

113. *Sapogenins. Part VII. The Structure of Quillaic Acid and its Relation to Echinocystic Acid.*

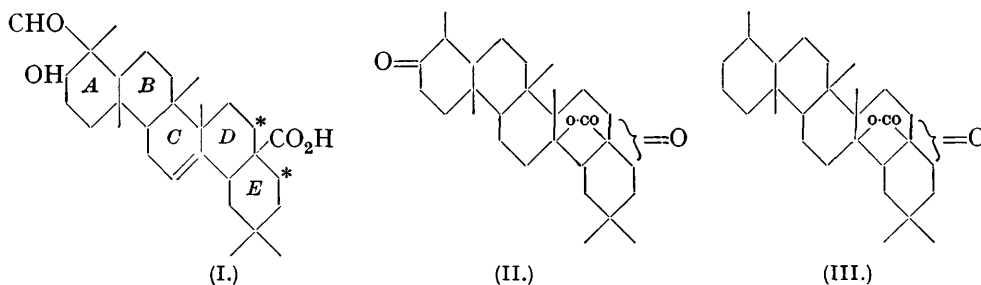
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In continuation of previous work (J., 1939, 1130) it is shown that the second hydroxyl group of quillaic acid, which is not part of the group $\text{CH}(\text{OH})\cdot\text{CMe}\cdot\text{CHO}$, is attached to a carbon atom immediately adjacent to the quaternary carbon atom carrying the carboxyl group, as in echinocystic acid (Noller and White, *J. Amer. Chem. Soc.*, 1939, 61, 983). Quillaic acid has been converted, by the replacement of the aldehyde group by methyl, into *deoxyquillaic acid* and this has been compared with echinocystic acid. In spite of the great similarity between the two acids and their derivatives they are for the present regarded as distinct.

IN Part VI (Elliott and Kon, J., 1939, 1130) it was shown that the triterpene quillaic acid is a dihydroxy-aldehyde and that one hydroxyl is part of the group $\text{CH}(\text{OH})\cdot\text{CMe}\cdot\text{CHO}$, which is also present in gypsogenin. On the grounds of analogy it may be assumed that quillaic acid belongs to the β -amyrin group of triterpenes and is a hydroxygypsogenin (I). It is hoped to obtain support for this assumption as the result of degradation experiments

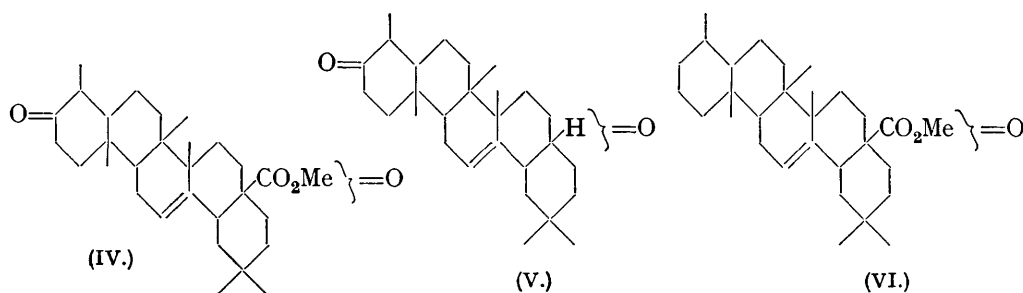
which are still in progress; in the meantime it is desirable to place on record observations bearing on the position of the second hydroxyl group.

It has already been pointed out by Elliott and Kon that the comparatively ready hydrolysis of quillaic ester is probably due to the proximity of this hydroxyl group to the carbomethoxyl group. Moreover, this group occupies a sterically protected position. Thus, the diketo-lactone (II) previously obtained (*loc. cit.*), in which this group has been oxidised to carbonyl, only forms a monosemicarbazone and condenses with one molecule of 2 : 4-dinitrophenylhydrazine. It is now found that of the two carbonyl groups in this compound one resists reduction by Clemmensen's method, whereas the other is very readily reduced, giving rise to the *keto-lactone* (III). The carbonyl group which is reduced is doubtless that in ring A, the structure of which is, if our assumption is justified, identical with that of methyl hedragone. The reduction of the latter compound has been carried out by Jacobs and Gustus under similar mild conditions (*J. Biol. Chem.*, 1926, **61**, 641).



* Possible positions of second OH group.

