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Novel cytotoxic acylphenol dimers of *Myristica gigantea*; enzymatic synthesis of giganteones A and B

Van Cuong Pham,[a] Akino Jossang,[a] Thierry Sévenet[b] and Bernard Bodo[a,*]

[a]*Laboratoire de Chimie des Substances Naturelles, ESA 8041 CNRS, Muséum National d'Histoire Naturelle, 63 rue Buffon, 75005 Paris, France*

[b]*Institut de Chimie des Substances Naturelles, 1206 CNRS, 91198 Gif sur Yvette Cedex, France*

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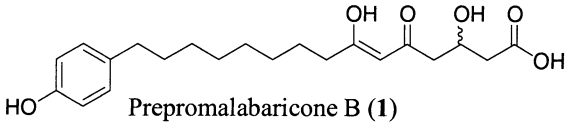
Abstract—Three new compounds, prepromalabaricone B (**1**) and two dimeric acylphenols, giganteones A (**2**) and B (**3**) were isolated from EtOAc extract of the fruits of *Myristica gigantea* (Myristicaceae), together with known promalabaricone C (**4**), malabaricones A–C (**5**–**7**) and maingayone (**8**). The structures were determined by mass and 2D NMR spectral analysis, and the enzymatic synthesis of giganteones A and B was performed from malabaricone C. Both **2** and **3** possess significant cytotoxic activity in vitro against human nasopharynx KB cells.

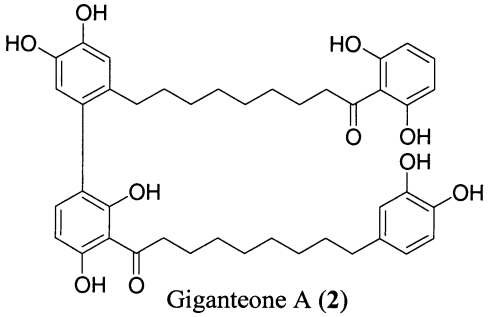
1. Introduction

The Myristicaceae family is an uniform archaic group, consisting of 19 genera and nearly 440 species, found in the lowland rain forests in the Asian tropics, tropical America, Africa and Madagascar.[1] In this family, *Myristica fragrans* and some *Myristica* are used in folk medicine in Asia.[2] A number of lignans, neolignans and acylphenols have been described from different *Myristica* species and several show antioxidant,[3] antifungal[4,5] or antileishmanial[6] activities. Additionally, alkaloids were isolated from different genera such as *Virola* which provides an Amazonian drug 'snuff', containing *N*,*N*-dimethyltryptamine[7] and β-carbolines, and *Horsfieldia* which yields the oxindole, horsfiline.[8] Recently, antihyperglycemic terpenoid-quinones were isolated from *Pycnanthus angolensis*.[9] We previously reported the isolation from *Myristica maingayi*, of the cytotoxic acylphenols, maingayone, promalabaricones B and C, malabaricones A–C, together with maingayic acids B and C,[10] and knerachelins A and B from *Knema furfuracea*.[11]

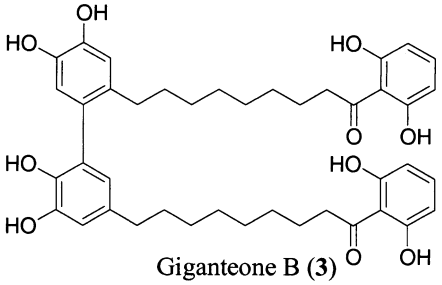
In search of natural antitumor agents, we examined the fruits of *M. gigantea*, collected in Malaysia. From the EtOAc extract that exhibited a cytotoxic activity on human nasopharynx KB cells (81% inhibition at 10 μg/ml), three new compounds, prepromalabaricone B (**1**), and two dimeric acylphenols, giganteones A (**2**) and B (**3**) were isolated,



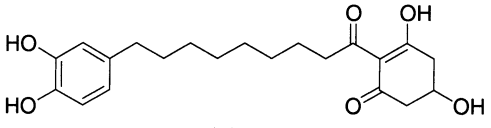
Prepromalabaricone B (**1**)



Giganteone A (**2**)



Giganteone B (**3**)



Promalabaricone C (**4**)

Keywords: *Myristica gigantea*; Myristicaceae; dimeric acylphenols; cytotoxicity; enzymatic synthesis; prepromalabaricone B; giganteone A; giganteone B.

