Two New, 1-Oxygenated *ent*-Kaurane-Type Diterpenes from *Croton kongensis*

by **Wei Chen**[a])[b]), **Xiao-Dong Yang***[a]), **Jing-Feng Zhao**[a]), **Hong-Bin Zhang**[a]), and **Liang Li***[a])

[a]) Key Laboratory of Medicinal Chemistry for Natural Resource (Yunnan University), Ministry of Education, School of Chemical Science and Teleology, Yunnan University, Kunming 650091, P. R. China (phone: +86-871-5033644-8899; fax: +86-871-5035538; e-mail: liliang5758@hotmail.com)

[b]) Institute of Material Medica, Yangtze River Pharmacy Group, No. 1 South Yangtze River Road, Taizhou 225321, P. R. China

One new *ent*-8,9-secokaurane diterpene, kongensin D (**1**), and one new *ent*-kaurane diterpene, kongensin E (**2**), along with one known compound, (7α)-7,18-dihydroxy-*ent*-kaur-16-en-15-one 18-acetate (**3**), were isolated from the aerial parts of *Croton kongensis*. The structures of the new compounds were elucidated by means of HR-MS and in-depth NMR experiments, and by comparison with literature data. Compounds **1** and **2** showed an unusual oxygenation pattern with an OH or AcO group at C(1).

Introduction. – The genus *Croton* (Euphorbiaceae) is distributed throughout the southern part of China. It grows in sandy and wet soils, and on damp areas near river banks [1]. Several species are being used in traditional Chinese medicine to alleviate dysmenorrhea (fruits), as a purgative (seeds), and to treat dyspepsia (bark) and malaria (leaves) [2]. The plant *C. kongensis*, collected in Thailand, has been investigated by *Thongtan et al.* [3]. Interestingly, we found that the same species, when collected in China, contained different constituents, possibly due to geographical variation.

Our previous work [4] on this species has resulted in the isolation of four *ent*-8,9-secokaurane diterpenes, kongensins A–C and rabdoumbrosanin, and one *ent*-kaurane diterpene, (7α,14β)-7,14-dihydroxy-*ent*-kaur-16-en-15-one. In the present paper, we describe the isolation and structure elucidation of two new 1-oxygenated *ent*-kaurane-type diterpenes named kongensins D and E (**1** and **2**). These constituents were isolated, together with the known compound (7α)-7,18-dihydroxy-*ent*-kaur-16-en-15-one 18-acetate (**3**) [5], from Chinese *C. kongensis*.

Results and Discussion. – Kongensin D (**1**) was isolated in the form of colorless needles, and was optically active. The molecular formula of **1** was determined as $C_{20}H_{28}O_4$ by HR-ESI-MS ($[M+Na]^+$ at m/z 355.1877). The UV spectrum of **1** showed an absorption maximum at 246 nm (log $\varepsilon = 3.85$). The IR spectrum indicated the presence of OH (3466 cm^{-1}) and α,β-unsaturated C=O (1687 and 1652 cm^{-1}) functions. From the ^{1}H- and ^{13}C-NMR data (*Table*), their comparison with those of rabdoumbrosanin [6] and 2D-NMR experiments (*Fig. 1*), kongensin D (**1**) was identified as (1β,7α)-1,7-dihydroxy-*ent*-8,9-secokaura-8(14),16-diene-9,15-dione.

