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CYTOTOXIC BIFLAVONOIDS FROM *SELAGINELLA WILLDENOWII*

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Key Word Index—*Selaginella willdenowii*; Selaginellaceae; pteridophyte; leaves; cytotoxic biflavones; 2″,3″-dihydroisocryptomerin; ^{13}C NMR.

Abstract—Bioactivity-guided fractionation of the leaves of *Selaginella willdenowii* afforded three known biflavones, 4′,7″-di-*O*-methylamentoflavone, isocryptomerin and 7″-*O*-methylrobustaflavone, that were significantly cytotoxic against a panel of human cancer cell lines. Non-cytotoxic isolates were also obtained, namely, amentoflavone, bilobetin, robustaflavone and 2″,3″-dihydroisocryptomerin, a new dihydrobiflavone. The structure for the new biflavonoid was unambiguously assigned by a combination of spectroscopic methods.

INTRODUCTION

Several *Selaginella* species are used in traditional medicine in various countries to treat a variety of diseases such as cancer [1], cardiovascular problems [1], diabetes [2], gastritis [3], hepatitis [4], skin diseases [5] and urinary tract infections [6]. Extracts from some *Selaginella* species have shown activity as anti-inflammatory [7], antimutagenic [8], antispasmodic [9], cytotoxic [10] and immunostimulant and RNA reverse-transcriptase inhibitory agents [11]. However, only a few studies on the bioactive components of species in this genus have been performed. A previous investigation on *S. doederleinii* demonstrated that its cytotoxic activity against L929 murine carcinoma cells was correlated to its lignan constituents [1], although several biflavones also isolated from this species were found to be inactive in this same assay. Thus, the folkloric use of many of these species to treat cancer may not be entirely explained by the presence of lignans, since such compounds have been isolated thus far from only the above-mentioned *Selaginella* species.

As part of our ongoing project on the discovery of natural anticancer agents, *Selaginella willdenowii*, a species chosen for study by analysis of the data contained in the NAPRALERT® computer system [12, 13], was subjected to bioactivity-guided fractionation after an ethyl acetate-soluble extract was found to exhibit significant cytotoxic activity for a colon cancer cell line (Col2). Among the active and inactive biflavonoids obtained was the novel compound 2″,3″-dihydroisocryptomerin (**1**), whose characterization is described herein. Unambiguous ^{13}C NMR data have been obtained for the first time for several of the *S. willdenowii* biflavone isolates.

RESULTS AND DISCUSSION

An ethyl acetate extract of *S. willdenowii* that exhibited an ED_{50} of 10.3 $\mu g\,ml^{-1}$ when tested against a human colon cancer cell line was fractionated by silica gel column chromatography. During this process, several fractions afforded insoluble yellow precipitates which, in addition to mother liquors and other fractions, were evaluated for cytotoxic activity. Four fractions [F14 (ED_{50} 4.3 $\mu g\,ml^{-1}$), F16 (ED_{50} 9.1 $\mu g\,ml^{-1}$), F36 (ED_{50} 5.0 $\mu g\,ml^{-1}$) and F43 (ED_{50} 4.6 $\mu g\,ml^{-1}$)] showed activity against the Col2 cell line, and were chosen for further fractionation. From F14, the known biflavone 4′,7″-di-*O*-methylamentoflavone (**2**, 20.7 mg) was obtained; the precipitate associated with F14 was found to be a mixture of the cytotoxic biflavones, 4′,7″-di-*O*-methylamentoflavone (**2**) and isocryptomerin (**3**) (2:1). These compounds were also purified from the precipitate from F16 which gave **2** (44.6 mg) and **3** (24.4 mg). Fractions F36 and F43 contained two unidentified hemolytic saponins that were not subjected to further investigation since further purification showed only marginal cytotoxic activity.

