Two New Clerodane Diterpenes from *Dodonaea viscosa*

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Two new clerodane diterpenes **4** and **3**, along with four known constituents, including neocleroda-3,13-diene-15,16-olide-18-oic acid (**1**), stictic acid (**2**), hautriwaic acid (**5**), and 15,16-epoxy-19-hydroxy-1,3,13(16),14-clerodatetraen-18-oic acid (**6**), were isolated from stems and leaves of *Dodonaea viscosa.* Their structures were elucidated by spectroscopic methods, including one- and two-dimensional NMR experiments (COSY, HMBC, HMQC, and ROESY).

Key words: Clerodane Diterpene, *Dodonaea viscosa*

Introduction

Dodonaea viscosa, belonging to the Sapindaceae family, is a staff bushy plant mainly distributed in southern and southwest China [1]. As recorded in the pharmacopoeia of China, it has been used as a Chinese traditional medicine for wound healing and treatments of detumescence and detoxification [2]. Recently, it has been reported that the extract of *Dodonaea viscose* has anti-inflammatory, antimicrobial and antifungal and smooth muscle relaxant activities [3 – 6]. Previous studies revealed that this plant mainly contain flavones and clerodane diterpenes [7, 8]. As part of our continuing research on novel biologically active compounds from Chinese traditional medicine, two new clerodane diterpenes and four known compounds were isolated from the leaves and stems of this plant (Fig. 1). In this paper, we describe the isolation and structure elucidation of the new clerodane diterpenes **3** and **4**.

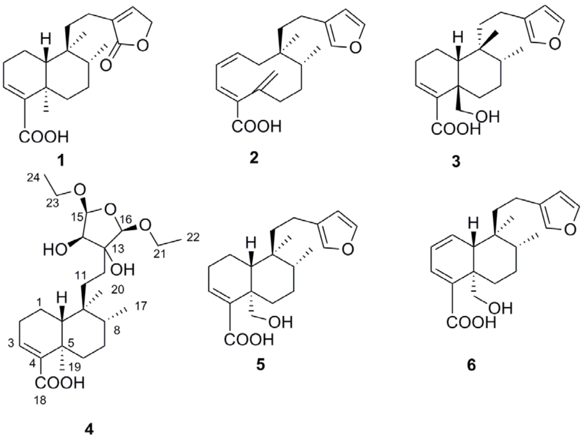

Fig. 1. Structures of compounds **1** – **6** isolated from *D. viscosa.*

Results and Discussion

Compound **4** was isolated as colorless needle-shaped crystals. The molecular formula was determined to be $C_{24}H_{40}O_7$ by a combination of negative ESI-MS, ^1H and ^{13}C NMR and distortionless enhancement by polarization transfer (DEPT) spectra, and further confirmed by HR-ESI-MS (m/z = 439.2709, calcd. 439.2701, $[M-1]^-$). The IR spectrum of **4** showed characteristic absorption bands due to hydroxy groups (3411cm^{-1}) and a carboxyl function (1678 cm^{-1}). In the ^1H NMR spectrum (Table 1), one methyl doublet of H-17 (δ = 0.82, J = 6.6 Hz), two methyl triplets of H-22 (δ = 1.19, J = 7.2, 4.9 Hz) and H-24 (δ = 1.17, J = 7.2, 4.9 Hz) and two methyl singlets of H-20 (δ = 0.76) and H-19 (δ = 1.26) were observed. In addition, two oxymethylene signals of H-21 (δ = 3.74, 3.47, m) and H-23 (δ = 3.74, 3.47, m) were observed, and one α, β-unsaturated proton was seen at δ = 6.71 (m). The ^{13}C NMR spectrum of **4** together with the DEPT and ^1H NMR spectra revealed 24 carbon signals that were classified into five methyl carbons (δ_C = 16.5, C-17; 19.8, C-20; 21.7, C-19; 17.1, C-22; 16.2, C-24), six methylene carbons (δ_C = 18.6, C-1; 28.7, C-2; 37.7, C-6; 28.9, C-7; 28.6, C-11; 32.6, C-12), five methine carbons (δ_C = 37.7, C-8; 48.8, C-10; 81.9, C-14; 108.4,

Table 1. NMR spectral data for compounds **3** – **5**[a].

