Regular Article

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

Susumu Kawakami, a Katsuyoshi Matsunami, b Hideaki Otsuka, a,c Masanori Inagaki, a Yoshio Takeda, a Masatoshi Kawahata, a and Kentaro Yamaguchi a

a Department of Natural Products Chemistry, Faculty of Pharmacy, Yasuda Women’s University; 6–13–1 Yashigahashi, Asaminami-ku, Hiroshima 731–0153, Japan; b Department of Pharmacognosy, Graduate School of Biomedical and Health Sciences, Hiroshima University; 1–2–3 Kasumi, Minami-ku, Hiroshima 734–8553, Japan; and c Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, Tokushima Bunri University Kagawa Campus; 1314–1 Shido, Sanuki, Kagawa 769–2193, Japan.

Received August 16, 2015; accepted September 28, 2015

From the 1-BuOH-soluble fraction of a methanol (MeOH) extract of the leaves of Croton cascarilloides, crotolfolean: crotocascarsins 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; crotolfolean; nor-crotofolane; isocrotofolane

A diterpenoid, phorbol 12-myristate 13-acetate, isolated from Croton oil is known to have a potent tumor-promoting effect1,2) 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 C. cascarilloides provided eight rare crotolfolean-type and two rearranged crotolfolean-type diterpenoids. In this study, from the 1-BuOH-soluble fraction of a MeOH extract of the leaves of C. cascarilloides, three crotolfolean-type diterpenoids, named crotocascarsins 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.

The structures of the new compounds isolated were elucidated from spectroscopic evidence and that of crotocascarin D (9) was determined to be C20H24O6, which is one oxygen more than in the case of crotocascarin D (9). The absolute structure of 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 (Δε +1.42) in the circular dichroism (CD) spectrum to that observed in crotocascarin D (9). Crotocascarin J (2), [α]D 24 +32.0, was isolated as an amorphous powder and its elemental composition was determined to be C30H32O6, 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 hydroxyl and γ-lactone functional groups. The UV absorption band at 223 nm indicated the presence of an α,β-unsaturated ketone moiety. In the 1H-NMR spectrum, signals for one singlet methyl at δH 1.02, two doublet methyls at δH 1.05, two doublet methyls at δH 1.05 (1H, s) and 5.06 (1H, s) were observed. The 13C-NMR spectrum was similar to that 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 δH 5.49 (crotocascarin D) to δH 4.20 in the 1H-NMR spectrum, and the C-1 signal shifted up from δC 74.5 (crotocascarin D) to δC 73.6 in the 13C-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 (Δε +1.42) in the circular dichroism (CD) spectrum to that observed in crotocascarin D (9).

Crotocascarin J (2), [α]D 24 +9.1, was isolated as an amorphous powder and its elemental composition was determined to be C30H32O6, which is one oxygen more than in the case of 1. The 13C-NMR spectrum was similar to that of 1, and this
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 $\delta_{C} 108.0$, with the simultaneous disappearance of the oxygenated methine signal at $\delta_{C} 82.3$ 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 $\delta_{H} 5.24$, and H-18b and 20b at $\delta_{H} 5.09$, 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. 1. The Structures of Compounds Isolated and Reference Compounds

Fig. 2. Important HMBC Correlations of Crotocascarin K (3)
The structure has crystallographic numbering.
placed the hydroxy group at the 5-position in a \( \beta \)-orientation, and those between H-7 and H-320, and H-5 and H-20 the hydroxy group at the 5-position also in a \( \beta \)-orientation and the 20-methyl group in an \( \alpha \)-orientation, as shown in Fig. 6. Those between H-5 and H-3a (\( \delta_H 3.16 \)), H-3b (\( \delta_H 2.35 \)) and H3-19 and then H-2 (\( \delta_H 2.45 \)) and H-3a placed the 19-methyl group in a \( \beta \)-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 C18H26O11 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 anomic protons at \( \delta_H 5.52 \) and 5.69 on \( \delta_C 102.8 \) 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 [\( \delta_H 6.90 \) (dd, \( J=8.1, 2.0 \) Hz)], and those on anomic protons, \( \delta_H 5.52 \) 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 \( \beta \)-D-(6\( -\)-O-\( \beta \)-D-apiofuranosyl) glucopyranoside, as shown in Fig. 1.

Isocrotofolane glucopyranoside (5) is a diterpene, having a new carbon skeleton. Biosynthesis of crotofolane is expected to start from geranylgeranylpyrophosphate and then proceed via embrane, casbane, lathyrane and then jatrophone (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 diterpeneoid skeleton, isolated from \( C. cascarilloides \).

### Experimental

#### General Experimental Procedure

Melting point (mp)

| C   | 1  | 2  | 3  | 4\(^a\) | 5\(^b\) | 5a\(^b\) |
|-----|----|----|----|--------|--------|--------|
| 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  |
| 5   | 57.9| 58.2| 71.1| 75.8   | 82.3   | 75.1   |
| 6   | 56.1| 56.6| 145.1| 89.8  | 76.1   | 75.8   |
| 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   |

\( a \) Data for CD\(_2\)OD. \( b \) \( \Delta \delta_5-\delta_5b \).
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. 1H- and 13C-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.

