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Salvihispin A and its glycoside, two *neo*-clerodane diterpenoids with neurotrophic activities from *Salvia hispanica* L.

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ABSTRACT

A *neo*-clerodane diterpenoid, salvihispin A (**1**), as well as its glycoside, salvihispin A-2-*O*-*β*-D-3-keto-glucopyranoside (**2**) were isolated from the aerial parts of *Salvia hispanica*. Compound **2** possessed an unusual 3-keto-glucopyranoside moiety. Their structures and absolute configurations were elucidated by extensive spectroscopic methods and confirmed by single crystal X-ray diffraction. Salvihispin A (**1**) and its glycoside (**2**) enhanced the neurite outgrowth of NGF-mediated PC12 cells at a concentration of 10 μM.

Clerodane diterpenes are a large group of natural metabolites that found in several hundreds of plant species from various families and in organisms from several taxonomic groups, such as fungi, bacteria, and marine sponges.[1] Some of them have shown interesting biological properties, such as antifeedant,[2] antiprotozoal,[3] anti-inflammatory,[4] cytotoxic activities,[5] as well as NGF-potentiating.[6] In particular, sulfotanshinone sodium injection, as the clinically available tanshinone IIA agent, has been widely used for the treatment of cardiovascular and cerebrovascular diseases.[7] In addition, salvinorin A, a major active clerodane diterpenoid of *Salvia divinorum*, has attracted great interest from chemical biologists and has been used as novel opioid receptor probe,[8] opening additional areas for chemical investigation.[9]

Salvia hispanica L. (Chia), belonging to Lamiaceae family, is native to southern Mexico and northern Guatemala.[10] Traditionally, its seeds were used by Aztecs and Mayas people for the preparation of food, canvases, and folk medicines, which was a source of natural lipid antioxidants.[11] Since most previous research of chemical components and biological activities on *S. hispanica* has focused on its seeds, there is no prior systematic study on the aerial parts of this species. As a continuation of our studies to identify bioactive components from *Salvia* species, the chemical constituents of *S. hispanica* "Chia" were investigated. As a consequence, a new clerodane diterpenoid, salvihispin (**1**), and its glycoside (**2**) with an unusual carbohydrate unit (3-keto-glucopyranoside) (Fig. 1) were isolated. It is worth noting that the absolute configuration of the sugar unit, which was quite rare in nature, was verified by single crystal X-ray diffraction for the first time. Herein, we described the isolation, structure elucidation, and neurotrophic activities of **1** and **2**.

Aerial parts of *S. hispanica* were collected from Kunming Botanical Garden, Yunnan Province, which were cultivated and identified by Prof. Xiao Cheng of Kunming Institute of Botany, Chinese Academy of Sciences (voucher No. 2015-10A-09B). The air-dried powder of the aerial parts of *S. hispanica* (26 kg) was extracted with acetone (50 L × 3, each 24 h) to yield 1.5 kg of a crude extract, which was subjected to silica gel chromatography column (CC), and eluted with petroleum ether/acetone (100:0–0:100) to give five fractions. Fr.1-Fr.5. Of these, Fr.4 (270 g) was chromatographed over repeated silica gel columns (petroleum ether/$CHCl_3$/EtOAc) to afford **1**[12] (10.4 mg), and Fr.5 (220 g) was further purified by silica gel CC (petroleum ether/$CHCl_3$/EtOAc) and semipreparative HPLC (CH_3OH-H_2O, 45:55) to yield **2**[13] (15.5 mg).

Compound **1** was isolated as colorless prism crystals. Its HRESIMS peak established a molecular formula of $C_{20}H_{24}O_5$ via a sodium adduct ion at *m*/*z* 367.1513 (calcd 367.1516), implying nine degrees of unsaturation. The IR absorption bands at 3401 and 1766 cm^{-1} suggested the presence of hydroxy and carbonyl functionalities, respectively. In the ^1H NMR spectrum of **1** (Table 1), characteristic signals attributable to one methyl group (δ_H 1.14, CH_3-20), one typical *β*-substituted furan ring [δ_H 6.34 (dd, *J* = 1.6, 0.7 Hz, H-14), 7.39 (dd, *J* = 1.6, 1.6 Hz, H-15), and 7.34 (dd, *J* = 1.6, 0.7 Hz, H-16)] were observed. The ^{13}C NMR and DEPT spectra (Table 1) exhibited 20 carbon resonances comprising one methyl,

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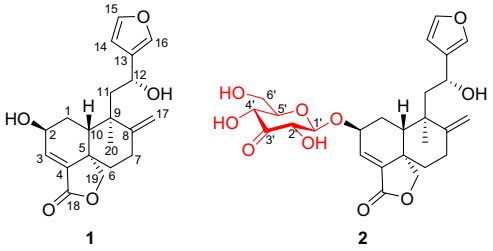

Fig. 1. Chemical structures of **1** and **2**.

