

STUDIES IN THE CHEMISTRY OF LIMONIN

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SUMMARY

The following original contributions were made to the chemistry of limonin:

1. The reactions of hexahydrolimonini acid with acids were elucidated (see section 6[@])
2. The isomerization of tetrahydrolimonin was examined (see section 2).
3. The reaction of tetrahydrodesoxylimonin with acetic anhydride and hydroiodic acid was explained (see section 7).
4. Tetrahydrodesoxylimonin was degraded, giving the substitution pattern at C₈ and C₉ (see section 5).
5. Limonilic acid was degraded (see section 11).
6. The structure of citrolin was determined (see section 12).

The following original contributions were made to the chemistry of obacunone and nomilin (see section 15) :

1. The degree of unsaturation of

[@] This , and all subsequent internal references, refer to subsections of the main discussion sections.

obacunonic acid was determined.

2. Some derivatives were prepared by degradation of lactone D of obacunonic acid, showing close analogy to limonin.

3. iso-Obacunonic acid was prepared.

4. The positive iodoform test given by obacunonic acid was verified and interpreted by means of model experiments.

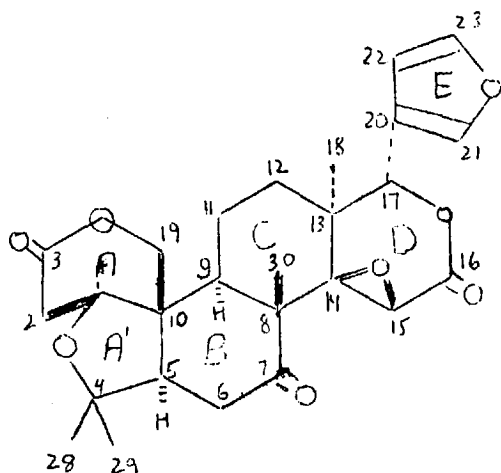
5. A plausible structure for obacunonic acid was postulated.

In addition , a number of model investigations was carried out concerned with new methods of decarboxylation (see sections 16 and 17).

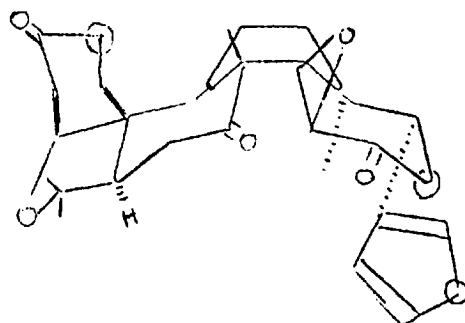
The above results and all chemical reactions of limonin , are discussed and interpreted in terms of the structure which was proposed on the basis of chemical evidence and fully confirmed by X-ray crystallographic analysis. A plausible biogenetic scheme for limonin, obacunone and nomilin is proposed on the basis of accepted biogenetic schemes for triterpenes.

INTRODUCTION

Limonin, $C_{26}H_{30}O_8$, the chief bitter principle of citrus fruit, was first isolated by Bernay¹ in 1841 and has since been studied by American, Australian, British, German, Indian, Italian, Japanese and Swiss workers who have described their findings in over thirty publications. In addition, the chemistry of limonin has been investigated by three teams working in collaboration and headed respectively by Prof. D. H. R. Barton (Imperial College, London), Prof. E. J. Corey (University of Illinois) and Prof. O. Jeger (E. T. H., Zurich). The findings of the combined groups resulted in publication² of the full structure of limonin (I), which was confirmed by the results of X-ray crystallographic analysis³ carried out by Prof. J. M. Robertson, in collaboration with the team at Imperial college. The latter work also elucidated the stereochemistry of the molecule. The full experimental details of the chemical work - including the contribution of the present author - are awaiting publication.



(I)



In the present work an attempt has been made to discuss all known chemical reactions of limonin in terms of structure (I) , rather than to deduce (I) rigidly on the basis of the chemical evidence alone. It is hoped that the work has gained in clarity and brevity what it might have lost in subtlety.

Accordingly, following a brief summary of the published work, the main discussion section is devoted to the interpretation of the chemistry of limonin. The order of presentation was chosen to minimise the number of crossreferences to work yet not discussed. After the various aspects of the chemistry of limonin are described in the first thirteen subsections, the chemical evidence is

summarised in section 13B and the biogenetic implications of the work are discussed in section 14.

Work dealing with the chemistry of the two minor bitter principles of citrus fruit, obacunone and nomilin , is discussed in section 15 which also contains a full description of published work on this topic.

A number of model experiments is described in the following two sections , and the thesis closes with the account of the experimental work carried out by the author.

SUMMARY OF PUBLISHED WORK

The original discoverer of limonin, Bernay, classified the material as an alkaloid¹ on the basis of a spurious test for nitrogen. This error was rectified by Schmidt⁴ who also noted that while limonin was resistant to oxidation, it was degraded to low molecular weight products once oxidation started.

As early as 1879, Paterno and Ogliolero⁵ interpreted their analytical results in terms of the correct formula $C_{26}H_{30}O_8$, but it did not gain universal acceptance until much later. Hoffman⁶ isolated limonin among a number of plant products, but did not investigate its constitution. He considered that it was probably a glycoside. Another preliminary investigation was described by Peters and Frierichs⁷ who carried out unsuccessful attempts to methylate, oxidise and estimate the methoxyl groups in limonin.

A great deal of confusion arose from the fact that limonin isolated by numerous workers from several sources was often described as a separate

bitter principle due to the poor characterisation of the material. Thus Fujita and Wada⁸ isolated "obaculactone" , Chen and Chen⁹ "evodin" and Feist and Schulte¹⁰ "citrolimonin" , all of which were eventually proved to be identical with limonin largely due to the efforts of Schechter and Haller¹¹ who in another contribution¹² also investigated the toxicity of limonin-containing extracts to insects. The question was further complicated by the isolation of similar , but genuinely different bitter principles obacunone and nomilin from citrus fruit, which have since been investigated by Emerson^{13,14} and Dean and Geissman¹⁵.

Limonin was also isolated by Hookerjee¹⁶, Nolte and Loesecke¹⁷ and Higby¹⁸, but these authors did not make any major contributions to the chemical investigations.

Serious attempts to discover the structure of limonin date from the work of Koller and Czerny^{19,20}. In spite of working with the wrong molecular formula, $C_{23}H_{28}O_7$, these workers made important contributions to limonin chemistry. By hydrogenating over palladium-carbon they obtained tetrahydrolimonin and hexahydro-

limoninic acid to which they assigned wrong formulae, although they observed the correct hydrogen uptake. They also obtained 1:2:5 trimethyl naphthalene and isophthalic acid by alkali fusion and benzene penta-carboxylic acid by oxidation with manganese dioxide and sulphuric acid.

In 1946 , Geissman and Tulagin²¹ summarised all the previous work and prepared the novel derivatives limonilic acid (by the action of alkaline manganate) and citrolin (by the action of hydroiodic acid). From the latter reaction they also isolated a small yield of another important derivative, desoxy-limonin. They also showed that acetone was formed as a result of alkali fusion of limonin and that neither of the two lactones present can be opened by methylation.

The optical, crystallographic and X-ray properties of limonin were studied by Jones and Palmer²². The X-ray data suggested that the molecule was rather flat and the comparison of the calculated and determined densities of limonin and its acetic acid solvate gave an accurate check of its molecular weight.

The hydrogenation of limonin was re-

investigated by Rosenfeld and Hofman²³, who confirmed the earlier results of Koller and Czerny²⁰ and also found that the acidic product, hexahydrolimoninic acid, could be decarboxylated by heating at 240°C under nitrogen.

Brachvogel²⁴ also obtained tetrahydrolimonin by hydrogenation over palladium catalysts and further obtained a compound $C_{26}H_{30}O_8$, which contained one active hydrogen atom but was not acidic, by hydrogenation over platinum oxide. The latter was most likely tetrahydrolimonol. She also produced an independent proof of the degree of unsaturation of limonin by measuring the uptake of bromine. Brachvogel further found 1:2:5 trimethyl naphthalene among the products of zinc distillation and acetone and ortho-cresol among the products of alkaline fusion of limonin. The latter reaction also gave rise to an unidentified "lactone" $C_{13}H_{14}O_2$.

Chandler and Kefford^{25,26,27,28} proved that limonin had a ketonic function by preparing a 2:4 dinitrophenylhydrazone as well as an oxime, reducing the ketone to an alcohol (limonol) with aluminium isopropoxide and assigning the carbonyl

frequencies in the infrared spectrum. They also isolated another natural product , a bitter principle obtainable from limonin by permanganate oxidation , and named it limonexic acid.

In 1952, Emerson²⁹ prepared and thoroughly characterised tetrahydrolimonin, hexahydrolimoninic acid, tetrahydrolimonin oxime, limonilic acid, methyl limonilate oxime, tetrahydrolimonilic acid, a di-acid with both limonilic and limoninic functions, citrolin and various derivatives of the above acids. He interpreted correctly that hydrogenolysis opened one lactone ring and limonilic acid change the other one and investigated the chemistry of citrolin by hydrogenation and alkaline decomposition.

Fujita and Hirose³⁰ produced "dihydrolimonin" (probably limonol) by reducing limonin with sodium amalgam and "dehydrolimonin" (probably desoxylimonin) by action of iodine in acetic acid. They also showed that limonin oxime could be converted back to limonin , i.e. that it was a simple ketonic derivative, and prepared citrolinonic acid³¹ by ozonolysis.

Evidence for the presence of a β -substituted , isolated, furan nucleus was produced by

Fujita and Akatsuka³² and Kubota and Kotoroyama³³ and finally confirmed by NMR measurements carried out by Corey³⁴. This gave a rational explanation for the hydrogenation and hydrogenolysis behaviour of limonin and explained the nature of limonexic and etiolimononic acids.

In 1957 , Jeger and his co-workers³⁵ , re-investigated all the reduction and hydrogenation products of limonin and carried out and interpreted the complex reaction which limonol undergoes with alkali. They also described an investigation into the alkaline degradation of limonin which has since yielded a number of interesting results.

However, even in this work, only the relation between the furan and the lactone ring D could be deduced with any degree of certainty.

DISCUSSION

Note on spectroscopic results

As in the case of most complex molecules, the infrared spectra of limonin and its derivatives proved to be far too complicated for the interpretation of the majority of the bands. A further complication arose from the comparatively large number of carbonyl groups present which made the interpretation of the frequencies occurring in the carbonyl stretch region more difficult than usual. Some spectra could not be fully resolved and in others it appeared possible that some displacement of peak frequencies occurred due apparently to purely "algebraic" interaction. Nevertheless, valuable information was obtained from the study of carbonyl frequencies because of often remarkable consistency in certain series of reactions (e.g. acid isomerisation of hexahydrolimoninic acid, hydroiodic acid degradation of limonin, limonilic acid and its derivatives).

A further limitation on any generalisations which could be derived from the infrared spectra of limonin and its derivatives is due to the fact that

most of the spectra were obtained by using Nujol mulls. However, in all cases where spectra were also obtained in solution (limonin, desoxylimonin, octahydroacetate of limonin, cisoid diene acid, methyl limonilyl ketone) the differences from mull spectra were negligible. This may be due to the fact that intermolecular interactions in large and complex molecules may be less important than it is generally realised.

Unless otherwise stated, all interpretations of infrared spectra are based on the work of Bellamy³⁶ and of ultraviolet spectra on Gillam and Stern³⁷ or Fieser and Fieser³⁸.

Nuclear magnetic resonance spectra , were kindly determined by Dr L.M. Jackman and his collaborators.

1. THE FURAN AND LACTONE FUNCTIONS OF LIMONIN

a. Reactions of the furan nucleus

The considerable evidence for the presence of a β -substituted furan ring in limonin has already been reviewed in the literature^{35,34} and only a brief summary is presented here. The furan ring is the only unsaturated structure in limonin; no evidence of other unsaturation has ever been obtained.

The ultraviolet absorption spectrum of limonin shows a flat peak at about 208m μ (ϵ :7,000) which is characteristic of a furan. Phillips³⁹ identified a number of bands in the infrared spectrum of limonin as due to the characteristic vibrations of the furan group. The peaks at 1505-1502 cm⁻¹(m) and at 875 cm⁻¹(s) have proved to be of diagnostic value. Corey³⁴ interpreted the proton magnetic resonance spectrum of limonin as showing the presence of a β -substituted furan nucleus.

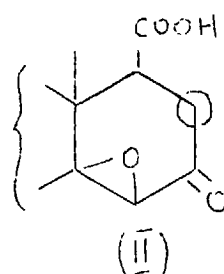
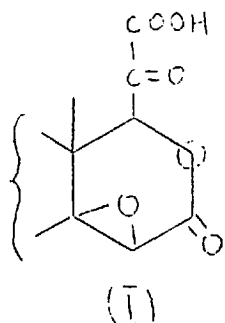
Hydrogenation of limonin over rhodium-carbon⁴⁰ gives almost exclusively a neutral tetrahydro derivative (tetrahydrolimonin, C₂₆H₃₄O₈) whose infrared spectrum shows the absence of the characteristic furan bands and which has no strong absorption

in the ultraviolet. Formation of tetrahydrolimonin can thus be interpreted as a straightforward hydrogenation of the furan ring.

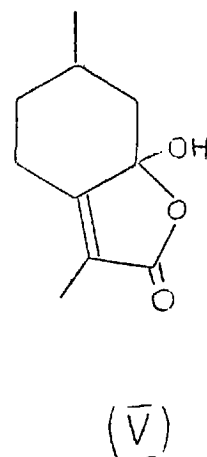
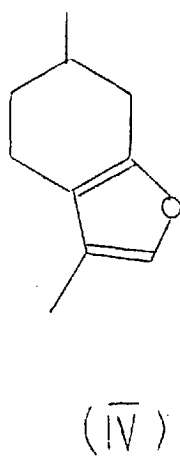
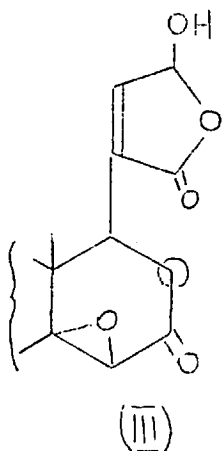
When limonin is hydrogenated over palladium-carbon , it yields some tetrahydrolimonin, but the main product^{20,29} is an acidic hexahydro derivative ,whose chemistry is discussed in sections 1b and 2c.

Hydrogenation in the presence of platinum oxide³⁰ may cause the reduction of the ketone (section 3) to the alcohol, as well as the saturation of the furan ring, but may also result in the uptake of four moles of hydrogen to give a neutral , dihydroxylic compound named hexahydrolimondiol³⁵. As this compound undergoes the merolimonol change (see section 8) to give a product identical with that obtained from limonol, it is likely that the second hydroxyl group is generated by a hydrogenolytic cleavage of the tetrahydrofuran ring - possibly while the furan is partially hydrogenated to the unstable dihydro derivative. Hexahydrolimondiol was also obtained in the course of the present work when limonin was hydrogenated over a rhodium-carbon catalyst at about 125 atmospheres.

The furan ring of limonin undergoes a number of oxidation reactions. Ozonolysis^{32,35} yields two products ; aetiolumonic acid (I) and trisnor acid (II) , both are the expected products from a β -substituted furan nucleus.

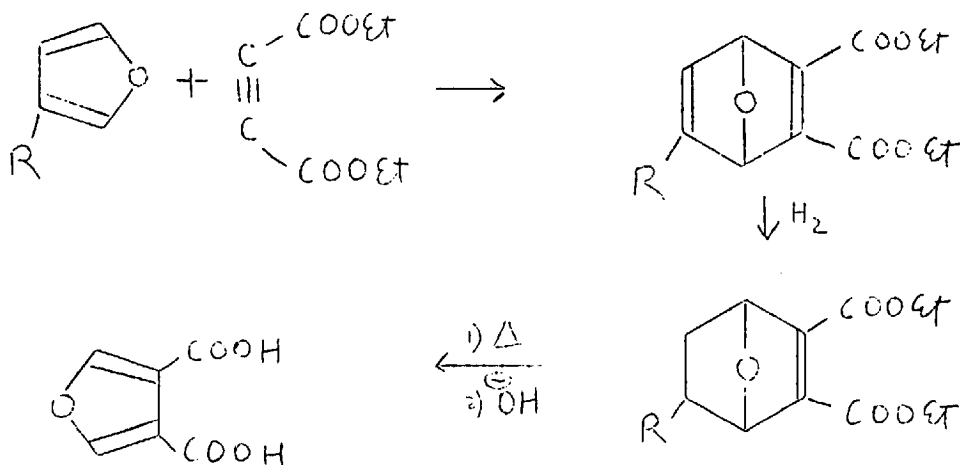


Oxidation of limonin with gaseous oxygen in presence of ultraviolet light and eosin³⁵, or alternatively oxidation with either performic acid or permanganate^{26,28} gives limonexic acid (III).



In accordance with the above structure, the ultraviolet absorption spectrum of limonexic acid shows a maximum at 209 m μ (ϵ :11,800). The infrared spectrum shows bands at 3255 cm^{-1} (hydroxyl) and 1803 cm^{-1} (α - β unsaturated γ -lactone), but none of the bands assigned to furan. An analogous oxidation of menthofuran (IV) to the lactone (V) by hydrogen peroxide in acetic acid, chromic acid, or oxygen in the presence of ultraviolet light has been described by Woodward and Eastman⁴¹.

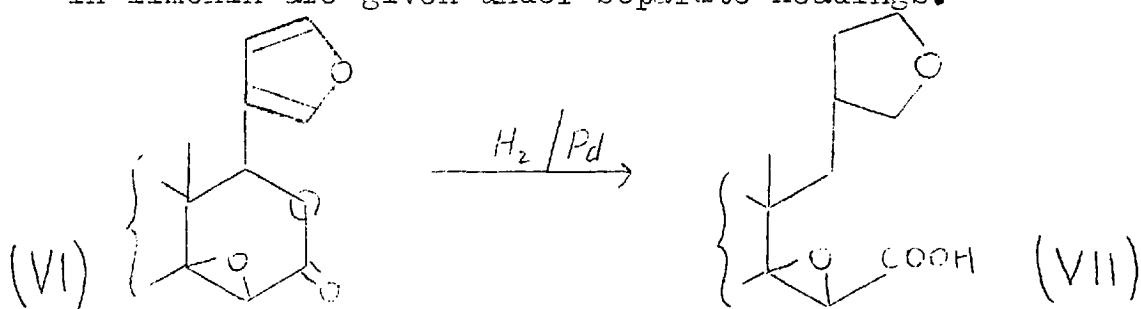
Kubota and Tokoroyama³³ carried out the following series of reactions on a pentoal derived from limonin by lithium aluminium hydride reduction, thus providing further evidence for the presence of a furan ring unsubstituted in either of the α positions in limonin.



b. The lactone rings

The fact that limonin contained two lactone rings was established very early⁷. These appear to be δ constituted from infrared data (bands near 1755 cm^{-1} in limonin itself and varying from 1760 to 1740 cm^{-1} in various derivatives retaining unaltered lactone functions).

Hydrogenolysis of limonin (VI) to a hexahydro acid, hexahydrolimoninic acid $\text{C}_{26}\text{H}_{36}\text{O}_8$ (see section 1a), suggests that the alcoholic oxygen of one of the lactone rings is situated allylically with respect to the furan ring. The fact that tetrahydrolimonin cannot be further hydrogenolysed confirms this. As hexahydrolimoninic acid is abnormally strong ($\text{pK}:3.2$) an oxygen function has to be placed α to the carbonyl group. Further work described in sections 3, 6 and 8 gave good evidence that hexahydrolimoninic acid possesses the part structure (VII). An exactly analogous situation has been described in the chemistry of columbin⁴². Other reactions of the lactone groups in limonin are given under separate headings.



2. REACTIONS OF THE EPOXIDE

a. Reduction

Corey⁴⁰ obtained desoxylimonin, $C_{26}H_{30}O_7$, by reduction of limonin, $C_{26}H_{30}O_8$, with chromous chloride. This result was confirmed in the course of the present work. Desoxylimonin had been previously obtained^{21,29} as a byproduct of citrolin (section 12) by the action of hydroiodic acid on limonin. Modification of the latter procedure by Pradhan⁴³, gave desoxylimonin in good yield.

Desoxylimonin is a neutral dilactone retaining the ketone function (oxime^{39,43}) and the furan ring (bands at 1503 and 875 cm^{-1} in the infrared spectrum). The ultraviolet spectrum of desoxylimonin shows a maximum at 213 $m\mu$ (ϵ :15,800); after subtracting the spectrum of limonin a maximum remains at 218 $m\mu$ (ϵ :10,500), characteristic of an α - β unsaturated lactone or ester. The infrared spectrum of desoxylimonin gives further evidence for the presence of such grouping; the carbonyl bands being situated at 1746 cm^{-1} (δ lactone) and at 1715 cm^{-1} (α - β unsaturated δ lactone and ketone), the second band being much stronger than the first. In contrast, the infra-

red spectrum of limonin itself shows the major band near 1755 cm^{-1} (two δ lactones) and the minor band at 1709 cm^{-1} (ketone).

As a confirmation, the tetrahydro derivative of desoxylimonin was prepared⁴³, both by the action of hydroiodic acid on tetrahydrolimonin and by hydrogenation of desoxylimonin (see also section 7). As expected, the ultraviolet spectrum of tetrahydrodesoxylimonin, $\text{C}_{26}\text{H}_{34}\text{O}_7$, shows a maximum at $218.5\text{ m}\mu$ ($\epsilon:10,600$) and the infrared spectrum shows carbonyl bands at 1752 cm^{-1} (δ lactone) and at $1720\text{--}1707\text{ cm}^{-1}$ ($\alpha\text{--}\beta$ unsaturated lactone and ketone), but no bands characteristic of the furan.

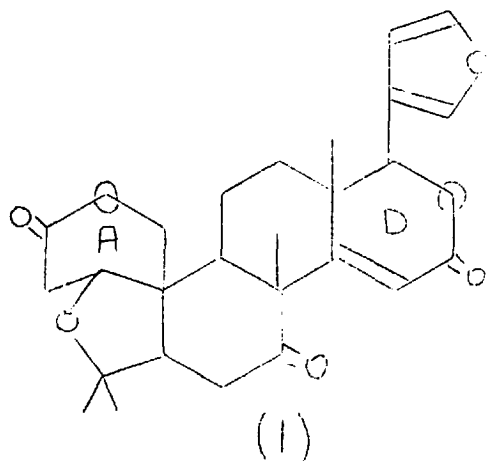
Tetrahydrodesoxylimonin was also prepared by Corey⁴⁰, by the action of chromous chloride on tetrahydrolimonin, who also converted it to a dihydro derivative by hydrogenating over a rhodium catalyst and oxidising the dihydro-tetrahydrodesoxylimonol formed with chromic acid in pyridine.

The following evidence can be advanced to show that it is the lactone ring D which is involved in the reduction of limonin to desoxylimonin, rather than the lactone ring A:

(i) The low pK value of hexahydrolimoninic acid²⁹ (section 1b) requires the presence of an α -negative substituent in the lactone D. It is the reduction of such a group which could be expected to give rise to an α -unsaturated lactone function, in particular if the α -oxygen was a part of an epoxide ring (see below).

(ii) Emerson²⁹ has shown that a dicarboxylic acid could be formed either by hydrogenolysis of limonilic acid (where lactone A is involved, see section 11) or by iodine/alkali oxidation of hexahydrolimoninic acid thus proving that different lactones were involved in hydrogenolysis and in limonilic acid formation. As lactone D has the α -oxygen function, it is unlikely that lactone A is involved in the formation of desoxylimonin.

(iii) A desoxylimonin analogue of limon-



ilic acid was prepared by Pradhan⁴³ and showed the same spectroscopic properties as desoxylimonin itself. That is it is an α - β unsaturated lactone, $\lambda_{\max}$: 213-4 m μ (ϵ : 15,400). This

also indicated that the carboxylic group of lactone A , which had been opened by the limonic acid reaction , was not involved in the formation of desoxylimonin.

(iv) The fact that desoxylimonin does not give any acidic product when hydrogenated over palladium carbon (see preparation of tetrahydro-desoxylimonin) , also indicates that lactone D was altered in some way in the transformation of limonin to desoxylimonin.

The degree of substitution at the double bond of the α - β unsaturated lactone in desoxylimonin is indicated by the nature of the transformation products of tetrahydrolimonin with acid (see section 2b) and the nature of decarboxyhexahydrolimonic acid (see section 2c). The position of the maximum in the ultraviolet spectrum of tetrahydrodesoxylimonin (page 20) is also consistent with the presence of a trisubstituted double bond.

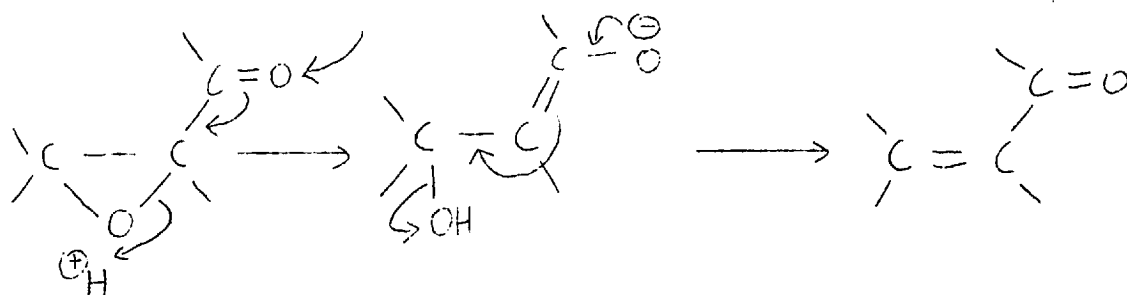
On the basis of the above evidence , structure (I) was assigned to desoxylimonin.

Besides evidence discussed in sections 2b and 2c, the following evidence can be advanced

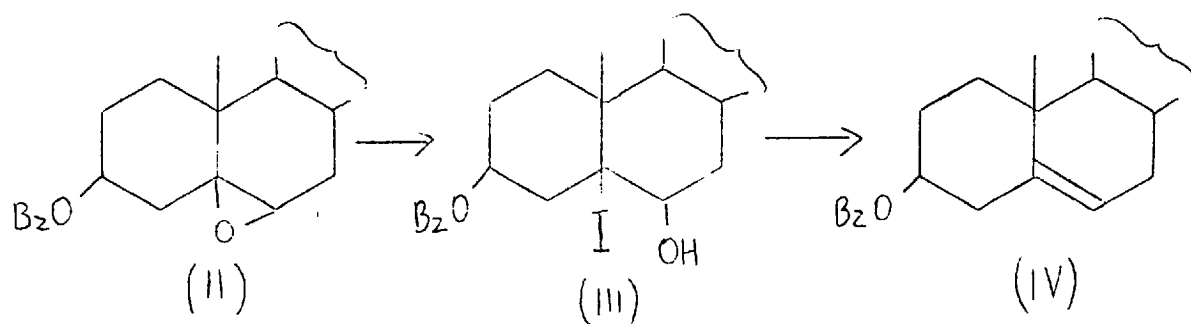
to show that the unsaturated lactone in desoxylimonin has been derived from a reduction of an epoxide α - β to the carbonyl group of lactone D in limonin:

(i) The occurrence and course of the merolimonol reaction (see section 8) requires that an epoxide of the postulated degree of substitution be present in the lactone D of limonin.

(ii) Chromous chloride is a specific reducing agent for epoxides α - β to a carbonyl group⁴⁴, the following mechanism being postulated for the reaction:



(iii) Epoxides are also known to be reduced by hydroiodic acid⁴⁵. E.g. it was shown that cholesteryl benzoate β -oxide (II) may be converted to the iodohydrin (III) and thence to cholesteryl benzoate (IV) by treatment with hydroiodic acid in chloroform.



From the structure (I), it would appear possible to epoxidise desoxylimonin (or its tetrahydro derivative) back to limonin (or tetrahydrolimonin) respectively. Numerous attempts to react tetrahydrodesoxylimonin with either perbenzoic or peracetic acids were made both by Pradhan⁴³ and in the course of the present work, but in all cases the starting material was recovered unchanged.

b. Isomerisation of tetrahydrolimonin with acid

Treatment of tetrahydrolimonin (V), $C_{26}H_{34}O_8$ with a mixture of concentrated hydrochloric acid and acetic acid, yielded⁴³ an isomeric substance, which will be referred to as iso-tetrahydrolimonin. The ultra-violet spectrum of iso-tetrahydrolimonin showed a maximum at 257 m μ (ϵ :5,500) which shifted to 290 m μ (ϵ :4,200) in the presence of alkali. The neutral monoacetate showed a maximum at 218 m μ (ϵ :11,000).

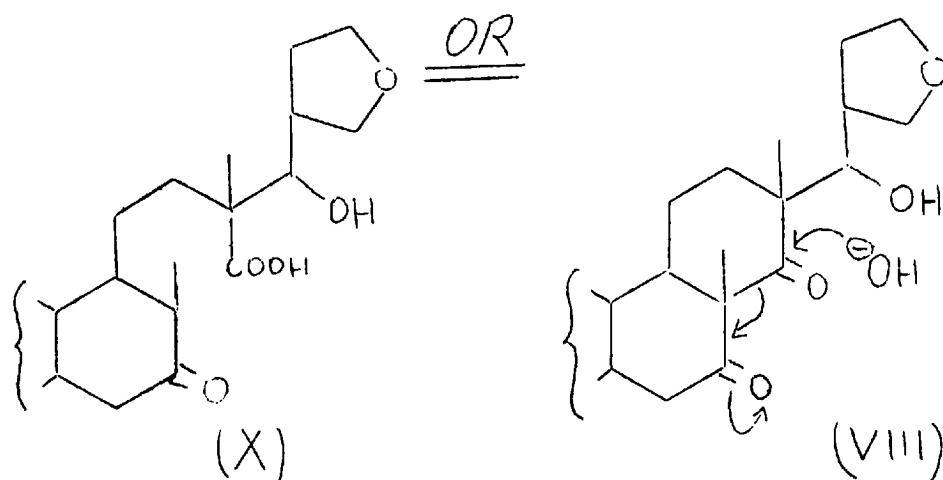
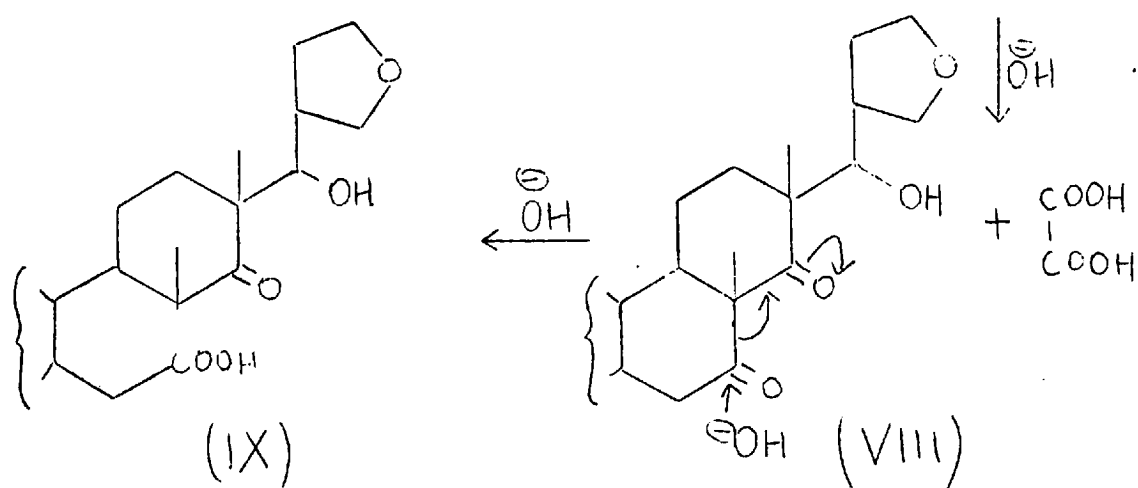
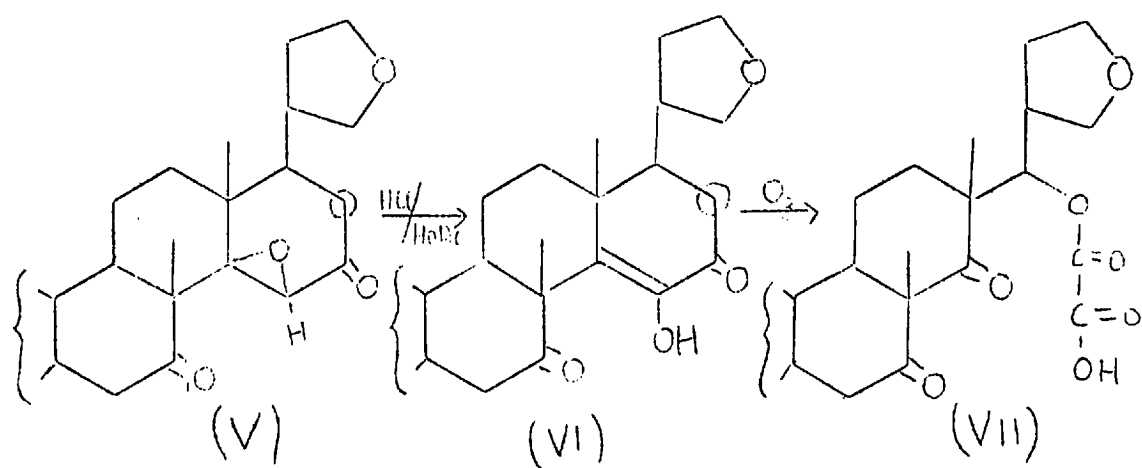
iso-Tetrahydrolimonin is an enolic substance, i.e. it is soluble in dilute alkali but not in sodium carbonate solutions and gives a positive ferric chloride test. The infrared spectrum of iso-tetrahydrolimonin shows a band at 3250 cm^{-1} (enolic hydroxyl).

In the course of the present work, iso-tetrahydrolimonin was ozonised and treated with alkali to give oxalic acid and an amorphous acidic material containing two carboxylic (and/or lactonic) groups.

Pradhan⁴³ demonstrated that the reaction leading to iso-tetrahydrolimonin involves the epoxide because desoxytetrahydrolimonin (where the epoxide had been reduced to a double bond, see section 2a) can be recovered unchanged from a mixture of concentrated hydrochloric and acetic acids.

On the basis of the above data, iso-tetrahydrolimonin was assigned the structure (VI). The amorphous acidic product of ozonolysis and hydrolysis of (VI) is probably a mixture of (IX) and (X) which are likely hydrolysis products of an intermediate β -diketone (VIII), the expected major hydrolysis product of the primary ozonolysis product (VII).

The acid isomerisation of tetrahydrolimonin provided evidence for the presence of an epoxide α - β to the carbonyl of lactone D and for the presence of a hydrogen at C₁₅ (see section 2a).



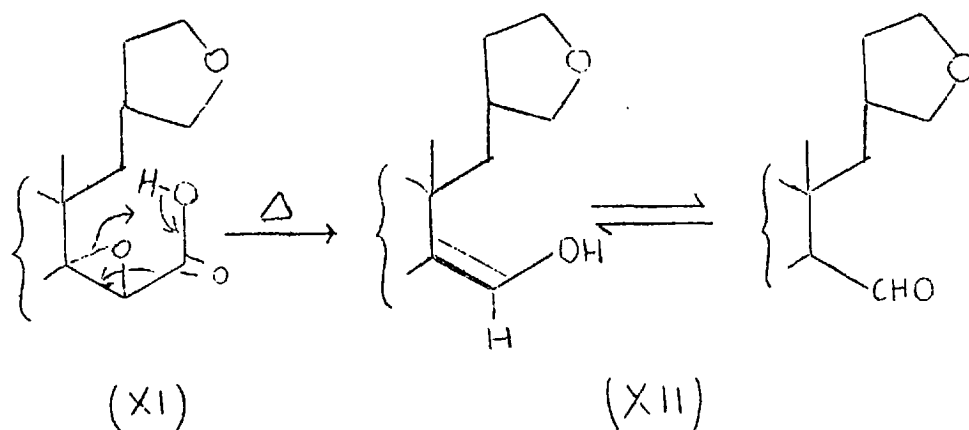
Earlier work³⁹ indicated that limonin itself undergoes an analogous reaction in the presence of concentrated hydrochloric acid and acetic acid. An amorphous product was isolated whose ultraviolet spectrum showed a maximum near 212 m μ (ϵ : 7,000 , furan) and at 258 m μ (ϵ :9,700). The latter maximum shifted to 291 m μ (ϵ :8,000) in presence of alkali. The infrared spectrum showed bands at 3400cm⁻¹ (hydroxyl), 1743 cm⁻¹ (δ lactone) , 1712 cm⁻¹ (α - β unsaturated lactone and ketone) , 1607 cm⁻¹ (conjugated system) and furan bands. Unfortunately no crystalline derivatives could be obtained.

c. Decarboxylation of hexahydrolimoninic acid

Hexahydrolimoninic acid (XI) , C₂₆H₃₆O₈ (see section 1b) loses carbon dioxide on heating to about 240°C to give a neutral compound²³. Bis-oxime and bis-dinitrophenylhydrazone derivatives^{40,46} of this compound have been prepared, showing that a new carbonyl group has been generated.

