THE CHEMISTRY OF BYSSOCHLAMIC ACID

A thesis presented by

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Abstract

The acetate hypothesis of biosynthesis of mould metabolites is reviewed. The evidence relating to configurational correlations is described critically. The previous chemistry of byssochlamic acid is discussed and the following original contributions to this field are presented.

(1) A method for the degradation of maleic anhydrides to ketones and its application to the structural problem of byssochlamic acid.

(2) The chemical proof of structure and absolute configuration of byssochlamic acid. The rationalisation of the further reactions of byssochlamic acid on the basis of this structure.

(3) The relative configuration of glauconic acid at C_3 and C_{12}.

(4) A novel autoxidation reaction of disubstituted maleic anhydrides.

(5) The course of metal hydride reduction of maleic anhydrides.

(6) A method for the preparation of thioanhydrides.

Acknowledgements

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The Acetate Theory of Biosynthesis

The earlier, speculative, structural correlations [1,2], concerning the biosynthesis of mould metabolites have more recently been rendered into a concise form which has become known as the acetate theory.[3] This general hypothesis, that many natural products are produced *in vivo* by the linking of two carbon units, has received much experimental confirmation.[4] It has been studied especially in moulds both because it seems to be an important route in these organisms and also because of the relative ease of feeding and isolation of metabolites. Much of the simplicity of the original hypothesis has been lost as more evidence has been accumulated, but at the same time a deeper insight into the mechanisms used by the living systems is appearing.

Although the earliest biogenetic experiments were conducted with radioactively labelled acetate it has since been shown that the chain extending species is malonate,[5,6] in the form of the coenzyme-A-half ester.[7] This is chemically reasonable in view of the greater enolising power of the malonyl residue as opposed to that of the acetyl species. It has been shown that the chain extension process may be visualised as a simultaneous condensation and decarboxylation,[8] as in the figure (1).

In this way a poly-β-ketone chain may be constructed. Lynen[7] sees the conversion of acetate to malonate as an enzymic carboxylation reaction, which explains the facile incorporation of acetate.

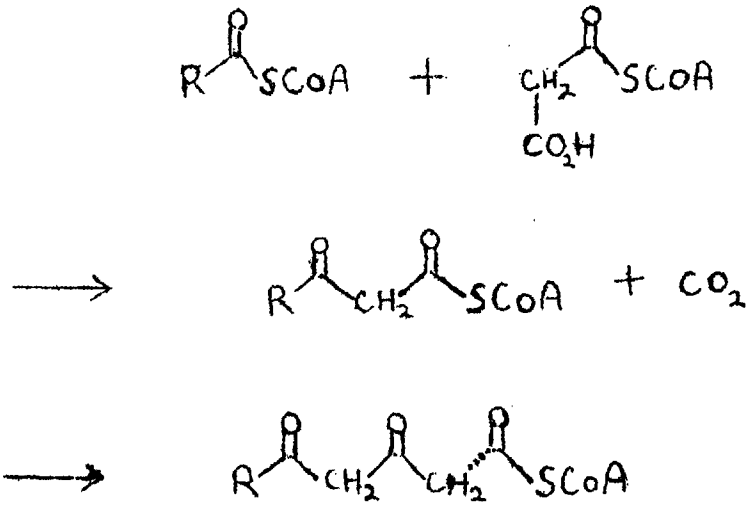


fig.(1)

The chain initiating species may be any one of a large number of natural carboxylic acids, again as the coenzyme-A-ester. The most common "starter" species is, of course, acetyl coenzyme-A, which fact rationalises the previously observed higher incorporation of acetate into the chain terminal positions, as compared with the in-chain positions.

The usual processes of C- and O- alkylation by methionine[9] and isopentenyl pyrophosphate[10] have experimental justification

and occur at chemically acceptable sites, i.e. anionic positions, fig.(2).

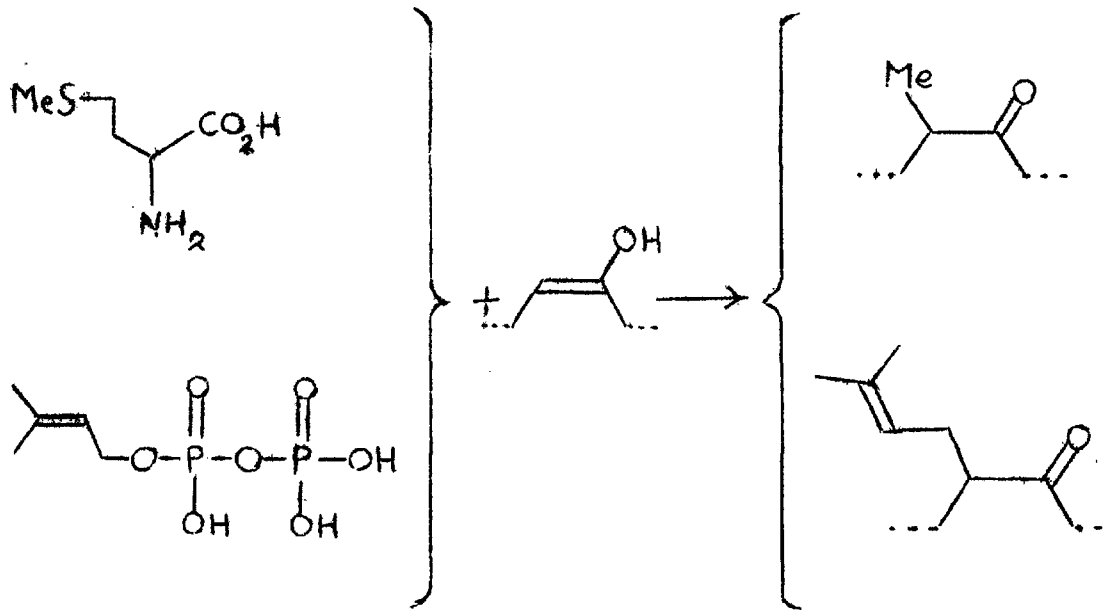


fig.(2)

Chain branching is often accomplished by suitable linkage of two distinct chains, and one further modification to the general theory is the use of propionate[11] in certain cases, as a chain extending residue.

All the above mentioned facets of the acetate pathway have withstood reasonable experimental examinations and so may constitute a basis for further speculation. It should, however, be pointed out at this stage that none of the

proposed poly-β-ketone intermediates have been isolated from living organisms.[12] Whilst recent studies by Birch[13] on the chemical properties of such compounds suggest that their inherent instability may be the reason for their absence from metabolic extracts it has been suggested by Woodward[14] that condensation to a poly-β-ketone and subsequent transformation to more stable products may take place in an enzyme bound condition. Such a process would of course preclude the isolation of the intermediates by present techniques. It was also argued that the aglycone portions of the macrolide antibiotics, such as magnamycin, fig.(3) represent the closest structural approach to the hypothetical open chain poly-β-ketones, which are presently available.

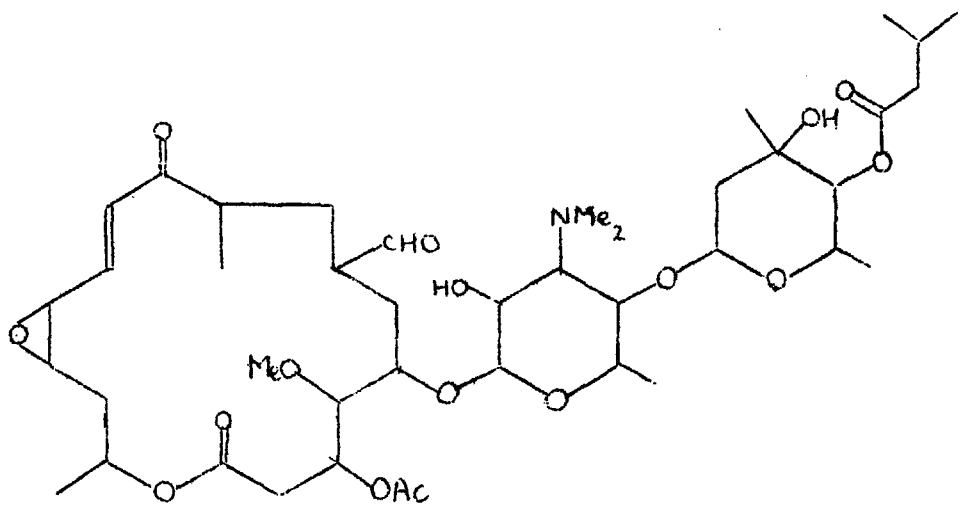


fig.(3)

Another hypothesis, which would explain the lack of polyketones, is that the chain is modified as each unit is added. That is, it may undergo reduction of the carbonyl groups in the case of non-aromatic genesis, for example the fatty acids,[15] or alternatively, the chain may be cyclised in groups of three or four units to the correspondingly more stable aromatic compounds. In this way, the generation of a tricyclic molecule such as islandicin[16] might be visualised in two, distinct, ways.

(1) The formation of an enzyme bound poly-β-ketone, comprised of eight units, followed by self condensation, fig.(4).

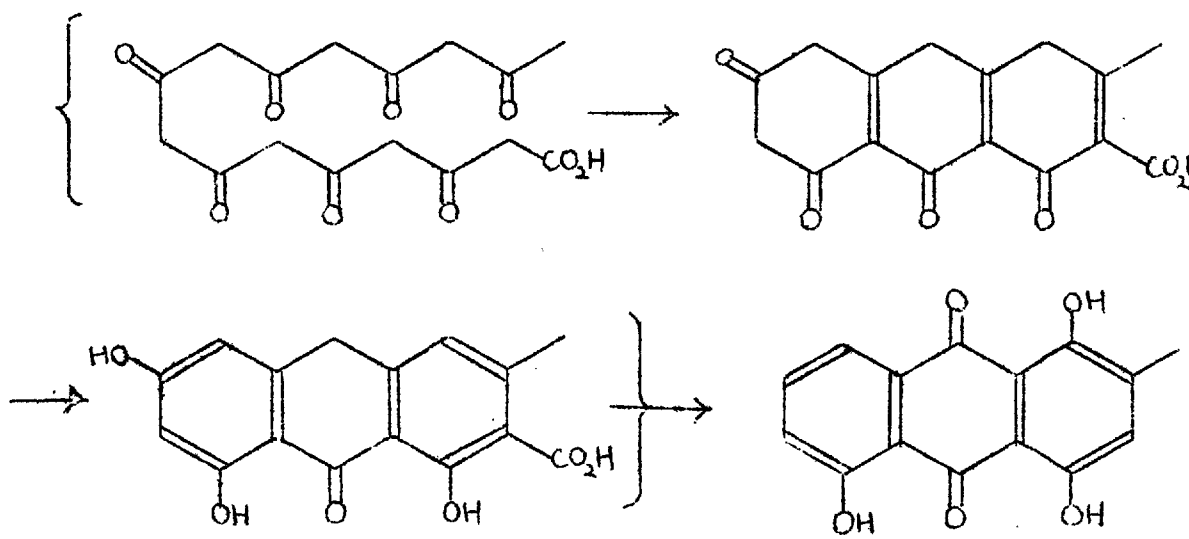


fig.(4)

Oxidation and decarboxylation steps complete the synthesis. The parentheses in the figure serve to represent the "bound" condition of the substrate.

(2) The initial formation from four units of a benzenoid compound, followed by a dimerisation of the same. This process results in the elaboration of the anthrone residue which may be further oxidised and decarboxylated to islandicin, fig.(5).

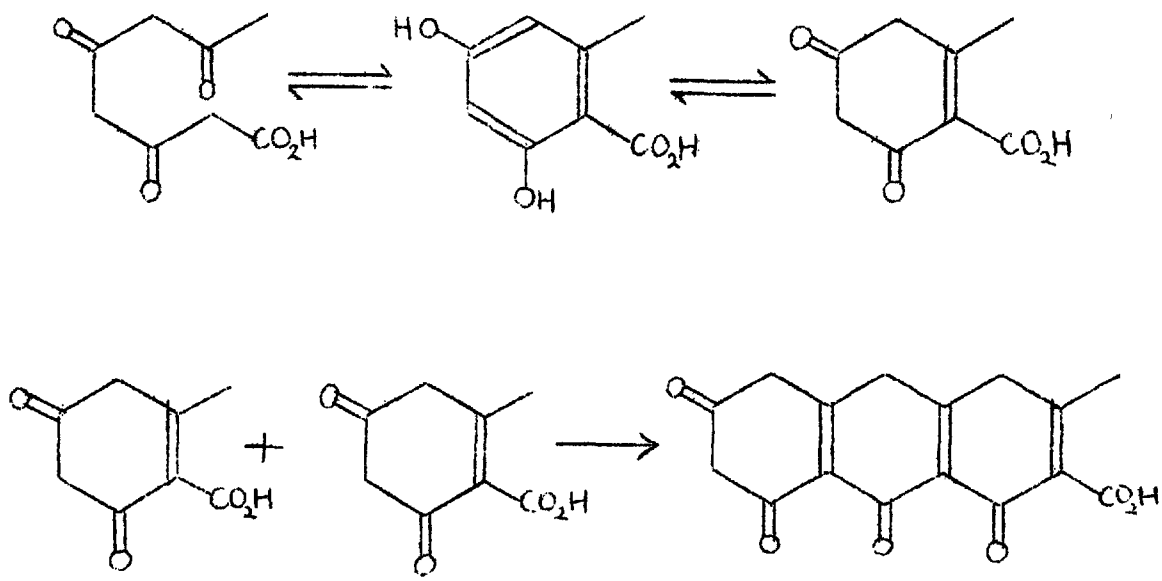


fig.(5)

The latter is chemically less likely since the postulated intermediate, orsellinic acid, does not dimerise under physiological conditions of acidity and temperature. However, both schemes would produce the same radioactive labelling pattern when derived from a precursor such as acetate. Alternatives, such as the above, can be differentiated by an accurate comparison of the levels of incorporation at each labelled position in the molecule. Thus Gatenbach[17] has shown that P.islandicum readily produced labelled islandicin when radioactive $2^{-14}C$ malonate was added to the culture medium. He has degraded the molecule and shown that only the C-Me group shows the lower activity, expected of the initial degradation of malonate to acetate, which then becomes the chain starter. This result favours the first scheme as the mode of genesis of islandicin.

With respect to the size of the molecules which are derived by this pathway most of them contain nine or less C_2 units, and although there are exceptions to this rule most of these can be rationalised on the basis of the dimerisation of a smaller unit. Thus corynomycolic acid[18] from Corynebacterium diphtheriae, could be derived by an aldol condensation of two C_{16} chains, fig.(6). However, several bacterial fatty acids have chain lengths of over 20 carbon

atoms, for example phthioic acid[19] from Mycobacterium tuberculosis, fig.(7).

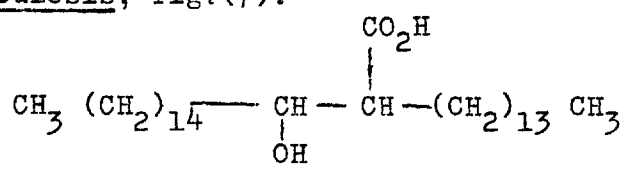


fig.(6)

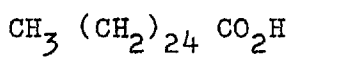


fig.(7)

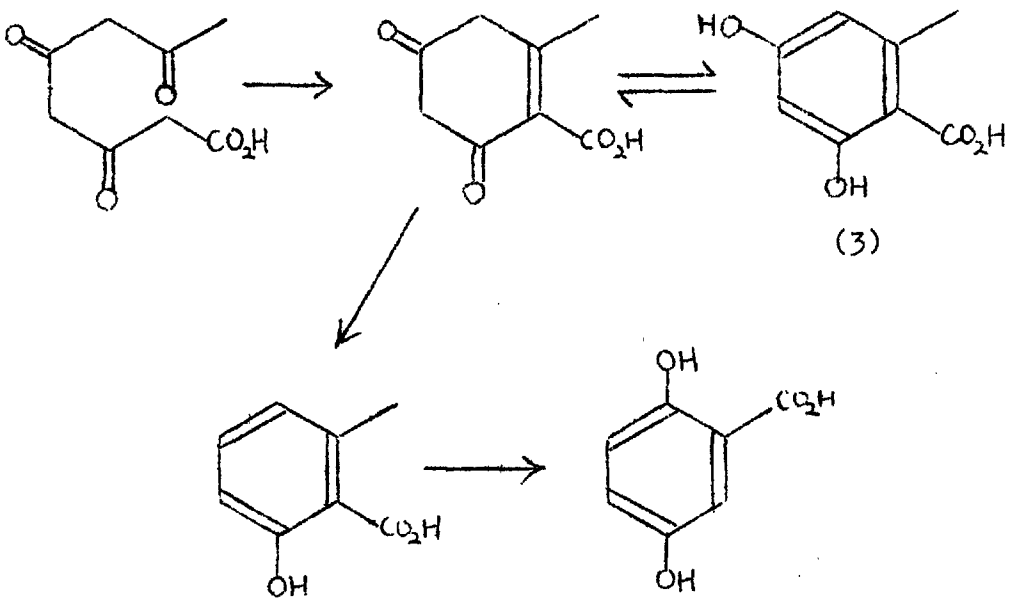
It is of interest to note that the majority of terpenoids seem to be formed by head-to-tail linking of four isoprene units or less i.e. a chain length of 16 carbon atoms, which must correspond roughly in size to the acetate limit of 18 carbon atoms.

Although it is not possible to discuss here the wide range of mould metabolites in detail some examples of the experimental work and the conclusions drawn therefrom will be given in order to illustrate the more general points made above.

1. Metabolites derived from C_2 and C_1 units

There is a small group of metabolites constructed from four C_2 units which seem to have a more general role as intermediates in the biosynthesis of other compounds.

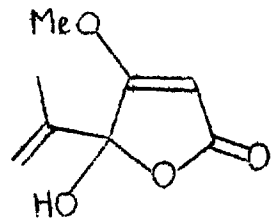
These are 6-Methyl salicylic acid[7](1)(P.griseofulvum),gentisic acid[20](2) (P.urticae), and orsellinic acid[21](3) (P.patulum). Each of these compounds has been obtained radioactively labelled from precursors such as $1^{-14}C$ acetate and $2^{-14}C$ malonate. Degradation of the compounds gave the labelling pattern expected of a β -tetracarbonyl precursor, which is cyclised and subjected to the reasonable transformations shown in the figure, fig.(8).



(1) (2)

fig.(8)

In the moulds P.barnensis and P.cyclopium, as well as orsellinic acid, there occurs another metabolite, penicillic acid[22](4).



(4)

Mosbach[23] and Bentley[24] have both been able to show that labelled orsellinic acid is a precursor of this metabolite under conditions in which there seemed to be no intermediate degradation of orsellinic acid to acetate. This was proved by examination of the activity of the fatty acid fractions from the mycelium. Further, by the use of orsellinic acid labelled as in fig.(9), Mosbach degraded the isolated penicillic acid in such a manner as to prove the point of fission of the benzene nucleus.

The mould P.patulum produces, along with gentisyl alcohol, an antibiotic called patulin[25](5). It was first hypothesised by Birkinshaw[2] that patulin might be derived from gentisaldehyde. Bu'Lock and Ryan[26] have now shown that

labelled 6-Methyl salicylic acid is a precursor of this metabolite and have determined the labelling pattern of the derived patulin, fig.(10)

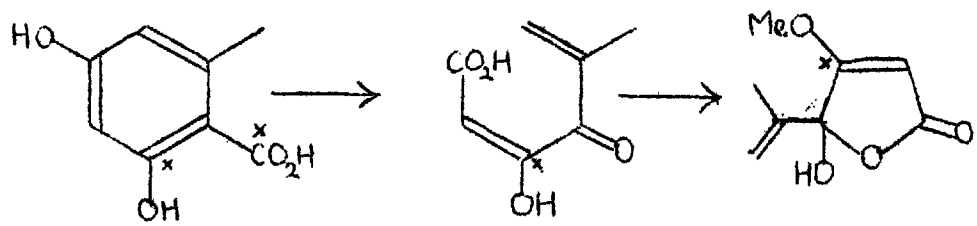
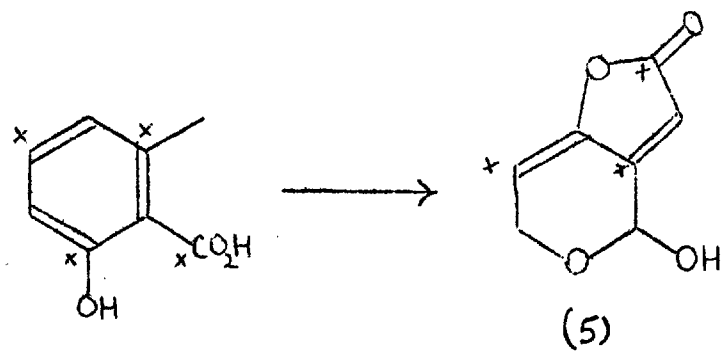


fig.(9)

In another investigation Tannenbaum and Basset[27] found that gentisic acid was not incorporated into patulin and also that the 6-Methyl salicylic acid, produced by P.patulum from labelled acetate, is generally of lower activity than the patulin. To explain this anomaly these authors suggested a reversible process, in which some intermediate, W, may be transformed into patulin or 6-Methyl salicylic acid. fig.(11).

Although from a chemical point of view, the cleavage of an aromatic nucleus at first seems unlikely, when one considers the possibilities of keto-enol tautomerism in these

phenols we can represent the process by the following mechanism, fig.(12).



(5)

fig.(10)

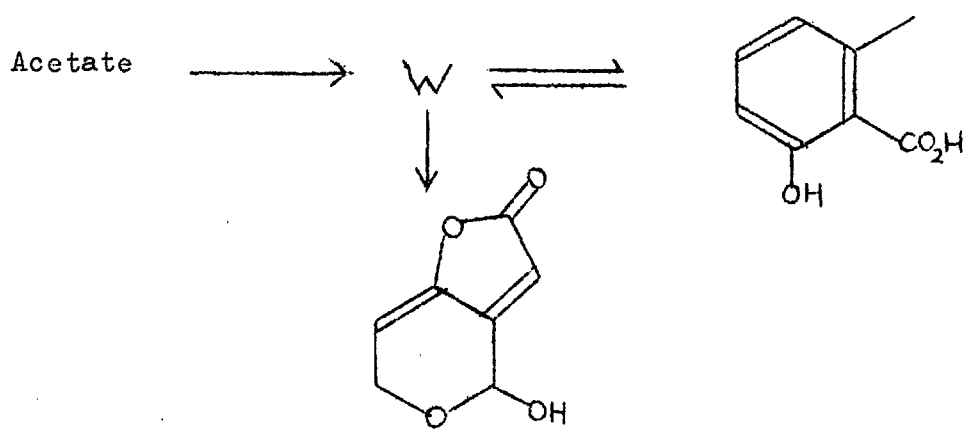


fig.(11).

The 6-methyl salicylic acid in this system could readily be transformed to gentisyl alcohol by decarboxylation and oxidation as in fig.(8).

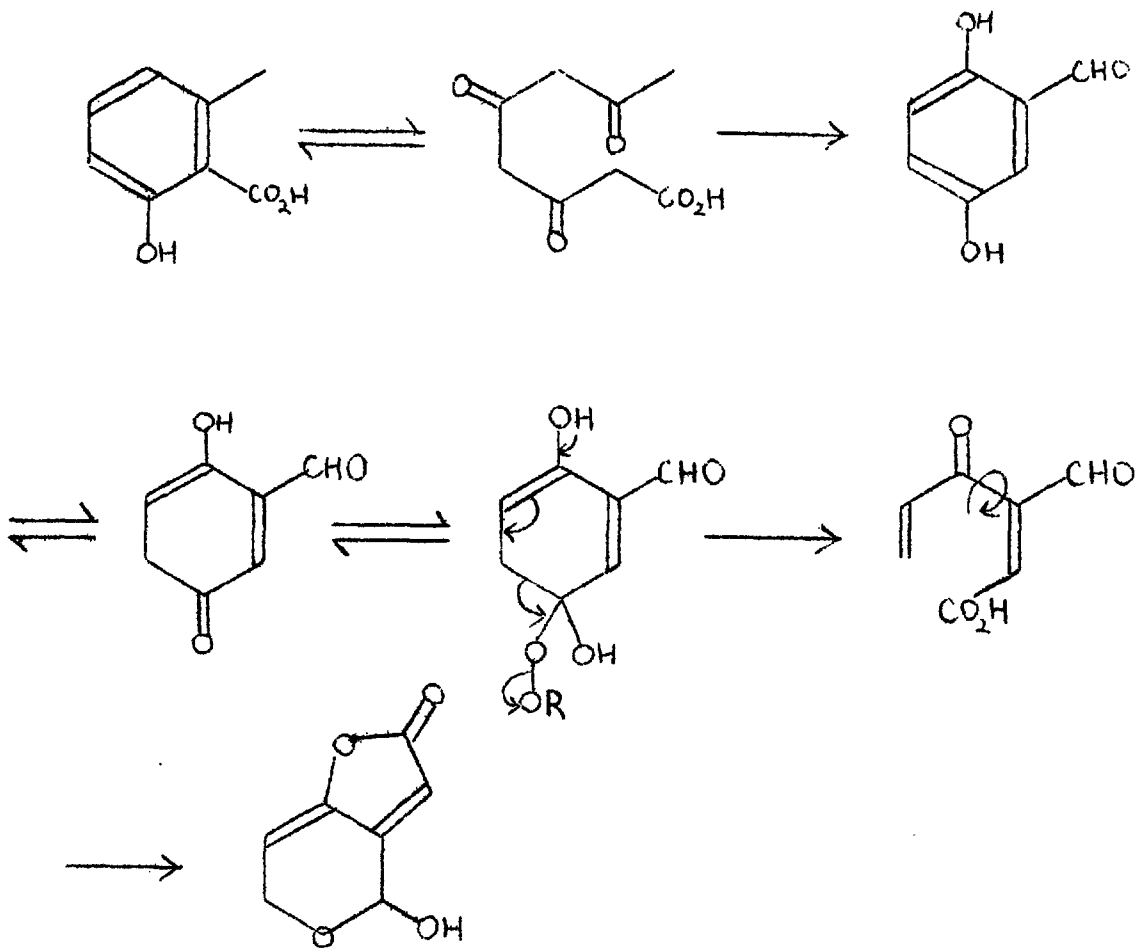


fig.(12)

A slight modification of the above scheme enables a simple mechanistic picture of the formation of penicillic acid from orsellinic acid to be drawn.

This process, fig.(13), involves the same type of Baeyer-Villiger cleavage of the ring as is envisaged in the generation of patulin.

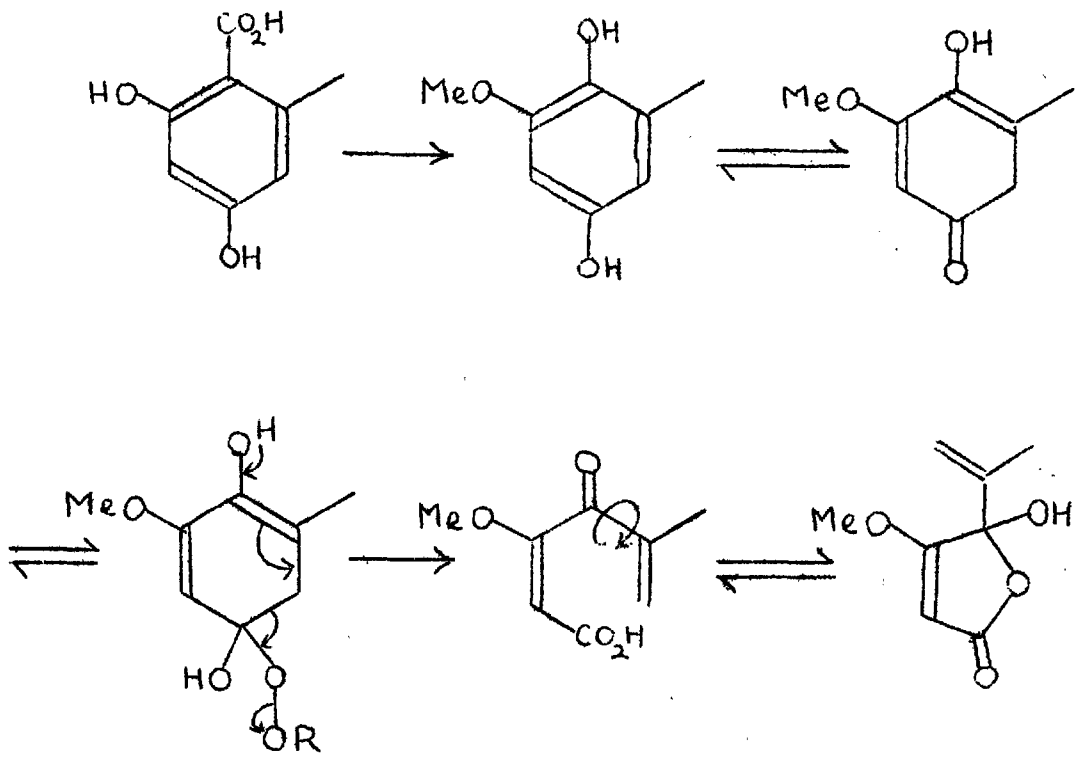
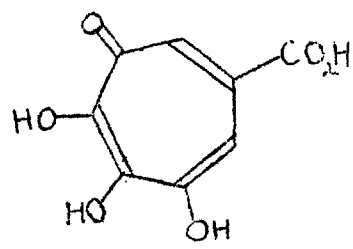


fig.(13)

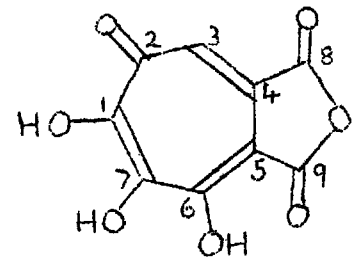
The mould tropolones represent an interesting group of compounds, both from the viewpoint of their unusual structures and also because of their apparently mysterious biosynthesis.

There are four compounds, conveniently grouped in pairs

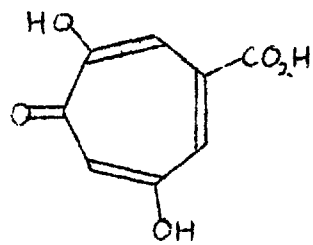
according to their respective origins, puberulic[28](6) and puberulonic (7) acids[29] (P.aurantiovirens), stipitatic[30](8) and stipitatonic (9) acids[31] (P.stipitatum).



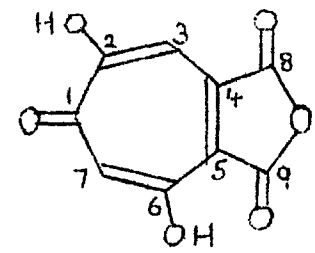
(6)



(7)



(8)



(9)

fig.(14)

Their mode of biosynthesis has been the result of much speculation. Thus Birch[32] has proposed a route from shikimic acid, and Seshadri[33] suggested the ring expansion of an aromatic precursor. The mono-carboxylic acid in the P.stipitatum metabolites has been shown to originate by

enzymatic decarboxylation of the dicarboxylic acid,[34] a process which is easily accomplished <u>in vitro</u> and it is reasonable to assume occurs in the other series.

Richards[35] has obtained labelled puberulonic acid from acetate and formate precursors and suggests the following labelling scheme for the decarboxylated material, fig.(15).

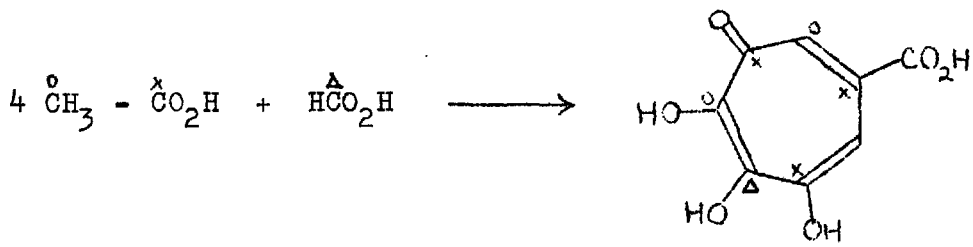


fig.(15)

A recent report indicates that C_9 of puberulonic acid, the labile carboxyl group, is derived from a C_1 unit, possibly formate.[36]

Bentley[37,38] in an extensive study of the <u>P.stipitatum</u> metabolites has derived a labelling pattern which is summarised in fig.(16).

It should be understood that the degradations undertaken by the various authors in this series are ambiguous to some extent, in that most of the activities of skeletal

carbon atoms are derived by difference values. However, the best rationalisation of the facts at present available is that the mould tropolones are derived from aromatic precursors by ring expansions and this hypothesis is described in the following scheme, fig.(17).

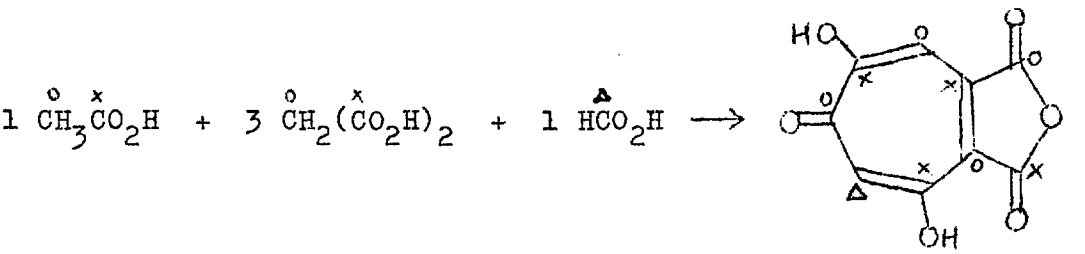


fig.(16)

In this scheme the supposed precursor is orsellinic acid, which is alkylated by formaldehyde or formate once for the P.stipitatum compounds and twice for the P.puberulum series. The ring expansion has several chemical analogies,[39] but of course the acceptance of the scheme must await further experiments with the labelled precursors. It is perhaps significant that this also gives rise to the observed labelling pattern in cyclopaldic acid[40](10), which occurs along with the reduced form, cyclopolic acid (11) in P.cyclopium.[41] (Westling).

The addition of C_1 units, postulated in the following biosynthetic scheme for the tropolone acids, is substantiated by many analogies, thus the widely occurring product citrinin[42] (12) (P.citrinum) has been shown by Birch[43] and his co-workers to be derived from five acetate units and three C_1 units, the latter from methionine, fig.(18).

