PERGAMON

Phytochemistry 50 (1999) 273–277

PHYTOCHEMISTRY

Flavanones from *Baeckea frutescens*

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Received 30 March 1998; revised 16 July 1998

Abstract

Three novel flavanones, named BF-4, BF-5 and BF-6, were isolated from the leaves of *Baeckea frutescens*. The structures of these flavanones were determined by their mass, ^{1}H and ^{13}C NMR spectral data. BF-4 and BF-5 showed strong cytotoxic activity (IC_{50} = 0.2–0.5 μg/ml) against leukemia cells (L1210) in tissue culture.

Keywords: *Baeckea frutescens*; Myrtaceae; Flavanone; Cytotoxicity

1. Introduction

In the previous paper (Fujimoto, Usui, Makino, & Sumatra, 1996), we reported the isolation and the structural elucidation of two new phloroglucinol derivatives (BF-1 and BF-2) together with a known compound baeckeol (Hems & Todd, 1940) (BF-3), from the leaves of *Baeckea frutescens* L. which have been used as a folk medicine for the alleviation of fever in southeast Asia (Jiangsu New Medical College, 1977). Further studies on the constituents of this plant led to the isolation of three new cytotoxic flavanones, named BF-4 (**1**), BF-5 (**2**) and BF-6 (**3**) from the leaves of *Baeckea frutescens* (Fig. 1).

2. Results and discussion

BF-4 (**1**) was obtained as crystals (MeOH), $[\alpha]_D$ 0°[1], mp 155–156°. Its high resolution MS spectrum indicated the molecular ion peak at *m/z* 488.2187 (M^+) corresponding to the molecular formula $C_{30}H_{32}O_6$. The ^{1}H NMR spectrum of **1** showed the presence of two secondary methyl groups (δ 0.68 and 0.72 (3H each, d, J = 6.8 Hz)), two tertiary methyl groups (δ 1.29 and 1.33 (3H each, s)), two vinyl methyl groups (δ 2.08 and 2.20 (3H each, s)), a methoxyl group (δ 3.85), five aromatic protons (δ 7.38–7.42 (5H, m)) and a hydrogen bonded hydroxyl group (δ 12.18 (1H, s)).

The ^{13}C NMR DEPT spectra exhibited the presence of twelve sp^3 carbon signals due to seven methyls, a methylene, three methines, a quaternary carbon and eighteen sp^2 carbon signals including two carbonyl carbon signals (δ 197.1 and 200.1) (Table 1). These spectral data and the ^{1}H–^{1}H and ^{13}C–^{1}H COSY spectra of **1** disclosed the presence of the partial structures A, B, C and D. In order to investigate the connectivities of the partial structures and the functional groups, we analyzed the HMBC spectrum of **1**. The geminal methyl signals at δ 1.29 and 1.33 in the partial structure A revealed long-range correlations with the carbon signals at δ 50.3 (C-14), 200.1 (C-15) and 168.6 (C-13) which showed cross-peaks with the methoxyl proton signal at δ 3.85 and the methyl proton signal at δ 2.08 (H-20) through C-12 (δ 110.1). The proton signal at δ 4.24 (H-9) in the partial structure B showed long-range couplings with carbon signals at δ 200.1 (C-15), 157.4 (C-8a), 108.9 (C-10), 104.3 (C-8), 156.8 (C-7) and 164.0 (C-11) which exhibited long-range correlation with the methyl proton signal at δ 2.08 (H-20). Thus, the partial structure A was extended to A-1.

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[1] BF-4, BF-5 and BF-6 were obtained as racemates. It is considered that the chiral centers at C-2 and C-9 are readily isomerized through flavanone–chalcone interconversion and the carbanion stabilized by resonance with the benzene ring and the double bond at C-10.

