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Hypersubones A and B, New Polycyclic Acylphloroglucinols with Intriguing Adamantane Type Cores from *Hypericum subsessile*

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Supporting Information

ABSTRACT: Hypersubones A and B (**1**, **2**), two adamantane type polycyclic polyprenylated acylphloroglucinols possessing an unprecedented *seco*-adamantane architecture and a tetracyclo-[6.3.1.1^{3,10}.0^{4,8}]-tridecane core combined with a peroxide ring, respectively, were isolated from *Hypericum subsessile* together with three analogues (**3**–**5**). Their structures were determined by extensive NMR spectroscopic analysis, ECD calculations, and single-crystal X-ray diffraction. Compound **2** exhibited significant cytotoxicities against four human cancer lines in vitro (IC$_{50}$ 0.07–7.52 μM).

Adamantane, known as the "chemist's diamond", has a highly rigid and caged tricyclo-[3.3.1.1^{3,7}]-decane core and has been studied extensively in the last 50 years.[1−5] In 1964, amantadine, the most famous member of the adamantane family, was found to exhibit potent anti-influenza A properties.[2,3] The significance of the adamantyl group in drug design is multidimentional.[4−6] For instance, an adamantyl-based compound can be sufficiently lipophilic to increase partition coefficients, and the adamantyl group can positively modulate the therapeutic index.[5,6] In recent years, the adamantyl group was present in seven compounds in current clinical use and is being incorporated into many more compounds that are in development as potential therapeutics.[5] These adamantanes are all synthetic entities.[2] However, there are also natural products that incorporate the adamantane hydrocarbon scaffold, some of which also display interesting bioactivities. In 1996, plukenetione A,[7] a polycyclic polyprenlated acylphloroglucinols (PPAP) type metabolite possessing the first adamantane skeleton, was isolated from *Clusia plukenetii*.

PPAPs are a group of structurally fascinating natural products that have only been isolated from plants of the family Guttiferae,[8,9] most of which contined an *endo*-bicyclic polyprenylated acylphloroglucinols (*endo*-BPAPs) with a bicyclo-[3.3.1]-nonane-2,4,9-trione core.[8,10] The adamantane PPAPs consist of 62 members, and are presumably derived from the *endo*-BPAPs via secondary cyclizations (Scheme 1) and can be divided into four structural types.[7,11] To the best of our knowledge, the adamantane PPAPs are the only source of natural hydrocarbon scaffold adamantane derivatives.[2,8] These metabolites show a variety of bioactivities including antitumor, antimicrobial, anti-HIV, antioxidant, and antidepressant activities.[8,9,12] In our systematic study of the PPAP metabolites, five adamantane PPAPs including three new ones (hypersubones A–C, **1**–**3**) were isolated from *Hypericum subsessile* and characterized to possess four different carbon skeletons including two unusual ones (**1** and **2**, Figure 1). Their structures were elucidated by extensive NMR spectroscopic methods, ECD calculations, and single-crystal X-ray diffraction. To our knowledge, hypersubone A (**1**) was the first *seco*-adamantane PPAP that could be biosynthetically formed by the cleavage of the C-1/C-9 bond of normal adamantane. Compound **2** was elucidated to possess a tetracyclo-[6.3.1.1^{3,10}.0^{4,8}]-tridecane carbon skeleton bearing an unusual peroxide ring, and exhibited significant cytotoxicity against four human cancer cell-lines in vitro (IC$_{50}$ 0.07–7.52 μM). This article presents the structural elucidation, proposed briosynthetic pathway, and the evaluation of anticancer activity of the new isolates.

The molecular formula of hypersubone A (**1**) was determined to be $C_{36}H_{54}O_5$ by its HR-EI-MS (*m/z* 566.3962, [M]$^+$, calcd 566.3971) and ^{13}C NMR data. Its IR spectrum showed absorption bands that were consistent with hydroxyl (3440 cm^{-1}) and carbonyl groups (1720 and 1738 cm^{-1}). Analysis of ^{13}C NMR and DEPT spectra revealed that **1** possessed 36 carbons (Supporting Information (SI), Table S3), in which 23 signals could be assigned to a geranyl, one prenyl, an isobutyryl, and one isobutenyl. The remaining 13 carbon signals consisted of seven quaternary carbons, two methines, one methylene, and three methyls, nine of which were ascribed to a nonconjugated carbonyl at δ_C 206.1 (C-4), three

