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Benzopyran derivatives from the aerial parts of *Eriostemon rhomboideus*

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Abstract

Twelve compounds have been isolated from the aerial parts of *Eriostemon rhomboideus*, six of which are novel. The novel compounds have been characterised as the flavones, 3,5,7,8,3′,4′-hexahydroxyflavone-3-*O*-β-glucopyranoside-7-methyl ether and 3,5,7,8,3′,4′,-hexahydroxyflavone-3-*O*-(6-*p*-coumaroyl)-β-glucopyranoside-7-methyl ether, the 2-alkylbenzopyrans, 5,7-dihydroxy-2-heptacosanylbenzopyran-4-one and 2ξ,5,7-trihydroxy-2-heptacosanyl-2,3-dihydrobenzopyran-4-one, glyceryl-1-tetracosanoate and the coumarin, isobaisseoside-3′-*p*-coumarate {esculetin-7-*O*-(3-*p*-coumaroyl-6-α-rhamnopyranosyl)-β-glucopyranoside}. The known compounds were eriostoic acid, eriostemoic acid, hesperidin, sitosterol-3-*O*-β-glucopyranoside and a mixture of catechin and epicatechin. The chemotaxonomic importance of the isolation of a coumarin glycoside is noted.

Keywords: *Eriostemon rhomboideus*; Rutaceae; Glyceryl-1-tetracosanoate; Flavonol-3-*O*-glucosides; 2-Alkyl-5,7-dihydroxybenzopyran-4-ones; Coumarin glycoside

1. Introduction

Eriostemon rhomboideus P.G. Wilson (Rutaceae) is an undershrub endemic to the southwest part of Western Australia (Wilson, 1970). No phytochemical work has previously been recorded on this species. As part of our ongoing phytochemical and chemotaxonomic survey of the genus *Eriostemon* (Ghisalberti, 1998), we now wish to communicate our findings for the aerial parts of this species.

2. Results and discussion

A petroleum ether (bp 40–60°C) extract of the aerial parts of *E. rhomboideus* yielded eriostoic acid, eriostemoic acid, both of which are common in the genus, and a new compound identified as glyceryl-1-tetracosanoate (**1**). Further extraction with ethyl acetate yielded five novel compounds, two 2-alkylbenzopyran-4-ones (**2**, **3**), a coumarin glycoside (**4**) and two flavone glycosides (**5**, **6**), together with daucosterol and the flavanone glycoside hesperidin. Finally, a mixture of catechin and epicatechin was obtained from the ethyl acetate soluble part of the MeOH extract. The known compounds were characterised 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 characterised by spectroscopic methods.

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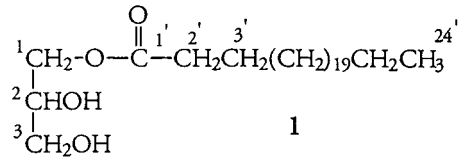

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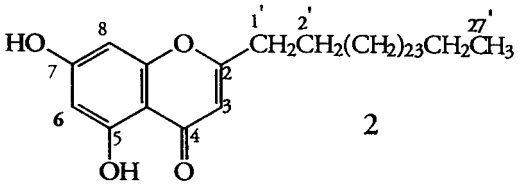

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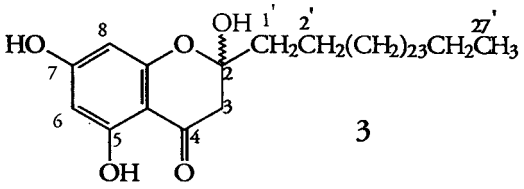

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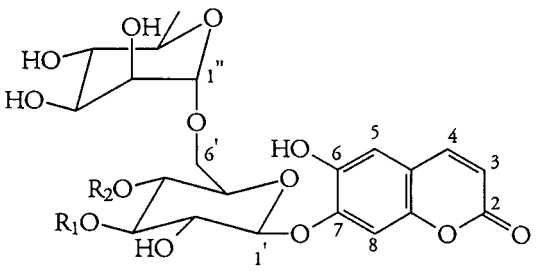
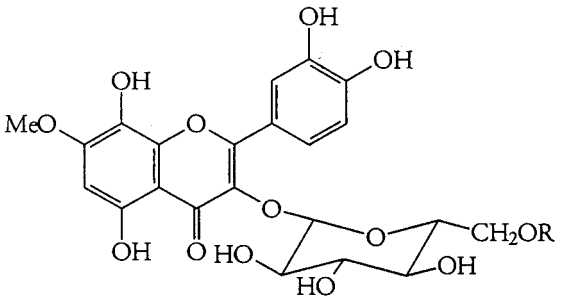


