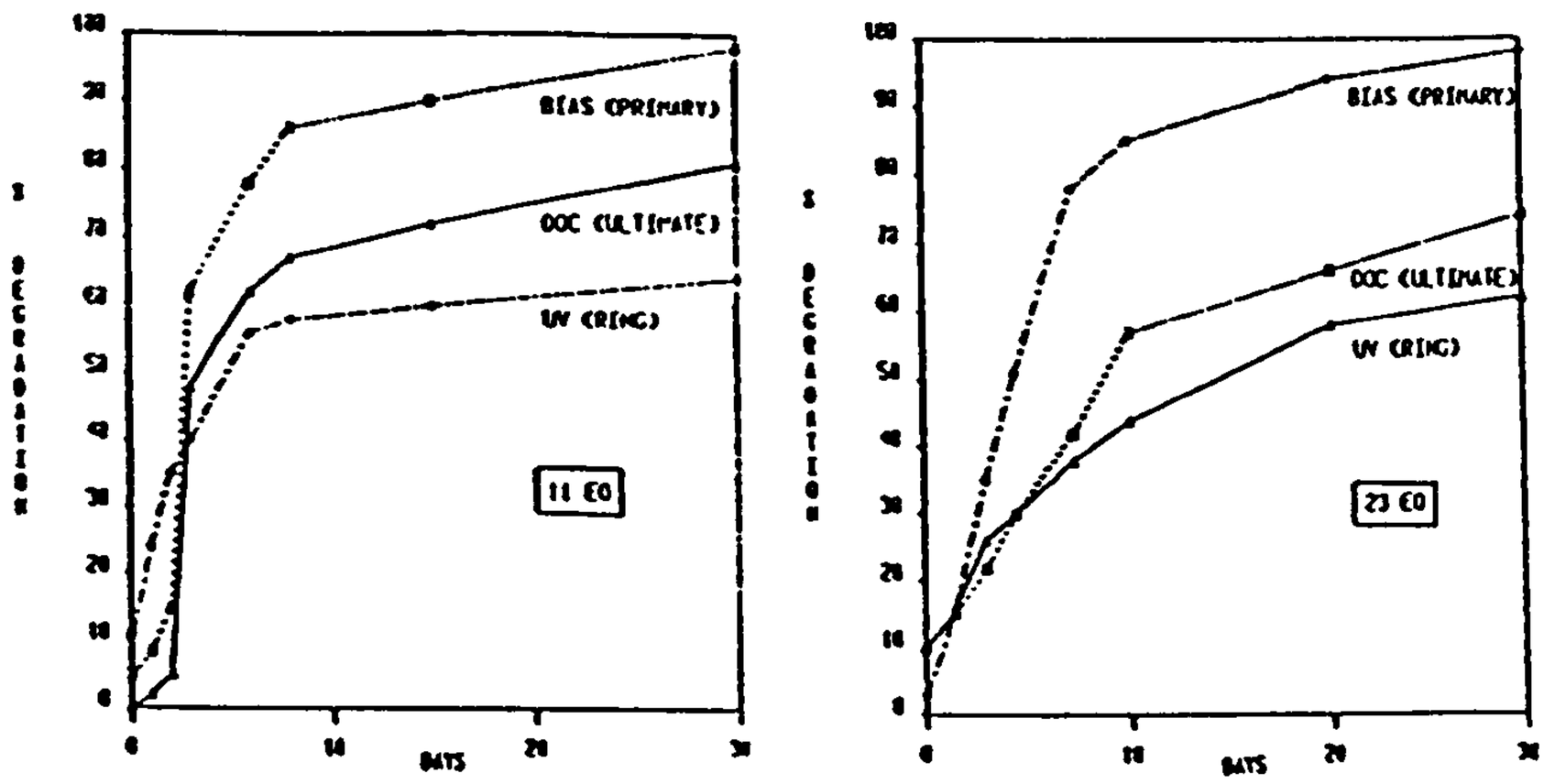

Figure 19. Primary and ultimate biodegradation of nonyl phenol polyethoxylates.

The importance of adequate acclimation of bacterial inocula in studying the biodegradation of surfactants has been mentioned in the literature[68, 73, 74]. River die-away studies by Rieff[75] have shown slower bacterial acclimation times as well as slower primary biodegradation rates for a commercial APE compared to two commercial AE. Slower biodegrading surfactants may require considerably longer acclimation times. In a sewage treatment plant which has experienced a period of upset, such as a bacterial kill or excess storm runoff, reacclimation and recovery of the plant to normal operating conditions would be expected to depend on acclimation time.

1.8.2 Ultimate Biodegradation.

In recent studies, the rate at which surfactants are converted into their ultimate biodegradation products, CO_2 and water, has been examined. A pioneering study of the biodegradation of nonionic surfactants by CO_2 evolution has been published by Sturm[78]. In this paper he describes how he tested the biodegradability of a number of surfactants on acclimated sewage sludge by measuring the CO_2 evolved. The CO_2 test apparatus was arranged in such a way that 8 materials and a positive control were tested simultaneously (Figure 20).

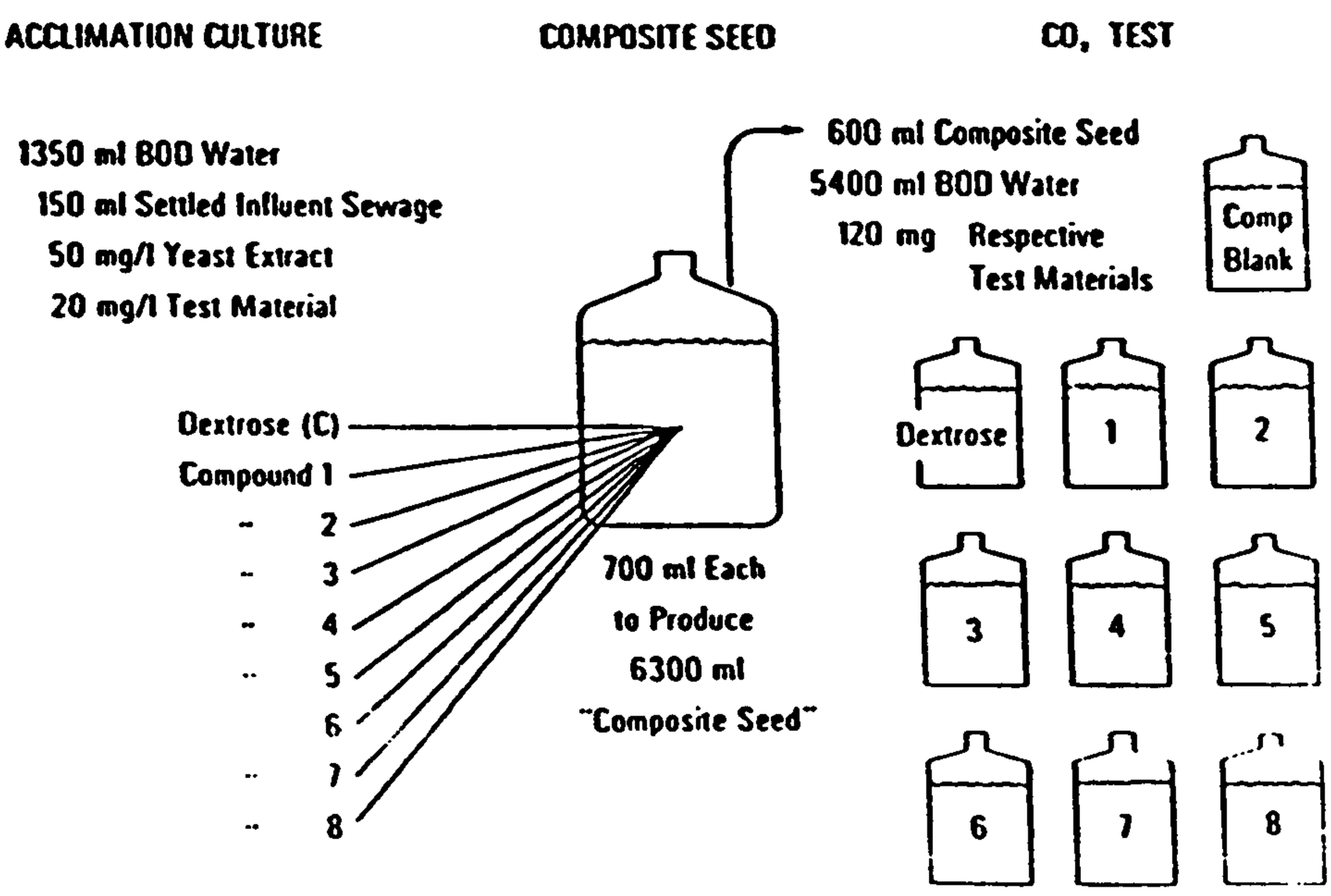

Figure 20. General protocol for a 10 unit CO_2 production test[78].

The nine individual acclimation cultures - each combining settled raw sewage as a source of microorganisms, yeast extract as an easily assimable nutrient source, BOD water as a diluent and a source of inorganic nutrients, and a test material - were permitted to sit in the dark for 14 days. At the end of that time, equal aliquots from each of the cultures were used to make the composite seed (a combined acclimated inoculum) for use in the CO_2 test. The final composition in each test vessel included 600ml of the composite seed and 120mg of the test material diluted to 6l with BOD water. The use of a common composite seed had two advantages (a) it allowed the use of one blank for eight test units, and (b) it provided a bacterial population acclimated to a variety of materials, including the test compound.

During the test CO_2 - free air was bubbled through the test units (Figure 21), and the effluent gas was passed through a series of CO_2 absorbers containing barium hydroxide.

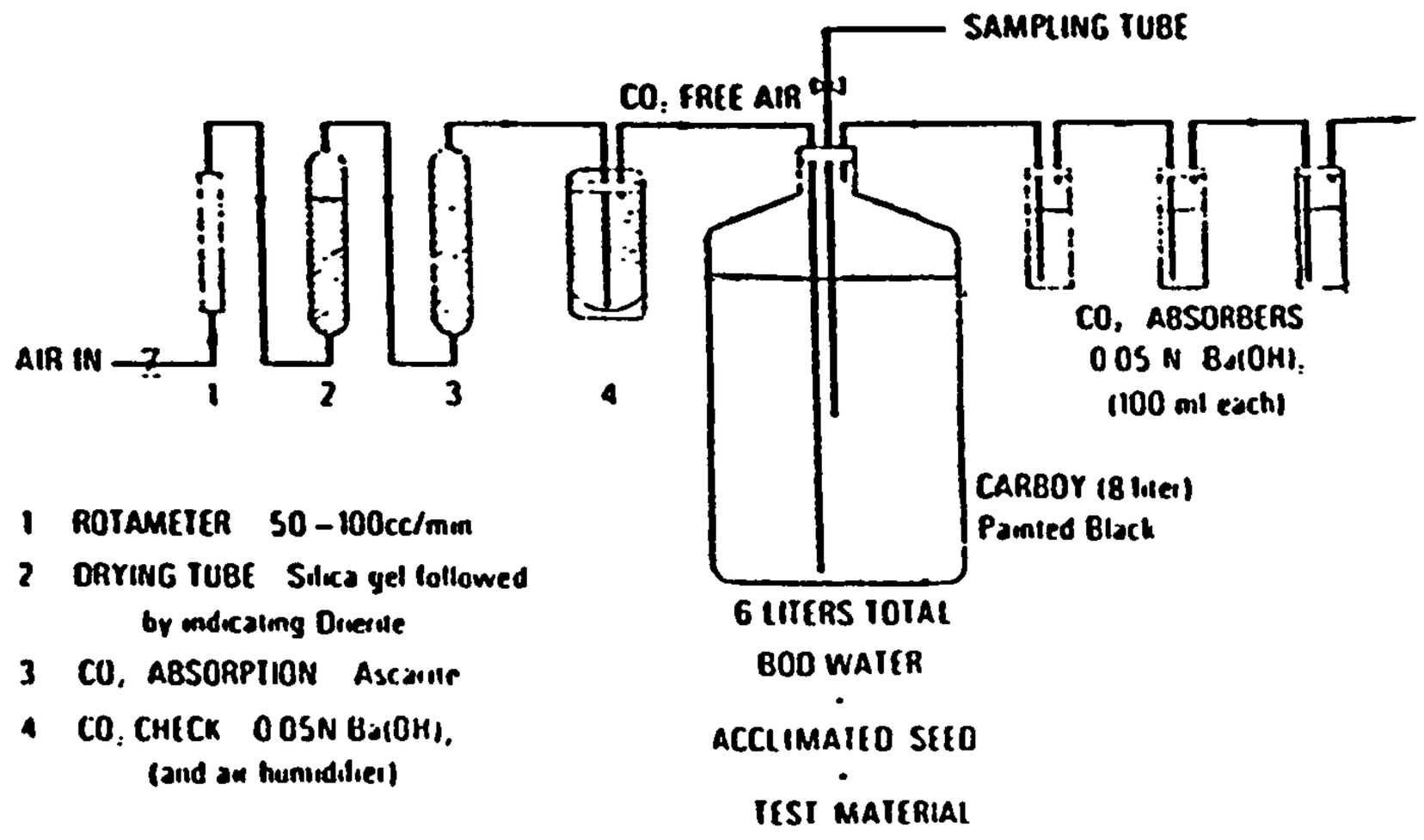

Figure 21. Diagram of individual CO_2 production test unit.

Periodically, the proximal absorber was removed for titration. The remaining two absorbers were each moved one place closer to the test vessel and a new absorber placed at the distal end of the series. A positive control was tested along with each group of test materials as a means of measuring the variability of the tests, which may have been attributable to the "strength" of the raw sewage seed. CO_2 production from dextrose exceeded 80% of theoretical and the tests were continued for 26 days. The 26

day time period was based mainly on the loss of usefulness of the positive control due to a plateau of CO_2 production.

A wide range of nonionic surfactants were screened for biodegradability by this method and the results, in general, were in agreement with those of earlier investigators[50, 79-82]. Ethylene oxide chain length did effect biodegradability and the role of the polyethoxy chain was studied by testing a series of polyethylene glycols (PEG) ranging in molecular weight from 300 - 4000 (Figure 22). As can be seen, PEG 300, 400, and 600 appear to be readily biodegradable, yielding CO_2 production figures of 87, 80, and 90% of theoretical, respectively. PEG's of higher molecular weight appear to be resistant to biodegradation.

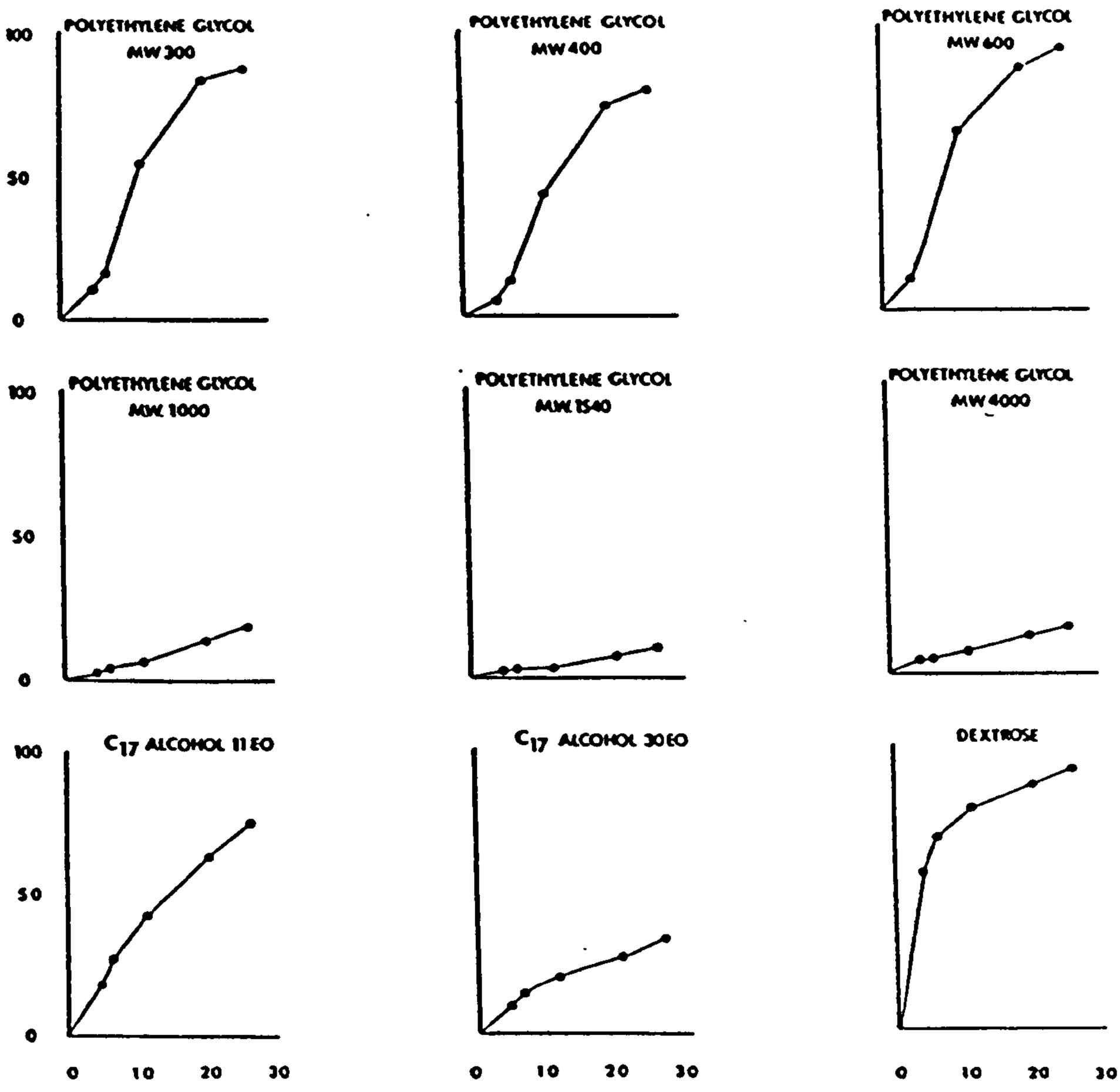

Figure 22. Biodegradation - CO_2 production, effect of molecular weight on biodegradability of polyethylene glycols[78].

Comparisons of the effect of hydrophobe structure on biodegradability were made between primary and secondary alcohol polyethoxylates, straight and partially branched alkyl nonionics and alkylphenol polyethoxylates (Figure 23). From

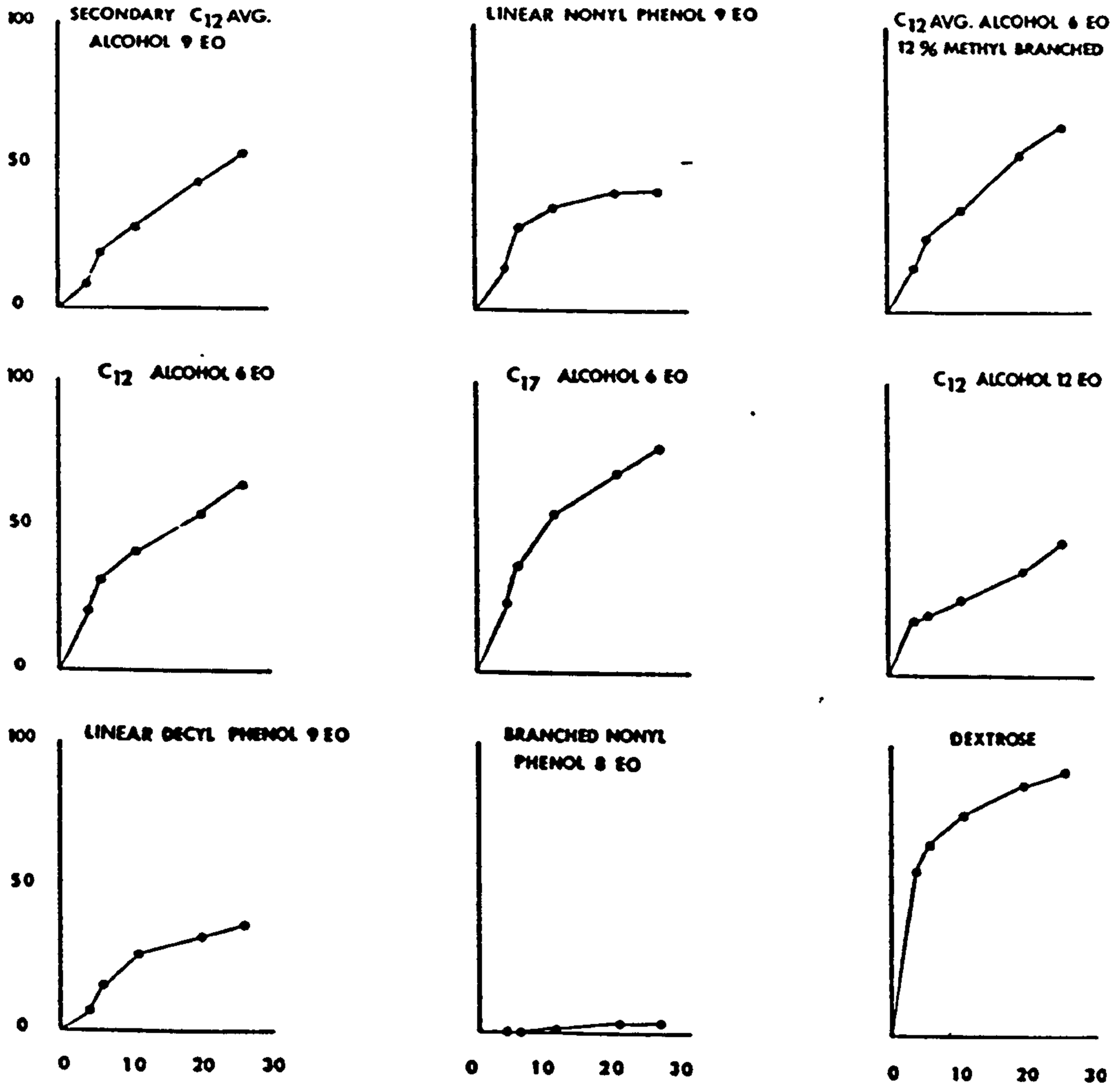

Figure 23. Biodegradation - CO_2 production: comparison of biodegradability of poyethoxylated nonionic surfactants of various hydrophobe structures.

these results it was found that primary alcohol polyethoxylates were slightly more degradable than secondary alcohol polyethoxylates and slight (12%) methyl branching in a primary alcohol polyethoxylate appeared to have no significant effect. The presence of a phenolic group in the molecule, however, appeared to reduce the biodegradability of the nonionics tested (linear nonyl and linear decyl phenol

polyethoxylates), and the presence of a branched alkyl chain in a nonyl phenol polyethoxylate reduced biodegradability even further.

Shake flask results from more recent studies[66] showed the much slower rate at which a branched octylphenol polyethoxylate evolved CO_2 compared to an essentially linear AE containing an alkyl chain in the C_{12-15} range and approximately the same average ethoxylate chain length as the octylphenol polyethoxylate (Figure 24). Disappearance of organic carbon from the aqueous medium also showed the much slower rate at which the APE biodegraded. Gledhill[67] found less than 20% biodegradation of a branched octylphenol polyethoxylate as measured by CO_2 evolution in a modified shake flask test.

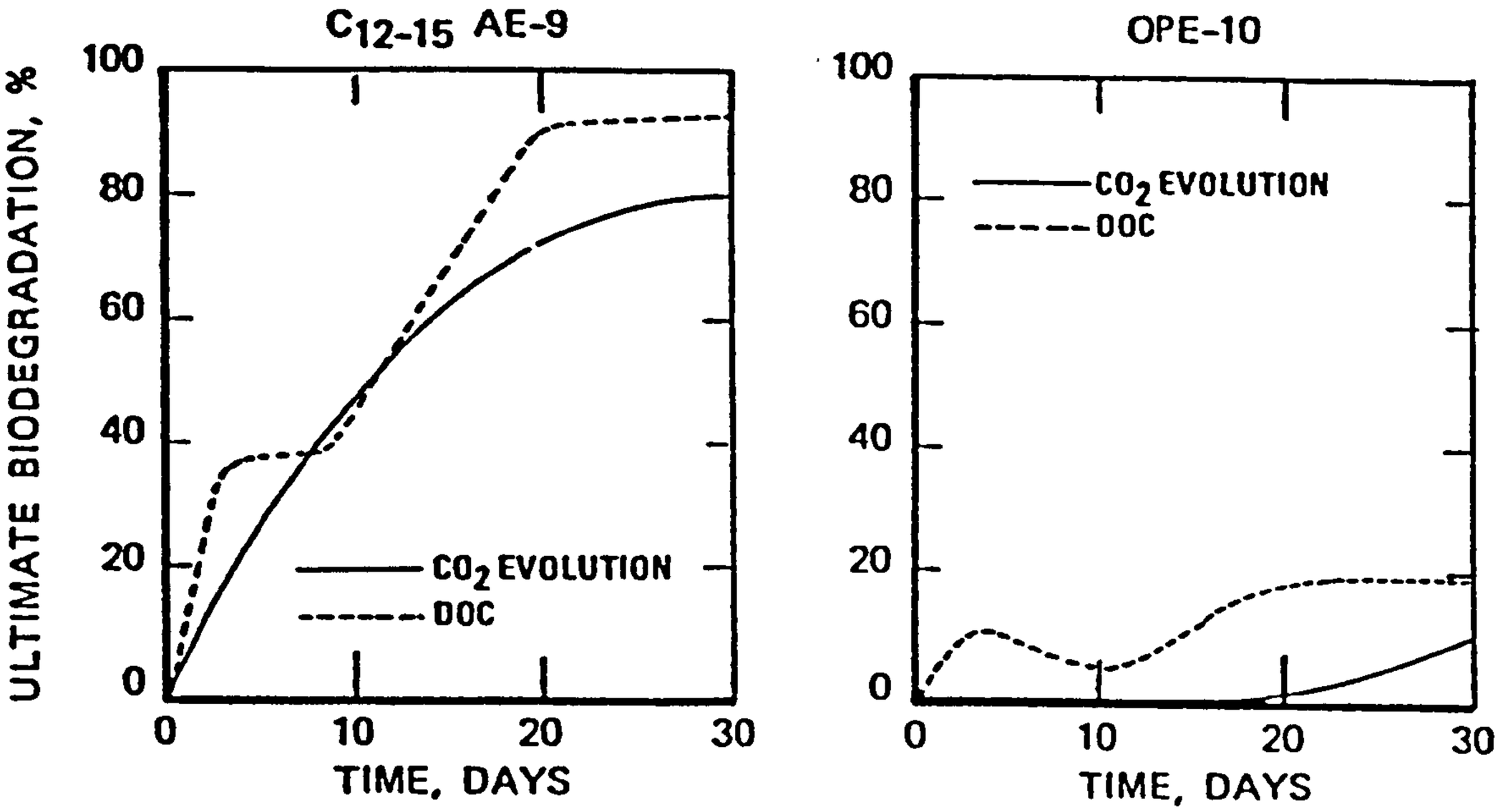

Figure 24. Ultimate biodegradation determined by CO_2 evolution and by dissolved organic carbon (DOC)[66].

1.8.3 Field Tests.

Studies in a trickling filter plant have only indicated 20% removal of APE compared to greater than 90% removal for AE under cool water conditions as measured by primary biodegradation criteria[76]. Although the removal of APE after the onset of the warmer

summer months to ca. 80%, it never consistently reached the greater than 90% levels of AE.

Abram *et al*[83] reported greater than 95% primary biodegradation of AE which had been fed into a trickling filter plant at 5 - 10°C at concentration levels of 10 and 25mg/l.

The results of a field test on the effect of an AE on the operation of an activated sludge treatment plant in Ohio recently have been reported[84,85]. In this test the plant effluent was dosed with 10mg/l of the AE under both summer and winter conditions. Plant performance was followed by sampling specific locations throughout the treatment facility before, during and after dosing. Results of analyses for such parameters as surfactant concentration, 5 - day BOD, COD, sludge volume index and sludge retention time indicated that the AE was 90% removed and its presence had no adverse effects on plant performance or on aquatic life in a receiving stream.

1.8.4 Effect of Structure on Biodegradability.

The effect of up to 55% branching on the alkyl chain apparently has a marginal negative effect on the biodegradability rates of primary alcohol polyethoxylates[66] at 25°C. A slight decrease has been observed in the initial rate of CO_2 evolution for a 55% 2-alkyl branched primary AE_9 compared to 25% 2-alkyl branched AE_9 (Figure 26). A 100% linear secondary AE having approximately the same alkyl and polyethylene oxide chain lengths degraded at a slightly slower rate than the branched primary AE products already discussed.

The effect of the polyethylene oxide chain length on the biodegradation of linear primary alcohol polyethoxylates containing an average of 7, 18, 30 and 100 ethylene oxide units/mole has been evaluated[86] using CO_2 evolution, DOC, CTAS and HBr - GC criteria. The results of this study indicate a significantly lower level of biodegradation only for the AE containing 100 EO units/mole.

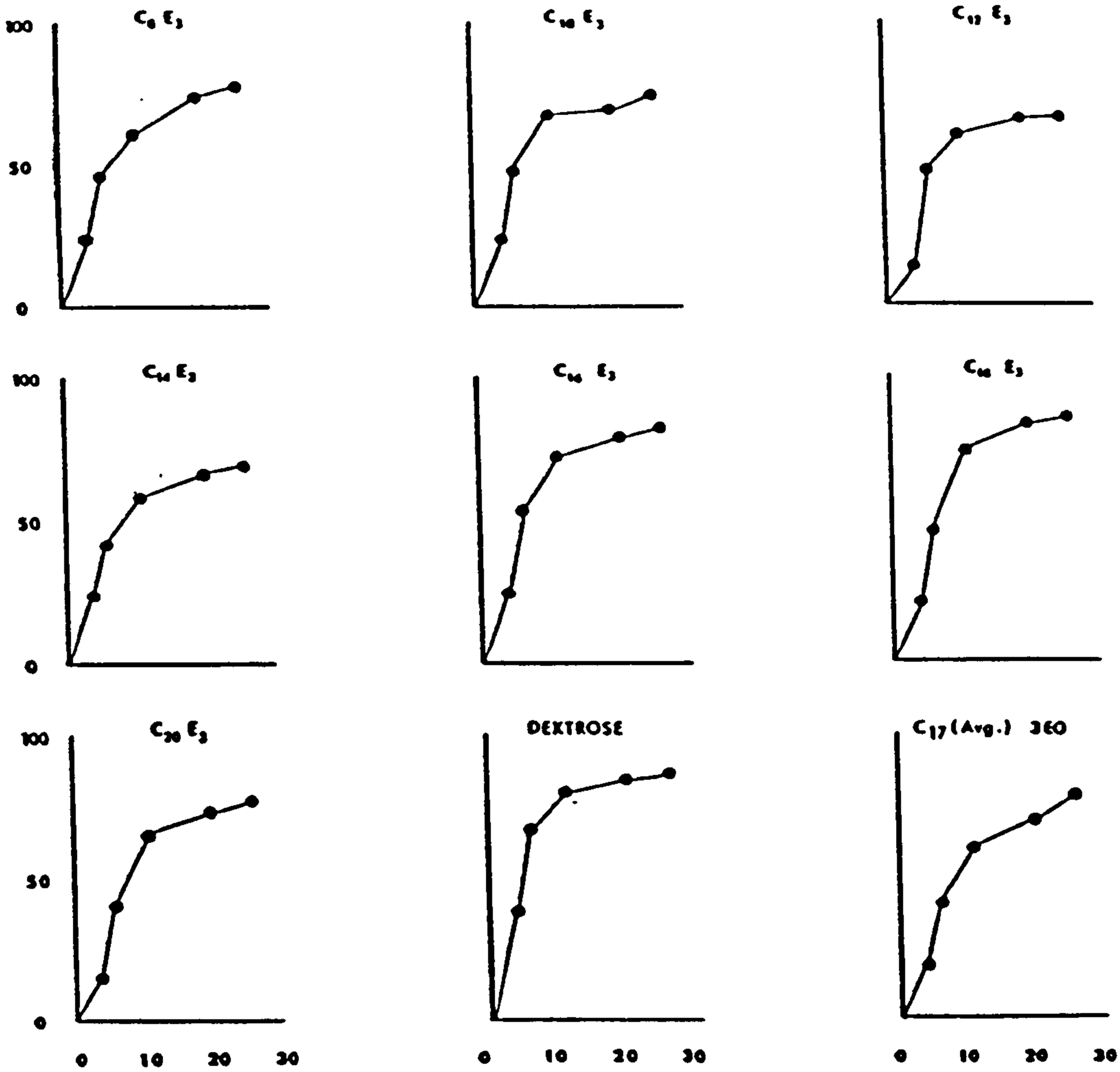

Figure 25. Effects of alkyl structure on AE ultimate biodegradation by CO_2 evolution[78].

Replacing the branched nonyl chain found in most commercial APE with a linear nonyl chain has been reported to increase the biodegradation rate to some extent[78]. The branched APE had reached a 5% theoretical yield of CO_2 evolution after 26 days in a laboratory activated sludge system whereas the linear APE had attained a 40% yield. AE having essentially equivalent ethylene oxide content produced greater than 65% CO_2 yields in equivalent tests. These results indicate that both the branching and the aromatic structure of APE decrease its biodegradation rates.

1.8.5 Effect of Temperature.

Mann and Reid[76] have reported that low winter temperatures have a significant negative effect on the biodegradation of branched APE but little effect on 2% branched primary AE. A primary biodegradation study[87] using commercial samples of 55% branched AE and a branched APE showed both of these products biodegraded slower at 3 - 4°C than at 20 - 23°C. However, the negative effect of temperature was much more pronounced for the APE. It would appear from these results that low temperatures begin to exert a pronounced negative biodegradation effect for AE which have branching in excess of 25%.

1.8.6 Aquatic Toxicity.

Commercial surfactants used commonly in the home and in industrial applications exhibit little toxicity to mammals but are relatively toxic to fish[88]. Since these surfactants generally pass through sewage treatment plants before entering receiving waters containing aquatic life, the ability of the surfactants to be rendered harmless as a result of treatment is of major concern.

The effect of AE on a sewage treatment plant and the resulting loss of aquatic toxicity[85] have been mentioned. In an aquatic toxicity study comparing AE with APE under unacclimated river die away conditions, Reiff[77] reported that AE, at 20mg/l initial concentration, became nontoxic to rainbow trout (*Salmo gairdneri*) in 10 - 14 days. under these conditions the APE required ca. 70 days to be nontoxic to the trout. Loss of toxicity of both these surfactants was accomplished by decreasing response to analysis for primary biodegradation (BIAS). The intermediate products of AE and APE biodegradation were less toxic than their respective intact precursors.

1.9 Environmental Concerns for Nonionic Surfactants.

With increasing regulatory implementation of environmental legislation by the EEC and in the USA by the Environmental Pollution Agency (EPA), there is active interest in the environmental fate of nonionics. The surfactant industry through its technical committee, is expending considerable effort to ascertain the effect of surfactants on the environment. Areas being considered for further study include the following.

1.9.1 Monitoring of nonionics in sewage plant outfalls and receiving waters by primary biodegradation criteria such as CTAS.

A project to accomplish this study is currently under way and methodology is being developed for monitoring AE. A selective analytical method for AE is required in order to properly understand the primary biodegradation monitoring results.

1.9.2 Standardisation of Biodegradation Test Methodology.

The EPA has already suggested a test protocol in a guidance document issued in 1979. The EEC has recently issued a directive in which test methodology and a minimal standard of 80% primary biodegradability for nonionic surfactants has been stated.

1.9.3 Coupling of Biodegradation Tests to Aquatic Toxicity.

Few results based on testing nonionic surfactants by biodegradability/toxicity coupled tests have been reported. The effects on the intact surfactants and their biodegradation intermediates on aquatic organisms is an area of continuing study.

1.9.4 Effects of Surfactants on Amended Soils Derived from Waste Sludge.

Increasing amounts of waste sludge are being used for agricultural purposes. Data may be required on the effects of nonionics and their biodegradation products which are present in these sludges.

1.10 Conclusions

a) Branched alkyl-aryl polyethoxylates only degrade slowly.

b) Linear alkyl-aryl polyethoxylates degrade at a faster rate.

c) Acceptable rates of biodegradation are only just being established.

d) It is possible that the products of biodegradation mechanisms are themselves biodegradable, but in general the faster a compound degrades, the better.

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CHAPTER TWO

NONIONIC SURFACTANTS FROM NATURALLY OCCURRING PHENOLIC LIPIDS

-

2.2. Types of non-isoprenoid phenolic lipids

2.2.1 Background

In the last decade, increasing numbers of this class have been isolated from a wide variety of living sources. These lipids arise from a replenishable source rather than a fossil-derived one and a new dimension of interest has been raised in studies on their chemistry as a biological resource of environmental interest. The growing awareness of the limited life expectation of petroleum sources and of petrochemically derived intermediates has concentrated attention on these alternative renewable sources.

The non-isoprenoid phenolic lipids have dual aromatic and acyclic character and, partly as a consequence, have remained comparatively little known. They occur in several different botanical families, notably *Anacardiaceae*, and exist in tropical, sub-tropical and temperate climates in certain trees, shrubs, small plants as well as in some bacterial sources. As benzenoid derivatives they are conveniently grouped, for chemical purposes, into the classes of phenolic acids, polyhydric, dihydric and monohydric phenols.

The cashew tree, *Anacardium occidentale* is the most widely distributed source of the phenolic lipid (cashew nut-shell liquid, CNSL), which is a by-product of industrial processing primarily to produce the cashew kernel[3,4]. Although originally indigenous to Brazil, the cashew tree is now grown in many parts of the equatorial and sub-equatorial regions including countries such as Thailand and Indonesia. Wide aspects of the cultivation of the cashew have been discussed[5]. The unusual nature of the raw cashew-nut, an external seed of the cashew apple, is shown in Figure 1.

The seed is kidney-shaped and approximately 2 - 3cm in length. The shell comprises of some 50% of the weight of the raw nut, the kernel represents 25% and the remaining 25% consists of natural cashew nut-shell liquid. This is a reddish-brown liquid

composed of the anacardic acids (1), (5 - 7), the cardols (8 - 10 where R = H) and a smaller

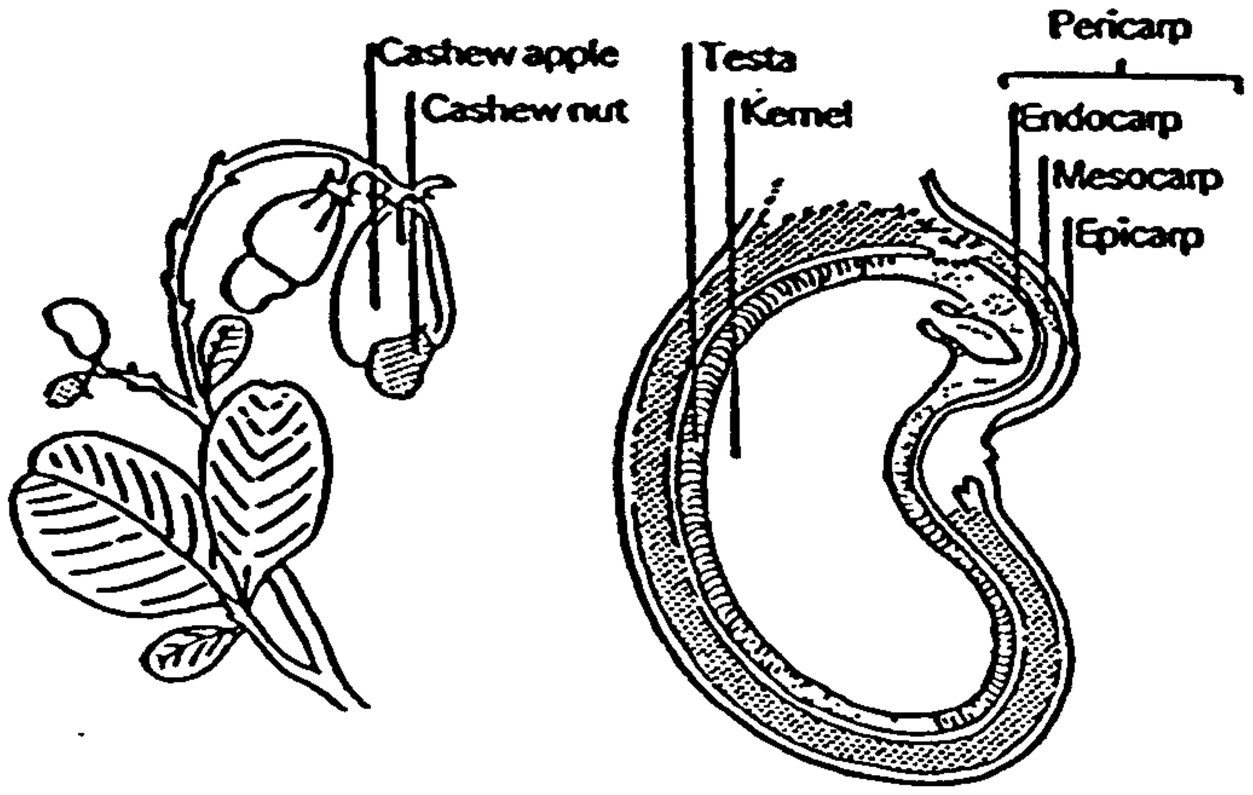
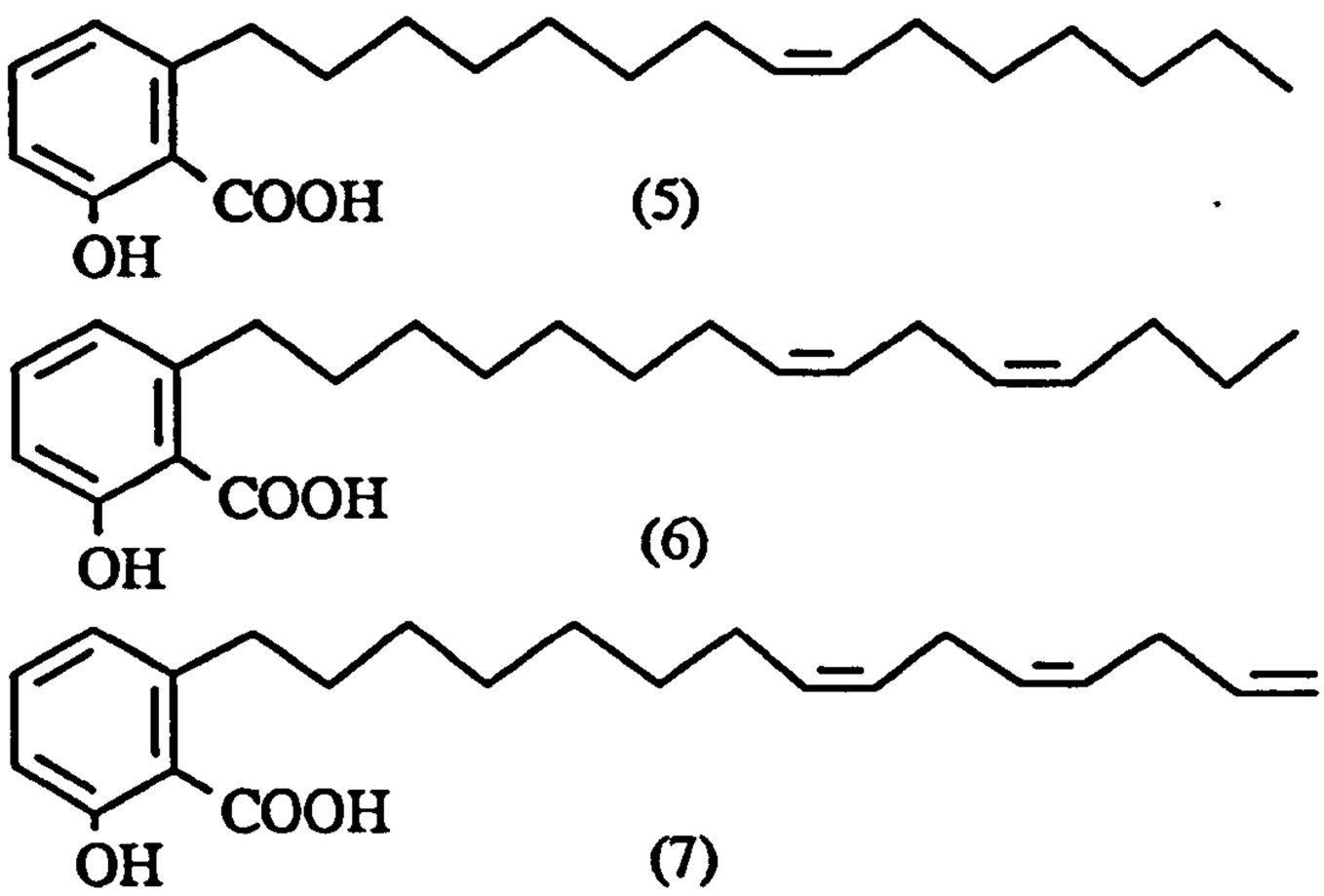
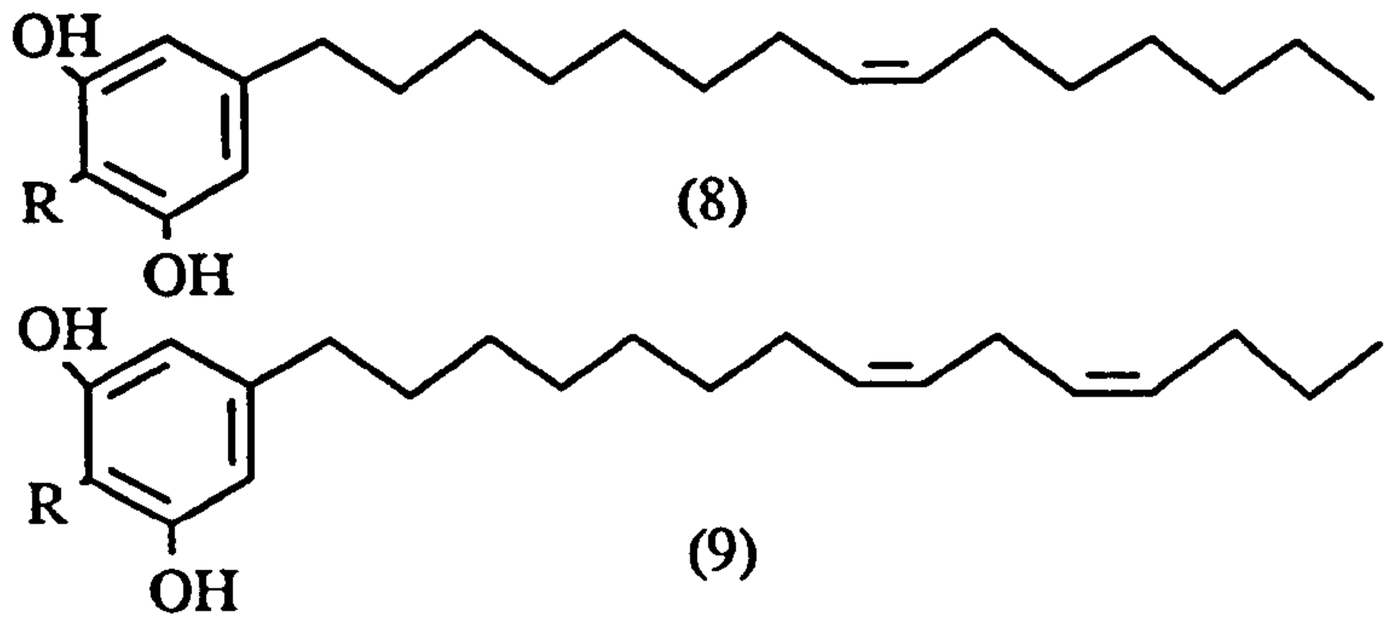
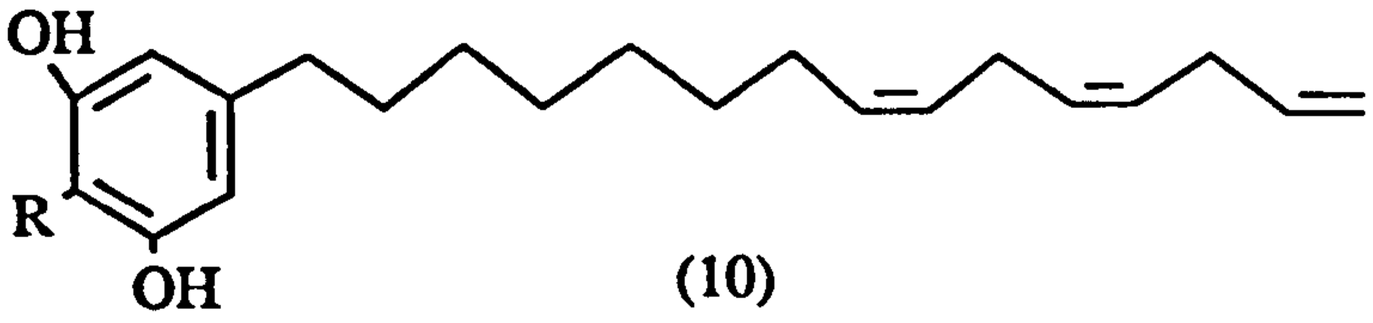


Figure 1. Cashew nut



portion of the 2-methylcardols (8 - 10 where R = Me), cardanols (11 - 14) and a little polymeric material.





