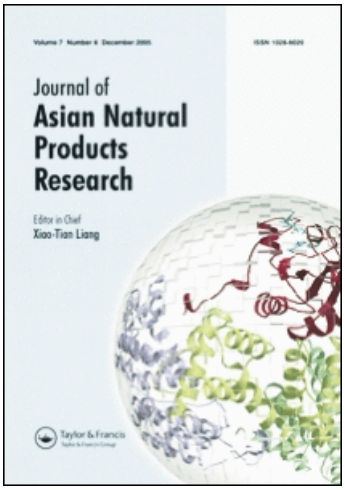

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A NEW TRITERPENOID FROM *ENTODON OKAMURAE* BROTH

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One new triterpenoid, entokamurol (**1**), was isolated from *Entodon okamurae* Broth, together with other nine compounds, namely dryocrassol (**2**), chrysophamol (**3**), physcion (**4**), 10-nonacosamnol (**5**), *n*-hexadecanol (**6**), phthalic acid isodibutyl ester (**7**), curcumol (**8**), β-sitosterol (**9**) and daucosterol (**10**). Their structures were elucidated on the basis of extensive NMR (DEPT, DQF-COSY, HMQC, HMBC and NOESY), IR and MS studies. All the compounds were isolated and identified from the genus of *Entodon* for the first time, and it is also the first report of a guaiane-type sesquiterpenoid and compounds with anthraquinone skeleton in mosses.

Keywords: *Entodon okamurae* Broth; Entokamurol; Triterpenoid; Sesquiterpenoid; Anthraquinone

INTRODUCTION

Many mosses have long been used in traditional medicine for their biological properties such as antibacterial, diuresis and antivirus and so on. As a special type of botany, mosses, which contain plentiful active chemical constituents, are a rich source of new compounds [1]. In recent years, increasing attention has been focused on chemical and biological research into mosses. In this paper, ten compounds (**1**–**10**) are reported to have been isolated from *Entodon okamurae* Broth. On the basis of spectroscopic data and their chemical and physical analysis, their structures were determined, including two triterpenoids: entokamurol (**1**) and dryocrassol (**2**) [2–4]; two anthraquinonoids: chrysophamol (**3**) [5] and physcion (**4**) [6–8]; two lipidols: 10-nonacosamnol (**5**) [9,10] and *n*-hexadecanol (**6**) [11–13]; one benzenoid: phthalic acid isodibutyl ester (**7**) [14–18]; one sesquiterpenoid: curcumol (**8**) [19,20]; two steroids: β-sitosterol (**9**) and daucosterol (**10**). All the compounds were isolated from the genus of *Entodon* for the first time, and it is also the first report of a guaiane-type sesquiterpenoid and compounds with anthraquinone skeleton in mosses. Entokamurol (**1**) is a new compound.

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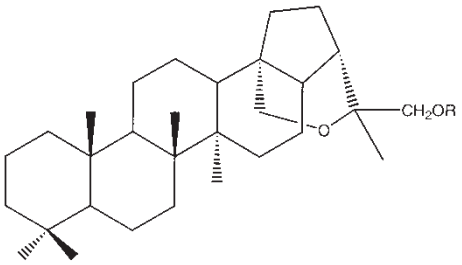

FIGURE 1 Structures of **1** and **1a**.

RESULTS AND DISCUSSION

Entokamurol (**1**) (Fig. 1) was obtained as a white amorphous powder. Its molecular formula $C_{30}H_{50}O_2$ was determined by the [MH^+] ion peak at *m/z* 443 in EIMS and at *m/z* 442.3823 by HRMS (calcd. for $C_{30}H_{50}O_2$ is 442.3811) and by its ^{13}C NMR spectral data.

