'THE SYNTHESIS OF THE MACROLIDE ANTIBIOTIC (±)-PYRENOPHORIN'.

A THESIS

PRESENTED TO THE UNIVERSITY OF GLASGOW FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

BY

THOMAS ANDREW PURCELL.

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

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Thomas Andrew Purcell.

To my wife and my parents.

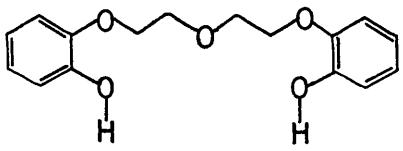
Summary

The synthesis of the macrolide antibiotic (±)-pyrenophorin (1) is described. The molecular sieve promoted cyclisation of the hydroxy methyl ester (135) failed in our hands but the hydroxy-acid (190) was successfully converted to the dithioacetal of (1) using N, N' carbonyldiimidazole (207) and 1, 5 diazabicyclo(4,3,0)-non-5-ene (147).

A novel method of removing p-toluenesulphonylethyl esters (166) is reported as is a new simple technique of hydroxyl alkylation involving molecular sieves and sodium hydrogen carbonate.

Contents.

INTRODUCTION.



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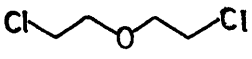

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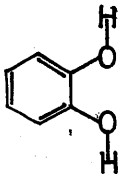

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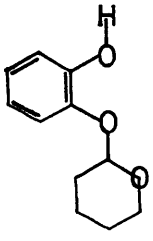

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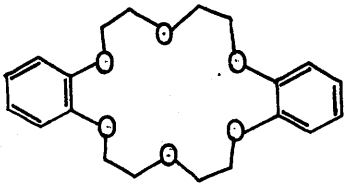

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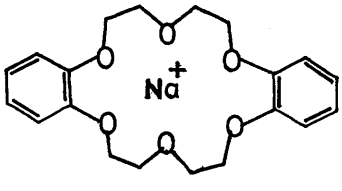

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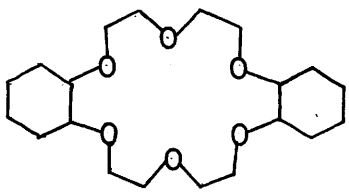
In 1967 Pedersen [1,2] reported that in preparing bis(2-(o-hydroxyphenoxy)ethyl)ether (1) from bis(2-chloroethyl)ether (2) and the sodium salt of 2-(o-hydroxyphenoxy)tetrahydropyran (3) he had isolated from the reaction mixture a very small quantity of a crystalline by-product. The compound was only slightly soluble in methanol at room temperature yet addition of sodium hydroxide increased its solubility therein severalfold. This apparently trivial observation assumed much greater status when the compound was identified as the non-acidic cyclic ether (4) arising from reaction of (2) with a small quantity of catechol (5) present in (3). The mechanism whereby sodium hydroxide could enhance the solubility, in methanol, of a non-acidic molecule like (4) was elucidated by the discovery that most methanol soluble salts of the alkali and alkaline earth metals had the same effect. Thus it was not the hydroxide ion which altered the solubility of (4) but the positively charged sodium ion which, Pedersen suggested, interacted electrostatically with the lone pairs of the six ring oxygens of the macrocycle to form a large soluble complex cation (6). The need for more evidence to test this theory and to develop it prompted the syntheses of large numbers of different macrocyclic polyethers.

To facilitate the very cumbersome nomenclature of these cyclic molecules Pedersen christened them crown ethers and adopted the following system. In parenthesis is the total number of ring atoms in the macrocycle; this is followed by the term crown to define the macrocyclic nature of the compound; the term crown is suffixed by the number of oxygen atoms which appear in the ring. The whole is anteceded by the identity of the substituents on the



2,5,8,15,18,21-hexaoxatricyclo(20.4.0.$0^{9,14}$)hexacosane

perhydrodibenzo(18)crown-6

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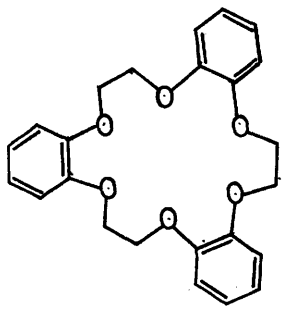

2,5,12,15,22,25-hexaoxatetracyclo(24.4.0^{6,11}.0^{16,21})triaconta-6(11),7,9,16(21),17,19,26(1),27,29-nonaene

tribenzo(18)crown-6

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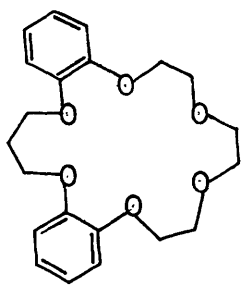


(9)

2,3,14,15-dibenzo-1,4,7,9,13,16,-hexaoxacyclononadecane

asym-dibenzo(19)crown-6

macrocycle; a symmetrical substitution calls only for the identity and number of the groups, while an asymmetrical substitution demands, in addition, the prefix asym-. The prefix asym- also describes an asymmetric ether oxygen pattern. (See 7 - 9).

From the large family of crown ethers synthesised, numbering over sixty and containing four to twenty oxygen atoms each, it has been possible to generalise their physical properties as follows[1,2,3,4]. Those with aromatic substituents, for example (8), are colourless and crystalline, with the melting point for each polyether ring increasing with the number of these substituents. This class of polyethers shows poor solubility in protic solvents but high solubility in methylene chloride and chloroform. Their saturated analogues (eg 7),on the other hand are colourless oils or low melting solids, dissolving in all solvents more readily than their aromatic counterparts. These observations are explicable if it is assumed,and reasonably so, that the aromatic polyethers can pack better in a crystal lattice and therefore show a higher lattice energy than their perhydrogenated relatives.

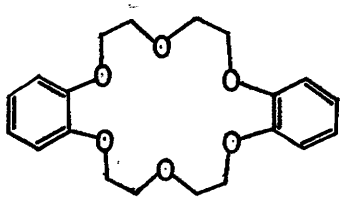
Unlike the saturated compounds, which do not show an absorption above 220 nm, the aromatic ones show absorption bands, in methanol, near 275 nm, which are characteristic for catechol and its ethers, with extinction coefficients of around 2200 for each benzene ring. Both classes of polyethers absorb infra-red radiation near 1100 cm^{-1} in keeping with the presence of an aliphatic ether link, though the unsaturated molecules, with their aromatic-aliphatic ether bonds, also absorb near 1230 cm^{-1}.

All the crown ethers are thermally stable in an inert atmosphere.

The large and diverse accumulation of data so far collected from the various crown ethers strongly favour Pedersen's hypothesis that these macrocycles can effect complexation of suitable cations. The initial criterion for complexation was the ability of the crown ethers to solubilise ionic compounds in organic solvents; frequently addition of a crown ether caused dissolution of salts and related compounds in solvents in which they were otherwise substantially insoluble.

This was readily explicable in terms of the molecular structure of the polyether whose inner surfaces were polar and electron rich but whose outer surfaces were mainly hydrocarbon in nature and, accordingly, organophilic. Thus a cation could electrostatically bind to the inside of the macrocycle to form a structure whose exterior was still relatively organophilic and dissolvable in non-polar solvents.

Experimentation subsequently showed that the best way to realise a salt solution was to dissolve the crown ether and the salt in methanol, remove the solvent in vacuo and dissolve the residue in the chosen solvent. The observation that in several cases the methanol was not totally removed, and indeed strongly retained, incurred the suggestion that the methanol served to complete the solvation sphere of the ether complexed cation or helped to solvate the anion. It was in fact found that addition of small quantities of methanol greatly increased the amount of salt which dissolved directly in solvents containing crown ether. In assessing the degree of complexation by this method, attention, justifiably, had to be paid to the anion. The complexed cation, its charge buried deep in a structure whose exterior is organo-



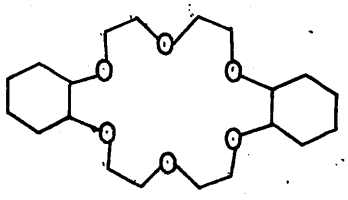
(4)

philic, can be easily accommodated in a non-polar environment. Interaction, however, between the anion and a non-polar solvent is much less favourable especially for small ions of high charge density and low polarisability. In accordance with this view, it was shown that salts of such hard anions as fluoride or sulphate were not solubilised appreciably by cyclic polyethers but that salts of soft anions as iodide, thiocyanate, picrate, and fatty acid anions were readily solubilised.

Pedersen[1] provided spectroscopic evidence for the existence and nature of these complexes by comparing the infra-red and ultra-violet spectra of the free polyether with those of the polyether in the presence of a solubleisable salt. In examining the infra-red spectrum of the potassium thiocyanate/dibenzo(18) crown-6 (4) couple and that of the parent (4) alone he noticed that the spectra differed most strikingly in the 9-14 mμ region. This region contains bands attributable to the wag, twist, and rock modes of vibration of methylene groups: these modes are opposed by the adjoining groups and changes in the bonding character of these groups affect these modes of vibration. The two medium intensity bands at 10.04 mμ and 10.71 mμ in the parent are closed up to 10.41 mμ and 10.60 mμ and increased in intensity in the potassium system. Pedersen attributed this to decreased restriction on the coupling of these vibrational modes in the salt system. This decrease in restriction he accounted for by postulating that the oxygen atoms are showing less effect on these modes because they are bonded to some extent with the potassium ion. The significant variation in the aromatic bands between 13 and 14 mμ is the shift to shorter wavelengths in the salt

system. Since these bands are attributable to the C-H out of plane bending mode of vibration, it is apparent that the potassium ion is bonded in such a way as to cause this C-H vibration to be more restricted.

All the cyclic polyethers with aromatic substituents have a characteristic ultra-violet absorption maximum at 275 nm in methanol. Addition of a cation incurs changes in this band, generally by the appearance of a second peak at about 280 nm, at other times by a hypsochromic shift and changed absorbance of the main band. Thus, for dibenzo(18)crown-6 (4) the ultra-violet spectrum shows a peak[1] at about 275 nm. The spectrum of the same ether with potassium ion shows a second peak about 6 nm to the longer wavelength side of the major peak. If the ultra-violet spectrum is taken while adding potassium iodide, it is observed that the peak is fully developed at about 1.0-1.3 equivalents of potassium iodide. The change in the ultra-violet spectrum is consistent with a change in the environment of the polyether; this change could be the formation of a cationic complex. If a complex is formed between (4) and potassium ion then the ultra-violet spectrum run while adding potassium iodide to (4) shows that this complex is only slightly dissociated even at concentrations of 0.000214 moles/litre. For sodium methoxide, the peak is not fully developed until 2.3 equivalents of salt is added. Aside from this, the second peak for sodium is less pronounced than that for potassium. Hence, if cationic complexes are formed, then there seems to be a connection between the stability of the complex and the magnitude of the second peak observed in solutions containing the salts in excess.



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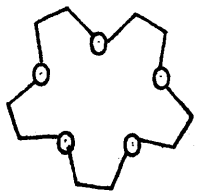
Frensdorff[5] has employed conductivity experiments to show the existence and composition of crown ether-cation complexes. In methanol, the dissociation of potassium chloride is essentially complete, and complexing, by increasing the size, decreases the mobility of the cation. This explains the reduction in conductivity of methanol solutions of potassium chloride on addition of perhydrodibenzo(18)crown-6 (7). However, in 90% chloroform-methanol, the conductance increases on addition of (7). Frensdorff interprets this by noting that potassium chloride, in this solvent system, is largely undissociated, but complexing, by surrounding the cation with the polyether ring, shields its charge and thus greatly increases dissociation of the ion pairs, which results in an appreciable conductance increase.

Conclusive evidence for the formation of complexes between crown ethers and salts derives from the isolation of crystalline complexes. Numerous well defined, sharp melting crystalline complexes of salts and cyclic polyethers have been prepared. Although salts with high lattice energy, such as fluorides, nitrates, and carbonates, form complexes with the crown ethers in alcoholic solution, they cannot be isolated in the solid state because one or other of the uncomplexed components precipitates when the solution is concentrated. The complexes do not all show 1:1 stoichiometry - 2:1 and even 3:2 complexes have been isolated from some cation/macrocycle systems.

Having established the existence of the cationic complexes it was natural to try to evaluate their stability constants which reflect directly the stability of the complexes. The stability constant of a particular complex is merely the equilibrium



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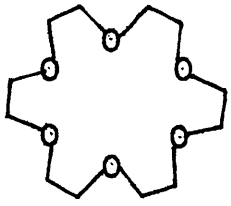

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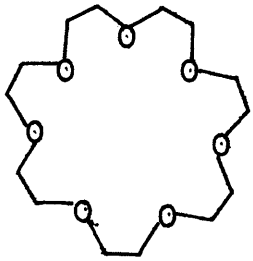

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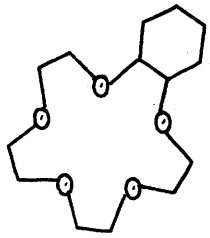

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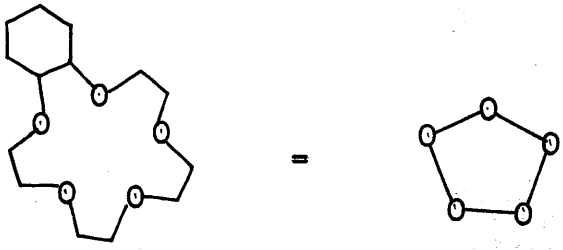


Polyether ring	Hole * diameter(Å)[4]	Ion	Ionic diameter in crystal(Å)
(14)Crown-4	1.2-1.5	Li^+	1.36
(15)Crown-5	1.7-2.2	Na^+	1.94
(18)Crown-6	2.6-3.2	K^+	2.66
(21)Crown-7	3.4-4.3	Rb^+	2.94
		Cs^+	3.34
		NH_4^+	2.86
		Ag^+	2.52
		Ba^{2+}	2.68

* Lower values estimated from Corey-Pauling-Kolton atomic models, higher values from Fisher-Hirschfelder-Taylor models.

Table I.

constant for its formation from its components. These stability constants have been measured in a variety of ways[6,7,8]. In general, the stability constants are three to four powers of ten higher in methanol than in water because the alcohol does not solvate cations as strongly as water. This, indeed, makes methanol a particularly convenient solvent for complexing measurement which permits the highly important study of the effect of polyether ring size, and cation size, on the strength of complexation.

Pedersen showed that the stability constant for each cation goes through a maximum with increasing polyether ring size: between (15)crown-5 (10) and (18)crown-6 (11) for sodium ion; at (18)crown-6 (11) for potassium ion; and between (18)crown-6 (11) and (21)crown-7 (12) for caesium ion. In calculating the size of the hole in each of the above crown ethers using molecular models[1,4] (the values in reference 1 were revised in reference 4) he found he could easily explain the above observation since these optimum ring sizes were found to provide the closest fit between the cation and the hole (Table I)[9]. Rationalisation of the existence of the 2:1 and 3:2 polyether complexes was also realised. Thus, for example, the 2:1 complexes, in solution, of perhydrobenzo(15)crown -5 (13) with potassium ion (K^+) and caesium ion (Cs^+), and dibenzo(18)crown-6 (4) were all cases of the cation being too large to fit into the hole and forming, probably, a sandwich molecule (14) instead.

What would happen if the hole was too big? X-ray studies of crystalline complexes of the 15-18 membered crown ether rings, have shown these rings to be nearly flat with the cation usually slightly above this plane - hence the need for additional ligands

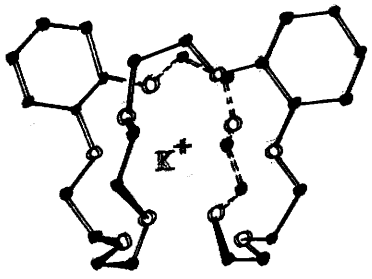
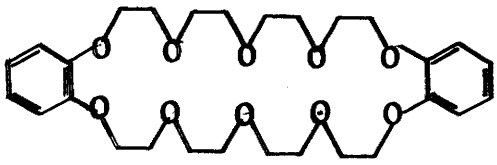


Figure I



(15)

O = Oxygen

● = Carbon

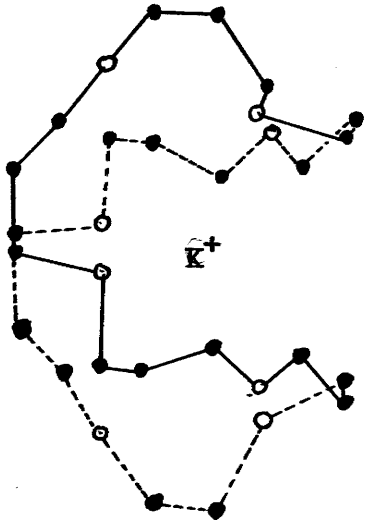
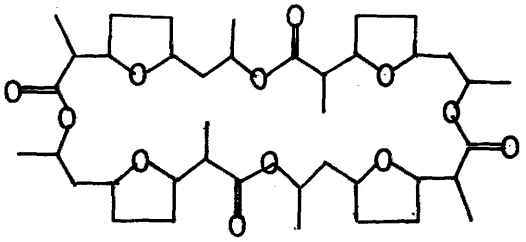


Figure II



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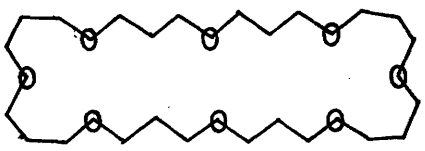

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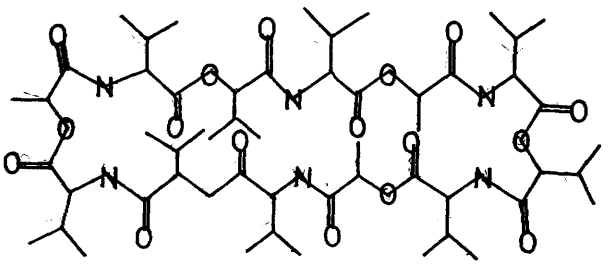

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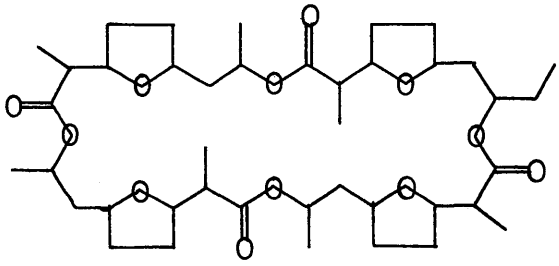

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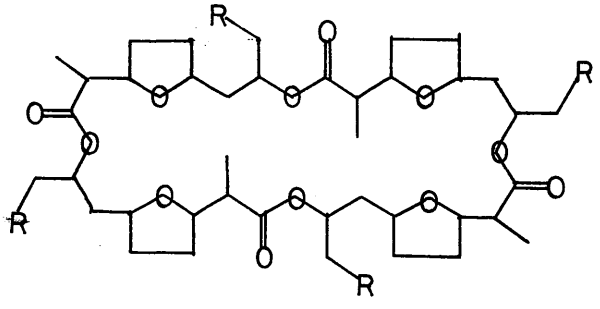
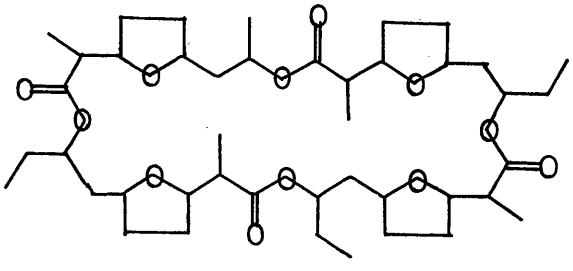


Two R = CH_3

Two R = H

(20)



(21)

to complete the solvation sphere of the cation[10,11,12, 13] . A really large polyether ring, sufficiently flexible and with enough oxygen atoms, might completely envelop a cation. Such a complex, KI-dibenzo(30)crown-10 (15),has been prepared and its X-ray analysis[13]shows that this complex indeed contains a cation completely enclosed by the polyether and coordinated with all ten oxygen atoms.(Fig I).

Now the architecture of this KI-dibenzo(30)crown-10 couple is markedly similar to a complex derived from a large naturally occurring macrocycle, nonactin (16), and potassium thiocyanate[14]. (Fig II). Examination of this macrocycle (16) by itself reveals a structure which might be described as hailing from a (32)crown-8 (17) type of polyether. This is most interesting, for in 1964 Moore and Pressman[15] in analysing the observation[16] that the antibiotic valinomycin (18) produced uncoupling of oxidative phosphorylation and activation of ATP-ase in rat liver mitochondria at very low concentrations, presented evidence that the mode of action of the depsipeptide (18) was mediated by an ion transport phenomenon viz. the energy dependent accumulation of K^+. Lardy[17] remarks that valinomycin (18) is only one member of a general class of antibiotics which induce the transport of alkali metal cations into mitochondria against a concentration gradient[18]. This class includes certain neutral depsipeptides, polypeptides and, most importantly here, the macrotetralides nonactin (16), monactin (19), dinactin (20), and trinactin (21). It appears then that the macrotetralides can affect the permeability of lipid membranes to alkali metal cations. Biological membranes[19], however, are about 100 Å thick and comprise lipids and proteins

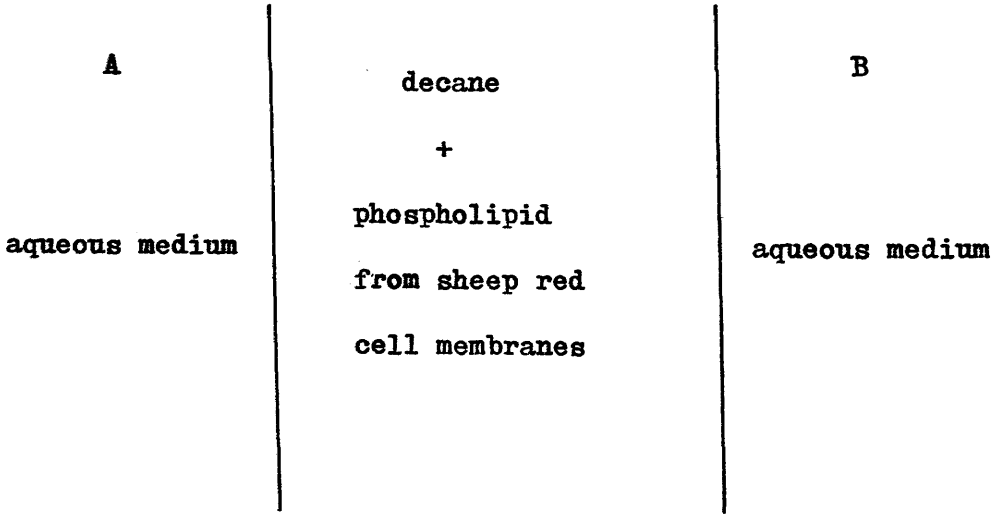
in a more or less ordered arrangement. The lipid molecules, forming a bilayer, are oriented such that their polar head-groups are in contact with the aqueous phases, and the hydrocarbon chains are directed towards the interior of the membrane, constituting a hydrocarbon medium. Because hydrocarbons have a low dielectric constant, the energy required to bring a small ion, such as sodium (Na^+) or potassium (K^+), from the aqueous medium into the membrane is many times the mean thermal energy of each cation. This means that the lipid portions of the membrane represent an extremely high barrier for the passage of these ions. We have, nevertheless, seen how salts can be solubilised in organic media using the crown ethers. The inference, then, is that the macrotetralides, with their crown ether like hydrophilic interiors and hydrophobic exteriors, function similarly to the crown ethers and carry ions, via complexes, through cell membranes.

It appears[15], further, that potassium ion is the principal passenger across mitochondrial walls, which phenomenon demands a high specificity by the carrier or ionophore for this ion. We observed above that (18)crown-6 (11) provided the most closely fitting two dimensional hole for K^+. Do the macrotetralides provide the most closely fitting three dimensional hole? Fortunately a comparison of the ion binding properties of the macrocyclic antibiotic nonactin (16) to Na^+, K^+, and Cs^+ has been made[20].

Nuclear magnetic resonance (nmr) spectroscopy at 220 MHz has been used to study the complexes in anhydrous acetone-d_6, and acetone-d_6/water mixtures containing as much as 0.5 mole fraction of water. Complex formation constants of 7×10^4, 7×10^4, and 1×10^4

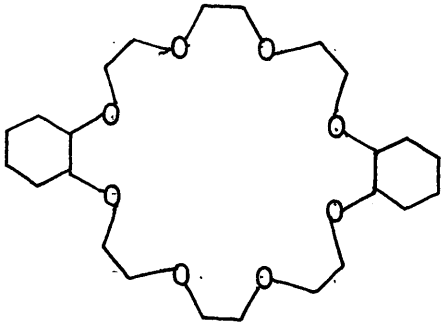
are obtained for the Na^+, K^+, and Cs^+ complexes, respectively, in dry acetone; in wet acetone the respective constants are 210, $2x10^4$, and 400. Thus the three ions bind to nonactin with nearly equal affinity in dry acetone, but the binding constants are greatly altered when the solvent system is altered by addition of appreciable amounts of water. It is significant that the reduction in their magnitude is far greater for Na^+ and Cs^+ than for K^+ making the binding to nonactin highly favoured in the more aqueous medium. Analysis of the nmr data also indicates that the nonactin ring undergoes sizeable conformation changes on incorporation of these alkali ions. The extent of this conformational change is slightly different depending on the ion but, on the whole, the three complexes studied appear to exhibit quite similar structures. The results so far indicate that nonactin is a flexible molecule capable of binding a range of alkali ions with nearly equal affinity, at least in acetone solution, leaving it with <u>little inherent propensity</u> for selective binding. When, however, the authors plotted standard free energies of hydration and of ion complexation with ionic radius, on the same graph, they noticed that energy changes in passing from the hydration to the complexation curve were most favourable at the radius corresponding to K^+. Thus, accepting the proposed inability of nonactin to accommodate an ion with its hydration sphere, the selective binding of potassium ion by the macrotetralide nonactin (16) could be explained.

Having shown the specificity, in aqueous media, of macrotetralides for potassium ion can we substantiate the inference that these macrocycles carry ions through cell membranes in the


A
aqueous medium
decane
+
phospholipid
from sheep red
cell membranes
B
aqueous medium

form of large hydrocarbon soluble complex cations? If it were true then it would be reasonably expected that Pedersen's synthetic crown ethers would operate on cell membranes in a fashion similar to their natural analogues. Happily, comparitive experiments have been described by Lardy[17] and Tosteson[21]; Lardy[17] reveals that the synthetic crown ethers exhibit specific influences on cation transport in rat liver mitochondria but that these effects appear to be inconsistent. Thus dicyclohexyl(18)crown-6 (7) induces mitochondria to accumulate this cation (K^+), an absent property of the closely related dibenzo(18)crown-6 (4). The latter molecule (4), in fact, prevents valinomycin (18) and monazomycin (22), but not dinactin (20), from inducing K^+ uptake; it has no effect on the retention of endogenous K^+ by mitochondria but causes the loss of this cation after it has been accumulated under the influence of valinomycin (18), dinactin (20), or monazomycin (22). Tosteson[21] presents a more direct comparison: he firstly contrasts the effects of the natural valinomycin and tetralides with the synthetic crown ether perhydrodibenzo(18)-crown-6 (7) towards thick artificial membranes. In the apparatus shown opposite, the total electrical resistance in going from A to B is compounded in series of a surface resistance, referable to monolayers at the decane-water interfaces, a bulk resistance of the lipid/decane medium, and another surface resistance. This compound resistance was confirmed in two ways:-

a) the extrapolated value of the resistance of this thick lipid membrane in the presence of an infinite concentration of sheep red cell lipids is not zero as would be expected for an ideal salt solution but about 10^8 ohm cm^2 when expressed as a



(23)

Compound	Concentration (Molar)	Red cell cation content (m. moles/original litre red cells)	
		K^+	Na^+
None (control)		87	39
(15)Crown-5	10^{-3}	75	50
(18)Crown-6	10^{-3}	68	38
(24)Crown-8	10^{-3}	65	47

Table II

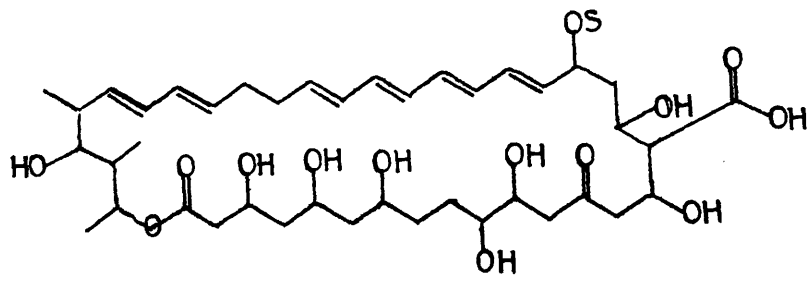
surface resistivity,

b) the extrapolated value of membrane resistance at zero membrane thickness is independent of lipid concentration and again equal to about 10^8 ohm cm^2 when expressed as a surface resistivity. (Note: it is helpful to consider that in the medium the phospholipids, by virtue of their randomly oriented polar groups, lower the dielectric sothat the bulk resistance is directly proportional to their concentration, which is found. On the other hand, at each monolayer, the phospholipids are not randomly oriented but are arranged side by side such as to form a hydrocarbon wall of high electrical resistance).

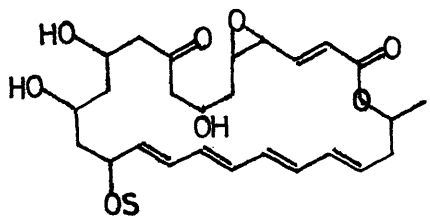
It was found that all the macrocycles had the same effect, that of markedly decreasing the surface resistance and not the bulk resistance. All transported K^+ selectively.

He further studied the effect of Pedersen's crown ethers on the cation permeability of whole HK and LK sheep cells (Table II). Thus perhydrobenzo(15)crown-5 has a greater effect on Na^+ permeability but perhydrodibenzo(18)crown-6 (7) and perhydrodibenzo(24)crown-8 (23) affect the K^+ more than the Na^+ permeability. All had a greater effect on the membranes of whole cells than on artificial bilayers. Of the polyethers tested only (7) substantially modified the ionic permeability properties of thin lipid membranes prepared with lipids extracted from sheep red cells.

These studies[17, 21], though not exhaustive , seem to lend some credence to the indication that the macrotetralides alter membrane permeabilities to K^+ by carrying the ion in the form of a fat soluble complex.



(24)



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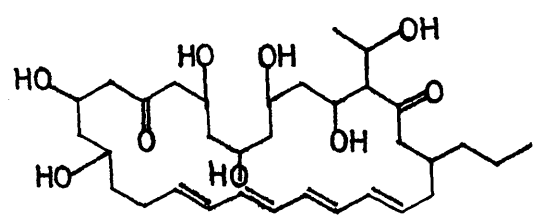
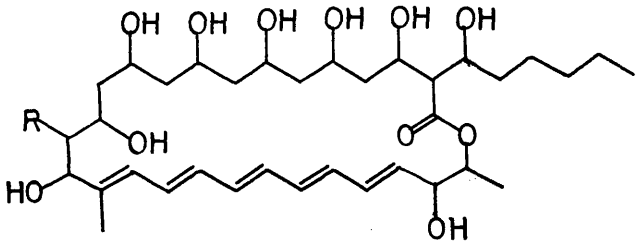
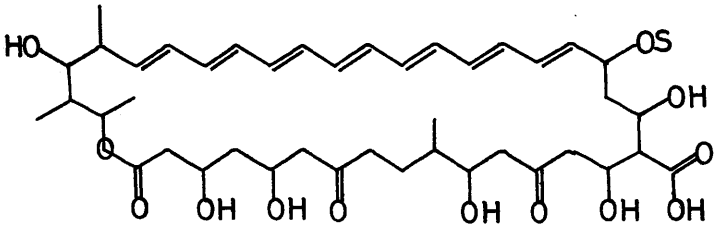


(26)

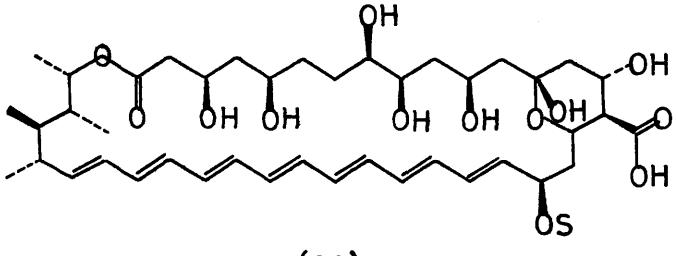


(28), R = OH.

(27), R = H.



(29)



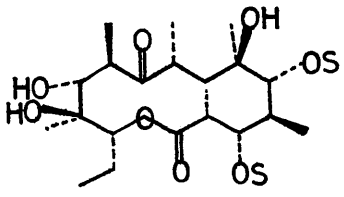
(30)

The macrotetralides are, of course, only a small representative body of the general class of macrocyclic lactones which Woodward[22] generically christened macrolides. Though vast quantities of biological and clinical data on macrolides are currently being published, there is still not nearly enough evidence to support the popular assumption that all macrolides act similarly to the large macrotetralides. To effectively show this the following extremely brief description of apparent chemical trends demands as usual the apportioning of the entire class into two groups, one comprising the polyene macrolides, the other, the non-polyene macrolides.

The polyene macrolides[23] were first recognised by their characteristic polyene ultra-violet and visible absorption spectra. Widely produced as metabolites of soil microorganisms, the actinomycetes, they were believed to be the first examples of highly conjugated, non-isoprenoid chromophores to be described as microbial products. Thus, on the basis of their ultra-violet spectra were identified the tetraenes, nystatin (24), pimaricin (25), amphotericin A, rimocidin (26), the pentaenes filipin (27), lagosin (28), the hexaenes flavacid, mediocidin, and the heptaenes candidin (29), candicidin, amphotericin B (30), trichomycin, and perimycin. All gained widespread interest because of their high degree of activity against a wide variety of fungi and yeasts, and their characteristic inactivity towards bacteria. Experiments involving the heptaenes showed that interference at the hydrophilic zones did not effect complete deactivation of the antibiotics; disruption of the lipophilic polyene fragment, however, incurred a total loss of activity.

Coupled with the apparent increase in activity in passing from the tetraenes to the heptaenes, the latter observation led to the postulate that the polyene chromophore is involved[24,25] in binding the molecule to the lipophilic constituents of cell membranes to alter the latter's permeability function. Lampen further evidenced [24,25] that the action of amphotericin B (30) and nystatin (24) depends on the binding of the antibiotic with the sterols in the fungal protoplast to alter the permeability of the cell wall, causing leakage of K^+ and sugar with the resultant death of the cell. Sadly, and to totally confuse matters, however, any apparent similarities between the action of the macrotetralides and the polyene antibiotics were soon dashed when Andreoli and Monahan[26] showed that nystatin (24) differed markedly from the macrotetralides in its effect on the ionic permeability of thin lipid membranes. They indeed demonstrated that the tetraene failed to discriminate between Na^+ and K^+ and, remarkably, produced selectivity for anions over cations!

The non-polyene macrolides are described as large lactone rings wherein no more than two double bonds are conjugated. It is interesting to note the predominance of 14-membered and 16-membered ring systems in this category[27]. In his determination of the conformations of macrocyclic hydrocarbons by the use of space filling models, Dale[28] has found that no hydrocarbon ring system from cycloheptane through cyclotridecane can exist in a strain free conformation. This results from the fact that all ring sizes from 7 through 13 contain a number of internal interactions caused by intra-annular hydrogens attempting to occupy the same spatial positions in the 'ideal' diamond lattice arrangement of



(31)

S = Sugar

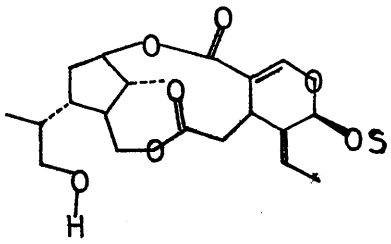
atoms. The inability of odd membered rings to attain this 'ideal' system explains the notable absence of odd membered macrolides.

