Chinese Chemical Letters xxx (2019) xxx–xxx

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Communication

Polycyclic polyprenylated acylphloroglucinol with an unprecedented spirocyclic core from *Hypericum patulum*

Yansong Ye[a,c,1], Nana Jiang[a,c,1], Xianwen Yang[b], Gang Xu[a,*]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China and Yunnan Key Laboratory of Natural Medicinal Chemistry, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China
[b] Key Laboratory of Marine Biogenetic Resources, Third Institute of Oceanography, Xiamen 361005, China
[c] University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

Article history:
Received 15 March 2020
Received in revised form 14 April 2020
Accepted 15 April 2020
Available online xxx

Keywords:
Spirocyclic PPAP
Hypericum patulum
Structural elucidation
Derivatives
Cytotoxic activities

ABSTRACT

Spirohypatone A (**1**), a spirocyclic PPAP (polycyclic polyprenylated acylphloroglucinol) bearing an unprecedented hexahydro-1′H-spiro[cyclohexane-1,2′-pentalene]-2,4,6-trione core and a new homologue (spirohypatone B, **2**) were isolated from *Hypericum patulum* together with two known biosynthetic precursors. Compound **1** represents the first spirocyclic PPAP possessing a 5/5/6 carbon ring system, biogenetically derived from the intermediate **3** (attack from C-3 to C-12), which was differed from normal spirocyclic PPAPs (attack from C-3 to C-11). In addition, through extensive spectroscopic analysis, an interconversion mechanism of keto-enol of **1** was postulated and confirmed by its methylated reaction. The structures and absolute configurations of **1** and **2** were determined by comprehensive spectroscopic and chemical derivatized methods and X-ray crystallography. Compounds **1**, **2**, and **4** were tested to exhibit cytotoxic activities against several cancer cell lines.

Polycyclic polyprenylated acylphloroglucinols (PPAPs) with highly oxygenated dearomatized isoprenylated acylphloroglucinol cores represent a special class of secondary metabolites formed by hybridization the mevalonate/methylerythritol phosphate and polyketide biosynthetic pathways [1]. Due to complicated structures and a broad range of biological activities, this type of metabolites has become a hot topic in the research of natural products. Structurally, PPAPs can be divided into bridged-cyclic with bicyclo[3.3.1]nonane-2,4,9-trione cores, adamantane, *homo*-adamantane, spirocyclic, and some other complicated architectures according to their different scaffolds [2]. To date, more than 50 spirocyclic PPAPs have been reported and they are mainly share an octahydrospiro[cyclohexan-1,5-indene]-2,4,6-trione 5/6/6 ring system or its *seco*-cyclic skeletons [2,3].

Hypericum patulum, a traditional medicinal plant in China, was used for treatment of hepatitis tonsillitis and bruises [4]. Recently, hyperinoids A and B, two PPAPs that showed significant antiinflammatory effects were reported from this plant [5]. Our group has been devoted to the study of bioactive PPAPs from *Hypericum* and *Garcinia* genus for a long time and reported a lot of PPAPs with various skeletons such as hypersubone A,hyphenrone A, and garsubelone A [6]. Recently, our team have found a series of PPAPs as the most potent $Ca_v3.1$ agonist and antagonist [7]. As part of our ongoing discovery for bioactive PPAPs with novel structures, spirohypatone A (**1**), a spirocyclic PPAP bearing an unprecedented hexahydro-1′*H*-spiro[cyclohexane-1,2′-pentalene]-2,4,6-trione core, was characterized from *H. patulum* together with a new normal spirocyclic PPAP with 5/6/6 ring system (spirohypatone B, **2**) and two known biosynthetic precursors (**3** and **4**) (Fig. 1). Structurally, spirohypatone A (**1**) represents the first spirocyclic PPAP with a 5/5/6 carbon ring system. It is noteworthy that the NMR spectra of **1** exhibited two sets of signals since the existence of a keto-enol tautomerism. And its structure was determined by the detailed analysis of the high-quality single crystals and clear spectra of the methylated derivatives (**1a** and **1b**). Biosynthetically, **1** could be derived from the key intermediate **3** (attack from C-3 to C-12), which was distinctively differed from normal spirocyclic PPAPs (attack from C-3 to C-11) with 5/6/6 skeleton such as compound **2**.