The ^1H NMR spectrum of this compound showed typical characteristic features of a saturated triterpenoid, and suggested the presence of six tertiary methyl groups from signals at δ 0.79, 0.82, 0.85, 0.98, 0.99 and 1.33 (each s, respectively, H_3-24, H_3-25, H_3-23, H_3-26, H_3-27, H_3-29). In the downfield region, two $-CH_2O$ groups of geminal coupling protons were found at δ 3.20 (1H, d, $J = 11.4$), 4.03 (1H, d, $J = 11.4$) and 3.29 (1H, d, $J = 10.8$), 3.56 (1H, d, $J = 10.8$), which were further determined by the two corresponding carbons at δ 65.68 and 69.96 in HMBC. One hydroxymethine (CH_2OH) group was demonstrated by the appearance of one free OH at (υ_{max} 3500 cm^{-1} in the IR spectrum and by the disappearance of this signal after formation of **1a** after acetylation. In HMBC (Fig. 2), the chemical shift of this CH_2OH was then deduced to be δ 69.96 in **1** and 70.72 in **1a** for the correlation of H_2-30 (4.00, 1H, d, $J = 11$ Hz, 3.88, 1H, d, $J = 11$ Hz) with the carbonyl at δ 171.03. Also, one tertiary carbon at δ 76.08 was found to be correlated with the above two hydroxymethine signals and one methyl signal at δ 1.33 s. From the correlation signals between the tertiary methyls and the corresponding carbons in HMBC, the skeleton structure of **1** can be deduced as depicted in Fig. 2 (bold bonds). Accordingly, the hopane-type triterpenoid structure of **1** was finally decided.

The relative stereochemistry of **1** was resolved by observation of the NOE correlations (Fig. 3) between different protons in NOESY. The NOEs between H_{25} and H_{24}, H_{26}, and the NOEs between H_{27} and $H_{7\alpha}$, $H_{16\alpha}$ as well as no NOEs between H_{27} and H_{26} indicated that C_{24}, C_{25} and C_{26} are in β positions while C_{27} is in the α position. The NOEs between H_{13} and H_{17}, $H_{19\beta}$, H_{26}; H_{17} and H_{13}, $H_{16\beta}$, $H_{19\beta}$, H_{21} suggested that both H_{17} and H_{21} are in β positions. The NOE between H_{28} and $H_{16\alpha}$, H_{27}, H_{29} showed that C_{22} and C_{28} are

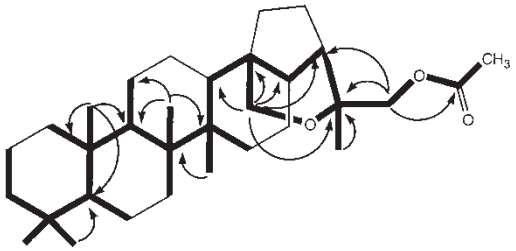

FIGURE 2 Skeleton linkage determined by HMBC of **1a** (→ refers to the correlations between H and C observed in HMBC).

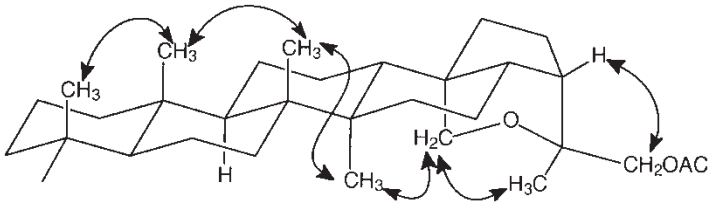

FIGURE 3 Main NOE correlations (↔) between protons observed in NOESY of **1a**.

in α positions. H_{29} (δ1.33) was deduced from its NOE correlation with $H_{16\alpha}$ and H_{28} while the hydroxyl substitution is at the H_{30} position. Thus compound **1** was finally elucidated as hopane-22,28-epoxy-30-ol, and designated as entokamurol.

Acetylation of **1** afforded **1a**. Both the ^{1}H NMR and ^{13}C NMR spectra of **1a** showed very close similarity to that of compound **1** except for two more carbon signals at δ_C 20.9 and δ_C 171.0 in the ^{13}C NMR spectrum, and one more tertiary methyl signal at δ_H 2.07 and pronounced downfield shifts of H_{28} from δ_H 3.29 and δ_H 3.56 to δ_H 3.88 and δ_H 3.99, respectively in the ^{1}H NMR spectrum. Its 2DNMR spectra displayed the same correlations as those of compound **1**.

