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Scaparvin A, A Novel Caged *cis*-Clerodane with an Unprecedented C-6/C-11 Bond, and Related Diterpenoids from the Liverwort *Scapania parva*

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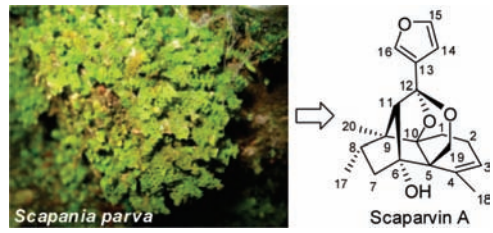
ABSTRACT



A novel caged *cis*-clerodane diterpenoid, scaparvin A, possessing an unprecedented C-6/C-11 bond and a ketal ring, as well as four new *cis*-clerodane derivatives, scaparvins B−E, were isolated from the Chinese liverwort *Scapania parva*. Their absolute structures were elucidated by analysis of NMR and CD data coupled with electronic circular dichroism (ECD) calculations. It was proposed that an enzymatic intramolecular aldol reaction was the key step in the biogenetic pathway of scaparvin A.

Liverworts have been rich sources of terpenoids and aromatic compounds, which are particularly valuable as chemosystematic and genetic markers.[1] Also, many of the secondary metabolites exhibited interesting biological properties, such as cytotoxic, antioxidative, antifungal, and insect antifeedant activities.[2] Previous studies on liverworts of the genus *Scapania* have afforded several kinds of di- and sesquiterpenoids, including clerodane, labdane, and verrucosane diterpenoids, as well as cadinane, longipinane, and muurolane sesquiterpenoids.[3] In this study, scaparvin A (**1**), a novel caged *cis*-clerodane diterpenoid intraring bridged with an unprecedented C-6/C-11 bond and intramolecular ketalization

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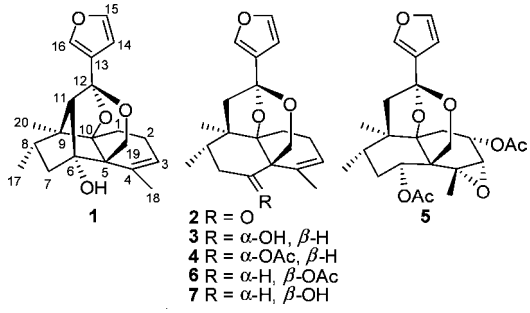
at C-12, four new related *cis*-clerodanes, scaparvins B−E (**2**−**5**), as well as two knowns, parvitexins B (**6**) and C (**7**),[3f] were isolated from the epilithic liverwort *Scapania parva* Steph., collected from Mount Leigong, Guizhou Province, P. R. China. Herein, we report the structural elucidation of compounds **1**−**5** and propose a biosynthetic pathway. This work represents the first phytochemical study on the title plant.

The liverwort was authenticated by Prof. Yuan-Xin Xiong (College of Life Sciences, Guizhou University, P. R. China). A voucher specimen (no. 200907-8) has been deposited in the Department of Natural Products Chemistry, School of Pharmaceutical Sciences, Shandong University, P. R. China. The air-dried powder of the plant material of *S. parva* (280 g) was extracted with 90% EtOH at room temperature (3 × 1.5 L). The obtained crude extract (15 g) was chromatographed on an MCI gel column (CHP20P, 70−150 μm, MeOH/H_2O, 4:6 to 9:1) to give seven fractions 1−7. Fraction 3 (1.2 g) was separated on a silica gel column [200−300 mesh, petroleum ether (60−90 °C)/Me_2CO, 150:1 to 0:1] to give eight subfractions 3a−3h. Fraction 3b (45 mg) was further purified over HPLC [Agilent 1100 isopump, Agilent 1100 VWD detector (210 nm), and Phenomenex Luna 5 μm C18(2) column (250 × 4.60 mm), MeOH/H_2O 69:31, 0.65 mL/min] to yield **1** (1.5 mg), **2** (10.1 mg), **4** (0.8 mg), and **6** (1.2 mg). Separation of fraction 4 (0.5 g) following a procedure similar to fraction 3 yielded **3** (2.5 mg), **5** (15.6 mg), and **7** (2.1 mg).



