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Norascyrononones A and B, 2,3,4-*nor*-Polycyclic Polyprenylated Acylphloroglucinols from *Hypericum ascyron*

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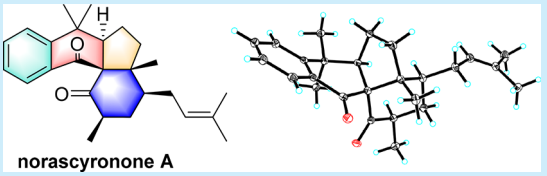
Supporting Information

ABSTRACT: Phytochemical study of *Hypericum ascyron* led to the characterization of norascyrononones A−C (**1**−**3**), metabolites derived from bicyclic polyprenylated acylphloroglucinols by losing eight carbons (C-2/3/4 of core and the isoprenyl at C-3). Compounds **1**/**2** with an unprecedented 6/6/5/6 ring system should be generated via [4 + 2] intramolecular cyclization of **3**. Their structures were determined by spectroscopic and X-ray diffraction data. Compounds **1** and **2** showed cytotoxicities against the SK-BR-3 cell line (IC_{50} 4.3 and 7.8 μM).



Polycyclic polyprenylated acylphloroglucinols (PPAPs), possessing highly oxygenated acylphloroglucinol cores densely substituted by prenyl or geranyl side chains, are a group of structurally fascinating and synthetically challenging natural products that collectively exhibit a broad range of biological activities.[1,2] Up to date, more than 540 PPAPs were reported exclusively from the plant of families *Hypericaceae* and *Clusiaceae*,[1,2] of which the majority is bicyclic polyprenylated acylphloroglucinols (BPAPs) featuring a bicyclo[3.3.1]nonane-2,4,9-trione core, as exemplified by hyperforin and garcinol.[1,2] Biosynthetically, BPAPs may not only further cyclize to caged PPAPs with the adamantane or homoadamantane skeleton but also be oxidized to open the ring to generate *seco*-BPAPs, such as hyphenrone A.[2,3] Besides, a small class of structurally related natural products with a simpler cyclohexanone core,[4−11] *nor*-BPAP derivatives, were considered to be derived from BPAPs via degradation of C-2, C-2/3, or C-2/3/4 (together with the C-3 side chain), respectively. According to a literature survey, a total of 18 *nor*-BPAPs with such architecture have been reported.[4−12] As a part of our systematic search for bioactive PPAPs from *Hypericum* plants,[3,13−21] the chemical constituents of *Hypericum ascyron*[17,22−27] were further investigated, and three new *nor*-BPAP derivatives, norascyrononones A−C (**1**−**3**), were characterized (Figure 1). Interestingly, compounds **1** and **2** sharing an intriguing 6/6/5/6 ring system could be formed from precursor **3** via a [4 + 2] intramolecular radical cyclization. The absolute configurations of **1** and **2** were defined by X-ray diffraction data of **1** and experimental and calculated electronic circular dichroism (ECD) spectra of **2**. Herein, the isolation, structure elucidation, biosynthetic discussion, and cytotoxic activities of **1**−**3** were presented.

Norascyronone A (**1**) was isolated as a colorless crystal. Its molecular formula was established to be $C_{26}H_{34}O_2$ from its ^{13}C NMR and HRESIMS data (*m*/*z* 401.2454 $[M + Na]^+$, calcd for 401.2457), corresponding to 10 degrees of unsaturation. The

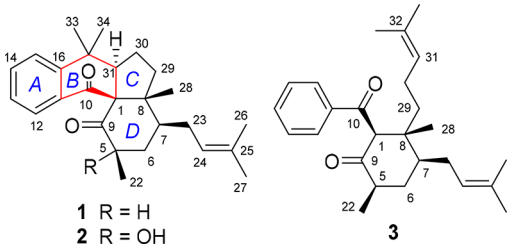


Figure 1. Structures of norascyrononones A−C (**1**−**3**).

