

Studies on the Constituents of Asclepiadaceae Plants. XLIII.¹⁾
Component of *Marsdenia tomentosa* DECNE.
Structure of Tomentomin

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A new polyoxypregnane derivative, tomentomin (12 β -O-cinnamoyl-20 α -O-nicotinoyl-tomentogenin), was isolated from the leaf of *Marsdenia tomentosa*. Tomentomin is the first tomentogenin derivative with nicotinic acid in ester-linking to be isolated from Asclepiadaceae plants.

Keywords—*Marsdenia tomentosa*; Asclepiadaceae; leaf; polyoxypregnane; tomentomin; 12 β -O-cinnamoyl-20 α -O-nicotinoyltomentogenin

Our previous papers reported the structures of several ester-type polyoxypregnane derivatives^{1,3)} from the stem of *Marsdenia tomentosa* DECNE (Asclepiadaceae). In this paper, we report some finding on the component of the leaf of this plant.

The aglycone mixture, obtained by a mild acid hydrolysis of the crude glycoside,⁴⁾ was separated and purified by silica gel column chromatography and preparative thin-layer chromatography (TLC). These procedures yielded four fine crystalline substances, tentatively named compounds-A, -B, -C, and -D, which were main components of the aglycone mixture. Among these, compounds-A, -B, and -C were identical with penupogenin^{4a,5)} (I), 20-O-acetylpenupogenin^{3e)} (II), and gagaminin⁶⁾ (III), respectively, from the comparison of spectral data and mixed mp with authentic samples.

Compound-D (IV) showed mp 155–157° and $[\alpha]_D^{20} + 137^\circ$ ($c = 0.40$, in CHCl_3). The molecular formula $\text{C}_{36}\text{H}_{45}\text{O}_7\text{N}$ was given for IV from its elemental analysis and mass spectrum (M^+ -nicotinic acid at m/e 480). The infrared (IR) spectrum of IV showed absorption for hydroxyl groups at 3400 and 1030 cm^{-1} , and α, β -unsaturated esters at 1720, 1710, 1690, 1640, 1590, 1170, and 1150 cm^{-1} . The nuclear magnetic resonance (NMR) spectrum of IV showed signals for two tertiary methyl groups at δ 0.76 (s) and 1.46 (s), one secondary methyl group at 1.36 (d, $J = 6$ Hz), three hydroxy-methines at 3.50 (m), 4.76 (d.d, $J = 6, 11$ Hz), and 4.82 (q, $J = 16$ Hz), and eleven olefinic protons at 5.98 (1H, d, $J = 16$ Hz), 7.20 (6H, m), 7.38 (1H, d, $J = 6$ Hz), 8.04 (1H, d, $J = 8$ Hz), 8.64 (1H, d, $J = 6$ Hz), and 9.10 (1H, s).

Hydrolysis of IV with 5% methanolic potassium hydroxide afforded tomentogenin^{4c,7,8)} (V) as a neutral product. Prominent mass spectral peaks of IV indicative of cinnamate and

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2) Location: Kita-12-jo, Nishi-6-chome, Kita-ku, Sapporo 060, Japan.

3) a) H. Seto, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **23**, 1552 (1975); b) H. Seto, K. Hayashi, and H. Mitsuhashi, *ibid.*, **23**, 2397 (1975); c) H. Seto, K. Hayashi, and H. Mitsuhashi, *ibid.*, **24**, 443 (1976); d) H. Seto, K. Hayashi, and H. Mitsuhashi, *ibid.*, **24**, 1552 (1976); e) H. Seto, T. Sasaki, K. Hayashi, and H. Mitsuhashi, *ibid.*, **24**, 2185 (1976).

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nicotinate functional groups were observed at m/e 131 and 106, respectively. Further evidence was secured from the mass spectral peaks of IV since there were no parent ion at m/e 603 but other fragments at m/e 480 (M^+ -nicotinic acid), 462 (M^+ -nicotinic acid- H_2O), 455 (M^+ -cinnamic acid), 453 (M^+ - $CHO \cdot C_6H_4ON \cdot Me$),⁹ 437 (M^+ -cinnamic acid- H_2O), 435 (M^+ - $CHO \cdot C_6H_4ON \cdot Me - H_2O$), 314 (M^+ -cinnamic acid-nicotinic acid), 131 (cinnamoyl cation), and 106 (nicotinoyl cation). The presence of cinnamate and nicotinate functional groups was also supported by ultraviolet (UV) absorptions at 218 ($\log \epsilon$ 4.48), 222 (4.39), and 273 (4.20) nm. These evidences suggest that IV is a diester of tomentogenin (V) with cinnamic acid and nicotinic acid. The peak at m/e 453 definitely indicated that the nicotinate moiety was at C-20 of tomentogenin (V), thus placing the cinnamate group at C-12.