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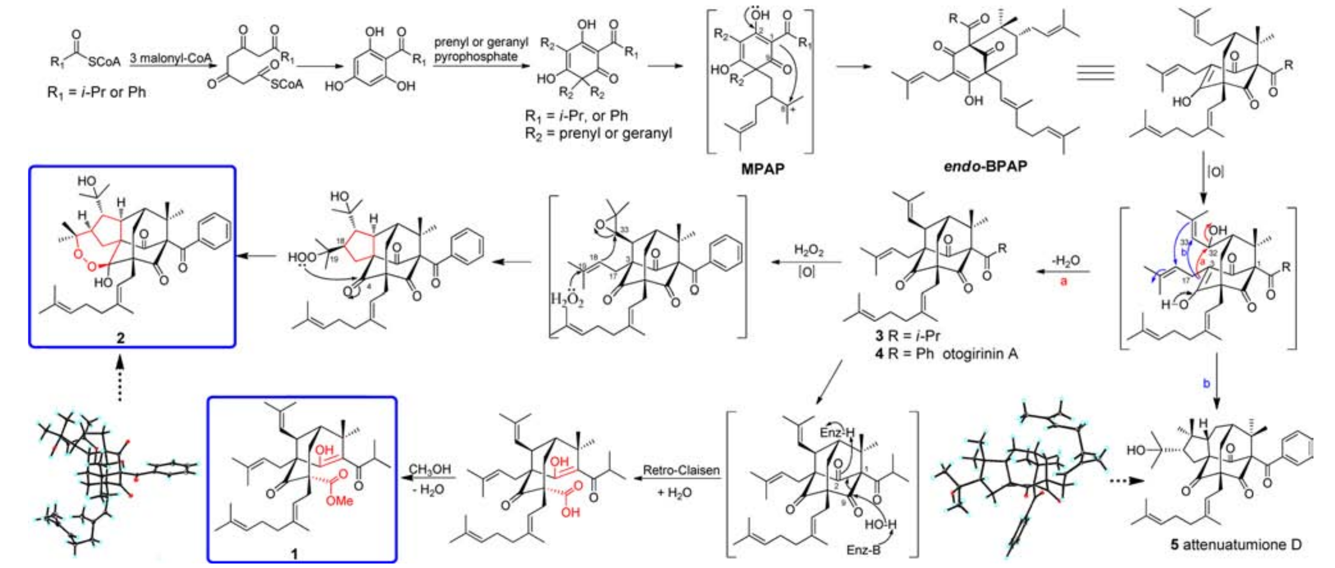
Scheme 1. Putative Biosynthesis Pathways to 1−5



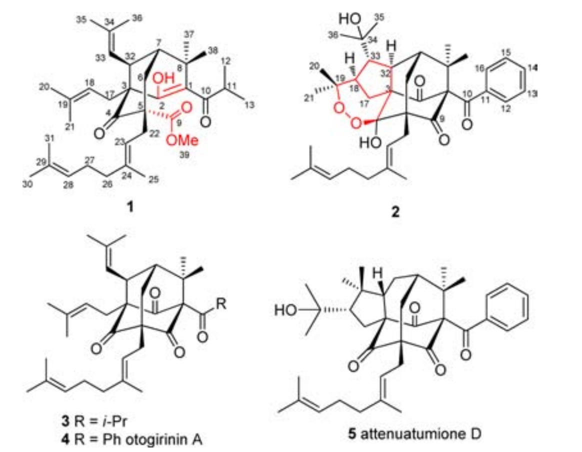


Figure 1. Structures of compounds **1**−**5**.

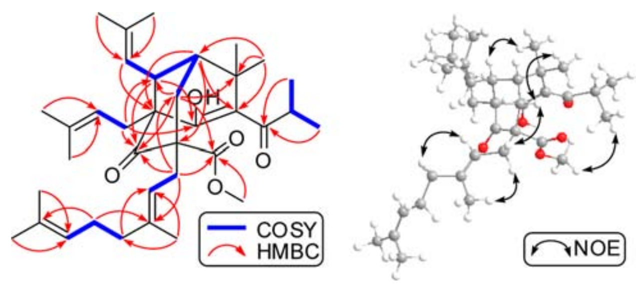


Figure 2. Key HMBC, $^1H-^1H$ COSY, and key NOE correlations of compound **1**.

