Eight New Diterpenoids and Two New Nor-Diterpenoids from the Stems of *Croton cascarilloides*

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From the stems of *Croton cascarilloides*, eight new diterpenoids, named crotocascarins A–H (1–8), having a crotofolane skeleton were isolated along with two new nor-diterpenoids (9 and 10), named crotocascarins *α* and *β*, derived through rearrangement of the crotofolane skeleton. The structures of these compounds were elucidated by means of extensive one- and two-dimensional NMR spectroscopic analyses. The absolute structures of the diterpene moiety were determined by application of the circular dichroism (CD) rule for the *γ*-lactone ring. The relative structures of the two crotofolanes (1 and 2) and one rearranged compound (9) were confirmed by X-ray crystallographic analyses. Compounds 1, 2 and 9 possessed 2-methylbutyric acid in their molecules, the absolute configuration of which was found to be 2*S* by comparison of its HPLC behavior with that of an authentic sample. Therefore, the absolute structures of these crotocascarins (1, 2 and 9) were unambiguously determined. The absolute structures of crotofolanes are reported for the first time in this paper.

Key words *Croton cascarilloides*; Euphorbiaceae; crotocascarin; crotofolane; diterpenoid

Crotofolane-type diterpenoids have fused 5-, 6- and 7-membered rings, and are expected to be biosynthesized from cembranes *via* lathyrane through cross annular cyclization.[1)] In 1975, this type of diterpenoid was first found in Jamaican *Croton* species, *C. corylifolius* Lamarck as crotofolin A as shown in Fig. 1.[1,2)] Only two other species, Kenyan *C. dichogamus* Pax[3)] and Congolese *C. haumanianus* J. Léonard,[4)] were also found to contain rare crotofolanes. Genus *Croton* (Euphorbiaceae) comprises about 600 species and is distributed in tropical areas of both hemispheres. Croton oil obtained from seeds of *C. tiglium* Linné has a strong purgative effect and the occurrence of cocarcinogenic agents; fatty acid esters of diterpene phorbol in this plant has attracted our interest to investigate this genus.[5)] *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. Leaves are oblong-lanceolate to oblong-oval, and their undersurface is covered by shiny white ramenta.[6)] Our phytochemical investigation of the stems (14.5 kg) of *C. cascarilloides* collected in the Okinawa Islands led to the isolation of eight new crotofolane-type diterpenoids, given trivial names crotocascarins A–H (**1**–**8**), and two new nor-diterpenoids with a new skeleton, given trivial names crotocascarins *α* and *β* (**9**, **10**). The absolute configuration of crotofolanes was first determined in this study.

Results and Discussion

Using several types of chromatography, diterpenoids (**1**–**8**) and nor-diterpenoids (**9**, **10**) were isolated from a CH_2Cl_2-soluble fraction prepared from the MeOH extract of the stems of *C. cascarilloides* (Fig. 2).

Crotocascarin A (**1**), $[\alpha]_D^{26}$ +16.4, was isolated as colorless rods and its elemental composition was determined to be $C_{25}H_{32}O_7$ by high-resolution (HR)-electrospray ionization (ESI)-mass spectrometry. The IR spectrum showed absorption bands for ester carbonyl and lactone carbonyl groups (1763, 1739 cm^{-1}), and double bonds (1650 cm^{-1}). In the ^{1}H-NMR spectrum, signals for two singlet methyls, two doublet methyls and one triplet methyl together with ones for two olefinic protons (δ_H 5.06, 5.09) and two oxygenated methine protons (δ_H 3.17, 5.48) were observed (Table 1). The ^{1}H–^{1}H correlation spectroscopy (COSY) and heteronuclear multiple bond correlation (HMBC) spectra (Fig. 3) together with one-dimensional ones indicated the presence of a 2-methylbutanoic acid moiety, the remaining 20 ^{13}C-NMR signals comprising those of three methyls, three methylenes, five methines, one tetra- and one disubstituted double bonds, three oxygenated tertiary carbons, one carbonyl carbon and one hemiketal carbon (δ_C 107.5) (Table 2). The ten degrees of unsaturation, based on the results of HR-ESI-MS, other than two carbonyl groups and two double bonds, required six ring systems in the skeleton. Precise inspection of two-dimensional NMR spectra led to the conclusion that compound **1** was a diterpenoid with

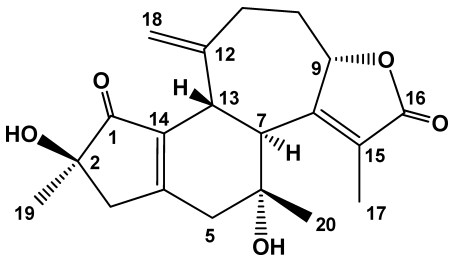

Fig. 1. Structure of Crotofolin A, Isolated from *C. corylifolius*

The authors declare no conflict of interest.

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Table 1. ^{1}H-NMR Spectroscopic Data for Crotocascarins A–H (**1**–**8**), and α and β (**9**, **10**) (400 MHz, $CDCl_3$)