Since biflavones **2** and **3** displayed significant cytotoxicity (Table 1), the biflavonoids present in the remaining precipitates were purified and tested. Thus, the precipitate from F18 was filtered through Sephadex LH-20 using MeOH, and two pure compounds were obtained and identified as bilobetin (**4**, 4.7 mg) and 7″-*O*-

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Table 1. Cytotoxicity of compounds **1–8** and isocalycopterone with human cancer cell lines

Compound	Cell line*/ED_{50}† values ($\mu g\,ml^{-1}$)									
	BC1	HT-1080	Lu1	Col2	KB	KB-V +	KB-V −	LNCaP	ZR-75-1	U373
1	> 20	8.9	10.9	6.4	> 20	> 20	> 20	NT	12.2	> 20
2	5.7	11.8	12.0	2.5	> 20	7.0	> 20	> 20	16.2	3.8
3	1.5	0.6	0.9	1.8	1.6	1.5	2.1	2.1	0.58	3.5
4	10.4	4.8	19.9	9.1	> 20	> 20	> 20	NT	10.2	9.7
5	3.3	0.9	0.4	6.0	3.6	8.9	7.5	3.7	1.4	0.7
6	> 20	9.2	18.2	> 20	> 20	> 20	> 20	> 20	> 20	> 20
7	> 20	5.3	13.5	> 20	> 20	16.4	14.8	> 20	> 20	> 20
8‡	2.1	1.9	1.3	4.1	2.3	1.5	1.4	3.3	1.8	1.1
Calycopterone§	5.4	1.8	1.2	0.4	0.8	9.6	15.7	0.5	0.5	0.5

*Cytotoxicity assays were performed according to established protocols [26]. Key to human cancer cell lines: BC1, breast; HT-1080, fibrosarcoma; Lu1, lung; Col2, colon; KB, oral epidermoid carcinoma; KB-V +, drug-resistant KB assessed in the presence of vinblastine (1 $\mu g\,ml^{-1}$); KB-V −, drug-resistant KB assessed in the absence of vinblastine; LNCaP, hormone-dependent prostate; ZR-75-1, hormone-dependent breast; U373, glioblastoma.

†A value of $ED_{50} \leqslant 4.0\ \mu g\,ml^{-1}$ is considered to be of significant cytotoxicity [30].

‡Tetraacetate derivative of **3**.

§Included as a reference biflavonoid [29].

methylrobustaflavone (**5**, 32.8 mg). The precipitate from fraction F19 consisted solely of 7″-*O*-methylrobustaflavone (**5**), while the filtrates from fractions F23 and F24 afforded amentoflavone (**6**, 24.7 mg) and robustaflavone (**7**, 22.7 mg), respectively. The physical and spectral data for compounds **2–7** were in agreement the literature data, as follows: 4′,7″-di-*O*-methylamentoflavone (**2**) [14], isocryptomerin (**3**) [15, 16], bilobetin (**4**) [15, 17], 7″-*O*-methylrobustaflavone (**5**) [18], amentoflavone (**6**) [15, 16, 19, 20] and robustaflavone (**7**) [15, 16, 19, 20], respectively. Although ^{13}C NMR spectral data for **4** have been published, the assignments for many carbon atoms remained ambiguous [15]. Accordingly, by employing 1D HETCOR and selective INEPT NMR techniques, all of the ^{13}C NMR signals were unambiguously assigned for **4** (Table 2). In a similar manner, the ^{13}C NMR spectra of 4′,7″-di-*O*-methylamentoflavone (**2**) and 7″-*O*-methylrobustaflavone (**5**), which have not been previously reported, were assigned (Table 2). Previously unpublished and/or unassigned ^1H NMR data for compounds **2** and **3** were determined and reassigned in the present investigation.

