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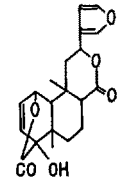
The Constitution of Limonin

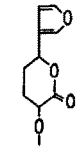
By D. ARIGONI[1], D. H. R. BARTON[2], E. J. COREY[3], and O. JEGER[1], in collaboration with L. CAGLIOTI[1], SUKH DEV[3], P. G. FERRINI[1], E. R. GLAZIER[3], A. MELERA[1], S. K. PRADHAN[2], K. SCHAFFNER[1], S. STERNHELL[2], J. F. TEMPLETON[2], and S. TOBINAGA[3]

The citrus bitter principle limonin was first isolated in 1841[4], but intensive investigation of its constitution did not commence until the last decade. The earlier literature has recently been summarised in detail[5] and we shall make reference here only to such of the earlier work as is especially pertinent to the derivation of the structure. The present communication represents a summary of the chemical evidence and conclusions to be reached from the work of three groups of investigators[1–3]. Experimental details of this work will be published separately in due course. Commencing in 1956 there has been in progress at the University of Glasgow a determination of the constitution and stereochemistry of limonin by the X-ray crystallographic method. This work, carried out under the leadership of Professor J. M. ROBERTSON, F.R.S., has been brought to a definitive conclusion and is summarised in the following communication. Professor ROBERTSON and his colleagues have at no time been informed of the progress of the chemical work until an exchange of letters dated September 11 1959 between one of us (D. H. R. B.) and the Glasgow crystallography group (see further below).

Before commencing an exposition of the chemical investigations it is essential to make a brief reference to what is known in the published literature about the constitution of limonin. Limonin, $C_{26}H_{30}O_8$ contains[5] two lactone rings, which can be opened reversibly, a β-substituted furan ring, a ketonic oxygen atom, and two ethereal oxygen rings. From infrared data both lactone rings are δ-constituted. Hydrogenation of limonin affords tetrahydrolimonin, $C_{26}H_{34}O_8$, and hexahydrolimoninic acid, $C_{26}H_{36}O_8$. These compounds both have the furan ring saturated and the acid is formed by hydrogenolysis of one of the lactone rings. Since tetrahydrolimonin is not an intermediate in the genesis of the acid one must conclude that the ether oxygen of the lactone that is cleaved is secured allylicly with respect to the furan ring. This is the same situation[6] as pertains in columbin (I), the main bitter principle of Colombo root. At first a relationship between limonin and columbin was suspected but, as shown in the sequel, limonin is a triterpenoid, not a diterpenoid, derivative and the supposed relationship is, in fact, misleading. Hexahydrolimoninic acid is an abnormally strong acid[7] and it is plausible to explain this by placing[5] an oxygen substituent α to the carboxylic acid group to give the part formula (II)[5] for limonin.



(I)



(II)

The ketone group of limonin has an infrared frequency which corresponds to that of a six (of higher) membered ketone. Reduction by the Ponndorff-Meerwein method affords[8] limonol, whereas borohydride reduction gives the epimeric epilimonol[5]. Since the latter compound is also formed by sodium amalgam reduction[5,9], a method which affords the more stable of a pair of stereoisomers, it can be assigned an equatorial orientation. Limonol, itself, must then be the axial isomer in agreement[10] with its mode of formation. We have converted[2] epilimonol into its chloro-acetate and thence into its iodoacetate. It is the latter compound which has been used for the X-ray crystallographic study of Professor J. M. ROBERTSON *et al.* The latter (see further below) confirm the equatorial character of the hydroxyl group in epilimonol.

[1] Organisch-chemisches Laboratorium, Eidg. Technische Hochschule, Zürich. This work was carried out with the financial support of the Schweiz. Nationalfonds zur Förderung der wissenschaftlichen Forschung.

[2] University of Glasgow and Imperial College, London.

[3] University of Illinois, Urbana; present address of E. J. C.: Harvard University, Cambridge (Mass.).

[4] BERNAYS, Liebigs Ann. *40*, 317 (1841).

[5] A. MELERA, K. SCHAFFNER, D. ARIGONI, and O. JEGER, Helv. chim. Acta *40*, 1420 (1957).

[6] D. H. R. BARTON and D. ELAD, J. chem. Soc. *1956*, 2085.

[7] O. H. EMERSON, J. Amer. chem. Soc. *74*, 688 (1952).

[8] B. V. CHANDLER and J. F. KEFFORD, Austr. J. Sci. *14*, 24 (1951).

[9] A. FUJITA and Y. HIROSE, J. pharm. Soc. Japan *74*, 365 (1954); *76*, 129 (1956).

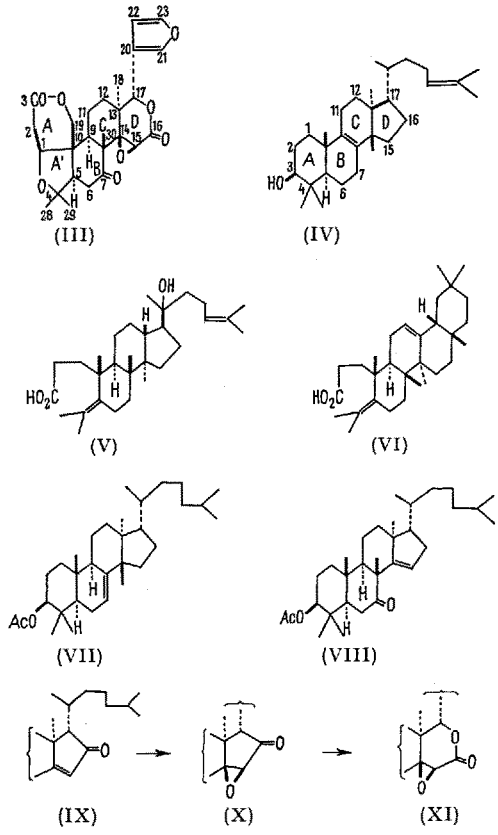
[10] D. H. R. BARTON, J. chem. Soc. *1953*, 1027.

Treatment of limonin with hydriodic acid[11] gives first desoxylimonin, $C_{26}H_{30}O_7$ and then citrolin, $C_{26}H_{28}O_6$. The former contains an $\alpha\beta$-unsaturated δ-lactone system[2,3] and has played an important role in the further elucidation of the constitution of limonin.

