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Dioxasampsones A and B, Two Polycyclic Polyprenylated Acylphloroglucinols with Unusual Epoxy-Ring-Fused Skeleton from *Hypericum sampsonii*

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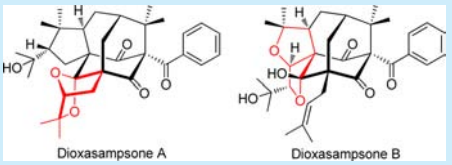
Supporting Information

ABSTRACT: Dioxasampsones A and B (**1** and **2**), two new polycyclic polyprenylated acylphloroglucinols with an unusual epoxy-ring-fused skeleton by new ways of cyclization, along with a new nor-PPAPs hypersampson R (**3**) with the loss of C-31−33 in isopentenyl, were isolated from the aerial parts of *Hypericum sampsonii*. **1** possessed an unexpected hexacyclic skeleton with a rare 2,7-dioxabicyclo[2.2.1]heptane moiety, and **2** featured a unique tetrahydrofuro-[3,4-*b*]furan-fused tricycle[4.3.1.1^{5,7}]undecane skeleton. The gross structures of the new compounds were determined by extensive NMR spectroscopic methods. Their absolute configurations were deduced by single-crystal X-ray diffraction and ECD calculations.



Investigations on the chemical constituents of plants from the Guttiferae family have revealed a series of complex caged polycyclic polyprenylated acylphloroglucinols (PPAPs), which featured a highly oxygenated and densely substituted bicyclo[3.3.1]nonane-2,4,9-trione core adorned with prenyl or geranyl side chains.[1,2] Secondary cyclizations of the decorated prenyl or geranyl groups led to pyrano-fused structures with a bicyclo[3.3.1]nonane skeleton, homoadamantanes with a tricyclo[4.3.1.1^{5,7}]undecane skeleton, adamantanes, or other cyclized structures.[2−12] PPAPs have attracted much attention from natural product researchers because of their fascinating chemical structures and their intriguing biological activities. There have been reports in the past few decades revealing that PPAPs exhibit a wide variety of biological activities such as antimicrobial, antidepressant, antioxidant, cytotoxic, and anti-HIV activities.[2]

Hypericum sampsonii, as a member of the Guttiferae family, has been traditionally used in China for the treatment of blood stasis and swelling reduction.[13] In addition, it was considered to be a promising antitumor herb in Taiwan.[14] In our preliminary research on the aerial parts of *H. sampsonii*, four new decarbonyl PPAPs were discovered,[11] with five new homoadamantanyl-type PPAPs.[11,12] Further investigation on this plant led to the isolation of two novel PPAPs with unusual epoxy-ring-fused skeletons (**1** and **2**) and one nor-PPAPs hypersampson R (**3**) with the loss of C-31−33 in isopentenyl (Figure 1). Compound **1** possessed an unexpected hexacyclic skeleton with a rare 2,7-dioxabicyclo[2.2.1]heptane moiety, and compound **2** featured a unique tetrahydrofuro[3,4-*b*]furan-fused tricyclo[4.3.1.1^{5,7}]-

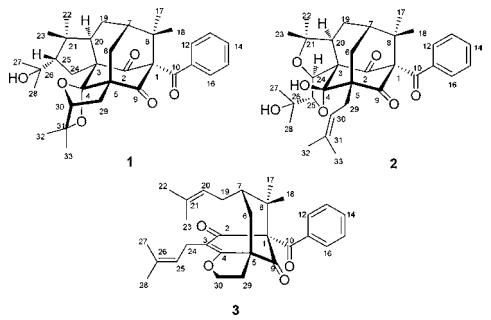


Figure 1. Structures of **1**−**3**.

undecane skeleton. In this paper, we report the isolation, structure elucidation, and biological activity evaluation of these new compounds.

