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NOTE

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Sesquiterpenes from *Blumea balsamifera*

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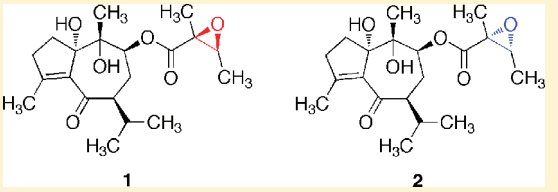
Supporting Information

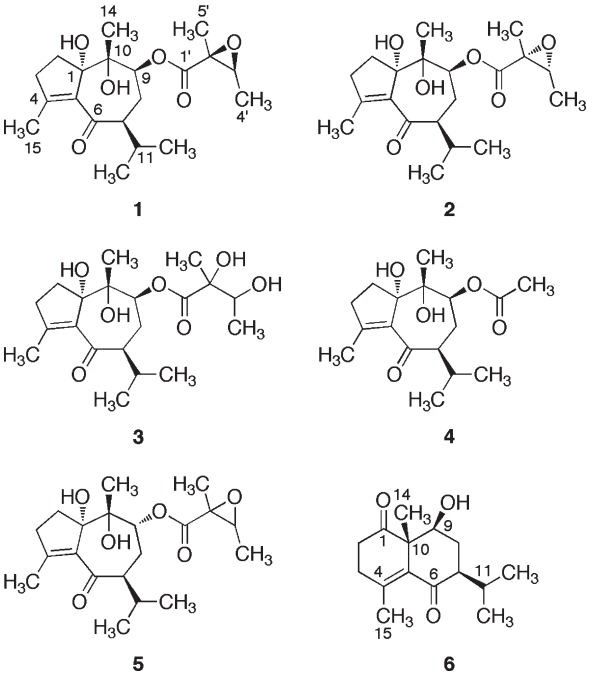
ABSTRACT: Five new guaiane sesquiterpenes, blumeaenes E1 (**1**), E2 (**2**), K (**3**), L (**4**), and M (**5**), and one new eudesmane sesquiterpene, samboginone (**6**), along with three known compounds, cryptomeridiol, 3,3′,5,7-tetrahydroxy-4′-methoxyflavanone, and austroinulin, were isolated from the leaves of the Philippine medicinal herb sambong, *Blumea balsamifera*. The absolute configuration of the new guaiane core was determined as 1*S*,7*S*,9*S*,10*R* by employing the modified Mosher's method. In the structure of **1**, the absolute configuration of the epoxyangelic acid moiety was identified as 2*S*,3*S* using (*R*)-PGME as a chiral anisotropic auxiliary.



The Philippines archipelago, with some 8000 higher plant species, is one of the eight most plant-rich areas in Asia.[1] The vegetative pattern of the Philippine area suggests that most of these plants have considerable and distinct medicinal values. Further, some species are being screened for their pharmacological importance. However, only a few are recognized in the standard herbal pharmacopoeia of the Philippines.

Sambong (Blumeae Balsamiferae Folium) is one of the most commonly used medicinal herbs in the Philippines and is the fresh or dried leaf of *Blumea balsamifera* (L.) DC (Asteraceae). The plant is an erect, suffrutescent, pubescent shrub with strong aromatic properties that grows up to 3 m high on roadsides and in fields, lowlands, and mountainous regions in India, Myanmar, South China, Taiwan, Thailand, Malaysia, Indonesia, and the Philippines. It has several common names, such as sambong (in Tagalog), lakdabulan (in Bikol), subsusob (in Ilokano), and blumea camphor (in English) and is traditionally used for arthritis, rheumatism, and chest pain; the crushed leaves mixed with coconut oil are applied on the affected area. A decoction of the leaves is ingested as a tea for cough and gas pain in adults and children older than seven. Crushed leaves are mixed with oil and applied on the abdomen of children to relieve gas pain. Doctors in the Philippines now routinely prescribe sambong for the dissolution of kidney stones. Sambong is also known as a diuretic and is used in cases of hypertension and mild to moderate congestive heart failure. Previous chemical investigations of this plant led to the isolation of several sesquiterpenoids and flavonoids.[2−14]

We investigated the chemical constituents of the Philippine sambong to be used as reference compounds for TLC analysis of monographs of Philippine herbal drugs. Chromatography of a leaf extract of *B. balsamifera* afforded six new sesquiterpenes (**1**−**6**) along with three known compounds, cryptomeridiol,[15] 3,3′,5,7-tetrahydroxy-4′-methoxyflavanone,[2] and austroinulin.[16]



Compounds **1** and **2** were obtained as amorphous solids. Their identical molecular formula, $C_{20}H_{30}O_6$, was established by HRFABMS data of *m*/*z* 349.1995 $[M + H - H_2O]^+$. The ^1H NMR spectra of both compounds (Table 1) showed similar chemical shifts and coupling constants; three methyl doublets,

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Table 1. ^{1}H NMR Spectroscopic Data (400 MHz, $CDCl_3$) for 1−6