* Corresponding author. Tel.: +33-1-40793129; fax: +33-1-40793135; e-mail: bodo@mnhn.fr

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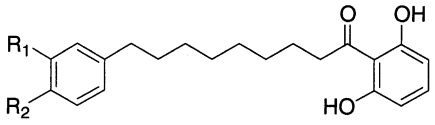
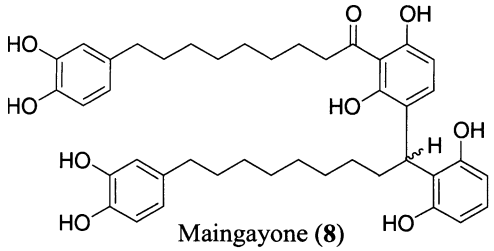
together with previously described promalabaricone C (**4**), malabaricones A–C (**5**–**7**), maingayone (**8**).[10] Furthermore, we synthesized the two dimeric acylphenols **2** and **3** by enzymatic oxidative dimerisation of malabaricone C, and examined the cytotoxicity against human nasopharynx KB cells.



Malabaricone A: $R_1 = R_2 = H$ (**5**)
Malabaricone B: $R_1 = H$; $R_2 = OH$ (**6**)
Malabaricone C: $R_1 = R_2 = OH$ (**7**)



Maingayone (**8**)

2. Results and discussion

Eight constituents were isolated from the EtOAc extract of the dried fruits of *M. gigantea* by bioassay-guided fractionation. Promalabaricone C (**4**) and malabaricones A–C (**5**–**7**) were separated by column chromatography (cc) on silica gel. The more polar fractions were further separated on Sephadex LH 20 column, and final purification was achieved with semipreparative C-8 RP HPLC to yield **1**–**3** and **8**.

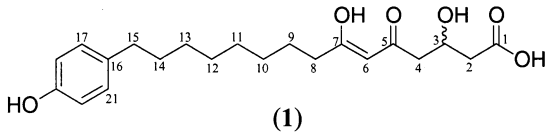
Compound **1** was obtained as colourless crystals (mp 163–164°C) with optical activity, $[\alpha]_D^{20} = -48.0°$ (*c* 0.8, MeOH). Its ESI-MS spectrum showed the $[M-H_2O+Na]^+$ ion at *m/z* 383.1821, (calcd 383.1834 for $C_{21}H_{28}O_5Na$), suggesting the molecular formula $C_{21}H_{30}O_6$. The IR spectrum showed a broad hydroxyl band at ν_{max} 3388, methylene bands at 2929 and 2856, acid and conjugated carbonyl bands at 1722 and 1648 cm^{-1}, respectively.

The ^1H and ^{13}C NMR spectra (Table 1) indicated a typical spin system for a 1,4-disubstituted benzene ring and signals for 10 methylenes. Two methylenes at δ 2.53 and 2.78 (CH_2-2 and -4), vicinal to two carbonyl groups, were linked to the same methine group at δ 4.81 (CH-3), giving thus an AA′XBB′ spin system and the other methylenes formed an octyl linear chain. In the HMBC spectrum, the sp^2 quaternary carbon at δ 134.8 (C-16) was, on one hand, correlated to the aromatic protons at δ 6.68 (H-18 and -20) and, on the other hand, to the methylenes at δ 1.56 and 2.50 (CH_2-14 and -15): the *n*-octyl chain was thus linked to the *p*-hydroxybenzene ring. The carbonyl at δ 173.8 (C-1) was correlated to the methine at δ 4.81 (H-3) and to methylene at δ 2.78 (CH_2-2), depicting the terminal carboxylic acid structure. The carbonyl at δ 195.5 (C-5), typical of a conjugated one, was correlated to the singlet proton at δ 5.36 (H-6) and to the methylene at δ 2.53 (CH_2-4), as well as to the methine at 4.81 (H-3) of the above AA′XBB′ system. The quaternary carbon at δ 180.6 (C-7) was correlated to the methylenes at δ 1.56 and 2.28 (CH_2-9 and -8), ending the *n*-octyl chain on its remaining side. It was also correlated to the proton at δ 5.36 (H-6), thus determining the enol form for carbon C-7. From complete analysis of the HMBC spectrum, the structure of prepromalabaricone B (**1**) was determined as 3,7-dihydroxy-15-(4-hydroxyphenyl)-5-oxo-6-pentadecenoic acid.

