(5 ml.) N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-proline *p*-nitrophenyl ester (98 mg.) was added, followed by Et_3N to keep the solution slightly alkaline and after 1 day, added more the N-protected dipeptide *p*-nitrophenyl ester (30 mg.). After 2 days at room temperature, the reaction mixture was diluted with 1*N* NH_4OH (2 ml.), stirred for 1 hr., and then diluted with EtOAc containing MeOH. The EtOAc solution was washed successively with 1*N* NH_4OH, H_2O, 1*N* HCl, and H_2O. The EtOAc containing MeOH solution was dried over $MgSO_4$ and concentrated to small volume. Petroleum ether was added to the residue, the precipitate thereby formed was reprecipitated from hot MeOH and ether; yield 129 mg. (65%), m.p. 135~138°, $[\alpha]_D^{22}$ −64.2° (c=0.7, AcOH), *Anal.* Calcd. for $C_{66}H_{84}O_{18}N_{18}\cdot 3\,H_2O$: C, 53.87; H, 6.17; N, 17.13. Found : C, 54.00; H, 6.40; N, 17.15. Deblocked peptide ester : Rf^1 0.77, Rf^2 0.92, single ninhydrin positive spot.

L-Arginyl-L-prolyl-L-prolyl-β-alanyl-L-phenylalanyl-β-alanyl-L-prolyl-L-phenylalanyl-L-arginine Triacetate (XV)——The fully protected nonapeptide (XIV) (94 mg.) was hydrogenated in 10:5 mixture of AcOH and H_2O (15 ml.) in the presence of 10% Pd-C for 40 hr. The catalyst was removed by filtration with the aid of Cellite. The solution was evaporated to dryness in vacuum and the residue was dried over KOH pellets in vacuum. The solution of the hydrogenated product in H_2O (10 ml.) was added to a (2.0×6.0 cm.) CM-cellulose column which was eluted with a linear gragient elution from H_2O (300 ml.) in a mixing chamber to 0.15*M* pyrdinium acetate buffer solution (pH 5.10) (300 ml.) in a reservoir. Fractions 13 ml. each were collected at a flow rate of 3 to 4 ml./min. with an automatic fraction collector. Arginine-containing peptide was located in the eluate by Sakaguchi reaction. The eluates in tubes No. 26 to 38 containing the nonapeptide were pooled, evaporated to dryness, and lyophilized to constant weight; yield 61 mg. (72%), $[\alpha]_D^{22}$ −81.6 (c=0.6, H_2O), Rf^1 0.45, Rf^2 0.48, single ninhydrin and Sakaguchi positive spot, amino acid ratios in acid hydrolysate : Arg 2.0, Pro 3.0, β-Ala 2.0, Phe 2.1.

The authors thank Prof. T. Kameyama, and his co-workers of this College, for biological assay, and the staffs of Central Analysis Laboratory, Department of Chemistry, Faculty of Sciences, University of Tohoku, for elemental analysis.

Summary

The synthesis of 6-β-alanine, 4-β-alanine, and 4,6-di-β-alanine-bradykinin is described. The biological activity of three analogs were compared with that of bradykinin.

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UDC 615.89 : 581.19 : 547.597 : 582.893

141. Shoji Shibata, Isao Kitagawa,*1 and Haruhiro Fujimoto : The Chemical Studies on Oriental Plant Drugs. XV. On the Constituents of *Bupleurum* Spp. (2).*2 The Structure of Saikogenin A, a Sapogenin of *Bupleurum falcatum* L.*3

(*Faculty of Pharmaceutical Sciences, University of Tokyo**4)

As reported in the previous paper,[1] the root of *Bupleurum falcatum* L. (Mishima-saiko) (Umbelliferae) which is currently used as an important drug (Saiko : Chai-hu) in Chinese medicine contains a considerable amount of saponins,*5 whose thin-layer

*1 Present address : Faculty of Pharmaceutical Sciences, Osaka University (Toneyama, Toyonaka, Osaka).
*2 Part XIV (1) : S. Shibata, I. Kitagawa, R. Takahashi, H. Fujimoto : Yakugaku Zasshi, in press.
*3 Preliminary report of this paper appeared in Tetrahedron Letters, No. 42, 3783 (1965).
*4 Hongo, Tokyo (柴田承二, 北川 勲, 藤本治宏).
*5 S. Ezawa reported[2] the existence of saponin in the *Bupleurum* root.
1) S. Shibata, I. Kitagawa, R. Takahashi, H. Fujimoto : Yakugaku Zassai, in press.

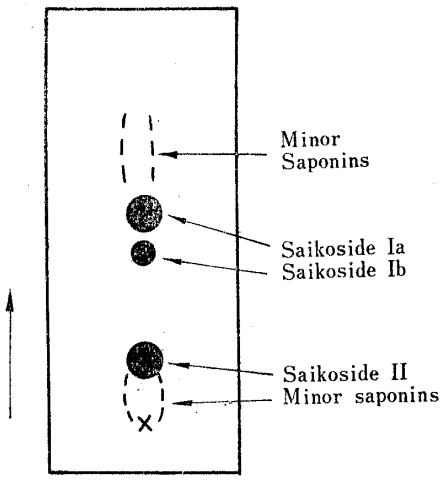


Fig. 1.
Silica Gel G Solvent: $CHCl_3$-MeOH (3:1)

chromatogram is shown in Fig. 1, and the main components have now been named saikosides Ia, Ib, and II. The present paper concerns with the chemical structure of saikogenin A (I), which was obtained by the hydrolysis of the mixture of saikosides Ia and Ib.

Saikogenin A (I), $C_{30}H_{48}O_4$, m.p. 283~285° (decomp.), seems to be a genuine sapogenin as it shows in the UV spectrum a characteristic heteroannular diene absorption[3] (λ_{max} (log ε) 242 (4.43), 250 (4.48), 260 mμ (4.29)), which is also given by the mother saponins. The IR spectrum of the sapogenin reveals that it possesses hydroxyl group (IR^{Nujol}_{max} cm^{-1}: 3358) and no carbonyl.

The genin exhibits the positive Liebermann-Burchard reaction (deep red to deep purple). Acetylation of saikogenin A with acetic anhydride in pyridine gave a tetraacetate (UV λ_{max} mμ: 240, 250, 260; IR $^{CCl_4}_{max}$: no OH, and acetyl carbonyl (1746 cm^{-1}); NMR: 4 acetyl CH_3 peaks at 7.96 (3H), 7.98 (3H), 8.02 (6H)) which, though unsuccessful in getting in a crystalline form, could easily be deacetylated with dil. alkali to regenerate the starting genin. These facts agree to assume all the oxygen-functions of saikogenin A, as being alcoholic hydroxyls.

