A crotofolane-type diterpenoid and a rearranged nor-crotofolane-type diterpenoid with a new skeleton from the stems of *Croton cascarilloides*

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ABSTRACT

From the stems of *Croton cascarilloides* collected in the Okinawa Islands, a structurally rare crotofolane-type diterpenoid (1) and a rearranged nor-crotolalane, a new skeletal diterpenoid (2), were isolated. The structures were determined by X-ray crystallographic analyses, establishing their absolute stereostructures for the first time. Compound 2 was probably biosynthesized from 1 through several steps, such as decarboxylation, oxidation, C-C bond migration, etc.

*Keywords:*

*Croton cascarilloides*

Euphorbiaceae

Crotolalane

Diterpenoid
Crotolofane-type diterpenoids have fused 5-, 6- and 7-membered rings and are expected to be biosynthesized from cembrane via lathyrane through cross annular cyclization. These diterpenoids have been found in only three Croton species, Jamaican *C. corylifoius*, Kenyan *C. dichogamus*, and Congolese *C. humanianus*.

Our phytochemical investigation of the stems (14.5 kg) of *Croton cascarilloides* Räuschel, collected in the Okinawa Islands, led to the isolation of a crotofolane-type diterpenoid and a rearranged nor-molecular species of it having a new skeleton. A MeOH extract of branches of *C. cascarilloides* was washed with *n*-hexane and then evaporated to a gummy mass, which was then suspended in H$_2$O and extracted with CH$_2$Cl$_2$. The CH$_2$Cl$_2$-soluble fraction was separated by normal and reversed-phase silica gel column chromatographies, Sephadex LH-20 column chromatography, and then HPLC to afford compounds 1 and 2 (30.0 mg and 3.5 mg, respectively).

Compound 1 was isolated as colorless plates and its elemental composition was determined to be C$_{25}$H$_{32}$O$_7$. The IR spectrum of 1 showed absorptions for ester carbonyl and lactone carbonyl groups. $^{13}$C-NMR of compound 1 revealed 25 resonances, five of which were assignable to 2-methylbutanoic acid. The remaining 20 signals comprised those of three methyls, two methylenes, seven methines, one tetra- and one disubstituted double bond, respectively, and three quaternary carbons. Precise inspection
of two-dimensional NMR spectra led to the conclusion that compound 1 was a diterpenoid with an unusual carbon skeleton. Thus, X-ray crystallographic analysis of 1 was performed and the relative stereostructure of 1 was established to be a derivative of crotofolane-type diterpenoid (Figs. 1 and 2).\textsuperscript{7} The positive Cotton effect in the CD spectrum empirically indicated the absolute configuration at the 9-position was S\textsuperscript{8} and chirality analysis of the 2-methylbutanoic acid moiety by HPLC established the absolute configuration of 1, as shown in Fig. 1.\textsuperscript{9} This is the first report of the absolute structure of a crotofolane and the absolute configuration of the pentanolide portion, presumed based on the empirical rule for the CD spectrum, was proved to be correct.

Compound 2\textsuperscript{6} was isolated as colorless plates and its elemental composition was determined to be C\textsubscript{24}H\textsubscript{32}O\textsubscript{8}. The \textsuperscript{13}C-NMR spectrum displayed 24 signals, including five attributable to 2-methylbutanoic acid. Thus, the core skeleton was constituted of 19 carbons. X-ray crystallographic analysis revealed that compound 2 has a new skeleton, such as that of a rearranged mononor-crotofolane, as shown in Figs. 1 and 3.\textsuperscript{10} Compound 2 was probably derived from some crotofolane, like compound 1, through several steps, such as decarboxylation, oxidation, C-C bond migration, etc.

