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HYDROPIPEROSIDE, A NOVEL COUMARYL GLYCOSIDE FROM THE ROOT OF *POLYGONUM HYDROPIPER*

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Key Word Index—*Polygonum hydropiper*; Polygonaceae; hydropiperoside; β-D-(1,3,6-tri-*p*-coumaryl)-fructofuranosyl-α-D-glucopyranoside; anthraquinone; ellagic acid 3,3′-di-*O*-methyl ether; gallic acid; quercetin 3-*O*-glucoside; quercetin 3-*O*-rhamnoside; antifertility activity; allelopathy.

Abstract—From the methanol extract of the root of *Polygonum hydropiper*, a novel coumaryl glycoside hydropiperoside was isolated together with anthraquinone, ellagic acid 3,3′-di-*O*-methyl ether, gallic acid, two quercetin glycosides and an unidentified aromatic δ-lactone possessing antifertility activity. The structure of hydropiperoside was established as β-D-(1,3,6-tri-*p*-coumaryl)-fructofuranosyl-α-D-glucopyranoside by combination of extensive ^{1}H NMR and ^{13}C NMR spectra, and the FD/MS spectrum.

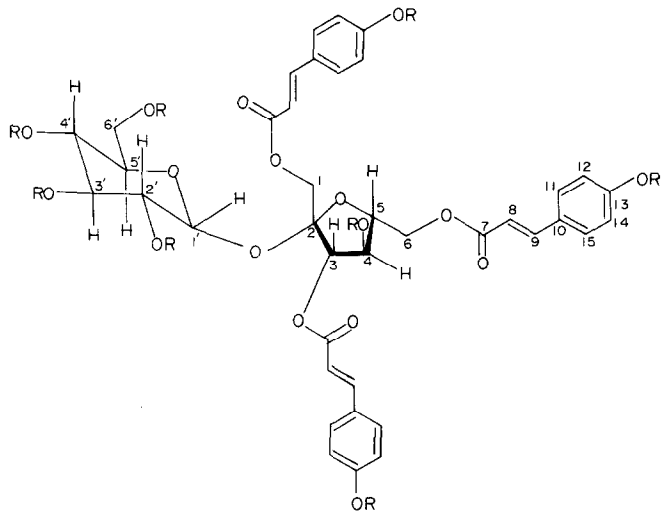
INTRODUCTION

The folk medicinal plant, *Polygonum hydropiper* L., which is used against cancer [1], elaborates the intense pungent sesquiterpene dialdehyde, polygodial, which shows anti-complement and plant growth regulatory activities, and the related drimane-type sesquiterpenoids in leaf and seed [2–4]. Recently, we reported isolation of an additional potent pungent sesquiterpene dialdehyde, warburganal [5], which has the intense cytotoxic (0.01 μg/ml, KB) and antifeedant properties (0.1 ppm/cm^2) against African army worm [6–9]. Garg *et al.* [10, 11] reported that an ethanolic extract of the root of *P. hydropiper* showed antifertility activity against female albino rats. When *P. hydropiper* is planted in a garden, neighbouring plants gradually die, indicating that it has allelopathic effects. As a part of a systematic study of medicinal plants, we investigated the chemical constituents of the root of *P. hydropiper* and isolated a novel phenylpropanoid glycoside hydropiperoside (**1**), which is described in the present communication.

RESULTS AND DISCUSSION

Hydropiperoside (**1**)

The compound (**1**), $C_{39}H_{40}O_{17}$ (FD/MS: $[M+K]^+$, 819; $[M+Na]^+$, 803) was colorless and amorphous. The UV and IR spectra of **1** showed the presence of a hydroxyl group (3350 cm^{-1}) aromatic rings (1600, 1517 cm^{-1}; λ_{max} 228, 315 nm) and an aromatic ester carbonyl group



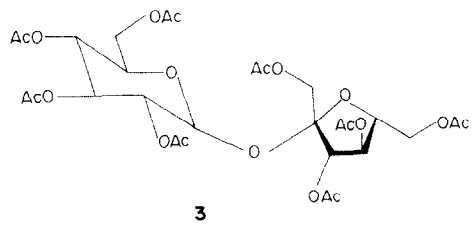
1 R = H
2 R = Ac

Table 1. ^{1}H NMR spectral data* (400 MHz) of hydropiperoside (**1**) and its acetate (**2**)