Compound **1** was obtained as colorless crystals {[α]$_D^{23}$ +16.8 (c = 0.50, $CHCl_3$)}. Its molecular formula was determined as $C_{33}H_{42}O_6$ by HR-ESI MS at m/z 535.3061 [M + H]$^+$ (calcd 535.3060), suggesting the existence of 13 degrees of unsaturation. ^{13}C NMR and DEPT spectra showed 33 carbon signals including two carbonyl groups [δ_C 206.6, 204.9], a benzoyl group [δ_C 194.9, 137.3, 132.0, 129.6 × 2, 127.6 × 2], eight methyls, four methylenes, four methines, and eight quaternary carbons. All these functional groups accounted for seven degrees of

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unsaturation, requiring six rings in **1**. Extensive comparison of the 1D and 2D NMR data of **1** (Table 1) with those of plukenetion

Table 1. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR Data for 1 and 2 in $CDCl_3$ (δ in ppm, J in Hz)

	1		2	
no.	δ_H	δ_C	δ_H	δ_C
1		82.9		82.5
2		206.6		203.6
3		63.5		77.4
4		112.3		106.9
5		64.0		57.1
6α	2.29 (m)	45.1	2.22 (dd, 14.4, 6.0)	41.5
6β	1.85 (m)		2.44 (m)	
7	1.81 (m)	44.8	1.99 (m)	44.8
8		50.0		52.3
9		204.9		207.7
10		194.9		194.6
11		137.3		136.8
12,16	7.90 (dd, 7.2, 1.5)	129.6	7.56 (br d, 7.2)	129.8
13,15	7.24 (tt, 7.2, 1.5)	127.6	7.24 (br t, 7.2)	127.9
14	7.37 (tt, 7.2, 1.5)	132.0	7.36 (tt, 7.2, 1.5)	132.1
17	1.32 (s)	23.4	1.46 (s)	23.1
18	1.35 (s)	26.4	1.37 (s)	27.1
19	2.10 (m) 1.63 (m)	29.0	2.05 (m), 2.02 (m)	29.1
20	2.01 (m)	54.1	2.47 (m)	50.2
21		42.9		86.9
22	1.19 (s)	27.9	1.33 (s)	32.2
23	1.18 (s)	28.2	1.33 (s)	24.3
24	2.31 (m), 2.23 (m)	32.5	5.06 (d, 3.0)	83.2
25	2.27 (m)	56.0	4.00 (d, 3.0)	89.6
26		73.6		70.0
27	1.35 (s)	30.7	1.25 (s)	27.5
28	1.28 (s)	30.2	1.30 (s)	26.5
29	2.74 (d, 13.2), 1.22 (m)	29.9	2.84 (dd, 14.7, 10.5), 2.49 (m)	28.9
30	4.49 (d, 5.4)	86.1	5.46 (tdd, 10.5, 5.4, 1.5)	120.3
31		80.6		137.4
32	1.25 (s)	21.7	1.72 (s)	18.2
33	1.26 (s)	27.6	1.75 (s)	26.5

B[4] (Scheme 1) suggested that **1** was also a PPAPs derivative possessing a tetracyclic core structure. However, clear upfield shift of C-4 (δ_C 112.3) and significant downshift of C-30 (δ_C 86.1) and C-31 (δ_C 80.6) with two additional rings indicated that **1** differed from plukenetion B with respect to the carbonyl at C-4 being transformed to a ketal with hydroxy groups at C-30 and C-31. The observation was further confirmed by the correlations observed in the HMBC spectrum (Figure 2). HMBC correlations from H-29 to C-5/C-9/C-31, H-30 to C-4, and H_3-32/H_3-33 to C-30/C-31 demonstrated that the 2,7-dioxabicyclo[2.2.1]heptane moiety was fused to C-4 and C-5 of the core structure. Thus, the gross structure of **1** was deduced.

In the NOESY spectrum, the correlations of H_3-18/H-6α and H_3-17/H-20 suggested the α-orientation of H-20. The NOESY correlations of H-6β/H_3-23 and H_3-22/H_3-27 indicated that H_3-23 and H-25 were situated in β-orientation. However, the configurations of C-4 and C-30 could not be deduced according to the NOESY spectrum. Fortunately, crystals suitable for a single-crystal X-ray diffraction were obtained, which was performed with Cu Kα (Figure 3). The Flack parameter of 0.04(13) allowed unambiguous assignment of the absolute configuration as 1*R*,3*S*,4*S*,5*S*,7*S*,20*R*,25*S*,30*R*. Moreover, the quantum chemical ECD calculation method was used to further confirm the absolute configuration of **1**. Calculated ECD curves of **1a** (1*R*,3*S*,4*S*,5*S*,7*S*,20*R*,25*S*,30*R*) and its enantiomer **1b** (1*S*,3*R*,4*R*,5*R*,7*R*,20*S*,25*R*,30*S*) with an experimental ECD curve are shown in Figure 4. Theoretically calculated ECD data of **1a** agreed with the experimental ECD data, which supported the assignment of the absolute configuration of **1** as 1*R*,3*S*,4*S*,5*S*,7*S*,20*R*,25*S*,30*R*.

