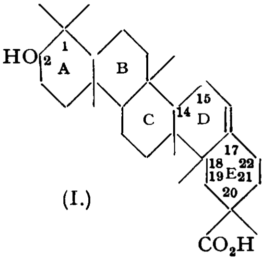
161. *Sapogenins. Part XVII. The Position of the Carboxyl Group in Oleanolic and Glycyrrhetic Acids.*

By George A. R. Kon and Walter C. J. Ross.

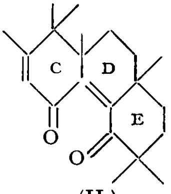
Methyl acetyloleanolate and methyl acetyldeoxoglycyrrhetate on treatment with selenium dioxide give isomeric acetyl dehydrodiketo-esters, both of which undergo alkaline hydrolysis with the loss of the carbomethoxy-group. Both are therefore *β*-ketonic esters; this shows that the carboxyl group in the parent acids must occupy the same position in the molecule and cannot be attached to a ring junction, as already deduced from measurements of surface films (Bilham, Kon, and Ross, this vol., p. 535). The experiments thus afford an independent chemical confirmation of the earlier results and incidentally also of the formulæ recently put forward (*loc. cit.*, p. 532).

The appearance of a paper by Ruzicka and Jeger (*Helv. Chim. Acta*, 1942, **25**, 775) makes it desirable to place on record certain experiments, which, though still incomplete, afford a purely chemical confirmation of the formulæ recently put forward for deoxoglycyrrhetic and oleanolic acids, both of which are represented by formula (I) and differ by the configuration of the groups attached to C_{20} (Bilham, Kon, and Ross, this vol., p. 535).

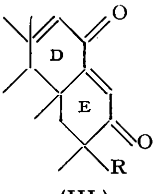


(I.)

The compound known as Jacobs's keto-diol has recently been shown to be a hydroxy-dione (Ruzicka and Jeger, *Helv. Chim. Acta*, 1941, **24**, 1257) and the formation of its esters from β-amyrin esters takes place in three ways: (i) by the permanganate oxidation of the thio-compounds formed from β-amyrin or its benzoate and sulphur (Jacobs and Fleck, *J. Biol. Chem.*, 1930, **88**, 137; Simpson, J., 1938, 1313), (ii) by the action of selenium dioxide on amyradienol I (Picard and Spring, J., 1941, 35), and (iii) as a by-product in the action of selenium dioxide on β-amyrin acetate, amyradienol II being formed at the same time (Ruzicka and Jeger, *loc. cit.*); the same compound is also formed by the futher action of selenium dioxide on amyradienol II (Ruzicka, Jeger, and Norymberski, *ibid.*, 1942, **25**, 457).* Ruzicka and Jeger conclude from the reaction of the compound with hydrazine that it is a 1 : 4-diketone and formulate it as (II) on the basis of the Haworth triterpene formula; on the assumption of the new formula (I) it will be represented by the partial structure (III, R = Me).



(II.)



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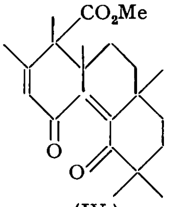
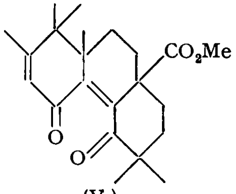


(IV.)



(V.)

Compounds analogous to Jacobs's hydroxy-dione can also be derived from methyl oleanolate and from methyl deoxoglycyrrhetate; both will be represented by the same formula (III, R = CO_2Me) if (I) correctly represents the parent acids, and will be β-ketonic esters. The older formulations would, on the other hand, lead to the structures (IV) and (V) respectively.

An ester with properties similar to those of Jacobs's hydroxy-dione acetate is formed in good yield on treatment of methyl acetyldeoxoglycyrrhetate with selenium dioxide in acetic acid solution; it becomes the sole product of the reaction if the amount of selenium dioxide is increased, otherwise it is accompanied by methyl acetyldehydrodeoxoglycyrrhetate (compare Bilham, Kon, and Ross, *loc. cit.*); it is also produced from the latter compound by the action of selenium dioxide. The diketo-ester is readily hydrolysed by alkali to the *acid* $C_{30}H_{42}O_5$, which is accompanied by a neutral *diketone* $C_{29}H_{42}O_3$, formed by the spontaneous decarboxylation of the acid; the same diketone is also produced by the decarboxylation of the acid on heating. These results clearly indicate that the parent compound must be a β-ketonic ester; an account of them was ready for