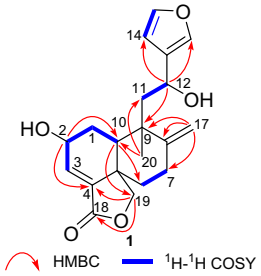

Fig. 2. Key ^{1}H–^{1}H COSY and HMBC correlations of **1**.

six methylenes (one oxygenated and one olefinic), seven methines (two oxygenated and four olefinic), and six quaternary carbons (one ester carbonyl and three olefinic). The ^{1}H–^{1}H COSY spectrum showed the existence of four fragments: –CH–CH_2–CH–CH– (for C10/C1/C2/C3), –CH_2–CH_2– (for C6/C7), –CH_2–CH– (for C11/C12), –CH–CH– (for C14/C15). The HMBC correlations from H_2-19 to the ester carbonyl (C-18) verified the 18,19-lactone unit. The aforementioned spectroscopic data showed that **1** was a clerodan-18,19-olide *neo*-clerodane diterpenoid similar to 7α,12α-dihydroxyhautriwaic acid-19-lactone,[14,15] which previously isolated from *Heteropappus altaicus*. However, taking the molecular formula into account, the observed differences between **1** and 7α,12α-dihydroxyhautriwaic acid-19-lactone[15] were the presence of an exocyclic double bond (δ_C 112.4, 153.4; δ_H 5.09, 5.04) in **1** instead of the methyl group (δ_H 0.99) and one methine (δ_H 1.88) in the latter, and that one hydroxy group attached to C-2 in **1** was observed instead of that located at C-7 in 7α,12α-dihydroxyhautriwaic acid-19-lactone, which were confirmed through the HMBC correlations (Fig. 2) from H_2-17 (δ_H 5.09, 5.04) to C-7 (δ_C 28.8), C-8 (δ_C 153.4), C-9 (δ_C 42.6), and H-2 (δ_H 4.47) to C-3 (δ_C 132.8), C-4 (δ_C 141.9), C-10 (δ_C 42.1), respectively. Thus, the planar structure of **1** was established.

The relative configuration of **1** was established by a ROESY experiment (Fig. 3). The distinctive ROESY correlations of H_3-20/H_2-19, H-19a/H-6a and H-6b/H-10 suggested that H_2-19 and H_3-20 were α-oriented, as well as H-10 was β-orientated. However, it is difficult to determine the configurations of C-2 and C-12 by the ROESY spectrum, which required the growth of high quality single crystal. Finally, a single crystal was obtained from methanol solution and was suitable for X-ray crystallography (Fig. 4). Final refinement of the diffraction data resulted in a small Flack parameter 0.00(6), allowing the assignment of the absolute configuration of **1** as 2*S*,5*S*,9*R*,10*R*,12*R*.[16] Therefore, compound **1** was established and named as salvihispin A. Crystallographic data of **1** have been deposited at the Cambridge Crystallographic Data Centre (CCDC) (deposition No. 1495554).

Compound **2** was obtained as colorless plate crystals. The UV spectrum revealed the absorption maximum at 206 nm. Absorption bands at 3426 and 1766 cm^{-1} in the IR spectrum of **2** were

Table 1
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of **1** and **2** in CD_3OD (δ in ppm, *J* in Hz).

