Furanocembranoids from the Stem Bark of *Croton oblongifolius*

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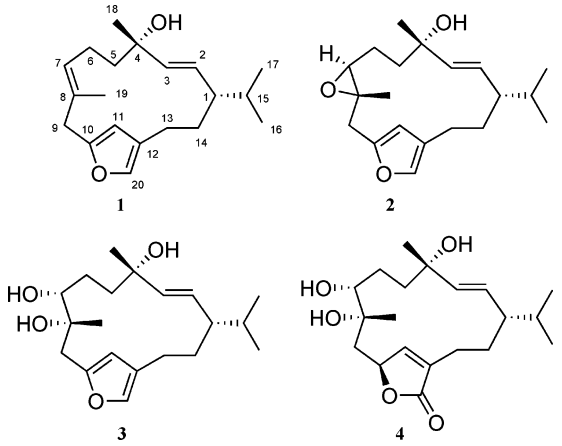
Four novel furanocembranoids (**1**−**4**) were isolated from the stem bark of *Croton oblongifolius*. Their structures were elucidated on the basis of spectroscopic analysis, mainly NMR and MS. Compounds **1**, **3**, and **4** exhibited good cytotoxicity against several human tumor cell lines.

Croton oblongifolius Roxb. (Euphorbiaceae) has been used as a traditional medicine for applications such as for dysmenorrhea, as a purgative, and to treat dyspepsia and dysentery. This plant has also been used as a folk medicine in conjunction with *Croton sublyratus* to treat grastric ulcers and gastric cancers. In earlier work, we identified several cytotoxic diterpenoids from *C. oblongifolius*.[1−5] As part of our ongoing efforts to isolate biologically active compounds from *C. oblongifolius*, the chemical constituents of *C. oblongifolius* collected from Phu Roe, Loei Province, were investigated, and four new furanocembranoids (**1**−**4**) were isolated from the hexane extract. Cytotoxicities of **1**−**4** against BT474 (human breast ductol carcinoma), CHAGO (human undifferentiated lung carcinoma), Hep-G2 (human liver hepatoblastoma), KATO-3 (human gastric carcinoma), and SW-620 (human colon adreno carcinoma) are also reported.



Compound **1** was isolated as a yellow oil. The molecular formula was determined to be $C_{20}H_{30}O_2$ by HRFABMS, indicating the existence of six degrees of unsaturation and two oxygen atoms. The planar structure of **1** was deduced to be as shown by analysis of ^{1}H and ^{13}C data, including COSY, HSQC, and HMBC experiments. The ^{1}H NMR spectrum (Experimental Section) showed the characteristic signals of an isopropyl group, five olefinic protons, one tertiary methyl connected to an oxygen-bearing carbon, and one vinylic methyl group. Analysis of ^{13}C NMR and HSQC experiments revealed the presence of 20 nonequivalent carbons, of which 12 were sp^3- and eight were sp^2-hybridized carbons. With four C−C double bonds present, as inferred from the eight sp^2 carbon atoms, compound **1** was clearly bicyclic. The ^{1}H and ^{13}C NMR spectra indicated the presence of a furan ring (δ_H 5.99 and 7.14). From these data, compound **1** was determined to be a furanocembranoid.

The partial structures elucidated from the ^{1}H−^{1}H COSY study are indicated by bold lines and the crucial long-range ^{1}H−^{13}C correlations shown by arrows in Figure 1. On the basis of HMBC experiments, the cross-peak observed between H_2-13 and the carbon atoms C-20, C-12, and C-11 and between H_2-9 and the carbon atoms C-8, C-10, and C-11 allowed us to connect H_2C-13 and H_2C-9 to C-10 and C-12 of the furan ring, respectively. These results, together with the lack of coupling between the two hydrogen atoms of the furan at δ_H 5.99 and 7.14, demonstrated that the furan ring was 2,4-disubstituted. The configuration of the Δ^{13} double bond was assigned as *E* by the coupling constant of the signals at δ_H 5.22 and 5.39 with $J = 15.2$ Hz from the ^{1}H NMR spectrum. Likewise the *E* configuration of another olefin at Δ^8 was deduced by the presence of a NOESY cross-peak between H_2C-9 at δ_H 3.21 and the downfield triplet at δ_H 4.83 (H-7). The ^{13}C NMR chemical shift of the C-19 methyl group (δ_C 17.3) was similar to the chemical shifts of the methyl groups *trans* to vinylic protons such as those reported in isoneocembrene A.[6,7] The relative stereochemistry of **1** was determined by the NOE spectrum as depicted in Figure 2. The compound exhibited NOEs between H-1 and H-13, between H-3 and Me-18, and between H-3 and Me-19, indicating that all these protons are located on the β-face, while the NOE response between H-2 and Me-16/Me-17 would then allocate these protons to the α-face such as those reported in cembranoids from Greek tobacco.[8] The absolute stereochemistry at C-4 in **1** was thus established as C-4*S* as previously reported.

