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# Studies on the Constituents of Asclepiadaceae Plants. XLI.[1] Component of *Cynanchum caudatum* Max. Structure of Glycopenupogenin

Hideo Bando, Takashi Yamagishi, Koji Hayashi, and Hiroshi Mitsuhashi

*Faculty of Pharmaceutical Sciences, Hokkaido University*[2]

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A new polyoxypregnane having 5α,6β-hydroxyl groups, glycopenupogenin (I), was isolated from *Cynanchum caudatum* Max., and its structure was elucidated on the bases of physical data and chemical reactions. I was found to be identical with a derivative obtained by the glycolation of penupogenin (IV), which had been isolated from the same plant.

Several polyoxypregnane derivatives having 5α,6β-hydroxyl groups have been isolated from *Cynanchum caudatum* Max, (Asclepiadaceae). Our previous paper[3] reported the isolation and structural elucidation of glycocaudatin, and the present paper deals with the structure determination of glycopenupogenin (I).

Glycopenupogenin (I), $[\alpha]_D$ +15.9°, was separated by preparative thin-layer chromatography (TLC) from the crude aglycone fraction which contained glycocynanchogenin,[4] 12-O-cinnamoyl-20-*O*-acetylglycosarcostin,[5] and glycocaudatin.[3] Infrared (IR) spectrum of I showed the absorption of hydroxyl groups at 3400 $cm^{-1}$, conjugated ester group at 1700, 1635, and 1160 $cm^{-1}$, and an aromatic group at 1575 $cm^{-1}$. Mass spectrum of I showed no molecular ion peak but *m/e* 398 ($M-C_9H_8O_2$), *m/e* 148 ($C_9H_8O_2$), and *m/e* 147 ($C_9H_7O_2$) as a base peak, and also indicated ion peaks due to the loss of $H_2O$ as a characteristic fragmentation of polyoxypregnane derivatives[6] at *m/e* 380 ($398-H_2O$), 362 ($398-2H_2O$), 344 ($398-3H_2O$), 326 ($398-4H_2O$), 308 ($398-5H_2O$), and 290 ($398-6H_2O$). Nuclear magnetic resonance (NMR) spectrum of I showed a secondary methyl at δ 1.21 (d, $J$=6 Hz, 21-$CH_3$), two tertiary methyls at δ 1.56 (s, 19-$CH_3$) and 1.97 (s, 18-$CH_3$), three hydroxyl methines at δ 3.87 (q, $J$=6 Hz, 20-H), 4.13 (broad s, 6α-H), and 4.79 (m, 3α-H), and one methine geminal to the ester group at δ 5.10 (m), and two *trans*-olefinic protons at δ 6.84 (d, $J$=16 Hz) and 8.23 (d, $J$=16 Hz).

Hydrolysis of I gave glycosarcostin[5] (III), mp 294—299°, and cinnamic acid which was identified by gas-liquid chromatography (GLC) as its methyl ester. The structure of cinnamoylglycosarcostin was, therefore, assigned to I, and this structure was also supported by its ultraviolet (UV) spectrum $\lambda_{max}$ at 217 nm (log ε 4.20), 223 (4.14), and 280 (4.20), and the ester group was suggested to be located at C-12β from the biogenetic analogy and the comparison of NMR spectra of I and III.

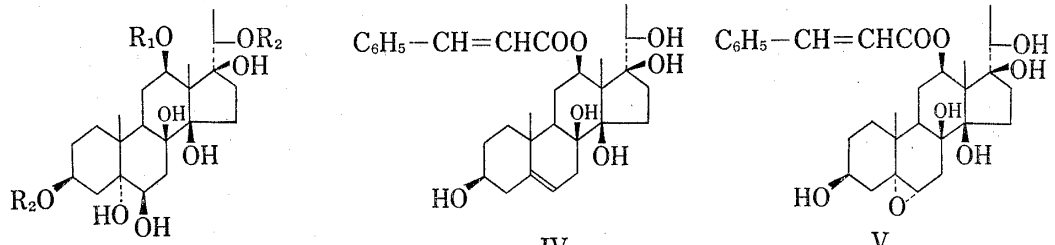
Acetylation of I with acetic anhydride–pyridine at room temperature gave 3,20-diacetate (II), mp 202—204°,, without acetylation of 6β-axial hydroxyl group because of steric hindrance, and NMR spectrum of II showed a singlet at δ 2.04 (6H, $COCH_3$) and a multiplet at δ 3.56 (1H, 6α-H).