Unlike the polyenes, members of this class are generally active against mycoplasma and bacteria. Erythromycin A (31) has been shown to inhibit bacterial protein synthesis in intact cells and cell free systems, binding specifically to the 50S subunit of Staphylococcus aureus ribosomes[30]. This 1:1 complex formation requires the presence of NH_4^+ or K^+ ions, which ions, notably, are of similar size. Though nothing appears to have been said about the function of these ions, it seems likely that they are complexed with the macrolide to hold it in a particular conformation which allows strongest binding to the ribosome. Despite this specific action,however, it admittedly remains difficult to stray very far from involvement with cell membranes; the antibacterial activity of known non-polyene macrolide antibiotics differs from their antimycoplasma activity[31]. This is assumed to be due to the difference in membrane structure between the two kinds of microorganisms - mycoplasma has no cell wall and is said to be enveloped only with a cell membrane[32].

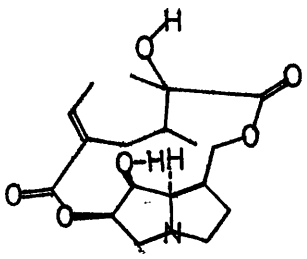
Though a fuller account of macrolide biochemistry appears in Appedix A there is sufficient indication in this small discussion to show that although a common trend of ion transport across cell membranes by macrolides might lurk in the background, the data so far presented do not consolidate this trend as a general property of the macrolide group of antibiotics.

Nevertheless, a mere scan of all the structures of the macrolides does bring to light a very real general property.

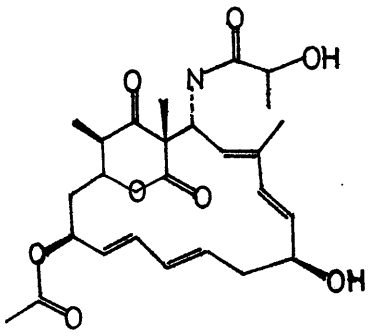
Uniformly, the macrolide group of antibiotics have presented the synthetic organic chemist with a severe problem. For their successful construction these outrageous assemblies of atoms demand an efficient synthesis of a medium to very large lactone ring, which, in most cases, must preserve a concatenation of functionality and contiguity of asymmetry hitherto unobserved in organic chemistry. To emphasise this a bare scan of the structures and, where possible, the stereochemistry of the more recent macrolides is proposed. Those compounds which have been shown to possess antibiotic activity are marked with an asterisk. Only the most recent references to each macrolide are given. (Note: earlier structures can be found in Celmer's review of macrolide stereochemistry[33] and in Berry's more general review[34]). Throughout this structural review the letter 'S' denotes a sugar or a sugar chain.



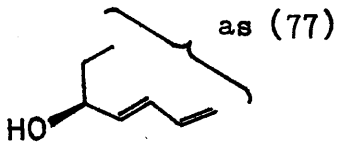
Jasminin (75)[68]



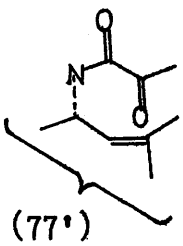
Madurensine (76)[69]



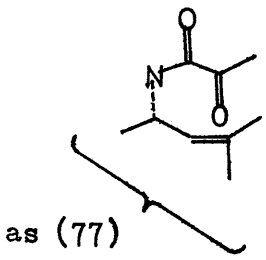
T-2636 D (77)[70]



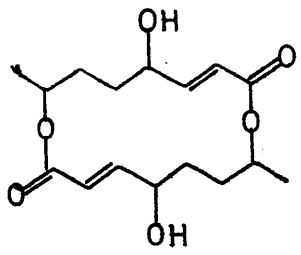
T-2636 F (77')[70]



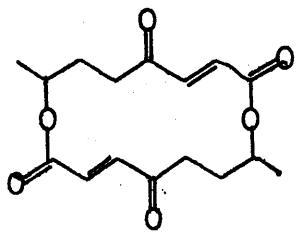
Bundlin A (78)[71, 72, 73]



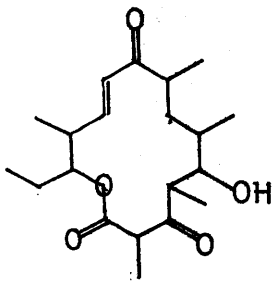
Bundlin B (79)[71, 72, 73]



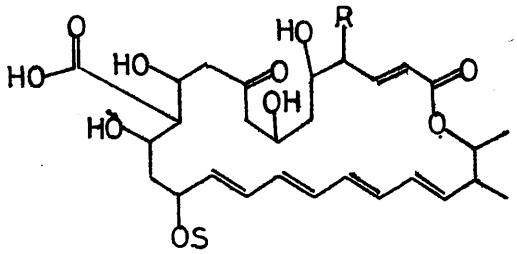
Pyrenophorol (80)[74]



Pyrenophorin*(81)[75]

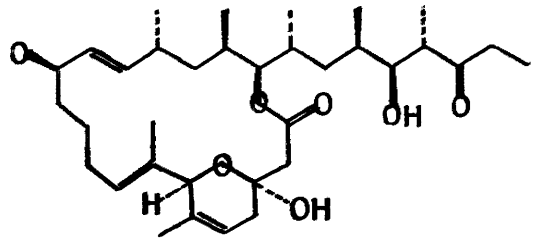


Narbonolide (82)[76]

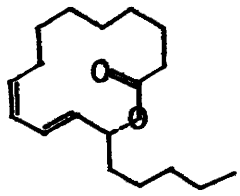


Tetrin A*(83)[77], R = H.

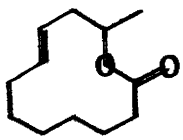
Tetrin B*(84)[78], R = OH.



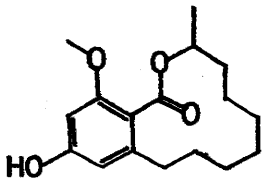
Aglycone of Venturicidin A*(85) and B*(86)[79, 80]



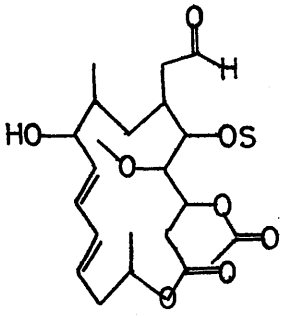
Coriolide (87)[81]



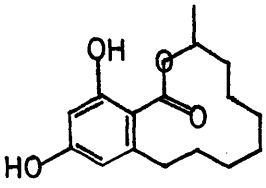
11-Hydroxydodec-8-enoic acid lactone (88)[82]



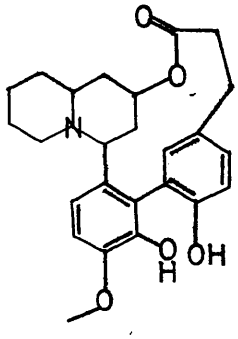
Lasiodiplodin (89)[83]



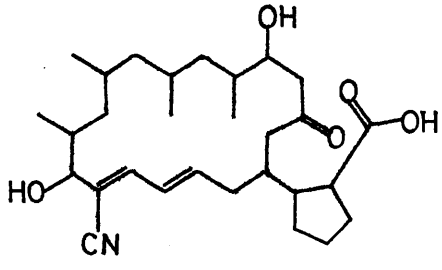
Aglycone of YL-704 A*(90) and YL-704 B*(91)[84]



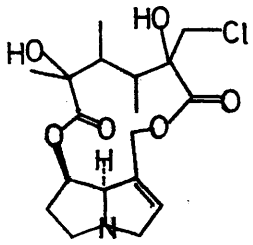
De-O-methyl-Lasiodiplodin (92)[85]



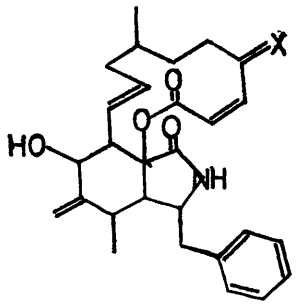
Dihydroverticillatine (93)[86]



Borrelidin (94)[87]

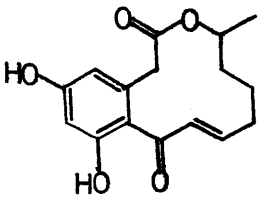


Chlorodeoxysceleratine (95)[88]

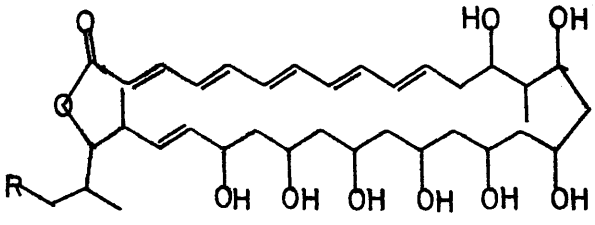


Cytochalasin A (96)[89], X = O.

Cytochalasin B (97)[90], X = H, OH.

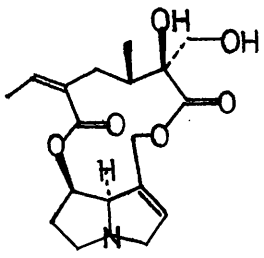


α,β-Dehydrocurvularin (98)[91]



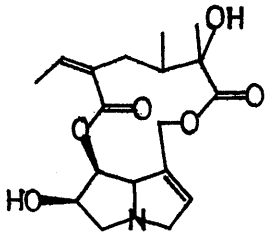
Mycotocin A*(99)[92], R = H.

Mycotocin B*(100)[92], R = CH_3.



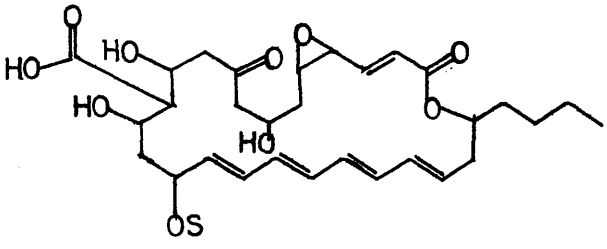
Retrorsine (101)[93] (cis)

Usaramine (102)[94] (trans)

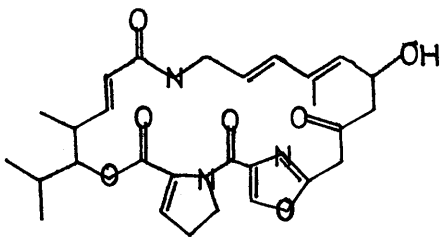


Anacrotine (103)[95] (cis)

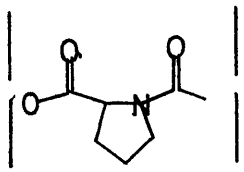
Madurensine (105)[96] (trans)



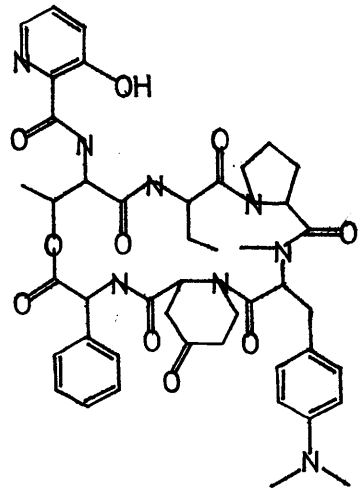
Lucenomycin (Etruscomycin) (104)[97]



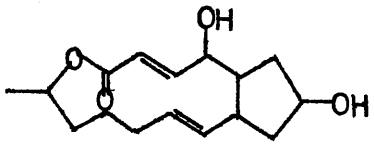
Ostreogrycin A*(106)[98]



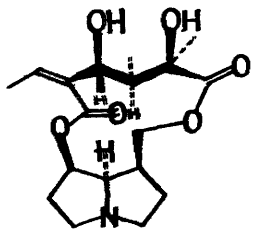
Ostreogrycin G*(107)[99]



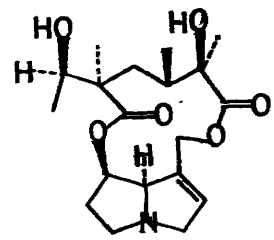
Ostreogrycin B*(108)[100]



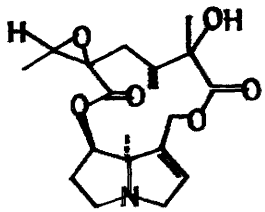
Brefeldin A (Cyanein, Decumbin) (109)[101]



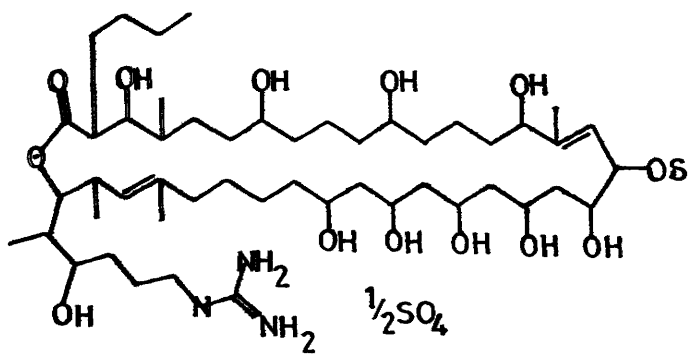
Hygrophylline (110)[102]



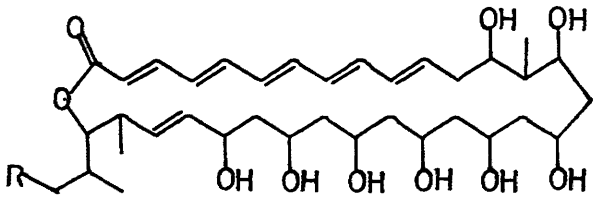
Jacoline (111)[103]



Jacozine (112)[104]



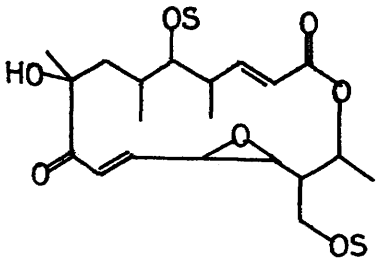
Primycin (major component) (113)[105]



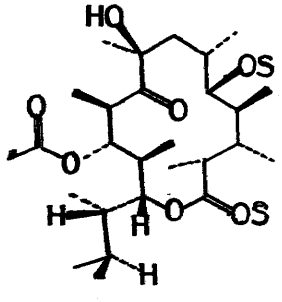
Flavofungin (114)[106]

Two components, i) R = H.

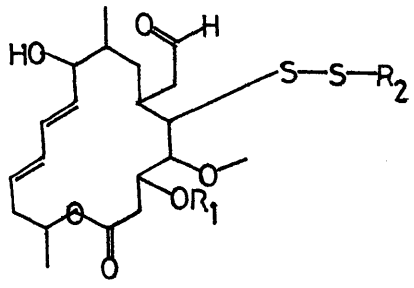
ii) R = CH_3.



Chalcomycin (115)[107]



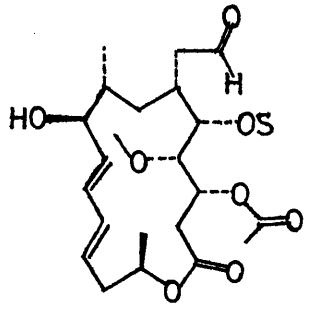
Lankamycin*(116)[108]



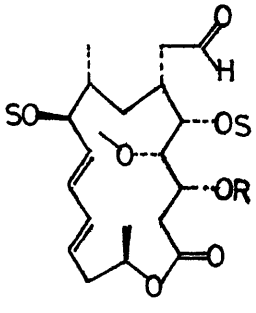
Leucomycin*(117)[109, 110]

	R_1	R_2
A_1	H	Isovaleryl
A_3	Ac	Isovaleryl
A_4	Ac	n-Butyryl
A_5	H	n-Butyryl
A_6	Ac	Propionyl
A_7	H	Propionyl
A_8	Ac	Ac
A_9	H	Ac

Ac = Acetyl.



Leucomycin A_3[111, 112]

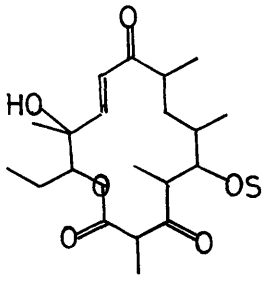


Spiramycin*(118)[113]

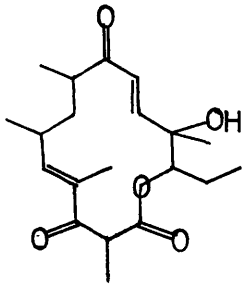
I, R = H

II, R = Acetyl

III, R = Propionyl

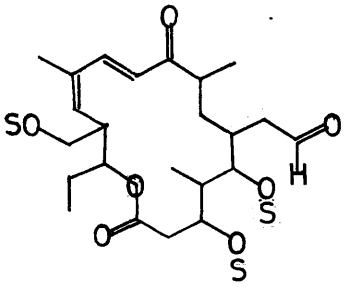


Pikromycin*(119)[114, 115]

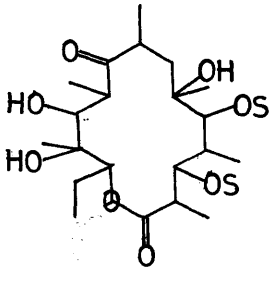


Kromycin (120)[115]

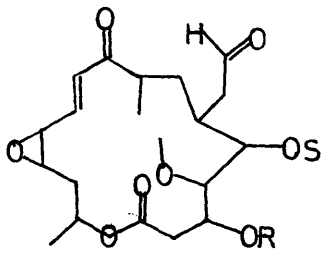
(hydrolysis product of Pikromycin)



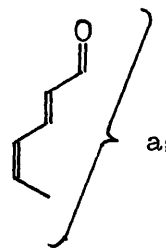
Tylosin*(121)[116]



Erythromycin C*(122)[117]



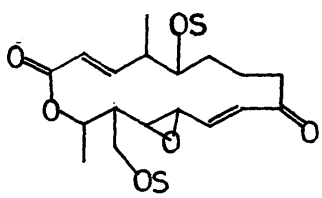
Magnamycin*(123)[118, 119], R = Acetyl.



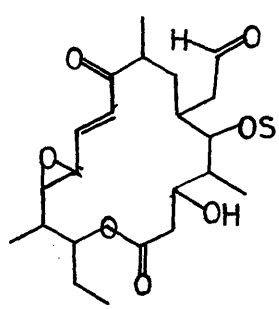
as (123)

Magnamycin B*(124)[118, 119], R = Acetyl.

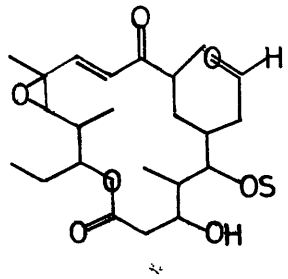
Niddamycin*(125)[120], R = H.



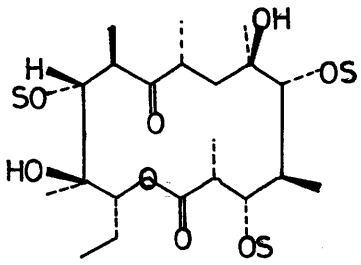
Neutramycin*(126)[121]



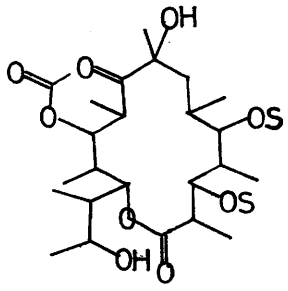
Cirramycin A_1* (127)[122]



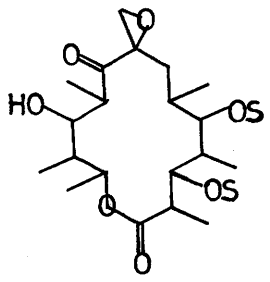
B-58941*(128)[123]



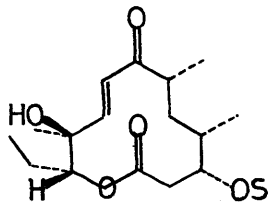
Megalomicin A*(129)[124]



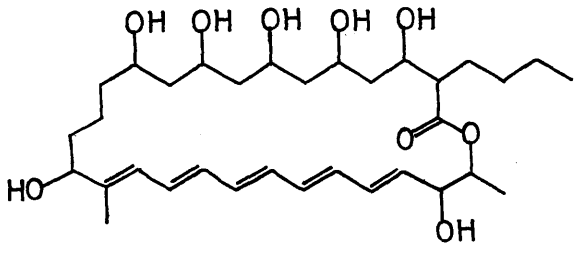
Kujimycin A*(130)[125]



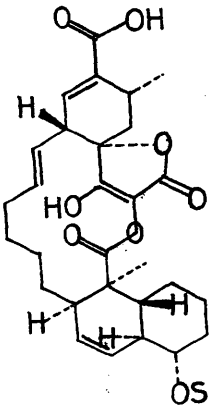
O-Demethyloleandomycin*(131)[126]



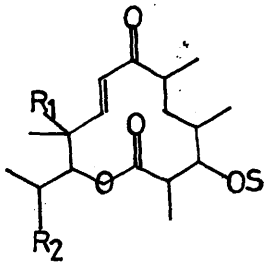
Methymycin*(132)[127]



Chainin*(133)[128]



Chlorothricin*(134)[129]

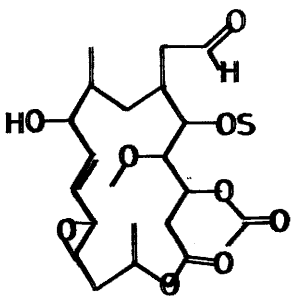


YC-17*(135)[130]

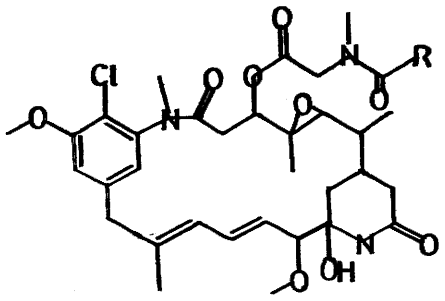
I. R_1 = H, R_2 = H,

II. R_1 = OH, R_2 = H,

III. R_1 = H, R_2 = OH.



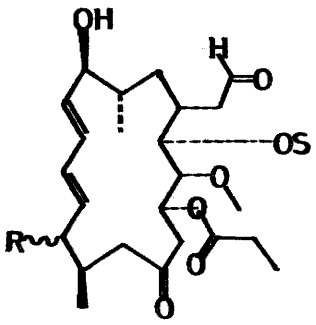
Maridomycin II*(136)[131]



Maytansine*(137), R = CH_3 [132]

Maytanprine*(138), R = CH_2CH_3 [133]

Maytanbutine*(139), R = $CH(CH_3)_2$ [133]



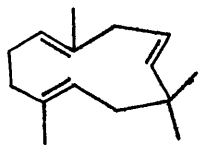
SF 837*(140)[134]

I, R = H, R_1 = H,

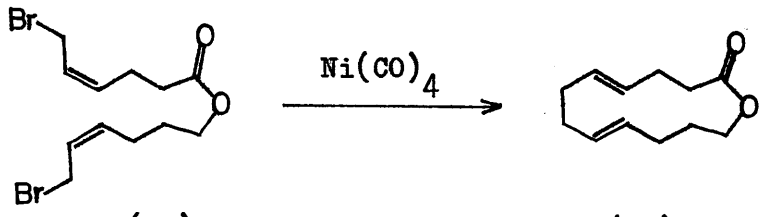
II. R = H, R_1 = Propionyl,

III. R = OH, R_1 = H.

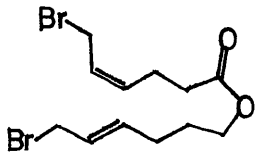
See also, Nystatin*(24)[135], Pimaricin*(25)[136], Rimocidin*(26)[137], Filipin*(27)[128, 138], Lagosin*(28)[128], Candidin*(29)[139], Amphotericin B*(30)[140, 141].



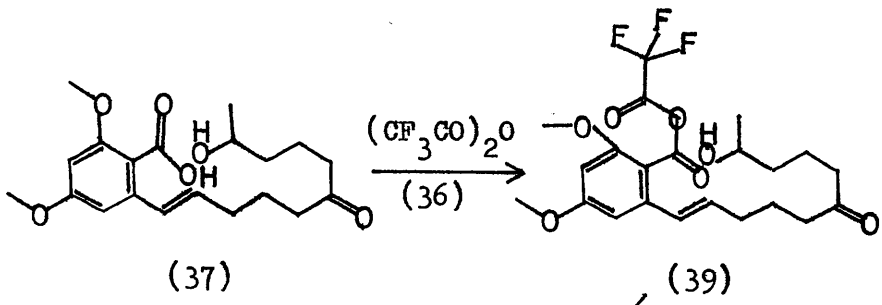
(32)



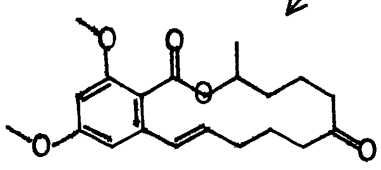
(33) (34)



(35)



(37) (39)



(38)

How Nature is believed to create the macrolides is presented in Appendix B, and how man has attempted to create them is discussed below. General methods for preparing large carbocyclic rings have been reviewed elsewhere[35,36,37] so only those ideas specifically oriented towards synthesising macrolides will be entertained.

To date chemists have envisaged two possible modes of entry into the macrocyclic skeleton. These I define as:-

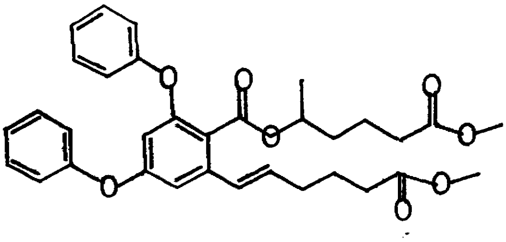
a) the acyclic approach which involves the closure at some point in the synthesis of a long acyclic molecule,

and b) the polycyclic approach whereby the bridging atoms of the polycycle are, at some stage, cleaved or removed leaving behind the intact ester containing periphery.

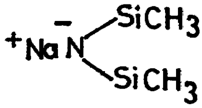
Corey's contribution to the acyclic mode manifests itself in the shape of bis π-allyl nickel complexes. Originally presented in his synthesis of humulene (32)[38] the method was later successfully applied to the construction of macrocyclic lactones[39]. Thus gradual addition of the Z,Z allylic dibromide isomer (33) to six equivalents of nickel carbonyl in N-methylpyrrolidone (c=0.2M) at 50° under argon gave a 75% yield of the E,E diene macrolide (34). The E,Z dibromide isomer (35) also gave rise to the E,E diene macrolide.

Taub[40] used trifluoroacetic anhydride (36) to activate the long chain hydroxy acid (37) towards cyclisation to the dimethylether of Zearalenone (38). Intermediated by the mixed anhydride (39) the reaction gave rise to the benzolactone in 80% conversion yield.

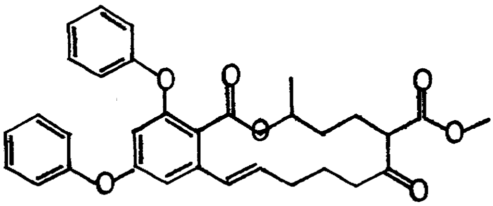
A similar assembly was also prepared [41] via the Dieck-



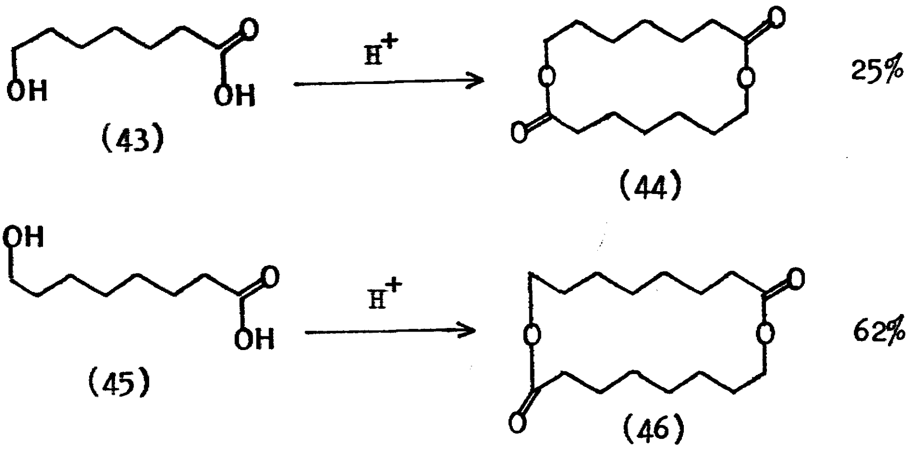
(40)



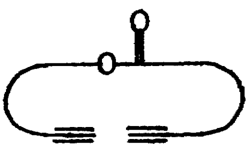
(42)



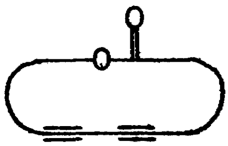
(41)



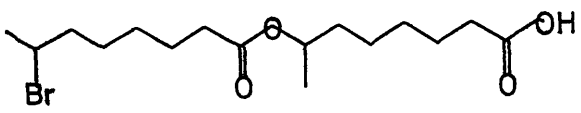
Scheme (1).



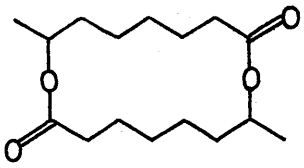
(47)



(48)

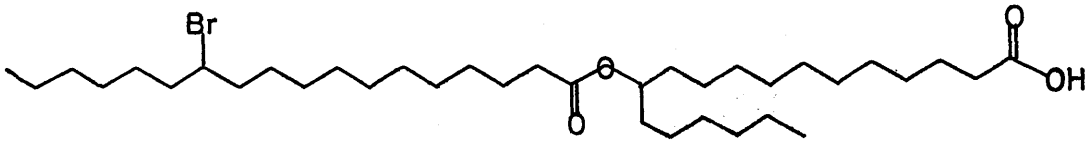


(49)

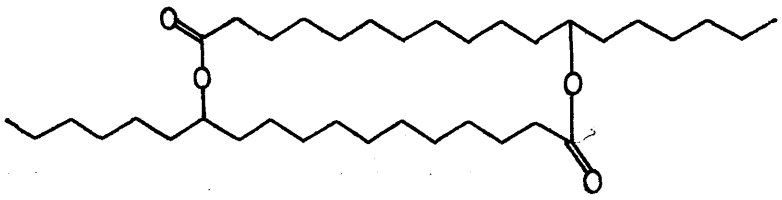


(50)

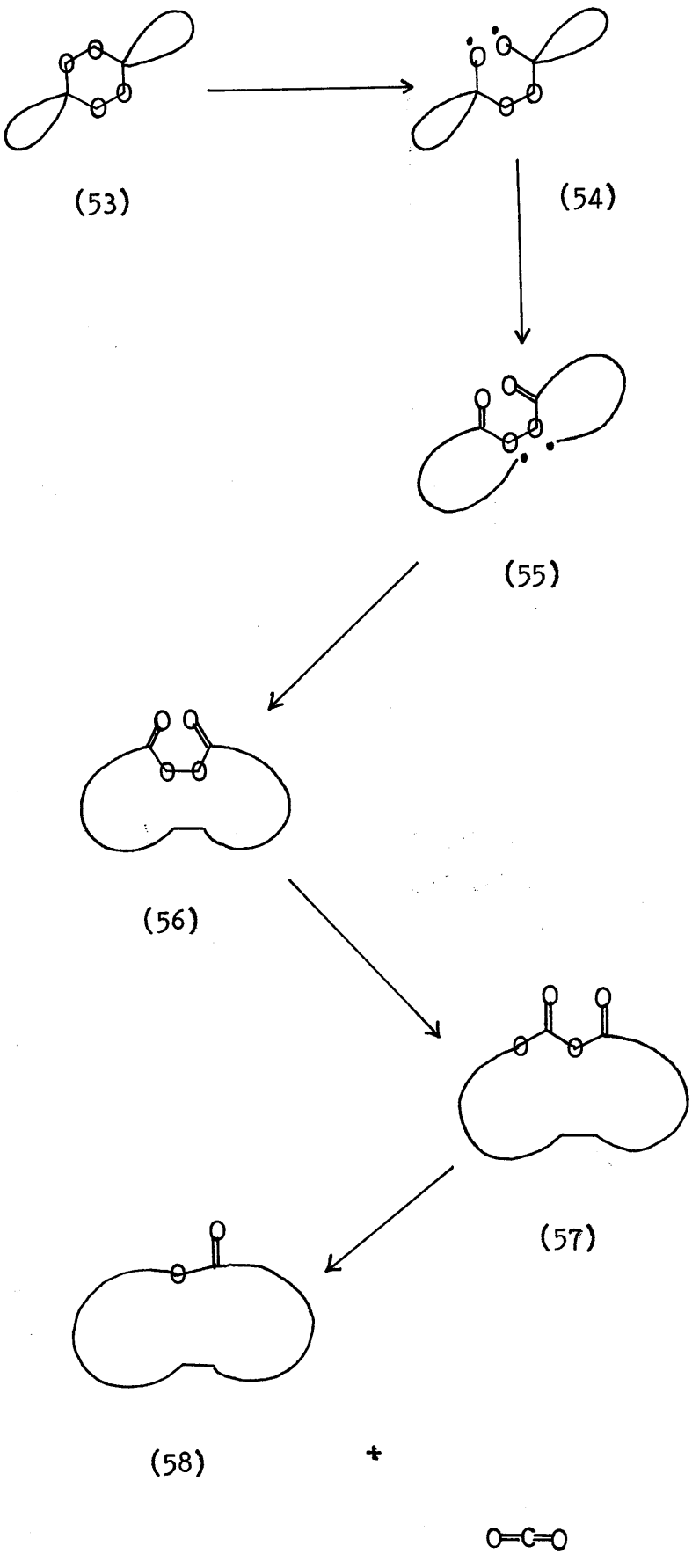
Scheme (2)



(51)



(52)



Scheme (3)

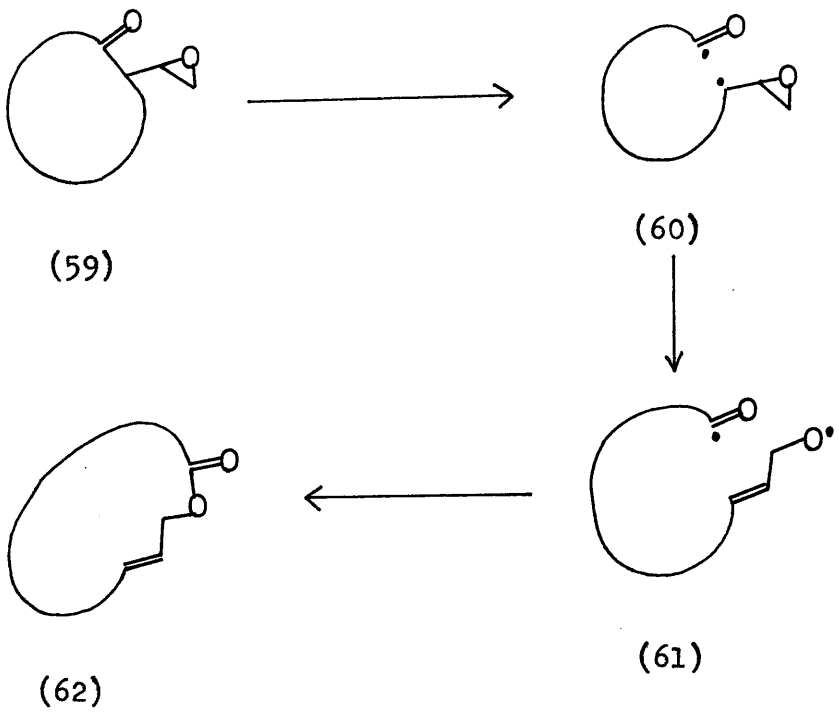
mann cyclisation of the diester (40) to (41). The reaction had been unsuccessful using bases like potassium tert-butoxide but employment of the organic soluble amide (42) in hot ether, under conditions of high dilution, effected ready cyclisation in a yield of 77%.

