Regular Article

Crotocascarins I–K: Crotofolane-Type Diterpenoids, Crotocascarin γ, Isocrotofolane Glucoside and Phenolic Glycoside from the Leaves of *Croton cascarilloides*

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From the 1-BuOH-soluble fraction of a methanol (MeOH) extract of the leaves of *Croton cascarilloides*, crotofolanes: crotocascarins I–K, nor-crotofolane: crotocascarin γ, isocrotofolane glucoside and phenolic glycoside were isolated by a combination of various separation techniques. Their structures were elucidated mainly from the NMR spectroscopic evidence. The structure of crotocascarin K was first elucidated by spectroscopic analysis and then was confirmed by X-ray crystallographic analysis. Its absolute structure was finally determined by the modified Mosher's method. Isocrotofolane glucoside was found to possess a new skeleton, however, its absolute structure remains to be determined.

Key words *Croton cascarilloides*; Euphorbiaceae; crotocascarin; crotofolane; nor-crotofolane; isocrotofolane

A diterpenoid, phorbol 12-myristate 13-acetate, isolated from Croton oil is known to have a potent tumor-promoting effect[1,2] and is often employed in biomedical research to activate protein kinase C.[3] Thus, *Croton* species plants attracted our attention. *C. cascarilloides* Räuschel is an evergreen shrubby tree that grows on elevated coral reefs of the Okinawa Islands, Taiwan, southern China, the Malay Peninsula and Malaysia. The leaves are oblong-lanceolate to oblong-oval, and their undersurface is covered by shiny white ramenta.[4] In a previous study,[5] investigation of the constituents of the stems of *Croton cascarilloides* provided eight rare crotofolane-type and two rearranged crotofolane-type diterpenoids. In this study, from the 1-BuOH-soluble fraction of a MeOH extract of the leaves of *C. cascarilloides*, three crotofolane-type diterpenoids, named crotocascarins I–K (**1**–**3**), a nor-diterpenoid, crotocascarin γ (**4**), a diterpenoid glucoside with a new skeleton, which was named isocrotofolane glucoside (**5**), and one phenolic glycoside (**6**) along with two known compounds (**7**, **8**) were isolated. The structures of the new compounds isolated were elucidated from spectroscopic evidence and that of crotocascarin K (**3**) was determined by X-ray crystallographic analysis, followed by application of the modified Mosher's method to clarify its absolute structure. The known compounds were identified as angelicoidenol 2-*O*-β-D-glucopyranoside (**7**)[6] and senecrassidiol (**8**)[7] by comparison of the physico-chemical data with those reported in the literature.

Results and Discussion

From the 1-(butanol) BuOH-soluble fraction of a MeOH extract of the leaves of *C. cascarilloides*, three crotofolane-type diterpenoids, named crotocascarins I–K (**1**–**3**), a nor-diterpenoid, crotocascarin γ (**4**), a diterpenoid glucoside with a new skeleton, which was named isocrotofolane glucoside (**5**), and a phenolic glycoside (**6**) were isolated by means of a combination of various chromatographic techniques.

Crotocascarin I (**1**), $[\alpha]_D^{26}$ +32.0, was isolated as an amorphous powder and its elemental composition was determined to be $C_{20}H_{24}O_5$ by high-resolution (HR)-electrospray ionization (ESI)-mass spectrometry (MS). The IR spectrum showed absorption bands at 3478 cm^{-1} and 1739 cm^{-1} assignable to hydroxy and γ-lactone functional groups. The UV absorption band at 223 nm indicated the presence of an α,β-unsaturated ketone moiety. In the ^{1}H-NMR spectrum, signals for one singlet methyl at δ_H 1.05, two doublet methyls at δ_H 1.02 (J=7.2 Hz) and 1.91 (J=1.2 Hz), and two olefinic protons at δ_H 5.06 (1H, s) and 5.07 (1H, s) were observed. The ^{13}C-NMR spectrum together with distortionless enhancement polarization transfer (DEPT) results revealed a total of 20 resonances, which comprised those of three methyls, three methylenes, three oxygenated methines, three methines, three oxygenated tertiary carbons, tetrasubstituted and *exo*-cyclic double bonds, and one carbonyl carbon. Except for the absence of a 2-methylbutyrate moiety, these functionalities were the same as those of crotocascarin D (**9**), which was isolated from the branches of the title plant.[5] When compared the NMR spectra of **1** and **9**, the H-1 signal shifted up from δ_H 5.49 (crotocascarin D) to δ_H 4.20 in the ^{1}H-NMR spectrum, and the C-1 signal shifted up from δ_C 74.5 (crotocascarin D) to δ_C 73.6 in the ^{13}C-NMR spectrum. Therefore, the structure of crotocascarin I (**1**) was elucidated to be as shown in Fig. 1, namely the deacyl form of crotocascarin D (**9**). The absolute structure of **1** was the same as that of crotocascarin D (**9**), as judged from a similar positive Cotton effect at 252 nm ($\Delta\varepsilon$ +1.42) in the circular dichroism (CD) spectrum to that observed in crotocascarin D.[5]

Crotocascarin J (**2**), $[\alpha]_D^{24}$ +9.1, was isolated as an amorphous powder and its elemental composition was determined to be $C_{20}H_{24}O_6$, which is one oxygen more than in the case of **1**. The ^{13}C-NMR spectrum was similar to that of **1**, and this