H	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	5.48 d 5	5.38 d 5	5.34 d 5	5.49 d 5	5.43 d 5	5.48 d 5	5.39 d 5	5.35 d 4	5.78 d 5	5.82 d 5
2	2.18 m	2.18 dqdd 8, 7, 7, 5	2.18 m	2.21 m	2.16 m	2.20 m	2.18 m	2.18 m	2.22 m	2.22 m
3	2.47 dd 14, 7 1.65 dd 14, 10	2.49 dd 14, 8 1.70 dd 14, 10	2.50 dd 13, 7 1.72 dd 13, 10	2.48 dd 14, 7 1.64 dd 14, 10	2.48 dd 14, 7 1.69 dd 14, 10	2.47 dd 14, 7 1.65 dd 14, 10	2.50 dd 14, 7 1.70 dd 14, 10	2.50 dd 14, 7 1.71 dd 14, 10	1.56 dd 14, 10 2.34 dd 14, 7	1.52 dd 14, 10 2.36 dd 14, 7
4	—	—	—	—	—	—	—	—	—	—
5	3.17 s	3.19 s	3.20 s	3.15 s	3.18 s	3.16 s	3.19 s	3.20 s	4.39 s	4.36 s
6	—	—	—	—	—	—	—	—	—	—
7	2.99 dd 13, 1	3.06 br d 12	3.00 dd 12, 1	3.10 d 13	2.95 dd 12, 1	2.99 d 13	3.06 d 13	2.99 dd 12, 1	2.92 d 13	3.01 d 13
8	—	—	—	—	—	—	—	—	—	—
9	—	5.14 ddd 13, 4, 2		4.85 dddd 11, 4, 2, 2	—	—	5.14 br d 11	—	—	—
10	2.46 m 1.57 ddd 13, 13, 5	2.73 ddd 13, 4, 4 1.27 ddd 13, 13, 4	2.76 dd 14, 3 1.70 dd 14, 3	2.51 m 1.17 m	3.04 dd 16, 2 1.45 ddd 16, 5, 2	2.44 m 1.58 ddd 13, 13, 5	2.73 ddd 14, 4, 4 1.30 br d 14	2.75 dd 15, 3 1.71 ddd 15, 3, 2	2.07 dd 15, 10 2.46 dd 15, 7	2.17 m 2.36 m
11	2.48 m 2.14 ddd 13, 13, 5	4.53 ddd 4, 4, 2	4.61 dd 3, 3	2.52 m 2.44 m	4.43 ddd 5, 2, 2	2.44 m 2.11 ddd 13, 13, 5	4.52 dd 3, 3	4.60 ddd 3, 3, 2	4.17 br dd 10, 7	2.09 m 2.17 m
12	—	—	—	—	—	—	—	—	—	—
13	3.05 d 13	3.15 d 13	3.45 d 12	2.56 d 13	3.52 d 12	3.04 d 13	3.15 d 13	3.45 d 12	3.11 br d 13	2.99 br d 13
14	—	—	—	—	—	—	—	—	—	—
15	—	—	—	—	—	—	—	—	—	—
16	—	—	—	—	—	—	—	—		
17	1.90 3H d 1	1.90 3H br s	1.89 3H br s	1.91 3H br s	1.88 3H d 1	1.91 3H d 1	1.90 3H d 1	1.89 3H d 1	2.39 3H, s	2.39 3H s
18	5.09 s 5.06 br s	5.20 s 5.17 s	5.28 s 5.23 br s	5.09 s 5.07 br s	5.23 s 5.17 br s	5.09 s 5.06 br s	5.21 s 5.16 br s	5.28 s 5.23 br s	5.23 br s 5.39 s	4.92 s 5.20 br s
19	0.92 3H d 7	0.98 3H d 7	1.00 3H d 7	0.92 3H d 7	0.96 d 7	0.91 3H d 7	0.97 3H d 7	0.99 3H d 7	0.92 3H, d 7	0.90 3H d 7
20	1.17 3H s	1.06 3H s	1.17 3H s	1.07 3H s	1.14 3H s	1.17 3H s	1.06 3H s	1.17 3H s	1.27 3H, s	1.29 3H s
1′	—	—	—					—	—	—
2′	2.43 m	2.49 qdd 7, 7, 7	2.49 qdd 7, 7, 7	2.49 m	2.47 qdd 7, 7, 7	2.62 septet 7	2.65 septet 7	2.66 septet 7	2.38 m	2.36 m
3′	1.72 dqd 14, 7, 7 1.48 dqd 14, 7, 7	1.74 dqd 14, 7, 7 1.50 dqd 14, 7, 7	1.72 dqd 14, 7, 7 1.49 dqd 14, 7, 7	1.73 dqd 14, 7, 7 1.49 dqd 14, 7, 7	1.75 dqd 14, 7, 7 1.50 dqd 14, 7, 7	1.21 3H s 1.21 3H s	1.21 3H s 1.20 3H s	1.20 3H s 1.19 3H s	1.43 dqd 14, 7, 7 1.68 dqd 14, 7, 7	1.42 dqd 14, 7, 7 1.64 dqd 14, 7, 7
4′	0.94 3H t 7	0.93 3H t 7	1.19 3H t 7	0.94 3H t 7	0.93 3H t 7				0.90 3H t 7	0.89 3H t 7
5′	1.19 3H d 7	1.17 3H d 7	1.17 3H d 7	1.18 3H d 7	1.19 3H 6				1.12 3H d 7	1.10 3H d 7
–OCH_3					3.57 3H s					
9-OH						3.49 br s				
11-OH		2.44 dd 2, 2			2.42 dd 2, 2			3.05 dd 2, 2		2.26 d 6