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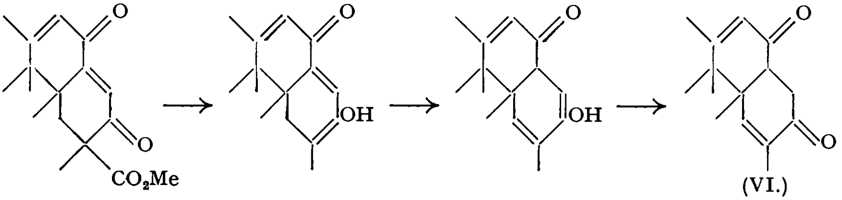
* The authors are greatly indebted to Professor Ruzicka for reprints of this and the preceding paper, which have only recently become available in this country.

publication when it was anticipated by the appearance of Ruzicka and Jeger's last paper, in which the diketo-ester and its hydrolysis products are described, the experimental data being in excellent agreement with ours.

Now an *ester* exactly similar to the above is also formed from methyl acetyloleanolate. A compound of the expected composition has already been briefly mentioned by Ruzicka, Grob, and Sluys-Veer (*Helv. Chim. Acta*, 1939, **22**, 788), who obtained it as a by-product in the preparation of the dehydro-ester, but did not investigate it. It has also been prepared by us in this way and by the action of selenium dioxide on the dehydro-ester; the pure compound melts somewhat higher than found by the Swiss authors, namely, at 247—248° (uncorr.), and is lævorotatory, $[\alpha]_D$ −144°. Their figure for $[\alpha]_D$, 146°, must, we think, be due to a misprint, as is that for the dehydro-ester, which also is lævorotatory. The absorption spectrum of this ester is entirely similar to that of its isomeride (maxima at 2790 A. and 2250 A., log $\varepsilon_{max.}$ 4·11 and 3·84); on alkaline hydrolysis it yields an acid $C_{30}H_{42}O_5$ and a neutral compound $C_{29}H_{42}O_3$. These two compounds are identical with the hydrolysis products obtained by Jacobs and Fleck (*J. Biol. Chem.*, 1932, **96**, 341) from the benzoate of an ester obtained essentially by route (i) above. The presence of a 1 : 4-diketone grouping in these compounds follows from the conversion of the C_{30}-acid into a *pyridazine* derivative on heating with hydrazine hydrate. This compound is evidently formed with the elimination of the carboxyl group and has the formula $C_{29}H_{42}ON_2$; this, and the production of the neutral diketone on hydrolysis of the ester, shows that the latter must be a β-ketonic ester, a conclusion already tentatively expressed by Jacobs and Fleck. It follows that the carboxyl group of oleanolic and of glycyrrhetic acid must occupy the same position in the molecule, in agreement with formula (III), but not (IV) or (V).

There is an interesting difference between the hydroxy-diones derived from the two epimeric esters: the compound obtained from acetyldeoxoglycyrrhetic ester is in every way similar to the diketo-ester from which it is derived, has a similar absorption spectrum and can be described as the lower homologue of Jacobs's hydroxy-dione. The isomeride derived from acetyloleanolic ester is much less soluble and, unlike the lævorotatory parent compound, it is strongly dextrorotatory. Also, its absorption spectrum is quite different: the maximum lies at 2390 A., not *ca.* 2790 A., as in all the other compounds of this type, and the intensity is appreciably higher (log $\varepsilon_{max.} = 4{\cdot}27$). A secondary maximum at 3100 A. (log $\varepsilon_{max.} = 2{\cdot}30$) shows that the selective absorption is attributable to an αβ-unsaturated ketone grouping or groupings (compare, *e.g.*, the absorption of cholestenone; Jones, Wilkinson, and Kerlogue, this vol., p. 391). It is clear that the chromophore originally present in both the β-ketonic esters and in Jacobs's hydroxy-dione must have undergone a rearrangement. This only takes place after removal of the blocking carbomethoxy-group and it is reasonable to suppose that it involves the carbon atom bearing this group; it may be pictured as a process of enolisation, followed by rearrangement, thus:

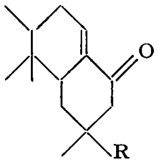


(VI.)

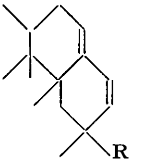
The absorption spectrum of the compound is such as would be expected from the structure (VI); the occurrence of the rearrangement suggests that, of the two epimerides, oleanolic acid has the less stable stereochemical arrangement about C_{20}. It might have been supposed that, as the isomerisation of the hydroxy-dione appears to involve this asymmetric centre, the *same* compound would be produced from either of the epimeric diketo-esters, but this has not yet been experimentally realised.