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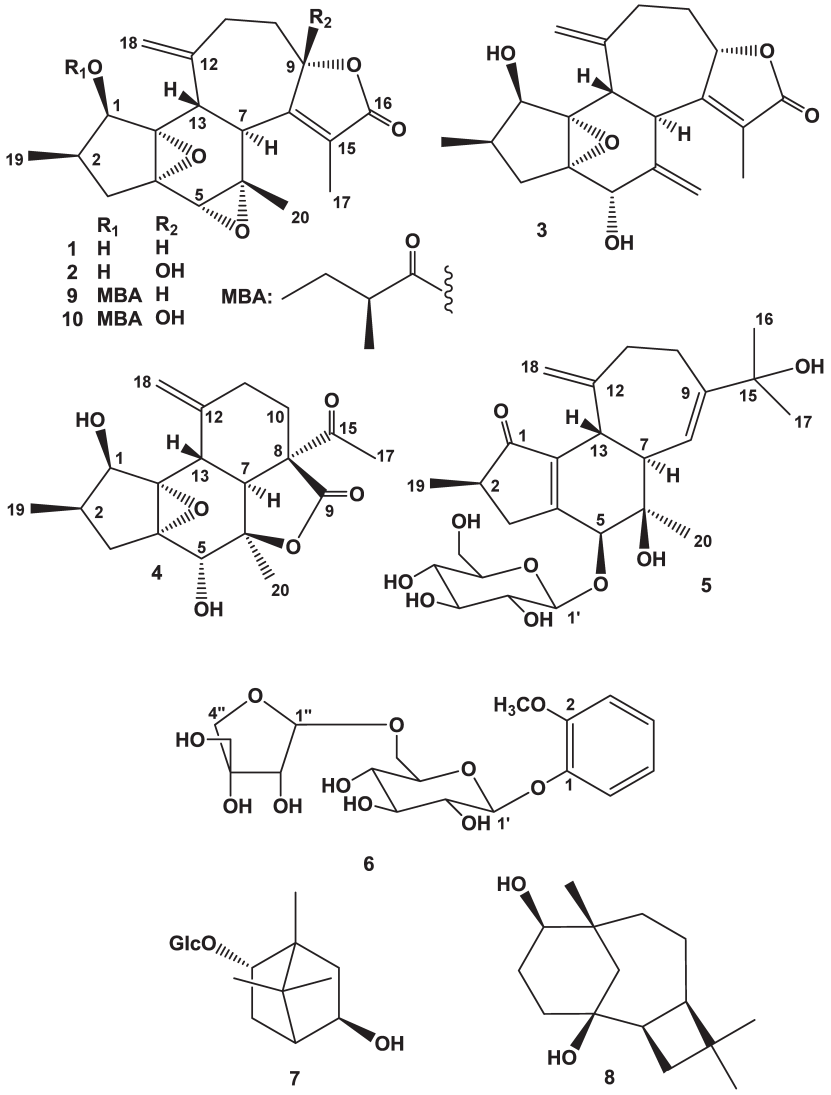

Fig. 1. The Structures of Compounds Isolated and Reference Compounds

together with the absence of an oxygenated methine proton at the 9-position means the 9-position must have been replaced by a hydroxy functional group to form a ketal, which was supported by the appearance of δ_C 108.0, with the simultaneous disappearance of the oxygenated methine signal at δ_C 82.3 observed for **1**. Therefore, the structure of crotocascarin J (**2**) was elucidated to be as shown in Fig. 1, namely the deacyl form of crotocascarin A (**10**). The absolute structure of **2** was the same as that of crotocascarin A, as judged from a similar positive Cotton effect at 255 nm ($\Delta\varepsilon$ +2.54).[5)]

Crotocascarin K (**3**), $[\alpha]_D^{24}$ +96.7, was isolated as colorless plates and its elemental composition was determined to be $C_{20}H_{24}O_5$ by HR-ESI-MS. The ^{13}C-NMR spectrum displayed 20 carbon signals, which showed close resemblance to those of crotocascarin I (**1**), except for the presence of one more *exo*-cyclic double bond and a secondary alcohol, and the disappearance of an epoxy ring at C-5 and C-6. In the ^{1}H-NMR spectrum, although *exo*-cyclic methylene protons resonated at the same frequencies, H-18a and 20a at δ_H 5.24, and H-18b and 20b at δ_H 5.09, significant heteronuclear multiple-bond connectivity (HMBC) cross peaks between these *exo*-methylene protons and C-5, C-6 and C-7 suggested that the position of the new *exo*-cyclic double bond was at C-6 and the secondary hydroxy group at C-5 (Fig. 2). The CD spectrum of **3** exhibited a strong Cotton effect at 226 nm ($\Delta\varepsilon$ +16.6), however, the spectrum profile was different from those of **1** and **2**, probably due to the closeness of the new *exo*-cyclic double bond to another double bond between C-8 and C-15.[8,9)] Thus, application of the CD spectrum rule to the stereochemistry of the 9-position was too ambiguous to draw a correct conclusion.

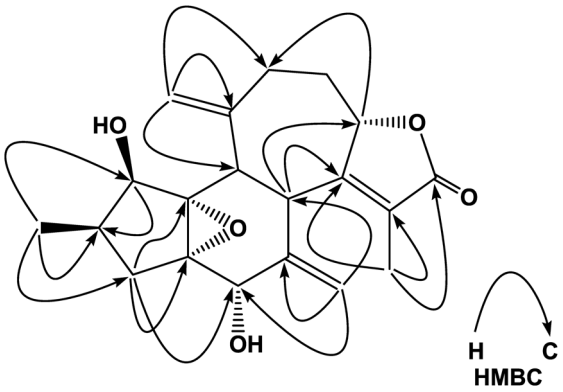

Fig. 2. Important HMBC Correlations of Crotocascarin K (**3**)

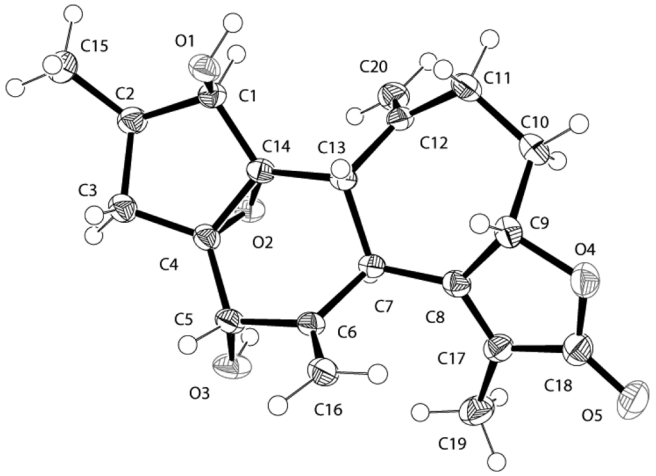

Fig. 3. ORTEP Drawing of Crotocascarin K (**3**)
The structure has crystallographic numbering.

