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COUMARIN GLYCOSIDES FROM TWO SPECIES OF *ERIOSTEMON*

SATYAJIT D. SARKER, PETER G. WATERMAN,*

Phytochemistry Research Laboratories, Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow, G1 1XW, Scotland, UK

and JAMES A. ARMSTRONG

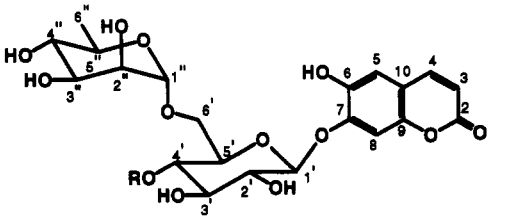
Department of Conservation and Land Management, Hackett Drive, Crawley, Perth, Western Australia 6009

ABSTRACT.—Examination of the aerial parts of *Eriostemon cymbiformis* led to the isolation of two novel coumarin glycosides, isobaisseoside [esculetin-7-(6-α-rhamnopyranosyl)-β-glucopyranoside] [**1**] and isobaisseoside-4′-*p*-coumarate [**2**], along with the common flavonoid glycoside hesperidin from the methanolic extract, and the dihydrocinnamic acid derivative eriostemoic acid from the *n*-hexane extract. The methanolic extract of the aerial parts of *Eriostemon wonganensis* also produced **1**, while the petroleum ether extract of the same yielded two other dihydrocinnamic acid derivatives, eriostoic acid and the novel methyl ester of eriostemoic acid [**3**]. The chemotaxonomic implications of these compound identifications are discussed.

Eriostemon cymbiformis P.G. Wilson (1) and *Eriostemon wonganensis* P.G. Wilson (2) (Rutaceae) are sub-shrubs found only in Western Australia. Neither has previously been studied for their secondary metabolite profiles. As part of an ongoing phytochemical and chemotaxonomic survey of the genus *Eriostemon* (3–11), we have undertaken a thorough investigation of these species.

By a combination of vlc, cc, prep. tlc, and hplc, eriostemoic acid (12) was isolated from the *n*-hexane extract of *E. cymbiformis*, and hesperidin (13,14) and the two novel coumarin glycosides [**1**, **2**] obtained from the MeOH extract. Similar treatment of the MeOH extract of *E. wonganensis* also yielded one of the novel coumarin glycosides, while the petroleum ether extract produced two further dihydrocinnamic acid derivatives, eriostoic acid (15) and the methyl ester of eriostemoic acid [**3**]. The known compounds were characterized by direct comparison of their physical and spectroscopic characteristics with those published in the literature and with samples previously isolated in our laboratory. The novel compounds were characterized by spectroscopic means.

Both compounds **1** and **2** were visualized on tlc as bluish-white fluorescent spots under uv light (366 nm). The uv, ir, and ^{1}H-nmr data suggested that they were coumarins (16). The fabms spectrum of **1** indicated the empirical formula $C_{21}H_{26}O_{13}$. The ^{1}H-nmr spectrum (Table 1) displayed, in addition to the



1 R=H

2 R=

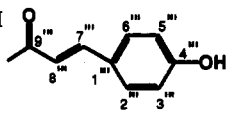


TABLE 1. ^{1}H- and ^{13}C-Nmr Data for **1**.

Position	δ ^{1}H[a]	δ ^{13}C[b]	Position	δ ^{1}H[a]	δ ^{13}C[b]
2		162.7	1′	5.62 (1H, d, J=7.5 Hz)	103.8
3	6.27 (1H, d, J=9.5 Hz)	114.3	2′	4.38 (1H, m)	75.1
4	7.61 (1H, d, J=9.5 Hz)	144.7	3′	4.37 (1H, m)	79.1
5	7.23 (1H, s)	114.2	4′	4.08 (1H, m)	72.1
6		146.7	5′	4.23 (1H, m)	78.0
7		151.3	6′	4.10 (1H, m)	68.0
				4.39 (1H, m)	
8	7.72 (1H, s)	106.2	1″	5.45 (1H, s)	102.6
9		149.0	2″	4.67 (1H, m)	72.9
10		114.7	3″	4.76 (1H, d, J=9.0 Hz)	73.1
			4″	4.29 (1H, m)	74.1
			5″	4.34 (1H, m)	70.4
			6″	1.62 (3H, d, J=6.0 Hz)	19.2

[a]Solution in C_5D_5N referenced to pyridine at δ 8.74 (400 MHz).
[b]Solution in C_5D_5N referenced to pyridine at δ 150.35 (100 MHz).