1) Part XL: H. Seto, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **24**, 2457 (1976).
2) Location: *Kita-12-jo, Nishi-5-chome, Kita-ku, Sapporo, 060, Japan.*
3) H. Bando, T. Yamagishi, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **24**, 1842 (1976).
4) T. Yamagishi, K. Hayashi, R. Kiyama, and H. Mitsuhashi, *Tetrahedron Letters*, **1972**, 4005.
5) T. Yamagishi, K. Hayashi, and H. Mitsuhashi, *Tetrahedron Letters*, **1973**, 4735.
6) M. Fukuoka and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **16**, 553 (1968); *idem, ibid.*, **17**, 2448 (1969); K. Hayashi, and H. Mitsuhashi, *ibid.*, **20**, 2065 (1972).

The structure of I was confirmed by glycolation of penupogenin (IV), which was isolated from the same plant and also from *Marsdenia tomentosa* Decne by Sasaki, *et al.*[7]

Epoxidation of IV with *m*-chloroperbenzoic acid yielded an α-epoxide (V), mp 210—212.5°, which was converted to glycopenupogenin (I), $[\alpha]_D$ +21.0°, by *trans*-cleavage with perchloric acid. Glycopenupogenin (I) obtained through such a glycolation was also acetylated to 3,20-diacetate (II), mp 198.5—203.5°, under the condition as above.

Comparison of the natural compound (I) and its diacetate (II) with the glycolated penupogenin and its diacetate, respectively, showed identical *Rf* value on TLC, coloration to $SbCl_3$, and other physical properties based on UV, IR, NMR, and mass spectra, and the structure of I was defined as 12β-*O*-cinnamoyloxy-3β,5α,6β,8β,14β,17β,20α-heptahydroxypregnane.[8]




I : $R_1=C_6H_5-CH=CHCO$, $R_2=H$
II : $R_1=C_6H_5-CH=CHCO$, $R_2=CH_3CO$
III : $R_1=R_2=H$

IV V

Fig. 1

## Experimental

All melting points were determined on a Kofler hot stage and are uncorrected. Optical rotations were measured in MeOH solution on a Hitachi S115-4 polarimeter; NMR spectra were taken at 100 MHz in $CDCl_3$ and pyridine-$d_5$ solution with a JEOL PS-100 spectrometer using tetramethylsilane as an internal standard and abbreviations used are s=singlet, d=doublet, q=quartet, and m=multiplet; IR spectra were in Nujol and $CHCl_3$ with a Hitachi 215 spectrometer and UV spectra in MeOH solution with a Hitachi EPS-3T spectrometer. GLC was carried out on a Shimadzu GC-4BPF using a glass column (3 m×3 mm) packed with SE-30 on Chromosorb-W (AW-DMCS) under $N_2$ carrier gas flow control (1.6 kg/cm²). TLC was performed on silica gel $HF_{254}$ and $PF_{254}$ (Merck, Type 60).

**Glycopenupogenin (I)**——From the crude aglycone fraction (840 mg) obtained glycocaudatin in the previous work, I was separated as an amorphous powder (80 mg), $[\alpha]_D$ +15.9° ($c$=0.44), by preparative TLC using benzene–acetone and $CHCl_3$–MeOH as solvent system. Mass Spectrum *m/e*: 398, 362, 344, 326, 308, 290, 148, 147, 77. IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3400, 1700, 1635, 1575, 1160. NMR (δ) pyridine-$d_5$+$D_2O$: 1.21 (3H, d, $J$=6 Hz), 1.56 (3H, s), 1.97 (3H, s), 3.87 (1H, q, $J$=6 Hz), 4.13 (1H, broad s), 4.79 (1H, m), 5.10 (1H, m), 6.84 (1H, d, $J$=16 Hz), 7.22—7.48 (5H, m), 8.23 (1H, d, $J$=16 Hz). UV $\lambda_{max}^{MeOH}$ nm (log ε): 217 (4.20), 223 (4.18), 280 (4.20).

**Alkaline Hydrolysis of Glycopenupogenin (I)**——1) A solution of 20 mg of I in 2 ml of 5% KOH–MeOH was allowed to stand for 20 hr at room temperature and 20 ml of $H_2O$ was added. This reaction mixture was extracted with ether continuously. Evaporation of ether and crystallization of the extract from MeOH–$H_2O$ afforded III as prisms (9.5 mg), mp 294—299°, identical with the authentic glycosarcostin, mp and mixed mp 290.5—297°.

2) Glycopenupogenin (5 mg) was also hydrolyzed in the same way as above, the reaction mixture was acidified with 10% $H_3PO_4$ solution, and extracted with ether. Ether extract was methylated with $CH_2N_2$, and was found by GLC to contain methyl cinnamate by comparison of the retention time (5.3 min at column temperature of 150°) with that of the authentic standard.

