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Activity-guided isolation of cytotoxic constituents from the bark of *Aglaia crassinervia* collected in Indonesia

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Abstract—Activity-guided fractionation of a $CHCl_3$-soluble partition of the MeOH extract of the bark of *Aglaia crassinervia* collected in Indonesia led to the isolation of three new glabretal-type triterpenoids, aglaiaglabretols A–C (**1**–**3**), as well as nine known compounds, 3-*epi*-cabraleahydroxylactone (**4**), cabraleahydroxylactone (**5**), rocaglaol (**6**), 2β,3β-dihydroxy-5α-pregn-17(20)-(*E*)-16-one, scopoletin, and mixtures of cabraleadiol and epicotillol and of β-sitosterol and stigmasterol. The structures of compounds **1**–**3** were determined on the basis of spectroscopic and chemical methods. The structure of aglaiaglabretol A (**1**) was confirmed by single-crystal X-ray analysis, and the absolute stereochemistry of this isolate was established by the Mosher ester method. The cytotoxic activity of all isolates and several chemical transformation products obtained in the present study was evaluated. The known cyclopenta[*b*]benzofuran, rocaglaol (**6**), was found to be significantly active and comparable in potency to the positive controls, paclitaxel and camptothecin. Aglaiaglabretol B (**2**) was further tested in an in vivo hollow fiber model.

1. Introduction

In the course of our collaborative research program directed toward the discovery of novel anticancer agents of plant origin,[1] several rocaglate derivatives have been isolated from a number of *Aglaia* species as potent cytotoxic constituents.[2–4] *Aglaia* Lour., the largest genus of the subtropical and tropical angiosperm family Meliaceae, consists of about 130 species distributed mainly in the Indo-Malaysian region, southern mainland China, and the Pacific Islands.[5] The taxonomic classification of *Aglaia* species has presented more difficulty than any other genus of the family Meliaceae.[6] To date, the chemical components of over 30 *Aglaia* species have been studied. The major compounds isolated from this genus are benzo[*b*]oxepines, bisamides, cyclopenta[*b*]benzofurans, cyclopenta[*bc*]benzopyrans, lignans, pregnane steroids, sesquiterpenoids, and triterpenoids.[3,5,7–10] The cyclopenta[*b*]benzofurans and their structurally related compounds, the benzo[*b*]oxepines and the cyclopenta[*bc*]benzopyrans, are possibly derived through the coupling and rearrangement reactions from a flavonoid moiety and a cinnamic acid unit.[11] Therefore, 'flavagline' has been proposed[12] as a classification name for these structurally unique natural products isolated only from the genus *Aglaia*. However, this name has not yet been widely adopted. Cyclopenta[*b*]benzofurans and related compounds are commonly known as rocaglamide (possessing an amide functionality, generally at C-2) or rocaglate (having no amide group) derivatives. Several studies have shown that cyclopenta[*b*]benzofurans represent a group of potent insecti-

Keywords: *Aglaia crassinervia*; Meliaceae; Glabretal triperpenoids; Aglaiaglabretols A–C; Single-crystal X-ray analysis; Mosher ester method; Cytotoxicity; Hollow fiber assay.
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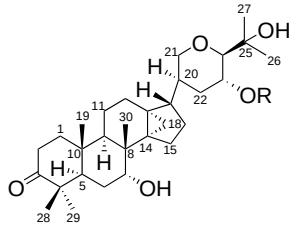
cides, for example.[12,13] In addition, some members of this class of natural products were found to be significantly cytotoxic against human cancer cell lines.[2–4,14] Cyclopenta[*b*]benzofurans have also been found to be highly potent and specific inhibitors of TNF-α or PMA-induced NF-κB activity in different mouse and human T cell lines.[15] Recently, NF-κB was suggested to be a potential target for cancer prevention in chronic inflammatory diseases, since it is essential for promoting inflammation-associated cancer.[16]

The chloroform-soluble partition part of the methanol extract of the bark of *Aglaia crassinervia* Kurz. ex Hiern was found to exhibit cytotoxic activity against several human cancer cell lines. No phytochemical or biological studies have been reported on this plant previously. Bioassay-guided fractionation of this extract using the Lu1 (human lung cancer) cell line to monitor fractionation resulted in the isolation of three new glabretal-type triterpenoids, aglaiaglabretols A–C (**1**–**3**), as well as nine known compounds. Among these isolates, the known cyclopenta[*b*]benzofuran, rocaglaol (**6**), was found to be highly cytotoxic and comparable in potency to the positive controls, paclitaxel and camptothecin. Aglaiaglabretol B (**2**) was further tested in an in vivo hollow fiber model. The structure of aglaiaglabretol A (**1**) was confirmed by a single-crystal X-ray analysis, and the absolute stereochemistry of this new triterpene was determined by a convenient Mosher ester method. We report herein the isolation and structure elucidation of compounds **1**–**3**, the cytotoxicity evaluation of all isolates and chemical transformation products obtained against several human cancer cell lines, and the in vivo hollow fiber biological testing results on aglaiaglabretol B (**2**).

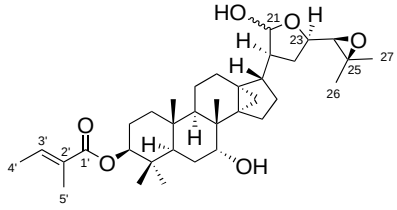
2. Results and discussion

2.1. Structure and absolute stereochemistry determination of aglaiaglabretol A (1)

Aglaiaglabretol A (**1**) was obtained as colorless needles, mp 185–187 °C. A molecular formula of $C_{30}H_{48}O_5$ was assigned to **1** on the basis of its HRESIMS (found *m*/*z* 511.3383, calcd for $C_{30}H_{48}O_5Na$, *m*/*z* 511.3394). The ^1H NMR spectrum of **1** (in $CDCl_3$, Table 1) displayed signals for six singlets characteristic of tertiary methyl groups (CH_3-19, 26, 27, 28, 29, and 30), five oxygenated methine and methylene protons (H-7, H_2-21, H-23, and H-24), and a number of overlapping protons for aliphatic methines and methylenes. In addition, two protons of an aliphatic methylene (confirmed by ^1H–^1H COSY and HMQC correlations) were observed in a relatively high-field region at δ_H 0.69 (1H, br d, J = 4.3 Hz, H-18a) and 0.48 (1H, d, J = 4.6 Hz, H-18b). Consistent with the determined molecular formula, 30 carbon signals appeared in the ^{13}C NMR spectrum (in $CDCl_3$, Table 1)



1 R = H
1r R = (*R*)-MTPA
1s R = (*S*)-MTPA



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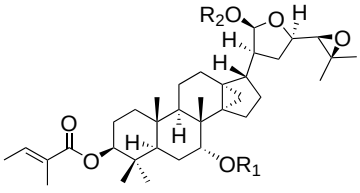
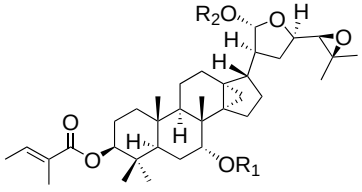
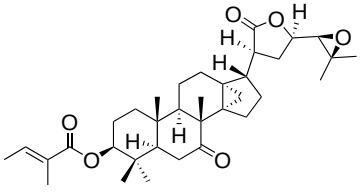


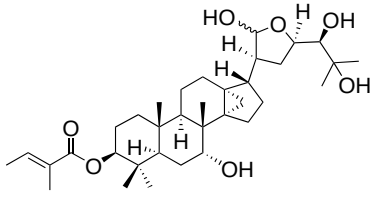
2a R_1 = H R_2 = Ac
2b R_1 = R_2 = Ac



2c R_1 = H R_2 = Ac
2d R_1 = R_2 = Ac



2e



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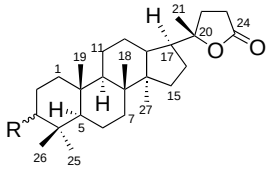

4 R = β-OH
5 R = α-OH

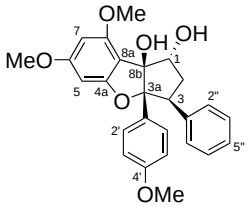

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Table 1. NMR spectroscopic data of compound **1**[a]