	1†	2‡	Shift value
H-1	4.34 *d*, *J* = 12.0	4.33 *d*, *J* = 12.1	+0.01
	4.36 *d*, *J* = 12.0	4.43 *d*, *J* = 12.1	−0.07
H-3	5.54 *d*, *J* = 8.0	5.67 *d*, *J* = 5.9	−0.13
H-4	4.45 *dd*, *J* = 8.0, 8.0	5.54 *dd*, *J* = 5.9, 5.9	−1.09
H-5	4.19 *ddd*, *J* = 8.0, 7.0, 4.0	4.35 *ddd*, *J* = 6.6, 5.9, 4.8	−0.16
H-6	4.50 *dd*, *J* = 12.0, 7.0	4.49 *dd*, *J* = 11.8, 6.6	+0.01
	4.52 *dd*, *J* = 12.0, 4.0	4.54 *dd*, *J* = 11.8, 4.8	−0.02
H-1′	5.50 *d*, *J* = 4.0	5.78 *d*, *J* = 3.7	−0.28
H-2′	3.44 *dd*, *J* = 9.0, 4.0	4.95 *dd*, *J* = 10.3, 3.7	−1.51
H-3′	3.36 *dd*, *J* = 9.0, 9.0	5.50 *dd*, *J* = 10.3, 9.9	−2.14
H-4′	3.40 *dd*, *J* = 9.0, 9.0	5.05 *dd*, *J* = 9.9, 9.6	−1.65
H-5′	3.97 *ddd*, *J* = 12.0, 9.0, 3.0	4.40 *ddd*, *J* = 9.6, 4.4, 2.2	−0.43
H-6′	3.79 *dd*, *J* = 12.0, 5.0	4.17 *dd*, *J* = 12.5, 2.2	−0.38
	3.85 *dd*, *J* = 12.0, 3.0	4.24 *dd*, *J* = 12.5, 4.4	−0.39
H-8	6.27 *d*, *J* = 15.8	6.44 *d*, *J* = 16.2	—
	6.33 *d*, *J* = 15.8	6.45 *d*, *J* = 16.2	
	6.38 *d*, *J* = 15.8	6.47 *d*, *J* = 16.2	
H-12	6.78 *d*, *J* = 8.8	7.09 *d*, *J* = 8.8	—
H-14	6.82 *d*, *J* = 8.8	7.10 *d*, *J* = 8.8	—
	6.83 *d*, *J* = 8.8	7.12 *d*, *J* = 8.8	
H-11	7.36 *d*, *J* = 8.8	7.55 *d*, *J* = 8.8	—
H-15	7.42 *d*, *J* = 8.8	7.56 *d*, *J* = 8.8	—
	7.43 *d*, *J* = 8.8	7.60 *d*, *J* = 8.8	
H-9	7.64 *d*, *J* = 15.8	7.72 *d*, *J* = 16.2	—
	7.65 *d*, *J* = 15.8	7.72 *d*, *J* = 16.2	
	7.71 *d*, *J* = 15.8	7.77 *d*, *J* = 16.2	
Ac	—	1.85 *s* (3H)	—
		1.97 *s* (3H)	
		2.06 *s* (3H)	
		2.07 *s* (3H)	
		2.12 *s* (3H)	
		2.31 *s* (9H)	

*All assignments were confirmed by double resonance experiments.
†In DMSO-d_6. *J* values in Hz.
‡In $CDCl_3$. *J* values in Hz.

(1700 cm^{-1}). Hydrolysis of **1** with 0.2% sodium hydroxide gave *p*-coumaric acid. The ^{1}H NMR spectrum (400 MHz) (Table 1) showed the presence of the typical proton signals of three *p*-coumaryl moieties [δ6.78, 6.82, 6.83 (each 2H, *d*, *J* = 8.8 Hz); 7.36, 7.42, 7.43 (each 2H, *d*, *J* = 8.8 Hz); 6.27, 6.33, 6.38 (each 1H, *d*, *J* = 15.8 Hz); 7.64, 7.65, 7.71 (each 1H, *d*, *J* = 15.8 Hz)], showing that **1** was esterified with three *p*-coumaric acids. Acetylation of **1** with acetic anhydride–pyridine gave the octa-acetate (**2**), mp 84–85.5°, $C_{55}H_{56}O_{25}$ (FD/MS: M^+, 1116; 1742 cm^{-1}), whose ^{1}H NMR spectrum (Table 1) exhibited the presence of five aliphatic acetate groups (δ1.85, 1.97, 2.06, 2.07, 2.12) and three acetate groups (δ2.31) for each *p*-coumaric acid residue. The EI/MS spectrum of **2** showed the fragment ions at *m/z* 331 and 769, assignable to glucose tetra-acetate (**4**) and the fragment **5**, with one aliphatic acetate and three acetylated coumaryl groups. The presence of a glucose group in hydropiperoside (**1**) was further supported by the fragment ion at *m/z* 477 (M^+ + Na-coumaryl-H-gluc) in the FD/MS spectrum of **1**. Furthermore, the presence of the following partial structures (**A**–**C**) in **2** were confirmed by the extensive proton spin decoupling experiments (Table 1). On the basis of ^{1}H NMR and mass spectra of **2**, it was suggested that the partial structure **A** might be glucose which was not esterified by *p*-coumaric acids. Thus, three *p*-coumaric acids might be esterified by three of five hydroxyl groups in the partial structures **B** (–CH_2O–) and **C** (–$(CHO)_3$–CH_2O). The arrangement of the position of



