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TWO *P*-COUMAROYL GLYCERIDES FROM *JUNCUS EFFUSUS*

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Key Word Index—*Juncus effusus*; Juncaceae; *p*-coumaroyl glyceride; juncusyl ester A; juncusyl ester B.

Abstract—From the medullae of *Juncus effusus*, 13 compounds were isolated: a (2*S*)-2,3-isopropylidene-1-*O*-*p*-coumaroyl glyceride (juncusyl ester A), 2-*O*-*p*-coumaroyl glyceride (juncusyl ester B), (2*S*)-1-*O*-*p*-coumaroyl glyceride, 5α-spinasterol, β-sitosterol, β-sitosteryl-β-D-glycoside, effusol, *p*-coumaric acid, isoscutellarein pentamethyl ether, nobiletin, quercetin, rutinose and vanillic acid. Juncusyl esters A and B are new compounds.

INTRODUCTION

Juncus effusus L. ranges from the tropical and subtropical zones to the frigid zone and is often found in wetlands and coastal marshes. The species is one of the traditional medicines recorded in the Chinese Pharmacopoeia [1]. The medullae can be utilized to cure irritability and insomnia. Antioxidant and antiviral activities have recently been reported in an ethyl-acetate extract [2]. The present paper deals with the structural elucidation of two new *p*-coumaroyl glycerides in the *n*-butanol fraction of 90% ethanol extract of *J. effusus* medullae.

RESULTS AND DISCUSSION

Compound **1** was obtained as crystals. The high-resolution mass spectrum displayed a molecular ion at *m/z* 278.1151, corresponding to the formula $C_{15}H_{18}O_5$ (calculated 278.1154). The ^{1}H NMR spectrum showed two *ortho*-coupled doublets at δ6.84 (2H, $J = 8.4$ Hz) and 7.39 (2H, $J = 8.4$ Hz) in an AB system, and two *trans*-coupled doublets at δ6.27 (1H, $J = 15.9$ Hz) and 7.63 (1H, $J = 15.9$ Hz). Infrared absorption bands at 3300, 1700, 1600 and 1580 cm^{-1} indicated the presence of hydroxyl groups, a carbonyl group and an alkene group conjugated with an aromatic ring, respectively. These data suggested that **1** had a *p*-coumaroyl moiety, which was supported by detection of a singlet at δ167.2 in the ^{13}C NMR spectrum and characteristic fragment ions at *m/z* 164, 147 and 119 in the EIMS. The ^{13}C NMR spectrum showed signals at δ73.8, 66.3 and 64.8, indicating the presence of two CH_2-O groups and one CH-O group in **1**. This was supported by the five protons that gave rise to two double doubles at δ3.81 (1H, $J = 8.4$, 6.2 Hz) and 4.13 (1H, $J = 8.4$, 6.2 Hz), a double doublet triplet at δ4.42 (1H, $J = 6.3$, 4.3, 6.2 Hz), and two double doublets at δ4.30 (1H, $J = 11.6$, 4.3 Hz) and 4.42 (1H, $J = 11.6$, 6.3 Hz) in the ^{1}H NMR spectrum, suggesting the sequence of the groups to be O-CH_2-CH(O)-CH_2-O. Comparison with ^{13}C NMR spectrum of **1** and (2*S*)-1-O-*p*-coumaroyl glyceride [3] indicated that the *p*-coumaroyl moiety was attached to C-1 of the glycerol moiety. The ^{1}H NMR spectrum further showed two methyl signals at δ1.40 and 1.47, and the ^{13}C NMR three signals at δ110.0 (*s*), 25.3 (*q*) and 26.6 (*q*), indicating that **1** contained an isopropylidene moiety which was located at C-2 and C-3. Therefore, **1** was 2,3-isopropylidene-1-O-*p*-coumaroyl glyceride. To determine the stereochemistry at C-2, **1** was treated with 10% HCl-Me to give (2*S*)-(+)-1-O-*p* coumaroyl glyceride (**3**). Jung and coworkers showed that the configuration of a glycerol acetonide is retained on hydrolysis [4]. The configuration of C-2 can then be deduced by the optical activity of the hydrolysis product. (2*S*)-(+)-1-O-*p*-Coumaroyl glyceride has $[\alpha]_D + 12.8°$ ($c = 0.6$, MeOH) [5] which is in good agreement with the $[\alpha]_D + 11.6°$ of our hydrolysis product, indicating that the stereochemistry at C-2 is *S*. Hence **1** is (2*S*)-2,3-isopropylidene-1-O-*p*-coumaroyl glyceride, to which we have given the common name juncusyl ester A. Compound **3** was also found in the plant (see Experimental).

