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PHLOROGLUCINOLS FROM *BAECKEA FRUTESCENS*

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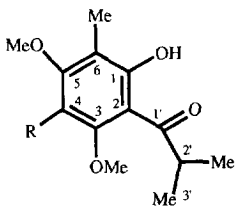
Key Word Index—*Baeckea frutescens*; Myrtaceae; phloroglucinols; cytotoxic activity.

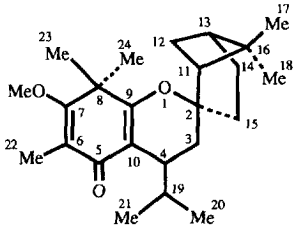
Abstract—Two new phloroglucinols, named BF-1 and BF-2, were isolated from the dried leaves of *Baeckea frutescens*. Their structures were determined by their ^1H and ^{13}C NMR and mass spectral data. BF-2 exhibited strong cytotoxic activity (IC_{50} = 5.0 $\mu g\,ml^{-1}$) against leukaemia cells (L 1210) in tissue culture.

INTRODUCTION

Baeckea frutescens L. is a medicinal plant which has been used as an anti-febrile in southeast Asia and China. Studies on the constituents of this plant were carried out in China and some sesquiterpenes have been isolated [1]. In our biological studies on the alcoholic extracts of the leaves of this plant, we found that the extracts exhibited strong cytotoxic activity against leukaemia cells (L 1210). In this paper, we describe the isolation and structural elucidation of two new cytotoxic phloroglucinol derivatives, BF-1 (**1**) and BF-2 (**2**), along with a known phloroglucinol, baeckeol (**3**) [2], from dried leaves of *B. frutescens*.



R
1 Me
3 H



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RESULTS AND DISCUSSION

BF-1 (**1**) was obtained as an oil. Compound **1** exhibited the molecular ion peak at *m/z* 252.1346 corresponding to $C_{14}H_{20}O_4$ and the base peak at *m/z* 209 $[M - C_3H_7]^+$. Its ^1H NMR spectrum (Table 1) indicated the presence of an isopropyl group [δ1.18 (6H, *d*, *J* = 6.6 Hz), δ3.84 (1H, *m*)], two methyl groups [δ2.13 and 2.16 (3H each, *s*)] on an aromatic ring, two methoxyl groups [δ3.70 and 3.74 (3H each, *s*)] and a hydrogen bonded hydroxyl group at δ12.58. The ^{13}C NMR spectrum of **1** (Table 2) disclosed the presence of fully substituted benzene ring (δ110.7, 115.3, 115.6, 158.4, 160.6 and 163.0) and a carbonyl group at δ212.1, which showed an IR absorption band at 1615 cm^{-1}, suggesting the formation of hydrogen bonding with the hydroxyl group. The substitution pattern on the benzene ring and the connection of the carbonyl group with the isopropyl group was elucidated as shown in Fig. 1 by analysis of the ^{13}C–^1H long range COSY spectrum of **1**. These spectral data are explained satisfactorily by the structure **1**.

Table 1. ^1H NMR spectral data for BF-1 (**1**) (400 MHz, $CDCl_3$)

H	δ (*J*, Hz)
MeO-3	3.70
Me-4	2.16
MeO-5	3.74
Me-6	2.13
HO–	12.58 *s*
2′	3.84 *m*
3′	1.18 *d* (6.6)

BF-2 (**2**), mp 131–133°, $[\alpha]_D$ + 166.5° ($CHCl_3$, *c* 0.1), exhibited the molecular ion peak at *m/z* 372.2683 corresponding to $C_{24}H_{36}O_3$. Its ^1H NMR spectrum (Table 3) showed the presence of four quaternary methyl groups [δ0.98 (3H), 1.28 (3H) and 1.3 (6H)], a vinyl methyl group [δ1.88], a methoxyl group [δ3.84] and an isopropyl group [δ0.55 (3H, *d*, *J* = 6.8 Hz), 0.92 (3H, *d*, *J* = 6.8 Hz) and 2.95 (1H, *m*)]. The ^{13}C NMR spectrum of **2** (Table 4) exhibited an oxygen-bearing quaternary carbon signal (δ83.2), four sp^2 carbon signals (δ112.1, 117.7, 169.8 and 171.7) and a conjugated carbonyl carbon signal (δ188.0).

These spectral data and the ^1H NMR decoupling experiments suggested the presence of the partial structure

Table 2. ^{13}C NMR spectral data for BF-1 (1) (100 MHz, $CDCl_3$)

C	δ
1	160.6
2	110.7
3	158.4
4	115.3
5	163.0
6	115.6
1′	212.1
2′	39.0
3′	19.6
MeO-3	62.3
Me-4	9.2
MeO-5	60.0
Me-6	8.7

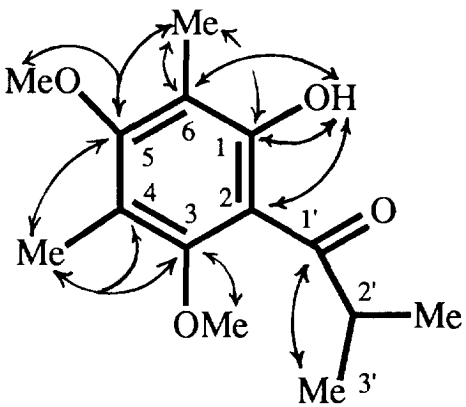

Fig. 1. Long range correlations (denoted by arrows) observed in the HMBC spectrum of 1.