	1	2	3	4	5	6
position	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
2α	1.73, m	1.71, ddd (6.2, 8.9, 14.3)	1.78, ddd (5.5, 9.0, 14.2)	1.72, ddd (6.0, 8.8, 14.3)	1.62, m	2.38, ddd (3.7, 5.7, 13.7)
2β	2.71, ddd (3.8, 8.8, 14.3)	2.71, ddd (3.9, 8.8, 14.3)	2.66, ddd (4.5, 8.7, 14.2)	2.70, ddd (3.9, 8.8, 14.3)	2.61, ddd (5.0, 6.8, 12.8)	2.79, ddd (7.7, 10.8, 13.7)
3α	2.52, m	2.53, dddd (1.0, 3.9, 9.0, 18.6)	2.57, dddd (1.0, 4.5, 9.0, 18.5)	2.57, br-dddd (4.0, 9.0, 18.7)	2.46, m	2.50, dddd (1.0, 5.7, 10.8, 25.2)
3β	2.39, m	2.39, m	2.39, m	2.39, m	2.42, m	2.58, ddt (3.7, 7.7, 25.2)
7	2.79, ddd (1.3, 3.7, 11.6)	2.80, ddd (1.3, 3.9, 11.7)	2.85, ddd (1.3, 4.2, 11.8)	2.85, ddd (1.1, 3.8, 11.5)	2.52, m	2.17, ddd (4.0, 6.2, 12.0)
8α	1.77, ddd (1.4, 3.2, 14.2)	1.86, ddd (1.3, 3.2, 14.2)	1.86, ddd (1.3, 3.5, 14.1)	1.83, ddd (1.1, 3.2, 14.3)	1.66, br-dt (2.0, 10.9)	2.13, ddd (4.6, 6.2, 23.8)
8β	1.51, dt (11.7, 14.2)	1.50, dt (11.7, 14.2)	1.47, dt (11.8, 14.1)	1.45, dt (11.7, 14.3)	2.51, m	1.67, dd (12.0, 23.8)
9	5.56, dd (3.2, 11.8)	5.52, dd (3.2, 11.8)	5.54, dd (3.5, 11.8)	5.54, dd (3.2, 11.7)	5.00, br d (9.2)	4.25, dd (4.6, 12.0)
11	2.35, m	2.34, m	2.31, dq (4.2, 7.0)	2.34, m	2.51, m	2.32, dq (4.0, 6.9)
12	0.83, d (6.8)	0.84, d (6.8)	0.86, d (6.8)	0.84, d (6.8)	0.82, d (6.6)	0.90, d (6.8)
13	0.93, d (7.0)	0.95, d (7.0)	0.95, d (7.0)	0.95, d (7.0)	0.92, d (6.8)	0.95, d (7.0)
14	1.15, s	1.15, s	1.17, s	1.13, s	1.41, s	1.22, s
15	2.10, s	2.11, s	2.12, s	2.11, s	2.06, s	1.81, s
OH (1)	2.50, s	2.19, s	2.44, s	2.44, s	1.82, s	
OH (9)						4.28, br s
OH (10)	2.61, s	2.47, s	2.63(*s*)	2.63, s	2.09, s	
2′				2.14, s		
3′	3.08, q (5.5)	3.09, q (5.5)	4.36, q (6.7)		3.09, q (5.4)	
4′	1.38, d (5.5)	1.38, d (5.5)	1.38, d (5.5)		1.36, d (5.4)	
5′	1.62, s	1.60, s	1.62, s		1.60, s	
OH (2′)			3.39, s			

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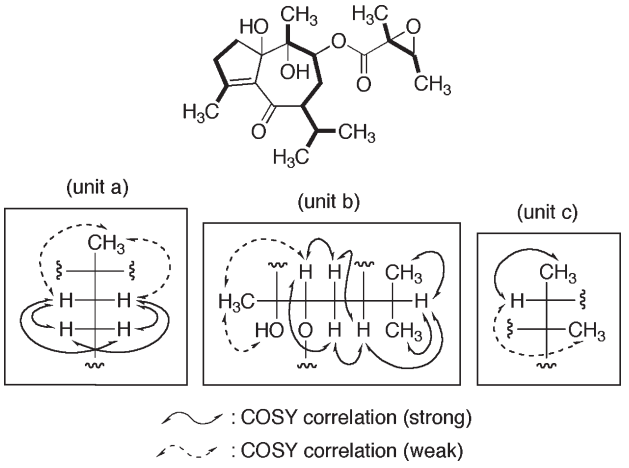

Figure 1. COSY correlations of **1** and **2**.