The positions of ester groups in IV were also examined by the NMR spin decoupling experiments. Irradiation of 21-Me group protons (δ 1.36, 3H, d, $J=6$ Hz), collapsed the quartet at δ 4.82 to a singlet, but the double-doublet at δ 4.78 showed no change. This signal at δ 4.78 corresponds to 12α -H.¹⁰ On the basis of these results, the structure of compound-D (IV) was determined as 12β -O-cinnamoyl-20 α -O-nicotinoyltomentogenin and was named tomentomin. This is the first example of a tomentogenin derivative with an ester-linking with nicotinic acid to be isolated from a plant of Asclepiadaceae family.

The isolation of tomentomin (IV) and gagaminin (III) from only the leaf portion of *M. tomentosa* is very interesting from a biogenetical point of view, namely the site of nicotinoylation at C-20 of polyoxypregnane, since they could not be detected from the stem and other portions of this plant even on TLC analysis with several solvent systems.

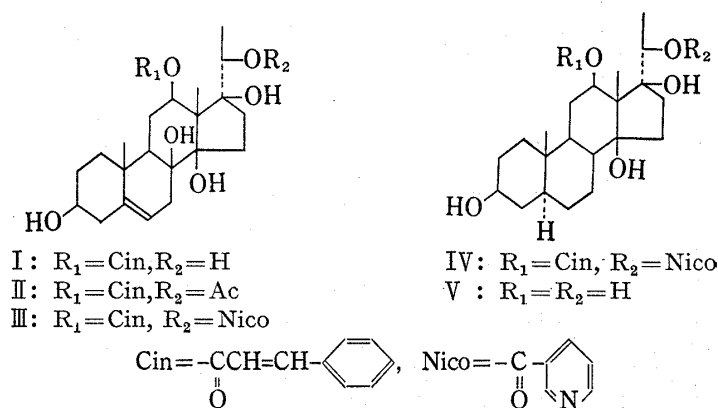


Fig. 1

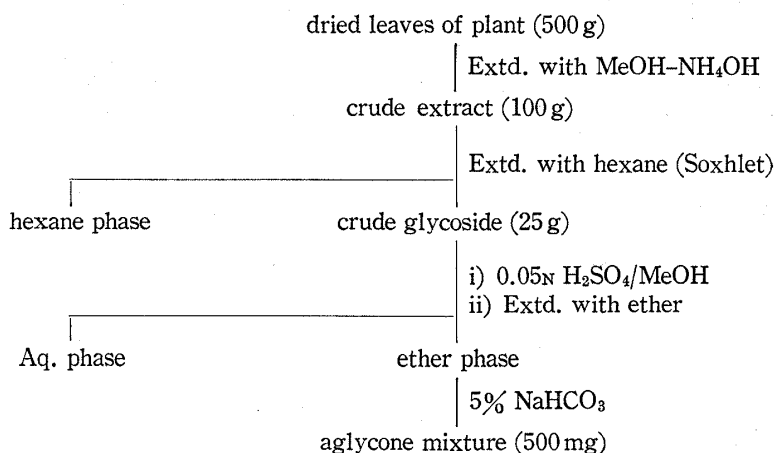


Chart 1

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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. TLC was performed on silica gel HF₂₅₄ (Merck, Type 60), and silica gel 0.05–0.2 mm (Merck, 70-325 mesh ASTM) was used for column chromatography.

Isolation of Aglycone Mixture—The dried and powdered leaf (500 g) of *M. tomentosa*, collected in April 1975 at Owase, Mie Prefecture, was used as the material. The ammoniacal MeOH extract (100 g) was treated with hexane to yield the crude glycoside (25 g). A solution of 24 g of the crude glycoside dissolved in 120 ml of MeOH was refluxed for 30 min with 120 ml of 0.1N H_2SO_4 on a water bath, 120 ml of H_2O was added, MeOH was evaporated *in vacuo*, and the residual aqueous solution was heated at 60° for 30 min. The resulting mixture was extracted five times with a total of 200 ml of ether, which was washed with 5% NaHCO_3 solution and H_2O , and dried over anhydrous Na_2SO_4 to yield 500 mg of an ester-type aglycone mixture. The ester-type aglycone mixture (500 mg) was chromatographed over 50 volumes of silica gel, and the column was eluted successively with CHCl_3 , CHCl_3 -MeOH (99:1), CHCl_3 -MeOH (97:3), CHCl_3 -MeOH (95:5), and CHCl_3 -MeOH (9:1).