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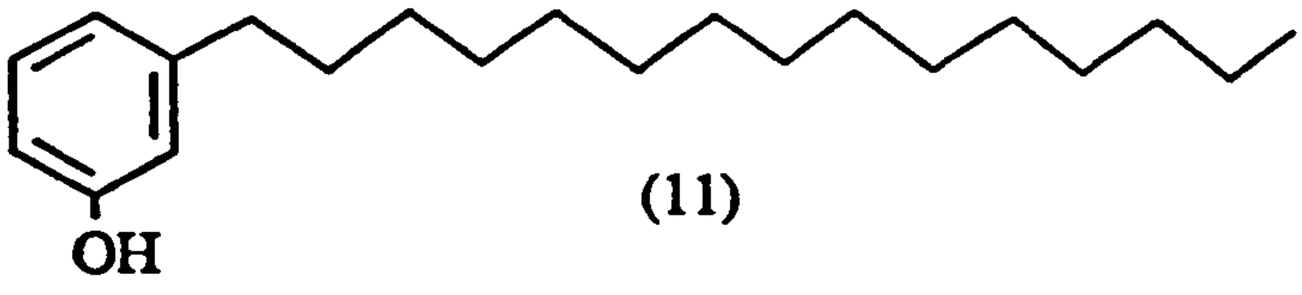

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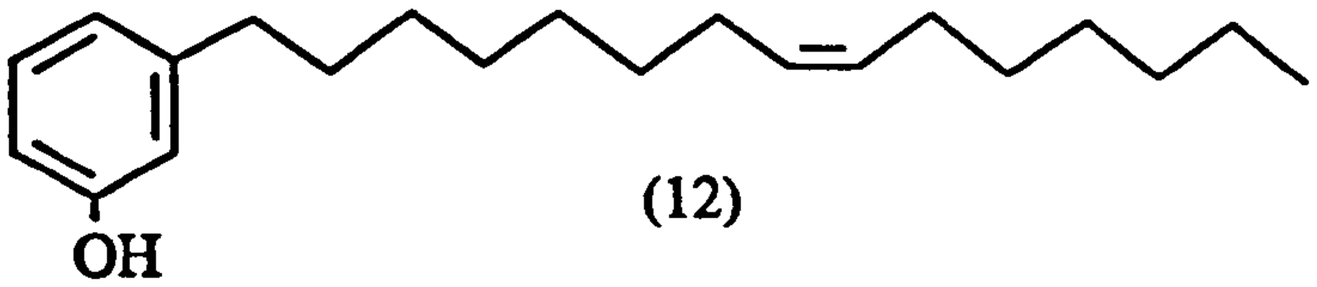

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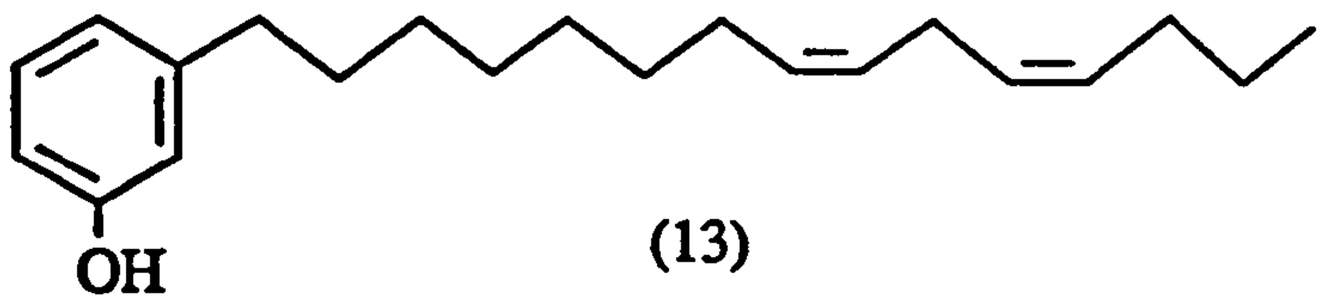

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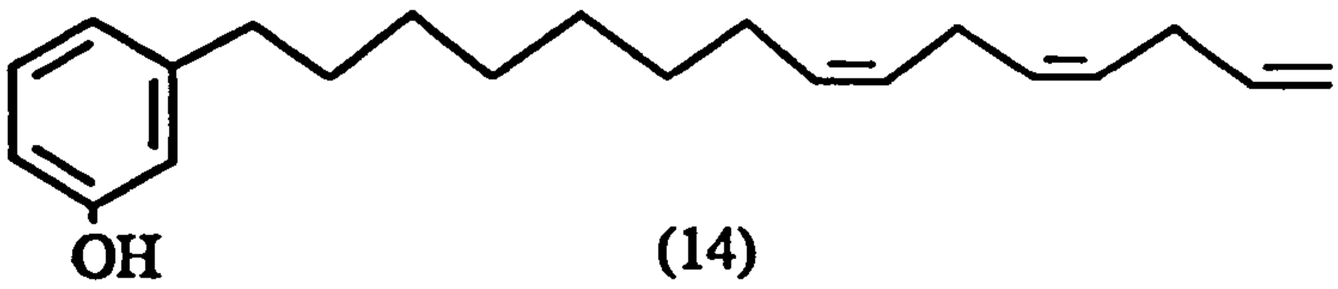

(14)

The kernel itself contains a rich source of protein and a glyceride oil chiefly containing the fatty palmitic and oleic acids. In world production the quantity of cashew produced is comparable to that of almond and hazel nuts. Table 1 gives projected estimates of the world production of cashew[5].

Year	World	Mozambique	Tanzania	India	Brazil	Kenya	Madagascar
1955	125	54	18	47	2	2	-
1960	160	63	37	50	2	5	-
1955	280	119	65	80	4	8	-
1970	370	115	110	95	31	13	1
1975	470	166	115	110	44	22	5
1980	535	145	122	130	84	30	10
1985	750	215	135	130	207	35	15
1990	910	235	222	130	229	40	20
1995	1,000	255	240	140	260	45	25
2000	1,120	300	250	150	284	50	30
2005	1,260	350	280	175	290	50	40

Table 1. Estimated world production of raw cashew nuts (1,000 tons) excluding home consumption.

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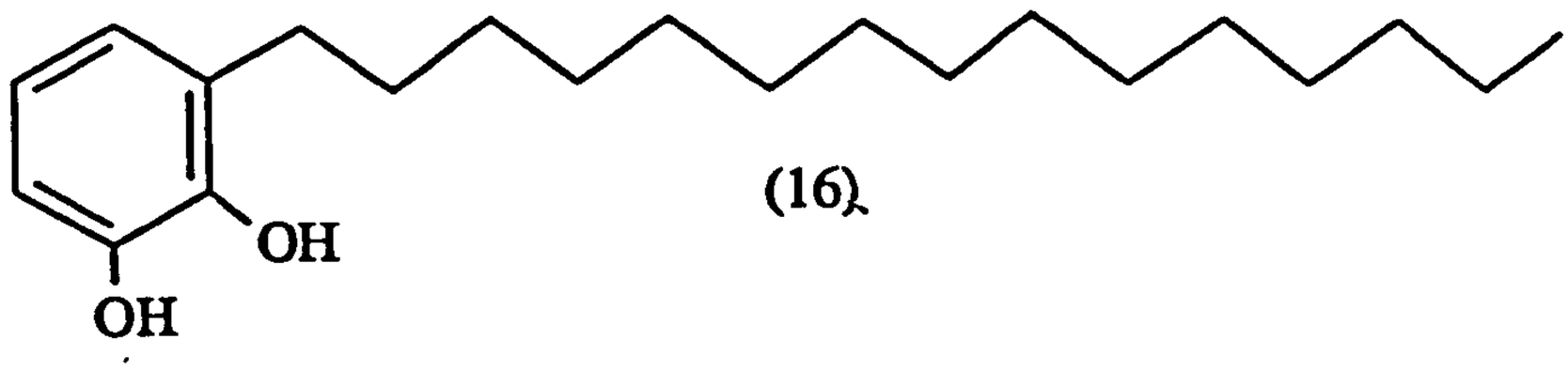
Among the catechols, the urushiols (eg. **16**) in *Rhus Vernicifera* and *Rhus toxicodendron*



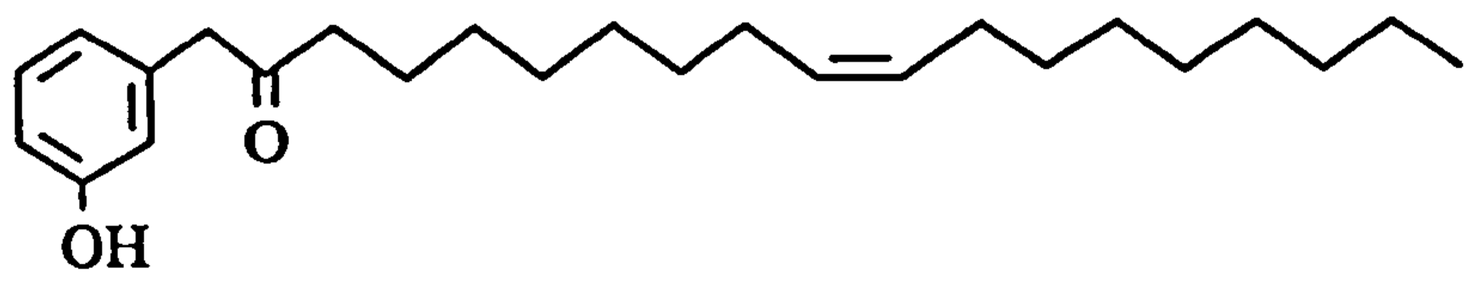
(16)

have been well studied in the extensive research of Majima[9]. Japanese lac from *Rhus vernicifera* is probably the oldest known cultivated source of phenolic lipid, having been used from the 6th century. The structures and composition of lacs from other countries have been less well studied. Alkyl catechols with a terminal phenyl group in the side chain have been isolated from the Burmese lac tree[10].

Highly unsaturated alkyl resorcinols and phloroglucinols have been found in certain algae[11, 12]. The latter is one of the few examples of an alternative biosynthetic pathway resulting in 2, 4, 6-hydroxy substitution rather than the usual 3- or 1,3-hydroxy pattern. Comparatively few alkylhydroquinones have so far been located in natural sources.

2.2.4 Monohydric Phenols

All the members are 3-alkenylphenols sometimes, as with campnospermanol (17), containing a keto function in the side chain. The highly unsaturated substance, flexirubin (18) is a phenolic lipid and appears to have the ability to cause allergenic reactions and to have vesicant action[13 - 15], often very specifically. It was partly this action which led to Australian research into the structure of certain *Greviaali* species and American research with technical cashew nut-shell liquid. Probably the effect is more pronounced with the urushiols, the poisonous constituent of the North American ground ivy. Remarkably, the historical use of Japanese lacquer since the sixth century does not seem to have been impeded by the physiological action of the raw material.



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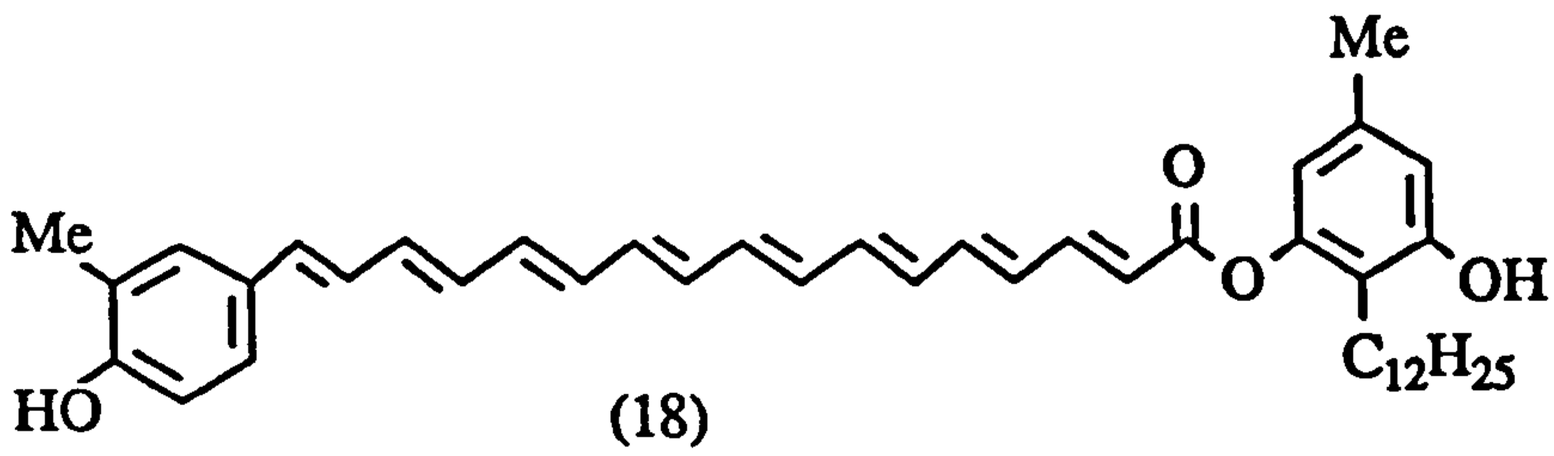


(18)

2.3 Extraction

2.3.1 Industrial Processes

The phenolic lipid *Anacardium occidentale* has been commercially exploited[16]. Technical cashew nut-shell liquid (CNSL) from industrial processing has been employed as a phenolic source for copolymerisation with formaldehyde. The polymer products as compounded are the basis for friction dusts, widely used throughout the world in vehicle brake and clutch linings[17].

The primary objective of cashew processing has been to recover the nutritious kernel, and the by-product cashew nut-shell liquid only later became of interest as a raw material for friction dusts; these exhibit superior properties to earlier materials which were based on composites of phenol/formaldehyde resins with highly unsaturated glyceride oils. Most CNSL in India and East Africa is now extracted (cracked) by an automatic process[3, 18] by the 'hot CNSL bath' process in which raw humidified cashew nuts are submerged in technical CNSL on a slowly-travelling conveyor belt before heating to 180 - 190°C. The scheme used is illustrated in Figure 2.

The anacardic acids in the natural CNSL of the raw nut shell are decarboxylated and the cardanol liberated, through the bursting of the outer shell, supplements the hot CNSL in the bath and the excess flows continuously out of the system. The spent processed

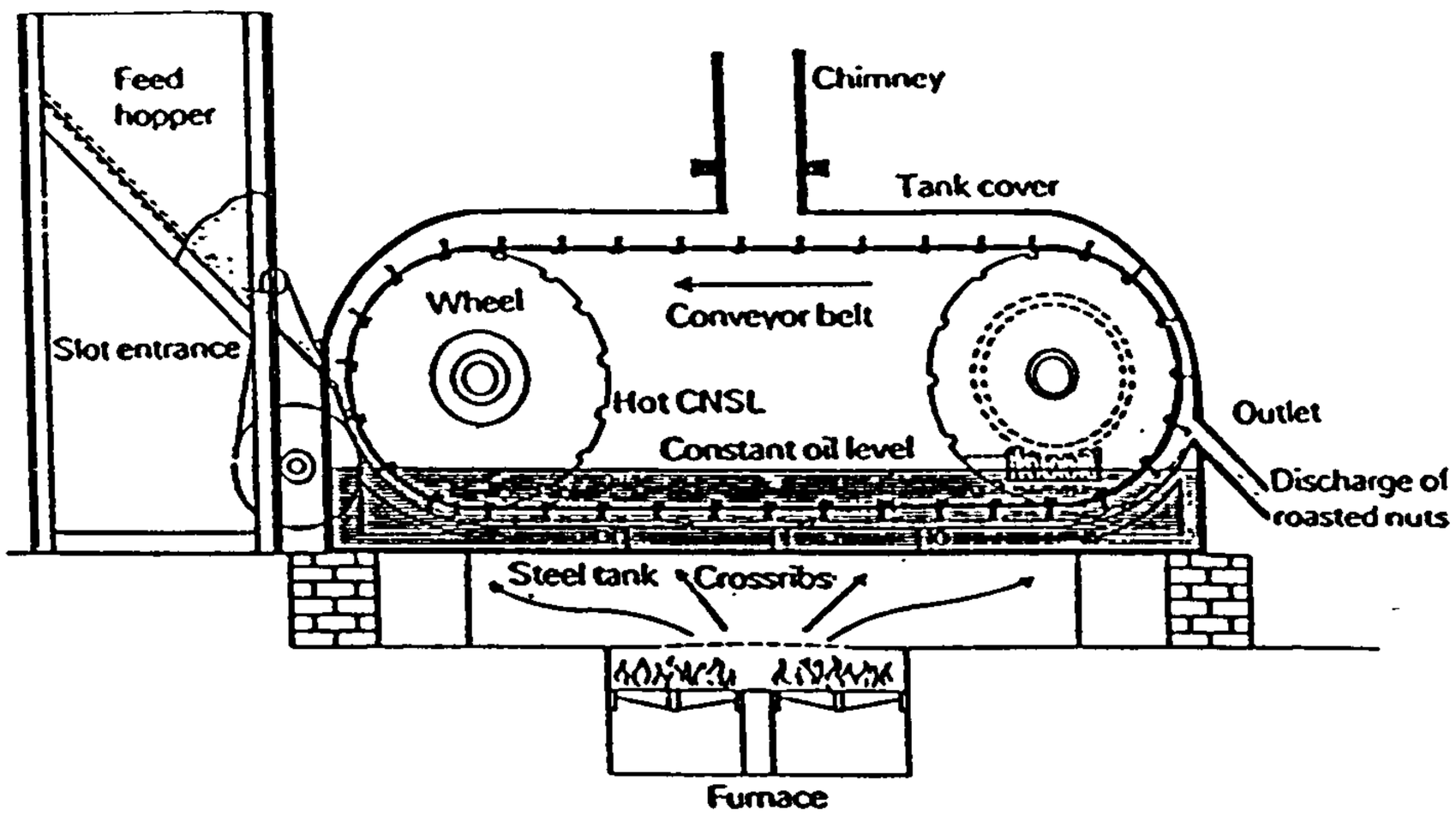


Figure 2. An early hot CNSL plant for processing cashews.

nuts with the inner shell still intact also pass out of this system and, after removal of the remaining CNSL by centrifugation and adsorption, are shelled.

In recent years, attention has been turned to cold cutting of the cashews[19]. By solvent extraction of the cominuted shell and catalytic decarboxylation to avoid polymerisation[20], technical CNSL can be recovered in an overall yield of 25% compared with approximately only 10% by the hot oil bath process.

2.3.2 Separation of cashew phenols

Phenolic lipids have been separated from natural sources for compositional studies and structural determination either by solvent extraction or by chromatographic techniques. The individual component phenols of the major phenolic lipid (CNSL) from *Anacardium occidentale* have assumed some significance in certain chemical applications and detailed purification processes have been developed. Although for some industrial uses of the natural or technical product these operations may be superfluous and the mixture of phenols is quite satisfactory, for surface coatings and in some synthetic chemical applications the dark colour of technical CNSL is unacceptable. The dark colour is partly due to brown impurities dissolved from the outer shell case by the solvent action of the phenols during processing.

Molecular distillation with a multi-stage still provides the only method for the separation of the cardanol and cardol[21]. Conventional fractional distillation and spinning band techniques are ineffective since the prolonged heating involved merely results in extensive polymerisation and poor throughput. Using wide-bore equipment and short-path stills, excellent recoveries may be made by high vacuum distillation, giving products substantially free of colour although the extent of separation of the two major phenols is by no means complete.

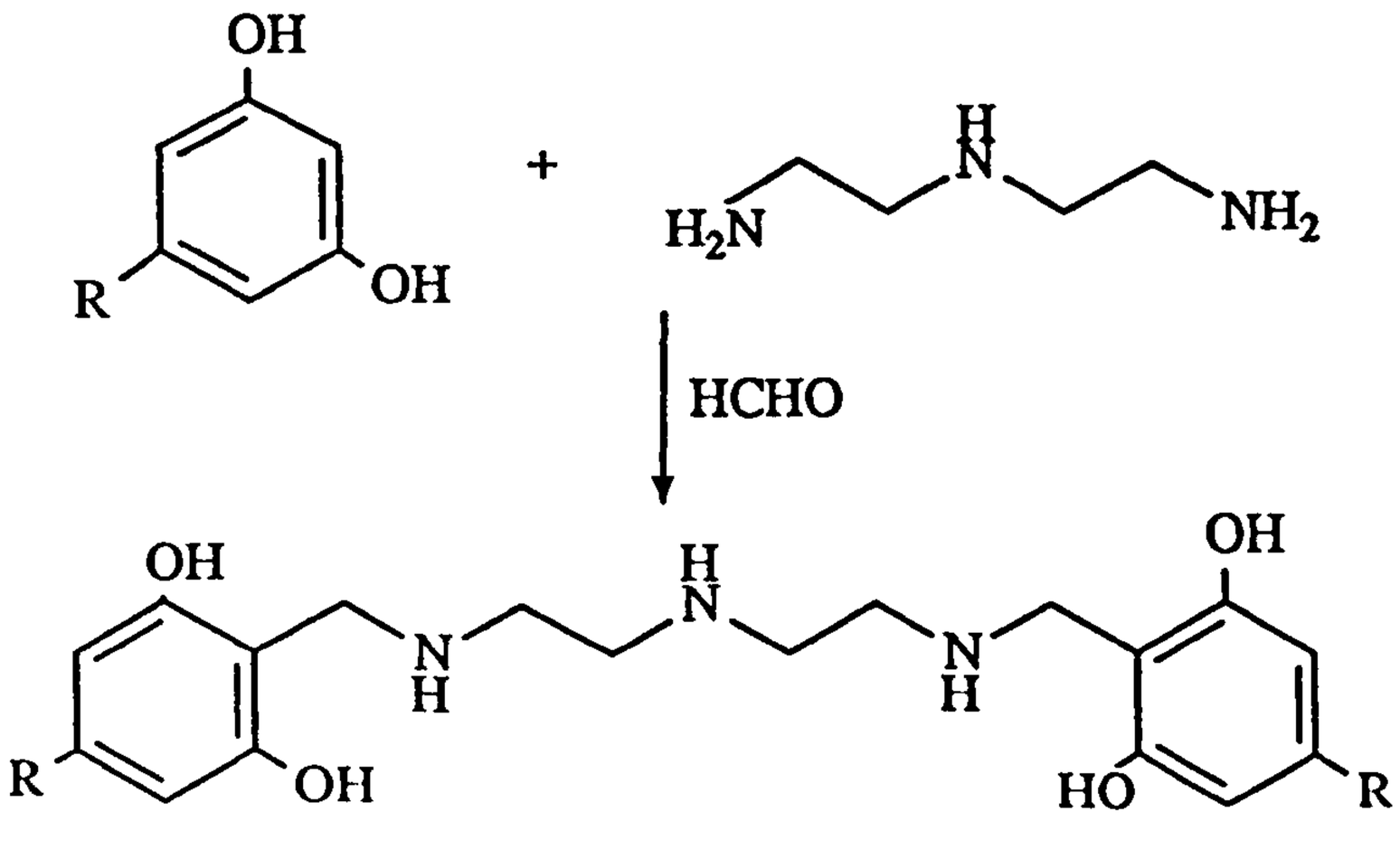
2.3.3 Chemical purification/vacuum distillation techniques

For the separation of cardanol from technical CNSL, without resorting to molecular distillation, it was found necessary to use a combined chemical purification/vacuum distillation technique[22].

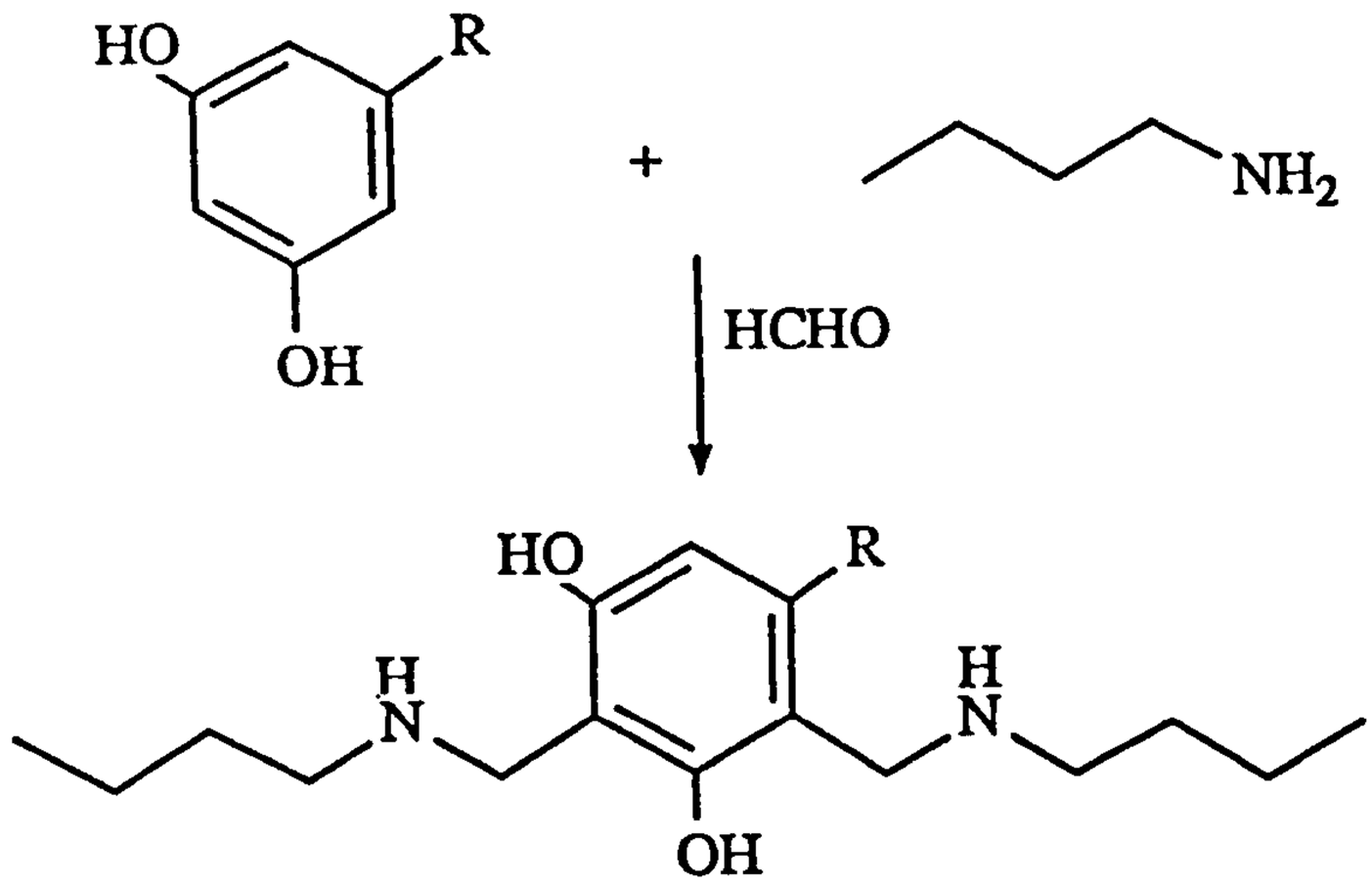
This technique takes into account the greater acidity of the cardols by subjecting the technical CNSL to a Mannich type reaction. The Mannich reaction involves the reaction of the substrate with formaldehyde and diethylene triamine (or *n*-butylamine) in methanolic solution. Under these conditions, the dihydric phenol, cardol, forms a high molecular weight solid polymer while the cardanol remains substantially unreacted, Separation by decanting gives good material (analysed by HPLC[23], Table 2).

While it is uncertain what reactions occur, the following reactions have been postulated[22].

With diethylene triamine



With *n*-butylamine



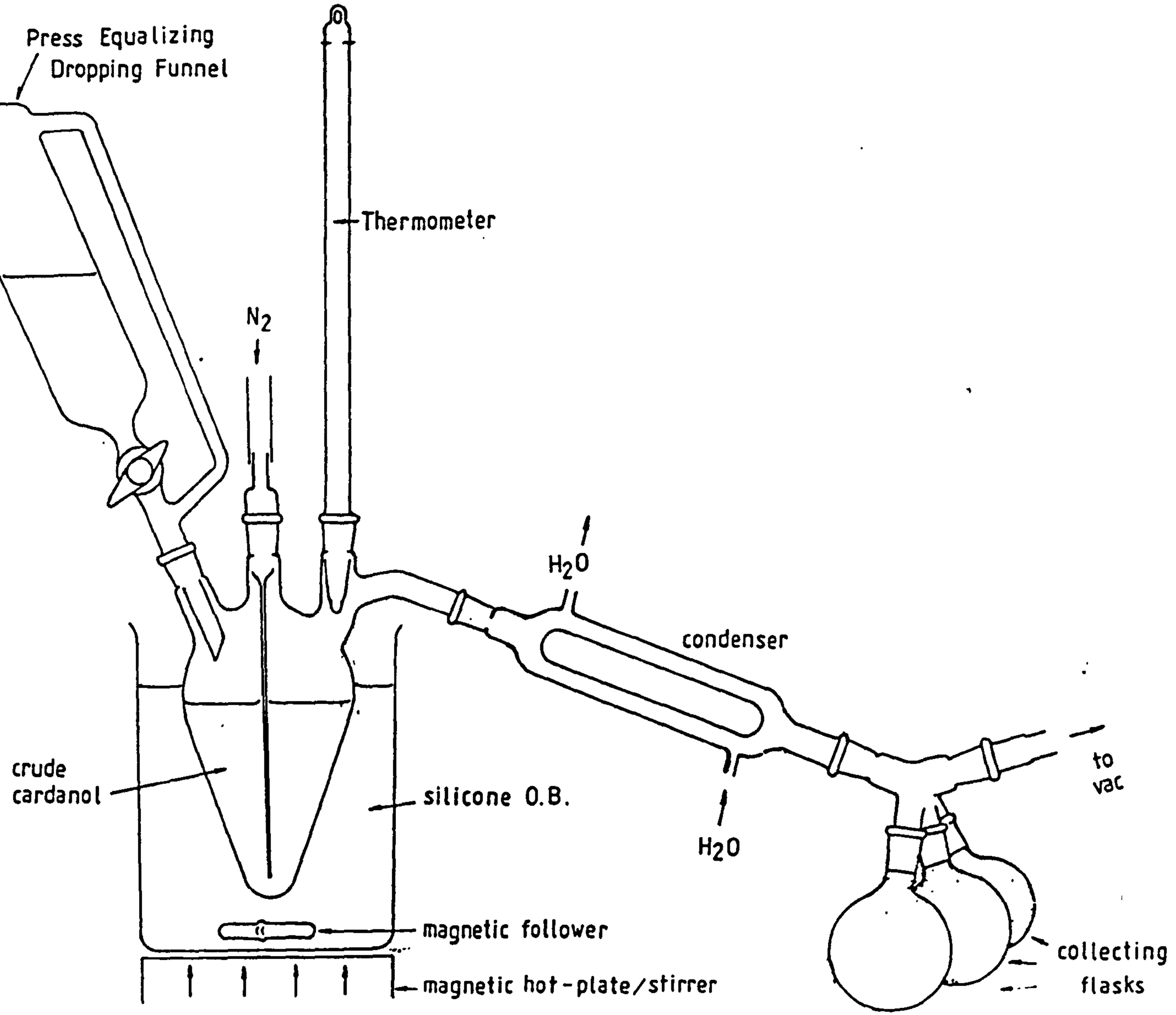
Where R = $C_{15}H_{31-n}$ (n = 0, 2, 4, 6)

A reduction in the amount of the formaldehyde solution with diethylene triamine used in the reaction has a negative effect, *ie*, the phase separation is not as distinct and the amount of cardol removed is lowered (Table 2). Therefore, any reduction in the 1.2 molar excess of formaldehyde to CNSL decreases both the purity and yield of crude cardanol obtained.

	Formaldehyde: CNSL (Yield %)		
Component	**1.2:1 (58%)**	**0.6:1 (50%)**	**0.3:1 (30%)**
Cardol	1.02%	3.21%	4.63%
Cardanol	76.51%	76.32%	74.97%
Polymer	21.47%	20.46%	19.72%

Table 2 Showing how the variation of molar ratio of formaldehyde:CNSL effects the yield and purity of crude cardanol.

After recovery of solvents the crude cardanol was vacuum distilled using the apparatus shown in Figure 3. The crude cardanol was heated for as short as time as possible to


Press Equalizing
Dropping Funnel
Thermometer
N_2
H_2O
condenser
crude
cardanol
silicone O.B.
H_2O
to
vac
magnetic follower
magnetic hot-plate/stirrer
collecting
flasks

2.3.4 Phase Separation

It was of interest to look at a simple method of purification whereby both cardanol and cardol could be isolated. By using a phase separation technique, cardanol was separated from cardol and 2-methylcardol, both of which were also recovered. The technique involves the use of a two phase system, namely, a petroleum ether phase and a diol phase. A number of diols were tested for their extractability of technical CNSL and the petroleum ether phase was analysed by HPLC (Table 3) from each diol. In theory, the dihydric phenols should remain in the lower diol phase and the monohydric phenol, cardanol, should be present in the petrol phase.

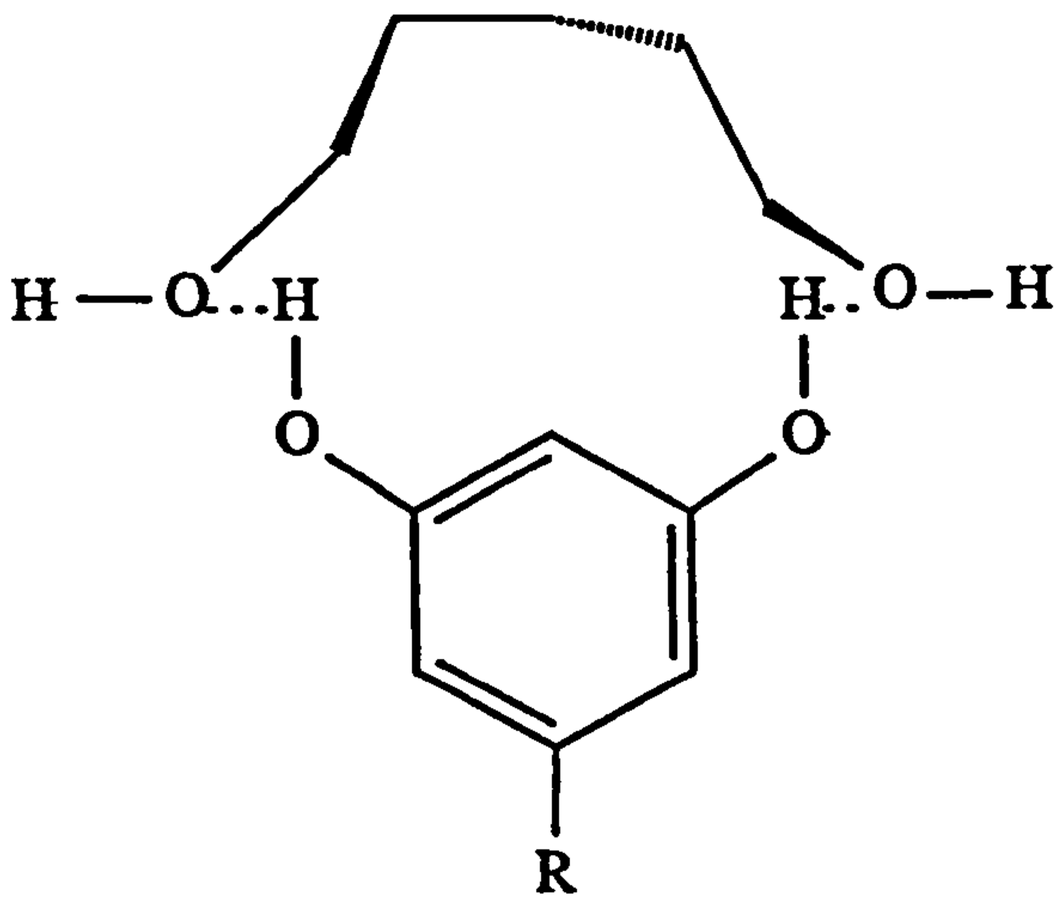
Diol	**wt% in petrol phase**	**%cardol**	**%cardanol**	**%polymer**
Ethane-1,2-diol	95%	3.68	69.33	26.99
Propane-1,2-diol	90%	1.40	65.84	30.80
Propane-1,3-diol	89%	2.13	72.47	24.51
Butane-2,3-diol	54%	1.07	68.86	30.38
Butane-1,3-diol	46%	0.82	64.38	33.79
Butane-1,4-diol	84%	0.65	70.24	29.26
Pentane-1,5-diol	93%	0.11	56.91	41.97
Diethylene glycol	41%	3.64	66.83	29.52
Polyethylene-glycol	7%	0.85	58.4	37.08

Table 4 HPLC analysis of petrol phase upon extraction with various aliphatic diols.

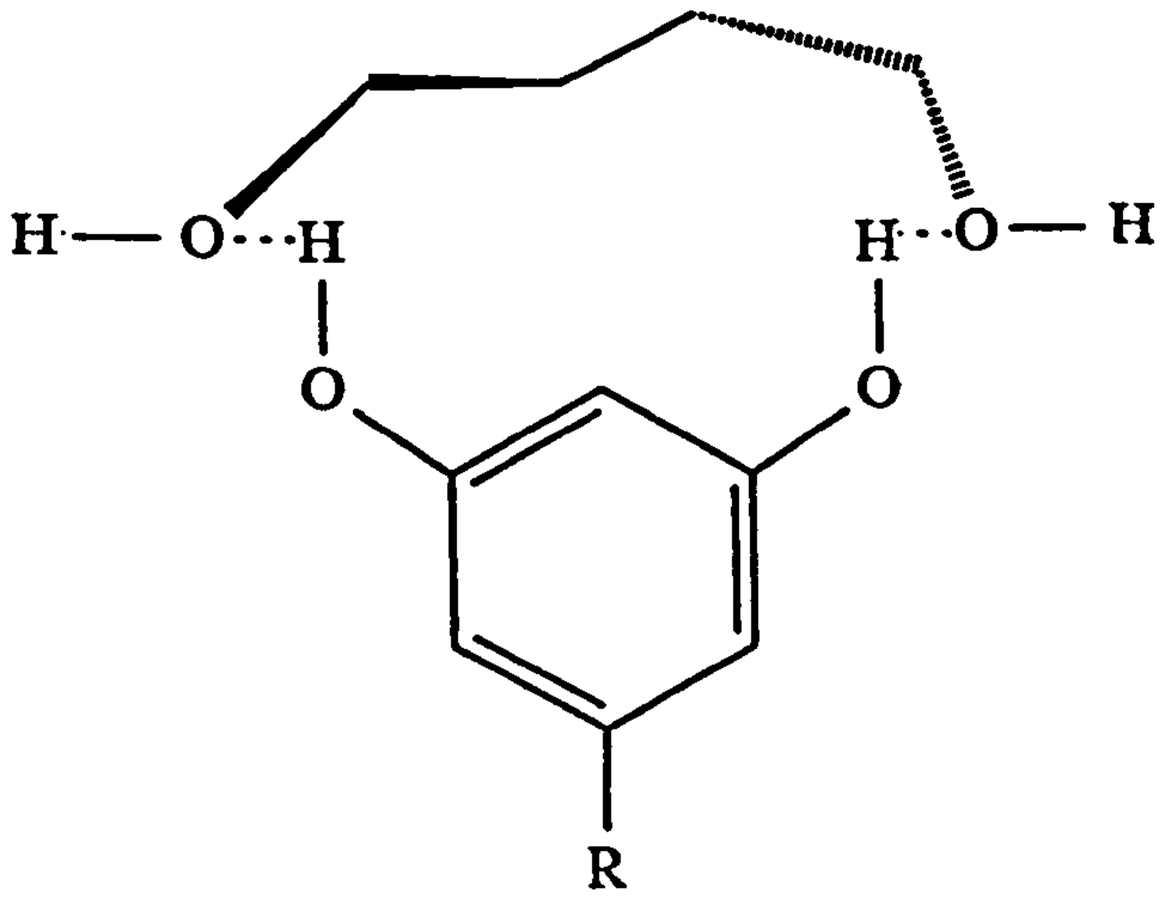
It can be seen from these analyses that the best diol, in terms of retention of the dihydric phenols, is pentane-1,5-diol. But the composition is about 50:50 cardanol:polymer, so in effect there is only 53% recovered cardanol, while for butane-1,4-diol there is approximately 83% recoverable cardanol in the petrol phase. This

appears to be a very simple method for the separation of cardanol, and as both solvents and dihydric phenols can be recovered by vacuum distillation this is a very efficient technique.

One possible reason why these diols are selective for the dihydric phenols is that a spacing of 4 or 5 carbon units between the two hydroxyl groups is the optimum for the required hydrogen bonding of both hydroxy groups of the phenol with both hydroxy groups of the diol. Longer chain diols were not used due to the fact that they are solids at room temperature.



Proposed binding of pentane-1,5-diol with a 1,3-dihydric phenol



Proposed binding of butane-1,4-diol with a 1,3-dihydric phenol.

2.3.5 Isolation of the Anacardic Acids from Natural CNSL

The anacardic acids were required as starting materials for synthetic work and were recovered from the shells using the following technology.

In the two stage recovery of anacardic acids[1,5-7] from the raw shells of cashew nuts, much work has been carried out to obtain the best yield[20].

At the first stage (solvent extraction), prior to communition, the yields from intact half shells are considerably less than that from manually processed (crushed) shells due to extensive internal fracturing of the shell structure and greater penetration in the latter case. Static solvent extraction of macerated shells has been shown to give the same yield as Soxhlet extraction but the filtration stage was reported to be difficult and large volumes of solvent were required. Soxhlet solvent extraction or ultrasound/solvent extraction of manually processed shells at ambient temperature gave good yields and economy of solvent usage. Both have been shown to be much superior to mechanical agitation.

In this case the method using the ultrasound/solvent extraction method was used whereby the raw half shells were initially crushed coarsely using a commercial blender and extracted into carbon tetrachloride, filtered and further crushed and extracted to give a high overall yield of the natural CNSL (41%), which is considerably higher than that in the traditional recovery of technical CNSL by the hot oil bath industrial method. Previous work[25] has established that natural CNSL contains some polymeric material and so freshly crushed shells were used because some deterioration could be expected at the surface of half shells after storage.

The anacardic acids were precipitated as their lead salts with lead hydroxide in ethanolic solution. The lead anacardate was collected by filtration and the acid regenerated by acidification. This lead to good overall yields of the anacardic acid (58%), 24% of the raw shell weight.

2.3.6 Separation of cardol from Technical CNSL[26]

The practical separation of cardol from technical cashew nut-shell liquid was effected by liquid chromatography on silica gel, with solute/adsorbent ratios in the range 1:5 - 1:6.

The column was packed by slurrying the silica gel with a known volume of light petroleum and consolidation by light tapping during filling. Light petroleum, with the progressive addition of diethyl ether, was preferred to light petroleum/ethyl acetate mixtures and also aided solvent recovery by distillation. Approximately 50g of technical CNSL was loaded on to 250g of silica and the column was reused four times before its resolution started to be adversely affected. Fractions were collected after the first 200ml of solvent had eluted, and the fractions were monitored by TLC. The combined fractions were analysed by HPLC and the results are given in Table 5.

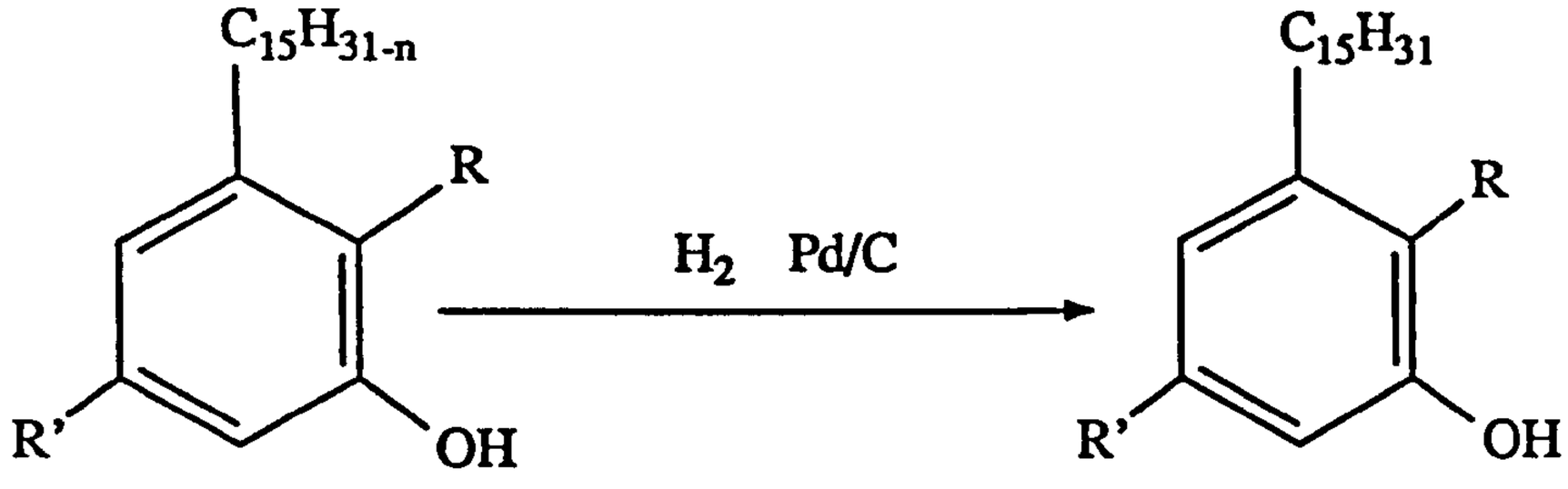
		Weight g (% yield)		
Column	**Amount loaded**	**Cardanol**	**Cardol**	**2-methyl cardol**
1	52.17	41.64 (80%)	6.14 (12%)	1.10 (2%)
2	52.39	40.16 (77%)	5.16 (10%)	1.06 (2%)
3	50.57	37.76 (75%)	4.5 (9%)	0.98 (2%)
4	51.27	37.85 (74%)	3.69 (7%)	0.85 (2%)
Total	206.36	157.45 (76%)	19.50(10%)	3.99 (2%)

Table 5 Results from the HPLC analysis of column chromatographed Technical CNSL.

As can be seen from these results, the amounts of pure material isolated decreases, this is due to the number of mixed fractions increasing with the number of times the column was used, *ie* the resolution deteriorates with each run.

2.3.7 Reduction of unsaturated phenolic lipids[27]

Cardanol, cardol and anacardic acid were reduced to 3-pentadecyl phenol (11) 3,5-dihydroxy pentadecyl benzene (19) and 2-hydroxy-6-pentadecyl



n = 0, 2, 4, 6

(1) R = CO_2H; R' = H

(11) R = R' = H

(19) R = H; R' = OH

benzoic acid (1) respectively. This was achieved by catalytic reduction with 5% Pd on charcoal and hydrogen at room temperature. The reactions are normally carried out at high pressure but it was found that low pressure apparatus gave equally high yields (98% - 99%) even though reaction times were slightly longer. This reduction can also be achieved chemically[28] with hydrazine hydrate in the presence of air by the di-imide method but the yields are slightly lower.

2.4 Synthesis of 3-Pentadecyl Phenol and Cardanol Polyethoxylates

The aims of this project were to synthesize, characterise and follow the biodegradation of various naturally occurring phenolic lipids as their polyethoxylated derivatives. It was decided that the best method for the characterisation of the polyethoxylates was by high performance liquid chromatography. In order to achieve this, several members of each series were required in order that solutions of the particular polyethoxylate could be 'spiked' with a single polyethoxylate of known chain length. Thus n, the average

number of ethylene oxide units, could be calculated for every synthetic alkylphenol polyethoxylate produced.

Several methods were attempted to synthesize these reference materials and these will be discussed here.

Preparation of phenol ethers is an important synthetic procedure for which a wide variety of methods have been developed[29]. Most of the commonly used methods involve alkylation of the parent phenol or the derived phenoxide ion, with the latter type of reaction being far more important. Use of dialkyl sulphate esters as alkylating agents often allows both generation and alkylation of the phenoxide ion to be carried out in aqueous solution and leads to excellent yields of the desired ethers. Use of the more readily accessible alkyl halides as alkylating agents, on the other hand, almost always necessitates operation in dry organic solvents. The phenoxide ion is generated by treatment of the phenol with a base such as sodium, sodium hydride or sodium amide in a solvent such as benzene, toluene or dioxane; alkylation with the appropriate alkyl halide is normally carried out in the same solvent. This method is again usually highly efficient, although some care must be exercised in the choice of solvent in order to avoid both O- and C- alkylated products[30,31].

There are few useful procedures available for the conversion of phenols into phenol ethers which do not necessitate the initial formation of the corresponding phenoxide ion. Direct alkylation with diazomethane is the most widely used of these[29] but is seldom the method of choice because of the hazardous nature of the reagent. Alkylation has also been accomplished with alkyl *ortho*-carbonate esters[32], using dialkyl oxalate esters[33] and by the treatment of phenols with alcohols in the presence of dicyclohexylcarbodiimide[34]. None of these methods is, however, very general with respect to the variety of alkyl groups which can be introduced into the alkyl-aryl ether, and they have usually been employed under special circumstances.