The pyrolytic decomposition of an α -oxy acid to give a new carbonyl function has been interpreted as evidence for the presence of an α -glycidic

system ⁴⁷ in lactone D of limonin. The course of the reaction is envisaged as follows:



The only evidence for the presence of an aldehydic function in the decarboxylated hexahydrolimoninic acid (XII) was obtained from its NMR spectrum⁴⁶ but this result has been disputed⁴⁰. It is possible, however that group migration may have taken place to give a ketonic rather than an aldehydic carbonyl function⁴⁷.

3. THE REACTIONS OF THE CARBONYL GROUP

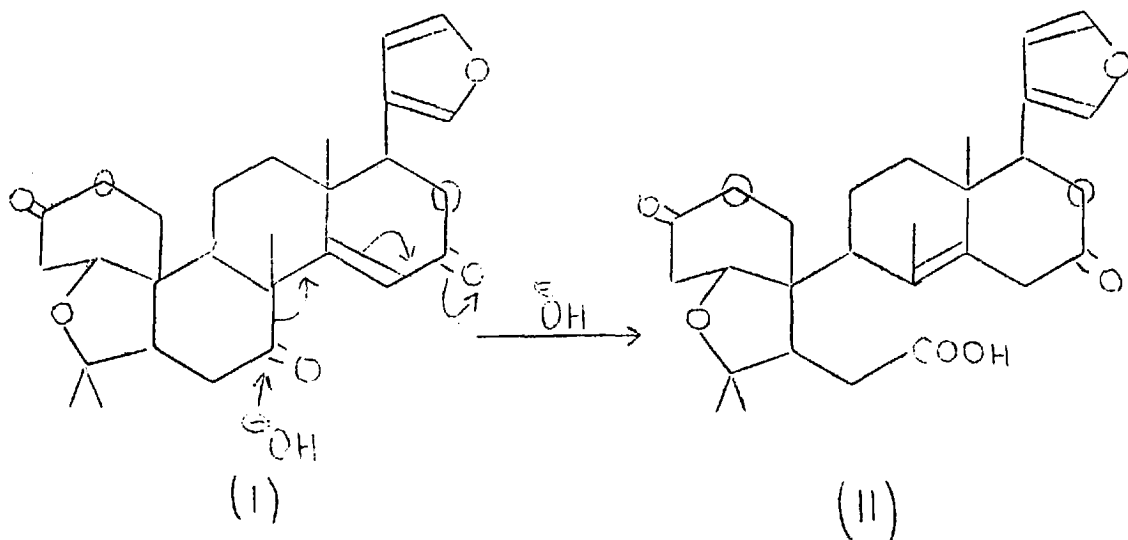
The presence of a ketonic carbonyl in limonin is indicated by the band at 1709cm^{-1} in the infrared and a maximum at $285\text{m}\mu$ ($\epsilon:33$) in the ultra-violet spectrum. These suggest that the ketone is present in a six membered (or larger) ring.

Although the typical derivatives of the ketonic carbonyl group proved difficult to crystallize, the dinitrophenylhydrazone²⁶ and the oxime^{26,29} were eventually prepared. The carbonyl group can also be reduced with aluminium iso-propoxide²⁶ or with either sodium borohydride³⁵ or sodium amalgam³⁰ to give two isomeric alcohols limonol and epi-limonol, thus confirming that the ketone group of limonin was present in a ring. The ease of acetylation of epi-limonol and the choice of the reagents used for its preparation indicate that epi-limonol is the equatorial and limonol the axial derivative. Both can be oxidised back to limonin with chromic oxide in pyridine³⁵.

4. DESOXYLIMONIC ACID

The reaction of desoxylimonin with alkali described below, gave an indication of the relative position of the ketonic carbonyl group with respect to the lactone D . This was also independently confirmed by the merolimonol reaction (see section 3).

Treatment of desoxylimonin (I), $C_{26}H_{30}O_7$, (see section 2a) with aqueous alkali, gave a dilactone carboxylic acid⁴³ (II), $C_{26}H_{32}O_9$ which no longer possessed a ketonic function. The ultraviolet spectrum of this product, which was named desoxylimonic acid, showed no maximum near 214 m μ but had instead a strong end absorption indicating the presence of an isolated double bond. The course of the reaction may be envisaged as follows:

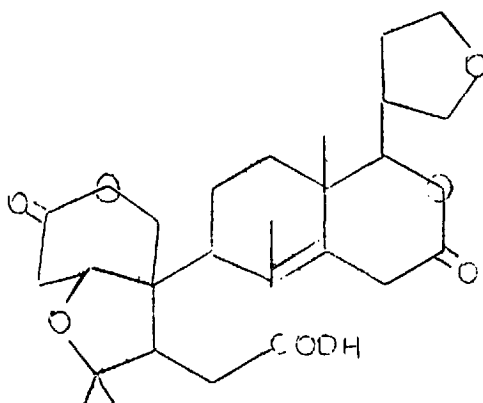


It can be seen that the reaction is in fact an "acid" hydrolysis of a vinylogue of a β -keto ester. The mechanism, which requires both the presence of a carbonyl and an α - β unsaturated lactone, is confirmed in that neither limonin nor desoxylimonin oxime undergo the above reaction in the presence of alkali⁴³.

It is well known that the occurrence of an "acid" hydrolysis of a β -keto ester is governed by the relative ease of attack of the hydroxyl ions on the carbon carrying the ketonic function and the hydrolysis of the ester group. In the case of the desoxy derivative of limonilic acid (see section 11), the carbon carrying the ketonic function appears to be sterically hindered by the presence of the α -oxygen, and thus desoxylimonilic acid was recovered unchanged⁴³ from treatment with alkali.

In the course of the present work, the tetrahydro analogue of desoxylimononic acid was prepared by the action of alkali on tetrahydrodesoxylimonin, by hydrogenation of desoxylimononic acid and by the action of alkali on tetrahydrodesoxyiodoacetate (see section 7). The properties of this compound were in full agreement with the structure expected (III), i.e.

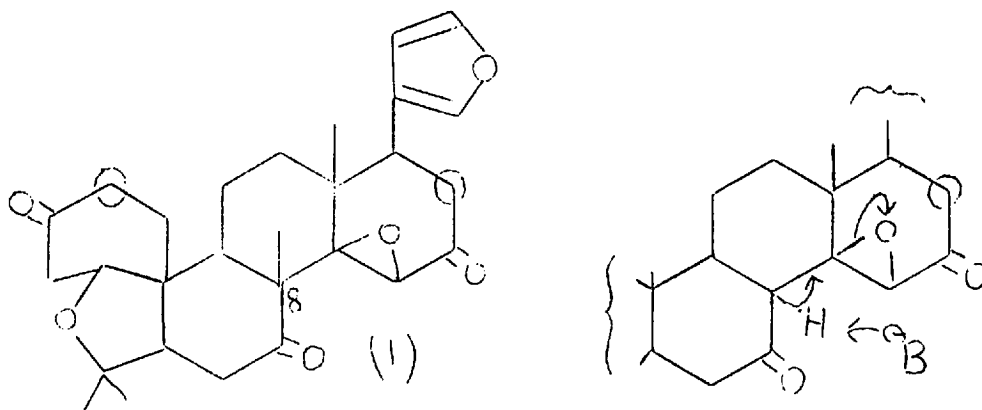
it was a dilactone carboxylic acid and its ultra-violet spectrum showed no maximum but a strong end-absorption (ϵ :10,000 - 11,000) at 202m μ . The infrared spectrum showed diffuse bands in the 3500-3000 cm^{-1} region (carboxylic hydroxyl), 1755 cm^{-1} (δ lactone A), 1725 cm^{-1} -1715 cm^{-1} (δ lactone D and carboxylic acid) and no furan bands,.



(III)

5. DEGRADATION OF TETRAHYDRODESOXYLIMONIC ACID

Pradhan⁴³ proposed that the position C₈ in the structure of limonin (I) could not have a hydrogen substituent, as such a molecule would be expected to undergo β -elimination in the manner indicated below on treatment with alkali to give a γ -hydroxy- α - β unsaturated ketone, while limonin was known to be stable to refluxing 6% methanolic potassium hydroxide.⁴³

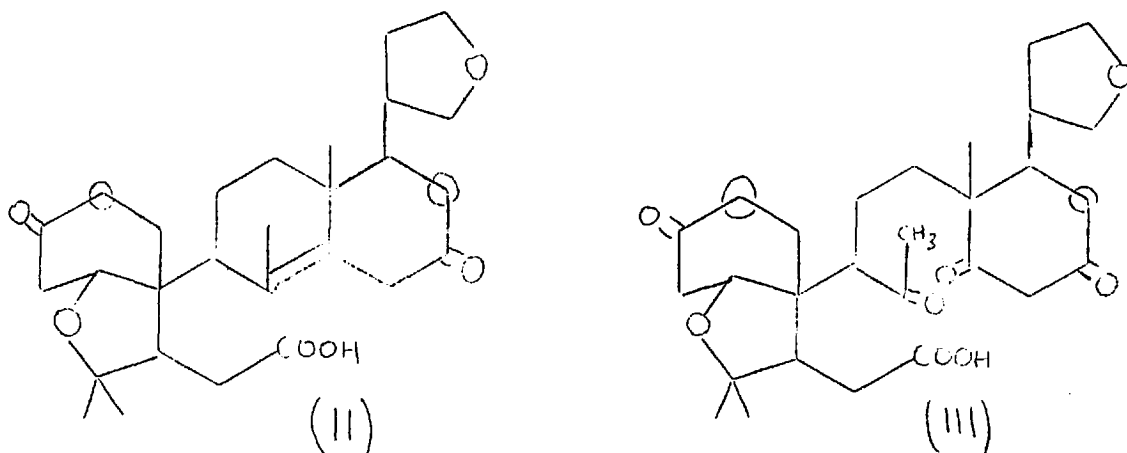


In the course of the present work, it was shown by degradation of tetrahydrodesoxylimononic acid (see section 4) that C₈, in fact, bears a methyl substituent.

a. Ozonolysis

Ozonolysis of tetrahydrodesoxylimononic acid (II), C₂₆H₃₆O₈, yielded an amorphous product whose

ultraviolet spectrum showed a maximum at 273m μ in the presence of alkali (ϵ :5,500) and which gave an immediate iodoform test. These properties are entirely consistent with structure (III):

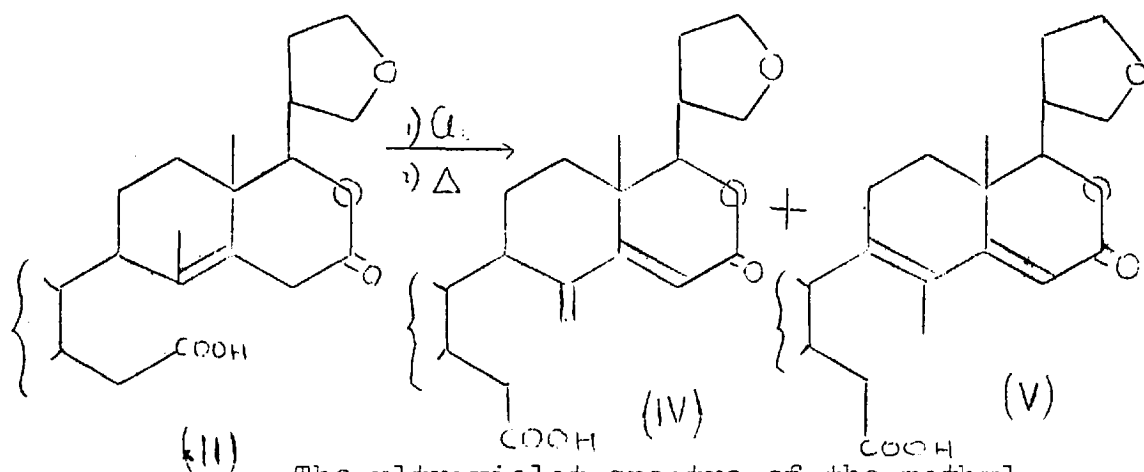


b. Chlorination and dehydrochlorination

Although tetrahydrodesoxylimonic acid can be recovered unchanged from solutions of bromine in either carbon tetrachloride or acetic acid, chlorination by a solution of chlorine in acetic acid followed by heating under moderate vacuum at 100°C, yielded two isomeric products, the cisoid (IV) and transoid (V) diene acids, C₂₆H₃₄O₈.

No chlorine containing material could be isolated in pure state, but a study of the ultraviolet spectra of the material during the course

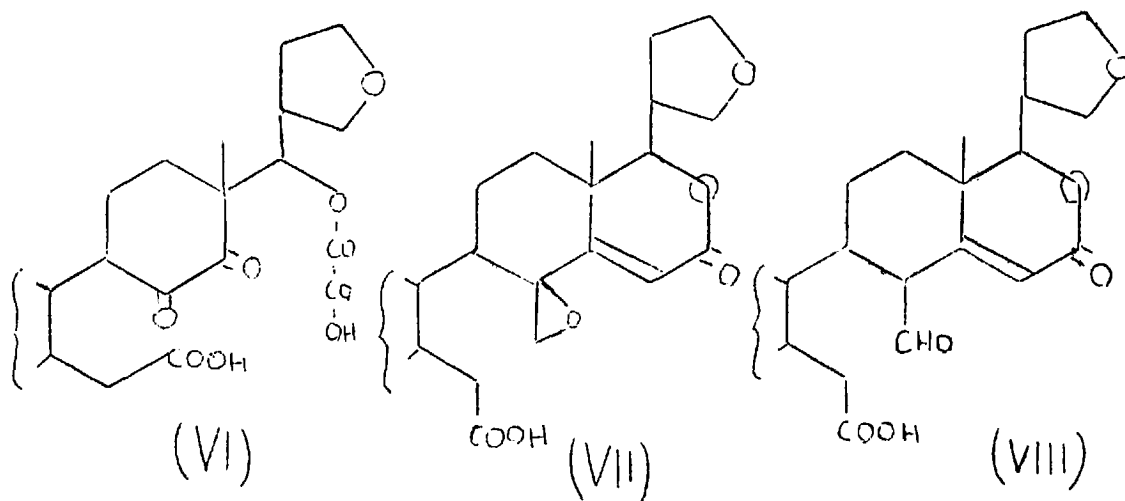
of thermal dehydrochlorination indicated that the double bond conjugated with the carbonyl group of lactone D was formed first. The ultraviolet spectrum of the cisoid diene acid showed a broad maximum at 255 mμ (ϵ :7,800) while that of the transoid diene acid had a maximum at 284 mμ (ϵ :16,400) and a subsidiary maximum at 230 mμ (ϵ :6,300). The infrared spectrum of the cisoid isomer showed two bands at 1620 and 1603 cm^{-1} , that of the transoid isomer had a single band at 1594 cm^{-1} .



The ultraviolet spectra of the methyl esters of both isomers were nearly identical with those of the parent acids. The relative intensities and positions of all the relevant spectroscopic features agree well with the structures (IV) and (V).

Ozonolysis of (IV) yielded significant

quantities of formaldehyde in confirmation of the presence of an exocyclic double bond. The method of estimation of formaldehyde was checked by using blanks and pyrethrosin⁴⁸. The main ozonolysis product could not be obtained in a crystalline form, but its ultraviolet spectrum in the presence of alkali (λ_{max} :384 m μ , ϵ :2000) indicated that the expected enolisable product (VI) could be responsible, although the position of the maximum was at a somewhat high wave length.



Treatment of the cisoid diene acid (IV) with peracetic acid, gave an oxide , $\text{C}_{26}\text{H}_{34}\text{O}_9$, whose ultraviolet spectrum had a maximum at 217-8 m μ (ϵ :9,000) showing that an α - β unsaturated lactone system was present, i.e. that the epoxide formed on the exocyclic

double bond in accordance with the structure (VII). On treatment with boron trifluoride, (VII) yielded an amorphous material whose ultraviolet spectrum showed a maximum at 390 m μ (ϵ :2000) in presence of alkali. This material could be an enolisable aldehyde of structure (VIII).

Chlorination of the cisoid diene acid (IV) resulted in the uptake of one mole of chlorine to give an amorphous product containing an α - β unsaturated lactone grouping.

Further evidence for the presence of a methyl group at C₈ was obtained from proton magnetic resonance spectra. It was found that while tetrahydro-desoxylimonic acid (II) had no peaks in the region associated with vinylic hydrogen, the cisoid diene acid (IV) had three such peaks (τ : 4.60, 4.38, 3.97 ppm) in this region.

The above evidence leaves little doubt about the presence of a methyl at C₈ and of at least one hydrogen atom at C₉.

The predominant formation of the energetically unfavourable cisoid structure can be readily explained by postulating that the intermediate chloro-

compound is either wholly or partially in a configuration where the chlorine at C₈ and the hydrogen at C₉ are in relative positions unfavourable to dehydrohalogenation.

Tetrahydrodesoxylimonic acid remained unaffected by selenium dioxide, perphthalic acid . peracetic acid and strong mineral acid. Some of this indicates a high degree of hindrance in the region of the tetra-substituted double bond, which is quite apparent on construction of a model.

6. SOME TRANSFORMATIONS OF HEXAHYDROLIMONINIC ACID

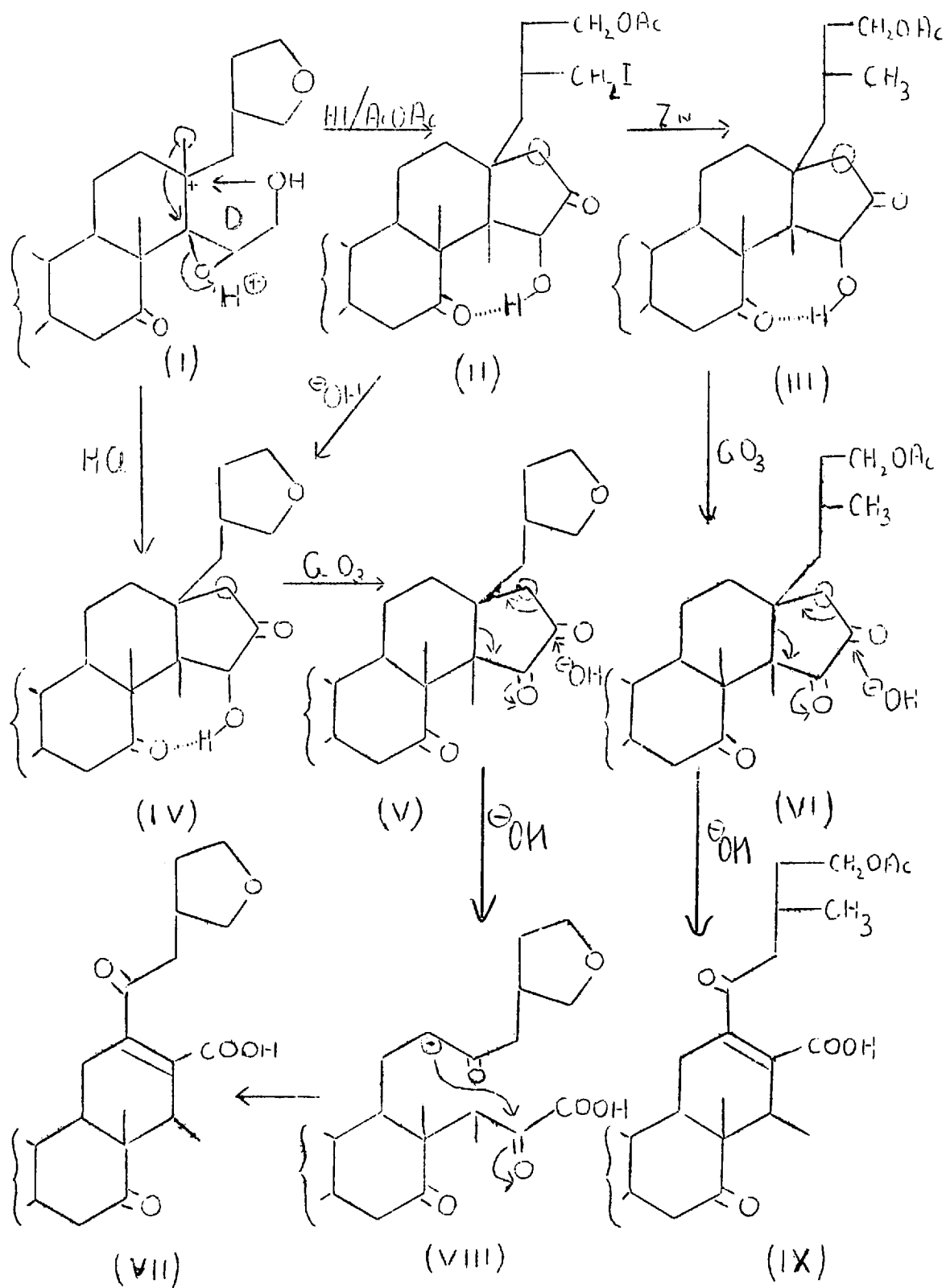
The reaction of hexahydrolimoninic acid with hydroiodic acid and acetic anhydride in acetic acid and also with hydrochloric acid in dioxane was first described by Pradhan⁴³ and was repeated and extended in the course of the present investigation.

Hexahydrolimoninic acid (I) , $C_{26}H_{36}O_8$, yields a neutral iodo-monoacetate (II) , $C_{28}H_{39}O_9I$, by the action of hydroiodic acid and acetic anhydride in acetic acid. The infrared spectrum of this compound indicated that it contained a γ lactone and a hydroxyl group , as well as a displaced ketonic carbonyl (bands at 1789,1755,3300 and 1677 cm^{-1}). The ultraviolet spectrum showed a peak at 290 m μ (ϵ :26) , showing that the ketonic function was retained and that no α - β unsaturated carbonyl group had been introduced. On treatment with zinc , the iodo-monoacetate (II) gave a desiodo compound (III) , $C_{28}H_{40}O_9$, whose infrared spectrum was very similar to that of (II). By the action of alkali , (II) lost iodine and acetyl to give a neutral isomer of hexahydrolimoninic acid (IV) whose infrared spectrum retained bands at 1789,3300 and 1686 cm^{-1} .

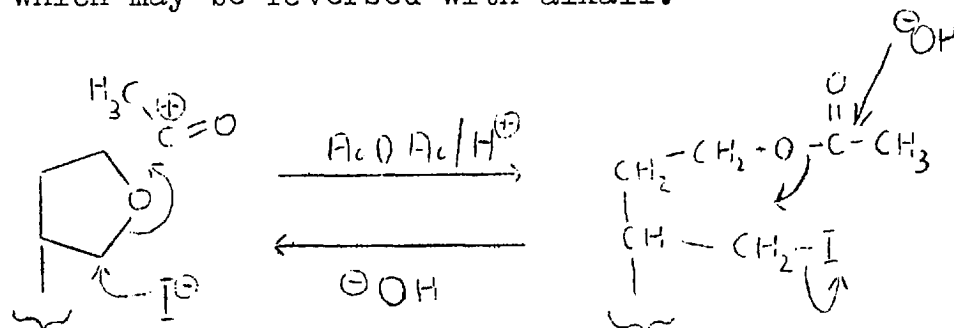
The action of concentrated hydrochloric acid in dioxane on (I) gave (IV) directly. The presence of free hydroxyl groups in (III) and (IV) was confirmed by active hydrogen determinations and by oxidation with chromic acid and pyridine, when analogous α -keto γ -lactones (V), $C_{26}H_{34}O_8$, and (VI), $C_{28}H_{38}O_9$, were obtained. The infrared spectra of (V) and (VI) no longer showed any bands in the O-H stretching region, but had bands near 1790 cm^{-1} (γ lactone), 1775 cm^{-1} (non enolised cyclopentanone α -to lactone carbonyl) and 1720 cm^{-1} (non hydrogen-bonded ketone).

The lack of evidence for an enolic grouping in (V) and (VI) requires C_{14} to be tertiary in these compounds, and by implication also in (II), (III) and (IV), as α -keto- γ -lactones with a hydrogen in the β position are fully enolised^{49,50}.

The fact that limonin does not undergo an analogous reaction with acids indicated that the hydrogenolysed ring D lactone and/or the tetrahydro furan ring were participating.



The introduction of iodine and acetate is considered to be an opening of the tetrahydrofuran ring, which may be reversed with alkali:

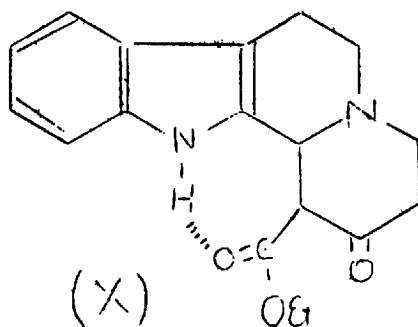


An analogous reaction takes place with tetrahydrodesoxylimonin (see section 7). The relative positions of the acetate and iodine in (II) were not investigated and the order shown in (II), (III), (VI) and (IX) is arbitrary.

The principal reaction (I) to (IV) was postulated to involve an opening of the epoxide by protonation, followed by methyl migration from C₁₃ to C₁₄ and the closing of a γ lactone on the C₁₃ carbonium ion. No exact analogy could be found in the literature.

The abnormally low carbonyl frequencies in (II), (III) and (IV) are probably due to intramolecular hydrogen bonding with the hydroxyl group at C₁₅. Although a seven membered ring is involved,

this hypothesis receives strong support from the observation that the abnormally low carbonyl band does not appear in the spectra of (V) and (VI) where the hydroxyl group is oxidised to a ketone, or in the spectrum of an acetate of (IV) which was also prepared. The bands near 1680 cm^{-1} cannot be due to an α - β unsaturated carbonyl function as neither (III) nor (IV) show any strong absorption in the ultraviolet. Intermolecular hydrogen bonding is unlikely, as the infrared spectrum of (IV) was the same in chloroform solution as in Nujol mull. An ana-



logy for a strong hydrogen bonding effect involving a 7 membered ring can be found in the infrared spectrum of (X)⁵¹, which shows bands at 1709 cm^{-1} (ketone) and 1695 cm^{-1}

(hydrogen bonded ester). The abnormally low ester band cannot be due to an enolic structure, as a crystalline enolic form of (X) was isolated independently.

The α -keto- γ -lactones (V) and (VI) react with alkali to give amorphous acidic products with

very similar ultraviolet spectra. The acid derived from (V) shows a maximum at 244 m μ (ϵ : 3,800) in the ultraviolet and bands near 1755 (δ lactone), 1715 (carboxylic acid) and 1670 cm⁻¹ (α - β unsaturated ketone) in the infrared. The nature of the products could not be established as no crystalline derivative was obtained, but the structure (VII) derived from (V) via the intermediate (VIII) offers an interesting hypothesis, apparently in full agreement with the facts available and involving a plausible mechanism. Some support for the structure (VII) can be derived from the fact that the intensity of the band near 250 m μ can be considerably reduced by boiling with zinc in acetic acid, while the ultraviolet spectrum was unaffected by the action of alkaline hydrogen peroxide, indicating the absence of an α -keto carboxylic acid.

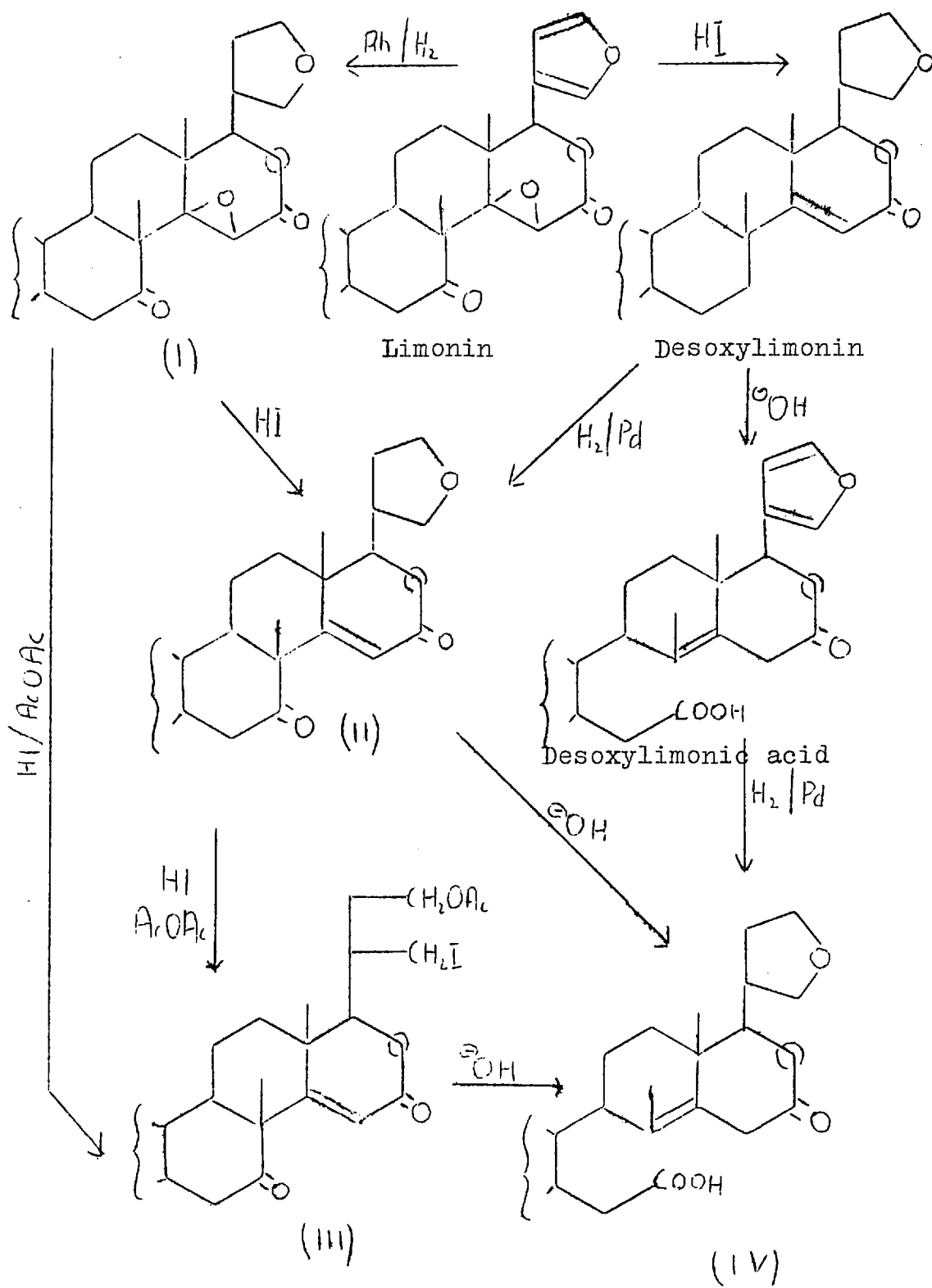
Structure (IX) represents the analogous product obtained by treatment of (VI) with alkali.

7. IODOACETYLATION OF TETRAHYDROLIMONIN

Pradhan⁴³ obtained a neutral iodoacetate by the action of hydroiodic acid and acetic anhydride in acetic acid on either tetrahydrolimonin (I) or tetrahydrodesoxylimonin (II) (see section 2a).

In the course of the present work it was established that this iodomonoacetate (III), $C_{28}H_{37}O_8I$, could be converted to a dilactone carboxylic acid, tetrahydrodesoxylimonic acid (IV) (see section 4). The ultraviolet absorption spectrum of (III) shows a maximum at 219 mμ (ϵ :12,700), which, together with the fact that it was also obtained from tetrahydrodesoxylimonin (II), indicates that it contains an α - β unsaturated lactone system in the ring D.

The structure of the acid (IV), was established independently (see section 4) and thus it becomes apparent that (III) differs from (II) only by the opening of an oxide ring to give an iodoacetate, the oxide being regenerated by the action of alkali. By analogy with the reactions described in section 6, the tetrahydrofuran is probably involved. Again, the relative positions of iodine and acetyl are arbitrary.



8. MEROLIMONOL AND ITS DERIVATIVES

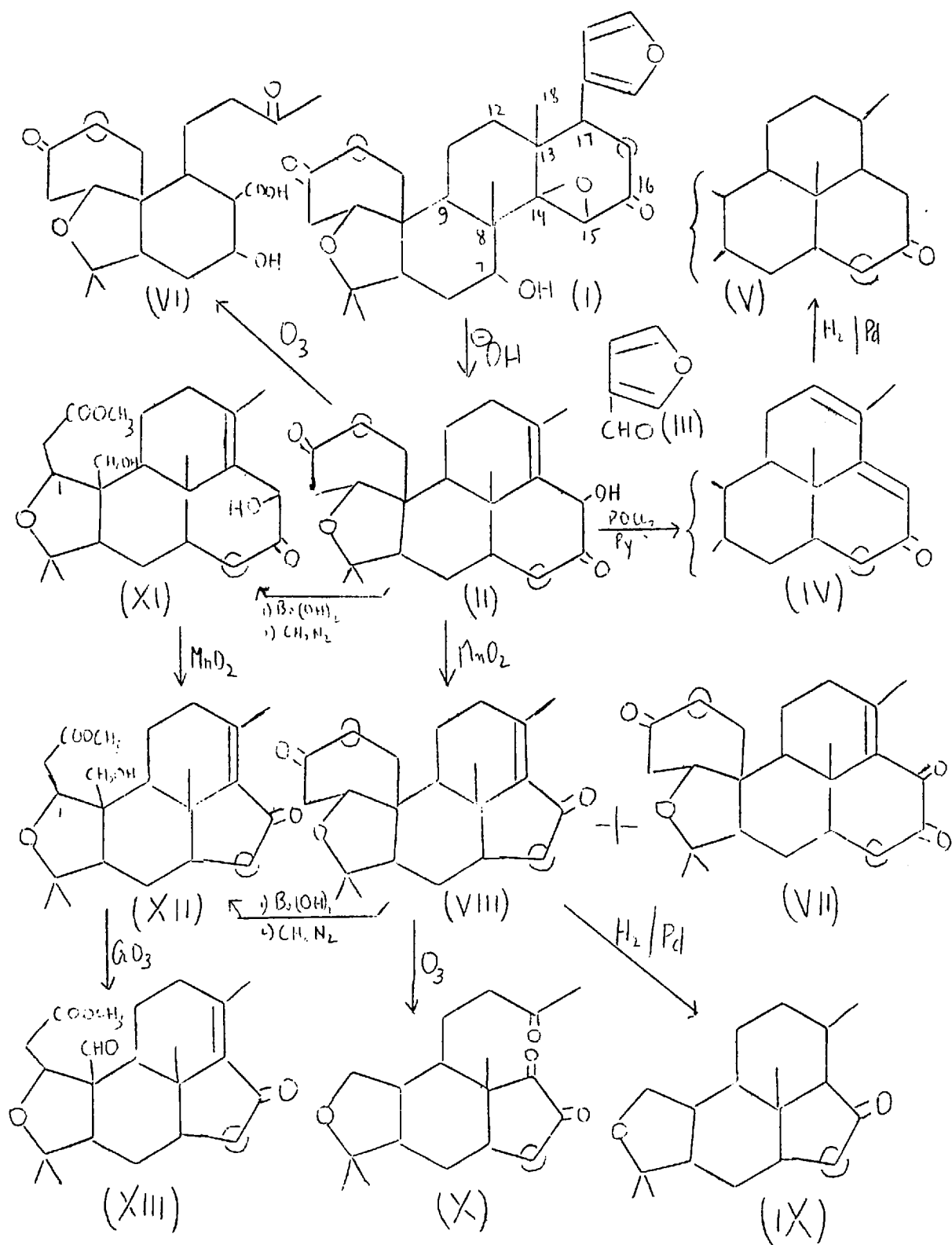
The work summarized in this and the following section was performed almost entirely by the Zurich group⁴⁶, although contributions to the chemistry of merolimonol were also made by Corey⁴⁰. Some of the earlier results in this series have already been published³⁵.

Limonol(I) (see section 3), but not the epimeric epi-limonol, reacts with alkali to give merolimonol (II) and a smaller fragment identified as furan-3-aldehyde (III). Merolimonol, which was further characterised by its monoacetate, can be easily dehydrated to a doubly unsaturated conjugated lactone (IV) which may be converted to a tetrahydro derivative (V). Merolimonol is cleaved by ozonolysis to give the keto-acid (VI), characterised by its methyl ester, methyl ester acetate and methyl ester oxime. (VI) Gives a positive iodoform test, thus proving the presence of a methyl substituent at C₁₃. Oxidation of merolimonol with manganese dioxide in benzene solution gives the expected ketone (VII) and a decarboxylated derivative (VIII), whose

infrared and ultraviolet spectra show the presence of a δ lactone and of an α - β unsaturated γ lactone. Hydrogenation of (VIII) gives the saturated γ lactone (IX) which could also be obtained by hydrogenating merolimonol to dihydromerolimonol and oxidising with pyridine/chromic oxide. Ozonolysis, or alternatively hydroxylation followed by leadtetraacetate cleavage, of (VIII) affords the α -keto- γ -lactone (X) which is a methyl ketone, thus confirming the presence of a methyl group at C₁₃. Furthermore, (X) is a non-enolisable α -keto- γ -lactone^{49,50}, thus showing that C₈ cannot have a hydrogen substituent (see sections 5 and 6).

