Clerodane Diterpenoids from *Kinostemon alborubrum*

by **Deng Ye**[a]), **Peng Shu-Lin***[a]), **Zhang Qiang**[b]), **Liao Xun**[a]), and **Ding Li-Sheng**[a])

[a]) Chengdu Institute of Biology, The Chinese Academy of Sciences, Chengdu 610041, P.R. China
[b]) School of Pharmacy, West China University of Medical Sciences, Chengdu 610041, P.R. China
(Fax: +86-28-522-3843; e-mail: peng shulin@163.net)

Three new clerodane diterpenoids, kinalborin A (**1**), B (**2**), and C(**3**), together with six known diterpenoids, teuperin A (**4**), montanin E (**5**), teulamifin B (**6**), teuflin (**7**), montanin D (**8**), and teupernin B (**9**), have been isolated from the whole plant of *Kinostemon alborubrum* (HEMSL.) C. Y. WU et S. CHOW, a Chinese endemic herb. Their structures were established by spectral methods, and those of kinalborins A (**1**) and C (**3**) were confirmed by X-ray diffraction.

Introduction. – *Kinostemon alborubrum* (HEMSL.) C. Y. WU et S. CHOW (Labiatae), an endemic herb from the Southwest of China, is used for the treatment of rheumatism in China [1]. To the best of our knowledge, no chemical studies have been previously reported on this plant. During the search for its bioactive components, three new diterpenoids, called kinalborin A (**1**), B (**2**), and C (**3**) and the six known diterpenoids **4**–**9** (see *Exper. Part*) were obtained from the EtOH extract of the whole plant. We report herein the isolation and structure elucidation of these compounds.

Results and Discussion. – Kinalborin A (**1**) was isolated as colorless orthorhombic crystals. Its molecular formula was determined as $C_{20}H_{20}O_7$ by HR-EI-MS (*m*/*z* 372.1203, calc. 372.1209). The IR absorption indicated the presence of an OH group (3520 cm^{-1}), a furan ring (3140, 1600, 1505, 875 cm^{-1}), and an α,β-unsaturated γ-lactone (1750, 1670 cm^{-1}). The structure of **1**, (12*S*)-15,16-epoxy-8β-12,19-trihydroxy-6-oxoneoclerodane-3,13(16),14-triene-18,20-dioic acid 18,19 : 20,12-dilactone[1]) (*Fig. 1*) was established by ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*), by HMQC, HMBC, and ^{1}H,^{1}H COSY experiments, by comparison with known compounds and finally by X-ray diffraction (*Fig. 2*).

The presence of a β-substituted furan ring in **1** was supported by the ^{1}H-NMR (two furan H–C(α) at δ 7.75 and 7.94, and one furan H–C(β) at δ 6.70) and ^{13}C-NMR data (δ 108.8, 125.5, 141.0, 144.9). The *ABX* system in the ^{1}H-NMR at δ 3.11 (*dd*, *J* = 14.8, 9.6 Hz, H–C(11a)), 2.76 (*dd*, *J* = 14.8, 7.2 Hz, H–C(11b)), and 5.75 (*dd*, *J* = 9.6, 7.2 Hz, H–C(12)) and the ^{13}C-NMR signals at δ 177.7 (C(20)) and 72.3 (C(12)) were similar to those of *ent*-clerodane diterpenoids carrying a γ-lactone moiety at C(20), C(12). Comparison of the ^{1}H-NMR and ^{13}C-NMR data of **1** with those of teupernin A (**4**) [2] (*Fig. 3*) suggested that **1** has a similar structure, which was confirmed by HMBC experiments showing correlations of H–C(12) with C(11), C(13), C(14), and C(15). The relatively high-field shift of the two olefinic C-atoms at δ 133.1 (C(4)) and 136.6(C(3)) and C(18) at δ 168.0 were similar to those of vittagraciliolide, indicating that the second γ-lactone moiety in **1** was α,β-unsaturated [3]. In the HMBC spectrum, the olefinic H–C(3) showed correlation with C(5) and C(18). The correlations of H–C(7) (δ(H) 2.78 and 4.10) with C(6), C(8), and C(17) were also observed.

