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Cytotoxic constituents from the roots of *Tovomita brevistaminea*

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Abstract

Two known xanthones, trapezifolixanthone and manglexanthone were isolated as cytotoxic constituents from the $CHCl_3$ extract of the roots of *Tovomita brevistaminea* by bioassay-guided fractionation using the KB cell line. In addition, a new compound, tovophenone C, and two known compounds, tovophenones A and B which are benzophenones, were found to be inactive constituents in this investigation. The structure of the new isolate was determined by detailed analysis of spectroscopic parameters including its 1D and 2D NMR spectroscopy data.

Keywords: *Tovomita brevistaminea*; Guttiferae; Stems; Prenylated xanthones; Prenylated benzophenones; Tovophenone C; Cytotoxic activity

1. Introduction

As a part of an ongoing collaborative program to discover novel antineoplastic agents of plant origin, the roots of *Tovomita brevistaminea* Engl. (Guttiferae) were collected in Brazil and investigated further, since its chloroform-soluble extract exhibited significant cytotoxic activity against the KB cell line. Teas prepared from the flowers of other *Tovomita* species such as *T. brasiliensis* and *T. laurina* have been reported to be helpful in controlling diarrhea (Schultes, 1983). Although *T. brevistaminea* has not yet been investigated phytochemically, secondary metabolites such as betulinic acid (de Oliveira, Mesquita, de Lima, Gottlieb & Gottlieb, 1984; Filho, Miranda, Gottlieb & Magalhaes, 1982; Marta et al., 1976; Mesquita, de Oliveira, Neiva & Gottlieb, 1975), coumarins (Filho et al., 1982), various xanthones (de Oliveira et al., 1972; de Oliveira, Gottlieb & Mesquita, 1984; Filho et al., 1982; Gabriel & Gottlieb, 1972; Marta et al., 1976; Mesquita et al., 1975) and benzophenones (delle Monache, della Monache, Bettolo, Lyra & Lwande, 1984) were previously reported from several related *Tovomita* species.

In the present investigation, bioassay-guided fractionation utilizing the KB cell cytotoxicity led to the isolation of two known xanthones, trapezifolixanthone (**1**) (Somanathan & Sultanbawa, 1974) and manglexanthone (**2**) (Marta et al., 1976) as active constituents. In addition, a new compound, tovophenone C (**5**) and two known compounds, tovophenones A (**3**) and B (**4**) (delle Monache et al., 1984), which are benzophenones, were found to be inactive in the KB cell line. We report herein the isolation and structure elucidation of the new compound **5**. The detailed ^{1}H- and ^{13}C-NMR assignments for compounds **2**, **3** and **4**, and the ^{13}C-NMR assignments for **1** are reported for the first time.

2. Results and discussion

Compounds **1** ($C_{23}H_{22}O_5$), **2** ($C_{24}H_{24}O_6$), **3** ($C_{29}H_{36}O_5$) and **4** ($C_{29}H_{36}O_6$) were identified as the

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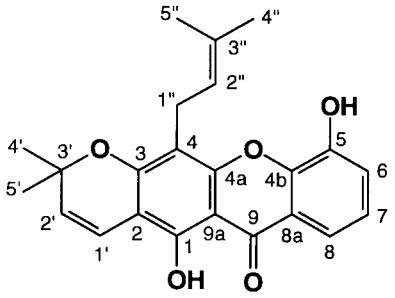
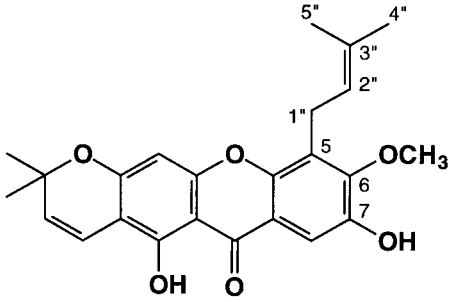
known compounds, trapezifolixanthone (Somanathan & Sultanbawa, 1974), manglexanthone (Marta et al., 1976), tovophenone A (delle Monache et al., 1984) and tovophenone B (delle Monache et al., 19), respectively, by comparison of their physical and spectral data with reported values. Their ^{13}C-NMR signals were unambiguously assigned using HMQC- and HMBC-NMR spectra.