Compound **2**, juncusyl ester B, was obtained as crystals. High resolution MS, $[M]^+$ *m/z* 238.0841, gave the molecular formula $C_{12}H_{14}O_5$ (calculated, 238.0842). The NMR spectral data of **2** were very similar to those of (*S*)-(+)-1-O-*p*-coumaroyl glyceride except for those of the glycerol unit. In the ^{13}C NMR spectrum, the glyceride carbons appeared at δ76.1 (*d*) and 61.2 (*t*) while in the

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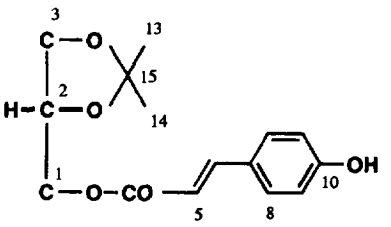
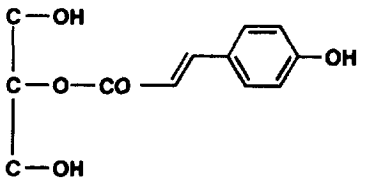


1



2

^{1}H NMR spectrum signals appeared at δ 3.74 (4H, *m*) and 4.89 (1H, *m*), which indicated that **2** had a symmetrical structure. Therefore, the *p*-coumaroyl moiety was connected at C-2 and juncusyl ester B was thus 2-O-*p*-coumaroyl glyceride (**2**).

EXPERIMENTAL

General. Mps: uncorr; ^{1}H and ^{13}C NMR: 500 (^{1}H) and 125 (^{13}C) MHz, TMS as int. standard; MS: JEOL JMSD, 300 type mass spectrometer.

Plant material. Dry medullae of *J. effusus* were purchased from the Nanjing Pharmaceutical Company, China, and the voucher specimen is deposited in the herbarium of China Pharmaceutical University.

Extraction and isolation. The medullae (6 kg) were extracted with 90% EtOH (15 l × 3) at room temp. After concn of the extract, a certain vol of H_2O was added and agitated thoroughly to form a suspension, which was partitioned with EtOAc and *n*-BuOH, successively. The EtOAc fraction was chromatographed on silica gel eluted with petrol–EtOAc (5:2) and then $CHCl_3$–MeOH (5:2) to give 5-α-spinasterol (10 mg), sitosterol (2 g) and effusol [6] (40 mg). The *n*-BuOH fraction was also subjected to silica gel CC to give isoscutellarein tetramethyl ether (5,7,8,4′-tetramethoxyflavone) (15 mg), nobiletin (5,6,7,8,′3′4′-hexamethoxyflavone) [7] (15 mg), juncusyl ester A (**1**) (50 mg), sitosteryl-β-D-glucoside (60 mg), (2*S*)-1-O-*p*-coumaroyl glyceride (**3**) (50 mg) and juncusyl ester B (**2**) (30 mg), respectively. Another sample of the same material (4 kg) was extracted with water (5 l × 2) at room temp. The extract was partitioned with petrol. After concn, the petrol fraction was subjected to silica gel CC eluted with $CHCl_3$–MeOH–EtOAc (13:7:2) to give quercetin (20 mg), rutinose (20 mg), *trans-p*-coumaric acid (100 mg), and vanillic acid (20 mg), respectively. The structures of known compounds were determined on the basis of their physicochemical data and spectroscopic analysis.

Juncusyl ester A (**1**). Crystals, mp 121–123° (petrol–EtOAc), $[\alpha]_D^{22}$ + 6.6° (*c* = 0.64, MeOH). HR-MS *m/z*: 278.1151, calcd for $C_{15}H_{18}O_5$: 278.1154; EI-MS *m/z* (rel. int.): 278 $[M]^+$ (9), 263 (41), 220 (69), 164 (29), 159 (10), 147 (100), 131 (2), 101 (41); UV λ_{max} (EtOH) nm γ_{max}^{kBr} (log ε): 211 (3.11), 228 (3.24), 313 (3.52); IR ν_{max} KBr cm^{-1}: 3300, 3000, 1700, 1600, 1580, 1520, 1450, 1270, 1220, 1040, 990, 840; ^{1}H and ^{13}C NMR: Tables 1 and 2.

Acidic methanolysis of **1** *followed by acid hydrolysis.* Compound **1** (50 mg) was treated with 10% HCl–MeOH (5 ml) at 60° for 2 hr. The reaction mixture was concd to give a residue. The residue was subjected to silica gel CC eluted with $CHCl_3$–MeOH (5:1) to give **4** (30 mg). **4**: mp 118–120°, $[\alpha]_D^{22}$ + 11.6° (*c* = 0.2, MeOH). EI-MS *m/z* (rel. int.): 238 $[M]^+$ (10), 207 (6), 164 (33), 147 (100), 119 (20); ^{1}H NMR ($CDCl_3$, 360 MHz) δ: 3.60 (1H, *dd*, *J* = 3.7, 11.0 Hz, H-3), 3.63 (1H, *dd*, *J* = 3.0, 11.0 Hz, H-3′), 3.90 (1H, *dddd*, *J* = 3.0, 3.7, 4.4, 6.6 Hz, H-2), 4.20 (1H, *dd*, *J* = 6.6, 11.7 Hz, H-1), 4.23 (1H, *dd*, *J* = 4.4, 11.7 Hz, H-1′), 6.35 (1H, *d*, *J* = 16.0 Hz, H-5), 6.80 (2H, *d*, *J* = 8.8 Hz, H-9, 11), 7.45 (2H, *d*, *J* = 8.8 Hz, H-8), 7.65 (1H, *d*, *J* = 16.0 Hz, H-6). These physicochemical and spectroscopic data are identical to those of (2*S*)-(+)-1-O-*p*-coumaroyl glyceride.

