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PARISHINS B AND C FROM RHIZOMES OF *GASTRODIA ELATA*

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Key Word Index—*Gastrodia elata*; Orchidaceae; rhizomes; glucoside; parishin, parishins B and C.

Abstract—Two new glucosides, 1,2- and 1,3-bis[4-(β-D-glucopyranosyloxy)benzyl]citrate, named parishins B and C, together with tris [4-(β-D-glucopyranosyloxy)benzyl] citrate (parishin) have been isolated from the rhizomes of *Gastrodia elata*, and their structures elucidated on the basis of spectroscopic evidence.

INTRODUCTION

Rhizomes of *Gastrodia elata* (Chinese name: Tien-ma) are recorded in Shen-nung-pen-tsao-ching as a high-grade drug. It is used for the treatment of headache, dizziness, vertigo, convulsions epilepsy, etc. [1]. Phytochemical studies of this species have revealed the presence of vanillyl alcohol [2], 4-hydroxybenzaldehyde, 4-hydroxybenzyl alcohol, 4-hydroxybenzyl methyl ether, 4-(4′-hydroxybenzyloxy)benzyl methyl ether, bis(4-hydroxybenzyl) ether, bis(4-hydroxybenzyl)ether mono-β-D-glucopyranose, 4-(β-D-glucopyranosyloxy) benzyl alcohol and tris[4-(β-D-glucopyranosyloxy) benzyl] citrate (parishin) [3].

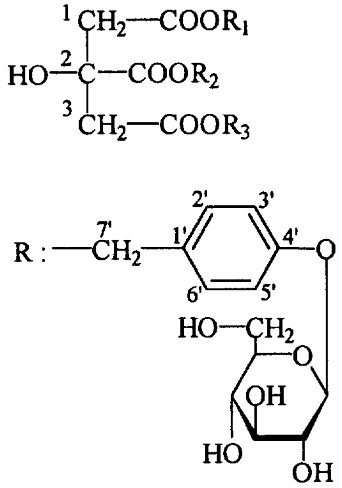
Upon investigation of marker substances for chemical evaluation of Gastrodiae rhizomes, we have isolated three highly polar components (**1**–**3**). Among them, compound **1** was identified as parishin [3] and the other two compounds were analogous to **1**. They were new and named parishins B and C, respectively. In this paper we describe the isolation and structural elucidation of the new compounds.

RESULTS AND DISCUSSION

Rhizomes of *G. elata* were ground and extracted with 70% methanol. The extract was chromatographed on charcoal and successively eluted with H_2O, ethanol and chloroform. The ethanol and chloroform eluates, after concentration, were suspended in H_2O and partitioned against chloroform and ethyl acetate. The aqueous mother liquid was subjected to MCI gel CHP 20P column chromatography to give compounds **2**, **3** and **1**, successively. These compounds were detected in the original 70% methanol extract by HPLC.

Compound **1** was obtained as a white amorphous powder, $C_{45}H_{56}O_{25} \cdot 1/2H_2O$, $[\alpha]_D^{28}$ −73.9°. The IR spectrum showed absorption bands at 1736 and 1234 cm^{-1}, indicating the presence of an ester group. The ^1H and ^{13}C NMR spectra showed signals assignable to those of parishin (**1**) and the negative ion FAB mass spectrum exhibited a $[M - H]^-$ peak at m/z 995, which correlated as well. The structure of **1** was further confirmed by comparing the NMR data for its acetate derivative with values reported in the literature [3]. Parishin was first isolated from *Vanda parishi* by Dahmen and Leander in 1976 [4] and from *G. elata* by Taguchi *et al.* in 1981 [3], but no ^1H and ^{13}C NMR data were presented in detail.

Both parishins B (**2**) and C (**3**) were obtained as white amorphous powders and showed the same for-



1. $R_1=R_2=R$, $R_3=H$

2. $R_1=R_3=R$, $R_2=H$

3. $R_1=R_2=R_3=R$

mula, $C_{32}H_{40}O_{19} \cdot 1/2H_2O$, by elemental analysis. The ^{1}H and ^{13}C NMR spectra of **2** showed patterns similar to those of **1**. Inspection of the ^{1}H NMR spectrum of **2**, showed signals arising from the citrate moiety [δ 2.73, 2.89 (each 1H, *d*, $J = 15.7$ Hz, $-CH_2-$), 2.80 and 2.95 (each 1H, *d*, $J = 15.4$ Hz, $-CH_2-$)] and from the glucopyranosyloxybenzyl alcohol moiety [δ 4.88 and 4.89 (each 1H, *d*, $J = 7.5$ Hz, anomeric H); 4.98 and 5.02 (each 2H, *s*, Ph–CH_2O–); 7.05, 7.07, 7.23 and 7.26 (each 2H, *d*, $J = 8.1$ Hz, 2 × A_2B_2 type H)], with a molar ratio of 1:2. This was consistent with the negative ion FAB mass spectrum of **2**, which showed a prominent $[M-H]^-$ at *m/z* 727, corresponding to one glucopyranosyloxybenzyl alcohol moiety less than **1**.