McKillop *et al*[35] reported the results of an investigation into the use of phase-transfer catalysis for the preparation of phenol ethers.

2.4.1 Phase Transfer Catalysis

Phase-transfer catalysis, also referred to as 'ion-pair partition' or 'extractive alkylation', is a technique which has been the subject of much interest[36]. A number of very useful synthetic applications of this method have been reported[37]. As applied to the alkylation of phenols, the process can be represented schematically as shown in Figure 5 ($Q^+ = R_4N^+$). In practice, the phenol is added to a two phase system consisting of an

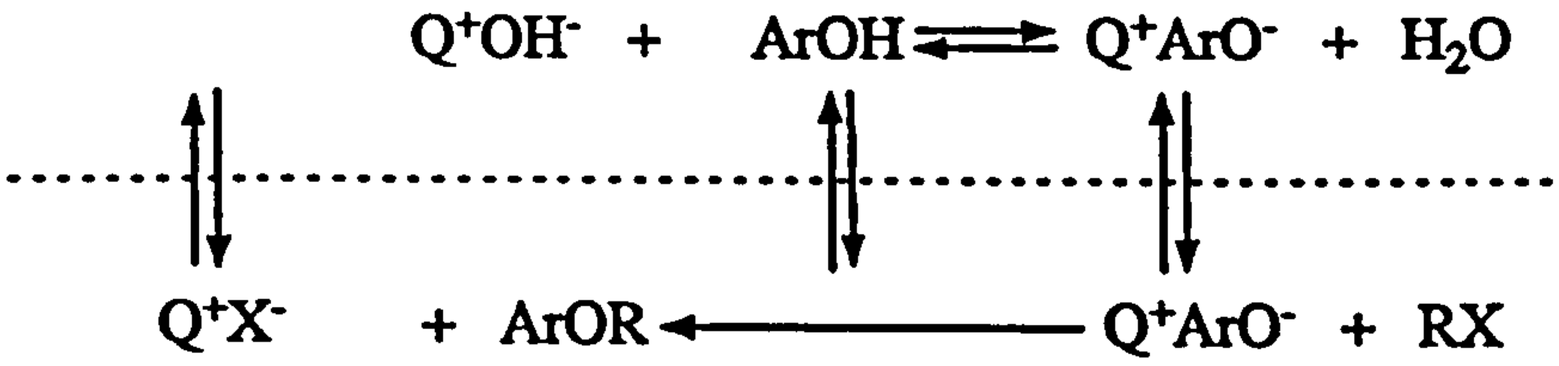
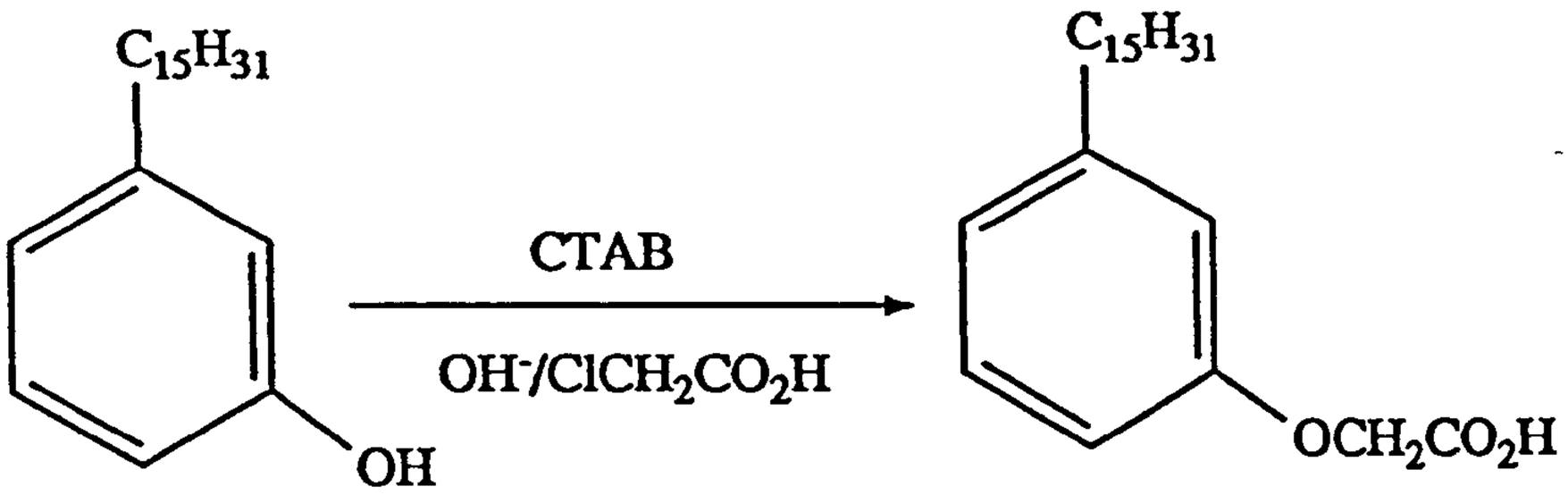


Figure 5. Schematic diagram showing the principle of phase transfer catalysis.

aqueous solution of the quaternary ammonium hydroxide and a dichloromethane solution of the alkylating agent. The phenol, which in most cases is partitioned naturally between the two phases, is converted into the quaternary ammonium phenoxide in the aqueous phase. This latter salt has a discrete solubility in the organic phase; consequently, transport of the phenoxide ion into the methylene chloride solution is followed by rapid irreversible alkylation and formation of the phenol ether.

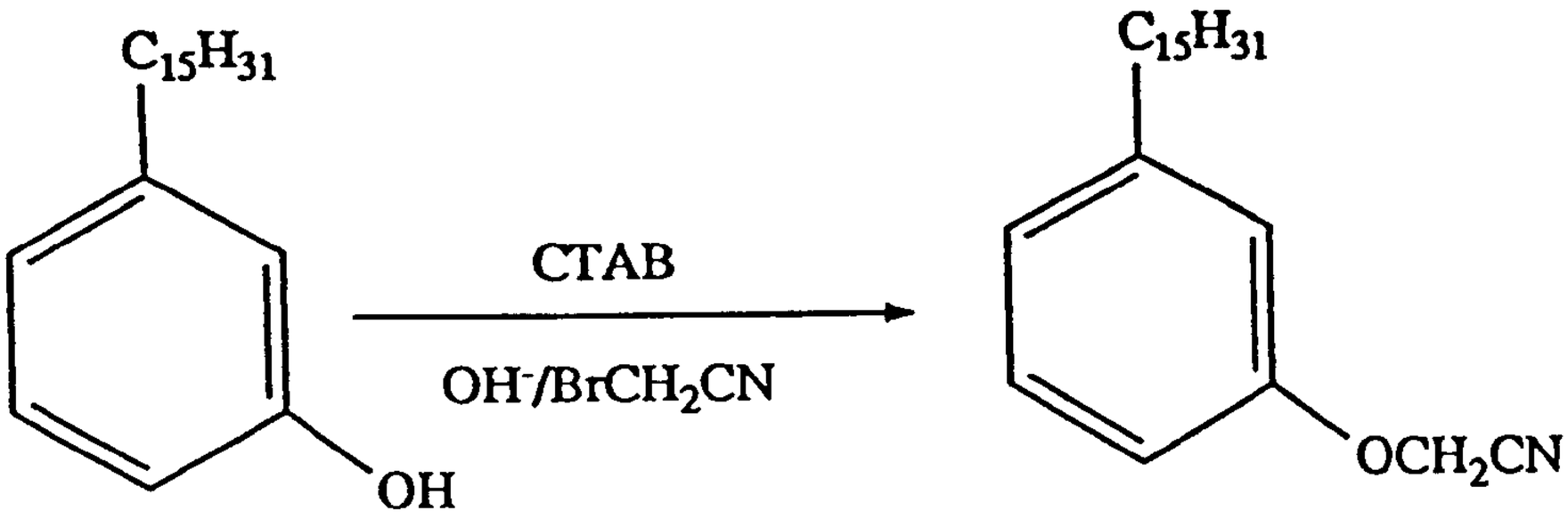
The quaternary ammonium salt, cetyl trimethylammonium bromide (CTAB) was used in an attempt to synthesize 2-(3-pentadecylphenoxy) acetic acid (20) from 3-pentadecyl phenol and 2-chloroacetic acid, which could then be reduced to the alcohol.



(20)

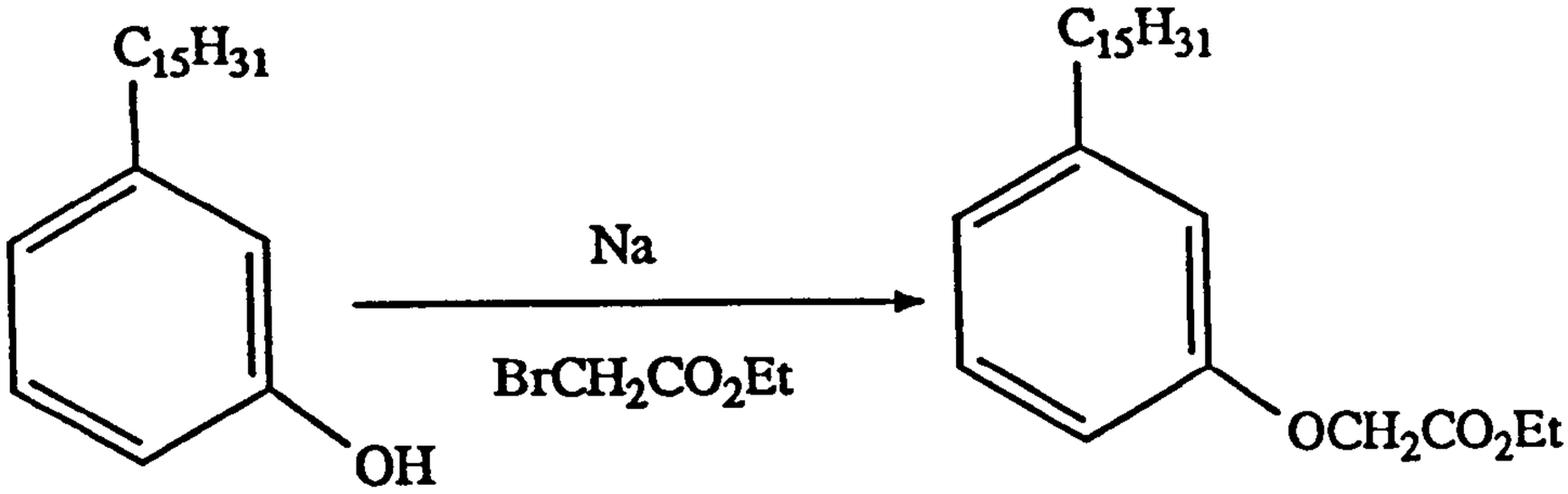
The reaction was unsuccessful and thus, several attempts were made to obtain the required product by altering slightly the reaction conditions by (a) using 2-bromo- and 2-iodo acetic acids and (b) by raising the reaction temperature. These changes appeared to have no effect. There are three possible explanations as to why this reaction does not work; (i) the reaction conditions are not forcing enough to give the phenoxide ion (ii) the phenoxide ion, due to the long alkyl chain, is insoluble in the aqueous phase or (iii) the carboxylate anion is formed, not the phenoxide.

In order to prove or disprove the last of these assumptions the same reaction conditions were used with 3-pentadecyl phenol and 1-bromoacetonitrile in an attempt to form 3-pentadecylphenoxy acetonitrile (21) which could then undergo acid hydrolysis to the



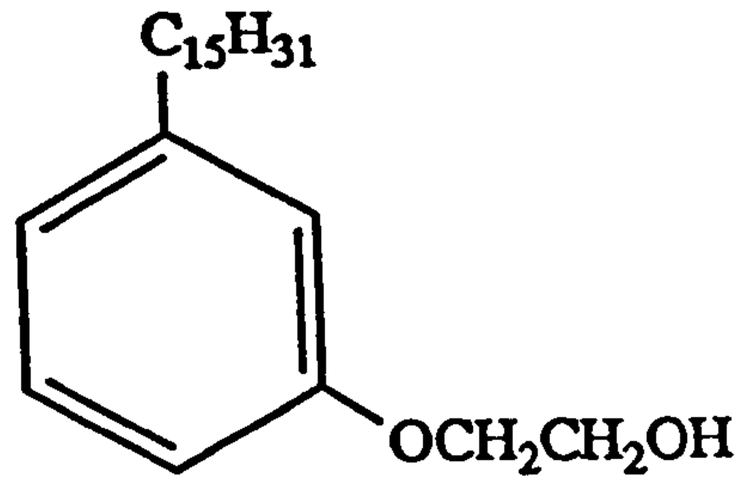
(21)

carboxylic acid. This reaction was also unsuccessful and the phase transfer catalysed method was abandoned in favour of the use of a stronger base, direct formation of the phenoxide ion by the use of sodium metal. This was employed to synthesize both the 3-pentadecylphenoxy acetonitrile **(21)** and 2-(3-pentadecylphenoxy) ethyl acetate (22). The solid phenoxide ion was formed after 3 hours stirring in benzene under reflux.



(22)

Addition of the ester or nitrile to the refluxing benzene suspension led to a slow dissolution of the solid and precipitation of a suspension of sodium halide. This gave yields of 67% and 61% of **(21)** and **(22)** respectively. Base hydrolysis of the ester gave the corresponding acid **(20)** in 93% yield, and reduction to the alcohol **(23)** with $LiAlH_4$ proceeded in 78% yield.



(23)

The overall yield of the alcohol from the starting phenol was only ~40% after 3 reaction steps and in order to synthesize further members of the series, a large amount of cardanol and other reagents would be needed. Thus, a simple 1-step reaction was required to synthesize these ethoxylates. This was achieved by adaptation of the method described by Abe and Watanabe[38]. The paper was concerned with the synthesis

of decyl, dodecyl and tetradecyl monoethers of hexa- to nona-ethylene glycol and nonylphenol monoethers of hexa- to octa-ethylene glycol.

The preparation of the phenyl ethers involve two steps; firstly the pentadecyl phenol was converted to the phenoxide, in the absence of solvent, by heating to 90°C in the presence of potassium metal for 9 hours which was then condensed with 2-bromoethanol, di- or tri-ethylene glycol monochloride. These can be again reacted with potassium metal to give the alkoxide which can be further reacted with the chloro- or bromo-glycol ethers. By the proper combination of the alkoxide and the chloro-glycol ethers in the second step, phenyl ethers of mono- to hexa-ethylene glycol were obtained. The combinations were as follows:

$$C_{15}E_0 \xrightarrow{E_2} C_{15}E_2$$

$$C_{15}E_0 \xrightarrow{E_3} C_{15}E_3$$

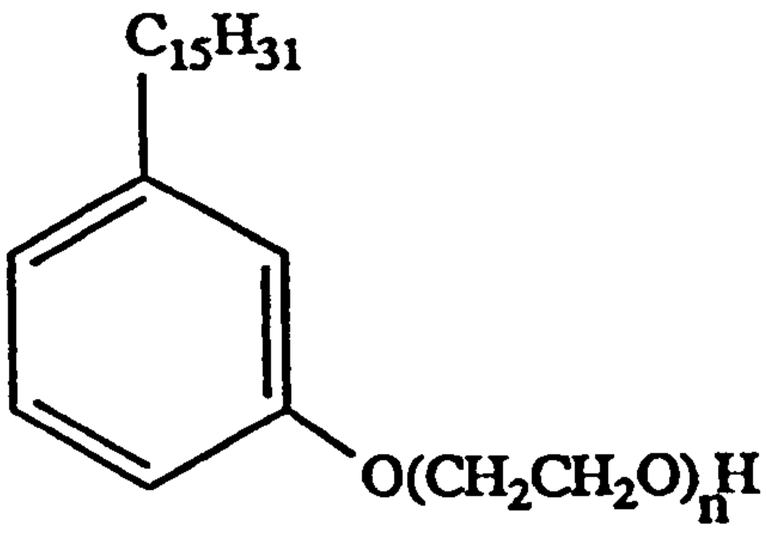
$$C_{15}E_1 \xrightarrow{E_3} C_{15}E_4$$

$$C_{15}E_3 \xrightarrow{E_2} C_{15}E_5$$

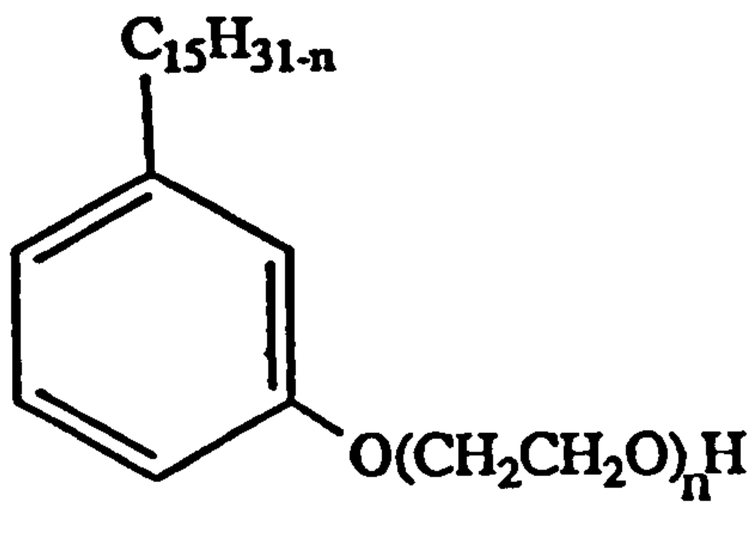
$$C_{15}E_3 \xrightarrow{E_3} C_{15}E_6$$

C_{15} = Pentadecyl phenol

E_x = Number of ethylene oxide units



(23) n = 1
(25) n = 2 (45%)
(27) n = 3 (46%)
(29) n = 4 (36%)
(31) n = 5 (42%)
(33) n = 6 (46%)



(24) n = 1 (63%)
(26) n = 2 (42%)
(28) n = 3 (43%)
(30) n = 4 (38%)
(32) n = 5 (40%)
(34) n = 6 (47%)

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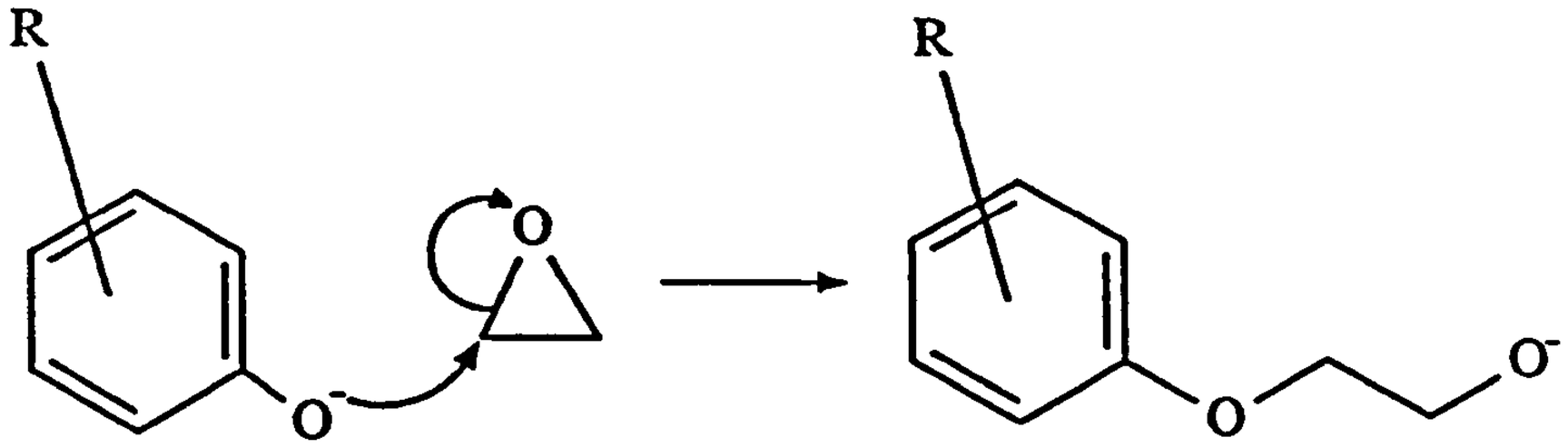
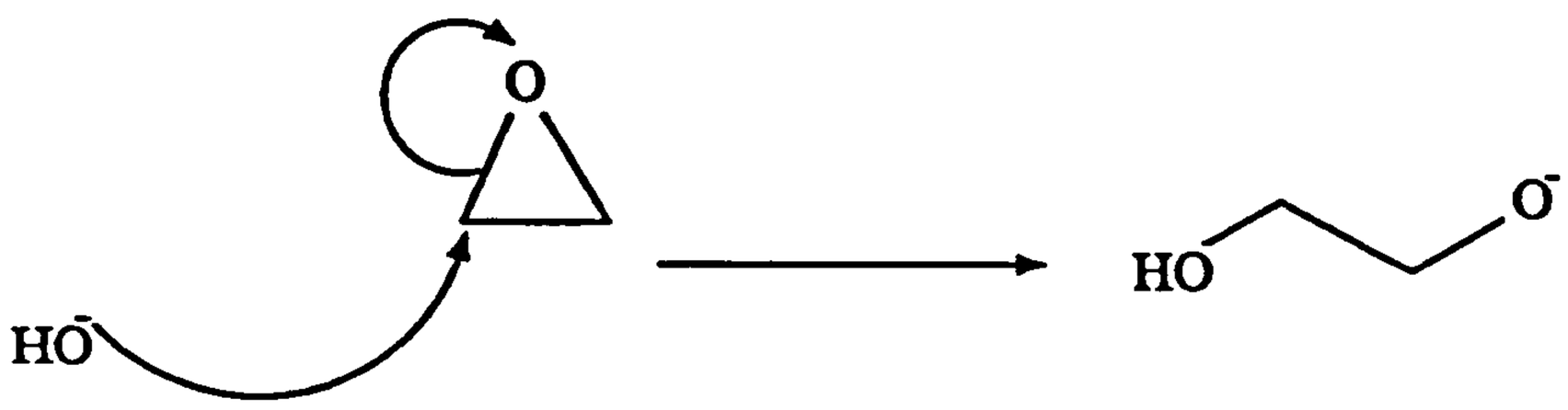
these samples were analysed, after neutralisation of the basic catalyst, by both nmr and HPLC.

The mechanism for the polyethoxylation reaction is a very simple nucleophilic attack of the phenolate anion at one of the carbons of the ethylene oxide, resulting in ring



opening of the ethylene oxide molecule, and generation of the alcoholate anion which can react with a further ethylene oxide molecule. There is a competing reaction in this system, that of the monoethylene glycol anion formed by the reaction of an ethylene oxide molecule with a hydroxyl anion which can react with further ethylene oxide



to form polyethylene glycols. The latter reaction appears, by nmr (Figure 7), to only be significant during the first hour of reaction possibly due to the difficulty in reacting the phenolate anion, as shown earlier. Thus, as the phenol gradually reacts the conjugated alcoholate, which appears to be more reactive than the phenolate ion, reacts with ethylene oxide in preference to the glycol.

Ethyl anacardate could not be polyethoxylated under these conditions and cardanol was obtained at the end of the reaction. This would imply that the ethyl anacardate had been

de-esterified and decarboxylated by the base resulting in its consumption, leaving no base remained to catalyse the polyethoxylation reaction.

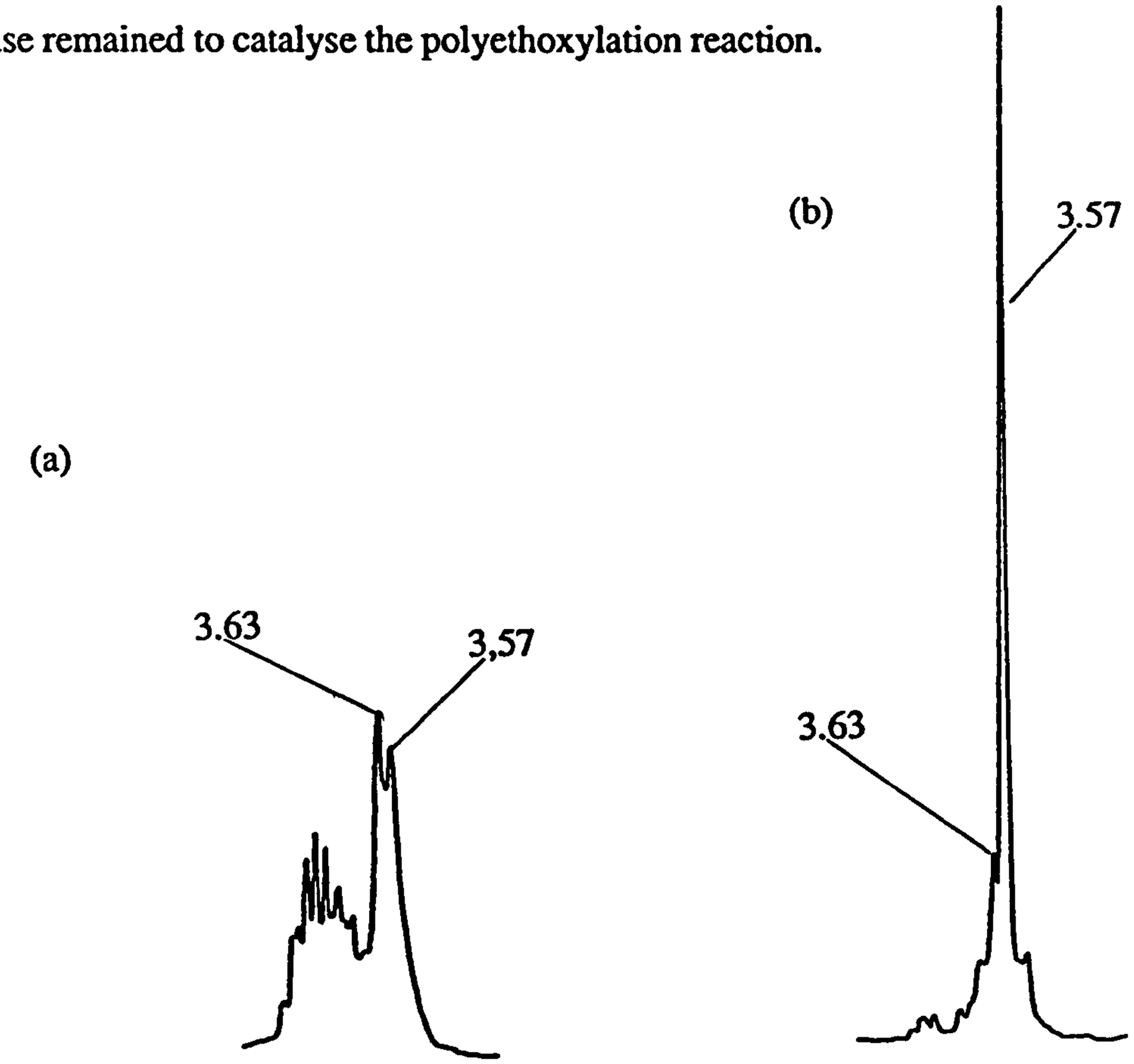


Figure 7. NMR spectra of cardanol during the polyethoxylation reaction showing the peaks at 3.63 (polyethylene glycol) and 3.57 (cardanol polyethoxylate) after (a) 30 min and (b) 210 min.

2.6 NMR Analysis of Ethylene Oxide Adducts.

NMR analysis of polyethoxylated phenols gives a rapid indication of degree of polyethoxylation. All analyses were carried out in a 1% solution in deuteriochloroform at 90MHz.

The nmr spectrum of cardanol shows the four aromatic protons between 6.4 and 7.2ppm, the olefinic protons between 4.8 and 6.0ppm and the aliphatic protons between 0.8 and 2.9ppm as multiplets (Figure 8). The ethylene oxide adducts of cardanol and cardol retained the general features of the cardanol spectrum and in addition a new

multiplet appeared in the region 3.2 and 4.3ppm accounting for the newly introduced polyethoxy and hydroxy group protons (Figure 9)

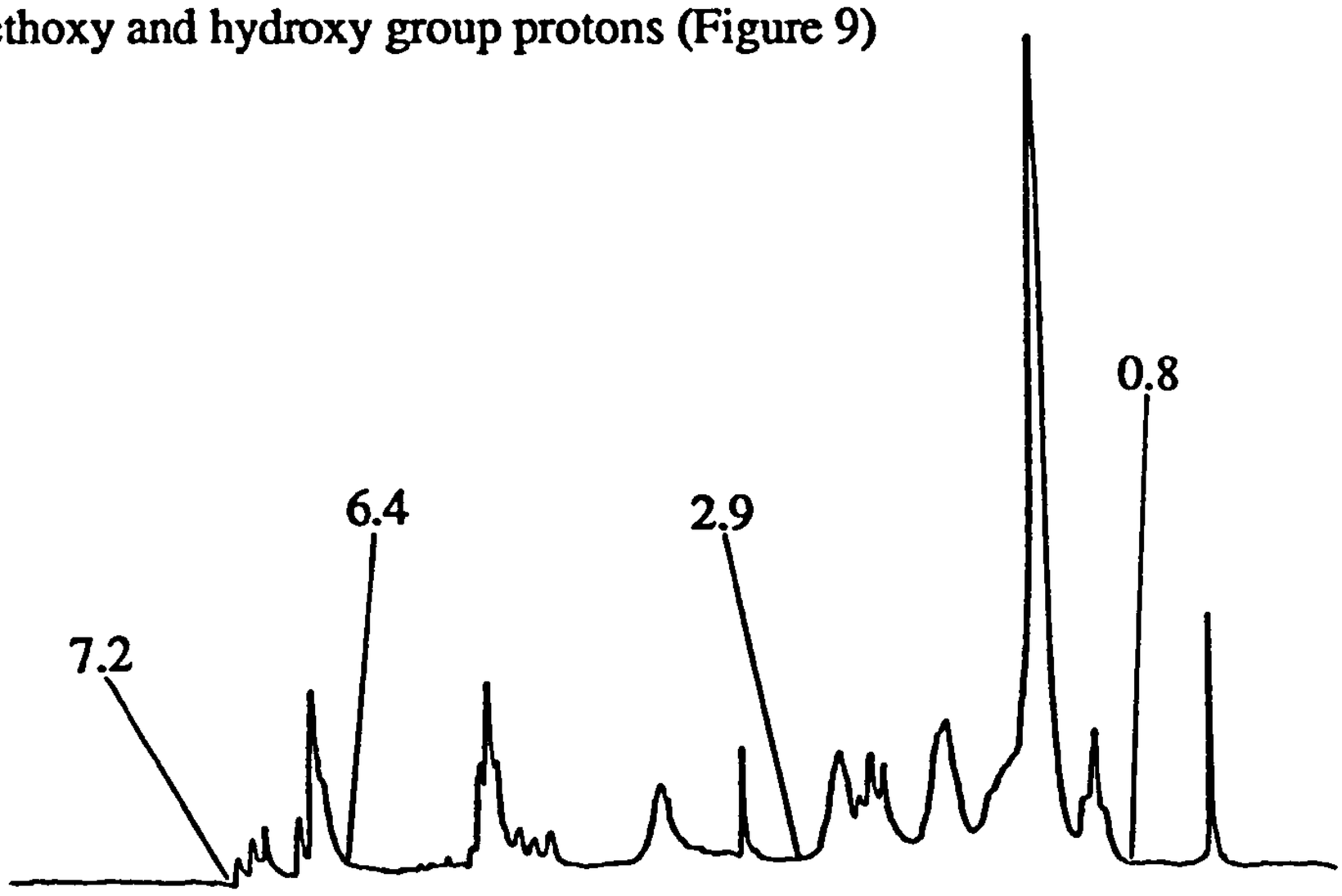


Figure 8. NMR spectrum of cardanol.

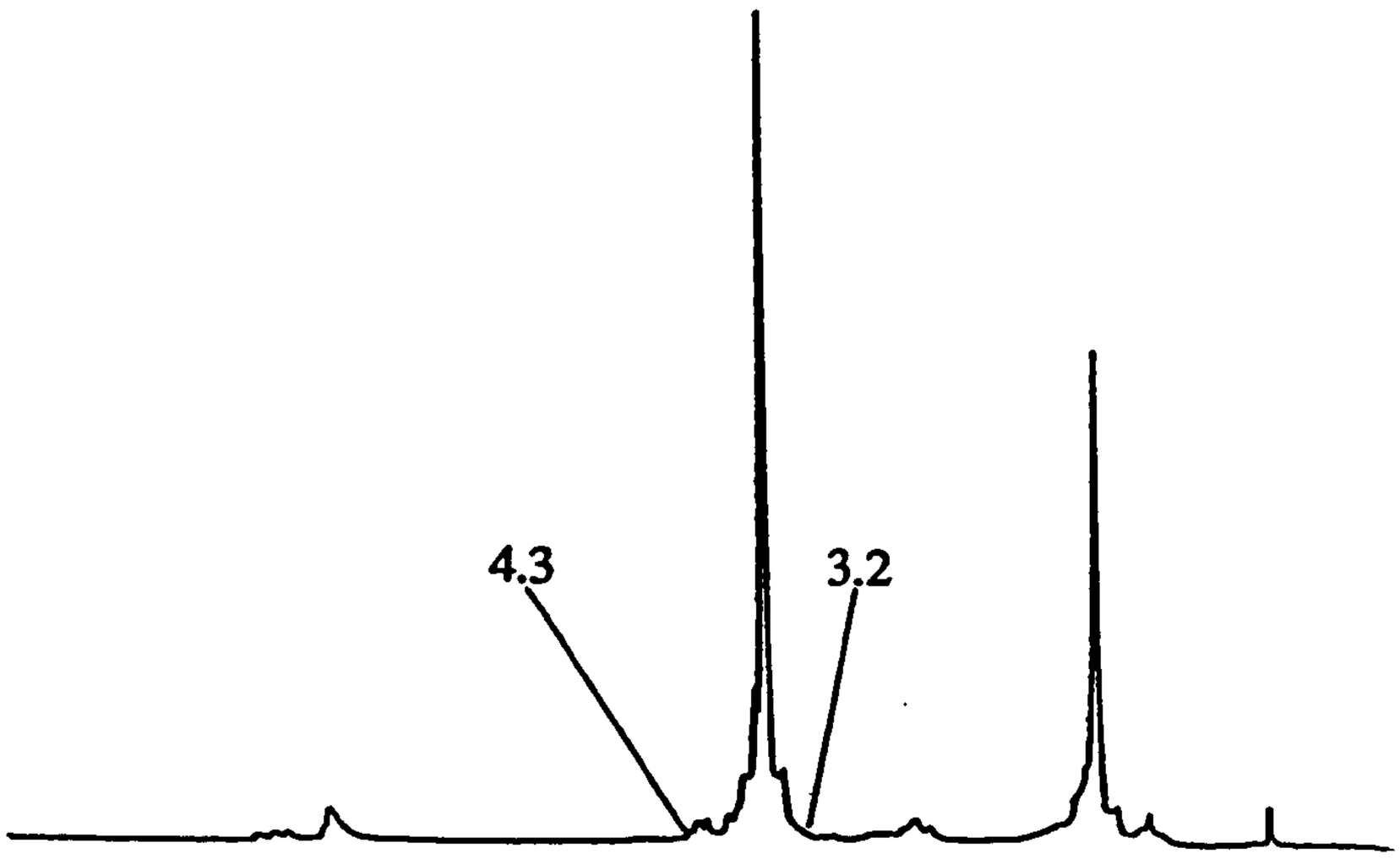


Figure 9. NMR spectrum of an ethylene oxide adduct of cardanol.

The integration curve for the area under this signal was used to calculate the number of protons of the ethoxy group and hydroxy group in comparison with the integration curve for the four aromatic protons in the region between 6.4 and 7.2ppm. The total number of protons resulting from the multiplet between 3.2 and 4.3ppm, less one hydroxyl proton, gave the number of ethoxy protons from which the average number of ethoxy groups was calculated for cardanol (Table 6), cardol (Table 7) and pentadecyl phenol (Table 8).

Time (mins)	Average number of EO units/molecule	Time (mins)	Average number of EO units/molecule
30	0.6	270	18.3
60	1.0	300	21.1
90	1.8	330	25.9
120	5.5	360	28.9
150	7.2	390	32.6
180	10.7	420	37.2
210	13.5	450	42.2
240	16.9	480	48.0

Table 6. Time of reaction of cardanol with ethylene oxide and the average number of moles of ethylene oxide/molecule as calculated by nmr.

Time (mins)	Average number of EO units/molecule	Time (mins)	Average number of EO units/molecule
30	0.2	270	11.4
60	0.6	300	14.8
90	0.9	330	17.3
120	2.3	360	19.9
150	3.1	390	22.6
180	4.9	420	25.1
210	6.7	450	29.3
240	9.3	480	34.7

Table 7. Time of reaction of cardol with ethylene oxide and the average number of moles of ethylene oxide/molecule as calculated by nmr.

Time (mins)	Average number of EO units/molecule	Time (mins)	Average number of EO units/molecule
30	0.7	270	10.0
60	1.2	300	15.2
90	1.7	330	18.5
120	2.0	360	24.0
150	2.2	390	26.9
180	2.4	420	32.8
210	4.0	450	39.5
240	6.5	480	47.1

Table 8. Time of reaction of pentadecyl phenol with ethylene oxide and the average number of moles of ethylene oxide/molecule as calculated by nmr.

2.7 HPLC Analysis of Ethylene Oxide Adducts.

2.7.1 General modes of HPLC analysis

a) Adsorption systems

Separations in which surface adsorption is the predominant sorption process depend upon polarity differences between solute molecules. Those which are highly symmetrical or consist of atoms with similar electronegativities are relatively non-polar, eg C_5H_{12}, C_6H_6, CCl_4. The presence of functional groups leads to an increase in polarity. The more polar a molecule, the more strongly it will be adsorbed by a polar surface. The approximate order of increasing strength of adsorption is: paraffins < olefins < ethers < esters < ketones < aldehydes < amines < alcohols < phenols < acids. During the separation process there is competition for adsorption between solute molecules and those of the mobile phase. Solute and solvent molecules are continually being adsorbed and desorbed as the mobile phase travels through the system. Solutes of low polarity spend proportionally more time in the mobile phase than those that are

highly polar. Consequently the components of a mixture are eluted in order of increasing polarity (increasing distribution ratio).

Stationary phase - Almost any polar solid can be used, the most common choices being silica gel or alumina. Silica gel and alumina are highly polar materials that adsorb molecules strongly, and are said to be active adsorbents. Activity is determined by the overall polarity and by the number of adsorption sites. In silica gel the adsorption sites are on the oxygen atoms and silanol groups (Si-OH) which readily form hydrogen bonds with polar molecules. Adsorption sites of different types are present on the surface of alumina but, unlike silica gel, a proportion of them are hydroxyl groups. The amount of water present on the surface has a profound effect on the activity by blocking adsorption sites. If the water is progressively removed by oven baking, the material correspondingly becomes more active.

The choice of stationary phase and its degree of activity is determined by the nature of the sample. If samples are adsorbed too strongly they may be difficult to elute or chemical changes may occur. Weakly polar solutes should be separated on highly active adsorbents otherwise they may elute too rapidly with little or no resolution. Strongly polar solutes are better separated on adsorbents of low polarity. Silica gel can be prepared with a wider range of activities than alumina and is less likely to induce chemical changes.

Mobile phase - The eluting power of a solvent is determined by its overall polarity, the polarity of the stationary phase and the nature of the sample components. In practice, better separations are achieved with the least polar solvent possible or mixtures of solvents.

It is important that a given solvent should not contain impurities of a more polar mixture as resolution is impaired. Certain solvent-adsorbent combinations can be chemically unstable, eg acetone is polymerised by basic alumina

The technique of adsorption is employed in column chromatography, Thin-layer chromatography (TLC) and Gas chromatography (GC).

b) Partition systems

In a partition system the stationary phase is generally a liquid coated on to a solid support. Silica gel, kieselguhr (diatomacious earth) or cellulose powder are the most frequently used. Conditions closely resemble those of counter-current distribution so that in the absence of adsorption by the solid support, solutes move through the system at rates determined by their relative solubilities in the stationary and mobile phases.

Stationary and mobile phases - There is a very wide range of pairs of stationary and mobile phases. It is not necessary for them to be totally immiscible, but low mutual solubility is desirable. A hydrophilic liquid may be used as the stationary phase with a hydrophobic mobile phase or *vice versa*. The latter situation is referred to as a 'reversed phase' system. Water, aqueous buffers and alcohols are suitable mobile phases for the separation of very polar mixtures whilst hydrocarbons, in combination with ethers, esters *etc*, would be chosen for less polar materials.

The partition system is used in Gas-liquid chromatography (GLC) and some HPLC applications.

c) Ion-Exchange systems

Ion-exchange separation systems are limited to ionized or partially-ionized solutes. The stationary phase consists of an insoluble, resinous material which contains fixed charge-carrying groups and mobile counter ions can be reversibly exchanged with those of a solute which carry a like charge as the mobile phase travels through the system. Variations in the affinity of the stationary phase for different ionic species is responsible for different rates of migration. Separations are often enhanced by eluting with a mobile phase containing a complexing agent.

d) Gel-Permeation systems

Molecules that differ in size can be separated by passing the sample solution through a stationary phase consisting of a porous cross-linked polymeric gel. The pores of the gel exclude molecules with a certain critical size whilst smaller molecules can permeate the gel structure. Excluded molecules pass through the system more rapidly than smaller ones which can diffuse into the gel. Diffusion within the gel also varies with molecular size and shape because the pores are of different dimensions and are distributed throughout the gel structure in a random manner. The smaller molecules are eluted at rates dependant on their rate of permeation into the gel and components of a mixture therefore elute in order of decreasing size and molecular weight.

2.7.2 Chromatographic Analysis of Ethylene Oxide Adducts

Many chromatographic techniques, such as thin-layer chromatography, gas chromatography and size-exclusion chromatography have been employed for the analysis of oligomeric mixtures but they have severe limitations.

With TLC[41, 42], poor reproducibility is obtained and the quantification is cumbersome. With GC[43] a very limited number of oligomers are usually eluted from the column, even if the volatility of the sample is increased by derivatization[44, 45]. Size-exclusion chromatography (SEC) on both rigid and soft gels is suitable for separation of oligomeric mixtures, but is less selective than gradient elution HPLC[46]. On the other hand SEC on soft gels is an excellent method for preparative separations (10 - 100mg)[47, 48] and SEC on rigid gels may be rapid[49].

Sample retention in liquid chromatography depends strongly on the composition of the mobile phase. In many applications of LC to the separation of complex mixtures, sometimes containing widely dissimilar components, it is frequently necessary to change the composition of the mobile phase in order to elute all of the components present in the sample satisfactorily. Snyder[50] described this situation as the 'general

elution problem'. To overcome this problem, gradient elution or solvent programming may be applied.

The successive components of an oligomeric mixture generally only show a slight difference in chromatographic behaviour; on the other hand, the number of oligomers in one sample is often considerable. Therefore, gradient elution HPLC appears to be promising for the analysis of such mixtures.

a) Amino-Propyl Column.

Van der Maeden *et al*[46] have shown how gradient elution HPLC can be used to separate the individual oligomers of polyethoxylated octyl phenol up to the 20-mer using the following conditions:

Column	250 x 4.6mm ID μ-Bondpack semipolar NH_2
Solvent	A : 20% THF in *n*-hexane
	B : 10% water in *iso*-propanol
Flow rate	1ml/min
UV	280nm

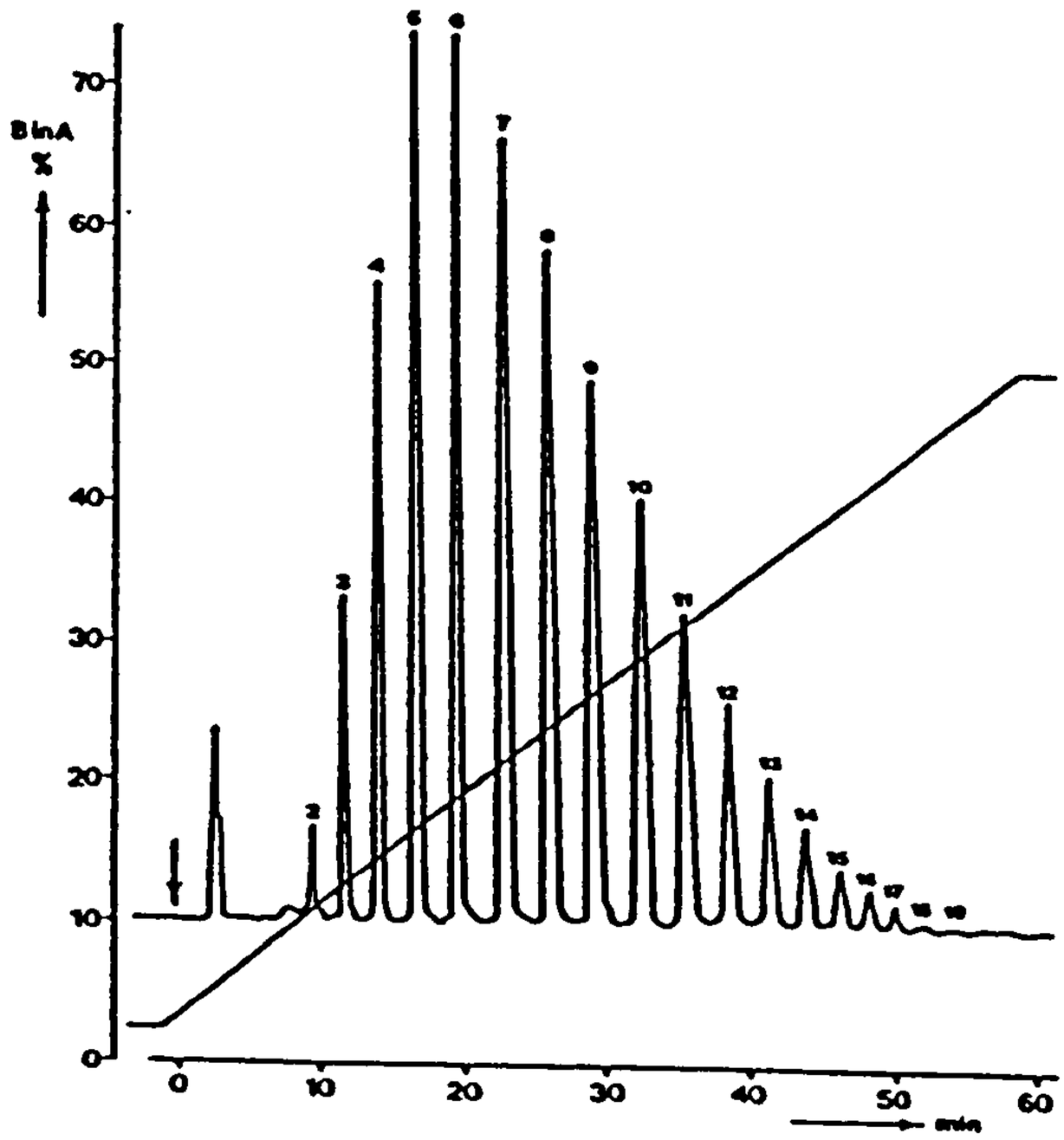


Figure 10. HPLC chromatogram of a polyethoxylated octyl phenol using the above conditions

As can be seen from Figure 10, this system permits accurate quantification up to the 20-mer, it has also been reported that separations up to the 30-mer are possible.

b) Bonded Diol Phases

Some workers have used bonded diol phases for the separation of polyethoxylated surfactants[51] and eluting with mobile phases comprising of *n*-hexane, *iso*-propanol, water and acetic acid in various ratios:

A, 75 : 125 : 10 : 1

B, 90 : 110 : 10 : 1

C, 105 : 95 : 10 : 1

D, 120 : 80 : 10 : 1

E, 125 : 75 : 10 : 1

F, 140 : 60 : 10 : 1

Columns 1. 250 x 4.6mmID LiChrosorb DIOL 10µm

2. 250 x 4.6mmID LiChrosorb DIOL 5µm

Flow rate 1ml/min for Column 1

0.8ml/min for Column 2

Many separations of polyethoxylated alcohols and phenols were carried out with this system and with differing degrees of resolution, *eg* Figures 11 and 12.

