Triterpenoids. Part XV. The Constitution of Icterogenin, a Physiologically Active Triterpenoid.*

By D. H. R. Barton and P. de Mayo.

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The constitution and stereochemistry of icterogenin, the jaundice-producing constituent of *Lippia rehmanni* Pears, have been elucidated. Icterogenin, $C_{35}H_{52}O_6$, is a carboxylic acid containing a ketone group, a free primary hydroxyl group, and also a secondary hydroxyl group esterified with angelic acid. Mild treatment with alkali provokes elimination of formaldehyde. On mechanistic grounds, confirmed by subsequent degradation, this shows that the primary hydroxylic function (CH_2·OH) is secured α with respect to the ketone grouping. The attachment of the angelic acid residue β with respect to the carboxyl group has been demonstrated by the appropriate hydrolysis, oxidation, and decarboxylation experiments. By alkali-induced elimination of the primary hydroxylic function and pyrolytic removal of the angeloyloxy-residue followed by hydrogenation, icterogenin methyl ester has been converted into methyl hedragonate.† Icterogenin methyl ester and methyl hederagonate † have been transformed into hexahydrobenzoates differing only in configuration at $C_{(4)}$.

This investigation is concerned with the jaundice-producing principle of *Lippia rehmanni* Pears (Rimington, Quin, and Roets, *Onderstepoort J. Vet. Sci.*, 1937, **9**, 225). This compound, which has been named icterogenin, is one of the substances known to produce "*geeldikkop*" (yellow thick head), a complex syndrome including jaundice and photosensitivity in sheep. A probably related compound, lantadene A, with the same physiological activity, has been isolated by Louw (*ibid.*, 1943, **18**, 197; 1948, **23**, 233; 1949, **22**, 321, 329) from *Lantana camara* L.

The isolation of pure icterogenin and a preliminary survey of its chemistry were reported in the excellent paper by Rimington, Quin, and Roets (*loc. cit.*). Through the courtesy of Professor C. Rimington, to whom we express our best thanks, it became possible to undertake an extensive re-investigation of the chemistry of this compound, initially with material extracted from the leaves of the plant.

Rimington *et al.* (*loc. cit.*) originally reported the isolation of three icterogenins designated A, B, and C. We find that icterogenin C is a solvated form of icterogenin A. Icterogenin B was found by Rimington *et al.* to afford icterogenin A acetate on acetylation and, although we have not yet encountered icterogenin B, we believe that it is also a different crystal form of icterogenin A. The constitutional problem is therefore simplified to a consideration of the chemistry of icterogenin A, henceforth designated icterogenin.

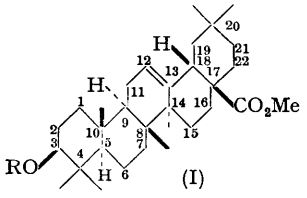
By extraction of *Lippia* leaves by the method of Rimington *et al.* (*loc. cit.*) icterogenin was obtained in small yield with constants in good agreement with those reported previously. Combustion data on icterogenin and its derivatives, as well as the extensive series of degradational experiments reported below, showed that the true molecular formula was $C_{35}H_{52}O_6$. In agreement with Rimington *et al.* (*loc. cit.*) the presence of a carboxyl group was shown by titration and by the preparation of a methyl ester. The equivalent, determined potentiometrically, was in satisfactory agreement with the above molecular formula. Likewise in agreement with the earlier work, the presence of a reactive carbonyl function was demonstrated by the formation of a 2 : 4-dinitrophenylhydrazone both by icterogenin and by its methyl ester. Since icterogenin was not a reducing substance it was tentatively, and correctly (see below), concluded that it was a ketone, not an aldehyde. The ultra-violet absorption spectra of the 2 : 4-dinitrophenylhydrazones were also in agreement with this view (cf. Braude and Jones, *J.*, 1945, 498). Acetylation of icterogenin gave an acetate with constants in agreement with those reported by Rimington *et al.* (*loc. cit.*).

* Part XIV, *J.*, 1953, 3111.

† It should be noted that the two slightly different names, methyl hederagonate and methyl hedragonate, represent different compounds, *viz.* (II; R = H) and (III), respectively.

As additional evidence for the presence of a hydroxyl group, a methyl ester acetate and a methyl ester benzoate, further characterised as the oxime, were prepared. The remaining two oxygen atoms of icterogenin could not be characterised directly, but were recognised to be part of an αβ-unsaturated ester or lactone function by the ultra-violet absorption spectrum [$\lambda_{max.}$ 212 mμ (ε 12,000)]. More extensive justification for this conclusion is presented later.

Pure icterogenin was initially available to us from the leaves of the plant only by wasteful fractional crystallisation. It seemed desirable therefore to seek alternative methods of purification. The study of these processes also had the added objective of confirming the homogeneity of icterogenin, a homogeneity concealed by the multiplicity of crystal forms. Chromatography of the methyl ester acetates gave the icterogenin derivative in only poor yield. From the earlier fractions, however, there was isolated in small amount methyl oleanolate acetate (I; R = Ac), the identity of which was confirmed by hydrolysis to methyl oleanolate (I; R = H). Even smaller quantities of a third



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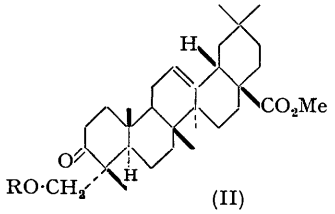

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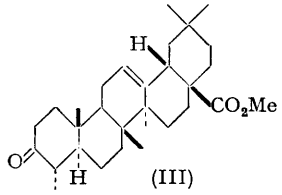

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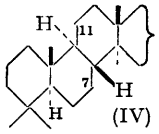

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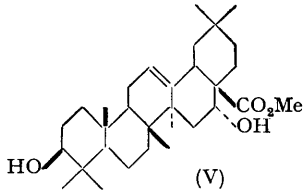


(V)

component were also isolated. In contrast, chromatography of the methyl ester benzoates (cf. Brooks, Klyne, and Miller, *Biochem. J.*, 1953, **54**, 212) provided a convenient method of isolation and proof of homogeneity; much of the initial structural work was carried out, therefore, on icterogenin methyl ester benzoate. From the earlier fractions a small amount of methyl oleanolate benzoate (I; R = Bz) was isolated, whilst from later fractions two other apparently homogeneous compounds, also benzoates, were obtained, likewise in minor amount.

Attention was next directed to a determination of the number of ethylenic linkages in icterogenin. Palladium-catalysed hydrogenation of icterogenin in neutral solution afforded dihydroicterogenin. The latter showed no αβ-unsaturated function, in the ultra-violet absorption spectrum, but retained the functionally reactive carbonyl group. Similarly icterogenin methyl ester benzoate gave a dihydro-derivative characterised as the oxime. Further hydrogenation, with a platinum catalyst, furnished dihydroicterogenin methyl ester hexahydrobenzoate. Hydrogenation with a platinum catalyst in ethanol and acetic acid afforded tetrahydroicterogenin methyl ester hexahydrobenzoate, unchanged on further attempted hydrogenation. In the tetrahydro-derivative the carbonyl group had been reduced, as shown by the absence of a maximum at 280 mμ and the failure to give ketonic derivatives. All hydrogenated derivatives of icterogenin gave positive tetranitromethane tests indicating the retention of at least one non-hydrogenatable double bond. Titration of icterogenin methyl ester benzoate with perbenzoic acid gave indefinite results, but titration of the dihydro-derivative indicated the presence of only one ethylenic linkage. We were thus led to conclude that icterogenin had two ethylenic linkages, one of which was αβ-unsaturated to an ester or lactone group. On the former assumption there must, then, be five alicyclic rings. This conclusion was confirmed in the following way. Treatment of icterogenin with methanolic bromine gave a bromo-lactone, $C_{35}H_{51}O_6Br$, which regenerated its precursor on digestion with zinc dust and acetic acid. The bromo-lactone retained the

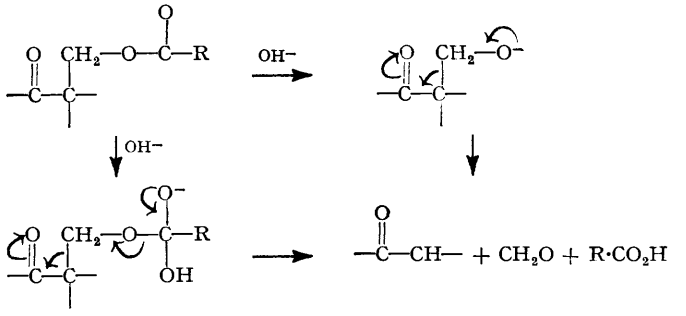
αβ-unsaturated function as shown by the ultra-violet absorption maximum at 218 mμ (ε 8400),* but no longer gave a colour with tetranitromethane. The bromo-lactone function must be derived from the unconjugated non-hydrogenatable ethylenic linkage, leaving the conjugated, but easily hydrogenated, ethylenic linkage untouched.

