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Phenethyl alcohol glycosides and isopentenol glycoside from fruit of *Bupleurum falcatum*

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Abstract

Investigation on the constituents of the fruit of *Bupleurum falcatum* L. resulted in the isolation of the three new glycosides, phenethyl alcohol 8-*O*-β-D-glucopyranosyl-(1 → 2)-*O*-β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranoside, phenethyl alcohol 8-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside and isopentenol 1-*O*-β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranoside along with five known glycosides, icariside D_1, icariside F_2, saikosaponin a, saikosaponin c and saikosaponin d. The structures of these compounds were elucidated on the basis of interpretation of chemical and spectral data. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Bupleuri Radix; *Bupleurum falcatum*; Umbelliferae; Phenethyl alcohol glycoside; Isopentenol glycoside; Triterpene glycoside

1. Introduction

Bupleurum falcatum L. (Umbelliferae) is cultivated in Asia, and its root, Bupleuri Radix, is one of the most important crude drugs in Japan. A large number of investigations on its chemical constituents have been reported so far; especially, triterpene oligoglycosides, saikosaponins, which have been reported as major bioactive components of this crude drug (Shibata, Kitagawa, & Fujimoto, 1966; Aimi & Shibata, 1966; Kubota & Hinoh, 1966a, 1966b, 1968a, 1968b; Kubota, Tonami, & Hinoh, 1967; Kubota & Tonami, 1967; Aimi, Fujimoto, & Shibata, 1968; Shimaoka, Seo, & Minato, 1975; Ishii, Seo, Tori, Tozyo & Yoshimura, 1977; Ishii et al., 1980; Koji, Amagaya, & Ogihara, 1985; Nose, Amagaya, Takeda, & Ogihara, 1989; Ebata, Nakajima, Taguchi, & Mitsuhashi, 1990). In the course of our studies on the constituents of the fruit of the Umbelliferae (Ono, Ito, Kinjo, Yahara, & Nohara, 1995; Ono et al., 1996; Ono et al., 1997), we now report the isolation and structure determination of two new phenethyl alcohol glycosides (**1** and **2**) and a new isopentenol glycoside (**3**) along with five known glycosides (**4**–**8**) from the fruit of *Bupleurum falcatum*.

2. Results and discussion

The MeOH extract of the fruit of *B. falcatum* L. was purified by DAIAION HP 20, Sephadex LH-20 and silica gel column chromatographies (CC) as well as HPLC on ODS to afford eight compounds (**1**–**8**).

Compounds **4**–**8** were identified as icariside D_1, icariside F_2, saikosaponin a, saikosaponin c and saikosaponin d, respectively, based on their physical and spectral data (Koji et al., 1985; Miyase, Ueno, Takizawa, Kobayashi, & Oguchi, 1987; Ono et al., 1996).

Compound **1** exhibited an $[M–H]^-$ ion peak at m/z 577 together with a fragment ion peak at m/z 415 $[M–H–hexose]^-$ in the negative FAB-MS and an $[M+Na]^+$ ion peak at m/z 601, which was 162 mass