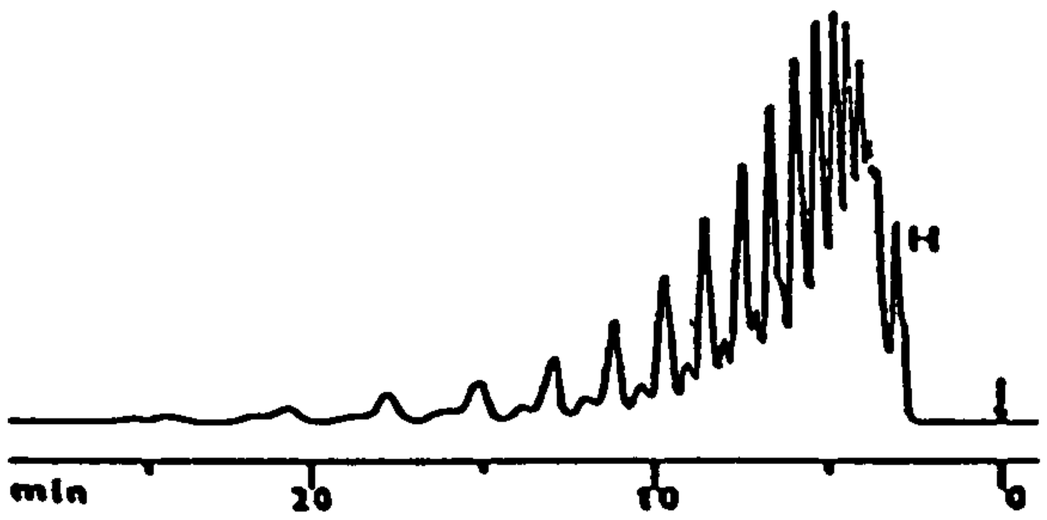


Figure 11. Separation of polyethoxylated lauryl alcohol using Column 1 and mobile phase F.

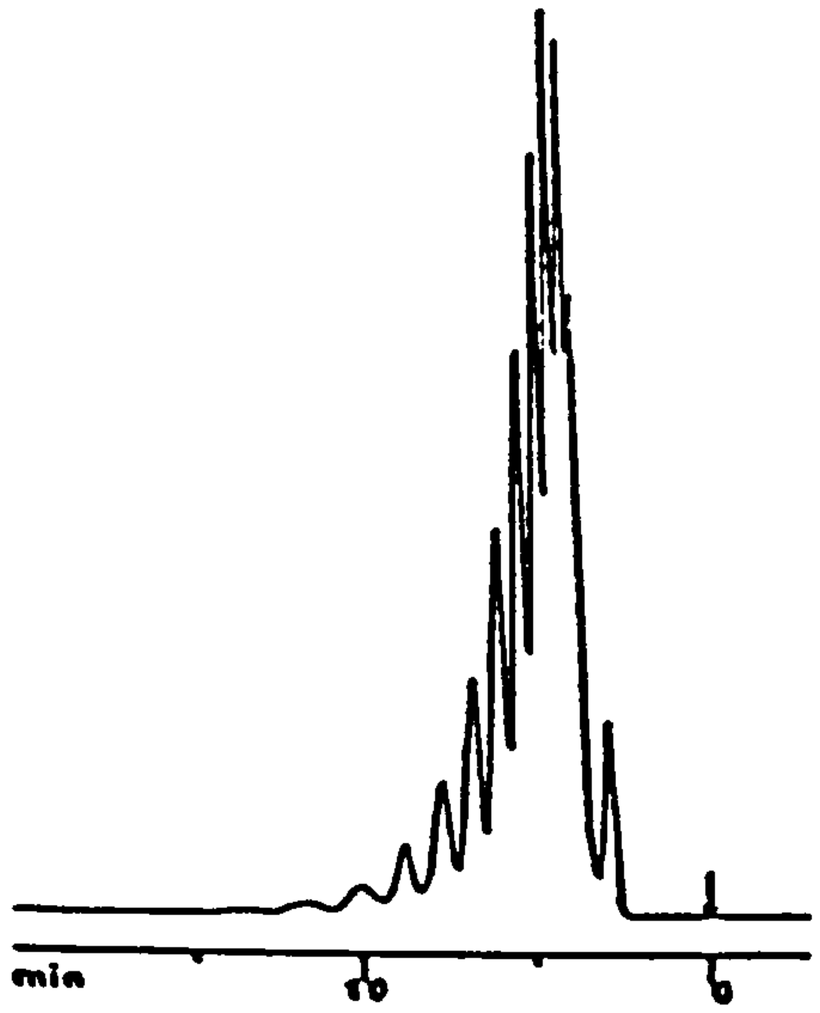


Figure 12. Separation of polyethoxylated dodecyl phenol using Column 2 and mobile phase F.

As can be seen from the above chromatograms the resolution is not as good as in the previous system but this would appear to be a useful procedure for the analysis of nonionic surfactants by isocratic elution.

c) Reversed-phase systems

Reversed-phase chromatography of polyethylene glycols and its derivatives have received little attention, the usual methods being with polar adsorbents. Melander *et al*[52] have shown that polyethoxylated phenol and octylphenol can be successfully separated into the various oligomers using the following conditions:

Column	1. 250 x 4.6mmID Zorbax ODS 5µm
	2. 250 x 4.6mmID LiChrosorb RP-8 5µm
Solvents	A : Acetonitrile
	B : Water
Flow rate	2ml/min
UV	200 and 275nm

Thus, it can be seen from Figure 13 that reverse-phase chromatography can be used to obtain high resolution of homologues for phenyl polyethoxylates but separation is not as good for the alkylphenol derivatives of ethylene oxide.

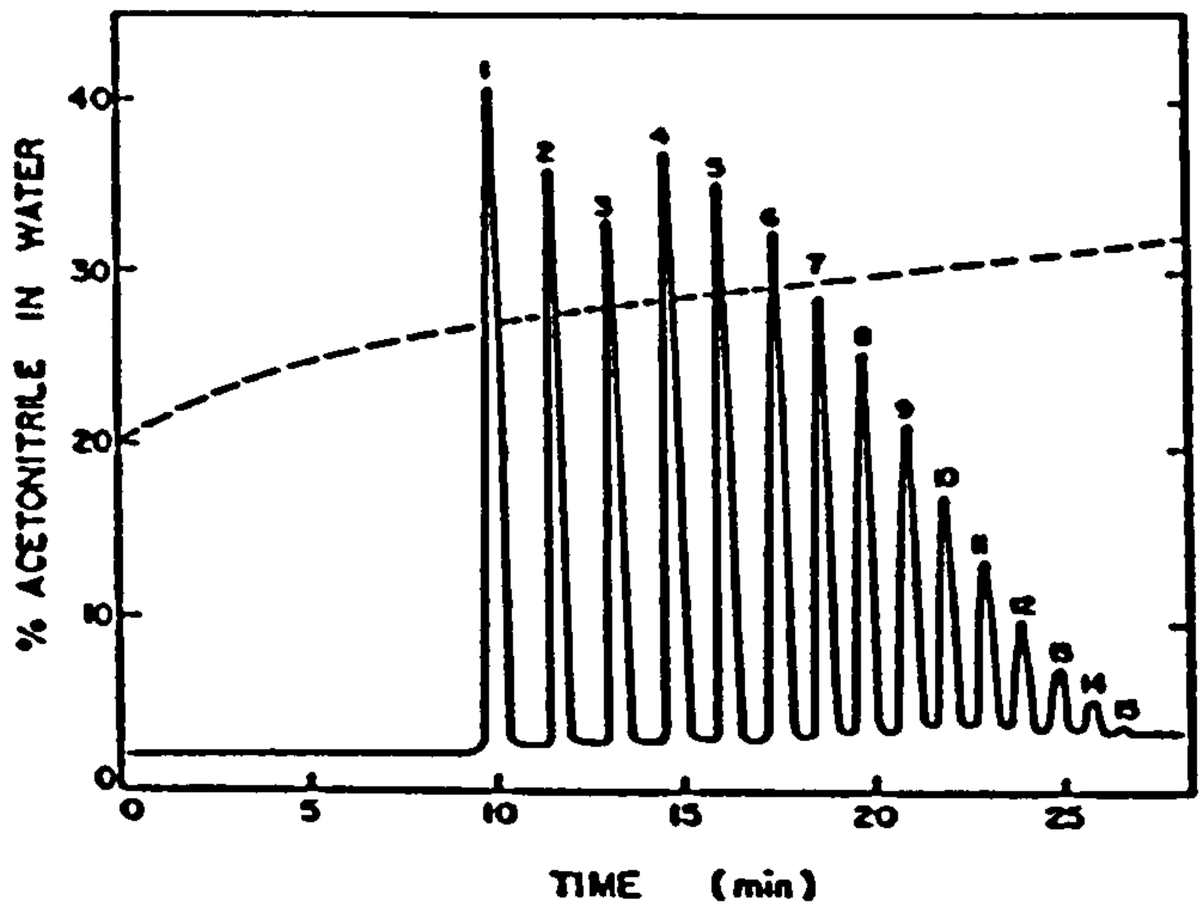


Figure 13. HPLC chromatogram of polyethoxylated phenol using a reverse-phase column and gradient elution.

d) Derivatization

HPLC analysis of polyethoxylated aliphatic surfactants has also been carried out using a Flame-Ionisation Detector (FID)[53] but was found to be inconvenient for routine work because the detector was delicate and required much skill to obtain reproducible results. The HPLC methods using UV detectors are easier than FID methods, so derivatization of the aliphatic polyethylene oxide condensates with a UV absorber would be necessary.

Nozawa and Ohnuma[54] showed that the esters of these condensates with 3,5-dinitrobenzoyl chloride were separated satisfactorily by HPLC under certain conditions and were easily detected by UV. The aliphatic alcohol they chose for the polyethoxylation reaction was dodecanol and the following conditions were used.

Column	250 x 4.6mmID LiChrosorb RP-8
Solvent	Acetonitrile : Water (6 : 4)
Flow rate	0.8ml/min
UV	254nm

2.7.3 Ethoxylate Numbers.

The correct assignment of ethoxylate numbers is critical for their characterisation and for this purpose a reference material of known ethoxylate chain length R-$PhO(CH_2CH_2O)_nH$, eg n = 3[54] is normally employed. The reference materials can be used to 'spike' the nonionic surfactant solutions to show the elution positions of the oligomers and thus, the weight-average ethoxylate number can be calculated.

The calculation of the weight-average ethoxylate number involves multiplying the peak areas by the ratio of the molecular weights of the ethoxylate oligomer and the monoethoxylate component. These values are then multiplied by the relevant ethoxylate number. The two sets of numbers are then summed.

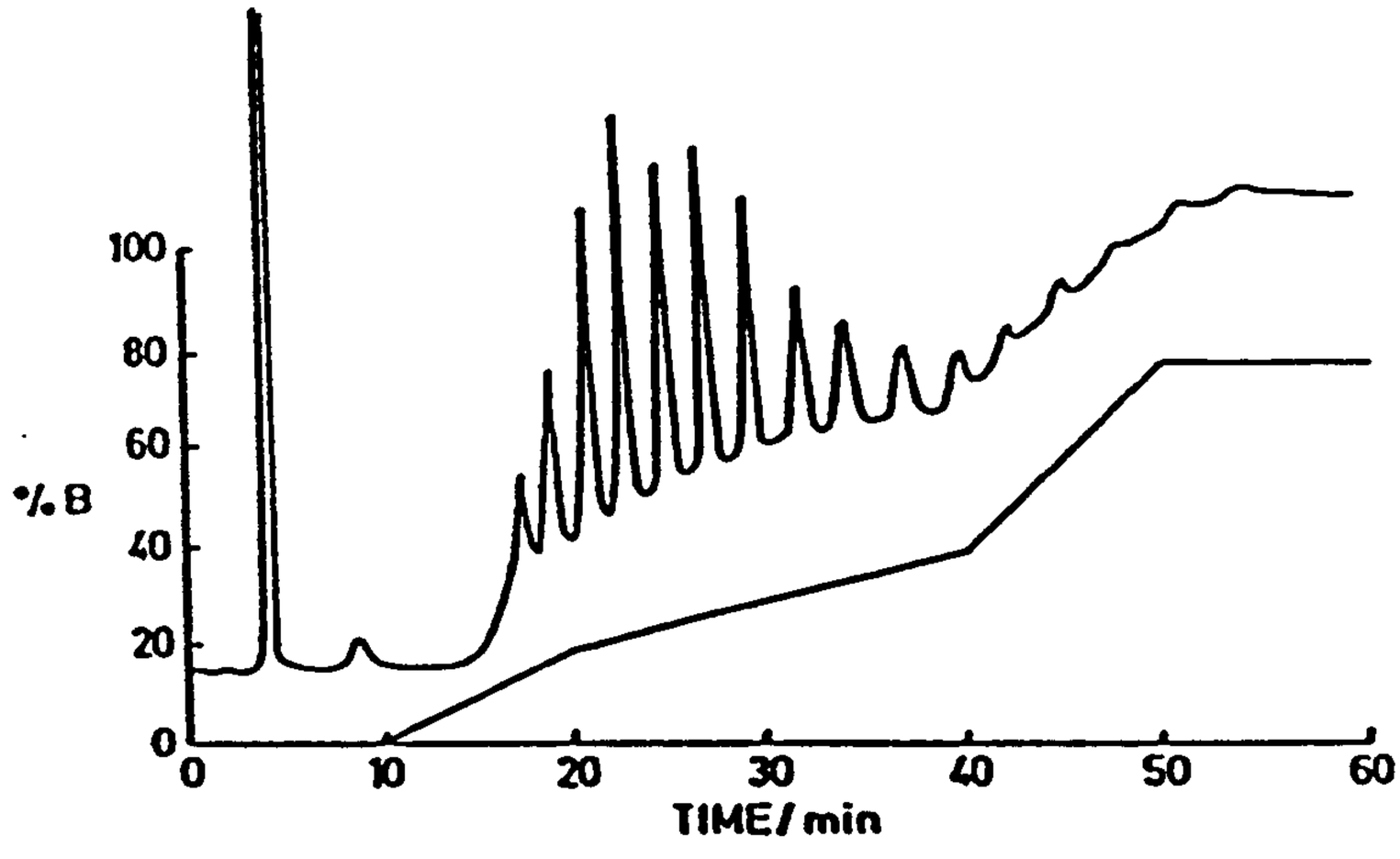
E_1 = Sum of (peak area x molecular weight ratio)

E_2 = Sum of (peak area x MWt x ethoxylate number)

E_2/E_1 = Weight-Average Ethoxylate Number.

The molar average ethoxylate number is obtained in a similar way, but omitting the molecular weight ratio. An example of the analysis of a nonionic surfactant material is shown in Figure 15 and the resultant graphs for the weight and molar ethoxylate distribution are also given. It has been shown by other workers that the molar

extinction coefficients of the ethoxylate oligomers are independent of the ethoxylate chain length and therefore the peaks are directly proportional to the mole percent of the components. The weight distribution results give some additional information which is also useful for surfactant characterisation.



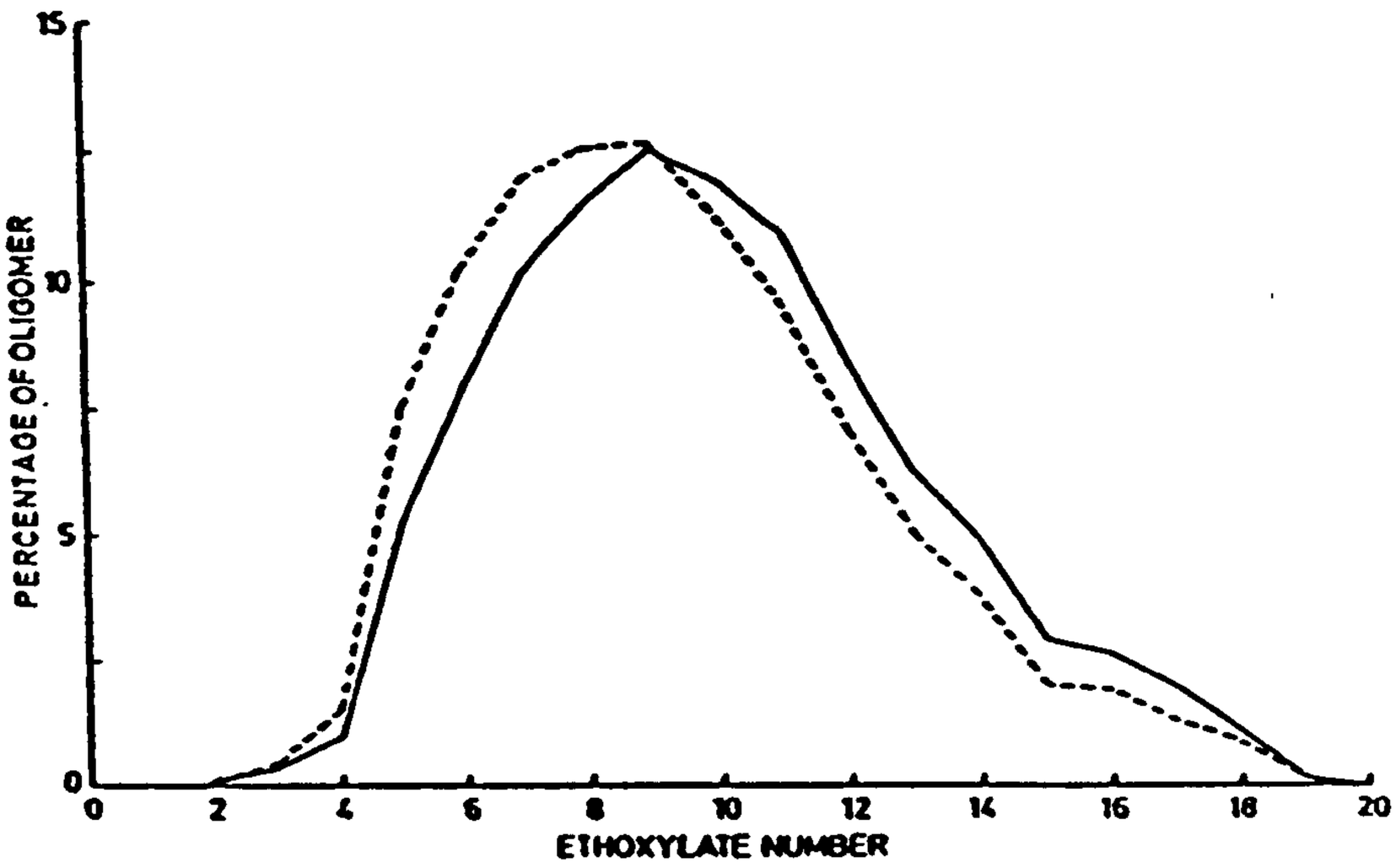


Figure 15. a) Chromatogram of nonionic surfactant R-PhO$(CH_2CH_2O)_nH$; b) Weight and molar distribution curves for R-PhO$(CH_2CH_2O)_{10}H$.

2.7.4 Results from the HPLC Analysis of Polyethoxylated Alkyphenols

The use of the Amino-Propyl column[46] was employed to analyse polyethoxylated cardanol, cardol and pentadecyl phenol and the whole range of samples were characterised. Also a commercial sample of a nonyl phenol polyethoxylate ('Synerponic NP-8') was also characterised in order to have a reference sample for later biodegradation studies. It was found that the ideal loading for the best resolution was 200µg of sample in 20µl of ether, and chromatograms for all the samples were obtained as typified by Figure 16.

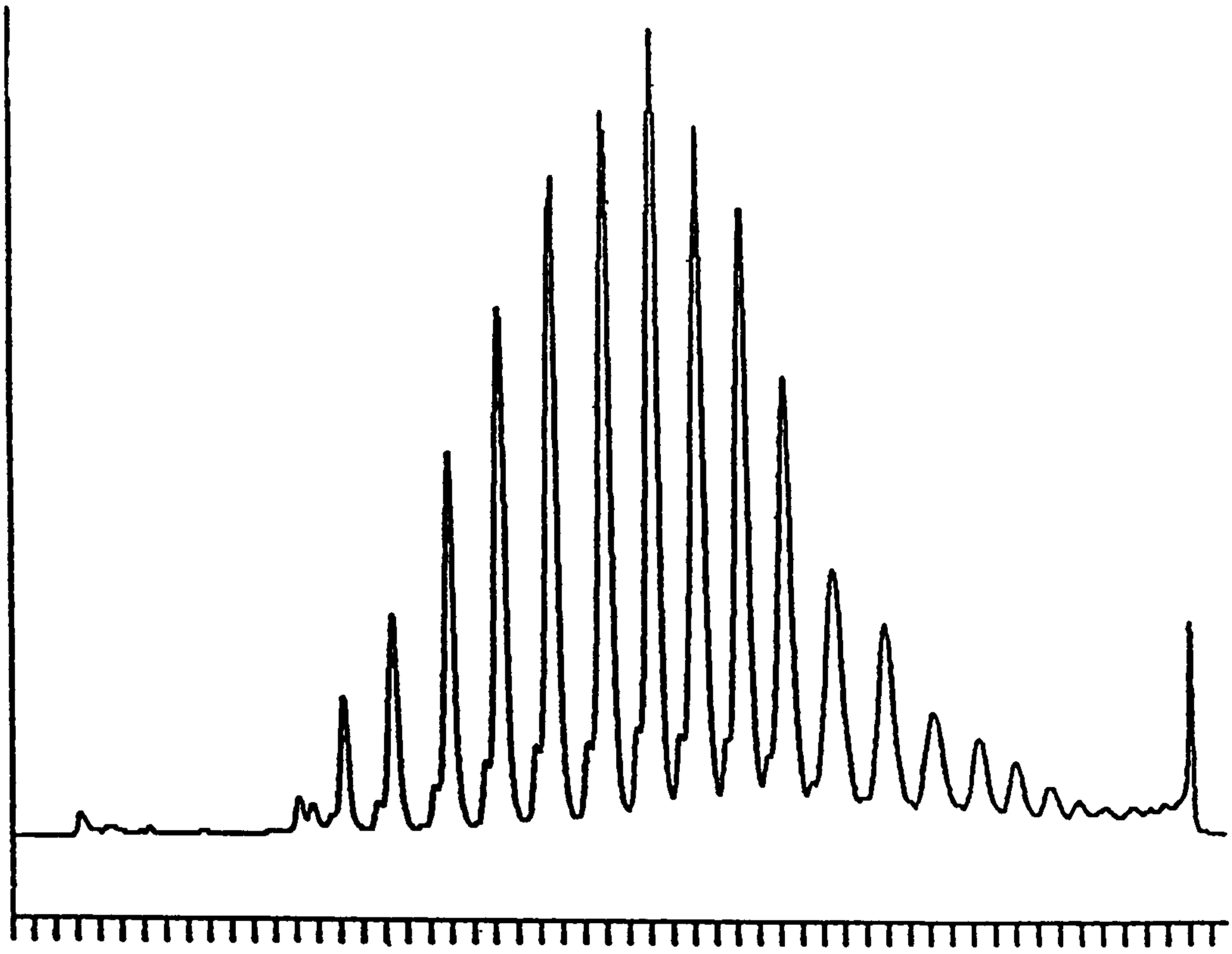


Figure 16. HPLC chromatogram of polyethoxylated cardanol with no reference material present.

The small peaks present before each of the major cardanol ethoxylate peaks is due to 2-methyl cardol polyethoxylate (35) as 2-methyl cardol is present to a small degree in the starting cardanol, even after distillation.

Retention time (min)	Area %	Ethoxylate number
15.24	1.24	2
17.47	2.63	3
20.01	4.59	4
22.36	7.76	5
24.64	10.23	6
26.99	11.56	7
29.14	12.79	8
31.32	11.97	9
33.45	10.65	10
35.45	8.29	11
37.73	5.41	12
40.16	3.83	13
42.45	2.18	14
44.59	1.35	15
46.30	0.81	16

Table 9. An example of ethoxylate number, retention time and percentage of each oligomer for a cardanol polyethoxylate.

The numbering of the individual oligomers was achieved by introducing a small sample of the standard of known ethoxylate number into a solution of the polyethoxylated cardanol and a chromatogram was obtained where identification was made possible (Figure 17). In this way the weight-average ethoxylate numbers and the molar average ethoxylate numbers were calculated for every sample of cardanol (Table 10), Cardol (Table 11) and pentadecyl phenol (Table 12).

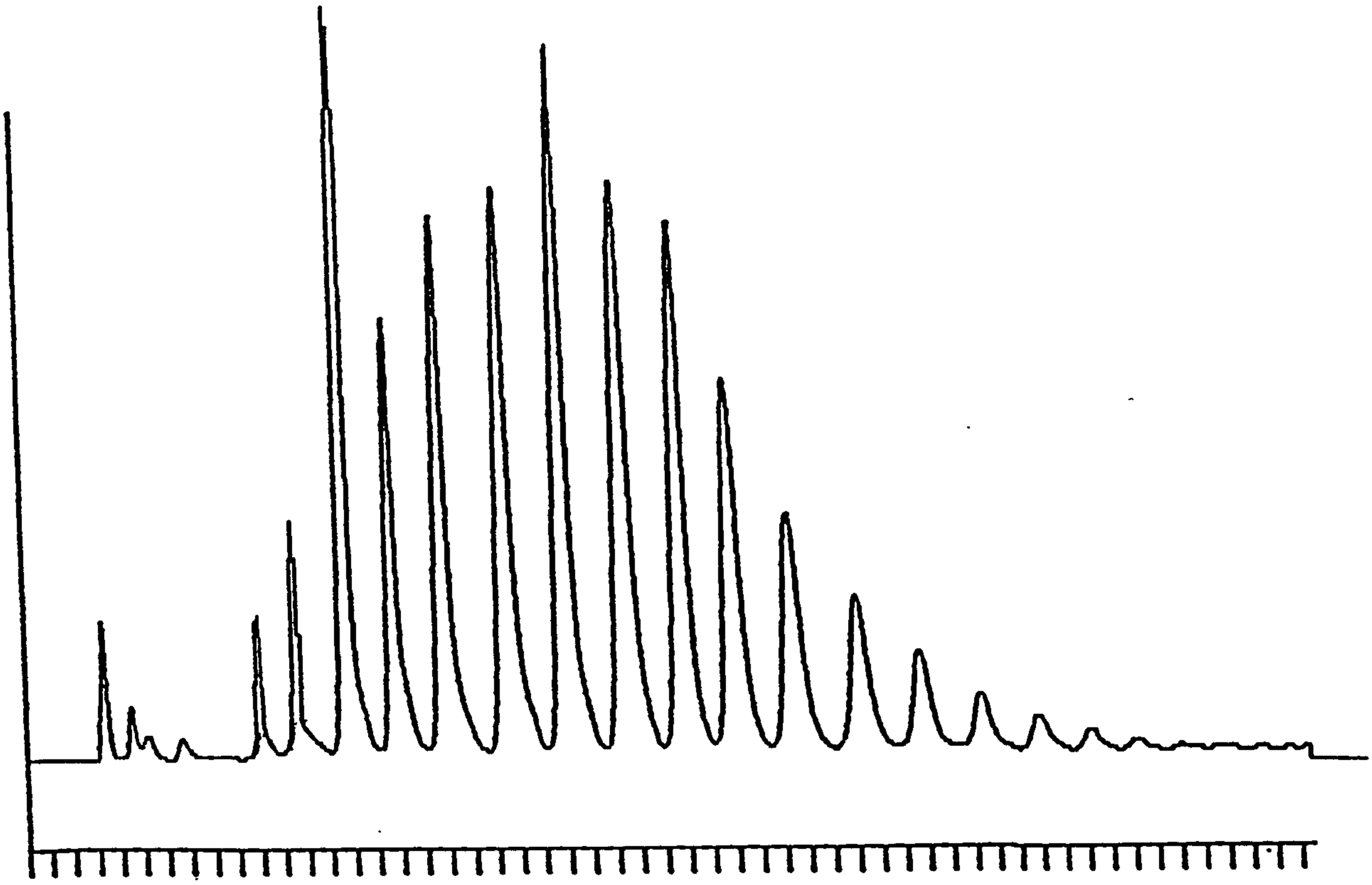


Figure 17. A 'spiked' solution of a cardanol polyethoxylate.

As can be seen from Tables 12 - 14 the results from the nmr analyses are in good agreement with those obtained for the molar-average ethoxylate number by HPLC, varying from 1 - 5% error. Thus, nmr analysis of these polyethoxylated products gives a rapid and fairly accurate indication of the number of mole of ethylene oxide per mole of alkylphenol. From these results it is possible to give an indication of the ideal chain length to give the best hydrophobe-lipophobe balance (HLB) which in turn will give the best chain length for the reduction in surface tension, a measure of how good a surfactant is.

Time (min)	Weight-Average Ethoxylate Number	Molar-average Ethoxylate Number	Average Number of EO units/mol (nmr)
30	0.93	0.62	0.6
60	1.47	1.14	1.0
90	2.14	1.86	1.8
120	6.14	5.71	5.5
150	8.38	7.52	7.2
180	11.62	10.98	10.7
210	14.21	13.44	13.5
240	17.59	16.98	16.9
270	19.67	18.55	18.3
300	22.61	21.33	21.1
330	27.72	27.24	25.9
360	30.11	28.47	28.2
390	35.16	33.02	32.6
420	39.27	37.17	37.2
450	44.56	42.37	42.2
480	50.17	48.51	48.0

Table 10. Time of reaction of cardanol with ethylene oxide, the weight-average and molar-average ethoxylate numbers as calculated from HPLC results and the average number of ethylene oxide units/mole of cardanol as calculated from nmr data.

Time (min)	Weight-Average Ethoxylate Number	Molar-Average Ethoxylate Number	Average Number of EO units/mol (nmr)
30	0.42	0.27	0.2
60	1.03	0.66	0.6
90	1.44	0.91	0.9
120	2.61	2.38	2.3
150	3.71	3.21	3.1
180	5.28	4.87	4.9
210	7.41	6.89	6.7
240	10.96	10.31	9.3
270	12.68	11.92	11.4
300	16.23	15.22	14.8
330	18.94	17.81	17.3
360	20.47	19.28	19.9
390	25.56	24.21	22.6
420	26.57	25.17	25.1
450	31.04	29.64	29.3
480	38.11	36.49	34.7

Table 11. Time of reaction of cardol with ethylene oxide, the weight-average and molar-average ethoxylate numbers as calculated from HPLC results and the average number of ethylene oxide units/mole of cardol as calculated by nmr.

Time (min)	Weight-Average Ethoxylate Number	Molar-Average Ethoxylate Number	Average Number of EO units/mol (nmr)
30	0.82	0.71	0.7
60	1.47	1.28	1.2
90	2.16	1.82	1.7
120	1.33	1.96	2.0
150	2.51	2.03	2.2
180	3.39	2.74	2.4
210	4.67	4.39	4.0
240	7.18	6.21	6.5
270	11.59	10.12	10.0
300	15.99	14.81	15.2
330	20.03	18.66	18.5
360	25.83	24.29	24.1
390	28.91	27.13	26.9
420	36.62	34.61	32.8
450	40.71	38.68	39.5
480	49.56	47.22	47.1

Table 12. Time of reaction of pentadecyl phenol with ethylene oxide, the weight-average and molar average ethoxylate numbers as calculated from HPLC results and the average number of ethylene oxide units/mole of pentadecyl phenol as calculated from nmr data.

2.7.5 Surface Tension,

The surface tension, in this case, determines the force required to detach a metal ring from the surface of a liquid. This has been used for many years as a method for measuring surface tension, with the wire ring connected either to one arm of a balance (in place of the pan) or to a light beam carried on a horizontal torsion wire whose

constants are known. The simplest theoretical interpretation of the result equates the pull required to detach a ring from the surface to the total perimeter of the ring multiplied by the surface tension: the total perimeter of the ring is twice the length, since the meniscus pulls on each side of the wire (Figure 18). Equating the vertical pull *f* on the ring, at the moment of the ring breaking away from the surface, to the radius r we have:

$$f = 4\pi r\gamma$$

or

$$\gamma = \frac{f}{4\pi r}$$

This assumes that the surface tension acts vertically and that the contact angle is zero, *ie* that the liquid completely wets the ring; to ensure this, the platinum ring was readily cleaned by gentle flaming. With such a platinum ring attached to a torsion wire the instrument is known as a du Nouy tensiometer.

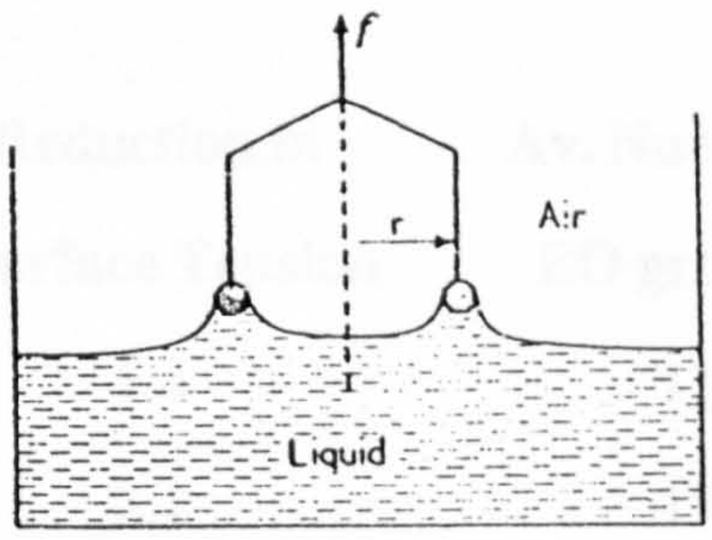


Figure 18. Du Nouy Tensiometer (Ring Method)

The surface tension of distilled water was measured by this method and was taken as standard. A 1% solution of each of the surfactants, in water from the same source, was also measured and the reduction in surface tension tabulated (Tables 13 - 15) and can also be graphically represented in order to determine the polyethoxylate chain length of optimum surface tension reduction and thus the best surfactant properties. Nonyl

phenol with an average of 8 ethylene oxide units/mole reduced the surface tension of water by $33Nm^{-1}$

Av. Number of EO groups	Reduction in Surface Tension	Av. Number of EO groups	Reduction in Surface Tension
0.62	4.6	18.55	28.6
1.14	7.9	21.33	26.0
1.86	8.9	26.24	24.5
5.71	16.4	28.47	23.5
7.52	24.0	33.02	23.0
10.98	31.4	37.17	22.4
13.44	34.2	42.37	21.7
16.98	30.8	48.51	20.2

Table 13. Average number of ethylene oxide groups/mole of cardanol and the reduction in surface tension (Nm^{-1}) of a 1% solution with respect to water at 25°C.

Av. Number of EO groups	Reduction in Surface Tension	Av. Number of EO groups	Reduction in Surface Tension
0.27	1.4	11.92	23.6
0.66	2.1	15.22	22.1
0.91	2.9	17.81	21.7
2.38	7.4	19.28	20.3
3.21	11.1	24.21	18.9
4.87	17.5	25.17	17.8
6.89	19.0	29.64	16.3
10.31	24.2	36.49	15.1

Table 14. Average number of ethylene oxide units/mole of cardol and the reduction in surface tension of a 1% solution with respect to water at 25°C.

Av. Number of EO groups	Reduction in Surface Tension	Av. Number of EO groups	Reduction in Surface Tension
0.71	3.3	10.12	28.4
1.28	4.2	14.81	33.9
1.82	5.2	18.66	29.5
1.96	8.3	24.29	25.2
2.03	8.5	27.13	24.2
2.74	10.0	34.61	22.8
4.39	15.2	38.68	22.0
6.21	19.9	47.22	20.9

Table 15. Average number of ethylene oxide units/mole of pentadecyl phenol and the reduction in surface tension of a 1% solution with respect to water at 25°C.

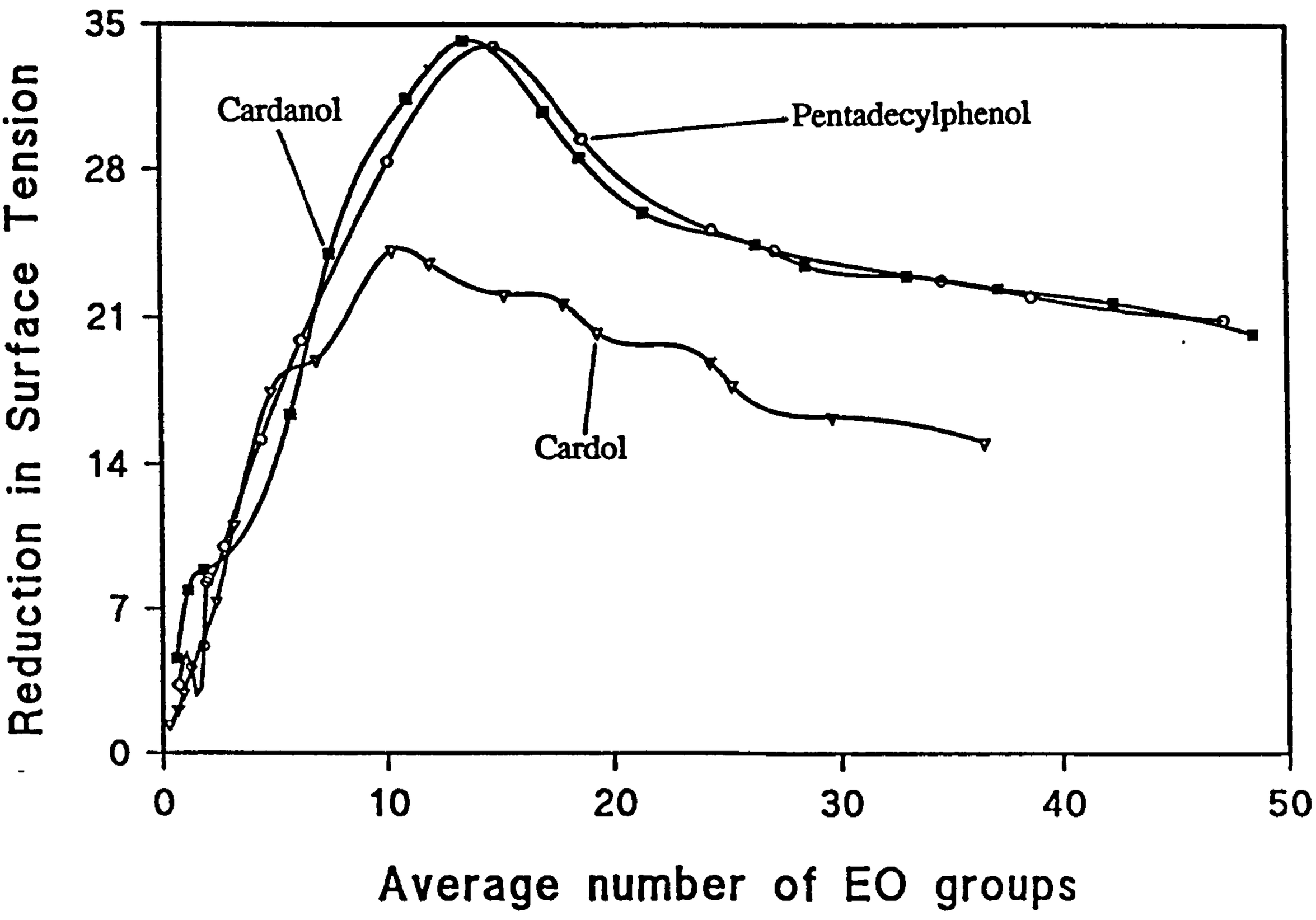


Figure 19. Variation in surface tension with ethylene oxide chain length.

As can be seen from the graphic plots (Figure 19) of ethoxylate number against the reduction in surface tension, the best surfactant properties are apparent when the average number of ethylene oxide units/mole is 14 for both cardanol and pentadecyl phenol and 10.5 for cardol. Thus the samples of cardanol and cardol with 13.44 and 10.33 units/mole respectively were used for the biodegradation studies. In order that unsaturated and saturated samples had exactly the same average number of ethylene oxide units/mole, a small sample of both cardanol 13.44EO and cardol 10.33EO were hydrogenated as previously described.

2.8 Biodegradation Studies of Nonionic Surfactants

2.8.1 Modified OECD Screening Test for the Determination of Biodegradability

The modified OECD screening test is designed to pick out substances which are readily biodegradable. It employs a low concentration (10^2/ml) of microorganisms and has a maximum duration of 28 days. Any substance which requires a period greater than 18 days for acclimation of the relevant bacteria or the presence of another substrate (co-metabolism) will be found to be poorly or non-biodegradable. However this does not necessarily mean that the substance will not be adequately degraded in sewage treatment, but merely that further testing is necessary. Substances passing this test (> 70% DOC removal) can be assumed to be extensively degraded in sewage treatment and will be rapidly removed from bodies of water, and thus do not need further work on them.

Principle - The test compound was dissolved in an inorganic medium at a concentration of 40 - 100mg C/l, the medium was inoculated with a relatively low concentration of microorganisms from a mixed population and aerated at constant temperature for not more than 28 days. Biodegradation was followed by DOC analysis. Control flasks were run in parallel to determine the DOC blanks. The procedure was checked by means of a reference substance of known biodegradability.

Interferences - Any chemical substance in the air, or in solution may adversely affect the growth of microorganisms, examples are: organic solvents, toxic metals, strong alkali and biocides.

The concentration of test substance chosen may be inhibitory to growth of microorganisms. If this was the case then the test should be repeated using a lower concentration (to a minimum of 5mg C/l). Intermediates or products of biodegradation of the test substance may also be inhibitory. Substances interfering in the chemical analytical method used may give false results.

Hygiene - As the inoculum originated from a soil sample, precautions were taken to avoid the risk of infection from possible pathogenic organisms present in the unknown mixed population. Asceptic techniques were employed throughout this work.

Reagents - The water used was free from toxic substances such as copper. In view of the need to determine DOC in the range 1 - 100mg C/l the water had a low organic carbon content. It was suggested that the water contain not more than 10% of the DOC introduced via the test material and that the same batch of water should be used for a test series, so one batch of carbon filtered water was used throughout.

DOC Analysis - There exist few reliable methods for the analysis of low levels of carbon in aqueous solutions. The most reliable and accurate is a carbon analyser which measures levels of dissolved organic carbon down as far as the ppb range. The oxidation of the carbon present in the sample with dichromate is another method and the CO_2 evolved when acid is present can be trapped by aqueous barium hydroxide, which can then be titrated with dilute acid[57]. The use of an Inductively Coupled Plasma (ICP) spectrometer was attempted to measure the carbon present but it was found that at the low concentrations used in these experiments the errors encountered were too great, sometimes up to 100% for the lower concentrations. Thus, the dichromate oxidation method was used to determine the levels of DOC in the samples.

2.8.2 Biodegradation Results.

All the samples were tested in duplicate in case the inoculum failed to grow in one of the test samples. The results from the DOC analyses are shown in Tables 16 and 17 and the average DOC removal worked out as a percentage of the total DOC from Time = 0 days.

Time (days)	Sample 1 % DOC	Sample 2 % DOC	Average % DOC	Glucose Reference
0	100	100	100	100
4	60	64	63	46
8	43	51	47	35
12	30	30	30	25
16	24	27	26	20
20	24	32	28	14
24	21	26	24	11
28	15	18	17	5

Table 16. Biodegradation of cardanol 13.4EO as followed by DOC analysis

The results can be plotted, *ie* time against %DOC which shows how rapidly both cardanol and pentadecyl phenol 13.4EO degrade over the 28 day time period. There is only 17% and 25% respectively of the initial DOC present after 28 Days but cardol and saturated cardol 10.5EO degrade somewhat slower, 37% and 46% respectively but even these are far more biodegradable, in this test, than nonyl phenol 8EO which only degrades to 77% of its original DOC concentration.

The results indicate that the linear hydrophobic chains in the natural phenol samples aid the degradation process more than the branched nonyl phenol samples, as was found by Swisher[58]. The slightly higher biodegradation of the unsaturated phenols compared with the saturated equivalents is probably due to the increased number of biochemical

mechanisms that can operate on the unsaturated members compared to the saturated ones.

Time (days)	Cardol 10.5EO	Saturated cardol 10.5EO	Pentadecyl phenol 13.4EO	Nonyl phenol 8EO
0	100	100	100	100
4	86	90	67	96
8	74	76	44	90
12	61	77	34	91
16	54	57	29	81
20	49	51	34	81
24	41	47	27	79
28	37	46	25	77

Table 17. Biodegradation of cardol 10.5EO, saturated cardol 10.5EO and pentadecyl phenol as followed by DOC analysis.

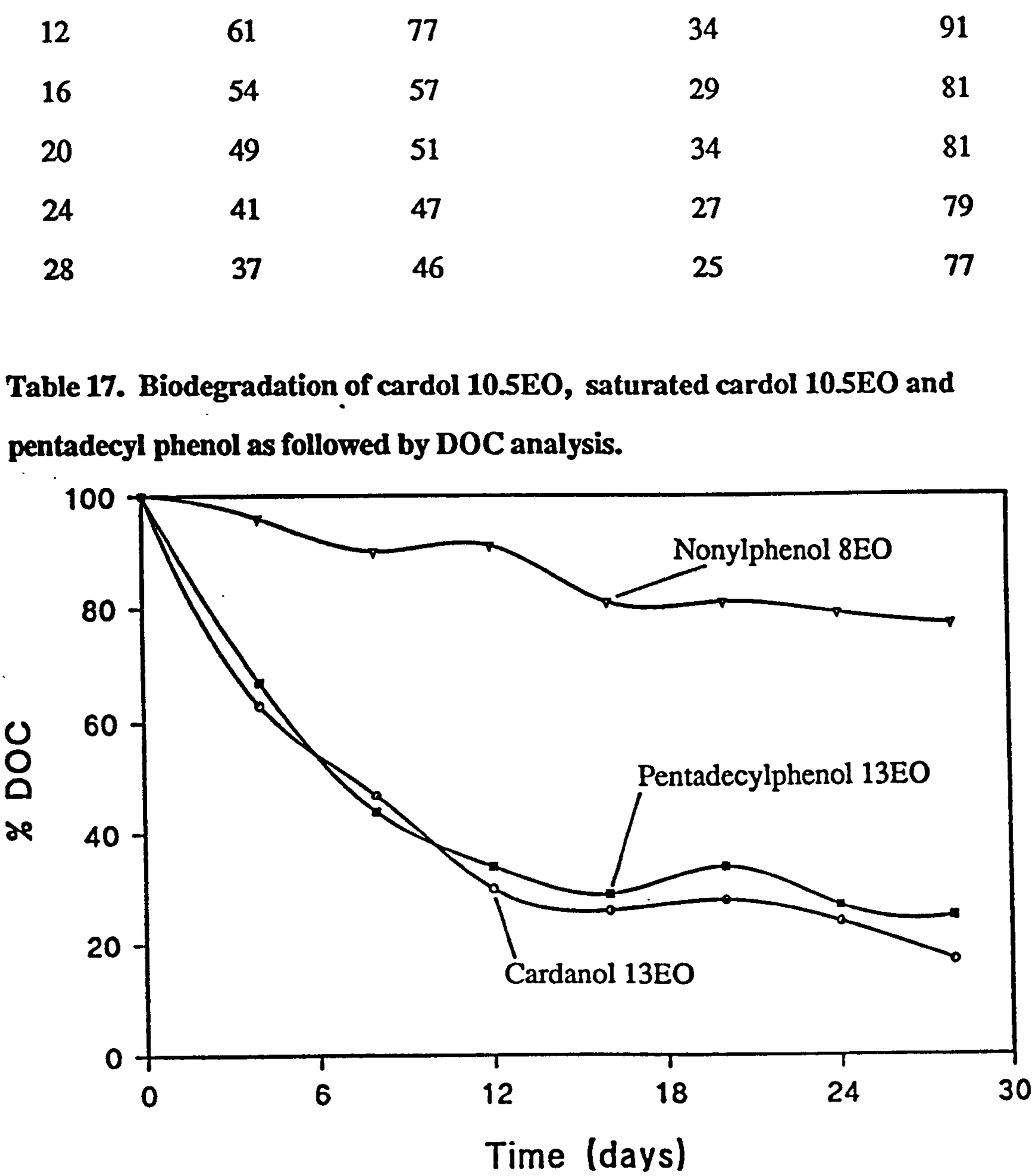


Figure 20. Biodegradation of cardanol and pentadecyl phenol 13.4EO and nonyl phenol 8EO

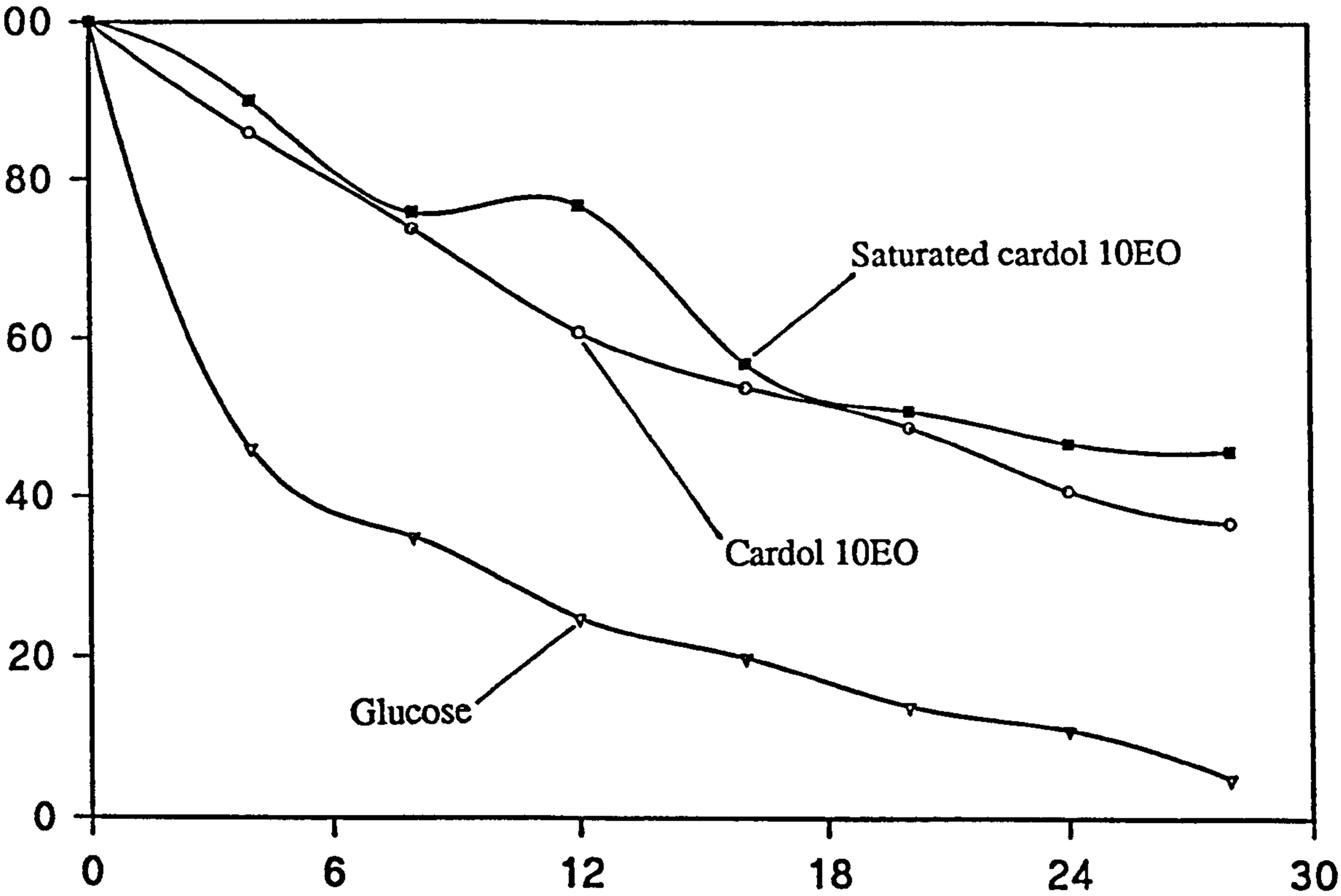


Figure 21 Biodegradation of cardol and saturated cardol 10.5EO and nonyl phenol 8EO

2.9 Conclusions

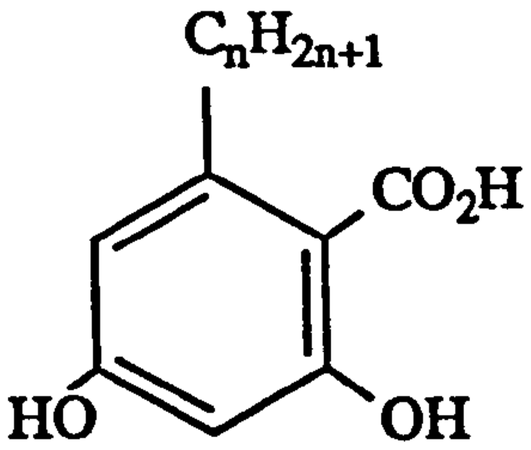
The major phenolic components of technical CNSL were isolated and their base catalysed reaction with ethylene oxide yielded a series of four nonionic surfactants. These were analysed by both nmr and HPLC and their surfactant properties were investigated in terms of their surface tension measurements. From these results, cardanol 13.4EO and cardol 10.5EO gave the best reduction in surface tension. These were tested to find how biodegradable they were by means of a modified 'shake flask' method and their biodegradation followed by DOC removal. They were found to be highly degradable, up to 80% DOC removal after 28 days compared with 23% DOC removal of branched nonyl phenol polyethoxylate.

