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Sarglaperoxides A and B, Sesquiterpene−Normonoterpene Conjugates with a Peroxide Bridge from the Seeds of *Sarcandra glabra*

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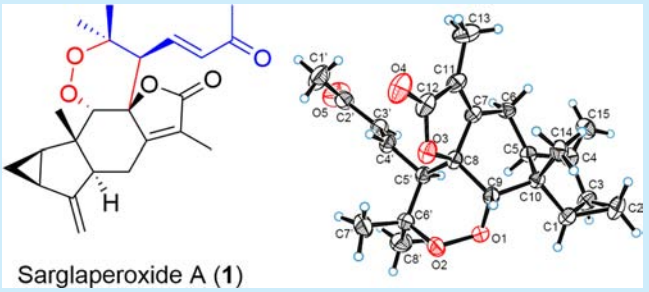
Supporting Information

ABSTRACT: Sarglaperoxides A (**1**) and B (**2**), a pair of unusual sesquiterpene−normonoterpene conjugates with a peroxide bridge, were isolated from the seeds of *Sarcandra glabra*. The structures and absolute configurations of **1** and **2** were determined on the basis of spectroscopic data analysis, including MS, NMR, CD, and XRD. The two compounds were screened in antimicrobial, anti-inflammatory, and cytotoxic bioassays and showed moderate bioactivities.



Sarcandra glabra (Thunb.) Nakai (Chloranthaceae), a folk medicinal herb widely used in China and other East and Southeast Asian countries for the treatment of inflammation and traumatic injuries,[1] was verified to contain abundant eudesmanes, linderanes, and linderane dimers that were demonstrated to be the bioactive substances corresponding to the traditional application of *S. glabra*.[2a] Among them, the linderane dimers were the characteristic metabolites of the plants of Chloranthaceae, and most of them were constructed by two linderane monomers via a [4 + 2] cycloaddition between $\Delta^{15(4),5(6)}$ and $\Delta^{8'(9')}$. Some linderane dimers were esterified further with small organic acids to form an 18-membered ester ring. Besides, there were some other dimers that were formed by [2 + 2] cycloaddition, radical C−C bond coupling, and other pathways to present diverse oligomeric skeletons,[2] which evoked a wide interest of synthetic chemists in their biomimetic syntheses.[3] The linderane dimers were also reported to have potent anti-inflammatory,[4] antitumor,[5] anti-HIV-1,[6] and other bioactivities,[7] which inspired us to find more structurally attractive and bioactive sesquiterpene oligomers.

In our previous research of the linderane dimers from the plants of Chloranthaceae, novel compounds have been isolated and reported.[4,8,9] Unexpectedly, a pair of structurally related terpene lactones sarglaperoxides A (**1**) and B (**2**) were obtained in our recent investigation of the constituents of *Sarcandra glabra* (Figure 1). In spite of 23 carbons, a linderane unit and a normonoterpene unit could be distinguished in their molecules and offered a pair of sesquiterpene−normonoterpene conjugates. To our knowledge, few sesquiterpene−monoterpene conjugates were found in the nature,[10] which are biogenetically differentiated from the sesterterpenes derived from geranylfarnesyl pyrophosphate (GFPP). Being of our great interest, sarglaperoxides A and B, assembled from a linderane lactone and an eight-carbon normonoterpene, contain a peroxide bridge, reminiscent of many natural peroxides having potent bioactivities.[11] Herein, we report the isolation, structural elucidation, possible biogenetic pathway, and biological activities of **1** and **2**.

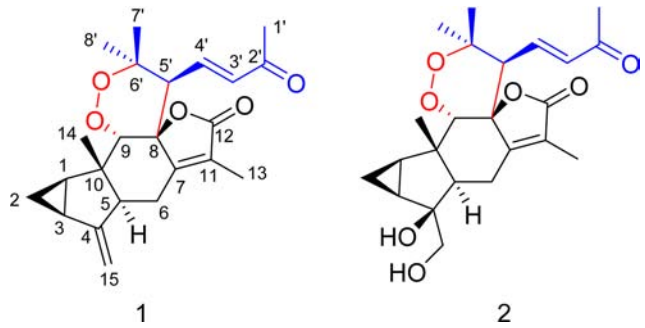


Figure 1. Structures of sarglaperoxides A (**1**) and B (**2**).