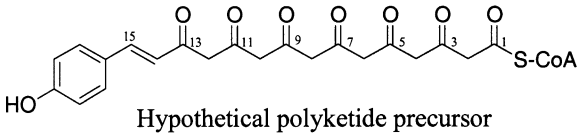
Table 1. NMR data for prepromalabaricone B (**1**), ^1H: 400.13 MHz, ^{13}C: 75.47 MHz, CD_3OD

C. No.	δC	δH *J* (Hz)
1	173.8	–
2	40.1	2.78 m
3	77.2	4.81 m
4	41.0	2.53 m
5	195.5	–
6	104.7	5.36 s
7	180.6	–
8	35.7	2.28 m
9	27.4	1.56 m
10–13	30.0–30.4	1.32 br
14	33.0	1.56 m
15	36.0	2.50 t 7.5
16	134.8	–
17	130.2	6.97 m 8.4
18	116.0	6.68 m 8.4
19	156.2	–
20	116.0	6.68 m 8.4
21	130.2	6.97 m 8.4

Interestingly, compound **1** gave information on the biosynthetic pathway of malabaricones. We previously proposed[10] that malabaricones A, B and C were biosynthesized from the same precursors as those used by chalcone synthase in the flavonoids biosynthesis pathway, starting from a cinnamoyl CoA derivative (originating from shikimic acid), but elongated here with six acetyl-CoA units, instead of three. The cyclisation of the last three acetyl units of the polyketide intermediate (Fig. 1) yields a phloroglucinol type aromatic ring. The isolation of prepromalabaricone B (**1**) suggested that either, the reduction of the carbonyl-3 of the polyketide into a secondary alcohol, occurred prior to the ring closure, or that, it is a by-product in the malabaricone biosynthesis. Since the cyclisation



(**1**)



Hypothetical polyketide precursor

Figure 1. Prepromalabaricone B (**1**) and its biogenetic hypothetical precursor.

Table 2. NMR data for giganteones A (**2**) and B (**3**), ^{1}H: 400.13 MHz, ^{13}C: 75.47 MHz, CD_3OD

