ChemComm

Cite this: *Chem. Commun.*, 2012, **48**, 5998–6000

www.rsc.org/chemcomm

COMMUNICATION

Hypercohin A, a new polycyclic polyprenylated acylphloroglucinol possessing an unusual bicyclo[5.3.1]hendecane core from *Hypericum cohaerens*†

Xing-Wei Yang,[ab] Xu Deng,[ab] Xia Liu,[ab] Chun-Yan Wu,[a] Xiao-Nian Li,[a] Bin Wu,[a] Huai-Rong Luo,[a] Yan Li,[a] Hong-Xi Xu,[c] Qin-Shi Zhao[a] and Gang Xu*[a]

Received 1st April 2012, Accepted 18th April 2012
DOI: 10.1039/c2cc32352d

Hypercohin A (1), an unprecedented polycyclic polyprenylated acylphloroglucinol featuring with an unusual bicyclo[5.3.1]hendecane core, was isolated from *Hypericum cohaerens*. Its structure and absolute configurations were elucidated by extensive NMR methods and crystal X-ray diffractions of its *p*-bromobenzoate ester (2).

Natural polycyclic polyprenylated acylphloroglucinols (PPAPs), with highly oxygenated and densely substituted bicyclo[3.3.1]-nonane-2,4,9-trione or other related core structures decorated with prenyl or geranyl side chains, are a special class of complex natural products that have been only isolated from plants of the family Guttiferae so far.[1] This kind of metabolites show a wide variety of biological activities such as antitumor, antimicrobial, anti-HIV, antioxidant, and especially in the CNS as modulators of neurotransmitters associated with neuronal damage and depression.[1,2] In recent years, the fascinating chemical structures and intriguing biological activities of PPAPs have attracted widespread attention from phytochemical, organic synthetic, and pharmacological endeavors.[3]

The plants of genus *Hypericum* (Guttiferae), occurring widely in temperate regions, have been used as traditional medicine in many countries all over the world.[4] *H. cohaerens* N. Robson is an endemic plant distributed in Guizhou and Yunnan provinces, P. R. China,[5] and has not been phytochemically studied so far. As a part of our systematic search for new and bioactive natural PPAPs from Guttiferae plants,[6] the chemical constituents of this plant were studied and hypercohin A (**1**), an unprecedented PPAP featured with an unusual bicyclo[5.3.1]-hendecane core, was isolated (Fig. 1). The structure of **1** was elucidated using a combination of NMR spectroscopy and the crystal X-ray diffractions of its *p*-bromobenzoate ester (**2**). In the bioactive studies, both **1** and **2** showed moderate inhibitory activities on acetylcholinesterase (AChE). In addition, compound **1** displayed moderate cytotoxic activities against five human cancer cell lines *in vitro* (IC_{50} 10.4–24.7 μM).

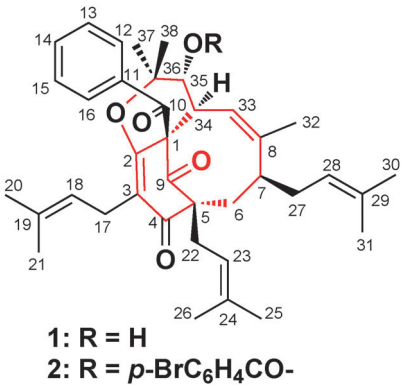


Fig. 1 Structures of hypercohin A (**1**) and its derivative (**2**).