1



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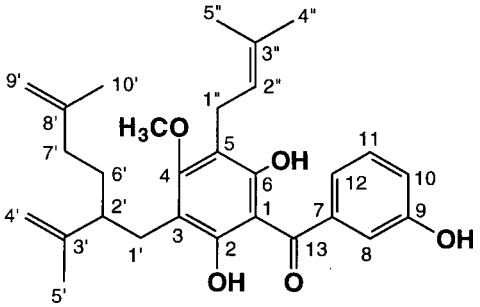

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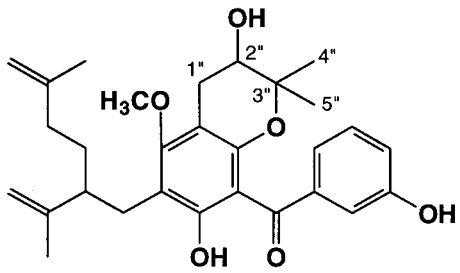

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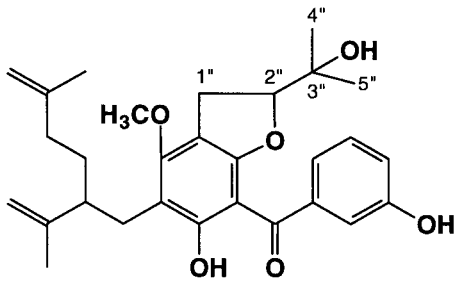

5

The elemental formula of compound **5** was deduced as $C_{29}H_{36}O_6$ by HREIMS which showed a molecular ion peak at *m/z* 480.2507. The IR spectrum showed an absorption band at 3595 cm^{-1} for one or more hydroxyl groups and 1626 cm^{-1} for a conjugated carbonyl functionality. A hydroxyl proton which was hydrogen-bonded with the carbonyl at C-13, appeared at δ_H 12.10 (1H, s) in the ^{1}H-NMR spectrum of **5**. The ^{13}C-NMR spectrum showed a signal for the carbonyl of C-13 at δ_C 198.5. A ^{1}H-NMR signal at δ_H 3.92 (3H, s) which was correlated to the ^{13}C-NMR resonance at δ_C 58.5 in the HMQC-NMR spectrum, was attributable to a methoxyl group affixed to a phenyl group (delle Monache et al., 1984; de Oliveira et al., 1984; Marta et al., 1976; Mesquita et al., 1975). ^{1}H-NMR signals for a disubstituted phenyl group appeared at δ_H 6.98 (1H, d, J = 2.0 Hz, H-8), 6.90 (1H, dd, J=7.0 and 2.0 Hz, H-10), 7.25 (1H, t, J = 7.7 Hz, H-11) and 7.06 (1H, d, J = 7.7 Hz, H-12) and showed one-bond connectivities to the ^{13}C-NMR

