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Flavonoids from the aquatic plant *Eriocaulon buergerianum*

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Abstract

Four flavonoids including (2*S*)-3′,4′-methylenedioxy-5,7-dimethoxyflavan and hispidulin 7-(6-*E*-*p*-coumaroyl-β-D-glucopyranoside), and one tocopherol were isolated from the capitulum of *Eriocaulon buergerianum* KOERN. Their structures were established by spectral and chemical evidence.

Keywords: *Eriocaulon buergerianum*; Eriocaulaceae; Flavonoids; Flavans; Ophthalmology

1. Introduction

In search of bioactive constituents of crude drugs derived from aquatic plants, the constituents of *Eriocaulon buergerianum* KOERN. (Eriocaulaceae), were investigated, since this plant is used as an ophthalmic and anti-inflammatory medicine in Taiwan. The genus *Eriocaulon* consists of some 250 species, eight of which were found in Taiwan (Chang, 1976). Some species have been shown to contain flavonoids, including patuletin, quercetagetin and quercetagetin derivatives (Bate-Smith and Harborne, 1969). In this paper we report the isolation and structural elucidation of a new flavan, (2*S*)-3′,4′-methylenedioxy-5,7-dimethoxyflavan (**1**), and hispidulin 7-(6-*E*-*p*-coumaroyl-β-D-glucopyranoside) (**2**), together with three known compounds, hispidulin (**3**), hispidulin 7-*O*-glucoside (**4**) and γ-tocopheryl acetate (**5**) from the capitulum of *E. buergerianum*.

2. Results and discussion

Repeated separation of the methanol extract from the capitulum of *E. buergerianum* resulted in the isolation of five compounds. Through physical and spectral comparisons three of them were found to be hispidulin (**3**) (Hase et al., 1995; Cui et al., 1993; Krishnaveni and Rao, 2000), hispidulin 7-*O*-glucoside (**4**) (Abdalla et al., 1983; Alam et al., 1986; Hase, et al., 1995) and γ-tocopheryl acetate (**5**) (Attygalle et al., 1996; Koyama et al., 1995). The other two compounds (**1–2**) were characterized by spectroscopic methods.

The molecular formula of **1** was determined as $C_{18}H_{18}O_5$ on the basis of HREIMS. The broad band decoupled ^{13}C NMR spectrum showed 18 carbon signals. A DEPT experiment indicated that compound **1** contained seven quaternary, six tertiary, three secondary and two methoxyl carbons. The IR spectrum showed absorption bands at 1590 and 1490 cm^{-1} which were assignable to an aromatic ring. The above data and UV spectrum (λ_{max}) at 230 nm and 283 nm (*sh*) suggested a flavan nature for compound **1** (Jayaprakasam et al., 1999), which was further evidenced by the analysis of the ^1H NMR and ^1H–^1H COSY spectra. The ^1H NMR spectrum showed AM-type proton signals at δ 6.07 and 6.11. The AMX-type proton signals were at δ 6.79, 6.87 and 6.92. The H-2, H_2-3 and H_2-4 signals were at (δ 4.87), (δ 1.96, 2.13) and (δ 2.61, 2.73), respectively. Additionally, the ^1H NMR spectrum showed two methoxy singlets at δ 3.74, 3.78 and one methylenedioxy signal at δ 5.94. The ^{13}C NMR and HMQC spectra revealed C_6–C_3 signals at δ 19.3 (C-4), 29.5 (C-3), 77.7 (C-2), 91.4 (C-6), 93.4 (C-8), 103.0 (C-10), 156.2 (C-9), 158.5, 159.4 (C-5,7) and C_6 signals at δ 106.7 (C-2′), 108.1 (C-5′), 119.6 (C-6′), 135.6 (C-1′), 147.2, 147.8

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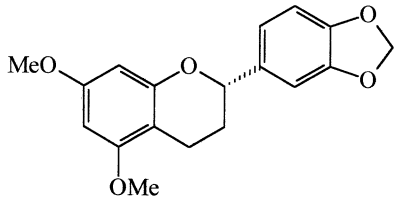

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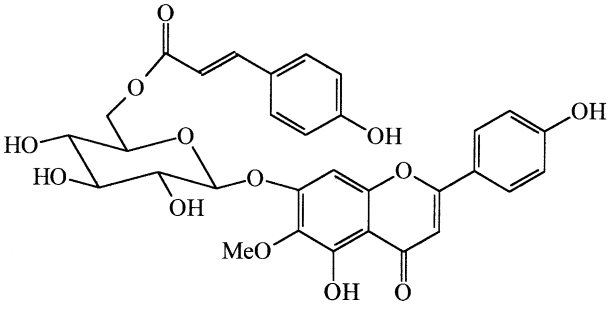

2

(C-3′, 4′). In addition, two methoxyl signals were found at δ 55.3 (C-OMe), 55.4 (C-OMe) and one methylenedioxy signal was at δ 101.0. A typical RDA-fragment ion at m/z 148 (59%) from EI-MS spectrum suggested one methylenedioxy unit in ring B.