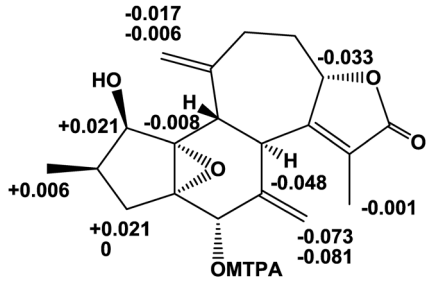

Fig. 4. Results with the Modified Mosher's Method for Crotocascarin K (**3**)

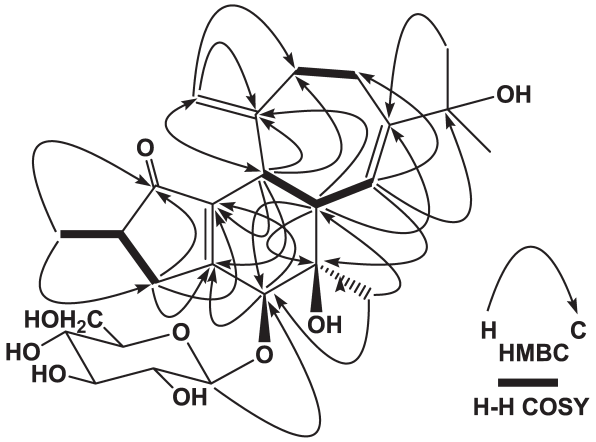

Fig. 5. ^{1}H–^{1}H COSY and Diagnostic HMBC Correlations of **5**
Dual arrow curves denote HMBC correlations were observed in both directions.

Therefore, X-ray crystallographic analysis was performed to confirm the structure, Fig. 3 showing an ORTEP drawing of **3**, followed by application of the modified Mosher's method to establish the absolute structure[10] (Fig. 4). As a result, the spectroscopically deduced structure of **3** was proved to be correct and the absolute stereochemistry was found to be the same as that of other crotofolanes isolated from *C. cascarilloides* (Fig. 1).

Crotocascarin γ (**4**), $[\alpha]_D^{23}$ +21.8, was isolated as an amorphous powder and its elemental composition was determined to be $C_{19}H_{24}O_6$ by HR-ESI-MS. The NMR spectroscopic data were similar to those of crotocascarin β, except for the absence of a 2-methylbutyric acid moiety. Namely, **4** was assigned as deacyl crotocascarin β, as shown in Fig. 1.

1-Oxo-5,6,15-trihydroxyisocrotofola-4 (**14**), 8,12(18)-triene 5-*O*-β-D-glucopyranoside (isocrotofolane glucoside) (**5**), $[\alpha]_D^{24}$ −63.6, was isolated as an amorphous powder and its elemental composition was determined to be $C_{26}H_{38}O_9$ by HR-ESI-MS. The IR spectrum exhibited absorptions assignable to hydroxy (3378 cm^{-1}) and carbonyl (1690 cm^{-1}) groups, and double bonds (1646 cm^{-1}), and the UV spectrum showed an absorption for an α,β-unsaturated ketone at 234 nm. In the ^{1}H-NMR spectrum, signals for three singlet methyls, one doublet methyl, three olefinic protons [δ_H 4.28 (s), 4.62 (s), 6.29 (d, J=6.0 Hz)], one doublet methine proton [δ_H 4.60 (d, J=1.1 Hz)] with an oxygen atom and an anomeric proton [δ_H 4.49 (d, J=7.3 Hz)] were observed. In the ^{13}C-NMR spectrum together with DEPT revealed six typical signals assignable to terminal glucopyranoside, the remaining 20 signals comprising those of four methyls, three methylenes, three methines, one oxygenated methine, two oxygenated tertiary carbons, tetrasubstituted, trisubstituted and *exo*-cyclic double bonds, and a carbonyl carbon signals. On ^{1}H–^{1}H correlation spectroscopy (COSY), the presence of three proton chains, *i.e.*, CH_3–CH–CH_2–, –CH–CH–CH= and –CH_2–CH_2–, was confirmed. From the degree of unsaturation, **5** must have a tetracyclic structure, and to establish the structure HMBC correlations were precisely inspected. Correlations between H_3-19 (δ_H 1.11) and C-1 (δ_C 213.3), C-3 (δ_C 38.1), and δ_H 2.35 and 3.16 on C-3 and C-4 (δ_C 174.1) and C-14 (δ_C 139.4) established a five membered-ring, which must involve an α,β-unsaturated ketone (Fig. 5). Further correlations H-5 and C-4, C-14, H-7 and C-5, C-6, C-13, and H-13 and C-4 and C-14 established a six-membered ring fused at the C-4 and C-14 tetrasubstituted double bond. When C-18, isopropyl (C-15, -16, -17) and methyl (C-20) groups were excluded as *exo*-cyclic carbons, the remaining four carbons must form a third seven-membered ring in crotofolanes. The HMBC correlations H-7 and C-9, C-12, C-13, H-8 and C-6, C-10, H-13 and C-11, C-12, C-18, H_2-18 and C-11, C-12 and C-13, and H_3-20 and C-5 and C-7 were supportive of the structure shown in Fig. 1. The hydroxy isopropyl group was placed at C-9 based on the ^{1}H–^{1}H COSY correlation from H-7 to H-8, and the HMBC correlations between H-7 and C-9, H-8 and C-15, and H_3-16 (or 17) and C-9 (Fig. 5), thus, a different skeleton from the crotofolane was formed, *i.e.*, a new one named the isocrotofolane. The position of the sugar linkage was also confirmed by the HMBC spectrum and the mode of linkage was β as judged from the coupling constant of the anomeric proton (J=7.3 Hz). HPLC analysis revealed that the glucose is in the D-series. Although compound **5** was enzymatically hydrolyzed to give **5a**, to which the β-D-glucopyranosylation-induced shift-trend rule could be applied,[11] ^{13}C-NMR chemical shift changes of C-4 and C-6 from **5** to **5a** did not observe the typical rule (C-4: $\Delta\delta$ −0.9 ppm and C-6: $\Delta\delta$ +0.3) (Table 1). Furthermore, an attempt to prepare α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) esters was not successful, probably due to steric hindrance around the C-5 region. Assuming that the absolute stereochemistries of the ring junctures at the C-7 and C-13 positions were the same as those of other crotofolanes isolated from the title plant, phase-sensitive (PS) nuclear Overhauser enhancement (NOESY) spectroscopic correlation H-5 and H-7

placed the hydroxy group at the 5-position in a β-orientation, and those between H-7 and H_3-20, and H-5 and H_3-20 the hydroxy group at the 5-position also in a β-orientation and the 20-methyl group in an α-orientation, as shown in Fig. 6. Those between H-5 and H-3a (δ_H 3.16), H-3b (δ_H 2.35) and H_3-19 and then H-2 (δ_H 2.45) and H-3a placed the 19-methyl group in a β-orientation. Therefore, the structure of **5** was tentatively elucidated to be as shown in Fig. 1.

