Two New Sesquiterpenes from the Resin of *Toxicodendron vernicifluum*

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A phytochemical investigation of the resin of *Toxicodendron vernicifluum* resulted in the isolation of two new sesquiterpenoids, toxicodenanes D (**1**), endowed with a new carbon skeleton, and E (**2**), along with six known sesquiterpenoids (*Fig. 1*). Their structures were determined by spectroscopic analysis, including 1D- and 2D-NMR and mass spectrometry.

Introduction. – *Toxicodendron vernicifluum* is a traditional Chinese medicine used for the treatment of gastritis, stomach cancer, and atherosclerosis [1–3]. In pharmacological studies, it was found that it has anti-inflammatory activities [4], anti-oxidant properties [5], and a protective effect against pancreatic β-cells [6]. Recently, we reported that sesquiterpenoids from this species possess beneficial effects concerning diabetic nephropathy [7]. In the course of our continuing efforts concerning chemical constitutes from *T. vernicifluum*, we isolated two new sesquiterpenoids, one of which has a new C_{15}-carbon skeleton. In this article, we describe the isolation and structural identification of the two new secondary metabolites.

Results and Discussion. – Compound **1** was obtained as colorless gum. The molecular formula of **1** was determined by HR-ESI-MS (neg.) at *m*/*z* 237.1850 ($[M-H]^-$, $C_{15}H_{25}O_2^-$; calc. 237.1854), suggesting three degrees of unsaturation. The ^{1}H-NMR spectrum of **1** (*Table*), revealed the presence of four *singlet* Me groups (δ(H) 0.89 (*s*, Me(13)), 0.92 (*s*, Me (15)), 0.97 (*s*, Me(14)), 2.14 (*s*, Me(10))) and of a vinyl moiety (δ(H) 5.72 (*dt*, J = 16.5, 10.5, 1 H), 4.97 (*dd*, J = 16.5, 2.5, 1 H), and 5.05 (*dd*, J = 10.5, 2.5, 1 H)). The ^{13}C-NMR and DEPT spectra of **1** (*Table*) showed 15 C-atom signals, ascribed to four Me, five CH_2, two CH groups (one of the latter O-bearing), and four quaternary C-atoms. Inspection of the ^{1}H- and ^{13}C-NMR spectra of **1** indicated a sesquiterpenoid. In addition, the IR spectrum showed the presence of a OH group (3453 cm^{-1}), a CO group (1713 cm^{-1}), and of a C=C group (1633 cm^{-1}). The C=C bond and the CO group matched with two degrees of unsaturation, the remaining parameters require the presence of one ring.

Analysis of 2D-NMR spectra, including HSQC, HMBC, and ^{1}H,^{1}H-COSY, allowed the establishment of the constitutional formula of **1** (*Fig. 1*). The ^{1}H,^{1}H-COSY spectrum (*Fig. 2*) disclosed the spin systems corresponding to H–C(1)/CH_2(2), H–C(4)/H–C(11)/CH_2(12), and CH_2(6)/CH_2(7)/CH_2(8). In the HMBC spectrum, correlations Me(13,14)/C(2,3,4) indicate that Me(13) and Me(14) are both connected

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

Position	**1**[a])		**2**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)
1	4.06 (*t*, *J* = 6.0)	78.5 (*d*)	1.86 (overlap)	49.9 (*d*)
2	1.91 (*dd*, *J* = 13.0, 6.0), 1.52 (*dd*, *J* = 13.0, 6.0)	48.5 (*t*)	2.14–2.21 (*m*), 1.35 (*m*)	33.3 (*t*)
3		38.7 (*s*)	4.36 (*dd*, *J* = 9.2, 7.2)	73.7 (*d*)
4	2.01 (*d*, *J* = 10.5)	65.1 (*d*)		45.3 (*s*)
5		48.0 (*s*)	1.61 (*dd*, *J* = 13.0, 7.2), 1.38–1.45 (*m*)	26.3 (*t*)
6	1.31 (*dt*, *J* = 13.0, 6.0), 1.23 (*dt*, *J* = 13.0, 6.0)	37.0 (*t*)	1.90–1.94 (*m*), 1.37–1.43 (*m*)	28.5 (*t*)
7	1.53–1.59 (*m*)	18.7 (*t*)	4.44 (br. *s*)	76.1 (*d*)
8	2.39 (*t*-like, *J* = 5.5)	44.5 (*t*)		151.7 (*s*)
9		209.0 (*s*)	2.59 (*s*)	35.4 (*t*)
10	2.14 (*s*)	31.1 (*q*)		92.8 (*s*)
11	5.72 (*dt*, *J* = 16.5, 10.5)	136.0 (*d*)	1.82–1.88 (*m*)	29.8 (*d*)
12	5.05 (*dd*, *J* = 10.5, 2.5), 4.97 (*dd*, *J* = 16.5, 2.5)	117.3 (*t*)	0.86 (*d*, *J* = 6.4)[c])	20.6 (*q*)[d])
13	0.89 (*s*)	25.8 (*q*)	0.96 (*d*, *J* = 6.4)[c])	23.7 (*q*)[d])
14	0.97 (*s*)	29.9 (*q*)	0.75 (*s*)	18.6 (*q*)
15	0.92 (*s*)	20.4 (*q*)	5.02 (*s*), 4.82 (*s*)	104.1 (*t*)