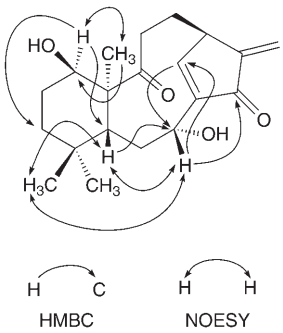

Fig. 1. *Key correlations in the HMBC and NOESY plots of kongensin D* (**1**)

Table. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, $CDCl_3$) *of Compounds* **1** *and* **2**. δ in ppm, J in Hz.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	3.54 (br. *s*)	71.3 (*d*)	4.91 (br. *s*)	73.8 (*d*)
CH_2(2)	1.80–1.90 (*m*)[a]), 1.32–1.45 (*m*)[a])	28.0 (*t*)	2.00–2.04 (*m*), 1.63–1.70 (*m*)[a])	23.6 (*t*)
CH_2(3)	1.68–1.80 (*m*)[a]), 1.16–1.25 (*m*)[a])	33.9 (*t*)	1.47–1.53 (*m*)[a]), 1.20–1.30 (*m*)[a])	35.9 (*t*)
C(4)		34.7 (*s*)		33.9 (*s*)
H–C(5)	1.33 (*d*, J = 4.0)[a])	38.8 (*d*)	1.61 (*dd*, J = 4.3, 1.2)[a])	48.2 (*d*)
CH_2(6)	1.73–1.83 (*m*)[a]), 1.15–1.24 (*m*)[a])	37.1 (*t*)	1.83–1.90 (*m*)[a]), 1.90–1.98 (*m*)[a])	25.3 (*t*)
H–C(7)	4.62 (*dd*, J = 11.6, 4.1)	64.3 (*d*)	5.31 (*dd*, J = 11.9, 4.3)	74.7 (*d*)
C(8)		148.7 (*s*)		61.9 (*s*)
C(9) or H–C(9)		214.6 (*s*)	1.84–1.97 (*m*)[a])	49.4 (*d*)
C(10)		57.5 (*s*)		44.0 (*s*)
CH_2(11)	2.52–2.60 (*m*), 1.90–2.02 (*m*)[a])	36.3 (*t*)	1.53–1.60 (*m*)[a]), 1.32–1.39 (*m*)[a])	17.4 (*t*)
CH_2(12)	2.52–2.60 (*m*), 1.64–1.77 (*m*)[a])	26.1 (*t*)	2.12–2.18 (*m*), 1.81–1.88 (*m*)[a])	33.4 (*t*)
H–C(13)	3.51 (br. *s*)	42.7 (*d*)	3.08 (br. *s*)	45.2 (*d*)
H–C(14)	7.20 (br. *s*)	160.1 (*d*)	6.08 (*s*)	75.9 (*d*)
C(15)		195.5 (*s*)		204.9 (*s*)
C(16)		146.5 (*s*)		146.6 (*s*)
CH_2(17)	6.04 (br. *s*), 5.36 (br. *s*)	116.7 (*t*)	6.17 (br. *s*), 5.41 (br. *s*)	118.6 (*t*)
Me(18)	1.00 (*s*)	33.9 (*q*)	0.98 (*s*)	33.9 (*q*)
Me(19)	0.90 (*s*)	23.6 (*q*)	0.92 (*s*)	22.4 (*q*)
Me(20)	0.92 (*s*)	18.0 (*q*)	1.34 (*s*)	19.5 (*q*)
AcO–C(1)			2.03 (*s*)	22.1 (*q*), 171.1 (*s*)
AcO–C(7)			1.98 (*s*)	22.2 (*q*), 170.4 (*s*)
AcO–C(14)			2.06 (*s*)	22.2 (*q*), 171.6 (*s*)

[a]) Assignment confirmed by COSY and HMQC experiments.

The ^{1}H-NMR spectrum of **1** showed three Me *s* at δ(H) 0.90, 0.92, and 1.00, two oxygenated CH at δ(H) 3.54 (br. *s*) and 4.62 (*dd*, J = 11.6 and 4.1 Hz), an exocyclic CH_2= moiety at δ(H) 5.36, 6.04 (each br. *s*), and a trisubstituted C=C bond at δ(H) 7.20 (br. *s*). In the ^{13}C-NMR spectrum, the signals of two C=O, an exocyclic CH_2=, a trisubstituted C=C, and two oxygenated CH groups were present, together