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PII: S0031-9422(98)00534-2

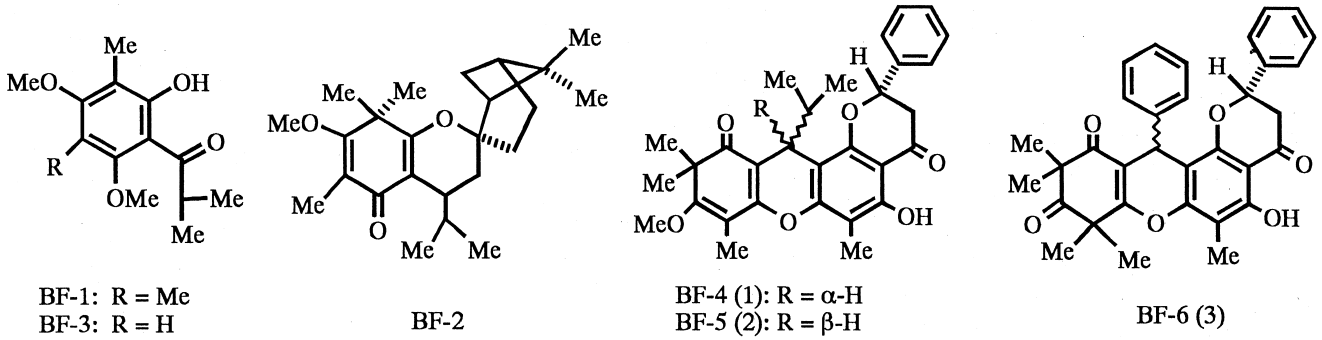

Fig. 1.

The carbon signal at δ 156.8 (C-7) was found to have a long-range correlation with the methyl proton signal at δ 2.20 (H-16) which showed cross peaks with the carbon signal at δ 106.1 (C-6) and 159.3 assignable to a hydroxyl-bearing carbon (C-5). The hydroxyl proton at δ 12.18 (C-5–OH) also showed cross peaks with the carbon signal at δ 105.2 (C-4a) as well as the C-5 and C-6 signals. The chemical shift values of C-7 (δ 156.8) and C-11 (δ 164.0) suggested the presence of an ether linkage between these two carbons. Thus, the partial

Table 1
^{1}H and ^{13}C NMR data for BF-4, BF-5 and BF-6 in $CDCl_3$

Position	^{1}H NMR (δ, 400 MHz)			^{13}C NMR (δ, 100 MHz)		
	BF-4	BF-5	BF-6	BF-4	BF-5	BF-6
2	5.42 dd (3.0, 13.0)	5.34 dd (2.9, 13.9)	5.44 dd (3.7, 12.5)	79.1	79.4	79.4
3α	3.08 dd (13.0, 17.0)	3.08 dd (13.9, 17.6)	2.93 dd (12.5, 17.6)	43.8	44.2	43.7
3β	2.90 dd (3.0, 17.0)	2.86 dd (2.9, 17.6)	2.80 dd (3.7, 17.6)	43.8	44.2	43.7
4				197.1	197.2	196.7
4a				105.2	105.1	105.2
5				159.3	159.1	159.5
6				106.1	105.8	105.6
7				156.8	157.06[a]	154.6
8				104.3	104.8	104.6
8a				157.4	157.11[a]	157.0
9	4.24 d (4.2)	4.19 d (3.7)	5.16 s	31.6	31.9	33.7
10				108.9	108.7	112.8
11				164.0	164.0	165.1
12				110.1	110.1	47.4
13				168.6	168.5	211.5
14				50.3	50.3	56.3
15				200.1	200.1	196.9
16	2.20 s	2.20 s	2.24 s	7.3	7.3	7.2
17	1.81 m	1.91 m	1.56 s H_3C	35.1	35.6	24.9
18	0.68 d (6.8)	0.77 d (6.6)	1.66 s H_3C C-12	19.0	19.39	25.1
19	0.72 d (6.8)	0.81 d (6.6)	1.11 s H_3C	19.4	19.44	24.3
20	2.08 s	2.08 s	1.31 s H_3C C-14	9.9	9.9	23.3
21	1.29 s	1.27 s		23.7	23.5	
22	1.33 s	1.32 s		25.0	25.0	
OCH_3	3.85	3.85		62.1	62.1	
1′				138.3	135.5	138.2
2′ and 6′	7.37–7.48 m		7.17–7.40 m	125.9	125.9	125.9
3′ and 5′	7.37–7.48 m		7.17–7.40 m	128.9	128.8	128.2[b]
4′	7.37–7.48 m		7.17–7.40 m	128.7	128.7	128.8[c]
OH	12.18 s		12.05			
1″						144.2
2″ and 6″			7.14–7.40 m			128.7[c]
3″ and 5″			7.14–7.40 m			128.3[b]
4″			7.14–7.40 m			126.6

[a–c]May be interchanged in each column.