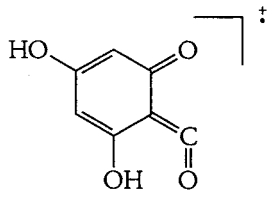
4 R_1 = p coumaric acid; R_2 = H

9 R_1 = H; R_2 = p coumaric acid



5 R = p coumaric acid

6 R = H



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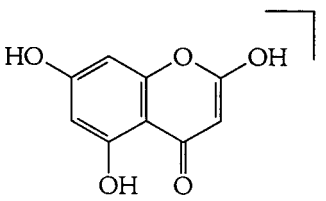

Table 1
NMR data for compound **1** (400 MHz, δ values in $CDCl_3$)

Position	^{1}H	^{13}C	^2J	^3J
1-H_a	4.23 (dd, J = 4.6, 11.6 Hz)	65.4	C-2	C-1′, C-3
1-H_b	4.17 (dd, J = 6.1, 11.6 Hz)			
2	3.94 (q, J = 5.9 Hz)	70.5		
3-H_a	3.71 (dd, J = 4.0, 11.4 Hz)	63.6		
3-H_b	3.60 (dd, J = 5.8, 11.4 Hz)			
1′		174.6		
2′	2.35 (t, J = 7.48 Hz)	34.4	C-1′, C-3′	C-1
3′	1.64 (q, J = 7.12 Hz)	25.2		
4′-22′	1.29 (br)	29.5–32.3		
23′	1.29 (br)	22.9		
24′	0.90 (t, J = 7.04 Hz)	14.3	C-23′	

The HREI mass spectrum of **1** showed a molecular ion m/z 442, which solved for $C_{27}H_{54}O_4$ with an intense fragment at m/z 323 due to the loss of $C_4H_7O_4$. The ^{1}H-NMR spectrum (Table 1) showed oxymethylene proton signals for an –O–CH_2–CH(O)–CH_2–O spin system attributable to glycerol. The ^{13}C-NMR spectrum (Table 1) showed an ester carbonyl signal at δ 174.6. In an HMBC experiment (Table 1) the methylene protons (H-1) at δ 4.23/4.17 showed a ^3J correlation with the carbonyl carbon so revealing the single point of esterification. As the NMR spectra revealed a single linear alkyl chain this must be C-24 and on this basis **1** must be glyceryl-1-tetracosanoate.

The FAB mass spectrum of **2** gave a quasi-molecu-

Table 2
NMR data for compound **2**, (400 MHz, δ values in $CDCl_3 + CD_3OD$)

Position	^1H	^{13}C	^2J	^3J
2		170.9		
3	5.87 (s)	107.45	C-2, C-4	C-4a
4		182.6		
4a		104.3		
5		161.5		
6	6.12 (d, J = 2.1 Hz)	99.1	C-5, C-7	C-4a, C-8
7		164.1		
8	6.20 (d, J = 2.1 Hz)	94.1	C-7, C-8a	C-4a, C-6
8a		158.4		
1′	2.45 (t, J = 7.5 Hz)	34.1	C-2, C-2′	C-3
2′	1.57 (q, J = 7.3 Hz)	26.7		
3′-25′	1.06-1.27 m	28.8–31.8		
26′	1.06 m	22.5		
27′	0.75 (t, J = 6.64 Hz)	13.8		

