

# C<sub>28</sub> Terpenoids from Lamiaceous Plant *Perovskia scrophulariifolia*: Their Structures and Anti-neuroinflammatory Activity

Naonobu Tanaka, Kanji Niwa, Seita Kajihara, Daisuke Tsuji, Kohji Itoh, Nilufar Z. Mamadalieva, and Yoshiki Kashiwada\*



**ABSTRACT:** Structurally unique  $C_{28}$  terpenoids, perovsfolins A (1) and B (2), were isolated from the aerial parts of an Uzbek medicinal plant, *Perovskia scrophulariifolia* (Lamiaceae). Their chemical structures including an unprecedented 6/8/6/6/6 pentacyclic carbon skeleton with a  $C_6-C_3$  ester moiety were elucidated on the basis of spectroscopic analyses aided by density functional theory calculations as well as chemical evidence. Perovsfolin B (2) exhibited an anti-neuroinflammatory activity.

he Perovskia plants, a small group of the Lamiaceae family, are aromatic shrubs growing in the arid region of Central Asia.<sup>1</sup> Various diterpenes and related terpenoids such as abietane diterpene glycosides,<sup>2</sup> 20-norabietane diterpenes,<sup>3</sup> diterpene dimers,<sup>4</sup> and a C<sub>23</sub> terpene<sup>5</sup> have been isolated from P. atriplicifolia, whereas abietane and 20-norabietane diterpenes<sup>6</sup> and conjugates of icetexane diterpene and monoterpene have also been isolated from another species, P. abrotanoides. The aerial parts of P. scrophulariifolia have been used as a traditional herbal medicine to treat dermatitis and human intestinal parasites in Uzbekistan, from which abietane diterpenes have been reported to date.<sup>8</sup> As part of our research for specialized metabolites possessing unique chemical structures from traditional herbal medicines collected around the world including Japan,<sup>9</sup> China,<sup>10</sup> Mongolia,<sup>11</sup> and Uzbekistan,<sup>12</sup> the aerial parts of *P. scrophulariifolia* have been investigated to furnish two novel C28 terpenoids, perovsfolins A (1) and B (2). We describe herein the isolation, structure elucidation, and evaluation of biological activity of 1 and 2.

The MeOH extract from the aerial parts of *P. scrophulariifolia* was partitioned with EtOAc and water. The EtOAcsoluble materials were dissolved in 90% MeOH aq. and then partitioned with *n*-hexane. The defatted materials were repeatedly separated by chromatographies to give perovsfolins A (1, 3.7 mg) and B (2, 2.7 mg) together with a known compound (3), which was identified as methyl rosmarinate.<sup>13</sup>

Perovsfolin A (1), a colorless amorphous solid, was optically active {[ $\alpha$ ]<sub>D</sub> +251.1 (*c* 0.1, MeOH)}. UV absorptions at 249, 288, and 335 nm revealed that 1 had a conjugated system. The

<sup>1</sup>H NMR spectrum displayed the resonances due to eight aromatic or olefinic protons, five sp<sup>3</sup> methines, of which two were oxygenated, three sp<sup>3</sup> methylenes, and three tertiary methyls, including one methoxy methyl, and two secondary methyls (Table S1). The <sup>13</sup>C NMR spectrum showed 38 signals including one sp<sup>3</sup> quaternary, one oxygenated tertiary, 20 aromatic or olefinic, and three carbonyl carbon signals. These NMR data and an ion peak at m/z 677.2360 ([M + Na]<sup>+</sup>, calcd for C<sub>38</sub>H<sub>38</sub>O<sub>10</sub>Na<sup>+</sup>, 677.2357) obtained by the high-resolution electrospray ionization mass spectrometry (HRESIMS) suggested the molecular formula of **1** to be C<sub>38</sub>H<sub>38</sub>O<sub>10</sub>.

2D NMR analysis implied that 1 consisted of two partial structures (units A and B). The presence of one 1,2,4,5-tetrasubstituted benzene (C-1'-C-6') and one 1,2,4-trisub-stituted benzene (C-10'-C-15') was revealed by analysis of the 1D NMR and heteronuclear multiple bond correlation (HMBC) spectra (Figure 1), whereas the <sup>13</sup>C resonances of C-3' ( $\delta_{\rm C}$  148.5), C-4' ( $\delta_{\rm C}$  147.1), C-12' ( $\delta_{\rm C}$  147.1), and C-13' ( $\delta_{\rm C}$  146.3) implied the existence of hydroxy groups at these aromatic carbons. The spin systems of H-7'/H-8' and H<sub>2</sub>-16'/

Received: August 25, 2020





Figure 1. Selected 2D NMR correlations and partial structures (units A and B) of perovsfolin A (1).

