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Bioactive Acylphloroglucinols with Adamantyl Skeleton from *Hypericum sampsonii*

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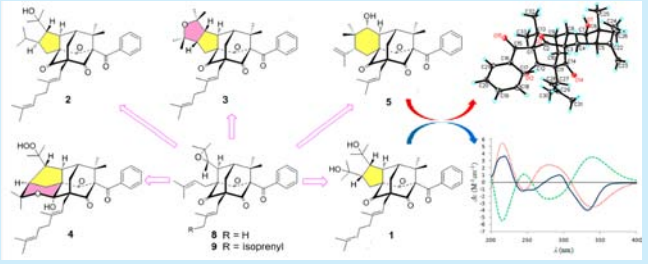
Supporting Information

ABSTRACT: Hyperisampsins A–D (**1**–**4**), with tetracyclo-[6.3.1.1^{3,10}.0^{3,7}]tridecane skeletons and seven biogenetically related congeners (**5**–**11**), were isolated from *Hypericum sampsonii*. Their structures were elucidated by comprehensive spectroscopic techniques. The absolute configuration of **1** was established by ECD calculations, and those of **5** and **9** were confirmed by single X-ray crystallographic analyses. Hyperisampsins A and D showed potent anti-HIV activities with EC_{50} of 2.97 and 0.97 μM and selectivity index of 4.80 and 7.70, respectively.



Polycyclic polyprenylated acylphloroglucinols (PPAPs) are usually characterized by a highly oxygenated and densely substituted bicyclo[3.3.1]nonane-2,4,9-trione or other related core structures that were decorated with isoprenyl, geranyl, and acyl groups.[1] Over the past two decades (especially in recent years), more than 200 structurally complex PPAPs endowed with different skeletons have been reported, such as [3.3.1]-type, adamantyl-type, adamantyl-like-type, spiro-type, hyperuralones, hyphenrones, etc. (Supporting Information, SI, Figure S1).[1b,2] PPAPs are mainly isolated from the plants of the Guttiferae family and exhibit a wide variety of bioactivities, including antitumor, antibacterial, antioxidant, anti-HIV, and anti-neurodegenerative.[1] For instance, hyperforin, a representative PPAP obtained from St. John's wort (*H. perforatum*), was found to possess antidepressant and antibiotic activities;[3] guttiferone I, isolated from *Garcinia humilis*, was reported to be a ligand of liver X receptors.[4] In light of their complex structures and potential biological activities, the PPAP family has attracted great interest from the organic synthetic community.[5]

H. sampsonii (Guttiferae) has been used as a traditional Chinese medicine to treat backache, burns, diarrhea, and swelling.[6] Previous chemical and medicinal investigations on this species have led to the isolation of a series of metabolites including anthraquinones, flavonoids, xanthones, and PPAPs.[7] In our current study, seven novel PPAPs, termed hyperisampsins A–G (**1**–**6** and **8**, Figure 1), along with four known

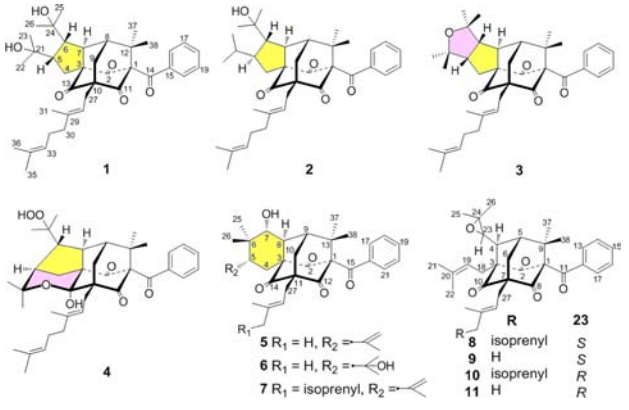


Figure 1. Structures of compounds **1**–**11**.

analogues, sampsoniones I (**7**),[2b] Q (**9**),[7a] J (**10**),[2b] and 28,29-epoxyplukenetione A (**11**),[8] were isolated from the aerial parts of *H. sampsonii*. Hyperisampsins A–D (**1**–**4**) represent the first set of examples of PPAPs with a rigid caged tetracyclo-[6.3.1.1^{3,10}.0^{3,7}]tridecane-2,11,13-trione skeleton carrying a five-membered ring on the western hemisphere. Herein, we present the isolation, structure elucidation, and bioactivity evaluation of

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these new compounds. In addition, a plausible biogenetic pathway of **1**−**4** is also proposed.