Compound **2** was isolated as a colorless oil, and its molecular formula was assigned as $C_{20}H_{30}O_3$ on the basis of HRFABMS data, possessing one more oxygen atom than **1**. Comparison of the ^{1}H and ^{13}C NMR spectra of **2** with those of **1** revealed them to be very similar, with the only difference being the appearance of a double doublet at δ_H 2.60 in the ^{1}H NMR spectrum, coupled in the HSQC spectrum to a newly appearing oxymethine resonance at δ_C 59.7, while the C-7,C-8 double bond signal at δ_C 124.9 and 132.5 had disappeared. The ^{1}H−^{1}H COSY and HMBC experiments indicated that this oxymethine is attached to H_2C-6. Thus, the gross structure was proposed as depicted. The relative configuration of the epoxide moiety in **2** was established by analysis of the NOESY spectrum. The lack of NOESY correlation between H-7 and Me-19 indicated that the epoxide was *trans*.

Compound **3** was also isolated as a colorless oil that analyzed for $C_{20}H_{32}O_4$ by HRFABMS, one oxygen and two hydrogen atoms more than in the molecular formula of **2**. NMR spectroscopic data of **3** were very similar to those obtained from **2**, suggesting that these two compounds had the same skeletal arrangement and a similar oxygenation pattern. The only significant difference was the upfield shift of the C-9 proton by 0.24 ppm in the ^{1}H NMR

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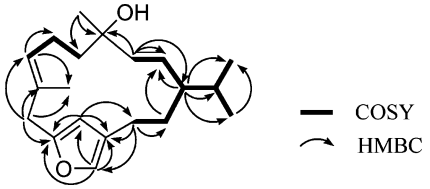

Figure 1. Key HMBC and COSY correlations for **1**.

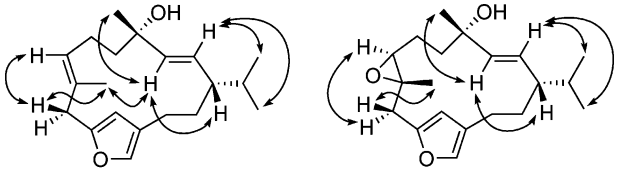

Figure 2. Selected NOESY correlations of compounds **1** and **2**.

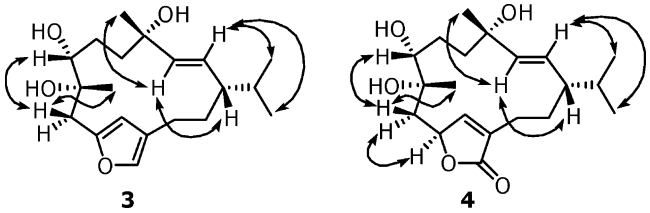

Figure 3. Selected NOESY correlations of compounds **3** and **4**.

spectrum of **2**. Also, the absence of resonance bands near δ_C 61.2 (C) and 59.7 (CH) indicated the lack of an oxirane ring in this compound, a feature found in **2**. Therefore, compound **3** was concluded to be the corresponding glycol derivative of **2**. Assignment of the relative stereochemistry of the two hydroxyl groups in **3** was also accomplished by analysis of the NOESY spectrum. The observed NOE between H-7 and H_β-9 but not with Me-19 suggested that H-7 and Me-19 were on the opposite face and *syn* diol at C-7 and C-8 (Figure 3).