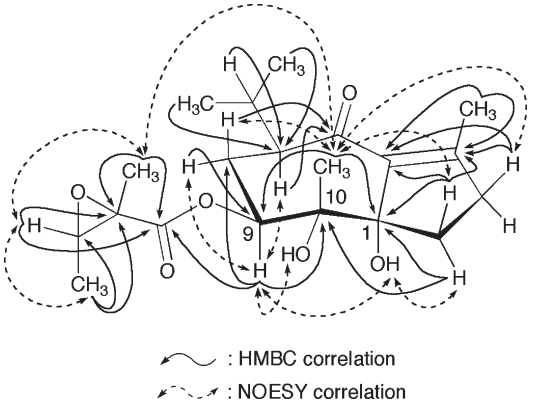

Figure 2. Principal HMBC and NOESY correlations of **1** and **2**.

three methyl singlets, one oxymethine, two D_2O exchangeable signals, and nine methine and/or methylene signals between 1.4 and 3.2 ppm were observed. The differences between **1** and **2** were restricted to the chemical shifts of two D_2O exchangeable signals and one methylene proton at δ_H 1.77 (ddd, J = 1.4, 3.3, 14.2 Hz) for **1** and δ_H 1.86 (ddd, J = 1.3, 3.2, 14.2 Hz) for **2**. The ^{13}C NMR spectra of the two compounds (Table 1) were similar, consisting of one ketone carbonyl, one ester carbonyl, one set of tetrasubstituted double bonds, two oxygen-attached quaternary carbons, one oxymethine, and six methyl carbons, along with the remaining three methylene, three methine, and one quaternary carbon in each compound. The analysis of the H−H COSY spectra of both compounds revealed the same three units, **a**−**c**, as shown in Figure 1. Construction of the gross structure for **1** and **2** was performed by HMBC analysis (Figure 2). In the ^1H NMR spectrum of **1**, a methyl singlet at δ_H 2.10 (H_3-15) of unit **a** showed HMBC correlations with two olefinic carbons at δ_C 137.1 (C-5) and 160.6 (C-4) and one ketone carbonyl carbon at δ_C 201.6 (C-6). This carbonyl carbon correlated with one methine proton at δ_H 2.35 (H-11) of unit **b**, thus confirming the connection between units **a** and **b** through an α,β-unsaturated carbonyl group. Similarly, the methyl singlet at δ_H 1.15 (H_3-14) of unit **b** had an HMBC correlation with an oxygen-substituted quaternary carbon at δ_C 90.4 (C-1). This quaternary carbon correlated with one set of methylene protons at δ_H 1.37/2.71 (H_2-2) of unit **a**. This set of methylene protons also gave correlations with the olefinic carbon at δ_C 137.1 (C-5). The same HMBC correlations were obtained in **2**. Thus, 1,9,10-trihydroxyguaian-4-en-6-one was deduced as a basic core of **1** and **2**. The relative configuration of the guaiane skeleton was determined by means of NOESY analysis as shown in Figure 2. The relative configuration of **1** and **2** was the same. The remaining unit **c** was found to be a glycidic acid moiety, which could be derived from an angelic acid or a tiglic acid, connected to the guaiane skeleton with an ester linkage at C-9 based on HMBC analysis. This glycidic acid moiety was derived from an angelic acid in both **1** and **2**, as a NOESY correlation was observed between a methine proton at δ_H 3.08/3.09 (H-3′) and singlet methyl protons at δ_H 1.38/1.38 (H_3-4′) of **1** and **2**. Therefore, the structural difference between **1** and **2** should be the configuration of the glycidic acid moiety. To determine the configuration of the glycidic acid and the guaiane core, the following strategy was employed. Angelic acid was oxidized to obtain glycidic acid (epoxyangelic acid) as an enantiomeric pair. This glycidic acid was attached to (*R*)-(−)-phenylglycine methyl ester (PGME),[17] which acted as a chiral anisotropic auxiliary, and products were separated by ODS HPLC to obtain each diastereomer in pure form. NMR analysis of these two diastereomers revealed that the first-eluting isomer on the ODS HPLC should have a 2*R*,3*R*-configuration concerning the epoxy ring, whereas the slow-eluting isomer should have a 2*S*,3*S*-configuration because an anisotropic effect was observed on the H-4 methyl group (δ_H 1.10) of the 2*R*,3*R*-isomer (the first-eluting isomer), and corresponding NOESY correlations for each stereoisomer were also observed as shown in Figure 3. Compound **1** was hydrolyzed to obtain the glycidic acid (epoxyangelic acid) and the guaiane core. Although NMR measurements of the (*R*)-PGME derivative of the glycidic acid from **1** failed because of the limited amount of the derivative and the water solubility of the glycidic acid, the retention time on the ODS HPLC of the (*R*)-PGME derivative was identical to that of the slow-eluting isomer of the reference compound. Thus the configuration of the glycidic acid moiety of **1** was confirmed as 2′*S*,3′*S*. In contrast, the remaining guaiane core was successfully subjected to the modified Mosher's method to establish the configuration as 1*S*,7*S*,9*S*,10*R* (Figure 4).[18] Therefore, diastereomer **2** should have a (2*R*,3*R*)-epoxyangelic acid moiety. After our investigation was completed, we found that Chen et al. recently reported the isolation and structural determination of 10 sesquiterpenoid esters, blumeaenes A−J, from the same plant material.[14] We realized that blumeaene E, one of their isolated compounds, had a similar relative configuration to that of **1** and **2**; however, the absolute configuration of both the sesquiterpene core and the glycidic acid moiety was not determined. Therefore, compounds **1** and **2** were named blumeaenes E1 and E2, respectively.

