[Chem. Pharm. Bull. 24(8)1842—1844(1976)]

UDC 547.918.02 : 581.192

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

Hideo Bando, Takashi Yamagishi, Koji Hayashi, and Hiroshi Mitsuhashi

Faculty of Pharmaceutical Sciences, Hokkaido University[2]

(Received December 5, 1975)

A new 5,6α-glycolic compound, glycocaudatin, isolated from *Cynanchum caudatum*, in addition to glycocynanchogenine and 12β-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 5α,6β-hydroxyl groups, glycocynanchogenine (I) and 12β-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 preceeding manner[4] 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 $C_{28}H_{44}O_9$ 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 ($C_7H_{12}O_2$) ester[5] group at *m/e* 396 ($M^+-C_7H_{12}O_2$) and 111 ($C_7H_{11}O$, base peak). The mass spectrum of III also indicated ion peaks due to the loss of H_2O as a characteristic fragmentation of polyoxypregnane derivatives[6] at *m/e* 378 ($396-H_2O$), 360 ($396-2H_2O$), 342 ($396-3H_2O$), 335 ($396-COCH_3-H_2O$), 317 ($335-H_2O$), 299 ($335-2H_2O$), 281 ($335-3H_2O$), and 263 ($335-4H_2O$). 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 glycocynanchogenine[3] (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 evidence[6] that the ion peak at 481 (M^+-COCH_3) 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.

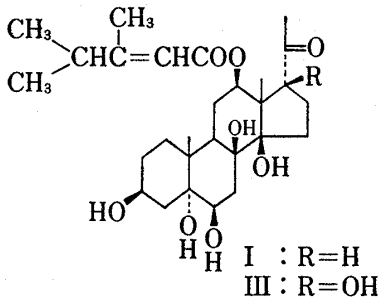
With respect to other functional groups, the presence of a methyl ketone was also suggested by the ions peaks at *m/e* 481 (M^+-COCH_3) and 353 ($M^+-C_7H_{17}O_2-COCH_3$) in the mass spectrum of III as shown above. On the basis of these spectral data, the compound (III) was

1) Part XXXVII: H. Seto, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **24**, 1552 (1976).
2) Location: *Kita-12-jo, Nishi-5-chome, Kita-ku, Sapporo, 060, Japan.*
3) T. Yamagishi, K. Hayashi, R. Kiyama, and Y. Mitsuhashi, *Tetrahedron Letters*, **1972**, 4005; T. Yamagishi, K. Hayashi, and H. Mitsuhashi, *Tetrahedron Letters*, **1973**, 4735.
4) H. Mitsuhashi and H. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **8**, 313 (1960).
5) T. Yamagishi and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **20**, 625 (1972).
6) M. Fukuoka and Y. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **16**, 553 (1968); *idem*, *ibid.*, **17**, 2448 (1969); K. Hayashi and H. Mitsuhashi, *ibid.*, **20**, 2065 (1972).

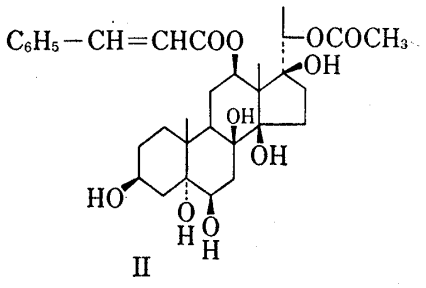
assumed to be 5,6-glycolyl-caudatin. In order to elucidate its structure, we attempted to derive III from caudatin (IV) which had been isolated from *C. caudatum* by Yamagishi and Mitsuhashi.[5]

The reaction of IV with hydrogen peroxide in formic acid[7] 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 acid[8] 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]

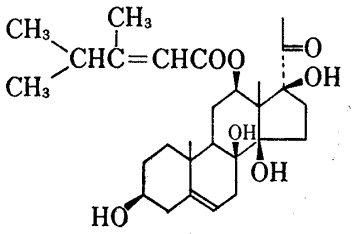
As a result of comparison of natural compound (III) with the product obtained through these synthetic processes, the *Rf* value on TLC, coloration to $SbCl_3$, absorption in the infrared (IR) spectrum, and fragmentation of the mass spectrum were completely identical between these two compounds. In addition,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



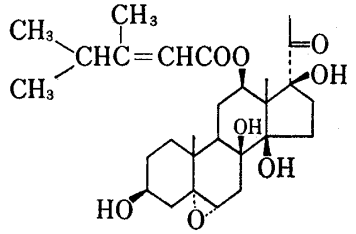
I : R=H
III : R=OH



II



IV



V

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 HF_{254} (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°

7) L.F. Fieser and S. Rajagopalan, *J. Am. Chem. Soc.*, **71**, 3938 (1949).
8) A. Bowers, E. Denot, R. Uraquija, and L.M. Sanches-Hidalgo, *Tetrahedron*, **8**, 116 (1960).
9) J.E. Bridgeman, P.C. Cherry, A.S. Cleggy, J.M. Evans, Sir Ewart R.H. Jones, A. Kasal, V. Kumar, G.D. Meakins, Y. Morisawa, E.E. Richards, and P.D. Woodgate, *J. Chem. Soc. Ser., C*, **1970**, 250.

(c=0.31). Mass Spectrum m/e: 524, 481, 396, 378, 360, 353, 342, 335, 317, 299, 281, 263, 213, 171, 128, 111. IR ν_{max}^{Nujol} cm^{-1}: 3480, 3390, 1708, 1700, 1645, 1235, 1165. *Anal.* Calcd. for $C_{28}H_{44}O_9$: C, 64.10; H, 8.45. Found: C, 63.91; Y, 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° 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 degradate 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 $LiAlH_4$ dy 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°, $[\alpha]_D$ −11.8° (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°, $[\alpha]_D$ −25° (c=0.6). Yield, 76%. IR ν_{max}^{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, 6β-H), 3.64 (m, 1H, 3α-H), 4.42 (m, 1H, 12α-H), 5.46 (broad, s, 1H, 2′-H). *Anal.* Calcd. for $C_{28}H_{42}O_8$: 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 $(CH_3)_2CO$ (10 ml) at room temperature for 13 hr. After neutralization with 5% NaOH, a small volume of water was added, $(CH_3)_2CO$ 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°, $[\alpha]_D$ = −16.6° (c=0.5). Yield, 75%. *Anal.* Calcd. for $C_{28}H_{42}O_9$: C, 64.10; H, 8.40. Found: C, 63.81; H, 8.57. IR ν_{max}^{Nujol} cm^{-1}: 3480, 3390, 1708, 1700, 1645, 1235, 1165. NMR (δ) $C_5D_5N+D_2O$: 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, 6α-H), 4.76 (m, 1H), 4.94 (m, 1H), 5.75 (m, 1H).

Acknowledgement The authors are grateful to Mr. J. Mizutani for collecting the plants, Miss T. Okayama for NMR spectral measurements, Miss H. Kakizaki and Miss T. Obara for elemental analyses, and Miss M. Takahashi for mass spectral measurements.