Neocrotocembranal from *Croton oblongifolius*

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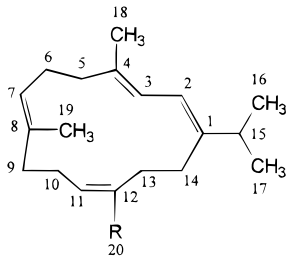
A new cembranoid diterpene, neocrotocembranal (**3**), was isolated from the stem bark of *Croton oblongifolius*. Its structure was established on the basis of spectroscopic analysis. This compound inhibited platelet aggregation induced by thrombin, with an IC_{50} value of 47.21 μg/mL, and exhibited cytotoxicity against P-388 cells in vitro, with an IC_{50} value of 6.48 μg/mL.

Crotocembraneic acid (**1**) and neocrotocembraneic acid (**2**) have been isolated from the stem bark of *Croton oblongifolius* Roxb. (Euphorbiaceae).[1] As a continuation of this work, we report the isolation of an aldehyde analogue of neocrotocembraneic acid from a recollection this plant. The structure of this compound (**3**) was determined by NMR techniques including ^{1}H, ^{13}C NMR, COSY, NOESY, DEPT, HMQC, and HMBC.



(1) R = COOH, $\Delta^{11} = Z$

(2) R = COOH, $\Delta^{11} = E$

(3) R = CHO, $\Delta^{11} = E$

Neocrotocembranal (**3**) was obtained from hexane crude extract from the stem bark of *C. oblongifolius* by Si gel column chromatography, eluting with hexane. The molecular formula of **3** was determined by HREIMS and elemental analysis as $C_{20}H_{30}O$. It showed a molecular ion peak $[M]^+$ at *m*/*z* 286 in EIMS. The IR absorption peak at 2704 cm^{-1} indicated the C−H stretching vibration of the aldehyde functional group. The strong absorption peak at 1690 cm^{-1} suggested an α,β-unsaturated carbonyl group. A carbon−carbon double-bond stretching vibration was also observed at 1639 cm^{-1}.

The ^{1}H NMR spectrum suggested that compound **3** possesses an isopropyl group (δ 1.04), two vinylic methyl groups (δ1.67 and 1.68), four olefinic protons (δ 5.13, 5.84, 5.96, and 6.39), and one aldehyde proton (δ 9.28). The ^{13}C NMR and DEPT data showed the presence of 19 nonequivalent carbons, of which 10 are sp^3 and eight are sp^2 hybridized carbon, together with an aldehyde carbon. The molecular formula of $C_{20}H_{30}O$ indicated this compound to have six degrees of unsaturation; therefore, **3** must consist of one ring in addition to the four double bonds and one carbonyl group. The spectral data of **3** were similar to those of neocrotocembraneic acid (**2**) except for the presence of an aldehyde group and the absence of the carboxyl group. The structure of compound **3** was confirmed by ^{1}H and ^{13}C NMR information, including HMQC, HMBC, and COSY experiments (Table 1). The NOESY experiment of **3** suggested the configuration of all double bonds. The downfield doublet at δ 5.96 (H-2) indicated NOESY cross-peaks with the isopropyl protons (H_3-16, H_3-17 at δ 1.04 and H-15 at δ 2.40), but not with H-3 (δ 5.84). This showed that the Δ^1 olefin has the *E* configuration (the isopropyl group was on the same side as H-2). The configuration of the Δ^3 olefin was also assigned to be *E* by the presence of a cross-peak between H-3 and H-5 (δ 2.12), which suggested that two groups were on the same side of the olefin. Similarly, the configuration of the Δ^7 olefin was determined to be *E* by the presence of a cross-peak between H-7 and H-9 (δ 2.24). The ^{13}C NMR chemical shifts of the C-18 and C-19 methyl groups (δ 17.2 and 18.0, respectively) were similar to those of the methyl groups *trans* to vinylic protons in isoneocembrene A.[2−4]

The presence of a NOESY cross-peak between the vinylic H-11 (δ 6.39) and H-20 of the aldehyde proton (δ 9.28), but not with H-13 (δ 2.32), indicated that the configuration of