Position	In $CDCl_3$ (300/75 MHz)		In pyridine-d_5 (360/90 MHz)	
	δ_H	δ_C	δ_H	δ_C
1	1.80–1.92, m; 1.48, m	39.4 t	1.69–1.73, m; 1.37, m	39.8 t
2	2.47, m	33.9 t	2.50, m	34.2 t
3		218.1 s		216.7 s
4		46.7 s		47.0 s
5	2.11, m	45.3 d	2.40–2.46, m	45.7 d
6	1.67, br dd (7.0, 2.1)	25.4 t	1.69–1.73, m	27.0 t
7	3.83, br s	74.0 d	3.96, br s	73.6 d
8		37.3 s[b]		37.8 s[b]
9	1.26–1.35, m	43.0 d	1.50–1.55, m	43.4 d
10		36.6 s		36.9 s
11	1.26–1.35, m	17.2 t	1.30, m; 1.14, m	17.7 t
12	1.80–1.92, m	28.14 t[c]	2.17, m; 1.69–1.73, m	28.8 t[c]
13		28.5 s		28.4 s
14		38.5 s[b]		39.0 s[b]
15	1.80–1.92, m; 1.53, m	26.1 t	1.80–1.91, m; 1.69–1.73, m	26.4 t
16	2.05, m; 0.90, m	28.08 t[c]	1.80–1.91, m; 0.86, m	28.7 t[c]
17	2.22, br q (7.8)	45.8 d	2.40–2.46, m	46.5 d
18	0.69, br d (4.3); 0.48, d (4.6)	14.3 t	0.98,d (5.2); 0.59, d (5.3)	15.4 t
19	0.95, s	16.0 q	0.90, s	16.0 q
20	1.55, m	40.4 d	1.50–1.55, m	41.1 d
21	4.09, br d (10.6)		4.29, br d (11.6)	
	3.42, br d (10.6)	70.7 t	3.56, dd (11.5, 2.4)	71.2t
22	2.00, m; 1.52, m	36.4 t	2.26, m;1.80–1.91, m	37.5 t
23	3.83, m	64.8 d	4.34, m	65.2 d
24	2.90, d (9.1)	86.6 d	3.37, d (9.2)	87.8 s
25		74.1 s		73.5 s
26	1.31, s	28.5 q	1.66, s	24.9 q
27	1.29, s	24.0 q	1.65, s	29.2 q
28	1.03, s	20.9 q	1.08, s	21.2 q
29	1.09, s	26.7 q	1.18, s	26.8 q
30	1.09, s	19.9 q	1.02, s	20.3 q
OH-7	2.57, br s		4.67, br s	
OH-23	3.66, br s		6.34, br s	
OH-25	3.05, br s		6.53, br s	

[a] TMS was used as internal standard; chemical shifts are shown in the δ scale with J values (Hz) in parentheses. Assignments are based on ^1H–^1H COSY, HMQC, and HMBC spectra.

[b,c] Assignments are interchangeable in the same column.

of **1**. The DEPT135 NMR spectroscopic data indicated that the thirty carbons in the molecule of **1** comprised six methyl groups, 10 methylenes, seven methines, and seven quaternary carbons, which were consistent with its ^1H NMR spectroscopic data analysis. All of the above-described evidence, in combination with a consideration of the chemotaxonomy of the genus *Aglaia*,[3–5,7] suggested that compound **1** is a triterpene. Based on the observed ^{13}C NMR chemical shifts, it was apparent that one saturated ketone (δ_C 218.1, C-3), one oxygenated methylene carbon (δ_C 70.7, C-21), three oxygenated methine carbons (δ_C 64.8, C-23; 74.0, C-7; 86.6, C-24), and one oxygenated quaternary carbon (δ_C 74.1, C-25) were present in the molecule of **1**. In the HMBC spectrum of this compound, correlations were observed from the proton signals of both CH_3-28 and CH_3-29 to C-3, C-4, and C-5, from CH_3-19 to C-1, C-5, C-9, and C-10, from CH_3-30 to C-7, C-8, C-9, and C-14, and from H-7 to C-5, C-9, and CH_3-30. These correlations were used to determine that four of the six methyl groups were in rings A and B of the molecule of **1**. This partial structure is similar to those of many common triterpenoids.[17] On further interpretation of the HMBC spectrum of **1**, correlations from the notably high-field methylene protons at δ_H 0.69 (H-18a) and 0.48 (H-18b) to C-8, C-12, C-13, C-14, C-15, and C-17 were observed. These correlations, in combination with the chemical shifts of H-18a, H-18b, and C-18, and the coupling constant between H-18a and H-18b,[18,19] suggested the presence of a cyclopropyl methylene group in the molecule of **1** located between C-13 and C-14. Therefore, compound **1** was assigned as a glabretal-type triterpene.[18] Generally, triterpenoids possessing a cyclopropyl methylene group isolated from *Aglaia* species are of the cycloartane type,[20,21] in which the cyclopropyl methylene group is between C-9 and C-10. The presence of a tetrahydropyran ring in the side chain was assigned based on the interpretation of the observed correlations in the ^1H–^1H COSY, HMQC, and HMBC spectra of **1**, which was also required by the determined unsaturation value.

As shown in Table 1, some of the overlapping signals of **1** in $CDCl_3$ were separated using pyridine-d_5. For example, the signals of CH_3-29 and CH_3-30 of **1** overlapped at δ_H 1.09 in the ^1H NMR spectrum recorded in $CDCl_3$,

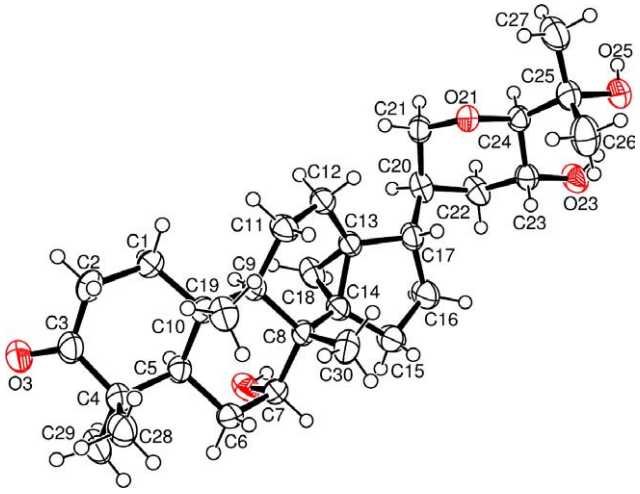

Figure 1. ORTEP drawing of aglaiaglabretol A (**1**).

Table 2. Selected ^{1}H NMR spectroscopic data of (*R*)- and (*S*)-MTPA ester derivatives (**1r** and **1s**) of compound **1**[a]

Position	δ_R	δ_S	δ_S–δ_R
7	3.945, br s	3.946, br s	+0.001
18	0.932[b]	0.975, d (5.4)	+0.043
	0.539, d (5.3)	0.602, d (5.4)	+0.063
20	1.836, m	1.875, m	+0.036
21	4.112, dd (11.0, 6.2)	4.155, dd (11.1, 5.3)	+0.033
	3.788, dd (11.1, 5.0)	3.786, dd (11.0, 4.3)	−0.002
22	2.213, m	2.292, m	+0.079
	1.844, m	1.880, m	+0.036
23	5.965, m	5.906, m	−0.059
24	3.628, d (5.4)	3.537, d (6.2)	−0.091

[a] Spectra were acquired in pyridine-d_5 at 360 MHz from the reaction NMR tubes directly; chemical shifts are shown in the δ scale with *J* values (Hz) in parentheses. Assignments are based on the observed ^{1}H–^{1}H COSY correlations.

[b] Overlapped with the signal of CH_3-30.

while these two resonances were clearly separated at δ_H 1.18 and 1.02 in pyridine-d_5. On interpretation of the NOESY spectra of **1** acquired in both $CDCl_3$ and pyridine-d_5, correlations were observed from H-5 to H-9 and CH_3-29, from H-7 to CH_3-30, and from H-9 to H-18a. These suggested that H-5, H-9, and the cyclopropyl methylene group are located on the same side of the molecule in **1**. The relative configurations of H-20, H-23, and H-24 of the tetrahydropyran ring in the side chain were determined as α, β, and α, respectively, based on the observed NOESY correlations from both H-20 and H-24 to H-21α, and from H-23 to CH_3-26 and CH_3-27. However, the relative stereochemistry of the tetrahydropyran ring of the side chain and the skeletal rings of **1** was difficult to discern, since the C-17/C-20 bond has a free rotation. Accordingly, single-crystal X-ray diffraction analysis (Fig. 1) was performed to confirm the structure and to establish the relative stereochemistry of the entire molecule of **1**. The Flack parameter of −0.03 (19) was determined for this X-ray structure and permits the assignment of the absolute stereochemistry of aglaiaglabretol A (**1**) as shown (Fig. 1). This assignment was consistent with the determination of 23*R* by analysis of the ^{1}H NMR spectroscopic data of **1r** and **1s** (Table 2) obtained using a convenient Mosher ester method.[22]

2.2. Structure elucidation and confirmation analysis of aglaiaglabretol B (2)

Aglaiaglabretol B (**2**), obtained as a white amorphous powder, mp 132–135 °C, showed a sodiated molecular ion peak at *m*/*z* 593.3802 in the HRESIMS, indicating a molecular formula of $C_{35}H_{54}O_6$ (calcd for $C_{35}H_{54}O_6Na$, 593.3818). Both the ^{1}H and ^{13}C NMR data (Table 3) of **2** were very similar to those of **1**, and suggested that compound **2** is also a glabretal triterpene.[18] When compared to **1**, the ^{1}H and ^{13}C NMR spectra of **2** exhibited signals typical for a tigloyloxy substituent at δ_H 6.83 (1H, dq, J = 7.1, 1.3 Hz, H-3′), 1.83 (3H, br s, H-5′), and 1.78 (3H, d, J = 7.1 Hz, H-4′), and δ_C 167.79 (s, C-1′), 136.48 (d, C-3′), 129.27 (s, C-2′), 14.31 (q, C-4′), and 12.09 (q, C-5′).[18,23] In the HMBC spectrum of **2**, the proton signals of both CH_3-28 and CH_3-29 correlated with a carbon signal at δ_C 80.71 (d, C-3), suggesting the presence of an oxygenated methine at C-3 in the molecule of **2** instead of the ketone group at the same position in **1**. On further interpretation of the HMBC spectrum of **2**, a correlation from H-3 to the carbonyl carbon of tigloyloxy group at δ_C 167.79 (s, C-1′) was observed. This correlation was used to establish that the tigloyloxy group was located at C-3 in **2**. The ^{13}C NMR spectrum of this compound displayed signals characteristic for a hemi-acetal group at δ_C 98.25 (d, C-21) and a trisubstituted epoxy group at δ_C 67.66 (d, C-24) and 58.05 (s, C-25). Based on the interpretation of the observed correlations in the ^{1}H–^{1}H COSY, HMQC, and HMBC spectra, the side chain of **2** was determined to possess a tetrahydrofuran ring containing a hemi-acetal group at C-21. In the same manner as for previously reported analogs,[18,24,25] aglaiaglabretol B (**2**) was isolated as an epimeric mixture. The ratio of the major to minor epimers was about 5 to 2. The NMR data for both epimers were assigned and are given in Table 3.