Hypercohin A (**1**) was obtained as colorless oil. Its molecular formula $C_{38}H_{48}O_5$ was established by the positive HRESIMS (*m*/*z* 585.3578, $[M + H]^+$), indicating 15 degrees of unsaturation. The IR spectrum showed obvious absorption bands for hydroxyl (3436 cm^{-1}), carbonyl (1716, 1686, and 1651 cm^{-1}), and olefinic (1618 cm^{-1}) groups, respectively. The ^1H NMR spectrum revealed the presence of a monosubstituted benzene ring (δ_H 7.74, 2H, d, J = 8.3 Hz; 7.52, 1H, t, J = 7.5 Hz; and 7.34, 2H, dd, J = 8.3, 7.5 Hz), four olefinic protons (δ_H 5.89, 1H, d, J = 9.8 Hz; 5.13, 1H, t, J = 7.5 Hz; 4.99, 1H, t, J = 6.6 Hz; 4.73, 1H, t, J = 7.9 Hz), and nine methyls (δ_H 1.28–1.73, s). The ^{13}C NMR and DEPT spectra (Table 1) resolved 38 carbon signals due to eight quaternary carbons (including two ketones, three olefinic, and an oxygenated one), four methines (one oxygenated and one olefinic), one methylene, three methyls, and 22 other signals assignable to a benzoyl and three isoprenyl groups. Careful analysis of these data indicated that the characteristic signals for a PPAP type metabolite of a nonconjugated carbonyl (δ_C 206.2, C-9), an enolized 1,3-diketone group (δ_C 168.3, C-2; 125.9, C-3; 201.2, C-4), two quaternary carbons at δ_C 75.9 (C-1) and 63.9 (C-5), a methine at δ_C 40.2 (C-7), and

[a] *State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China. E-mail: xugang008@mail.kib.ac.cn; Tel: +86-871-5217971*
[b] *Graduate University of the Chinese Academy of Sciences, Beijing 100049, P. R. China*
[c] *Shanghai University of Traditional Chinese Medicine, Shanghai 201203, P. R. China*

† Electronic supplementary information (ESI) available: 1D, 2D NMR spectra, detailed experimental procedures of **1**. CCDC 872701 (**2**). For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2cc32352d

Table 1 ^{1}H and ^{13}C NMR data for **1** in CD_3OD[a]

No.	δ_H (mult, J in Hz)	δ_C	No.	δ_H (mult, J in Hz)	δ_C
1		75.9	20	1.73 (s)	26.3
2		168.3	21	1.64 (s)	18.2
3		125.9	22	2.69 (dd, 13.2, 7.9)	40.9
4		201.2		2.36 (overlapped)	
5		63.9	23	4.73 (t, 7.9)	119.4
6	2.35 (br d, 12.8)	44.3	24		137.0
	1.40 (t, 12.8)		25	1.57 (s)	26.6
7	2.61 (m)	40.2	26	1.39 (s)	17.9
8		144.4	27	2.08 (m)	33.8
9		206.2		2.01 (m)	
10		196.4	28	4.99 (t, 6.6)	123.5
11		138.3	29		134.3
12	7.74 (d, 8.3)	129.6	30	1.71 (s)	26.0
13	7.34 (dd, 8.3, 7.5)	129.4	31	1.62 (s)	18.2
14	7.52 (t, 7.5)	133.9	32	1.63 (s)	19.2
15	7.34 (dd, 8.3, 7.5)	129.4	33	5.89 (d, 9.8)	127.2
16	7.74 (d, 8.3)	129.6	34	3.67 (dd, 10.9, 9.8)	52.5
17	3.22 (dd, 13.6, 7.5)	23.4	35	4.28 (d, 10.9)	73.9
	3.18 (dd, 13.6, 7.5)		36		92.8
18	5.13 (t, 7.5)	121.9	37	1.28 (s)	20.6
19		134.1	38	1.29 (s)	28.7

[a] Data were recorded on a Bruker AM-600 spectrometer.

a methylene at δ_C 44.3 (C-6) can be easily distinguished.[6,7] These observations, conjugated with the fact that a number of PPAPs have been isolated from *Hypericum* species, indicated that **1** could be ascribed as being a PPAP type derivative.