Compound **4** was obtained as a colorless oil. The HRFABMS together with the NMR data were used to determine the molecular formula of **4** to be $C_{20}H_{32}O_5$. Also, the ^{1}H and ^{13}C NMR signals of **4** were virtually identical to those of **3**. The absence of a signal for a furan ring, along with the observation of resonances for an additional carbonyl (δ_C 173.9) and an oxymethine (δ_C 80.4), suggested that the furan ring was converted into a lactone by the addition of a carbonyl at C-20. This was confirmed by HMBC correlations of HC-11 (δ_H 7.19) with C-20 (δ_C 173.9) and HC-10 (δ_H 5.19) with C-11 (δ_C 150.2). The relative configuration of diol at C-7 and C-8 was also assigned to be *syn* by NOESY data analysis, and the α-oriented H-10 was supported by a key cross-peak between H-10 and H_α-9 (Figure 3).

Pure isolated compounds (**1**−**4**) were evaluated for cytotoxic effects against five further cell lines—BT474, CHAGO, Hep-G2, KATO-3, and SW-620—by the MTT [3-(4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide] colorimetric method.[9,10] Results are presented in Table 1. Compounds **1**, **3**, and **4** showed broad cytotoxic activity for all cell lines tested, while compound **2** did not exhibit cytotoxic activity against the CHAGO and Hep-G2 cell lines. It should be noted that natural products possessing a cembranoid skeleton are widely distributed among lower marine creatures[11−13] and that some have been found in terrestrial plants such as tobacco (*Nicotiana* species),[8] *Croton* species,[1,2] and *Echinodorus* species.[14] Many of these compounds exhibit biological activities, such as cytotoxicity,[12,13] ichthyotoxicity,[14] and insecticidal activity.[15]

Table 1. Cytotoxicity of **1**−**4** against Human Tumor Cell Lines[a]

	cell lines[b]				
compound	BT474	CHAGO	Hep-G2	KATO-3	SW-620
1	7.8	7.0	5.6	5.9	6.3
2	9.5	>10	>10	6.8	9.9
3	9.6	7.1	5.7	8.2	5.6
4	9.6	9.3	6.1	8.1	6.0
adeblastin	8.9	5.4	3.9	>10	3.3

[a] Results are expressed as IC_{50} values (μg/mL). [b] BT474: human breast ductol carcinoma ATCC No. HTB 20; CHAGO: human undifferentiated lung carcinoma; Hep-G2: human liver hepatoblastoma; KATO-3: human gastric carcinoma ATCC No. HTB 103; SW-620: human colon adeno carcinoma ATCC No. CCL 227.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Shimadzu FTIR-8300. Mass spectra were obtained by a JEOL JMS DX-303 mass spectrometer. The NMR spectra were recorded on a Varian YH400 NMR spectrometer at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR.

Plant Material. The stem bark of *C. oblongifolius* Roxb. was collected from Phu Roe, Loei Province, Thailand, in June 2003. The plant specimen was compared against the voucher specimen No. BKF 084729 in the herbarium of the Royal Forest Department of Thailand, Bangkok, Thailand.

Extraction and Isolation. The powdered, air-sundried stem bark of *C. oblongifolius* (1 kg) was extracted successively with hexane, EtOAc, and MeOH. The hexane extract (32 g) was subjected to column chromatography over silica gel using hexane and hexane−EtOAc mixtures of increasing polarity to afford fractions I−VIII. Fraction III was rechromatographed over silica gel eluted with hexane−EtOAc (9:1) to yield compound **1** (9.8 mg). Fraction V was further subjected to flash column chromatography on silica gel (benzene−EtOAc, 8:2), followed by preparative TLC (benzene−EtOAc, 8:2) to afford compound **2** (6.0 mg) and compound **3** (20.3 mg). Fraction VII was rechromatographed on SiO_2 flash column chromatography eluted with benzene−EtOAc (7:3) and then purified by preparative TLC (MeOH−$CHCl_3$, 1:9) to give compound **4** (5.4 mg).

