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Hyperjapones F–I, terpenoid polymethylated acylphloroglucinols from *Hypericum japonicum*

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ABSTRACT

Hyperjapones F–I (**1**–**4**), novel terpenoid polymethylated acylphloroglucinols (TPAPs) with three unusual carbon skeletons, were characterized from *Hypericum japonicum*. Their structures were determined on the basis of comprehensive MS and NMR spectroscopic data. Compounds **1** and **2** could be formed by hybridization of a trimethylated acylphloroglucinol core with a sabinene-type monoterpenoid unit, while compounds **3** and **4** contained a pinene-type monoterpenoid unit in their molecules. Biosynthetically, a key hetero-Diels–Alder mechanism was proposed for the generation of compounds **1**–**4**.

Recently, meroterpenoids of phloroglucinols incorporating with terpenoid units with diverse structures and bioactivities have attracted considerable interests in isolation and synthetic efforts.[1,2] These metabolites have been mainly isolated from the plants of family Myrtaceae, as well as several fungi.[1,3] Interestingly, meroterpenoids with similar hybrid pattern have been found in *Hypericum japonicum* Thunb. (Guttiferae) for the first time in our previous study,[4] which was thought to produce another kind of metabolites, polycyclic polyprenylated acylphloroglucinols (PPAP) with fascinating chemical structures.[5] As a result, hyperjapones A–E, five terpenoid polymethylated acylphloroglucinols (TPAPs) incorporating with a trimethylated acylphloroglucinol core and a sesquiterpenoid unit were characterized from this plant instead of PPAPs.[4] Their structures shared a common dearomatized acylphloroglucinol core featuring with an enol-β-triketone system and C-5 dimethylation. Shortly after we reported the structures, hyperjapones A–E have been synthesized elaborately by Dr. J.H. George' Lab using a biomimetic, oxidative hetero-Diels–Alder reaction.[6]

Further phytochemical study of this plant led the isolation of four novel TPAPs, hyperjapones F–I (**1**–**4**, Fig. 1), with three unusual carbon skeletons. Compounds **1** and **2** could be formed by hybridization of a trimethylated acylphloroglucinol core with a sabinene-type monoterpenoid unit, while compounds **3** and **4** contained a pinene-type monoterpenoid unit in their molecules. In this Letter, we report the isolation, structural elucidation, and proposed biosynthetic pathways of the new isolates.

Hyperjapone F (**1**)[7] was obtained as a colorless gum, $[\alpha]_D^{24}$ −13 (*c* 0.15, MeOH). Its molecular formula $C_{23}H_{32}O_4$ was established by its HR-ESIMS (*m*/*z* 373.2374, $[M+H]^+$, calcd 373.2373) and ^{13}C NMR data. The UV (243, 282, and 328 nm) and IR (3421, 1656,

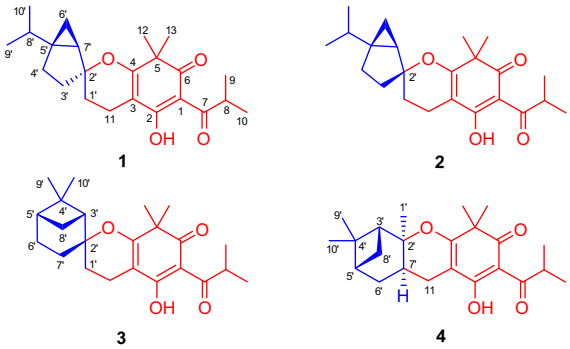

Fig. 1. Structure of hyperjapones F–I (**1**–**4**).

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Table 1
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data (δ in ppm) of **1** and **2** in acetone-d_6.