Compound **6**, $[\alpha]_D^{25}$ −93.6, was isolated as an amorphous powder and its elemental composition was determined to be $C_{18}H_{26}O_{11}$ by HR-ESI-MS. The IR spectrum showed absorption bands for hydroxy groups (3364 cm^{-1}) and an aromatic ring (1503 cm^{-1}), and the UV absorption bands also supported the presence of the aromatic ring. The ^{1}H-NMR spectrum along with ^{1}H–^{1}H COSY and heteronuclear single quantum coherence spectra showed four aromatic proton signals in series, two anomeric protons at δ_H 5.52 and 5.69 on δ_C 102.8 and 111.2, respectively and a methoxy signal (Table 2). The ^{13}C-NMR spectrum showed five signals assignable to a terminal apiofuranose and six for a hexopyranose unit, and HPLC analysis of the hydrolyzate of **6** gave two peaks for D-apiose and D-glucose. In difference NOE experiments, on irradiation of the methoxy signal, significant signal enhancement was observed at H-3 [δ_H 6.90 (dd, J=8.1, 2.0 Hz)], and those on anomeric protons, δ_H 5.52 and 5.69, NOE enhancement was observed at H-6 and H_2-6′ protons, respectively. Therefore, the structure of compound **6** was elucidated to be 2-methoxyphenol β-D-(6′-*O*-β-D-apiofuranosyl)glucopyranoside, as shown in Fig. 1.

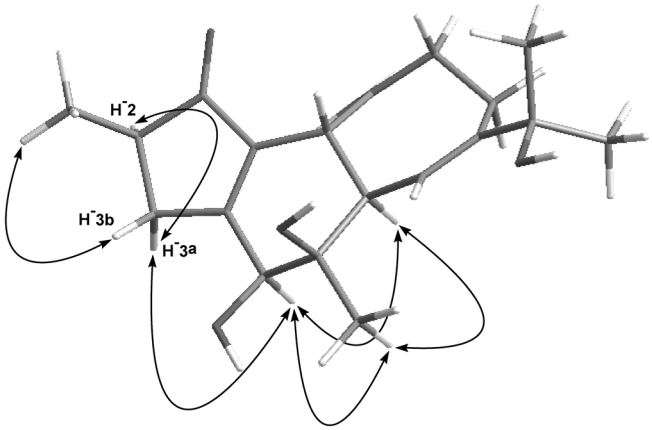

Fig. 6. Important PS NOESY Correlations of **5**
Glucose is omitted for clarity.

Isocrotofolane glucopyranoside (**5**) is a diterpene, having a new carbon skeleton. Biosynthesis of crotofolane is expected to start from geranylgeranylpyrophosphate and then proceed *via* cembrane, casbane, lathyrane and then jatropholane (Fig. 7). The final step is opening of the cyclopropane ring. In pathway 1, the cleavage of the C-9 and C-15 bond forms crotofolane, however, that in pathway 2 between the C-8 and C-15 bond may lead to the formation of a new skeleton, named the isocrotofolane. This is the second example of a new diterpenoid skeleton, isolated from *C. cascarilloides*.

Experimental

General Experimental Procedure Melting point (mp)

Table 1. ^{13}C-NMR Spectroscopic Data for Crotocascarins I–K (**1**–**3**), Crotocascarin γ (**4**), and Isocrotofolane Glucoside (**5**) (100 MHz, $CDCl_3$)

C	1	2	3	4[a]	5[a]	5a[a]
1	73.6	73.7	73.1	76.0	213.3	213.0
2	33.7	33.7	33.2	35.9	42.1	42.1
3	36.2	36.2	35.8	34.7	38.1	36.7
4	60.2	60.4	67.6	67.8	174.1	175.0 (−0.9)[b]
5	57.9	58.2	71.1	75.8	82.3	75.1 (+7.2)[b]
6	56.1	56.6	145.1	89.8	76.1	75.8 (+0.3)[b]
7	43.8	44.1	39.5	49.3	50.5	51.1
8	162.2	159.7	163.1	63.8	125.4	125.8
9	82.3	108.0	82.6	177.3	151.3	151.2
10	37.7	41.8	37.2	28.3	30.5	30.6
11	36.3	35.2	37.0	28.4	39.6	39.7
12	147.1	147.7	147.2	145.2	154.6	154.8
13	40.2	39.3	43.3	35.5	43.7	43.9
14	69.9	70.0	73.0	67.8	139.4	139.3
15	128.3	130.1	127.2	204.2	74.3	74.3
16	173.2	171.3	173.6	—	28.7	28.7
17	9.6	9.6	9.3	25.5	28.7	28.8
18	114.5	114.4	114.3	109.2	108.2	108.0
19	12.1	12.2	12.2	12.7	15.8	15.8
20	19.5	20.3	114.1	22.9	21.1	24.1
1′					105.9	
2′					75.8	
3′					78.3	
4′					72.0	
5′					77.7	
6′					63.1	

a) Data for CD_3OD. *b*) $\Delta_{\delta 5-\delta 5b}$.

was measured with a Yanagimoto micro melting point apparatus and is uncorrected. Optical rotations were measured on a JASCO P-1030 digital polarimeter. IR and UV spectra were measured on Horiba FT-710 and JASCO V-520 UV/Vis spectrophotometers, respectively. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL JNM α-400 spectrometer at 400 MHz and 100 MHz, respectively, with tetramethylsilane as an internal standard. CD spectra were obtained with a JASCO J-720 spectropolarimeter. Positive-ion HR-ESI-MS was performed with an Applied Biosystems QSTAR® XL NanoSpray™ System.

Table 2. NMR Spectroscopic Data for Compound **6** (^{13}C: 100 MHz, ^{1}H: 400 MHz; C_5D_5N)

	C	H
1	150.3	—
2	148.3	—
3	113.3	6.90 (dd, *J*=8.1, 2.0 Hz)
4	122.7	6.94 (ddd, *J*=8.1, 7.9, 2.0 Hz)
5	121.7	7.06 (ddd, *J*=7.9, 7.9, 2.0 Hz)
6	117.4	7.69 (dd, *J*=7.9, 2.0 Hz)
–OCH_3	56.0	3.67 (3H, s)
1′	102.8	5.52 (d, *J*=7.3 Hz)
2′	74.9	4.29 (overlapped)
3′	78.4	4.29 (overlapped)
4′	71.4	4.07 (dd, *J*=9.2, 9.0 Hz)
5′	77.4	4.22 (ddd, *J*=9.2, 7.0, 1.3 Hz)
6′	69.0	4.79 (dd, *J*=11.0, 1.3 Hz)
		4.13 (dd, *J*=11.0, 7.0 Hz)
1″	111.2	5.69 (d, *J*=2.4 Hz)
2″	77.7	4.72 (d, *J*=2.4 Hz)
3″	80.4	—
4″	75.1	4.55 (d, *J*=9.3 Hz)
		4.31 (d, *J*=0.3 Hz)
5″	65.7	4.16 (2H, s)