The above series of reactions provide independent evidence (see also section 4) for the relation between C₁₄, C₁₅ and C₁₆ with C₇ and C₈, and also for the relation of C₁₂ and C₁₃ with C₁₄ etc.

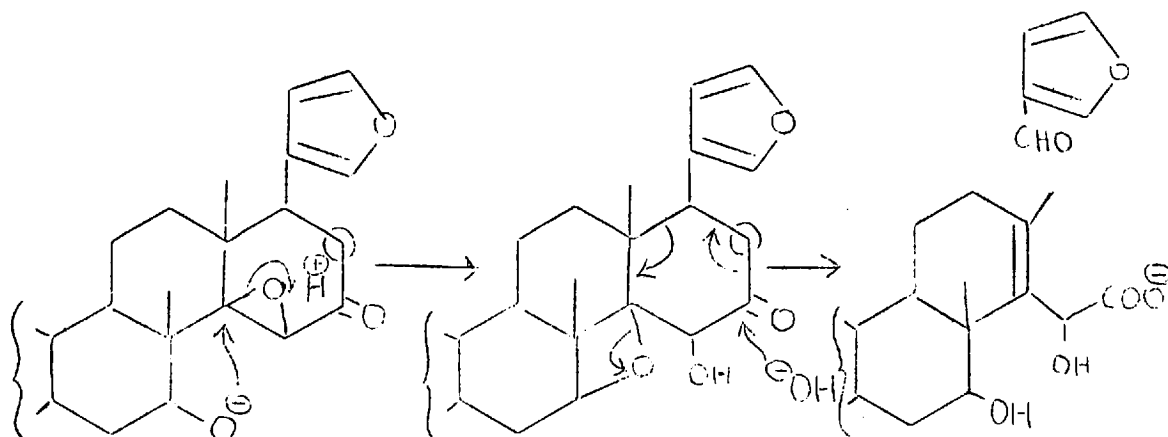
When merolimonol is treated with barium hydroxide under the conditions of formation of limoclastic acid (section 9), it yields an amorphous acid which has been characterised by its crystalline methyl ester (XI). This acid cannot be lactonised, in contrast with the behaviour of limonin. This phenomenon



can be explained by postulating that an inversion can occur at C_1 in the course of a reversible β -elimination and addition of the ethereal oxygen of ring A'. (see section 9). Oxidation of (XI) with manganese dioxide gives a γ -lactone (XII) which may also be obtained by baryta cleavage of (VIII) followed by esterification with diazomethane. The hydroxyl function of (XII) can be converted to an aldehyde (XIII) thus showing the position of the terminus of lactone A (see also sections 9, 10 and 11).

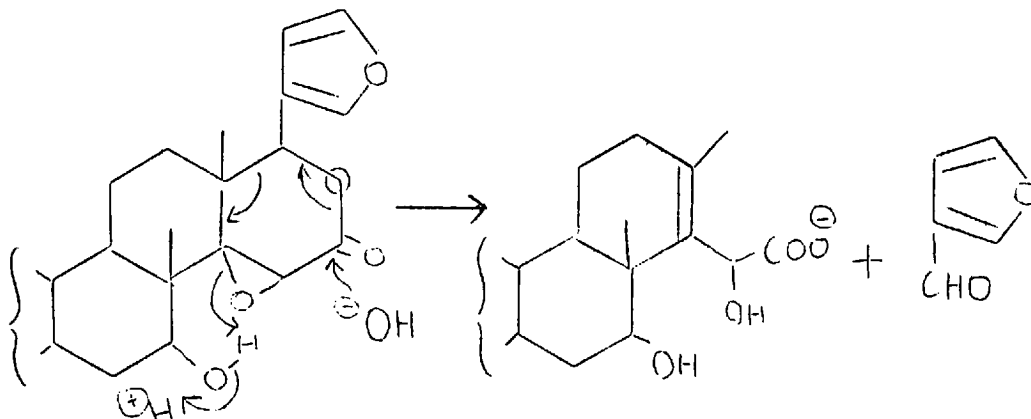
Two mechanisms have been suggested to explain the formation of merolimonol from limonol.

In mechanism (i) below:



the epoxide is opened by a displacement effected by the oxygen of limonol.

In the alternative mechanism (ii):



the epoxide is opened by hydrogen bonding with the hydroxyl of limonol. In both cases alkaline cleavage results in the loss of a five carbon fragment and the production of a hydroxy acid which lactonises on acidification to give merolimanol.

Of the two mechanisms , (i) appears preferable , as a study of a model reveals that an axial hydroxyl group at C₇ is well positioned for this type of displacement , while the formation of a strong hydrogen bond between the epoxide and the axial C₇ hydroxyl appears unlikely.

9. ALKALINE DEGRADATION OF LIMONIN - THE LIMOCLASTIC ACID SERIES

When limonin is treated with barium hydroxide solution under relatively vigorous conditions, an acidic substance is formed of the molecular formula $C_{20}H_{28}O_6$, which was designated as limoclastic acid.

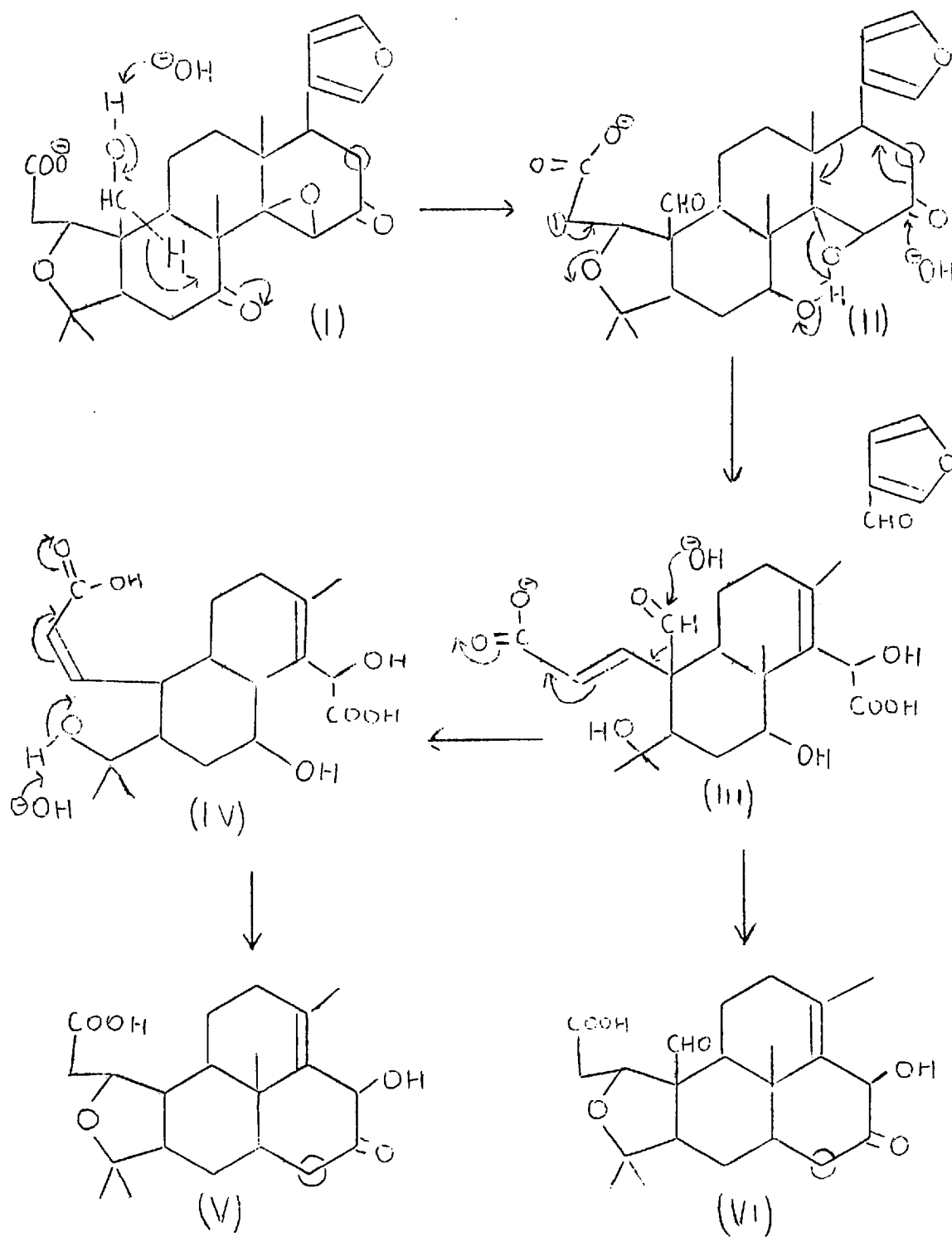
As limoclastic acid appeared to undergo all the reactions characteristic of merolimonol and its derivatives (see below and see section 8), a change analogous to the merolimonol transformation was considered as having taken place during the formation of limoclastic acid. The stoichiometry of the reaction then requires that formic acid must be lost as a minor fragment besides furan-3-aldehyde.

As a reduction of the ketone group of limonin (I) to an alcohol is necessary before a mero-limonol type of change could occur, and as the carboxylic acid group of limoclastic acid was considered to be derived from lactone A, it was reasonable to suppose that an intramolecular hydride transfer⁵² between the alcoholic group of lactone A and the ketone

has taken place, aided no doubt, by favourable stereochemical configuration. This gives a limonol-type compound (II) , in which the ether ring A' could be reversibly opened to provide an electron sink for a conventional deformylation in the form of an α - β unsaturated acid (III). Such a compound could also undergo the merolimonol change to give (IV) and finally , a re-closure of ring A' was postulated (see sections 11e and 15b) to give limoclastic acid formulated as (V). Support for the above scheme was obtained when an aldehyde corresponding in all its properties to (VI) was also isolated as a minor product of the reaction.

Limoclastic acid undergoes the following series of reactions which confirm structure (V) assigned to it and are closely analogous to the reactions of merolimonol (see section 8).

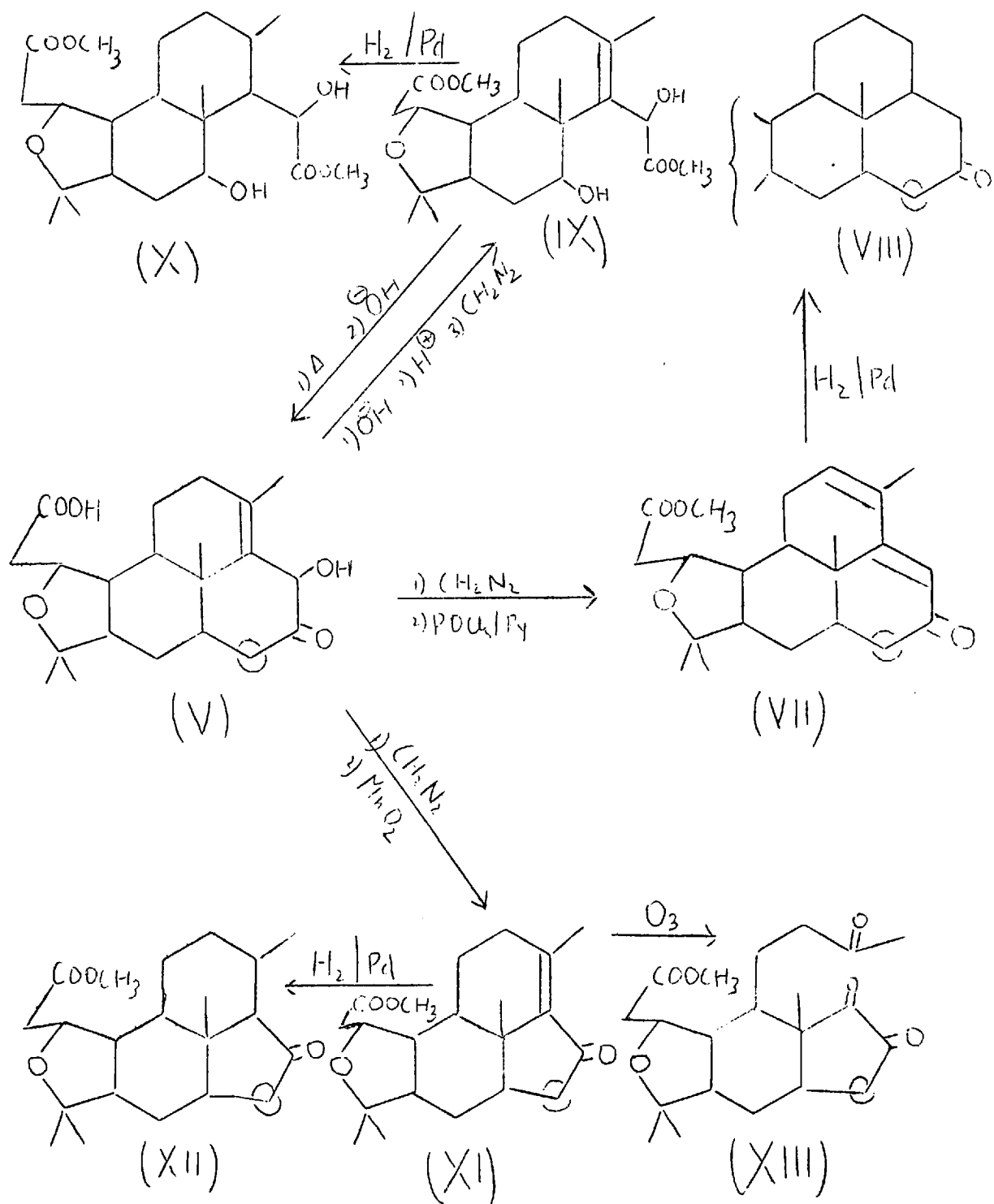
The methyl ester of limoclastic acid (V) may be dehydrated to an anhydro-derivative (VII) , which on hydrogenation followed by hydrolysis furnishes the tetrahydro acid (VIII). The monoacetate of methyl limoclastate also furnishes an analogous dihydro derivative.



Alkaline hydrolysis of limoclastic acid and esterification prior to lactonisation gives a diester (IX) , characterised as a mono-acetate, and capable of being converted to methyl limoclastate on gentle pyrolysis. Hydrogenation of (IX) yields a saturated dihydro derivative (X), characterised as a diacetate.

Oxidation of methyl limoclastate with manganese dioxide gives the nor compound (XI), which can be converted to the saturated γ -lactone (XII) by hydrogenation. Ozonolysis of (XI) gives an α -keto- γ -lactone (XIII) which gives a positive iodoform test , but does not enolise.

The chemistry of limoclastic acid confirms all the conclusions reached on the basis of the chemistry of merolimonol and in addition provides independent evidence for the nature of the alcoholic group of lactone A (primary) and - if the genesis of limoclastic acid is considered as proceeding by the mechanism outlined above - also gives a unique relationship between C_1, C_2, C_3 and C_{10} and the ether group of ring A'.

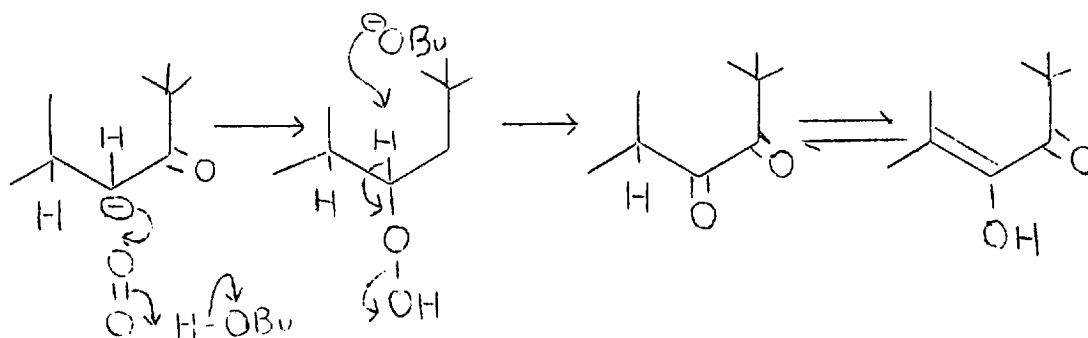


10. THE METHYLENE GROUP ADJACENT TO THE KETONE
CARBONYL - THE DIOSPHEOLS AND THEIR DEGRADATION

Limonin, tetrahydrolimonin and desoxy-limonin have been shown⁵³ to undergo analogous reactions with oxygen in the presence of potassium tertiary butoxide, resulting in the uptake of one atom of oxygen and the loss of two atoms of hydrogen. The ultraviolet spectra of the products are characteristic⁵⁴ of diosphenols, e.g. limonin diosphenol has $\lambda_{\max}$ at 282 m μ (ϵ :10,400), which changes to $\lambda_{\max}$: 335 m μ (ϵ :5,000) in the presence of alkali. The infrared spectrum, which shows bands at 3480 cm^{-1} (hydroxyl) 1748 cm^{-1} and 1736 cm^{-1} (δ lactones) and at 1689 cm^{-1} and 1661 cm^{-1} (the system $-\text{CO}-\text{C}(-\text{OH})=\text{C}$), also agree with the presence of such a feature. The diosphenols derived from limonin dissolve in sodium hydroxide solutions but not in sodium bicarbonate solutions and give positive colour tests with ferric chloride. Neutral monoacetates of the diosphenols were prepared in the usual manner and showed no hydroxyl bands in their infrared spectra. The ultraviolet spectrum of the

acetate of the diosphenol derived from limonin itself showed a maximum at 244 m μ (ϵ : 11,200).

The following mechanism has been postulated for the formation of diosphenols under the conditions described above:



This novel reactions has since been extended to other systems⁵³ containing a methylene group adjacent to a ketonic carbonyl.

The occurrence of this reaction provided evidence for the presence of a methylene group at C₆, α - to the ketone in limonin, and of at least one (from the position of the maximum in the ultra-violet, of only one) hydrogen in the β - position at C₅. Thus on the basis of the above evidence, and considering the nature of desoxylimononic acid (see sections 4 and 5), limonin should have two hydrogen

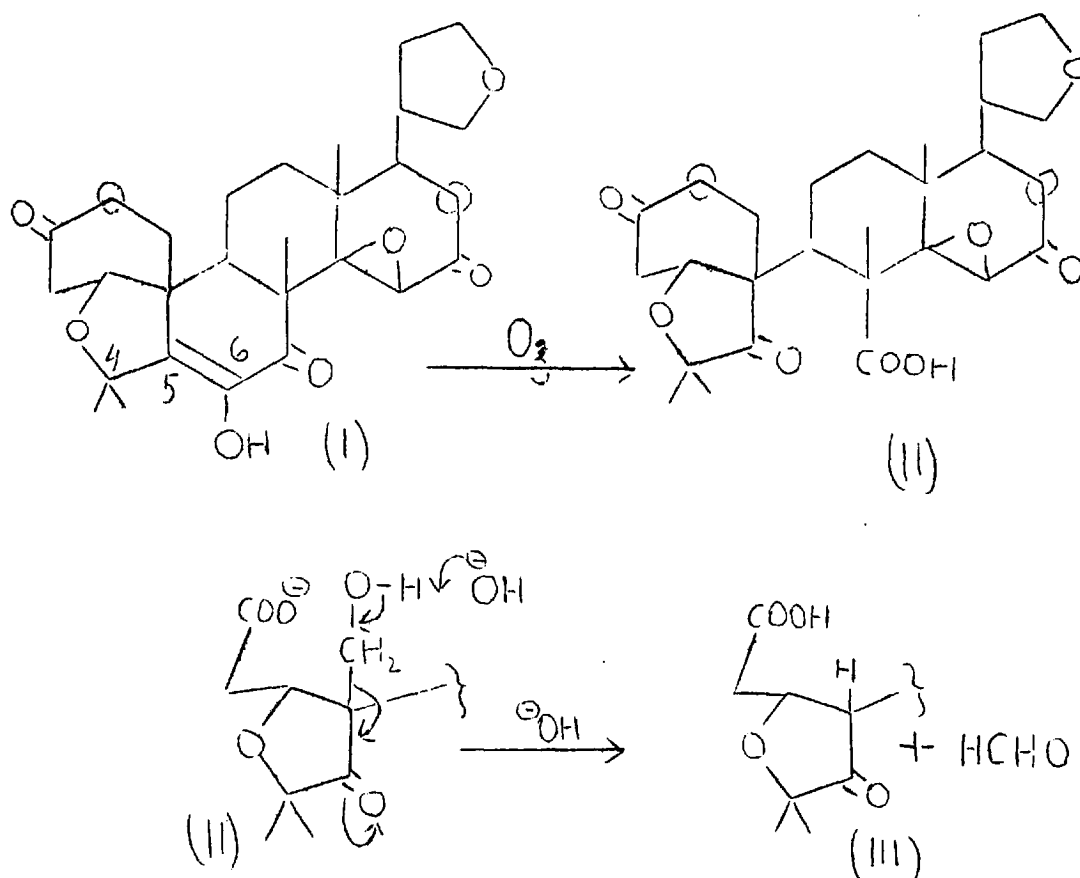
atoms activated by the presence of the ketonic carbonyl , which is in agreement with early deuteration studies^{39,40}.

The diosphenol derived from tetrahydrolimonin , which on the basis of the above was assigned the structure (I), yields a keto-acid on ozonolysis, which analyses well for (II) and is accordingly a dilactone monocarboxylic acid. The infrared spectrum (bands at 1760,1735,1725(sh) cm^{-1}) and the ultraviolet spectrum (λ_{max} :296m μ , ϵ :35) of this substance also agree with the structure assigned. The infrared spectrum of the tri-sodium salt of (II) shows a single band at 1748 cm^{-1} as compared with a band at 1690 cm^{-1} for the di-sodium salt of limonin. This can be interpreted as indicating the presence of a ketone group in a five membered ring with a negative (oxygen) substituent in the α position.

As expected, (II) reacts with zinc and acetic acid , but no crystalline derivative could be isolated.

On action of mild alkali, (II) undergoes a retro-aldol reaction ⁵⁵ by the mechanism shown below, to give significant quantities of formaldehyde

and an amorphous acid, presumably (III).



The de-formylation reaction of (II) provided evidence for the nature (primary hydroxyl) and mode of attachment of the alcoholic oxygen of lactone A (see also sections 9 ,11 and 12), although it did not exclude C_4 as a possible position for attachment of the $-CH_2OH$ group.

11. LIMONILIC ACID AND ITS DERIVATIVES

a. The structure of limonilic acid

Oxidation of limonin with potassium manganate²¹ or with iodine and alkali²⁹, yields limonilic acid, $C_{26}H_{30}O_9$, which is a monolactone monocarboxylic acid and retains the ketonic function of limonin²¹. A tetrahydro analogue has also been prepared, either by hydrogenation of limonilic acid or by the action of iodine and alkali on tetrahydrolimonin^{21,29,40,43}.

No hydroxyl groups, additional unsaturation or new carbonyl groups have been generated by the transformation of limonin into limonilic acid, as shown by the following evidence:

(i). The infrared spectrum of methyl limonilate shows no bands attributable to hydroxyl groups⁴³.

(ii). There is no evidence for the presence of double bonds in tetrahydrolimonilic acid, i.e. it does not take up hydrogen in the presence of palladium-carbon and its ultraviolet spectrum shows only very small end absorption,

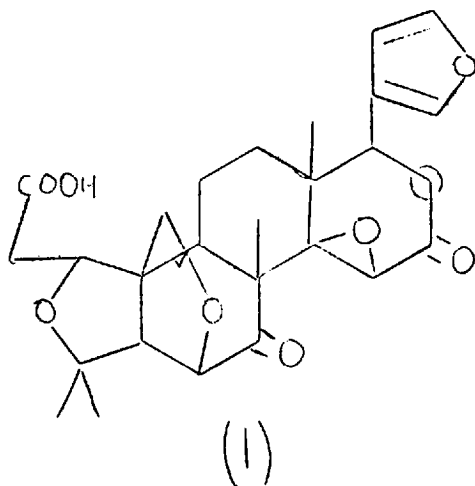
while its infrared spectrum shows no bands between 1690 and 1500 cm^{-1} ⁴³.

(iii). The ultraviolet spectrum of methyl limonilate oxime shows no carbonyl band ⁴³ and the NMR spectrum of methyl limonilate ⁴⁶ shows no aldehyde protons.

The behaviour of methyl limonilate ²⁹ on hydrogenation was analogous to that of limonin (see section 1) , a neutral tetrahydro and an acidic hexahydro derivative being formed, showing that the furan ring was not involved in the limonilic acid transformation and that the carboxylic acid group of limonilic acid was derived from lactone A rather than from lactone D. This was confirmed by Emerson ²⁹, who showed that hexahydro-limoninic acid reacted with iodine and alkali to give a diacid identical with the acidic product of hydrogenation of methyl limonilate followed by hydrolysis. The formation of a desoxy compound from limonilic acid ⁴³ (see section 2a) provides further evidence that lactone D was not involved in the formation of limonilic acid.

The ketonic group of limonilic acid is

of a different type than that present in limonin:
The ultraviolet spectrum of limonilic acid⁴³
showed a maximum at 309 m μ (ϵ :37) as compared with
limonin (λ_{max} :285 m μ , ϵ :33). In the present work,
the comparison of the infrared spectra of limonilic
acid, tetrahydrolimonilic acid, limonilyl chloride
and methyl limonilyl ketone (see below) with those
of limonin and its simple derivatives tetrahydro-



limonin and hexahydro-
limoninic acid, indicated that the carbonyl
absorption band due to
the ketone carbonyl in
limonilic acid and its
simple derivatives occurs
near 1722 cm^{-1} as compared
with 1709 cm^{-1} for limonin

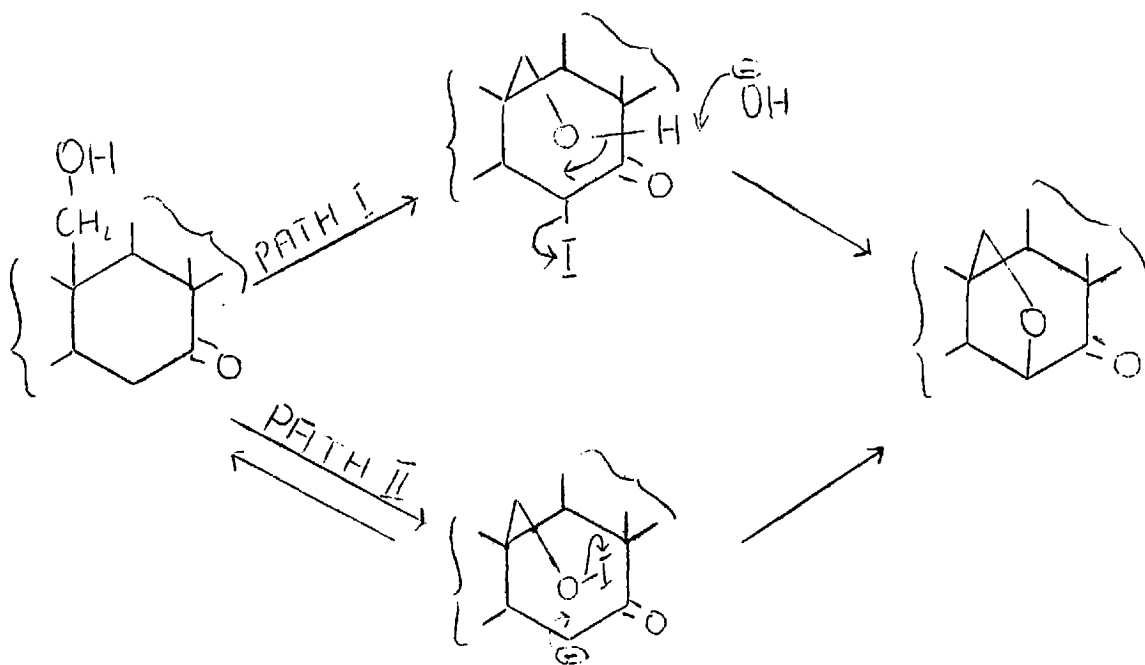
and its simple derivatives. The nature and magnitude
of these spectral changes is in accord with the pre-
sence of an α -negative (i.e. oxygen) substituent in
limonilic acid ketone. The sodium salts of limonin
and limonilic acid , where only the ketonic absorption
remains in the carbonyl region, also showed marked

differences in the frequency of the carbonyl band , which occurred at 1690cm^{-1} and 1717cm^{-1} respectively. Failure of desoxylimonilic acid to undergo the desoxylimononic acid change (see section 4) also indicates that the environment of the ketone was altered by the limonilic acid transformation , while the failure of desoxylimononic acid and of epi-limonol to react with iodine and alkali indicates that the presence of the ketonic carbonyl group is essential for the reaction⁴³. Furthermore, it was shown⁴³ that the oxime of limonilic acid was much more difficult to prepare than that of limonin and that limonilic acid does not undergo the diosphenol reaction (see section 10).

The above evidence indicates that the limonilic acid transformation involves the condensation of the hydroxyl group derived from hydrolysis of lactone A with a carbon α to the ketone group, to form a new ether ring. In view of the structure of limonin , limonilic acid may thus be represented by structure (I). Strong confirmation of this was obtained , when it was found that limonilic acid could be reduced back to limonin by the action of aluminium amalgam⁴⁶ or lithium amalgam⁵³.

This formulation of the limonilic acid transformation placed obvious steric restrictions on the structure of limonin, thus excluding many possible structures.

The limonilic acid change appears to be a novel reaction without precedent in the literature, probably made possible only by the unusual steric arrangement present in limonin. The reaction mechanism may involve either an initial iodination of the ketone (path I) or a carbanion displacement at the "alkyl hypoiodide" (path II). The latter mechanism is supported by the extreme rapidity of the reaction.



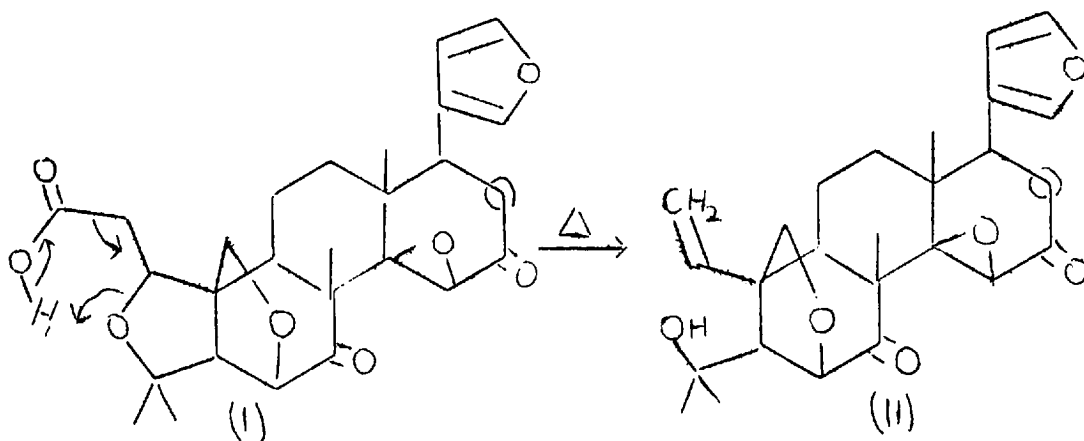
b. Rate of hydrolysis of methyl limonilate

The rate of alkaline hydrolysis of methyl limonilate relative to those of the methyl esters of some model primary, secondary and secondary α -oxygen substituted acids was measured by Templeton⁵³, sufficient precautions for complete hydrolysis of lactone D being taken. The hydrolysis rate of methyl limonilate was found to be close to those of secondary and secondary α -oxygenated esters but considerably slower than that of a primary ester. This misleading result may be due to steric hindrance by more removed groups in limonin.

c. Decarboxylation of limonilic acid

Emerson²⁹ observed that limonilic acid lost carbon dioxide at its melting point. Templeton⁵³ isolated two crystalline compounds from the action of heat alone or boiling quinoline on limonilic acid. One of these has been characterised as $C_{25}H_{30}O_7$, a neutral monolactone retaining the ketone and furan functions and in addition containing a hydroxyl group. (Bands in infrared at 3340, 1742, 1715, 1500 and 875cm^{-1} , λ_{max} : 306-12, ϵ : 43 and λ_{max} : 208, ϵ : 7,000).

The unsaturated alcohol (II) was postulated as the structure of this compound, which could be derived from limonilic acid (I) in the following manner (see also section 16):

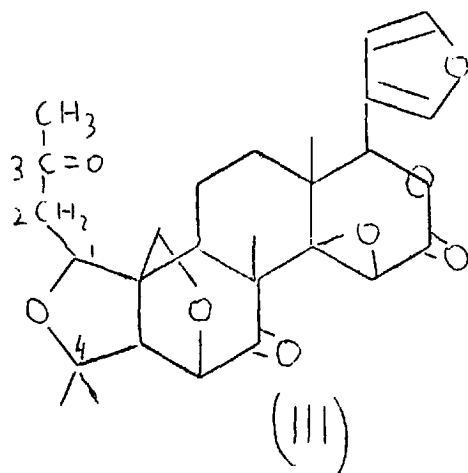


Although no direct evidence of unsaturation could be obtained (negative TNM test, lack of absorption larger than that due to furan in the ultraviolet), the compound gave a dihydro derivative on hydrogenating over palladium carbon, which retained the furan function. The hydroxyl group of (II) proved unreactive to acetylation and oxidation with chromic oxide in pyridine.

d. Preparation and properties of methyl-limonilyl ketone

A Bayer-Villager degradation of limonilic acid via methyl-limonilyl ketone (see below) was

The properties of methyl-limonilyl ketone, $C_{27}H_{32}O_8$, agree with structure (III). Thus its ultraviolet spectrum shows an extended low intensity (ϵ : approx.50) peak between 288 and 310 m μ and a high intensity peak at 207 m μ (ϵ :7,000 , furan). Its infrared spectrum



shows discrete bands at 1735 cm^{-1} (δ lactone), 1722 cm^{-1} (ketone with an α -negative substituent) and 1712 cm^{-1} (methyl ketone). The position of the new maximum in the ultraviolet and the new carbonyl band in the infrared (1712 cm^{-1}

both in Nujol and in chloroform) agree with the absence of an α - oxygen substituent at C_2 (see derivation of structure, section 13b).

Methyl-limonilyl ketone gives an instantaneous iodoform test ; the main acidic product could not be crystallized but its infrared spectrum is very similar to that of the expected limonilic acid. As expected, methyl-limonilyl ketone failed to react with zinc and acetic acid in accordance with the absence

of a new, α oxygen substituted ketone (see above). However, this piece of negative evidence is of very doubtful value, as limonilic acid itself also failed to react with zinc and acetic acid⁴³.

Although (III) would be expected to undergo β -elimination with alkali, methyl-limonilyl ketone was recovered unchanged from 1N aqueous sodium hydroxide solution after several hours heating. This can be easily explained, when the position of equilibrium between obacunonic and iso-obacunonic acids is considered (see section 15.b.iv).

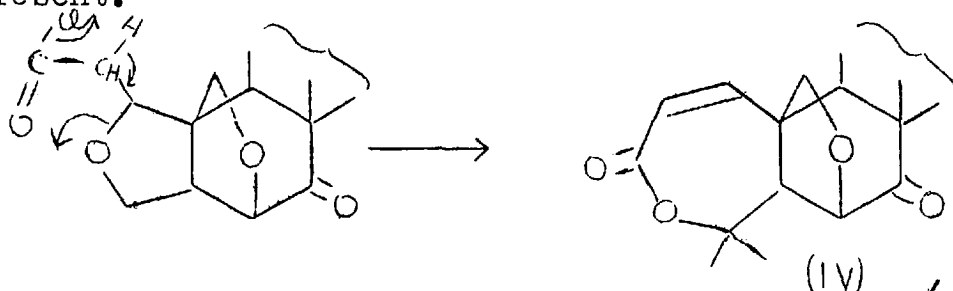
The formation of a monobenzylidene derivative was indicated by the development of absorption at 293 m μ (ϵ :3,000) in an alkaline solution of methyl-limonilyl ketone and benzaldehyde.