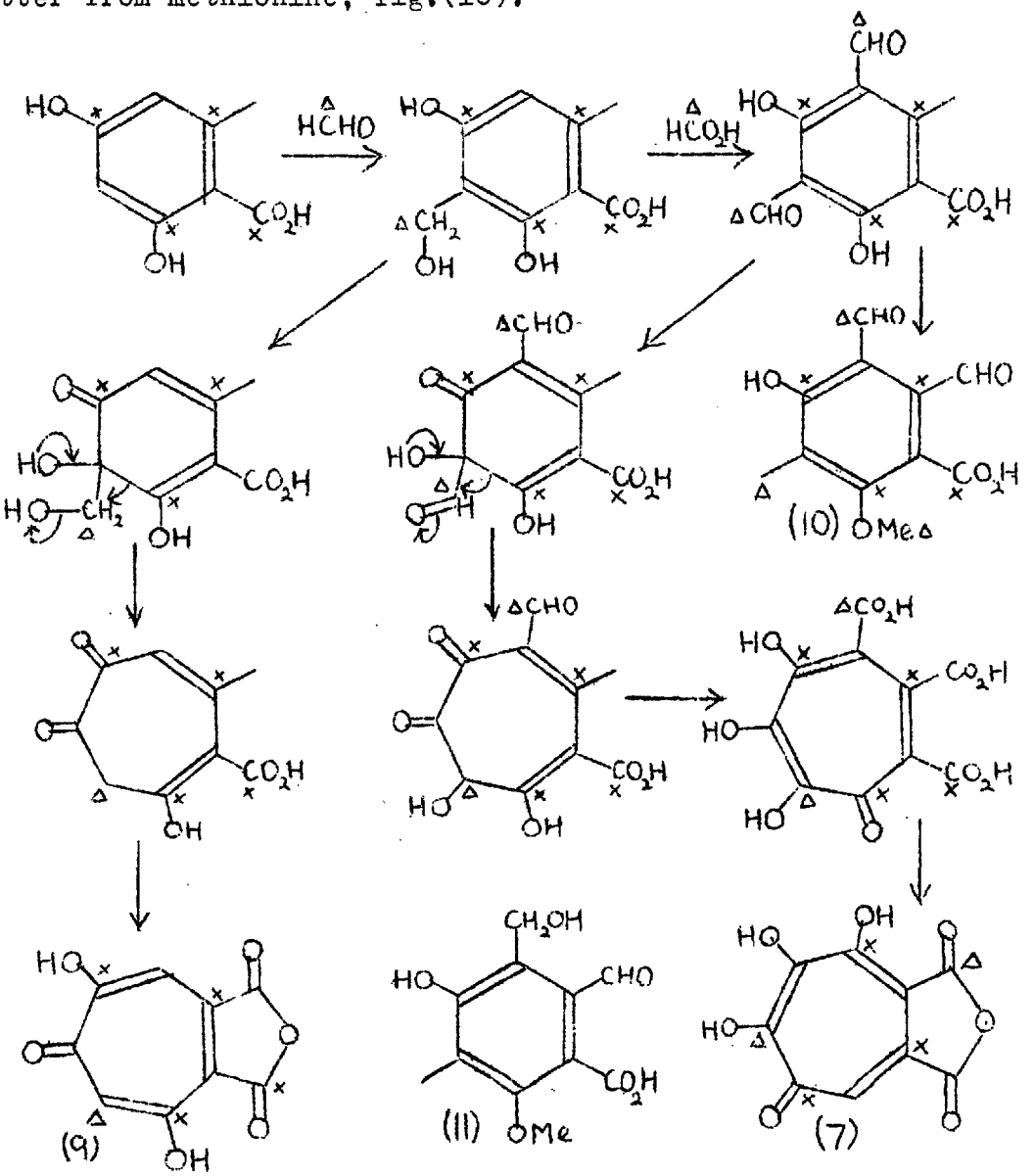
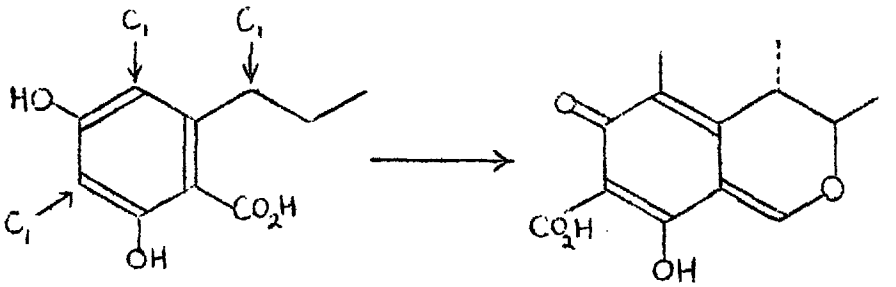


fig.(17)

As an example of a route which has been established as proceeding by the condensation of two chains, the work of Gatenbach and Mosbach[44] on the metabolite citromycetin (13) (P.frequentans) is of particular interest.

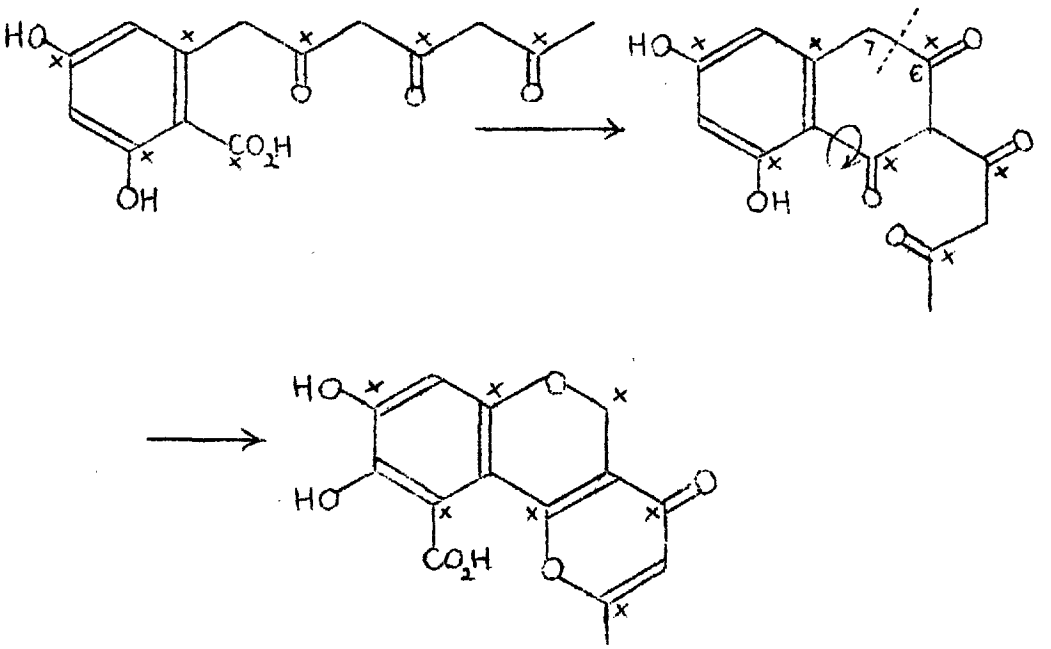


(12)

fig.(18)

This compound also represents a situation where the first order labelling pattern may be interpreted in several ways, which may only be differentiated by more accurate work. Thus Whalley,[45] on the basis of labelling experiments, postulated the route of fig.(19), a pathway which was in accord with the observed labelling pattern. This involved a bond fission between C_6 and C_7 followed by the formation of an ether linkage.

However, more recent work[44] has shown that citromycetin derived from $2^{-14}C$ malonate shows a lower incorporation of label into both the carboxyl and the methyl group.



(13)

fig.(19)

Gatenbach has rationalised this observation by postulating genesis from two chains, fig.(20).

This scheme yields the same labelling pattern as observed by Whalley and is chemically much simpler.

A somewhat different linkage between this, seemingly, ubiquitous orsellinic acid-type unit and a C_6-polyketone chain

readily explains the observed labelling pattern of palitantin[46] (14) (P.freqentans) and further offers a simple hypothesis concerning the biosynthesis of two structurally similar metabolites, fulvic acid[47](15) (P.griseofulvum) and fusarubin[48] (16) (Fusarium solani).

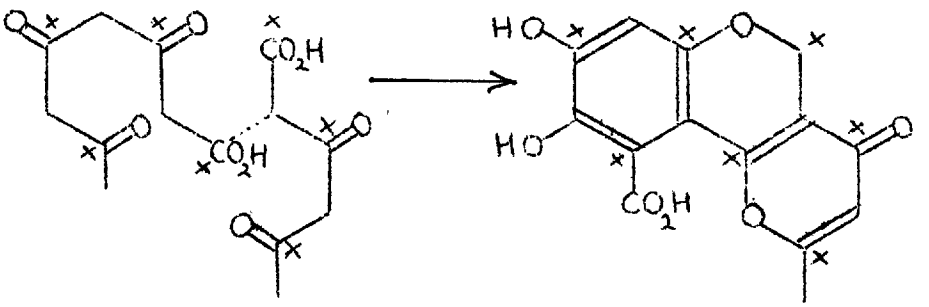
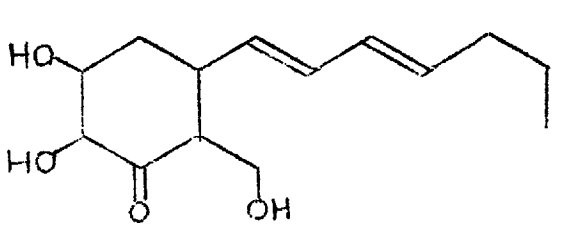
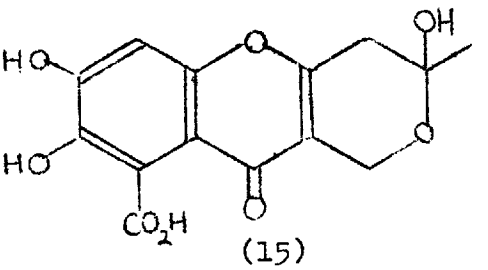


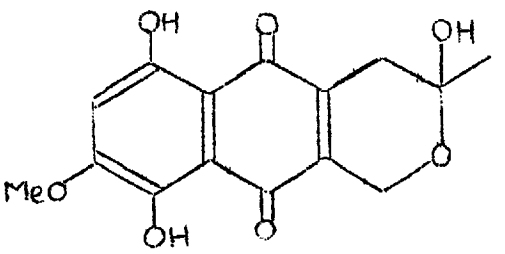
fig.(20)



(14)



(15)



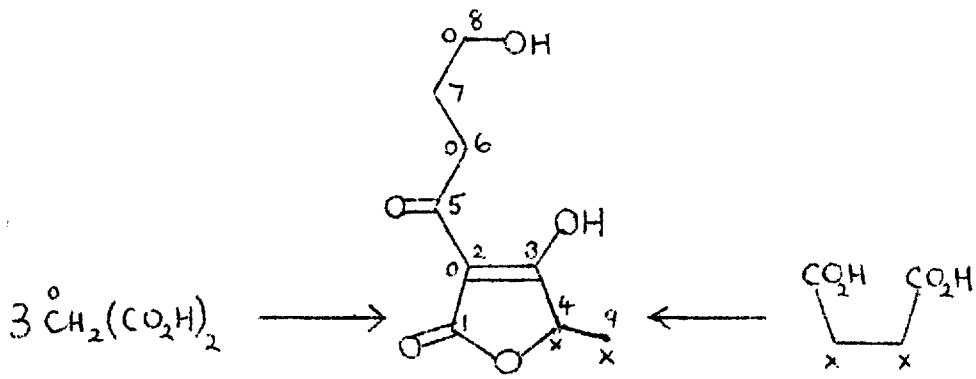
(16)

2. Metabolites involving citric acid cycle intermediates

The condensation of two acetate derived chains, as elaborated above, can, in principle, be modified to a condensation between an acetate derived chain and another acid, which could arise from some other metabolic pathway in the organism. There are now a number of examples of this process in which the secondary component appears to be derived from the citric[49] or glyoxylic acid cycle.[50] Amongst the known mould tetronic acids[51] there is a group of compounds of striking similarity whose structures were deduced by Raistrick and his co-workers. The biosynthesis has been shown to involve the coupling of an acetate derived chain of six or eight units with an intermediate of the citric acid cycle, probably oxalacetic acid. It has been shown that carolic acid[52,53,54] (18) (P.charlesii)[55] is derived in carbons 1,2 and 5-8 from malonate whereas the residual three carbon atoms showed a much lower incorporation of this precursor, as would have been expected by the operation on malonate of the tricarboxylic acid cycle, prior to incorporation, fig.(21).

Bentley has shown that $2^{-14}C$, $3^{-14}C$ succinic acid is incorporated into C_4 and C_9 of carolic acid without redistribution of the labelled atoms. Since succinic acid is but shortly removed from oxalacetic acid in the citric

acid cycle, a process which has been identified in moulds, it is possible to rationalise the genisis of a whole group of these tetronic acids.



(18)

fig.(21)

Thus it may be suggested that the acids, carlosic (20) (P.charlesii),[55] dehydrocarolic acid (21) (P,cinerascens),[57] carlic acid (22) (P.charlesii)[55], carolinic acid (23)[56] (P.charlesii)[53,55], as well as carolic acid (18), are all derived from a common precursor (19) which is the result of an aldol condensation between β-ketohexanoic acid and oxalacetic acid. For clarity the acids are shown in fig.(22) in their hydrated forms.

There is another well defined group of metabolites which seem to fit into this general thesis but at present there are no published accounts relating to experiments with labelled precursors for these compounds, so the following remarks are entirely speculative.

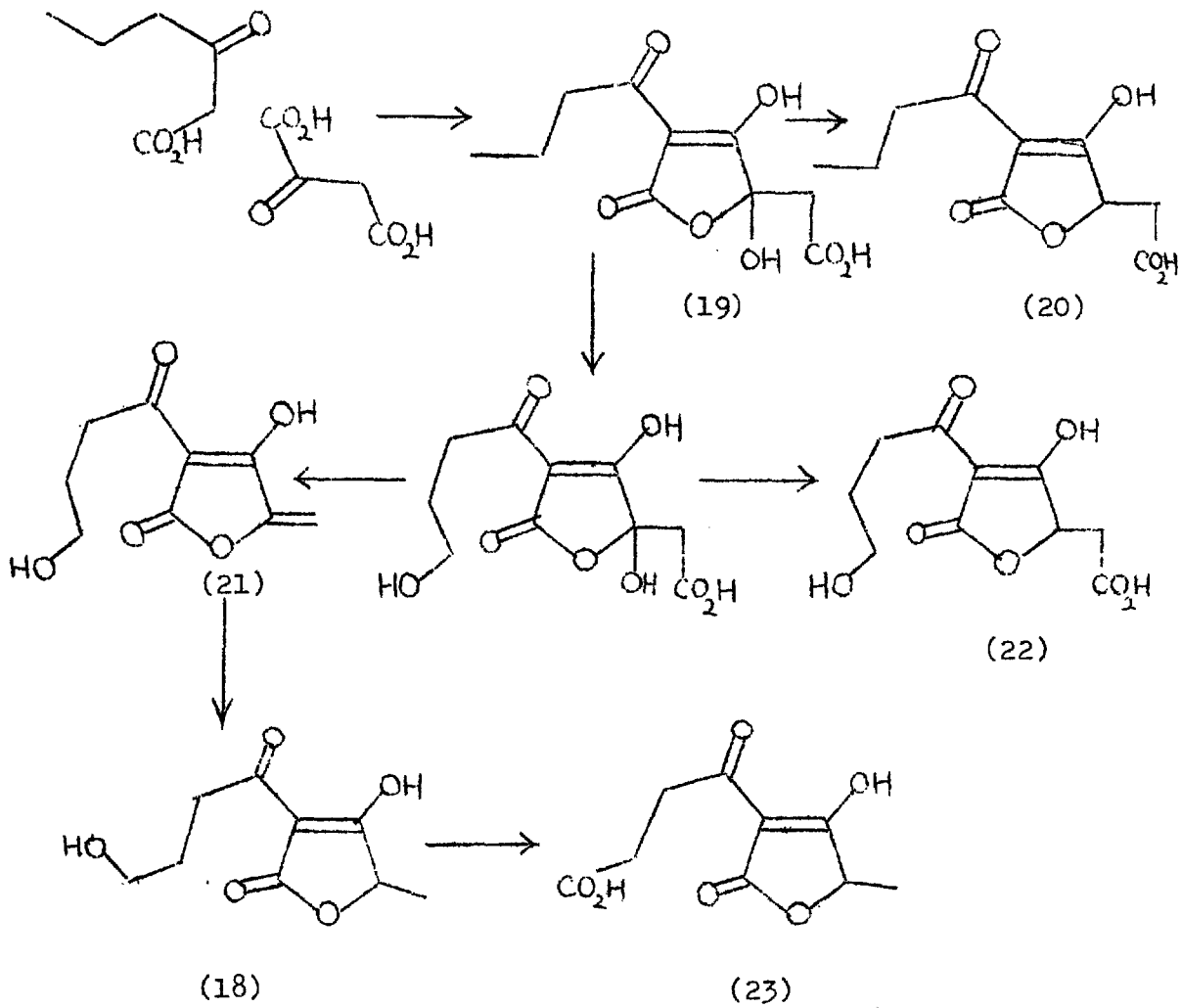


fig.(22)

These may be subdivided into two groups.

(a) Oxalacetate derived.

As in the case of the tetronic acids, above, these examples may be constructed by the condensation of a long chain β -hydroxy fatty acid, probably in the form of the carboxylated malonyl derivative, with oxalacetate. In this case the condensation takes place at the keto-group whereas the tetronic acids were derived by condensation at the carboxyl residue, fig.(23).

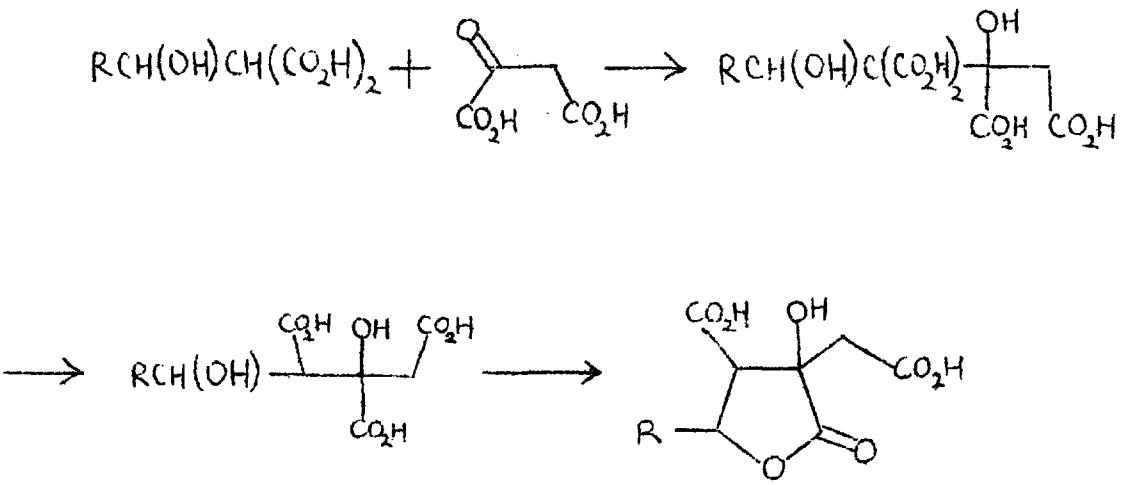
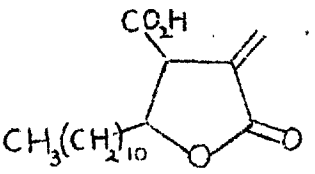


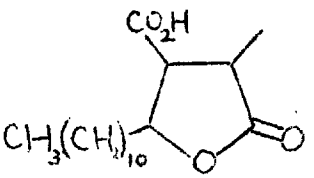
fig.(23)

Subsequent biochemically well established processes such as decarboxylation, dehydration, and saturation of conjugated double bonds lead to the following compounds, fig.(24).

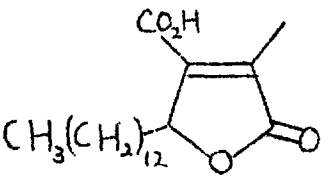
Nephrosterinic acid[58] (24) and nephrosteranic acid[58] (25) are clearly related and occur together in Nephromopsis-endocrocea.



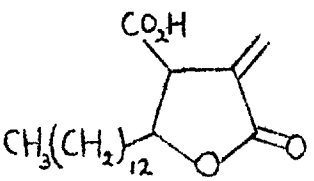
(24)



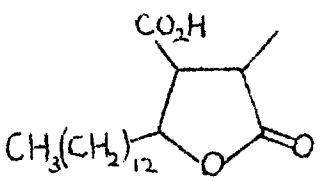
(25)



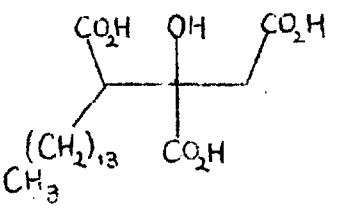
(26)



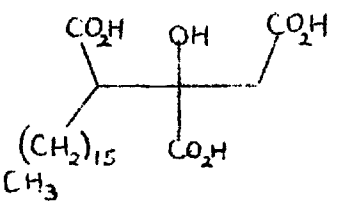
(27)



(28)



(29)

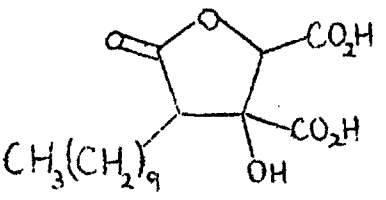


(30)

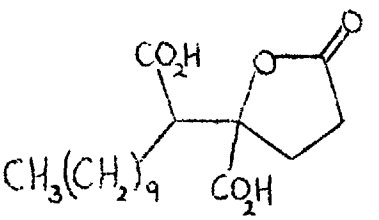
fig.(24)

Lichesterinic acid (26) (Cetraria islandica)[59] and d-proto-Lichesterinic acid (27) (Cetraria islandica)[60] are simply double bond isomers and are derived from a C_{16} acetate chain. Nephromopsic acid (28)[61] (Nephromopsia Stracheyi) is the dihydro-derivative of (27) and (26). The last two acids, nor-caperatic acid (29)[62] (Parmelia caperata) and agaricic acid (30)[63] (Polyporus officinalis) are open chain forms of the same precursors, lacking, however, the β-hydroxyl group necessary for lactonisation.

(b) Mineoluteic acid (31)[64] (P.mineoluteum) and spiculisporic acid (32)[65] (P.spiculisporum) appear, from their structures, to involve a different condensing acid, fig.(25).



(31)



(32)

fig.(25)

It might have been postulated that the former be derived from citric acid, as its coenzyme-A monoester, condensing onto a C_{10} - acetate derived unit. However, the structural relationships previously described seem to indicate that the more likely process is the reverse i.e. the condensation of an alkyl malonic acid onto a citric acid cycle intermediate. In this way mineoluteic acid (31) would be derived from a hydroxy-oxalacetate, a substance which is not an intermediate in the citric or glyoxylic acid cycles. The second compound, spiculisporic acid (32) offers no such difficulties in a condensation reaction between dodecanoic acid and α-keto glutaric acid, a well known intermediate of the tricarboxylic acid cycle.[49]

At this point it is convenient to discuss the biosynthesis of the compound which is the subject of this thesis, byssochlamic acid (33) and the related glauconic (34) and glaucanic acids (35). A preliminary report has appeared which indicates that glauconic acid is derived, at least in part, from acetate.[66]

An examination of the structures of these metabolites, fig.(26), suggests their common origin in the condensation of two essentially equivalent units.

It appeared very likely that the individual units were derived by pathways similar to those which led to the

tetronic acids or, speculatively, to the other mould acids discussed above.

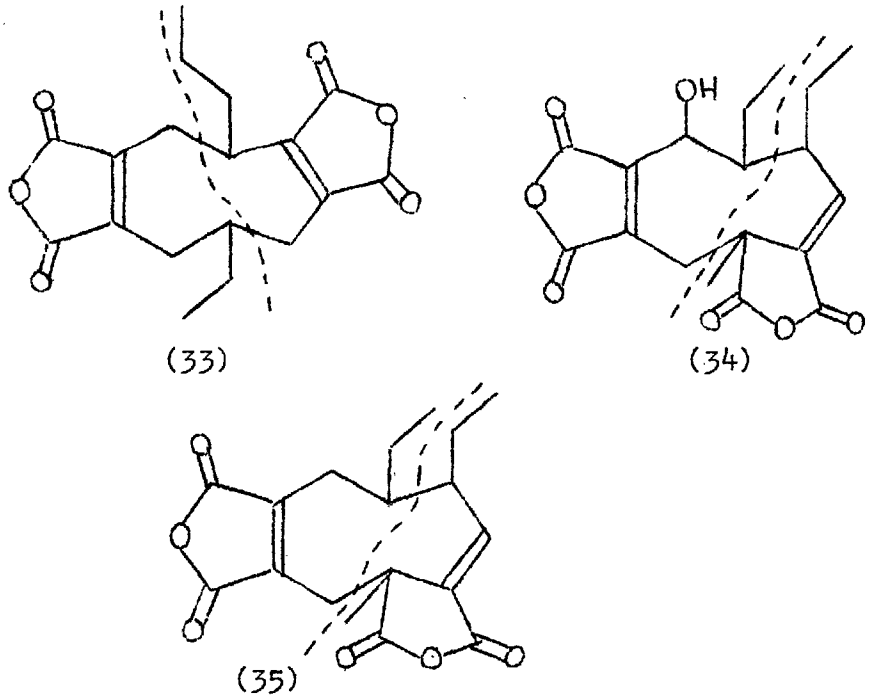
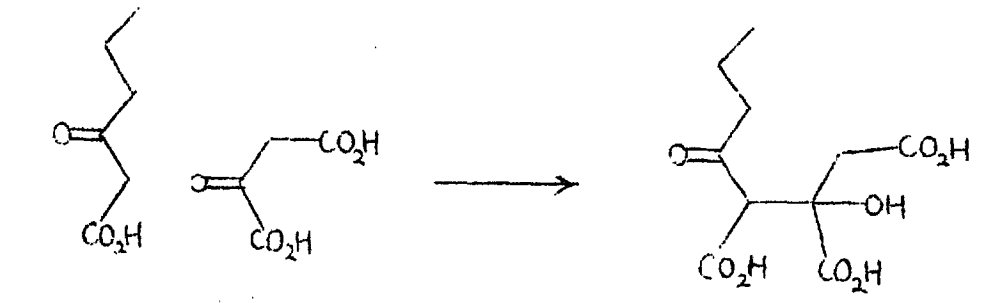


fig.(26)

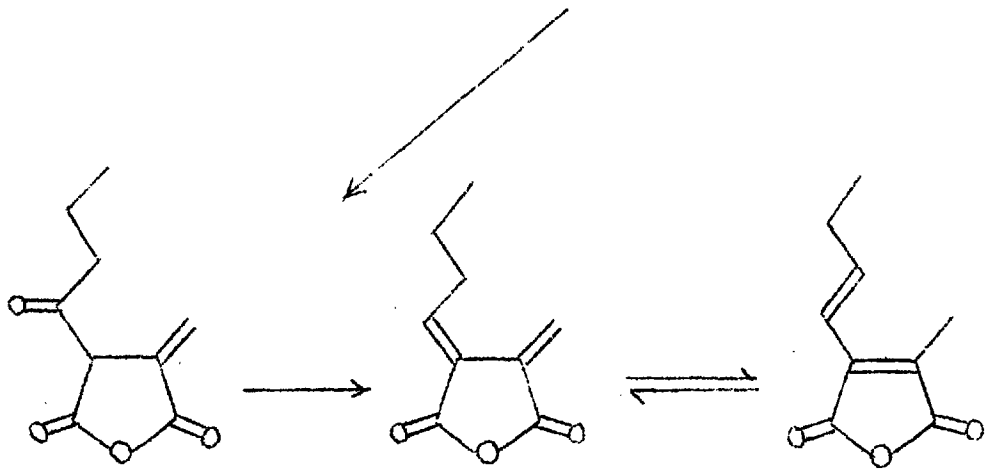
Thus, in this case, the condensation of a six carbon chain with oxalacetate at the carbonyl group would lead to an intermediate (36) which is arranged for anhydride formation, fig.(27). The product may then undergo a concerted loss of water and carbon dioxide to yield a substance (37), from which in principle it is possible to derive the carbon skeletons of the above three acids, as well as their double bond positions.

The postulated cyclisation scheme involves chemically acceptable mechanisms but is naturally, at present, only a

working hypothesis, fig.(28).



(36)



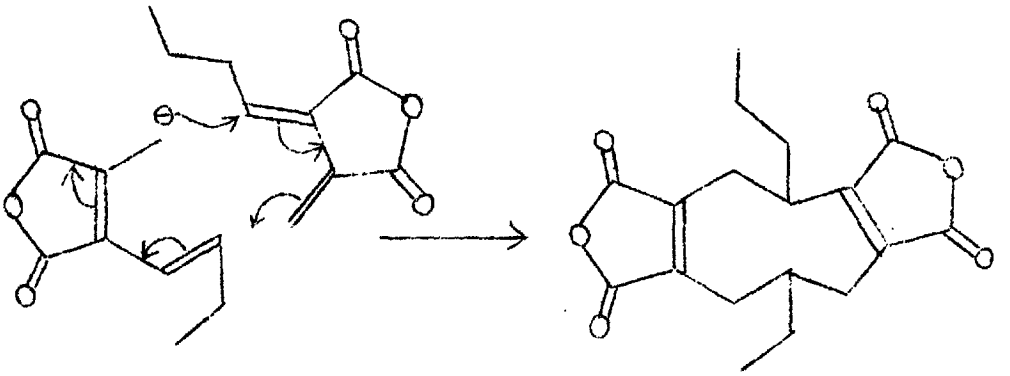
(37)

fig.(27)

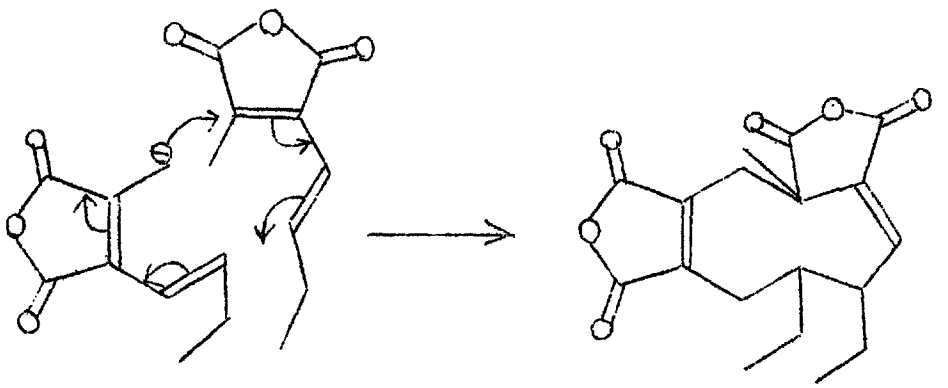
Glauconic acid (34) may be envisaged as the biochemical hydroxylation product of glaucanic acid (35).

Many of the metabolites described here have been described as shunt or overflow metabolites.[2] There are indications that they arise by the inhibition of an enzyme or also possibly the gain of some new enzymic activity,[49]

processes which are both probably initiated by the somewhat 'unnatural' growth conditions, i.e. high glucose concentration, under which the moulds have been cultivated.



(33)



(35)

fig.(28)

The above discussion is concerned with some of the results of speculation and experiment into the biosynthesis of natural products. Apart from their intrinsic interest, the usefulness of such results in structural work is becoming increasingly apparent, for they offer a simple method for the

derivation of hypothetical structures, which may be tested by experiment. This in fact occurred in the present work and is developed below.

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Configurational Correlations

The absolute configuration of organic compounds is based upon the X-ray analysis of sodium rubidium tartrate[1] and iso-leucine hydrobromide.[2] The configurations of the parent compounds and their directions of rotation are shown here, fig.(29).

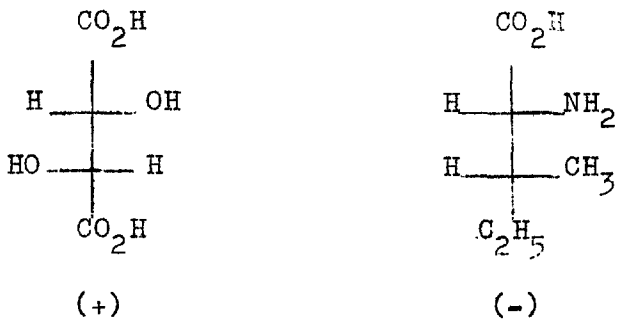
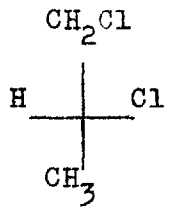


fig.(29)

Theoretical studies, notably those of Eyring[3] and Kirkwood[4] have resulted in the a priori calculation of the sign of rotation of several asymmetric molecules. The latter worker, in particular, has given a positive sign of rotation to 1, 2-dichloropropane (38), in the configuration shown, fig.(30). Since this had previously been related to D(+)-glyceraldehyde, albeit by processes which involved impure materials at several stages, it was found that the absolute assignment of Kirkwood confirmed the original selection of Fischer,[5,6].



(+) (38)

fig.(30)

Although no great reliance may be placed upon these theoretical studies, it is encouraging to note their agreement with the X-ray work.

The correlation between D(+)-glyceraldehyde and compounds containing an asymmetric tertiary carbon atom has been the result of much painstaking research. At present there are two pathways by which configurational relationships have been established.

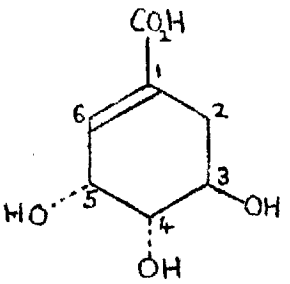
(1) Direct chemical correlation

H.O.L. Fischer has converted shikimic acid (39) into 2-desoxy-D-gluconic acid[7] (40), this compound having been derived from D-glucose by Levene.[8]

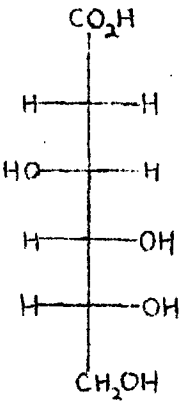
This transformation fixed the absolute configuration of shikimic acid as that which is indicated in fig.(31). Furthermore, Fischer has also obtained a dihydroshikimic acid which yielded an optically active δ-lactone on pyrolysis, this last compound being resistant to lead tetraacetate, and hence may be formulated as (41)?[9]

The lactone formation defines the relative configuration of carbons 1 and 4 of dihydroshikimic acid (It is worthy

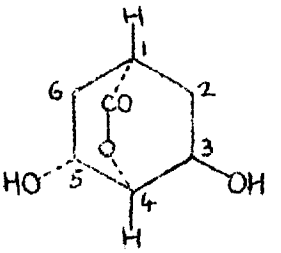
of note that the conversion of the lactone back into dihydroshikimic acid does not seem to have been attempted.)



(39)



(40)



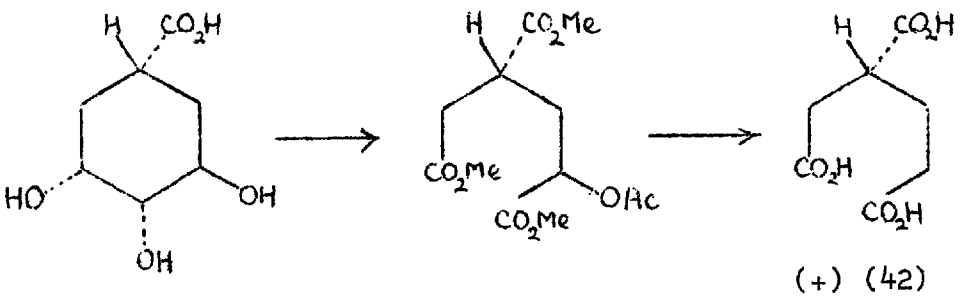
(41)

fig.(31)

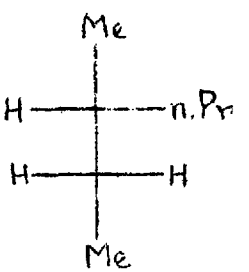
It is this C_1 of dihydroshikimic acid lactone which has been used by Freudenberg in his configurational correlation with the terpenes. By a series of quite unequivocal transformations Freudenberg and his collaborators have transformed dihydroshikimic acid into (+) β -carboxy-adipic acid (42)[10,11]. which was converted into the hydrocarbon (+) 3-methyl-hexane (43) by way of reduction of the corresponding tribomo-compound. fig.(32).[12,13].

The hydrocarbon had the same boiling point and refractive index as authentic racemic material. Clearly any monocyclic

hydrocarbons resulting from the reductive cyclisation of the tribromide would be symmetrical; and although Freudenberg and others,[14,16] who have prepared this compound, produce no proof of its homogeneity, the constancy of the physical properties which have been recorded stand in it's favour.



(+) (42)



(+) (43)

fig.(32)

(+) 3-methyl hexane (43) has been correlated with (+) citronellal (44) by the combined efforts of Kishner,[15] Levene and Marker,[16,17] and Markwald.[14] The route is outlined here, fig.(33).

