

## Crotonkinins A and B and Related Diterpenoids from *Croton tonkinensis* as Anti-inflammatory and Antitumor Agents

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Received July 31, 2007

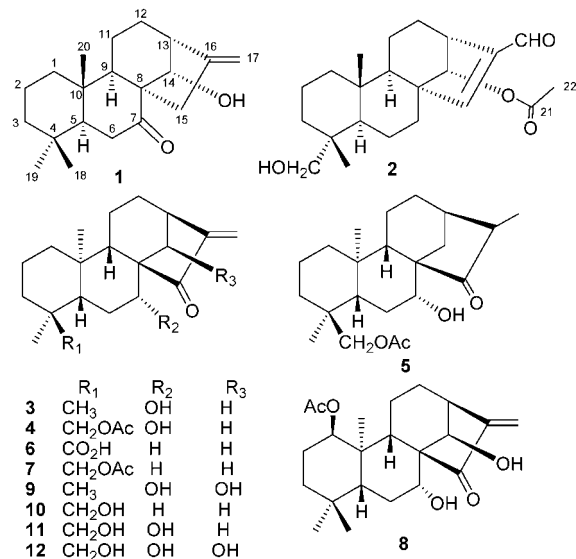
Cytotoxicity-guided phytochemical investigation of a methanolic extract of *Croton tonkinensis* afforded two new kaurane diterpenoids (**1**, **2**) and 10 known *ent*-kaurane-type diterpenoids (**3**–**12**). The structures of **1** and **2** were based on analysis of spectroscopic and mass spectral data. Compounds **3**–**12** were identified by comparison of their spectroscopic and physical data with those reported in the literature. Selected compounds from this plant were examined for cytotoxic and anti-inflammatory activities. Compounds **4** and **9** showed the highest cytotoxic activity against the tested tumor cell lines. Compounds **3**, **4**, **6**, **8**, **9**, and **11** had IC<sub>50</sub> values less than 5 μM and were more potent than the nonspecific NOS inhibitor L-NAME in inhibiting LPS-induced NO production.

The genus *Croton* (Euphorbiaceae) includes about 300 species that are widely distributed throughout tropical regions.<sup>1</sup> *C. tonkinensis* Gagnep. is a tropical shrub native to Northern Vietnam, where it has been commonly used to treat stomachache, abscesses, impetigo, gastric and duodenal ulcers, malaria, urticaria, leprosy, psoriasis, and genital organ prolapse.<sup>2–4</sup> Prior phytochemical investigations showed that this species contains steroids and *ent*-kaurane diterpenoids.<sup>5–10</sup> The crude extract of *C. tonkinensis* was also found to show significant cytotoxicity against MCF-7, NCI-H460, and SF-268 tumor cell lines. On the basis of the above studies, we selected *C. tonkinensis* as a target of our integrated program aimed at new drug discovery. Cytotoxicity-monitored fractionation of CH<sub>2</sub>Cl<sub>2</sub>-solubles of a MeOH extract from the whole plant of *C. tonkinensis* led to the isolation of two new kaurane diterpenoids, crotonkinins A (**1**) and B (**2**), together with 10 known diterpenoids. Herein, we report the structural determination of the new diterpenoids, evaluation of cytotoxic and anti-inflammatory bioactivities, and structure–cytotoxicity relationships of the isolated compounds.

### Results and Discussion

Air-dried and powdered whole plants of *C. tonkinensis* were extracted with hot MeOH and concentrated. The MeOH extract was suspended in MeOH–H<sub>2</sub>O (90:10) and partitioned with *n*-hexane. The MeOH–H<sub>2</sub>O fraction was further fractionated between CH<sub>2</sub>Cl<sub>2</sub> and H<sub>2</sub>O to afford CH<sub>2</sub>Cl<sub>2</sub>- and H<sub>2</sub>O-solubles, respectively. Cytotoxicity-guided purification of the CH<sub>2</sub>Cl<sub>2</sub> fraction yielded two new compounds (**1** and **2**) and 10 known diterpenoids (**3**–**12**).