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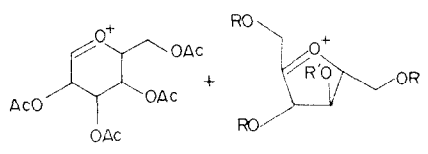

m/z 331 *m/z* 769

4

5 R = COCH=CH–C_6H_4–OAc, R′ = Ac

the three *p*-coumaryl moieties was established by comparison of ^{1}H NMR spectra between **1** and **2** (Table 1). The chemical shifts of the AB type protons corresponding to the partial structure **B** did not change (δ4.34 → 4.33; 4.36 → 4.43) after being acetylated, indicating that O-1 of the partial structure was not acetylated. In partial structure **C**, only the signal of H-4 was largely shifted (δ4.45 → 5.54) and the other protons, H-3, H-5 and H-6 did not shift significantly, showing that O-4 was acetylated and the other oxygen functions at C-3, C-5 and C-6 were not acetylated but esterified by *p*-coumaric acids. The above estimation was further supported by the fragment ion, **5**, at *m/z* 769 in the EI/MS of **2**. The presence of sucrose in hydropiperoside was confirmed by the excellent agreement of the chemical shifts of the ^{13}C NMR spectra (Table 2) between authentic sucrose octa-acetate (**3**) and the octa-acetate (**2**) of hydropiperoside (**1**) and the detection of sucrose on TLC after hydrolysis of **1**. On the basis of the spectral and chemical evidence the structure of hydropiperoside was established as β-D-(1,3,6-tri-*p*-coumaryl)-fructofuranosyl-α-D-glucopyranoside (**1**).

Table 2. ^{13}C NMR spectral data* (100 MHz) of hydropiperoside octa-acetate (**2**) and sucrose octa-acetate

Carbon atom	2	3
1	64.3	63.1
2	104.5	104.2
3	70.1	69.9
4	75.9	75.3
5	69.0	68.7
6	64.3	63.8
1′	90.6	90.2
2′	70.6	70.5
3′	76.5	76.0
4′	68.9	68.5
5′	79.6	79.3
6′	62.3	62.0
7	165.5	—
	166.2	
	166.6	
8	117.0	—
	117.6	
	117.9	
9	145.0	—
	145.2	
	146.2	
10	131.8	—
	132.2	
	132.4	
11, 15	122.5	—
12, 14	129.7	—
13	152.8	—
	152.9	
	153.0	
$\underline{C}H_3CO$	20.6	20.6
	20.8	
	20.9	
	21.3	
$CH_3\underline{C}O$	169.2	169.6
	169.9	169.8
	170.1	170.0
	170.6	170.2
	170.9	170.5
		170.7

*In $CDCl_3$.

In addition to **1**, the root of *P. hydropiper* contained the previously known ellagic acid 3,3′-di-*O*-methyl ether [12, 13], anthraquinone, quercetin 3-*O*-glucoside, quercetin 3-*O*-rhamnoside, gallic acid, and an unidentified aromatic δ-lactone, $C_{12}H_{12}O_4$ (M^+ 220), which showed antifertility activity against female mouse and plant growth inhibitory activity. No drimane-type sesquiterpenoids have been isolated from the root of *P. hydropiper* although the leaf and the seed contain a large quantity of pungent polygodial and its related drimane-type sesquiterpenoids [2–5]. The detailed structure of the δ-lactone with antifertility activity and the biological activity of **1** will be reported elsewhere.

EXPERIMENTAL

The solvents used for the spectral determinations were TMS–$CDCl_3$, DMSO-d_6 [^{1}H NMR (400 MHz); ^{13}C NMR (100 MHz)]; $CHCl_3$ (IR); and UV (MeOH) unless otherwise stated. TLC: (analyt. and prep.): precoated Si gel (0.25 mm) F_{254}; spots were visualized by UV light (254 nm) and 30% $CeSO_4$–H_2SO_4.