Stoll has published experiments[42] wherein he describes the oligomerisation of various hydroxyacids (Scheme 1). The interesting feature of these reactions is that he can regulate his conditions to obtain a high preponderance of the biscondensation product.

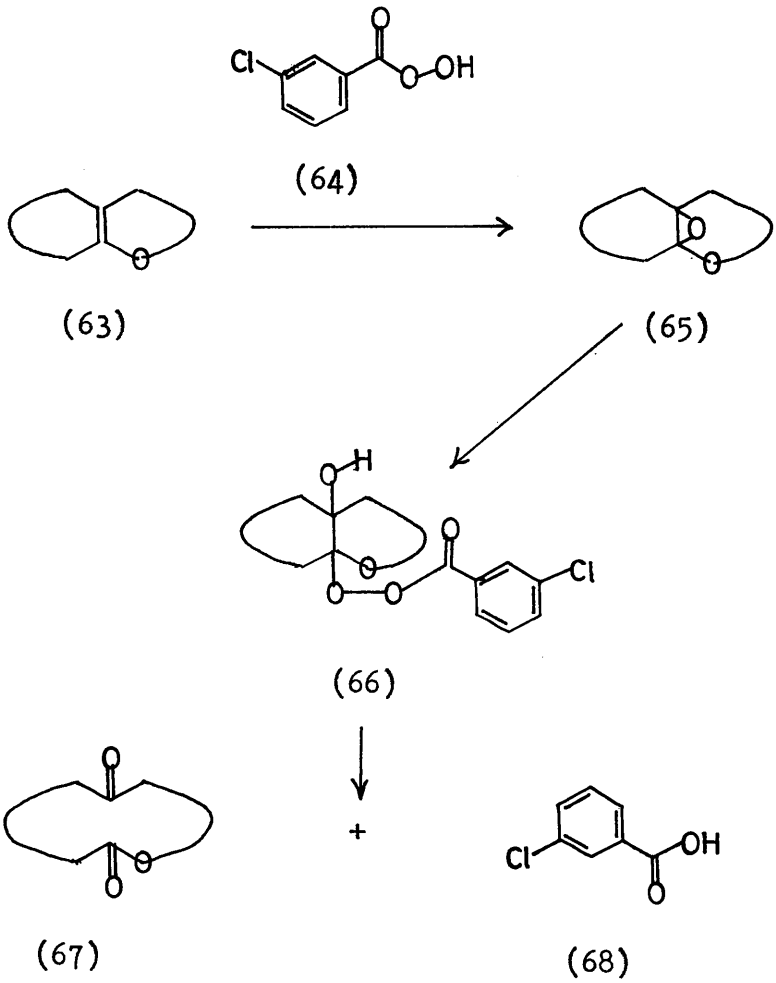
Bergelsen[43,44,45] has cyclised ω,ω' diacetylenic esters (47) at high dilution in a mixture of pyridine and diethylether under the action of anhydrous copper acetate to afford the macrolides (48).

Several authors[46,47,48] have effected intramolecular displacement of halide by carboxylate anion to yield, in excess of 40%, the desired product (Scheme 2)[47]. A 26-membered dilactone (51) has been synthesised using this technique of heating the parent acid (52) in ethylmethylketone with a vast excess of potassium carbonate.

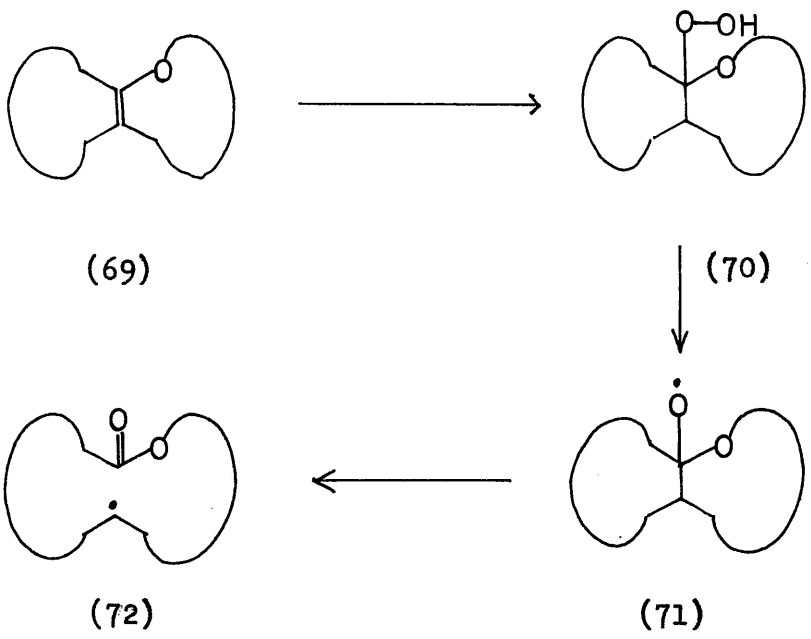
Scheme (3) depicts a remarkable acyclic approach to large ring lactones whereby Story[51] merely thermolyses or photolyses cyclic bis-peroxides to give the monocyclic esters in yields of about 20%. The reaction is believed to proceed by rupture of the weak oxygen-oxygen bond in (53), followed by a twin β-cleavage of the resulting diradical (54) to give the cyclisation precursor (55). Radical combination in (55) gives the peranhydride (56) which, via a carboxy inversion reaction[52], is envisaged to rearr-



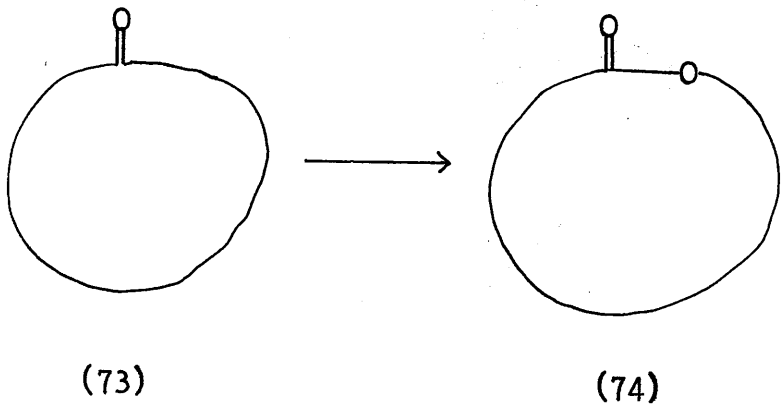
Scheme (4)



Scheme (5)



Scheme (6)



Scheme (7)

ange to the ketocarbonate (57) whose decarboxylation would result in the macrolide (58).

The photolysis[67] of 2-(oxiranyl)cycloalkanones (58) has been shown to generate macrolides by a three atom photochemical ring expansion (Scheme 4). Thus, α-cleavage in the parent ketone (58) delivers the cyclopropylmethylene radical (59) whose rearrangement to (60) is followed by radical combination to the unsaturated macrolide (61).

The polycyclic mode of synthesis has been dominated by the oxidative cleavage of the olefinic portion of bicyclic enol ethers.

Borowitz[49] accomplishes this reaction using a three fold excess of m-chloroperbenzoic acid (64) in dichloromethane (Scheme 5). The intermediary epoxide (65) is trapped as the hydroxyperester (66) which fragments to the ketolactone (67) and the carboxylic acid (68). Had he not used an excess of peracid (64) the product acid (68) would have competed with (64) in opening the remaining epoxide (65) thereby diverting the planned course of the reaction.

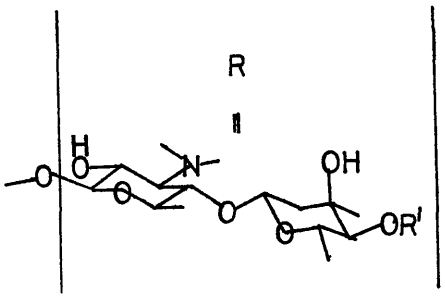
Becker and Ohloff[50], on the other hand, rely on the breakdown of the hydroperoxide (70) of the enol ether (69) to give macrocyclic lactones (Scheme 6). The hydroperoxide is generated using 30% hydrogen peroxide and 50% sulphuric acid dissolved in acetic acid.

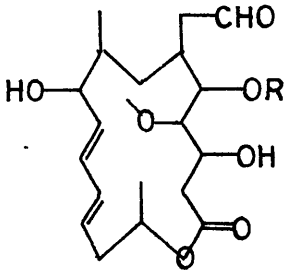
Finally, macrocyclic lactones issue from the Baeyer-Villager oxidation of macrocyclic ketones (73)[53], which ketones are derivable from either route (Scheme 7).

Several points arose from an examination of the contemporary techniques. To prepare a really large ring by the polycyclic routes would most likely require the synthesis of a macrocyclic precursor. Thus, despite their superbness in concept and undoubted value in 10-membered ring synthesis, they seemed to include the problem that they were intended to solve. Further, the fate of really complex precursors, as required in macrolide synthesis, was thought uncertain under application of the polycyclic techniques. People invariably equate the problem of cyclising a long chain macrolide hydroxyacid precursor with the proven difficulty in cyclising unfunctionalised long chain hydroxyacids[29]. It was felt in these laboratories that this view was unjustified for the complex functionality in the macrolide precursor might assist, via intramolecular hydrogen bonding, the internal esterification. Moreover, the evidence that macrolides complexed with ions promoted the view that these same ions might act as templates around which the hydroxyacid might cyclise.

Thus was initiated, in these laboratories, an attempt to estimate more fully the feasibility of the acyclid route to the macrolide group of antibiotics.

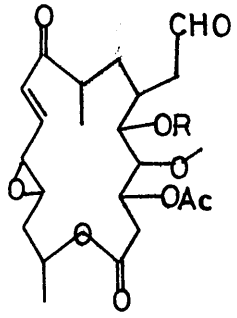
APPENDIX A.



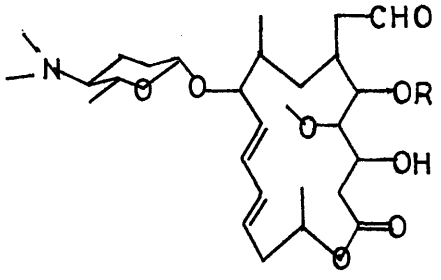


(141) Leucomycin A_1

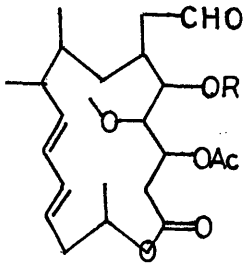
R'= Isovaleryl



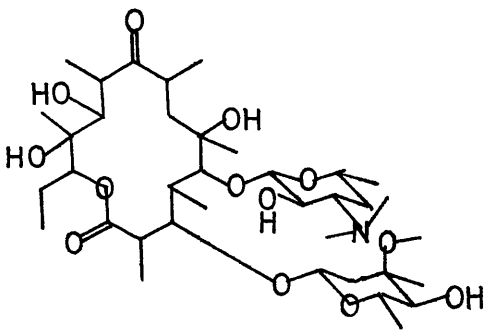
(143) Carbomycin A R'= Isovaleryl.



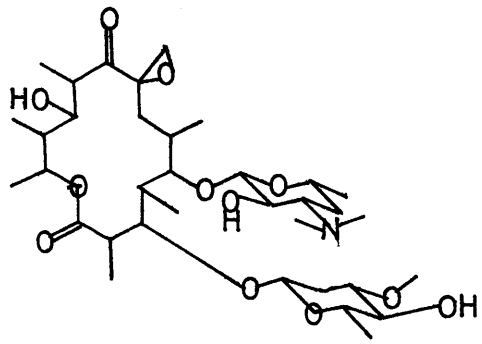
(142) Spiramycin I R' = H



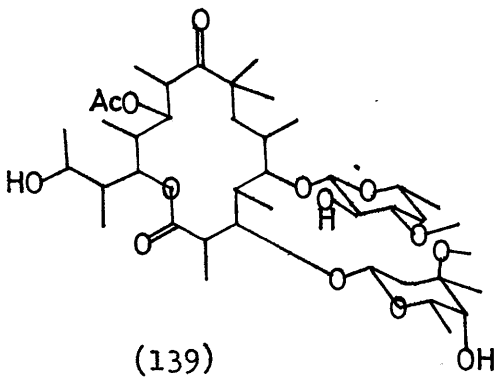
(138) Josamycin R' = Isovaleryl



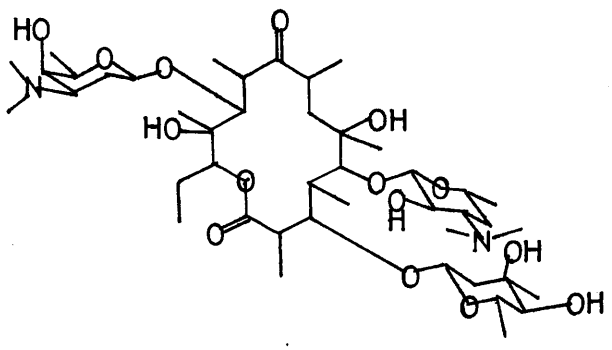
(136)



(137)



(139)



(140)

In protein synthesis in intact cells, the peptidyl-tRNA molecule occupies a site on the ribosomal complex which is often termed the 'donor' site, because the growing peptide chain resides here at the time the peptidyl chain is donated to the incoming amino acyl-tRNA species. Culmination of this step finds the new, elongated peptide chain attached to the 'acceptor' site of the ribosomal complex, via the tRNA of the latest amino acid. The ensuing migration of the new peptidyl-tRNA molecule back to the 'donor' site, to allow the synthetic cycle to be repeated with further amino acids, is called the 'translocation' process and is distinct from the first half of the cycle which is catalysed by 'peptidyl synthase'.[142]

This complex machinery, however, was found to break down on introduction of the macrolide antibiotics and many very interesting experiments have since been designed to reveal their fundamental mode of action.

Thus, erythromycin A was shown to bind, specifically, to the 50S subunit of Staphylococcus aureus ribosomes forming a 1 : 1 complex, the formation requiring NH_4^+ and K^+ ions. Though experimental correlations were poor, this binding was shown to be a prerequisite for antibiotic activity. On binding, its effect on polylysine synthesis was to cause an accumulation of dilysine and some accumulation of trilysine. Very little tetralysine was produced[130]. This was explained by assuming that erythromycin A (136) blocked a reaction after the synthesis of the first peptide bond, thus allowing only the formation of dilysine. Such a high accumulation of dilysine could overcome the inhibitory effect of the macrolide to incur some production of trilysine.

There are several steps between the synthesis of the first and second peptide bonds.

i) The release of tRNA from the donor site.

ii) The movement of the ribosome in relation to the dipeptidyl-tRNA-mRNA complex, so that dipeptidyl-tRNA moves from the acceptor site on the ribosome to the donor site.

iii) Hydrolysis of GTP.

iv) Transmission of energy to the movement.

v) The binding of the third amino acyl-tRNA to the acceptor site.

vi) The synthesis of a second peptide bond.

i - iv Correspond to the 'translocation' process.

The effects of erythromycin A on each step have been examined and, though yet incomplete, have engendered the suggestions that the antibiotic,

a) inhibits the release of peptidyl-tRNA from the acceptor site [30],

b) binds to the 50S subunit of the ribosomes and changes the activity of peptidyl transferase, most likely through an allosteric effect, to stimulate the first peptide bond formation and inhibit the second and subsequent peptide bond formations[30],

c) is a specific inhibitor of the translocation step.[30]

Biochemical studies of the non-polyene macrolides were further accelerated by the discovery of a high incidence of macrolide resistant strains in various bacteria.[143]

There are two types of resistance to macrolides in staphylococci, inducible and constitutive. The inducible type of res-

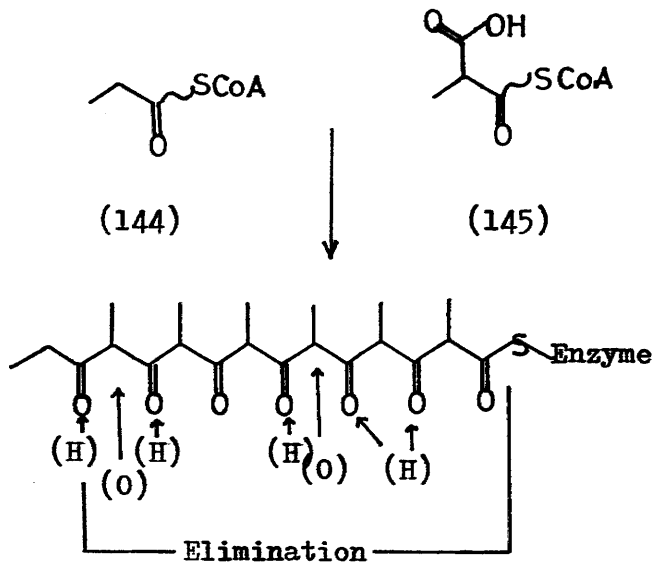
istance is raised after prior treatment of the organism with sub-inhibitory concentrations of the inducer, such as erythromycin A (136) or oleandomycin (137), and the induced population acquires a high resistance to both macrolides. The resistance of the induced cells is lost when they are again grown in a drug free medium. Constitutive resistance is an innate property of a constitutive resistant bacterium.

After wide scale use of erythromycin, more than 30% of staphylococci became resistant to the antibiotic. Josamycin (138) is effective against most of them. Further studies showed that kujimycin (139) and megalomicin (140) were also inducers but that the leucomycins (141), spiramycins (142), and carbomycins (143) were not. The two groups have definite structural differences.[144] The most prominent characteristics of the non-inducing type are the chain formation of two sugars, and the aldehyde group. The sugars in both groups appear to be essential for activity and Corcoran[145] has thus suggested that the intact antibiotics might act by mimicking subunits of peptidyl-tRNA. Of the second group, the aldehyde moiety in spiramycins[146] and leucomycins[147] has been shown to have a very great influence on antibacterial activity, a property not shared by the conjugated double bonds.

APPENDIX B

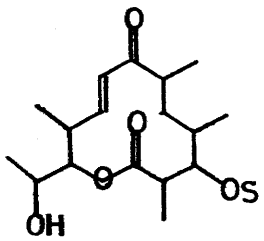
In 1956 Gerzon[55] greatly broadened the horizons of biosynthetic theory when he announced that the completely regular arrangement of the C-methyl groups on the aglycone of erythromycin (31) could be explained by postulating its biogenetic derivation from seven propionate units. This 'propionate rule' was extended to other macrolides and it was assumed that in the case of macrolides with fewer C-methyl groups, the lactone comprised propionate and acetate units[22]. Birch[56], on the other hand, considered the possibility that an unbranched, acetate derived, poly-β-keto chain might be methylated at the reactive methylene groups by well established methyl donors.

Labelling studies[57, 58, 59] showed unequivocally that Gerzon's hypothesis was valid and that propionate could serve as a biological building block in the same manner as acetate. Paralleling fatty acid synthesis it was assumed that the actual precursors of the lactone ring are one molecule of propionyl-CoA as a starter molecule and six molecules of methylmalonyl-CoA (145). Indeed, evidence was compiled that propionic acid did serve mainly as a starter molecule forming C atoms 1-12 of (31). The observation that in erythromycin the chiral centres between C-1 and C-8 are mirrored by those between C-10 and C-13 and similar observations in neomethymycin (147) and picromycin (148) led Djerassi[62] to the interesting speculation that the macrolide rings comprise two large fragments coupled at a late stage in the biosynthesis. Grisebach[54], however, points out that two different large precursors would be unlikely to possess the same activity which is in contrast with the observation that the activity in the lactone is evenly distributed throughout.

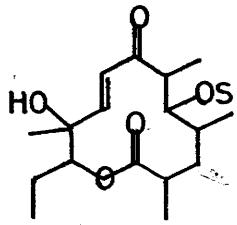


Erythromycin (31)

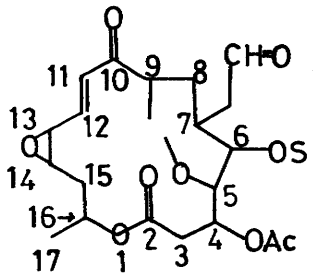
Diagram A



(147)



(148)



(123)

Further, two starter molecules would be required which also opposes experimental results.

The poly-β-keto acid supposedly formed according to the 'propionate rule' must subsequently undergo a series of oxidations and reductions (Diagram A) probably on the surface of an enzyme. Theoretically, picromycin (148), oleandomycin (137), lankamycin (116) and narbomycin (82) can be similarly treated. Methymycin (132), nevertheless, was reported, from labelling studies, as the product of five propionate units and one acetate unit,[63] but Bentley[64] has shown that the distribution of activity can be accommodated within the 'propionate rule' if it is assumed that the methyl group originally at C-8 has been lost, for instance, by oxidation and decarboxylation. Thus, is explained the poor incorporation of acetate into methymycin (132).

Magnamycin (123) does not conform. The methyl group at C-9 has been shown to originate from propionic acid[65] but carbon atoms 10-17 are derived from acetate[66]; carbon atoms 2-7 appear to involve precursors, other than acetate, which are readily formed from glucose or from succinic acid.

Though magnamycin was the first example of a macrolide which was not formed solely from acetate and propionate units other examples have since come to light; thus tylosin (121), acumycin (---), and the spiramycins (118) appear to be similar to magnamycin (123).

The polyene macrolides can all be visualised, at least, as deriving from propionate and acetate units.

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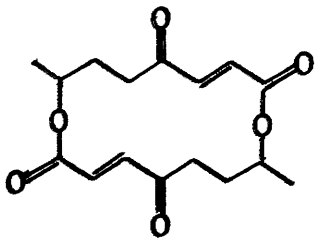
DISCUSSION.

FOREWORD.

Within the following discussion, the author has taken great pains to reproduce as accurately as possible the way in which synthetic schemes to pyrenophorin came into being; thus the transition of the initial and hazy inner eye's project to its more realistic and carefully examined successor is presented as free as possible of mechanistic dichotomies. Such mechanistic data as are deemed relevant appear in the discussion of the actual experimental work.

T. A. P.



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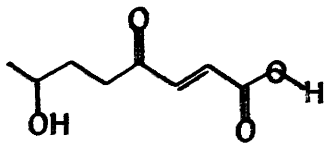

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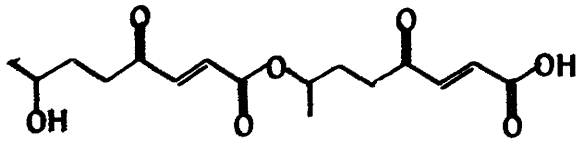

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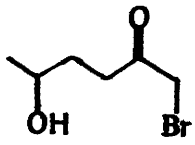

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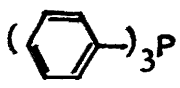

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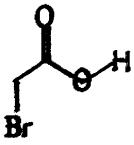

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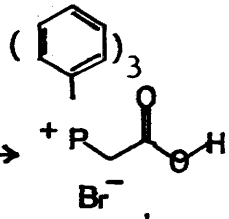

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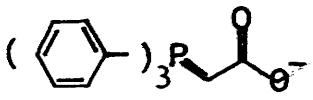

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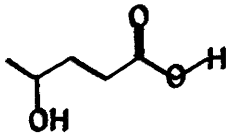
Scheme (1)



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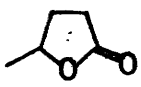

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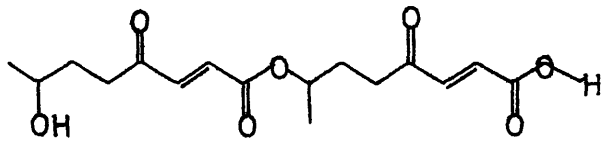
It was to explore the acyclic approach to macrolides that the synthesis of pyrenophorin[1] (1) was undertaken. Stereochemically the simplest of the macrolide antibiotics - it has but two asymmetric centres - this bislactone provided an excellent model for studies of the cyclisation of long chain hydroxyacids in the presence of a variety of functionalities. The proposed synthetic route to pyrenophorin was developed according to the usual methodology of fragmenting the molecule in a stepwise fashion to readily available and cheap starting materials with the prerequisite that each step be potentially capable of reversal in the laboratory. Application of this concept to the structure in question gave rise to the following events and arguments.

Broadly, and in keeping with the acyclic mode of synthesis, it was thought that the diester (1) could be a cyclisation product of (2). This long acycle is merely the monocondensed dimeric ester of 7-hydroxy-4-oxo-oct-2-enoic acid (3) which structure was envisaged as the hypothetical protonated product of an abnormal Wittig reaction between the bromomethyl ketone (4) and the phosphorous ylid (5). Such ylids might be prepared from bromoacetic acid (6) and triphenylphosphine (7) under well documented conditions (Scheme 1). Similarly, standard methods for preparing bromomethyl ketones from carboxylic acids inferred that the parent of (4) would be 4-hydroxypentanoic acid (9), the hydrolysis product of γ-valerolactone (10). Aside from its low cost, this lactone, being available in a resolved form[2], offered the tempting prospect of a totally stereospecific synthesis of natural (-)-pyrenophorin.

Quite reasonably it was expected that this breakdown would



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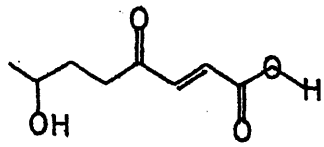

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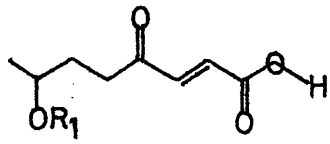

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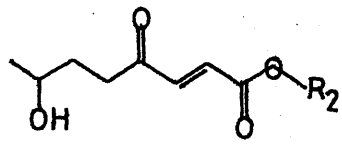

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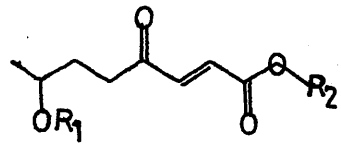


(13)

lend itself well to reversal in the laboratory although the exact way by which these fragments could be reassembled was, of course, the subject of a rigorous inspection of each step to uncover and eliminate all possible pitfalls. Great care was taken to ensure all steps were mutually compatible.

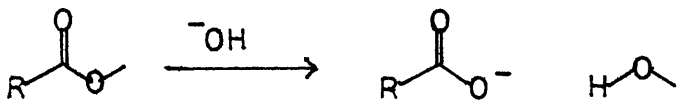
In view of the symmetrical bislactonic nature of pyrenophorin the acyclic mode of synthesis from a hydroxyacid like (3) was further divided into two subgroups which were defined as :-

(a) the 'one-step' process wherein both esterifications occur in a single reaction vessel,

and (b) the 'two-step' process where each esterification occurs in a different reaction vessel.

Thus, in the Stoll [3] type 'one-step' process long acyclic intermediates are not isolated whereas in the 'two-step' process long acyclic precursors are isolated.

Analogy with the work of Stoll [3] suggested that the 'one-step' dimerisation of (3), passing through the transient intermediate (2), to the macrocycle was feasible. However, to avoid polymerisation, a true 'two-step' process must involve at the first esterification, the condensation of intermediates like (11) and (12) where R_1 and R_2 are easily removable blocking groups which make the second discrete esterification accessible. To preclude two separate and time consuming syntheses, (11) and (12) should be daughter molecules of a structure (13) which adds the constraint that R_1 and R_2 be independently removable. Alternatively, one should be easily derived from the other. Unfortunately, amidst the existing plethora of protecting groups, remarkably few are designed for the blocking of carboxylic acids. Of the meagre



(14) (15)

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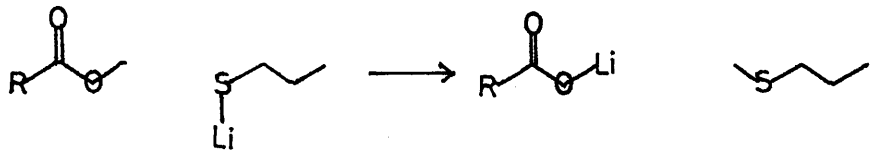
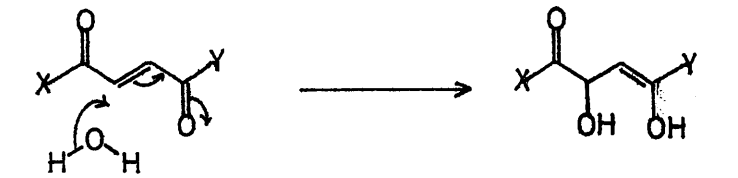


(14) (16) (15)

(14) (17) (15)

Scheme (3)



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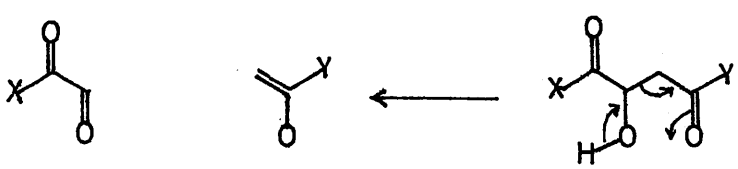
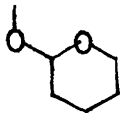


(24) (23) (22)

Scheme (4)



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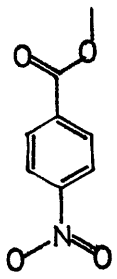

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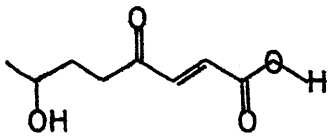

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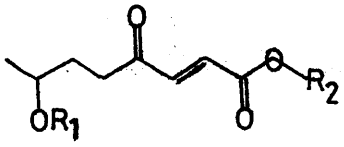

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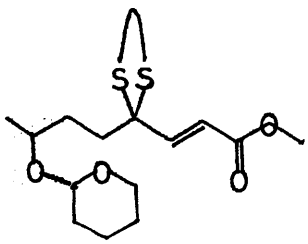

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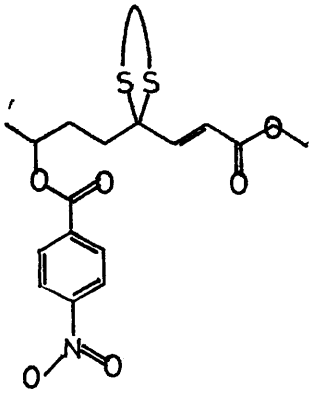
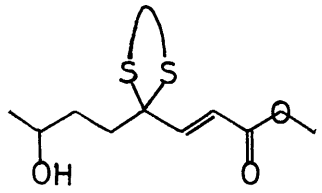


(27)

choice available the most versatile group appeared to be the methyl ester (R_2=CH_3) which is removable via acyl-oxygen fission using fairly mild alkali (Scheme 2) or by alkyl-oxygen fission (Scheme 3) using either lithiumpropylmercaptide[4] (16) in hexamethylphosphorictriamide at room temperature or lithium iodide[5] (17) in sym-collidine at temperatures in excess of 100°C. Adherence to such a group meant that OR_1 could be acid labile or very mild base labile which properties were shown respectively by the tetrahydropyranyl ether (18) and the p-nitrobenzoyl ester (19).

This assembly of protecting groups was considered, amongst all, to be the most useful but, when applied to the molecule in question, (13), begat a new problem. Any combination of the removal conditions of these blocking devices involves the use of aqueous acid or base yet enedione moieties (20) are notoriously amenable towards retroaldol conditions (Scheme 4). Thus if the choice of R_1 as methyl was to be pursued the ketonic carbonyl of (13) would have to be blocked. Three reasons more than justified the additional steps of attachment and subsequent removal of this most recent protective function. Firstly, the versatility of the original assembly of blocking groups; secondly, the convenient existence of the thioacetal protecting group (25) for ketones, which group can be added and removed in very high yield and independently of the other blocks; thirdly, the methyl ester could itself be used as a carboxyl activating group in molecular sieve[6] catalysed esterifications.

Thus an inspection of the conditions necessary to effect a 'two-step' reassembly of pyrenophorin from a fragment like (3), showed that (3) should be converted or replaced by (26) or (27).



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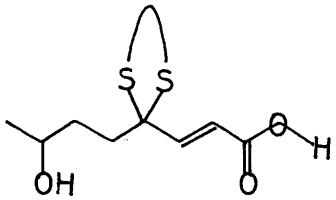

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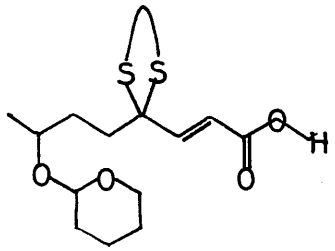

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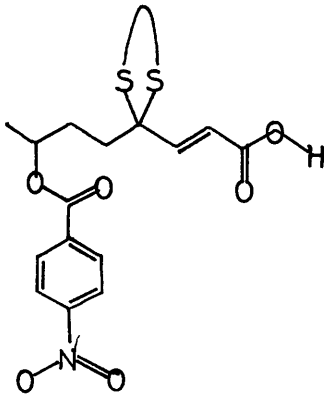

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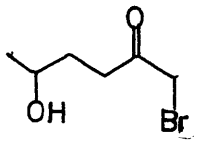

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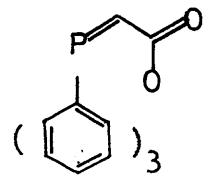

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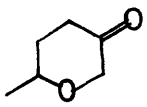

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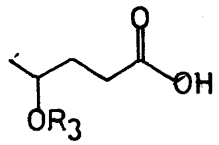

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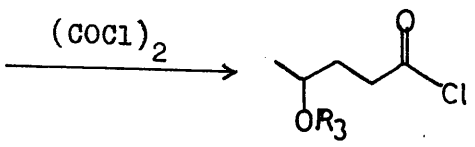

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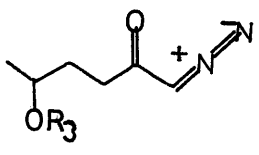

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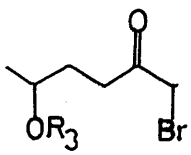


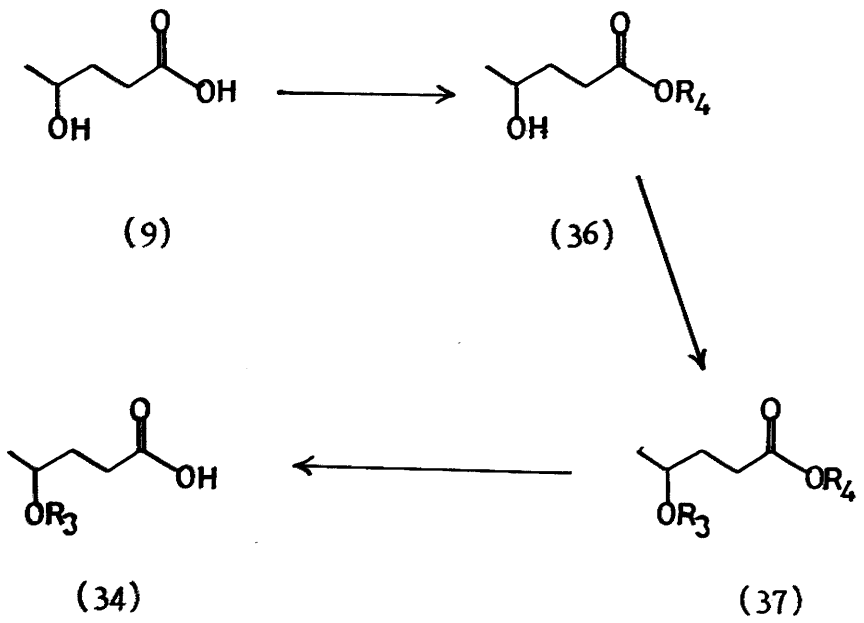
(33)

Scheme (5)

Continuing along the threads of our previously defined methodology it seemed reasonable that both (26) and (27) could be derived from (28) which is the simple methyl ester of the hydroxy-acid (29). In view of the apparent reversible nature of the Michael addition it was hoped that standard thioacetalisation conditions would give (29) from (3) making the conversion of the latter molecule to the fully protected monomer, (26) or (27), a very real possibility. It should be noted that although the addition sequence of the protecting groups to (3) could be varied it was not expected that the alcohol group could be blocked in the presence of a free carboxylic acid group to give the ultimately desired monomers (30) and (31) directly from (3) without ever needing to go through the fully protected form (26) or (27).