signals for H-3 and H-4, two aromatic singlets assignable to H-5 and H-8 and the signals for the protons of two hexose sugars. A rhamnose could be assumed from the occurrence of a 3H doublet at δ 1.62 (J=6 Hz) for the methyl group (C-6″) and two anomeric protons appeared as a doublet at δ 5.62 (J=7.5 Hz) and a singlet at δ 5.45. COSY-45 and TOCSY nmr spectra revealed all the ^{1}H-^{1}H couplings among the sugar protons and thus aided in the assignment of all the protons in the glycone moiety, which were attributable to glucose and rhamnose monomers. The ^{13}C-nmr spectrum (Table 1) revealed all 21 carbons and an HC-COBI dec. spectrum (Table 2) displayed the direct H-C couplings. In the HMBC nmr spectrum (Table 2) (17), the rhamnose anomeric proton (H-1″) showed ^3J correlation to C-6′ of glucose, indicating a 1→6 linkage between rhamnose and glucose to form the rutinose skeleton. Attachment of this glycone part at C-7 of the coumarin nucleus was evident from the ^3J H-C coupling between H-1′ and C-7 (δ 151.3). This was further confirmed from a COSY-ir nmr spectrum showing a cross-peak due to the ^{1}H-^{1}H long-range coupling between H-1′ and H-8, and again from the NOESY nmr spectrum (Figure 1), where a nOe be-

TABLE 2. Major HC-COBI Dec. and HMBC Correlations in **1**.

Proton	^{13}C		
	Direct	^2J	^3J
H-3	114.3	162.7 (C-2)	114.7 (C-10)
H-4	144.7	114.3 (C-3)	162.7 (C-2), 114.2 (C-5), 149.0 (C-9)
H-5	114.2	146.7 (C-6)	144.7 (C-4), 149.0 (C-9), 151.3 (C-7)
H-8	106.2	149.0 (C-9), 151.3 (C-7)	114.7 (C-10), 146.7 (C-6)
H-1′	103.8		151.3 (C-7)
H-2′	75.1		72.1 (C-4′)
H-3′	79.1	75.1 (C-2′)	103.8 (C-1′)
H-5′	78.0	68.0 (C-6′), 72.1 (C-4′)	
H-1″	102.6	72.9 (C-2″)	73.1 (C-3″), 68.0 (C-6′), 70.4 (C-5″)
H-2″	72.9		74.1 (C-4″)
H-3″	73.1	72.9 (C-2″), 74.1 (C-4″)	102.6 (C-1″)
H_3-6″	19.2	70.4 (C-5″)	74.1 (C-4″)

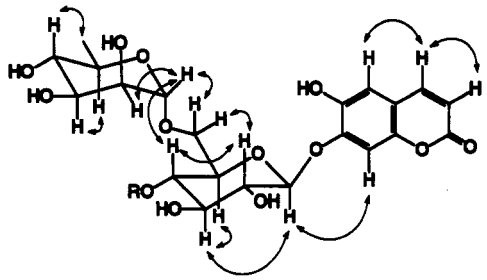


FIGURE 1. NOe interactions of **1**.

tween these two protons was also revealed. The structure of this coumarin glycoside was thus assigned unambiguously as **1**. This structure is isomeric with baisseoside (18) in which the rutinose moiety is attached to C-6, and, on this basis, **1** was named isobaisseoside.

The fabms spectrum of **2** revealed the empirical formula $C_{30}H_{32}O_{15}$, suggesting that in **2** a $C_9H_6O_2$ moiety was present in addition to the skeleton present in **1**. In the ir spectrum absorption bands at 1706 and 1688 cm^{-1} accounted for two carbonyl groups, one of which was the coumarin lactone carbonyl and the other a carbonyl group as part of the extra $C_9H_6O_2$ moiety. In the ^{1}H-nmr spectrum (Table 3), in addition to the signals present

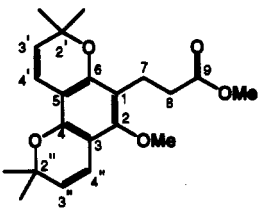
TABLE 3. ^{1}H- and ^{13}C-Nmr Data for **2**.