No.	**1**		**2**	
	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	105.0, C		105.0, C	
2	189.6, C		189.7, C	
3	103.4, C		103.1, C	
4	174.0, C		175.2, C	
5	49.1, C		49.2, C	
6	196.7, C		196.7, C	
7	207.9, C		208.0, C	
8	35.7, CH	3.96, sept (6.6)	35.7, CH	3.96, sept (6.6)
9	19.3, CH_3	1.09, d (6.6)	19.2, CH_3	1.09, d (6.6)
10	19.2, CH_3	1.08, d (6.6)	19.2, CH_3	1.08, d (6.6)
11	17.2, CH_2	2.45, dt (16.2, 6.0)	16.2, CH_2	2.50, m
		2.29, m		2.44, m
12	25.4, CH_3	1.29, s	25.1, CH_3	1.29, s
13	24.7, CH_3	1.32, s	24.4, CH_3	1.26, s
1′	28.2, CH_2	1.88, m	30.1, CH_2	1.96, m
		1.86, m		1.84, m
2′	91.8, C		89.1, C	
3′	33.1, CH_2	1.76, dd (14.4, 8.4)	32.9, CH_2	1.76, m
		1.38, m		1.57, m
4′	25.8, CH_2	1.94, m	25.1, CH_2	1.75, m
		1.65, dd (12.0, 7.8)		
5′	35.2, C		34.3, C	
6′	13.1, CH_2	0.53, dd (8.3, 5.4)	12.0, CH_2	0.90, overlap
		0.47, dd (5.4, 4.0)		0.49, dd (6.8, 5.4)
7′	31.1, CH	1.32, overlap	30.0, CH	1.30, m
8′	33.1, CH	1.44, sept (6.6)	33.1, CH	1.39, sept (6.6)
9′	20.4, CH_3	0.95, d (6.6)	19.8, CH_3	0.96, d (6.6)
10′	20.0, CH_3	1.02, d (6.6)	19.7, CH_3	0.90, d (6.6)

1619 cm^{-1}) spectra indicated the presence of an enolic 1,3-diketone system.[4,8] A shielded olefinic carbon at δ_C 105.0 (C-1) and three carbonyls at δ_C 189.6 (C-2), 196.7 (C-6), and 207.9 (C-7) in the ^{13}C NMR spectrum (Table 1) suggested the presence of an enol-β-triketone system. In the HMBC spectrum, the correlations of a *gem*-dimethyl at δ_H 1.29 (Me-12) and 1.32 (Me-13) with three quaternary carbons at δ_C 49.1 (C-5), 174.0 (C-4), and C-6 suggested the fragment of C-4/C-5/C-6. Moreover, the correlations from H_2-11 (δ_H 2.45 and 2.29) to δ_C 103.4 (C-3), C-2, and C-4 indicated the linkage of C-2/C-3/C-4. An isopropyl linked to C-7 was deduced by the correlations of both δ_H 1.09 (Me-9) and 1.08 (Me-10) with δ_C 35.7 (C-8) and C-7 (Fig. 2). These fragments, combined with the established enol-β-triketone system, constructed a trimethylated acylphloroglucinol moiety of **1** (the red part in Fig. 1), the same to those of hyperjapones A–E.[4]

Besides the aforementioned 13 carbon signals in the ^{13}C and DEPT NMR spectra of **1**, the remaining 10 resonances assignable to two methyls (δ_C 20.4 and 20.0), four methylenes (δ_C 33.1, C-3′, 28.2, C-1′, 25.8, C-4′, and 13.1, C-6′), two methines (δ_C 33.1, C-8′, and 31.1, C-7′), and two quaternary carbons (δ_C 91.8, C-2′, and 35.2, C-5′), indicated a sabinene-type monoterpenoid moiety (the blue part in Fig. 1). This assumption was further evidenced by the correlations of H_2-3′/H_2-4′ and H-6′/H_2-7′ in the ^{1}H–^{1}H COSY plot, combined with the HMBC correlations from two doublet (J = 6.6 Hz) methyls at δ_H 0.95 (Me-9′) and 1.02 (Me-10′) to C-5′, from δ_H 1.44 (sept, H-8′) to C-4′ and C-6′, from δ_H 1.76 and 1.38 (H-3′) to C-5′, and from δ_H 1.88 and 1.86 (H-1′) to C-2′, C-3′, and C-7′ (Fig. 2). The connection of C-11/C-1′ was deduced by the cross-peak between H-11 and H-1′, which combined the acylphloroglucinol and monoterpenoid moieties. The oxa-spiro[4.5] ring was finally deduced by the degree of unsaturation along with the downfield chemical shifts of C-4 (δ_C 174.0) and C-2′ (δ_C 91.8).

