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Studies on the Constituents of Asclepiadaceae Plants. XLIV.[1] Components of *Cynanchum caudatum* Max. Structure of 20-*O*-Cinnamoylsarcostin, 12-*O*-Cinnamoylikemagenol and 20-*O*-Cinnamoylikemagenol

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Three new polyoxypregnane derivatives were isolated from the rhizomes of *Cynanchum caudatum*, and their structures were established as 20-*O*-cinnamoylsarcostin (VI), 12-*O*-cinnamoylikemagenol (X) and 20-*O*-cinnamoylikemagenol (XI) on the basis of chemical and spectral evidences. In the elucidation of their structures, an acyl migration of C-12β ester group to C-20α was found and used for structural studies.

Keywords——*Cynanchum caudatum*; Asclepiadaceae; polyoxypregnane; 20-*O*-cinnamoylsarcostin; 12-*O*-cinnamoylikemagenol; 20-*O*-cinnamoylikemagenol; sarcostin; ikemagenol; acyl migration

In the previous paper, the authors reported the isolation and structural elucidation of glycocaudatin[3] and glycopenupogenin[4] from the rhizomes of *Cynanchum caudatum* Max. (Asclepiadaceae).

This paper deals with the structure determination of three new polyoxypregnane derivatives isolated from the same plant. The crude chloroform extract previously reported[3] was repeatedly chromatographed over silica gel to give 20-*O*-cinnamoylsarcostin (VI), 12-*O*-cinnamoylikemagenol (X) and 20-*O*-cinnamoylikemagenol(XI).

20-*O*-Cinnamoylsarcostin (VI) formed colorless needles, mp 230—232°,[5] $[\alpha]_D$ +118°, $C_{30}H_{40}O_7$. The infrared (IR) spectrum of VI suggested the presence of a conjugated unsaturated ester group at 1690, 1635 and 1270 cm^{-1} and an aromatic group at 1570 cm^{-1}. The mass spectrum did not show the molecular ion but the following ions were observed at *m/e* 364 (M^+-cinnamic acid), 148 (cinnamic acid) and 131 (cinnamoyl cation, base peak). Proton magnetic resonance (PMR) of VI exhibited a secondary methyl at δ 1.31 (d, J=6 Hz, 21-CH_3), two tertiary methyls at δ 1.15 and 1.36, two methines at δ 3.48 (d, d, J=4 Hz, 10 Hz, 12α-H) and 3.50 (m, 3α-H), one methine geminal to the ester group at δ 5.30 (q, J=6 Hz, 20-H) and an olefinic proton at δ 5.33 (m, 6-H) as well as a cinnamoyl group. This compound (VI) was hydrolyzed to a compound whose thin-layer chromatography (TLC) and color test with antimony trichloride showed it to be sarcostin (III). Moreover, VI was acetylated at room temperature to a mono-acetate (VII), whose melting point and IR spectrum were identical with those of the cinnamoyl derivative obtained from 3-*O*-acetylsarcostin[5] (IV). 20-*O*-Cinnamoylsarcostin (VI) had been isolated as an artifact from both penupogenin[6] (V) by an acyl migration and gagaminin (VIII) by hydrolysis with a concomitant acyl migration.[7]

1) Part XLIII: H. Seto, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **25**, 876 (1977).
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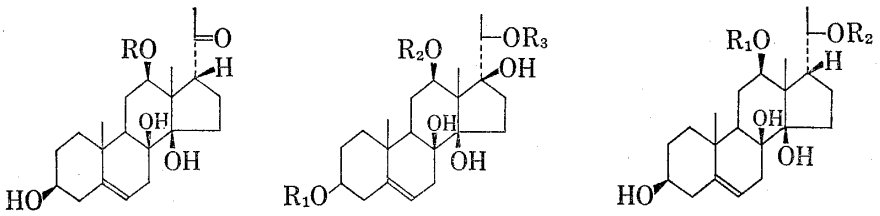
3) H. Bando, T. Yamagishi, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **24**, 1842 (1976).
4) H. Bando, T. Yamagishi, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **24**, 3085 (1976).
5) K. Hayashi and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **23**, 1845 (1975).
6) H. Mitsuhashi and Y. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **8**, 565 (1960); *idem, ibid.*, **10**, 725 (1962); T. Sasaki, K. Hayashi, and H. Mitsuhashi, *ibid.*, **20**, 628 (1972).
7) T. Yamagishi, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **20**, 2289 (1972).

