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THE STRUCTURE OF SAIKOGENIN A, A SAPOGENIN OF BUPLEURUM SPECIES

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The root of Bupleurum falcatum L. (Umbelliferae) (柴胡**) has been used for many centuries as an important drug in Chinese medicine. It has only been reported the isolation of some fatty acids[1,2] and neutral phytosterols, such as α-spinasterol, Δ^7-stigmastenol, Δ^{22}-stigmastenol and stigmasterol[3,4] from the root. The presence of saponin in the root was known[5,6] but any extensive chemical studies have not been reported.

We have isolated several saponins from the roots and seeds of Bupleurum falcatum L. and some other related Bupleurum spp. The saponins separated by chromatography were named saikosides Ia, Ib and II, while saikogenin A was obtained as a major sapogenin of saikosides Ia and Ib.

Saikogenin A (I), $C_{30}H_{48}O_4$, m.p. 283-285°(decomp.), $[\alpha]_D^{25}$ -54° (pyridine), showed in the U.V. spectrum a characteristic hetero-annular diene absorption (λ_{max} mμ (log ε): 242(4.43), 250(4.48), 260(4.29)), which can be observed also in the spectra of saikosides Ia and Ib. In the I.R. spectrum, saikogenin A showed

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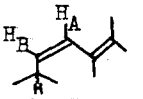
OH band (ν_{max}^{Nujol} 3358 cm^{-1}), but no absorption in the C=O region.

Saikogenin A afforded tetraacetate which cannot be obtained in a crystalline state, but it exhibited by U.V. spectrum the presence of heteroannular diene system, and by n.m.r. spectrum the existence of 2 primary and 2 secondary alcoholic acetate groupings, 6 tertiary methyls and 2 olefinic protons.

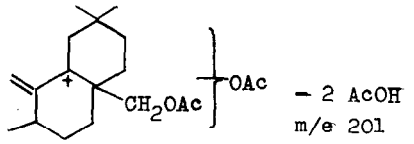
	$-\overset{|}{\underset{|}{C}}-CH_3$	CH_3COO-	$-\overset{|}{\underset{|}{C}}-CH_2OAc$	$-\overset{OAc}{\overset{|}{\underset{|}{C}}}-H$	
τ ($CDCl_3$)	9.20(9H) 9.03(6H) 8.89(3H)	8.02(6H) 7.98(3H) 7.96(3H)	6.26(2H,s) 5.75(2H,q)	5.24~4.89 (2H,m)	H_A {4.53 (1H) 4.36 (1H) H_B {3.74 (1H) 3.56 (1H)

On catalytic reduction , saikogenin A afforded a dihydro derivative (II), $C_{30}H_{50}O_4$, m.p. 288-289°(decomp.), which yielded mono- (IV) and di-acetonides(V), giving m.p. 219.5-220° and m.p. 211-214°, respectively, to reveal the relative disposition of 4 hydroxyls in saikogenin A.