The molecular formula of limonin, when coupled with knowledge of the functional groups (see above), requires that the molecule be bicarbocyclic. Since drastic degradation of limonin[12,13] gives 1,2,5-trimethylnaphthalene one can make, with considerable reservation, the assumption that the two carbocyclic rings are both six-membered.

For the sake of clarity we must now state that the constitution and configuration of limonin is correctly represented as in (III). This constitution can be deduced, in main part, from the chemical work and is firmly established by the X-ray investigations. The stereochemistry is entirely the result of the X-ray work.

Biogenesis.—Limonin is revealed as a triterpenoid of the euphol (IV)[14] type from which four carbon atoms at the end of the side chain have been removed and carbons C_{20} to C_{23} then converted into a furan ring. A comparable process must be involved in the biogenesis of the cardiac aglycones. Ring A of the triterpenoid skeleton has been oxidatively cleaved between C_3 and C_4 and the C_3 carboxyl thus formed oxidatively cyclised onto C_{19}. There is excellent precedent for a C_3–C_4 biogenetic cleavage of the triterpenoid skeleton in the recently revealed structures of dammarenolic (V)[15] and nyctanthic (VI)[15,16] acids. The constitution of limonin also requires a Baeyer-Villiger oxidative cleavage of a ring D 16-ketone to give the ring D lactone. Finally one methyl group must be migrated from C_{14} to C_8 of the euphol skeleton. It is not without interest that chromic acid oxidation[17] of dihydrobutyrospermol acetate (VII) furnishes the 7-ketone (VIII). This type of compound could well be oxidised further to a 16-ketone, [as (IX)] epoxidised to the oxido-ketone [as (X)] and then subjected to Baeyer-Villiger oxidation to give a product (as XI) of the same partial structure as limonin. The numbering and lettering of the limonin formula (III) follow from these ideas as to its biogenesis. As would be expected the absolute configuration of tetrahydrolimonin is comparable with that expected for the ketone (VIII). Thus the rotatory dispersion curve of limonin[18] has a strong negative Cotton effect and corresponds in type to that of a saturated steroidal 7-ketone.



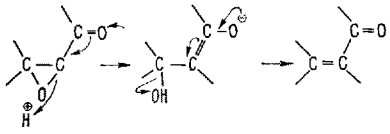
We now develop the detailed chemical evidence for the structure (III). For convenience we shall consider the molecule under sectional headings and recapitulate earlier work where appropriate and where it has been omitted from earlier reference above.

The Furan Ring.–The presence of a furan ring was first proposed by FUJITA and HIROSE[9]. Chemical confirmation of a β-substituted furan has been given by KUBOTA and TOKOROYAMA[19] and follows also from the following evidence. Treatment of limonol (XII) with alkali[1,3,5] gives a compound, $C_{21}H_{28}O_6$, designated[5] merolimonol (see further below). The loss of five carbons corresponds to the production of furan-3-aldehyde (XIII). This compound has now been isolated[3] and characterised as its 2,4-dinitrophenylhydrazone and by oxidation to 3-furoic acid. Finally, the nuclear magnetic resonance spectrum of limonin shows that it is a mono-β-substituted furan[20].

The Nature of Lactone Ring D.—The formation of the $\alpha\beta$-unsaturated lactone, desoxylimonin (XIV),

[11] T. A. GEISSMAN and V. TULAGIN, J. org. Chem. *11*, 760 (1946).
[12] G. KOLLER and H. CZERNY, Mh. Chem. *67*, 248 (1936).
[13] L. BRACHVOGEL, Arch. Pharmaz. *285*, 57 (1952).
[14] D. H. R. BARTON, J. F. MCGHIE, M. K. PRADHAN, and S. A. KNIGHT, Chem. & Ind. *1954*, 1325; J. chem. Soc. *1955*, 876. – D. ARIGONI, R. VITERBO, M. DÜNNENBERGER, O. JEGER, and L. RUZICKA, Helv. chim. Acta *37*, 2306 (1954); and references there cited.
[15] D. ARIGONI, D. H. R. BARTON, R. BERNASCONI, C. DJERASSI, J. S. MILLS, and R. WOLFF, Proc. chem. Soc. *1959*, 306.
[16] G. WHITHAM, Proc. chem. Soc. *1959*, 271.
[17] W. LAWRIE, W. HAMILTON, F. S. SPRING, and H. S. WATSON, J. chem. Soc. *1956*, 3272.
[18] C. DJERASSI, Bull. Soc. chim. France *1957*, 741; and references there cited. The rotatory dispersion curve of tetrahydrolimonin was determined in MeOH at the Postgraduate Medical School, London University. We thank Dr. W. KLYNE cordially for making this measurement and for helpful discussion.
[19] T. KUBOTA and T. TOKOROYAMA, Chem. & Ind. *1957*, 1298.
[20] E. J. COREY, G. SLOMP, SUKH DEV, S. TOBINAGA, and E. R. GLAZIER, J. Amer. chem. Soc. *80*, 1204 (1958).

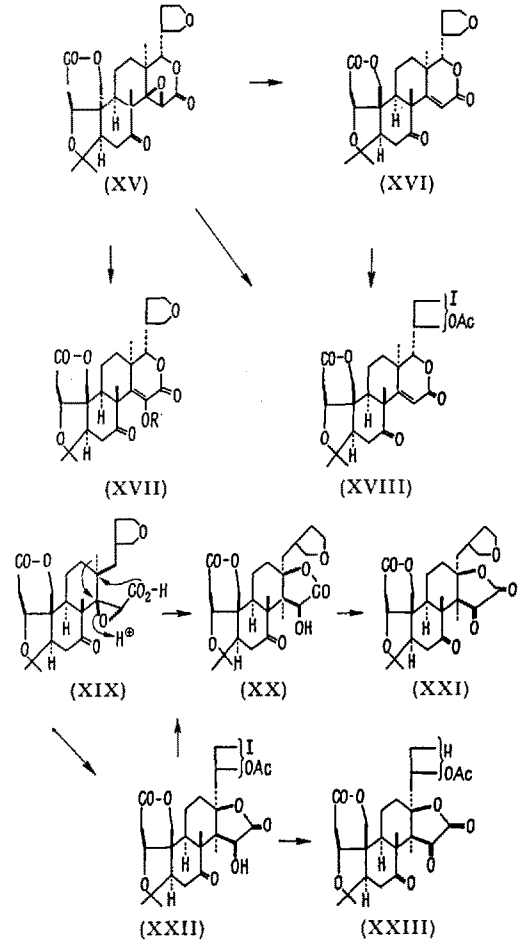
from the action of hydriodic acid on limonin suggests the possible presence of a 1,2-epoxide conjugated with the ring D lactone. Strong evidence for this grouping[3] is the fact that reduction of limonin with chromous chloride also gives desoxylimonin. Similarly tetrahydrolimonin (XV) furnishes desoxytetrahydrolimonin (XVI)[2,3]. Reductive elimination of 1,2-epoxides by chromous chloride is only possible if they are placed in conjugation with a carbonyl group[21] in accordance with the mechanism:



