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Constituents of Asclepiadaceae Plants. XXXV.[1] Component of *Marsdenia tomentosa* Decne. Structure of Tomentin and Dehydrotomentin

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Two new polyoxypregnane derivatives, tomentin (12β-*O*-,20-*O*-diacetyltomentogenin) and dehydrotomentin (12β-*O*,20-*O*-diacetylutendin), were isolated from the stem of *Marsdenia tomentosa*. Dehydrotomentin is a diester possessing the utendin skeleton to be isolated from the Asclepiadaceae plants for the first time. Very characteristic fragment pattern for tomentogenin derivatives was observed in the mass spectrum of tomentin.

We reported in our previous paper the isolation and characterization of tomentosin,[3] a new polyoxypregnane derivative possessing a tomentogenin skeleton, from the stem of *Marsdenia tomentosa* Decne and the presence of some unidentified ester-type substances, compounds A—H. We now report the isolation and structural elucidation of compounds D and E, new diesters of polyoxypregnane.

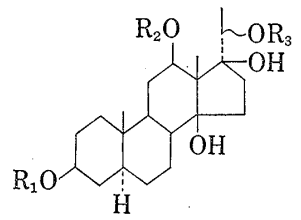
The ester-type aglycone mixture, obtained by a mild acid hydrolysis of the crude glycoside,[4] was separated by silica gel column chromatography and preparative thin-layer chromatography (TLC).

Two fine crystalline substances, compounds D and E, were obtained along with tomentosin from the same column chromatographic fraction, followed by preparative TLC. Compound D (I) showed the following properties: mp 137—141°, $[\alpha]_D^{11}$ +30° (c=0.6, $CHCl_3$); molecular formula of $C_{25}H_{40}O_7$ from elemental analysis and mass spectrum (M^+ at m/e 452). Infrared (IR) spectrum of I showed absorptions for hydroxyl groups at 3300 and 1040 cm^{-1}, and saturated esters at 1735, 1720, 1260, and 1240 cm^{-1}. The nuclear magnetic resonance (NMR) spectrum of I showed signals for two tertiary methyl groups at δ 0.76 (s) and 1.17 (s), one secondary methyl group at 1.21 (d, J=7 Hz), two acetyl groups at 1.92 (s) and 2.04 (s), three hydroxymethines at 3.52 (br. m), 4.52 (q, J=6 Hz), and 4.56 (d.d, J=6, 11 Hz), and no olefinic proton.

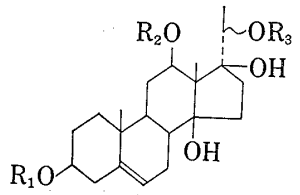
Hydrolysis of I with 5% methanolic potassium hydroxide afforded tomentogenin[4c,5] (II) as a neutral product. Prominent mass spectral peak indicative of acetate functional group was observed at m/e 43. Further evidence was secured from the mass spectral peaks of I since there were faint parent ion at m/e 452 and other fragments at m/e 434 (M^+-H_2O), 392 (M^+—AcOH), 374 (M^+—AcOH–H_2O), 365 (M^+—CHOAc·Me),[5] 332 (M^+—2AcOH), and 43 (acetyl cation). The peak at m/e 365 definitely suggested that one acetate moiety was at C-20 of tomentogenin, thus placing another at C-12. In addition, very characteristic fragments for tomentogenin skeleton analysis were observed at m/e 262, 249, 244, and 226,[4d] also confirmed by another tomentogenin type derivatives including aglycone, monoester- and diesters (Fig. 2). Acetylation of I with acetic anhydride–pyridine afforded an acetate (III),

1) Part XXXIV: K. Hayashi and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), 23, 1845 (1975).
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) *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).
5) H. Mitsuhashi, T. Sato, T. Nomura, and I. Takemori, *Chem. Pharm. Bull.* (Tokyo), **12**, 981 (1964).

mp 285—288°, which was identical with tri-*O*-acetyltomentogenin[4c,5] from mixed mp and the comparison of spectral data. From these evidences, compound D (I) was determined as 12β-*O*,20-*O*-di-acetyltomentogenin and was named tomentin. From the less polar fraction of tomentin, compound E (IV) was isolated and showed the following properties: mp 145—150°, $[\alpha]_D^{19}$ −9° (c=0.45, $CHCl_3$); molecular formula of $C_{25}H_{38}O_7$ from elemental analysis and mass



I: R_1=H, R_2=R_3=Ac (tometin)
II: R_1=R_2=R_3=H (tomentogenin)
III: R_1=R_2=R_3=Ac (tomentin acetate =tri-O-acetyltomentogenin)