lar ion at m/z 557 $[M+1]^+$ ($C_{36}H_{60}O_4$) and showed intense fragments at m/z 205, for the loss of a long alkyl chain, and at m/z 152 (**7**), suggestive of a 5,7-oxygenated benzopyran-4-one. The ^{1}H-NMR spectrum (Table 2) exhibited a pair of *meta*-coupled aromatic protons typical of H-6 and H-8 of a 5,7-dioxygenated flavonoid and an aromatic singlet attributable to H-3 of a flavone. However, no further aromatic signals were observed, the rest of the spectrum being made up of the resonances for an alkyl chain suggesting that **2** was not a flavone but a 5,7-dihydroxybenzopyran-4-one substituted at C-2 with a saturated alkyl group. The ^{13}C-NMR spectrum (Table 2) supported this hypothesis. In the HMBC spectrum (Table 2), the H-3 proton showed ^2J correlation with a carbonyl carbon (δ 182.6) and with an oxygen bearing quaternary carbon at δ 170.9 (C-2) and ^3J correlation with a quaternary carbon at δ 104.3 (C-4a). A methylene triplet at δ 2.45 (C-1′) showed ^2J correlation with δ 170.9 (C-2) and ^3J correlation with δ 107.4 (C-3) which confirmed attachment of the side chain at C-2. Compound **2** is therefore identified as 5,7-dihydroxy-2-heptacosanylbenzopyran-4-one.

Table 3
NMR data for compound **3** (400 MHz, δ values in Pyridine-d_5)

Position	^1H	^{13}C	^2J	^3J
2	3.63 (s, OH)	104.2		
3	3.24 (d, J = 16.8 Hz) 3.15 (d, J = 16.8 Hz)	46.6	C-2, C-4	
4		197.1		
4a		103.4		
5	12.82 (s, OH)	165.4	C-5	C-4a, C-6
6	6.47 (d, J = 2.1 Hz)	97.4	C-5, C-7	C-4a, C-8
7	9.10 (s, OH)	168.9		
8	6.49 (d, J = 2.1 Hz)	97.3	C-6, C-8a	C-4a, C-6
8a		162.4		
1′	2.20 (m)	42.2	C-2	C-3
2′	1.73 (q, J = 7.0 Hz))	32.6		
3′–26′	1.29–1.34 (br s)	23.4–30.6		
27′	0.89 (t, J = 6.8 Hz)	14.8		

Table 4
NMR data for compound **4** (400 MHz, δ values in CD_3OD)

Position	^1H	^{13}C	^2J	^3J
2		164.4		
3	6.32 (d, J = 9.4 Hz)	114.8	C-2	C-4a
4	7.84 (d, J = 9.5 Hz)	146.0		C-2, C-5, C-8a
4a		115.5		
5	7.04 (s)	114.1	C-6	C-4, C-7, C-8a
6		145.7		
7		150.6		
8	7.35 (s)	105.9	C-7, C-8a	C-4a, C-6
8a		149.7		
1′	5.05 (d, J = 7.9 Hz)	103.3		C-7
2′	3.84 (m)	73.4	C-1′, C-3′	
3′	5.22 (t, J = 9.3 Hz)	78.5	C-2′, C-4′	C-9‴
4′	3.59 (m)	70.4	C-3′	
5′	3.80 (m)	77.3		
6″	4.16 (dd, J = 1.8, 10.7 Hz) 3.57 (dd, J = 1.84, 9.8 Hz)	67.7		
1″	4.73 (d, J = 1.2 Hz)	102.1		C-3″, C-6′
2″	3.99 (dd, J = 3.4, 12.8 Hz)	72.5		C-4″
3″	3.95 (m)	72.3		
4″	3.42 (t, J = 9.4 Hz)	73.8	C-3″, C-5″	C-2″
5″	3.71 (m)	70.0		
6″	1.23 (d, J = 6.2 Hz)	18.2	C-5″	C-4″
1‴		127.4		
2‴/6‴	7.48 (d, J = 8.6 Hz)	131.3		C-4‴, C-7‴
3‴/5‴	6.81 (d, J = 8.6 Hz)	117.0	C-4‴	C-1‴
4‴		161.4		
7‴	7.68 (d, J = 15.9 Hz)	146.9		C-2‴/6‴, C-9‴
8‴	6.42 (d, J = 15.9 Hz)	115.5	C-9‴	C-1‴
9′		169.1		

The FAB mass spectrum of **3** showed a quasi-molecular ion at m/z 575 $[M+1]^+$ solving for $C_{36}H_{62}O_5$. A pair of major fragments at m/z 557 and m/z 556 indicated the facile loss of the elements of water and an intense fragment at m/z 194 (**8**) due to the loss of the side chain from the m/z 556 ion. The ^{1}H-NMR spectrum (Table 3) showed many similarities to **2** revealing *meta*-coupling aromatic protons signals for H-6 and H-8 and a long alkyl chain. However, the resonance for H-3 of **2** was missing and replaced by signals for a nonequivalent methylene centred at δ 3.20 (J = 16.8 Hz). The ^{13}C-NMR spectrum (Table 3) showed a triplet at δ 46.6 for the C-3 methylene and the resonance for C-2 was observed as a quaternary carbon resonating at 104.2. This can be attributed to a doubly oxygenated sp^3 carbon and the presence of a nonaromatic hydroxyl bonded to this carbon was supported by a resonance at δ 3.63 in the ^{1}H-NMR spectrum.

