Three New, 1-Oxygenated *ent*-8,9-Secokaurane Diterpenes from *Croton kongensis*

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

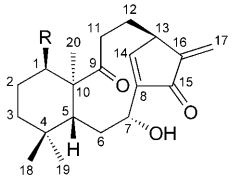
[a]) Key Laboratory of Medicinal Chemistry for Natural Resource (Yunnan University), Ministry of Education, Research School of Pharmacy, Yunnan University, Kunming 650091, Yunnan, P. R. China (phone: +86-871-5033644; fax: +86-871-5035538; e-mail: liliang5758@hotmail.com)

[b]) School of Environmental Science and Engineering, Kunming University of Science and Technology, Kunming 650093, Yunnan, P. R. China

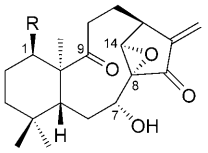
Three new *ent*-8,9-secokaurane diterpenes, kongensins A–C (**1**–**3**), were isolated from the aerial parts of *Croton kongensis*, together with two known compounds, rabdoumbrosanin (**4**) and (7α,14β)-7,14-dihydroxy-*ent*-kaur-16-en-15-one (**5**). The structures of the new compounds were elucidated by HR-MS as well as in-depth 1D- and 2D-NMR analyses. Compounds **1**–**3** showed an unusual oxygenation pattern, with an AcO or OH group at C(1), in combination with a $\Delta^{8(14)}$ unsaturation (**1**) or an 8,14-epoxide function (**2**, **3**).

Introduction. – The genus *Croton* belongs to the Euphorbiaceae family, and some 21 species are distributed throughout the southern part of China. It grows in sandy and wet sails, 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.

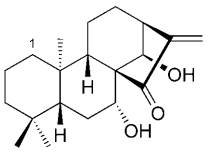
In this paper, we describe the isolation and structure elucidation of three new 1-oxygenated *ent*-8,9-secokaurane diterpenes named kongensins A–C (**1**–**3**). These constituents were isolated , together with the known compounds rabdoumbrosanin (**4**) [4] and (7α,14β)-7,14-dihydroxy-*ent*-kaur-16-en-15-one (**5**) [4], from Chinese *C. kongensis.*



1 R = OAc
4 R = H



2 R = OAc
3 R = OH



5

Results and Discussion. – Compound **1** was isolated in the form of colorless needles (m.p. 160–162°), and was optically active ($[\alpha]_D^{26} = -86.2$ ($c = 0.79$, $CHCl_3$)). The molecular formula of **1** was determined as $C_{22}H_{30}O_5$ by HR-ESI-MS (*m/z* 397.1990 ($[M+Na]^+$)).

The UV spectrum of **1** showed an absorption maximum at 245 nm (log $\varepsilon = 4.14$). The IR spectrum indicated the presence of OH (3420), ester C=O (1735), and α,β-unsaturated C=O (1690, 1641 cm^{-1}) functions. The ^{1}H-NMR spectrum of **1** (*Table 1*) showed three Me *singlet*s at δ(H) 0.96, 0.98, and 1.05, resp., an Ac group at δ(H) 1.98 (*s*), two oxygenated CH at δ(H) 4.72 (br. *s*) and 4.68 (*dd*, $J = 11.6$, 4.5 Hz), an *exo*-methylidene moiety at δ(H) 5.42, 6.13 (2 br. *s*), and a trisubstituted C=C bond at δ(H) 7.25 (*d*, $J = 2.6$ Hz). The ^{13}C-NMR spectrum (*Table 2*) showed signals due to two C=O groups, an *exo*-methylidene, an Ac, a trisubstituted C=C bond, and two oxygenated CH groups, 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 **4**, except for an additional AcO group in **1**. Several known 8,9-secokauranes have AcO groups at C(3) or C(11) [4][5], but NMR experiments suggested that this was not the case for **1**. We observed HMBC correlations from the oxygenated methine signal at δ(H) 4.72 (br. *s*, H–C(1)) to the signals at δ(C) 34.5 (C(3)), 39.4 (C(5)), and 169.8 (C=O of Ac), and from the signal at δ(H) 0.98 (*s*, Me(20)) to that at δ(C) 73.5 (C(1)), which unambiguously placed the AcO group at C(1). This assignment was further corroborated by ^{1}H,^{1}H-COSY correlations between H–C(1) and CH_2(2) [δ(H) 1.77–1.84, 1.56–1.63 (2*m*)].