Crotonkinin A (**1**) was isolated as optically active colorless needles (mp 175–178 °C, [α]<sub>D</sub><sup>25</sup> +90.5), and its molecular formula



was determined as C<sub>20</sub>H<sub>30</sub>O<sub>2</sub>, with six degrees of unsaturation as established by HRFABMS (*m/z* 303.2322 [M + H]<sup>+</sup>). IR absorption bands at 3363 and 1689 cm<sup>-1</sup> were assignable to hydroxy and carbonyl functionalities, respectively. Analysis of <sup>13</sup>C NMR (Table 1), DEPT-135, and HMQC spectroscopic data revealed that **1** contains one carbonyl group, four quaternary carbons, including one olefinic carbon, four methines, including one oxygenated carbon, eight methylenes, including one olefinic carbon, and three methyl groups. The <sup>1</sup>H NMR spectrum of **1** displayed characteristic signals for three methyl groups, two terminal olefinic protons, an oxygenated methine proton, two methylene groups, and a methine. The <sup>1</sup>H–<sup>1</sup>H COSY spectrum revealed connectivities for H-1 to H-3, H-5 to H-6, and H-9 to H-14. In the HMBC spectrum, long-range proton–carbon correlations from CH<sub>3</sub>-18 to C-3, -4, and -5; CH<sub>3</sub>-20 to C-1, -5, and -9; CH<sub>2</sub>-6 to C-5 and -7; and H-9 to C-8, -10, -11, and -14 established the ABC rings of the kaurane diterpenoid skeleton. These data also indicated the presence of a unique 14-hydroxy-7-one unit in the molecule. Moreover, HMBC <sup>2</sup>*J*- and <sup>3</sup>*J*-correlations of CH<sub>2</sub>-17 to C-13, -15, and -16; CH<sub>2</sub>-15 to C-7, -8,

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**Table 1.**  $^{13}\text{C}$  NMR Data of **1–3** (125 MHz,  $\text{CDCl}_3$ ,  $\delta$  in ppm)

	<b>1</b>	<b>2</b>	<b>3</b>
1	40.4	39.1	39.1
2	18.4	17.7	18.0
3	41.7	35.1	41.0
4	33.6	37.3	32.9
5	53.8	48.4	52.5
6	37.9	18.3	27.7
7	212.9	42.3	70.5
8	62.5	49.4	58.0
9	59.9	55.3	52.0
10	39.2	38.8	39.3
11	16.8	34.5	18.0
12	32.3	37.6	32.6
13	50.4	36.1	37.4
14	77.6	68.5	27.7
15	38.2	162.5	210.0
16	151.7	150.7	149.2
17	108.3	189.2	114.6
18	32.8	17.5	33.5
19	21.0	71.8	21.2
20	16.7	18.1	17.5
21		169.3	
22		21.4	

**Table 2.**  $\text{EC}_{50}$  Values of Tested Compounds against Human Tumor Cell Line Panel<sup>a</sup>

cmpd	A549	MCF-7	KB	KB-VIN
<b>1</b>	NA	NA	NA	NA
<b>3</b>	4.77	1.47	0.79	0.74
<b>4</b>	1.26	0.65	0.71	0.61
<b>5</b>	NA	NA	NA	NA
<b>6</b>	3.91	2.69	1.04	1.08
<b>7</b>	6.56	6.11	3.75	2.84
<b>8</b>	3.03	0.94	0.91	0.78
<b>9</b>	1.45	0.75	0.64	0.63
<b>10</b>	6.52	4.31	1.47	1.31
<b>11</b>	3.96	0.98	0.87	0.82
<b>12</b>	2.78	1.98	0.93	0.88

<sup>a</sup> "NA": did not reach 50% inhibition at 20  $\mu\text{g/mL}$ .