Giganteone A (**2**)						Giganteone B (**3**)					
Substructure **I**			Substructure **II**			Substructure **I**			Substructure **II**		
C. No.	δC	δH *J* (Hz)	C. No.	δC	δH *J* (Hz)	C. No.	δC	δH *J* (Hz)	C. No.	δC	δH *J* (Hz)
1	209.7	–	1′	210.1	–	1	209.0	–	1′	209.0	–
2	45.8	3.08 t 7.4	2′	45.9	3.14 t 7.4	2	45.8	3.08 m	2′	45.8	3.08 m
3	25.7	1.62 m	3′	26.0	1.68 m	3	25.8	1.61 m	3′	25.8	1.61 m
4	30.3	1.29 m	4′	30.4	1.34 m	4	30.2	1.14 m	4′	30.2	1.14 m
5	30.1	1.30 m	5′	30.1	1.30 m	5	30.4	1.29 m	5′	30.4	1.29 m
6	30.6	1.31 m	6′	30.6	1.31 m	6	30.6	1.32 m	6′	30.6	1.32 m
7	30.6	1.31 m	7′	30.6	1.31 m	7	30.6	1.32 m	7′	30.6	1.32 m
8	32.1	1.41 m	8′	32.9	1.55 m	8	31.9	1.41 m	8′	32.9	1.59 m
9	33.7	2.30 t 7.5	9′	36.3	2.43 t 7.5	9	33.8	2.34 t 7.8	9′	36.8	2.46 t 7.4
10	134.8	–	10′	135.8	–	10	134.5	–	10′	134.7	–
11	118.9	6.68s	11′	116.5	6.60 d 2.0	11	117.1	6.69s	11′	115.0	6.60 d 2.1
12	145.6	–	12′	146.0	–	12	146.0	–	12′	141.5	–
13	143.8	–	13′	144.0	–	13	145.4	–	13′	142.4	–
14	117.1	6.54s	14′	116.2	6.65 d 8.0	14	118.5	6.58s	14′	130.1	–
15	129.6	–	15′	120.6	6.46 dd 8.0; 2.0	15	130.6	–	15′	123.3	6.34 d 2.1
16	111.1	–	16′	111.4	–	16	111.4	–	16′	111.4	–
17	163.4	–	17′	161.2	–	17	163.8	–	17′	163.8	–
18	108.4	6.34 d 8.2	18′	122.4	–	18	108.3	6.32 d 8.2	18′	108.3	6.32 d 8.2
19	136.9	7.18 t 8.2	19′	138.9	7.05 d 8.3	19	136.8	7.18 t 8.2	19′	136.8	7.18 t 8.2
20	108.4	6.34	20′	107.4	6.41 d 8.3	20	108.3	6.32 d 8.2	20′	108.3	6.32 d 8.2
21	163.4	–	21′	161.4	–	21	163.8	–	21′	163.8	–

product, promalabaricone (**4**) has also been isolated from *M. maingayi* and *M. gigantea*, the former hypothesis seems more appropriate. In this way, the cinnamoyl polyketide undergoes reduction of the carbonyls at 9, 11, 13 into CH_2 groups, and of carbonyl-3 into a >CHOH group to yield prepromalabaricones and finally, the ring closure, dehydration of the secondary alcohol and enolisation lead to malabaricones.

Compound **2** was isolated by C-8 RP HPLC as a colourless solid (mp 150–151°C). Its IR spectrum displayed a broad hydroxyl absorption band at ν_{max} 3430, methylene bands at 2929 and 2856, and a conjugated carbonyl band at 1681 cm^{-1}. The HRFABMS showed the $[M+H]^+$ ion at *m/z* 715.3544 (calcd 715.3482 for $C_{42}H_{51}O_{10}$), indicating the molecular formula $C_{42}H_{50}O_{10}$. The ^{1}H, ^{13}C and 2D NMR spectra (Table 2), depicted four benzene rings: 1,2,3,4-tetrasubstituted (**b**′), 1,2,4,5-tetrasubstituted (**a**), 1,2,3-trisubstituted (**b**) and 1,3,4-trisubstituted (**a**′). Analysis of the aliphatic region indicated 16 methylene groups distributed into two *n*-octyl chains (**c** and **d**).

In the HMBC spectrum, the quaternary sp^2 carbon at δ 134.8 (C-10) was strongly correlated to the proton at δ 6.54 (H-14) of the **a**-ring and to the methylenes at δ 1.41 and 2.30 (CH_2-8 and -9) of the **c**-chain, thus linking C-10 to the *n*-octyl **c**-chain. The chemical shift of the methylene at δ 3.08 suggested that it was vicinal to a carbonyl, and those of the seven aromatic quaternary carbons between 143.8 and 163.4 ppm were linked to oxygen atoms. The carbonyl at δ 209.7 (C-1) was correlated to the methylenes at δ 3.08 and 1.62 (CH_2-2 and -3), and to the aromatic protons at δ 6.34 (H-18 and -20, ^{4}J W-coupling) of the **b**-ring, ending the *n*-octyl **c**-chain and thus, the substructure **I** was defined as a 15-substituted malabaricone C. In the same way, long range correlations connected the *n*-octyl **d**-chain to the **a**′-ring and to the carbonyl at δ 210.1 (C-1′) that was linked to the **b**′-ring. Substructure **II** was assigned to an 18′-substituted malabaricone C. The quaternary carbon at δ 129.6 (C-15) was correlated to the proton at δ 6.68 (H-11) of the **a**-ring, and the proton at δ 7.05 (H-19′) of the **b**′-ring, thus allowing the linkage at C-15 and C-18′ of substructures **I** and **II**, respectively (Fig. 2). The structure of giganteone A was determined as 1-(2,6-dihydroxyphenyl)-9-(2-{3-[9-(3,4-dihydroxyphenyl)-nonanoyl]-2,4-dihydroxyphenyl}-4,5-dihydroxyphenyl)-1-nonanone, which is thus a dimer of malabaricone C by the linkage of C-15 to C-18′.

