48. *Triterpenoids. Part XXV.* The Constitutions of Limonin and Related Bitter Principles.*

By D. H. R. Barton, S. K. Pradhan, S. Sternhell, and J. F. Templeton.

Extensive investigations on the constitution of the citrus bitter principle, limonin, are reported. By a series of stepwise reactions all the oxygen functions have been in turn inter-related, one of the more important processes used being autoxidation of the anion from the ketone group of limonin which affords the derived diosphenol. By summation of the work reported here with that of others the constitution of limonin has been deduced.[1]

The congeneric bitter principles nomilin and obacunone have been investigated by a study of the commonly derived obacunoic acid. The constitutions which we proposed earlier have received support.

Limonin is the characteristic bitter principle of citrus species and occurs in all parts of the plant. It was first isolated by Bernay in 1841.[2] Limonin represents one of the few remaining classical problems of plant structural chemistry. The literature on this subject has been detailed in a recent paper by Melera, Schaffner, Arigoni, and Jeger [3] and we shall cite in the present paper only those references especially pertinent to our own work.

Our investigations on the constitution of limonin were initiated in Glasgow in 1956 and have been continued to the present time. From the outset we collaborated with Professor J. Monteath Robertson, F.R.S., in an *X*-ray investigation which eventually (see below) made an important contribution to the problem.

Chemical work on the constitution of limonin has also been in progress in Zürich in the laboratories of Professor O. Jeger and Dr. D. Arigoni and at Urbana, Illinois, under the direction of Professor E. J. Corey. During the last three years there has been an exchange of information and free discussion of the problem, and the conclusions reached on the chemical side, summarised in a recent joint communication,[1] represent a collaborative effort. We express here our appreciation of this friendly international exchange. The present paper summarises the experimental work of the Glasgow–Imperial College group and the conclusions that could be drawn from it, with, of course, reference to the *X*-ray work at Glasgow [4] and to other chemical investigations where relevant.

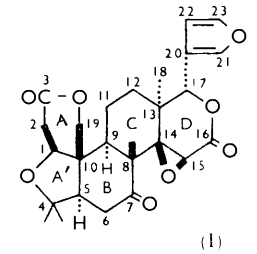
The constitution and stereochemistry of limonin, $C_{26}H_{30}O_8$, can be represented [1,4] as in (I). From the biogenetic point of view the compound is a tetracyclic triterpenoid of the euphol (II) type [5] from which four carbon atoms at the end of the side chain have been removed and carbon atoms 20—23 then converted into a furan ring. Ring A of the triterpenoid skeleton has been oxidatively cleaved between $C_{(3)}$ and $C_{(4)}$ and the 3-carboxyl group thus formed oxidatively cyclised on to $C_{(19)}$. There is excellent precedent for a $C_{(3)}$—$C_{(4)}$ biogenetic cleavage of the triterpenoid skeleton in the recently divulged structures of dammarenolic [6] (III) and nyctanthic acid [6,7] (IV). The constitution of limonin also requires an oxidative cleavage of the Bayer–Villiger type upon a 16-ketone in the skeleton of (II) to give the ring-D lactone. Finally one methyl group must be moved from $C_{(14)}$ to $C_{(8)}$. It is of interest that oxidation of dihydrobutyrospermyl acetate (V) by chromic

* Part XXIV, *J.*, 1958, 2239.

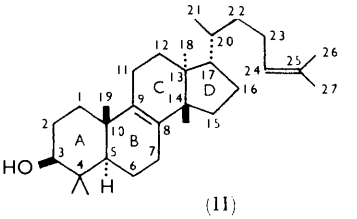
[1] Cf. Arigoni, Barton, Corey, and Jeger, and their collaborators, *Experientia*, 1960, **16**, 41.
[2] Bernay, *Annalen*, 1841, **40**, 317.
[3] Melera, Schaffner, Arigoni, and Jeger, *Helv. Chim. Acta*, 1957, **40**, 1420.
[4] Arnott, Davie, Robertson, Sim, and Watson, *Experientia*, 1960, **16**, 49.
[5] Barton, McGhie, Pradhan, and Knight, *Chem. and Ind.*, 1954, 1325; *J.*, 1955, 876; Arigoni, Viterbo, Dünnenberger, Jeger, and Ruzicka, *Helv. Chim. Acta*, 1954, **37**, 2306; and references there cited.
[6] Arigoni, Barton, Bernasconi, Djerassi, Mills, and Wolff, *Proc. Chem. Soc.*, 1959, 306; *J.*, 1960, 1900.
[7] Whitham, *Proc. Chem. Soc.*, 1959, 271; *J.*, 1960, 2016.

acid furnishes the compound [8] (VI) which has a 7-ketone group, a 14,15-ethylenic linkage, and a β-methyl group moved from $C_{(14)}$ to $C_{(8)}$. It is attractive to suppose that an equivalent mechanism operates in the plant in the biogenesis of limonin. The numbering and lettering systems in limonin (I) follow directly from these biogenetic considerations and are the same as in other tetracyclic triterpenoids.[9]

The absolute configuration of limonin, expected from the biogenetic arguments developed above, has been confirmed [1] by rotatory dispersion curves,[10] kindly measured by Professor W. Klyne (Postgraduate Medical School, London).



(I)



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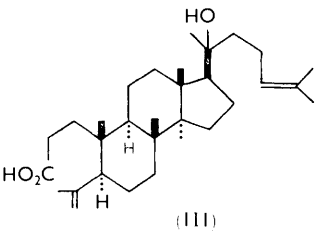

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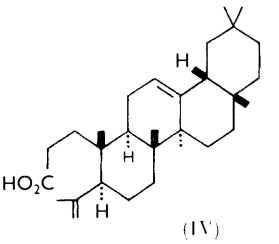

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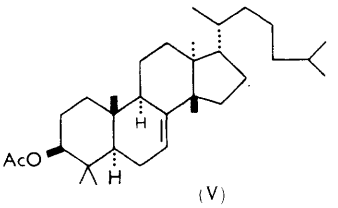

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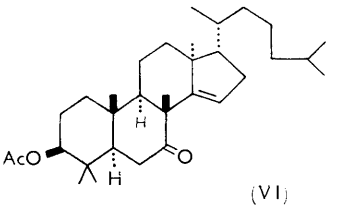


(VI)

Work published either before, or during, our own investigations had shown [1,3] that limonin contains two δ-lactone rings, which can be opened reversibly with alkali, a β-substituted furan residue, a ketonic oxygen present in a six-membered (or larger) ring, and two ethereal oxygen cycles. Hydrogenation of limonin gives tetrahydrolimonin, $C_{26}H_{34}O_8$, and hexahydrolimoninic acid, $C_{26}H_{36}O_8$. The furan ring is saturated in both compounds and the acid is formed by hydrogenolysis of one of the lactone rings. There is good evidence [1,3] that this lactone cleavage is caused by having the alkyl oxygen of the lactone in allylic relation to the furan ring. An exactly comparable situation is found in columbin.[11] Hexahydrolimoninic acid is abnormally strong,[12] a fact which is conveniently explained by attaching one of the ethereal oxygen atoms in the α-position. The partial formula (VII) is thus established.[3]

[8] Lawrie, Hamilton, Spring, and Watson, *J.*, 1956, 3272.
[9] Jones and Halsall, "Fortschritte der Chemie Organische Naturstoffe," Springer-Verlag, Vienna, 1955, Vol. XII, p. 44.
[10] Djerassi, *Bull. Soc. chim. France*, 1957, 741; "Optical Rotatory Dispersion," McGraw-Hill Book Co. Inc., New York, 1960.
[11] Barton and Elad, *J.*, 1956, 2085.
[12] Emerson, *J. Amer. Chem. Soc.*, 1952, **74**, 688.

The ketone group of limonin can be reduced stereospecifically by the Ponndorff–Meerwein procedure [13] to give the axial alcohol limonol (VIII; R = OH, R′ = H). On the other hand, reduction with borohydride or with sodium amalgam gives the equatorial epilimonol (VIII; R = H; R′ = OH). We have converted epilimonol into its chloroacetate and thence into the iodoacetate (VIII; R = H, R′ = O·CO·CH_2I). It is the latter compound which was used in the *X*-ray work of Robertson and his colleagues.[4] Convenient procedures for the preparation of cholesterol chloroacetate and iodoacetate are also reported in the Experimental section.

Treatment of limonin with hydriodic acid gives first, deoxylimonin,[14] $C_{26}H_{30}O_7$ (IX), and then citrolin,[12,14] $C_{26}H_{28}O_6$. Further reference to these compounds is made below.

The molecular formula of limonin, when coupled with knowledge of the functional groups, requires that the molecule be bicarbocylic. Since drastic degradation [15] of limonin affords 1,2,5-trimethylnaphthalene one can tentatively, and (as it turned out) correctly, assume that the two carbocycles are both six-membered.

We now develop our own arguments in support of the constitution (I). The formation of the αβ-unsaturated lactone deoxylimonin by the action of hydriodic acid on limonin suggests the presence of a 1,2-epoxide.[16] Conjugation of this epoxide with the ring-D lactone carbonyl group was proposed by Professor O. Jeger [17] in a lecture in Glasgow in 1957 in order to explain the conversion of limonol into merolimonol [3] and has since been thoroughly confirmed.[1] In agreement we found that treatment of tetrahydrolimonin (X) with hydrochloric–acetic acid mixture under controlled conditions gave isotetrahydrolimonin, an enolised α-keto-lactone (XI; R = H), characterised as its acetate (XI; R = Ac). The presence of the enolic system, established by a ferric reaction and by spectroscopic data, was confirmed by ozonolysis followed by mild hydrolysis, which afforded oxalic acid.

Treatment of tetrahydrolimonin with hydriodic acid under controlled conditions gave deoxytetrahydrolimonin [as (IX)], also prepared by selective hydrogenation of deoxylimonin (IX). Deoxytetrahydrolimonin was, as expected, stable to acid under the conditions specified above. Treatment of either tetrahydrolimonin or deoxytetrahydrolimonin with hydriodic acid in acetic acid–acetic anhydride at room temperature served to open the tetrahydrofuran ring to give an iodo-acetate (XII). No other change in the molecule was produced (see below), indicating that the second ethereal oxygen was probably not present as a 1,2- or 1,3-epoxide.

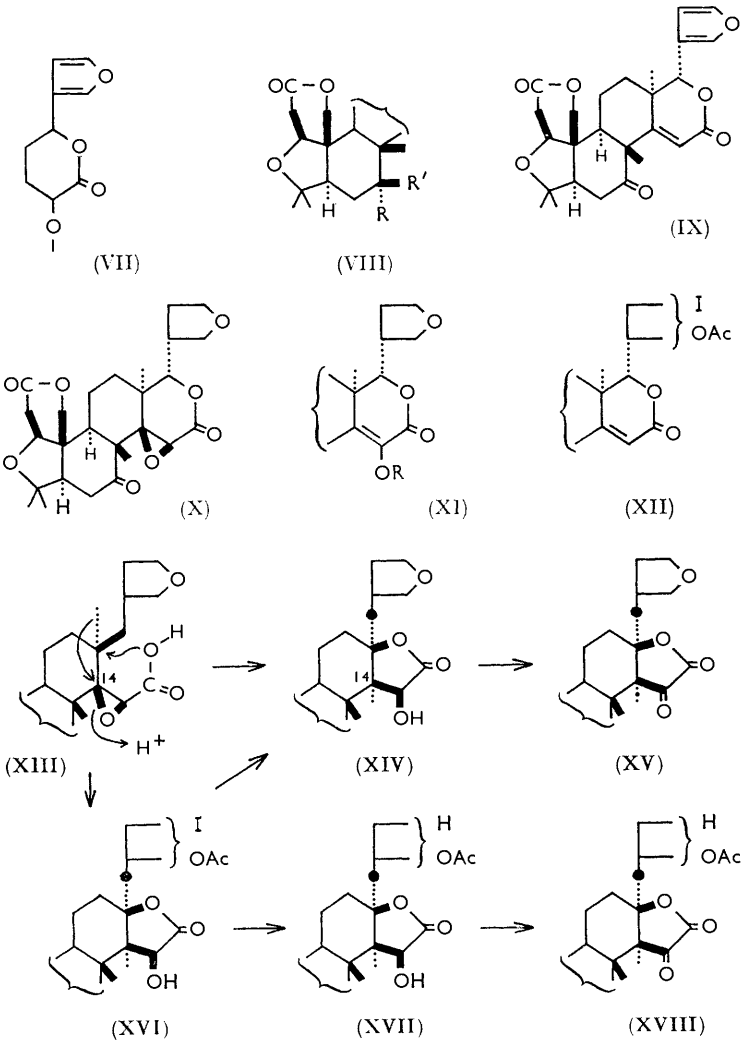
The action of hydrochloric acid–acetic acid on hexahydrolimoninic acid (XIII) gave a neutral isomer containing (from the infrared spectrum) a γ-lactone ring. This product is formulated as (XIV) and, in agreement, oxidation with pyridine–chromium trioxide afforded an α-keto-lactone (XV) which could *not* be made to enolise. One must conclude from this that the original isomerisation of (XIII) has placed a new carbon–carbon bond at $C_{(14)}$ in accordance with the formula (XIV). Our own work does not prove that the migrating group is methyl: this is, however, shown conclusively by work carried out at Zürich and Urbana.[1]

When hexahydrolimoninic acid (XIII) was treated with hydriodic acid in acetic acid–acetic anhydride at room temperature it also afforded a γ-lactone (XVI), the tetrahydrofuran ring being opened at the same time. On mild treatment with alkali this product (XVI) gave the γ-lactone (XIV), whilst on reduction with zinc dust it afforded the deiodo-compound (XVII). Oxidation of the latter furnished a non-enolisable α-keto-lactone (XVIII) comparable with the compound (XV) described above.

