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Studies on the Constituents of Asclepiadaceae Plants. XLIX.[1] Confirmation of the Structures of Antitumor-active Glycosides in Condurango Cortex. Chemical Transformation of the Aglycone Moiety[2]

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In order to confirm the structure of condurangogenin B, which is the aglycone of antitumor-active condurango glycoside (CG) B_0, the hypoiodite reaction of condurangogenin C 3-acetate, was carried out. A mixture of condurangogenin C 3-acetate, $Pb(OAc)_4$, I_2, $CaCO_3$, and diethyl ether was irradiated under nitrogen using a tungsten lamp. HPLC separation of the reaction products gave three compounds, P-1, P-2, and P-3.

The structures of these compounds were determined from the spectroscopic data. The PMR and mass spectra of P-1 were essentially identical with those of condurangogenin B 3-acetate. Thus, the ester linkage positions (11-cinnamoyl, 12-acetyl) and the ketal structure of conduragogenin B were confirmed. As CGD_0, 20-*O*-methyl-CGD_0, and 20-iso-*O*-methyl-CGD_0 could be converted chemically to CGB_0, these glycosides have the same positions of ester linkages as CGB_0.

Keywords——Condurango Cortex; antitumor-active glycosides; 18-oxygenated pregnane; positions of ester linkages; hypoiodite reaction

Pregnane glycosides from *Marsdenia cundurango* Reichenbach *fil.* were found to be active against Ehrlich ascites carcinoma *in vivo*. The structures of the glycosides were tentatively proposed on the basis of spectral data[3] (Chart 1).

However, the structures of the aglycone moieties of these glycosides have not been confirmed, except for condurango glycosides (CG) A_0 (**1**) and CGC_0 (**2**). As the aglycone moieties of CGB_0 (**3**), CGD_0 (**4**), 20-*O*-methyl-CGD_0 (**5**), and 20-iso-*O*-methyl-CGD_0 (**6**) were interconvertible, purification of their aglycones was not easy. Separation of the aglycone mixture of **3** by high performance liquid chromatography (HPLC) gave condurangogenin B (**7**), which was determined to be the genuine aglycone by comparison of the ^{13}C-nuclear magnetic resonance (CMR) spectra.[3] Nevertheless, the positions of ester linkages could not be determined from the spectral data.

Therefore, transformation to **7** from condurangogenin C (**8**),[4] whose structure has been established by chemical evidences, was attempted. Gainelli *et al.*[5] reported that the treatment of 3β-acetoxy-20β-hydroxy-5α-pregnane (**9**) with $Pb(OAc)_4$ in benzene afforded the 18,20-oxide (**10**) as the major product (Chart 2).

However, the reaction of condurangogenin C 3-acetate (**11**) without iodine yielded only a little of a 20-keto derivative (**12**). The presence of iodine has been shown to accelerate the oxidative ring formation between C-18 and C-20 of **9** by Ch. Mystre *et al.*[6]

A mixture of **11**, $Pb(OAc)_4$, I_2, and diethyl ether was irradiated under nitrogen using a tungsten lamp. In contrast with the oxidation of the 18-methyl group of C/D-*trans* steroids,[6] hypoiodite reaction of **11** gave relatively simple reaction product. The mixture was subjected to HPLC and three compounds, P-1, P-2, and P-3, were obtained in the ratio of 27: 32: 10 (Chart 3).

P-2 (**12**) was identified as condurangogenin A 3-acetate[7] by comparison with an authentic specimen.