Spirohypatone A (**1**) was obtained as colorless oil. Its molecular formula, $C_{26}H_{38}O_5$, was established by HRESIMS (*m/z* 453.2608, $[M+Na]^+$, calcd. 453.2611) indicating 8 degrees of unsaturation (Fig. S8 in Supporting information). The 1D NMR spectra exhibit two sets of signals as a result of tautomerism of keto-enol (Figs. S1 and S2 in Supporting information), which have been reported as a relatively common phenomenon in this type of metabolites [7,8].

* Corresponding author.
E-mail address: xugang008@mail.kib.ac.cn (G. Xu).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.cclet.2020.04.028
1001-8417/

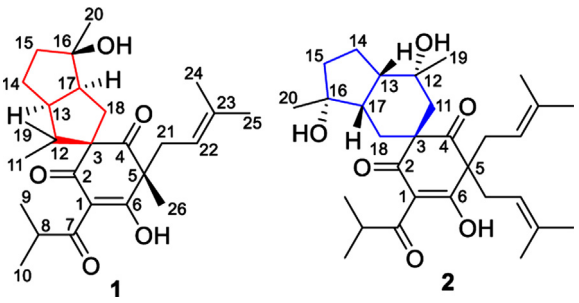


Fig. 1. Structures of **1** and **2**.

The planar structure of **1** could be elucidated by the major set of NMR data. The ^{1}H NMR spectrum (Table 1) exhibited resonances for one isopropyl group (δ_H 1.08, d, J = 6.5 Hz; 1.23, d, J = 6.5 Hz; 3.49, sept., J = 6.5 Hz), one olefinic proton (δ_H 4.57), and six methyl singlets. The ^{13}C and DEPT NMR (Table 2) spectra showed 26 carbon resonances including eight methyls, four methylenes, four methines, and ten quaternary carbons. In particular, a nonconjugated ketone (δ_C 213.6, C-4), a 1,3-diketone group (δ_C 113.7, C-1; 197.7, C-6; 193.4, C-2), two quaternary carbons at δ_C 78.2 (C-3) and 57.1 (C-5), and a isopropyl group (δ_C 20.8, C-9; 17.9, C-10; 35.0, C-8) are indicative of characteristic resonances of a disubstituted dearomatized acylphloroglucinol core at C-3 and C-5, respectively [9]. The HMBC correlations from Me-24/25 to C-22/C-23, Me-26/H-21 to C-4/C-5/C-6, together with the ^{1}H–^{1}H COSY correlation of H-21/H-22 confirmed that a methyl and an isoprenyl were attached at C-5 (Fig. 2 and Figs. S3–S5 in Supporting information).

Besides the acylphloroglucinol core and two substituents (a methyl and an isoprenyl) at C-5, the remaining 10 carbons (C-11∼C-20) could be a C10 unit derived from two isoprenyls. The planar structure of this C10 unit was deduced by the key ^{1}H-^{1}H COSY correlative interpretations of H-15/H-14/H-13/H-17/H-18 as well as the HMBC correlations from Me-20 to C-15, C-16, and C-17, and from Me-19/11 to C-12 and C-13. Finally, the linkage of acylphloroglucinol and the 10 carbons as shown in Fig. 2 was deduced by the HMBC correlations from Me-11, Me-19 and H_2-18 to C-2, C-3 and C-4. Hence, the planar structure of **1** was elucidated to be a spirocyclic PPAP possessing an hexahydro-1′*H*-spiro[cyclohexane-1,2′-pentalene]-2,4,6-trione core.

Although the planar structure of **1** can be characterized by 1D and 2D NMR data, due to overlapping NMR signals of tautomer, the confirmation of structure is a real challenge. In order to finally determine its structure and absolute configuration without any doubt, the methylated derivatives of **1** (**1a** and **1b**) were prepared with TMS−CHN_2 to elucidate its configuration and ensure its correctness of planar structure (Fig. 3). Eventually, high-quality single crystals of **1a** were obtained and prepared for X-ray analysis (CCDC: 1990172), which determined the absolute configuration of **1** as 3*S*,5*R*,13*S*,16*R*,17*S* undoubtedly (Fig. 4).

