Tabelle I. Werte der Chemischen Verscheibungen in ppm Einheiten für die Aromatenprotonen von IV, V und VI.

Verbindung		C-2	C-4	C-5	C-7
Physcion-monomethyläther-monoacetat (IV)	Gem.	7.15	7.75	7.70	6.93
	Ber.	7.05	7.70	7.60	6.95
Physcion-diacetat(V)		7.24	8.05	7.72	6.92
Emodin-trimethyläther (VI)		7.15	7.70	7.37	6.83

60 MHz; Konz. 10% (w/v) in $CDCl_3$; Standard: TMS

Für die weitere Klärung der Zucker-Haftstelle war das NMR-Spektrum sehr aufschlussreich. In Tabelle I sind die chemische Verschiebungen für Aromatenprotonen in $CDCl_3$ von Physcion-monomethyläther-monoacetat (Smp: 182.5—184.5°) (IV), Physcion-diacetat (V) sowie Emodin-trimethyläther (VI) zusammengestellt. Die δ-Werte der aromatischen C-7 und C-5 Protonen bzw. der C-2 und C-4 Protonen von (IV) sind denjenigen von Physcion-diacetat (V) bzw. von Emodintrimethyläther (VI), sehr ähnlich. In einer Arbeit von Ballantine, *et al.*[3] wurde die Additivität der Substituenten-Effekte n substituierten Phenolen in guter Näherung bewiesen. In der Tat finden wir eine schöne Übereinstimmung der berechneten mit den gemessenen Verschiebungen der Aromatenprotonen von (IV). Das NMR-Spektrum ist nur mit der Struktur (IV) für Physcion-monomethyläther-monoacetat (Smp: 182.5—184.5°) vereinbar, wie sich auch massenspektroskopisch belegen läßt.

Damit sind die Strukturen der Anthraglykoside A und B ermittelt: A ist Physcion-8-β-D-Glukosid (I), B ist Emodin-8-β-D-Glukosid (II).

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3) J.A. Ballantine und C.T. Pillinger, *Tetrahedron*, **23**, 1691 (1967).

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UDC 581.19 : 547.918.02 : 582.99

Sapogenins of the Roots of *Platycodon grandiflorum* A. De Candolle and the Stereochemistry of Polygalacic Acid[1]

Although the extensive studies on sapogenins and saponins of the roots of *Platycodon, grandiflorum* (Japanese name "Kikyo") have been reported by Tsujimoto[2] and Yamaguchi, *et al.*,[3] little has been known about their structures.

1) This paper excepting the stereochemistry of I was read at the 88th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April 1968,
2) M. Tsujimoto, *Nippon Nogeikagaku Kaishi*, **16**, 613 (1940) and the references cited therein.
3) K. Yamaguchi, M. Ito, M. Nishimoto, and S. Natori, *Shoyakugaku Zasshi*, **18**, 12 (1964).

The crude saponins isolated from the methanolic extracts of the roots of this plant were hydrolyzed with dil. mineral acid to give a complex mixture of sapogenins, which was separated by column chromatography on silica gel to afford several crystalline sapogenins.

One of the sapogenins (I), $C_{30}H_{48}O_6$, mp 308°, $[\alpha]_D^{25}$ +45° (pyridine), afforded a methyl ester (II), mp 245—245.5°, $[\alpha]_D^{25}$ +47.3° (ethanol), and a methyl ester tetra-acetate (III), mp 175—175.5°, $[\alpha]_D^{25}$ +6.3° (ethanol). This sapogenin was found to be identical with polygalacic acid which was isolated by Rondest and Polonsky from *Polygala paenea* L.[4] The identity was established by mixed melting point determination of III with authentic methyl tetra-O-acetylpolygalacate and the comparisons of the thin-layer chromatograms and infrared (IR) spectra of I and III with those of the respective authentic samples.