[a]) ^{1}H-NMR at 500 MHz and ^{13}C-NMR at 125 MHz. [b]) ^{1}H-NMR at 400 MHz and ^{13}C-NMR at 100 MHz. [c])[d]) Assignments may be interchanged.

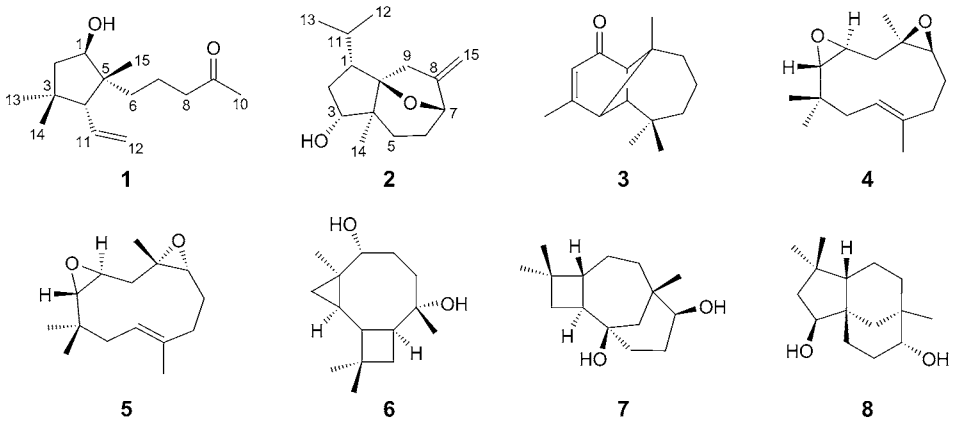

Fig. 1. *Compounds isolated from* Toxicodendron vernicifluum

to C(3). The HMBC from Me(15) to, CH_2(6)/C(5) suggest that Me(15) is linked to C(5). The correlations from Me(10) to C(9) and C(8) established the position of Me(10), the Me group attached to the C(9)=O group. The relative configuration of **1** was established by ROESY correlations (*Fig. 3*) between H–C(4)/Me(15) and H–C(4)/Me(14), indicating that H–C(4), Me(15), and Me(14) are on the same side

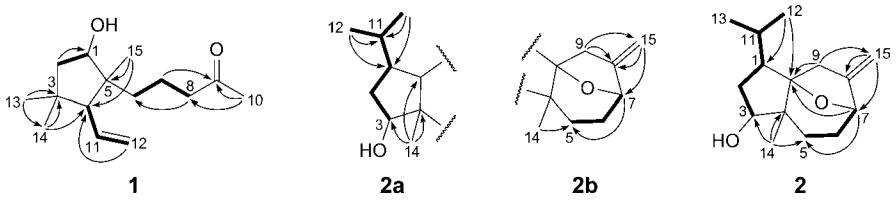

Fig. 2. *Key HMBC* (H → C) *and* $^1H,^1H$*-COSY* (—) *correlations of* **1** *and* **2**

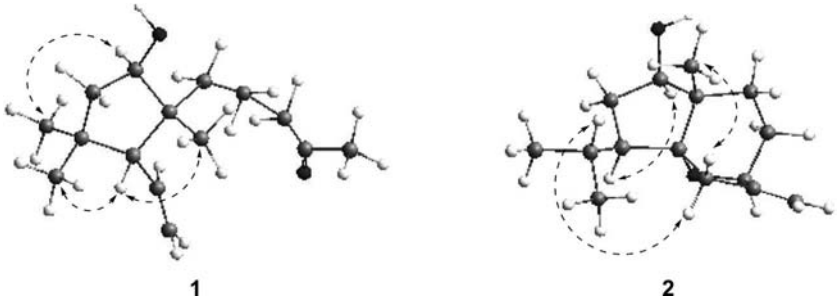

Fig. 3. *ROESY* (H ⇢ H) *correlations of* **1** *and* **2**

of the ring. The ROESY correlation of Me(13)/H–C(1) indicates that Me(13) and H–C(1) are at the same side. Therefore, the structure of **1** was established and tentatively named as toxicodenane D. The absolute configuration of compound **1** remains to be established.