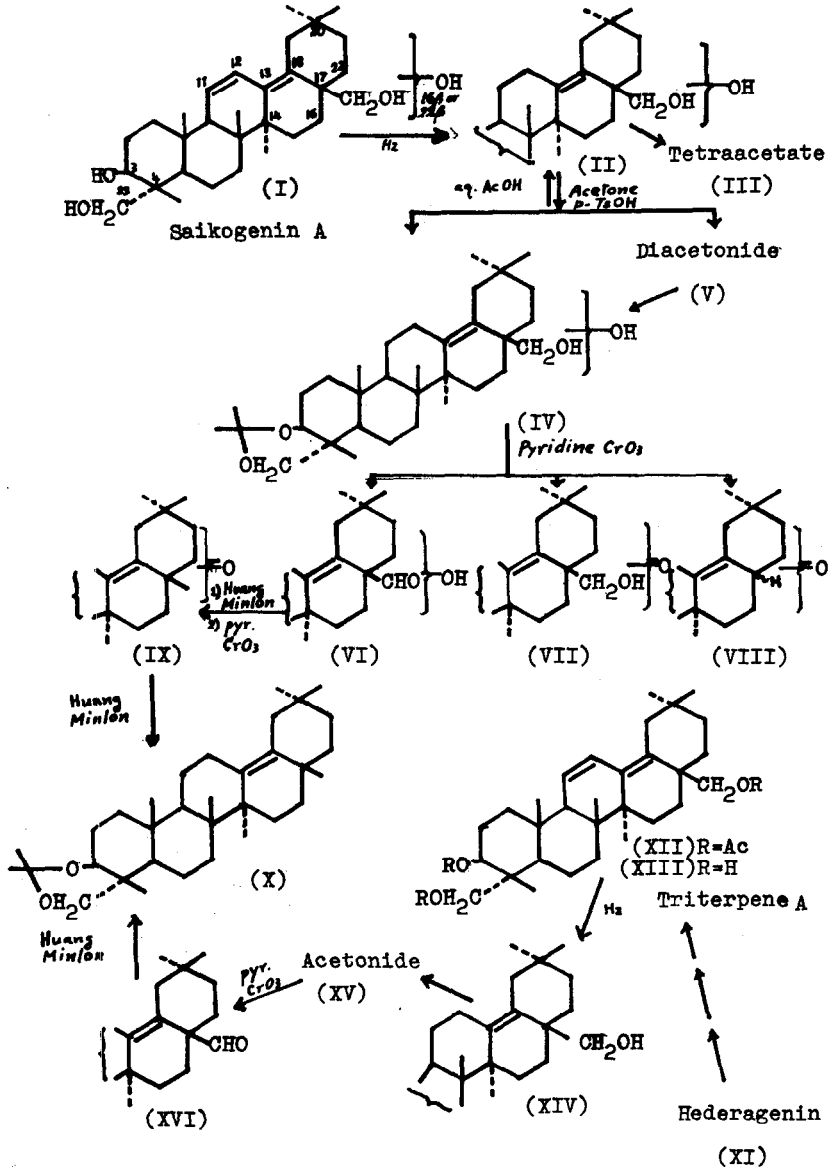
According to the findings of Gandemer, Polonsky and Wenkert[7] in the n.m.r. spectra of triterpenes, the CH_2OAc grouping of saikogenin A tetraacetate which shows higher τ-value of $-CH_2-$ signal could be assigned as equatorial, and one which gives the signal of lower τ-value as axial. In the n.m.r. spectrum of the same compound, the broad signals of 2 protons attached to carbon atoms bearing secondary alcoholic hydroxyl which appeared in rather higher field suggested that these hydrogen atoms are axial, otherwise 2 secondary hydroxyls are equatorial.[8,9]

This was supported by the similar findings in the n.m.r. spectra of dihydrosaikogenin A tetraacetate (III), triterpene A triacetate (XII)[10] and dihydrotriterpene A triacetate.

The fundamental skeleton of saikogenin A has been established to be belonged to the oleanane group on the basis of findings in the U.V. spectrum giving heteroannular diene absorption which suggested a $\Delta^{11,13(18)}$oleanadiene system[11,12], and in the mass spectrum of dihydrosaikogenin A tetraacetate, m.p. 181-181.5°, $[\alpha]_D^{29}$ -35°, which revealed the presence of $\Delta^{13(18)}$-α or β-amyrin structure by the base peak at m/e 201.[13,14]



Finally, a chemical evidence for the oleanane skeleton of saikogenin A has been provided by the following reactions: Dihydrosaikogenin A monoacetonide (IV), m.p. 219.5-220°, $[\alpha]_D^{33}$ -29°, was oxidized with pyridine-CrO_3 complex to afford an aldehydic compound (VI), m.p. 227-229°, and two ketonic compounds (VII and VIII). Monoacetonide of the aldehydic compound (VI) was reduced by the Huang-Minlon reaction and then oxidized with pyridine-CrO_3 to yield a ketonic compound (IX). The ketonic group was reduced into methylene by the Huang-Minlon reaction to give a compound, m.p. 242-243°, $[\alpha]_D^{30}$ -46°, m/e 482 (M^+), 205 (base peak), which was proved to be identical with $\Delta^{13(18)}$-oleanene-3β,23-diol monoacetonide (X). The compound X was prepared from hederagenin (XI) by several steps of unambiguous reactions. The compound XII which was prepared as an intermediate product of the above synthesis was proved to be identical with triacetate of triterpene A, which was isolated by Breton and Gonzalez[10] as a sapogenin of saponin in the leaves of Scrophularia smithii W.