Comparative analysis of its 1D NMR spectral data with those of the normal PPAPs with a bicyclo[3.3.1]nonane core displayed some significant differences. It is obvious that the characteristic signals for a normal PPAP at C-8 (usually present between δ_C 47–52 in the ^{13}C NMR spectrum) as well as the angular methyl (Me-32, δ_C 12–16, δ_H 0.9–1.2, s), the methylene (C-33, δ_C 35–38, δ_H 1.3–2.1), and the isoprenyl side chain substituted at C-8 were absent in **1**.[6,7] Instead, eight unusual signals including two quaternary carbons (δ_C 144.4 and 92.8), three methines (δ_C 127.2, δ_H 5.89, d, J = 9.8 Hz; δ_C 52.5, δ_H 3.67, dd, J = 10.9, 9.8 Hz; and δ_C 73.9, δ_H 4.28, d, J = 10.9 Hz), and three methyls (δ_C 19.2, δ_H 1.63; δ_C 20.6, δ_H 1.28; and δ_C 28.7, δ_H 1.29) were present. These observations indicated that the carbon skeleton of ring B should be different.

The 2D NMR data of **1** deduced the linkage of C-6/C-7/C-8/C-33/C-34/C-35/C-36/C-37(C-38) by the HMBC correlations from Me-32 to C-7, C-8 and C-33, from Me-37 and Me-38 to C-35 and C-36, and the ^{1}H–^{1}H COSY correlations of H-6/H-7 and H-33/H-34/H-35. The evidence mentioned above, conjugated with the HMBC correlations of H-34 with C-1, C-2, and C-9; H-6 with C-4, C-5, and C-9; as well as H-7 with C-5 and C-6 established the unique eight-membered ring B of **1**.

The ether ring C was formed by the connection of C-2 and C-36 through an oxygen atom, which was elucidated by the weak HMBC correlations from both Me-37 and Me-38 to C-2 coupled with the degrees of the unsaturation. In addition, the HMBC correlations of H-17 with C-2, C-3, and C-4; H-22 with C-4, C-5, C-6, and C-9; H-27 with C-6, C-7, and C-8, as well as H-34 with C-10, coupled with the proton spin systems H-17/H-18, H-22/H-23, and H-6/H-7/H-27/H-28 can all be found, which not only established the connection of the benzoyl and three isoprenyl groups at C-1, C-3, C-5, and C-7, respectively, but also confirmed the core structure of **1** furthermore. Then, the planar structure of **1** was elucidated as shown (Fig. 2).

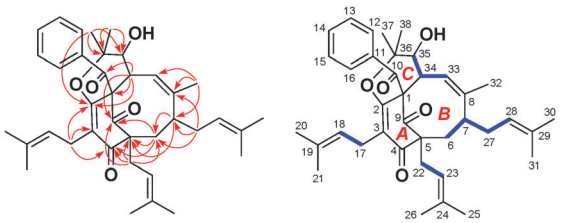


Fig. 2 Key HMBC (arrows) and ^{1}H–^{1}H COSY (bold) correlations of **1**.

The relative configurations of **1** were elucidated by analysis of the ROESY spectrum. Diagnostic cross peaks between H-16 and H-23 indicated that the benzoyl and isoprenyl groups at C-5 were both located on the same face of the molecule, which permitted the β-orientations of both C-10 and C-22. In addition, the ROESY correlations of H-17/Me-37, Me-37/H-34, and H-34/H-7 indicated that H-7, H-34, and Me-37 were α-oriented. Then, H-35 was determined to be β-oriented by its ROESY correlations with Me-38.

To determine the absolute configurations and confirm the unique structure of **1** furthermore, other solid evidence such as X-ray diffraction analysis was necessary. Since **1** was obtained as oil, the *p*-bromobenzoate derivative (**2**) was prepared by its esterification.[3] The single-crystal X-ray study of **2**‡ unambiguously confirmed the structure of **1** and clarified the absolute configurations of **1** as 1*S*, 5*R*, 7*S*, 34*S*, and 35*R* (Fig. 3).