Compound **3** was obtained as an amorphous solid, which had a molecular formula of $C_{20}H_{32}O_7$ according to HRMS. The ^1H NMR spectrum of **3** (Table 1) showed a similar spectroscopic pattern to that of **1** and **2**, except for the low-field shift of H-3′ (δ_H 3.08/3.09 for **1** and **2**; δ_H 4.36 for **3**) and the appearance of one additional D_2O exchangeable proton at δ_H 3.39. A low-field shift of C-2′ (δ_C 60.2/59.8 for **1** and **2**; δ_C 77.2 for **3**) was observed in the ^{13}C NMR spectrum. Therefore, cleavage of the epoxy rings in **1** and **2** was assumed for **3** from these spectroscopic features and was in good agreement with its molecular formula. Chemical shift assignments of **3** were performed by a combination of HSQC and HMBC spectroscopic analyses, and the similarity of the relative configuration of the guaiane core to those of **1** and **2** was confirmed by NOESY correlations.

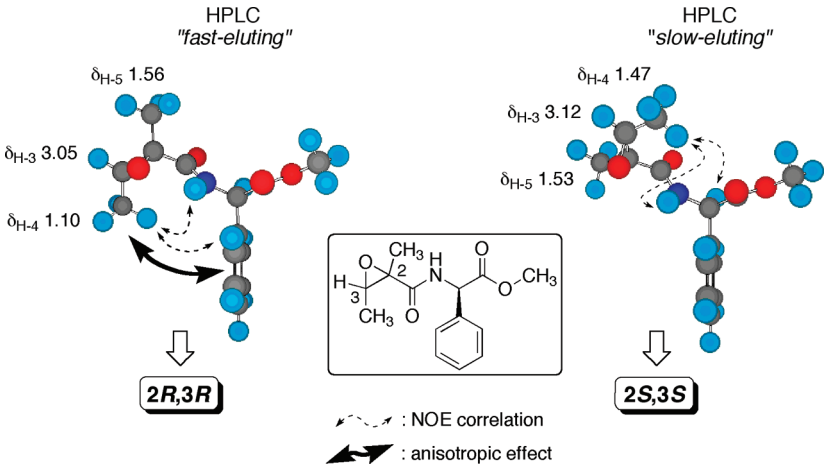

Figure 3. Absolute configuration of (2*R*,3*R*)- and (2*S*,3*S*)-epoxyangelic acid (*R*)-PGME conjugates.

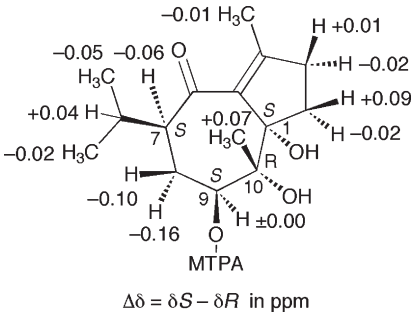

Figure 4. Absolute configuration of the guaiane core of **1** by means of the modified Mosher's method.

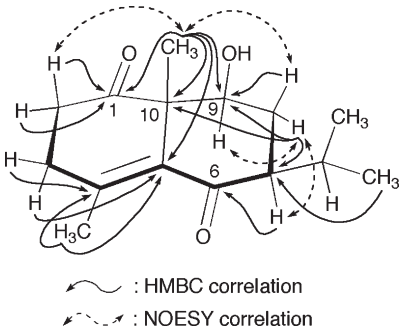

Figure 5. Principal HMBC and NOESY correlations of **6**.

Thus, the structure of **3** for blumeaene K was proposed as shown.

Compound **4**, an amorphous solid, had a molecular formula of $C_{17}H_{26}O_5$. The ^{1}H NMR spectra of **4** (Table 1) showed the same spectroscopic pattern of the guaiane core as those of **1**−**3** and an acetyl methyl proton signal instead of angelic acid derivative signals. Therefore, it was assumed that **4** was the acetyl derivative of the guaiane core, which we prefer to call the blumeaene core. Thus, the structure of **4** was proposed for blumeaene L. 2D NMR spectroscopic analyses fully agreed with the structure.