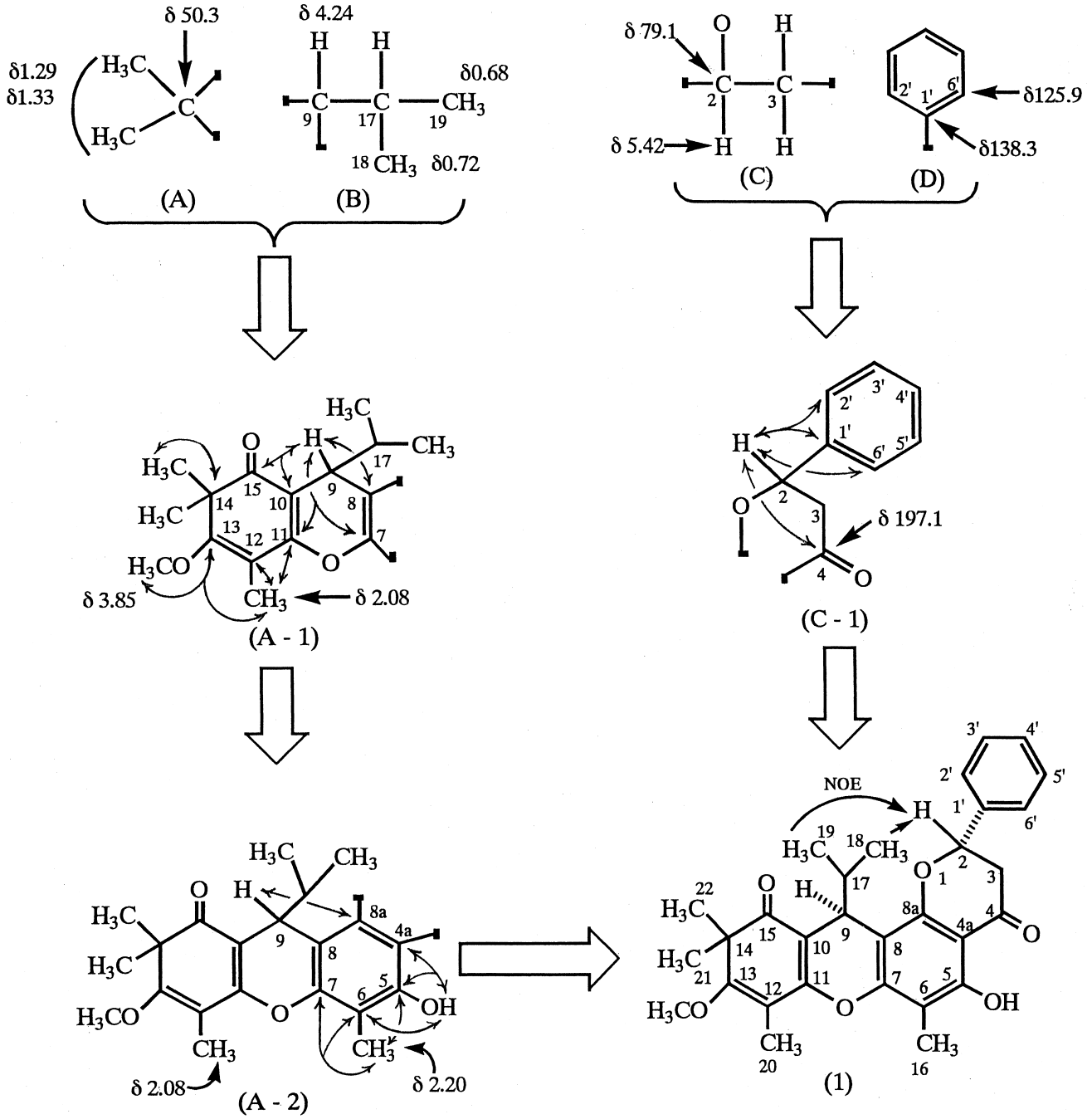

Fig. 2. Long range correlations (denoted by arrows) observed in the HMBC spectrum of **1**.