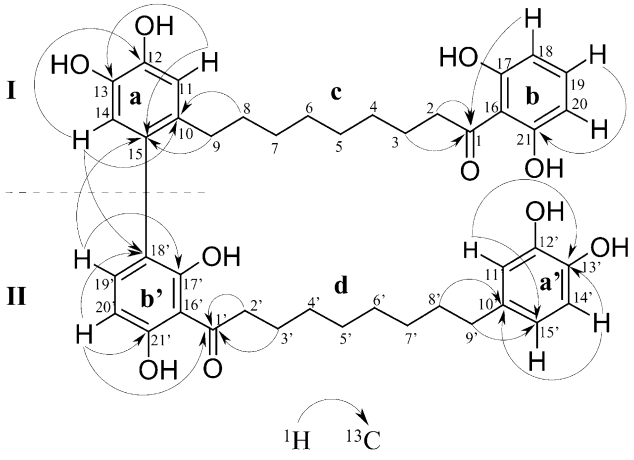


Figure 2. Selected HMBC correlations for giganteone A (**2**).

Compound **3** was obtained by HPLC as colourless crystals (mp 169–170°C). Its IR spectrum had a broad hydroxyl absorption band at 3387, methylene bands at 2928 and 2854 and a conjugated carbonyl band at 1655 cm^{-1}. The ESI-MS spectrum showed the $[M+Na]^+$ ion at *m/z* 737.3327 (calcd 737.3302 for $C_{42}H_{50}O_{10}Na$), suggesting the molecular formula $C_{42}H_{50}O_{10}$. The ^{1}H and ^{13}C NMR spectra (Table 2) were similar to those of giganteone A

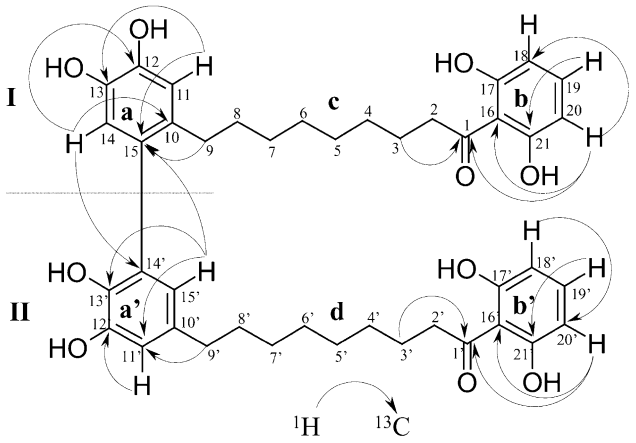


Figure 3. Selected HMBC correlations for giganteone B (**3**).

(**2**), with some differences in the aromatic region, showing the presence of 1,2,4,5-tetrasubstituted (**a**), 1,2,3,5-tetrasubstituted (**a**′), two 1,2,3-trisubstituted rings (**b** and **b**′), and two *n*-octyl chains (**c** and **d**).