-9, -16, and -17; and H-14 to C-16 suggested that the D ring possesses a 16-ene partial structure. On the basis of spectroscopic comparison of **1** with related compounds,<sup>5–10</sup> we proposed that **1** has a kaur-16-en-7-one skeleton with a hydroxy group located at C-14. In addition, the positive optical rotation<sup>11</sup> indicated that **1** is a kaurane-type diterpenoid. The relative stereochemistry of **1** was elucidated from NOESY data and comparison with kaurane-type diterpenoids.<sup>11</sup> The orientation of the hydroxy group at C-14 was assigned as  $\alpha$  because H-14 ( $\delta_{\text{H}}$  4.34) displayed a strong NOE correlation with  $\text{CH}_3$ -20 ( $\delta_{\text{H}}$  1.12). On the basis of the foregoing spectroscopic studies, the structure of **1** was fully established as 14 $\alpha$ -hydroxykaur-16-en-7-one, and **1** was given the trivial name crotonkinin A.

Crotonkinin B (**2**) was isolated as an optically active colorless syrup with  $[\alpha]_{\text{D}}^{25} +96.5$ , and its molecular formula was determined to be  $\text{C}_{22}\text{H}_{32}\text{O}_4$  on the basis of a pseudomolecular ion peak at  $m/z$  361.2376  $[\text{M} + \text{H}]^+$  in HRFABMS. The UV spectrum of **2** exhibited a strong absorption maximum at 230 nm, compatible with an  $\alpha,\beta$ -unsaturated carbonyl chromophore.<sup>7</sup> IR absorption bands at 3441 and 1731  $\text{cm}^{-1}$  suggested the presence of hydroxy and ester carbonyl functionalities, respectively. In addition, IR signals characteristic for a Fermi resonance of a formyl group were also found at 2862 and 1375  $\text{cm}^{-1}$ . Comparison of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **2** with those of the known compound *ent*-7 $\beta$ -hydroxy-16-kauran-15-one (**3**)<sup>12</sup> inferred that **2** was an analogue of 14-acetoxykauran-18-ol, with a variation in the D ring (Table 1). The presence of an  $\alpha,\beta$ -unsaturated formyl group, rather than an oxo functionality at C-15 and terminal methylene at C-16, in this kaurane-type diterpenoid was suggested by  $^{13}\text{C}$  signals at  $\delta_{\text{C}}$  189.2 (C-17), 162.5 (C-15), and 150.7 (C-16), as well as  $^1\text{H}$  signals at  $\delta_{\text{H}}$

9.74 (1H, s, H-17) and 6.60 (1H, s, H-15). Long-range HMBC  $^2J$ - and  $^3J$ -correlations of H-17 with C-13 and -16 and of H-15 with C-7, -8, -13, -16, and -17 further established the D ring as a 15-ene-17-formyl conjugated system, which was also consistent with the downfield shift of H-15 ( $\delta_{\text{H}}$  6.60). In the NOESY spectrum, H-14 displayed a NOE correlation with  $\text{CH}_3$ -20, indicating that **2** and **1** have the same stereochemistry at C-14. Consequently, the structure of **2** was determined as 14 $\alpha$ -acetoxyl-17-formylkaur-15-en-18-ol, and **2** was given the trivial name crotonkinin B. The isolation of crotonkinins A (**1**) and B (**2**) from the title plant is the first report of kaurane-type diterpenoids rather than *ent*-kauranes from this species.