IV: R_1=H, R_2=R_3=Ac (dehydrotomentin)
V: R_1=R_2=R_3=Ac (dehydrotomentin acetate =tri-O-acetylutendin)
VI: R_1=R_2=R_3=H (utendin)

Fig. 1

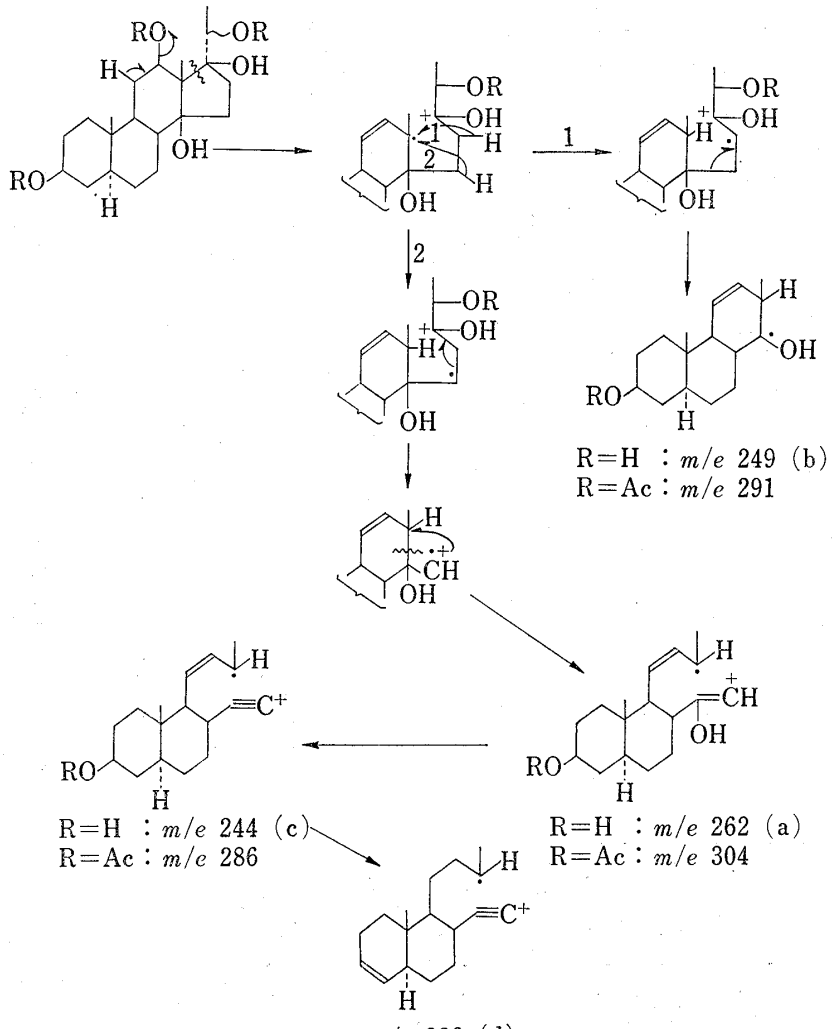


Fig. 2

spectrum (M^+ at *m/e* 450). IR spectrum of IV showed absorptions for hydroxyl groups at 3400 and 1040 cm^{-1}, and for saturated esters at 1735, 1710, 1260, and 1240 cm^{-1}. NMR spectrum of IV showed signals for two tertiary methyl groups at δ 0.99 (s) and 1.21 (s), one secondary methyl group at 1.23 (d, $J=7$ Hz), two acetyl groups at 1.99 (s) and 2.08 (s), three hydroxy-methines at 3.50 (br. m), 4.56 (q, $J=6$ Hz), and 4.60 (m), and one olefinic proton at 5.40. The mass spectrum of IV showed the presence of two acetyl groups at *m/e* 390 (M^+—AcOH), 363 (M^+—CHOAc·CH_3),[6] 330 (M^+—2AcOH), 312 (M^+—2AcOH–$2H_2O$), and 43 (acetyl cation). Acetylation of IV with acetic anhydride–pyridine afforded an acetate (V), mp 255—258° $[\alpha]_D^{19}$ −20°, which was identified with the authentic tri-*O*-acetylutendin,[7] prepared by Reichstein (reported mp 259—261° and $[\alpha]_D^{26}$ −15.9°), from spectral data.

On the basis of these data, it is determined that the structure of compound E (IV) is 12β-*O*,20-*O*-diacetylutendin and was named dehydrotomentin. This is the first example of a diester possessing utendin (VI) skeleton to be isolated from a plant of the Asclepiadaceae family.

