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# *seco*-KAURANE SKELETON DITERPENOIDS FROM *Croton oblongifolius*

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*A new* seco-*kaurane type diterpenoid,* ent-*3,4*-seco-*17-oxo-kaur-4(19),15(16)-dien-3-oic acid, and a known compound,* ent-*3,4*-seco-*kaur-4(19),16(17)-dien-3-oic acid, were isolated from the stem bark of* Croton oblongifolius. *The structures of these compounds were established on the basis of spectroscopic data.*

**Keywords**: *Croton oblongifolius*, diterpenoid, seco-kaurane.

*Croton oblongifolius* Roxb. (Euphorbiaceae) is widely distributed in Thailand and has been used as a medicinal plant for the treatment of dysmenorrhea, as a purgative, and to treat dyspepsia and dysenteria. This plant has also been used in conjunction with *C. sublyratus* to treat gastric ulcers and gastric cancers. The chemical constituents of *Croton oblongifolius* Roxb. were first reported by Seshadri et al. during 1968 to 1972 [1–5]. Due to the interest in the identification of biologically active compounds of this plant, we have investigated this plant in Thailand and found that it contains different chemical constituents. In a decade of our continuing investigation on the chemical constituents of *C. oblongifolius* in various parts of Thailand, we found that this plant is a rich source of diterpenoid compounds with various skeletons [6–18]. As part of our continuing search for novel diterpenoids in this plant, we investigated the chemical constituents of this plant by comparing its $^1$H NMR profile in hexane extracts with the $^1$H NMR data of our previous reported compounds. We found a new 3,4-*seco*-kaurane-type diterpenoid, *ent*-3,4-*seco*-17-oxo-kaur-4(19),15(16)-dien-3-oic acid (**2**), together with a known diterpenoid, *ent*-3,4-*seco*-kaur-4(19),16(17)-dien-3-oic acid (**1**). In this paper we describe the isolation and structure elucidation of **1** and **2**.

In comparison with the $^1$H NMR data of our previous report, the $^1$H NMR profile of the hexane extract of *C. oblongifolius* specimens collected from Amphoe Nong-Han, Udon-Thani Province, in the northeastern part of Thailand displayed mainly one compound that was similar to that of *ent*-kaur-16-en-19-oic acid. After isolation using silica gel column chromatography eluted with hexane–ethyl acetate in a stepwise fashion, compound **1** was obtained as a major component along with compound **2**.

The molecular formula of compound **1** was assigned as $C_{20}H_{30}O_2$ based on high-resolution mass spectrometric analysis, which exhibited an $[M + H]^+$ ion at *m/z* 303.2319. $^1$H and $^{13}$C NMR (Table 1) indicated the presence of two methyl carbons, eight $sp^3$-methylene carbons, two $sp^2$-methylene carbons, three $sp^3$-methine carbons, two $sp^3$-quaternary carbons, two $sp^2$-quaternary carbons, and a carbonyl carbon. Since the six degrees of unsaturation have been accounted for, we assume that **1** contains three rings. From the HMBC data (Table 1) with the assistance of COSY, TOCSY, and NOESY (Fig. 1), **1** was identified as *ent*-3,4-*seco*-kaur-4(19),16(17)-dien-3-oic acid. A search through the crossfire Beilstein database revealed that compound **1** was the known compound reported in [19]. A comparison of the specific optical rotation $\{[\alpha]_D^{20}$–45° (*c* 0.28, $CHCl_3)\}$ of **1** with that reported in the crossfire Beilstein database $\{[\alpha]_D^{20}$–33° (*c* 0.28, $CHCl_3)\}$ indicated that compound **1** is the same compound.

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TABLE 1. 1D and 2D NMR Data of **1** and **2**[a] (J/Hz)