The H-2 proton formed a doublet of doublets with *trans* coupling ^3J (10.5 Hz) and *cis* coupling ^3J (1.8 Hz) suggesting that this methine proton must be axial. The absolute configuration at position 2 was determined as 2*S* by comparing the negative Cotton effect at 281 nm ($\Delta\varepsilon_{281}$-0.50, MeCN, *c* 0.001) in CD experiment with the authentic (2*S*)-4′-hydroxy-5,7,3′-trimethoxyflavan which was isolated from *Mariscus psilostachys* (Garo et al., 1996). From the above evidence, compound **1** was determined to be (2*S*)-3′,4′-methylenedioxy-5,7-dimethoxyflavan.

The molecular formula of **2** was determined as $C_{31}H_{28}O_{13}$ on the basis of HRFABMS. The IR spectrum revealed hydroxyl (3350 cm^{-1}), conjugated ester (1700 cm^{-1}), α, β-unsaturated carbonyl (1660 cm^{-1}) and aromatic absorptions (1600, 1560, 1500 cm^{-1}). The UV spectrum in methanol exhibited absorptions (λ_{max}) at 338 nm, 275 nm (*sh*) and 230 nm (*sh*). The ^{1}H NMR, ^{1}H–^{1}H COSY, ^{13}C NMR and HMQC spectra displayed characteristic signals for the flavone, glucose and *p*-coumaroyl moieties.

The ^{1}H NMR and ^{1}H–^{1}H COSY spectra showed a methoxyl group at δ 3.66 (6-OMe), two singlet protons at δ 6.90 (1H, *s*, H-3), 7.08 (1H, *s*, H-8), three para substituted aromatic protons at δ 7.00 (2H, *d*, J=8.4, H-3′, 5′), 8.02 (2H, *d*, J=8.4, H-2′, 6′) and a hydrogen bonded hydroxyl group at δ 13.06 (1H, *br,s*, 5-OH). From the above data, the flavone moiety was suggested as hispidulin. Moreover, glucose moiety signals were found at δ 5.30 (1H, *d*, J=8.4, H-1″), 3.39 (1H, *m*, H-4″), 3.50 (2H, *m*, H-2″, 3″), 3.96 (1H, *br,t*, J=8.4, H-5″), 4.30 (1H, *dd*, J=11.2, 7.6, H-6″a) and 4.57 (1H, *br,d*, J=11.2, H-6″b). The anomeric hydrogen signal at δ 5.30 (1H, *d*, J=8.4 Hz, H-1″) supported the β-pyranoside configuration. In comparison with hispidulin glucoside (**4**) (Table 1), it showed that compound **2** revealed extra ^{1}H NMR signals at δ 7.33 (2H, *d*, J=8.4,

Table 1
^{13}C-NMR spectral data for compounds **2**, **3** and **4** (100 MHz, DMSO-d_6)

Carbon	2	3	4
2	164.5	164.1	164.4
3	102.7	102.6	102.7
4	182.4	182.3	182.3
5	152.7	152.9	152.5
6	132.6	131.6	132.6
7	156.3	157.6	156.5
8	94.4	94.5	94.4
9	152.2	152.7	152.2
10	106.0	104.3	105.8
1′	121.2	121.5	121.1
2′,6′	126.6	128.7	128.6
3′,5′	115.9	116.3	116.1
4′	161.5	161.4	161.4
OMe	60.6	60.7	60.4
Glucosyl			
1″	100.1		100.3
2″	73.2		73.3
3″	76.6		77.3
4″	70.4		69.7
5″	74.1		76.8
6″	63.7		60.7
Coumaroyl			
1‴	125.0		
2‴,6‴	130.0		
3‴,5‴	116.2		
4‴	159.8		
7‴	113.7		
8‴	145.2		
9‴	166.7		

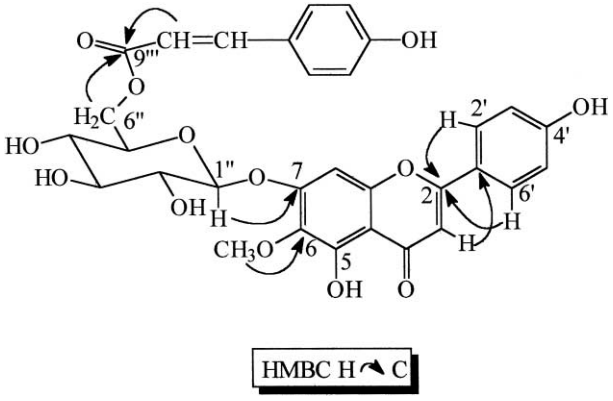

Fig. 1. HMBC correlations of **2**.