Penupogenin (V) was also found to give VI in 2% yield under the treatment with 0.05 N sulfuric acid, which was used for the transformation of a crude polyoxypregnane glycoside bearing deoxysugars into a crude ester-type aglycone mixture. From the same extract, both 105 mg of VI and 270 mg of V were isolated simultaneously. This quantity of VI was too large to consider as an artifact which was obtained from V during the isolation process, since the transformation of V into VI resulted in at most 2% under the acidic condition mentioned above. Therefore, most of VI isolated should originally exist in the crude aglycone mixture.

Treatment of V in basic condition, such as 5% methanolic potassium carbonate, has been reported to give the migration product VI in a poor yield and lead to sarcostin (III) through hydrolysis.[7] In the present study, however, it was found that treatment of V with sodium hydride in dimethylformamide (DMF) at low temperature gave VI in a good yield.

12-*O*-Cinnamoylikemagenol (X) formed a colorless crystalline powder, mp 145—150°, $[\alpha]_D$+30°. A molecular formula, $C_{30}H_{40}O_6$, of X was deduced from the mass spectrum, which did not show a molecular ion peak but following ions were observed at *m/e* 348 (M^+-cinnamic acid), 330 (*m/e* 348-H_2O), 312 (*m/e* 348-2H_2O), 148 (cinnamic acid), 147 and 131 (cinnamoyl cation, base peak) as the characteristics of the polyoxypregnane derivative, and the mass spectrum suggested 496 to a molecular weight. The IR spectrum showed a conjugated ester group at 1700, 1630 and 1160 cm^{-1}. The PMR spectrum exhibited a secondary methyl at δ 1.09 (d, J=6 Hz, 21-CH_3), two tertiary methyls at δ 1.16 and 1.53, one methine geminal to the ester group at δ 4.91 (d,d, J=4 Hz, 10 Hz, 12α-H), two methines at δ 3.55 (m) ascribed to the protons at C-3α and C-20 geminal to the hydroxyl group, a cinnamoyl group at δ 6.47 (d, J=16 Hz), 7.36—7.71 (m) and 7.76 (d, J=16 Hz). Four of six oxygenes were assigned above. If the remaining two were assumed to be hydroxyl groups, their position might be 8β, 14β or 17β by the biogenetic analogy with other polyoxypregnanes hitherto isolated from the same plant. The possibility at 17β was excluded from the mass spectrum, since most of the compounds having a hydroxyl group at 17β shows a typical fragmentation pattern due to a fission of the side chain[8] while this compound did not show any fragmentation like that. Therefore the compound X was assigned to be 12β-cinnamoyloxy-pregn-5-ene and its structure was established as follows. Hydrolysis of X gave ikemagenol (IX) which was identical with (20*S*)-dihydrolineolon in all respect.[5] The compound X was also identical with the reduction product of ikemagenin[9] (II) with sodium borohydride.

20-*O*-Cinnamoylikemagenol (XI) formed a colorless crystalline powder, mp 135—137°, $[\alpha]_D$+55°. A molecular formula, $C_{30}H_{40}O_6$, was deduced by the mass spectrum which resem-



I: R=H
II: R=Cin

III: $R_1=R_2=R_3=H$
IV: R_1=Ac, $R_2=R_3=H$
V: $R_1=R_3=H$, R_2=Cin
VI: $R_1=R_2=H$, R_3=Cin
VII: R_1=Ac, $R_2=H$, R_3=Cin
VIII: $R_1=H$, R_2=Cin, R_3=Nic

IX: $R_1=R_2=H$,
X: R_1=Cin, $R_2=H$
XI: $R_1=H$, R_2=Cin

Ac=acetyl, Cin=cinnamoyl, Nic=nicotinoyl

Fig. 1

8) M. Fukuoka and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **16**, 553 (1968); K. Hayashi and H. Mitsuhashi, *ibid.*, **20**, 2065 (1972).
9) T. Yamagishi and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **20**, 2070 (1972).

bled that of 12-*O*-cinnamoylikemagenol (X) closely. The IR spectrum also resembled that of X. The PMR spectrum showed two protons at δ 5.35 (m). One was assigned to be an olefinic proton at C-6 and another to be a methine proton geminal to an ester group at C-20. Therefore XI was assigned to be 20-cinnamoyloxy-3β, 8β, 12β, 14β-tetrahydroxy-pregn-5-ene, and the structure was established as follows. Treatment of X with sodium hydride in DMF afforded an acyl migrated compound, whose melting point, IR, PMR and mass spectra were identical with those of XI and there was no depression in mixed melting point between them.