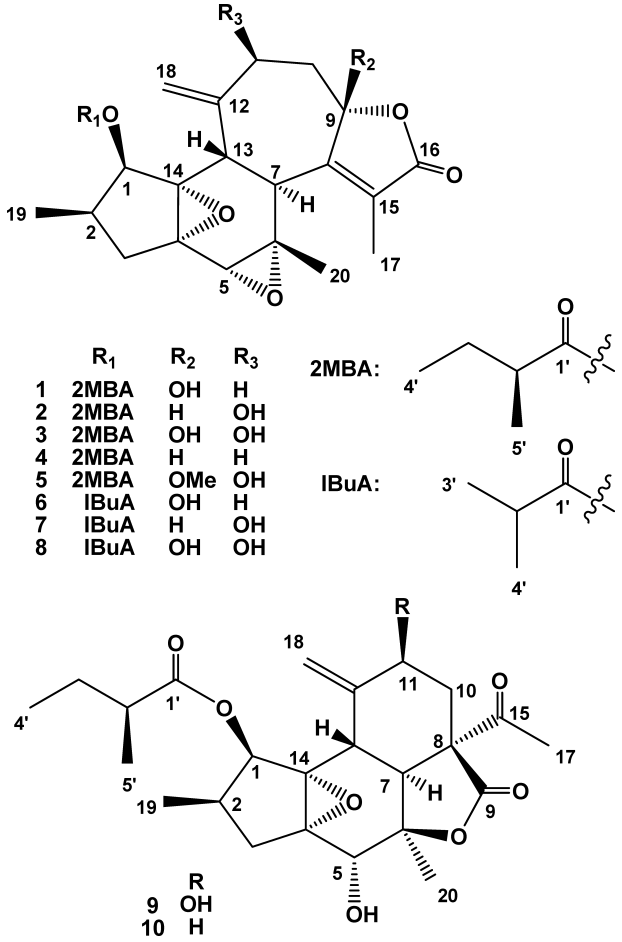

Fig. 2. Structures of New Compounds Isolated

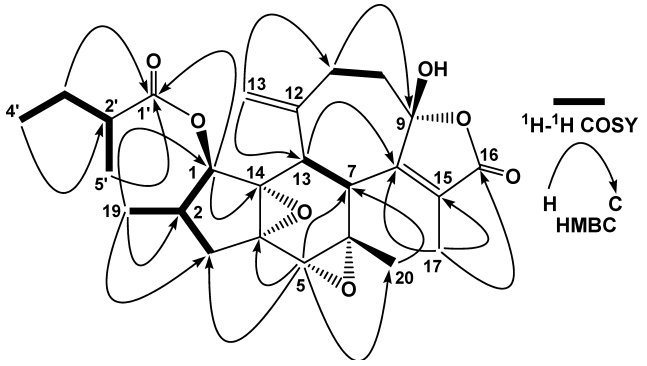

Fig. 3. ^{1}H–^{1}H COSY and Selected HMBC Correlation of **1**

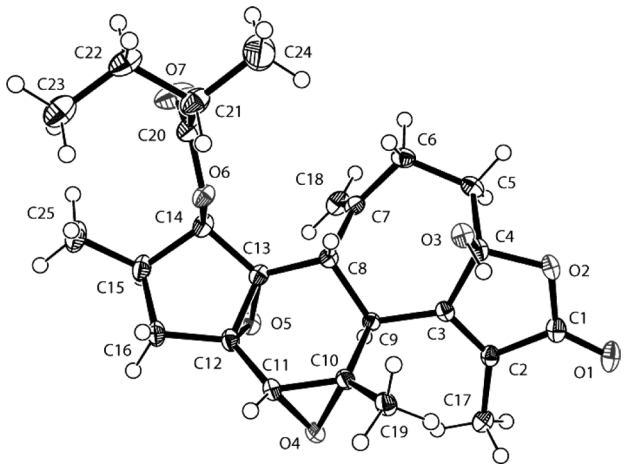

Fig. 4. ORTEP Drawing of **1**
The crystal structure has crystallographic numbering.

an unusual carbon skeleton (Fig. 2). To confirm this, X-ray crystallographic analysis of **1** was performed and the relative stereostructure of **1** was established to be that of a derivative of crotofolane-type diterpenoid (Fig. 4). The positive Cotton effect observable in the circular dichroism (CD) spectrum [$\Delta\varepsilon$ +3.41 (251)] empirically indicated the absolute configuration at the 9-position of the α,β-unsaturated γ-lactone ring was *S*,[7,8] and chirality analysis of the 2-methylbutanoic acid moiety by HPLC with an optical rotation detector established the absolute configuration of **1** to be shown in Fig. 2. This is the first report of the absolute structure of a crotofolane diterpene being disclosed and the absolute configuration of the α,β-unsaturated γ-lactone portion, presumed based on the empirical rule for the CD spectra, was proved to be correct.

Crotocascarin B (**2**), $[\alpha]_D^{26}$ +81.8, was isolated as colorless plates and its elemental composition was determined to be $C_{25}H_{32}O_7$ by HR-ESI-MS. NMR spectroscopic data indicated that **2** was a similar compound to **1**, except for the disappearance of the hemiketal signal, instead of which, two oxymethine signals (δ_H 5.14 on δ_C 78.4 and 4.53 on 72.7) were observed. Since these oxymethine protons were correlated in the ^{1}H–^{1}H COSY spectrum through methylene protons on C-10, the planar structure of **2** was established to be as shown in Fig. 2. To confirm this, X-ray crystallographic analysis of **2** was performed and the relative stereostructure of **2** was established to be that of a derivative of crotofolane-type diterpenoid[9] (Fig. 5). The positive Cotton effect observable in the CD spectrum [$\Delta\varepsilon$ +1.36 (249)] indicated the absolute configuration at the 9-position of the α,β-unsaturated γ-lactone ring was also *S*, and chirality analysis of the 2-methylbutanoic acid moiety by HPLC with an optical rotation detector established the absolute structure of **2** to be as shown in Fig. 2.

Crotocascarin C (**3**), $[\alpha]_D^{24}$ +88.9, and crotocascarin D (**4**), $[\alpha]_D^{24}$ +2.6, were also similar compounds to the aforementioned ones with the respective elemental compositions of $C_{25}H_{32}O_8$ and $C_{25}H_{32}O_6$. In the NMR spectra of **3** and **4**, a hemiketal carbon signal (C-9: δ_C 106.4) and an oxygenated carbon signal (C-11: δ_C 73.7) with δ_H 4.61 were observed in the former, and an oxygenated carbon signal (C-9: δ_C 82.2) and a methylene signal (C-11: δ_C 36.2) in the latter. A similar coupling pattern fot H-11 in **3** to that of **2** placed the hydroxy group at the 11-position in a β-orientation. Since the ^{1}H–^{1}H COSY spectrum of the former exhibited the proton chain from H_2-10 to H-11, along with the observation of a hemiketal carbon in the ^{13}C-NMR spectrum, and that of the latter one from H-9 to H_2-11 through H_2-10, their structures were established to be as shown in Fig. 2. A similar coupling pattern for H-11 in **3** to that of **2** placed the hydroxy group at the 11-position in a β-orientation and positive Cotton effects at 252 nm ($\Delta\varepsilon$ +4.47, +1.17, respectively) substantiated that the absolute configurations of the diterpene moieties were the same as those of aforementioned compounds. The absolute configuration of 2-methylbutanoic acid in **3** and **4** must also be the same as that in **1** and **2**, judging from the ^{13}C-NMR chemical shifts of

