Cytotoxic Prenylxanthones from *Garcinia bracteata*

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Bioassay-guided fractionation of a leaf extract of *G. bracteata* has yielded six new prenylxanthones, bractatin (**1**), isobractatin (**2**), 1-*O*-methylbractatin (**3**), 1-*O*-methylisobractatin (**4**), 1-*O*-methyl-8-methoxy-8,8a-dihydrobractatin (**5**), and 1-*O*-methylneobractatin (**6**). The structures of these compounds have been elucidated by spectroscopic means (NMR, MS), literature data, and X-ray crystallographic analysis of **2**. These compounds possess significant cytotoxicity against the KB cell line.

The genus *Garcinia* (Clusiaceae), which is encountered mainly in lowland rainforests of the tropical world, has been extensively investigated from phytochemical and biological points of view. Prenylated xanthones,[1−11] triterpenes,[12,13] and biflavonoids[14,15] have been isolated from African and Southeast Asian species. In the course of our ongoing search for anticancer agents from natural sources, an EtOAc extract of the leaves of *Garcinia bracteata* C. Y. Wu ex Y. H. Li (Clusiaceae), collected in Hoa Binh Province, Vietnam,[16] was cytotoxic against the KB cell line. Bioassay-guided fractionation of this extract afforded six new prenylated xanthones: bractatin (**1**), isobractatin (**2**), 1-*O*-methylbractatin (**3**), 1-*O*-methylisobractatin (**4**), 1-*O*-methyl-8-methoxy-8,8a-dihydrobractatin (**5**), and 1-*O*-methylneobractatin (**6**). Compounds of this type, except **6**, have previously been isolated from *G. morella*,[1,2,8] *G. hanburyi*,[9,10] *G. forbesii*,[17] *G. gaudichaudii* ,[18,19] and *G. lateriflora*.[20] In this paper, we report the isolation, structure elucidation, and biological activity of **1**−**6** (Chart 1).

Results and Discussion

Dried and powdered leaves of *G. bracteata* were extracted with EtOAc then EtOH. The EtOAc extract exhibited 50% growth inhibition of KB cells at 4 μg/mL. This extract was chromatographed on silica gel, and the most active fractions yielded **1**−**6**. The structures of **1**−**6** have been determined by a combination of ^{1}H and ^{13}C and 2D NMR techniques.

Bractatin (**1**) gave a major peak $[MH]^+$ at *m*/*z* 465.2299 (HRCIMS), which matched the molecular formula $C_{28}H_{33}O_6$. The EIMS of **1** showed a molecular peak at *m*/*z* 464, the major peak being *m*/*z* 436, due to the loss of 28 amu. In the COSY spectrum, a proton resonance at δ 7.45 (H-8) showed scalar couplings with the signal at δ 3.51 (H-7), this one being coupled with H_A-21 (δ 2.36). The other, H_B-21 at δ 1.32, is correlated with H-22 (δ 2.61), indicating the sequence $=CH-CH-CH_2-CH$. An isoprenyl chain was deduced from signals at δ 2.67 (2H-16), 4.40 (1H-17), 1.41 (3H-19), and 1.13 (3H-20). A 1,1-dimethylallyl group was indicated by the presence of one H (H-12) at δ 6.48 (dd, 10, 18 Hz), which correlated with one H (H-13) at δ 5.48 (d, 18 Hz) and one H (H-13) at δ 5.40 (d, 10 Hz). In the ^{1}H NMR spectrum, there are two H (2 × OH) at δ 12.95 (chelated) and 7.53, one aromatic H (s) at δ 6.03, and four methyl signals at δ 1.87, 1.73, 1.58, and 1.26. Analysis of the ^{13}C NMR spectrum and of HMBC correlations allowed unambiguous assignment of the various resonances (Tables 1 and 2).

Isobractatin (**2**), gave a major peak $[MH]^+$ at *m*/*z* 465.2298 (HRCIMS), which matched the molecular formula $C_{28}H_{33}O_6$. The ^{1}H NMR spectrum was very similar to that of **1**, except for the lack of protons of a vinyl group and the appearance of a $CHCH_3$ group at δ 4.4 (1H, C-12) and 1.40 (3H, C-13). This indicates that the 1,1-dimethylallyl group is cyclized into a dihydrofurane as observed previously in related compounds.[21]

A chemical correlation has been made between **1** and **2**. Compound **1** was refluxed in formic acid; under these conditions, a 1,1-dimethylallyl chain adjacent to a phenolic hydroxyl usually cyclizes to generate a trimethyldihydrofurane. In this case, two products were obtained, isobractatin (**2**) and a new compound (**7**). Compound **7** gave a major peak at $[MH]^+$ *m*/*z* 397 (CIMS), corresponding to the loss of the 1,1-dimethylallyl group. The ^{1}H NMR spectrum is closely related to that of **1**, except for the signals of the 1,1-dimethylallyl group, which are replaced by an aromatic singlet at δ 6.06. These data allow us to propose for **7** the structure of a 4-deprenylated compound, as it has already been described for rheediaxanthone B.[22]