In the HMBC spectrum, the quaternary sp^2 carbon at δ 130.6 (C-15) was correlated to the proton at δ 6.69 (H-11) on the **a**-ring and the proton at δ 6.34 (H-15′) on the **a**′-ring, indicating the linkage of **a** and **a**′-rings in a biphenyl system. The carbon at δ 134.8 (C-10) was correlated to the protons at δ 6.68 (H-11) and 2.30 (CH_2-9) of the **c**-chain, thus ending this chain on one side. One of the two carbonyls superposed at δ 209.0 (C-1) was correlated to the **b**-ring protons at δ 6.32 (H-18 and -20, ^4J W-coupling) and methylene protons at δ 3.08 (CH_2-2), thus ending the **c**-chain on the other side. Similarly, the quaternary carbon at δ 135.8 had correlations with protons at δ 6.60 (H-11′), 6.46 (H-15′) and 2.43 (CH_2-9′), ending the other *n*-octyl (**d**-chain) on one side. The **b**′-ring was linked with C-1′ at δ 209.0 and ended the **d**-chain (Fig. 3). From the foregoing studies, the structure of 1-(2,6-dihydroxyphenyl)-9-(2-{5-[1-(2,6-dihydroxyphenyl)-1-oxonona-9-yl]-2,3-dihydroxyphenyl} -4,5-dihydroxyphenyl)-1-nonanone was assigned to compound 3 and named giganteone B.

2.1. Hemisynthesis of giganteones A and B

Phenols are easily oxidized, both in vivo and in vitro, to give phenoxy radicals via one electron-transfers. The resulting neutral phenoxy radicals may be transformed into many other classes of compounds.[12,13] Several enzymes are able to catalyse this oxidation type, such as laccase,[14,15] horseradish peroxidase,[16–18] and tyrosinase.[19–22]

The structures of giganteones A and B suggested that they were biosynthezised by the oxidative coupling of two units of malabaricone C. It prompted us to investigate their synthesis by the oxidative dimerization of malabaricone C catalyzed by such enzymes as rhus laccase and horseradish peroxidase.

The primary oxidation process described in Fig. 4 involves phenoxyl radicals formation due to an electron-transfer, to give neutral radicals **III**, **III**′ and **V**–**VIII**, which undergo dimerisation by the coupling of two radicals.

Oxidation of malabaricone C was first processed by catalysis with laccase in MeOH/H_2O: 1/1 (v/v) at 33°C for 3 days, formed giganteones A and B, with low yields, 5% and 0.4% respectively. When using MeOH/H_2O: 7/3 (v/v), at the same temperature and time conditions, giganteones A and B were successfully formed with higher yields of 25 and 5%, respectively. This could be explained by the higher solubility of malabaricone C in these conditions. Treating with hydroxyperoxide in the presence of horseradish peroxidase in acetonitrile/phosphate buffer (pH 6, 0.05 M) 7/3 (v/v), malabaricone C was transformed into the same dimeric products, but once again with low yields for giganteones

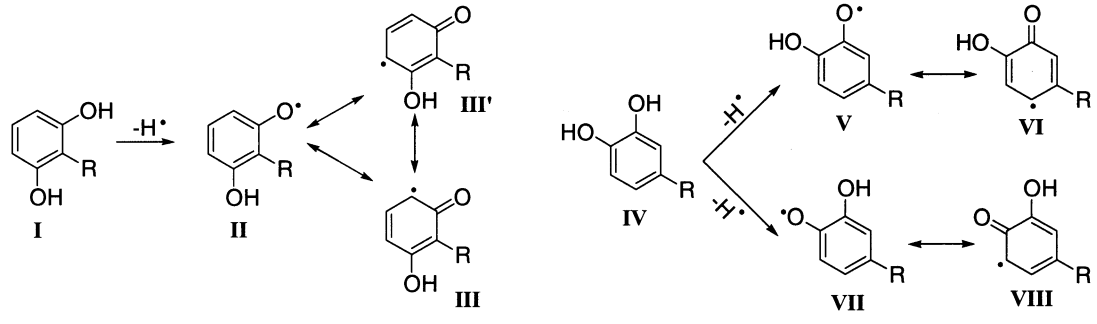
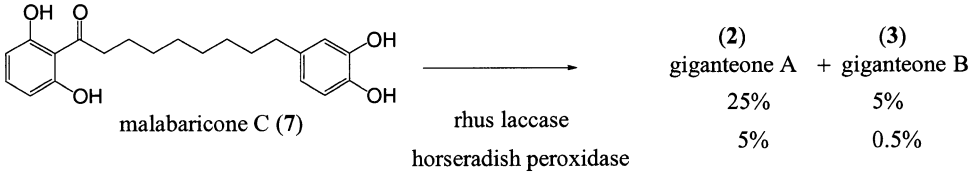


Figure 4. Selected radicals formation from 2,6- and 3,4-dihydroxyphenyl moieties.



