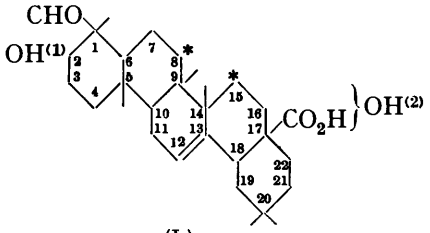
271. *Sapogenins. Part X. The Carbon Skeleton and the Position of the Second Hydroxyl Group of Quillaic Acid.*

By Philip Bilham and George A. R. Kon.

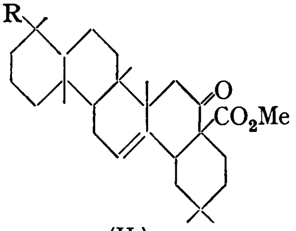
Surface-film measurements show that the second hydroxyl group of quillaic acid, $OH^{(2)}$, which had previously been shown to be attached to a carbon atom adjacent to the quaternary carbon atom carrying the carboxyl group, is situated on C_{16} in ring *D* and it can be inferred that the carboxyl group must be attached to C_{17} at the junction of rings *D* and *E*.

Deoxyquillaic acid, in which the aldehyde group of quillaic acid has been reduced to methyl, has been converted into a *hydrocarbon* $C_{29}H_{48}$, which is evidently a stereoisomeride of Winterstein and Stein's oleanene II (*Z. physiol. Chem.*, 1931, **202**, 222), from which it differs by the *trans*-locking of rings *D* and *E*. This has been converted into oleanene III (Winterstein and Stein, *Annalen*, 1933, **502**, 223), thus proving that the carbon skeleton of quillaic acid must be identical with that of oleanolic acid and gypsogenin. The formation of the new hydrocarbon is explained by assuming *cis*-locking of rings *D* and *E* in all these acids.

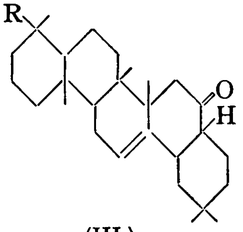
It was shown by Elliott, Kon, and Soper (this vol., p. 612) that one of the hydroxyl groups of quillaic acid, $OH^{(2)}$, occupies a position β with respect to the carboxyl group, which is attached to a quaternary carbon atom. On the assumption that the carbon skeleton of quillaic acid is similar to that of gypsogenin the formula (I) was put forward, the two alternative positions of $OH^{(2)}$ being C_{22} and C_{16}:



(I.)



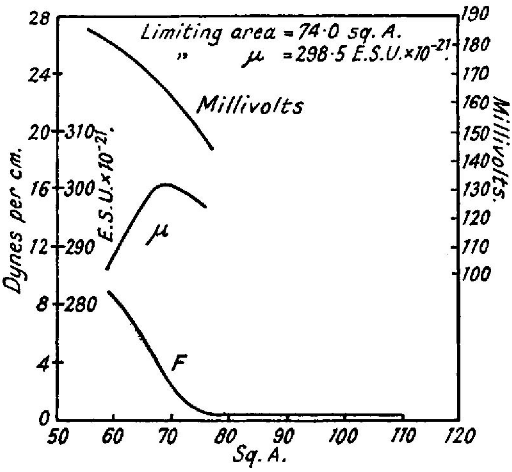
(II.)



(III.)

It is a matter of some difficulty to decide between the two possible positions of $OH^{(2)}$ by purely chemical means, although such a decision could be made on the basis of Blanc's rule; a dibasic acid produced by the opening of the oxgyen-containing ring should form a ketone on pyrolysis if the ring is terminal, but only an anhydride if the ring is *B*, *C*, or *D*. Apart from the difficulties encountered in attempting to open the oxygenated ring in the ester (II; R = H) (Kon and Soper, unpublished work), this method is not suitable for eliminating other possible, though less likely, positions of $OH^{(2)}$, such as those marked with asterisks in formula (I). Measurements of unimolecular films of the appropriate ketone or alcohol should, however, readily afford the required information, since large differences in limiting area are found, for example, in isomeric cholestanones and cholestanols differing by the position of the oxygen atom (compare Adam, Askew, and Danielli, *Biochem. J.*, 1935, **29**, 1786).