The structure of **2** was confirmed by single-crystal X-ray structure analysis. Compound **2** was recrystallized from a mixture of heptane/ethyl acetate as small yellow crystals showing two different morphological forms: form **A** as small plates with a diamond-shaped surface, and form **B** as elongated parallelepiped prisms. These two forms crystallize in the centrosymmetric space group P-1 of the triclinic system, implying that, in the solid-state, isobractatin (**2**) is racemic. The two unit-cells are different, giving for **B** a smaller volume (−18 Å^3). The two crystal forms are compared in Figure 1 in an identical orientation: the two molecules appear quite similar, except for the side chain fixed at C-5, bent differently at C-17. Thus, these two molecules are rotamers resulting from a rotation of 118° of the C-17−C-18 double bond around the C-16−C-17 bond (torsion angles: C-5−C-16−C-17−C-18 = 133.0(3)° in **A**, −109.4(2)° in **B**. The energy difference between these two conformers, estimated by the molecular mechanics calculations (Macromodel, MM2 forcefield)[23,24] starting from the X-ray coordinates, is 2.3 kcal/mol. The highest energy

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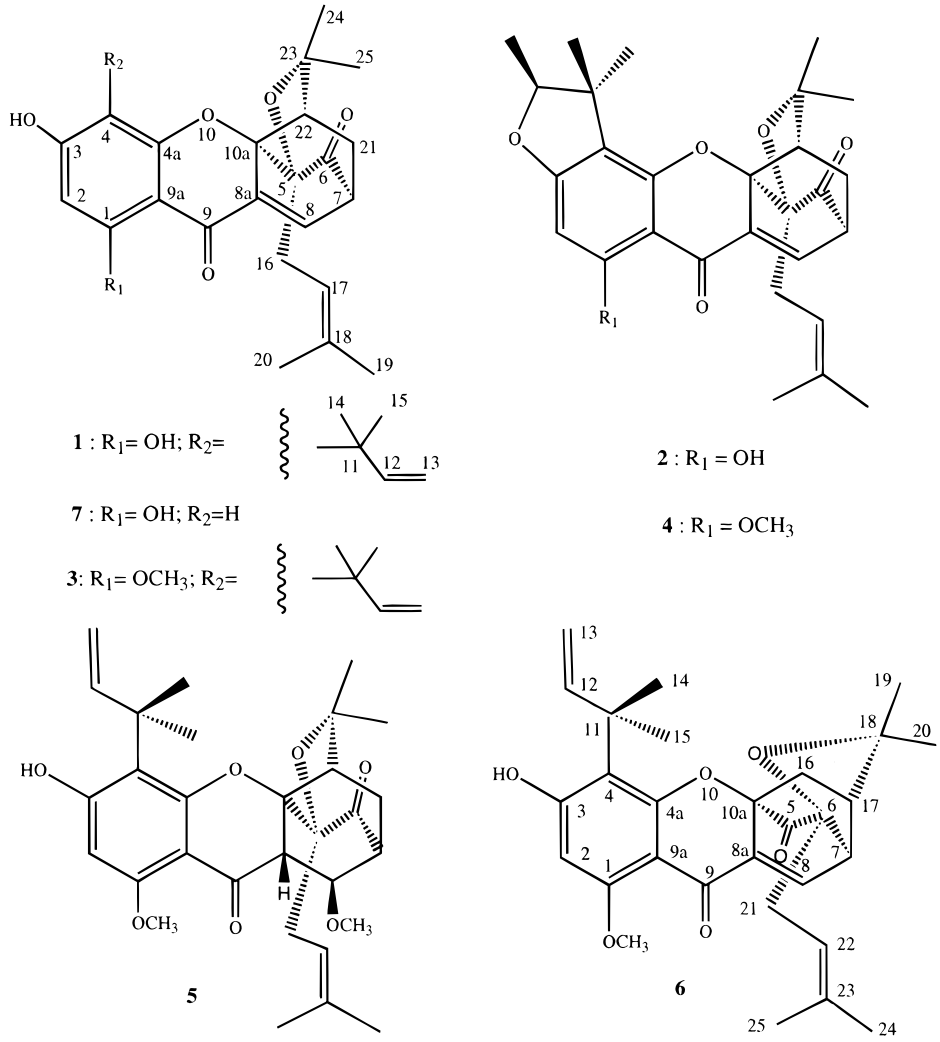
Chart 1