The structure of I has been proposed to be 2β,3β,16β,23-tetrahydroxyolean-12-en-28-oic acid by Rondest and Polonsky.[4] In the present study, II was acetylated with acetic anhydride in pyridine at 0° to give methyl 3, 23-di-O-acetylpolygalacate (IV), mp 128—132°, $[\alpha]_D^{28}$ +66.2° ($CHCl_3$), whose nuclear magnetic resonance (NMR) spectrum[5] clearly showed non-overlapping signals due to protons on carbon atoms bearing the oxygen functions, *i.e.* at 3.70 and 3.86 (a pair of AB type doublets, J=12 cps, $-C_{(23)}\underline{H}_2OAc$), at 4.93 (1H doublet, J=3 cps, $-C_{(3)}\underline{H}$-OAc), at 4.25 (1H, quartet-like, $W_{h/2}$=7.5 cps, -C$\underline{H}$-OH), and at 4.54 (1H, triplet-like, $W_{h/2}$=4cps, -C$\underline{H}$-OH). The coupling features of the latter two signals strongly suggest that the configurations of both hydroxyl groups remained unacetylated (at C-2 and C-16) should be assigned as axial. The mild acetylation of methyl 2,3-isopropylidenepolygalacate (V) with acetic anhydride in pyridine at −10° yielded its 23-O-acetyl derivative (VI), mp 190—194°, $[\alpha]_D^{25}$+65.6° ($CHCl_3$), IR $\nu_{max}^{CCl_4}$ 3630 (free OH) and 1740 cm^{-1} (-CO-O-). The absence of intramolecularly hydrogen bonded hydroxyl and ester absorption bands also supports the α (axial) configuration of the hydroxyl group at C-16 of I.[6] On oxidation with chromic acid in pyridine, VI gave the amorphous 16-keto derivative (VIII), IR $\nu_{max}^{CCl_4}$ 1745 (-CO-O-) and 1715 cm^{-1} (C=O),which was homogeneous by the thin-layer chromatography and showed ORD and CD curves with negative Cotton effect consisting with the presence of 16-ketone of triterpenes of this series.[4,7]

The β (equatorial) configuration of C-16-hydroxyl group of I was proposed on the basis of the fact that reduction with $NaBH_4$ followed by acid hydrolysis of the keto-aldehyde derivative (VII) prepared from V did not reproduce II, though the purity and the structure of this reaction product, mp 233—235°, were not detailed.[4] In order to re-examine their result of the reduction of 16-ketone, VIII was subjected to $NaBH_4$ reduction in aqueous methanol at room temperature to reproduce VI along with a small amount of V. Referring to the stereochemistry of $NaBH_4$ reduction of 16-ketone of olean-12-ene type triterpenes[8] coupled with the above mentioned evidences, it follows now that the configuration of the C-16 hydroxyl group of I should be revised to be α (axial).[9]

The second sapogenin (IX), $C_{30}H_{48}O_7$, mp 241—242°, $[\alpha]_D^{25}$ +35.3° (pyridine), IR ν_{max}^{KBr} near 1700 cm^{-1}, positive to tetranitromethane test, gave a methyl ester (X), $C_{31}H_{50}O_7$, mp

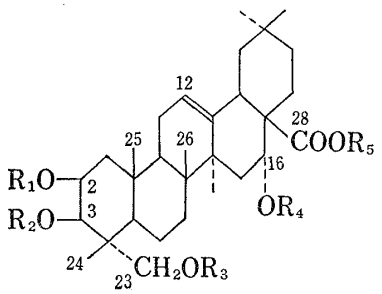
4) J. Rondest and J. Polonsky, *Bull. Soc. Chim. France*, **1963**, 1253.
5) NMR spectra were determined at 100 Mc in $CDCl_3$ solution unless otherwise stated. Chemical shifts (δ) are expressed in ppm from TMS.
6) The IR spectrum of methyl cochalate (methyl 3β,16β-dihydroxy-olean-12-en-28-oate)[8] in CCl_4 showed a free OH band at 3630, a hydrogen bonded OH band (16β-hydroxyl group) at 3530 (concentration independent), a free ester band at 1740 (weak) and a hydrogen bonded ester band at 1705 cm^{-1} (strong). Ref. A.R.H. Cole and G.T.A. Müller, *J. Chem. Soc.*, **1959**, 1224.
7) C. Djerassi, J. Osiecki, and W. Closson, *J. Am. Chem. Soc.*, **81**, 4587 (1959).
8) C. Djerassi, G.H. Thomas, and H. Monsimer, *J. Am. Chem. Soc.*, **77**, 3579 (1955).
9) According to the private communication from Dr. Kubota, Shionogi Research Laboratory, he and his co-worker recently isolated I from *P. grandiflorum* and also amended the configuration of C-16 hydroxyl group to be α by the chemical correlation of I with quillaic acid (T. Kubota and H. Kitatani, *Chem. Commun.*, **1968**, in press).