Degradation experiments on icterogenin were next attempted in an effort to inter-relate the various oxygenated functions. Attempted alkaline hydrolysis of icterogenin methyl ester benzoate under normal conditions gave only intractable amorphous products. Treatment in the cold with 4% aqueous-methanolic potassium hydroxide under carefully defined conditions furnished a high yield of a crystalline compound, m. p. 220°, $[\alpha]_D$ +93°, of the composition $C_{35}H_{52}O_5$, as well as formaldehyde and benzoic acid. The presence of the last-named was shown by isolation whilst the formaldehyde was detected by condensation with dimedone and by the chromotropic acid colour reaction. A similar elimination of formaldehyde was induced, in the same way, from icterogenin methyl ester acetate with concomitant formation of the same compound $C_{35}H_{52}O_5$. The latter retained the carbonyl group of icterogenin, shown by oxime formation, as well as the αβ-unsaturated ester function, but the original hydroxyl group had disappeared (failure of acylation or dehydration; no hydroxyl band in the infra-red). Palladium-catalysed hydrogenation gave a dihydro-derivative, $C_{35}H_{54}O_5$, m. p. 188°, $[\alpha]_D$ +86°, showing only isolated-double-bond absorption in the ultra-violet. The compound of m. p. 188° was also obtained from dihydroicterogenin methyl ester benzoate and hexahydrobenzoate under mild alkaline conditions. It was further characterised as the oxime and 2 : 4-dinitrophenylhydrazone and thus also retained intact the original ketonic grouping of icterogenin. That the presence of the latter functional group was a prerequisite for generation of formaldehyde was indicated by the failure of tetrahydroicterogenin methyl ester hexahydrobenzoate to eliminate formaldehyde under the usual conditions.

Icterogenin itself, treated under mild alkaline conditions, lost formaldehyde to give an acid, $C_{34}H_{50}O_5$, converted on methylation into the compound, m. p. 220°, referred to above. Palladium-catalysed hydrogenation afforded a dihydro-derivative, $C_{34}H_{52}O_5$, also obtained by elimination of formaldehyde from dihydroicterogenin. Methylation, as expected, gave the compound, m. p. 188°, referred to above.

The facts with regard to formaldehyde elimination seemed to find explanation in terms of a reversed aldol-type condensation, for example as in scheme (*A*).

(*A*)

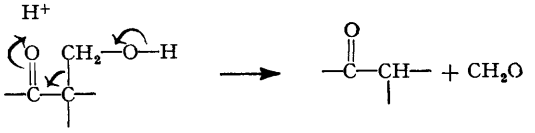


The following evidence provided support for this view. Although the compound, m. p. 220°, $C_{35}H_{52}O_5$, took up an indefinite amount of perbenzoic acid, its dihydro-derivative consumed only one mol., thus indicating only one unconjugated ethylenic linkage. The model compounds methyl hederagonate (II; R = H) and methyl hederagonate benzoate (II; R = Bz), on treatment under mild basic conditions identical with those applied to icterogenin, both gave methyl hedragonate (III) and formaldehyde. In the case of the

* The position of the maximum is displaced 6 mμ with resepct to that of icterogenin. The reason for this is that the maximum for the bromo-lactone represents the true maximum, whereas that reported for icterogenin itself is the summation of the band (at 218 mμ) due to the unsaturated ester function and that (at approx. 205 mμ on the sensitive Unicam S.P. 500 Spectrophotometer) due to the tri-substituted ethylenic linkage. The greater intensity of the maximum for icterogenin relative to that for the bromo-lactone illustrates also its composite character.

benzoate the formaldehyde was characterised both as the dimedone derivative and by the chromotropic acid colour test. In such compounds as methyl hederagonate elimination of formaldehyde can also be induced under acid conditions [mechanism (B)], as shown by the appropriate chromotropic acid test (see Experimental section). The elimination of formaldehyde from a similar system in a derivative of the triterpenoid asiatic acid during Wolff–Kishner reduction has been noted by Polonsky (*Compt. rend.*, 1951, **233**, 93, 671).

(*B*)



An even closer structural relationship to methyl hederagonate was suggested by the Zimmermann colour test (Zimmermann, *Z. physiol. Chem.*, 1935, **233**, 257; 1936, **245**, 47). We find that, amongst triterpenoid compounds with ketonic functions at position 3, 11, 12, 16, or 19 in the β-amyrin-type skeleton [see (I)] and at position 7 or 11 in the lanostane type skeleton [see (IV)], only those with the 3-keto-group give a colour. By applying this test to icterogenin and its derivatives, it was shown that the intensity and colour corresponded exactly to that observed with hederagonic acid and its derivatives, including methyl hedragonate (III). The positive Zimmermann test, coupled with the formaldehyde elimination, demonstrates the presence of the grouping (·CH_2·CO·C·CH_2·OH) in icterogenin and tentatively locates the keto-group at $C_{(3)}$ in an assumed triterpenoid skeleton.

Further experiments then revealed the nature of the postulated αβ-unsaturated ester function. Vigorous alkaline hydrolysis of the $C_{35}H_{54}O_5$ compound, m. p. 188° (see above), afforded a keto-alcohol, $C_{30}H_{46}O_4$, and a steam-volatile acid identified by its equivalent, by R_F value, and by preparation of the *p*-bromophenacyl ester, as α-methyl-*n*-butyric acid. Similar hydrolysis of the $C_{35}H_{52}O_5$ compound, m. p. 220° (see above), gave the same keto-alcohol and a steam-volatile acid identified by the ultra-violet absorption spectrum and by the *p*-bromophenacyl ester as tiglic acid. Five carbon atoms of icterogenin were thus accounted for as well as its αβ-unsaturated ester function. The postulate of five alicyclic rings also receives final confirmation.

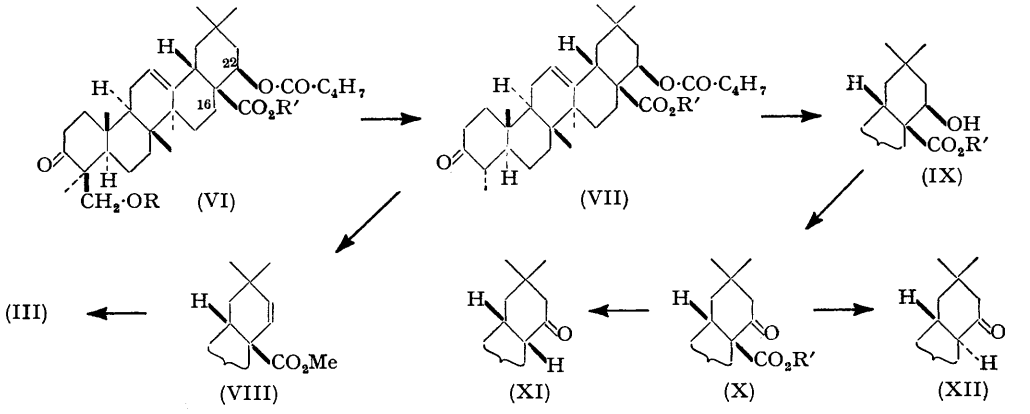
The keto-alcohol, $C_{30}H_{46}O_4$, was smoothly oxidised by chromic acid to a diketone, $C_{30}H_{44}O_4$, which on treatment with ethanolic potassium hydroxide gave, with concomitant hydrolysis and decarboxylation, a diketone $C_{28}H_{42}O_2$. An isomeric diketone was obtained in the following way. A by-product of the preparation of the keto-alcohol methyl ester, $C_{30}H_{46}O_4$, was the corresponding acid, $C_{29}H_{44}O_4$, reconverted into the methyl ester by diazomethane. This was more conveniently available by alkali-induced elimination of formaldehyde from icterogenin or dihydroicterogenin followed by more vigorous alkaline hydrolysis. Chromic acid oxidation gave an unstable diketo-acid which, when warmed in benzene solution, was decarboxylated to give the isomeric diketone, $C_{28}H_{42}O_2$. These results require that the unsaturated side-chain of icterogenin be attached through a secondary hydroxyl group β with respect to the carboxyl group. The formation of two isomeric C_{28} diketones requires that the carboxyl be tertiary in character in order to provide an asymmetric centre bearing an enolisable hydrogen α- to the adjacent ketone group. The environment of the carboxyl group was also indicated by the resistance of the methyl ester groups in icterogenin derivatives to alkaline hydrolysis. Thus the difficulty of hydrolysis was about the same as in methyl echinocystate (V). The hydrolysis was, however, easier than with methyl oleanolate (I; R = H). In both the former cases this must be due to facilitation by a β-hydroxyl group. One must conclude from these experiments that icterogenin contains the grouping $HO_2C{\cdot}\overset{|}{\underset{|}{C}}{\cdot}\overset{|}{\underset{|}{C}}H{\cdot}O{\cdot}CO{\cdot}CMe{:}CHMe$.