[1]) Neoclerodane is (1*S*,2*R*,3′*S*,4a*R*,5*S*,8a*R*)-1,2,3,4,4a,5,6,7,8,8a-decahydro-1,2,4a,5-tetramethyl-1-(3-methylpentyl)naphthalene, for systematic names, see *Exper. Part.*

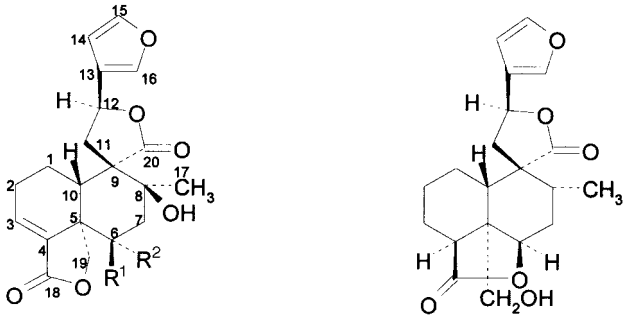

Fig. 1. *The structures of kinalborins A, B, and C*. Trivial numbering.

Table 1. *^{1}H-NMR Data for* **1**–**3**. δ in ppm, J in Hz. Trivial numbering

	1[a])	**2**[b])	**3**[b])
CH_2(1)	1.60, 2.10	1.44, 2.05	1.66, 2.11
CH_2(2)	2.00, 2.30	2.55	1.47
H–C(3)	6.88 (*dd*, *J* = 7.2, 6.8)	6.94 (*d*, *J* = 6.4)	1.83
H–C(4)	–	–	1.72
H–C(6)	–	4.24 (br. *s*)	4.61 (br. *s*)
CH_2(7)	2.78, 4.10	2.05, 2.63	2.09, 2.27
H–C(8)	–	–	1.94
H–C(10)	3.36 (*d*, *J* = 12)	3.07 (*d*, *J* = 12.8)	3.13 (*dd*, *J* = 6.8, 2.4)
CH_2(11)	2.76 (*dd*, *J* = 14.8, 7.2), 3.11 (*dd*, *J* = 14.8, 9.6)	2.46, 2.83	2.35, 2.45
H–C(12)	5.75 (*dd*, *J* = 9.6, 7.2)	5.37 (*dd*, *J* = 9.2, 8.0)	5.36 (*dd*, *J* = 9.6, 8.0)
H–C(14)	6.70 (*dd*, *J* = 2.0, 1.2)	6.42 (br. *s*)	6.38 (br. *s*)
H–C(15)	7.94 (*t*, *J* = 0.8)	7.46 (*d*, *J* = 1.6)	7.46 (br. *s*)
H–C(16)	7.75 (*t*, *J* = 1.6)	7.50 (br. *s*)	7.44 (br. *s*)
Me(17)	1.60	1.25	1.05 (*d*, *J* = 6.8)
CH_2(19)	4.42, 5.33	4.02, 4.56	3.68, 4.35

[a]) In C_5D_5N. [b]) In $CDCl_3$.

Kinalborin B (**2**) was isolated as colorless crystals. Its HR-EI-MS showed a molecular ion at *m*/*z* 374.1375 (calc. 374.1366 for $C_{20}H_{22}O_7$). A comparison of its ^{1}H- and ^{13}C-NMR data with those of **1** suggested that the structure of **2** was similar to **1**, except for the C(6) of **2** being an alcohol instead of a ketone group. HMQC, HMBC, and ^{1}H-^{1}H COSY experiments confirmed this conclusion. The β-configuration of OH–C(6) was determined by a NOESY spectrum in which the correlations H–C(6)/H–C(7a), H–C(7b), and H–C(19a) (*Fig. 4*) were found. Therefore, the structure of **2** was established as (12*S*)-15,16-epoxy-6β,8β,12,19-tetrahydroxyneoclerodane-3,13(16),14-triene-18,20-dioic acid 18,19 : 20,12-dilactone[1]) (*Fig. 1*).