The fresh seeds of *Sarcandra glabra* (10 kg) were roughly air-dried and extracted with 95% ethanol. After removing the solvent, the residue (460 g) was successively extracted by petroleum ether, ethyl acetate, and *n*-butanol to obtain three extracts (110, 70, and 50 g, respectively). To identify the main constituents of the extracts preliminarily, an HPLC−HRMS method was applied to analyze the extracts and revealed the

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enrichment of sesquiterpene monomers in PE extract and the enrichment of both monomers and dimers in EtOAc extract. Subsequent isolation of the compounds in the PE extract via column chromatography and preparative HPLC led to the discovery of the first sesquiterpene−normonoterpene conjugate sarglaperoxide A (**1**) (11.2 mg). In order to find more analogues of **1** in this plant, HPLC−HRMS was used as a guidance to assist the isolation, and fortunately, the second sesquiterpene−normonoterpene conjugate sarglaperoxide B (**2**) (3.8 mg) was obtained in the EtOAc extract.

Sarglaperoxide A (**1**)[12] was obtained as a colorless column. The molecular formula was determined as $C_{23}H_{28}O_5$ by HRESIMS (m/z 402.2274 $[M + NH_4]^+$, calcd. for $C_{23}H_{32}NO_5$, 402.2275). The ^{1}H NMR and HSQC spectra of **1** displayed a pair of characteristic upfield protons at δ_H 0.82 (H-2α, ddd, J = 8.6, 8.6, 5.5 Hz) and 0.71 (H-2β, m), diagnostic of the cyclopropane ring in a linderane,[2b] which represents the major form of the sesquiterpenes in *S. glabra*.[2a] The ^{1}H NMR and HSQC spectra also displayed an *E*-form double bond at δ_H 6.04 (H-3′, d, J = 16.0 Hz) and 6.35 (H-3′, d, J = 16.0 and 10.3 Hz), and a terminal double bond at δ_H 4.76 (H-15, br. s) and 5.07 (H-15, d, J = 1.5 Hz). Besides, totally five methyl groups could be observed in the ^{1}H NMR spectrum. The ^{13}C NMR spectrum of **1** displayed 23 carbons (Table 1) that were in accordance with the molecular formula of **1**, including a conjugated keto-carbonyl (δ_C 197.4), a conjugated ester carbonyl (δ_C 173.0), three pairs of double bonds (δ_C 162.0, 151.0, 139.7, 136.0, 124.9, and 107.2), three oxygenated carbons (δ_C 88.8, 88.3, and 81.6), and 12 other aliphatic carbons. Among them, the oxygenated carbons were apparently downfield-shifted compared with the normal ones, reminiscent of the existence of a peroxide bond.

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of Compounds 1 and 2 in $CDCl_3$

	1		2	
no.	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	1.91 (ddd, 7.9, 7.9, 3.9)	22.6	1.90 (ddd, 8.5, 7.8, 4.3)	24.3
2α	0.82 (ddd, 8.6, 8.6, 5.5)	15.9	0.66 (ddd, 8.8, 8.8, 5.7)	10.6
2β	0.71 (m)		1.17 (m)	
3	2.02 (m)	23.9	1.57 (ddd, 8.7, 7.6, 3.6)	29.2
4		151.0		79.0
5	3.35 (dd, 11.5, 7.9)	53.0	2.50 (dd, 12.9, 6.9)	53.5
6α	2.35 (m)	22.8	2.21 (m)	21.4
6β			2.64 (dd, 17.6, 12.9)	
7		162.0		162.6
8		88.8		88.5
9	4.49 (s)	88.3	4.36 (s)	88.7
10		43.4		45.7
11		124.9		124.7
12		173.0		173.2
13	1.76 (d, 1.0)	8.6	1.75 (d, 1.6)	8.5
14	0.53 (s)	19.6	0.82 (s)	20.4
15	5.07 (d, 1.5)	107.2	3.75 (d, 10.5)	70.2
	4.76 (br. s)		3.72 (d, 10.5)	
1′	2.20 (s)	26.6	2.19 (s)	26.5
2′		197.4		197.7
3′	6.04 (d, 16.0)	136.0	6.06 (d, 16.0)	136.1
4′	6.35 (dd, 16.0, 10.3)	139.7	6.34 (dd, 16.0, 10.3)	139.8
5′	2.87 (d, 10.3)	52.2	2.78 (d, 10.3)	52.3
6′		81.6		81.5
7′	1.29 (s)	21.9	1.28 (s)	21.9
8′	1.42 (s)	27.4	1.41 (s)	27.6