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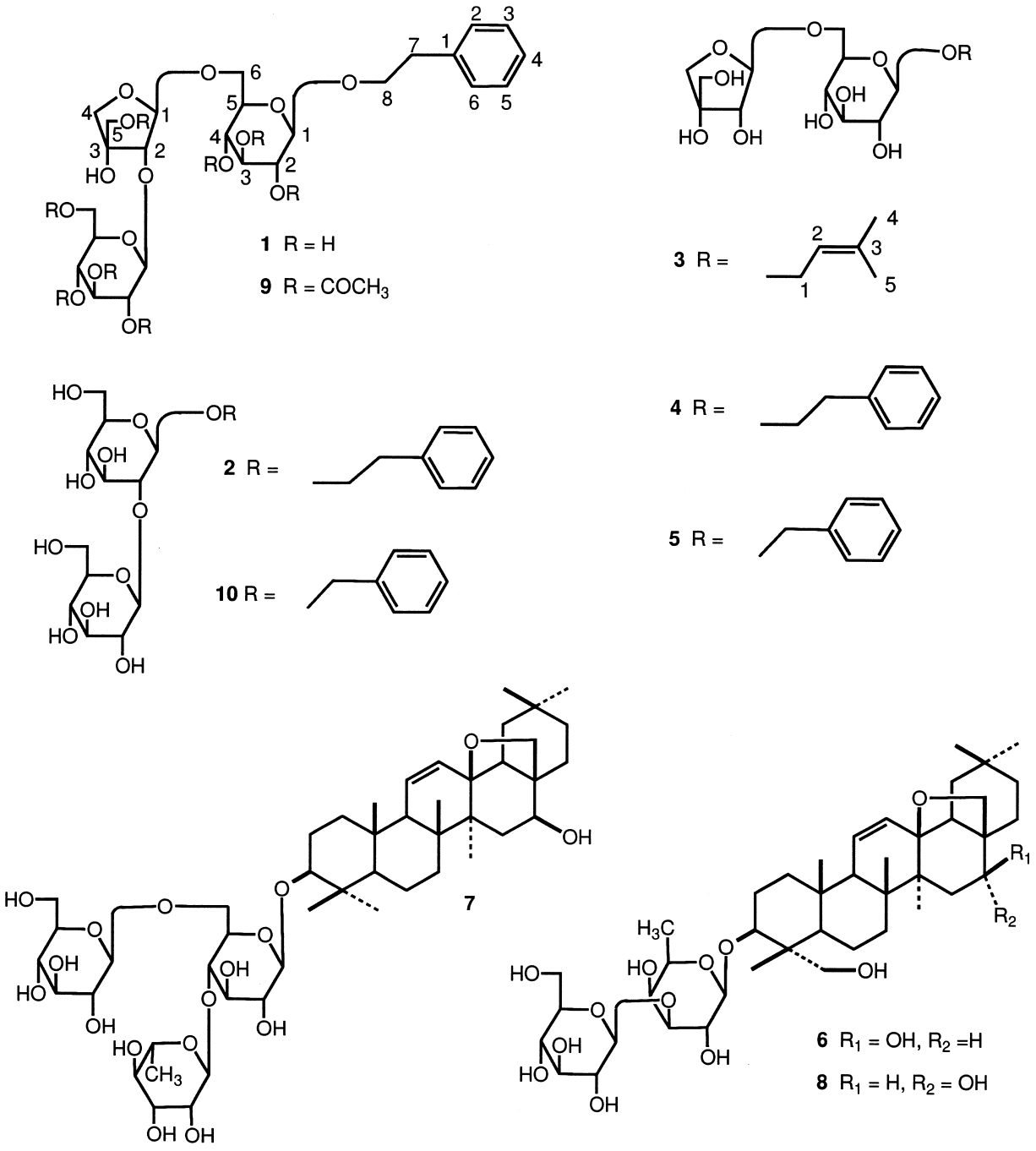

units (hexose unit) larger than that of **4**, in the positive FAB-MS. The molecular formula was determined as $C_{25}H_{38}O_{15}$ by HR positive FAB-MS. The ^1H NMR and ^{13}C NMR spectra of **1** showed three monosaccharide units and a phenethyl alcohol residue. Acid hydrolysis of **1** afforded glucose and apiose which were identified as the D-form on the basis of gas chromatographic (GC) analysis according to Hara, Okabe, and Mihashi, (1987). From these data and the coexistence of **4**, it was presumed that the sugar moiety of **1** might be attaching 1 mol glucose to **4**, and the coupling constant of anomeric proton signal indicated the mode of glycosidic linkage of this glucose unit to be β. This assumption was confirmed by the enzymatic hydrolysis of **1** with β-glucosidase from almonds to give **4** and glucose.

The ^1H NMR signals due to the sugar moiety of **1** could not be assigned because of overlap. Therefore, **1** was converted into the acetate (**9**), of which the ^1H-signals were assigned on the basis of COSY spectrum. In this spectrum, the signals due to 2-, 3- and 4-H of the inner glucose (Glc), 2-, 3- and 4-H of the terminal glucose (Glc′) and 5-H_2 of apiose were shifted downfield suggesting the sugar linkage of Glc′ to be located at

the 2-OH of apiose. This suggestion was supported by the difference nuclear Overhauser effect (NOE) spectra of **9**. In the NOE difference spectra, irradiation of the signal of the anomeric proton of Glc′ showed NOE enhancement of the signal of 2-H of apiose, and irradiation of the signal of 2-H of apiose gave NOE enhancement of the signal of anomeric proton of Glc′. Accordingly, the structure of **1** was concluded to be phenethyl alcohol 8-*O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside.