The ketone (III; R = H), a degradation product of quillaic acid in which the oxygen-containing groups in ring *A* and the carboxyl group have been eliminated, did not give a satisfactory film. The corresponding *alcohol* derived from deoxyquillaic acid (R = Me) proved to be suitable; it was chosen because the oxidation of methyl quillaate to the diketo-ester is attended with difficulty, whereas methyl deoxyquillaate gives an excellent yield of the diketo-ester. The carbonyl group in ring *A* is reduced by Clemmensen's method and the *monoketo-ester* (II; R = Me) is hydrolysed to the *ketone* (III; R = Me), m.p. 220—222°. An isomeric *ketone*, m. p. 160—161°, is also formed in varying amounts and differs from the strongly lævorotatory principal product in being feebly dextrorotatory. Reduction of the high-melting ketone with sodium in alcohol gives the corresponding secondary alcohol, which may be termed the β-epimeride.



The alcohol is soluble in 5 : 2 benzene-alcohol and is deposited from this solution on N/100-hydrochloric acid, forming a liquid, readily compressible film. This is moderately stable, withstanding pressures up to 8 dynes/cm. The limiting area found by extrapolation of the force-area curve to zero compression is 74 sq. A. and the value of μ at this area is 298 E.S.U. × 10^{-21} (see figure).

Examination of scale models gives the following values for the minimum areas on the assumption that the dipole (OH group) is vertical:

Position of OH *group.*	*Locking of rings D/E.* *trans.*	*cis.*
On C_{22} (= C_4 in sterols)	47 sq. A.	49·5
„ C_{16} (= C_6 „)	78 „	92
„ C_{15} (= C_7 „)	83 „	86

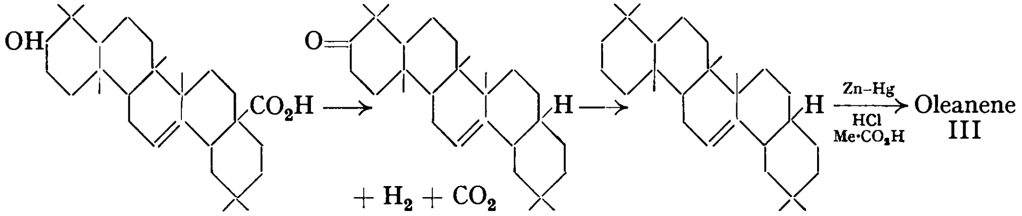
The value observed clearly excludes a hydroxyl on C_{22} (in ring *E*), but agrees well with a hydroxyl on C_{16} and a *trans*-locking of rings *D* and *E*; the latter point is further discussed below. The compound is thus analogous to 6-cholestanol and the values for μ for the two compounds are almost identical. The limiting area for 6-cholestanol is only 48 sq. A., but the presence of an additional ring in place of the flexible side chain and also of the *gem*-dimethyl group accounts for the larger area in the triterpene derivatives.

The evidence also appears to exclude other possible positions of the hydroxyl, such as C_{15} and C_8, which are analogous to C_7 in the sterols. This point is of considerable importance, because it constitutes the first proof that the carboxyl group of quillaic acid, which is attached to a carbon atom adjacent to the hydroxyl-bearing carbon atom, must necessarily be situated at the junction of rings *D* and *E*, a position first suggested (for acids of the gypsogenin group) by R. D. Haworth (*Ann. Reports*, 1937, 327 *et seq.*). It is hoped to support this conclusion by further measurements on suitable compounds.