In addition, 10 known compounds were identified as *ent*-7 $\beta$ -hydroxy-16-kauran-15-one (**3**),<sup>12</sup> *ent*-7 $\beta$ -hydroxy-15-oxokaur-16-en-18-yl acetate (**4**),<sup>5</sup> *ent*-(16*S*)-18-acetoxy-7 $\beta$ -hydroxykaur-15-one (**5**),<sup>9</sup> *ent*-kaur-16-en-15-one 18-oic acid (**6**),<sup>13</sup> *ent*-18-acetoxykaur-16-en-15-one (**7**),<sup>14</sup> *ent*-1 $\beta$ -acetoxyl-7 $\alpha$ ,14 $\beta$ -dihydroxykaur-16-en-15-one (**8**),<sup>7</sup> *ent*-7 $\alpha$ ,14 $\beta$ -dihydroxykaur-16-en-15-one (**9**),<sup>15</sup> *ent*-18-hydroxykaur-16-en-15-one (**10**),<sup>14</sup> *ent*-7 $\beta$ -hydroxy-15-oxokaur-16-en-18-ol (**11**),<sup>8</sup> and *ent*-18-acetoxy-7 $\alpha$ ,14 $\beta$ -dihydroxykaur-16-en-15-one (**12**)<sup>7</sup> by spectroscopic data comparison with published values.

Diterpenoids **1** and **3–12** were assayed for cytotoxic activity against A549, MCF-7, KB, and KB-VIN human tumor cell lines, as described previously,<sup>16</sup> and the  $\text{EC}_{50}$  values are summarized in Table 2. Compounds **4** and **9** showed the highest potency against the tested cell lines, with  $\text{EC}_{50}$  values ranging from 0.61 to 1.45  $\mu\text{g/mL}$ . The remaining tested isolates showed decreased activity against A549 tumor cells, although **3**, **8**, **11**, and **12** displayed strong cytotoxicity against MCF-7, KB, and KB-VIN cell lines ( $\text{EC}_{50}$  values  $<2.0$   $\mu\text{g/mL}$ ). Compounds **7** and **10** were active only against KB and KB-VIN cells, and **1** and **5** were not active against any cell line. These results demonstrate the importance of an  $\text{O}=\text{C}-\text{CH}=\text{CH}-$  system for cytotoxic activity, as this moiety is susceptible to nucleophilic attack by key regulatory enzymes.<sup>17,18</sup>

From the above results, the following structure–cytotoxicity relationships were deduced. Kaurane-type diterpenoids that do not possess the 16-en-15-one basic skeleton, such as **1** and **5**, do not display cytotoxic activity, and diterpenoids without hydroxyls at the 7-position, for example, **6**, **7**, and **10**, have decreased activity. In addition, 18-acetylation increased cytotoxicity against the A549 tumor cell line as shown by the decrease in  $\text{EC}_{50}$  from 3.96  $\mu\text{g/mL}$  for **11** (18- $\text{CH}_2\text{OH}$ ) to 1.26  $\mu\text{g/mL}$  for **4** (18- $\text{CH}_2\text{OAc}$ ). 14-Hydroxylated or -acetylated *ent*-kaurane-type diterpenoids are commonly found in isolates from *C. tonkinensis*, and in the present study, **4** and **12** differ only in the presence of an additional hydroxyl on position-14 in the latter compound. This substitution led to decreased cytotoxicity against A549, MCF-7, KB, and KB-VIN cell lines. Thus, minor structural factors, including 18-acetoxy and 14-hydroxy substitutions, can also contribute to the cytotoxic effect of this compound class.

Activated microglial cells play deleterious roles in mediating central nerve system (CNS) inflammatory responses by producing enormous amounts of NO and ROS through induction of inducible nitric oxide synthase (iNOS) and activation of NOX, which results in neuronal damage by NO, ROS, and the more toxic metabolite peroxynitrite ( $\text{ONOO}^-$ ).<sup>19–21</sup> We also reported that drugs with antioxidative and NO-reducing activity can prevent stroke-induced brain damage.<sup>22</sup> Therefore, inhibiting NO or ROS production is a useful strategy for treating inflammatory disorders, such as cardiovascular diseases and neurodegenerative disorders.<sup>20,23</sup> The anti-inflammatory potentials of **1**, **3–6**, **8**, **9**, and **11** were evaluated by examining their effects on LPS-induced iNOS-dependent NO production and NOX-dependent ROS production in microglial cells. Compounds **3**, **4**, **6**, **8**, **9**, and **11** were more potent ( $\text{IC}_{50} <5$   $\mu\text{M}$ ) than L-NAME ( $\text{IC}_{50}$  20.1  $\mu\text{M}$ ), a nonspecific NOS inhibitor, at