CHAPTER THREE

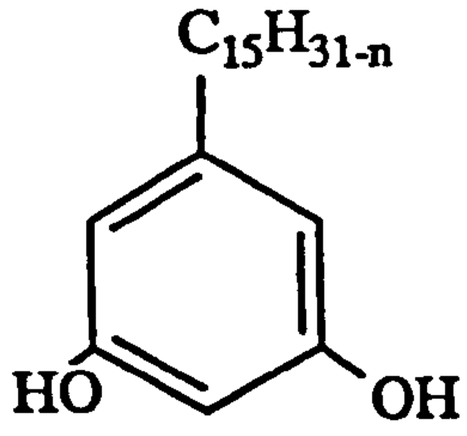
THE SYNTHESIS OF 2,4-DIHYDROXY-6-PENTADECYLBENZOIC ACID

3 The Synthesis of 2,4-Dihydroxy-6-pentadecylbenzoic Acid.

The orsellinic acids (35) have been postulated as biosynthetic intermediates in the phenolic lipid series[63, 64] particularly with reference to the formation of 5-alkyl resorcinols, such as cardols (36), since the acids are readily decarboxylated. In the merulinic acid series, a (15:1)-orsellinic acid (37) has been synthesized by a polyketide route[65] and C_{15}-orsellinic acids have been isolated from natural sources in biosynthetic experiments[64]. However, syntheses of the higher members have been achieved only in poor yields[66].



(35)



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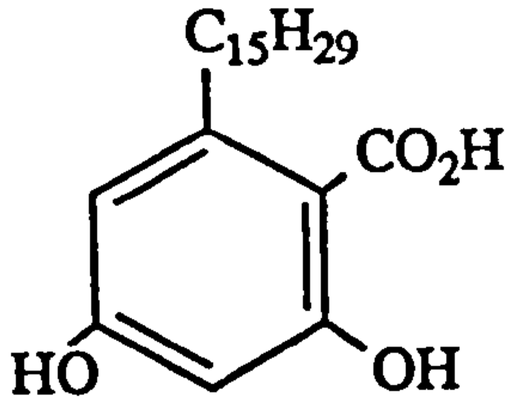
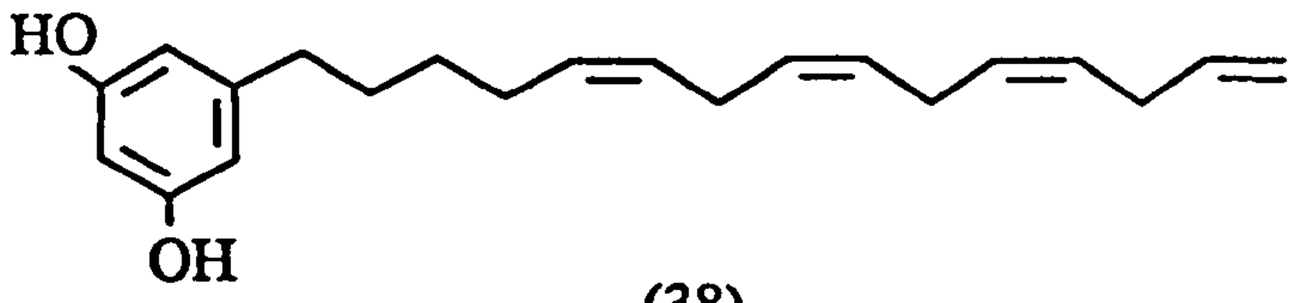


(37)

3.1 Biosynthesis.

The biosynthesis of phenolic lipids of the anacardic acid and orsellinic acid types have been investigated by the ^{14}C acetate method[64] and a polyketide pathway based on malonylcoenzyme A has been established. There had been earlier suggestions that the characteristic substitution pattern in the cashew phenols came about through such a route involving Knoevenagel cyclisation (crotonic) giving an orsellinic structure rather than a Claisen type of intramolecular cyclisation leading to a phloroacetophenone[63, 67, 68]. It was feasible to consider that the process would be similar to the formation of 2-hydroxy-6-methylbenzoic acid (6-methylsalicylic acid), the parent or first member of the anacardic acids, in *Penicilium griseofulvium*[69]. Also a study of the metabolism of *Penicilium madriti* led to the isolation from the culture filtrate of two metabolic products, penicillic acid and orsellinic acid[70]. Orsellinic acid had not previously been recognised as a fungal product although it had been postulated[71] as an intermediate in mould metabolism.

The occurrence of this product, the resorcinol (38) and phloroglucinol (39), indicates that in some species both mechanisms may operate.



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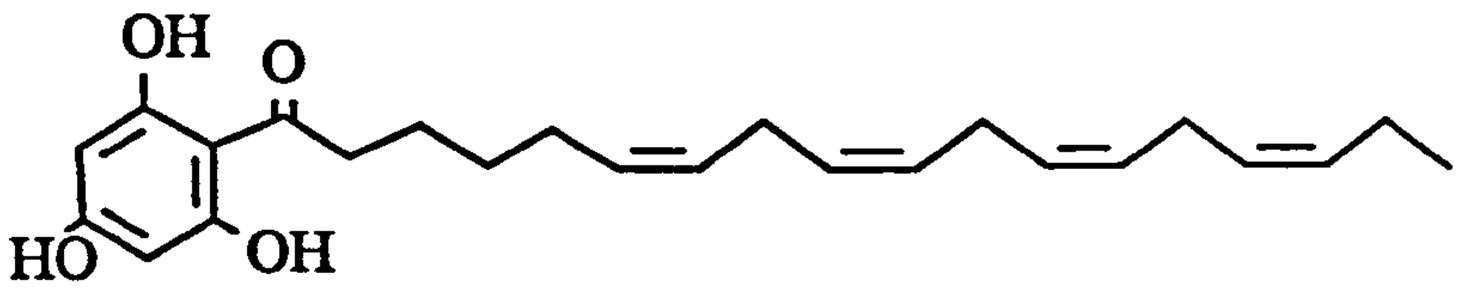

(39)

A full biosynthetic pathway has to account for the simultaneous formation of cardol, 2-methylcardol, anacardic acid, orsellinic acid and some cardanol and for their substitution patterns. The reductive mechanism probably proceeds as far as polyethenoid fatty acids but it is of interest that palmitoleic acid was not found to be incorporated[64]. The conclusion was made that in anacardic acid synthesis the side chain was in a different state of activation and/or site than in the case of common lipids which are found in the triglyceride oil of the cashew kernel.

The oxidative conversion of anacardic acid to urushiol has been suggested[72] although this conversion could not be realised *in vitro*[73]. A 'biological' Dakin reaction on anacardic aldehyde[13] seems possible although a hydroxylation mechanism involving cardanol appears to be feasible and it is of interest that cardanol has been found as a minor component in natural urushiol[74]. The folding mechanism of the polyketide at the intramolecular cyclisation stage has been studied[75] and portrayed schematically.

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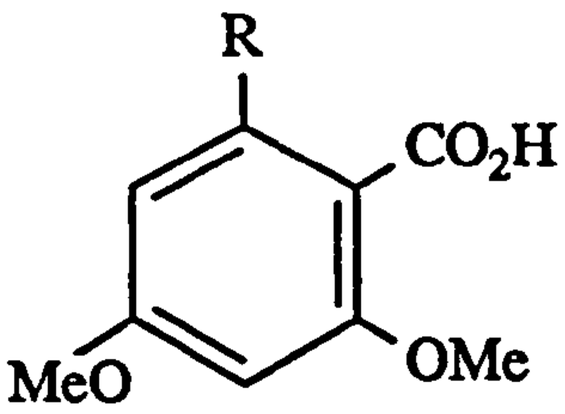
3.2 Synthesis of Orsellinic Acids

Orsellinic acid and its homologues, in the form of their monomethyl (or other protected) ethers **(40)**, are important components of the simple depsides[76] **(41)** and depsidones **(42)** as indeed are many other secondary metabolites of plant and fungal origin. *Ceratocystis ulmi*, the agent responsible for Dutch Elm Disease, contains the orsellinic acid derivative, 2,4-dihydroxy-6-(1-hydroxyacetonyl)benzoic acid[77].



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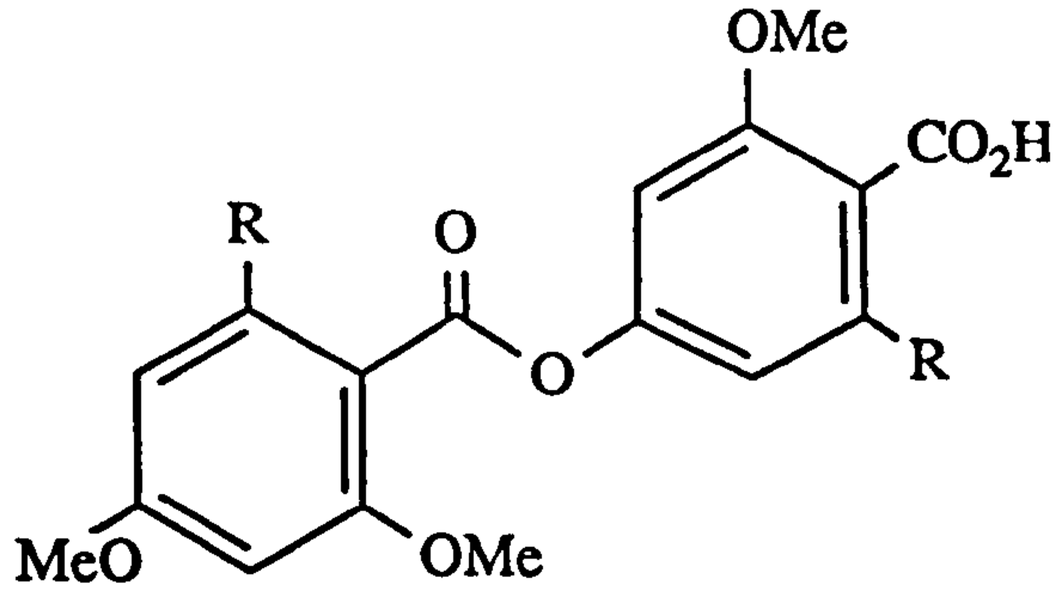

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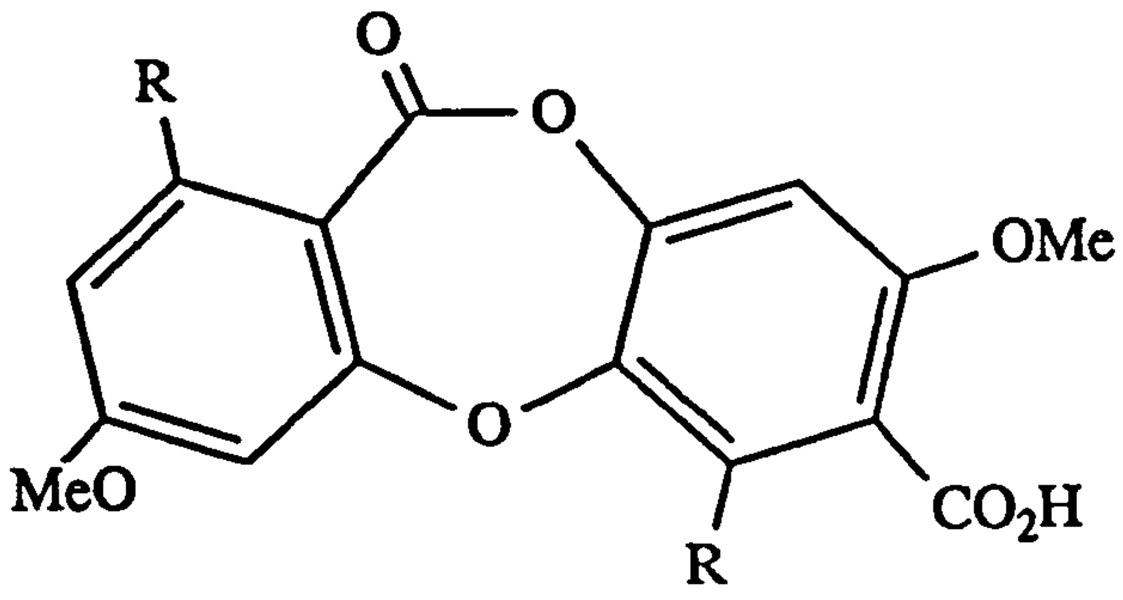


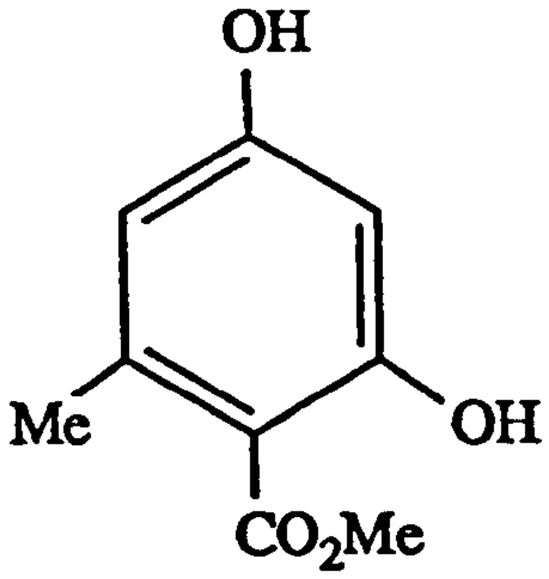
(42)

Although widely distributed, invariably as derivatives, orsellinic acids still remain comparatively little known as their decomposition, and that of their esters[78], complicates synthesis.

Methods have been based on the formylation of 5-alkyl resorcinols[79, 80], for which a variety of procedures are available[81], Michael addition reactions[82] and on biogenetic type models[83].

3.2.1 Synthesis by polyketide type reactions

Barton *et al*[84] considered an alternative method, that of condensation of dimethylmalonate with pentane-2,4-dione which should provide methyl orsellinate (43) and thus avoid the oxidation step (Section 3.2.3) which is often unsatisfactory[85]. This has an analogy in the reported condensation[86] of 3-acetoxyhex-2-en-4-one with dimethyl malonate giving methyl-β-orcinolcarboxylate (44) directly.



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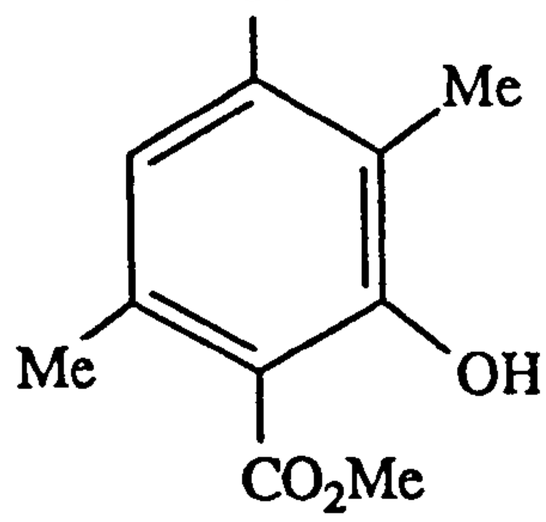
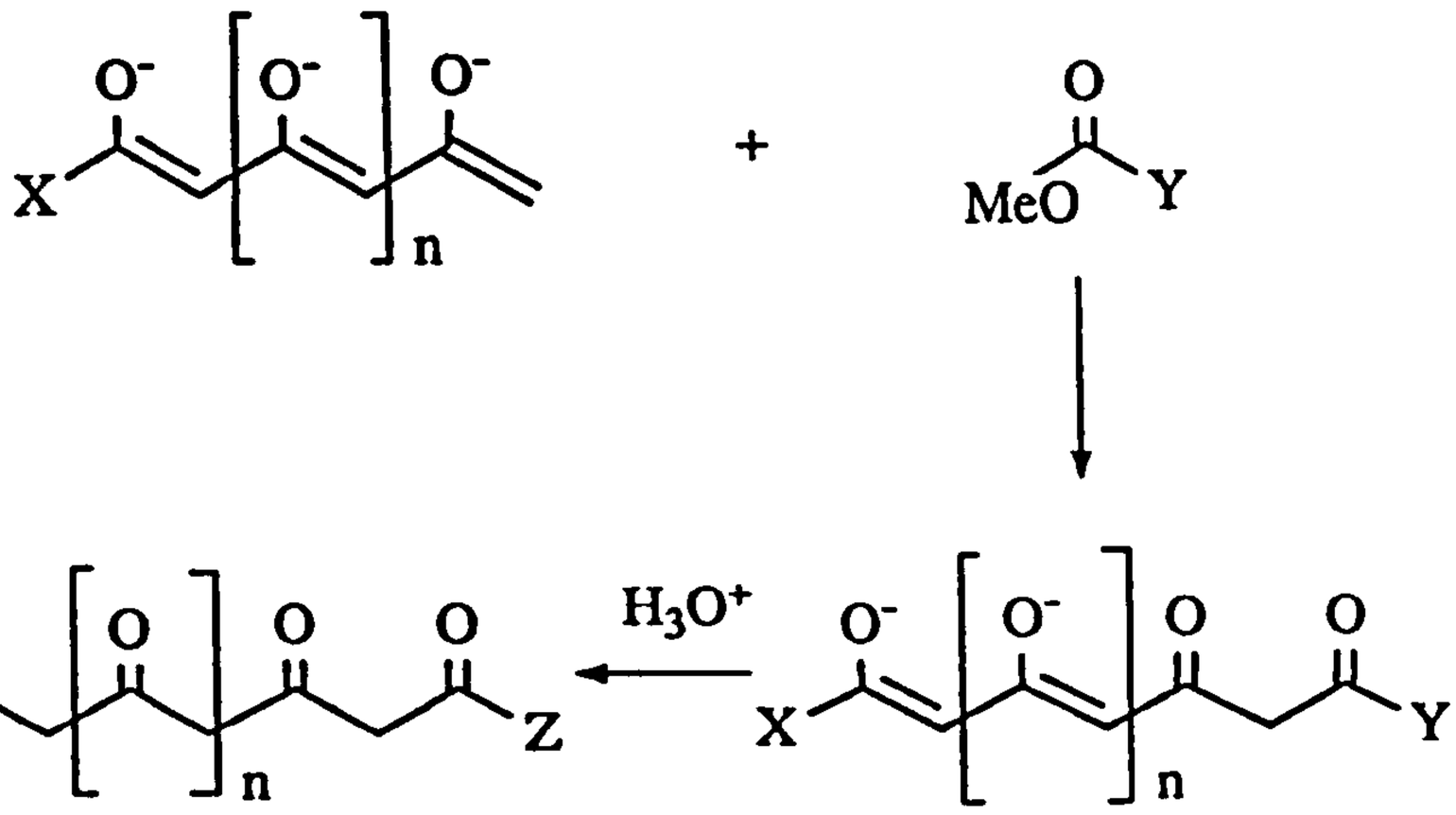


(44)

Harris[87] has reported the high yield conversion of methyl-3,5,7-trioxo-octanoate (45) into methyl orsellinate (43) although the experimental details have not been published.

The condensation of polyanions derived from polyketones or polyketoesters with electrophiles has been widely applied[87] in homologation (Figure 22)



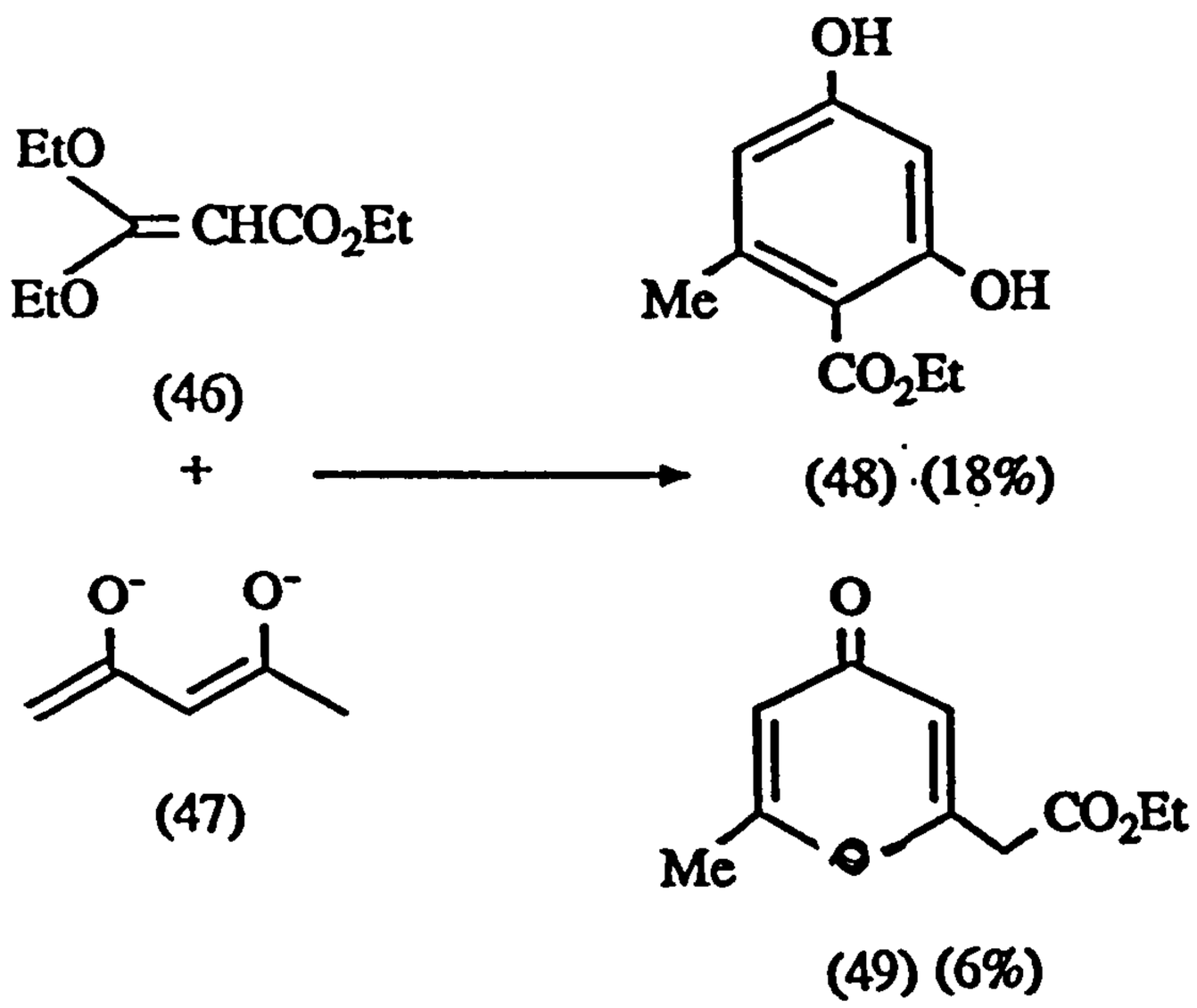
X = Me, MeO

Y = Me, MeO, CH=C(Me)O$^-$

Z = Me, MeO, CH_2COMe

Figure 22. For convenience, products are shown in non-enolised form.

It was considered[84] that the Harris methodology could be modified in order to improve the polyketo-ester route to alkyl-β-resorcylates. Initially, the condensation of the malonate derivative, ethyl 3,3-diethoxyprop-2-enoate (46)[88] with the pentane-2,4-dione dianion (47) was examined. This ester was chosen as a protected malonate to prevent proton transfer to the dianion which was generated with lithium di-*iso*propylamide or sodium hydride and *n*-butyllithium reacted with the ester to give, after treatment with pH 9.2 buffer, ethyl orsellinate (48) and 2-ethoxycarbonylmethyl-6-methyl-4-pyrone (49) presumably formed by cyclisation of the intermediate prior to ester regeneration.



Finally, the β-resorcylate derivatives (35 and 48) were prepared[84] in one-pot reactions. For example, pentane-2,4-dione was treated with sodium hydride, lithium hexamethyldisilazide, dimethyl carbonate, *n*-butyllithium and DMA in sequence to give methyl orsellinate in 21% yield. Similarly, 3-methylpentane-2,4-dione and pentane-2,4-dione gave (48) in 18% yield.

3.2.2 Synthesis by Michael Addition reactions

The use of open-chain precursors to the benzenoid ring in anacardic and orsellinic acids has proved a fruitful approach. Ethyl 2-methoxy-6-methyl benzoate (synthesized through the Michael addition of ethyl acetoacetate with but-2-en-1-al, followed by

through the Michael addition of ethyl acetoacetate with but-2-en-1-al, followed by cyclisation and aromatisation), has been alkylated in an aprotic solvent after formation of the carbanion with lithium di-*iso*propylamide (Figure 23a)[89 - 91]. In a similar way ethyl-2,4-dimethoxy-6-methyl benzoate (fully protected ethyl orsellinate formed from ethyl acetoacetate and ethyl crotonate followed by aromatisation and methylation)[82] can be alkylated (Figure 23b)[92]. Thus, in this way it may be possible to synthesise the C_{15}-orsellinic acid precursor to some of the component phenols in CNSL and this method may indirectly afford another route into the cardol series.

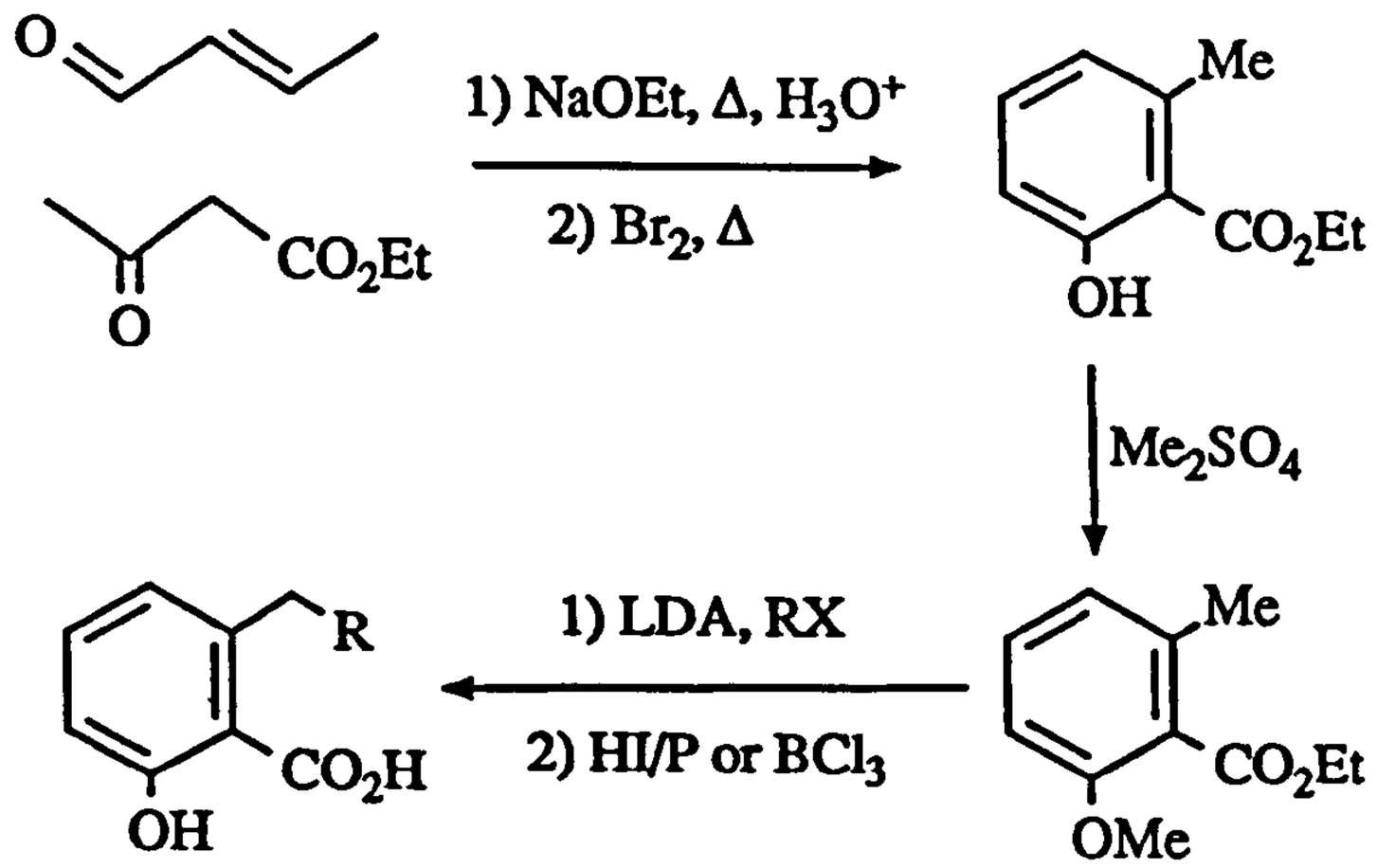


Figure 23a. Synthesis of a C_{15} salicylic acid

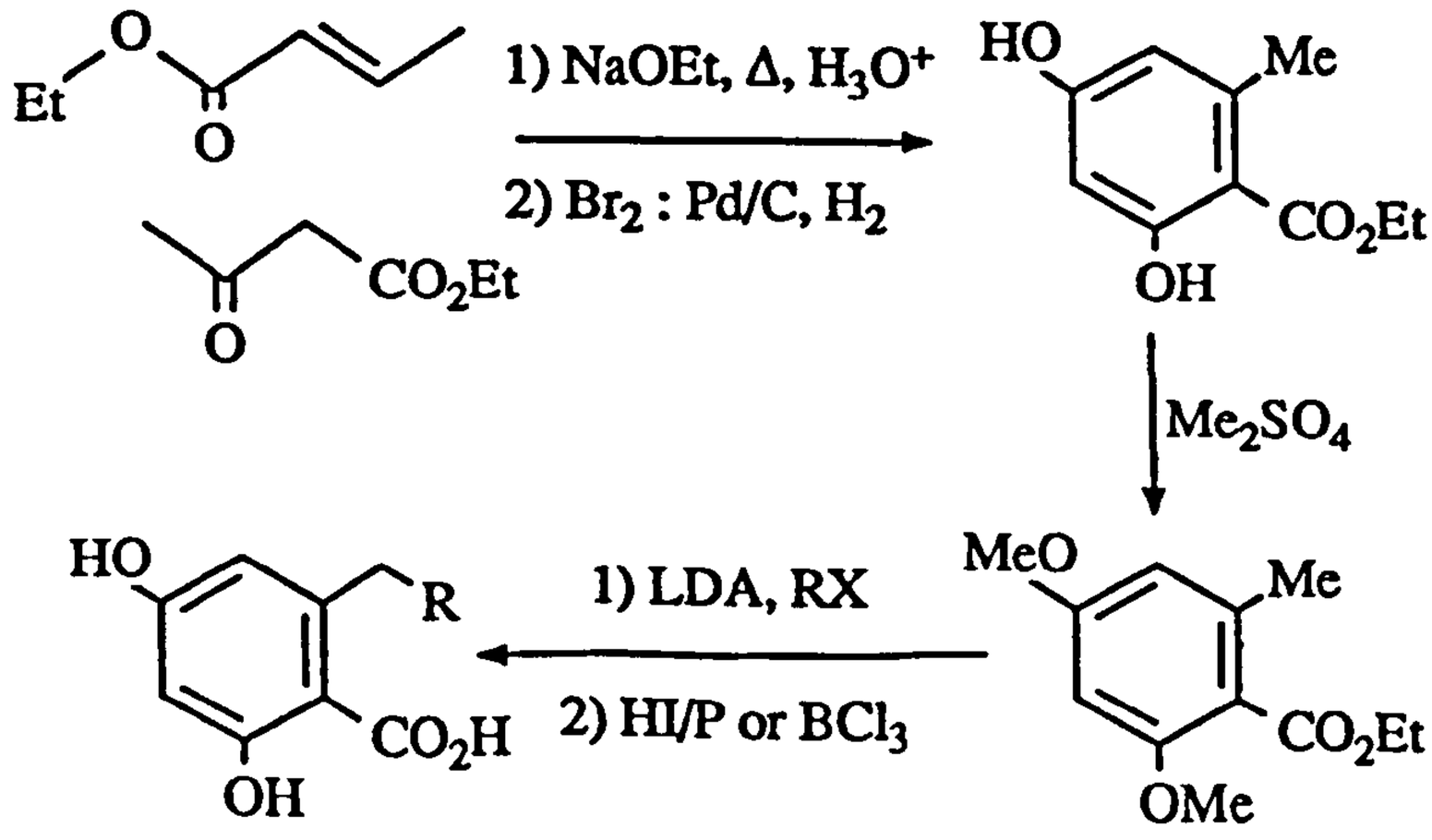
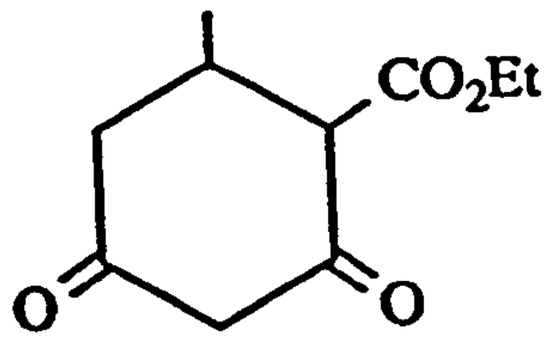


Figure 23b. Synthesis of a C_{15} orsellinic acid.

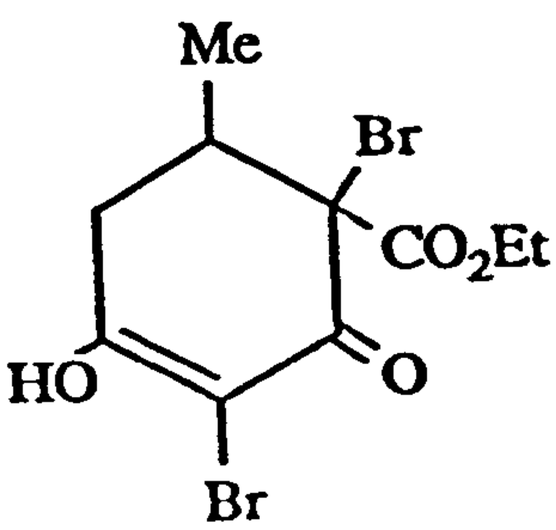
3.2.3 Synthesis of a C_{15} Orsellinic Acid

Route 1 - following essentially the method by Gaucher and Shepherd[82] ethyl crotonate was condensed with ethyl acetoacetate in the presence of sodium ethoxide to give ethyl dihydro-orsellinate **(50)** in 62% yield, slightly lower than reported. This reaction follows a Michael-type reaction.



(50)

The existing method for the conversion of the dihydro compound into ethyl orsellinate **(48)** was by bromination and subsequent debromination[78, 93]. Some confusion[93, 94] is apparent in the literature concerning the products of bromination of ethyl dihydro-orsellinate. Contrary to the results of Santesson[93], rapid bromination of the ethyl ester **(50)** with 2 molar equivalents of bromine gave an cyclic dibromo-compound formulated as **(51)** on the grounds of its nmr spectrum. If the mixture, resulting from the rapid addition of 2 molar equivalents of bromine to the ester **(50)**, was allowed to remain in contact with the hydrogen bromide generated in the reaction then the chief product isolated was the bromo-compound **(52)**, accompanied by a little of the dibromo-compound **(53)**[95]; evidently elimination occurs under these conditions.



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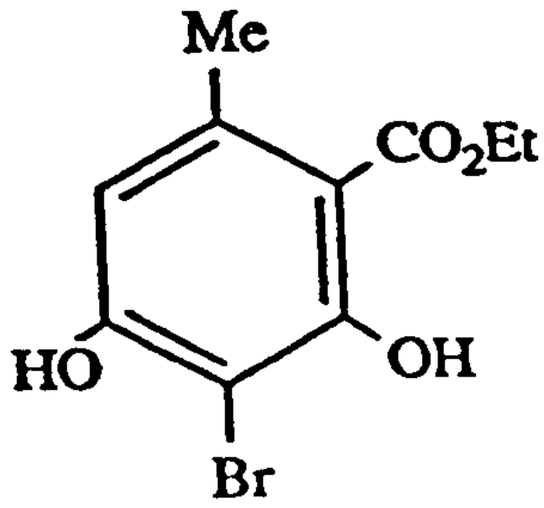

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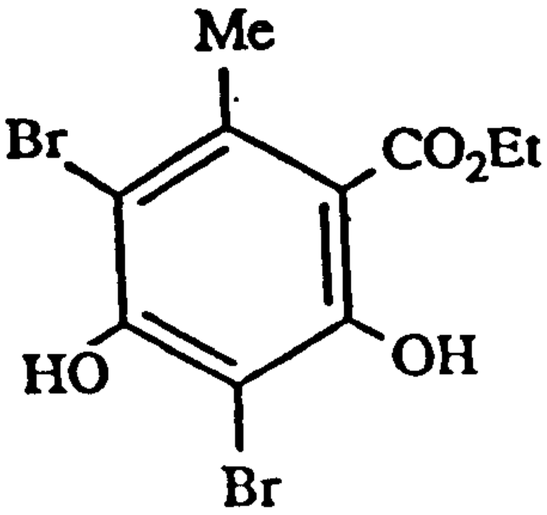
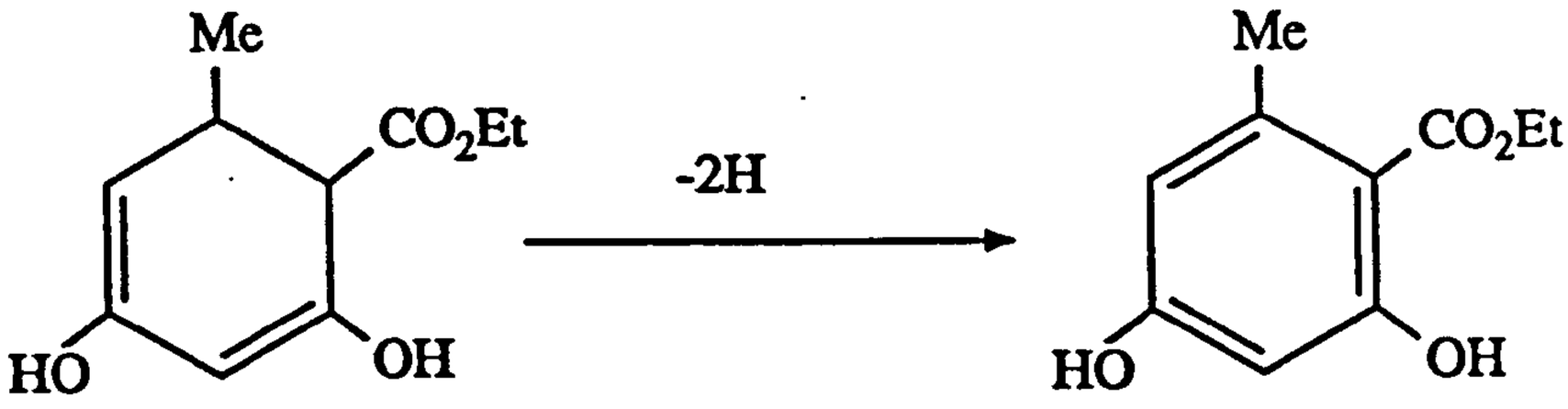


(53)

The structure of the bromo-compound **(52)** followed from its conversion into the known dimethyl ether[96]. Treatment of the dihydro-ester **(50)** with 3 molar equivalents of bromine for an extended period of time gave the dibromo-derivative **(53)** in agreement with the results of Ansell and Culling[95]. This underwent smooth debromination[93] and afforded ethyl orsellinate **(48)**.

As ethyl dihydro-orsellinate only requires the loss of two protons for it to become aromatic, two oxidising/dehydrogenating agents were used to try to affect this reaction. The reaction with selenium dioxide was unsuccessful.



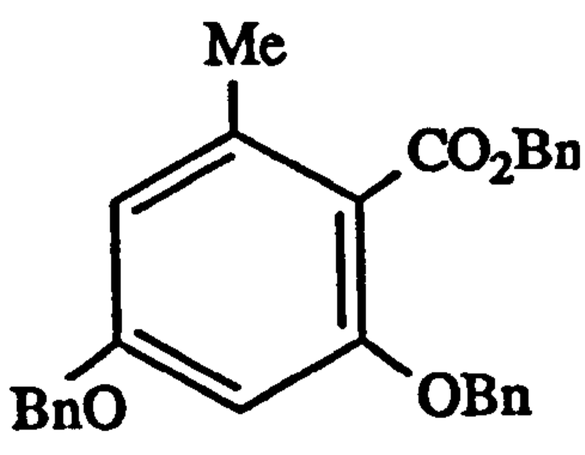
The facile dehydrogenation of chromanones to chromones has been reported[97], using the convenient reagent 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) as an oxidant. This reagent did convert the dihydro product into ethyl orsellinate but the overall yield was only 20 - 25% whereas via the bromination/debromination route the overall yield was found to be between 40 - 50%.

The next step in the reaction scheme was to protect both hydroxyl groups prior to the alkylation reaction. Two protecting groups were utilized at this stage, namely, methyl and benzyl groups. These were chosen because of their ease of removal especially the benzyl groups which could be removed by simple hydrogenolysis.

The transesterification of ethyl 2,4-dibenzyloxy-6-methyl benzoate to benzyl-2,4-dibenzyloxy-6-methyl benzoate **(54)** was also attempted by two methods:

(a) Reaction of the benzyl alcoholate anion with ethyl orsellinate via nucleophilic displacement of the ethoxy anion. Equilibrium can be shifted to the right by removal of any liberated ethanol. This was attempted but was found to be unsuccessful.

(b) A titanate mediated transesterification was also attempted. This method is extremely mild and is compatible with a large variety of functional groups without affecting either any acid and the alcohol components present. The ethyl dibenzyloxy orsellinate and tetraethyl titanate were heated to 100°C overnight with benzyl alcohol but upon work-up there was no trace of the required product **(54)**. This could be due to the space around the ester group being too crowded since the reaction intermediate is the species **(55)** and as such the benzyloxy group might not be able to get close enough thus making it impossible for reaction to occur.



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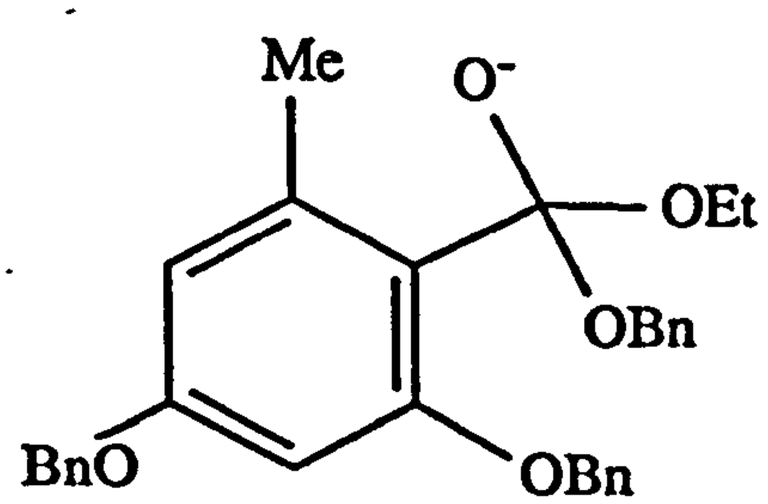

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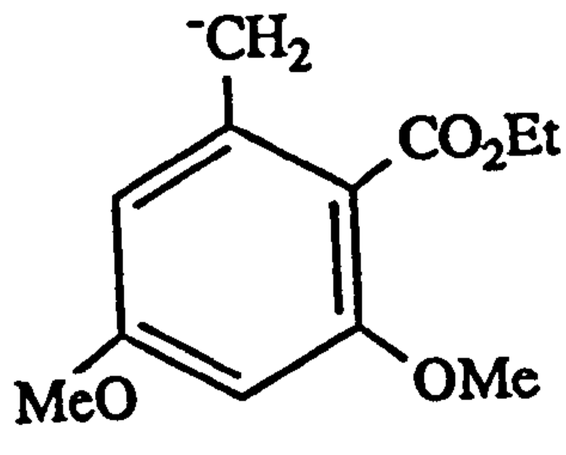
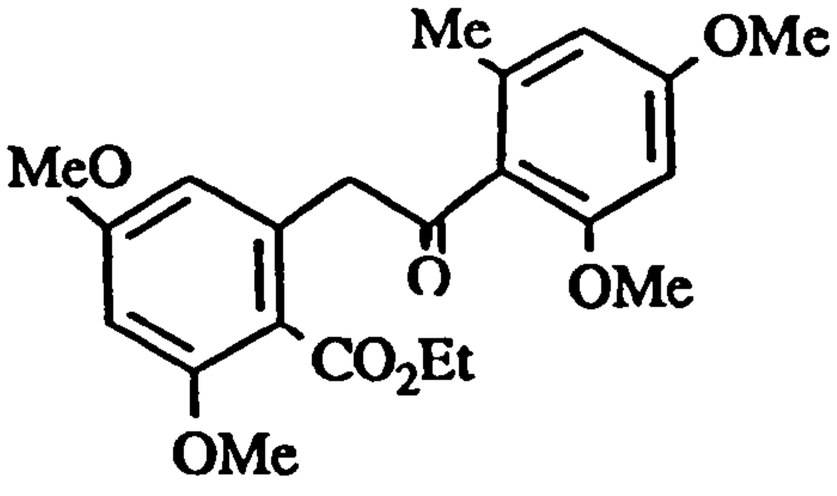


(56)

The next step in the reaction sequence was the alkylation of the 6-methyl position. Staunton *et al*[92] describe the formation of the anion **(56)** with lithium di-*iso*propylamide in an aprotic solvent such as HMPA or NMP. The conditions used were repeated, and as an examination to prove the anion was formed a small amount was quenched with D_2O giving levels of deuteration approaching 100% by nmr and MS. The anion is stable for a few hours if kept at -78°C but after longer periods or at higher temperature decomposition is observed. The decomposition products **(57)** and **(58)**, produced by self-condensation, and the dimer **(59)** can also be isolated as by-products of some of the reactions of the anion.