An alternative method of preparation of methyl-limonilyl ketone via a malonic ester synthesis was also investigated with partial success (see relevant experimental section).

e. Pyrolysis of limonilyl chloride

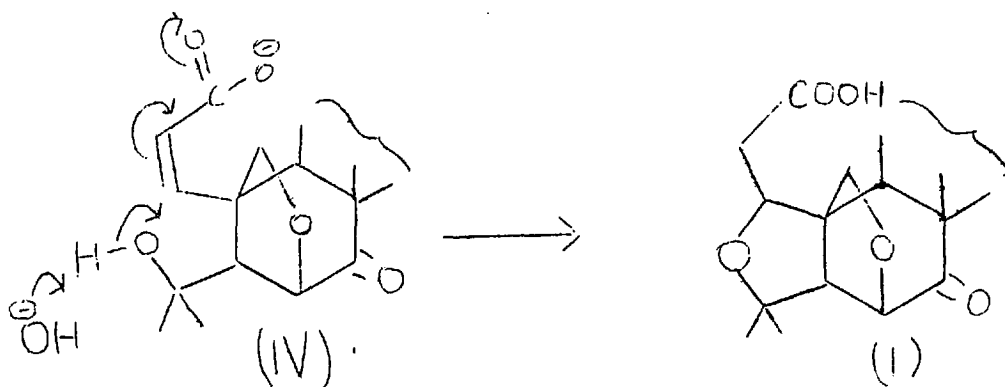
In the course of the present work, it was found that limonilyl chloride (see above) lost hydrogen

chloride on heating to about 230°C , giving rise to a neutral , amorphous dilactone which could be converted to an amorphous acid by mild alkali. On more prolonged treatment with alkali , limonilic acid was obtained. The ultraviolet spectrum of the crude lactone showed a peak at $207 - 10 \text{ m}\mu$ ($\epsilon:13,000$) and on subtraction of the spectrum of methyl-limonilyl ketone a peak remained at $210-15 \text{ m}\mu$ ($\epsilon:6,500$). The infrared spectrum of the crude lactone showed bands at 1750 , 1715 , 1505 and 875 cm^{-1} thus possibly indicating the presence of a δ lactone, an unsaturated lactone and ketone and of the furan ring. No hydroxyl bands were present.



Although the failure to obtain ~~crystalline~~ ^(IV) products makes accurate interpretation of the results impossible, the formation of a seven membered, α - β unsaturated lactone (IV) by the mechanism illustrated above agrees with the experimental results available.

The lactone (IV) could then be converted to limonilic acid under the influence of alkali in the following manner:



The close analogy of this reaction to the mechanism proposed for the formation of limonilic acid (see section 9) and iso-obacunic acid (see section 15) , makes this mechanism very attractive.

12. THE STRUCTURE OF CITROLIN

Citrolin has been obtained by the action of hydroiodic acid on limonin^{21,29,39,40} and by the action of hydroiodic acid on desoxylimonin³⁹. In the course of the present work, it was shown that citrolin may also be obtained by the action of hydrobromic acid on desoxylimonin.

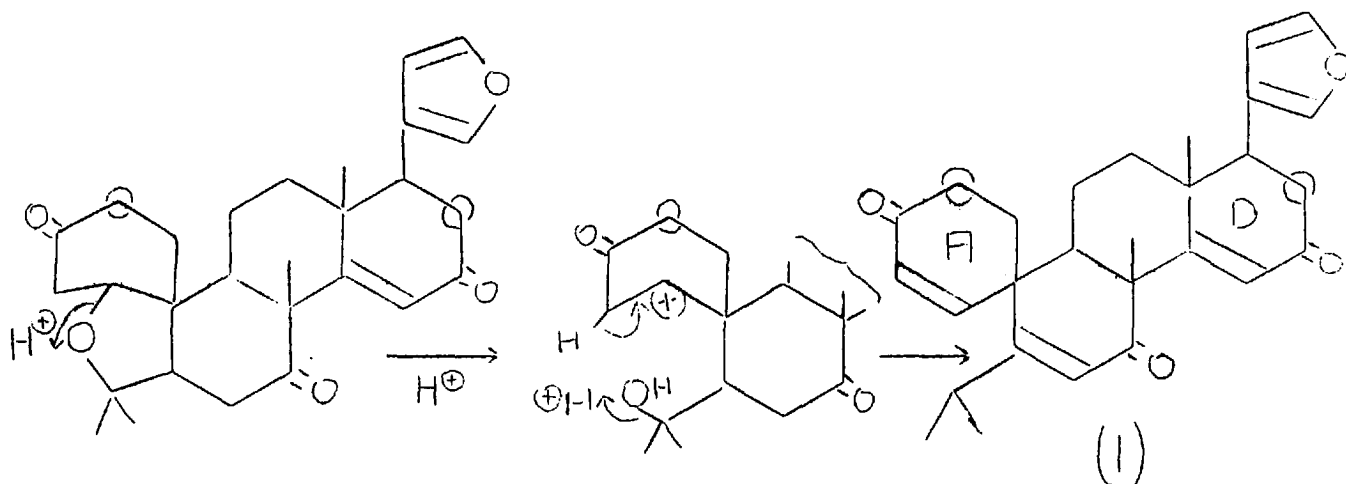
Although the analytical data published are conflicting, the best results^{29,39} indicate that citrolin has the formula $C_{26}H_{28}O_6$, i.e. that it is formed by the loss of a molecule of water from desoxylimonin $C_{26}H_{30}O_7$. The validity of the above formula received further support when citrolin was prepared by the action of hydrobromic rather than hydroiodic acid i.e. under conditions which would not normally lead to a reduction of the type which is involved in the formation of desoxylimonin from limonin (see section 2a).

Citrolin is a neutral dilactone retaining the furan and ketone functions^{21,29,39}. Emerson²⁹ showed that citrolin contains at least four double bonds on the basis of hydrogenation experiments, but

no crystalline derivatives could be isolated. Citrolin has been shown to contain all the four methyl groups present in limonin⁴⁰ as determined by NMR measurements (present work).

The ultraviolet spectrum of citrolin shows a maximum at 213.5 m μ (ϵ :25,200) and a shoulder near 240 m μ (ϵ :18,500). When the spectrum of desoxylimonin is subtracted from the spectrum of citrolin, two maxima appear at 213.5 m μ (ϵ :9,500) and at 241 m μ (ϵ :13,700). The infrared spectrum of citrolin shows a large band at 1724-16 cm⁻¹ and a smaller band at 1670 cm⁻¹ as compared with bands at 1746 cm⁻¹ and 1715 cm⁻¹ for desoxylimonin.

The above data indicate that citrolin has two α - β unsaturated lactones and an α - β unsaturated ketone. Having regard for the structure of desoxylimonin (section 2a), structure (I) was proposed for citrolin, which may be formed from desoxylimonin by the following mechanism:



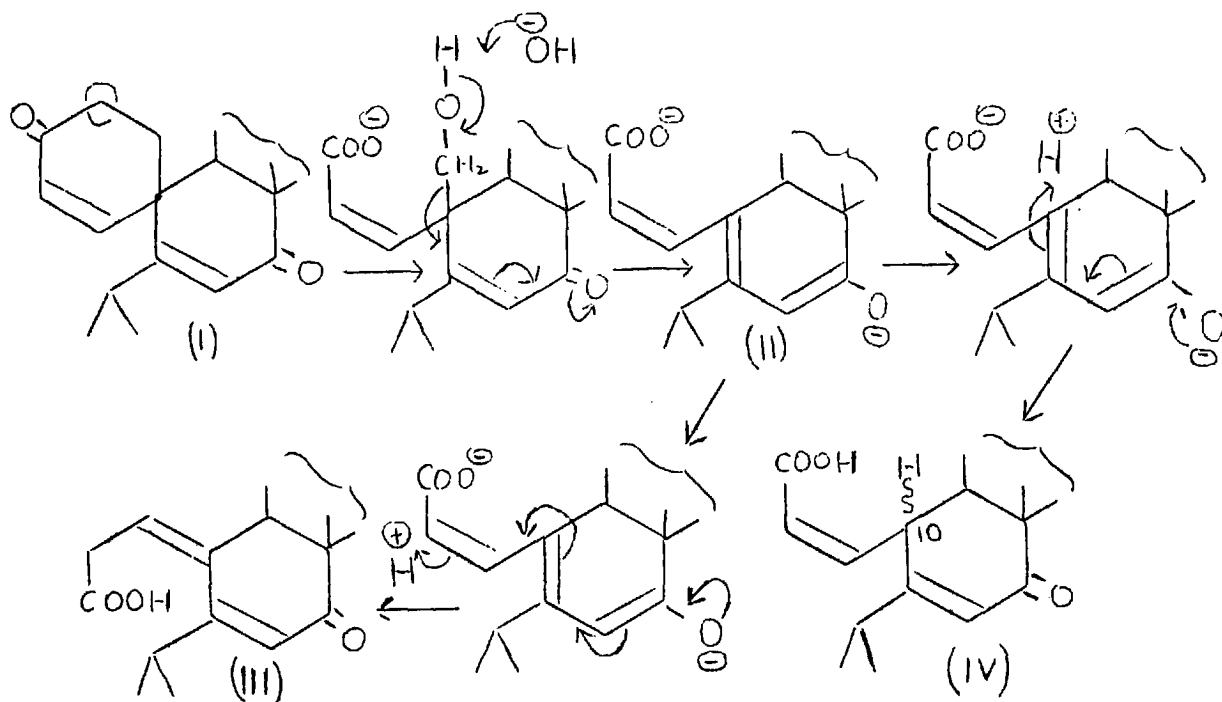
Hydrogenation experiments carried out in the course of the present work further confirm structure (I). A neutral dihydro derivative , $C_{26}H_{30}O_6$ was isolated following hydrogenation of citrolin over palladium-carbon. The ultraviolet spectrum of this substance (λ_{max} : 216 $m\mu$, ϵ : 17,200 and shoulder near 231-43 $m\mu$, ϵ : 14,700) together with the presence of ^{BANDS} at 1757, 1713 and 1668 cm^{-1} in the infrared and the furan bands, indicate that one of the two α - β unsaturated lactones has been hydrogenated. In confirmation , when the ultraviolet spectrum of desoxylimonin was subtracted from that of dihydrocitrolin, only a single peak remained at 248 $m\mu$ (ϵ : 10,600).

The α - β unsaturated lactone of desoxylimonin was not hydrogenated under similar conditions (see preparation of tetrahydridesoxylimonin) and the ultraviolet spectrum of dihydrocitrolin remained unaltered in the 280 m μ region on heating with 2N alkali (see below) , indicating that it was the lactone A which was hydrogenated.

A further neutral crystalline substance was isolated as a minor product of hydrogenation of citrolin . The infrared spectrum of this material was similar in the carbonyl region (bands at 1753, 1698 and 1663 cm⁻¹) to that of dihydrocitrolin, but the furan bands were no longer present. The ultraviolet spectrum showed a maximum at 238 m μ (ϵ :13,700) and a shoulder between 222 and 230 m μ (ϵ :13,000). These features indicate that this product differs from dihydrocitrolin only in the saturation of the furan ring , i.e. that it is hexahydrocitrolin. Unfortunately insufficient sample was available for elementary analysis.

Citrolin is unstable to alkali²⁹ yielding formaldehyde⁵³ and acidic products⁴⁰ which retain the ultraviolet spectrum of citrolin and analyse

for "deformylated" materials $C_{25}H_{28}O_6$. Two such acids were obtained by Corey⁴⁰. Under more severe conditions (heating with 2N alkali), citrolin gave rise to an acidic compound whose ultraviolet spectrum showed a maximum at $286-8\text{ m}\mu$ ⁵³ but which could not be obtained in a crystalline form. Phillips³⁹ isolated an acid of this kind, which showed two maxima in the ultraviolet spectrum (at $214\text{ m}\mu$, $\epsilon:14,000$ and at $280\text{ m}\mu$, $\epsilon:5,000$) and had bands in the infrared spectrum near $1720, 1687, 1630$ and 1608 cm^{-1} . The formation of all these products can be adequately explained by the scheme shown below:

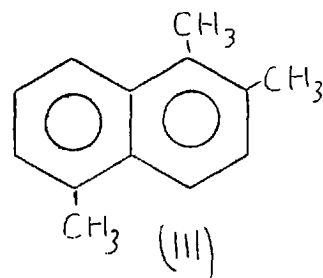
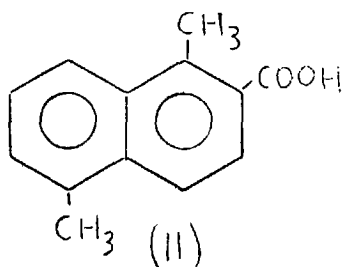
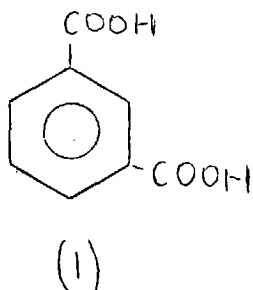


Thus , depending on the position of protonation of the transition state (II) one can obtain either a dienone (III) or an α - β unsaturated ketone not conjugated with an α - β unsaturated acid (IV). The structure (IV) which should correspond to the acids isolated by Corey⁴⁰ would be expected to show isomerism depending on the configuration at C₁₀.

13A. MISCELLANEOUS REACTIONS OF LIMONIN AND ITS DERIVATIVES

a. Potassium hydroxide fusion

Potassium hydroxide fusion of limonin^{20,24} yielded iso-phthalic acid (I) and a "lactone"²⁴ of composition $C_{13}H_{14}O_2$ or $C_{13}H_{12}O_2$ which is probably identical with 1:5dimethyl-2-naphthoic acid (II), also obtained from potash fusion of columbin⁴². Acetone, in yields of up to 0.7 mole, was also identified as one of the products of potassium hydroxide fusion of limonin^{20,24,21,29} and also of limonilic and hexahydrolimoninic acids²⁹. The formation of (I) cannot be easily explained in terms of the accepted structure of limonin, but (II) could be easily derived from rings B and C while the high yield of acetone provided some evidence for the substitution at C_4 .



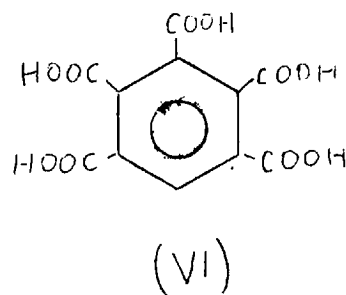
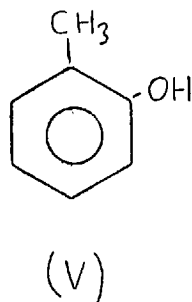
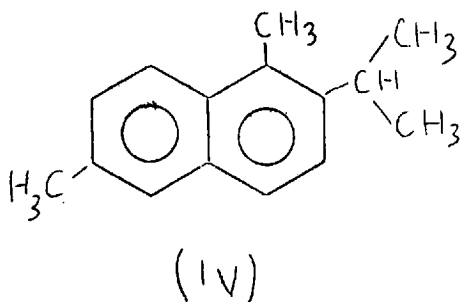
b. Selenium dehydrogenation

Selenium dehydrogenation of the non-volatile residue of potassium hydroxide fusion²⁰ or of zinc distillation residue²⁴ of limonin, gave 1:2:5:trimethyl naphthalene (III), probably derived from rings B and C of limonin.

Selenium dehydrogenation of merolimonol or limoclastic acid^{35,46} gave a naphthalene $C_{15}H_{18}$ which has been tentatively identified as 1:6:dimethyl-2-isopropyl naphthalene (IV). The identification of this compound provided direct evidence for the presence of the isopropyl group in limonin.

c. Zinc dust distillation

The only identifiable product of zinc dust distillation²⁴ of limonin, was o-cresol (V), probably derived from ring B of limonin.



d. Oxidation with manganese dioxide and sulphuric acid

Koller and Czerny²⁰ isolated benzene-pentacarboxylic acid (VI) by oxidation of limonin with manganese dioxide and sulphuric acid. To obtain this product from limonin, methyl migrations must be postulated. There is some precedent⁵⁸ for the appearance of too highly substituted benzene carboxylic acids by oxidation under strongly acidic conditions.

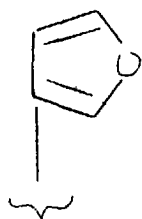
e. Lithium aluminium hydride reduction

Jeger and his co-workers^{35,46} isolated a product which still contained the furan function, from lithium aluminium hydride reduction of limonin and which appeared to be a heptol on the basis of the number of active hydrogens remaining in the tetra- and penta-acetates derived from it. This indicated that both the ether functions could be reductively cleaved and in view of the established structure of limonin, the cleavage of the ring A' ether can only be explained as due to the assistance of the β -carbonyl function of lactone A. The mechanism of such an assistance cannot be easily visualised.

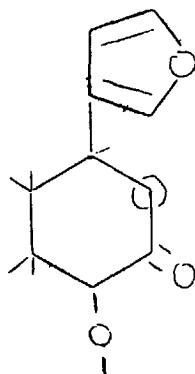
13B. SUMMARY OF CHEMICAL EVIDENCE FOR THE STRUCTURE OF LIMONIN

As already indicated in the introduction, a rigid derivation of the structure of limonin from chemical evidence alone was not attempted in the present work. In this section a brief summary will be presented of the way in which the accepted structure of limonin was in fact postulated on the basis of chemical evidence, prior to the results of the X-ray crystallographic analysis becoming known.

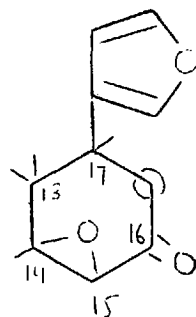
Spectroscopic and other evidence (section 1a) enables us to assign the β -mono substituted furan ring (I) as a part structure of limonin. The results of the hydrogenolysis experiments and the nature of hexahydrolimoninic acid (section 1b) makes it possible to expand this to structure (II):



(I)

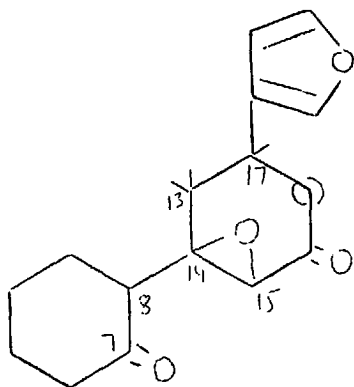


(II)

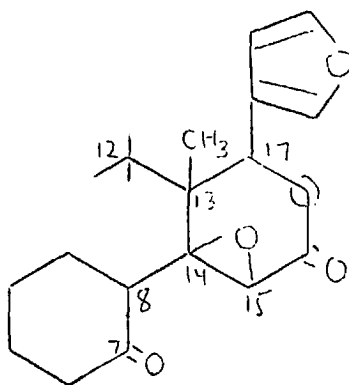


(III)

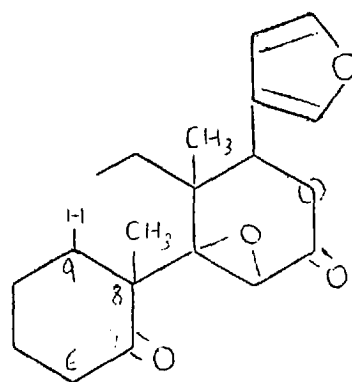
The nature of the negative substituent at C_{15} and the degree of substitution at C_{14} and C_{15} is revealed by experiments described in section 2, thus enabling us to write (III) as a partial structure of limonin. This can be further expanded to (IV) on the basis of interpretation of the formation of desoxylimononic acid (section 4). The nature of substitution at C_{15} , C_{14} and possibly C_{13} is also confirmed by the transformations of hexahydrolimoninic acid with acid (section 6). The formation of merolimonol (section 8) and the properties of its degradation products gave a further independent proof of the relation between the lactone ring D and the ketone at C_7 and furthermore revealed the nature of substitution at C_{13} and C_{17} and the presence of carbon at C_{12} , thus yielding the expanded partial structure (V).



(IV)



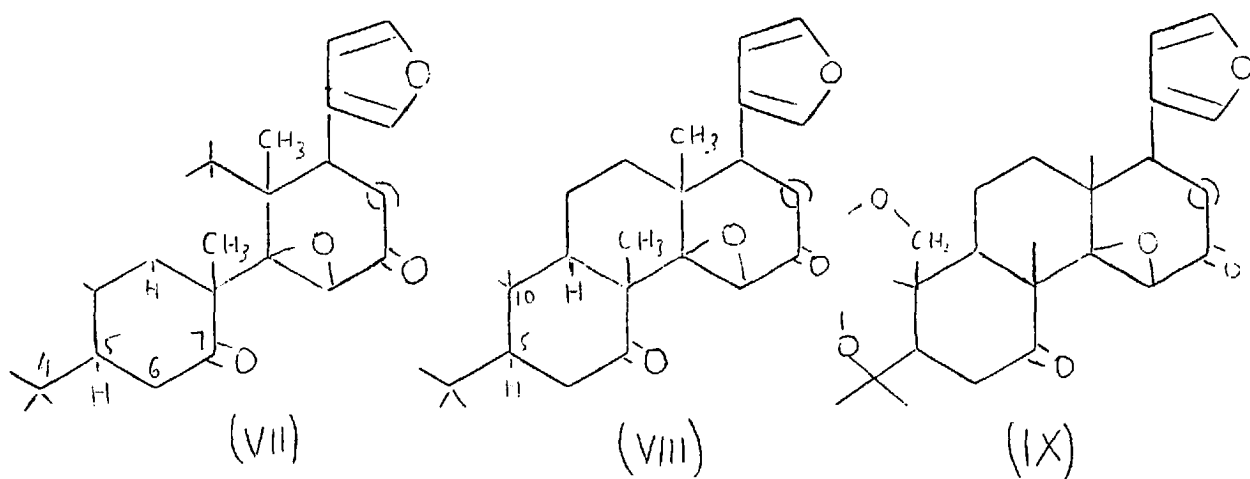
(V)



(VI)

This may be further expanded to (VI) from the consideration of the nature of degradation products of tetrahydrodesoxylimonic acid (section 5).

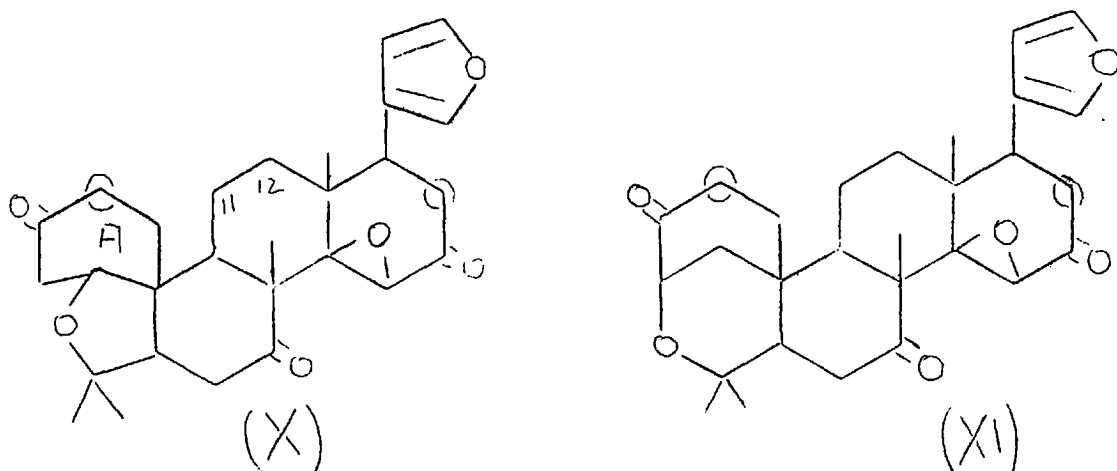
The nature of substitution at C_6 and the presence of hydrogen at C_5 and carbon at C_4 was revealed by the formation of limonin diosphenols (section 10) thus giving the partial structure (VII). The bicarbocyclic nature of limonin and the presence of several naphthalene derivatives among the drastic degradation products of limonin (section 13A) allows us to expand (VII) into (VIII).



The alkaline degradation of the ozonolysis products of tetrahydrolimonindiosphenol (section 10), the formation of limonilic acid (section 11) and the alkaline degradation of citrolin (section 12)

indicate that the group $-\text{CH}_2\text{OH}$, the terminus of lactone A, is placed either at C_{10} or at C_4 . This, together with the formation of isopropyldimethyl naphthalene and of acetone, by drastic degradation of limonin made structure (IX) extremely attractive.

Given the fact that lactone A is a δ lactone and considering the formation of citrolin (section 12), (IX) was expanded into either (X) or (XI):



Of these two structures (X) was favoured as it provided a plausible mechanistic basis for the formation of limoclastic acid (section 9) and also was more in accord with the nature of some degradation products of limonilic acid (section 11), and the limonin diosphenols (section 10). The main reasons why (X) could not be considered rigidly derived were: a) lack of evidence for the primary nature of limonilic acid, b) lack of knowledge about substitution at C_{11} and C_{12} .

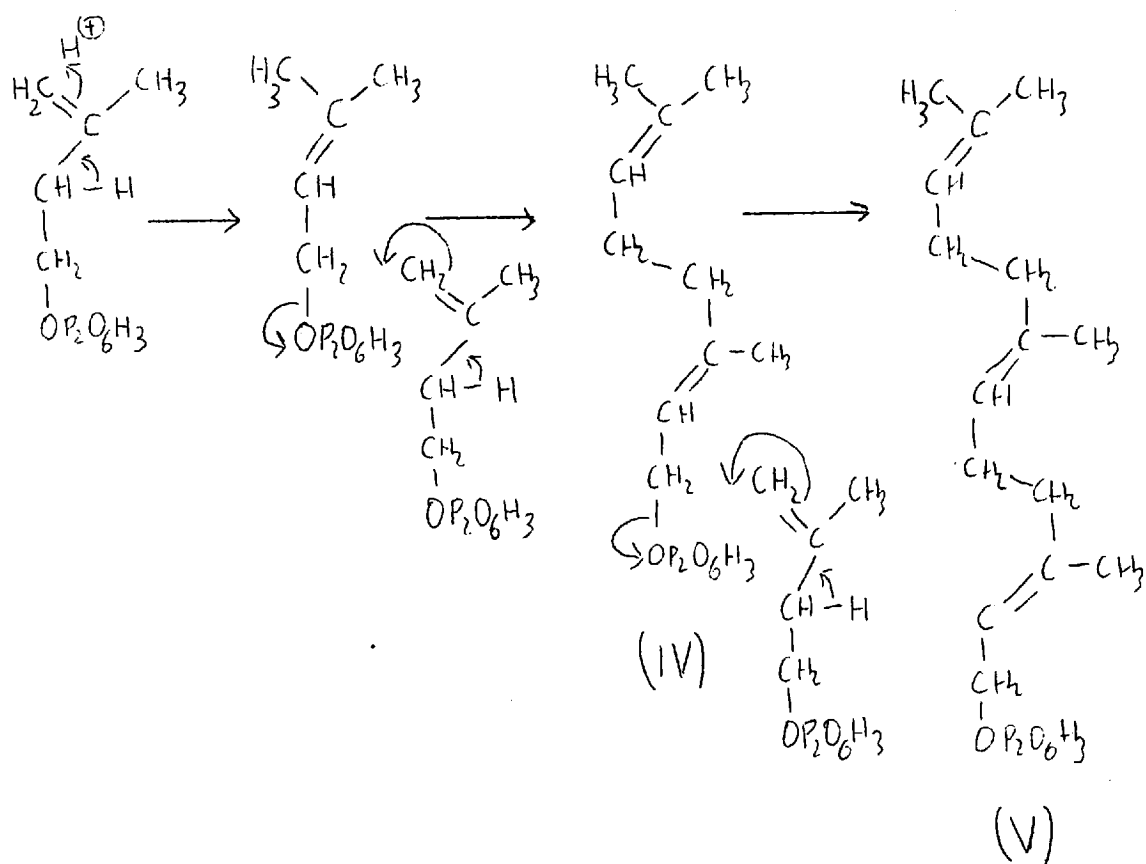
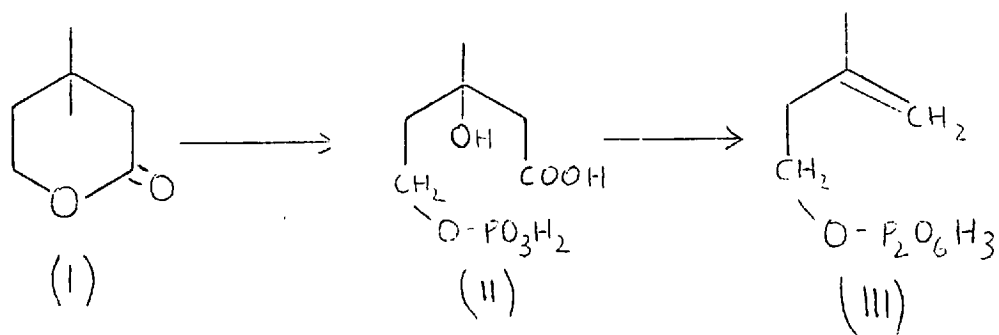
14. BIOGENESIS OF LIMONIN

a. Biogenesis of triterpenes

Regularities of structure among the terpenes were noted in the last century and, in fact, the simple "isoprene rule" holds for a considerable proportion of terpenes known⁵⁹.

Serious attempts to elucidate the biosynthesis of natural products in the living organisms date from the introduction of tracer analysis. The mechanism of biosynthesis has attracted considerable attention, has been reviewed recently^{60,61} and has also been the subject of an international symposium⁶².

Briefly, present knowledge indicates that the precursor of the five carbon ("isoprene") fragment is mevalonic lactone (I) which is in turn derived from acetate units by a process whose details are unknown^{63,64,65}. Mevalonic lactone is then converted by phosphorylation to (II) and then by decarboxylation and further phosphorylation to iso-pentenyl pyrophosphate (III) from which geranyl pyrophosphate (IV) and farnesyl pyrophosphate (V) may be derived as shown.⁶⁶



Further condensations could lead to geranyl-geraniol and squalene and hence by cyclisation⁶⁰ to mono-, sesqui-, di-, and triterpenes as well as steroids⁶². It should be noted that the SN2 reactions indicated may in fact proceed by a different mechanism.

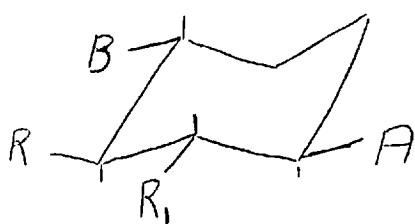
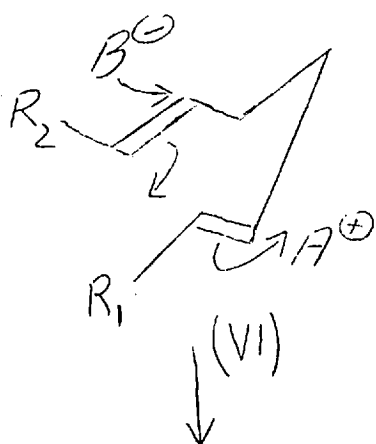
The experimental evidence for this scheme consists of isolation of radioactive terpenes and steroids, as well as of the above-mentioned intermediates, from biological systems into which labelled acetate or mevalonic lactone were introduced. Degradation of the various products obtained showed that the labelled carbon atoms were introduced in the positions predicted by the theory^{61,67,68,69,70,71,72,73,74}.

While geraniol, farnesol, geranylgeraniol and squalene are found in nature, there is no positive evidence suggesting that they are the immediate precursors of all mono-, sesqui-, di-, and triterpenes. However, schemes for the biogenesis of these classes of compounds based on the above acyclic precursors have been so fruitful⁶⁰ that it seems probable that these compounds or their "biological equivalents" are actually involved.

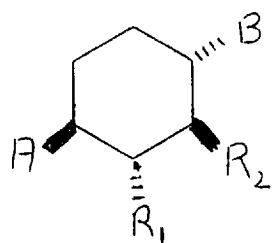
In particular , all known cyclic triterpenes including lanosterol - and hence steroids - may be derived from squalene by means of acid catalysed cyclisations and rearrangement of carbonium ions⁷⁵. As limonin has been postulated (see below) to be derived from a normal triterpene, it is pertinent to summarize the arguments of Ruzicka et. al.⁷⁵

Assuming trans addition to double bonds, two products could be expected from an acid (A^+) initiated cyclisation of a cyclohexadiene, depending on whether the acyclic precursor was in a "chair favouring" conformation (VI) or in a "boat favouring" conformation (VII) , viz. the "anti" and "syn" conformations shown. B^- denotes the "base" which neutralises the carbonium ion formed.

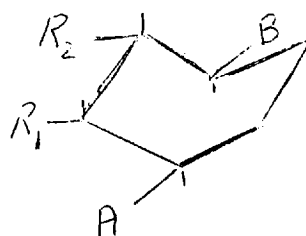
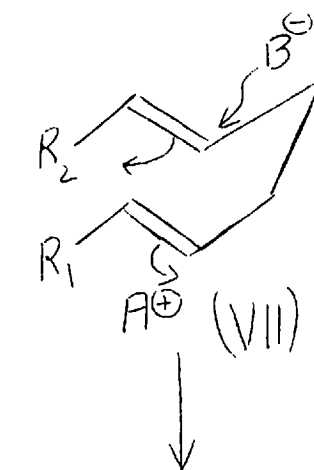
A further assumption made, concerned the trans nature of acid catalysed 1-2 rearrangements, which, like additions to double bonds are postulated to proceed via non-classical carbonium ions. As a consequence , the migrating group - usually methyl or proton - never crosses from the α to the β side.



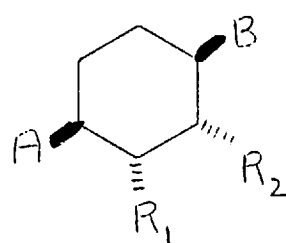
III



"ANTI"



III

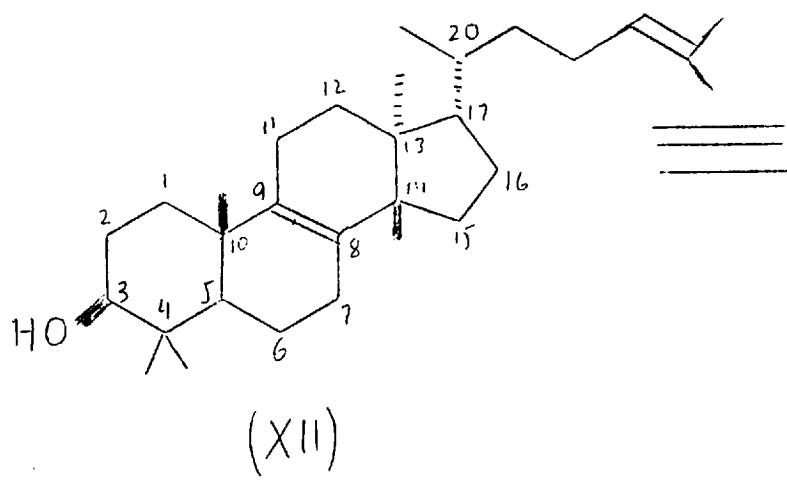
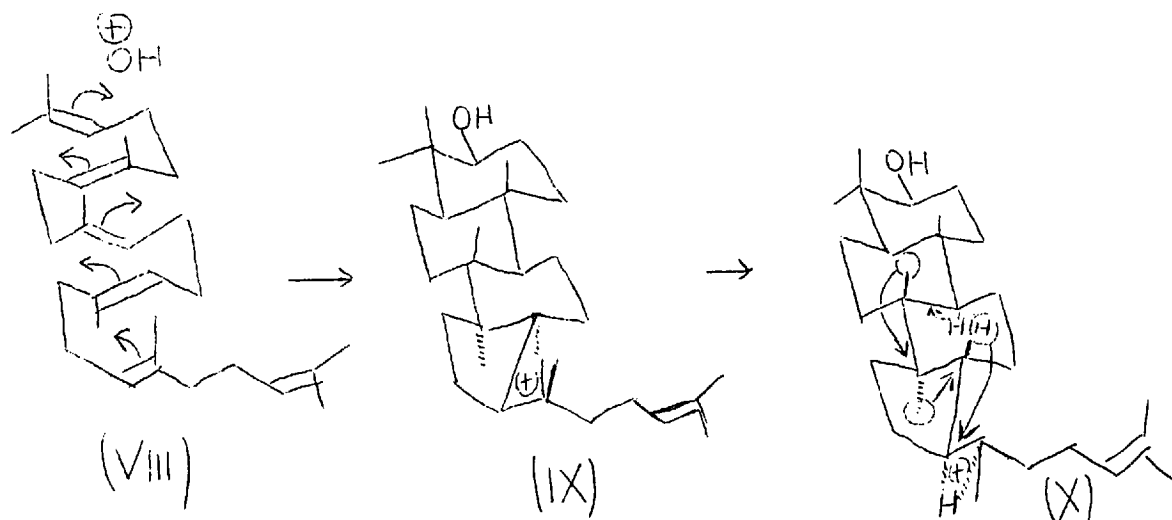


"SYN"

It was also necessary to assume that squalene - or its biological equivalent - was in an all trans configuration , an assumption for which there is some experimental basis⁷⁶.

As no stable intermediates, formed by neutralisation of the positive charge (by hydration or proton splitting) at some intermediate stage of cyclisation have been isolated, the authors have postulated that the formation of the naturally occurring triterpenes is a "non-stop" process, presumably due to the rigid adhesion of the reacting molecule on the enzyme surface.