Scaparvin A (**1**),[4] a white amorphous solid, showed the molecular formula of $C_{20}H_{24}O_4$ as determined by HRESIMS at *m*/*z* 351.1558 $[M + Na]^+$ (calcd 351.1567), requiring 9 double bond equivalents. The ^{13}C NMR resolved 20 carbon signals, which were classified by chemical shifts and an HSQC spectrum as 3 methyls, 4 methylenes (one oxygenated), 6 methines (four olefinic ones), and 7 quaternary carbons (three oxygenated and two olefinic carbons). Moreover, two tertiary methyls (δ_H 1.07 and 1.82), one secondary methyl (δ_H 0.95, d, J = 7.2 Hz), one oxygenated methylene (δ_H 3.94 and 3.99, d, J = 9.0 Hz; δ_C 63.3), one tertiary hydroxyl group (δ_H 3.38), and a β-substituted furan ring were identified by analysis of its NMR data (Table 1). The spectral data aforementioned indicated a clerodane framework with a Δ^3 double bond for **1**.[3f] The 2D NMR ($^1H-^1H$ COSY, HSQC, and HMBC, see Supporting Information) experiments revealed the planar structure of **1** as depicted. The most striking feature of **1** was a novel skeleton with a C-6/C-11 linkage, which was verified by the HMBC correlations of H-11/C-5, H-11/C-6, H-7α/C-11, and 6-OH/C-11 (Figure 1). Furthermore, the HMBC correlation of H-19α with the ketal carbon at C-12 (δ_C 100.3) implied the presence of an ether bridge between C-12 and C-19. The remaining one degree of unsaturation and the downfield shifted signal of C-10 (δ_C 90.4) suggested an ether linkage between C-10 and C-12.[5]

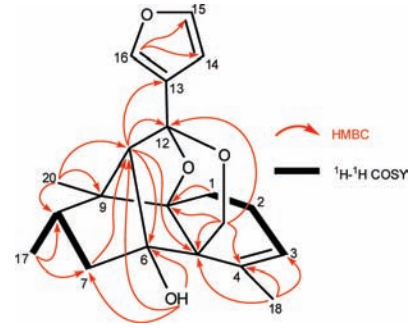


Figure 1. Key HMBC and $^1H-^1H$ COSY correlations of **1**.

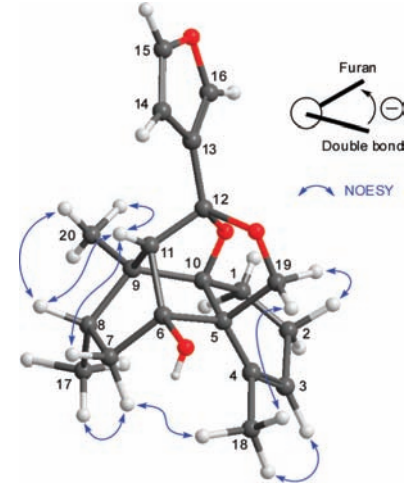


Figure 2. Key NOESY correlations of **1** and exciton coupling of the Δ^3 double bond and furan with negative chirality.

The NOESY correlations (Figure 2) of H-1α/H_3-17, H-2β/H-19β, H-3/H_3-18, H-7α/H_3-17, H-7α/H_3-18, H-7β/H-11, H-8/H-11, H-8/H_3-20, H-11/H_3-20, and H_3-18/H-19α determined the relative configuration of **1**.

