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Communication

Phthalide-derived oxaspiroangelioic acids A–C with an unprecedented carbon skeleton from an aqueous extract of the *Angelica sinensis* root head

Youzhe Chen, Chengbo Xu, Weiping Wang, Xiaoliang Wang, Qinglan Guo*, Jiangong Shi*

State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

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ABSTRACT

Three phthalide-derived analogues, oxaspiroangelioic acids A–C (**1**–**3**), were isolated as minor components of an aqueous extract of the *Angelica sinensis* root heads (guitou). Oxaspiroangelioic acids A and B were racemates separated into enantiomers by chiral HPLC. Their structures including absolute configurations were determined by spectroscopic data analysis, single crystal X-ray diffraction, exciton chirality method and electronic circular dichroism (ECD) calculation. These compounds share an undescribed carbon skeleton, for which biosynthetic pathways are proposed. Compound **1** and its enantiomers showed almost identical activity inhibiting Tandem of P domains in a weak inwardly rectifying K^+ channel 1 (TREK-1).

Danggui, the dried whole root of *Angelica sinensis* (Oliv.) Diels (Umbelliferae), is a popular traditional Chinese medicine for the treatment of various diseases [1]. It is also used as food/dietary supplement in the Western countries [2,3]. In traditional Chinese medicine, different parts of the root are described to have different medicinal functions. The root head (guitou) is hemostasis to induce blood up going, the root body (guishen) nourishes blood to guard heart, and the root tail (guiwei) activates blood to induce blood down going [1]. Extensive chemical and pharmacological studies were previously performed on crude extracts of the whole roots, and around 100 chemical constituents, including phthalides, phenylpropanoids, coumarins, lignans, alkynes, terpenes, sterols, alkaloids, fatty acids and polysaccharides [2–10], have been documented. Ferulic acid, *Z*-ligustilide and polysaccharides are considered as the main active constituents of the dried whole root (danggui). However, there is no specific investigation on the root head (guitou). Thus, as part of our program to access the chemical diversity of traditional Chinese medicines, focusing on the minor constituents [11–19], we investigated an aqueous extract of guitou, to keep relative consistency with classic utilization method of water decoctions of the drug materials. In a previous paper, we reported a pair of novel neolignan enantiomers from the extract [20]. A continuation of the study has resulted in isolation and structural determination of three phthalide-derived analogues with a novel carbon skeleton, oxaspiroangelioic acids A–C (**1**–**3**) (Fig. 1), from the same extract. Oxaspiroangelioic acids A and B (**1** and **2**) were racemates that were separated into enantiomers (+)-/(−)-**1** and (+)-/(−)-**2**, respectively, while oxaspiroangelioic acid C (**3**) was optical pure. Herein, are detailed isolation (Supporting information), structural elucidation, plausible biosynthesis and bioactivity of **1**–**3**.

Racemate **1** was obtained as colorless crystals. Its IR spectrum indicated the presence of carbonyls (1702 and 1667 cm^{-1}) in the molecule. The molecular formula was determined as $C_{16}H_{22}O_4$ with six degrees of unsaturation by (−)-HR-ESIMS at *m/z* 277.1444 $[M-H]^-$ (calcd. for $C_{16}H_{21}O_4$, 277.1445) and NMR spectroscopic data (Supporting information Table S1). The ^1H NMR spectrum of **1** in acetone-d_6 exhibited proton signals ascribable to a conjugated trisubstituted double bond at δ_H 7.43 (dd, J = 4.8 and 3.0 Hz, H-7) and two inequivalent propyls attaching to olefinic carbons at δ_H 2.48/2.11 (t, J = 7.2 Hz, H_2-2′/H_2-9), 1.65/1.46 (hex, J = 7.2 Hz, H_2-3′/H_2-10) and 0.96/0.88 (t, J = 7.2 Hz, H_3-4′/H_3-11), along with partially overlapping signals attributable to three vicinal coupling methylenes between δ_H 2.44 and 1.69 (6*H*, m, H_2-4, H_2-5 and H_2-6). The ^{13}C NMR and DEPT spectra of **1** showed 16 carbon signals (Table S1) corresponding to the above proton-bearing units and six quaternary carbons, including five sp^2 carbons and an oxygen-bearing sp^3 carbon. When compared with those of the previously reported

* Corresponding authors.
E-mail addresses: guonina@imm.ac.cn (Q. Guo), shijg@imm.ac.cn (J. Shi).