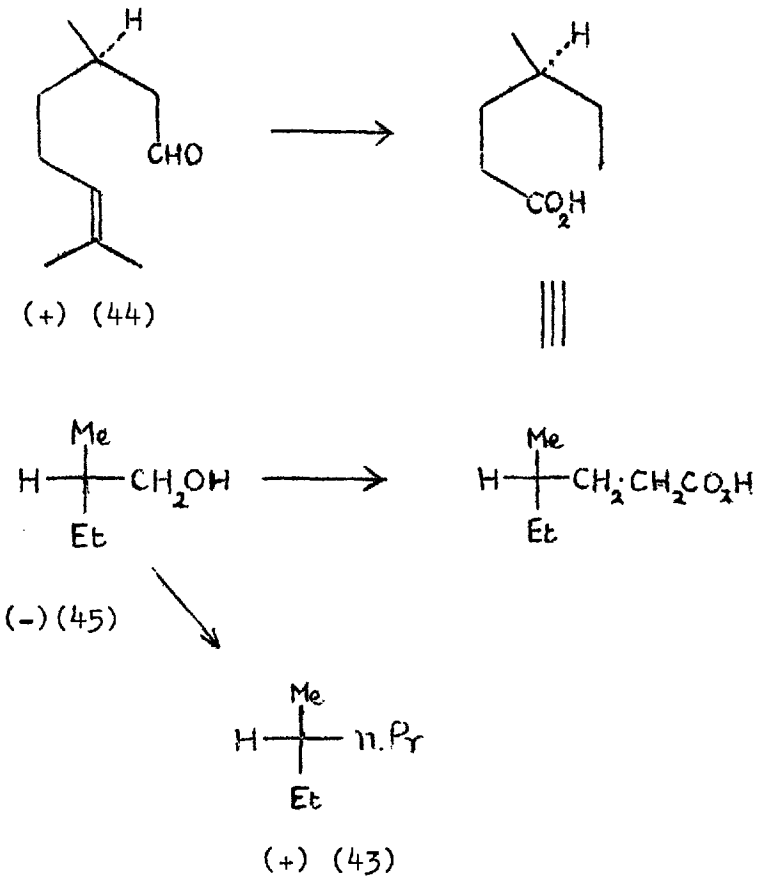
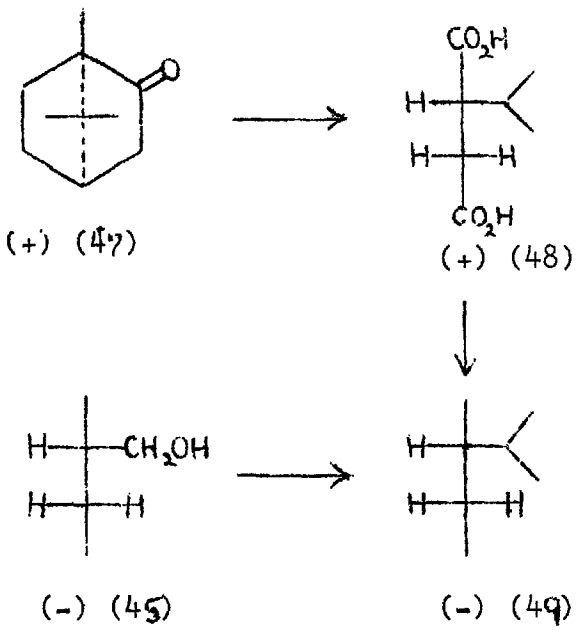


fig.(33)

This work establishes the absolute configuration of (+) citronellal (44). Freudenberg[13] has also converted the (-) amyl alcohol (45) to the hydrocarbon (-) 2,3-dimethyl

pentane (49) with a small amount of racemization. Since this compound was also obtained from (+) iso-propyl succinic acid (48), a well known degradation product of (+) camphor (47), these interconversions establish the absolute configuration of (+) camphor (47), fig.(39).



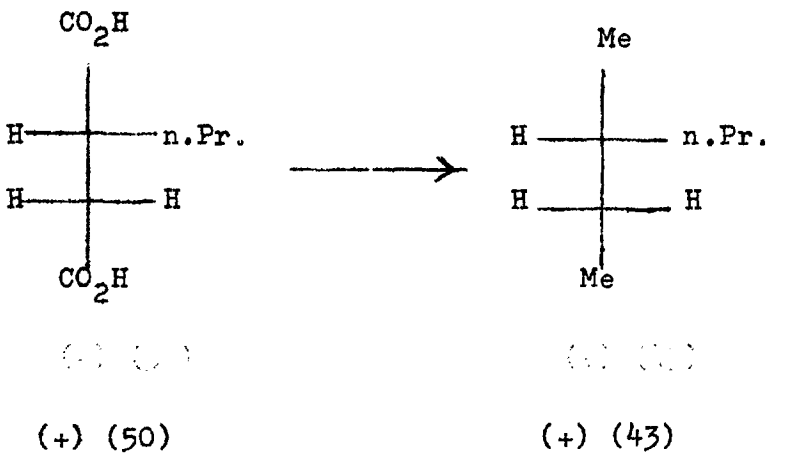
(+) (47) (+) (48)

(-) (45) (-) (49)

fig.(34)

This scheme is self consistent, since the configurational correlation of (+) camphor and (+) citronellal had been already achieved in earlier work.[18] Since the intermediate (-) amyl alcohol (45) has been obtained from natural (+) iso-leucine this result establishes another linkage between the X-ray work and the extensive chemical work.

The absolute configuration of (+) n-propyl succinic acid (50) has been determined by Freudenberg,[13] since he has converted this compound, through the diol and dibromide, respectively, to the optically active (+) 3-methyl hexane (43), fig.(35).



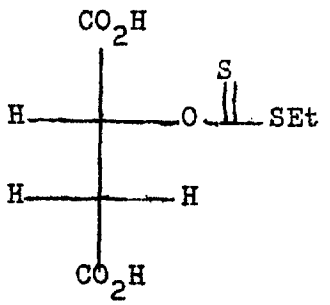
(+) (50) (+) (43)

fig.(35)

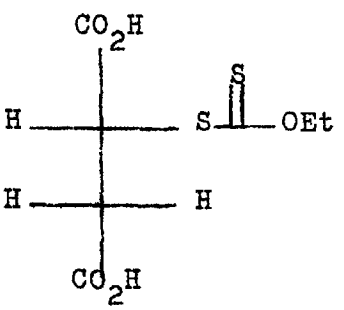
2. Thermal Method

The chemical work described above has received support from the thermal method of Fredga.[19] This worker has shown that (+) malic acid and (+) mercapto-succinic acid have the same configuration, by the

formation of quasi-racemates of two derivatives, the ethyl xanthate (51) and the ethylthione-carbonate (52), respectively.[20]



(51)

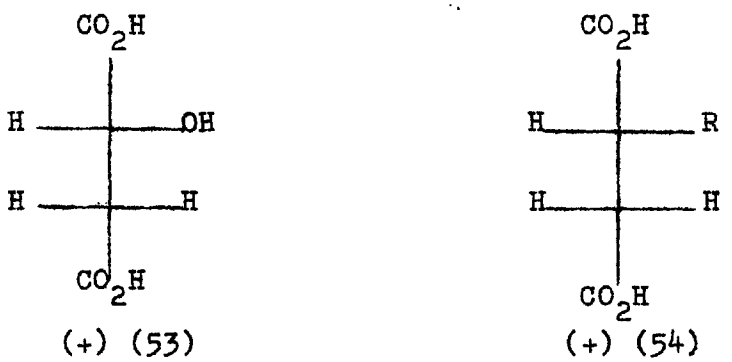


(52)

fig.(36)

Further he found, in collaboration with Matell[21], that the monoalkyl succinic acids, up to n-butyl, formed quasi-racemates with the s-alkyl mercaptosuccinic acids of the opposite sign of rotation. The components of the quasi-racemates differed only in the substitution of a methylene group for a sulphur atom. Since the S-alkyl succinic acids

were prepared by simple alkylation of mercaptosuccinic acid, then the statement that the (+) alkyl succinic acids (54) (R = Me to n-but..) have the same configuration as (+) malic acid (53) is justified, fig.(37).

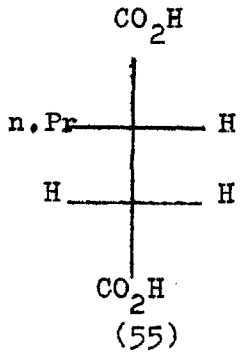


figl(37)

(+) Malic acid has been related to (+) tartaric acid and therefore a correlation exists between the alkyl succinic acids and the material originally used in the X-ray determination of the absolute configuration.[2]

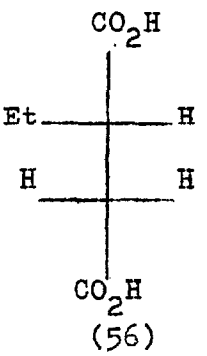
In summary we may write the configurations of the acids isolated in the various degradations of byssochlamic acid as follows, fig.(38), including therein the nomenclature of

Cahn, Ingold, Prelog,[22] fig.(38).



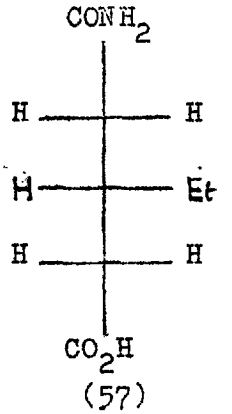
(55)

S(-) n-Propyl succinic acid



(56)

S(-) Ethyl succinic acid



(57)

S(+) 4-Carbamoyl-3-Ethyl-butyric acid.

fig.(38)

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The chemistry of Byssochlamic acid

The mould metabolite byssochlamic acid, m.p. 163°, $(\alpha)_D^{25}$ + 104°, was first isolated from the mould Byssochlamys fulva and described by Raistrick and Smith in 1933.[1] This work indicated a molecular formula $C_{18}H_{20}O_6$ and the presence of four, potential, acidic groups, as the compound titrated as a tetrabasic acid. Apart from the measurement of the physical properties and the preparation of some metal salts, no further properties were described at this stage.

Further investigations, conducted by Raistrick and his co-workers revealed some interesting properties of this compound.[2] Thus, the four acidic groups were correctly placed in two cyclic anhydride residues, on the basis of the lack of active hydrogen as well as the preparation of a bis-phenylhydrazide, m.p. 156-157°, and a bis-toluimide, m.p. 156-157°, the latter compounds being similar in their behaviour on titration and in colour reactions with the known compounds from phthalic anhydride.

Byssochlamic acid, although resistant to catalytic hydrogenation was found to yield upon zinc in acetic acid reduction, a compound $C_{18}H_{24}O_7$, m.p. 243-244°, which titrated in the cold as a tribasic acid. Of greater significance at this stage, was the discovery that on catalytic dehydrogenation of byssochlamic acid there was formed a compound,

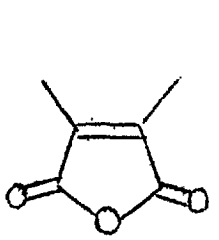
$C_{16}H_{18}O_3$, which titrated on heating as a dibasic acid. Fusion of byssochlamic acid with molten alkali gave rise to an oily hydrocarbon of somewhat indeterminate nature, whose constitution was suggested to be $C_{14}H_{24}$

Later unpublished work,[3,4] carried out in Glasgow, resulted in the transformation of the dehydrogenation product by dilute nitric acid oxidation, to benzene pentacarboxylic acid. The alkali fusion product was found to be resistant to hydrogenation and dehydrogenation. Also byssochlamic acid was transformed into an isomer, iso-byssochlamic acid, m.p. 149-150°, by sublimation from alumina. This latter compound was, unlike the natural product, converted by hydrolysis to a stable monohydrate, iso-byssochlamic dicarboxylic acid, $C_{18}H_{22}O_7$, m.p. 160-175°(d), which was catalytically hydrogenated to dihydro-iso-byssochlamic dicarboxylic acid, $C_{18}H_{24}O_7$, m.p. 185-200°(d).

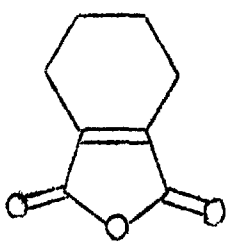
The above represented the available knowledge concerning this compound when we began our own investigations.

The molecular formula $C_{18}H_{20}O_6$ was confirmed by the determination of the molecular weight by mass spectrum, which gave the expected value of 332.[5] The infra-red spectrum, $\nu_{mx.}$ 1845 and 1770 $cm.^{-1}$ confirmed the presence of two anhydride rings, and further indicated that these were both

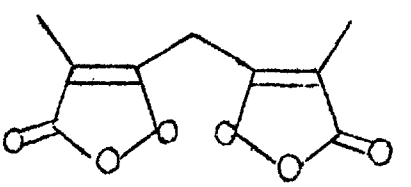
five-membered residues. For comparison dimethyl maleic anhydride (58) and Δ-1 tetrahydrophthalic anhydride (59) absorbed at the same wavelengths and with the same relative intensities. The ultra-violet spectrum was informative, having $\lambda_{mx.}$ (cyclohexane) 244 mμ. (ϵ 9,100). Comparison of this spectrum with those of suitable models further showed the presence of two disubstituted maleic anhydride residues, fig.(39).



(58)



(59)



(60)

fig.(39)

Byssochlamic acid (cyclohexane) $\lambda_{mx.}$ 244 mμ.(ϵ 9,100), Dimethyl maleic anhydride (58) (ethanol) 248 mμ.(ϵ 4,800), Δ-1 tetrahydrophthalic anhydride (59) (cyclohexane)

250 mμ (ε 3,540), Glauconin (60) (cyclohexane) 250 mμ. (ε 10,200)[6].

The n.m.r. spectrum of the compound, apart from showing the absence of vinylic hydrogen atoms and the presence of one or possibly more ethyl groups, was not of further use.

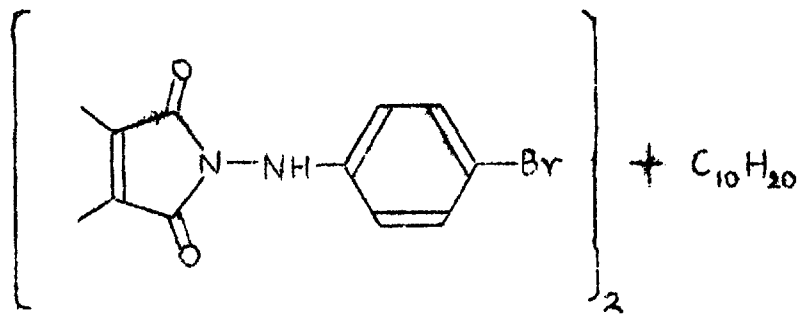
The above evidences indicated at this stage the presence of a monocarbocyclic dianhydride system, without further functionality.

Since it had been decided at the outset that an X-ray crystallographic determination of the structure should be undertaken simultaneously with the chemical work, it became necessary to obtain a derivative of byssochlamic acid containing a suitable heavy atom. After abortive attempts at bromination of the double bonds[7] use was made of the earlier observations that this molecule reacted with phenyl hydrazine to yield a crystalline bis-phenyl hydrazide. Thus byssochlamic acid was found to react smoothly with p-bromo-phenyl hydrazine at 0°C to yield a crystalline byssochlamic acid bis-p-bromophenyl hydrazide, $C_{30}H_{30}O_4N_4Br_2$, m.p. 164-166°, $(\alpha)_D^{22}$ - 97°(0.3).

The infra-red spectrum of this compound, having $\nu_{mx.}$ 1780, 1720 cm.$^{-1}$ was compatible with the partial formulation (61).

This compound was obtained, quite readily, in

rectangular prisms by slow crystallisation and was submitted for X-ray analysis.



(61)

Our approach to the chemical elucidation of the structure of byssochlamic acid was initially by the examination of the previously described dehydrogenation product, $C_{16}H_{18}O_3$. We repeated this preparation and obtained the material, hereafter called dehydrobyssochlamic acid.

It had m.p. 90-91°, $(\alpha)_D^{25} \pm 0^0$ (1.1) and the constitution $C_{16}H_{18}O_3$ was confirmed by elementary analysis and mass spectrometric molecular weight.[5] The infra-red spectrum, $\nu_{mx.}$ 1835, 1767 cm.$^{-1}$ placed the three oxygen atoms in a five-membered anhydride ring and the ultra-violet absorption, $\lambda_{mx.}$ 224 mμ. (ε 26,000), 232 mμ. (ε 18,000), was that characteristic of a substituted phthalic anhydride. The integrated n.m.r. spectrum confirmed the previous degradation to benzene pentacarboxylic acid[3] in that it showed one aromatic proton, as a singlet at τ 2.72, which was not significantly shifted from the line position of unsubstituted benzene. It is known[8] that aromatic protons ortho- to electronegative groups are shifted downfield, fig.(40).

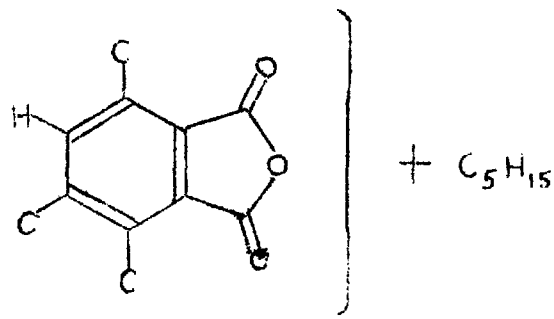
Substituent	Shift (ppm.)
- CO_2H	- 0.63
- COCl	- 0.90
- CO_2Me	- 0.93

fig.(40)

The absorption of this lone proton, therefore indicates that it bears a meta-para relationship to the anhydride group. The remaining atoms and single double bond equivalent must be incorporated into another ring since the compound shows no vinyl proton in its n.m.r. spectrum and does not react with

perphthalic acid.

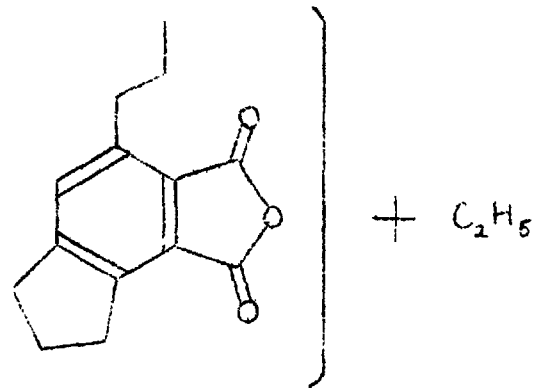
These arguments may be combined to produce the partial formula (62).



(62)

The n.m.r. spectrum of dehydrobyssochlamic acid shows complex structure in the region τ 7-8 but none of the sharp lines characteristic of aromatic methyl groups. The splitting pattern could be attributed in part to two ethyl groups, a fact which correlates with the two C-methyl groups obtained in Kuhn-Roth oxidations. This being so, the side chain at C_6 must at least be n-propyl in order to account

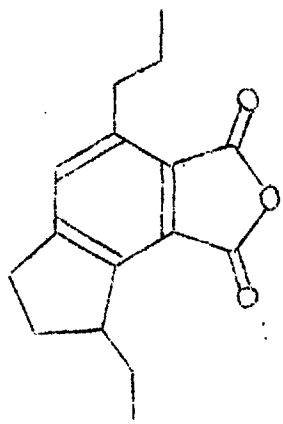
for a molecule of acetic acid under Kuhn-Roth conditions. The remaining atoms may be fitted into an indane structure (63), leaving one ethyl group to be placed.



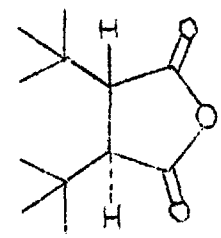
(63)

The alternative, methyl substituted tetralin, is rejected since this would be expected to dehydrogenate under the conditions of the reaction. The location of the remaining ethyl group is not possible with the evidence in hand but the close similarity in the ethyl resonances in the n.m.r. spectrum suggests the equivalence of environments represented by (64).

This location, which offers maximum hindrance to the anhydride ring also explains the observation of Clutterbuck, that in the cold this compound titrated as a monobasic acid. This is similar to the observations of other workers on sterically hindered 1,2-dicarboxylic acids, for example, trans-ditertiarybutyl succinic acid (65).[9]



(64)



(65)

During the preparation of dehydrobyssochlamic acid it was found that there was also formed a crystalline product, isomeric with the starting material, which we have

shown to be identical with the iso-byssochlamic acid described by Paton.[4] Our compound has m.p. 153°, $(\alpha)_D^{25}$ - 27° (0.91) and $\nu_{mx.}$ 1830, 1810, 1770 cm.$^{-1}$, this infra-red data being consistent with the presence of two five-membered anhydride rings in different environments.

The ultra-violet absorption at 236 mμ. (6,300) was indicative of a change in the chromophore from two maleic residues to one itaconic anhydride and also a saturated five-ring structure, fig.(41).

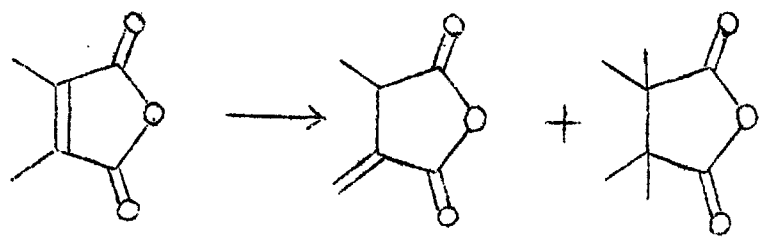


fig.(41)

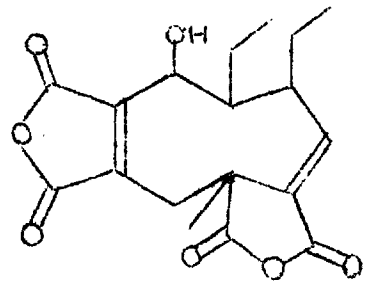
The n.m.r. spectrum was not very informative but clearly indicated the absence of vinylic hydrogen. That the conversion of byssochlamic acid to iso-byssochlamic acid

represented the transformation of a monocyclic di-unsaturated compound to a bicyclic-mono-unsaturated compound was confirmed by the ready hydrogenation of iso-byssochlamic acid to dihydro-iso-byssochlamic acid, $C_{18}H_{22}O_6$, m.p. 128-130°, $(\alpha)_D^{22}$ - 16° (0.89). The latter compound still contained both anhydrides, $\nu_{mx.}$ 1840, 1775 cm.$^{-1}$ but was essentially transparent to ultra-violet light, having $\epsilon_{220\,m\mu.}$ 245, and thus contained no unsaturation. It is therefore bicarbocyclic.

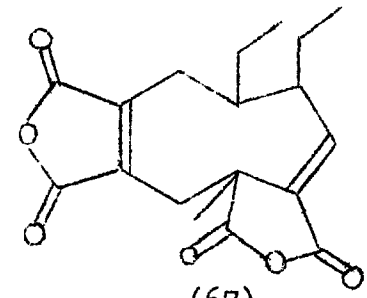
Now iso-byssochlamic acid was converted, on dehydrogenation to the same dehydrobyssochlamic acid, but in somewhat higher yield. With these facts in mind it seemed to us that their most likely explanation was in the formulation of byssochlamic acid as a nine-membered ring structure, the conversion to the dehydro-compound simply meant the transannular cyclisation of the medium ring to a hydrindane, which was then aromatised.

It had been suspected by ourselves that byssochlamic acid had some biogenetic relationship with the mould metabolites glauconic (66) and glaucanic acids (67).[6] The structures of these two compounds were elucidated in these laboratories before the structure of byssochlamic acid became known.

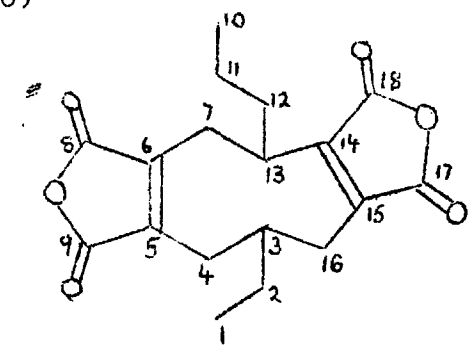
By using the hypothetical biogenetic scheme elaborated in the review, p.32 it was possible to combine this with the chemical evidence available at this point to produce the structure (68) for byssochlamic acid.



(66)



(67)



(68)

It was on this hypothetical structure for byssochlamic acid that we based our further degradative work.

Realising that the simplest proof of the above hypothesis lay in degrading the monocyclic compound by direct oxidative cleavage of the double bonds, we proceeded to investigate various reagents designed to accomplish this end.

We first studied the cleavage of such a bond in the model Δ-1 tetrahydrophthalic anhydride. The alkaline permanganate oxidation of this compound had been described earlier and was said to yield adipic acid.[10] In repeating this work we obtained adipic acid but also the further degradation products succinic acid and glutaric acid. Clearly this was not to be a method of choice and indeed we found that upon alkaline permanganate oxidation, byssochlamic acid was degraded to oxalic acid and carbon dioxide, the conditions required to initiate attack being so vigorous that the molecule was rapidly destroyed.

Ozonolysis of the model anhydride proceeded well, both as the anhydride in ethyl acetate and as the disodium salt in water. These procedures gave rise to almost quantitative yields of adipic acid, fig.(42).

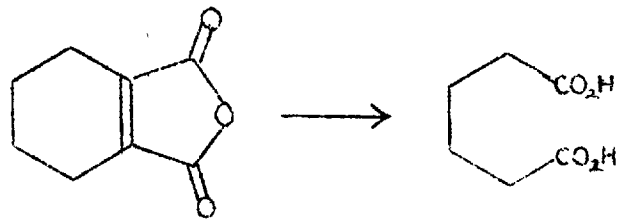


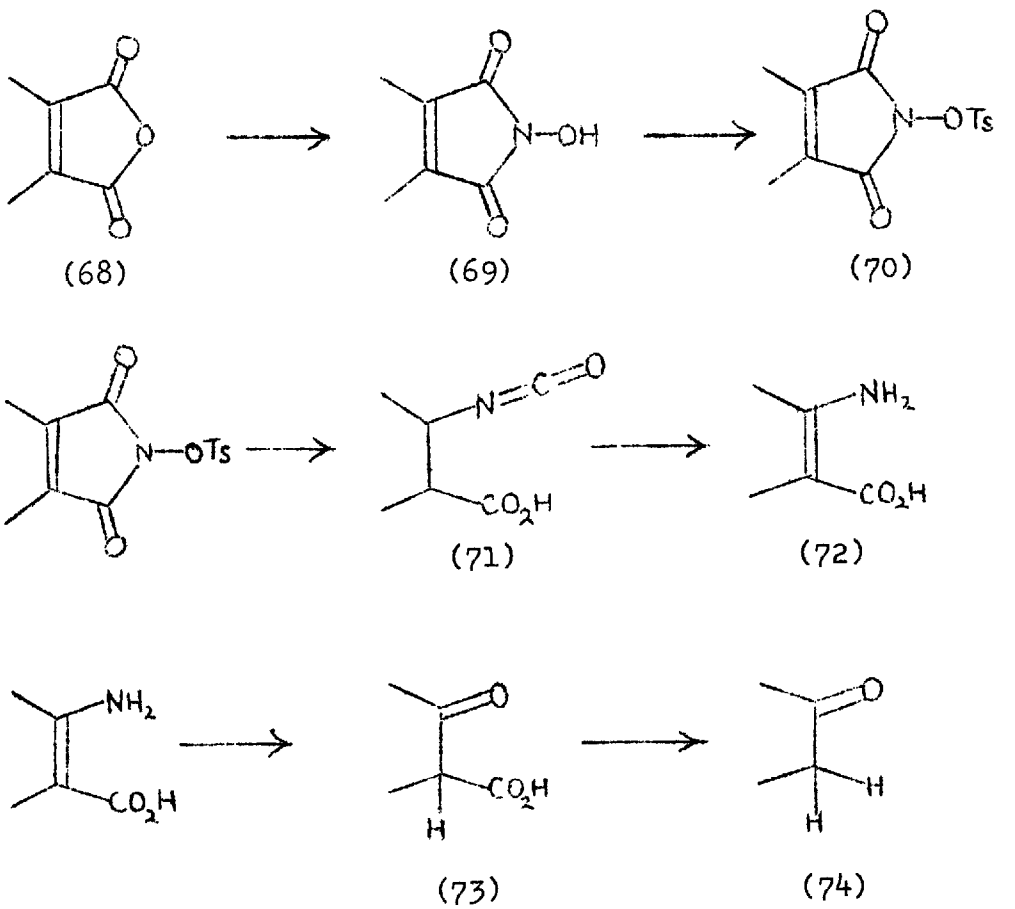
fig.(42)

However when this method was applied to the natural product we found that, as the dianhydride, it was essentially resistant to ozonolysis, being recovered largely unchanged after twelve hours in ethyl acetate, and as the tetrasodium salt it underwent a slow decomposition to a complex mixture of acids. Because of these difficulties, we examined the effects of other reagents, which are listed in the experimental part but had no useful effect upon the natural product.

It was clearly necessary to effect some modification to the very resistant anhydride residues in order that we might obtain a selective degradation. To this end the following hypothetical scheme, fig.(43), was envisaged for the conversion of the anhydride residue to a carbonyl group, losing two carbon atoms in the process, fig.(43).

The conversion of the anhydride (68) to the N-hydroxyimide (69) by reaction with hydroxylamine would be assisted by the known ease of ring closure of the anhydride. The formation of the hydroxylamine O-tosylate has ample precedent[11] and the following stages which should result from base treatment of the N-tosyloxyimide (70) are no more than a Lossen rearrangement[12], hydrolytic fission of the isocyanate (71) and the enamine (72), leading to the β-keto-acid (73), which is decarboxylated to the ketone (74).

In the event, we treated byssochlamic acid with one equivalent of hydroxylamine and were able to obtain a non-crystalline mono-N-hydroxyimide derivative whose properties were in accord with its formulation (75).



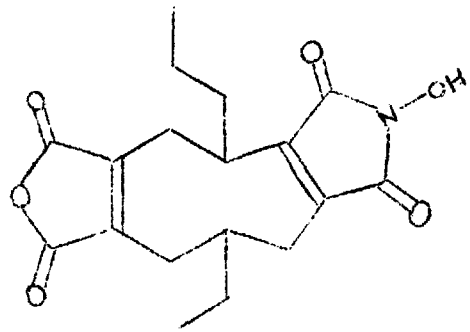
(68) (69) (70)

(71) (72)

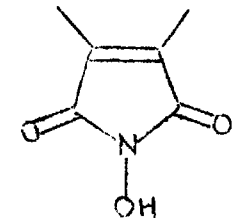
(73) (74)

fig.(43)

Thus, the anhydride residue was apparent in the infra-red spectrum, $\nu_{mx.}$ 1840, 1770 cm.$^{-1}$



(75)

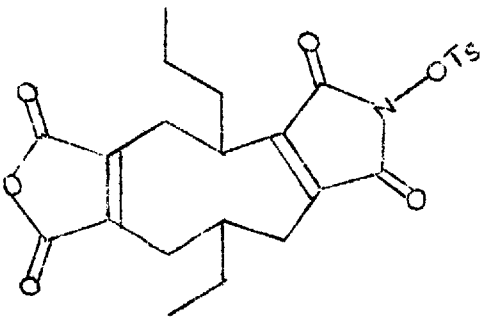


(76)

The N-hydroxyimide function absorbed at 1800 cm.,$^{-1}$ 1720 cm.,$^{-1}$ 1660 and 3300 cm.$^{-1}$ which data are in good agreement with those reported by Ames for N-Hydroxymaleimide (76)[13].

We were, of course, unable to differentiate between the two structures which arose by attack on one or other of the anhydride residues, but as will be seen in the sequel the product we obtained is that formulated here. Tosylation of this compound produced the N-tosyloxyimide (77), which

was again non-crystalline.

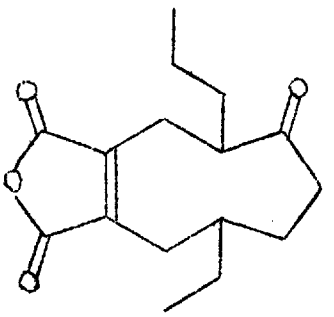


(77)

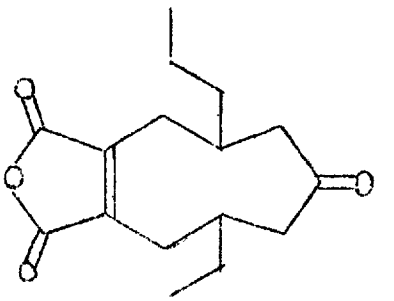
However its infra-red spectrum, $\nu_{mx.}$ 1820, 1760 (five ring anhydride), 1860, 1740 cm.$^{-1}$ (N-tosyloxymaleimide), showed the slight hypsochromic shift expected for the introduction of a more electronegative substituent onto nitrogen. Base treatment of this compound produced a crystalline reaction product which by chromatography on silica gel was resolved into two crystalline keto-anhydrides.

The first of these, Ketone-I (78), $C_{16}H_{22}O_4$, m.p. 110°, $(\alpha)_D^{25}$ - 79° (0.90), was obtained in 26% yield from

byssochlamic acid. The infra-red spectrum confirmed that we had succeeded in removing one of the maleic anhydride residues, for it absorbed at $\nu_{mx.}$ 1859, 1761, characteristic of a disubstituted maleic anhydride, and also at 1695 $cm.^{-1}$ an absorption attributed to a medium ring ketone.[14] The ultra-violet absorption at 252 $m\mu$. (ϵ 4,600) was consistent with the presence of one maleic anhydride function.



(78)



(79)

The second product, Ketone-II (79), $C_{16}H_{22}O_4$, m.p. 161°, $(\alpha)_D^{24}$ + 5.5° (0.95), was obtained in 11% overall yield.

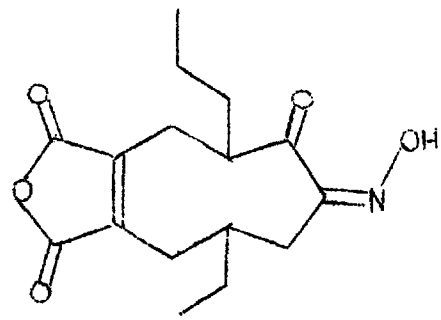
The spectral properties were similar to those of Ketone-I in that it had $\nu_{mx.}$ 1845, 1761 (five ring anhydride) and 1685 cm.$^{-1}$ (medium ring ketone) and exhibited $\lambda_{mx.}$ 255 mμ (ε 5,500), the latter again attributed to a disubstituted maleic anhydride.

That these compounds were not merely epimers, produced by an equilibration of a single precursor ketone, was proved by treatment of each with the original conditions of basicity employed in their preparation. They were both recovered unchanged, indicating positional isomerism. The proof of the formulations (78) and (79) given for these compounds is derived from their further degradation.

The compound produced in larger yield, Ketone-I, gave a positive Zimmerman test,[15] indicative of an enolisable α-methylene group. In this property we saw a potential degradation, for, if we were to use this enol to form a suitable derivative we would be able to cleave the bond linking the carbonyl group to its adjacent methylene. We decided on the second order Beckmann rearrangement of α-oximino ketones as the method of choice.[16]

Thus Ketone-I upon reaction with amyl nitrite under acidic catalysis[17] gave a highly crystalline α-oximino ketone (80), $C_{16}H_{21}NO_5$, m.p. 171-172°, $(\alpha)_D^{22}$ + 445 (1.09).

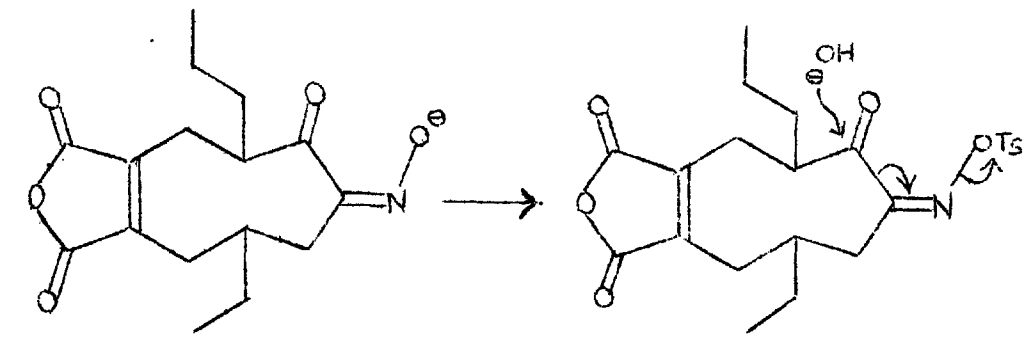
The infra-red absorptions, ν_{mx} 1845, 1775 (five ring anhydride), 3300 cm.$^{-1}$ (chelated hydroxyl group), as well as the ultra-violet absorptions λ_{mx} 239 mμ. (ε 8,500), 267 mμ (ε 4,200) were consistent with the proposal (80).



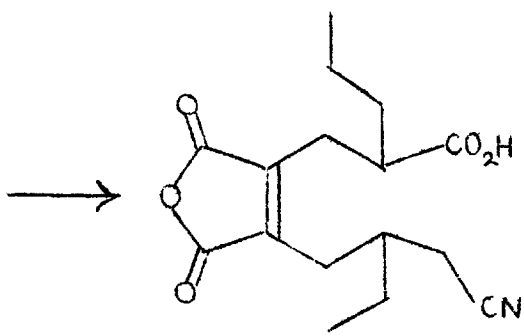
(80)

Although an oxime-tosylate could not be formed under normal tosylation conditions, that is, in pyridine with tosyl-chloride at 0°, presumably because of the strongly chelated nature of the hydroxyl group, we found that after dissolution in aqueous base as the yellow anion, addition of tosyl chloride to this solution resulted in a discharge of the colour.

The product was found to be the desired ring opened nitrile acid, which resulted from tosylation and subsequent base catalysed fragmentation of the oxime-tosylate (81), fig.(44).