UV spectrum showed conjugated groups by presenting maximum absorptions at 254 and 289 nm, and the IR spectrum exhibited absorption bands due to carbonyls (1703 and 1665 cm^{-1}). The ^1H NMR spectrum displayed signals of an *ortho*-disubstituted benzene (δ_H 7.86, d, H-12; 7.24, t, H-13; 7.42, t, H-14; 7.25, d, H-15, J = 7.6 Hz), one olefinic proton (δ_H 5.09), one doublet methyl (δ_H 1.05, J = 6.6 Hz), and five singlet methyls. The ^{13}C NMR and DEPT data presented a total of 26 carbon signals, including one conjugated (δ_C 195.9) and one nonconjugated (δ_C 213.3) carbonyl, three quaternary carbons (δ_C 73.4, 58.8, and 37.7), three methines, four methylenes, six methyls, and one phenyl group (Table 1).

In the HMBC spectrum, the correlations of two doublet aromatic protons (δ_H 7.86, H-12; 7.25, H-15) with the conjugated carbonyl (δ_C 195.9, C-10) and an upfield quaternary carbon (δ_C 37.7, C-32), respectively, indicated that the benzoyl group was involved in further cyclization. This structural fragment is unusual among the polyprenylated acylphloroglucinol-type metabolites from *Hypericum* plants, except for several PPAPs via [4 + 2] intramolecular cyclization.[2] The structure of the B-ring was established by the HMBC correlations from a

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Table 1. ^{13}C (150 MHz) and ^{1}H (600 MHz) NMR Spectroscopic Data of 1 and 2 in $CDCl_3$

	1		2	
no.	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	73.4		72.6	
5	41.7	2.84, m	77.5	
6	36.2	α: 1.92, m	35.9	β: 2.32, t (13.3)
		β: 1.45, q (12.8)		α: 1.65, overlap
7	42.0	1.84, brt (12.8)	45.4	1.50, overlap
8	58.8		56.4	
9	213.3		217.8	
10	195.9		197.5	
11	134.1		134.4	
12	127.9	7.86, d (7.6)	127.5	7.64, d (7.4)
13	126.6	7.24, t (7.6)	126.8	7.25, t (7.4)
14	133.1	7.42, t (7.6)	133.4	7.45, t (7.4)
15	125.0	7.25, d (7.6)	124.5	7.26, d (7.4)
16	148.8		150.2	
22	15.5	1.05, d (6.6)	26.8	1.51, s
23	29.4	1.98, brd (13.6)	29.5	1.91, m
		1.65, overlap		1.82, m
24	123.6	5.09, t (6.8)	122.8	5.03, t (7.0)
25	133.0		132.8	
26	25.9	1.68, s	25.8	1.64, s
27	17.9	1.55, s	17.9	1.52, s
28	16.9	0.96, s	18.8	0.72, s
29	37.4	α: 1.73, m	41.9	β: 1.67, m
		β: 1.03, overlap		α: 1.30, td (12.9, 5.9)
30	27.6	α: 2.07, m	28.9	α: 1.80, m
		β: 1.28, m		β: 1.10, m
31	54.8	3.12, dd (9.9, 8.3)	53.0	2.99, dd (12.7, 6.8)
32	37.7		36.1	
33	33.5	1.06, s	36.0	1.06, s
34	27.3	1.37, s	26.3	1.43, s
OH-5				2.75, s