Spirohypatone B (**2**) was purified as colorless crystals. The molecular formula was determined as $C_{30}H_{44}O_6$ by analysis of its HRESIMS (*m/z* 523.3033, $[M + Na]^+$, calcd. 523.3030) and ^{13}C NMR data (Fig. S32 in Supporting information and Table 2). The ^{1}H NMR spectrum showed two olefinic proton at δ_H 4.60 and 4.92, an isopropyl group (δ_H 1.12, d, J = 7.1 Hz; 1.22, d, J = 7.1 Hz; 3.67, sept, J = 7.1 Hz), and six singlet methyls (Table 1 and Fig. S25 in Supporting information). The ^{13}C NMR and DEPT spectra revealed 30 carbon signals corresponding to two nonconjugated carbonyls at δ_C 213.3 (C-4) and 209.0 (C-7), a 1,3-diketone group (δ_C 111.5, C-1; 196.4, C-6; 195.7, C-2), four sp^3 quaternary carbons (two oxygenated ones), two methyls, four methylenes, two methines, and 13 other resonances assignable to an isopropyl and two isoprenyls (Table 2 and Fig. S26 in Supporting information). Analysis of these data indicated the characteristic signals of a spirocyclic PPAP [9,10]. Detailed comparison of the NMR data of **2** with those of tomoeone A from *H. ascyron* revealed that they could

Table 1
The ^{1}H NMR data for compounds **1**,**1a**, **1b** and **2** in $CDCl_3$.

No.	**1**[a]		**1a**[b]	**1b**[a]	**2**[a]
8	3.49, sept (6.5)	3.60, sept (6.5)	3.05, sept (7.6)	3.02, sept (7.3)	3.67, sept (7.1)
9	1.08, d (6.5)	1.06, d (6.5)	1.11, d (7.6)	1.15, d (7.3)	1.12, d (7.1)
10	1.23, d (6.5)	1.21 d (6.5)	1.20, d (7.6)	1.20, d (7.3)	1.22, d (7.1)
11	0.78, s	0.76, s	0.82, s	0.93, s	a 2.18, d (15.7) b 1.85, d (15.7)
13	2.17, overlap	2.17, overlap	2.38, m	2.32, overlap	2.08, m
14	1.34, overlap 1.62, overlap	1.34, overlap 1.62, overlap	1.41, m 1.62, m	1.39, overlap 1.64, overlap	1.74, overlap
15	a 1.49, overlap b 1.83, overlap	a 1.49, overlap b 1.83, overlap	a 1.63, m b 1.87, m	a 1.59, m b 1.82, m	a 1.67, overlap b 1.55, overlap
17	2.63, m	2.69, m	2.55, overlap	2.72, overlap	1.48, m
18	a 2.48, overlap b 2.21, m	a 2.48, overlap b 2.30, m	a 2.01, dd (13.6, 9.6) b 2.62, dd (13.6, 9.6)	a 2.01, dd (13.3, 9.7) b 2.32, overlap	1.71, overlap
19	0.89, s	0.84, s	0.94, s	0.97, s	1.26, s
20	1.26, overlap	1.26, overlap	1.29, s	1.26, s	1.07, s
21	2.48, overlap 2.30, overlap	2.11, overlap 2.34, overlap	2.29, dd (12.9, 7.9) 2.38, overlap	2.32, overlap 2.68, overlap	2.46, m 2.57, m
22	4.57, t (7.7)	4.67, t (7.7)	4.85, t (7.6)	4.68, t (7.5)	4.60, t (7.3)
24	1.47, s	1.52, s	1.51, s	1.53, s	1.50, s
25	1.31, s	1.37, s	1.62, s	1.49, s	1.39, s
26	1.43, s	1.29, s	1.29, s	1.39, s	a 2.72, m b 2.82, m
27					4.92, t (6.5)
29					1.62, s
30					1.57, s
OCH_3			3.72, s	3.73, s	

[a] Recorded at 600 MHz.
[b] Recorded at 800 MHz.