Table 1
NMR spectral data of compound **5**

Carbon	δ_H (int. Mult. *J* in Hz)	δ_C[a]	^1H–^1H COSY	^1H–^{13}C HMBC
1		102.3		
2		160.9		
3		112.1		
4		105.1		
5		161.5		
6		161.5		
7		142.9		
8	6.98 (1H, *d*, 2.0)	114.7		C-13, C-12
9		155.6		
10	6.90 (1H, *dd*, 7.7, 2.0)	117.9	H-11	C-8, C-12
11	7.25 (1H, *t*, 7.7)	129.0	H-10, H-12	C-7, C-9
12	7.06 (1H, *d*, 7.7)	123.6	H-11	C-13, C-8, C-10
13		198.5		
1′	2.63 (2H, *d*, 8.0)	27.8	H-2′	C-3, C-4, C-2′, C-3′, C-6′
2′	2.38 (1H, *m*)	46.7	H-1′, H-6′	C-1′, C-3′, C-4′, C-5′, C-6′
3′		148.0		
4′	4.72 (1H, *s*)	111.0		C-2′, C-5′
	4.57 (1H, *brs*)			
5′	1.72 (3H, *s*)	18.2		
6′	1.55 (2H, *dd*, 16.0, 8.0)	30.5	H-2′, H-7′	C-1′, C-2′, C-3′, C-7′, C-8′
7′	1.96 (1H, *dt*, 15.5, 8.0)	35.6	H-6′	C-6′, C-8′, C-9′, C-10′
	1.87 (1H, *dt*, 15.5, 8.0)			
8′		146.4		
9′	4.72 (1H, *brs*)	109.2		C-7′, C-10′
	4.67 (1H, *brs*)			
10′	1.70 (3H, *s*)	22.4		
1″	3.16 (2H, *d*, 9.3)	29.4		C-2″, C-3″, C-5
2″	4.36, 4.35 (1H, *t*, 9.3)	90.3		
3″		71.7		
4″	0.96 (3H, *s*)	25.5		C-2″, C-3″, C-5″
5″	1.09 (3H, *s*)	23.6		C-2″, C-3″, C-4″
OCH_3	3.92 (3H, *s*)	58.5		C-4
OH-2	12.10 (1H, *s*)			C-1, C-2, C-3

[a] Obtained from ^1H–^{13}C HMQC-NMR data.

signals at δ_C 114.7, 117.9, 129.0 and 123.6, respectively, in the HMQC-NMR spectrum. The H-8 and H-12 protons displayed a three-bond connectivity with the carbonyl carbon at δ_C 198.5 (C-13) in the HMBC spectrum, indicating a benzophenone skeleton, by analogy to the known compounds **3** and **4**. A side chain comprising two methyls, two exomethylenes, three aliphatic methylenes and one methine was identified by the ^1H–^1H COSY and ^1H–^{13}C HMBC-NMR spectra as shown in Table 1, as well as comparison with reported data (delle Monache et al., 1984). A dihydrofuran ring with a hydroxy–isopropyl group appeared at δ_H 3.16/δ_C 29.4 (C-1″), 4.36/90.3 (C-2″), 1.09/23.6 (C-5″), 0.96/25.5 (C-4″) and 71.7 (C-3″). The ^1H-NMR signal for H-1″ (δ_H 3.16) was correlated to the ^{13}C-NMR signals at C-2″, C-3″ and C-5 in the HMBC spectrum, affording further evidence for the presence of a dihydrofuran ring with a hydroxy–isopropyl group, and in addition indicating that this ring is fused to C-5 and C-6. The side chain was assigned at C-3 since H-1′ showed a two bond-connectivity with C-3 which was also correlated with OH-2 as a three-bonded carbon. The methoxyl group exhibited a three-bond connectivity with C-4; in turn, this carbon was correlated to H-1′ as a three-bond connectivity, showing a stronger evidence for the position of the side chain. Therefore, the structure of **5** was assigned as the new compound tovophenone C (**5**).

Xanthones are of frequent occurrence in the family Guttiferae (delle Monache et al., 1984; de Oliveira et al., 1972, 1984; Filho et al., 1982; Gabriel & Gottlieb, 1972; Somanathan and Sultanbawa, 1974; Marta et al., 1976; Mesquita et al., 1975). The xanthones **1** and **2** are characterized by the presence of a prenyl side chain at positions C-4 and C-5, respectively. Cyclization of a precursor prenyl side chain with a hydroxyl group at C-3 may account for the pyran ring found in **1** and **2**. Compounds **3–5** are benzophenones characterized by side chains which were previously found in *T. magle* (delle Monache et al., 1984).

Compounds **1–5** were evaluated against the KB (human oral epidermoid) cancer cell line

Table 2
Cytotoxic activity of isolates from *T. brevistaminea* with the KB cell line

Compound	**1**	**2**	**3**	**4**	**5**
KB[a]	4.1	1.9	10.0	9.0	8.2

[a] KB = Oral epidermoid carcinoma. Results are expressed as EC_{50} values (μg ml^{-1}) and were obtained using standard protocols (Likhitwitayawuid et al., 1993).