	1		**2**	
No	δ_H	δ_C	δ_H	δ_C
1a	1.82 (dt, 13.6, 2.8)	30.1	2.14 (dt, 13.7, 2.5, 2.5)	28.5
1b	1.40 (td, 13.6, 2.8)		1.43 (td, 13.7, 13.7, 2.4)	
2	4.47 (m)	64.5	4.73 (m)	71.7
3	6.67 (d, 6.5)	132.8	6.76 (d, 6.4)	130.4
4		141.9		143.7
5		45.9		46.1
6a	1.97 (m)	34.1	1.97 (dt, 12.1, 9.7)	33.9
6b	1.59 (t, 11.9)		1.58 (t, 12.1)	
7a	2.53 (m)	28.8	2.56 (m)	28.7
7b	2.37 (m)		2.36 (dd, 13.8, 9.7)	
8		153.4		153.6
9		42.6		42.6
10	2.46 (dd, 13.6, 2.8)	42.1	2.56 (m)	42.0
11a	2.02 (dd, 14.5, 8.5)	47.8	2.07 (dd, 14.8, 8.1)	47.9
11b	1.75 (d, 14.5)		1.81 (dd, 14.8, 1.0)	
12	4.70 (d, 8.5)	64.1	4.71 (m)	64.6
13		132.5		132.6
14	6.34 (dd, 1.6, 0.7)	109.7	6.34 (dd, 1.7, 0.8)	109.8
15	7.39 (dd, 1.6, 1.6)	144.3	7.39 (dd, 1.7, 1.7)	144.3
16	7.34 (dd, 1.6, 0.7)	139.6	7.36 (dd, 1.7, 0.8)	139.7
17a	5.09 (s)	112.4	5.06 (s)	112.3
17b	5.04 (s)		5.03 (s)	
18		172.1		171.8
19a	4.25 (d, 7.8)	74.4	4.27 (d, 7.8)	74.3
19b	4.09 (dd, 7.8, 1.6)		4.11 (dd, 7.8, 1.6)	
20	1.14 (s)	20.5	1.14 (s)	20.4
1′			4.51 (d, 7.9)	104.9
2′			4.12 (dd, 7.9, 1.6)	78.4
3′				206.9
4′			4.20 (dd, 10.2, 1.2)	73.7
5′			3.37 (ddd, 10.2, 5.2, 1.4)	78.5
6′a			3.94 (dd, 12.0, 1.4)	62.5
6′b			3.76 (dd, 12.0, 5.2)	

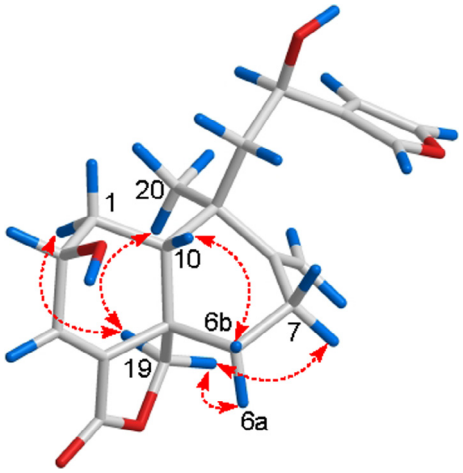

Fig. 3. Key ROESY correlations of **1**.

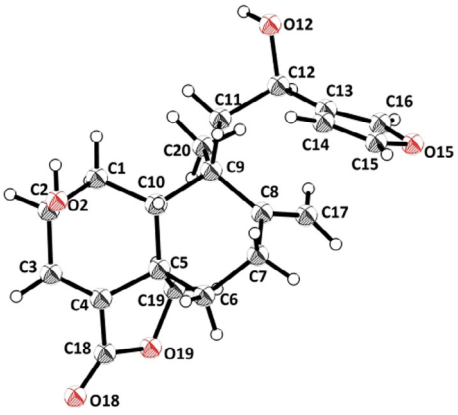

Fig. 4. X-ray single-crystal structures of **1**.

suggestive of hydroxy and carbonyl groups, respectively. Its molecular formula was determined to be $C_{26}H_{32}O_{10}$ on the basis of the HRESIMS at *m*/*z* 527.1890 $[M+Na]^+$ (calcd 527.1888), indicating 11 degrees of unsaturation. The ^{1}H NMR spectrum of **2** (Table 1) revealed the presence of one methyl group (δ_H 1.14, CH_3-20), a diagnostic β-substituted furan ring [δ_H 6.34 (dd, J = 1.7, 0.8 Hz, H-14), 7.39 (dd, J = 1.7, 1.7 Hz, H-15), and 7.36 (dd, J = 1.7, 0.8 Hz, H-16)]. The ^{13}C NMR and DEPT spectra (Table 1) showed resonances for 26 carbon signals. Of the 26 carbons, 20 were assigned to the aglycone and six carbons were attributable to the sugar moiety. With the aid of ^{1}H–^{1}H COSY, HSQC, HMBC, and ROESY experiments, the NMR signals of **2** could be assigned as shown in Table 1. A comparison of the 1D NMR data of **1** and **2** revealed that compound **1** and the aglycone moiety of compound **2** had the same planar structure.