In view of these considerations it appeared to be of special interest to link up the carbon skeleton of quillaic acid with that of other known triterpenes. It has already been shown that the keto-ester (II; R = H) could be reduced by the Kishner–Wolff method, with the simultaneous elimination of the carbomethoxy-group, to a well-defined hydrocarbon $C_{28}H_{46}$ (Kon and Soper, this vol., p. 1335). The corresponding ester derived from deoxyquillaic acid (II; R = Me) has now been similarly reduced to a *hydrocarbon* $C_{29}H_{48}$, m. p. 193—193·5°. Since the keto-ester is obtained from the diketo-ester by reduction by Clemmensen's method, which is known to cause isomerisation in some cases (compare

Winterstein and Stein, *Annalen*, 1933, **502**, 223), the parent diketo-ester was reduced by the Kishner–Wolff method and gave an equally good yield of the same hydrocarbon; and the same compound was also obtained both from the lævorotatory ketone (III ; R = Me) and from its dextrorotatory isomeride, in each case in almost quantitative yield.

The properties of the hydrocarbon, except for its lower rotation, suggested that it might be identical with oleanene II (Winterstein and Stein, *loc. cit.* ; *Z. physiol. Chem.*, 1931, **202**, 222), especially since Todd, Harris, and Noller (*J. Amer. Chem. Soc.*, 1940, **62**, 1624) have converted echinocystic acid, which is closely related to, or identical with, deoxyquillaic acid, into oleanene III.* The method used by the American authors is similar to that described above, except that the reduction of the ketone (norechinocystenone) was carried out by the Clemmensen method; and the previous work of Winterstein and Stein had already established that oleanene II passes into oleanene III under these conditions. Oleanene II was easily obtained by a variant of the method of Winterstein and Stein : oleanolic acid was heated with copper bronze, giving a good yield of oleanone (this reaction was first tried by Winterstein and Hämmerle, *Z. physiol. Chem.*, 1931, **199**, 56, who noted the formation of a crystalline product, but did not identify it) and this was reduced to oleanene II by the Kishner–Wolff method :



Oleanolic acid. Oleanone. Oleanene II.

Direct comparison showed that the hydrocarbon, m. p. 193—193·5°, was different from oleanene II and also from oleanene III. This at first suggested that the skeleton of deoxyquillaic acid differs from that of oleanolic acid, but consideration shows that such a result is to be expected.

The formation of two ketones by the hydrolysis of the homogeneous ester (II ; R = Me) clearly suggests that they are stereoisomerides differing in the mode of locking of rings *D* and *E* ; the surface-film measurements on the alcohol derived from the higher-melting ketone point to the latter having a *trans*-configuration. Since these compounds are α-decalones, the less stable *cis*-form would be expected to pass into the *trans* on treatment with such reagents as hot sodium ethoxide, *e.g.*, under the conditions of the Kishner–Wolff reaction, and this explains why only one hydrocarbon, doubtless the *trans*-compound, is formed from *either* ketone. On the other hand, the formation of the less stable *cis*-form of the ketone in varying amounts suggests that the parent ketonic ester is a *cis*-compound, which first gives rise to the *cis*-ketone and this is further isomerised to the *trans* in the strongly alkaline reaction medium.† Now oleanolic acid is presumably decarboxylated without change of configuration; if, like deoxyquillaic acid, it is a *cis*-compound, the products (oleanone and hence also oleanene II) would also have a *cis*-locking of rings *D* and *E*. These products cannot undergo further change, since there is no α-keto-group to provide the necessary mechanism (enolisation) ; *different* hydrocarbons should therefore be formed in the two cases :

As already mentioned, oleanene II passes into oleanene III under the conditions of the