Please cite this article in press as: Y. Ye, et al., Polycyclic polyprenylated acylphloroglucinol with an unprecedented spirocyclic core from *Hypericum patulum*, Chin. Chem. Lett. (2020), https://doi.org/10.1016/j.cclet.2020.04.028

Table 2
The ^{13}C NMR data for compounds **1**, **1a**, **1b** and **2** in $CDCl_3$.

No.	1[a]		1a[b]	1b[a]	2[a]
1	113.7	112.4	121.0	122.8	111.5
2	193.4	193.3	178.5	198.1	195.7
3	78.2	74.9	73.3	77.8	62.0
4	213.6	210.9	210.3	211.9	213.3
5	57.1	61.0	58.6	55.0	61.2
6	197.7	196.9	200.0	173.8	196.4
7	206.6	204.8	207.8	208.8	209.0
8	35.0	34.4	40.9	42.6	35.7
9	20.8	20.5	19.9	18.9	19.6
10	17.9	18.3	18.3	18.4	18.7
11	29.6	29.1	22.8	23.2	38.2
12	51.8	53.1	53.6	51.6	71.4
13	60.2	58.6	58.1	58.3	44.1
14	25.1	24.4	24.0	24.4	22.9
15	43.2	43.4	43.5	44.0	40.9
16	79.1	78.7	78.4	78.6	78.3
17	51.9	52.9	53.5	52.6	43.8
18	27.9	28.4	29.7	28.9	30.3
19	22.9	22.6	28.8	29.6	28.5
20	28.9	28.8	28.8	28.8	23.9
21	42.8	41.2	41.2	40.7	40.9
22	116.8	117.5	118.3	118.2	116.6
23	138.6	136.4	135.5	136.9	138.3
24	17.8	17.6	17.7	18.0	17.7
25	25.8	25.9	26.0	26.2	26.0
26	19.1 (overlap)	19.1 (overlap)	19.7	22.1	34.8
27					118.7
28					136.4
29					26.0
30					17.9
OCH_3			62.3	61.8	

[a] Recorded at 150 MHz.
[b] Recorded at 200 MHz.

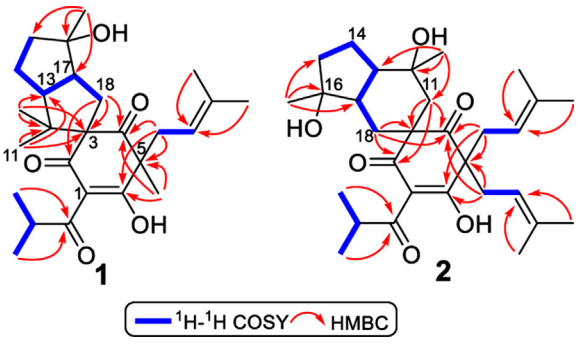


Fig. 2. Key ^{1}H-^{1}H COSY and HMBC correlations of **1** and **2.**

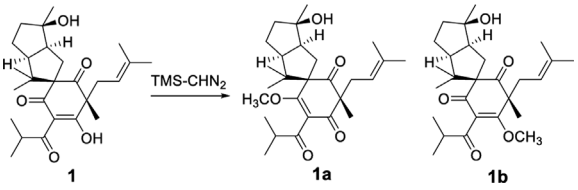


Fig. 3. Methylation of compound **1** to **1a** and **1b**.

share the same planar structure [10], which was confirmed by the ^{1}H-^{1}H COSY and HMBC spectra (Fig. 2 and Figs. S27–S29 in Supporting information). However, the different chemical shifts of C-4, C-17, and C-13 in **2** and tomoeone A suggested configurational difference of the two compounds. The NOE cross-peaks of

