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Spicachlorantins C–F, hydroperoxy dimeric sesquiterpenes from the roots of *Chloranthus spicatus*

Sang-Yong Kim [a], Yoshiki Kashiwada [a,*], Kazuyoshi Kawazoe [b], Kotaro Murakami [c], Han-Dong Sun [d], Shun-Lin Li [d], Yoshihisa Takaishi [a]

[a] Graduate School of Pharmaceutical Sciences, University of Tokushima, 1-78 Shomachi, Tokushima 770-8505, Japan
[b] Department of Clinical Pharmacy, Tokushima University Hospital, 2-50-1 Kuramoto, Tokushima 770-8503, Japan
[c] Faculty of Pharmaceutical Sciences, Sojo University, 4–22–1 Ikeda, Kumamoto 862-0082, Japan
[d] Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, China

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ABSTRACT

Spicachlorantins C–F (**1**–**4**), new lindenane–sesquiterpene dimers possessing a hydroperoxy group, were isolated from the roots of *Chloranthus spicatus*. Their structures, including the absolute stereostructures, were established by 1D and 2D NMR, as well as CD spectroscopic analyses. Spicachlorantins C–F were considered to be biogenetic precursors for the corresponding hydroxy derivatives of dimeric lindenane sesquiterpenes distributed in *Chloranthus* plants.

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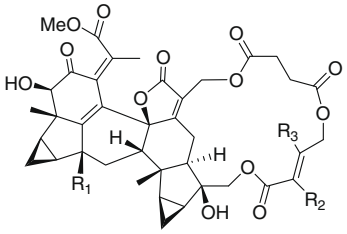
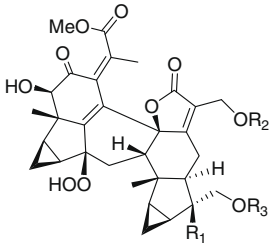
We have been investigating the traditional herbal medicines used by ethnic minority groups in Yunnan Province, China, designing to identify the natural products on which new therapeutic agents can be based. About 17,000 plant species grow wild in Yunnan, and many of these plants have been used by the minority groups in Yunnan as herbal medicines. In the course of our ethnopharmacological study, we found that the Va and the Dai ethnic minority groups[1] in Yunnan use *Chloranthus* plants medicinally. The genus *Chloranthus* (Chloranthaceae) has about 10 species distributed in the tropical zone of Asia and the temperate zone of East Asia.[2] Among them, *Chloranthus spicatus* (Thunb.) Makino is used to treat bone fracture by the Va ethnic group, while the Dai ethnic group use this plant to treat high blood pressure. As part of this study, we have previously examined the MeOH extracts from the roots of *C. spicatus* collected in Yunnan, and reported the isolation and structure elucidation of two new lindenane–sesquiterpene dimers, spicachlorantins A and B,[3] along with a known related compound, chloramultilide A (**5**).[4] Similar complex structures of lindenane–sesquiterpene dimers with unique biological activities, including inhibition on the delayed rectifier K^+ current,[5,6] inhibition of the expression of cell adhesion molecules,[7] and inhibition of tyrosinase,[8] have been reported from *Chloranthus* plants, and have been of interest to natural product chemist as leads for new therapeutic agents. Therefore, we further examined the constituents of *C. spicatus* cultivated in Japan, and has resulted in the isolation of four new lindenane-type sesquiterpene dimers, spicachlorantins C–F (**1**–**4**), possessing a hydroperoxy group at C-4 position. These compounds were considered to be biogenetic precursors of the corresponding hydroxy derivatives of dimeric lindenane sesquiterpenes distributed in the *Chloranthus* plants. This Letter describes the isolation and structural characterization of these compounds.

