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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 (12β-*O*-cinnamoyl-20α-*O*-nicotinoyltomentogenin), 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; 12β-*O*-cinnamoyl-20α-*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[4d,5] (I), 20-*O*-acetylpenupogenin[3e] (II), and gagaminin[6] (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° (c=0.40, in $CHCl_3$). The molecular formula $C_{36}H_{45}O_7N$ 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

1) Part XLII: H. Seto, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **25**, 611 (1977).
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).
4) *a*) H. Mitsuhashi, I. Takemori, Y. Shimizu, T. Nomura, and E. Yamada, *Chem. Pharm. Bull.* (Tokyo), **10**, 882 (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 (1968).
5) H. Mitsuhashi and Y. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **10**, 725 (1962).
6) T. Yamagishi, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **20**, 2289 (1972).
7) H. Mitsuhashi, T. Sato, T. Nomura, and I. Takemori, *Chem. Pharm. Bull.* (Tokyo), **12**, 981 (1964).
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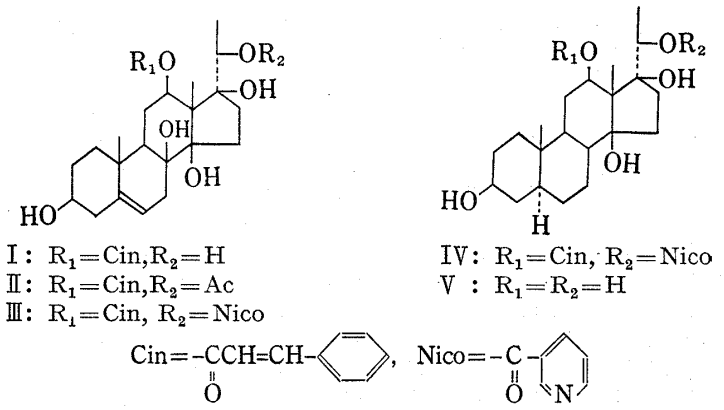
nicotinate functional groups were observed at *m/e* 131 and 106, respectively. Further evidence was secured from the mass spectral peaks of IV since there were no parent ion at *m/e* 603 but other fragments at *m/e* 480 (M^+-nicotinic acid), 462 (M^+-nicotinic acid-H_2O), 455 (M^+−cinnamic acid), 453 ($M^+-CHO.C_6H_4ON\cdot Me$),[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 ε 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-20α-*O*-nicotinoyltomentogenin and was named tomentomin. This is the first example of a tomentogenin derivative with an ester-linking with nicotinic acid to be isolted 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.



I: R_1=Cin, R_2=H
II: R_1=Cin, R_2=Ac
III: R_1=Cin, R_2=Nico
IV: R_1=Cin, R_2=Nico
V: R_1=R_2=H

Cin=−CCH=CH−(C6H5), Nico=−C(=O)−(C5H4N)

Fig. 1

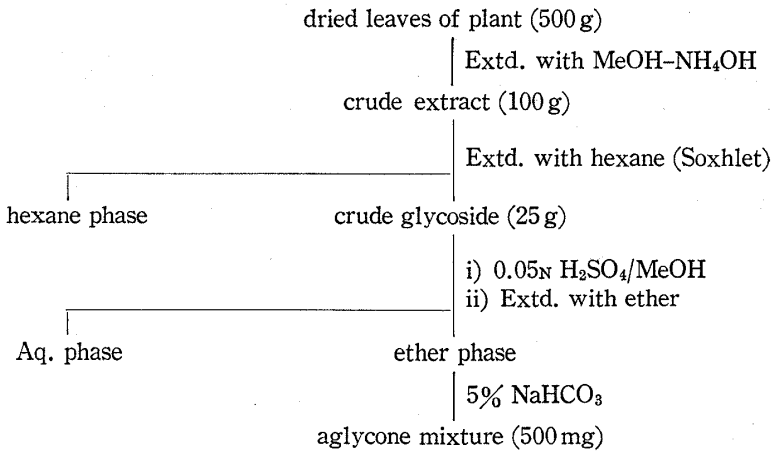


Chart 1

9) M. Fukuoka, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **19**, 1469 (1971).
10) N.S. Bhacca and D.H. Williams, "Application of NMR Spectroscopy in Organic Chemistry-Illustrations from Steroid Field," Holden-Day, Inc., San Francisco, 1964.

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 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 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° (c=0.40, $CHCl_3$). Mass Spectrum m/e: 480 (M^+—nicotinicacid), 462 (M^+—nicotinic acid—H_2O), 455 (M^+—cinnamic acid), 453 (M^+—CHO·C_6H_4ON·Me), 437 (M^+—cinnami cacid —H_2O), 435 (M^+—CHO·C_6H_4ON·Me–H_2O), 332 (M^+—nicotinic acid-cinnamic acid), 314 (M^+—nicotinic acid-cinnamic acid—H_2O), 305 (M^+—CHO·C_6H_4ON·Me—cinnamic acid), 299, 148, 147, 131 (base peak), 123, 106, 105, 103. IR ν_{max}^{Nujol} cm^{-1}: 3400, 1720, 1710, 1690, 1640, 1590, 1580, 1285, 1170, 1150, 1080, 1040, 1030. UV λ_{max}^{EtOH} nm (log ε): 218 (4.48), 222 (4.39), 273 (4.20). NMR $\delta_{ppm}^{CDCl_3}$ 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.d, J=6, 11 Hz, 12α-H), 4.82 (1H, q, J=6 Hz, 20β-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 $C_{36}H_{45}O_7N$: 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^+—$2H_2O$), 323 (M^+—CHOH·Me), 305 (M^+—CHOH·Me–H_2O, base peak), 287 (M^+—CHOH·Me—$2H_2O$), 269 (M^+—CHOH·Me—$3H_2O$).

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