A highly-porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Kagaku (Tokyo, Japan). Silica gel column chromatography (CC) was performed on silica gel 60 (E. Merck, Darmstadt, Germany), and octadecyl silanized silica gel (ODS) open CC on Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto, Japan) [Φ=50 mm, *L*=25 cm, linear gradient: MeOH–H_2O (1 : 9, 1 L)→(1 : 1, 1 L), fractions of 10 g being collected]. The droplet counter-current chromatograph (DCCC) (Tokyo Rikakikai, Tokyo, Japan) was equipped with 500 glass columns (Φ=2 mm, *L*=40 cm), the lower and upper layers of a solvent mixture of $CHCl_3$–MeOH–H_2O–*n*-propanol (9 : 12 : 8 : 2) being used as the stationary and mobile phases, respectively. Five-gram fractions were collected and numbered according to their order of elution with the mobile phase. HPLC was performed on an ODS column (Inertsil; GL Science, Tokyo, Japan; Φ=20 mm, *L*=250 mm, 6 mL/min), and the eluate was monitored with a UV detector at 254 nm and a refractive index monitor. Authentic D-apiose {$[\alpha]_D^{23}$ +9.4 (*c*=0.84, H_2O)} was obtained by chromatographic separation of a hydrolyzate of apiin, isolated from commercial parsley (*Petroselinum crispum*). D-Apiose was identified by NMR spectroscopy.[12] (*R*)- and (*S*)-α-MPTAs were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Plant Material Leaves of *C. cascarilloides* RÄUSCHEL (Euphorbiaceae) were collected in Kunigami-son, Kunigami-gun, Okinawa, Japan, in July 2004, and a voucher specimen was deposited in the Herbarium of Pharmaceutical Sciences, Graduate School of Biomedical and Health Sciences, Hiroshima University (04-CC-Okinawa-0628).

Extraction and Isolation Air-dried leaves of *C. cascarilloides* (6.53 kg) were extracted with MeOH (45 L) three times. The MeOH extract was concentrated to 6 L and then washed with *n*-hexane (6 L, 59.1 g). The methanolic layer was concentrated to a viscous gum. The gummy mass was suspended in H_2O (6 L), and then partitioned with EtOAc

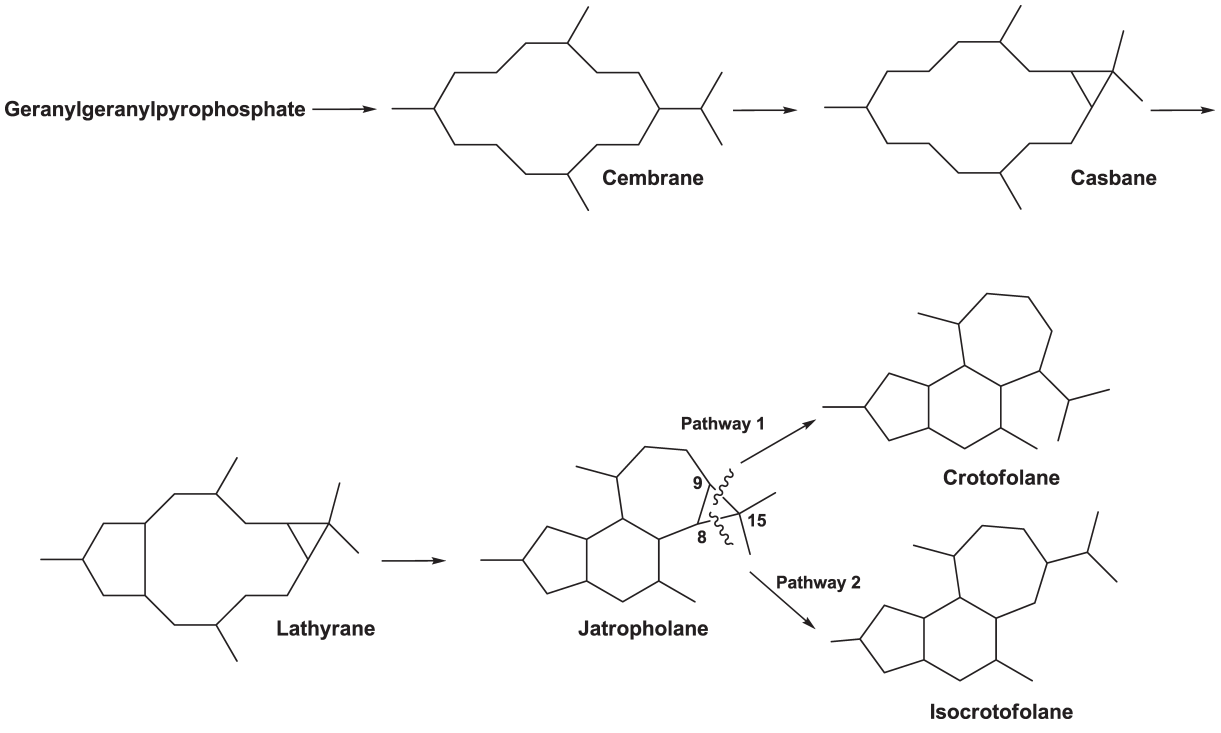

Fig. 7. Possible Biosynthetic Pathway for Isocrotofolane

(6 L) and 1-BuOH (6 L), successively, to give 100 g and 126 g of EtOAc and 1-BuOH-soluble fractions, respectively. The remaining water-layer was concentrated to give a H_2O-soluble-fraction (263 g). The 1-BuOH-soluble fraction was subjected to a Diaion HP-20 column (Φ=80 mm, L=57 cm), and eluted with H_2O–MeOH (4 : 1, 6 L), (3 : 2, 6 L), (2 : 3, 6 L), and (1 : 4, 6 L), and MeOH (6 L), 1 L-fractions being collected. The residue (6.38 g) in fractions 10–14 was subjected to silica gel CC (Φ=36 mm, L=57 cm), and eluted with $CHCl_3$ (1.5 L), $CHCl_3$–MeOH (99 : 1, 1.5 L), (97 : 3, 1.5 L), (19 : 1, 1.5 L), (37 : 3, 1.5 L), (9 : 1, 1.5 L), (7 : 1, 1.5 L), (17 : 3, 1.5 L), (33 : 7, 1.5 L), (4 : 1, 1.5 L), (3 : 1, 1.5 L), and (7 : 3, 1.5 L), and MeOH (1.5 L), 250 mL-fractions being collected. The residue (1.61 g) in fractions 59–79 was separated by ODS open CC to give 50.6 mg of crude **6** in fractions 58–64, which was finally purified by HPLC (H_2O–MeOH, 3 : 2) to afford 2.9 mg of pure **6** from the peak at 5.4 min.

