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Constituents of Asclepiadaceae Plants. XXXVII.[1] Component of *Marsdenia tomentosa* Decne: Structure of Deacetyltomentosin and Tomentidin

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Two new polyoxypregnane derivatives, deacetyltomentosin (12β-*O*-tigloyl-tomentogenin) and tomentidin (12β-*O*-acetyl-20-*O*-cinnamoyltomentogenin), were isolated from the stem of *Marsdenia tomentosa*. Deacetyltomentosin is a monoester possessing the tomentogenin skeleton to be isolated from the Asclepiadaceae plants for the first time.

In our previous papers we reported the isolation and characterization of tomentosin[3] (I), tomentin,[4] dehydrotomentin,[4] tomentonin,[1] tomentodin[1] (II), and dehydrotomentosin,[1] new polyoxypregnane derivatives possessing a tomentogenin or an utendin skeleton from the stem of *Marsdenia tomentosa* Decne and the presence of some unidentified ester-type compounds. This paper describes the isolation and structural elucidation of two new polyoxypregnane derivatives, tentatively named compounds G and H.

The aglycone mixture, obtained by a mild acid hydrolysis of the crude glycoside,[5] was separated and purified by silica gel or alumina column chromatography, and preparative thin-layer chromatography (TLC).

These procedures yielded two crystalline substances, compounds G and H. Compound G (III), $C_{26}H_{42}O_6$, mp 218—221°, $[\alpha]_D^{20}$ +41° (c=0.40, $CHCl_3$), m/e 450 (M^+). The infrared (IR) spectrum of III showed absorptions for hydroxyl groups at 3400 and 1035 cm^{-1}, and an α,β-unsaturated ester at 1710, 1690, 1650, and 1140 cm^{-1}, which was supported by ultraviolet (UV) absorption at 218 nm (log ε 4.12). The nuclear magnetic resonance (NMR) spectrum of III showed signals for two tertiary methyl groups at δ 0.82 (s) and 1.28 (s), one secondary methyl group at 1.10 (d, J=6 Hz), two vinyl-methyl groups at 1.82 (d, J=6 Hz) and 1.83 (s), three hydroxy-methines at 3.50 (br. m), 3.56 (q, J=6 Hz), and 4.60 (d.d, J=6, 11 Hz), and one olefinic proton at 6.86 (d, J=6 Hz).

Hydrolysis of III with 5% methanolic potassium hydroxide afforded tomentogenin[5c,6] (IV) as a neutral product. Prominent mass spectral peaks of III indicative of tiglate functional group were observed at m/e 83 (C_5H_7O) and 55 (C_4H_7). Further evidence was secured from the mass spectral peaks of III since there were a faint parent ion at m/e 450 and other fragments at m/e 432 (M^+-H_2O), 405 (M^+–CHOH·Me),[7] 378 (M^+-4H_2O), 350 (M^+–tiglic acid), 332 (M^+–tiglic acid–H_2O), and 83 (tigloyl cation). These evidences suggest that III is a monoester of tomentogenin (IV) with tiglic acid. The peak at m/e 405 definitely suggested that tiglate moiety was not at C-20 of tomentogenin. In order to confirm the position of the ester linkage of III, the NMR spin decoupling experiments were carried out. Irradiation of 21-Me group

1) Part XXXVI: H. Seto, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **24**, 443 (1976).
2) Location: *Kita-12-jo, Nishi-5-chome, Kita-ku, Sapporo, 060, Japan.*
3) H. Seto, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **23**, 1552 (1975).
4) H. Seto, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **23**, 2397 (1975).
5) *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).
6) H. Mitsuhashi, T. Sato, T. Nomura, and I. Takemori, *Chem. Pharm. Bull.* (Tokyo), **12**, 981 (1964).
7) M. Fukuoka, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **19**, 1469 (1971).