The β-orientation of the AcO group at C(1) of **1** was determined through the NMR coupling pattern of H–C(1), which appeared as a broad *singlet*, and based on a strong correlation between H–C(1) and Me(20) in the NOESY spectrum (*Figure*). The coupling pattern of H–C(1) is known to depend on the

Table 1. *^{1}H-NMR Data of Compounds* **1**–**3**. At 500 MHz in $CDCl_3$; δ in ppm, *J* in Hz.

Position	**1**	**2**	**3**
1	4.72 (br. *s*)	4.81 (br. *s*)	3.66 (br. *s*)
2[a])	1.77–1.84 (*m*)	1.84–1.90 (*m*)	1.84–1.89 (*m*)
	1.56–1.63 (*m*)	1.66–1.71 (*m*)	1.67–1.76 (*m*)
3[a])	1.47–1.54 (*m*)	1.63–1.66 (*m*)	1.47–1.54 (*m*)
	1.20–1.26 (*m*)	1.26–1.33 (*m*)	1.23–1.29 (*m*)
4			
5[a])	1.33 (*dd*, $J = 4.5$, 1.3)	1.62 (*dd*, $J = 5.0$, 1.5)	1.64 (*dd*, $J = 7.0$, 1.0)
6[a])	1.85–1.91 (*m*)	1.90–1.96 (*m*)	1.90–1.96 (*m*)
	1.25–1.30 (*m*)	1.15–1.20 (*m*)	1.06–1.12 (*m*)
7	4.68 (*dd*, $J = 11.6$, 4.5)	4.62 (*dd*, $J = 11.5$, 4.1)	4.64 (*dd*, $J = 11.5$, 3.9)
11[a])	2.44–2.50 (*m*)	2.80–2.91 (*m*)	2.89–3.00 (*m*)
	1.63–1.69 (*m*)	1.75–1.83 (*m*)	2.20 (*dd*, $J = 19$, 7.8)
12[a])	2.58 (*td*, $J = 13.0$, 3.4)	2.67 (*td*, $J = 13.7$, 3.5)	2.69 (*td*, $J = 13.9$, 3.4)
	1.67–1.74 (*m*)	1.66–1.71 (*m*)	1.98–2.09 (*m*)
13	3.56 (br. *d*, $J = 2.4$)	3.22 (br. *s*)	3.21 (br. *s*)
14	7.25 (*d*, $J = 2.6$)	3.66 (*s*)	3.68 (*s*)
17	6.13 (br. *s*)	6.28 (br. *s*)	6.28 (br. *s*)
	5.42 (br. *s*)	5.50 (br. *s*)	5.52 (*d*, $J = 1.2$)
18	1.05 (*s*)	1.13 (*s*)	1.14 (*s*)
19	0.96 (*s*)	1.00 (*s*)	1.00 (*s*)
20	0.98 (*s*)	1.05 (*s*)	1.00 (*s*)
Ac	1.98 (*s*)	1.99 (*s*)	–

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

Table 2. *^{13}C-NMR Data of Compounds* **1**–**3**. At 125 MHz in $CDCl_3$; δ in ppm.

Position	**1**	**2**	**3**
1	73.5 (*d*)	73.3 (*d*)	71.2 (*d*)
2	24.1 (*t*)	24.1 (*t*)	25.6 (*t*)
3	34.5 (*t*)	34.7 (*t*)	33.7 (*t*)
4	34.6 (*s*)	34.9 (*s*)	35.0 (*s*)
5	39.4 (*d*)	38.0 (*d*)	37.6 (*d*)
6	36.4 (*t*)	32.8 (*t*)	36.6 (*t*)
7	64.1 (*d*)	61.9 (*d*)	62.0 (*d*)
8	148.7 (*s*)	64.7 (*s*)	64.9 (*s*)
9	211.9 (*s*)	211.2 (*s*)	213.9 (*s*)
10	56.4 (*s*)	56.2 (*s*)	57.3 (*s*)
11	34.6 (*t*)	35.1 (*t*)	34.1 (*t*)
12	26.1 (*t*)	25.4 (*t*)	28.2 (*t*)
13	42.4 (*d*)	39.9 (*d*)	40.2 (*d*)
14	160.2 (*d*)	60.6 (*d*)	60.9 (*d*)
15	195.2 (*s*)	197.1 (*s*)	197.2 (*s*)
16	146.5 (*s*)	145.7 (*s*)	145.5 (*s*)
17	116.4 (*t*)	122.3 (*t*)	122.7 (*t*)
18	33.7 (*q*)	33.6 (*q*)	33.8 (*q*)
19	23.7 (*q*)	23.3 (*q*)	23.1 (*q*)
20	17.7 (*q*)	17.5 (*q*)	17.7 (*q*)
Me*C*O	169.8 (*s*)	169.6 (*s*)	–
*Me*CO	21.8 (*q*)	21.8 (*q*)	–

