Studies on the Constituents of Asclepiadaceae Plants. XXXVIII.¹⁾
Component of *Cynanchum caudatum* Max.
Structure of Glycocaudatin

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A new 5,6 α -glycolic compound, glycocaudatin, isolated from *Cynanchum caudatum*, in addition to glycocynanchogenine and 12 β -O-cinnamoyl-20-O-acetyl-glycosarcostin, was identical with the derivative obtained in a good yield *via* the epoxidation of caudatin, which had been isolated from the same plant.

Two novel compounds having 5 α ,6 β -hydroxyl groups, glycocynanchogenine (I) and 12 β -O-cinnamoyl-20-O-acetyl-glycosarcostin (II), and several polyoxypregnane derivatives were isolated from *Cynanchum caudatum* Max. (Asclepiadaceae) in previous investigation.³⁾

In this paper the structure determination of a new aglycone, glycocaudatin (III), from *C. caudatum* will be reported. Extraction and isolation procedures followed the preceding manner⁴⁾ and the aglycone mixture obtained from the extracts was subjected to column chromatography and preparative thin-layer chromatography (TLC) to give a crystalline compound (III), mp 286—293°, with a molecular formula of C₂₈H₄₄O₉ from its elemental analysis and mass spectrum (M⁺ at *m/e* 524). Infrared (IR) spectrum of III showed absorptions for hydroxyl group at 3480 cm⁻¹, carbonyl at 1700 cm⁻¹, and conjugated ester at 1708, 1640, and 1170 cm⁻¹. The ester group was also supported by the mass spectrum showing the presence of ikemaic acid (C₇H₁₂O₂) ester⁵⁾ group at *m/e* 396 (M⁺—C₇H₁₂O₂) and 111 (C₇H₁₁O, base peak). The mass spectrum of III also indicated ion peaks due to the loss of H₂O as a characteristic fragmentation of polyoxypregnane derivatives⁶⁾ at *m/e* 378 (396—H₂O), 360 (396—2H₂O), 342 (396—3H₂O), 335 (396—COCH₃—H₂O), 317 (335—H₂O), 299 (335—2H₂O), 281 (335—3H₂O), and 263 (335—4H₂O). Therefore, from the mass spectrum at least, five hydroxyl groups should be present. The two of them were assumed to be present at C-5 and C-6 since the compound (III) as well as glycocynanchogenine³⁾ (I) did not show characteristics of ordinary Δ^5 -pregnene derivatives which had an intensive peak at *m/e* 138 due to retro-Diels-Alder fragmentation. One of the remainder was at C-17, which was supported by the evidence⁶⁾ that the ion peak at 481 (M⁺—COCH₃) due to the loss of a methyl ketone was appeared intensively to some extent when the moiety with carbonyl group at C-20 and hydroxyl group at C-17 was present simultaneously. Other hydroxyl groups should be located at C-3, -8, and -14 from the biogenetic analogy of a number of pregnane derivatives from the same plant.

With respect to other functional groups, the presence of a methyl ketone was also suggested by the ions peaks at *m/e* 481 (M⁺—COCH₃) and 353 (M⁺—C₇H₁₇O₂—COCH₃) in the mass spectrum of III as shown above. On the basis of these spectral data, the compound (III) was

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assumed to be 5,6-glycolyl-caudatin. In order to elucidate its structure, we attempted to derive III from caudatin (IV) which had been isolated from *C. caudatum* by Yamagishi and Mitsuhashi.⁵⁾

The reaction of IV with hydrogen peroxide in formic acid⁷⁾ gave the compound (III) in a poor yield after chromatographic separation procedures. It had been found that the α -epoxide (V) from the reaction of caudatin (IV) with *m*-chloroperbenzoic acid followed by treatment with perchloric acid⁸⁾ converted to III in a good yield by the *trans*-cleavage, and the product showed a multiplet ($J=8$ Hz, $1/2$ HW) at δ 4.18 assigned to 6α -H, geminal to a hydroxyl group, in the nuclear magnetic resonance (NMR) spectroscopy.⁹⁾

As a result of comparison of natural compound (III) with the product obtained through these synthetic processes, the R_f value on TLC, coloration to $SbCl_3$, absorption in the infrared (IR) spectrum, and fragmentation of the mass spectrum were completely identical between these two compounds. In addition, there was no depression of mixed melting point, and the structure of III was defined as 12β -O-ikemaoyl- $3\beta,5\alpha,6\beta,8\beta,14\beta,17\beta$ -hexahydroxypregn-20-one.

Experimental

All melting points were determined on a Kofler hot stage and are uncorrected. Optical rotations were measured in MeOH solution on a Hitachi S115-4 polarimeter; NMR spectra were taken at 100 MHz in $CDCl_3$ and C_5D_5N solutions with a JEOL PS-100 spectrometer using tetramethylsilane as an internal standard and abbreviations used are s=singlet and m=multiplet; IR spectra were determined on a Hitachi 215 spectrometer in Nujol; and mass spectra were recorded with Hitachi RMU-7 mass spectrometer. TLC was performed on Silica gel HF₂₅₄ (Merck, Type 60) and column chromatography was run on silica gel (Merck, 70–230 mesh ASTM).

