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Spirochensilides A and B, Two New Rearranged Triterpenoids from *Abies chensiensis*

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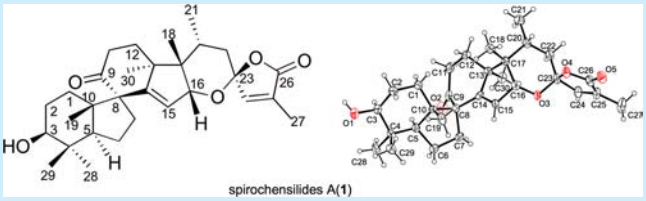
Supporting Information

ABSTRACT: Two new triterpenoids, spirochensilides A (**1**) and B (**2**) were isolated from *Abies chensiensis*. Comprehensive spectroscopic analysis revealed that **1** and **2** are the first example of triterpenoids possessing a unique 8,10-cyclo-9,10-seco and methyl-rearranged carbon skeleton. The single crystal X-ray diffraction analyses and computational methods allowed the absolute configuration assignments of the two compounds. A plausible biogenetic pathway of spirochensilide A (**1**) is also proposed.



The genus of *Abies* has been studied extensively because of structurally diverse and biologically active terpenoid constituents. Until now, more than 250 terpenoids and their analogues have been reported from this genus, and most of them are lanostane triterpenoids.[1−3] Furthermore, several types of lanostane-related carbon skeletons have been found in the *Abies* genus, such as spiroveitchionolide,[4] abiestetranes A and B,[5] and abibalsamins A and B.[6]

A. chensiensis is one of the Chinese endemic plants mainly distributed over Shanxi and Hubei provinces of the People's Republic of China.[7] A number of nature products have been found in the previous studies.[8,9] As part of a program to search for unique and bioactive secondary metabolites, two novel rearranged triterpenoids, spirochensilides A (**1**) and B (**2**), along with a known biogenetically related compound **3** (23-hydroxy-3-oxolanosta-8,24-dien-26,23-olide),[10] were isolated from the leaves and twigs of *A. chensiensis*. Herein, we report the isolation and structural elucidation of these novel compounds. In addition, the plausible biogenetic pathway of **1** and the anti-inflammatory activities of these compounds are also described.

Spirochensilide A (**1**, $[\alpha]^{20}_{D}$ −10.0) was obtained as a colorless crystal. The HRESIMS data suggested the molecular formula of **1** was $C_{30}H_{42}O_5$ (*m*/*z* 483.3115 $[M + H]^+$ calcd for 483.3105 $[M + H]^+$), requiring 10 degrees of unsaturation. Its IR spectrum exhibited absorptions at 3303 and 1736 cm^{-1}, assignable to hydroxyl and carbonyl, respectively. The ^{13}C NMR spectrum exhibited 30 carbon signals, which were classified as seven methyls, seven methylenes, six methines, and ten quaternary carbons with the aid of HSQC data, including one lactone carbonyl (δ_C 171.9), one keto carbonyl (δ_C 214.8), and two pairs of double bonds (δ_C 147.2, 132.1, 124.0, 160.2). These analyses suggested four degrees of unsaturation, and thus, six rings should be present in spirochensilide A (**1**).

Comprehensive analyses of the $^1H-^1H$ COSY spectrum of **1** revealed five spin-coupling systems, which were identified as follows: $C(1)H_2-C(2)H_2-C(3)H$; $C(5)H-C(6)H_2-C(7)H_2$; $C(11)H_2-C(12)H_2$; $C(15)H-C(16)H$; and $C(21)H_3-C(20)-H-C(22)H_2$ (Figure 2). The HMBC correlations from the gem-dimethyl protons to C-3 and C-5; from H_3-19 to C-1, C-5, and C-8 (Figure 2); and from H-6 to C-10 and C-8 constructed the rings A and B.

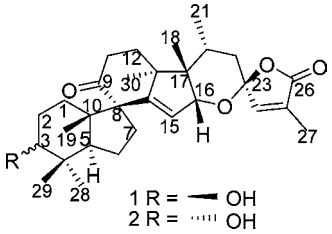


Figure 1. Structures of spirochensilides A (**1**) and B (**2**).

