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THE STRUCTURE OF SAIKOGENINS A, B, C AND D, TRITERPENOID ALCOHOLS OF BUPLEURUM FALCATUM L.*

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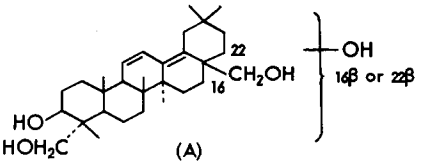
Most recently, Shibata et al. (1) have reported isolation of saikogenin A, a major sapogenin of the root of Bupleurum falcatum L., and proposed the partial structure (A) for it.



(A)

The report prompts us to describe our result on triterpenoid sapogenins of the same source. Acid hydrolysis of crude saponins afforded, in addition to saikogenin A, three new triterpenoid alcohols, saikogenin B, C and D, and longispinogenin (XIIIa) (2). The structure of four saikogenins is shown to be Ia, IIa, IIIa and IVa, respectively, in the present communication.

Crystallization of crude sapogenins from ether gave saikogenin A, $C_{30}H_{48}O_4$, m.p. 287-293°, $[\alpha]_D$ -43° (EtOH), λ_{max} 242, 251 and 260 mμ (ε 26,800, 30,400

* Satisfactory analyses were obtained for all compounds described. Unless otherwise stated, rotations were measured in chloroform and U. V. spectra were taken in ethanol solutions.

and 19,400) which was identified with a specimen isolated by Shibata et al. (1). Chromatography of the mother liquor over alumina afforded, in the order of elution, a sterol mixture, a mixture of saikogenin B and longispinogenin, saikogenins C, A and D. Fractionation of saikogenin B and longispinogenin was accomplished by preparative thin layer chromatography of acetates. Longispinogenin triacetate (XIIIb), m.p. 220-222°, was identified with an authentic sample supplied by Prof. Djerassi (2). The new sapogenins show the following constants:

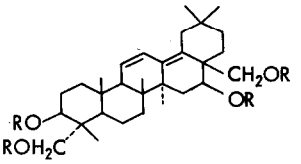
Saikogenin B, $C_{30}H_{48}O_3$, m.p. 267-269°, $[\alpha]_D$ +285°, λ_{max} 282 mμ (ϵ 9,000);

Saikogenin C, $C_{30}H_{48}O_3$, m.p. 291-294°, $[\alpha]_D$ -46°, λ_{max} 242, 251 and 260 mμ (ϵ 26,500, 31,000 and 19,400);

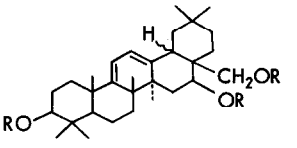
Saikogenin D, $C_{30}H_{48}O_4$, m.p. 261-266°, $[\alpha]_D$ -48° (EtOH), λ_{max} 242, 252 and 261 mμ (ϵ 25,300, 28,800 and 19,200).

These four saikogenins, in the I. R. spectra, show hydroxyl bands but no carbonyl absorption.

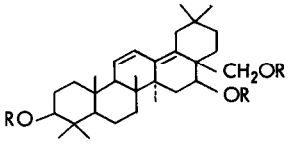
Discussion is begun with saikogenins A and D. Saikogenin A (Ia) is readily acetylated in mild conditions whereas saikogenin D (IVa) requires a more forcing condition for complete acetylation. At any rate, both afforded the respective amorphous acetates exhibiting no hydroxyl absorption in the I. R. spectrum. Their NMR spectra indicated that each of the acetates has the acetoxy groups of two primary and two secondary alcohols (TABLE I). In both of the tetracetates, Ib and IVb, one of the two CH_2OAc groups could be predicted as equatorial due to higher τ-value of the methylene signal adjacent to the acetoxy group and the other as axial from the signal of lower τ-value (3). The two secondary hydroxyl groups of saikogenin A were suggested to be equatorial by easy acetylation of them and by



