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Constituents of Asclepiadaceae Plants. XXXIII.[1] Component of *Marsdenia tomentosa* Decne. Structure of Tomentosin

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A new polyoxypregnane derivative, tomentosin (12β-*O*-tigloyl-20-*O*-acetyl-tomentogenin) was isolated from the stem of *Marsdenia tomentosa*. Tomentosin is the first example of the diester possessing a tomentogenin skeleton to be isolated from the Asclepiadaceae plant, and it did not undergo any internal acyl migration which occurrs on mild alkaline hydrolysis of C-12β, 20-diester of sarcostin and other polyoxypregnane derivatives.

The structures of several polyoxypregnane derivatives isolated from the stems of *Marsdenia tomentosa* Decne (Japanese name; "Kidjoran"), a plant of Asclepiadaceae family, have been reported previously.[3] Further examination of the aglycone resulted in the isolation of some polyoxypregnane derivatives, compound A, B, C and D. In this paper, we report the isolation and the structure of compound C (I), a new diester of polyoxypregnane.

The aglycone mixture, obtained by a mild acid hydrolysis of the crude glycoside,[3] was separated by silica gel column chromatography and preparative thin-layer chromatography (TLC). These procedures yielded a fine crystalline compound (I), mp 148—150°, $[\alpha]_D^{19}$ +43.5 (c=0.23, $CHCl_3$). The molecular formula of $C_{28}H_{44}O_7$ from elemental analysis and mass spectrum (M^+-H_2O at m/e 474) was given for I. Infrared (IR) spectrum of I showed absorptions for hydroxyl groups at 3400 and 1080 cm^{-1}, a saturated ester at 1735 and 1240 cm^{-1}, and an α,β-unsaturated ester at 1710, 1690, 1650 and 1150 cm^{-1}, which was supported by ultraviolet (UV) absorption at 216 nm (log ε, 4.14). The nuclear magnetic resonance (NMR) spectrum of I showed signals for two tertiary methyl groups at δ 0.78 and 1.22, one secondary methyl group at 1.27 (d, J=7 Hz), two vinyl-methyl groups at 1.84 (s) and 1.88 (d, J=6 Hz), one acetyl group at 1.90 (s), three hydroxy-methines at 3.65 (br, m), 4.54 (q, J=6 Hz), and 4.68 (d.d, J=6,11 Hz), and one olefinic proton at 6.84 (q, J=6 Hz). The mass spectrum of I showed the presence of a tigloyl group at m/e 392 ($M^+-C_5H_8O_2$), 83 (C_5H_7O), and an acetyl group at m/e 432 ($M^+-CH_3CO_2H$), 43 ($COCH_3$). Hydrolysis of I with 5% methanolic potassium hydroxide afforded tomentogenin (II)[3b,4] as a neutral product. These facts suggest that I is a diester of tomentogenin with acetic acid and tiglic acid.

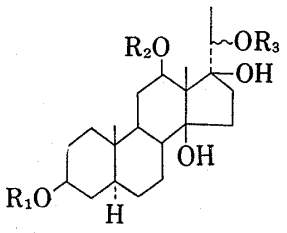
Acetylation of I with acetic anhydride-pyridine afforded a monoacetate (III), mp 192—197°. A new methyl signal appeared at δ 2.02 and a hydroxy-methine signal at δ 3.65 shifted to 4.60 in the NMR spectrum of this acetate (III). This result indicates that a hydroxy-methine of I at δ 3.65 is assigned as 3α-proton.

Mild alkaline hydrolysis of I with saturated methanolic porassium carbonate gave a monoester (IV), mp 210—215°, whose IR spectrum showed absorptions for an α,β-unsaturated ester at 1720, 1705, 1650 and 1165 cm^{-1}, which was supported by UV absorption at 213 nm

1) Part XXXII: K. Hayashi and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo) **23**, 139 (1975).
2) Location: *Kita-21-jo, Nishi-5-chome, Kita-ku, Sapporo, 060, Japan.*
3) *a*) H. Mitsuhashi, I. Takemori, Y. Shimizu, T. Nomura, and E. Yamada, *Chem. Pharm. Bull.* (Tokyo), **10**, 804 (1962); *b*) H. Mitsuhashi, T. Sato, T. Nomura, and I. Takemori, *ibid.*, **13**, 267 (1965); *c*) M. Fukuoka and H. Mitsuhashi, *ibid.*, **16**, 1634 (1968); *d*) T. Sasaki, K. Hayashi, and H. Mitsuhashi, *ibid.*, **20**, 628 (1972).
4) H. Mitsuhashi, T. Sato, T. Nomura, and I. Takemori, *Chem. Pharm. Bull.* (Tokyo), **12**, 981 (1972).