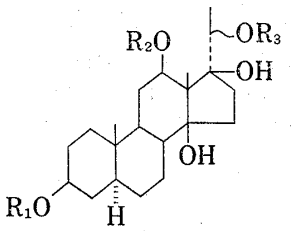
protons (δ 1.10, 3H, d, J=6 Hz) collapsed the quartet at δ 3.56 to a singlet but not the double-doublet at δ 4.60, so that the latter corresponds to 12α-H.[8]

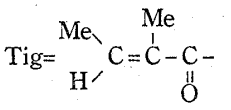
Acetylation of III with acetic anhydride–pyridine afforded a diacetate (V), mp 191—195°, which was identified with tomentosin acetate.[3] On the basis of these data, compound G (III) was determined as 12β-*O*-tigloyltomentogenin and was named deacetyltomentosin.

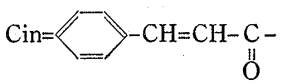
Compound H (VI), $C_{32}H_{44}O_7$, mp 148—150°, $[\alpha]_D^{20}$ +49° (c=0.20, $CHCl_3$), *m/e* 522 (M^+–H_2O). The IR spectrum of VI showed absorptions for hydroxyl groups at 3350 and 1070 cm^{-1}, a saturated ester at 1730 and 1280 cm^{-1}, and an α,β-unsaturated ester at 1710, 1695, 1635, and 1170 cm^{-1}. The NMR spectrum of VI showed signals for two tertiary methyl groups at 0.80 (s) and 1.32 (s), one secondary methyl group at 1.28 (d, J=6 Hz), one acetyl group at 2.20 (s), three hydroxy-methines at 3.60 (m), 4.24 (q, J=6 Hz), and 4.58 (d.d, J=6, 11 Hz), and seven olefinic protons at 6.26 (1H, d, J=16 Hz), 7.40 (5H, m), and 7.58 (1H, d, J=16 Hz). The mass spectrum of VI showed the presence of an acetyl group at *m/e* 480 (M^+–AcOH) and 43 (acetyl cation), and a cinnamoyl group at *m/e* 392 (M^+-cinnamic acid) and 131 (cinnamoyl cation). The presence of a cinnamoyl group was also supported by UV absorptions at 217 (log ε 4.27), 223 (4.25), and 278 (4.35) nm.

Hydrolysis of VI with 5% methanolic potassium hydroxide afforded tomentogenin (VI). These facts suggest that VI is a diester of tomentogenin with acetic acid and tiglic acid. Although tomentodin[1] (II), 12β-*O*-cinnamoyl-20-*O*-acetyl-tomentogenin, had already been isolated from the same plant, physical properties of VI was different from those of II. Acetylation of VI with acetic anhydride–pyridine afforded a monoacetate (VII), mp 174—176°, which was not identified with tomentodin acetate[1] (VIII) from mixed mp and the comparison of spectral data. From these evidences, it was concluded that the structure of compound H (VI) is 12β-*O*-acetyl-20-*O*-cinnamoyltomentogenin and was named tomentidin.

Deacetyltomentosin (II) is the first example of a monoester possessing tomentogenin skeleton to be isolated from a plant of the Asclepiadaceae family. Tomentidin (VI) can not be considered as an artifact produced during hydrolysis or separation procedure since any internal ester exchange or transesterification of polyoxypregnane derivatives was not observed under the condition used in the hydrolysis of the crude glycoside or purification of the aglycone.







I: R_1=H, R_2=Tig, R_3=Ac (tomentosin)
II: R_1=H, R_2=Cin, R_3=Ac (tomentodin)
III: R_1=R_3=H, R_2=Tig (deacetyltomentosin)
IV: R_1=R_2=R_3=H (tomentogenin)
V: R_1=R_3=Ac, R_2=Tig (deacetyltomentosin diacetate=tomentosin acetate)
VI: R_1=H, R_2=Ac, R_3=Cin (tomentidin)
VII: R_1=R_2=Ac, R_3=Cin (tomentidin acetate)
VIII: R_1=R_3=Ac, R_2=Cin (tomentodin acetate)

Experimental

Melting points were determined on a Kofler hot stage and are uncorrected. Optical rotations were measured in $CHCl_3$ solution on a Hitachi Model S115-4 polarimeter. NMR spectra were determined on a

8) N.S. Bhacca and D.H. Williams, "Application of NMR spectroscopy in Organic Chemistry-Illustrations from Steroid Field," Holden-Day, Inc. San Francisco, 1964.