The relationship between the ketone group of limonin and the lactone ring D was

[13] Chandler and Kefford, *Austral. J. Sci.*, 1951, **14**, 24.
[14] Geissman and Tulagin, *J. Org. Chem.*, 1946, **11**, 760.
[15] Koller and Czerny, *Monatsh.*, 1936, **67**, 248; see also Brachvogel, *Arch. Pharm.*, 1952, **285**, 57.
[16] Cf. Barton, Miller, and Young, *J.*, 1951, 2598.
[17] Jeger, Chem. Soc. Terpene Symposium, Glasgow, July, 1957.

disclosed by the following experiments (see also ref. 1). Treatment of deoxylimonin (IX) under lactone-titration conditions gave a non-conjugated carboxylic acid, $C_{26}H_{32}O_8$, which still retained the two lactone residues but no longer responded to spectroscopic or chemical tests for the ketone group. Deoxylimonin oxime is stable under the same conditions of alkalinity, so that it must be attack by hydroxide ion upon the ketone group which initiates the reaction. All these facts are consistent with a reaction course indicated



in (XIX; arrows) to give (XX) as the structure of the acid, now conveniently designated as deoxylimonic acid. Similarly deoxytetrahydrolimonin (as IX) gave deoxytetrahydrolimonic acid (XXI). This acid was also obtained under the same alkaline conditions from the iodo-acetate (XII) with reclosure of the tetrahydrofuran ring as well as by hydrogenation of deoxylimonic acid.

The further reactions of deoxytetrahydrolimonic acid have confirmed the structure assigned. It reacted with one mole of chlorine to give an adduct [(XXII) or an equivalent] which was too unstable to be isolated. Heating *in vacuo* afforded a mixture of two crystalline diene-acids, both characterised as their methyl esters. The major product

had a broad absorption band at 255 mμ and a low ε value (7800) in agreement with a *cisoid* diene structure (XXIII). It gave formaldehyde on ozonolysis and had three vinyl-type protons in its nuclear magnetic resonance spectrum, two (τ = 4·60 and 4·38) indicative of an exocyclic methylene group (or an equivalent structure), and one (τ = 3·97) consistent with the presence of a vinyl hydrogen α to a lactone grouping (as in ring D). The acid (XXIII) was further characterised as the epoxide (XXIV) which showed the spectrum of a normal αβ-unsaturated lactone comparable with that of deoxylimonin. The minor product obtained by heating the adduct (XXII) was a *transoid* diene lactone (XXV) as shown by its ultraviolet spectrum [$\lambda_{max.}$ 230 and 284 mμ (ε 6300 and 16,400 respectively)]. These two diene lactones not only confirm the relationship of the 7-ketone group of limonin to the ring-D lactone group, but also show the presence of an 8-methyl group and a 9-hydrogen atom.

The environment of the 7-ketone group of limonin was defined in a simple manner which may well have more extensive application.[18] Limonin in dry t-butyl alcohol containing potassium t-butoxide was rapidly autoxidised to the diosphenol (XXVI; R = H) characterised as its acetate (XXVI; R = Ac). Analogous diosphenols, (XXVII) and (XXVIII) respectively, were obtained from deoxylimonin (IX) and tetrahydrolimonin (X). These compounds show that limonin contains the system (–$\overset{\beta}{\underset{|}{C}}$H·$\overset{\alpha}{C}H_2$·CO). The position of the ultraviolet maximum near 280 mμ further indicated [19] that there was very probably only one hydrogen atom β with respect to the ketone group of limonin, as in the partial formula given below.

These diosphenol formulations were further confirmed by the ozonolysis of the derivative (XXVIII) from tetrahydrolimonin. This gave in good yield a nor-acid (XXIX) characterised as its methyl ester. Treatment of this acid with aqueous alkali afforded formaldehyde, a reaction comparable with those recorded in the chemistry of icterogenin [20] and iresin.[21] The presence of the grouping (–CO·O·CH$_2$·$\underset{|}{C}$·$\underset{|}{C}$=O) in the nor-acid (XXIX) is thus established. In its infrared spectrum the methyl ester of acid (XXIX) showed a carbonyl band at 1760 cm.$^{-1}$: this cannot be due to either of the lactones A and D, for the trisodium salt (two lactones opened and the carboxyl group neutralised) still showed a carbonyl band near 1750 cm.$^{-1}$; it must be ascribed to the new ketone function produced in the ozonolysis. This enhanced frequency is consistent with a cyclopentanone with an α-ethereal substituent.[22]

Limonilic acid,[12] $C_{26}H_{30}O_9$, was first obtained [14] by oxidation of limonin in alkaline solution with potassium permanganate, but it is more readily prepared in essentially quantitative yield by treatment of an alkaline solution of limonin with hypoiodite.[12] Limonilic acid is to be formulated as (XXX) on the basis of the following considerations.[1] Emerson had shown [12] that hexahydrolimoninic acid (XIII) gave a dicarboxylic acid (XXXI) on oxidation with hypoiodite. The lactone ring modified in the reaction was, therefore, lactone A and not that (lactone D) opened by hydrogenolysis. In confirmation, reduction of limonilic acid with hydriodic acid in the usual way gave a deoxylimonilic acid (as IX) with an αβ-unsaturated ring-D lactone group comparable with that in deoxylimonin itself (IX). We found that, contrary to earlier observations,[12] methyl limonilate contained no hydroxyl group (infrared spectrum). Bearing these facts in mind, and having regard to the stoicheiometry of the reaction,[12] we see that formation of

[18] See, *e.g.*, Bailey, Elks, and Barton, *Proc. Chem. Soc.*, 1960, 214; Davis and Weedon, *ibid.*, p. 182; Barton and (J.) Templeton, unpublished work.

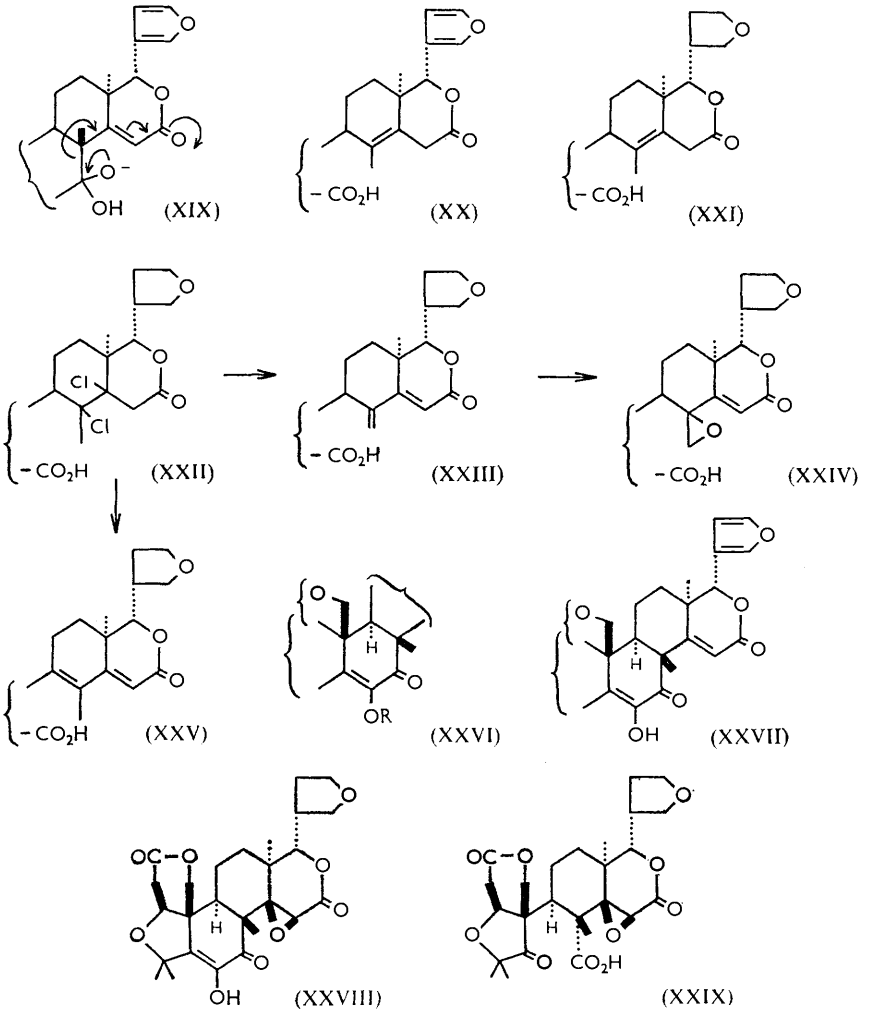
[19] See Fieser and Fieser, "Natural Products Related to Phenanthrene," 3rd edn., Reinhold Publ Corpn., New York, 1949, p. 195.

[20] Barton and de Mayo, *J.*, 1954, 887.

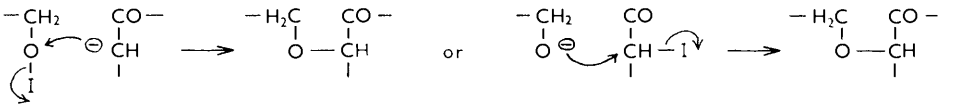
[21] Djerassi and Burstein, *J. Amer. Chem. Soc.*, 1958, **80**, 2593, and earlier papers there cited.

[22] Cf. Cookson and Dandegaonker, *J.*, 1955, 352; Baumgartner and Tamm, *Helv. Chim. Acta*, 1955, **38**, 441.

limonilic acid must involve conversion of the alkyl-oxygen of lactone A, already characterised (see above) as of the type $-CH_2{\cdot}O{\cdot}CO-$, into either an aldehyde or an ether grouping. Now the ketone group of limonin is also profoundly modified as a result of the limonilic

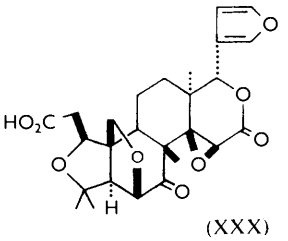


acid change. Thus it no longer gives an oxime or undergoes the diosphenol reaction (see above). Also it has displaced ultraviolet and infrared bands relative to those of limonin itself. These facts are consistent with the presence of an axially oriented ether substituent in the α-position (6β) with respect to the ketone grouping. There is no evidence for the presence of an aldehyde function in limonilic acid. We concluded, therefore, that the conversion of limonin into limonilic acid involved a change of the type



These views were confirmed by the important observation announced by Professor O. Jeger and his colleagues at the Glasgow Terpene Symposium [17] that reduction of limonilic acid with aluminium amalgam gave back limonin. The other properties of limonilic acid were likewise interpreted by Professor Jeger [17] as in the text above.

The genesis of limonilic acid from limonin represents a new procedure for the formation of an ethereal linkage facilitated, no doubt, by a specially favourable conformational situation.



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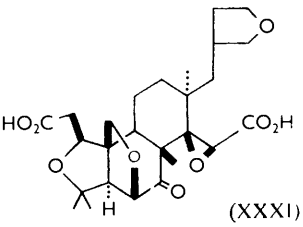
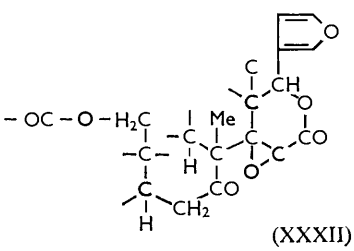


(XXXI)

The work of our group outlined above, coupled with earlier published work on limonin, makes it possible to write without ambiguity the partial structure (XXXII). If we accept a triterpenoid type of biogenesis [1] and have regard to the formation of acetone in high yield on fusion with potassium hydroxide [12] and to the formation of naphthalenic products [3,15] on dehydrogenation then one can write (XXXIII) as a plausible formula for limonin (see ref. 1). This was the position in September, 1959, when Professor Monteath Robertson was able to state from the *X*-ray evidence (see ref. 1) that limonin was either (I) or (XXXIV). The latter formula is, of course, incompatible with much chemical evidence and therefore formula (I) stands. In addition, the *X*-ray work provides the complete stereochemistry of the molecule.

