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Studies on the Constituents of Asclepiadaceae Plants. XL.[1] Absolute Configurations of Tomentogenin and Utendin

Hideo Seto, Koji Hayashi, and Hiroshi Mitsuhashi

Faculty of Pharmaceutical Sciences, Hokkaido University[2]

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The configuration at C-20 of tomentogenin and utendin was determined as s by means of the circular dichroism experiment of 20-*O*-*o*-nitrobenzoyl derivatives. From these results, the absolute configurations of tomentogenin and utendin were determined as 5α-pregnan-3β,12β,14β,17β,20α-pentol and pregn-5-ene-3β,12β,14β,17β,20α-pentol, respectively.

The tentative structure of tomentogenin[3] (I), isolated from the stem of *Marsdenia tomentosa* Decne, was proposed as 5α-pregnan-3β,12β,14β,17β,20-pentol with some ambiguity for the configuration of C-20 hydroxyl group, and the configuration at C-20 of utendin[3b,c,4] (II), pregn-5-ene-3β,12β,14β,17β,20-pentol, was confirmed to be the same as that of I. Several tomentogenin and utendin derivatives have been isolated from *Caralluma dalzielli* by Tschesche[5] and from *M. tomentosa* by us.[1,6]

Recently, Hayashi and Mitsuhashi proved the configuration at C-20 of sarcostin (III) to be s[7,8] on the basis of the optical rotatory dispersion (ORD) examination synchronized with X-ray analysis. In this paper, we report the absolute configurations of tomentogenin (I) and utendin (II).

Partial acetylation of I with 2.5 molar equiv. of acetic anhydride–pyridine afforded 3,12-diacetate (IV), mp 217—220°, $[\alpha]_D^{18}$ +31° (c=0.42, $CHCl_3$), as a main product, with 3-monoacetate (V), 3,20-diacetate (VI), and 3,12,20-triacetate (VII). The molecular formula of $C_{25}H_{40}O_7$ was given for IV from its elemental analysis and mass spectrum (M^+ at m/e 452). The infrared (IR) spectrum of IV showed absorptions for hydroxyl groups at 3550, 3490, 3420, and 1030 cm^{-1}, and esters at 1730, 1720, 1260, and 1240 cm^{-1}. The nuclear magnetic resonance (NMR) spectrum of IV showed signals for two tertiary methyl groups at δ 0.84 (s) and 1.22 (s), one secondary methyl group at 1.12 (d, J=6 Hz), two acetyl groups at 2.02 (s) and 2.10 (s), and three hydroxy-methines at 3.52 (q, J=6 Hz), 4.50 (d.d, J=6, 11 Hz) and 4.60 (m). Irradiation of 21-Me group protons (δ 1.12) collapsed the quartet at δ 3.52 assignable for C-20 proton

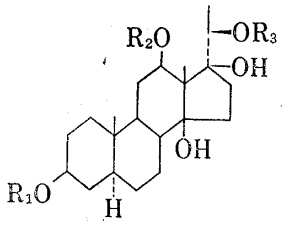
1) Part XXXIX: H. Seto, T. Sasaki, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **24**, 2185 (1976).
2) Location: *Nishi-6-chome, Kita-12-jo, Kita-ku, Sapporo, 060, Japan.*
3) *a*) H. Mitsuhashi, I. Takemori, Y. Shimizu, T. Nomura, and E. Yamada, *Chem. Pharm. Bull.* (Tokyo), **10**, 804 (1962); *b*) H. Mitsuhashi, T. Sato, T. Nomura, and I. Takemori, *Chem. Pharm. Bull.* (Tokyo), **12**, 981 (1964); *c*) H. Mitsuhashi, T. Sato, T. Nomura, and I. Takemori, *Chem. Pharm. Bull.* (Tokyo), **13**, 267 (1965); *d*) M. Fukuoka and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **16**, 1634 (1968).
4) E. Abisch, Ch. Tamm, and T. Reichstein, *Helv. Chim. Acta*, **42**, 1014 (1959).
5) R. Tschesche and G. Marwede, *Tetrahedron Letters*, **1967**, 1359.
6) *a*) H. Seto, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **23**, 1552 (1975); *b*) H. Seto, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **23**, 2397 (1975). In this paper, we gave names of tomentin and dehydrotomentin to 12β-*O*,20-*O*-diacetyltomentogenin and 12β-*O*,20-*O*-diacetylutendin, respectively. The name "tomentin" had been used for the coumarin aglycone isolated from *Prunus tomentosa* by Hasegawa [*Bot. Mag.* (Tokyo), **82**, 458 (1969)], and therefore, we discontinue to use these names and change them to tomentinin and dehydrotomentinin; *c*) H. Seto, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo) ,**24**, 443 (1976); *d*) H. Seto, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **24**, 1552 (1976).
7) K. Hayashi and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **23**, 1845 (1975).
8) T. Reichstein, *Naturwissenschaften*, **54**, 53 (1967).