On hydrogenation over Adams catalyst in acetic acid medium,*6 saikogenin A afforded dihydro-saikogenin A (II), $C_{30}H_{50}O_4$, m.p. 288~289° (decomp.), which showed only an end absorption in the UV spectrum, and gave a strong hydroxyl absorption band (IR $^{Nujol}_{max}$ cm^{-1}: 3323) in the IR spectrum. The dihydrosaikogenin A yielded the following crystalline derivatives: Tetraacetate (III), $C_{30}H_{46}(OCOCH_3)_4$, m.p. 181~181.5°, IR $^{Nujol}_{max}$ cm^{-1}: 1740, 1755 (shoulder), no OH, mol. wt. 642 (mass spectrum*7); monoacetonide (IV), $C_{33}H_{54}O_4$, m.p. 219.5~220°; IR $^{Nujol}_{max}$ cm^{-1}: 3336 (OH); diacetonide (V), $C_{36}H_{58}O_4$, m.p. 211~214° (no OH absorption in IR).

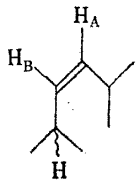
Dihydrosaikogenin A monoacetonide (IV) was led to a monoacetonide diacetate, which was failed to be obtained in a crystalline form. On treatment with hot 50% acetic acid, the latter was transformed into a crystalline diacetate (VI), $C_{30}H_{46}(OH)_2(OCOCH_3)_2$, m.p. 231~232°. The existence of two hydroxyl groups in IV was verified by forming crystalline ditosylate (VII), $C_{47}H_{66}O_8S_2$, m.p. 143~144° (no OH absorption in IR).

Nuclear magnetic resonance (NMR) study reveals the presence of two primary alcoholic groupings, two secondary alcohols, six tertiary methyls and two olefinic protons in saikogenin A. According to the NMR study of $-CH_2OH$ and $-CH_2OAc$ groups in terpenoid compounds by Gandemer, Polonsky and Wenkert,[4] one of the two primary alcoholic groupings with higher τ-value would be assigned to the equatorial bonding and the another one with lower τ-value to the axial. The broad signals of two protons attached to carbons bearing secondary alcoholic groups which appear in rather higher field would suggest that these hydrogens are axial.[5,6] The two hydroxyls, therefore, could be in equatorial and this is also supported by the ease of their acetylation and

*6 In the ethanolic solution, the hydrogenation proceeded rather slowly.
*7 The authors' thanks are due to Prof. K. Nakanishi, Tohoku Univ., for measurement of mass spectra.

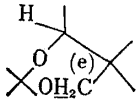
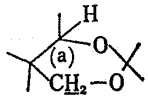
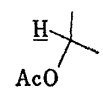
2) S. Ezawa: Report Central Res. Lab. Taiwan, **5**, 179 (1916).
3) D. H. R. Barton, C. J. W. Brooks: J. Chem. Soc., **1951**, 257.
4) A. Gandemer, J. Polonsky, E. Wenkert: Bull. soc. chim. France, 407 (1964).
5) M. Shamma, R. E. Glick, R. O. Mumma: J. Org. Chem., **27**, 4512 (1962).
6) J. N. Shoolery, M. T. Rogers: J. Am. Chem. Soc., **80**, 5121 (1958).

TABLE I. τ Value in Deuterochloroform

Compound	$-\underset{\vert}{C}-\underline{CH_3}$	$\underline{CH_3}COO-$	$-\underset{\vert}{C}-\underline{CH_2}-OAc$	$-\underset{\vert}{\overset{OAc}{\overset{\vert}{C}}}-\underline{H}$	 (a)
Saikogenin A tetraacetate	9.20(9H) 9.03(6H) 8.89(3H)	8.02(6H) 7.98(3H) 7.96(3H)	6.26(2H, br. s.)[b] 6.06, 5.89, 5.61, 5.44 (2H) AB type	5.24~4.89 (2H, m.)	H_A {4.53, 4.36 (1H) H_B {3.74, 3.56 (1H) unsharp
Dihydrosaikogenin A tetraacetate (III)[a]	9.26(3H) 9.19(3H) 9.06(12H)	7.99(3H) 7.98(6H) 7.94(3H)	6.38, 6.26, 6.20, 6.08 (2H) AB type 6.00, 5.88, 5.58, 5.45 (2H) AB type	5.31~4.97 (2H, m.)	
Triterpene A triacetate (XV)	9.26(3H) 9.22(3H) 9.16(3H) 9.04(9H)	8.00(3H) 7.98(3H) 7.96(3H)	6.25(2H, br. s.)[b] 6.15, 5.98, 5.90, 5.73 (2H) AB type	5.37~5.12 (1H, m.)	H_A {4.59, 4.39 (1H) H_B {3.75, 3.55 (1H) unsharp
Dihydrotriterpene A triacetate (XIX)[a]	9.25(3H) 9.18(3H) 9.12(3H) 9.07(9H)	7.98(3H) 7.94(3H) 7.92(3H)	6.34, 6.23, 6.16, 6.05 (2H) AB type 6.06, 5.95, 5.83, 5.72 (2H) AB type	5.27~5.07 (1H, m.)	

a) These NMR spectra were taken with Varian 100 Mc. NMR spectrometer. All the C-methyl signals given by the above compounds were confirmed to be uncoupled measuring under 60 Mc.
b) broad singlet.

TABLE II.

Compound	(e) $C\underline{H_2}OH$		(a) $C\underline{H_2}OH$		(a) $C\underline{H_2}OAc$	
IV		6.55(br. s)	6.26(br. s)			
V		6.49(br. s)		6.24(d)		
IV-diAc		6.50(br. s)			5.40, 5.58, 5.84, 6.01 AB type	5.20(m)
VI	6.44(d)				5.41, 5.60, 5.84, 6.03 AB type	5.18(m)

the deacetylation of the acetates. The quartet signals due to two olefinic protons, both in I-tetraacetate and synthetic triterpene A triacetate (XV), which disappeared in the corresponding dihydro derivatives (III and XIX), indicated AB type coupling. The lower signals showed weak couplings, which would strongly suggest the existence of the system (a) in saikogenin A as well as in XV. Furthermore, NMR analyses of dihydrosaikogenin A monoacetonide (IV), diacetonide (V), monoacetonide-diacetate, and diacetate (VI) revealed that four hydroxyls in saikogenin A are located in such a way that they could form diacetonides*[8] (Table II).

On the basis of all these findings a feasible partial structure for saikogenin A can be illustrated as follows (Chart 1).

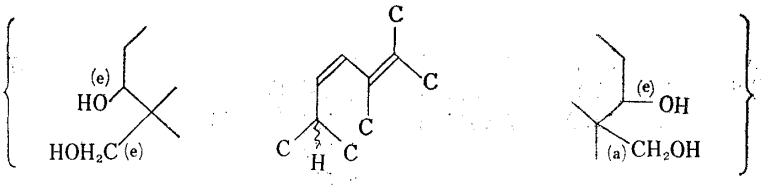


Chart 1.

