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Harrisotones A–E, five novel prenylated polyketides with a rare spirocyclic skeleton from *Harrisonia perforata*

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ABSTRACT

Five novel prenylated polyketides, harrisotones A–E (**1**–**5**) representing a rare spirocyclic skeleton, along with a new hydroperoxypolyketide harrisonol A (**6**), were isolated from the stems and leaves of *Harrisonia perforata*. The structures of harrisotones A–E (**1**–**5**) were extensively elucidated on the basis of spectroscopic analysis, especially 2D NMR and CD spectra. A plausible origin of compounds **1**–**5** was rationalized biogenetically, and traced back to harrisonol A (**6**). Harrisotones A–C (**1**–**3**) and harrisonol A (**6**) exhibited significant cytotoxicity against P-388 and/or A-549 tumor cell lines.

1. Introduction

Polyketides are a structurally diverse family of natural products with an extremely broad range of biological activities and pharmacological properties.[1] However, only a limited number of prenylated polyketides have been obtained so far.[2–4] Biogenetically, the prenylated polyketides can be considered as a polyketide consisting of several condensed C_2 units substituted with some isoprenyl chains, and the biosynthetic pathways of several polyketides were demonstrated by radioactive tracer experiment.[5]

The genus *Harrisonia* (Simaroubaceae) comprising four species are mainly distributed in the southeast of Asia, Africa, and Oceania.[6] *Harrisonia perforata* (Blanco) Merr., a shrub, is the only species of this genus growing in China, and is applied in Chinese folklore medicine for the treatment of malaria.[6] Chemical investigations on this plant collected from Vietnam and China have led to the isolation of several limonoids and quassinoids.[7] Previously, three prenylated polyketides have been obtained from *Harrisonia abyssinica* of the same genus.[2] In the current research, six novel prenylated polyketides, harrisotones A–E (**1**–**5**) and harrisonol A (**6**), some of which showed significant cytotoxicity against P-388 and/or A-549 tumor cell lines, were isolated from the stems and leaves of *H. perforata*. Harrisotones A–E (**1**–**5**) representing a rare spirocyclic skeleton were structurally elucidated on the basis of extensive spectroscopic analysis. The biogenetic pathway of **1**–**5** was postulated, and harrisonol A (**6**) played a crucial role to trace the key biosynthetic intermediates via a retro-synthesis approach (Scheme 1). We describe herein the isolation and characterization of harrisotones A–E (**1**–**5**) and harrisonol A (**6**).

2. Results and discussion

Harrisotone A (**1**) showed a molecular ion at *m*/*z* 472.2829 $[M]^+$ in HREIMS, consistent with the molecular formula of $C_{28}H_{40}O_6$ (calcd 472.2825) requiring nine double bond equivalents (DBE). The strong IR absorptions at 3425 and 1708 cm^{-1} indicated the presence of hydroxyl and carbonyl groups, respectively. The UV absorptions at 280 and 231 nm, and the IR absorption at 1650 cm^{-1} implied the presence of an enolized β-triketone system.[8] The ^{13}C NMR data of **1** (Table 2 in C_5D_5N) further confirmed the presence of the enolized β-triketone (at δ_C 196.6, 114.1, 195.6, and 200.4) and an isolated ketone group (at δ_C 209.7). Seven methyls at δ_H 2.55 (3H, s), 1.65 (3H, s), 1.60 (3H, s), 1.56 (3H, s), 1.50 (3H, s), 1.51 (3H, s), and 1.48 (3H, s), and two olefinic protons at δ_H 4.95 (1H, t, J=8.0 Hz) and 5.32 (1H, t, J=7.2 Hz) were identified by the initial examination of ^1H NMR data (Table 1 in C_5D_5N). All the 28 carbons in the molecule were resolved in the ^{13}C NMR, and were classified into three carbonyl carbons, four sp^2 quaternary carbons (one oxygenated), two sp^2 methines, four sp^3 quaternary carbons (two oxygenated), two sp^3 methines, six sp^3 methylenes, and seven methyls. As the

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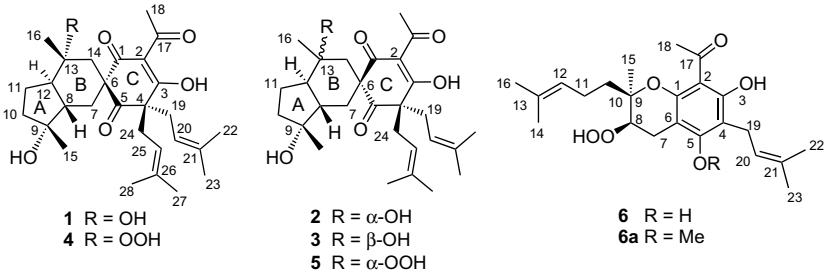