**Table 3.** Effects of Tested Compounds on NADPH Oxidase (NOX) and Nitric Oxide Synthase (NOS) Activity in Murine Microglial Cells<sup>a</sup>

cmpd	% inhibition in NOX (at 50 $\mu$ M)	IC <sub>50</sub> ( $\mu$ M) in NOS
<b>1</b>	11.2 $\pm$ 1.3*	46.2 $\pm$ 3.1*
<b>3</b>	23.1 $\pm$ 2.5*	4.0 $\pm$ 0.8*
<b>4</b>	24.8 $\pm$ 3.1*	1.3 $\pm$ 0.1*
<b>5</b>	9.5 $\pm$ 1.4*	42.5 $\pm$ 2.6*
<b>6</b>	14.2 $\pm$ 0.3*	2.6 $\pm$ 0.1*
<b>8</b>	29.4 $\pm$ 2.5*	1.3 $\pm$ 0.0*
<b>9</b>	29.4 $\pm$ 0.9*	1.2 $\pm$ 0.0*
<b>11</b>	20.6 $\pm$ 2.8*	1.4 $\pm$ 0.0*
DPI	94.3 $\pm$ 1.3	ND
L-NAME	ND	20.1 $\pm$ 1.8

<sup>a</sup> NOX and NOS activity were measured as ROS and NO production, respectively, in the presence of 0.1–50  $\mu$ M of drugs. DPI (diphenyleneidonium, a NOX inhibitor) and L-NAME (a NOS inhibitor) were included as positive control. Data were calculated as % inhibition or 50% inhibitory concentration (IC<sub>50</sub>) and expressed as the mean  $\pm$  SEM from 3–6 experiments performed on different days using cells from different passage. \**P* < 0.05 as compared with relative positive control (DPI or L-NAME), respectively. "ND", value not determined.

inhibiting LPS-induced NO production (Table 3). Compounds **1** and **5** were less effective (IC<sub>50</sub> 42–46  $\mu$ M).

NOX is the major ROS-producing enzyme in activated inflammatory cells.<sup>24</sup> We previously reported that drugs with anti-inflammatory activity also show potent NOX-inhibitory action.<sup>25,26</sup> Therefore, we evaluated the eight diterpenoids for effects on NOX activity in lysates of microglial cells. Our data suggest that **3**, **4**, **8**, **9**, and **11** (maximum inhibition of NOX activity at 50  $\mu$ M ranging from 20 to 29%) are ordinary inhibitors of NOX, as compared to the specific NOX inhibitor DPI (IC<sub>50</sub> 16  $\mu$ M) (Table 3). In contrast, compounds **1**, **5**, and **6** showed only slight (9.5–14.2%) inhibition of NOX activity, indicating that NOX might not be the direct target of these compounds. In addition, the free radical-scavenging capacities of these compounds were examined in a cell-free DPPH solution. However, none of these compounds showed considerable free radical-scavenging activity. The above-described effects were not due to cytotoxicity, because no significant cell death, as compared to the 0.1% DMSO solvent control (generally less than 5% cell death), was observed due to these compounds in the concentration ranges (0.1–50  $\mu$ M) examined (data not shown).

In conclusion, these results indicate that diterpenoids **3**, **4**, **6**, **8**, **9**, and **11** display potent NO-reducing activities in microglial cells. Thus, they have potential to be anti-inflammatory drugs for the treatment of NO-dependent neurodegenerative disorders.

