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PERILLOSIDE A, A MONOTERPENE GLUCOSIDE FROM *PERILLA FRUTESCENS*

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Key Word Index—*Perilla frutescens*; Labiatae; monoterpene glucoside; perilloside A; (−)perillyl alcohol; eugenyl *O*-β-D-glucopyranoside.

Abstract—A new monoterpene glucoside named perilloside A has been isolated from the fresh leaves of *Perilla frutescens*. Its structure has been characterized on the basis of spectral and chemical evidence.

INTRODUCTION

Perilla frutescens Britton *forma viridis* Makino (Japanese name; aojiso, white-flowering), an annual aromatic herb [1], is important in Japanese cooking, as a popular garnish. Previous studies of the constituents of the plant have reported in detail on the essential oils [2–5] and other less polar substances such as sterols [6] and fatty acids [7]. Furthermore, a few reports for polar substances of the plant have been found, whilst *P. frutescens* var. *acuta* Kudo (shiso, purple-flowering) has been especially studied [8, 9]. We have focused on the polar constituents of the plant, and have isolated a new monoterpene glucoside named perilloside A, as well as a known phenylpropanoid glucoside, eugenyl *O*-β-D-glucopyranoside (=citrusin C) [10–12]. We now report the isolation and structural elucidation of the glucosides by means of spectroscopic and chemical methods.

RESULTS AND DISCUSSION

The methanolic extract was separated using a combination of preparative TLC and HPLC to give two glycosidic compounds (**1**, **2**).

Compound **1**, named perilloside A, was obtained as needles and its IR spectrum showed absorption bands due to hydroxyl groups (3400, 1080 and 1045 cm^{-1}) and olefinic group (1640 cm^{-1}). The molecular formula was established as $C_{16}H_{26}O_6$ by the SI mass spectrum (*m/z* 315 $[M+H]^+$) and elemental analysis (found; C 61.04%, H 8.15%, required; C 61.13%, H 8.34%). The ^1H NMR spectrum revealed the presence of an isopropenyl moiety [δ1.73 (3H, *s*, Me) and 4.71 (2H, *s*, H-9)], another olefinic proton [δ5.76 (1H, *br s*, H-2)], and oxygenated methylene [δ4.02 and 4.22 (each 1H, *d*, $J=11.9$ Hz, $-CH_2O-$)]. Additionally, three signals due to a sugar moiety were observed at 3.67 (1H, *dd*, $J=5.2$, 11.9, H-6'a), 3.86 (1H, *dd*, $J=1.8$, 11.9, H-6'b), and 4.27 (1H, *d*, $J=7.9$, anomeric proton), of which the coupling constant indicated the β-linkage with the aglycone. Its ^{13}C NMR spectrum (Table 1) showed signals due to D-glucopyranoside. On enzymatic hydrolysis with β-glucosidase, **1** gave an aglycone which was characterized as (−)-perillyl alcohol [=(*S*)-1,8-*p*-menthadien-7-ol], whose optical rotation showed $[\alpha]_D^{25}$ −93.8° (MeOH; *c* 0.10). These were identified by TLC, GC and/or GC-MS. The sugar moiety was identified as D-glucose by GC comparison of trimethylsilyl derivatives of the hydrolysed product and authentic D-glucose. Acetylation of **1** yielded tetraacetate (**3**) as a powder. The molecular formula of **3** was confirmed by elemental analysis ($C_{24}H_{34}O_{10}$). The ^1H NMR spectrum of **3** exhibited four signals due to acetyl groups at δ1.94, 1.96, 1.98, 2.02 (each 3H, *s*) and anomeric proton signals at 4.45 (1H, *d*, $J=8.2$). Thus, perilloside A was characterized as (−)-perillyl 7-*O*-β-D-glucopyranoside; the structure of **1** was further confirmed by the following synthesis.