1 R = OH
4 R = OOH

2 R = α-OH
3 R = β-OH
5 R = α-OOH

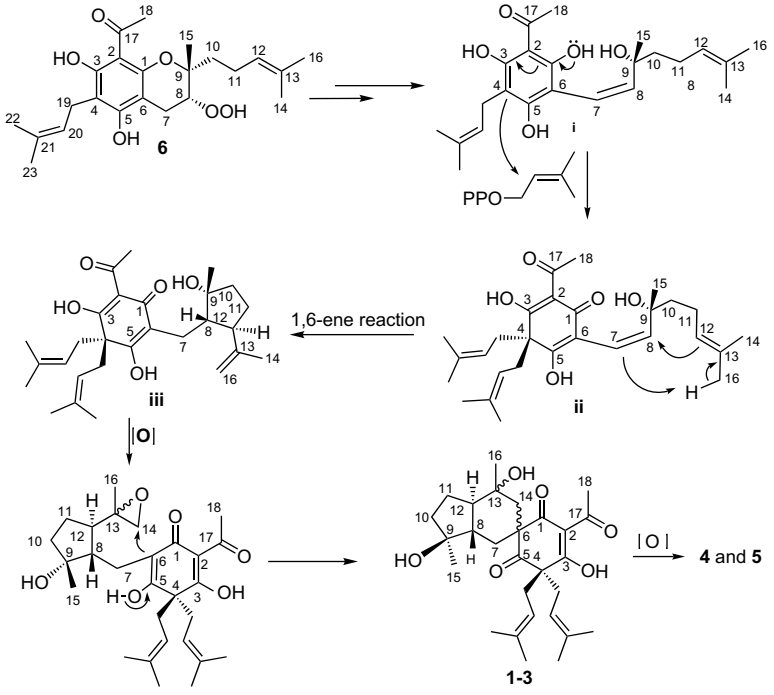
6 R = H
6a R = Me

aforementioned functional groups accounted for six out of nine DBE, the remaining three DBE required a tricyclic ring system for **1**. Careful analysis of the 1D and 2D NMR spectroscopic data (in C_5D_5N, Table 1) revealed the correlation sequences consistent with the structure of **1** as shown in Figure 1. A detailed account of these assignments is given below.

Three spin systems **a** (C_7–C_8–C_{12}–C_{11}–C_{10}), **b** (C_{19}–C_{20}), and **c** (C_{24}–C_{25}) highlighted in Figure 1A were defined by analysis of ^1H–^1H COSY (in C_5D_5N). The connectivity of the structural fragments **a**–**c**, the quaternary carbons, and the other functional groups was furnished by HMBC experiment (Fig. 1A). In the HMBC, the correlations from Me-15 to C-8, C-9, and C-10, and the correlations from H-8 and H_2-10 to C-9 furnished the five-membered ring A. The six-membered ring B was established by the HMBC correlations of Me-16/C-12, C-13, and C-14; H_2-7/C-6 and C-14; and H_2-14/C-6 and C-13. The C-1 at δ_C 196.6 assigned as one carbonyl of the enolized β-triketone was attached to C-6 by the ^3J HMBC correlations from H-7α and H_2-14 to C-1. The C-5 at δ_C 209.7 assigned for the isolated ketone carbonyl was also attached to C-6 by the ^3J HMBC correlations from H_2-7 and H_2-14 to C-5. The H_2-19 and H_2-24 correlating with C-3, C-4, and C-5 in the HMBC positioned the two prenyls at C-4, and allowed the establishment of the six-membered C-ring. The only remaining acetyl group was finally assigned to C-2 by the strong HMBC correlations from Me-18 to C-2 and C-17. Furthermore, when the HMBC spectrum of **1** was performed in $CDCl_3$, a severely down field shifted proton resonance at δ 18.1 (s) for a chelated hydroxyl correlating with C-2, C-3, C-4, and C-17 was attributable to the 3-OH, and required that the acetyl at C-2 was coplanar with the 3-OH to achieve the chelation. The long distant HMBC correlation between 3-OH and C-17 was also indicative of the formation of H-bond between the 3-OH and C-17 ketone group (as a pseudo ^2J), and supported the assignment of the C-ring. The planar structure of harrisotone A (**1**) was thus figured out.

The relative configuration of **1** was accomplished by ROESY spectrum recorded in C_5D_5N (Fig. 1). The ROESY correlations of Me-16/H-8 and H-14β; Me-15/H-8 and H-7β; and H-12/H-7α and H-14α indicated that A/B-rings were trans-fused, and that Me-16, Me-15, H-14β, H-8, and H-7β were co-facial, and were arbitrarily assigned in an β-orientation. In consequence, the H-12, H-7α, and H-14α were α-oriented. The ROESY correlations of H-20/H-7β, H-25/H-14β, and H-25/Me-16 clearly indicated that the C-4 to C-6 hemisphere of C-ring was at the upside of the C-6 spiro-center, and was β-oriented. Based on the ROESY spectral analysis, the five-membered A-ring and six-membered B-ring were thus assigned as an envelope and a chair conformation, respectively, and the six-membered C-ring containing the enolized β-triketone system was assigned as half-chair conformation, which was consistent with the β-triketone system reported in the literature.[4]

HREIMS analysis revealed that the molecular ions at *m*/*z* 472.2821 and 472.2822 of harrisotones B (**2**) and C (**3**), respectively,



Scheme 1. Biogenetic pathway proposed for compounds **1**–**5**.