The precipitate obtained from fraction F12 showed different TLC characteristics to the biflavones (**2–7**) present in the other fractions. After purification, the biflavonoid **1** (3.3 mg) was obtained. By CI-mass spectrometry, compound **1** showed a molecular ion at *m/z* 555 $[M + H]^+$, consistent, by high resolution-mass spectrometry, with a molecular formula of $C_{31}H_{22}O_{10}$. The ^1H NMR (DMSO-d_6) spectrum showed characteristic signals for a biflavonoid, with the signals at δ8.01 (2H, *d*, J = 8.6 Hz), 7.38 (2H, *d*, J = 8.6 Hz), 7.10 (2H, *d*, J = 8.6 Hz), and 6.82 (2H, *d*, J = 8.6 Hz) indicating the presence of two *para*-substituted B-rings, and two doublets at δ6.20 (H-6, J = 2.0 Hz) and 6.50 (H-8, J = 2.0 Hz) showing that one of the A-rings was substituted at C-5 and C-7. The other A-ring was additionally substituted at C-6″ or C-8″ and bore only one aromatic proton that appeared as a singlet (δ6.46). The remaining singlet corresponded to H-3 in one of the subunits (I or II), with an additional group of signals at δ5.59 (H-2‴, *dd*, J = 2.5 and 13.1 Hz) and 2.76 (H-3‴β, *dd*, J = 2.5 and 17.3 Hz) indicating that the C-2/C-3 double bond in one of the flavonyl units was saturated. However, the expected doublet of doublets for H-3‴α was found to be obscured by solvent peaks at *ca* δ3.21. A singlet at δ3.80 (3H) accounted for a methoxyl group, while all of the other substituents were found to be OH groups (δ12.9, 12.0, *ca* 10 *br* and 9.7). Based on the above data and on biogenetic considerations, **1** was hypothesized as being structurally related to isocryptomerin (**3**). The ^{13}C NMR spectrum of **1** showed signals characteristic of a flavanone unit at 197.7 (C-4″), 79.1 (C-2″) and 42.0 ppm (C-3″), and a OMe group carbon resonance at δ56.7 (Table 2). A set of carbon resonances was shown to be coincident with those for apigenin [15], and it was assigned to unit (I) of the dimer; hence unit (II) was assigned to the naringenin residue [15]. The interflavonyl linkage was determined as C-4′-O-C-6″ on the basis of the C-6″ chemical shift (δ122.5). It has been observed that the presence of a methoxyl group at C-7″ does not affect the chemical shift of C-6″, but shields C-8″ by *ca* − 2.6 ppm in the case of isocryptomerin (**3**) and hinokiflavone [15] (Table 2). If a calculated substituent effect of + 26 ppm on C-6″ (95.9 ppm) of naringenin is considered [15], a chemical shift of 121.9 ppm is obtained, which is close to the experimental value ($\Delta\delta$ = 0.6 ppm) observed for **1** (Table 2). On the other hand, if the residue were at C-8″ as in lanaroflavone [21], an isomer of hinokiflavone, a resonance at *ca* 118 ppm would be expected [15].

Table 2. ^{13}C NMR spectral data for biflavonoids **1**, **2**, **4** and **5***

C	1	2	4	5
2	163.1	163.3	163.3	163.7
2″	79.1	164.0	163.5	163.9
3	103.9	103.8	103.7	103.1
3″	42.0	103.1	102.6	102.8
4	181.8	181.7	181.7	181.6
4″	197.7	181.9	182.0	181.9
5	161.4	161.4	161.7	161.4
5″	157.8[a]	157.9	160.6	158.1
6	98.9	98.8	98.8	98.8
6″	122.5	90.8	98.5	109.3
7	164.3	164.1	161.4	164.1
7″	160.1	162.7	161.7	162.9
8	94.0	94.1	94.0	94.0
8″	92.5	103.5	103.6	90.6
9	157.3	157.3	157.4	157.3
9″	153.9	156.9	154.3	156.8
10	103.8	103.5	103.7	103.7
10″	102.6	104.6	103.6	104.6
1′	124.2	122.4	122.4	121.0
1‴	128.6	121.1	121.2	121.1
2′	128.4	130.2	130.9	130.7
2‴	128.5	128.6	128.2	128.5
3′	115.0	122.1	121.6	120.5
3‴	115.2	116.0	116.1	116.0
4′	160.8	160.5	160.4	159.4
4‴	160.2[a]	161.3	161.0	161.3
5′	115.0	111.7	111.7	116.1
5‴	115.2	116.0	115.8	116.0
6′	128.4	128.0	128.0	127.6
6‴	128.5	128.6	128.2	128.5
OMe	56.7	56.4–55.8	55.9	56.3

*In DMSO-d_6 at 90.8 MHz. Chemical shifts given in ppm using TMS as int. reference. Values bearing the same superscript may be interchanged.