Furthermore decarboxyhexahydrolimoninic acid[22], the pyrolysis product of hexahydrolimoninic acid (XIX), contains an aldehyde group[1] (n. m. r. spectrum), as would be expected from the pyrolysis of a glycidic acid. Evidence that it is the D ring lactone which is involved and not the A ring lactone is given later. The presence of the glycidic lactone grouping in ring D is also shown by the following experiments[2]. Treatment of tetrahydrolimonin (XV) with hydrochloric-acetic acid mixture under controlled conditions gives the enol-lactone (XVII; R=H) characterised as its acetate (XVII; R=Ac). The ultraviolet absorption properties of these compounds show the presence of the enolised α-keto-lactone grouping and in confirmation[2] ozonolysis of (XVII; R=H), followed by mild hydrolysis, affords oxalic acid. Desoxytetrahydrolimonin itself is stable under the same conditions of acidity[2]. Treatment of desoxytetrahydrolimonin (XVI), or of tetrahydrolimonin (XV), with hydriodic acid in acetic acid-acetic anhydride at room temperature[2] served to open the tetrahydrofuran ring to give an iodo-acetate (XVIII). No other change in the molecule was produced (see further below).

When hexahydrolimoninic acid (XIX) was treated with acid[2] under comparable conditions to those described above it rearranged to a neutral isomer (XX) containing a new γ-lactone ring (infrared spectrum). Oxidation of this isomer with pyridine-chromium trioxide gave an α-keto-γ-lactone (XXI) in which the new ketone group could not be enolised. One must conclude, therefore, that the original isomerisation has placed a new carbon-carbon bond at the β-position in the lactone ring in agreement with the formulae given above. A comparable acid catalysed isomerisation of the ketone (VIII) has already been reported[17].

When hexahydrolimoninic acid (XIX) was treated with hydriodic acid[2] under the conditions applied above to desoxytetrahydrolimonin it furnished an analogous compound (XXII) with the tetrahydrofuran ring opened. This gave a desiodo-compound with zinc, whioh was oxidised to the α-keto-γ-lactone (XXIII) (infrared spectrum) with pyridine-chromium trioxide and gave (XX) on treatment with alkali.



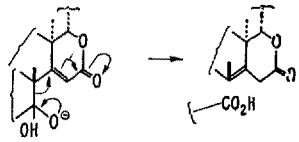
The Environment of the Ketone Group in Ring B.— The environment of the ketone group in ring B was defined in an unexpectedly simple manner[2,23]. Limonin (III), dissolved in dry tert.-butanol containing potassium tert.-butoxide was rapidly autoxidised with uptake of one mole of oxygen. The highly crystalline acidic product (XXIV; *R*=H) was characterised as its acetate (XXIV; R=Ac). The presence of the diosphenol chromophore was shown by the spectroscopic properties of these compounds as well as by the further degradation of the chromophore described later. Analogous diosphenols, (XXV) and (XXVI), were obtained in good yield from, respectively, desoxylimonin (XIV) and tetrahydrolimonin (XV). The environment of the ketone group, as disclosed by these experiments, is also in accord with deuteration experiments[2,3] under alkaline conditions at room temperature. Two atoms of deuterium are rapidly incorporated, a third more slowly.

[21] W. COLE and P. L. JULIAN, J. org. Chem. *19*, 131 (1954).

[22] R. S. ROSENFELD and K. HOFMANN, J. Amer. chem. Soc. *73*, 2491 (1951).

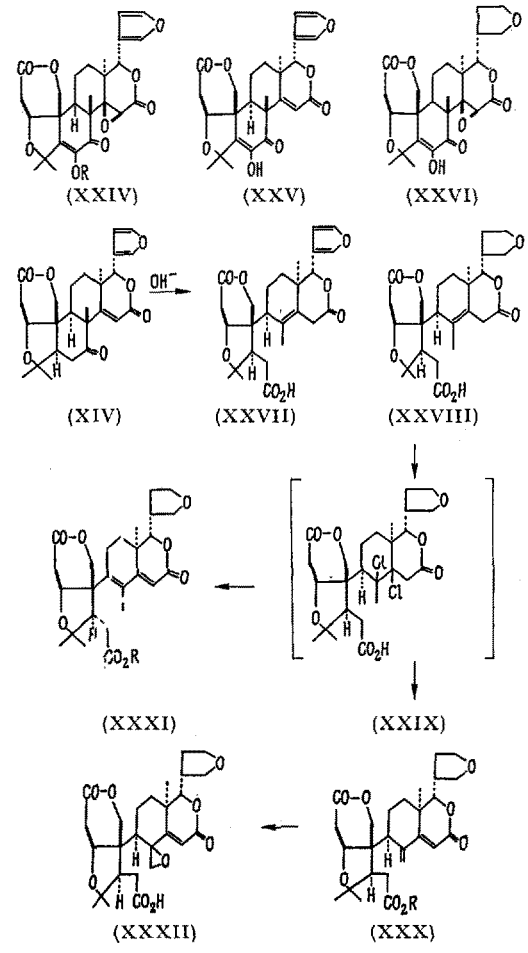
[23] The general scope of this reaction is currently being examined by two of us (D.H.R.B. and J.F.T.).

