Two New Cembranoids from *Croton oblongifolius*

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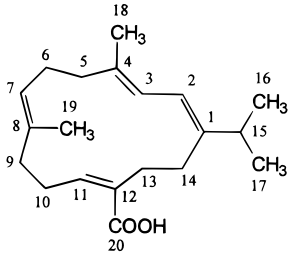
Two new cembranoids, crotocembraneic acid (**1**) and neocrotocembraneic acid (**2**), were isolated from the stem bark of *Croton oblongifolius*. Their structures were established on the basis of spectroscopic analysis.

Croton oblongifolius Roxb. (Euphorbiaceae), a medium-sized tree, is widely distributed throughout Thailand. It has been used as traditional medicine to alleviate dysmenorrhea (fruits), as a purgative (seeds), and to treat dyspepsia (bark) and dysentery (roots). Even though *C. oblongifolius* has been intensively investigated by Seshadri *et al.*,[1−5] we found that this same plant, when collected in Thailand, contains different constituents, possibly due to geographical variation. We now report the isolation and characterization of crotocembraneic acid (**1**) and neocrotocembraneic acid (**2**), two novel cembranoid diterpenes from *C. oblongifolius*.



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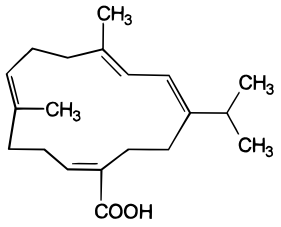

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Crotocembraneic acid (**1**) was obtained from a hexane-soluble crude extract from the stem bark of *C. oblongifolius* by Si gel column chromatography, eluting with hexane and ethyl acetate. The IR spectrum of **1** showed a broad absorption band between 3000 and 3400 cm^{-1} (OH stretching) and a strong absorption band at 1690 cm^{-1}, consistent with an α,β-unsaturated carboxylic carbonyl group. A carbon−carbon double bond stretching vibration was also observed at 1640 cm^{-1}. The molecular formula of $C_{20}H_{30}O_2$ was established for compound **1** from the elemental analysis, HREIMS, and ^1H and ^{13}C NMR data (Tables 1 and 2). ^1H and ^{13}C NMR spectra together with 2D NMR experiments allowed the complete structure of compound **1** to be established.

The ^1H NMR spectrum indicated that compound **1** possesses an isopropyl group (δ 1.04), two vinylic methyl groups (δ 1.54 and 1.73), and four olefinic protons (δ 5.10, 5.90, 6.01, and 6.03). The ^{13}C NMR spectrum and DEPT experiments revealed the presence of 19 nonequivalent carbons, of which 10 are sp^3 and eight are sp^2 hybridized carbons, together with a carbonyl carbon of carboxylic acid. These eight vinyl carbons were consistent with the presence of four double bonds in the molecule. The molecular formula, $C_{20}H_{30}O_2$, of compound **1** defined a degree of unsaturation of six; therefore, compound **1** must consist of one ring in addition to the four double bonds and a carboxyl group. These data indicated that compound **1** could be a novel cembranoid possessing a 14-membered ring diterpene skeleton.

Table 1. ^1H NMR Spectral Data of Compounds **1** and **2** (500 MHz $CDCl_3$)[a]

protons	**1**	**2**
H-1		
H-2	6.03 (1H, d, J = 11.0 Hz)	6.01 (1H, d, J = 11.0 Hz)
H-3	5.90 (1H, dd, J = 11.0, 0.9 Hz)	5.91 (1H, d, J = 11.0 Hz)
H-4		
H-5	2.15 (2H, m)	2.15 (2H, m)
H-6	2.20 (2H, m)	2.23 (2H, m)
H-7	5.10 (1H, dt, J = 6.4, 1.2 Hz)	5.14 (1H, t, J = 8.0, 2.2 Hz)
H-8		
H-9	2.15 (2H, m)	2.20 (2H, m)
H-10	2.70 (2H, m)	2.38 (2H, m)
H-11	6.01 (1H, t, J = 6.5 Hz)	6.89 (1H, t, J = 8.0 Hz)
H-12		
H-13	2.41 (2H, m)	2.36 (2H, m)
H-14	2.41 (2H, m)	2.26 (2H, m)
H-15	2.34 (1H, m)	2.39 (1H, m)
H-16	1.04 (3H, d, J = 6.7 Hz)	1.05 (3H, d, J = 7.0 Hz)
H-17	1.04 (3H, d, J = 6.7 Hz)	1.05 (3H, d, J = 7.0 Hz)
H-18	1.73 (3H, d, J = 0.9 Hz)	1.71 (3H, s)
H-19	1.54 (3H, br s)	1.68 (3H, s)
COOH		

[a] Spectra were recorded in $CDCl_3$, at 500 MHz with TMS as internal standard. Proton integrations, multiplicities, and coupling constants (J in Hz) are in parentheses.