Position	δ ^{1}H[a]	δ ^{13}C[b]
2		164.5
3	6.30 (1H, d, *J*=9.5 Hz)	114.2
4	7.86 (1H, d, *J*=9.5 Hz)	146.2
5	7.02 (1H, s)	114.5
6		147.1
7		151.3
8	7.31 (1H, s)	105.7
9		149.2
10		115.5
1′	5.00 (1H, d, *J*=7.7 Hz)	103.4
2′	3.70 (1H, m)	75.1
3′	3.52 (1H, m)	75.6
4′	4.97 (1H, t, *J*=9.0 Hz)	72.2
5′	3.64 (1H, m)	75.5
6′	3.33 (1H, m), 3.57 (1H, m)	67.4
1″	4.67 (1H, s)	102.1
2″	3.92 (1H, m)	72.6
3″	4.00 (1H, m)	72.5
4″	3.43 (1H, m)	73.7
5″	3.69 (1H, m)	70.1
6″	1.17 (3H, d, *J*=6.2 Hz)	18.2
1‴		127.1
2‴, 6‴	7.49 (1H, d, *J*=8.6 Hz)	131.3
3‴, 5‴	6.82 (1H, d, *J*=8.6 Hz)	117.1
4‴		161.9
7‴	7.71 (1H, d, *J*=15.9 Hz)	147.7
8‴	6.40 (1H, d, *J*=15.9 Hz)	114.6
9‴		168.5

[a]Solution in CD_3OD referenced to CH_3OH at δ 3.31 (400 MHz).
[b]Solution in CD_3OD referenced to CH_3OH at δ 49.15 (100 MHz).

in **1**, there were signals for trans olefinic protons (J=15.9 Hz) and for the protons of a para- disubstituted benzene ring, which together made up a *p*-coumaroyl moiety. The J-modulated ^{13}C-nmr spectrum (Table 3) also displayed all the resonances for the nine carbons of a *p*-coumaroyl unit, including a carbonyl carbon (δ 168.5), two other quaternary carbons, one oxygen-bearing (δ 161.9, 127.1), two olefinic methine carbons (δ 114.2, 147.7), and four aromatic methine carbons resonating at two δ values (δ 117.1, 131.3). Attachment of this *p*-coumaroyl unit at C-4′ of the glucose molecule was confirmed from the ^3J interaction between H-4′ (δ 4.97) and the coumaroyl carbonyl C-9‴ (δ 168.5) in the HMBC nmr spectrum (Table 4). An unambiguous assignment of all the protons and carbons was achieved via COSY-45 and HMBC nmr experiments (Table 4) and thus the structure of this compound was confirmed as **2** and it was named as isobaisseoside-4′-*p*-coumarate.

The novel dihydrocinnamic acid derivative **3** was detected as a dark quenching spot on tlc under uv light (254 nm). Spraying with vanillin/H_2SO_4 produced an instant red color that turned blue on heating. The uv and ir absorptions were



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similar to those published for eriostemoic acid (12) and suggested that this compound was a dihydrocinnamic acid derivative. The eims showed the molecular ion at *m/z* 358 which analyzed for $C_{21}H_{26}O_5$. The ^{1}H-nmr spectrum (Table 5) showed two quartets of J=10 Hz centered at δ 5.49, 6.61 and δ 5.49, 6.49, representing pyran ring protons, a 12H singlet (δ 1.42) for four methyls of two pyran rings, two deshielded 3H singlets, one for an aromatic methoxyl and one for an esterifying methoxyl attached to the side-chain, and two sets of multiplets for two adjacent non-equivalent methylene groups in the side-chain. The J-modulated ^{13}C-nmr spectrum (Table 5) revealed all 21 carbons including two methoxyls, four methyls, two methylenes, four methines, a carbonyl, and six quaternary carbons, of which three were oxygenated. The ^{1}H-nmr spectral data

TABLE 4. Major H-C-C-C HMBC Correlations in **2**.

Proton	^{13}C	
	^2J	^3J
H-3	164.5 (C-2)	115.5 (C-10)
H-4	114.2 (C-3), 115.5 (C-10)	164.5 (C-2), 114.5 (C-5), 149.2 (C-9)
H-5	147.1 (C-6)	146.2 (C-4), 149.2 (C-9), 151.3 (C-7)
H-8	149.2 (C-9), 151.3 (C-7)	115.5 (C-10), 147.1 (C-6)
H-1′		75.6 (C-3′), 151.3 (C-7)
H-2′	75.6 (C-3′)	72.2 (C-4′)
H-3′	75.1 (C-2′)	
H-4′		168.5 (C-9″)
H-1″		72.5 (C-3″), 67.4 (C-6′), 70.1 (C-5″)
H-2″		73.7 (C-4″)
H_3-6″	70.1 (C-5″)	73.7 (C-4″)
H-2‴, H-6‴	117.1 (C-3‴, C-5‴)	131.3 (C-2‴, 6‴), 147.7 (C-7‴), 161.9 (C-4‴)
H-3‴, H-5‴	131.3 (C-2‴, C-6‴), 161.9 (C-4‴)	127.1 (C-1‴), 117.1 (C-3‴, 5‴)
H-7‴	114.6 (C-8‴)	168.5 (C-9‴)
H-8‴	168.5 (C-9‴)	127.1 (C-1‴)

TABLE 5. ^{1}H- and ^{13}C-Nmr Data for **3**.