gem-dimethyl at δ_H 1.06 (Me-33) and 1.37 (Me-34) to C-16 (δ_C 148.8), C-31 (δ_C 54.8), and C-32 (δ_C 37.7), as well as from H-31 (δ_H 3.12) to C-1 (δ_C 73.4) and C-10. Likewise, the proton spin system of H-31/H_2-30 (δ_H 2.07 and 1.28)/H_2-29 (δ_H 1.73 and 1.03) in the $^{1}H-^{1}H$ COSY spectrum and the HMBC correlations from a singlet methyl at δ_H 0.96 (Me-28) to C-1, C-8 (δ_C 58.8), and C-29 (δ_C 37.4) indicated the cyclopentene C-ring. The existence of the D-ring was deduced by HMBC correlations from Me-28 to C-7 (δ_C 42.0), from Me-22 (δ_H 1.05) to the nonconjugated carbonyl at δ_C 213.3 (C-9), and from H-5 (δ_H 2.84) to C-1, together with $^{1}H-^{1}H$ COSY correlations of Me-22/H-5/H_2-6 (δ_H 1.92 and 1.45)/H-7 (δ_H 1.84). In addition, an isoprenyl group was attached to C-7 by the $^{1}H-^{1}H$ COSY correlations of H-7/H-23 (δ_H 1.98 and 1.65)/H-24 (δ_H 5.09) and HMBC correlations from singlet methyls at δ_H 1.68 (Me-26) and 1.55 (Me-27) to C-24 (δ_C 123.6). Hence, the planar structure of **1** was elucidated as shown (Figure 2).

In the ROESY spectrum (Figure S7, Supporting Information), the cross peaks of Me-28/H-30β (δ_H 1.28) and H-30α (δ_H 2.07)/H-31 suggested the α-orientation of H-31. Furthermore, the large coupling constant (12.8 Hz) of H-5 (δ_H 2.84)/H-6_{ax} (δ_H 1.45) and H-6_{ax}/H-7 (δ_H 1.84) indicated their 1,3-diaxial position in the six-membered D-ring with chair conformation. This evidence, conjugated with the NOE contact of Me-28/H-6_{ax}, defined the α-configuration of both H-5 and H-7. However, the configuration of C-1 was uncertain due to the lack of reliable NOE correlation. Fortunately, quality crystals of **1** were obtained, and the X-ray crystallographic data [Flack parameter = 0.04(5)] evidently confirmed the proposed structure and also determined its absolute configuration as 1*S*,5*R*,7*S*,8*R*,31*R* (Figure 2).[28]

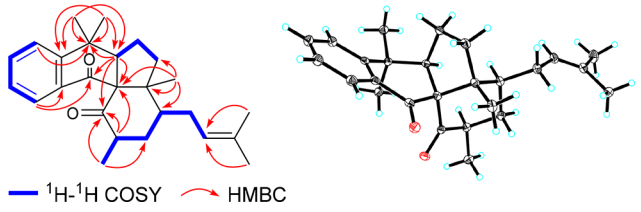

Figure 2. HMBC and $^{1}H-^{1}H$ COSY correlations and ORTEP drawing of **1**.

Norascyronone B (**2**) had a molecular formula of $C_{26}H_{34}O_3$, which was 16 mass units more than that of **1**. Comparison of its 1D NMR data (Table 1) with those of **1** indicated that the sp^3 methine at δ_C 41.7 (C-5) in **1** was replaced by an oxygenated quaternary carbon (δ_C 77.5), suggesting oxidation of C-5 in **2**. This assumption was supported by the HMBC correlations from OH-5 (δ_H 2.75) to C-5, C-9 (δ_C 217.8), and C-22 (δ_C 26.8) in the HMBC spectrum. The relative configuration of **2** was consistent with that of **1** (Figure 3), as indicated by the NOE correlations of Me-28 (δ_H 0.72)/H-29β (δ_H 1.67), H-29α (δ_H 1.30)/H-31 (δ_H 2.99), and Me-28/H-6β (δ_H 2.32) recorded in $CDCl_3$, in combination with NOE contacts of H-6α (δ_H 1.74)/OH-5 (δ_H 5.34)/H-7 (δ_H 1.97) recorded in DMSO-d_6.

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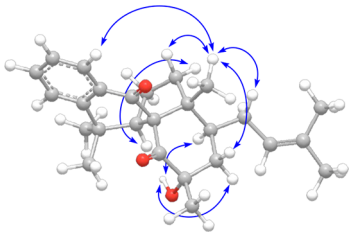


Figure 3. Key ROESY correlations of **2**.