Table 5
NMR data for compound **5** (400 MHz, δ values in DMSO-d_6)

Position	^{1}H	^{13}C	^2J	^3J
2		156.5		
3		132.8		
4		178.0		
4a		104.1		
5	12.13 (s, OH)	152.7	C-5	C-4a, C-6
6	6.38 (s)	95.3	C-5, C-7	C-4a, C-8
7		153.7		
8	9.99 (s, OH)	126.0		
8a		143.7		
1′		121.3		
2′	7.64 (d, J = 2.2 Hz)		C-3′	C-2, C-4′, C-6′
3′	9.25 (s, OH)	144.8		
4′	9.70 (s, OH)	148.6		
5′	6.83 (d, J = 8.6Hz)	115.1	C-6′	C-1′, C-3′
6′	7.62 (dd, J = 2.2, 7.3 Hz)	121.8		C-2, C-2′, C-4′
7-OMe	3.80 (s)	56.2		C-7
1″	5.52 (d, J = 7.4 Hz)	100.6		
2″	3.35	74.0		
3″	3.29	76.4		
4″	3.20	70.0		
5″	3.39	74.4		
6″	4.02 (dd, J = 6.9, 12.0 Hz) 4.26 (dd, J = 1.8, 11.8 Hz)	63.1		C-9‴
1‴		125.0		
2‴/6‴	7.34 (d, J = 8.6 Hz)	130.1		
3‴/5‴	6.77 (d, J = 8.6 Hz)	115.7	C-4‴	C-1‴
4‴	8.75 (s, OH)	159.7		
7‴	7.28 (d, J = 15.9 Hz)	144.5		C-2‴/6‴, C-9‴
8‴	6.10 (d, J = 15.9 Hz)	113.7	C-9‴	C-1‴
9‴		166.1		

Table 6
NMR data for compound **6** (400 MHz, δ values in DMSO-d_6)

Position	^{1}H	^{13}C	^2J	^3J
2		156.4		
3		133.1		
4		178.0		
4a		104.3		
5	12.16 (s, OH)	152.7	C-5	C-4a, C-6
6	6.55 (s)	95.4	C-5, C-7	C-4a, C-8
7		153.8		
8		126.0		
8a		143.7		
7-OMe	3.90 (s)	56.4		C-7
1′		121.4		
2′	7.68 (d, J = 2.2 Hz)	116.4	C-1′, C-3′	C-4′
3′		144.8		
4′		148.6		
5′	6.84 (d, J = 8.7 Hz)	115.1	C-6	C-1′, C-3′
6′	7.66 (dd, J = 2.2, 7.6 Hz)	121.9		C-2, C-2′, C-4′
1″	5.45 (d, J = 7.6 Hz)	100.9		C-3
2″	3.24 (bt, J = 7.3 Hz)	74.1	C-3″	
3″	3.26 (bt, J = 7.3 Hz)	77.5	C-2″, C-4″	
4″	3.04 (m)	69.9	C-3″	
5″	3.33 (m)	76.5		C-3″
6″	4.02 (dd, J = 2.4, 11.9 Hz) 3.58 (dd, J = 5.28, 11.9 Hz)	61.0		

The HMBC spectrum (Table 3) showed the H-3 methylene protons to couple with the C-4 carbonyl and C-2. Another methylene resonance (at δ 2.20) also showed correlation to (C-2) and to 46.6 (C-3), which confirmed the attachment of the side chain at C-2. Compound **3** is thus identified as 2ξ,5,7-trihydroxy-2-heptacosanyl-2,3-dihydrobenzopyran-4-one.