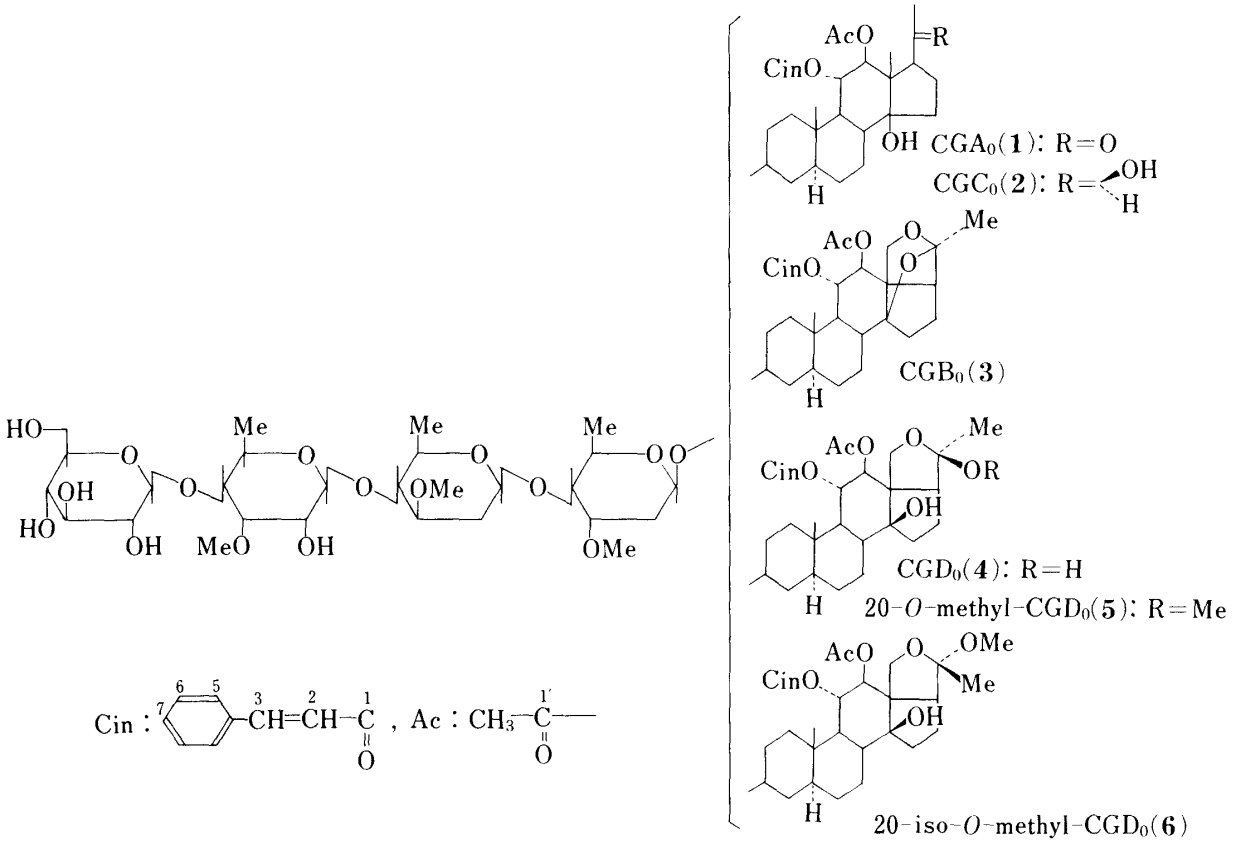

Chart 1

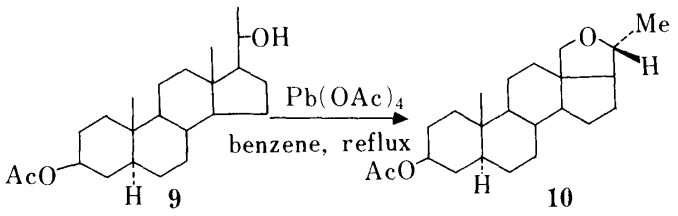

Chart 2

The ^{1}H-nuclear magnetic resonance (PMR) spectrum of P-3 (**13**) showed the disappearance of one angular methyl group and the appearance of oxygenated methylene signals at δ 4.04 and 4.16 as an AB type quartet (J=9 Hz). Irradiation of the methine signal at δ 3.87 collapsed the doublet signal at δ 1.07 to a singlet. These results suggest that P-3 is an 18, 20-oxide. The CMR spectrum also exhibited signals indicative of ether structure at δ 66.8 (C-18) and 82.6 (C-20).