The unequivocal assignment of compound **1** was established by the information from HMBC, HMQC, and COSY NMR experiments. The $^1H-^{13}C$ long-range correlations as determined from the HMBC spectrum allowed the connectivity of the structural fragments around each methyl group to be deduced. Crucial $^1H-^{13}C$ long-range correlations included the protons of the C-19 methyl group (H_3-19, δ 1.54) to C-8 (δ 134.0), C-7 (δ 125.7), and C-9 (38.6); the protons of the C-18 methyl group (H_3-18, δ 1.73) to C-4 (δ 135.2), C-3 (δ 121.6), and C-5 (δ 39.2); the isopropyl methyl protons at δ 1.04 (H_3-16, H_3-17) to C-15 (δ 33.8) and C-1 (δ 146.9); and the isopropyl methine proton at δ 2.34 (H-15) to C-16 and C-17 (δ 22.1), C-1 (δ 146.9), C-2 (δ 118.7), and C-14 (δ

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Table 2. ^{13}C NMR (125 MHz $CDCl_3$) and 2D Long-Range $^1H-^{13}C$ Correlations in the HMBC Spectra of Compounds **1** and **2**[a]

	1		**2**	
carbon	δ_C	correlated hydrogen	δ_C	correlated hydrogen
C-1	146.9 s[b]	H-3; H-14; H-15; H-16,17	146.5 s[b]	H-2; H-3; H-14; H-15; H-16,17
C-2	118.7 d	H-14; H-15	118.6 d	
C-3	121.6 d	H-5; H-18	120.0 d	H-14; H-15
C-4	135.2 s	H-2; H-5; H-6; H-18	135.6 s	H-2; H-18
C-5	39.2 t	H-3; H-6; H-18	37.7 t	H-2; H-5; H-6; H-18
C-6	25.1 t	H-5; H-7	24.7 t	H-3; H-6; H-18
C-7	125.7 d	H-6; H-9; H-19	127.8 d	H-5; H-7
C-8	134.0 s	H-9; H-10; H-19	134.8 s	H-6; H-9; H-19
C-9	38.6 t	H-7; H-10; H-11; H-19	38.5 t	H-9; H-10; H-19
C-10	26.4 t	H-9	30.5 t	H-7; H-10; H-19
C-11	146.3 d	H-9; H-10	145.7 d	H-11
C-12	130.9 s	H-10; H-13	132.1 s	H-9; H-10
C-13	33.6 t	H-14	26.7 t	H-10; H-11; H-13; H-14
C-14	28.7 t	H-2; H-13; H-15	29.1 t	H-14
C-15	33.8 d	H-2; H-16,17	34.6 d	H-2; H-13
C-16	22.1 q	H-15	22.1 q	H-2; H-14; H-16,17
C-17	22.1 q	H-15	22.1 q	H-15
C-18	17.0 q	H-3	18.0 q	H-15
C-19	15.8 q	H-7; H-9	17.4 q	H-3
COOH	174.1 s	H-11; H-13	173.5 s	H-7 H-11; H-13

[a] Chemical shifts are relative to the solvent signal ($CDCl_3$). [b] Multiplicities were established from HMQC and DEPT spectra (*s* = singlet, d = doublet, t = triplet, and q = quartet).

28.7) (Tables 1 and 2). The position of the C-20 carbonyl group was established by a $^{13}C-^1H$ three-bond correlation with H-11 and H-13. These partial structures were joined together from information obtained from the long-range $^{13}C-^1H$ correlation of vinylic or methylene protons and methylene carbons of each fragment and *vice versa*. These included the CH_2 protons at δ 2.20 (H-6) to C-5 (δ 39.2) and C-7 (δ 125.7), the CH_2 protons at δ 2.70 (H-10) to C-9 (δ 38.6), C-8 (δ 134.0), C-11 (δ 146.3), and C-12 (δ 130.9); and the CH_2 protons at δ 2.41 (H-13) to C-14 (δ 28.7) (Tables 1 and 2). A COSY experiment also established a correlation between the two vinylic protons at δ 5.90 (H-3) and δ 6.03 (H-2). This allowed the assignment of the complete structure of **1** as shown.