(81)

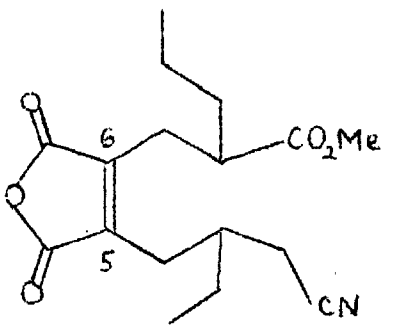


(82)

fig.(44)

The nitrile acid (82) so formed, was not crystalline and was converted to its mono-methyl ester by brief treatment with diazomethane. This compound was distilled in vacuum, as a colourless oil, Ketone I -anhydride-nitrile-ester (83),

$C_{17}H_{23}O_5N$, $(\alpha)_D^{25}$ - 6.8° (1.84). The spectral characteristics were those expected of structure (83), for it exhibited $\nu_{mx.}$ 1855, 1770, (five ring anhydride) 1730, 1280, (carbomethoxyl group) and 2300 cm.$^{-1}$ (nitrile). The ultra-violet absorption of 255 mμ. (ε 4,000) indicated the retention of the maleic anhydride chromophore.



(83)

We were now confronted with the fission of the remaining maleic anhydride residue between C_5 and C_6 to yield the two dicarboxylic acid fragments, whose skeletons constituted the medium ring of byssochlamic acid. In view of our earlier, facile, ozonolysis of Δ -1 tetrahydrophthalic anhydride we decided that the residual maleic anhydride unit, now removed from the special environment of

the medium ring, might yield to this degradation.

In the event, ozonolysis of the ester-nitrile in ethyl acetate and subsequent treatment of the product with alkaline hydrogen peroxide provided a non-crystalline acidic product. Examination of a specimen of methylated material (diazomethane) indicated the presence of two products, apart from some oxalic acid, which was removed by virtue of its insolubility in benzene.

The mixture was cleanly resolved by partition chromatography on silicic acid.

The major component was a crystalline solid, m.p. 103-104°, $(\alpha)_D^{25}$ - 18° (1.0), a portion of which, upon treatment with acetyl chloride, gave rise to an oily anhydride having $\nu_{mx.}$ 1783, 1864 cm.$^{-1}$. This was compared to the absorptions of succinic anhydride, $\nu_{mx.}$ 1786, 1868 cm.$^{-1}$ and also glutaric anhydride, $\nu_{mx.}$ 1765, 1810 cm.$^{-1}$. Clearly we were dealing with a substituted succinic acid. Confirmation of its identity as (-) n-propyl succinic acid came from a comparison of the infra-red spectrum with that of an authentic sample of (+) n-propyl succinic acid, m.p. 104-105°, $(\alpha)_D^{25}$ + 18° (1.0) and finally by the formation of (±) n-propyl succinic acid, m.p. 92°, $(\alpha)_D^{25}$ ± 0° (.5), by using equal amounts of the two samples of opposite rotation.

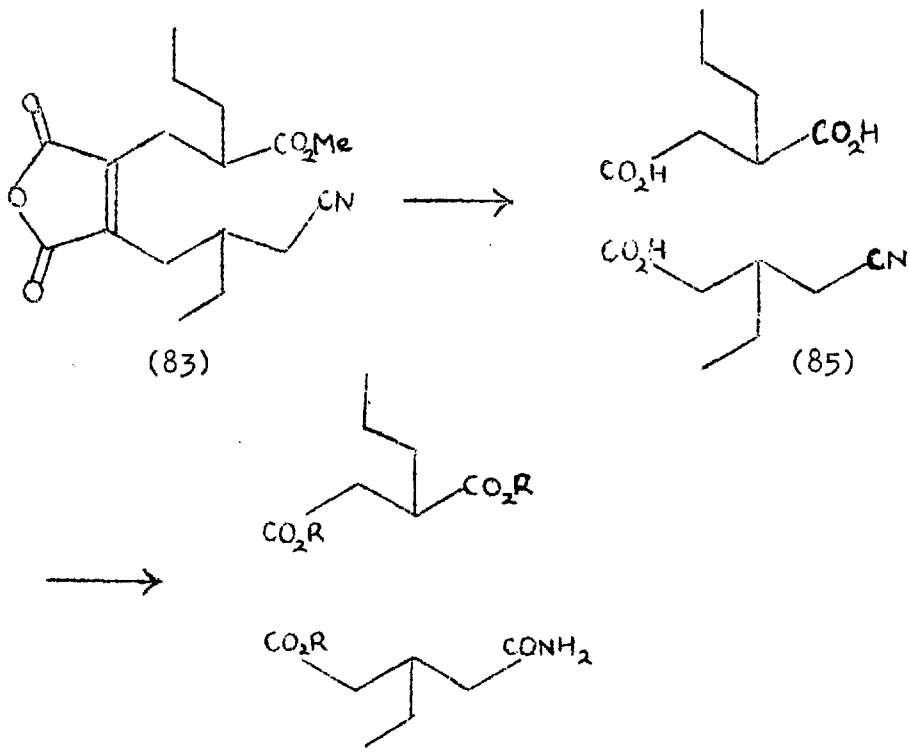
The identity of the racemic material was confirmed by mixed melting point and infra-red spectrum.

The second component of this mixture was a non-crystalline nitrile-acid. Since, on the basis of our working hypothesis we expected this compound to be β-ethyl glutaric acid half-nitrile, an as yet unknown compound, we decided in the first case to degrade the compound to the parent glutaric acid and confirm the identity before synthesising the nitrile-acid. By vigorous acid hydrolysis we obtained a crystalline compound, m.p. 71-73°, which did not depress the melting point of authentic β-ethyl glutaric acid, m.p. 72-73°, and had an identical infra-red spectrum. The β-ethyl glutaric acid was obtained by standard procedures described in the experimental section.

At this stage we were confident that we had the optically active half-nitrile of β-ethyl glutaric acid and so the ozonolysis was repeated and the product treated somewhat more vigorously with alkaline hydrogen peroxide. The acid mixture was converted to the mixture of p-phenyl phenacyl esters and these were separated by crystallisation and chromatography. Thus we obtained the bis-p-phenyl phenacyl ester of (-) n-propyl succinic acid, m.p. 144-147°, $(\alpha)_D^{29}$ + 34° (1.16), which upon admixture with an equal

amount of the derivative of the (+) acid yielded the racemic bis-ester, shown to be identical to an authentic specimen of the bis-phenyl phenacyl ester of racemic n-propyl succinic acid.

We also obtained the p-phenyl phenacyl ester of 4-carbamoyl 3-ethyl-butyric acid, m.p. 137-139°, $(\alpha)_D^{25}$ + 15° (0.57), $C_{21}H_{23}O_4N$. The infra-red absorptions at 1740, 1695, (phenacyl ester), 1660 and 1633 cm.$^{-1}$ (primary amide) were in full accord with its formulation, (87).



(83) (85)

(87)

fig.(45) (R = p-phenyl phenacyl)

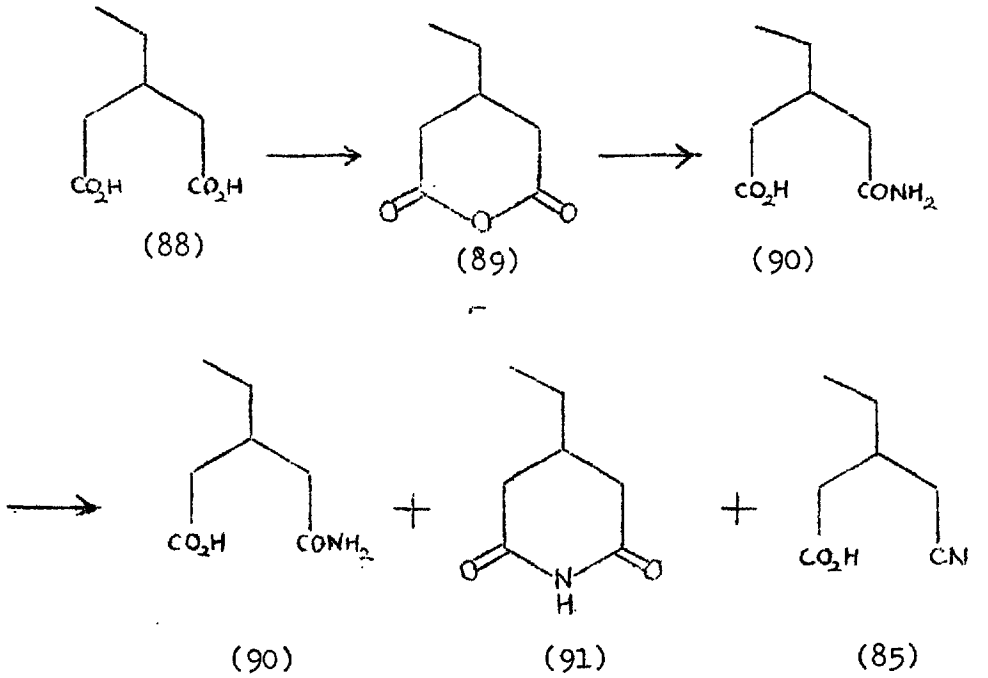
We express these transformations in fig.(45), without at present indication of stereochemistry.

It is clear at this stage why we chose the Beckmann transformation in the degradation of Ketone-I , for this procedure yields a β -ethyl glutaric residue which is optically active by virtue of the nitrile function; the diacid is, of course, symmetrical. Thus it remained to synthesize this material, to resolve it, and finally to prove its configuration. This was achieved by the following route.

The starting point was β-ethyl glutaric acid (88) which was readily available by Guareschi imide synthesis.[18] The acid was converted to the cyclic anhydride (89) with acetyl chloride. The oily anhydride reacted smoothly with ammonia to yield racemic 4-carbamoyl-3-ethyl butyric acid (90), m.p. 133-134°, fig.(46). All attempts to resolve this compound failed. We therefore transformed it into the corresponding nitrile acid (85), but not without complication. It was found that upon treatment of the free acid with p-toluene sulphonyl chloride in pyridine, a reagent which has been used to convert amides to nitriles under mild conditions,[19] there was obtained a mixture of amide, imide, and nitrile, fig.(46).

It was clear that the neighbouring carboxyl group was

responsible for this effect, probably through the formation of a mixed sulphonic anhydride.[20]



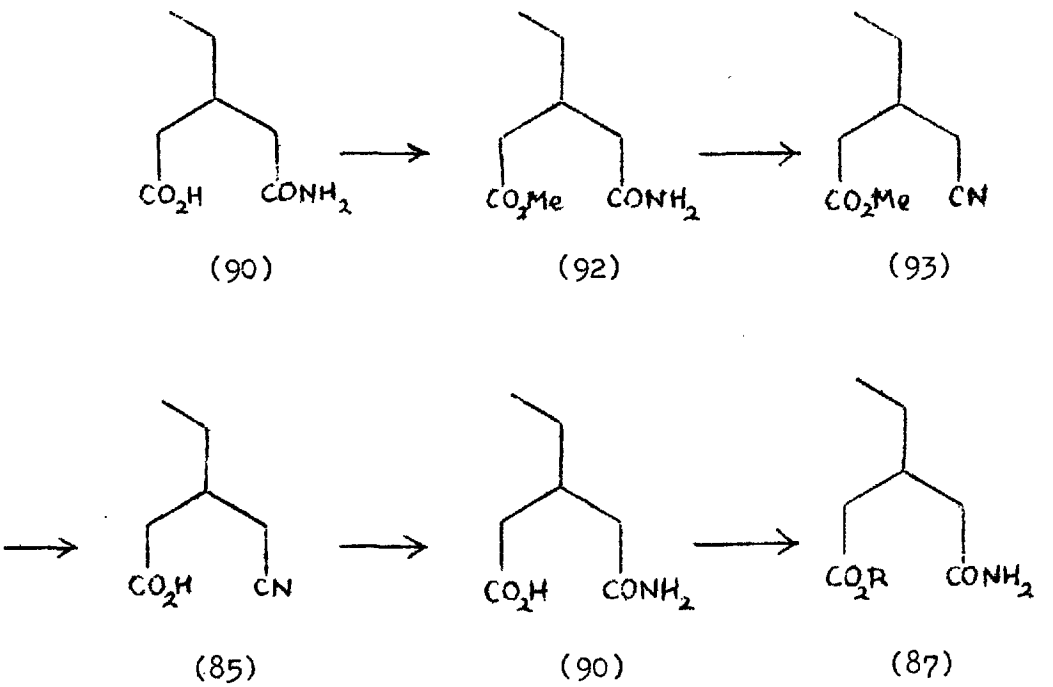
(88) (89) (90)

(90) (91) (85)

fig.(46)

We therefore protected the carboxyl group by using 4-carbamoyl-3-ethyl-butyric acid methyl ester (92). The methyl ester was smoothly transformed into the ester-nitrile (93) which was hydrolysed back to the acid-nitrile (85), although this hydrolysis at first presented some difficulty.

It was found with excess base (1N aqueous alkali) the compound was rapidly hydrolysed to β-ethyl glutaric acid, again probably through the influence of the neighbouring carboxyl group. However with a controlled amount of dilute ethanolic base (0.2 N) there was rapidly obtained, at room temperature, the required nitrile-acid (85), fig.(47).



(90) (92) (93)

(85) (90) (87)

fig.(47) (R= p-phenyl phenacyl)

The oily nitrile-acid was resolved through the quinine metho-salt,[21] which process gave (+) 4-cyano-3-ethyl-butyric

acid (85), $(\alpha)_D^{25}$ + 28° (5.22), which was transformed with alkaline peroxide[22] to (-) 4-carbamoyl-3-ethyl-butyric acid (90), m.p. 113-114°, $(\alpha)_D^{25}$ - 2° (1.16, water). This material was converted into the p-phenyl phenacyl ester (87), m.p. 137-139°, $(\alpha)_D^{25}$ - 15° (0.82).

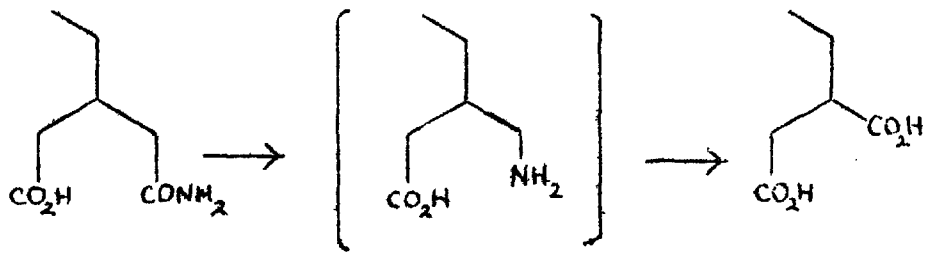
The infra-red spectrum of this material was identical with that obtained from the natural product, and identity was confirmed by using equal amounts of the two compounds and thereby obtaining the racemate of 4-carbamoyl-3-ethyl butyric acid p-phenyl phenacyl ester, m.p. 134-135°, undepressed on admixture with the authentic racemate and having an identical infra-red spectrum.

The absolute configuration of (-) 4-carbamoyl-3-ethyl-butyric acid.

The configuration of this compound was established by conversion to (-) ethyl succinic acid. Thus the free, optically active (-) acid was subjected to the Hofmann amide rearrangement[23] and the γ-amino acid (94), resulting from this process, was oxidised, without isolation, to the succinic acid (95), fig.(48).

There was obtained (-) ethyl succinic acid, m.p. 94-96°, $(\alpha)_D^{26}$ - 10° (1.1), whose identity was confirmed by conversion to the racemate, as in previous cases, since (+) ethyl

succinic acid was readily available from the (±) -acid by resolution with strychnine.[24]



(94) (95)

fig.(48)

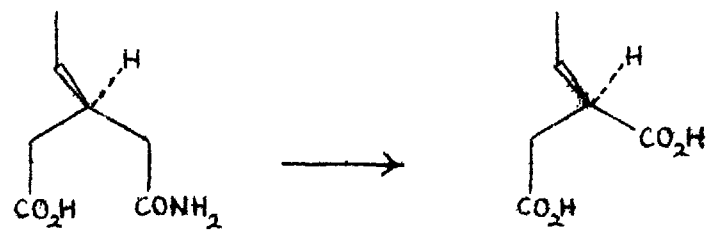
The configurations of (+) ethyl (96) and (-) propyl succinic (97) acids are as shown in the accompanying fig.(49).



(-) (97) (+) (96)

fig.(49)

It follows from the degradation of the (-) 4-carbamoyl-3-ethyl-butyric acid to (-) ethyl succinic acid that the natural (+) 4-carbamoyl-3-ethyl-butyric acid (98) must be related to the (+) ethyl succinic acid (96) as described by fig.(50).



(+) (98) (+) (96)

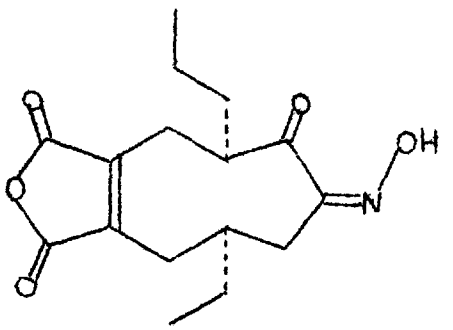
fig.(50)

These two fragments, that is (-) propyl succinic acid and (+) 4-carbamoyl-3-ethyl-butyric acid, may be used to reconstitute the precursor oximino-ketone in two ways, expressed by (99) and (100) respectively, fig.(51).

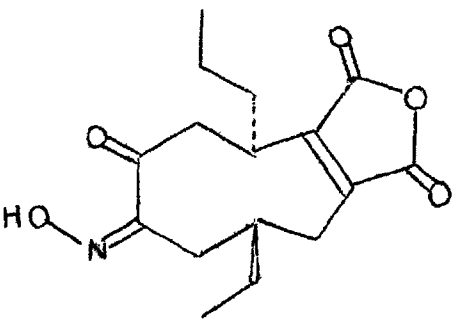
Each of these would yield a pair of structures for byssochlamic acid, thus (99) yields (101) and (102), whilst (100) may be extended to (103) and (104) respectively, fig.(52).

The expression (102) arises because of the possibility of epimerization taking place at the centre α to the carbonyl

group, for although the ketone is recovered unchanged by basic treatment, this possibility must still be considered.



(99)

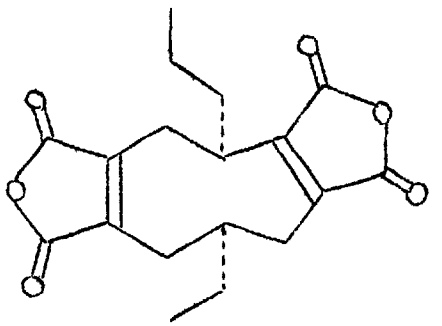


(100)

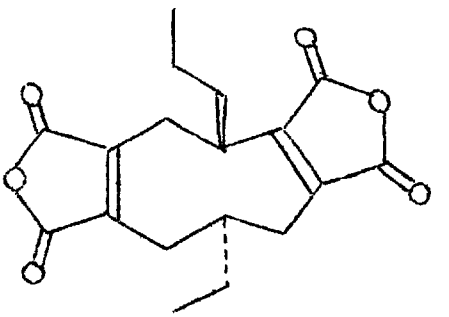
fig.(51)

With these facts in mind we decided that the best approach to a decision between these various possibilities lay in a re-examination of the original ozonolysis of byssochlamic acid. This was reasonable at this stage since we now had much greater faith in our hypothetical structure. By extended ozonolysis we were able to isolate, in pure condition, the acids (-) n-propyl succinic acid, ethyl succinic acid, and β -ethyl glutaric acid. This result confirmed our original structure (68) and indicated that there was no change in the configuration at the n-propyl centre, C_{13}, during the degradation.

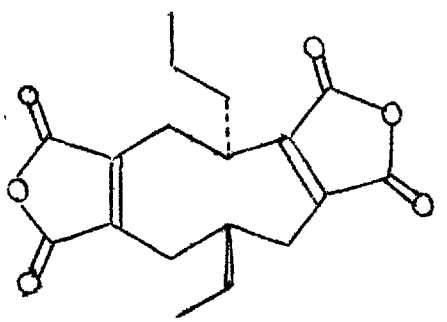
On this basis structures (102) and (104) were rejected.



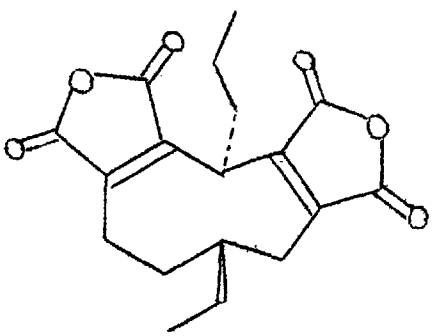
(101)



(102)



(103)



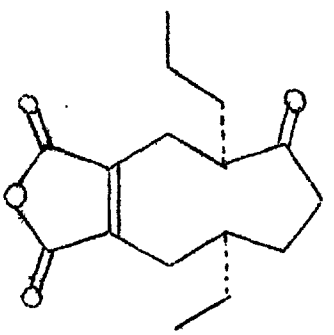
(104)

fig.(52)

Unfortunately, it is not at present possible to differentiate between the two remaining expressions, (101) and (103) on the basis of the chemical work alone. However, by the time these experiments were conducted the X-ray structural determinations had been completed and confirmed our original suggestion.[25]

This work gave a relative cis-orientation to the two alkyl side chains, but gave no information on the absolute stereochemistry. By using the latter result the structure and absolute configuration of byssochlamic acid is represented only by (101).

By extrapolation the structure of Ketone-I , used in our degradation, is defined as (105), and the other compounds in the series follow accordingly.



(105)

The structure of Ketone-II

This compound did not form an oximino-ketone under a variety of conditions. For an alternative degradation we chose the Baeyer-Villiger cleavage and accordingly when the above compound was treated with peroxy-trifluoacetic acid[26] there was obtained a crystalline lactone, $C_{16}H_{22}O_5$, m.p. 100-103°, $(\alpha)_D^{23}$ + 5° (1.0). The spectral data were consistent with the retention of the maleic anhydride chromophore and the carbonyl frequency of the ketone at 1685 $cm.^{-1}$ was replaced by a new absorption at 1706 $cm.^{-1}$ and 1160 $cm.^{-1}$ Such changes were to be expected in the formation of a ten-membered ring lactone. In accord with the observations of previous workers[27] such a lactone was hydrolysed with difficulty, but we found that after prolonged basic treatment we could obtain an oily hydroxy-anhydride acid. This material was methylated (diazomethane) and then acetylated, the resulting anhydride-ester-acetate being distilled in vacuum as an oil, $C_{19}H_{28}O_7$, $(\alpha)_D^{25}$ ± 0° (3.5).

This material was ozonised and the ozonide worked up in the previously described fashion, by treatment with alkaline hydrogen peroxide. The results were more complicated than in the previous case for gas-liquid chromatography indicated the presence of at least five components. These were initially identified by suitable mixed chromatograms

and the results are shown in the table, fig.(53).

Ethyl succinic acid	6%	Concentration expressed as a fraction of total volatile esters.
n-Propyl succinic acid	7%	
β-Ethyl glutaric acid	42%	
β-Propyl glutaric acid	32%	
Unknown compound	13%	

fig.(53)

The two major components were confirmed by isolation as crystalline material and comparison with authentic compounds.

The unknown compound was not separated in a gas-liquid chromatogram from one of the components in a mixture of α- and β-n-propyl butyrolactones. Before attempting to rationalise these facts we re-examined the anhydride-ester-acetate by nuclear magnetic resonance spectroscopy. This spectrum showed the absence of any vinylic hydrogen atom and thus discharged the possibility of an allylic rearrangement prior to ozonolysis, as is described by fig.(54).

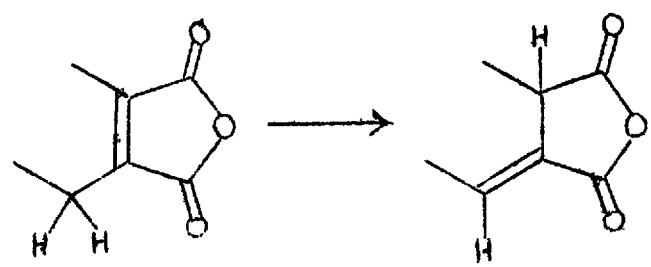


fig.(54)

The ultra-violet spectrum of the ester was consistent with is formulation as a substituted maleic anhydride, having $\lambda_{mx.}$ 253 mμ. (4,750). The simplest explanation of the formation of two glutaric acid residues is in their derivation from a mixture of lactones. Thus the formulation of Ketone-Ⅱ as before (106) and its degradation to the observed fragments is expressed by the transformations in fig.(55).

The formation of a mixture of lactones, m.p. 100-103°, (107a), is not perhaps surprising when the near electronic and steric symmetry of Ketone-Ⅱ (106) is considered. Since the homogeneity of Ketone-Ⅱ is not in doubt the above is the only structure which may accommodate the facts observed in the degradation.

Thus we are able to formulate Ketones-Ⅰ and -Ⅱ as (105) and (106), respectively, and see that they are derived by the degradation of the sterically more hindered anhydride.

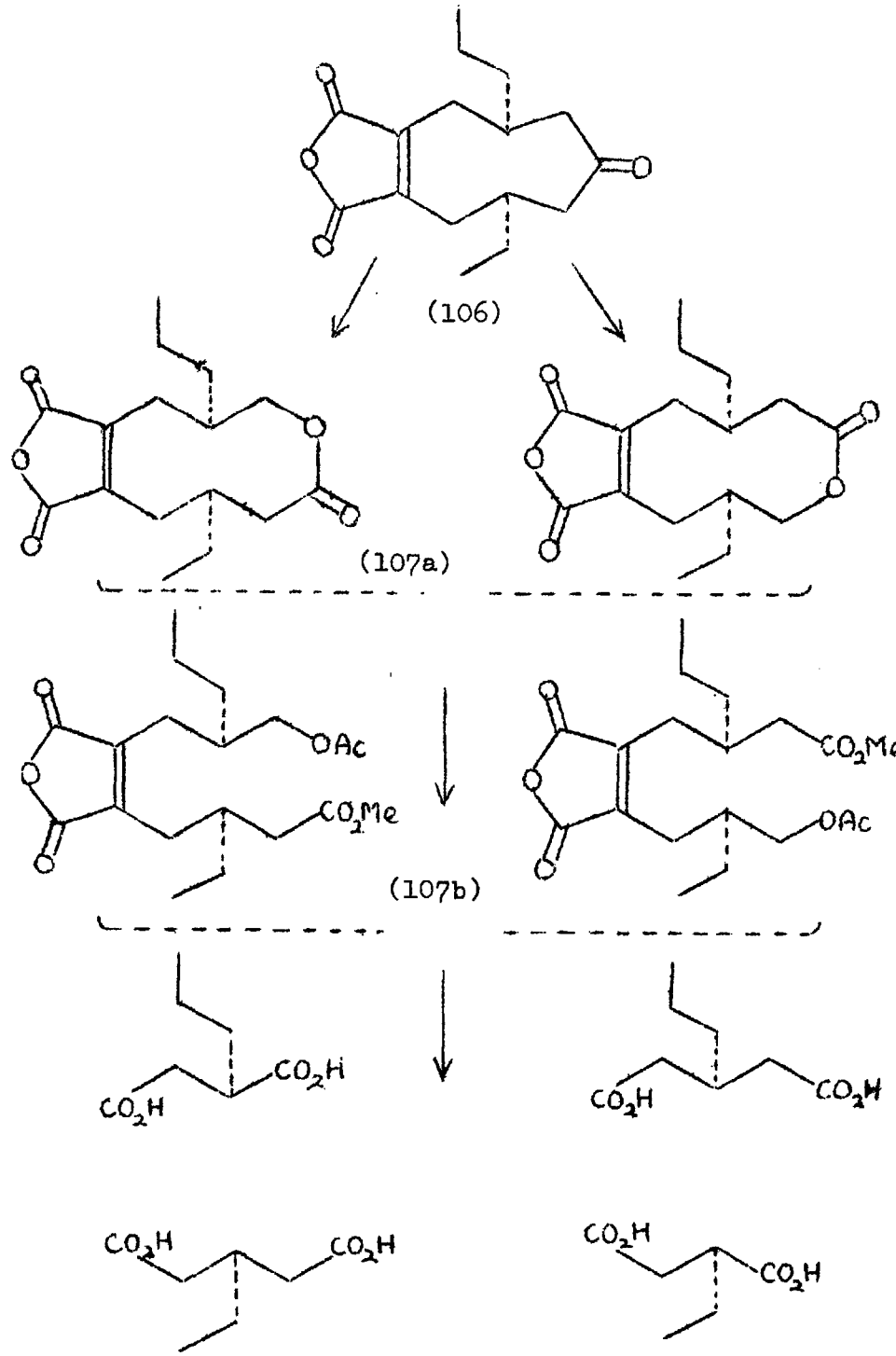

(106)
(107a)
OAc
CO2Me
CO2Me
OAc
(107b)
CO2H
CO2H
CO2H
CO2H
CO2H
CO2H
CO2H
CO2H

fig.(55)

Further reactions of byssochlamic acid

On the basis of the above, derived, structure of byssochlamic acid (101) it is possible to discuss the further reactions of this molecule.

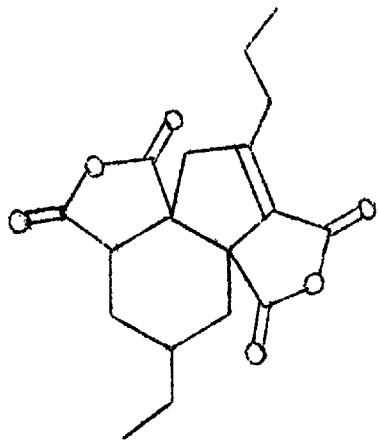
Iso-Byssochlamic Acid

The conversion of byssochlamic acid by pyrolysis into iso-byssochlamic acid was rationalised as a cyclisation reaction (p.61). Further, the transformation of iso-byssochlamic acid to the dehydrobyssochlamic acid, in higher yield than from the natural product, indicated at an early stage that iso-byssochlamic acid might contain the elements of a reduced indane structure. Bearing in mind the requirement that iso-byssochlamic acid contain no vinylic hydrogen atom, the unsaturation being contained in a substituted itaconic type anhydride, it was possible to write down three structures for the isomer, fig.(56).

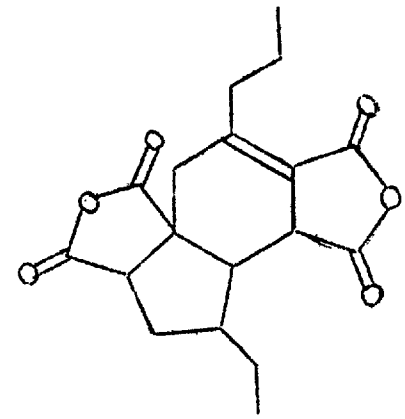
Evidence at present in hand favours expression (109). Thus this structure is an obvious precursor of dehydrobyssochlamic acid by a simple dehydrogenation and decarboxylation process. fig.(57).

The alternatives (108) and (110) both require a rearrangement step before aromatisation may take place.

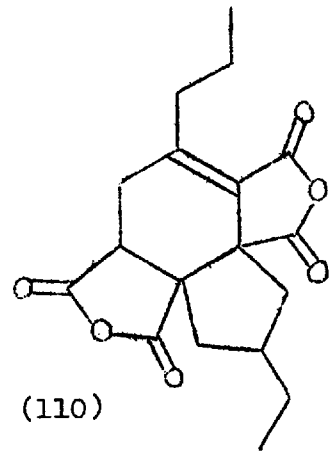
Further evidence in support of (109) comes from the reaction of iso-byssochlamic acid with N-bromosuccinimide.



(108)



(109)

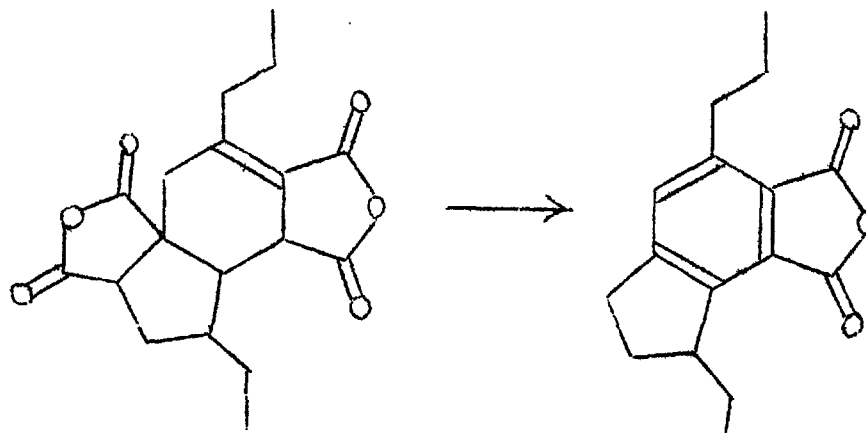


(110)

fig.(56)

This procedure led to the formation of a mono-bromo-iso-byssochlamic acid (111), $C_{18}H_{19}O_6Br$, m.p. 177-180°, $(\alpha)_D^{21}$ - 31° (1.0). This material retained intact the two anhydride residues, as was seen in the infra-red spectrum, which showed absorptions at 1830, 1775 $cm.^{-1}$ and also the ultra-violet absorption at $\lambda_{mx.}$ 240 mμ (ϵ 8,600) defined the presence of the itaconic anhydride

residue. The n.m.r. spectrum of this compound was informative, in that it showed a triplet at τ 4.14 (1 proton, J^1 = 4 cps.).

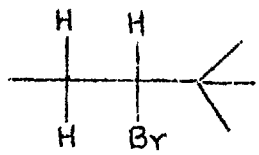


(109)

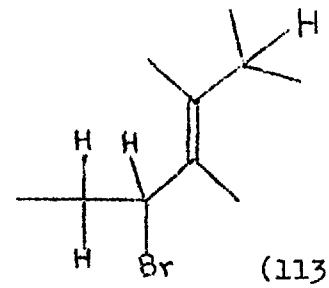
fig.(57)

Such an absorption is consistent with the part structure (112). Furthermore, under higher resolution, this triplet was resolved into a six line spectrum (J^{11} = 1 cps), the latter splitting derived from long range coupling with a single proton. Allylic coupling could result in a

coupling constant of this magnitude, and this is consistent with the part structure (113), fig.(58).



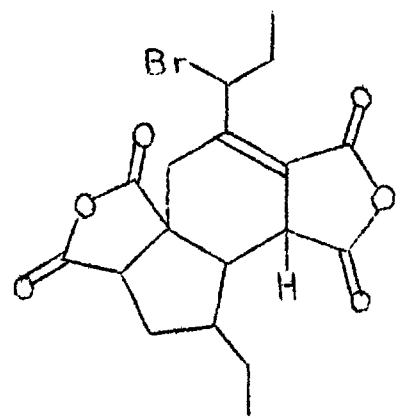
(112)



(113)

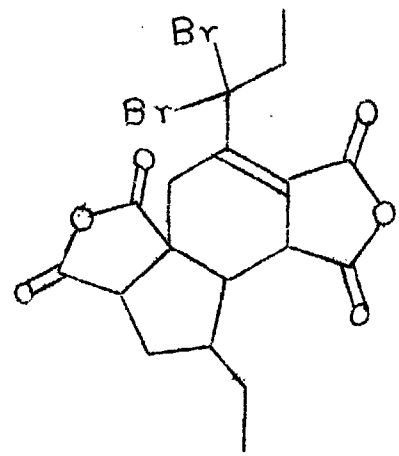
fig.(58)

Of the alternative formulations for iso-byssochlamic acid such a residue is contained only in the monobromo-derivative (111), derived from (109).



(111)

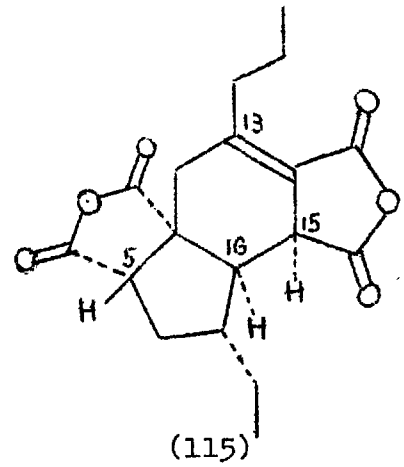
Reaction of iso-byssochlamic acid with two equivalents of N-bromosuccinimide resulted in the formation of a dibromo-iso-byssochlamic acid, $C_{18}H_{18}O_6Br_2$, m.p. 153-155°, $\nu_{mx.}$ 1840, 1775 cm.$^{-1}$ and $\lambda_{mx.}$ 258 mμ (ε 8,600), which is presently formulated as (114).



(114)

On the basis of expression (109) the stereochemistry of iso-byssochlamic acid may be resolved by a consideration of the mechanism of its formation. This process must involve the transfer of hydrogen from C_{13} of byssochlamic acid to C_5, simultaneously with a migration of hydrogen from C_{16} to C_{15}. If such a process is concerted then C_{16} must undergo an inversion of its stereochemistry, fig.(59).