The molecular formula of hyperisampsin A (**1**), $C_{38}H_{50}O_6$, with 14 degrees of unsaturation, was evidenced by the $[M + Na]^+$ ion at m/z 625.3489 in HRESIMS. The ^1H NMR spectrum of **1** (Table S1) showed signals of a phenyl group (δ_H 7.19, 2H, brd, J = 8.3 Hz; 7.29, 2H, brt, J = 7.8 Hz; 7.43, 1H, brt, J = 7.3 Hz), two olefinic protons (δ_H 5.19, 1H, t, J = 7.1 Hz; 5.05, 1H, t, J = 7.0 Hz), and nine methyl singlets (δ_H 1.32−1.66). The ^{13}C NMR of **1** (Table S1) revealed that **1** possessed 38 carbons, including 10 quaternary carbons (four carbonyls), four methines, two methylenes, six methyls, and another 16 signals assignable to a phenyl group and a geranyl moiety. These analyses indicated that **1** was likely a PPAP-containing tetracyclic system. The planar structure of **1** was established by extensive analyses of its HSQC, $^1H-^1H$ COSY, and HMBC spectra (Figure 2). The HMBC correlations from Me-37 and

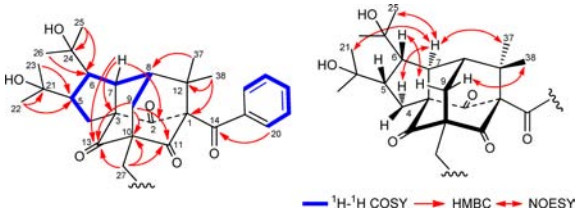

Figure 2. Key 2D NMR correlations of **1**.

Me-38 to C-1, C-8, and C-12, from H-7 to C-2, C-3, and C-13, from H-8 to C-1, C-3, and C-10, and from H-9 to C-7, C-10, C-11, C-12, and C-13 constructed the rigid adamantyl framework, which was comparable to that of sampsonione Q (**9**).[7a] The five-membered carbon ring was established by HMBC cross-peaks from H-4 to C-3, C-6, and C-7 and from H-5 and H-6 to C-7 and further was supported by the proton spin system of H-4/H-5/H-6/H-7 in the $^1H-^1H$ COSY spectrum. The HMBC correlations from Me-22 and Me-23 to C-5 and C-21 and from Me-25 and Me-26 to C-6 and C-24 revealed that two 2-hydroxyisopropyl moieties were located at C-5 and C-6, respectively. Moreover, the locations of the geranyl at C-10 and the benzoyl group at C-1 were elucidated to be the same as those of sampsonione J (**10**).[2b]

With respect to the conformation of the tetracyclic system, the relative configurations at C-1, C-3, C-8, and C-10 of the rigid adamantyl core were set up as those of **9**.[7a] The relative configurations of the remaining chiral centers at C-5, C-6, and C-7 of **1** were deduced by careful analysis of its NOESY spectrum (Figure 2). The NOESY correlations of Me-37/H-7, H-7/Me-25, H-7/Me-21, and H-7/H-4α revealed that H-7, Me-25, Me-21, and Me-37 were cofacial and α-oriented. Consequently, the NOESY correlations of Me-38/H-9a and H-9b/H-6 indicated the β-orientations of these protons. Thus, the relative configuration of **1** was determined as depicted in Figure 1.