The fundamental carbon skeleton of saikogenin A has been established to be belonged to the oleanane group by the following reasons. The typical heteroannular diene absorption in UV spectrum of saikogenin A would suggest a $\Delta^{11,13(18)}$-oleanadiene skeleton.[7,8] The mass spectral study of dihydrosaikogenin A tetraacetate (III) and dihydrotriterpene A triacetate (XIX) also supported the presence of $\Delta^{13(18)}$-amyrin (α- or β-) structure by their base peaks at m/e 201 and 203, respectively.

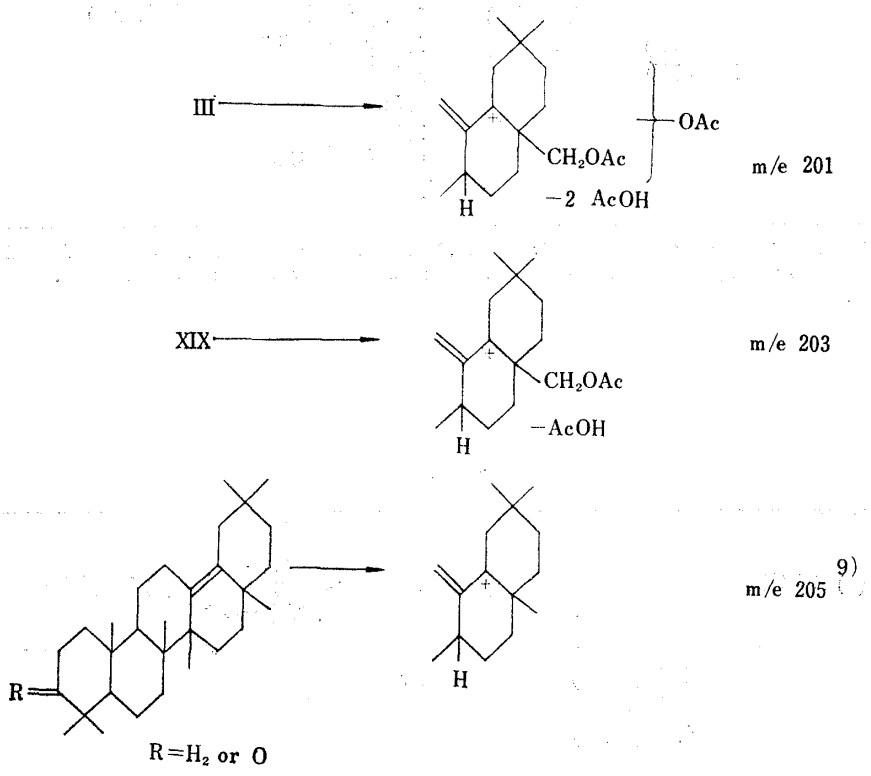


Chart 2.

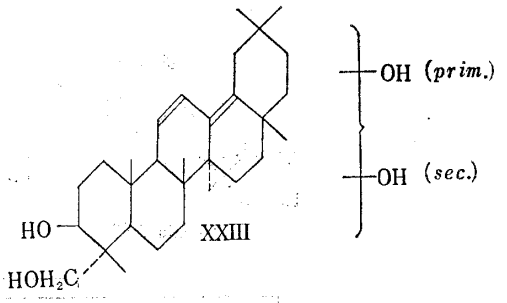
*8 The acetonide dimethyl signals appeared around 8.57~8.58 (τ).

7) A. Sandoval, A. Manjarrez, P. R. Leeming, G. H. Thomas, C. Djerassi: J. Am. Chem. Soc., **79**, 4468 (1957).
8) A. K. Barua, P. Chakrabarti: Tetrahedron, **21**, 381 (1965).

The comparison of these base peaks[9,10] provides a good evidence for the existence of an angular CH_2OAc, and >CHOAc in ring D or E of Ⅲ.

Finally, the chemical proof for the oleanane skeleton was attained by the following reactions. The pyridine-CrO_3 complex oxidation of Ⅳ under ice-cooling afforded Ⅷ, Ⅸ and a ketonic compound (Ⅹ). The monoacetonide-aldehyde (Ⅷ) was then transformed into Ⅺ by the Huang-Minlon reduction[11] followed by pyridine-CrO_3 oxidation. The Huang-Minlon reduction was adopted again to the monoketone (Ⅺ) to produce $\Delta^{13(18)}$-oleanene-3,23-diol monoacetonide (Ⅻ), which had been derived unambiguously from hederagenin (XIII) (obtained from Sapindus saponin[12]) through the reaction steps (XIII→XIV→XV→XVI→XVIII→XX→XXI→XII) as shown in Chart 5. The both samples of compound of structure Ⅻ derived from saikogenin A and hederagenin were proved to be identical in all respects (m.p., mixed m.p., IR (in CCl_4), $[\alpha]_D$), thus it has been established the gross structure of saikogenin A as being represented by XXIII.

On the other hand, the synthetic sample of compound (XV) was proved to be identical (m.p., mixed m.p., IR (KBr)) with triterpene A triacetate[13] (a sapogenin obtained from the saponin of *Scrophularia smithii* W. leaves) kindly supplied by Dr. Breton. Consequently the position of the primary alcoholic grouping was verified at C_{23}*[9] (equatorial).



The remaining primary alcoholic grouping of saikogenin A was established to be at position 17 (axial) by the evidence given below. The mass spectral data revealed that CH_2OH group is located possibly in ring D or E.

The NMR peaks ascribed to the methylene protons of axial CH_2OAc of saikogenin A tetraacetate (τ 5.75 (q)), Ⅲ (τ 5.74 (q)), dihydrosaikogenin A monoacetonide diacetate (τ 5.71 (q)), Ⅵ (τ 5.72 (q)), and of an axial CH_2OH of Ⅳ (τ 6.26 (s)) (in Table I, Ⅱ) could rule out the position 14*[10] as the site of the CH_2OH grouping.

Thus the position 17*[11] and 20 would be most possible for the location of the axial CH_2OH. The optical rotatory dispersion curves of the aldehydes Ⅷ and XXI showed

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*9 Breton and Gonzalez[13] proposed the structure (XVI) for triterpene A. The orientation of CH_2OH at the position 4 was mainly deduced from the infrared spectral analyses and the pyrolysis with copper obtaining formaldehyde.