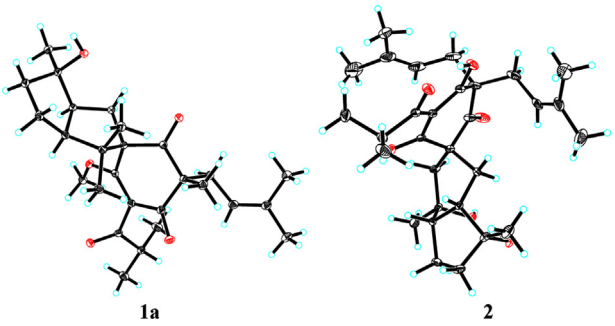
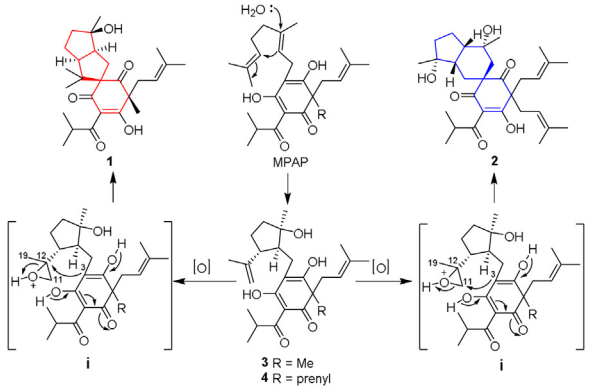


Fig. 4. X-ray crystal structures of **1a** and **2**.



Scheme 1. Hypothetical biosynthetic pathways of **1** and **2**.

H-17/H-11a/H-13, H-13/Me-19, H-17/Me-20 and H-27/H-11a in the ROESY spectrum (Fig. S30 in Supporting information) indicated that the relative configuration of **2** together with the fact that compound **2** was the C-13 epimer of tomoeone A. Finally, the X-ray crystallography of **2** result (CCDC: 1990149) not only confirmed **2** as epimer of tomoeone A at C-13 but also unambiguously determined its absolute configuration as 3*S*,12*R*,13*S*,16*R*,17*S* (Fig. 4).

Spirohypatone A (**1**) represent the first example of spirocyclic PPAP with 5/5/6 carbon ring system, which differed from the normal spirocyclic PPAP with 5/6/6 carbon ring skeleton. Biosynthetically, spirocyclic PPAPs could derived from MPAPs (monocyclic polyprenylated acylphloroglucinols) [1,2]. The geranyl side chain of MPAP may be cyclized to formed chinesin II (**3**) [11,12] and hypercalin C (**4**) [11], which have also been isolated in this study. Thereafter, the **3** undergoes a reaction to produce key intermediate **i**. Then, the attack from C-3 to C-11 of **i** form the normal spirocyclic PPAP (**2**) with the known octahydrospiro[cyclohexan-1,5-indene]-2,4,6-trione architecture, while attack from C-3 to C-12 form **1** with an unprecedented hexahydro-1′*H*-spiro[cyclohexane-1,2′-pentalene]-2,4,6-trione architecture (Scheme 1).

The cytotoxic effects of these isolates on several cancer cell lines were assayed. Compound **4** was tested to exhibited significant activities against NCI-H460, HCT-15, and MCF-7 cell lines with IC_{50} values of 1.96, 1.94, and 2.37 μmol/L, respectively. Moreover, **1** and **2** also tested to show weak and moderate cytotoxic effects against HL-60 (the IC_{50} values of 33.43 for **1** and 16.08 μmol/L for **2**) and A549 (IC_{50} 29.5 and 33.55 for **1** and **2**, respectively).

In summary, spirohypatone A (**1**), a PPAP type metabolites bearing an undescribed hexahydro-1′*H*-spiro[cyclohexane-1,2′-pentalene]-2,4,6-trione core, and a normal spirocyclic PPAP (spirohypatone B, **2**) with the known octahydrospiro[cyclohexan-1,5-indene]-2,4,6-trione core were isolated from *H. patulum* together with their known biosynthetic precursors. Compound **1**