Table 1
The ^{1}H NMR data of compounds **1**–**5** (recorded at 400 MHz)

No.	**1**		**2**		**3**		**4**	**5**
	δ_H,[a] *J* in Hz	δ_H,[b] *J* in Hz	δ_H,[a] *J* in Hz	δ_H,[b] *J* in Hz	δ_H,[a] *J* in Hz	δ_H,[b] *J* in Hz	δ_H,[b] *J* in Hz	δ_H,[b] *J* in Hz
7α	1.88 (t, 12.4)[c]	2.84 (m)[c]	1.93 (t,12.3)	2.80 (m)	1.36 (dt, 12.8, 1.9)	2.52 (m)[c]	2.81 (m)	2.78 (m)
7β	1.58 (m)[c]	2.12 (m)[c]	1.62 (m)[c]	2.17 (m)[c]	1.82 (d, 12.8)	2.33 (brd, 12.0)	2.14 (dd, 13.4, 4.0)	2.13 (m)
8	1.53 (m)	2.06 (dd, 12.7, 4.4)	1.73 (m)	2.14 (m)[c]	1.19 (m)	2.43 (m)	2.03 (m)	2.17 (m)
10α	1.77 (m)[c]	2.13 (m)[c]	1.78 (m)[c]	2.14 (m)[c]	1.69 (m)[c]	1.83 (m)[c]	2.08 (m)	2.06 (m)
10β	1.77 (m)[c]	1.92 (ddd, 12.5, 12.6, 4.1)	1.78 (m)[c]	1.95 (m)	1.69 (m)[c]	2.07 (m)	1.88 (m)	1.89 (ddd, 11.0, 11.0, 3.1)
11α	1.80 (m)[c]	2.14 (m)[c]	1.37 (m)[c]	2.16 (m)[c]	1.66 (m)[c]	1.85 (m)[c]	2.04 (m)	No[d]
11β	1.48 (m)[c]	1.78 (ddd, 11.3, 11.5, 5.3)	1.80 (m)[c]	1.67 (m)	1.67 (m)[c]	1.86 (m)[c]	1.70 (m)	
12	1.85 (m)[c]	2.64 (m)	1.76 (m)[c]	2.58 (m)	1.71 (m)[c]	2.15 (m)	2.79 (m)	2.76 (m)
14α	1.59 (m)[c]	2.26 (d,14.2)	1.17 (d, 13.7)	1.80 (d,13.4)	1.76 (dd, 15.0, 1.7)	1.61 (m)[c]	2.29 (d, 14.4)	2.15 (m)
14β	1.98 (d, 14.6)	2.36 (d,14.2)	2.09 (dd, 13.7, 1.7)	2.64 (m)	2.07 (d, 15.1)	2.58 (m)[c]	2.70 (d, 14.4)	2.88 (m)
15	1.25 (3H, s)	1.56 (3H, s)	1.37 (3H, s)[c]	1.64 (3H, s)	1.13 (3H, s)	1.59 (3H, s)[c]	1.53 (3H, s)	1.60 (3H, s)
16	1.10 (3H, s)	1.48 (3H, s)[c]	0.96 (3H, s)	1.31 (3H, s)	1.22 (3H, s)	1.29 (3H, s)	1.54 (3H, s)	1.36 (3H, s)
18	2.52 (3H, s)[c]	2.55 (3H, s)	2.47 (3H, s)	2.65 (3H, s)	2.54 (3H, s)	2.59 (3H, s)[c]	2.53 (3H, s)	2.68 (3H, s)
19a	2.49 (dd, 13.6, 7.0)	2.60 (m)	2.37 (dd, 13.2, 8.3)	2.47 (dd, 13.2, 8.0)	2.50 (dd, 16.8, 9.5)	2.54 (m)[c]	2.59 (dd, 14.0, 7.4)	2.49 (dd, 13.2, 7.9)
19b	2.68 (dd, 13.6, 8.8)	2.85 (m)[c]	2.60 (dd, 13.2, 8.3)	2.77 (dd, 13.2, 8.5)	2.66 (m)	2.80 (m)	2.85 (m)	2.77 (m)
20	4.74 (t, 8.4)	4.95 (t, 8.0)	4.63 (t, 8.2)	4.85 (t, 8.1)	4.70 (t, 7.4)	4.94 (t, 8.0)	4.94 (t, 8.0)	4.85 (t, 7.4)
22	1.50 (3H, s)	1.50 (3H, s)[c]	1.39 (3H, s)	1.38 (3H, s)	1.44 (3H, s)	1.44 (3H, s)	1.50 (3H, s)	1.36 (3H, s)
23	1.57 (3H, s)[c]	1.51 (3H, s)[c]	1.53 (3H, s)	1.52 (3H, s)	1.56 (3H, s)	1.55 (3H, s)	1.51 (3H, s)	1.50 (3H, s)
24a	2.53 (m)[c]	2.78 (dd, 14.1, 5.9)	2.68 (dd, 13.7, 9.1)	2.87 (m)	2.63 (m)	2.85 (m)[c]	2.80 (m)	2.88 (m)
24b	2.72 (dd, 13.9, 9.2)	3.02 (dd, 14.1, 8.8)	2.77 (dd, 13.7, 9.1)	2.95 (dd, 13.5, 9.9)	2.76 (dd, 16.8, 9.5)	2.91 (m)[c]	3.01 (dd, 14.0, 9.0)	2.96 (dd, 13.8, 9.5)
25	4.87 (t, 7.3)	5.32 (t, 7.2)	4.97 (t, 6.2)	5.28 (t, 7.7)	4.85 (t, 6.5)	5.27 (t, 8.4)	5.30 (t, 7.0)	5.32 (t, 7.6)
27	1.61 (3H, s)	1.60 (3H, s)	1.62 (3H, s)[c]	1.56 (3H, s)	1.59 (3H, s)	1.63 (3H, s)	1.65 (3H, s)	1.72 (3H, s)
28	1.52 (3H, s)[c]	1.65 (3H, s)	1.60 (3H, s)	1.60 (3H, s)	1.55 (3H, s)	1.66 (3H, s)	1.64 (3H, s)	1.65 (3H, s)
3-OH	18.1 (s)		17.9 (s)		18.2 (s)			
13-OH					5.13 (s)			

[a] Measured in $CDCl_3$.
[b] Measured in C_5D_5N.
[c] Overlapped signals in the same vertical column.
[d] Signals not detected.