quaternary carbons at δ_C 62.7 (C-3), 60.3 (C-5), and 36.9 (C-8), two methines at δ_C 51.2 (C-7) and 40.8 (C-32), one methylene at δ_C 30.9 (C-6), and two methyls at δ_C 33.0 (C-37) and 27.7 (C-38). The aforementioned signals implied that **1** possessed an adamantane PPAP core,[8,11] which was confirmed by the HMBC correlations from Me-37 (δ_H 1.47, s) and Me-38 (δ_H 1.59, s) to C-1 (δ_C 119.2), C-7, and C-8; from H-7 (δ_H 1.52, overlap) to C-1, C-3, C-5, C-6, C-8, C-32, and C-37; from H-32 (δ_H 3.37, brd, J = 9.5) to C-2 (δ_C 180.6), C-3, C-4, and C-7; and from H-6 to C-4, C-5, C-7, C-8, C-9, and C-32 coupled with the $^1H-^1H$ COSY correlations of H-6a/H-7/H-32 (Figure 2).

In general, the chemical shift of C-1 for normal adamantane PPAPs was always located at δ_C 81−88, and that of C-2 and C-9 were at δ_C 201−209, respectively.[7,10,11,15] However, the chemical shifts of the remaining three signals (δ_C 119.2, 173.5, and 180.6) in **1** ascribable to the three carbons were different. Furthermore, the methoxycarbonyl group was also present in the ^{13}C NMR spectrum. These evidence indicated a cleavage of the C-1/C-9 or C-1/C-2 bond of the adamantane core in **1**. The key HMBC correlations from H_2-6 to C-4, C-5, and the methoxycarbonyl group at δ_C 173.5 (C-9), and from H_2-22 to C-4, C-5, C-6, and C-9 confirmed that the *seco*-adamantane core of **1** was formed by the cleavage of the C-1/C-9 bond of the normal adamantane. The key HMBC correlations from Me-37/Me-38 to C-1 (δ_C 119.2), from H-32/H_2-17 to C-2 (δ_C 180.6) confirmed the presence of the enol moiety at C-1 (δ_C 119.2) and C-2 (δ_C 180.6). The isobutyryl, prenyl, geranyl, and isobutenyl were located at C-1, C-3, C-5, and C-32, respectively, on the basis of the detailed HMBC and $^1H-^1H$ COSY correlation analysis (Figure 2).

In the ROESY spectrum of **1**, the NOE correlations of H-6b/Me-38, H-6b/H_2-22, and Me-19/Me-13 indicated that Me-38 and C-22 were both β-oriented while Me-37 and C-9 were α-oriented. The α-orientation of H-32 was deduced by the NOE correlation of Me-37/H-32. Moreover, the geometry of the double bond in the geranyl group was elucidated to be 23*E* on the basis of the ROESY correlations of H_2-26/H-23 and Me-25/H_2-22 (Figure 2).

The absolute configuration of **1** was determined by the comparison of experimental and time-dependent Density Functional Theory (TDDFT) that calculated electronic circular dichroism (ECD) spectra. Conformational analysis using molecular mechanics calculations was perfomred in the Discovery Studio 3.5 Client with MM force field with 10 kcal/mol upper energy limit. Using the Gaussian 09 software package, the selected conformers were optimized at the B3LYP/6-31G(d,p) level. The theoretical calculation of ECD was performed using time dependent density functional theory (TDDFT) at B3LYP/6-31G(d,p) level in MeOH with PCM model. The ECD spectra of **1** matched well the experimental

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spectra (Figure 3). Thus, the absolute configuration of **1** was confirmed as 3*S*,5*S*,7*R*,32*S*.

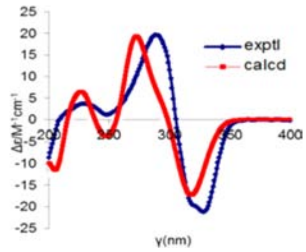

Figure 3. Calculated and experimental ECDs of **1** (red, calculated at the B3LYP-PCM/6-31G(d,p)//B3LYP/6-31G(d,p) level in CH_3OH; blue, experimental in CH_3OH).