The configuration of all double bonds was determined from a NOESY experiment on **1**. The lowfield doublet at δ 6.03 ppm (H-2) showed NOESY cross peaks with the isopropyl protons (H_3-16, H_3-17 at δ 1.04 and H-15 at δ 2.34) and the methyl group attached to C-4 (H-18, δ 1.73), but not with H-3 (δ 5.90). This indicated that Δ^1 has the *E* configuration (the isopropyl group was on the same side as H-2), and the Δ^3 conjugated olefin also has the *E* configuration. The latter was supported by the UV spectrum, which exhibited a λ_{max} at 249 nm, in good agreement with the value reported for isoneocembrene A [(1*E*,3*E*,7*E*,11*E*)-1-isopropyl-4,8,12-trimethylcyclotetradeca-1,3,7,11-tetraene].[6−8] The configuration of the Δ^3 olefin was also shown to be *E* by the presence of a cross peak between H-3 and H-5 (δ 2.15), which indicated that the two groups were on the same side of the olefin. Likewise the *E* configuration of another olefin at Δ^7 could be deduced by the presence of a cross peak between H-7 and H-9 (δ 2.15). The ^{13}C NMR chemical shifts of the C-18 and C-19 methyl groups (δ 17.0 and 15.8, respectively) were similar to the chemical shifts of methyl groups *trans* to vinylic protons such as those reported in isoneocembrene A.[7,8]

The presence of a NOESY cross peak between the vinylic H-11 (δ 6.02) and H-13/H-14 (δ 2.41) made it possible to identify unambiguously the configuration of the Δ^{11} olefin as *Z*. Furthermore, the H-11 proton resonance fell in the region expected for a vinylic proton *trans* to a carboxyl group in a trisubstituted olefin (calcd for δ_H *trans* = 6.19, found δ 6.01).[9] Thus, crotocembraneic acid (**1**) was assigned as (1*E*,3*E*,7*E*,11*Z*)-1-isopropyl-4,8-dimethylcyclotetradeca-1,3,7,11-tetraene-12-carboxylic acid.

Neocrotocembraneic acid (**2**), $C_{20}H_{30}O_2$, was isolated as colorless prisms from the same fraction as **1**. The molecular formula was established from elemental analysis, HREIMS, and NMR data (Tables 1 and 2). Using the same approach for **1** in terms of ^1H and ^{13}C NMR analysis, together with information obtained from 2D NMR experiments, the connectivities of the carbons and protons in **2** were deduced and found to be identical to **1**. The ^{13}C NMR chemical shifts of the C-18 and C-19 methyl groups (δ 18.0 and 17.4) again suggested the configuration of the double bond to be *E*. However, marked differences between their ^1H and ^{13}C NMR spectra were found in the region near the carboxylic acid. Therefore, compounds **1** and **2** were assigned as geometrical isomers around Δ^{11} conjugated to the carboxylic acid group. Because this functionality in **1** was assigned as *Z*; therefore, **2** was proposed as having Δ^{11} in the *E* configuration, from the chemical shift of H-11 at δ 6.89 (calcd δ_H, *cis* = δ 6.83).[9] Thus, compound **2** was assigned as (1*E*,3*E*,7*E*,11*E*)-1-isopropyl-4,8-dimethylcyclotetradeca-1,3,7,11-tetraene-12-carboxylic acid.

It is known that *cis-trans* isomerization of some natural products containing α,β-unsaturated carbonyl compounds could occur during the isolation process as pointed out by Bernart *et al.*[10] The possibility of isomerization of **1** to **2** or *vice versa* was ruled out by the fact that no change in the NMR spectra was observed when the chloroform solutions of **1** and **2** were heated at 60 °C for 2 h or by leaving them at room temperature for 4 weeks. Therefore, **1** and **2** both appear to be natural products.