(log ε, 4.13). The signal for the acetyl group at δ 1.90 disappeared and that for the hydroxy-methine at 4.54 (q, J=6 Hz) shifted to 3.65. From these results, the hydroxy-methine at δ 4.54 (q, J=6 Hz) was assigned as C-20 proton, which was supported by the mass spectrum at m/e 415 (M^+-45),[5] and the remaining hydroxy-methine at 4.68 (d.d, J=6, 11 Hz) was assigned as 12α-proton.[5]

In order to confirm the position of the ester linkages of I, the monoester (IV) was acetylated with acetic anhydride-pyridine to afford a diacetate, which was identical with compound C monoacetate (III) in mixed mp and spectral data.



I : R_1=H, R_2= Me Me / C=C-C- / H O, R_3=CH_3C- (tomentosin)

II : R_1=R_2=R_3=H (tomentogenin)

III : R_1=CH_3C-, R_2= Me Me / C=C-C- / H O, R_3=CH_3-C (tomentosin acetate)

IV: R_1=H, R_2= Me Me / C-C-C= / H O, R_3=H (12β-O-tigloyl-tomentogenin)

Fig. 1

From these evidences, the compound C (I) was determined as 12β-*O*-tigloyl-20-*O*-acetyltomentogenin and was named tomentosin. This is the first example of a diester possessing tomentogenin skeleton to be isolated from a plant of the Asclepiadaceae family. The internal acyl migration between C-12β and C-20-OH on mild alkaline hydrolysis has been proved with several polyoxypregnane derivatives,[6] but tomentosin (I) afforded only 12β-*O*-tigloyl monoester (IV) on mild alkaline hydrolysis with potassium carbonate. This result indicates that the internal acyl migration did not occur in tomentosin (I).

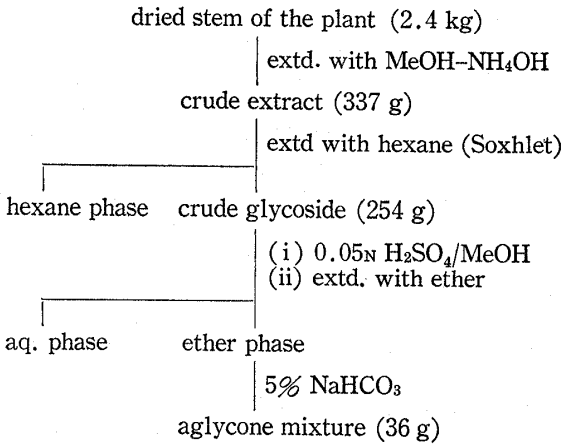


Chart 1. Extraction and Separation

Experimental

Melting points were determined on a Kofler hot stage and are uncorrected. Optical rotations were measured in $CHCl_3$ solution on a Hitachi S115-4 polarimeter. NMR spectra were determined on a JEOL PS-100 spectrometer operating at 100 MHz with tetramethylsilane (TMS) as an internal standard. Mass spectra were determined on a Hitachi RMU-7 mass spectrometer, IR spectra were taken in Nujol mull on a Hitachi 215 spectrometer, and UV spectra were determined in EtOH solution on a Hitachi EPS-3T spectrometer. Gas–liquid chromatography (GLC) was carried out on a Shimadzu CG-3BF gas chromatograph using a glass column (2 m×3 mm) packed with 25% DEGS on Chromosorb-W (60—80 mesh) with N_2 carrier gas flow-rate of 60 ml/min. TLC was performed on silica gel HF_{254} (Merck, Type 60) and silica gel 0.05—0.2 mm (Merck, 70—325 mesh ASTM) was used for column chromatography.