H-17' were found by <sup>1</sup>H-<sup>1</sup>H COSY analysis, and the connectivities of C-7' to C-1' and of C-16' to C-10' were assigned by HMBC analysis. In addition, HMBC cross-peaks of the methoxy signal and  $H_2$ -16' to one ester carbonyl carbon (C-18',  $\delta_{\rm C}$  170.4) and of H-7' and H-17' to the other carbonyl carbon (C-9',  $\delta_{\rm C}$  174.4) suggested the connectivity of a methoxy carbonyl group to C-17' and the presence of an ester linkage between C-9' and C-17'. Therefore, the partial structure corresponding methyl rosmarinate (C-1'-C-18')was assigned as unit B. Meanwhile, the interpretation of the <sup>1</sup>H-<sup>1</sup>H COSY and HMBC spectra lead to the structures of an  $\alpha$ -isopropyl- $\alpha$ , $\beta$ -unsaturated ketone moiety (C-12-C-17) and a tetrahydronaphthalene moiety (C-1-C-10) with a geminal dimethyl group at C-4. HMBC correlations for H-14 with C-7, C-8, and C-9 suggested the connectivity between C-8 and C-14. Thus the gross structure of unit A was assigned, as shown in Figure 1.

The linkage between C-1 in unit A and C-8' in unit B was evident from a  ${}^{1}\text{H}{-}^{1}\text{H}$  COSY cross-peak of H-1/H-8' and an HMBC correlation for H-8'/C-10 (Figure 2). Furthermore, the connectivities among C-9, C-12, and C-6' via an oxygenated tertiary carbon (C-11,  $\delta_{\rm C}$  96.7) and of C-7' and C-11 via an ether linkage were elucidated by HMBC cross-peaks of H-5' and H-7' to C-11, taking the molecular formula of 1 into consideration, to appear as a structure corresponding to a 20-norabietane diterpene (C-1–C-19). This assignment



Figure 2. Selected 2D NMR correlations and the planar structure of perovsfolin A (1).

was further supported by a rotating frame Overhauser effect spectroscopy (ROESY) correlation for  $H_3$ -16/H-5'. Thus the planar structure of perovsfolin A (1) was assigned, as shown in Figure 2.

The H-1/H-8'-anti and H-7'/H-8'-syn relationships of 1 were implied by the analysis of the coupling constants  $({}^{3}J_{\text{H-1/H-8'}} = 11.2 \text{ Hz and } {}^{3}J_{\text{H-7'/H-8'}} = 3.4 \text{ Hz})$ . The relative configurations were underpinned by ROESY correlations among protons on the  $\beta$ -side of the molecule (H-1/H-3 $\beta$  and H-1/H-7') as well as those on the  $\alpha$ -side (H-2 $\alpha$ /H<sub>3</sub>-18 and H-2 $\alpha$ /H-8') (Figure 3). In addition, the C-11R\* configuration of 1 was assigned by a steric restriction of the dihydrofuran ring (C-1', C-6', C-7', and C-11').



Figure 3. Selected ROESY correlations and the relative configuration of perovsfolin A (1). (C-9'-C-18') and protons of methyl groups are omitted.)

Perovsfolin B (2) was obtained as an optically active amorphous solid {[ $\alpha$ ]<sub>D</sub> -190.7 (*c* 0.1, MeOH)}, and its molecular formula was determined to be the same as 1 by HRESIMS analysis. The 1D NMR spectra of 2 were closely correlated with those of 1 (Table S1), suggesting that 2 was a stereoisomer of 1. The ROESY spectrum of 2 showed correlations similar to those of 1, implying that the relative configurations of C-1, C-11, C-7', and C-8' for 2 and 1 were the same.

The absolute configurations of C-17' in perovsfolins A (1)and B (2) were confirmed as follows (Scheme 1). Methylation of 1 and 2 with CH<sub>3</sub>I afforded permethyl derivatives (1a and 2a). Treatment of 1a and 2a with NaOH in MeOH/acetone gave terpenoid (1b and 2b, respectively) and  $C_6-C_3$  (1c and 2c, respectively) moieties in each case. The latter products, both of which were shown to have a carboxylic acid group, were further treated with TMS-CHN<sub>2</sub> to furnish trimethyltanshinols (1d and 2d). In contrast, the methylation of methyl rosmarinate (3) gave a rosmarinic acid permethylate (3a), which was subsequently treated with K<sub>2</sub>CO<sub>3</sub>/MeOH to give 3b. The R configuration of 3b was confirmed by the comparison of its specific rotation value {[ $\alpha$ ]<sub>D</sub> +11.7 (c 0.3,  $(CH_2Cl_2)$  with the literature value { $[\alpha]_D$  +10.6 (c 0.67,  $(CH_2Cl_2)$ }.<sup>14</sup> The racemate of **3b** was obtained according to the procedure described in the literature.<sup>15</sup> Thus Dess-Martin oxidation of (+)-3b followed by reduction with NaBH<sub>4</sub> gave  $(\pm)$ -3b. The separation of the enantiomers  $\{(\pm)$ -3b $\}$  on chiral high-performance liquid chromatography (HPLC) showed the separated peaks [ $t_R$  12.1 min for (-)-3b; 14.7 min for (+)-3b], whereas analyses of 1d and 2d under the same conditions gave a single peak at 14.7 min in each case. These findings clearly indicated the absolute configuration of C-17' in both 1 and 2 to be R.