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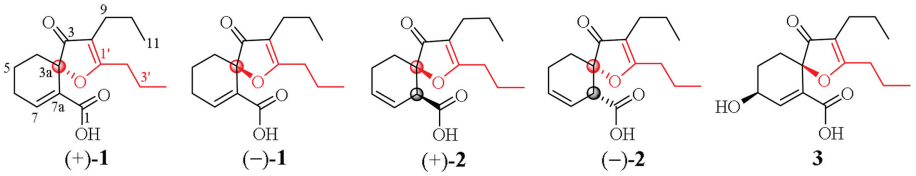


Fig. 1. Structure of compounds (+)/(−)-**1**, (+)/(−)-**2** and **3**.

constituents from *A. sinensis* [3–8], these spectroscopic data demonstrated that **1** was a phthalide analogue having an additional four carbon unit to give an abnormal structure, which was further determined by 2D NMR experiments (Fig. 2).

Analysis of the HSQC spectrum assisted assignment of the proton-bearing carbon and corresponding proton signals in the NMR spectra of **1**. The ^{1}H–^{1}H COSY spectrum showed homonuclear coupling cross-peaks of H_2-4/H_2-5/H_2-6/H-7, H_2-9/H_2-10/H_3-11 and H_2-2′/H_2-3′/H_3-4′, proving the presence of three vicinal proton coupling fragments (Fig. 2, bold lines). In the heteronuclear multiple bond correlation (HMBC) spectrum, the three-bond correlations from H_2-4 and H_2-6 to the quaternary sp^2 carbon (C-7a, δ_C 129.1), from H_2-5 and H-7 to the quaternary sp^3 carbon (C-3a, δ_C 83.4); and from H-7 to the sp^2 quaternary carbon (C-1, δ_C 165.6) (arrows in Fig. 2), along with their chemical shifts, demonstrated that there was a disubstituted cyclohexene-carboxylic acid moiety in **1**. The HMBC correlations from H_2-10 and H_2-2′ to the sp^2 quaternary carbon (C-8, δ_C 114.3) and from H_2-9 and H_2-3′ to the other sp^2 quaternary carbon (C-1′, δ_C 184.6) indicated that the two propyls connected to the two ends of a vinyl unit (C-8 and C-1′), respectively. In addition, the HMBC correlations from H_2-4 and H_2-9 to the remaining ketone carbonyl (C-3, δ_C 205.8) inserted the carbonyl between C-3a and C-8. To satisfy requirements of the molecular formula as well as substitution and chemical shifts of C-3a and C-1′, the two carbons must share an oxygen to form an oxaspiro[4,5]decane-carboxylic acid motif in **1**. Thus, the planar structure of **1** was elucidated as an undescribed phthalide derivative as shown. Crystallization of **1** in n-hexane afforded single crystals. Follow up X-ray diffraction analysis proved that **1** was a racemate with the P-1 space group, an ORTEP drawn crystal structure with the relative configuration shown in Fig. 3.

Separation of **1** by semi-preparative chiral HPLC (Fig. S1 in Supporting information) obtained (+)-**1** {$[\alpha]_D^{20}$ + 55.5 (*c* 0.29, MeOH)} and (−)-**1** {$[\alpha]_D^{20}$ − 59.2 (*c* 0.29, MeOH)}, with the ^{1}H NMR spectra identical to that of **1** before separation (Supporting information), but the circular dichroism (CD) curves mirrored each other (Fig. S8 in Supporting information). The CD spectra displayed typical exciton coupling Cotton effects (CEs) at λ_{max} 267.5 and 300.5 nm, arising from coupling between *n*-π^* transition moments of the two chromophores in the structures. By application of the exciton chirality method [21], the positive and negative exciton coupling CEs of (+)- and (−)-**1** predicted 3a*S*- and 3a*R*-configurations (Figs. S29 and S30 in Supporting information), respectively. The prediction was supported by consistency of the experimental CD and calculated electronic circular dichroism

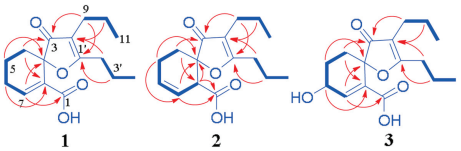


Fig. 2. The ^{1}H–^{1}H COSY (thick lines) and main two- and three-bond HMBC correlations (red arrows, from ^{1}H to ^{13}C) of **1**–**3**.