During the work outlined above a number of observations had been made which were indicative of a possible β-amyrin skeleton in icterogenin. The ready formation of a bromo-lactone is characteristic of oleanolic acid and its derivatives. The bromo-lactone from icterogenin had a γ-lactone band at 1786 cm.$^{-1}$, closely comparable with that (1779 cm.$^{-1}$) found by us for oleanolic bromo-lactone and that (1780 cm.$^{-1}$) for pyroquinovic bromo-lactone. Icterogenin is thus βγ- or γδ-unsaturated. The former possibility was rejected

since neither icterogenin nor any of its appropriate derivatives shows the ease of decarboxylation (cf. Barton and Brooks, *J.*, 1951, 257) characteristic of βγ-unsaturated acids. Icterogenin therefore contains the grouping $\rangle C{:}C\cdot\overset{|}{C}\cdot\overset{|}{C}\cdot CO_2H$). The reactivity of the isolated ethylenic linkage of icterogenin towards per-acid and towards chromic acid was also indicative of a β-amyrin-type skeleton. Oxidation of the $C_{35}H_{54}O_5$ compound, m. p. 188° (see above), with chromic acid at room temperature gave an αβ-unsaturated ketone, $C_{35}H_{52}O_6$, $\lambda_{max.}$ 249 mμ. Under the same conditions β-amyrin benzoate afforded 11-oxo-β-amyrin benzoate, $\lambda_{max.}$ 249 mμ.

The presence of a β-amyrin-type carbon skeleton was conclusively established in the following way. The $C_{35}H_{52}O_5$ compound, m. p. 220° (see above), was pyrolysed at low pressure (see Experimental section) to give the corresponding olefin, $C_{30}H_{44}O_3$, by unimolecular ester elimination (cf. Barton, Head, and Williams, *J.*, 1953, 1715). Hydrogenation of this compound, followed by reoxidation of the reduced ketone grouping, gave a substance identified as methyl hedragonate (III).

The summation of the evidence described above, coupled with further considerations outlined in the sequel, now lead us to propose formula (VI; R = R′ = H) as the constitution of icterogenin. The compound, $C_{35}H_{52}O_5$, m. p. 220°, is therefore (VII; R′ = Me), the derived pyrolysis product, $C_{30}H_{44}O_3$, is (VIII), the hydroxy-ester, $C_{30}H_{46}O_4$, is (IX; R′ = Me), the diketo-ester, $C_{30}H_{44}O_4$, is (X; R′ = Me) and the two derived diketones are (XI) and (XII).



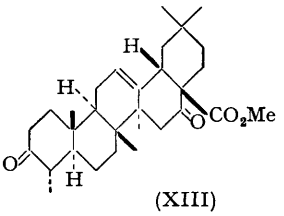
The isolation of tiglic acid as outlined above does not differentiate between a tiglate or an angelate side chain in icterogenin, since angelic acid is readily isomerised to tiglic acid. That the icterogenin side chain was in fact derived from angelic acid was shown by an examination of the acid produced in the pyrolysis of (VII; R′ = Me) (see above). This was shown to be pure angelic acid. Under the same pyrolytic conditions the thermodynamically more stable tiglic acid was, of course, recovered unchanged.

The work summarised above proves that the angelate side chain is secured β- with respect to the carboxyl group of icterogenin, but it does not distinguish between attachment at $C_{(16)}$ or at $C_{(22)}$. A decision in favour of $C_{(22)}$ is reached by exclusion. In their extensive work on the constitution of quillaic acid Kon and his collaborators (*J.*, 1939, 1130; 1940, 612, 1469; 1941, 552) prepared a diketo-ester (XIII), m. p. 193°, $[\alpha]_D$ +9°, which is clearly different from the corresponding icterogenin derivative [(X; R′ = Me), m. p. 234—235°, $[\alpha]_D$ +49°] although the methods of preparation of these two compounds as well as other considerations (see Experimental section) show that they both must have the more stable (α on the basis of conformational analysis: see Barton, *Experientia*, 1950, **6**, 316) configuration at $C_{(4)}$. Similarly the diketone (XIV) obtained by the alkali-induced conversion $CO_2Me \longrightarrow H$ of (XIII) had m. p. 197°. The corresponding icterogenin derivative [(XI) or (XII)], although probably still a mixture of stereoisomers, had m. p. 220—226°.

The configuration of the $C_{(22)}$ ester residue is regarded as β on the grounds that its

marked resistance to hydrolysis is more commensurate with the polar (β) than the equatorial (α) configuration (see Barton, *loc. cit.*; *J.*, 1953, 1027; Barton and Holness, *J.*, 1952, 78). This contention is supported by the fact that the keto-alcohol (IX; R′ = Me) was recovered unchanged after treatment with pyridine–acetic anhydride at room temperature overnight. So far as we are aware all equatorial hydroxyl groups are acetylated under these conditions.

The configuration of the CH_2·OH residue of icterogenin was shown to be β on the following evidence. Vogel, Jeger, and Ruzicka (*Helv. Chim. Acta*, 1951, **34**, 2321) have used molecular-rotation arguments to show that the $C_{(4)}$-configuration in methyl hederagonate (II; R = H) must be α. The changes in molecular rotation on proceeding from methyl hederagonate and its benzoate (II; R = H and Bz respectively) to methyl hedragonate (III) are +86° and +275° respectively. The corresponding changes for icterogenin derivatives (VI; R = H, R′ = Me; and R = Bz, R′ = Me), are −80° and +95°. The discrepancies are large enough to suggest a difference in configuration, especially as optical anomalies (cf. Barton and Cox, *J.*, 1948, 783) should not be serious here. The postulated



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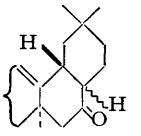

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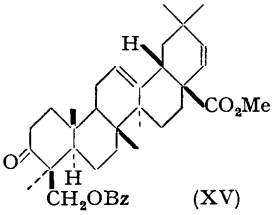

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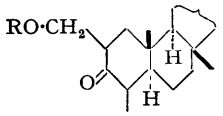

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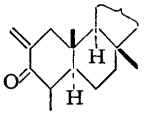

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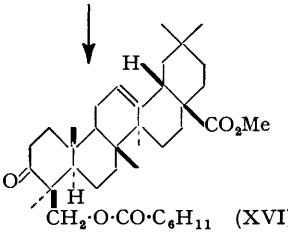

(XVI)

epimeric difference was confirmed by the fact that pyrolysis of icterogenin methyl ester benzoate (VI; R = Bz, R′ = Me) gave angelic acid and the benzoate (XV), hydrogenation of which, followed by reoxidation of the reduced ketonic grouping, gave a hexahydrobenzoate (XVI) *different* from the corresponding derivative (II; R = C_6H_{11}·CO·) of methyl hederagonate. The latter was prepared by direct hexahydrobenzoylation of methyl hederagonate. As would be expected both hexahydrobenzoates on treatment with aqueous-methanolic potassium hydroxide afforded methyl hedragonate (III).

The constitutional problem posed by icterogenin should not be left without mention of an alternative formulation (XVII; R = H) which could conceivably explain most of the relevant facts. This formulation is rejected (*a*) because it does not comply with the isoprene rule, (*b*) because treatment with alkali would be expected to give the methylene ketone (XVIII) (which would be negative in the Zimmermann test) rather than provoke the elimination of formaldehyde and (*c*) because pyrolysis of the appropriate benzoate (XVII; R = Bz) would also be expected to give compounds of the type (XVIII), especially as thermal elimination of benzoic acid occurs more readily that that of a lower aliphatic acid (cf. Barton and Rosenfelder, *J.*, 1949, 2459).

The infra-red spectra of icterogenin and many of its derivatives have been determined. It will be sufficient to state that these spectra are in consonance with the structures deduced as above on other evidence. The same applies to the internal consistency of the molecular-rotational correlations which can be made.

An examination of the roots of *Lippia rehmanni* revealed, in agreement with the earlier findings by Rimington *et al.* (*loc. cit.*), that these were a rich source of triterpenoid material. It proved impossible to obtain pure icterogenin simply by crystallisation, but by chromatography over silica gel (a procedure which should prove of general use in the fractionation of

triterpenoid carboxylic acids) it was possible to separate icterogenin from a new acid. In view of the origin of the latter we designate it rehmannic acid. It has the molecular formula $C_{35}H_{52}O_5$. The presence of a carboxyl group was indicated by its acidity and by the preparation of a methyl ester. A reactive carbonyl function was revealed by the preparation of an oxime and of a 2 : 4-dinitrophenylhydrazone. The position of the ultra-violet absorption maximum of the latter coupled with the non-reducing properties of the acid indicated that the carbonyl function was ketonic, not aldehydic. Work on the constitution of this acid is in progress and will be reported later.