conformer (form **B**) corresponds to the smallest unit-cell. Torsion angles show that the central ring atoms are coplanar with atoms C-7, C-8, C-11, and O-3. The ring described by atoms C-5, C-6, C-7, C-8, C-8a, C-10a is in a perfect boat conformation with atoms C-7 and C-10a as the apexes. The two five-membered rings involving the atoms O-3, C-3, C-4, C-11, C-12 and C-5, O-5, C-23, C-22, C-10a exhibit an envelope conformation with, respectively, atoms C-12 and C-10a as the flap, out of the mean plane of the other four atoms. In both crystal forms, an intramolecular hydrogen bond (average distance O-1···O-9 = 2.561 Å) is observed between the hydroxyl group O-1-H and the oxygen atom O-9. The atomic coordinates for the nonhydrogen atoms are given in the Supporting Information.

1-*O*-Methylbractatin (**3**) gave a major peak $[MH]^+$ at *m*/*z* 479.2429 (HRCIMS), which matched the molecular formula $C_{29}H_{35}O_6$. The ^1H NMR spectrum was similar to that of **1**. Compound **3** shows no chelated OH, but had a methoxyl signal at δ 3.85. These data, together with HMBC correlations, are consistent with 1-*O*-methylbractatin (**3**) (Tables 1 and 2).

1-*O*-Methylisobractatin (**4**) gave a major peak $[MH]^+$ at *m*/*z* 479.2441 ($C_{29}H_{35}O_6$) HRCIMS. Observations similar to those for compound **2** can be drawn from NMR data, and the data are consistent with structure **4**.

Compound **5** gave a major peak $[MH]^+$ at *m*/*z* 511.2686 (HRCIMS), which matched molecular formula $C_{30}H_{39}O_7$. The ^1H NMR spectrum was similar to the spectrum of **3**, but it lacked a signal for H-8 at δ 7.32. Two new signals also appeared at δ 4.45 and 3.16, and one methoxy signal appeared at δ 3.27. These data are consistent with structure **5** and are similar to those observed for isomoreollin B.[10] As the double bond C-8−C-8a is saturated, the C-ring of **5** is in the chair form, and the hydrogen at C-17 is deshielded from δ 4.51 to 5.32, which is no longer in the cone of anisotropy of the A-ring.

1-*O*-Methylneobractatin (**6**) gave a major peak $[MH]^+$ at *m*/*z* 479.2432 (HRCIMS) ($C_{29}H_{35}O_6$). The ^1H NMR spectrum was similar to that of **3**, but H-22 (corresponding to H-17 in **1**−**5**) was deshielded from δ 4.51 to 5.11. In the COSY spectrum, a proton resonance at δ 6.99 (H-8) showed scalar couplings with the resonance at δ 3.69 (H-7), this one being coupled with H-17 at δ 2.18. The resonance of H-17 correlated with a methylene (H_A δ 2.50 and H_B δ 1.93). This indicated a sequence =CH−CH−CH−CH_2. The methyl signals were also slightly shifted. NOESY correlations (Figure 2) existed between protons H-7, H-17, 3H-19, H-8, 2H-21, and H-22. Modeling studies (Macromodel) showed that these data are consistent with structure **6**, which is an isomer of 1-*O*-methylbractatin (**3**). HMBC correlations were also consistent with the proposed structure of **6**.

Biogenetic considerations in this type of molecule have already been hypothetized, but a more evident biogenetic route (Figure 3a in Supporting Information) from synthetic work has been partially demonstrated by Quillinan et al.[25]

Table 1. ^{1}H NMR Data for Compounds **1**−**7**[a] (δ ppm; *J* Hz) and HMBC Correlations for **1** nd **6**