Compound **5**, an amorphous solid having a molecular formula of $C_{20}H_{30}O_6$, showed similar patterns in both the ^{1}H and ^{13}C NMR spectra to those of **1** and **2** including a glycidic acid moiety. However, chemical shift differences were observed especially at H-14 (δ_H 3.08/3.09 for **1** and **2**; δ_H 4.36 for **5**), H-8β (δ_H 1.51/1.50 for **1** and **2**; δ_H 2.51 for **5**), and H-9 (δ_H 5.56/5.52 for **1** and **2**; δ_H 5.00 for **5**). NOESY analysis of **5** revealed that the blumeaene core was epimerized at C-9 because a NOESY correlation was observed between H-9 and H-14. This spectroscopic analysis also confirmed epoxyangelic acid as a glycidic acid moiety, which showed a NOESY correlation between H-3′ and H-4′, connected at C-9 of the blumeaene core via an ester linkage. Therefore, the structure of **5** was assigned as blumeaene M.

Compound **6**, an amorphous solid, had a molecular formula of $C_{15}H_{22}O_3$. The ^{1}H and ^{13}C NMR spectroscopic patterns were somewhat different from those of the other compounds, and it showed no acid moiety esterified to the sesquiterpene core. The COSY spectrum revealed similar connectivity with units **a** and **b** of the blumeaene core. In the HMBC spectrum, the methyl protons at δ_H 1.22 had long-range correlations with C-9 (δ_C 70.8), a carbonyl carbon at δ_C 217.5, a quaternary carbon at δ_C 52.2, and an olefinic carbon at δ_C 138.4; methylene protons H_2-2 (δ_H 2.38/2.79) and H_2-3 (δ_H 2.38/2.58) showed correlations with the carbonyl carbon. These long-range correlations revealed that the methyl group was attached to the quaternary carbon next to C-9, and the carbonyl carbon was located between the quaternary carbon and C-2. Thus, a eudesmane core, 9-hydroxyeudesman-4-ene-1,6-dione, was found as the skeleton of **6**, as shown in Figure 5. The relative configuration including the β-oriented methyl group at C-10 was established by NOESY analysis; thus the structure of **6** was proposed for samboginone.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a JASCO P-1030 polarimeter. UV, CD, and IR spectra were obtained with a JASCO V-560 UV/vis spectrophotometer, a JASCO J-820 spectropolarimeter, and a JASCO FT/IR-6300 spectrometer with an ATR option, respectively. 1D and 2D ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Plus 400 spectrometer at 300 K by using Varian standard pulse sequences. Phase-sensitive NOESY experiments were conducted with a mixing time of 800 ms. A 3.57 ms (140 Hz) delay was used to optimize one-bond coupling in the HSQC spectra and suppress them in the HMBC spectra, and the evolution delay for long-range couplings in the HMBC spectra was set at 62.5 ms (8 Hz). FABMS and HRFABMS spectra were obtained on a JEOL AX-505H spectrometer. ESITOF and HRESITOFMS spectra were obtained on a Q-Tof micro mass spectrometer (Micromass/Waters). Silica gel open-column chromatography was performed on silica gel 60 (Merck). MPLC

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was performed with a prepacked glass column (Ultra Pack: 26 mm i.d. × 300 mm; Yamazen Corp., Kyoto, Japan) packed with 40 μm of silica gel. HPLC was performed with an Inertsil PREP-ODS column (5 mm i.d. × 250 mm for analysis, 20 mm i.d. × 250 mm for preparative; GL Science Inc. Tokyo, Japan) packed with 10 μm of ODS. TLC was conducted on precoated silica gel 60 F_{254} (Merck) and/or RP-18 F_{254s} (Merck), and the spots were detected by heating after spraying with vanillin−sulfuric acid reagent.

Plant Material. Air-dried *B. balsamifera* (L.) DC (Asteraceae) leaves were obtained in August 2000 from the University of the Philippines Los Banos, Laguna. Plant identification was confirmed by Dr. Wilfredo Vendivil of the Herbarium in the Department of Botany, the National Museum of the Philippines. Voucher specimens (#2000.8-SBF) were deposited at the Division of Pharmacognosy and Phytochemistry, National Institute of Health Sciences, Japan, and Food-Drug Regulation Office, Bureau of Food and Drugs, Department of Health, Philippines.

Extraction and Isolation. *B. balsamifera* leaves (24.3 g) were powdered and extracted with MeOH (3 × ca. 100 mL) at 40−50 °C, and the MeOH solution was evaporated *in vacuo* at 37 °C to yield a MeOH extract (3.23 g). The extract was chromatographed over a silica gel open column eluted with *n*-hexane/EtOAc (9:1 to 0:1) and then EtOAc/MeOH (1:1 to 0:1) as stepwise gradient solvent systems to yield 10 fractions. Fraction 6 (199 mg) was separated by silica gel MPLC eluted with *n*-hexane/EtOAc (6:4, 1:1, and 4:6). Fraction 7 (209 mg) was separated by silica gel MPLC eluted with *n*-hexane/EtOAc (4:6 and 3:7). The MPLC-derived fractions were further purified by ODS HPLC with aqueous MeCN as the eluent to yield six new compounds: blumeaene E1 (**1**; 17.3 mg, 0.071% from dried leaves), blumeaene E2 (**2**; 2.1 mg, 0.009%), blumeaene K (**3**; 1.2 mg, 0.005%), and blumeaene L (**4**; 0.6 mg, 0.002%) from Fr. 7 and blumeaene M (**5**; 0.8 mg, 0.003%) and samboginone (**6**; 6.4 mg, 0.026%) from Fr. 6. The known compounds, cryptomeridiol (7.2 mg, 0.030%),[15] 3,3′,5,7-tetrahydroxy-4′-methoxyflavanone (20.2 mg, 0.083%),[2] and austroinulin (5.2 mg; 0.021%),[16] were also isolated from Frs. 9 and 10. All of these extraction and isolation experiments were performed in 2000−2001.