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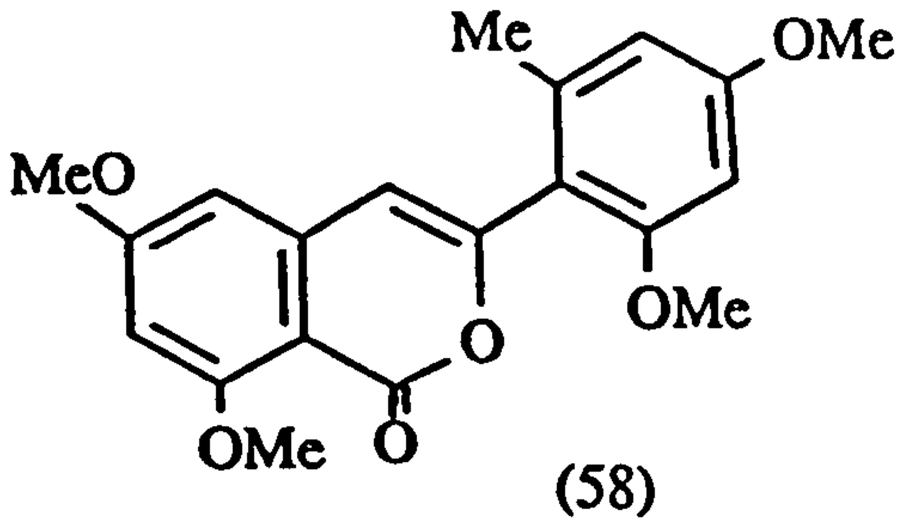

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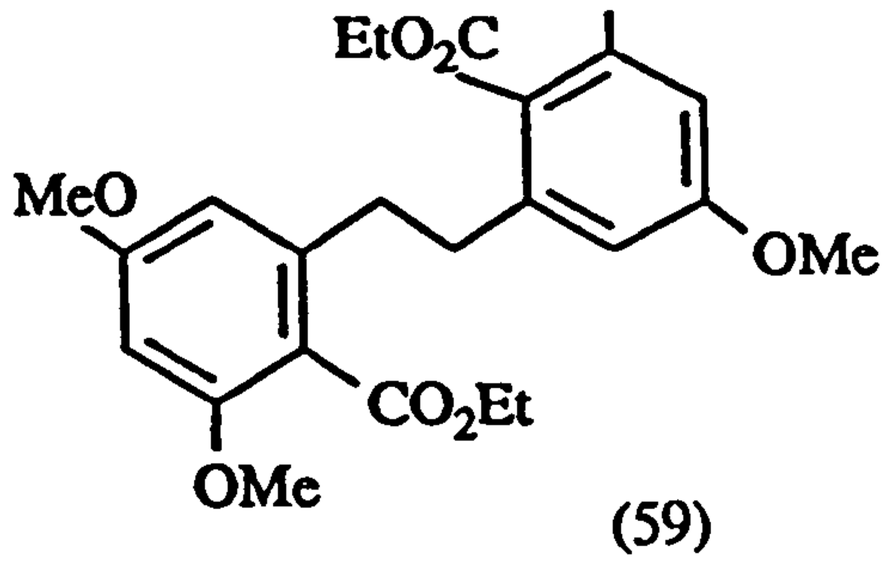
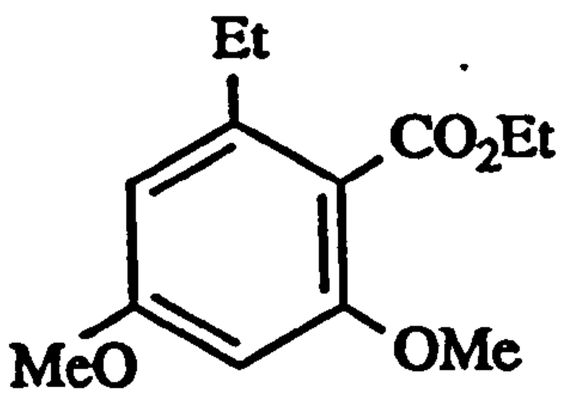


(59)

The anion is reported to react with a variety of electrophiles so initially the reaction was attempted with methyl iodide to give ethyl 2,4-dimethoxy-6-ethylbenzoate (60). The methyl iodide was added to the deep-red THF solution of the anion, at -78°C, with a little HMPA. After work-up the required product was isolated in good yield (61%) with some of the aforementioned side-reaction products (57 - 59).



(60)

The anion was then reacted with 1-bromotetradecane under the same conditions but only a low yield (3 - 5%) of the required ethyl 2,4-dimethoxy-6-pentadecylbenzoate was recovered. Attempts were made to try and improve the yield by using 1-iodotetradecane, formed by halogen exchange of 1-bromotetradecane, and using a different aprotic solvent, such as NMP, but these changes had little effect on the overall yield.

The deprotection steps were carried out as described[91], removal of the ester group by base hydrolysis followed by removal of the two methoxy-groups with dimethylboron bromide to give the desired C_{15} -orsellinic acid.

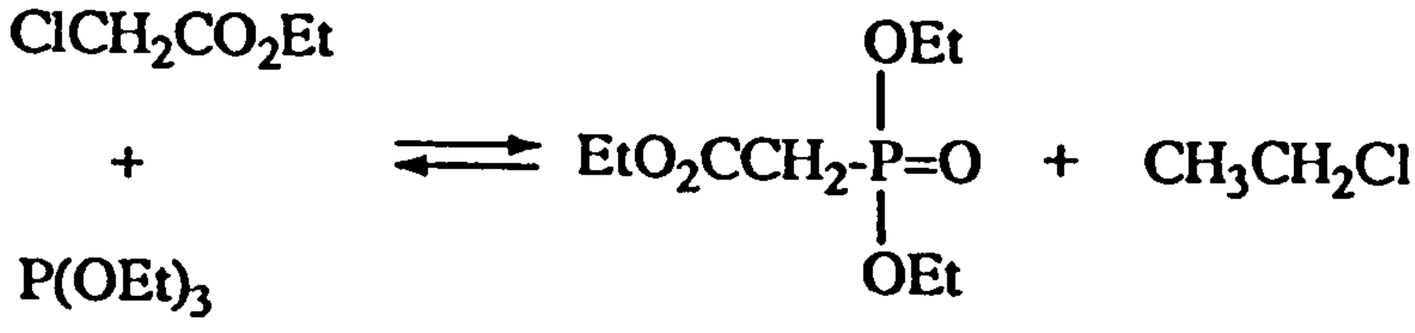
The attempted alkylation of ethyl 2,4-dibenzyloxy-6-methylbenzoate was unsuccessful giving no trace of the required product. This could be due to the LDA forming anions not only on the toluyl group but also on the benzyl methine positions giving a whole range of products.

This reaction scheme was not totally satisfactory as larger yields of the C_{15}-orsellinic acid were desirable, thus, a further method was attempted, a Horner-Emmons modification to the Wittig reaction.

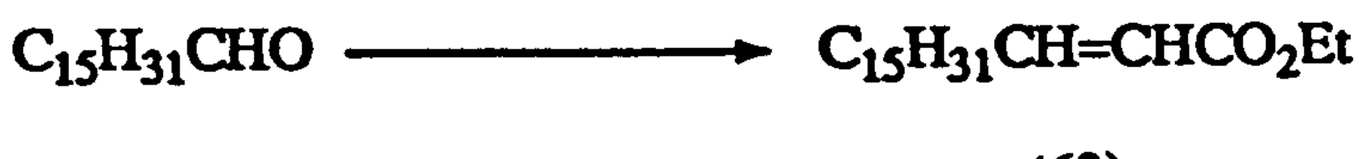
Route 2 - Marmor[99] reported improvements to the method described[78] which are of importance to workers requiring 5-alkyl resorcinols in pharmaceutical research and, in particular, in the preparation of Cannabis analogues. The outline of the synthesis can be seen in Scheme 3.

Before this reaction scheme could be used the aldehyde, hexadecan-1-al (**61**), had to be synthesized and as the aldehyde polymerises on standing it had to be made just prior to reaction. This was achieved by oxidation of hexadecan-1-ol with pyridinium chlorochromate (PCC), and the 'waxy' aldehyde used immediately in the next reaction step.

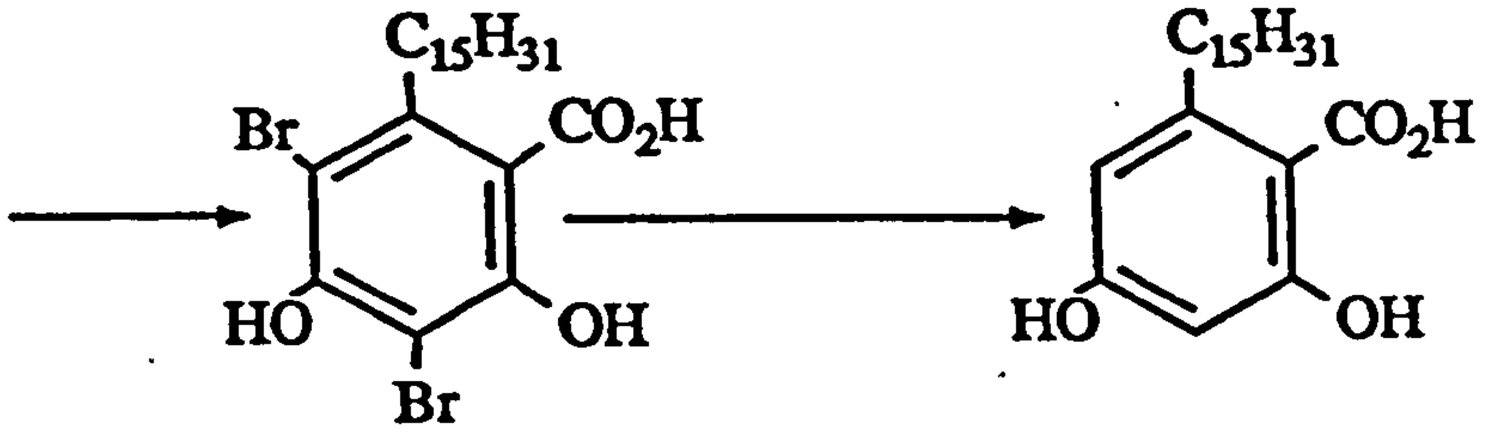
Prior to this reaction, triethyl phosphonacetate was synthesized by reaction of ethyl chloroacetate with triethyl phosphite at elevated temperature to drive off the excess ethyl chloride and therefore force the equilibrium to the right and the required product.



The next reaction in the scheme was the Horner-Emmons modification to the Wittig reaction, whereby the aldehyde was reacted with the sodium salt of triethyl phosphonoacetate. The ethyl β-alkylacrylate (62) was condensed in the same manner with ethyl acetoacetate as previously described. The bromination was also accomplished to the dibromoester followed by de-esterification with concentrated H_2SO_4 at 0^0C and debromination either by reduction or by warming in DMF, though the latter did cause a little decarboxylation. This yielded the required compound namely 2,4-dihydroxy-6-pentadecylbenzoic acid in better yields than had previously been reported.



(61) (62)



(63) (33)

Conclusion

A good overall yield (30%) of C_{15}-orsellinic acid was obtained by a route which incorporated the Horner-Emmons modification to the Wittig reaction and utilised existing methodology which has been well documented.

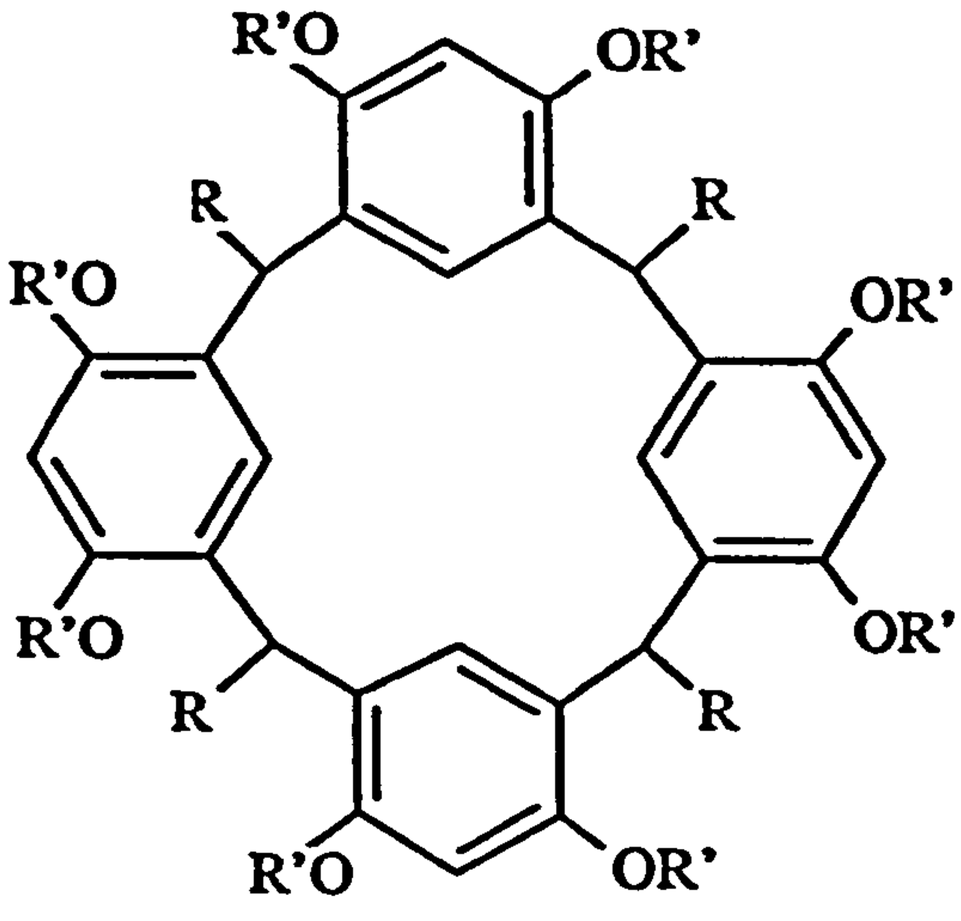
CHAPTER FOUR

CAVITANDS

4. Cavitands

The formation of crystalline, high melting products by the acid catalysed condensation of resorcinol with acetaldehyde[100-104] or higher aliphatic aldehydes[102, 103] or by the reaction of resorcinol with acetylene in the presence of mercuric salts[101, 105] is well known. At first, these products were thought to be of low molecular weight and were assigned various acetal[106], diphenylalkane[102, 105] or vinyl resorcinol[101] structures. Nierderl and Vogel[103] obtained a single product from the reaction of resorcinol with acetaldehyde in aqueous sulphuric acid and assigned it the macrocyclic structure **(64)**. The mass spectrum of an octamethyl ether, prepared by Erdtman *et al*[104], was in agreement with this structure **(65)**.

It has also been found that under similar conditions resorcinol reacts with several aromatic aldehydes such as benzaldehyde and *p*-bromobenzaldehyde to give macrocycles **(66)** and **(67)**.



(64) R = CH_3; R' = H

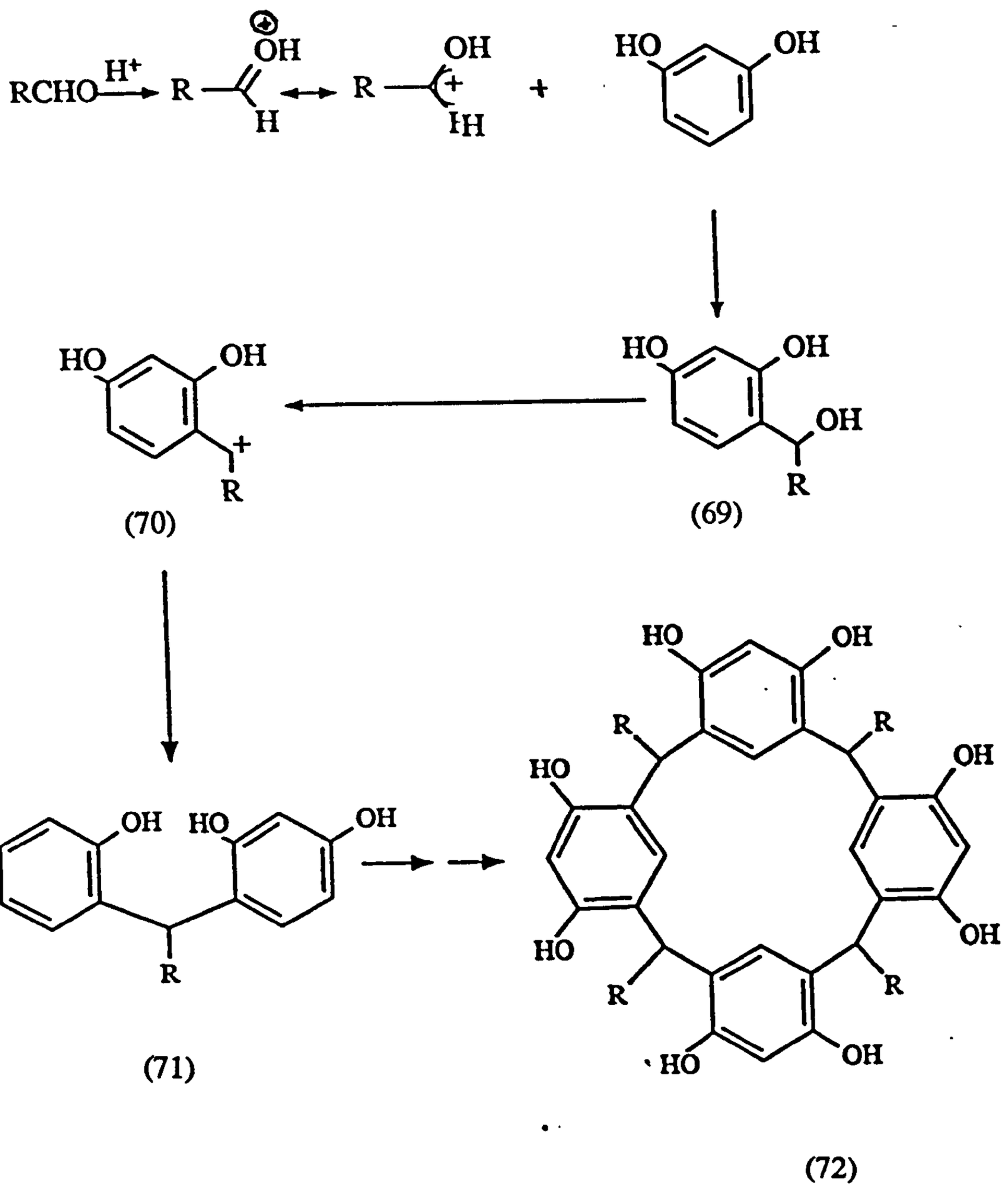
(65) R = R' = CH_3

(66) R = C_6H_5; R' = H

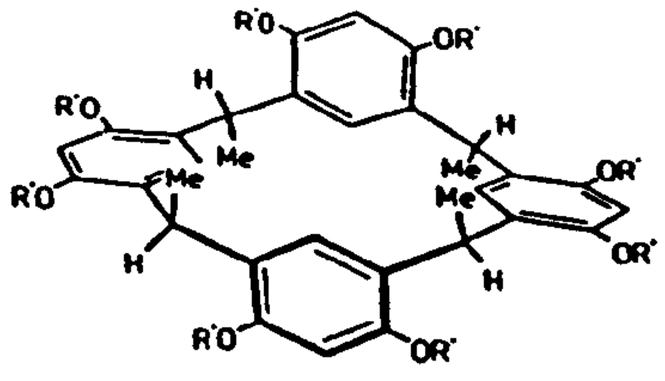
(67) R = C_6H_4Br; R' = H

The acid-catalysed condensation of resorcinol with aldehydes is most logically interpreted in terms of the cationic intermediates **(68)** and **(70)** and electrophilic aromatic substitution reactions to form **(69)**, **(71)** and **(72)** as portrayed in Figure 25. It is not known whether the cyclic tetramer forms by cyclodimerisation of a pair of hydroxydimethylated dimers derived from **(71)** or from a simple cyclisation of a

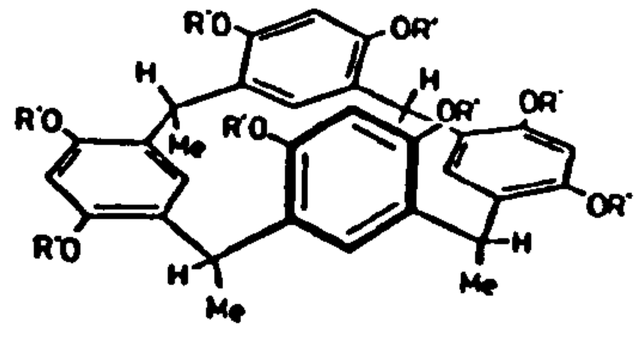
hydroxymethyl linear tetramer. It is not clear what the driving forces are for cyclisation. Although the eight extraannular -OH groups of these resorcinol-derived cyclotetramers cannot engage in circular hydrogen bonding (*cf.* calixarenes), pairwise hydrogen bonding is possible and may play a dominant role in organising the system for cyclisation even under acidic conditions.



The reaction of resorcinol with acetaldehyde in aqueous hydrochloric acid at 75°C for 1 hour gives a phenolic precipitate which can be acylated[107]. Fractional crystallisation of the acylation products gave the two isomers **(68a)** (13%) and **(68b)** (47%).



(a)



(b)

(64) R' = H
(68) R' = $COCH_3$

However, when reaction is carried out in a mixture of ethanol and concentrated hydrochloric acid, no precipitate is observed but on addition of water to the solution, a small amount of phenolic product precipitated which on acylation gave **(68b)** (12%) as the only product. When a mixture of ethanol and concentrated hydrochloric acid is used, only phenol **(64b)** is precipitated yielding 57% of the octaacetate upon acylation.

The symmetry properties and the temperature dependence of the two isomers are similar to those that have been discussed for resorcinol-benzaldehyde cyclotetramers[108]. Thus the cyclophane **(64a)** is assigned a *cis*, *trans*, *trans* configuration and a chair-like conformation with the four methyl groups in axial positions, know as C_{2v}, and the cyclophane **(64b)** an all-*cis* configuration and a boat-like conformation with the four methyl groups also in axial positions, known as C_{4v}. The activation energies for pseudorotation of the flexible octaesters **(68b)** and **(68b)** were found to be 60.3kJmol^{-1} (14,4kcalmol^{-1}) and 63.7kJmol^{-1} (15.2kcalmol^{-1}) respectively, as determined by the coalescence point approximation[109]. Apparently isomer **(64a)** does not precipitate from the more solubilizing ethanolic reaction mixtures. Since the condensation reactions are reversible[108], precipitation of the less soluble isomer **(64b)** serves as a thermodynamic sink, driving the reaction toward the formation of one macrocyclic end product.

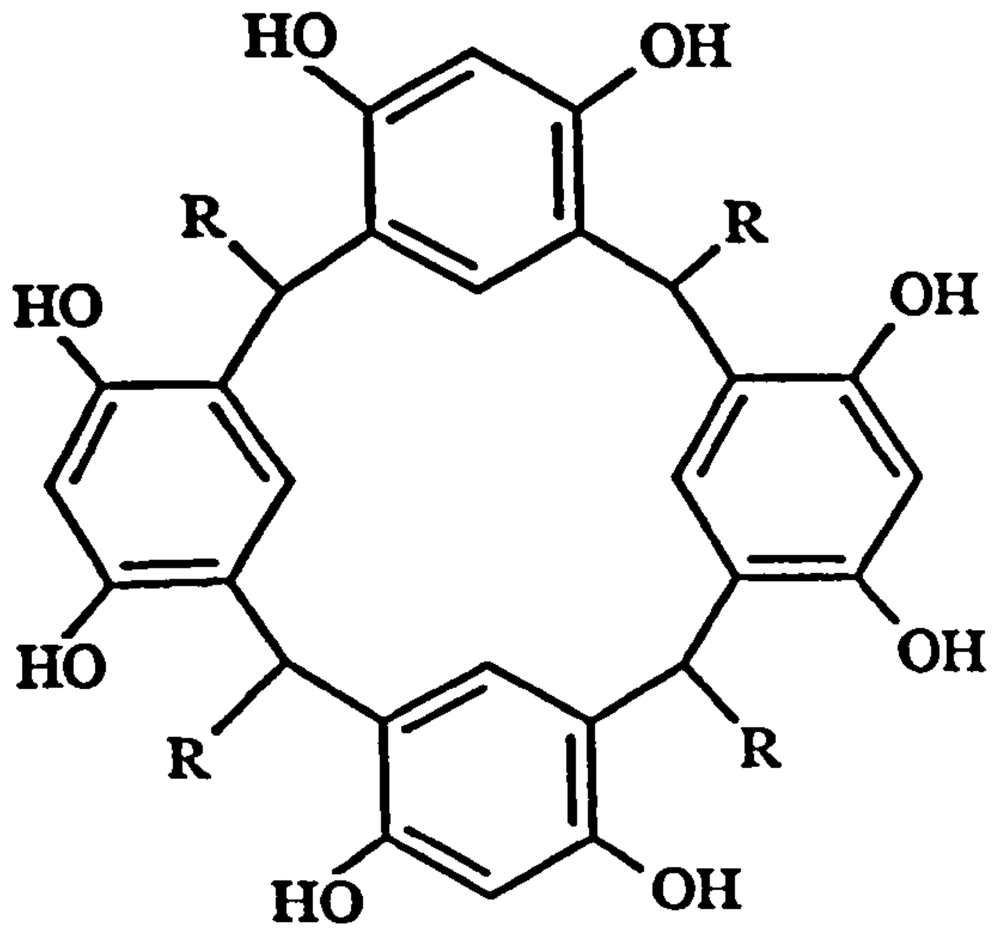
Previous work demonstrated that the four proximate oxygen atom pairs of the C_{2v} isomer **(64b)** can be bridged with four methylene, dimethylene or trimethylene[110], four dialkylsilylidene groups[111] or four 2,3-disubstituted-1,4-diazanaphthylene groups[112]. These bridges constrained the conformational mobility of the aryl groups originally present in **(64)** to produce bowl-shaped cavitands whose width and depth are partially

determined by the molecular dimensions of the bridges. The low solubility of these cavitands limit their usefulness as starting materials for making organic catalysts or carcerands[113]. This limited solubility of the cavitands and carcerands appears to be associated with their rigidity but conformationally mobile hydrocarbon chains such as R in **(64)** would increase the solubility of the derived cavitands and carcerands.

4.1 Synthesis of Cavitands.

Cardol **(8 - 10)** is a by-product from the purification of the more useful phenolic lipid, cardanol, from CNSL. Thus, as a 5-alkyl resorcinol it may form a cyclotetramer with a low molecular weight aldehyde such as formaldehyde or acetaldehyde. Initially, work was carried out on cyclotetramers based on resorcinol and several new structures were synthesized.

Resorcinol was condensed with three aldehydes, acetaldehyde, dodecanal and hexadecanal to give the products **(64)**, **(75)** and **(76)** respectively. The last of these was used in order to obtain the cyclotetramer with four C_{15} chains, as is found in the natural phenols from CNSL.

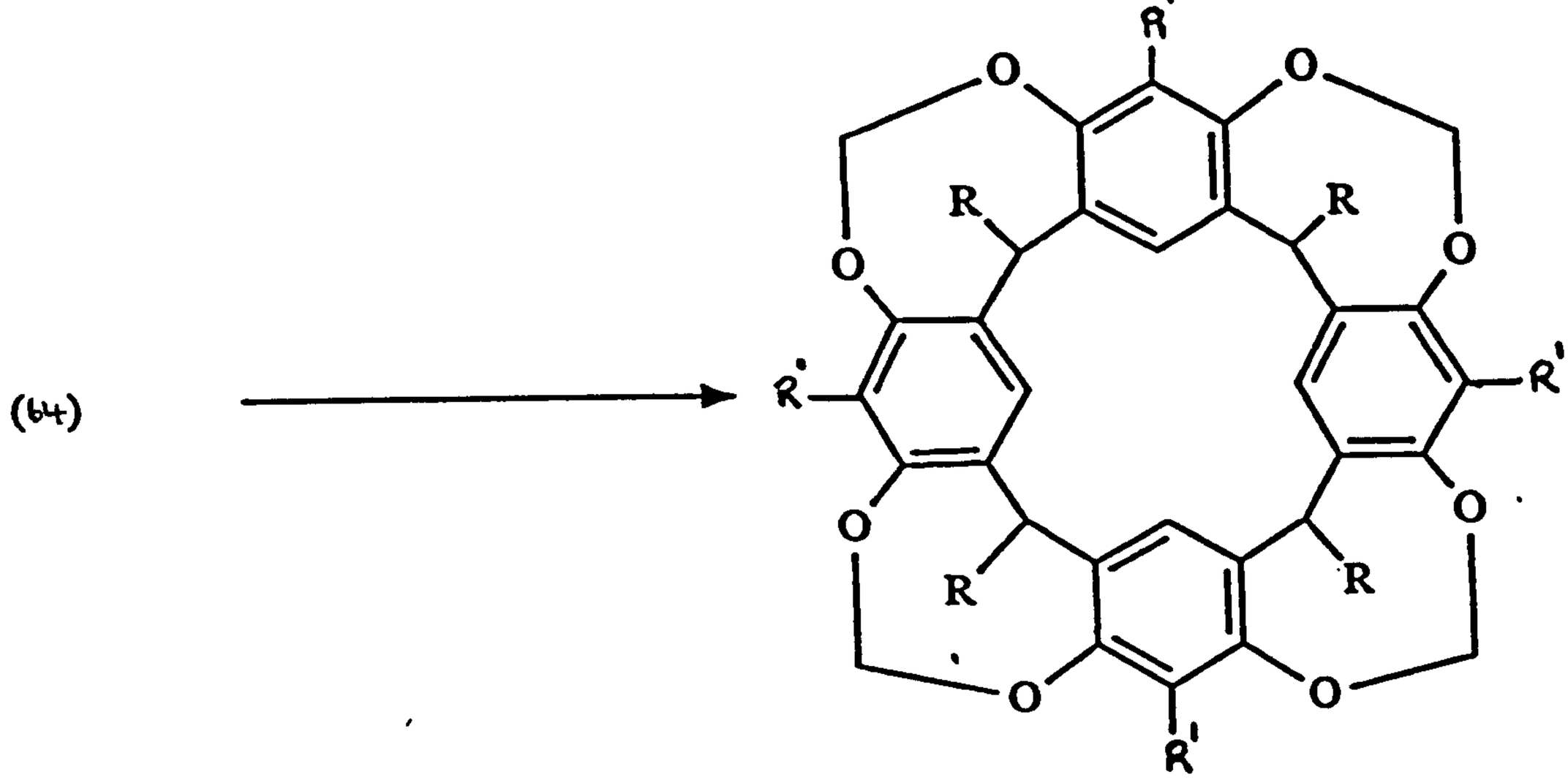


(75) $R = C_{11}H_{23}$

(76) $R = C_{15}H_{31}$

The same problems were encountered using hexadecanal as were earlier described (Section 3.2.2) *ie*, hexadecanal on standing polymerises, so it had to be synthesized from hexadecanol with PCC immediately prior to use. Yields for **(64)**, **(75)** and **(76)** were 59%, 68% and 38% respectively. The yield of **(76)** was low due to the fact that some of the hexadecanal polymerised prior to reaction and also the product needed to be recrystallised four times before submission for spectral analysis as some non-cyclised material was still apparent, by TLC, after two recrystallisations. Spectral properties of this novel cyclotetramer were similar to that of **(75)**[114] but the solubility of **(76)** was slightly greater in organic solvents and it was also discovered by Perry[115] that **(76)** formed a Langmuir-Blodgett film whereas **(75)** did not.

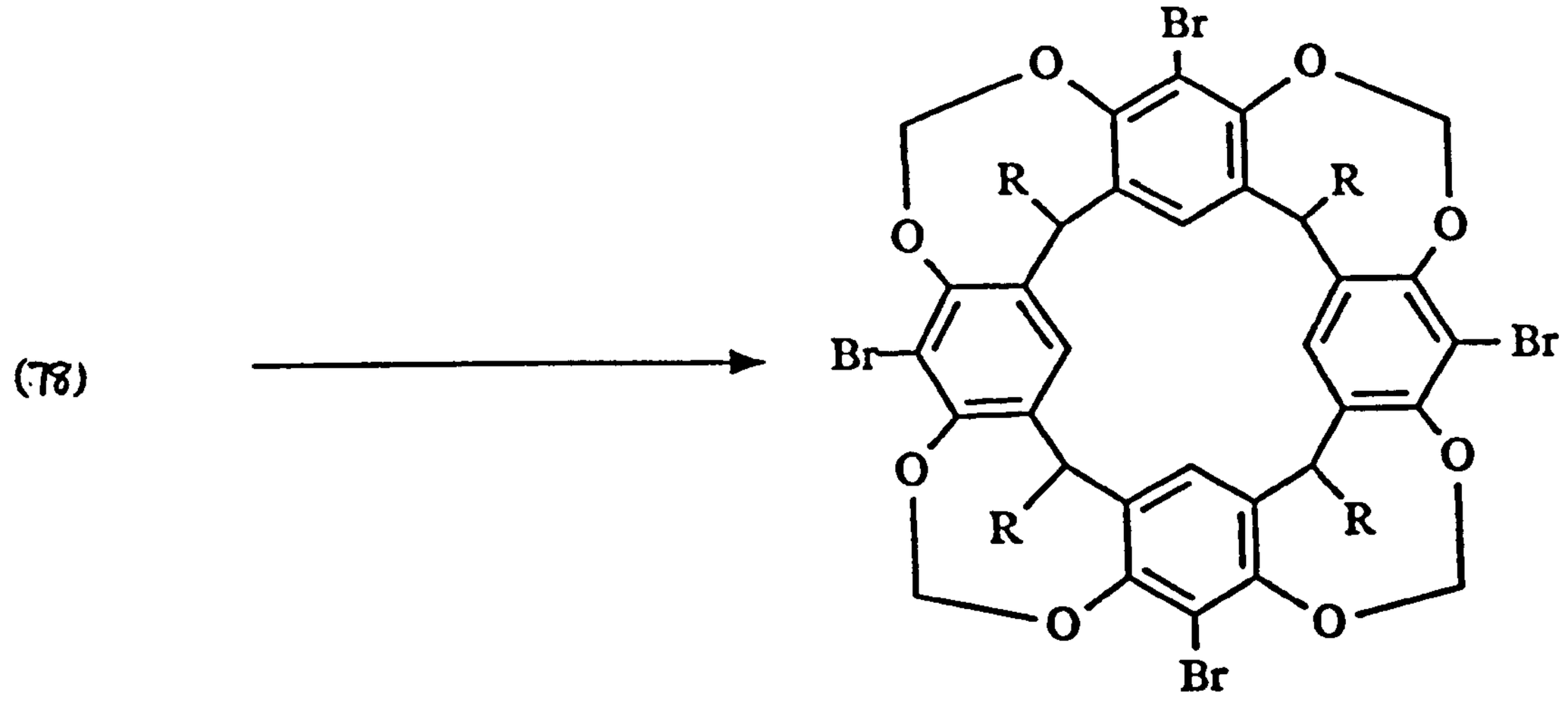
The cyclotetramer **(64)** was converted to the ring closed cavitand **(77)** by slow addition, four days, of the octol **(64)** and bromochloromethane in DMF to a suspension of potassium carbonate in DMSO which after a further day at 30°C only gave a 19% yield of **(77)**.



(77) R = Me

Tetrabromination of **(64)** with *N*-bromosuccinamide led to the octol **(78)** (78%) and this compound reacted with bromochloromethane at a much faster rate than **(64)** ie, 24 hours at 70°C to give a 55% yield of **(79)**. Cram[110] found that, generally, higher yields

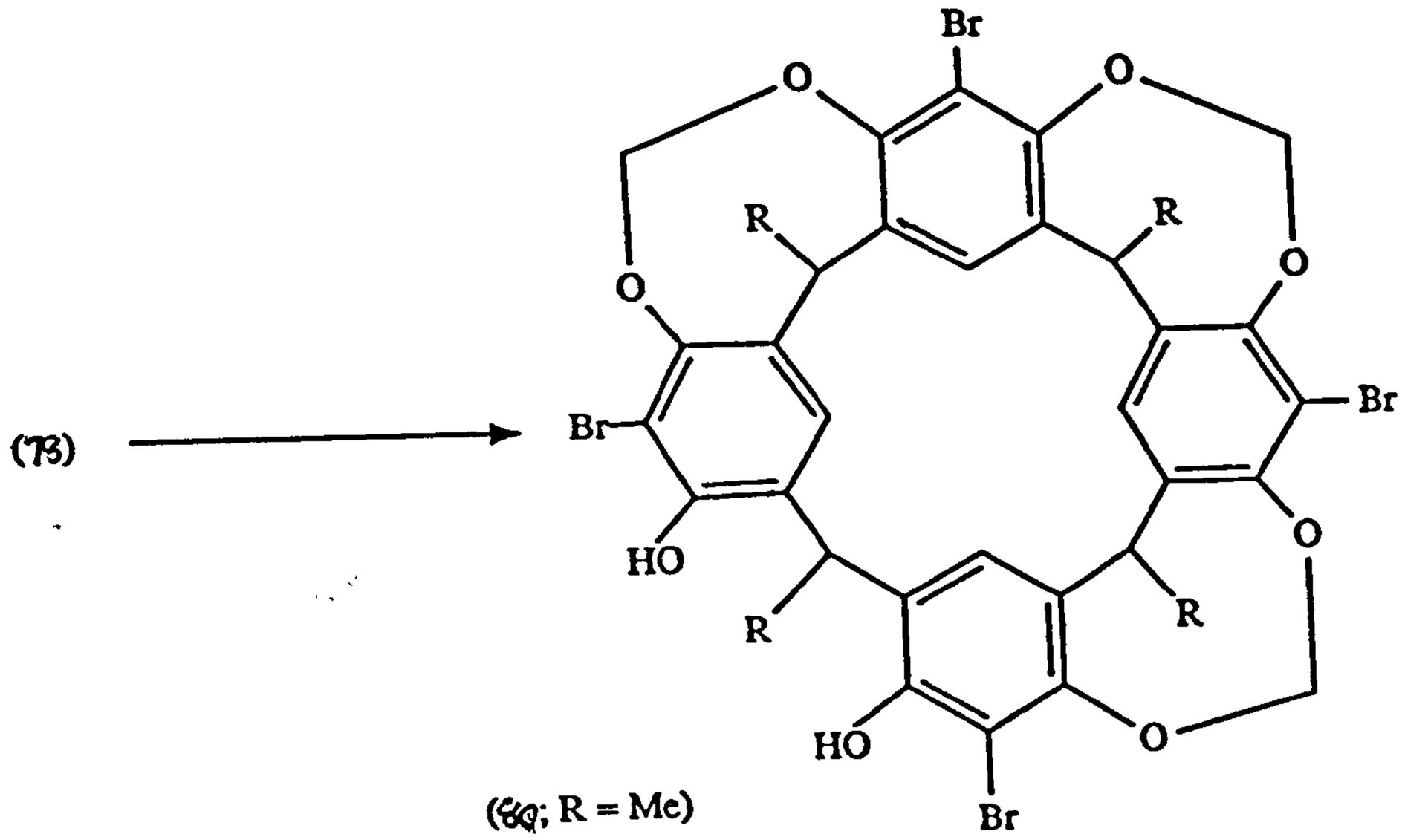
were obtained from phenols with R = CH_3 or Br than with R = H and suggested that it was probably because steric depression of intermolecular reaction rates leading to noncyclic oligomers was greater than that of their intramolecular counterparts leading to ring closure. He also found that the use of bromochloromethane gave better yields of the cavitands than either diiodomethane and dibromomethane.



(78)

(79) R = Me

When ring closing (78), the introduction of the fourth bridge occurred more slowly than the other three, so that the diphenol (80) containing three bridges was obtained as a product (11%).



(73)

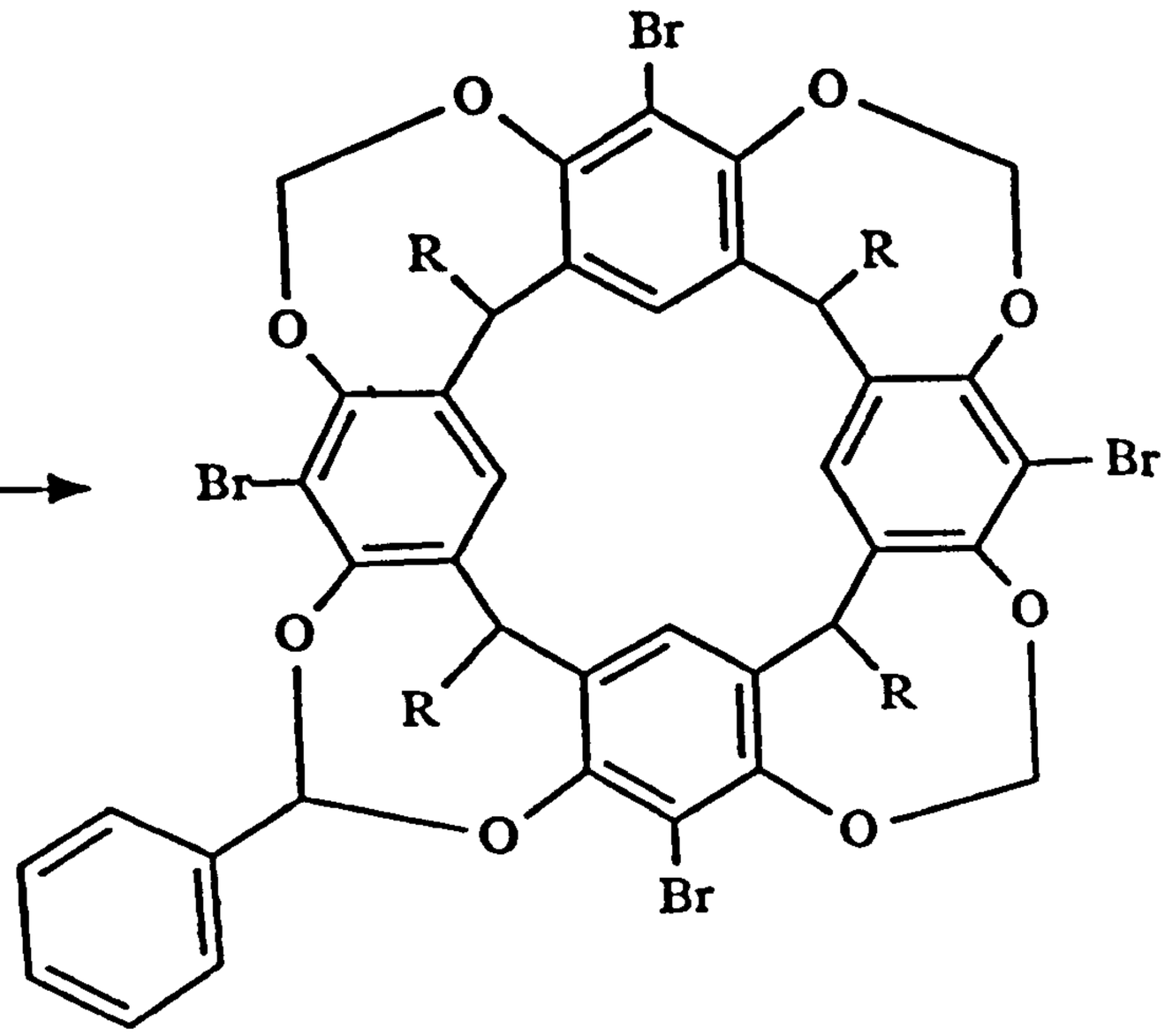
(80; R = Me)

This can be obtained as a major product (67%) by decreasing the amount of bromochloromethane employed.

The slower rate of addition of the fourth bridge probably reflects the incremental increase in rigidity of the cavitands with the addition of each bridge. Molecular models (CPK)[111] indicated that the tribridged phenol (80) provide much less conformational adaptability to accommodate geometric requirements of linear SN2 transition states than the mono- or di-bridged intermediate phenols. The first bridge introduced blocks the ring-inverting conformational interconversions, characteristic of the free phenols and the noncyclic derivatives. Results[111] show that practical amounts of mono-bridged and the two di-bridged analogues of the starting phenols could be obtained by proper manipulation of the reaction conditions. Access to such compounds and (80) is very welcome since they provide useful starting materials for synthesis of a variety of desired cavitands containing different types of substituents in the molecule.

Following this, it was found possible to form a cyclic acetal with benzaldehyde using concentrated sulphuric acid at 0°C. The product precipitated and on analysis was found to be the novel compound (81). This type of reaction can open up a whole range of novel cavitands and carcerands.

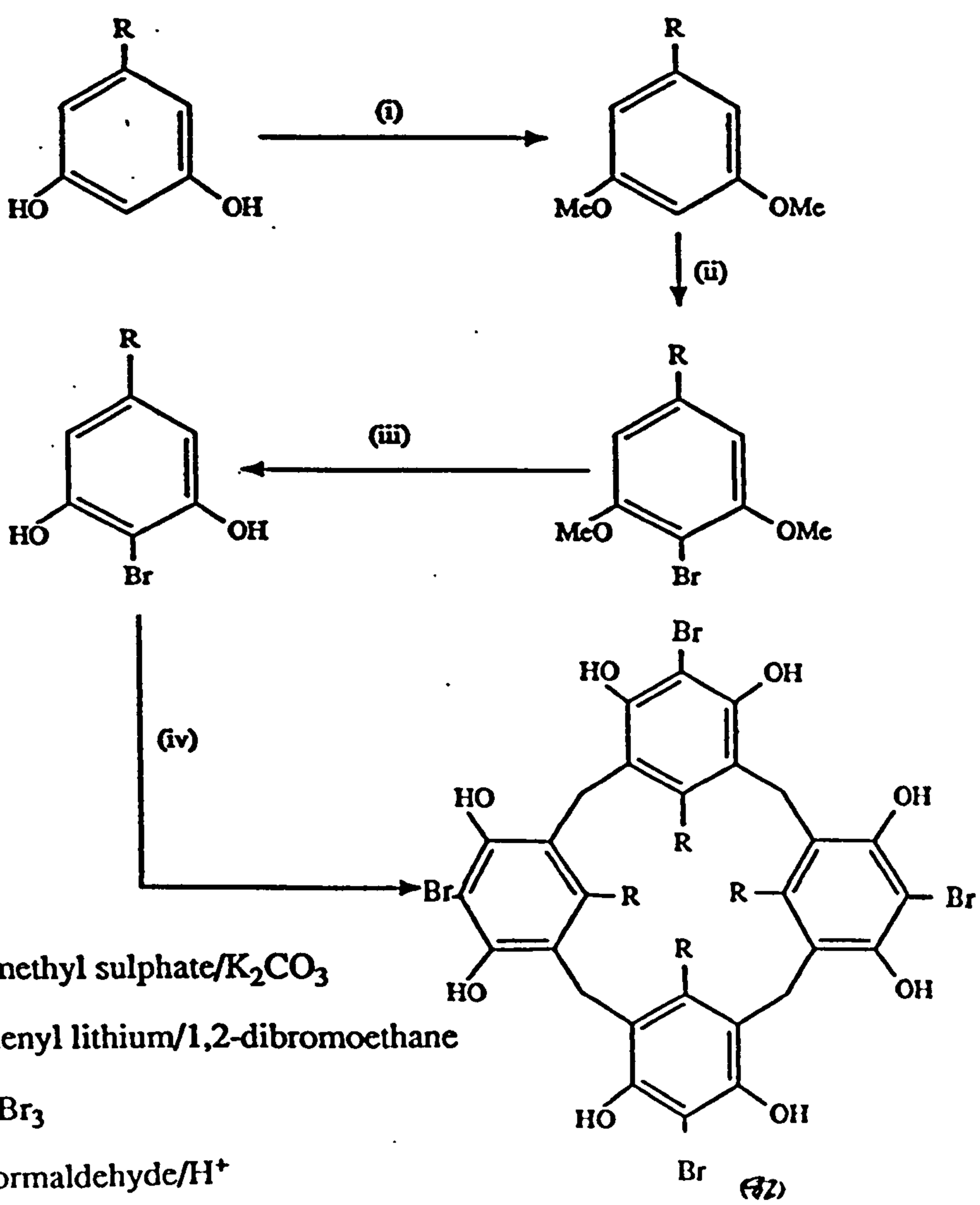
(80)



(81) R = Me

Synthesis of the cyclotetramers using 5-substituted resorcinols is slightly more difficult as there are several factors that may complicate the condensation with formaldehyde or acetaldehyde.

There are two different sites at which the aldehyde may attack, namely, between the two hydroxyl groups, which is more highly activated than in the case of resorcinol, or between the alkyl group and the hydroxyl group. To combat the former of these two situations, the 2-position was blocked by a bromine atom. This was achieved by following the reaction scheme shown in Figure 25[116]. This was carried out for both orcinol (5-methyl resorcinol) and for cardol. Reaction with formaldehyde in the presence of concentrated acid was then attempted and gave the required octol (82, R = Me) in the case of orcinol (34%) but failed to give any of octol (82, R = $C_{15}H_{31}$). This is probably due to steric hindrance, *ie* the four alkyl chains in (82, R = $C_{15}H_{31}$) were too bulky and thus prevent cyclisation.