An authentic sample of (−)-perillyl 7-*O*-β-D-glucopyranoside was prepared from (−)-perillyl alcohol and acetobromoglucose by modified Koenigs–Knorr synthesis [13], and then saponification of the corresponding synthetic tetraacetylglucoside with methanolic potassium hydroxide. The two pairs of compounds had the same melting point, which showed no depression on admixture, and were identical in every respect examined. Perilloside A was, thus, inferred to be (−)-perillyl 7-*O*-β-D-glucopyranoside.

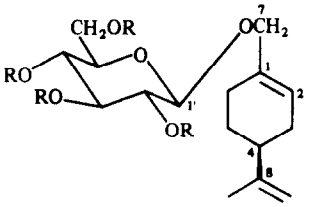
Compound **1** is new natural compound, which was found to hydrolyse slowly in the mouth with the evolution of (−)-perillyl alcohol. It appears to be present in the plant as a protected and stored form of (−)-perillyl alcohol.

Compound **2** was characterized from spectral data (see Experimental) as eugenyl *O*-β-D-glucopyranoside (=citrusin C) and this was confirmed by comparison with an authentic sample (HPLC, IR, SI-MS, ^1H and ^{13}C NMR) [10–12]. It has now been found for the first time in the genus *Perilla*.

EXPERIMENTAL

General. Mps were uncorr. Specific rotations MeOH. ^1H NMR: 270 MHz, using TMS as an int. standard. ^{13}C NMR: 67.5 MHz. SI-MS: matrix, glycerol. HPLC: Chemcosorb 5-ODS-H (4.6 mm i.d. × 150 mm) columns.

Plant material. Plant materials used in the experiment were a green variety (white-flowering) of *Perilla frutescens* Britton



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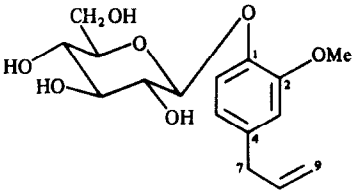

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forma viridis Makino. Mature leaves were harvested from plants growing on the commercial farm of Katano, Osaka Prefecture.

Isolation. The fresh leaves of *P. frutescens* (11 kg) were extracted ×3 with MeOH (100 l) at room temp. and the extract evapd *in vacuo*. The concd suspension was defatted with hexane (1 l × 5) and then extracted with $CHCl_3$ (1 l × 5). The $CHCl_3$ extract was concd *in vacuo* to give a residue (70.4 g), which was chromatographed over silica gel (3 kg) with $CHCl_3$–MeOH as eluant with increasing MeOH content. Fifty fractions were collected, and then each fraction was checked by TLC using a mixed solvent, $CHCl_3$–MeOH (5:1) to look for terpenoids and their glycosides which showed visible colourations by vanillin–H_2SO_4 reagent. As a result, 2 fractions, 23 (1.25 g) and 24 (0.98 g) were assumed to contain monoterpenoid glycosides. These fractions were subjected to prep. TLC ($CHCl_3$–MeOH, 5:1) to give crude monoterpene glycoside (R_f 0.3–0.4). The crude glycoside was rechromatographed by prep. HPLC using MeOH–H_2O (1:1) and MeOH–H_2O (9:11) on a reversed phase column (Chemcosorb 5-ODS-H), to afford two compounds, perilloside A (**1**, 432 mg) and eugenyl *O*-β-D-glucopyranoside (= citrusin C) (**2**, 105 mg).

Perilloside A (**1**). Needles, mp 114.5–115.0°, $[\alpha]_D^{22}$ −92.7° (MeOH; *c* 0.77). IR ν_{max}^{KBr} cm^{-1}: 3400, 1640, 1080 and 1045. SIMS *m/z*: 315 $[M+H]^+$. Elemental analysis: found; C 61.04%, H 8.15%, $C_{16}H_{26}O_6$ required; C 61.13%, H 8.34%. ^1H NMR (CD_3OD): δ 1.48 (1H, *m*, H-5ax), 1.73 (3H, *s*, H-10), 1.81 (1H, *m*, H-5eq), 3.67 (1H, *dd*, *J* = 5.2, 11.9 H-6′a), 3.86 (1H, *dd*, *J* = 1.8, 11.9, H-6′b), 4.02 (1H, *d*, *J* = 11.9 H-7a), 4.22 (1H, *d*, *J* = 11.9, H-7b), 4.27 (1H, *d*, *J* = 7.9, H-1′α), 4.71 (2H, *s*, H-9), 5.76 (1H, *br s*, H-2). ^{13}C NMR (CD_3OD): see Table 1.