(Likhitwitayawuid, Angerhofer, Cordell, Pezzuto & Ruangrungsi, 1993. Compounds **1** and **2** exhibited significant cytotoxic activity with EC_{50} 4.1 and 1.9 μg ml^{-1}, respectively, in the KB cell line, whereas **3–5** were found to be inactive (EC_{50} > 5 μg ml^{-1}) (Table 2).

3. Experimental

Mps. Uncorr. IR: $CHCl_3$. ^1H, ^{13}C, COSY, NOE, HMQC, HMBC-NMR: 500 MHz NMR instrument with TMS as int. standard. EIMS: direct probe.

3.1. Plant material

The roots of *T. brevistaminea* Engl. were collected in August 1993, in Recife, Pernambuco, Brazil, and identified by one of the authors (R.M.). A voucher specimen (voucher number B1035) has been deposited in the Field Museum of Natural History, Chicago, IL, USA.

3.2. Extraction and isolation

Dried roots of *T. brevistaminea* (775 g) were ground and extracted with MeOH (2 × 2 l) for 24 h by percolation. The MeOH extracts were concentrated, mixed with water (MeOH–H_2O = 9:1), and subsequently washed with hexane (300 ml). The aq. MeOH soln. (300 ml) was further partitioned with $CHCl_3$ (2 × 300 ml). The $CHCl_3$ fraction was washed with 1% saline solution, then evapd, affording 22 g of a $CHCl_3$ extract which showed cytotoxic activity against the KB cell line.

The $CHCl_3$ extract (22 g) mixed with celite (22 g), was subjected to silica gel CC (480 g) using $CHCl_3$–MeOH (gradient, 100:0 → 10:1) as a solvent system, providing eight fractions. Fraction 2 (256 mg) eluted with $CHCl_3$–MeOH (19.9:0.1) from the initial CC, was further separated by silica gel CC using hexane–EtOAc (10:1) as a solvent, thus, a pure compound **1** (43 mg, 0.005% w/w) was obtained. From fraction 3 (4 g) of the initial CC using $CHCl_3$–MeOH (99:1) as eluent, betulinic acid (500 mg) was precipitated, and the mother liquor was subjected to silica gel CC using hexane–acetone (gradient, 9:2 → 1:1) as a solvent, yielding compounds **2** (30 mg, 0.004% w/w) and **3** (70 mg, 0.009% w/w). Compound **3** was further purified by prep. HPLC using MeOH–H_2O (9:1) (R_t 21 min). Betulinic acid (200 mg) was also obtained from fraction 4 by precipitation with MeOH. After betulinic acid was removed by filtration, the mother liquor was further pretreated to silica gel CC using $CHCl_3$–MeOH (99:1), affording fractions containing compounds **4** and **5**. Final purifications were performed by preparative HPLC using MeOH–H_2O (88:12), yielding pure compounds **4** (R_t 20 min, 103 mg, 0.013% w/w) and **5** (R_t 30 min, 130 mg, 0.017% w/w).