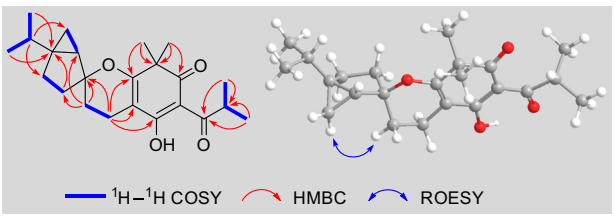


Fig. 2. Key ^{1}H–^{1}H COSY, HMBC, and ROESY correlations of **1**.

Due to ring strain of the cyclopropane ring in the monoterpenoid moiety, the relative configurations of the bridgehead carbons C-5′ and C-7′ were obviously indicated. Moreover, as shown in the molecular model (Fig. 2), the two rings near the C-2′ spirocyclic center were orthogonal. In the ROESY spectrum, the correlation of δ_H 0.47 (H-6′) and δ_H 1.88 (H-1′) suggested the relative configuration of the spirocyclic center carbon C-2′. Hence, the structure of **1** with relative configuration was elucidated (Fig. 1).

Hyperjapone G (**2**)[9] shared the same planar scaffold as **1** by detailed analysis of its HR-ESIMS, 1D and 2D NMR spectroscopic data. The ^{13}C NMR data of **2** (Table 1) were nearly the same to those of **1** except for the carbon resonances of C-1′ (δ_C 30.1), C-2′ (δ_C 89.1), and C-7′ (δ_C 30.0), which supposed **2** as 2′-epimer of **1**. The NOE correlation of δ_H 1.30 (H-7′) with δ_H 1.96 (H-1′) in the ROESY spectrum further supported the assumption.

Hyperjapone H (**3**)[10] was isolated as colorless gum. On the basis of the positive HR-ESIMS (m/z 373.2374 $[M+H]^+$, calcd 373.2373) and ^{13}C NMR spectroscopic data, its molecular formula was defined as $C_{23}H_{32}O_4$, the same as that of **1**. Analysis of its 1D (Table 2) and 2D NMR data revealed that **3** also shared a trimethylated acylphloroglucinol core, but possessed a different monoterpenoid moiety. A β-pinene-type monoterpenoid were further confirmed by the correlations from δ_H 1.96 and 1.79 (H-1′) to δ_C 86.2 (C-2′), 50.1 (C-3′), and 28.8 (C-7′), from both singlet methyls at δ_H 1.28 (Me-9′) and 1.01 (Me-10′) to δ_C 38.7 (C-4′), 41.1 (C-5′) and C-3′, and from δ_H 2.26 and 1.64 (H-8′) to δ_C 25.2 (C-6′) and C-2′ in the HMBC spectrum, coupled with the proton spin system of H-3′/H-8′/H-5′/H-6′/H-7′ in the ^{1}H–^{1}H COSY spectrum. The connection mode of the acylphloroglucinol and monoterpenoid moieties was similar to that of **1** as evidenced by the ^{1}H–^{1}H COSY contact of

Table 2
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data (δ in ppm) of **3** and **4** in acetone-d_6.

No.	**3**		**4**	
	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	104.8, C		104.9, C	
2	189.4, C		189.8, C	
3	103.0, C		100.3, C	
4	174.0, C		174.9, C	
5	49.1, C		49.2, C	
6	196.6, C		196.6, C	
7	207.9, C		207.5, C	
8	35.7, CH	3.96, sept (6.6)	35.6, CH	3.96, sept (6.6)
9	19.2, CH_3	1.07, d (6.6)	19.3, CH_3	1.08, d (6.6)
10	19.2, CH_3	1.06, d (6.6)	19.2, CH_3	1.07, d (6.6)
11	15.6, CH_2	2.37, dd (7.2, 6.4)	19.6, CH_2	2.44, dd (16.0, 2.0)
				2.38, dd (16.0, 6.6)
12	24.9, CH_3	1.28, s	26.1, CH_3	1.27, s
13	24.8, CH_3	1.23, s	23.9, CH_3	1.28, s
1′	32.4, CH_2	1.96, m	28.7, CH_3	1.42, s
		1.79, m		
2′	86.2, C		87.1, C	
3′	50.1, CH	2.13, brt (5.4)	55.0, CH	2.20, m
4′	38.7, C		40.3, C	
5′	41.1, CH	1.95, m	41.7, CH	1.92, m
6′	25.2, CH_2	1.99, m	35.8, CH_2	2.19, m
		1.89, m		1.29, overlap
7′	28.8, CH_2	1.98, overlap	31.1, CH	2.80, m
		1.90, overlap		
8′	26.9, CH_2	2.26, m	30.2, CH_2	2.22, m
		1.64, d (10.2)		0.90, dd (10.0, 6.8)
9′	27.6, CH_3	1.28, s	28.8, CH_3	1.32, s
10′	23.3, CH_3	1.01, s	22.9, CH_3	1.13, s