Hypersubone B (**2**) was obtained as colorless crystals. Its molecular formula, $C_{38}H_{50}O_7$, was established by the HR-ESI-MS (*m*/*z* 641.3458 $[M + Na]^+$, calcd 641.3454) and ^{13}C NMR, indicating 14 degrees of unsaturation. The IR spectrum showed absorptions attributable to hydroxyl (3434 cm^{-1}, br), carbonyl groups (1731 and 1698 cm^{-1}), and phenyl group (1599 and 1449 cm^{-1}), respectively. The ^1H NMR spectrum showed signals assignable to a monosubstituted benzene ring, two olefinic protons (δ_H 5.35, 1H, t, J = 7.4 Hz and 5.09, 1H, t, J = 7.0 Hz), and nine singlet methyl groups (δ_H 1.20−1.66) (SI, Table S3). The ^{13}C NMR and DEPT spectra exhibited 38 carbon resonances that corresponded to nine quarternary carbons (including two carbonyls, two oxygenated carbons and one hemiketal carbon), four methines, two methlenes, six methyls, and 17 other signals attributable to a benzoyl group and a geranyl group (SI, Table S3). By carefully analyzing the characteristic resonances, two nonconjugated carbonyls at δ_C 205.5 (C-2) and 207.4 (C-9), four quaternary carbons at δ_C 84.2 (C-1), 66.8 (C-3), 58.7 (C-5) and 56.3 (C-8), two methines at δ_C 46.1 (C-7) and 51.9 (C-32), one methylene δ_C 30.5 (C-6), and two methyls were clearly observed, which indicated that compound **2** possessed an adamantane type PPAP core.[8,11b] The core structure was confirmed by the HMBC correlations from H-6a (δ_H 2.20, dd, J = 2.2 and 14.3 Hz) to C-4 (δ_C 102.6), C-5, C-7, C-8, and C-9, from H-7 (δ_H 1.88, brs) to C-1, C-3, C-5, C-6, C-8, and C-32, from H-32 (δ_H 2.61, m) to C-2, C-3, C-4, and C-7, from Me-37 and Me-38 to C-1, C-7, and C-8, coupled with the proton spin system of H-6/H-7/H-32 (Figure 4).

Further analyses of the $^1H-^1H$ COSY spectrum revealed a continuous spin coupling system, H-17/H-18/H-33/H-32, in the structure of **2** (Figure 4). In the HMBC spectrum, the cross-peaks from H-32 to C-2, C-3, C-17 (δ_C 30.2), and C-18 (δ_C 50.4), from H-17β to C-2 and C-3, from H-17α (δ_H 2.51)

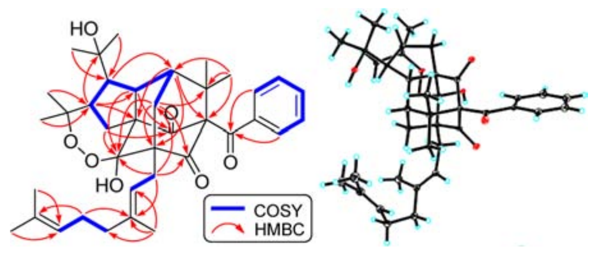

Figure 4. Key HMBC, $^1H-^1H$ COSY (left) correlations and single-crystal X-ray structure (right) of **2**.

to C-32, from H-18 to C-3, C-17, and C-32 were also observed (Figure 4). The aforementioned evidence suggested the presence of an unusual five-membered carbon ring coupled to the admantane core. The HMBC correlations from H_2-22 to C-4, C-5, C-6, and C-9 indicated that the geranyl and benzoyl groups were located at C-5 and C-1, respectively. On the basis of the HMBC correlations from Me-20/Me-21 to C-18/C-19, and from Me-35/Me-36 to C-33/C-34, two oxygenated isopropyl groups were elucidated as being located at C-18 and C-33, respectively.

The molecular formula of $C_{38}H_{50}O_7$ indicated 14 degrees of unsaturation for **2**. The presence of an adamantane core fused to a five-membered ring, two carbonyls, one benzoyl, and one geranyl only accounted for 13 degrees of unsaturation, which suggested the existence of one more ring in **2**. This additional ring should be located between C-4 and C-19 through a peroxide bond, since the chemical shift of C-19 and C-34 were located at a lower field region at δ_C 86.0 and 73.6, respectively. The 1-hydroxyl-isopropyl should therefore be located at C-33 (Figure 4).