3.3. Trapezifolixanthone (*1*)

Yellow needles (MeOH). Mp, UV, IR, EIMS and ^1H-NMR spectral ($CDCl_3$) data comparable with lit. values (Somanathan & Sultanbawa, 1974). ^1H-NMR (500 MHz; Me_2CO-d_6): δ 13.33 (1H, s, OH-1), 9.22 (1H, s, OH-5), 7.69 (1H, dd, *J* = 1.5, 8.0 Hz, H-8), 7.38 (1H, dd, *J* = 1.5, 8.0 Hz, H-6), 7.28 (1H, t, *J* = 8.0 Hz, H-7), 6.71 (1H, d, *J* = 10.0 Hz, H-1′), 5.77 (1H, d, *J* = 10.0 Hz, H-2′), 5.31 (1H, t, *J* = 6.0 Hz, H-2″), 3.57 (1H, d, *J* = 6.0 Hz, H-1″), 1.87 (3H, s, H-5″), 1.66 (3H, s, H-4″), 1.51 (6H, s, H-5′). ^{13}C-NMR (125 MHz; $CDCl_3$): δ 181.0 (C-9), 158.2 (C-3), 156.0 (C-1), 153.7 (C-4a), 144.4 (C-5), 144.2 (C-4b), 131.6 (C-3″), 127.4 (C-2′), 123.9 (C-7), 122.7 (C-2″), 120.9 (C-8a), 119.7 (C-6), 116.8 (C-8), 115.7 (C-1′), 107.0 (C-4), 104.7 (C-2), 103.3 (C-9a), 78.3 (C-3′), 28.3 (C-4′), 28.3 (C-5′), 25.5 (C-4″), 21.7 (C-1″), 17.9 (C-5″). ^1H-^{13}C HMBC-NMR (500 and 125 MHz; $CDCl_3$): H-6 → C-8, C-4b; H-7 → C-5, C-8, C-8a; H-8 → C-6, C-9, C-4b; H-1′ → C-1, C-3, C-3′; H-2′ → C-2, C-3′; H-4′ → C-5′, C-2′, C-3′; H-5′ → C-4′, C-2′, C-3′; H-1″ → C-3, C-4, C-4a, C-2″, C-3″; H-4″ → C-2″, C-3″, C-5″; H-5″ → C-4″; OH-1 → C-1, C-2, C-9a; OH-5 → C-6, C-4b.

3.4. Manglexanthone (*2*)

Yellow needles (MeOH). Mp, UV, IR, EIMS, and ^1H-NMR spectral data comparable with lit. values (Marta et al., 1976). ^{13}C-NMR (125 MHz; $CDCl_3$): δ 180.4 (C-9), 160.4 (C-3), 157.5 (C-4a), 157.3 (C-1), 151.3 (C-6), 149.3 (C-4b), 145.9 (C-7), 132.9 (C-3″), 127.3 (C-2′), 123.8 (C-5), 121.1 (C-2″), 117.2 (C-8a), 115.5 (C-1′), 107.5 (C-8), 104.3 (C-2), 103.3 (C-9a), 94.8 (C-4), 78.1 (C-3′), 61.8 (OCH_3), 28.4 (C-4′ and C-5′), 25.7 (C-4″), 23.3 (C-1″), 18.0 (C-5″). ^1H–^{13}C HMBC-NMR (500 and 125 MHz; $CDCl_3$): H-4 → C-3, C-4a, C-9a; H-8 → C-6, C-9; H-1′ → C-1, C-3, C-3′; H-2′ → C-2, C-3′; H-4′(5′) → C-4′(5′), C-7; H-1″ → C-6, C-2″; H-2″ → C-1″, C-4″, C-5″; H-4″ → C-2″, C-3″,

C-5″; H-5″ → C-2″, C-3″, C-4″; OH-1 → C-1, C-2; OCH_3 → C-6.