Experimental

For general experimental conditions see Part VII (*J.*, 1952, 2339). Rotations were determined in chloroform solution, unless specified to the contrary. All ultra-violet absorption spectra (except those for 2 : 4-dinitrophenylhydrazones) were determined in ethanol solution with the Unicam S.P. 500 Spectrophotometer. Infra-red spectra were kindly determined by Messrs. Glaxo Laboratories Ltd. using, unless stated to the contrary, carbon disulphide as solvent. Silica gel for chromatography was obtained from Messrs. Hopkin and Williams Ltd.

Examination of the Leaves of Lippia rehmanni.—The dried powdered leaves (500 g.) were steeped in 95% ethanol (2 l.) overnight. After filtration the leaves were further extracted with a similar volume of alcohol. The combined filtrates were evaporated *in vacuo* to *ca.* 700 ml. Ether (2 l.) was then added and the mixture diluted to 5 l. with water. After separation of the ethereal layer the aqueous phase was re-extracted with a further quantity (1 l.) of ether. The combined ethereal extracts were washed twice with water and then shaken with sodium carbonate solution (2·5%; 15 ml.). The dark aqueous phase was separated (viewing by reflected light from a 100-w bulb is recommended) and the ether further extracted with sodium carbonate solution (6 × 25 ml.). All extracts except the first were combined. Occasionally some sodium salt separated; this was collected separately. The alkaline extracts were washed twice with ether, and a final layer of ether was added. Solid sodium chloride (25 g.) was introduced with stirring and the mixture left in the icebox overnight. The separated sodium salt was collected by filtration [0·65 g.; m. p. 225—300° (decomp.)].

The total combined sodium salts were dissolved in alcohol (10 ml.), acidified to Congo-red paper with hydrochloric acid, and diluted, when hot, with water until the solution was cloudy. Crude icterogenin (0·35 g.; m. p. 200—220°) gradually separated. Repeated recrystallisation from hot methanol afforded in poor yield a product, m. p. 239—241°, crystallising in short rods. This corresponded to the icterogenin A of Rimington *et al.* (*loc. cit.*); it gave no depression in m. p. on admixture with the original specimen (m. p. 239—240°). If the product was allowed to crystallise slowly from cold aqueous methanol it separated as fine needles, m. p. 155—160°. This corresponded to the icterogenin C of Rimington *et al.* (*loc. cit.*) and, in agreement, it gave no depression in m. p. on admixture with the original specimen (m. p. 155—160°). If the icterogenin C (either specimen) was kept for a short time at 140° it then melted at 239—240°, undepressed on admixture with icterogenin A. If the m. p. was determined in the usual way the melt resolidified at 160° and then remelted at 225—230°. Crystallisation of icterogenin A from cold aqueous methanol afforded icterogenin C, and recrystallisation of the latter in the usual way gave back icterogenin A.

Icterogenin, isolated in this way, and also from the roots of the plant (see below), had: (*A*) m. p. 239—241°, $[\alpha]_D$ +64° (*c*, 1·01), +63° (*c*, 0·84) (both in EtOH), +108° (*c*, 1·43), $\lambda_{max.}$ 212 mμ (ε 12,000); (*C*) m. p. 155—160°, $[\alpha]_D$ +63° (*c*, 1·07) (in EtOH) [Found, on material crystallised from benzene: C, 73·8; H, 9·1%; equiv., (potentiometrically) 562, (phenolphthalein) 555. $C_{35}H_{52}O_6$ requires C, 73·9; H, 9·2%; equiv., 569. Icterogenin was not a reducing substance as shown by tests with Fehling's solution and with Tollens's reagent.

Icterogenin, treated with pyridine–acetic anhydride at room temperature overnight, gave icterogenin acetate, m. p. (from methanol; plates) 155—168°, (from boiling aqueous ethanol; needles) 140—143°, $[\alpha]_D$ +56° (*c*, 1·12), +57° (*c*, 1·29). The latter crystal form and m. p. correspond to those reported by Rimington *et al.* (*loc. cit.*).

Icterogenin 2 : 4-dinitrophenylhydrazone, prepared in the usual way and crystallised from methanol (needles), had m. p. 244—246°, $\lambda_{max.}$ 365 mμ (ε 22,000 in $CHCl_3$) (Found: N, 7·2. $C_{41}H_{56}O_9N_4$ requires N, 7·5%).

Icterogenin, treated with diazomethane, gave the *methyl ester* (from methanol) as fine solvated needles, m. p. *ca.* 160° (with solvent evolution), $[\alpha]_D$ +102° (*c*, 1·40) (Found: C, 73·25; H, 9·75. $C_{36}H_{54}O_6,\frac{1}{2}CH_3{\cdot}OH$ requires C, 73·25; H, 9·35%).

Icterogenin methyl ester was converted into the 2 : 4-*dinitrophenylhydrazone* in the usual way. Recrystallised from acetone–light petroleum (b. p. 60—80°) this had m. p. 230—231° (Found : N, 7·5. $C_{42}H_{58}O_9N_4$ requires N, 7·35%).

Icterogenin acetate (see above), treated with diazomethane, afforded *icterogenin methyl ester acetate*. Recrystallised from methanol this had m. p. 161—163°, $[\alpha]_D$ +59° (*c*, 1·97), $\lambda_{max.}$ 214 mμ (ε 12,100) (Found : C, 71·5, 71·8; H, 9·25, 9·15. $C_{38}H_{56}O_7,CH_3{\cdot}OH$ requires C, 71·3; H, 9·2%).

Icterogenin methyl ester with pyridine–benzoyl chloride at room temperature overnight gave *icterogenin methyl ester benzoate*. Recrystallised from methanol this had m. p. 159—161°, $[\alpha]_D$ +60° (*c*, 0·95) ,+61° (*c*, 1·18), $\lambda_{max.}$ 228 mμ (ε 20,000) (Found : C, 75·45, 75·1; H, 8·3, 8·15. $C_{43}H_{58}O_7$ requires C, 75·2; H, 8·5%). The compound gave a yellow colour with tetranitromethane. The benzoate (50 mg.) was treated with an equal weight of hydroxylamine hydrochloride in pyridine at room temperature overnight. Crystallisation from methanol furnished the *oxime*, m. p. 221—223° (Found : C, 73·55; H, 8·2. $C_{43}H_{59}O_7N$ requires C, 73·55; H, 8·5%).

Icterogenin (30 mg.) in methanol (5 ml.) was treated with the theoretical amount of bromine in methanol solution (freshly prepared). The colour was rapidly discharged. The product of reaction was entirely neutral. Crystallisation from chloroform–methanol gave *icterogenin bromo-lactone*. This had m. p. 239—242° (decomp.), $[\alpha]_D$ +81° (*c*, 0·86), $\lambda_{max.}$ 218 mμ (ε 8,400) (Found : Br., 12·1. $C_{35}H_{51}O_6Br$ requires Br, 12·3%). The bromo-lactone gave no colour with tetranitromethane. The bromo-lactone (40 mg.) in " AnalaR " acetic acid (10 ml.) with addition of zinc dust (5 g.) was heated on the steam-bath with occasional shaking for 2 hr. Crystallisation of the product from aqueous methanol gave back icterogenin (m. p., mixed m. p., and negative Beilstein test).

Separation of Methyl Ester Benzoates.—The crude acids (20 g.), from the extraction of 7 kg. of powdered leaves by the process outlined above, were methylated with diazomethane, benzoylated (pyridine–benzoyl chloride at room temperature), and chromatographed over alumina (500 g.), to give 57 fractions. Elution with 1 : 1 benzene–light petroleum (b. p. 40—60°) (4 fractions; total 60 mg.) gave methyl oleanolate benzoate (from chloroform–methanol), m. p. 253—255°, $[\alpha]_D$ +84° (*c*, 1·30), undepressed in m. p. on admixture with an authentic specimen {m. p. 256—257°, $[\alpha]_D$ +85° (*c*, 1·10)}. Elution with 3 : 2 benzene–light petroleum (b. p. 40—60°) (4 fractions; total 30 mg.) gave a compound (from chloroform–methanol), m. p. 265—266° $[\alpha]_D$ −80° (*c*, 1·32) $\lambda_{max.}$ 228 mμ ($E^{1\%}_{1cm.}$ 314) (Found : C, 78·25; H, 8·7%). Elution with benzene gave material (3 fractions; total 75 mg.) which furnished a compound (from aqueous methanol), m. p. 219—222°, $[\alpha]_D$ +91° (*c*, 1·00), $\lambda_{max.}$ 228 mμ ($E^{1\%}_{1cm.}$ 400) (Found : C, 75·0; H, 8·05%). Elution with benzene and 19 : 1 benzene–ether (15 fractions) gave, after crystallisation from methanol, icterogenin methyl ester benzoate (see above) (2·2 g.). The homogeneity of this compound was further established by prolonged fractional crystallisation and by rechromatography.