carbon	**1**	HMBC[b] (**1**)	**2**	**3**	**4**	**5**	**6**	HMBC[b] (**6**)	**7**
OH-1	12.95 s	1, 2, 3, 9a, 9	13.00 s						12.5 s
MeO-1				3.85 s	3.88 s	3.89 s	3.82 s	1, 2	
2	6.03 s	1, 3, 4, 9a	6.03 s	6.08 s	6.10 s	6.13 s	6.07 s	1, 3, 4, 9, 9a, 11	6.03 d (2)
OH-3	7.53 s	1, 2, 3, 4		7.53 s		7.01s	7.44 s	1, 2, 3, 4, 4a	6.25 s
4									6.06 d (2)
7	3.51 dd (6, 7)	6, 8a, 22	3.50 dd (5, 7)	3.43 dd (5, 7)	3.42 dd (5, 7)	2.77 t (7)	3.69 dd (5, 7)	5, 6, 8, 8a, 9, 16, 17, 21	3.52 dd (6.5, 4)
8	7.45 d (7)	6, 7, 8a, 9	7.48 d (7)	7.32 d (7)	7.35 d (7)	4.45 dd (4, 1)	6.99 d (7)	5, 6, 7, 8a, 9, 10a, 16, 17	7.44 d (6.5)
8a						3.16 bs			
MeO-8						3.27s			
12	6.48 dd (10,18)	11, 15	4.40 q (6)	6.45 dd (10;18)	4.40 q (6)	6.28dd (10,18)	6.49 dd (10, 18)	4, 11, 14, 15	
13	5.48 d (18) 5.40 d (10)	11, 1211	1.40 d (7)	5.47 d (18) 5.38 d (10)	1.41 d (7)	5.38 d (18) 5.32 d (10.5)	5.47 d (18) 5.34 d (10)	11, 12, 14, 1511, 12, 14, 15	
14	1.87 s	4, 11, 12, 15	1.18s	1.85 s	1.19 s	1.63 s	1.74 s	11, 12, 15	
15	1.58 s	4, 11, 12, 14	1.60 s	1.61 s	1.63 s	1.70 s	1.61 s	11, 12, 14	
16	2.67 m	5, 17, 18	2.62 m	2.68 m	2.57 m	2.67 dd (8.5, 15) 2.90 dd (6, 15)	2.46 d (14) 1.88 dd (10, 14)	5, 7,8a,10a, 175,7, 8a, 10a, 17	2.60 d (8)
17	4.40 t (9)	16, 19, 20	4.36 m	4.51 t (9)	4.46 m	5.32 m	2.18 dd (4, 10)	6, 7, 16, 18	4.45 t (8)
19	1.41 s	17, 18, 20	1.37 s	1.42 s	1.37 s	1.67 s	1.32 s	17, 18,20	1.44 s
20	1.13 s	17, 18, 19	1.09 s	1.19 s	1.10 s	1.61 s	1.36 s	17, 18, 19	1.32 s
21	2.36 dd (5, 13) 1.32 m	5, 7, 8, 23, 10a22, 23, 8a	2.34 dd (5, 13) 1.32 m	2.31 dd (5, 13) 1.35 m	2.28 dd (5, 14) 1.31 m	1.88 dd (6, 15) 1.48 dd (8,15)	2.50 dd (8, 15) 1.93 dd (8, 15)	6, 7, 22, 236, 7, 22, 23	2.34 dd (4, 12.5) 1.30 m
22	2.61 m	7, 10a	2.54 m	2.54 m	2.51 m	2.48 dd (9, 13)	5.11 t (8)	21, 24, 25	2.45 d (9)
24	1.26 s	22, 23, 25	1.28 s	1.24 s	1.26 s	1.12 s	1.74 s	22, 23, 25	1.42 s
25	1.73 s	22, 23, 24	1.74 s	1.71 s	1.73 s	1.38 s	1.58 s	22, 23, 24	1.70 s

[a] $CDCl_3$. [b] Carbon atoms correlated with H on position number.

Table 2. ^{13}C Assignments of Isolated Compounds[a]

carbon	**1**	**2**	**3**	**4**	**5**	**6**
1	163.5	166.2	160.8	163.7	160.7	160.5
OCH_{3-1}			55.9	56.3	56.3	55.9
2	99.0	92.6	95.4	91.9	96.0	95.8
3	165.2	168.5	160.8	165.9	163.1	163.1
4	110.9	113.6	111.7	114.2	112.3	112.7
4a	160.3	156.0	162.1	158.4	162.1	161.8
5	85.0	84.6	84.8	84.5	87.3	200.6
6	204.2	203.7	204.6	204.3	209.8	78.9
7	47.5	47.1	47.1	46.9	46.6	44.1
8	134.3	134.2	132.0	132.3	74.7	131.2
OCH_{3-8}					56.6	
8a	133.2	135.3	135.8[b]	134.5	48.7	138.3
9	179.9	178.9	175.4	174.0	188.1	175.0
9a	101.8	101.4	105.1	104.6	106.5	106.0
10a	91.9	90.9	91.6	84.7	88.1	83.7
11	40.8	43.2	40.9	43.7	41.8	41.4
12	149.9	91.0	150.0	89.0	150.0	150.3
13	113.7	13.5	112.6	13.4	112.1	111.9
14	28.4	24.0	28.6	23.9	30.8	28.4
15	24.6	21.1	25.7	20.8	27.3	27.3
16	29.0	29.0	28.6	28.6	28.1	32.2
17	118.1	117.6	117.6	117.4	118.0	42.5
18	135.4	133.7	134.8[b]	136.0	132.8	83.6
19	25.7	25.6	25.5	25.5	25.9	26.8
20	17.1	17.0	17.2	16.9	18.3	29.5
21	26.8	26.0	27.2	26.3	19.8	29.7
22	49.9	49.4	49.4	49.3	43.8	117.3
23	83.2	83.2	82.8	82.7	81.4	136.0
24	29.4	29.1	29.2	28.9	27.9	18.1
25	30.8	30.8	30.8	30.8	30.8	25.9

[a] In $CDCl_3$. [b] Interchangeable values.

