Dauricoside, a New Glycosidal Alkaloid Having an Inhibitory Activity against Blood-Platelet Aggregation

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Dauricoside (1), a new glycosidal alkaloid, was ioslated from the rhizomes of *Menispermum dauricum* DC. along with dauricine (2), daurisoline (3), dauriporphine (4), menisporphine (5), and 6-*O*-demethylmenisporphine (6), and its structure was determined by means of spectroscopic methods. Compounds 1, 2, and 3 inhibited blood-platelet aggregation induced by adenosine 5′-diphosphate (ADP).

Keywords dauricoside; *Menispermum dauricum*; blood-platelet aggregation inhibitor; artavenustine-11-*O*-β-D-glucopyranoside; tetrahydroprotoberberine-type alkaloid; Menispermaceae

In a previous paper, we reported that a Chinese crude drug, "Bei-Dou-Gen (北豆根)" (rhizomes of *Menispermum dauricum* DC.), showed a moderate inhibitory activity toward rabbit blood-platelet aggregation induced by adenosine 5′-diphosphate (ADP).[1] In a continuation of that work, we have examined the active components of the plants and isolated a new alkaloid named dauricoside (**1**). This paper deals with the structure elucidation of **1**.

An ethanol extract of air-dried rhizomes of *Menispermum dauricum* DC. was treated with 10% citric acid solution and the citric acid-soluble portion was basified with aqueous ammonia and extracted successively with chloroform and butanol to give a chloroform extract and a butanol extract, respectively. The chloroform extract was treated as shown in Chart 2 to give dauricine (**2**),[2] daurisoline (**3**),[3] dauriporphine (**4**),[4] menisporphine (**5**),[5] and 6-*O*-demethylmenisporphine (**6**).[5] On the other hand, the butanol extract was subjected to silica gel column chromatography with methanol–chloroform to give a new alkaloid named dauricoside (**1**).

Dauricoside was obtained as a hydrochloride (**1**·HCl), colorless prisms, mp 216—217 °C, $[\alpha]_D^{22}$ −185.5° (MeOH), and its molecular formula was determined to be $C_{24}H_{29}NO_9 \cdot HCl$ by elemental analysis and the FAB-MS measurement (*m/z* 476, $[1+H]^+$). The UV spectrum of **1**·HCl exhibited absorption maxima at 209 (log ε, 4.47), 224 (4.07), and 287 nm (3.78) and the IR spectrum showed absorptions due to hydroxyl group(s) at 3500—3310 (br) cm^{-1} and benzene ring(s) at 1600, 1530, and 1440 cm^{-1}.

The ^{1}H-NMR spectrum of **1**·HCl in methanol-d_4 revealed signals due to a methoxyl (δ 3.89, 3H, s), four isolated aromatic protons (δ 7.16, 6.93, 6.72, 6.68, each 1H, s), and a glucose group (δ 4.80, 1H, d, *J*=8.3 Hz, 1′-H; δ 3.50, 1H, t, *J*=8.3 Hz, 2′-H; δ 3.47, 1H, t, *J*=8.3 Hz, 3′-H; δ 3.37, 1H, dd, *J*=9.5, 8.3 Hz, 4′-H; δ 3.45, 1H, ddd, *J*=9.5, 6, 2 Hz, 5′-H; δ 3.91, 1H, dd, *J*=12, 2 Hz, 6′-H; δ 3.70, 1H, dd, *J*=12, 6 Hz, 6′-H), but some of the methine and methylene signals were too broad to be analyzed, probably because of slow interconversion between the B/C-*cis* and B/C-*trans* isomers.[6] However, in pyridine-d_5–methanol-d_4 (3 : 1) the spectrum gave a well resolved pattern and showed ^{1}H-signals due to a methine proton (δ 4.50, 14-H) and four methylene protons (δ 2.92, 3.42, 5-H_2; 3.32, 3.66, 6-H_2; 4.50, 4.57, 8-H_2; 3.19, 3.72, 13-H_2) along with four isolated aromatic protons (δ 7.42, 12-H; 7.00, 1-H; 6.98, 9-H; 6.92, 4-H), a methoxyl group (δ 3.85), and a glucose group. The ^{13}C-NMR spectrum in pyridine-d_5–methanol-d_4 (3 : 1) also showed distinct signals corresponding to those groups. These ^{1}H- and ^{13}C-signals were analyzed by the use of ^{1}H–^{1}H shift correlation spectroscopy (COSY), ^{1}H-detected heteronuclear multiple-quantum correlation (HMQC), and ^{1}H-detected heteronuclear multiple-quantum multiple-bond correlation (HMBC) spectra (Chart 3), and it was proved that dauricoside is a tetrahydroprotoberberine-type alkaloid having a methoxyl group and a β-glucopyranosyl group.