(4) Scaparvin A (**1**): white amorphous solid; $[\alpha]^{20}_D$ −61.9° (*c* 0.095, MeOH); UV (MeOH) λ_{max} (log ε) 199 (4.63) nm; CD (MeOH) 201 ($\Delta\varepsilon$ + 0.73), 218 ($\Delta\varepsilon$ − 0.26) nm; IR (KBr) ν_{max} 3481, 2968, 1344, 1137, 1109, 1078, 876, 795, 599 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; positive ESIMS *m*/*z* (relative intensity) 329.5 $[M + H]^+$ (100), 351.4 $[M + Na]^+$ (90), 367.3 $[M + K]^+$ (9); positive HRESIMS *m*/*z* 351.1558 $[M + Na]^+$ (calcd for $C_{20}H_{24}O_4Na$, 351.1567).

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Table 1. ^{1}H and ^{13}C NMR Data of **1** and **2** (in $CDCl_3$)[a]

	1		**2**	
position	δ_H (mult, *J*)	δ_C	δ_H (mult, *J*)	δ_C
1	1.87 (2H, m)	21.3 t	α 1.47 (ddd, 13.6, 11.7, 6.2)	27.9 t
			β 2.12 (dd, 14.1, 5.6)	
2	2.17 (2H, m)	22.1 t	α 2.07 (m)	22.9 t
			β 2.30 (m)	
3	5.69 (br s)	126.8 d	5.83 (br s)	126.7 d
4		132.8 s		131.3 s
5		49.7 s		55.8 s
6		83.3 s		210.5 s
7α	1.55 (dd, 12.0, 4.8)	37.2 t	2.36 (dd, 15.8, 12.5)	44.2 t
7*β*	2.13 (t, 12.0)		2.44 (dd, 15.8, 4.9)	
8	2.06 (ddq, 12.0, 7.2, 4.8)	34.0 d	2.64 (ddq, 12.5, 7.0, 4.9)	35.1 d
9		56.3 s		45.4 s
10		90.4 s		86.2 s
11	2.47 (s)	68.6 d	α 2.26 (d, 14.1)	56.8 t
			β 2.46 (d, 14.1)	
12		100.3 s		102.2 s
13		125.9 s		126.9 s
14	6.48 (br s)	108.4 d	6.42 (br s)	108.3 d
15	7.40 (br s)	143.5 d	7.38 (t, 1.5)	143.1 d
16	7.51 (br s)	140.1 d	7.50 (br s)	139.8 d
17	0.95 (3H, d, 7.2)	15.7 q	1.11 (3H, d, 7.0)	18.4 q
18	1.82 (3H, s)	19.6 q	1.98 (3H, br s)	22.1 q
19α	3.99 (d, 9.0)	63.3 t	4.21 (d, 12.3)	62.8 t
19*β*	3.94 (d, 9.0)		4.28 (d, 12.3)	
20	1.07 (3H, s)	14.5 q	1.21 (3H, s)	22.5 q
6-OH	3.38 (s)			

[a] Recorded at 600 MHz (^{1}H NMR) or 150 MHz (^{13}C NMR). *J* in Hz. ^{13}C multiplicities were determined by HSQC experiments.

Scaparvin B (**2**),[6] obtained as a colorless oil, was assigned the molecular formula of $C_{20}H_{24}O_4$ as determined by HRESIMS at *m*/*z* 351.1555 $[M + Na]^+$ (calcd 351.1567). The ^{1}H and ^{13}C NMR data of **2** (Table 1) resembled those of **1**, except for the resonances of C-6 and C-11. A ketone carbonyl (δ_C 210.5) and a methylene (δ_H 2.26 and 2.46, d, $J = 14.1$ Hz; δ_C 56.8) in **2** replaced the C-6 oxygenated quaternary carbon and the C-11 methine in **1**, respectively, which was deduced by comprehensive analysis of the HSQC and HMBC spectra of **2** (Supporting Information). Accordingly, the structure of **2** was determined as shown.