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 75C18-OPN (Nacalai Tesque, Kyoto, Japan) [Φ=50 mm, L=25 cm, linear gradient: MeOH–H2O (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 CHCl3–MeOH–H2O–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 ([α]D23 +9.4 (c=0.84, H2O)) 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 RAUSCHEL (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 H2O (6 L), and then partitioned with EtOAc

Table 2. NMR Spectroscopic Data for Compound 6 (13C: 100 MHz, 1H: 400 MHz; C5D5N)

| C     | H        |
|-------|----------|
| 1     | 150.3    |
| 2     | 148.3    |
| 3     | 113.3    |
| 4     | 122.7    |
| 5     | 121.7    |
| 6     | 117.4    |
| CH3   | 56.0     |
| 1’    | 102.8    |
| 2’    | 74.9     |
| 3’    | 78.4     |
| 4’    | 71.4     |
| 5’    | 77.4     |
| 6’    | 69.0     |
| OCH3  | 56.0     |
| 1”    | 111.2    |
| 2”    | 77.7     |
| 3”    | 80.4     |
| 4”    | 75.1     |
| 5”    | 65.7     |

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 H2O-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 H2O–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 (6.90 g) in fractions 15–18 obtained on Diaion HP-20 was subjected to silica gel CC (Φ=36 mm, L=57 cm), and eluted with CHCl3 (99:1, 1.5 L), CHCl3–MeOH (97:3, 1.5 L), (97:1, 1.5 L), (7:1, 1.5 L), (7:1, 1.5 L), (37:3, 1.5 L), (9:1, 1.5 L), (17:3, 1.5 L), (33:7, 1.5 L), (4:1, 1.5 L), (3:1, 1.5 L), and MeOH (1.5 L), 250 mL-fractions being collected. The residue (1.61 g) in fractions 58–79 was separated by ODS open CC to give 50.6 mg of crude 5 in fractions 87–97, which was finally purified by HPLC (H2O–MeOH, 3:2) to afford 2.9 mg of pure 5 in fractions 87–97, which was finally purified by HPLC (H2O–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 was subjected to silica gel CC (Φ=36 mm, L=57 cm), and eluted with CHCl3 (1.5 L), CHCl3–MeOH (99:1, 1.5 L), (97:3, 1.5 L), (97:1, 1.5 L), (7:1, 1.5 L), (7:1, 1.5 L), (37:3, 1.5 L), (9:1, 1.5 L), (17:3, 1.5 L), (33:7, 1.5 L), (4:1, 1.5 L), (3:1, 1.5 L), and MeOH (1.5 L), 250 mL-fractions being collected. The residue (592 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 1.3 mg of pure 3 and 4.3 mg of 4 from the peaks at 22 min and 24 min, respectively. Crotocascarins J (2) and K (3)

Crotocascarin J (2)

Amorphous powder, [α]D24 +9.1 (c=0.64, CHCl3); IR νmax (KBr) cm−1: 3433, 2929, 2874, 1745, 1645, 1415, 1384, 907; UV λmax (MeOH) nm (log ε): 220 (3.54); 1H-NMR (400 MHz, CDCl3) 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-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), 1.34 (3H, s, H-20), 1.03 (3H, d, J=7.2 Hz, H-19); 13C-NMR (100 MHz, CDCl3); Table 1; CD Δε (nm): +2.54 (255), −2.71 (223) (c=1.78×10−3 M, MeOH); HR-ESI-MS (positive-ion mode) m/z: 383.1464 [M+[Na]+] (Calcd for C20H24O5Na: 383.1465).  
Crotocascarin K (3)

Colorless plates, mp 244–245°C; [α]D24 +96.7 (c=0.15, CHCl3); IR νmax (KBr) cm−1: 3435, 2924, 2857, 1739, 1634, 1406, 1384, 904; UV λmax (MeOH) nm (log ε): 218 (3.99); 1H-NMR (400 MHz, CDCl3) 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, dd, 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-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-19); 13C-NMR (100 MHz, CDCl3); Table 1; CD Δε (nm): +16.6 (226) (c=4.45×10−3 M, MeOH); HR-ESI-MS (positive-ion mode) m/z: 367.1516 (Calcd for C20H24O5Na: 367.1515).  

Isocrotofolane Glucoside (5)

Amorphous powder, [α]D25 −63.6 (c=0.36, MeOH); IR νmax (film) cm−1: 3378, 2930, 2876, 1690, 1457, 1380, 1077, 897; UV λmax (MeOH) nm (log ε): 234 (3.84), 211 (3.79); 1H-NMR (400 MHz, CDOD) δ: 6.29 (1H, d, J=6.0 Hz, H-8), 4.62 (1H, s, H-18a), 4.60 (1H, d, J=11.1 Hz, H-5), 4.49 (1H, d, J=7.3 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-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, C3), 1.00 (3H, d, J=7.2 Hz, H-19); 13C-NMR (100 MHz, CDOD); Table 1; HR-ESI-MS (positive-ion mode) m/z: 371.1475 (Calcd for C18H22O5Na: 371.1465).
Table 2; HR-ESI-MS (positive-ion mode) m/z: 441.1363 [M+Na]⁺ (Caled for C₈H₁₃O₃Na: 441.1367).