The Relationship of Ring B to Lactone Ring D.— Treatment of desoxylimonin (XIV) under mild alkaline conditions[2,3] causes cleavage of ring *B* to give a carboxylic acid (XXVII), conveniently designated desoxylimonic acid. Desoxylimonin oxime is stable[2] under the same conditions so that it is the carbonyl group of desoxylimonin that triggers the cleavage. Desoxylimonic acid, characterised as its methyl ester[2,3], showed only terminal absorption in the ultraviolet so that the conjugated δ-lactone ring of desoxylimonin has disappeared in the reaction. Similarly, desoxytetrahydrolimonin with aqueous alkali gives desoxytetrahydrolimonic acid (XXVIII). This acid is also obtainable from the iodo-acetate (XVIII) under the same conditions with reclosure of the tetrahydrofuran ring. The mechanism of this alkaline cleavage reaction, which does not, of course, occur with limonin, can be represented as follows:



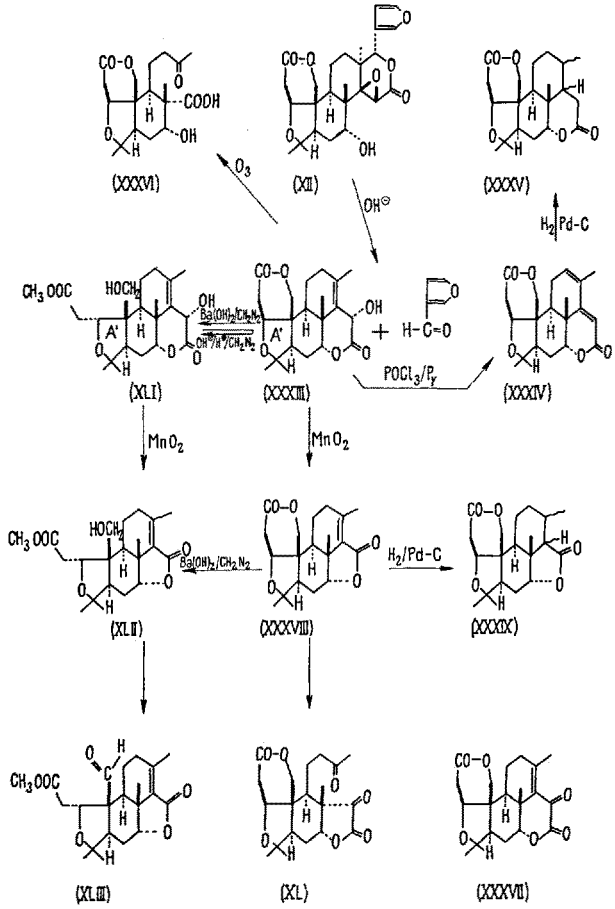
The further reactions of desoxytetrahydrolimonic acid have confirmed[2] the structure assigned. This acid reacts with one mole of chlorine to give an adduct (XXIX) (or equivalent) which is too unstable to be isolated. Heating *in vacuo* affords a mixture of two crystalline diene-acids. The major product has a broad absorption band with a maximum at 255 mμ and a low ε (7,800) in agreement with a *cisoid* diene as in (XXX; R=H). The minor product shows λ_{max} 230 and 284 mμ (ε=6,300 and 16,400 respectively) in agreement with the *transoid* diene formulation (XXXI; R=H). Both compounds were characterised as their methyl esters (XXX and XXXI; R=Me). The *cisoid* diene gives with per-acid an epoxide (XXXII) which has the normal spectrum of an $\alpha\beta$-unsaturated lactone. On ozonolysis (XXX; R=H) gives formaldehyde. This shows by chemical means the presence of a methyl group at C_8 [see (III)]. The formation of the *transoid* diene (XXXI) demonstrates also the presence of hydrogen at C_9.

Merolimonol and its Derivatives.— The relationship between the ketogroup of limonin and the lactone ring D indicated by these experiments can be proved independently from the chemistry of merolimonol[1,3,5], now assigned the constitution (XXXIII). Brief reference has been made to this compound before. As already reported it gives an acetate[5], now characterised[3] as a monoacetate, and is easily dehydrated to a doubly unsaturated conjugated lactone (XXXIV) which on hydrogenation affords a saturated tetrahydro-derivative (XXXV). We can now report[1] that ozonolysis of merolimonol gives a keto-acid (XXXVI) characterised as the methyl ester, the methyl ester acetate and as the methyl ester oxime. This compound is a methyl ketone since it affords iodoform with alkaline hypoiodite. Oxidation of merolimonol with manganese dioxide in chloroform[1] or in benzene[3] solution has given interesting results[1,3]. Besides the expected ketone[3] (XXXVII), there is formed[1,3] the



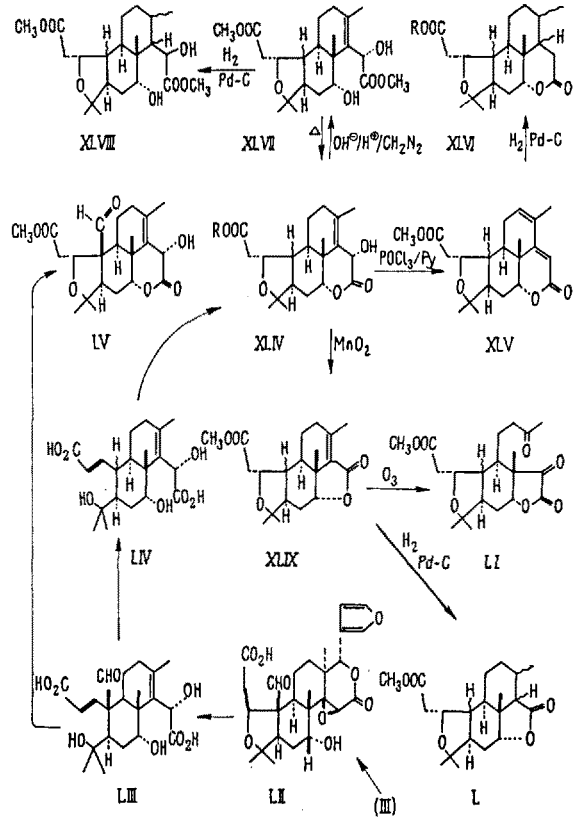
decarbonylated derivative (XXXVIII). The infrared and ultraviolet spectra of this compound show the presence of δ-lactone and $\alpha\beta$-unsaturated γ-lactone groupings. On hydrogenation it affords[1,3] a saturated dihydro-derivative (XXXIX) in which the γ-lactone could easily be seen in the infrared spectrum. This same compound was also obtained[3] by hydrogenating merolimonol to dihydromerolimonol followed by oxidation with pyridine-chromium trioxide. Decarbonylation is again involved[3] in this interesting reaction. Ozonolysis[1] or hydroxylation with osmium tetroxide followed by lead tetra-acetate cleavage[3] of the lactone (XXXVIII) affords an α-keto-lactone (XL) which is of importance for two reasons. First, the compound gives a positive iodoform test thus indicating a methyl ketone (see further under the discussion on n. m. r. spectra below); secondly, the α-keto-lactone does not enolise thus indicating (see also above) the presence of a substituent at C_8. As a whole, the results with merolimonol[1,3] characterise beyond question C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, and C_{18} in their relationship with C_7 and C_8.