Acetylation of perilloside A. Compound **1** (9.9 mg) on acetylation with Ac_2O–pyridine afforded tetraacetate as a powder (**3**, 12.2 mg); mp 68.5–70.0°, found: C 59.55%; H 6.92%. $C_{24}H_{34}O_{10}$ requires: C 59.74%; H 7.10%. IR ν_{max}^{KBr} cm^{-1}: 1750, 1640, 1370, 1240 and 1045. ^1H NMR ($CDCl_3$): δ 1.67 (3H, *s*, H-10); 1.94, 1.96, 1.98, 2.02 (each 3H, *s*, each Ac); 3.61 (1H, *ddd*, *J* = 2.4, 4.6, 9.8, H-5′); 3.91 (1H, *d*, *J* = 11.9, H-7a); 4.07 (1H, *dd*, *J* = 2.4, 12.5, H-6′b); 4.12 (1H, *d*, *J* = 11.9, H-7b); 4.20 (1H, *dd*, *J* = 4.6, 12.5, H-6′a); 4.45 (1H, *d*, *J* = 8.2, H-1′α); 4.65 (1H, *s*, H-9a); 4.67 (1H, *s*, H-9b); 4.95 (1H, *dd*, *J* = 7.9, 9.5, H-2′); 5.03 (1H, *dd*, *J* = 9.5, 9.8, H-4′); 5.15 (1H, *dd*, *J* = 9.5, 9.5, H-3′); 5.64 (1H, *br s*, H-2). ^{13}C NMR ($CDCl_3$): see Table 1.

Enzymatic hydrolysis of perilloside A. To a soln of **1** (23.2 mg) in H_2O (5 ml), β-glucosidase (10 mg) was added. The mixture was stirred for 5 hr at 37°, and then extracted with Et_2O. The extract was worked-up as usual and the residue was purified by prep. TLC to give (−)-perillyl alcohol as an oil (9.6 mg): $[\alpha]_D^{25}$ −93.8° (MeOH; *c* 0.10), EI-MS *m/z* 152 $[M]^+$.

Identification of sugar moiety. A supernatant (1 ml) of the water layer was evapd and dried in a desiccator to give a residue. The residue was treated with 1-(trimethylsilyl)-imidazole at 80° for 1 hr and then H_2O was added to the reaction mixture to decompose the excess reagent. The reaction product was extracted with hexane (2 ml). The hexane soln was subjected to GC for an identification of the sugar moiety.

Table 1. ^{13}C NMR spectral data of compounds **1**, **3** and **4** ($CDCl_3$)

C	**1** (CD_3OD)	**3**	**4**
1	136.2 *s*	133.0 *s*	137.2 *s*
2	126.7 *d*	125.8 *d*	122.5 *d*
3	32.4 *t*	30.4 *t*	30.4 *t*
4	43.1 *d*	40.8 *d*	41.1 *d*
5	29.5 *t*	27.2 *t*	27.5 *t*
6	28.2 *t*	26.1 *t*	26.1 *t*
7	75.1 *t*	73.5 *t*	67.3 *t*
8	151.7 *s*	149.5 *s*	149.8 *s*
9	110.0 *t*	108.8 *t*	108.6 *t*
10	21.8 *q*	20.7 *q*	20.8 *q*
1′	103.6 *d*	98.9 *d*	
2′	75.7 *d*	71.2 *d*	
3′	78.8 *d*	72.9 *d*	
4′	72.3 *d*	68.4 *d*	
5′	78.5 *d*	71.6 *d*	
6′	63.5 *t*	61.9 *t*	
–CO		170.7 *s*	
		170.3 *s*	
		169.4 *s*	
		169.2 *s*	
		20.72 *q*	
–Me		20.65 *q*	
		20.60 *q*	
		20.57 *q*	

4: (−)-Perillyl alcohol.