The formulæ (I) and (IX) for limonin and deoxylimonin respectively permit the formulation of citrolin as in (XXXV). The earlier work of Emerson [12] had established that citrolin contained an αβ-unsaturated ketone function as well as an αβ-unsaturated lactone and a furan ring. A study of the hydrogenation of citrolin [12] appeared to show that these were the only unsaturated groupings present. We noted, however, that the ultraviolet and infrared spectra of citrolin were in better accord with the presence of *two* αβ-unsaturated lactone groups. This was confirmed by the selective hydrogenation of citrolin (under conditions which do not attack the unsaturation of ring D) to give a dihydrocitrolin (XXXVI). In this compound the presence of a saturated δ-lactone ring could be



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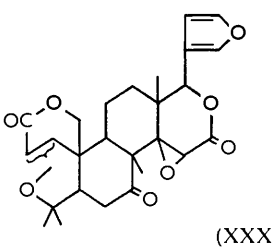

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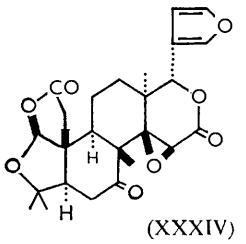

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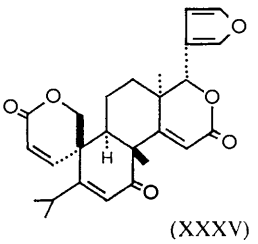

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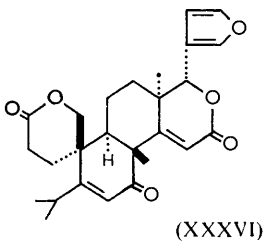


(XXXVI)

seen directly in the infrared spectra and indirectly by differential ultraviolet spectroscopy with respect to deoxylimonin (IX). The nuclear magnetic resonance spectrum of citrolin confirmed the presence of 4 *C*-methyl groups.

Other aspects of limonin chemistry which can be interpreted in terms of formula (I), but to which we have made no experimental contribution, have already been discussed in the joint communication [1] and, therefore, do not merit repetition here.

The two minor bitter principles of citrus species are [23] nomilin, $C_{28}H_{34}O_9$, and obacunone, $C_{26}H_{30}O_7$. They have recently been investigated further by Dean and Geissman.[24] Both compounds contain two lactone rings, one of which can be opened reversibly and the other irreversibly, a furan ring, a somewhat unreactive ketone group, and an ethereal ring. Nomilin is a β-acetoxy-lactone which, on treatment with a suitable hot tertiary amine, is converted into the αβ-unsaturated lactone obacunone with elimination of acetic acid. Upon consideration of these and other published facts discussed in more detail below, and having regard to a probable biogenetic relation to limonin, one of us (D. H. R. B.) proposed [1] the formulæ (XXXVII) and (XXXVIII) for nomilin and obacunone respectively. The work that we summarise in the sequel, whilst not providing a complete proof of the correctness of these structures, is nevertheless in full accord with them and confirms many of the relationships of the functional groups. In addition we have resolved certain anomalies in the earlier work [24] which were in apparent disagreement with our proposed formulæ.

Both nomilin and obacunone on mild treatment [23,24] with alkali afford obacunoic acid, $C_{26}H_{32}O_8$ (XXXIX; R = H), in which the ring-A lactone has been opened irreversibly as expected of a seven-membered ring. Obacunoic acid has two active hydrogen atoms, in keeping with the presence of a normal alcoholic hydroxyl group, a feature about which there was some confusion earlier.[24] Methyl obacunoate [24] (XXXIX; R = Me) likewise showed a hydroxyl band of normal type in the infrared spectrum. The nuclear magnetic resonance spectrum of this ester confirmed the presence of four normal *C*-methyl groups (τ = 8·88, 8·97, 9·008, and 9·043), one *C*-methyl group (τ = 8·618) of the type at $C_{(13)}$ in limonin (that is, close to the furan ring), and a *cis*-β-substituted acrylic ester [*A*–*B* quartet at τ 4·24 and 4·49 (α-H) and at τ 3·798 and 4·08 (β-H); J_{AB} = 13·4 c./sec.; see ref. 25). In addition, two α-hydrogen (τ = 2·506) and one β-hydrogen atom (τ = 3·658) attached to a furan ring [26] could be clearly characterised. One proton with a τ value of 4·63 indicated >CH·O– at position 17, a feature seen in the nuclear magnetic resonance spectra of limonin and its derivatives.

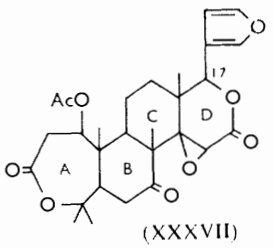
On hydrogenation in acetic acid over palladised charcoal, obacunoic acid consumed 3·74 mols. of hydrogen. Methyl obacunoate behaved similarly (3·86 mols. uptake) and gave a crystalline acid ester (XL) through hydrogenolysis of the D-ring lactone just as in limonin (see above). The consumption of hydrogen in our hands is one mol. less than that observed earlier.[24] We conclude, therefore, that obacunone and nomilin are bicarbocyclic, like limonin, not monocarbocyclic as proposed [24] earlier on the basis of the hydrogenation evidence.

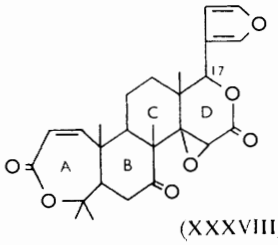
Obacunoic acid behaved like limonin on reduction with chromous chloride [1] and afforded a crystalline deoxyobacunoic acid. This contained an αβ-unsaturated lactone ring regarded as δ because of its infrared frequency. A similar conclusion as to the size of the reversibly opened (D-ring) lactone can be reached from the infrared spectra of all obacunoic acid derivatives (for details see Experimental section). Deoxyobacunoic acid behaved like deoxylimonin (see above) in that mild treatment with base gave a product (XLI) which, on spectroscopic evidence, lacked the αβ-unsaturated lactone ring. It did not crystallise but was clearly an analogue of deoxylimonic acid (XX).

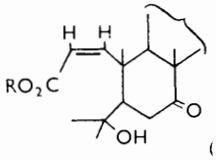
Another analogy between limonin and obacunoic acid could be seen in the following transformation. Treatment of the methyl ester acid (XL) with dioxan–hydrochloric acid under the conditions needed to transform hexahydrolimoninic acid (XIII) into its rearranged isomer (XIV) gave an acidic product which, from its infrared spectrum, was

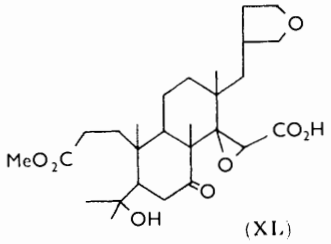
[23] Emerson, *J. Amer. Chem. Soc.*, 1948, **70**, 545; 1951, **73**, 2621; and references there cited.
[24] Dean and Geissman, *J. Org. Chem.*, 1958, **23**, 596.
[25] Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London, 1959.
[26] Corey, Slomp, Sukh Dev, Tobinaga, and Glazier, *J. Amer. Chem. Soc.*, 1958, **80**, 1204.

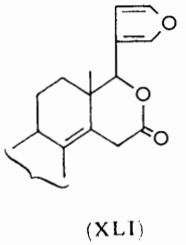
also a γ-lactone (band at 1785 cm.$^{-1}$). In addition this substance showed αβ-unsaturated ketone absorption in the ultraviolet region, but with an infrared carbonyl frequency which was abnormally low (1630 cm.$^{-1}$). The same anomalously low carbonyl frequency is found in compound (XIV) (1686 cm.$^{-1}$) and must be due to hydrogen bonding with the

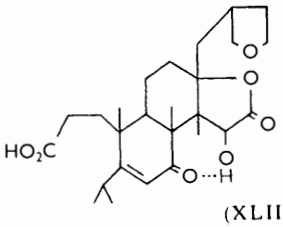

(XXXVII)

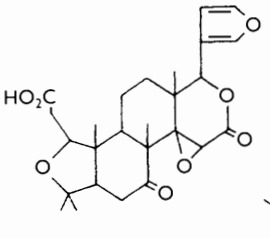

(XXXVIII)

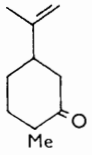

(XXXIX)

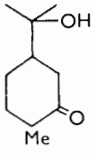

(XL)

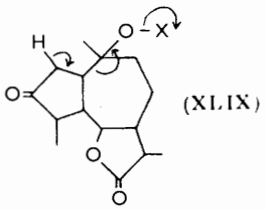

(XLI)

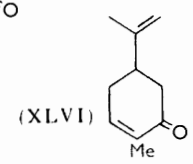

(XLII)

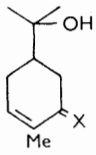

(XLIII)

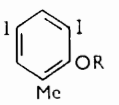

(XLIV)


(XLV)


(XLIX)


(XLVI)


(XLVII)


(XLVIII)

15-hydroxyl group. In (XIV) the λ-lactone frequency is also somewhat exalted (at 1789 cm.$^{-1}$). The reaction product is, therefore, formulated as (XLII) although it too did not crystallise.

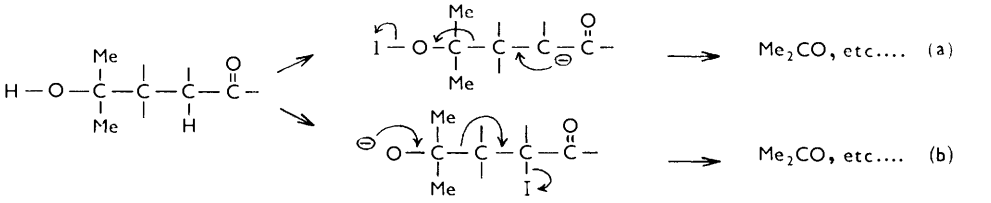
On mild treatment with sodium methoxide, methyl obacunoate was converted into iso-obacunoic acid (XLIII). This substance lacked the αβ-unsaturated acid function of obacunoic acid and also (see below) the hydroxyl group. The facts are nicely explained by formula (XLIII) and, indeed, the formation of such an iso-acid is in full accord with the proposed [1] (see also above) biogenesis of limonin.

Dean and Geissman [24] made the interesting observation that obacunone gives a positive iodoform test. Under standard conditions [27] the following yields of iodoform were obtained: acetophenone 30, acetonedicarboxylic ester 16, 21, obacunoic acid 15, limonin 0, iso-obacunoic acid 0, cinnamic acid 0, crotonic acid 0%. These results show that it is the hydroxyisopropyl grouping of obacunoic acid which is responsible for the test. There are two obvious mechanisms which would explain this.

Route (a) appears the more probable, but we do not have any decisive experiment to

[27] Fieser, " Experiments in Organic Chemistry," Heath and Co. Inc., Boston, 1955.

distinguish between the two. We have shown, however, that model compounds react in the same way. Thus, whilst dihydrocarvone [28] (XLIV) gave no iodoform, its hydrate [29] (XLV) gave 4%. Similarly carvone (XLVI) furnished no iodoform, but its hydrate [30] (XLVII; X = O) gave 6%. From the latter reaction the expected 4,6-di-iodo-*o*-cresol (XLVIII; R = H), characterised as its 3,5-dinitrobenzoate, could be isolated. α-Terpineol (XLVII; X = H_2) afforded no iodoform, but dihydroisophotosantonic lactone [31]



(XLIX; X = H), where an analogous mechanism indicated in (XLIX; X = I; see arrows) can operate, gave 4%. These iodoform experiments, taken as a whole, suggest that obacunoic acid has a hydroxyisopropyl group separated by two carbon atoms from the ketone group as already written into formula (XXXIX).

The environment of the ketone group of obacunoic acid was further defined by autoxidation experiments of the kind described above for limonin and its derivatives. Alkaline autoxidation of obacunoic acid gave a diosphenol, characterised as its acetate, in the manner expected. However, the ε values for these compounds were significantly lower than for compounds of the limonin series, and both the diosphenol and its acetate analysed for one H_2O less than expected. In addition, both compounds were neutral, not acidic. Formulæ which explain these facts are (L), (LI), and (LIII) according to which a normal diosphenol acid (L) is first formed but then rearranges, if only fleetingly, to (LI), collapse of which [(LI); see arrows, or equivalent displacements] gives the diosphenol (LIII; R = H).

Autoxidation of iso-obacunoic acid gave an acidic diosphenol with the normal spectroscopic and chemical properties expected of a structure (LII). However, it did not crystallise.