Juncusyl ester B (**2**). Crystals, mp. 110–114° ($CHCl_3$–MeOH) HR-MS *m/z*: 238.0841, calcd for $C_{12}H_{14}O_5$: 238.0842; EI-MS *m/z* (rel. int.): 238 $[M]^+$ (9),

Table 1. ^{1}H NMR spectral data for compounds **1**, **2** and **3** ($CDCl_3$, δ, Hz)

H	1	2	3
1	4.42 (1H, *dd*, *J* = 11.6, 6.3)	3.74 (4H, *m*)	4.20 (1H, *dd*, *J* = 11.7, 6.6)
	4.30 (1H, *dd*, *J* = 11.6, 4.3)		4.23 (1H, *dd*, *J* = 11.7, 4.4)
2	4.42 (1H, *ddt*, *J* = 6.3, 4.3, 6.2)	4.98 (1H, *m*)	3.90 (1H, *dddd*, *J* = 3.0, 3.7, 4.4, 6.6)
3	3.81 (1H, *dd*, *J* = 8.4, 6.2)	3.74 (4H, *m*)	3.60 (1H, *dd*, *J* = 11.1, 3.0)
	4.13 (1H, *dd*, *J* = 8.4, 6.2)		3.63 (1H, *dd*, *J* = 11.1, 3.7)
5	6.27 (1H, *d*, *J* = 15.9)	6.37 (1H, *d*, *J* = 15.8)	6.37 (1H, *d*, *J* = 16.0)
6	7.63 (1H, *d*, *J* = 15.9)	7.66 (1H, *d*, *J* = 15.8)	7.65 (1H, *d*, *J* = 16.0)
8, 12	7.39 (2H, *d*, *J* = 8.4)	7.46 (2H, *d*, *J* = 8.3)	7.45 (2H, *d*, *J* = 8.8)
9, 11	6.84 (2H, *d*, *J* = 8.4)	6.84 (2H, *d*, *J* = 8.3)	6.80 (2H, *d*, *J* = 8.8)

Table 2. ^{13}C NMR (DEPT) spectral data for compounds **1**, **2** and **3** ($CDCl_3$, δ)

C	1	2	3
1	73.8 (*t*)	61.2 (*t*)	71.3 (*t*)
2	66.3 (*d*)	76.1 (*d*)	66.5 (*d*)
3	64.8 (*t*)	61.2 (*t*)	64.1 (*t*)
4	167.2 (*s*)	168.3 (*s*)	169.2 (*s*)
5	114.6 (*d*)	114.9 (*d*)	115.0 (*d*)
6	145.3 (*d*)	146.0 (*d*)	115.0 (*d*)
7	126.9 (*s*)	126.7 (*s*)	127.2 (*s*)
8	130.1 (*d*)	130.5 (*d*)	131.2 (*d*)
9	115.9 (*d*)	116.2 (*d*)	116.8 (*d*)
10	130.1 (*d*)	130.5 (*d*)	131.2 (*d*)
11	115.9 (*d*)	116.5 (*d*)	116.8 (*d*)
12	158.1 (*s*)	160.6 (*s*)	161.2 (*s*)
13	25.3 (*q*)		
14	26.6 (*q*)		
15	110.0 (*s*)		

207 (2), 164 (33), 147 (100), 119 (17), 91 (13), 65 (8), 31 (3), UV λ_{max} (EtOH) nm (log ε): 211 (3.81), 312 (3.72); IR ν_{max}^{KBr} cm^{-1}: 3400, 1680, 1640, 1600, 1520, 1450, 1180, 830; ^1H and ^{13}C NMR: Tables 1 and 2.

(2*S*)-1-O-*p*-*Coumaroyl glyceride* (**3**). Crystals, mp 118–120° ($CHCl_3$–MeOH), $[\alpha]_D^{20}$ + 12.0° (*c* = 0.1 MeOH). UV λ_{max} (EtOH) nm (log ε): 208 (4.04), 225 (4.08), 300 (4.17); IR ν_{max} (KBr) cm^{-1}: 3440, 2980, 1710, 1680, 1630, 1600, 1520, 1450, 830; ^1H and ^{13}C NMR: Tables 1 and 2.

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