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

Susumu Kawakami [a], Katsuyoshi Matsunami [a], Hideaki Otsuka [a,*], Takakazu Shinzato [b], Yoshio Takeda [c], Masatoshi Kawahata [d], Kentaro Yamaguchi [d]

[a] *Department of Pharmacognosy, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, Japan*

[b] *Subtropical Field Science Center, Faculty of Agriculture, University of the Ryukyus, 1 Senbaru, Nishihara-cho, Nakagami-gun, Okinawa 903-0213, Japan*

[c] *Faculty of Pharmacy, Yasuda Women's University, 8-13-1 Yasuhigashi, Asaminami-ku, Hiroshima 731-0153, Japan*

[d] *Department of Analytical Chemistry, Faculty of Pharmacaeutical Sciences, Tokushima Bunri University, Kagawa, 1314-1 Shido, Sanuki City, Kagawa 769-2193, Japan*

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* Corresponding author.
E-mail address: hotsuka@hirohisma-u.ac.jp (H. Otsuka).

ABSTRACT

From the stems of *Croton cascarilloides* collected in the Okinawa Islands, a structurally rare crotofolane-type diterpenoid (**1**) and a rearranged nor-crotofalane, 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

Crotofolane

Diterpenoid

Crotofolane-type diterpenoids have fused 5-, 6- and 7-membered rings and are expected to be biosynthesized from cembrane via lathyrane through cross annular cyclization.[1] These diterpenoids have been found in only three *Croton* species, Jamaican *C. corylifoius*,[1,2] Kenyan *C. dichogamus*,[3] and Congolese *C. humanianus*.[4]

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_2O and extracted with CH_2Cl_2. The CH_2Cl_2-soluble fraction was separated by normal and reversed-phase silica gel column chromatographies, Sephadex LH-20 column chromatogaraphy, and then HPLC to afford compounds **1**[5] and **2** (30.0 mg and 3.5 mg, respectively).[6]

Compound **1**[5] 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).[7] The positive Cotton effect in the CD spectrum empirically indicated the absolute configuration at the 9-position was *S*[8] and chirality analysis of the 2-methylbutanoic acid moiety by HPLC established the absolute configuration of **1**, as shown in Fig. 1.[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**[6] was isolated as colorless plates and its elemental composition was determined to be $C_{24}H_{32}O_8$. The ^{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.[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

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5. Compound **1**: colorless plates (2-PrOH), m.p. 152-153 ºC, $[\alpha]_D^{26}$ +81.8 (c 1.52, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}:3478, 2972, 2929, 2879, 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): 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, H_3-17), 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, H_3-5'), 1.06 (3H, s, H_3-20), 0.98 (3H, d, *J* = 7 Hz, H_3-19), 0.93 (3H, dd, *J* = 7, 7 Hz, H_3-4'). ^{13}C-NMR ($CDCl_3$, 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 Δε (nm): +1.36 (249), −1.27 (210) (c 4.31 × 10^{-5}, MeOH). HR-ESI-MS (positive-ion mode) *m*/*z*: 467.2017 $[M + Na]^+$ ($C_{25}H_{32}O_7Na$ requires 467.2040).

6. Compound **2**: colorless plates ($CHCl_3$), m.p. 202-203 ºC, $[\alpha]_D^{26}$ +78.7 (c 0.13, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}:3479, 2968, 2926, 2855, 1761, 1721, 1634, 1461, 1193, 804. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ5.78 (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): δ202.0 (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 $[M + Na]^+$ ($C_{24}H_{32}O_8Na$ requires 471.1689).

7. X-ray diffraction study on compound **1**: $C_{25}H_{32}O_7 \cdot C_3H_8O$, 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. The data were measured using a Bruker APEX II CCD diffractometer, using Mo$K\alpha$ graphite-monochromated radiation (λ = 0.71073 Å) in the range of $3.14 < 2\theta < 53.4$. 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.[11] The refinement and all further calculations were carried out using SHELXL-97.[11] 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 $I > 2\sigma(I)$ 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.

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9. Compound **1** (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 ºC 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% CH_3CN in H_2O, containing 0.5% trifluoroacetic acid. A peak appeared at 16.4 min which showed positive chirality and was indentified as that of authentic (*S*)-(+)-2-methylbutanoic acid.

10. X-ray diffraction study on compound **2**: 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) º, V = 1123.9(2) $Å^3$, Z = 2, D_c = 1.325 Mg/m^3, F(000) = 1088. Of 5560 reflections collected in the range of 3.28º < 2θ < 54.1º, 2416 were unique (R_{int} = 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 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 base on all data. The largest difference peak and hole were 0.285 and −0.208 $eÅ^{-3}$, respectively.