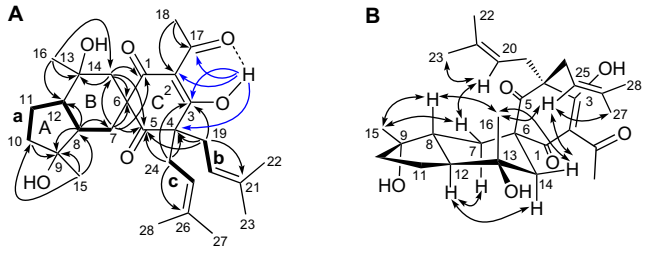


Figure 1. A: ^{1}H–^{1}H COSY (▬) and selected HMBC (H→C, blacks in C_5D_5N, blues in $CDCl_3$) of **1**. B: Key NOESY correlations (↔) of **1**.

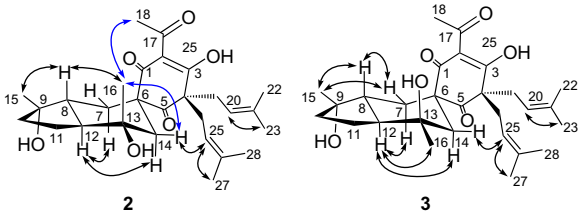


Figure 2. Key NOESY correlations of **2** and **3** (↔); blacks in C_5D_5N, blues in $CDCl_3$.

were consistent with a molecular formula of $C_{28}H_{40}O_6$ (calcd 472.2825), indicating that they were isomers of harrisotone A (**1**). Comprehensive analyses of UV, IR, 1D and 2D NMR spectra (Tables 1 and 2, Fig. 1, Supplementary data) of harrisotones B (**2**) and C (**3**) showed that they shared a common planar structure with that of **1**, suggesting that harrisotones A–C (**1**–**3**) were stereoisomers. The structural elucidation of compounds **2** and **3** were thus focused on the determination of their relative configurations.

As a few of the important proton resonances of **2** were overlapped to a certain extent either in the solvent C_5D_5N or $CDCl_3$, the ROESY experiments of **2** were therefore performed in both solvents for a cross check. Comprehensive analysis of the ROESY spectra of **2** measured in two different solvents revealed that it was the C-6 stereoisomer of **1**. In the ROESY spectrum (Fig. 2), the correlations of H-12/H-14α, H-12/H-7α, H-8/Me-15, and H-8/Me-16 indicated that the relative configurations at C-8, C-9, C-12, and C-13, and the conformations of A, B-rings of **2** were identical with those of **1**. This assignment was substantiated by the fact that the chemical shifts of the corresponding carbon signals of C-8, C-9, C-12, and C-13 in both compounds were very close (the differences were less than 0.8 ppm, Table 2). The only stereo difference between two compounds was occurred at the C-6 spiro-center as judged by the strong ROESY correlation between Me-18 and Me-16 in **2**, which located the C-1 to C-3 hemisphere of the C-ring at the upside of the C-6 spiro-center. In comparison with **1**, the carbon resonance of C-6 in **2** was down field shifted ca. 2.4 ppm (in both solvents, see Table 2) consistent with the above assignment. The totally opposite Cotton effects of **1** and **2** (Fig. 3) aroused by the different stereochemistry at the C-6 spiro-center further corroborated the relative configurations of **1** and **2**.

The CD spectrum (Fig. 3) of **3** showed high similarity with that of **2**, indicating that they shared the same stereochemistry at the C-6 spiro-center. In the ROESY spectrum of **3** (in C_5D_5N, Fig. 2), the correlations from H-12 to H-7α, H-14α, and Me-16 indicated that they were in the same side of the ring B, and were randomly assigned as α-configuration. Subsequently, the ROESY correlations from Me-15 to H-8 and H-7β revealed that they were co-facial and β-oriented. The aforementioned analysis revealed that **3** was a 13-epimer of **2**. The significant pyridine-induced chemical shift of H-8 ($\Delta\delta CDCl_3-C_5D_5N=\delta_H 1.19-\delta_H 2.43=-1.24$ ppm) was obviously indicative of a 1,3-diaxial relationship between 13β-OH and H-8,[9]

Table 2
The ^{13}C NMR data of compounds **1**–**5** (recorded at 100 MHz)