(i) Dimethyl sulphate/K_2CO_3

(ii) Phenyl lithium/1,2-dibromoethane

(iii) BBr_3

(iv) Formaldehyde/H^+

(82)

4.2 Transport Studies.

The resorcinol-dodecanal cyclotetramer (**75**) has recently been shown to form hydrogen bonded complexes with some sugars, such as ribose, in apolar organic media[117]. Tanaka *et al*[118] showed how D-fructose could be completely extracted into CCl_4 and then reextracted back into aqueous solution and that there was no extraction of the sugar in the absence of the cyclotetramer. Following this it was decided to attempt to use the cyclotetramers (**75**) and (**76**) in sugar transport experiments.

The apparatus was designed as shown in Figure 26. Vigorous stirring of the CCl_4 solution of the cyclotetramer (1×10^{-2}M) with an aqueous solution (3.5M) of D-fructose at 25°C for 7 days resulted in transport of the sugar to the alternate side of the apparatus. The increase in sugar concentration over the 7 day time period was followed by polarimetry and the results can be seen in Table 18 and represented graphically in Figure 27.

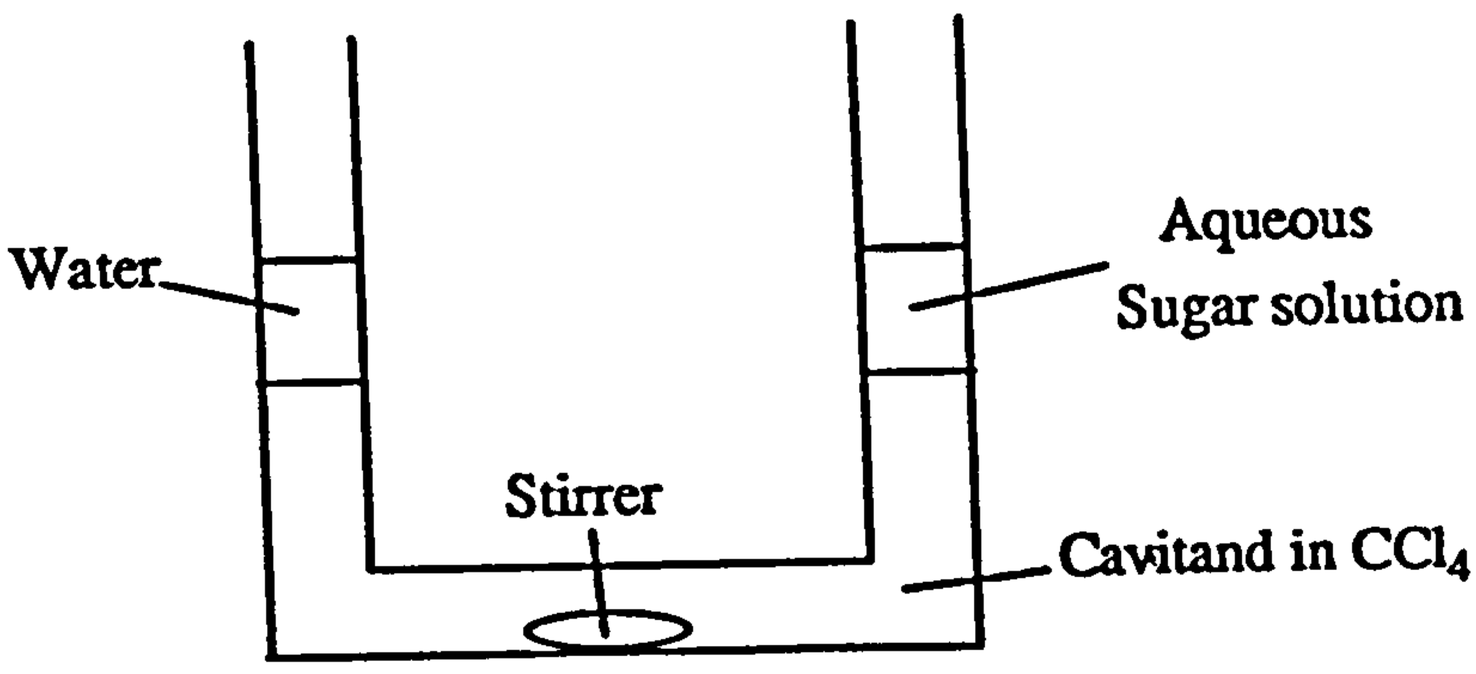


Figure 26. Apparatus for measuring sugar transport.

Time (days)	Concentration of D-fructose in (b)	
	(75)	(76)
0	0	0
1	0.525	0.725
2	0.900	0.825
3	1.250	1.150
4	1.500	1.375
5	1.675	1.525
6	1.680	1.625
7	1.680	1.650

Table 18. Time against increase in concentration of D-fructose in (b)

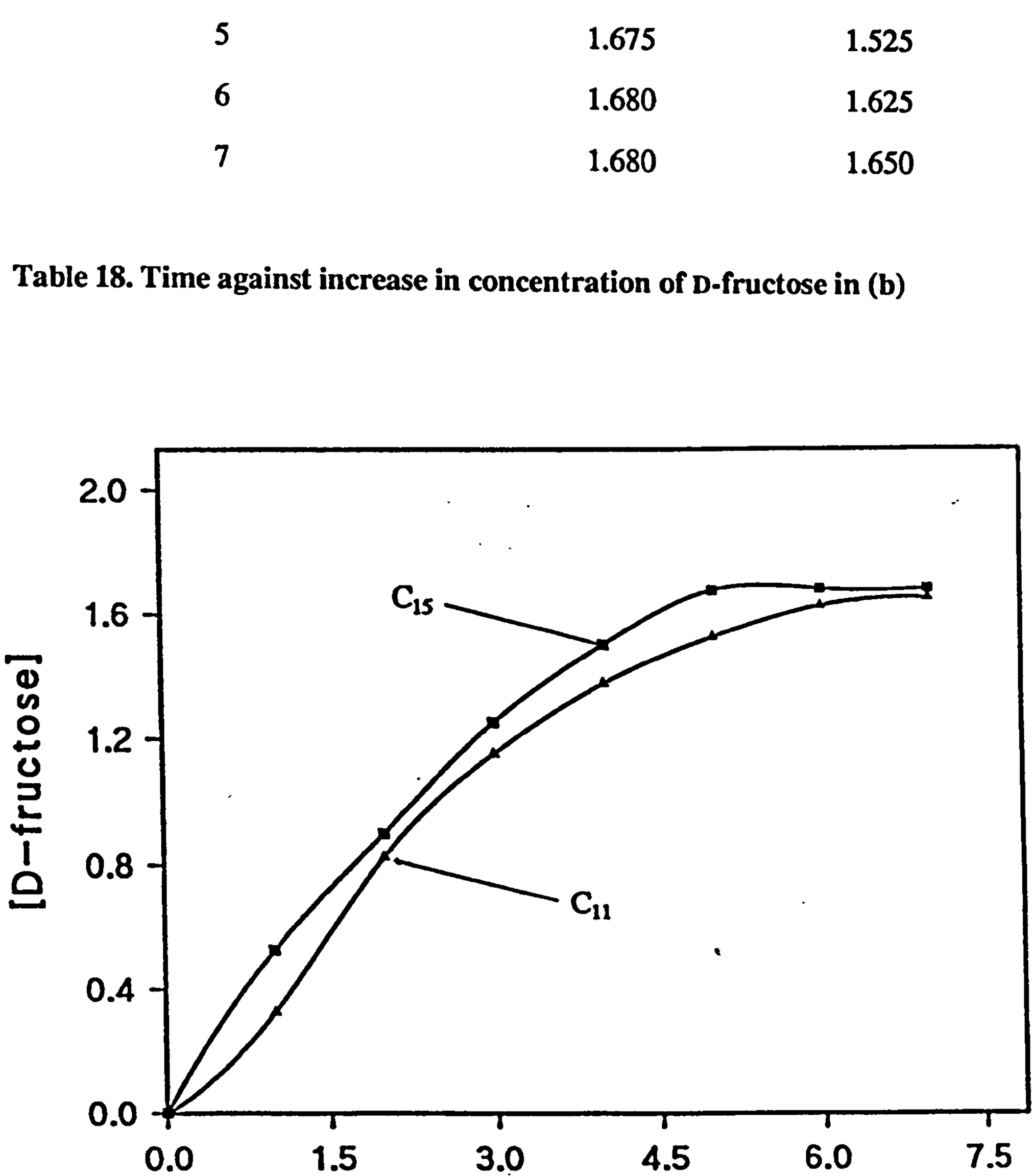


Figure 27. Graph of Time vs increase in concentration of D-fructose in (b)

As can be seen from these results, both octols **(75)** and **(76)** can reversibly bind D-fructose and transport it across an organic barrier. The octol with the longer chain (C_{15}) transports at a higher rate than the shorter chained octol.

CHAPTER FIVE

EXPERIMENTAL

General Experimental Techniques.

All melting points were recorded at atmospheric pressure and are uncorrected.

Micro-analytical determinations were performed at the Butterworths micro-analytical Laboratories and more recently at MEDAC Ltd micro-analytical laboratory on a CEC model 240XA CHN analyser with a furnace temperature of 950°C.

Infrared spectra were recorded on a Pye-Unicam SP3-100 spectrophotometer or, on a Perkin Elmer 1420 ratio recording spectrophotometer. Spectra were recorded as films (for liquids) or as potassium bromide disks (for solids).

Proton nuclear magnetic resonance spectroscopy was carried out on a Varian CF-T 20 (80 MHz, Fourier Transform) instrument or a Jeol JNM FX-200 (200 MHz) instrument depending on the expected complexity of the spectra and the peak resolution required. Spectra were recorded in either deuteriochloroform, hexadeuterioacetone or hexadeuteriomethylsulphoxide relative to tetramethylsilane as internal standard.

Mass spectra were recorded on an EMI EM902 double focusing spectrometer. All spectra reported are electron impact measured with a variable temperature direct insertion probe set at 70eV.

Thin-layer chromatography was carried out on 0.25mm silica gel UV_{254} pre-coated glass plates. Column chromatography was carried out on either Kieselgel 60 (70 - 230 mesh ASTM) or, where improved resolution was required, Kieselgel 60 (230 - 400 mesh ASTM). In the latter case, columns were normally slurry packed. Preparative high performance liquid chromatogrphy was carried out on a Gilson modular auto-prep system with a 21.4mm internal diameter column pre-packed with functionalised silica gel (5µm particle diameter). Commercially available HPLC-grade solvents were filtered and degassed prior to use. Peak detection was at 254 and 275nm. Solvents for

column chromatography were redistilled prior to use. Solvent ratios are described as volumes before mixing. Light petroleum refers to the fraction of boiling range 40 - 60°C and ether refers to diethyl ether throughout.

Solvents and reagents were purified according to standard procedures[119,120].

All apparatus used in moisture sensitive reactions were oven dried before assembly, whilst hot, in a stream of dry, oxygen free nitrogen.

Separation of cardanol from technical CNSL[22] (11 - 14)

Technical CNSL (60.92g, 200mmol), formaldehyde solution (40%, 20ml, 26mmol) and diethylene triamine (2.5g, 25mmol) were mixed together in methanol (250ml) in a beaker. This solution was allowed to stand at room temperature for 30min after which time two layers had formed, an upper, slightly reddish, solution and a lower phase which was solid and dark in colour. The upper phase was decanted and treated with water (40ml) followed by extraction with petroleum ether (3 x 50ml). The petroleum ether extract was evaporated under reduced pressure to leave a reddish residue (37.41g) which from TLC and HPLC contained cardanol, no cardol, small amounts of 2-methyl cardol and some polymeric material (Table 1). The crude cardanol was then distilled under reduced pressure to give three fractions; Fraction 1, 3.42g (Bpt up to 160°C/11mm of Hg), Fraction 2, 11.27g (160 - 180°C), Fraction 3, 13.24g (180 - 220°C). The total yield was 27.93g (46% of the CNSL used). These fractions were examined by HPLC before combining.

λ_{max}(nm), 201.2 (ε = 16582), 273.1 (ε = 1356). δ(^{1}H $CDCl_3$), 7.14 - 6.81 (m, 1H), 6.80 - 6.63 (m, 3H), 5.42 - 5.35 (m, 2H), 2.56 (t, 2H; *J*, 7.4Hz), 1.59 - 0.88 (m, 27H). Requires m/z = 304.514, 302.499, 300.483, 298.468; Found m/z = 304.1, 302.1, 300.1, 298.1.

Separation of cardanol from CNSL (Diol method).

Technical CNSL (ca. 1g) was dissolved in the diol under examination (10ml) and then extracted into light petroleum (10ml). The two phases separated and the upper petrol layer was analysed by TLC and HPLC (Table 4).

Separation of anacardic acid from natural CNSL[20] (1, 5 - 7).

Whole cashew nuts (500g) were frozen in liquid nitrogen and cracked open with a hammer to obtain the shells (348.7g) free from the kernels. The shells were then crushed coarsely using a blender and natural CNSL extracted by stirring the shells with carbon tetrachloride (1000ml) for 6h. The shells were filtered and crushed further. These were then placed in an ultrasonic bath with further carbon tetrachloride (1500ml)

extraction for 12h and then filtered again. The filtrates were combined and the solvent evaporated *in vacuo* to give a viscous brown oil. Wt = 145.7g (41% of the cashew nut shells).

Lead hydroxide was prepared by the reaction of sodium hydroxide (17.0g, 424mmol) in distilled water (250ml) and lead nitrate (70.6g, 212mmol) in distilled water (400ml). The resulting white precipitate was filtered, washed with distilled water (400ml) and then IMS (2 x 300ml). The natural CNSL (145.7g), in IMS (300ml), was added to the lead hydroxide and this was left to stir at room temperature for 16h. The precipitated lead anacardate was filtered, washed with IMS (100ml) and anacardic acid liberated with dilute hydrochloric acid (3M, 200ml). This was then extracted with ether (3 x 100ml), dried and evaporated to dryness *in vacuo* to give 84.1g (58% of the natural CNSL).

λ_{max}(nm), 207(ε = 12126), 240(3312), 304(1298). δ(^{1}H $CDCl_3$), 6.87 - 6.71 (m, 3H), 5.42 - 5.35 (m, 2H), 2.41 (t, 2H; *J*, 7.4Hz), 1.59 - 0.88 (m, 27H). Requires m/z = 348.525, 346.509, 344.493, 342.477; Found m/z = 348.2, 346.2, 344.2, 342.2.

<u>Separation of cardol and 2-methyl cardol (8 - 10) from technical CNSL</u>[26]

Technical CNSL (50g) was loaded on to a wide bore column (id 6.5cm x 50cm length) containing silica gel H (250g, TLC grade, type 60, article 7736), stepwise elution using petroleum ether (60 - 80)/diethyl ether mixtures was used in the following sequence:-

P; P/E (12:1); P/E (10:1)	650ml, 650ml, 650ml
P/E (7:1)	1300ml
P/E (3:1)	1300ml
P/E (1:1)	2600ml
Methanol	1000ml
Ethyl Acetate	1000ml
E	1000ml
P	1000ml

Collection of 20ml fractions started after 600ml had eluted. Every 5 fractions were monitored by TLC (Solvent system D) and bulked accordingly. The results of HPLC analysis of these combined fractions can be found in Table 3. This procedure was repeated several times to obtain sufficient quantities of both cardol and 2-methyl cardol for synthetic work.

Mixed Cardol

λ_{max}(nm), 207(ε = 3306), 273(209), 278(169). δ(^{1}H $CDCl_3$), 6.43 (s, 1H), 6.34 - 6.31 (m, 2H), 2.54 (t, 2H; *J*, 7.4Hz), 1.59 - 0.89 (m, 29H). Requires m/z = 320.508, 318.492, 316.476, 314.461; Found m/z = 320.2, 318.2, 316.2, 314.2.

Mixed 2-methyl cardol

λ_{max}(nm), 207(ε = 4399), 273(324), 278(420). δ(^{1}H $CDCl_3$), 6.35 (s, 2H), 2.98 (s, 3H), 2.54 (t, 2H; *J*, 7.4Hz), 1.59 - 0.89 (m, 29H). Requires = 334.535, 332.519, 330.503, 328.487; Found 334.4, 332.4, 330.4, 328.4.

3-Pentadecyl phenol[27] **(11)**

Cardanol (10.32g, 3.21mmol) and palladium/carbon (5%, 1.5g) catalyst were mixed with absolute ethanol (100ml) in a 250ml low pressure hydrogenation flask. This suspension was then shaken in the presence of hydrogen (1680ml) for 8h, filtered, dried and evaporated to dryness under reduced pressure to give 3-pentadecyl phenol. Wt = 10.28g (99%).

λ_{max}(nm), 207(ε = 1603), 212(1705), 268(456), 275(397). δ(^{1}H $CDCl_3$), 7.18 - 7.09 (m, 1H), 6.81 - 6.63 (m, 3H), 2.53 (t, 2H; J,7.4Hz), 1.33 - 0.89 (m, 29H). Requires m/z 304.514; Found m/z = 304.2.

1-Hydroxy-6-pentadecyl benzoic acid[27] (1)

Anacardic acid (10.13g, 29.4mmol) was reduced in the same manner as described previously with palladium/carbon (5%, 1.43g) in ethanol (100ml) requiring 1642ml of hydrogen to give the *title compound*. Wt = 10.99g (98%).

λ_{max}(nm), 207(ε = 7681), 240(1681), 304(1045). δ(^{1}H $CDCl_3$), 10.99 (s, 1H), 7.36 (t, 1H; *J*, 8.06Hz), 6.89 (d, 1H; *J*, 7.33Hz), 6.79 (d, 1H; *J*, 7.32Hz), 2.94 (t, 2H; *J*, 7.41Hz), 1.60 - 0.84 (m, 29H). Requires m/z = 348.525; Found m/z = 348.4.

1,3-dihydroxy-5-pentadecyl benzene[27] (8)

Cardol (1.03g, 3.21mmol) was dissolved in ethanol (50ml) and to this was added Pd/C (5%, 0.21g). This was then shaken, at room temperature and atmospheric pressure, in the presence of hydrogen until hydrogen uptake ceased (4 h). The reaction mixture was then filtered and evaporated to dryness *in vacuo* to give the required product. Wt = 0.98g(96%).

λ_{max}(nm), 207(ε = 5473), 273(530), 279(525). δ(^{1}H $CDCl_3$), 6.43 (s, 1H), 6.35 - 6.30 (s, 2H), 2.55 (t, 2H; *J*, 7.4Hz), 1.59 - 0.88 (m, 29H). Requires m/z = 320.508; Found m/z = 320.4.

Ethyl anacardate

Anacardic acid (4.23g, 12.1mmol) was refluxed in absolute ethanol (100ml) in the presence of H_2SO_4 (18M, 2ml) for 24h. The reaction mixture was then evaporated to dryness, dissolved in ether (20ml) and washed with sodium bicarbonate solution (5%, 50ml), brine and dried. This was then evaporated to dryness *in vacuo* to give the *title compound*. Wt = 3.97g (87%). Recrystallised from methanol/water to give white crystals.

δ(^{1}H $CDCl_3$) 7.24 - 7.17 (m, 2H), 6.71 (m, 1H), 4.45 (q, 2H; *J*, 7.1Hz), 2.89 (t, 2H; *J*, 7.4Hz), 1.41 (t, 3H; *J*, 7.1Hz), 1.58 - 0.89 (m, 29H). Requires m/z = 374.417; Found m/z = 376.1.

2-(3-Pentadecylphenoxy) acetic acid[35] **(20)**

3-Pentadecyl phenol (3.55g, 118mmol) was dissolved in dichloromethane (50ml). Sodium hydroxide (0.78g, 95mmol) and chloracetic acid (2.96g, 3.14mmol) were dissolved in water (50ml). These were combined and the interface agitated using a 'Vibromix' apparatus for 6h.

The aqueous phase was then separated and extracted with dichloromethane (2 x 20ml). The combined organic extracts were evaporated under reduced pressure, water added (100ml) and then re-extracted with diethyl ether (3 x 50ml). The organic phase was then washed with aqueous NaOH solution (3M, 20ml), dried and evaporated to dryness.

TLC and nmr indicated that no reaction had taken place.

3-Pentadecylphenoxy acetonitrile[35] **(21)**

3-Pentadecyl phenol (3.07g, 10.2mmol) and 1-bromoacetonitrile (0.79g, 10.31mmol) were dissolved in dichloromethane (50ml). KOH (0.95g, 16.2mmol), cetyltrimethyl ammonium hydroxide (0.346g, 0.99 mmol) were dissolved in water (50ml). These two solutions were added together and refluxed, whilst being vigorously agitated by a mechanical overhead stirrer, for 2h. The aqueous phase was separated and extracted with dichloromethane (2 x 20ml). The organic extracts were combined and evaporated to dryness under reduced pressure.

TLC and MS suggested no reaction had occurred.

2-(3-Pentadecylphenoxy) ethyl acetate **(22)**

3-Pentadecyl phenol (10.045g, 33.0mmol) was refluxed in benzene (100ml) with sodium metal (0.761g, 33.7mmol) for 3h. Ethyl bromoacetate (7.521g, 45.1mmol) was then added dropwise and the reaction mixture allowed to stir under reflux for 18 h. The solid NaBr was filtered off and the benzene removed under reduced pressure. TLC (2% ethyl acetate in chloroform) indicated the presence of some starting material (R_f =

0.76) and some less polar material ($R_f = 0.92$). These were separated by flash chromatography, eluting with 2% ethyl acetate in chloroform, yielding the *title compound*. Wt = 7.86g (61%). Recrystallisation from IMS gave white needles. Mpt = 37 - 38°C.

λ_{max}(nm), 207($\varepsilon = 3407$), 273(490). δ(^{1}H $CDCl_3$), 7.25 - 7.15 (m, 1H), 6.83 - 6.69 (m, 3H), 4.61 (s, 2H), 4.33 - 4.22 (q, 2H; *J*, 7.7Hz), 2.60 - 2.53 (t, 3H; *J*, 7.4Hz), 1.33 - 0.89 (m, 31H). Requires m/z = 390.605; Found m/z = 390.5. Found C = 76.95; H = 10.59; $C_{25}H_{42}O_3$ requires C = 76.87; H = 10.84.

2-(3-Pentadecylphenoxy) acetic acid **(20)**

Pentadecylphenoxyethyl acetate (1.11g, 2.87mmol) was heated under reflux with magnetic stirring, in the presence of 1,4-dioxan (10ml) and potassium hydroxide (1.23g, 21.0mmol), for 18h. When cooled, the organic layer solidified and was then acidified with hydrochloric acid (3M, 5ml) then extracted with diethyl ether (3 x 20ml). The ether extract was then dried, filtered and evaporated to dryness under reduced pressure. TLC (2% ethyl acetate in chloroform) indicated that no starting material was present, only one baseline component ($R_f = 0.02$). Wt = 0.97g (93%). Recrystallised from petroleum ether to give white needles, Mpt = 102 - 104°C.

λ_{max}(nm), 207($\varepsilon = 5436$), 273(1022). δ(^{1}H $CDCl_3$), 12.31 (s, 1H), 7.25 - 7.16 (m, 1H), 6.83 - 6.69 (m, 3H), 4.83 (s, 2H), 2.53 (t, 2H; *J*, 7.4Hz), 1.33 - 0.89 (m, 29H). Requires m/z = 362.551; Found m/z = 362.2. Found C = 76.47; H = 10.39; $C_{23}H_{38}O_3$ requires C = 76.24; H = 10.49.

3-Pentadecylphenoxy acetonitrile **(21)**

3-Pentadecyl phenol (1.06g, 3.81mmol) and potassium carbonate (1.05g, 7.62mmol) were refluxed in benzene (10ml) for 30min. To this was added 2-bromoacetonitrile (0.48g, 4.02mmol) and the mixture refluxed for 36h. Hydrochloric acid (3M, 20ml) was added, to remove any remaining potassium carbonate, followed by extraction with

benzene (3 x 20ml). The combined organic extracts were washed with water, dried and evaporated to dryness under reduced pressure to yield a mixture of starting material and some less polar material. Flash chromatography was used to separate the mixture (2% ethyl acetate in chloroform) to yield the *title compound*. Wt = 0.58g (67%). Recrystallised from IMS to give white crystals, Mpt = 39 - 41°C. λ_{max}(nm), 207(ε = 2285), 212(2500), 268(464), 275(428). δ(^{1}H $CDCl_3$), 7.23 - 7.15 (m, 1H), 6.72 - 6.75 (m, 3H), 4.61 (s, 2H), 2.51 (t, 2H; *J*, 7.4Hz), 1.33 - 0.87 (m, 29H). IR (KBr / cm^{-1}) = 2250. Requires m/z = 343.518; Found m/z = 343.1. Found C = 80.31; H = 10.49; $C_{23}H_{37}NO$ requires C = 80.19; H = 10.71.

<u>2-(3-Pentadecylphenoxy) ethylamine</u>

To a stirred solution of 3-pentadecylphenoxy acetonitrile (0.099g, 0.289mmol) in THF (5ml) was added $LiAlH_4$ (0.084g, 2.21mmol) which was stirred for 2h at room temperature. After reaction was complete ethyl acetate (1ml), water (0.25ml) and aqueous sodium hydroxide (3M, 0.25ml) were added slowly in sequence to remove excess $LiAlH_4$ from the reaction mixture. The organic phase was separated, dried and evaporated to dryness *in vacuo* to yield, by TLC, starting material and some baseline material (2% ethyl acetate in chloroform). Flash chromatography was used to separate the mixture (5% ethyl acetate in chloroform) to give the *title compound*. Wt = 0.066g (64%).

Recrystallisation from petroleum ether to give white needles. Mpt = 115 -116°C. λ_{max}(nm), 207(ε = 3224), 268(544), 275(467). δ(^{1}H $CDCl_3$), 7.23 - 7.29 (m, 1H), 6.78 - 6.73 (m, 3H), 4.09 - 3.87 (m, 4H), 2.52 (t, 2H; *J*, 7.4Hz), 1.51 - 0.89 (m, 29H). Requires m/z = 347.550; Found m/z = 347.2. Found C = 79.34; H = 11.52; $C_{23}H_{41}NO$ requires C = 79.22; H = 11.78.

2-(3-Pentadecylphenoxy) ethanol **(23)**

2-(3-Pentadecylphenoxy) acetic acid (4.05g, 11.2mmol) was dissolved in dry THF (12ml) under nitrogen. To this was added $LiAlH_4$ (2.09g, 55.0mmol), and this was stirred at room temperature for 18h. Ethyl acetate (1ml), aqueous NaOH (3M, 1ml) and water (2ml) were then added slowly to the reaction mixture to destroy any unreacted $LiAlH_4$ before extraction with ether (2 x 10ml). The ether extract was dried, filtered and evaporated to dryness under reduced pressure. This afforded the *title compound*. Wt = 3.27g (78%).

λ_{max}(nm), 207(ε = 2544), 268(358), 275(465). δ(^{1}H $CDCl_3$), 7.21 - 7.05 (m, 1H), 6.79 - 6.70 (m,, 3H), 4.07 - 3.93 (m, 4H), 2.52 (t, 2H; *J*, 7.4Hz), 1.59 (s, 1H), 1 .50 - 0.88 (m, 29H). Requires m/z = 348.569; Found m/z = 348.4. Found C = 78.20; H = 11.76; $C_{23}H_{40}O_2$ requires C = 78.41; H = 11.57.

Mixed cardanol phenoxyethyl acetate

Mixed cardanol (3.03g, 10.1mmol) dissolved in NMP (10ml) was reacted with sodium (0.27g, 11.7mmol) and ethyl bromoacetate (1.74g, 10.41mmol) as described for 2-(3-pentadecylphenoxy) ethyl acetate. The stirred reaction mixture was heated at 100°C for 3h, then allowed to cool to ambient. Addition of *t*-butanol (3ml) afforded the safe removal of any unreacted sodium metal. The reaction mixture was then poured into water (20ml) and extracted with diethyl ether (3 x 20ml), dried, filtered and evaporated to dryness under reduced pressure, separated from unreacted starting material by flash chromatography eluting with 2% ethyl acetate in chlorofom, to give the *title compounds*. Wt = 2.79g (71%).

δ(^{1}H $CDCl_3$), 7.14 - 6.81 (m, 1H), 6.80 - 6.63 (m, 3H), 5.42 - 5.35 (m, 2H), 4.57 (s, 2H), 4.38 (q, 2H; *J*, 7.7Hz), 2.84 (m, 2H), 2.65 (t, 2H; *J*, 7.4Hz), 2.092 (m, 2H), 1.37 - 0.80 (m, 21H). Requires m/z = 390.605, 388.589, 386.573, 384.587; Found m/z = 390.5, 388.5, 386.5, 384.5. IR (liquid film, cm^{-1}) 1780

Mixed cardanol phenoxy ethanol **(24)**

Mixed cardanol phenoxyethyl acetate (6.07g, 15.5 mmol) was dissolved in dry THF (10ml) under nitrogen. $LiAlH_4$ (1.77g, 46 mmol) was added and the suspension left to stir at room temperature for 30min. The reaction mixture was worked up in the same manner as decribed previously and purified by chromatography eluting with 2% ethyl acetate in chloroform to give the *title compounds*. Wt = 3.37g (63%).

λ_{max}(nm), 201(ε = 15463), 273(1275). δ(^{1}H $CDCl_3$), 7.22 - 7.14 (m, 1H), 6.80 - 6.71 (m, 3H), 5.42 - 5.35 (m, 2H), 4.60 - 3.93 (m, 4H), 2.78 (m, 2H), 2.57 (t, 2H; *J*, 7.4Hz), 2.23 (m, 2H), 1.59 - 0.88 (m, 21H). Requires m/z = 348.569, 346.553, 344.537, 342.522; Found m/z = 348.0, 346.0, 344.0, 342.0.

6-(3-Pentadecylphenyl)-3,6-dioxapentanol[38] **(25)**

3-Pentadecyl phenol (1.02g, 3.35mmol) and potassium metal (0.13g, 3.41mmol) were heated together, with stirring, at 90°C for 10h under nitrogen. 2-chloro(2-ethoxy) ethanol (0.42g, 3.34mmol) was then added dropwise and the reaction mixture heated for a further 10h at 100°C. The solid KCl was filtered and the product separated from the less polar starting material by using a 'Chromatotron' (5% ethyl acetate in chloroform). Wt = 0.59g (45%).

λ_{max}(nm), 207(ε = 2467), 275(479). δ(^{1}H $CDCl_3$), 7.26 - 7.14 (m, 1H), 6.82 - 6.69 (m, 3H), 4.11 - 3.92 (m, 8H), 2.51 (t, 2H; *J*, 7.4Hz), 1.51 (s, 1H; D_2O exch.), 1.48 - 0.97 (m, 29H). Requires m/z = 392.620; Found m/z = 392.1. Found: C = 76.34; H = 11.31; $C_{25}H_{44}O_3$ requires C = 76.51; H = 11.22.

Mixed cardanol phenoxy(2-ethoxy) ethanol[38] **(26)**

Cardanol (1.42g, 4.73mmol), potassium metal (0.19g, 4.85mmol) and 2-chloro(2-ethoxy) ethanol (0.56g, 4.56mmol) were reacted together and worked up in the same manner as described in the previous experiment, to give the *title compounds*. Wt = 0.77g (42%).

λ_{max}(nm), 201(ε = 15433), 275(1388). δ(^{1}H $CDCl_3$), 7.25 - 7.13 (m, 1H), 6.80 - 6.68 (m, 3H), 5.42 -5.35 (m, 2H), 4.12 - 3.91 (m, 8H), 2.51 (t, 2H; *J*, 7.4Hz), 1.59 - 0.88 (m, 27H). Found m/z = 392.2, 390.2, 388.2, 386.2; Requires m/z = 392.616, 390.600, 388.585, 386.569.

9-(3-Pentadecylphenyl)-3,6,9,-trioxaoctanol[38] (27)

3-Pentadecyl phenol (2.116g, 6.96mmol) and potassium (0.304g, 6.72mmol) were heated together, with stirring, at 90°C for 10h under nitrogen. 2-chloro-(2-ethoxy[2-ethoxy]) ethanol (0.867g, 7.02mmol) was then added dropwise and heated for a further 10h at 100°C. The solid residue was then filtered, evaporated to dryness and the product separated from the less polar starting material by flash chromatography etution with 5% ethyl acetate in chloroform, to give the *title compound.* Wt = 1.39g (46%). λ_{max}(nm), 207(ε = 2383), 275(490). δ(^{1}H $CDCl_3$), 7.25 - 7.15 (m, 1H), 6.82 - 6.69 (m, 3H), 4.10 - 3.93 (m, 12H), 2.51 (t, 2H; *J*, 7.4Hz), 1.52 (s, 1H), 1.50 - 0.88 (m, 29H). Found m/z = 436.2; Requires m/z = 436.664. Found: C = 74.11; H = 10.85; $C_{27}H_{48}O_4$ requires C = 74.31; H = 11.01.

Mixed cardanolphenoxy(2-ethoxy[2-ethoxy]) ethanol[38] (28)

Cardanol (1.37g, 4.56mmol), potassium metal (0.18g, 4.60mmol) and 2-chloro(2-ethoxy[2-ethoxy]) ethanol (0.76g, 4.53mmol) were reacted together and worked up in the same manner as previously described to give the *title compounds.* Wt = 0.86g (43%).

λ_{max}(nm), 201(ε = 15392), 275(1462). δ(^{1}H $CDCl_3$), 7.25 - 7.14 (m, 1H), 6.80 - 6.69 (m, 3H), 5.42 - 5.35 (m, 2H), 4.13 - 3.90 (m, 12H), 2.51 (t, 2H; *J*, 7.4Hz), 1.58 - 0.88 (m, 27H). Found m/z = 436.1 434.1, 432.1, 430.1; Requires m/z = 436.664, 434.648, 432.633, 430.617.

12-(3-Pentadecylphenyl)-3,6,9,12-tetraoxaundecanol[38] (29)

3-Pentadecylphenoxy ethanol (1.06g, 2.76mmol) and potassium metal (0.11g, 2.82mmol) were heated together, with stirring, at 90°C for 5h under an atmosphere of nitrogen. 2-chloro(2-ethoxy[2-ethoxy]) ethanol (0.46g, 2.73mmol) was added dropwise and the reaction mixture heated for a further 10h at 100°C. The solid KCl was filtered and the product separated from the starting material using a 'Chromatotron' eluting with 7% ethyl acetate in chloroform to give the *title compound*. Wt = 0.48g (37%).

λ_{max}(nm), 207(ε = 2309), 275(507). δ(^{1}H $CDCl_3$), 7.26 - 7.16 (m, 1H), 6.82 - 6.70 (m, 3H), 4.09 - 3.94 (m, 16H), 2.51 (t, 2H; *J*, 7.4Hz), 1.50 - 0.88 (m, 29H). Found m/z = 480.2; Requires m/z = 480.712. Found: C = 72.61; H = 10.39; $C_{29}H_{52}O_5$ requires C = 72.50; H = 10.62..

Mixed cardanol phenoxy(2-ethoxy(2-ethoxy(2-ethoxy))) ethanol[38] (30)

Mixed cardanol phenoxy ethanol (0.98g, 3.26mmol), potassium metal (0.13g, 3.33mmol) and 2-chloro(2-ethoxy[2-ethoxy]) ethanol (0.54g, 3.20mmol) were reacted together and worked up in the same way as described previously to give the *title compounds*. Wt = 0.59g (38%).

λ_{max}(nm), 201(ε = 14360), 275(1492). δ(^{1}H $CDCl_3$), 7.25 - 7.14 (m, 1H), 6.81 - 6.69 (m, 3H), 5.42 - 5.35 (m, 2H), 4.13 - 3.90 (m, 16H), 2.51 (t, 2H; *J*, 7.4Hz), 1.58 - 0.88 (m, 27H). Found m/z = 480.1, 478.1, 476.1, 474.1; Requires = 480.712, 478.696, 476.680, 474.664.

15-(3-Pentadecylphenyl)-3,6,9,12,15-pentaoxatetradecanol[38] (31)

9-(3-Pentadecylphenyl)-3,6,9-trioxaoctanol(1.34g, 3.06mmol) and potassium metal (0.12g, 3.08mmol) were heated together, with stirring, at 90°C for 5h under a nitrogen atmosphere. 2-chloro(2-ethoxy) ethanol (0.38g, 3.05mmol) was added dropwise and the reaction mixture was heated for a further 10h at 100°C. The solid KCl was filtered

and the product separated from the starting material using a 'Chromatotron' eluting with 10% ethyl acetate in chloroform to give the *title compound.* Wt = 0.67g (42%).

λmax(nm), 207(ε = 2247), 275(534). δ(^{1}H CDCl$_3$), 7.25 - 7.14 (m, 1H), 6.81 - 6.70 (m, 3H), 4.13 - 3.91 (m, 20H), 2.51 (t, 2H; *J*, 7.4Hz), 1.55 - 0.88 (m, 29H). Found m/z = 524.9; Requires m/z = 524.759. Found: C = 71.01; H = 10.32; $C_{31}H_{56}O_6$ requires C = 70.86; H = 10.48.

Mixed cardanol phenoxy(2-ethoxy(2-ethoxy(2-ethoxy(2-ethoxy)))) ethanol[38] **(32)**

Cardanol phenoxy(2-ethoxy[2-ethoxy]) ethanol (1.56g, 3.60mmol), potassium metal (0.15g, 3.84mmol) and 2-chloro(2-ethoxy) ethanol (0.44g, 3.53mmol) were reacted together and worked up in the same way as described previously to give the *title compounds.* Wt = 0.75g (40%).

λ_{max}(nm), 201(ε = 13126), 275(1537). δ(^{1}H $CDCl_3$), 7.25 - 7.19 (m, 1H), 6.81 - 6.69 (m, 3H), 5.41 - 5.32 (m, 2H), 4.13 - 3.90 (m, 20H), 2.51 (t, 2H; *J*, 7.4Hz), 1.58 - 0.89 (m, 27H). Found m/z = 524.1, 522.1, 520.1, 518.1; Requires m/z = 524.759, 522.744, 520.728, 518.718.

18-(3-Pentadecylphenyl)-3,6,9,12,15,18-hexaoxaoctadecanol[38] **(33)**

9-(3-Pentadecylphenyl)-3,6,9-trioxaoctanol (0.87g, 2.34mmol) and potassium metal (0.10g, 2.56mmol) were heated together, with stirring, at 90°C for 4h under a nitrogen atmosphere. 2-chloro(2-ethoxy[2-ethoxy]) ethanol (0.37g, 2.19mmol) was added dropwise and the reaction mixture heated at 100°C for a further 10h. The solid residue was filtered and the product separated from unreacted starting material using a 'Chromatotron' eluting with 15% ethyl acetate in chloroform to give the *title compound.* Wt = 0.61g (46%).

λ_{max}(nm), 207(ε = 2163), 275(550). δ(^{1}H $CDCl_3$), 7.25 - 7.14 (m, 1H), 6.81 - 6.70 (m, 3H), 4.13 - 3.91 (m, 24H), 2.51 (t, 2H; *J*, 7.4Hz), 1.55 - 0.88 (m, 29H). Found m/z =

568.9; Requires m/z = 568.807. Found: C = 69.77; H = 10.29; $C_{33}H_{58}O_7$ requires C = 69.72; H = 10.38.

Mixed cardanol phenoxy(2-ethoxy(2-ethoxy(2-ethoxy(2-ethoxy(2-ethoxy))))) ethanol[38] **(34)**

Mixed cardanol phenoxy(2-ethoxy[2-ethoxy]) ethanol (1.23g, 2.85mmol), potassium metal (0.11g, 2.87mmol) and 2-chloro(2-ethoxy[2-ethoxy]) ethanol (0.47g, 2.87mmol) were reacted together and worked up in the same manner as described previously to give the *title compounds*. Wt = 0.71g (43%).

λ_{max}(nm), 201(ε = 12643), 275(1604). δ(^{1}H $CDCl_3$), 7.25 - 7.19 (m, 1H), 6.81 - 6.69 (m, 3H), 5.41 - 5.32 (m, 2H), 4.13 - 3.90 (m, 24H), 2.50 (t, 2H; *J*, 7.4Hz), 1.58 - 0.88 (m, 27H). Found m/z = 568.9, 566.9, 564.9, 562.9; Requires m/z = 568.807, 566.791, 564.775, 562.760.

Polyethoxylation of mixed cardanol[39, 40]

Cardanol (21.1g, 7.03mmol) and sodium hydroxide (0.023g, 0.57mmol) were stirred together under nitrogen at 180°C for 30min. After this time the flow of nitrogen was stopped and ethylene oxide allowed to pass over the reaction mixture. Samples (ca. 1g) of the reaction mixture were taken every 30min by stopping the flow of ethylene oxide and flushing the system with nitrogen, taking a sample, the restarting the flow of ethylene oxide. This procedure was continued for 10h and gave a whole range of polyethoxylated cardanols which were then analysed by nmr (Table 6) and HPLC (Table 10).

λ_{max}(nm), 203(ε = 970), 220(934), 257(449), 264(594), 275(478). δ(^{1}H $CDCL_3$), 7.51 - 7.21(m, 1H), 6.77 - 6.68(m, 2H), 5.42 - 5.30(m, 2H), 4.04 - 3.61(m, 4*n*H; max at 3.57), 2.56(t, 2H; *J*, 7.4Hz), 1.42 - 0.87(m, 27H; max at 1.30).

3-(Pentadecyl)phenoxypolyethoxylate[39, 40]

3-Pentadecyl phenol (20.6g, 6.97mmol) was polyethoxylated in the same manner as described above. All samples were again analysed by nmr (Table 8) and HPLC (Table 12).

λ_{max}(nm), 203(ε = 1404). δ(^{1}H $CDCl_3$), 7.26 - 7.15(m, 1H), 6.72 - 6.68(m, 2H), 4.02 - 3.51(m, 4*n*H; max at 3.61), 2.51(t, 2H), 1.59 - 0.85(m, 29H; max at 1.23).

Polyethoxylation of cardol[39, 40]

Cardol (10.27g, 3.25mmol) and sodium hydroxide (0.26g, 6.5mmol) were heated together under nitrogen at 160°C for 30min. The flow of nitrogen was stopped and ethylene oxide allowed to pass over the reaction mixture. Samples (ca. 0.5g) were taken as previously described. These samples were analysed by nmr (Table 7) and HPLC(Table 11).

λ_{max}(nm), 207(ε = 430), 275(1243). δ(^{1}H $CDCl_3$), 6.43 (s, 1H), 6.33 (s, 2H), 4.05 - 3.60 (m, 8*n*H; max at 3.61), 2.54 (t, 2H; *J*, 7.4Hz), 1.59 - 0.88 (m, 29H; max at 1.23).

1,3-Polyethoxy-5-pentadecyl benzene[39, 40]

To a solution of Cardol polyethoxylate (5.63g, *n* = 13.8, 5.36mmol) in ethanol (100ml) was added Pd/C (5%, 1.43g). This was hydrogenated, using low pressure hydrogenation apparatus, at room temperature requiring 723ml of hydrogen. The catalyst was filtered and the filtrate evaporated to dryness under reduced pressure. Wt = 5.24g (93%).

δ(^{1}H $CDCl_3$), 7.29 - 7.22 (m, 1H), 7.18 - 7.08 (m, 2H), 3.86 - 3.65 (m, 130H), 2.61 (t, 2H; *J*, 7.4Hz), 1.54 - 0.81 (m, 29H).

Attempted polyethoxylation of ethyl anacardate[39, 40]

Ethyl anacardate (20.61g, 58.1mmol) and sodium hydroxide (0.025g, 0.6mmol) were stirred together under nitrogen at 180°C for 30min. After this time the flow of nitrogen was stopped and ethylene oxide allowed to pass over the reaction mixture. Samples (ca. 1g) were taken every 30 min and after 2h it became apparent that no reaction was taking place, and the loss of the ester signals in the nmr spectrum indicated that ethyl anacardate hed de-esterified and de-carboxylated. The reaction was abandoned.

Isomerisation of mixed cardanol

Cardanol (5.30g, 17.6mmol) was stirred in the presence of potassium hydroxide (2.51g, 44.8mmol) and triethanolamine (1.31g, 8.79mmol) in diethylene glycol (2ml). The reaction mixture was heated to 180 - 190°C, using an isomantle, in a nitrogen atmosphere. The solution was cooled to ambient and acidified with sulphuric acid (3M, 5ml) and extracted with ether (2 x 20ml). The organic layer was washed free of acid with bicarbonate solution (2 x 20ml), dried and evaporated to dryness *in vacuo*. Wt = 4.92g (89%).

λ_{max}(nm), 201(ε = 11039), 220.3(10227), 273.2(3755). δ(^{1}H $CDCl_3$), 7.21 - 7.01 (m, 1H), 6.76 - 6.55 (m, 3H), 6.41 - 5.40 (m, 4H), 5.32 (t, 1H; *J*, 4.1Hz), 4.47 (s, 1H; D_2O exch.), 2.53 (t, 2H; *J*, 7.1Hz), 2.17 (s, 2H), 1.29 - 0.87 (m, 20H). Found m/z = 304.5, 302.5, 300.5, 298.5; Requires m/z = 304.515, 302.499, 300.483, 298.467.

Isomerised cardanol methyl ether

Isomerised cardanol (0.83g, 2.76mmol) and powdered NaOH (0.165g, 4.14mmol) were mixed together in NMP (10ml) at room temperature. Dimethyl sulphate (0.351g, 2.81mmol) was then added and the reaction mixture stirred at ambient for 30min. After this time the reaction mixture was poured into water (50ml) and extracted with ether (3 x 50ml). The organic phase was washed thoroughly with water (6 x 50ml), to remove any unreacted dimethyl sulphate, dried and evaporated to dryness *in vacuo* and

separated from starting material using prep. TLC (2% ethyl acetate in chloroform). Wt = 0.47g (55%).

δ(^{1}H $CDCl_3$), 7.29 - 7.17 (m, 1H), 6.79 - 6.66 (m, 3H), 5.65 - 5.30 (m, 2H), 3.80 (s, 3H), 2.61 (t, 2H; *J*, 7.1Hz), 2.08 (m, 2H), 1.79 - 0.93 (m, 20H). Found m/z = 318.3, 316.3, 314.3, 312.3; Requires m/z = 318.542, 316.436, 314.420, 312.404.

Ethyl crotonate

Crotonic acid (104.2g, 1.19mol) was dissolved in ethanol (300ml) and sulphuric acid (18M, 2ml) was added. The reaction mixture was heated under reflux in the presence of molecular seive (4A, 20g) for 16h. The reaction mixture was then filtered and evaporated to dryness *in vacuo*. The residue was pored into a saturated solution of sodium bicarbonate and extracted into chloroform (3 x 70ml), dried, filtered and evaporated to dryness, then distilled at atmospheric pressure and the fraction boiling at 137 - 140^{0}C was collected. Wt = 107.14 (78%).

δ(^{1}H $CDCl_3$), 7.07 - 6:62 (m, $^1/_2$H), 5.83 - 5.57 (2 x q, $^1/_2$H; *J*, 1.7Hz), 4.18 - 3.91 (q, 2H; *J*, 7.1Hz), 1.79 - 1.68 (2 x d, 1H; *J*, 1.6Hz), 1.06 (t, 3H; *J*, 7.1Hz). Found m/z = 116.2; Requires m/z = 116.159.

Benzyl crotonate

Benzyl alcohol (29.5ml, 287mmol) and crotonic acid (64.5g, 750mmol) were mixed together in a 500ml rbf. Sulphuric acid (18M, 1ml) and molecular seive (4A, 10g) were introduced and the mixture refluxed with stirring for 16h. The reaction mixture was filtered, then poured into water (200ml) and carbon tetrachloride (10ml), to eliminate emulsion formation. The lower organic phase was separated and washed successively with water, sodium bicarbonate solution, and water, then dried, filtered and evaporated to dryness and vacuum distilled (Bpt = 98 - 99°C, 2mm). Wt = 18.68g(38%).

λ_{max}(nm), 211(ε = 5294). δ(^{1}H $CDCl_3$), 7.42 - 7.35(s, 5H), 7.26 - 6.89(dd, 1H; *J*, 6.90, 8.60Hz), 6.00 - 5.79(dq, 1H; *J*, 6.90, 1.60Hz), 5.18(s, 2H), 1.93 - 1.82(dd, 3H; *J*, 8.60, 1.60Hz). IR(liq. film cm^{-1}), 1720. Found m/z = 176.2; Requires m/z = 176.214.

Ethyl dihydroorsellinate[93]

Ethyl crotonate (67.12g, 578mmol) was added to a mechanically stirred solution of sodium ethoxide (40.12g, 581mmol) in ethanol (250ml) in the presence of ethyl acetoacetate (76.13g, 585mmol). This was then heated under reflux for 2h, heating was then discontinued and the reaction mixture allowed to cool to room temperature. The pH was adjusted to 7 with sulphuric acid (3M, 15ml), and the precipitated Na_2SO_4 was filtered. The filtrate was adjusted to pH4 with hydrochloric acid (3M) and then extracted with chloroform (3 x 50ml), dried, filtered and evaporated to dryness *in vacuo* to give a cream precipitate. Wt = 70.14g (62%).