Kinalborin C (**3**) was isolated as lamellar crystals. The molecular formula was determined as $C_{20}H_{24}O_6$ by HR-EI-MS (*m*/*z* 360.1569, calc. 360.1573). Its IR spectrum showed characteristic absorptions for an OH group (3440 cm^{-1}), a furan ring (3140,

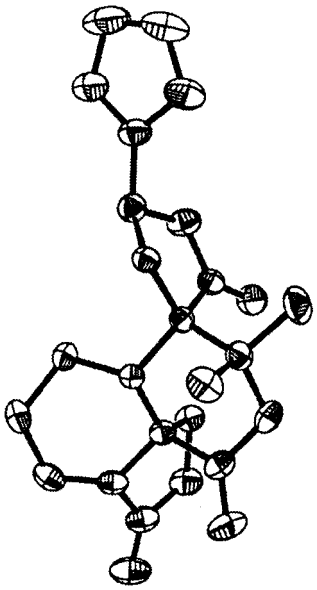

Fig. 2. *X-Ray structure of* **1**

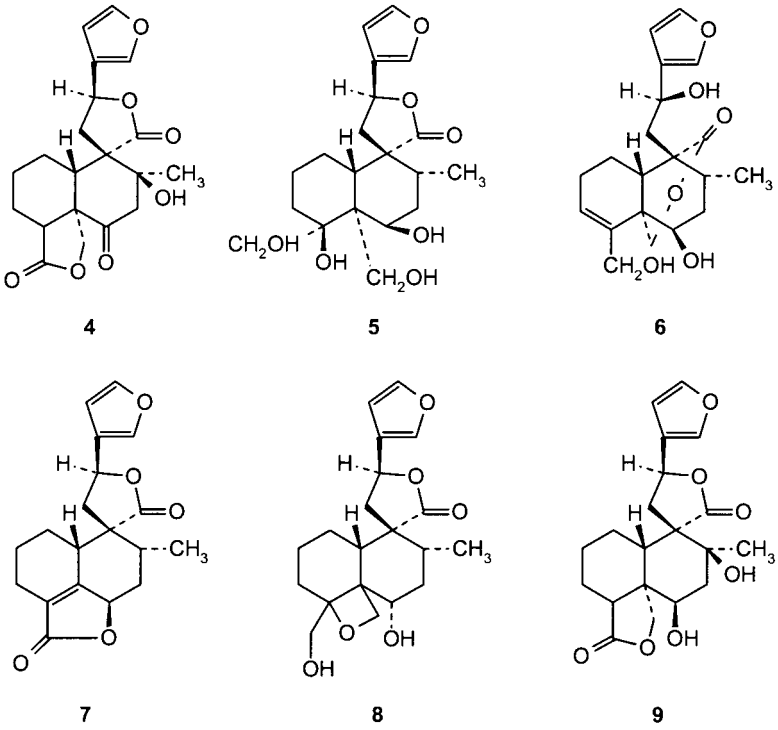

Fig. 3. *Structures of the known diterpenoids teupernin A* (**4**), *montanin E* (**5**), *teulamifin B* (**6**), *teuflin* (**7**), *montanin D* (**8**), *and teupernin B* (**9**)