Moreover, the ^{1}H and ^{13}C chemical shifts of **2** were calculated at the B3LYP-SCRF/6-31G(d,p)//M062X/def2-SVP level of theory in chloroform with the IEFPCM solvent model, and the good consistency between the theoretical and experimental chemical shifts strongly supported the above established structure of **2** (Figure S1 and Tables S2−S3, Supporting Information). Subsequently, TDDFT ECD calculation was run on one of the two possible enantiomers (**2a** and **2b**, Figure 4) of

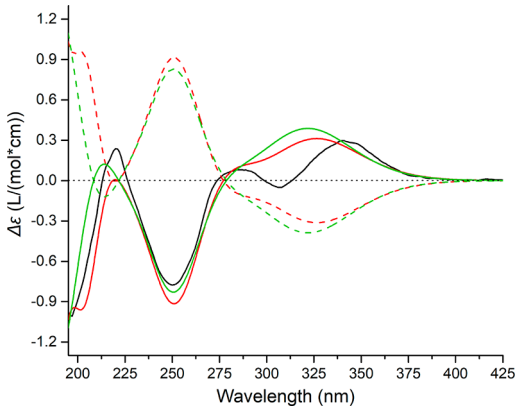


Figure 4. Experimental ECD spectrum of **2** (black). Calculated ECD spectrum of **2a** (UV correction = +15 nm, red) and **2b** (UV correction = 15 nm, red dash) at the CAM-B3LYP-SCRF/def2-SVP level of theory in MeOH with IEFPCM solvent model. Calculated ECD spectrum of **2a** (UV correction = 10 nm, green) and **2b** (UV correction = 10 nm, green dash) at the ωB97X-D-SCRF/def2-SVP level of theory in MeOH with IEFPCM solvent model.

2 using two methods, and both of the calculated ECD curves of **2a** were found to agree well with their experimental counterpart, thus confirming the absolute configuration of **2** to be the same with **1** as 1*R*,5*S*,7*S*,8*R*,31*R*.

Norascyronone C (**3**), a biogenetically related precursor of **1** and **2**, was also isolated in this study. Compound **3** was assigned the molecular formula of $C_{26}H_{36}O_2$ from its ^{13}C NMR (Table 2) and HRESIMS data. On the basis of analysis of its 1D and 2D NMR data, compound **3** was shown to possess the same backbone and relative configuration as hyperibrin A,[11] a known *nor*-BPAP obtained from *H. ascyron* as well (Figure 5). The structural novelty of **3** involves the presence of a C-1 benzoyl rather than an isobutyryl group.

Biosynthetically, these *nor*-BPAP derivatives (**1**−**3**) should be derived from BPAPs via degradation of C-2/3/4 of the phloroglucinol core, accompanied with loss of the C-3 isoprenyl side chain. This process may be realized in plants through a cascade of similar Retro-Claisen and decarboxylation reactions.[8,9] Inspired by George's biomimetic syntheses of PPAPs using radical cyclization,[29−31] we proposed a similar [4 + 2] cyclization mechanism for the biosynthesis of compounds **1** and

Table 2. ^{13}C (150 MHz) and ^{1}H (600 MHz) NMR Data of 3 in $CDCl_3$

no.	δ_C	δ_H (*J* in Hz)	no.	δ_C	δ_H (*J* in Hz)
1	63.7	4.55, s	22	14.4	0.98, d (6.6)
5	45.4	2.55, m	23	26.9	2.11, m
6	37.1	2.13, m			1.70, overlap
		1.25, q (12.3)	24	123.0	5.09, t (7.2)
7	42.8	1.94, m	25	132.9	
8	46.3		26	25.9	1.68, s
9	209.6		27	17.9	1.56, s
10	196.8		28	17.6	1.07, s
11	138.5		29	36.4	1.50, m
12	127.5	7.71, d (7.8)	30	22.0	1.91, overlap
13	128.6	7.35, t (7.8)			1.65, m
14	132.7	7.44, t (7.8)	31	123.6	4.80, t (7.0)
15	128.6	7.35, t (7.8)	32	131.7	
16	127.5	7.71, d (7.8)	33	25.5	1.44, s
			34	17.3	1.27, s