Separation of Methyl Ester Acetates.—The crude acids (6·0 g.), from the extraction of 3 kg. of powdered leaves by the process outlined above, were methylated, acetylated (pyridine–acetic anhydride), and chromatographed over alumina (200 g.) to give 21 fractions. Elution with benzene (2 fractions; total 70 mg.) and crystallisation from chloroform–methanol gave methyl oleanolate acetate, m. p. 215—217°, $[\alpha]_D$ +71° (*c*, 1·02), undepressed in m. p. on admixture with an authentic specimen (m. p. 218°, $[\alpha]_D$ +70°). Hydrolysis with 5% methanolic potassium hydroxide gave methyl oleanolate, m. p. 195—196°, $[\alpha]_D$ +77° (*c*, 0·27), undepressed in m. p. on admixture with an authentic specimen (m. p. 195—196°, $[\alpha]_D$ +75°). Further elution with benzene (2 fractions; total 30 mg.) gave a compound (from chloroform–methanol), m. p. 243—244°, $\lambda_{max.}$ 216 mμ ($E^{1\%}_{1cm.}$ 270) (Found : C, 74·15, 73·6; H, 9·85, 9·05%). Further elution with benzene (6 fractions; total 1·01 g.) and repeated crystallisation from methanol gave, in poor yield only, icterogenin methyl ester acetate.

Examination of the Roots of Lippia rehmanni.—The finely powdered whole roots (10 kg.) were extracted with methanol (20 l.) as in the corresponding treatment of the leaves (see above). After three extractions the combined extracts were evaporated *in vacuo* to approx. 3 l. A heavy oil separated and, on cooling, the supernatant liquid could be decanted easily. The residue was almost entirely acidic and on crystallisation from chloroform–methanol gave mixed acids (10 g.) m. p. 253—256°, $[\alpha]_D$ +74° (*c*, 1·12 in EtOH), +103° (*c*, 2·35), not resolved by further crystallisation. Isolation of the acids from the supernatant liquid in the manner recorded above for treatment of the leaves gave a further 6 g. of mixed acids.

Although icterogenin and rehmannic acid could not be separated by crystallisation, the mixture (1·0 g.) was readily resolved on chromatography in benzene solution over silica gel

(80 g.) (15 fractions). Elution with 4 : 1 benzene–ether (five fractions; total 265 mg.) and crystallisation from methanol gave *rehmannic acid*, m. p. 295—300° (decomp.), $[\alpha]_D$ +84° (*c*, 1·05), $\lambda_{max.}$ 212 mμ (ε 12,400) (Found : C, 75·7; H, 9·25. $C_{35}H_{52}O_5$ requires C, 76·05; H, 9·5%). Elution with 1 : 1 benzene–ether (five fractions; total 575 mg.) and crystallisation from benzene afforded icterogenin, identical in all respects with that obtained from the leaves.

Rehmannic acid, treated with pyridine–hydroxylamine hydrochloride at room temperature overnight, afforded its *oxime*. Recrystallised from methanol this had m. p. 270—272° (decomp.) (Found : C, 73·95; H, 9·25. $C_{35}H_{53}O_5N$ requires C, 74·05; H, 9·4%).

Rehmannic acid was converted into the 2 : 4-*dinitrophenylhydrazone* in the usual way. Recrystallised from chloroform–methanol this had m. p. 273—274° (decomp.), $\lambda_{max.}$ 368 mμ (ε 21,000 in $CHCl_3$) (Found : C, 66·9; H, 7·55; N, 7·35. $C_{41}H_{56}O_8N_4$ requires C, 67·2; H, 7·7; N, 7·65%).

Methyl rehmannate was prepared from rehmannic acid (50 mg.) by methylation with diazomethane. Recrystallised from methanol in a solvated form this had m. p. *ca.* 140° (with evolution of solvent), $[\alpha]_D$ +86° (*c*, 1·37) (Found : C, 74·9; H, 9·9. $C_{36}H_{54}O_5,\frac{1}{2}CH_3{\cdot}OH$ requires C, 75·25; H, 9·7%).

Hydrogenation Products of Icterogenin.—Icterogenin (50 mg.) in ethyl acetate (10 ml.) was hydrogenated with a palladised charcoal catalyst (5%; 100 mg.) for 1 hr. Recrystallisation of the product from benzene gave *dihydroicterogenin*, m. p. 233—235°, $[\alpha]_D$ +126° (*c*, 1·07), no absorption max. at 212 mμ (Found : C, 73·6; H, 9·4. $C_{35}H_{54}O_6$ requires C, 73·65; H, 9·55%). There was a 10° depression in m. p. on admixture with icterogenin.

Icterogenin methyl ester benzoate (100 mg.) in ethanol (50 ml.) was hydrogenated with a palladised strontium carbonate catalyst (5%; 100 mg.) for 15 min. Recrystallisation of the product from methanol furnished *dihydroicterogenin methyl ester benzoate*, m. p. 180—182°, $[\alpha]_D$ +56° (*c*, 3·25) (Found : C, 74·85; H, 9·05. $C_{43}H_{60}O_7$ requires C, 74·9; H, 8·8%). This dihydrocompound (50 mg.), treated with pyridine–hydroxylamine hydrochloride overnight at room temperature, gave the *oxime*, m. p. 266—267° (from methanol) (Found : C, 73·15; H, 8·35. $C_{43}H_{61}O_7N$ requires C, 73·3; H, 8·75%). The benzoate and its oxime gave positive tetranitromethane tests.

Icterogenin methyl ester benzoate (20 mg.) in ethanol (50 ml.) was hydrogenated with a platinum catalyst (50 mg.) for 1 hr., to give *dihydroicterogenin methyl ester hexahydrobenzoate* (from aqueous methanol), m. p. 191—193°, $\lambda_{max.}$ 287 mμ (ε 30), no max. at 230 mμ (Found : C, 74·05; H, 9·4. $C_{43}H_{66}O_7$ requires C, 74·3; H, 9·55%), which gave a positive test with tetranitromethane.

Icterogenin methyl ester benzoate (70 mg.) in ethanol (50 ml.) was hydrogenated with a platinum catalyst (50 mg.) for 24 hr. Acetic acid (1 ml.) was then added and the mixture reduced for a further 15 min. Working up gave *tetrahydroicterogenin methyl ester hexahydrobenzoate* (from methanol), m. p. 200—202°, $[\alpha]_D$ +57° (*c*, 1·09), no carbonyl max. in the ultraviolet region (Found : C, 73·7; H, 10·05. $C_{43}H_{68}O_7$ requires C, 74·1; H, 9·85%), which gave a positive test with tetranitromethane.

Elimination of Formaldehyde in the Icterogenin Series.—Icterogenin methyl ester benzoate (100 mg.) in methanol (70 ml.) was treated with potassium hydroxide (4 g.) in water (30 ml.) and left overnight at room temperature. The deposited crystals (75 mg.) were almost pure *methyl* 22β-*angeloyloxyhedragonate*. Recrystallised from methanol, this had m. p. 220—221°, $[\alpha]_D$ +93° (*c*, 1·68), $\lambda_{max.}$ 212 mμ (ε 9000) (Found : C, 75·95; H, 9·45. $C_{35}H_{52}O_5$ requires C, 76·05; H, 9·5%). This compound gave a positive test with tetranitromethane. It was recovered unchanged after attempted acetylation, benzoylation, and dehydration (with pyridine–phosphorus oxychloride). The compound (50 mg.) was treated with pyridine–hydroxylamine hydrochloride overnight at room temperature, to give the *oxime* (from methanol), m. p. 219—221° (Found : C, 73·75; H, 9·4. $C_{35}H_{53}O_5N$ requires C, 74·05; H, 9·4%). The alkali treatment was repeated at reflux temperature for 30 min., but no crystalline product could be isolated.

