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A BIFLAVONOID FROM *DYSOXYLUM LENTICELLARE* GILLESPIE

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Key Word Index—*Dysoxylum lenticellare*; Meliaceae; leaves; biflavonoids.

Abstract—A novel biflavonoid, robustaflavone 4′,7″-dimethyl ether, has been isolated from the leaves of *Dysoxylum lenticellare*, in addition to two known compounds, isoginkgetin and bilobetin. Their structures were established by spectroscopic data and chemical modification.

INTRODUCTION

The genus *Dysoxylum* of the family Meliaceae is composed of about 60 species of trees native to Polynesia and Indo-Malaysia. Alkaloids [1–5] and terpenoids [6, 7] have been reported from *Dysoxylum lenticellare* Gillespie. In continuation of our studies of *D. lenticellare* we now report the structures of a novel biflavonoid, robustaflavone 4′,7″-dimethyl ether, together with the known compounds isoginkgetin and bilobetin from the hydroalcoholic extract of the leaves of this species, which is endemic to the Fiji Islands.

RESULTS AND DISCUSSION

Compound **1**, mp > 300°, was isolated as a yellow powder which gave a positive reaction for flavonoids with diphenylboric acid 2-aminoethyl ester. The high-resolution mass spectrum of 566.1221 for **1** gave a molecular formula of $C_{32}H_{22}O_{10}$ (calculated 566.1206) corresponding to a biflavonoid.

The UV spectrum of **1** in methanol exhibited maxima with band I at 331 nm and band II at 265 nm. Addition of sodium methoxide caused band I to shift to 382 nm, indicating the presence of a free 4′ or 4‴-hydroxyl group. Addition of sodium acetate caused a partial bathochromic shift of band II (about 6 nm) indicating the presence of a free 7 or 7″ hydroxyl group. The $AlCl_3$, $AlCl_3$/HCl, and $NaOAc/H_3BO_3$ spectral data established the absence of a 3′,4′-orthodihydroxyl system [8].

The ^1H NMR spectrum of **1** showed the presence of an AMX coupling system with signals at δ 7.83 (H-2′, *d*, $J = 2.5$ Hz), 8.06 (H-6′, *dd*, $J = 8.8$, 2.5 Hz), and 7.22 (H-5′, *d*, $J = 8.8$ Hz), indicating that C-3′ was the position where two flavonoid units linked together. Two meta-coupled protons of H-6 and H-8 at δ 6.18 and δ 6.47 (2H, *d* each, $J = 2.1$ Hz), and an AA′XX′ coupling system with the signals at δ 7.99 (H-2‴, H-6‴ *d*, $J = 8.9$ Hz) and δ 6.94 (H-3‴, 5‴, *d*, $J = 8.9$ Hz) excluded the possibility of the linkage between the two flavone moieties at C-6, C-8, C-3‴, C-5‴, C-2‴, and C-6‴. The proton signal appearing at δ 6.94 (1H, *s*) was assigned to be the H-8″ proton, which was proved by NOESY spectrum where a cross-peak was observed between H-8″ and 7″-OCH_3 at δ 3.80. The result suggested that another connecting position for the two flavones was at the C-6″. This assignment was supported from the ^{13}C NMR spectrum where the C-6″ signal was observed at δ 109.1 [9]. In the proton-coupled ^{13}C NMR spectrum of **1**, a broad singlet peak for C-6″ and a doublet peak for C-8″ ($^1J_{CH} = 165$ Hz) were observed, while C-6 and C-8 displayed broad doublets, which also suggested that C-6″ was substituted [10, 11]. To confirm the proposed linkage position of the two flavonoid units, a HMBC spectrum was measured. The observed long-range carbon proton couplings between H-2′ and C-6″, H-8″ and C7″, and also 5″-OH and C-6″ were further proof of the above suggestion (Fig. 1). These data indicated that **1** was a biflavonoid having a C-3′–C-6″ interflavonoid linkage corresponding to the robustaflavone series [9].