Compound **2** showed an $[M–H]^-$ ion peak at *m/z* 445 in the negative FAB-MS and an $[M+Na]^+$ ion peak at *m/z* 469 in the positive FAB-MS. ^1H NMR and ^{13}C NMR spectra indicated **2** to be a diglycoside of phenethyl alcohol which, on acid hydrolysis, gave D-glucose. Furthermore, the ^1H NMR and ^{13}C NMR spectral data of the sugar moiety of **2** were quite similar to those of zizybeoside I (**10**) (Ono et al., 1996). The structure of **2** was therefore defined as phenethyl alcohol 8-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

Compound **3** gave an $[M–H]^-$ ion peak at *m/z* 379 in the negative FAB-MS and an $[M+Na]^+$ ion peak at *m/z* 403 in the positive FAB-MS. The NMR spectral data and acid hydrolysis of **3** indicated that the sugar moiety of **3** was identical to that of **4**. Furthermore, the ^1H NMR spectrum showed signals of two methyl groups (δ 1.58, 1.60), two aliphatic protons (δ 4.39, ca 4.59) and one olefinic proton (δ 5.49), and the ^{13}C NMR spectrum indicated signals of two methyl carbons (δ 18.0, 25.7), two sp^2 carbons (δ 121.8, 136.8), and one oxymethylene carbon (δ 65.6) for the signals of aglycone moiety, suggesting the aglycone of **3** to be isopentenol. Accordingly, the structure of **3** was concluded to be isopentenol 1-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside.

As far as we know, **1**–**3** are novel glycosides, and the isolation of **4**–**8** from the fruit of *B. falcatum* L. is described here for the first time.

3. Experimental

3.1. General

^1H NMR: 500 MHz; ^{13}C NMR: 100 and 125 MHz; NOE:400 MHz (TMS as int. standard). CC: Diaion HP 20 (Mitsubishi Chemical Industries Co., Ltd), Sephadex LH-20 (Pharmacia Fine Chemicals) and silica gel 60 (230–400 mesh, Merck). HPLC: YMC pack S-5 120A ODS (250 mm × 20 mm i.d., YMC Co., Ltd.). GC: silicone OV-1 (30 m × 0.32 mm i.d., Ohio Valley Specially Chem.). TLC: silica gel 60 (Art. 5554, Merck).

3.2. Plant material

Fruit of *B. falcatum* L. was purchased from Kikuka town office, Kumamoto prefecture, Japan.

3.3. Extraction and isolation

Fruit of *B. falcatum* L. (1212 g) was extracted with MeOH (2 L × 2) under reflux. The MeOH extract (84.7 g) was subjected to Diaion HP 20 CC (H_2O, 20% MeOH, 40% MeOH, 60% MeOH, 80% MeOH, MeOH, acetone) to give seven frs. (fr. 5 was crude saponin (23. 2 g)). Fr. 1 was partitioned between BuOH and H_2O. The BuOH fr. was subjected to silica gel CC ($CHCl_3$–MeOH–H_2O, 10:2:0.1, 8:2:0.2, 7:3:0.5, 6:4:1, 0:1:0) and HPLC (80% MeOH and 85% MeOH, flow rate 4.5 ml/min) to yield **6** (7 mg), **7** (10 mg) and **8** (71 mg). Fr. 2 was successively subjected to Sephadex LH-20 CC (MeOH), silica gel CC ($CHCl_3$–MeOH–H_2O, 8:2:0.2, 7:3:0.5, 6:4:1) and HPLC (50% MeOH, flow rate 3.0 ml/min) to give **1** (18 mg), **2** (6 mg), **3** (14 mg), **4** (16 mg) and **5** (17 mg).

Phenethyl alcohol 8-*O*-β-D-glucopyranosyl-(1 → 2)-*O*-β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranoside (**1**). Powder, $[\alpha]_D^{26}$ −56.6° (MeOH; *c* 1.7). FAB-MS (negative) *m/z* (rel. int): 577 (100) $[M–H]^-$, 415 (20) [M–H–hexose]$^-$; FAB-MS (positive) *m/z*: 601 $[M+Na]^+$; HR FAB-MS (positive) *m/z*: 601.2111 $[M+Na]^+$ (Calcd for $C_{25}H_{38}O_{15}Na$: 601.2108). ^1H NMR (pyridine-d_5): see Table 1. ^{13}C NMR (in pyridine-d_5): see Table 2.