Scheme 1. Chemical Conversions of Perovsfolins A (1) and B (2) and Methyl Rosmarinate (3)



The absolute configurations of the terpenoid moieties (1b and 2b) were assigned by electron circular dichroism (ECD) analysis. The ECD spectra of 1b and 2b indicated that they were enantiomers, and the spectrum of 1b was well correlated with the time-dependent density functional theory (TDDFT) calculated spectrum of a possible enantiomer with the 1*R*, 11*R*, 7'*R*, and 8'*R* configurations (Figure 4). Accordingly, the 1*R*,



Figure 4. Experimental and TDDFT calculated ECD spectra of the terpenoid moieties (1b and 2b) of perovsfolins A (1) and B (2).

11*R*, 7'R, 8'R, and 17'R configurations of perovsfolin A (1) and the 1*S*, 11*S*, 7'S, 8'S, and 17'R configurations of perovsfolin B (2) were elucidated. It is noteworthy that 1 and 2 showed Cotton effects similar to those of 1b and 2b, respectively (Figure S1).

The investigation of the aerial parts of an Uzbek medicinal plant *Perovskia scrophulariifolia* resulted in the isolation of two terpenoids, perovsfolins A (1) and B (2), possessing a  $C_{28}$  terpenoid moiety with an unprecedented 6/8/6/6/6 pentacyclic carbon skeleton. Perovsfolins A (1) and B (2) were

presumed to be generated by the condensation of a 20norabietane diterpene, dehydromiltirone,<sup>16</sup> and methyl rosmarinate (3) (Scheme S1), where spontaneous (nonenzymatic) reactions may be involved. 20-Norabietane diterpenes are recognized as constituents of Lamiaceous plants belonging to the genera *Salvia*<sup>17</sup> and *Perovskia*.<sup>3,6</sup> In contrast, rosmarinic acid is a caffeic acid ester of tanshinol widely distributed in the plant kingdom and shows various biological activities such as anti-inflammatory, antioxidant, and neuroprotective activities.<sup>18</sup> Methyl rosmarinate (3) has also been reported to be a plant metabolite possessing an anti-inflammatory activity.<sup>19</sup>

Letter

In our search for natural products to be a potential lead for a therapeutic agent for neuroinflammation-related diseases,<sup>11</sup> perovsfolins A (1) and B (2) were evaluated for their antiinflammatory activity on microglial cells. Thus perovsfolin B (2) demonstrated an inhibitory effect on IL-1 $\beta$  production from lipopolysaccharide (LPS)-stimulated microglial cells (EC<sub>50</sub> 38.4  $\mu$ M) without cytotoxicity against microglial cells (IC<sub>50</sub> > 50  $\mu$ M). Their antiproliferative activity against human cancer cell lines (A549, HeLa, and MCF-7) was also evaluated, and 2 showed cytotoxicity with IC<sub>50</sub> values of 32.1, 26.5, and 29.6  $\mu$ M, respectively. In contrast, neither anti-inflammatory nor antiproliferative activities were found in 1.

## ASSOCIATED CONTENT

#### **Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c02855.

Experimental section and 1D and 2D NMR spectra of perovsfolins A and B (PDF)

#### AUTHOR INFORMATION

#### **Corresponding Author**

Yoshiki Kashiwada – Graduate School of Pharmaceutical Sciences, Tokushima University, Tokushima 770-8505, Japan; orcid.org/0000-0002-5429-3211; Email: kasiwada@ tokushima-u.ac.jp

#### Authors

- Naonobu Tanaka Graduate School of Pharmaceutical Sciences, Tokushima University, Tokushima 770-8505, Japan; orcid.org/0000-0002-9281-7098
- Kanji Niwa Graduate School of Pharmaceutical Sciences, Tokushima University, Tokushima 770-8505, Japan
- Seita Kajihara Graduate School of Pharmaceutical Sciences, Tokushima University, Tokushima 770-8505, Japan
- Daisuke Tsuji Graduate School of Pharmaceutical Sciences, Tokushima University, Tokushima 770-8505, Japan
- Kohji Itoh Graduate School of Pharmaceutical Sciences, Tokushima University, Tokushima 770-8505, Japan
- Nilufar Z. Mamadalieva Institute of the Chemistry of Plant Substances, Academy Sciences of of the Republic of Uzbekistan, Tashkent 100170, Uzbekistan

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.orglett.0c02855

#### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

This work was partly supported by JSPS KAKENHI grant number JP17K08337.

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