| C atom | **1** | | | **2** | | |
|---|---|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ | HMBC (H→C) | $\delta_C$ | $\delta_H$ | HMBC (H→C) |
| 1 | 33.6 | 1.65 m | 3, 5 | 33.6 | 1.71 m | 2, 3, 9, 10 |
| | | 1.70 m | | | 1.73 m | |
| 2 | 28.5 | 2.15 (ddd, J = 7.4, 11.6, 16) | 1, 3 | 28.2 | 2.14 (ddd, J = 6.4, 11.6, 16) | 1, 3 |
| | | 2.31 (ddd, J = 5.2, 12.8, 16) | 1, 3 | | 2.33 (ddd, J = 6, 10.8, 16) | 1, 3 |
| 3 | 180.0 | – | | 178.1 | – | |
| 4 | 147.4 | – | | 146.9 | – | |
| 5 | 50.4 | 1.97 (dd, J = 2, 9) | 4, 10, 18, 19, 20 | 50.1 | 2.00 (br.d, J = 11.2) | |
| 6 | 26.2 | 1.43 m | | 36.6 | 1.70 m | 8, 10 |
| | | 1.41 m | | | 1.66 m | 8, 10 |
| 7 | 39.5 | 1.41–1.51 m[b] | 8 | 24.7 | 1.48 m | |
| | | | | | 1.56 m | |
| 8 | 43.9 | – | | 50.5 | – | |
| 9 | 46.0 | 1.20 (br.d, J = 6.8) | 1, 8, 10, 11, 12, 20 | 37.3 | 1.25 (br.d, J = 7.6) | 1, 8, 10, 11, 12, 14, 15, 20 |
| 10 | 40.8 | – | | 41.1 | – | |
| 11 | 18.2 | 1.49 m | 8, 13 | 18.5 | 1.52–1.55 m[b] | |
| | | 1.64 m | | | | |
| 12 | 32.9 | 1.50 m | | 24.9 | 1.54–1.62 m[b] | |
| | | 1.63 m | | | | |
| 13 | 43.7 | 2.65 br.s | 8 | 37.8 | 3.07 br.s | |
| 14 | 39.4 | 1.95 (d, J = 10.4) | 8, 12, 13, 15, 16 | 42.9 | 2.15 (d, J = 10.8) | 7, 15, 16 |
| | | 1.15 (dd, J = 4, 10.4) | 7, 8, 9, 12, 13 | | 1.53 m | 8, 12 |
| 15 | 49.0 | 2.01–2.13 m[b] | 8, 9, 16, 17 | 161.1 | 6.59 s | 8, 13, 14, 16, 17 |
| 16 | 155.3 | – | | 148.6 | – | |
| 17 | 103.2 | 4.80 s | 13, 15 | 189.4 | 9.74 s | 13, 16 |
| | | 4.74 s | 13, 15 | | | |
| 18 | 23.3 | 1.73 s[c] | 4, 5, 19 | 23.2 | 1.74 s[c] | 4, 5, 19 |
| 19 | 113.6 | 4.87 s | 5, 18 | 114.0 | 4.89 s | 5, 18 |
| | | 4.64 s | 5, 18 | | 4.67 s | 5, 18 |
| 20 | 21.8 | 1.00 s[c] | 1, 5, 9, 10 | 21.7 | 1.06 s[c] | 1, 5, 9, 10 |

[a]Data were obtained at 400 MHz for $^1$H and 100 MHz for $^{13}$C NMR with chemical shifts (δ) in ppm and were referenced to residual solvent signals with resonances at $\delta_H$ 7.26 and at $\delta_C$ 77.0.
[b]Intensity of two protons; [c]intensity of three protons.

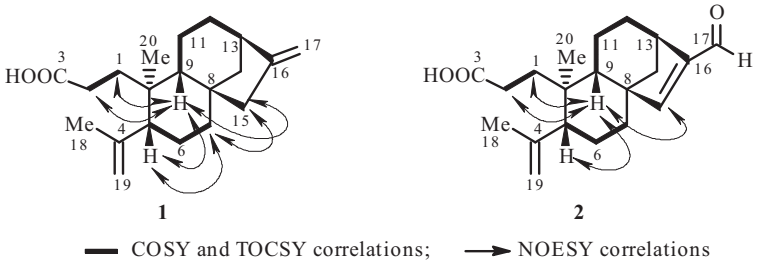


Fig. 1. COSY, TOCSY, and NOESY correlations of **1** and **2**.