In the PMR spectrum of P-1 (**14**), C-18 methylene signals appeared at δ 3.97 and 4.17, and the C-20 methine signal was absent. The mass spectrum of **14** showed the characteristic fragment peak indicating the presence of a ketal moiety (Chart 4).

On hypoiodite reaction, P-3 (**13**) was converted into P-1 (**14**) quantitatively. Therefore, the configuration at C-20 of P-3 was determined as ***R*** and the ketal structure of P-1 was also confirmed.

Condurangogenin B (**7**) was acetylated in the usual manner and the PMR spectrum of the product was compared with that of P-1 (**14**). The PMR spectra of the two compounds were identical and the structure of **7** was confirmed, including the 11-cinnamoyloxy and 12-acetyloxy groups.

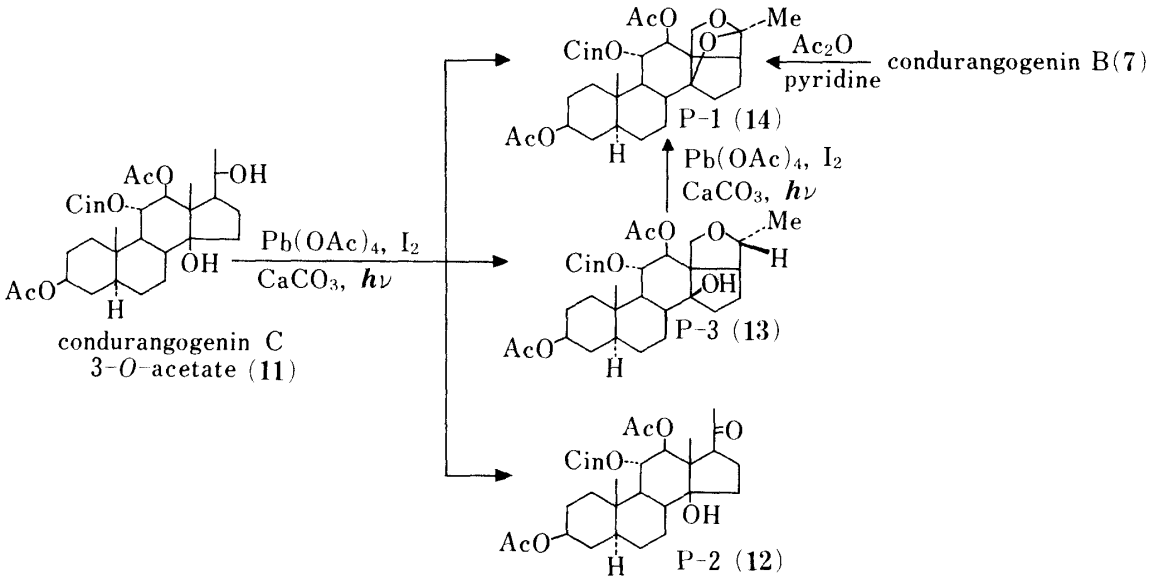

Chart 3

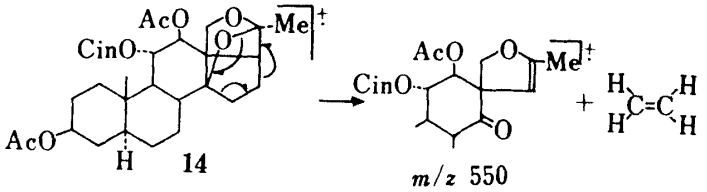

Chart 4

As CGD_0 (**4**), 20-*O*-methyl-CGD_0 (**5**), and 20-iso-*O*-methyl-CGD_0 (**6**) have been converted into CGB_0 (**3**) by treatments with $ZnCl_2$ in benzene,[3] these glycosides also have the same positions of ester linkages as CGB_0.

Thus, the structures of the four antitumor-active glycosides from condurango cortex were established.