No.	**1**		**2**		**3**			**4**	**5**
	δ_C[a]	δ_C[b]	δ_C[a]	δ_C[b]	δ_C[a]	δ_C[b]	δ_C[c]	δ_C[b]	δ_C[b]
1	196.7	196.6	198.0	199.2	200.6	198.4	200.7	196.0	199.2
2	113.7	114.1	114.3	115.0	113.6	115.7	116.5	113.9	114.9
3	195.3	195.6	196.3	196.7	196.1	194.2	195.2	195.1	196.4
4	61.0	61.8	61.5	62.0	61.3	61.6	62.8	61.6	62.0
5	208.9	209.7	207.4	208.0	209.0	208.9	210.3	209.6	208.3
6	62.4	63.4	64.8	65.7	64.5	64.8	66.0	62.6	64.9
7	26.9	26.9	24.2	25.2	33.6	26.5	29.1	26.4	25.7
8	47.6	48.8	48.1	48.9	44.6	45.4	46.3	48.3	48.7
9	78.9	78.2	79.2	78.2	79.6	78.3	80.5	77.6	77.8
10	40.6	41.3	39.8	40.9	40.5	40.8	41.3	41.1	41.0
11	21.6	22.9	21.7	22.9	21.0	21.8	22.3	22.7	23.0
12	51.0	52.3	51.8	53.0	51.4	51.3	52.3	46.6	47.4
13	72.7	72.9	73.0	72.3	69.5	70.5	71.7	84.8	84.2
14	44.4	47.3	50.2	51.8	41.1	49.0	48.3	41.0	46.0
15	26.5	27.3	26.7	27.4	26.5	27.1	26.8	26.9	27.2
16	23.3	23.2	20.8	21.4	29.4	29.2	29.6	19.0	17.6
17	201.0	200.4	199.0	198.7	202.0	199.3	201.5	200.5	199.2
18	27.2	26.6	26.0	25.6	28.0	25.9	27.1	26.6	25.9
19	38.0	39.5	40.7	41.1	39.8	40.1	40.4	39.1	40.7
20	117.8	118.4	116.8	117.7	117.2	118.2	119.3	118.1	117.8
21	137.3	137.3	138.1	137.8	138.2	137.1	138.7	136.9	137.7
22	17.8	17.8	17.7	17.6	17.9	17.7	18.4	17.6	17.8
23	26.0	25.9	25.9	25.9	26.1	25.9	26.7	25.7	25.9
24	37.0	35.7	34.4	34.5	36.2	35.6	37.8	35.9	34.9
25	118.0	119.7	119.1	120.2	118.7	120.5	120.9	119.6	119.9
26	137.0	135.4	135.7	135.0	136.6	135.1	137.5	135.5	135.8
27	26.0	25.9	25.8	25.8	26.1	25.9	26.7	25.7	26.0
28	17.9	18.0	17.9	17.9	18.0	17.9	18.4	17.8	18.0

[a] Measured in $CDCl_3$.
[b] Measured in C_5D_5N.
[c] Measured in CD_3OD.

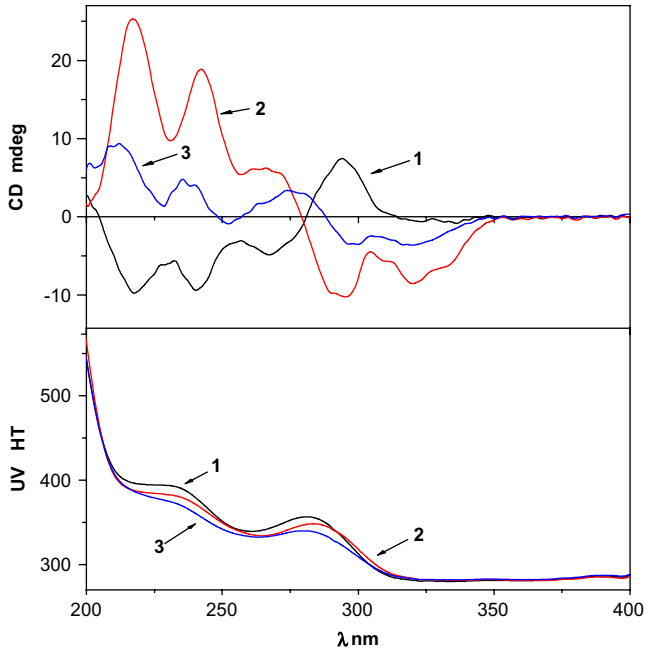


Figure 3. CD and UV spectra of **1**–**3** (in MeOH).

thus supporting the relative structure of **3** as established by ROESY spectrum.

In the ^{1}H NMR of **3** recorded in $CDCl_3$, the chelated hydroxyl proton of 13-OH at δ 5.13 (s) as assigned by HMQC spectrum required the spatial proximity to the C-1 ketone group to form a hydrogen bond. The C-1 carbon resonance of **3** thus shifted down field ca. $\Delta\delta$ 2.6 (in $CDCl_3$) as compared with that of **2**. In addition, the occurrence of the chelating H-bond between 13-OH and C-1 carbonyl resulted in the severe deshielding and shielding effects on the C-7 and C-14 of **3**, respectively (Fig. 4, Table 2), implying that the C-1 to C-3 hemisphere of C-ring was more close to the C-14 (far away from C-7) in **3**. These deshielding and shielding effects were alleviated in the polar solvents such as C_5D_5N and CD_3OD (Table 2) since the intramolecular H-bond was weakened in the polar solvents.

The HRESIMS of **4** gave a pseudo-molecular ion at *m*/*z* 511.2694 $[M+Na]^+$ (calcd 511.2672) corresponding to the molecular formula of $C_{28}H_{40}O_7$, with one more O-atom than **1**. The ^{13}C NMR data of **4** was very similar to that of **1** except that the differences scattered around C-13. The carbon resonances of C-12, C-13, C-14, and C-16 of **4** were shifted by $\Delta\delta_C$ −5.7, +11.9, −6.3, and −4.2 ppm, respectively, with respect to the corresponding carbon resonances of **1**, indicating that **4** possessed a hydroperoxy group at C-13.[10] The relative configuration of **4** was identical with that of **1** as assigned by ROESY experiment, and by comparing its NMR data with those of **1**. Compound **4** was partially converted into **1** when it was exposed to the air for several days (as checked by HPLC-MS with co-injection with pure sample of **1**). This further confirmed that compound **4** was the C-13 hydroperoxide of **1**.