Compound **2**, colorless oil {$[\alpha]_D^{23}$ +77.0 (c = 0.50, $CHCl_3$)}, has the molecular formula $C_{33}H_{42}O_7$, determined by its HR-ESI MS (m/z 551.3008 $[M + H]^+$, calcd 551.3009), requiring 13 degrees of unsaturation. Extensive analysis of the NMR data of **2** suggested that it was also a PPAPs derivative with two carbonyl groups [δ_C 207.7, 203.6], a benzoyl group [δ_C 194.6, 136.8, 132.1, 129.8 × 2, 127.9 × 2], an isopentenyl group [δ_C 28.9, 120.3, 137.4, 18.2, 26.5], six methyls, two methylenes, four methines, and seven quaternary carbons. NMR data of **2** were similar to those of **1** with the same core structure of the tricyclo[4.3.1.1^{5,7}]undecane skeleton. HMBC correlations from H-29 to C-4/C-5/C-9 and OH-4 to C-3/C-4/C-5 allowed the linkage of the isopentenyl group and the hydroxyl group to the C-5 and C-4 of the core structure, respectively. By now, the gross structure accounting for 11 degrees of unsaturation was deduced. The remaining two degrees of unsaturation indicated another two rings in **2**. Taking the characteristic chemical shifts of C-4 (106.9), C-21 (δ_C 86.9), C-24 (δ_C 83.2), and C-25 (δ_C 89.6) into account, a furo-furan moiety was deduced. HMBC correlations from H-20 to C-2/C-4/C-21/C-22, H_3-22/H_3-23 to C-20/C-21, H-24 to C-2/C-4/C-25/C-26, H-25 to C-24 with those from H_3-27/H_3-28 to C-25/C-26 indicated that a rare tetrahydrofuro[3,4-*b*]furan moiety was fused with C-3, C-4, and C-20 of the core structure.

The relative configuration of **2** was determined by NOESY experiments. NOESY correlations (Figure 2) of H_3-18/H-6α, H_3-17/H-20, and H-20/H-24 suggested the α-orientation of H-20 and H-24. In addition, the NOESY correlations of H-6β/OH-4 and OH-4/H-25 demonstrated that OH-4 and H-25 were situated in a β-orientation. The absolute configuration of **2** was determined by ECD calculations. A pair of enantiomers (1*R*,3*R*,4*S*,5*R*,7*S*,20*S*,24*S*,25*S*)-**2a** and (1*S*,3*S*,4*R*,5*S*,7*R*,20*R*,24*R*,25*R*)-**2b** were calculated for their ECD spectrum based on the known relative configuration. As a result, the overall pattern of the calculated ECD curve of **2a** was consistent with the experimental data of **2** (Figure 5). Thus, the absolute configurations of chiral carbons in **2** were assigned as 1*R*,3*R*,4*S*,5*R*,7*S*,20*S*,24*S*,25*S*.

The molecular formula of **3** was determined to be $C_{30}H_{36}O_4$ by its HR-ESI MS (m/z 461.2691 $[M + H]^+$, calcd 461.2692). Extensive analysis of the NMR spectra (Supporting Information) revealed that **3** was similar to sampsonione L,[5] except for the loss of a 1-hydroxyl-1-methylethyl group at C-30. It was further confirmed by the correlations observed in the ^{1}H−^{1}H COSY and HMBC spectra (Figure 6). The relative stereochemistry of the chiral carbon at C-7 was deduced from the $\Delta\delta_H$ between the chemical shifts of the two hydrogens of the CH_2-6 and the δ_C for C-7, C-17, and C-18.[15,16] In compound **3**, $\Delta\delta_H$ (0.14) between the two hydrogens of the CH_2-6 with the δ_C for C-7 (48.0), C-17 (27.2), and C-18 (22.5) indicated the axial orientation of the isopentenyl group at C-7. The absolute configuration of **3** was deduced by ECD calculations. The calculated ECD curves of **3a** (1*R*,5*R*,7*S*) and **3b** (1*S*,5*S*,7*R*) were compared with the experimental one. This comparison revealed good agreement