Methyl 22β-angeloyloxyhedragonate (200 mg.) in ethanol (70 ml.) was hydrogenated with a palladised strontium carbonate catalyst (100 mg.) for 1 hr., to give *methyl* 22β-(α-*methyl*-n-*butyroyloxy*)*hedragonate*. Recrystallised from methanol this had m. p. 187—188° $[\alpha]_D$ +86° (*c*, 1·37), no αβ-unsaturated ester band in the ultra-violet region (Found : C, 75·5; H, 9·7. $C_{35}H_{54}O_5$ requires C, 75·75; H, 9·8%). It gave a positive test with tetranitromethane and was recovered unchanged on being refluxed in 1% methanolic potassium hydroxide. On treatment (40 mg.) with pyridine–hydroxylamine hydrochloride it afforded the corresponding *oxime* (from methanol), m. p. 205—207° (Found : C, 73·6; H, 9·35. $C_{35}H_{55}O_5N$ requires C, 73·45; H, 9·7%). The ketone gave its 2 : 4-*dinitrophenylhydrazone* (from chloroform–methanol), m. p. 236—238°,

$\lambda_{max.}$ 369 mμ (ε 24,400 in $CHCl_3$) (Found: C, 67·35; H, 7·65. $C_{41}H_{58}O_8N_4$ requires C, 67·0; H, 7·95%).

Dihydroicterogenin methyl ester benzoate (100 mg.) in methanol (36 ml.) was treated with potassium hydroxide (2·5 g.) in water (12 ml.) and left for 3 hr. Filtration of the deposited crystals in 1 : 1 benzene–light petroleum (b. p. 40—60°) solution through a short column of alumina and crystallisation from methanol furnished methyl 22β-(α-methyl-*n*-butyroyloxy)-hedragonate, identified by m. p., mixed m. p., and rotation. The identity was confirmed by conversion into the oxime and 2 : 4-dinitrophenylhydrazone (see above) and by the appropriate mixed m. p.s thereon.

Dihydroicterogenin methyl ester hexahydrobenzoate (6 mg.) in methanol (8 ml.) was treated with potassium hydroxide (400 mg.) in water (3 ml.) during 4 hr. The product, isolated with ether, was converted into the 2 : 4-dinitrophenylhydrazone which had m. p. (from chloroform–methanol) 229—233°, undepressed on admixture with the corresponding derivative (see above) of methyl 22β-(α-methyl-*n*-butyroyloxy)hedragonate.

Icterogenin methyl ester acetate (10 mg.), treated in the same way, gave methyl 22β-angeloyloxyhedragonate, identified by m. p. and mixed m. p. Tetrahydroicterogenin methyl ester hexahydrobenzoate (9 mg.), also treated in the same way, was recovered unchanged (m. p. and mixed m. p.).

Icterogenin (350 mg.) in aqueous methanol (70%; 200 ml.) containing potassium hydroxide (8 g.) was set aside for 3 hr. The product, isolated with ether, was chromatographed over silica gel (8 g.) in 8 fractions. Elution with 9 : 1 benzene–ether (2 fractions; total 200 mg.) gave 22β-*angeloyloxyhedragonic acid*. Recrystallised from methanol as plates, this had m. p. 256—259°, $[\alpha]_D$ +92° (*c*, 1·41) (Found: C, 75·4; H, 9·05. $C_{34}H_{50}O_5$ requires C, 75·8; H, 9·35%). Elution with 1 : 1 benzene–ether (2 fractions) gave back a small amount of unchanged icterogenin (m. p. and mixed m. p.). On methylation with diazomethane 22β-angeloyloxyhedragonic acid gave the known methyl ester (see above), identified by m. p. and mixed m. p.

22β-Angeloyloxyhedragonic acid (120 mg.) in ethyl acetate (20 ml.) was hydrogenated for 1 hr. with a palladised charcoal catalyst (5%; 100 mg.), to give 22β-(α-*methyl*-n-*butyroyloxy*)-*hedragonic acid*, plates (from methanol) m. p. 250—253°, $[\alpha]_D$ +96° (*c*, 1·34) (Found: C, 75·3; H, 9·6. $C_{34}H_{52}O_5$ requires C, 75·5; H, 9·7%). The same compound (m. p. and mixed m. p.) was obtained on subjecting dihydroicterogenin (10 mg.) to the alkaline conditions used with icterogenin. Methylation with diazomethane gave methyl 22β-(α-methyl-*n*-butyroyloxy)-hedragonate identical (m. p. and mixed m. p.) with the compound described under the same name above.

Elimination of Formaldehyde in the Hederagone Series.—Methyl hederagonate, m. p. 213—215°, $[\alpha]_D$ +78° (*c*, 1·12), +100° (*c*, 1·14 in $COMe_2$) (230 mg.) (Jacobs, *J. Biol. Chem.*, 1925, **63**, 631), was converted into the *benzoate* by the action of pyridine–benzoyl chloride overnight at room temperature. Recrystallised from methanol, this had m. p. 160—161°, $[\alpha]_D$ +32° (*c*, 2·18) (Found: C, 77·35; H, 8·75. $C_{38}H_{52}O_5$ requires C, 77·5; H, 8·9%).

Methyl hederagonate benzoate (100 mg.) in methanol (70 ml.) was treated with a solution of potassium hydroxide (4 g.) in water (30 ml.). After 45 min. the crystals deposited (61 mg.) were filtered off and recrystallised from methanol, to give methyl hedragonate (Jacobs and Gustus, *ibid.*, 1926, **69**, 641), identified by m. p., mixed m. p., and rotation {$[\alpha]_D$ +101° (*c*, 1·62), +102° (*c*, 1·11)}. The authentic specimen had $[\alpha]_D$ +102° (*c*, 1·08) and m. p. 203—205°. The m. p. is lower than that recorded by Jacobs and Gustus but chromatography and crystallisation failed to raise it.

Methyl hederagonate (43 mg.) in aqueous methanol (70%; 40 ml.) containing potassium hydroxide (1·5 g.) was set aside overnight at room temperature. The deposited crystals (24 mg.) gave, on crystallisation from methanol, methyl hedragonate, identified by m. p. and mixed m. p.; there was a pronounced m. p. depression on admixture with starting material.

Methyl hedragonate (200 mg.) in methanol (50 ml.) containing potassium hydroxide (2·5 g.) was refluxed with two mols. of formaldehyde for 4 hr. The product of reaction (part of which had separated; m. p. 270—275°) was an amorphous intractable solid even on chromatography. Methyl hedragonate treated similarly but without the addition of formaldehyde was recovered unchanged (m. p. and mixed m. p.).

Isolation of Benzoic Acid.—The mother-liquors remaining after the elimination of formaldehyde from icterogenin methyl ester benzoate (165 mg.) in the usual way (see above) were extracted with ether, and the ether discarded. The aqueous phase was then acidified and again extracted. Removal of the ether and crystallisation from hot water gave benzoic acid (14 mg.) identified by m. p., mixed m. p., and absorption spectrum.

Detection of Formaldehyde.—The aqueous–methanolic filtrate from the treatment of methyl hederagonate benzoate (see above) was acidified to Congo-red paper with sulphuric acid and then distilled. To the distillate dimedone (70 mg.) was added. After 30 min. the mixture was distilled to 20 ml. The dimedone–formaldehyde compound (12·3 mg., 24·8%) gradually separated. It was identified by m. p., mixed m. p., and crystal form (fine needles). A similar experiment on icterogenin methyl ester benzoate (25 mg.) likewise afforded the dimedone–formaldehyde compound. A control experiment, without addition of any triterpenoid compound, yielded none of the compound.

The elimination of formaldehyde was also detected by the chromotropic acid method (Bricker and Johnson, *Ind. Eng. Chem. Anal.*, 1945, **17**, 400). Solutions of methyl hederagonate (38·4 mg.), icterogenin (36·7 mg.), and icterogenin methyl ester benzoate (48·6 mg.), each in ethanol (10 ml.), were treated at 21° with ethanolic potassium hydroxide (10%; 10 ml.). At various intervals 1-ml. portions were withdrawn and added each to water (1 ml.) containing concentrated sulphuric acid (0·2 ml.) in a 15-ml. centrifuge tube. Chloroform (2 ml.) was added to each tube and, after mixing and centrifuging, the chloroform layer was withdrawn by pipette. A further 2 ml. of chloroform were added and the extraction procedure was repeated. The residual aqueous layer was then treated in the manner described by Bricker and Johnson (*loc. cit.*). Trial experiments with added formaldehyde (but no triterpenoid) served to demonstrate that the extraction and estimation technique was adequate for the determination of more than 90% of the formaldehyde present (as shown by the standard curve). Runs with no formaldehyde and no triterpenoid gave no chromotropic acid colour (relative to the control). Failure to remove triterpenoid material by the chloroform-extraction procedure (see above) gave fallacious results since the concentrated sulphuric acid used in the last stage of Bricker and Johnson's procedure provoked formaldehyde elimination by an acid-catalysed mechanism. Thus methyl hederagonate (2·3 mg.) in ethanol (1 ml.), treated with standard chromotropic acid–sulphuric acid reagent, gave formaldehyde (14%). The following results (in terms of % of formaldehyde eliminated) were obtained after leaving the various solutions in contact with the alkaline reagent (see above) for 5 min.: methyl hederagonate 17 ± 3, icterogenin 13 ± 3, and methyl icterogenin benzoate 7 ± 2%