Thus, Claisen rearrangement involving migration of the allyloxy group in C-6 to the ortho position leads to an intermediate that undergoes a Diels−Alder cyclization of the double bond C-22−C-21 on C-10a and C-7, respectively (compounds **1**−**5**). Another mechanism may be proposed (Figure 3b in Supporting Information), in which the Claisen rearrangement involves the allyloxy group at C-5, which gives an isoprenyl substituent on C-6, followed by a Diels−Alder cyclization of the double bond C-16−C-17 on C-10a and C-7, respectively (compound **6**). The rearrangement conforms to the modeling studies done on the hypothetical precursors. Such a Claisen reaction would normally lead to a racemic molecule as it is observed by X-ray of compound **2**. To verify this assumption, we have submitted all the compounds to HPLC on OD chiral column and observed the presence of two peaks corresponding to the two enantiomers. The α_D values of all the compounds were very low, except for compound **1**, which could mean that the enantiomers are in equal amounts. Compound **1** has been purified on a preparative OD column, and we successfully isolated two identical products possessing opposite values of $[\alpha]_D$, the first being −414°, the second +414°. The $[\alpha]_D$ observed for **1** is −85°, which means that the two enantiomers were in a ratio of 60:40, this ratio varying with various crystallizations.

Compounds **1**−**7** are highly cytotoxic against KB cells (Table 3), the most cytotoxic compounds being **1**, **3**, and **6**. Surprisingly, the two enantiomers **1a** and **1b** have the same activity as racemic **1**, which could mean that the cytotoxic effect is not specific. Biological activity has also been evaluated on Farnesyl−Protein transferase (FPTase), showing that **1** and **2** also exhibit good activity (Table 3). Other similar compounds isolated from various *Garcinia* also exhibit high cytoxic activity against various cell lines.[9,10,18,19]

From a chemotaxonomic point of view, *G. bracteata* contains compounds with a rearranged xanthone skeleton,

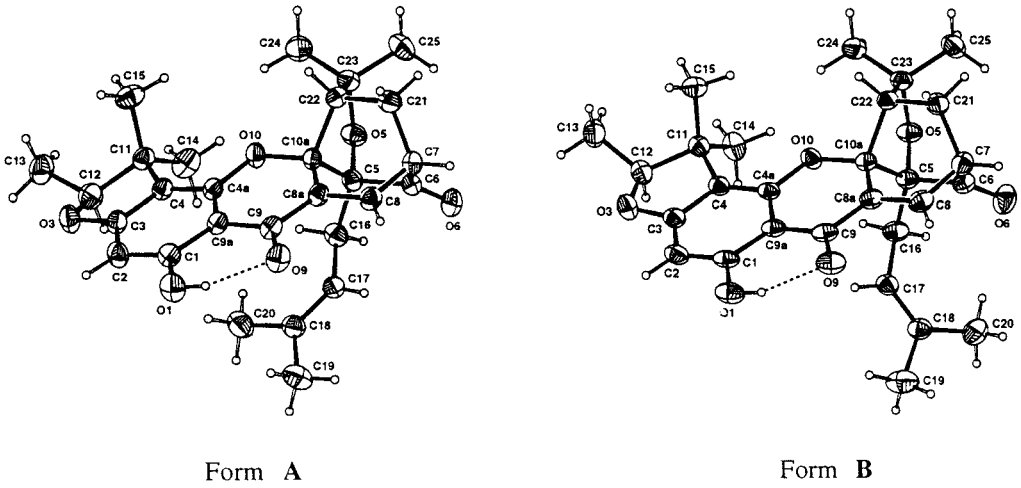


Figure 1. ORTEP diagram (30% probability ellipsoids) showing the crystallographic numbering scheme and solid state conformation of compound **2** in the two crystal forms A and B.

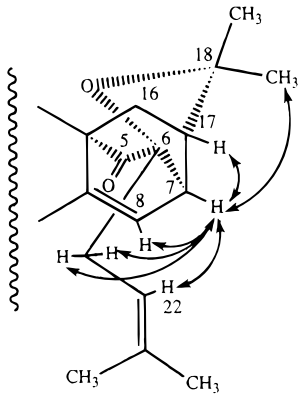


Figure 2. Partial NOESY correlations for **6**.