The HMBC cross peaks of H-11 with C-8 and C-13; of H-12 with C-9, C-14, and C-30; of H-15 with C-8, C-13, and C-17; and of H-16 with C-13, C-14, and C-18 revealed a partial structure that resembled the 6/5 fused C/D rings of triterpenoid. However, unlike a typical lanostane-type triterpenoid, the successive methyl-rearrangement at C-13 and C-14 occurred in the C/D ring systems, which might be caused by enzymic oxidation of 17-C biogenetically. Moreover, the cross peaks of H-6, H-11, H-15, and H_3-19 with C-8 suggested that the substructures A/B and C/D first joined at C-8, forming an unprecedented spiro-[5, 6] system.

The typical NMR data (δ_H 1.92, s, 6.75, brs; δ_C 10.5, 105.4, 147.2, 132.1, 171.9) indicated the presence of an *α,β*-

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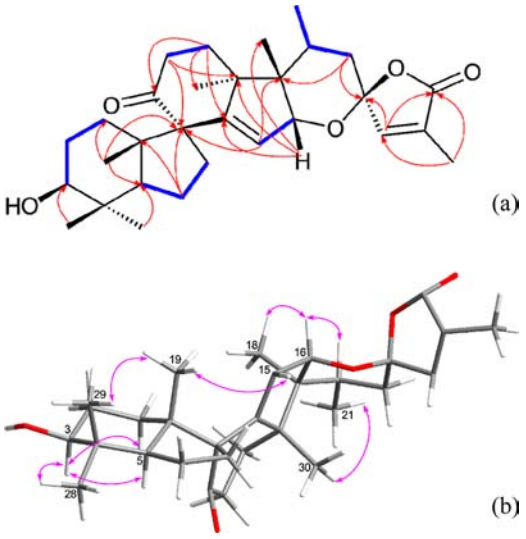

Figure 2. Key (a) ^{1}H−^{1}H COSY (—), HMBC (H → C), and (b) NOESY correlations of **1**.

unsaturated-γ-lactone moiety (ring F),[10] which was verified by the following HMBC data: H-24 with C-23 and C-26; and H-27 with C-26 and C-24. In addition, the HMBC cross peaks from H-22 to C-17 as well as from H-22 to C-23, taking the remaining oxygen atoms into consideration, revealed that rings D and F were connected via C(20)−C(22) bond and a rare oxygen bridge between C-16 and C-23 forming ring E. The rings E and F were linked via a dioxygen-bearing sp^3 quaternary carbon (C-23). Thus, the planar structure of **1** was elucidated as depicted in Figure 1.

The relative configuration of **1** was determined partially based on the NOESY data. For rings A and B, the cross peaks of H-3/H-1α, H-3/H$_3$-28, and H-3/H-5 revealed a cofacial relationship of H-3, 28-Me, and H-5 and was assigned as α-oriented. The β-orientation of Me-19 and Me-29 was assigned by the correlation of H$_3$-19/H$_3$-29. The relative stereochemistry around substructure C/D/E was also assigned by the NOESY correlations of H$_3$-30/H-12α, H$_3$-18/H-20, H-16/H$_3$-18, and H$_3$-18/H-12β, indicating that Me-18 and H-16, and Me-21 and Me-30 resided on the opposite side of the plane defined by the C/D/E rings. The cross-peak observed from H$_3$-19 to H-15 demonstrated that a dihedral angle near 90° among substructures A/B and C/D/E was necessary to keep an anticoplanar conformation, and the C-14 was on the upside of the rings A/B. However, because of the absence of certain evidence, the relative configuration of C-23 was left unassigned.

The single-crystal X-ray diffraction experiment (Cu Kα radiation) further corroborated the planar structure and fully determined the assignment of its absolute configuration as (3*S*, 5*R*, 8*R*, 10*S*, 13*R*, 16*R*, 17*S*, 20*R*, 23*S*) [with a Flack parameter of 0.051(133)] (Figure 3).

Spirochensilide B (**2**, $[\alpha]^{20}_D$ +22.2) was isolated as a colorless oil. The molecular formula ($C_{30}H_{42}O_5$) of compound **2** was deduced to be the same as compound **1** on the basis of HRESIMS data (*m*/*z* 505.2917 [M + Na]$^+$ calcd for 505.2924 [M + Na]$^+$). A comparison of the NMR data of compounds **1** and **2** revealed that **2** shared the similar A, B, C, D, E, and F ring systems as those of **1**, with the only difference occurring in ring A. ^{1}H−^{1}H COSY spectrum (Supporting Information Figure S14) showed a correlation from H-3 (3.38, brs) to H-2, which demonstrated the presence of a hydroxyl located at C-3.