orientation of the 1-OH (or AcO) group [6], and H_β–C(1) appeared as a double *doublet*, with a large coupling constant to the CH_2(2) H-atoms, whereas H_α–C(1) showed a broad *singlet* [7]. The coupling constants for H–C(7) were similar in compounds **1** and **4**. In the case of **1**, the signal appeared at δ(H) 4.68 (*dd*), with *J* values of 11.6 and 4.5 Hz; in the case of **7**, the resonance appeared at δ(H) 4.65 (*dd*), with *J* values of 11.9 and 4.5 Hz. Further, there were strong NOESY correlations between H–C(7) and both Me(18) at δ(H) 1.05 (*s*) and H_β–C(5) at 1.33 (*dd*, $J = 4.5$, 1.3 Hz). This indicated that H–C(7) was β-oriented. Thus, the 7-OH group had to be α-oriented.

From the above data, and by complete NMR assignment of all H- and C-atoms through 2D-NMR experiments, compound **1** was identified as (1β,7α)-7-hydroxy-9,15-dioxo-*ent*-8,9-secokaura-8(14),16-dien-1-yl acetate, and named *kongensin A*.

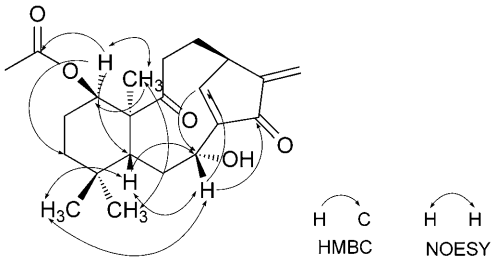


Figure. *Key correlations in the HMBC and NOESY spectra of kongensin A* (**1**)

The optically active compound **2** was isolated in the form of colorless needles (m.p. 224–226°; $[\alpha]_D^{26} = -67.6$ ($c = 1.08$, $CHCl_3$)). Its molecular formula was determined as $C_{22}H_{30}O_6$ by HR-ESI-MS (*m/z* 413.1929 ($[M+Na]^+$)). Comparison of the spectroscopic data of **2** with those of **1** indicated that both compounds had the same basic skeleton.

In the ^{1}H-NMR spectra, there was a remarkable NMR upfield shift for H–C(14) from δ(H) 7.25 for **1** to 3.66 for **2** (*Table 1*). In the ^{13}C-NMR (DEPT) spectra, the signals at δ(C) 148.7 (C(8)) and 160.2 (C(14)) for **1** were replaced by new signals at 60.6 (*d*) and 64.7 (*s*) for **2** (*Table 2*). These data, together with evidence from HR-ESI-MS experiments, indicated that **2** was the 8,14-epoxide of **1**. The relative configuration of **2** was assumed to be the same as for **1**, based on the similar (negative) rotations, and corroborated by a NOESY experiment. The NOESY correlations between H–C(14) at δ(H) 3.66 (*s*) and both H–C(18) at 1.13 (*s*) and H–C(7) at 4.62 (*dd*, $J = 11.5, 4.1$ Hz) revealed that the epoxide function was α-oriented.

On the basis of the above spectroscopic data, compound **2** was, thus, identified as (1β,7α)-8,14-epoxy-7-hydroxy-9,15-dioxo-*ent*-8,9-secokaur-16-en-1-yl acetate, and named *kongensin B*.

Compound **3** was also optically active, and isolated as a colorless oil ($[\alpha]_D^{26} = -35.5$ ($c = 0.52$, $CHCl_3$)). Its molecular formula was determined as $C_{20}H_{28}O_5$ by HR-EI-MS (*m/z* 371.1884 ($[M+Na]^+$)). The ^{1}H-NMR spectrum of **3** (*Table 1*) was very similar to that of **2**, expect for *1*) a remarkable NMR upfield shift for H–C(1) (from δ(H) 4.81 in **2** to 3.68 in **3**), and *2*) the absence of the Me signal of the 1-AcO group.

In the ^{13}C-NMR (DEPT) spectrum of **3**, the signals at δ(C) 169.6 (*s*) and 21.8 (*q*) for **2** were absent. These data, together with evidence from HR-EI-MS, indicated that **3** was a hydrolysis product of **2**. The H- and C-atoms of **3** could be completely assigned by analysis of its 2D-NMR spectra. The compound also exhibited a negative $[\alpha]_D$ value, and NOESY correlations similar to those for **2**, which indicated identical relative configurations. Moreover, the observed coupling patterns for **3** with respect to H–C(1) at δ(H) 3.68 (br. *s*) and H–C(7) at δ(H) 4.64 (*dd*, $J = 11.5$, 3.9 Hz) were very similar to those of **2**, which additionally supported the proposed *ent*-kaurane configuration.