If the difference between the major and minor epimers results from the configuration of the hemi-acetal hydroxyl groups, the coupling constants of H-20α/H-21α and H-20α/H-21β should generally vary, while the splitting patterns of H-23 of the two epimers should be similar. However, the signals of H-21 of the two epimers overlapped at δ_H 5.43 in the ^{1}H NMR spectrum of **2**, since these two hemi-acetal protons had almost identical chemical shifts in $CDCl_3$. Therefore, any differences in the coupling constants of H-20α/H-21α and H-20α/H-21β of these two epimers were not clear. On the other hand, both the chemical shifts and the splitting patterns of H-23 in these epimers were clearly different (Table 3). Based on these spectroscopic observations, it was also thought possible that the relative configuration of C-23 could be different in these epimers, although this was more likely at the hemi-acetal carbon from a chemical stability standpoint. Therefore, aglaiaglabretol B (**2**) was acetylated using acetic anhydride and pyridine. Four acetylation

Table 3. NMR spectroscopic data of compound **2**[a]

Position	Major isomer		Minor isomer	
	δ_H	δ_C	δ_H	δ_C
1	1.53–1.75, m; 1.03–1.09, m	38.27 t	1.53–1.75, m; 1.03–1.09, m	38.17 t
2	1.69–1.75, m; 1.53–1.67, m	23.51 t	1.69–1.75, m; 1.53–1.67, m	23.51 t
3	4.56, dd (11.4, 4.5)	80.71 d	4.56, dd (11.4, 4.5)	80.66 d
4		37.58 s[b]		37.58 s[b]
5	1.53–1.67, m	46.09 d	1.53–1.67, m	46.16 d
6	1.69–1.75, m; 1.53–1.67, m	24.20 t	1.69–1.75, m; 1.53–1.67, m	24.20 t
7	3.76, br s	74.39 d	3.76, br s	74.25 d
8		38.86 s		38.91 s
9	1.21–1.36, m	44.17 d	1.21–1.36, m	43.99 d
10		37.18 s[b]		37.25 *s*[b]
11	1.21–1.36, m	16.37 t	1.21–1.36, m	16.22 t
12	1.53–2.15, m	27.51 t[c]	1.53–2.15, m	25.94 t[c]
13		28.99 s		28.58 s
14		36.88 s		36.01 s
15	1.53–2.15, m	25.29 t[c]	1.53–2.15, m	25.29 t[c]
16	1.53–1.75, m; 0.84–0.91, m	26.34 *t*[c]	1.41, m; 0.94, m	25.98 *t*[c]
17	2.21, br q (7.6)	44.83 d	2.05, m	48.22 d
18	0.67, br d (4.0); 0.48, d (4.2)	13.77 t	0.75, br d (4.2); 0.50, d (4.4)	13.59 t
19	0.91, s	15.97 q	0.90, s	15.85 q
20	1.85–1.91, m	49.35 d	2.08–2.18, m	50.66 d
21	5.43, m	98.25 d	5.43, m	102.16 d
22	1.90–2.01, m; 1.66–1.74, m	30.83 t	2.03–2.11, m; 1.38–1.42, m	32.82 t
23	3.88, ddd (7.4, 7.4, 7.2)	78.43 d	3.95, m	77.34 d
24	2.84, d (7.5)	67.66 d	2.70, d (7.6)	65.35 *d*
25		58.05 s		57.25 s
26	1.30, s	25.03 q	1.35, s	24.94 q
27	1.31, s	19.21 q	1.31, s	19.42 q
28	0.90, s	16.88 q	0.90, s	16.88 q
29	0.88, s	27.82 q	0.88, s	27.82 q
30	1.04, s	19.53 q	1.03, s	19.47 q
OH-7	2.52, br s		2.46, br s	
OH-21	3.06, br s		3.02, br s	
1′		167.79 s		167.79 s
2′		129.27 s		129.27 s
3′	6.83, dq (7.1, 1.3)	136.48 d	6.83, dq (7.1, 1.7)	136.48 d
4′	1.78, d (7.1)	14.31 q	1.78, d (7.1)	14.31 q
5′	1.83, br s	12.09 q	1.83, s	12.09 q

[a] ^1H and ^{13}C NMR spectra were acquired in $CDCl_3$ at 500 and 125 MHz, respectively; TMS was used as internal standard; chemical shifts are shown in the δ scale with J values (Hz) in parentheses. Assignments are based on ^1H–^1H COSY, HMQC, and HMBC spectra.
[b,c] Assignments are interchangeable in the same column.

products, two 7,21-diacetates (**2b** and **2d**) and two 21-monoacetates (**2a** and **2c**), were produced. The coupling constants between H-20 and H-21 in all the acetylation products (**2a**–**2d**) were very close, in a range of 2.7–3.2 Hz. These results still did not indicate whether the epimeric site in aglaiaglabretol B (**2**) is at C-21 or C-23. Hence, aglaiaglabretol B (**2**) was oxidized using CrO_3, leading to only one oxidation product, 7,21-dehydroaglaiaglabretol B (**2e**), which established that the epimers in aglaiaglabretol B (**2**) varied at C-21. Based on the above-described chemical transformation results, as well as the splitting patterns and coupling constants of H-21 and H-23 of compounds **2** (Table 3) and **2a**–**2d**, it could be concluded that the tetrahydrofuran ring has a different conformation when OH-21 or AcO-21 is α- or β-oriented (Fig. 2). In both cases, the angles between H-21/C-21 and H-20/C-20 are closely comparable, and therefore, the coupling constants of H-21/H-20 of all of the isomers are almost the same (2.7–3.2 Hz) (Fig. 2).

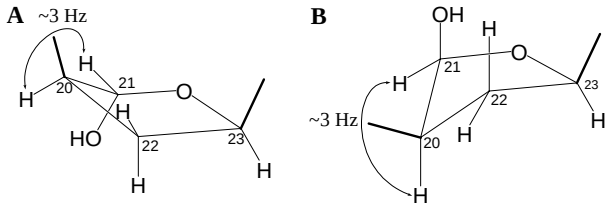

Figure 2. Possible confirmations of the tetrahydrofuran ring in aglaiaglabretol B (**2**) when H-20 and H-21 are *trans*-fused (A) and *cis*-fused (B), respectively.

In the same manner as for aglaiaglabretol A (**1**), H-17 and H-20 of all previously reported glabretal triterpenes[18,19,26,27] have been assigned as β and α, respectively. This information, in combination with the observed NOESY correlation from H-20 to H-23, suggested that both H-20 and H-23 of **2** are α-oriented. On biogenetic grounds, the relative stereochemistry of C-24 of **2** was

assigned as *R*, which is the same as that of **1**. The chemical shift of the hemi-acetal carbon (C-21) of glabretal triterpenes is in a more up-field region when OH-21 or AcO-21 group has a β-orientation.[27] The H-21 protons of **2a** and **2b**, and of **2c** and **2d**, were determined to be α and β, respectively, based on the comparison of the chemical shifts of C-21 of these acetylation products.[27]

2.3. Structure elucidation of aglaiaglabretol C (3)

Both the ^{1}H and ^{13}C NMR data of aglaiaglabretol C (**3**) (Table 4) were very similar to those of **2**. The correlations observed in the 2D NMR spectra (^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY) also suggested the presence of a β-tigloyloxy group at C-3 and a α-hydroxy group at C-7 in the molecule of **3**, the same as **2**. The chemical shifts of C-24 (δ_C 67.66) and C-25 (δ_C 58.05) of **2** were observed in a more downfield region (δ_C 74.80 and 73.74) in the ^{13}C NMR spectrum of **3**. This indicated that a 24,25-dihydroxy functionality in **3** had replaced the 24,25-epoxy group in **2**. The HRESIMS (*m*/*z* 611.3903) was used to assign the molecular formula of **3** as $C_{35}H_{56}O_7$ (calcd for $C_{35}H_{56}O_7Na$, 611.3924), one H_2O unit more than **2**. Aglaiaglabretol C (**3**) was also isolated as an epimeric mixture, and the ratio of the major and minor isomers was about 5 to 1. Only the ^{1}H and ^{13}C NMR data of the major epimer are given in Table 4, since the 2D NMR correlations observed for the minor isomer were not clear enough for complete assignments to be made. In a similar manner as the assignment made for **2** described above, the relative stereochemistry of C-20, C-23, and C-24 of **3** was determined as *S*, *R*, and *R*, respectively.