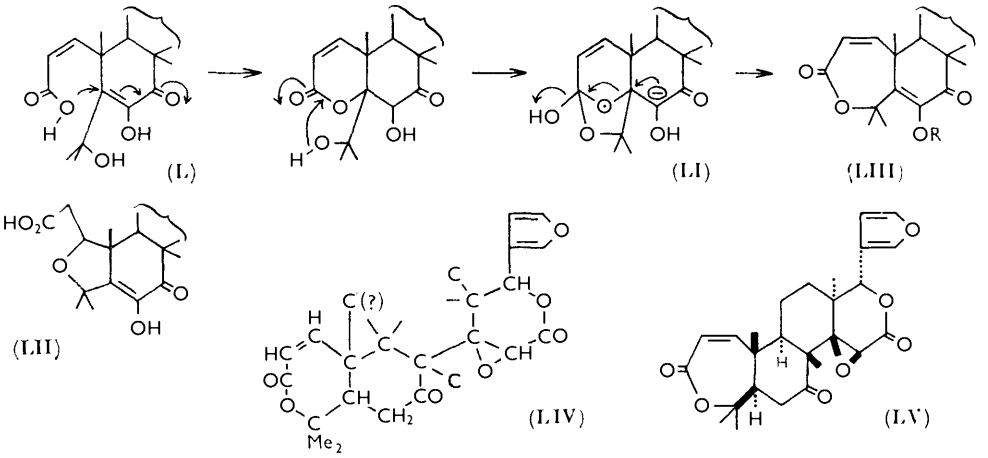
If all the facts on obacunoic acid are summarised and the interpretations made in the deduction [1] of the limonin structure are repeated then, by assuming also that the ring-A lactone of obacunone is 7-membered, the partial expression (LIV) for obacunone can be derived fairly rigidly.

The stereochemistry of obacunone is not defined by the above experiments. However, the change in $[M]_D$ between limonin and epilimonol is almost exactly the same as that between obacunone and α-obacunol, the borohydride reduction product of obacunone,[24] indicating an analogous stereochemical environment. The change in $[M]_D$ from obacunoic acid to deoxyobacunoic acid is not, however, in good agreement with the corresponding change in the limonin series. We assumed that obacunone was related, stereochemically as well as constitutionally, to limonin and was thus (LV). An attempt to inter-relate limonin and obacunone was, therefore, initiated along the following lines.

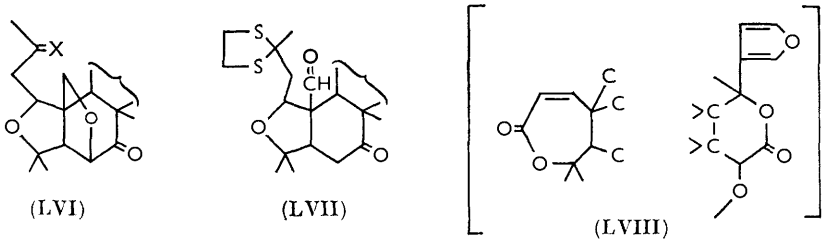
Limonilic acid (XXX) was transformed by a standard series of reactions [32] into the corresponding methyl ketone (LVI; X = O) which was then converted into the dithioketal [LVI; X = ·S·CH_2·)$_2$]. Preliminary experiments showed that limonilic acid could be reduced back efficiently to limonin by the use of lithium amalgam (for large-scale preparation of this amalgam, see the Experimental section) and that a typical dithioketal

[28] Wallach and Schrader, *Annalen*, 1894, **279**, 377.
[29] Rupe and Liechtenhan, *Ber.*, 1906, **39**, 1124.
[30] Knoevenagel and Samel, *Ber.*, 1906, **39**, 677.
[31] Barton, de Mayo, and Shafiq, *J.*, 1957, 929.
[32] See, *e.g.*, Wolfrom and Brown, *J. Amer. Chem. Soc.*, 1943, **65**, 1516; Barton, Campos-Neves, and Scott, *J.*, 1957, 2698.

was not reduced by this reagent under the experimental conditions employed. Treatment of the thioketal (LVI) in this way and oxidation of the product with pyridine–chromium trioxide then furnished the keto-aldehyde (LVII). Conversion of the aldehyde group into methyl should afford a derivative of iso-obacunoic acid. So far, however, it has not been possible to reduce the aldehyde group of (LVII) without damaging other functional groups in the molecule. A further report will be presented if we ultimately attain this objective.



After the completion of our experiments there appeared a preliminary communication by Kubota, Kamikawa, Takoroyama, and Matsuura [33] in which independent evidence is



presented for the partial structure (LVIII) for obacunane. All the observations of the Japanese authors are in agreement with our proposed formula (XXXVIII) for this bitter principle.

Experimental

M. p.s were taken on the Kofler block. Ultraviolet absorption spectra are for EtOH solutions unless stated otherwise. Rotations were determined for $CHCl_3$ solutions except where otherwise specified. Infrared spectra were taken for Nujol mulls unless stated to the contrary. Light petroleum refers to the fraction of b. p. 40—60°. Microanalyses were by Mr. J. M. L. Cameron (Glasgow) and Miss J. Cuckney (Imperial College) and their respective associates. Neutral alumina, graded according to the Brockmann scale of activity, was used in chromatography. For general experimental procedures see earlier work.[34]

Nuclear magnetic resonance spectra were kindly determined and interpreted by Drs. L. M. Jackman and J. W. Lown to whom we express our best thanks. The spectra were taken in $CDCl_3$ using a Varian Associates spectrometer model V.4311 at a fixed frequency of 56·445 Mc./sec. Line positions were measured by the conventional side-band technique with a Muirhead Decade oscillator (model D 695-A) and with tetramethylsilane as internal reference. For general information on definition of τ and other symbols see ref. 25.

[33] Kubota, Kamikawa, Tokoroyama, and Matsuura, *Tetrahedron Letters*, 1960, No. 8, p. 1.
[34] Barnes, Barton, Fawcett, and Thomas, *J.*, 1952, 2339.

Extraction of Limonin (with Mr. G. F. PHILLIPS).—The following procedure represents a convenient method for the extraction of limonin. Crushed citrus seeds (3 kg.) and commercial acetone (17 l.) were left at room temperature with occasional stirring for 2—3 weeks. The extract was decanted and filtered. The residual meal was washed with further acetone (1 l.) and processed similarly. The total acetone solution was concentrated to a syrup and then mixed with good stirring with light petroleum (b. p. 60—80°; 2·5 l.). With commercial acetone two layers may be formed, with solid at the interface; with a good grade of acetone the crude limonin is precipitated without separation of the solvent into two layers. The acetone–light petroleum (b. p. 60—80°) mixture was left at room temperature for 1—3 days and then filtered. The solid was twice made into a slurry with ethanol (150 ml.) and filtered each time. The filtrate after the second washing was almost colourless. A solution of crude limonin (suitably 24 g.) in methylene dichloride (250 ml.) was filtered, concentrated to about 100 ml., and treated with propan-2-ol (200 ml.). The solution was concentrated to the first sign of crystallisation and then left for several hours at room temperature. This gave pure limonin (12 g.), m. p. 298°, $[\alpha]_D$ −125° (in acetone), −132°, +36° (in N-ethanolic potassium hydroxide), $\lambda_{max.}$ 207 and 285 mμ (ε 7000 and 38 respectively), $\nu_{max.}$ 3124, 1603, 1505, and 876 (furan), 1755—1760 (δ-lactones) and 1706 (ketone) cm.$^{-1}$.

Various sources of limonin were investigated with the results indicated in the Table. The outstanding advantage of grapefruit seeds will be obvious.

Species and origin of citrus seeds	Limonin isolated (%) Average	Best
Valencia orange (S. Rhodesia)	0·32	0·41
Orange (S. Rhodesia)	0·42	0·54
Lemon (unknown source)	0·08	—
Lime (Jamaica)	0·20	0·21
Grapefruit (Trinidad)	0·66	0·86

Limonin was characterised as the oxime,[23] m. p. 236—239°, $[\alpha]_D$ −115° (*c* 1·00 in acetone), $\lambda_{max.}$ 201 mμ (ε 7200).

Deoxylimonin (with Mr. G. F. PHILLIPS).—Limonin (300 mg.) in " AnalaR " acetic acid (7 ml.) and concentrated aqueous hydriodic acid (5 ml.) was kept at 55° for 4 hr. (optimum time). Dilution with water, extraction into chloroform, and washing with aqueous sodium hydrogen sulphite and then aqueous sodium hydrogen carbonate gave deoxylimonin [14] (30%). Recrystallised from acetone–methanol this had m. p. 331—336°, $[\alpha]_D$ −39° (*c* 1·08), $\lambda_{max.}$ 214 mμ (ε 17,000), $\nu_{max.}$ 1747 (δ-lactone), 1714 (αβ-unsaturated δ-lactone), 1699 (ketone) cm.$^{-1}$, as well as the usual furan bands. Deoxylimonin (100 mg.) in dry pyridine (2 ml.) was treated with hydroxylamine hydrochloride (100 mg.) in ethanol (1·0 ml.) and left for 7 days at room temperature. Recrystallisation of the product from aqueous ethanol gave *deoxylimonin oxime*, m. p. 258—259°, $[\alpha]_D$ +31° (*c* 1·00 in acetone), $\nu_{max.}$ 3300 (OH), 1735 (δ-lactone), 1709 (αβ-unsaturated δ-lactone) and 1626 (C=N) cm.$^{-1}$ (Found: C, 66·45; H, 6·55; N, 3·4. $C_{26}H_{31}NO_7$ requires C, 66·5; H, 6·65; N, 3·0%).

Reduction of deoxylimonin under more vigorous conditions [14] gave citrolin, identical with material obtained in the same way by direct reduction of limonin. Recrystallised from benzene–ether this had m. p. 297—298°, $[\alpha]_D$ −140° (*c* 1·00), $\lambda_{max.}$ 212, 238, and 338 mμ (ε 23,200, 18,300, and 150 respectively), $\nu_{max.}$ 1717 (lactones), and 1667 (αβ-unsaturated ketone) cm.$^{-1}$ as well as the usual furan bands.

Citrolin was also obtained conveniently by the following procedure. Deoxylimonin (716 mg.) in hydrogen bromide in glacial acetic acid (50% w/v; 10 ml.) was refluxed for 40 min. Crystallisation of the neutral product from acetone and from tetrahydrofuran–propan-2-ol gave pure citrolin (392 mg.), identified by m. p., mixed m. p., and infrared spectrum.

Dihydrocitrolin.—Citrolin (71·4 mg.) in dioxan (10 ml.) was hydrogenated over pre-reduced palladised charcoal (10% w/w; 114 mg.) until just over 1 mol. of hydrogen had been absorbed. The neutral product, crystallised from methylene dichloride–propan-2-ol or from acetone–ethanol, gave *dihydrocitrolin* (XXXVI) (50 mg.), m. p. 282—286°, $[\alpha]_D$ −106° (*c* 1·00), $\lambda_{max.}$ 216 mμ (ε 17,200), $\lambda_{shoulder}$ 231—243 mμ (ε 14,700), $\nu_{max.}$ 1756 (δ-lactone), 1712 (αβ-unsaturated δ-lactone), 1667 and 1500 (αβ-unsaturated cyclohexanone) cm.$^{-1}$ (Found: C, 71·4; H, 7·15. $C_{26}H_{30}O_6$ requires C, 71·2; H, 6·9%).

Cholesteryl Iodoacetate.—Cholesterol (100 mg.) in dry dioxan (5 ml.) was treated with chloroacetyl chloride (0·5 ml.) at room temperature overnight. Addition of water gave the

chloroacetate which, after recrystallisation from chloroform–ethanol (80 mg.), had m. p. 156—159°, $[\alpha]_D$ −39° (*c* 1·43). Diels and Stamm [35] give m. p. 162°.

The chloroacetate (100 mg.) and finely powdered potassium iodide (400 mg.) in acetone (8 ml.) were heated under reflux for 3 hr. Crystallisation of the product from chloroform–methanol furnished *cholesteryl iodoacetate* (90 mg.), m. p. 121—123°, $[\alpha]_D$ −22° (*c* 2·06) (Found: C, 62·8; H, 8·05; I, 23·25. $C_{29}H_{47}IO_2$ requires C, 62·8; H, 8·55; I, 22·9%).

Epilimonol Iodoacetate.—Reduction of limonin with potassium borohydride essentially as described by Melera, Schaffner, Arigoni, and Jeger [3] gave epilimonol, m. p. 262—266°, $[\alpha]_D$ +9° (*c* 1·12 in acetone) (Found: C, 66·15; H, 7·05. Calc. for $C_{26}H_{32}O_8$: C, 66·1; H, 6·85%). The derived acetate had m. p. 298—301° (Found: C, 65·55; H, 6·75. Calc. for $C_{28}H_{34}O_9$: C, 65·35; H, 6·65%). The derived *methanesulphonate*, obtained from treating epilimonol (440 mg.) in dry pyridine (14 ml.) with methanesulphonyl chloride (5 ml.) at room temperature overnight, had, after crystallisation from acetone–light petroleum (300 mg.), m. p. 218°, $[\alpha]_D$ −10° (*c* 1·51) (Found: C, 58·85; H, 6·15; S, 5·95. $C_{27}H_{34}O_{10}S$ requires C, 58·9; H, 6·2; S, 5·8%).