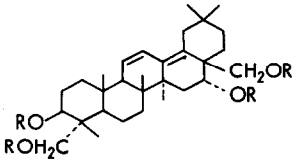
Ia R=H
b R=Ac



IIa R=H
b R=Ac



IIIa R=H
b R=Ac



IVa R=H
b R=Ac

TABLE I

NMR Signals of Acetates of Saikogenin A, B, C and D
[in $CDCl_3$, 60 Mc., τ]

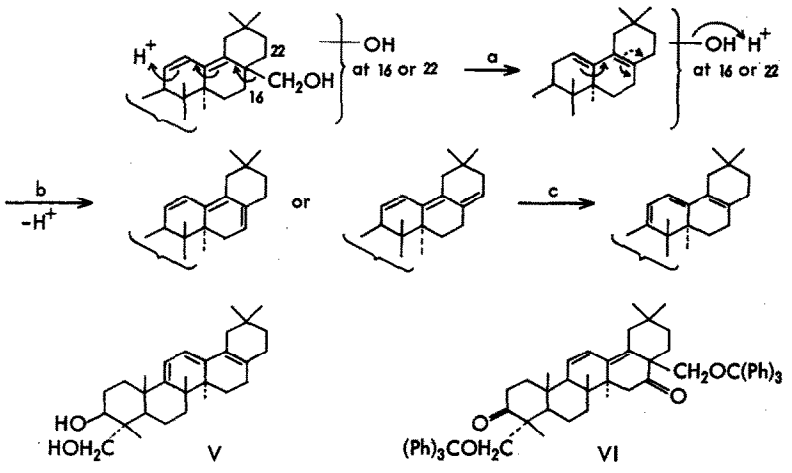
	$-O-\overset{O}{\overset{\|}{C}}-\underline{CH_3}$	$-\overset{\|}{\underset{\|}{C}}-\underline{CH_2}OAc$	$-\overset{OAc}{\overset{\|}{C}}-\underline{H}$	Olefinic <u>H</u>
A	7.98 (6H, s) 7.95 (3H, s) 7.93 (3H, s)	6.20 (2H, s) 5.68 (2H, q)	5.33 { (2H, m) 4.72	4.38 (1H, d) 3.56 (1H, q)
B	7.97 (6H, s) 7.93 (3H, s)	5.88 (2H, q)	5.50 (1H, m) 4.60 (1H, m)	4.42 (2H, s)
C	7.95 (9H, s)	5.68 (2H, q)	5.50 (1H, m) 4.90 (1H, m)	4.34 (1H, d) 3.56 (1H, q)
D	8.00 (3H, s) 7.97 (3H, s) 7.95 (6H, s)	6.21 (2H, s) 5.90 (2H, q)	5.20 (1H, m) 4.82 (1H, t)	4.40 (1H, d) 3.56 (1H, q)

a broad multiplet signal appeared for the two protons on acetoxy-bearing carbon atoms in the tetracetate (4). On the other hand, one of the two secondary alcohol groups of saikogenin D could be settled as axial from the facts that the one hydroxyl group resists acetylation under a mild condition and that one of the protons on two carbon atoms bearing secondary acetoxyl groups of the tetracetate appears as a rather sharp triplet at 4.82 τ (J=3 cps) (4). Saikogenins A and D, on treatment with acetone and sulphuric acid in ether, afforded the respective diacetonides, m.p. 213-214°, $[\alpha]_D$ -35° and m.p. 266-278°, $[\alpha]_D$ +224°. These results are suggestive of the relative locations of their hydroxyl groups.

Both the sapogenins, in the U. V. spectrum, show characteristic absorption of a heteroannular diene and the NMR spectra of these tetracetates exhibit the signals of two olefinic protons and of six tertiary methyl groups. Saikogenins A and D are inferred to be oleana-11,13(18)-dienetetrols.