3.5. Tovophenone A (**3**)

Yellow oil (MeOH). $[\alpha]_D$ −18.4° (*c* 0.96, MeOH). UV, IR, and EIMS data comparable with lit. values (delle Monache et al., 1984). ^{1}H-NMR (500 MHz; $CDCl_3$): δ 8.86 (1H, s, OH-2), 8.77 (1H, s, OH-6), 7.33 (1H, t, *J* = 8.0 Hz, H-12), 7.18 (1H, d, *J* = 8.0 Hz, H-13), 7.09 (1H, t, *J* = 2.0 Hz, H-9), 7.02 (1H, dd, *J* = 8.0, 2.0 Hz, H-11), 5.17 (1H, t, *J* = 6.5 Hz, H-2″), 4.70 (1H, s, H-4′), 4.66 (1H, s, H-9′), 4.63 (1H, s, H-9′), 4.62 (1H, s, H-4′), 3.75 (3H, s, OCH_3), 3.30 (2H, d, *J* = 6.5 Hz, H-1″), 2.63 (2H, d, *J* = 7.2 Hz, H-1′), 2.42 (1H, dt, *J* = 15.5, 7.2 Hz, H-2′), 1.95 (1H, dt, *J* = 15.3, 8.0 Hz, H-7′), 1.85 (1H, dt, *J* = 15.3, 8.0 Hz, H-7′), 1.74 (3H, s, H-4″, interchangeable with H-5″), 1.69 (3H, s, H-5″, interchangeable with H-4″), 1.69 (3H, s, H-5′), 1.67 (3H, s, H-10′), 1.51 (2H, dd, *J* = 15.5, 8.0 Hz, H-6′). ^{13}C-NMR (125 MHz; $CDCl_3$): δ 198.4 (C-7), 164.2 (C-4), 157.8 (C-2), 157.3 (C-6), 156.2 (C-10), 148.1 (C-3a), 146.3 (C-8a), 141.5 (C-8), 133.3 (C-3″), 130.2 (C-12), 122.4 (C-2″), 120.2 (C-13), 119.5 (C-11), 114.7 (C-9), 114.1 (C-3), 113.6 (C-5), 111.4 (C-4′), 109.3 (C-9′), 107.4 (C-1), 61.3 (OCH_3), 46.7 (C-2′), 35.6 (C-7′), 30.5 (C-6′), 28.6 (C-1′), 25.6 (C-5″, interchangeable with C-4″), 22.9 (C-1″), 22.4 (C-10′), 18.5 (C-5′), 17.8 (C-4″, interchangeable with C-5″). ^{1}H–^{13}C HMBC-NMR (500 and 125 MHz; $CDCl_3$): H-9 → C-7, C-11, C-13; H-11 → C-9, C-13; H-12 → C-8, C-10; H-13 → C-7, C-9, C-11; H-1a → C-2, C-3, C-4, C-2′, C-3′, C-6′; H-2′ → C-1′, C-3′, C-4′, C-5′, C-6′; H-4′ → C-2′, C-5′; H-6′ → C-1′, C-2′, C-3′, C-7′, C-8′; H-7′ (δ 1.95) → C-2′, C-6′, C-8′, C-9′; H-7′ (δ 1.85) → C-6′, C-8′, C-9′; H-9′ → C-2′, C-7′; H-1″ → C-4, C-5, C-6, C-2″, C-3″; H-2″ → C-4″, C-5″; OCH_3 → C-4; OH-6 → C-1, C-5, C-6; OH-2 → C-1, C-2, C-3.