(I)
Saikogenin A
Hz
(II)
Tetraacetate
(III)
aq. AcOH
Acetone
p-TsOH
Diacetonide
(V)
(IV)
Pyridine CrO3
(IX)
(VI)
(VII)
(VIII)
1) Huang Minlon
2) pyr. CrO3
Huang Minlon
(X)
(XII)R=Ac
(XIII)R=H
Triterpene A
Hz
Acetonide
(XV)
pyr. CrO3
(XVI)
(XIV)
Hederagenin
(XI)

In regard to the established structure of triterpene A, the position of a primary alcohol was verified at $C_{(23)}$(equatorial). The remaining primary hydroxyl must be located in the ring D or E on the basis of mass spectral analysis of dihydrosaikogenin A tetraacetate, and the n.m.r. signals (τ 5.75(q)) ascribed to the methylene protons of axial CH_2OAc of the compound ruled out the possibility of location of the group CH_2OAc at the position $C_{(14)}$[8]. A large molecular amplitude of the strong negative Cotton effect in the ORD curve of aldehyde (VI) (- 496) suggested an $\beta\gamma$-unsaturated carbonyl system[15], which led the possibility of location of aldehyde group at $C_{(17)}$ rather than $C_{(20)}$. The aldehyde group of VI was shown to be hydrogen bonded with the neighbouring secondary hydroxyl by the I.R. absorption of C=O at 1718 cm^{-1}(XVI showed aldehyde C=O band at 1728 cm^{-1})(in $CHCl_3$).

Consequently , all the evidence mentioned above have led us to formulate saikogenin A as (I).

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REFERENCES

1) T. Araki and Y. Miyashita: J. Taiwan Med.Soc.,No.296,1(1929).
2) Yuoh-Fong Chi and Chi-Ming Ma: J.Chinese Chem.Soc.,3 ,78 (1935) (C.A. 29 , 4515 (1935)).
3) K. Takeda, K. Hamamoto and T. Kubota: Yakugaku Zasshi(J. Pharm.Soc. Japan),73 , 272 (1953).
4) K.Takeda and T. Kubota: Chem.Pharm.Bull.,(Tokyo),6 , 536 (1958).

5) T. Ezawa: Report of Taiwan Central Res.Lab., 5 , 179(1956).
6) T.H. Tang and C.C. Peng: J. Pharm.Soc. China, 1, 17 (1957).
7) A. Gandemer, J. Polonsky and E. Wenkert: Bull.Soc. chim. France, 407 (1964).
8) M. Shamma, R.E. Glick and R.C. Mumma: J.Org. Chem.,27, 4512 (1962).
9) J.N. Shoolery and M.T.Rogers: J.Am.Chem.Soc., 80, 5121 (1958).
10) J.L. Breton, and A.G. Gonzalez: J. Chem.Soc.,1401 (1963).
11) A. Sandoval, A. Manjarrez, P.R. Leeming, G.H. Thomas and C. Djerassi: J.Am.Chem.Soc., 79 , 4468 (1957).
12) A.K. Barua, P. Chakrabarti: Tetrahedron, 21 , 381 (1965).
13) H. Budzikiewicz, C. Djerassi and D.H. Williams:"Structural Elucidation of Natural Products by Mass Spectrometry" Vol. II, pp. 128 (Holden-Day,1964).
14) H. Budzikiewicz, J.M. Wilson and C. Djerassi: J.Am.Chem. Soc., 85 , 3688 (1963).
15) A. Moscowitz, K. Mislow, M.A.W. Glass and C.Djerassi: J. Am.Chem.Soc., 84 , 1945 (1962).