The conjunction of the two glucopyranosyloxybenzyl alcohol moieties with the citrate moiety was determined by interpretation of ^{1}H and ^{13}C NMR spectra. The chemical shifts of the two methylene groups of the citrate moiety in the ^{1}H NMR spectrum and three carbonyl groups of the citrate moiety (δ 171.1, 173.2 and 174.6) in the ^{13}C NMR spectrum were presented separately, indicating that the citrate moiety existed in an asymmetrical situation. Thus, **2** was established as 1,2-bis[4-β-D-glucopyranosyloxy)benzyl] citrate.

Although the ^{1}H NMR spectrum of parishin C also showed signals assignable to the citrate and glucopyranosyloxybenzyl alcohol moieties in a ratio of 1:2, signals arising from the two methylene groups of the citrate moiety [δ 2.83 and 2.95 (each 2H, *d*, $J = 13.0$ Hz, 2 × $-CH_2-$)] and those from the two glucopyranosyloxybenzyl alcohol moieties [δ 4.90 (2H, *d*, $J = 7.5$ Hz, 2 × anomeric H), 5.04 (4H, *s*, 2 × Ph–CH_2–O–), 7.06 and 7.29 (each 4H, *d*, $J = 8.7$ Hz, 2 × A_2B_2 type H)] were overlapped. This observation indicated that the two glucopyranosyloxybenzyl alcohol moieties were connected to 1,3-carbonyl groups of the citrate moiety, forming a symmetrical molecule. The ^{13}C NMR spectrum of **3** showed a signal due to two carbonyl groups [δ 171.2 (2C, $-COOR_1$ and $-COOR_3$)] and also showed two methylene groups [δ 44.3 (2C, 2 × $-CH_2-$)] of the citrate moiety; the other two methylene groups belonged to the two glucopyranosyloxybenzyl alcohol moieties [δ 67.2 (2C, 2 × Ph–CH_2O–] with the same chemical shift, respectively. That was in agreement with the above suggestion, and **3** was thus concluded to be 1,3-bis[4-β-D-glucopyranosyloxy)benzyl] citrate.

EXPERIMENTAL

Plant material. Rhizomes of *G. elata* Blume were purchased in Taipei, Taiwan, and verified by Dr Hsien-Chang Chang, Brion Research Institute of Taiwan. A voucher specimen is deposited in the National Laboratories of Foods and Drugs, Department of Health, Executive Yuan, Republic of China.

Extraction and isolation. Dried and ground rhizomes (2.0 kg) were extracted with 70% MeOH (5 l × 5) at 80°. The extract, after removal of MeOH by evapn, was subjected to charcoal CC. Elution was carried out with H_2O (3 l) followed by EtOH (7.5 l) and $CHCl_3$ (4 l). The EtOH and $CHCl_3$ eluates were combined and concd. The residue was suspended in H_2O and partitioned against organic solvents to give $CHCl_3$-, EtOAc- and H_2O-soluble frs. The aq. layer was chromatographed over MCI gel CHP 20P [H_2O–MeOH (1:0 → 3:2)] to give **2** (6.77 g), **3** (1.41 g) and **1** (9.92 g), successively.

Each isolation step was checked by HPLC, which was carried out on an ODS column (4.6 × 150 mm), 222-nm UV detector and eluted by 1% H_3PO_4–MeOH (gradient: 0 min, 17:3 → 15 min, 3:1 → 30 min, 7:3) at 1 ml min^{-1}. The contents of these marker substances in crude material was detected by HPLC under the same conditions.

Compound **1** (*parishin*). Amorphous solid. $[\alpha]_D^{28}$ −73.9° (MeOH; *c* 1.0). Found: C, 54.01; H, 5.65, Calc. for $C_{45}H_{56}O_{25} \cdot 1/2H_2O$, C, 53.73, H, 5.71%. Negative-ion FAB MS *m/z* (rel. int.): 995 $[M-H]^-$ (54), 727 $[M-268]^-$ (100), 459 $[M-2\times268]^-$ (23). UV λ_{max}^{EtOH} nm: 276, 270, 222. IR ν_{max}^{KBr} cm^{-1}. 3393, 2924, 1736, 1234, 1047. ^{1}H NMR (300 MHz, MeOH d_4): δ 2.77, 29.4 (each 2H, *d*, $J = 15.2$ Hz, 2 × $-CH_2-$), 3.68 (3H, *dd*, $J = 4.7$, 12.0 Hz, 3 × Glc H-6), 3.87 (3H, *dd*, $J = 1.8$, 12.0 Hz, 3 × Glc H-6), 4.88 (3H, *d*, $J = 7.5$ Hz, 3 × anomeric H), 4.90 (2H, *s*, H-7′), 4.97 (4H, *s*, 2 × H-7′;), 7.03 (2H, *d*, $J = 8.6$ Hz, H-2′, 3′, 5′, 6′), 7.06 (4H, *d*, $J = 8.6$ Hz, 2 × H-3′, 5′), 7.15, 7.24, 7.25, (each 2H, *d*, $J = 8.6$ Hz, 3 × H-2′, 6′). ^{13}C NMR (75 MHz, MeOH-d_4): δ 44.7 (2C, C-1, 3), 62.5 (3C, 3 × Glc C-6), 67.3 (2C, 2 × C-7′), 68.2 (C-7′), 71.4 (3C), 74.7, 74.9 (2C), 77.9 (3C), 78.1 (3C) (3 × Glc-2′, 3′, 4′, 5′), 102.3 (3C, 3 × Glc C-1), 117.8 (6C, 3 × 3′, 5′), 130.7 (3C, 3 × C-1′), 131.0 (6C, 3 × C-2′, 6′), 159.1 (3C, 3 × C-4′), 170.9 (2C, $-COOR_1$, $-COOR_3$), 174.3 ($-COOR_2$).