Some additional data pertaining to the chemistry of merolimonol can now be interpreted[1]. On treatment with barium hydroxide merolimonol gives an amorphous acid furnishing a crystalline methyl ester (XLI) with diazomethane. In this acid lactone ring A has been opened irreversibly in contrast to the behaviour of limonin and limonin derivatives in general. This can be simply explained by supposing that there is a reversible β-elimination and addition of the ethereal oxygen of ring A', thus permitting inversion at C_1 as already formulated in (XLI). Manganese dioxide oxidation of (XLI) affords[1] the expected γ-lactone (XLII) which on further oxidation with chromium trioxide gives the aldehyde (XLIII) (n. m. r. spectrum). This is important evidence that C_{19}, or other equivalent methyl carbon, is the terminus of lactone A (see further below). The mechanism which permits the merolimonol cleavage of limonol will be discussed in our[1,3] full papers.



The Chemistry of Limoclastic Acid.—When limonin is treated with alkali[5] under conditions significantly more vigorous than those required for the limonol → merolimonol change it affords, with a total loss of six carbon atoms, an acid, $C_{20}H_{28}O_6$. This substance is now assigned the structure (XLIV; R=H) and conveniently designated limoclastic acid[1]. Prior knowledge[5] of limoclastic acid can be summarised as follows. Its methyl ester is readily dehydrated by phosphorous oxychloride and pyridine to an anhydro-derivative (XLV) which has the same ultraviolet absorption spectrum as anhydromerolimonol (XXXIV). The anhydro-derivative, like (XXXIV), affords a satured tetrahydro-derivative (XLVI; R=Me) on hydrogenation which on alkaline hydrolysis furnishes the corresponding acid (XLVI; R=H). Methyl limoclastate is further characterised[5] as the monoacetate, giving a saturated dihydro-derivative on hydrogenation. Alkaline hydrolysis of methyl limoclastate (XLIV; R=Me) and esterification of the acidic product prior to relactonisation[1] affords a dimethyl ester (XLVII), characterised as a monoacetate, and giving back (XLIV; R=Me) on gentle pyrolysis. Hydrogenation of (XLVII) furnishes[1] a saturated dihydro-derivative (XLVIII) characterised as a diacetate. These experiments establish the functional groups of limoclastic acid and show that it is bicarbocyclic.

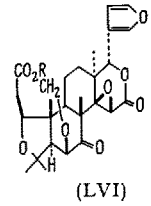
On oxidation with manganese dioxide methyl limoclastate (XLIV; R=Me) shows the same peculiar reactivity as merolimonol (see above) and affords a nor-compound (XLIX). On hydrogenation this gives a saturated dihydro-derivative (L) in which the γ-lactone grouping can be clearly discerned (infrared spectrum). Ozonolysis of (XLIX) also proceeds as with the merolimonol derivative (XXXVIII) and gives an α-keto-γ-lactone (LI) which does not enolise but does give the iodoform test in agreement with the presence of a methyl ketone grouping.



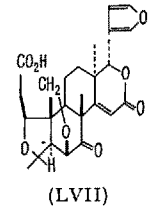
The stoichiometry of the limonin-limoclastic acid change requires that, if one fragment is furan-3-aldehyde (see above), a second fragment must be formic acid (or equivalent). Since the chemistry of limoclastic acid parallels exactly (see above) that of merolimonol we considered that the compounds were comparably formulated. Thus the ketone group of limonin must be converted by alkali induced intramolecular hydride transfer to a limonol type compound [as (LII)] which can now undergo the limonol-merolimonol change to furnish a compound [as (LIII)] which permits the deformylation process to occur by a conventional mechanism[24]. This gives in principle a structure [as (LIV)] which by re-addition of the tert.-hydroxyl to the $\alpha\beta$-unsaturated acid and lactonisation would afford limoclastic acid (XLIV; R=H). Interestingly enough a minor product from the alkaline degradation of limonin[1] is the aldehyde (LV). A direct connection between the limoclastic acid and merolimonol series of compounds has been secured as follows. The aldehyde (XLIII), to which reference has already been made above, is converted by the action of barium hydroxide followed by methylation of the product, into the lactone-ester (XLIX). Since (XLIII) is *stable under pyrolytic conditions*, the alkali must exert a special function in the decarbonylation reaction. The mechanism outlined above explains this in a unique manner and shows that ring A of limonin must be a δ-lactone with at least one α-hydrogen to permit reversible elimination of a β-placed ethereal oxygen. The terminus of the lactone ether-oxygen has already been established (see above).

Limonilic Acid and Derivatives. — Limonilic acid, $C_{26}H_{30}O_9$[7], was first obtained[11] by oxidation of limonin in alkaline solution with potassium manganate, but it is more readily available[7] in essentially quantitative yield by treatment of an alkaline solution of limonin with hypoiodite. Limonilic acid is now formulated as (LVI; R=H) on the basis of the following evidence. EMERSON[7] had shown that both tetrahydrolimonin (XV) and hexahydrolimoninic acid (XIX) gave limonilic acid type derivatives. The lactone ring modified in the limonilic acid reaction must therefore be lactone A and not lactone D (the hydrogenolysable lactone). In confirmation limonilic acid with either chromous chloride[3] or hydriodic acid[2] gives desoxylimonilic acid (LVII) in which the ring D lactone has been converted to an $\alpha\beta$-unsaturated lactone in the customary manner (see above). Furthermore[1] hydrogenation of limonilic acid followed by esterification affords the diester (LVIII; R=R'=Me) equally available from the hypoiodite oxidation[7] of hexahydrolimoninic acid and esterification.