Table 1. ^{1}H, ^{13}C NMR Data for Neocrotocembranal (**3**) (in $CDCl_3$)

position	^{1}H	^{13}C[a]
1		146.2 s
2	5.96 (1H, d, 11.0)	118.4 d
3	5.84 (1H, dd, 1.4, 11.0)	119.8 d
4		135.4 s
5	2.12 (2H, m)	37.4 t
6	2.22 (2H, m)	24.8 t
7	5.13 (1H, t, 6.4)	128.0 d
8		134.0 s
9	2.24 (2H, m)	38.5 t
10	2.50 (2H, m)	30.0 t
11	6.39 (1H, t, 7.8)	155.0 d
12		143.9 s
13	2.32 (2H, m)	24.1 t
14	2.20 (2H, m)	28.7 t
15	2.40 (1H, heptet, 6.7)	34.0 d
16, 17	1.04 (6H, d, 6.7)	22.0 q
18	1.67 (3H, s)	18.0 q
19	1.68 (3H, s)	17.2 q
20	9.28 (1H, s)	194.7 s

[a] Assignments confirmed by DEPT, COSY, HMBC, and NOESY experiments.

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the Δ^{11} olefin was *E*. Furthermore, the H-11 proton resonance fell in the region expected for a vinylic proton *cis* to an aldehyde group in a trisubstituted olefin.[5] Thus, neocrotocembranal (**3**) is (1*E*,3*E*,7*E*,11*E*)-1-isopropyl-4,8-dimethylcyclotetradeca-1,3,7,11-tetraene-12-carboxaldehyde.

The effect of **1**, **2**, and **3** on platelets was examined. Only compound **3** (47.21 μg/mL) markedly inhibited platelet aggregation induced by thrombin (0.25 unit/mL). The effect of **3** on platelets is probably due to the reactive aldehyde functionality. In addition, **3** (6.48 μg/mL) and **2** (41.47 μg/mL) exhibited cytotoxic activity against P-388 cell culture, although **1** was inactive. It should be mentioned that many cembranoids exhibit cytotoxic activity, especially those highly functionalized cembranoids obtained from marine sources.[6−8]

Experimental Section

General Experimental Procedures. All commercial grade solvents were distilled prior to use. 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. ^{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 was 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 May 1998. 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 (8.2 kg) of *C. oblongifolius* was repeatedly extracted with hexane (3 × 8 L). The hexane crude extract was obtained as a yellowish green oil (350 g) after evaporation. The crude hexane extract (200 g) was fractionated by Si gel column chromatography using Merck Si gel 60 (500 g). The column was eluted with hexane−$CHCl_3$ gradient in a stepwise fashion. Compound **3** was eluted with 10% $CHCl_3$ in hexane, which was monitored by TLC using 2,4-dinitrophenylhydrazine solution as visualizing agent. Similar fractions were combined and the solvent removed by rotary evaporation. Rechromatography of the residual mixture on SiO_2 with 5% $CHCl_3$ in hexane gave **3** (1.26 g, 0.63%). Elution with 20% $CHCl_3$ in hexane gave **1** (28.45 g, 14.23%) and **2** (15.40 g, 7.7%), respectively.

Neocrotocembranal (3): pale yellow oil; optically inactive; UV (EtOH) λ_{max} 246sh (log ϵ 4.21) nm; IR ν_{max} 2704 (C−H, aldehyde), 1690 (C═O), 1639 (C═C) cm^{-1};^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125.65 MHz), see Table 1; EIMS *m*/*z* 286 [M$^+$] (100), 243 (33), 175 (96), 136 (98), 121 (100); HREIMS *m*/*z* found 286.2290, calcd for $C_{20}H_{30}O$, 286.2291.

Pharmacological Tests. The platelet aggregation was determined by a standard turbidometric method using the protocol described by Ohizumi *et al*.[9,10] Cytotoxicity against P-388 cell culture *in vitro* was performed by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric method.[11−12]

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