

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

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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 5 α -pregnan-3 β ,12 β ,14 β ,17 β ,20 α -pentol and pregn-5-ene-3 β ,12 β ,14 β ,17 β ,20 α -pentol, respectively.

The tentative structure of tomentogenin³⁾ (I), isolated from the stem of *Marsdenia tomentosa* DECNE, was proposed as 5 α -pregnan-3 β ,12 β ,14 β ,17 β ,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-3 β ,12 β ,14 β ,17 β ,20-pentol, was confirmed to be the same as that of I. Several tomentogenin and utendin derivatives have been isolated from *Caralluma dalzielli* by Tschesche⁵⁾ 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°, $[\alpha]_D^{18} + 31^\circ$ ($c=0.42$, CHCl₃), as a main product, with 3-monoacetate (V), 3,20-diacetate (VI), and 3,12,20-triacetate (VII). The molecular formula of C₂₅H₄₀O₇ 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

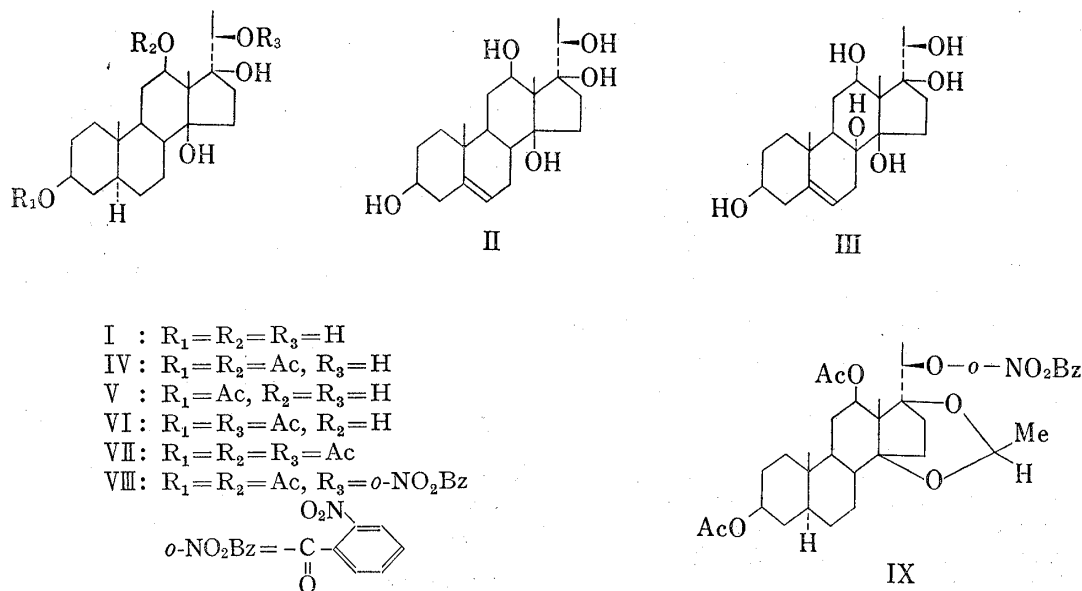
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- 2) Location: *Nishi-6-chome, Kita-12-jo, Kita-ku, Sapporo, 060, Japan.*
- 3) a) H. Mitsuhashi, I. Takemori, Y. Shimizu, T. Nomura, and E. Yamada, *Chem. Pharm. Bull.* (Tokyo), **10**, 804 (1962); b) H. Mitsuhashi, T. Sato, T. Nomura, and I. Takemori, *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 12 β -*O*,20-*O*-diacetyltomentogenin and 12 β -*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).
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to a singlet. 3,20-Diacetate (VI), $C_{25}H_{40}O_7$ (M^+ at m/e 452) showed mp 207—209°, $[\alpha]_D^{25} +13^\circ$ ($c=0.50$, $CHCl_3$). 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-12 β 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 ($[\theta] -2555$).

In order to mask the 17 β -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 ($[\theta] +5563$).

Conversion of the Cotton effect of 20-*C*-*o*-nitrobenzoate from a negative to a positive on masking C-17 β hydroxyl group was already established in the experiment on sarcostin⁷ (III).