Isolation of the Aglycone Mixture——The stem (2.4 kg) of *M. tomentosa*, collected at Owasa, Mie Prefecture, in November 1973, was extracted with ammoniacal MeOH to yield a crude extract (337 g), and the crude glycoside (254 g) was obtained from this extract after hexane treatment (Soxhlet). A solution of 240 g of the crude glycoside dissolved in 1.2 liters MeOH was refluxed for 30 min with 1.2 liters of 0.1 N H_2SO_4 on a water bath, 1.2 liters of H_2O was added, MeOH was evaporated *in vacuo*, and the residual aqueous solution

5) M. Fukuoka, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **19**, 1469, (1971).
6) T. Yamagishi, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **20**, 2289 (1972).

was heated at 60° for 30 min. The resulting mixture was extracted five times with 3 liters of ether, which was washed with 5% $NaHCO_3$ solution and H_2O, and dried over anhydrous Na_2SO_4 to yield 36 g of an ester-type aglycone mixture.

Tomentosin (I)——From 15 g of the ester-type aglycone mixture, 413 mg of tomentosin (I) was obtained by column chromatography and preparative TLC. I was recrystallized from hexane–acetone to afford needles, mp 148—150°, $[\alpha]_D^{19}$+43.5 (c=0.23, $CHCl_3$). Mass Spectrum m/e: 474 (M^+-H_2O), 456 (M^+-2H_2O), 432 (M^+-acetic acid), 414 (M^+-acetic acid-H_2O), 396 (M^+-acetic acid-2H_2O), 392 (M^+-tiglic acid), 374 (M^+-tiglic acid-H_2O), 356 (M^+-tiglic acid-2H_2O), 332 (M^+-tiglic acid-acetic acid), 314 (M^+-tiglic acid-acetic acid-H_2O), 296(M^+-tiglic acid-acetic acid-2H_2O), 83 (base peak), 55, 43. IR ν_{max}^{Nujol} cm^{-1}: 3400, 1735, 1710, 1650, 1270, 1240, 1150, 1080, 1040. UV λ_{max}^{EtOH} 216 nm (log ε, 4.14). NMR (δ) $CDCl_3$: 0.78 (3H, s, 18-Me), 1.22 (3H, s, 19-Me), 1.24 (3H, d, J=7 Hz, 21-Me), 1.84 (3H, s, vinyl-Me), 1.88 (3H, d, J=6 Hz, vinyl-Me), 1.90 (3H, s, OAc), 3.65 (1H, m, 3α-H), 4.54 (1H, q, J=6 Hz, 20-H), 4.68 (1H, d.d, J=6,11 Hz, 12α-H), 6.84 (1H, q, J=6 Hz). *Anal.* Calcd. for $C_{28}H_{44}O_7$: C, 68.26; H, 9.00. Found: C, 68.03; H, 9.04.

Acetylation of Tomentosin (I)——A solution of 72 mg of tomentosin, 1 ml of Ac_2O, and 1 ml of pyridine was allowed to stand for 24 hr at room temperature, and poured into ice-water. A white powder that appeared was collected and purified by preparative TLC ether. Recrystallization from acetone–hexane afforded 58 mg of tomentosin monoacetate (III) as needles, mp 192—196°. Mass Spectrum m/e: 516 (M^+-H_2O), 456 (M^+-acetic acid-H_2O), 438 (M^+-acetic acid-2H_2O), 434 (M^+-tiglic acid), 416 (M^+-tiglic acid-H_2O), 398 (M^+-tiglic acid-2H_2O), 396 (M^+-2×acetic acid-H_2O), 374 (M^+-tiglic acid-acetic acid), 83 (base peak), 55, 43. IR ν_{max}^{Nujol} cm^{-1}: 3530, 3450, 1740, 1725, 1710, 1650, 1260, 1240, 1160, 1080, 1030. NMR (δ) $CDCl_3$: 0.80 (3H, s, 18-Me), 1.22 (3H, s, 19-Me), 1.24 (3H, d, J=7 Hz, 21-Me), 1.84 (3H, s, vinyl-Me), 1.88 (3H, d, J=6 Hz, vinyl-Me), 1.90 (3H, s, OAc), 2.02 (3H, s, OAc), 4.52 (1H, q, J=6 Hz, 20-H) 4.60 (1H, m, 3α-H), 4.68 (1H, d.d, J=6,11 Hz, 12α-H), 6.84 (1H, q, J=6 Hz).