*10 Ref. 5

	CH_2OAc at the position 14
A_1-barrigenol pentaacetate	4.92 (τ-value)
7β-hydroxy-A_1-barrigenol hexaacetate	4.82
11-keto-A_1-barrigenol pentaacetate	4.80

*11 The methylene protons of CH_2OAc at the position 17 of longispinogenin triacetate exhibited a singlet at 6.00.[5]

*12 Gummosogenin[15] (=Δ^{12}-oleanene-3β, 16β-dihydroxy-28-al) exhibits (−) Cotton effect with [A] = −50.9. Pachygenin ($\Delta^{9(11)}$-19 al-5α-digitoxigenin) has (−) Cotton effect with [A]= about −380° (calculated from a figure in the literature : W. Schmid, H.P. Uehlinger, Ch. Tamm, T. Reichstein : Helv. Chim. Acta, **42**, 72 (1959)) while antiarigenin (5β,11α-dihydroxy-19al-digitoxigenin) shows a weak (−) Cotton effect (trough at 320 mμ : −4) : R. P. Martin, Ch. Tamm : Helv. Chim. Acta, **42**, 696 (1959).

9) H. Budzikiewicz, C. Djerassi, D. H. Williams : "Structural Elucidation of Natural Products by Mass Spectrometry," Vol. Ⅱ, pp. 128 (Holden-Day, Inc., 1964).
10) H. Budzikiewicz, J. M. Wilson, C. Djerassi : J. Am. Chem. Soc., **85**, 3688 (1963).
11) C. Djerassi, L. E. Geller, A.J. Lemin : *Ibid.*, **76**, 4089 (1954).
12) W. A. Jacobs : J. Biol. Chem., **64**, 379 (1925).
13) J. L. Breton, A. G. Gonzalez : J. Chem. Soc., **1963**, 1401.

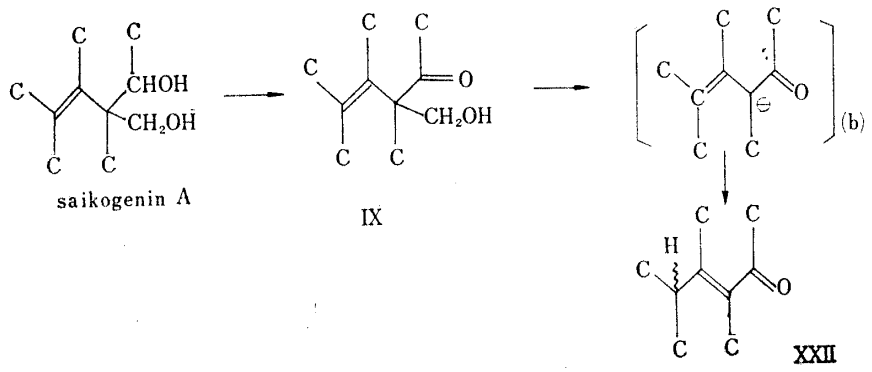

Chart 3.

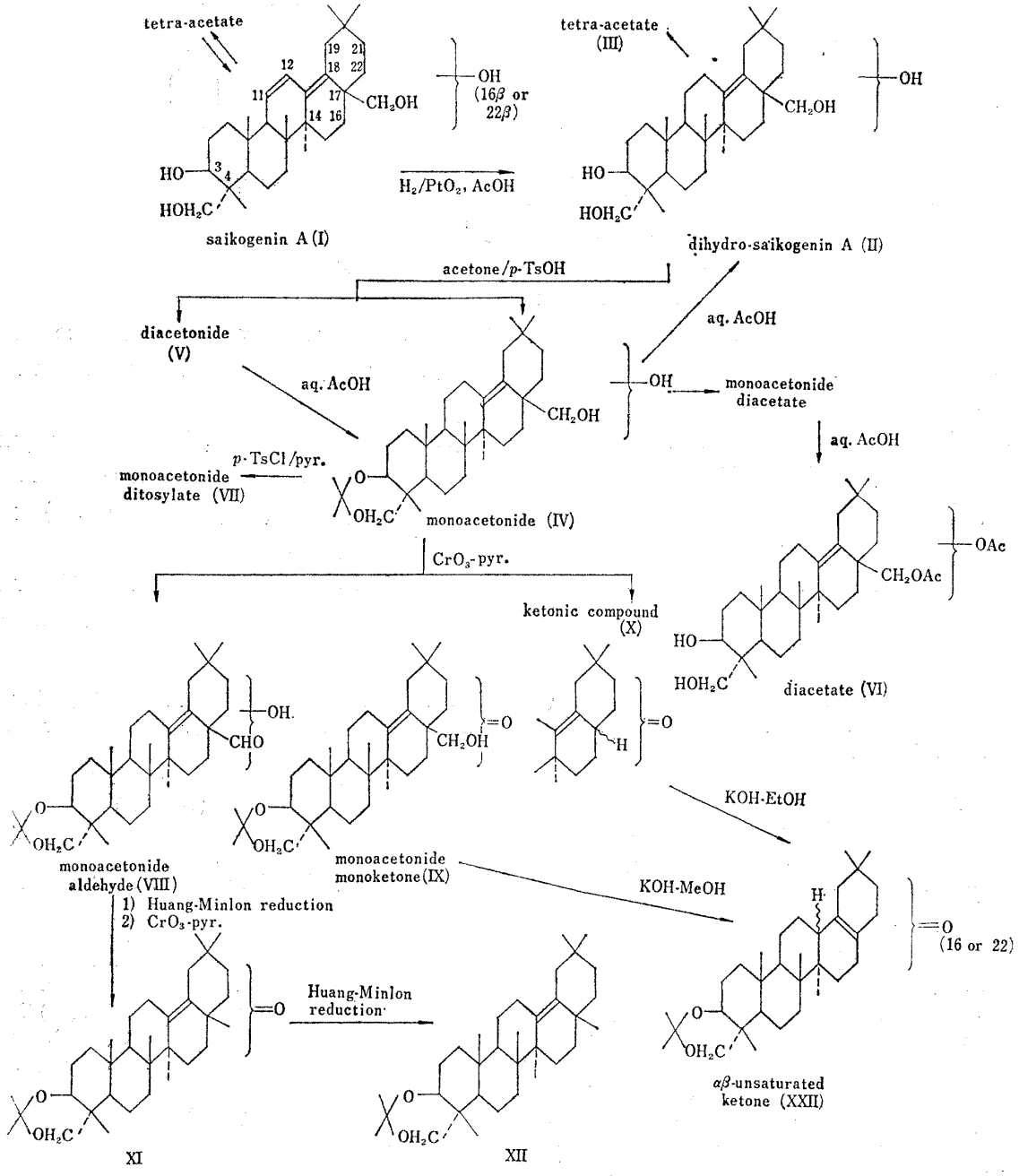

Chart 4.

strong negative Cotton effects with large amplitudes of (−) 496 and (−) 117, respectively indicating the β,γ-unsaturated carbonyl environment.*[12,14]

Especially, the extremely large molecular amplitude of Ⅷ could be attributable to the limited direction of carbonyl bonding due to the hydrogen bond with the neighboring secondary OH group, which has been proved already to exist in the proximity to form acetonide linkage. Carbonyl absorption bands in IR spectra of Ⅷ and XXI (1718 cm^{-1}, 1728 cm^{-1}, respectively) corroborate this assumption. If the aldehyde groups of Ⅷ and XXI were located at the position 20, these large molecular amplitudes would not be observed.