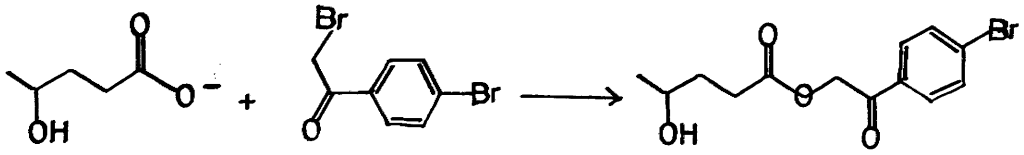
The next step in the initial fragmentation scheme was the suggestion that (3) be the hypothetical protonated product of an abnormal Wittig reaction between the bromomethyl ketone (4) and the triphenylphosphorane (5). There were, sadly, practical objections to using these synthons as they were; in the first instance the bromomethyl ketone would most likely undergo base catalysed cyclisation under the reaction conditions of hot benzene and basic ylid to form 2-methyl-5-oxo-tetrahydropyran (32). The alcohol really had to be protected by an as yet undetermined group R_3 as in (33). The questions of whether or not to attach this group before or after synthesising (4) and of the nature of the group were answered by looking further back in our fragmentation scheme. The route supposedly destined to arrive at the general molecule (33) is depicted in Scheme 5.

If we let R_3 be protium then the starting material appears



(9) (36) (34) (37)

Scheme (6)

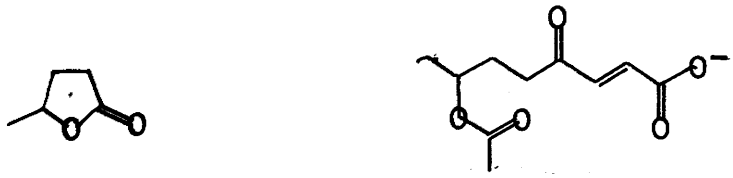


(38) (34) (41)

Scheme (7)

(38) (40) (42)

Scheme (8)



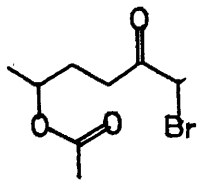
(10) (43)

as 4-hydroxypentanoic acid (9); characteristically unstable relative to γ-valerolactone (10), this hydroxyacid spontaneously cyclises to the pentanolide under carboxyl activation albeit by the presence of a mere trace of a general acid. Such a molecule would never survive the first step in Scheme (5) which demands a hydroxy protected precursor.

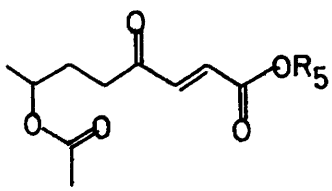
In view of the inherent instability of (9) the successful protection of its hydroxyl group posed a little problem. Despite extensive sorties into the literature it was concluded that to effect this requisite would require blocking the carboxylic function of (9) with R_4, to give (36), as the first step, then protecting the alcohol with R_3, as in (37), and finally removing R_4, forming (34), as in Scheme (6). Fortunately, the enhanced nucleophilicity of the carboxylate anion in (38) over the free acid (9), and the stability of (38) towards cyclisation made the first event in Scheme (6) a reasonable proposition: Thus the carboxylate anion could displace bromide from p-bromophenacylbromide[7] (39) and, with assistance from Ag^+, from benzylbromide[8] (40) (Schemes 7 and 8).

Thus the point of entry of R_3 into the projected scheme was established although the identity of the group had yet to be decided till the restraints imposed by the removal conditions of R_4 had been realised.

The case of R_4 as p-bromophenacyl was considered first; the use of zinc and acetic acid to cleave off this moiety immediately ruined the lucrative prospects of equating R_1 and R_3. The stabler acetate was a more realistic choice for R_3 in this case but its use would give as the abnormal Wittig product (43). To return to the main scheme this acetate, after protonation and protection of



(54)



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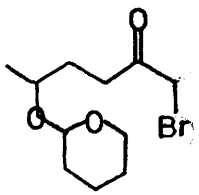

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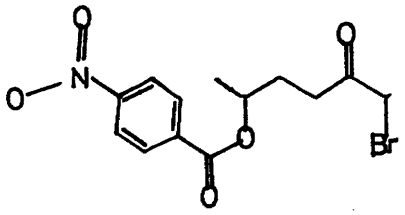

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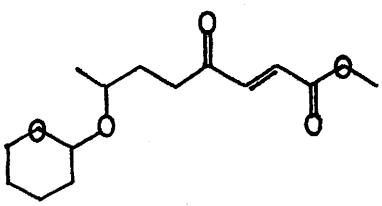
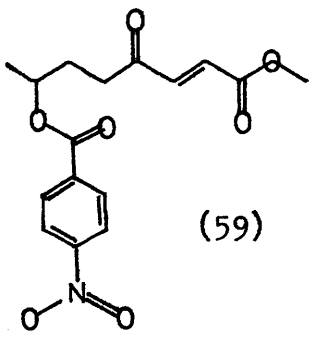


(58)



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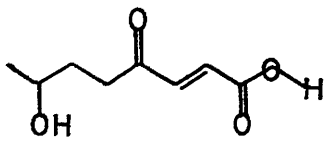

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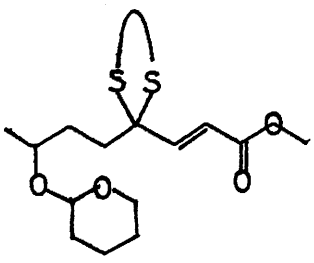
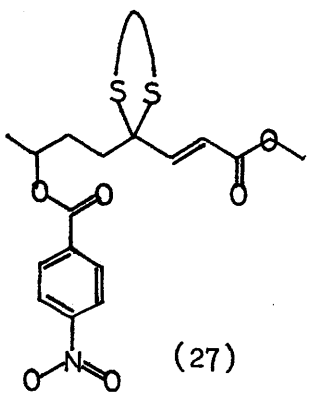


(26)



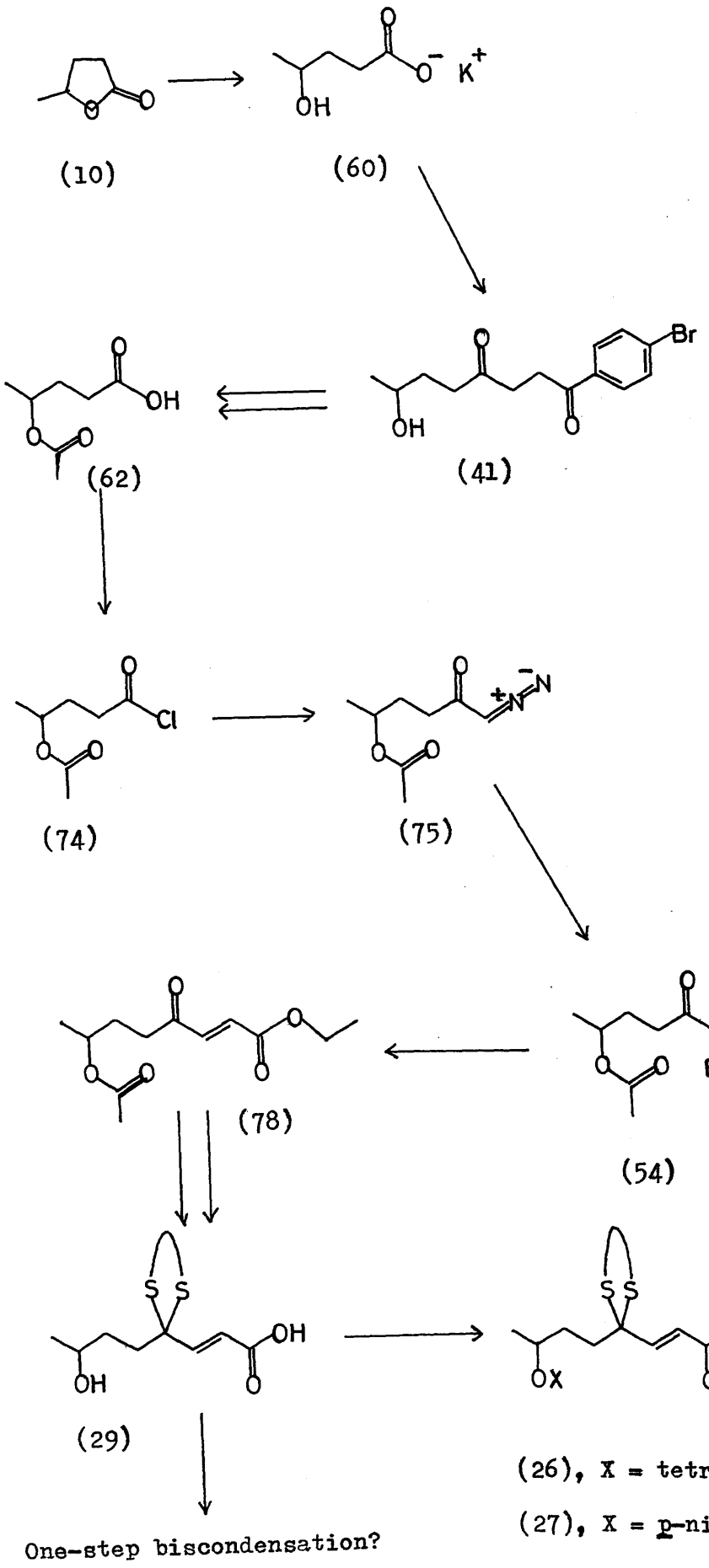
(27)

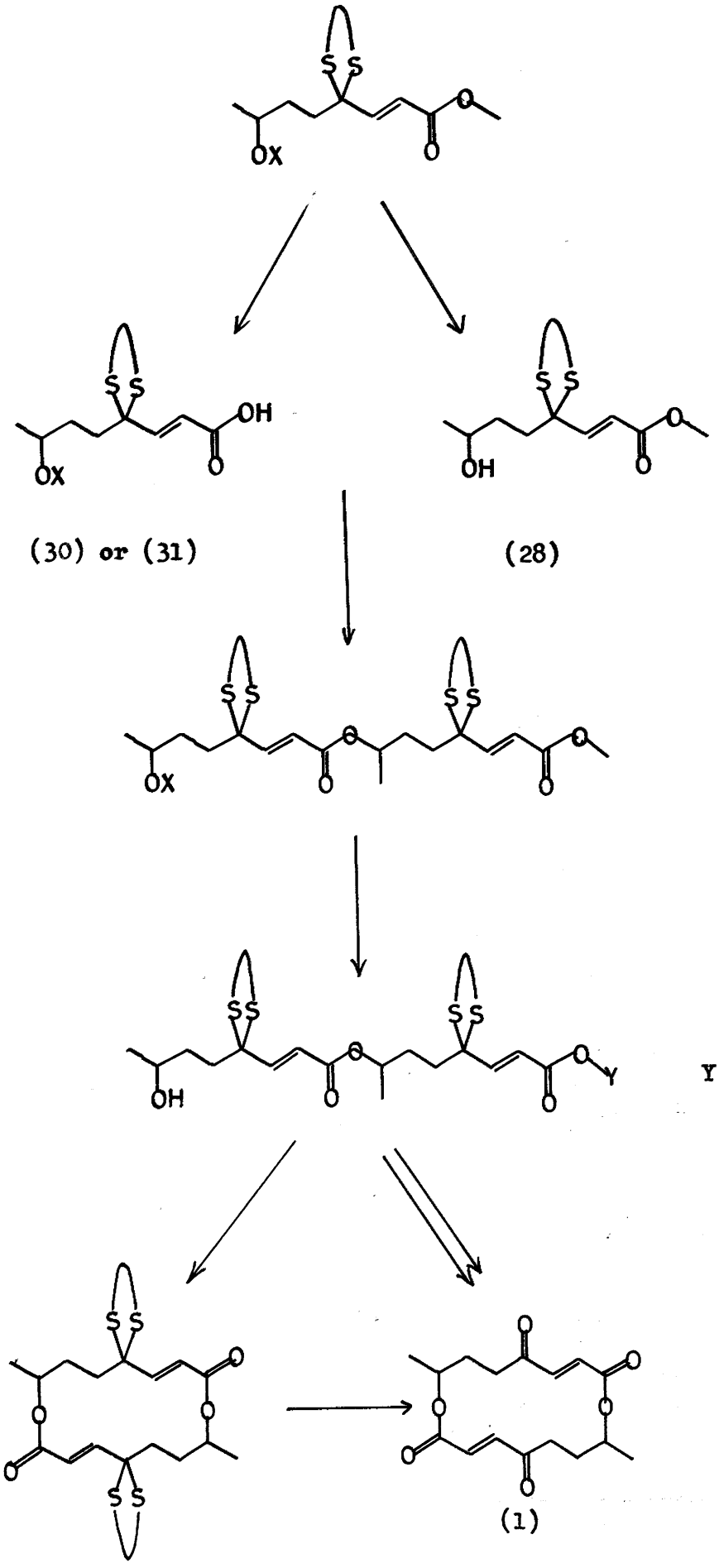
the ketone, would have to be hydrolysed off thereby adding another step to the overall projected synthesis.

The fate of the protecting group R_1 as tetrahydropyranyl or p-nitrobenzoyl under hydrogenolytic cleavage of R_4 as benzyl was less certain.

It is of significant relevance to digress at this point to consider another objection to the initially presented abnormal Wittig reaction. In their studies of the preparation and reactions of some phosphobetaines, Denny and Smith[9] showed that although the phosphonium chloride salt (45) could be prepared by reaction of triphenylphosphine (7) and chloroacetic acid (44) it was unstable, not surprisingly, in base, giving carbon dioxide (47) and methylenetriphenylphosphorane (48) (Scheme 9). Probably it was the stability of carbon dioxide and not, as the authors suggested, of the ylid (48) which caused rapid decarboxylation of the transient intermediate (46). Reaction of triphenylphosphine (7) with bromoacetic acid (6), on the other hand, gave triphenylphosphine oxide (49) and acetyl bromide (50). It has, however, been demonstrated many times[10], that ylids of type (53), where R_5 is a small alkyl group, are very readily formed as depicted in Scheme (10) and, unable to fragment as in Scheme (9), are stable over long periods.

Now use of (53) in conjunction with the phenacyl derived system (54) would give a product (55) but compared to the original scheme still only one step would be added since both esters, after protection of the carbonyl group, could be cleaved in one step to give (29). Of course, with R_5 as methyl, use of (53) with the possible benzyl derived systems (56) and (57) might give (58) and


(10)
(60)
(62)
(41)
(74)
(75)
(78)
(54)
(29)
One-step biscondensation?
(26), X = tetrahydropyranyl.
(27), X = p-nitrobenzoyl.



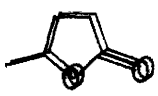
Route A

(59) directly; after thioacetalisation this would satisfy the alternative proposition of replacing (3) by (26) or (27) in the synthetic scheme.

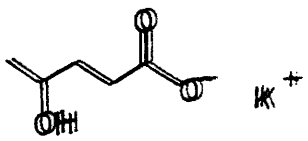
Two reasons justified the dismissal of the benzyl system: Firstly, it was considered doubtful that R_3 as tetrahydropyranyl or p-nitrobenzoyl would survive the reaction conditions from removal of the carboxylic acid blocking group R_4 to the abnormal Wittig reaction, particularly the hydrobromination conditions in Scheme (5). Secondly, experience in these laboratories of silver carboxylates had shown them difficultly handleable and indeed the entire benzylation and debenzylation procedure to be quite tedious.

Finally, to complete the projected scheme the preparation of the carboxylate anion (38) would be effected by simple alkaline hydrolysis of γ-valerolactone (10).

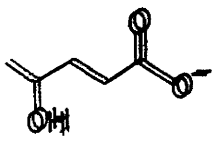
This projected route, shown collectively in Route (A), had the distinct advantage of encompassing, en route, both 'one-step' and 'two-step' subgroups via (29) and (26) or (27) respectively. Furthermore, its maintainance of stereochemistry at the alcoholic centre, of course, lent the project well to a possible synthesis of natural (-)-pyrenophorin.



(10)



(60)



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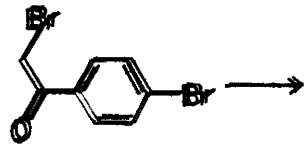
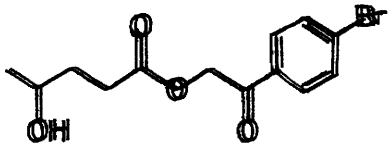
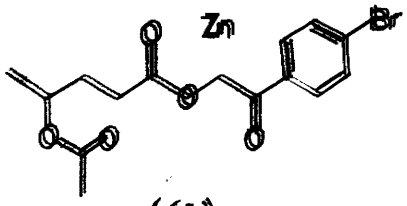


(39)

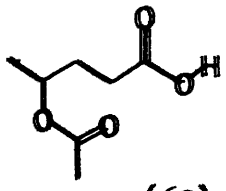


(41)

Scheme (7)

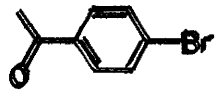


(61)



(62)

+



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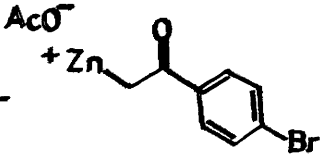
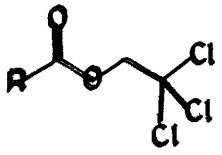


(64)

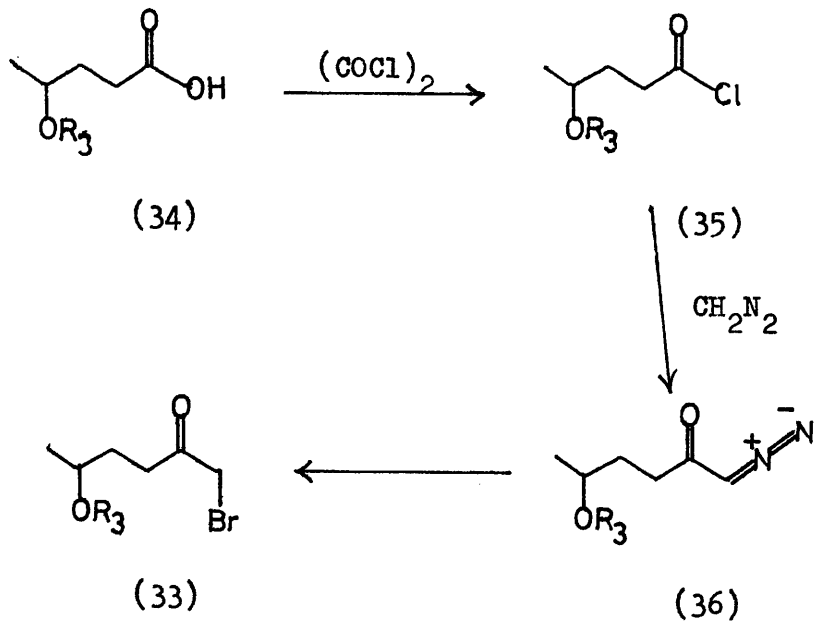
Scheme (11)



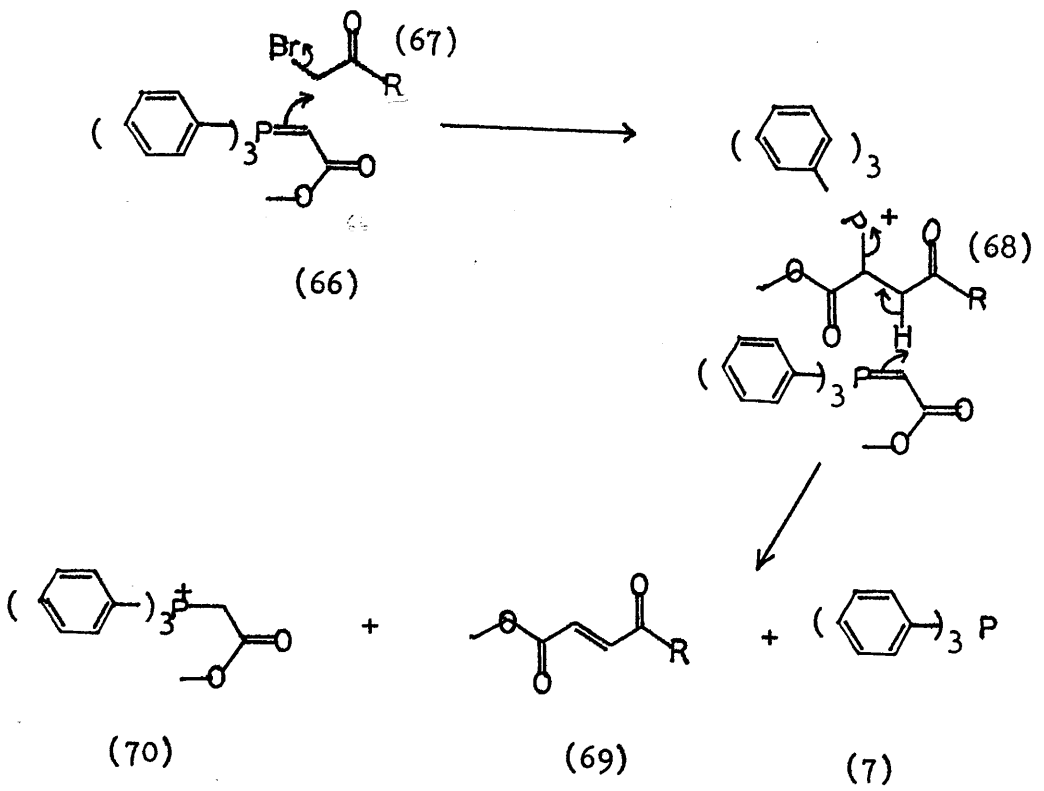
(63)

Thus, freshly redistilled γ-valerolactone (10) was hydrolysed to potassium 4-hydroxypentanoate (60) with 10 Normal potassium hydroxide and the reaction mixture divorced of water after adjustment of the pH to between eight and nine. The reaction of this dry potassium salt (60) with p-bromophenacylbromide (39) in dry dimethylformamide was devoid of problems provided the salt was in excess; this condition circumvented the observed difficulty of freeing the product ester (41) from any unreacted p-bromophenacylbromide via crystallisation. Acetylation of the product, to (61), and reductive cleavage of the carboxyl blocking group, giving (62), were equally effortless demonstrating the inherent usefulness of this protecting group recently offered by Hendrickson and Kandall[7] as an alternative to Woodward's[11] zinc removable β-trichloroethyl ester (63). In their paper these authors successfully pointed out the advantage of phenacyl esters over their rival in that they are readily synthesised under conditions which do not involve attack on the carboxyl carbonyl since they are formed by S_N2 attack of carboxylate anion on the phenacylbromide (Scheme 7). To further promote their new protecting group they showed that phenols may also be protected and freed with the same releasing agents, glacial, or dilute aqueous, acetic acid and an excess of zinc dust (Scheme 11). p-Bromophenacylbromide was described as having the advantage over other phenacylbromides, offering higher melting derivatives with clearer (AB) nuclear magnetic resonance patterns.

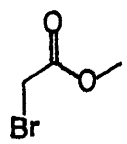
Unfortunately, extraction of the released carboxylic acid (62) by standard techniques was complicated by its very high water solubility. These difficulties, however, were made redundant when it was found that (62) could be distilled under reduced press-



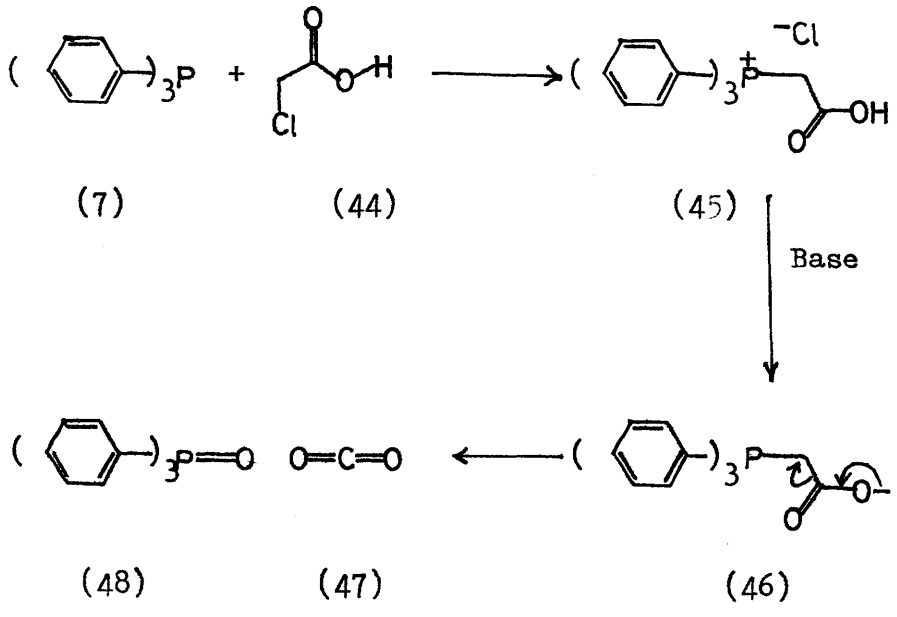
Scheme (5)



Scheme (12)



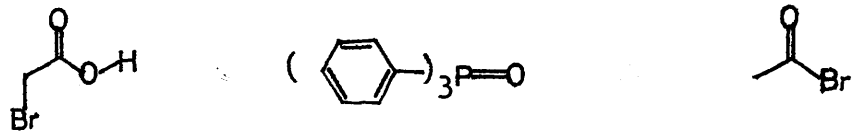
(71)



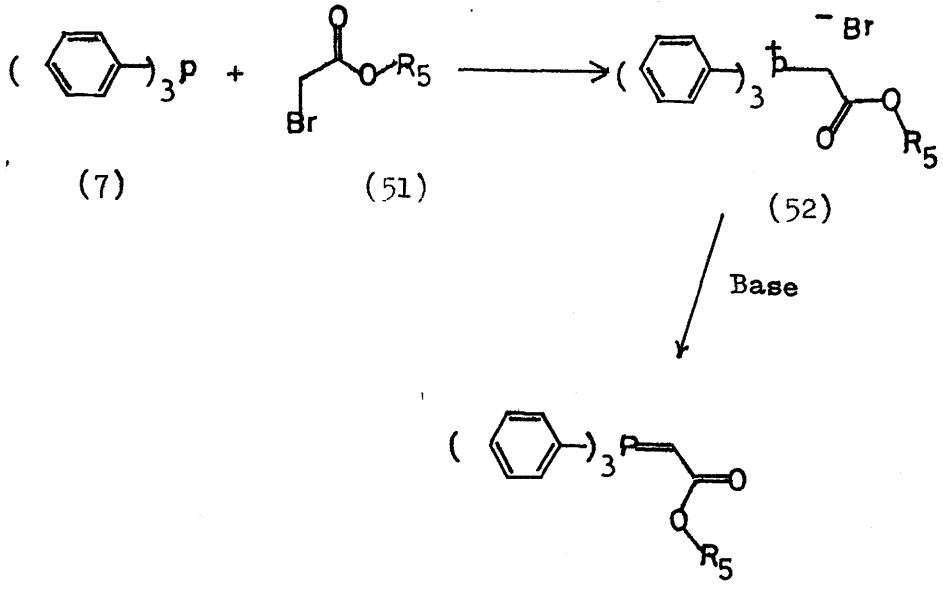
(7) (44) (45)

(48) (47) (46)

Scheme (9)



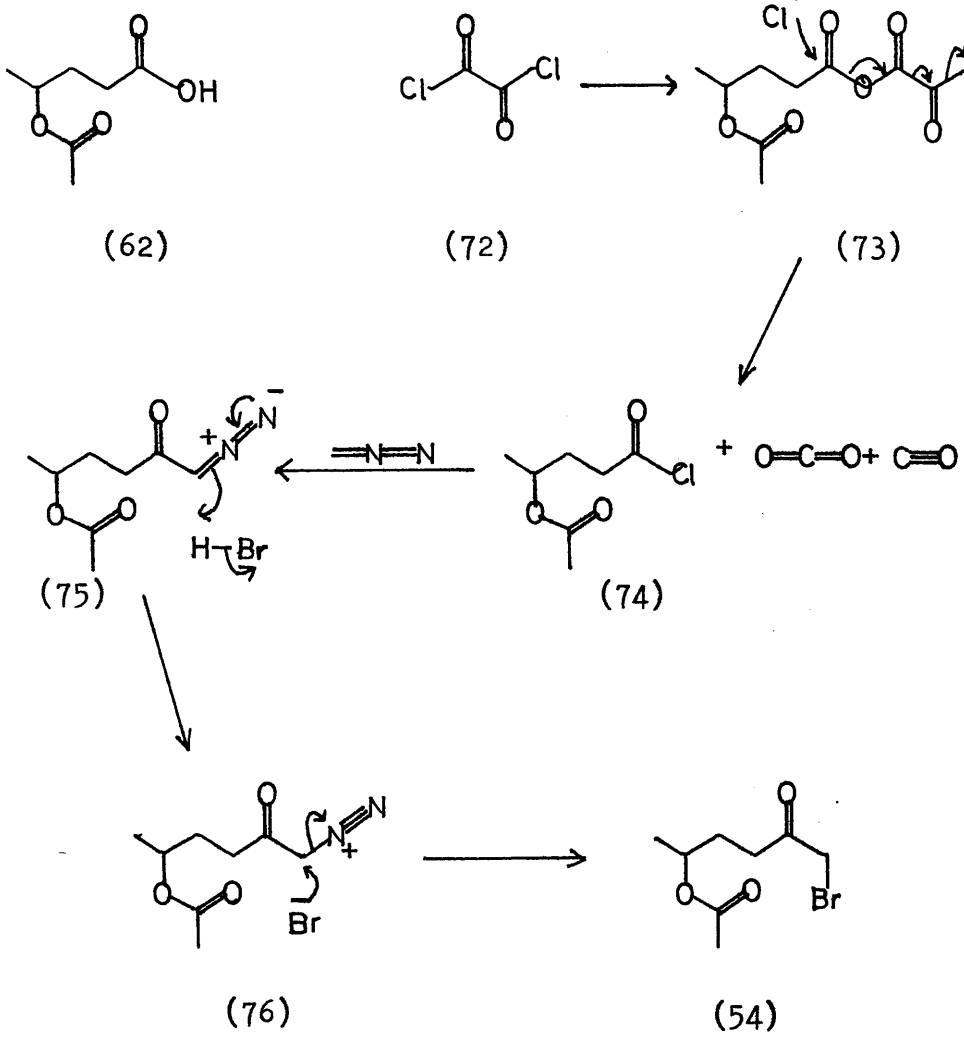
(6) (49) (50)



(7) (51) (52)

(53)

Scheme (10)



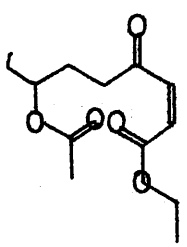
(62) (72) (73)

(75) (74)

(76) (54)

Scheme (13)

(77) (78)



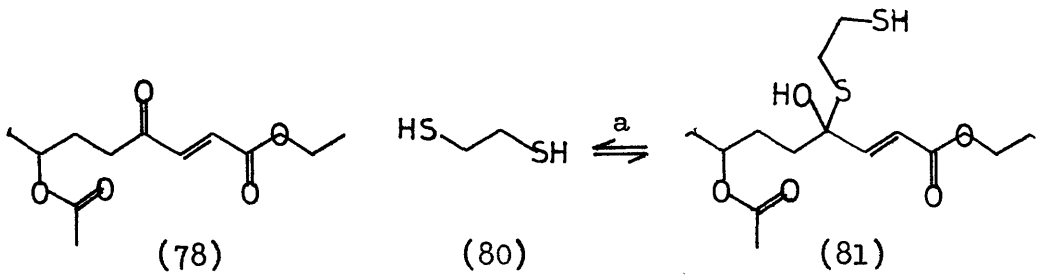
(79)

ure from the filtered reaction mixture at temperatures below 140°C: Temperatures above this were accompanied by very rapid decomposition of the product.

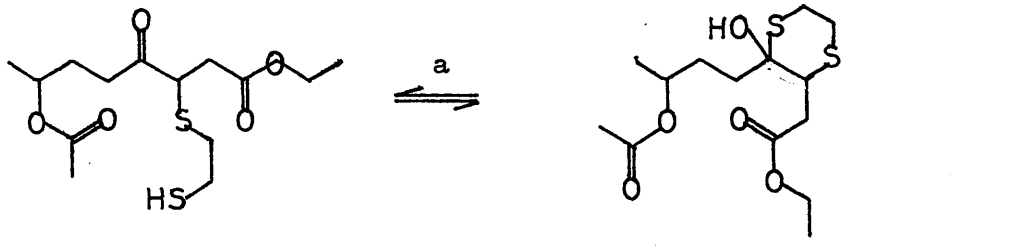
In 1960, Weygand and Bestmann[12] published in their review of diazoketones an extract from 'Neuere Methoden der Praparativen Organischen Chemie 1', Verlag Chemie, Weinheim 1944 S359, describing the formation of bromomethyl ketones from carboxylic acid chlorides. The process involved treating an ethereal solution of the carboxylic acid chloride (cf 35 in Scheme 5) with diazomethane to furnish the diazoketone (cf 36). Hydrobromination of the diazoketone solution with dry hydrogen bromide caused evolution of nitrogen and production of the bromomethyl ketone (cf 33).

Bestmann[13], in 1963, furthered this one carbon homologation by another two units by reacting the general bromomethyl ketone (67) with a several fold excess of methoxycarbonylmethylenetriphenylphosphorane (66). They found that in benzene the carbanion (66) displaced bromide from (67) in an S_N2 fashion to produce the phosphonium bromide (68) (Scheme 12) which, with the excess (66) acting as base underwent a 1,2 elimination to the enedione (69). The triphenylphosphine also produced was precipitated as the insoluble phosphonium bromide (70) on addition of methylbromoacetate (71).