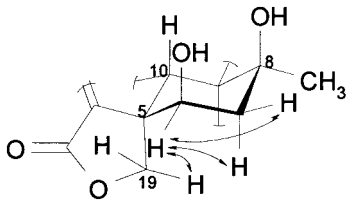

Fig. 4. *Important NOESY correlations of* **2**

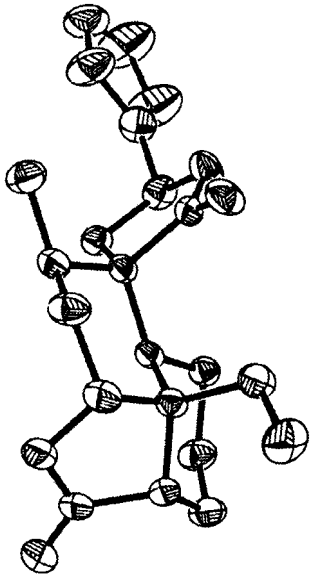

Fig. 5. *X-Ray structure of* **3**

1600, 1510, 870 cm^{-1}), and γ-lactone moiety (1760 cm^{-1}). Its ^{1}H-NMR and ^{13}C-NMR spectra showed the presence of a β-substituted furan ring and a γ-lactone at C(20), C(12), just as in **1**. The structure of **3** was found to be similar to that of teuscorodonin [4], except that C(3) of **3** was saturated. This conclusion was supported by 2D-NMR. Thus, the structure of **3** was elucidated as (12*S*)-15,16-epoxy-6β,12,19-trihydroxyneoclerodane-13(16),14-diene-18,20-dioic acid 18,6:20,12-dilactone[1]) (*Fig. 1*), which was confirmed by X-ray-diffraction analysis (*Fig. 5*).

Experimental Part

General. Column chromatography (CC): silica gel (170–200 mesh; *Qingdao Haiyang Chemical Group Co.*), *Lobar LiChroprep RP-8* (40–68 μm; *Merck*), *Lobar LiChroprep Si-60* (40–68 μm; *Merck*), and *MCI-Gel CHP-20P* (75–150 μm; *Mitsubishi Chemical Corporation*). M.p.: *XRC-1* apparatus; uncorrected. Optical rotations: *PE-241* polarimeter. IR Spectra: *Nicolet MX-1* spectrometer. NMR Spectra: *Varian-Unity Inova-400* spectrometer; $SiMe_4$ as internal standard. HR-EI-MS: *VG-AutoSpec-3000* spectrometer. ESI-MS: *Finnigan LCQ^{DECA}* spectrometer.

Plant Material. The whole plants of *K. alborubrum* were collected from Nanchuan, Chongqing, China, in July 1999 and identified by Prof. *Liu Zheng-Yu*. A voucher specimen (1999-50) was deposited in the Herbarium of Chengdu Institute of Biology, The Chinese Academy of Sciences.

Table 2. *^{13}C-NMR* (DEPT) *Data for* **1**–**9**. δ in ppm. Trivial numbering

	1[a])	**2**[b])	**3**[b])	**4**[a])	**5**[a])	**6**[c])	**7**[b])	**8**[b])	**9**[c])
C(1)	21.2	21.0	19.5	24.3	22.0	20.1	23.3	16.5	24.3
C(2)	27.2	27.7	21.3	24.1	23.1	25.0	22.9	20.9	22.8
C(3)	136.6	139.6	24.0	24.2	31.1	132.5	18.3	29.6	24.6
C(4)	133.1	135.4	46.3	42.1	78.6	137.9	123.4	88.0	43.9
C(5)	59.2	51.1	47.1	56.4	47.2	41.3	165.9	47.1	48.5
C(6)	206.2	69.7	77.6	208.6	67.9	69.8	76.1	69.3	69.5
C(7)	49.7	37.0	29.8	48.7	35.4	36.1	31.4	32.7	36.5
C(8)	76.6	76.7	34.3	76.2	32.7	30.5	35.5	31.7	78.1
C(9)	55.8	56.1	50.5	56.0	52.8	49.8	50.6	51.8	57.1
C(10)	45.4	38.8	46.7	44.7	43.7	36.4	42.7	37.6	38.7
C(11)	38.5	38.5	41.7	38.7	46.2	35.2	42.4	41.3	39.2
C(12)	72.3	72.1	72.1	72.5	72.1	62.2	71.2	71.9	72.8
C(13)	125.5	124.6	124.6	125.9	126.7	130.3	123.9	124.7	125.2
C(14)	108.8	108.2	107.9	108.9	109.0	108.5	107.5	107.7	108.4
C(15)	144.9	144.3	144.3	145.0	144.7	143.1	143.9	143.7	144.5
C(16)	141.0	139.9	139.8	141.1	140.4	138.3	139.5	139.2	140.1
C(17)	25.4	25.0	16.2	26.1	17.0	16.1	17.1	16.2	25.4
C(18)	168.0	169.0	178.4	178.0	68.0	63.6	177.6	66.0	178.7
C(19)	71.4	72.0	58.4	69.4	60.1	74.6	–	71.4	70.7
C(20)	177.7	177.8	178.0	177.6	178.9	173.7	173.3	177.6	180.0