Table 4. NMR spectroscopic data of compound **3**[a]

Position	**3** (360/90 MHz)	
	δ_H	δ_C
1	1.51–1.74, m; 1.02–1.06, m	38.25 t
2	1.51–1.74, m	23.45 t
3	4.49–4.58, m	80.72 d
4		37.53 s[b]
5	1.51–1.74, m	46.03 d
6	1.51–1.74, m	24.12 t
7	3.77, br s	74.35 d
8		38.84 s[b]
9	1.21–1.32, m	44.07 d
10		36.92 s[b]
11	1.21–1.32, m	16.30 t
12	1.86–2.00, m; 1.51–1.74, m	26.25 *t*[b]
13		28.84 s
14		37.11 s[b]
15	1.51–1.74, m; 1.21–1.32, m	25.67 t[b]
16	1.21–1.32, m; 0.84–0.91, m	27.46 t
17	2.19, m	44.91 d
18	0.67, br d (3.9); 0.47, *d* (4.5)	13.73 t
19	0.90, s	15.97 q
20	1.86–2.00, m	48.61 d
21	5.36, br s	97.29 d
22	1.86–2.00, m; 1.81, m	29.44 t
23	4.49–4.58, m	78.75 d
24	3.14, d (7.6)	74.80 d
25		73.74 s
26	1.26, s	26.72 q
27	1.30, s	26.78 q
28	0.90, s	16.86 q
29	0.88, s	27.80 q
30	1.03, s	19.51 q
OH-7	2.55, br s[b]	
OH-21	5.08, br s[b]	
1′		167.88 s
2′		129.16 s
3′	6.83, dq (7.1, 1.2)	136.66 d
4′	1.79, d (7.1)	14.36 q
5′	1.83, s	12.09 q

[a] ^{1}H and ^{13}C NMR spectra were acquired in $CDCl_3$ at 360 and 90 MHz, respectively; TMS was used as internal standard; chemical shifts are shown in the δ scale with *J* values (Hz) in parentheses. Assignments are based on ^{1}H–^{1}H COSY, HMQC, and HMBC spectra.

[b] Assignments are interchangeable in the same column.

2.4. Known compounds isolated from the bark of *Aglaia crassinervia*

Compounds **4** and **5** displayed very similar ^{1}H and ^{13}C NMR spectra, and the same molecular formula, $C_{27}H_{44}O_3$, was established for both substances based on their HREIMS data. NMR chemical shift differences between these two compounds were evident for the protons and carbons of ring A, especially the splitting pattern of H-3. Interpretation of the observed 2D NMR correlations indicated that compounds **4** and **5** have the same gross structure as certain dammarane-type nortriterpenoids.[28] The H-3 signal was observed as a doublet of doublets at δ_H 3.20 (J = 10.9, 5.2 Hz) and a broad singlet at δ_H 3.39 in the ^{1}H NMR spectra of **4** and **5**, respectively. This suggested that compounds **4** and **5** are epimeric at C-3, which was confirmed by their NOESY spectroscopic correlations. Compound **4**, 3-*epi*-cabraleahydroxylactone, was reported recently as a new natural product from the seed oil of *Camellia japonica*, apparently after hydrolysis of its acetate purified from an acetylated fraction.[29] Compound **5**, cabraleahydroxylactone, was isolated as one of the major components in the present study. This compound was previously reported from *Cabralea polytricha*.[30] Complete ^{1}H and ^{13}C NMR data of **4** and **5** assigned by their 2D NMR spectroscopic correlations are given in Section 4 for the first time.

The other known compounds obtained in this study, rocaglaol (**6**),[31] cabraleadiol,[32] 2β,3β-dihydroxy-5α-pregn-17(20)-(*E*)-en-16-one,[33] epiocotillol,[8] scopoletin,[34] and a mixture of β-sitosterol[35] and stigmasterol,[35] were identified by comparing their physical and spectroscopic data ($[\alpha]_D$, ^{1}H NMR, ^{13}C NMR, DEPT, 2D NMR, and MS) with published values. The H-2α and H-2β signals of rocaglaol (**6**) were misassigned previously.[31] The revised ^{1}H and ^{13}C NMR data assigned by interpretation of the 2D NMR correlations of this rocaglate derivative are given in Section 4.

2.5. Biological activity evaluation

All isolates and chemical transformation products obtained in the present investigation were evaluated for their cytotoxic activity against several human cancer

cell lines (Table 5).[36] Among these 12 isolates, two new glabretal triterpenes, aglaiaglabretols B (**2**) and C (**3**), and the known rocaglate derivative, rocaglaol (**6**), were found to be active principles, while all other compounds were inactive (ED_{50} > 5 μg/ml). As shown in Table 5, rocaglaol (**6**) was demonstrated to be a potent cytotoxic agent. The ED_{50} values of rocaglaol (**6**) against the cancer cell lines (Lu1, LNCaP, and MCF-7) used were comparable to those of the positive controls, paclitaxel (Taxol®) and camptothecin. Moreover, rocaglaol (**6**) was found to be selectively (>330) active against these three cancer cell lines, when compared with the non-tumorigenic HUVEC cell line (Table 5). The limited quantity of compound **6** isolated did not permit any additional biological testing to be carried out as part of the present investigation.

As described above in the description of the structure elucidation and confirmation analysis of aglaiaglabretol B (**2**), in order to confirm the structure and analyze the confirmation of the tetrahydrofuran ring, this compound was acetylated and oxidized. Interestingly, two of the four acetylation products obtained, **2c** and **2d**, in which OAc-21 is α-oriented, were found to be active or marginally active (Table 5). In contrast, two other epimers, **2a** and **2b**, were inactive. The oxidation product **2e** was also inactive against the cell lines used.

Aglaiaglabretol B (**2**) was further evaluated in an in vivo murine hollow fiber assay.[36,37] This assay was developed initially at the U.S. National Cancer Institute (NCI) to prioritize cytotoxic compounds for possible further testing in murine xenograft models.[38,39] Aglaiaglabretol B (**2**) showed discernible inhibitory activity only for the human breast cancer cells (MCF-7) among the three cell types used (human lung cancer, Lu1; hormone-dependent human prostate cancer, LNCaP; and MCF-7) (Fig. 3). At doses of 6.25, 12.5, and 25 mg/kg body weight, aglaiaglabretol B (**2**) showed inhibitions of 37.4%, 41.2%, and 66.2%, respectively, in the growth of MCF-7 cells implanted in the intraperitoneal (ip) compartment. However, at a dose of 50 mg/kg body weight, two mice died due to the toxicity of this compound. In contrast, no significant inhibitory effects were observed in the subcutaneous (sc) compartment of mice using MCF-7 cells with compound **2**. Also at these doses (6.25, 12.5, 25, and 50 mg/kg body weight), no significant inhibitory effects were observed in either the ip or sc compartments of mice for the two other cell types used, Lu1 and LNCaP. Insufficient quantities of **2** remained for further in vivo testing in a murine xenograft model.

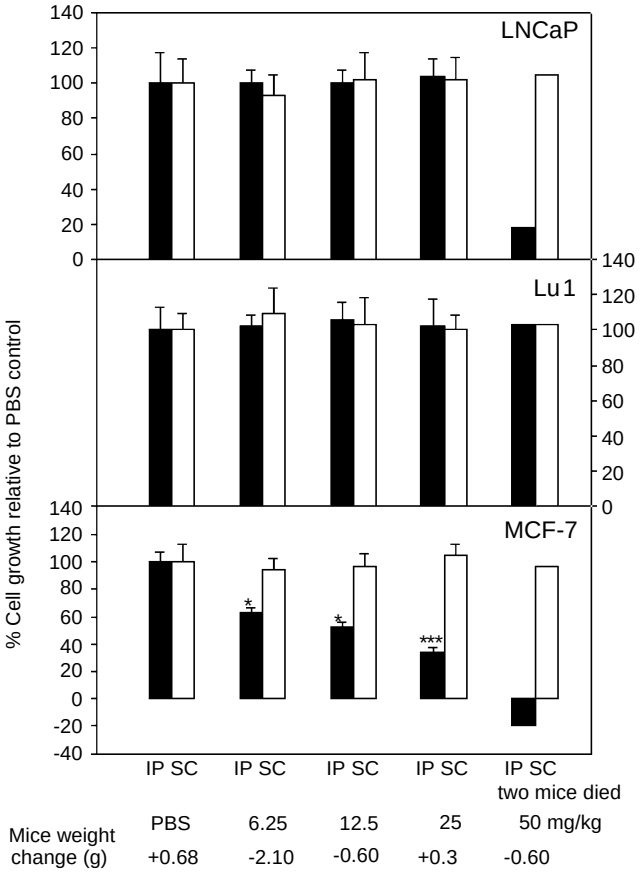

Figure 3. Effect of aglaiaglabretol B (**2**) on the growth of LNCaP, Lu1, and MCF-7 cells implanted at the i.p. (solid column) and the s.c. (open column) compartments of NCr *nu/nu* mice. The animals were treated with PBS (control) or the indicated doses of aglaiaglabretol B (**2**) once daily by intraperitoneal injection from day 3 to 6 after implantation. On day 7, mice were sacrificed, and fibers were retrieved and analyzed. Results are shown as the average percentage of cell growth relative to control, ±SE (bars). Changes in mouse body weight at the end of the experiment are listed at the bottom of the figure. *Treatment groups were significantly different from the control group ($p < 0.01$); ***Treatment groups were significantly different from the control group ($p < 0.0001$) using Student's t test, with $n = 6$ for the control group and $n = 3$ for treatment groups.

Table 5. Cytotoxic activity of compounds **2**, **2c**, **2d**, **3**, and **6**[a]

Compound	Cell line[b]			
	Lu1	LNCaP	MCF-7	HUVEC
2	2.6	0.5	1.7	3.4
2c	6.2	3.8	2.5	8.7
2d	13.9	9.0	12.1	16.3
3	3.6	4.7	3.1	6.8
6	0.006	0.01	0.004	3.3
Paclitaxel[c]	0.002	0.004	0.0006	0.09
Camptothecin[c]	0.01	0.01	0.01	0.09

[a] All other isolates and chemical transformation products obtained in the present investigation are inactive (ED_{50} > 5 μg/mL).
[b] Results are expressed as ED_{50} values (μg/mL). Key to cell lines used: Lu1, human lung cancer; LNCaP, hormone-dependent human prostate cancer; MCF-7, human breast cancer; HUVEC, human umbilical vein endothelial cells.
[c] Used as positive control substances.