The planar structure of **1** was established by its HMBC spectrum. The correlations from CH_3-1′ to C-3′ and C-4′, from H-3′ to C-5′, from H-4′ to C-2′, and from CH_3-7′ and CH_3-8′ to C-5′ revealed an 8-carbon correlation system in the molecule, representing the monoterpene monomer, or normonoterpene more precisely. The remaining 15 carbons constructed a linderane lactone that could be easily established by the HMBC correlations, representing the sesquiterpene monomer. The connections between two monomers were resolved by the key HMBC correlations from H-4′ to C-8 and from H-5′ to C-7 (Figure 2). In addition, a peroxide bridge was believed to lie between C-9 and C-6′ according to their ^{13}C shifts and the molecular formula of **1**, which had two additional oxygen atoms except the two constructed monomers.

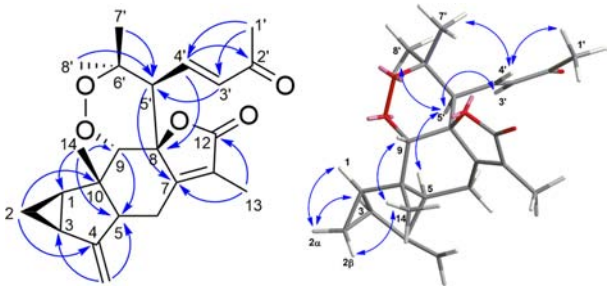

Figure 2. Key HMBC and ROESY correlations of sarglaperoxide A (**1**).

The relative configuration of compound **1** was determined on the basis of a ROESY experiment (Figure 2). The correlations of H-2β/CH_3-14 and H-9/CH_3-14 indicated their cofacial orientation that was arbitrarily assigned as β. Therefore, H-1, H-2α, H-3, and H-5 were assigned as α based on the ROESY correlations of H-1/H-2α and H-3/H-2α, as well as invisible correlation between H-5 and CH_3-14 (or H-9). As a result, the linderane lactone moiety exhibited the same configuration as the analogues in this plant.[4] The cross peaks of H-5/H-5′ and H-5′/CH_3-8′ in the ROESY spectrum demonstrated the α-orientations of H-5′ and CH_3-8′. The configuration of the double bond $\Delta^{3'(4')}$ was assigned as *E*-form according to the ROESY correlations of H-3′/H-5′ and H-4′/CH_3-1′. Consequently, the relative configuration of **1** was established.

In the molecule of compound **1**, there are two adjacent α,β-unsaturated carbonyl chromophores, suggesting a possible exciton chirality for this molecule.[4] Therefore, the CD spectrum of **1** was recorded in methanol, showing a standard positive exciton chirality (Figure 3), with a positive first Cotton effect at 241 nm ($\Delta\varepsilon$ + 3.44) and a negative second Cotton effect at 218 nm ($\Delta\varepsilon$ − 5.32). Thus, a right-handed helicity of two coupling chromophores could be derived from the CD spectrum, demonstrating the absolute configuration of **1** as 1*R*, 3*S*, 5*S*, 8*S*, 9*S*, 10*S*, and 5′*S* (Figure 1). Subsequently, the single crystals of **1** were obtained in methanol and subjected to an X-ray diffraction experiment with Cu Kα radiation. The XRD result confirmed the planar structure and the absolute configuration (Flack parameter x = −0.01 (6)) of **1** (Figure 4).