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

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References and Notes


5. Compound 1: colorless plates (2-PrOH), m.p. 152-153 °C, [α]D26 +81.8 (c 1.52, CHCl3). IR (KBr) νmax cm⁻¹: 3478, 2972, 2879, 1769, 1739, 1659, 1457, 1185, 1143, 1014, 804. UV (MeOH) λmax nm (log ε): 218 (4.00). ¹H-NMR (CDCl3, 400 MHz): δ5.38 (1H, d, J = 5 Hz, H-1), 5.20 (1H, s, H-18a), 5.17 (1H, s, H-18b), 5.14 (1H, dddd, J = 13, 4, 2, 2 Hz, H-9), 4.53 (1H, ddd, J = 4, 2, 2 Hz, H-13), 3.19 (1H, s, H-5), 3.15 (1H, d, J = 13 Hz, H-13), 3.06 (1H, br d, J = 12 Hz), 2.49 (1H, dd, J = 14, 8 Hz, H-3a), 2.73 (1H, ddd, J = 13, 4, 4 Hz, H-10a), 2.49 (1H, qdd, J = 7, 7, 7 Hz, H-2'), 2.44 (1H, dd, J = 2, 2 Hz, -OH), 2.18 (1H, dqdd, J = 8, 7, 7, 5 Hz, H-2), 1.90 (3H, br s, H3-17), 1.74 (1H, ddq, J = 14, 7, 7 Hz, H-3'a), 1.70 (1H, dd, J = 14, 10 Hz, H-2b), 1.50 (1H, ddq, J = 14, 7, 7 Hz H-3'b), 1.27 (1H, dddd, J = 13, 13, 4, 2 Hz, H-10b), 1.17 (3H, d, J = 7 Hz, H3-5'), 1.06 (3H, s, H3-20), 0.98 (3H, d, J = 7 Hz, H3-19), 0.93 (3H, dd, J = 7, 7 Hz, H3-4'). ¹³C-NMR (CDCl3, 100 MHz): δ178.0 (C-1’), 173.4 (C-16), 162.0 (C-8), 148.9 (C-12), 128.2 (C-15), 115.2 (C-18), 78.4 (C-9), 75.8 (C-1), 72.7 (C-11), 68.8 (C-14), 60.1 (C-4), 57.8 (C-5), 55.9 (C-6), 44.4
(C-7), 44.1 (C-10), 41.2 (C-2'), 36.4 (C-3), 32.7 (C-2), 31.7 (C-13), 26.6 (C-3'), 19.3 (C-20), 16.2 (C-5'), 12.3 (C-19), 11.4 (C-4'), 9.7 (C-17). CD $\Delta \varepsilon$ (nm): +1.36 (249), −1.27 (210) (c $4.31 \times 10^{-5}$, MeOH). HR-ESI-MS (positive-ion mode) $m/z$: 467.2017 [M + Na]$^+$ ($C_{25}H_{32}O_7$Na requires 467.2040).

6. Compound 2: colorless plates (CHCl$_3$), m.p. 202-203 °C, $[\alpha]_D^{26} +78.7$ (c 0.13, CHCl$_3$). IR (KBr) $\nu_{\text{max}}$ cm$^{-1}$: 3479, 2968, 2926, 2855, 1761, 1721, 1634, 1461, 1193, 804. $^1$H-NMR (CDCl$_3$, 400 MHz): $\delta$: 7.78 (1H, d, $J = 5$ Hz, H-1), 5.39 (1H, br s, H-18a), 5.23 (1H, s, H-18b), 4.39 (1H, d, $J = 6$ Hz, H-5), 4.17 (1H, br t-like, $J = 8$ Hz, H-11), 3.11 (1H, br d, $J = 13$ Hz, H-13), 2.92 (1H, d, $J = 13$ Hz, H-7), 2.46 (1H, dd, $J = 15$, 7 Hz, H-10a), 2.39 (3H, s, H$_3$-17), 2.38 (1H, overlapped, H-2'), 2.34 (1H, dd, $J = 14$, 7 Hz, H-3a), 2.26 (1H, d, $J = 6$ Hz, -OH at C-5), 2.22 (1H, m, H-2), 2.07 (1H, dd, $J = 15$, 10 Hz, H-10b), 1.68 (1H, ddq, $J = 14$, 7, 7 Hz, H-3'a), 1.60 (1H, br d, $J = 3$ Hz, -OH at C-11), 1.56 (1H, dd, $J = 14$, 10 Hz, H-3b), 1.43 (1H, ddq, $J = 14$, 7, 7 Hz, H-3'b), 1.27 (3H, s, H$_3$-20), 1.12 (3H, d, $J = 7$ Hz, H$_3$-5'), 0.92 (3H, d, $J = 7$ Hz, H$_3$-19), 0.90 (3H, dd, $J = 7$, 7 Hz, H$_3$-4'). $^{13}$C-NMR (CDCl$_3$, 100 MHz): $\delta$: 202.0 (C-9), 175.5 (C-1'), 174.7 (C-15), 146.1 (C-12), 113.8 (C-18), 88.0 (C-6), 75.5 (C-1), 75.4 (C-5), 67.9 (C-11), 66.0 (C-14), 64.8 (C-4), 60.1 (C-8), 48.0 (C-7), 41.2 (C-2'), 35.8 (C-10), 34.4 (C-2), 34.3 (C-3), 32.4 (C-13), 26.6 (C-3'), 26.0 (C-17),
22.0 (C-20), 16.9 (C-5'), 12.6 (C-19), 11.8 (C-4'). HR-ESI-MS (positive-ion mode)