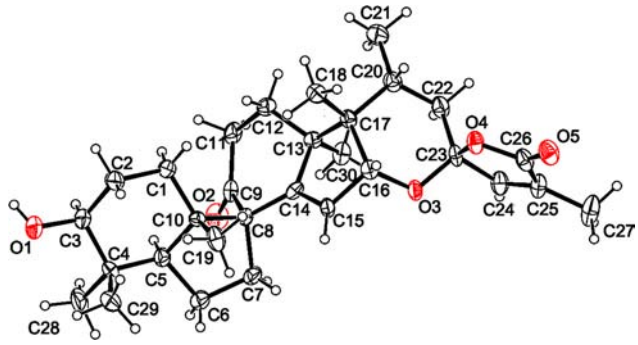

Figure 3. X-ray crystallographic structure of **1**.

However, little difference can be found in the ^{1}H and ^{13}C NMR data (Table 1): H-3 shifted downfield 0.2 ppm, while C-3 upfield 4.5 ppm, suggesting a different orientation of 3-OH between **1** and **2**.[11] This conclusion was further confirmed by

Table 1. ^{1}H and ^{13}C NMR Data of 1 and 2[a]

	1		2		
	δ_H mult. (*J* in Hz)	δ_C	δ_H mult. (*J* in Hz)	δ_C	
1α	1.56 m	32.9	1.72 m	26.9	CH_2
1β	1.30 m		2.12 m		
2α	1.67 m	28.8	1.66 m	27.9	CH_2
2β	1.67 m		1.91 m		
3	3.17 dd (10.2, 6.0)	78.9	3.38 brs	74.5	CH
4		38.6		38.0	C
5	1.58 m	51.7	1.94 m	44.7	CH
6α	2.37 m	36.0	1.61 m	34.7	CH_2
6β	1.70 m		1.61 m		
7α	1.69 m	20.9	1.62 m	22.7	CH_2
7β	1.55 m		1.52 m		
8		66.9		67.6	C
9		214.9		214.6	C
10		49.5		49.6	C
11α	2.58 m	37.6	2.58 m	37.8	CH_2
11β	2.39 m		2.44 m		
12α	1.72 m	26.2	1.74 m	26.2	CH_2
12β	2.08 m		2.15 m		
13		51.3		51.2	C
14		160.2		160.5	C
15	5.53 d (3.6)	123.9	5.51 s	123.4	CH
16	4.51 d (3.0)	86.1	4.50 s	86.0	CH
17		44.9		45.0	C
18	1.08 s	25.3	1.09 s	25.3	CH_3
19	1.04 s	18.4	1.00 s	17.8	CH_3
20	2.35 m	34.2	2.33 m	34.1	CH
21	1.19 d (7.2)	16.3	1.18 d (6.6)	16.3	CH_3
22a	1.57 m	37.6	1.60 m	37.6	CH_2
22b	2.07 m		2.08 m		
23		105.4		105.7	C
24	6.75 brs	147.2	6.75 brs	147.4	CH
25		132.0		132.3	C
26		171.9		171.9	C
27	1.92 s	10.5	1.91 s	10.6	CH_3
28	0.96 s	29.0	0.91 s	21.7	CH_3
29	0.90 s	15.8	0.92 s	28.8	CH_3
30	1.22 s	24.9	1.22 s	24.9	CH_3

[a]Data were measured in $CDCl_3$ at 600 MHz (^{1}H) and 125 MHz (^{13}C). Chemical shifts (δ) are in ppm being relative to TMS.

