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New Acylphloroglucinol Derivatives with Diverse Architectures from *Hypericum henryi*

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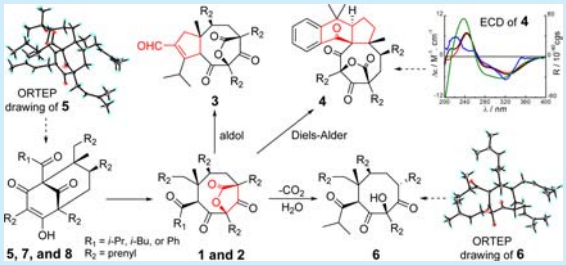
Supporting Information

ABSTRACT: Hyphenrones A–F (**1**–**6**), six polycyclic polyprenylated acylphloroglucinol derivatives with four architectures including three unprecedented cores as exemplified by **1**, **3**, and **4**, were isolated from *Hypericum henryi*. Compounds **3** and **4** possess two unique 5/8/5 and 6/6/5/8/5 fused ring systems, respectively. Their absolute configurations were defined by experimental and calculated ECD of **4** and X-ray diffractions of **5** and **6**, coupled with their putative biosynthetic origins. Three compounds exhibited interesting AChE inhibitory activities.



Plants of the genus *Hypericum*, widely used as traditional medicine in many countries,[1] are a rich source of polycyclic polyprenylated acylphloroglucinols (PPAPs) with diverse structures and significant bioactivities.[2] PPAPs are a group of structurally fascinating and synthetically challenging natural products possessing highly oxygenated acylphloroglucinol-derived cores densely decorated with prenyl substituents, which collectively exhibit a remarkably broad range of biological activities such as tumor inhibitive, antimicrobial, HIV preventative, antioxidant, and antidepressant.[2,3] Since the characterization of hyperforin, a typical PPAP type molecule with cognitive-enhancing and memory-facilitating properties as well as neuroprotective effects against Alzheimer's disease,[4] approximately 200 PPAPs have been reported, most of which are *endo*-bicyclic polyprenylated acylphloroglucinols (*endo*-BPAPs) with a bicyclo[3.3.1]nonane-2,4,9-trione core.[2]

Hypericum henryi is a traditional Chinese medicinal plant used for the treatment of hepatitis in Yunnan, China.[5] As a part of our systematic search for new and bioactive natural PPAPs from the *Hypericum* genus,[6] hyphenrones A–F (**1**–**6**), six PPAP-type derivatives with four architectures including the three unprecedented cores as exemplified by **1**, **3**, and **4**, together with three known precursors **7**–**9**, were isolated from *H. henryi* (Figure 1 and Scheme 1). Their absolute configurations were defined by experimental and calculated electronic circular dichroism (ECD) spectra of **4** and the X-ray diffractions (Cu Kα) data of **5** and **6** coupled with the presumed close relationship in their biosynthetic pathway. Compounds **1** and **2** possess an unprecedented *seco*-PPAP skeleton formed by cleavage of the C-1/C-9 bond of the normal *endo*-BPAPs. Compounds **3** and **4**, featuring fascinating

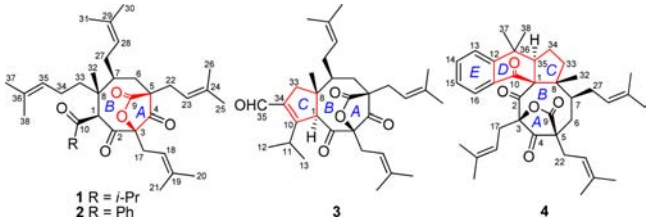


Figure 1. Structures of compounds **1**–**4**.

5/8/5 and 6/6/5/8/5 fused ring systems, were presumably synthetically derived from **1** and **2** via key aldol condensation and Diels–Alder cycloaddition reactions, respectively. Compounds **1**, **3**, and **4** exhibited interesting AChE inhibitory activities. Herein, the structural elucidation including absolute configurational analysis and bioactive evaluation of **1**–**6** are reported.