Epilimonol (1·5 g.) in dry chloroform (100 ml.) was refluxed with chloroacetyl chloride (35 ml.) in the presence of pyridine (3 drops) for 5½ hr. Careful addition of water and separation of the chloroform layer gave *epilimonol chloroacetate* (1·10 g.), m. p. (from aqueous acetone) 265—268°, $[\alpha]_D$ +16° (*c* 1·44 in acetone) (Found: C, 59·1; H, 6·1. $C_{28}H_{33}ClO_9,H_2O$ requires C, 59·3; H, 6·2%).

Epilimonol chloroacetate (100 mg.) and sodium iodide (1·0 g.) in acetone (20 ml.) were refluxed for 4 hr. Crystallisation of the product from aqueous acetone gave *epilimonol iodoacetate*, m. p. 211—214° decomp., $[\alpha]_D$ +22° (*c* 1·54 in acetone) (Found: I, 19·9. $C_{28}H_{33}IO_9$ requires I, 19·8%).

Limonol Methanesulphonate.—Limonol [3,13] (200 mg.) in dry pyridine (16 ml.) was treated with methanesulphonyl chloride (4 ml.) at 0° and left at 0° for 3 days. Crystallisation of the product from acetone–methanol afforded the *ester* (89 mg.), m. p. 205—210° (decomp.), $[\alpha]_D$ −74° (*c* 1·07 in acetone) (Found: C, 59·1; H, 6·05. $C_{27}H_{34}O_{10}S$ requires C, 58·9; H, 6·2%).

Deoxyepilimonol.—Deoxylimonin in dioxan was reduced with potassium borohydride essentially as for the preparation of epilimonol (see above). Crystallisation of the product from acetone–light petroleum furnished *deoxyepilimonol*, m. p. 300—305°, $[\alpha]_D$ +61° (*c* 0·70 in acetone), $\lambda_{max.}$ 212 mμ (ε 17,300), $\nu_{max.}$ 3390 (OH) and 1730 and 1706 (δ-lactones) cm.$^{-1}$ (Found: C, 68·55; H, 7·1. $C_{26}H_{32}O_7$ requires C, 68·4; H, 7·05%). Treatment of deoxyepilimonol with an excess of methanesulphonyl chloride in pyridine overnight at room temperature afforded the *methanesulphonate*. Recrystallised from acetone–ethanol this had m. p. 245—246° (decomp.), $[\alpha]_D$ +62° (*c* 0·72 in acetone), $\lambda_{max.}$ 213—214 mμ (ε 17,200) (Found: C, 60·75; H, 6·45; S, 6·1. $C_{27}H_{34}O_9S$ requires C, 60·65; H, 6·4; S, 6·0%).

The Diosphenol (XXVIII) *from Tetrahydrolimonin and its Congeners.*—Tetrahydrolimonin (finely powdered; 2·5 g.) suspended in t-butyl alcoholic N-potassium t-butoxide (200 ml.) was shaken with oxygen in a standard hydrogenation apparatus for 1—2 hr. (uptake of 1 mol. of oxygen). Water (200 ml.) was added and then 6N-hydrochloric acid (50 ml.). The solution was extracted with chloroform (3 × 100 ml.), and the extracts were washed with saturated aqueous sodium hydrogen carbonate (2 × 25 ml.) and then water (100 ml.). The chloroform solution was then shaken with aqueous 4N-sodium hydroxide (2 × 25 ml.). Acidification of this extract with aqueous 6N-hydrochloric acid gave a white precipitate which was extracted in chloroform (3 × 50 ml.) again. The chloroform solutions were washed with water (2 × 50 ml.), and the solvent was removed (70—90% of acidic material). Crystallisation from methylene dichloride–ethanol gave the *diosphenol* (XXVIII) (60%), m. p. 157—161°, $[\alpha]_D$ −223° (*c* 1·26 in acetone), $\lambda_{max.}$ 278 mμ (ε 11,000), $\lambda_{max.}$ 336—340 mμ (ε 6300 in 0·1N-sodium hydroxide; reversible shift), $\nu_{max.}$ 3484 (OH), 1754 and 1739 (δ-lactones), and 1695 and 1653 (diosphenol cm.$^{-1}$ (Found: C, 63·05; H, 7·25. $C_{26}H_{32}O_9,C_2H_6O$ requires C, 62·9; H, 7·15%). The compound gave an immediate ferric chloride test.

In a similar way limonin (1·0 g.) was converted into the corresponding *diosphenol* (XXVI; R = H) (470 mg.). Recrystallised from acetone–ethanol this had m. p. 273—288°, $[\alpha]_D$ −200° (*c* 1·00 in acetone), $\lambda_{max.}$ 207 and 278 mμ (ε 7900 and 10,100), shifted reversibly in alkali to $\lambda_{max.}$ 336 mμ (ε 6150), $\nu_{max.}$ 3484 (OH), 1748 and 1736 (δ-lactones), and 1689 and 1661 (diosphenol)

[35] Diels and Stamm, *Ber.*, 1912, **45**, 2228.

cm.$^{-1}$ (Found: C, 64·3; H, 6·1. $C_{26}H_{28}O_9$ requires C, 64·45; H, 5·85%). This diosphenol (200 mg.) was treated with excess of pyridine–acetic anhydride (1 : 1) overnight at room temperature. Crystallisation of the product from methylene dichloride–ethanol furnished the *diosphenol acetate* (XXVI; R = Ac) (184 mg.), m. p. 292—299°, $[\alpha]_D$ −101° (*c* 1·20), $\lambda_{max.}$ 210 and 245 mμ (ε 8800 and 11,000 respectively), $\nu_{max.}$ 1751 and 1739 (δ-lactones and diosphenol acetate), 1695 (αβ-unsaturated ketone), and 1650 (conjugated ethylenic linkage) cm.$^{-1}$ (Found: C, 63·3; H, 6·1; Ac, 8·15. $C_{28}H_{30}O_{10}$ requires C, 63·8; H, 5·7; Ac, 8·75%).

The same autoxidation process converted deoxylimonin (300 mg.) into its *diosphenol* (XXVII). Recrystallised from acetone–ethanol this (175 mg.) had m. p. 310—317°, $[\alpha]_D$ −112° (*c* 0·93 in acetone), $\lambda_{max.}$ 282 mμ (ε 10,400), 335 mμ (ε 5200 in N-ethanolic potassium hydroxide), $\nu_{max.}$ 3480 (OH), 1764 and 1733 (δ-lactones) and 1681 (diosphenol) cm.$^{-1}$ (Found: C, 66·55; H, 6·1. $C_{26}H_{28}O_8$ requires C, 66·65; H, 6·0%). Treatment with pyridine–acetic anhydride overnight at room temperature gave the corresponding *acetate*, m. p. (from acetone–ethanol) 262—264°, $[\alpha]_D$ −74° (*c* 1·53 in acetone), $\lambda_{max.}$ 213—214 and 246 mμ (ε 18,000 and 13,000 respectively), $\nu_{max.}$ 1764, 1733, and 1706 (δ-lactones and acetate), 1684 (αβ-unsaturated ketone), and 1605 (conjugated ethylenic linkage) cm.$^{-1}$ (Found: C, 65·6, 66·05; H, 6·05, 6·25. $C_{28}H_{30}O_9$ requires C, 65·85; H, 5·9%).

Degradation of the Diosphenol (XXVIII) *from Tetrahydrolimonin.*—The diosphenol (500 ml.) in chloroform (50 ml.) was ozonised at −20° until the diosphenol chromophore had been destroyed (20 min.). Water (25 ml.) was added, the chloroform removed on the steam-bath, and heating continued for 15 min. Excess of sodium hydrogen carbonate was added and the solution extracted with chloroform to remove traces (10 mg.) of neutral material. Acidification with 6N-hydrochloric acid and extraction into chloroform afforded the *nor-acid* (XXIX). Recrystallised from methylene dichloride–ethanol, this (75%) had m. p. 208—212° (decomp.), $[\alpha]_D$ −200° (*c* 0·80 in acetone), $\lambda_{max.}$ 296 mμ (ε 37), $\nu_{max.}$ 1760 (exalted cyclopentanone), 1735 and 1725 (δ-lactones and carboxylic acid) cm.$^{-1}$ (Found: C, 61·2; H, 6·35. $C_{25}H_{28}O_{10}$ requires C, 61·45; H, 6·35%). Treatment with an excess of ethereal diazomethane in a little dioxan gave the corresponding *methyl ester*, m. p. (from acetone–light petroleum) 215—221°, $[\alpha]_D$ −190° (*c* 1·56 in acetone), $\nu_{max.}$ 1761 (exalted cyclopentanone), and 1739 and 1727 (δ-lactones and methoxycarbonyl group) cm.$^{-1}$ in $CHCl_3$ (Found: C, 61·4; H, 6·75. $C_{26}H_{30}O_{10}$ requires C, 61·65; H, 6·75%).

The nor-acid (163 mg.) was left in aqueous 4N-sodium hydroxide (3·0 ml.) at room temperature overnight under oxygen-free nitrogen. The solution was acidified with 6N-sulphuric acid (2 ml.) and distilled into aqueous dimedone (2 × theor.). This gave the dimedone–formaldehyde condensation product (13·8 mg., 23%), identified by m. p. and mixed m. p. The appropriate control gave none of this derivative.

The nor-acid (170 mg.) was treated as described above except that the distillate was collected in a solution of 2,4-dinitrophenylhydrazine in methanolic sulphuric acid. Filtration in benzene solution through alumina (grade III) gave formaldehyde 2,4-dinitrophenylhydrazone (17%), identified by m. p., mixed m. p., and ultraviolet absorption spectrum. A control experiment gave none of this derivative.

Limonilic Acid and Its Derivatives.—Limonilic acid was prepared according to Emerson's method.[12] Recrystallised from propan-2-ol–light petroleum it had m. p. 290—292°, $[\alpha]_D$ +104° (*c* 1·92 in acetone). The derived methyl ester (diazomethane–ether), crystallised from the same solvent mixture, had m. p. 231—233°, $[\alpha]_D$ +111° (*c* 1·01 in acetone), $\lambda_{max.}$ 307 mμ (ε 37), $\nu_{max.}$ 1735 and 1715 (δ-lactones and ketone) cm.$^{-1}$. There was no sign of a hydroxyl band in the infrared spectrum.

When epilimonol was treated with iodine and alkali under the conditions used for making limonilic acid [12] it was recovered unchanged in high yield.

Limonilic acid was recovered unchanged after being shaken under oxygen with potassium t-butoxide in t-butyl alcohol as in the preparation of the series of diosphenols (see above).

The rate of alkaline hydrolysis of methyl limonilate was compared with that of other esters in the following way. The methyl ester (0·02 milliequiv.) in dioxan (5 ml.) and aqueous 0·04N-sodium hydroxide (5 ml.) was kept at 25° and the uptake of alkali determined in the usual way by removal of 1 ml. aliquot portions. Preliminary experiments with methyl limonilate showed that the lactone ring was opened essentially instantaneously. Correction was made, therefore, for this by the appropriate adjustment of the concentration of aqueous sodium hydroxide used. The same applies for the hexahydro-derivative of methyl limonilate (which has a free carboxyl group). The times for half-hydrolysis, in parentheses, were: (i) methyl propionate (8·5 min.),

(ii) methyl isobutyrate (24 min.), (iii) methyl α-methoxy-α-methylpropionate (26 min.), (iv) hexahydro-derivative of methyl limonilate (31 min.), (v) methyl limonilate [approx. as for (iv)].

Isotetrahydrolimonin.—Tetrahydrolimonin (880 mg.) in (3 : 2) acetic acid–concentrated hydrochloric acid (20 ml.) was heated on the steam-bath for 1½ hr. Crystallisation of the product from acetone–ethanol gave *isotetrahydrolimonin*, m. p. 263—266°, $[\alpha]_D$ −102° (*c* 0·96 in acetone), $\lambda_{max.}$ 256 mμ (ε 5300), changing to $\lambda_{max.}$ 290 mμ (ε 4600) on addition of 1 drop of N-ethanolic potassium hydroxide, $\nu_{max.}$ 1740 and 1710 (δ-lactones and ketonic carbonyl group) and 1660 (conjugated ethylenic linkage) cm.$^{-1}$ (Found: C, 65·6; H, 7·55. $C_{26}H_{34}O_8$ requires C, 65·8; H, 7·2%). Treatment with excess of pyridine–acetic anhydride overnight at room temperature afforded *isotetrahydrolimonin acetate*. Recrystallised from aqueous ethanol this had m. p. 192—196°, $[\alpha]_D$ −150° (*c* 1·00 in acetone), $\lambda_{max.}$ 218 mμ (ε 11,000), $\nu_{max.}$ 1745 and 1720 (δ-lactones, enolic acetate and ketonic carbonyl group), and 1630 (conjugated ethylenic linkage) cm.$^{-1}$ (Found: C, 63·0; H, 7·35. $C_{28}H_{36}O_9,H_2O$ requires C, 62·9; H, 7·15%).