Table 2. ^{13}C-NMR Spectroscopic Data for Crotocascarins A–H (**1**–**8**), and α and β (**9**, **10**) (100 MHz, $CDCl_3$)

C	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	74.6	75.8	76.6	74.5	75.4	74.6	76.7	76.3	75.5	75.7
2	33.3	32.7	32.5	33.2	32.6	33.4	32.8	32.5	34.4	34.5
3	36.9	36.4	36.3	36.9	36.4	36.9	36.3	36.3	34.3	34.3
4	60.4	60.1	60.2	60.5	60.2	60.4	60.1	60.2	64.8	65.2
5	57.9	57.8	57.9	57.6	58.0	57.8	57.7	57.9	75.4	75.6
6	56.5	55.9	56.5	56.1	56.4	56.4	55.9	56.5	88.0	87.9
7	44.3	44.4	44.4	44.1	44.7	44.2	44.2	44.4	48.0	47.4
8	159.0	162.0	158.6	161.7	159.0	158.7	161.9	158.5	60.1	62.2
9	107.5	78.4	106.4	82.2	109.5	107.2	78.3	106.4	174.7	175.1
10	41.9	44.1	45.7	37.7	40.4	42.0	44.1	45.8	35.8	27.1
11	34.8	72.7	73.7	36.2	73.7	34.8	72.6	73.7	67.9	27.2
12	146.8	148.9	148.4	146.0	149.3	146.6	148.7	148.3	146.1	141.0
13	39.5	31.7	31.8	40.7	31.4	39.5	31.7	31.6	32.4	34.7
14	68.9	68.8	68.8	68.7	68.8	68.8	68.8	68.7	66.0	65.9
15	130.4	128.2	129.8	128.5	129.9	130.6	128.2	129.9	202.0	201.7
16	170.8	173.4	170.3	173.0	170.7	170.5	173.3	170.2		
17	9.6	9.7	9.6	9.6	9.7	9.7	9.7	9.6	26.0	25.4
18	115.1	115.2	116.3	115.2	115.9	115.1	115.2	116.3	113.8	110.4
19	12.7	12.3	12.2	12.7	12.3	12.5	12.1	12.1	12.6	12.7
20	20.2	19.3	19.7	19.5	20.1	20.3	19.3	19.7	22.0	22.1
1′	175.4	178.0	178.8	175.2	178.1	175.6	178.1	179.0	175.5	175.0
2′	41.2	41.2	41.4	41.2	41.5	34.2	34.3	34.4	41.2	41.6
3′	26.7	26.6	26.6	26.8	26.5	19.3	19.3	19.2	26.6	26.6
4′	11.7	11.4	11.5	11.7	11.5	19.4	18.7	18.9	11.8	11.7
5′	17.2	16.2	16.6	17.1	16.5				16.9	17.3
$-OCH_3$					52.8					

the acid moiety and the five-membered ring.

Crotocascarin E (**5**), $[\alpha]_D^{25}$ +95.2, was isolated as an amorphous powder and its elemental composition was determined to be $C_{26}H_{34}O_8$ by HR-ESI-MS. The NMR spectroscopic data were essentially the same as those of crotocascarin C (Tables 1, 2), except for the presence of a methoxy signal [δ_H 3.57 (3H, s)], which crossed the ketal carbon one (δ_C 109.5) in the HMBC spectrum. Therefore, the structure of **5** was assigned, as shown in Fig. 1. The methoxy derivative is probably an artifact formed during the extraction and isolation processes.

Crotocascarins F (**6**), $[\alpha]_D^{25}$ +16.8, and G (**7**), $[\alpha]_D^{24}$ +81.7, were isolated as amorphous powders, and crotocascarin H (**8**), $[\alpha]_D^{24}$ +94.2, as colorless needles, and their elemental compositions were determined to be $C_{24}H_{30}O_7$, $C_{24}H_{30}O_7$ and $C_{24}H_{30}O_8$, respectively, by HR-ESI-MS. The ^{13}C-NMR spectral data for their diterpeneoid regions were essentially superimposable on those of crotocascarins A (**1**), B (**2**), and C (**3**), respectively. The common acyl moiety of these diterpenoids comprised four carbons, *i.e.*, two doublet methyls, one methine, whose proton was coupled as a septet, and a carbonyl carbon. Therefore, the structure of the acyl moiety was expected to be isobutanoic acid and the gross structures of **6**–**8** were shown to be as in Fig. 1. The absolute configurations of **6**, **7** and **8** were expected to be the same as those of crotocascarins A (**1**), B (**2**), and C (**3**), respectively, from similar respective optical rotation values and similar positive Cotton effect, [**6**: +4.66 (251), **7**: +1.41 (248) and **8**: +8.73 (252), respectively].

Crotocascarin α (**9**), $[\alpha]_D^{26}$ +78.7, was isolated as colorless plates and its elemental composition was determined to be $C_{24}H_{32}O_8$ by HR-ESI-MS. In the IR spectrum, absorption bands for carbonyl groups (1761, 1721 cm^{-1}) and a double bond (1634 cm^{-1}) were observed. The NMR spectroscopic data