Furanocembranoid 1: light yellow oil; $[\alpha]_D^{20}$ −31.5 (*c* 0.5, MeOH); IR (film) ν_{max} 3380, 3056, 1643, 1616, 1119 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 Hz) δ 7.14 (1H, s, H-18), 5.99 (1H, s, H-11), 5.39 (1H, dd, J = 10.0, 15.2 Hz, H-2), 5.22 (1H, d, J = 15.2 Hz, H-3), 4.83 (1H, t, J = 6.4 Hz, H-7), 3.21 (2H, s, H-9), 2.63 (1H, dt, J = 3.2, 13.6 Hz, H-13b), 2.25 (1H, ddd, J = 3.2, 13.6, 16.4 Hz, H-13a), 2.20 (1H, m, H-6b), 2.08 (1H, m, H-6a), 1.80 (1H, m, H-14b), 1.73 (1H, m, H-5b), 1.71 (3H, s, CH_3-19), 1.67 (1H, m, H-15), 1.59 (1H, m, H-5a), 1.56 (1H, m, H-1), 1.45 (1H, m, H-14a), 1.22 (3H, s, CH_3-18), 0.90 (3H, d, J = 6.8 Hz, CH_3-17), 0.84 (3H, d, J = 6.8 Hz, CH_3-16); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 154.0 (C, C-10), 137.8 (CH, C-18), 137.6 (CH, C-3), 132.5 (C, C-8), 129.6 (CH, C-2), 124.9 (CH, C-7), 125.7 (C, C-12), 109.6 (CH, C-11), 73.0 (C, C-4), 47.0 (CH, C-1), 42.6 (CH_2, C-5), 36.4 (CH_2, C-9), 33.7 (CH_2, C-14), 33.4 (CH, C-15), 31.2 (CH_3, C-18), 24.8 (CH_2, C-13), 22.8 (CH_2, C-6), 20.7 (CH_3, C-17), 19.7 (CH_3, C-16), 17.3 (CH_3, C-19); HRFABMS *m*/*z* 302.2244 (calcd for $C_{20}H_{30}O_2$, 302.2246).

Furanocembranoid 2: colorless oil; $[\alpha]_D^{20}$ −15.5 (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$, 400 Hz) δ 7.13 (1H, s, H-20), 5.94 (1H, s, H-11), 5.40 (1H, dd, J = 8.4, 15.6 Hz, H-2), 5.33 (1H, d, J = 15.6 Hz, H-3), 2.99 (1H, d, J = 15.6 Hz, H-9b), 2.94 (1H, d, J = 15.6 Hz, H-9a), 2.60 (1H, dd, J = 3.6, 8.8 Hz, H-7), 2.57 (1H, m, H-13b), 2.25 (1H, ddd, J = 3.2, 13.6, 16.4 Hz, H-13a), 1.88 (1H, m, H-6b), 1.82 (1H, m, H-14b), 1.79 (1H, m, H-5b), 1.68 (1H, m, H-5a), 1.66 (1H, m, H-1), 1.56 (1H, m, H-15), 1.49 (1H, m, H-14a), 1.43 (1H, m, H-6a), 1.37 (3H, s, CH_3-19), 1.26 (3H, s, CH_3-18), 0.88 (3H, d, J = 6.8 Hz, CH_3-17), 0.82 (3H, d, J = 0.68 Hz, CH_3-16); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 151.4 (C, C-10), 138.4 (CH, C-20), 137.7 (CH, C-3), 130.1 (CH, C-2), 125.3 (C, C-12), 109.6 (CH, C-11), 72.7 (C, C-4), 61.2 (C, C-8), 59.7 (CH, C-7), 46.5 (CH, C-1), 39.3 (CH_2, C-5), 35.3 (CH_2, C-9), 33.6 (CH, C-15), 33.3 (CH_2, C-14), 31.9 (CH_3, C-18), 24.4 (CH_2, C-14), 23.0 (CH_2, C-6), 20.7 (CH_3, C-17), 19.5 (CH_3, C-16), 19.4 (CH_3, C-16); HRFABMS *m*/*z* 318.2203 (calcd for $C_{20}H_{30}O_3$, 318.2195).