With the proof of the position of the carboxyl group now given, the arguments advanced in Part XIII (this vol., p. 532) in support of the new position of the double bond in β-amyrin and the related acids gain additional weight, since they are to a great extent contingent on the position of the carboxyl group in the latter compounds.

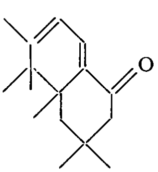
In Part XIV (this vol., p. 535) it was suggested that the carbonyl group of glycyrrhetic acid occupies a position on C_{22} because the acid is reduced by sodium and alcohol to the lævorotatory dehydro-acid (VII ⟶ VIII, R = CO_2H), exactly like keto-oleanolic acid, in which the position of the carbonyl group is established by the surface-film method and also by its relationship with siaresinolic acid. It was hoped to obtain additional evidence on this point from the most recent experiments of Ruzicka and Jeger (*loc. cit.*), who succeeded in introducing an additional double bond into methyl acetylglycyrrhetate by the method previously used with keto-β-amyrin (Picard and Spring, *loc. cit.*).



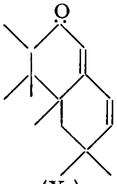
(VII.)



(VIII.)



(IX.)



(X.)

Our own attempts to achieve this had failed because a bromine-containing compound was obtained (Bilham, Kon, and Ross, *loc. cit.*); this is exclusively formed under mild conditions and is now found to be a saturated *dibromide*, resulting from the addition of bromine to the double bond. This reaction, which may be of some interest from the point of view of triterpene chemistry, is analogous to the formation of the dibromide from 6-keto-3-acetoxy-Δ^4-cholestene (Jackson and Jones, J., **1938**, 1406); the compound itself is remarkable owing to its unusually high rotation ($[\alpha]_D$ +521°). Under vigorous conditions the dehydro-ester described by Ruzicka and Jeger is also obtained, and it also results from the debromination of the dibromide with pyridine.

The introduction of an additional double bond into the structure (VII) will give rise to a compound (IX) with two double bonds in one ring, as in amyradienol I. This, and other similar compounds, is characterised by a high positive rotation. The new dehydro-compound has $[\alpha]_D$ +321° (as against 282°, the figure given by Ruzicka and Jeger) and amyradienonyl acetate has $[\alpha]_D$ +336° (Ruzicka, Jeger, and Norymberski, *loc. cit.*), in agreement with this formulation. However, the absorption spectra of these compounds are in better agreement with the alternative formula (X), as is the formation of amyradienonol from amyradienol II on oxidation with lead tetra-acetate; the formation of amyrenonyl acetate from amyradienyl II acetate (Picard and Spring, *loc. cit.*) also becomes intelligible if the carbonyl group is placed on C_{15}.

A simple way of settling these discrepancies would be the elimination of the carbonyl group in the dehydro-ester, which should give rise to the known acetyldehydrodeoxoglycyrrhetic ester (VIII, $R = CO_2Me$) if it has the structure (X), or to a new ester analogous to amyradienol I. Reduction of the ester with hydrazine and sodium ethoxide gave uncrystallisable products. Catalytic reduction gave a crystalline product, but this proved to be a mixture; it had no selective absorption of light, showing that the unsaturated system had undergone reduction. Finally, reduction by the Clemmensen method proceeded easily, but the elimination of the carbonyl group was accompanied by the reduction of one of the double bonds, a phenomenon recently observed in other cases (Parts XV and XVI, this vol., pp. 540, 544). These experiments are therefore inconclusive and further work is in progress.

EXPERIMENTAL.

Unless otherwise stated, analysis specimens were dried for 2 hours at 100°/1—2 mm.; m. p.'s were determined in sealed capillaries and are uncorrected; rotations were determined in chloroform solution, and absorption spectra in alcoholic solution.

Diketo-dehydro-ester from Oleanolic Acid (III).—300 Mg. of methyl acetyldehydro-oleanolate (compare Bilham, Kon, and Ross, *loc. cit.*) were boiled overnight with 150 mg. of selenium dioxide and 20 c.c. of AnalaR acetic acid. The solution was diluted with *ca.* 4 vols. of benzene, extracted with alkali, washed, dried, and percolated through an 8 cm. column of activated alumina, the latter being washed with benzene until no more solid was recovered on evaporation; this gave some 40 mg. of unchanged dehydro-ester contaminated with selenium.