The molecular formula of hyperisampsin B (**2**) was determined to be $C_{38}H_{50}O_5$ as evidenced by HRESIMS at m/z 609.3529. The ^1H and ^{13}C NMR data (Table S1) of **2** were found to closely resemble those of **1**, except for the absence of a quaternary carbon and the presence of an additional methine (δ_C 28.5, C-21), which suggested the structural similarities of both compounds. The replacement of the 2-hydroxylisopropyl at C-6 in **1** by an isopropyl in **2** was revealed by the presence of two methyl doublets in the ^1H NMR spectrum (δ_H 0.94 and 0.96, d, J = 6.9 Hz) and further confirmed by $^1H-^1H$ COSY and HMBC spectra. The relative configuration of **2** through NOESY spectrum (Figure S2) and coupling constant analyses was found to be identical to those of **1** except for the orientation of H-5, which was confirmed by NOESY correlation between H-7 and H-5.

Hyperisampsin C (**3**), with a molecular formula of $C_{38}H_{48}O_5$ and 15 degrees of unsaturation, was deduced from HRESIMS $[M + Na]^+$ m/z 607.3373. The ^1H and ^{13}C NMR data (Table S1) of **3** closely resembled those of **1**. 2D NMR experiments disclosed that **3** and **1** possessed similar structures with the same connected mode of carbon rings. Comparison of the ^{13}C NMR data of **3** with that of **1** revealed that their major differences were the deshielded signals of C-21 and C-24 (δ_C 81.7 and 79.2 for **3**, δ_C 75.3 and 70.9 for **1**). These findings, along with the deduced degrees of unsaturation, implied that C-21 and C-24 were connected by an oxygen atom to form an additional furan ring. The NOESY (Figure S3) spectrum revealed that **3** had the same relative configuration as **1**.

The molecular formula of **4** was determined as $C_{38}H_{50}O_7$ by the accurate molecular ion peak at m/z 641.3433 $[M + Na]^+$ in HRESIMS, indicating 14 degrees of unsaturation identical to that of **1**. Comparison of the NMR data (Table S1) of **4** with those of **1**−**3** revealed their structural similarities. However, an apparent difference of ^{13}C NMR at δ_C 202.0 in **1** and δ_C 101.9 in **4** indicated that the carbonyl group (C-13) of **1** was replaced by a hemiketal group in **4**. Extensive analyses of the 2D NMR indicated that **4** featured the same carbon skeleton as that of **1**. Considering the degrees of unsaturation and the absence of the carbonyl group, **4** should possess an additional ring formed between C-13 and C-21 or C-24 (Figure S4, **4a** and **4b**). By comparison of the calculated and experimental ^{13}C NMR data (Table S2, Figure S4), it could be deduced that the six-membered pyran ring was formed by the linkage of C-13 and C-21 via an oxygen atom. The characteristic chemical shift of C-24 (δ_C 85.5)[7b] combined with the molecular formula deduced by HRESIMS suggested the presence of the hydroperoxy group at C-24. By careful analysis of the NOESY spectrum (Figure S5) of **4**, orientations of H-5, H-6, and H-7 were assigned the same as those of **2** by cross-peaks of Me-38/H9b, H-9a/H-6/Me-23, and Me-37/H-7/Me-25. The configuration of C-13-OH (δ_H 4.46, s) takes the axial orientation as determined by NOESY correlations from C-13-OH to H-4ax and H-28. The α-orientation of the 2-hydroperoxyisopropyl moiety at C-6 further confirmed the position of the pyran ring, which was located between C-21 and C-13. Thus, the structure of **4** was established.

To determine the absolute configurations of **1**−**4**, the electronic circular dichroism (ECD; Figure 3; for detailed procedures, see SI) of **1** [enantiomers: **1A** (1*R*,3*R*,5*S*,6*R*,7*R*,8*R*,10*S*); **1B** (1*S*,3*S*,5*R*,6*S*,7*S*,8*S*,10*R*)] were calculated using time-dependent density functional theory (TDDFT).[9] Six stable conformations of **1** (**1a** 25.9%, **1b** 12.4%, **1c** 0.6%, **1d** 34.3%, **1e** 1.0%, **1f** 25.8%) were yielded at the B3LYP/6-31G(d,p) level, and these conformations were then used for ECD calculations at the B3LYP-PCM/6-311++G(d,p)//B3LYP/6-31G(d,p) level. There is an overall agreement between the experimental CD and the calculated ECD curve of **1A**. Therefore, the absolute configuration of **1** was established as 1*R*,3*R*,5*S*,6*R*,7*R*,8*R*,10*S*.