The residue (6.90 g) in fractions 15–18 obtained on Diaion HP-20 CC was subjected to silica gel CC (Φ=36 mm, L=57 cm), and eluted with $CHCl_3$ (1.5 L), $CHCl_3$–MeOH (99 : 1, 1.5 L), (97 : 3, 1.5 L), (19 : 1, 1.5 L), (37 : 3, 1.5 L), (9 : 1, 1.5 L), (7 : 1, 1.5 L), (17 : 3, 1.5 L), (33 : 7, 1.5 L), (4 : 1, 1.5 L), (3 : 1, 1.5 L), and (7 : 3, 1.5 L), and MeOH (1.5 L), 250 mL-fractions being collected. The residue (921 mg) in fractions 42–52 was separated by ODS open CC and the residue (45.7 mg) in fractions 188–193 was then purified by DCCC to give 9.6 mg of crude **5** in fractions 87–97, which was finally purified by HPLC (H_2O–MeOH, 3 : 2) to afford 5.4 mg of pure **5** from the peak at 20.0 min.

The residue (11.7 g) in 19–24 fractions obtained on Diaion HP-20 CC was subjected to silica gel CC (Φ=50 mm, L=57 cm), and eluted with $CHCl_3$ (3 L), $CHCl_3$–MeOH (99 : 1, 3 L), (97 : 3, 3 L), (19 : 1, 3 L), (37 : 3, 3 L), (9 : 1, 3 L), (7 : 1, 3 L), (17 : 3, 3 L), (33 : 7, 3 L), (4 : 1, 3 L), (3 : 1, 3 L), and (7 : 3, 3 L), and MeOH (3 L), 500 mL-fractions being collected. The residue (598 mg) in fractions 19–24 was subjected to ODS open CC and the residue (32.5 mg) in fractions 118–124 was purified by HPLC (H_2O–MeOH, 3 : 2) to afford the residue from the peak at 22 min, which was then purified again by HPLC (Inertsil Ph-3, H_2O–MeOH, 13 : 7) to give 1.3 mg of **4** and 3.4 mg of **3** from the peaks at 22 min and 24 min, respectively. Crotocascarins J (**2**) (59.8 mg) and I (**1**) (6.3 mg) were obtained from fractions 147–155 and 155–162, respectively.

Crotocascarin I (**1**)

Amorphous powder, $[\alpha]_D^{26}$ +32.0 (c=0.65, $CHCl_3$); IR ν_{max} (KBr) cm^{-1}: 3478, 2939, 2873, 1739, 1644, 1452, 1014, 904; UV λ_{max} (MeOH) nm (logε): 223 (3.75); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.07 (1H, s, H-18a), 5.06 (1H, s, H-18b), 4.92 (1H, ddq, J=12.0, 3.6, 1.2 Hz, H-9), 4.20 (1H, d, J=5.4 Hz, H-1), 3.11 (1H, s, H-5), 3.08 (1H, d, J=12.6 Hz, H-7), 2.90 (1H, d, J=12.6 Hz, H-13), 2.58 (1H, dddd, J=12.6, 4.2, 3.6, 3.6 Hz, H_2-10a), 2.55 (1H, ddd, J=13.2, 3.6, 3.6 Hz, H-11a), 2.41 (1H, dd, J=13.8, 7.2 Hz, H-3a), 2.19 (1H, ddd, J=13.2, 12.6, 4.2 Hz, H-11b), 2.02 (1H, ddqd, J=10.6, 7.2, 7.2, 5.4 Hz, H-2), 1.91 (3H, d, J=1.2 Hz, H_3-17), 1.65 (1H, dd, J=13.8, 10.6 Hz, H-3b), 1.21 (1H, dddd, J=13.2, 12.6, 12.0, 4.2 Hz, H_2-10b), 1.05 (3H, s, H_3-20), 1.02 (3H, d, J=7.2 Hz, H_3-19); ^{13}C-NMR (100 MHz, $CDCl_3$): Table 1; CD $\Delta\varepsilon$ (nm): +1.42 (252) (c=1.91×10^{-5} M, MeOH); HR-ESI-MS (positive-ion mode) m/z: 367.1514 $[M+Na]^+$ (Cacld for $C_{20}H_{24}O_5Na$: 367.1515).

Crotocascarin J (**2**)

Amorphous powder, $[\alpha]_D^{24}$ +9.1 (c=0.64, $CHCl_3$); IR ν_{max} (KBr) cm^{-1}: 3433, 2929, 2874, 1745, 1645, 1415, 1384, 907; UV λ_{max} (MeOH) nm (logε): 220 (3.54); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.06 (1H, s, H-18a), 5.04 (1H, s, H-18b), 4.18 (1H, d, J=5.1 Hz, H-1), 3.35 (1H, d, J=12.6 Hz, H-13), 3.11 (1H, s, H-5), 2.97 (1H, dd, J=12.6, 1.1 Hz, H-7), 2.53 (1H, m, H-10a), 2.41 (1H, m, H-11a), 2.39 (1H, dd, J=13.5, 7.0 Hz, H-3a), 2.33 (1H, ddd, J=14.8, 13.4, 6.4 Hz, H-11b), 2.04 (1H, m, H-2), 1.89 (3H, d, J=0.9 Hz, H_3-17), 1.68 (1H, dd, J=13.5, 10.2 Hz, H-3b), 1.56 (1H, ddd, J=13.6, 13.2, 5.7 Hz, H-10b), 1.14 (3H, s, H_3-20), 1.03 (3H, d, J=7.2 Hz, H_3-19); ^{13}C-NMR (100 MHz, $CDCl_3$): Table 1; CD $\Delta\varepsilon$ (nm): +2.54 (255), −2.71 (223) (c=1.78×10^{-5} M, MeOH); HR-ESI-MS (positive-ion mode) m/z: 383.1464 $[M+Na]^+$ (Cacld for $C_{20}H_{24}O_6Na$: 383.1465).