Hyphenrone A (**1**) was obtained as a light yellow gum. Its molecular formula $C_{35}H_{52}O_5$ was established by ^{13}C NMR and HR-ESI-MS data (*m*/*z* 575.3704, $[M + Na]^+$) indicating 10 indices of hydrogen deficiency. The ^{13}C NMR and DEPT spectra revealed 35 carbon resonances corresponding to six quaternary carbons (including one oxygenated and three carbonyls), two methines, two methylenes, one methyl, and 24 other resonances assignable to an isobutyryl and four prenyl groups (Table S7, Supporting Information). Analysis of these data indicated the characteristic resonances of three non-conjugated carbonyls at δ_C 197.9 (C-2), 206.9 (C-4), and 206.6

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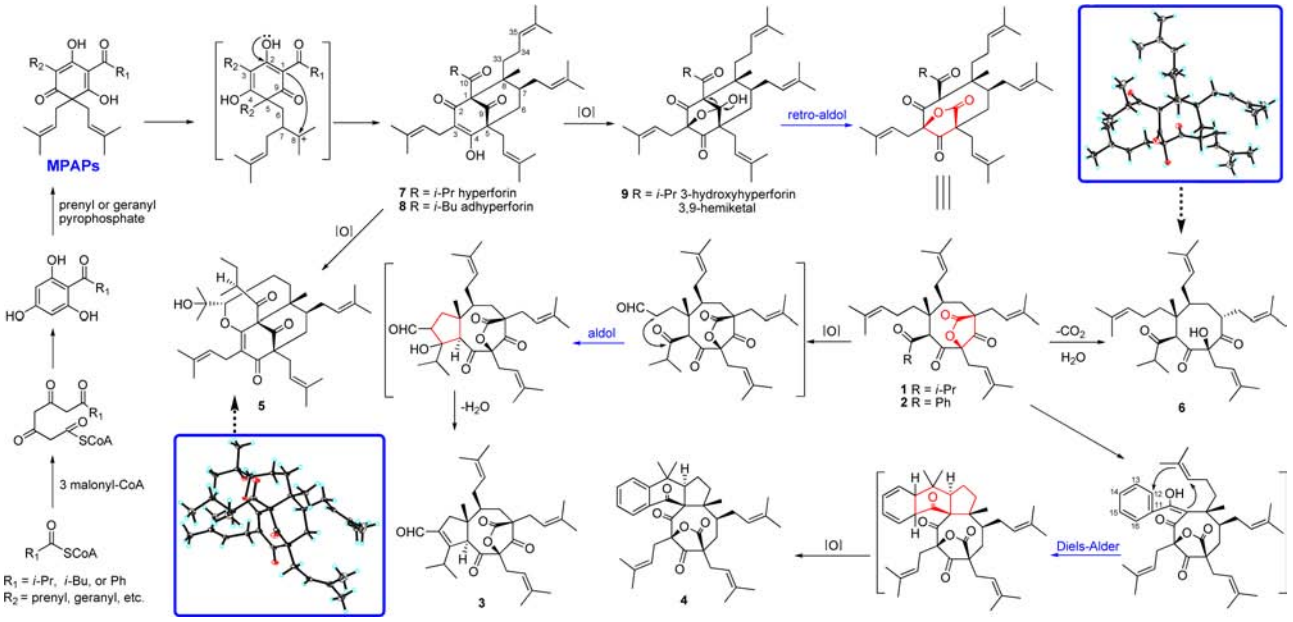
Scheme 1. Putative Biosynthesis Pathways to 1−9[a]



[a]Key: red type, newly formed rings in unprecedented skeletons; blue rectangles, ORTEP drawings.

(C-10), two quaternary carbons at δ_C 57.3 (C-5) and 48.7 (C-8), one methine at δ_C 40.7 (C-7), and one methylene at δ_C 37.1 (C-6) of a PPAP type metabolite.[6] Comparison of its 1D NMR data with those of related normal *endo*-BPAPs such as 3-hydroxyhyperforin-3,9-hemiketal (**9**, Scheme 1),[7] a known PPAP that was also isolated in this study, revealed some significant differences. The resonances of the hemiketal carbon (C-9, δ_C 108.1) and the characteristic quaternary carbon (C-1, δ_C 71.4) in **9** were replaced by the resonances of an ester carbonyl (δ_C 172.0) and a methine (δ_C 63.2, δ_H 4.54), respectively, in **1**. Analysis of the 2D NMR data indicated that these two resonances could be attributed to C-9 and C-1, respectively, due to the HMBC correlations from the methine proton at δ_H 4.54 (s) to C-2, C-3 (δ_C 95.6), C-7, and C-8, from H_2-22 (δ_H 2.55 and 2.10) to C-4, C-5, C-6, and the ester carbonyl (δ_C 172.0), and from Me-32 (δ_H 1.09, s) to C-1 and C-8. An internal ester bridge between C-3 and C-5 was indicated by the indices of hydrogen deficiency along with the characteristic chemical shift of C-3 at δ_C 95.6. On the basis of these results, compound **1** was presumably formed by cleavage of the C-1/C-9 bond of **9** followed by the formation of a five-membered lactone moiety. Additional 2D NMR data including ^{1}H−^{1}H COSY and HMBC confirmed the structure (Figure 2). Therefore, the molecular structure of **1** possesses an unprecedented *seco*-PPAP skeleton.