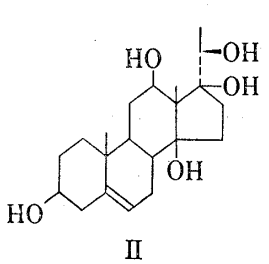
to a singlet. 3,20-Diacetate (VI), $C_{25}H_{40}O_7$ (M^+ at *m/e* 452) showed mp 207—209°, $[\alpha]_D^{15}$ +13° (*c*=0.50, $CHCl_3$). The IR spectrum of VI showed absorptions for hydroxyl groups at 3550, 3400, 1080, 1045, and 1030 cm^{-1}, and esters at 1730, 1710, 1270, and 1250 cm^{-1}. The NMR spectrum of VI showed signals for two tertiary methyl groups at δ 0.84 (s) and 1.12 (s), one secondary methyl group at 1.26 (d, J=6 Hz), two acetyl groups at 2.04 (s), and three hydroxy-methines at 3.36 (d.d, J=6, 11 Hz), 4.68 (m), and 5.14 (q, J=6 Hz). Irradiation of 21-Me group protons (δ 1.26) collapsed the quartet at δ 5.14 assignable for C-20 proton to a singlet. The yield-ratio between IV and VI on this condition was 8: 1, which indicates that the acetylation of C-12β hydroxy group occurred more easily than that of C-20 hydroxyl group in spite of a relatively less hindered position of the latter compared to the former.

o-Nitrobenzoylation of IV with *o*-nitrobenzoyl chloride–pyridine afforded 20-*O*-*o*-nitrobenzoate (VIII), mp 275—278°. The molecular formula of $C_{32}H_{43}O_{10}N$ was given for VIII from its elemental analysis and mass spectrum (M^+ − *o*-nitrobenzoic acid at *m/e* 434). The IR spectrum of VIII showed absorptions for hydroxyl groups at 3500, 3425, 1070, and 1030 cm^{-1}, acetyl esters at 1730, 1250, and 1240 cm^{-1}, and an *o*-nitrobenzoyl ester at 1710, 1540, 1360, and 1145 cm^{-1}. The NMR spectrum of VIII showed signals for two tertiary methyl groups at δ 0.80 (s) and 1.16 (s), one secondary methyl group at 1.44 (d, J=6 Hz), two acetyl groups at 2.04 (s), three hydroxy-methines at 4.60 (m), 4.62 (d.d, J=6, 11 Hz), and 4.92 (q, J=6 Hz), and four aromatic protons at 7.70 (m). The circular dichroism (CD) spectrum of VIII showed a negative Cotton effect at 330 nm ([θ] −2555).