Experimental

Optical rotations were measured in $CHCl_3$ solutions on a Perkin–Elmer 241 digital polarimeter. PMR spectra were determined at 200 MHz with a Varian XL 200 spectrometer, using tetramethylsilane (TMS). as an internal standard (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet). CMR spectra were recorded at 22.5 and 25.0 MHz using JNM FX 90Q and FX 100 spectrometers, respectively. Mass spectra were determined on a Hitachi M-60 machine. Ultra violet (UV) spectra were measured on a Hitachi 356 dual-wavelength double-beam spectrophotometer.

Acetylation of Condurangogenin C (8)——Acetic anhydride (0.16 ml) was added to a solution of **8** (670 mg) in 3 ml of dry pyridine. The solution was kept at room temperature for 24 h. The solution was concentrated *in vacuo* and the residue was subjected to HPLC (mobile phase, MeOH–$CHCl_3$–hexane=1: 4: 6; Wakogel LC-5H, 8 mmϕ×250 mm) to yield 495 mg of condurangogenin C 3-acetate (**11**), $[\alpha]_D$ +48.9° (c=0.67, $CHCl_3$). PMR δ ($CDCl_3$): 0.99 (3H, s, 19-CH_3), 1.20 (3H, d, J=6 Hz, 21-CH_3), 1.34 (3H, s, 18-CH_3), 1.85 (3H, s, Ac), 2.01 (3H, s, Ac), 3.83 (1H, m, 20-H), 4.69 (1H, m, 3α-H), 4.85 (1H, d, J=11 Hz, 12α-H), 5.33 (1H, t, J=11 Hz, 11β-H), 6.43+7.71 (2H, ABq, J=16 Hz).

Oxidation of Condurangogenin C 3-Acetate (11)——A mixture of **11** (400 mg), freshly prepared $Pb(OAc)_4$ (2.0 g), $CaCO_3$ (440 mg), I_2 (600 mg) and dry diethyl ether (16 ml) was irradiated under nitrogen using a tungsten lamp at 4—15°C for 4 h. The reaction mixture was passed through a short column of celite. The eluate was treated with 5% sodium thiosulfate solution and water successively and dried over $MgSO_4$. Evaporation of the solvent yielded 400 mg of product, which was separated by HPLC under the same conditions as **11** to provide P-1 (108 mg), P-2 (127 mg), and P-3 (41 mg).

P-1 (14)——Amorphous, $[\alpha]_D$ +28.6° (c=0.67, $CHCl_3$). PMR δ ($CDCl_3$): 1.04 (3H, s, 19-CH_3), 1.41

(3H, s, 21-CH_3), 1.91 (3H, s, Ac), 2.04 (3H, s, Ac), 3.97+4.17 (2H, ABq, J=9 Hz, 18-CH_2), 4.69 (1H, m, 3α-H), 5.13 (1H, d, J=10 Hz, 12α-H), 5.25 (1H, t, J=10 Hz, 11β-H), 6.41 (1H, d, J=16 Hz), 7.73 (1H, d, J=16 Hz). MS m/z: 578 (M^+), 550 (M^+−ethylene), 458 (M^+−2AcOH), 131 (cinnamoyl cation, base peak). UV λ_{max}^{EtOH} nm (log ε): 218 (4.34), 223 (4.27), 280 (4.53). *Anal.* Calcd for $C_{34}H_{42}O_8$: C, 70.56; H, 7.32. Found: C, 70.72; H, 7.30. CMR δ (pyridine-d_5): 12.5 (q, C-19), 16.5 (q, C-21), 18.0 (t, C-15), 21.3 (q, Ac), 21.6 (q, C-2′), 28.3 (t, C-2), 28.9 (t, C-7), 29.9 (t, C-6+C-16), 35.0 (t, C-4), 35.2 (d, C-8), 37.6 (t, C-1), 38.3 (s, C-10), 45.0 (d, C-5), 51.3 (d, C-9), 51.6 (d, C-17), 57.8 (s, C-13), 64.4 (t, C-13), 72.6 (d, C-11), 72.9 (d, C-12), 73.0 (d, C-3), 90.2 (s, C-14), 113.0 (s, C-20), 118.0 (d, Cin-2), 128.8 (d, Cin-6), 129.4 (d, Cin-5), 131.0 (s, Cin-4), 135.0 (d, Cin-7), 146.3 (d, Cin-3), 166.2 (s, Cin-1), 170.3 (s, C-1′).