The molecular formula of compound **2** was assigned as $C_{20}H_{28}O_3$ based on high-resolution mass spectrometric analysis, which exhibited an $[M + Na]^+$ ion at *m/z* 339.1941. The $^1$H NMR spectrum of **2** showed a sharp singlet signal of an aldehyde proton at δ 9.74. The presence of the aldehyde functional group was also supported by the $^{13}$C NMR spectrum, which showed a signal at δ 189.4. The $^1$H and $^{13}$C NMR data of **2** (Table 1) are similar to those of **1** except that compound **2** has an aldehyde function ($\delta_H$ 9.74 and $\delta_C$ 189.4) at C-17. $^1$H and $^{13}$C NMR (Table 1) indicated the presence of two methyl carbons, seven $sp^3$-methylene carbons, one $sp^2$-methylene carbon, three $sp^3$-methine carbons, two $sp^3$-quaternary carbons, one $sp^2$-methine carbon, two $sp^2$-quaternary carbons, and two carbonyl carbons. Since the seven degrees of unsaturation have been accounted for, we assume that **2** contains three rings. The HMBC analysis (Table 1) with the assistance of COSY,

TOCSY, and NOESY (Fig. 1) led to the structure of **2**. The relative stereochemistry at C-5, C-8, C-9, and C-10 were assigned on the basis of the cross-peaks observed between H-9 and H-1, between H-9 and H-2, between H-9 and H-5, between H-9 and H-15, and between the methyl protons of C-18 and the methyl protons of C-20 in the NOESY analysis. Thus, compound **2** was identified as a novel compound. The absolute configuration of **2** was tentatively assumed to have the same *ent* configuration of *ent*-kaur-16-en-19-oic acid [15] due to close similarity of their structures and based on their negative optical rotation sign.

## EXPERIMENTAL

**General Experimental Procedures**. $^1$H and $^{13}$C NMR spectra were recorded on a Varian Mercury +400 MHz NMR spectrometer ($^1$H at 400 MHz and $^{13}$C at 100 MHz). Chloroform-d ($CDCl_3$) was used in NMR experiments and chemical shifts (δ) were referenced to the signals of residual solvents at δ 7.26 ppm ($^1$H) and 77.0 ppm ($^{13}$C). HR-ESI-MS spectra were recorded on Micromass LCT (LC/MS). Optical rotations were measured on a Perkin–Elmer 341 polarimeter using a sodium lamp at wavelength 589 nm. FT-IR spectra were recorded on a Perkin–Elmer model 1760X Fourier transform infrared spectrophotometer. Melting points were examined using a Fisher-John melting point apparatus. All solvents used for column chromatography were of commercial grade and were distilled prior to use. TLC were carried out on precoated silica gel 60 $F_{254}$ (Merck), and spots were detected under UV (254 and 365 nm) before and after spraying with a vanillin/sulfuric acid solution followed by heating the plate. Isolations were carried out using column chromatography (CC) [silica gel 60 (Merck, 0.040–0.063 mm)].

**Plant Material.** The stem bark of *C. oblongifolius* was collected from Amphoe Nong-Han, Udon-Thani Province, Thailand. Botanical identification was achieved through comparison with a voucher specimen No. BKF 084729 in the Herbarium collection of the Royal Forest Department of Thailand.

**Extraction and Isolation of 1 and 2**. The powdered, sun-dried stem bark (12.8 g) of *C. oblongifolius* was extracted with hexane (5 × 500 mL). The hexane extract was filtered and evaporated in vacuo to obtain a viscous greenish yellow residue (125 mg). The hexane extract (120 mg) was fractionated by silica gel column chromatography and eluted with hexane–EtOAc in a stepwise fashion. Similar fractions were combined on the basis of TLC with detection by UV light and vanillin–$H_2SO_4$ reagent. Fractions 10–15, eluted with 5% ethyl acetate in hexane, were further purified by silica gel column chromatography eluting with 5% ethyl acetate in hexane to afford compound **1** as a clear film (20 mg). Fraction 17–20, eluted by 10% ethyl acetate in hexane, was further purified by silica gel column chromatography eluting with 10% ethyl acetate in hexane to afford compound **2** as a clear film (3 mg).

***ent*-3,4-*seco*-Kaur-4(19),16(17)-dien-3-oic Acid (1)**. $[\alpha]_D^{20}$ –45° (*c* 0.28, $CHCl_3$). IR ($\nu_{max,}$ neat, $cm^{-1}$): 3400 br, 1698, and 1637. $^1$H NMR (400 MHz, $CDCl_3$), see Table 1; $^{13}$C NMR (100 MHz, $CDCl_3$), see Table 1. HR-ESI-MS *m/z* 303.2319 ($[M + H]^+$ calcd for $C_{20}H_{30}O_2$ + H, 303.2324).