3.6. Tovophenone B (**4**)

Yellow powder (MeOH and H_2O). $[\alpha]_D$ −19.0° (*c* 0.50, MeOH). UV, IR, and EIMS data comparable with lit. values (delle Monache et al., 1984). ^{1}H-NMR (500 MHz; $CDCl_3$): δ 11.7 (1H, s, OH-2), 7.24 (1H, t, *J* = 8.0 Hz, H-12), 7.06 (1H, d, *J* = 8.0 Hz, H-13), 6.94 (2H, m, H-9 and H-11), 4.68 (1H, s, H-4′), 4.65 (1H, s, H-9′), 4.63 (1H, s, H-9′), 4.61 (1H, s, H-4′), 3.77 (3H, s, OCH_3), 3.63 (1H, br d, *J* = 5.5 Hz, H-2″), 2.87 (1H, m, H-1″), 2.69 (2H, m, H-1′), 2.63 (1H, m, H-1″), 2.49 (1H, dt, *J* = 14.5, 7.2 Hz, H-2′), 1.96 (1H, dt, *J* = 15.5, 7.5 Hz, H-7′), 1.86 (1H, dt, *J* = 15.5, 7.5 Hz, H-7′), 1.72 (3H, s, H-5′), 1.67 (3H, s, H-10′), 1.53 (2H, dd, *J* = 14.5, 7.5 Hz, H-6′), 0.99 (3H, s, H-5″, interchangeable with H-4″), 0.92 (3H, s, H-4″, interchangeable with H-5″). ^{13}C-NMR (125 MHz; $CDCl_3$): δ 200.1 (C-7), 163.9 (C-4), 161.6 (C-2), 153.0 (C-6), 155.0 (C-10), 147.8 (C-3′), 146.4 (C-8′), 143.8 (C-8), 129.0 (C-12), 119.9 (C-13), 117.6 (C-11), 114.5 (C-9), 114.4 (C-3), 111.3 (C-4′), 109.3 (C-9′), 107.6 (C-1), 103.1 (C-5), 77.7 (overlapped with $CDCl_3$ peak, C-3″: it showed at δ 79.1 in acetone-d_6) 68.7 (C-2″), 60.4 (OCH_3), 46.6 (C-2′), 35.6 (C-7′), 30.3 (C-6′), 27.8 (C-1′), 25.7 (C-1″), 24.2 (C-5″), 22.5 (C-10′), 21.0 (C-4″), 18.3 (C-5′). ^{1}H-^{1}H COSY (500 MHz; $CDCl_3$) H-11 → H-12; H-12 → H-11, H-13; H-13 → H-12; H-2′ → H-6′, H-1″; H-6′ → H-2′, H-7′; H-7′ → H-6′; H-1″ (δ 2.87) → H-1″ (δ 2.63), H-2″; H-2″ → H-1″ (δ 2.87, 2.63). ^{1}H-^{13}C HMBC-NMR (500 and 125 MHz; $CDCl_3$): H-9 → C-7, C-11; H-11 → C-9; H-12 → C-8, C-10; H-13 → C-7, C-9, C-11; H-1′ → C-2, C-3, C-4; H-4′ → C-2′, C-5′; H-5′ → C-3′: H-6′ → C-3′, C-8′; H-7′ → C-8′, C-9′; H-9′ → C-10′; H-10′ → C-8′; H-1″ → C-5; OCH_3 → C-4; OH-2 → C-1, C-2, C-3.

3.7. Tovophenone C (**5**)

Yellow oil (MeOH). $[\alpha]_D$ −25.2°C (*c* 0.54, MeOH). UV λ_{max}^{MeOH} nm (log ε): 304 (4.08), 240 (sh) (3.87), 210 (4.34). IR ν_{max} ($CHCl_3$) cm^{-1}: 3595, 3025, 1626, 1592, 1499, 1459, 1425, 1388, 1318, 1123, 1097, 889. ^{1}H-NMR (500 MHz; $CDCl_3$) see Table 1. ^{13}C NMR (125 MHz; $CDCl_3$) see Table 1. EIMS *m*/*z*: $[M]^+$ 480 (1), 357 (100), 341 (3), 339 (6), 297 (3), 285 (6), 255 (1), 263 (2), 245 (3), 215 (1), 205 (2), 191 (6), 177 (1), 163 (2), 121 (26), 93 (8). HREIMS *m*/*z*: 480.2507 (calcd for $C_{23}H_{22}O_5$, 480.2512) (see Fig. 1).

3.8. Betulinic acid

NMR data were comparable with lit. values (Sholichin, Yamasaki, Kasai & Tanaka, 1980).

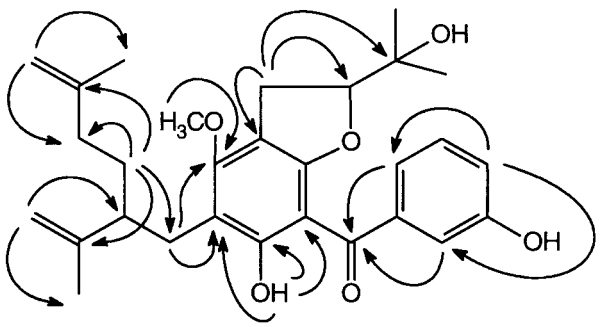

Fig. 1. Important HMBC correlations for compound **5**.

3.9. Cytotoxicity testing

Compounds **1–5** were evaluated for cytotoxic activity against the KB cell line as described previously (Likhitwitayawuid et al., 1993). Compounds were considered significantly active if they showed EC_{50} values of < 5 μg ml^{-1}.

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