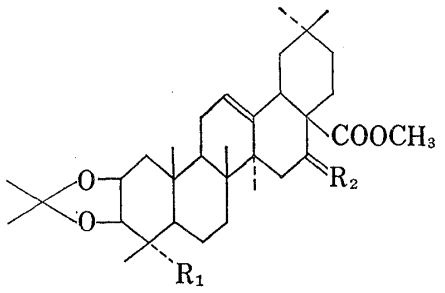
246°, $[\alpha]_D^{25}$ +45° (pyridine), NMR (in pyridine-d_5) 3.65 (3H singlet, -COOC$\underline{H}_3$), and an amorphous methyl ester penta-acetate (XI) (homogeneous by the thin-layer chromatography) IR $\nu_{max}^{CCl_4}$ 1750 cm^{-1} and no OH band, NMR 3.63 (3H singlet, -COOC$\underline{H}_3$), 1.99 (3H singlet, -OCOC$\underline{H}_3$), 2.05 (3H singlet, -OCOC$\underline{H}_3$), and 2.08 (9H singlet, 3×-OCOC$\underline{H}_3$). The mild acetylation of X afforded its triacetate, $C_{37}H_{56}O_{10}$, mp 189—190°, $[\alpha]_D^{15}$ +47.2° ($CHCl_3$). This sapogenin (IX) seems to be identical with platycodigenin isolated by Tsujimoto,[2] though the direct comparison has not been carried out.

Referring to the studies on the retro Diels–Alder cleavage of the olean-12-ene type triterpenes,[10] the comparison of the mass spectrum of X with that of II led to suggest that X would possess the olean-12-ene skeleton and the functional groups on its D and E rings would be the same as those of II.

The NMR spectrum of XI revealed that IX has one trisubstituted double bond, three secondary alcohols, and two primary alcohols. The chemical shifts and the coupling patterns of the NMR signals of XI attributable to the protons on a double bond and on carbon atoms bearing the secondary acetoxyl groups were quite similar to those of III. The close relationship between I and IX was further demonstrated by the comparisons of the tertiary methyl signals of XI with that of III, *i.e.*, XI 0.73, 0.93, 0.98 (3H each, singlets), and 1.22 (6H, singlet); III, 0.74, 0.94, 0.99, 1.04 (3H each, singlets), and 1.22 (6H, singlet). According to the study on the effect of 28-carbomethoxyl group to the tertiary methyl signals of the olean-12-ene series,[11] the highest methyl signal of III at 0.74 (0.73 in case of XI) can be assigned to its 26-methyl protons.

These evidences together with the formation of a bromolactone, $C_{30}H_{47}O_7Br$, mp 203—206°, IR ν_{max}^{KBr} 1760 cm^{-1}, from IX led to suggest that this sapogenin (IX) would be formulated as 2β,3β,16α,23,24-, 2β,3β,16α,23,25-, or 2β,3β,16α,24,25-pentahydroxyolean-12-en-28-oic acid. Further studies to establish the structures of IX and other sapogenins are under progress.





I: $R_1=R_2=R_3=R_4=R_5=H$

II: $R_1=R_2=R_3=R_4=H$, $R_5=CH_3$

III: $R_1=R_2=R_3=R_4Ac$, $R_5=CH_3$

IV: $R_1=R_4=H$, $R_2=R_3=Ac$, $R_5=CH_3$

V: $R_1=CH_2OH$, $R_2=$

VI: $R_1=CH_2CH_2OAc$, $R_2=R_2=$ 

VII: $R_1=CHO$, $R_2=\ =O$

VIII: $R_1=CH_2OAc$, $R_2=\ =O$

Chart. 1

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Addendum in proof (Received September 14, 1968): Very recently, the structure of the sapogenin (IX) has been established to be 2β,3β,16α,23,24-pentahydroxyolean-12-en-28-oic acid by the X-ray analysis of its bromolactone, mp 203—206°. (T. Akiyama, Y. Iitaka, and O. Tanaka, *Tetrahedron Letters*, **1968**, submitted).