Sarglaperoxide B (**2**)[13] was obtained as a colorless gum. Its molecular formula was determined as $C_{23}H_{30}O_7$ by HRESIMS (m/z 436.2329 $[M + NH_4]^+$, calcd. for $C_{23}H_{34}NO_7$, 436.2330). The ^{1}H and ^{13}C NMR spectra of **2** displayed similar signals to those of **1**, except for the data at C-4 (δ_C 79.0), C-15 (δ_C 70.2),

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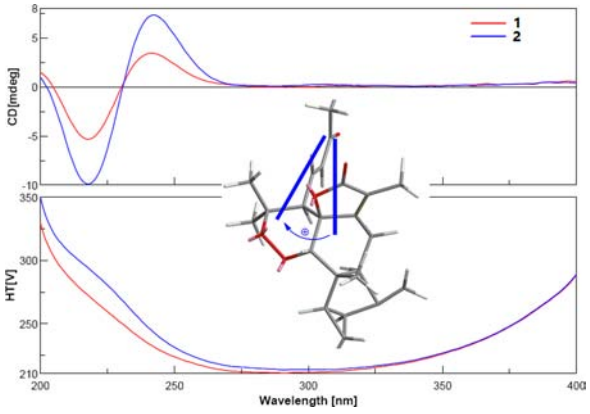


Figure 3. CD spectra of sarglaperoxides A (**1**) and B (**2**) and the stereoview of **1**.

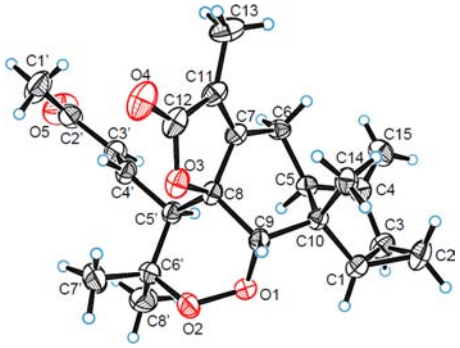
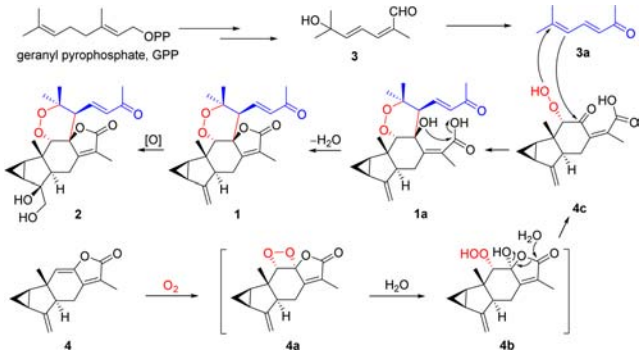


Figure 4. Crystal structure of sarglaperoxide A (**1**).

and H-15 (δ_H 3.75, d, J = 10.5 Hz; 3.72, d, J = 10.5 Hz). According to the change in the NMR spectra, as well as the obvious hydroxyl absorption in its IR spectrum, the molecule of **2** has two hydroxylated carbons replacing the double bond at C-4 and C-15 in compound **1**. Analysis of the 2D NMR spectra of **2** (HSQC, HMBC) confirmed the above proposal and assigned the NMR data of all protons and carbons in the molecule of compound **2** (Table 1). The relative configurations of most chiral centers were characterized to be the same as those of **1** on the basis of ROESY experiment. As for the stereochemistry of C-4, the key ROESY correlations of H-15/H-3 and H-15/H-5 indicated that OH-4 was β-oriented. The CD spectrum of compound **2** was predicted to be identical to that of **1** since they had almost the same excitons in the molecules. As a result, the CD experiment of compound **2**, as expected, afforded a positive exciton chirality with a positive first Cotton effect at 242 nm ($\Delta\varepsilon$ + 7.34) and a negative second Cotton effect at 218 nm ($\Delta\varepsilon$ − 9.91) (Figure 4). Therefore, the absolute configuration of **2** was determined to be 1*R*, 3*S*, 4*S*, 5*R*, 8*S*, 9*S*, 10*S*, and 5′*S* (Figure 1).