[a]) In C_5D_5N. [b]) In $CDCl_3$. [c]) In $CDCl_3/CD_3OD$.

Extraction and Isolation. The air-dried and powdered whole plant of *K. alborubrum* (4.5 kg) was extracted with EtOH at r.t. (3×10 days). After filtration, the solvent was evaporated to give 455 g of extract. The extract (455 g) was suspended in H_2O (1000 ml) and extracted with petroleum ether (3×500 ml), AcOEt (3×500 ml), and BuOH saturated with H_2O (3×500 ml). The AcOEt extract (19 g) was fractionated by CC (*MCI-Gel*, 3.2×25 cm column, MeOH/H_2O 1:1 (400 ml), MeOH/H_2O 7.5:2.5 (400 ml), and MeOH (400 ml): *Fr. I* (14 g), *II* (2.7 g), and *III* (0.15 g). *Fr. I* (14 g) was submitted to CC (silica gel (140 g), 3.0×40 cm column, $CHCl_3$/MeOH gradient $30:1 \rightarrow 2:1$): *Fr. I.1* with $CHCl_3$/MeOH 30:1 (600 ml and 25:1 (600 ml), *Fr. I.2* with $CHCl_3$/MeOH 20:1 (600 ml), *Fr. I.3* with $CHCl_3$/MeOH 15:1 (600 ml) and 10:1 (600 ml), and *Fr. I.4* with $CHCl_3$/MeOH 5:1 (600 ml) and 2:1 (600 ml). *Fr. I.1* was submitted to CC (*Lobar LiChroprep RP-8*, MeOH/H_2O 80:20 (400 ml): **1** (26 mg) and **2** (40 mg), after purification by recrystallization from MeOH and $CHCl_3$, respectively. Compound **3** (60 mg) was obtained from *Fr. I.2* by recrystallization from pyridine. *Fr. I.3* was submitted to CC (*Lobar LiChroprep RP-8*, MeOH/H_2O 70:30 (600 ml) and 75:25, 600 ml)): **4** (310 mg), **5** (6 mg), and **6** (30 mg). *Fr. II* (2.7 g) was submitted to CC (silica gel (50 g), 2.0×35 cm column, $CHCl_3$/MeOH 40:1 (600 ml)): *Fr. II.1* and *II.2*. *Fr. II.1* was submitted to CC (silica gel (35 g), 2.0×25 cm column $CHCl_3$/MeOH 45:1 (200 ml)) and recrystallized from $CHCl_3$: **7** (80 mg). *Fr. II.2* was recrystallized from MeOH: **8** (25 mg). Compound **9** (15 mg) was obtained from the BuOH extract (4.5 g) by CC (silica gel (50 g), 2.2×25 cm column, *Lobar LiChroprep Si-60* column).

Kinalborin A (=*(3*S*,5*S*,6′a*S*,8′S,10′a*R*)-5-(Furan-3-yl)-4,5,6′,6′a,8′,9′-hexahydro-8′-hydroxy-8′-methylspiro-[furan-3(2*H*),7′(10′*H*)-[1*H*]naphtho[1,8a-*c*]furan]-2,3′,10′(5′*H*)-trione*; **1**). Colorless crystals (26 mg). M.p. 208–209° (MeOH). $[\alpha]_D^{20} = +38$ ($c = 0.10$, MeOH). IR (KBr): 3630, 3520, 3140, 2920, 1745, 1710, 1665, 1600, 1505, 1195, 1165, 1020, 985, 875. ^{1}H-NMR: *Table 1*. ^{13}C-NMR: *Table 2*. HR-EI-MS: 372.1203 ($C_{20}H_{20}O7^+$; calc. 372.1209).