Blumeaene E1 (1):. amorphous solid; $[\alpha]^{22}_{D}$ −41 (*c* 0.09, MeOH);[14] UV (MeCN) λ_{max} (log ε) 247 (3.92) nm; CD (MeCN) λ_{max} ($\Delta\varepsilon$) 330 (−0.9), 289 (−0.2), 253 (−3.1), 203 (+1.8) nm; IR (ATR) 3466, 2959, 1728, 1673, 1607, 1447, 1369, 1266, 1161, 1110, 1082, 972, 876, 839, 764, 576 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 2; FABMS *m*/*z* 389.2 (16, $[M + Na]^+$), 349.2 (21, $[M + H - H_2O]^+$), 307.2 (17), 233.2 (57), 191.2 (27), 154.1 (100), 136.1 (71); HRFABMS *m*/*z* 349.1995 (calcd for $C_{20}H_{29}O_5$, 349.2015).

Blumeaene E2 (2):. amorphous solid; $[\alpha]^{22}_{D}$ −41 (*c* 0.11, MeOH);[14] UV (MeCN) λ_{max} (log ε) 247 (3.90) nm; CD (MeCN) λ_{max} ($\Delta\varepsilon$) 331 (−1.0), 283 (−0.2), 251 (−2.8), 200 (+1.1) nm; IR (ATR) 3389, 2957, 1747, 1660, 1609, 1468, 1432, 1385, 1266, 1160, 1110, 1081, 973, 876, 841, 825, 758, 648, 575 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 2; FABMS *m*/*z* 389.2 (3, $[M + Na]^+$), 349.2 (8, $[M + H - H_2O]^+$), 307.2 (20), 233.2 (17), 191.2 (7), 154.1 (100), 136.1 (66); HRFABMS *m*/*z* 349.2008 (calcd for $C_{20}H_{29}O_5$, 349.2015).

Blumeaene K (3):. amorphous solid; $[\alpha]^{23}_{D}$ −20 (*c* 0.10, MeOH); UV (MeCN) λ_{max} (log ε) 246 (3.91) nm; CD (MeCN) λ_{max} ($\Delta\varepsilon$) 330 (−0.9), 285 (−0.2), 252 (−2.8), 202 (+1.2) nm; IR (ATR) 3396, 2958, 1727, 1660, 1611, 1443, 1371, 1267, 1161, 1109, 1080, 973, 877, 842, 759, 648 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 2; FABMS *m*/*z* 425.2 (4, $[M + Na]^+$), 385.2 (6, $[M - H_2O + H]^+$), 307.2 (17), 233.2 (21), 154.1 (100), 136.1 (70); HRFABMS *m*/*z* 385.1792 (calcd for $C_{20}H_{32}O_7$, 385.1804).

Blumeaene L (4):. amorphous solid; $[\alpha]^{23}_{D}$ −18 (*c* 0.05, MeOH); UV (MeCN) λ_{max} (log ε) 246 (3.91) nm; CD (MeCN) λ_{max} ($\Delta\varepsilon$) 330 (−0.8), 285 (−0.2), 252 (−2.8), 202 (+1.5) nm; IR (ATR) 3428, 2929, 1720, 1666, 1606, 1462, 1431, 1370, 1242, 1107, 1038, 977, 877, 837, 669, 617 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 2; ESITOFMS *m*/*z* 333.2 (100, $[M + Na]^+$), 282.3 (41), 233.2 (78), 215.1 (32), 191.1 (33); HRESI-TOFMS *m*/*z* 333.1690 (calcd for $C_{17}H_{26}O_5Na$, 333.1678).

Blumeaene M (5):. amorphous solid; $[\alpha]^{23}_{D}$ +48 (*c* 0.01, MeOH); UV (MeCN) λ_{max} (log ε) 247 (3.90) nm; CD (MeCN) λ_{max} ($\Delta\varepsilon$) 319 (−0.4), 296 (−0.2), 279 (−0.4), 247 (+3.1), 204 (−3.2) nm; IR (ATR) 3445, 2960, 1727, 1667, 1612, 1445, 1372, 1264, 1112, 877, 760, 668 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 2; ESITOFMS *m*/*z* 389.2 (100, $[M + Na]^+$), 331.2 (17), 233.2 (61), 215.1 (45), 191.1 (27); HRESITOFMS *m*/*z* 389.1944 (calcd for $C_{20}H_{30}O_6Na$, 389.1940).