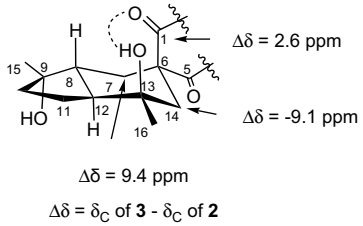


Figure 4. The H-bond induced ^{13}C NMR data changes of **3** versus **2** in $CDCl_3$.

Compound **5** displayed a pseudo-molecular ion at *m*/*z* 511.2659 $[M+Na]^+$ consistent with a molecular formula of $C_{28}H_{40}O_7$, suggesting that it was an isomer of **4**. The ^{13}C NMR data of **5** showed high similarity to that of **2** except that the carbon resonances of C-12, C-13, C-14, and C-16 of **5** were shifted by −5.6, +11.9, −5.8, and −3.8 ppm, respectively, as compared with those of **2**. This evidence indicated that the hydroxyl group at C-13 in **2** was replaced by a hydroperoxy group in **5**. Compound **5** was thus assigned the C-3 hydroperoxide of **2**, and this was confirmed by the fact that compound **5** was partially converted into **2** when kept in laboratory for several days (as checked by HPLC-MS with co-injection with pure sample of **2**).

Harrisonol A (**6**) gave a molecular formula of $C_{23}H_{32}O_6$ as determined by HREIMS at *m*/*z* 404.2196 $[M]^+$. The IR spectrum of **6** implied the presence of hydroxyls (3408 cm^{-1}) and a chelating ketone carbonyl (1628 cm^{-1}).[11] The ^{1}H and ^{13}C NMR data of **6** showed high similarity with those of a known compound (**6a**),[11] except for the presence of a C-5-OH in **6** instead of the C-5-OMe in **6a**. The structure of **6** was finally confirmed by 2D NMR spectra, including HMQC, ^{1}H–^{1}H COSY, HMBC, and ROESY.

2.1. Plausible biogenetic pathway proposed for harrisotones A–E (1–5)

From chemical view, the co-existence of harrisonol A (**6**) in this plant endowed us with a sudden inspiration to hypothetically trace the key biosynthetic intermediate (**iii**) via retro-synthesis approach (Scheme 1). Hydrolysis and loss of a hydroperoxide from **6** produced a pyran ring opened intermediate **i**, which would be alkylated with a dimethylallyl diphosphate at C-4 to yield an intermediate **ii**. The intramolecular cyclization of **ii** via a 1,6-ene reaction would form the key intermediate **iii**, which then underwent the epoxidation and spirocyclization to yield **1**–**3**. Subsequently, **1** and **2** could be further oxidated to afford **4** and **5**, respectively.

Since the intermediate **iii** was biosynthetically and structurally related with chinesins I and II,[4] whose absolute stereochemistry were determined by chemical means,[12] the absolute configurations at C-8, C-9, and C-12 in compounds **1**–**5** were presumed to be same as those in chinesins I and II. On the biogenetic consideration, the absolute configuration of harrisotones A–E (**1**–**5**) could be therefore proposed as depicted. The proposed absolute configuration of compounds **1**–**5** remains to be finally determined by synthetic chemistry or any other solid data.

2.2. Evaluation of cytotoxicity

The cytotoxic activities of compounds **1**–**3** and **6** against P-388 and A-549 tumor cell lines were evaluated by follow the standard protocols MTT[13] and SRB[14] methods, respectively. In these tests, pseudolaric acid B[15] (with IC_{50} values of 0.74 and 1.99 against P-388 and A-549, respectively) was used as positive control. Harrisotones A, C and harrisonol A (**1**, **3**, and **6**) exhibited significant cytotoxic activity against P-388 tumor cell line with IC_{50} values of 1.56, 2.35, and 0.27 μM, respectively. Harrisotone A (**1**) and harrisonol A (**6**) also showed moderate activity against A-549 tumor cell line with IC_{50} of 24.5 and 26.6 μM, respectively.

3. Experimental section

3.1. Plant material

The plant material of *H. perforata* was collected from Hainan province of PR China, and authenticated by Prof. Shi-Man Huang, Department of Biology, Hainan University of PR China. A voucher specimen has been deposited in Shanghai Institute of Materia

Medica, Chinese Academy of Science (Accession number HAPE-2004-1Y).

3.2. Extraction and isolation

The dried powder of stems and leaves of *H. perforata* (2.8 kg) was extracted with 95% EtOH for three times to give 300 g crude extract, which was suspended in 1.5 L water and then partitioned with ethyl acetate to give ethyl acetate soluble fraction I (120 g). Fraction I was subjected to a silica gel column chromatography (CC) eluted with an increasingly gradient of acetone in petroleum ether to obtain four fractions Fr1–4 according to TLC monitor. Fr 1 (10 g) was separated by silica gel CC eluted with petroleum ether/isopropyl alcohol (20:1) to yield **3** (6 mg). Fr 2 (50 g) was further separated on a silica gel column eluted with increasingly gradient of ethyl acetate in petroleum ether to obtain subfractions Frs 2a–2f. Fr 2d was purified on a reversed phase C18 silica gel column eluted with MeOH/H_2O (7:3, v/v) to afford compounds **2** (25 mg) and **5** (8 mg). Fr 2e was also purified on a reversed phase C18 silica gel column eluted with MeOH/H_2O (7:3, v/v) to give compounds **1** (20 mg) and **4** (6 mg). Fr 2f (500 mg) was separated on a Sephadex LH-20 column eluted with MeOH/H_2O (6:4, v/v) to yield compound **6** (20 mg).