Kinalborin B (=*(3*S*,5*S*,6′a*S*,8′S,10′*R*,10′a*R*)-5-(Furan-3-yl)-4,5,6′,6′a,9′,10′-hexahydro-8′,10′-dihydroxy-8′-methylspiro[furan-3(2*H*),7′(8′*H*)-[1*H*]naphtho[1,8a-*c*]furan-2,3′(5′*H*)-dione*; **2**). Colorless crystals (40 mg). M.p. 134–137° ($CHCl_3$). $[\alpha]_D^{20} = +31$ ($c = 0.10$, MeOH). IR (KBr): 3425, 2925, 1755, 1635, 1505, 1420, 1190, 1115, 1025, 990, 875. ^{1}H-NMR: *Table 1*. ^{13}C-NMR: *Table 2*. HR-EI-MS 374.1375 ($C_{20}H_{22}O_7^+$; calc. 374.1366.

Kinalborin C (=*2′*S*,3′*R*,5*S*,5′a*S*,7′*R*,8′a*R*,8′b*R*)-5-(Furan-3-yl)-3′,4,4′,5,5′,5′a,7′,8′,8′a,8′b-decahydro-8′b-(hydroxymethyl)-7′-methylspiro[furan-3(2*H*),6′-[6*H*]naphtho[1,8-*bc*]furan]-2,2′(2′a*H*)-dione*, **3**). Colorless

crystals (60 mg). M.p. 170–172° (pyridine). $[\alpha]_D^{20} = +82$ ($c = 0.10$, MeOH). IR (KBr): 3560, 3440, 3140, 2935, 1600, 1510, 1385, 1220, 1155, 870. ^{1}H-NMR: *Table 1*. ^{13}C-NMR: *Table 2*. HR-EI-MS: 360.1569 ($C_{20}H_{24}O_6^+$; calc. 360.1573).

X-Ray Crystal Structures. Crystallographic data for **1** and **3** have been deposited with the *Cambridge Crystallographic Data Centre* as supplementary publication nos. CCDC 162029 and 162030. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033; e-mail: deposit@ccdc.cam.ac.uk).

Teupernin A (**4**) [2]. Colorless crystals (310 mg). M.p. 261–265° ($CHCl_3$). $[\alpha]_D^{20} = +9$ ($c = 0.094$, $CHCl_3$). ^{13}C-NMR: *Table 2*.

Montanin E (**5**). [5]. Colorless crystals (6 mg). M.p. 215–220° (MeOH). $[\alpha]_D^{20} = +22$ ($c = 0.040$, $CHCl_3$). ^{13}C-NMR: *Table 2*.

Teulamifin B (**6**) [6]. Colorless crystals (30 mg). M.p. 201–206° ($CHCl_3$). $[\alpha]_D^{20} = -47$ ($c = 0.062$, $CHCl_3$). ^{13}C-NMR: *Table 2*.

Teuflin (**7**) [4]. Colorless crystals (80 mg). M.p. 211–214° ($CHCl_3$). $[\alpha]_D^{20} = +18$ ($c = 0.136$, $CHCl_3$). ^{13}C-NMR: *Table 2*.

Montanin D (**8**) [3]. Colorless crystals (25 mg). M.p. 211–214° (MeOH). $[\alpha]_D^{20} = -5$ ($c = 0.108$, $CHCl_3$). ^{13}C-NMR: *Table 2*.

Teupernin B (**9**) [2]. Colorless crystals (15 mg). M.p. 234–237° (MeOH). $[\alpha]_D^{20} = 47$ ($c = 0.020$, $CHCl_3$). ^{13}C-NMR: *Table 2*.

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