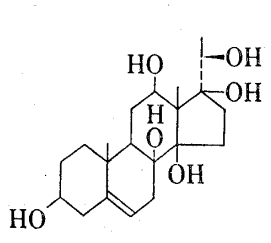
In order to mask the 17β-hydroxyl group, VIII was treated in paraldehyde–pyridine with boron trifluoride etherate as a catalyst to afford a cyclic *O*-ethylidene derivative (IX), mp 203—205°. The molecular formula of $C_{34}H_{45}O_{10}N$ was given for IX from its elemental analysis and mass spectrum (M^+ at *m/e* 627). The IR spectrum of IX showed absorptions for acetyl esters at 1735, 1250, and 1235 cm^{-1}, an *o*-nitrobenzoyl ester at 1725, 1545, 1360, and 1140 cm^{-1}, a cyclic *O*-ethylidene at 1290 and 1120 cm^{-1}, and no hydroxyl group. The NMR spectrum of IX showed signals for two tertiary methyl groups at δ 0.82 (s) and 1.22 (s), two secondary methyl groups at 1.33 (d, J=6 Hz) and 1.38 (d, J=5 Hz), two acetyl groups at 2.02 (s), three hydroxy-methines at 4.70 (d.d, J=6,11 Hz), 4.72 (m), 4.96 (q, J=6 Hz), one proton at 5.18 (q, J=5 Hz), and four aromatic protons at 7.70 (m). The CD spectrum of IX showed a positive Cotton effect at 323 nm ([θ]+5563).

Conversion of the Cotton effect of 20-*C*-*o*-nitrobenzoate from a negative to a positive on masking C-17β hydroxyl group was already established in the experiment on sarcostin[7] (III).



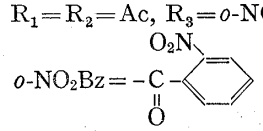


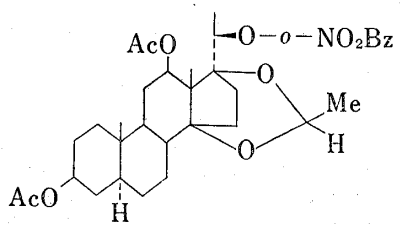
II



III

I : R_1=R_2=R_3=H
IV : R_1=R_2=Ac, R_3=H
V : R_1=Ac, R_2=R_3=H
VI : R_1=R_3=Ac, R_2=H
VII : R_1=R_2=R_3=Ac
VIII : R_1=R_2=Ac, R_3=*o*-NO_2Bz





IX

Chart

Catalytic hydrogenation of utendin (II) with platinum afforded a dihydro derivative, which was identical with tomentogenin[3b,c] (I) from the comparison of their spectral data and mixed mp with an authentic sample.

From these facts, the configuration at C-20 of tomentogenin (I) and utendin (II) was determined as s, so that the absolute configurations of I and II were finally determined as 5α-pregnan-3β,12β,14β,17β,20α-pentol and pregn-5-ene-3β,12β,14β,17β,20α-pentol, respectively.

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 on a JEOL PS-100 spectrometer operating at 100 MHz with tetramethylsilane (TMS) as an internal standard, mass spectra on a Hitachi RMU-7 mass spectrometer, and IR spectra were taken in Nujol mull on a Hitachi 215 spectrometer. CD spectra were measured in MeOH solution on a JASCO J-20 spectropolarimeter. Thin-layer chromatography (TLC) was performed on silica gel HF_{254} (Merck, Type 60). Tomentogenin and utendin used were obtained from *M. tomentosa*.