3.2.1. Harrisotone A (**1**)

Obtained as a pale solid; UV (CH_3OH) λ_{max} (log ε) 280 (4.16), 231 (4.21) nm; $[\alpha]_D^{20}$ +2.0 (*c* 0.27, MeOH); IR (KBr) ν_{max} cm^{-1} 3425, 2923, 2871, 1708, 1650, 1632, 1585, 1446, 1382, 1114; ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) see Table 1 and Supplementary data; EIMS 70 eV *m*/*z* (rel int) 472 [M^+] (8), 454 (24), 436 (20), 418 (11), 403 (13), 385 (97), 367 (100), 349 (87), 307 (57), 247 (56), 193 (26), 69 (89); HREIMS *m*/*z* 472.2829 ($C_{28}H_{40}O_6$, calcd 472.2825).

3.2.2. Harrisotone B (**2**)

Obtained as a pale solid; UV (CH_3OH) λ_{max} (log ε) 282 (4.11), 232 (4.18) nm; $[\alpha]_D^{20}$ +25.4 (*c* 1.30, MeOH); IR (KBr) ν_{max} cm^{-1} 3438, 2922, 1716, 1674, 1578, 1448, 1379, 1118, 1057; ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) see Table 1 and Supplementary data; EIMS 70 eV *m*/*z* (rel int) 472 [M^+] (6), 454 (17), 436 (18), 418 (11), 403 (58), 385 (83), 367 (100), 349 (80), 307 (52), 247 (74), 193 (22), 69 (49); HREIMS *m*/*z* 472.2821 ($C_{28}H_{40}O_6$, calcd 472.2825).

3.2.3. Harrisotone C (**3**)

Obtained as a pale solid; UV (CH_3OH) λ_{max} (log ε) 278 (4.05), 230 (4.16) nm; $[\alpha]_D^{20}$ +11.1 (*c* 0.80, MeOH); IR (KBr) ν_{max} cm^{-1} 3427, 2922, 2850, 1709, 1632, 1581, 1448, 1394, 1080; ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) see Table 1 and Supplementary data; EIMS 70 eV *m*/*z* (rel int) 472 [M^+] (9), 454 (15), 436 (6), 418 (5), 403 (9), 385 (70), 367 (46), 349 (38), 247 (37), 193 (24), 69 (100); HREIMS *m*/*z* 472.2822 ($C_{28}H_{40}O_6$, calcd 472.2825).

3.2.4. Harrisotone D (**4**)

Obtained as a pale solid; UV (CH_3OH) λ_{max} (log ε) 283 (3.86), 234 (4.02) nm; $[\alpha]_D^{20}$ +4.1 (*c* 0.12, MeOH); IR (KBr) ν_{max} cm^{-1} 3423, 2966, 2931, 2874, 1716, 1673, 1620, 1448, 1377, 1119; ^1H NMR and ^{13}C NMR (C_5D_5N) see Table 1; ESIMS *m*/*z* 487.3 [M−H]$^-$ (50), 997.7 2[M−H]$^-$+Na^+ (100); HRESIMS *m*/*z* 511.2694 [M+Na]$^+$ ($C_{28}H_{40}O_7Na$, calcd 511.2672).

3.2.5. Harrisotone E (**5**)

Obtained as a pale solid; UV (CH_3OH) λ_{max} (log ε) 285 (4.15), 234 (4.06) nm; $[\alpha]_D^{20}$ +22.1 (*c* 0.09, MeOH); IR (KBr) ν_{max} cm^{-1} 3425, 2972, 2931, 2874, 1716, 1674, 1620, 1448, 1379, 1109; ^1H NMR and ^{13}C NMR (C_5D_5N) see Table 1; ESIMS *m*/*z* 511.3 [M+Na]$^+$ (100), 487.3 [M−H]$^-$ (98); HRESIMS *m*/*z* 511.2659 [M+Na]$^+$ ($C_{28}H_{40}O_7Na$, calcd 511.2672).