Recrystallised from ethanol/water to give white needles. Mpt = 88 - 90°C (Lit. Mpt = 89 - 91°C).

δ(^{1}H $CDCl_3$), 2.73 (s, 2H), 2.56 - 2.38 (q, 2H; *J*, 5.2Hz), 2.00 (d, 2H; *J*, 2.5Hz), 1.35 - 1.10 (m, 1H), 1.06 (d, 1H; *J*, 2.5Hz), 0.57 (t, 3H; *J*, 5.2Hz), 0.38 (d, 3H; *J*, 4.0Hz). Found m/z = 198.1; Requires m/z = 198.218.

Attempted dehydrogenation of ethyl dihydroorsellinate

Ethyl dihydroorsellinate (5.16g, 26.1mmol) and selenium dioxide (3.47g, 31.3mmol) were dissolved in water/dioxan (4%, 20ml) and heated to 100°C under nitrogen. TLC and nmr indicated that a complex reaction mixture had formed, and the reaction was abandoned.

Dehydrogenation of ethyl dihydroorsellinate[97]

A solution of ethyl dihydroorsellinate (1.22g, 6.2mmol) in dry dioxan (10ml) was treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, DDQ, (1.58g, 6.7mmol) and heated on a water bath for 24h. The 2,3-dichloro-5,6-dicyano-1,4-hydroquinone that separates was filtered and washed with chloroform (3 x 20ml). The filtrate and chloroform washings were combined and washed with water (2 x 20ml), dried, filtered and evaporated to dryness *in vacuo*. The product was separated by column chromatography eluting with chloroform to give the ethyl orsellinate (46). Wt = 0.38g (18%).

Recrystallised from Ethanol/water to give white needles. Mpt = 197 - 198°C (Lit[82] = 196 - 198°C)

See later prep. for spectral details.

Ethyl dibromodihydroorsellinate[93] (55)

Ethyl dihydroorsellinate (46.61g, 235mmol) was dissolved in glacial acetic acid (300ml). Then with the temperature held at 40°C, bromine (27.3g, 502mmol) was added slowly with mechanical stirring. The reaction mixture was then stirred at ambient for 20h, and the precipitated dibromo-ester was collected by vacuum filtration and was air dried overnight. Wt = 56.92g (68%)

Recrystallised from ethanol/water to give white needles. Mpt = 143 - 145°C (Lit.[93] = 143 - 144°C).

δ(^{1}H $CDCl_3$), 4.59 - 4.32 (q, 2H; *J*, 7.2Hz), 2.68 (s, 3H), 1.43 (t, 3H; *J*, 7.1Hz).

Found m/z = 342.1; Requires m/z = 341.985.

2,4-Dihyroxy-3,5-dibromo-6-methyl benzoic acid[93]

Ethyl (2,4-dihydroxy-3,5-dibromo-6-methyl) benzoate (11.04g, 31mmol) was dissolved in sulphuric acid (18M, 30ml) and stirred at room temperature for 1h. The solution was the poured into ice-water (100ml) and a thick, white precipitate resulted.

This was extracted into ether (3 x 20ml) and the combined ether extracts were in turn extracted with a solution of bicarbonate (0.5M, 3 x 40ml). Acidification of the combined aqueous extracts to pH3 with hydrochloric acid (6M) resulted in the rapid precipitation of a thick, white solid which was filtered, and dried in a vacuum dessicator overnight. Wt = 9.15g(90%).

Recrystallised from methanol to give white needles. Mpt = 212 - 214°C, (Lit.[82] = 212 - 213°C).

λ_{max}(nm), 265 (ε = 8650), 313 (5850). δ(^{1}H $CDCl_3$), 10.14 (s, 1H; D_2O exch.), 4.57 (s, 2H; D_2O exch.), 2.73 (s, 3H). Found m/z = 326.0; Requires m/z = 325.950. Found C = 29.21; H = 2.47; $C_8H_6Br_2O_4$ requires C = 29.24; H = 2.40.

2,4-dihydroxy-6-methylbenzoic acid (Orsellinic acid)[93] (35, n = 15)

In a low pressure hydrogenation flask, 2,4-Dihydroxy-3,5-dibromo-6-methyl benzoic acid (23.79g, 72mmol) was slowly dissolved in acetic acid (250ml) with warming. After addition of sodium acetate (11.50g, 138mmol) and Pd/C (5%, 5.18g) the reaction mixture was shaken for 6h in a low pressure hydrogenation rig until no further uptake of H_2 occured (2,604ml). The reaction mixtuer was then poured into ether (250ml) and the entire reaction mixture was filtered. The organic phase was then washed well with water (7 x 100ml), dried and evaporated to dryness *in vacuo* to give *Orsellinic acid.*

Wt = 6.37g (54%).

Recrystallised from acetic acid/water to give white needles. Mpt = 172 - 173°C, (Lit.[93] = 172 - 174°C)

δ(^{1}H $(CD_3)_2CO$), 10.01 (s - v. broad, 3H; D_2O exch.), 6.27 - 6.15 (m, 2H), 2.50 (s, 3H). Found C = 57.23; H = 4.80; $C_8H_8O_4$ requires C = 57.14; H = 4.62.

Ethyl(2,4-dihydroxy-6-methyl) benzoate (Ethyl orsellinate)[93] (48)

Ethyl (2,4-dihydroxy-3,5-dibromo-6-methyl) benzoate (2.23g, 6.25mmol) was dissolved in glacial acetic acid (25ml) with warming. To the solution was added

sodium acetate (0.51g, 6.30mmol) and Pd/C (5%, 1.01g) and this was hydrogenated at atmospheric pressure and room temperature (requiring 457ml of H_2). The reaction mixture was then filtered, poured into water (50ml) and extracted into ether (3 x 30ml). The combined ether extracts were washed with a saturated solution of sodium bicarbonate, dried, filtered and evaporated to dryness *in vacuo*. Wt = 0.63g (53%). Recrystallised from ethanol/water to give white needles. Mpt = 197 - 198°C, (Lit.[93] = 196 - 198°C).

δ(^{1}H $CDCl_3$), 6.30 - 6.18 (m, 2H), 5.47 (s, 1H; D_2O exch.), 4.51 - 4.25 (q, 2H; *J*, 7.0Hz), 2.49 (s, 3H), 1.73 (s, 1H; D_2O exch.), 1.40 (t, 3H; *J*, 7.0Hz). Found C = 61.19; H = 6.34; $C_{10}H_{12}O_4$ requires C = 61.22; H = 6.12.

<u>Attempted transesterification of ethyl orsellinate</u>

Benzyl alcohol (1.32g, 12.2mmol) and sodium metal (0.015g, a.65mmol) were stirred together under nitrogen to form the benzyl alcoholate anion. to this was then added ethyl orsellinate (0.12g, 0.61mmol) and this was stirred under reduced pressure, to remove any liberated ethanol, for 12h. The reaction mixture was pored into water (20ml), acidified with acetic acid (3ml) and extracted into ether (3 x 20ml). The combined organic extracts were then washed with NaOH (3M, 2 x 40ml), brine and dried. There was no indication of the required product either by TLC or nmr, but the starting ethyl orsellinate was recovered. Wt = 0.082g (68%).

<u>Attempted transesterification of ethyl orsellinate with tetraethyl titanate</u>

To a solution of ethyl orsellinate (12.38g, 63.2mmol) in benzyl alcohol (200ml) was added tetraethyl titanate (5.00g, 22mmol) and the mixture was heated to 100°C overnight. The cooled reaction mixture was quenched with hydrochloric acid (3M, 100ml) and extracted with ether (2 x 200ml). The combined organic extracts were washed with saturated aqueous sodium bicarbonate (100ml) and saturated brine solution (100ml), dried and evaporated to dryness in vacuo (65°C/2mm for benzyl alcohol). No

apparent reaction had taken place and so the reaction was abandoned after recovery of the starting material (76%).

Ethyl(2,4-dimethoxy-6-methyl) benzoate

Ethyl (2,4-dihydroxy-6-methyl) benzoate (5.2g, 26.8mmol) waqs stirred at room temperature in acetone (100ml) with potassium carbonate (10.14g, 82.5mmol) for 30min. Then dimethyl sulphate (9.08g, 72.8mmol) was added to the reaction mixture and was heated under reflux for 12h. The reaction mixture was then filtered and evaporated to dryness. Purified by the extraction of an ether solution of the crude reaction mixture with a solution of sodium hydroxide (3M, 4 x 10ml), to remove any unreacted phenol. The ether phase was then dried, filtered and evaporated to dryness *in vacuo* to give the required product as a colourless oil. Wt = 4.82g (81%).

$\delta(^1H\ CDCl_3)$, 6.28(s, 2H), 4.47 - 4.20 (q, 2H; *J*, 7.4Hz), 3.77 (s, 6H), 2.28 (s, 3H), 1.35 (t, 3H; *J*, 7.3Hz). Found m/z = 224.2; Requires m/z = 224.256. Found C = 62.31; H = 7.47; $C_{12}H_{16}O_4$ requires C = 62.26; H = 7.55.

Ethyl (2,4-dibenzyloxy-6-methyl) benzoate

Ethyl (2,4-dihydroxy-6-methyl) benzoate (1.27g, 6.47mmol) and potassium carbonate (2.02g, 14.6mmol) were stirred together in acetone (50ml) for 30min. Benzyl bromide (1.56g, 13.2mmol) was then added and the reaction mixture refluxed for 16h. The potassium carbonate was then filtered and the filtrate evaporated to dryness. Any unreacted phenol was removed in the same manner as previously described. Excess benzyl bromide was removed by passing the crude reaction mixture through a short column of silica eluting the benzyl bromide with 100% petroleum ether, and the *title compound* with 100% ethyl acetate. Wt = 1.93g (79%).

Recrystallised from ethanol/water to give white needles. Mpt = 82 - 83°C.

λ_{max} (nm), 209(ε = 9294), $\delta(^1H\ CDCl_3)$, 7.23 (s, 10H), 6.43 (s, 2H), 5.05 (s, 4H), 4.41 - 4.28 (q, 2H; *J*, 7.0Hz), 2.28 (s, 3H), 1.30 (t, 3H; *J*, 7.0Hz). Requires m/z =

364.404; Found m/z = 364.5. Found C = 76.47; H = 6.61; $C_{23}H_{24}O_4$ requires C = 76.75; H = 6.42.

Ethyl (2,4-dimethoxy-6-ethyl) benzoate[92] **(62)**

To dry THF (2ml), containing a small crystal of triphenylmethane under nitrogen, was added *n*-butyl lithium (1.6M in hexane) until after a few drops a persistent pink colour indicated complete dryness. To this was added di-*iso*-propylamine (0.127g, 1.27mmol) followed by *n*-butyl lithium (2.1ml, 3.36mmol) and this was allowed to stir at ambient for 15min. After cooling to -78°C ethyl (2,4-dimethoxy-6-methyl) benzoate (0.293g, 1.31mmol) in dry THF (5ml) was added dropwise. This was left ot stir at -78°C for 30min, then methyl iodide (0.047g, 3.30mmol) was added. The reaction mixture was allowed to warm to room temperature and ethanol (15ml) was added, and then left to stir overnight. The reaction mixture was then poured in to an excess of hydrochloric acid (3M, 20ml) and then extracted with ether (2 x 20ml) and ethyl acetate (2 x 20ml). The combined organic extracts were washed with a saturated solution of potassium carbonate, dried, filtered and evaporated to dryness *in vacuo*. The product was separated from the starting material by using the chromatotron (100% chloroform eluant) to give the *title compound*. Wt = 0.191g (61%).

δ(^{1}H $CDCl_3$), 6.28 (s, 2H), 4.41 - 4.28 (q, 2H; *J*, 7.4Hz), 3.77 (s, 6H), 2.35 - 2.23 (q, 2H; *J*, 7.2Hz), 1.33 (t, 3H; *J*, 7.4Hz), 1.12 (t, 3H; *J*, 7.2Hz). Requires m/z = 226.272; Found m/z = 226.1. Found C = 63.89; H = 7.74; $C_{12}H_{18}O_4$ requires C = 63.70; H = 8.02.

Ethyl (2,4-dimethoxy-6-pentadecyl) benzoate[92]

To the anion of ethyl (2,4-dimethoxy-6-methyl) benzoate (3.41g, 14.1mmol) in dry THF (10ml) was formed with *n*-butyl lithium (8.8ml, 14.1mmol) and di-*iso*-propylamine (0.532g, 5.32mmol). To this anion, stirred at -78°C, was added 1-bromotetradecane (3.91g, 14.0mmol) in THF (10ml) and the reaction mixture allowed

to warm to room temperature, ethanol (30ml) added and left to stir overnight. The reaction mixture was worked up as previously describedand the *title compound* separated by column chromatography eluting with 2% ethyl acetate in petroleum ether. Wt = 0.177g (3%).

δ(^{1}H $CDCl_3$), 6.48 (s, 2H), 4.48 - 4.21 (q, 2H; *J*, 7.1Hz), 3.84 (s, 6H), 2.36 (t, 2H; *J*, 7.4Hz), 1.59 - 0.89 (m, 32H). Requires m/z = 420.631; Found m/z = 420.5. Found C = 74.38; H = 44.17; $C_{26}H_{44}O_4$ requires C = 74.24; H = 44.35.

Attempted preparation of ethyl (2,4-dibenzyloxy-6-pentadecyl) benzoate[92]

To dry THF (2ml), containing a small crystal of triphenylmethane under nitrogen, was added *n*-butyl lithium (1.6M in hexane) until after a few drops a persistent pink colour indicated complete dryness. To this was added di-*iso*-propylamine (0.128g, 1.28mmol) followed by *n*-butyl lithium (2.1ml, 3.36mmol) and this was allowed to stir at ambient for 15min. After cooling to -78°C, ethyl (2,4-dibenzyloxy-6-methyl) benzoate (0.493g, 1.32mmol) in dry THF (5ml) was added dropwise. This was left to stir at -78°C for 30min, then 1-bromotetradecane (0.914g, 3.31mmol) was added. The reaction mixture was allowed to warm to room temperature and the ethanol (15ml) was added, and allowed to stir overnight. The reaction mnixture was poured into an excess of dilute hydrochloric acid (3M, 20ml) and then extracted into ether (2 x 20ml) and then extracted with ethyl acetate (2 x 20ml). The combined organic extracts were washed with a saturated solution of sodium bicarbonate (2 x 20ml), dried, filtered and evaporated to dryness *in vacuo*. TLC and nmr indicated that complex mixture of products were present in the reaction mixture and the reaction was abandoned.

Triethyl phosphonacetate[99]

Ethylchloroacetate (100.10g, 815mmol) and triethylphosphite (135.48g, 816mmol) were thoroughly mixed and placed in an RBF fitted with a condenser, thermometer and dry nitrogen supply. The reaction mixture was stirred, under a nitrogen atmosphere,

and heated to 125°C then heating was discontinued for 30min whereupon the evolution of chloroethane occurred. The reaction mixture was then heated to 160°C over 1h and stirred at this temperature for 8h then allowed to cool to room temperature overnight. The product was distilled under vacuum and the *title compound* collected at 119 - 121°C (0.2mm). Wt = 131.77g (72%).

δ(^{1}H $CDCl_3$), 4.33 - 3.96 (2q, 4H; *J*, 7.2Hz and *J*, 6.7Hz), 3.07 - 2.80 (d, 2H; *J*, 21.7Hz), 1.42 - 1.19 (2t, 6H; *J*, 7.1Hz and *J*, 6.7Hz). Requires m/z = 224.193; Found m/z = 224.2.

Hexadecan-1-al **(61)**

1) Pyridinium chlorochromate.

To hydrochloric acid (6M, 184ml) was added chromium (IV) oxide (100.29g, 1.0mol) rapidly with stirring. After 5min the homogeneous solution was cooled to 0°C and pyridine (79.1g, 1.0mol) was added over a 10min period. Re-cooling to 0°C gave an orange-yellow solidwhich was collected on a sintered glass funnel and dried at ambient *in vacuo*. Wt = 180.8g (84%).

2) Hexadecan-1-al

Pyridinium chlorochromate (170.33g, 790mmol) was suspended in dichloromethane (1000ml). Hexadecan-1-ol (133.1g, 550mmol) in dichloromethane (600ml) was added in one portion to the magnetically stirred suspension. After 1.5h at room temperature, ether (500ml) was added, and the supernatant liquid decanted from the black gum. The organic phase was then passed through a large silica plug to give, after separation of the solvent, a pale yellow liquid which solidified on cooling. Wt = 79.42g (62%). Mpt = 30 - 31°C

IR (melt) cm^{-1}, 1725.

Ethyl octadec-2-ene-1-oate[99] (64)

To dry ether (250ml) was added sodium hydride (60% dispersion in mineral oil, 10.13g, 224mmol), under nitrogen. This stirred suspension was cooled to 1 - 2°C and triethyl phosphonacetate (50.11g, 223mmol) added over a period of 1.5h. This was then heated to reflux for 1.5h and then cooled in an ice/salt bath and then a solution of hexadecan-1-al (57.41g, 238mmol) in ether (100ml) was added over 1h. The reaction mixture was heated to reflux for 10min and then allowed to cool to room temperature. A thick precipitate of sodium diethyl phosphate formed, which was allowed to settle and the upper ether phase decanted. The lower phase was dissolved in warm water and the upper organic layer was separated. The aqeous layer was further extracted with ether (2 x 100ml). The combined ether extracts were washed with a saturated solution of sodium bicarbonate, dried, filtered and evaporated to dryness *in vacuo* and then distilled, bp = 184 - 186°C (0.04mm), to give the *title compound.* Wt = 25.13g (35%). δ(^{1}H $CDCl_3$), 7.02 - 6.82 (dd, 1H; *J*, 15.7Hz), 5.87 - 5.64 (dt, 1H; *J*, 15.7Hz), 4.29 - 4.02 (q, 2H; *J*, 7.1Hz), 2.38 - 2.22 (m, 2H), 1.36 - 1.06 (m, 29H; max at 1.25), 1.00 - 0.87 (t, 3H; *J*, 7.4Hz). IR (liq. film) cm^{-1}, 1725, 1650.

Ethyl (2,4-dione-6-pentadecyl) cyclohexanoate[99] (65)

To a solution of sodium metal (1.63g, 71mmol) in ethanol (130ml) was added ethyl acetoacetate (10.06g, 77mmol). the solution was stirred at reflux for 30min. Ethyl octadec-2-ene-1-oate (20.92g, 67mmol) was added over a period of 1h to the refluxing solution. This was then refluxed in a nitrogen atmosphere for 20h. The reaction mixture was then cooled in an ice/water bath and sulphuric acid (3M) added until pH 7 was reached. The solid sodium sulphate was filtered and the filtrate adjusted to pH 4 with hydrochloric acid (3M) and then extracted into chloroform (3 x 50ml), dried, filtered and evaporated to dryness *in vacuo* to give an off-white solid. Wt = 15.31g (58%). Recrystallised from ethanol/water to give white needles. Mpt = 58 - 60°C.

λ_{max} (nm) = 205 (ε = 640), 257 (1667), 279 (1417). δ(^{1}H $CDCl_3$), 4.37 - 4.10 (q, 2H; *J*, 7.0Hz), 3.59 - 3.42 (m, 2H), 2.68 - 2.14 (m, 3H), 1.63 - 1.12 (m, 32H; max at 1.24), 0.87 (t, 3H; *J*, 7.1Hz). Requires m/z = 394.593; Found m/z = 394.5.

Ethyl (2,4-dihydroxy-3,5-dibromo-6-pentadecyl) benzoate[99] (66)

Ethyl (2,4-dione-6-pentadecyl) cyclohexanoate (10.19g, 25.8mmol) was dissolved in glacial acetic acid (150ml). To this magnetically stirred solution was added bromine (9.07g, 57mmol) with the reaction temperature maintained at 40 - 50°C. When addition was complete the reaction mixture was stirred at room temperature for 18h. This was then poured into water (500ml) and extracted with ether (4 x 50ml). The combined ether extracts were washed with a solution of saturated sodium bicarbonate, dried, filtered and evaporated to dryness *in vacuo*. The crude product was purified by chromatography (10% ether/petroleum ether). Wt = 9.32g (66%).

λ_{max}(nm), 265(ε = 15463), 313(6328). δ(^{1}H $CDCl_3$), 4.45 - 4.27 (q, 2H; *J*, 7.0Hz), 3.09 (t, 2H; *J*, 7.2Hz), 1.81 - 0.74 (m, 32H; max at 1.25). Requires m/z = 550.380; Found m/z = 550.1. Found C = 52.44; H = 6.72; Br = 28.91; $C_{24}H_{38}Br_2O_4$ requires C = 52.36; H = 6.96; Br = 29.04.

2,4-Dihydroxy-3,5-dibromo-6-pentadecyl benzoic acid[93] (67)

Ethyl (2,4-dihydroxy-3,5-dibromo-6-pentadecyl) benzoate (5.21g, 9.41mmol) was stirred in sulphuric acid (18M, 20ml) at room temperature for 1h. The solution was poured into crushed-ice which resulted in the formation of a thick-white precipitate. The entire reaction mixture was then extracted into ether (3 x 20ml) which were combined and in turn washed with a solution of sodium bicarbonate (0.5M, 3 x 20ml). Acidification of the combined aqueous extracts to pH 3 with hydrochloric acid (6M, 15ml) resulted in the rapid precipitation of a white solid . This was vacuum filtered and dried overnight at room temperature in a vacuum dessicator. Wt = 4.25g (86%).

λ_{max} (nm), 265(ε = 8942), 313(5770). δ(^{1}H $CDCl_3$), 4.57 (s, 3H; D_2O exch.), 2.41 (t, 2H; *J*, 7.4Hz), 2.37 (s, 3H), 1.58 - 0.88 (m, 29H). Requires m/z = 522.326; Found m/z = 478.1. Found C = 50.41; H = 6.61; Br = 34.21; $C_{22}H_{34}Br_2O_4$ requires C = 50.58; H = 6.56; Br = 34.43.

2,4-dihydroxy-6-pentadecyl benzoic acid[93] (35; n = 15)

In a low pressure hydrogenation flask, 2,4-dihydroxy-3,5-dibromo-6-pentadecyl benzoic acid (2.47g, 4.71mmol) was slowly dissolved in glacial acetic acid (30ml) with warming. After the addition of sodium acetate (0.4g, 4.8mmol) and Pd/C (5%, 2.50g) the reaction mixture was shaken under a hydrogen atmosphere for 6h. The reaction mixture was washed intoa beaker containing ether (30ml) and, after thorough stirring, the entire mixture was gravity filtered. To the clear filtrate was added water (120ml) and the two phase mixture separated. The aqueous phase was extracted further with ether (2 x 60ml). The combined ether extracts were washed with water (2 x 30ml), dried, and evaporated to dryness in vacuo yielding a white solid. Recrystallised from acetic acid to give white needles. Mpt = 128 - 129°C

λ_{max} (nm), 265(ε = 16548), 313(5386). δ(^{1}H $CDCl_3$), 10.07 (s, 3H; D_2O exch.), 6.28 - 6.15 (m, 2H), 2.47 (t, 2H; *J*, 7.4Hz), 1.60 - 0.89 (m, 29H). Requires m/z = 364.524; Found m/z = 320.5. Found C = 72.31; H = 10.21; $C_{22}H_{36}O_4$ requires C = 72.49; H = 9.95.

Ethyl (2,4-dihydroxy-6-pentadecyl) benzoate[93]

In a low-pressure hydrogenation flask, ethyl (2,4-dihydroxy-3,5-dibromo-6-pentadecyl) benzoate (3.17g, 5.73mmol) was slowly dissolved in glacial acetic acid (30ml) with warming. To the solution was added sodium acetate (0.47g, 5.74mmol) and Pd/C (5%, 3.20g), and this was then shaken in a hydrogen atmosphere at room temperature for 10h. The reaction mixture was then carefully filtered and the clear filtrate poured into water (60ml) and extracted with ether (3 x 30ml). The combined organic extracts were

neutralised with solid sodium bicarbonate, dried, filtered and evaporated to dryness *in vacuo*. Purified by column chromatography (2% ethyl acetate in chloroform), to give a white solid. Wt = 1.64g (73%). Recrystallised from ethanol/water to give white needles. Mpt = 107 - 109°C.

λ_{max} (nm), 265(ε = 17436), 311(6352). δ(^{1}H $CDCl_3$), 6.33 - 6.20 (m, 2H), 5.52 (s, 2H; D_2O exch.), 4.52 - 4.26 (q, 2H; *J*, 7.2Hz), 2.41 (t, 2H; *J*, 7.4Hz), 1.75 (s, 1H; D_2O exch.), 1.58 - 0.87 (m, 32H). Requires m/z = 392.578; Found m/z = 392.0. Found C = 73.12; H = 10.49; $C_{24}H_{40}O_4$ requires C = 73.42; H = 10.27.

2,8,14,20-Tetramethylpentacyclo[19.3.1.1^{3,7}.1^{9,13}.1^{15,19}]octacosa-1(25),3,5,7(28),9,11,13(27),15,17,19(26),21,23-dodecaene-4,6,10,12,16,18,22,24-octol[100] (68)

To a solution of resorcinol (55.05g, 503mmol) in ethanol (250ml) was added water (250ml) and hydrochloric acid (18M, 125ml) under nitrogen. The stirred solution, under nitrogen, was cooled to 15°C and acetaldehyde (22.01g, 505mmol) was added dropwise over a 30min period. The mixture was then stirred at 50°C for 1h and then allowed to cool to 25°C. After 4days at 25°C a precipitate was produced. The solid was triturated twice with water, filtered, and recrystallised four times from ethanol-water to give the required product. Wt = 41.34g (61%).

Further recrystallisation fror acetonitrile to give single crystals. Mpt = < 360°C (Lit[100] Mpt = < 360°C)

δ(^{1}H $(CD_3)_2CO$), 7.62 (s, 4H), 6.22 (s, 4H), 4.66 - 4.40 (q, 4H; *J*, 7.3Hz), 1.81 - 1.72 (d, 12H; *J*, 7.3Hz). δ(^{13}C $(CD_3)_2CO$) 152.249(s), 126.026(d), 125.122(d), 103.514(d), 29.813(d), 20.264(q). Found C = 64.48; H = 5.85; $C_{32}H_{32}O_8.3H_2O$ requires C = 64.21; H = 6.15.

2,8,14,20-Tetraundecylpentacyclo[19.3.1.1^{3,7}.1^{9,13}.1^{15,19}]octacosa-1(25),3,5,7(28),9,11,13(26),21,23-dodecaene-4,6,10,12,16,18,22,24-octol[100] (79).

A solution of resorcinol (19.87g, 181mmol) in ethanol (75ml) and hydrochloric acid (18M, 25ml) was cooled to 2°C. Dodecanal (33.22g, 184mmol) in ethanol (50ml) was added dropwise over a period of 2h to the stirred solution under nitrogen. The resulting solution was allowed slowly to warm to 25°C and then heated to 75°C for 21h. The precipitate formed was filtered and repeatedly washed with cold methanol to give the required product. Wt = 34.2g (68%).

Recrystallised three times from methanol to give white needles. Mpt = 285°C dec.

δ(H^1 $(CD_3)_2CO$) 8.44(s, 8H), 7.54(s, 4H), 6.24(s, 4H), 4.31(t, 4H; *J*, 7.82Hz), 2.30(m, 8H), 1.30(s, 72H), 0.89(t, 12H; *J*, 6.45Hz). Found C = 78.01; H = 10.31; $C_{72}H_{112}O_8$ requires C = 78.21; H = 10.21.

2,8,14,20-Tetrapentadecylpentacyclo[19.3.1.^{13,7}.1^{9,13}.1^{15,19}]octacosa-1(25),3,5,7(28),9,11,13(27),15,17,19(26),21,23-dodecaene-4,6,10,12,16,18,22,24-octol[100] (80)

A solution of resorcinol (20 14g, 183mmol) in a solution of ethanol (75ml) and hydrochloric acid (12M, 25ml) was cooled to 2°C. Hexadecan-1-al (43.92g, 183mmol) was added dropwise to the solution stirred under nitrogen over a period of 2h. The resulting solution was slowly warmed to room temperature and then heated to 70°C for 21h. The precipitate that separated was filtered and washed repeatedly with ice-cold methanol and dried. Wt = 15.71g (38%). This was twice recrystallised from methanol to give a white solid. Mpt = 290°C (dec.).

δ(^{1}H $CDCl_3$), 8.45 (s, 8H), 7.54 (s, 4H), 6.24 (s, 4H), 4.32 (t, 4H; *J*, 7.7Hz), 2.31 (m, 8H), 1.59 - 0.89 (m, 116H). Requires m/z = 1330.101; Found m/z = 1330.102. Found C = 79.31; H = 11.14; $C_{88}H_{144}O_8$ requires C = 79.47; H = 10.91.

1,21,23,25-Tetramethyl-2,20:3,19-dimetheno-1*H*,21*H*,23*H*,25*H*-bis[1,3]dioxcino[5,4-*i*:5',4'-*i*']benzo[1,2-*d*:5,4-*d*']bis[1,3]benzodiocin[110] (81)

To a mixture, stirred at 30°C under nitrogen, of dry DMSO (250ml) and finely ground potassium carbonate (27.25g, 150mmol) were added over 4 days (by means of a a dropping funnel whose end was drawn into a capillary) dried octol (68) (7.22g, 12mmol) and bromochloromethane (7.15g, 110mmol) dissolved in dry DMF (40ml). The reaction mixture was stirred for a further day at 30°C under nitrogen and then poured into an aqeous solution of sodium chloride (2M, 500ml). The mixture was agitated for 1h and the precipitate that separated was filtered and washed with water. The solid material was suspended in dichloromethane (150ml), stirred for 5h and filtered. The solid was then suspended in acetone/dichloromethane (10%, 100ml) for a further 3h and filtered again. The combined organic filtrates were washed with aqeous sodium hydroxide (2M, 200ml) and water (100ml), dried, filtered and evaporated to dryness *in vacuo*. The residue was washed through a column of silica with dichloromethane and the residue was evaporated to a volume of 50ml and diluted with ethanol (100ml), cooled to 0°C and the product crystallised. This was collected and washed with cold dichloromethane to give, after drying, the title compound. Wt = 1.50g (19%). The associated dichloromethane was removed by repeated evaporation of chloroform solutions of the compound. Mpt = < 360°C.

δ(^{1}H $CDCl_3$), 7.25 (s, 4H), 6.47 (s, 4H), 5.75 (d, 4H; *J*, 7.2Hz), 4.96 (q, 4H; *J*, 7.4Hz), 4.45 (d, 4H; *J*, 7.2Hz), 1.77 (d, 12H; *J*, 7.4Hz). Requires m/z = 592.644; Found m/z = 592.1. Found C = 72.80; H = 5.51; $C_{36}H_{32}O_8$ requires C = 72.86; H = 5.44.

5,11,17,23-Tetrabromo-2,8,14,20-tetramethylpentacyclo[19.3.1.1^{3,7}.1^{9,13}.1^{15,19}]octacosa-1(25),3,5,7(28),9,11,13(27),15,17,19(26),21,23-dodecaene[110] (82)

To a stirred orange solution of of dried octol (68) (25.80g, 47.4mmol) in 2-butanone (600ml) was added *N*-bromosuccinamide (67.81g, 380mmol) in portions. After 10min the product started to crystallise. The mixture was stirred for 4h and the product was

collected by filtration and washed with hot dichloromethane and dried under vacuum at 100°C to give the *title compound* as a white solid. Wt = 31.38g (78%). A sample was recrystallised from DMF/dichloromethane to give white needles. Mpt = < 360°C (dec.). δ(^{1}H (CDC_3)$_2$SO), 8.28 (s, 8H; D_2O exch.), 6.78 (s, 4H), 4.72 - 4.45 (q, 4H; *J*, 7.0Hz), 1.43 - 1.35 (d, 12H; *J*, 7.0Hz). Required m/z = 860 204; Found m/z = 860.200. Found C = 44.76; H = 3.28; $C_{32}H_{28}Br_4O_8$ requires C = 44.49; H = 3.28.

7,11,15,28-Tetrabromo-1,21,23,25-tetramethyl-2,20:3,19-dimetheno-1*H*,21*H*,23*H*,25*H*-bis[1,3]dioxocino[5,4-*i*:5',4'-*i*']benzodioxocin (83) and 4,8,12,16-Tetrabromo-20,22,24,25-tetramethyl-2,18-methano-20*H*,22*H*,24*H*-dibenzo[*d*,*d*'][1,3]dioxocino[5,4-*i*:7,8-*i*]bis[1,3]-benzodioxocin-3,17-diol[110] (84)

A mixture of dry octol (82) (1.02g, 1.12mmol), caesium carbonate (6.06g, 18.6mmol) and bromochloromethane (1.23g, 9.53mmol) in dry DMSO (50ml) were stirred together under nitrogen at 70°C for 24h. The mixture was cooled and filtered through 'Celite' and the solvent was removed from the filtrate under vacuum. The residue was dissolved in dichloromethane (30ml) and the solution washed with water and brine and then dried. The solvent was removed *in vacuo* to give a yellow solid which was a mixture of two compounds by TLC. This material was chromatographed, eluting with dichloromethane to give first (83), Wt = 0.62g (55%), followed by (84), Wt = 0.12g (11%).

(83) was recrystallised from dichloromethane/hexane (1:1). Mpt = > 360°C

δ(^{1}H $CDCl_3$), 7.21 (s, 4H), 5.92 (d, 4H; *J*, 7.2Hz), 5.05 (q, 4H; *J*, 7.4Hz), 4.38 (d, 4H; *J*, 7.2Hz), 1.75 (d, 12H; *J*, 7.4Hz). Requires m/z = 912.280; Found m/z = 912.280. Found C = 47.61; H = 3.28; $C_{36}H_{28}Br_4O_8$ requires C = 47.60; H = 3.10.

(84) was recrystallised from dichloromethane/acetonitrile. Mpt = 335 - 340°C (dec.).

δ(^{1}H $CDCl_3$), 7.18 (s, 2H), 7.12 (s, 2H), 5.93 (d, 2H; *J*, 7.2Hz), 5.91 (d, 1H; J, 7.2Hz), 5.05 (q, 3H; J, 7.4Hz), 5.00 (q, 1H; J, 7.4Hz), 4.40 (d, 2H; J, 7.2Hz), 4.38 (d, 1H; J,

7.2Hz), 1.77 (d, 3H; J, 7.4Hz), 1.76 (d, 6H; J, 7.4Hz), 1.72(d, 3H; J, 7.4Hz). Requires m/z = 900.269; Found m/z = 900.267. Found C = 47.10; H = 3.44; $C_{35}H_{28}Br_4O_8$ requires C = 47.39; H = 3.31.

4.8.12.16-Tetrabromo-20.22.24.25-tetramethyl-2.18-metheno-3.17-benzyloxo-20*H*.22*H*.24*H*-dibenzo[*d*.*d*']1.3] dioxocino[5.4-*i*:7.8-*i*]bis[1.3] benzodoxocin (85)

To a stirred solution of benzaldehyde (0.062g, 0.06mmol), at 0°C in dichloromethane (1ml), was added concentrated sulphuric acid (18M, 1 drop). To this yellow solution was added dry (84) (0.0135g, 0.015mmol). This was allowed to warm to room temperature and stirred at 25°C for 8h. The precipitate that formed was collected and purified by preparative TLC eluting with chloroform. Wt = 0.0124g (84%). δ(^{1}H $CDCl_3$) 7.27 - 7.11 (m, 7H), 6.04 (s, 1H), 5.96 (d, 2H; *J*, 7.2Hz), 5.94 (d, 1H; *J*, 7.2Hz), 5.11(q, 3H; *J*, 7.4Hz), 5.04 (q, 1H: *J*, 7.4Hz), 4.42 (d, 2H; *J*, 7.2Hz), 4.40 (d, 1H; *J*, 7.2Hz), 1.78 (d, 3H; *J*, 7.4Hz), 1.76 (d, 6H; *J*, 7.4Hz), 1.72 (d, 3H; *J*, 7.4Hz). Found C = 50.81; H = 3.02; $C_{42}H_{29}Br_4O_8$ requires C = 51.04; H = 2.86.

3.5-Dimethoxytoluene

To a mechanically stirred solution of orcinol (40.43g, 284mmol) and potassium carbonate (78g, 625mmol) in acetone (100ml) was added dimethyl sulphate (78.16g, 625mmol). This was stirred under reflux for 24h. After filtration and evaporation of the solvent the residue was dissolved in ether (50ml) and washed successively with sodium hydroxide (2M, 2 x 50ml), ammonia solution (0.88M, 100ml) and a saturated solution of brine. The ether phase was then dried, filtered and evaporated to dryness *in vacuo*, and the product distilled at 106 - 107°C (12mm). Wt = 31.13g (72%).

4-bromo-3,5-dimethoxytoluene[116]

To a stirred solution of 3,5-dimethoxy toluene (2.00g, 13mmol), under nitrogen in dry ether (20ml), was added phenyl lithium (2M in hexane, 6ml, 13.5mmol). The mixture was left to stir in the dark at room temperature for 100h. 1,2-dibromoethane (2.46g, 13mmol) in dry ether (20ml) was then added over a peiod of 2h. This solution was the stirred at room temperature for 3h. The reaction was quenched with dilute hydrochloric acid (3M, 50ml) and the organic phase was then neutralised, dried and evaporated to dryness in vacuo. Precipitation of the product was acheived by the addition of petroleum ether (60 - 80) and cooling. Wt = 2.20 (71%). Recrystallised from ether/petroleum ether to give white needles. Mpt = 74 - 750C.

$\delta(^1H\ CDCl_3)$, 6.25 (s, 2H), 2.78 (s, 6H), 2.39 (s, 3H). Requires m/z = 151.185; Found m/z = 151.0.

4-Bromo-3,5-dihydroxytoluene[116]

To a stirred solution, under nitrogen, of 4-bromo-3,5-dimethoxytluene (1.60g, 6.93mmol) in dry dichloromethane (20ml), at 0°C, was added a solution of boron tribromide (2.2M, 0.4ml) in dichloromethane. This was then stirred under reflux for 2h. The reaction mixture was then poured into ice-water and extracted into dichloromethane (2 x 20ml). The organic extracts were dried, filtered and evaporated to dryness. The product was purified by column chromatography eluting with dichloromethane. Wt = 1.37g (98%). Mpt = 110°C (sublimes).

$\delta(^1H\ CDCl_3)$, 6.44 (s, 2H), 5.30 (s, 2H; D_2O exch.), 2.24 (s, 3H). Requires m/z = 203.041; Found m/z = 203.1.

2,4,14,20-tetrahydro-25,26,27,28-tetramethylpentacyclo[19.3.1.1^{3,7}.1^{9,13}.1^{15,19}]octacosa-1(25),3,5,7(28),9,11,13(27),15,17,19(26),21,23-dodecaene-4,6,10,12,16,18,22,24-octol (86; R = Me)

4-Bromo-3,5-dihydroxytoluene (5.27g, 26mmol) was dissolved in ethanol (20ml) and water (20ml), and concentrated hydrochloric acid (37%, 2ml). To this stirred solution was added formaldehyde (37%, 2.2ml, 27mmol). The reaction was immediately cooled in an ice-water bath to control the exotherm. The reaction mixture was then slowly heated to 70°C and maintained at that temperature for 16h. The reaction mixture was then neutralised with sodium hydroxide (3M, 4ml) and extracted into ether (3 x 30ml). This was then dried, filtered and evaporated to dryness *in vacuo*. The product was isolated from the reaction mixture by column chromatography eluting with 20% ethyl acetate in chloroform. Wt = 0.67g (12%).

δ(^{1}H $(CD_3)_2CO$), 5.42 (s, 8H; D_2O exch.), 4.62 (s, 8H), 4.17 - 4.02 (q, 12H; *J*, 7.1Hz). Requires m/z = 860.204; Found m/z = 860.203. Found C = 44.39; H = 3.52; $C_{32}H_{28}Br_4O_8$ requires C = 44.68; H = 3.28.

3,5-Dimethoxy pentadecyl benzene

To a mechanically stirred solution of 3,5-dihydroxy pentadecyl benzene (10.17g, 32mmol) and potassium carbonate (8.78g, 65mmol) in acetone (25ml), dimethyl sulphate (7.99g, 65mmol) was added. This was stirred under reflux for 24h. After filtration and evaporation, the residue was dissolved in ether (20ml) and washed successively with sodium hydroxide (2M, 2 x 20ml), ammonia solution (0.88M, 50ml) and a saturated solution of brine. The ether phase was then dried, filtered and evaporated to dryness *in vacuo*, and the product separated by chromatography, eluting with chloroform. Wt = 8.62g (78%).

δ(^{1}H $CDCl_3$), 6.32 (s, 1H), 6.28 - 6.25 (m, 2H), 3.73 (s, 6H), 2.54 (t, 2H; *J*, 7.4Hz), 1.54 - 0.89 (m, 29H). Requires m/z = 348.548; Found m/z = 348.1.

4-Bromo-3,5-dimethoxy pentadecyl benzene

To a stirred solution of 3,5-dimethoxypentadecyl benzene (7.21g, 21mmol), under nitrogen, in dry ether (30ml) was added phenyl lithium (2M in hexane, 10ml, 22mmol). The mixture was allowed to stir in the dark at room temperature for 100h. 1,2-Dibromoethane (4.11g, 22mmol) in dry ether (30ml) was then added over a period of 2h. This solution was then stirred at room temperature for a further 3h. The reaction mixture was then quenched with dilute hydrochloric acid (3M, 50ml) and the organic phase was then neutralised, dried and evaporated to dryness *in vacuo*. The product was then separated by column chromatography, eluting with chloroform. Wt = 6.81g (77%).

$\delta(^1H\ CDCl_3)$, 6.27 (s, 2H), 3.21 (s, 6H), 2.51 (t, 2H; *J*, 7.4Hz), 1.55 - 0.89 (m, 29H). Requires m/z = 427.449; Found m/z = 427.2.

4-Bromo-3,5-dihydroxy pentadecyl benzene

To a stirred solution, under nitrogen, of 4-bromo-3,5-dimethoxy pentadecyl benzene (6.13g, 14mmol) in dry dichloromethane at 0°C was added a solution of boron tribromide (2.2M, 1ml) in dichloromethane. This was then stirred under reflux for 2h. The reaction mixture was poured into ice-water and extracted into dichloromethane (2 x 30ml). The combined organic extracts were dried, filtered and evaporated to dryness *in vacuo*. The product was purified by column chromatography eluting with dichloromethane. Wt = 5.41g (94%).

$\delta(^1H\ CDCl_3)$, 6.51 (s, 2H), 2.60 (t, 2H; *J*, 7.4Hz), 1.59 - 0.88 (m, 29H). Requires m/z = 401.411; Found m/z = 401.2. Found C = 62.77; H = 8.92; $C_{21}H_{35}BrO_2$ requires C = 62.84; H = 8.79; Br = 19.91.

Attempted preparation of 2,4,14,20-tetrahydro-25,26,27,28-tetrapentadecylpentacyclo[19,3,1,1^{3,7},1^{9,13},1^{15,19}]octacosa1(25),3,5,7(28),9,11,13(27),15,17,19(26),21,23-dodecaene-4,6,10,12,16,18,22,24-octol (86; R = $C_{15}H_{31}$)

4-Bromo-3,5-dihydroxy pentadecyl benzene (5.03g, 12.5mmol) was dissolved in water (20ml), ethanol (20ml) and concentrated hydrochloric acid (37%, 2ml). To this stirred solution was added formaldehyde (37%, 1.1ml, 13.3mmol). The reaction was immediately cooled in an ice-water bath to control the exotherm. The reaction mixture was then slowly heated to 70^{0}C and maintained at that temperature for 16h. The reaction mixture was then neutralised with sodium hydroxide solution (3M, 4ml) and extracted into ether (3 x 30ml). This was then dried, filteredand evaporated to dryness *in vacuo*. Ther appeared to be no trace of the title compound in the reaction mikxture by either TLC or nmr. The reaction was abandoned.

HPLC Analysis Conditions

Analysis of Cardanol Fractions

Equipment - a Perkin-Elmer LC-55 variable-wavelength ultraviolet spectrophotometer, 2 Altex metering pumps (Model 110A), a Rikadenki recorder and a Columbia Scientific Instruments Supergrator 3A computing integrator and more recently a Jones Chromatography JC6000 computer integrating system. The reversed-phase partition experiments were conducted with 5µm Magnusphere and Spherisorb bonded with ODS in 250 x 4.6mm stainless steel columns.

Conditions - for the detection of the phenols, the wavelength chosen for detection was 275nm. The solutes were made up in ethereal solution and generally 5 - 10µl of a 5% solution was used. It was found essential to use a gradient elution system according to the following two programmes, with Solvent A - acetonitrile:water:acetic acid (66:33:2) and Solvent B - tetrahydrofuran (100%).

1.

Time	Flow (ml/min)	Composition
0	2.7	A(100%)
25	2.7	A(70%) B(30%)
38	2.7	A(0%) B(100%)
52	2.7	A(100%)

2.

Time	Flow (ml/min)	Composition
0	2.0	A(100%)
20	2.0	B(100%)
25	2.0	A(100%)

At the end of a series of runs the column was purged with water to remove any remaining acetic acid, followed by pumping through with methanol or acetonitrile.

After a succession of many runs it was found essential to clean the column by passing through solvent in the order tetrahydrofuran, water, methanol, acetonitrile, dichloromethane, *iso*-octane followed by a reverse of this sequence.

Analysis of Polyethoxyleted Phenols[46]

The baseline separation of polyethoxylated phenols was achieved by using the same basic equipment as previously described, but the column used in this case was a Dynamax 5µm amino-propyl reverse phase column. Solvents used in this case were Solvent A - 20% tetrahydrofuran in *n*-hexane and Solvent B - 10% water in *iso*-propanol. Samples were made up in Solvent A and were generally 200µg in 20µl. UV detection was again at 275nm and the gradient employed was as follows:

Time	Flow (ml/min)	Composition
0	1.0	A(98%) B(2%)
60	1.0	A(50%) B(50%)
65	1.0	A(98%) B(2%)
70	1.0	A(98%) B(2%)

After a succession of runs it was deemed necessary to clean the column as previously described. This improved both the sharpness of the peaks and the reproducibility of the retention times.

Modified OECD Screening Test For the Determination of Biodegradability.

Deionised or Distiled Water - the water used in these experiments was carbon-filtered and was free of toxic substances, such as copper.

Nutrient Solution - to water (900ml), each of the following solutions, a) to f) (1ml) were added, in the order given, mixing between additions, and then made up to 1l.

a) Calcium chloride dihydrate (18.2g) in water (500ml)

b) Magnesium sulphate heptahydrate (11.25g) in water (500ml)

c) Ferric chloride hexahydrate (0.25g) in water (1000ml)

d) Potassium dihydrogen phosphate (4.25g)
Dipotassium hydrogen phosphate (10.85g)
Disodium hydrogen phosphate dihydrate (16.70g)
Ammonium chloride (10.00g)
Dissolved in water (500ml)

e) Trace element solution
Manganous sulphate tetrahydrate (40mg)
Boric acid (57.2mg)
Zinc sulphate heptahydrate (42.8mg)
Ammonium molybdate (34.7mg)
Iron chelate ($FeCl_3$.EDTA) (100mg)
Dissolved in water (1000ml)

f) Vitamin solution
Biotin (0.2mg)
Nicotinic acid (2.0mg)
Thiamine (1.0mg)
p-Aminobenzoic acid (1.0mg)

Pantothenic acid (1.0mg)

Pyridoxamine (5.0mg)

Cyanocobalamine (2.0mg)

Folic acid (5.0mg)

Dissolved in water (100ml)

The stock solutions a) to e) were stored at 0°C in the dark.

Stock solution of test substance

A solution of the test substance containing 1g C/l was prepared. From this a second solution containing the chosen test concentration (~100mg C/l) was prepared to check the concentration by analysis. Thus the DOC of the test substance was determined.

Stock solution of reference

A solution containing 100mg C/l of the reference compound (glucose) was prepared as described above and analysed accordingly.

Inoculation

Soil (100g) (fertile, not sterile and not recently treated with herbicides, fertilisers, etc.) was suspended in water (1000ml). After stirring, the suspension was allowed to settle for 30min. The supernatant was filtered through coarse filter paper, the first 200ml being discarded. The filtrate was aerated immediately until use. The inoculum was used on the day of collection.

Procedure

The test materials were evaluated simultaneously in duplicate, together with a duplicate reference.

To the nutrient solution (200ml) a suitable volume of solution of test substance was added to give the required DOC concentration (100mg C/l) and inoculum (0.1ml). This was repeated for the test sample. For the controls (blank) no reference or test substance was added but they were inoculated in the same way.

A suitable volume (10ml) of test, reference and control solutions were withdrawn and membrane filtered and the initial concentration of the DOC were determined in duplicate.

Each solution was divided into two reaction flasks, and the necks were covered with loose fitting metal caps so that the passage of air was not impeded.

The flasks were placed on a shaker and agitated gently for 28 days. The temperature was kept at 25^0C during the test and the flasks were shielded from light.

During the biodegradation test the DOC concentration were determined in duplicate every 4 days until the 28th day.

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