Plant material. Polygonum hydropiper L. identified by Y. A. and T. T. was deposited in the Herbarium of Institute of Pharmacognosy, Tokushima Bunri University.

Extraction and isolation. The MeOH extract (450 g) of the root of *P. hydropiper*, collected in Oct. 1978, was partitioned between EtOAc and H_2O to obtain an EtOAc-soluble portion (90 g). The EtOAc fraction (50 g) was chromatographed on Si gel using *n*-hexane–C_6H_6–$CHCl_3$–MeOH and divided into six fractions: fraction 1 (*n*-hexane, 100%); 2(C_6H_6, 100%); 3 (C_6H_6–EtOAc, 4:1); 4(C_6H_6–EtOAc, 1:1); 5(EtOAc, 100%) and 6 ($CHCl_3$–MeOH, 7:3). The second fraction (3.3 g) was chromatographed on Si gel (C_6H_6–EtOAc, 7:3) to give anthraquinone (2 mg) and an aromatic δ-lactone (150 mg), $C_{12}H_{12}O_4$ (M^+ 220). The third fraction (24 g) was rechromatographed on Si gel (C_6H_6–EtOAc gradient) to give phytosterols (stigmasterol and sitosterol) (502 mg) and ellagic acid 3,3′-di-O-methyl ether (200 mg), $C_{16}H_{10}O_8$, mp 330° (lit. 330–331° [13]); IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1715 (aromatic δ-lactone); ^{1}H NMR: δ4.06 (6H, *s*, OMe), 7.54 (2H, *s*), 8.31 (2H, *br s*, OH); MS *m/z* (rel. int.) 330 $[M]^+$ (100), 315 (42), 287 (11). The sixth fraction (10 g) was rechromatographed on Si gel ($CHCl_3$–MeOH) and divided into 37 fractions. Fractions 15–19 were further purified by HPLC [μ Bondapak C_{18} (7.8 × 300 mm), MeOH–H_2O (1:1), 3.5 ml/min] to obtain hydropiperoside (R_t 25.6 min) (**1**) (100 mg) as an amorphous substance. UV λ_{max} nm: 228 (ε 15 000), 315 (26 000); IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1700 (COO), 1630 (C=C), 1600, 1517 (aromatic ring), 1150, 809; FD/MS *m/z* (rel. int.) 819 $[M^+ + K]^+$(50), 803 $[M + Na]^+$ (100), 657 $[803 - p\text{-coumarate-H}]^+$ (67), 511 $[657 - p\text{-coumarate-H}]^+$ (12), 477 $[803 - p\text{-coumarate-H-gluc}]^+$ (8), 164 $[HOC_6H_4CH=CHCOOH]^+$ (11). Fractions 25 and 26 (250 mg) were purified by HPLC [μ Bondapak C_{18} (7.8 × 300 mm) MeOH–H_2O (48:53), 3 ml/min] to give quercetin 3-*O*-glucoside (R_t 7.2 min) (15 mg) and quercetin-3-*O*-rhamnoside (R_t 9.0 min) (30 mg) whose spectral data were identical to those of authentic samples. Fractions 32–37 contained gallic acid (200 mg).

Hydrolysis of **1**. To a MeOH (0.5 ml) soln of **1** (0.4 mg) was added 0.2% NaOH (0.1 ml) and it was then stirred for 20 hr at room temp. The reaction mixture was acidified by 1 N HCl and extracted with Et_2O. The residue after removal of the solvent contained *p*-coumaric acid (R_f 0.63, C_6H_6–EtOAc–HOAc,

5:5:0.1) which was identified by TLC and MS. The presence of sucrose in the unsaponifiable portion was detected by TLC ($CHCl_3$–MeOH–H_2O, 7:3:0.5).

Acetylation of **1**. To a pyridine (0.1 ml) soln of **1** (5 mg) was added Ac_2O (0.2 ml) and it was allowed to stand overnight. Work-up as usual gave pure acetate (**2**) (5.1 mg) as colorless prisms, mp 84–85.5°; UV λ_{max} nm: 214 (ε 11 700), 278 (23 400); IR ν_{max} cm^{-1}: 1742 (OAc), 1700 (COO), 1630 (C = C), 1595, 1500 (aromatic ring), 1370, 1200, 1010; FD/MS *m/z* (rel. int.): 1116 $[M]^+$ (100), EI/MS *m/z* (rel. int.): 769 $[M - 347]^+$ (5), 727 (7), 331 (32), 189 (55), 169 (65), 164 (20), 147 (76), 109 (25), 43 (100).

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