In the ROESY spectrum of **1**, diagnostic cross-peaks observed for Me-32/H_2-27, H_2-33/H-1, and H-1/H-7 demonstrated that Me-32 and CH_2-27 were both β-oriented while H-1, H-7, and C-33 were all α-oriented. The NOE correlations of H-1/H-17, Me-32/H-6β, and H-6α/H-22 defined the α-orientations of CH_2-22 and CH_2-17. The same orientations of the prenyl groups at C-3 and C-5 were in agreement with the presence of a five-membered lactone moiety (Figure 3).

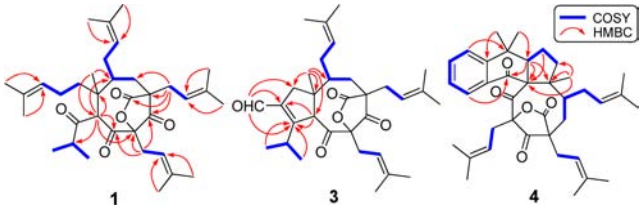


Figure 2. Key HMBC and ^{1}H−^{1}H COSY correlations of **1**, **3**, and **4**.

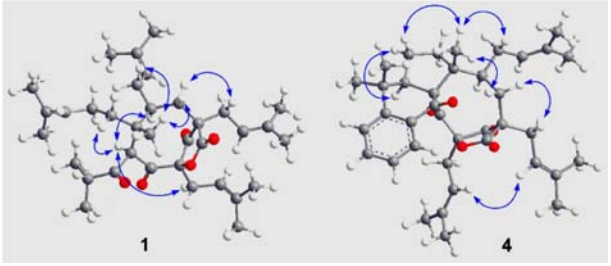


Figure 3. Key ROESY correlations of **1** and **4**.

Analyses of the MS and NMR data (Table S8, Supporting Information) showed that hyphenrone B (**2**) shared the same carbon skeleton and relative configuration as **1**. The isopropyl group in **1** was replaced by a phenyl group in **2**.

The molecular formula of hyphenrone C (**3**) was determined to be $C_{32}H_{44}O_5$ by its HR-ESI-MS (m/z 531.3080, $[M + Na]^+$) and ^{13}C NMR data. Analysis of its 1D and 2D NMR data (Table S9, Supporting Information) revealed that **3** was similar to **1**, especially the resonances of the eight-membered core and lactone moieties (rings A and B). However, comparison of their NMR data indicated that the C-10 carbonyl resonance (δ_C 206.6) and the C-33 isoprenyl unit in **1** were absent in **3**. Instead, an unusual α,β-unsaturated formyl group (δ_C 190.0, δ_H 10.12, s; δ_C 140.0, C-α, and δ_C 168.2, C-β) was observed in **3**. This observation implied that **3** might be a trinor derivative of **1**, which was most likely formed via oxidative cleavage of the C-35/C-36 double bond in **1** followed by aldol condensation between C-34 and C-10 to form the unusual cyclopentene C-ring in **3**. This assumption was confirmed by analysis of its 2D

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NMR data. The cyclopentene C-ring was elucidated on the basis of the HMBC correlations of Me-32 (δ_H 0.79) with C-1 (δ_C 66.5), C-8 (δ_C 55.0), and C-33 (δ_C 44.7), the formyl proton (δ_H 10.12, H-35) with C-10, C-33, and C-34, and H-1 (δ_H 4.32, d, J = 3.0 Hz) with the α (C-34) and β (C-10) carbons of the α,β-unsaturated formyl group. An isopropyl group at C-10 was indicated by the HMBC correlations from both Me-12 and Me-13 (δ_H 1.30 and 1.23, respectively, d, J = 7.2 Hz) to C-10 as well as the proton spin system consisting of Me-12/H-11/Me-13 present in the ^{1}H−^{1}H COSY spectrum (Figure 2). On the basis of similar correlations observed in their ROESY spectra, the relative configurations of C-1, C-3, C-5, C-7, and C-8 of **3** and **1** were identical. Thus, the structure of **3** possesses a new scaffold featuring a 5/8/5 fused ring system (Figure 2).