with signals for three Me, five CH_2, and two CH groups, as well as two quaternary C-atoms. The ^{1}H- and ^{13}C-NMR spectra of **1** were almost identical to those of the known compound rabdoumbrosanin [6], except for an additional oxygenated CH group (δ(H) 3.54 (br. *s*)) in **1**. This data, together with evidence from HR-ESI-MS experiments, indicated that **1** had an additional OH group as compared to rabdoumbrosanin. Several known 8,9-secokauranes have OH (or AcO) groups at C(3) or C(11) [6][7], but the HMBC experiment suggested that this was not the case in **1**. We observed HMBC (*Fig. 1*) from the OCH signal at δ(H) 3.54 (br. *s*, H–C(1)) to the signals at δ(C) 33.9 (C(3)) and 38.8 (C(5)), and from the signal at δ(H) 0.92 (*s*, Me(20)) to that at δ(C) 71.3 (C(1)), which unambiguously placed the OH group at C(1). This assignment was further corroborated by the ^{1}H,^{1}H-COSY cross-peaks H–C(1)/CH_2(2) (δ(H) 1.32–1.45 and 1.80–1.90 (2*m*)). The β-orientation of the OH group at C(1) of **1** was determined by the ^{1}H-NMR coupling pattern of H–C(1), which appeared as a broad *s*, and based on a strong correlation H–C(1)/Me(20) in the NOESY plot (*Fig. 1*). The coupling pattern of H–C(1) is known to depend on the orientation of OH–C(1) [8], H_β–C(1) appearing as a *dd* with a large coupling constant to the 2 H–C(2), whereas H_α–C(1) shows a broad *s* [9]. The coupling constants for H–C(7) were similar in **1** and rabdoumbrosanin. In the case of **1**, the signal appeared at δ(H) 4.62 (*dd*), with *J* values of 11.6 and 4.1 Hz; in the case of rabdoumbrosanin, the resonance appeared at δ(H) 4.68 (*dd*), with *J* values of 12 and 5 Hz [6]. Further, there were strong NOESY correlations H–C(7)/Me(18) (δ(H) 1.00 (*s*)) and H_β–C(5) δ(H) 1.33 (*d*, $J = 4.0$ Hz)). This indicated that H–C(7) was β-orientated.

The optically active kongensin E (**2**) was isolated as a colorless oil. Its molecular formula was determined as $C_{26}H_{36}O_7$ by HR-ESI-MS ($[M + Na]^+$ at *m*/*z* 483.2358). It showed an absorption maximum at 240 (3.48) nm in the UV spectrum. The IR spectrum indicated the presence of ester C=O (1736 cm^{-1}) and C=C (1650 cm^{-1}) functions. On the basis of the ^{1}H- and ^{13}C-NMR data (*Table*), their comparison with those of (3β,7α,14β)-3,7,14-trihydroxy-*ent*-kaur-16-en-15-one 3,7,14-triacetate [10] and 2D-NMR experiments (*Fig. 2*), kongensin E (**2**) was identified as (1β,7α,14β)-1,7,14-trihydroxy-*ent*-kaur-16-en-15-one 1,7,14-triacetate.

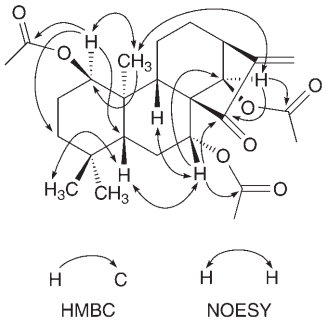

Fig. 2. *Key correlations in the HMBC and NOESY plots of kongensin E* (**2**)