Confirmation of this view is afforded by the oxidation of the methyl ester of quillaic acid with chromic acid or with copper bronze. The product is the *diketo-ester* (IV), C₃₀H₄₄O₄, formed with the loss of carbon dioxide and degradation of the groups in ring A as in the formation of the diketo-lactone (II). One carbonyl group in this ester occupies a position β with respect to the carbomethoxyl group, because the latter is readily eliminated as potassium carbonate on boiling with alcoholic potassium hydroxide, with the production of the *diketone* (V); there is evidence to show that this may be a mixture of stereoisomerides. Like the diketo-lactone (II), the ester (IV) is reduced by Clemmensen's method with the elimination of one carbonyl group to the *ester* (VI); this still retains the inert carbonyl group in the β -position to the carbomethoxyl group, because it undergoes hydrolysis to carbon dioxide and a *monoketone*, C₂₈H₄₄O. The carbonyl group in the monoketone is no



longer inert and reacts with 2 : 4-dinitrophenylhydrazine, whereas the parent ester does not form derivatives with ketonic reagents. The ketonic esters (IV) and (VI) do not give colours with ferric chloride and it follows from this and the ready elimination of the carbomethoxyl group that the latter is attached to a quaternary carbon atom. For the moment no attempt will be made to decide between the two possible positions of the second hydroxyl group, because it is hoped to obtain decisive evidence shortly.

The reactions of the keto-esters outlined above are entirely parallel to those of the oxidation products of methyl echinocystate (Noller and White, *J. Amer. Chem. Soc.*, 1939, **61**,

983) and suggest that quillaic acid and echinocystic acid might be related in the same way as gypsogenin and oleanolic acid, in which case echinocystic acid should be formed from quillaic acid by conversion of the aldehyde group into methyl.

The reduction of quillaic acid itself by Clemmensen's method had been attempted in these laboratories some years ago by Dr. J. S. Fitzgerald, who found that the carbonyl group is not reduced and the only product is the diacetyl-lactone. The latter compound is also not reduced, but undergoes a partial isomerisation to a higher-melting compound. The methyl ester of quillaic acid is almost completely reduced, but we were unable to purify the product. The *semicarbazone* of the ester is, however, smoothly reduced by the Kischner-Wolff method to *deoxyquillaic acid*, which appears to have all the properties of echinocystic acid; the most characteristic is the fact that it crystallises from *isopropyl* alcohol in plates and from ethyl alcohol in fine needles (compare Bergsteinsson and Noller, *J. Amer. Chem. Soc.*, 1934, **56**, 1403); the rotations of the two acids are also similar. Certain discrepancies in the properties of derivatives have, however, been noted: the *methyl* ester of deoxyquillaic acid melts very sharply some 4° too low and the *diketo-ester* prepared from it by oxidation with chromic acid melts at 152–153° or 15° lower than the compound described by Noller and White (*loc. cit.*), and its *oxime* melts 12° too low. Unlike the *diketo-ester* described by Noller and White, our compound does not give off methane under the conditions of the Zerevitinov test.

These facts were communicated to Prof. C. R. Noller, who kindly sent us pure specimens of echinocystic acid and its methyl ester and a partially purified specimen of the *diketo-ester*; at the same time he informed us that all the melting points recorded in his papers (*loc. cit.*) are corrected and therefore necessarily higher than ours.

We found that the melting points of the two acids were almost identical, but no great importance can be attached to this because the acids decompose at temperatures below the melting point and the latter is very dependent on the extent to which the bath is preheated. On the other hand, the melting points of methyl echinocystate and of the *diketo-ester* determined by us were distinctly higher than those of our compounds (3° and 6° respectively). The preparation of the *diketo-ester* from echinocystic acid was repeated; the ester melted at 158–159° after two crystallisations, but, contrary to Noller and White's observation, gave no methane under the conditions of the Zerevitinov test. Mixtures of echinocystic and quillaic derivatives melted in every case at intermediate temperatures and a super-saturated solution of methyl echinocystate crystallised immediately on being seeded with a crystal of methyl deoxyquillate. We nevertheless hesitate to conclude that the two series of compounds are identical, because differences in rotations were observed under exactly comparable conditions; for instance, methyl deoxyquillate had $[\alpha]_D + 33.9^\circ$ in alcohol and methyl echinocystate $[\alpha]_D + 31.4^\circ$. We were unable to dissolve anhydrous echinocystic acid in alcohol and the rotations were compared in pyridine; deoxyquillaic acid again had the higher rotation (+13.5° as against +7.8°); the very marked influence of the solvent may be noted in passing. The differences may perhaps be attributable to partial racemisation of the acids in the course of preparation and it is hoped to examine the matter further.

