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FLAVAN-3-OLS AND DIHYDROPHENANTHROPYRANS FROM *PLEIONE BULBOCODIOIDES*

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Key Word Index—*Pleione bulbocodioides*; Orchidaceae; tubers; flavan-3-ol; dihydrophenanthrene; dihydrophenanthropyran; shanciols A–D.

Abstract—From tubers of *Pleione bulbocodioides*, two flavan-3-ols and two stilbenoids (shanciols A–D) were isolated, together with the known compounds, bletilols A and C. The new structures were elucidated to be 4′-hydroxy-3′,5′,7-trimethoxy-5-(3″-hydroxyphenethyl)flavan-3-ol, 4′-hydroxy-3′,7-dimethoxy-5-(3″-hydroxyphenethyl)flavan-3-ol, 4-hydroxy-11-methoxy-3-(4′-hydroxy-3′,5′-dimethoxyphenyl)-3,4,5,6-tetrahydro-2H-phenanthro[2,1-b] pyran-8-ol and 4-hydroxy-6-methoxy-3-(4′-hydroxy-3′,5′-dimethoxyphenyl)-3,4,10,11-tetrahydro-2H-phenanthro[2,3-b]pyran-8-ol, respectively, on the basis of spectroscopic data and chemical correlations.

INTRODUCTION

In our previous papers, the isolation and structural determination of some stilbenoids and lignans in *Pleione bulbocodioides* were described [1–4]. Further investigation of the same source has resulted in the isolation of two new flavan-3-ols shanciols A (**1**) and B (**2**), and two stilbenoids, shanciols C (**3**) and D(**4**), along with two known compounds, bletilols A (**5**) and C (**6**).

RESULTS AND DISCUSSION

Shanciol A (**1**) was obtained as colourless needles and the UV spectrum showed absorption maxima at 250 and 350 nm. The IR spectrum exhibited absorptions at 3300 (OH), 1610 and 1595 cm^{-1} (benzenoids). The mass spectrum exhibited a $[M]^+$ at *m/z* 452 ($C_{26}H_{28}O_7$), a base peak at *m/z* 257 and a prominent peak at *m/z* 196, which were completely in agreement with the rationality of a Retro-Diels–Alder fission, as shown in Scheme 1, suggesting **1** to be a flavan-3-ol. The ^{13}C NMR spectrum displayed signals for all 26 carbons in the molecule: one ethylene, one methylene, three methoxyls and two methines, along with 18 aromatic carbons, of which eight were protonated, four quaternary and six bearing oxygen. Based on HMBC and ^{13}C–^1H COSY experiments, all carbon signals of **1** were assigned as shown in Table 2. Acetylation of **1** afforded a triacetate ($[M]^+$ *m/z* 578) in whose ^1H NMR spectrum appeared two signals at δ 1.96 (3H) and 2.25 (6H), suggesting the presence of one secondary and two phenolic hydroxyl groups. In the ^1H NMR spectrum (Table 1), the presence of a flavan-3-ol moiety was confirmed by the appearance of the signals due to one methylene at δ 2.61 and 2.88, two bearing oxygen methines at δ 4.02 and 4.62, and four aromatic protons. Two of them appeared as a pair of doublets at δ 6.31 and 6.37 due to H-8 and H-6 in tetrasubstituted A ring and the remaining two protons appeared as one singlet at δ 6.70, which correlated with C-2 in the long-range HMBC spectrum, assignable to H-2′ and H-6′ suggested the symmetrically substituted B ring. Furthermore, the ^1H NMR spectrum of **1** revealed the presence of a phenethyl moiety as shown by the signals at δ 6.59, 6.63 and 7.05 assignable to H-2″, H-4″, H-6″ and H-5″ in 1″,3″-disubstituted D ring, together with the signals of one multiplet at δ 2.80 and four protons of an ethylene. This phenethyl moiety was concluded to be at C-5, because an enhancement of H-6 was observed on irradiation of the ethylene in a NOE experiment. All signal assignments and the location of the functional groups were performed by ^1H–^1H COSY and NOE experiments. Irradiation of the methoxyl group at δ 3.71 led to the enhancements of both H-6 (6%) and H-8 (10%). Irradiation of the other at δ 3.84 (6H) caused enhancement of the H-2′ and H-6′. These results indicated that three methoxyl and one hydroxyl groups were located at C-3′, C-5′, C-7 and C-4′ on