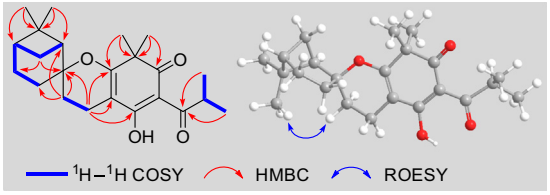


Fig. 3. Key $^{1}H–^{1}H$ COSY, HMBC, and ROESY correlations of **3**.

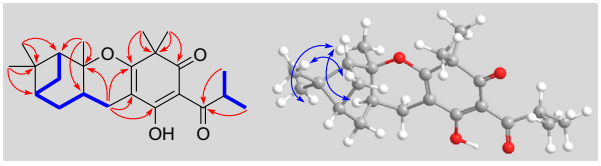
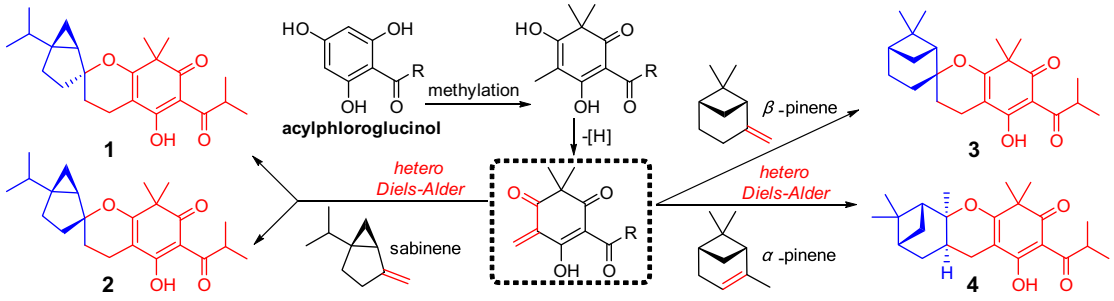


Fig. 4. Key $^{1}H–^{1}H$ COSY, HMBC, and ROESY correlations of **4**.

H-11/H-1′, downfield chemical shifts of C-4 (δ_C 174.0) and C-2′ (δ_C 86.2), and the indices of hydrogen deficiency. The NOE correlation of δ_H 1.01 (Me-10′) with δ_H 1.79 (H-1′) obtained in the ROESY spectrum, in combination with the molecular model (Fig. 3), could define the relative configuration of **3** (Fig. 1).

Hyperjapone I (**4**)[11] was determined to be a structural isomer of **3** by analyzing its HR-ESIMS, 1D and 2D NMR spectroscopic data. An α-pinene-type monoterpenoid was hybridized to the trimethylated acylphloroglucinol in **4** as supported by the key HMBC correlations of Me-1′ (δ_H 1.42) with C-2′ (δ_C 87.1) and C-7′ (δ_C 31.1) and the $^{1}H–^{1}H$ COSY correlations of H-7′ (δ_H 2.80) with H_2-11 (δ_H 2.44 and 2.38) and H-6′ (δ_H 2.19 and 1.29). Other parts of the monoterpenoid moiety were elucidated to be the same as those of **3** by detailed 2D NMR data (Fig. 4). In the ROESY spectrum, the cross-peaks of H-7′/Me-1′, Me-1′/Me-10′, and Me-9′/H-8′ (δ_H 0.90) suggested the relative configuration of **4** (Fig. 4). Hence, the structure of **4** was elucidated as shown.