Table 3. Bioassays on the Isolated Compounds (DMSO)

compound	KB (IC_{50} μg/mL)	FPTase (μg/mL)
EtOAc leaf extract	4	
EtOH leaf extract	9	9.5
1	0.4	4.7
1a	0.4	nt[a]
1b	0.4	nt
2	1.5	5.6
3	0.3	>10
4	0.8	>10
5	1.5	>10
6	0.2	nt
7	0.8	nt

[a] nt = not tested.

similar to those of *G. hanburyi*,[9] *G. morella*,[1,2,8] and *G. gaudichaudii* [18,19] and very different from the other *Garcinia* spp. The genus could exist in two sections, the first one including species with classical xanthones, and the other one including species with molecules having the rearranged skeleton, the so-called caged-xanthanoids,[18,19] as in *G. bracteata*, *G. morella*, *G. forbesii*, *G. gaudichaudii*, and *G. hanburyi*.

Experimental Section

General Experimental Procedures. Optical rotations at 20° were measured on a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Perkin-Elmer spectrum BX FT-IR spectrometer and UV on a Shimadzu UV-161 UV-vis spectrophotometer; HRCIMS were obtained on a Kratos MS 9 mass spectrometer; EIMS were recorded on a Kratos MS 50. The NMR spectra were recorded on a Bruker AC-250 and AC 300 or AM 400 instrument, in $CDCl_3$, using TMS as internal standard. Column chromatography was performed using Si gel Merck H60.

Plant Material. Leaves of *G. bracteata* were collected at Pà Co, Mai Chau, Hoa Binh Province, 150 km west of Hanoi, North Vietnam, in November 1995. Identification was provided by A. Gramain and Nguyen Huu Hien (Institute of Ecology, NCST, Hanoi). Voucher specimens (VN 027) are deposited in the Herbarium of the Institute of Ecology and Biological Resources, NCST, Hanoi, Vietnam.

Extraction and Isolation. The dried, ground leaves of *G. bracteata* (2.88 kg) were extracted in a Soxhlet at room temperature with EtOAc, and the extract was evaporated under vacuum (62 g, yield 2,15%). Repeated column chromatography on Si gel afforded (from heptane/EtOAc to EtOAc/MeOH) isobractatin (**2**) (0.7 g, heptane/EtOAc, 5:5), bractatin (**1**) (1.6 g, heptane/EtOAc, 2:8), 1-*O*-methylisobractatin (**4**) (0.080 g, EtOAc/MeOH, 9.5:0.5), 1-*O*-methylbractatin (**3**) (0.050 g, EtOAc/MeOH, 9:1), 1-*O*-methylneobractatin (**6**) (0.025 g, EtOAc/MeOH, 9:1), 1-*O*-methyl-8-methoxy-8,8a-dihydrobractatin (**5**) (0.037 g, EtOAc/MeOH, 9:1).

Bractatin (**1**): yellow crystals from heptane/EtOAc, 2:8, mp 215−217°; $[\alpha]_D$ −85° (*c* 0.102, $CHCl_3$); *anal.* C 72.31%, H 6.91%, O 20.51%, calcd for $C_{28}H_{32}O_6$ C 72.41%, H 6.90%, O 20.69%; IR ν_{max} ($CHCl_3$) 3389, 1738 (unconjugated carbonyl group), 1641 (α,β-unsaturated ketone), 1589 cm^{-1}; UV λ_{max} nm (ϵ) (EtOH) 357 (9837), 330 (7888); +NaOH, 411 (14384); ^1H and ^{13}C NMR, Tables 1 and 2. EIMS *m/z* $[M]^+$ 464 (17), 436 (100), 421 (43), 339 (73), 297 (60); HRCIMS $[MH]^+$*m/z* 465.2299 (calcd for $C_{28}H_{33}O_6$, 465.2277).

Separation of the two enantiomers of **1** (15 mg) was performed by HPLC on an OD chiral semipreparative column (hexane/2-propanol/acetic acid 95:5:0.1). Compounds **1a** {(7 mg), $[\alpha]_D$ +414° (*c* 0.70, $CHCl_3$)} and **1b** {(7 mg), $[\alpha]_D$ -414° (*c* 0.70, $CHCl_3$)} were obtained.

Isobractatin (2): yellow crystals from heptane/EtOAc, 5:5, mp 188−190°; $[\alpha]_D$ −3° (*c* 0.58, $CHCl_3$); *anal.* C 72.31%, H 7.01%, O 20.95%, calcd for $C_{28}H_{32}O_6$ C 72.41%, H 6.90%, O 20.69%; IR ν_{max} ($CHCl_3$) 1740 (unconjugated ketone), 1638 (α,β-unsaturated ketone), 1590 cm^{-1}; UV λ_{max} nm (ϵ) (EtOH) 353 (16 866), 328 (sh) (12 064); +NaOH, 416 (6844), 345 (12 226), 320 (11 136); ^1H and ^{13}C NMR, Tables 1 and 2; EIMS *m/z* $[M]^+$ 464 (10), 436 (100), 421 (21), 339 (18), 297 (12), 215 (10), 69 (25); HRCIMS $[MH]^+$*m/z* 465.2298 (calcd for $C_{28}H_{33}O_6$, 465.2277).