Elution of the column with ether gave the *diketo*-ester as a yellow oil which rapidly solidified and was practically free from selenium; it crystallised from methyl alcohol or from petroleum–benzene in plates (Found: C, 73·8; H, 8·8. $C_{33}H_{46}O_6$ requires C, 73·6; H, 8·6%); the properties of the compound are given on p. 742.

Several grams of residues from the preparation of the dehydro-ester were similarly purified and subjected to separation by the chromatographic method, a long column being used. After elution of the diketo-ester an even more strongly adsorbed fraction was obtained in small amount; this was repeatedly recrystallised from methyl alcohol, forming needles, m. p. 198—199°, consisting of methyl oleanolate, evidently formed by the hydrolysis of its acetate (Found: C, 78·9; H, 10·7. Calc.: C, 79·1; H, 10·7%). The diketo-ester was purified as before (Found: C, 73·5; H, 8·5%); it gave a very faint yellow colour with tetranitromethane.

Hydrolysis. 500 Mg. of the ester were boiled for 4 hours with 1·5 g. of potassium hydroxide in 1·5 c.c. of water and 20 c.c. of alcohol; a deep yellow colour gradually developed. The hot solution was gradually diluted, and the crystalline precipitate recrystallised from slightly diluted alcohol, forming fine needles (250 mg.), m. p. 286—289°, $[\alpha]_D$ +204° (c = 1·67 in pyridine; compare Jacobs and Fleck, *loc. cit.*); it was rather sparingly soluble in acetone, the solvent recommended by Jacobs and Fleck (Found: C, 79·2, 79·1; H, 10·2, 9·9. Calc.: C, 79·4; H, 9·7%).

The alkaline mother-liquors from which the above hydroxy-diketone had been removed were acidified; the hydroxy-acid then deposited decomposed very easily even on drying at 80°. A specimen crystallised from aqueous methyl alcohol had m. p. 262—264° (lit., 268—269°) and an absorption maximum at *ca.* 2800 A. (Found for a specimen dried at room temperature in a high vacuum: C, 74·0; H, 9·2. Calc.: C, 74·7; H, 8·8%); when melted, the acid was converted into the hydroxy-diketone, which was identified by its m. p., mixed m. p., and absorption spectrum.

Pyridazine derivative. 60 Mg. of the above acid were heated overnight in a sealed tube at 200° with 1 c.c. of 98% hydrazine hydrate and 5 c.c. of alcohol. The *pyridazine* derivative, recovered by extraction with ether and evaporation of the washed and dried extract, crystallised from ethyl acetate–light petroleum in felted needles, m. p. 263—265°; the crude compound rapidly assumed a reddish tinge on exposure to light (Found: C, 80·0; H, 10·2. $C_{29}H_{42}ON_2$ requires C, 80·1; H, 9·7%).

Oxidation of the hydroxy-ketone. 550 Mg. of the hydroxy-diketone in 20 c.c. of AnalaR acetic acid were treated with 300 mg. of chromic acid in 1 c.c. of water and 5 c.c. of acetic acid. After ½ hour the solution was warmed to 60° and gradually diluted, the crystalline precipitate being collected and washed with water. The *triketone* crystallised from dilute alcohol in small needles (220 mg.) melting rather indefinitely above 300° (decomp.) (Found: C, 79·3; H, 9·3. $C_{29}H_{40}O_3$ requires C, 79·8; H, 9·3%). The triketone was reduced by Clemmensen's method, but a homogeneous product could not be isolated.

Diketo-dehydro-ester from Deoxoglycyrrhetic Acid.—This preparation was carried out as described above and the product was purified in the same way. It was obtained free from the dehydro-ester and in 75% yield when 2 g. of acetyldeoxoglycyrrhetic ester were boiled overnight with 3 g. of freshly sublimed selenium dioxide in 100 c.c. of AnalaR acetic acid. The *diketo*-ester crystallised from light petroleum (b. p. 60—80°), containing a little benzene, in plates, m. p. 236—237° (Found: C, 73·8; H, 9·2. $C_{33}H_{46}O_6$ requires C, 73·6; H, 8·6%).