EXPERIMENTAL

General Experimental Procedures

Melting points (uncorrected) were measured on a micro-melting apparatus. IR spectra were run on a NICOLET NEXUS 470 FT-IR spectrometer. NMR spectra were obtained with Varian UNITY INOVA 600 and Bruker Advance DEX 500 FT spectrometers from $CDCl_3$ solutions using a cryoprobe, with $CDCl_3$ itself as internal standard. EIMS spectra were taken on a HP 5989A mass spectrometer. Column chromatography: Silica gel 230–400 mesh (Merck), Sephadex LH-20 (Pharmacia); TLC and prepared TLC were from Qingdao Sea Chemical Co. Ltd.

Plant Material

The *Entodon okamurae* Broth. was collected from Mount Lu, Shandong Province of China, in July 2000, and identified as *Entodon okamurae* Broth. by Dr Xue-sun Wen of the College of Pharmacy, Shandong University. A voucher specimen is deposited at the College of Pharmacy, Shandong University.

Extraction and Isolation

Dried *Entodon okamurae* Broth. (4.9 kg) was refluxed three times with 95% EtOH for two hours. The extract was concentrated *in vacuo* to yield 310 g of extract, which was suspended in warm water (1.0 l). The suspension was extracted with light petroleum (60–90°C) (1.0 l × 3) followed by ethyl acetate (1.0 l × 3). The light petroleum fraction (139 g) was subjected to silica gel chromatography and eluted with Petrol–Me_2CO (99:1–97:3) (Petrol = light petroleum) to give Fr. A–G fractions. Repeated chromatography over silica gel afforded compound **1** (12 mg) from Fr. A, **2** (10 mg) from Fr. C, **5** (100 mg) from Fr. D, **6** (30 mg) from Fr. E, **8** (4 mg) from Fr. F, **9** (2 g) from Fr. G. Fraction B was subjected to silica gel chromatography and yield fractions Fr. B_1–B_3.Compound **3** (8 mg) from Fr. B_1, **4** (6 mg)

from Fr. B_2, **7** (4 mg) from Fr. B_3 were afforded after purifying over Sephadex LH-20 and prepared by TLC. The ethyl acetate fraction (14.5 g) was repeatedly chromatographed over silica gel column with the mixture of Petrol–Me_2CO to give **10** (10 mg).

Entokamurol (1)

The compound is a white amorphous powder, mp ($CHCl_3$) 224–227 °C, EIMS 443 [MH^+] (4.6), 425 [443 − H_2O] (11.0), 411 [442 − CH_2OH] (100.0), 393 (2.1), 367 (2.4), 272 (4.0), 233 (4.8), 219 (8.8), 205 (12.0), 191 (18.0). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: Table I, ^{13}C NMR ($CDCl_3$, 125 MHz) δ: Table I.

29-Acetylentokamurol(1a)

Colorless needles, mp (EtOH) 164–167°C. EIMS 484 [M^+], ^{1}H NMR ($CDCl_3$, 500 MHz) δ: Table I, ^{13}C NMR ($CDCl_3$, 125 MHz) δ: Table I.

Dryocrassol (2)

White cluster powder, mp ($CHCl_3$) 211.5–213.4°C. EIMS *m/z*: 428 [M^+] (2.3). ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 0.72 (3H, s, H-28), 0.79 (3H, s, H-24), 0.82 (3H, s, H-25), 0.85 (3H, s, H-23), 0.96 (6H, s, H-26, H-27), 1.05 (3H, d, 6.6 Hz, H-29), 3.39 (1H, dd, J = 10.6, 6.6, H-30), 3.63 (1H, dd, J = 10.6, 6.6, H-30). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 16.00(C-25), 16.1(C-11), 21.8(C-24), 22.8(C-16), 24.2(C-12), 27.4(C-20), 33.4(C-4), 33.5(C-7), 33.6 (C-23), 33.8(C-15), 37.8(C-10), 39.8(C-22), 40.6(C-1), 41.9(C-19), 41.9(C-9), 42.0(C-14), 42.3(C-3), 42.8(C-21), 44.6(C-18), 49.5(C-13), 50.7(C-9), 54.5(C-17), 56.4(C-5), 68.0(C-30).