Isotetrahydrolimonin (249 mg.) in chloroform (50 ml.) was ozonised at −20° until the absorption at $\lambda_{max.}$ 256 mμ was negligible (25 min.). Water (15 ml.) was added and the chloroform distilled off. The resultant clear aqueous solution was kept on the steam-bath for 30 min. The water was removed *in vacuo* to give the product, which did not crystallise. The crude product was warmed with 0·4N-sodium hydroxide for 20 min. (standard procedure for equivalent determination) and found to give an equiv. of 138 (Calc. for 3 acid groups, 169). The neutralised solution, treated with saturated aqueous calcium nitrate, gave an immediate precipitate of calcium oxalate (80%) identified by crystal form and infrared spectrum.

Deoxylimonic Acid and its Derivatives.—Deoxylimonin (470 mg.) was heated with aqueous 2N-sodium hydroxide (40 ml.) for 1½ hr. on the steam-bath. The clear alkaline solution was acidified with hydrochloric acid and extracted into ether. Crystallisation of the acidic product from propan-2-ol gave *deoxylimonic acid* (260 mg.), m. p. 247—250°, $[\alpha]_D$ −166° (*c* 1·26 in acetone) [Found: C, 66·2; H, 6·55%; equiv. (excess of 4N-aqueous sodium hydroxide on steam-bath for 10 min.; then back-titration to phenolphthalein), 153, 158. $C_{26}H_{32}O_8$ requires C, 66·1; H, 6·85%; equiv. (3 acidic groups), 157]. Limonin showed an equiv. of 234 (theor., 235) under the same conditions. *Methyl deoxylimonate*, prepared with diazomethane in the usual way, had, after crystallisation from propan-2-ol, m. p. 164—167°, $[\alpha]_D$ −154° (*c* 1·25 in acetone), ε = 11,400 at 208 mμ, $\nu_{max.}$ 1720—1755 (δ-lactones and carbomethoxyl) cm.$^{-1}$, no hydroxyl band (Found: C, 66·6; H, 6·85; OMe, 6·15. $C_{27}H_{34}O_8$ requires C, 66·65; H, 7·05; OMe, 6·35%). The ester gave no oxime under the conditions used for the preparation of deoxylimonin oxime (see above). Deoxylimonic acid did not undergo a limonilic-type reaction with alkaline hypoiodite.

Deoxylimonin oxime was treated with alkali under the conditions used (see above) for making deoxylimonic acid but was recovered unchanged (193 mg. from 220 mg.).

Deoxylimonilic Acid and Derivatives.—Limonilic acid (150 mg.) in " AnalaR " acetic acid (3·5 ml.) and concentrated aqueous hydriodic acid (2·5 ml.) was kept at 55° for 4 hr. Crystallisation of the acidic product from propan-2-ol–light petroleum afforded *deoxylimonilic acid* (87 mg.), m. p. 275—278°, $[\alpha]_D$ +47° (*c* 1·56 in acetone) (Found: C, 66·0; H, 6·25. $C_{26}H_{30}O_8$ requires C, 66·35; H, 6·45%). The derived *methyl ester*, prepared with ethereal diazomethane in the usual way, had, after crystallisation from propan-2-ol–light petroleum, m. p. 213—215°, $[\alpha]_D$ +46° (*c* 1·64 in acetone), $\lambda_{max.}$ 213—214 mμ (ε 15,400), broad infrared band at 1700—1730 cm.$^{-1}$ (δ-lactones and carbomethoxyl group) (Found: C, 66·45; H, 6·65; OMe, 6·15. $C_{27}H_{32}O_8$ requires C, 66·9; H, 6·65; OMe, 6·4%).

Deoxylimonilic acid, treated with alkali under the conditions used (see above) for the preparation of deoxylimonic acid, did not go into solution and was recovered unchanged. The acid (100 mg.) was, therefore, refluxed with methanolic 5% potassium hydroxide (10 ml.) for 30 min. The methanol was removed *in vacuo*, excess of aqueous hydrochloric acid added, and the product separated into acidic and neutral (negligible) fractions. Crystallisation of the acidic fraction from propan-2-ol–light petroleum gave back deoxylimonilic acid (62 mg.), identified by m. p., mixed m. p., and ultraviolet absorption spectrum.

Deoxytetrahydrolimonin.—(a) *From tetrahydrolimonin.* Tetrahydrolimonin (100 mg.) in " AnalaR " acetic acid (3·5 ml.) and concentrated aqueous hydriodic (2·5 ml.) was kept at 55° for 3½ hr. Crystallisation of the product from methylene dichloride–ethanol afforded *deoxytetrahydrolimonin*, m. p. 293—297°, $[\alpha]_D$ −88° (*c* 1·30 in $CHCl_3$), $\lambda_{max.}$ 219 mμ (ε 10,800), $\nu_{max.}$ 1752, 1720 (sh) and 1710 (δ-lactones and ketonic carbonyl) cm.$^{-1}$ (Found: C, 68·15; H, 7·4.

$C_{26}H_{34}O_7$ requires C, 68·1; H, 7·45%). On crystallisation from methanol this compound melted at 178—182°.

(b) *From deoxylimonin.* Deoxylimonin (1·0 g.) was dissolved in warm acetic acid (100 ml.) and then hydrogenated over 10% palladised charcoal (1·0 g.) (1 mol. uptake). Crystallisation of the product from methylene dichloride–ethanol afforded deoxytetrahydrolimonin identical with the compound described above.

After treatment with acid as for the preparation of isotetrahydrolimonin (see above) deoxytetrahydrolimonin was recovered unchanged in high yield.

Iodoacetate (XII) *from Ring-cleavage of Deoxytetrahydrolimonin.*—(a) *From tetrahydrolimonin.* Tetrahydrolimonin (367 mg.) was warmed at 55° with a mixture of " AnalaR " acetic acid and concentrated hydriodic acid (10 ml.) for 3½ hr. The neutral product, crystallised from ethanol, gave the *iodoacetate*, m. p. 238—239°, $[\alpha]_D$ −105° (*c* 0·85), −118° (*c* 1·03 in acetone), $\lambda_{max.}$ 219 mμ (ε 12,700), $\nu_{max.}$ 1710 and 1732 (δ-lactones, acetate and ketonic carbonyl) cm.$^{-1}$ (Found: C, 53·8; H, 6·0; Ac, 6·9, 7·3. $C_{28}H_{37}IO_8$ requires C, 53·5; H, 5·9; Ac, 6·85%).

(b) *From deoxytetrahydrolimonin.* Deoxytetrahydrolimonin (260 mg.) in 1 : 4 acetic anhydride–acetic acid (10 ml.) was treated dropwise at room temperature with concentrated hydriodic acid (3 ml.) and left for 16 hr. Crystallisation of the product from ethanol–light petroleum gave the above iodoacetate (40 mg.), identified by m. p., mixed m. p., and ultraviolet and infrared spectra.

Deoxytetrahydrolimonic Acid.—(a) *From deoxytetrahydrolimonin.* Deoxytetrahydrolimonin (150 mg.) was heated on the steam-bath for 2 hr. with aqueous 2N-sodium hydroxide (30 ml.). Acidification with 6N-hydrochloric acid gave *deoxytetrahydrolimonic acid.* Recrystallised from aqueous acetone this had m. p. 276—284°, $[\alpha]_D$ −89° (*c* 1·10 in pyridine), $\lambda_{max.}$ 202 mμ (ε 11,300), $\nu_{max.}$ 1758, 1730, and 1716 (δ-lactones and carbonyl group) cm.$^{-1}$ (Found: C, 65·5; H, 7·8. $C_{26}H_{36}O_8$ requires C, 65·55; H, 7·6%).

(b) *From deoxylimonic acid.* Deoxylimonic acid (572 mg.) was hydrogenated in acetic acid (75 ml.) over 10% palladised charcoal (1·0 g.) (uptake 1 mol.). Crystallisation of the product from aqueous acetone gave deoxytetrahydrolimonic acid, identified by m. p., mixed m. p., and infrared spectrum.

(c) *From the iodoacetate* (XII) *from deoxytetrahydrolimonin.* The iodoacetate (XII) (272 mg.) was shaken with potassium hydroxide (3 g.) in water (20 ml.) until dissolved and then left at room temperature for 19 hr. Acidification with 2N-sulphuric acid gave deoxytetrahydrolimonic acid (184 mg.), identified, after crystallisation from aqueous acetone, in the usual way.

Derivatives of Deoxytetrahydrolimonic Acid.—Deoxytetrahydrolimonic acid (720 mg.) in acetic acid (100 ml.) was treated with chlorine (0·20 mmole) in carbon tetrachloride (28 ml.) and left in the dark at room temperature for 16 hr. The mixed solvent was removed *in vacuo* and the residue heated at 110° for 3 hr. also *in vacuo*. Crystallisation from acetone–ether gave the cisoid *diene-acid* (XXIII) (390 mg.), m. p. 259—269°, $[\alpha]_D$ +231° (*c* 0·80), $\lambda_{max.}$ 255 mμ (ε 7800) in a broad band, $\nu_{max.}$ 1760, 1714, and 1703 (δ-lactones and carboxyl), and 1619 and 1600 (C=C) cm.$^{-1}$ [Found: C, 65·7; H, 7·4%; equiv. (saponification and back-titration), 174. $C_{26}H_{34}O_8$ requires C, 65·8; H, 7·2%; equiv. (3 acidic functions), 160]. The crude material obtained after removal of the mixed solvents but before the heating period (see above) was also examined. It had $\lambda_{max.}$ *ca.* 230 mμ (ε 6000) and a shoulder at *ca.* 250 mμ (ε 4000). Crystalline *cisoid* diene-acid (XXIII) was obtained from this product in poor yield. During the heating period the spectrum slowly changed to that expected for a mixture of *cisoid* and *transoid* (see below) diene-acids. Ethanolic potassium hydroxide or collidine gave similar results to heating but the products were not so clean. On a spectroscopic basis the ratio of *cisoid* to *transoid* isomers was between 2 : 1 and 4 : 1.

The *cisoid* diene-acid (XXIII) was characterised in the following way. The *methyl ester*, prepared with ethereal diazomethane (45 min. at room temperature) had, after crystallisation from acetone–ether, m. p. 200—204°, $[\alpha]_D$ +226° (*c* 1·30), $\lambda_{max.}$ 255 mμ (ε 7700) in a broad band (Found: C, 66·2; H, 7·35. $C_{27}H_{36}O_8$ requires C, 66·35; H, 7·45%). Treatment of the acid (XXIII) (74 mg.) with 80% peracetic acid (2 ml.), previously saturated with sodium acetate, for 16 hr. at 0° gave, after crystallisation of the product from acetone–ether, the *epoxide* (XXIV) (35 mg.), m. p. 243—249°, $[\alpha]_D$ +88° (*c* 0·40), $\lambda_{max.}$ 217—218 mμ (ε 9100), $\nu_{max.}$ 1767, 1715, and 1708 (δ-lactones and carbonyl group) cm.$^{-1}$ (Found: C, 63·85; H, 7·15. $C_{26}H_{34}O_9$ requires C, 63·65; H, 7·0%).

The *cisoid* diene-acid (133 mg.) in chloroform (20 ml.) was ozonised at −20° for 15 min.

(spectroscopic control). Zinc dust (500 mg.) and water (8 ml.) were added and the mixture was distilled. The distillate was treated with excess of aqueous dimedone solution and the chloroform distilled off. After 20 hr. the formaldehyde derivative was filtered off (18·5 mg., 13%) and identified by m. p. and mixed m. p. Under identical conditions pyrethrosin [36] (110 mg.) gave 13·4% of the formaldehyde–dimedone derivative. The appropriate controls were also run.

The mother-liquors from the crystallisation of the *cisoid* diene-acid (see above) were concentrated and left to crystallise. This gave the isomeric transoid *diene-acid* (XXV) (80 mg.), m. p. (from acetone–ether) 260—271°, $[\alpha]_D$ +260° (*c* 1·20 in CH_2Cl_2), $\lambda_{max.}$ 230 and 284 mμ (ε 6300 and 16,400 respectively), $\nu_{max.}$ 1748, 1730, and 1704 (δ-lactones and carbonyl group), and 1594 (C=C) cm.$^{-1}$ (Found: C, 64·75; H, 7·0. $C_{26}H_{34}O_8,0{\cdot}5H_2O$ requires C, 64·6; H, 7·3%). The derived *methyl ester*, prepared as for the *cisoid* ester (see above) and crystallised from acetone–ether, had m. p. 207—212°, $\lambda_{max.}$ 229 and 283 mμ (ε 5400 and 18,600 respectively) (Found: C, 65·1; H, 7·2. $C_{27}H_{36}O_8,0{\cdot}5H_2O$ requires C, 65·7; H, 7·5%).