Position	**4** (CD_3COCD_3)		**3** ($CDCl_3$)		**5** ($CDCl_3$)	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1	β 1.83 (m)	18.6	α 1.72 (m)	19.6	β 1.68 (m)	16.9
	α 1.43 (m)		β 1.08 (m)		α 1.54 (m)	
2	α 2.29 (m)	28.7	α 2.38 (m)	27.6	α 2.40 (m)	26.7
	β 2.16 (m)		β 2.17 (m)		β 2.28 (m)	
3	6.71 (m)	138.9	6.76 (t, 3.8)	135.6	6.75 (t, 3.8)	139.6
4		143.8		138.6		140.7
5		39.9		45.6		41.9
6	α 2.44 (d, 12.8)	37.7	α 1.93 (d, 12.8)	34.5	α 2.44 (d, 13.0)	31.2
	β 1.08 (m)		β 1.26 (m)		β 1.16 (m)	
7	α 1.38 (m)	28.9	α 1.65 (m)	27.8	α 1.46 (m)	26.7
	β 1.49 (m)		β 1.52 (m)		β 1.38 (m)	
8	1.51 (m)	37.7	1.72 (m)	36.5	1.61 (m)	36.2
9		39.2		38.8		38.7
10	1.26 (m)	48.8	1.77 (m)	48.1	1.55 (m)	46.3
11	1.67,	32.6	1.59 (m)	37.3	1.59 (m)	38.5
	1.46 (m)		1.72 (m)		1.65 (m)	
12	1.45 (m)	28.4	2.15 (m)	18.1	2.31 (m)	18.3
	1.49 (m)		2.40 (m)		2.13 (m)	
13		81.8		124.9		125.3
14	3.87 (d, 4.2)	81.9	6.26 (m)	110.8	6.23 (m)	110.9
15	4.91 (d, 4.2)	110.8	7.36 (t, 1.5)	142.9	7.35 (t, 1.5)	142.8
16	4.80 (s)	108.3	7.22	138.4	7.20	138.4
17	0.82 (d, 6.6)	17.1	0.85 (d, 6.6)	15.6	0.85 (d, 6.6)	15.8
18		169.2		169.4		171.9
19	1.26 (s)	21.7	3.92 (d, 10.2)	71.8	3.76 (d, 10.2)	65.1
			4.31 (d, 10.2)		4.20, (d, 10.2)	
20	0.76 (s)	19.8	0.61 (s)	17.7	0.75 (s)	18.6
21	3.75 (m)	64.0				
	3.47 (m)					
22	1.10 (s)	16.5				
23	3.75 (m)	65.4				
	3.47 (m)					
24	1.17 (s)	16.2				

[a] Chemical shift values δ in ppm, coupling constants J in Hz (in parentheses).

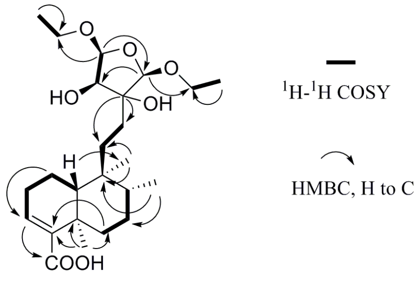

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Fig. 2. ^1H-^1H COSY and selected HMBC correlations for **4**.

C-16; 110.8, C-15), three quaternary carbons (δ_C = 39.9, C-5; 39.2, C-9; 81.8, C-13), two olefinic carbons (δ_C = 138.9, C-3; 143.8, C-4), two oxygenated methylene carbons (δ_C = 64.0, C-21; 65.4, C-23), and one carboxyl carbon at δ_C = 169.2 (C-18). Close similarities of the chemical shifts from C-1 to C-10 and C-17 to C-20 for **4** with reported values for related compounds comfirmed compound **4** possessing an *ent*-clerodane-type diterpene skeleton (Table 1) [9 – 11]. HMBC cross peaks (Fig. 2) were observed between H-22 and C-21, between H-24 and C-23, between H-16 and C-14 and C-21, and between H-15 and C-13 and C-23. The observation of ^1H-^1H COSY correlations between Me-21 and H-22, between Me-24 and H-23, and between H-15 and H-14, together with the HMBC data, led to the identification of an oxidized furan ring.