All the above evidences have led us to formulate saikogenin A as I, whose remaining one secondary alcohol grouping must be located either at 16β or 22β.*[13]

The position of 21β (axial) (see footnote*[13]), on the other hand, can be ruled out when the monoketone (Ⅸ) was treated with potassium hydroxide methanol producing

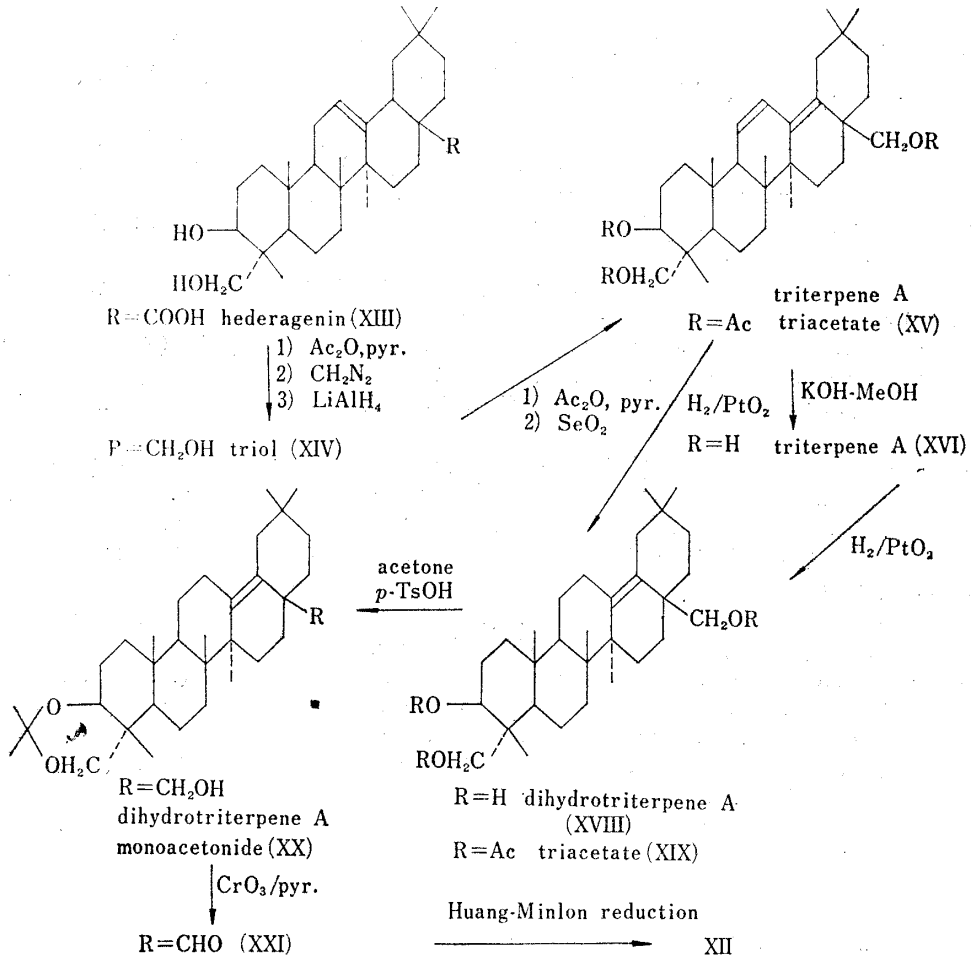

Chart 5.

*[13] By the dreiding model it is revealed that the hydroxyl which can form acetonide linkage with 17-CH_2OH must locate at such a position 15β (axial), 16β (equatorial), 19β (axial), 21β (axial), or 22β (equatorial). Among them, 15β can be eliminated by the acetylation experiment,[16] and 19β cannot be reasonable because of no significant UV absorption of Ⅸ and a ketonic compound (X).

*[14] A ketonic compound (X) also gave XXII on treatment with KOH-EtOH (proved by thin-layer chromatography and UV absorption). An intermediate (b) if protonated, therefore, might be identical with X.

14) A. Moscowitz, K. Mislow, M. A. W. Glass, C. Djerassi: J. Am. Chem. Soc., **84**, 1945 (1962).
15) C. Djerassi, J. Osiecki, W. Closson: *Ibid.*, **81**, 4582 (1959).
16) C. Djerassi, C.H. Robinson, D. B. Thomas: *Ibid.*, **78**, 5687 (1956).

an α,β-unsaturated ketone*14 (XXII, λ_{max} 254 mμ (log ε 4.01)). As reported by Djerassi and Monsimer[17] the above result requires the existence of 1,3-glycol system in saikogenin A as shown below (Chart 3).

Experimental*15

Saikogenin A (I) from the Crude Saponin——The crude saponin[1] (50.0 g.) was first chromatographed on Al_2O_3 (Brockmann, 600 g.) using methanol (total, 6.8 L.), *n*-butanol saturated with water (total, 6.2 L.) successively as the eluents. The methanol and early part of *n*-butanol fractions gave the saponin mixture containing mainly saikosides Ia and Ib (yield ca. 22 g.). After washing with ether, the saponin mixture was submitted to acid hydrolysis.

A mixture of the above saponin (41.0 g.) in methanol (450 ml.) and 10% H_2SO_4 (450 ml.) was refluxed for 4 hours. The dark brown mixture was diluted with water (1 L.), extracted with ether. The combined ether extracts (total 1.2 L.) were then treated under usual manner, and concentrated to a small volume, when the crystalline precipitates (crude saikogenin A) were separated.

The crude saikogenin A was then washed with ether giving practically pure saikogenin A. (Total yield, 3.15 g.)

The analytical sample, m.p. 283~285° (decomp.), $[\alpha]_D^{25}$ −54° (c=0.68, pyridine) was obtained by repeated recrystallization with acetone. IR ν_{max}^{Nujol} cm^{-1} 3358 ; UV λ_{max} mμ (log ε) : 242 (4.43), 250 (4.48), 260 (4.29). *Anal.* Calcd. for $C_{30}H_{48}O_4$ (472.68)*16 : C, 76.22; H, 10.24. Found : C, 76.19; H, 10.15.

On standing overnight at room temp., a mixture of saikogenin A (0.10 g.) in pyridine (2 ml.), Ac_2O (1 ml.) gave a crude acetate which by a chromatography on silica gel (5 g.) eluting with benzene and benzene-ether (9:1) produced a tetraacetylsaikogenin A. Though unsuccessful in getting it in a crystalline form, the tetraacetate showed a single spot on a TLC plate (solvent : benzene-ether (4:1)). IR $\nu_{max}^{CCl_4}$ cm^{-1} : 1746, no OH; UV λ_{max} mμ : 240, 249, 260.