Isomerisation of Hexahydrolimoninic Acid.—Hexahydrolimoninic acid (500 mg.) in dioxan (6 ml.) and concentrated hydrochloric acid (6 ml.) was heated for 14 hr. on the steam-bath. Crystallisation of the neutral product from methylene dichloride–acetone afforded *isohexahydrolimoninic acid* (XIV), chars at 315°, $[\alpha]_D$ −82° (*c* 0·58), end-absorption only in the ultraviolet region, infrared bands at 3333 (OH), 1789 (γ-lactone), 1755 (δ-lactone), and 1686 (hydrogen-bonded carbonyl) cm.$^{-1}$ (Found: C, 65·4; H, 7·7; active H, 0·22. $C_{26}H_{36}O_8$ requires C, 65·55; H, 7·6; active H, 0·21%).

Isohexahydrolimoninic acid (226 mg.) was left at room temperature for 22 hr. with a ground mixture of chromium trioxide (700 mg.) in pyridine (6 ml.). Water (20 ml.) was added and the excess of oxidant destroyed with sulphur dioxide. The neutral product (181 mg.), recrystallised from methylene dichloride–ethanol or from acetone–ethanol, gave *dehydroisohexahydrolimoninic acid* (XV), m. p. 245—253°, $[\alpha]_D$ −43° (*c* 1·50), no high-intensity ultraviolet absorption, $\nu_{max.}$ 1792 (γ-lactone), 1777 (α-keto-γ-lactone), 1760 (δ-lactone), and 1720 (non-hydrogen-bonded ketonic carbonyl) cm.$^{-1}$ (Found: C, 66·0; H, 7·25. $C_{26}H_{34}O_8$ requires C, 65·8; H, 7·2%).

Iodoacetate (XVI) *from Ring-cleavage of Hexahydrolimoninic Acid.*—Hexahydrolimoninic acid (640 mg.) in " AnalaR " acetic acid (16 ml.), acetic anhydride (4 ml.), and concentrated hydriodic acid (6 ml.) was left at room temperature for 24 hr. Working up gave a neutral product (751 mg.) which, on repeated crystallisation from ethanol, afforded the *iodoacetate* (XVI), m. p. 227—229°, $[\alpha]_D$ −59° (*c* 1·50 in acetone), $\lambda_{max.}$ 255 mμ (ε 500), $\nu_{max.}$ 3220 (OH), 1790 (γ-lactone), 1755 (δ-lactone), 1730 (OAc), and 1677 (hydrogen-bonded ketonic carbon) cm.$^{-1}$ (Found: C, 52·5, 52·5, 52·45; H, 5·95, 6·15, 5·9; I, 21·25; Ac, 6·75. $C_{28}H_{39}IO_9$ requires C, 52·15; H, 6·05; I, 19·65; Ac, 6·65%).

This iodoacetate (500 mg.) in " AnalaR " acetic acid (50 ml.) was heated on the steam-bath with zinc powder (3 g.) for 10 min. with constant shaking. Crystallisation of the product from methylene dichloride–ethanol gave the *γ-lactone acetate* (XVII), m. p. 246—256°, $[\alpha]_D$ −76° (*c* 1·31 in acetone), $\lambda_{max.}$ 290 mμ (ε 26), $\nu_{max.}$ 3300 (OH), 1790 (γ-lactone), 1751 (δ-lactone), 1730 (OAc) and 1683 (hydrogen-bonded ketonic carbonyl) cm.$^{-1}$ (Found: C, 64·3; H, 7·9; Ac, 8·5; active H, 0·18. $C_{28}H_{40}O_9$ requires C, 64·6; H, 7·75; Ac, 8·25; active H, 0·19%).

The iodoacetate (XVI) (200 mg.) was heated on the steam-bath for 30 min. with aqueous N-sodium hydroxide (15 ml.). Crystallisation of the neutral product from methylene dichloride–ethanol furnished isohexahydrolimoninic acid, identified especially by its infrared spectrum.

The γ-lactone acetate (XVII) (290 mg.) in pyridine (5·5 ml.) was slowly added to a ground suspension of chromium trioxide (364 mg.) in pyridine (3 ml.) and left at room temperature overnight. Addition of water, destruction of the excess of oxidant with sulphur dioxide, and extraction in the usual way gave a neutral product (284 mg.) which, recrystallised from methylene dichloride–ethanol and then acetone–ethanol, furnished the *α-keto-γ-lactone* (XVIII), m. p. 235—239°, $[\alpha]_D$ −45° (*c* 1·60 in acetone), $\nu_{max.}$ 1792 (γ-lactone), 1775 (α-keto-γ-lactone), 1750 with shoulder (δ-lactone and acetate) and 1717 (non-hydrogen-bonded ketonic carbonyl) cm.$^{-1}$ (Found: C, 64·7; H, 7·55; Ac, 8·1. $C_{28}H_{38}O_9$ requires C, 64·85; H, 7·4; Ac, 8·3%).

Methyl Norlimonilyl Ketone (LVI; X = O).—Limonilic acid (2·06 g.) in tetrahydrofuran (30 ml.) and oxalyl chloride (5 ml.) was heated under reflux for 1 hr. The mixed solvents were removed *in vacuo* and the residual limoniloyl chloride crystallised from benzene–light petroleum and dried *in vacuo* at 50°. Reliable analytical data were not secured for this compound but it

[36] Barton and de Mayo, *J.*, 1957, 150.

showed infrared bands at 1791 (acid chloride), 1740 (δ-lactone), and 1722 (cyclohexanone with α-ether group) cm.$^{-1}$ and gave methyl limonilate, identified by m. p. and mixed m. p., (214 mg. from 234 mg. of acid), on dissolution in methanol. The crystalline chloride, in dry tetrahydrofuran (30 ml.), was poured slowly into a solution of diazomethane in dry ether (prepared from 25 g. of *N*-methylnitrosourea) with cooling to 0°. The solution was left at room temperature for 1 hr. and then the ether was removed *in vacuo* at 30—40°. The residue was dissolved in chloroform (25 ml.) and shaken with hydriodic acid (*d* 1·7; 6 ml.) for 5 min. The neutral product in ethanol (30 ml.) was refluxed with aqueous 0·4N-sodium hydroxide (30 ml.) on the steam-bath for 30 min. with gradual removal of the ethanol. After cooling, the aqueous solution was extracted with chloroform (discarded) and then acidified, and the neutral product (1·58 g.) was extracted in benzene. After drying (Na_2SO_4), this extract was filtered through alumina (grade I; 6 g.). Crystallisation from benzene–light petroleum then afforded *methyl norlimonilyl ketone* (782 mg.), m. p. 110—112° (decomp., $[\alpha]_D$ +85° (*c* 1·10 in acetone), $\nu_{max.}$ 1736 (δ-lactone), 1722 (cyclohexanone with α-ether group), and 1712 (Me ketone) cm.$^{-1}$ (Found: C, 73·25; H, 6·9%; sap. equiv., 600. $C_{27}H_{32}O_8,2C_6H_6$ requires C, 73·1; H, 6·9%; equiv., 640). The presence of 2 mols. of benzene of crystallisation was confirmed by quantitative ultraviolet absorption and by the presence of the appropriate infrared bands. Precipitation of the compound from acetone with light petroleum gave benzene-free amorphous material, m. p. *ca.* 145°, showing no benzene absorption but only end-absorption, λ 207 mμ (ε 7000). Methyl norlimonilyl ketone did not crystallise except as the benzene solvate.

Methyl norlimonilyl ketone (71 mg.) was heated on the steam-bath with aqueous N-sodium hydroxide (6 ml.) for 1 hr. Working up gave back starting material (64 mg.), identified as the benzene solvate by m. p., mixed m. p., and rotation.

Methyl norlimonilyl ketone (41 mg.) in aqueous N-sodium hydroxide (10 ml.) was treated with excess of iodine in potassium iodide solution and left at room temperature. Iodoform was precipitated immediately and identified by m. p. and mixed m. p.

Derivatives of Methyl Norlimonilyl Ketone.—The ketone (2·31 g.) in ethanedithiol (50 drops) and tetrahydrofuran (5 ml.) was left at room temperature with toluene-*p*-sulphonic acid (3 g.) for 50 min. The solution was diluted with chloroform, extracted with aqueous N-sodium hydroxide, and evaporated. The crude product was filtered in benzene (25 ml.) through alumina (grade V; 10 g.), elution being with more benzene (50 ml.). Crystallisation from benzene–light petroleum afforded *methyl norlimonilyl ketone ethylene dithioketal* [LVI; X = (·S·CH_2·)$_2$] (1·53 g.), m. p. 264—272°, $[\alpha]_D$ +57° (*c* 1·00 in acetone), $\nu_{max.}$ 1742 (δ-lactone) and 1729 (exalted cyclohexanone) cm.$^{-1}$ (Found: C, 62·15; H, 6·75; S, 11·65. $C_{29}H_{36}O_7S_2$ requires C, 62·1; H, 6·75; S, 11·65%). Limonin, treated under the same conditions, was recovered unchanged (520 mg. from 557 mg.).

In a preliminary experiment 3β-acetoxyallopregnane-11,20-dione (kindly provided by Messrs. Glaxo Laboratories Ltd.) (465 mg.) was left with ethanedithiol (10 drops) and boron trifluoride–ether complex (15 drops) at room temperature for 5 min. (crystalline deposit). Addition of methanol and working up in the usual way furnished 3β-*acetoxyallopregnane*-11,20-*dione* 20-(*ethylene dithioketal*) (554 mg.). Recrystallised from methylene dichloride–ethanol this (300 mg.) had m. p. 234—237°, $[\alpha]_D$ +22° (*c* 1·50), $\nu_{max.}$ 1725 (OAc) and 1695 (11-ketone) cm.$^{-1}$ (Found: C, 66·05; H, 8·5; S, 14·1. $C_{25}H_{38}O_3S_2$ requires C, 66·65; H, 8·5; S, 14·2%). This dithioketal (237 mg.) in ethanol (50 ml.) was refluxed with Raney nickel (activity W1, 2 g.) for 16 hr. Crystallisation of the product from aqueous methanol gave 3β-*acetoxyallopregnan*-11-*one*, m. p. 159—162°, $[\alpha]_D$ +38° (*c* 1·30), +40° (*c* 1·90) (Found: C, 76·6; H, 10·3; Ac, 11·85. $C_{23}H_{36}O_3$ requires C, 76·6; H, 10·05; Ac, 11·95%). In contrast, treatment of the dithioketal (125 mg.) in methanol (20 ml.), tetrahydrofuran (15 ml.), and water (2 ml.) with 3% lithium amalgam (5 g.) at room temperature with agitation for 20 hr. caused only hydrolysis of the acetate residue to furnish, after crystallisation from aqueous methanol, 3β-*hydroxyallopregnane*-11,20-*dione* 20-(*ethylene dithioketal*) (50 mg.), m. p. 242—246°, $[\alpha]_D$ +36° (*c* 0·60), $\nu_{max.}$ 3200 (OH) and 1697 (11-ketone) cm.$^{-1}$ (Found: C, 66·95; H, 8·65; S, 16·3. $C_{23}H_{36}O_2S_2$ requires C, 67·6; H, 8·85; S, 15·6%).

Relatively large quantities of lithium amalgam were prepared by the following safe procedure. Mercury (1 kg.) and lithium (33 g.) were heated at 350° for 12 hr. in a stainless-steel autoclave (500 ml. capacity). After cooling, the hard frothy amalgam was chipped out with a chisel and stored under light petroleum.

The dithioketal of methyl norlimonilyl ketone (1·04 g.) in ethanol (40 ml.), diluted with

aqueous 0·4N-sodium hydroxide (25 ml.) and water (10 ml.), was left at room temperature with agitation in the presence of 3% lithium amalgam (65 g.) for 20 hr. Working up gave the diol (880 mg.). Without purification this was taken up in pyridine (10 ml.), added to a suspension of chromium trioxide (2 g.) in pyridine (20 ml.), and left at room temperature for 20 hr., then poured into water. Destruction of the excess of oxidant with sulphur dioxide (cooling) and working up in the usual way gave neutral material (495 mg.) that was chromatographed over alumina (grade V, acid-washed; 8 g.) in benzene. Crystallisation from benzene–light petroleum then afforded the required *aldehyde* (LVII) (170 mg.), double m. p. 135°, 196—206°, $[\alpha]_D$ −132° (*c* 1·20 in benzene), $\lambda_{max.}$ 207—209 mμ (ε 8000), $\nu_{max.}$ 1749 (δ-lactone) and 1713 (CHO and 7-ketone) cm.$^{-1}$ (Found: C, 61·95; H, 6·7; S, 10·95. $C_{29}H_{36}O_7S_2$ requires C, 62·1; H, 6·45; S, 11·45%). The aldehyde group showed the characteristic τ value of −0·27 in its nuclear magnetic resonance spectrum.