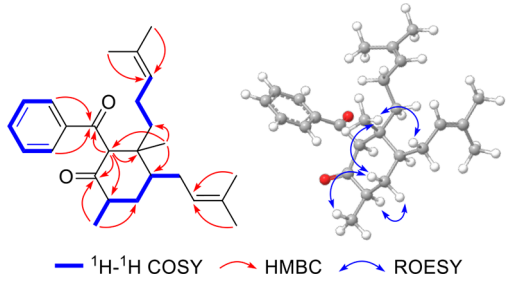
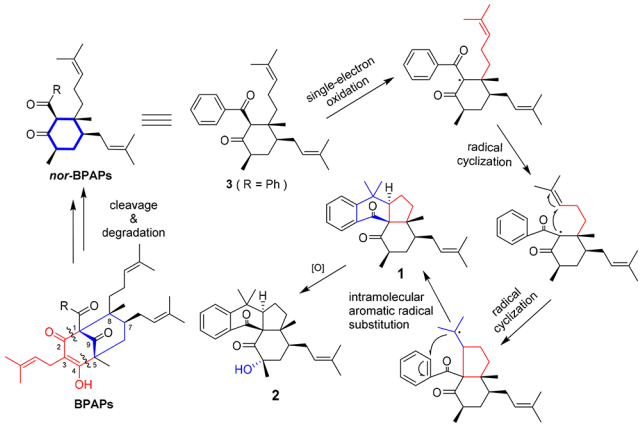


Figure 5. Key 2D NMR correlations of **3**.

2. As shown in Scheme 1, single electron oxidation of **3** would give rise to a stabilized α-diketo radical, which could undergo

Scheme 1. Plausible Biosynthetic Pathway to 1−3



consecutive radical cyclization followed by a terminating aromatic substitution reaction to generate compounds **1** and **2** with an intriguing 6/6/5/6 ring system. In fact, free radical addition generally does not yield stereoselective products, and there should be four stereoisomers. However, we obtained only one stereoisomer in this study.

Since some prenylated acylphloroglucinols are reported to be antitumor agents,[1,2] compounds **1**−**3** were tested for their cytotoxic activities against three human tumor cell lines (SK-BR-3, PANC-1, and ECA-109). As a result, compound **1** showed moderate activities against SK-BR-3 and PANC-1 with IC_{50}

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values of 4.3 and 8.4 μM, respectively, while compound **2** exhibited activities to SK-BR-3 and ECA-109 cell lines (IC_{50} 7.8 and 12.7 μM).

In conclusion, norascyrononones A and B (**1** and **2**), two cytotoxic *nor*-BPAP derivatives with an unprecedented tetracyclic carbon skeleton, were characterized from *H. ascyron*, together with their plausible precursor, norascyronone C (**3**). The loss of C-2/3/4 from the core of BPAPs (to give **3**) followed by the [4 + 2] intramolecular radical cyclization of the isoprenyl side chain plays a significant role in the construction of **1** and **2**. Considering the existence of carbonyls, double bonds, as well as the isoprenyl side chains in the molecules of PPAPs, it is expected that more PPAP derivatives with intriguing architectures might be found further.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b04022.

Details of isolation and biological experimental procedures and original MS and NMR spectra (PDF)

Accession Codes

CCDC 1885429 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

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(28) Crystal data for **1**: $C_{26}H_{34}O_2$, M = 378.53, a = 8.25410(10) Å, b = 11.9609(2) Å, c = 21.6670(4) Å, α = 90°, β = 90°, γ = 90°, V = 2139.11(6) Å^3, T = 100(2) K, space group $P212121$, Z = 4, μ(Cu Kα) = 0.555 mm^{-1}, 13 575 reflections measured, 3952 independent reflections (R_{int} = 0.0222). The final R_1 values were 0.0306 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0797 ($I > 2\sigma(I)$). The final R_1 values were 0.0309 (all data). The final $wR(F^2)$ values were 0.0800 (all data). The goodness of fit on F^2 was 1.051. Flack parameter = 0.04(5).
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