The relative stereochemistry of compound **4** was elucidated by the ROESY experiment (Fig. 3). ROESY correlations observed for H-10/H-6β, H-10/H-8, H-6β/H-8, and H-19/H-20, suggested that two six-membered rings were *trans*-oriented and existed in twist-chair and chair conformations. The H-10 proton was in β-position and Me-19, Me-20 and Me-17

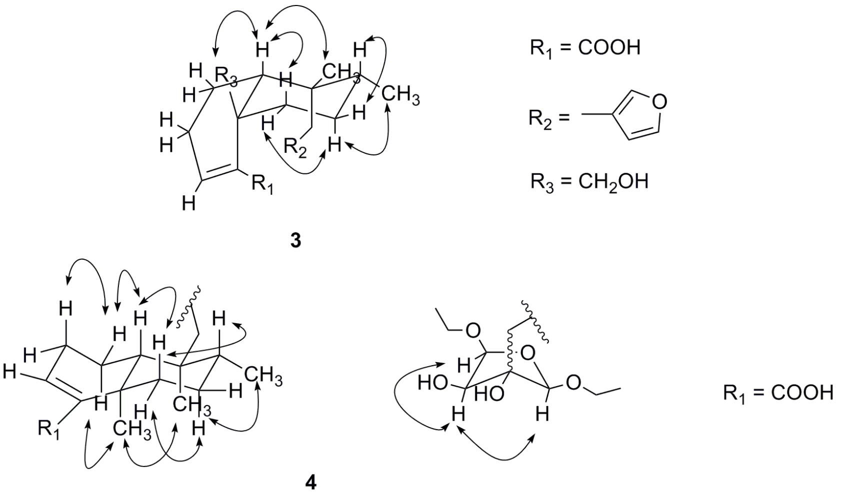

Fig. 3. Selected ROESY correlations for **3** and **4**.

were in α-position. These assignments were consistent with the configurations of H-10, Me-19, Me-20, and Me-17 of *ent*-clerodane-type diterpenes reported in the literature [12]. Furthermore, the evident ROEs of H-12/H-14, H-14/H-15, and H-16/H-12 implied that H-12, H-14 and H-15 are located on the same side of the furan ring, and ROE correlations H-14/H-11 and H-11/H-6α indicated that H-14, H-15 and H-16 have α-orientation. Thus, compound **4** was elucidated as a new *ent*-clerodane diterpene and named as visclerodol acid.

Compound **3** was obtained as colorless needle-shaped crystals and found to possess the same molecular formula as **3** based on the HR-ESI-MS (*m/z* = 331.1918, calcd. 331.1915, $[M-1]^-$), ^{1}H and ^{13}C NMR data. ^{1}H-^{1}H COSY, HMQC and HMBC experiments established that **3** had the same planar structures as the previously reported compound hautriwaic acid [13]. The distinct differences were that the signal of C-19 at δ_C = 71.8 in the ^{13}C NMR spectrum is shifted downfield, and the one of H-20 at δ_H = 0.61 is shifted upfield in the ^{1}H NMR spectum compared to the corresponding signal of the known compound (δ_C = 65.1, δ_H = 0.75 resp.) due to different configurations at C-19 and C-20. ROE correlations were observed between H-6β and H-7β, H-7β and H-20, H-7β and H-19, H-10 and H-20, as well as between H-7β and H-19, revealing that the two six-membered rings were fused with a *cis*-orientation and H-19, H-20, and H-10 were all β-configurated. Thus, compound **3** was defined as vishautriwaic acid. Compound **3** is a new stereoisomer of the known compound hautriwaic acid.