Acid Transformation of Bractatin (1). A sample of **1** (20 mg) was heated at 80 °C for 90 min in HCOOH (2 mL). The acid medium was then extracted with CH_2Cl_2 (3 × 10 mL) and the organic solution washed with a dilute $NaHCO_3$ solution (5%, 3 × 10 mL) and evaporated to dryness. The reaction mixture was separated on Si gel plates (heptane/acetone, 7:3)

to give isobractatin (**2**) (7.7 mg) and a new compound **7** (5 mg) corresponding to a 4-deprenylbractatin.

Compound 7: amorphous powder; $[\alpha]_D$ −10° (*c* 0.72, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3587, 1738 (unconjugated ketone), 1642 (α,*β*-unsaturated ketone), 1594 cm^{-1}; UV λ_{max} nm (ϵ) (EtOH) 394 (sh) (15 840), 353 (24 047), 325 (sh) (18 480), 203 (67 547); +HCl, 347 (26 003), 320 (sh) (23 760), 208 (69 187); +NaOH, 386 (27 217); ^{1}H NMR, Table 1; CIMS *m/z* $[MH]^+$ 397; HRCIMS $[MH]^+$*m/z* 397.1640 (calcd for $C_{23}H_{25}O_6$, 397.1651).

1-*O*-Methylbractatin (3): amorphous powder, $[\alpha]_D$ −50° (*c* 0.42, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3399, 1737 (unconjugated ketone), 1655 cm^{-1}(α,*β*-unsaturated ketone); UV λ_{max} nm (ϵ) (EtOH) 340 (8962), 326 (9560), 300 (8962); +NaOH, 400 (10 349), 351 (11 878); ^{1}H and ^{13}C NMR, Tables 1 and 2; EIMS *m/z* $[M]^+$ 478 (26), 450 (100), 435 (30), 343 (26); HRCIMS $[MH]^+$*m/z* 479.2429, (calcd for $C_{29}H_{35}O_6$, 479.2434).

1-*O*-Methylisobractatin (4): amorphous powder, $[\alpha]_D$ −6° (*c* 0.52, $CHCl_3$); IR ν_{max} ($CHCl_3$) 1738 (unconjugated ketone), 1656 (α,*β*-unsaturated ketone), 1611 cm^{-1}; UV λ_{max} nm (ϵ) (EtOH) 337 (9105), 320 (8843), 214 (23 135) +NaOH, no change; ^{1}H and ^{13}C NMR, Tables 1 and 2; EIMS *m/z* $[M]^+$ 478 (15), 450 (100), 435 (63), 381 (47), 353 (99), 339 (38), 311 (52), 285 (28); HRCIMS $[MH]^+$*m/z* 479.2441 (calcd for $C_{29}H_{35}O_6$, 479.2434).

1-*O*-Methyl-8-methoxy-8,8a-dihydrobractatin (5): amorphous powder, $[\alpha]_D$ −7° (*c* 2.6, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3413, 1740 (unconjugated ketone), 1670 (α,*β*-unsaturated ketone), 1603 cm^{-1}; UV λ_{max} nm (ϵ) (EtOH) 331 (4250), 292 (12 661), 216 (17 467) +NaOH, 340 (17 340), 331 (16 957), 258 (5843); ^{1}H and ^{13}C NMR, Tables 1 and 2; EIMS *m/z* $[M]^+$ 510 (100), 495 (9), 482 (20), 413 (96), 285 (43), 235 (56); HRCIMS $[MH]^+$ 511.2686 (calcd for $C_{30}H_{39}O_7$, 511.2696).

1-*O*-Methylneobractatin (6): amorphous powder, $[\alpha]_D$ +2° (*c* 0.20, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3412, 1774 (weak) (unconjugated ketone), 1663 (α,*β*-unsaturated ketone), 1616 cm^{-1}; UV λ_{max} nm (ϵ) (EtOH) 317 (9775), 296 (9607), 224 (22 561), 206 (29 540), +NaOH, 344 (10 826), 270 (19 191); ^{1}H and ^{13}C NMR, Tables 1 and 2; EIMS *m/z* $[M]^+$ 464 (28), 450 (100), 435 (18), 381 (99), 353 (74), 339 (31), 325 (18), 311 (20); HRCIMS $[MH]^+$ 479.2432 (calcd for $C_{29}H_{35}O_6$, 479.2434).