Furanocembranoid 3: colorless oil; $[\alpha]_D^{20}$ −10.3 (*c* 0.5, MeOH); IR (film) ν_{max} 3454, 3024, 1662, 1620, 1243, 1118 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 Hz) δ 7.08 (1H, s, H-20), 6.07 (1H, s, H-11), 5.45 (1H, dd, J = 7.6, 16.0 Hz, H-2), 5.21 (1H, d, J = 16.0 Hz, H-3), 2.96 (1H,

d, $J = 14.8$ Hz, H-9b), 2.77 (1H, d, $J = 14.8$ Hz, H-9a), 2.67 (1H, brd, $J = 14.8$ Hz, H-13b), 2.32 (1H, m, H-13a), 1.93 (1H, m, H-14b), 1.83 (1H, m, H-5b), 1.75 (1H, m, H-1), 1.71 (1H, m, H-6b), 1.64 (1H, m, H-15), 1.62 (1H, m, H-6a), 1.60 (1H, m, H-14a), 1.56 (1H, m, H-5a), 1.33 (3H, s, CH_3-18), 1.24 (3H, s, CH_3-19), 0.82 (6H, d, $J = 6.8$ Hz, CH_3-16 and CH_3-17); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 153.0 (C, C-10), 137.7 (CH, C-20), 134.3 (CH, C-3), 132.2 (CH, C-2), 125.1 (C, C-12), 112.4 (CH, C-11), 80.8 (C, C-4), 79.0 (CH, C-9), 74.3 (C, C-8), 47.3 (CH, C-1), 40.3 (CH_2, C-9), 35.0 (CH_2, C-5), 33.6 (CH, C-15), 29.9 (CH_2, C-14), 28.5 (CH_3, C-18), 25.8 (CH_2, C-6), 24.7 (CH_2, C-13), 20.5 (CH_3, C-19), 19.6 (CH_3, C-17), 19.0 (CH_3, C-16); HRFABMS *m*/*z* 336.2386 (calcd for $C_{20}H_{32}O_4$, 336.2301).

Furanocembranoid 4: colorless oil; $[\alpha]_D^{20}$ −3.0 (*c* 0.5, MeOH); IR (film) ν_{max} 3450, 3034, 1748, 1682, 1618, 1256, 1098 cm^{-1}; ^1H NMR ($CDCl_3$, 400 Hz) δ 7.19 (1H, s, H-11), 5.65 (1H, dd, $J = 9.6$, 15.6 Hz, H-14), 5.47 (1H, d, $J = 15.6$ Hz, H-3), 5.19 (1H, brd, $J = 6.4$ Hz, H-10), 3.23 (1H, t, $J = 7.6$ Hz, H-7), 2.56 (1H, dd, $J = 8.4$, 18.8 Hz, H-13b), 2.36 (2H, m, CH_2-7), 2.12 (1H, dd, $J = 10.0$, 18.8 Hz, H-13a), 1.92 (1H, m, H-14b), 1.79 (1H, m, H-5b), 1.78 (2H, m, CH_2-6), 1.73 (1H, m, H-1), 1.68 (1H, m, H-14a), 1.62 (2H, m, H-5a and H-15), 1.34 (3H, s, CH_3-18), 1.15 (3H, s, CH_3-19), 0.93 (3H, d, $J = 6.4$ Hz, CH_3-17), 0.89 (3H, d, $J = 6.4$ Hz, CH_3-16); ^{13}C NMR ($CDCl_3$, 100 Hz) δ 173.9 (CO, C-20), 150.2 (CH, C-11), 137.1 (CH, C-3), 134.1 (CH, C-2), 133.4 (C, C-12), 81.8 (C, C-4), 80.4 (CH, C-10), 79.4 (CH, C-7), 74.4 (C, C-8), 51.8 (CH, C-1), 39.1 (CH_2, C-9), 38.0 (CH_2, C-5), 32.3 (CH, C-15), 29.4 (CH_3, C-18), 27.7 (CH_2, C-14), 26.1 (CH_2, C-6), 24.9 (CH_2, C-13), 21.8 (CH_3, C-19), 20.9 (CH_3, C-17), 19.6 (CH_3, C-16); HRFABMS *m*/*z* 352.2296 (calcd for $C_{20}H_{32}O_5$, 352.2250).

Cytotoxicity Assays. Bioassay of cytotoxicity against human cell cultures *in vitro* was performed by the MTT [3-(4,5)-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide] colorimetric method. Each isolate was evaluated for cytotoxicity against breast carcinoma (BT474), lung carcinoma (CHAGO), human hepatocarcinoma (HEP-G2), gastric carcinoma (KATO-3), and colon carcinoma (SW-620). Adeblastin was used as a positive control.

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