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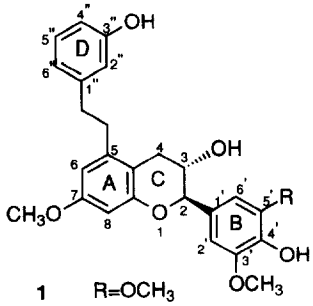
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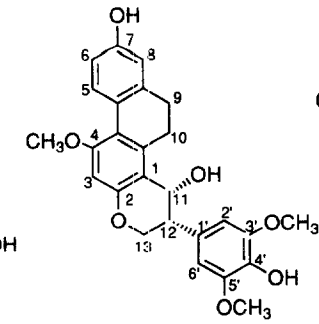
2 R=H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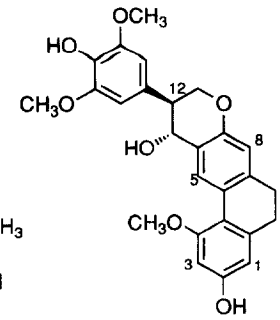
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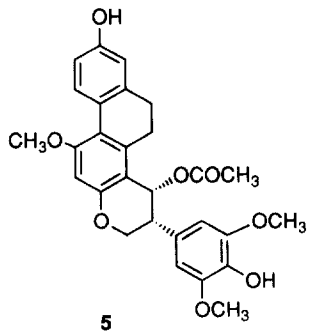
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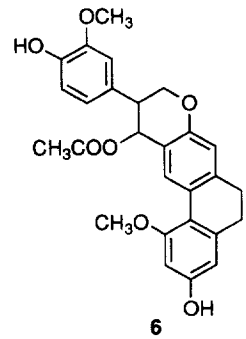
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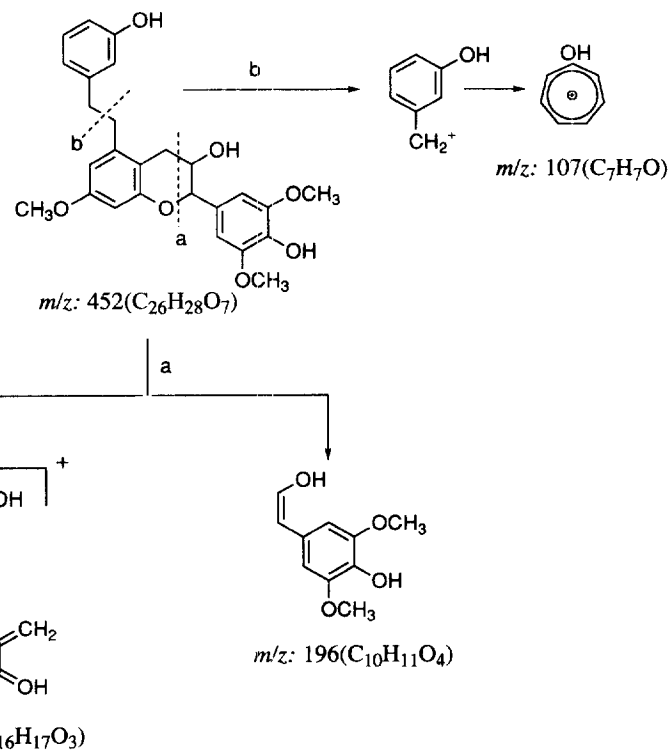
b
a
m/z: 452(C26H28O7)
m/z: 107(C7H7O)
m/z: 257(C16H17O3)
m/z: 196(C10H11O4)

Scheme 1.

Table 1. ^{1}H NMR data of shanciols A (**1**) and B (**2**), and their acetates*