Selective INEPT and 1D HETCOR NMR experiments [22–24] were performed in order to confirm the proposed structure and to unambiguously assign the ^1H and ^{13}C NMR spectra. By 1D HETCOR, irradiations of the singlet at δ6.87 and 6.46 produced enhancements at δ103.9 [C-3 (I)] and 92.5 [C-8″ (II)]. As a result of selective INEPT experiments, irradiation at C-8″ enhanced the signal at δ102.6, characteristic for C-10 of flavanones [25], and at δ160.1 ppm (C-7″). The latter signal was also enhanced when the singlet for the methoxyl group was irradiated, hence confirming the position of the methoxyl group at C-7″. In addition, C-8″ (δ92.5) exhibited an upfield shift of −2.1 ppm, as expected [15]; by irradiating the doublets at δ6.20 (H-6) and 6.50 (H-8) an enhancement at δ103.8 (C-10) was obtained, consequently confirming the structure of **1** as 2″,3″-dihydroisocryptomerin (Table 3).

All of the isolated biflavonoids were tested against a panel of human cancer cell lines according to established protocols [26] (Table 1). Although the new biflavonoid, 2″,3″-dihydroisocryptomerin (**1**), was inactive, the parent molecule, isocryptomerin (**3**), displayed significant activity against the HT-1080 and Lu1 cell lines (Table 1), demonstrating that the C-2″/C-3″ double bond is required to mediate cytotoxic activity in this compound class. The methoxyl group at C-7″ seems to have little relevance regarding cytotoxicity, since the ED_{50} reported for hinokiflavone in KB (ED_{50} 2.0 $\mu g\,ml^{-1}$) [27] is comparable to that found for **3**. The tetraacetyl derivative of **3**, (**8**) [28], was prepared and tested, and a general decrease in cytotoxic activity was observed compared with the parent substance. Among the remaining biflavone isolates, amentoflavone (**6**) was found to be inactive, consistent with previous assay data [27], and bilobetin (**4**) displayed only marginal activity against the HT-1080 cell line. However, the presence of two methoxyl groups, as in 4′,7″-di-*O*-methylamentoflavone (**2**),

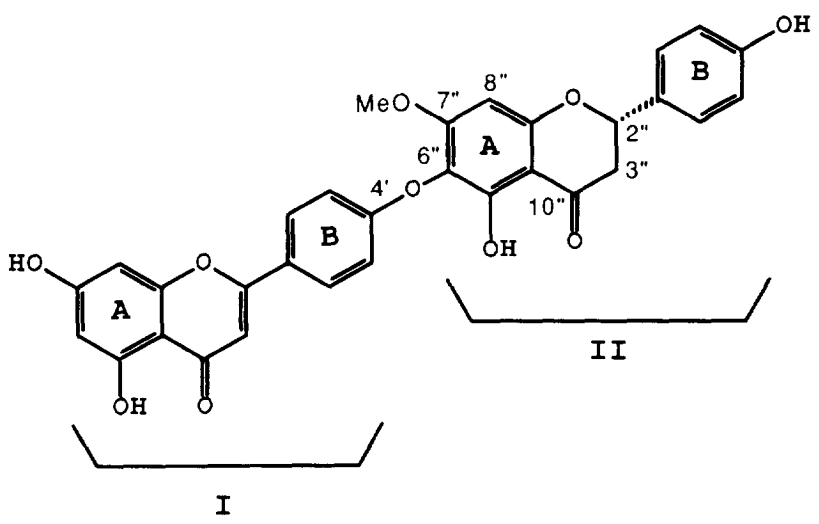

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Table 3. Selective INEPT* and 1D HETCOR experiments on 2″,3″-dihydroisocryptomerin (1)†

Proton irradiated	δ	Carbon enhanced (ppm)
OMe	3.80	C-7″ (160.1)
6	6.20	C-5 (161.4), C-10 (103.8)
6′‡	6.20	C-6 (98.9)
8	6.50	C-7 (160.2), C-10 (103.8), C-6″ (122.5), C-7″ (160.1), C-9″ (157.3), C-10″ (102.6)
8″	6.46	
3′, 5′‡	6.50	C-3′ and C-5′ (115.0)
3	6.87	C-2 (163.1), C-10 (103.8)
3‡	6.87	C-3 (103.9)
2‴,6‴‡	7.39	C-2‴ and C-6‴ (128.5)
2′,6′‡	8.01	C-2′ and C-6′ (128.4)