Please cite this article in press as: Y. Ye, et al., Polycyclic polyprenylated acylphloroglucinol with an unprecedented spirocyclic core from *Hypericum patulum*, Chin. Chem. Lett. (2020), https://doi.org/10.1016/j.cclet.2020.04.028

represents the first spirocyclic PPAP with 5/5/6 ring system and can be considered as a new type of spirocyclic PPAP. Biosynthetically, although **1** and **2** derived from intermediates with the same skeleton (**3** and **4**), the C-3 attacks different positions (C-11 and C-12) and eventually leads to produce distinct spirocyclic PPAPs. In the bioactive assay, compounds **1** and **2** were tested to show inhibitory effects on HL-60 and A549, while **4** was found to exhibited significant cytotoxicities against NCI-H460, HCT-15, and MCF-7 cell lines. Altogether, these findings enrich the diversity of structural type of PPAPs.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The work was financially supported by the NSFC-Joint Foundation of Yunnan Province (No. U1902213), the Second Tibetan Plateau Scientific Expedition and Research (STEP) Program (No. 2019QZKK0502020303), Southeast Asia Biodiversity Research Institute, CAS (No. 2017CASSEABRIQG003), and the Natural Sciences Foundation of Yunnan Province (No. 2019FA003).

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.2020.04.028.

References

[1] R. Ciochina, R.B. Grossman, Chem. Rev. 106 (2006) 3963–3986.
[2] X.W. Yang, R.B. Grossman, G. Xu, Chem. Rev. 118 (2018) 3508–3558.
[3] (a) Y.T. Duan, J. Zhang, Y.Z. Lao, et al., Tetrahedron Lett. 59 (2018) 4067–4072; (b) Z.N. Wu, Q.W. Niu, Y.B. Zhang, et al., RSC Adv. 9 (2019) 7961–7966; (c) J.J. Zhang, X.W. Yang, J.Z. Ma, et al., Nat. Prod. Bioprospect. 4 (2014) 73–79.
[4] State Administration of Traditional Chinese Medicine of the People's Republic of China, Chinese Materia Medica, Vol. 9, Shanghai Scientific and Technical Publishers, Shanghai, 1999, pp. 594–608.
[5] X. Jia, Y. Wu, C. Lei, et al., Chin. Chem. Lett. (2019), doi:http://dx.doi.org/10.1016/j.cclet.2019.10.014.
[6] (a) Y. Liao, X. Liu, J. Yang, et al., Org. Lett. 17 (2015) 1172–1175; (b) Y.L. Wang, Y.S. Ye, W.W. Fu, et al., Org. Lett. 21 (2019) 1534–1537; (c) X.W. Yang, Y.Q. Ding, J.J. Zhang, et al., Org. Lett. 16 (2014) 2434–2437.
[7] Y.S. Ye, W.Y. Li, S.Z. Du, et al., J. Med. Chem. 63 (2020) 1709–1716.
[8] N. Zhang, Z. Shi, Y. Guo, et al., Org. Chem. Front. 6 (2019) 1491–1502.
[9] (a) S. Abe, N. Tanaka, J. Kobayashi, J. Nat. Prod. 75 (2012) 484–488; (b) S. Yin, X. Chen, Z.S. Su, et al., Tetrahedron 65 (2009) 1147–1152; (c) W. Xin, X. Man, C. Zheng, et al., Fitoterapia 83 (2012) 1540–1547; (d) N. Tanaka, S. Abe, J. Kobayashi, Tetrahedron Lett. 53 (2012) 1507–1510; (e) H. Zhu, C. Chen, J. Liu, et al., Phytochemistry 115 (2015) 222–230.
[10] W. Hashida, N. Tanaka, Y. Kashiwada, et al., Phytochemistry 69 (2008) 2225–2230.
[11] L.A. Decosterd, H. Stoeckli-Evans, J.C. Chapuis, B. Sordat, K. Hostettmann, Helv. Chim. Acta 72 (1989) 1833–1845.
[12] Y. Aramaki, K. Chiba, M. Tada, J. Chem. Soc. Perkin Trans. I 1 (1995) 683–688.

Please cite this article in press as: Y. Ye, et al., Polycyclic polyprenylated acylphloroglucinol with an unprecedented spirocyclic core from *Hypericum patulum*, Chin. Chem. Lett. (2020), https://doi.org/10.1016/j.cclet.2020.04.028