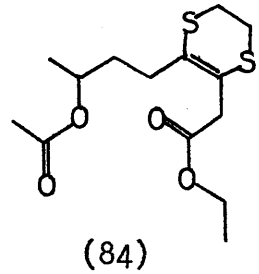
Using the same conditions acetoxy acylchloride (74), formed from the parent acid (62) and oxalyl chloride (72), via (73), was successfully converted to the bromomethyl ketone (54) (Scheme 13). This product was not purified but was carried successfully on into the abnormal Wittig reaction of Bestmann[13], with ethoxycarbonylmethylenetriphenylphosphorane (77) to give in 54% yield



(78) (80) (81)



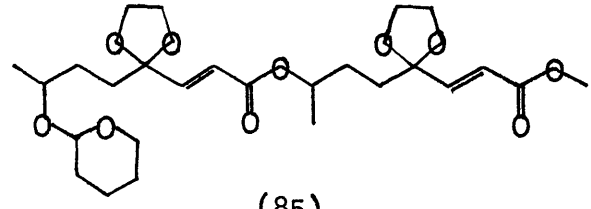
(83) (82)



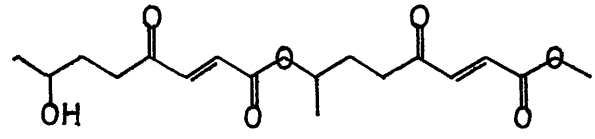
(84)

a=BF_3-etherate

Scheme (14)



(85)

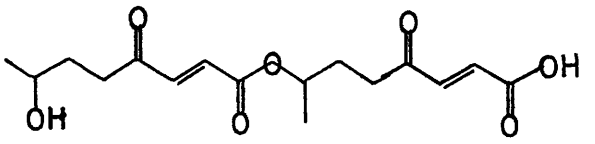


(86)

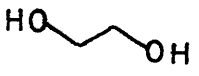
from 4-acetoxypentanoic acid (62), the desired trans enedione (78) as a pale yellow oil. This molecule, however, suffered the restriction of having to be kept in the dark as it isomerised in light to its cis isomer[14] (79).

The first deviation from the projected scheme occurred when an attempt was made to protect the ketone with ethanedithiol (80). Standard thioacetalisation conditions gave rise to an unstable product whose rather poor nuclear magnetic resonance spectrum showed the absence of a double bond and a methylene pattern (δ 1.8) characteristic of the ketonic carbon's ability to deshield being reduced. Deuterium exchange technique revealed no exchangeable proton. This product's permanent state of impurity forbade its characterisation but the main peaks of the spectrum can be accounted for by a structure (84) derived possibly by the route depicted in Scheme (14).

A proper synthetic plan, of course, should not be so rigid that failure of a particular step would terminate the entire project. Instead, it should be sufficiently manipulative as to allow most problems to be bypassed albeit by less desirable routes. An alternative to the thioacetal was its oxygen analogue, the acetal: Admittedly inferior to the thioacetal in that it could not be used independently of the tetrahydropyranyl moiety, it was recognised that this distinction was really only important in preparing the long acycle (85) for cyclisation. Conditions for removing the alcohol blocking group, itself an acetal, would probably remove the acetals protecting the ketones in the oxygen analogue (85) to give the cyclisation precursor (86). It was hoped that the enedione moieties would survive the non aqueous



(2)



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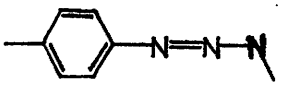

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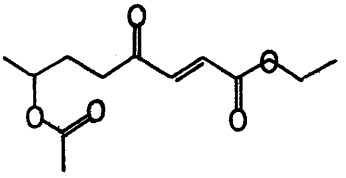

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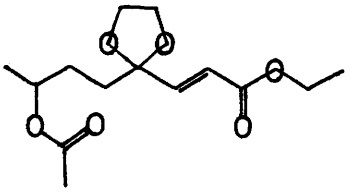

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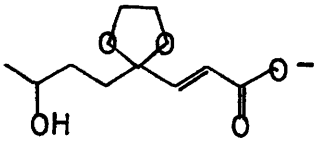

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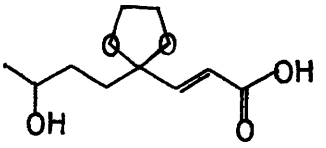

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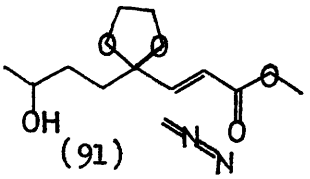
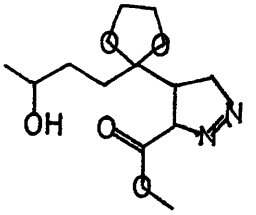


(91)

(92)



(93)

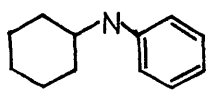
Scheme (15)

cyclisation conditions or those required to prepare and cyclise the parent acid (2).

Happily, ethylene glycol (87), under acid catalysis, added to the abnormal Wittig product (78) to afford the acetal (88) in reasonable yield. This diester was subsequently hydrolysed in four hours using .1 Normal sodium hydroxide, and a little heat, to the hydroxy carboxylate (89). After careful acidification at $0^{\circ}C$, the very water soluble hydroxyacid (90) was rapidly salted out into ether and, to prevent polymerisation, immediately methylated to the methyl ester (91).

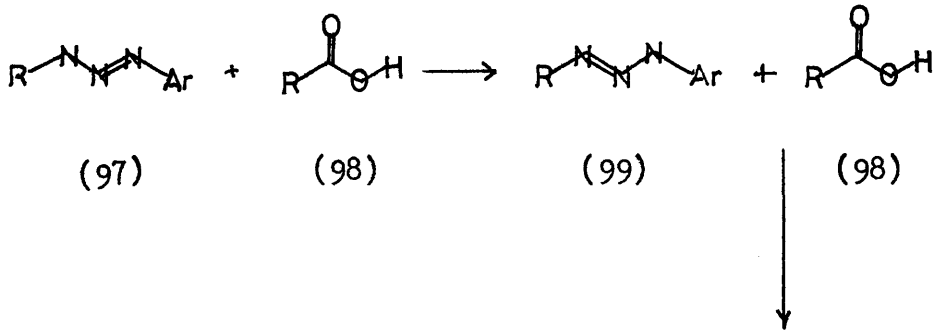
Two methylation techniques were attempted. The most obvious neutral methylation reagent, diazomethane,(92), was tried and a fair yield of the methyl ester was obtained; unfortunately, significant amounts of a more polar product were also obtained, the quantity increasing with length of exposure of the hydroxyacid to diazomethane. It is believed that the latter product is (93), formed by addition of diazomethane to the conjugated double bond[14] (Scheme 15).

In efforts to circumvent this problem, another methylation reagent, 1-methyl-3-p-tolyltriazene (94),was prepared. White, Baum and Ettel[15] have shown that the alkylation of acids with triazenes is superior to alkylation with diazomethane and other diazoalkanes in view of their crystallinity, stability, and ease of preparation and storage. Further, their alkylations proceed without addition to strained or conjugated double bonds as are often observed in corresponding reactions with diazoalkanes. Many solvents may be used as the reaction media although the reaction rate is greater in non-polar solvents, which solvents, as described by



(95)

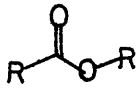
(96)



(97) (98) (99) (98)

(100)

(101)



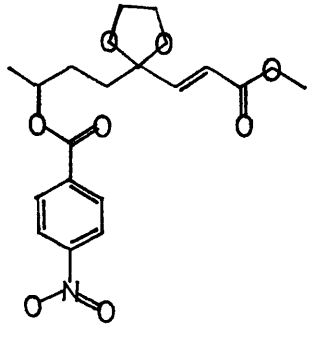
(102)

$N \equiv N$

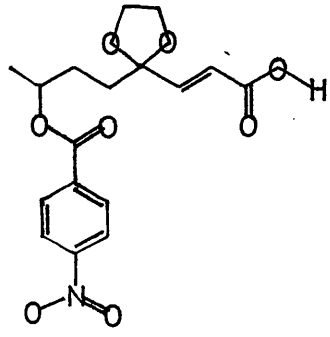
$ArNH_2$

(103)

Scheme (16)



(104)



(105)

White and Scherred[16], also inhibit skeletal rearrangement of the migrating alkyl group. The latter authors showed too that the principal side reactions lead to olefins and, in the case of secondary carbinamines, to secondary amines. Thus, (95) is a by-product of (96).

These reaction characteristics can be accounted for by the mechanism described in Scheme (16).

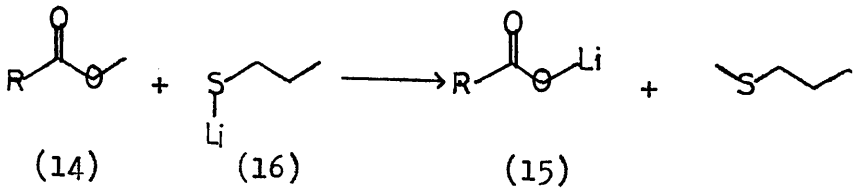
The triazenes have also been used in conjunction with aluminium alcoholate catalysts to alkylate certain alcohols, phenols and thiols.

Despite all these advantages, the triazene (94) reacted with the hydroxyacid (90) producing very little of the desired product (91). Thus, reluctantly, diazomethane was employed forthwith to methylate the hydroxyacid (90).

The hydroxy methyl ester (91), of course, was projected to become one half of the acyclic dimer, and to be the building block for the other half. Note that this satisfies the other condition for a 'two-step' process, i.e. that one half of the dimer be easily derived from the other.

In view of the identical chemical characteristics of the tetrahydropyranyl ether and the acetals, this alcohol blocking group was relegated in favour of the p-nitrobenzoyl ester. Accordingly, after facile p-nitrobenzoylation of the alcohol, attempts were made to secure demethylation of this product (104) to yield the carboxylic acid (105). At hand were two relevant methods.

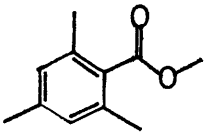
In his studies of the demethylation of carbomethoxy steroidal acetates, Eschenmoser[5] showed that lithium iodide in sym-collidine at 172°C could promote alkyl-oxygen fission at the



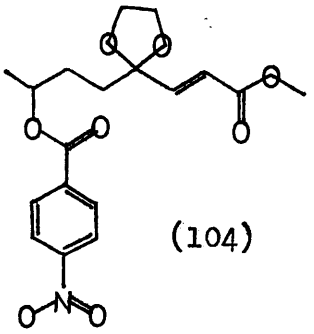
(14) (16) (15)

(14) (17) (15)

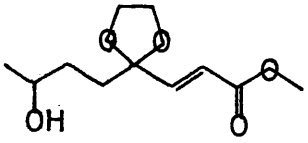
Scheme (3)



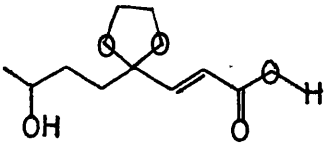
(106)



(104)



(91)



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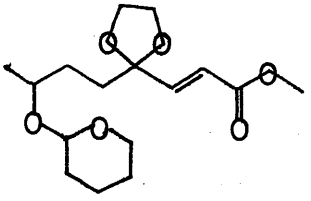


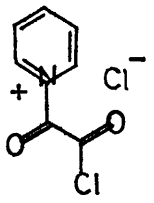
(107)

methyl ester in high yield and without removing the acetate, (Scheme 3). Experiments using 2,6 lutidine instead of sym-collidine were less effective in displacing carboxylate and more effective in removing acetate. Later chemists[18] used dimethyl-formamide as solvent.

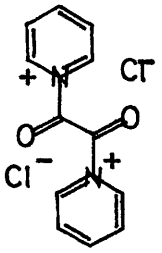
In 1970, Johnson[4] offered a tempting alternative in the form of a lithiumpropylmercaptide/ hexamethylphosphorictriamide couple thereby putting into practice an observation by Vaughan and Baumann[19] that sodiumpropylmercaptide in dimethylformamide effects ready cleavage of esters by an S_N2 process. Use of lith-ium instead of sodium, and hexamethylphosphorictriamide instead of dimethylformamide raised the rate of cleavage of methyl mesitoate (106) by a factor of about fifty seven. Its selectivity appeared similar to lithium iodide in sym-collidine but it used room temp-erature as opposed to 172°C (Scheme 3).

When applied to the structure in question, (104), both of these methods gave a common product in low yield. Diazomethylation of this product afforded the hydroxy methyl ester (91) showing that the product from the reactions was (90) and that the p-nitro-benzoyl group, if not the entire molecule, was unstable to the con-ditions of these reactions, probably as a result of its highly electrophilic carbonyl group.

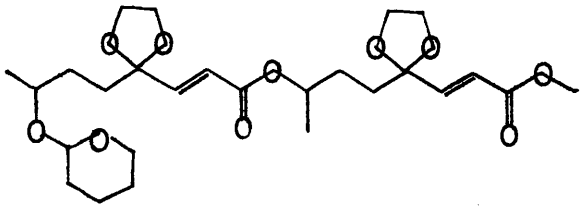
Thus the choice of the tetrahydropyranyl moiety as the alc-ohol protecting group thrust upon us. Using phosphorousoxychloride as catalyst, the hydroxy methyl ester (91) added across dihydropy-ran in moderate yield to give the structure (107). This ester was hydrolysed with 1 Normal sodium hydroxide, then, with the aqueous mixture saturated with sodium chloride at all times, the



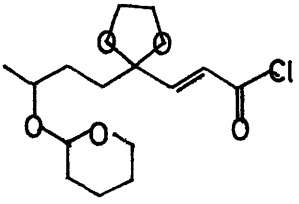
(113)



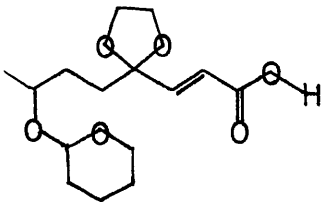
(114)



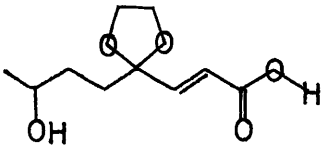
(85)



(115)



(108)



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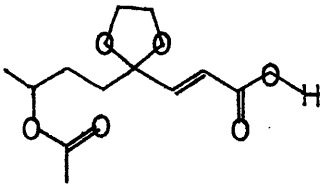
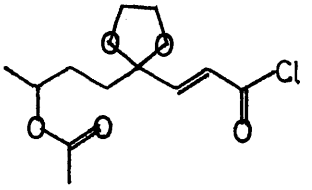
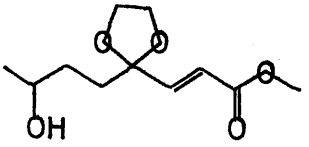


(109)



(110)



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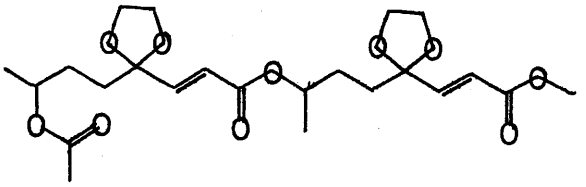

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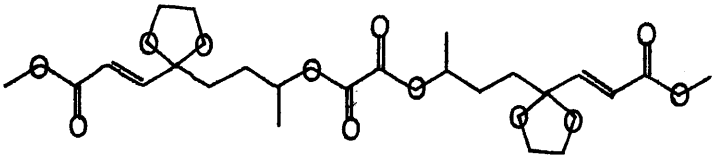
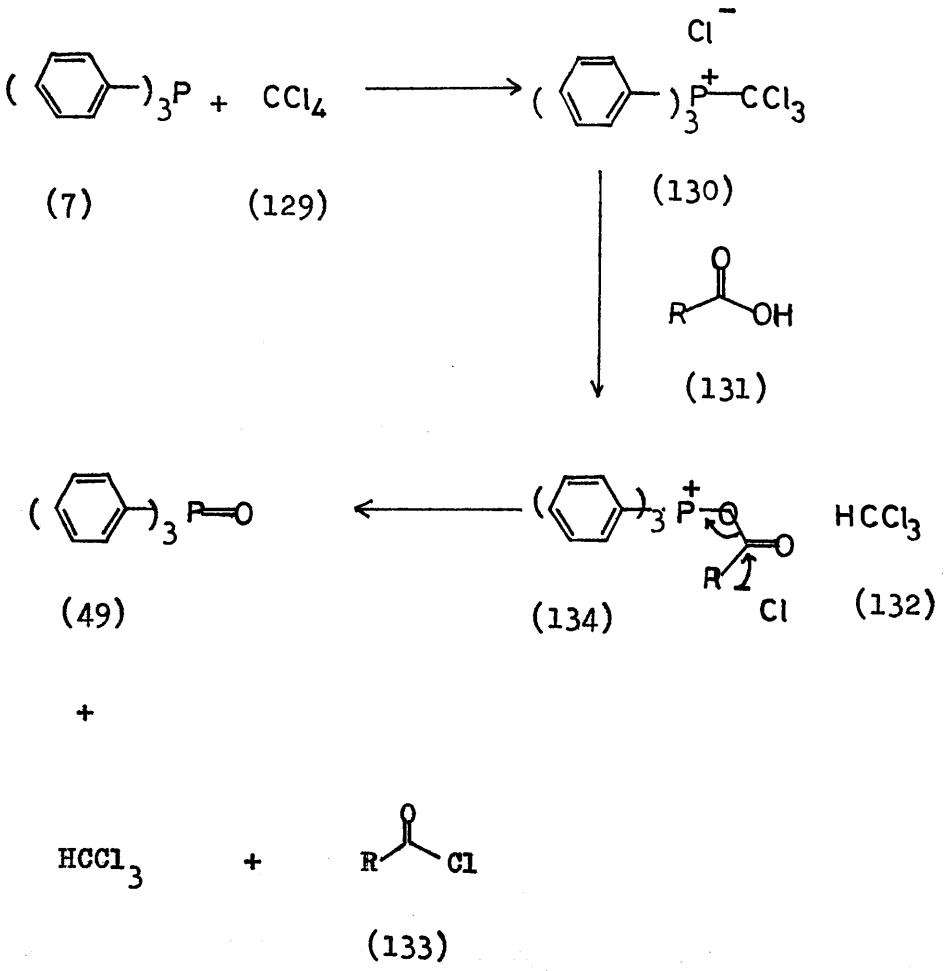


(112)

whole carefully acidified with 0.25 Normal sulphuric acid at 0°C. Immediately the salt saturated aqueous phase was extracted three times with diethylether and small portions of each diazomethylated. Consistently it was found that the first extract was exclusively (108), the second, (108) together with some (90), and the third, (108) heavily contaminated with (90). The first two extracts were combined and chromatographed on a reversed phase Sephadex column to give the pure carboxylic acid (108) in a yield of 50% from the prehydrolysis material.

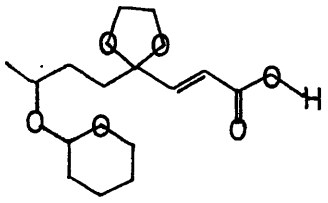
Model studies[14] had shown that (109), when treated with a slight excess of oxalyl chloride in benzene and an excess of pyridine formed the acid chloride (110) which, in chloroform, reacted with the hydroxy methyl ester (91) to give, in about 30-40% yield, the desired dimer (111). It was hoped that the diacetal acid (108) would react accordingly, but experimentation along the same lines produced only a product whose infra-red and nuclear magnetic resonance spectra were consistent with a structure (112). Obviously the involatile oxalyl chloride/ pyridine complex, (113) or (114), had not reacted with the acid (108) but with the subsequently added hydroxy methyl ester (91). In support of this, the same product was obtained on adding the hydroxy methyl ester (91) to a chloroform solution of (113) or (114). Variation of reaction times did not induce the desired coupling to the dimer (85).

Thus other methods were sought which might persuade the carboxylic acid (108) to form its chloride (115); the most attractive appeared to be the triphenylphosphine/ carbon tetrachloride couple. Like so many other aspects of chemistry this reagent couple was developed from a rogue reaction: Kosolapoff[20] in 1947

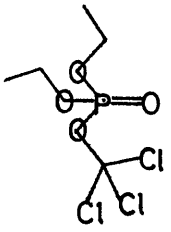


(7) (129) (130) (131) (49) (134) (132) (133)

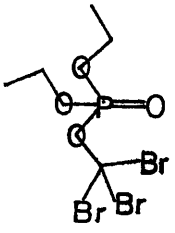
Scheme (18)



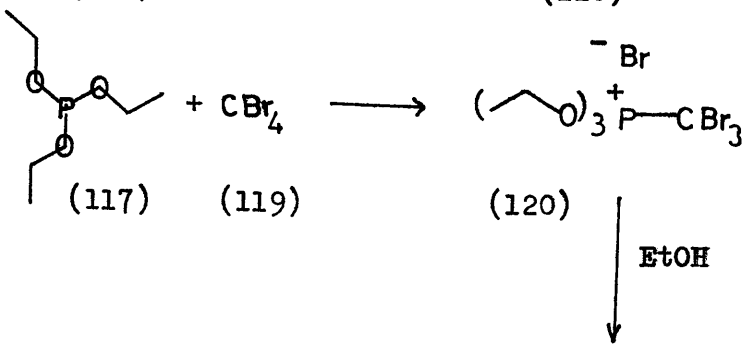
(108)



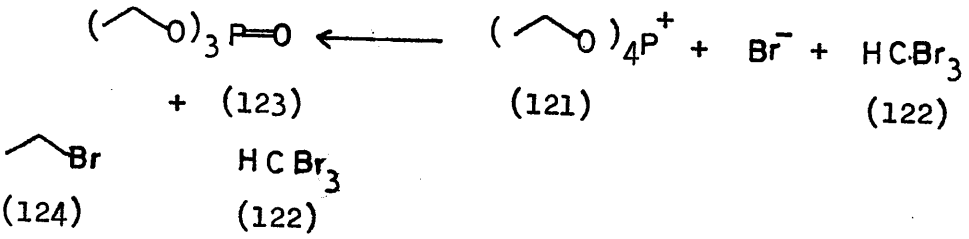
(116)



(118)



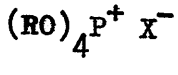
(117) (119) (120)



(123) (121) (122)

(124) (122)

Scheme (17)



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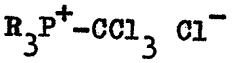

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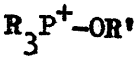
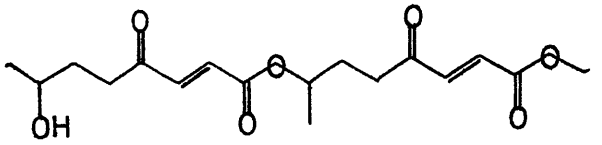


(128)

and Kameii and Egorova[21] in 1946, showed that diethyltrichloromethylphosphonate (116) is formed in high yield by reacting triethylphosphite (117) with an excess of carbon tetrachloride. In attempting to prepare the tribromomethyl analogue (118) Crofts and Downie[22] in 1963 found that the intermediate reacted with ethanol (125) to give ultimately bromoform (122), ethyl bromide (124), and triethylphosphate (123), presumably via the quasi-phosphonium ions (120) and (121). As shown in Scheme (17), (121) appears to undergo an Arbusov reaction. Triethylphosphite and ethanol underwent a similar reaction with carbon tetrachloride (129). Recognising the potential use of this reaction and of the disadvantages of having intermediates like (126) where final Arbusov reactions would produce mixed halides the authors reasoned that phosphines might react similarly via (127) to intermediates like (128), where only one Arbusov reaction can take place to give one halide.[24] Indeed they found that analogous reactions using triphenylphosphine, rather than triethylphosphite, proceeded rapidly at moderate temperatures with primary or secondary alcohols to give the halides in good yield.

In 1966 Lee[23] advanced the usefulness of this reaction by showing that carboxylic acid (131) hydroxyl groups could replace ordinary hydroxyl groups to give acyl halides (133) via (134) also in good yield (Scheme 18).

Rather distressingly, when applied to the tetrahydropyranyl carboxylic acid (108) this reaction, as judged by infra-red spectroscopy, failed to produce significant amounts of acyl chloride even after extended reaction periods. Either the bulky tetrahydropyranyl group is blocking access to the carboxyl carbonyl group or



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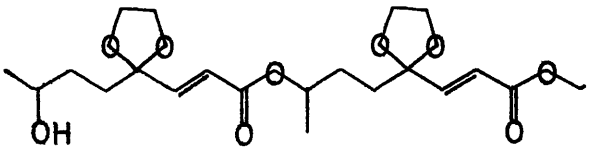

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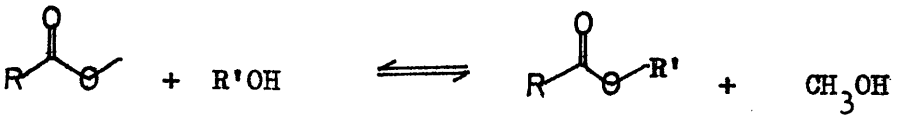
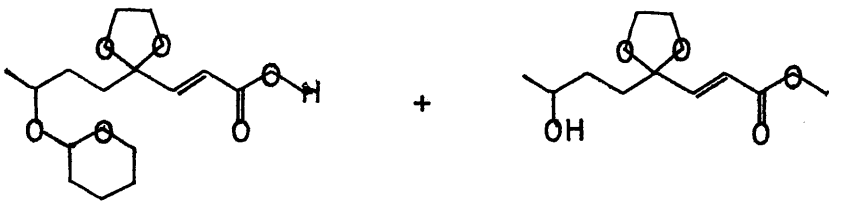
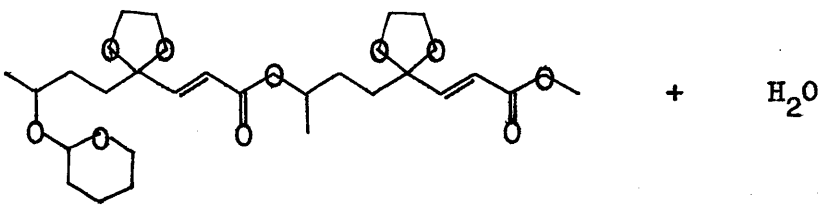


(136) (137) (138) (139)

Scheme (19)



(108) (91)



(85) (140)

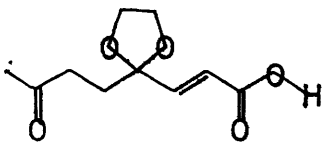
Scheme (20)

the carboxylic acid is being stabilised by hydrogen bonding into the tetrahydropyranyl group.

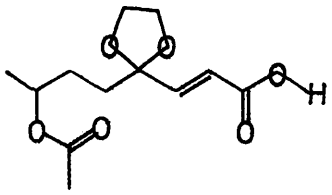
Whilst considering the requirements for making this coupling a reality it seemed a good idea to employ an equilibrating system where the last step, forming the dimer, could be made irreversible. Thus even if the normal equilibrium did not favour the product it could, thereby, be dragged to that end. This, indeed, was the very same principle by which it was hoped to cyclise the long acycle (86) or its diacetal (135) using molecular sieves.

How this principle could be put into practice was first shown by Roelofsen, Hagendoorn, and van Bekkum[6] in their paper entitled "Ester Interchange Technique using Molecular Sieves". They observed that, although uniformly pore-sized adsorbents were widely used for separating purposes, little data could be found in the literature concerning the use of these molecular sieves in shifting chemical equilibria by selective adsorption of one of the products. Upon studying the equilibrium depicted in Scheme (19), rapidly established by alkoxide catalysis, they found that the reaction proceeded smoothly to completion by adsorbing the methanol (139) selectively on a suitable molecular sieve. Investigations further showed that the use of sieve type 3A permitted reaction of any alcohol R'OH (137), use of type 4A prohibited R'OH as ethanol, and that type 5A was well suited for R'OH as a secondary or tertiary or branched primary alcohol.

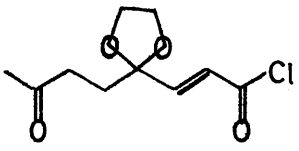
It was therefore considered that if an acid catalysed equilibrium (Scheme 20) could be set up, the above principle might be applied and the water (140) removed from the reaction media by a



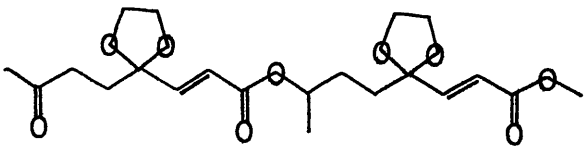
(142)



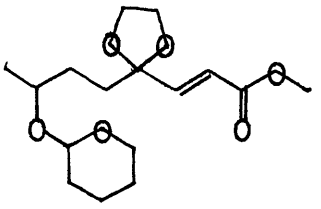
(109)



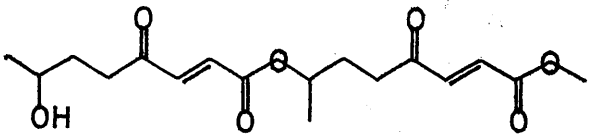
(143)



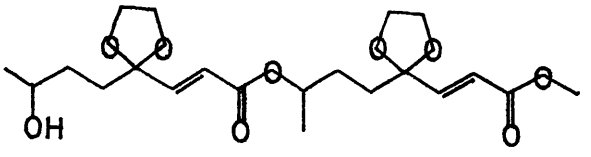
(144)



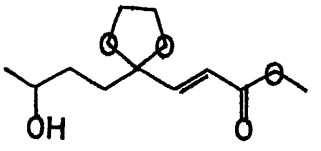
(107)



(86)



(135)



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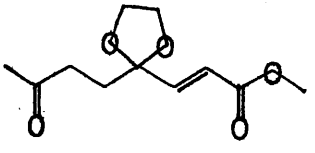


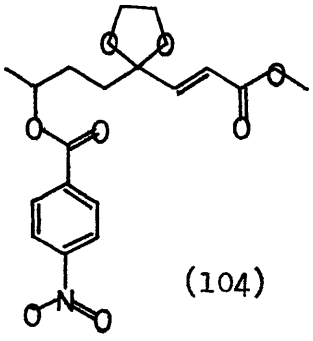
(141)

type 3A molecular sieve. The attempted experiment used dried protonated Amberlite 120 resin as acid catalyst and Linde type 3A molecular sieves in dry benzene in the presence of two control experiments, one using only sieves, the other only resin. After six days the only distinct product, from the experiment using both sieves and resin, was, tentatively, the transesterification product (107).

It was at that time considered that in view of the uncertainty involved in the final cyclisation and of the possibility of a protracted experimental study of the first coupling to the acyclic dimer, that time would be better spent on a rapid non-stereospecific synthesis of the cyclisation precursor,(86) or (135), to examine its potential.

Thus the hydroxy methyl ester (91) was oxidised, using Cornforth's reagent, to the keto methyl ester (141). The Pfitzner Moffat oxidation[25] was also employed here having the advantage of speed and of a much easier work-up. The keto methyl ester (141) was hydrolysed in the usual way to the crystalline carboxylic acid (142). Happily this acid behaved similarly to the acetoxy acid (109) used in model studies and reacted, via its chloride (143), with the alcohol (91) to furnish the acyclic dimer (144) in fair yield. Borohydride reduction of this ketone (144) proceeded smoothly and with no complications save some purification to (135).

Before long it was realised that the decision to adopt a rapid non-stereospecific synthesis had been well founded for no experimental conditions could be found to induce this molecule (135) to cyclise. No products were obtained from any attempts to



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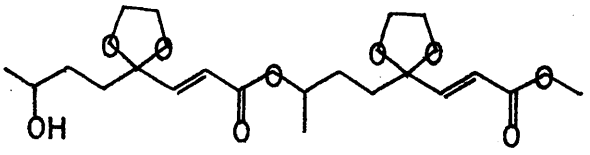

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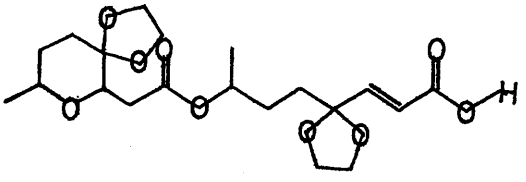

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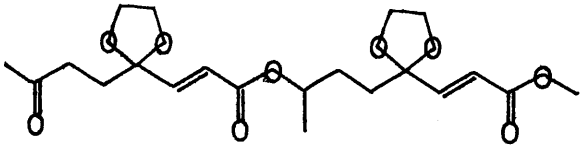
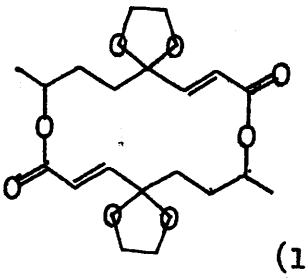
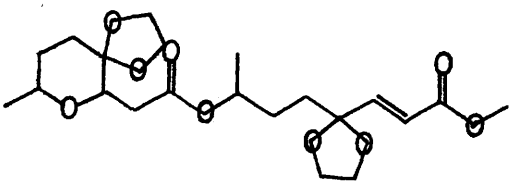


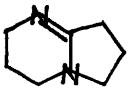
(144)



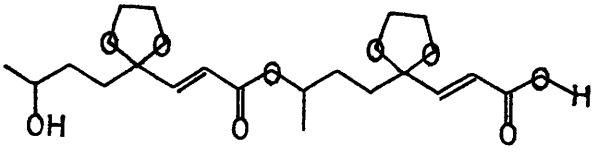
(145)



(146)



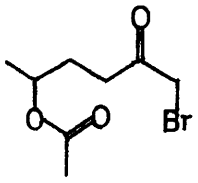
(147)



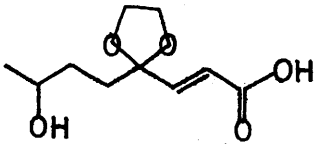
(148)

form the macrocycle (145) other than the intramolecular Michael adduct (146) from the 1,5 diazabicyclo (4,3,0) non-5-ene (147)/ sieve catalysed reaction. It will be observed in the experimental section that amongst catalysts used were sodium methoxide, sodium hydride, and potassium tert-butoxide . It had been hoped, albeit in vain, that either sodium or potassium would form a template around which the molecule would fold to enhance the possibility of cyclisation.

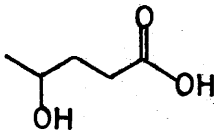
Attention was thus drawn to the hydroxy acid (148) and to the prospects of inducing this molecule to cyclise to the diacetal of pyrenophorin (145). The lack of success, however, of the alkyl-oxygen fission of the methyl ester (104) by iodide and mercaptide made its application to (135) an unhealthy proposition so mild hydrolysis conditions were sought after to obtain (148). Using (146) as a model compound it was found that the molecule could be successfully demethylated to (149) using a five-fold excess of decinormal sodium carbonate, or much less cleanly but faster using decinormal sodium hydroxide. The same conditions applied to the ketone (144) and the alcohol (135) were totally unsuccessful.



(54)



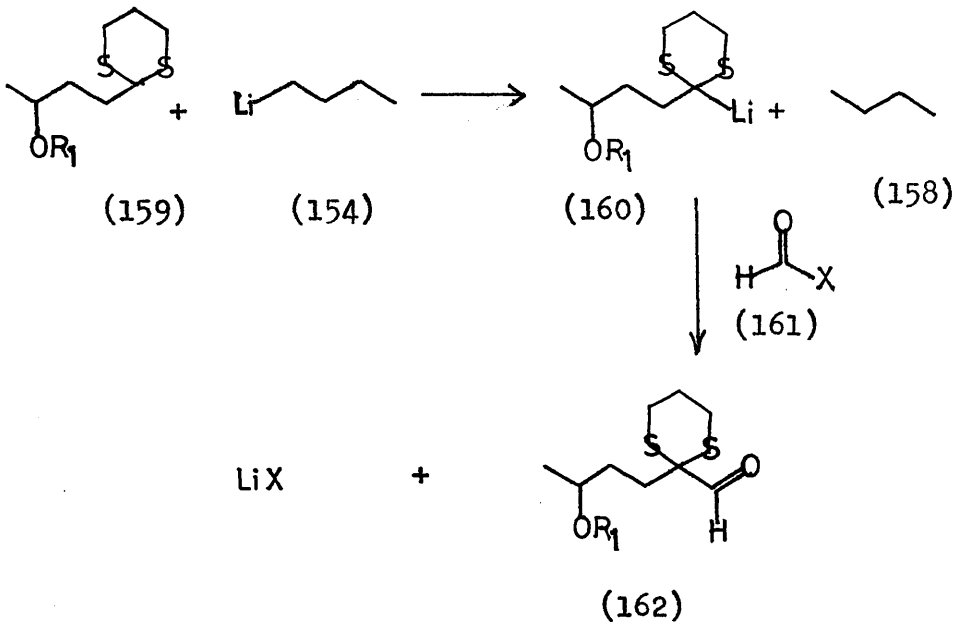
(90)



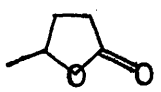
(9)

In retrospect the methyl ester had not behaved as once hoped in the molecular sieve catalysed reactions; furthermore, conditions for its removal were too harsh for the rest of the molecule to remain intact. Its replacement by another more labile group was inevitable but the attendant problem was at what stage in the scheme was this group to be introduced? The route to the bromomethyl ketone (54) required the protection of the alcohol as its acetate whose alkaline removal conditions spoiled any chance of inserting a base labile carboxyl protecting group at the abnormal Wittig stage. The carbonyl acetalisation step likewise prohibited the presence of an acid labile protecting group. The next point of entry for the new carbonyl protective function was at the realisation of the hydroxyacid (90). The problems associated with this structure were similar to those of 4-hydroxypentanoic acid (9) in that the molecule (90) was prone to polymerise under carboxyl activation. Benzylation of its carboxylate was expected to give experimental difficulty and p-bromophenacylation was rendered unsuitable in view of its subsequent removal conditions. Any later entries than this hydroxyacid (90) would have engendered a rather tedious, and therefore unsavoury, labyrinthine synthetic scheme.