Dihydrosaikogenin A (II)——A mixture of saikogenin A (I) (1.416 g.) and PtO_2 (0.602 g.) in glacial acetic acid (35 ml.) was hydrogenated at room temp. under atmospheric pressure for 1.5 hr. A trituration of the product with acetone afforded a practically pure dihydrosaikogenin A (II). (Yield, 1.00 g.)

The analytical sample of II melted at 288~289° (decomp.) with $[\alpha]_D^{23}$ −41° (c=0.64, pyridine), IR ν_{max}^{Nujol} cm^{-1}: 3323, UV : end absorption only. *Anal.* Calcd. for $C_{30}H_{50}O_4$: C, 75.90; H, 10.62. Found : C, 76.01; H, 10.63.

Dihydrosaikogenin A Tetraacetate (III)——Acetylation of II (0.10 g.) with pyridine (2 ml.) and Ac_2O (1 ml.) mixture produced dihydrosaikogenin A tetraacetate (III). Recrystallization with aq. methanol yielded the analytical sample of melting at 181~181.5°, $[\alpha]_D^{29}$ −35° (c=0.78), IR ν_{max}^{Nujol} cm^{-1} : 1740, 1755 (shoulder), no OH. *Anal.* Calcd. for $C_{38}H_{58}O_8$ (642.84); C, 70.99; H, 9.09. Found : C, 71.15; H, 9.22. Molecular ion at m/e 642, base peak at m/e 201.

Acetonide Formation of Dihydro-saikogenin A (IV, V)——A suspension of II (1.00 g.) and *p*-toluenesulfonic acid monohydrate (0.20 g.) in dry acetone (200 ml.) was stirred at room temp. for 40 minutes. After 5 minutes, the suspension turned into a colorless solution. After adding K_2CO_3 (0.20 g.) under cooling, the solution was concentrated into a syrup which was diluted with water. The colorless precipitate was collected by filtration, washed with water, dried and chromatographed on Al_2O_3 (6.0 g.). The eluation with benzene afforded diacetonide (V) (0.086 g.), and benzene-ether (1:1) mixture eluted monoacetonide (IV) (0.52 g.).

The analytical sample of V was obtained by recrystallization with acetone, m.p. 211~214°, $[\alpha]_D^{34}$ −3° (c=0.67), IR $\nu_{max}^{CCl_4}$ cm^{-1} : no OH, 1167, 1153, 1110, 1058, 864. *Anal.* Calcd. for $C_{36}H_{58}O_4$: C, 77.93; H, 10.54. Found : C, 78.23; H, 10.54.

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*15 All melting points under 270° were determined on Yanagimoto Micro Melting-point Apparatus and recorded as read. Optical rotations were measured with Yanagimoto Photo-Magnetic Direct Reading Polarimeter Model OR-20 in chloroform unless mentioned otherwise. The ultraviolet absorption spectra were measured in ethanol solution with Cary self-recording spectrophotometer Model 11, infrared spectra with Japan Spectroscopic Co. Model DS-402 G Infrared Spectrophotometer. The NMR spectra were taken in deuterochloroform with Japan Electron Optics Lab. JNM-3H-60 spectrometer. Optical rotatory dispersion curves were taken in methanol with an automatic recording ORD/UV-5 Spectropolarimeter of Japan Spectroscopic Manufac. Co. Alumina of activity grade II (Woelm. neutral) were used unless otherwise stated.

*16 Molecular weight determination by mass spectrometry gave indefinite results depending upon the conditions for running spectra. A spectrum taken by the direct inlet method with 190° of both ion source and evaporation temp. gave a molecular ion at m/e 472. (taken by Mr. H. Sato, Hitachi Co., Ltd.)

17) C. Djerassi, H. G. Monsimer : J. Am. Chem. Soc., **79**, 2901 (1957).

Recrystallization of IV with acetone-*n*-hexane gave the analytical sample of melting at 219.5~220°, $[\alpha]_D^{33}$ −29° (c=0.80), IR ν_{max}^{Nujol} cm^{-1}: 3336, 1162, 1108, 1067, 856. *Anal.* Calcd. for $C_{33}H_{54}O_4$: C, 76.99; H, 10.57. Found: C, 76.78; H, 10.57.

Dihydrosaikogenin A Diacetate (VI)——Diacetyl derivative of dihydrosaikogenin A monoacetonide prepared from IV (0.08 g.) with pyridine (2 ml.), Ac_2O (1 ml.) mixture, was treated with 50% aq. AcOH (8 ml.) in a boiling water bath for 40 minutes. After diluting the mixture with water, the colorless precipitates were collected by filtration, washed, dried, and recrystallized from acetone repeatedly to yield VI, m.p. 231~232°, α_D^{31} −48 (c=0.63), IR ν_{max}^{Nujol} cm^{-1}: 3376, 1739. *Anal.* Calcd. for $C_{34}H_{54}O_6$: C, 73.08; H, 9.74. Found: C, 72.88; H, 9.81.

Dihydrosaikogenin A Monoacetonide Ditosylate (VII)——A solution of IV (0.20 g.) and *p*-toluenesulfonyl chloride (1.0 g.) in dry pyridine (10 ml.) was kept at room temp. for 2 days and treated with ice-water as usual. The crude product was then chromatographed on Al_2O_3 (8 g.) using benzene as a developing solvent. Ditosylate (VII) was recrystallized with acetone giving prisms melting at 143~144° (decomp.), $[\alpha]_D^{31}$ −52° (c=0.73), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1375, 1189, 1177, no OH. *Anal.* Calcd. for $C_{47}H_{66}O_8S_2$: C, 68.59; H, 8.08; S, 7.78. Found: C, 68.83; H, 8.11; S, 7.72.

Pyridine-Chromium Trioxide Complex Oxidation of Dihydrosaikogenin A Monoacetonide yielding VIII, IX and X——To an ice-cooled suspension of pyridine (5 ml.)-CrO_3 (0.50 g.) complex, was added dropwise a solution of IV (0.65 g.) in pyridine (8 ml.) during a period of 20 minutes. Additional one ml. of pyridine was used to rinse the flask. The total mixture (dark yellow-brown) was stirred continuously under ice-cooling for further 4 hr., treated with methanol (1 ml.), diluted with water and extracted with ether. An oily product obtained on evaporation of the solvent was chromatographed on Al_2O_3 (110 g.) eluting with (a) benzene (1.1 L.), (b) benzene-ether (9:1) (2 L.), (c) benzene-ether (8:2) (1.9 L.), (d) ether (0.3 L.), (e) ether-methanol (9:1) (0.3 L.), successively.

The early part of (a) afforded crystalline material (from acetone) (X, 8 mg.), and a further crop (2 mg.) was obtained by the repeated chromatography (silica gel, benzene and benzene-$CHCl_3$ (1:1)) of the combined benzene fraction and the mother liquor of the above crystals.