Treatment of saikogenin A or D with concentrated hydrochloric acid in boiling methanol in a N_2 atmosphere yielded an identical conjugated triene, $C_{29}H_{44}O_2$, m.p. 246-249° (dec.), $[\alpha]_D$ +439°, λ_{max} 319 mμ (ϵ 19,100). The product readily formed an acetonide, m.p. 223-230°, and an amorphous diacetate, whose NMR spectrum shows the signals of a singlet at 4.28 τ for two olefinic protons, an AB quartet centered at 6.17 τ due to the CH_2 of a CH_2OAc group, and of a broad multiplet centered at 5.13 τ due to a proton on a carbon atom bearing secondary acetoxy group. Although the two surviving hydroxyl groups can be predicted to be located at 3β and 23 from the NMR data of the diacetate, the existence of the hydroxyl groups at 3β and 23 in saikogenin A has already been confirmed by Shibata et al. (1). The structure of the triene is thus represented as 28-noroleana-9(11),12,17-triene-3β,23-diol (V) arising from (a) elimination of formaldehyde from the allylic hydrox-

methyl group followed by (b) dehydration of a resulted allylic hydroxy group and (c) double bonds migration. Consequently, out of the four hydroxyl groups existing in

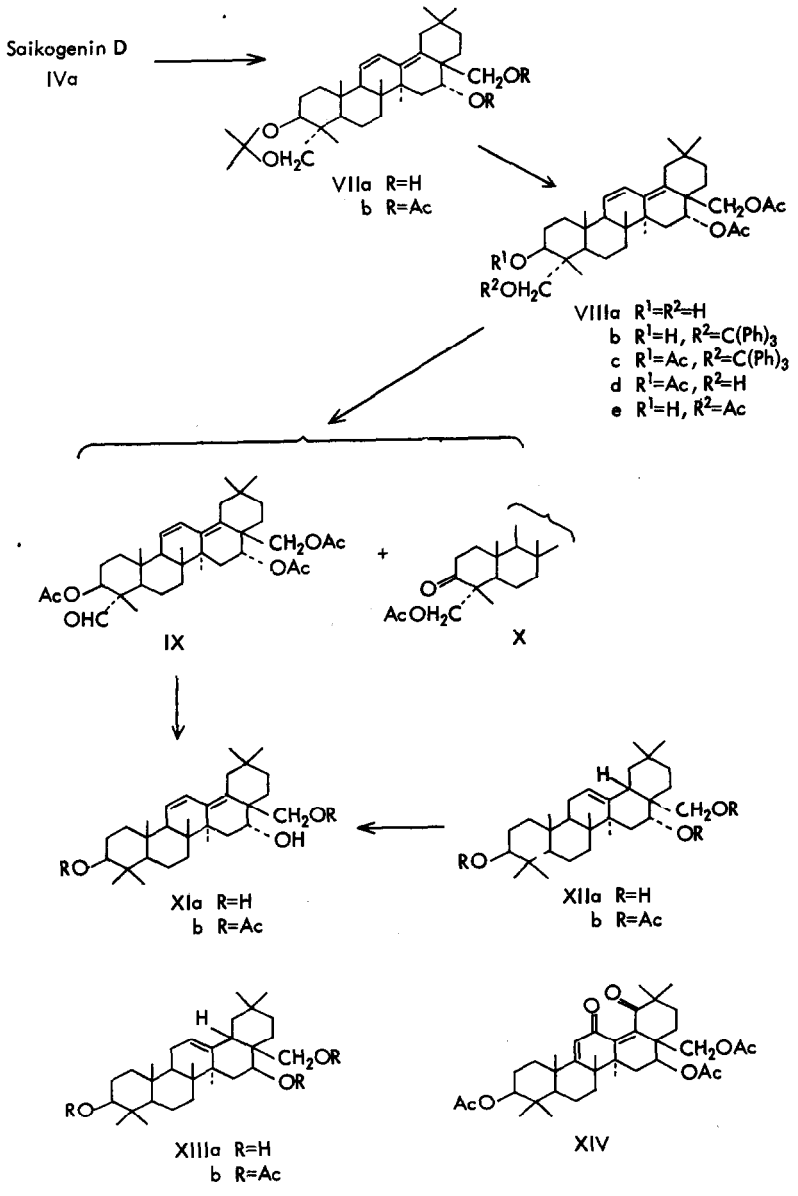


each of saikogenins A and D, three have been proved to occupy the common positions, 3β, 23 and 28, and the both sapogenins differ only in the location of the fourth hydroxyl group at C_{16} or C_{22}. In view of the acetonide linkage with the C_{28}-hydroxy group, the possible location of the hydroxyl group in question is limited to 16β and 22β for equatorial hydroxy group of saikogenin A and to 16α for axial one of saikogenin D. The structure of saikogenin D might be concluded to be oleana-11,13(18)-diene-3β,16α,23,28-tetrol (IVa). Of the two possibilities for saikogenin A, 16β hydroxy group epimeric with saikogenin D seemed to be more likely from the biogenetic standpoint. Saikogenins A and D were converted to the respective ditrityl ethers being connected with the primary hydroxy groups by treatment with trityl chloride in pyridine. Oxidation of both the ditrityl ethers with Kiliani's reagent in acetone afforded an identical ditrityl ether-diketone (VI), m.p. 241-245°, $[\alpha]_D$ -58°. The epimer onship of the hydroxy group between saikogenins A and D has been