structure A-1 was developed to the partial structure A-2. The connectivities of the partial structures C and D were also determined by detection of the C–H long-range correlations in the HMBC spectrum. The proton signal at δ 5.42 (H-2) in the partial structure C showed cross peaks with the carbon signals at δ 138.3 (C-1′) and 125.9 (C-2′ and C-6′) in the benzene ring (D) and the carbon signal at δ 197.1 assignable to carbonyl carbon (C-4). Therefore, the partial structures C and D must be connected as shown in the partial structure (C-1). Consideration from these spectral data and the molecular formula, BF-4 should be a pentacyclic flavanone (**1**) consisting of partial structures (A-2) and (C-1). The stereochemical relationship of H-2 and the isopropyl group at C-9 was determined to be *syn* by NOE experiments. Irradiation of the methyl signals (δ 0.68 or 0.72) of the isopropyl group produced significant enhancement of the H-2 signal (δ 5.42). Thus, the

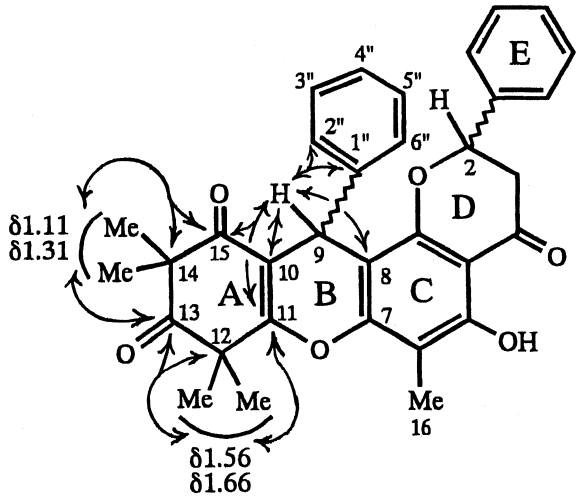

Fig. 3. Selected long range correlations observed in the HMBC spectrum.

structure of BF-4 was represented by the formula **1** shown in Fig. 2.

BF-5 (**2**) was obtained as crystals (MeOH), $[\alpha]_D$ 0°, mp 81–83°. Its high resolution CI–MS spectrum showed a $[M + H]^+$ ion peak at *m/z* 489.2287 corresponding to the molecular formula $C_{30}H_{32}O_6$. The ^1H and ^{13}C NMR spectral data of **2** showed close similarity with those of **1** (see Table 1) and its HMBC spectrum indicated that the structure of **2** was very similar to that of **1**. In NOE experiments on **2**, irradiation at δ 4.19 (H-9) caused significant enhancement of the H-2 signal (δ 5.34), while irradiation of methyl signals (H-18 or H-19) produced the enhancement of H-2′ and H-6′ signals. Therefore, BF-5 was the C-9 isomer of **1**.