*J_{C-H} value considered for all of the irradiations was 8 Hz.
†Experiments run in DMSO-d_6 at 90.8 MHz.
‡Results for 1D HETCOR experiments.

was observed to enhance the cytotoxicity of the compound, as in the case of the Col2 and U373 cell lines. Although there was a tendency to increase the activity with increasing numbers of methoxyl groups in the molecule (Table 1), 7,7″-di-*O*-methylamentoflavone and 7,4′,7″,4‴-tetra-*O*-methylamentoflavone have been found by others to be inactive against L929 murine cells [1]. Only two compounds of the robustaflavone series were isolated and tested. Consistent with earlier literature [27], robustaflavone (**7**) showed a lack of significant cytotoxicity, but its monomethyl derivative, 7″-*O*-methylrobustaflavone (**5**) was found to be a potent cytotoxic agent against the HT-1080, Lu1 and U373 cell lines (Table 1). Its *in vitro* potency is comparable to that of calycopterone, a new type of biflavonoid recently isolated by Wall *et al.* [29].

Selaginella willdenowii was chosen for the present study by an analysis of the published literature using the NAPRALERT® database. The extensive use of species in this genus to treat a variety of diseases in traditional medicine in several countries, coupled with the fact that only limited literature information on the pharmacological activity of its constituents was available, had suggested this investigation. The present results indicate that a computer-based selection of plants for study may be an effective approach to natural product drug discovery.

EXPERIMENTAL

General. The ^1H and ^{13}C NMR spectra, selective INEPT and ID HETCOR experiments were performed in DMSO-d_6 with TMS as an int. standard, using a Nicolet 360 MHz spectrometer. The CIMS spectra were obtained on a Finnigan MAT-90 spectrometer. Pre-coated silica gel plates were used for analytical TLC using toluene–pyridine–acetic acid (10:1:1) and (20:1:1) as required.

Plant material. The leaves of *Selaginella willdenowii* (Desv. ex Poir) Baker were collected in Campana Province, Panama on October 11, 1992. The taxonomic identification was established by Prof. Mireya Correa, Director of the Herbarium of Panama, where a voucher specimen (FLORPAN 1239) is deposited.

Extraction and isolation. Powdered, dry leaves (1 kg) were extracted with MeOH (2 × 6 l) and the extracts combined, dried and resuspended in MeOH–H_2O (8:2). The methanolic aq. layer was defatted with hexane several times (total of 4 l) and then partitioned with EtOAc (4 l). The EtOAc extract (14.34 g, ED_{50} 10.3 μg ml^{-1} against the Col2 cancer cell line) was absorbed on silica gel 60 (100 g) and chromatographed on the same adsorbent (600 g) using $CHCl_3$, $CHCl_3$–MeOH mixts of increasing polarity (5% up to 50%), and MeOH as solvents, and collecting a total of 60 frs of 250 ml each. Frs 12–24 afforded ppts that were filtered, and their mother liquors combined into 6 frs according to their TLC profile; the remaining frs were combined in the same fashion. Samples of the mother liquors and subsequent frs were assessed for cytotoxic potential with cultured Col2 cells. Four frs were found to be active: the mother liquors of F14 (604.9 mg, ED_{50} 4.3 μg ml^{-1}) and F16 (512.4 mg, ED_{50} 9.1 μg ml^{-1}); and frs F36 (1.403 g, ED_{50} 5.0 μg ml^{-1}) and F43 (1.697 g, ED_{50} 4.6 μg ml^{-1}). The mother liquor from F14 was fractionated over Sephadex LH-20 using, in turn, MeOH, MeOH–Me_2CO (1:1) and $CHCl_3$; subfr. F39 gave a pure biflavonoid identified as 4′,7″-di-*O*-methylamentoflavone (**2**). The precipitate from F14 (276.3 mg, ED_{50} 1.9 μg ml^{-1}) was analysed by ^1H NMR and found to be a mixt. (2:1) of **2** and biflavonoid **3**. The ppt from F16 (102.7 mg) showed two spots by TLC, and was purified by Sephadex LH-20 using MeOH and $CHCl_3$–MeOH (1:1) as solvents; subfr. F4 contained biflavone **2** (44.6 mg) and F37 gave biflavone **3**, identified as isocryptomerin (**3**, 24.4 mg). A portion of **3** (16 mg) was acetylated and the product (**8**)