Phenethyl alcohol 8-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**2**). Powder, $[\alpha]_D^{26}$ −17.7° (MeOH; *c* 0.7). FAB-MS (negative) *m/z* 445 $[M–H]^-$; FAB-MS (positive) *m/z*: 469 $[M+Na]^+$; HR FAB-MS (positive) *m/z*: 469.1681 $[M+Na]^+$ (Calcd for $C_{20}H_{30}O_{11}Na$: 469.1686). ^1H NMR (pyridine-d_5): see Table 1. ^{13}C NMR (in pyridine-d_5): see Table 2.

Isopentenol 1-*O*-β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranoside (**3**). Powder, $[\alpha]_D^{26}$ −66.2° (MeOH; *c* 2.0). FAB-MS (negative) *m/z* 379 $[M–H]^-$; FAB-MS (positive) *m/z*: 403 $[M+Na]^+$; HR FAB-MS (positive) *m/z*: 403.1581 $[M+Na]^+$ (Calcd. for $C_{16}H_{28}O_{10}Na$: 403.1580). ^1H NMR (pyridine-d_5): see Table 1. ^{13}C NMR (in pyridine-d_5): see Table 2.

3.4. Acid hydrolysis of *1*–*3*

Compounds **1** (1 mg), **2** (1 mg) and **3** (1 mg) in 2 N HCl (0.5 ml) were heated at 95°C for 1 h and the reaction mixture was neutralized with 1 N NaOH. After removal of the solvent under red. pres., the residue was extracted with MeOH (1 ml). The MeOH extracts were subjected to GC analyses as trimethylsilyl ethers of the methyl thiazolidine 4(*R*)-carboxylate derivatives according to Hara et al. (detector: FID, column temp.:

Table 1
^{1}H NMR spectral data for **1**–**4** and **9** (in pyridine d_5, 500 MHz)

	1	**2**	**3**	**4**	**9**
Aglycone					
1a			ca 4.59		
1b			4.39 dd (7.9, 11.6)		
2	7.31 d(7.3)	ca 7.27	5.49 br t (7.9)	7.28 d (6.9)	ca 7.35[a]
3	7.27 dd (7.3, 7.3)	ca 7.27		7.26 dd (6.9, 6.9)	ca 7.24[a]
4	7.16 t (7.3)	7.18 m	1.60 s	7.17 t (6.9)	ca 7.35
5	7.27 dd (7.3, 7.3)	ca 7.27	1.58 s	7.26 dd (6.9, 6.9)	ca 7.24[a]
6	7.31 d (7.3)	ca 7.27		7.28 d (6.9)	ca 7.35[a]
7a	3.02 t (7.3)	3.10 m		2.99 t (7.3)	2.97 t (6.7)
7b	3.02 t (7.3)	3.10 m		2.99 t (7.3)	2.97 t (6.7)
8a		ca 4.21		4.34 dt (9.8, 7.3)	4.30 dt (9.8, 6.7)
8b		3.83 m		3.90 dt (9.8, 7.3)	3.87 dt (9.8, 6.7)
Glucose (Glc)					
1	4.83 d (7.9)	4.92 d (7.9)	4.85 d (7.3)	4.83 d (7.9)	4.90 d (8.5)
2		4.18 dd (7.9, 8.5)	4.03 dd (7.3, 8.5)	4.00 dd (7.9, 9.3)	5.43 dd (8.5, 9.5)
3		ca 4.35	4.22 dd (8.5, 8.5)	ca 4.20	5.70 dd (9.5, 9.5)
4		ca 4.20	ca 4.05	ca 4.03	5.43 dd (9.5, 9.5)
5		3.89 m	ca 4.06	ca 4.07	4.14 ddd (2.4, 6.1, 9.5)
6a		4.52 dd (1.8, 12.2)	ca 4.75	ca 4.74	4.10 dd (2.4, 11.0)
6b		ca 4.35	ca 4.15	ca 4.20	3.92 dd (6.1, 11.0)
Apiose					
1	5.86 s		5.80 s	5.79 d (2.4)	5.64 d (1.8)
2	5.00 s		4.76 s	4.75 d (2.4)	4.56 d (1.8)
4a			4.58 d (9.5)	4.57 d (9.2)	4.28 d (9.8)
4b			4.35 d (9.5)	4.34 d (9.2)	4.19 d (9.8)
5a			4.16 s	4.14 s	4.61 d (11.6)
5b			4.16 s	4.14 s	4.54 d (11.6)
Glucose (Glc′)					
1	5.26 d (7.9)	5.36 d (7.9)			5.39 d (7.9)
2		4.14 dd (7.9, 8.5)			5.49 dd (7.9, 9.5)
3		4.26 dd (8.5, 8.5)			5.78 dd (9.5, 9.5)
4		4.28 dd (8.5, 8.5)			5.53 dd (9.5, 9.5)
5		3.99 m			4.21 ddd (2.0, 3.0, 9.5)
6a		4.55 dd (1.8, 11.9)			4.61 dd (3.0, 12.2)
6b		4.42 dd (4.9, 11.9)			4.49 dd (2.0, 12.2)
$COCH_3$					
					2.08 s
					2.06 s
					2.05 s
					2.03 s
					2.02 s
					2.01 s
					2.01 s
					1.97 s