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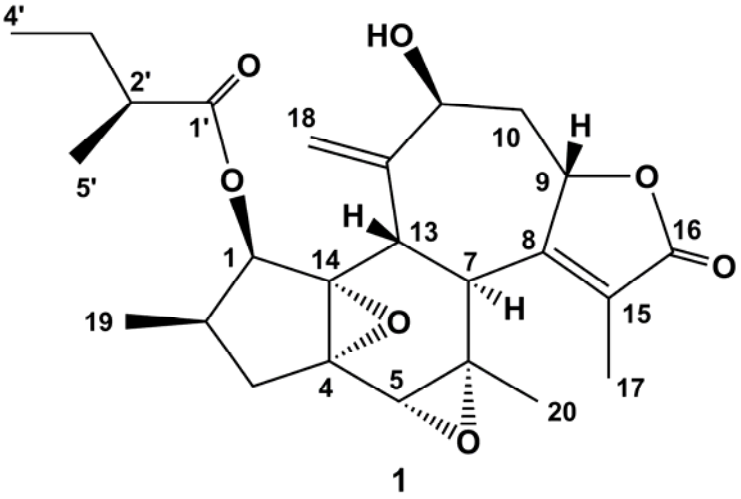

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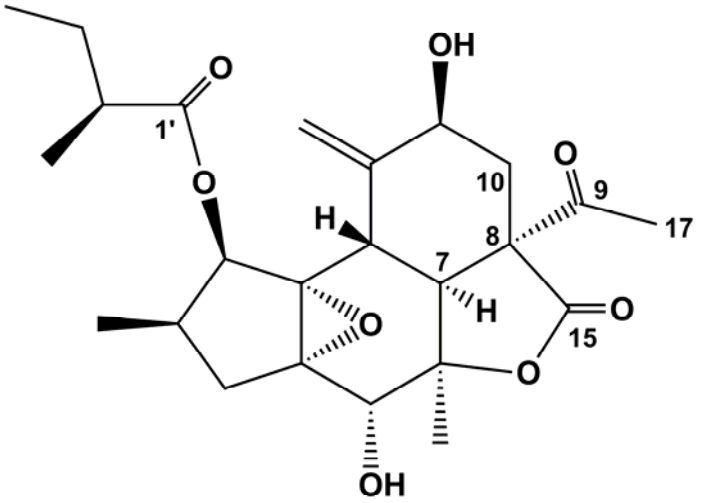

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Fig. 1 Structures of Compounds **1** and **2**