Compound **4** was visualised on TLC as a bluish–white fluorescent spot under UV light (366 nm). The UV, IR and ^{1}H-NMR data suggested that it was a coumarin (Murray, Mendez & Brown, 1982). The FAB mass spectrum showed a quasi-molecular ion $[M+1]^+$ at 633 suggesting $C_{30}H_{32}O_{15}$. The ^{1}H-NMR spectrum (Table 4) displayed, in addition to the signals for H-3 and H-4 of a coumarin, two aromatic singlets attributable to H-5 and H-8, signals for the protons of two sugar hexose units, identified as glucose and rhamnose, and a *para*-coumaric acid esterifying group. The ^{13}C-NMR spectrum (Table 4) confirmed the presence of the two hexose units sugars and *p*-coumaroyl moiety. The anomeric proton of the glucose unit showed a ^3J correlation to a carbon of the coumarin (either C-7 or C-6) and the phase sensitive NOESY experiment showed the correlation between the same anomeric proton and H-8, so indicating attachment of the glycoside group at position C-7.

Compound **4** was recognised as being similar to isobaisseoside-4′-*p*-coumarate (**9**) reported from *Eriostemon cymbiformis* (Sarker, Waterman & Armstrong, 1995) but the NMR spectra differed significantly in the resonances for the glucose unit. Attachment of the *p*-coumaroyl ester at C-3 of the glucose was confirmed from a ^3J interaction between H-3 of the glucose (δ 5.22) and the *p*-coumaroyl carbonyl carbon (δ 169.1) in the HMBC spectrum (Table 4). The rhamnose anomeric proton showed correlation to the methylene carbon of glucose, indicating 1–6 linkage between rhamnose and glucose to form the rutinose skeleton. This coumarin can, on these findings, be unambiguously identified as isobaisseoside-3-*p*-coumarate (esculetin-7-(3-*para*-coumaroyl-6-α-rhamnopyranosyl)-β-glucopyranoside, **4**).

The FAB mass spectrum of **5** showed m/z 641 $[M+1]^+$, suggesting the molecular formula $C_{31}H_{28}O_{15}$ with a base peak m/z 333 due to the loss of hexose and *p*-coumaric acid moieties. The ^{1}H-NMR spectrum (Table 5), confirmed the presence of glucose and *p*-coumarate and showed other signals suggestive of a flavonol mono-methyl ether with 3′,4′-substitution in the B-ring and a single A-ring proton at C-6 or C-8. Analysis of the UV spectrum utilising shift reagents (Mabry, Markham & Thomas, 1970) indicated the pre-

sence of hydroxyl groups at C-5, C-3′ and C-4′. These data suggested that **5** was a hexahydroxyflavone oxygenated at 3, 5, 7, 3′, 4′ and either 6 or 8. The ^{13}C-NMR spectrum (Table 5) showed a methoxyl carbon resonating at δ 56.2, requiring at least one adjacent unsubstituted position (Panichpol & Waterman, 1978), which can only be C-7. The phase sensitive NOESY experiment confirmed this by showing an interaction between the methoxyl group and the A-ring proton.

The position of substituents were confirmed by long-range heteronuclear correlation studies using HMBC experiment and through phase sensitive NOESY. In the HMBC spectrum (Table 5), the H-bonded 5-hydroxyl proton showed correlations with C-4a, C-5 and C-6, the latter being a methine at δ 95.3, so requiring C-6 to be unsubstituted and placing the final A-ring hydroxy substituent at C-8. The anomeric proton of glucose (δ 5.52) showed a correlation with C-3 of the flavonol, so placing the glycoside, and similarly the methylene (C-6) protons of the glucose correlated with the *p*-coumaric acid carbonyl, so establishing esterification at C-6 of the hexose. This flavonol is thus identified as 3,5,7,8,3′,4′-hexahydroxyflavone 3-*O*-(6-*p*-coumaroyl-)-β-glucopyranoside-7-methyl ether, (**5**).

A second flavonol gave a quasi-molecular ion at *m*/*z* 455 by FAB mass spectrum, suggesting $C_{22}H_{22}O_{13}$, with a base peak at *m*/*z* 332 due to loss of a hexose. The UV shift analysis was comparable to that of **5** and the NMR spectra (Table 6) were identical to **5** except for the absence of the *p*-coumaric acid group. An unambiguous assignment of all the protons and carbons was achieved using COSY, NOESY and HMBC and identified the flavonoid as 3,5,7,8,3′,4′-hexahydroxyflavone-3-*O*-β-glucopyranoside-7-methyl ether (**6**).