The planar structure of hexose residue was determined by interpretation of 2D NMR spectra. The ^{1}H–^{1}H COSY spectrum analysis yielded two isolated spin systems: **a**, –CH–CH– (for C-1′/C-2′); **b**, –CH–CH–CH_2– (for C-4′/C-5′/C-6′). The spin systems **a** and **b** were connected through a ketone group based on the HMBC correlations of H-2′, H-4′ and H-5′ to C-3′ (δ_C 206.9). The HMBC correlations from H-1′ to C-3′ and C-5′ (δ_C 78.5), H-2′ to C-4′ (δ_C 73.7), and H-4′ to C-6′ (δ_C 62.5) (Fig. 5), indicated the presence of a keto sugar moiety, which was further confirmed to be 3-keto-glucopyranoside by comparison of its observed and reported NMR data.[17] Additionally, the attachment of the sugar moiety to C-2 of the aglycone was established by an HMBC correlation between the anomeric proton

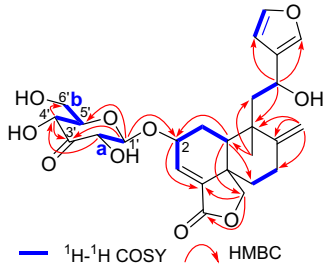

Fig. 5. Key ^{1}H–^{1}H COSY and HMBC correlations of **2**.

H-1′ and C-2, which was further supported by the downfield shift of C-2 from δ_C 64.5 to δ_C 71.7 as compared to **1**.

The ROESY correlations (Fig. 6) observed in the spectrum of **2** revealed that the relative configuration at the stereogenic centers of the aglycone was identical to that of **1**. Moreover, the relative configuration of the sugar moiety was determined by analyzing ROESY correlations. Clear ROESY correlations of H-1′/H-5′, H-2′/H-4′ suggested the α-orientation for H-1′ and H-5′, while the β-orientation for H-2′ and H-4′ (Fig. 6). The β-configuration of the sugar unit was deduced from the coupling constant (J = 7.9 Hz) of the anomeric proton at δ_H 4.51 in the ^{1}H NMR spectrum (Table 1). Therefore, the sugar unit was determined to be β-3-keto-glucopyranoside.

As a consequence, the absolute configuration of sugar unit was confirmed by an X-ray diffraction analysis (Fig. 7, CCDC 1544686) as β-D-3-keto-glucopyranoside.[18] In addition, the final refinement of the Cu Kα data resulted in a small Flack parameter of −0.14 (14), allowing an unambiguous assignment of the absolute configurations of **2** as 1′*R*,2′*S*,4′*R*,5′*R*,2*S*,5*S*,9*R*,10*R*,12*R* (Fig. 7), and **2** was named as salvihispin A-2-*O*-β-D-3-keto-glucopyranoside.

Genus *Salvia* comprises many species traditionally used as brain-enhancing tonics.[19] Since the seeds of *S. hispanica* have been used as the part of human food for about 5500 years for energy, endurance and strength needed under extreme conditions by Aztecs and Mayas people,[11] compounds **1** and **2** were evaluated for their neurotrophic activities on PC12 cells. As a result, compounds **1** and **2** had obviously increasing activity of neuronal differentiation after 72 h at the concentration of 10 μM with the differentiation rate of 9.30% and 10.96%, respectively, compared with 4.29% of the negative control.

Compounds **1** and **2** were also evaluated for in vitro cytotoxicity against five human cancer cell lines (HL-60, A-549, SMMC-7721, MCF-7, and SW480) and for AChE inhibitory activities using the Ell-

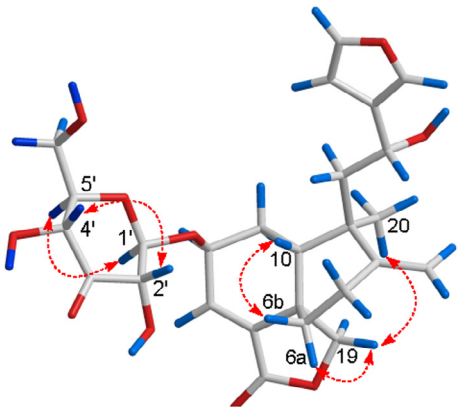

Fig. 6. Key ROESY correlations of **2**.