The compound (X) of melting at 197~200° (unsharp) is probably a mixture of 17-H epimers. The thin-layer chromatogram of the material showed an elongate spot (solvent: EtOAC-cyclohexane-water: 30:70:0.1). UV: end absorption only, IR ν_{max}^{KBr} cm^{-1}: 1714, IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1718, ORD (c=0.052): $[\alpha]_{268}$ +1154, $[\alpha]_{291}$ 0, $[\alpha]_{306}$ −1038, [A]=−98.5 (as mol. wt.=482).

The later part of (b) and early part of (c) gave VIII (70 mg.). Recrystallization with acetone yielded the analytical sample of VIII, melting at 227~229°, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3464, 2712, 1718, ORD (c=0.138): $[\alpha]_{284}$ +4964, $[\alpha]_{308}$ 0, $[\alpha]_{331}$ −4710, [A]=−496. *Anal.* Calcd. for $C_{33}H_{52}O_4$: C, 77.29; H, 10.22. Found: C, 77.69; H, 10.16.

Repeated chromatography of (e) on Al_2O_3 (20 g.) eluted with (e-a) benzene, (e-b) benzene-ether (9:1), (e-c) benzene-ether (3:1), (e-d) benzene-ether (1:1), (e-e) ether-methanol (9:1), successively afforded IX (39 mg. from fraction (e-b)) and the starting acetonide (IV) recovered (54 mg. from fraction (e-c)).

The analytical sample of IX, m.p. 240~242°, was obtained by recrystallization with benzene, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3466, 1707, ORD (c=0.091): $[\alpha]_{273}$ +648, $[\alpha]_{290}$ 0, $[\alpha]_{308}$ −813, [A]=−74.9. *Anal.* Calcd. for $C_{33}H_{52}O_4$: C, 77.29; H, 10.22. Found: C, 77.18; H, 10.18.

$\Delta^{13(18)}$-Oleanene-3β,23-diol Acetonide (XII) from VIII——A solution of VIII (50.7 mg.) in ethanol (2 ml.), diethyleneglycol (2 ml.) and 80% hydrazine hydrate (0.50 ml.) was refluxed for 30 minutes (bath temp. 128~130°). Potassium hydroxide (0.20 g.) was added to the mixture which was refluxed for additional 15 minutes, and then the condenser was fitted downward, and the oil bath temp. was raised to 227°. During the period, effervescence of the mixture was observed. On refluxing for 2.5 hr., the solution was poured into water, and extracted with benzene (3 times). The benzene extract yielded a solid residue, which was oxidized with pyridine (0.3 ml.)-CrO_3 (30 mg.) complex in pyridine (1 ml.) by keeping at room temp. for 2 days. Ether extraction of the product yielded a colorless solid (m.p. 218~222°, unsharp). A part of the solid was crystallized from acetone giving XI, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1715, no OH. The rest of the product was submitted to the Huang-Minlon reduction again as described above. The benzene extracts of the reaction product afforded a crystalline residue, which was recrystallized with acetone yielding XII of melting at 234~236° (unsharp).

A chromatography of the product on Al_2O_3 (4 g.) followed by recrystallization with acetone gave the analytical sample of XII, m.p. 242~245°, IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, no carbonyl, 1169, 1114, 1068, 864, mass spectrum: m/e 482 (m^+), 205 (base peak).[9]

XII from XXI——Forty mg. of XXI was treated under the Huang-Minlon procedure (ethanol 2 ml., diethyleneglycol 2 ml., 80% hydrazine hydrate 0.6 ml., KOH 0.20 g.) as for VIII. The benzene extracts afforded XII by recrystallization with acetone. The analytical sample melted at 241~242°, $[\alpha]_D^{30}$ −46 (c=0.37). *Anal.* Calcd. for $C_{33}H_{54}O_2$: C, 82.07; H, 11.27. Found: C, 81.65; H, 11.27.

The identification of doth samples of XII obtained through VIII and XXI was achieved by mixed melting point and comparison of their infrared spectra (CCl_4 solution) and optical rotations.*17

α,β-Unsaturated Ketone (XXII) from IX——A solution of IX (18 mg.) in 1% KOH-MeOH (5 ml.) was refluxed for one hour, diluted with water, and extracted with ether. Recrystallization of the product with methanol yielded XXII (8 mg.) of melting at 239~241°, UV λ_{max} mμ (log ε): 254 (4.01), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1666, 1627 (w), no OH.

XXII from X——A few mg. of X was refluxed for 1 hour in dilute KOH-EtOH solution. The product was confirmed to be identical with above sample of XXII by comparing their UV spectra and TLC (Solvent: EtOAc-cyclohexane=1:6).

Δ^{12}-Oleanene-3β,23,28-triol (XIV)——Reduction of hederagenin methyl ester diacetate (3.87 g.) in ether (110 ml.) with $LiAlH_4$ (1.5 g.) in ether (100 ml.) by refluxing for 3 hr., produced practically pure XIV which can be used for further reaction without recrystallization. A part of the product was recrystallized from aq. methanol to yield the needles, melting at 260~261°,*18 $[\alpha]_D^{29}$ +48 (c=0.44), IR ν_{max}^{Nujol} 3383, 3217 (broad) cm^{-1}.

Triterpene A Triacetate (XV)——The above triol (XIV) was acetylated with pyridine (60 ml.), acetic anhydride (30 ml.) by the usual method. The crude acetate (3.61 g.) was refluxed with SeO_2 (3.6 g.) in glacial acetic acid (150 ml.) for 1 hour. Evaporation of the light brown filtrate gave brown solid residue which was purified by Al_2O_3 (100 g.)-chromatography eluting with benzene, yielding triacetate (2.24 g.). The analytical sample, m.p. 181.5~182°, $[\alpha]_D^{28}$ −85 (c=0.89), was obtained by recrystallization with methanol, UV λ_{max} mμ (log ε): 242 (4.46), 250 (4.50), 260 (4.31), IR ν_{max}^{KBr} cm^{-1}: 1743. *Anal.* Calcd. for $C_{36}H_{54}O_6$ (582.79): C, 74.19; H, 9.34. Found: C, 74.22; H, 9.31.

The compound was confirmed to be identical with triterpene A triacetate provided by Dr. Breton by mixed melting point, comparing their UV and IR spectra, and optical rotations.

Triterpene A (XVI)——To a warm solution of XV (1.37 g.) in methanol (100 ml.) was added 2*N* NaOH (30 ml.). The suspension was continued to reflux for one hour. After cooling, the crystalline product (XVI) was collected by filtration. Yield, 1.03 g.