**Acetylation of Glycopenupogenin (I)**——A solution of I (50 mg) in 1 ml of $Ac_2O$ and 1 ml of pyridine was allowed to stand for 20 hr at room temperature and poured into ice-water. The precipitate that formed was

7) T. Sasaki, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **20**, 628 (1972).
8) K. Hayashi and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **23**, 1845 (1975).

collected and purified by preparative TLC (benzene: acetone 2: 1). Recrystallization of the precipitate from $CHCl_3$–MeOH–hexane afforded 36 mg of a diacetate (II) as a crystalline powder, mp 202—204°. *Anal.* Calcd. for $C_{34}H_{46}O_{11}$: C, 64.74; H, 7.35. Found: C, 64.95; H, 7.40. Mass Spectrum *m/e*: 482 (M−cinnamic acid), 422 (482−acetic acid), 362 (422−acetic acid), 148, 147, 131, 77, 43 (base peak). IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3450—3400, 1720 (shoulder), 1705, 1635, 1250, 1180. NMR ($\delta$) $CDCl_3$: 1.22 (3H, d, $J$=6 Hz), 1.33 (3H, s), 1.48 (3H, s), 2.03 (6H, s), 3.58 (1H, broad s), 4.63 (1H, q, $J$=6 Hz), 4.78 (1H, m), 5.14 (1H, m), 6.25 (1H, d, $J$=16 Hz), 7.29—7.48 (5H, m), 7.59 (1H, d, $J$=16 Hz).

**Epoxidation of Penupogenin (IV)**——A solution of *m*-chloroperbenzoic acid (123 mg) in $CH_2Cl_2$ (2 ml) was added during 1 hr to a stirring solution of IV (200 mg) in $CH_2Cl_2$ (2 ml) under cooling with ice-water, and excess peracid was destroyed by the addition of 10% $Na_2SO_3$ solution. The reaction mixture was extracted with $CHCl_3$, and the $CHCl_3$ layer was washed consecutively with 5% $NaHCO_3$, $H_2O$, and saturated NaCl solution. The organic layer was dried over anhydrous $Na_2SO_4$ and evaporated *in vacuo*. The residue was purified by preparative TLC ($CHCl_3$: MeOH, 10: 1) to give the epoxide (V) (179 mg), which was recrystallized from benzene–hexane to needles, mp 210—212.5°. *Anal.* Calcd. for $C_{30}H_{40}O_8$: C, 68.16; H, 7.63. Found: C, 68.06; H, 7.66. IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3430, 1700, 1630, 1575, 1180, 1075. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 217 (4.18), 223 (4.11), 280.5 (4.30). NMR ($\delta$) $CDCl_3$: 1.11 (3H, d, $J$=6 Hz), 1.14 (3H, s), 1.53 (3H, s), 3.40 (1H, broad s, $J$ 1/2 HW=5 Hz), 3.51 (1H, q, $J$=6 Hz), 3.72 (1H, m), 4.65 (1H, d.d, $J$=4 Hz, 11 Hz), 6.50 (1H, d, $J$=16 Hz), 7.32—7.65 (5H, m), 7.83 (1H, d, $J$=16 Hz). Mass Spectrum *m/e*: 483 (M−$C_2H_5O$), 380 (M−cinnamic acid), 148, 147, 131 (base peak).

**Cleavage of Penupogenin Epoxide (V) with Perchloric Acid**——A solution of 7% $HClO_4$ (0.3 ml) was added to a stirring solution of the epoxide (V) (120 mg) in tetrahydrofuran (10 ml) at room temperature during 3 hr. After neutralization with 5% $NaHCO_3$, a small volume of $H_2O$ was added, tetrahydrofuran was evaporated *in vacuo*, and the resulting mixture was filtered to give I as an amorphous powder (88 mg), $[\alpha]_D$ +21.0° ($c$= 0.57). *Anal.* Calcd. for $C_{30}H_{42}O_9 \cdot 1/2H_2O$: C, 64.85; H, 7.79. Found: C, 64.77; H, 7.73. All spectral data were identical with those of glycopenupogenin (I).

**Acetylation of Glycolated Penupogenin**——A solution of glycolated penupogenin (50 mg) in $Ac_2O$ (1 ml) and pyridine (1 ml) was allowed to stand for 20 hr at room temperature, and worked up in the same way as for the acetylation of glycopenupogenin (I) to yield an amorphous powder (40 mg), which was recrystallized from $CHCl_3$–ether–hexane to a crystalline powder, mp 198.5—203.5°, whose spectral data were identical with those of glycopenupogenin 3,20-diacetate (II) mp 202—204°. Mixed mp 198—203°.

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