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. 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 stem (2.4 kg) of *M. tomentosa*, collected in November 1973 at Owase, Mie Prefecture, was used as the material. The ammoniacal MeOH extract (337 g) was treated with hexane to yield the crude glycoside (254 g). A solution of 240 g of the crude glycoside dissolved in 1.2 liters of MeOH was refluxed for 30 min with 1.2 liters of 0.05 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 was heated at 60° for 30 min. The resulting mixture was extracted five times with total of 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.

Tomentin (I)——From 15 g of the ester-type aglycone mixture, 53 mg of tomentin (I) was obtained by column chromatography and preparative TLC. I was recrystallized from hexane–acetone to needles, mp 137—141°, $[\alpha]_D^{11}$ +30° ($c=0.6$, $CHCl_3$). Mass Spectrum: *m/e* 452 (M^+), 434 (M^+–H_2O), 416 (M^+–$2H_2O$), 392 (M^+–acetic acid), 374 (M^+–acetic acid–H_2O), 365 (M^+–CHOAc·Me) 356 (M^+–acetic acid–$2H_2O$), 296 (M^+–2 × acetic acid–$2H_2O$), 262, 249, 244, 242, 226, 43 (base peak). IR ν_{max}^{Nujol} cm^{-1}: 3300, 1735, 1720, 1640, 1260, 1240, 1040, 1020. NMR (δ) $CDCl_3$: 0.76 (3H, s, 18–Me), 1.17 (3H, s, 19–Me), 1.21 (3H, d, $J=7$ Hz, 21–Me), 1.92 (3H, s, OAc), 2.04 (3H, s, OAc), 3,52 (1H, m, 3α-H), 4.52 (1H, q, $J=6$ Hz, 20–H), 4.56 (1H, d.d, $J=6,11$ Hz, 12α–H). *Anal.* Calcd. for $C_{25}H_{40}O_7$: C, 66.34; H, 8.91. Found: C, 66.04; H, 9.06.

Alkaline Hydrolysis of Tomentin (I)——A solution of 20 mg of tomentin (I) in 5 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 (MeOH: $CHCl_3$, 1: 19). Recrystallization of I from MeOH–acetone gave 15 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^+–CHOH·Me), 305 (M^+–CHOH·Me–H_2O, base peak), 287 (M^+–CHOH·Me–$2H_2O$), 269 (M^+–CHOH·Me–$3H_2O$). IR ν_{max}^{Nujol} cm^{-1}: 3400, 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).

Acetylation of Tomentin (I)——A solution of 20 mg of tomentin (I), 1 ml of Ac_2O, and 1 ml of pyridine was allowed to stand for 18 hr at room temperature, and poured into ice water. A white powder that appeared was collected and recrystallized from acetone–MeOH to afford 18 mg of tomentin acetate (III) as needles, mp 285—288°, $[\alpha]_D^{11}$ +26° ($c=0.8$, $CHCl_3$). Mass Spectrum *m/e*: 476 (M^+–H_2O), 434 (M^+–acetic acid), 416 (M^+–acetic acid–H_2O), 407 (M^+–CHOAc·Me), 398 (M^+–acetic acid–$2H_2O$), 389 (M^+–CHOAc·Me-H_2O), 374 (M^+–2 × acetic acid), 356 (M^+–2 × acetic acid–H_2O), 339 (M^+–CHOAc·Me–acetic acid), 314 (M^+–3 × acetic acid), 304, 291, 286, 226, 43 (base peak). IR ν_{max}^{Nujol} cm^{-1}: 3470, 3400, 1740, 1710, 1275, 1250, 1235, 1050, 1040, 1020. NMR (δ) $CDCl_3$: 0.82 (3H, s, 18-Me), 1.22 (3H, s, 19-Me), 1.26 (3H, d, $J=7$ Hz, 21-Me), 1.97 (3H, s, OAc), 2.02 (3H, s, OAc), 2.08 (3H, s, OAc), 4.54 (1H, q, $J=6$ Hz, 20-H), 4.60 (1H, d.d, $J=6$, 11 Hz, 12α-H), 4.62 (1H, m, 3α-H). *Anal.* Calcd. for $C_{27}H_{42}O_8$: C, 65.56; H, 8.56. Found: C, 65.59; H, 8.64.

6) M. Fukuoka, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **19**, 1469 (1971).
7) E. Abisch, Ch. Tamm, and T. Reichstein, *Helv. Chim. Acta*, **42**, 1014 (1959).