The analytical sample of melting at 294~295° (decomp.) was obtained by recrystallization with methanol, $[\alpha]_D^{31}$ −80° (c=0.81, pyridine), UV λ_{max} mμ (log ε): 243 (4.42), 251 (4.48), 261 (4.28); IR ν_{max}^{KBr} cm^{-1}: 3414. *Anal.* Calcd. for $C_{30}H_{48}O_3$: C, 78.89; H, 10.59. Found: C, 78.84; H, 10.73.

Triterpene A Monoacetonide (XVII)——A suspension of XVI (0.15 g.), *p*-toluenesulfonic acid monohydrate (0.04 g.) in dry acetone (30 ml.) was continued to stir at room temp. for 3 hours. In 20 minutes, the suspension turned into colorless solution. The solution was evaporated *in vacuo* under cooling, and then diluted with water. The colorless precipitates collected by filtration were dissolved in benzene (0.07 g. of starting material was removed as insolubility), and chromatographed on Al_2O_3 (4 g.). The benzene-ether (3:2) eluate afforded XVII (0.05 g.). The analytical sample (from methanol) melted at 197~198°, $[\alpha]_D^{33}$ −52 (c=0.31), IR ν_{max}^{Nujol} cm^{-1}: 3494, 1160 (br.), 1104, 1066, 857. *Anal.* Calcd. for $C_{33}H_{52}O_3$: C, 79.78; H, 10.55. Found: C, 79.55; H, 10.72.

Dihydrotriterpene A (XVIII)——A mixture of XVI (1.02 g.), PtO_2 (0.44 g.) in glacial acetic acid (50 ml.) was shaken under hydrogen atmosphere for 4 hours. The product (no diene absorption in UV spectrum) was pure enough for further reaction. For the analytical sample, the product was recrystallized with methanol, m.p. 292~293° (decomp.) $[\alpha]_D^{29}$ −46 (c=0.53, pyridine), IR ν_{max}^{Nujol} cm^{-1}: 3308. *Anal.* Calcd. for $C_{30}H_{50}O_3$: C, 78.55: H, 10.99. Found: C, 78.34; H, 10.86.

Dihydrotriterpene A Triacetate (XIX)——XV (100 mg.) was hydrogenated with PtO_2 (34 mg.) in glacial acetic acid (10 ml.) as above. Recrystallization from aqueous methanol gave the analytical sample (XIX), m.p. 160.5~162°; $[\alpha]_D^{28}$ −39° (c=0.66), IR $\nu_{max}^{CCl_4}$ cm^{-1} 1744. *Anal.* Calcd. for $C_{36}H_{56}O_6$ (584.81); C,73.93; H, 9.65. Found: C, 73.95; H, 9.78. Mass spectrum; m/e 584 (M^+), 203 (base peak).

Dihydrotriterpene A Monoacetonide (XX)——XVIII (1.03 g.) was treated with *p*-toluenesulfonic acid monohydrate (0.20 g.) in dry acetone (200 ml.) as for XVII. After addition of K_2CO_3 (anhydr.) (0.20 g.) under ice-cooling, the suspension was stirred for further 10 minutes, concentrated *in vacuo* into paste, and treated with water. The product was then chromatographed on Al_2O_3 (40 g.) eluting with (a) benzene, (b) benzene-ether (9:1) mixture, successively. The fractions of (a) and early part of (b) yielded XX. The analytical sample (cryst. from acetone) melted at 218~219°, $[\alpha]_D^{28.5}$ −50° (c=0.66), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3352, 1162, 1112, 1065, 859. *Anal.* Calcd. for $C_{33}H_{54}O_3$: C, 79.46; H, 10.92. Found: C, 79.15; H, 10.84.

$\Delta^{13(18)}$-Oleanene-3β,23-diol-28-al Monoacetonide (XXI)——To a stirred suspension of pyridine (3 ml.)-CrO_3 (0.15 g.) complex was added a suspension of XX (0.20 g.) in pyridine (3 ml.). (One additional ml. of pyridine was used to rinse flask). The total mixture was stirred continuously for 50 minutes at room temp., treated with 20 drops of methanol, and worked up as usual. The ether extract gave an amorphous residue, which was chromatographed on Al_2O_3 (20 g.) using benzene as eluent.

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*17 Shortage of the sample from VIII made impossible to take its rotation in an adequate concentration, so that the comparison of their rotations of both samples (XII) from VIII and XXI was made in c=0.2, which showed good agreement with −75° and −80°, respectively.

*18 M.p. 250°, α_D +77 (c=0.42, chloroform) by F.E. King, *et al.*: J. Chem. Soc., **1958**, 2830.

Trituration of the product with acetone yielded XXI (91 mg.). Further recrystallization with acetone gave the analytical sample, m.p. 205~207°, $[\alpha]_D^{29}$ −94° (c=0.67), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2790, 2732 (w), 2704 (w), 2664, 1728. ORD (c=0.061): $[\alpha]_{290}$ +734, $[\alpha]_{302}$ 0, $[\alpha]_{328}$ −1615, $[\alpha]_{337}$ −1615, [A]=−117. *Anal.* Calcd. for $C_{33}H_{52}O_3$: C, 79.78; H, 10.55. Found: C, 79.75; H, 10.51.

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Summary

A sapogenin, for which the name saikogenin A is proposed, has been obtained from saponins of *Bupleurum falcatum* L (Mishimasaiko). The present study has given the structural formula (I) for saikogenin A,

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142. Shojiro Uyeo,*1 Hideaki Shirai,*2 Akira Koshiro,*1 Tamotsu Yashiro,*2 and Kengo Kagei*1 : Galanthamine Chemistry. VII.*3 Synthesis of Analogues of Galanthamine.

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Galanthamine, an alkaloid isolable from some species of the Amaryllidaceae, due to its anticholinesterase activity,[1] has become popular in the clinical treatment of poliomyelitis. Furthermore this alkaloid bears some resemblance to morphine in the ring system and has been shown to have analgesic action, the duration of effect approaching that of morphine and codeine.[2] The purpose of our present investigation was to synthesize compounds structurally related to galanthamine (I), in particular II, and to test their biological effects, especially their anticholinesterase activities. The compound (II) differed from galanthamine only in the absence of the oxide ring and the double bond in the cyclohexane ring. Compound (III), an isostere of II, was a by-product obtained in the course of synthesis.

Michael reaction of *m*-methoxybenzyl cyanide (IV)[3] with methyl acrylate in the presence of Triton B yielded methyl γ-cyano-γ-(*m*-methoxyphenyl)pimelate (V) which gave the keto-ester (VI) after a Dieckmann cyclization. Saponification of VI followed by decarboxylation resulted in the formation of the keto-nitrile (VII).

Ketalization of the keto-nitrile (VII) and hydrolysis of the nitrile group in VII afforded the ketal-acid (IX) which on treatment with lithium aluminum hydride gave the ketal-alcohol (X). Chromic acid oxidation of the alcohol (X) gave the aldehyde (XI) which was

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3) R.B. Woodward: *Ibid.*, **62**, 1478 (1940).