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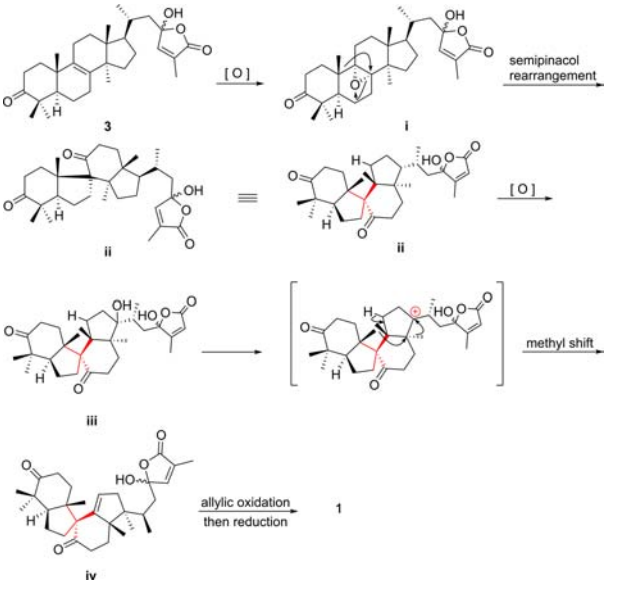
the different peak patterns of H-3 in **1** (dd, $J_{2,3}$ 10.2, 6.0 Hz) and **2** (brs), which showed an α orientation of 3-OH in **2**. Thus, compound **2** was proposed as a C-3 epimer of **1**. Furthermore, the consistent ECD spectra of **1** and **2** (a negative $\pi-\pi^*$ Cotton effect at 225 nm and a positive $n-\pi^*$ Cotton effect at 250 nm) provided more evidence in support of the assignment, and based on the biosynthetic point of view, the absolute configuration of compound **2** was elucidated as (3*R*, 5*R*, 8*R*, 10*S*, 13*R*, 16*R*, 17*S*, 20*R*, 23*S*).

It is doubted that spirochensilides A (**1**) and B (**2**) had the same absolute configuration except for C-3, while their optical rotation values were much different. To figure out this query, quantum mechanical calculations were carried out. The IEFPCM solvation model was employed for the optical rotation calculation, and the chloroform was used as the solvent that was in agreement with the experiment condition. The calculated optical rotation for (3*S*, 5*R*, 8*R*, 10*S*, 13*R*, 16*R*, 17*S*, 20*R*, 23*S*) configuration is −10.6, while for (3*R*, 5*R*, 8*R*, 10*S*, 13*R*, 16*R*, 17*S*, 20*R*, 23*S*) the configuration is +19.9, which were close to the experimental values of compounds **1** and **2**, respectively. Moreover, ECD calculation was conducted, and the calculated ECD spectrum of the conformer (3*R*, 5*R*, 8*R*, 10*S*, 13*R*, 16*R*, 17*S*, 20*R*, 23*S*) is in good accordance with the experimental ECD spectrum of **2**. Thus, the absolute configuration of **2** was further unambiguously established.

Spirochensilides A (**1**) and B (**2**) are the first of a new rearranged triterpenoid skeleton featuring an unprecedented spiro-[5,6] ring systerm. Biosynthetically, spirochensilide A (**1**) might be traced back to **3** (Scheme 1), a lanostane triterpene

Scheme 1. Proposed Biosynthetic Pathway of Compound 1



lactone that is common in the genus of *Abies*. In brief, **3** would be oxidized to produce compound **i**, which was followed by an acid induced epoxy opening/1,2-shift reaction[12] to produce compound **ii**. Compound **ii** would undergo an enzymatic region-selective oxidation in biological systems and two Wagner−Meerwein type reactions[13] to give compound **iv**. Then intramolecular allylic oxidation and etherification of the compound **iv** followed by a reduction of the carbonyl group would construct compound **1**.

In order to demonstrate the biogenetic pathway experimentally, compound **3** was first oxidized by m-CPBA to produce epoxide **i** in a highly chemoselective manner. Although the similar 1,2-migration involving the concurrent epoxy opening has been realized previously,[12] our initial attempts to induce the above proposed rearrangement using various Lewis acids have been proved unsuccessful.[14] The undesired compound **v** generated by the elimination of epoxide **i** was always obtained as the major product, and the frustrated experimental result may be attributed to the decreased driven force of the ring-contraction due to the steric hindrance in forming two consecutive quaternary carbon centers. Although the experiment was unsuccessful, we hope that the process would shed more light on the future synthesis of the triterpenoids with an unprecedented spiro-[5,6] ring system.

Because of the scarce amounts obtained of compound **2**, only **1** and **3** were assessed for the biological evaluations. Because of the cytotoxicity of compound **3** at the concentration of 12.5 μg/mL, only spirochensilide A (**1**) was further tested for the inhibitory activities on NO production. Spirochensilide A (**1**) showed relatively weak inhibitory effect, and the inhibition of NO production is 30% at the concentration of 12.5 μg/mL.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures; crystallographic data of **1**; 1D and 2D NMR, IR spectra, and HRESIMS of spirochensilides A and B (**1** and **2**). The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01166.

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Notes

The authors declare no competing financial interest.

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