established. The chemical evidences for the constitution assigned to the two sapogenins is now provided by correlation with the known triterpene.

Treatment of saikogenin D (IVa) with acetone and a catalitic amount of p-toluenesulphonic acid yielded exclusively the monoacetonide (VIIa), m.p. 264-268°, $[\alpha]_D$ -54°, which on acetylation with heated acetic anhydride and pyridine gave the acetonide-diacetate (VIIb). Treatment in a warm aqueous acetic acid resulted in hydrolysis of the acetonide group giving the diacetate (VIIIa), which on trityl ether formation of the primary alcohol at C_{23} followed by acetylation of the 3β-hydroxyl afforded the trityl ether-triacetate (VIIIc), $C_{55}H_{68}O_7$, m.p. 145-147°, $[\alpha]_D$ -24°. Hydrolysis of the trityl ether with an aqueous acetic acid gave a mixture of oily triacetates recognized as two spots close together on thin layer chromatography. It was presumed that the one is the expected 3β,16α,28-triacetate (VIIId) and the other might be the 16α,23,28-triacetate (VIIIe) arising from acetyl migration from 3β to 23. Without purification, the mixture was oxidized with Kiliani's reagent in acetone to give a mixture of the carbonyl compounds, which was separated into the two fractions by a preparative thin layer chromatographic method. One of them, the amorphous triacetate-aldehyde (IX) exhibiting the I. R. band at 2709 cm^{-1} and the NMR signal at 0.72 τ characteristic of an aldehyde, was subjected to Huang-Minlon reduction yielding oleana-11,13(18)-diene-3β,16α,28-triol (XIa), $C_{30}H_{48}O_3$, m.p. 241-246°, $[\alpha]_D$ -45°. The product was identified, as expected, with a specimen prepared by dehydrogenation of authentic primulagenin A triacetate (XIIb) (5) with selenium dioxide followed by saponification. Mild acetylation of both the specimens afforded the identical 3,28-diacetate (XIb),** m.p. 266-271°, $[\alpha]_D$ -98°, λ_{max} 243, 251 and 261

** For this compound, Barton et al. (6) reported m.p. 165-166°, $[\alpha]_D$ -52°, λ_{max} 250 mμ (ε 22,800). We suppose the values might be attributed to contamination with the precursor, olean-12-ene-3β,16α,28-triol 3β,28-diacetate.