Dried roots of *C. spicatus* were extracted with Et_2O. The Et_2O extract was subjected to silica gel chromatography (hexane/EtOAc) followed by repeated column chromatographies over Sephadex LH-20 (benzene/MeOH), Toyopearl HW-40 (benzene/MeOH), MCI gel CHP-20P (MeOH/H_2O), YMC ODS A (MeOH/H_2O), Asahipak GS-310 on HPLC (MeOH), reversed phase HPLC (MeOH/H_2O), and a silica gel HPLC ($CHCl_3$/EtOAc/2-PrOH) to give spicachlorantins C (**1**) (22.5 mg; 0.0043% yield), D (**2**) (5.2 mg; 0.00188%), E (**3**) (5.5 mg; 0.00046%), and F (**4**) (3.9 mg; 0.00033%) (Fig. 1).



	R_1	R_2	R_3
1	OOH	Me	H
2	OOH	H	Me
5	OH	Me	H



	R_1	R_2	R_3
3	OH	Ac	Tig*
4	H	H	Ac

*tigloyl group =

Figure 1.

* Corresponding author. Tel./fax: +81 88 633 7276.
E-mail address: kasiwada@ph.tokushima-u.ac.jp (Y. Kashiwada).

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Spicachlorantin C (**1**)[9] was obtained as a white amorphous powder. The molecular formula of **1** was determined as $C_{40}H_{44}O_{15}$ by HRESIMS (*m*/*z* 787.2588 $[M+Na]^+$). The ^1H NMR spectrum showed the presence of two vinylic methyl groups (δ_H 1.94 and 1.83), two tertiary methyl groups (δ_H 1.05 and 0.93), and a methoxy group (δ_H 3.74), together with an olefinic proton signal (δ_H 6.57) and three hydroxymethyl signals [δ_H 5.25, 4.56 (each 1H, d, J = 12.2 Hz), 5.12 (1H, ddd, J = 4.9, 14.5 Hz), 4.63 (1H, ddd, J = 7.0, 14.5 Hz), 4.41, 3.86 (each 1H, d, J = 11.7 Hz)]. It also exhibited a characteristic singlet signal at extremely low-field (δ_H 8.23), which did not show any correlations in the HSQC spectrum. The occurrence of two sets of proton spin systems of a 1,2-substituted cyclopropane ring moiety revealed by the ^1H–^1H COSY spectrum was distinctive of a lindenane–sesquiterpene dimer.[4–8] The ^{13}C NMR spectrum with DEPT experiments displayed the presence of 40 carbons ascribable to six carbonyl, including a ketone group and five ester carbonyl groups, eight olefinic, four methyl, nine sp^3 methylene, seven sp^3 methine, two sp^3 quaternary, a methoxy, and three oxygen-bearing sp^3 quaternary carbons (Table 1). The existence of a succinyl and a 4-hydroxy-2-methylbut-2-enoyl groups was also provided by the ^1H–^1H COSY correlations of H-3″–H_2-4″ and H-2‴–H-3‴, combined with the HMBC correlations of Me-5″ with C-1″, C-2″, and C-3″, and of H-3‴ with C-1‴ and C-4‴. These spectral features were quite similar to those of chloramultilide A (**5**)[4] except for the characteristic singlet signal at δ_H 8.23 in the ^1H NMR spectrum as well as the oxygen-bearing quaternary carbon resonance at low-field (δ_C 90.5) in the ^{13}C NMR spectrum, which were indicative of the presence of a hydroperoxy group in **1**.[10] The ^{13}C resonance for C-4 showed downfield shift (+10.4 ppm), whereas the neighboring C-3, C-5, and C-15 were shifted upfield (−2.2, −4.4, and −4.6 ppm, respectively) as compared with those of chloramultilide A (**5**), suggesting the location of the hydroperoxy group at C-4. This was further confirmed by the HMBC correlations of H-2 and H-15 with C-4. The other key COSY and HMBC correlations shown in Figure 2 provided the planar structure of **1** (Fig. 2).