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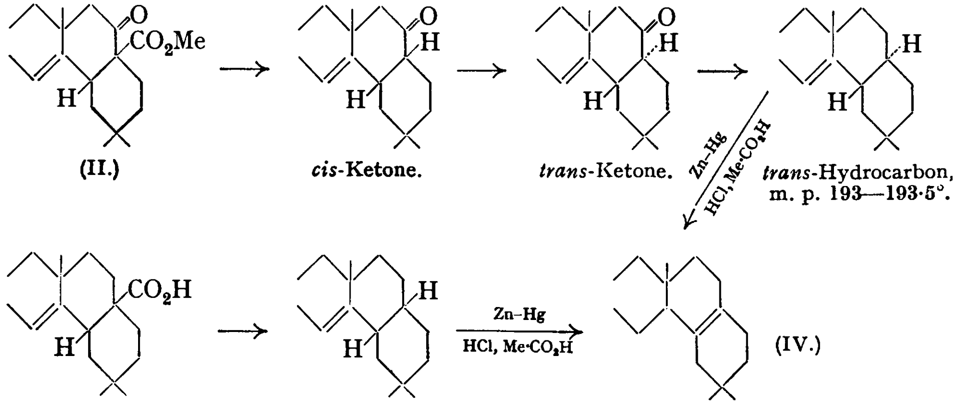
* Prof. C. R. Noller very kindly communicated these results to one of us before publication.

† It appears very probable that Noller and White's *iso*echinocystenedione (dextrorotatory) and echinocystenedione (lævorotatory) are related in the same way. The *iso*-compound is produced by the direct oxidation of echinocystic acid and should be the *cis*-compound. Echinocystenedione is formed in the alkaline hydrolysis of the diketo-ester and should have the *trans*-configuration ; it is also obtained from the *iso*-dione by boiling with alkali (Jones, Todd, and Noller, *J. Amer. Chem. Soc.*, 1939, **61**, 2421).

Clemmensen reaction; it is now found that the hydrocarbon, m. p. 193—193·5°, is also rapidly isomerised to oleanene III under these conditions; moreover, the *trans*-ketone is converted into the latter hydrocarbon on reduction by Clemmensen's method. It can therefore be concluded that oleanene II and the new hydrocarbon are the *cis*- and the *trans*-form of the same compound; and it follows that the carbon skeleton of deoxyquillaic acid is identical with that of oleanolic acid. Oleanene III must differ from the other two hydrocarbons by the position of the double bond and since it is formed with equal ease from both, the double bond doubtless occupies (or passes through) a position involving the bridge head, such as (IV). This formulation would necessarily dispose of triterpene formulæ in which a methyl group occupies this bridge head.



(II.) *cis*-Ketone. *trans*-Ketone. *trans*-Hydrocarbon, m. p. 193—193·5°.

Oleanolic acid. Oleanene II (*cis*). Oleanene III. (IV.)

Experimental.

(M. p.'s are uncorrected; specimens for analysis were dried for 2 hours at 100°/1—2 mm.)

Deoxyquillaic Acid.—1 G. of quillaic acid was heated in a sealed tube for 12 hours at 200° with 1·25 g. of sodium in 25 c.c. of alcohol and 3 c.c. of 95% hydrazine hydrate. The crude acid was isolated in quantitative yield by dilution with water, acidification, and extraction with ether. Crystallisation from *iso*propyl alcohol was somewhat wasteful, but up to 0·7 g. of material was obtained.

When ethyl alcohol was replaced by benzyl alcohol and the reaction mixture was refluxed, the yield of deoxyquillaic acid from 1 g. of quillaic acid was only 300 mg. and the isolation proved somewhat difficult. The same reaction was carried out with 4 g. of quillaic acid and the benzyl alcohol was removed by distillation in steam; practically no acidic material was isolated. There was, however, a quantity of a neutral product, which crystallised from acetic acid in felted needles melting rather indefinitely at 147—150°; this appeared to be a dihydric alcohol formed by loss of carbon dioxide from quillaic acid; the name *quillaol* is suggested for it (Found: C, 78·7; H, 10·2; active OH, 1·86. $C_{29}H_{46}O_3$ requires C, 78·7; H, 10·5%).