Synthesis of perilloside A. Compound **1** was prepared from (−)-perillyl alcohol (d_4^{20} 0.959, n_D^{20} 1.5010, α_D −102.1°), which was rectified with fine distillation, according to the modified method given in the literature [13]. (a) 2,3,4,6-Tetra-*O*-acetyl-β-D-glucoside (**3**). To a soln of acetobromoglucose (26.67 g) and (−)-perillyl alcohol (9.86 g) in C_6H_6, mercuric cyanide (17.1 g) was added, and then the mixture was stirred for 90 min at 50°. The reaction mixture was filtered off and the ppt. washed with EtOAc (50 ml). The filtrate was washed with H_2O (30 ml) and satd Na_2CO_3 aq. soln (30 ml) and then H_2O. The extract was dried over dry Na_2SO_4 and evapd *in vacuo*. The residue was rechromatographed on silica gel with hexane–EtOAc (5:1) to give **3** as a pale yellow paste (16.0 g). Crystallization from

Et_2O–petrol furnished **3** (15.0 g) as needles mp 69.0–70.0°, $[\alpha]_D^{25}$ −60.3° (MeOH; *c* 0.62). Other spectral data of **3** coincided with these of tetraacetylperilloside A. (b) Deacetylation of compound **3**. To a solution of **3** (7.5 g) in MeOH (20 ml), 10% methanolic KOH (40 ml) was added, and then the mixture was stirred for 1 hr at room temp. After the MeOH was distilled away, the residue was dissolved in H_2O and the soln extracted with EtOAc. The extract was worked-up as usual and the residue chromatographed on silica gel with $CHCl_3$–MeOH (9:1) to give **1** as a pale yellow paste (3.67 g). Crystallization from $CHCl_3$–Et_2O furnished **1** (3.29 g) as needles, mp 114.5–115.0°, $[\alpha]_D^{25}$ −85.5° (MeOH; *c* 0.38). Other spectral data of **1** were identical with these of perilloside A.

Eugenyl O-β-D-glucopyranoside (=Citrusin C, **2**). Powder, mp 129–130° (lit. 130–131°) [10, 11], $[\alpha]_D^{22}$ −50.7° (MeOH; *c* 0.13) (lit. $[\alpha]_D^{20}$ −54.0°) [10], IR ν_{max}^{KBr} cm^{-1}: 3450, 1510, 1260, 1220, 1070, and 1020 UV λ_{max}^{MeOH} nm: 276 (ε5800), SI-MS *m/z* 327 $[M+H]^+$, ^1H NMR (CD_3OD): δ3.32 (2H, *d*, *J* = 6.7, H-7); 3.68 (1H, *dd*, *J* = 4.7, 11.9, H-6′a); 3.83 (3H, *s*, OMe); 3.87 (1H, *d*, *J* = 11.9, H-6′b); 4.84 (1H, *d*, *J* = 7.3, H-1′α); 5.03 (1H, *dd*, *J* = 1.5, 9.7, H-9a); 5.05 (1H, *dd*, *J* = 1.5, 17.1, H-9b); 5.95 (1H, *dd*, *t*, *J* = 6.7, 10.1, 17.1, H-8); 6.72 (1H, *dd*, *J* = 1.5, 8.2, H-5); 6.82 (1H, *d*, *J* = 1.5, H-3); 7.08 (1H, *d*, *J* = 8.2, H-6). ^{13}C NMR (CD_3OD): δ147.1 (*s*, C-1); 151.5 (*s*, C-2), 119.0 (*d*, C-3), 139.8 (*s*, C-4), 122.9 (*d*, C-5), 114.9 (*d*, C-6), 41.6 (*t*, C-7), 137.2 (*d*, C-8), 116.7 (*t*, C-9), 57.4 (*q*, C-10), 103.8 (*d*, C-1′), 75.7 (*d*, C-2′), 79.0 (*d*, C-3′), 72.1 (*d*, C-4′), 78.6 (*d*, C-5′), 63.3 (*t*, C-6′).

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