The presence of the coumarin **4** is of systematic interest. *E. rhomboideus* is placed by Wilson (1970; 1982) in the section Nigrostipulae of *Eriostemon*. Many species of this section produce pyranocoumarins but there is now emerging a second group of taxa, including *E. cymbiformis* and *E. wonganensis*, and now also *E. rhomboideus*, which produce simple coumarin glycosides and not pyranocoumarins.

3. Experimental

3.1. General experimental procedures

Mps: Uncorr. EIMS at 70 eV. FABMS spectra were obtained with a nitrobenzoyl alcohol or glycerol matrix. NMR spectra were recorded on a Bruker AMX-400 instrument, with chemical shift data reported in ppm relative to the solvent used. Vacuum liquid chromatography (VLC) and column chromatography (CC) were performed on Merck (7736) silica gel 60H (0.04–0.05 mm) and Merck (7734) silica gel (0.063–0.2 mm), respectively. Analytical TLC and PTLC were performed on precoated Merck F_{254} silica gel plates and visualised by spraying with anisaldehyde-H_2SO_4. Gel filtration chromatography (GFC) was performed on Sephadex LH-20 (0.25–1 mm).

3.2. Plant materials

Aerial parts of *Eriostemon rhomboideus* were collected from shrublands in the Lake King area, some 250 km ENE of Albany, southwest Western Australia, in September 1991. A voucher specimen (PERTH 01656309) has been deposited at the Western Australian Herbarium in Perth.

3.3. Extraction and isolation

The dried, ground, plant material (362 g) was extracted in a Soxhlet separately and successively with petroleum ether (40–60°C), EtOAC and MeOH and the extracts were concentrated using a rotary evaporator at a maximum temperature of 40°C. The MeOH extract was subsequently partitioned between EtOAc and H_2O. The petroleum ether extract (11.18 g) was fractionated by VLC over silica gel eluting with solvents of increasing polarity. The fraction obtained with 18–20% EtOAc in *n*-hexane was subsequently subjected to GFC eluting with $CHCl_3$ and then $CHCl_3$:MeOH mixtures to give eriostoic acid (251 mg). The VLC eluate from 25 to 30% EtOAc in *n*-hexane yielded eriostemoic acid (265 mg) and the fraction from 45 to 75% EtOAc in *n*-hexane, after GLF and then CC gave **1** (14.9 mg).

The EtOAc extract (9.34 g) was fractionated by VLC and the eluate obtained from 20% EtOAc in *n*-hexane subjected to GFC, eluting with $CHCl_3$, to yield compounds **2** (15.9 mg) and **3** (82.1 mg). The VLC fraction eluted with 80–100% EtOAc in *n*-hexane and then GFC gave **4** (12.6 mg). The VLC fraction eluted with 3–4% MeOH in EtOAc was clarified by GFC and then further fractionated by CC to yield dacusterol (35.9 mg) and **5** (24.7 mg). The VLC fraction from 5 to 9% MeOH in EtOAc was subjected to GFC using 10% MeOH in $CHCl_3$ and then by CC eluting with EtOAc:MeOH to yield **6** (40.8 mg). Finally, the VLC eluate of 10–20% MeOH in EtOAc, after the same GFC and CC clean up gave hesperidin (255 mg).

VLC of the EtOAc soluble part of the MeOH extract (3.59 g) was performed as before. The fraction eluted with 90–100% EtOAc in $CHCl_3$ was clarified by GFC eluting with 5% MeOH in $CHCl_3$ to yield a mixture of catechin and epicatechin (34.3 mg).

3.4. Glyceryl-1-tetracosanoate (*1*)

Amorphous. Found M^+ 442.4039, $C_{27}H_{54}O_4$

requires 442.40221. IR ν_{max}: (KBr) cm^{-1}: 3432, 1709, 1472, 1463, 1432, 1410, 1300, 930, 729, 719. EIMS *m/z* (rel. int.): 442 $[M]^+$ (9), 411 (23), 383 (18), 368 (28), 351 (38), 323 (31), 154 (25), 112 (34), 98 (100). ^{1}H-NMR, ^{13}C-NMR ($CDCl_3$) — see Table 1.