Utilising these simple assumptions, the Swiss workers were able to account for the structures and stereochemistry of all the known types of triterpenes by starting from different arrangements of "chair favouring" and "boat favouring" portions of the squalene chain. For example , the "chair-chair-chair-boat-straight chain" conformation of squalene (VIII), may give rise to the tetracyclic triterpene euphol (see section 14b) by the following mechanism:

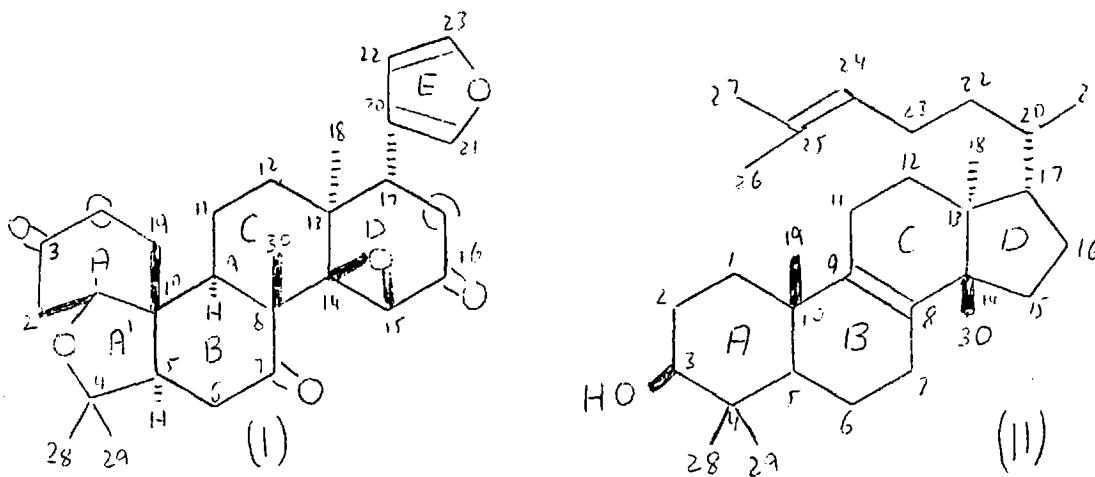


Cyclisation is accompanied by the rearrangement of the non-classical carbonium ion (IX) to (X) and by a concerted transfer of proton from C₁₃ to C₁₇ (on β side), transfer of methyl from C₁₄ to C₁₃ (α side), transfer of methyl from C₈ to C₁₄ (β side) and elimination of proton from C₉ (from α side), to give (XI), which can be conventionally represented as euphol (XII).

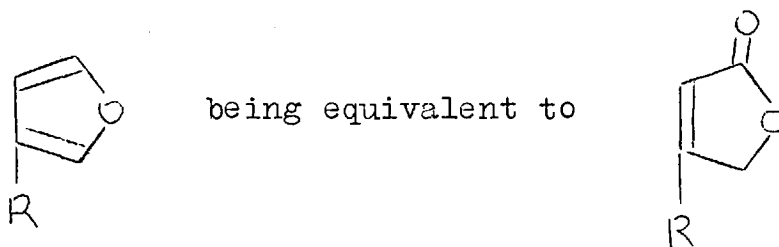
Conversion of (X) to (XI) is here indicated as a series of 1-2 shifts, but the Swiss workers also considered the possibility of 1-3 shifts in some cases. In the case of cyclisation of squalene to lanosterol^{61,77}, it has been shown that 1-2 shifts actually occur.

b. BIOGENESIS OF LIMONIN

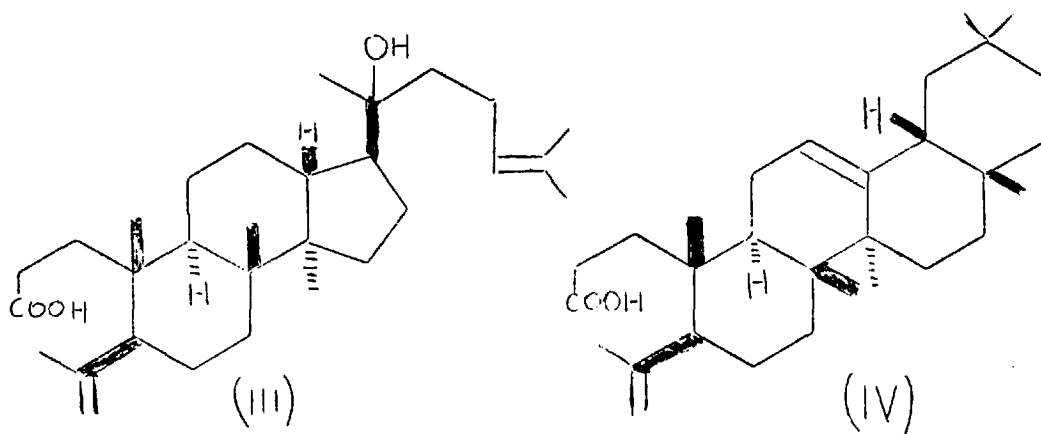
Limonin (I) appears to be closely related to the tetracyclic triterpene euphol^{78,79} (II). Three series of steps must be postulated for a biogenesis of limonin from a euphol type compound:



(i) Four carbon atoms are removed from the end of the side chain and carbons C_{20} to C_{23} are converted to a furan ring. A similar process appears to be involved in the formation of cardiac glycosides



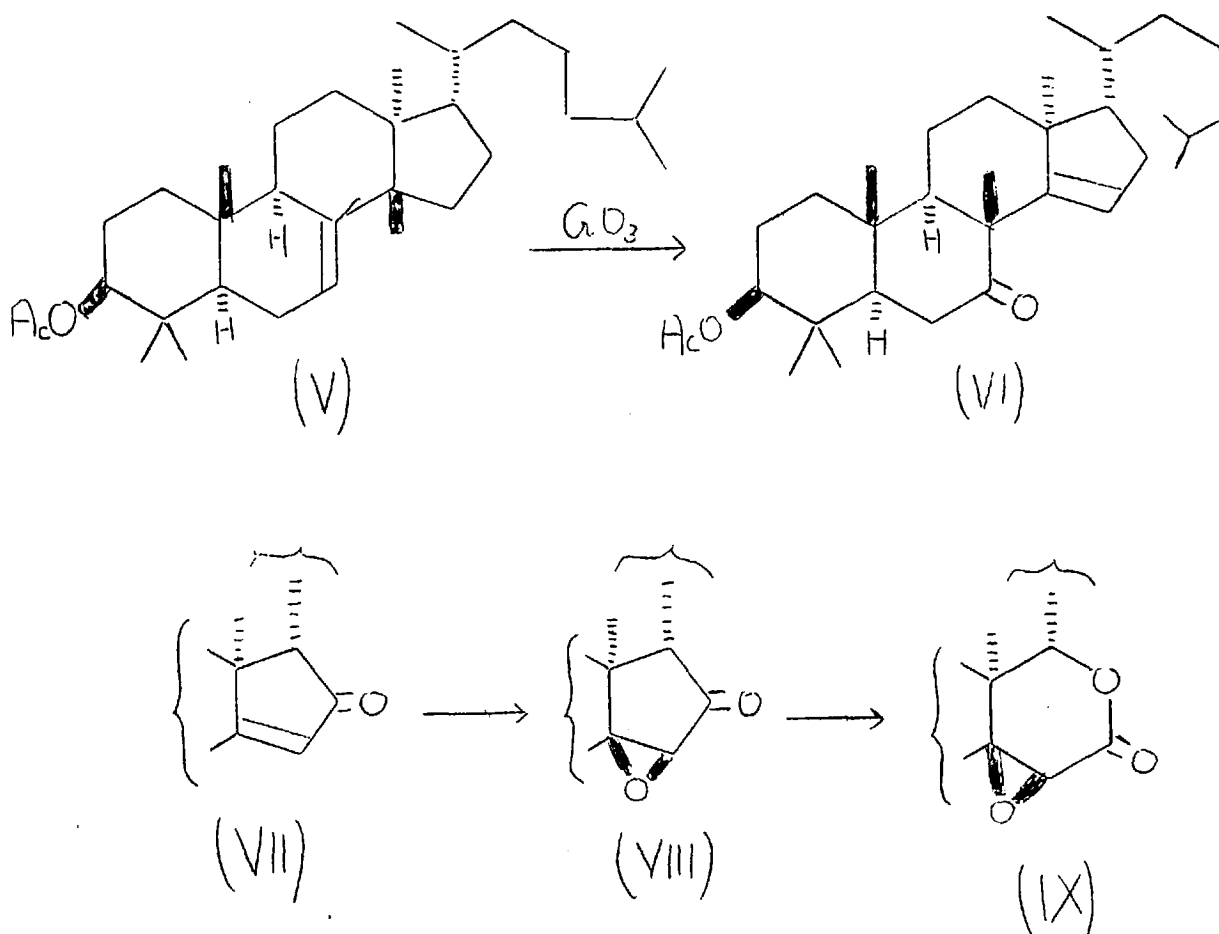
(ii) The ring A of the triterpenoid skeleton is cleaved oxidatively between C₃ and C₄ and the C₃ carbonyl thus formed is oxidatively cyclised onto C₁₉. The recently described structures of dammarenolic⁸⁰ (III) and nycanthic⁸¹ (IV) acids afford a precedent for a C₃ - C₄ biogenetic cleavage of the triterpenoid skeleton.



No analogy for an oxidative closure on a methyl group is known, but recent work⁸² indicates that similar reactions may occur under the influence of ultraviolet light.

(iii). The ring D of a euphol type terpene is oxidised to a lactone by a Beyer Villager type of reaction and a methyl migration from C₁₄ to C₈ must be postulated.

A possible analogy may be found in the chromic acid oxidation of dihydrobutyrospermol acetate⁸³ (V) to give the 7-ketone (VI). This type of compound could be oxidised further to a 16 ketone (VII), epoxidised to an oxido-ketone (VIII) and then give a limonin type structure (IX) by a Beyer Villager oxidation.



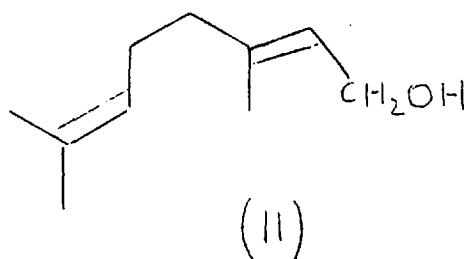
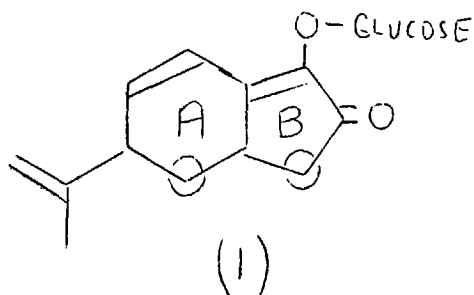
The numbering of the carbon atoms in the structure of limonin follows from the above ideas of its biogenesis.

On the basis of the above, the absolute configuration of limonin would be expected to be the same as that of the ketone (VI). The rotary dispersion curve⁸⁴ of tetrahydrolimonin shows a strong, negative Cotton effect and corresponds to the type shown by saturated steroidal 7-ketones , thus providing support for the above hypothesis.

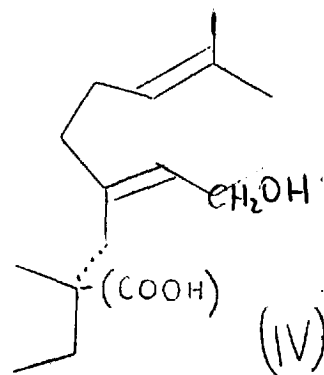
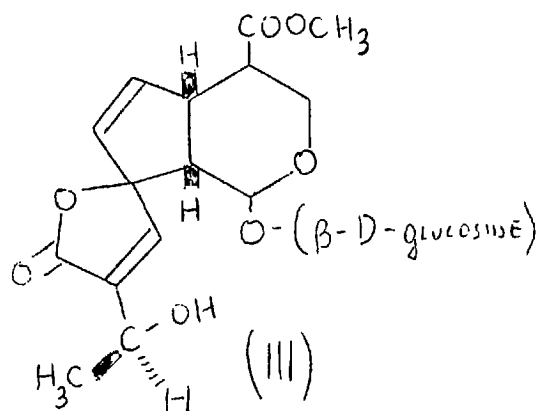
c. The biogenesis of some lactonic bitter principles

Lactonic bitter principles form a chemically ill-defined group, being best classified as non-alkaloidal, bitter plant products containing lactone groups. A review of the chemistry of bitter principles has appeared recently⁸⁵. The majority of the known bitter principles are terpenoid, and although they are often of considerable chemical complexity, their biogenesis is relatively straightforward. In the present section some of the more chemically interesting bitter principles are presented from the point of view of their biogenesis.

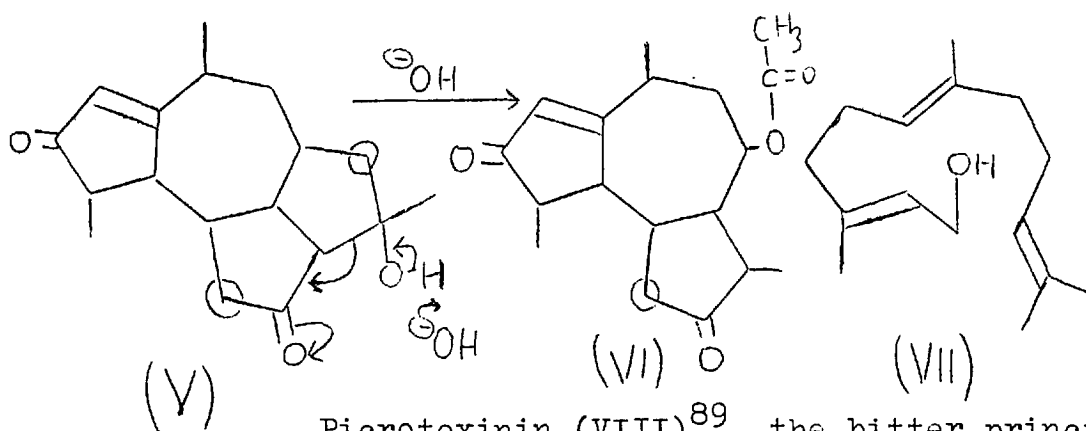
Gentiopikrin (I)⁸⁶, the bitter principle of gentiana lutea has the geraniol (II) skeleton. If the monoterpene character of gentiopikrin is in fact correct, then the cyclisation of lactone B provides an example of oxidative cyclisation on a methyl group (see section 14 b).



Plumerid⁸⁷ (III) was postulated to be derived from geraniol and an "acetoacetate unit"(IV):

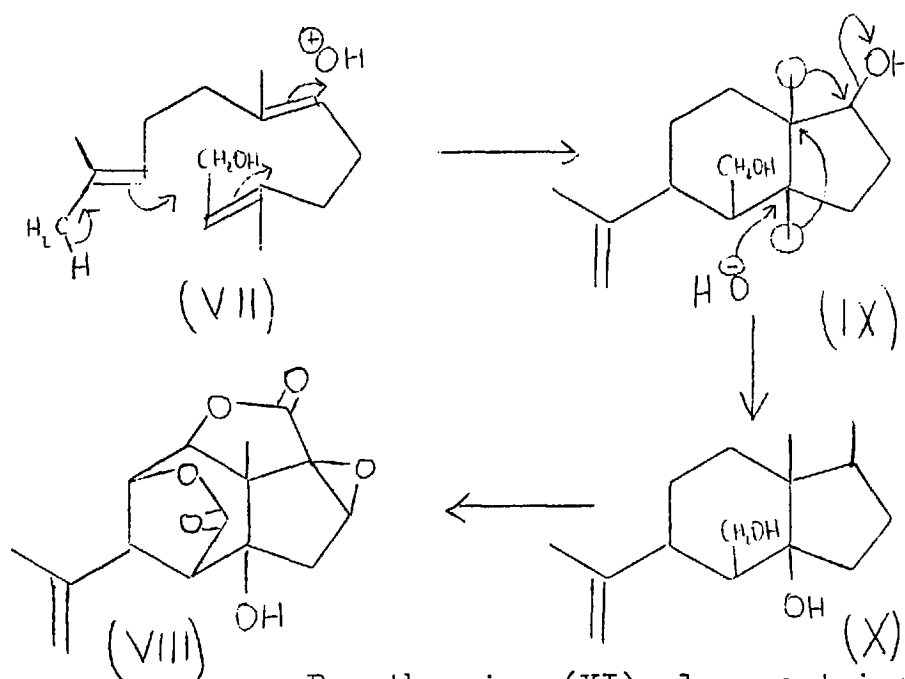


Tenulin (V) , a sesquiterpenoid bitter principle⁸⁸ of *helenium tenuifolium* and the isomeric iso-tenulin (VI) both contain the unrearranged farnesol skeleton (VII):

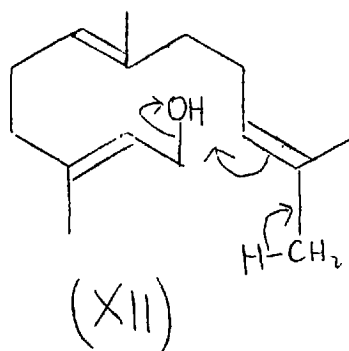
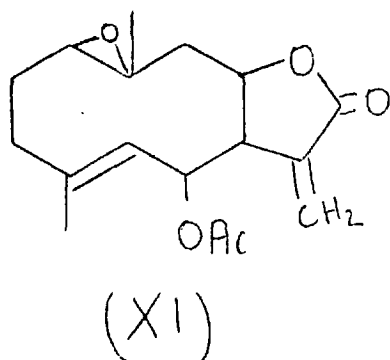


Picrotoxinin (VIII)⁸⁹, the bitter principle from *anamirta cocculus* can also be derived from

farnesol (VII) via the intermediates (IX) and (X):



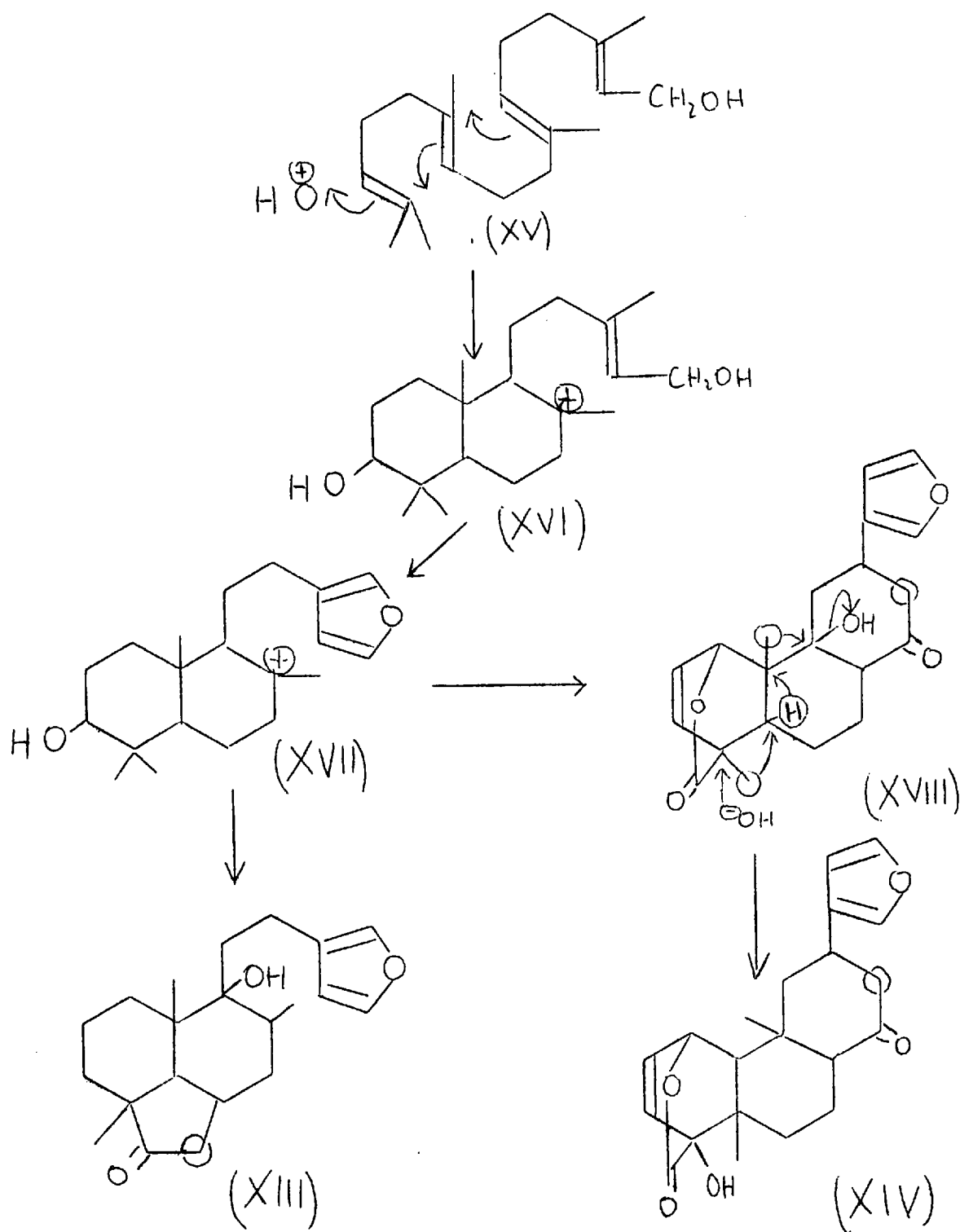
Pyrethrosin, (XI) also contains the unrearranged farnesol chain (XII), and is of special interest⁴⁸ as the first ten membered sesquiterpene isolated.



It must be noted, that the biogenetic reactions of the sesquiterpenes shown here are purely schematic. The actual intermediates postulated^{91,90} in the formation of sesquiterpenes are ten and eleven membered cyclic cations resulting from rearrangements of non-classical carbonium ions formed from either cis or trans farnesol. The "large ring hypothesis" was introduced to account for various stereochemical features found in naturally occurring sesquiterpenes.

Both marrubiin (XIII)⁹², the bitter principle of marrubium vulgare and columbin (XIV)⁴², a bitter principle of colombo root, are diterpenes, but while marrubiin retains the skeleton of geranylgeraniol (XV) and of the intermediates (XVI) and (XVII), in the case of columbin a rearrangement of (XVIII) has been postulated to account for the abnormal diterpene skeleton.

So far, limonin, nomilin and obacunone are the only well established triterpenoid bitter principles.



15. THE CHEMISTRY OF OBACUNONE AND NOMILIN

a. Summary of previous work

i. Isolation of obacunone and nomilin

Obacunone was isolated by Kaku and Ri⁹³ from the bark of phellodendron amurense and is identical⁹⁴ with cassimirolid, which was isolated earlier from cassimiroa edulis, another member of the rutacea family, by Power and Callan⁹⁵.

Obacunone, together with the entirely new bitter principle nomilin, was later isolated by Emerson^{13,14} from immature navel oranges and from citrus seed oil. Later work^{15,40} showed that both nomilin and obacunone occur in grapefruit seed oil and in the course of the present work, it was shown that either or both of the bitter principles may be obtained from grapefruit seeds in approximately 0.03% yield. Some evidence⁵³ exists that the bitter principle involved is in fact nomilin.

ii. Relation of obacunone to nomilin

Reliable characterisation of obacunone and nomilin is due to Emerson^{13,14} who also elucidated

the relationship between them. Both bitter principles were shown to be dilactone ketones by equivalent determinations and preparation of oximes and furthermore nomilin, $C_{28}H_{34}O_9$, was shown to contain one acetoxy group which was lacking in obacunone, $C_{26}H_{30}O_7$. On mild alkaline hydrolysis both materials yielded obacunoic acid, $C_{26}H_{32}O_8$, thus showing that nomilin was acetoxy obacunone and that the acetoxy group was capable of being eliminated under alkaline conditions. In confirmation it was also found possible to convert nomilin to obacunone by heating in the presence of pyridine.

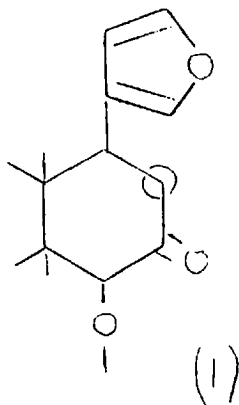
iii. The functional groups of obacunone and nomilin

Besides the work of Emerson^{13,14}, only one other investigation has been described into the chemistry of nomilin and obacunone¹⁵, from which all the information in this subsection has been abstracted.

(1): Degree of unsaturation: On the basis of hydrogenation experiments, Dean and Geissman¹⁵ assigned four double bonds to obacunone and obacunoic acid and three double bonds to nomilin. The amounts of hydrogen uptake by obacunoic acid and methyl

obacunoate , quoted by the above authors, were found incorrect in the course of the present work (see below) and it can be stated with reasonable certainty that obacunoic acid has only three double bonds.

Hydrogenation over palladium-carbon also caused hydrogenolysis with formation of an abnormally strong carboxylic acid - a situation completely analogous to the hydrogenolysis of limonin and columbin (see section 1). The infra-



red spectra of obacunone and nomilin show the characteristic furan frequencies ,identical with those detected in limonin (especially bands at 1505 and 875 cm^{-1}) and their NMR (see below) and ultraviolet spectra are also

in accord with the presence of a β -substituted furan nucleus. On the basis of the above, (I) becomes an attractive partial structure for obacunone and nomilin. The presence of a β -substituted furan in obacunone was also indicated by experiments³³ analogous to those described on page 17. In the course of the

present work further evidence has been obtained in support of (I). (see below).

(2) The nature of the lactone groups: On the basis of infrared and ultraviolet spectra both lactones in obacunone were assigned the δ configuration, one of them being α - β unsaturated. It is the unsaturated lactone which is hydrolysed in the formation of obacunoic acid, which is of normal acid strength.

(3) The nature of the ketonic carbonyl: Two isomeric obacunols can be obtained by reduction of the ketonic carbonyl in a manner analogous with limonin (see section 3). This, and the infrared frequency of the ketonic carbonyl absorption suggests that the ketonic function of obacunone is present in a ring, probably six membered or larger. The assignement of all the carbonyl frequencies was further supported by careful quantitative studies of the infrared spectra of a number of derivatives and model compounds.

(4) Obacunoic acid: As expected, obacunoic acid is a mono lactone mono carboxylic acid^{13,14} and the infrared spectrum of methyl obacunoate shows the

presence of a hydroxyl group. Emerson¹⁴, Kaku and Ri⁹³ and Callan and Power⁹⁵ were able to prepare an acetyl derivative of obacunonic acid by heating with acetic anhydride and sodium acetate, but this experiment could not be repeated by Dean and Geissman¹⁵, suggesting that the hydroxyl group in obacunonic acid is difficult to acetylate. There is no evidence for the presence of any hydroxyl groups in either obacunone or nomilin.

(5) Miscellaneous : Unlike limonin, nomilin appears to give no acetone on fusion with alkali. Also unlike limonin, obacunone gives a positive iodoform test.

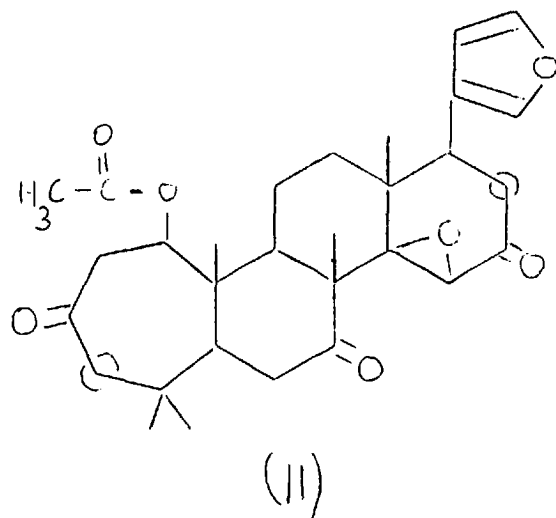
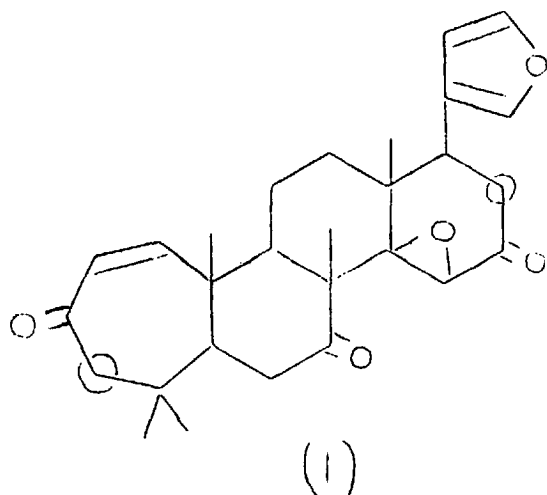
Obacunone gives a hydrochloride, which can be re-converted to the starting material by warming with pyridine. Spectroscopic evidence suggests that the double bond involved is conjugated with a lactone carbonyl. The same double bond appears to be capable of being selectively hydrogenated to give dihydro obacunone.

α -Obacunol appears to undergo a complex change with alkali to give an amorphous product. This suggests an analogy with the merolinonol change (see section 8).

b. The present work

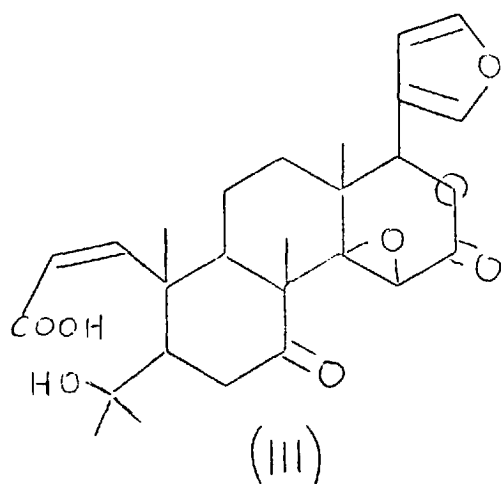
i. Biogenetically attractive structures of obacunone and nomilin

On the basis of published work (see section 15a) and the known structure of limonin, Barton² proposed structure (I) for obacunone and (II) for nomilin. It follows, that the common derivative of obacunone and nomilin, obacunonic acid, should have structure (III).



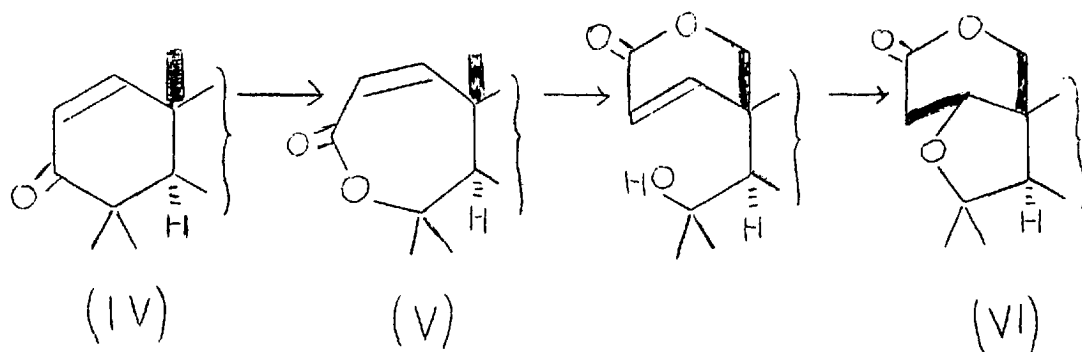
It can be seen that the part structure proposed for obacunone (V) is , in fact, an inter-

mediate in the postulated transformation of the normal terpene structure (IV) to limonin (VI). (see section 14b). Limonin, may then be derived by acetoxylation of (V).



Besides being attractive biogenetically,

structures (I),(II) and (III) , agree with all the known properties of obacunone, nomilin and obacunoic acid (see section 15a) except:



- (a) Experiments of Dean and Geissman¹⁵ suggested that obacunone and nonilin had one more double bond each than shown in structures (I) and (II).
- (b) Obacunonic acid was reported¹⁵ to give a positive iodoform test.

In the course of the present work, it was shown that obacunonic acid contains only three double bonds and that structures similar to that postulated for obacunonic acid (III) can give rise to iodoform.

Other evidence was also introduced in favour of structures (I), (II) and (III). All the work described in the remainder of this section was performed by the present author with the exception of oxidation of obacunonic acid with oxygen in the presence of potassium tertiary butoxide⁵³.

ii. Hydrogenation of obacunonic acid and methyl obacunonate

When hydrogenated over palladium-carbon, obacunonic acid was found to take up 3.74 moles of hydrogen to give an amorphous acid. As hydrogenation over palladium always leads to hydrogenolysis of lactone D in limonin, this result indicated that

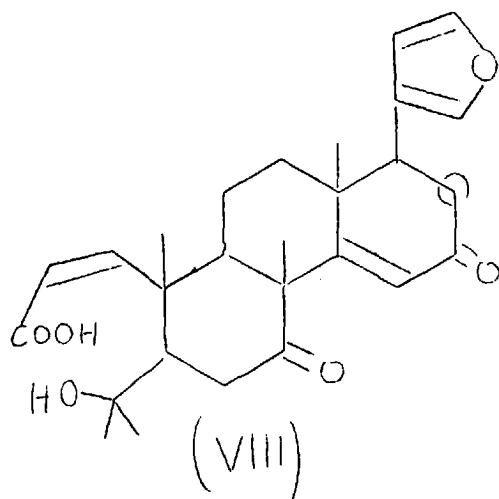
only three moles of hydrogen were taken up by unsaturated structures in obacunoic acid.

More important, it was found that hydrogenation of methyl obacunoate over palladium-carbon also resulted in the uptake of 3.75 moles of hydrogen to give methyl-hydrogenoctahydroobacunoninate identical in its properties with the material described by Dean and Geissman¹⁵, who however recorded a higher hydrogen uptake in an identical experiment. On the basis of structure (III) for obacunoic acid, methyl-hydrogenoctahydroobacunoninate should be (VII) (see below).

ii. Reactions of obacunoic acid analogous to limonin

(1) Desoxyobacunoic acid: Treatment of obacunoic acid, $C_{26}H_{32}O_8$ with chromous chloride^{44,96} resulted in the formation of a desoxy acid $C_{26}H_{32}O_7$ whose

ultraviolet spectrum showed a peak at 206-208 mμ (ϵ :22,000) which is in agreement with structure (VIII), the expected product of a reaction analogous to the formation of desoxylimonin from limonin.

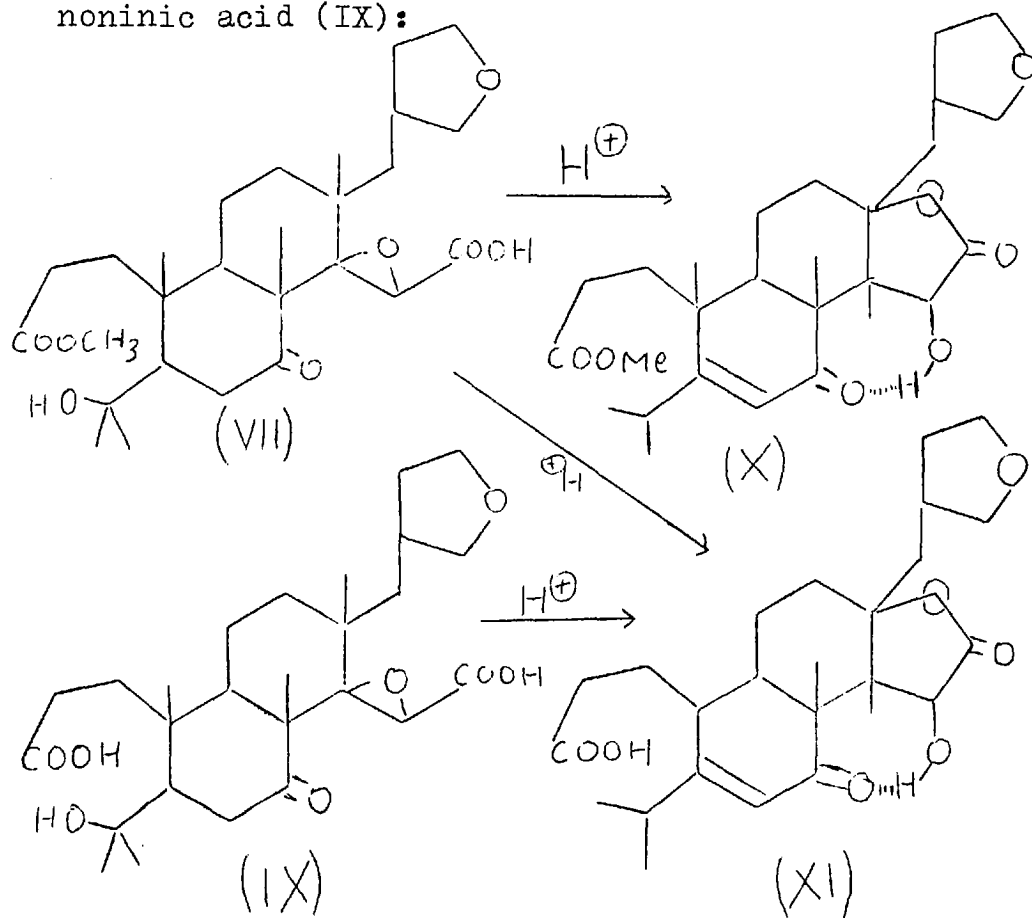


The infrared spectrum of desoxyobacunoic acid, had no bands above 1715 cm^{-1} in the carbonyl region, in accordance with the presence of an α - β unsaturated lactone, an α - β unsaturated acid and a cyclohexanone. Treatment of (VIII) with alkali followed by acidification resulted in the formation of an acidic material which exhibited no maximum in the ultraviolet but retained strong end absorption, a reaction analogous to the formation of desoxylimonic acid from desoxylimonin (see section 4), as expected from structure (VIII).