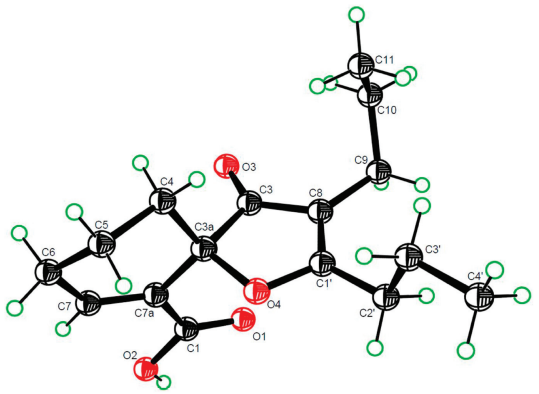


Fig. 3. An ORTEP drawn crystal structure of **1**.

(ECD) spectra between (+)-**1** and 3a*S*-**1** as well as between (−)-**1** and 3a*R*-**1**, respectively (Fig. S8). Therefore, the structures of (+)- and (−)-**1** were determined and trivially designated as (+)-(3a*S*)- and (−)-(3a*R*)-oxaspiroangelioic acids A, respectively.

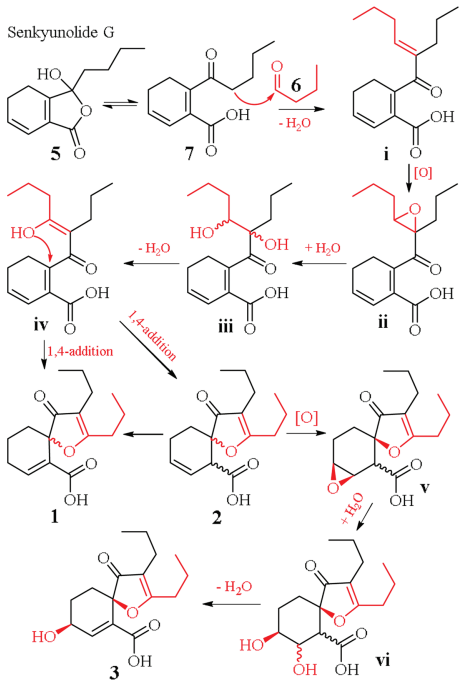
Racemate **2** was an isomer of **1** as shown by spectroscopic data. Comparison of the NMR spectroscopic data of the two compounds (Table S1) revealed that one methylene and one quaternary sp^2 carbon in **1** were replaced by a sp^2 methine [δ_H 5.85 (m, H-6) and δ_C 127.3 (C-6)] and a sp^3 methine [δ_H 3.56 (brs, H-7a) and δ_C 46.3 (C-7a)] in **2**, respectively. In addition, C-4 and C-7 in **2** were shielded significantly by $\Delta\delta_C$ −4.0 and −24.6, whereas C-1 and C-5 were deshielded by $\Delta\delta_C$ +5.2 and +3.6, respectively. These variations suggested that the double bond at C-7 in **1** migrated to C-6 in **2**. The suggestion was proved by 2D NMR data analysis of **2**, especially by the ^{1}H–^{1}H COSY cross-peaks of H_2-4/H_2-5/H-6/H-7/H-7a and the HMBC correlations of H_2-5/C-3a and C-7 (Fig. 2). In addition, in the ^{1}H NMR spectrum of **2**, H-7a appeared as a broad singlet, indicating that the dihedral angle between H-7 and H-7a was nearly perpendicular and that the cyclohexene ring had a preferential half/twist chair conformation with H-7a occupying a pseudo-axial position. This was supported by a NOE cross-peaks between H-7a and H-4A (Fig. S26 in Supporting information). The racemate **2** was separated by semi-preparative chiral HPLC (Fig. S2 in Supporting information) into (+)-**2** {$[\alpha]_D^{20}$ + 150.0 (*c* 0.08, MeOH)} and (−)-**2** {$[\alpha]_D^{20}$ − 145.0 (*c* 0.08, MeOH)}. Because the relative configuration could not be deduced from the above spectroscopic data, the ECD spectra of two pairs of the possible enantiomers of **2** were calculated (Figs. S9 and S12 in Supporting information). The calculated ECD curves of the (3a*R*,7a*S*)- and (3a*S*,7a*R*)-enantiomers were fully consistent with the measured CD spectra of (+)- and (−)-**2** (Fig. S9), respectively. In contrast, in the low wavelength region the calculated ECD curves of the (3a*R*,7a*R*)- and (3a*S*,7a*S*)-enantiomers completely differed from the measured CD spectra of (+)- and (−)-**2** (Fig. S12). Thus, stereochemistry of (+)- and (−)-**2** was assigned as 3a*R*,7a*S* and 3a*S*,7a*R*, respectively. The assignment was consistent with that predicted by the exciton

chirality method (Figs. S43 and S44 in Supporting information). Therefore, the structures of (+)- and (−)-**2** were determined and named (+)-(3a*R*,7a*S*)- and (−)-(3a*S*,7a*R*)-oxaspiroangelioic acids B, respectively.