Crotocascarin K (**3**)

Colorless plates, mp 244–245°C; $[\alpha]_D^{24}$ +96.7 (c=0.15, $CHCl_3$); IR ν_{max} (KBr) cm^{-1}: 3435, 2924, 2857, 1739, 1634, 1406, 1384, 904; UV λ_{max} (MeOH) nm (logε): 218 (3.99); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.24 (2H, s, H-18a, 20a), 5.09 (2H, s, H-18b, 20b), 5.06 (1H, m, H-9), 4.42 (1H, s, H-5), 4.15 (1H, d, J=5.2 Hz, H-1), 3.55 (1H, dd, J=11.7, 0.7 Hz, H-7), 2.88 (1H, d, J=11.7 Hz, H-13), 2.60 (2H, m, H-10a, 11a), 2.41 (1H, dd, J=13.7, 7.2 Hz, H-3a), 2.27 (1H, m, H-11b), 2.03 (1H, m, H-2), 1.69 (3H, d, J=1.1 Hz, H_3-17), 1.53 (1H, dd, J=13.7, 10.2 Hz, H-3b), 1.27 (1H, m, H-10b), 1.00 (3H, d, J=7.1 Hz, H_3-19); ^{13}C-NMR (100 MHz, $CDCl_3$): Table 1; CD $\Delta\varepsilon$ (nm): +16.6 (226) (c=4.45×10^{-5} M, MeOH); HR-ESI-MS (positive-ion mode) m/z: 367.1516 (Calcd for $C_{20}H_{24}O_5Na$: 367.1515).

Crotocascarin γ (**4**)

Amorphous powder, $[\alpha]_D^{23}$ +21.8 (c=0.07, $CHCl_3$); IR ν_{max} (film) cm^{-1}: 3420, 2957, 2930, 1757, 1711, 1454, 1150, 891; ^{1}H-NMR (400 MHz, CD_3OD) δ: 5.19 (1H, brs, H-18a), 4.91 (1H, s, H-18b), 4.39 (1H, d, J=5.0 Hz, H-1), 4.28 (1H, s, H-5), 3.15 (1H, brd, J=13.6 Hz, H-13), 2.85 (1H, d, J=13.6 Hz, H-7), 2.50 (1H, m, H-11a), 2.36 (3H, s, H_3-17), 2.20 (3H, overlapped, H-10a, b, 11b), 2.19 (1H, dd, J=13.8, 7.3 Hz, H-3a), 1.95 (1H, m, H-2), 1.51 (1H, dd, J=13.8, 10.6 Hz, H-3b), 1.20 (3H, s, H_3-20), 1.00 (3H, d, J=7.2 Hz, H_3-19); ^{13}C-NMR (100 MHz, CD_3OD): Table 1; HR-ESI-MS (positive-ion mode) m/z: 371.1475 (Calcd for $C_{19}H_{24}O_6Na$: 371.1465).

Isocrotofolane Glucoside (**5**)

Amorphous powder, $[\alpha]_D^{24}$ −63.6 (c=0.36, MeOH); IR ν_{max} (film) cm^{-1}: 3378, 2930, 2876, 1690, 1646, 1457, 1380, 1077, 897; UV λ_{max} (MeOH) nm (logε): 234 (3.84), 211 (3.79); ^{1}H-NMR (400 MHz, CD_3OD) δ: 6.29 (1H, d, J=6.0 Hz, H-8), 4.62 (1H, s, H-18a), 4.60 (1H, d, J=1.1 Hz, H-5), 4.49 (1H, d, J=7.3 Hz, H-1′), 4.28 (1H, s, H-18b), 3.93 (1H, dd, J=11.9. 1.8 Hz, H-6′a), 3.69 (1H, dd, J=11.9, 5.9 Hz, H-6′b), 3.33 (4H, m, H-2′, 3′, 4′, 5′), 3.16 (1H, m, H-3a), 2.65 (1H, brd, J=8.8 Hz, H-13), 2.56 (1H, m, H-11a), 2.51 (1H, m, H-10a), 2.45 (1H, m, H-2), 2.35 (1H, m, H-3b), 2.27 (1H, dd, J=8.8, 6.0 Hz, H-7), 2.00 (2H, m, H-10b, 11b), 1.39 (3H, s, H_3-20), 1.35 (3H, s, H_3-17), 1.34 (3H, s, H_3-16), 1.11 (3H, d, J=7.3 Hz, H_3-19); ^{13}C-NMR (100 MHz, CD_3OD): Table 1; HR-ESI-MS (positive-ion mode) m/z: 517.2385 $[M+Na]^+$ (Calcd for $C_{26}H_{38}O_9Na$: 517.2408).

Compound **6** (**6**)

Amorphous powder, $[\alpha]_D^{25}$ −93.6 (c 0.19, MeOH); IR ν_{max} (film) cm^{-1}: 3364, 2928, 1503, 1255, 1069; UV λ_{max} (MeOH) nm (logε): 333 (2.74), 269 (3.25), 220 (3.57); ^{1}H-NMR

(400 MHz, C_5D_5N): Table 2; ^{13}C-NMR (100 MHz, C_5D_5N): Table 2; HR-ESI-MS (positive-ion mode) *m/z*: 441.1363 $[M+Na]^+$ (Calcd for $C_{18}H_{26}O_{11}Na$: 441.1367).

X-Ray Crystallographic Analysis of Crotocascarin K (3) $C_{20}H_{24}O5_7$, M=344.39, crystal size: 0.23×0.20×0.12 mm^3, space group: orthorhombic, $P2_12_12_1$, T=150 K, a=10.2738(14) Å, b=11.3380(15) Å, c=14.764(2) Å, V=1719.8(4) Å^3, Z=4, D_c=1.330 Mg/m^3, F(000)=736. The data were measured using a Bruker SMART 1000 CCD diffractometer, using Mo$K\alpha$ graphite-monochromated radiation (λ=0.71073 Å) in the range of 4.52<2θ<54.3. Of 8399 reflections collected, 2019 were unique (R_{int}=0.0204, data/restraints/parameters 2019/0/2368). The structure was solved by a direct method using the program SHELXTL-97.[13] The refinement and all further calculations were carried out using SHELXTL-97. The absorption correction was carried out utilizing the SADABS routine.[14] The H atoms were included at calculated positions and treated as riding atoms using the SHELXTL default parameters. The non-H atoms were refined anisotropically using weighted full-matrix least-squares on F^2. Final goodness-of-fit on F^2=1.047, R_1=0.0288, wR_2=0.0737 based on $I>2\sigma(I)$, and R_1=0.0323, wR_2=0.0757 based on all data. The largest difference peak and hole were 0.158 and −0.157 eÅ^{-3}, respectively. Supplementary X-ray crystallographic data for **3** (CCDC 1417185) can be obtained free of charge *via* www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