(2). Action of strong acids on methyl-hydrogenoctohydroobacunoninate and octahydroobacunoninic acid

Treatment of methyl-hydrogenoctohydroobacunoninate with strong acid under conditions analogous to those described in section 6, gave an amorphous, neutral material (X) whose infrared spectrum showed bands at 3200 cm^{-1} (hydroxyl), 1787 cm^{-1} (γ lactone), 1732 cm^{-1} (methyl ester) and 1631 cm^{-1} (very strong) (hydrogen bonded, α - β unsaturated ketone). The presence of the α - β unsaturated ketone was confirmed by the ultraviolet spectrum which showed a maximum at $247\text{ m}\mu$ (ϵ :8,000). An acidic product (X'),

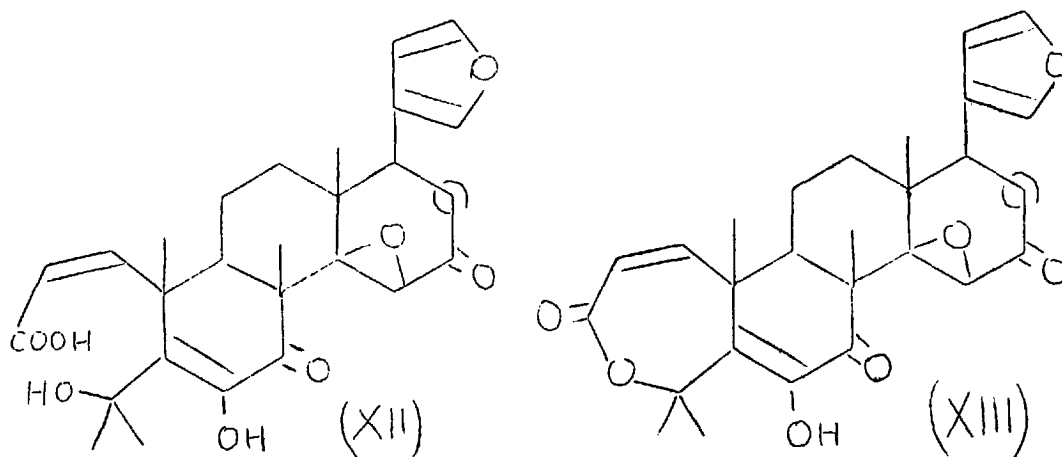
was also isolated, whose infrared spectrum showed bands near $3400\text{--}3000\text{ cm}^{-1}$ (hydroxyl), 1785 cm^{-1} (γ lactone), 1720 cm^{-1} (saturated carboxylic acid) and $1631\text{ (v.s.) cm}^{-1}$ (hydrogen bonded, α - β unsaturated ketone). The ultraviolet spectrum of the acidic product was very similar to that of the neutral material. An amorphous acidic material identical in its spectroscopic properties with the acidic material (XI) was also obtained by the action of strong acid on the amorphous hydrogenation product of obacunonic acid itself, probably octahydroobacunanonic acid (IX):



The above reactions fit well with the structure (VII) for methyl-hydrogenoctohydroobacunoninate, the products being analogous to those obtained from hexahydrolimoninic acid (see section 6), but accompanied by the elimination of hydroxyl from C₄ and conjugation of the resulting double bond with the ketone at C₇. In particular the frequency of the very strong absorption at 1631 cm⁻¹ is very strongly indicative of a hydrogen bonded, α - β unsaturated ketone.

(3).Oxidation of obacunonic acid with oxygen in the presence of potassium tertiary butoxide: Using conditions identical to those employed for the oxidation of limonin (see section 10), Templeton⁵³ oxidised obacunonic acid to a material which showed the diosphenol chromophore, nearly identical with that shown by limonin diosphenol, in the ultraviolet and infrared. However, instead of the composition C₂₆H₃₀O₉ expected for the predicted acid (XII), the product analysed for C₂₆H₂₈O₈, was insoluble in sodium bicarbonate solutions and was shown to be a dilactone. A plausible structure for this material is the lactone (XIII), which may be formed from the initial product

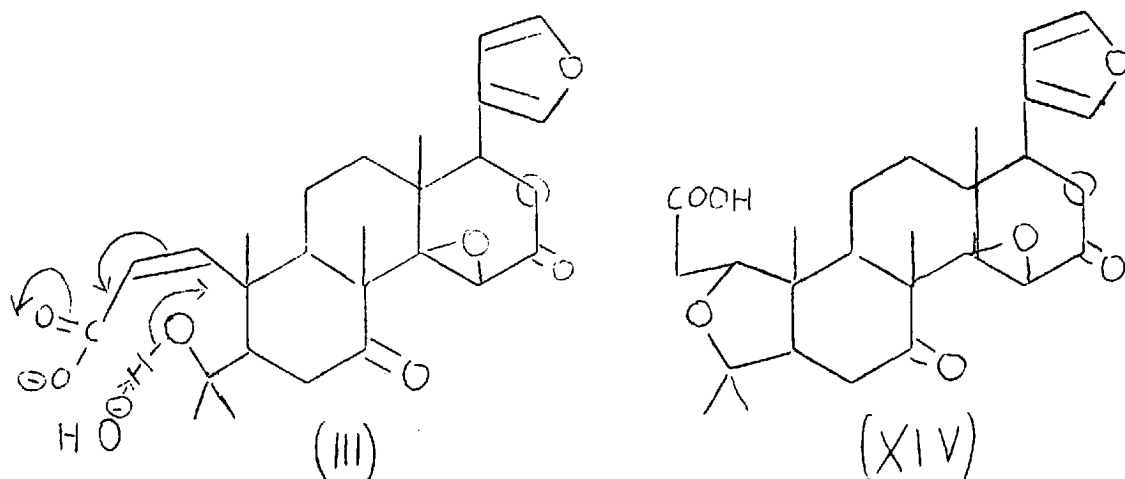
(XII) by an indirect mechanism:



iv. Formation of iso-obacunoic acid

Treatment of methyl obacunoate or obacunoic acid with methanolic sodium methoxide gave an isomeric acid, iso-obacunoic acid (XIV). In accordance with structure (XIV), this material had a considerably lower absorption in the ultraviolet (λ_{max} : 208 m μ , ϵ : 5,200) than obacunoic acid, and lacked the band at 1630 cm $^{-1}$ in the infrared which was assigned to the double bond conjugated with the carboxylic acid carbonyl in the spectrum of obacunoic acid. Bands at 3500-3000 cm $^{-1}$ (carboxylic hydroxyl), 1741 cm $^{-1}$ (lactone D), 1716 cm $^{-1}$ (ketone and carboxylic acid), as well as furan bands in the infrared spectrum of iso-obacunoic acid support structure (XIV).

The mechanism proposed for the formation of iso-obacunoic acid from obacunoic acid is shown below. It can be seen that it is analogous to that which may be involved in the regeneration of limonic acid from pyrolysed limonilyl chloride (section 11e) and to that proposed for the formation of limo-clastic acid (section 9):



v. Interpretation of the positive iodoform test
given by obacunoic acid

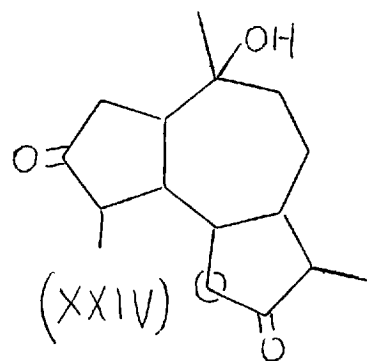
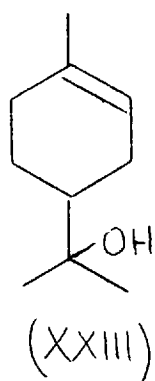
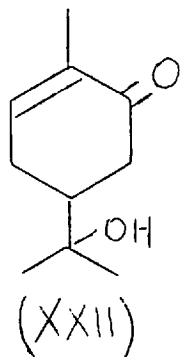
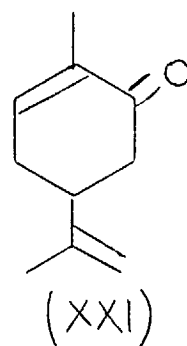
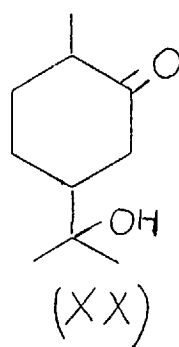
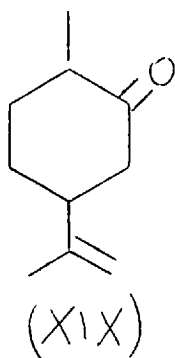
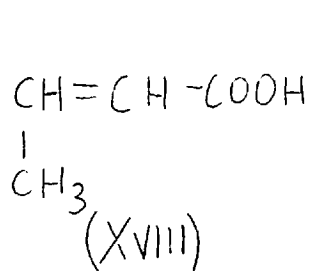
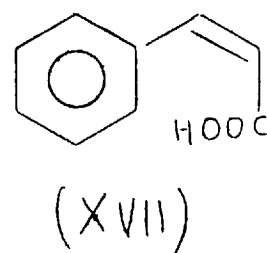
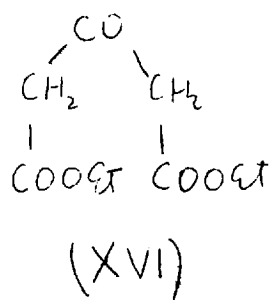
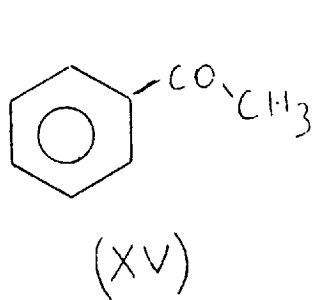
Dean and Geissman¹⁵ reported that obacunoic acid gave a positive iodoform test. This finding was confirmed in the course of the present work, when it was found that a highly purified sample of obacunoic acid also gave a positive iodoform test, while iso-obacunoic acid failed to react with iodine and

alkali and was recovered unchanged from the reaction mixture.

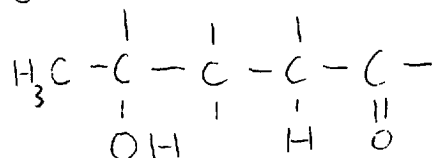
Iodoform tests were then carried out on a number of model compounds using a standard procedure⁹⁷:

<u>STRUCTURE</u>	<u>SUBSTANCE</u>	<u>% YIELD IODOFORM</u>
XV	Acetophenone.....	30
XVI	Acetonedicarboxylic ester.....	16,21
III	Obacunoic acid.....	15
XIV	<u>iso</u> -Obacunoic acid.....	nil
	Limonin	nil
XVII	Cinnamic acid.....	nil
XVIII	Crotonic acid.....	nil
XIX	Dihydrocarvone ⁹⁸	nil
XX	Dihydrocarvone hydrate ⁹⁹	4
XXI	Carvone.....	nil
XXII	Carvonehydrate ¹⁰⁰	6
XXIII	α -Terpineol.....	nil
XXIV	Dihydro- <u>iso</u> -photosantonin lactone ¹⁰¹ ...	4

From the consideration of the structures below, it can be seen that a positive iodoform test can be obtained not only with methyl ketones (XV), or with substances which hydrolyse to methyl ketones (XVI).

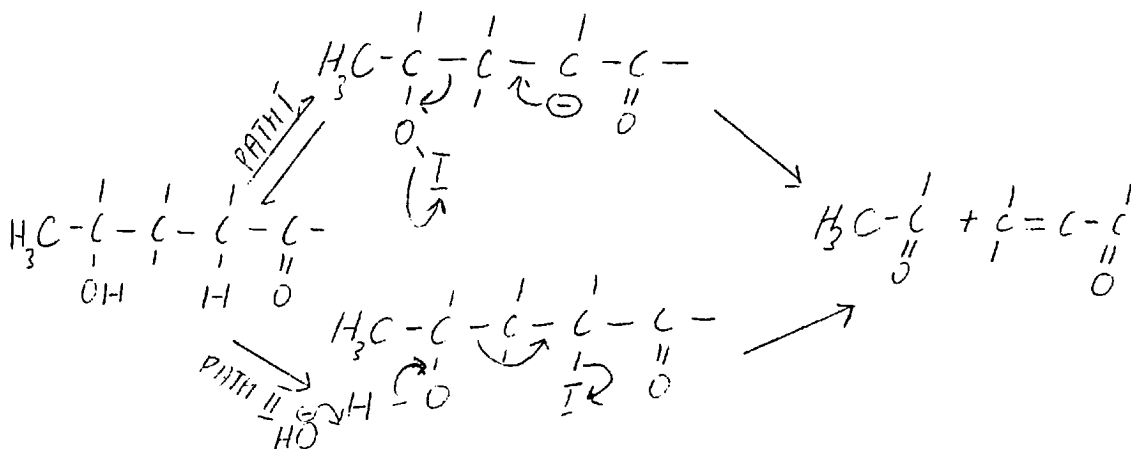


but also with substances (XX), (XXII), (XXIV) containing the grouping



and as such a grouping has been postulated for obacunoic acid (III) but not for iso-obacunoic acid (XIV), the positive iodoform test obtained with the former and the negative result with the latter provides some evidence for the correctness of the structure assigned to obacunoic acid and hence to obacunone.

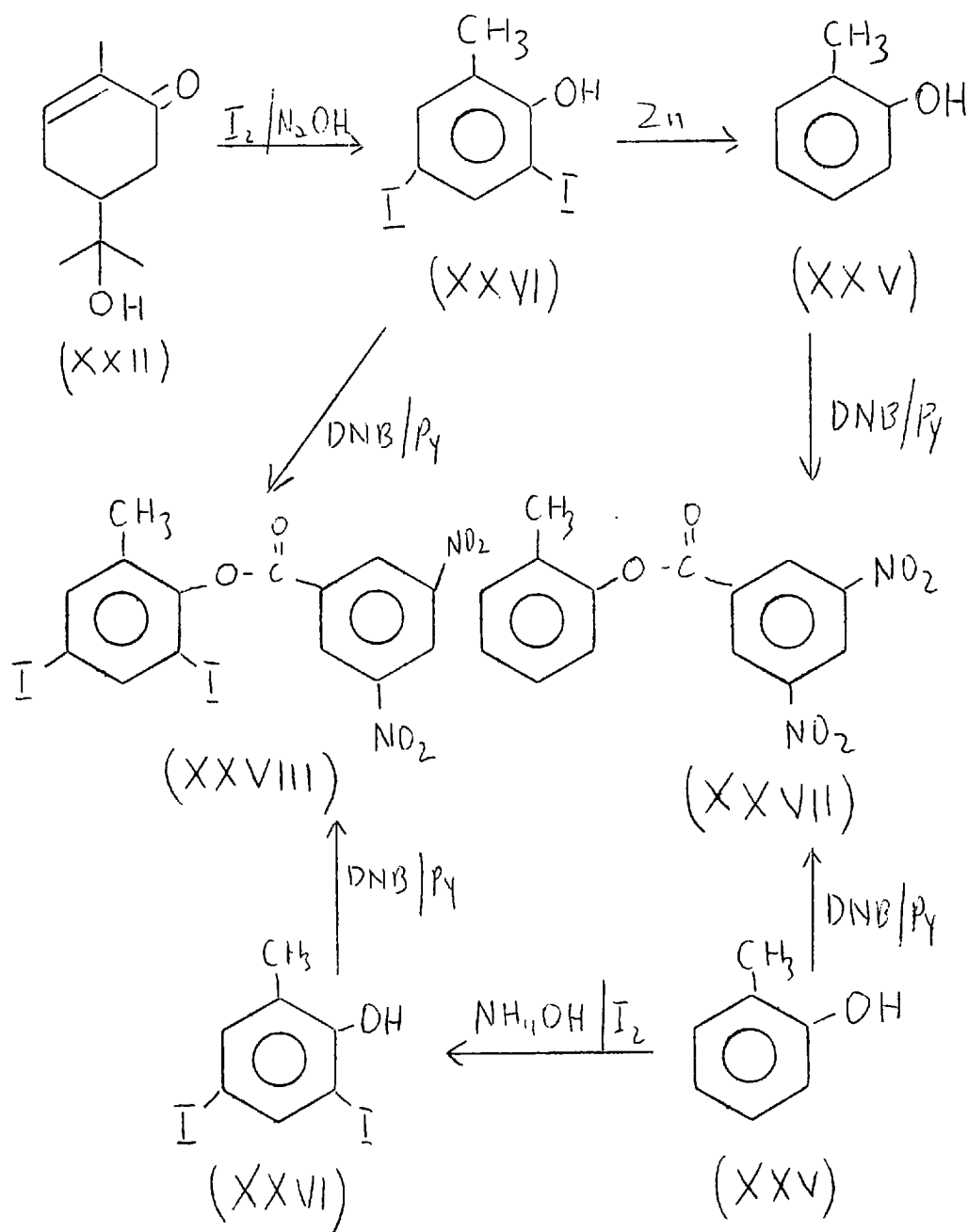
Two mechanisms may be postulated for the reaction:



Path I involves a preliminary equilibrium with the hapohalide while path II involves a preliminary α iodination of the ketone, (see section 11), and the methyl ketone formed then gives iodoform in the usual manner.

The major product of such a reaction should be an α - β unsaturated ketone. No chromophore assignable to such a structure could be found in the crude reaction product of obacunoic acid with iodine and alkali, but as the reaction which gives rise to iodoform takes place only to a small extent, it is possible that the α - β unsaturated ketone was further oxidised, or that it is undetectable in small concentration.

In the case of carvone hydrate, the expected product, orthocresol (XXV) , was isolated in a small yield following treatment with iodine and alkali and de-iodination of the phenolic fraction of the product. When the de-iodination step was omitted, di-iodo-o-cresol (XXVI) (probably the 2:4 diiodo isomer)¹⁰² was isolated. The cresols were identified by the comparison of their 3:5:dinitrobenzoates, (XXVII) and (XXVIII), with those prepared from authentic specimens. (Note: DNB stands for 3:5 dinitrobenzoyl chloride).



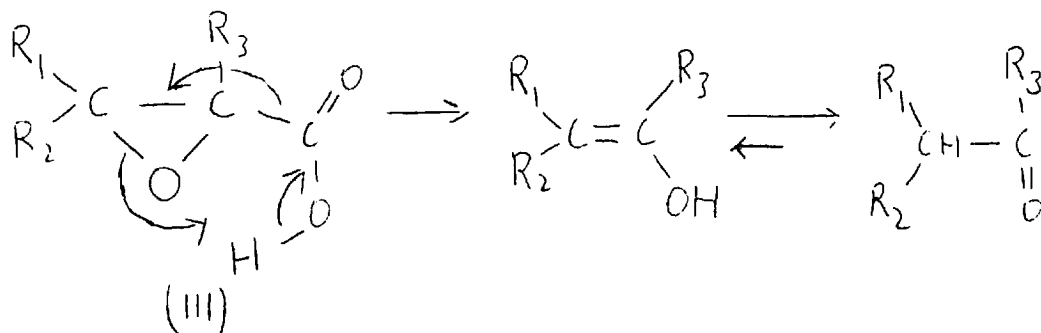
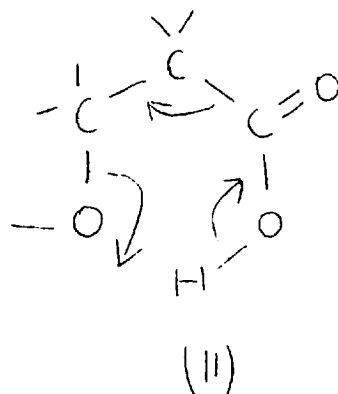
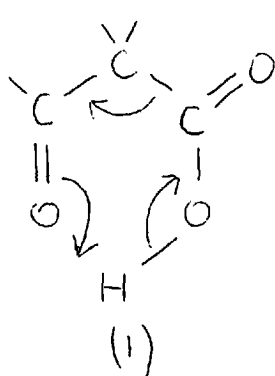
(vi) The NMR spectrum of methyl obacunoate

The table below lists some of the features of a high resolution proton magnetic resonance spectrum of methyl obacunoate , together with the assignments made. In particular , the AB quartet (J: 12 c.p.s.) is almost identical with that shown by the spectrum of cis methyl crotonate¹⁰³ and five distinct methyl peaks may be seen as compared with four in the spectrum of limonin and citrolin. In general, this evidence lends strong support for the assignment of structure (III) to obacunoic acid.

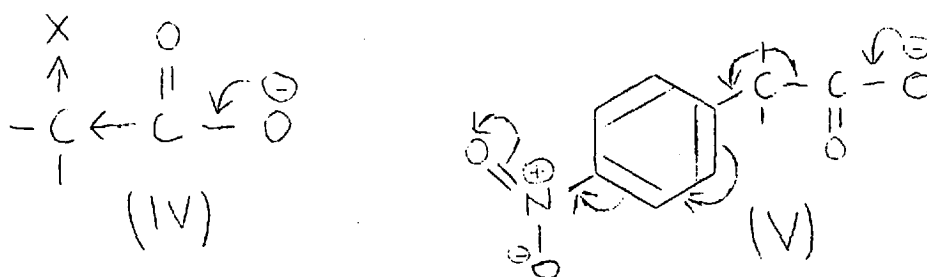
<u>Tau (p.p.m.)</u>	<u>Assignment</u>
9.043 9.008	{ Four angular methyl groups at C ₁₉ , C ₂₈ , C ₂₉ and C ₃₀
8.97 8.88	
8.618	Methyl group adjacent to furan (C ₁₃)
6.33	Methyl ester group
4.63	Proton at C ₁₇
4.490 4.24	{ αH } A-B quartet at C ₁ -C ₂
4.08 3.798	
	{ β H }
3.668	β hydrogen of furan ring
2.506	α hydrogens of furan ring

16. ATTEMPTED THERMAL DECARBOXYLATION OF SOME
DERIVATIVES OF CARBOXYLIC ACIDS

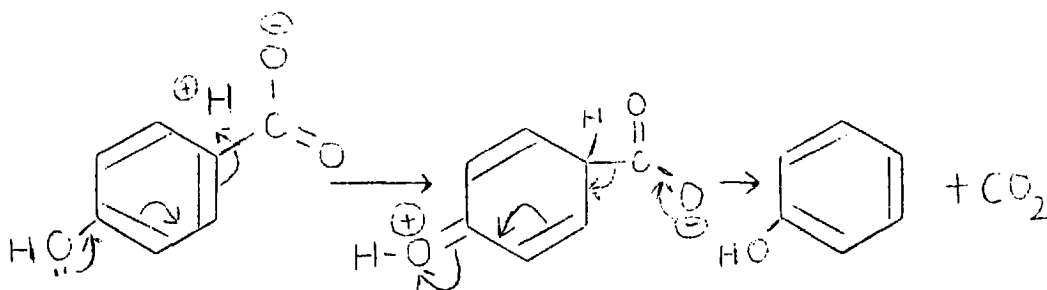
In general, uncatalysed thermal decarboxylation of carboxylic acids can be accomplished under relatively mild conditions, only if the environment of the carboxylic carbonyl group is favourable¹⁰⁴. Thus β -keto acids¹⁰⁵ (I) and β -oxy acids (II) are believed to decarboxylate by cyclic mechanisms shown below. An interesting example of the latter, is the decarboxylation of glycidic acids¹⁰⁶ (III), which is the final step in Darzen's glycidic ester synthesis⁴⁷.



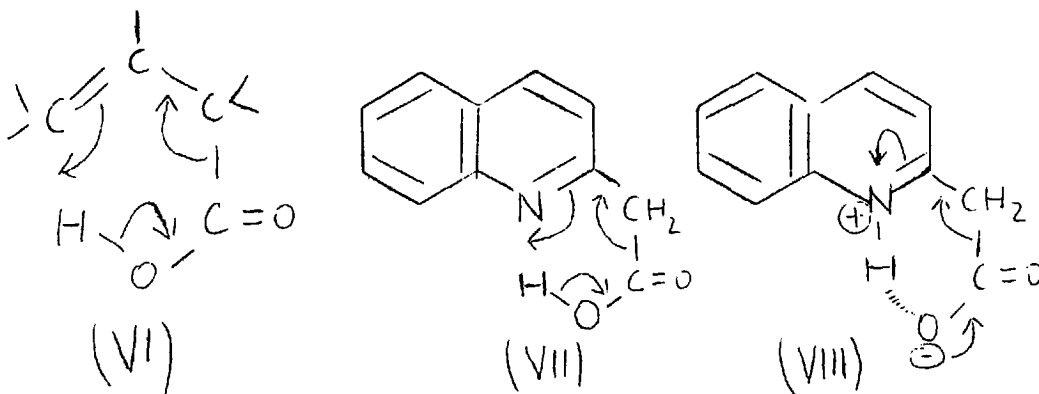
The presence of a negative substituent in the α position to the carboxylic carbonyl can also facilitate thermal decarboxylation - which often proceeds via the carboxylate ion - either by an inductive effect (IV) or by electron shift (V):



The decarboxylation of hydroxy benzoic acids has been formulated¹⁰⁷ as proceeding via an electrophilic substitution by a proton - which is still available in the mildly alkaline solution used - followed by decarboxylation proper according to the scheme set out below:

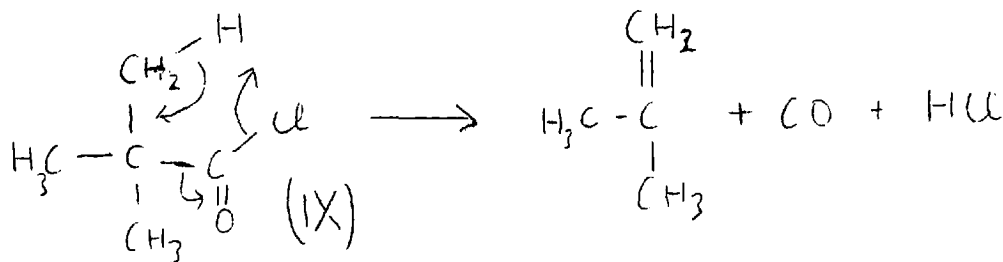


A cyclic intermediate was also postulated for the thermal decarboxylation¹⁰⁸ of β - γ unsaturated carboxylic acids (VI), and it was also suggested that α - β unsaturated carboxylic acids are decarboxylated after a prior isomerisation of the double bond to the β - γ position¹⁰⁹.



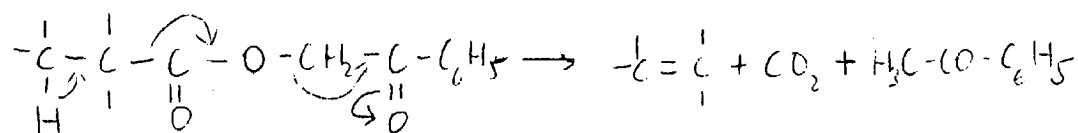
It is also known¹⁰⁸ that some heterocyclic acids (e.g. 2-quinolyl acetic acid) decarboxylate readily. Again, attractive cyclic transition states involving either the acid itself (VII) or its zwitterion (VIII) can be postulated.

An interesting example of decarboxylation is the decarbonylation of pivalic acid chloride (IX)¹¹⁰, possibly involving the following mechanism, although catalysis by aluminium chloride suggests that carbonium ions are involved:

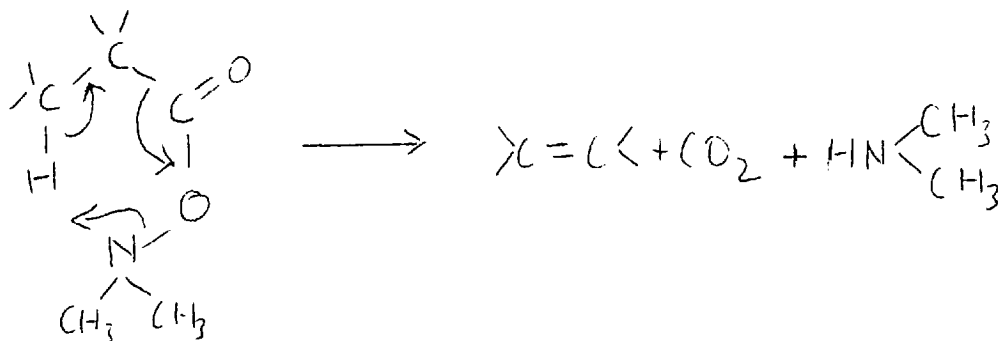


In the course of the present work, an attempt was made to produce structures electronically favourable for decarboxylation by a suitable choice of R' in ester-type structures R-CO-O-R'.

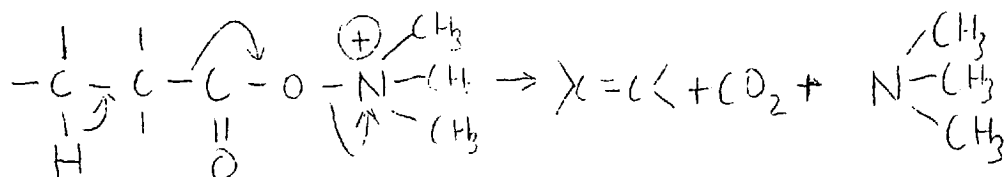
It appeared possible that phenacyl esters of carboxylic acids which had a hydrogen in the β position to the carboxylic carbonyl may decompose thermally in the following manner to give unsaturated compounds:



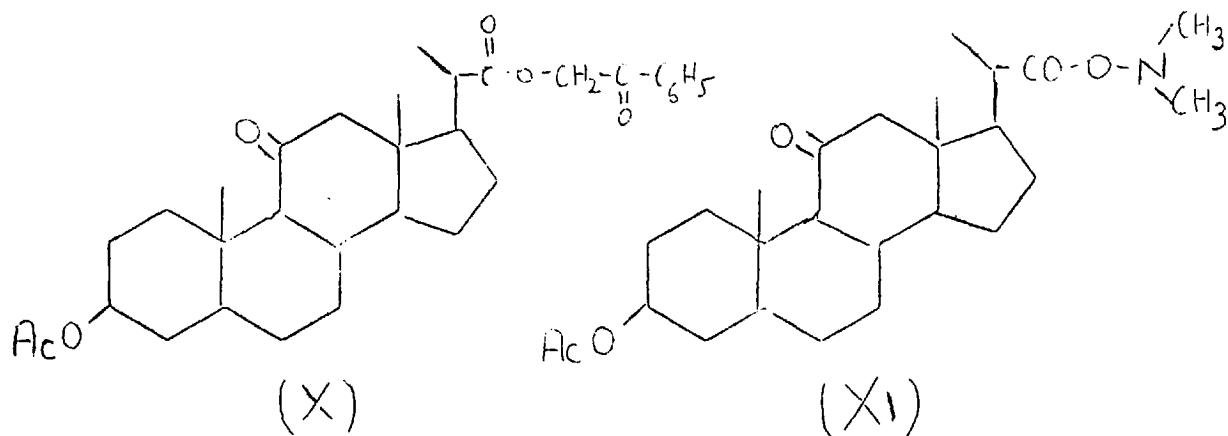
Another route to decarboxylation appeared in a thermal decomposition of O-acyl-NN-dimethyl hydroxylamines^{111,112,113}, possibly involving a cyclic transition state:



An even more favourable case would be presented by the thermal decomposition of quarternised O-acyl-NN-dimethyl hydroxylamines:



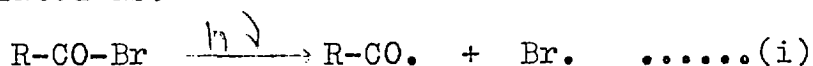
The desired intermediates (X) and (XI) derived from the model 3- β -acetoxy-11-keto-bisnor-allo-cholanic acid¹¹⁴ were prepared, but all efforts to decarboxylate them thermally were unsuccessful. Quarternisation of (XI) followed by pyrolysis also did not yield any decarboxylated material.



17. IRRADIATION OF ACYL CHLORIDES WITH ULTRA-VIOLET LIGHT

The irradiation of acyl chlorides in solution was studied as a possible general method for decarboxylation of carboxylic acids.

A literature search revealed that no such studies have been described, although the photolysis of a simple acyl bromide in the gas phase has been described¹¹⁵. From the consideration of the nature of the products obtained, which included carbon monoxide, dialkyl, diacyl, paraffin and alkyl halide species, the primary process was postulated as:



The acyl radicle could then dissociate into an alkyl radicle and carbon monoxide:

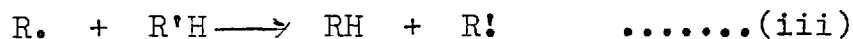


and reactions of the various radicles formed can account for all the products observed.

In the present investigation, 3- β -acetoxy-11-keto-bisnor-allo-cholanolic acid chloride (II), derived from the known acid¹¹⁴ (I) was found

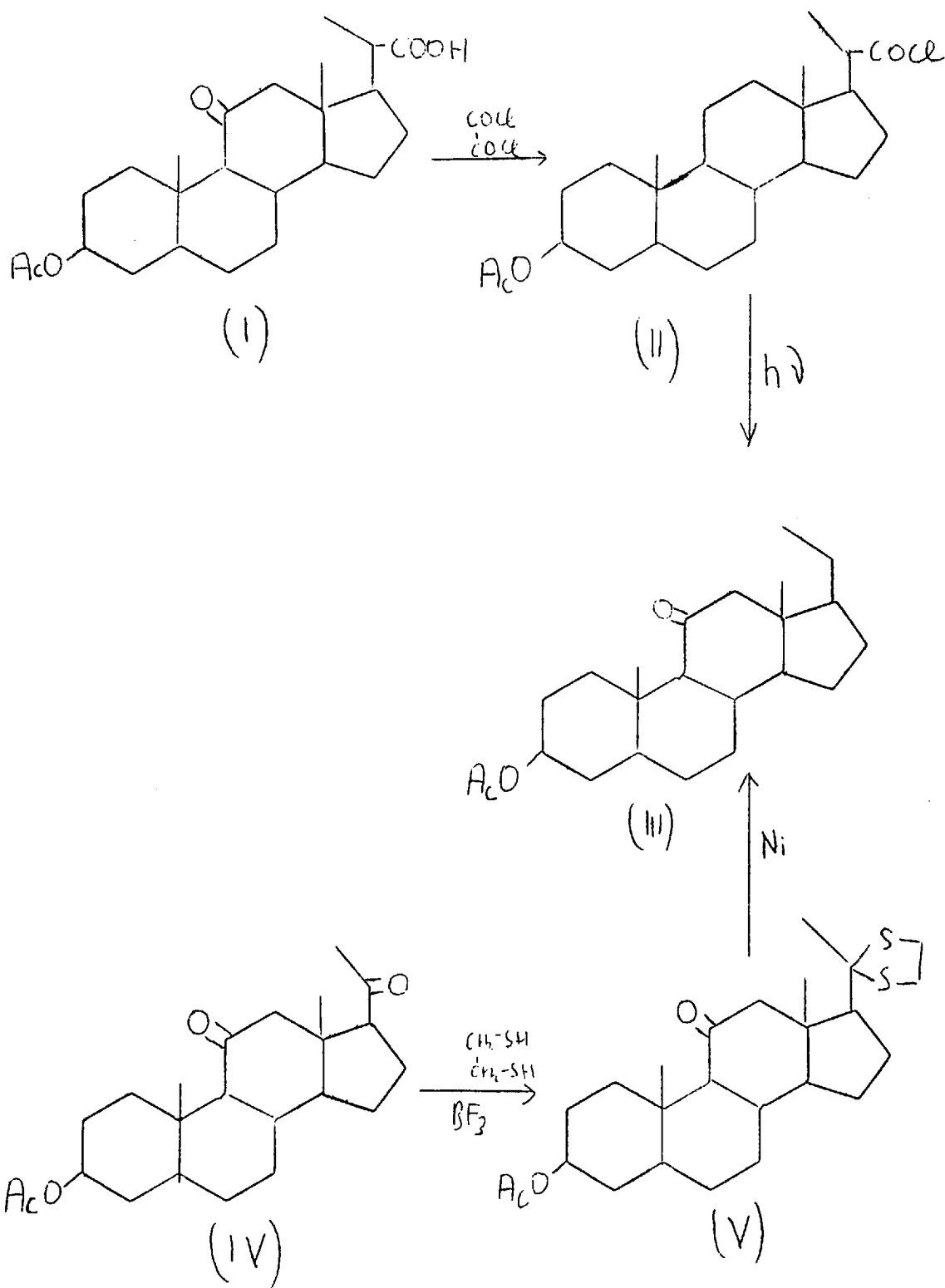
to yield a neutral product of composition corresponding to (III) on irradiation in dioxane solution. The structure of (III) was independently proved by synthesis from the known^{116,117} 3- β -acetoxy-11:20-diketo-allo-pregnane (IV) via the 20-dithioketal¹¹⁸. The formation of the 20 rather than of the 11 derivative was assumed on grounds of better steric accessibility of the 20 position. A Huang-Minlon reduction of (IV) resulted in the formation of the known^{119,120} 3- β -hydroxy-allo-pregnane.