These arguments uniquely define the stereochemistry of iso- byssochlamic acid (115).

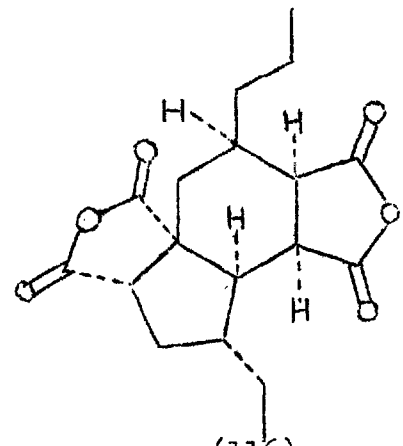


(115)

fig.(59)

As mentioned above, in the discussion of the dehydrogenation reaction, iso-byssochlamic acid was readily hydrogenated to dihydro-iso-byssochlamic acid, a compound which contained two anhydride rings and was fully saturated. As an examination of a model shows the double bond of iso-byssochlamic acid is seriously hindered from that side which is cis- with respect to the five membered carbocycle. Thus hydrogenation would be expected to proceed from the

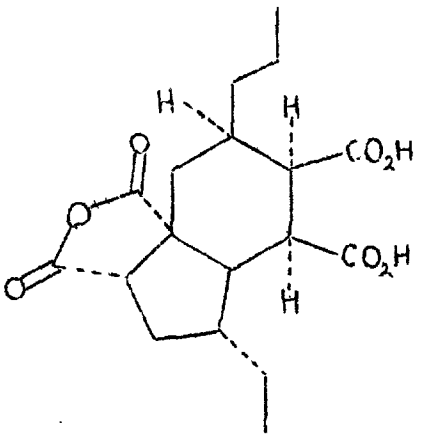
less hindered face to yield the structure (116).



(116)

The monohydrate, derived by dissolution in alkali and acidification, as dihydro-iso-byssochlamic dicarboxylic acid, $C_{18}H_{24}O_7$, m.p. 190-197° (d), had infra-red absorptions consistent with the presence of an intact five-membered anhydride ring at 1828, 1776 $cm.^{-1}$ and the characteristic carbonyl frequency due to two carboxyl groups at 1701 and 1689 $cm.^{-1}$ On the basis of the previous observation that it is generally the least substituted anhydride which opens upon hydrolysis[9] it is possible to formulate this hydrate

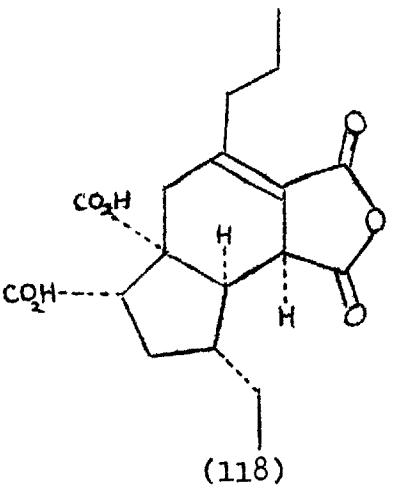
by the expression (117) which is consistent with its ready transformation, by heating above the melting point, back into dihydro-iso-byssochlamic acid.



(117)

The corresponding hydrolysis products of iso-byssochlamic acid are similarly formulated. Thus iso-byssochlamic dicarboxylic acid (118), $C_{18}H_{22}O_7$, m.p. 173-183°, $(\alpha)_D^{21}$ + 24° (1.34), which had $\nu_{mx.}$ 1840, 1780, 1700 and 1690 cm.$^{-1}$ was clearly an anhydride-dicarboxylic acid. It was readily dehydrated to yield iso-byssochlamic acid on sublimation. The question as to which anhydride was opened was resolved by an examination

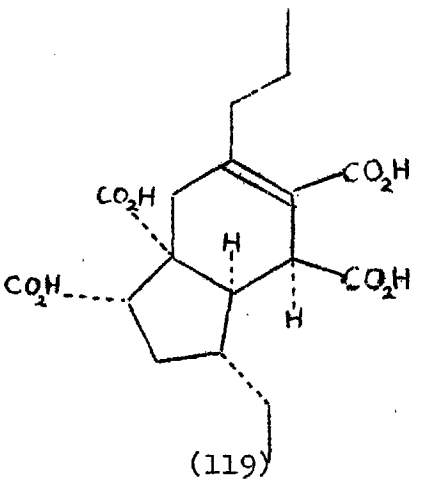
of the ultra-violet spectrum which showed $\lambda_{mx.}$ 234 mμ (ϵ 3,300), an absorption of the itaconic type. This evidence is in accord with its formulation as (118).



(118)

The dihydrate, iso-byssochlamic dicarboxylic acid m.p. 165-168°, $(\alpha)_D^{21}$ + 8° (1.2) had only the absorptions of a polycarboxylic acid at 1718 and 1680 $cm.^{-1}$. Its ultra-violet spectrum, $\lambda_{mx.}$ 221 mμ (ϵ 6,400), was that of an unsaturated acid and its ready conversion on heating to iso-byssochlamic acid is in accord with the expression (119).

Although the above discussion, represents an account of the chemistry of iso-byssochlamic acid, it is clearly in need of further support. To this end a highly crystalline bis-p-bromo-phenyl hydrazide has been submitted for X-ray analysis.



(119)

Dihydrobyssochlamic Acid

As the previous workers had observed, when reduced with zinc and acetic acid,[2] byssochlamic acid was converted to a compound dihydrobyssochlamic dicarboxylic acid, $C_{18}H_{24}O_7$, m.p. 233-240°, $(\alpha)_D^{22}$ + 14° (0.9). That this compound was an anhydride-dicarboxylic acid was apparent

from its infra-red spectrum, $\nu_{mx.}$ 1830, 1780, 1700 cm.$^{-1}$. It was fully saturated, having $\epsilon_{220m\mu}$ 450 and showed no tetranitromethane colour. The formation, on reduction of a saturated dihydro-compound is consistent with a reductive cyclisation process, in which the two maleic anhydride units become linked by a carbon to carbon bond, as described by fig.(59).

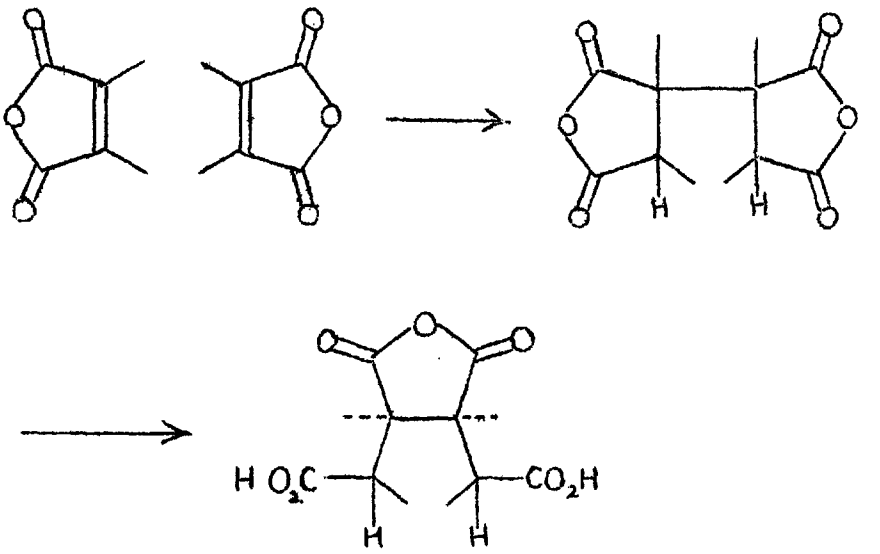


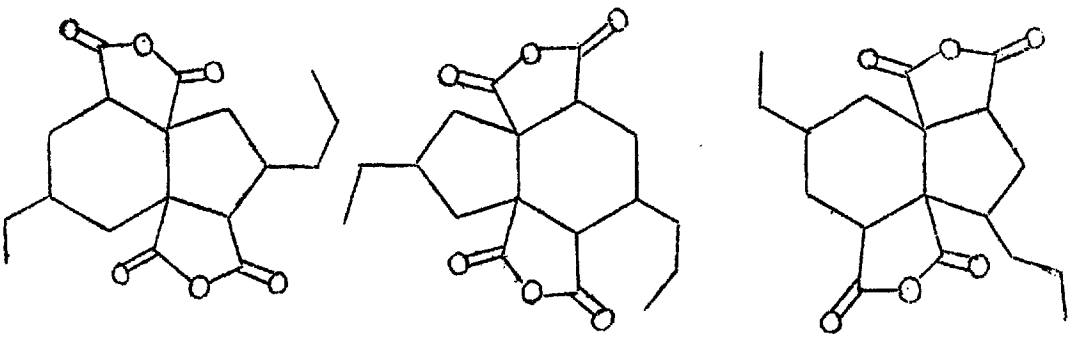
fig.(59)

The interchange of anhydride functions, depicted here, is understandable in view of the great readiness with which tetra-substituted succinic acids are dehydrated.[9]

Unlike the di- and tetra-carboxylic acids, derived from iso-byssochlamic acid, this compound was not

decomposed by heating and could be sublimed, largely unchanged, at 200°. Refluxing in acetyl chloride, however, effected a dehydration to yield dihydro-byssochlamic acid, $C_{18}H_{22}O_6$, m.p. 120°, $(\alpha)_D^{25}$ - 54° (1.00), $\nu_{mx.}$ 1840, 1775 cm.$^{-1}$ and $\epsilon_{220m\mu.}$ 350. The spectral data here were consistent with a saturated dianhydride. Hydrolysis of this last compound with dilute alkali yielded the original dihydrobyssochlamic dicarboxylic acid.

If only structures involving six and five membered rings are considered then there are three possibilities for the dianhydride, described by (120), (121) and (122), fig.(60).



(120) (121) (122)

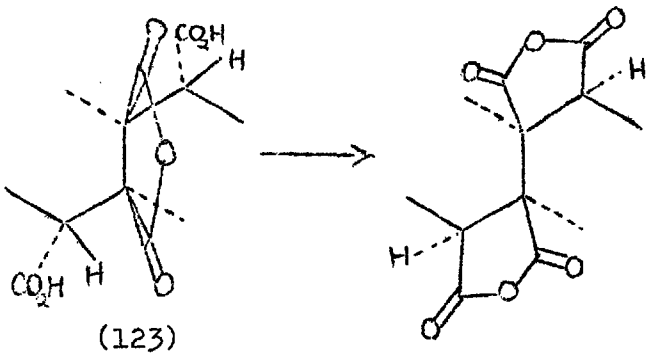
fig.(60)

Upon attempted dehydrogenation of the dicarboxylic acid this compound was converted into an isomer of dihydrobyssochlamic acid, pyro-dihydrobyssochlamic acid, $C_{18}H_{22}O_6$, m.p. 168-170°, $(\alpha)_D^{23}$ + 72° (1.1). That this compound was a saturated dianhydride was apparent from its infra-red absorptions at 1840, 1775 $cm.^{-1}$ and its transparency to ultra-violet light, $\epsilon_{220m\mu.}$ 445.

This change is best explained as a thermal epimerisation of the two, free, carboxyl groups in the molecule to a configuration which is more suitable for anhydride formation. If this is so then the original acid must have contained carboxyl groups in a transoid disposition (123), which fact was responsible for the lack of anhydride formation. Thus the pyrolytic reaction may be expressed by fig.(61).

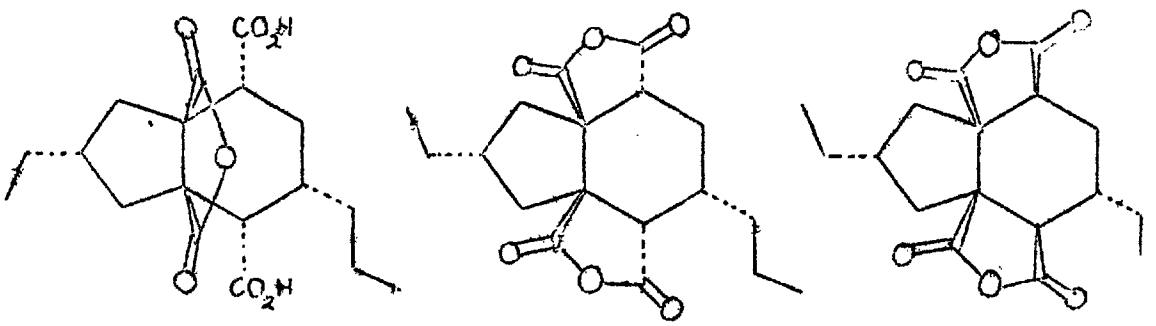
Since the original formation of a dianhydride, with acetyl chloride, involved no epimerisation (the same dicarboxylic acid was recovered upon hydrolysis), then these facts tend to support the formulation (121), above. This is the only structure in which a trans-anhydride is not fused to a five membered ring, cyclopentane-trans-1: 2-dicarboxylic acid does not give a trans-anhydride.[28] The mechanism of formation expressed by fig.(59) and the

argument concerning the relative configuration of the carboxyl groups lead to the structures for the three compounds, fig.(62), dihydro-byssochlamic dicarboxylic acid (124), dihydro-byssochlamic acid (125), and pyro-dihydro byssochlamic acid (126).



(123)

fig.(61)



(124) (125) (126)

fig.(62)

Photobyssochlamic Acid

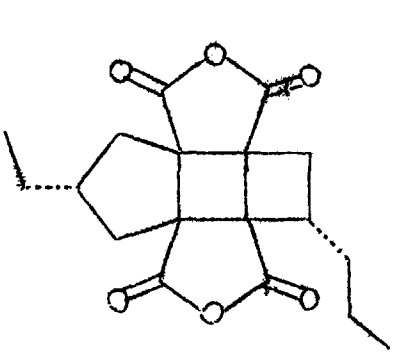
In view of the reported dimerisation of maleic anhydride and it's derivatives,[29] under photochemical conditions, to various isomers of cyclobutane tetracarboxylic acid, it was of interest to study the action of ultra-violet light on byssochlamic acid. Upon irradiation in tetrahydrofuran solution the natural product was rapidly transformed into a crystalline isomer, photobyssochlamic acid, $C_{18}H_{20}O_6$, m.p. 210-220°, $(\alpha)_D^{23}$ + 6° (1.0).

The molecular weight was determined by mass spectroscopy,[5] which gave a value of 332. The spectral data, $\epsilon_{220m\mu.}$ 725 and $\nu_{mx.}$ 1842, 1821, 1764 cm.$^{-1}$, were consistent with the presence of two saturated five-ring anhydrides. The compound showed no tendency to react with bromine or tetranitromethane and so was supposed to be fully saturated, which was in accord with the lack of vinyl protons in the n.m.r. spectrum.

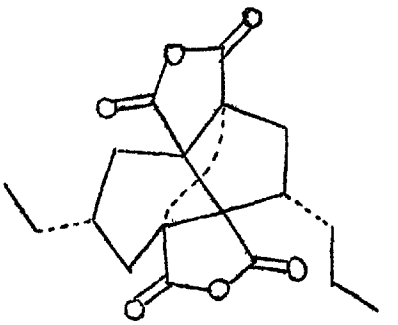
The compound titrated in hot solution, as a dicarboxylic acid and acidification produced the starting material, no hydrates of this compound being obtainable.

The constitution and lack of unsaturation indicates a tricyclic formulation for this material and such a

requirement is accommodated by structures (127) and (128).



(127)



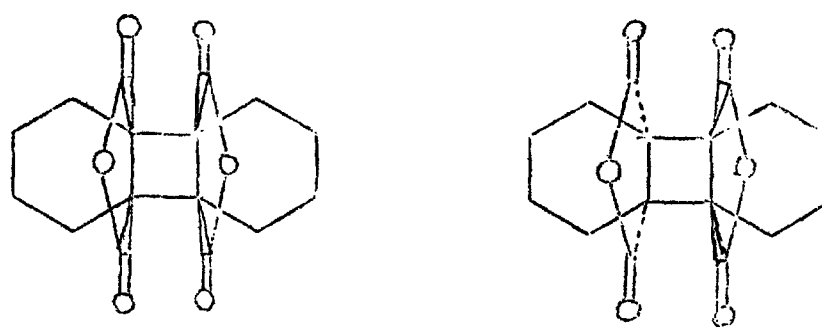
(128)

fig.(63)

All attempts at degradation of this compound were unsuccessful. The substance was stable to pyrolysis at 310°, to catalytic dehydrogenation, to acidic and basic treatment. Lead tetracetate in pyridine, a reagent which has been reported [30] to attack 1,2-dicarboxylic anhydrides did not affect this compound.

In order to obtain a simple system for study, the photolysis of the Δ-1 tetrahydrophthalic anhydride was carried out. A crystalline dimer was thereby produced, $C_{16}H_{16}O_6$, m.p. 277-283°. The molecular weight determination

of 313 ($C_{16}H_{16}O_6$ requires 304) indicated the dimeric formulation. That it was a tricyclic saturated dianhydride was deduced from its spectra, these exhibiting $\lambda_{mx.}$ 238 (780), $\nu_{mx.}$ 1865, 1835, and 1790 cm.$^{-1}$. The n.m.r. spectrum showed the absence of vinylic hydrogen atoms. By analogy with the reported reaction of maleic anhydride[29] two structures are possible for this compound, and these result from cis-(129) and trans-(130) addition respectively, fig.(64).



(129) (130)

fig.(64)

As in the case of photobyssochlamic acid the compound titrated in hot solution, only as a dicarboxylic acid and upon acidification gave a tetracarboxylic acid, which gave a crystalline methyl ester, $C_{20}H_{28}O_8$, m.p. 132-135° $\nu_{mx.}$ 1738, 1728 cm..$^{-1}$

The tetracarboxylic acid upon heating was dehydrated to the original anhydride.

The photodimer was surprisingly resistant to pyrolysis but it was possible to obtain a small yield of the original Δ-1 tetra-hydrophthalic anhydride by pyrolysis at 300° for 11 hours, fig.(65).

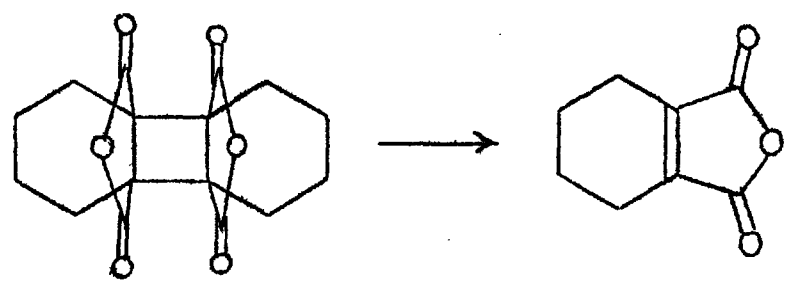


fig.(65)

This latter reaction establishes the retention of the original carbon skeleton and supports the structural proposals (129) or (130). No choice may be made between these possibilities on the basis of the available evidence and the same statement applies to structures (127) and (128) for photo-byssochlamic acid.

The related mould metabolites glauconic acid, glaucanic acids were also subjected to ultra-violet irradiation. Although the chromophores were rapidly destroyed by the treatment no crystalline materials could be isolated.

Contribution to the stereochemistry of glauconic acid

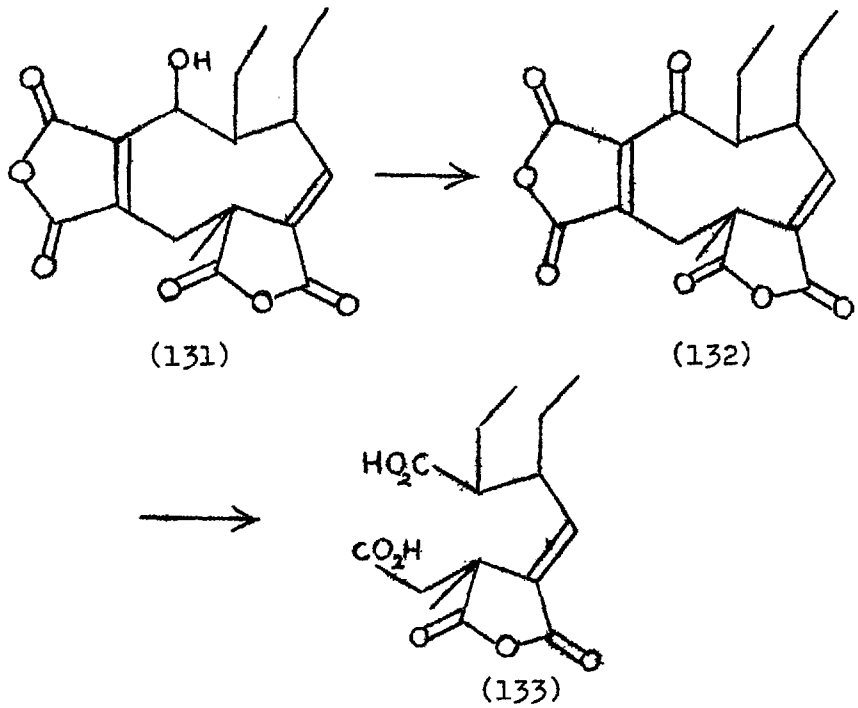
During the course of the work on byssochlamic acid it became necessary to carry out some oxidative degradations on glauconic acid. The result of this work is reported here.

Previous experiments by Dr. Sutherland had led to the isolation of a degradation product of glauconic acid, the so-called "C_{15}" acid, $C_{15}H_{20}O_7$. A structure had been proposed for this compound on the basis of its composition and spectral data.[6] When the work was repeated the "C_{15}" acid was obtained by stepwise oxidation with chromic acid of glauconic acid (131) to glauconic acid ketone (132) and thence to the "C_{15}" (133), fig.(66).

The "C_{15}" acid (133) had spectral properties in accord with its proposed structure, $\nu_{mx.}$ 1845, 1750 (five membered anhydride), and 1730, 1690 (dicarboxylic acid) and $\lambda_{mx.}$ 224 mμ (ϵ 7,450). Upon concentration of the mother liquor from this oxidation there was obtained a further crystalline material, m.p. 194-197°, which had only carboxylic absorptions in the infra-red spectrum.

This material had no selective ultra-violet absorption. The properties were those expected of some further degradation product and the possibility of its being an

alkylated dicarboxylic acid was apparent.



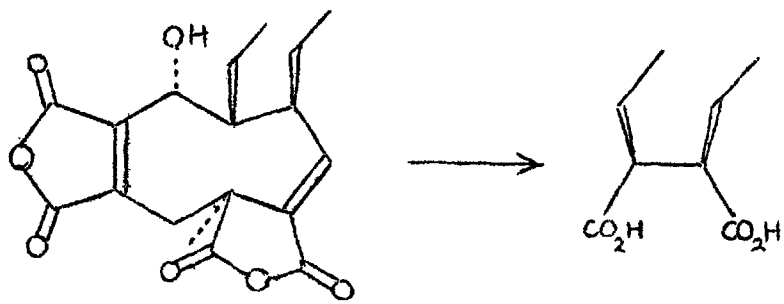
(131) (132) (133)

fig.(66)

In the event it was shown to possess the same paper chromatographic behaviour as meso-diethyl succinic acid (134). The latter compound was separated from a mixture with the racemic acid, as is described in the experimental section.

The identity was confirmed by melting point, mixed melting point and infra-red spectrum. The isolation of this fragment enabled the assignment of a cis-relationship between the two ethyl groups of glauconic acid to be made. fig.(67).

This assignment was later confirmed by the X-ray analysis of glauconic acid -meta-iodobenzoate,[31] which indicated the relative stereochemistry of the parent compound to be that expressed in (131).



(131) (134)

fig.(67)

Although the "C_{15}" acid was resistant to ozonolysis it was slowly degraded by chromic acid to meso-diethyl succinic acid, whose identity was confirmed by paper chromatography. This observation constitutes further support for the proposed structure (133) for the "C_{15}" acid.

The autoxidation of anhydrides

In an attempt to convert Ketone-II to a diosphenol using the procedure of Barton,[32] it was found that the product no longer contained the characteristic ultra-violet absorption of a substituted maleic anhydride. In order to ascertain whether this residue was stable under these conditions the model Δ-1 tetra-hydrophthalic anhydride was shaken with potassium tert-butoxide in the presence of oxygen. One mole of oxygen was quickly absorbed and the product, obtained by careful treatment of the reaction mixture was a crystalline, acidic, solid, m.p. 154-155° (d).

Analysis indicated a constitution of $C_{12}H_{18}O_6$ and the equivalent weight determination demonstrated the presence of one carboxyl group and also one peroxide residue, the latter by iodometric titration.

The spectral data were consistent with a carboxylic acid (1685 cm.$^{-1}$) and a free hydroxyl group (3400 cm.$^{-1}$). A peak at 1715 cm.$^{-1}$ was attributed to an ester function. The absorption at 217 mμ. (ϵ 8,000) was characteristic of an α-β unsaturated acid or ester.

Upon treatment with hot, dilute, sulphuric acid there was obtained a good yield of cyclohexenone, characterised as its 2:4-dinitrophenylhydrazone.

Brief treatment with diazomethane yielded a crystalline mono-methyl ester, $C_{13}H_{20}O_6$, m.p. 80-81°, which still gave a positive peroxide reaction and absorbed at 3500 $cm.^{-1}$

As in the case of the parent compound, hot, dilute, acid degraded it to cyclohexenone.

The C_{12} constitution of the peroxyacid indicated the inclusion of an extra four carbon atoms into the original C_8 formulation. It seemed reasonable to conclude that these were derived from the t-butoxide reagent.

The formation of cyclohexenone on acid treatment suggested that the six-membered ring of the starting material remained intact. With these facts in mind it seemed most likely to us, that the appearance of the peroxide could be explained by the attack of oxygen upon the enolate anion produced from the anhydride with strong base. This is analogous to the mechanism of autoxidation of enolate anions[33] derived from ketones, fig.(68).

In this case, if it is presumed that a preliminary enolisation takes place, to yield the anion (135), then in the oxidation step it is possible to visualize the formation of a tertiary hydroperoxide (136), fig.(69).

The alternative secondary hydroperoxide would be expected to immediately collapse to the ketone, under these strongly basic conditions.

The presence of a hydroperoxide in the product suggests a tertiary environment.

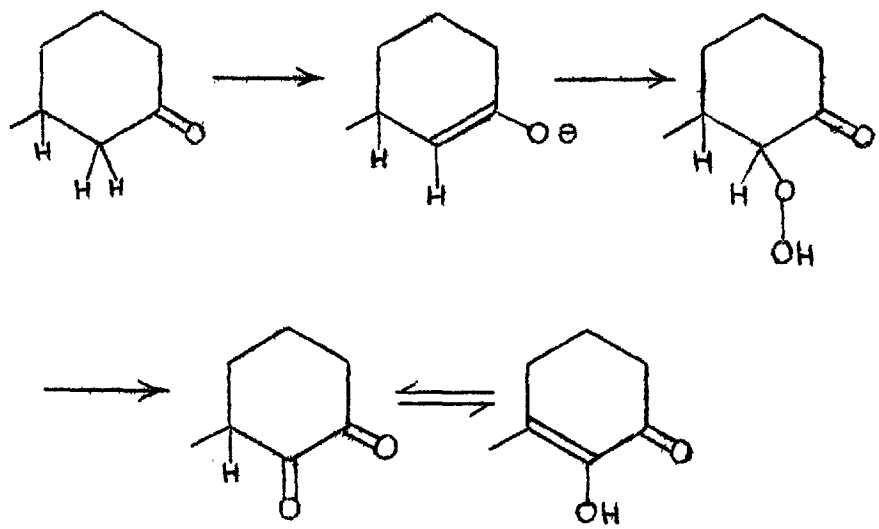
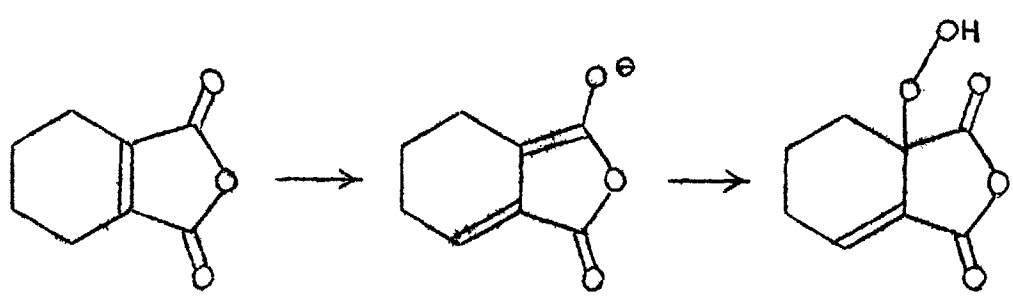


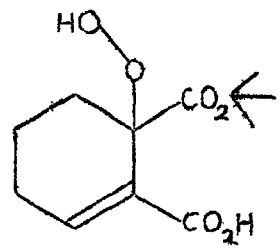
fig.(68)



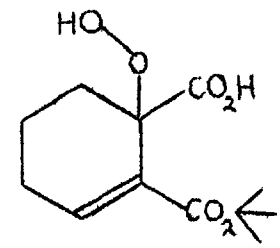
(135) (136)

fig.(69)

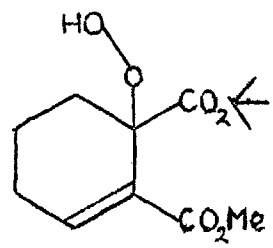
The intermediate (136) may now be opened in either of two directions by attack of t-butoxide anion to yield a compound with the required constitution (137) or (138). The derived methyl ester being (139) and (140), respectively, fig.(70).



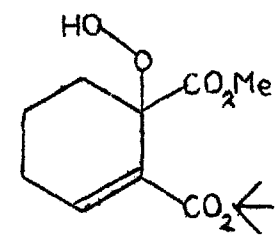
(137)



(138)



(139)



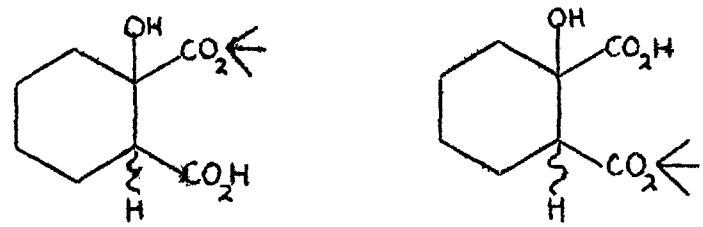
(140)

fig.(70)

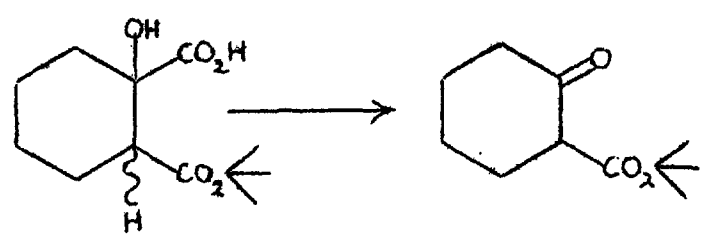
An examination of the n.m.r. spectrum of the methyl ester substantiated the essential correctness of these conclusions. Thus this compound exhibited resonances at τ 2.62 (triplet, 1 vinyl proton), 6.27 (singlet, 3 protons of a

methoxyl group), 8.53 (singlet, 9 protons of t - butoxy group).

In order to locate the positions of the t - butoxy group in the original peroxide it was reduced catalytically to a dihydro-desoxy compound, $C_{12}H_{20}O_5$, m.p. 97-99°. This compound titrated as a monocarboxylic acid and so accordingly was given two possible formulations (141) and (142), fig.(71).



(141) (142)

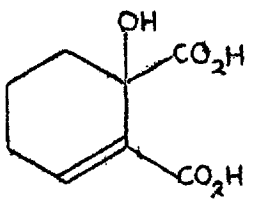


(142) (143)

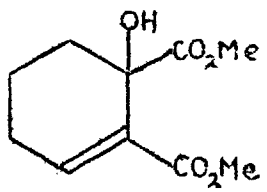
fig.(71)

It was hoped to cleave this compound with lead tetra-acetate to the corresponding keto-ester (143). However under conditions in which malic acid was readily cleaved this material was unaffected, thus supplying negative evidence in favour of (141).

Sodium borohydride reduction of the peroxide resulted in both reduction and hydrolysis to yield a dicarboxylic acid, m.p. 175-180° (d), further characterised as the dimethyl ester $C_{10}H_{14}O_5$, m.p. 91-92°.
The acid and the ester only have one formulation from either precursor, these are (144) and (145), fig.(72).



(144)



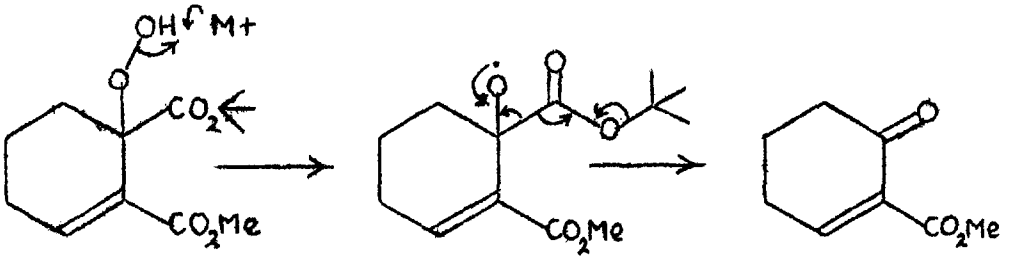
(145)

fig.(72)

The n.m.r. spectrum of the ester was in accordance with this expression, having τ 2.74 (triplet, 1 vinyl proton), 6.23 (doublet, 6 protons in two methoxyl groups).

Although the dicarboxylic acid reacted readily with lead tetracetate under mild conditions the product could not be isolated as a crystalline derivative.

Since both of the above attempts to locate the position of the t - butyl residue had failed it seemed possible that an application of the well known radical decomposition of peroxides[34], brought about by transition metals, coupled with the relative ease of formation of t - butyl radicals might constitute a possible selective degradation of this system. Thus a fragmentation might be achieved which would leave undisturbed the adjacent carboxyl group. This is expressed by fig.(73).



(146)

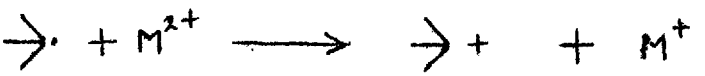


fig.(73)

The free t - butyl radical could be oxidised by the higher valence state of the metal to the carbonium ion. Thus the overall reaction should be catalysed by a suitable metal ion. If the adjacent carboxyl group were suitably marked, say as the ester, then the isolation of the ester-ketone (146), would constitute evidence for a unique structure (137) of the peroxide.

When the peroxide-methyl-ester was treated with ferrous ammonium sulphate at 0°C, there was isolated as the 2:4-dinitro-phenyl hydrazone, 2-methoxycarbonyl-cyclohex-2-enone (146), $C_{14}H_{14}N_4O_6$, m.p. 200-206°. This was as yet an unknown compound. The absorption at 1720 $cm.^{-1}$ was consistent with the presence of an unsaturated ester function and the ultra-violet absorptions at 254 mμ. (ε 13,600), 370 mμ. (ε 23,200) were identical with those reported by Brenner[35] for the corresponding ethyl ester. Further confirmation came from the n.m.r. spectrum of this derivative, which showed, as well as the usual aromatic protons, a triplet at τ 2.98 (1 vinyl proton), and a singlet at τ 6.11 (3 protons in a methoxyl group). Thus the peroxy-acid may be formulated only as (137).

Since the mechanism of its formation has been previously rationalised it remains to justify the degradation to cyclohexenone.

This is understandable when the known lability of t - butyl esters towards acids is remembered, thus a preliminary hydrolysis to a hydroperoxy-acid yields a system (147) which is ideally arranged for a decarboxylative elimination, fig.(74).