The oxidation of the diacetyl-lactone of quillaic acid has been re-examined in the hope of discovering analogies with the behaviour of the *isoacetyl-lactone* of gypsogenin under the same conditions (Kon and Soper, following paper). The formation of the lactone (II) is, indeed, entirely analogous to that of hedragone lactone and the properties of the two compounds are very similar. The nature of the acidic products is, however, different. Elliott and Kon (*loc. cit.*) had obtained three acids, designated as A₁, A₂ and B. Of these, A₂ may have been the keto-hydroxy-acid C₂₉H₄₄O₆ analogous to the C₂₉ acid from gypsogenin and A₁ appeared to be C₂₇H₄₀O₆, but the amounts of these acids were so small as to preclude further examination. The yield of the acid B has, on the other hand, been improved and it has also been isolated in the form of a *hydrate* (?), which gives the same methyl ester as the acid B, although it could not be dehydrated to the latter without decomposition. The ester reacts, though slowly and incompletely, with methylmagnesium iodide, giving 0.7 mol. of methane. The absorption spectrum in alcoholic solution reveals a low intensity band at 2920 Å. ($\epsilon = 18.6$) attributable to a carbonyl, like that of the ester

of the C_{29} acid from gypsogenin. Repeated analyses of the acid, its ester and the 2:4-dinitrophenylhydrazone of the latter suggest, however, that the acid is not the expected C_{29} acid, but has the composition $C_{31}H_{48}O_7$. Now this acid is obtained by the oxidation of a C_{30} compound, in the course of which a sterically protected carboxyl group of the latter is eliminated; the formation of a C_{31} compound can only be explained by assuming that a group such as acetyl is introduced at some period. We have, however, failed to detect this group by analytical methods; for example, the ester of the acid B is completely hydrolysed by boiling for an hour with methyl-alcoholic potassium hydroxide, but the product is the hydrate of the original acid; similarly, the acid is unaffected by prolonged boiling with aqueous alkali. For the present we are unable to give a satisfactory explanation of the nature of these compounds.

EXPERIMENTAL.

M. p.'s are uncorrected; unless otherwise stated, the specimens for analysis were dried at $100^\circ/1$ mm. for 2 hours.

Oxidation Products of the Diacetyl-lactone.—The oxidation of the diacetyl-lactone to the C_{30} acid was carried out as described by Elliott and Kon (*loc. cit.*). In the oxidation of the C_{30} acid it was found preferable to use an equivalent amount of Kiliani's solution in place of chromic acid and to introduce it more rapidly ($3\frac{1}{2}$ hours for 5 g. of acid). In this way the yield of acid B was increased to 25%, whilst the amounts of the acids A_1 and A_2 became negligible and could be readily removed by extraction with ethyl acetate. The acid B had the properties previously described; the analysis figures recorded (*loc. cit.*) for the acid agree perfectly with those calculated for $C_{31}H_{48}O_7$ (Calc. : C, 69.9; H, 9.1%) and those for the ester with $C_{32}H_{50}O_7$ (Calc. : C, 70.3; H, 9.2%). When the crude acid was crystallised from dilute methyl alcohol, the hydrate (?) was obtained, forming long needles melting indefinitely at 170 – 180° (Found : C, 67.7; H, 9.4. $C_{31}H_{48}O_7 \cdot H_2O$ requires C, 67.6; H, 9.2%). All attempts to obtain the acid B from the hydrate were unsuccessful, although various degrees of dehydration were achieved. Sublimation in a high vacuum gave an unsaturated acid which could not be purified; the analytical figures correspond roughly with the loss of a molecule of acetic acid and one of water from the hydrate (Found : C, 74.4; H, 9.5. $C_{29}H_{42}O_5$ requires C, 73.6; H, 9.4%). On esterification with diazomethane the hydrate gave the ester of the acid B, which crystallised in characteristic, sparingly soluble rods; the ester prepared from the hydrate melted 4° higher than that from the anhydrous acid; a mixture of the two melted at an intermediate temperature (Found : C, 70.1, 70.1; H, 9.4, 9.5%). An identical 2:4-dinitrophenylhydrazone, m. p. 283° (decomp.), was prepared from both specimens of the ester and crystallised from acetic acid in iridescent orange-yellow plates (Found : C, 62.9; H, 7.5; N, 7.5, 7.6. $C_{38}H_{54}O_{10}N_4$ requires C, 62.8; H, 7.5; N, 7.7%). A specimen of the ester was boiled for an hour with an excess of 25% methyl-alcoholic potassium hydroxide, and the alcohol then evaporated; water was added, but no ester was precipitated. On acidification the hydrated acid was obtained and identified by reversion into the characteristic ester, m. p. 210° .