Compound **2** was obtained as colorless gum. The molecular formula of **2** was determined as $C_{15}H_{24}O_2$ based on its HR-EI-MS at *m*/*z* 236.1778 (M^+, calc. for $C_{15}H_{24}O_2^+$, 236.1776), suggesting four degrees of unsaturation. The ^{1}H-NMR spectrum of **2** displayed signals for two terminal olefinic H-atoms (δ(H) 5.02 and δ(H) 4.82 (*s*, H_α–C(15), H_β–C(15))), and for three Me groups. In addition, 15 C-atoms including three Me, five CH_2, four CH (two O-bearing) groups, and three quaternary C-atoms were observed in the ^{13}C-NMR and DEPT spectra (*Table*). The IR spectrum exhibited the presence of a OH group (3420 cm^{-1}) and of a C=C bond (1665 cm^{-1}). Taking this C=C group into account, the remaining three degrees of unsaturation in **2** suggest the presence of three rings.

Careful interpretation of 2D-NMR data resulted in the elucidation of the fragments **2a** and **2b** (*Fig. 2*). The partial structure of **2a** was revealed by ^{1}H,^{1}H-COSY correlations of H–C(1)/CH_2(2)/H–C(3), H–C(11)/Me(12,13) and HMBC cross peaks of Me(12) (δ(H) 0.86 (*d*, *J* = 6.4)), Me(13) (δ(H) 0.96 (*d*, *J* = 6.4))/C(11) (δ(C) 29.8), C(1) (δ(C) 49.9); and Me(14)/C(3,4,5). Further, the observed ^{1}H,^{1}H-COSY correlations of CH_2(6)/CH_2(5)/CH_2(7) and HMBCs of CH_2(15)/C(7,8,9) suggested fragment **2b**. Finally, the HMBC of H–C(7)/C(10) showed that C(7) is linked to C(10) *via* an O-atom, Me(14) correlating with C(5) suggests the connection C(4)–C(5). Accordingly, the constitutional formula of **2** was proposed as shown (*Fig. 1*). The relative configuration of **2** was established by ROESY experiments (*Fig. 3*). The ROESY

correlations of H–C(11)/Me(14), and of Me(14)/CH_2(9), suggested that H–C(11) and Me(14) are both α-oriented. The correlation H–C(1)/H–C(3) indicated that H–C(1) and H–C(3) are β-oriented. According to a molecular model, the presence of the C(7)–C(10) O-bridge makes the skeleton of **2** very rigid, requiring H–C(7) to be α-oriented. Consequently, the structure of **2**, excluding its absolute configuration, was identified as shown, and named as toxicodenane E.

The known compounds were identified as vulgarone B (**3**) [8], (2*E*,6*R**,7*R**,9*S**,10*S**)-humulene 6,7,9,10-diepoxide (**4**) [9] and (2*E*,6*S**,7*S**,9*S**,10*S**)-humulene 6,7,9,10-diepoxide (**5**) [9], tricyclohumuladiol (**6**) [10], caryolane-1,9β-diol (**7**) [11], and clovane-2β,9α-diol (**8**) [11] by comparison of their NMR data with the literature data.

Experimental Part

General. Thin-layer chromatography (TLC): silica gel (SiO_2) *60 F_{254}* on glass plates (*Qingdao Marine Chemical Inc.*, P. R. China) using various solvent systems and spots were visualized by heating the SiO_2 plates sprayed with 10% H_2SO_4 in EtOH. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh; *Qingdao Marine Chemical Inc.*, P. R. China), C_{18} reverse-phase silica gel (40–60 μm; *Daiso Co.*, Japan), *MCI* gel *CHP 20P* (75–150 μm, Tokyo, Japan), and *Sephadex LH-20* (*Amersham Pharmacia*, Sweden). Optical rotations: *Horiba SEPA-300* polarimeter. IR Spectra: *Tensor 27* spectrometer, with KBr pellets; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker AV-400* or *DRX-500* spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI-MS and HR-ESI-MS: *API QSTAR Pulsar 1* spectrometer; in *m*/*z*.