To better understand the experimental CD curve of **1** and further determine the absolute configurations of **2**−**4**, the molecular orbital (MO) analysis (Figure 4) of **1** was performed

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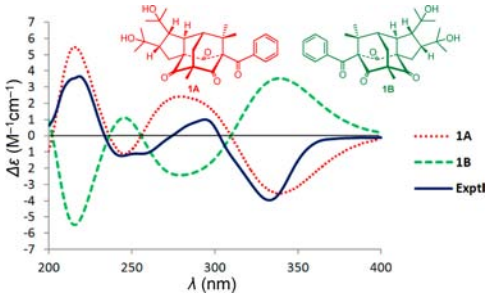

Figure 3. Experimental CD (in MeOH) and calculated ECD spectra of **1A** and **1B** in MeOH.

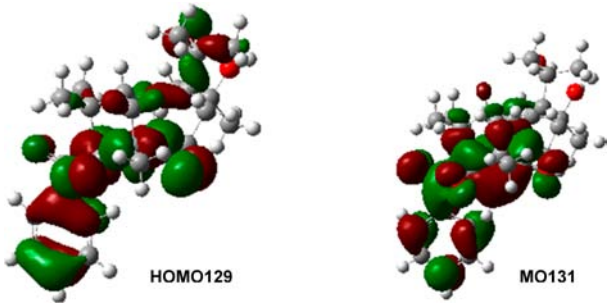

Figure 4. Most important orbitals involved in the key transitions of the conformer **1d** in MeOH using PCM model.

by using the conformer **1d** at the B3LYP/6-311++G(d,p) level in MeOH with PCM model. As shown in Figure 3, the ECD Cotton effect (CE) at 335 nm could be assigned to the experimental CE at 333 nm, which was caused by the electronic transitions from HOMO129 to MO131 involving an n−π* transition of the C═O double bond and π−π* transition of the benzene ring.

To the best of our knowledge, the absolute configuration of C-1 for all naturally occurring adamantyl and homoadamantyl PPAPs appeared as 1*R* in the literature.[2f,5f,10] In this study, the absolute configurations of hyperisampsin E (**5**) and the known compound sampsonione Q (**9**) were first determined by X-ray diffractions with Cu Kα radiation (Figures S5 and S6, CCDC

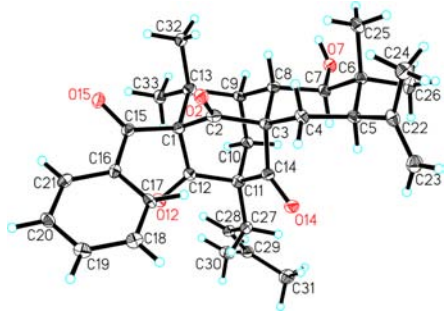

Figure 5. X-ray crystal structure of hyperisampsin E (**5**).

997655 and 912992), and the absolute configurations of hyperisampsins F and G (**6** and **8**) and the known compounds **7**, **10**, and **11** were unambiguously confirmed by comparing their experimental CD curves with those of **5** and **9** (Figure 6); all of them featured 1*R* configurations (see SI for the structure elucidations of **5**, **6**, and **8**). These findings, coupled with the aforementioned TDDFT ECD calculation, and MO analysis of **1**, which parallel previous reports of the absolute configuration