	1	1 Acetate	2	2 Acetate
2	4.62 *d* (7.7)	5.07 *d* (6.0)	4.61 *d* (7.8)	5.06 *d* (6.6)
3	4.02 *m*	5.30 *m*	4.02 *ddd* (8.3, 7.8, 5.6)	5.33 *dt* (11.5, 6.6)
4	2.61 *dd* (15.8, 8.6)	2.65 *m*	2.62 *dd* (15.7, 8.3)	2.66 *dd* (16.2, 6.6)
	2.88 *dd* (15.8, 5.3)	2.65 *m*	2.89 *dd* (15.7, 5.6)	2.86 *m*
6	6.37 *d* (2.6)	6.39 *d* (3.0)	6.36 *d* (2.6)	6.42 *d* (2.6)
8	6.31 *d* (2.6)	6.38 *d* (3.0)	6.29 *d* (2.6)	6.41 *d* (2.6)
2′	6.70 *s*	6.69 *s*	6.97 *d* (1.9)	6.95 *d* (1.7)
5′	—	—	6.80 *d* (8.1)	6.91 *d* (8.3)
6′	6.70 *s*	6.69 *s*	6.85 *dd* (8.1, 1.9)	6.92 *dd* (8.3, 1.7)
2″	6.59†	6.85†	6.59†	6.99 *d* (3.0)
4″	6.59†	6.85†	6.59†	7.01 *d* (8.1)
5″	7.05 *t* (7.7)	7.18 *t* (7.7)	7.05 *t* (7.7, 7.3)	7.25†
6″	6.63 *d* (7.7)	6.91 *d* (7.3)	6.64 *d* (7.7)	7.01 *d* (8.1)
—CH_2—CH_2—	2.80 *m*	2.83 *m*	2.79 *m*	2.80 *m*
7-OMe	3.71 *s*	3.72 *s*	3.70 *s*	3.72 *s*
3′-OMe	3.84 *s*	3.75 *s*	3.84 *s*	3.80 *s*
5′-OMe	3.84 *s*	3.75 *s*	—	—
OCOMe	—	1.96 *s*, 2.25 *s*, 2.25 *s*	—	1.98 *s*, 2.29 *s*, 2.29 *s*

* Coupling constant (J in Hz) are given in parentheses.
† Unresolved.

Table 2. ^{13}C NMR data of shanciols A (**1**) and B (**2**)

	1	2
2	83.2	83.0
3	69.1	69.1
4	31.6	31.6
4a	112.5	112.5
5	143.3	143.3
6	109.5	109.4
7	160.4	160.4
8	100.5	100.5
8a	156.6	156.6
1′	136.7	132.1
2′	105.9	112.0
3′	149.3	149.0
4′	131.2	147.7
5′	149.3	116.5
6′	105.9	120.9
1″	144.6	144.6
2″	116.5	116.1
3″	158.5	158.5
4″	113.9	114.0
5″	130.4	130.4
6″	120.9	121.4
CH_2CH_2	35.9, 37.8	35.9, 37.8
OCH_2	55.7, 56.9	55.7, 56.5

flavan-3-ol, respectively and, hence, the remaining hydroxyl group was at C-3″ on phenethyl. The location of the 3″-hydroxyl group was also supported by the appearance of acetylation shifts of H-2″ and H-4″ in ^{1}H NMR spectrum of **1** acetate (Table 1) and the fragment peak at m/z 107 due to the hydroxytropylium in the mass spectrum (Scheme 1).

The relative stereochemistry of the C-2 and C-3 substituents was assumed to be *trans* from the coupling constant (J = 7.8 Hz) between H-2 and H-3 [5]. On the basis of above findings, the structure of **1** was decided to be 4′-hydroxy-3′,5′,7-trimethoxy-5-(3″-hydroxy-phenethyl)flavan-3-ol.

Shanciol B (**2**) showed UV maxima at 280 and 387 nm and the IR spectrum showed the presence of hydroxyl groups and benzenoids. The mass spectrum exhibited a $[M]^+$ at m/z 422 ($C_{25}H_{26}O_6$), 30 mu less than that of **1** but the same fragmentation patterns. Shanciol B also gave a triacetate by acetylation. The ^{1}H NMR spectrum of **2** (Table 1) showed that the signals of the phenethyl group depended on the same substitution patterns as those of **1**, while the signals of the B ring of flavan-3-ol moiety appeared as an ABX system at δ 6.80 (*d*, J = 8.1 Hz, H-5′), 6.85 (*dd*, J = 8.1, 1.9 Hz, H-6′) and 6.97 (*d*, J = 1.9 Hz, H-2′). Only two methoxyls at δ 3.70 and 3.84 were observed, indicating that **2** was a demethoxy derivative of **1**. This assumption was further supported by NOE enhancements. In these experiments with irradiation of the methoxyl group at δ 3.70, enhancements of H-6 (5%) and H-8 (8%) were observed. On irradiation of the other at δ 3.84, an expected NOE enhancement of H-2′ (11%) was observed, confirming the two methoxyl groups at C-3′ and C-7. Thus, the structure of **2** was established as 4′-hydroxy-3′,7-dimethoxy-5-(3″-hydroxyphenethyl)flavan-3-ol.