Table 1
^{13}C NMR data of compounds **1**–**4** in $CDCl_3$

	1	2	3	4
1	26.0	25.8	26.0	25.8
2	8.1	8.1	8.1	8.1
3	27.7	27.7	27.6	27.8
4	90.5	90.6	90.5	90.8
5	158.4	158.5	158.5	158.4
6	126.7	126.9	126.8	126.9
7	142.5	142.8	142.9	143.0
8	199.0	198.9	198.7	198.9
9	77.8	77.8	77.8	77.8
10	50.1	50.2	50.1	50.2
11	129.5	129.3	129.0	128.9
12	169.4	169.7	169.6	170.2
13	20.7	20.8	21.0	21.3
14	15.1	15.1	15.2	15.3
15	36.8	37.0	36.7	36.4
1′	27.3	27.2	27.3	26.3
2′	10.1	10.4	10.3	16.4
3′	28.6	28.6	29.2	22.5
4′	77.1	77.8	77.5	44.4
5′	56.9	57.4	54.8	53.4
6′	23.5	24.3	22.4	25.5
7′	172.4	172.2	170.2	166.2
8′	87.1	87.4	87.2	87.6
9′	52.5	52.5	52.4	52.1
10′	45.4	44.9	45.1	44.6
11′	124.7	124.5	125.0	128.0
12′	171.9	171.4	171.5	172.7
13′	54.0	55.4	55.1	55.0
14′	23.8	23.8	24.0	22.6
15′	73.2	71.3	70.7	65.8
1″	166.7	166.1	168.1	171.2
2″	129.4	113.2	127.9	20.6
3″	135.6	154.2	138.9	
4″	61.3	66.1	14.6	
5″	12.8	15.8	12.1	
1‴	171.4	171.4	170.4	
2‴	29.2	29.1	20.4	
3‴	28.7	29.0		
4‴	171.7	171.7		
OMe	52.5	52.7	52.6	52.8

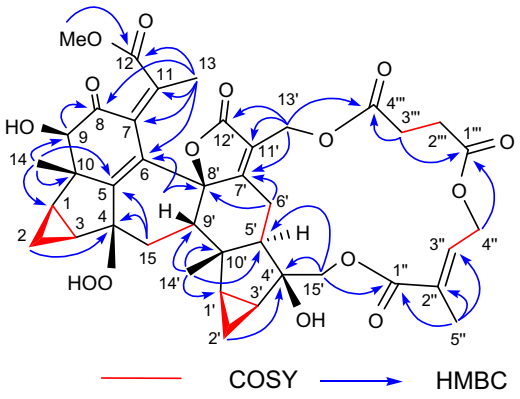

Figure 2. Selected COSY and HMBC correlations of **1**.

The relative stereochemistry of **1** was elucidated from ROESY correlations as shown in Figure 3. The ROESY correlations of H-1/H-2α, H-1/H-9, H-3/H-15β, H-9/H-5′, H-15β/H-1′, H-3′/H_2-15′, and H-6′α/H_2-15′ indicated that they are located mutually on the same α-side, and therefore the relative stereochemistries of H-1 (α), H-3 (α), H-1′ (α), H-3′ (α), H-5′ (α) as well as OOH-4 (β) and OH-4′ (β) were assigned. In contrast, the relative stereochemistries of Me-14 (β), H-9′ (β), Me-14′ (β) and C-O-8′ (β) were concluded from the ROESY cross-peaks of Me-13/Me-14, H-2β/Me-14, H-2′β/Me-14′, H-6′β/Me-14′, H-9′/Me-14′, and H_2-13′/Me-14′. The geometry of the double bond in the 4-hydroxy-2-methylbut-2-enoyl group was also elucidated to be *E* by the ROESY correlation of H-4″ and Me-5″ (Fig. 3). Consequently, the relative stereostructure of **1** was elucidated as illustrated in Figure 3.