Partial Acetylation of Tomentogenin (I)——A solution of 201 mg of tomentogenin (I) in 0.14 ml of Ac_2O and 2 ml of pyridine was allowed to stand for 25 hr at 40° and the reaction mixture was separated by preparative TLC ($CHCl_3$: MeOH=97: 3) to afford 25 mg of 3-monoacetate (V), 13 mg of 3,20-diacetate (VI), 108 mg of 3,12-diacetate (IV), and 43 mg of 3,12,20-triacetate (VII). 3-Monoacetate (V) was recrystallized from hexane–acetone to plates, mp 204—208°, $[\alpha]_D^{18}$ +40° (c=0.30, $CHCl_3$). Mass Spectrum *m/e*: 410 (M^+) 392 (M^+-H_2O), 374 (M^+-2H_2O), 365 (M^+—CHOH·Me),[9] 356 (M^+-3H_2O), 350 (M^+—AcOH), 347 (M^+—CHOH-Me $-H_2O$), 338 (M^+-4H_2O), 332 (M^+—AcOH—H_2O), 329 (M^+—CHOH·Me—$2H_2O$), 314 (M^+—AcOH—$2H_2O$), 305 (M^+—CHOH·Me—AcOH), 304, 291, 287 (M^+—CHOH·Me—AcOH–H_2O), 286, 269 (M^+—CHOH·Me—AcOH—$2H_2O$), 226, 43 (base peak). IR ν_{max}^{Nujol} cm^{-1}: 3350, 1730, 1245, 1030. NMR $\delta_{ppm}^{CDCl_3}$: 0.84 (3H, s, 19-Me), 1.12 (3H, s, 18-Me), 1.20 (3H, d, J=6 Hz, 21-Me). 2.04 (3H, s, OAc), 3.44 (1H, d.d, J=6, 11 Hz, 12α-H), 4.00 (1H, q, J=6 Hz, 20β-H), 4.66 (1H, m, 3α-H). *Anal.* Calcd. for $C_{23}H_{38}O_6$: C, 67.29; H, 9.33. Found: C, 67.52; H, 9.59.

3,20-Diacetate (VI) was recrystallized from acetone to needles, mp 207—209°, $[\alpha]_D^{18}$ +13° (c=0.50, $CHCl_3$). Mass Spectrum *m/e*: 452 (M^+), 434 (M^+-H_2O), 416 (M^+-2H_2O), 392 (M^+—AcOH), 374 (M^+—AcOH—H_2O), 365 (M^+—CHOAc·Me),[9] 356 (M^+—AcOH—$2H_2O$), 347 (M^+—CHOAc·Me—H_2O), 338 (M^+–AcOH—$3H_2O$), 332 (M^+—2AcOH), 329 (M^+—CHOAc·Me—$2H_2O$), 314 (M^+—2AcOH—H_2O), 311 (M^+—CHOAc·Me—$3H_2O$), 304, 291, 286, 226, 43 (base peak). IR ν_{max}^{Nujol} cm^{-1}: 3550, 3400, 1730, 1710, 1270, 1250, 1080, 1045, 1030. NMR $\delta_{ppm}^{CDCl_3}$: 0.84 (3H, s, 19-Me), 1.12 (3H, s, 18-Me), 1.26 (3H, d, J=6 Hz, 21-Me), 2.04 (6H, s, 2× OAc), 3.36 (1H, d.d, J=6, 11 Hz, 12α-H), 4.68 (1H, m, 3α-H), 5.14 (1H, q, J=6 Hz, 20β-H). *Anal.* Calcd for $C_{25}H_{40}O_7$: C, 66.34; H, 8.91. Found: C, 66.12; H, 9.02.