Material. The resin of *Toxicodendron vernicifluum* was purchased from Yunnan Corporation of Materia Medica, Yunnan Province, P. R. China, and identified by Mr. *Hong-Yan Sun*, at Yunnan Corporation of Materia Medica, in July 2008. A voucher specimen (CHYX-0470) was deposited with the State Key Laboratory of Photochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, P. R. China.

Extraction and Isolation. The dried and powdered resin (17 kg) was extracted with 80% EtOH (3 × 15 l) at r.t. The extracts were combined and concentrated *in vacuo* to furnish a dark residue, which was suspended in H_2O, followed by successive extraction with AcOEt (3 × 5 l). The AcOEt extract (220 g) was separated by a SiO_2 column (200–300 mesh, 2.5 kg) eluted with a gradient of $CHCl_3$/MeOH to afford *Frs. 1–10*. *Fr. 2* (3.1 g) was subjected to SiO_2 column (200 g), eluted with a gradient petroleum ether (PE)/Me_2CO (90:10 → 50:50) to yield *Frs. 2.1–2.3*. *Fr. 2.2* (1.1 g) was gel filtrated on *Sephadex LH-20* ($CHCl_3$/MeOH, 1:1) to yield **3** (34.6 mg). *Fr. 4* (17.5 g) was subjected to *MCI* gel *CHP 20P* column with gradient aq. acetone (40–100%) as eluents to produce *Frs. 4.1–4.8*. *Fr. 4.4* (1.5 g) was submitted to gel filtration over *Sephadex LH-20* ($CHCl_3$/MeOH, 1:1) to produce *Fr. 4.4.1* (200 mg). *Fr. 4.4.1* was subjected to SiO_2 column (100 g) eluted with gradient PE/Me_2CO (100:1 → 50:50) to give a mixture of **4** and **5** (40.0 mg) which was further purified by semi-prep. HPLC (MeOH/H_2O, 65:35) to yield **4** (22.0 mg) and **5** (8.0 mg). *Fr. 5* (12.8 g) was purified over in *MCI* gel *CHP 20P* column with gradient aq. acetone (40–100%) to produce *Frs. 5.1–5.5*. *Fr. 5.1* (2.6 g) was fractionated by gel filtrated over *Sephadex LH-20* ($CHCl_3$/MeOH, 1:1) to furnish **1** (13.0 mg). *Fr. 5.3* (300 mg) was purified over a SiO_2 column (100 g) to obtain **2** (12.0 mg). *Fr. 7* (13.0 g) was purified over an *MCI* gel *CHP 20P* column with gradient aq. MeOH (30–100%) to produce *Frs. 7.1–7.4*. *Fr. 7.1* (1.1 g) was further separated over *Sephadex LH-20* (MeOH) to yield **6** (300 mg). *Fr. 7.3* (3.1 g) was purified over C_{18} reverse-phase silica gel with MeOH/H_2O (50%) as the mobile phase to afford **7** (90.0 mg). *Fr. 7.4* (400 mg) was further purified over *Sephadex LH-20* (MeOH) to afford **8** (230 mg).

Toxicodenane D (= rel-*5-[(1*R,*2*R,*5*R*)*-2-Ethenyl-5-hydroxy-1,3,3-trimethylcyclopentyl]pentan-2-one*; **1**). Colorless gum. $[\alpha]_D^{25} = -4.1$ ($c = 0.26$, $CHCl_3$). IR (KBr): 3453, 2955, 2870, 1713, 1633, 1463, 1364, 1173, 1124, 1030, 912. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz): see *Table*. ESI-MS (neg.): 237 ($[M-H]^-$). HR-ESI-MS (neg.): 237.1850 ($[M-H]^-$), $C_{15}H_{25}O_2^-$; calc. 237.1854).

Toxicodenane E (= rel-*(1*R*,3*R*,3a*R*,6*S*,8a*S*)-Octahydro-8a-methyl-5-methylidene-3-(propan-2-yl)-1*H*-3a,6-epoxyazulen-1-ol*; **2**). Colorless gum. $[\alpha]_D^{25} = -7.0$ ($c = 0.14$, $CHCl_3$). IR (KBr) 3420, 3259, 2925, 2852, 1665, 1462, 1377, 1043, 1033, 886. ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz): see *Table*. EI-MS (70 eV): 236 (M^+). HR-EI-MS: 236.1778 (M^+, $C_{15}H_{24}O_2^+$; calc.236.1776).

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