## Experimental Section

**General Experimental Procedures.** UV spectra were obtained on a GBC Cintra 101 UV–vis spectrophotometer, and IR spectra were recorded on a Varian Scimitar FTS-2000 FT-IR spectrophotometer. Optical rotations were measured using a Jasco DIP-370 digital polarimeter. Melting points were determined using a Yanagimoto MP-S3 micro melting point apparatus without correction. <sup>1</sup>H and <sup>13</sup>C NMR, COSY, HMQC, HMBC, and NOESY spectra were recorded on a Bruker AV-500 NMR spectrometer, using tetramethylsilane (TMS) as the internal standard. Standard pulse sequences and parameters were used for the NMR experiments, and all chemical shifts were reported in parts per million (ppm,  $\delta$ ). The low- and high-resolution FAB mass spectra were obtained on a JEOL JMS-700 spectrometer. TLC was conducted on precoated Kieselgel 60 F 254 plates (Merck), and the spots were detected either by examining the plates under a UV lamp or by treating the plates with a 10% methanolic solution of *p*-anisaldehyde acid followed by heating at 110 °C.

**Plant Material.** The whole plant of *C. tonkinensis* (Euphorbiaceae) was collected in Vietnam, and the plant material was identified and authenticated by Assoc. Prof. Dr. Vu Xuan Phuong, Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology. A voucher specimen was deposited in the herbarium of the Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology, Hanoi, Vietnam.

**Extraction and Isolation.** Air-dried and powdered whole plants of *C. tonkinensis* (10 kg) were extracted with MeOH six times (6  $\times$  20 L) under reflux for 8 h and concentrated to give a brown syrup (740 g). The extract was suspended in MeOH–H<sub>2</sub>O (90:10) and partitioned with *n*-hexane to afford *n*-hexane-solubles (220 g). The solvent was removed *in vacuo* from the MeOH–H<sub>2</sub>O fraction, and the residue was fractionated between CH<sub>2</sub>Cl<sub>2</sub> and H<sub>2</sub>O to afford CH<sub>2</sub>Cl<sub>2</sub>-solubles (325 g), H<sub>2</sub>O fraction (125 g), and precipitates (70 g), respectively. At 30  $\mu$ g/mL, the crude extract and the CH<sub>2</sub>Cl<sub>2</sub> fraction showed ca. 100% inhibition against MCF-7, NCI-H460, and SF-268 tumor cell lines, respectively. Accordingly, the CH<sub>2</sub>Cl<sub>2</sub>-solubles were subjected to column chromatography over silica gel, eluted using a step gradient of *n*-hexane–acetone (50:1 to 1:1), to obtain nine fractions (F1–F9) based on TLC profile. Each fraction was concentrated *in vacuo* and monitored by an *in vitro* cytotoxicity assay. The active fractions (F2, F3, F4, F5, and F7) were subjected to additional chromatography. F2 gave four subfractions after column chromatography over silica gel using gradient mixtures of benzene–acetone (from 200:1 to 1:1). Subfraction 2 was further subjected to column chromatography over silica gel using a stepwise gradient of *n*-hexane–EtOAc (20:1 to 1:1) to afford **2** (1.0 mg) and **3** (30.0 mg). Further purification of subfraction F3 by repeated column chromatography followed by TLC gave **1** (50.0 mg). Repeated silica gel column chromatography with benzene–acetone gradient mixtures (from 200:1 to 1:1) and preparative TLC purification of active fraction F3 yielded **4** (500.0 mg). The fourth and fifth active fractions (F4 and F5) were combined due to the similarity in the TLC profile and were purified by silica gel column chromatography using a stepwise gradient of CH<sub>2</sub>Cl<sub>2</sub>–acetone (from 100:1 to 1:1) followed by preparative TLC with *n*-hexane–EtOAc (5:1) to afford **5** (12.0 mg), **6** (250.0 mg), **7** (5.0 mg), **8** (200.0 mg), and **9** (7.2 mg). Active fraction F7 was further chromatographed over silica gel eluted with CH<sub>2</sub>Cl<sub>2</sub>–MeOH (from 30:1 to 1:1) and then purified by preparative TLC with *n*-hexane–acetone (3:1) to yield **10** (1.5 mg), **11** (100.0 mg), and **12** (2.6 mg).