Structurally, compounds **1** and **2** present an unprecedented skeleton that is constructed by a linderane lactone and a linear normonoterpene via a six-membered ring. More interestingly, they feature a peroxide bond between the two units. The biogenetic pathways of compounds **1** and **2** attracted our attention and were proposed in Scheme 1. The viewpoint of sesquiterpene−normonoterpene conjugates reminded the formation of two monomers first of all. After excluding Diels−Alder cycloaddition, the retrosynthetic analysis presumed that compounds **3a** and **4c** in Scheme 1 might be the nearest monomers to produce the scaffold of **1** and **2**. An attempt in search of the presumed intermediates **3a** and **4c** led to the

Scheme 1. Plausible Biosynthesis of Compounds 1 and 2



isolation of shizukanolide B (**4**) and another normonoterpene **3** from the PE extract of *S. glabra*. Compound **3** was a nine-carbon normonoterpene and, apparently, could easily convert to **3a** by dehydroxylation, double bond migration, oxidation, and decarboxylation. Compound **4**, considered as the precursor of **4c**, was abundant in the essential oil of the seeds of *S. glabra*. Compound **4** was reported to be unstable on storage and tend to undergo an epoxidation at the enolic double bond (C8 and C9).[14] On this account, **4** was supposed to have a [2 + 2] addition with O_2 to produce **4a** first. A following hydrolysis of the peroxide bond in **4a** created the intermediate **4b** that had a hydroperoxyl group. A further hydrolysis of the lactone ring of **4b** afforded the sesquiterpene monomer **4c**. Finally, **3a** and **4c** constructed the skeleton of the conjugates through a nucleophilic cycloaddition and produced **1a**, which performed a lactonization to form the final product **1**. Oxidation of the exocyclic double bond of **1** could produce **2**.

As a component of traditional Chinese medicine (TCM), *S. glabra* has undergone numerous biological investigations that revealed the antitumor, antibacterial, and antifungal activities of the essential oil of *S. glabra*.[2a] Our previous research also found that some linderane dimers isolated from *S. glabra* had inhibitory effects on NO production in LPS-induced macrophages.[9] As far as **1** and **2** were concerned, the unique structures, especially the peroxide bond, inspired us to investigate their biological activities since many natural peroxides exhibiting significant bioactivities attributed those to their peroxide bonds.[11] The 2015 Nobel Prize for Physiology or Medicine was awarded to Professor Tu for her discovery of artemisinin, a famous antimalarial agent with an active peroxide bond.[15] Accordingly, **1** and **2** received a wide and preliminary investigation on their bioactivities, and as a result, **1** showed moderate antibacterial and anti-inflammatory effects that matched the traditional applications of *S. glabra*. Compound **1** exhibited 64.5% inhibitory effect against *Staphylococcus aureus* at 25 μg/mL and displayed 53.6% inhibitory effects on NO production in LPS-induced RAW264.7 at 25 μM. Neither **1** nor **2** had marked antifungal activity or cytotoxicity.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00112.

Detailed experimental procedures, full spectroscopic data (NMR, MS, and CD) of compounds **1** and **2** (PDF)

Data for compound **1** (CIF)

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Notes

The authors declare no competing financial interest.

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(12) Sarglaperoxide A (**1**): colorless column (CH_3OH); mp 233−235 °C; [α]23 D − 10.3 (*c* 0.14, MeOH); UV (CH_3OH) λ_{max} (log ε) 228 (4.16) nm; CD (MeOH, $\Delta\varepsilon$) λ_{max} 241 (+3.44), 218 (−5.32) nm; IR (KBr) ν_{max} 2927, 2899, 1748, 1675, 1384, 1252, 999 cm^{-1}; HRESIMS *m*/*z* 402.2274 [M + NH_4] $^+$, calcd. for $C_{23}H_{32}NO_5$, 402.2275).

(13) Sarglaperoxide B (**2**): colorless gum; [α]23 D + 21.2 (*c* 0.16, MeOH); UV (CH_3OH) λ_{max} (log ε) 229 (4.21) nm; CD (MeOH, $\Delta\varepsilon$) λ_{max} 242 (+7.34), 218 (−9.91) nm; IR (KBr) ν_{max} 3442, 2976, 2933, 1747, 1677, 1384, 1255, 1020 cm^{-1}; HRESIMS *m*/*z* 436.2329 [M + NH_4] $^+$, calcd. for $C_{23}H_{34}NO_7$, 436.2330).

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