[a] Assignments may be interchanged in the same column. *J* values (in Hz) in parentheses.

230°C, injector temp.: 270°C, detector temp.: 270°C, carrier gas: He) (Hara et al., 1987). The retention times of these products for **1** and **3** were each identical with those of D-glucose and D-apiose derivatives, and the retention time of the product for **2** corresponded to that of D-glucose derivative.

3.5. Enzymatic hydrolysis of *1*

Compound **1** (4 mg) was dissolved in AcOH–AcONa buffer (pH 6.22, 1 ml) and β-glucosidase (from almonds, lot 102H4008, Sigma Chemical Co., 7 mg) was added. The mixture was left to stand at 36°C for 5

Table 2
^{13}C NMR spectral data for **1**–**4** and **10** (in pyridine-d_5)

	1 (100 MHz)	**2** (100 MHz)	**3** (125 MHz)	**4** (125 MHz)	**10*** (100 MHz)
Aglycone					
1	139.4	139.4	65.6	139.4	138.8
2	128.6[a]	128.7[a]	121.8	128.7[a]	127.8
3	129.4[a]	129.6[a]	136.8	129.4[a]	128.5
4	126.4	126.5	18.0	126.4	127.6
5	129.4[a]	129.6[a]	25.7	129.4[a]	128.5
6	128.6[a]	128.7[a]		128.7[a]	127.8
7	36.6	36.7		36.7	70.8
8	70.5	70.9		70.6	
Glucose (Glc)					
1	104.7[b]	103.1	103.2	104.7	102.3
2	75.3[c]	84.2	75.1	75.0	84.3
3	78.3[d]	78.2[b]	78.6[a]	78.5[b]	78.0[a]
4	71.5[e]	71.6[c]	71.9	71.8	71.3[b]
5	76.8	78.4[b]	77.2[a]	77.2[b]	78.4[a]
6	68.4	62.6[d]	69.0	68.9	62.4[c]
Apiose					
1	109.5		111.2	111.2	
2	84.9		77.9[a]	77.8[b]	
3	81.3		80.5	80.5	
4	75.4		75.0	75.0	
5	65.7		65.6	65.6	
Glucose (Glc′)					
1	104.6[b]	106.6			106.6
2	75.0[c]	76.9			76.8
3	78.5[d]	78.0[b]			78.0[a]
4	71.1[e]	71.3[c]			71.1[b]
5	78.5[d]	78.8[b]			78.6[a]
6	62.0	62.8[d]			62.5[c]

[a,b,c,d,e] Assignments may be interchanged in the same column.
* Taken from Ono et al. (1996).

days. After removal of the solvent under red. pres., the residue was extracted with MeOH, and the MeOH extract was chromatographed over silica gel CC ($CHCl_3$–MeOH–H_2O, 8:2:0.2, 7:3:0.5, 6:4:1) to give **1a** (1 mg) and **1b** (1 mg). The ^1H NMR spectrum of **1a** was identical to that of **4**. Compound **1b** was identified as D-glucose by GC under the same conditions as above.

3.6. Acetylation of *1*

Compound **1** (4 mg) in Ac_2O–pyridine (1:1, 2 ml) was allowed to stand at room temp. overnight. After removal of the solvent of the reagent under a stream of N_2, the residue was partitioned between diethyl ether (1 ml) and H_2O (1 ml). The diethyl ether layer was concentrated to afford **9** (4 mg), syrup, ^1H NMR (in pyridine-d_5): see Table 1.

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