Experimental

Melting points were determined by a Kofler hot stage apparatus and are uncorrected. Optical rotations were taken with a JASCO DIP-4 digital polarimeter. IR spectra were recorded on a Hitachi 215 spectrometer. PMR spectra were measured at 100 MHz with a JNM-FX 100 spectrometer with tetramethylsilane as an internal standard (s, singlet; d, doublet; q, quartet; m, multiplet). Mass spectra (MS) were determined on a Hitachi RMU-7 mass spectrometer. Column chromatography and TLC were carried out using silica gel (70—230 mesh, Merck and HF_{254}, type 60, Merck, respectively).

Isolation Procedure——From the crude aglycone (250 g) which was previously reported,[3] 20-*O*-cinnamoylsarcostin (VI, 105 mg), 12-*O*-cinnamoylikemagenol (X, 5 mg), and 20-*O*-cinnamoylikemagenol (XI, 2 mg), were isolated by silica gel column chromatography and preparative TLC with ethyl acetate–hexane, chloroform–hexane–acetone and so on as an developing solvent systems.

20-*O*-Cinnamoylsarcostin (VI)——Colorless needles from ethyl acetate–hexane, mp 230—232°, $[\alpha]_D$ +118° (c=0.3, MeOH). *Anal.* Calcd. for $C_{30}H_{40}O_7$: C, 70.19; H, 7.89. Found: C, 69.79; H, 7.71. IR ν_{max}^{Nujol} cm^{-1}: 3375, 1690, 1635, 1570, 1270, 1050. PMR $(\delta)_{CDCl_3}$: 1.15 (3H, s), 1.31 (3H, d, J=6 Hz), 1.36 (3H, s), 3.48 (1H, d.d, J=4 Hz, 10 Hz), 3.50 (1H, m), 5.30 (1H, q, J=6 Hz), 5.33 (1H, m), 6.40 (1H, d, J=16 Hz), 7.28—7.54 (5H, m), 7.64 (1H, d, J=16 Hz). MS m/e: 494 (M$^+$—H_2O), 364 (M$^+$—cinnamic acid), 346 (M$^+$—cinaamic acid-H_2O), 328 (M$^+$—cinnamic acid-2H_2O), 313 (M$^+$—cinnamic acid-2H_2O-CH_3), 310 (M$^+$—cinnamic acid-3H_2O), 148, 147, 131 (base peak).

12-*O*-Cinnamoylikemagenol (X)——A crystalline powder from benzene–hexane, mp 145—150°, $[\alpha]_D$ +30° (c=0.04, MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3570, 1700, 1630, 1575, 1160. PMR $(\delta)_{CDCl_3}$: 1.09 (3H, d, J=6 Hz), 1.16 (3H, s), 1.53 (3H, s), 3.55 (2H, m), 4.91 (1H, d.d, J=4 Hz, 10 Hz), 5.36 (1H, broad s), 6.47 (1H, d, J=16 Hz), 7.36—7.71 (m), 7.76 (1H, d, J=16 Hz). MS m/e: 348 (M$^+$—cinnamic acid), 330 (M$^+$—cinnamic acid-H_2O), 312 (M$^+$—cinnamic acid-2H_2O), 297 (M$^+$—cinnamic acid-2H_2O-CH_3), 148, 147, 131 (base peak). Mixed mp with the sodium borohydride reduction product (mp 145—151°) of ikemagenin was 145—151°.

20-*O*-Cinnamoylikemagenol (XI)——A crystalline powder from benzene–hexane, mp 135—137°, $[\alpha]_D$ +55° (c=0.04, MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 1690, 1635, 1575, 1135, 1105. PMR $(\delta)_{CDCl_3}$: 1.15 (3H, s), 1.28 (3H, d, J=6 Hz), 1.31 (3H, s), 3.59 (2H, m), 5.35 (2H, m), 6.42 (1H, d, J=16 Hz), 7.30—7.60 (m), 7.68 (1H, d, J=16 Hz). MS m/e: 348 (M$^+$—cinnamic acid), 330 (M$^+$—cinnamic acid-H_2O), 312 (M$^+$—cinnamic acid-2H_2O), 297 (M$^+$—cinnamic acid-2H_2O-CH_3), 148, 147, 131 (base peak). Mixed mp with the acyl migrated compound (mp 134—136°) derived from X was 134—137°.