Locations of the methoxyl and the β-glucopyranosyl group were determined based on the results of difference nuclear Overhauser effect (NOE) experiments. Irradiation of the methoxyl protons at δ 3.85 showed an NOE increase

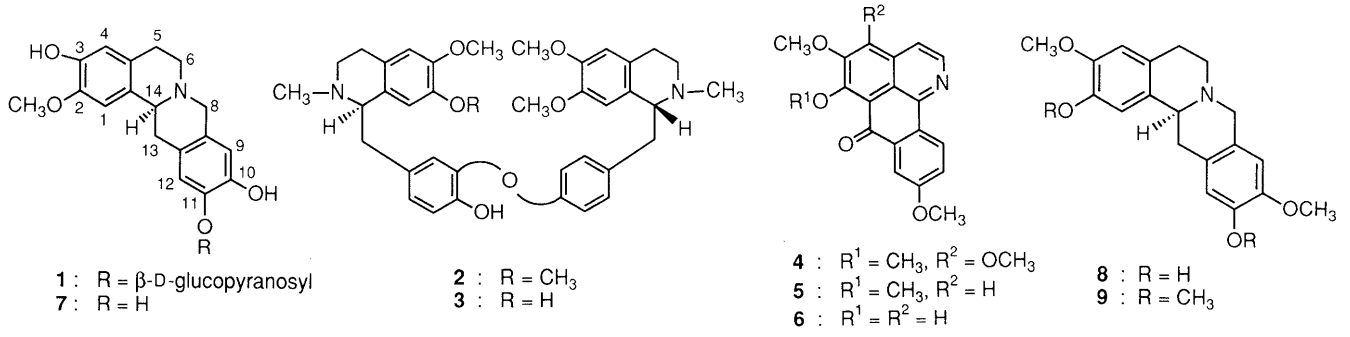
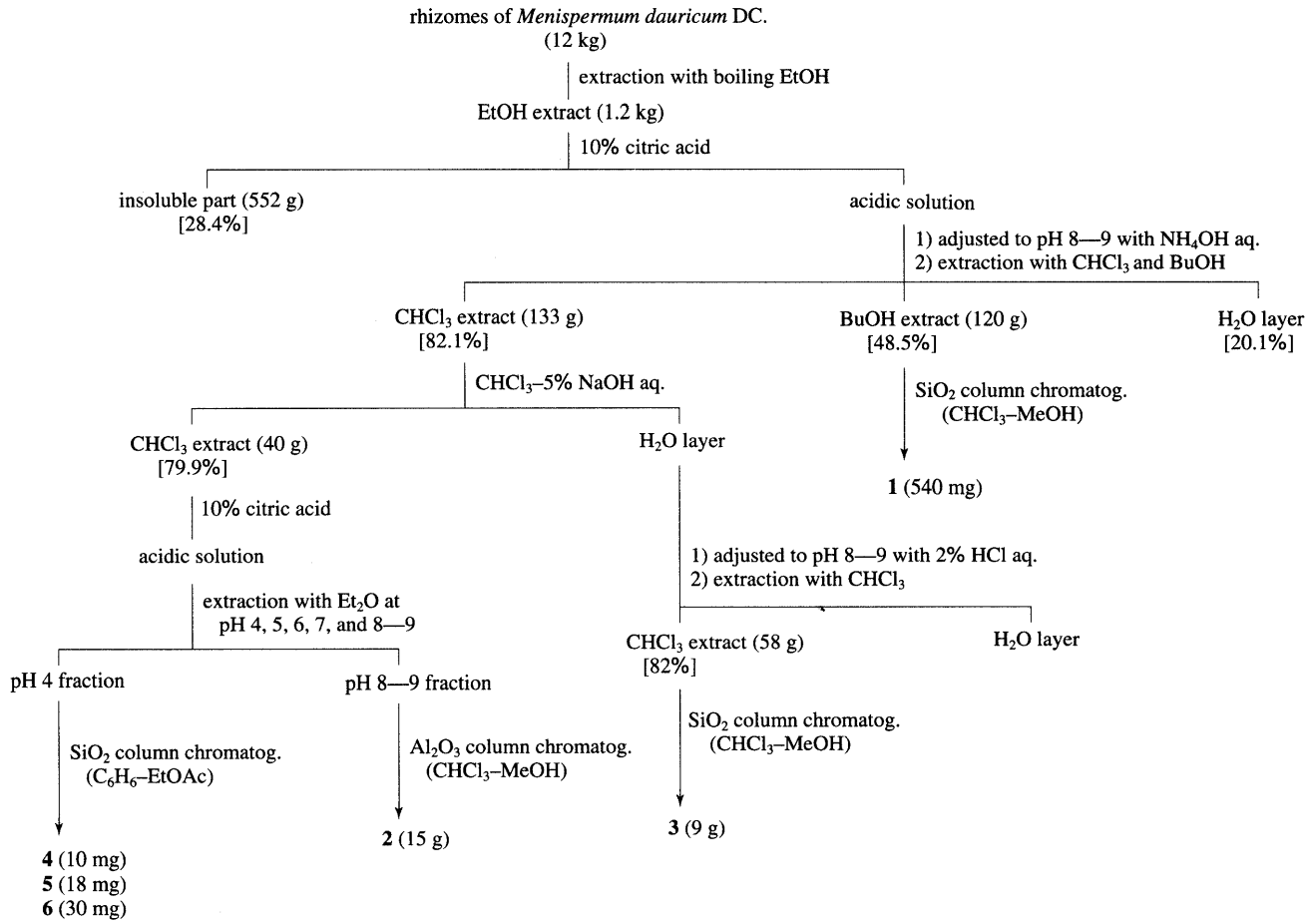