To account for the formation of the saturated decarboxylated derivative (III), one must postulate an exchange reaction of the type:



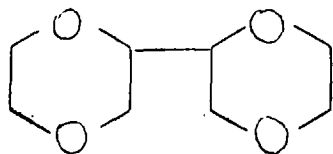
which is a common process in photochemical and free radical reactions¹²¹. In the present case, $R'H$ could be a solvent molecule or another molecule of the photolysed species.

The irradiation of another model compound¹¹, 3- β -11- α -diacetoxy-bisnor-allo-cholanic acid chloride, and of limonilyl chloride resulted only in the formation of amorphous neutral materials whose infrared spectra showed that decarboxylation has taken place.



Thus it appears that although acid chlorides can be generally decarboxylated by irradiation in dioxane , the method suffers from practical difficulties , possibly due to the large number of side reactions possible.

It was also observed , that irradiation of dioxane itself resulted in the formation of



(VI)

a product whose elementary composition and molecular weight corresponded to $C_8H_{14}O_2$ and whose infra-red spectrum had no bands in either the O-H stretching, the carbonyl or the double

bond regions. The NMR spectrum showed only a single broad band in the same position as that shown by the spectrum of dioxane. On the basis of the above, structure (VI) was assigned to this material.

The reaction leading to di-dioxane (VI), was not investigated in detail, but preliminary results indicate that traces of oxygen in the nitrogen which was passed through all the irradiated mixtures , might be responsible.

EXPERIMENTAL

General

a) Unless otherwise stated all ultraviolet spectra were determined in ethanol on a Unicam model 500 spectrophotometer.

b) Unless otherwise stated all infrared spectra were determined in a Nujol mull on a Perkin-Elmer model 21 spectrophotometer.

c) The phrase "working up" indicates that the reaction mixture was poured into chloroform, washed with water and extracted with a solution of sodium bicarbonate. The chloroform extract was evaporated to dryness to yield the crude neutral fraction. The bicarbonate solution was acidified, extracted with chloroform and the extract was evaporated to dryness to yield the crude acidic fraction. In the case of reactions where hydroiodic acid was used the original chloroform extract was washed with saturated sodium sulphite solution. Where pyridine was used, the chloroform extract was washed with dilute hydrochloric acid.

d) All melting points were determined on a Koffler block.

Extraction of limonin from grapefruit seeds

Freshly ground grapefruit seeds (2.5 kg) were soaked in acetone (12 l) for 8 days with occasional stirring. The extract was filtered and the crushed seeds were washed with a further portion of acetone (3 l). The combined extracts were distilled under atmospheric pressure until the temperature of the liquid reached 110°C . The syrupy residue was poured into petroleum ether (Bp. $60-80^{\circ}\text{C}$, 2.5 l) and cooled for two days at 0°C . The precipitate was collected, washed with methanol (2 x 100 ml) and crystallized from methylene dichloride/ iso-propanol to give limonin (21.4 g).

Mp.: $290-2^{\circ}\text{C}$

$(\alpha)_{\text{D}}$: -124° (c:1.3, acetone)

U.V.: λ_{max} : 207-10 m μ , ϵ :6,800

I.R.: 1761, 1709, 1504, 876 cm^{-1}

I.R. in chloroform: 1755, 1715, 1505, 875 cm^{-1}

I.R. of disodium salt : 1690 cm^{-1}

Hydrogenation of limonina) Over rhodium-carbon at atmospheric pressure

Limonin (10 g) was dissolved in glacial

acetic acid (500 ml) and hydrogenated at atmospheric pressure over 5% rhodium-carbon (10 g) for 8 hours when uptake of hydrogen ceased. The reaction mixture was filtered , the solvent was distilled under vacuum and the product was worked up as usual. On recrystallization from methylene-dichloride/methanol the neutral fraction gave tetrahydrolimonin (6.1 g), Mp.: 280-93°C.

Acidification of the bicarbonate extract followed by prolonged cooling yielded crystalline hexahydrolimoninic acid (1.34 g), Mp.: 165-8°C , not depressed by mixing with an authentic specimen.

b) Over palladium-carbon at atmospheric pressure

Limonin (7.0 g) in glacial acetic acid (500 ml) was hydrogenated over 10% palladium-carbon (7.0 g) for 3 hours when uptake of hydrogen ceased. The reaction mixture was filtered , the solvent was distilled under vacuum and the residue was worked up as usual except that a saturated solution of sodium carbonate was used to extract the acidic component . Acidification of the carbonate extract followed by prolonged cooling gave crystalline

hexahydrolimoninic acid (5.4 g) ,Mp.: 163-71°C .

I.R.: broad band near 3400,1750,1710-1700 cm⁻¹

Crystallization of the neutral fraction from methylene dichloride/methanol yielded tetra - hydrolimonin (0.6 g), Mp.:285°C.

c)Over rhodium-carbon at high pressure

Limonin (10 g) in glacial acetic acid (500 ml) was hydrogenated over 5% rhodium-carbon (10 g) for 12 hours at 125 atmospheres with stirring. The reaction mixture was filtered, the solvent was distilled under vacuum and the product worked up as usual. On crystallization from methylene dichloride/methanol, the neutral fraction yielded hexahydrolimondiol (4.46 g) , Mp.:299-302°C (α)_D: -50° (c: 1.0 , pyridine).

Acidification of the bicarbonate extract followed by prolonged cooling gave hexahydrolimoninic acid (0.7 g) , Mp.: 161-6°C not depressed by mixing with an authentic specimen.

Desoxylimonin

a) By action of hydroiodic acid on limonin

Limonin (10 g) in a mixture of glacial

acetic acid (80 ml) and hydroiodic acid (S.g. 1.7, 40 ml) was warmed at 50-60°C for 3½ hours. Working up as usual gave neutral fraction (6.9 g) which on crystallization from methylene dichloride/methanol yielded desoxylimonin (5.23 g).

Mp.: 325-33°C

U.V.: λ_{max} : 213.5 mμ , ϵ : 15,800

I.R.: 1746, 1715, 1695(sh), 1503, 875 cm⁻¹

I.R.: in chloroform: 1755, 1715 cm⁻¹

b) By action of chromous chloride on limonin

Limonin (530 mg) in glacial acetic acid (25 ml) and acetone (25 ml) was treated with a solution of chromous chloride (1M , 14 ml) under nitrogen. The mixture was allowed to stand at room temperature for 44 hours, acetone was distilled under vacuum and the residue was poured into a large volume of water. The crystalline precipitate which appeared on cooling was collected and recrystallized from methylene dichloride/methanol to give desoxylimonin (444 mg) , Mp.: 327-35°C identified by its infrared spectrum.

Tetrahydordesoxylimonin

Desoxylimonin (514 mg) in glacial acetic acid (50 ml) was hydrogenated over 10% palladium - carbon (500 mg) until hydrogen uptake ceased (66ml at 20°C). Working up as usual gave neutral material (517 mg) which was crystallized from methylene di - chloride/ethanol to give tetrahydordesoxylimonin. The analytical sample was dried under vacuum at 145°C for 40 hours.

Calculated for $C_{26}H_{34}O_7$: C:68.10% H:7.47%

Found: C:68.16% H:7.46%

Mp.: 292-300°C

(α)_D: -81° (c : 1.2 , chloroform)

U.V.: λ_{max} : 218.5 m μ ϵ : 10,600

I.R.: 1752, 1720, 1707 cm⁻¹

Isotetrahydrolimonin

Tetrahydrolimonin (2.08 g) in glacial acetic acid (30 ml) and concentrated hydrochloric acid (20 ml) was warmed on the steam bath for 90 minutes. Working up as usual gave neutral (1.38 g) and acidic (593 mg) fractions . The neutral fraction was found to be partially soluble in cold dilute

sodium hydroxide solution. The remaining neutral material (225 mg) was identified by its melting point and infrared spectrum as unreacted tetra - hydrolimonin. The alkali soluble material was worked up as usual to give neutral (844 mg) and acidic (118 mg) fractions. The neutral fraction was crystallized from methylene dichloride/ethanol to yield isotetrahydrolimonin.

Mp.: 260°C

U.V.: λ_{max} :257 m μ ϵ :5,460

U.V. in presence of alkali: λ_{max} :290 m μ ϵ :4,200

I.R. a weak broad band near 3300, 1751, 1723 cm⁻¹

The two acidic fractions could not be crystallized. They had very similar infrared spectra and no maxima in the ultraviolet spectra. The second acidic fraction was found to have an equivalent weight of 221.

Ozonolysis of isotetrahydrolimonin

Isotetrahydrolimonin (249 mg) was dissolved in chloroform (50 ml), cooled to the temperature of a carbon tetrachloride/dry ice mixture and ozonised oxygen was passed through

the solution. After 25 minutes the absorption maximum at 257 m μ fell to a negligible value , water (15 ml) was added , chloroform was boiled off and the clear aqueous solution was warmed on the steam bath for 30 minutes. The solution was evaporated to dryness and the total residue was found to be soluble in sodium bicarbonate solution. It could not be crystallized.

The crude product was found to have an equivalent weight of 138 and addition of a solution of calcium nitrate to the solution of the hydrolysed material obtained from the determination of equivalent weight caused the immediate precipitation of calcium oxalate which was identified by its infrared spectrum. The major hydrolysis product was acidic and could not be crystallized.

Desoxylimonic acid

Desoxylimonin (2.0 g) was dissolved in 2N sodium hydroxide solution (50 ml) and heated on the steam bath for 100 minutes. The mixture was acidified with concentrated hydrochloric acid and worked up as usual to give acidic product (1.76 g) which

on crystallization from methylene dichloride /
iso-propanol yielded desoxylimonic acid.

Mp.: 250-2°C

(α)_D: -139° (c:1.0 , acetone)

I.R.: diffuse band near 3200, 1750(sh), 1734, 1719,
1503, 874 cm⁻¹.

Desoxylimonic acid was converted to
its methyl ester by treatment with excess ethereal
diazomethane in a dioxane solution. Mp.: 161-6°C
not depressed by admixture with an authentic specimen.

Tetrahydrodesoxylimonic acid

a) From tetrahydrodesoxyiodoacetate

Tetrahydrodesoxyiodoacetate (272 mg)
was shaken with a solution of potassium hydroxide
(3 g) in water (20 ml) until a homogenous solution
was obtained. The mixture was allowed to stand at
room temperature for 19 hours . Acidification with
dilute sulphuric acid caused precipitation of
crystalline tetrahydrodesoxylimonic acid (184 mg)
which was recrystallized from aqueous acetone. The
analytical sample was dried under vacuum at 105°C

for 18 hours.

Calculated for $C_{26}H_{36}O_8$: C:65.53% H:7.61%

Found: C:65.49% H:7.78%

Mp.: 276-84°C

(a)_D: -89° (c: 1.1 , pyridine)

Equivalent weight: 168

U.V.: end absorption at 202 mμ ε: 11,000

I.R.: diffuse 3200,1758,1730,1716 cm⁻¹

b)From tetrahydrodesoxylimonin

Tetrahydrodesoxylimonin (150 mg) was warmed on the steam bath with 2N sodium hydroxide solution (30 ml) for two hours. The solution was cooled and acidified with dilute hydrochloric acid. The crystalline precipitate was collected and recrystallized from aqueous acetone to give tetrahydrodesoxylimonic acid ,Mp.: 275-80°C whose infrared spectrum was identical with spectrum of the material prepared by the method a).

c)From desoxylimonic acid

Desoxylimonic acid (572 mg) in glacial acetic acid (75 ml) was hydrogenated over 10% palladium-carbon (1.0 g) which was previously prehydrogenated. The material took up 51 ml of hydrogen and working up as usual gave acidic material

(620 mg) which on crystallization from aqueous acetone yielded tetrahydrodesoxylimonic acid Mp.: 266-72°C whose infrared spectrum was identical with that of a specimen prepared by method a).

Ozonolysis of tetrahydrodesoxylimonic acid

In a typical experiment , tetrahydrodesoxylimonic acid (130 mg) was ozonised in a mixture of glacial acetic acid (30 ml) and chloroform (45 ml) at -13°C. Aliquots were removed at intervals and worked up by addition of water and distillation of solvents under vacuum. The ultraviolet spectra of materials thus obtained showed no maxima in ethanol solution but on addition of alkali a peak appeared at 273 mμ which disappeared on re-acidification. The intensity was found to vary with the time of ozonolysis reaching a maximum of ε:5,500 after 10-15 minutes and decreasing sharply after longer periods. The materials thus obtained could not be crystallized although a variety of ozonolysis procedures was used including reactions with calculated quantities of ozone as estimated by an iodometric method.

The crude ozonolysis products gave immediate positive iodoform tests. A model substance, diethylacetonedicarboxylic ester, also gave a positive iodoform test but not immediately.

Attempted bromination of tetrahydrodesoxylimonic acid

Tetrahydrodesoxylimonic acid (44 mg) in glacial acetic acid (4 ml) was treated with excess bromine in glacial acetic acid. The mixture was allowed to stand at room temperature for two hours, was warmed briefly on the steam bath and the solvent was distilled under vacuum. The crude residue (50 mg) crystallized on addition of a drop of acetone to give unchanged starting material identified by its melting point (275-80°C), mixed melting point and infrared spectrum.

Treatment of tetrahydrodesoxylimonic acid with acid

Tetrahydrodesoxylimonic acid (51 mg) was warmed on the steam bath with a mixture of concentrated hydrochloric acid (3 ml) and dioxan (3 ml) for 18 hours. Working up as usual gave crude

acidic product (45 mg) which had infrared and ultraviolet spectra similar to those of the starting material but which could not be crystallized.

Chlorination and dehydrochlorination of tetrahydro-desoxylimonic acid

In a typical experiment, tetrahydro-desoxylimonic acid (720 mg) was dissolved in glacial acetic acid (100 ml) and a solution of chlorine (0.20 milimole) in carbon tetrachloride (28 ml) was added. The mixture was allowed to stand in the dark at room temperature for 16 hours, the solvents were distilled under vacuum and the solid residue was heated under vacuum for 3 hours at 110°C. Crystallization from acetone/ether gave cisoid diene acid (390 mg).

Calculated for $C_{26}H_{34}O_8$: C:65.80% H:7.22%

Found: C:65.70% H:7.40%

Mp.: 259-69°C

$(\alpha)_D$: +231° (c:0.8, chloroform)

U.V.: λ_{max} : 255 m μ ϵ : 7,750 (a broad band)

I.R.: 1760, 1714, 1703, 1619, 1600 cm^{-1}

I.R. in chloroform : almost identical

Cisoid diene acid was converted to its methyl ester by treatment with excess ethereal diazomethane at room temperature for 45 minutes ,working up as usual and crystallization from acetone/ether.

Calculated for $C_{27}H_{36}O_8$: C:66.37% H:7.43%

Found: C:66.21% H:7.34%

Mp.:200-204°C

$(\alpha)_D$: +226 (c: 1.3 , chloroform)

U.V.: λ_{max} : 255 m μ ϵ :7,700 (a broad band)

Further slow crystallization of the residue from the preparation of the cisoid diene acid from acetone/ether gave transoid diene acid (80 mg).

Calculated for $C_{26}H_{34}O_8 \cdot \frac{1}{2}H_2O$: C:64.60% H:7.30%

Found: C:64.76% H:7.02%

M.p.:260-71°C

$(\alpha)_D$: +260 (c:1.2 methylene dichloride)

U.V.: λ_{max} :230 m μ ϵ :6,300 and λ_{max} :284m μ ϵ :16,400

I.R.:1752,1731,1704,1594 cm^{-1}

The transoid diene acid was converted to itsmethyl ester by treatment with excess ethereal diazomethane at room temperature for 45 minutes, working up as usual and crystallization from

acetone/ether.

Calculated for $C_{27}H_{36}O_8 \cdot \frac{1}{2}H_2O$: C:65.68% H:7.50%

Found: C:65.09% H:7.19%

Mp.:207-12°C

$(\alpha)_D$: +234° (c: 1.8 , chloroform)

U.V.: λ_{max} :229m μ ϵ :5,400 and λ_{max} :283 m μ ϵ :18,650

Note: The crude material obtained after chlorination but before heating had the following ultraviolet spectrum: λ_{max} :230 m μ ϵ :6000 and a shoulder approximately at 250 m μ ϵ :4000. All attempts of crystallization resulted only in the cisoid diene acid being isolated. On heating the spectrum of the material changed slowly to the one expected from a mixture of cisoid and transoid diene acids. Alcoholic potassium hydroxide or collidine had an effect similar to heating but the products were darker and difficult to crystallize. Although only small amounts of the transoid diene acid were actually isolated , the ultraviolet spectra of the mother liquors indicated that the actual proportions of the cisoid/transoid isomers formed were between 2/1 and 4/1.

Ozonolysis of cisoid diene acid

Cisoid diene acid (133 mg) was dissolved in chloroform (20 ml) and ozonised at -20°C for 15 minutes. Zinc dust (0.5 g) and water (8 ml) were added and the mixture was distilled. The aqueous layer of the distillate was separated, treated with excess aqueous dimedone reagent, allowed to stand for 20 hours and the dimedone-formaldehyde adduct (identified by its melting point and mixed melting point) was collected, dried and weighed. Yield: 18.5 mg (13.2% of theory).

Under identical conditions, pyrethrosin ($\text{C}_{17}\text{H}_{22}\text{O}_5$, 110 mg) gave 14.8 mg of the dimedone-formaldehyde adduct (13.4% of theory). Blanks using tetrahydrolimonin (100 mg) gave less than 2 mg of precipitate with the dimedone reagent.

The main ozonolysis product could not be crystallized. The ultraviolet spectrum of the crude material showed a small (ϵ : 2,000) peak at 384 m μ in presence of alkali which disappeared on re-acidification.

Cisoid diene acid oxide

Cisoid diene acid (74 mg) was dissolved in peracetic acid (80% , 2 ml) previously saturated with sodium acetate. The mixture was allowed to stand for 16 hours at 0°C and the excess peracetic acid was destroyed with sodium sulphite. Working up as usual and crystallization from acetone/ether gave cisoid diene acid oxide (35 mg). The analytical sample was dried under vacuum for 28 hours at 110°C.

Calculated for $C_{26}H_{34}O_9$: C:63.66% H:6.99%

Found: C:63.87% H:7.13%

Mp.: 243-9°C

$(\alpha)_D$: +88° (c: 0.4 , chloroform)

U.V.: λ_{max} : 217-8 mμ ϵ :9,100

I.R.: 1767,1718,1708 cm^{-1}

Treatment of cisoid diene acid oxide with boron trifluoride

Cisoid diene acid oxide (3.8 mg) was dissolved in boron trifluoride-ether (45%,0.5 ml) and the mixture was allowed to stand at room temperature for 15 hours. The mixture was poured into water and worked up as usual to give an amorphous

material whose ultraviolet spectrum showed no maxima in ethanol but a maximum appeared at 390 m μ (ϵ :2000) following the addition of a drop of alkali and disappeared on re-acidification.

Chlorination of cisoid diene acid

Cisoid diene acid (28.5 mg) was dissolved in glacial acetic acid (20 ml) and a solution of chlorine in carbon tetrachloride (3 ml, approx. 0.05 mmole of chlorine) was added. The mixture was allowed to stand in the dark at room temperature for 16 hours. Blank determinations and the determination of excess chlorine in the reaction mixture showed that 1.2 moles of chlorine were taken up. Working up as usual gave an acidic product which could not be crystallized. U.V.: λ_{max} :225 m μ ϵ : 7,000.

Attempted isomerisation of cisoid diene acid

Samples of cisoid diene acid were heated on the steam bath in 6N hydrochloric acid perchloric acid/acetic acid, hydrochloric acid/dioxane, and hydrochloric acid/acetic acid. In no case a shift in the maximum in the ultraviolet spectrum larger

than 7 μ could be detected.

Treatment with sulphuric acid/acetic acid caused the maximum to shift to 284 μ but the intensity remained unchanged and no material could be recovered from the solution.

Isomer of hexahydrolimoninic acid

a) By action of hydrochloric acid in dioxane on hexahydrolimoninic acid

Hexahydrolimoninic acid (616 mg) was heated on the steam bath with a mixture of dioxane (10 ml) and concentrated hydrochloric acid (10 ml) for 17 hours. Working up as usual yielded neutral material (524 mg) which on crystallization from methylene dichloride/ethanol gave the isomer of hexahydrolimoninic acid.

Calculated for $C_{26}H_{36}O_8$: Active hydrogen: 0.212%

Found: Active hydrogen: 0.223%

Mp.: above 350°C

I.R.: 3280, 1787, 1754, 1634 cm^{-1}

b) By action of hydrochloric acid in methanol on hexahydrolimoninic acid

Hexahydrolimoninic acid (395 mg) was refluxed on the steam bath in a mixture of methanol (10 ml) and concentrated hydrochloric acid (10 ml). Aliquots were withdrawn at intervals and the specific rotation was determined. After $5\frac{1}{2}$ hours the specific rotation was -74.5° as compared with the initial value of -28.4° . The mixture was worked up as usual to give neutral material (227 mg) which on crystallization from methylene dichloride/ethanol yielded the isomer of hexahydrolimoninic acid
Mp.: above 345°C , identified by its infrared spectrum.

Acetylation of the isomer of hexahydrolimoninic acid

The isomer of hexahydrolimoninic acid (90 mg) was dissolved in a mixture of acetic anhydride (5 ml) and pyridine (5 ml) and allowed to stand at room temperature for 17 hours. The mixture was poured into water and worked up in the usual manner to give neutral material (70 mg) which on crystallization from ethanol yielded the acetate of the isomer of hexahydrolimoninic acid.

Mp.: $187-92^{\circ}\text{C}$

I.R.: 1788, 1755, 1710, 1235 cm^{-1} .

Action of hydroiodic acid and acetic anhydride
on limonin

Limonin (517 mg) was dissolved in a mixture of acetic anhydride (5 ml) , glacial acetic acid (20 ml) and hydroiodic acid (S.g. 1.94 , 12 ml) and allowed to stand at room temperature for 40 hours. Working up as usual gave neutral product (280 mg) which on crystallization from chloroform/ethanol yielded desoxylimonin , Mp.:312-28°C identified by its infrared spectrum.

Heptahydroiodoacetate

Hexahydrolimoninic acid (640 mg) was dissolved in a mixture of acetic acid (16 ml) and acetic anhydride (4 ml) and hydroiodic acid (S.g. 1.94 , 6 ml) was added with cooling. The mixture was allowed to stand at room temperature for 24 hours. Working up as usual yielded neutral product (751 mg) which on crystallization from ethanol gave heptahydroiodoacetate.The analytical sample was dried under vacuum for 48 hours at 80°C.

Calculated for $C_{28}H_{39}O_9I$: C:52.17% H:6.04% OAc:6.66%
Found: C:52.48% H:5.93% OAc:6.77%

Mp.: 218-21°C

I.R.: 3260, 1790, 1755, 1730, 1677, 1240 cm^{-1}

Octahydroacetate

Heptahydroiodoacetate (500 mg) was dissolved in glacial acetic acid (50 ml) and heated on the steam bath with zinc powder (3 g) for 10 minutes with constant shaking. Working up as usual and crystallization from methylene dichloride/ethanol gave octahydroacetate. The analytical sample was dried under vacuum at 120°C for 15 hours.

Calculated for $\text{C}_{28}\text{H}_{40}\text{O}_9$: OAc: 8.25% Active H: 0.194%

Found: OAc: 8.50% Active H: 0.183%

Mp.: 246-56°C

I.R.: 3280, 1790, 1751, 1730, 1683, 1240 cm^{-1} .

α -keto- γ -lactone

Isomer of hexahydrolimoninic acid (226 mg) was allowed to stand at room temperature with a well ground mixture of chromic oxide (700 mg) and pyridine (6 ml) for 22 hours. Water was added and sulphur dioxide was passed into the reaction mixture

until it turned green. Working up as usual gave neutral product (181 mg) which on crystallization from acetone/ethanol yielded α -keto- γ -lactone. The analytical sample was dried under vacuum for 7 days at 50°C.

Calculated for $C_{26}H_{34}O_8$: C:65.80% H:7.22%

Found: C:66.04% H:7.26%

Mp.:246-53°C

(α)_D: -43° (c: 1.5 ,chloroform)

U.V.:no strong absorption. On addition of alkali
a peak appeared slowly at 252 m μ (ϵ :5,700),
not reversible on re-acidification.

I.R.:1790,1775,1755,1720 cm^{-1} .

α -keto- γ -lactone acetate

Octahydroacetate (290 mg) in pyridine (5.5 ml) was slowly added to a well ground suspension of chromic oxide (364 mg) in pyridine (3 ml). The mixture was allowed to stand at room temperature overnight , water was added and sulphur dioxide was passed in until a green colour was obtained. Working up as usual gave neutral material (284 mg) which on crystallization from acetone/ethanol gave

α -keto- γ -lactone acetate. The analytical sample was dried under vacuum for 50 hours at 120°C.

Calculated for $C_{28}H_{38}O_9$: C:64.85% H:7.39% OAc:8.29%

Found: C:64.69% H:7.55% OAc:8.10%

Mp.: 235-9°C

$(\alpha)_D$: -45° (c: 1.6, acetone)

U.V.: no strong absorption. On addition of alkali

a peak appeared slowly at 250m μ (ϵ :4,500)

which could not be reversed with acid.

I.R.: 1792, 1775, 1750, 1730(sh), 1717(sh), 1235 cm^{-1}

Action of alkali on α -keto- γ -lactone

α -keto- γ -lactone (69 mg) was dissolved in a mixture of ethanol (35 ml) and 0.4N sodium hydroxide (2.00 ml) and allowed to stand for 20 minutes at 40°C. Backtitration with standard acid compared with a blank showed that the equivalent weight of the original substance was 246. Working up as usual gave an acidic product (32 mg) which could not be crystallized.

U.V.: λ_{max} : 244 m μ ϵ :3,800

I.R.: 1755, 1715, 1670 cm^{-1} .

The crude product (16 mg) was refluxed

in glacial acetic acid (3 ml) with zinc dust (100 mg) for 7 hours. Working up as usual gave an acidic product (13 mg) which could not be crystallized.

U.V.: $\lambda_{\max}$: 250 m μ ϵ : 2,200 .

Action of alkaline hydrogen peroxide on α -keto- γ -lactone

α -keto- γ -lactone (28 mg) was allowed to stand in 0.4 N sodium hydroxide solution (2.5 ml) for 25 minutes at room temperature. Hydrogen peroxide (100 volume, 0.5 ml) was added and the mixture was allowed to stand at room temperature for a further 15 minutes. Working up as usual gave an acidic product (10 mg) which could not be crystallized.

U.V.: $\lambda_{\max}$: 244 m μ ϵ : 4,300

Tetrahydrodesoxyiodoacetate

Tetrahydrolimonin (367 mg) in a mixture of glacial acetic acid (15 ml) and hydroiodic acid (S.g. 1.94 , 10 ml) was warmed at 55-58°C for 3½ hours. Working up as usual gave neutral product (438 mg) which on crystallization from ethanol yiel-

ded tetrahydrodesoxyiodoacetate . The analytical sample was dried under vacuum for 48 hours at 90°C. Calculated for $C_{28}H_{37}O_8I$: C:53.50% H:5.89% OAc:6.86% Found: C:53.81% H:5.99% OAc:6.89%
 Mp.: 238-9°C
 $(\alpha)_D$: -105° (c: 0.9 , chloroform)
 U.V.: λ_{max} : 219 m μ ϵ : 12,700
 I.R.: 1748, 1726, 1715, 1238 cm^{-1}

Limonilic acid

Limonin (10.0 g) was dissolved in 4N sodium hydroxide solution (250 ml) and a solution of iodine (6 g) and potassium iodide (6 g) in water (100 ml) was added at room temperature. The mixture was allowed to stand for 25 minutes, some solid sodium sulphite was added and the mixture was acidified with concentrated hydrochloric acid and allowed to cool for 2 hours. The resulting precipitate was collected, washed with water and re-crystallized from aqueous acetone to give limonilic acid (9.9 g).
 Mp.: 282-7°C
 I.R.: diffuse 3,200, 1741, 1721, 1702(sh), 1502, 874 cm^{-1} .
 I.R. of disodium salt: 1717 cm^{-1} .

Tetrahydrolimonilic acid

Tetrahydrolimonin (1.36 g) was dissolved in 4N sodium hydroxide solution (50 ml) and a solution of iodine (1 g) and potassium iodide (1 g) in water (20 ml) was added at room temperature. The mixture was allowed to stand for 20 minutes, some solid sodium sulphite was added and the mixture was acidified with concentrated hydrochloric acid and allowed to cool for 2 hours. The resulting precipitate was collected, washed with water and recrystallized from aqueous acetone to give tetrahydrolimonilic acid (750 mg).

Mp: 267-73°C (sinters at 180°C)

I.R.: 1742, 1725, 1695 cm^{-1}

Limonilyl chloride

Limonilic acid (1.93 g) in tetrahydrofuran (30 ml) was refluxed on the steam bath with oxalyl chloride (5 ml) for 1 hour. The mixture was evaporated to a syrupy consistency under vacuum and crystallized from benzene/petroleum ether to give limonilyl chloride (2.26 g). Beilstein test: +ve.

Mp.: 220-4°C

$(\alpha)_D: +84^\circ$ (c: 1.3 , chloroform)

I.R.: 1791, 1740, 1722, 1500, 872 cm^{-1}

No reliable analytical figures could be obtained as the material was prone to hydrolysis.

Methyl limonilate from limonilyl chloride

Limonilic acid (234 mg) in tetrahydrofuran (6 ml) was refluxed on the water bath with oxalyl chloride (1 ml) for one hour. Methanol (10 ml) was added and reflux continued for 10 minutes. The solvents were evaporated under vacuum and the mixture was worked up as usual to give neutral material (214 mg) which on crystallization from methanol yielded methyl limonilate , Mp.: $229-33^\circ\text{C}$ not depressed by admixture of an authentic specimen prepared by the action of ethereal diazomethane on limonilic acid. The two samples had identical infrared spectra.

Limonilyl malonic ester

Limonilic acid (680 mg) was refluxed in tetrahydrofuran (15 ml) with oxalyl chloride (3 ml) on the steam bath for 1 hour. The solvents

were distilled under vacuum until the mixture reached a syrupy consistency.

Sodium (300 mg) was dissolved in diethyl malonate (6 ml) in tetrahydrofuran (10 ml) by refluxing on the steam bath and the solution was added to the syrupy limonilyl chloride and reflux continued for one hour. Working up as usual gave a neutral material (704 mg) which could not be crystallized.

U.V.: $\lambda_{\max}$: 209 m μ ϵ : 6,800 and $\lambda_{\max}$: 263 m μ ϵ : 5,600

U.V. in presence of alkali: $\lambda_{\max}$: 272 m μ ϵ : 11,200

Methyl-limonilyl ketone

Limonilic acid (2.06 g) in tetrahydrofuran (30 ml) was refluxed on the steam bath with oxalyl chloride (5 ml) for 1 hour. The solvents were distilled under vacuum until the material reached a syrupy consistency and the crude limonilyl chloride was crystallized from benzene/petroleum ether and dried under vacuum at 50°C. The crystalline material was dissolved in tetrahydrofuran (30 ml) and poured slowly into ethereal diazomethane (prepared from 25 g of nitroso-N-methyl urea) with cooling in an

ice bath. The mixture was allowed to stand at room temperature for 1 hour and ether was distilled under vacuum at room temperature. The residue was dissolved in chloroform, shaken with hydroiodic acid (S.g. 1.7, 6 ml) for 5 minutes and worked up as usual. The neutral material was dissolved in ethanol (30 ml) and boiled with 0.4N sodium hydroxide solution (30 ml) on the steam bath for 30 minutes the ethanol being allowed to distill. The solution was cooled, extracted with chloroform, the extract was discarded and the alkaline solution was worked up as usual to give neutral material (1.58 g). This was dissolved in benzene (50 ml), filtered and chromatographed on alumina (grade I, 6 g). The material washed through with benzene (100 ml) and with 7% acetone/benzene (200 ml) was combined and crystallized from benzene petroleum ether to give methyl-limonilyl ketone (782 g).

Calculated for $C_{27}H_{32}O_8 \cdot 2C_6H_6$: C:73.10% H:6.92%

Found: C:73.26% H:6.88%

Mp: Benzene solvate: 110-112°C. Amorphous material precipitated from acetone with petroleum ether: 145°C.

(α)_D: +85° (c:1.1, acetone)

U.V. of benzene solvate: fine structure with principal maximum at 255 m μ (ϵ :357). Also $\lambda_{\max}$:288m μ (ϵ :54) and an inflexion near 310 m μ (ϵ : 40).

U.V. of material freed from benzene: $\lambda_{\max}$:207m μ
 ϵ :7,000.

Equivalent weight of benzene solvate: 600

I.R. of benzene solvate: 1735,1722,1712,1500,876,691cm⁻¹.
 (1735,1722,1713,1500,875 in cm⁻¹ in CCl₄ solution)

Action of zinc and acetic acid on methyl-limonilyl ketone

Methyl-limonilyl ketone (60 mg) was refluxed in glacial acetic acid (10 ml) with powdered zinc (1 g, previously activated by shaking with dilute aqueous copper sulphate, washing and drying) for 17 hours. The mixture was filtered, the solution was evaporated to dryness under vacuum and the residue was worked up as usual to give neutral material (54 mg) which yielded crystalline methyl-limonilyl ketone on addition of a drop of benzene, Mp.: 110°C, identified by its infrared spectrum.

Action of alkali on methyl-limonilyl ketone

Methyl limonilyl ketone (71 mg) was

warmed on the water bath with 1N sodium hydroxide solution (6 ml) for 1 hour. Working up as usual gave neutral material (64 mg) which crystallized on addition of a drop of benzene and had melting point and rotation identical with that of the starting material.

In another experiment , methyl-limonilyl ketone (0.630 mg) was heated on the steam bath in 1N sodium hydroxide solution (5 ml) and the ultra-violet spectrum was examined at intervals. No change was observed during 2 hours.

Reaction of methyl-limonilyl ketone with benzaldehyde in the presence of alkali

Methyl-limonilyl ketone (100 mg) in ethanol (10 ml) and 4N sodium hydroxide (3 ml) was allowed to stand at room temperature with benzaldehyde (1 ml) for 17 hours. Working up as usual gave neutral material (88 mg) which could not be crystallized.

U.V.: end absorption at 208 m μ : ϵ :16,000 and $\lambda_{\max}$:
293 m μ ϵ :3,100.

Oxidation of methyl-limonilyl ketone with iodine
and potassium hydroxide

Methyl-limonilyl ketone (41 mg) was dissolved in 1N sodium hydroxide (10 ml), excess iodine in potassium iodide solution was added and the mixture was allowed to stand at room temperature for 20 minutes. A copious precipitate of iodoform appeared as soon as the reagents were mixed. This was collected and crystallized from ethanol, Mp.: 121-3°C not depressed by mixing with an authentic specimen. The alkaline solution was worked up as usual to give an acidic product (25 mg) which could not be crystallized. The infrared spectrum of this material was very similar to that of limonilic acid. $(\alpha)_D^{25} : +86^\circ$ (c:0.9 , acetone)

Pyrolysis of limonilyl chloride

In a typical experiment , limonilyl chloride (33 mg) was heated under a stream of nitrogen at 234°C and the gas was passed into water and titrated with sodium hydroxide solution. In 8 minutes hydrochloric acid equivalent to 3.5 ml of 0.1 N alkali was evolved (63% of theory). Only neut-

ral material could be isolated from the residue on working up as usual.

Larger batches of limonilyl chloride were pyrolysed at 234°C under vacuum (0.5 mm Hg). The pyrolysis was virtually complete after 6 minutes and only neutral material could be isolated from the reaction mixture by working up in the usual manner. The material could not be crystallized.