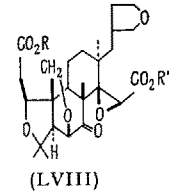
Contrary to earlier views[7] methyl limonilate[2] and the diester (LVIII; R=R'=Me)[1] have no hydroxyl group (infrared spectrum). With this fact established and, having regard to the stoichiometry of the reaction[7], limonilic acid must have either a second ketone or aldehyde function or else an ether grouping. Now the original ketone group of limonin, the presence of which is essential for a limonilic-type reaction, is profoundly modified in the limonilic acid reaction. It no longer exchanges readily with deuterium oxide in alkaline solution[3], no longer undergoes[2] the diosphenolation reaction (see above), has an ultraviolet maximum displaced to longer wavelengths and an infrared band at 1722 cm^{-1} displaced from its normal position near 1710 cm^{-1}. These facts are consistent with the presence of an axially oriented[25] ether substituent in the α-position with respect to the ketone. In agreement with this, reduction of limonilic acid with aluminium amalgam[1] gives back limonin. Furthermore[2], the degree of hindrance of the ketone group to carbonyl addition reagents has also been increased in limonilic acid, because its derivative, desoxylimonilic acid (LVII) no longer is subject to facile cleavage under mild alkaline conditions.



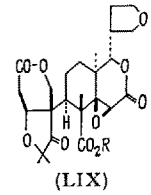
(LVI)



(LVII)

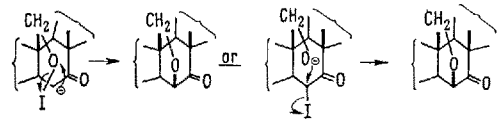


(LVIII)



(LIX)

On the basis of all this evidence limonilic acid must be formulated as already indicated in (LVI). The mechanism of its formation is presumably as follows:



The formation of an ether linkage is facilitated, no doubt, by a favourable steric situation. It will be of interest to see if such a process is of more general applicability.

Further Degradation of Ring B[2]. — The diosphenol (XXVI) from tetrahydrolimonin can be degraded smoothly by ozonolysis. This produces a crystalline nor-acid (LIX; R=H) further characterised as its

[24] Of course, the reaction conditions are alkaline so that anions are involved. This intricate process will be discussed in more detail elsewhere[1].

[25] R. C. COOKSON and S. H. DANDEGAONKER, J. chem. Soc. *1955*, 352. – G. BAUMGARTNER and C. TAMM, Helv. chim. Acta *38*, 441 (1955).

methyl ester (LIX; R=Me). When this nor-acid is treated with alkali it gives formaldehyde by a reversed aldol-type elimination. This proves that there is a $-CH_2-O-CO-$ group in limonin attached at either C_{10} or C_4.

The infrared spectrum of the methyl ester (LIX; R=Me) showed a band at 1760 cm^{-1}. This cannot be due to either lactones A or D and must be ascribed to the new ketone present in ring A'. This enhanced frequency for the ketone group is consistent with a cyclopentanone with an α-ethereal substituent such as is actually present.

Application of Nuclear Magnetic Resonance (n.m.r.) Spectroscopy. – Throughout this work one of the groups of investigators[3] has made extensive use of n. m. r. spectroscopy. In some cases this has suggested structures later confirmed by chemical methods and in others it has supplied valuable supporting evidence. A special section in this communication is clearly justified.

The n. m. r. signals which are characteristic of the β-monosubstituted furan ring of limonin[20] are invariably absent from those derivatives lacking this unit (e.g. hexahydrolimoninic acid, merolimonol, methyl etiolimonate[9], and methyl limoclastate) and present in the remainder (e. g. citrolin, methyl limonilate).

At the other extreme of the n. m. r. spectrum lies the absorption due to the tertiary methyl groups which possess the most shielded protons. These signals are also extremely informative in the case of most limonin derivatives. With limonin ($CDCl_3$ solution) three sharp methyl peaks are observed at shift values (in parts per million from external methylene chloride as reference,

$$\frac{H - H_{CH_2Cl_2}}{H_{CH_2Cl_2}} \times 10^6$$

of + 4·16, + 4·29 and + 4·36 with a signal ratio of 1:2:1 and a total signal intensity of ca. 12 protons, a clear indication of the presence of four C-methyl groups.

In contrast, the n. m. r. spectrum of merolimonol shows absorption from only three methyl groups in this region. Three peaks are manifested at + 4·18, + 4·24, and + 4·35 which are cleanly separated with relative intensity 1:1:1 and total intensity of nine protons. Evidently one methyl group has been displaced in merolimonol and in agreement one finds a sharp band at + 3·4 (intensity ca. 3 protons) in the spectrum which corresponds to a methyl attached to a double bond. This conclusion is substantiated by the n. m. r. spectra of the γ, δ-dilactone XXXVIII (methyl peaks at + 3·08, + 4·07, + 4·20, and + 4·28), acetyl merolimonol and anhydromerolimonol (XXXIV). These data allow the assignment of an angular methyl substituent to C_{13} and are confirmed by the degradation of merolimonol to the diketo-dilactone (XL) which shows n. m. r. absorption characteristic of a methyl ketone at + 3·22 (acetone value + 3·25).

The n. m. r. spectrum of anhydromerolimonol (XXXIV) also exhibits a single peak at – 0·477 which is due to *two* olefinic protons. Since the diene system of XXXIV must possess a proton α to the carbonyl and a methyl at the γ-carbon, the remaining olefinic proton must be attached to the δ-carbon.

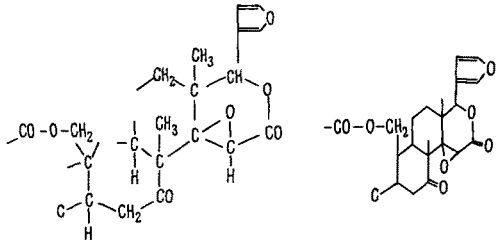
Strong evidence for the presence of an angular methyl substituent at C_8 came from the spectrum of methyl desoxylimonate (XXVII, methyl ester). The n. m. r. spectrum taken at 60 mc shows four sharp, cleanly resolved methyl peaks at + 3·67, + 4·07, + 4·17, and + 4·27 and no peaks due to olefinic protons (excluding the furan absorption). This shows definitely that the non-conjugated double bond in this substance is tetrasubstituted and that one of the attached groups is methyl (corresponding to the displaced peak at + 3·67). This methyl group can only be attached to C_8 since C_{14} substitution has been defined completely by the data pertaining to lactone ring D and merolimonol.

In this connection, it is also of interest that the n. m. r. spectra of desoxylimonin (XIV) and desoxytetrahydrolimonin show the attachment of only a single hydrogen to the olefinic linkage of ring D (sharp peak at – 1·42).