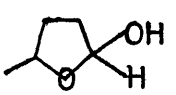
It will also be recalled that the first esterification in the 'two-step' project had been so far unsuccessful using designed precursors though this was not regarded as an insurmountable difficulty. Thus, it was judged that a more direct and flexible synthesis required formulation.



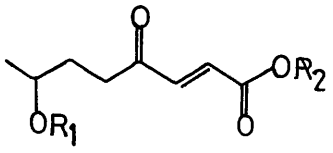
Scheme (22)



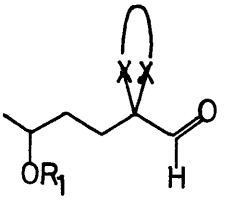
(10)



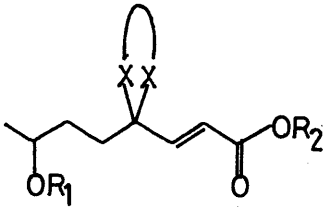
(163)



(13)



(151)



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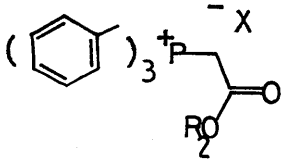

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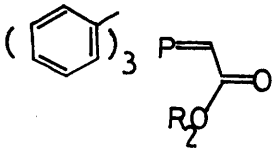

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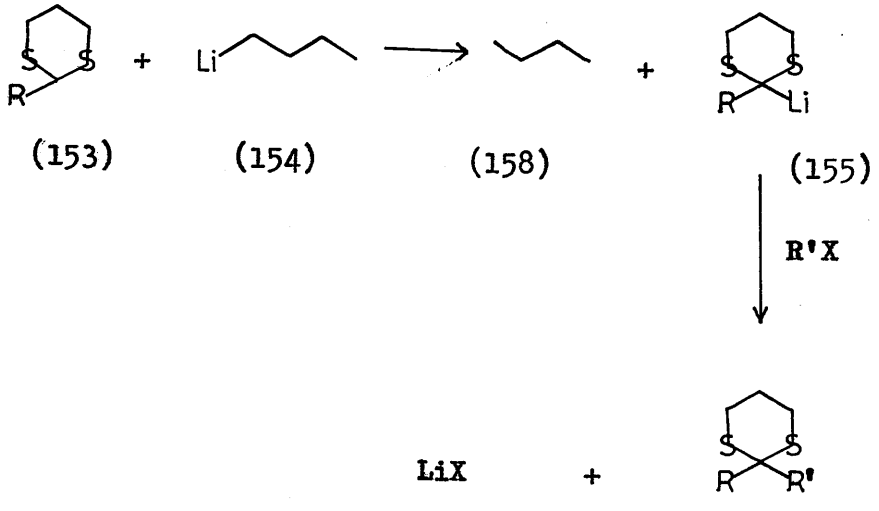


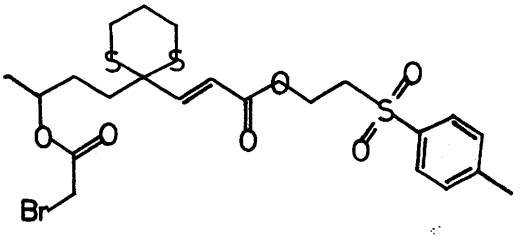
(153) (154) (158) (155)

Scheme (21)

The failure of the 'one-step' process[14] allowed all efforts to be concentrated on the 'two-step' version: As will be remembered, the requisite molecule for this process was regarded as (13), but the high likelihood of this molecule or a derivative being exposed to aqueous acid or base now forced it to be replaced by the more general assembly (150) where all the protecting groups, R_1, R_2, -X-X-, were independently removable.

Applying the usual methodology we imagined the double bond formed from a Wittig condensation of (151) and (152). This proved tobe an extremely interesting fragmentation, because letting -X-X- be $-S(CH_2)_3S-$ opened the door to an invaluable aspect of synthon chemistry lately enunciated by Seebach and Corey[26] - the 1,3 dithiane synthon. Thus, as in Scheme (21) the general propylenethioacetal (153) could be lithiated by butyllithium (154) to (155), and subsequently alkylated to (157) by (156). Hence analogy suggested that the aldehyde (162) be derived from a reaction scheme such as Scheme (22). The thioacetal (159) is merely a masked version of γ-valerolactol (163), the initial reduction product of γ-valerolactone (10). The triphenylphosphorane (152) was to be generated in the normal way.

The restraints on protecting groups were now as follows. The very nature of the scheme demanded that the ketone's blocking group be the propylenethioacetal. The lithiation step required R_1 to be stable to butyllithium, which limited the choice of R_2 to base labile moieties, but it was noted that R_2 had to be sufficiently base stable to allow for ylid formation from the deprotonation of the phosphonium salt (164). After much scanning of the literature a protecting group developed by Miller and Stirling[27]



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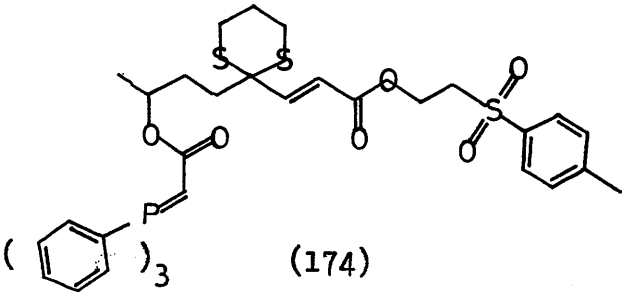

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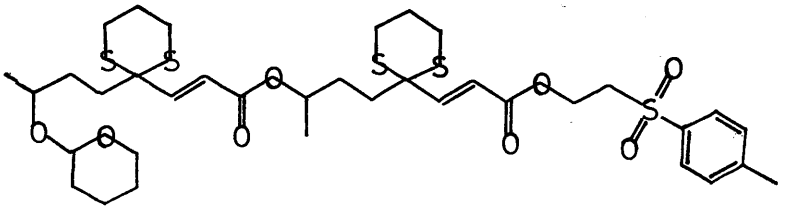

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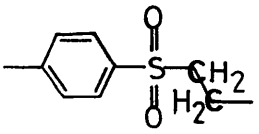

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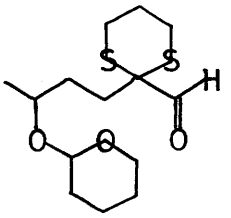

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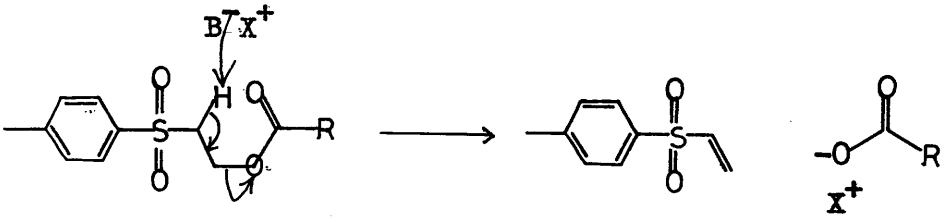
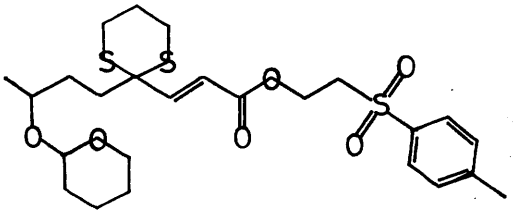


(166) (167) (168)

Scheme (31)



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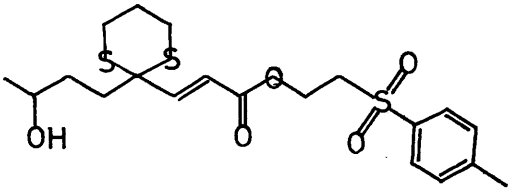

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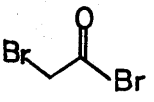

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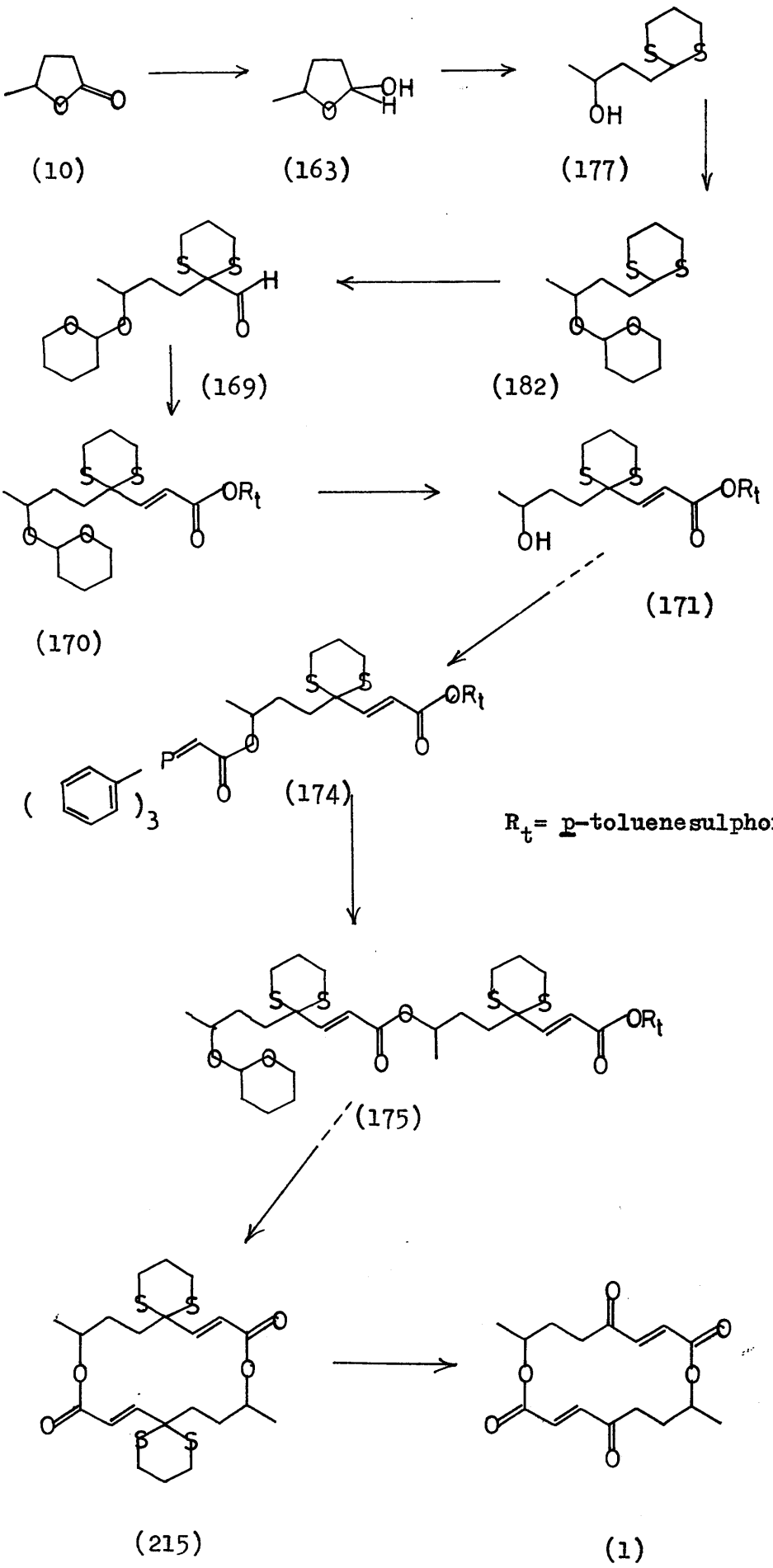
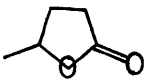

(10)
(163)
(177)
(169)
(182)
(170)
(171)
(174)
(175)
(215)
(1)
R_t= p-toluenesulphonylethyl.

Route B

offered itself for scrutiny. Describing it as the p-toluene-sulphonylethyl group (165) these authors had shown that the ester (166), in dioxan, when treated with 10% excess of uninormal aqueous sodium hydroxide, gave, in three minutes, an 80 to 90% yield of free acid after work up. It was also shown to be removable with potassium cyanide or sodium carbonate (Scheme 31). In view of this enhanced base lability over the methyl ester it was decided to include this group in the latest projected synthetic scheme. Again R_1 was chosen to be the tetrahydropyranyl fragment.

Because of the presence of the aldehyde (169) it was realised that a ready alternative to the first esterification rendered itself available. Thus removal of the tetrahydropyranyl grouping from (170) would give the alcohol (171) which might react with bromoacetyl bromide (172) to furnish (173). If an ylid, say (174) could be formed from (173) then its reaction with the aldehyde might result in the desired dimer (175).

With this alternative in mind the new projected synthesis, shown collectively in Route B, was embarked on.



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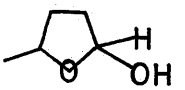

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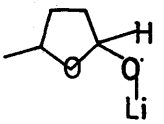
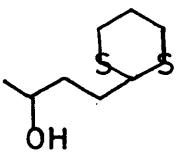
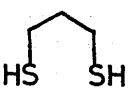


(176)



(177)

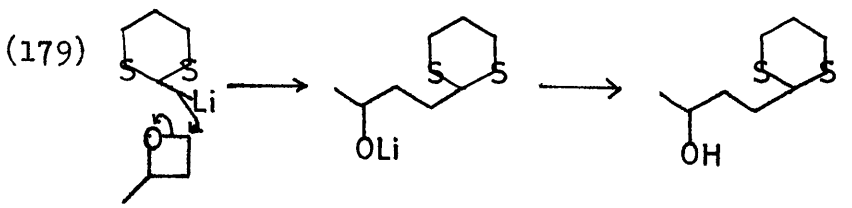


(178)

The literature was pregnant with ways of reducing lactones to lactols but the most convenient method was described in a paper by Arth[28]. Citing γ-valerolactone as an example, his technique involved the addition of one equivalent of lithium aluminium hydride in dry tetrahydrofuran, to a solution of valerolactone in the same solvent, at about -15°C, thereafter leaving the mixture to warm up to room temperature over one hour. Despite many variations in temperature, reaction time, rate of addition of hydride and mode of work up this experiment could not be repeated in these laboratories.

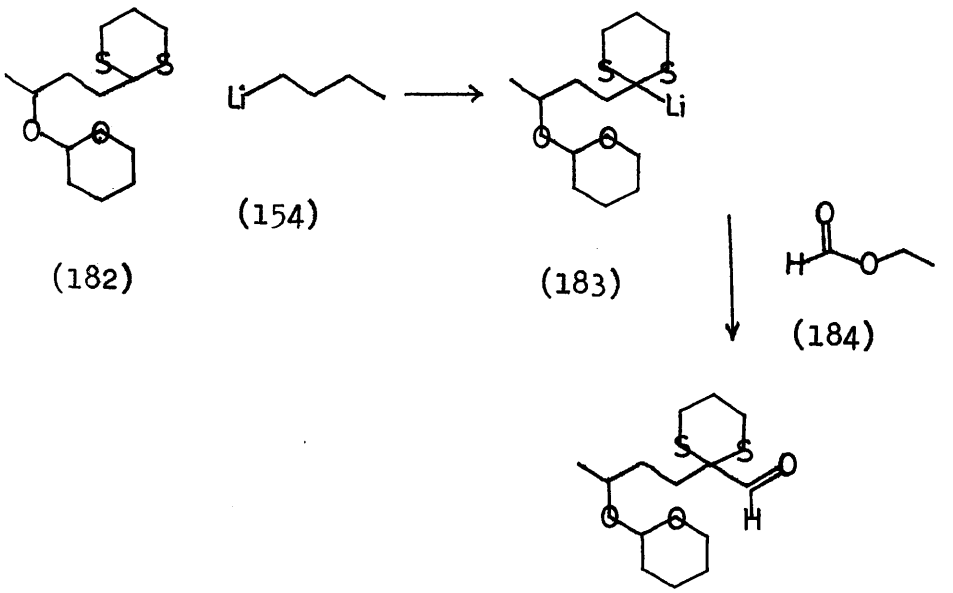
In 1963, however, Zakharkin[29] in the U.S.S.R. showed that acyclic esters could be reduced to aldehydes using an equivalent of sodium aluminium hydride, and, indeed, using their conditions, with a modified work up, γ-valerolactone (10) was successfully reduced to γ-valerolactol (163).

The criterion of these reductions is that the initial reduction product (176) be less soluble in the reaction medium than the starting lactone. And, since homogeneous reactions are faster than heterogeneous ones, the lactones would be preferentially attacked by the soluble hydride. It is well known that the lithium-oxygen bond is more covalent in nature than the sodium-oxygen bond making lithium alkoxides more soluble in organic solvents than their sodium counterparts; thus it would be expected that the reduction of γ-valerolactone with sodium aluminium hydride would be easier than with lithium aluminium hydride. The lactol thus obtained was not normally purified but was immediately converted to its 1,3 propylenethioacetal (177) using 1,3 propanedithiol (178) and boron trifluoride etherate in chloroform solution. The same



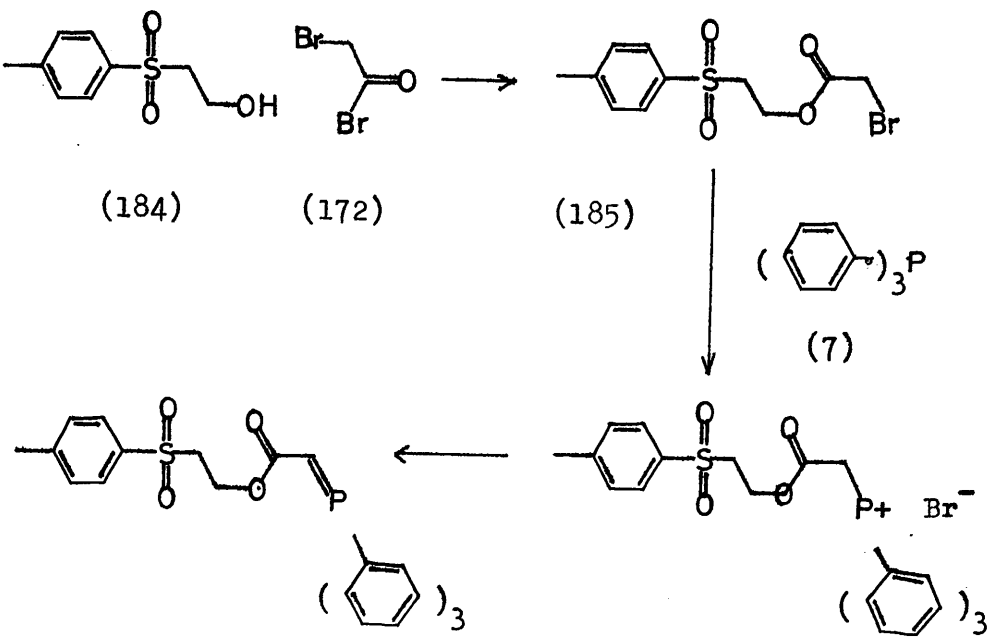
(179) (180) (181) (177)

Scheme (23)



(182) (154) (183) (184) (169)

Scheme (24)



(184) (172) (185) (7) (186) (187)

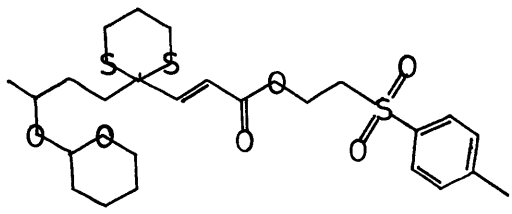
Scheme (25)

molecule (177) was also synthesised by opening 2-methyloxetane (180) with 2-lithio-1,3-dithiane (179), the anion preferring to attack at the less hindered carbon atom to give predominantly, the secondary alcohol (Scheme 23). The latter method, of course, would be of less value in any synthesis using optically active precursors.

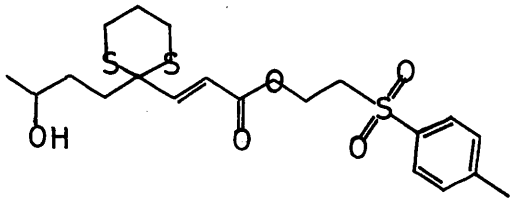
Again, without purification, the alcohol was converted to its tetrahydropyranyl ether (182) in the usual way and purified by column chromatography on silica gel. The purified product (182) was lithiated to (183) using n-butyllithium in dry tetrahydrofuran, after five hours at -30°C, and immediately formylated to (169) overnight, at -30°C, by addition of a ten-fold excess of ethyl formate (184) (Scheme 24).

The ylid (187) was readily synthesised by reacting p-toluenesulphonylethanol[30] (184) with bromoacetyl bromide (172) in the presence of anhydrous sodium hydrogen carbonate, to remove the hydrogen bromide, and Linde type 3A molecular sieves to remove the water thus produced (Scheme 25). Filtration of the solution and addition of triphenylphosphine saw the precipitation of the phosphonium salt (186) as a pale yellow gum which was subsequently taken up in cold water. In view of the base lability of the alkoxy group the aqueous solution was titrated with 4N sodium hydroxide till the pH began to deviate from neutrality. At this point the precipitated ylid was taken up in ethyl acetate and this solution washed with water till neutral, then brine. The solvent was then removed to leave a 60-70% yield of ylid as a yellow solid.

The aldehyde (169) and the ylid (187) were heated under reflux together in benzene for about 24 hours to give the fully



(170)



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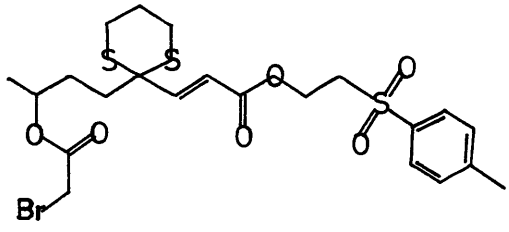
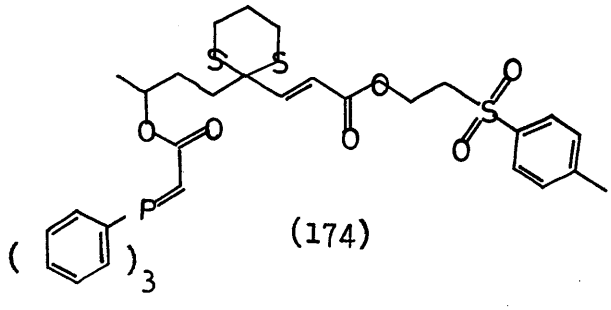
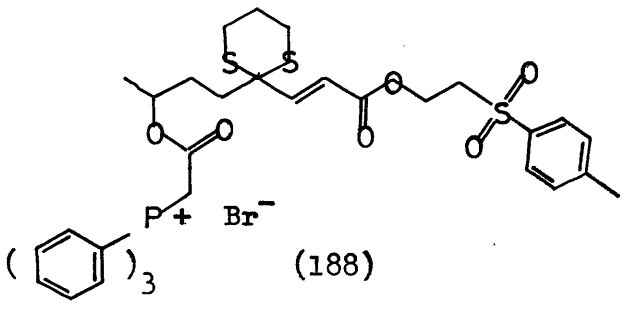


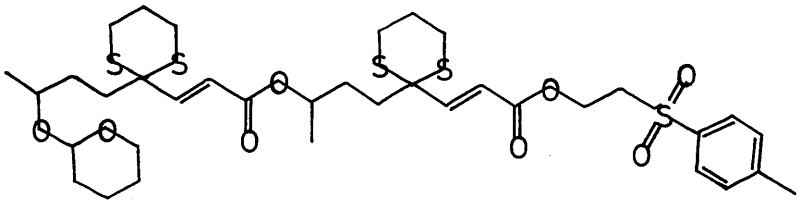
(173)



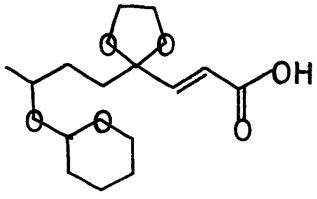
(174)



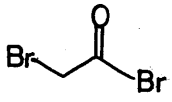
(188)



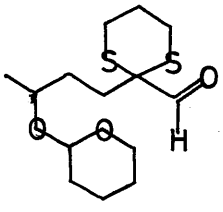
(175)



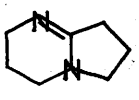
(108)



(172)



(169)



(147)

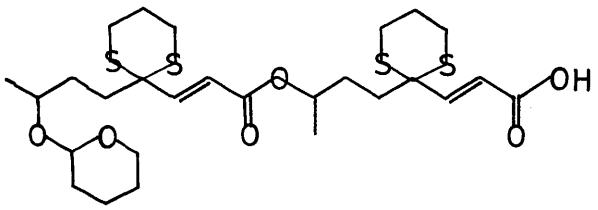
protected monomer (170) as the sole product. The trans stereochemistry of the olefinic linkage was defined by the nuclear magnetic resonance spectrum which displayed an olefinic coupling constant of 16Hz.

The success of the Wittig reaction encouraged an attempt at the alternative, described previously, to the hitherto unsolved activation and reaction of the carboxylic acid (108).

As projected, removal of the tetrahydropyranyl ether with methanolic hydrochloric acid gave the free alcohol (171) in excellent yield. Treatment of this alcohol (171) in dry benzene with bromoacetyl bromide (172), sodium hydrogen carbonate, and Linde type 4A molecular sieves furnished, after filtration, a solution of the bromoacetate (173) from which, after addition of triphenylphosphine, the phosphonium salt (188) precipitated. A complication arose here, however, because the salt was only slightly water soluble, but, by shaking the very gummy salt with an ethyl acetate /water mixture and titrating with sodium hydroxide as before, an ethyl acetate solution of ylid (174) was eventually prepared.

Refluxing this ylid (174) with the aldehyde (169) in benzene successfully afforded the all trans dimer (175) as the sole product in 35% yield. The applicability of the carboxyl protecting group was immediately examined. Horror of horrors! The removal of the protecting group under the conditions recommended by Miller and Stirling also effected hydrolytic fission of the central ester link.

This problem, however, was circumvented by the adoption of the non-nucleophilic strong organic base, 1,5 diazabicyclo (4,3,0) non-5-ene (147). Using this base in dry benzene, the



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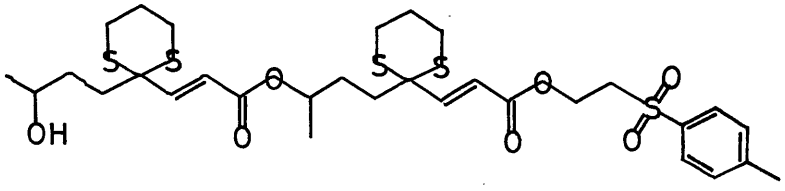

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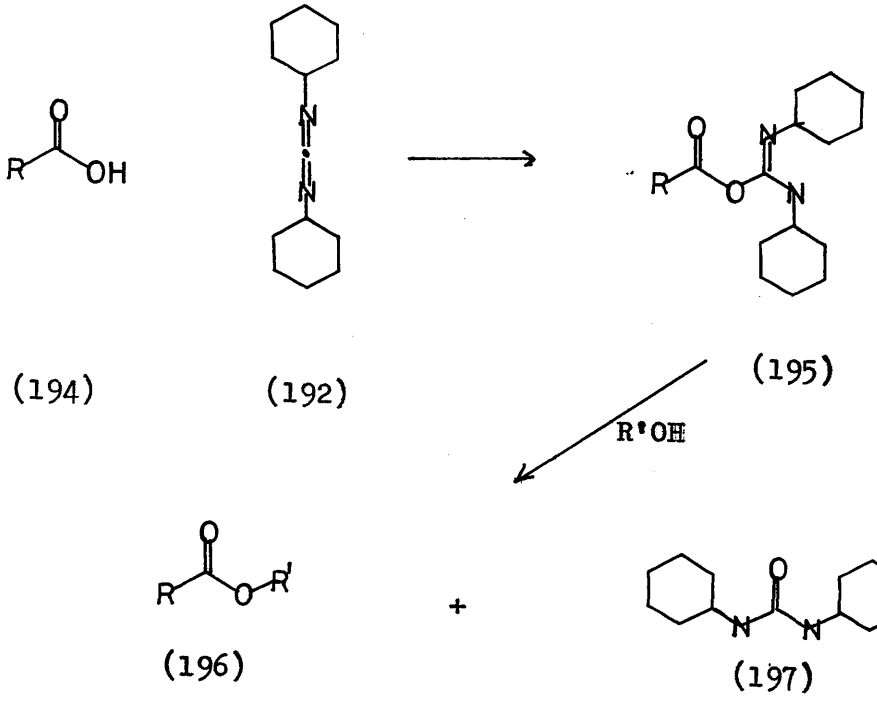
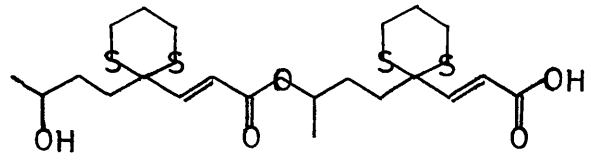


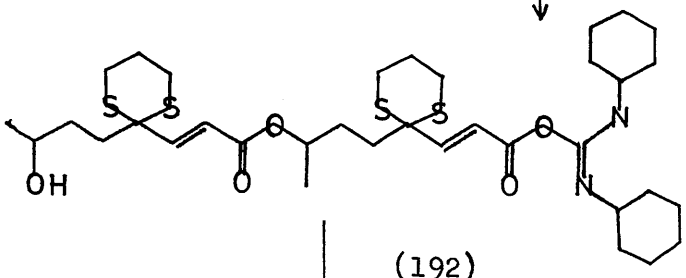
(194) (192) (195) (196) (197)

Scheme (26)

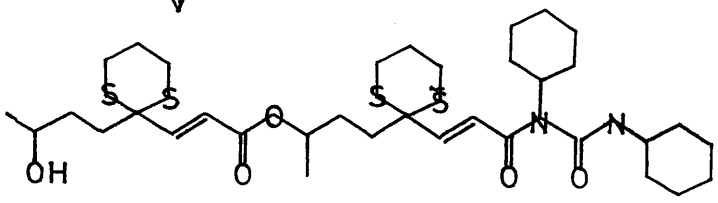


(190)

Dicyclohexylcarbodiimide (192)

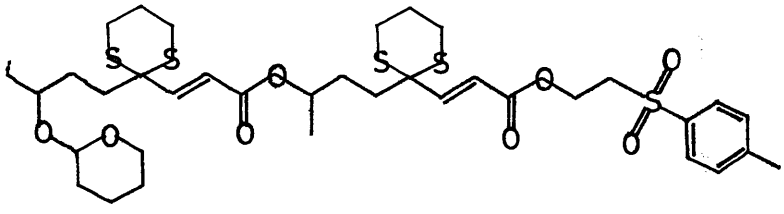


(192)



(198)

Scheme (27)



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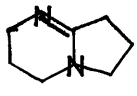

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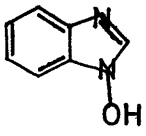


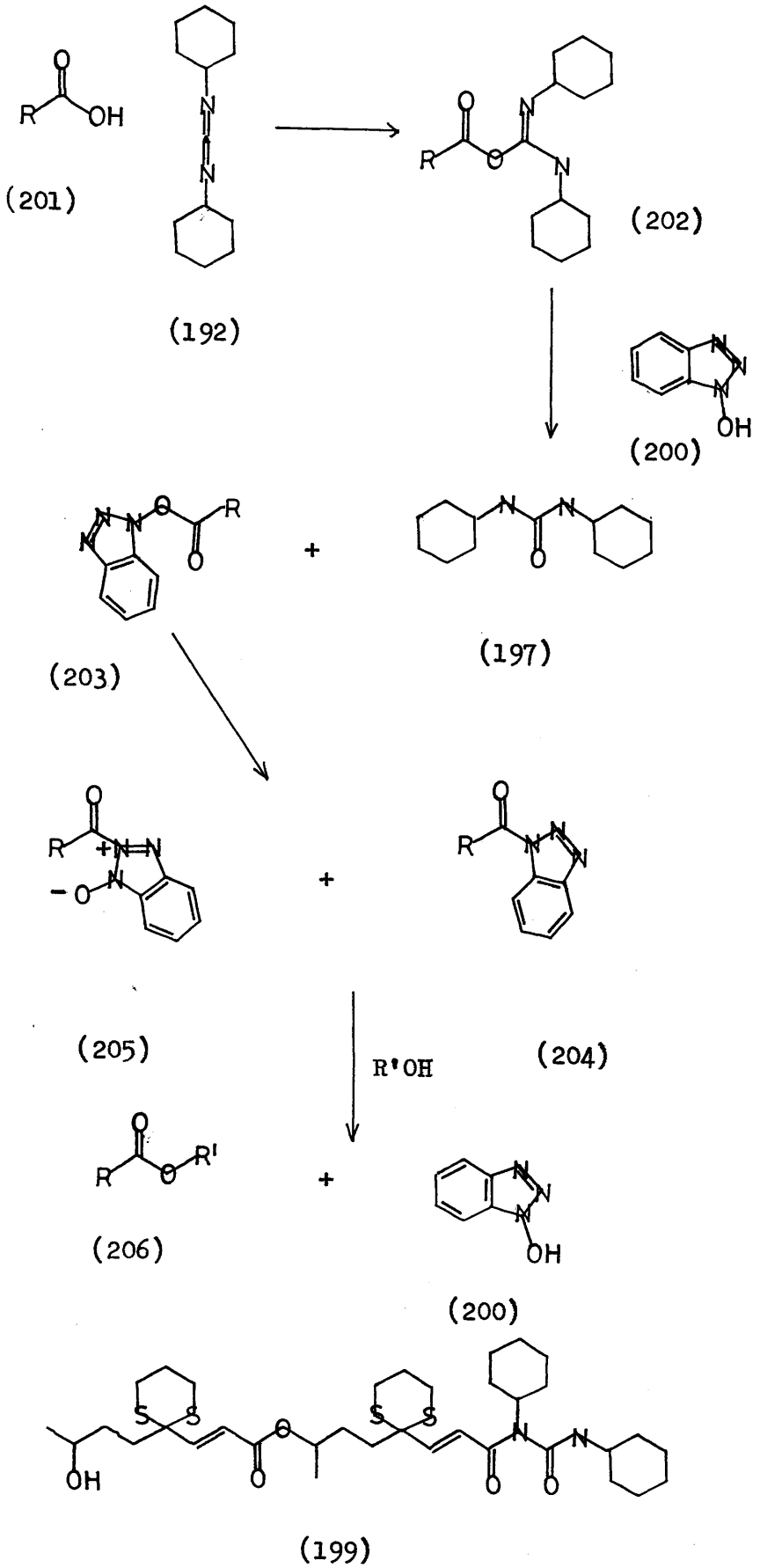
(200)

p-toluenesulphonylethyl protecting group was easily and quantitatively removed at ambient temperature to give after protonation, the acid (189).