Equivalent weight: 226, 233. After working up, the alkali treated material was acidic.

U.V.: λ_{max} : 207-10 m μ ϵ : 13,000. When the spectrum of methyl-limonilyl ketone was subtracted a maximum near 210-15 m μ remained (ϵ : 6,500).

I.R.: 1750, 1715, 1502, 875 cm^{-1} .

Treatment of pyrolysed limonilyl chloride with alkali

The crude neutral product of pyrolysis of limonilyl chloride (313 mg) was heated on the steam bath in acetone (10 ml) and 2N sodium hydroxide (5 ml) for 30 minutes. Working up as usual gave an acidic product (174 mg) which on crystallization from aqueous acetone yielded limonilic acid (50 mg) identified by its melting point and infrared spectrum.

Citrolin from desoxylimonin

Desoxylimonin (716 mg) was refluxed with hydrogen bromide in glacial acetic acid (50% weight/volume , 10 ml) for 40 minutes. The mixture was cooled, poured into water and worked up as usual to give only neutral material. Crystallization from tetrahydrofuran/iso-propanol yielded citrolin (392 mg).

Mp.: 294-8°C

U.V.: λ_{max} : 213.5 m μ ϵ : 25,200 and shoulder near 240 m μ ϵ : 18,500. When the spectrum of desoxylimonin was subtracted from that of citrolin two maxima appeared: 206-15m μ (ϵ : 9,500) and 244 m μ (ϵ : 13,700)

I.R.: 1724-1716, 1670, 1504, 875 cm⁻¹.

Hydrogenation of citrolin over palladium-carbon

In a typical experiment , citrolin (71.4 mg) in dioxan (10 ml) was hydrogenated over previously pre-hydrogenated palladium-carbon (10%, 114 mg) until 4.2 ml of hydrogen (approximately 1.05 mole) was absorbed. The mixture was filtered, the solution was evaporated to dryness under vacuum

and the residue was worked up as usual to give only neutral material. Crystallization from acetone/ethanol yielded dihydrocitrolin (50 mg). The analytical sample was dried under vacuum for 14 hours at 100°C.

Calculated for $C_{26}H_{30}O_6$: C:71.21% H:6.90%

Found: C:71.41% H:7.13%

Mp.: 282-6°C

$(\alpha)_D$: -106° (c:1.0, chloroform)

U.V.: λ_{max} : 216 m μ ϵ :17,200 and shoulder near 231-243 m μ (ϵ :14,700). When the spectrum of desoxylimonin was subtracted from that of dihydrocitrolin a maximum remained at 248 m μ ϵ :10,550

I.R.: 1756, 1712, 1667, 1500, 874 cm^{-1} .

In other experiments, when larger volumes of hydrogen were absorbed under similar conditions, a second crystalline neutral product, hexahydrocitrolin, could be isolated in a small yield besides dihydrocitrolin. Insufficient sample was available for analysis but material crystallised from ethanol/acetone had the following properties:

Mp.: 290°C

U.V.: λ_{max} : 238 m μ ϵ : 13,700 and shoulder near 225m μ
(ϵ : 13,000)

I.R.: 1753, 1698, 1663 cm^{-1} (no furan bands).

Treatment of dihydrocitrolin with alkali

Dihydrocitrolin (3.00 mg) in ethanol (5.0 ml) and 2N sodium hydroxide (25 ml) was heated on the steam bath under nitrogen. Ultraviolet spectra of the reaction mixture were determined at intervals both in alkaline and acidified solution. No significant changes could be observed in the region between 245 and 300 m μ in a period up to 2 hours.

Obacunoic acid

Crushed grapefruit seeds (5 kg) were extracted for limonin in the usual way. The mother liquors remaining after crystallization of limonin (35.2 g) were evaporated to dryness and worked up to give a crude neutral residue (24 g) which was boiled with acetone (100 ml) and 1N sodium hydroxide solution (200 ml) for 1 hour, acetone being allowed to evaporate. Working up as usual gave an acidic material (12 g) which on crystallization from acetone/ether yielded obacunoic acid (1.8 g). The analytical sample was dried under vacuum for 74 hours at 93°C.

Calculated for $C_{26}H_{32}O_8$: C:66.03% H:6.83% activeH(2):4.24%

Found: C:65.82% H:6.86% activeH: 4.26%

Mp.: 205-8°C

(α)_D: -103° (c:1.2, tetrahydrofuran)

U.V.: λ_{max} : 204-5m μ (ϵ :18,000). At 210m μ , ϵ :14,000

I.R.: 3220, diffuse absorption between 3400-2800, 1745, 1710, 1621, 1503, 874 cm^{-1} .

Methyl obacunoate

(i) By action of ethereal diazomethane on obacunoic acid

Obacunoic acid (460 mg) in tetrahydrofuran

(25 ml) was treated with excess ethereal diazomethane at room temperature for 1 minute. The mixture was evaporated to dryness under vacuum at room temperature and the residue was crystallized from ether to give methyl obacunoate (400 mg). Part of the sample was purified and dried under vacuum for 41 hours at 95°C. Mp.: 173-6°C

(α)_D: -94° (c: 1.2, methylene dichloride)

U.V.: λ_{max} : 203 m μ (ϵ : 7,100), at 210 m μ , ϵ : 6,700

I.R.: 3540, 1738, 1716, 1623, 1503, 1204, 875 cm⁻¹.

(ii) By action of methyl iodide on obacunoic acid

Obacunoic acid (98 mg) in acetone (30 ml) was treated with 0.10 N sodium hydroxide solution (5.50 ml) and methyl iodide (5 ml). The mixture was allowed to stand at room temperature for 17 hours. Working up as usual and crystallization from ether gave methyl obacunoate (66 mg), Mp.: 168-73°C, whose infrared spectrum was identical with that of a sample prepared by the action of diazomethane on obacunoic acid.

Hydrogenation of obacunoic acid

Obacunoic acid (105 mg) was hydrogenated over previously prehydrogenated palladium-carbon (10%,

150 mg) in glacial acetic acid (10 ml). Uptake of hydrogen ceased when 20.25 ml (3.74 moles) were absorbed at 24°C. Working up as usual gave an acidic material (109 mg) ,which could not be crystallized.

Methyl-hydrogen octahydroobacunoninate

Methyl obacunoate (328 mg) in acetic acid (25 ml) was hydrogenated over previously pre-hydrogenated palladium-carbon (10%, 690 mg) , suspended in acetic acid (10 ml). The uptake of hydrogen ceased when 63.5 ml were absorbed at 22°C (3.86 moles). Working up as usual gave acidic material (220 mg) which was crystallized from aqueous methanol to yield methyl-hydrogen octahydroobacunoninate (150 mg).

M.p.:173-5°C

(α)_D: +120° (c:1.1 , methanol)

I.R.:3470,3230,1741,1720,1705 cm⁻¹.

In another experiment , methyl obacunoate (19.4 mg) was found to absorb accurately determined 3.75 moles of hydrogen under the same conditions. Working up as usual and crystallization from aqueous

methanol gave methyl-hydrogen octahydroobacunoninate which was identified by its mixed melting point and infrared spectrum.

Desoxyobacunoic acid

Obacunoic acid (136 mg) was dissolved in a mixture of acetone (16 ml) and acetic acid (6 ml) and a solution of chromous chloride (1N, 5.0 ml) was added under nitrogen. The mixture was shaken under nitrogen for 43 hours at room temperature, acetone was evaporated under vacuum and water (100 ml) was added. Working up as usual and crystallization from methylene dichloride/benzene gave desoxyobacunoic acid (107 mg). The analytical sample was dried under vacuum for 54 hours at 95°C.

Calculated for $C_{26}H_{32}O_7$: C:68.40% H:7.07%

Found: C:68.33% H:7.17%

Mp.: 158-79°C

$(\alpha)_D$: - 101° (c:1.0, methylene dichloride)

U.V.: λ_{max} : 206-8m μ (ϵ :22,600), at 210m μ ϵ :21,800

I.R.: 3360, 1715, 1695, 1636, 1502, 875 cm^{-1} .

Treatment of desoxyobacunoic acid with alkali

Desoxyobacunoic acid (3.8 mg) was refluxed on the steam bath in a mixture of methanol (2 ml) and 2N sodium hydroxide solution (2 ml) for 90 minutes. Working up as usual gave an acidic material (1.2 mg), which could not be crystallized.

U.V.: at 206m μ ; ϵ :10,000 , at 210 m μ ; ϵ :7,700

Treatment of methyl-hydrogen octahydroobacunoninate with strong acid.(i) Action of concentrated hydrochloric acid in dioxan

Methyl-hydrogen octahydroobacunoninate (83 mg) was heated on the steam bath with a mixture of concentrated hydrochloric acid (2 ml) and dioxane (2 ml) for 16 hours. Working up as usual gave a neutral fraction (30 mg) and an acidic fraction (49mg) neither of which could be crystallized. The crude materials had the following spectroscopic properties:

Neutral: I.R.:3200,1732,1631,1787 cm⁻¹

U.V.: $\lambda_{\max}$:247 m μ (ϵ : 8,000)

Acid: I.R.:3200,diffuse bands between 3400-3000,
1785,1720,1631 cm⁻¹.

U.V.: $\lambda_{\max}$:250.5 m μ (ϵ :9,200)

(ii) Action of concentrated hydrochloric acid in methanol

Methyl-hydrogen octahydroobacunoninate (2.57 mg) was refluxed on the steam bath with a mixture of concentrated hydrochloric acid (12.5 ml) and methanol (12.5 ml). Aliquots were withdrawn at intervals and their ultraviolet spectra were determined. A peak appeared at 250 m μ after 40 minutes of refluxing (ϵ :4,400) , increased to ϵ :12,000 after approximately 12 hours and remained unaltered after a total reflux time of 25 hours.

The experiment was repeated with 71 mg of the starting material but both the neutral (52 mg) and the acidic (20 mg) fractions which were obtained could not be crystallized.

Treatment of hydrogenated obacunonic acid with strong acid.

The crude acidic material obtained by hydrogenation of obacunonic acid over palladium-carbon (91 mg) was heated on the steam bath with a mixture of concentrated hydrochloric acid (2 ml) and dioxane (2 ml) for 17 hours. Working up as usual gave an acidic material (62 mg) which could not be crystallized.

I.R.: Diffuse bands in the 3500-3000 region, 1785, 1720, 1630 cm^{-1} .

U.V.: λ_{max} : 250 $\text{m}\mu$, ϵ : 10300

The ultraviolet spectrum of a sample of the crude product heated in 2N sodium hydroxide solution for 100 minutes on the steam bath and worked up as usual, remained unaltered in the region between 230 and 320 $\text{m}\mu$.

iso-Obacunoic acid

Methyl obacunoate (122 mg) in dry methanol (8 ml) was added under nitrogen to a solution of sodium (0.5 g) in dry methanol (5 ml). The mixture was refluxed under nitrogen for 1 hour, water (10 ml) was added and reflux was continued for a further 30 minutes. Acidification and working up as usual, gave only acidic material (prolonged shaking with bicarbonate solution was necessary to effect extraction), which on crystallization from methylene dichloride/benzene yielded iso-obacunoic acid (70 mg). The analytical sample was dried under vacuum for 45 hours at 95°C.

Calculated for $\text{C}_{26}\text{H}_{32}\text{O}_8$: C: 66.08% H: 6.83%

Found: C: 65.95% H: 6.93%

Mp.: 155-61°C

(α)_D: -41° (c:1.2 in methylene dichloride)

U.V.: λ_{max} : 208 m μ (ϵ :5,200)

I.R.: Diffuse bands between 3500 and 3000 ,1741,1716
1704,1505,876 cm⁻¹.

When obacunonic acid was treated with a solution of sodium methoxide under identical conditions, iso-obacunonic acid was also obtained but large losses were incurred during crystallization. The purified product was identified by its melting point, mixed melting point and infrared spectrum.

Hydrogenation of iso-obacunonic acid

iso-Obacunonic acid (19.5 mg) in glacial acetic acid (10 ml) was hydrogenated over previously prehydrogenated palladium-carbon (10% , 65 mg), until hydrogen uptake ceased. The volume of hydrogen taken up at 20°C was 2.96 ml (2.94 moles). Working up as usual gave an acidic product which could not be crystallized.

Treatment of obacunonic acid with iodine and alkali

Obacunonic acid (analytical purity, 55 mg)

was dissolved in 2N sodium hydroxide solution (3 ml) and was treated with excess aqueous iodine and potassium iodide. A precipitate appeared, was collected, purified by sublimation at 100°C and identified as iodoform by its melting point and mixed melting point with an authentic sample. The weight of the crude dried precipitate was 7 mg (approximately 15% of theory). The filtrate was worked up as usual to give an acidic product (46 mg) which could not be crystallized.

I.R.: Diffuse bands between 3500 and 3000, 1745-1705, 1630, 1500, 875 cm^{-1} .

U.V.: End absorption at 208 $\text{m}\mu$, ϵ : 12,000

Treatment of iso-obacunoic acid with iodine and alkali

iso-Obacunoic acid (44 mg) was dissolved in sodium hydroxide solution (2N, 2.5 ml) and treated with excess aqueous iodine and potassium iodide. No precipitate appeared on standing at room temperature for 30 minutes. Working up as usual and crystallization from benzene gave iso-obacunoic acid (30 mg), Mp.: 150-155°C, whose infrared spectrum was identical with that of an authentic sample.

Dihydrocarvone

Carvone (50 ml) in ethanol (300 ml) was added to a suspension of zinc dust (100 g) in water (300 ml) and sodium hydroxide (50 g). The mixture was refluxed for 16 hours, ethanol was allowed to distill and the residue was filtered and extracted with ether. The extract was dried, ether was evaporated off and the residue was distilled under reduced pressure. The fraction boiling between 134-144°C at 60 mm Hg was collected (26 g) and identified as di-hydrocarvone.

$n_d^{23^\circ\text{C}}$: 1.4713

I.R.: 1705, 1640, 895 cm^{-1} (c.f. carvone: 1680, 1640, 895 cm^{-1})

U.V.: No strong absorption between 210-300 mμ

Dihydrocarvonehydrate

Dihydrocarvone (14 g) was mixed with cooling with dilute sulphuric acid (40% w/w, 120g) and the mixture was shaken at room temperature for 24 hours. Sodium hydroxide solution (4N, 200 ml) was added with cooling and the mixture was extracted with ether. The extract was dried, ether was distilled off and the residue was distilled under reduced pressure.

The fraction boiling between 136-41°C at 10 mm Hg (7.2 g) was collected and redistilled to give pure dihydrocarvonchhydrate.

$n_d^{21^\circ\text{C}}$: 1.4773

I.R.: 3400, 1705 cm^{-1}

A GLC run showed that the sample was at least 98% pure.

Carvonehydrate

Carvone (100 ml) was added to a solution of sodium metabisulphite (anhydrous, 90 g) in water (200 ml). The mixture was boiled until homogenous (45 minutes) and cooled to 0°C. A mixture of sulphuric acid (80 g) and water (80 ml) was added with cooling and the pale, homogenous solution was allowed to stand at room temperature for 6 days. Sodium hydroxide solution (6N, 300 ml) was added with cooling and the mixture was extracted with ether. The extract was dried, ether was evaporated off and the residue was distilled under reduced pressure. The fraction boiling between 152-4°C at 10mm Hg (64 g) was collected and redistilled to give carvonehydrate (39 g).

I.R.: 3400, 1680 cm^{-1}

Ferric chloride test: negative.

Treatment of carvonehydrate with iodine and alkali

Carvonehydrate (4.95 g) in dioxane (50 ml) was treated with sodium hydroxide solution (4N, 100 ml) and a solution of iodine (30 g) and potassium iodide (40 g) in water (100 ml). A dense precipitate of iodoform formed and was removed by filtration. The filtrate was acidified and worked up as usual. The neutral fraction was divided into material insoluble in dilute sodium hydroxide solution and enolic material (1.68 g), which was a dark oil with a strong phenolic odour and gave a green colouration with ferric chloride. The crude phenolic material was warmed on the steam bath with 3:5:dinitrobenzoyl chloride (2 g) and pyridine (20 ml) for 30 minutes. Working up as usual and chromatography on alumina (grade III) gave diiodo-o-cresyl-3:5:dinitrobenzoate (900 mg i.e. 5.6% of theory) which did not depress the melting point of a specimen prepared from authentic diiodo-o-cresol. The two samples had identical infrared spectra.

Calculated for $C_{14}H_8O_6N_2I_2$: C:30.35%, H:1.45%, N:5.07%, I:45.8%

Found: C:31.16% H:1.55% N:5.17% I:45.6%

Mp.: 206-13°C, from ethanol.

In another experiment, the phenolic fraction (1.69 g), which was obtained in the manner described above from carvonehydrate (5.48 g) , was dissolved in 2N sodium hydroxide solution (30 ml) and refluxed under nitrogen with zinc dust (10 g) and powdered iron (2 g) for 2 hours. Working up as usual gave an oil (770 mg) which had a strong phenolic odour and gave a negative Beilsten test.

A portion of the material (460 mg) in benzene (20 ml) was refluxed with 3:5dinitrobenzoyl chloride (2.2 g) and pyridine (2 ml) for 20 minutes. Working up as usual and crystallization from ethanol gave o-cresyl-3:5dinitrobenzoate (207 mg i.e. 3.5% of theory) , Mp.:135-3°C, not depressed by admixture of authentic material. The two samples had identical infrared spectra.

Diiodo-o-cresol

o-Cresol (5 g) in aqueous ammonia (20N, 80 ml) was treated with a solution of iodine (20 g) and potassium iodide (25 g) in water (40 ml) at room temperature. A precipitate was formed immediately, which on crystallization from glacial acetic acid gave diiodo-o-cresol (4.5g), Mp.:65-9°C, I.R.:3,400,860,855 cm^{-1} .

3- β -Acetoxy-11-keto-bisnor-allo-cholanic acid

The crude mixture of 3- β -acetoxy- and 3- β -hydroxy-11-keto-bisnor-allo-cholanic acids (3.97 g) was dissolved in pyridine (10 ml) and acetic anhydride (10 ml) and the mixture was allowed to stand at room temperature for 20 hours. The reaction mixture was poured into water and the precipitate was collected and crystallized from aqueous acetone to give 3- β -acetoxy-11-keto-bisnor-allo-cholanic acid (3.39 g).

Mp.: 189-98°C

I.R.: 1727, 1709, 1683, 1250 cm^{-1} .

3- β -Hydroxy-11-keto-bisnor-allo-cholanic acid

The crude mixture of 3- β -acetoxy- and 3- β -hydroxy-11-keto-bisnor-allo-cholanic acids (5.78 g) was heated on the steam bath with ethanol (25 ml) and 2N sodium hydroxide (25 ml) for 1 hour. The mixture was acidified and the precipitate was collected and crystallized from aqueous methanol to give 3- β -hydroxy-11-keto-bisnor-allo-cholanic acid (4.68 g). Mp; 252-60°C. $(\alpha)_D^{25} + 27^\circ$ (c: 4.8, chloroform). I.R.: 3350, 1702, 1680 cm^{-1} .

Phenacyl ester of 3- β -acetoxy-11-keto-bisnor-
allo-cholanic acid

The crude mixture of 3- β -acetoxy and 3- β -hydroxy 11-keto-bisnor-allo-cholanic acids (3.06 g) was dissolved in a solution of sodium carbonate (670 mg) in water (15 ml). Phenacyl bromide (2.4 g) in ethanol (35 ml) was added and the mixture was heated on the water bath for 2 hours. Working up as usual gave only neutral material, which was heated with a mixture of acetic anhydride (10 ml) and pyridine (10 ml) on the steam bath for 1 hour. The reaction mixture was poured into water and worked up as usual. Crystallization from aqueous methanol gave the phenacyl ester of 3- β -acetoxy-11-keto-bisnor-allo-cholanic acid (2.88 g). The analytical sample was dried under vacuum for 15 hours at 85°C.

Calculated for $C_{32}H_{42}O_6$: C:73.53% H:8.10% OAc:8.24%

Found: C:73.47% H:8.35% OAc:8.18%

Mp.: 159-60°C

(α)_D: +16.7° (c:3.4, chloroform)

U.V.: λ_{max} : 242m μ ϵ : 12,700

I.R.: 1744, 1724, 1710, 1694, 1599 cm^{-1}

Heating of phenacyl ester of 3- β -acetoxy-11-keto-bisnor-allo-cholanic acid with collidine

The ester (246 mg) was refluxed with collidine (10 ml) for 2 hours. Working up as usual and crystallization from aqueous methanol gave the starting material (185 mg) , Mp.:159-60°C whose infrared spectrum was identical with that of an authentic sample.

In other experiments , the phenacyl ester was heated with collidine in a sealed tube for 12 hours at 250°C and with specially dried collidine for 15 hours at 260°C. In both cases the starting material was recovered in the form of the 3- β -hydroxy-11-keto-bisnor-allo-cholanic acid , following hydrolysis with dilute alkali.

Dimethyl cyclohexylamine oxide

Dimethyl cyclohexylamine (63 g) was dissolved in a mixture of acetone (50 ml) and hydrogen peroxide (100 volume , 67 ml). The mixture warmed up spontaneously. After the initial reaction subsided , the mixture was refluxed on the steam bath for 5½ hour and excess reagents and solvents were distilled

at 100°C under approximately 30 mm Hg. The remaining material solidified on cooling and was crystallised from benzene to give dimethyl cyclohexylamine oxide
Sublimation point: 100-110°C.

N-N-Dimethyl hydroxylamine

Dimethyl cyclohexylamine oxide (50 g) was pyrolysed at 150-200°C and the fraction of distillate boiling between 94 and 97°C was collected and re-distilled to give N-N-dimethyl hydroxylamine (16.3 g) characterised by its hydrochloride Mp.: 99-105°C.

3-β-Acetoxy-11-keto-bisnor-allo-cholanic acid chloride

3-β-Acetoxy-11-keto-bisnor-allo-cholanic acid (1.78 g) was refluxed in tetrahydrofuran (8 ml) with oxalyl chloride (3 ml) for 1 hour. The mixture was evaporated under vacuum to a syrupy consistency and crystallized from petroleum ether to give 3-β-acetoxy-11-keto-bisnor-allo-cholanic acid chloride (1.26 g).

Calculated for $C_{24}H_{35}O_4Cl$: C: 68.14% H: 8.34% Cl: 8.38% OAc: 10.1

Found: C: 66.73% H: 7.95% Cl: 7.98% OAc: 11.7

Mp.: 161-4°C $(\alpha)_D$: +17.6° (c: 3.1, chloroform)

I.R.: 1780, 1730, 1706, 1248 cm^{-1} .

O-Dimethyl hydroxylamine ester of 3-β-acetoxy-11-keto-bisnor-allo-cholanic acid

3-β-Acetoxy-11-keto-bisnor-allo-cholanic acid chloride (480 mg) was dissolved in tetrahydrofuran (2.5 ml) and N-N-dimethyl hydroxylamine (0.2 ml) was added. The mixture was allowed to stand at room temperature for 80 minutes. Working up as usual and crystallization from aqueous methanol gave O-dimethyl hydroxylamine ester of 3-β-acetoxy-11-keto-bisnor-allo-cholanic acid (500 mg). The analytical sample was dried under vacuum for 12 hours at 90°C.

Calculated for $C_{26}H_{41}O_5N$: C:69.76% H:9.23% N:3.13% OAc:9.60%

Found: C:70.04% H:9.34% N:3.06% OAc:9.43%

Mp.:148-52°C

(α)_D: +26° (c: 1.1, chloroform)

I.R.: 1756, 1731, 1704, 1247 cm^{-1} .

Attempted decarboxylation of O-dimethylhydroxylamine ester of 3-β-acetoxy-11-keto-bisnor-allo-cholanic acid
a) By pyrolysis

The ester was pyrolysed (i) at 200°C and (ii) at 238°C, under a stream of nitrogen and the gas

was passed through a solution of barium hydroxide. No precipitate of barium carbonate appeared in 30 minutes and the residual material gave , after hydrolysis with dilute alkali and working up as usual, high yields of 3- β -hydroxy-11-keto-bisnor-allo-cholan-ic acid ,which was identified by its melting point and infrared spectrum.

b) By quaternization followed by pyrolysis

The ester (112 mg) was dissolved in dry methyl iodide (2.5 ml), the mixture was refluxed for several hours with exclusion of moisture, evaporated to dryness and heated at 100°C for several minutes. Hydrolysis with dilute alkali and working up as usual gave 3- β -hydroxy-11-keto-bisnor-allo-cholan-ic acid (91 mg) , Mp.:256-9°C, whose infrared spectrum was identical with that of an authentic sample.

The above experiment was repeated with the following modifications:

(i) The ester was heated with dry methyl iodide in a sealed tube at 147°C for 1½ hours.

(ii) The ester was refluxed with dry methyl iodide, the mixture was evaporated to dryness and the residue was refluxed in dry collidine for 1½ hours..

(iii) The ester was heated with dry methyl iodide in a sealed tube at 170°C for 4 hours.

In all cases the 3- β -hydroxy-11-keto-bisnor-allo-cholanic acid was isolated from the reaction mixtures following hydrolysis with dilute alkali and working up as usual. Substitution of benzyl bromide for methyl iodide in an experiment in which the ester was heated in a sealed tube at 150°C resulted in an intractable tar.

O-Benzyl-N-oxy-piperidine

Benzoyl peroxide (7.5 g) was dissolved in ether (300 ml) and shaken at room temperature with piperidine (6 ml) for 12 hours. An oily layer separated out and was discarded. The ethereal layer was saturated with hydrogen chloride and the precipitate which formed was collected after cooling. The crude hydrochloride was decomposed with water, extracted with ether, washed with bicarbonate solution and evaporated to dryness. Crystallization from petroleum ether gave O-benzyl-N-oxy piperidine (1.92 g) Mp.: 59-62°C.

Treatment of 3- β -acetoxy-11-keto-bisnor-allo-cholanic acid chloride with triethylamine in the presence of air.

3- β -Acetoxy-11-keto-bisnor-allo-cholanic acid (1.22 g) was refluxed in tetrahydrofuran (10 ml) with oxalyl chloride (0.60 ml) for 1 hour. The mixture was cooled to room temperature, dry air was passed through it and triethylamine (2.50 ml) in tetrahydrofuran (20 ml) was added. Air was passed through the mixture for 22 hours, water was added and the mixture was worked up as usual to give a neutral material (630 mg) which on crystallization from methylene dichloride/petroleum ether yielded 3- β -acetoxy-11-keto-bisnor-allo-cholanic anhydride. The analytical sample was dried under vacuum for 45 hours at 85°C.

Calculated for $C_{48}H_{70}O_9$: C:72.88% H:8.92% OAc:10.87%

Found: C:73.12% H:9.19% OAc:11.07%

Mp.: 237-44°C

$(\alpha)_D$: +35° (c:1.5, chloroform)

I.R.: 1813, 1744, 1731, 1704, 1242 cm^{-1} .

Irradiation of 3- β -acetoxy-11-keto-bisnor-allo-cholanic acid chloride .

The acid chloride (951 mg) in dioxane (60 ml) was irradiated[@] for 3 hours. Aliquots were withdrawn at intervals , poured into water and titrated with 0.1 N sodium hydroxide solution. At the end of the irradiation period the titre fell to 20% of the original value and was decreasing only very slowly. Working up as usual gave the crude neutral product (897 mg) which was dissolved in a mixture of petroleum ether (40 ml) and benzene (20 ml) and filtered through a column of alumina (grade III, 8 g). The material washed through and that elluted by a further portion of the same solvent mixture (60 ml) was combined (460 mg) and crystallized from aqueous methanol to give 3- β -acetoxy-11-keto-allo-pregnane which was identical in all respects with a sample prepared from 3- β -acetoxy-11-20-diketo-allo-pregnane.

Calculated for $C_{23}H_{36}O_3$: C:76.62% H:10.07% OAc:11.93%

Found: C:76.60% H:10.28% OAc:11.87%

Mp.: 159-62°C

(α)_D: +38° (c: 1.3 , chloroform)

I.R.: 1731, 1705, 1250 cm^{-1}

U.V.: end absorption at 208 $\text{m}\mu$, ϵ : 500

@Standard irradiation conditions: The solution was placed in a quartz flask fitted with a reflux condenser and irradiated with a mercury lamp (150 w) placed as closely as possible to the flask. Dry, oxygen free nitrogen was passed through the solution throughout the irradiation period.

Treatment of 3- β -acetoxy-11-20-diketo-allo-pregnane with ethane dithiol.

3- β -Acetoxy-11-20-diketo-allo-pregnane (465 mg) was treated with ethane dithiol (10 drops) and boron trifluoride (45% in ether, 15 drops) at room temperature. The mixture, which formed a homogeneous solution, set to a solid after one minute, was allowed to stand at room temperature for five minutes and was poured into methanol. Some sodium bicarbonate was added and the mixture was worked up as usual. Crystallization from methylene dichloride/ethanol gave 3- β -acetoxy-11-keto-20-dithioketal-allo-pregnane (300 mg).

Calculated for $\text{C}_{25}\text{H}_{38}\text{O}_3\text{S}_2$: C:66.64% H:8.50% S:14.2%

Found: C:66.03% H:8.49% S:14.1%

Mp.: 234-7°C

(α)_D: + 21.5° (c: 1,5 in chloroform)

I.R.: 1725,1695,1249 cm⁻¹.

3- β -Acetoxy-11-keto- α -pregnane

3- β -Acetoxy-11-keto-20-dithioketal- α -pregnane (237 mg) in absolute ethanol (50 ml) was refluxed with Raney nickel (activity W1, 2g) for 16 hours. The mixture was filtered, evaporated to dryness and worked up as usual to give 3- β -acetoxy-11-keto- α -pregnane, Mp. 160-3°C (from aqueous methanol) not depressed by admixture of material prepared by irradiation of 3- β -acetoxy-11-keto-bisnor- α -cholanic acid chloride. The two samples had identical infrared spectra.

(α)_D : +40° (c: 1.9 , chloroform).

Huang-Minlon reduction of 3- β -acetoxy-11-20-diketo- α -pregnane.

The ketone (683 mg) in diethylene glycol (20 ml) was refluxed with hydrazine (aqueous, 64% w/w, 2.0 ml) and potassium hydroxide (3 g) for 90 minutes

under nitrogen. Water was allowed to distill until the temperature of the liquid rose from an initial 140°C to 200°C. Reflux was then continued for a further 4 hours and the mixture was cooled and worked up as usual to give a neutral material (580 mg) which crystallized on addition of methanol to yield 3-β-hydroxy-allo-pregnane. The analytical sample was dried under vacuum for 45 hours at 30°C.

Calculated for $C_{21}H_{36}O$: C:82.83% H:11.92%

Found: C:82.77% H:12.19%

Mp.: 139-40°C

$(\alpha)_D$: +21° (c:1.3, chloroform)

IR: 3200 cm^{-1} , no bands in 1800-1500 cm^{-1} region.

Another crystalline reduction product (Mp.: 168-72°C) was isolated from the mother liquors in small yield. The infrared spectrum of this material was very similar to that of the major product.

3-β-11-α-Dihydroxy-bisnor-allo-cholanic acid

The crude mixture of 3-β-acetoxy and 3-β-hydroxy 11-keto-bisnor-allo-cholanic acids (12 g) was dissolved in ethanol (350 ml), and sodium (35 g) was added slowly to the mixture while it was refluxed

on the steam bath. A thick paste was formed as all the sodium dissolved. Water (125 ml) was added and the homogenous mixture was refluxed on the steam bath for 30 minutes, cooled and acidified with hydrochloric acid. The precipitate was collected and crystallized from aqueous methanol to give 3- β -11- α -dihydroxy-bisnor-allo-cholanic acid (9.1g).

The analytical sample was dried under vacuum for 51 hours at 100°C.

Calculated for $C_{22}H_{36}O_4$: C:72.49% H:9.96%

Found: C:72.28% H:9.86%

Mp.: 277-81°C

(α)_D: -36° (c: 0.8, chloroform)

I.R.: 3400, 3220, 2600, 1667 cm^{-1}

3- β -11- α -Diacetoxy-bisnor-allo-cholanic acid

3- β -11- α -dihydroxy-bisnor-allo-cholanic acid (9.0 g) was allowed to stand at room temperature for 21 hours with a mixture of pyridine (22 ml) and acetic anhydride (22 ml). Working up as usual and crystallization from acetone/petroleum ether gave 3- β -11- α -diacetoxy-bisnor-allo-cholanic acid (8.38g).

The analytical sample was dried under vacuum for

27 hours at 100°C.

Calculated for $C_{26}H_{40}O_6$: C:69.61% H:8.99% OAc:19.2%

Found: C:69.35% H:9.21% OAc:19.6%

Mp.: 206-8°C

$(\alpha)_D$: -40° (c:1.7, chloroform)

I.R.: 3000 (diffuse), 1735, 1705, 1251 cm^{-1} .

3- β -Hydroxy-11- α -acetoxy-bisnor-allo-cholanic acid

3- β -11- α -diacetoxy-bisnor-allo-cholanic acid (320 mg) was heated on the steam bath in a mixture of tetrahydrofuran (30 ml) and 1N sodium hydroxide solution (10 ml) for 1 hour. Working up as usual and crystallization from methanol gave 3- β -hydroxy-11- α -acetoxy-bisnor-allo-cholanic acid (303 mg).

Calculated for $C_{24}H_{38}O_5$: C:70.90% H:9.42% OAc:10.6%

Found: C:71.20% H:9.72% OAc:10.6%

Mp.: 308-14°C

$(\alpha)_D$ - 47° (c: 0.9, pyridine)

I.R.: 3340, 1723, 1698, 1286 cm^{-1} .

3- β -11- α -Diacetoxy-bisnor-allo-cholanic acid chloride

3- β -11- α -diacetoxy-bisnor-allo-cholanic acid (675 mg) was refluxed with oxalyl chloride (1 ml)

in tetrahydrofuran (10 ml) for 1 hour. The solution was evaporated to dryness under vacuum and the residue was crystallized from benzene/petroleum ether to yield 3- β -11- α -diacetoxy-bisnor-allo-cholanic acid chloride (321 mg).

Mp.: 147-55°C

I.R.: 1785, 1731, 1250 cm^{-1} .

Irradiation of 3- β --11- α -diacetoxy-bisnor-allo-cholanic acid chloride

The acid chloride (300 mg) , in dry dioxane (15 ml) was irradiated for 4 hours. Aliquots were withdrawn at intervals, poured into water and titrated with 0.1 N sodium hydroxide solution. At the end of the irradiation period the titre fell to 30% of the original value and was decreasing only very slowly. Water (5 ml) was added to the reaction mixture which was then heated on the steam bath for 20 minutes . Working up as usual gave a neutral product (186 mg) which could not be crystallized. The crude neutral material was heated on the steam bath with 4N sodium hydroxide solution (5 ml) and ethanol (10 ml) for 1 hour and worked up

as usual to give a neutral material whose infrared spectrum had very strong bands in the O-H stretch region and no bands between 1800 and 1500 cm^{-1} .

Irradiation of limonilyl chloride

Limonilyl chloride (1.31 g) in dioxane (18 ml) was irradiated for 60 minutes, when the infrared spectrum of an aliquot ceased to show a separate band near 1790 cm^{-1} . Water (5 ml) was added and the solution was allowed to stand at room temperature for 2½ hours. Working up as usual gave acidic material (450 mg), which crystallized from aqueous acetone to yield limonilic acid identified by its infrared spectrum and mixed melting point, and neutral material (450 mg), which could not be crystallized even after chromatography on alumina. The crude neutral material eluted from alumina with benzene/acetone had the following infrared spectrum: no bands in the O-H stretch region, 1745, 1726, 1503 and 875 cm^{-1} .

Di-dioxan

Dioxan (50 ml) was irradiated for 3 hours,

the solvent was evaporated to dryness and the solid residue (33 mg) was sublimed to give di-dioxane.

Calculated for $C_8H_{14}O_4$: C:55.16% H:8.10% Mol.wt. 174

Found: C:55.05% H:8.03% Mol.wt. 172

N.M.R.:Single broad band (tau: 6.33-6.50) (c.f. dioxane at 6.43)

I.R.:2924,2850,1360,1340 (hexachlorobutadiene mull)

1300,1280,1234,1125,1066,956,924,900,879,688 cm^{-1}

(nujol mull)

Sublimation point: 110°C.

In other experiments the following yields of di-dioxane were obtained under the following conditions:

(i).Dioxane (50 ml) was distilled without prior irradiation. No solid material was obtained.

(ii).Dioxane (50 ml) was irradiated under nitrogen for 18 hours. Yield of di-dioxane:33 mg.

(iii). Dioxane (50 ml) was irradiated without nitrogen flushing, for 20 hours. Yield of di-dioxane: 58 mg.

(iv).Dioxane (25 ml) was irradiated under a stream of dry air for 22 hours. The yellow solution contained undistillable residue (2.5 g),which could not be crystallized.

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