A plausible biogenetic pathway for **1** was proposed (Scheme 1). Biogenetically, **1** should be derived from the normal PPAPs such as uralodins B and C which have been also isolated in this study.[7c] An intermediate (**M**) was derived from oxidation and dehydration of uralodin B or C, which was further proceeded by a key C[1, 3]σ migration rearrangement reaction to obtain an expanded octatomic ring B, and then followed by dehydration, keto–enol tautomerism, and further cyclization to afford **1**.

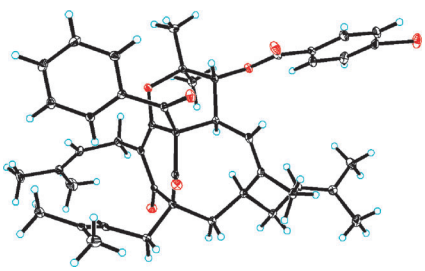
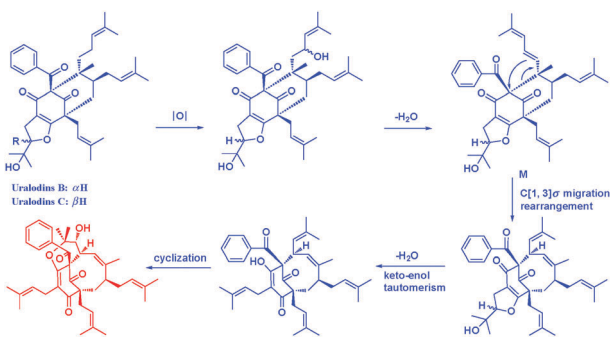


Fig. 3 X-ray crystallographic structure of **2** (displacement ellipsoids are drawn at the 30% probability level).



Scheme 1 Plausible biogenetic pathway of **1**.

Table 2 Cytotoxicity of **1** against human cancer and normal cell lines

	IC_{50}/μM					
	HL-60	SMMC-7721	A-549	MCF-7	SW480	Beas-2B
1	13.4	13.8	10.4	24.1	24.7	17.8
2	> 40	> 40	> 40	> 40	> 40	—
DDP[a]	3.3	9.6	10.0	24.6	23.5	8.6

[a] Positive control.

The AChE inhibitory activity of **1** and **2** was assayed using the Ellman method.[8] Both **1** and **2** showed moderate inhibitory activities (inhibition percentages were 41.7% and 23.0%, respectively) at the concentration of 50 μM. In addition, compounds **1** and **2** were also tested for their cytotoxic effects against five human cancer cell lines, HL-60, A-549, SMMC-7721, MCF-7, SW480, and the human normal bronchial epithelial cell line (Beas-2B), using the MTT method described previously.[9] Interestingly, **1** showed moderate toxicities, while **2** exhibited no inhibitory activity (Table 2).

To the best of our knowledge, compound **1** can be seen as the first PPAP featured with an eight-membered ring B. The core structure of **1** is an unusual bicyclo[5.3.1]hendecane accompanied by a six-membered ether ring, which is really rare in natural products. Only two classes of famous diterpenoids, *i.e.*, taxol and pleuromutilin, together with their analogues have been reported to possess a similar bicyclo[5.3.1]hendecane core so far.[10] As for the oxygen-bridge between C-2 and C-36, it has been also never reported to be present in natural PPAPs before.