Scheme 1.

A (5%) and B (0.5%). In all cases, only the coupling of radical species **III** (or **III′**) with **VI**, and **VI** with **VIII** were observed. The synthesis of giganteones was more efficiently catalysed by laccase than by peroxidase. The dimeric compounds, either natural or hemisynthetic, had no optical activity, indicating that there is no atropoisomerism in this series (Scheme 1).

The cytotoxicity assay of compounds **1**–**3** against human tumoral KB cells showed that giganteone B was more active (IC_{50} 1.8 μg/ml, 2.6×10^{-6} M; vinblastine showed a IC_{50} of 0.01 μg/ml), than giganteone A (IC_{50} 11.4 μg/ml, 1.6×10^{-5} M) and prepromalabaricone B (18.9 μg/ml, 5×10^{-5} M). The cytotoxicity of the known compounds **5**–**8** were previously reported,[10] with IC_{50} between 9×10^{-6} and 15×10^{-5} M.

3. Experimental procedures

3.1. General

Melting points were determined on a Reichert microscope and are uncorrected. Optical rotations were measured on a Perkin–Elmer 341 polarimeter, UV spectra were recorded on an Uvikon 930 Kontron spectrophotometer, and IR spectra (KBr) on a Nicolet Impact 400 D spectrophotometer. ESI-MS were performed on an API Q-STAR PULSAR i of Applied Biosystem and FABMS on a ZAB-HF instrument of VG analytical. ^{1}H spectra were recorded on an Avance 400 Bruker, and ^{13}C on a Bruker AC 300 spectrometers operating at 400.13 and 75.47 MHz, respectively. ^{1}H chemical shifts were referenced relative to central peak of CHD_2OD at 3.31 ppm and ^{13}C chemical shifts to the central peak of CD_3OD at 49.0 ppm. For the HMBC, the delay was optimised for a coupling constant of 7 Hz.

3.2. Extraction and isolation

The dried ground fruits of *M. gigantea* King (600 g) were extracted with EtOAc at rt and gave after evaporation of the solvent a residue (19 g), which was chromatographed on a silica gel column. Elution with a CH_2Cl_2/MeOH mixture starting from 5 to 100% MeOH, yielded 28 fractions (F1–F28). Fractions F3, F11 and F13 were composed of pure malabaricones A (**5**, 60 mg), B (**6**, 110 mg) and C (**7**, 84 mg), respectively. Fractions F16–F18 were combined (2.34 g) and further chromatographed on silica gel with a CH_2Cl_2/MeOH mixture (from 10 to 100% MeOH) giving 10 fractions (f1–f10): fraction f7 yielded promalabaricone C (**4**, 31 mg). The fractions F19–F21 (1.2 g) were chromatographed on Sephadex LH 20 (MeOH) and further purified by HPLC (RP8 Chromasil, 85/15, MeOH/H_2O), to give prepromalabaricone B (**1**, 6 mg), giganteones A (**2**, 7 mg), B (**3**, 3 mg) and maingayone (**8**, 3 mg).

3.3. Enzymatic hemisynthesis of giganteones A and B

(a) Rhus laccase (10 mg) was added to a solution of malabaricone C (60 mg) in 0.8 ml of MeOH/H_2O: 7/3 (v/v). The reaction mixture was stirred in the presence of air at 33°C for 3 days. After removing the solvent under reduced pressure, the residue was chromatographed on a Sephadex LH 20 column (MeOH) to give 21 mg of a mixture of dimeric compounds, together with 30 mg of unreacted malabaricone C. The mixture was separated by HPLC on a C-18 RP column, (MeOH/H_2O: 85/15), to yield 15 mg (25%) and 3 mg (5%) of giganteones A and B, respectively.