Saikogenin D
IVa
VIIa R=H
b R=Ac
VIIIa R¹=R²=H
b R¹=H, R²=C(Ph)₃
c R¹=Ac, R²=C(Ph)₃
d R¹=Ac, R²=H
e R¹=H, R²=Ac
IX
X
XIa R=H
b R=Ac
XIIa R=H
b R=Ac
XIIIa R=H
b R=Ac
XIV

mμ (ε 27,500, 31,600 and 19,800). The structure of saikogenin D has now been confirmed as oleana-11,13(18)-diene-3β,16α,23,28-tetrol (IVa) and accordingly saikogenin A is oleana-11,13(18)-diene-3β,16β,23,28-tetrol (Ia).

The constitution of saikogenin C was deduced to be oleana-11,13(18)-diene-3β,16β,28- or 3β,22β,28-triol from the results of examinations analogous to those mentioned above for saikogenins A and D. In an earlier stage of this investigation, out of the two possibilities, the latter had been tentatively assigned to saikogenin C because of great discrepancy between the constants, m.p. 213-215°, $[\alpha]_D$ -70°, observed for the triacetate, and m.p. 178-179°, $[\alpha]_D$ -29°, described for oleana-11,13(18)-diene-3β,16β,28-triol triacetate by Djerassi (2). Experiments carried out based on the above presumption will be described shortly in a full paper whereas the present communication deals with only the facts essential for the elucidation of structure.

Reductive elimination of the hydroxyl group at C_{23} in saikogenin A (Ia) was processed in the same sequence as carried out on saikogenin D (IVa). Saikogenin A on acetonide formation under the mild condition afforded the mono and diacetonides. The monoacetonide, m.p. 269-271°, $[\alpha]_D$ -56°, readily formed the acetonide-diacetate, which was converted to the trityl ether-triacetate, m.p. 176-181°, $[\alpha]_D$ -12°, by acetonide cleavage, trityl ether formation and acetylation. Treatment of the trityl ether-triacetate with an aqueous acetic acid yielded a mixture of the two triacetates as observed in the derivative of saikogenin D. Oxidation and separation of the products by preparative thin layer chromatography gave the triacetate-aldehyde, which on Huang-Minlon reduction afforded a triol, $C_{30}H_{48}O_3$, m.p. 291-294°. The triol was identified with saikogenin C in all respects and thus the structure of saikogenin C should be concluded to be oleana-11,13(18)-diene-3β,16β,28-triol (IIIa).

Based on this finding, the correlation of saikogenin C with the known longispinogenin (XIIIa) (2) was carried out. Oxidation of longispinogenin triacetate (XIIIb) with selenium dioxide in acetic acid afforded, after separation from the unchanged starting material, the dehydrogenated product melting at 213-215°. The m.p. was different from that recorded by Djerassi (2) but identical with that of saikogenin C triacetate (IIIb). Identity of the product with saikogenin C triacetate was established by the mixed m.p. and by the I. R. spectra.

Saikogenin B showed, in the U. V. spectrum, an absorption characteristic of a homoannular diene and was readily acetylated giving a triacetate, m.p. 209-210°, $[\alpha]_D$ +213°, which exhibits a singlet signal at 4.42 τ due to two olefinic protons in the NMR spectrum. The sapogenin, therefore, was expected to be an oleana-9(11),12-dienetriol. Oxidation of the triacetate with selenium dioxide in acetic acid gave, as a sole product, $C_{36}H_{50}O_8$, m.p. 208-209°, $[\alpha]_D$ -94°, λ_{max} 278 mμ (ε 13,600), which was characterized as a triacetoxyoleana-9(11),13(18)-diene-12,19-dione (XIV). The same compound was also obtained from oxidation of saikogenin C triacetate (IIIb) with selenium dioxide in benzyl acetate and it has been established that the three hydroxyl groups of saikogenin B are located at the same positions as those of saikogenin C. The constitution of saikogenin B has now been elucidated as oleana-9(11),12-diene-3β, 16β,28-triol (IIa) with reservation of the configuration at C_{18}.

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