3,12-Diacetate (IV) was recrystallized from acetone to needles, mp 217—220°, $[\alpha]_D^{18}$ +31° (c=0.42, $CHCl_3$). Mass Spectrum *m/e*: 452 (M^+), 434 (M^+-H_2O), 416 (M^+-2H_2O), 407 (M^+—CHOH·Me), 392 (M^+—AcOH), 389 (M^+—CHOH·Me—H_2O), 374 (M^+—AcOH—H_2O), 371 (M^+—CHOH·Me—$2H_2O$), 356 (M^+—AcOH—$2H_2O$), 347 (M^+—CHOH·Me—AcOH), 338 (M^+—AcOH—$3H_2O$), 332 (M^+—2AcOH), 329 (M^+—CHOH·Me-AcOH—H_2O), 314 (M^+—2AcOH—H_2O), 311 (M^+—CHOH·Me—AcOH—$2H_2O$), 304, 291, 286, 226, 43 (base peak). IR ν_{max}^{Nujol} cm^{-1}: 3550, 3490, 3420, 1730, 1720, 1260, 1240, 1030. NMR $\delta_{ppm}^{CDCl_3}$: 0.84 (3H, s, 19-Me), 1.12 (3H, d, J=6 Hz, 21-Me), 1.22 (3H, s, 18-Me), 2.02 (3H, s, OAc), 2.10 (3H, s, OAc), 3.52 (1H, q, J=6 Hz, 20β-H), 4.50 (1H, d.d, J=6, 11 Hz, 12α-H), 4.60 (1H, m, 3α-H). *Anal.* Calcd. for $C_{25}H_{40}O_7$: C, 66.34; H, 8.91. Found: C, 66.59; H, 9.04.

3,12,20-Triacetate (VII) was recrystallized from MeOH–acetone to prisms, mp 285—288°, $[\alpha]_D^{11}$ +26° (c=0.80, $CHCl_3$). Mass Spectrum *m/e*: 476 (M^+), 434 (M^+—AcOH), 416 (M^+—AcOH—H_2O), 407 (M^+—CHOAc·Me), 398 (M^+—AcOH—$2H_2O$), 389 (M^+—CHOAc·Me—H_2O), 374 ($M^+-2\times$AcOH), 356 ($M^+-2\times$AcOH—H_2O), 339 (M^+—CHOAc·Me—AcOH), 314 ($M^+-3\times$AcOH), 304, 291, 286, 226, 43 (base peak). IR ν_{max}^{Nujol} cm^{-1}: 3470. 3400, 1740, 1710, 1275, 1250, 1235, 1050, 1040, 1020. NMR $\delta_{ppm}^{CDCl_3}$: 0.82 (3H, s, 19-Me), 1.22 (3H, s, 18-Me), 1.26 (3H, d, J=6 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, 20β-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.

***o*-Nitrobenzoylation of Tomentogenin 3,12-Diacetate (IV)**——A solution of 151 mg of tomentogenin 3,12-diacetate (IV) and 232 mg of *o*-nitrobenzoyl chloride in 2 ml of pyridine was stirred for 20 hr at room temperature. The reaction mixture was purified by preparative TLC to afford 65 mg of *o*-nitrobenzoate (VIII), recrystallized from MeOH–acetone–hexane to needles, mp 275—278°. Mass Spectrum *m/e*: 434 (M^+—*o*-nitrobenzoic acid), 416 (M^+—*o*-nitrobenzoic acid—H_2O), 407 (M^+—CHO·$C_7H_4O_3N$·Me), 374 (M^+—*o*-nitrobenzoic acid—AcOH), 356 (M^+—*o*-nitrobenzoic acid—AcOH—H_2O), 346, 341, 314 (M^+—*o*-nitrobenzoic acid-2× AcOH),

9) M. Fukuoka and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **17**, 2448 (1969).

304, 291, 286, 226, 167, 43 (base peak). IR ν_{max}^{Nujol} cm^{-1}: 3500, 3425, 1730, 1710, 1580, 1540, 1360, 1250, 1240, 1145, 1070, 1030. NMR $\delta_{ppm}^{CDCl_3}$: 0.80 (3H, s, 19-Me), 1.16 (3H, s, 18-Me), 1.44 (3H, d, $J=6$ Hz, 21-Me), 2.04 (6H, s, $2\times$OAc), 4.60 (1H, m, 3α-H), 4.62 (1H, d.d, $J=6$, 11 Hz, 12α-H). 4.92 (1H, q, $J=6$ Hz, 20β-H), 7.70 (4H, m, aromatic protons). CD: $[\theta]_{330}$ -2555 ($c=3.3\times10^{-3}$ M, MeOH). *Anal.* Calcd. for $C_{32}H_{43}O_{10}N$: C, 63.88; H, 7.20; N, 2.33. Found: C, 63.74; H, 7.13; N, 2.45.