HRESIMS of scaparvin C (**3**) exhibited an $[M + Na]^+$ ion peak at *m*/*z* 353.1714 (calcd 353.1723), corresponding to a molecular formula of $C_{20}H_{26}O_4$, which suggested one less degree of unsaturation than **2**. The C-6 ketone carbonyl in **2** was reduced to an oxymethine group (δ_H 3.45, t, $J = 7.0$ Hz; δ_C 67.6) in **3**, which was supported by the $^{1}H-^{1}H$ COSY correlation of H-6 with both protons on C-7 (δ_H 1.47 and 2.12). The α-orientation of OH-6 was furnished by the NOESY correlation of H-6 with H_2-19 (δ_H 3.89 and 4.20, d, $J = 12.8$ Hz). Both **3** and **7** were reduction products of the C-6 carbonyl group of **2**, with different relative configurations at C-6. The *O*-acetyl derivative of **3**, scaparvin D (**4**), with the molecular formula $C_{22}H_{28}O_5$ from its HRESIMS (*m*/*z* 395.1817 $[M + Na]^+$; calcd 395.1829), was also obtained and identified by its 2D NMR experiments (Supporting Information).

Scaparvin E (**5**) was obtained as a colorless oil. A molecular formula of $C_{24}H_{30}O_8$ was assigned for **5** from the $[M + Na]^+$ ion peak at *m*/*z* 469.1816 (calcd 469.1833) in the HRESIMS. The similarity of the ^{1}H and ^{13}C NMR spectroscopic data of **5** (Supporting Information) to those of **4** indicated that **5** possessed the same *cis*-clerodane skeleton with a characteristic ketal moiety and a *β*-substituted furan ring. Two acetyls were placed at C-2 and C-6 on the basis of the corresponding HMBC correlations. In addition, the Δ^3 double bond in **4** was replaced by a 3,4-epoxide in **5**. The acetoxyl group at C-2 was α-configured as deduced by the coupling constants of $J_{1\alpha,2} = 7.9$ Hz and $J_{1\beta,2} = 3.8$ Hz.[7] The coupling pattern and coupling constant of H-3 (δ_H 3.22, d, $J = 3.5$ Hz) exhibited a 3,4-α-epoxide,[8] which was consistent with the NOESY correlations of H-2/H-3, H-6/H_3-18, and H_3-18/H-19α. The NOESY correlations of H-1α/H_3-17, H-3/H_3-18, H-6/H-19α, H-6/H-19*β*, H-7α/H_3-17,

(6) Scaparvin B (**2**): colorless oil; $[\alpha]^{20}_D$ +52.5° (*c* 0.122, MeOH); UV (MeOH) λ_{max} (log ε) 195 (4.62) nm; CD (MeOH) 215 ($\Delta\varepsilon$ − 1.46), 246 ($\Delta\varepsilon$ + 0.77), 301 ($\Delta\varepsilon$ + 1.13) nm; IR (KBr) ν_{max} 2974, 1708, 1233, 1133, 1109, 1068, 876, 798, 602 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; positive ESIMS *m*/*z* (relative intensity) 329.5 $[M + H]^+$ (100), 351.4 $[M + Na]^+$ (83), 367.3 $[M + K]^+$ (35); positive HRESIMS *m*/*z* 351.1555 $[M + Na]^+$ (calcd for $C_{20}H_{24}O_4Na$, 351.1567).

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H-7β/H-19β, H-8/H-11β, and H-11β/H-19β confirmed the relative configurations of **5** as depicted.