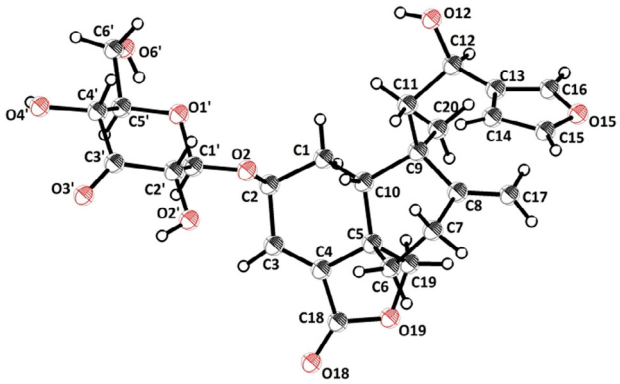

Fig. 7. X-ray single-crystal structures of **2**.

man method, but showed no activity after two repeated experiments.

In conclusion, two *neo*-clerodane diterpenoids, including a diterpenoid glycoside (**2**) with a 3-keto-glucopyranoside moiety, which was unusual as a natural sugar were isolated from the aerial parts of *S. hispanica* "Chia", and its structure and absolute configuration was elucidated by X-ray diffraction experiments for the first time. Additionally, salvihispin A (**1**) and salvihispin A-2-*O*-*β*-D-3-keto-glucopyranoside (**2**) showed moderate neurotrophic activity, which might be the potential lead compounds to treat neurodegenerative diseases, including Parkinson's disease and Alzheimer's disease.

Acknowledgment

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.tetlet.2017.12.010.

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12. Salvihispin A (**1**). Colorless prism crystals (MeOH); mp 247–248 °C; $[\alpha]_D^{25}$ −168.6 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.18) nm; IR (KBr) ν_{max} 3401, 2932, 1766, 1188, 1121, 1034, 790, 600 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1, respectively; HRESIMS *m*/*z* 367.1513 $[M+Na]^+$ (calcd for $C_{20}H_{24}O_5Na$, 367.1516).
13. Salvihispin A-2-*O*-*β*-D-3-keto-glucopyranoside (**2**). Colorless plate crystals (MeOH); mp 138–139 °C; $[\alpha]_D^{25}$ −155.4 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 374 (1.65), 206 (4.10) nm; IR (KBr) ν_{max} 3426, 2933, 1766, 1638, 1317, 1048, 875, 768, 601 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 527.1890 $[M+Na]^+$ (calcd for $C_{26}H_{32}O_{10}Na$, 527.1888).
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16. Crystal data of salvihispin A (**1**): $C_{20}H_{24}O_5$, M = 344.39, a = 7.5775(2) Å, b = 8.9612(2) Å, c = 24.9985(7) Å, α = 90°, β = 90°, γ = 90°, V = 1697.49(8) Å^3, T = 100(2) K, space group *P*212121, Z = 4, μ(CuKα) = 0.786 mm^{-1}, 9702 reflections measured, 3045 independent reflections (R_{int} = 0.0420). The final R_1 values were 0.0490 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1238 ($I > 2\sigma(I)$). The final R_1 values were 0.0491 (all data). The final $wR(F^2)$ values were 0.1239 (all data). The goodness of fit on F^2 was 1.109. Flack parameter = 0.00(6). Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 1495554).
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18. Crystal data of salvihispin A-2-*O*-*β*-D-3-keto-glucopyranoside (**2**): $C_{26}H_{32}O_{10}$, M = 504.51, a = 19.2801(6) Å, b = 7.7242(3) Å, c = 20.6958(7) Å, α = 90°, β = 114.830(2)°, γ = 90°, V = 2797.17(17) Å^3, T = 100(2) K, space group *P*21, Z = 4, μ(CuKα) = 0.772 mm^{-1}, 24772 reflections measured, 8279 independent reflections (R_{int} = 0.0492). The final R_1 values were 0.1300 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.3192 ($I > 2\sigma(I)$). The final R_1 values were 0.1372 (all data). The final $wR(F^2)$ values were 0.3281 (all data). The goodness of fit on F^2 was 1.422. Flack parameter = −0.14(14). Crystallographic data for the structure of **2** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 1544686).
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