In the ROESY spectrum, diagnostic cross-peaks observed for H-6a/H-33 and H-6b/Me-38 demonstrated that Me-38 and H-33 were both β-oriented while Me-37 was α-oriented. The NOE correlations of Me-37/H-32 and H-32/H-18 indicated the α-orientation of H-32 and H-18. In addition, the geometry of the double bond in the geranyl group was deduced to be 23*E* by the ROESY correlation for H_2-26/H-23. Thus, hypersubone B (**2**) was elucidated to possess an unusual tetracyclo-$[6.3.1.1^{3,10}.0^{4,8}]$-tridecane carbon skeleton bearing an unusual peroxide ring. In addition, we were lucky to obtain crystals suitable for a single-crystal X-ray diffraction analysis (CCDC 1037457), which further confirmed the planar construction and determined the absolute configuration as 1*R*,3*S*,4*R*,5*S*,7*R*,18*R*,32*R*,33*R* [the Flack parameter is −0.01(12) and the Hooft parameter is 0.09(6) for 2560 Bijvoet pairs][16,17] (Figure 4).

Hypersubone C (**3**) was assigned the molecular formula $C_{35}H_{50}O_4$ on the basis of its HR-ESI-MS (*m*/*z* 557.3616, $[M + Na]^+$ calcd 557.3606) and ^{13}C NMR. Extensive analysis of its 1D and 2D NMR data (SI, Table S3) showed that hypersubone C (**3**) shared a similar carbon skeleton and relative configuration with the known analogue, otogirinin A[11b] (**4**). The novelty for **3** was assigned to the substitution of an isobutyryl at C-1, instead of a benzoyl group in **4**. In addition, the biosynthesis of **3**, **4**, and **5** (CCDC 1049339) are presumably closely related to that of **1** and **2** as shown in Scheme 1. Thus, the absolute configuration of these analogs should show a considerable degree of consistency with each other, and this deduction was consisted with the fact that the absolute configuration of C-1 was *R* for all natural adamantane and *homo*-adamantane PPAPs so far.[15,18−20]

From a biogenetic point of view, adamantane PPAPs are presumably derived from *endo*-BPAPs through C−C radical coupling (Scheme 1).[8] The *endo*-BPAPs are probably biosynthesized from monocyclic polyprenylated acylphloroglucinols (MPAPs) via one cyclization, which are possibly generated through the "mixed" prenylation/polyketide biosynthetic pathway (Scheme 1).[8,13] Hypersubone A (**1**) is the first *seco*-adamantane PPAP biogenetically derived by the cleavage of the C-1/C-9 of the normal adamantane such as **3** through one Retro-Claisen reaction and then an esterification (Scheme 1).[14,15] Hypersubone B (**2**) was determined to possess a tetracyclo-$[6.3.1.1^{3,10}.0^{4,8}]$-tridecane carbon skeleton

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bearing an unusual peroxide ring, which was possibly generated from otogirinin A (**4**) via C−C radical coupling of C-18 and C-33, and then one nucleophilic addition reaction of OOH-19 and C-4 (Scheme 1).

Compounds **1**−**3** were tested for their cytotoxic effects against four human cancer cell lines (i.e., HepG2, Eca109, HeLa, and A549) by the MTT reagent assay as described previously.[21] It is worthy to note that compound **2** showed promising toxicities against the four human cancer cell lines (IC_{50} 0.07−7.52 μM) (Table 1).

Table 1

	HepG2	Eca109	HeLa	A549
1	17.74	13.54	42.46	>50
2	1.58	**0.07**	3.54	7.52
3	9.74	6.71	9.33	17.23
etoposide[a]	>20	8.04	21.02	>20

[a]Positive control.

In conclusion, five adamantane PPAPs including three new ones (hypersubones A−C, **1**−**3**) were isolated in this study. The five isolates were elucidated to possess four different carbon skeletons including two unusual ones as exemplified by **1** and **2**. Biosynthetically, all five of the adamantane PPAPs are presumably derived from *endo*-BPAPs via different reactions. The discovery of hypersubone A (**1**), the first *seco*-adamantane PPAP, enriches the research of natural adamantane metabolites, and also provides a new challenging target for chemists. Hypersubone B (**2**) was defined as possessing a tetracyclo-$[6.3.1.1^{3,10}.0^{4,8}]$-tridecane carbon skeleton bearing an unusual peroxide ring, and exhibited promising cytotoxicities against four human cancer cell-lines in vitro (IC_{50} 0.07−7.52 μM), and therefore has marked therapeutic potential.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures, physical-chemical properties, MS and NMR spectra for all new compounds, ^{1}H and ^{13}C NMR data of **1**−**3**. Computational details of **1**. Key HMBC, ^{1}H−^{1}H COSY and NOE correlations of **3**. 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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