Chrysophamol (3)

Yellow cluster crystals, mp 166.8–168.5°C ($CHCl_3$–Petrol), EIMS *m/z*: 254 [M^+] (100.0). ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 2.46 (3H, s, CH_3), 7.09 (1H, s, H-2), 7.28 (1H, d, J = 8.4 Hz, H-7), 7.64 (1H, s, H-4), 7.66 (1H, t, J = 8.4 Hz, 6.6 Hz, H-6), 7.81 (1H, d, J = 6.6 Hz, H-5). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 22.3(CH_3), 113.8(C-9α), 115.9(C-8α), 119.9(C-5), 121.4(C-4), 124.4(C-7), 124.6 (C-2), 133.3(C-4α), 133.7(C-10α), 137.0(C-6), 149.4(C-3), 162.5(C-1), 162.8(C-8), 182.0(C-10), 192.7(C-9).

Physcion (4)

Yellow cluster crystals, mp 162–165°C ($CHCl_3$–Petrol), EI-MS *m/z*: 284 [M^+] (100.0). ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 2.45 (3H, s, CH_3), 3.94 (3H, s, OCH_3), 6.69 (1H, s, H-7), 7.09 (1H, s, H-2), 7.38 (1H, d, J = 1, 8 Hz, H-5), 7.64 (1H, s, H-4). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 22.2(CH_3), 56.1(OCH_3), 106.8(C-7), 108.3(C-5), 110.5(C-8α), 113.9(C-9α), 121.3(C-4), 124.5(C-2), 133.5(C-4α), 135.3(C-10α), 148.5(C-3), 162.6(C-1), 165.3(C-8), 166.6(C-6), 182.0(C-10), 190.9(C-9).

10-Nonacosamnol (5)

White amorphous powder, mp 79–80.2°C ($CHCl_3$–Petrol), IR ν_{max} (KBr) (cm^{-1}): 3328, 2955, 2917, 2848, 2872, 1470, 1091, 721. EIMS *m/z*: 423 [M^+ − H](44). ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 0.88 (6H, t, 7.2 Hz, CH, H-1, 29), 3.58 (1H, q, 4.8 Hz, H-10). ^{13}C NMR