On the basis of the above data and considerations, compound **3** was, thus, identified as (1β,7α)-8,14-epoxy-1,7-dihydroxy-*ent*-8,9-secokaur-16-ene-9,15-dione, and named *kongensin C*.

Experimental Part

General. M.p.: *XT-4* melting-point apparatus, and are uncorrected. UV Spectra: *Shimadzu UV-210A* spectrometer; λ_{max} (log ε) in nm. Optical rotations: *Jasco 20C* digital polarimeter. 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_4Si, *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 Prof. *Yu 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, and the resulting residue was suspended in H_2O, and then extracted with petroleum ether (PE), AcOEt, and BuOH, in this order. The AcOEt extract (86 g) was subjected to column chromatography (CC) (SiO_2; PE/acetone 60 : 1 → 0 : 1): six frac-

tions (*Fr. 1–6*). *Fr. 3* was further purified by CC (1. SiO_2, PE/AcOEt 20:1 → 1:1; 2. *Sephadex LH-20*, MeOH) to yield **1** (65 mg), **2** (110 mg), **4** (78 mg), and **5** (165 mg). *Fr. 4* was purified by CC (1. SiO_2, $CHCl_3$/AcOEt 20:1 → 2:1; 2. *Sephadex LH-20*, MeOH) to yield **3** (8 mg).

Kongensin A (= *(1β,7α)-7-Hydroxy-9,15-dioxo*-ent-*8,9-secokaura-8(14),16-dien-1-yl Acetate*; **1**). Colorless needles. M.p. 160–162° ($CHCl_3$). UV ($CHCl_3$): 245 (4.14). $[\alpha]_D^{26} = -86.2$ ($c = 0.79$, $CHCl_3$). IR (KBr): 3420, 2962, 2927, 1735, 1690, 1641, 1616, 1260, 1243, 1038, 802. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. ESI-MS: 397 ($[M+Na]^+$). HR-ESI-MS: 397.1990 ($[M+Na]^+$, $C_{22}H_{30}NaO_5^+$; calc. 397.1990).

Kongensin B (= *(1β,7α)-8,14-Epoxy-7-hydroxy-9,15-dioxo*-ent-*8,9-secokaur-16-en-1-yl Acetate*; **2**). Colorless needles. M.p. 224–226° ($CHCl_3$). UV ($CHCl_3$): 241 (3.96). $[\alpha]_D^{26} = -67.6$ ($c = 1.08$, $CHCl_3$). IR (KBr): 3450, 2964, 2939, 1725, 1691, 1640, 1369, 1252, 1033, 942, 807. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. ESI-MS: 413 ($[M+Na]^+$). HR-ESI-MS: 413.1929 ($[M+Na]^+$, $C_{22}H_{30}NaO_6^+$; calc. 413.1940).

Kongensin C (= *(1β,7α)-8,14-Epoxy-1,7-dihydroxy*-ent-*8,9-secokaur-16-ene-9,15-dione*; **3**). Colorless oil. UV ($CHCl_3$): 241 (3.78). $[\alpha]_D^{26} = -35.5$ ($c = 0.52$, $CHCl_3$). IR (neat): 3453, 2964, 2926, 1728, 1685, 1642, 1412, 1262, 1098, 1021, 800. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. EI-MS: 348 (1), 330 (1), 315 (5), 312 (4), 297 (7), 249 (13), 211 (20), 195 (17), 163 (23), 149 (25), 137 (53), 121 (100), 107 (78), 95 (70), 81 (76), 69 (50), 55 (73). HR-EI-MS: 371.1884 ($[M+Na]^+$, $C_{20}H_{28}NaO_5^+$; calc. 371.1834).

This work was partially supported by the Center for Advanced Studies of Medicinal and Organic Chemistry of Yunnan University.

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 & Technology Press, Shanghai, 1977, p. 502–506.
[3] J. Thongtan, P. Kittakoop, N. Puangrungsi, J. Saenboonrueng, Y. Thebtaranonth, *J. Nat. Prod.* **2003**, *66*, 868.
[4] N. B. Perry, E. J. Burgess, S. H. Baek, R. T. Weawers, W. Geis, A. B. Mauger, *Phytochemistry* **1999**, *50*, 423.
[5] M. Node, N. Ito, I. Uchida, E. Fujita, K. Fuji, *Chem. Pharm. Bull.* **1985**, *33*, 1029.
[6] Y. Takeda, T. Fujita, A. Ueno, *Phytochemistry* **1983**, *22*, 2531.
[7] 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.

Received December 7, 2005