**Crotonkinin A (1):** colorless needles; mp 175–178 °C (acetone); [ $\alpha$ ]<sub>D</sub><sup>25</sup> +90.5 (c 0.7, CHCl<sub>3</sub>); IR (Nujol)  $\nu_{\max}$  3363, 2927, 1689, 1373, 1304 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  5.01 (2H, br s, CH<sub>2</sub>-17), 4.34 (1H, br s, H-14), 3.32 (1H, dt, *J* = 17.3, 2.4 Hz, H-15a), 2.68 (1H, br s, H-13), 2.52 (1H, dd, *J* = 14.4, 14.0 Hz, H-6a), 2.47 (1H, dd, *J* = 14.4, 3.7 Hz, H-6b), 2.05 (1H, br s, D<sub>2</sub>O exchangeable, OH-14), 2.01 (1H, d, *J* = 17.3 Hz, H-15b), 1.90 (1H, m, H-12), 1.81 (1H, d, *J* = 12.9 Hz, H-1), 1.30 (1H, dd, *J* = 13.4, 3.8 Hz, H-5), 1.12 (3H, s, CH<sub>3</sub>-20), 0.87 (6H, s, CH<sub>3</sub>-18 and -19); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) see Table 1; FABMS *m/z* 303 [M + H]<sup>+</sup> (100), 285 (60), 267 (45); HRFABMS *m/z* 303.2322 [M + H]<sup>+</sup> (calcd for C<sub>20</sub>H<sub>31</sub>O<sub>2</sub>, 303.2324).

**Crotonkinin B (2):** colorless syrup; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +96.5 (c 0.07, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 230 (3.18, sh) nm; IR (CHCl<sub>3</sub>)  $\nu_{\max}$  3441, 2928, 2862, 1731, 1451, 1375, 1244 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  9.74 (1H, s, H-17), 6.60 (1H, s, H-15), 5.17 (1H, d, *J* = 5.7 Hz, H-14), 3.44 (1H, d, *J* = 10.8 Hz, H-18a), 3.11 (1H, d, *J* = 10.8 Hz, H-18b), 2.98 (1H, br s, H-13), 1.87 (3H, s, CH<sub>3</sub>-22), 1.08 (3H, s, CH<sub>3</sub>-20), 0.75 (3H, s, CH<sub>3</sub>-19); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) see Table 1; FABMS *m/z* 361 [M + H]<sup>+</sup> (1); HRFABMS *m/z* 361.2376 [M + H]<sup>+</sup> (calcd for C<sub>22</sub>H<sub>33</sub>O<sub>4</sub>, 361.2379).

**Cytotoxicity Assay.** The cytotoxicities of compounds toward three human cancer cell lines, MCF-7, NCI-H460, and SF-268, were measured with an MTS assay procedure reported in the literature.<sup>27</sup>

**In Vitro Cytotoxicity Assay.** Cytotoxic potential of antofine against L-1210, P-388, A-549, and HCT-8 cell lines was determined as described previously.<sup>28</sup>

**Measurement of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical-Scavenging Capacity.** DPPH radical-scavenging capacity assay was performed as in our previous report.<sup>25</sup>

**Microglial Cell Culture and Measurements of Nitric Oxide (NO).**

The murine microglial cell line (BV2) was cultured and production of NO was measured by the methods as described in our prior report.<sup>22</sup>

**Measurement of NADPH Oxidase (NOX) Activity.** NADPH oxidase activity was measured as described previously.<sup>22</sup>

**Acknowledgment.** The authors are grateful for financial support from the National Science Council, Taiwan, Republic of China (NSC 95-2323-B-006-003) awarded to T.S.W. and are also thankful to the National Research Institute of Chinese Medicine, Taiwan, Republic of China, for partial financial support of this research. Thanks are also due in part to support from NIH grant CA17625 awarded to K.H.L.

**Supporting Information Available:** Significant HMBC and NOE-SY correlations for compounds **1** and **2**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References and Notes

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NP070383F