The yield of neutral product in the oxidation of the C_{30} acid is always poor; it consists of the diketo-lactone (II) previously described. We find that this compound crystallises best from acetic acid.

Reduction of the Diketo-lactone (II).—500 Mg. of the diketo-lactone were boiled for $\frac{1}{2}$ hour with 32.5 c.c. of acetic acid, 7.5 c.c. of hydrochloric acid, and 12.5 g. of amalgamated zinc wool (compare Jacobs and Gustus, *loc. cit.*). The reaction, which was very vigorous at first, gradually subsided; the solution was diluted with water and extracted with ether. The extract was washed with water, then with alkali, again with water, dried, and evaporated; an almost theoretical yield of crude reduction product was obtained. It crystallised from acetic acid, in which it was much less soluble than the diketo-lactone, in iridescent plates, m. p. 293 – 295° , depressed by admixture of the diketo-lactone. The yield of recrystallised keto-lactone (III) was 75% (Found : C, 78.9; H, 10.3. $C_{29}H_{44}O_3$ requires C, 79.1; H, 10.1%).

Diketo-ester (IV).—1 G. of methyl quillaate was intimately ground with 3 g. of copper bronze (Kahlbaum) and heated at 270° until no more gas was evolved ($\frac{1}{2}$ hour), then distilled at $170^\circ/0.006$ mm. The pale yellow glass crystallised from methyl alcohol in stout needles (150 mg.), m. p. 192° after two crystallisations. A better yield (25–30%) was obtained by treating a mechanically stirred solution of 2 g. of ester in 100 c.c. of acetic acid and a few drops of water dropwise with 8.5 c.c. of Beckmann's solution diluted with 20 c.c. of acetic acid at about 10° . The solvent was distilled off under reduced pressure, water added, and the reaction products

extracted with ether. Extraction of the ethereal solution with alkali removed 700 mg. of uncrystallisable acidic material, which has not yet been investigated. The neutral material recovered by evaporation of the ether was purified by percolation of a benzene solution through a column of activated alumina, and the solid obtained crystallised from chloroform-methyl alcohol; m. p. 193° , $[\alpha]_D + 8.9^{\circ}$ ($c = 4.024$ in chloroform). The analysis specimen was dried for 12 hours at $80^{\circ}/0.002$ mm. and 1 hour at $110^{\circ}/0.002$ mm. (Found : C, 76.6, 76.6; H, 9.3, 9.4. $C_{30}H_{44}O_4$ requires C, 76.9; H, 9.5%).

Diketone (V).—200 Mg. of the above ester were boiled with 10 c.c. of 5% alcoholic potassium hydroxide for $2\frac{1}{2}$ hours. A yellow colour developed almost at once and darkened to deep orange; a deposit of potassium carbonate soon formed. The solution was poured into water and extracted with ether; the yellow extract became colourless on treatment with a drop of mineral acid. On evaporation a solid crystallising from acetone in small plates, m. p. 197° , together with a little unchanged diketo-ester, could be isolated. On a larger scale 1.15 g. of ester were similarly hydrolysed for $3\frac{1}{2}$ hours. No starting material was recovered; the neutral material was purified by percolation of a benzene solution through alumina, but it was found to melt lower than before (yield, 250 mg.). After several recrystallisations from acetone it formed needles melting to an opaque liquid at 185° and clearing at 210° , not depressed by admixture of the compound of m. p. 197° (Found : C, 81.8; H, 10.1. $C_{28}H_{42}O_4$ requires C, 81.9; H, 10.3%). It appears probable that a mixture of stereoisomerides is formed in this hydrolysis.

Keto-ester (VI).—(i) Reichstein's procedure (*Helv. Chim. Acta*, 1937, **20**, 1050) was followed : 500 mg. of the diketo-ester (IV) in 80 c.c. of acetic acid and 70 c.c. of hydrochloric acid were heated on the steam-bath with 30 g. of amalgamated zinc wool, a slow stream of hydrogen chloride being passed through the liquid for 3 hours. The reaction mixture was poured into water and extracted with ether, the extract washed with alkali and evaporated, and the residue crystallised from chloroform-methyl alcohol. It formed short prisms (150 mg.), m. p. 178° , $[\alpha]_D + 5.2^{\circ}$ ($c = 2.656$ in chloroform) (Found : C, 79.1; H, 10.1. $C_{30}H_{46}O_3$ requires C, 79.2; H, 10.2%). (ii) A yield of 300 mg. of the same compound was obtained when the reduction was carried out exactly as described on p. 615.