From the biosynthetic viewpoint, it is noteworthy that a flavan-3-ol with a phenethyl group was identified. It might play an important role in forming the

dihydrophenanthropyran shanciol [1], recently obtained from our laboratory and this is the first report of their isolation from a natural source.

Shanciol C (**3**) showed UV absorption maxima at 250, 281 and 320 sh nm, and IR absorptions at 3250 (OH), 1600 and 1500 cm^{-1} (benzenoids). The mass spectrum exhibited a $[M]^+$ at *m/z* 450 ($C_{26}H_{26}O_7$) and a significant peak at *m/z* 432 $[M-H_2O]^+$. The ^1H NMR spectrum showed that **3** had a dihydrophenanthropyran moiety, *viz.*, one multiplet at δ 2.59–2.71 (4H) due to H-9 and H-10, an ABX system at δ 6.61, 6.62, 8.00 due to H-8, H-6, H-5 and a singlet at δ 6.56 due to H-3, along with one methine at δ 5.64 due to H-11, one up-field shifted methine at δ 3.46 due to H-12 and one down-field shifted methylene at δ 3.59 and 3.86 due to H-13. Additionally, the ^1H NMR spectrum of **3** showed the presence of a 3′,4′,5′-trisubstituted phenyl group at δ 6.63 (2H) and three methoxyl groups at δ 3.79 (6H) and 3.85. The chemical shifts and splitting patterns of all signals were very similar to those of the known dihydrophenanthropyran, bletilol A (**5**) [6], except for the absence of a signal due to one acetyl group. Furthermore, acetylation of **3** afforded a triacetate ($[M]^+$ *m/z* 576), in whose ^1H NMR spectrum appeared three signals at δ 2.09, 2.30 and 2.31, suggesting the presence of one more hydroxyl than in **5**. It is presumed that the hydroxyl at C-11 is instead of the acetyl in **5**. This assumption was further supported by the results that the spectral data and the R_f values on TLC of **3** were consistent with those of deacetylate of **5**. Thus, shanciol C was established to be structure **3**.

Shanciol D (**4**) showed UV and IR spectra similar to those of **3**. Its spectrum exhibited the same $[M]^+$ at *m/z* 450 ($C_{26}H_{26}O_7$) and fragment peaks as those in **3**. Comparison between the ^1H and ^1H–^1H COSY spectra of **4** and **3** showed that the only difference between them was in the substitution pattern of the functional groups on dihydrophenanthrene, whose aromatic signals were observed as two singlets at δ 8.06 and 6.69 due to H-5 and H-8, and one pair of doublets at δ 6.31 and 6.41 due to H-1 and H-3. In a NOE experiment, irradiation of the methoxyl group at δ 3.82 caused enhancements of H-3 (10%) and H-5 (1%). Thus, the methoxyl group on the dihydrophenanthrene was confirmed at C-4. Furthermore, in the ^1H NMR of **4** acetate, the marked down-field shifts of H-1 (Δ 0.32) and H-3 (Δ 0.23) indicated that **4** has a hydroxyl group at C-2. That is to say, the pyran ring was formed by C-6 and O-7 of the dihydrophenanthrene instead of C-1 and O-2 as in **3**. From these observations, the structure of shanciol D was assigned to be **4**. It is noteworthy that shanciol C (**3**) and D (**4**) have different relative stereochemistry of H-11 and H-12, with *cis* in the former and *trans* in the latter.

In order to compare spectral data with other bibenzyls and phenanthrenes, we have used their numbering systems instead of systematic nomenclature. Thus shanciol C and D should be called 4-hydroxy-11-methoxy-3-(4′-hydroxy-3′,5′-dimethoxyphenyl)-3,4,5,6-tetrahydro-2H-phenanthro[2,1-b]pyran-8-ol and 4-hydroxy-6-methoxy-3-(4′-hydroxy-3′,5′-dimethoxyphenyl)-3,4,10,11-tetrahydro-2H-phenanthro[2,3-b]pyran-8-ol, respectively. The known compounds, bletilol A (**5**) and C (**6**) were identified by direct comparison with authentic samples [6].

EXPERIMENTAL

Mps: uncorr. IR: KBr. UV: MeOH. ^1H and ^{13}C NMR: 500 and 125 MHz, respectively, MeOH-d_4 with TMS. EIMS: 70 eV. CC and TLC: Merck silica gel.