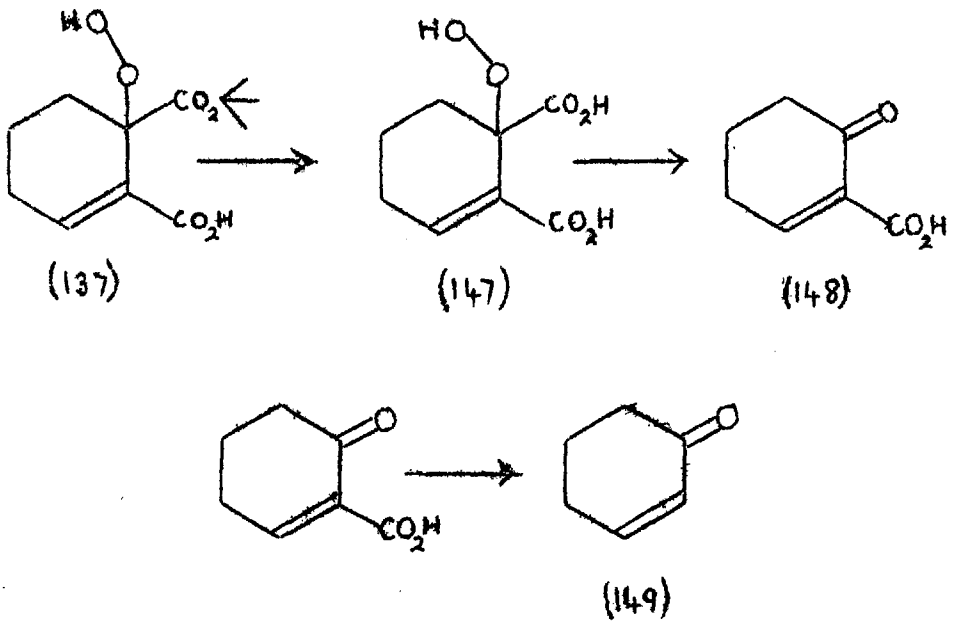
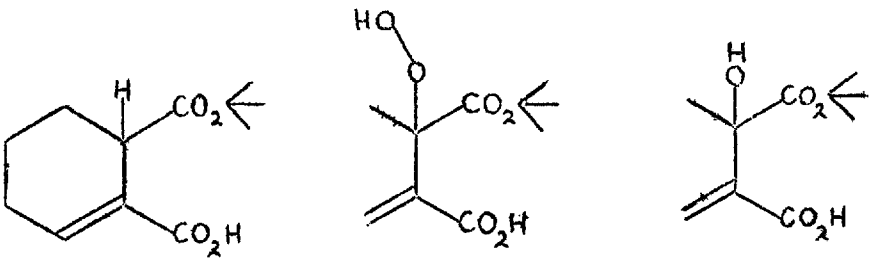


fig.(74)

The unsaturated keto-acid (148) could easily be decarboxylated via the $\beta\gamma$-unsaturated isomer.

That the preliminary enolisation process required by the mechanism of formation of the peroxide is a facile process was shown by the dissolution of the anhydride in t - butoxide under an atmosphere of nitrogen.

There was obtained a crystalline solid, 1-t-butoxy-carbonyl-2-carboxy-cyclohex-2-ene (150), m.p. 127-128°. This material was also autoxidised under the condition used for the anhydride, but the reaction proceeded at a much slower rate and in lower yield. This is in keeping with the presence of a free carboxylate anion in this product.



(150) (151) (152)

fig.(75)

To extend the generality of this reaction, dimethyl maleic anhydride was submitted to the same conditions of reaction and there was obtained a crystalline, non-peroxidic, material, $C_{10}H_{16}O_5$, m.p. 104-105°. This material titrated as a monobasic acid and had $\lambda_{mx.}$ 210 mμ. (ε 4,700) and

$\nu_{max.}$ 3400, 1720, 1690, 880 cm.$^{-1}$. This data being consistent with the presence of a hydroxyl group, an ester and an acidic residue. That the compound was the reduced form (152) of the expected peroxide (151) was made clear by the n.m.r. spectrum, which was characterised by τ 3.74 (doublet, 2 vinyl protons), τ 8.46 (singlet, 1 quaternary C - methyl group), τ 8.56 (singlet, nine protons assigned to the t-butyl group). The molecule readily absorbed one mole of hydrogen by catalytic hydrogenation.

The presence of the reduced form of the peroxide probably arises via intermolecular oxidation of the sensitive vinyl function.

Further examples studied were citraconic anhydride and cis-hexahydrophthalic anhydride but neither led to any characterisable material. The reaction seems to be restricted to dialkyl substituted maleic anhydrides.

Miscellaneous Reactions of Anydrides

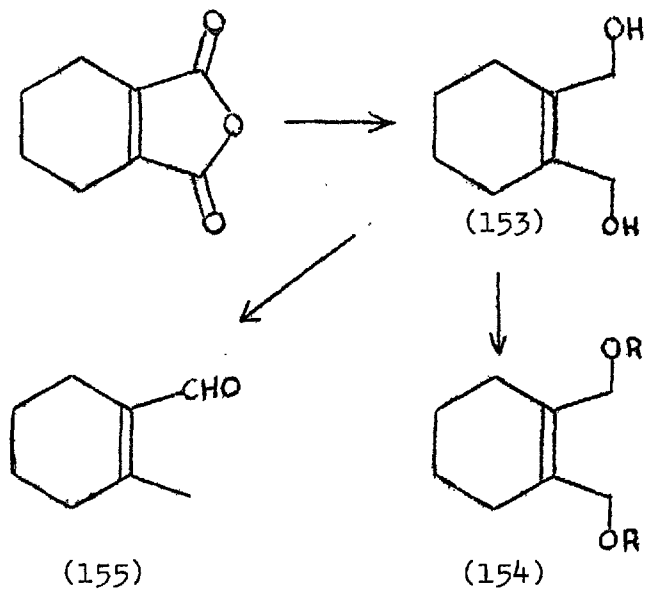
Two further reactions of cyclic anhydrides have been studied on model compounds but have had no useful application in the structural work on the natural product.

1) Hydride reductions of disubstituted maleic anhydrides

A potential degradation of byssochlamic acid was the reduction of that compound to a tetrol. The reduced material, lacking the strongly electron withdrawing carboxyl groups was presumed to be more susceptible to ozonolysis than the original anhydride. The reduction with lithium aluminium hydride of Δ-1 tetrahydrophthalic anhydride resulted in the formation of an oily, hydroxylic, material, which however still contained a carbonyl absorption at 1725 cm.,$^{-1}$ The material was divided into two portions by extraction with alkali.

The alkali-insoluble part, which showed only hydroxylic absorption in the infra-red spectrum, was shown to be 1:2 - dihydroxymethyl -cyclohexene (153) by conversion under mild conditions, to the crystalline bis- 3:5 - dinitrobenzoate (154), $C_{22}H_{18}N_4O_{12}$, m.p. 158-160°. This diol also gave a non-crystalline diacetate. Furthermore, when the original diol was warmed with acidic 2:4 - dinitrophenylhydrazine solution there was obtained the 2:4 -dinitrophenylhydrazone of 2-methyl-cyclohexene-

-carboxaldehyde (155), m.p. 190-191°. This was a known compound[36] and the n.m.r. spectrum and ultra violet absorption ($\lambda_{mx.}$ 388 mμ. (ϵ 30,400)) were in accord.

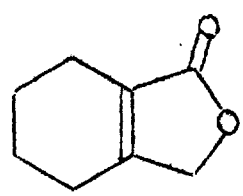


(R= 3:5-dinitrobenzoyl)

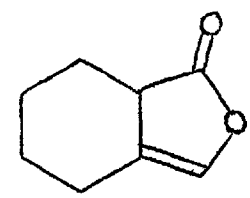
fig.(76)

The alkali soluble part, upon acidification, produced an oily mixture of lactones, whose infra-red spectrum, $\nu_{mx.}$ 1720, 1760 cm.,$^{-1}$ was in accord with their formulation as a mixture of $\alpha\beta$- and $\beta\gamma$-unsaturated - γ - lactones, (156) and (157) respectively, fig.(77).

The mechanistic rationalisation of these products is that there exists two routes by which the product of addition of two hydride ions (158), to the unsaturated anhydride, may decompose.



(156)



(157)

fig.(77)

The first way (I), by which the bulk of the material reacts, simply leads to the intermediacy of the dialdehyde (159) which is further reduced to the diol. The second route (II), involves the elimination of an oxygen atom, presumably as the aluminium alkoxide, to yield the aluminium enolate of a 1-hydroxy furan (160). The latter anion would be resistant to further reduction and hence on work up would yield the α/β-unsaturated lactone, fig.(78).

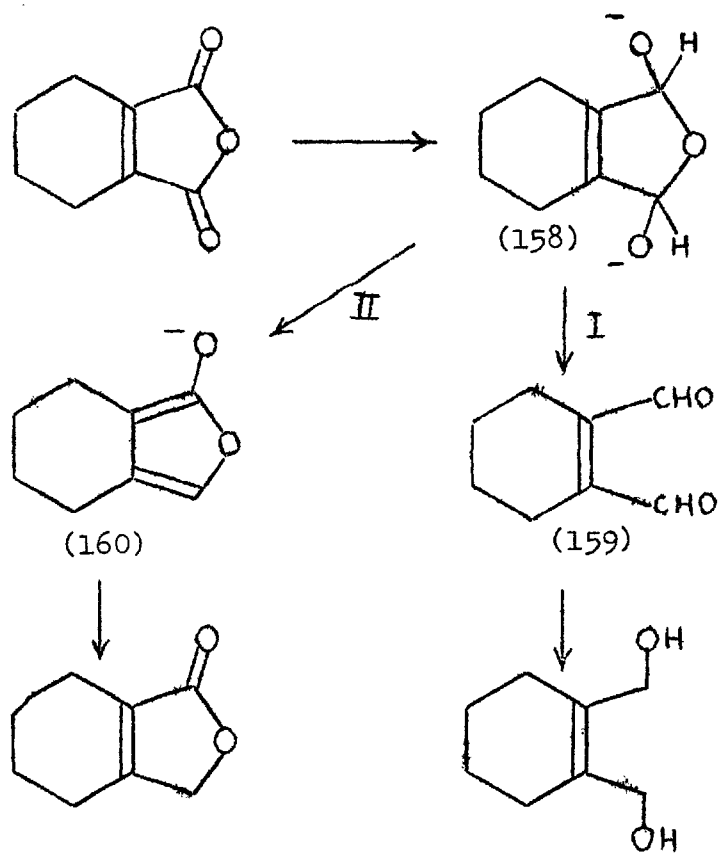
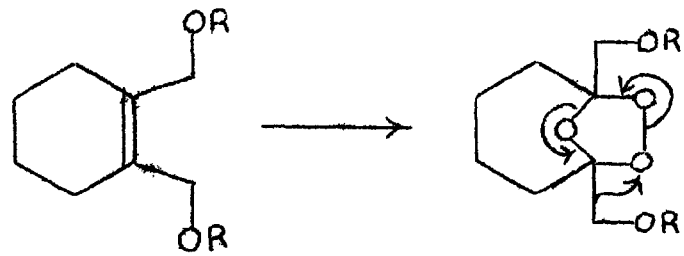
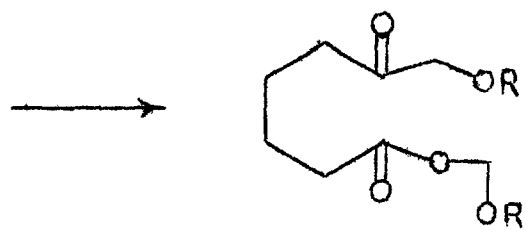


fig.(78)

In confirmation of the original expectation, the bis- 3:5 -dinitrobenzoate was smoothly ozonised to a crystalline product, $C_{22}H_{18}O_{15}N_4$, m.p. 186-188°. The absorptions at 1750, 1730 $cm.^{-1}$ were consistent with its formulation as a ketol ester (161), by way of the mechanism illustrated, fig.(79).



(154)



(161)

(R=3:5-dinitrobenzoyl.)

fig.(79)

2) <u>The preparation of cyclic thioanhydrides</u>

A report that the mixed anhydrides of carboxylic acids and dithiocarbonic acids, upon irradiation, underwent fragmentation reactions,[37] fig.(80), suggested a photochemical degradation of anhydrides.

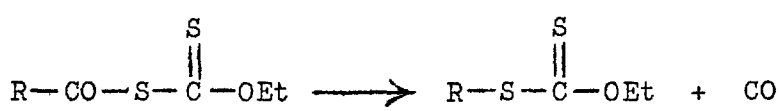


fig.(80)

It was postulated that a cyclic thioanhydride, upon irradiation, would decompose in such a manner, fig.(81), as to yield the olefine.

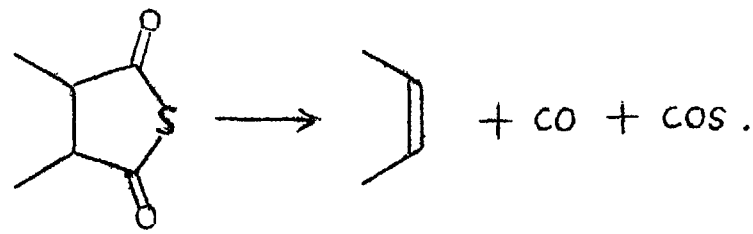


fig.(81)

For this reaction a suitable route to cyclic thioanhydrides was required and so the following procedure was used to meet this requirement.

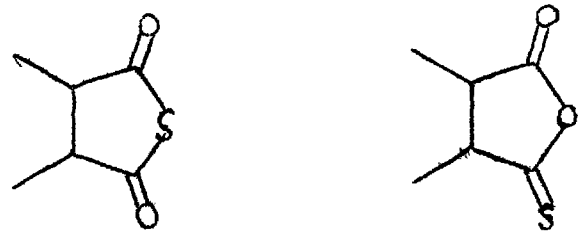
The corresponding cyclic carboxylic anhydride was shaken with a solution of phosphorus pentasulphide in pyridine for three days at room temperature. Work up under anhydrous conditions gave rise to the corresponding thioanhydrides. Thus from model compounds the following examples were prepared.

(i) Phthalic thioanhydride, m.p. 114°,

(ii) Δ -4 tetrahydrophthalic thioanhydride, m.p. 142-143°,

(iii) cis-hexahydrophthalic thioanhydride, an oil.

All of these compounds showed two closely spaced absorptions at about 1710 $cm.^{-1}$ in the infra-red. Such an absorption is more in accord with the symmetrical structure (162) than the alternative (163).



(162) (163)

fig.(82)

As would be expected these compounds were all very sensitive to acids and bases, being readily hydrolysed back to the corresponding dicarboxylic acids, with loss of hydrogen sulphide.

Experimental Part

Melting points were determined on a Kofler block and are uncorrected.

Rotations were measured in chloroform, unless otherwise stated, on a photoelectric polarimeter.

Ultra-violet spectra were recorded, in absolute ethanol solution, on a Unicam SP.700 instrument. Infra-red spectra were recorded on a Perkin Elmer 137 and also a Unicam SP.200 instrument.

Nuclear magnetic resonance spectra were kindly determined by Dr. Foster and Mrs. Boston on a Varian A.60. Micro-analyses were performed by the staff of the micro-analytical laboratory (Imperial College).

Gas-liquid chromatograms were run on a Pye Argon Chromatograph, using a polyethyleneglycol-adipate column. Chromatograms were run at 150° with a flow rate of 20 ml. p.min..

Work up of reactions by extractive procedures involved the washing of the organic phase with saturated sodium chloride solution, after which it was dried with sodium sulphate and evaporated under reduced pressure.

Acidification of reaction mixtures was, unless otherwise stated, with hydrochloric acid (2.0N), (congo red).

Preparation of Byssochlamic Acid Ketones

Byssochlamic acid (1.0 g) was dissolved by warming with aqueous sodium hydroxide (N 0.2, 100 ml.). The solution was cooled to room temperature and titrated with hydrochloric acid (N 4.0) to pH 10. To this solution was added hydroxylamine hydrochloride (0.50 g, 2 m) and the rapidly darkening solution allowed to stand overnight. It was then cautiously acidified with hydrochloric acid (N 4.0) and thoroughly extracted with chloroform. Unchanged byssochlamic acid was removed by shaking the chloroform solution with saturated sodium bicarbonate, when the N-hydroxyimide passed into the bicarbonate phase as a cherry red anion. The product was recovered by acidification and re-extraction with chloroform (200 ml.). After drying over sodium sulphate, careful evaporation at 50° gave byssochlamic acid mono-N-hydroxyimide (75) as a foam (1.1 g.).

This gave no ferric chloride colour and behaved as a single compound upon chromatography over silica-gel. This product showed absorptions in the infra-red and ultra-violet regions compatible with its formulation as an N-hydroxyimide.

Byssochlamic Acid -N-hydroxyimide	N-hydroxy-dimethyl maleimide[13]	Assignment
1840, 1770 cm.$^{-1}$	-	5-membered anhydride
1800, 1720, 1660, 3200 cm.$^{-1}$	1792, 1730 1667, cm.$^{-1}$	N-hydroxyimide
232 mμ.(ε 12,500)	225 mμ. (ε 14,500)	
214 mμ. (ε 9,800)	214 mμ. (ε 16,700)	

This compound was converted to its N-tosyloxy derivative by dissolving in dry pyridine (distilled from potassium hydroxide) and then adding to this ice-cold solution finely powdered p-toluene sulphonyl chloride (1.8 g.). The initially yellow solution was allowed to stand at 0° overnight. It was poured into ice-water (250 ml.) and after two hours was acidified with dilute hydrochloric acid (N 1.0) and extracted with chloroform (200 ml.). The solution was washed with water, dried with sodium sulphate, and upon careful evaporation at 50° gave byssochlamic acid mono-N-tosyloxyimide as a foam (1.3 g.).

This compound was homogeneous by chromatography over silica-gel and had the following spectral characteristics, $\nu_{mx.}$ 1860, 1820, 1760, 1740, 1600 cm.,$^{-1}$

consistent with the presence of one anhydride ring and one N-tosyloxyimide residue.

The hydrolysis to the mixture of ketones was conducted by dissolution of the tosylate in absolute alcohol (12 ml.) and then aqueous potassium hydroxide was added (N 1.0, 50 ml.) and the solution heated on the steam bath in a stream of nitrogen for about three hours, when all the ammonia evolution had ceased. The solution was cooled to room temperature and extracted with chloroform (100 ml.) and this extract discarded. The aqueous phase was acidified and again extracted with chloroform (200 ml.). After washing and drying the organic phase, evaporation produced a crystalline mixture of ketones (0.55 g.). The mixture was chromatographed on silica-gel (B.D.H. acid washed, 35 g.) elution with benzene gave Ketone-I (105), recrystallised from ligroin or aqueous ethanol (0.22 g.), m.p. 110°, $(\alpha)_D^{25}$ - 79° (c0.90). (Found : C, 68.98; H, 8.03, $C_{16}H_{22}O_4$ requires C, 69.04; H, 7.97%). ν_{mx} 1859, 1761, 1667, 1695 $cm.^{-1}$ $\lambda_{mx.}$ (cyclohexane) 215 mμ.(ε 5,010), 252 mμ.(ε 4,600). This compound gave a positive Zimmerman test.[15]

Further elution with benzene containing ether (15% v/v.) gave Ketone-II (106), recrystallised from aqueous ethanol to m.p.161°, (90 mg.), $(\alpha)_D^{24}$ + 5.5

(c 0.95). (Found: C, 68.77; H, 7.55; $C_{16}H_{22}O_4$ requires C, 69.04; H, 7.97%).

$\nu_{mx.}$ 1845, 1761, 1658, 1685 cm.$^{-1}$

$\lambda_{mx.}$ (cyclohexane) 215 mμ. (ε 4,600), 255 mμ. (ε 5,500).

This did not give the Zimmerman test.

Attempted isomerisation of the Ketones

1) Base catalysed. The ketone (20 mg.) was refluxed with freshly distilled triethylamine (5 ml.) for three hours. Evaporation of the solvent and crystallisation of the residue gave material which was identical with the starting material in each case, m.p. mixed m.p., and infra-red spectrum.

The same result was obtained by subjecting the ketone (20 mg.) to the original conditions of formation. Work up by the same procedure yielded unchanged starting material.

2) Acid catalysed. The ketone (20 mg.) was refluxed with acetic acid (glacial,A.R. 8.0 ml.) and concentrated hydrochloric acid (2.0 ml.) for 22 hours. The reaction was followed by the ultra-violet spectrum.

Ketone- II was recovered quantitatively in this experiment as was shown by m.p., mixed m.p., and infra-red spectrum.

Ketone-I was slowly converted into a non-crystalline acid-ketone, $\nu_{mx.}$ 1700, 2600, 3300 cm.,$^{-1}$ no selective absorption in the ultra-violet spectrum. This material was smoothly converted to a non-crystalline anhydride-ketone by warming with acetyl chloride, $\nu_{mx.}$ 1840, 1775, 1705 cm.,$^{-1}$ no selective absorption in the ultra-violet spectrum.

Byssochlamic Acid- α -Oximino-Ketone-I (99)

Ketone-I (243 mg.) in dry ether (30 ml.) was stirred at 0° while passing a stream of dry hydrogen chloride into the solution. After five minutes a solution of amyl nitrite (freshly distilled, 0.5 ml.) in dry ether (10 ml.) was added, dropwise, over one hour.[17] The passage of hydrogen chloride was continued for another hour and then the solution was evaporated to dryness and the residue crystallised from chloroform-petroleum ether (b.p. 40-60°) to yield byssochlamic acid- α -oximino-ketone-I as needles (200 mg.), m.p. 171-172°, $(\alpha)_D^{22}$ + 445° (c 1.0), (Found; C, 63.06; H, 6.87. $C_{16}H_{21}NO_5$ requires C, 62.52; H, 6.88%).
$\nu_{mx.}$ (Nujol) 1845, 1775, 1680, 1630, 3300 cm.,$^{-1}$
$\lambda_{mx.}$ 207 mμ.(ε 9,000), 239 mμ.(ε 8,500), 267 mμ.(ε 4,200), shifted with one drop of potassium hydroxide (N 0.1)

to $\lambda_{mx.}$ 230 mμ.(ε 8,500).

Byssochlamic Acid-Nitrile-Acid (82)

The oximino-ketone (99) (50 mg.) in aqueous potassium hydroxide (N 0.5, 4.0 ml.) was treated at room temperature with finely powdered p-toluene sulphonyl chloride (104 mg. 3 m.) and shaken for 15 hours. The initial yellow colour of the solution was gradually discharged. The solution was filtered, saturated with sodium chloride, acidified with dilute hydrochloric acid and extracted with ether (150 ml.) to yield the nitrile-acid (82) as an oil (30 mg.)

$\nu_{mx.}$ 2600-3300, 2300, 1823, 1767, 1708 cm.$^{-1}$

$\lambda_{mx.}$ 210 mμ.(ε 9,000), 251 mμ.(ε 3,900).

This was converted into the monomethyl ester by treatment with ethereal diazomethane over three minutes. The ester (83) was distilled in high vacuum (95°, 10^{-4}mm.Hg.) as a colourless oil, $(\alpha)_D^{25}$ - 7° (c 1.8). (Found: C, 63.71; H, 7.33. $C_{17}H_{23}O_5N$ requires C, 63.53; H, 7.21%).

$\nu_{mx.}$ 1820, 1855, 1770, 1730, 1280, 2300 cm.,$^{-1}$

$\lambda_{mx.}$ 210 mμ.(ε 9,000), 255 mμ(ε 4,000).

Ozonolysis of Ketone- I -anhydride-nitrile-ester (83)

After preliminary experiments had shown that the rate of ozonolysis, as determined by the rate of disappearance of the maximum at 255 mμ., was extremely slow

at -20°, the ozonolysis was conducted at 0°C.

Thus the compound (83) (97mg.) in ethyl acetate (A.R. 20 ml.) was cooled to 0° and a stream of ozonised, dry, oxygen blown through the solution, the reaction being followed by the intensity of the peak at 255 mμ.. After seven hours the reaction had proceeded to about 90% and so the ozone supply was disconnected and the solvent removed under vacuum at 0°. The work up from this point proceeded by two possible routes.

1) The ozonide was treated with an ice-cold mixture of aqueous potassium hydroxide (N 1.0, 3.0 ml.) and hydrogen peroxide (30%, 2.0 ml.) and then allowed to stand at room temperature for 20 minutes. A trace of platinic oxide was added to destroy excess peroxide and the reaction mixture allowed to stand for a further ten hours. At the end of this time it was warmed on the steam bath for 30 minutes and then acidified, saturated with salt and continuously extracted with ether for 24 hours to yield a mixture of acids (54 mg.). A small portion (3 mg.) was converted to the methyl ester (diazomethane) and examined by gas-liquid chromatography. This indicated the presence of two components, in the ratio 65:35 (M), as well as a little oxalic acid, ($<$ 5%).

These components were separated by chromatography of the bulk of the material on silicic acid.

Partition Chromatography.[38,39]

Silica-gel (B.D.H. chromatographic grade, 45 g.) was ground in a mortar with slow addition of water (12 ml.) until a finely divided, free flowing, powder was obtained. This was suspended in chloroform (100 ml.) and poured into a suitable column. The silicic acid was allowed to settle with gentle vibration of the column and also with a slight compression applied at every centimeter of the column's length. It was possible, in this way to obtain a fairly reproducible column, however, it was noted that differences between various samples of silica-gel caused changes in the column performance. The sample was introduced in the minimum volume of chloroform and the chromatogram developed by elution with chloroform containing a steadily increasing concentration of n-butanol, the eluant having been saturated with water before use.

The chromatogram could be followed in two ways, that is by titration of the eluate with dilute alkali or by evaporation of the eluate and subsequent weighing of the residues. For small samples, say less than 10 mg.

the former method is more reliable for location of samples in the eluate, but of course involves the regeneration of the acid from the salt, so obtained. This method was applied to the problem in hand, the minor component (B) being eluted with chloroform containing 2% n-butanol, whilst the major component (A) was removed with 3% n-butanol.

Component A. The solution of the potassium salt, obtained from the chromatogram titration, was acidified and extracted with ether continuously, to yield a crystalline solid (30 mg.). This was divided into two portions.

(i) The smaller portion (5 mg.) was warmed with acetyl chloride (redistilled, 3 drops) on the steam bath for three minutes. It was evaporated under reduced pressure and the residual oil distilled in high vacuum (80-90°, 10^{-4}mm Hg.) as an oil, $\nu_{mx.}$ 1783, 1864 cm.$^{-1}$. Authentic succinic anhydride had $\nu_{mx.}$ 1786, 1868 cm..$^{-1}$ Authentic glutaric anhydride had $\nu_{mx.}$ 1765, 1810 cm..$^{-1}$ The relative intensities of the two bands were identical to those of succinic anhydride.

(ii) The remainder was sublimed in high vacuum (95°, 10^{-4} mm.Hg.) as a crystalline solid (21 mg.) which after two crystallisations from benzene-ligroin mixture had m.p. 103-104°, $(\alpha)_D^{25}$ - 18° (c 0.6), and whose infra-red

spectrum was identical with that of authentic (+) n-propyl succinic acid,[40] m.p. 104-105°, $(\alpha)_D^{25}$ + 18°.

A small portion (2 mg.) was converted to the dimethyl ester (diazomethane) and was not separated in a mixed gas-liquid chromatogram from authentic (±) - dimethyl n-propyl succinate. The identity of the material (3.83 mg.) was finally confirmed by mixing with a quantity of authentic (+) n-propyl succinic acid (3.84 mg.) in benzene solution. Recrystallisation from benzene-petrol (40-60° b.p.) produced racemic n-propyl succinic acid (5 mg.), m.p. 92°, identified by mixed m.p. and infra-red spectrum (Nujol).

Component B. The solution of the potassium salt was taken to dryness and suspended in dry methanol (10 ml.) and saturated with hydrogen chloride for two hours at room temperature. Evaporation of the solution produced a gum (20 mg.), which showed amide absorption at 1670 $cm.^{-1}$ but no nitrile band in the infra-red spectrum. This material was hydrolysed with concentrated hydrochloric acid (2.0 ml.) at 95° for three hours. After evaporation to dryness and extraction of the residue with chloroform there was obtained a gum (7 mg.) which was distilled in high vacuum (95°, 10^{-4} mm.Hg.). The oily, crystalline, product was crystallised from benzene-ligroin to m.p. 71-73°, mixed m.p. with authentic β-ethyl glutaric acid

was 71-73°, m.p. of authentic material was 72-73°.

A small portion (1 mg.), after conversion to the dimethyl ester (diazomethane) was not separated in a mixed chromatogram from the dimethyl ester of β-ethyl glutaric acid.

2) To the gummy ozonide was added a cold mixture of aqueous potassium hydroxide (N 1.0, 3.0 ml.) and hydrogen peroxide (30%. 2.0 ml.) and then the reaction mixture was warmed on the steam bath, cautiously, until the initial vigorous reaction was complete and then held at steam bath temperature for eight hours. The solution was cooled, acidified, and then continuously extracted with ether for 24 hours to yield a mixture of acids (60 mg.).

The gum was dissolved in absolute ethanol (5.0 ml.) and titrated with aqueous potassium hydroxide (0.72 ml., 0.1 N.). To the warmed solution, adjusted to pH. 5-6, was added p-phenyl phenacylbromide (198 mg., 1 m.) in ethanol and the mixture heated on the steam for three hours. Slow cooling produced the bis-p-phenyl phenacyl ester of (-) n-propyl succinic acid, which was chromatographed on alumina (G III , neutral, 6 g.) in benzene to yield the pure bis-p-phenyl phenacyl ester of (-) n-propyl succinic acid (20 mg.). m.p. 144-147°,

$(\alpha)_D^{29}$ + 34°, (c 1.2). (Found: C, 77.11; H, 5.88, $C_{35}H_{32}O_6$ requires C, 76.6; H, 5.88%).

The mother liquor from the esterification reaction was diluted with water, and extracted with chloroform to yield a gum (90 mg.) which was chromatographed on alumina (G III, neutral, 6 g.) in benzene. Elution with benzene-chloroform mixture (2:1 v/v) gave a crystalline solid (15 mg.), recrystallised from benzene-petrol (40°- 60° b.p.) to m.p. 137-139°, $(\alpha)_D^{25}$ + 15°, (c 0.6), (Found: C, 71.57; H, 6.56; $C_{21}H_{23}O_4N$ requires C, 71.37; H, 6.56%). This was the p-phenyl phenacyl ester of (+) 4-carbamoyl-3-ethyl-butyric acid, $\nu_{mx.}$ (Nujol) 1740, 1695, 1660, 1633, 1600 $cm.^{-1}$.

The infra-red spectrum of this acid was identical to that obtained from the ester of (-) 4-carbamoyl -3-ethyl-butyric acid. The identity was confirmed in the following way. The ester from the natural product (1.73 mg.) was mixed in benzene solution with the synthetic material of opposite configuration (1.74 mg.) and the mixture so obtained was evaporated and crystallised from benzene to yield, as fine needles (2.5 mg.), p-phenyl phenacyl ester of (±) 4-carbamoyl -3-ethyl-butyric acid, m.p. 134-135°, identical with an authentic

specimen, as was demonstrated by m.p., mixed m.p., and infra-red spectrum.

Ketone- II -lactone (107a)

Ketone- II (200 mg.) was suspended in trifluoacetic anhydride[26] (4.55 g. 3.0 ml.) and cooled to -12°, with stirring. A solution of hydrogen peroxide (80%, 1.12 g. 0.9 ml.) was added slowly over 30 minutes. This reaction was autocatalytic and so the addition of hydrogen peroxide was made initially with great caution. It was advantageous to add one drop of peroxide and then remove the cooling bath until the reaction had started, and then to replace the bath immediately. Thereafter additions could be made with confidence. The reaction mixture became clear. The cooling bath was replaced by an ice-bath and stirring continued for 30 minutes, and then the reaction was allowed to stand for 48 hours at 0°. It was poured into ice-water and extracted with chloroform, the residue obtained by evaporation of the solvent was recrystallised from aqueous ethanol as ketone- II -lactone (107a), (83 mg.), m.p. 100-103°, $(\alpha)_D^{23}$ + 5° (c 1.0). (Found: C, 65.18; H, 7.23. $C_{16}H_{22}O_5$ requires C, 65.29; H, 7.53%).

This material had $\nu_{max.}$ 1835, 1773, 1706, 1160 cm.$^{-1}$

$\lambda_{mx.}$ (cyclohexane) 209 mμ. (ε 4,300), 256 mμ. (ε 4,500).

Hydrolysis of Ketone-Π-lactone (107a)

The lactone (83 mg.) was dissolved at room temperature, under nitrogen, in aqueous sodium hydroxide (N 4.0) and the resulting solution warmed on the steam bath for five hours, allowed to cool overnight to room temperature, acidified and extracted thoroughly with chloroform (150 ml.). Evaporation of the solvent gave an oily hydroxy-acid (70 mg.), $\nu_{mx.}$ 3300, 1820, 1760, 1705 cm.$^{-1}$ $\lambda_{mx.}$ 210 mμ.(ε 6,000), 253 mμ.(ε 4,500). This material was treated with dry diazomethane in ether for one minute and then evaporated to a clear gum, ketone-Π-hydroxy-ester, which had $\nu_{mx.}$ 3500, 1810, 1760, 1720 cm.$^{-1}$.

This compound was acetylated by dissolving in acetic anhydride. (A.R. 0.6 ml.), with a little chloroform to assist solution (1.0 ml.), cooled to 0° and treated with pyridine (2 drops). After standing for six hours at 0° the mixture was poured into ice-water, allowed to stand for 30 minutes and then acidified with hydrochloric acid (IN). Extraction with chloroform gave a gum, distilled in vacuum (110°, 10^{-4} mm.Hg.) as a colourless oil (75 mg.),

Ketone- II -ester-acetate (107b), $(\alpha)_D^{25} \pm 0^\circ$ (c 3.5). (Found: C, 62.43; H, 8.26. $C_{19}H_{28}O_7$ requires C, 61.94; H, 7.66%), $\nu_{mx.}$ 1820, 1760, 1725, 1620 cm.$^{-1}$ $\lambda_{mx.}$ 253 mμ. (ε 4,500).

Ozonolysis of Ketone- II -ester-acetate (107b)

The oily product (75 mg.) was dissolved in ethyl acetate (A.R. 30 ml.) and ozonised at 0°, following the reaction by the decrease in the maximum at 253 mμ. . After four hours this absorption had disappeared and the reaction was stopped, the solvent removed (in vacuo) at room temperature. The residue was treated with a prmmixed solution of aqueous sodium hydroxide (N 1.0, 3.0 ml.) and aqueous hydrogen peroxide (30%, 3.0 ml.). The reaction mixture was cautiously warmed on the steam bath and then heated at 95° for two hours. The solution was cooled, acidified, and saturated with salt and then extracted continuously with ether for twelve hours. The product was an acidic oil (53 mg.).

A small portion of this oil (5 mg.) was methylated, (diazomethane) and examined by gas-liquid chromatography, which indicated the presence of five components:-

1) Ethyl succinic acid, 6%, proved by mixed chromatogram with authentic (±) dimethyl ethyl succinate.

2) n-Propyl succinic acid, 7%, proved by mixed chromatogram with authentic material.

3) β-Ethyl glutaric acid, 42%, proved by a mixed chromatogram with authentic material.

4) β-n-Propyl glutaric acid, 32%, proved by a mixed chromatogram with authentic material.

5) Unknown compound, 13%, having the same retention time as one of the components in a equimolar mixture of α- and β-n-propyl butyrolactone.

The presence of the two β-alkyl glutaric acids was confirmed by their isolation, as follows.

Isolation of β-ethyl glutaric acid and β-n-propyl from Ketone-II degradation

The total, combined, ozonolysis product of three runs (132 mg.) was placed on a column of silicic acid (175 g. containing 12% of N 0.5 hydrochloric acid, packed as described before) in chloroform.

β-Ethyl glutaric acid (14 mg.) was obtained by elution with chloroform containing 7% n-butanol (v/v.) and was distilled in high vacuum (100°, 10^{-4} mm.Hg.) and then recrystallised from carbon tetrachloride as small plates (3 mg.), m.p. 72-73°, its identity with an

authentic specimen of β-ethyl glutaric acid was confirmed by mixed m.p. 72-73°, and infra-red spectrum.

β-n-propyl glutaric acid (12 mg.) was obtained as a gum by elution with chloroform containing 6% n-butanol (v/v.) and was distilled as a colourless oil (11 mg.) (95-100°, 10^{-4}mm.Hg.) which could not be induced to crystallise. The glutaric acid nature of this compound was demonstrated by the treatment of the total distillate with acetyl chloride (0.3 ml.), with warming on the steam bath for five minutes. After evaporation of the excess reagent the oily anhydride was examined by infra-red spectroscopy. It showed $\nu_{mx.}$ 1811, 1764 cm.$^{-1}$ The anhydride was titrated with sodium hydroxide solution (N 0.1) and then, after adjustment to pH.6 with a drop of dilute hydrochloric acid, was treated with p-phenyl phenacylbromide (35 mg.) in ethanol at reflux for three hours. The product, obtained by extraction with chloroform, did not crystallise and so was chromatographed on alumina (G III , neutral, 6 g.) in benzene. Elution with benzene produced some unchanged reagent, followed by the diester (2 mg.) which was recrystallised from benzene-ligroin mixture to m.p. 103-104°. Mixed m.p. with an authentic sample of β-n-propyl glutaric acid bis-p-phenyl phenacyl ester of m.p. 104-105° was 103-105°.