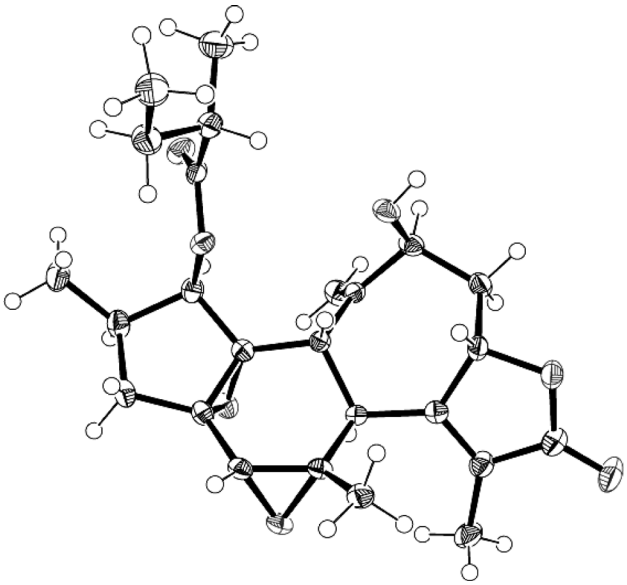

Fig. 5. ORTEP Drawing of **2**

The crystallization solvent is omitted from the structure.

indicated the presence of 2-methylbutanoic acid as an acyl substituent, thus the terpenoid region comprised 19 carbons, *i.e.*, three methyls, two methylenes, three oxymethines, three methines, three oxygenated tertiary carbons, one quaternary carbon, an exomethylene moiety and two carbonyl carbons. The presence of the 2-methylbutanoic acid moiety and other functionalities, observed in one-dimensional NMR spectra, implied that crotocascarin α (**9**) was a compound related to crotofolanes. Since extensive examination of two-dimensional NMR data unfortunately did not lead to a structure which

satisfied all the spectroscopic data, an attempt was made to solve the structure by X-ray crystallographic analysis and an ORTEP drawing of **9** is presented in Fig. 6.[9] Crotocascarin *α* (**9**) has a new skeleton and was probably derived from some crotofolane, like crotocascarin B (**2**), through several steps, such as decarboxylation, C–C bond migration, oxidation, *etc.* (Fig. 7). The absolute configuration of the 2′-position was determined to be *S* by the same method used for **1** and **2**. Therefore, the structure of **9** is shown in Fig. 2, including the absolute one.

Crotocascarin *β* (**10**), $[\alpha]_D^{23}$ +35.0, was isolated as an amorphous powder and its elemental composition was determined to be $C_{24}H_{32}O_7$, which was one oxygen atom less than that of **9**. The NMR spectroscopic data indicated that crotocascarin *β* (**10**) was a congeneric compound to **9** with three methylene carbons. One of the oxymethine protons at δ_H 4.17, which was observed in the NMR spectrum of **9** was obviously replaced by methylene protons, with H-1 and H-5 remaining intact. Therefore, the structure of **10** was elucidated to be as shown in Fig. 2, namely 11-deoxycrotocascarin *α*. The absolute configuration of 2-methylbutanoic acid must also be the same as that in **1**, **2** and **8**, judging from the ^{13}C-NMR chemical shifts of the acid moiety and the five-membered ring.

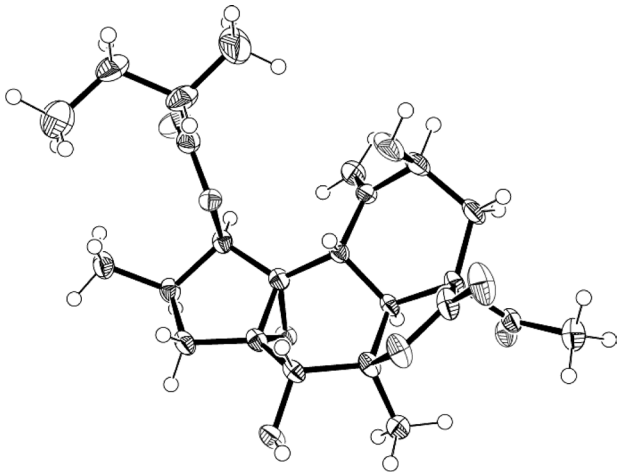

Fig. 6. ORTEP Drawing of **9**

Only seven crotofolane-type diterpenoids have been isolated so far, four from Jamaican *C. corylifolius*,[1,2] two from Kenyan *C. dichogamus*,[3] and one from Congolese *C. haumanianus*.[4] In these studies, without exception, the authors used an X-ray crystallographic method to come to a the final conclusion as to the relative structure. In this investigation on *C. cascarilloides*, a series of crotofolanes was isolated and two nor-diterpenes having a new skeleton probably derived from a crotofolane through the postulated biosynthetic scheme in Fig. 7. The structures of **1** and **2** were solved by X-ray crystallography using a direct method, and assignment of the absolute configuration of the acyl moiety, 2-methylbutanoic acid, obtained on chemical degradation of crotocascarins A (**1**) and B (**2**) as *S* provided information on the absolute structure of a crotofolane for the first time.