P-2 (12)——Amorphous, MS m/z: 580 (M^+), 432 (M^+−cinnamic acid–acetic acid–H_2O), 131 (cinnamoyl cation, base peak), 43 (acetyl cation). PMR δ ($CDCl_3$): 0.98 (3H, s, 19-CH_3), 1.10 (3H, s, 18-CH_3), 1.85 (3H, s, Ac), 2.01 (3H, s, Ac), 2.15 (3H, s, 21-CH_3), 4.64 (1H, m, 3α-H), 4.84 (1H, d, J=10 Hz, 12α-H), 5.34 (1H, t, J=10 Hz, 11β-H), 6.44 (1H, d, J=16 Hz), 7.75 (1H, d, J=16 Hz).

P-3 (13)——Amorphous, $[\alpha]_D$ +13.1° (c=0.67, $CHCl_3$), PMR δ ($CDCl_3$): 0.92 (3H, s, 19-CH_3), 1.07 (3H, d, J=6 Hz, 21-CH_3), 1.87 (3H, s, Ac), 2.01 (3H, s, Ac), 3.87 (1H, m, 20-H), 4.04+4.16 (2H, ABq, J=9 Hz, 18-CH_2), 4.68 (1H, m, 3α-H), 4.97 (1H, t, J=10 Hz, 11β-H), 5.13 (1H, d, J=10 Hz, 12α-H), 6.47 (1H, d, J=16 Hz), 7.76 (1H, d, J=16 Hz). MS m/z: 562 (M^+−H_2O), 502 (M^+−H_2O−AcOH), 414 (M^+−2H_2O−2AcOH), 131 (cinnamoyl cation, base peak). UV λ_{max}^{EtOH} nm (log ε): 218 (4.36), 222 (4.27), 280 (4.60). CMR δ (pyridine-d_5): 12.2 (q, C-19), 20.3 (q, C-21), 21.3 (q, Ac), 21.6 (q, Ac), 24.6 (t, C-15), 28.3 (t, C-2), 29.3 (t, C-6+C-7), 35.0 (t, C-4), 37.2 (t, C-1), 37.9 (s, C-10), 38.3 (t, C-16), 43.1 (d, C-8), 45.0 (d, C-5), 50.0 (d, C-9), 57.2 (d, C-17), 66.0 (s, C-13), 66.8 (t, C-18), 73.2 (d, C-3), 74.0 (d, C-11), 76.7 (d, C-12), 82.6 (s+d, C-14+C-20), 118.5 (d, Cin-2), 128.8 (d, Cin-6), 129.4 (d, Cin-5), 130.9 (s, Cin-4), 135.0 (d, Cin-7), 146.3 (d, Cin-3), 167.0 (s, Cin-1), 170.3 (s+s, ester carbonyl). *Anal.* Calcd for $C_{34}H_{44}O_8$: C, 70.32; H, 7.64. Found: C, 70.41; H, 7.58.

Conversion of P-3 (13) to P-1 (14)——A mixture of **13** (20 mg), $Pb(OAc)_4$ (100 mg), $CaCO_3$ (22 mg), iodine (30 mg), and diethyl ether (3 ml) was irradiated under nitrogen using a tungsten lamp at 4—15°C for 4 h. The reaction mixture was treated by the same method as in the case of **11**. The spectral data for the product were identical with those of P-1 (**14**).

Acetylation of Condurangogenin B (7)——Condurangogenin B (10 mg) was acetylated with acetic anhydride (0.2 ml) in 2 ml of dry pyridine. After 2 h, the solution was concentrated *in vacuo*, and the residue was purified by HPLC. The PMR spectrum of condurangogenin B 3-acetate was identical with that of **14**.

References and Notes

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