Isolation of Obacunoic Acid.—Crushed grapefruit seeds (3 kg.) were extracted for limonin in the usual way. The mother-liquors remaining were taken to dryness (13·8 g.), dissolved in the minimum of acetone (about 20 ml.), and heated with an excess (about 150 ml.) of N-aqueous sodium hydroxide on the steam-bath for 1 hr., the acetone being allowed to evaporate. The reaction mixture was cooled, acidified with N-hydrochloric acid, and extracted with chloroform. The chloroform extract was shaken with excess of saturated sodium hydrogen carbonate solution and then discarded. The sodium hydrogen carbonate extract was acidified with N-hydrochloric acid and extracted with chloroform. The latter furnished an acid fraction (4·48 g.) which, on crystallisation from aqueous acetone or acetone–ether, gave obacunoic acid (1·4 g.), m. p. 205—208°, $[\alpha]_D$ −99° (*c* 1·06 in acetone), −103° (*c* 1·20 in tetrahydrofuran), $\lambda_{max.}$ 204—205 mμ (ε 18,000), λ_{210} mμ (ε 14,000), $\nu_{max.}$ 3220 (OH), diffuse absorption at 2800—3400 (CO_2H), 1745 (δ-lactone), 1710 (cyclohexanone and αβ-unsaturated CO_2H), and 1621 (C=C) cm.$^{-1}$ as well as the usual furan absorption (Found: C, 65·8; H, 6·85; active H, 0·4. Calc. for $C_{26}H_{32}O_8$: C, 66·1; H, 6·85; 2 active H, 0·45%).

Treatment of obacunoic acid (460 mg.) in tetrahydrofuran (25 ml.) with excess of ethereal diazomethane for 1 min. at room temperature (destruction of excess of diazomethane with one drop of formic acid) gave methyl obacunoate. Recrystallised from ether this (400 mg.) had m. p. 173—176°, $[\alpha]_D$ −94° (*c* 1·20 in CH_2Cl_2), −97° (*c* 0·73 in acetone), $\lambda_{max.}$ 205—206 mμ (ε 8400), $\nu_{max.}$ 3540 (OH), 1738 (δ-lactone), 1716 (αβ-unsaturated ester and cyclohexanone), and 1623 (C=C) cm.$^{-1}$ as well as the usual furan bands. Methyl obacunoate was also prepared by using 0·1N-sodium hydroxide and methyl iodide in acetone.

Obacunoic acid of analytical purity (5 mg.) in aqueous 2N-sodium hydroxide (3 ml.) was treated with an excess of aqueous iodine–potassium iodide solution. Iodoform (7 mg.) was precipitated; it was identified by sublimation and m. p. and mixed m. p. of the sublimate. The main acidic product (46 mg.) did not crystallise.

Hydrogenation of Obacunoic Acid and of its Methyl Ester.—Obacunoic acid (105 mg.) was hydrogenated over prereduced 10% palladised charcoal (150 mg.) in glacial acetic acid (10 ml.) (3·74 mols. uptake). The acidic product did not crystallise.

Methyl obacunoate (328 mg.) in acetic acid (25 ml.) was hydrogenated over prereduced 10% palladised charcoal (690 mg.) suspended in acetic acid (10 ml.) (3·86 mols. uptake). Crystallisation of the acidic product (220 mg.) from aqueous methanol furnished methyl hydrogen octahydro-obacunoninate (150 mg.), m. p. 173—175°, $[\alpha]_D$ +120° (*c* 1·10 in MeOH), $\nu_{max.}$ 3470 (OH), 3230 (CO_2H) 1741 and 1720 (saturated ester and CO_2H) and 1705 (cyclohexanone) cm.$^{-1}$. This methyl ester acid (83 mg.) was heated on the steam-bath with dioxan (2 ml.) and concentrated hydrochloric acid (2 ml.) for 16 hr. Working up in the usual way gave acidic (49 mg.) and neutral (30 mg.) fractions, neither of which crystallised. The acidic fraction showed $\lambda_{max.}$ 250 mμ (ε 9200) and had infrared bands at 1785 (γ-lactone), 1720 (CO_2H) and 1630 (hydrogen-bonded αβ-unsaturated cyclohexanone) cm.$^{-1}$. The neutral fraction had almost identical constants. Similar results were obtained with hydrogenated obacunoic acid (see above) as starting material.

Deoxyobacunoic Acid.—Obacunoic acid (136 mg.) in acetone (16 ml.) and acetic acid (6 ml.) was treated with aqueous N-chromous chloride (5 ml.) under nitrogen for 43 hr. at room temperature with constant agitation. The acetone was removed *in vacuo* and water (100 ml.) was added. Working up as usual gave, after crystallisation from methylene dichloride–benzene, *deoxyobacunoic acid* (107 mg.), m. p. 158—179°, $[\alpha]_D$ −98° (*c* 1·00 in CH_2Cl_2), $\lambda_{max.}$ 206—208 mμ (ε 22,600), λ_{210} mμ (ε 21,800), $\nu_{max.}$ 3360 (OH), 1715 and 1695 (αβ-unsaturated δ-lactone, CO_2H,

and cyclohexanone) cm.$^{-1}$ as well as the usual furan bands (Found: C, 68·35; H, 7·15. $C_{26}H_{32}O_7$ requires C, 68·4; H, 7·05%).

Deoxyobacunoic acid (3·8 mg.) in methanol (2 ml.) and aqueous 2N-sodium hydroxide (2 ml.) was heated on the steam-bath for 90 min. Working up in the usual way gave acidic material which did not crystallise. It showed λ 206 (ε 10,000), 210 mμ (ε 7700).

Iso-obacunoic Acid.—Methyl obacunoate (122 mg.) in dry methanol (8 ml.) was added under nitrogen to a solution from sodium (500 mg.) in dry methanol (5 ml.) and refluxed for 1 hr. Water (10 ml.) was then added and the refluxing continued for 30 min. Acidification with N-hydrochloric acid and working up in the usual way (prolonged shaking with saturated sodium hydrogen carbonate was necessary to effect extraction) gave *iso-obacunoic acid* (XLIII). Recrystallised from methylene dichloride–benzene this (70 mg.) had m. p. 155—161°, $[\alpha]_D$ −41° (*c* 1·20 in CH_2Cl_2), $\lambda_{max.}$ 208 mμ (ε 5200), $\nu_{max.}$ 3000—3500 (diffuse CO_2H absorption), 1741 (δ-lactone), 1716 (CO_2H) and 1704 (cyclohexanone) cm.$^{-1}$ as well as the usual furan bands (Found: C, 65·95; H, 6·95. $C_{26}H_{32}O_8$ requires C, 66·1; H, 6·85%). Treatment of obacunoic acid under the same conditions also afforded iso-obacunoic acid but in inferior yield.

Iso-obacunoic acid (19·5 mg.) in glacial acetic acid (10 ml.) was hydrogenated over pre-reduced 10% palladised charcoal (65 mg.) until saturated (2·94 mols. uptake). The acidic product did not crystallise.

Iso-obacunoic acid (44 mg.) was treated under the usual conditions for the iodoform test (see under obacunoic acid above). No iodoform was produced and iso-obacunoic acid was recovered (30 mg.; correct m. p. and mixed m. p., and infrared spectrum).

Autoxidation of Obacunoic Acid.—Obacunoic acid (106 mg.) in t-butyl-alcoholic N-potassium t-butoxide was shaken under oxygen for 2 hr. (uptake of just over 1 mol. of oxygen). The product was worked up as in the comparable experiments in the limonin series (see above) to give the derived *diosphenol* (LIII; R = H) (103 mg.). Recrystallised from acetone–ethanol this (35 mg.) had m. p. 280—283°, $[\alpha]_D$ −160° (*c* 1·31 in acetone), $\lambda_{max.}$ 277 mμ (ε 4600), shifting reversibly in alkaline solution to $\lambda_{max.}$ 335 mμ (ε 3100), $\nu_{max.}$ 3484 (OH), 1737 and 1750 (lactones), 1690 (diosphenol) cm.$^{-1}$ as well as the usual furan bands (Found: C, 66·25; H, 6·45. $C_{26}H_{28}O_8$ requires C, 66·65; H, 6·02%). This diosphenol was neutral to sodium hydrogen carbonate solution. After treatment with diazomethane it was recovered unchanged. The diosphenols in the limonin series (see above) are also resistant to diazomethane and neutral to sodium hydrogen carbonate solution.

This diosphenol (73 mg.), treated with pyridine–acetic anhydride overnight at room temperature, gave an *acetate* (LIII; R = Ac). Recrystallised from acetone–ethanol this (52 mg.) had m. p. 277—279°, $[\alpha]_D$ −124° (*c* 1·26 in acetone), $\lambda_{max.}$ 210 mμ (ε 7100), 244 mμ (ε 3200), $\nu_{max.}$ 1751 (enol acetate), 1718 and 1704 (lactones), and 1658 (conjugated cyclohexanone) cm.$^{-1}$ (Found: C, 66·2; H, 6·1; Ac, 8·95. $C_{28}H_{30}O_9$ requires C, 65·85; H, 5·9; Ac, 8·4%). The acetate was recovered unchanged after treatment with diazomethane.

When iso-obacunoic acid (118 mg.) was autoxidised in the same way (see above) (rapid uptake of 1 mol. of oxygen) the product did not crystallise. However, it showed a normal diosphenol absorption [$\lambda_{max.}$ 276 mμ (ε 7700) changing reversibly to $\lambda_{max.}$ 340 mμ (ε 4700) in alkaline solution] and gave an acetyl derivative, also non-crystalline, which had $\lambda_{max.}$ 242 mμ (ε 10,000). The diosphenol gave a normal test with saturated aqueous sodium hydrogen carbonate and could be methylated with diazomethane to a non-crystalline methyl ester without affecting the diosphenol chromophore.

Treatment of Carvone Hydrate with Hypoiodite.—Carvone hydrate [30] (XLVII; X = O) (4·95 g.) in dioxan (50 ml.) was treated with aqueous 4N-sodium hydroxide (100 ml.) and iodine (30 g.) in water (100 ml.) containing potassium iodide (40 g.). The precipitated iodoform (6%) was removed and identified after sublimation by m. p. and mixed m. p. The filtrate was acidified and worked up as usual. The neutral fraction was divided into material insoluble in dilute sodium hydroxide, and enolic material (1·68 g.) (a dark oil with a strong phenolic odour and giving a green ferric chloride test). The crude phenolic fraction in pyridine (20 ml.) was heated on the steam-bath with 3,5-dinitrobenzoyl chloride (2·0 g.) for 30 min. Chromatography of the product over alumina gave 4,6-di-iodo-*o*-tolyl 3,5-dinitrobenzoate (XLVIII; R = dinitrobenzoyl) (900 mg., 5·6%). Recrystallised from ethanol this had m. p. and mixed m. p. 206—213° and an infrared spectrum identical with that of the authentic specimen described below.

In a second experiment the phenolic fraction (1·69 g.), obtained from carvone hydrate

(5·48 g.) as described above, was refluxed in aqueous 2N-sodium hydroxide (30 ml.) under nitrogen with zinc dust (10 g.) and iron powder (2 g.) for 2 hr. Working up in the usual way gave a phenolic oil (770 mg.) (Beilstein test negative). A portion of this material (460 mg.) in benzene (20 ml.) and pyridine (2 ml.) was refluxed with 3,5-dinitrobenzoyl chloride (2·2 g.) for 20 min. Crystallisation of the neutral product from ethanol gave *o*-tolyl 3,5-dinitrobenzoate (207 mg.), identified by m. p., mixed m. p., and infrared spectrum.

o-Cresol (5 g.) in aqueous 20N-ammonia (80 ml.) was treated with iodine (20 g.) and potassium iodide (25 g.) in water (40 ml.) at room temperature.[37] The precipitated 4,6-di-iodo-*o*-cresol was purified by crystallisation from glacial acetic acid (4·5 g.), m. p. 65—69°, then converted into the 3,5-*dinitrobenzoate* (see above) in the usual way. Recrystallised from ethanol this had m. p. 206—213° (Found: C, 31·15; H, 1·55; N, 5·15; I, 45·6. $C_{14}H_8I_2N_2O_6$ requires C, 30·35; H, 1·45; N, 5·05; I, 45·8%).

We thank the Government Grants Committee of the Royal Society and Imperial Chemical Industries Limited for financial assistance. This work was aided by the Tropical Products Institute (formerly the Colonial Products Research Council) through the supply of citrus fruit seeds over a number of years and by fellowship support extended to S. K. Pradhan. Fellowships are also acknowledged with gratitude by J. F. Templeton (Rothermere Foundation, Newfoundland) and S. Sternhell (C.S.I.R.O., Australia). Messrs. Glaxo Laboratories gave us valuable help in the isolation of limonin on a large scale. We thank Mr. G. F. Phillips for his collaboration in the early stages of this investigation and particularly for his help in working out the procedure for the extraction of limonin.

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[*Received, July 14th,* 1960.]

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