**X-Ray Crystallographic Analysis of Crotocascarin K (3)**

C₂₀H₂₄O₅₇, M=3443.39, crystal size: 0.23×0.15×0.15 mm, space group: orthorhombic, P₂₁₂₁₂, T=150 K, a=10.2738(14)Å, b=11.338(15)Å, c=174.64(2)Å, V=1719.8(4)Å³, Z=4, Dₛ=1.330 Mg/m³, F(000)=736. The data were measured using a Bruker SMART 1000 CCD diffractometer, using MoKα graphite-monochromated radiation (λ=0.71073 Å) in the range of 4.52≤2θ≤54.5. Of 3893 reflections collected, 2019 were unique (Rₑ=0.020, 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. 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 F². Final goodness-of-fit on F²=1.047. R₁=0.0288, wR₂=0.0737 based on I=2σ(I), and Rₑ=0.0323, wR₂=0.0757 based on all data. The largest difference peak and hole were 0.185 and −0.157eÅ⁻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 CHCl₃ 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 CHCl₃ (1.5 mL), the reaction mixture was successively washed with H₂O (1 mL), 1M HCl (1 mL), NaHCO₃-saturated H₂O (1 mL), and brine (1 mL). The organic layer was dried with Na₂SO₄ 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 CHCl₃–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; ¹H-NMR (400 MHz, CDCl₃) 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-3), 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-17), 0.99 (3H, d, J=6.8 Hz, H-3b), 0.157 eÅ⁻³ (Caled for C₂₀H₂₄O₅₇Na: 355.1887 [M+Na]⁺).

**Sugar Analysis**

About 500 µg each of 5 and 6 was hydrolyzed with 1M HCl (0.1 mL) at 90°C for 2 h. The reaction mixtures werepartitioned with an equal amount of EtOAc (0.1 mL), and the water layers were analyzed by HPLC with a chiral detector (JASCO OR-2090plus) on an amino column [Asahipak NH₂-P-50 4E, CH₃CN–H₂O (4:1), 1 mL/min]. A hydrolzate of 5 gave a peak for α-glucose at 20.0 min, and one of 6 gave peaks for β-apiose and α-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.

**Acknowledgments**

The authors are grateful for access to the superconducting NMR instrument (JEOL JNM α-400) at the Analytical Center of Molecular Medicine of the Hiroshima University Faculty of Medicine, and an Applied Biosystem QSTAR XL system ESI (Nano-Spray)-MS at the Analysis Center of Life Science of the Graduate School of Biomedical Sciences, Hiroshima University. This work was supported in part by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and the Japan Society for the Promotion of Science (Nos. 22590006, 23590130 and 15H04651). Thanks are also due to the Research Foundation for Pharmaceutical Sciences and the Takeda Science Foundation for the financial support.

**Conflict of Interest**

The authors declare no conflict of interest.

**References**

1) Castagna M., Takai Y., Kaibuchi K., Sano K., Kikkawa U., Nishizuka Y., J. Biol. Chem., 257, 7847–7851 (1982).
2) Hecktor E., Naturwiss., 54, 282–284 (1967).
3) Van Duuren B. L., Prog. Exp. Tumor Res., 11, 31–68 (1969).
4) Hatushima S., “Flora of the Ryukyus. Added and Corrected,” The Biological Society of Okinawa, Naha, Japan, 1975, p. 364.
5) Kawakami S., Toyoda H., Harinantenaina L., Matsunami K., Otsuka H., Shinzato T., Takeda Y., Kawahata M., Yamaguchi K., Chem. Pharm. Bull., 57, 1053.
6) Inoshiri S., Saiki M., Kohda H., Otsuka H., Yamasaki K., *Phytochemistry*, **27**, 2869–2871 (1988).

7) Bohlmann F., Ziesche J., *Phytochemistry*, **20**, 469–472 (1981).

8) Snatzke G., *Angew. Chem.*, **7**, 14–25 (1968).

9) Fragoso-Serrano M., Gibbons S., Pereda-Miranda R., *Planta Med.*, **71**, 278–280 (2005).

10) Ohtani I., Kusumi T., Kashman Y., Kakisawa H., *J. Am. Chem. Soc.*, **113**, 4092–4096 (1991).

11) Kasai R., Suzuo M., Asakawa J., Tanaka O., *Tetrahedron Lett.*, **18**, 175–178 (1977).

12) Ishii T., Yanagisawa M., *Carbohydr. Res.*, **313**, 189–192 (1998).

13) Sheldrick G. M., *Acta Crystallogr. A*, **64**, 112–122 (2008).

14) Sheldrick G. M., SADABS, program for empirical absorption correction of area detector data. University of Göttingen, Germany, 1996.