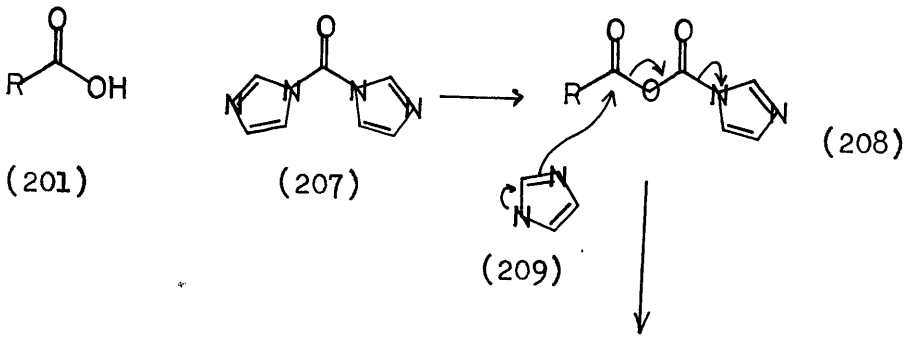
Removal of the tetrahydropyranyl ether from (189) under normal conditions to (190) rather surprisingly took $2\frac{1}{2}$ days to effect. Possibly coiling of the molecule via intramolecular hydrogen bonding stabilised the molecular structure, or merely shielded the protecting group from hydrolytic attack, or even afforded a stable protonated species. The reasonable assumption that the large hydrophobic p-toluenesulphonylethyl group would be loth to interact via coiling with the rest of the molecule in (175) supports the above hypothesis for inversion of the order of protecting group removal saw complete freeing of the alcohol (191) from its ether (175) in about thirty minutes. Using 1,5 diazabicyclo (4,3,0) non-5-ene (147), the ester (191) readily furnished the free hydroxyacid (190) in a few minutes.

How to cyclise the hydroxyacid (190)? The most widely used mild esterifying reagent appeared to be dicyclohexylcarbodiimide[31] (192) which couples the general alcohol (193) and acid (194) according to Scheme (26) to furnish the ester (196) and dicyclohexylurea (197) which is insoluble in most organic solvents and can usually be filtered from the reaction mixture. When applied to the long hydroxyacid (190) the only product obtained was the N-acylurea (199) formed from the activated molecule (198) (Scheme 27).

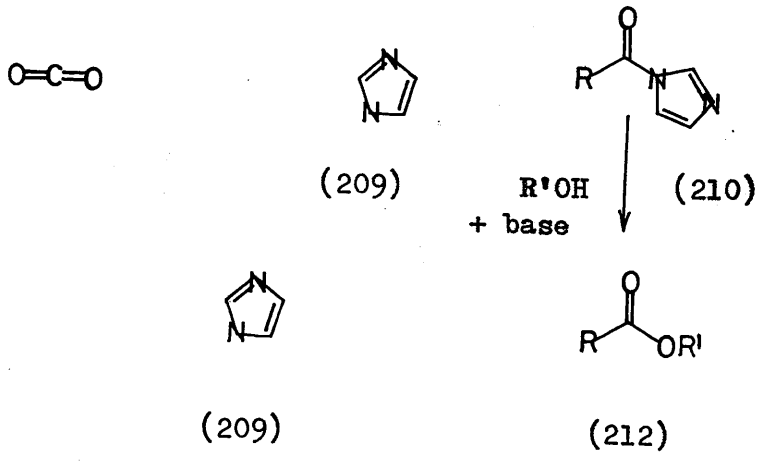
In 1970, however, Konig and Geiger[32] showed that adding 1-hydroxybenzotriazole (200) to the dicyclohexylcarbodiimide, acid and alcohol mixture, decreased racemisation of the acid's



Scheme (28)



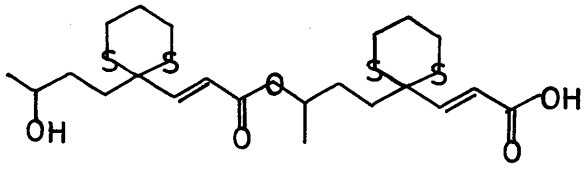
(201) (207) (208) (209)



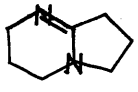
(209) (210) (209) (212)

R'OH + base

Scheme (29)



(190)



(147)

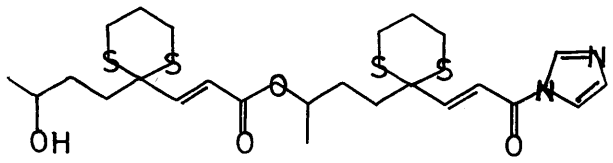
α-carbon, increased yields of coupling and, most importantly here, tended to prohibit the formation of N-acylurea.

Thus, as in Scheme (28), the 1-hydroxybenzotriazole, very rapidly attacked the activated acid (202) to afford the ester (203) which rearranged to the amides (204) or (205). The activated carbonyl group of these three compounds (203), (204), and (205), went on to react with the alcohol to afford the ester (206) and refurnish (200).

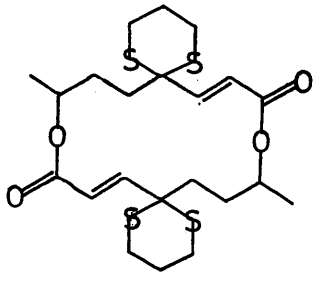
Application of this method to the hydroxyacid (190) persistently afforded the N-acylurea (199) despite variations in reaction times and temperatures.

In 1962, Staab and Mannschreck[33] introduced a delightful method of activating carboxylic acids towards ester formation with alcohols. Their method involved reaction of the carboxylic acid, in dry tetrahydrofuran, with N,N' carbonyldiimidazole (207) to furnish, via the ester (208), the imidazolide (210), imidazole (209), and carbon dioxide according to Scheme (29). The evolution of carbon dioxide, of course, guaranteed irreversibility. Addition of the appropriate alcohol (211) and a catalytic amount of base secured reaction to give the ester (212) and imidazole (209); most likely imidazolide (213)was the active catalyst, formed between the imidazole present and the added base.

Thus one equivalent of N,N' carbonyldiimidazole (207) was added to a 5M solution of the hydroxyacid (190) in dry tetrahydrofuran. After leaving overnight the system was diluted with dry benzene, to afford high dilution conditions, and a catalytic amount of 1,5 diazabicyclo (4,3,0) non-5-ene (147) added to effect a smooth and speedy cyclisation of the intermediate imidazolide



(214)



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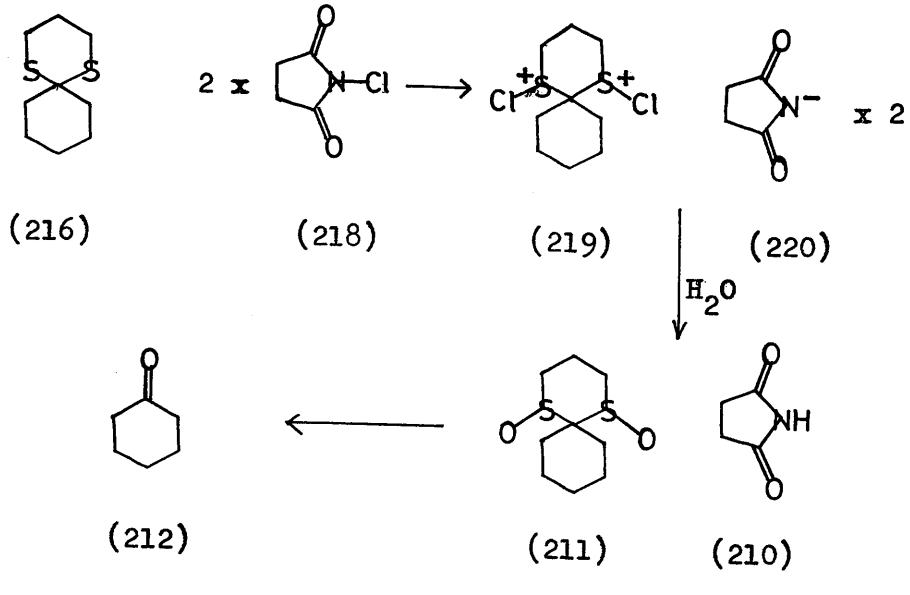


(216) (218) (219) (220)

(212) (211) (210)

Scheme (30)

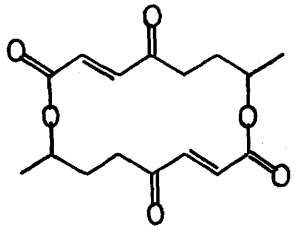
(214) to a 1:1 mixture of diastereomeric bislactones (215) in a total yield of not less than 60%. Isolation of these macrocycles proved difficult but the elusive molecules were eventually obtained as pure white crystals by quickly passing the reaction mixture through a short column of grade II neutral alumina, collecting small fractions and eluting with fresh benzene and removing the solvent.

The difficulty of isolation of the bisthioacetal (215) implied a degree of instability which frowned on all dethioacetalisation procedures save the mildest. As literature methods referred generally to the ethylenethioacetal it was considered most pertinent to test their applicability to a compound bearing a 1,3 propylenethioacetal. The model chosen (216) soon informed us that the system of choice was that prescribed by Corey[34] (Scheme 30).

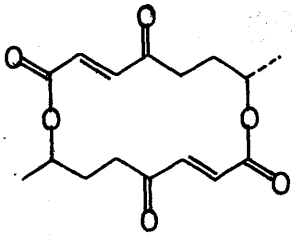
The bisthioacetal (215) was immediately subjected to Corey's conditions.

The frustration wrought by the inadequacies of small thin layer chromatograms was, when a larger chromatogram condescended to complete its term of duty, displaced by a rather curious sense of pessimistic optimism at the appearance of a non-polar compound from the reaction mixture, whose R_f was identical with that of an authentic sample of (-)-pyrenophorin. It was accompanied, in comparable quantity, by a more polar compound.

Various gas-liquid chromatography systems reinforced an obvious hope. Nuclear magnetic resonance spectroscopy, mass spectroscopy, and infra-red spectroscopy confirmed it. Of the 1:1 mixture of products from the dethioacetalisation reaction,



(1)



(217)

the less polar compound was identical in all respects, save rotation, with naturally occurring (-)-pyrenophorin (1). The more polar compound, although chromatographically different, showed spectroscopic properties virtually identical with the less polar molecule, and hence was assigned the structure of the meso diastereomer (217).

EXPERIMENTAL.

General Experimental and Abbreviations.

Melting points are uncorrected and were determined on a Kofler hot-stage apparatus. Microanalyses were obtained by Mr. J. M. L. Cameron, Miss F. Cowan and their staff. Mass spectra were recorded by Mr. A. Ritchie on A. E. I. - G. E. C./ MS 12 and A. E. I. -G. E. C./ MS 902S mass spectrometers. Infra-red spectra were recorded by Mrs. F. Lawrie and her staff on a Unicam SP 100 Mark II spectrometer. Routine infra-red spectra were recorded on a Unicam SP 1000 instrument and were mainly liquid film in nature: the remainder were recorded in chloroform solution and are denoted herein by an asterisk. Nuclear magnetic resonance spectra were recorded by Mr. A. Haetzman or Mr. J. Gall on a Varian T-60 or a Varian HA 100 spectrometer using tetramethylsilane as an internal standard.

Kieselgel G (Merck) was used for preparative thin layer chromatography. Light petroleum refers to the fraction of boiling point 60-80°. Analytical thin layer chromatography plates were stained with iodine vapour and/or ceric ammonium sulphate followed by heating to approximately 150°.

All dilute mineral acids were 6N unless otherwise stated.

All organic solutions, unless otherwise indicated, were dried over anhydrous magnesium sulphate.

Dihydropyran was dried by refluxing with metallic sodium and subsequent distillation. Tetrahydrofuran was heated under reflux with lithium aluminium hydride and distilled prior to use. Dioxan and t-butanol were heated under reflux and distilled. Methylene chloride was dried by passing it through a column of grade I basic alumina.

The following abbreviations and symbols have been employed in the experimental section,

t.l.c.	thin layer chromatography,
i.r.	infra-red,
n.m.r.	nuclear magnetic resonance,
s	singlet,
d	doublet,
m	multiplet,
b	broad,
sex	sextet,
g.l.c.	gas liquid chromatography,
M^+	molecular ion.

p-Bromophenacyl-4-hydroxypentanoate (41).- To γ-valerolactone (10) (15 g, 150 m.moles) was added 10N aqueous potassium hydroxide (15 ml, 150 m.moles). The mixture was stirred vigorously till homogeneity obtained and titrated with dilute hydrochloric acid till the pH of the solution was within the range of eight to nine pH units. After extraction of the aqueous layer with diethylether the water was removed as its benzene azeotrope leaving behind the potassium 4-hydroxypentanoate (60) as a white solid. Dry dimethylformamide (90 ml) and p-bromophenacylbromide (39) (36.4 g, 131 m.moles) were added and the mixture stirred overnight. The yellow solution was poured onto water (1000 ml) and the precipitated solid mass taken up in diethylether (3 500 ml). The combined ether layers were washed with water, brine, and dried. Removal of solvent *in vacuo* yielded a yellow solid mass from which the ester (41) was crystallised as colourless needles (35 g, 111 m.moles, 85%, m.p. 74° ex diethylether/light petroleum).

ν^*_{max} 3430, 1680, and 1590 cm^{-1}, δ ($CDCl_3$) 1.20 (3H; $C\underline{H}_3$-C, d, J 6 Hz), 1.88 (3H; -CH(O$\underline{H}$)-C$\underline{H}_2$-, m, one H exchanges with D_2O), 2.62 (2H; -CH_2-C(O)-, t, J 7.5 Hz), 3.88 (1H; CH_3-C$\underline{H}$(OH)-, sex, J 6 Hz), 5.26 (2H; -O-C$\underline{H}_2$-C(O)-, s), 7.62 (4H; arom, q, J 10 Hz), (Found: C, 49.61; H, 4.71. $C_{13}H_{15}O_4Br$ requires C, 49.54; H, 4.80%)

p-Bromophenacyl-4-acetoxypentanoate (61).- p-Bromophenacyl 4-hydroxypentanoate (41) (50 g, 158 m.moles) was dissolved in acetic anhydride (200 ml) and pyridine (3 ml). After 12 hours, ice was added andthe mixture stirred until the organic layer had assumed a constant volume. The resulting oil was taken up in diethylether and the whole transferred to a separating funnel, wherein

the ether layer was washed with dilute hydrochloric acid, saturated sodium hydrogen carbonate, water and brine. The solution was dried and the solvent removed in vacuo, to give the acetate (61) as a clear oil which crystallised on standing. One recrystallisation gave an analytically pure sample as white-silver plates (53.3 g, 149 m.moles, 94%, m.p. 50-51° ex diethylether/light petroleum), ν^{*}_{max} 1740, and 1260 cm^{-1}, (Found: C, 50.32; H, 4.83. $C_{15}H_{17}O_5Br$ requires C, 50.44; H, 4.80%).

4-Acetoxypentanoic acid (62).- The parent phenacyl ester (61) (44 g, 123 m.moles) was dissolved in glacial acetic acid (150 ml) and an excess of zinc powder added in portions over ten minutes. After 2.5 hours the solids were filtered off and the acetic acid removed in vacuo leaving a colourless oil from which (62) could be distilled as a clear mobile liquid (17 g, 106 m.moles, 86%, b.p. 102-4° at 0.1 mm Hg), ν_{max} 3700-2500, 1730, and 1280 cm^{-1}, δ ($CDCl_3$) 1.24 (3H; $C\underline{H}_3$-C, d, J 6.4 Hz), 1.90 (2H; $C\underline{H}_2$-CH_2-C(O) m), 2.02 (3H; -O-C(O)-$C\underline{H}_3$, s), 2.40 (2H; CH_2-$C\underline{H}_2$-C(O)-, t, J 6.4 Hz), 4.92 (1H; CH_3-$C\underline{H}$-, sex, J 6.4 Hz).
The methyl ester of (62) furnished the following data, ν_{max} 1740, 1260 cm^{-1}, (Found: C, 55.18; H, 8.25. $C_8H_{14}O_4$ requires C, 55.16; H, 8.10%).

4-Acetoxypentanoyl chloride (74).- Oxalyl chloride (15 ml, 176 m.moles) was added to 4-acetoxypentanoic acid (62) (5 g, 31 m.moles) in dry benzene (200 ml). After refluxing for 2.5 hours the volatiles were removed in vacuo leaving the acid chloride (74) as a pale yellow oil, ν_{max} 1790, and 1730 cm^{-1}.

1-Diazo-5-acetoxyhexan-2-one (75).- The acid chloride (74) was added to a diethylether solution of diazomethane (ex 27 g of nitrosan) and left for 16 hours. After boiling to half volume, the ether solution was freed, by filtration, from polymethylene. The product was not isolated but the solution was used immediately in the next step.

1-Bromo-5-acetoxyhexan-2-one (54).- Dry hydrogen bromide was bubbled through the above solution of the diazoketone (75) until no more was absorbed: The solution was then poured into a separating funnel, washed with water till neutral, then brine, and dried. The diethylether was removed in vacuo leaving (54) as a dark red liquid (5.5 g, 23.2 m.moles), ν_{max} 1720 cm^{-1}.

trans Ethyl 4-oxo-7-acetoxyoct-2-enoate (78).- 1-Bromo 5-acetoxyhexan-2-one (54) (5.5 g, 23.2 m.moles) from the previous experiment was taken up in dry benzene (125 ml) and freshly recrystallised ethoxycarbonylmethylenetriphenylphosphorane (77) (16 g, 46 m.moles) added all at once. After heating under reflux for 2.5 hours the cooled solution was filtered and ethyl bromo-acetate added (2.8 ml, 31 m.moles). After boiling for a further 2.5 hours, the dark red solution was cooled, filtered, and the benzene solvent removed in vacuo. The ensuing gum (8.8 g) was extracted with diethylether several times. Evaporation of the ethereal solution gave a yellow oil which was chromatographed on a silica gel column yielding the desired trans keto ester (78) (4.05 g, 16.7 m.moles, 54% from (62)), ν_{max} 1720, and 1700 cm^{-1}, $\delta(CDCl_3)$ 1.22 (3H; CH_3-C, d, J 6.4 Hz), 1.30 (3H; -O-CH_2-CH_3, t,

J 7.0 Hz), 1.90 (2H; $-C\underline{H}_2-CH_2-C(O)-$, m), 2.20 (3H; $CH_3-C(O)-$, s), 2.70 (2H; $-CH_2-C\underline{H}_2-C(O)-$, t, J 7.8 Hz), 4.26 (2H; $-O-\underline{CH}_2-CH_3$, q, J 7.0 Hz), 4.94 (1H; $CH_3-C\underline{H}-$, sex, J 6.4 Hz), 6.62 (1H; $-C(O)-CH=CH-C(O)-O-$, d, J 16.6 Hz), 7.10 (1H; $-C(O)-CH=CH-C(O)-O-$, d, J 16.6 Hz), (Found: C, 59.24; H, 7.53. $C_{12}H_{18}O_5$ requires C, 59.49; H, 7.49%).

Attempted formation of trans ethyl 7-acetoxy-4-(ethylenedithio)oct-2-enoate (208).- To the enone (78) (530 mg, 2.19 m.moles) were added ethane-1,2-dithiol (80) (0.75 ml, 8.9 m.moles) boron trifluoride etherate (0.75 ml) and, after ten minutes, dry chloroform (5 ml). The mixture was left standing overnight whereupon one major product was identified on t.l.c. (30% ethyl acetate/light petroleum, developing solvent). The mixture, diluted with chloroform (100 ml), was washed with water (4 100 ml), and brine (2 100 ml), then dried over anhydrous sodium sulphate. The chloroform was removed in vacuo and the product, in diethylether solution, passed slowly through a column of alumina to remove the dithiol (80). The crude product thus obtained defied all attempts to purify it, always decomposing to a more polar product. It was, however, tentatively assigned the structure (84) from its n.m.r. spectrum, δ ($CDCl_3$) 1.20 (3H; $C\underline{H}_3-CH-C$, d, J 6.5 Hz), 1.26 (3H; $C\underline{H}_3CH_2-$, t, J 6.5 Hz), 1.84 (4H; $-C-C\underline{H}_2-C\underline{H}_2-C-$ m) 2.00 (3H; $C\underline{H}_3-C(O)-$, s), 2.80 (4H; $-S-C\underline{H}_2-C\underline{H}_2-S-$, m), 3.26 (2H; $C=C-C\underline{H}_2-C(O)-$, s), 4.20 (2H; $CH_3-C\underline{H}_2-$, q, J 6.5 Hz), 4.84 (1H; $CH_3-C\underline{H}-C$, m).

trans Ethyl 7-acetoxy-4-(ethylenedioxy)oct-2-enoate (88).- A solution of the enone (78) (2.0 g, 8.3 m.moles), ethylene glycol (87) (10 ml, 180 m.moles), and p-toluenesulphonic acid (100 mg, 0.58 m.moles) in dry benzene (250 ml) was heated under reflux for 48 hours in a Dean and Stark apparatus. The solution was cooled, washed with dilute aqueous sodium hydrogen carbonate, water, brine and dried over anhydrous sodium sulphate. After removal of the solvent in vacuo, the product oil (2.0 g) was chromatographed on a silica gel column to give the pure product as a pale yellow oil (1.7 g, 6.0 m.moles, 72%, ν_{max} 1720 cm^{-1}, δ ($CDCl_3$) 1.18 (3H; CH_3-C, d, J 6.0 Hz), 1.28 (3H; -O-CH_2-CH_3, t, J 7.0 Hz) 1.70 (4H; C-CH_2-CH_2-C, m) 2.00 (3H; CH_3-C(O)-, s) 3.84 (4H; bs, O-CH_2-CH_2-O) 4.08 (2H; -O-CH_2-CH_3, q, J 7.0 Hz), 4.84 (1H; CH_3-CH-m), 5.96 (1H; -CH=CH-C(O)-, d, J 16.0 Hz), 6.66 (1H; -CH=CH-C(O)-, d, J 16.0 Hz), (Found: C, 58.92; H, 7.94. $C_{14}H_{22}O_6$ requires C, 58.73; H, 7.75%).

trans Methyl 7-hydroxy-4-(ethylenedioxy)oct-2-enoate (91).- To the ethyl ester (88) (1.0 g, 3.5 m.moles) was added water (15 ml) and 4N aqueous sodium hydroxide (5 ml, 20 m.moles). After stirring, with heat, for 4 hours the resulting homogeneous solution was cooled and extracted with ether. The aqueous layer, cooled to 0^{o}, was saturated with sodium chloride, carefully acidified with 0.25N sulphuric acid, and rapidly extracted with diethylether (3x20 ml). An excess of diazomethane in diethylether was added all at once to the combined, and undried, ether extracts; after five minutes, the excess diazomethane was boiled off, and the ether solution washed with dilute aqueous sodium

hydrogen carbonate, water, brine and dried over anhydrous sodium sulphate. Evaporation of the solvent gave a crude product (827.3 mg) which yellow oil was chromatographed on silica gel to give the pure methyl ester (603.4 mg, 2.6 m.moles, 74%), ν_{max} 3500, and 1720 cm^{-1}, δ ($CDCl_3$) 1.20 (3H; $C\underline{H}_3$-C, d, J 6.0 Hz), 2.08 (1H; CH_3-CH(O$\underline{H}$)-, s, exchanges with D_2O), 3.72 (3H; $C\underline{H}_3$-O-, s), 3.94 (5H; -O-$C\underline{H}_2$-$C\underline{H}_2$-O-, CH_3-$C\underline{H}$, bs + m), 6.06 (1H; -CH=CH-C(O)- d, J 15.5 Hz), 6.72 (1H; -CH=CH-C(O)-, d, J 15.5 Hz), (Found: C, 57.58; H, 8.01. $C_{11}H_{18}O_5$ requires C, 57.38; H, 7.88%).

trans Methyl 7-p-nitrobenzoyl-4-(ethylenedioxy)oct-2-enoate (104).- The methyl ester (91) (100 mg, 0.43 m.moles) was dissolved in dry pyridine (8 ml) and p-nitrobenzoyl chloride (100 mg, 0.5 m. moles) added. After 3 hours the mixture was added to diethylether (100 ml) and the whole washed with 0.25N sulphuric acid till no pyridine remained. The ether layer was then washed with dilute aqueous sodium hydrogen carbonate, water, brine, and dried. Removal of the solvent in vacuo gave the p-nitrobenzoate (104) as a viscous oil (155 mg, 0.40 m.moles, 93%), δ($CDCl_3$) 1.32 (3H; $C\underline{H}_3$-C, d, J 6.5 Hz), 1.84 (4H; -C-$C\underline{H}_2$-$C\underline{H}_2$-C-, m), 3.78 (3H; CH_3-O-, s) 3.92 (4H; -O-$C\underline{H}_2$-$C\underline{H}_2$-O-, bs), 5.20 (1H; CH_3-$C\underline{H}$, m), 6.10 (1H; -CH=CH-C(O)-, d, J 16 Hz), 6.78 (1H; -CH=CH-C(O)-, d, J 16 Hz), 8.26 (4H; aromatic, bs).

Attempted formation of trans 7-p-nitrobenzoyl-4-(ethylene-dioxy)oct-2-enoic acid (105).- I. To lithium iodide (17) (1 g, 7.46 m.moles) which had been vacuum dried at 150° was added the methyl ester (104) (100 mg, 0.26 m.moles) in dimethylformamide

(25 ml). The mixture was heated under reflux overnight, cooled, then poured onto water (100 ml) from which mixture the dimethylformamide was extracted with diethylether. The aqueous phase was cooled to 0°, carefully acidified with 0.25N sulphuric acid, saturated with sodium chloride, and extracted with diethylether (2 50 ml). The ether extracts were washed with brine, dried, and the solvent removed *in vacuo*, to give 40 mg of product. Methylation of this product with diazomethane gave mainly the hydroxy methyl ester (91).

II. Lithiumpropylmercaptide (16) was prepared by titrating lithium metal in deoxygenated hexamethylphosphorictriamide under dry nitrogen. For example, to deoxygenated hexamethylphosphorictriamide (10 ml), under dry nitrogen, was added lithium metal (77 mg, 11.1 m.moles) and propanethiol was dropped in slowly from a syringe till the blue colour had been totally discharged leaving behind a pale yellow solution of the lithium salt (16). Experiments using an equivalent or an excess of this reagent at no time gave the desired product.

trans Methyl 7-tetrahydropyranyloxy-4-(ethylenedioxy)oct-2-enoate (107).- The hydroxy methyl ester (91) (125 mg, 0.54 m.mole) was dissolved in dry benzene (3 ml) and freshly purified dihydropyran (1.5 equivalents) added together with one drop of phosphorusoxychloride. After 15 hours, the solution was washed with dilute aqueous sodium hydrogen carbonate, water, brine, and dried over anhydrous sodium sulphate; the solvent was removed *in vacuo*. Chromatography of the product oil (150 mg) on Grade II neutral alumina gave pure tetrahydropyranylether (107) (80 mg,

0.26 m.moles, 48%), ν^{*}_{max} 1720, 1380, and 980 cm^{-1}, δ ($CDCl_3$) 1.20 + 1.64 (13H; $-CH_2-CH_2-CH_2-CH_2-O-$, $CH_3-CH(OTHP)-CH_2-CH_2-$, m + m), 3.48 (2H; $-CH_2-O-$, q, J 7.0 Hz), 3.74 (3H; CH_3-O-, s), 3.90 (5H; $-O-CH_2-CH_2-O-$, CH_3-CH, bs + m), 4.66 (1H; $-O-CH-O-$, bm) 6.02 (1H; -CH=CH-C(O)-, d, J 16.0 Hz), 6.76 + 6.78 (1H; -CH=CH-C(O)-, d of d, J 16.0 Hz).

Note: THP = tetrahydropyranyl.

trans 7-Tetrahydropyranyloxy-4-(ethylenedioxy)oct-2-enoic acid (108).- To the tetrahydropyranylether (107) (0.26 m.moles, 80 mg) were added 4N sodium hydroxide (0.4 ml, 1.6 m.moles) and water (2.4 ml). After 30 minutes, at 40°, the homogeneous solution was cooled to 0°, extracted with diethylether, and saturated with sodium chloride. The aqueous layer was then acidified carefully with 0.25N sulphuric acid in the presence of solid sodium chloride, and extracted rapidly with diethylether (3x10 ml). The third fraction was usually discarded and the first two combined, washed with brine, and dried. Removal of solvent, in vacuo, gave almost pure carboxylic acid (108) (46 mg, 0.15 m.moles, 58%). Chromatography on a reversed phase Sephadex column using 90% methanol/benzene as eluent gave pure carboxylic acid (108) (40 mg, 0.13 m.moles, 50%), ν^{*}_{max} 3500-2500, 1697, 1655, 980 cm^{-1}, δ ($CDCl_3$) 1.16 + 1.60 (13H; $-CH_2-CH_2-CH_2-CH_2-O-$, $CH_3-CH(OTHP)-CH_2-CH_2-$, m + m), 3.48 (2H; $-CH_2-O-$, J 7.0 Hz) 3.94 (5H; $-O-CH_2-CH_2-O-$, CH_3-CH-, bs + bm) 6.04 (1H; -CH=CH-C(O)-, d, J 15.0 Hz), 4.64 (1H; $-O-CH-O-$, bm), 6.88 (1H; -CH=CH-C(O)-, a sightly broadened doublet, J 15.0 Hz), 8.60 (1H; $-C(O)-OH$, bs, exchanges with D_2O).

Attempted preparation of 7'-(trans 1'-carbomethoxy-4'-(ethylenedioxy)oct-2'-enyl)trans 7-tetrahydropyranyl-4-(ethylenedioxy)oct-2-enoate (85).- I. To the carboxylic acid (108) (50 mg, 0.17 m.moles) dissolved in dry benzene (5 ml) were added dry pyridine (0.1 ml, 1.24 m.moles) and oxalyl chloride (72) (0.08 ml, 0.94 m.moles). After stirring for 6.5 hours the benzene was removed in vacuo and the alcohol (91) (50 mg, 0.22 m.moles) added in dry chloroform (1 ml) and dry pyridine (0.05 ml, 0.62 m.moles). The stirred reaction mixture was left in the dark overnight then taken up in diethylether, washed with dilute aqueous sodium hydrogen carbonate, brine and dried over anhydrous sodium sulphate. After removal of the solvent in vacuo the product mixture was chromatographed via t.l.c. (developing solvent: 50% ethyl acetate/light petroleum) to give 25 mg of a pure colourless oil. Its n.m.r. spectrum was inconsistent with that expected for the desired product, but consistent with a structure (112). This same product was obtained by adding the alcohol (91) (50 mg, 0.22 m.moles) to dry pyridine (0.1 ml, 1.24 m.moles), chloroform (1 ml) and oxalyl chloride (72) (0.08 ml, 0.94 m.moles);
ν^{*}_{max} 1722, 1740, and 1760 cm^{-1}, δ ($CDCl_3$) 1.32 (6H; CH_3-C, d, J 6.0 Hz) 1.80 (8H; -C-CH_2-CH_2-C-, m) 3.74 (6H; CH_3-O-, s) 3.92 (8H; -O-CH_2-CH_2-O-, bs) 5.10 (2H; CH_3-CH-, bm) 6.06 (2H; -CH=CH-C(O)-, d, J 16.0 Hz) 6.74 (2H; -CH=CH-C(O)-, d, J 16.0 Hz).

II. Triphenylphosphine (7) (80 mg, 0.31 m.moles) was added to the carboxylic acid (108) (90 mg, 0.30 m.moles) in carbon tetrachloride (1 ml). The solution was refluxed gently and after 15 minutes became cloudy; after a further hour the solution was

cooled to 0°, filtered, and divorced of solvent *in vacuo*. To the clear, pale yellow, residue was then added a solution of the alcohol (91) (80 mg, 0.35 m.moles) in pyridine (0.3 ml, 3.72 m.moles) and chloroform (1 ml). A further 0.4 ml of chloroform washings were added. After several days in the dark no product was observed. The attempted formation of the acid chloride was examined using the same proportions of reagents and it was found that even over several days the acid chloride was not formed to any great extent if at all.

III. (i). To the carboxylic acid (108) (25 mg, 0.08 m.moles) dissolved in dry benzene (2.5 ml) were added Linde type 3A molecular sieves (2 g) and the alcohol (91) (25 mg, 0.11 m.moles) in dry benzene (0.5 ml).

(ii). To the carboxylic acid (108) (25 mg, 0.08 m.moles) dissolved in dry benzene (2.5 ml) were added Linde type 3A molecular sieves (2 g), Amberlite 120 resin (0.5 g) (in its protonated form), and the alcohol (91) (25 mg, 0.11 m.moles) in dry benzene (0.5 ml).

(iii). To the carboxylic acid (108) (25 mg, 0.08 m.moles) dissolved in dry benzene (2.5 ml) were added Amberlite 120 resin (1 g) (in its protonated form) and the alcohol (91) (25 mg, 0.11 m.moles) in dry benzene (0.5 ml).

After six days the reactions were each filtered and stripped of solvent *in vacuo*. Analytical t.l.c. (developing solvent 50% ethyl acetate/ light petroleum) revealed only one distinct product in sizeable quantity and that from the second

experiment. Preparative t.l.c. afforded 5 mg of this product whose sub-standard n.m.r. spectrum suggested the transesterification product (107).

trans Methyl 7-oxo-4-(ethylenedioxy)oct-2-enoate (141).- I. Cornforth's solution was prepared by adding pyridine (15 ml) to an ice-cold solution of chromium trioxide (1.5 g, 15 m.moles) in water (0.9 ml).

To the methyl ester (91) (400 mg, 1.74 m.moles) in dry pyridine (1 ml) was added Cornforth's reagent (5.3 ml, 5 m. moles) and the mixture stirred for 48 hours. The mixture was poured onto diethylether (50 ml) and the solid residue washed thoroughly with more diethylether (50 ml). The combined ether extracts were washed with dilute aqueous copper sulphate, water, brine, and dried. Removal of the solvent in vacuo gave nearly pure ketone (316 mg, 1.38 m.moles, 80%).