3. Conclusion

Twelve compounds were isolated and identified from the bark of *A. crassinervia* in the present study. These were consistent with other studies showing that the characteristic chemical constituents of *Aglaia* species include triterpenoids and rocaglate derivatives.[5] The structure of aglaiaglabretol A (**1**) was confirmed by single-crystal X-ray analysis, and the absolute stereochemistry of this

new triterpene was determined by a convenient Mosher ester method. Glabretal triterpenoids generally possess a hemi-acetal functionality containing a tetrahydropyran or tetrahydrofuran ring in their side chain. Therefore, these triterpenoids are usually isolated as epimeric mixtures. Based on the NMR study of the acetylation and oxidation products of aglaiaglabretol B (**2**), the conformation of the tetrahydrofuran ring was suggested to be different when the hemi-acetal hydroxyl groups have different orientations.

All isolates and chemical transformation products obtained in the present investigation were evaluated for their cytotoxic activity against several human cancer cell lines. The acetylation products **2c** and **2d** were demonstrated to be active or marginally active in the cell lines tested (Table 5). However, **2a** and **2b**, the epimeric isomers of **2c** and **2d**, were found to be inactive for the same cell lines. This suggested that the cytotoxic activity of the acetylation products (**2a**–**2b**) of aglaiaglabretol B (**2**) is related to the configuration of the acetoxy group at C-21. Only one cyclopenta[*b*]benzofuran derivative was isolated in the present investigation, the known rocaglaol (**6**), which was highly cytotoxic and comparable in potency to the positive controls, paclitaxel and camptothecin. Furthermore, **6** was demonstrated to be selectively (>330) cytotoxic toward the cancer cell lines in this study as compared to the non-tumorigenic HUVEC cell line (Table 5). The insecticidal[31] and antifungal[40] activities of **6** have been reported previously. This rocaglate derivative was also demonstrated to be an inhibitor of protein synthesis.[41] Very recently, **6** was indicated to be a potent inhibitor of IL-1β-, TNFα-, and LPS-induced expression of various cytokines and chemokines in human endothelial and/or murine glial cells.[42] This compound was found also to possess neuroprotective activity in vitro and in animal models of Parkinson's disease and traumatic brain injury.[42] The biological results of our present investigation and previous studies[31,40–42] suggest that rocaglaol (**6**) and related cyclopenta[*b*]benzofurans have excellent potential for further investigation as biologically active natural products.

4. Experimental

4.1. General experimental procedures

Melting points were determined with a Thomas Hoover capillary melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 automatic polarimeter. UV spectra were obtained with a Beckman DU-7 spectrometer, and the IR spectra were run on an ATI Mattson Genesis Series FT-IR spectrophotometer. NMR spectroscopic data were recorded at room temperature on a Bruker Avance DPX-300, 360, or DRX-500 MHz spectrometer with tetramethylsilane (TMS) as an internal standard. Standard pulse sequences were employed for the measurement of 2D NMR spectra (^{1}H–^{1}H COSY, HMQS, HMBC, and NOESY). HRESIMS and EIMS were performed on a JEOL GCmate II mass spectrometer, while FABMS was obtained on a VG 7070E-HF sector-field mass spectrometer. Column chromatography was carried out with Si gel G (Merck, 70-230 or 230-400 mesh). Analytical thin-layer chromatography (TLC) was performed on 250 μm thickness Merck Si gel 60 F_{254} aluminum plates, while preparative thin-layer chromatography was performed on precoated 500 or 1000 μm thick Merck Si gel 60 F_{254} glass plates.

X-ray crystallographic analysis data collection for aglaiaglabretol A (**1**) was carried out on a Rigaku RAPID area detector using Cu Kα radiation. The WinGX package[43] was used for completing the structure determination, the direct method, SIR-92,[44] was used to locate non-hydrogen atoms, and ORTEP[45,46] was used to generate Figure 1.

4.2. Plant material

The bark (850 g) of *A. crassinervia* was collected in Lombok Island, Indonesia, in October 2001, and identified by S.R. A voucher specimen (collection No. SR-040) has been deposited at the Herbarium Bogoriense, Indonesia Institute of Science, Bogor, Indonesia.

4.3. Cytotoxicity evaluation procedures

The cytotoxic activity of extracts, chromatographic fractions, and pure compounds was evaluated against a panel of human cancer cell lines (Table 5), according to established protocols.[36]

4.4. In vivo hollow fiber evaluation of aglaiaglabretol B (2)

Aglaiaglabretol B (**2**) was evaluated for its biological potential in the murine hollow fiber in vivo model as described previously.[36,37]

4.5. Extraction and isolation

The dried and milled bark (850 g) was extracted using MeOH (3 × 3 L) at rt, for two days each. After filtration and evaporation of the solvent under reduced pressure, the combined crude methanolic extract was suspended in H_2O (700 mL), which was then partitioned, in turn, with petroleum ether (3 × 500 mL), $CHCl_3$ (3 × 500 mL), and EtOAc (3 × 500 mL), to afford dried petroleum ether- (5.2 g), $CHCl_3$- (20.0 g), EtOAc- (1.5 g), and H_2O-soluble (ca. 14.6 g) extracts. The $CHCl_3$-soluble extract was found to be active in the Lu1 cell line with an ED_{50} value of 0.29 μg/mL. This extract was therefore chromatographed over a Si gel column (7 × 40 cm, 460 g 230–400 mesh), and eluted with gradient mixtures of $CHCl_3$–MeOH (from 50:1 to 2:1), to afford seven fractions (F01–F07). These fractions were again evaluated in the Lu1 cell line, and the ED_{50} (μg/mL) values were 1.6, 0.19, 1.4, 1.7, 16.4, >20, and >20, respectively.

Fractions F01 and F02 were combined and chromatographed over a Si gel column (5.5 × 40 cm) and eluted with petroleum ether–acetone (12:1 to 1:1), to give seven subfractions (F0101–F0107). Fraction F0102 was purified by Si gel column (4.0 × 40 cm) chromatography

using $CHCl_3$–acetone (60:1 to 10:1) as solvents, to give a mixture of β-sitosterol and stigmasterol (250 mg), and a mixture of cabraleadiol and epiocotillol (125 mg), in order of polarity. The ^1H NMR integration suggested the ratio of β-sitosterol and stigmasterol was about 3:2, while the ratio of cabraleadiol and epiocotillol was about 1:1. These two mixtures of natural products could not be separated by HPLC and were not investigated further. Compound **5** (520 mg) was obtained as a white amorphous powder from a $CHCl_3$–acetone (ca. 12:1) solution of F0103. Subfraction F0104 was chromatographed over a Si gel column (3.8 × 45 cm) and eluted with $CHCl_3$–acetone (30:1 to 10:1), yielding an additional amount of **5** (6 mg) and a mixture of **4** and **5** (58 mg). This mixture was then purified by preparative TLC (Merck 60 Å Si gel, 20 × 20 cm, 500 μm), developed with $CHCl_3$–MeOH (40:1), to afford **5** (R_f = 0.62; 9.0 mg), and its *epi*-isomer, compound **4** (R_f = 0.60; 15.2 mg). Subfraction F0105 was purified over a Sephadex LH-20 column (3.5 × 45 cm) and eluted with MeOH, to give four further subfractions (F010501–F010504). Aglaiaglabretol B (**2**, 85 mg) was obtained as a white amorphous powder from a *n*-hexane–EtOAc (ca. 1:1) solution of F010502. Fraction F010503 was purified over a Si gel column (4.0 × 30 cm) and eluted with $CHCl_3$–MeOH (100:1), to give aglaiaglabretol A (**1**, 78 mg). Fraction F010504 was chromatographed over a Si gel column (4.0 × 30 cm) and eluted with $CHCl_3$–MeOH (80:1 to 60:1), and afforded a mixture (18 mg) and 2β,3β-dihydroxy-5α-pregn-17(20)-(*E*)-en-16-one (20 mg), in order of polarity. This mixture was then purified by preparative TLC (Merck 60 Å Si gel, 20 × 20 cm, 1000 μm), developed with $CHCl_3$–acetone (5:2), to yield **6** (R_f = 0.56; 10.0 mg).

The other two active fractions from the initial column chromatography, F03 and F04, were combined and fractionated over a Si gel column (4.0 × 40 cm) using petroleum ether–acetone (8:1 to 1:1) as eluant, and afforded six subfractions (F0301–F0306). Fraction F0302 was purified over a Si gel column (4.0 × 30 cm) and eluted with $CHCl_3$–MeOH (100:1), to give an additional amount of aglaiaglabretol A (**1**, 13.5 mg) and scopoletin (15.0 mg). Fraction F0304 was purified over a Sephadex LH-20 column (3.5 × 45 cm) and eluted with $CHCl_3$–MeOH (1:1), to give five further subfractions (F030401–F030405). Aglaiaglabretol C (**3**, 38 mg) was obtained as a white amorphous powder from *n*-hexane–EtOAc (ca. 1:2) solution of F030402.