Chart 1



[%], inhibition rate (%) at a final concentration of 0.83 mg/ml

Chart 2

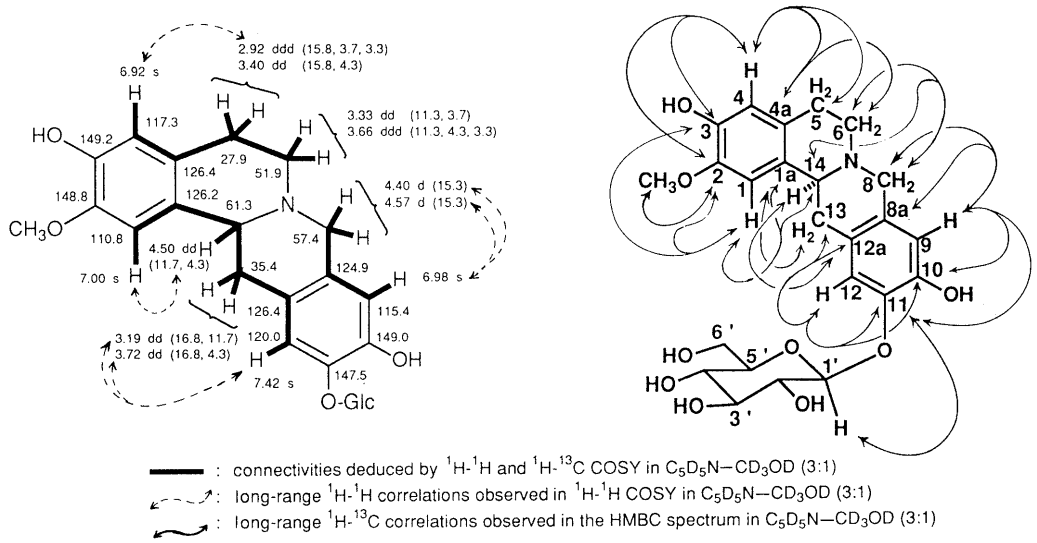


Chart 3

at 1-H (δ 7.00), while irradiation of the anomeric proton at δ 5.33 caused an NOE increase at 12-H (δ 7.42). Thus, the methoxyl group should be located at the C-2 position and the glucopyranosyl group at the C-11 position, and **1** was concluded to be 11-*O*-β-glucopyranosylartavenustine.

The circular dichroism (CD) spectrum of dauricoside hydrochloride (**1**·HCl) showed negative Cotton effects at 289, 233, and 213 nm ([θ], −7700, −30700, −36000), which were parallel with those of 14*S*-(−)-coreximine hydrochloride (**7**·HCl) and 14*S*-(−)-xylopinine hydrochloride (**8**·HCl).[7] This showed the *S*-configuration at the C-14 position, and thus dauricoside was determined to be (−)-artavenustine-11-*O*-β-D-glucopyranoside (**1**), which is the first example of a glycoside of a tetrahydroproto-

berberine-type alkaloid.

Among the compounds obtained, **1**, **2**, and **3** showed moderate inhibitory effects toward rabbit blood-platelet aggregation induced by ADP (IC_{50}: **1**, 0.81 mM; **2**, 0.07 mM; **3**, 0.10 mM), being more potent than aspirin (IC_{50}, 0.95 mM).

Experimental

Melting points (uncorrected) were obtained with a Yanagimoto micro melting point apparatus. Optical rotation was measured with a JASCO DIP-140 digital polarimeter. UV, IR, and CD spectra were taken with a Shimadzu 202 UV spectrometer, a JASCO IRA-2 spectrometer, and a JASCO J-500C spectropolarimeter, respectively. NMR spectra were measured with a JEOL GX-400 spectrometer and MS were taken with a JEOL JMS D-300 spectrometer.