Experimental

General Melting points were measured on a Yanagimoto

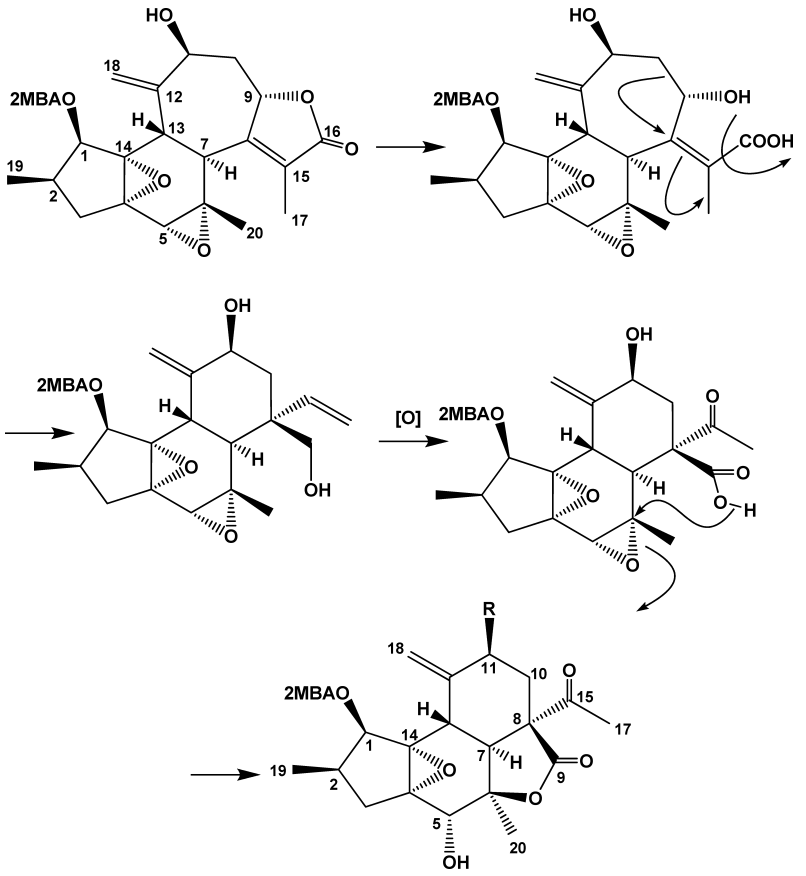

Fig. 7. Possible Biosynthetic Pathway from **2** to **9**

micro melting point apparatus and are 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 at 400 MHz and 100 MHz 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. Silica gel column chromatography (CC) was performed on Kiesel Gel (silica gel 60) (70–230 mesh) (E. Merck, Darmstadt, Germany) and reversed-phase octadecylsilanized (ODS) open CC on Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto, Japan) (Φ=50 mm, L=25 cm). HPLC was performed on an ODS column (Inertsil ODS-3; GL Science, Tokyo, Japan; Φ=6 mm, L=25 cm, 1.6 mL/min), and the eluate was monitored with UV (210 nm) and refractive index monitors. (*S*)-(+)-2-Methylbutanoic acid was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Plant Material Stems of *C. cascarilloides* were collected at Okinawa in June 2004, and a voucher specimen was deposited in the Herbarium of the Department of Pharmacognosy, Graduate School of Biomedical and Health Sciences, Hiroshima University (04-CC-Okinawa-0628).

Extraction and Isolation Stems (14.5 kg) of *C. cascarilloides* were extracted with MeOH (15 L×3) for a week at 25°C. The combined extract was concentrated to 6 L and then partitioned with *n*-hexane (6 L, *n*-hexane extract: 92.1 g). The methanolic layer was concentrated and the resulting residue was suspended in 6 L of H_2O. The H_2O layer was partitioned with 6 L each of CH_2Cl_2, EtOAc and 1-BuOH to give 39.1 g, 10.5 g and 52.2 g of the respective residues.

The residue (39.1 g) of the CH_2Cl_2-soluble fraction was subjected to silica gel CC (400 g) (Φ=60 mm, L=30 cm) with $CHCl_3$ (5 L), $CHCl_3$–MeOH (15 : 1, 7 L and 12 : 1, 5 L), and MeOH (2 L). Fractions of 500 mL were collected. The residue (3.11 g) in fraction 14 was separated by two runs of ODS open CC [H_2O–MeOH (1 : 1, 1 L)→(1 : 9, 1 L) and then H_2O–MeOH (1 : 9, 250 mL)→MeOH (250 mL)], fractions of 10 g being collected. The residue (116 mg) in fractions 137–152 obtained on the first run of ODS open CC was again subjected to silica gel CC (Φ=10 mm, L=40 cm) with *n*-hexane–EtOAc [(9 : 1, 100 mL), (17 : 3, 100 mL), (4 : 1, 100 mL), (7 : 3, 100 mL), (3 : 2, 100 mL) and (1 : 1, 100 mL)], and EtOAc (100 mL). Fractions of 2 mL were collected, and 3.1 mg of **5** was obtained in fractions 135–144.

The residue (166 mg) in fractions 45–58 obtained on the second run of ODS open CC was again subjected to silica gel CC (Φ=10 mm, L=40 cm) with *n*-hexane (250 mL) → *n*-hexane–EtOAc (1 : 1, 250 mL), and then *n*-hexane–EtOAc (1 : 1, 250 mL). Fractions of 2 mL were collected. The residue (16.4 mg) in fractions 270–290 was finally purified by HPLC (H_2O–MeOH, 1 : 1) to give 3.5 mg of **9** from the peak at 35 min.

The residue (101 mg) in fractions 64–72 obtained on the second run of ODS open CC was again subjected to silica gel CC (Φ=10 mm, L=40 cm) with *n*-hexane (250 mL)→*n*-hexane–EtOAc (1 : 1, 250 mL), and then *n*-hexane–EtOAc (1 : 1, 250 mL). Fractions of 2 mL were collected. The residue (22.2 mg) in fractions 123–139 was purified by HPLC (H_2O–MeOH, 1 : 1) to give 12.3 mg of **7** from the peak at 76 min. From fractions 140–151, 10.5 mg of **8** was obtained in a crystalline state. The residue (12.2 mg) in fractions 152–151 was purified by HPLC [Inertsil (Ph-3), H_2O–MeOH, 1 : 1; 1.6 mL/min] to afford a further amount (5.1 mg) of **8** from the peak at 41 min.

The residue (155 mg) in fractions 73–85 obtained on the second run of ODS open CC was again subjected to silica gel CC (Φ=10 mm, L=40 cm) with *n*-hexane (250 mL)→*n*-hexane–EtOAc (1 : 1, 250 mL), and then *n*-hexane–EtOAc (1 : 1, 250 mL). Fractions of 2 mL were collected. The residue (2.3 mg) in fractions 106–113 was purified by HPLC (H_2O–MeOH, 2 : 3) to give 0.7 mg of **10** from the peak at 21 min. The residue (64.9 mg) in fractions 124–134 was purified by HPLC (H_2O–MeOH, 3 : 7) to yield 8.6 mg of **6** and 5.4 mg of **2** from the peaks at 10 min and 11 min. respectively. From fractions 135–147, 2.5 mg of **3** was obtained in a crystalline state.