Extraction Procedure—The dried rhizomes (50 kg) of *C. caudatum* were extracted with $CHCl_3$ and the crude extract was treated with MeOH followed by hexane to yield a crude glycoside mixture (2.7 kg). This glycoside mixture (440 g) was refluxed on a water bath for 1 hr in MeOH (2.4 liters) with an equivalent volume of 0.1 N H_2SO_4 solution, water (2.4 liters) was added, MeOH was evaporated *in vacuo*, and the residual aqueous solution was heated at 60° for 30 min. The resulting mixture was extracted 5 times with $CHCl_3$ (2.4 liters in total), which was washed with 5% $NaHCO_3$ solution and H_2O , and dried over anhydrous Na_2SO_4 to give an aglycone mixture (250 g) on evaporation of the solvent.

Glycocaudatin—From the crude aglycones (250 g), glycocaudatin (III) (5 mg) was separated by column chromatography and preparative TLC, and crystallized from MeOH to prisms, mp 286 – 293° , $[\alpha]_D -22.3^\circ$

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($c=0.31$). Mass Spectrum m/e : 524, 481, 396, 378, 360, 353, 342, 335, 317, 299, 281, 263, 213, 171, 128, 111. IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 3480, 3390, 1708, 1700, 1645, 1235, 1165. Anal. Calcd. for $\text{C}_{28}\text{H}_{44}\text{O}_9$: C, 64.10; H, 8.45. Found: C, 63.91; H, 8.48.

Peroxidation of Caudatin (IV) with Hydrogen Peroxide—A solution of caudatin (200 mg) dissolved in 88% HCO_2H (2 ml) was heated at $70-80^\circ$ for 5 min, cooled, 30% H_2O_2 (0.2 ml) was added and allowed to stand at 37° for 4 hr. The reaction mixture was poured into a large volume of H_2O , FeSO_4 was added to degrade excess H_2O_2 , and resulting solution was evaporated *in vacuo* to yield a dried material, which was reduced with excess LiAlH_4 in tetrahydrofuran. After deactivating excess LiAlH_4 by filtration of the tetrahydrofuran. After deactivating excess LiAlH_4 by filtration of the tetrahydrofuran solution through silica gel with water-saturated ether, MeOH, and H_2O , the precipitate was extracted with MeOH, which was evaporated *in vacuo* to give a massive mixture. This mixture was separated by preparative TLC to yield prisms (21 mg), which were recrystallized from MeOH, mp $286-292^\circ$, $[\alpha]_D -11.8^\circ$ ($c=0.66$). Yield, 10%.

Epoxidation of Caudatin (IV) with *m*-Chloroperbenzoic Acid—A solution of *m*-chloroperbenzoic acid (120 mg) in CH_2Cl_2 (2 ml) was added to a stirring solution of III (200 mg) dissolved in CH_2Cl_2 (2 ml) under cooling during 1 hr, and excess peracid was destroyed by the addition of 10% Na_2SO_3 . The reaction mixture was extracted with CHCl_3 which was washed consecutively with 5% NaHCO_3 , H_2O , and saturated NaCl solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was purified by preparative TLC to give the epoxide (V) (157 mg), which was recrystallized from benzene-hexane, needles, mp $218-221^\circ$, $[\alpha]_D -25^\circ$ ($c=0.6$). Yield, 76%. IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 3560, 3450, 3300, 1710, 1700, 1640, 1230, 1170, 1150. NMR (δ) CDCl_3 : 1.03 (s, 3H), 1.13 (s, 6H), 1.43 (s, 3H), 2.13 (s, 3H), 2.18 (s, 3H), 3.38 (m, 1H, J 1/2 HW=5 Hz, 6 β -H), 3.64 (m, 1H, 3 α -H), 4.42 (m, 1H, 12 α -H), 5.46 (broad, s, 1H, 2'-H). Anal. Calcd. for $\text{C}_{28}\text{H}_{42}\text{O}_8$: C, 65.40; H, 8.36. Found: C, 65.34; H, 8.39.

Cleavage of Epoxide Ring with Perchloric Acid—A solution of 7% HClO_4 (0.3 ml) was added to a stirring solution of the epoxide (IV) (109 mg) in $(\text{CH}_3)_2\text{CO}$ (10 ml) at room temperature for 13 hr. After neutralization with 5% NaOH, a small volume of water was added, $(\text{CH}_3)_2\text{CO}$ was evaporated *in vacuo*, and the resulting solution was filtered to give an amorphous powder, which was crystallized from MeOH to prisms (85 mg), mp $288-293^\circ$, $[\alpha]_D -16.6^\circ$ ($c=0.5$). Yield, 75%. Anal. Calcd. for $\text{C}_{28}\text{H}_{49}\text{O}_9$: C, 64.10; H, 8.40. Found: C, 63.81; H, 8.57. IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 3480, 3390, 1708, 1700, 1645, 1235, 1165. NMR (δ) $\text{C}_5\text{D}_5\text{N} + \text{D}_2\text{O}$: 0.95 (s, 3H), 1.02 (s, 3H), 1.65 (s, 3H), 1.88 (s, 3H), 2.26 (s, 3H), 2.46 (s, 3H), 4.18 (m, 1H, J 1/2 HW=8 Hz, 6 α -H), 4.76 (m, 1H), 4.94 (m, 1H), 5.75 (m, 1H).

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