4.6. Aglaiaglabretol A (1)

Colorless needles, mp 185–187 °C; $[\alpha]_D^{23}$ +42.6° (*c* 3.0, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 210 (3.45) nm; IR υ_{max} (film) 3383, 1699, 1453, 1386, 1067 cm^{-1}; HRESIMS *m*/*z* 511.3383 (calcd for $C_{30}H_{48}O_5Na$, 511.3394); for ^1H and ^{13}C NMR data, see Table 1.

4.7. X-ray crystallographic data of aglaiaglabretol A (1)

A colorless crystal was obtained from *n*-hexane–EtOAc (ca. 2:1). The data were collected at 150 K. Cell parameters: a = 20.9018 (14) Å; b = 7.4919 (5) Å; c = 17.7797 (13) Å; β = 101.993 (5)°; V = 2723.4 (3) $Å^3$, at 150 K; space group C_2, Z = 4; D_{calc} = 1.192 g/cm^3; λ = 1.5418 Å; μ(CuKα) = 0.624 mm^{-1}; F(000) = 1072. Data collection yielded 13,503 reflections of which 4442 were considered unique. Full-matrix least-squares refinement led to a final R (all), $R(I > 2\sigma)$, and GOF values of 0.0486, 0.0470, and 2.362. The Flack parameter was −0.03 (19) to assign the correct absolute stereochemistry from anomalous dispersion. Crystallographic data (excluding structure factors) for the structure of this compound have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 273768. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

4.8. Preparation of (*R*)- and (*S*)-MTPA esters (1r and 1s) of aglaiaglabretol A (1) using a convenient Mosher ester method

The (*R*)- and (*S*)-MTPA ester derivatives (**1r** and **1s**) of aglaiaglabretol A were prepared as described previously.[24] Briefly, two portions of aglaiaglabretol A (**1**) were transferred into two clean NMR tubes (each 1 mg). After the samples were dried completely under the vacuum of an oil pump, deuterated pyridine was added to both tubes (each 0.5 mL). Then, (*R*)-(−)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (5 μL) was added to one tube, while (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (5 μL) was added to another tube. The reaction NMR tubes were stood at rt and their ^1H NMR spectra were monitored every hour. The reactions were found to be completed after 4 h. Selected ^1H NMR spectroscopic data of **1r** and **1s** are given in Table 2.

4.9. Aglaiaglabretol B (2)

White amorphous powder, mp 132–135 °C; $[\alpha]_D^{23}$ +21.3° (*c* 2.0, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 210 (very broad) (3.78) nm; IR υ_{max} (film) 3422, 1705, 1652, 1389, 1269, 1026 cm^{-1}; ESIMS *m*/*z* (rel. int.): 593.5 $[M+Na]^+$ (52), 553.2 (18), 481.3 (13), 263.3 (14); HRESIMS *m*/*z* 593.3802 (calcd for $C_{35}H_{54}O_6Na$, 593.3818); for ^1H and ^{13}C NMR data, see Table 3.

4.10. Preparation of 7,21-diacetates (2b and 2d) and 21-monoacetates (2a and 2c) of aglaiaglabretol B

Aglaiaglabretol B (18 mg) was dissolved in anhydrous pyridine (0.5 mL) in a vial (4 mL), and acetic anhydride (0.3 mL) was added. After standing at rt overnight, the reaction mixture was evaporated to dryness under reduced pressure at ca. 45 °C. The products were then purified by preparative TLC (20 × 20 cm, 500 μm) developed with *n*-hexane–EtOAc–MeOH (70:70:3), to give four separated bands, namely, two 7,21-diacetates of aglaiaglabretol B, **2d** (R_f 0.80; 3.0 mg) and **2b** (R_f 0.78; 1.7 mg), and two 21-monoacetates of aglaiaglabretol B, **2c** (R_f 0.60; 5.5 mg) and **2a** (R_f 0.56; 4.5 mg), in order of polarity.

2a: white amorphous powder, mp 198–200 °C; $[\alpha]_D^{23}$ +72.1° (*c* 0.38, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, TMS) δ 6.83 (1H, dq, J = 7.0, 1.2 Hz, H-3′), 6.29 (1H, d, J = 3.2 Hz, H-21), 4.56 (1H, dd, J = 11.4, 4.6 Hz, H-3), 3.89 (1H, m, H-23), 3.75 (1H, br s, H-7), 2.67 (1H, d, J = 7.6 Hz, H-24), 2.01–2.11 (3H, m, H-17, H-20, and H-22a), 2.08 (3H, s, OAc-21), 1.95 (1H, br dd, J = 20.4, 11.6 Hz, H-15a), 1.83 (3H, s, CH_3-5′), 1.78 (3H, d, J = 7.0 Hz, CH_3-4′), 1.66–1.76 (5H, m, H-2a, H-6a, H-12a, H-16a, and H-22b), 1.56–1.64 (6H, m, H-1a, H-2b, H-5, H-6b, H-12b, and H-15b), 1.32 (3H, s, CH_3-27), 1.25–1.31 (3H, m, H-9, H-11a, and H-11b), 1.28 (3H, s, CH_3-26), 1.07 (1H, m, H-1b), 1.02 (3H, s, CH_3-30), 1.01 (1H, m, H-16b), 0.904 (3H, s, CH_3-28), 0.895 (3H, s, CH_3-19), 0.86 (3H, s, CH_3-29), 0.73 (1H, br d, J = 4.9, H-18a), 0.44 (1H, d, J = 5.0 Hz, H-18b); ^{13}C NMR (125 MHz, $CDCl_3$, TMS) δ 169.9 (OAc-21, s), 167.8 (C-1′, s), 136.5 (C-3′, d), 129.3 (C-2′, s), 97.6 (C-21, d), 80.6 (C-3, d), 79.8 (C-23, d), 74.2 (C-7, d), 66.7 (C-24, d), 57.1 (C-25, s), 47.9 (C-20, d), 46.2 (C-5, d), 44.8 (C-17, d), 43.9 (C-9, d), 39.0 (C-8, s), 38.1 (C-10, s), 37.6 (C-1, t), 37.3 (C-4, s), 36.6 (C-14, s), 30.6 (C-22, t), 28.9 (C-13, s), 27.8 (CH_3-29, q), 27.5 (C-16, t), 26.3 (C-15, t), 25.1 (C-2, t), 24.9 (CH_3-27, *q*), 24.2 (C-6, t), 23.5 (C-12, t), 21.6 (OAc-21, q), 19.4 (CH_3-30, q), 19.3 (CH_3-26, q), 16.9 (CH_3-28, q), 16.3 (C-11, t), 15.8 (CH_3-19, q), 14.3 (CH_3-4′, q), 13.3 (C-18, t), 12.1 (CH_3-5′, q); ESIMS *m*/*z* (rel. int.) 635.2 $[M+Na]^+$ (60), 569.9 (7), 481.2 (14), 463.1 (5), 263.3 (8), 315.2 (3).

2b: colorless gum, $[\alpha]_D^{23}$ +42.2° (*c* 0.14, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, TMS) δ 6.84 (1H, dq, J = 7.0, 1.4 Hz, H-3′), 6.26 (1H, d, J = 2.7 Hz, H-21), 4.99 (1H, br s, H-7), 4.57 (1H, dd, J = 11.2, 4.8 Hz, H-3), 3.88 (1H, m, H-23), 2.66 (1H, *d*, J = 7.6 Hz, H-24), 1.99–2.06 (3H, m, H-17, H-20, and H-22a), 2.08 (3H, s, OAc-21), 2.03 (3H, s, OAc-7), 1.95 (1H, br dd, J = 20.4, 11.6 Hz, H-15a), 1.83 (3H, s, CH_3-5′), 1.79 (3H, d, J = 7.0 Hz, CH_3-4′), 1.66–1.76 (5H, m, H-2a, H-6a, H-12a, H-16a, and H-22b), 1.58–1.73 (10H, m, H-1a, H-2a, H-2b, H-6a, H-6b, H-12a, H-15a, H-15b, H-16a, and H-22b), 1.42 (1H, m, H-5), 1.32 (3H, s, CH_3-27), 1.25–1.30 (3H, m, H-9, H-11a and H-11b), 1.27 (3H, s, CH_3-26), 1.05–1.11 (1H, m, H-1b), 1.08 (3H, s, CH_3-30), 0.92 (3H, s, CH_3-19), 0.84–0.89 (1H, m, H-16b), 0.87 (3H, s, CH_3-28), 0.77 (3H, s, CH_3-29), 0.65 (1H, br d, J = 5.5, H-18a), 0.35 (1H, d, J = 5.6 Hz, H-18b); ^{13}C NMR (125 MHz, $CDCl_3$, TMS) δ 170.3 (OAc-7, s), 170.0 (OAc-21, s), 167.8 (C-1′, s), 136.7 (C-3′, d), 129.2 (C-2′, s), 97.7 (C-21, d), 80.3 (C-3, d), 79.9 (C-23, d), 76.0 (C-7, d), 66.8 (C-24, d), 57.1 (C-25, s), 48.1 (C-17 and C-20, d), 47.5 (C-5, d), 45.2 (C-9, d), 38.3 (C-1, t), 38.1 (C-8, s), 37.6 (C-4, s), 37.1 (C-10, s), 36.9 (C-14, s), 30.5 (C-22, t), 29.1 (C-13, s), 27.7 (CH_3-29, q), 27.5 (C-12 or C-15, t), 26.5 (C-16, t), 25.7 (C-12 or C-15, t), 24.9 (CH_3-27, q), 23.5 (C-6, t), 23.2 (C-2, *t*), 21.6 (OAc-21, q), 21.5 (OAc-7, q), 19.5 (CH_3-30, q), 19.3 (CH_3-26, *q*), 16.7 (C-11, t), 16.6 (CH_3-19, and CH_3-28, q), 14.5 (C-18, t), 14.3 (CH_3-4′, q), 12.1 (CH_3-5′, q); ESIMS *m*/*z* (rel. int.) 677.4 $[M+Na]^+$ (100), 595.1 (12), 535.3 (5), 523.2 (14), 463.3 (13), 435.2 (6), 363.3 (8).