Hydrolysis of the Ester (VI).—This was carried out exactly as described above. The solution remained practically colourless throughout; the product formed needles, m. p. 185 — 187° , after repeated crystallisation from methyl alcohol (Found : C, 84.4; H, 11.1. $C_{28}H_{44}O$ requires C, 84.8; H, 11.2%). The 2 : 4-dinitrophenylhydrazone formed brownish-yellow, silky needles from acetic acid, m. p. 268° (decomp.).

Attempted Reduction of Quillaic Acid Derivatives.—Attempts to reduce quillaic acid by Clemmensen's method in acetic acid solution gave the diacetyl-lactone. The diacetyl-lactone was reduced by the method of Jacobs and Gustus (*loc. cit.*) as described on p. 615. The product contained, in addition to unchanged diacetyl-lactone, a more soluble isomeride, which crystallised from ethyl or methyl alcohol in needles, m. p. 272 — 274° , depressed by admixture of quillaic lactone, which might have been produced by deacetylation (Found : C, 71.3, 71.1; H, 9.0, 8.9. $C_{34}H_{50}O_7$ requires C, 71.5; H, 8.9%). The methyl ester of quillaic acid was reduced by the same method, but the product did not crystallise even after sublimation in a high vacuum, although it gave analytical figures approximating to those required for a deoxy-ester.

Methyl quillaate was kept for 48 hours with a solution prepared by grinding a 15% excess of semicarbazide hydrochloride with sodium acetate and methyl alcohol (compare Ruzicka and Giacomello, *Helv. Chim. Acta*, 1936, **19**, 1136). The solvent was then evaporated, leaving an oily mass, which solidified on grinding with water. It was collected, washed, and dried. The semicarbazone was repeatedly crystallised from dilute methyl alcohol, but a preparation with a satisfactory melting point could not be obtained : it sintered at 186° and melted between 200° and 220° ; it was, however, analytically pure (Found : N, 7.5. $C_{32}H_{51}O_6N_3$ requires N, 7.4%).

2.6 G. of the semicarbazone were heated in a sealed tube with 1.5 g. of sodium in 25 c.c. of alcohol for 36 hours at 160 — 170° . The contents of the tube were diluted with 300 c.c. of water and thoroughly extracted with ether, which removed only a small amount of neutral material. The aqueous layer contained a considerable amount of an insoluble sodium salt; it was acidified with hydrochloric acid and extracted with ether. The dried extract gave on evaporation about 1.5 g. of solid *deoxyquillaic acid*. This had a great tendency to separate from solvents in a gelatinous form, but crystallised when its solution in isopropyl alcohol was concentrated. It formed fine plates, m. p. 302° with previous sintering, $[\alpha]_D + 34^{\circ}$ ($c = 1.768$ in 95% alcohol).*

* We were unable to obtain a satisfactory reading for the green mercury line, but on the assumption that the dispersion of the acid is of the same order as that of related compounds, the above value is in good agreement with the rotation of echinocystic acid found by Bergsteinsson and Noller (*loc. cit.*).

[1940] *Sapogenins. Part VIII. The Sapogenin of Fuller's Herb.* 617

The analysis specimen was dried for 3 hours at 150—160° in a high vacuum (Found : C, 76·5; H, 10·3. $C_{30}H_{48}O_4$ requires C, 76·2; H, 10·2%). The *methyl* ester, prepared with the aid of diazomethane, formed needles from methyl alcohol, m. p. 209—210° (Found : C, 76·3; H, 10·6. $C_{31}H_{50}O_4$ requires C, 76·5; H, 10·4%).

The methyl ester of deoxyquillaic acid was oxidised to the *diketo-ester* according to the directions of Noller and White (*loc. cit.*); the ester, obtained in excellent yield, crystallised from dilute methyl alcohol in needles, m. p. 152—153°; it was recovered unchanged after another treatment with chromic acid (Found : C, 76·9; H, 9·7. $C_{31}H_{46}O_4$ requires C, 77·1; H, 9·6%). The *oxime* was prepared as described by Noller and White; it was sparingly soluble in methyl alcohol, from which it separated in needles, m. p. 246—247° (Found : C, 75·2; H, 9·8. $C_{31}H_{47}O_4N$ requires C, 74·8; H, 9·5%).

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