Alkaline Hydrolysis of Tomentosin (I)——a) A solution of 35 mg of tomentosin (I) in 5 ml of 5% MeOH–KOH was allowed to stand for 36 hr at room temperature and the reaction mixture was purified directly by preparative TLC (MeOH:$CHCl_3$, 1:19). Recrystallization from MeOH–acetone gave 20 mg of tomentogenin (II) as prisms, mp 263—267°. Mass Spectrum m/e: 368 (M^+), 350 (M^+-H_2O), 332 (M^+-2H_2O), 323 (M^+-45), 305 (M^+-45-H_2O, base peak), 287 (M^+-45-2H_2O), 269 (M^+-45-3H_2O). IR ν_{max}^{Nujol} cm^{-1}: 3600—3100, 1040. NMR (δ) pyridine-d_5: 0.76 (3H, s, 18-Me), 1.54 (3H, d, J=7 Hz, 21-Me), 1.64 (3H, s, 19-Me), 3.74 (1H, d.d, J=6, 11 Hz, 12α-H), 3.80 (1H, m, 3α-H), 4.38 (1H, q, J=6 Hz, 20-H).

b) A solution of 8.0 mg of tomentosin (I) in 1 ml of 5% MeOH–KOH was allowed to stand for 48 hr at room temperature. The reaction mixture was acidified with 10% H_3PO_4 solution and extracted with ether. The ether extract was found by GLC to be composed of AcOH and tiglic acid by the comparison of retention times (AcOH, 1.8 min at 120°; tiglic acid, 5.2 min at 140°) with those of authentic standards (AcOH, 1.8 min at 120°, tiglic acid, 5.0 min at 140°).

Partial Hydrolysis of Tomentosin (I)——A solution of 200 mg of I in 10 ml of saturated MeOH–K_2CO_3 was allowed to stand for 12 hr at room temperature. After addition of 10 ml of H_2O, MeOH was evaporated *in vacuo*. The residual aqueous solution was extracted with ether, and the ether phase was purified by preparative TLC (MeOH:$CHCl_3$, 1.19) to afford 20 mg of monoester (IV) and 120 mg of I. IV was recrystallized from acetone–hexane to give needles, mp 210—215°. Mass Spectrum m/e: 414 (M^+-2H_2O), 405 (M^+-45), 396 (M^+-3H_2O), 387 (M^+-45-H_2O), 350 (M^+-tiglic acid), 332 (M^+-tiglic acid-H_2O), 314 (M^+-tiglic acid-2H_2O), 296 (M^+-tiglic acid-3H_2O), 83 (base peak) 55. IR ν_{max}^{Nujol} cm^{-1}: 3575, 3475, 1705, 1650, 1265, 1160, 1085. UV λ_{max}^{EtOH}: 213 nm (log ε 4.13). NMR (δ) pyridine-d_5: 0.82 (3H, s, 18-Me), 1.52 (3H, d, J=7 Hz, 21-Me), 1.58 (3H, s, 19-Me), 1.88 (3H, d, J=6 Hz, vinyl-Me), 1.96 (3H, s, vinyl-Me), 3.65 (1H, m, 3α-H), 3.70 (1H, q, J=6 Hz, 20-H), 4.70 (1H, d.d., J=6, 11 Hz, 12α-H), 7.02 (1H, q, J=6 Hz).

Acetylation of Monoester (IV)——A solution of 20 mg of IV in 1 ml of pyridine and 1 ml of Ac_2O was allowed to stand for 48 hr at room temperature and worked up in the same way as for the acetylation of I to yield 15 ml of an amorphous product, which was recrystallized from acetone–hexane to give needles, mp 190—195° and mixed mp with III 190—193°. All spectral data were identical with those of III.

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