Experimental Section

General

Optical rotations were measured on a Horiba SEPA-300 polarimeter. IR spectra were recorded on a Bruker Tensor 27 spectrometer with KBr pellets. Both 1D and 2D NMR experiments were performed on a Bruker AM-400 or DRX-500 spectrometer with tetramethylsilane (TMS) as the internal standard. The ESI-MS was recorded with a VG Autospec-3000 spectrometer. The HR-ESI-MS was recorded with an API QSTAR Pulsar 1 spectrometer. Column chromatography was performed on silica gel (200 – 300 mesh; Qingdao Marine Chemical Ltd., Qingdao, People's Republic of China) and Sephadex LH-20 (Amersham Biosciences, Sweden). TLC analysis was carried out on silica gel GF$_{254}$ precoated plates (0.20 – 0.25 mm; Qingdao) with detection by heating silica gel plates sprayed with 10 % H_2SO_4 in ethanol.

Plant material

The aerial parts of *Dodonaea viscosa* were collected in Yunnan (China) in August 2008 and identified by Prof. Yu Chen, Kunming Institute of Botany, the Chinese Academy of Sciences. The voucher specimen of this plant has been deposited at the South China Agriculture University, Guangzhou, China.

Extraction and isolation

The air-dried plants (9.5 kg) were extracted with 95 % ethanol at r. t. for 3 × 24 h and filtered. The filtrate was concentrated and partitioned with EtOAc. The EtOAc part was evaporated to give 400 g of a residue, which was subjected to column chromatography over silica gel and eluted with petroleum ether/ethyl acetate (from 1 : 0 to 0 : 1). By combining the fractions with TLC (GF$_{254}$), seven fractions were obtained. Then fraction 4 (61 g) was subjected to column chromatography (CC) on silica gel, eluted with $CHCl_3$/MeOH (from 200 : 1 to 25 : 1), respectively, to give five fractions D_1–D_5. Subfraction D_3 was subjected to Sephadex LH-20 and silica gel chromatography using $CHCl_3$/MeOH (30 : 1) to afford compounds **1** (32 mg) and **2** (16 mg). Subfraction D_4 was repeatedly applied to a silica gel column eluted with $CHCl_3$/acetone (50 : 1, 25 : 1) and passed over Sephadex LH-20 to yield compound **3** (23 mg). Subfraction D_5 was chromatographed on silica gel eluted with $CHCl_3$/acetone (30 : 1) followed by RP-18 gel chromatography eluted with MeOH/H_2O (75 : 25) to give compound **4** (10 mg). Fraction 6 was chromatographed repeatedly on silica gel using $CHCl_3$/MeOH (50 : 1) and further purified by RP-18 gel chromatography eluted with MeOH/H_2O (75 : 25) to afford compounds **5** (120 mg) and **6** (13 mg).

*Visclerodol acid **4***: colorless needle-shaped crystals, m. p. 141 – 142 °C. – $[\alpha]_D^{26.9} = -41.05$ (c = 0.0003, acetone). – IR (KBr): ν = 3411, 2971, 2919, 2870, 1678, 1672, 1416, 1382, 1263, 1117, 978 cm^{-1}. – ^1H NMR and ^{13}C NMR (CD_3COCD_3): see Table 1. – MS ((–)-ESI): *m/z* = 439 [M–1]$^-$, 879 [2M−1]$^-$. – HRMS ((–)-ESI): *m/z* = 439.2709 (calcd. 439.2701 for $C_{24}H_{40}O_7$, [M−1]$^-$).

*Vishautriwaic acid **3***: colorless needle-shaped crystals, m. p. 102 – 103 °C. – $[\alpha]_D^{26.9} = -96.00$ (c = 0.0014, acetone). – ^1H NMR and ^{13}C NMR ($CDCl_3$): see Table 1. – MS ((–)-ESI): *m/z* = 331 [M–1]$^-$, 663 [2M–1]$^-$. – HRMS ((–)-ESI): *m/z* = 331.1918 (calcd. 331.1915 for $C_{20}H_{28}O_4$, [M−1]$^-$).

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