\[ m/z: 471.1937 \ [M + Na]^+ \] (C_{24}H_{32}O_{8}Na requires 471.1689).

7. X-ray diffraction study on compound I: C_{25}H_{32}O_{7} \cdot C_{3}H_{8}O, M = 504.60, crystal size:

\[ 0.50 \times 0.30 \times 0.15 \text{ mm}^3, \text{ space group: orthorhombic, } P2_12_12_1, T = 120 \text{ K}, a = 10.1775(10) \ \text{Å}, b = 10.4348(10) \ \text{Å}, c = 25.908(3) \ \text{Å}, V = 2751.5(5) \ \text{Å}^3, Z = 4, D_c = 1.218 \ \text{Mg/m}^3, F(000) = 1088. \] The data were measured using a Bruker APEX II CCD diffractometer, using MoKα graphite-monochromated radiation (λ = 0.71073 Å) in the range of 3.14 < 2θ < 53.4. Of 13566 reflections collected, 3212 were unique (R_{int} = 0.0224), data/restraints/parameters 3212 / 0 / 334. The structure was solved by a direct method using the SHELXS-97. The refinement and all further calculations were carried out using SHELXL-97. The H atoms were included at calculated positions and treated as riding atoms using the SHELXL 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= 1.048, R_1 = 0.0344, wR_2 = 0.0868 based on I > 2σ(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.


9. Compound I (2 mg) was dissolved in 100 µL of 50% aqueous 1,4-dioxane and then
100 μL of a 10% KOH solution was added. The reaction mixture was kept at 100 °C for 3 h and then the cooled solution was neutralized by the addition of IR-120B (H⁺) ion-exchange resin. An aliquot (20 μL) was analyzed by a HPLC system equipped with an optical rotation detector on an ODS column with a solvent system of 20% CH₃CN in H₂O, containing 0.5% trifluoroacetic acid. A peak appeared at 16.4 min which showed positive chirality and was identified as that of authentic (S)-(+)2-methylbutanoic acid.

10. X-ray diffraction study on compound 2: C₂₄ H₃₂ O₈, M = 448.50, crystal size: 0.30 × 0.15 × 0.15 mm³, space group: monoclinic, P2₁, 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) Å³, Z = 2, Dc = 1.325 Mg/m³, F(000) = 1088. Of 5560 reflections collected in the range of 3.28° < 2θ < 54.1°, 2416 were unique (Rint = 0.0154), data/restraints/parameters 2416 / 1 / 296. The structure was solved in a similar manner to as for compound 1. Final goodness-of-fit on F² = 1.056, R₁ = 0.0315, wR₂ = 0.0794 based on I > 2σ(I) and R₁ = 0.0335, wR₂ = 0.0809 base on all data. The largest difference peak and hole were 0.285 and −0.208 eÅ⁻³, respectively.

Fig. 1 Structures of Compounds 1 and 2
Fig. 2 ORTEP drawing of compound 1
Fig. 3 ORTEP drawing of compound 2