Plant materials

See Ref. [1].

Extraction and isolation

See Ref. [1]. Fr. 5 chromatographed repeatedly over silica gel with $CHCl_3$–EtOAc, followed by LH-20 with MeOH–$CHCl_3$ to give **1** (2 mg) and **2** (10 mg). Fr. 6 was rechromatographed over silica gel, LH-20 and Cosmosil C_{18} (MeOH–H_2O) to give **3** (17 mg) and **4** (5 mg).

Compound **1**

Colourless needles from hexane–EtOAc, mp 146–148°. $[\alpha]_D$ 2.0 (MeOH, *c* 0.1). IR ν_{max} cm^{-1}: 3300, 1610, 1595. UV λ_{max} nm (log ε): 250 (4.04), 350 (4.09), 387 (3.54). ^1H NMR: Table 1. ^{13}C NMR: Table 2. MS *m/z* (rel. int.): 452 (81), 257 (100), 196 (50), 107 (14). *Triacetate*. Powder. ^1H NMR: Table 1. MS *m/z* (rel. int.): 578 $[M]^+$ (41), 536 (9), 494 (4), 476 (100), 434 (14).

Compound **2**

Powder. $[\alpha]_D$ 1.0 (MeOH, *c* 0.48). IR ν_{max} cm^{-1}: 3300, 1605, 1595. UV λ_{max} nm (log ε): 280 (4.06), 387 (3.32). ^1H NMR ($CDCl_3$): Table 1. ^{13}C NMR: Table 2. MS *m/z* (rel. int.): 422 (60), 257 (100), 166 (27), 107 (15). *Triacetate*. Powder. ^1H NMR ($CDCl_3$): Table 1. MS *m/z* (rel. int.): 548 $[M]^+$ (49), 488 (51), 446 (58), 429 (100).

Compound **3**

Oil. $[\alpha]_D$ −8.2 (MeOH, *c* 0.19). IR ν_{max} cm^{-1}: 3250, 1600, 1500. UV λ_{max} nm (log ε): 250 (4.21), 281 (4.39), 320 sh (4.11). ^1H NMR: δ 2.59–2.71 (4H, *m*, —CH_2—CH_2—, H-9, 10), 3.46 (1H, *ddd*, *J* = 9.0, 3.8, 3.2 Hz, H-12), 3.59 (1H, *dd*, *J* = 11.1, 9.0 Hz, H-13 ax), 3.79 (6H, *s*, 3′,5′-OMe), 3.85 (3H, *s*, 4-OMe), 3.86 (1H, *dd*, *J* = 11.1, 3.8 Hz, H-13 eq), 5.64 (1H, *d*, *J* = 3.2 Hz, H-11), 6.56 (1H, *s*, H-3), 6.61 (1H, *d*, *J* = 2.8 Hz, H-8), 6.62 (1H, *dd*, *J* = 9.2, 2.8 Hz, H-6), 6.63 (2H, *s*, H-2′, H-6′), 8.00 (1H, *d*, *J* = 9.2 Hz, H-5). ^{13}C NMR: δ 28.0 (C-9 or C-10), 30.9 (C-9 or C-

10), 54.9 (C-12), 56.3 (4-OMe), 56.9 (3′,5′-OMe), 65.0 (C-13), 88.8 (C-11), 93.9 (C-3), 103.9 (C-2′,6′), 113.8 (C-6), 115.1 (C-8), 116.8 (C-4a), 118.2 (C-1), 126.2 (C-4′), 130.2 (C-5), 135.0 (C-1′), 136.3 (C-1a), 137.7 (C-5a), 140.3 (C-8a), 149.5 (C-3′,5′), 156.3 (C-7), 159.5 (C-2), 160.6 (C-4). MS *m/z* (rel. int.): 450 (100), 432 (55), 158 (66). *Triacetate*. Powder. ^{1}H NMR ($CDCl_3$): δ 2.09 (3H, *s*, OAc), 2.30 (3H, *s*, OAc), 2.31 (3H, *s*, OAc), δ 2.71–2.74 (4H, *m*, —CH_2—CH_2—, H-9, 10), 3.73 (1H, *ddd*, J = 9.4, 4.3, 3.4 Hz, H-12), 3.78 (6H, *s*, 3′,5′-OMe), 3.88 (3H, *s*, 4-OMe), 4.14 (1H, *dd*, J = 11.1, 9.4 Hz, H-13 ax), 4.48 (1H, *dd*, J = 11.1, 4.3 Hz, H-13 eq), 5.60 (1H, *dd*, J = 3.4 Hz, H-11), 6.55 (1H, *s*, H-3), 6.58 (2H, *s*, H-2′, H-6′), 6.94 (1H, *m*, H-6), 6.96 (1H, *d*, J = 2.6 Hz, H-8), 8.20 (1H, *d*, J = 8.1 Hz, H-5). MS *m/z* (rel. int.): 576 $[M]^+$ (100), 534 (27), 492 (5), 474 (60), 432 (34).