II. To the methyl ester (91) (1726 mg, 7.50 m.moles), dissolved in dry dimethyl sulphoxide (30 ml) and dry benzene (30 ml), were added dicyclohexylcarbodiimide (192) (4.50 g, 21.84 m.moles) and pyridinium trifluoroacetate (750 mg, 3.88 m.moles). The mixture was stirred for 18 hours then filtered, diluted with ethyl acetate (200 ml), and washed with water (5×200 ml), brine, and dried. Evaporation of the solvent in vacuo gave 4.30 g of a mixture which on chromatography on silica gel afforded the pure ketone (141) (1.20 g, 5.26 m.moles, 70.1 %), ν_{max} 1720 cm^{-1}, $\delta(CDCl_3)$ 2.14 (3H; $C\underline{H}_3$-C(O)-, s), 2.08 + 2.58 (4H; -C-$C\underline{H}_2$-$C\underline{H}_2$-C, m + m), 3.72 (3H; $C\underline{H}_3$-O-, s) 3.88 (4H; -O-$C\underline{H}_2$-$C\underline{H}_2$-O-, bs) 6.02

(1H; -CH=CH-C(O)-, d, J 15.0 Hz) 6.68 (1H; -CH=CH-C(O)-, d, J 15.0 Hz), (Found: C, 57.89; H, 7.07. $C_{11}H_{24}O_6$ requires C, 57.88; H, 7.07%).

trans 7-Oxo-4-(ethylenedioxy)oct-2-enoic acid (142).- To the keto methyl ester (141) (600 mg, 2.63 m.moles) were added 4N aqueous sodium hydroxide (1.50 ml, 6.00 m.moles) and water (4 ml). After 30 minutes of stirring the mixture became homogeneous and was extracted with diethylether. The aqueous phase was cooled to 0° and acidified, in the presence of solid sodium chloride, with 0.25N sulphuric acid. It was then extracted with diethylether, and the ether extracts washed with brine and dried. Evaporation of the ether in vacuo gave the keto acid as a pale yellow solid (142) (513 mg, 2.40 m.moles, 91%, m.p.92 $^{\circ}$ ex diethylether/light petroleum), ν^{*}_{max} 3500-2500, and 1707 cm^{-1}, $\delta(CDCl_3)$ 2.16 (3H; $\underline{CH}_3$-C(O) s), 2.00 + 2.56 (4H; -C-$\underline{CH}_2$-$\underline{CH}_2$-C-, m + m), 3.92 (4H; -O-$\underline{CH}_2$-$\underline{CH}_2$-O-, bs), 6.10 (1H; -CH=CH-C(O)-, d, J 16.0 Hz), 6.88 (1H; -CH=CH-C(O)-, d, J 16.0 Hz), 9.52 (1H; -C(O)-O$\underline{H}$, bs).

Diazomethylation of a portion of this acid (142) regenerated the starting methyl ester (141).

7'-(trans 1'-Carbonylmethoxy-4'-(ethylenedioxy)oct-2'-enyl)-trans 7-oxo-4-(ethylenedioxy)oct-2-enoate (144).- To the keto acid (142) (250 mg, 1.17 m.moles) in dry benzene (10 ml) were added oxalyl chloride (0.65 ml, 7.60 m.moles) and dry pyridine (0.98 ml, 12.10 m.moles). The mixture was stirred in the dark for 6 hours when it was filtered and evaporated, in vacuo, to dryness. More pyridine (0.4 ml, 5.0 m.moles) was added, followed by the alcohol

(91) (150 mg, 0.65 m.moles) in dry, alcohol free, chloroform (4 ml) and the reaction left in the dark for 13 hours. The mixture was then washed with water and the whole azeotroped with benzene to give 234 mg of crude product. Preparative t.l.c. (developing solvent 20% methanol/benzene) gave the pure dimer (144) (127 mg, 0.3 m.moles, R_f 0.7, 25%), ν^*_{max} 1722 cm^{-1}, $\delta(CDCl_3)$ 1.22 (3H; $C\underline{H}_3$-CH d, J 6.0 Hz), 1.72 (4H; CH_3-CH(-O-)-$C\underline{H}_2$-$C\underline{H}_2$-, m), 2.00 + 2.50 (4H; CH_3-C(O)-$C\underline{H}_2$-$C\underline{H}_2$-, m + m), 2.14 (3H; $C\underline{H}_3$-C(O)-, s), 3.76 (3H; $C\underline{H}_3$-O-, s), 3.94 (8H; -O-$C\underline{H}_2$-$C\underline{H}_2$-O-, bs), 5.00 (1H; CH_3-$C\underline{H}$-, bm), 6.00 + 6.06 (2H; -CH=CH-C(O)-, d + d, J 16.0 Hz), 6.68 + 6.74 (2H; -CH=CH-C(O)-, d + d, J 16.0 Hz).

7'-(trans 1'-Carbonylmethoxy-4'-(ethylenedioxy)oct-2'-enyl) -trans 7-hydroxy-4-(ethylenedioxy)oct-2-enoate (135).- The ketonic dimer (144) (127 mg, 0.30 m.moles) was dissolved in ethanol (5 ml) and enough water was added to give a turbid solution (about 2 ml); an excess of sodium borohydride was added. After 15 minutes the mixture was added to water (50 ml) and the aqueous solution thoroughly extracted with ethyl acetate. The combined organic phases were washed with brine, dried, and subjected to solvent removal in vacuo. Preparative t.l.c. (developing solvent 20% methanol/benzene) of the 122.3 mg of product gave pure alcohol dimer (135) (91 mg, 0.21 m.moles, R_f 0.4, 70%), ν_{max} (CCl_4), 3500, 1720 cm^{-1}, $\delta(CDCl_3)$ 1.20 + 1.26 (6H; $C\underline{H}_3$-C, d + d, J 6.0 Hz), 1.76 (9H; -C-$C\underline{H}_2$-$C\underline{H}_2$-C-, C-O$\underline{H}$, m, one H exchanges with D_2O), 3.78 (3H; $C\underline{H}_3$-O-, s), 3.92 (9H; -O-$C\underline{H}_2$-$C\underline{H}_2$-O-, CH_3-$C\underline{H}$(OH)-, bs + bm), 5.00 (1H; CH_3-$C\underline{H}$(OC(O)-)-, bm), 6.00 + 6.06 (2H; -CH=CH-C(O)-, d + d, J 16.0 Hz), 6.72 (2H; -CH=CH-C(O)-, bd, J 16.0 Hz),

Attempted cyclisation of (135) to the diacetal of pyrenophorin (145).-I.To the dimer (135) (4.5 mg, 0.01 m.moles) was added 1 ml of a suspension of potassium t-butoxide (10 mg, 0.09 m.moles) in dry benzene (100 ml). Oven dried and ground Linde type 5A molecular sieves (200 mg) were added and the mixture stirred under reflux. No identifiable product appeared after several days though the starting material ultimately disappeared. The reaction was repeated on larger scales, for example using 30 mg and 50 mg of dimer (135) in the hope of finding small quantities of a distinct product. Sieve quantities of up to 0.5 g were also employed.

II. To the dimer (6 mg, 0.014 m.moles) was added one ml of a suspension of sodium hydride (8 mg, 0.2 m.moles, 60% dispersion in mineral oil) in dry benzene (100 ml). Oven dried and ground Linde type 5A molecular sieves (200 mg) were added; after stirring under reflux for three hours the starting material had vanished, forming no isolable product.

III. To the dimer (7.2 mg, 0.017 m.moles) were added 1 ml of a suspension of sodium methoxide (9.2 mg, 0.17 m.moles) in dry benzene (100 ml) and oven dried and ground Linde type 5A molecular sieves (200 mg). Stirring, under reflux, at no time gave an identifiable product.

IV. To the dimer (60 mg, 0.14 m.moles) dissolved in dry benzene (12 ml) were added oven dried and ground Linde type 5A

molecular sieves (2 g) and 1,5 diazabicyclo (4,3,0) non-5-ene (147) (8 drops). After 48 hours the reaction mixture was filtered and the filtrate washed with water, brine and dried. Preparative t.l.c. (developing solvent: 50% ethyl acetate/light petroleum) of the solvent freed reaction product mixture gave a non-polar material subsequently identified as (146) (20 mg, 0.046 m.moles, 33%, R_f 0.45), ν_{max} 1723 cm^{-1}, $\delta(CDCl_3)$ 1.16 + 1.20 (6H; CH_3-C, d + d J 6.0 Hz), 1.72 (8H; C-$C\underline{H}_2$-$C\underline{H}_2$-C, m), 2.52 (2H; -$C\underline{H}_2$-C(O)-O-, m), 3.76 (3H; CH_3-O-, s), 3.92 (9H; -O-$C\underline{H}_2$-$C\underline{H}_2$-O-, CH_3-$C\underline{H}$(-O-CH)-, bs + bm), 5.00 (2H; CH_3-$C\underline{H}$(-O-C(O)-)-, CH_3-CH(-O-$C\underline{H}$)-, bm), 6.06 (1H; -CH=CH-C(O), d, J 15.0 Hz), 6.72 (1H; -CH=CH-C(O), d, J 15.0 Hz).

Selective hydrolytic cleavage of the methyl ester of (146).

I. To the dimer (146) (6 mg, 0.014 m.moles) in methanol (0.5 ml) was added water (0.25 ml) and 0.1N aqueous sodium hydroxide (0.70 ml, 0.07 m.moles). After 3 hours the starting material had disappeared so the mixture was added to water (2 ml), extracted with ethyl acetate, acidified, saturated with salt, and re-extracted with fresh ethyl acetate. The latter organic extracts were washed with brine and diazomethylated. Removal of the solvent *in vacuo* gave 4 mg of crude starting material (146).

II. The above experiment was repeated using decinormal sodium carbonate instead of sodium hydroxide and given a reaction time of 6 hours. This method gave 4 mg of pure starting material after re-esterification.

Attempted preparation of 7'-(trans 1'-carbonylhydroxy-4'-(ethylenedioxy)oct-2'-enyl)-trans 7-hydroxy-4-(ethylenedioxy)oct-2-enoate (148).- I. To the dimer (135) (6 mg, 0.014 m.moles) in methanol (0.5 ml) was added water (0.25 ml) and 0.1N aqueous sodium hydroxide (0.70 ml, 0.07 m.moles). When the starting material had disappeared the mixture was added to water (2 ml), extracted with ethyl acetate, acidified, saturated with salt, and re-extracted with fresh ethyl acetate. The latter organic extracts were washed with brine and diazomethylated. Analytical t.l.c. did not show the starting ester (135).

II. The above experiment was repeated using decinormal sodium carbonate instead of sodium hydroxide but again the acidified hydrolysate gave no evidence of (148).

III. Experiment I was repeated using 0.1N potassium hydrogen carbonate but hydrolysis, under these conditions, failed to occur.

Similar unsuccessful attempts were made to selectively demethylate (144).

1-(Propylene-1,3-dithio)pentan-4-ol (177).- I. To a solution of 1,3-dithiane (153, R=H) (1.32 g, 11.0 m.moles) in dry tetrahydrofuran (50 ml) at -30° under an atmosphere of dry nitrogen was added n-butyllithium (6.05 ml, 12.1 m.moles, 2.1M in hexane). After one hour, 2-methyloxetane (180) (1 ml, 11 m.moles) at -30° was added from a pre-cooled syringe. After 5 days the mixture was poured onto water and the whole extracted with diethylether: The organic layer was washed with water, brine and dried. Removal of the volatiles, in vacuo, yielded a yellow-brown liquid which, after chromatography on silica gel, furnished a 9:1 mixture of isomers (177) and (218), (177) predominating, (1.25 g, 6.51 m.moles, 59.1%). It was later found that (177) could be prepared in a pure form (Method II).

2-Hydroxy-5-methyltetrahydrofuran (163).- To -valerolactone (10) (40 g, 400 m.moles) in dry tetrahydrofuran (300 ml) at -78° under an atmosphere of dry nitrogen was added, with stirring, a suspension of sodium aluminium hydride (6.0 g, 111.1 m.moles) in dry tetrahydrofuran (20 ml). The stirred mixture was allowed to reach -20° then left standing at -20° for 5 hours. Upon allowing the mixture to come to ambient temperature, water was added till no more precipitate was formed. After centrifugation, the supernatant liquid was removed and the solids washed with diethylether; the ether layer, after centrifugation, was combined with the tetrahydrofuran solution and the whole dried over anhydrous sodium sulphate. The solvent was removed in vacuo leaving a clear oil which was immediately used in the next experiment. A small portion was, however, subjected to infra-red spectroscopy showing ν_{max}

3510, and 1720 cm^{-1}.

1-(Propylene-1,3-dithio)pentan-4-ol (177).- II. To the crude lactol (163) from the previous experiment were added propane-1,3-dithiol (178) (41 ml) and boron trifluoride etherate (37 ml) and the mixture left for ten minutes. After addition of dry chloroform (300 ml) the solution was left standing for 18 hours whereupon 5N aqueous sodium hydroxide (300 ml) was added and the mixture stirred vigorously till no free thiols remained. The separated organic layer was washed with water (600 ml) and with brine until the washings were neutral. Removal of the solvent in vacuo gave 26.1 g of crude product which was used per se in the following experiment. A small sample, however, was purified for characterisation purposes. Thus preparative t.l.c. (developing solvent: 40% ethyl acetate/light petroleum) gave pure alcohol (177) (R_f 0.5), ν_{max} 3430 cm^{-1}, $\delta(CDCl_3)$ 1.20 (3H; CH_3-C, d, J 6.0 Hz), 1.74 (7H; -S-CH_2-CH_2-CH_2-S-, -C-OH, -C-CH_2-CH_2-C-, bm, one H exchanges with D_2O), 2.88 (4H; -S-CH_2-CH_2-CH_2-S- m) 4.00 (2H; CH_3-CH-, -S-CH-S-, m), (Found: C, 50.02; H, 8.64. $C_8H_{16}OS_2$ requires C, 49.96; H, 8.39%).

1-(Propylene-1,3-dithio)-4-tetrahydropyranyloxypentane (182) To the crude alcohol (177) from the previous experiment, in dry benzene (300 ml), were added dihydropyran (15 ml) and phosphorous-oxychloride (3 drops). After standing for 18 hours the organic layer was washed with dilute aqueous sodium hydrogen carbonate, water and brine; after drying over anhydrous sodium sulphate, the solvent was evaporated in vacuo to give 35 g of crude product.

Chromatography on grade II neutral alumina yielded the pure ether as a clear oil (182) (27.6 g, 100 m.moles, 25% from (10)), ν_{max} 2950 cm^{-1}, $\delta(CDCl_3)$ 1.14 + 1.28 (3H; CH_3-C, d + d, J 6.0 Hz), 1.70 (12H; -S-CH_2-CH_2-CH_2-S-, -CH_2-CH_2-CH_2-CH_2-O-, CH_3-CH(OTHP)-CH_2-CH_2-, bm), 2.90 (4H; -S-CH_2-CH_2-CH_2-S-, m), 3.90 (4H; -CH_2-O-, -S-CH-S-, CH_3-CH, bm), 4.70 (1H; -O-CH-O-, m), (Found: C, 56.66; H, 9.00. $C_{13}H_{24}O_2S_2$ requires C, 56.48; H, 8.75%).

2-(Propylene-1,3-dithio)-5-tetrahydropyranyloxyhexanal (169)

To a stirred solution of the tetrahydropyranyl ether (182) (1.89 g, 6.85 m.moles) in dry tetrahydrofuran (50 ml) at -30° under an atmosphere of dry nitrogen was added n-butyllithium (3.64 ml, 6.85 m.moles, 2.1M in hexane) and the whole kept at -30° for five hours when pre-cooled ethyl formate (184) (5.2 g, 70 m.moles) was quickly added. After 18 hours the mixture was allowed to reach ambient temperature then poured onto water, and the whole extracted with diethylether. The organic layer was washed with water, brine and dried over anhydrous sodium sulphate. Removal of the volatiles *in vacuo* gave an oil which after chromatography on grade II neutral alumina afforded the pure aldehyde (169) (1.6 g, 5.26 m.moles, 76%), ν_{max} 2810, and 1710 cm^{-1}, $\delta(CDCl_3)$ 1.10 + 1.24 (3H; CH_3-C, d + d, J 6.0 Hz), 1.62 (12H; -S-CH_2-CH_2-CH_2-S-, -CH_2-CH_2-CH_2-CH_2-O-, CH_3-CH(OTHP)-CH_2-CH_2-, bm), 2.2 - 4.0 (7H; -S-CH_2-CH_2-CH_2-S-, -CH_2-O-, CH_3-CH, complex multiplets), 4.62 (1H; -O-CH-O-, bs), 8.94 (1H; -CHO, a pair of very close singlets)

p-Toluenesulphonylethyl bromoacetate (185).- To a stirred suspension of p-toluenesulphonylethanol (184) (7.2 g, 36.0 m.moles) in dry benzene (250 ml) were added anhydrous sodium hydrogen carbonate (3.36 g, 40.0 m.moles), Linde type 3A molecular sieves (20 g), and bromoacetyl bromide (3.36 g, 36.0 m.moles). Stirred for 18 hours, the mixture was filtered and the filtrate used for the next experiment. A small portion was, however, retained and the solvent removed, in vacuo, leaving (185) as a nearly pure oil, δ ($CDCl_3$) 2.42 (3H; CH_3-aromatic, s), 3.44 (2H; $-C\underline{H}_2-CH_2-S(O)_2-$, t, J 6.5 Hz), 3.62 (2H; $-C\underline{H}_2-Br$, s), 4.46 (2H; $-CH_2-C\underline{H}_2-S(O)_2-$, t, J 6.5 Hz), 7.34 (2H; aromatic, d, J 9.0 Hz), 7.76 (2H; aromatic, d, J 9.0 Hz).

Carbonyl-p-toluenesulphonylethoxymethylenetriphenylphosphonium bromide (186).- To the stirred benzene solution of the bromoacetate (185) from the previous experiment was added triphenylphosphine (7) (10.5 g, 40.0 m.moles) whereupon a reddish oil soon separated from the solvent. After three hours the benzene was decanted and the oil washed with fresh benzene. The phosphonium bromide (186) thus prepared was used immediately in the next experiment.

Carbonyl-p-toluenesulphonylethoxymethylenetriphenylphosphorane (187).- The red oil from the previous experiment was dissolved in water and carefully titrated with 5N sodium hydroxide. The precipitated ylid was taken up in ethyl acetate and the organic solution quickly washed with water, brine, and dried over anhydrous sodium sulphate. Removal of the solvent in vacuo gave

the ylid as a gummy solid (187) (11.8 g, 28.0 m.moles, 70%), δ ($CDCl_3$) 2.28 (3H; CH_3-aromatic, s), 3.28 (2H; -$C\underline{H}_2$-CH_2-$S(O)_2$-, t, J 6.5 Hz), 4.20 (2H; -CH_2-$C\underline{H}_2$-$S(O)_2$-, t, J 6.5 Hz), 7.44 (19H; aromatic, bm), 2.60 (1H; -C(O)-C<u>H</u>=P-, bs).

<u>trans</u> p-<u>Toluenesulphonylethyl</u>-7-<u>tetrahydropyranyloxy</u>-4-(<u>propylene</u> 1,3-<u>dithio</u>)<u>oct</u>-2-<u>enoate</u> (170).- To the aldehyde (169) (690 mg, 2.27 m.moles) in hot dry benzene (10 ml), was added the ylid (187) (1.2 g, 2.55 m.moles) and the solution stirred for 36 hours. The solvent was removed and the product mixture chromatographed on a thin layer of silica (developing solvent: 50% ethyl acetate/light petroleum) to return the starting aldehyde (169) (300 mg, 1.0 m.moles, 44%) and the pure <u>trans</u> olefin (170) (600 mg, 1.13 m.moles, R_f 0.5, 50%), ν^*_{max} 1720, 1643, 1598, and 1320 cm^{-1}, δ ($CDCl_3$) 1.10 + 1.20 (3H; $C\underline{H}_3$-C, d + d, J 6.0 Hz), 1.70 (12H; CH_3-CH(OTHP)-$C\underline{H}_2$-CH_2, -$C\underline{H}_2$-$C\underline{H}_2$-$C\underline{H}_2$-CH_2-O-, -S-CH_2-$C\underline{H}_2$-CH_2-S-, bm), 2.54 (3H; CH_3-aromatic, s), 2.72 (4H; -S-$C\underline{H}_2$-CH_2-$C\underline{H}_2$-S-, m), 3.56 (5H; -$(CH_2)_3$-$C\underline{H}_2$-O, CH_3-C<u>H</u>, -O-$C\underline{H}_2$-CH_2-$S(O)_2$-, m + t, J 6.0 Hz), 4.56 (3H; -O-C<u>H</u>-O-, -O-CH_2-$C\underline{H}_2$-$S(O)_2$-, m + t, J 6.0 Hz), 6.00 (1H; -CH=CH-C(O)-, d, J 16.0 Hz), 6.90 (1H; -CH=CH-C(O)-, d, J 16.0 Hz), 7.40 (2H; aromatic, d, J 7.0 Hz), 7.88 (2H; aromatic, d, J 7.0 Hz), (Found M^+, 528.166483 or 528.166985. $C_{25}H_{36}O_6S_3$ requires 528.166387).

<u>trans</u> p-<u>Toluenesulphonylethyl</u>-7-<u>hydroxy</u>-4-(<u>propylene</u>-1,3-<u>dithio</u>)<u>oct</u>-2-<u>enoate</u> (171).- To the ether (170) (1.12 g, 2.0 m.moles) in methanol (8 ml) was added 1N hydrochloric acid (8 drops) and the solution stirred for one hour. The reaction mixture was poured onto ethyl acetate and the whole washed with dilute aqueous sodium hydrogen carbonate, water, brine, and dried over anhydrous

ium hydrogen carbonate, water, brine, and dried over anhydrous sodium sulphate. Removal of solvent in vacuo gave the almost pure alcohol as a gum (171) (943 mg). A small portion was purified via preparative t.l.c. (developing solvent: 50% ethyl acetate/light petroleum, R_f 0.45), ν^*_{max} 3530, 3610, 1720, 1640 1595, and 1320 cm^{-1}, δ ($CDCl_3$) 1.20 (3H; CH_3-C, d, J 6.0 Hz), 1.80 (7H; -C-CH_2-CH_2-C-, -S-CH_2-CH_2-CH_2-S-, CH_3-C(OH)-, m, one H exchanges with D_2O), 2.48 (3H; CH_3-aromatic, s), 2.78 (4H; -S-CH_2-CH_2-CH_2-S-, m), 3.52 (3H; CH_3-CH, -O-CH_2-CH_2-$S(O)_2$-, m + t, J 7.0 Hz), 4.54 (2H; -O-CH_2-CH_2-$S(O)_2$-, t, J 7.0 Hz), 6.00 (1H; -CH=CH-C(O)-, d,J 15.0 Hz), 6.90 (1H; -CH=CH-C(O)-, d, J 15.0 Hz) 7.44 (2H; aromatic, d, J 8.0 Hz), 7.90 (2H; aromatic, d, J 8.0 Hz).

trans p-Toluenesulphonylethyl-7-bromoacetoxy-4-(propylene-1,3-dithio)oct-2-enoate (173).- To the crude alcohol (171) (943 mg) from the previous experiment, in dry benzene (10 ml), were added anhydrous sodium hydrogen carbonate (250 mg, 3.0 m.moles), Linde type 3A molecular sieves (2.0 g), and bromoacetyl bromide (172) (0.19 ml, 2.20 m.moles). After stirring for 18 hours, the mixture was filtered and the filtrate used in the next step.

Carbonyl(trans p-toluenesulphonylethyl-7-oxy-4-(propylene-1,3-dithio)oct-2-enoate)methylenetriphenylphosphonium bromide (188).- To the stirred solution of (173) from the above experiment was added triphenylphosphine (655 mg, 2.5 m.moles). After stirring for 30 minutes the temperature was raised to about 50° and the mixture stirred for a further two hours; cooled to ambient temperature, the benzene was then decanted and the residual

gummy salt (188) washed with fresh benzene. All the benzene solutions were extracted with water and the aqueous extracts, containing some (188), retained.

Carbonyl(trans p-toluenesulphonylethyl-7-oxy-4-(propylene-1,3-dithio)oct-2-enoate)methylenetriphenylphosphorane (174).- The phosphonium salt (188) and the aqueous extracts from the above experiment were shaken with a similar volume of ethyl acetate whilst titrating the salt with 4N sodium hydroxide. When the pH began to deviate from neutrality the phases were separated and the aqueous phase extracted with fresh ethyl acetate. All the organic solutions were then combined, washed with water, and brine and dried over anhydrous sodium sulphate. Removal of the solvent *in vacuo* gave the ylid as a clear yellow gum (174) (1.4 g, 1.87 m. moles, 94% from (170).

7'-(trans 1'-Carbonyl p-toluenesulphonylethoxy-4'-(propylene-1,3-dithio)oct-2'-enyl)-trans 7-tetrahydropyranyloxy-4-(propylene-1,3-dithio)oct-2-enoate (175).- A stirred solution of the ylid (174) (1.4 g, 1.94 m.moles) and the aldehyde (169) (1.0 g, 3.2 m.moles) in dry benzene was heated under reflux for 36 hours. After cooling the solvent was removed *in vacuo* and the gummy product mixture chromatographed on grade II neutral alumina to give the pure all trans dimer (175) (540 mg, 0.7 m.moles, 35% from (174)), (yields varied from 30% to 50% for this step), ν_{max} 1710 1638, 1592, and 1315 cm^{-1}, δ ($CDCl_3$) 1.22 (6H; CH_3-C, m), 1.72 (18H; CH_3-C(O-)-CH_2-CH_2-, -S-CH_2-CH_2-CH_2-S-, -C-CH_2-CH_2-CH_2-CH_2-O, bm), 2.44 (3H; CH_3-aromatic, s), 2.68 (8H; -S-CH_2-CH_2-CH_2-S-, m),

3.44 (2H; -O-$C\underline{H}_2$-CH_2-S(O)$_2$-, t, J 6.5 Hz), 3.80 (3H; CH_3-$C\underline{H}$(OTHP), -CH_2-O-, m), 4.44 (3H; -O-$C\underline{H}_2$-CH_2-S(O)$_2$-, -O-$C\underline{H}$-O-, t + m, J 6.5 Hz), 4.86 (1H; CH_3-$C\underline{H}$(O-C(O)-)-, m), 5.82 (1H; -CH=CH-C(O)-, d, J 16.0 Hz), 6.02 (1H; -CH=CH-C(O)-, d, J 16 Hz), 6.72 (1H; -CH=CH-C(O)-, d, J 16.0 Hz), 6.80 (1H; -CH=CH-C(O)-, d, J 16.0 Hz) 7.24 (2H; aromatic, d, J 8.0 Hz), 7.70 (2H; aromatic, d, J 8.0 Hz) (Found M^+, 688.17156. $C_{31}H_{44}O_7S_5$ requires 688.16905, fragment corresponds to loss of the tetrahydropyranyl moiety, possibly as dihydropyran).

7'-(trans 1'-Carbonyl p-toluenesulphonylethoxy-4'-(propylene-1,3-dithio)oct-2'-enyl)-trans 7-hydroxy-4-(propylene-1,3-dithio)oct-2-enoate (191).- The dimer (175) (2.26 g, 2.92 m.moles) was dissolved in 50% ethyl acetate/methanol (50 ml) and 5N aqueous hydrochloric acid (4 drops) added. After stirring for one hour the reaction mixture was poured onto dilute aqueous sodium hydrogen carbonate/ethyl acetate. The separated organic layer was washed with water, brine, and dried over anhydrous sodium sulphate. Removal of solvent in vacuo gave 2.12 g of product. Purification via preparative t.l.c. (developing solvent: 50% ethyl acetate/light petroleum) of a small portion of the slightly impure product gave pure alcohol (191), ν^*_{max} 3500, 1720, 1650, 1605, and 1310 cm^{-1} δ ($CDCl_3$) 1.20 (3H; $C\underline{H}_3$-C, d, J 6.0 Hz), 1.24 (3H; $C\underline{H}_3$-C, d, J 6.0 Hz), 1.87 (12H; -C-$C\underline{H}_2$-$C\underline{H}_2$-C-, -S-CH_2-$C\underline{H}_2$-CH_2-S-, m), 2.33 (1H; CH_3-C(O$\underline{H}$)-, bs, exchanges with D_2O) 2.50 (3H; CH_3-aromatic, s), 2.77 (8H; -S-$C\underline{H}_2$-CH_2-$C\underline{H}_2$-S-, m), 3.53 (3H; CH_3-$C\underline{H}$(OH)-, -O-$C\underline{H}_2$-CH_2-S(O)$_2$-, t + m, J 6.5 Hz), 4.60 (2H; -O-CH_2-$C\underline{H}_2$-S(O)$_2$-, J 6.5 Hz), 5.00 (1H; CH_3-$C\underline{H}$(O-C(O)-)-, m), 6.03 (1H; -CH=CH-C(O)-,

d, J 16.0 Hz), 6.42 (1H; -CH=CH-C(O)-, d, J 16.0 Hz), 6.98 (1H; -CH=CH-C(O)-, d, J 16.0 Hz), 7.07 (1H; -CH=CH-C(O)-, d, J 16.0 Hz) 7.53 (2H; aromatic, d, J 8.0 Hz), 8.00 (2H; aromatic, d, J 8.0 Hz).

7'-(trans 1'-Carbonylhydroxy-4'-(propylene-1,3-dithio)oct-2'-enyl)-trans 7-hydroxy-4-(propylene-1,3-dithio)oct-2-enoate (190).- The dimer (191) (150 mg of product from previous experiment) was dissolved in dry benzene (2 ml) and 1,5 diazabicyclo-(4,3,0) non-5-ene added (7 drops). After about five minutes the mixture was dissolved in ether/water and the ether extracts quickly discarded. The aqueous layer was rapidly acidified and extracted thoroughly with ethyl acetate. The organic phase was washed with water, brine and dried over anhydrous sodium sulphate to give 100 mg of pure hydroxyacid (190) (0.197 m.moles ,90% from (175), ν^*_{max} 2500-3500, 1710, 1635 cm^{-1}, δ ($CDCl_3$) 1.26 (6H; CH_3-C, m), 1.84 (12H; -C-CH_2-CH_2-C-, -S-CH_2-CH_2-CH_2-S-, m), 2.74 (8H; -S-CH_2-CH_2-CH_2-S-, m), 3.80 (1H; CH_3-CH(OH)-, m), 5.00 (1H; CH_3-CH(O-C(O)-), m), 6.10 (1H; -CH=CH-C(O)-, d, J 16.0 Hz), 6.14 (1H; -CH=CH-C(O)-, d, J 16.0 Hz), 6.88 (1H; -CH=CH-C(O)-, d, J 16.0 Hz), 6.94 (1H; -CH=CH-C(O)-, d, J 16.0 Hz).

Attempted preparation of 7'-(trans 1'-carbonylhydroxy-4'-(propylene-1,3-dithio)oct-2'-enyl)-trans 7-hydroxy-4-(propylene-1,3-dithio)oct-2-enoate lactone (215).- The hydroxyacid (190) (23 mg, 0.045 m.moles) was dissolved in dry benzene (1.5 ml) and dicyclohexylcarbodiimide (192) (12 mg, 0.058 m.moles) added. After stirring overnight (14 hours) the mixture was filtered free of dicyclohexylurea (197) and the solvent removed in vacuo. Preparative t.l.c. (developing solvent: 50% ethyl acetate/light

Preparative t.l.c. (developing solvent: 50% ethyl acetate/light petroleum) of the product mixture gave a compound assigned the structure (199) (10 mg, .01 m.moles, 34 %, R_f 0.45), δ ($CDCl_3$) 1.26 (6H; $C\underline{H}_3$-C, m), 1.94 (33H; -S-CH_2-$C\underline{H}_2$-CH_2-S-, cyclohexyl methylenes, CH_3-CH(O$\underline{H}$)-, bm), 2.80 (8H; -S-$C\underline{H}_2$-CH_2-$C\underline{H}_2$-S-, m), 3.80 (4H; CH_3-C$\underline{H}$(OH)-, -N$\underline{H}$-, -N-C$\underline{H}$, m), 5.00 (1H; CH_3-C$\underline{H}$(-O-C(O)-, m), 6.10 (1H; -CH=CH-C(O)-, d, J 16.0 Hz), 6.44 (1H; -CH=CH-C(O)-, d, J 16.0 Hz), 6.80 (1H; -CH=CH-C(O)-, d, J 16.0 Hz), 6.88 (1H; -CH=CH-C(O)-, d, J 16.0 Hz).

II. 1-Hydroxybenzotriazole (200) (54 mg, 0.4 m.moles) was added to a solution of the hydroxyacid (190) (94 mg, 0.19 m.moles) and the stirred solution cooled in an ice-bath. After cooling, dicyclohexylcarbodiimide (192) (45 mg, 0.22 m.moles) was added and the solution left for 16 hours at room temperature. The mixture was added to ethyl acetate, filtered, washed with water and brine and dried over anhydrous sodium sulphate. Removal of the solvent *in vacuo* gave 161 mg of product which on preparative t.l.c. (developing solvent: 50% ethyl acetate/light petroleum) gave, once more the amide (199) (45 mg, .06 m.moles, 34 %).

7'-(*trans* 1'-Carbonylhydroxy-4'-(propylene-1,3-dithio)oct-2'-enyl)-*trans* 7-hydroxy-4-(propylene-1,3-dithio)oct-2-enoate lactone (215).- To the hydroxyacid (190) (184 mg, 0.36 m.moles) in dry tetrahydrofuran was added N,N' carbonyldiimidazole (207) (71.28 mg, 0.44 m.moles). After 18 hours the solution was diluted with dry benzene (5 ml) and 1,5 diazabicyclo(4,3,0)non-5-ene (147) (1 drop) added. After a further 18 hours the volume

was reduced and the solution passed quickly through a short column of grade II neutral alumina, eluting with dry benzene. Combination of the two ml fractions according to analytical t.l.c. evidence afforded, after removal of solvent in vacuo, the pure white crystalline macrocycle (215) (107 mg, 0.22 m.moles, 60%), ν^{*}_{max} 1705 cm^{-1}, δ ($CDCl_3$) 1.20 (6H; CH_3-C, m), 1.90 (12H; $-CH_2-CH_2-C-CH_3$, $-S-CH_2-CH_2-CH_2-S-$, m), 2.70 (8H; $-S-CH_2-CH_2-CH_2-S$ m), 5.0 (2H; CH_3-CH, m), 6.10 (1H; -CH=CH-C(O)-, d, J 16.0 Hz), 6.12 (1H; -CH=CH-C(O)-, d, J 16.0 Hz), 6.73 (1H; -CH=CH-C(O)-, d, J 16.0 Hz), 6.75 (1H; -CH=CH-C(O)-, d, J 16.0 Hz), (M, low resolution mass spectrum, 488. Calculated 488.).

(±)-Pyrenophorin and meso-pyrenophorin (1 and 217 respectively).- A mixture of silver nitrate (183.6 mg, 1.08 m.moles), freshly prepared N-chlorosuccinimide ($_{218}$) (128.2 mg, 0.96 m.moles) in acetonitrile (5.2 ml) and water (2.1 ml) was cooled to 0° under an atmosphere of dry nitrogen. The dithioacetal (215) (59 mg, 0.12 m.moles) in acetonitrile (2.6 ml) was added, followed by a 0.5 ml rinse of acetonitrile, and the mixture stirred for 25 minutes. Sodium hydrogen carbonate (80 mg, 0.96 m.moles) was added in portions over the first ten minutes of the latter period. Dimethylsulphoxide (0.52 ml) was then added and the mixture stirred for a further 30 minutes.

The solution was poured onto water and extracted with ethyl acetate; the organic layer was further washed with water and brine and dried over anhydrous sodium sulphate. Preparative t.l.c. (developing solvent 30 % ethyl acetate/light petroleum) of the solvent freed product mixture gave a less polar component

(5 mg), and a more polar component (5 mg). The less polar compound was identical in all respects (n.m.r., i.r., mass spectrum, t.l.c., and g.l.c.) save rotation with the naturally occurring (-)-pyrenophorin, and was assigned the structure of (±)-pyrenophorin (1) (5 mg, 0.016 m.moles, 13%, m.p. 124-125° ex diethylether/light petroleum). The more polar compound, though chromatographically different, showed a mass spectrum indistinguishable from that of the less polar compound; since other spectral properties were virtually identical, it was assigned the structure of the meso diastereomer (217) (5 mg, 0.016 m.moles, 13%, m.p. 118-119° ex diethylether/light petroleum).

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