Biosynthetically, hyperjapones F–I (**1**–**4**) could be derived from a "mixed" biosynthetic pathway (Scheme 1). Methylation of the acylphloroglucinol core affords trimethylated acylphlorogluci-



Scheme 1. Proposed biosynthetic pathways to compounds **1**–**4**.

nols.[3a,12] Then, dehydrogenation of the intermediates may form an α,β-unsaturated ketone moiety, which may further cyclize with sabinene, β-pinene, or α-pinene to form **1**–**4**, respectively, by a key hetero-Diels-Alder machanism.[6,13] Although the putative precursors were not isolated, the monoterpenoids and acylphloroglucinol derivatives with similar structures have been reported from this plant and other plants of *Hypericum*.[14]

In summary, four monoterpenoid-based TPAPs were characterized in this study to possess three unusual carbon skeletons. Although meroterpenoids (such as euglobal Ib, guadials B and C) with similar hybrid pattern have been previously isolated from the plants of family Myrtaceae,[1,3a] the highly functionalized acylphloroglucinol core of TPAPs are distinct from those of the reported meroterpenoids. Dimethylation of C-5 breaks up the aromatic feature of the phloroglucinol in TPAPs, accompany with the formation of an enol-β-triketone system. In addition, the hetero Diels-Alder mechanism proposed for the biosynthesis of hyperjapones A–E in our previous study has been successfully verified in the total synthesis.[6] Therefore, we predict that existence of diverse terpenoids allowed more acylphloroglucinol derivatives with novel scaffolds via [4+2] cycloadditions in *Hypericum* plants. Our finding presents challenging natural products for organic synthesis and also expands botanic resource for diverse meroterpenoids.

The antitumor activity of the isolates were evaluated *in vitro*, but all the compounds did not show activity against five human tumor cell lines AGS, Hela, HepG2, HCT116, and MDA-MB-468.

Acknowledgments

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A. Supplementary data

Supplementary data (detailed experimental procedures, original MS, 1D and 2D NMR spectra for **1**–**4**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.11.058.

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7. Hyperjapone F (**1**): colorless gum; $[\alpha]_D^{24}$ −13 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 200 (3.71), 243 (3.81), 281 (3.63), 328 (3.83) nm; IR (KBr) ν_{max} 3421, 2928, 2866, 1656, 1619, 1525, 1469, 1438, 1384, 1240, 1214, 1170, 1161, 1092, 884 cm^{-1}; CD (0.003 M, MeOH) λ_{max} (Δε) 200 (+0.9), 230 (−0.3), 355 (−0.6) nm; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 373 $[M+H]^+$; HR-ESIMS *m*/*z* 373.2374 $[M+H]^+$ (calcd for $C_{23}H_{33}O_4$, 373.2373).
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9. Hyperjapone G (**2**): colorless gum; $[\alpha]_D^{24}$ +17 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 201 (3.59), 243 (3.68), 284 (3.51), 327 (3.69) nm; IR (KBr) ν_{max} 3420, 2928, 2867, 1655, 1618, 1525, 1469, 1439, 1384, 1241, 1214, 1172, 1161, 1092, 888 cm^{-1}; CD (0.003 M, MeOH) λ_{max} (Δε) 205 (+0.7), 245 (+0.8), 345 (−0.4) nm; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 373 $[M+H]^+$; HR-ESIMS *m*/*z* 373.2371 $[M+H]^+$ (calcd for $C_{23}H_{33}O_4$, 373.2373).
10. Hyperjapone H (**3**): colorless gum; $[\alpha]_D^{24}$ +3 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 200 (3.39), 243 (3.46), 285 (3.30), 328 (3.48) nm; IR (KBr) ν_{max} 3429, 2927, 2871, 1655, 1619, 1623, 1525, 1472, 1386, 1312, 1191, 1167, 1028 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; ESIMS *m*/*z* 371 $[M-H]^-$; HR-ESIMS *m*/*z* 373.2374 $[M+H]^+$ (calcd for $C_{23}H_{33}O_4$, 373.2373).
11. Hyperjapone I (**4**): colorless gum; $[\alpha]_D^{24}$ +51 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 200 (3.60), 244 (3.67), 285 (3.53), 329 (3.73) nm; IR (KBr) ν_{max} 3428, 2928, 2871, 1655, 1618, 1622, 1525, 1472, 1387, 1312, 1192, 1167, 1022 cm^{-1}; CD (0.003 M, MeOH) λ_{max} (Δε) 205 (−4.5), 238 (−1.7), 280 (+1.3), 320 (+1.3) nm; ^1H and ^{13}C NMR data, see Table 2; ESIMS *m*/*z* 373 $[M+H]^+$; HR-ESIMS *m*/*z* 373.2375 $[M+H]^+$ (calcd for $C_{23}H_{33}O_4$, 373.2373).
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