Identity of the infra-red spectrum completed the comparison.

Attempted oxidations of byssochlamic acid

The following reagents were used on byssochlamic acid with no successful results, the material being either completely degraded or else recovered unchanged.

Alkaline Permanganate, Permanganate-Periodate mixture, Osmium Tetroxide, Pertrifluoacetic Acid, Hydrogen Peroxide in Sulphuric Acid, Concentrated Nitric Acid, and Chromic in Acetic Acid.

Ozonolysis of Δ-1 tetrahydrophthalic anhydride

(i) As the anhydride:

Δ-1 tetrahydrophthalic anhydride (110 mg.) in ethyl acetate (A.R. 10 ml.) was cooled to 0° and a stream of ozonised oxygen blown into the solution. The reaction was followed spectrophotometrically ($\lambda_{mx.}$ 252 mμ.) and was complete in one hour. The solvent was removed in vacuum at room temperature and the gummy residue treated with a mixture of aqueous potassium hydroxide (N 1.0, 2.0 ml.) and hydrogen peroxide (30%, 1.0 ml.) and then heated on the steam bath for one hour. After cooling and acidification, continuous extraction with ether produced

a crystalline solid (72 mg.), m.p. 130-145°, which was shown to be adipic acid, with traces ($<$ 5%) of glutaric acid by paper chromatography. The identity was confirmed by conversion to the p-phenyl phenacyl derivative m.p. 146-147° (from ethanol), mixed m.p. with an authentic sample was undepressed.

(ii) As the dipotassium salt:

Δ-1 tetrahydrophthalic anhydride (303 mg.) was dissolved in warm aqueous potassium hydroxide (N 1.0, 5.0 ml.) and back titrated with hydrochloric acid to pH.10. The solution was made up to 50 ml. with water, cooled to 0° in ice and a stream of ozonised oxygen passed in. The reaction was followed spectrophotometrically with the absorption at 231 mμ ., due to the dianion. Reaction was complete after three hours and so was worked up by adding hydrogen peroxide (30%, 1.0 ml.) and aqueous potassium hydroxide (N 1.0) dropwise until the pH. 9-10. The mixture was heated on the water bath for one hour, cooled, acidified and extracted continuously with ether. This procedure yielded a crystalline solid (250 mg.), m.p. 145-150°, recrystallised from ethyl acetate to m.p. 148-150°, mixed m.p. with authentic adipic acid was 148-150°.

Ozonolysis of Byssochlamic acid, as the tetrasodium salt.

Byssochlamic acid (444 mg.) in solution in aqueous sodium hydroxide (N 1.0, 7.0 ml.) was titrated to pH.10 with dilute hydrochloric acid. The volume of the solution was adjusted to 75 ml. and then cooled to 0°. A stream of ozonised oxygen was passed through the solution, and the reaction followed by the change in the absorption at 232 mμ.. It was essentially complete after 20 hours. To the reaction solution was added a mixture of aqueous sodium hydroxide (N 1.0, 6 ml.) and hydrogen peroxide (30%, 6 ml.) and the resulting mixture heated on the steam bath for two hours. After cooling and acidification the solution was saturated with sodium chloride and continuously extracted with ether, to yield a yellow oil. On treatment of the latter with chloroform, oxalic acid crystallised out (60 mg.) and the chloroform solubles were concentrated for further purification.

This procedure was repeated until the degradation product of 2.5 g. of byssochlamic acid had been accumulated.

A small portion (15 mg.) of the combined chloroform solubles (1.5 g.) was examined by gas-liqiid chromatography, which indicated the presence of small quantities of propyl and ethyl succinic acid and also β-ethyl glutaric acid.

The isolation of these acids was achieved as follows.

The acid mixture, dissolved in the minimum volume of chloroform, was applied to a column of silicic acid (silica gel, B.D.H. 300 g. containing 75 ml. of hydrochloric acid (N 0.5)) and eluted with chloroform containing an increasing proportion of n-butanol. The chromatogram was followed by the weight of the evaporated fractions and each of these was examined by gas-liquid chromatography. Oxalic acid appeared in all fractions up to 8% n-butanol and the three dicarboxylic acids appeared in those fractions of 6-8% n-butanol. These, when combined, constituted a yellow gum (200 mg.).

This was subjected to a further chromatography on silicic acid (silica gel, 120 g., hydrochloric acid 30 ml., (0.5N)) which was followed by the same technique as before. By elution with chloroform containing 4% n-butanol there was obtained a yellow gum (100 mg.) consisting of a mixture of n-propyl succinic acid and β-ethyl glutaric acid. Elution with chloroform containing 5% n-butanol gave a small amount of ethyl succinic acid which crystallised from benzene-ligroin mixture as needles (10 mg.), m.p. 75-95°. This was not separated from authentic material in a mixed gas-liquid chromatogram of the dimethyl esters.

To effect separation of the propyl succinic acid from the β -ethyl glutaric acid it was necessary to change the irrigating solvent, as attempts to separate authentic mixtures of these two compounds by the following methods were unsuccessful:-

(i) Partition chromatography over silicic acid with the chloroform-n-butanol system.

(ii) Fractional vacuum sublimation.

(iii) Preparative thin layer chromatography.[41]

It was found that a partition chromatogram over silicic acid using a carbon tetrachloride-n-butanol solvent system gave a reasonable resolution of the authentic mixture and so this was applied to the mixture from byssochlamic acid.

The mixture (100 mg.) in the minimum volume of carbon tetrachloride was applied to a column of silicic acid (silica gel, B.D.H. 100 g. hydrochloric acid, (N 0.5) 36 ml.) and eluted with carbon tetrachloride containing an increasing proportion of n-butanol. Elution with 7% n-butanol gave a crystalline solid (15 mg.), which after sublimation in high vacuum, followed by crystallisation from benzene-ligroin had m.p. 99-103°, $(\alpha)_D^{25}$ - 17° (c 0.63), which on further crystallisation gave

(-) n-propyl succinic acid (10 mg.), m.p. 104-105°, $(\alpha)_D^{25}$ - 18° (c 1.0). Mixed m.p. with the material from the degradation of Ketone-I was 104-105°.

Further elution with same solvent mixture produced a slowly crystallising oil, which after distillation (95°, 10^{-4}mm.Hg.), gave on crystallisation from carbon tetrachloride, β-ethyl glutaric acid (5 mg.), m.p. 72-73°, mixed m.p. with authentic material was 72-73°.

Synthesis of n-propyl succinic acid.[40]

The homogeneity of all liquid products was checked by gas-liquid chromatography.

Diethyl malonate (redistilled, 33 g.) was alkylated in refluxing ethanol, containing one equivalent of sodium ethoxide, with freshly distilled ethyl chloracetate (1.1 m, 28 g.).

The product was isolated by pouring the reaction mixture into water, acidification, and extraction with ether. Distillation under reduced pressure gave diethyl α-ethoxycarbonyl-succinate, b.p. 164-170° (22 mm.Hg.), (32 g.).

The tricarboxylic ester (31 g.) was run into a solution of sodium ethoxide (1.1 m.) in dry ethanol and then dry propyl iodide (freshly distilled, 24 g.) cautiously added.

The reaction mixture was refluxed for four hours and worked up as before to yield diethyl α-ethoxycarbonyl-α-propyl-succinate, b.p. 110-120°, (1.5 mm.Hg.), (33 g.).

This material was saponified by refluxing under nitrogen with stirring, in a mixture of potassium hydroxide (30 g.), water (20 ml.), and ethanol (100 ml.) for twelve hours. Most of the ethanol was removed by distillation and the alkaline solution extracted with ether, the neutral fraction being discarded. The aqueous phase was acidified with concentrated hydrochloric acid and extracted thoroughly with ether (500 ml.) to yield as a crystalline solid α-carboxy- α-propyl succinic acid, m.p. 153-155°, (21 g.).

The tricarboxylic acid was decarboxylated by pyrolysis at 200° for 15 minutes under a slow stream of nitrogen. The solid, so obtained, was heated on the steam bath with dilute hydrochloric acid (10 ml. 2N.) and the acids extracted with ether (250 ml.), dried and evaporated to yield (±) n-propyl succinic acid, (16 g.) recrystallised from benzene-ligroin or from water to m.p. 91-92°.

Resolution of n-propyl succinic acid

The acid (1 g.) and strychnine (4.2 g.) were heated

in water (60 ml.) for 45 minutes and filtered hot from some undissolved strychnine. The filtrate on slow cooling to 0°, precipitated a crystalline salt (3 g.). This was recrystallised three times from water and the (+) n-propyl succinic acid liberated by treatment of an aqueous solution of the salt with ammonium hydroxide (15 ml. s.g. 0.88), filtration of the precipitated strychnine and acidification followed by continuous extraction gave (+) n-propyl succinic acid, recrystallised from benzene-ligroin to m.p. $104\text{-}105^{\circ}$, (0.2 g.), $(\alpha)_{D}^{25}$ + 22° (c 0.9, water), $(\alpha)_{Hg}^{25}$ + 28° (c 0.9, water), $(\alpha)_{D}^{27}$ + 18° (c 1.7).

The mother liquors, from the first precipitation of the strychnine salt, gave the (-) n-propyl succinic acid (0.4 g.), $(\alpha)_{D}^{25}$ - 14° (c 1.0, water). This gave a crystalline quinine salt, which however did not effect any increase in the rotation of the acid upon repeated crystallisation.

Synthesis of ethyl succinic acid

This was carried out exactly as described for n-propyl succinic acid with the exception that ethyl iodide was used to alkylate the intermediate tricarboxylic ester. Hydrolysis and decarboxylation steps

proceeded as before and yielded (±) ethyl succinic acid, recrystallised from water to m.p. 98-100°.

Resolution was achieved via the strychnine salt[24]. Thus ethyl succinic acid (1.0 g.) in water (18 ml.) was treated with excess strychnine (4.5 g.) and heated on the steam bath for one hour. The mixture was filtered hot and on cooling deposited a crystalline salt (2.4 g.) which was recrystallised four times from water until the salt had m.p. 124-132°, $(\alpha)_D^{25}$ - 50° (c 0.7, ethanol.). The acid was recovered as before to yield (+) ethyl succinic acid (100 mg.), m.p. 94-95°, $(\alpha)_D^{25}$ + 27° (c 0.7, acetone), $(\alpha)_D^{25}$ + 13° (c 1.2).

Synthesis of β-ethyl glutaric acid[18]

(i) Cyanoacetamide. Ethyl cyanoacetate (100 g.) and aqueous ammonia (75 ml., s.g. 0.88) was shaken in a stoppered, wide mouthed, bottle with cooling during the first hour. It was then allowed to stand at room temperature for twelve hours. Filtration gave a white solid (70 g.) which was recrystallised from ethanol to yield cyanacetamide (58 g.), dried in an oven at 70°, m.p. 118-120°.

(ii) Condensation. The amide (30 g.) in water (400 ml.) was cooled in ice and then propionaldehyde (freshly distilled, 7.65 ml.) was added with swirling, followed by

aqueous potassium hydroxide (50% w/v. 0.6 ml.). The reaction was allowed to stand at + 5° in the cold room for eighteen hours. It was filtered from a white crystalline solid, which was dried in vacuum at room temperature, m.p. 130-150°, (20 g.).

(iii) Hydrolysis. The crystalline mixture from the condensation was dissolved in hydrochloric acid (N 6.0, 100 ml.) and refluxed for 18 hours. It was cooled, diluted to dissolve precipitated ammonium chloride and extracted with ether (500 ml.) to yield β-ethyl glutaric acid (14 g.), recrystallised from benzene-ligroin or from carbon tetrachloride, m.p. 72-73°.

β-ethyl glutaric anhydride

β-ethyl glutaric acid (15 g.) was refluxed for one hour with acetyl chloride (70 ml.). The reaction mixture was distilled in vacuum to yield β-ethyl glutaric anhydride (11 g.) b.p. 98° (0.1 mm.Hg.) as a colourless liquid, which did not crystallise. $\nu_{mx.}$ (film) 1810. 1768 cm..$^{-1}$

4-Carbamoyl -3-ethyl-butyric acid (90)

β-ethyl glutaric anhydride (1.0 g.) was dissolved in benzene (10 ml.) and then added dropwise to an ice-cold, stirred, solution of aqueous ammonia (14 ml. 2.0N.).

The resultant mixture was acidified with dilute hydrochloric acid, cooled in ice, and the precipitate filtered (1.0 g.). This was recrystallised from water as fine needles of 4-Carbamoyl-3-ethyl-butyric acid, m.p. 132-133°. A sample was sublimed in high vacuum (120°, 10^{-4}mm.Hg.) to m.p. 133-134°. (Found: C, 53.10; H, 8.11; N, 8.99. $C_7H_{13}NO_3$ requires C, 52.81; H, 8.23; N, 8.80%).

$\nu_{mx.}$ (Nujol) 1708, 1670, 1590, 2600-3509 cm.$^{-1}$

This compound was further characterised as its p-phenyl phenacyl ester. The acid (313 mg.) was treated, as it's potassium salt, at pH. 6-7 in aqueous ethanol with p-phenyl phenacyl bromide (578 mg. 1.0 m.) under reflux for three hours. The solution was diluted with water, warmed, and then allowed to cool slowly to room temperature. The crystalline product (650 mg.) was recrystallised three times from benzene as fine needles, m.p. 134-135°, (Found: C, 71.8; H, 6.92; $C_{21}H_{23}NO_4$ requires C, 71.4; H, 6.52%).

$\nu_{mx.}$ 3400, 3200, 1725, 1685, 1640, 1600 cm.$^{-1}$

Methyl 4-carbamoyl-3-ethyl-butyrate (92)

The acid (30 g.) in methanolic solution was treated with excess ethereal diazomethane until the colour remained. The solvents were removed in vacuum

and the product was crystallised from ether-petroleum ether (40°- 60°) as plates of methyl 4-carbamoyl-3-ethyl -butyrate, m.p. 55-58°. (Found: C, 55.25; H, 8.79; N, 7.98. $C_8H_{15}NO_3$ requires C, 55.47; H, 8.73; N, 8.09%). $\nu_{mx.}$ 1725, 1680, 1600, 3450, 3600 cm.$^{-1}$

Methyl 4-cyano-3-ethyl-butyrate (93)

The ester (31 g.) was dissolved in dry pyridine (distilled from potassium hydroxide, 200 ml.) and cooled to 0°C. This solution was treated with p-toluene sulphonyl chloride (freshly crystallised 175 g. 5 m.) and allowed to stand at 0°C for 90 minutes, at the end of which time much solidification had taken place. The mixture was warmed to room temperature and after 2.5 hours was poured into ice-water (1 ℓ .) and allowed to stand for a further two hours. The solution was cooled to 0° and acidified by dropwise addition of ice-cold, concentrated hydrochloric acid to the stirred liquid. The product was extracted with ether to yield a pale yellow oil (25 g.), $\nu_{mx.}$ 1735, 2260 cm.$^{-1}$

A small portion (500 mg.) was distilled twice in high vacuum (60°, 10^{-4}mm.Hg.) as a colourless liquid, (Found: C, 61.94; H, 8.43; N, 8.75; $C_8H_{13}O_2N$ requires C, 61.91; H, 8.44; N, 9.03%), $\nu_{mx.}$ (film) 1735, 2260 cm.$^{-1}$

4-cyano-3-ethyl butyric acid (85)

This compound was sensitive to aqueous alkali and initial attempts to selectively hydrolyse the ester function of methyl 4-carbamoyl-3-ethyl butyrate were unsuccessful. Thus hydrolysis with aqueous potassium hydroxide (N 1.0) at 95° for one hour gave rise to a quantitative yield of β-ethyl glutaric acid, m.p. 72-73°, mixed m.p. with authentic material was undepressed.

For this reason, the following experiment was conducted to determine optimum conditions for the hydrolysis. Methyl 4-cyano-3-ethyl-butyrate (201 mg.) was dissolved in ethanol (4.0 ml.) and treated with aqueous potassium hydroxide (1.0 N, 2.6 ml., 2 equivs.) and made up to 10 ml. with water. A sample (0.5 ml.) was removed at intervals and titrated with hydrochloric acid (N 0.1).

Time	Solution (ml.)	Blank (ml.)	(ml.)
0	0.87	1.32	0.45
15	0.72	1.31	0.59
60	0.68	1.28	0.60
120	0.72	1.31	0.59

Theoretical uptake = 0.65 ml.

Thus hydrolysis was well advanced even on mixing the two solutions.

The solution was carefully neutralised with hydrochloric acid (N 1.0) and evaporated to dryness in high vacuum and the residue acidified and extracted with ether to yield an oily product, 4-cyano-3-ethyl-butyric acid (120 mg.) $\nu_{mx.}$ 1710, 2300 cm.$^{-1}$.

This procedure was applied to the bulk material. The crude ester, from the above preparation, (31 g.), was dissolved in ethanol (400 ml.) and then water (400 ml.) added. To the stirred mixture, cooled in ice, was added over thirty minutes, aqueous potassium hydroxide (1.0 N, 400 mls, 2 m.) and the resulting solution stirred for a further thirty minutes. The ice-cold solution was cautiously acidified with cold concentrated hydrochloric acid and then extracted with ether (1.5 ℓ.), washed, dried, and evaporated under reduced pressure to yield a pale yellow oil. This oil was subjected to a short path distillation at 95-105° (10^{-4}mm.Hg.) to yield 4-cyano-3-ethyl-butyric acid (21 g.), n_D^{24} 1.4511, $\nu_{mx.}$ (film) 1705, 2280, 2700-3300 cm.$^{-1}$.

The oil became slightly yellow on standing and so was stored at 0°C.

The constitution of the nitrile acid was proved by its reconversion to 4-Carbamoyl-3-ethyl-butyric acid.

Thus 4-cyano-3-ethyl butyric acid (112 mg.) was suspended in a little water and treated with aqueous sodium hydroxide (1.0 N. 2.0 ml.) and then aqueous hydrogen peroxide (30%, 1.0 ml.). The solution was warmed to 60° for four hours and then cooled to room temperature, acidified with dilute hydrochloric acid (1.0 N.). All the water was removed in vacuum and the white residue was recrystallised twice from the minimum volume of water, which effected a separation of the acid from the more soluble sodium chloride, to yield a crystalline acid (54 mg.) m.p. 132-133°, m.p. was undepressed by admixture with authentic 4-carbamoyl-3-ethyl-butyric acid.

Resolution of 4-cyano-3-ethyl-butyric acid

A solution of quinine methohydroxide was prepared by shaking quinine methochloride[42] (14 g.) and silver oxide (15 g.) in water (70 ml.) for three hours. The mixture was filtered through celite and the filtrate so obtained, added from a burette to a solution of 4-cyano-3-ethyl-butyric acid (6 g.) in ethanol (60 ml.) to pH. 9-10. The resulting solution was evaporated in high vacuum to a sticky white solid which was dried by azeotropic distillation of benzene. The residue was dissolved in acetonitrile (70 ml.), filtered through celite and allowed to stand at room temperature.

There was formed a crystalline precipitate (6 g.), m.p. 162-166°, $(\alpha)_D^{25}$ - 157° (c 1.2, water).

This material was recrystallised from acetonitrile three times to yield a salt (4 g.), m.p. 166-168°, $(\alpha)_D^{25}$ - 154° (c 1.4, water).

The salt was dissolved in water (8 ml.) and acidified with sulphuric acid (1.0 N.), the initially precipitated quinine metho-sulphate redissolved as the bisulphate on addition of excess acid. The solution was extracted with ether (75 ml.) and the ethereal solution evaporated to (+) 4-cyano-3-ethyl-butyric acid, which was distilled in high vacuum (95-100°, 10^{-4}mm.Hg.),(1.1 g.), $(\alpha)_D^{25}$ + 28° (c 5.2), $\nu_{mx.}$ (film) 1705, 2280 cm..$^{-1}$

Formation of (-) 4-carbamoyl-3-ethyl-butyric acid

The redistilled (+) acid (491 mg.) was suspended in water (2.5 ml.) and treated at room temperature with a mixture of aqueous sodium hydroxide (N 1.0, 10 ml.) and hydrogen peroxide (30%, 5.0 ml.). An exothermic reaction set in immediately and after 15 minutes the mixture was warmed to 60° in a water bath and held at that temperature for three hours. The solution was cooled, acidified to pH.2 with dilute hydrochloric acid (2.0 N.) and the water evaporated in vacuum. The solid residue was recrystal-

lised from water to yield crude (-) 4-carbamoyl-3-ethyl butyric acid (300 mg.), m.p. 97-104°.

This material was purified by extracting the finely ground solid with two portions of boiling benzene (25 ml.) for thirty minutes. The residue (250 mg.) was crystallised from water at 0°C to yield 4-carbamoyl-3-ethyl-butyric acid, m.p. 113-114°, $(\alpha)_D^{25}$ - 2° (c 1.1 water), $(\alpha)_D^{25}$ - 2° (c 0.8 ethanol). (Found :: C, 52.98; H, 8.38; N, 8.90, $C_7H_{13}NO_3$ requires C, 52.81; H, 8.23; N, 8.80%).

The compound was further characterised by conversion to the p-phenyl phenacyl ester. The acid (75 mg.) was titrated with aqueous potassium hydroxide (1.0 N) in aqueous ethanol, adjusted to pH.7 with a few drops of dilute hydrochloric acid and refluxed for three hours with p-phenyl phenacyl bromide (124 mg. 1 m.). Cooling produced a crystalline solid (153 mg.) which was chromatographed on alumina (G III , neutral, 5 g.) in benzene solution. Elution with benzene-chloroform (1:1 v/v) gave a crystalline solid (70 mg.) recrystallised from benzene, m.p. 137-139°, $(\alpha)_D^{25}$ - 15° (c 0.8). (Found : C, 71.99; H, 6.36; N, 3.86; $C_{21}H_{23}O_4N$ requires C, 71.37; H, 6.56; N, 3.96%).

$\nu_{mx.}$ (Nujol) 1740, 1695, 1660, 1633, 1600, 3400, 3200 $cm.^{-1}$

Conversion of (-) 4-carbamoyl-3-ethyl-butyric acid to (-) ethyl succinic acid

(-) 4-carbamoyl-3-ethyl-butyric acid (144 mg.) was dissolved in aqueous potassium hydroxide (1.0 N, 4.0 ml.) and treated with bromine (166 mg. 1.2 m.). The solution was kept at room temperature for thirty minutes and then heated on a steam bath for two hours. To the cooled solution was added, at room temperature with stirring, aqueous potassium permanganate (1.0 N, 3.0 ml.) over 1.5 hours.

After destruction of the excess oxidant with sulphur dioxide the acidified solution was saturated with sodium chloride and extracted with ether to yield an acidic gum, which was chromatographed on silicic acid (silica gel B.D.H. 55 g. hydrochloric acid (0.5 N.) 15 ml.) by elution with chloroform-n-butanol mixtures. A crystalline solid (47 mg.) was obtained by elution with chloroform containing 8% n-butanol (v/v), which was sublimed in vacuum (95°, 10^{-4}mm.Hg.) and recrystallised from benzene-ligroin as white needles (27 mg.) m.p. 94-96°, $(\alpha)_D^{26}$ - 10° (c 1.1).

A small portion (2 mg.) was converted to the dimethyl ester, subjected to gas-liquid chromatography,

when it was not separated in a mixed chromatogram with authentic ethyl succinic acid dimethyl ester.

The infra-red spectrum (Nujol) was identical with that of authentic (+) ethyl succinic acid and identity was finally confirmed by mixing an equal weight (1.57 mg.) of authentic (+) ethyl succinic acid with the material from the degradation. Recrystallisation of the mixture from benzene-ligroin gave needles (2.3 mg.), m.p. 98-99°. The m.p. was not depressed on admixture with authentic (±) ethyl succinic acid, m.p. 98-99°, and the infra-red spectra of the two materials were identical.

Preparation of β-ethyl adipic acid

The preparation of this compound is described in the literature but since all but one carbon atom of the skeleton was already available in the β-ethyl glutaric acid it was found that the following method was convenient for the preparation of β-ethyl adipic acid.

4-cyano-3-ethyl-butyric acid (1.0 g.) was titrated with aqueous sodium hydroxide (1.0 N, 7.40 ml.) to pH.10. The solution was taken to dryness, under reduced pressure, and heated in vacuum at 60°C for eight hours. The residue was broken up with a spatula and dried by

azeotropic distillation of benzene (3 x 50 ml.). The sodium salt was a deliquescent white solid, which was suspended in dry petroleum (40-60° b.p.) and cooled to 0° and oxalyl chloride (3.22 ml.) added with stirring. The mixture was placed in the cold room overnight and then taken to dryness in vacuum. The acid chloride, so formed, was dissolved in dry ether, filtered from sodium chloride, and added to an ice-cold, stirred solution of diazomethane (prepared from 20 g. of N-nitroso-methyl urea and distilled from potassium hydroxide) in ether. After two hours the solution was cautiously taken to dryness at room temperature to give the diazo-ketone as a yellow oil, ν_{mx} (film) 2300, 2130, 1640 cm.$^{-1}$. This oil was dissolved in a mixture of tetrahydrofuran (100 ml.) and water (25 ml.) and irradiated at room temperature in a pyrex flask for 14 hours under nitrogen, following the reaction by the intensity of the diazo-ketone absorption (2130 cm.$^{-1}$) in the infra-red spectrum. The bulk of the tetrahydrofuran was evaporated, the residue diluted with water and made just alkaline with sodium hydroxide (1.0 N., 5 ml.) and then extracted with ether. The acid fraction, obtained by acidification of the aqueous phase and extraction with ether, was a somewhat yellow oil (510 mg.).

This was hydrolysed in sulphuric acid (50%) under reflux for 14 hours. The product obtained by extraction with ether was an almost colourless oil, which rapidly crystallised (400 mg.). This material was crystallised with some loss at -20° from ether-ligroin mixture to m.p. 56-58°, reported 58-59°.[43]

Reactions of Byssochlamic acid

Dehydrogenation of Byssochlamic acid

Formic acid (90%, 4 ml.) was added to a boiling solution of platinic chloride (2 g.) in water (1.5 l.). The solution was stirred for ten minutes on the steam bath and then potassium hydroxide (10%, 100 ml.) was added and the solution stirred for a further two hours at the same temperature. The black precipitate was collected, washed with water and dried in vacuum.

Byssochlamic acid (423 mg.) was mixed with this platinum black (84 mg.) and heated under a slow stream of nitrogen at 300-310° for two hours, by which time all gas evolution from the melt had ceased. The product was dissolved in ether and filtered, the ether evaporated and the resinous residue extracted with boiling ligroin (2 x 25 ml.) and the concentrated petrol solubles were chromatographed on silica gel (B.D.H. acid washed, 15 g.). Elution with benzene-petroleum (70:40 v/v) gave an oily crystalline solid (52 mg.) which was recrystallised from petroleum ether (40-60° b.p.) to yield the pure dehydrobyssochlamic acid (20 mg.) m.p. 90-91°, $(\alpha)_D^{25} \pm 0°$ (c 1.1), (Found: C, 74.67; H, 7.13; $C_{16}H_{18}O_3$ requires C, 74.39; H, 7.03%).

Molecular weight (Rast) 247, $C_{16}H_{18}O_3$ requires 258, Kuhn-Roth oxidation indicated 1.90 C-Me.

$\nu_{mx.}$ 1835, 1767 cm.$^{-1}$ $\lambda_{mx.}$ 224 mμ. (ε 26,000), 232 mμ. (ε 18,000).

This compound gave no colour with tetranitromethane.

Perphthalic acid titration of dehydrobyssochlamic acid

The compound (5 mg.) was dissolved in chloroform (A.R. 2.0 ml.) and treated with a solution of monoperphthalic acid (1 ml.) and then placed in the cold room for six hours. The uptake of oxidant was measured by addition of potassium iodide and titration of the liberated iodine with standard thiosulphate. There was no uptake of perphthalic acid under conditions where cyclohexene readily absorbed one mole.

Preparation of iso-byssochlamic acid (115)

Byssochlamic acid (513 mg.) was sealed under vacuum (1.0 mm.Hg.) in a pyrex tube which was placed in a small electric furnace at 210°. After 24 hours the tube was removed, cooled, and the contents dissolved in chloroform. The solution was concentrated and treated with ether (2 vols.) to produce iso-byssochlamic acid as fine needles, (289 mg.), m.p. 153°, $(\alpha)_D^{25}$ - 27°, (c 0.9). (Found: C, 64.85; H, 6.29; $C_{18}H_{20}O_6$ requires C, 65.02; H, 6.07%).

$\nu_{mx.}$ 1830, 1810, 1770, 1650 cm.$^{-1}$ $\lambda_{mx.}$ 207 mμ. (ε 6,000), 236 mμ. (ε 6,300).

Dehydrogenation of iso-byssochlamic acid

Iso-byssochlamic acid (50 mg.) and platinum black (50 mg.) was sealed into an evacuated tube (10^{-4}mm.Hg.) and placed in a small electric furnace. The temperature was slowly raised to 300°C and held at this level for 7.5 hours. The tube was cooled, the contents dissolved in chloroform and filtered free of catalyst. The gum so obtained was distilled in high vacuum as oily crystals. Recrystallisation from petroleum ether (40°-60° b.p.) gave the same compound as was obtained from byssochlamic acid, (4 mg.), m.p. 90-91°, mixed m.p. with the material from byssochlamic acid was 90-91°.

Hydrolysis of iso-byssochlamic acid

Iso-byssochlamic acid (100 mg.) was dissolved in aqueous hydroxide (1.0 N, 5.0 ml.) by warming gently on the steam bath. The solution was cooled to room temperature and acidified with concentrated hydrochloric acid to pH.2. Continuous extraction with ether over 17 hours gave a colourless gum which slowly crystallised from acetonitrile-benzene as needles of iso-byssochlamic dicarboxylic acid, m.p. 173-183°, (d), $(\alpha)_D^{21}$ + 24° (c 1.3 acetone), $\lambda_{mx.}$ 234 mμ. (ε 3,300)

$\nu_{mx.}$ (Nujol) 1840, 1780, 1700, 1690 cm.$^{-1}$

Dehydration of iso-byssochlamic dicarboxylic acid

A small sample of the hydrate (10 mg.) was heated in a Wood's metal bath at 190° for five minutes, until no further gas evolution occurred. The residue was crystallised from chloroform-ether mixture to m.p. 153°, mixed m.p. with authentic iso-byssochlamic acid was undepressed.

Iso-byssochlamic tetracarboxylic acid (119)

The hydrolysis of iso-byssochlamic acid (75 mg.) was repeated and the solution cooled to 0° and then cautiously acidified with ice-cold hydrochloric acid (2 N.). There was obtained after continuous ethereal extraction a solid (86 mg.) as rectangular plates of iso-byssochlamic tetracarboxylic acid, m.p. 165-168° $(\alpha)_D^{21}$ + 8° (c 1.2 acetone). $\lambda_{mx.}$ 221 mμ. (ε 6,400), $\nu_{mx.}$ (Nujol) 1718-1680 cm..$^{-1}$

On heating this material at 150-160° in vacuum, it quickly reverted to iso-byssochlamic acid, m.p., mixed m.p., infra-red spectrum.

Dihydro-iso-byssochlamic acid (116)

Iso-byssochlamic acid (126 mg.) in glacial acetic acid (A.R. 15.0 ml.) was hydrogenated in the presence of palladium on charcoal catalyst (10%, 117 mg.).

The catalyst was removed by filtration and the solvent evaporated. The product was crystallised from benzene-ether mixture as dihydro-iso-byssochlamic acid, m.p. 128-130°, (75 mg.), $(\alpha)_D^{22}$ - 16° (c 0.9). (Found; C, 64.69; H, 6.82; $C_{18}H_{22}O_6$ requires C, 64.65; H, 6.63%). $\nu_{mx.}$ (Nujol) 1840, 1775 cm.$^{-1}$

ϵ 220 mμ. 245.

This compound gave no colour with tetranitromethane.

Dihydro-iso-byssochlamic acid was further characterised by hydrolysis with aqueous potassium hydroxide (N 1.0, 3.0 ml.) on the steam bath for 50 minutes. The solution was cooled, acidified with hydrochloric acid (pH.2, 2.0 N) and extracted with ethyl acetate to yield a gum (27 mg.) crystallised from ethyl acetate as dihydro-iso-byssochlamic dicarboxylic acid (117), m.p. 190-197° (d), (Found: C, 61.52; H, 6.72; $C_{18}H_{24}O_7$ requires C, 61.35; H, 6.86%). $\nu_{mx.}$ 1828, 1776, 1701, 1689 cm.$^{-1}$ $\epsilon_{220\ m\mu.}$ 630.

Dehydration of dihydro-iso-byssochlamic dicarboxylic acid

The dicarboxylic acid (10 mg.) was heated at 190° in a Wood's metal bath for five minutes, cooled, and the residue recrystallised from benzene-ether mixture to m.p. 128-130°, undepressed on admixture with authentic dihydro-iso-byssochlamic acid.

Monobromo-iso-byssochlamic acid (111)

Iso-byssochlamic acid (202 mg.) was dissolved by refluxing in carbon tetrachloride (60 ml. A.R. freshly distilled from N-bromosuccinimide). The solution was cooled slightly and N-bromosuccinimide (124 mg. 1.1 m., freshly crystallised from hot water) was added and the solution refluxed for 11 hours. On cooling to 0° for one hour a precipitate of succinimide appeared, which was filtered (61 mg.) and the residue taken to dryness at room temperature. This was induced to crystallise by trituration with ether and the product was purified by recrystallisation from benzene-ether mixture, as monobromo-iso-byssochlamic acid m.p. 177-180°, (85 mg.) $(\alpha)_D^{21}$ - 31° (c 1.0).

(Found: C, 52.23; H, 4.63; Br, 20.60; $C_{18}H_{19}O_6Br$ requires C, 52.52; H, 4.62; Br, 19.46%). $\nu_{mx.}$ (Nujol) 1830, 1775, 1665 cm.$^{-1}$ $\lambda_{mx.}$ (cyclohexane) 240 mμ. (ε 8,600).

Dibromo-iso-byssochlamic acid (114)

Iso-byssochlamic acid (494 mg.) in carbon tetrachloride (200 ml.) was treated with N-bromosuccinimide (314 mg.). The mixture was refluxed for 13 hours and then a further portion of N-bromosuccinimide (200 mg.) was added and the reaction continued for a further 15 hours.

After cooling the reaction mixture and removal of the succinimide by filtration, the solvent was removed at room temperature. The gummy residue was chromatographed an silica gel (15.0 g. acid washed). Elution with benzene (80 ml.) produced a solid, recrystallised from benzene-ether as long needles of dibromo-iso-byssochlamic acid (50 mg.), m.p. 153-155°. (Found: C, 44.39; H, 3.96; $C_{18}H_{18}O_6Br_2$ requires C, 44.10; H, 3.71%). $\nu_{mx.}$ (Nujol) 1840, 1775 cm.$^{-1}$ $\lambda_{mx.}$ (cyclohexane) 258 mμ. (ε 8,600).

Further elution of the chromatogram produced the monobromo-derivative (150 mg.) m.p. 177-180°.

Iso-byssochlamic acid bis-p-bromophenyl hydrazide

Iso-byssochlamic acid (206 mg.) and p-bromophenyl hydrazine (260 mg. 2.2 m., freshly prepared from the hydrochloride) were refluxed in dry benzene (20 ml.) under nitrogen for three hours.

The solvent was removed by evaporation under reduced pressure and the residue taken into chloroform solution and extracted with dilute hydrochloric acid (N 1.0, 25 ml.) and then with sodium bicarbonate (50% satd., 2 x 25 ml.). After washing with water the chloroform was removed and the residue crystallised

from a mixture of chlorofrom and petrol as large hexagonal plates of iso-byssochlamic acid bis-p-bromophenyl hydrazide (125 mg.), m.p. 140-145°, $(\alpha)_D^{27}$ - 8° (c 0.9), (Found: C, 54.2; H, 4.48; N, 8.78; Br, 24.5; $C_{30}H_{30}N_4O_4Br_2$ requires C, 53.7; H, 4.48; N, 8.35; Br, 23.8%), $\nu_{mx.}$ (Nujol) 3300, 1770 1720, 1660 cm.$^{-1}$.

A sample, suitable for crystallographic studies, was obtained by a slow crystallisation from chloroform-petrol mixture at room temperature.

The bicarbonate soluble fraction was isolated by acidification with dilute hydrochloric acid (2 N.) and extraction with chloroform (4 x 20 ml.), dried and evaporated to a gum which gave an amorphous solid (80 mg.) from acetonitrile-benzene mixture, m.p. 196-200°, $\nu_{mx.}$ 2700-3300, 1780, 1720, 1690, 3400 cm.$^{-1}$ (Found: C, 50.55; H, 5.01%).