Hyphenrone D (**4**), a colorless gum, exhibited a molecular ion at m/z 584.3497 in its positive HR-EI-MS, which in conjunction with the ^{13}C NMR data, indicated a molecular formula of $C_{38}H_{48}O_5$, i.e., two hydrogens fewer than **2**. The ^{1}H and ^{13}C NMR spectra of **4** (Table S10, Supporting Information) differed from those of **2** despite some similarities in the resonances of rings A and B along with their substituents. An *ortho*-disubstituted benzene ring in **4** was evident from resonances at δ_H 7.37 (d, J = 7.8 Hz), 7.53 (t, J = 7.8 Hz), 7.38 (t, J = 7.8 Hz), and 7.87 (d, J = 7.8 Hz) compared to the monosubstituted benzene ring in **2**. In addition, the resonances characteristic of C-1 (δ_C 57.9, d; δ_H 5.53, s) and the double bond (δ_C 123.7, C-35, and 135.3, C-36) of the C-8 homoprenyl group in **2** were replaced by two quaternary carbons at δ_C 78.4 and 37.4 and a methine carbon (δ_C 56.6; δ_H 3.34, dd, J = 11.0, 9.0 Hz) in **4**. These observations suggested that **4** might be derived from **2** via an intramolecular cyclization, leading to the formation of two extra carbocycles, such cyclization occurring between C-1, C-35, C-36, and the carbons of the benzoyl group. This assumption was confirmed by the 2D NMR data. The HMBC correlations from Me-32 (δ_H 1.29, s) to C-1, C-8 (δ_C 56.3), and C-33 (δ_C 40.5), from H-35 (δ_H 3.34) to C-1 and C-8, and from H-34 to C-1 coupled with the ^{1}H−^{1}H COSY correlations of H-33/H-34/H-35 established the presence of the cyclopentene C-ring (Figure 2). The presence of the D-ring was confirmed by the HMBC correlations of H-35 with a benzoyl carbonyl carbon at δ_C 199.7 (C-10), of Me-37 and Me-38 (δ_H 1.43 and 0.98) with C-35 (δ_C 56.6), C-36 (δ_C 37.4), and C-12 (δ_C 151.5), of H-16 (δ_H 7.87) with C-10, and of H-13 (δ_H 7.37) with C-36. Thus, the molecular structure of **4** possesses an unprecedented carbon skeleton comprising a 6/6/5/8/5 fused ring system.

The relative configurations of C-5, C-7, and C-8 of **4** were the same as in compounds **1**−**3**, based on the ROESY correlations of Me-32/H-27, Me-32/H-6β, and H-6α/H-22, that indicated β-orientations of Me-32 and CH_2-27 and an α-orientation of CH_2-22. In addition, the correlation between the olefinic protons H-18 (δ_H 4.12) and H-23 (δ_H 4.82) confirmed the α-orientations of the isoprenyl groups at C-3 and C-5. The NOE correlations of Me-32/H-34β and H-34α/H-35 indicated the α-orientation of H-35 (Figure 3). However, the definition of the C-1 configuration was more problematic and had to be derived from its putative biosynthesis pathway. The α-orientation of the C-8 homoprenyl group in precursor **2** necessitated the β,β-junction of ring D to the C-ring, which indicated the relative configuration of C-1 (Scheme 1).

The absolute configuration of **4** was confirmed by comparison of experimental and TD-DFT caculated ECD spectra.[8] First, an arbitrarily assigned 1*R*,3*S*,5*R*,7*S*,8*R*,35*R* configuration was employed for the conformationally random search using the MMFF94s force field in the MacroModel software package. By using the Gaussian09 software package, 23 conformers, which possess the lowest energies from the conformational search with an energy cutoff of 17 kJ/mol (approximately 4 kcal/mol), were included for full geometry optimization at the B3LYP/6-31G** level in the gas phase where 18 conformers were relocated. Further calculations were performed at the B3LYP/6-311++G** and B3LYP-SCRF-(COSMO)/6-311++G**//B3LYP/6-311++G** levels in the gas phase and in MeOH, respectively. Conformational analysis and ECD calculations were performed at the same levels in the gas phase and in MeOH, respectively. The calculated individual and weighted ECD spectra of the predominant conformers were similar and match very well with the experimentally observed spectra at the above levels in both the gas phase and in MeOH (Figure 4). Therefore, the absolute configuration of **4** was defined to be 1*R*,3*S*,5*R*,7*S*,8*R*,35*R*.