*Detection of α-Methyl-*n*-butyric and of Tiglic Acids.*—Methyl 22β-(α-methyl-*n*-butyroyloxy)-hedragonate (see above) (250 mg.) in ethanolic potassium hydroxide (10%; 20 ml.) was refluxed for 3 hr. The resulting solution was diluted with water and separated, by ether-extraction, into neutral and acid fractions. The aqueous phase (acid fraction) was acidified with 50% aqueous sulphuric acid to pH 1—2 and distilled. The distillate consumed 0·048N-sodium hydroxide solution equivalent to 43 mg. of α-methyl-*n*-butyric acid (theor., 45·5 mg.). The distillate had no selective ultra-violet absorption above 205 mμ. A small portion of the acid was run on ascending partition chromatograms in *n*-butanol–1·5N-ammonia (method of Reid and Lederer, *Biochem. J.*, 1952, **50**, 60), development being with *p*-bromocresol-purple in ethanol–formaldehyde. The R_c of the volatile acid (where $R_c = \frac{R_F \text{ of volatile acid}}{R_F \text{ of } n\text{-butyric acid}}$) was found to be 1·45. Under the same conditions valeric acid had R_c 1·7 and *iso*valeric acid R_c 1·4. This suggested that the volatile acid was a branched C_5 acid, and this was confirmed by conversion of the remainder of the material into the *p*-bromophenacyl ester. This had m. p. (from aqueous methanol) 56—57°, undepressed on admixture with an authentic specimen of the *p*-bromophenacyl ester of α-methyl-*n*-butyric acid (Found: C, 52·65; H, 5·45. Calc. for $C_{13}H_{15}O_3Br$: C, 52·2; H, 5·05%). Higher and lower homologues have widely different compositions. The ester was depressed in m. p. by *ca.* 10° on admixture with the *p*-bromophenacyl ester (m. p. 68°) of *iso*valeric acid.

Methyl 22β-angeloyloxyhedragonate (200 mg.) was hydrolysed and the volatile acid distilled as in the procedure detailed above. The distillate contained acid equivalent to 25 mg. of tiglic acid (theor., 36·5 mg.) and showed $\lambda_{max.}$ 217 mμ (ε *ca.* 7000). The acid was converted into its *p*-bromophenacyl ester which, after two crystallisations from aqueous methanol, had m. p. 62—65°, melting at 67—69° on admixture with authentic ester (m. p. 69—70°) from tiglic acid, but at 55—58° on admixture with authentic ester (m. p. 68—69°) from angelic acid. A mixed m. p. curve of the two authentic esters was constructed as indicated (percentage of tiglate, m. p. range, median m. p.): 95%, 64—70°, 66·5°; 90%, 59—65°, 62·5°; 70%, 52—61°, 56·5°; 60%, 51—57·5°, 54·5°; 44%, 49—54°, 51·5°; 31%, 49—55·5°, 52°. This indicated that the ester isolated contained about 93% of the tiglate (Found: C, 52·0; H, 4·65. Calc. for $C_{13}H_{13}O_3Br$: C, 52·55, H, 4·4%).

Degradation of Methyl 22β-(α-*Methyl-*n*-butyroyloxy)hedragonate.*—The methyl ester (100 mg.) in ethanolic potassium hydroxide (4%; 20 ml.) was refluxed for 6 hr. The neutral product was

chromatographed over alumina (8 fractions). Elution with 1 : 1 benzene–light petroleum (b. p. 40—60°) (6 fractions) gave *methyl* 22β-*hydroxyhedragonate*. Recrystallised from methanol this either had m. p. 242—245° or (solvated form) *ca.* 110° (decomp.), $[\alpha]_D$ +90° (*c*, 1·25) (Found : C, 76·75; H, 9·5. $C_{30}H_{46}O_4$ requires C, 76·55; H, 9·85%). It gave a positive test with tetranitromethane. The same compound (m. p. and mixed m. p.) was obtained from the alkaline hydrolysis of methyl 22β-angeloyloxyhedragonate under the same conditions. The compound (23 mg.) was left with acetic anhydride and pyridine overnight at room temperature. Crystallisation of the product from chloroform–light petroleum gave unchanged starting material (m. p. and mixed m. p.).

22β-(α-Methyl-*n*-butyroyloxy)hedragonic acid (40 mg.), refluxed with the ethanolic potassium hydroxide in the same way (4% ; 10 ml.) for 6 hr., gave 22β-*hydroxyhedragonic acid*. Recrystallised from methanol, this had m. p. 267—271° (decomp.), $[\alpha]_D$ +97° (*c*, 1·36) (Found : C, 76·0; H, 9·7. $C_{29}H_{44}O_4$ requires C, 76·25; H, 9·7%). The same compound, identified by m. p. and mixed m. p., was obtained by similar hydrolysis of 22β-angeloyloxyhedragonic acid and as the by-product in the hydrolysis of methyl 22β-(α-methyl-*n*-butyroyloxy)- and 22β-angeloyloxyhedragonate referred to above. Methylation with diazomethane gave methyl 22β-hydroxyhedragonate, identified by m. p. and mixed m. p.

Methyl 22β-hydroxyhedragonate (100 mg.) in " AnalaR " acetic acid (10 ml.) was treated with chromium trioxide (18 ml.) in the minimum of water and left at room temperature overnight. Chromatography of the product over alumina (2 g.; 7 fractions) gave *methyl* 22-*oxohedragonate*, eluted with 1 : 1 benzene–light petroleum (b. p. 40—60°) (6 fractions; all identical). Recrystallised from methanol this had m. p. 234—235°, $[\alpha]_D$ +49° (*c*, 1·03) (Found : C, 76·7; H, 9·75. $C_{30}H_{44}O_4$ requires C, 76·90; H, 9·45%). It gave a positive test with tetranitromethane. The m. p. was depressed to 185—188° on admixture with an authentic specimen of the diketo-ester (m. p. 193°) from quillaic acid (Elliott, Kon, and Soper, *J.*, 1940, 612). Both diketo-esters were recovered unchanged (m. p. and mixed m. p.) after 5 minutes' refluxing with 5% methanolic potassium hydroxide.

Methyl 22-oxohedragonate (70 mg.) was hydrolysed by refluxing ethanolic potassium hydroxide (20% ; 10 ml.) for 2 hr. The product, which was entirely neutral, was chromatographed on alumina (2 g.). Elution with 2 : 5 benzene–light petroleum (b. p. 40—60°) gave the *more* stable diketone. Recrystallised from methanol this melted unsharply at 220—226° (Found : C, 81·0; H, 10·35. Calc. for $C_{28}H_{42}O_2$: C, 81·9; H, 10·3%). In view of probable inhomogeneity of this material and of its unsatisfactory carbon analysis an alternative approach was indicated as outlined below.

22β-Hydroxyhedragonic acid (65 mg.) in " AnalaR " acetic acid (3 ml.) was treated with chromium trioxide (11 mg.) in " AnalaR " acetic acid (1 ml.) at room temperature overnight. The product was separated into acid and neutral (trace) fractions, and the acid fraction in benzene (10 ml.) heated on the steam-bath to effect decarboxylation. Separation into acid and neutral fractions gave, in the latter fraction, the *less* stable diketone. Purified by filtration in benzene solution through alumina and crystallisation from methanol, this had m. p. 212—217°, $[\alpha]_D$ +51° (*c*, 0·96) (Found : C, 82·1; H, 10·25%). It gave a marked depression in m. p. on admixture with the *more* stable diketone reported above. There were similar marked depressions in m. p. on admixture of either of the diketones from icterogenin with the analogous diketone (m. p. 185°) from quillaic acid (Elliott, Kon, and Soper, *loc. cit.*).

Pyrolysis of Methyl 22β-*Angeloyloxyhedragonate.*—The ester (205 mg.) was pyrolysed by passage through a Pyrex glass tube (40 cm. × 11 mm.) at 500°/0·5—0·7 mm. in a stream of oxygen-free nitrogen (approx. ½ l. per hr. at room temperature and atmospheric pressure). The product (140 mg.) was refluxed with methanolic potassium hydroxide (5% ; 10 ml.) for 2 hr. and the neutral material (133 mg.) chromatographed over alumina. In this way any unchanged angeloyloxy-compound was converted into a relatively strongly adsorbed alcohol. Elution with 1 : 1 benzene–light petroleum (b. p. 40—60°) afforded *methyl hedragon*-21-*enate*. Recrystallised from methanol, this had m. p. 190—191°, $[\alpha]_D$ +39° (*c*, 1·39) (Found : C, 79·3; H, 9·6. $C_{30}H_{44}O_3$ requires C, 79·6; H, 9·8%).