Preparation of (*R*)- and (*S*)-MTPA Esters (3a, b) from 3 A solution of **3** (0.55 mg) in 0.5 mL of dry CH_2Cl_2 was reacted with (*R*)-MTPA (25.4 mg) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (15.8 mg) and *N*,*N*-dimethyl-4-aminopyridine (4-DMAP) (15.5 mg). The mixture was then occasionally stirred at 37°C for 24 h. After the addition of $CHCl_3$ (1.5 mL), the reaction mixture was successively washed with H_2O (1 mL), 1 M HCl (1 mL), $NaHCO_3$-saturated H_2O (1 mL), and brine (1 mL). The organic layer was dried with Na_2SO_4 and evaporated under reduced pressure. The residue was purified by preparative TLC [silica gel (0.25 mm thickness), being applied for 8 cm width, with development with *n*-hexane–EtOAc (1 : 1) for 9 cm and then eluting with $CHCl_3$–MeOH (9 : 1)] to furnish an ester **3a** (0.2 mg) at *Rf*=0.38. Through the same procedure, **3b** (0.3 mg, *Rf*=0.37) was prepared from **3** (0.55 mg) using (*S*)-MTPA (21.5 mg), EDC (13.4 mg), and 4-DMAP (16.1 mg). (*R*)-MTPA ester (**3a**): amorphous powder; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.38–7.57 (5H, m, aromatic protons), 5.77 (1H, s, H-5), 5.31 (1H, s, H-20b), 5.22 (1H, s, H-18b), 5.08 (1H, s, H-18a), 5.04 (1H, m, H-9), 4.78 (1H, s, H-20a), 4.13 (1H, m, H-1), 3.58 (1H, d, *J*=11.2 Hz, H-7), 3.54 (3H, s, -OMe), 2.88 (1H, d, *J*=11.2 Hz, H-13), 2.42 (1H, dd, *J*=13.7, 6.8 Hz, H-3b), 2.00 (1H, m, H-2), 1.62 (1H, dd, *J*=13.7, 10.2 Hz, H-3a), 1.26 (3H, s, H_3-17), 0.99 (3H, d, *J*=7.3 Hz, H_3-19); HR-ESI-MS (positive-ion mode) *m/z*: 583.1912 $[M+Na]^+$ (Calcd for $C_{30}H_{31}O_7F_3Na$, 583.1920). (*S*)-MTPA ester (**3b**): amorphous powder; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.37–7.60 (5H, m, aromatic protons), 5.78 (1H, s, H-5), 5.24 (2H, s, H_2-18a, 20b), 5.08 (1H, s, H-18a), 5.01 (1H, m, H-9), 4.70 (1H, s, H-20a), 4.15 (1H, m, H-1), 3.53 (1H, d, *J*=11.7 Hz, H-7), 3.66 (3H, s, -OMe), 2.88 (1H, d, *J*=11.7 Hz, H-13), 2.42 (1H, dd, *J*=13.7, 7.3 Hz, H-3b), 2.02 (1H, m, H-2), 1.65 (1H, dd, *J*=13.7, 10.3 Hz, H-3a), 1.26 (3H, s, H_3-17), 0.99 (3H, d, *J*=6.8 Hz, H_3-19); HR-ESI-MS (positive-ion mode) *m/z*: 583.1904 $[M+Na]^+$ (Calcd for $C_{30}H_{31}O_7F_3Na$, 583.1920).

Enzymatic Hydrolysis of 5 Isocrotofolane glucoside (**5**) (4.1 mg) in H_2O (1 mL) was hydrolyzed with β-glucosidase (4.0 mg) at 37°C for 4 d. The reaction mixture was evaporated to dryness, and then the remaining residue was subjected to silica gel CC (Φ=2 cm, L=15 cm) with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$–MeOH (9 : 1; 50 mL), (7 : 3; 100 mL) and (1 : 1; 100 mL)] 5 mL-fractions being collected. An aglycone (**5a**) (2.5 mg) was obtained in fractions 17–24 and the sugar (1.2 mg) in 51–70. Compound **5a**: Amorphous powder, $[\alpha]_D^{24}$ −115.0 (*c* 0.17, $CDCl_3$); IR ν_{max}(film) cm^{-1}: 3321, 2927, 2871, 1683, 1650, 1455, 1373, 1054, 827; UV λ_{max} (MeOH) nm (logε): 231 (3.98), 214 (3.93); ^{1}H-NMR (400 MHz, CD_3OD) δ: 6.29 (1H, d, *J*=6.0 Hz, H-8), 4.61 (1H, s, H-18a), 4.30 (1H, brs, H-5), 4.25 (1H, s, H-18b), 2.95 (1H, dd, *J*=18.7, 6.1 Hz, H-3a), 2.65 (1H, brd, *J*=9.0 Hz, H-13), 2.59 (1H, m, H-10a), 2.51 (1H, m, H-11a), 2.47 (1H, m, H-2), 2.42 (1H, dd, *J*=18.7, 3.6 Hz, H-3b), 2.21 (1H, dd, *J*=9.0, 6.0 Hz, H-7), 2.00 (2H, m, H-10b, 11b), 1.35 (3H, s, H_3-16), 1.34 (3H, s, H_3-17), 1.32 (3H, s, H_3-20), 1.16 (3H, *d*, *J*=7.3 Hz, H_3-19); ^{13}C-NMR (100 MHz, CD_3OD): Table 1; HR-ESI-MS (positive-ion mode) *m/z*: 355.1887 $[M+Na]^+$ (Calcd for $C_{20}H_{28}O_4Na$: 355.1879).

Sugar Analysis About 500 μg each of **5** and **6** was hydrolyzed with 1 M HCl (0.1 mL) at 90°C for 2 h. The reaction mixtures were partitioned with an equal amount of EtOAc (0.1 mL), and the water layers were analyzed by HPLC with a chiral detector (JASCO OR-2090*plus*) on an amino column [Asahipak NH_2P-50 4E, CH_3CN–H_2O (4 : 1), 1 mL/min]. A hydrolyzate of **5** gave a peak for D-glucose at 20.0 min, and one of **6** gave peaks for D-apiose and D-glucose at 6.4 min and 20.0 min, respectively, with positive optical rotation signs. The peaks were identified by co-chromatography with authentic samples.

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