Compound **4**

Oil. $[\alpha]_D$ 2.0 (MeOH, *c* 0.30). IR ν_{max} cm^{-1}: 3300, 1605, 1430. UV λ_{max} nm (log ε): 250 (4.18), 281 (4.36), 300 (4.30). ^{1}H NMR: δ 2.62–2.69 (4H, *m*, —CH_2—CH_2—, H-9, 10), 3.47 (1H, *m*, H-12), 3.77 (1H, *dd*, J = 10.9, 7.7 Hz, H-13 ax), 3.81 (6H, *s*, 3′,5′-OMe), 3.82 (3H, *s*, 4-OMe), 3.88 (1H, *dd*, J = 10.9, 5.3 Hz, H-13 eq), 5.50 (1H, *d*, J = 6.0 Hz, H-11), 6.31 (1H, *d*, J = 2.1 Hz, H-1), 6.41 (1H, *d*, J = 2.1 Hz, H-3), 6.68 (2H, *s*, H-2′,6′), 6.69 (1H, *s*, H-8), 8.06 (1H, *s*, H-5). ^{13}C NMR: δ 31.6 (C-9 or C-10), 31.9 (C-9 or C-10), 55.4 (C-12), 56.0 (4-OMe), 56.9 (3′,5′-OMe), 65.8 (C-13), 88.9 (C-11), 99.5 (C-3), 104.3 (C-2′,6′), 108.4 (C-1), 109.0 (C-8), 117.0 (C-1a), 125.5 (C-5), 125.9 (C-4a), 127.5 (C-4′), 134.7 (C-6), 136.5 (C-5a), 140.4 (C-1′), 142.0 (C-8a), 149.5 (*s*, C-3′,5′), 157.7 (C-7), 159.0 (C-2), 159.2 (C-4). MS *m/z* (rel. int.): 450 (16), 432 (100), 420 (27). *Triacetate*. Powder. ^{1}H NMR ($CDCl_3$): δ 2.07 (3H, *s*, OAc), 2.30 (3H, *s*, OAc), 2.32 (3H, *s*, OAc), δ 2.62–2.69 (4H, *m*, —CH_2—CH_2—, H-9, 10), 3.79 (1H, *m*, H-12), 3.81 (6H, *s*, 3′,5′-OMe), 3.87 (3H, *s*, 4-OMe), 4.37 (1H, *dd*, J = 11.1, 8.1 Hz, H-13 ax), 4.50 (1H, *dd*, J = 11.1, 5.6 Hz, H-13 eq), 5.46 (1H, *d*, J = 6.8 Hz, H-11), 6.63 (1H, *d*, J = 2.1 Hz, H-1), 6.64 (1H, *d*, J = 2.1 Hz, H-3), 6.66 (2H, *s*, H-2′, H-6′), 6.78 (1H, *s*, H-8), 8.11 (1H, *s*, H-5). MS *m/z* (rel. int.): 576 $[M]^+$ (100), 534 (14), 516 (14), 492 (4), 474 (61), 432 (53).

REFERENCES

1. Bai, L., Yamaki, M., Yamagata, Y. and Takagi, S., *Phytochemistry*, 1996, **41**, 625.
2. Bai, L., Yamaki, M. and Takagi, S., *Phytochemistry*, 1996, **42**, 853.
3. Bai, L., Yamaki, M. and Takagi, S., *Phytochemistry*, 1996, **44**, 341.
4. Bai, L., Yamaki, M. and Takagi, S., *Phytochemistry*, 1996, **44**, 1565.
5. Yamaguchi, S., Ito, S., Nakamura, A. and Inoue, N., *Bull. Chem. Soc. Jpn*, 1965, **38**, 2187.
6. Yamaki, M., Bai, L., Kato, T., Inoue, K. and Takagi, S., *Phytochemistry*, 1993, **32**, 427.