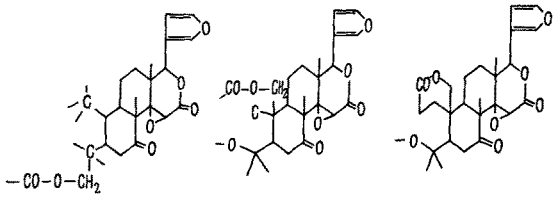
The Derivation of the Constitution of Limonin from Chemical Evidence. – The evidence outlined above provides conclusive proof for the presence of the system (LX) in limonin. If one has regard to the bicarbocyclic nature of the molecule and the formation of 1,2,5-trimethylnaphthalene on degradation (see above)[26] it is a possible assumption that two six-membered rings are present so that the rigidly derived partial structure can be expanded to (LXI) or (LXII). Structures like (LXI) and (LXII) immediately suggest a biogenesis from a triterpenoid (see above) and if one has regard to this and to the formation of acetone from the alkaline fusion of limonin and derivatives[7] then the constitution (LXI) can be expanded to (LXIII). Provided that there has been no reformation of carbon-carbon bonds in the biogenetic process we can then write (LXIV) as the constitution of limonin. Two formulae, (LXV) and (LXVI), derived from (LXIV) had been debated by us during the last six months. The origin of limoclastic acid, the properties of the nor-acid methyl ester (LIX; R=Me) (see above) and the course of attempts to degrade limonilic acid through its carboxyl group[2,3] tended to favour (LXVI) as against

[26] Dehydrogenation of merolimonol or of anhydrolimoclastic acid affords[5] a dimethylisopropylnaphthalene in which, from the ultraviolet absorption spectrum[1], the substituents are placed in positions 1, 2, and 6. From the constitution (III) of limonin this hydrocarbon should be 1,6-dimethyl-2-isopropylnaphthalene. Further work is in hand to confirm this[1].

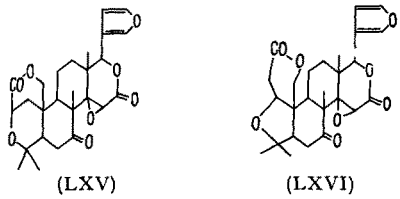
(LXV). The chemical evidence, however, still did not exclude some more exotic biogenesis of limonin, for example a condensation onto C_{11}, when the constitution from X-ray crystallographic work was announced by Professor M. ROBERTSON in the exchange of letters already referred to above. At that time nothing was known from the chemical point of view as to the stereochemistry of limonin. The helpful collaboration of Professor M. ROBERTSON *et al.* in making substantial contributions to our knowledge of limonin is gratefully appreciated by us.



(LX) (LXI)



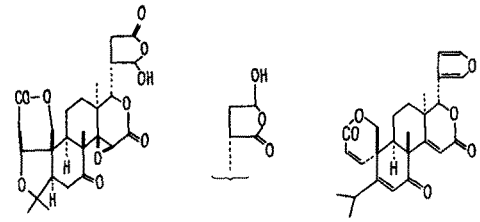
(LXII) (LXIII) (LXIV)



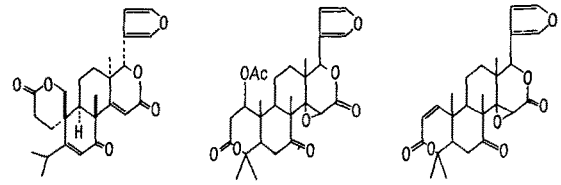
(LXV) (LXVI)

Other Aspects of Limonin Chemistry. — Limonexic acid[5,27], an oxidation product of limonin, must now be formulated as (LXVII) or (LXVIII). The relationship between this substance and limonin has already been established[5].

Citrolin, $C_{26}H_{28}O_6$, the product of vigorous hydriodic acid treatment of limonin (see above) can be obtained more conveniently by the action of hydrobromic acid[2] on desoxylimonin. Citrolin contains[7] the $\alpha\beta$-unsaturated lactone of desoxylimonin and has an $\alpha\beta$-unsaturated ketone grouping now to be placed in ring B. The most plausible formula for citrolin is (LXIX). In agreement the ultraviolet absorption curve of desoxylimonin, substrated from that of citrolin, gives a curve with maxima at 244 mμ ($\varepsilon = 14{,}000$) and 215 mμ ($\varepsilon = 8{,}000$), the latter possibly indicative of an additional $\alpha\beta$-unsaturated lactone grouping[2]. The infrared spectrum of citrolin is in much better agreement with the presence of two $\alpha\beta$-unsaturated δ-lactones than with one such lactone and one non-conjugated δ-lactone. Finally, selective catalytic hydrogenation of citrolin gives[2] a dihydro-derivative (LXX) shown by spectroscopic measurements to have one unsaturated and one saturated δ-lactone.



(LXVII) (LXVIII) (LXIX)



(LXX) (LXXI) (LXXII)

Some Related Bitter Principles. — It is probable that there are a number of bitter principles analogous in structure to limonin. We would mention especially nomilin[28], $C_{28}H_{34}O_9$, and the related obacunone[28,29], $C_{26}H_{30}O_7$. Recently[30] the functional groups of these compounds have been studied in some detail. It would appear that the formulae (LXXI) and (LXXII) are plausible and biogenetically attractive representations of nomilin and obacunone respectively.

Zusammenfassung

Limonin, ein Bitterstoff der Zusammensetzung $C_{26}H_{30}O_8$, kann aus verschiedenen Citrus-Arten isoliert werden. Frühere Untersuchungen haben gezeigt, dass der Naturstoff zwei carbocyclische Ringe, einen β-substituierten Furankern, zwei sechsgliedrige Lactone, ein Ketoncarbonyl und zwei intramolekulare Ätherguppierungen enthält. Neuere Abbauresultate, die in drei verschiedenen Laboratorien erzielt worden sind, erlauben, zusammen mit biogenetischen Betrachtungen, für diesen komplexen Naturstoff die Formel (LXVI) aufzustellen. Die gleiche Struktur, zusammen mit der noch unbekannten Stereochemie (vgl. III), ist ohne Kenntnis der chemischen Abbauresultate von J. M. ROBERTSON *et al.* auf röntgenographischem Wege ermittelt worden. Auf Grund der Formel (III) kann Limonin als das Produkt eines weitgehenden oxydativen Abbaus des tetracyclischen Triterpens Euphol (IV) aufgefasst werden.