Keto-ester (II; R = Me) (*Methyl* 16-*Keto-oleanolate*).—Methyl deoxyquillaate was oxidised to the diketo-ester according to the instructions of Noller and White (*J. Amer. Chem. Soc.*, 1939, **61**, 983) for the oxidation of methyl echinocystate; the yield was almost quantitative. The diketo-ester was recrystallised once before reduction, which was carried out on 1·67 g. by the process of Jacobs and Gustus (*J. Biol. Chem.*, 1926, **61**, 641; Elliott, Kon, and Soper, *loc. cit.*), giving an almost theoretical yield of the crude *keto-ester*. This was very sparingly soluble in methyl alcohol, unlike the diketo-ester, and was purified for hydrolysis by partial dissolution in this solvent (50 c.c.); both the undissolved material and that which crystallised on cooling melted above 195° (yield, 1·40 g.). It was purified for analysis by recrystallising alternately from methyl and ethyl alcohol and formed fine needles, m. p. 204—205° (Found: C, 79·3; H, 10·3. $C_{31}H_{48}O_3$ requires C, 79·4; H, 10·3%).

Ketone III (16-*Keto*-$\Delta^{12:13(?)}$-*oleanene*).—1·3 G. of the keto-ester were boiled for 4 hours with 4 g. of potassium hydroxide in 75 c.c. of 95% alcohol and water was then added until the liquid was permanently turbid. The mixture of ketones (0·94 g.) which separated on cooling was recrystallised from acetone, not methyl alcohol as recommended by Noller and White (*loc. cit.*). The first crop (0·4 g.) melted above 218°, and gave flattened needles, m. p. 220—222° after another crystallisation, consisting of the trans-*ketone*, $[\alpha]_D - 146{\cdot}9°$ ($c = 2{\cdot}830$ in chloroform) (Found : C, 85·0; H, 11·6. $C_{29}H_{46}O$ requires C, 84·8; H, 11·3%). The mother-liquor from the first crystallisation of the mixed ketones was concentrated and gave a second crop of crystals (0·32 g.) similar in appearance to the *trans*-ketone, but melting at 157—160°. Repeated crystallisation from methyl alcohol, in which the compound was much more soluble than the *trans*-ketone, gave fine needles, m. p. 160—161°, not depressed by admixture of the *trans*-ketone, $[\alpha]_D + 13°$ ($c = 1{\cdot}00$ in chloroform) (Found : C, 84·8; H, 11·4%).

16-*Hydroxy*-$\Delta^{12:13(?)}$-*oleanene*.—A solution of the *trans*-ketone in 50 c.c. of alcohol was refluxed while sodium was gradually added until no more would dissolve. The solution was diluted with water; ether then extracted the *product*, which crystallised on rubbing with acetone and separated from this solvent in fine needles, m. p. 179° (Found : C, 84·3; H, 11·5. $C_{29}H_{48}O$ requires C, 84·6; H, 11·7%).

trans-$\Delta^{12:13(?)}$-*Oleanene*.—140 Mg. of the keto-ester, 0·3 g. of sodium in 6 c.c. of alcohol, and 0·3 c.c. of 95% hydrazine hydrate were heated in a sealed tube at 300° for 12 hours. The *hydrocarbon* was isolated by dilution with water and extraction with ether; the yield was nearly quantitative. It was alternately crystallised from acetic acid and acetone, forming long flattened needles, m. p. 193—193·5°. After two crystallisations the specimen had $[\alpha]_D + 23°$ ($c = 0{\cdot}8537$ in chloroform) (Found : C, 87·9; H, 12·2. $C_{29}H_{48}$ requires C, 87·8; H, 12·2%). A specimen similarly prepared from the high-melting *trans*-ketone had $[\alpha]_D + 27°$ ($c = 0{\cdot}8690$) and from the *cis*-ketone, $[\alpha]_D + 24{\cdot}5°$ ($c = 1{\cdot}389$); the specimens were identified by m. p. and mixed m. p. determinations.