Reaction of *o*-Nitrobenzoate (VIII) with Paraldehyde——The suspension of 52 mg of *o*-nitrobenzoate (VIII) in 2.5 ml of paraldehyde and three drops of BF_3-ether was stirred for 1 hr at room temperature. After addition of 100 mg of K_2CO_3, the reaction mixture was filtered to remove excess K_2CO_3 and concentrated *in vacuo*. The residue was purified by preparative TLC ($CHCl_3$) to afford 39 mg of cyclic *O*-ethylidene derivative (IX). Cyclic *O*-ethylidene derivative (IX) was recrystallized from acetone–hexane to needles, mp 203—205°. Mass Spectrum *m/e*: 627 (M^+), 612 (M^+-Me), 597 ($M^+-2\cdot Me$), 567 (M^+-AcOH), 541, 523, 507 ($M^+-2AcOH$), 460 (M^+-o-nitrobenzoic acid), 304, 291, 286, 226, 150, 120, 43 (base peak). IR ν_{max}^{Nujol} cm^{-1}: 1735, 1725, 1600, 1580, 1545, 1360, 1290, 1250, 1235, 1140, 1120, 1070, 1030. NMR $\delta_{ppm}^{CDCl_3}$: 0.82 (3H, s, 19-Me), 1.22 (3H, s, 18-Me), 1.33 (3H, d, $J=6$ Hz, 21-Me), 1.38 (3H, d, $J=5$ Hz), 2.02 (6H, s, $2\times$OAc), 4.70 (1H, d.d, $J=6$, 11 Hz, 12α-H), 4.72 (1H, m, 3α-H), 4.96 (1H, q, $J=6$ Hz, 20β-H), 5.18 (1H, q, $J=5$ Hz), 7.70 (4H, m, aromatic protons). CD: $[\theta]_{323}$ $+5563$ ($c=3.2\times10^{-3}$ M, MeOH). *Anal.* Calcd. for $C_{34}H_{45}O_{10}N$: C, 65.05; H, 7.23; N, 2.23. Found: C, 64.94; H, 7.20; N, 2.24.

Hydrogenation of Utendin (II)——A solution of 15 mg of utendin (II) in 10 ml of EtOAc–MeOH (4: 1) and one drop of AcOH was hydrogenated over 30 mg of PtO_2 under atmospheric pressure at 20°. It consumed 1 mol. equiv. of H_2 (2 ml) during 3 hr. After the reaction mixture was diluted with H_2O with cooling, the catalyst was removed. The product was purified by preparative TLC ($CHCl_3$: MeOH=9: 1) to yield 9 mg of dihydroderivative, which showed mp 265—268° and mixed mp with I, 263—266°. Mass Spectrum *m/e*: 368 (M^+), 350 (M^+-H_2O), 332 (M^+-2H_2O), 323 ($M^+-CHOH\cdot Me$), 305 ($M^+-CHOH\cdot Me-H_2O$, base peak), 287 ($M^+-CHOH\cdot Me-2H_2O$), 269 ($M^+-CHOH\cdot Me-3H_2O$), 262, 249, 244, 226. IR ν_{max}^{Nujol} cm^{-1}: 3400, 1040. NMR $\delta_{ppm}^{pyridine\text{-}d_5}$: 0.76 (3H, s, 19-Me), 1.54 (3H, d, $J=6$ Hz, 21-Me), 1.64 (3H, s, 18-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, 20β-H). *Anal.* Calcd. for $C_{21}H_{36}O_5$: C, 68.44; H, 9.85. Found: C, 68.35; H, 9.99.

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