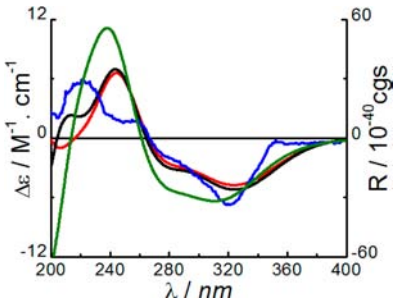

Figure 4. Calculated and experimental ECD spectra of **4** (red and black, at the B3LYP/6-31G** and B3LYP/6-311++G** levels in the gas phase, respectively; olive, at the B3LYP-SCRF(COSMO)/6-311++G**//B3LYP/6-311++G** level in MeOH; blue, experimentally observed in MeOH).

Hyphenrone E (**5**) was determined to be a homologue of oxepahyperforin via analysis of the MS and NMR data (Table S11, Supporting Information).[7] The isopropyl moiety in oxepahyperforin was replaced by an isobutyl group in **5** (Scheme 1).

It is challenging to assign the absolute configuration of PPAPs because they are typically isolated as gums. However, crystals of **5** (CCDC 962624) and **6** (CCDC 962625) suitable for X-ray diffraction analysis were obtained from methanol. The final refinement on the Cu Kα data of the crystal of **5** [the Flack parameter is 0.09(19) and the Hooft parameter is 0.11(5) for 2319 Bijvoet pairs] and **6** [the Hooft parameter is 0.11(7) for 1815 Bijvoet pairs] allowed an unambiguous assignment of the absolute structures as shown in Scheme 1,[9] indicating that the absolute configurations of C-1, C-3, C-5, C-7, and C-8 in **5** and **6** were consistent with the conclusions derived from the experimental and calculated ECD spectra of **4** and those of hyperforin established previously.[2] In addition, the biosyntheses of the members in Scheme 1 are presumably closely related (vide infra). Thus, the absolute configurations of these PPAPs should show a considerable degree of similarity.

Biosynthetically, PPAPs are presumably derived from a "mixed" prenylation/polyketide biosynthesis pathway (Scheme 1). Their acylphloroglucinol core structure may be derived from a characteristic polyketide-type biosynthesis involving the condensation of one acyl-CoA and three malonyl-CoA units.[2,10]

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Prenylation of this core moiety would afford monocyclic polyprenylated acylphloroglucinols (MPAPs), which may be further cyclized to PPAP type metabolites with diverse carbon skeletons.[2,10] In this study, hyperforin (**7**) and adhyperforin (**8**) represented the normal *endo*-BPAPs with a bicyclo[3.3.1]-nonane-2,4,9-trione core and should be the precursors of the 2,35-epoxy derivative hyphenrone E (**5**) and hemiketal derivative **9**. Compounds **1** and **2** were likely formed by cleavage of the C-1/C-9 bond of hemiketal derivatives such as **9** via a retro-aldol mechanism and subsequent formation of a five-membered lactone moiety. The intriguing structure of hyphenrone C (**3**) possessing a new scaffold featuring a 5/8/5 fused ring systems may be derived from **1** via oxidative cleavage of the C-35/C-36 double bond followed by formation of the cyclopentene ring via aldol condensation. The structure of hyphenrone D (**4**), featuring an unprecedented 6/6/5/8/5 fused ring system, was presumbly formed via an intermolecular Diels–Alder cycloaddition of **2**. In addition, the known hyphenrone F (**6**), possessing a 9-nor-PPAP skeleton, may be derived from **1** via sequential hydrolysis and decarboxylation steps.[11] Therefore, the four architectures of these PPAPs are explicable in terms of their presumed biosynthetic origins, and their absolute configurations should accordingly be interrelated.

Several cytotoxic PPAPs have been previously isolated in our laboratory,[6,12] and these types of metabolites are associated with neurodegenerative diseases, such as Alzheimer's disease,[4] we examined the inhibitory activities of the aforementioned compounds against acetylcholinesterase (AChE) and the five human tumor cell lines HL-60, A-549, SMMC-7721, MCF-7, and SW480. Compounds **4** and **5** exhibited moderate cytotoxic activities (IC_{50} 4.7–25.5 μM, Table S6, Supporting Information) against the cancer cell lines using the MTT method.[13] In the AChE inhibition assay using the Ellman method,[14] compound **5** exhibited weak AChE inhibitory activity with an IC_{50} value of 25.4 μM. Notably, the PPAPs that possess a lactone moiety (**1**, **3**, and **4**) exhibited significant negative inhibitory activities (approximately −100% for each) at 50 μM (Table S4, Supporting Information).

■ ASSOCIATED CONTENT

Supporting Information

Computational details of **4**, experimental procedures, physical–chemical properties, MS and NMR spectra for all new compounds, crystallographic data (CIF) for **5** and **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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