3.2.6. Harrisonol A (**6**)

Obtained as a yellow amorphous solid; UV (CH_3OH) λ_{max} (log ε) 334 (3.43), 287 (4.26), 228 (4.17) nm; $[\alpha]_D^{20}$ +52.4 (*c* 0.34, MeOH); IR (KBr) ν_{max} cm^{-1} 3408, 2920, 2850, 1628, 1572, 1439, 1375, 1319, 1238, 1080; ^1H NMR (in $CDCl_3$) δ_H (*J* in Hz): 3.06 (2H, dd, 8.3, 2.8, H-7), 4.73 (1H, t, 9.8, H-8), 1.56 (2H, m, H-10), 2.12 (2H, m, H-11), 5.12 (1H, br t, 5.6, H-12), 1.69 (3H, s, H-14), 1.28 (3H, s, H-15), 1.62 (3H, s, H-16), 2.63 (3H, s, H-18), 3.27 (2H, d, 7.3, H-19), 5.24 (1H, br t, 6.0, H-20), 1.76 (3H, s, H-22), and 1.81 (3H, s, H-23); ^{13}C NMR ($CDCl_3$) δ_C: 157.2 (C-1), 105.7 (C-2), 161.2 (C-3), 101.2 (C-4), 164.2 (C-5), 104.0 (C-6), 26.9 (C-7), 90.5 (C-8), 73.8 (C-9), 37.1 (C-10), 22.0 (C-11), 124.0 (C-12), 132.3 (C-13), 25.7 (C-14), 22.6 (C-15), 17.7 (C-16), 203.3 (C-17), 32.8 (C-18), 22.4 (C-19), 121.3 (C-20), 135.5 (C-21), 25.8 (C-22), and 17.9 (C-23); EIMS 70 eV *m*/*z* (rel int) 404 [M^+] (0.3), 388 (66), 371 (9), 333 (9), 303 (47), 287 (11), 247 (55), 231 (34), 247 (37), 206 (100), 109 (28), 69 (34); HREIMS *m*/*z* 404.2196 ($C_{23}H_{32}O_6$, calcd 404.2199).

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.11.068.

References and notes

1. (a) Tang, Y.; Lee, T. S.; Khosla, C. *PLoS Biol.* **2004**, *2*, 227–238; (b) Stierle, D. B.; Stierle, A. A.; Hobbs, J. D.; Stokken, J.; Clardy, J. *Org. Lett.* **2004**, *6*, 1049–1052; (c) Cane, D. E.; Walsh, C. T.; Khosla, C. *Science* **1998**, *282*, 63–68.
2. Balde, A. M.; Pieters, L.; Apers, S.; De Bruyne, T.; Van den Heuvel, H.; Claeys, M.; Vlietinck, A. *J. Nat. Prod.* **1999**, *62*, 364–366.
3. Kralj, A.; Kehraus, S.; Krick, A.; Eguereva, E.; Kelter, G.; Maurer, M.; Wortmann, A.; Fiebig, H. H.; Konig, G. M. *J. Nat. Prod.* **2006**, *69*, 995–1000.
4. Hashida, W.; Tanaka, N.; Kashiwada, Y.; Sekiya, M.; Ikeshiro, Y.; Takaishi, Y. *Phytochemistry* **2008**, *69*, 2225–2230.
5. Simpson, T. J. *Nat. Prod. Rep.* **1984**, *1*, 281–297.
6. Hainan Institute of Botany. *Hainan Flora (Hainan Zhiwu Zhi)*; Science: Beijing, 1974; Vol. 3, pp 54–55.
7. (a) Khuong-Huu, Q.; Chiarona, A.; Riche, C.; Nguyen-Ngoc, H.; Nguten-Viet, K.; Khuong-Huu, F. *J. Nat. Prod.* **2000**, *63*, 1015–1018; (b) Kamiuchi, K.; Mitsunaga, K.; Koike, K.; Ouyang, Y.; Ohmoto, T.; Nikaido, T. *Heterocycles* **1996**, *43*, 653–664; (c) TranVan, S.; Nguyen-Minh, P.; Kamperdick, C.; Adam, G. *Phytochemistry* **1995**, *38*, 213–215; (d) Byrne, L. T.; Van Tri, M.; Nguyen-Minh, P.; Sargent, M. V.; Skelton, B. W.; White, A. H. *Aust. J. Chem.* **1991**, *44*, 165–169; (e) Wei, X.; Pan, Z.; Gu, X.; Wang, M.; Zhu, Y. *Jiegou Huaxue* **1985**, *4*, 281–287.
8. (a) Winkelmann, K.; Heilmann, J.; Zerbe, O.; Rali, T.; Sticher, O. *J. Nat. Prod.* **2000**, *63*, 104–108; (b) Weng, J. R.; Tsao, L. T.; Wang, J. P.; Wu, R. R.; Lin, C. N. *J. Nat. Prod.* **2004**, *67*, 1796–1799.
9. Demarco, P. V.; Farkas, E.; Doddell, D.; Mylari, B. L.; Wenkert, E. *J. Am. Chem. Soc.* **1968**, *90*, 5480–5486.
10. (a) Ono, M.; Tsuru, T.; Abe, H.; Eto, M.; Okawa, M.; Abe, F.; Kinjo, J.; Ikeda, T.; Nohara, T. *J. Nat. Prod.* **2006**, *69*, 1417–1420; (b) Arciniegas, A.; Perez-Castorena, A.-L.; Reyes, S.; Contreras, J. L.; Vivar, A. R. de. *J. Nat. Prod.* **2003**, *66*, 225–229.
11. Liu, H. W.; Kusumi, T.; Nakanishi, K. *J. Chem. Soc., Chem. Commun.* **1981**, 1271–1272.
12. Aramaki, Y.; Chiba, K.; Tada, M. *J. Chem. Soc., Perkin Trans. 1* **1995**, 683–688.
13. Liu, W. J.; Jiang, J. F.; Xiao, D.; Ding, J. *Biochem. Pharmacol.* **2002**, *64*, 1677–1687.
14. Xiao, D.; Zhu, S. P.; Gu, Z. L. *Acta Pharmacol. Sin.* **1997**, *18*, 280–283.
15. Pan, J. P.; Li, Z. L.; Hu, C. Q.; Chen, K.; Chang, J. J.; Lee, K. H. *Planta Med.* **1990**, *56*, 383–385.