The ^{13}C NMR spectrum of **1** showed two methoxyl signals at δ 55.9 and δ 56.4. The two carbonyl signals near δ 182 in the ^{13}C NMR excluded the location of the methoxyl groups in either the positions 5 and 5″. If this was the case, the signals for C-4 and C-4″ would appear around δ 177 [12]; the two downfield proton signals at δ 12.94 and δ 13.12 could also be assigned as corresponding to the 5- and 5″-hydroxyl groups chelated with the 4- and 4″-carbonyl groups. Furthermore, it was determined that 7- and 4‴-hydroxyls were not likely the positions of methylation because the

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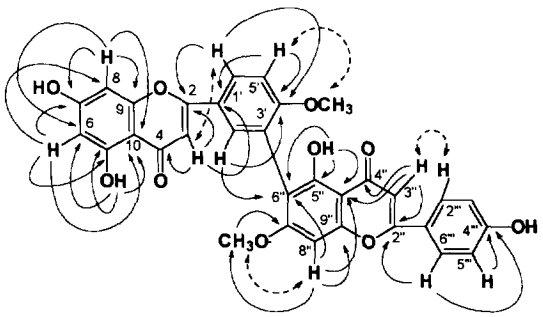

Fig. 1. HMBC correlations (solid arrows) and NOE correlations (dotted arrows) for compound **1**.

carbons of 6, 8, 2‴, 6‴ and 3‴, 5‴ showed identical values to those corresponding to robustaflavone in the ^{13}C NMR spectrum. Based on this information, the only positions available as the sites for methylation were those corresponding to carbons 4′ and 7″. This was further supported by a detailed comparison of ^{1}H and ^{13}C NMR data for **1** with those reported for robustaflavone.

When the ^{1}H NMR spectrum of **1** was compared with that of robustaflavone it was observed that **1** had 0.18 and 0.31 ppm downfield shifts for H-5′ and H-8″, respectively. In the ^{13}C NMR spectra, it was observed that, while all chemical shifts exhibited similar values for these two compounds, only C-5′ and C-8″ in **1** displayed 5.0 and 2.8 ppm upfield shifts, respectively when comparing with those of robustaflavone. In the NOESY spectrum, cross-peaks between H-8″ at δ 6.94 and 7″-OCH_3 at δ 3.80, as well as H-5′ at δ 7.22 and 4′-OCH_3 at δ 3.78, also support the above suggestion (Fig. 1). To confirm the deductions, an acetyl derivative of **1** was prepared. By comparing the ^{1}H NMR spectrum of **1** and its diacetyl derivative (**1a**), it was found that only H-6, H-8, H-3‴, and H-5‴ showed downfield shifts of 0.37, 0.37, 0.19, and 0.19 ppm, respectively. These data indicated that the 7 and 4‴ hydroxyls were acetylated.

The mass spectrum of **1** exhibited some characteristic flavonoid fragments which included the $[M]^+$ *m/z* 566 and ions such as 551 $[M - CH_3]^+$, 535 $[M - CH_3O]^+$, and 283 $[M]^{2+}$. The ion *m/z* 153 corresponded to the A-ring fragment obtained by RDA fragmentation. Fragments characteristic for the E-ring were observed at *m/z* 121 and *m/z* 118. An $[M - CH_3 - OCH_3]^+$ ion at *m/z* 520 was evidence for a molecule containing a methoxyl group ortho to the interflavonoid linkage, which was indicative of the presence of a 4′-*O*-methylation [13].

Based on the above deductions, **1** was elucidated as the novel robustaflavone 4′,7″-dimethyl ether. Assignment of the ^{13}C NMR spectrum of **1** was based on the HMBC experiment (Fig. 1). Two other compounds were identified as isoginkgetin and bilobetin, based on comparisons of their NMR data with those reported in the literature [9] and by co-chromatography with authentic samples.

EXPERIMENTAL

General. Mps uncorr.; NMR: DMSO 250 MHz for ^{1}H, 62.5 MHz for ^{13}C, and 125 MHz for proton-coupled ^{13}C, 500 HMz for NOE with mixing time of 0.4 sec, and 300 MHz for HMBC with average CH long-range coupling of 8 Hz. MS: 70 eV. See ref. [1] for the description of the plant material and extraction method.