Position	δ ^{1}H[a]	δ ^{13}C[b]
1	—	107.7
2	—	154.9
MeO-2	3.72 (3H, s)	62.7
3	—	107.0
4	—	147.9
5	—	114.0
6	—	152.3
CH_2-7	2.87 (2H, m)	34.2
CH_2-8	2.59 (2H, m)	19.2
9	—	178.7
MeO-9	3.68 (3H, s)	50.9
2′	—	76.5
Me-2′	1.42 (6H, s)	28.0
3′	5.49 (1H, d, J=10 Hz)	127.3
4′	6.61 (1H, d, J=10 Hz)	116.8
2″	—	76.2
Me-2″	1.42 (6H, s)	28.0
3″	5.49 (1H, d, J=10 Hz)	127.5
4″	6.49 (1H, d, J=10 Hz)	117.8

[a]Solution in $CDCl_3$ referenced to $CHCl_3$ at δ 7.27 (400 MHz).
[b]Solution in $CDCl_3$ referenced to $CHCl_3$ at δ 77.23 (100 MHz).

were very similar to those published for eriostemoic acid (12) with the exception that an extra methoxyl group was present. On this basis the compound was assigned as **3**.

This is the first report of coumarin glycosides from the genus *Eriostemon*. Both *E. cymbiformis* and *E. wonganensis* are placed in *Eriostemon* section *Nigrostipulae* (1,2) and the co-occurrence of these coumarin glycosides indicates a close affinity between them. In a recent hplc-based study of *Eriostemon* (S.D. Sarker, University of Strathclyde, Glasgow, UK, unpublished results), it was observed that species in *Eriostemon* section *Nigrostipulae* could be divided into chemical groups, one of which produces avicennol/avicennin-type angular pyranocoumarins (3,5,6). *E. cymbiformis* and *E. wonganensis* do not accumulate any angular pyranocoumarins and are clearly distinct from the other species examined previously. Hesperidin occurs widely in the Rutaceae (19) and dihydrocinnamic acid derivatives seem to be present in most species of *Eriostemon*.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir and uv spectra were recorded on Mattson Genesis Series Ft-ir and Perkin-Elmer 552 spectrophotometers, respectively. The eims were recorded on an AEI MS 902 spectrometer. Fabms spectra were obtained on a VG ZAB-E spectrometer with a nitrobenzoyl alcohol or glycerol matrix. Nmr spectra were obtained on a Bruker AMX-400 instrument using standard Bruker microprograms. Chemical shifts were reported in ppm relative to solvent ($C_5D_5N/CD_3OD/CDCl_3$). For HMBC experiments, ^2J and ^3J coupling was set for approximately 7 Hz. Si gel 60H (Merck 7736), Si gel 60-PF254 (Merck 7749), and Si gel (Merck 7734) were used for vlc, tlc, and cc, respectively. Hplc separation was performed in a Gilson model 811 hplc equipped with SPD-6AV uv-vis detector (Shimadzu) and using a semi-prep. C_{18} silica column (Spherisorb 5 ODS 2, 250×10.0 mm). Petroleum ether refers to petroleum ether, bp 40–60°.

PLANT MATERIALS.—*Eriostemon cymbiformis* was collected from Fitzgerald River National Park, ca. 11.5 km south of Jerramungup to Ravensthorpe Road, along Rabbit Proof Fence Road, Western Australia (voucher: PERTH 01655728). *Eriostemon wonganensis* was collected from the Gap, Wongan Hills, 10 km west-northwest of Wongan Hills Township, longitude 116°36′ E, latitude 30°50′S,

Western Australia (voucher: PERTH 01642375). Vouchers have been deposited at the Western Australian Herbarium, Perth.

EXTRACTION AND ISOLATION.—Powdered plant materials [*E. cymbiformis*, 340 g and *E. wonganensis*, 150 g] were each extracted (Soxhlet) with, successively, *n*-hexane (petroleum ether for the latter), $CHCl_3$, and MeOH, and the extracts were concentrated using a rotary evaporator at a maximum temperature of 40°.