JEOL PS-100 spectrometer operating at 100 MHz, in $CDCl_3$ solution with tetramethylsilane (TMS) as an internal standard, mass spectra on a Hitachi RMU-6E spectrometer, IR spectra in Nujol mull on a Hitachi 215 spectrometer, and UV spectra in EtOH solution on a Hitachi EPS-3T 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) and alumina (Merck, neutral II—III) were used for column chromatography.

Deacetyltomentosin (III)——From 15 g of the ester-type aglycone mixture obtained by the same procedure as reported previously,[3] 58 mg of deacetyltomentosin (III) was obtained by silica gel and alumina column chromatography, and preparative TLC ($CHCl_3$: MeOH=19: 1). III was recrystallized from acetone–hexane to prisms, mp 218—221°, $[\alpha]_D^{20}$ +41° (c=0.40, $CHCl_3$). Mass Spectrum *m/e*: 450 (M^+), 432 (M^+–H_2O), 414 (M^+–$2H_2O$), 405 (M^+–CHOH·Me), 396 (M^+–$3H_2O$), 378 (M^+–$4H_2O$), 350 (M^+–tiglic acid), 332 (M^+–tiglic acid–H_2O), 314 (M^+–tiglic acid–$2H_2O$), 305 (M^+–tiglic acid–CHOH·Me), 262, 249,[4] 105, 83, 55 (base peak). IR ν_{max}^{Nujol} cm^{-1}: 3400, 1710, 1690, 1645, 1270, 1140, 1070, 1035. UV λ_{max}^{EtOH} 218 nm (log ε 4.12). NMR $\delta_{max}^{CDCl_3}$ 0.82 (3H, s, 19-Me), 1.10 (3H, d, J=6 Hz, 21-Me), 1.28 (3H, s, 18-Me), 1.82 (3H, d, J=6 Hz, vinyl-Me), 1.83 (3H, s, vinyl-Me), 3.50 (1H, m, 3α-H), 3.56 (1H, q, J=6 Hz, 20-H), 4.60 (1H, d.d, J=6, 11 Hz, 12α-H), 6.86 (1H, d, J=6 Hz). *Anal.* Calcd. for $C_{26}H_{42}O_6$: C, 69.30; H, 9.40. Found: C, 69.20; H, 9.20.

Alkaline Hydrolysis of Deacetyltomentosin (III)——A solution of 10 mg of deacetyltomentosin (III) in 2 ml of 5% MeOH–KOH was allowed to stand for 26 hr at room temperature and the reaction mixture was purified directly by preparative TLC ($CHCl_3$: MeOH=9: 1). Recrystallization from MeOH–acetone gave 6 mg of tomentogenin (IV) 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–$3H_2O$).

Acetylation of Deacetyltomentosin (III)——A solution of 20 mg of deacetyltomentosin (III), 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 recrystallized from acetone–hexane to afford 20 mg of deacetyltomentosin diacetate as plates, mp 191—195°, and mixed mp with tomentosin acetate 190—193°. 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), 304,[4] 291, 286, 83 (base peak), 55, 43. IR ν_{max}^{Nujol} cm^{-1}: 3530, 3450, 1740, 1725, 1710, 1650, 1260, 1240, 1160, 1080, 1030. NMR $\delta_{max}^{CDCl_3}$: 0.80 (3H, s, 19-Me), 1.22 (3H, s, 18-Me), 1.24 (3H, d, J=6 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, d, J=6 Hz). *Anal.* Calcd. for $C_{30}H_{42}O_8$: C, 67.39; H, 8.67. Found: C, 67.21; H, 8.57.