In the structural elucidation of natural PPAPs, determining the absolute configurations was always a tough task to complete. Biosynthetically, PPAPs were derived from monocyclic polyprenylated acylphloroglucinols (MPAPs) by the cyclization of the prenyl group at C-5 to C-1, which permitted the different configurations of C-1 and C-5.[1] In addition, natural PPAPs were usually obtained as oil or gum since polyprenyl substitutions existed in the molecules, which led to the difficulties in the crystallization of these metabolites. Based on the detailed investigation, the appropriate single crystals of less than ten PPAPs for X-ray diffraction analysis have been reported to be achieved, and only one of these X-ray studies has determined the absolute configurations of the target molecule.[6c,11] Despite the absolute configurations of two normal PPAPs have been determined by comparison of their electronic circular dichroism measurements with those values predicted by DFT calculations,[2d] X-ray analysis is still the most effective and powerful method. For the compounds obtained as oil or gum, preparing derivatives has been proved to be a useful method to make the products or intermediates more crystallizable.[12] In 2010, the absolute configurations of two adamantane derivatives, which can be derived by further cyclization of PPAPs, have been published in "*J. Am. Chem. Soc.*" by preparing their *p*-bromobenzoate ester.[3] This method was also used in this study, which can be seen as the first use of this method in the determination of absolute configurations of natural PPAPs.

This work was supported financially by the foundations from NSFC (20972167 and 81173485), the 973 programs (2009CB522300 and 2011CB915503), the Young Academic Leader Raising Foundation of Yunnan Province (No. 2009CI073), and the foundation from CAS to Dr G. Xu.

Notes and references

‡ Crystal data for **2**: $C_{45}H_{51}BrO_6$, M = 767.77, triclinic, a = 9.0921(12) Å, b = 9.5542(12) Å, c = 12.8573(17) Å, α = 72.794(2)°, β = 70.787(2)°, γ = 78.211(2)°, V = 1000.4(2) Å^3, T = 100(2) K, space group $P1$, Z = 1, μ(MoKα) = 1.074 mm^{-1}, 13 227 reflections measured, 9544 independent reflections (R_{int} = 0.0217). The final R_1 values were 0.0357 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1030 ($I > 2\sigma(I)$). The final R_1 values were 0.0391 (all data). The final $wR(F^2)$ values were 0.1042 (all data). The goodness of fit on F^2 was 1.037. Flack parameter = 0.013(5). CCDC 872701.

1 (*a*) R. Ciochina and R. B. Grossman, *Chem. Rev.*, 2006, **106**, 3963–3986; (*b*) I. P. Singh and S. B. Bharate, *Nat. Prod. Rep.*, 2006, **23**, 558–591; (*c*) I. P. Singh, J. Sidana, S. B. Bharate and W. J. Foley, *Nat. Prod. Rep.*, 2010, **27**, 393–416.

2 (*a*) L. J. Zhang, C. T. Chiou, J. J. Cheng, H. C. Huang, L. M. Y. Kuo, C. C. Liao, K. F. Bastow, K. H. Lee and Y. H. Kuo, *J. Nat. Prod.*, 2010, **73**, 557–562; (*b*) R. A. Hussain, A. G. Owegby, P. Parimoo and P. G. Waterman, *Planta Med.*, 1982, **44**, 78–81; (*c*) A. L. Piccinelli, O. Cuesta-Rubio, M. B. Chica, N. Mahmood, B. Pagano, M. Pavone, V. Barone and L. Rastrelli, *Tetrahedron*, 2005, **61**, 8206–8211; (*d*) U. M. Acuna, M. Figueroa, A. Kavalier, N. Jancovski, M. J. Basile and E. J. Kennelly, *J. Nat. Prod.*, 2010, **73**, 1775–1779; (*e*) T. N. Griffith, L. Varela-Nallar, M. C. Dinamarca and N. C. Inestrosa, *Curr. Med. Chem.*, 2010, **17**, 391–406.

3 (*a*) J. T. Njardarson, *Tetrahedron*, 2011, **67**, 7631–7666; (*b*) Q. Zhang, B. Mitasev, J. Qi and J. A. Jr. Porco, *J. Am. Chem. Soc.*, 2010, **132**, 14212–14215; (*c*) J. Qi, A. B. Beeler, Q. Zhang and J. A. Jr. Porco, *J. Am. Chem. Soc.*, 2010, **132**, 13642–13644; (*d*) J. H. George, M. D. Hesse, J. E. Baldwin and R. M. Adlington, *Org. Lett.*, 2010, **12**, 3532–3535.