(b) Horseradish peroxidase was added to a solution of 100 mg (0.28 mmol) malabaricone C in a mixture of CH_3CN (6 ml) and phosphate buffer (3 ml, pH 6, 0.05 M). Hydroperoxide 5% (0.4 ml) was slowly added at 20°C in 30 min. The reaction mixture was stirred at the same temperature for 1 day. After removing CH_3CN, the residue was extracted with *n*-BuOH (5 ml×3). The organic phases were combined and evaporated, and the residue chromatographed on Sephadex LH 20 (MeOH), to yield 9 mg of a mixture of dimers and 70 mg of recovered malabaricone C. This mixture was further purified by HPLC (RP18 Chromasil, MeOH/H_2O: 85/15), giving 5 mg (5%) and 0.5 mg (0.5%) of giganteones A and B, respectively.

3.4. Cytotoxic activity

KB cells were maintained in Dulbecco's D-MEM medium, supplemented with 10% fetal calf serum, L-glutamine (2 mM), penicillin G (100 UI/mL), streptomycin (100 μg/mL) and gentamycin (10 μg/mL). Stock solutions of compounds were prepared in DMSO/H_2O (1/9), and the cytotoxicity assays were carried out in 96-well microtiter plates in triplicate against human nasopharynx carcinoma KB cell lines (3×10^3 cells/mL) using a modification of the published method.[23] After 72 h incubation at 37°C in air/CO_2 (95/5) with or without test compounds, cell growth was estimated by colorimetric measurement of stained living cells by neutral red. Optical density was determined at 540 nm on a Titertek Multiscan photometer. The IC_{50} value was defined as the concentration of sample necessary to inhibit the cell growth to 50% of the control.

3.4.1. Prepromalabaricone B (1). 3,7-dihydroxy-15-(4-hydroxyphenyl)-5-oxo-6-pentadecenoic acid: $C_{21}H_{30}O_6$; mp 163–164°C (MeOH); $[\alpha]_D^{25}=-48.0°$ (*c* 0.8, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3388, 2929, 2856, 1722, 1648, 1597, 1515, 1405, 1246; UV (MeOH) λ_{max} (log ε): 204 (3.9), 239 (3.3), 267 (3.8); ESI-MS: calcd 383.1834 for $C_{21}H_{28}O_5Na$, $[M-H_2O+Na]^+$, found 383.1821.

3.4.2. Giganteone A (2). 1-(2,6-dihydroxyphenyl)-9-(2-{3-[9-(3,4-dihydroxyphenyl)nonanoyl]-2,4-dihydroxyphenyl}-4,5-dihydroxyphenyl)-1-nonanone: $C_{42}H_{50}O_{10}$; mp 150–151°C (MeOH); IR (KBr): ν_{max} (cm^{-1}) 3430, 2929, 2856, 1681, 1629, 1596, 1453, 1207, 1143, 1026; UV (MeOH) λ_{max} (log ε): 210 (4.1), 224 (4.0), 274 (3.9), 355 (3.3), 471 (3.1); HRFABMS: calcd 715.3482 for $C_{42}H_{51}O_{10}$, $[M+H]^+$, found 715.3544.

3.4.3. Giganteone B (3). 1-(2,6-dihydroxyphenyl)-9-(2-{5-[1-(2,6-dihydroxyphenyl)-1-oxonona-9-yl]-2,3-dihydroxyphenyl}-4,5-dihydroxyphenyl)-1-nonanone: $C_{42}H_{50}O_{10}$; mp 169–170°C (MeOH); IR (KBr): ν_{max} (cm^{-1}) 3387, 2929, 2854, 1655, 1628, 1596, 1511, 1461, 1231, 1043; UV (MeOH) λ_{max} (log ε): 213 (3.8), 275 (3.3), 357 (3.0); ESI-MS: calcd 737.3302 for $C_{42}H_{50}O_{10}Na$, $[M+Na]^+$, found 737.3327.

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