Tomentodin (VI)——From the same column chromatographic fraction as above, 15 mg of tomentidin (VI) was obtained by repeated preparative TLC (ether, MeOH: $CHCl_3$=1: 99). VI was recrystallized from acetone–hexane to needles, mp 148—150°, $[\alpha]_D^{20}$ +49° (c=0.20, $CHCl_3$). Mass Spectrum *m/e*: 522 (M^+–H_2O), 480 (M^+–acetic acid), 462 (M^+–H_2O–acetic acid), 444 (M^+–$2H_2O$–acetic acid), 392 (M^+–cinnamic acid), 365 (M^+–CHO·C_9H_7O·Me), 332 (M^+–cinnamic acid–acetic acid), 305 (M^+–CHO·C_9H_7O·Me–acetic acid), 262, 249, 244, 148, 147, 131 (base peak), 103, 43. IR ν_{max}^{Nujol} cm^{-1}: 3350, 1720, 1710, 1695, 1635, 1280, 1170, 1070, 1040. UV λ_{max}^{EtOH} nm (log ε): 217 (4.27), 223 (4.25), 278 (4.35). NMR $\delta_{max}^{CDCl_3}$ 0.80 (3H, s, 19-Me), 1.28 (3H, d, J=6 Hz, 21-Me), 1.32 (3H, s, 18-Me), 2.20 (3H, s, OAc), 3.60 (1H, m, 3α-H), 4.24 (1H, q, J=6 Hz, 20-H), 4.58 (1H, d.d, J=6, 11 Hz, 12α-H), 6.26 (1H, d, J=16 Hz), 7.40 (5H, m, aromatic protons), 7.58 (1H, d, J=16 Hz). *Anal.* Calcd. for $C_{32}H_{44}O_7$: C, 71.08; H, 8.20. Found: C, 71.14; H, 8.44.

Alkaline Hydrolysis of Tomentidin (VI)——A solution of 5 mg of tomentidin (VI) in 0.5 ml of 5% MeOH–KOH was allowed to stand for 24 hr at room temperature and worked up in the same manner as in the alkaline hydrolysis of III to afford 3 mg of tomentogenin (IV), as prisms, mp 265—268°. Mass spectral datum was identical with that of alkaline hydrolysed product of IV.

Acetylation of Tomentidin (VI)——A solution of 10 mg of tomentidin (VI), 1 ml of Ac_2O, and 1 ml of pyridine was allowed to stand for 18 hr at room temperature and worked up in the usual manner to afford 8 mg of tomentidin acetate (VII) as needles from acetone–hexane, mp 174—176°. Mass Spectrum *m/e*: 564 (M^+–H_2O), 522 (M^+–acetic acid), 462 (M^+-2×acetic acid), 434 (M^+–cinnamic acid), 416 (M^+-cinnamic acid–H_2O), 407 (M^+–CHO·C_9H_7O·Me), 389 (M^+–CHO·C_9H_7O·Me–H_2O), 374 (M^+–cinnamic acid-acetic acid), 314 (M^+–cinnamic acid-2×acetic acid), 304,[4] 291, 286, 148, 147, 131 (base peak), 103, 43. IR ν_{max}^{Nujol} cm^{-1}: 3400, 1730, 1710, 1690, 1650, 1270, 1250, 1170, 1075, 1035. NMR $\delta_{max}^{CDCl_3}$ 0.82 (3H, s, 19-Me), 1.28 (3H, d, J=6 Hz, 21-Me), 1.32 (3H, s, 18-Me), 2.04 (3H, s, OAc), 2.20 (3H, s, OAc), 4.28 (1H, q, J=6 Hz, 20-H), 4.58 (1H, d.d, J=6, 11 Hz, 12α-H), 4.60 (1H, m, 3α-H), 6.24 (1H, d, J=16 Hz), 7.40 (5H, m, aromatic protons), 7.56 (1H, d, J=16 Hz).

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