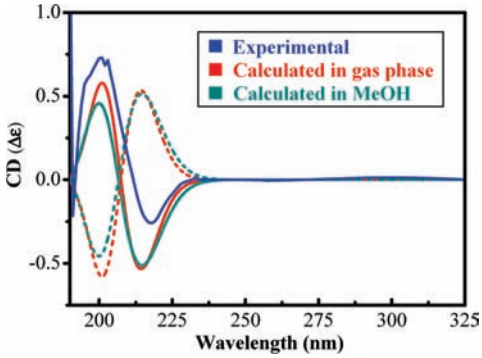
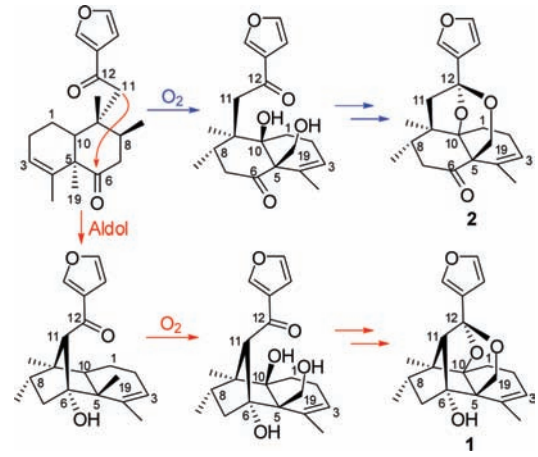


Figure 3. ECD spectra of **1** (solid) and its enantiomer (dash): experimental ECD (blue), calculated ECD in the gas phase (red), and in MeOH (cyan).

The absolute configurations of **1**−**4** were determined by applying the CD exciton chirality method.[9] The CD spectrum of **1** (Figure 3) exhibited a negative split resulting from the excition coupling between the two different chromophores of the furan ring (218 nm, $\Delta\varepsilon$ −0.26, $\pi\rightarrow\pi^*$ transition)[10] and the Δ^3 double bond (201 nm, $\Delta\varepsilon$ +0.73, $\pi\rightarrow\pi^*$ transition),[11] indicating that the transition dipole moments of the two chromophores were oriented in a counterclockwise manner[12] (Figure 2). Thus the absolute configuration of the seven chiral centers in **1** was determined as 5*S*, 6*S*, 8*S*, 9*S*, 10*R*, 11*S*, and 12*R*, which was further supported by the calculation result of electronic circular dichroism (ECD) using time-dependent density functional theory (TDDFT)[13] (Figure 3). The CD split manners of compounds **2**−**4** (Supporting Information) resembled that of **1** at ca. 218 and 201 nm, and their absolute configurations were thus defined as shown. With the absence of the Δ^3 double bond, the absolute structure of **5** could not be deduced by the CD exciton chirality method, which was solved by using the TDDFT ECD calculation (Supporting Information). The calculation result confirmed that the absolute configuration of **5** was consistent with those of **1**−**4**.

Scaparvin A (**1**) was the first caged *cis*-clerodane diterpenoid with an endotricyclo[6.2.1.0^{2,7}]undecane framework, formed by an unprecedented C-6/C-11 linkage. Considering the highly rigid structure of compound **2**, it is quite difficult to transform **2** into **1**, directly. Therefore, a plausible biogenetic pathway for **1** was proposed as shown in Scheme 1. A precursor with carbonyls at C-6 and C-12 would be transformed into the key intermediate by an enzymatic aldol reaction followed by oxidation and intramolecular ketalization to yield **1**. Oxidation of the same precursor followed by intramolecular ketalization would generate **2**.

Scheme 1. Plausible Biosynthetic Pathways of **1** and **2**



The isolated compounds **1**−**7** were all evaluated for their cytotoxicity against the KB, Hela, MCF-7, and PC3 cell lines by using the MTT assay[14] and with adriamycin as a positive control (IC_{50} values of 0.63, 0.79, 1.19, and 1.50 μM, respectively). Unfortunately, all compounds were inactive (IC_{50} > 10 μM) against all of the cell lines tested.

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Supporting Information Available: Experimental procedures, detailed HMBC correlations (in tables and figures), detailed NOESY correlations (in figures), 1D and 2D NMR, ESIMS, HRESIMS, IR, CD, and UV spectra of scaparvins A−E (**1**−**5**). This material is available free of charge via the Internet at http://pubs.acs.org.

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