10) H. Budzikiewicz, J.M. Wilson, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 3688 (1963); J. Karliner and C. Djerassi, *J. Org. Chem.*, **31**, 1945 (1966).

11) R. Savoir, B. Tursch, and M. Kaisin, *Tetrahedron Letters*, **1967**, 2129; B. Tursch, R. Savoir, R. Ottinger, and G. Chiurdoglu, *ibid.*, **1967**, 539.

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X-Ray Analysis of an Unusual Amino Acid Isolated from the Hydrolysate of a New Antibiotic, Enduracidin

Enduracidin is a new antibiotic peptide[1-3] produced by a strain of *Streptomyces fungicidicus*, No. B-5477. It contains non-ionic chlorine atoms in an unusual amino acid component, which was obtained on hydrolysis of the antibiotic with 6N hydrochloric acid and successive chromatographic fractionation of the hydrolysate.

For the elucidation of the structure of this unusual amino acid (tentatively named K2), X-ray analysis was undertaken in parallel with chemical synthetic study.[4] Compound K2 ($C_8H_7NO_4 \cdot Cl_2$) exists in two crystalline modifications in compliance with different stage of crystallization. Needles, mp 208—210° (decomp.), obtained from an aqueous methanolic solution at an early stage of crystallization are trigonal, whereas prisms or pillars, mp 211—212 °(decomp.), grown slowly from the mother liquor, are monoclinic. A preliminary study indicated that the latter, which contains one molecule of water of crystallization in the asymmetric unit, is more suitable for X-ray analysis than the former.

The unit cell of the latter with cell dimensions, $a=11.93$, $b=10.08$, $c=8.76$Å and $\beta=95°30'$, contains four molecules of K2, the space group being $P2_1/a$ (C^5_{2h}). The crystals are, therefore, of the racemic K2. Some degree of racemization had occurred during the acid hydrolysis.

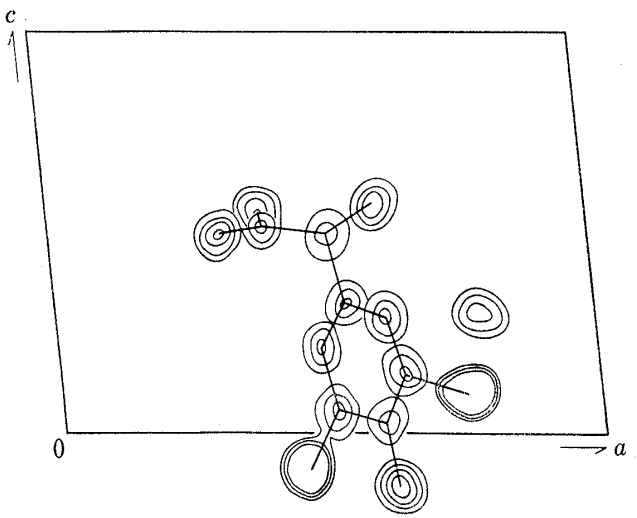

Fig. 1. The Third Three-dimensional Electron Density Distribution Projected on (010)

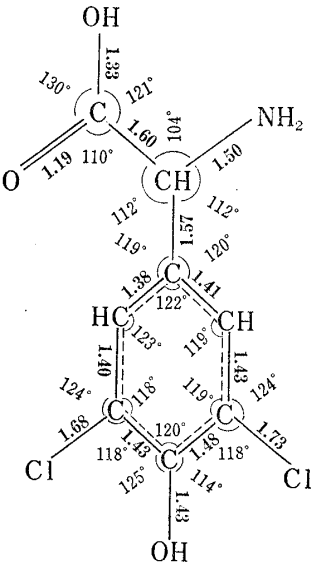

Fig. 2. Interatomic Distances and Angles

1) E. Higashide, K. Hatano and M. Shibata, *J. Antibiotics*, 21, 126 (1968).
2) M. Asai, M. Muroi, N. Sugita, H. Kawashima, K. Mizuno and A. Miyake, *J. Antibiotics*, 21, 138 (1968).
3) K. Tsuchiya, M. Kondo, T. Oishi and T. Yamazaki, *J. Antibiotics*, 21, 147 (1968).
4) J. Ueyanagi and H. Iwasaki, unpublished data.