2c: white amorphous powder, mp 184–185 °C; $[\alpha]_D^{23}$ + 8.3° (*c* 0.46, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, TMS) δ 6.83 (1H, dq, J = 7.0, 1.2 Hz, H-3′), 6.29 (1H, d, J = 3.2 Hz, H-21), 4.56 (1H, dd, J = 11.4, 4.6 Hz, H-3), 3.91 (1H, ddd, J = 10.1, 7.3, 5.8 Hz, H-23), 3.77 (1H, br s, H-7), 2.74 (1H, d, J = 7.5 Hz, H-24), 2.34 (1H, ddd, J = 17.2, 8.5, 3.6 Hz, H-20), 2.05-2.13 (2H, m, H-17, and H-22a), 2.07 (3H, s, OAc-21), 1.94 (1H, br dd, J = 20.4, 11.8 Hz, H-15a), 1.83 (3H, s, CH_3-5′), 1.79 (3H, d, J = 7.0 Hz, CH_3-4′), 1.66-1.75 (4H, m, H-2a, H-6a, H-12a, and H-16a), 1.55–1.63 (6H, m, H-1a, H-2b, H-5, H-6b, H-12b, and H-15b), 1.47 (1H, br q, J = 12.3 Hz, H-22b), 1.33 (3H, s, CH_3-27), 1.23–1.32 (3H, m, H-9, H-11a and H-11b), 1.31 (3H, s, CH_3-26), 1.06 (1H, m, H-1b), 1.03 (3H, s, CH_3-30), 0.95 (1H, m, H-16b), 0.91 (3H, s, CH_3-19), 0.90 (3H, s, CH_3-28), 0.87 (3H, s, CH_3-29), 0.72 (1H, br d, J = 4.7, H-18a), 0.49 (1H, d, J = 5.0 Hz, H-18b); ^{13}C NMR (125 MHz, $CDCl_3$, TMS) δ 170.5 (OAc-21, s), 167.8 (C-1′, s), 136.5 (C-3′, d), 129.3 (C-2′, s), 101.4 (C-21, d), 80.6 (C-3, d), 79.3 (C-23, d), 74.3 (C-7, d), 64.9 (C-24, d), 57.0 (C-25, s), 48.5 (C-20, d), 48.1 (C-17, d), 46.1 (C-5, d), 44.0 (C-9, d), 38.8 (C-8, s), 38.3 (C-1, t), 37.6 (C-4, s), 37.2 (C-10, s), 36.2 (C-14, s), 31.9 (C-22, t), 28.3 (C-13, *s*), 27.8 (CH_3-29, q), 26.0 (C-15, t), 25.8 (C-12, and C-16, t), 24.9 (CH_3-27, q), 24.2 (C-6, t), 23.5 (C-2, t), 21.4 (OAc-21, q), 19.5 (CH_3-26, and CH_3-30, q), 16.9 (CH_3-28, q), 16.3 (C-11, t), 15.9 (CH_3-19, q), 14.3 (CH_3-4′, q), 13.9 (C-18, t), 12.1 (CH_3-5′, q); ESIMS *m*/*z* (rel. int.) 635.3 $[M+Na]^+$ (63), 569.9 (8), 481.1 (16), 463.2 (6), 263.3 (8).

2d: colorless gum, $[\alpha]_D^{23}$ −20.0° (*c* 0.25, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, TMS) δ 6.84 (1H, dq, J = 7.0, 1.4 Hz, H-3′), 6.25 (1H, d, J = 3.2 Hz, H-21), 5.01 (1H, br s, H-7), 4.57 (1H, dd, J = 11.2, 4.8 Hz, H-3), 3.89 (1H, ddd, J = 10.2, 7.3, 5.9 Hz, H-23), 2.74 (1H, d, J = 7.5 Hz, H-24), 2.29 (1H, ddd, J = 16.8, 8.5, 3.0 Hz, H-20), 2.09 (1H, m, H-22a), 2.06 (3H, s, OAc-21), 2.03 (3H, s, OAc-7), 2.00 (1H, m, H-17), 1.81–1.87 (2H, m, H-12a, and H-15a), 1.83 (3H, s, CH_3-5′), 1.79 (3H, d, J = 7.0 Hz, CH_3-4′), 1.57-1.71 (7H, m, H-1a, H-2a, H-2b, H-6a, H-6b, H-12b, and H-16a), 1.40-1.48 (3H, m, H-5, H-15b, and H-22b), 1.33 (3H, s, CH_3-27), 1.30 (3H, *s*, CH_3-26), 1.25–1.29 (3H, m, H-9, H-11a and H-11b), 1.05–1.11 (1H, m, H-1b), 1.09 (3H, s, CH_3-30), 0.92 (3H, s, CH_3-19), 0.88 (3H, s, CH_3-28), 0.84 (1H, m, H-16b), 0.78 (3H, s, CH_3-29), 0.65 (1H, br d, J = 5.5, H-18a), 0.35 (1H, d, J = 5.6 Hz, H-18b); ^{13}C NMR (125 MHz, $CDCl_3$, TMS) δ 170.5 (OAc-21, s), 170.2 (OAc-7, s), 167.9 (C-1′, s), 136.8 (C-3′, d), 129.1 (C-2′, s), 101.3 (C-21, d), 80.3 (C-3, d), 79.3 (C-23, d), 76.1 (C-7, *d*), 64.9 (C-24, d), 57.0 (C-25, s), 48.8 (C-20, d), 48.1 (C-17, d), 47.4 (C-5, d), 45.3 (C-9, d), 38.4 (C-1, t), 38.0 (C-8, s), 37.6 (C-4, s), 37.1 (C-10, s), 36.5 (C-14, s), 32.0 (C-22, t), 28.5 (C-13, *s*), 27.7 (CH_3-29, q), 26.5 (C-12, C-15, or C-16, t), 26.2 (C-12, C-15, or C-16, t), 26.0 (C-12, C-15, or C-16, t), 24.9 (CH_3-27, q), 23.5 (C-6, *t*), 23.2 (C-2, t), 21.4 (OAc-21, q), 21.5 (OAc-7, q), 19.6 (CH_3-30, q), 19.5 (CH_3-26, q), 16.8 (C-11, t), 16.6 (CH_3-28, q), 16.1 (CH_3-19, q), 15.2 (C-18, t), 14.3 (CH_3-4′, q), 12.1 (CH_3-5′, q);

ESIMS *m*/*z* (rel. int.) 677.3 $[M+Na]^+$ (100), 617.4 (13), 596.0 (10), 523.1 (12), 463.3 (12), 435.3 (7), 363.2 (18), 315.2 (7).

4.11. Oxidation of aglaiaglabretol B

Aglaiaglabretol B (6.0 mg) was dissolved in anhydrous pyridine (0.5 mL) in a vial (4 mL), and CrO_3 (10.0 mg) was then added. After standing at rt overnight, the reaction mixture was filtered through a small silica gel (1 g) column (0.5 × 4.2 cm), eluted with $CHCl_3$–MeOH (8:1, 10 mL). The elution was evaporated to dryness under reduced pressure at ca. 45 °C, and then purified by preparative TLC (20 × 20 cm, 500 μm) developed with *n*-hexane–EtOAc (4:1), to give the oxidation product of aglaiaglabretol B, 7,21-dehydroaglaiaglabretol B (**2e**, R_f 0.65; 3.8 mg). **2e**: colorless gum, $[\alpha]_D^{23}$ −46.7° (*c* 0.30, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, TMS) δ 6.84 (1H, dq, *J* = 7.0, 1.4 Hz, H-3′), 4.52 (1H, dd, *J* = 11.6, 4.3 Hz, H-3), 4.17 (1H, ddd, *J* = 11.2, 7.1, 4.3 Hz, H-23), 2.91 (1H, ddd, *J* = 11.5, 8.5, 4.2 Hz, H-20), 2.81 (1H, d, *J* = 7.5 Hz, H-24), 2.61 (1H, br t, *J* = 14.0 Hz, H-6a), 2.47 (1H, ddd, *J* = 11.2, 7.1, 4.3 Hz, H-17), 2.25 (1H, dd, *J* = 13.3, 2.2 Hz, H-6b), 2.22 (1H, m, H-22a), 2.00 (1H, dd, *J* = 9.2, 8.2 Hz, H-15a), 1.91 (3H, m, H-12a, H-22b, and H-15b), 1.83 (3H, d, *J* = 0.9 Hz, CH_3-5′), 1.80 (3H, dd, *J* = 7.0, 0.8 Hz, CH_3-4′), 1.77 (2H, m, H-1a, and H-2a), 1.70 (1H, m, H-2b), 1.51–1.62 (2H, m, H-12b, and H-16a), 1.41 (1H, m, H-11a), 1.38 (3H, s, CH_3-27), 1.36 (3H, s, CH_3-26), 1.34 (1H, m, H-11b), 1.31 (3H, s, CH_3-30), 1.24 (1H, m, H-5), 1.10 (3H, s, CH_3-19), 1.06 (1H, br d, *J* = 10.0 Hz, H-9), 0.98 (1H, m, H-16b), 0.94 (3H, s, CH_3-28), 0.87 (3H, s, CH_3-29), 0.57 (1H, d, *J* = 5.9, H-18a), 0.38 (1H, br d, *J* = 5.8 Hz, H-18b); ^{13}C NMR (125 MHz, $CDCl_3$, TMS) δ 214.5 (C-7, s), 178.0 (C-21, s), 167.7 (C-1′, s), 137.0 (C-3′, d), 129.0 (C-2′, s), 79.9 (C-3, d), 78.5 (C-23, d), 64.4 (C-24, d), 57.3 (C-25, s), 54.9 (C-5, d), 51.5 (C-9, d), 50.0 (C-8, s), 47.9 (C-20, d), 44.8 (C-17, *d*), 38.5 (C-1, t, and C-4, s), 37.0 (C-10, s), 35.7 (C-6, t), 33.7 (C-14, s), 28.7 (C-13, s), 28.3 (C-15, t), 27.5 (CH_3-29, q), 27.0 (C-12, t), 26.4 (C-22, t), 24.8 (CH_3-27, q), 23.3 (C-2, t), 21.3 (C-16, t), 19.5 (CH_3-26, q), 18.8 (CH_3-30, q), 17.2 (C-11, t), 16.2 (CH_3-19, and CH_3-28, q), 15.2 (C-18, t), 14.4 (CH_3-4′, q), 12.1 (CH_3-5′, q); ESIMS *m*/*z* (rel. int.) 567.3 $[M+H]^+$ (45), 549.1 (5), 567.2 (18), 449.4 (5), 354.0 (3), 252.2 (3), 198.9 (2).