Table 3. ^{1}H NMR spectral data for BF-2 (2) (400 MHz, $CDCl_3$)

H	δ (*J*, Hz)
3α	1.92 *d* (8.0, 16.0)
3β	1.33 *brd* (16.0)
4	2.74 *m*
11	2.02 *dd* (5.5, 5.8)
12α	1.64 *m*
12β	2.28 *m*
13	1.96 *m*
14	1.79–1.92 *m*
15α	1.67 *m*
15β	1.82 *m*
17	1.30
18	0.98
19	2.95 *m*
20	0.55 *d* (6.8)
21	0.92 *d* (6.8)
22	1.88
23	1.28
24	1.30
OMe	3.84

Table 4. ^{13}C NMR spectral data for BF-2 (2) (100 MHz, $CDCl_3$)

C	δ
2	83.2
3	33.9
4	32.2
5	188.0
6	117.7
7	171.7
8	42.9
9	169.8
10	112.1
11	53.1
12	27.0
13	40.6
14	25.0
15	26.4
16	38.2
17	27.6
18	23.4
19	32.2
20	20.5
21	15.4
22	9.7
23	23.8
24	23.9
OMe	61.7

(A) and the carbon sequence of C-11 ~ C-13. The sequence was developed to the partial structure (B) by detection of the long range correlations shown in Fig. 2.

The presence of the partial structure (C) was revealed from considerations of the ^{1}H and ^{13}C NMR data and the analysis of the HMBC spectrum of **2**. The chemical shift values of C-9 (δ169.8) and C-2 (δ83.2) suggested the presence of an ether linkage between C-9 and C-2. Further analyses of the HMBC spectrum enabled us to confirm the plane structure of BF-2. In the HMBC spectrum of **2**, the long range correlations of the proton signal at δ2.74 (H-4) with the carbon signals at δ112.1 (C-10) and 169.8 (C-9) and the proton signal at δ2.02 (H-11) with the carbon signal at δ33.9 (C-3) were observed, suggesting the connectivities of the C-4 to the C-10 and the C-2 to the C-3. Finally, the stereostructure of **2** was determined as shown in the formula from the results of the NOE experiments (Table 5). BF-1 and BF-2 showed cytotoxic activities [BF-1 (IC_{50} = 50 μg ml); BF-2 (IC_{50} = 5 μg ml^{-1})] against leukaemia cells in tissue culture. The details on the biological activities of these compounds will be published elsewhere.

EXPERIMENTAL

The ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ containing TMS as int. standard. The mass spectra were recorded on a Hitachi RMU-6M. The leaves of *B. frutescens* L. were collected in Jakarta, Indonesia. The plant was identified by Dr. M. Sundaru and a

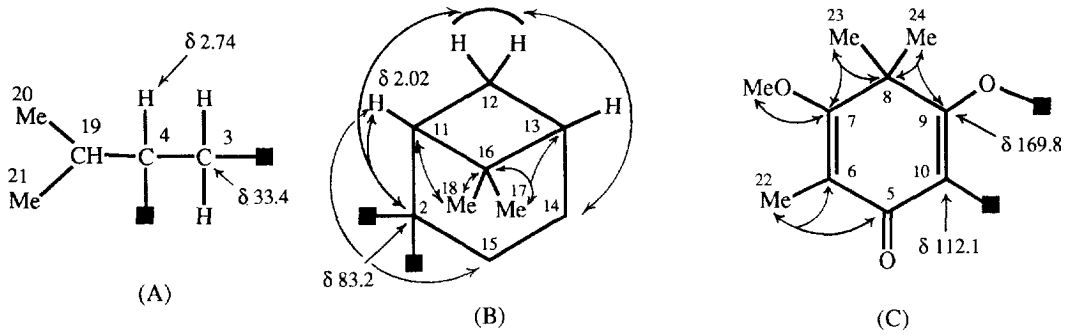

Fig. 2. Long range correlations (denoted by arrows) observed in the HMBC spectrum of **2**.

Table 5. Selected NOE difference spectral data for **2**

Saturation	Observed NOE
H-4	H-3α, H-19
H-11	H-3β, H-12β, H-17
H-17	H-11, H-12β, H-13, H-18
H-18	H-3α, H-14β, H-17

herbarium specimen has been deposited at the Botanical Garden, Bogor.

Isolation of BF-1 (**1**), *BF-2* (**2**) *and BF-3* (**3**). The dried leaves (500 g) of the plant were extracted with EtOH (11 × 3) under ultrasonication. After concn of the EtOH soln, the crude extracts were chromatographed on HP-20 resin (Nippon Rensui), eluted successively with 1.5 l each of 20% MeOH, 40% MeOH, 60% MeOH, 80% MeOH, MeOH and Me_2CO. The MeOH fr. (15.9 g) was subjected to CC on silica gel eluted successively with 1.5 l each of *n*-hexane–EtOAc (10:1), *n*-hexane–EtOAc (5:1), *n*-hexane–EtOAc (2:1), *n*-hexane–EtOAc (1:1) and EtOAc. The *n*-hexane–EtOAc (5:1) fr. was further purified by HPLC [Shodex SIL-SE, 10 × 250 mm, *n*-hexane–EtOAc (15:1); flow rate, 3 ml min^{-1}] to give 20 mg **1** (R_t, 8.0 min), 280 mg **2** (R_t, 13.5 min) and 650 mg **3** (R_t, 17.0 min).

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