was purified with Sephadex LH-20. The biflavonoids present in the remaining ppts were purified with Sephadex LH-20 using MeOH as follows: F12 (25.1 mg, $ED_{50} > 20\ \mu g\ ml^{-1}$) showed a main spot by TLC, 2″,3″-dihydroisocryptomerin (**1**, 3.3 mg) was obtained after further purification; F18 afforded bilobetin (**4**, 4.7 mg) and 7″-*O*-methylrobustaflavone (**5**, 32.8 mg); F19 afforded **5** (61.8 mg); F23 ($ED_{50} > 20\ \mu g\ ml^{-1}$) was found to contain amentoflavone (**6**, 24.7 mg); and F24 after purification gave robustaflavone (**7**, 22.7 mg).

2″,3″-Dihydroisocryptomerin (**1**). Amorphous yellow powder, m.p. 213–215° dec.; $[\alpha]_D^{25}$ + 24° (DMSO; $c = 0.31$); CIMS *m/z*: 555 $[M + H]^+$; HRMS: 555.1284 $[M + H]^+$, calc. for $C_{31}H_{23}O_{10}$: 555.1291; ^{1}H NMR (DMSO-d_6): δ8.01 (2H, *d*, $J = 8.6$ Hz, H-2′ and H-6′), 7.38 (2H, *d*, $J = 8.6$ Hz, H-2‴ and H-6‴), 7.10 (2H, *d*, $J = 8.6$ Hz, H-3′ and H-5′), 6.82 (2H, *d*, $J = 8.6$ Hz, H-3‴ and H-5‴), 6.87 (1H, *s*, H-3), 6.50 (1H, *d*, $J = 2.0$ Hz, H-8), 6.46 (1H, *s*, H-8″), 6.20 (1H, *d*, $J = 2.0$ Hz, H-6), 5.59 (1H, *dd*, $J = 2.5$ and 13.1 Hz, H-2‴), 3.80 (3H, *s*, OMe), 2.76 (1H, *dd*, $J = 2.5$ and 17.3 Hz, H-3‴β), (the H-3‴ α signal was obscured by solvent peaks at *ca* δ3.21). The ^{13}C NMR data are presented in Table 2.

4′,7″-Di-O-methylamentoflavone (**2**). Amorphous yellow powder, m.p. 246–248°; $[\alpha]_D^{25}$ 0° (DMSO; $c = 0.81$); CIMS *m/z*: 567 $[M + H]^+$; ^{1}H NMR (DMSO-d_6): δ13.1, 13.0, 10.8 and 10.4 (1H each, *s*, OH-5″, OH-5, OH-7 and OH-7″, respectively, assigned by 1D HETCOR), 8.06 (1H, *dd*, $J = 2.2$ and 9.0 Hz, H-6′), 8.01 (2H, *d*, $J = 8.6$ Hz, H-2‴ and H-6‴), 7.86 (1H, *d*, $J = 2.2$ Hz, H-2′), 7.22 (1H, *d*, $J = 9.0$ Hz, H-5′), 6.95 (2H, *d*, $J = 8.6$ Hz, H-3‴ and H-5‴), 6.93 (1H, *s*, H-6″[a]), 6.90 (1H, *s*, *H*-3′[a]), 6.85 (1H, *s*, H-3″[a]), 6.48 (1H, *d*, $J = 1.2$ Hz, H-8), 6.20 (1H, *d*, $J = 1.2$ Hz, H-6), 3.81 and 3.80 (3H each, *s*, 2 OMe) (values bearing the same superscript may be interchanged); ^{13}C NMR data in Table 2.