4.12. Aglaiaglabretol C (3)

Colorless gum; $[\alpha]_D^{23}$ +22.4° (*c* 1.5, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 212 (very broad) (3.83) nm; IR υ_{max} (film) 3443, 1704, 1650, 1465, 1389, 1269, 1156 cm^{-1}; ESIMS *m*/*z* (rel. int.) 1199.3 $[2M+Na]^+$ (100), 611.5 $[M+Na]^+$ (52), 571.3 (25), 471.1 (8), 453.3 (15), 363.3 (6), 319.4 (3), 213.1 (2); HRESIMS *m*/*z*: 611.3903 (calcd for $C_{35}H_{56}O_7Na$, 611.3924); for ^1H and ^{13}C NMR data of major isomer, see Table 4.

4.13. 3-*epi*-Cabraleahydroxylactone (4)

White amorphous powder, mp 206–208 °C; $[\alpha]_D^{23}$ +19.8° (*c* 0.54, $CHCl_3$); IR υ_{max} (film) 3454, 1767, 1457, 1387, 1191, 1075 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, TMS) δ 3.20 (1H, dd, *J* = 10.9, 5.2 Hz, H-3), 2.50–2.67 (1H, m, H-23), 2.07–2.16 (1H, m, H-22a), 1.80–2.00 (3H, m, H-16a, H-17, and H-22b), 1.72–1.79 (1H, m, H-11a), 1.55–1.71 (5H, m, H-1a, H-2a, H-2b, H-13, and H-15a), 1.46–1.60 (4H, m, H-6a, H-7a, H-12a, and H-12b), 1.38–1.48 (1H, m, H-6b), 1.25–1.38 (1H, m, H-16b), 1.36 (3H, s, CH_3-21), 1.30–1.34 (1H, m, H-9), 1.21–1.29 (3H, m, H-7b, H-11b, and H-15b), 0.90–0.99 (1H, m, H-1b), 0.97 (3H, s, CH_3-25), 0.96 (3H, s, CH_3-18), 0.89 (3H, s, CH_3-27), 0.84 (3H, s, CH_3-19), 0.77 (3H, s, CH_3-26), 0.73 (1H, br d, *J* = 11.3 Hz, H-5); ^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ 176.9 (C-24, s), 90.2 (C-20, s), 78.9 (C-3, d), 55.9 (C-5, d), 50.6 (C-9, d), 50.2 (C-8, s), 49.4 (C-17, d), 43.2 (C-13, d), 40.4 (C-14, s), 39.1 (C-4, s), 39.0 (C-1, t), 37.2 (C-10, s), 35.2 (C-7, t), 31.2 (C-15 or C-22, t), 31.1 (C-15 or C-22, t), 29.2 (C-23, t), 28.0 (CH_3-25, q), 27.4 (C-2, t), 26.8 (C-11, t), 25.5 (CH_3-21, q), 25.1 (C-16, t), 21.4 (C-12, t), 18.3 (C-6, t), 16.3 (CH_3-27, q), 16.2 (CH_3-19, *q*), 15.5 (CH_3-18, q), 15.4 (CH_3-26, q); EIMS *m*/*z* (rel. int.) 416 $[M]^+$ (6), 398 (34), 383 (12), 317 (4), 299 (8), 207 (12), 190 (42), 189 (100), 175 (13), 147 (10), 121 (23), 81 (27), 67 (15); HREIMS *m*/*z* 416.3306 (calcd for $C_{27}H_{44}O_3$, 416.3291).

4.14. Cabraleahydroxylactone (5)

White amorphous powder, mp 242–245 °C; $[\alpha]_D^{23}$ +21.5° (*c* 1.0, $CHCl_3$); IR υ_{max} (film) 3483, 1762, 1457, 1386, 1249, 1196, 1072 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, TMS) δ 3.39 (1H, br s, H-3), 2.50–2.71 (1H, m, H-23), 2.07–2.17 (1H, m, H-22a), 1.80–2.00 (4H, m, H-2a, H-16a, H-17, and H-22b), 1.72–1.82 (1H, m, H-11a), 1.47–1.63 (6H, m, H-2b, H-9, H-12a, H-13, H-15a, and H-16b), 1.31–1.47 (4H, m, H-1a, H-1b, H-6a, and H-6b), 1.36 (3H, s, CH_3-21), 1.20–1.29 (5H, m, H-5, H-7a, H-7b, H-11b, and H-12b), 1.08–1.20 (1H, m, H-15b), 0.96 (3H, *s*, CH_3-18), 0.94 (3H, s, CH_3-26), 0.90 (3H, s, CH_3-27), 0.85 (3H, s, CH_3-19), 0.84 (3H, s, CH_3-25); ^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ 176.8 (C-24, s), 90.2 (C-20, s), 76.1 (C-3, d), 50.3 (C-8, s; and C-9, d), 49.4 (C-5 or C-17, d), 49.3 (C-5 or C-17, d), 43.1 (C-13, d), 40.5 (C-14, s), 37.6 (C-4, s), 37.2 (C-10, s), 35.1 (C-7, t), 33.6 (C-1, *t*), 31.2 (C-15 or C-22, t), 31.1 (C-15 or C-22, t), 29.2 (C-23, t), 28.3 (CH_3-26, q), 26.8 (C-11, t), 25.4 (C-2, t), 25.3 (CH_3-21, q), 25.0 (C-16, t), 22.1 (CH_3-25, q), 21.2 (C-12, t), 18.2 (C-6, t), 16.3 (CH_3-27, *q*), 16.0 (CH_3-19, q), 15.5 (CH_3-18, q); EIMS *m*/*z* (rel. int.): 416 $[M]^+$ (2), 398 (39), 383 (15), 317 (4), 299 (8), 207 (17), 203 (10), 189 (100), 175 (16), 161 (12), 147 (10), 135 (41), 121 (30), 95 (44), 81 (35), 67 (25), 55 (46).

4.15. Rocaglaol (6)

^1H NMR (300 MHz, $CDCl_3$, TMS) δ 7.05–7.13 (5H, m, H-2′, 6′, 3″, 4″, and 5″), 6.98–7.00 (2H, m, H-2″ and 6″), 6.68 (2H, d, *J* = 8.9 Hz, H-3′ and 5′), 6.29 (1H, d, *J* = 1.9 Hz, H-5), 6.14 (1H, d, *J* = 1.9 Hz, H-7), 4.81 (1H, d, *J* = 6.0 Hz, H-1), 4.01 (1H, *dd*, *J* = 14.1, 6.6 Hz, H-3), 3.90 (3H, s, OMe-8), 3.84 (3H, s, OMe-6), 3.71 (3H, s, OMe-4′), 3.30 (1H, *br s*, OH-1 or 8b),

2.75 (1H, ddd, $J = 13.8, 13.8, 6.4$ Hz, H-2β), 2.20 (1H, dd, $J = 13.6, 6.6$ Hz, H-2α), 1.71 (1H, br s, OH-1 or 8b); ^{13}C NMR data (75 MHz, $CDCl_3$, TMS) δ 163.9 (C-6), 161.0 (C-4a), 158.6 (C-4′), 157.0 (C-8), 138.7 (C-1″), 128.9 (C-3″ and 5″), 128.1 (C-2′ and 6′), 127.7 (C-2″ and 6″), 126.8 (C-1′), 126.3 (C-4″), 112.7 (C-3′ and 5′), 107.8 (C-8a), 103.5 (C-3a), 94.8 (C-8b), 92.5 (C-7), 89.4 (C-5), 79.1 (C-1), 55.8 (OMe-8), 55.7 (OMe-6), 55.1 (OMe-4′), 53.2 (C-3), 36.4 (C-2); ESIMS *m/z* (rel. int.) 890.9 $[2M+Na]^+$ (100), 457.2 $[M+Na]^+$ (45), 417 (10), 373.3 (5), 313.3 (45), 284.3 (20), 269.3 (4), 235.2 (3).

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