X-Ray Structure Analysis of Isobractatin. Crystal data and results for form A: yellow plate crystal (0.30 × 0.40 × 0.52 mm) with a diamond-shaped surface: C_{28} H_{32} O_6, M_w = 464.54, mp 189 °C; triclinic system, space group *P*1, *Z* = 2, *a* = 10.303(4) Å, *b* = 10.480(4) Å, *c* = 13.068(4) Å, α = 111.18(2)°, *β* = 111.49(2)°, *γ* = 71.54(2)°, *V* = 1196.3 Å^3, d_c = 1.290 g cm^{-3}, *F*(000) = 496, λ(Cu Kα) = 1.5418 Å, *μ* = 0.73 mm^{-1}; 6082 measured data (−12 ≤ *h* ≤ 11, −12 ≤ *k* ≤ 11, −11≤ *l* ≤ 15), 4220 unique (R_{int} = 0.048); no decay, absorption ignored. Refinement converged to $R_1(F)$ = 0.0525 for the 3272 observed F_o with $F_o \geq 4\sigma(F_o)$ and $wR_2(F^2)$ = 0.1495 for all the 4220 data with goodness-of-fit *S* = 1.053. An intramolecular hydrogen bond is observed between the hydroxyl O-1-H and the oxygen atom O-9 (distances O-1-H = 0.82, O-1···O-9 = 2.569, H···O-9 = 1.84 Å, angle O−H−O = 147.8°). In the final difference map, the residual electron density was found between −0.19 and 0.48 e Å^{-3}.

Crystal data and results for form B: yellow parallelepiped crystal (0.30 × 0.50 × 0.80 mm); C_{28} H_{32} O_6, M_w = 464.54, mp = 189 °C; triclinic system, space group *P*1, *Z* = 2, *a* = 8.747(3) Å, *b* = 11.935(4) Å, *c* = 13.404(4) Å, α = 116.19(2)°, *β* = 80.74(2)°, *γ* = 110.07(2)°, *V* = 1179.3 Å^3, d_c = 1.308 g cm^{-3}, *F*(000) = 496, λ(Cu Kα) = 1.5418 Å, *μ* = 0.74 mm^{-1}; 5041 measured data (−10 ≤ *h* ≤ 10, −14 ≤ *k* ≤ 12, −9 ≤ *l* ≤ 15), 4213 unique (R_{int} = 0.030); no decay, absorption ignored. Refinement converged to $R_1(F)$ = 0.0471 for the 3593 observed F_o with $F_o \geq 4\sigma(F_o)$ and $wR_2(F^2)$ = 0.1362 for all the 4213 data with goodness-of-fit *S* = 1.088. The same intramolecular hydrogen bond is observed between the atoms O-1-H and O-9 (distances O-1···O-9 = 2.553, H···O-9 = 1.82 Å, angle O−H−O = 148.5°). In the final difference map, the residual electron density was found between −0.18 and 0.24 eÅ^{-3}. This second crystalline form is, thus, more compact than the former.

Intensity data were measured on a CAD4-Nonius diffractometer using graphite monochromated Cu Kα radiation and the (*θ*−2*θ*) scan technique up to *θ* = 68°. Cell parameters were refined from 25 well-centered reflections. The structures were solved by direct methods using program *SHELXS86*[26] and refined by full-matrix least-squares based upon unique F_o^2 with program *SHELXL93*[27]. The hydrogen atoms located in difference Fourier maps were fitted at theoretical positions and assigned an isotropic displacement parameter equivalent to that of the bonded atom plus 20% (30% for those of hydroxyl and methyl groups). For both structures, only normal van der Waals contacts are observed in the packing of the molecules.

KB Cytotoxicity Assay. Experiments were performed in 96-well microtiter plates (2 × 10^5cells mL^{-1}). Cell growth was estimated by colorimetric assay based on conversion of tetrazolium dye to a blue formazan product using live mitochondria.[28] Eight determinations were performed for each concentration. Control growth was estimated at 16 determinations. Optical density at 570 nm corresponding to solubilized formazan was read for each well on a Titertek Multiskan MKII.

FPTase Assay. FPTase was partially purified by ion exchange.[29,30] FPTase activity was measured by fluorimetry, based on fluorescence enhancement of a dansyl group attached to the peptide GCVLS due to changes in the hydrophobic environment by the binding of farnesyl pyrophosphate to the cysteine residue.[31]

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Supporting Information Available: Figures 3a and 3b of proposed biogenetic mechanism of formation of compounds **1**−**6.** Table 3 of X-ray data of crystal forms A and B of compound **2**. This material is available free of charge via the Internet at http://pubs.acs.org. Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: +44(0)1223-336033 or E-mail: deposit@ccdc.cam.ac.uk].

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