The inhibitory effect of each fraction and compound obtained was examined by the method of Born and Crose[8] as reported in a previous paper.[1]

Extraction and Isolation Air-dried rhizomes of *Menispermum dauricum* DC. (12 kg) were cut into small pieces and extracted with boiling EtOH. After concentration of the EtOH solution *in vacuo*, the residue (1.2 kg) was treated with 10% citric acid solution. The citric acid-soluble portion was basified with aqueous NH_4OH and extracted successively with $CHCl_3$ and BuOH to give a $CHCl_3$ extract (133 g) and a BuOH extract (120 g), respectively. The BuOH extract was chromatographed over silica gel (2500 g) with $CHCl_3$–MeOH. Fractions eluted with $CHCl_3$–MeOH (10 : 1 and 10 : 2) (620 mg) were further purified by silica gel column chromatography, and a fraction containing dauricoside was converted to the hydrochloride and treated with MeOH to give dauricoside hydrochloride (**1**·HCl, 540 mg) as colorless prisms, mp 216—217 °C. On the other hand, the $CHCl_3$ extract was treated as shown in Chart 2 to give dauricine (**2**, 15 g),[2] daurisoline (**3**, 9 g),[3] dauriporphine (**4**, 10 mg),[4] menisporphine (**5**, 18 mg),[5] and 6-*O*-demethylmenisporphine (**6**, 30 mg).[5]

Dauricoside Hydrochloride (1·HCl) Colorless prisms, mp 216—217 °C (MeOH), $[\alpha]_D^{22}$ −185.5° (c = 0.5, MeOH). UV λ_{max}^{MeOH} nm (log ε): 209 (4.47), 224 (4.07), 287 (3.78). IR ν_{max}^{KBr} cm^{-1}: 3500—3310 (br, OH), 1600, 1530, 1440 (benzene ring). ^{1}H-NMR [C_5D_5N–CD_3OD (3 : 1)] δ: 3.85 (3H, s, 2-OC$\underline{H}_3$), 3.90 (1H, ddd, J = 11.0, 5.3, 2.4 Hz, 5′-H), 4.06 (1H, dd, J = 11.0, 9.0 Hz, 4′-H), 4.09 (1H, dd, J = 9.0, 7.5 Hz, 2′-H), 4.11 (1H, t, J = 9.0 Hz, 3′-H), 4.19 (1H, dd, J = 12.5, 5.3 Hz, 6′-H), 4.36 (1H, dd, J = 12.5, 2.4 Hz, 6′-H), 5.33 (1H, d, J = 7.5 Hz, 1′-H), and Chart 3. ^{13}C-NMR [C_5D_5N–CD_3OD (3 : 1)] δ: 105.6 (d, C-1′), 79.9 (d, C-5′), 79.1 (d, C-3′), 75.9 (d, C-2′), 72.2 (d, C-4′), 63.3 (t, C-6′), 57.4 (q, 2-O$\underline{C}H_3$), and Chart 3. FAB-MS (matrix, glycerol) m/z: 476 $[\mathbf{1}+H]^+$. *Anal.* Calcd for $C_{24}H_{29}NO_9 \cdot HCl \cdot H_2O$: C, 54.39; H, 6.09; N, 2.64. Found: C, 54.15; H, 5.91; N, 2.8. CD (c = 0.005, MeOH) $[\theta]^{22}$ (nm): −7700 (289), −30700 (233), −36000 (213).

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References and Notes

1) G.-X. Qu, N.-L. Wang, Y.-X. Sang, X.-S. Yao, *J. Shenyang College of Pharmacy*, **6**, 16 (1989).
2) M. Tomita, Y. Okamoto, *Yakugaku Zasshi*, **84**, 1030 (1964).
3) X.-W. Zheng, Z.-D. Min, S.-X. Zhao, *Ke Xue Tong Bao*, **24**, 285 (1979).
4) M. Takani, Y. Takasu, K. Takahashi, *Chem. Pharm. Bull.*, **31**, 3091 (1983); J. Kunitomo, S. Kaede, M. Satoh, *ibid.*, **33**, 2778 (1985).
5) J. Kunitomo, M. Satoh, *Tetrahedron*, **39**, 3261 (1983).
6) N. Takao, K. Iwasa, M. Kamigauchi, M. Sugiura, *Chem. Pharm. Bull.*, **25**, 1426 (1977).
7) B. Ringdahl, R. P. K. Chan, J. C. Craig, R. H. F. Manske, *J. Nat. Prod.*, **44**, 75 (1981).
8) G. V. R. Born, M. J. Crose, *J. Physiol.*, **168**, 178 (1963).