4 P. Avato, in *Studies in Natural Products Chemistry*, ed. A.Rahman, Elsevier, Netherlands, 2005, vol. 30, pp. 603–634.

5 Y. H. Li, in *Flora of China*, ed. Z. Y. Wu, Science Press, Beijing, 1990, vol. 50, p. 12.

6 (*a*) G. Xu, C. Feng, Y. Zhou, Q. B. Han, C. F. Qiao, S. X. Huang, D. C. Chang, Q. S. Zhao, K. Q. Luo and H. X. Xu, *J. Agric. Food Chem.*, 2008, **56**, 11144–11150; (*b*) G. Xu, W. L. T. Kan, Y. Zhou, J. Z. Song, Q. B. Han, C. F. Qiao, C. H. Cho, J. A. Rudd, G. Lin and H. X. Xu, *J. Nat. Prod.*, 2010, **73**, 104–108; (*c*) X. Q. Chen, Y. Li, K. Z. Li, L. Y. Peng, J. He, K. Wang, Z. H. Pan, X. Cheng, M. M. Li, Q. S. Zhao and G. Xu, *Chem. Pharm. Bull.*, 2011, **59**, 1250–1253.

7 (*a*) L. Verotta, G. Appendino, J. Jakupovic and E. Bombardelli, *J. Nat. Prod.*, 2000, **63**, 412–415; (*b*) M. D. Shan, L. H. Hu and Z. L. Chen, *J. Nat. Prod.*, 2001, **64**, 127–130; (*c*) X. Q. Chen, Y. Li, X. Cheng, K. Wang, J. He, Z. H. Pan, M. M. Li, L. Y. Peng, G. Xu and Q. S. Zhao, *Chem. Biodiversity*, 2010, **7**, 196–204.

8 G. L. Ellman, K. D. Courtney, V. Jr. Andres and R. M. Featherstone, *Biochem. Pharmacol.*, 1961, **7**, 88–95.

9 M. C. Alley, D. A. Scudiero, A. Monks, M. L. Hursey, M. J. Czerwinski, D. L. Fine, B. J. Abbott, J. G. Mayo, R. H. Shoemaker and M. R. Boyd, *Cancer Res.*, 1988, **48**, 589–601.

10 (*a*) D. G. I. Kingston, *Phytochemistry*, 2007, **68**, 1844–1854; (*b*) Y. Z. Tang, Y. H. Liu and J. X. Chen, *Mini-Rev. Med. Chem.*, 2012, **12**, 53–61.

11 (*a*) Y. H. Zeng, K. Osman, Z. Y. Xiao, S. Gibbons and Q. Mu, *Phytochem. Lett.*, 2012, **5**, 200–205; (*b*) Y. H. Zeng, Q. Mu, Z. Y. Xiao, Y. Xu, M. M. Rahman and S. Gibbons, *Chem. Lett.*, 2009, 440–441; (*c*) F. T. Martins, D. M. Assis, M. H. Santos, I. Camps, M. P. Veloso, M. A. Juliano, L. C. Alves and A. C. Doriguetto, *Eur. J. Med. Chem.*, 2009, **44**, 1230–1239; (*d*) F. T. Martins, I. Camps, A. C. Doriguetto, M. H. Santos, J. Ellena and L. C. A. Barbosa, *Helv. Chim. Acta*, 2008, **91**, 1313–1325; (*e*) Z. Y. Xiao, Q. Mu, W. K. P. Shiu, Y. H. Zeng and S. Gibbons, *J. Nat. Prod.*, 2007, **70**, 1779–1782.

12 E. A. Couladouros, M. Dakanali, K. D. Demadis and V. P. Vidali, *Org. Lett.*, 2009, **11**, 4430–4433.