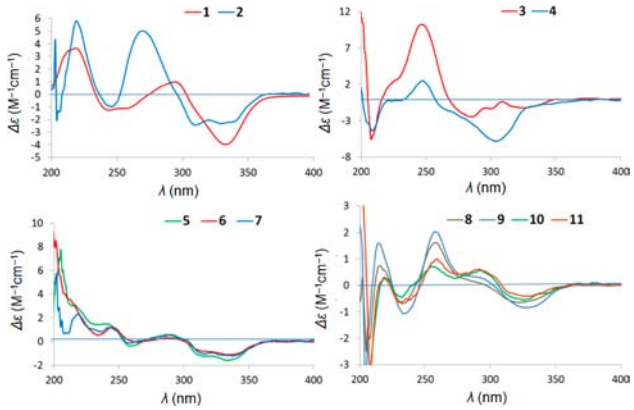

Figure 6. CD spectra of compounds **1**−**11**.

of C-1 of adamantyl and homoadamantyl PPAPs,[2f,5f,10] allowed us to analyze the CEs of **1**−**11** at 333 nm (Figure 6). When the MO analysis of **1** was taken into consideration, the negative CE at 333 nm indicated the *R* configuration for C-1 of benzoyl-substituted adamantyl and homoadamantyl PPAPs, which can serve as a benchmark for the determination of the absolute configurations of other adamantyl and homoadamantyl PPAPs. Since all CD spectra of compounds **2**−**4** showed negative CEs around 333 nm, the absolute configurations of C-1 of **2**−**4** were thus determined as *R*.

Hyperisampsins A−D (**1**−**4**) represent the first set of examples of natural PPAPs with rigid caged tetracyclo-[6.3.1.1^{3,10}.0^{3,7}]tridecane-2,11,13-trione skeletons. In addition, compound **3** featured an additional furan ring on the western hemisphere, and compound **4** was characterized by an additional pyran ring incorporated into the tetracyclo-[6.3.1.1^{3,10}.0^{3,7}]tridecane ring systems, which are unique in natural adamantyl PPAPs. To the best of our knowledge, only a few adamantyl-like PPAPs, such as sampsoniones A and B,[11] were found to possess a hemiketal carbon in the core skeleton, thus **4** represents the first example of adamantyl PPAPs with an unusual hemiketal carbon in the adamantyl skeleton. A plausible biogenetic pathway (Scheme S1) of **1**−**4** was postulated to explain their origins, which biogenetically confirmed the consistency of their absolute configurations. A series of oxidation, nucleophilic addition, dehydration, and aldol condensation reactions were involved in this hypothetical pathway, and the nucleophilic addition was the key step for the generation of compounds **1**−**4** from **8**.

Furthermore, compounds **1**−**6** and **8** were tested for cytotoxic activities (Table S5) against five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7, SW480) and the immortalized noncancerous human pulmonary epithelial cell line (Beas-2B) by the MT method,[12] in vitro, with cisplatin as the positive control. The results displayed that almost all the tested compounds are active against the above cancer cells, with IC_{50} values of 5.95−40 μM. Compounds **1** and **4** were also evaluated for anti-HIV-1 activities (Table S6) with the reported p24 assays,[13] and both compounds showed remarkable activities to prevent the cytopathic effects of HIV-1 in MT-4 cells with EC_{50} values of 2.97 and 0.97 μM and selectivity index of 4.80 and 7.70, respectively.

In conclusion, we have discovered a series of highly functionalized PPAPs, hyperisampsins A−G (**1**−**6** and **8**), from *H. sampsonii*. Among them, **1**−**4** featured unprecedented tetracyclo[6.3.1.1^{3,10}.0^{3,7}]tridecane adamantyl cores. In terms of

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the structure elucidation, the absolute configurations of these complex PPAP derivatives were determined by a combination of X-ray diffraction analysis, comparison of experimental CD and quantum chemical ECD calculations, and MO analysis. We provided a method using a CD spectrum to determine the absolute configurations of adamantyl and homoadamantyl PPAPs with a benzoyl group at C-1. In addition to the fascinating structural complexity, **4** possesses promising anti-HIV-1 activity, which makes it outstanding in the structure family of natural PPAPs and will be a challenging target for both chemists and biologists in the future.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, 1D and 2D NMR, MS, UV, and IR spectra for compounds **1**−**6** and **8**, and X-ray crystallographic data of **5** and **9** in CIF format were included. 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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