Samboginone (6):. amorphous solid; $[\alpha]^{23}_{D}$ −13 (*c* 0.114, MeOH); UV (MeCN) λ_{max} (log ε) 237 (3.78) nm; CD (MeCN) λ_{max} ($\Delta\varepsilon$): 306 (−0.8), 242 (+4.9), 202 (−1.6) nm; IR (ATR) 3428, 2959, 1694, 1445, 1371, 1263, 1236, 1161, 1067, 1007, 917, 803, 759, 669, 541 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 2; ESITOFMS *m*/*z* 233.2 (100, $[M - H_2O + H]^+$), 215.2 (52); HRESITOFMS *m*/*z* 233.1539 (calcd for $C_{15}H_{21}O_2$, 233.1542).

Preparation of Epoxyangelic Acids and Their (*R*)-PGME Derivatives. To an MeOH solution (60 mL) of angelic acid methyl ester (3.5 g; 30 mmol) was added 35 mL of 1 mol/L LiOH, and the mixture stirred overnight. The resulting solution was concentrated, acidified with 1 mol/L H_2SO_4, saturated with NH_4Cl, and then extracted with DCM five times. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to obtain angelic acid as a colorless, crystalline solid (2.15 g; 71.5%): ^1H NMR ($CDCl_3$, 400 MHz) δ 1.92 (3H, dq, *J* = 1.6, 1.6 Hz), 2.05 (3H, dq, *J* = 1.6, 7.2 Hz), 6.23 (1H, qq, *J* = 1.6, 7.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.1 (q), 20.4 (q), 127.2 (s), 141.2 (d), 173.9 (s).

To a mixture of angelic acid (573 mg; 5.7 mmol) and $Na_2WO_4 \cdot H_2O$ (190 mg; 0.57 mmol) was added 6 mL of 1 mol/L KOH. The resulting aqueous solution was acidified to pH 5−6 with 1 mol/L H_2SO_4, and then 0.6 mL of 10 mol/L H_2O_2 was added and the mixture stirred for 30 min. The reaction mixture was acidified to pH 2−3 with 1 mol/L H_2SO_4, saturated with NH_4Cl, and then extracted with DCM five times. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to obtain epoxyangelic acid as a colorless oil (515.4 mg; 77.8%): ^1H NMR ($CDCl_3$, 400 MHz) δ 1.40 (3H, d, *J* = 5.6 Hz), 1.61 (3H, s), 3.15 (1H, q, *J* = 5.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.5 (q), 18.6 (q), 59.7 (s), 60.6 (d), 174.8 (s).

To a mixture of epoxyangelic acid (43 mg; 0.37 mmol), (*R*)-(−)-PGME·HCl (85 mg; 0.41 mmol), 1-hydroxybenzotriazole (65 mg; 0.42 mmol), and benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (218 mg; 0.42 mmol) in 1 mL of DMF was added 135 μL of *N*-methylmorpholine (1.22 mmol), and the mixture stirred overnight. The resulting mixture was diluted with EtOAc and washed with 1 mol/L HCl, saturated $NaHCO_3$, and brine sequentially. The organic layer was concentrated and applied to preparative HPLC purification to obtain one set of pure diastereomers of epoxyangelic acid (*R*)-PGME derivative (the first eluted peak: 27.5 mg, 28.2%; the second eluted peak: 27.1 mg, 27.8%).

(*R*,*R*)-Epoxyangelic acid (*R*)-PGME derivative (the first eluted peak):. amorphous powder; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.10 (3H, d, *J* = 5.5 Hz), 1.56 (3H, s), 3.05 (1H, q, *J* = 5.5 Hz), 3.74 (3H, s), 5.57 (1H, d, *J* = 7.7 Hz), 7.22 (1H, br s, *J* = 7.7 Hz), 7.31−7.36 (5H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.4 (q), 18.8 (q), 52.8 (q), 56.0 (d), 61.4 (d), 61.8 (s), 127.4 (d × 2), 128.7 (d), 129.0 (d × 2), 136.3 (s), 169.2 (s), 170.8 (s); ESITOFMS *m*/*z* 286.1 (100, $[M + Na]^+$); HRESITOFMS *m*/*z* 286.1051 (calcd for $C_{14}H_{17}NO_4Na$, 286.1055).