3.5. 5,7-Dihydroxy-2-heptacosanylbenzopyran-4-one (**2**)

Amorphous. UV λ_{max} (EtOH) nm: 248, 293. IR ν_{max} (liquid film) cm^{-1}: 3539, 3368, 1659, 1633, 1593, 1472, 1432, 1390, 1367, 1302, 1172, 1158, 1011, 950, 835, 800, 764, 716, 644 and 631. FABMS: *m/z* 557 $[M+1]^+$, 205, 152. ^{1}H-NMR, ^{13}C-NMR — see Table 2.

3.6. 2ξ,5,7-Trihydroxy-2-heptacosanyl-2,3-dihydrobenzopyran-4-one (**3**)

Amorphous. λ_{max} (EtOH) nm: 287. IR ν_{max} (KBr) cm^{-1}: 3551, 3441, 1614, 1471, 1369, 1349, 1312, 1193, 1162, 1134, 1067, 1009, 960, 880, 848 and 718. FABMS *m/z* 575 $[M+1]^+$, 557, 556, 194. ^{1}H-NMR, ^{13}C-NMR — see Table 3.

3.7. Isobaisseoside-3″-p-coumarate (**4**)

Yellow amorphous. UV λ_{max} (MeOH) nm: 227, 296, 315 nm. IR ν_{max} (KBr) cm^{-1}: 3411, 1691, 1627, 1604, 1565, 1514, 1442, 1375, 1328, 1288, 1203, 1168, 1068, 982, 943, 832. FABMS: *m/z* 633 $[M+1]^+$. ^{1}H-NMR, ^{13}C-NMR — see Table 4.

3.8. 3,5,7,8,3′,4′-Hexahydroxyflavone-3-O-(6-p-comaroyl)-β-glucopyranoside)-7-methyl ether (**5**)

Yellow amorphous. UV λ_{max} (MeOH) nm: 281, 312; (+2M NaOH): 245,299, 363; (+5% $AlCl_3$), 223, 287, 315, 456; (+NaOAc) 281, 312; (+NaOAc-H_3BO_3) 281, 327. IR ν_{max} (KBr) cm^{-1}: 3412, 1680, 1660, 1629, 1605, 1562, 1514, 1442, 1393, 1350, 1275, 1259, 1196, 1168, 1061, 1009, 945, 893, 829. FABMS; *m/z* 641 $[M+1]^+$, 333 [M-*p*-coumaroylglucose]$^+$. ^{1}H-NMR, ^{13}C-NMR — see Table 5.

3.9. 3,5,7,8,3′,4′-Hexahydroxyflavone-3-O-β-glucopyranoside-7-methyl ether (**6**)

Yellow amorphous. UV λ_{max} (MeOH) nm: 279, 340; (+2M NaOH) 250, 293, 362; (+$AlCl_3$) 284, 450; (+NaOAc) 281; (+NaOAc+ H_3BO_3) 270, 387. IR ν_{max} (KBr) cm^{-1}: 3434, 1734, 1679, 1657, 1605, 1518, 1443, 1350, 1288, 1265, 1198, 1165, 1071, 1007, 945, 892, 816. FABMS; *m/z* 455 $[M+1]^+$, 332 [M-glucose]$^+$. ^{1}H-NMR, ^{13}C-NMR — see Table 6.

3.10. Daucosterol

Amorphous solid, UV, IR, ^{1}H-NMR, ^{13}C-NMR identical to authentic sample.

3.11. Hesperidin

Amorphous, UV, IR, ^{1}H-NMR, ^{13}C-NMR in agreement with literature values (Markham & Ternai, 1976).

3.12. Eriostemoic acid

Needles from *n*-hexane:EtOAc, m.p. 102–104°C. HREIMS *m/z* 344.1627 (calcd. 344.1624), UV, IR, NMR and MS data in agreement with literature values (Sarker et al., 1995).

3.13. Eriostoic acid

Needles from *n*-hexane EtOAc, m.p. 174–175°C. HREIMS *m/z* 344.1590 (calcd. 344.1624). UV, IR, NMR and EIMS data in agreement with literature values (Sarker et al., 1995).

3.14. Catechin and epicatechin

Amorphous, UV, IR, NMR and EIMS data in agreement with literature values (Markham & Ternai, 1976).

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