Conversion into oleanene III. About 30 mg. of the above hydrocarbon in 16 c.c. of acetic acid were boiled for 4 hours with 4 g. of amalgamated zinc wool and 4 c.c. of hydrochloric acid. The hydrocarbon was recovered by dilution and extraction with light petroleum; after one crystallisation from ethyl acetate it formed plates, m. p. 219—220°, not depressed by admixture of oleanene III prepared from oleanone.

Oleanene III ($\Delta^{17:18(?)}$-*Oleanene*).—200 Mg. of the ketone, m. p. 220—222°, were dissolved in 32 c.c. of acetic acid and boiled with 12·5 g. of amalgamated zinc wool and 7·5 c.c. of hydrochloric acid. As some solid remained undissolved, a further 16 c.c. of acetic acid and 4 c.c. of hydrochloric acid were added after 1½ hours. The product was isolated after 6 hours by dilution with water and extraction with light petroleum. After evaporation of the solvent the residue crystallised from hot ethyl acetate in plates (100 mg.), m. p. 213—215°. Another crystallisation from the same solvent gave long flattened needles, m. p. 219—220°, not depressed by admixture of oleanene III prepared from oleanone. The m. p. did not change on further crystallisation. A specimen crystallised four times had $[\alpha]_D + 32{\cdot}9°$ ($c = 2{\cdot}505$ in toluene); the rotation was determined in toluene (compare Todd, Harris, and Noller, *loc. cit,*) owing to the sparing solubility of the compound in chloroform (Found : C, 87·5; H, 12·3. Calc. for $C_{29}H_{48}$: C, 87·8; H, 12·2%).

Oleanone.—3·5 G. of oleanolic acid, prepared from cloves by the method of Winterstein and Stein (*Z. physiol. Chem.*, 1931, **202**, 222), were ground with 15 g. of copper bronze and heated in a sausage flask in a mixed nitrate bath at 330—340° for 20 mins. The flask was then evacuated with a mercury diffusion pump; a clear yellow oil distilled into the side arm and solidified to a gum. Crystallisation set in on treatment with acetone; after one crystallisation from this solvent, about 1 g. of oleanone was obtained in felted needles, m. p. 167—169° after repeated crystallisation. The compound is best recrystallised from ethyl alcohol (Found : C, 84·9; H, 11·4. Calc. : C, 84·8; H, 11·3%).

Oleanene II (cis-$\Delta^{12:13(?)}$-*Oleanene*).—Oleanone was reduced with sodium ethoxide and hydrazine as described on p. 1472. The hydrocarbon was isolated in almost quantitative yield and after one crystallisation from acetic acid and one from acetone it formed fern-like aggregates of needles, m. p. 188—189°, not changed by further crystallisation. The rotation was somewhat higher than that recorded by Winterstein and Stein (*loc. cit.*) for a specimen prepared by the reduction of oleanylene : $[\alpha]_D + 69{\cdot}0°$ ($c = 2{\cdot}475$ in chloroform).

Conversion into oleanene III. This was carried out as described for the *trans*-compound, but the reaction mixture was boiled for 6 hours. The product after one crystallisation from ethyl acetate had m. p. 217—218° and after a further one from acetone, 219—220°, not depressed by admixture of oleanene III prepared from deoxyquillaic acid.

Oleanene III ($\Delta^{17:18(?)}$-*Oleanene*).—Oleanone was reduced with amalgamated zinc exactly as described above; no solid separated. The hydrocarbon was isolated as before; it required three crystallisations from ethyl acetate and one from acetone to bring the m. p. up to the constant value of 219—220° and the yield was consequently poor.

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