The residue (126 mg) in fractions 86–95 obtained on the second run of ODS open CC was again subjected to silica gel CC (Φ=10 mm, L=40 cm) with *n*-hexane (250 mL)→*n*-hexane–EtOAc (1 : 1, 250 mL), and then *n*-hexane–EtOAc (1 : 1, 250 mL), fractions of 2 mL being collected. The residue (13.1 mg) in fractions 114–123 was purified by HPLC (H_2O–MeOH, 3 : 7) to give 6.5 mg of **1** and 2.9 mg of **4** from the peaks at 14 min and 15 min, respectively.

Crotocascarin A (**1**): Colorless rods (MeOH), mp 220–221°C, $[\alpha]_D^{26}$ +16.4 (c=0.95, $CHCl_3$); IR ν_{max} (KBr) cm^{-1}: 3399, 2966, 2930, 1763, 1739, 1650, 1456, 1180, 1146, 1018, 802; UV λ_{max} (MeOH) nm (log ε): 223sh (3.87), 208 (4.21); ^{1}H-NMR (400 MHz, $CDCl_3$): Table 1; ^{13}C-NMR (100 MHz, $CDCl_3$): Table 1; CD $\Delta\varepsilon$ (nm): +3.41 (251), −8.39 (224) (c=2.02×10^{-5} M, MeOH); HR-ESI-MS (positive-ion mode) *m/z*: 467.2046 $[M+Na]^+$ (Calcd for $C_{25}H_{32}O_7Na$: 467.2040).

Crotocascarin B (**2**): Colorless plates (2-PrOH), mp 152–153°C, $[\alpha]_D^{26}$ +81.8 (c=1.52, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3478, 2972, 2929, 1769, 1739, 1659, 1457, 1185, 1143, 1014, 804; UV (MeOH) λ_{max} nm (log ε): 218 (4.00); ^{1}H-NMR ($CDCl_3$, 400 MHz): Table 1; ^{13}C-NMR ($CDCl_3$, 100 MHz): Table 2; CD $\Delta\varepsilon$ (nm): +1.36 (249), −1.27 (210) (c 4.31×10^{-5} M, MeOH); HR-ESI-MS (positive-ion mode) *m/z*: 467.2017 $[M+Na]^+$ (Calcd for $C_{25}H_{32}O_7Na$: 467.2040).

Crotocascarin C (**3**): Colorless plates (MeOH), mp 203–205°C, $[\alpha]_D^{24}$ +88.9 (c=0.82, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3448, 2974, 2938, 1759, 1734, 1649, 1140, 1081, 877; UV (MeOH) λ_{max} nm (log ε): 214 (3.85); ^{1}H-NMR ($CDCl_3$, 400 MHz): Table 1; ^{13}C-NMR ($CDCl_3$, 100 MHz): Table 2; CD $\Delta\varepsilon$ (nm): +4.47 (252), −10.61 (226) (c=1.78×10^{-5} M, MeOH); HR-ESI-MS (positive-ion mode) *m/z*: 483.1985 $[M+Na]^+$ (Calcd for $C_{25}H_{32}O_8Na$: 483.1989).

Crotocascarin D (**4**): Amorphous powder, $[\alpha]_D^{24}$ +2.6 (c=0.19, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3463, 2972, 2932, 1794, 1748, 1651, 1457, 1161, 1112, 1062, 903; UV (MeOH) λ_{max} nm (log ε): 219 (4.00); ^{1}H-NMR ($CDCl_3$, 400 MHz): Table 1; ^{13}C-NMR ($CDCl_3$, 100 MHz): Table 2; CD $\Delta\varepsilon$ (nm): +1.17 (252), −3.21 (212) (c=2.26×10^{-5} M, MeOH); HR-ESI-MS (positive-ion mode) *m/z*: 451.2085 $[M+Na]^+$ (Calcd for $C_{25}H_{32}O_6Na$: 451.2091).

Crotocascarin E (**5**): Amorphous powder, $[\alpha]_D^{25}$ +95.2 (c=0.15, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3480, 2970, 2934, 1767, 1739, 1457, 1190, 1139, 1085, 802; UV (MeOH) λ_{max} nm (log ε): 216 (3.92); ^{1}H-NMR ($CDCl_3$, 400 MHz): Table 1; ^{13}C-NMR ($CDCl_3$, 100 MHz): Table 2; CD $\Delta\varepsilon$ (nm): +4.23

(251), −12.68 (224) (c=3.06×10^{-5} M, MeOH); HR-ESI-MS (positive-ion mode) m/z: 497.2129 $[M+Na]^+$ (Calcd for $C_{26}H_{34}O_8Na$: 497.2145).

Crotocascarin F (**6**): Amorphous powder, $[\alpha]_D^{25}$ +16.8 (c=0.22, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3466, 2973, 2932, 1765, 1739, 1651, 1459, 1337, 1191, 1154, 1066, 895; UV (MeOH) λ_{max} nm (log ε): 219 (3.88); ^{1}H-NMR ($CDCl_3$, 400 MHz): Table 1; ^{13}C-NMR ($CDCl_3$, 100 MHz): Table 2; CD $\Delta\varepsilon$ (nm): +4.66 (251), −10.18 (225) (c=2.56×10^{-5} M, MeOH); HR-ESI-MS (positive-ion mode) m/z: 453.1897 $[M+Na]^+$ (Calcd for $C_{24}H_{30}O_7Na$: 453.1889).

Crotocascarin G (**7**): Amorphous powder, $[\alpha]_D^{24}$ +81.7 (c=0.82, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3436, 1741, 1634, 1459, 1195, 1157, 1072, 887; UV (MeOH) λ_{max} nm (log ε): 220 (4.08); ^{1}H-NMR ($CDCl_3$, 400 MHz): Table 1; ^{13}C-NMR ($CDCl_3$, 100 MHz): Table 2; CD $\Delta\varepsilon$ (nm): +1.41 (248), −4.22 (212) (c=1.91×10^{-5} M, MeOH); HR-ESI-MS (positive-ion mode) m/z: 453.1888 $[M+Na]^+$ (Calcd for $C_{24}H_{30}O_7Na$: 453.1883).