The structures of **1** and **2** resemble poilaneic acid, previously isolated from *Croton poilanei*, a native Thai plant, by Sato and co-workers.[11] Crotocembraneic acid (**1**) and neocrotocembraneic acid (**2**) contain conjugated

olefins at Δ^1 and Δ^3, while those of poilaneic acid are at Δ^2 and Δ^4. Thus, poilaneic acid has a chiral center, while compounds **1** and **2** are achiral. Recently, echinoic acid, a new cembrane was isolated from *Echinodorus grandiflorus*.[12] Compounds **1** and **2** can be regarded as analogues of echinoic acid in which a different methyl group is biogenetically oxidized to the carboxylic acid. It should be noted that natural products possessing a cembranoid skeleton are widely distributed among lower marine creatures[6−8,13] and some have been found in terrestrial plants such as tobacco (*Nicotiana* species),[14] *Croton* species,[11] and *Echinodorus* species.[12] Many of these compounds exhibit biological activities, such as cytotoxicity[15] and ichthyotoxicity.[16] The biological activities of compounds **1** and **2** have not yet been determined.

Experimental Section

General Experimental Procedures. All commercial grade solvents were distilled prior to use. Melting points were determined on a Fisher−Johns melting point apparatus and are reported uncorrected. The optical rotation was determined on a JASCO DIP-370 digital polarimeter. Measurements of UV spectra were carried out on a Milton−Roy Spectronic 3000 Array UV/vis spectrophotometer. IR spectra were recorded on a Perkin−Elmer model 1760X FT-IR spectrophotometer. Spectra of solid samples were recorded as KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded at 500.00 and 125.65 MHz, respectively, on a JEOL JNM-A500 NMR spectrometer. LREIMS were obtained with a Fisons Instruments model Trio 2000 mass spectrometer at 70 eV. HREIMS spectra were obtained with a Bruker model CX47 FTMS mass spectrometer.

Plant Material. The *C. oblongifolius* sample used in this study was collected from Amphur Bungsamphan, Petchaboon Province, Thailand, in October 1994. The plant specimen was compared against voucher specimen no. 9607 in the herbarium of the Royal Forest Department of Thailand, Bangkok, Thailand.

Extraction and Isolation. The powdered, sun-dried stem bark (5.0 kg) of *C. oblongifolius* was repeatedly extracted with MeOH (10 × 5 L). The MeOH extract was filtered and evaporated under reduced pressure to obtain a dark-red gummy residue that was repeatedly extracted with hexane. The hexane crude extract was obtained as a yellowish green oil (223 g) after evaporation. The crude hexane extract (50 g) was fractionated by Si gel column chromatography using Merck Si gel 60 (art. 7734.1000, 70−230 mesh ASTM) as adsorbent. The column was eluted with a hexane−EtOAc gradient in a stepwise fashion. Compounds **1** and **2** were eluted with 10% EtOAc in hexane. Similar fractions were combined, and the solvents were removed by rotary evaporation. Compound **2** crystallized first and was recrystallized from hexane (1.00 g). The mother liquor was concentrated, and additional chromatography with ether−hexane (gradient elution) afforded compound **1** with 15% ether−hexane. Recrystallization from hexane gave pure **1** (550 mg).

Crotocembraneic acid (1): white crystalline solid (550 mg, 1.1%); mp 109−111 °C; $[\alpha]^{25}_D$ +0.2° (*c* 2.28 $CHCl_3$); UV (EtOH) λ_{max} 249 sh (log ϵ 11 000) nm; IR ν_{max} 3400−3000 (OH), 1690 (C=O), 1640 (C=C) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (125.65 MHz, $CDCl_3$), see Table 2; EIMS *m/z* 302 [M$^+$] (35), 152 (49), 136 (62), 121 (100), 93 (77); HREIMS *m/z* found 302.2230, calcd for $C_{20}H_{30}O_2$, 302.2240; *anal*. C 79.47%, H 9.66%, calcd for $C_{20}H_{30}O_2$, C 79.47%, H 9.93%.

Neocrotocembraneic acid (2): white crystalline solid (1.00 g, 2.0%); mp 127−129 °C; $[\alpha]^{25}_D$ −0.3° (*c* 2.55 $CHCl_3$); UV (EtOH) λ_{max} 243 sh (log ϵ 9000) nm; IR ν_{max} 3400−3050 (OH), 1684 (C=O), 1635 (C=C) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (125.65 MHz, $CDCl_3$), see Table 2; EIMS *m/z* 302 [M$^+$] (28), 152 (20), 136 (64), 121 (100), 93 (68); HREIMS *m/z* found 302.2230, calcd for $C_{20}H_{30}O_2$, 302.2240; *anal*. C 79.41%, H 9.99%, calcd for $C_{20}H_{30}O_2$, C 79.47%, H 9.93%.

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