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 5 α -pregnan-3 β ,12 β ,14 β ,17 β ,20 α -pentol and pregn-5-ene-3 β ,12 β ,14 β ,17 β ,20 α -pentol, respectively.

Experimental

Melting points were determined on a Kofler hot stage and are uncorrected. Optical rotations were measured in CHCl₃ 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 HF₂₅₄ (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 Ac₂O and 2 ml of pyridine was allowed to stand for 25 hr at 40° and the reaction mixture was separated by preparative TLC (CHCl₃: 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°, $[\alpha]_D^{18} + 40^\circ$ ($c=0.30$, CHCl₃). Mass Spectrum m/e : 410 (M⁺) 392 (M⁺–H₂O), 374 (M⁺–2H₂O), 365 (M⁺–CHOH·Me),⁹⁾ 356 (M⁺–3H₂O), 350 (M⁺–AcOH), 347 (M⁺–CHOH·Me–H₂O), 338 (M⁺–4H₂O), 332 (M⁺–AcOH–H₂O), 329 (M⁺–CHOH·Me–2H₂O), 314 (M⁺–AcOH–2H₂O), 305 (M⁺–CHOH·Me–AcOH), 304, 291, 287 (M⁺–CHOH·Me–AcOH–H₂O), 286, 269 (M⁺–CHOH·Me–AcOH–2H₂O), 226, 43 (base peak). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3350, 1730, 1245, 1030. NMR $\delta_{\text{ppm}}^{\text{CDCl}_3}$: 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, 12 α -H), 4.00 (1H, q, $J=6$ Hz, 20 β -H), 4.66 (1H, m, 3 α -H). Anal. Calcd. for C₂₃H₃₈O₆: 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°, $[\alpha]_D^{18} + 13^\circ$ ($c=0.50$, CHCl₃). Mass Spectrum m/e : 452 (M⁺), 434 (M⁺–H₂O), 416 (M⁺–2H₂O), 392 (M⁺–AcOH), 374 (M⁺–AcOH–H₂O), 365 (M⁺–CHOAc·Me),⁹⁾ 356 (M⁺–AcOH–2H₂O), 347 (M⁺–CHOAc·Me–H₂O), 338 (M⁺–AcOH–3H₂O), 332 (M⁺–2AcOH), 329 (M⁺–CHOAc·Me–2H₂O), 314 (M⁺–2AcOH–H₂O), 311 (M⁺–CHOAc·Me–3H₂O), 304, 291, 286, 226, 43 (base peak). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3550, 3400, 1730, 1710, 1270, 1250, 1080, 1045, 1030. NMR $\delta_{\text{ppm}}^{\text{CDCl}_3}$: 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, 12 α -H), 4.68 (1H, m, 3 α -H), 5.14 (1H, q, $J=6$ Hz, 20 β -H). Anal. Calcd. for C₂₅H₄₀O₇: 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°, $[\alpha]_D^{18} + 31^\circ$ ($c=0.42$, CHCl₃). Mass Spectrum m/e : 452 (M⁺), 434 (M⁺–H₂O), 416 (M⁺–2H₂O), 407 (M⁺–CHOH·Me), 392 (M⁺–AcOH), 389 (M⁺–CHOH·Me–H₂O), 374 (M⁺–AcOH–H₂O), 371 (M⁺–CHOH·Me–2H₂O), 356 (M⁺–AcOH–2H₂O), 347 (M⁺–CHOH·Me–AcOH), 338 (M⁺–AcOH–3H₂O), 332 (M⁺–2AcOH), 329 (M⁺–CHOH·Me–AcOH–H₂O), 314 (M⁺–2AcOH–H₂O), 311 (M⁺–CHOH·Me–AcOH–2H₂O), 304, 291, 286, 226, 43 (base peak). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3550, 3490, 3420, 1730, 1720, 1260, 1240, 1030. NMR $\delta_{\text{ppm}}^{\text{CDCl}_3}$: 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, 20 β -H), 4.50 (1H, d.d, $J=6, 11$ Hz, 12 α -H), 4.60 (1H, m, 3 α -H). Anal. Calcd. for C₂₅H₄₀O₇: 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°, $[\alpha]_D^{11} + 26^\circ$ ($c=0.80$, CHCl₃). Mass Spectrum m/e : 476 (M⁺), 434 (M⁺–AcOH), 416 (M⁺–AcOH–H₂O), 407 (M⁺–CHOAc·Me), 398 (M⁺–AcOH–2H₂O), 389 (M⁺–CHOAc·Me–H₂O), 374 (M⁺–2 × AcOH), 356 (M⁺–2 × AcOH–H₂O), 339 (M⁺–CHOAc·Me–AcOH), 314 (M⁺–3 × AcOH), 304, 291, 286, 226, 43 (base peak). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3470, 3400, 1740, 1710, 1275, 1250, 1235, 1050, 1040, 1020. NMR $\delta_{\text{ppm}}^{\text{CDCl}_3}$: 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, 20 β -H), 4.60 (1H, d.d, $J=6, 11$ Hz, 12 α -H), 4.62 (1H, m, 3 α -H). Anal. Calcd. for C₂₇H₄₂O₈: 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–H₂O), 407 (M⁺–CHO·C₇H₄O₃N·Me), 374 (M⁺–*o*-nitrobenzoic acid–AcOH), 356 (M⁺–*o*-nitrobenzoic acid–AcOH–H₂O), 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 $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 3500, 3425, 1730, 1710, 1580, 1540, 1360, 1250, 1240, 1145, 1070, 1030. NMR $\delta_{\text{ppm}}^{\text{CDCl}_3}$: 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, $2 \times \text{OAc}$), 4.60 (1H, m, $3\alpha\text{-H}$), 4.62 (1H, d.d, $J=6, 11$ Hz, $12\alpha\text{-H}$), 4.92 (1H, q, $J=6$ Hz, $20\beta\text{-H}$), 7.70 (4H, m, aromatic protons). CD: $[\theta]_{330} -2555$ ($c=3.3 \times 10^{-3}$ M, MeOH). Anal. Calcd. for $\text{C}_{32}\text{H}_{43}\text{O}_{10}\text{N}$: 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 ($\text{M}^+ - \text{Me}$), 597 ($\text{M}^+ - 2 \cdot \text{Me}$), 567 ($\text{M}^+ - \text{AcOH}$), 541, 523, 507 ($\text{M}^+ - 2\text{AcOH}$), 460 ($\text{M}^+ - o\text{-nitrobenzoic acid}$), 304, 291, 286, 226, 150, 120, 43 (base peak). IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 1735, 1725, 1600, 1580, 1545, 1360, 1290, 1250, 1235, 1140, 1120, 1070, 1030. NMR $\delta_{\text{ppm}}^{\text{CDCl}_3}$: 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, $2 \times \text{OAc}$), 4.70 (1H, d.d, $J=6, 11$ Hz, $12\alpha\text{-H}$), 4.72 (1H, m, $3\alpha\text{-H}$), 4.96 (1H, q, $J=6$ Hz, $20\beta\text{-H}$), 5.18 (1H, q, $J=5$ Hz), 7.70 (4H, m, aromatic protons). CD: $[\theta]_{323} +5563$ ($c=3.2 \times 10^{-3}$ M, MeOH). Anal. Calcd. for $\text{C}_{34}\text{H}_{45}\text{O}_{10}\text{N}$: 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 ($\text{M}^+ - \text{H}_2\text{O}$), 332 ($\text{M}^+ - 2\text{H}_2\text{O}$), 323 ($\text{M}^+ - \text{CHOH} \cdot \text{Me}$), 305 ($\text{M}^+ - \text{CHOH} \cdot \text{Me} - \text{H}_2\text{O}$, base peak), 287 ($\text{M}^+ - \text{CHOH} \cdot \text{Me} - 2\text{H}_2\text{O}$), 269 ($\text{M}^+ - \text{CHOH} \cdot \text{Me} - 3\text{H}_2\text{O}$), 262, 249, 244, 226. IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 3400, 1040. NMR $\delta_{\text{ppm}}^{\text{pyridine-}d_5}$: 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, $12\alpha\text{-H}$), 3.80 (1H, m, $3\alpha\text{-H}$), 4.38 (1H, q, $J=6$ Hz, $20\beta\text{-H}$). Anal. Calcd. for $\text{C}_{21}\text{H}_{36}\text{O}_5$: C, 68.44; H, 9.85. Found: C, 68.35; H, 9.99.

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