***ent*-3,4-*seco*-17-Oxo-kaur-4(19),15(16)-dien-3-oic Acid (2)**. $[\alpha]_D^{20}$ –54° (*c* 0.03, MeOH). IR ($\nu_{max,}$ neat, $cm^{-1}$): 3400 br, 2705, 1699, 1690, and 1638. $^1$H NMR (400 MHz, $CDCl_3$), see Table 1; $^{13}$C NMR (100 MHz, $CDCl_3$), see Table 1. HR-ESI-MS *m/z* 339.1941 ($[M + Na]^+$ calcd for $C_{20}H_{28}O_3Na$, 339.1936).

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## REFERENCES

1. P. S. Rao, G. P. Sachdev, T. R. Seshadri, and H. B. Singh, *Tetrahedron Lett*., **9**, 4685 (1968).
2. V. N. Aiyar and T. R. Seshadri, *Tetrahedron*, **26**, 5275 (1970).
3. V. N. Aiyar and T. R. Seshadri, *Indian J. Chem*., **9**, 1028 (1971).

4. V. N. Aiyar and T. R. Seshadri, *Phytochemistry*, **11**, 1473 (1972).
5. V. N. Aiyar and T. R. Seshadri, *Curr. Sci.,* **41**, 839 (1972).
6. S. Roengsumran, S. Achayindee, A. Petsom, K. Pudhom, P. Singtothong, C. Surachetapan, and T. Vilaivan, *J. Nat. Prod*., **61**, 652 (1998).
7. S. Roengsumran, P. Singtothong, K. Pudhom, N. Ngamrochanavanich, A. Petsom, and C. Chaichantipyuth, *J. Nat. Prod*., **62**, 1163 (1999).
8. K. Pudhom, T. Vilaivan, N. Ngamrojanavanich, S. Dechangvipart, D. Sommit, A. Petsom, and S. Roengsumran, *J. Nat. Prod*., **70**, 659 (2007).
9. S. Roengsumran, A. Petsom, D. Sommit, and T. Vilaivan, *Phytochemistry*, **50**, 449 (1999).
10. S. Roengsumran, A. Petsom, N. Kuptiyanuwat, T. Vilavan, C. Ngamrojnavich, C. Chaichantiyouth, and S. Puthong, *Phytochemistry,* **56**, 103 (2001).
11. S. Roengsumran, N. Jaiboon, N. Chaichit, D. Sommit, D. Pattamadilok, C. Chaichantipyuth, and A. Petsom, *J. Chem. Crystallogr*., **32**, 511 (2002).
12. C. Chaichantipyuth, A. Petsom, P. Taweechotipatr, N. Muangsin, N. Chaichit, S. Puthong, S. Roengsumran, M. Kawahata, T. Watanabe, and T. Ishikawa, *Heterocycles,* **65**, 809 (2005).
13. S. Roengsumran, K. Musikul, A. Petsom, T. Vilaivan, P. Sangvanich, S. Pornpakakul, S. Puthong, C. Chaichantipyuth, N. Jaiboon, and N. Chaichit, *Planta Med*., **68**, 274 (2002).
14. C. Chaichantipyuth, N. Muangsin, N. Chaichit, S. Roengsumran, A. Petsom, T. Watanabe, and T. Ishikawa, *Z. Kristallogr. - New Cryst. Struct.,* **219**, 111 (2004).
15. N. Ngamrojnavanich, S. Sirimongkon, S. Roengsumran, A. Petsom, and H. Kamimura, *Planta Med*., **69**, 555 (2003).
16. S. Roengsumran, S. Pornpakakul, N. Muangsin, P. Sangvanich, T. Nhujak, P. Sintothong, N. Chaichit, S. Puthong, and A. Petsom, *Planta Med*., **70**, 87 (2004).
17. S. Roengsumran, P. Pata, N. Ruengraweewat, J. Tummatorn, S. Pornpakakul, P. Sangvanich, S. Puthong, and A. Petsom, *Chem. Nat. Comp.,* **45**, 641 (2009).
18. S. Suwancharoen, W. Tommeurd, C. Phurat, N. Muangsin, N. Chaichit, and S. Pornpakakul, *Acta Crystallogr., E*, **66**, o1531 (2010).
19. G. Palazzino, E. Federici, P. Rasoanaivo, C. Galeffi, and F. D. Monache, *Gazz. Chim. Ital*., **127**, 311 (1997).