Compound **3** was obtained as a white amorphous powder with $[\alpha]_D^{20}$ +81.0 (*c* 0.21, MeOH). The spectroscopic data of **3** demonstrated that it was an optical active derivative of **1**. When compared the NMR spectroscopic data of **3** and **1** (Table S1), replacement of one methylene in **1** by an oxygen-bearing methine [δ_H 4.42 (ddd, *J* = 5.4, 4.2, 3.6 Hz, H-6) and δ_C 64.8 (C-6)] and significant deshielded shift of C-5 ($\Delta\delta_C$ +9.4) in **3** revealed that **3** was the 6-hydroxy derivative of **1**. The deduction was proved by the ^1H–^1H COSY cross-peaks of H_2-4/H_2-5/H-6/H-7 and the HMBC correlation of H-6/C-7a. The coupling constants of H-6 ($J_{5A,6}$ = 4.2 Hz, $J_{5B,6}$ = 5.4 Hz and $J_{6,7}$ = 3.6 Hz) revealed that this proton had the pseudo-equatorial orientation on the cyclohexene ring with the half chair conformation in **3** (Fig. S27 in Supporting information). By comparison of the measured CD and calculated ECD spectra of all the possible stereoisomers (Figs. S13 and S15 in Supporting information), the configuration of **3** was assigned as 3a*R*,6*S*, which was supported by prediction of the exciton chirality method (Fig. S57 in Supporting information). Thus, the structure of compound **3** was determined and named (+)-(3a*R*,6*S*)-oxaspiroangelioic acid C.

It is worth noting that **2** was unstable to generate a main product when stored under an ambient condition. Using the aforementioned methods, the product was isolated, separated into enantiomers and structurally determined (Supporting information Scheme S1) as (+)-(3a*S*)- and (−)-(3a*R*)-6-oxo-oxaspiroangelioic acids C, respectively. This indicated that **2** was readily oxidized by air (Scheme S1).

According to the unique structures, consisting of the phthalide-derived motif and the additional four carbon units, the biosynthetic pathways of **1**–**3** are proposed in Scheme 1. The abundant co-occurring senkyunolide G (**5**) [3] and butanal (**6**) are preferential precursors of **1**–**3**. Isomerization of **5** gives a ketene acid **7**, which undergoes Aldol condensation with **6** to yield an intermediate (**i**).



Scheme 1. The plausible biosynthetic pathway of **1**–**3**.

An enzymatic epoxidation of the intermediate, followed by epoxide ring opening of the product (**ii**), affords a diol (**iii**). Subsequent dehydration of **iii** generates **iv**, which goes intramolecular cyclization *via* 1,4-addition to produce **1** and **2**, while **1** would be produced from **2** *via* double bond migration rearrangement. An enantiomer of **2** goes sequentially epoxidation, epoxide ring opening and dehydration through **v** and **vi** to yield **3**. Because **3** was optically active but **1** and **2** inactive, the 1,4-addition of **iv** might be non-enzymatic or enzyme-uncontrolled reactions. Alternatively, **1**–**3** may be produced from Aldol condensation between (*Z*)-ligustilide and butanal (Supporting information Scheme S2).

In a cell-based preliminary bioassay, among the isolates, only **1** and (+)- and (−)-**1** inhibited Tandem of P domains in a weak inwardly rectifying K^+ channel 1 (TREK-1) with half maximal inhibitory concentration (IC_{50}) values of 29.4, 28.1 and 31.3 μmol/L, respectively (the positive control fluoxetine, IC_{50} 27.4 μmol/L) (Fig. S25 in Supporting information). Subsequent methylation of the carboxylic acid or hydrogenation of the cyclohexene ring in **1** (Supporting information) led to loss of activity. The result suggested that the conjugated cyclohexene-carboxylic acid moiety was required by the activity, whereas a change of chirality at C-3a had little influence on the activity.

In conclusion, compounds **1**–**3** were characterized as the minor constituents of the aqueous extract of guitou. These compounds have the undescribed carbon skeleton biogenetically deriving from Aldol condensation of phthalides and butanal. The conjugated cyclohexene-carboxylic acid moiety was essential for the TREK-1 inhibition of this group of compounds. The minor components may have contributions to clinic effects of guitou. The structural novelty enriches the diversity of phthalides and provided a model architecture for synthetic and biosynthetic chemists to obtain enough amount of the samples for further evaluation.

Declaration of competing interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cclet.2021.03.004.

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