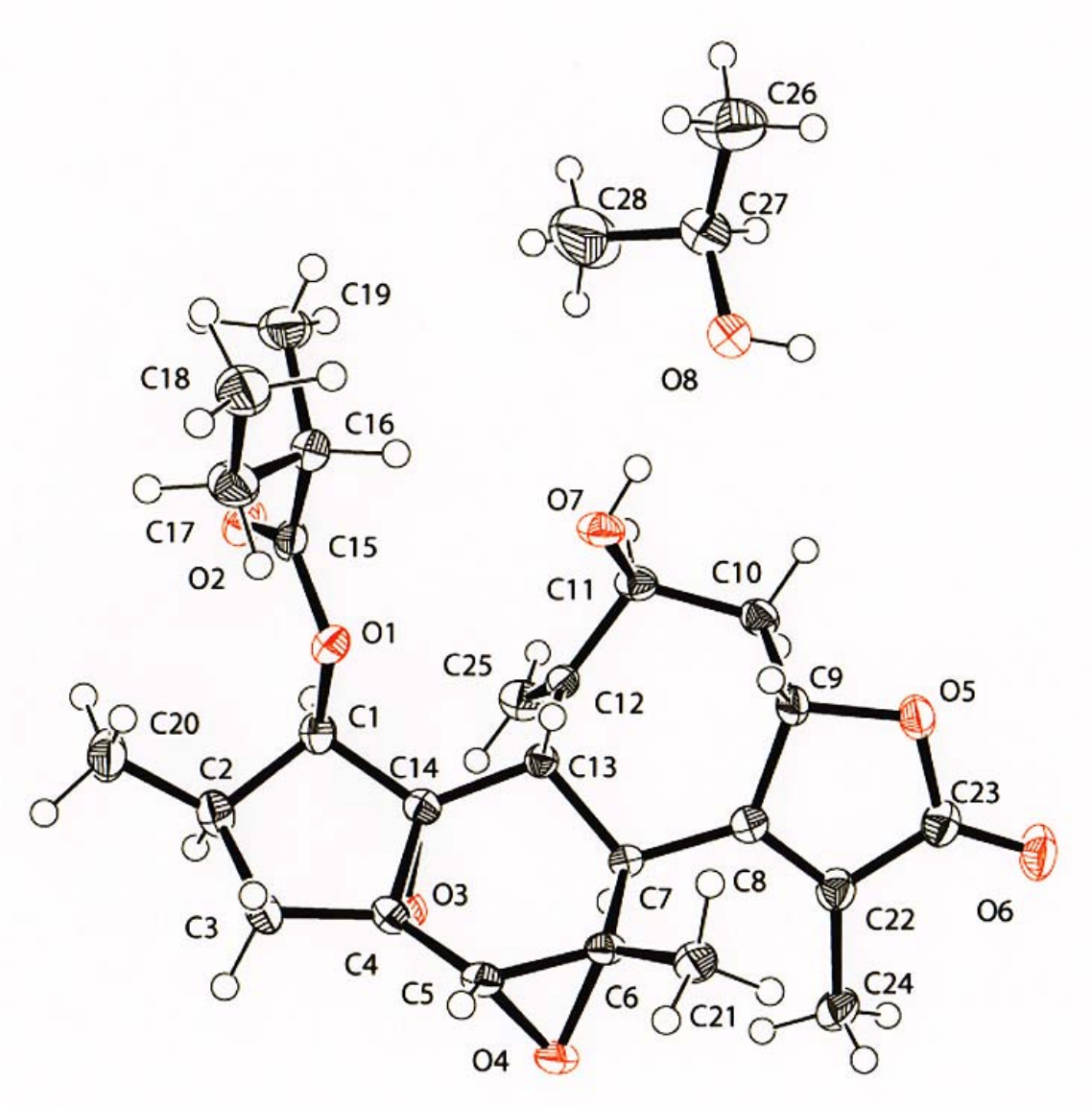

Fig. 2 ORTEP drawing of compound **1**

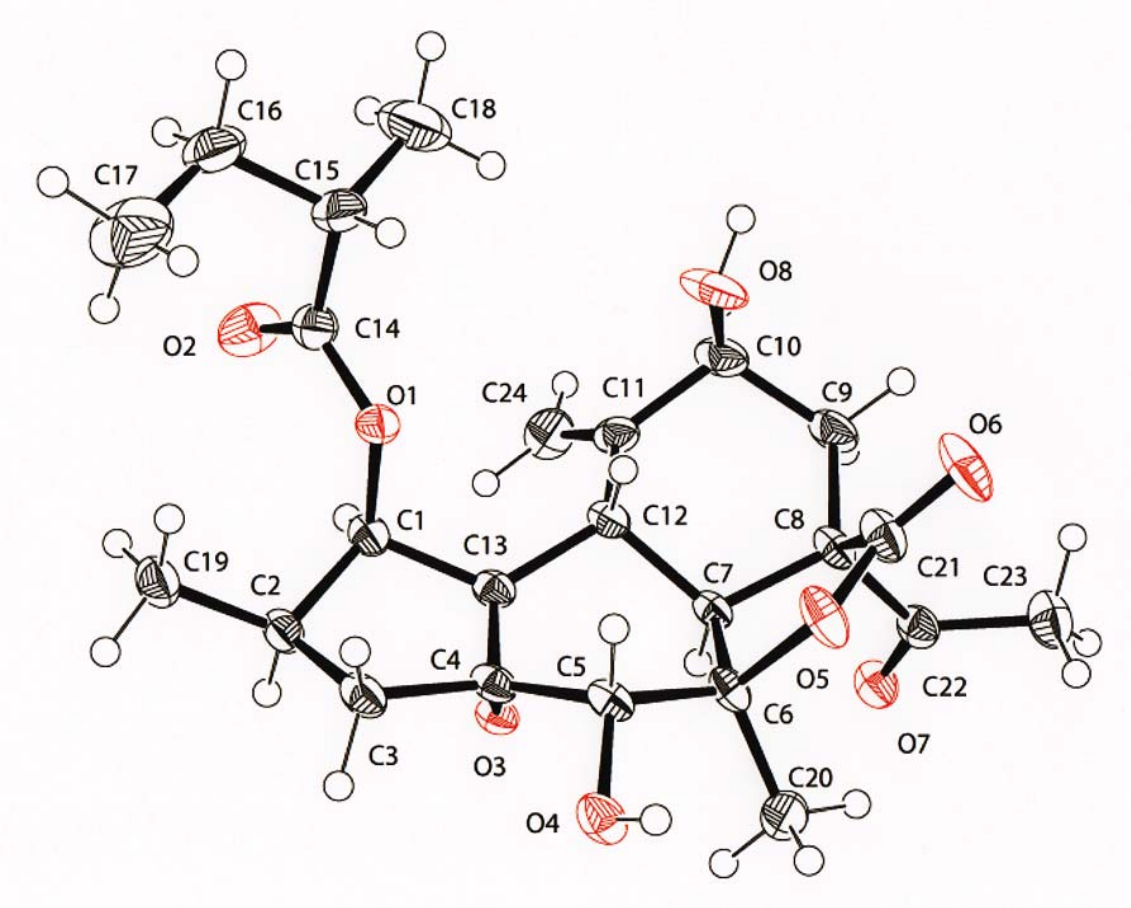

Fig. 3 ORTEP drawing of compound **2**