TABLE I

No.	Solvent system	Volume (ml)	Constituents	Weight (mg)
1	CHCl_3	1000		
2	CHCl_3 :MeOH (99:1)	1000		
3	CHCl_3 :MeOH (97:3)	500	I, II	50
4	CHCl_3 :MeOH (95:5)	500	III, IV	80
5	CHCl_3 :MeOH (9:1)	1000	residue	

Tomentomin (IV)—From the fraction No. 4, 17 mg of tomentomin (IV) was obtained with 13 mg of gagaminin (III) by repeated preparative TLC (CHCl_3 :MeOH=95:5). IV was recrystallized from EtOAc-hexane to plates, mp 155–157° and $[\alpha]_D^{20} + 137^\circ$ ($c=0.40$, CHCl_3). Mass Spectrum m/e : 480 (M^+ —nicotinic acid), 462 (M^+ —nicotinic acid— H_2O), 455 (M^+ —cinnamic acid), 453 (M^+ — $\text{CHO}\cdot\text{C}_6\text{H}_4\text{ON}\cdot\text{Me}$), 437 (M^+ —cinnamic acid— H_2O), 435 (M^+ — $\text{CHO}\cdot\text{C}_6\text{H}_4\text{ON}\cdot\text{Me}\cdot\text{H}_2\text{O}$), 332 (M^+ —nicotinic acid-cinnamic acid), 314 (M^+ —nicotinic acid-cinnamic acid— H_2O), 305 (M^+ — $\text{CHO}\cdot\text{C}_6\text{H}_4\text{ON}\cdot\text{Me}$ —cinnamic acid), 299, 148, 147, 131 (base peak), 123, 106, 105, 103. IR $\nu_{\text{max}}^{\text{NaCl}}$ cm^{-1} : 3400, 1720, 1710, 1690, 1640, 1590, 1580, 1285, 1170, 1150, 1080, 1040, 1030. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 218 (4.48), 222 (4.39), 273 (4.20). NMR $\delta_{\text{ppm}}^{\text{CDCl}_3}$: 0.76 (3H, s, 19-Me), 1.36 (3H, d, $J=6$ Hz, 21-Me), 1.40 (3H, s, 18-Me), 3.50 (1H, m), 4.78 (1H, d, $J=6$, 11 Hz, 12 α -H), 4.82 (1H, q, $J=6$ Hz, 20 β -H), 5.98 (1H, d, $J=16$ Hz), 7.20 (6H, m), 7.38 (1H, d, $J=16$ Hz), 8.04 (1H, d, $J=8$ Hz), 8.64 (1H, d, $J=6$ Hz), 9.10 (1H, s). Anal. Calcd. for $\text{C}_{36}\text{H}_{45}\text{O}_7\text{N}$: C, 71.61; H, 7.51; N, 2.32. Found: C, 69.94; H, 7.25; N, 2.13.

Alkaline Hydrolysis of Tomentomin (IV)—A solution of 7 mg of tomentomin (IV) in 1 ml of 5% MeOH-KOH was allowed to stand for 24 hr at room temperature and the reaction mixture was purified directly by preparative TLC (CHCl_3 :MeOH=9:1). Recrystallization from MeOH-acetone gave 3 mg of tomentogenin (V) as prisms, mp 263–267°. Mass Spectrum m/e : 368 (M^+), 350 (M^+ — H_2O), 332 (M^+ — $2\text{H}_2\text{O}$), 323 (M^+ — $\text{CHOH}\cdot\text{Me}$), 305 (M^+ — $\text{CHOH}\cdot\text{Me}\cdot\text{H}_2\text{O}$, base peak), 287 (M^+ — $\text{CHOH}\cdot\text{Me}\cdot 2\text{H}_2\text{O}$), 269 (M^+ — $\text{CHOH}\cdot\text{Me}\cdot 3\text{H}_2\text{O}$).

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