H-2‴, 6‴), 6.66 (2H, *d*, *J* = 8.4, H-3‴, 5‴), 6.36 (1H, *d*, *J* = 15.6, H-8‴), 7.54 (1H, *d*, *J* = 15.6, H-7‴) and extra ^{13}C NMR signals at δ 125.0, 130.0, 116.2, 159.8, 113.7, 145.2, 166.7. These extra NMR signals gave the evidences of the existence of an *E*-*p*-coumaroyl group in compound 2. The downed field shifted H-6″ resonance suggested that the *E*-*p*-coumaroyl moiety was adjacent to C-6″.

Finally, through HMBC results, the attachment positions were determined. The methoxyl signal at δ 3.66 (*s*, 6-OMe) showed a ^3J correlation to a carbon at δ 132.6 (C-6) suggesting the methoxyl group was connected to C-6 position in hispidulin moiety. The anomeric proton of the glucose unit (δ 5.30, H-1″) showed a ^3J correlation to a carbon of hispidulin (δ 157.6, C-7) indicating the attachment of the glucoside group at position C-7. Further, a ^3J interaction between H-6″ of the glucose (δ 4.30, H-6″) and the *p*-coumaroyl carbonyl carbon (δ 166.7, C-9‴) suggested the attachment of the *p*-coumaroyl ester at C-6″ of the glucose moiety (Fig. 1). From the above evidences, the structure of **2** was determined to be hispidulin 7-(6-*E*-*p*-coumaroyl-β-D-glucopyranoside).

3. Experimental

3.1. General

MPs were determined on a Yanaco micro-melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-360 digital polarimeter, whereas CD spectra were obtained with a JASCO J-720 spectropolarimeter. EI-MS were recorded with a JMS-HX-100 instrument and FAB-MS with a Jeol LMS-SX 102 system. IR spectra were recorded on a JASCO FT-IR-110 infrared spectrophotometer. UV spectra were recorded on a Perkin Elmer Lambda 5 UV/vis spectrophotometer, whereas ^{1}H and ^{13}C NMR spectra were acquired on Bruker AM-400 NMR and Bruker DMX-600 NMR spectrometers, respectively. CC was performed using silica gel (230–400 mesh, Merck), Diaion HP-20 (Pharmacia) and charcoal (Wako). TLC was conducted on precoated Kiesel gel 60 F_{254} plates (0.25 mm, Merck), spots were located by UV illumination and by spraying with $FeCl_3$ reagent or 10% H_2SO_4 followed by heating. MPLC was carried out on a Buchi MPLC system (pump, Buchi 688; detector, KAUER)

3.2. Plant material

The dry capitulum of *E. buergerianum* (7.2 kg) was collected in Taiwan and then identified by Professor H.C. Chang. A voucher specimen was deposited at the Department of Chemical Engineering, Ta-Hwa Institute of Technology, Hsinchu of Taiwan, ROC.

3.3. Extraction and isolation

The powdered material was successivey extracted with hot MeOH (50–60 °C) for 4–6 h (40 l×6) and concentrated to give a deep brown syrup (350 g), which was partitioned between 1:1 EtOAc/H_2O. The EtOAc layer was concentrated to give a brown residue (98.0 g) and then applied to a silica gel column eluted with *n*-hexane–EtOAc (1:1) to furnish five fractions. The second fraction was subjected to MPLC (silica gel, *n*-hexane–EtOAc gradient) to yield γ-tocopheryl acetate (25 mg). The third fraction was applied to a porous polymer Diaion HP-20 (H_2O–MeOH gradient) followed by MPLC (silica gel, CH_2Cl_2–MeOH gradient) to give **1** (26 mg), **2** (160 mg) and hispidulin (**3**) (300 mg). The water layer was partitioned between *n*-BuOH/H_2O (1:1), following which the *n*-BuOH layer was concentrated to give a brown residue (50.0 g) then subjected to CC on a charcoal column (H_2O–MeOH gradient) to afford hispidulin 7-*O*-glucoside (**4**) (5.6 g).