The ^{1}H-NMR spectrum of **2** showed three Me *s* at δ(H) 0.92, 0.98, and 1.34, three Ac groups at δ(H) 1.98, 2.03, and 2.06 (each *s*), three oxygenated CH at δ(H) 4.91 (br. *s*), 5.31 (*dd*, $J = 11.9$ and 4.3 Hz), and 6.08 (br. *s*), and an exocyclic CH_2= moiety at δ(H) 5.41 and 6.17 (each br. *s*). In the ^{13}C-NMR spectrum, the *s* of one C=O, an exocyclic CH_2=, three Ac, and three oxygenated CH groups were present, together with signals of three Me, five CH_2, and three CH groups, as well as three quaternary C-atoms. The ^{1}H- and ^{13}C-NMR spectra of **2** were almost identical to those of the known compound (3β,7α,14β)-3,7,14-trihydroxy-*ent*-kaur-16-en-15-one 3,7,14-triacetate [10], except for the different ^{1}H-NMR coupling pattern of the oxygenated CH(1) (δ(H) 4.91 (br. *s*)) in **1**. We placed two AcO groups at C(7) and C(14) by comparison of the spectroscopic data of **2** with those of (3β,7α,14β)-3,7,14-trihydroxy-*ent*-kaur-16-en-

15-one 3,7,14-triacetate [10]. There were strong HMBC (*Fig. 2*) from the OCH signal at δ(H) 4.91 (br. *s*, H–C(1)) to the signals at δ(C) 35.9 (C(3)), 48.2 (C(5)), and 171.1 (C=O of Ac), and from the signal at δ(H) 1.34 (*s*, Me(20)) to that at δ(C) 73.8 (C(1)). These data unambiguously placed the third AcO group at C(1), which was further confirmed by the ^{1}H,^{1}H-COSY H–C(1)/CH$_2$(2) (δ(H) 1.63–1.70 and 2.00–2.04 (2*m*)).

There were strong correlations H–C(1)/Me(20), H–C(7) (δ(H) 5.31 (*dd*, J = 11.9 and 4.3 Hz))/H$_\beta$–C(5) (δ(H) 1.61 (*dd*, J = 4.3 and 1.2 Hz)) and H$_\beta$–C(9) (δ(H) 1.84–1.97 (*m*)), and H–C(14) (δ(H) 6.08 (*s*))/Me(20) in the NOESY plot (*Fig. 2*). These indicated that H–C(1) and H–C(14) were α-oriented and H–C(7) was β-oriented.

This work was supported by the *Natural Science Foundation of Yunnan Province* (2005B0001Q) and *Yunnan Education Department* (06Z018A), which are gratefully acknowledged.

Experimental Part

General. M.p.: *XT-4* melting-point apparatus: uncorrected. Optical rotations: *Jasco-20C* digital polarimeter. UV Spectra: *Shimadzu UV-210A* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad FTS-135* spectrometer; in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker DRX-500* instrument; δ in ppm rel. to Me$_4$Si, J in Hz. EI-MS: *VG Auto-Spec-3000* mass spectrometer; in *m*/*z*.

Plant Material. The aerial parts of *C. kongensis* were collected in Simao Country, Yunnan Province, P. R. China, in April 2003. The plant was identified by associate researcher *Y.-U. Chen*, Kunming Institute of Botany, Chinese Academy of Sciences, P. R. China. A voucher specimen (No. 03-C001) was deposited at the Department of Phytochemistry, School of Pharmacy, Yunnan University, Yunnan, P. R. China.

Extraction and Isolation. The powdered, air-dried leaves (8 kg) of *C. kongensis* were extracted with 95% EtOH (5 × 25 l) at r.t. The EtOH extract was evaporated to yield a residue, which was suspended in H$_2$O and then partitioned successively with petroleum ether, AcOEt, and BuOH. The AcOEt extract (86 g) was subjected to column chromatography (CC) (SiO$_2$, petroleum ether/acetone 60:1 → 0:1): *Fractions 1–6*. *Fr. 2* was further purified by CC (SiO$_2$, petroleum ether/AcOEt 20:1 → 3:1): **1** (25 mg) and **3** (32 mg). *Fr. 4* was purified by CC (SiO$_2$, CHCl$_3$/AcOEt 20:1 → 1:1) and resubjected to CC (*Sephadex LH-20*, MeOH): **2** (10 mg).