BF-6 (**3**) was obtained as crystals (MeOH), $[\alpha]_D$ 0°, mp 89–93°. Its high resolution MS spectrum exhibited the molecular ion peak at *m/z* 522.2041 corresponding to molecular formula $C_{33}H_{30}O_6$. The ^1H and ^{13}C NMR spectra of **3** showed the presence of five tertiary methyl groups (δ 1.11, 1.31, 1.56, 1.66 and 2.24), two benzene rings (δ 7.15–7.3 (brm, 10 H)), a hydrogen bonded hydroxyl group (δ 12.05) and three carbonyl groups (δ 196.7, 196.9 and 211.5). Comparison of the ^1H and ^{13}C NMR spectra of **3** with those of **1** and **2** suggested that BF-6 was a flavanone derivative having the same B, C, D and E ring system as those of **1** and **2** (see Table 1). In the HMBC spectrum of **3**, H-9 signal (δ 5.16) exhibited cross peaks with the carbon signals at δ 112.8 (C-10), 165.1 (C-11), 196.9 (C-15) as well as δ 128.7 (C-2″ and C-6″) and 144.2 (C-1″) assignable to aromatic carbons from the ^{13}C–^1H COSY spectrum, thereby suggesting the presence of a benzene ring at C-9 and the carbonyl group at C-15. Furthermore, the presence of two geminal dimethyl groups at C-12 and C-14, and the carbonyl group at C-13 was confirmed by the detection of long-range correlations in the HMBC spectrum of **3**. As shown in Fig. 3, methyl proton signals at δ 1.56 and 1.66 exhibited cross peaks with carbon signals at δ 211.5 (C-13), 165.1 (C-11) and 47.4 (C-12), suggesting the presence of geminal dimethyl group at C-12. On the other hand, the two methyl proton signals at δ 1.11 and 1.31 showed long-range couplings with two carbonyl carbon signals at δ 211.5 (C-13), 196.9 (C-15) and a quaternary carbon signal at δ 56.3 (C-14), indicating the presence of geminal dimethyl group at C-14. Although the relative stereochemistry at C-2 and C-9 could not be elucidated because the NOE experiments on **3** did not give reliable information, the ^1H and ^{13}C NMR spectral data are explained satisfactorily by structure **3**. BF-4 and BF-5 exhibited strong cytotoxic activity (IC_{50} = 0.25 μg/ml) against leukemia cells (L1210) in tissue culture[2], while BF-6 showed relatively low cytotoxicity (IC_{50} = 10.0 μg/ml).

3. Experimental

The ^1H and ^{13}C NMR spectra were measured in $CDCl_3$ containing TMS as internal standard. The mass spectra were recorded on a Hitach RMU-6M. The leaves of *B. frutescens* L. were collected in Jakarta, Indonesia. The plant was identified by Dr. Suhardjono and a herbarium specimen has been deposited at the Botanical Garden, Bogor.

*3.1. Isolation of BF-4 (**1**), BF-5 (**2**) and BF-6 (**3**)*

The dried leaves (940 g) of the plant were extracted with EtOH under ultrasonication. After concentration of the EtOH solution, the crude extracts were chromatographed on HP-20 resin (Nippon Rensui), eluted successively with stepwise gradients of MeOH–H_2O (4:6, 7:3 and MeOH) and then acetone. An acetone fraction (14.9 g) was further fractionated on a silica gel column eluted successively with stepwise gradients of hexane–EtOAc (10:1, 5:1, 2:1), EtOAc and MeOH. The hexane–EtOAc (10:1) fraction was further separated by high performance liquid chromatography (HPLC) (Shodex SiL, 10 × 250 mm, hexane:EtOAc = 7:1, flow rate: 2.5 ml/min). The fractions with retention time 12–16 min were collected and purified by reverse phase HPLC (Shodex C_{18}-5E, 10 × 250 mm, 90%MeOH, flow rate: 3.0 ml/min) to give BF-4 (8 mg), BF-5 (6 mg) and BF-6 (3 mg).

[2] Leukemia cells (L 1210) were obtained from National Cancer Center Research Institute, 5-1-1 Tsukiji, Chyuo-ku, Tokyo 104, Japan.

Acknowledgements

We gratefully acknowledge financial support from The Suzuken Memorial Foundation.

References

Fujimoto, Y., Usui, S., Makino, M., & Sumatra, M. (1996). *Phytochemistry, 41,* 923.

Hems, B. A., Todd, A. R. (1940). *J. Chem. Soc.*, 1208.

Jiangsu New Medical College (1977). *Chinese drug dictionary* (p. 1121). Shanghai: Shanghai Science and Technology Co.