Wegweisend für die chemische Ableitung der Formel (LXVI) sind die Ergebnisse der alkalischen, sauren und oxydativen Behandlung des Limonins, welche einen Einblick in das reaktive Zusammenspiel der verschiedenen Sauerstoffunktionen gewähren. Die Natur und Umgebung des Lactonringes D folgt aus der Überführung von Limonin (III) in Desoxylimonin (XIV) sowie in eine Reihe von Produkten, in welchen der Epoxydring aufgespalten

[27] B. V. CHANDLER and J. F. KEFFORD, Austr. J. Sci. *13*, 112 (1951); *16*, 28 (1953).

[28] O. H. EMERSON, J. Amer. chem. Soc. *70*, 545 (1948); *73*, 2621 (1951).

[29] F. SONDHEIMER, A. MEISELS, and F. A. KINCL, J. org. Chem. *24*, 870 (1959).

[30] F. M. DEAN and T. A. GEISSMAN, J. org. Chem. *23*, 596 (1958).

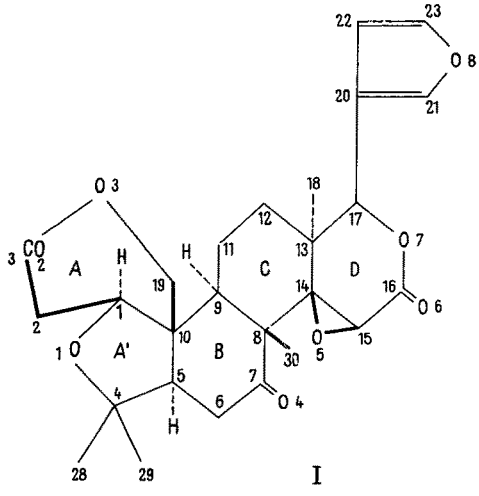
worden ist. Die Umwandlung von Desoxylimonin (XIV) in Desoxylimonsäure (XXVII) gestattet, die Beziehung zwischen dem Lactonring D und dem Ketoncarbonyl festzulegen. Zu gleichen Schlussfolgerungen führt auch die Untersuchung des Merolimonols (XXXIII), welches als Produkt des alkalischen Abbaus von Limonol (XII) anfällt. Der oxydative Abbau von Ring B gibt Auskunft über die Substitution der C-Atome 5 und 6, während die Beziehung zwischen dem Ketoncarbonyl und dem Lactonring A durch die Bildung der Limonilsäure (LVI) festgelegt ist. Der drastische Abbau des Limonins mit Alkali führt zur Limoclastsäure (XLIV). Aus dieser Umwandlung ergibt sich die Natur des Lactonringes A sowie eine Haftstelle der verbleibenden Sauerstoffunktion. Die Integration der somit abgeleiteten Strukturelemente mit früheren Dehydrierungsversuchen erlaubt die für Limonin in Frage kommenden Strukturen auf wenige Möglichkeiten zu beschränken, unter welchen lediglich (LXVI) biogenetisch plausibel erscheint.

Anschliessend werden andere Aspekte der Chemie des Limonins und verwandter Bitterstoffe kurz erläutert.

The Structure of Limonin

By S. ARNOTT, A. W. DAVIE, J. M. ROBERTSON, G. A. SIM, and D. G. WATSON[1]

X-ray studies in this department have led to the elucidation of the crystal structure of *epi*-limonol iodoacetate and hence to the derivation of the molecular structure of limonin, $C_{26}H_{30}O_8$, the bitter principle of citrus fruits. Our results are summarised in formula I for limonin and in Figure 5 which shows the arrangement of the atoms in the crystal asymmetric unit.



I

The X-ray work commenced in 1956 and at that time our knowledge of the chemical structure was confined to an enumeration of the functional groups and the fact that two carbocyclic rings were necessary. There was insufficient basis on which to build any plausible trial structure, and it was clear that we could only hope to proceed by means of some phase determining heavy atom technique[2,3].

Professor BARTON *et al.* supplied us with a variety of derivatives, and a preliminary study of these[4] indicated that the esters of *epi*-limonol were the most promising. The chloroacetate, which has two molecules in a monoclinic cell, space group $P2_1 - C_2^2$, was first studied. However, the presence of a screw axis introduces the ambiguity of a false symmetry centre between the two phase determining chlorine atoms, and work on this derivative was later abandoned in favour of the iodo-acetate of *epi*-limonol, $(C_{26}H_{31}O_8)COCH_2I$. This crystallises in the monoclinic system with cell dimensions $a = 15{\cdot}03$, $b = 12{\cdot}36$, $c = 15{\cdot}93$ Å, $\beta = 95° 12'$. There are four molecules in the unit cell, $D_m = 1{\cdot}426$, $D_x = 1{\cdot}441$ g·cm^{-3}, and the space group is again $P2_1 - C_2^2$.

In this space group the general positions are twofold, and hence, with four molecules in the unit cell, the asymmetric crystal unit consists of two chemical molecules. This is a formidable complication, because it means that the coordinates of 76 atoms other than hydrogen must be determined from the X-ray data. At a later stage of our analysis, however, this circumstance has been helpful. There is no symmetry relationship between the two molecules and the positions of all the atoms have been found and refined independently. The fact that these positions are now found to conform quite precisely to two chemically identical but differently oriented molecules is an important verification of the structure and the stereochemistry.

MoKα radiation was employed and the reflections were recorded photographically with Weissenberg and precession cameras from specimens measuring about $1{\cdot}0 \times 0{\cdot}2 \times 0{\cdot}02$ mm. Absorption corrections are small and were not applied. The extremely thin, flaky crystals made the collection of accurate data a very lengthy and tedious operation, but in the end 2927 structure factors were evaluated.

The positions of the iodine atoms were determined initially by means of two-dimensional and three-dimensional sharpened Patterson syntheses. A section of the three-dimensional Patterson function at $y = {}^1/_2$ is shown in Figure 1.

[1] Chemistry Department, The University, Glasgow (Scotland).
[2] J. M. ROBERTSON, J. chem. Soc. *1935*, 615; *1936*, 1195.
[3] J. M. ROBERTSON and I. WOODWARD, J. chem. Soc. *1937*, 219; *1940*, 36.
[4] S. ARNOTT and J. M. ROBERTSON, Acta cryst. *12*, 75 (1959).