Crotocascarin H (**8**): Colorless needles ($CHCl_3$), mp 242–244°C, $[\alpha]_D^{24}$ +94.2 (c=0.33, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3440, 2978, 2938, 1760, 1739, 1648, 1444, 1183, 1141, 1082, 880; UV (MeOH) λ_{max} nm (log ε): 214 (4.16); ^{1}H-NMR ($CDCl_3$, 400 MHz): Table 1; ^{13}C-NMR ($CDCl_3$, 100 MHz): Table 2; CD $\Delta\varepsilon$ (nm): +8.73 (252), −23.36 (226) (c=1.47×10^{-5} M, MeOH); HR-ESI-MS (positive-ion mode) m/z: 469.1831 $[M+Na]^+$ (Calcd for $C_{24}H_{30}O_8Na$: 469.1832).

Crotocascarin α (**9**): Colorless plates ($CHCl_3$), mp 202–203°C, $[\alpha]_D^{26}$ +78.7 (c=0.13, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3479, 2968, 2926, 1761, 1721, 1634, 1461, 1193, 804; ^{1}H-NMR ($CDCl_3$, 400 MHz): Table 1; ^{13}C-NMR ($CDCl_3$, 100 MHz): Table 2; HR-ESI-MS (positive-ion mode) m/z: 471.1973 $[M+Na]^+$ (Calcd for $C_{24}H_{32}O_8Na$: 471.1989).

Crotocascarin β (**10**): Amorphous powder, $[\alpha]_D^{23}$ +35.0 (c=0.04, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3467, 2926, 1741, 1714, 1654, 1460, 1162, 889; ^{1}H-NMR ($CDCl_3$, 400 MHz): Table 1; ^{13}C-NMR ($CDCl_3$, 100 MHz): Table 2; HR-ESI-MS (positive-ion mode) m/z: 455.2044 $[M+Na]^+$ (Calcd for $C_{24}H_{32}O_7Na$: 455.2040).

X-Ray Ccrystallographic Analysis of Crotocascarin A (1) $C_{25}H_{32}O_7$, M=444.51, crystal size: 0.38×0.20×0.10 mm^3, space group: orthorhombic, $P2_12_12_1$, T=90 K, a=6.1970(15) Å, b=15.124(4) Å, c=25.145(6) Å, V=2356.6(10) Å^3, Z=4, D_c=1.253 Mg/m^3, F(000)=952. 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 3.04<2θ<56.7. Of the 14241 reflections collected, 5548 were unique (R_{int}=0.0421, data/restraints/parameters 5548/0/298. The structure was solved by a direct method using the program SHELXTL-97.[10] The refinement and all further calculations were carried out using SHELXTL-97.[11] The absorption correction was carried out utilizing the SADABS routine.[10] The H atoms were included at the 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=1.078, R_1=0.0408, wR_2=0.0997 based on $I>2\sigma(I)$, and R_1=0.0496, wR_2=0.1114 based on all data. The largest difference peak and hole were 0.645 and −0.402 eÅ^{-3}, respectively.

X-Ray Crystallographic Analysis of Crotocascarin B (2) $C_{28}H_{40}O_8$, M=504.60, crystal size: 0.50×0.30×0.15 mm^3, space group: orthorhombic, $P2_12_12_1$, T=120 K, a=10.1775(10) Å, b=10.4348(10) Å, c=25.908(3) Å, V=2751.5(5) Å^3, Z=4, D_c=1.218 Mg/m^3, F(000)=1088. Of the 13566 reflections collected in the range of 3.14<2θ<53.4, 3212 were unique (R_{int}=0.0224), data/restraints/parameters 3212/0/334. The structure was solved in a similar manner to as that for compound **1**. Final goodness-of-fit on F^2=1.048, R_1=0.0344, wR_2=0.0868 based on $I>2\sigma(I)$, and R_1=0.0378, wR_2=0.0891 based on all data. The largest difference peak and hole were 0.335 and −0.228 eÅ^{-3}, respectively.

Alkaline Hydrolysis of Crotocascarins A (1), B (2) and α (8) Crotocascarins A (**1**) (2.3 mg), B (**2**) (2.5 mg) and α (2.0 mg), and authentic (*S*)-(+)-2-methylbutyric acid were (500 μL) each dissolved in 1 mL of a 1:1 mixture of 10% KOH in H_2O and 50% aqueous dioxane, and then heated for 3 h at 100°C. The cooled reaction mixtures were neutralized with Amberlite IR-120B (H^+) and then the filtrates were evaporated. The four residues were analyzed by HPLC (column: Inertsil ODS-3, 6 mm×250 mm; solvent: 20% acetonitrile in H_2O containing 0.5% trifluoroacetic acid; flow rate: 1.6 mL/min) with a chiral detector (JASCO OR-2090*plus*) to give a peak of (*S*)-(+)-2-methylbutyric acid at 17.5 min with a positive optical rotation sign.

X-Ray Crystallographic Analysis of Crotocascarin α (10) $C_{24}H_{32}O_8$, M=448.50, crystal size: 0.30×0.15×0.15 mm^3, space group: monoclinic, $P2_1$, T=120 K, a=9.9294(12) Å, b=9.1267(11) Å, c=12.5443(15) Å, β=98.650(1)°, V=1123.9(2) Å^3, Z=2, D_c=1.325 Mg/m^3, F(000)=1088. Of the 5560 reflections collected in the range of 3.28<2θ<54.1, 2416 were unique (R_{int}=0.0154), data/restraints/parameters 2416/1/296. The structure was solved in a similar manner to as that for compound **1**. Final goodness-of-fit on F^2=1.056, R_1=0.0315, wR_2=0.0794 based on $I>2\sigma(I)$, and R_1=0.0335, wR_2=0.0809 based on all data. The largest difference peak and hole were 0.285 and −0.208 eÅ^{-3}, respectively.

Supplementary Data Supplementary X-ray crystallographic data for **1** (CCDC 894968), **2** (CCDC 761004), and **10** (CCDC 761005) 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, U.K.; fax: (+44) 1223–336–033; or deposit@ccdc.cam.ac.uk).

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