Kongensin D (=(1β,7α)-1,7-Dihydroxy-ent-*8,9-secokaura-8(14),16-diene-9,15-dione = (1*R*,4a*R*,6*R*,10*R*,13a*S*)-1,2,3,4,4a,5,6,9,10,11,12,13a-Dodecahydro-1,6-dihydroxy-4,4,13a-trimethyl-9-methylene-10,7-metheno-7*H*-benzocycloundecene*; **1**): Colorless needles. M.p. 194–196° (CHCl$_3$). $[\alpha]_D^{26} = -85.5$ (c = 0.64, CHCl$_3$). UV (CHCl$_3$): 246 (3.85). IR (KBr): 3466, 3128, 1687, 1652, 1400, 1260, 1037, 934, 808. ^{1}H- and ^{13}C-NMR: *Table.* EI-MS: 332 (1, M^+), 314 (10), 299 (9), 281 (8), 258 (15), 240 (12), 215 (19), 192 (31), 179 (71), 151 (40), 136 (58), 122 (100), 107 (66), 93 (55), 81 (48), 55 (58). HR-ESI-MS: 355.1877 ([M + Na]$^+$, $C_{20}H_{28}O_4Na^+$; calc. 355.1885).

Kongensin E (=(1β,7α,14β)-1,7,14-Trihydroxy-ent-*kaur-16-en-15-one 1,7,14-Triacetate*; **2**): Colorless oil. $[\alpha]_D^{26} = -17.0$ (c = 0.71, CHCl$_3$). UV (CHCl$_3$): 240 (3.48). IR (neat): 3125, 2957, 2866, 1736, 1650, 1398, 1233, 1167, 1024, 879, 803. ^{1}H- and ^{13}C-NMR: *Table.* ESI-MS: 483 ([M + Na]$^+$). HR-ESI-MS: 483.2358 ([M + Na]$^+$, $C_{26}H_{36}O_7Na^+$; calc. 483.2358).

REFERENCES

[1] Institute of Botany, Chinese Academy of Science, 'Flora Reipublicae Sinicae', Science Press, Beijing, 1996, Vol. 44(2), p. 123–127.

[2] Jiangsu New Medical Insititute, 'Dictionary of Chinese Traditional and Herbal Drugs', Shanghai Science and Technology Press, 1977, p. 502–506.

[3] J. Thongtan, P. Kittakoop, N. Puangrungsi, J. Saenboonrueng, Y. Thebtaranonth, *J. Nat. Prod.* **2003**, *66*, 868.

[4] W. Chen, X. D. Yang, J. F. Zhao, J. H. Yang, H. B. Zhang, Z. Y. Li, L. Li, *Helv. Chim. Acta* **2006**, *89*, 537.

[5] P. T. Son, P. M. Giang, W. C. Taylor, *Aust. J. Chem*. **2000**, *53*, 1003.
[6] N. B. Perry, E. J. Burgess, S.-H. Baek, R. T. Weavers, W. Geis, A. B. Mauger, *Phytochemistry* **1999**, *50*, 423.
[7] M. Node, N. Ito, I. Uchida, E. Fujita, K. Fuji, *Chem. Pharm. Bull.* **1985**, *33*, 1029.
[8] Y. Takeda, T. Fujita, A. Ueno, *Phytochemistry* **1983**, *22*, 2531.
[9] A. J. Hou, Q. S. Zhao, M. L. Li, B. Jiang, Z. W. Lin, H. D. Sun, Y. P. Zhou, Y. Lu, Q. T. Zheng, *Phytochemistry* **2001**, *58*, 179.
[10] L. Ding, Z. J. Zhang, G. A. Liu, D. J. Yang, G. C. Guo, H. Wang, K. Sun, *Helv. Chim. Acta* **2005**, *88*, 2502.

Received March 16, 2007