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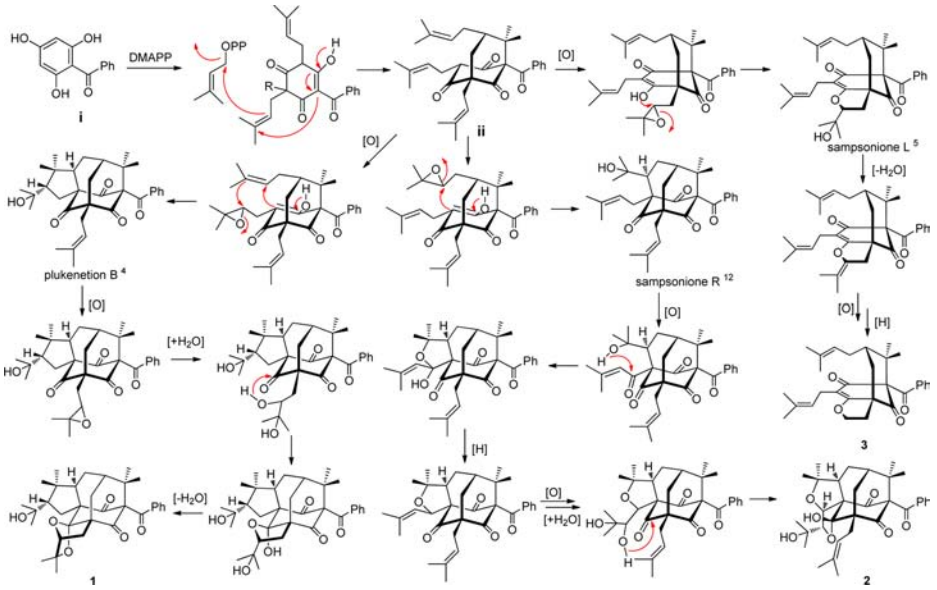
Scheme 1. Plausible Biogenetic Pathway for 1−3



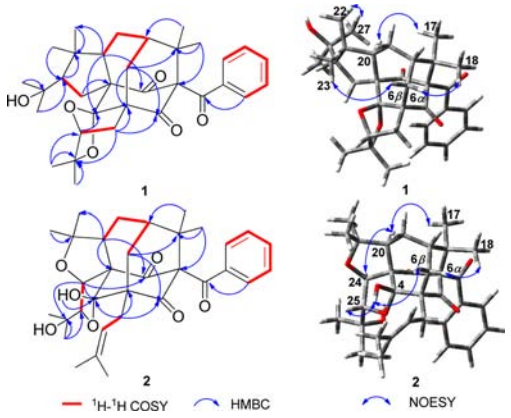


Figure 2. ^{1}H−^{1}H COSY, key HMBC, and NOESY correlations of **1** and **2**.

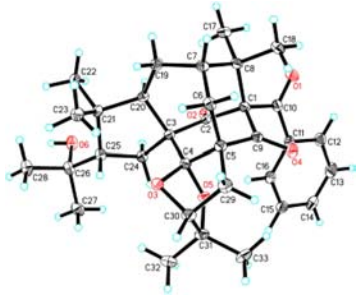


Figure 3. Single-crystal X-ray structure of **1**.

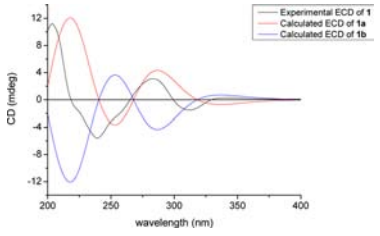


Figure 4. Calculated and experimental ECD spectra of **1**.

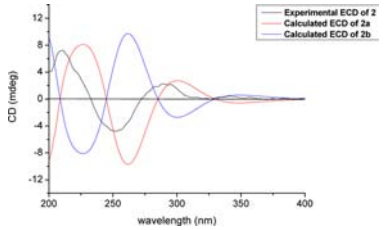


Figure 5. Calculated and experimental ECD spectra of **2**.