Isolation of the compounds. A total of 5 g of the flavonoid-positive material obtained as described in the extraction procedure in ref. [1] was filtered from the hydroalcoholic layer and was subjected to repetitive CC using silica gel with CH_2Cl_2–MeOH 50:1. The frs were further purified on prep. TLC with CH_2Cl_2–MeOH (20:1) which led to the isolation of robusta-

Table 1. ^{1}H and ^{13}C NMR assignments and proton coupling constants for compound **1**

3	6.85, *s*	2	163.3 *s**	2″	164.0 *s*
6	6.18, *d*, 2.1	3	103.2† *d*	3″	103.6† *d*
8	6.47, *d*, 2.1	4	181.8 *s*	4″	182.0 *s*
2′	7.83, *d*, 2.5	5	161.4 *s*	5″	157.9 *s*
5′	7.22, *d*, 8.8	6	98.9 *d*	6″	109.1 *s*
6′	8.06, *dd*, 8.8, 2.5	7	164.2 *s*	7″	162.8 *s*
3″	6.89, *s*	8	94.1 *d*	8″	90.8 *d*
8″	6.94, *s*	9	156.9 *s*	9″	156.4 *s*
2‴, 6‴	7.99, *d*, 8.9	10	103.8 *s*	10″	104.6 *s*
3‴, 5‴	6.94, *d*, 8.9	1′	122.2 *s*	1‴	121.1 *s*
5-OH	12.94, *s*	2′	130.2 *d*	2‴	128.6 *d*
5″-OH	13.12, *s*	3′	122.5 *s*	3‴	116.0 *d*
7,4‴-OH	10.82, *s*, 10.41, *s*	4′	160.5 *s*	4‴	161.3 *s*
4′-OCH_3	3.78, *s*	5′	111.8 *d*	5‴	116.0 *d*
7″-OCH_3	3.80, *s*	6′	128.0 *d*	6‴	128.6 *d*
		OCH_3	55.9 *q*	OCH_3	56.4 *q*

*Multiplicity was determined by DEPT.

†Exchangeable assignments.

flavone 4′,7″-dimethyl ether (35 mg), isoginkgetin (17 mg), and bilobetin (27 mg).

Robustaflavone 4′,7″-dimethyl ether (**1**). Yellow powder, mp > 300°. UV λ_{max} (nm): MeOH: 265, 331; NaOMe: 271, 382; $AlCl_3$: 271, 295, 343, 370 (sh); $AlCl_3$/HCl: 273, 298, 344, 375 (sh); NaOAc: 272, 327; NaOAc/H_3BO_3: 269, 327. IR ν^{KBr} cm^{-1}: 3400–2600, 1670, 1604, 1491, 1350, 1241, 1178, 1161, 826. ^1H NMR and ^{13}C NMR: Table 1. EI-MS *m/z* (rel. int.): 566 $[M]^+$ (38), 535 (100), 520 (5), 283 (4), 268 (13), 153 (4), 121 (3), and 118 (3).

7,4‴-diacetyl-4′,7″-dimethyl-robustaflavone (**1a**), $C_{36}H_{26}O_{12}$ mp 246–247°. ^1H NMR ($CDCl_3$, δ ppm): 7.93 (1H, *dd*, *J* = 8.8, 2.4 Hz, H-6′), 7.80 (1H, *d*, *J* = 2.4 Hz, H-2′), 7.30 (1H, *d*, *J* = 8.8 Hz, H-5′), 7.96 (2H, *d*, *J* = 8.8 Hz, H-2‴, 6‴), 7.13 (2H, *d*, *J* = 8.8 Hz, H-3‴, 5‴), 6.55 (1H, *d*, *J* = 2.2 Hz, H-6), 6.84 (1H, *d*, *J* = 2.0 Hz, H-8), 6.65, 6.68, 6.70 (3H, *s* each, H-8″, 3, 3″), 12.86, 12.92 (2H, *s* each, 5, 5″-OH), 3.88 (6H, *s* each, 4′,7″-OCH_3), 2.32, 2.36 (6H *s* each, 7,4‴-OAc). EI-MS *m/z* (rel. int.): 650 $[M]^+$ (18), 619 (29), 608 (26), 577 (73), 566 (13), 535 (100), 520 (10), 297 (2), 283 (9), 268 (12), 153 (2), 121 (2), and 118 (2).

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