3.4. (2S)-3′,4′-Methylenedioxy-5,7-dimethoxyflavan (*1*)

Pale yellow oil. $[\alpha]_D^{25}$ −7.4° ($CDCl_3$; *c* 0.5). HRFAB-MS *m*/*z*: 314.1145 (M^+, calc. for $C_{18}H_{18}O_5$; 314.1154). EI-MS *m*/*z* (rel. int.): 314 [M^+], (100), 283 (8), 166 (9), 148 (59), 147 (26), 138 (14). IR (neat) ν_{max} cm^{-1}: 3060, 2940, 2840, 1620, 1595, 1495, 1440, 1250, 1200, 1140, 1100. UV λ_{max}^{MeOH} nm (log ε): 283 (3.83), 230 (5.22). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.96 (1H, *m*, H-3a), 2.13 (1H, *m*, H-3e), 2.61 (1H, *m*, H-4a), 2.73 (1H, *m*, H-4e), 3.74 (OMe), 3.78 (OMe), 4.87 (1H, *dd*, *J* = 10.5, 1.8, H-2), 5.94 (2H, *s*, methylenedioxy), 6.07 (1H, *d*, *J* = 2.3, H-6), 6.11 (1H, *d*, *J* = 2.3, H-8), 6.79 (1H, *d*, *J* = 7.9, H-5′), 6.87 (1H, *dd*, *J* = 7.9, 1.3, H-6′), 6.92 (1H, *d*, *J* = 1.3, H-2′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.3 (C-4), 29.5 (C-3), 55.3 (OMe), 55.4 (OMe), 77.7 (C-2), 91.4 (C-6), 93.4 (C-8), 101.0 (methylenedioxy), 103.0 (C-10), 106.7 (C-2′), 108.1 (C-5′), 119.6 (C-6′), 135.6 (C-1′), 147.2, 147.8 (C-3′, 4′), 156.2 (C-9), 158.5, 159.4 (C-5, 7). CD data: $\Delta\epsilon_{243}$-0.49, $\Delta\epsilon_{281}$-0.50 (MeCN, *c* 0.012).

3.5. Hispidulin 7-(6-E-p-coumaroyl-β-D-glucopyranoside) (*2*)

Amorphous yellowish powder, mp 288–289 °C. $[\alpha]_D^{25}$ −46.4 (MeOH *c* 0.5). HR FAB-MS (negative) *m*/*z*: 608.1573 (M^+, Calc. for $C_{31}H_{28}O_{13}$; 608.1529); HR FAB-MS (negative) *m*/*z*: 607.1452 [$(M-H)^-$, calc. for $C_{31}H_{27}O_{13}$: 607.1452). EI-MS *m*/*z* (rel. int.): 300 (100), 285 (68), 257 (63), 167 (12), 164 (13), 147 (24), 139 (15), 119 (22), 69 (50). FABMS (negative) *m*/*z* (rel. int.): 608 [M^+], (3), 577 (M^+-OMe, 20). IR (neat) ν_{max} cm^{-1}: 3350, 1700, 1660, 1600, 1565, 1510, 1495, 1300, 1260, 1189, 1080. UV λ_{max}^{MeOH} nm (log ε): 338 (4.46), 275 (4.26), 230 (4.23). ^{1}H NMR (400 MHz, DMSO-d_6): δ hispidulin moiety: 3.66 (6-OMe), 6.90 (1H, *s*, H-3), 7.08 (1H, *s*, H-8), 7.00 (2H,

d, $J=8.4$, H-3′, 5′), 8.02 (2H, *d*, $J=8.4$, H-2′, 6′), 13.06 (1H, *br,s*, 5-OH), glucose moiety: 5.30 (1H, *d*, $J=8.4$, H-1″), 3.39 (1H, *m*, H-4″), 3.50 (2H, *m*, H-2″, 3″), 3.96 (1H, *br;t*, $J=8.4$, H-5″), 4.30 (1H, *dd*, $J=11.2$, 7.6, H-6″a), 4.57 (1H, *br,d*, $J=11.2$, H-6″b), coumaroyl moiety: 7.33 (2H, *d*, $J=8.4$, H-2‴, 6″), 6.66 (2H, *d*, $J=8.4$, H-3″, 5″), 6.36 (1H, *d*, $J=15.6$, H-7″), 7.54 (1H, *d*, $J=15.6$, H-8″), 5.41, 5.52, 5.67 (3×OH). ^{13}C NMR (100 MHz, DMSO-d_6): Table 1.

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