Byssochlamic acid-bis-p-bromophenyl hydrazide

p-Bromophenyl hydrazine hydrochloride (160 mg.) was dissolved in water (10 ml.), filtered from a little insoluble matter, and then basified with aqueous sodium hydroxide (0.1 N, 6.35 ml.). The base was extracted with ether, washed and dried and then evaporated to a yellow crystalline solid.

This was dissolved in chloroform (2.0 ml.) and the solution added to a solution of byssochlamic acid (60 mg.) in chloroform (1.0 ml.). The mixture was stored in the cold room for two days and the pale yellow precipitate so formed was filtered, washed with chloroform, and then dried in vacuum (82 mg.).

This material was crystallised, slowly, from ethanol-petrol (60-80° b.p.) mixture as large plates, m.p. 164-166°, $(\alpha)_D^{22}$ - 97° (c 0.3). (Found: C, 54.1; H, 5.04; N, 7.8; Br, 23.3; $C_{30}H_{30}O_4N_4Br_2$ requires C, 53.7; H, 4.5; N, 8.4; Br, 23.8%). $\nu_{mx.}$ (Nujol) 1780, 1720 cm.$^{-1}$ $\lambda_{mx.}$ 236 mμ. (ε 41,000), 292 mμ. (ε 3,100).

Crystals, suitable for crystallographic studies, were obtained by slow cooling of filtered solutions of the above compound in ethanol-petroleum ether mixtures.

Dihydrobyssochlamic acid experiments

Dihydrobyssochlamic dicarboxylic acid (124)

(i) Byssochlamic acid (400 mg.) was dissolved in acetic acid (A.R. 6.0 ml.) and refluxed with zinc dust (4.0 g.). At intervals of one hour portions of hydrochloric acid (1 0 N, 1.0 ml.) were added until a total of 8.0 ml., the reaction was continued for a further one hour and then filtered hot and the filter funnel washed with a

little hot acetic acid. On standing at 0° for two days the filtrate precipitated a crystalline solid, m.p. 212-225°, (86 mg.) which was recrystallised from the minimum volume of cold water as cubes of dihydrobyssochlamic dicarboxylic acid, m.p. 233-240°, $(\alpha)_D^{22}$ + 14° (c 0.9, acetone). $\nu_{mx.}$ (Nujol) 2700, 1820, 1775, 1705 cm.$^{-1}$ $\epsilon_{220\ m\mu.}$ 450.

This material did not depress the melting point of a specimen due to Paton and had an identical infra-red spectrum, (Nujol).

(ii) Byssochlamic acid (296 mg.) in acetic acid (35 ml.) was mixed with zinc dust (3.1 g.) and shaken for 24 hours at room temperature. The solution was filtered and the filtrate evaporated to a gum which was carefully chromatographed on silica gel (15.0 g, acid washed). Elution with benzene containing ether (20%, v/v) produced only dihydrobyssochlamic dicarboxylic acid, m.p. 223-233°, having an infra-red spectrum identical with that of the authentic material.

Dihydrobyssochlamic acid-dimethyl ester

On treatment of a solution of dihydrobyssochlamic dicarboxylic acid in methanol with ethereal diazomethane there was formed the corresponding dimethyl ester, which could not be crystallised. $\nu_{mx.}$ 1840, 1770, 1730 cm..$^{-1}$

Dihydrobyssochlamic acid (125)

Dihydrobyssochlamic dicarboxylic acid (103 mg.) was refluxed with acetyl chloride (redistilled, 5 ml.) for four hours. The excess reagent was removed in vacuum and the gummy residue crystallised from benzene-petroleum ether (60-80° b.p.) as dihydrobyssochlamic acid (87 mg.) m.p. 120°, $(\alpha)_D^{25}$ - 54° (c 1.0).
(Found: C, 64.48; H, 6.65; $C_{18}H_{22}O_6$ requires C, 64.65; H, 6.63%). $\nu_{mx.}$ (Nujol) 1840, 1775 cm.$^{-1}$
$\epsilon_{220\ m\mu.}$ 350.

Hydrolysis of Dihydrobyssochlamic acid

Dihydrobyssochlamic acid (53 mg.) was warmed on the steam bath for one hour with aqueous potassium hydroxide (4N, 1.5 ml.). The solution was cooled and acidified with dilute hydrochloric acid (6N.) and the liquid continuously extracted with ether for 18 hours. The product (55 mg.) was recrystallised from dioxan to m.p. 220-225°, and did not depress the melting point of dihydrobyssochlamic dicarboxylic acid and had an identical infra-red spectrum.

Pyrodihydrobyssochlamic acid (126)

Dihydrobyssochlamic dicarboxylic acid (50 mg.) was pyrolysed in a sealed tube (10^{-4} mm.Hg.) at 300° for eight hours.

The product was crystallised from benzene-ether as pyrodihydrobyssochlamic acid (30 mg.) m.p. 168-170°, $(\alpha)_D^{23}$ + 72° (c 1.1).

(Found: C, 65.12; H, 6.61; $C_{18}H_{22}O_6$ requires C, 64.65; H, 6.63%). $\nu_{mx.}$ (Nujol) 1840, 1775 cm.$^{-1}$ $\epsilon_{220\ m\mu.}$ 445.

Photo-Byssochlamic acid Experiments

Photo-Byssochlamic acid (127,128)

Byssochlamic acid (129 mg.), dissolved in tetrahydrofuran (250 ml. freshly distilled from lithium aluminium hydride), was irradiated with ultra-violet light (Hg. high pressure lamp) under a stream of dry nitrogen. The reaction was followed by spectrophotometric control and was stopped after one hour, when all absorption at 250 mμ had disappeared. The solvent was removed in vacuum and the product crystallised from a mixture of chloroform and ether to yield photobyssochlamic acid (91 mg.) m.p. 210-220°, $(\alpha)_D^{23}$ + 6° (c 1.0).

(Found: C, 65.10; H, 6.16; $C_{18}H_{20}O_6$ requires C, 65.03; H, 6.07%). $\nu_{mx.}$ (Nujol) 1842, 1821, 1764 cm.$^{-1}$ $\epsilon_{220\ m\mu.}$ 725.

Molecular weight determination by mass spectrum was 332. The latter measurement also indicated the presence of a compound having a molecular weight of 408, albeit in trace amounts.

Further experiments carried out under essentially identical conditions but at high (5 mg.p.ml.) and low (0.1 mg.p.ml.) concentrations gave only the above compound, m.p. and infra-red spectrum were identical.

Hydrolysis of Photobyssochlamic acid

Photobyssochlamic acid (40 mg.) was dissolved in hot aqueous potassium hydroxide (1.0 N, 1.9 ml.) and back titrated to phenolphthalein with dilute hydrochloric acid (0.1 N, 7.5 ml.).

Equivalent weight 160; calculated for dibasic acid 166. The solution was strongly acidified with hydrochloric acid (concentrated) and extracted with chloroform, to yield a solid (35 mg.) crystallised for chloroform-ether to m.p. 200-215°. This did not depress the m.p. of the starting material and had an identical infra-red spectrum.

Pyrolysis of Photo-byssochlamic acid

Photo-byssochlamic acid (22 mg.) was pyrolysed at 310° in a sealed, evacuated, tube for three hours. After cooling and dissolving in chloroform the product was crystallised by addition of ether to yield a solid (15 mg.), m.p. 195-210°, which did not depress the m.p. of starting material and had an identical infra-red spectrum.

Photolysis of Glauconic acid

Glauconic acid (132 mg.) was irradiated in tetrahydrofuran (250 ml.) under nitrogen with ultra-violet light (Hg. high pressure lamp). The reaction was followed by the ultra-violet spectrum and was stopped after two hours, at which time the spectrum showed no further change with time. The product was a colourless gum which was chromatographed on silica gel (5.0 g.) and eluted from the column as a gum. This material could not be crystallised and had $\nu_{mx.}$ 1830, 1775 $cm.^{-1}$ and no selective absorption in the ultra-violet spectrum. Glauconic acid acetate and glaucanic acid followed essentially the same course as the previous experiment, giving rise to materials which could not be crystallised.

Photolysis of byssochlamic acid in the presence of maleic anhydride

Byssochlamic acid (91 mg.) and maleic anhydride (resublimed, 45 mg. 1.5 m.) in tetrahydrofuran (100 ml.) were irradiated with ultra-violet light (Hg. high pressure lamp) under nitrogen for thirty minutes. The product was isolated by evaporation and crystallisation from chloroform -ether mixture as photo-byssochlamic acid (45 mg.), m.p. 210-220°.

Photodimer of Δ -1 tetrahydrophthalic anhydride (129,130)

The anhydride (1.2 g.) in cyclohexane (A.R. 200 ml.) was irradiated with ultra-violet light for three hours under nitrogen, following the reaction by the ultra-violet absorption at 250 mμ.. The solvent was evaporated and the product crystallised from benzene as cubes of Δ -1 tetrahydrophthalic anhydride photodimer (376 mg.) m.p. 277-283° (capillary). (Found: C, 63.39; H, 5.53; $C_{16}H_{16}O_6$ requires C, 63.15; H, 5.30% Mol. wgt. 304). Molecular weight (ebullioscopic method in benzene) 313.

$\nu_{mx.}$ 1865, 1835, 1790 cm.$^{-1}$

$\lambda_{mx.}$ 238 mμ. (ε 780).

Hydrolysis of Δ -1 tetrahydrophthalic anhydride photodimer

The photodimer (95 mg.) was dissolved on the steam bath, in aqueous potassium hydroxide (N 1.0, 2.0 ml.) cooled and titrated with aqueous hydrochloric acid (1N) to phenolphthalein. Equivalent weight 140.

The solution was strongly acidified with hydrochloric acid (6N) and extracted with ether to yield a foam (99 mg.), $\nu_{mx.}$ 1695 cm.$^{-1}$ A portion was converted to the tetramethyl ester, with diazomethane, obtained as a crystalline solid from benzene-petrol, m.p. 132-135°.

(Found: C, 61.79; H, 7.23; $C_{20}H_{28}O_8$ requires C, 60.59; H, 7.12%). $\nu_{mx.}$ 1738, 1728 cm.$^{-1}$

Another portion was sublimed in vacuum (150°, 10^{-4}mm.Hg.) as a crystalline solid, identical with the original photodimer in m.p., mixed m.p., and infra-red spectrum.

Pyrolysis of the photodimer (128, 130)

The dimer (80 mg.) was pyrolysed at 300° in a sealed, evacuated tube for 11 hours. The residue in the tube was dissolved in chloroform and treated with cyclohexane to precipitate starting material. This was repeated to yield, in all, the original photodimer (50 mg.). The mother liquor was concentrated and sublimed in high vacuum (75°, 10^{-4}mm.Hg.) to yield Δ-1 tetrahydrophthalic anhydride (10 mg.), m.p. 71-72°, which did not depress the melting point of the authentic material, m.p. 74-76°, and had an identical infra-red spectrum.

Experiments with Glauconic Acid

Preparation of glauconic acid ketone (132)

Glauconic acid (1.0 g.) was dissolved in acetone (A.R. 20 ml.) and cooled to -10°. Then a solution of chromium trioxide (7.75 N,(prepared from chromium trioxide (17.5 g.), sulphuric acid (14.8 ml.), in water to 50 ml.) 0.83 ml.) was added dropwise with stirring over 2.5 hours, the temperature being held at -5°. The reaction was poured into water (40 ml.) and after addition of some crystalline sodium metabisulphite was extracted with chloroform (400 ml.) washed and dried to yield glauconic acid ketone as a foam (800 mg.) $\nu_{max.}$ 1840, 1773, 1710 $cm.^{-1}$

This ketone was used without further purification.

Chromic acid oxidation of glauconic acid ketone

The ketone (726 mg.) was set aside at room temperature for 24 hours with chromium trioxide in acetic acid (prepared from chromium trioxide (2.27 g.) water (1.6 ml.) and glacial acetic acid (32 ml.)) in a loosely stoppered flask. The reaction was poured into excess, aqueous, sodium metabisulphite and extracted continuously with ether for 24 hours. The dried extract was evaporated to a gum which was taken up into the minimum volume of

acetonitrile and allowed to stand at 0°, whereupon after 24 hours was obtained a small crop of the "C_{15}" acid (133) (20 mg.), recrystallised from ethyl acetate-petrol (60-80° b.p.) to m.p. 200-205°, $(\alpha)_D^{25}$ + 61° (c 1.2).

$\nu_{mx.}$ 1845, 1750, 1730, 1690 cm.$^{-1}$

$\lambda_{mx.}$ 224 mμ. (ε 7,450).

Concentration of the mother liquor and further standing at 0° gave rise to meso-diethyl succinic acid (45 mg.) as needles, recrystallised from ethyl acetate-petrol (60-80° b.p.), m.p. 194-197° (capillary, rapid heating). Mixed m.p. with authentic meso-diethyl succinic acid (m.p. 194-197°) was undepressed and the two samples had identical infra-red spectra (KCl. disc.) and behaviour on paper chromatography.

Separation of meso- and dl-diethyl succinic acid

The mixture of these two acids was resolved by two procedures,

1. Treatment with concentrated hydrochloric acid[44]

The crude mixture (244 mg.) was mixed with concentrated hydrochloric acid (s.g. 1.2, 3.0 ml.) in a sealed, thick walled pyrex tube and heated to 200° in a small furnace for eight hours. The tube was allowed to cool slowly to room temperature and the crystalline solid, so obtained, recrystallised twice from water as meso-diethyl

succinic acid (75 mg.), m.p. 197-200° (cap.).

2. Crystallisation from benzene[45]

The mixture (192 mg.) was dissolved by refluxing in benzene (10 ml.) and allowed to cool slowly to room temperature. The first crop, m.p. 135-140°, was recrystallised from benzene as meso-diethyl succinic acid (40 mg.) m.p. 194-197° (cap.). The infra-red spectrum was identical to that obtained by the previous method and the discrepancy in the melting point was probably due to anhydride formation before melting. All these alkylated succinic acids have melting points dependent upon the rate of heating.[9]

Ozonolysis of the "C_{15}" acid (133)

The acid (41 mg.) was dissolved in glacial acetic acid (A.R. 10 ml.) and a stream of ozonised oxygen blown through this solution at room temperature, following the reaction by the ultra-violet spectrum. There was no change in the spectrum after three hours and evaporation of the solvent gave back the starting material.

Chromium trioxide oxidation of the "C_{15}" acid (133)

The acid (40 mg.) was treated with chromium trioxide ((2.0 ml.) of a solution prepared from chromium trioxide (1.71 g.), water (1.50 ml.), and acetic acid (23.5 ml.)) at room temperature for four days.

To the mixture was added saturated sodium metabisulphite solution and then it was extracted with ether to yield a gum, which crystallised from ethyl acetate-benzene at 0^{o} to yield "C_{15}" acid (13 mg.) the infra-red spectrum was identical with that of starting material.

The mother liquor was concentrated and examined by paper chromatography, which indicated the presence of meso-diethyl succinic acid.

Note on paper chromatographic technique[46]

The chromatograms were run on Whatman No.1 paper pre-washed with acetic acid (2N), distilled water, and finally the solvent system, which was a mixture of n-propanol (distilled from potassium hydroxide) and ammonium hydroxide (G.P.R. 2 N) (25:7 v/v).

Chromatograms were generally run over 24 hours and were developed by spraying the dried papers with bromocresol green in ethanol-formaldehyde at pH 5.2, when acids appeared as blue spots on a yellow background. These spots were not permanent and so were marked immediately.

Autoxidation Experiments

Autoxidation of Δ-1 tetrahydrophthalic anhydride (59)

The anhydride (1.0 g.) was suspended in a solution of potassium t-butoxide in t-butanol (N 1.0, 63 ml.) and shaken in the presence of dry oxygen. The solution became yellow in colour and quickly absorbed the gas (180 ml., 1.0 m.). The liquid was acidified with acetic acid and evaporated at room temperature, to remove most of the alcohol, and then diluted largely with water and extracted with ether. This yielded a crystalline solid (900 mg.) which was recrystallised from benzene-ether mixture as 1-t-butoxycarbonyl-2-carboxy-1-hydroperoxy-cyclohex-2-ene (137) (460 mg.) m.p. 154-155° (d). (Found: C, 55.18; H, 6.87; $C_{12}H_{18}O_6$ requires C,55.8; H, 7.03% molecular weight 258). $\lambda_{max.}$ 217 mμ. (ε 8,000).

Equivalent weight (acid-base titration) 252.

Equivalent weight (iodometric titration) 290.

$\nu_{max.}$ 3400, 2700-3000, 1715, 1685, 1650 $cm.^{-1}$

The mother liquor from the crystallisation of the peroxide was subjected to chromatography on silica gel (70 g. acid washed) in benzene solution. By elution with benzene containing ether (5% v/v) there was obtained a crystalline solid (30 mg.) shown to be Δ-1 tetrahydrophthalic anhydride by m.p., mixed m.p., and infra-red spectrum.

1-t-butoxycarbonyl-1-hydroperoxy-2-methoxycarbonyl-cyclohex-2-ene (139)

The acid peroxide (137) (100 mg.) in ether (10 ml.) was treated with a dry, freshly distilled, ethereal solution of diazomethane until the yellow colour remained. The solvent was removed in vacuum and the residue recrystallised from petroleum-ether (60-80 b.p.) as large prisms, m.p. 80-81°, of 1-t-butoxycarbonyl-1-hydroperoxy-2-methoxycarbonyl-cyclohex-2-ene (139).

(Found: C, 57.34; H, 7.40; $C_{13}H_{20}O_6$ requires C, 57.34; H, 7.42%)

$\nu_{mx.}$ (Nujol) 3500, 1715, 1640 $cm.^{-1}$

$\lambda_{mx.}$ 215 mμ. (ε 10,000).

Acid catalysed degradation of the peroxide (137)

The acid peroxide (137) (264 mg.) in dilute sulphuric acid (4N, 12 ml.) was heated to 95° for two or three minutes and then steam blown into the reaction mixture, the distillate being collected in aqueous 2:4-dinitrophenyl hydrazine sulphate. Distillation was continued until a total volume of 100 ml. had been collected and the yellow precipitate was filtered, washed with water and dried (189 mg.). This derivative was chromatographed over bentonite (9 g.) and celite (2 g.) in benzene solution to yield a solid, recrystallised from chloroform-ethanol mixture to m.p. 164-166°. This did not depress the melting point of an authentic sample of

cyclohex-2-enone-2:4-dinitrophenyl hydrazone, m.p. 164-167°, and had an identical infra-red spectrum.

Acid catalysed degradation of the peroxide methyl ester (139)

The ester (139) (117 mg.) was treated with dilute sulphuric acid (4N, 12 ml.) in a current of steam, the distillate being passed into aqueous 2:4-dinitrophenyl hydrazine sulphate solution. After thirty minutes the derivative was filtered off and dried in vacuum (88 mg.). Recrystallisation from chloroform-ethanol gave cyclohex-2-enone-2:4-dinitrophenyl hydrazone, m.p. 164-166°, undepressed on admixture with authentic material and having an identical infra-red spectrum.

Catalytic reduction of the peroxide (137)

The peroxide (137) (475 mg.) was hydrogenated in ethyl acetate (100 ml.) in the presence of palladium on carbon (5%, 240 mg.). The compound absorbed 82 ml. of hydrogen (2 m.). Filtration through celite and evaporation of the solvent produced an crystalline tetrahydro-compound, which was recrystallised from ethyl acetate-petroleum ether (60-80° b.p.) as 1-t-butoxycarbonyl-2-carboxy-1-hydroxy cyclohexane (141). (342 mg.) m.p. 97-99°

(Found: C, 59.02; H, 8.32; $C_{12}H_{20}O_5$ requires C, 59.00; H, 8.25% molecular weight 244).

Equivalent weight (acid-base titration) 230.

$\nu_{mx.}$ 3500, 1715, 1700 cm.$^{-1}$

$\epsilon_{220\ m\mu.}$ 350.

Attempted Hydrolysis of the reduction product (141)

The compound was recovered unchanged on treatment with aqueous potassium hydroxide (1N) at room temperature for twelve hours, m.p., mixed m.p., and infra-red spectrum.

Lead tetraacetate oxidation of the reduction product (141)

The acid (52 mg.) in acetic acid (A.R. 1.0 ml.) was treated with a solution of lead tetraacetate in acetic acid (0.13N, 1.8 ml.) and the reaction followed by adding portions to potassium iodide solution and titrating the liberated iodine with standard thiosulphate. There was no measurable uptake of the oxidant over five hours. A model experiment, conducted on malic acid in the same manner, showed a rapid uptake of lead tetraacetate (1.0 m.).

Sodium borohydride reduction of the peroxide (137)

The peroxide (137) (74 mg.) was dissolved in aqueous alkali (0.1 N, 4.00 ml.) and titrated to pH. 9-10 with dilute acid. Then a solution of sodium borohydride (210 mg.) in the minimum volume of cold water, was added and this solution allowed to stand at room temperature for twelve hours. Acidification and ether extraction produced a crystalline solid (45 mg.) which was crystallised only with difficulty from ethyl acetate, containing a drop of methanol as 1-hydroxy-1,2-dicarboxy-cyclohex-2-ene (144), m.p. 175-180°(d).

$\nu_{mx.}$ (Nujol) 1720, 1695 $cm.^{-1}$

$\lambda_{mx.}$ 217 mμ. (ϵ 8,000).

This compound was more readily manipulated as the dimethyl ester prepared by reaction with excess, ethereal, diazomethane, and recrystallised from petroleum ether (60-80°b.p.) as 1-hydroxy-1,2-dimethoxycarbonyl-cyclohex-2-ene (145) m.p. 91-92°.
(Found: C, 56.19; H, 6.70; $C_{10}H_{14}O_5$ requires C, 56.07; H, 6.59%). $\nu_{mx.}$ 3500, 1720 $cm.^{-1}$ $\lambda_{mx.}$ 223 mμ. (ε 5,000).

Lead tetraacetate oxidation of 1-hydroxy-1,2-dicarboxy-cyclohex-2-ene (144)

The acid (144) (47 mg.) was dissolved in glacial acetic acid (A.R. 1.0 ml.) and then treated with a solution of lead tetraacetate in the same solvent (0.14N, 3.0 ml.). The reaction was followed as before. After three hours the reaction was essentially complete, (0.98 m. oxidant) and poured into water and thoroughly extracted with ether (75 ml.). The extract was cautiously evaporated at room temperature and the residue shaken with aqueous 2:4-dinitrophenyl hydrazine sulphate. On standing for three hours a gummy red solid separated, m.p. 70-110°(d), which on attempted crystallisation from chloroform and ethanol decomposed to a tar.

2-Methoxycarbonyl-cyclohex-2-enone (146)

The peroxide methyl ester (139) (100 mg.) in aqueous ethanol (50%, 8 ml.) was stirred at 0° and a solution of

ferrous ammonium sulphate (4.096 g. in 50 ml. with a drop of dilute hydrochloric acid, 2.90 ml.) was added dropwise over one hour. The solution became reddish brown in colour and was stirred for a further one hour. After dilution with water and extraction with ether (100 ml.) there was obtained a colourless gum (64 mg.).

$\nu_{mx.}$ 1725, 1660, 1620 $cm.^{-1}$

$\lambda_{mx.}$ 269 mμ.(ε 3,800) 327 mμ.(ε 1,700).

This was dissolved in a little methanol and treated with a methanolic solution of 2:4-dinitrophenyl hydrazine sulphate at room temperature. After 15 minutes a crystalline precipitate was collected (21 mg.) which chromatographed on bentonite (10 g.) in benzene solution and the purified material recrystallised from chloroform-benzene as yellow needles of 2-methoxycarbonyl-cyclohex-2-enone-2:4-dinitrophenyl hydrazone m.p. 200-206°.

(Found: C, 50.07; H, 4.46; N, 16.63; $C_{14}H_{14}N_4O_6$ requires C, 50.30; H, 4.22; N, 16.76%).

$\nu_{mx.}$ 1720, 1610, 1580 $cm..^{-1}$

$\lambda_{mx.}$ 254 mμ,(ε 13,600), 370 mμ.(ε 23,200).

Preparation of 1-t-butoxycarbonyl-2-carboxy-cyclohex-2-ene (150).

Δ-1-tetrahydrophthalic anhydride (282 mg.) was dissolved in potassium t-butoxide in t-butyl alcohol (1N, 10 ml.) under nitrogen and allowed to stand for thirty minutes at room temperature.

Then the solution was acidified with acetic acid and most of the alcohol removed by evaporation under reduced pressure and the product isolated by dilution with water and extraction with ether (150 ml.) as a crystalline solid (335 mg.), which was recrystallised from benzene – ligroin . as 1-t-butoxy-carbonyl-2-carboxy-cyclohex-2-ene (150).

m.p. 127-128° (Found: C, 63.57; H, 7.99; $C_{12}H_{18}O_4$ requires C, 63.70; H, 8.02%)

$\nu_{mx.}$ 1710, 1690, 1650 $cm.^{-1}$

$\lambda_{mx.}$ 217 mμ.(ε 8,000).

Autoxidation of 1-t-butoxycarbonyl-2-carboxy-cyclohex-2-ene

Δ-1 tetrahydrophthalic anhydride (100 mg.) was shaken with a solution of potassium t-butoxide in t-butyl alcohol (1N, 10 ml.) under nitrogen for 60 minutes. The apparatus was evacuated and oxygen introduced. There was smooth absorption of gas (16.0 ml. 0.98 m.), and the reaction was worked up as before, yielding a colourless gum (108 mg.) which on trituration with benzene and acetone mixture produced a crystalline solid (13 mg.), 1-t-butoxycarbonyl-2-carboxy-1-hydroperoxy-cyclohex-2-ene, having same melting point and infra-red spectrum as authentic material.

The rate of oxygen uptake in this experiment was found to be about half that of the anhydride.

Autoxidation of Δ-1 tetrahydrophthalic acid dimethyl ester

The anhydride (250 mg.) was dissolved, by standing at room temperature, in aqueous potassium hydroxide (1N, 5.0 ml.). After one hour the solution was cooled to 0° and acidified with dilute hydrochloric acid (6N.) at the same temperature. The solution was saturated with salt and extracted with ethyl acetate to yield Δ-1 tetrahydrophthalic acid as a crystalline solid, which readily reverted to the anhydride. This material was dissolved in ether (15 ml.) and cooled to 0° and then treated with excess, ethereal, diazomethane to yield dimethyl- Δ -1 tetrahydrophthalate as an oil, $\nu_{mx.}$ 1730, 1650 cm.$^{-1}$.

A portion of this ester (100 mg.) was shaken with potassium t-butoxide in t-butanol at room temperature, whereupon it rapidly absorbed 1.06 m. of the gas. However, work up by the procedure described for the previous autoxidations gave no crystalline materials.

Autoxidation of Dimethyl maleic anhydride

The anhydride (m.p. 91-93°, 550 mg.) in potassium t-butoxide in t-butylalcohol (1N, 30 ml.) was oxygenated at room temperature and worked up as in all previous autoxidation experiments. This yielded a crystalline solid, recrystallised from benzene-petrol (60-80° b.p.) as colourless plates of 3-t-butoxycarbonyl-3-hydroxy-2-methylene-butyric acid (152) (103 mg.), m.p. 104-105°.

(Found: C, 55.55; H, 7.53; $C_{10}H_{16}O_5$ requires C, 55.54; H, 7.46% molecular weight 216)

$\nu_{mx.}$ 3400, 1720, 1690, 1625 cm.$^{-1}$ 880 cm.$^{-1}$.

$\lambda_{mx.}$ 210 mμ.(ε 4,700).

Equivalent weight (acid-base titration) 204.

Hydrogenation of 3-t-butoxycarbonyl-3-hydroxy-2-methylene-butyric acid

The acid (152) (28 mg.) in ethyl acetate (10 ml.) was hydrogenated in the presence of palladium on charcoal (5%, 20 mg.). The solution absorbed hydrogen (3.19 ml. 1 n.) and was filtered and evaporated to a gum, which could not be crystallised, having $\nu_{mx.}$ 1712, 1705, 3500 cm.$^{-1}$.

Autoxidation of citraconic anhydride and cis-hexahydrophthalic anhydride

These two compounds, when subjected to the conditions described above, each absorbed oxygen (ca. 0.5 m.) but yielded no crystalline material.

Attempted autoxidation of natural products

When byssochlamic acid and glauconic acid were exposed to the conditions, indicated above, there was no absorption of oxygen and the materials were recovered unchanged.

Hydride reduction of Anhydrides

Lithium aluminium hydride reduction of Δ -1 tetrahydrophthalic anhydride

The anhydride (1.0 g.) dissolved in dry tetrahydrofuran (freshly distilled from lithium aluminium hydride, 10 ml.) was added dropwise to a refluxing suspension of lithium aluminium hydride (2.5 g.) in tetrahydrofuran (70 ml.). After 12 hours the reaction mixture was cooled to room temperature and the excess reagent destroyed by careful addition of ethyl acetate. The mixture was treated with saturated sodium sulphate and filtered, the filter being carefully washed with warm tetrahydrofuran. By evaporation of the filtrate, dissolution in ether, and after drying and evaporation, there was obtained a colourless oil (940 mg.).

$\nu_{mx.}$ 3400, 1725 cm.$^{-1}$

Experiments performed on this oil were as follows.

1) 1,2-bis-(3:5-dinitrobenzoyloxymethyl)-cyclohexene (154)

The oil (500 mg.) in pyridine (dry, 2 ml.) was added, at 0° to an ice-cold solution of 3:5-dinitrobenzoic acid (864 mg.) and tosyl chloride (755 mg.) and the resultant mixture allowed to stand for twelve hours. The solution was poured into water and extracted with chloroform and then quickly washed with dilute hydrochloric acid (1N) and

then with saturated sodium bicarbonate, until the washings were colourless. Evaporation of the chloroform gave a yellow crystalline solid, recrystallised from benzene-petrol (40-60°b.p.) as 1,2-bis(3:5-dinitro-benzoyloxymethyl)-cyclohexene (361 mg.), m.p. 156-157°. A portion was chromatographed on alumina (G III , neutral) in benzene and recrystallised to m.p. 158-160°.

(Found: C, 49.85; H, 3.79; N, 10.46; $C_{22}H_{18}N_4O_{12}$ requires C, 49.82; H, 3.42; N, 10.56%)

$\nu_{mx.}$ 1730, 1630, 1600 $cm.^{-1}$

$\lambda_{mx.}$ 209 mμ.(ε 53,000), 224 mμ.(ε 40,500).

2) 1,2-bis(Acetoxymethyl)-cyclohexene

The oil (300 mg.) was acetylated with acetic anhydride and pyridine at 0° for 12 hours. The product was obtained by pouring into water and extraction with ether as an oil, $\nu_{mx.}$ 1740 $cm.^{-1}$ which could not be crystallised.

3) 2-Methyl-cyclohexene-carboxaldehyde [36]

The oil (436 mg.) was dissolved in ethanol (10 ml.) and heated with ethanolic 2:4-dinitrophenylhydrazine sulphate for 2.5 hours on the steam bath and then cooled and filtered from a red crystalline solid (319 mg.). This material was chromatographed on kieselguhr (3 g.) and bentonite (12 g.) in benzene and was thereby obtained as 2-methyl-cyclohexene-carboxaldehyde-2:4-dinitrophenyl hydrazone, as

red needles (75 mg.) m.p. 190-191° (Found: C, 54.86; H, 5.13; N, 8.26; $C_{14}H_{16}N_8O_4$ requires C, 55.25; H, 5.30; N, 8.41%). $\lambda_{mx.}$ 388 mμ.(ε 30,400).

4) Δ -1 and Δ -2 tetrahydrophthalide

The oil (256 mg.) was dissolved in aqueous potassium hydroxide (4N, 5.0 ml.) under nitrogen at room temperature for three hours and then extracted with ether (150 ml.). The neutral fraction (120 mg.) was an oil exhibiting no carbonyl absorption in the infra-red spectrum. The acidic fraction (50 mg.) was also an oil showing bands at 1720 and 1760 $cm.^{-1}$ in the infra-red. These were attributed to the presence of Δ -1 and Δ -2 tetrahydrophthalides.

Ozonolysis of 1,2-bis (3:5-dinitrobenzoyloxymethyl)-cyclohexene

The diester (101 mg.) in chloroform (A.R. 10 ml.) was cooled to -20° and a stream of ozonised oxygen blown through the solution for two hours. At the end of this time petroleum ether (A.R. 40-60°b.p.) was added with swirling until the solution was cloudy. It was allowed to warm to room temperature and then to stand overnight. The yellow crystalline solid (86 mg.) was filtered and recrystallised from acetic acid as small yellow rectangular prisms (161) (50 mg.), m.p. 186-188°, (Found: C, 46.62; H, 3.09; N,9.92; $C_{22}H_{18}O_{15}N_4$ requires C, 45.65; H, 3.11; N, 9.68%).

$\nu_{mx.}$ (Nujol) 1750, 1730 $cm.^{-1}$

Formation of Thioanhydrides

Δ-4 tetrahydrophthalic thioanhydrides

Δ-4 tetrahydrophthalic anhydride (900 mg. finely powdered) was mixed with phosphorus pentasulphide (467 mg. finely powdered)and the mixture dissolved in dry pyridine (10 ml.) and refluxed for four hours. The pyridine was removed under reduced pressure and the brownish residue dissolved in chloroform and separated from a black tar. Upon evaporation of the solvent the residue slowly crystallised and was recrystallised from petroleum ether (60-80°b.p.) and chloroform mixture, to yield Δ-4 tetrahydrophthalic thioanhydride (300 mg.), m.p. 142-143°, (Found: C, 56.80; H, 4.69; S, 19.16; $C_8H_8O_2S$ requires C, 57.10; H, 4.76; S, 19.0%) $\nu_{mx.}$ 1712, 1695 cm.$^{-1}$ $\lambda_{mx.}$ 228 mμ.(ε 5,000), 209 mμ.(ε 3,400).

Phthalic thioanhydride

(i) A solution of phosphorus pentasulphide (3.0 g.) in pyridine (100 ml., dried over potassium-hydroxide) was mixed with phthalic anhydride (2.66 g.) at room temperature and shaken for 48 hours. The pyridine was removed at 80-90° and the solid residue was treated with ether (25 ml.) and chloroform (25 ml.) and allowed to stand overnight. The yellow extract was evaporated to a crystalline mass (3.0 g.) which was recrystallised from petroleum ether (60-80°b.p.)

as needles of phthalic thioanhydride (2.0 g.), m.p. 114° (Found: C, 59.1; H, 2.77; S, 19.5; $C_8H_4O_2S$ requires C, 59.5; H, 2.40; S, 19.5%). $\nu_{mx.}$ 1786, 1730, 1704 cm.$^{-1}$ $\lambda_{mx.}$ (cyclohexane) 221 mμ. (ε 28,000), 230 mμ. (ε 25,000), 299 mμ. (ε 1,950).

(ii) Phthalic anhydride (2.0 g.) was ground in a mortar with sodium sulphide (Na_2S. 9 H_2O, 4.0 g.) until a yellow liquid was obtained. This was filtered into excess hydrochloric acid (4N, 12 ml.) at -10° and the organic material extracted quickly with ether, washed with sodium bicarbonate (50% satd.) dried and evaporated to yield a mixture of phthalic anhydride and phthalic thioanhydride (600 mg.) m.p. 110-130°. This mixture was readily resolved by chromatography on silica gel (acid washed, 42 g.). Elution with benzene produced phthalic thioanhydride (350 mg.) recrystallised from petroleum ether (60-80°b.p.) as colourless needles[47] m.p. 114°. Mixed m.p. with sample from phosphorus pentasulphide method was 114°.

Further elution with benzene-chloroform (1.1 v/v) gave phthalic anhydride, m.p. 127-131° (200 mg.).

Cis-hexahydrophthalic anhydride.

A solution of phosphorus pentasulphide (3.0 g.) in pyridine (dry, 100 ml.) was treated with cis-hexahydrophthalic anhydride (2.5 g.) and shaken at room temperature for two days.

The pyridine was removed in vacuum and the gum extracted with a mixture of chloroform and ether (1:1 v/v, 80 ml.), overnight in the shaker. Evaporation of this extract produced a yellow oil (2.25 g.) which was chromatographed on silica gel (acid washed, 110 g.). Elution with benzene-chloroform (3:1 v/v) gave an oil (0.93 g.) which was distilled in vacuum to yield cis-hexahydrophthalic thioanhydride as an oil.

(Found: C, 56.33; H, 5.88; S, 20.61; $C_8H_{10}O_2S$ requires C, 56.50; H, 5.88; S, 18.8%), $\nu_{mx.}$ 1710 cm.$^{-1}$

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