Table 2. ^{13}C NMR Spectroscopic Data (100 MHz, $CDCl_3$) for 1−6

	1	2	3	4	5	6
position	δ_H, mult.	δ_H, mult.	δ_H, mult.	δ_H, mult.	δ_H, mult.	δ_H, mult.
1	90.4, C	90.4, C	90.1, C	90.1, C	89.7, C	52.2, C
2	36.5, CH_2	36.6, CH_2	36.2, CH_2	36.6, CH_2	38.1, CH_2	35.3, CH_2
3	36.8, CH_2	36.8, CH_2	36.9, CH_2	36.9, CH_2	37.4, CH_2	33.1, CH_2
4	160.6, C	160.8, C	162.8, C	160.7, C	160.5, C	136.6, C
5	137.1, C	137.0, C	137.2, C	137.0, C	138.9, C	138.4, C
6	201.6, C	201.4, C	201.3, C	201.8, C	204.1, C	204.2, C
7	51.6, CH	51.6, CH	51.3, CH	51.6, CH	56.3, CH	53.8, CH
8	27.9, CH_2	27.7, CH_2	27.7, CH_2	27.7, CH_2	26.0, CH_2	28.0, CH_2
9	79.4, CH	79.8, CH	81.2, CH	78.7, CH	82.2, CH	70.8, CH
10	78.6, C	78.5, C	78.5, C	78.5, C	78.8, C	217.5, C
11	26.7, CH	26.8, CH	26.8, CH	26.8, CH	29.1, CH	25.6, CH
12	17.9, CH_3	18.0, CH_3	18.1, CH_3	17.9, CH_3	18.6, CH_3	17.9, CH_3
13	20.7, CH_3	20.8, CH_3	20.8, CH_3	20.8, CH_3	20.8, CH_3	20.7, CH_3
14	15.8, CH_3	16.0, CH_3	16.9, CH_3	16.1, CH_3	19.7, CH_3	17.8, CH_3
15	17.8, CH_3	17.8, CH_3	17.8, CH_3	17.8, CH_3	17.9, CH_3	20.6, CH_3
1′	170.0, C	170.0, C	174.2, C	171.3, C	170.0, C	
2′	60.2, C	59.8, C	77.2, C	21.3, CH_3	59.9, C	
3′	60.0, CH	60.2, CH	62.7, CH		60.1, CH	
4′	13.8, CH_3	13.7, CH_3	23.1, CH_3		13.8, CH_3	
5′	19.3, CH_3	19.3, CH_3	18.0, CH_3		19.2, CH_3	

(*S*,*S*)-Epoxyangelic acid (*R*)-PGME derivative (the second eluted peak):. amorphous, oily solid; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.47 (3H, d, J = 5.6 Hz), 1.53 (3H, s), 3.12 (1H, q, J = 5.6 Hz), 3.72 (3H, s), 5.54 (1H, d, J = 7.7 Hz), 7.11 (1H, br s, J = 7.7 Hz), 7.32−7.42 (5H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.4 (q), 18.7 (q), 52.7 (q), 56.1 (d), 61.8 (d), 61.9 (s), 127.4 (d × 2), 128.8 (d), 129.2 (d × 2), 135.6 (s), 169.2 (s), 170.9 (s); ESITOFMS m/z 286.1 (100, $[M + Na]^+$); HRESITOFMS m/z 286.1056 (calcd for $C_{14}H_{17}NO_4Na$, 286.1055).

Hydrolysis of Blumeaene E1 (1). To a solution of blumeaene E1 (ca. 1.0 mg; 2.7 μmol) in MeOH was added 0.5 mol/L LiOH (10 μL; 5 μmol), and the mixture stirred for 2 h at rt. The reaction mixture was diluted with H_2O and extracted with DCM three times and with *n*-hexane one time, and the combined organic extract was concentrated to obtain the blumeaene core. The remaining aqueous phase was acidified with 1 mol/L HCl and extracted with DCM three times and with EtOAc one time, and then the combined organic extract was concentrated to obtain epoxyangelic acid.

(*R*)- and (*S*)-MTPA Esterification of Blumeaene Core. To a mixture of the blumeaene core (<ca. 3 μmol), (*R*)-MTPA (ca. 2 mg; 8.5 μmol), EDC (ca. 2 mg; 10 μmol), and DMAP (ca. 0.1 mg; 0.8 μmol) was added CH_2Cl_2 (0.5 mL), and the mixture stirred overnight at rt. The reaction mixture was concentrated and applied to preparative TLC to obtain the (*R*)-MTPA ester of the blumeaene core (ca. 1.5 mg; 3 μmol). The (*S*)-MTPA ester of the blumeaene core was prepared in a similar manner.

Preparation of the (*R*)-PGME Amide of Epoxyangelic Acid from Blumeaene E1. To a mixture of epoxyangelic acid (<ca. 3 μmol) from the hydrolysis of **1**, (*R*)-(−)-PGME·HCl (13 mg; 64 μmol), HOBt (8 mg; 50 μmol), and PyBOP (20 mg; 50 μmol) in 0.5 mL of DMF was added 8 μL of NMM (75 μmol), and the mixture stirred overnight. The resulting mixture was diluted with EtOAc and sequentially washed with 1 mol/L HCl, saturated $NaHCO_3$, and brine. The organic layer was concentrated and applied to an analytical HPLC to identify the diastereomer of epoxyangelic acid (*R*)-PGME amides.

■ ASSOCIATED CONTENT

Supporting Information. NMR spectra of all new compounds (**1**−**6**). This material is available free of charge via the Internet at http://pubs.acs.org.

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■ DEDICATION

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