Compourd **2** (*pairishin B*). Amorphous solid. $[\alpha]_D^{28}$ −64.6° (MeOH; *c* 1.0). Found: C, 52.16; H, 5.76. $C_{32}H_{40}O_{19} \cdot 1/2H_2O$ requires: C, 52.10, H, 5.60%. Negative-ion FAB MS *m/z* (rel. int.): 727 $[M-H]^-$ (100), 459 $[M-268]^-$ (57), 191 $[M-2\times268]^-$ (9). UV λ_{max}^{EtOH} nm: 276, 270, 222. IR ν_{max}^{KBr} cm^{-1}: 3391, 2926, 1736, 1232, 1074. ^{1}H NMR (300 MHz, MeOH-d_4): δ 2.73, 2.89 (each 1H, *d*, $J = 15.7$ Hz, $-CH_2-$), 2.80, 2.95 (each 1H, *d*, $J = 15.4$ Hz, $-CH_2-$), 3.68 (2H, *dd*, $J = 4.9$, 12.1 Hz, 2 × Glc H-6), 3.87 (2H, *d*, $J = 12.1$ Hz, 2 × Glc H-6), 4.88, 4.89 (each 1H, *d*, $J = 7.5$ Hz, 2 × anomeric H), 4.98, 5.02 (each 2H, *s*, 2 × *H*-7′), 7.05, 7.07 (each 2H, *d*, $J = 8.1$ Hz, 2 × H-3′, 5′), 7.23, 7.26 (each 2H, *d*, $J = 8.1$ Hz, 2 × H-2′, 6′). ^{13}C NMR (75 MHz, MeOH-d_4): δ 44.2, 44.5 (C-1, 3), 62.5 (2C, 2 × Glc C-6), 67.3, 68.2 (2 × C-7′), 74.6 (C-1′), 71.4, 74.9, 77.9, 78.1 (each 2C, 2 × Glc C-2, 3, 4, 5), 102.3 (2C, 2 × Glc C-1), 117.8 (4C, 2 × C-3′, 5′), 130.8 (2C, 2 × C-1′), 131.0 (4C, 2 × C-2′, 6′), 159.1 (2C, 2 × C-4′), 171.1 ($-COOR_1$), 173.2 ($-COOR_3$), 174.6 ($-COOR_2$).

Compound **3** (*parishin C*). Amorphous solid. $[\alpha]_D^{28}$ −21.6° (MeOH; *c* 1.0). Found: C, 52.03, H, 5.49. $C_{32}H_{40}O_{19} \cdot 1/2H_2O$ requires: C, 52.10, H, 5.60%. Negative-ion FAB MS *m/z* (rel. int.): 727 $[M-H]^-$

(72), 459 $[M-268]^-$ (100), 191 $[M-2\times268]^-$ (59). UV λ_{max}^{EtOH} nm: 276, 270, 222. IR ν_{max}^{KBr} cm^{-1}: 3420, 2924, 1732, 1232, 1074. ^1H NMR (300 MHz, MeOH-d_4): δ 2.83, 2.95 (each 2H, *d*, J = 13.0 Hz, 2 × $-CH_2-$), 3.69 (2H, *dd*, J = 4.9, 12.0 Hz, 2 × Glc H-6), 3.88 (2H, *dd*, J = 1.6, 12.0 Hz, 2 × Glc H-6), 4.90 (2H, *d*, J = 7.5 Hz, 2 × anomeric H), 5.04 (4H, *s*, 2 × H-7′), 7.06 (4H, *d*, J = 8.1 Hz, 2 × H-3′, 5′), 7.29 (4H, *d*, J = 8.1 Hz, 2 × H-2′, 6′). ^{13}C NMR (75 MHz, MeOH-d_4): δ 44.3 (2C, C-1, 3), 62.5 (2C, 2 × Glc C-6), 67.2 (2C, 2 × C-7′), 74.3 (2C), 71.4 (2C), 74.9 (2C), 78.0, 78.2 (2 × Glc C-2, 3, 4, 5), 102.3 (2C, 2 × Glc C-1), 117.7 (4C, 2 × C-3′, 5′), 130.8 (2C, C-1′), 131.2 (4C, 2 × C-2′, 6′), 159.1 (2C, 2 × C-4′), 171.2 (2C, $-COOR_1$, $-COOR_3$), 176.3 ($-COOR_2$).

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