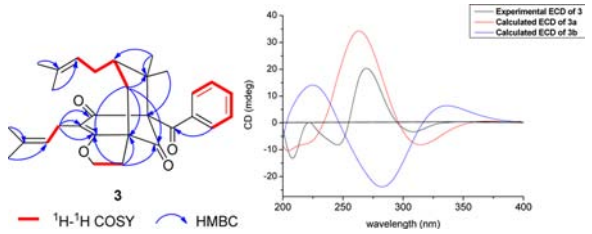


Figure 6. ^{1}H−^{1}H COSY, key HMBC, and calculated and experimental ECD spectra of **3**.

between the calculated ECD curve of **3a** and experimental one (Figure 6). Thus, the absolute configurations of chiral carbons in **3** were assigned as 1*R*,5*R*,7*S*.

Recently, the synthesis of PPAPs has been a vibrant area of natural product research,[17−21] driven by their fascinating and challenging structures. The unexpected hexacyclic skeleton with a rare 2,7-dioxabicyclo[2.2.1]heptane moiety in **1** and the unique tetrahydrofuro[3,4-*b*]furan-fused tricyclo[4.3.1.1^{5,7}]undecane skeleton of **2** further enriched the structural diversity of complex caged PPAPs. The epoxy-bridged 2,7-dioxabicyclo[2.2.1]-heptane substructure has also been found in other natural products, such as chuktabularins A−T,[22,23] isogosterones A and D,[24] loukacinol B,[25] and salpichrolide J.[26] However, this was the first time this rare substructure was discovered in naturally derived PPAPs. The bicyclic ketal skeleton is also known as a class of versatile intermediates for the stereocontrolled preparation of cyclic ethers. The ether bridge locks the conformation of the carbohydrate ring and simultaneously

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protects two of the hydroxy groups, which may be important in the synthetic process.[27,28] Compound **2** also set the first example of tetrahydrofuro[3,4-*b*]furan-fused PPAPs structures.

Most of the structurally related PPAPs isolated from *H. sampsonii* are probably biosynthesized from the biogenetically acceptable 2,4,6-trihydroxybenzophenone **i**. It is further decorated with dimethylallyl diphosphate to obtain intermediate **ii**, which is the common precursor of diverse PPAPs.[5,12] Compounds **1**−**3** were presumably biosynthesized from **ii** through a series of reactions, such as epoxidation, intramolecular cyclization, oxidation, dehydration, reduction, and so on.[5,12] A possible biosynthetic pathway of **1**−**3** is proposed in detail in Scheme 1.

Retinoid X receptor-α (RXRα) represents a unique intracellular target for pharmacologic interventions. Altered expression and function of RXRα is implicated in the development of a number of diseases and cancer.[29] The effects on RXRα transcriptional−inhibitory activity and cytotoxicity against HeLa cells of **1**−**3** were investigated. As a result, **2** (5−20 μM) showed mild RXRα transcriptional−inhibitory activities in a dose-dependent manner (Figure 7), and compounds **1** and **3** inhibited cell proliferation in HeLa cells at a concentration of 20 μM (Figure 8).

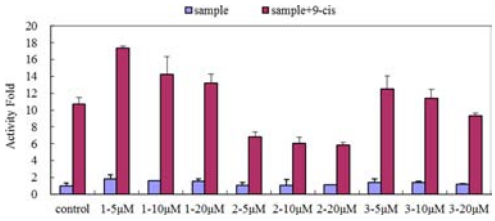

Figure 7. Effects of compounds **1**−**3** (5, 10, and 20 μM) on the transcriptional activities of RXRα.

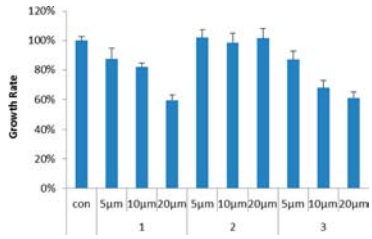

Figure 8. Effects of compounds **1**−**3** (5, 10, and 20 μM) on the cytotoxic effects.

■ ASSOCIATED CONTENT

Supporting Information

General experimental procedures; physicochemical properties; UV, IR, HR-ESI-MS, 1D, and 2D NMR spectra of compounds **1**−**3**; X-ray data of **1**; and ECD calculation method of **1**−**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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