TABLE I I NMR data of compounds **1** and **1a**

		1		**1a**				**1**		**1a**	
No		δ_H*	δ_C†	δ_H*	δ_C†	No		δ_H*	δ_C†	δ_H*	δ_C†
1	α	0.76 dd (3.6, 9.3)	40.37	0.73 dd (11.5, 1.5)	40.38	17		1.27 m	49.44	1.28 dd (11.0, 2.0)	49.17
	β	1.64 dd (12.6)		1.64 dd (11.5, 1.5)		18			43.54		43.43
2	α	1.39 m	18.75	1.38 m	18.75	19	α	1.86 dd (4.3, 9.3)	35.99	1.88 dd (9.2, 4.0)	36.07
	β	1.53 m		1.52 m			β	1.15 dd (4.8, 9.3)		1.15 dd (9.2, 4.0)	
3	α	1.12 dd (4.2, 13.5)	42.11	1.12 dd (3.8, 13.2)	42.12	20	α	1.41 d (4.2)	25.40	1.42 m	25.46
	β	1.37 dd (4.2, 13.5)		1.36 dd (3.8, 13.2)			β	1.84 m		1.75 ddd (6, 4, 3.5)	
4			33.51		33.27	21		1.82 m	44.64		44.52
5		0.71 dd (1.8, 12.0)	56.22	0.72 dd (1.6, 12.0)	56.24	22			76.08	1.89 m	75.14
6		1.32 m	18.69	1.34 m	18.68	23		0.85 s	33.39		33.52
7	α	1.46 dd (4.8, 7.2)	33.29	1.28 m	33.37	24		0.79 s	21.59	0.84 s	21.56
	β	1.26 dd (3.6, 7.2)		1.46 m		25		0.82 s	15.97	0.79 s	15.96
8			41.93		41.92	26		0.98 s	16.58	0.82 s	16.56
9		1.24 m	50.58	1.26 m	50.61	27		0.99 s	17.07	0.98 s	16.90
10			37.47		37.49	28		3.20 d (11.4)	65.68	0.99 s	65.82
11	α	1.26 m	21.07	1.52 m	21.07			4.03 d (11.4)		3.21 d (11.5)	
	β	1.52 m				29		1.33 s	21.14	4.04 d (11.5)	21.65
12	α	1.29 m	23.50	1.28 m	23.50	30		3.30 d (10.8)	69.79	1.31 s	70.72
	β	1.46 m		1.48 m				3.56 d (10.8)		3.99 d (11.0)	
13		1.52 dd (4.0, 7.0)	47.90	1.52 m	47.82	OAc				3.88 d (11.0)	20.93
14			41.99		41.99					2.06 s	171.03
15		1.28 m	32.68	1.30 m	36.28						
16	α	2.11 m	23.31	2.12 m	23.52						
	β	1.70 m		1.70 m							

* ^{1}H NMR data assigned according to g-COSY; data in parenthesis are coupling constants in Hz.
† ^{13}C NMR data assigned on the basis of HMQC and HMBC.

($CDCl_3$, 150 MHz) δ: 14.1(C-1, C-29), 22.7(C-2, C-28), 25.7(C-8, C-12), 32.0(C-3, C-27), 37.6(C-9, C-11), 72.1(C-10).

n-Hexadecanol (6)

White amorphous powder, mp 74.7–76.8°C (Petrol–$CHCl_3$), IR ν_{max} (KBr) (cm^{-1}): 3300, 2956, 2917, 2849, 1473, 1463, 1061, 730, 719. EIMS *m/z*: 223 [$M^+ - 19$](1.0). ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 0.88 (3H, t, CH_3, H-16), 1.54 (2H, m, H-2), 3.64 (2H, t, H-1).

Phthalic Acid Isodibutyl Ester (7)

Red needles. EI-MS *m/z* 279 [MH^+] (13.7). ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 0.99 (6H, d, $J = 7.2$ Hz, CH_3, H-3′, H-1″), 2.04 (1H, m, $J = 7.2$ Hz, 7.2 Hz, H-2′), 4.09 (2H, d, $J =$ 7.2 Hz, H-1′), 7.53 (1H, m, $J = 6.0$ Hz, 6.0 Hz, H-4, 5), 7.72 (1H, m, $J = 6$ Hz, 6 Hz, H-2, 6). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 19.2(C-3′, 1″), 27.2(C-2′), 71.8(C-1′), 128.8(C-4, 5), 130.9(C-2, 6), 132.4(C-1, 2), 167.7(C═O).

Curcumol (8)

Colourless needles, mp 103.5–104.8°C (Petrol–$CHCl_3$). EI-MS *m/z*: 236 [M^+] (19.7). ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 0.87 (3H, d, $J = 6.6$ Hz, CH–CH_3, H-15), 1.00, 1.01 (3H × 2, each d, 6.6 Hz, $CH(CH_3)_2$, H-13, 14), 2.52, 2.57 (1H × 2, each d, $J = 15.0$ Hz, H-9), 4.88 (2H, s, H-1). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 12.3(C-15), 21.4(C-14), 23.0(C-13), 28.2(C-3), 28.7(C-12), 30.9(C-10), 34.7 (C-6), 8.8(C-3), 39.4(C-8), 54.5(C-11), 56.5(C-5), 88.3(C-7), 104.5(C-4), 112.8(C-1), 144.7(C-2).