Isocryptomerin (**3**). Amorphous yellow powder, m.p. > 300°; CIMS *m/z*: 553 $[M + H]^+$; ^{1}H NMR (DMSO-d_6): δ8.02 (2H, *d*, $J = 8.9$ Hz, H-2′ and H-6′) 8.02 (2H, *d*, $J = 9.2$ Hz, H-2‴ and H-6‴), 7.13 (1H, *s*, H-8″), 7.04 (2H, *d*, $J = 8.9$ Hz, H-3′ and H-5′), 6.97 (2H, *d*, $J = 9.2$ Hz, H-3‴ and H-5‴), 6.95 (1H, *s*, H-3 or H-3″), 6.87 (1H, *s*, H-3″ or H-3), 6.49 (1H, *d*, $J = 1.8$ Hz, H-8), 6.20 (1H, *d*, $J = 1.8$ Hz, H-6), 3.90 (3H, *s*, OMe); ^{13}C NMR data and assignments as in ref. [15].

Bilobetin (**4**). Amorphous yellow powder, m.p. 227–230°; $[\alpha]_D^{25}$ 0° (DMSO; $c = 3.1$); CIMS *m/z*: 553 $[M + H]^+$; ^{1}H and ^{13}C NMR (DMSO-d_6) data were consistent with those in the literature [15]; all of the ^{13}C NMR resonances were unambiguously assigned by selective INEPT and ID HETCOR NMR experiments (Table 2). The chemical shift assignment for C-2′ was reassigned based on 1D HETCOR experiments.

7″-O-Methylrobustaflavone (**5**). Amorphous yellow powder, m.p. > 300°; $[\alpha]_D^{25}$ 0° (DMSO; $c = 3.7$); CIMS *m/z*: 553 $[M + H]^+$; ^{1}H NMR (DMSO-d_6): as in ref. [18] except for: δ6.79 (1H, *s*, H-3), 6.89 (1H, *s*, H-3″) and 6.89 (1H, *s*, H-8″); ^{13}C NMR data in Table 2.

Amentoflavone (**6**). Amorphous dark yellow powder, m.p. 230–232°; $[\alpha]_D^{25}$ 0° (DMSO; $c = 2.3$); CIMS *m/z*: 539 $[M + H]^+$; ^{1}H NMR (DMSO-d_6): as in the literature [16, 19] except for 6.86 (1H, *s*, H-3 or H-3″); ^{13}C NMR data as in the literature [15, 20].

Robustaflavone (**7**). Amorphous yellow powder, m.p. 202–206° dec.; resolidifies at 220°; $[\alpha]_D^{25}$ 0° (DMSO; $c = 2.5$); CIMS *m/z*: 539 $[M + H]^+$; ^{1}H NMR and ^{13}C NMR (DMSO-d_6) data as in the literature [15, 16, 19, 20].

Tetraacetyl isocryptomerin (**8**). Compound **8** was prepared as described in the literature [28], whereby a sample of **3** (16 mg) was reacted overnight with pyridine (0.32 ml) and acetic anhydride (0.32 ml). A complex mixt. was obtained and **8** was purified by CC with Sephadex LH-20; ^{1}H NMR (DMSO-d_6): δ8.19 (2H, *d*, $J = 8.6$ Hz, H-2‴ and H-6‴), 8.03 (2H, *d*, $J = 8.6$ Hz, H-2′ and H-6′), 7.61 (2H, *d*, $J = 8.6$ Hz, H-3‴ and H-5‴), 7.39 (2H, *d*, $J = 8.6$ Hz, H-3′ and H-5′), 7.08 (2H, *s*, H-3 or H-3″, H-8″), 7.05 (1H, *s*, H-3″ or H-3), 6.91 (1H, $J = 2.0$ Hz, H-8), 6.85 (1H, *d*, $J = 2.0$ Hz, H-6), 3.93 (3H, *s*, OMe), 2.50 (3H, *s*, 5″-OAc), 2.33 (6H, *s*, 7 and 4‴-OAc) and 2.21 (3H, *s*, 5-OAc).

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