Acetylation of VI——A solution of 20 mg of VI, 1 ml of acetic anhydride and 1 ml of pyridine was allowed to stand for 12 hr at room temperature, and poured into ice-water. The mixture was extracted with chloroform and the organic layer was worked up usually. The residue was purified with preparative TLC and crystallized from benzene-hexane to afford 6 mg of colorless needles (VII), mp 237—241°, $[\alpha]_D$ +60° (c=0.21, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 3450, 1730, 1705, 1690 (shoulder), 1635, 1580, 1265, 1185. PMR $(\delta)_{CDCl_3}$: 1.16 (3H, s), 1.34 (3H, d, J=6 Hz), 1.38 (3H, s), 2.04 (3H, s), 3.50 (1H, m), 4.55 (1H, m), 5.36 (2H, m), 6.44 (1H, d, J=16 Hz), 7.28—7.56 (m), 7.68 (1H, d, J=16 Hz).

Cinnamoylation of IV——A solution of 10 mg of IV, 0.5 ml of pyridine and 5.3 mg of cinnamoyl chloride was stirred for 20 hr at room temperature and poured into ice-water. The mixture was extracted with chloroform and the organic layer was worked up usually. The residue was purified with preparative TLC and crystallized from benzene–hexane to yield 8 mg of colorless needles, mp 240—244.5°. The IR and PMR spectra were identical with those of acetylated compound mentioned above, and there was no depression of mixed mp, 240—243°.

Conversion of Penupogenin (V) to 20-*O*-Cinnamoylsarcostin (VI)——i) A solution of 50 mg of V, 5 ml of MeOH and 5 ml of 0.1 N H_2SO_4 was refluxed for 1 hr, and worked up as an acid hydrolysis of a crude glycoside.[3] The resulting mixture was separated with preparative TLC to afford 45 mg of starting material and 1 mg of VI.

ii) A solution of 10 mg of V, 1 ml of DMF and 3 mg of sodium hydride was allowed to stand for 15 min under cooling with ice-water, and was neutrallized with 5% aqueous hydrochloric acid in MeOH and a small amount of water was added, following extraction with chloroform. The organic layer was worked up usually for purification with preparative TLC to afford 3 mg of starting material and 5 mg of VI. In those procedure i) and ii), mp and IR spectra of the migrated products were identical with those of the authentic standard.

Alkaline Hydrolysis of 12-*O*-Cinnamoylikemagenol (X)——A solution of 4 mg of X and 0.5 ml of 5% KOH–MeOH was refluxed for 1 hr. The resulting mixture was purified with preparative TLC and crystallized from MeOH–H_2O to afford 2.5 mg of colorless needles, mp 221—229°, $[\alpha]_D$ +43° (c=0.07, MeOH). PMR $(\delta)_{pyridine-d_5}$: 1.37 (3H, d, J=6 Hz), 1.45 (3H, s), 1.93 (3H, s), 3.74 (3H, m), 5.39 (1H, broad s). MS *m/e*: 366 (M^+), 348, 330, 315, 286, 170, 155, 147, 120. The mass spectrum was agreed to that of 20-dihydrolineolon,[5] and there was no depression of mixed mp, 221—229°.

Reduction of Ikemagenin (II) with $NaBH_4$——35 mg of $NaBH_4$ was added to a stirring solution of 150 mg of II dissolved in 5 ml of MeOH. After 3.5 hr, the solution was neutrallized with 5% aqueous HCl and a small amount of water was added. The reaction mixture was extracted with chloroform and the organic layer was worked up usually. The residue was crystallized from benzene–hexane to afford 108 mg of a colorless powder, mp 145—151°, $[\alpha]_D$ +20° (c=0.11, MeOH). *Anal.* Calcd. for $C_{30}H_{40}O_6$: C, 72.55; H, 8.12. Found: C, 72.06; H, 8.11. IR and PMR spectra of the product were identical with those of X.

Conversion of 12-*O*-Cinnamoylikemagenol (X) to 20-*O*-Cinnamoylikemagenol (XI)——50 mg of X was dissolved in 1 ml of DMF and 6 mg of NaH was added under cooling with ice-water, and after 15 min the resulting mixture was neutrallized with 5% aqueous hydrochloric acid in MeOH and a small amount of water was added, following extraction with chloroform. The organic layer was worked up usually for purification with preparative TLC to afford 14 mg of starting material and 12 mg of XI, mp 134—137°, $[\alpha]_D$ +58° (c=0.1, MeOH). IR and PMR spectra of the product were identical with those of 20-*O*-cinnamoylikemagenol isolated from natural source, and there was no depression of mixed mp, 134—137°.

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