Hydrolysis. This was carried out as described above, but owing to its greater solubility the neutral compound did not separate from the solution. This was therefore evaporated under reduced pressure, and the residue extracted with water; acidification of the aqueous solution gave the *acid*. The sandy insoluble material was recrystallised from dilute acetone (yield, 15%), forming plates, m. p. 248—249°, $[\alpha]_D$ −39° (c = 0·46). Light absorption: maxima at 2785 and 2250 A. (log $\epsilon_{max.}$ 4·11 and 3·74) (Found: C, 79·4; H, 9·7. $C_{29}H_{42}O_3$ requires C, 79·4; H, 9·8%). The same *hydroxy-diketone* was also obtained when the acid mentioned above was melted, but the purification of the product proved very wasteful.

The acid obtained above (yield, 60%) could be crystallised from dilute acetone; it melted partially at 184—185° and completely at *ca.* 245°, the latter being the m. p. of the hydroxy-diketone (Found : C, 74·4; H, 9·1. $C_{30}H_{42}O_5$ requires C, 74·7; H, 8·8%).

Bromination of Methyl Acetylglycyrrhetate.—This was carried out as described in Part XIV (*loc. cit.*), a 5% solution of bromine being used. At *ca.* 85°, or essentially under the conditions used by Ruzicka and Jeger, the only product formed was the bromo-ester described, but not analysed, in our previous paper. The product was conveniently precipitated by gradual dilution of the hot solution, separating in a crystalline condition. After repeated crystallisation from methyl alcohol the *dibromo*-ester decomposed at 215—220°, $[\alpha]_D$ +521° (*c* = 0·925); it gave no colour with tetranitromethane and had no selective absorption of light (Found : C, 58·5; H, 7·2. $C_{33}H_{48}O_5Br_2$ requires C, 57·9; H, 7·1%). When the bromination was conducted at the b. p. and as rapidly as possible, the product was usually a mixture, from which the less soluble dibromo-ester separated first, the dehydro-ester being obtained from the later crops. On one occasion it was the only product formed. It was also obtained by boiling the dibromo-ester with 20 parts of dry pyridine for ½ hour and precipitation with dilute acid; the coloured product was recrystallised from dilute methyl alcohol (charcoal) and formed small needles, m. p. 241—242°, $[\alpha]_D$ +321° (*c* = 1·425); the absorption spectrum showed a maximum at 2795 A., log $\epsilon_{max.}$ 4·10 (Found : C, 75·2; H, 9·5. Calc. : C, 75·5; H, 9·2%).

Reduction of the Dehydro-ester.—(i) 500 Mg. of the ester in 16 c.c. of hot acetic acid were added to 6 g. of amalgamated zinc. On the addition of 4 c.c. of hydrochloric acid a vigorous reaction occurred; after a few minutes' boiling most of the *ester* had separated from the solution. After ½ hour it was isolated by dilution and dissolved in benzene, the solution washed with alkali, dried, and percolated through a column of activated alumina, which was then well washed with benzene. The ester recovered by evaporation of the benzene solution was recrystallised from methyl alcohol, forming small plates, m. p. 258—259°; it gave a yellow colour with tetranitromethane and was optically transparent, $[\alpha]_D$ +127° (*c* = 1·13) (Found : C, 77·1; H, 10·4. $C_{33}H_{52}O_4$ requires C, 77·3; H, 10·2%).

(ii) The dehydro-ester was shaken with 100 parts of AnalaR acetic acid and ⅕ of it weight of Adams's catalyst, which had been previously reduced, for 18 hours. The ester, isolated in the usual way, was a crystalline solid, but it was evidently a mixture of reduction products; it was therefore dissolved in benzene, and the solution percolated through a column of activated alumina. Practically the whole of the product was found in the portion which had not been adsorbed, although a little material was recovered when the column was eluted with ether. The main portion was crystallised alternately from slightly diluted methyl alcohol and from petroleum; after numerous crystallisations nacreous plates still melting rather indefinitely at 222—224° with previous sintering were obtained, having practically no optical rotation and no selective absorption of light (Found : C, 75·9, 75·7; H, 10·0, 9·9%) and giving a yellow colour with tetranitromethane.

(iii) 500 Mg. of the dehydro-ester were heated overnight to 200° with 1·3 c.c. of 98% hydrazine hydrate and 10 c.c. of 5% sodium ethoxide solution. The acid recovered on acidification and extraction was a pale yellow gum; it was esterified with diazomethane, and the ester acetylated by warming with 10 parts of acetic anhydride and 10 parts of dry pyridine for a short time. After several hours the acetylated product was recovered by dilution and purified by percolation of a benzene solution through activated alumina. The ester was recovered as a colourless gum which did not solidify and gave a brownish-yellow colour with tetranitromethane.

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