Acetylation of Tomentogenin (II)——A solution of 43 mg of tomentogenin (II), 1 ml of Ac_2O, and 1 ml of pyridine was allowed to stand for 24 hr at room temperature and worked up in the same manner as in the acetylation of (I) to afford 35 mg of an amorphous substance. Recrystallization from acetone–MeOH gave needles (III), mp 287, $[\alpha]_D^{11}$ +25° (c=1.0 $CHCl_3$). Mass Spectrum *m/e*: 494 (M^+), 434 (M^+–acetic acid), 416 (M^+–acetic acid–H_2O), 407 (M^+–CHOAc·Me), 389 (M^+–CHOAc·Me–H_2O), 374 (M^+–2×acetic acid), 356 (M^+–2×acetic acid–H_2O), 339 (M^+–CHOAc·Me–acetic acid) 314 (M^+–3×acetic acid), 304, 291, 286, 226, 43 (base peak). NMR (δ) $CDCl_3$: 0.78 (3H, s, 18-Me), 1.18 (3H, s, 19-Me), 1.22 (3H, d, J=7 Hz, 21-Me), 1.92 (3H, s, OAc), 1.98 (3H, s, OAc), 2.04 (3H, s, OAc), 4.50 (1H, q, J=6 Hz, 20-H), 4.56 (1H, d.d, J=6, 11 Hz, 12α-H), 4.60 (1H, m, 3α-H).

Dehydrotomentin (IV)——From the less polar fraction of tomentin (I), 25 mg of dehydrotomentin (IV) was obtained by repeated preparative TLC (ether, MeOH: $CHCl_3$=1: 99). IV was recrystallized from hexane–acetone to needles, mp 145—150°, $[\alpha]_D^{19}$ −9° (c=0.6, $CHCl_3$). Mass Spectrum *m/e*: 450 (M^+), 432 (M^+–H_2O), 414 (M^+–2H_2O), 396 (M^+–3H_2O), 390 (M^+–acetic acid), 372 (M^+–acetic acid–H_2O), 363 (M^+–CHOAc·Me), 354 (M^+–acetic acid–3H_2O), 345 (M^+–CHOAc·Me–H_2O), 330 (M^+–2×acetic acid), 312 (M^+–2×acetic acid–2H_2O), 294 (M^+–2×acetic acid–2H_2O), 261, 242, 145, 43 (base peak). IR ν_{max}^{Nujol} 3400, 1735, 1710, 1260, 1240, 1040, 1020. NMR (δ) $CDCl_3$: 0.99 (3H, s, 18-Me), 1.21 (3H, s, 19-Me), 1.23 (3H, d, J=6 Hz, 21-Me), 1.99 (3H, s, OAc), 2.08 (3H, s, OAc), 3.50 (1H, m, 3α-H), 4.56 (1H, q, J=7 Hz, 20-H), 4.60 (1H, d.d, J=6, 11 Hz, 12α-H), 5.40 (1H, Δ^5olefinic proton). *Anal.* Calcd. for $C_{25}H_{38}O_7$: C, 66.64; H, 8.50. Found: C, 66.48; H, 8.34.

Acetylation of Dehydrotomentin (IV)——A solution of 18 mg of dehydrotomentin (IV), 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 12 mg of dehydrotomentin acetate (V), prisms, from acetone–MeOH, mp 255—258°, $[\alpha]_D^{19}$ −20° (c=0.32, $CHCl_3$). Mass Spectrum *m/e*: 492 (M^+), 474 (M^+–H_2O), 456 (M^+–2H_2O), 432 (M^+–acetic acid), 414 (M^+–acetic acid–H_2O), 372 (M^+–2×acetic acid), 354 (M^+–2×acetic acid–H_2O), 336 (M^+–2×acetic acid–2H_2O), 312 (M^+–3×acetic acid) 294 (M^+–3×acetic acid–H_2O), 276 (M^+–3×acetic acid–2H_2O), 261, 226, 145, 43 (base peak). IR ν_{max}^{Nujol} cm^{-1}: 3350, 1735, 1710, 1270, 1240, 1040, 1020. NMR (δ) $CDCl_3$: 0.99 (3H, s, 18-Me), 1.21 (3H, s, 19-Me), 1.23 (3H, d, J=7 Hz, 21-Me), 1.99 (3H, s, OAc), 2.03 (3H, s, OAc), 2.08 (3H, s, OAc), 4.52 (1H, q, J=6 Hz, 20-H), 4.56 (1H, d.d, J=6, 11 Hz, 12α–H), 4.60 (1H, m, 3α-H). *Anal.* Calcd. for $C_{27}H_{40}O_8$: C, 65.83; H, 8.19. Found: C, 66.01; H, 8.27.

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