β-Sitosterol (9)

Colourless needles, mp 134–136°C (MeOH). TLC and IR spectrum were identical with that of a standard compound.

Daucosterol (10)

White amorphous powder, mp 304–309°C ($CHCl_3$–MeOH); TLC and IR spectrum were identical with that of a standard compound.

Acknowledgements

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References

[1] Asakawa, Y. (1995), *Chemical Constituents of the Bryophytes in Progress in the Chemistry of Organic Natural Products* (Springer-Verlag, Weinheim, New York).
[2] Ageta, H., Shiojima, K., Arai, Y., Kasama, T. and Kajii, K. (1975), *Tetrahedron Lett.* **16**(38), 3297–3298.
[3] Shiojima, K., Arai, Y. and Ageta, H. (1990), *Phytochemistry* **29**(4), 1079–1082.
[4] Kamaya, R., Tanaka, Y., Hiyama, R. and Ageta, H. (1990), *Chem. Pharm. Bull.* **38**(8), 2130–2132.

[5] Dass, A., Joshi, T. and Shukla, S. (1984), *Phytochemistry* **23**(11), 2689–2691.
[6] Coskun, M., Tanker, N., Sakushima, A., Kitagawa, S. and Nishibe, S. (1984), *Phytochemistry* **23**(7), 1485–1487.
[7] Sadtler Research Laboratories, Division of Bio-rad Laboratories (1994), *Sadtler Standard Carbon-13 NMR Spectra* (Sadtler Research Laboratories, Philadelphia, USA), N 37701.
[8] *The Mass Spectrometry Data Centre. Eight Peak Index of Mass Spectra* (The Royal Society of Chemistry, Cambridge), 3rd Edn., Vol. 1, Part 2, P809
[9] Luo, S.D. and Wu, S.B. (1982), *Acta. Pharm. Sin.* **17**(9), 699–701.
[10] Chang, X.R., Wang, H.X., Zhou, G.Z. and Ma, G.E. (1982), *Chin. J. Pharm. Anal.* **2**(5), 273–277.
[11] Sadtler Research Laboratories, Inc. (1977), *Nuclear Magnetic Resonance Spectra* (Sadtler Research Laboratories, Philadelphia, USA), N 24001.
[12] Sadtler Research Laboratories (1969), *Sadtler Standard Infrared Grating Spectra* (Sadtler Research Laboratories, Philadelphia, USA) Vol. 16, N 15263.
[13] *The Mass Spectrometry Data Centre. Eight Peak Index of Mass Spectra* (The Royal Society of Chemistry, Cambridge), 3rd Edn., Vol. 1, Part 2, P662
[14] Sadtler Research Laboratories, Inc. (1966), *Nuclear Magnetic Resonance Spectra* (Sadtler Research Laboratories, Philadelphia, USA), N 709.
[15] Sadtler Research Laboratories, Division of Bio-rad Laboratories (1979), *Sadtler Standard Carbon-13 NMR Spectra* (Sadtler Research Laboratories, Philadelphia, USA), N 2883.
[16] Fenner, R.A. (1984), *Appl. Spectrosc.* **38**(1), 84–86.
[17] Hayashi, S., Asakawa, Y., Ishida, T. and Matsuura, T. (1967), *Tetrahedron Lett.* **8**(50), 5061–5063.
[18] *The Mass Spectrometry Data Centre. Eight Peak Index of Mass Spectra* (The Royal Society of Chemistry, Cambridge), 3rd Edn., Vol. 1, Part 2, P790
[19] Inayama, S., Gao, J.F., Harimaya, K., Kawamata, T., Iitaka, Y. and Guo, Y.T. (1984), *Chem. Pharm. Bull.* **32**(9), 3783–3786.
[20] Hu, I.H., Han, X.W. and Yang, Z.Y. (1986), *Chin. J. Magn. Reson.* **3**(8), 241–248.