Methyl hedragon-21-enate (29 mg.) in " AnalaR " acetic acid (10 ml.) was hydrogenated overnight at room temperature with a platinum catalyst (50 mg.). After removal of the catalyst by filtration and of the acetic acid by evaporation under reduced pressure, the product was refluxed with methanolic potassium hydroxide (5% ; 10 ml.) for 1 hr. The neutral material, in " AnalaR " acetic acid (1 ml.), was treated with chromium trioxide (4·3 mg.) in " AnalaR " acetic acid (1 ml.) at room temperature overnight. Filtration of the product in benzene through a short column of alumina and recrystallisation from methanol gave methyl hedragonate, m. p.

and mixed m. p. 200—203°, $[\alpha]_D$ +98° (*c*, 0·42). The identity was confirmed by conversion into the 2 : 4-dinitrophenylhydrazone (m. p. and mixed m. p.). The authentic specimen of *methyl hedragonate* 2 : 4-*dinitrophenylhydrazone* was recrystallised from chloroform–methanol and had m. p. 270° (Found : C, 67·9; H, 7·75; N, 8·7. $C_{36}H_{50}O_6N_4$ requires C, 68·1; H, 7·95; N, 8·85%).

Pyrolysis of Icterogenin Methyl Ester Benzoate.—The benzoate (500 mg.) was pyrolysed in 100 mg. batches as in the procedure outlined above. The pyrolysate was chromatographed over alumina (51 g. : 16 fractions). Elution with 1 : 1 benzene–light petroleum gave (10 fractions) *methyl* 4-epi*hederagon*-21-*enate benzoate*. Recrystallised from methanol this had m. p. 171—172°, $[\alpha]_D$ +3° (*c*, 1·52) (Found : C, 77·9; H, 8·55. $C_{38}H_{50}O_5$ requires C, 77·75; H, 8·6%). Elution with benzene (4 fractions) afforded unchanged (m. p. and mixed m. p.) icterogenin methyl ester benzoate (65 mg.).

Methyl 4-*epi*hederagon-21-enate benzoate (95 mg.) in " AnalaR " acetic acid (40 ml.) was hydrogenated overnight with a platinum catalyst. After crystallisation from light petroleum (b. p. 60—80°), the product (45 mg.) in " AnalaR " acetic acid (2 ml.) was treated with chromium trioxide (4·5 mg.) in the same solvent (0·5 ml.) overnight at room temperature, to give *methyl* 4-epi*hederagonate hexahydrobenzoate* (from methanol), m. p. 187°, $[\alpha]_D$ +42° (*c*, 1·02) (Found : C, 77·1; H, 10·0. $C_{38}H_{58}O_5$ requires C, 76·7; H, 9·85%). The hexahydrobenzoate (7 mg.) in aqueous methanol (70%; 10 ml.) containing dissolved potassium hydroxide (400 mg.) was left overnight at room temperature. Crystallisation of the product from aqueous methanol gave methyl hedragonate, identified by m. p. and mixed m. p. The m. p. was depressed by 40° on admixture with starting material.

Methyl Hederagonate Hexahydrobenzoate.—Hexahydrobenzoyl chloride (prepared from hexahydrobenzoic acid and thionyl chloride and purified by distillation *in vacuo*) (1·0 g.) was added to a solution of methyl hederagonate (1·0 g.) in pyridine (10 ml.), and the whole left overnight at room temperature. Chromatography of the product over alumina (22 g.; 10 fractions), and elution with 1 : 3 benzene–light petroleum (b. p. 40—60°), gave *methyl hederagonate hexahydrobenzoate*, rhombs [from light petroleum (b. p. 40—60°)], m. p. 154—155°, $[\alpha]_D$ +65° (*c*, 1·38) (Found : C, 76·55; H, 10·05%). This derivative of hederagonic acid, like the benzoate, could only be crystallised with difficulty even when carefully purified. The mixed m. p. with methyl 4-*epi*hederagonate hexahydrobenzoate was depressed to 134—140°. Treatment of the hexahydrobenzoate (10 mg.) with aqueous methanol (70%; 10 ml.) containing dissolved potassium hydroxide (400 mg.) overnight at room temperature afforded methyl hedragonate, identified by m. p. and mixed m. p.

Oxidation of Methyl 22β-(α-*Methyl*-n-*butyroyloxy*)*hedragonate with Chromic Acid.*—The ester (60 mg.) in " AnalaR " acetic acid (10 ml.) was treated with chromium trioxide (18 mg.) in " AnalaR " acetic acid (3 ml.) at room temperature overnight, to give *methyl* 22β-(α-*methyl*-n-*butyroyloxy*)-11-*oxohedragonate*. Recrystallised from methanol, this had m. p. 209—212°, $\lambda_{max.}$ 249 mμ (ε 8500) (Found : C, 73·7; H, 8·95. $C_{35}H_{52}O_6$ requires C, 73·9; H, 9·2%). When β-amyrin benzoate was oxidised under these conditions it likewise afforded 11-oxo-β-amyrin benzoate.

Perbenzoic Acid Titrations.—Derivatives of icterogenin were subjected to perbenzoic acid titration in chloroform solution at ice-box temperature in the usual way. The results are summarised in the Table.

Derivatives of icterogenin	Perbenzoic acid (M)	Perbenzoic acid (mols.) added	consumed 20 hr.	consumed 2 days	consumed 4 days
Icterogenin methyl ester benzoate	0·071	5	1·2	2·5	3·2
Dihydroicterogenin methyl ester benzoate	0·050	6	0·7	0·95	0·9
Methyl 22β-angeloyloxyhedragonate	0·071	6	0·8	2·0	2·6
Methyl 22β-(α-methyl-*n*-butyroyloxy)hedragonate	0·02	1·6	0·2	—	0·4 *

* 0·8 in 15 days.

Rates of Alkaline Hydrolysis.—The following esters were refluxed with ethanolic potassium hydroxide (20%; 10 ml.) for 3 hr. and the amount of hydrolysis determined by weighing the amounts of (non-volatile) acid produced. The amounts of ester taken are indicated in parentheses. Methyl echinocystate (18·5 mg.), 60%; methyl oleanolate (50 mg.), 5%; methyl 22β-(α-methyl-*n*-butyroyloxy)hedragonate (120 mg.), 60%.

Detection of Angelic Acid.—The volatile acids formed in the pyrolysis of (i) icterogenin methyl ester benzoate and (ii) methyl 22β-angeloyloxyhedragonate were collected in a U-tube cooled in

an alcohol–solid carbon dioxide bath in the form of beautiful crystals condensed on the walls of the U-tube just above the level of the refrigerant. Microscopically the crystals in each case resembled angelic acid. This was confirmed by a mixed m. p. determination. The m. p.s of the volatile acids and their mixed m. p.s with (*a*) angelic acid (m. p. 44°) and (*b*) tiglic acid (m. p. 64°) were respectively: (i) 38—42°, 39—43°, below room temp.; (ii) 38—41°, 39—43°, below room temperature. Tiglic acid, subjected to pyrolysis under the same conditions, was recovered in the U-tube unchanged (m. p. and mixed m. p.s with tiglic acid and with angelic acid).

The authentic angelic acid used in these experiments was prepared by the method of Buckles and Mock (*J. Org. Chem.*, 1950, **15**, 680).

Zimmermann Tests.—The method used was that of Callow, Callow, and Emmens (*Biochem. J.*, 1938, **32**, 1312). The compound (*ca.* 0·5 mg.) was dissolved in 1 ml. of 2N-potassium hydroxide in absolute ethanol and 1 ml. of 1% *m*-dinitrobenzene in absolute ethanol. After 10 min. the mixture was diluted to 10 ml. with absolute ethanol. Tested in this way icterogenin methyl ester benzoate, icterogenin, methyl 22β-angeloyloxyhedragonate, methyl hedragonate, methyl hederagonate, methyl oleanonate, and α-amyrenone all gave a violet colour which faded after dilution. No colour was developed by the following compounds: methyl 12-oxo-oleananolate acetate, 12-oxo-α-amyranyl benzoate, methyl 12:19-dioxo-18β-oleananolate (Barton, Holness, Overton, and Rosenfelder, *J.*, 1952, 3751), methyl 11-oxo-oleananolate acetate (Barton and Holness, *J.*, 1952, 78), 7:11-dioxolanostan-3β-yl acetate, and 16-oxo-28-norolean-12-ene (Bilham and Kon, *J.*, 1940, 1469).

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BIRKBECK COLLEGE, LONDON, W.C.1. [*Received, October 13th*, 1953.]