Vlc of the concentrated *n*-hexane extract of *E. cymbiformis* (11.0 g), eluting with solvents of increasing polarity starting from *n*-hexane (100%) via EtOAc to MeOH (100%), yielded several fractions. Prep. tlc ($CHCl_3$-EtOAc, 8:2) of the vlc fractions (10–20% EtOAc in *n*-hexane) yielded eriostemoic acid (72 mg).

Cc of the concentrated MeOH extract of the same plant (16.0 g), eluting with solvents of increasing polarity starting from 100% $CHCl_3$ to 100% EtOH, yielded a number of fractions. Cc fractions (30% EtOH in $CHCl_3$), on standing produced precipitation and the residue was filtered off to obtain hesperidin (91 mg). Prep. tlc ($CHCl_3$-MeOH, 9:1; double development) of column fractions (40% EtOH in $CHCl_3$ and 50% EtOH in $CHCl_3$) produced impure **2** and **1**, respectively. Both compounds were further purified via hplc using an isocratic elution with a MeOH/H_2O solvent mixture (45:55) and finally produced 20 mg of pure **1** and 3.8 mg of pure **2**.

The petroleum ether extract of *E. wonganensis* (4.4 g) was subjected to cc eluting with a petroleum ether/EtOAc solvent mixture of increasing polarity. Prep. tlc ($CHCl_3$-EtOAc, 8:2) of the cc fraction (20–30% EtOAc in petroleum ether) yielded eriostoic acid (4.5 mg) and **3** (5.2 mg). The MeOH extract (5.5 g) of this plant was treated in the same way as the MeOH extract of *E. cymbiformis* and compound **1** (25 mg) was isolated.

Isobaisseoside [*esculetin-7-(6-α-rhamnopyranosyl)-β-glucopyranoside*] [**1**].—Yellow gum; uv λ max (EtOH) (log ε) 240 (3.82), 262 (3.84), 281 (3.87), 304 (3.95), 336 (4.10), 382 (3.97) nm; ir ν max 3379, 1698, 1605, 1515, 1443, 1392, 1288, 1170, 834 cm^{-1}; ^{1}H- and ^{13}C-nmr data, see Table 1; fabms *m/z* $[M+H]^+$ 487, $[M+Na]^+$ 509, analyzed for $C_{21}H_{26}O_{13}$.

Isobaisseoside-4'-p-coumarate [**2**].—Yellow gum; uv λ max (EtOH) (log ε) 257 (3.86), 297 (3.88), 312 (3.99), 379 (3.97) nm; ir ν max 3446, 1706, 1688, 1652, 1559, 1498, 1457, 1388, 1289, 1066, 834 cm^{-1}; ^{1}H- and ^{13}C-nmr data, see Table 3; fabms *m/z* $[M+H]^+$ 633, $[M+Na]^+$ 655, analyzed for $C_{30}H_{32}O_{15}$.

Methyl ester of eriostemoic acid [**3**].—Colorless gum; uv λ max (EtOH) (log ε) 254 (3.96), 278 (3.98), 330 (3.94), 348 (3.88) nm; ir ν max 1726, 1630, 1590, 1466, 1410, 1365, 1300, 1265, 1225, 1200, 1175, 1130, 1100, 1050, 990, 910, 880, 744 cm^{-1}; ^{1}H- and ^{13}C-nmr data, see Table 5; eims *m/z* $[M]^+$ 358.1751 (calcd 358.1780 for $C_{21}H_{26}O_5$; major fragment ions *m/z* 358 $[M]^+$ (1), 344 $[M-CH_2]^+$ (23), 329 $[344-CH_3]^+$ (100), 269 (8).

Hesperidin.—Amorphous; uv, ir, ^{1}H-nmr, and ^{13}C-nmr data in agreement with literature values (13,14).

Eriostemoic acid.—Colorless gum; eims *m/z* $[M]^+$ 344.1623 (calcd 344.1624 for $C_{20}H_{24}O_5$); uv, ir, ^{1}H-nmr, and eims data in agreement with literature values (12).

Eriostoic acid.—Needles from *n*-hexane/EtOAc; mp 173°–174°; eims *m/z* $[M]^+$ 344.1621 (calcd 344.1624 for $C_{20}H_{24}O_5$); uv, ir, ^{1}H-nmr, and eims data in agreement with literature values (12,15).

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