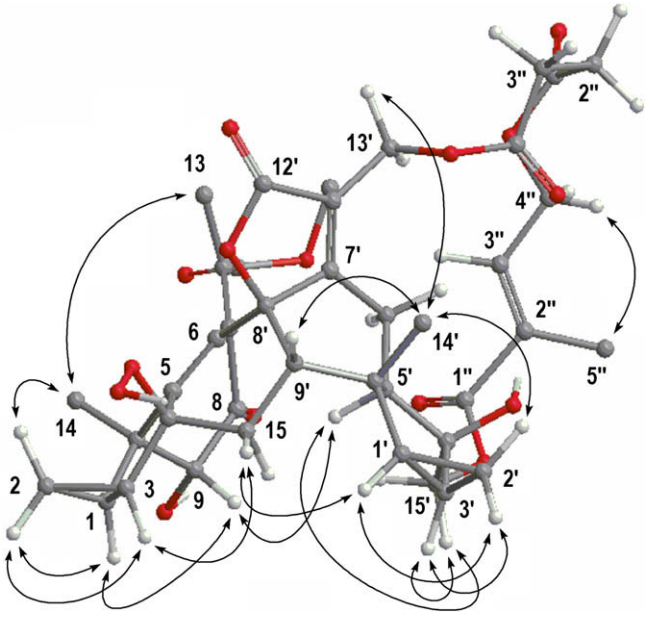

Figure 3. Key ROESY correlations of **1**.

Table 2
^{1}H NMR data for compounds **1**–**4** in $CDCl_3$ (*J* in Hz)

	1	2	3	4
1	2.05 (1H m)	2.04 (1H, m)	2.03 (1H, ddd, 8.4, 6.2, 4.4)	2.04 (1H, m)
2	1.28 (1H, m)	1.28 (1H, m)	1.27 (1H, dt, 5.6, 4.4)	1.29 (1H, m)
	0.97 (1H, dt, 8.9, 5.9)	0.97 (1H, dt, 8.3, 5.9)	0.97 (1H, m)	0.97 (1H, dt, 8.2, 5.8)
3	1.84 (1H, m)	1.85 (1H, ddd, 8.3, 6.4, 3.8)	1.85 (1H, m)	1.88 (1H, ddd, 8.2, 6.1, 3.8)
9	3.74 (1H, s)	3.80 (1H, s)	3.75 (1H, s)	3.85 (1H, s)
13	1.83 (3H, s)	1.79 (3H, s)	1.77 (3H, s)	1.79 (3H, s)
14	1.05 (3H, s)	1.04 (3H, s)	1.03 (3H, s)	1.04 (3H, s)
15	3.05 (1H, dd, 14.2, 7.2)	3.01 (1H, dd, 14.2, 7.2)	3.06 (1H, dd, 14.2, 7.2)	3.06 (1H, dd, 14.2, 7.2)
	1.62 (1H, m)	1.64 (1H, dd, 14.2, 10.0)	1.64 (1H, m)	1.69 (1H, dd, 14.2, 10.1)
1′	1.60 (1H, m)	1.57 (1H, m)	1.60 (1H, m)	1.44 (1H, ddd, 8.6, 7.4, 4.1)
2′	1.27 (1H, m)	1.27 (1H, m)	1.21 (1H, dt, 5.6, 4.0)	0.72 (1H, dt, 8.6, 5.5)
	0.65 (1H, dt, 8.9, 5.8)	0.63 (1H, dt, 8.9, 5.6)	0.62 (1H, dt, 8.9, 5.6)	0.65 (1H, m)
3′	1.48 (1H, m)	1.44 (1H, m)	1.61 (1H, m)	1.18 (1H, m)
4′				1.54 (1H, m)
5′	1.58 (1H, m)	1.60 (1H, m)	1.62 (1H, m)	1.67 (1H, m)
6′	2.90 (1H, dd, 17.9, 13.1)	2.95 (1H, m)	2.90 (1H, dd, 17.8, 13.0)	2.56 (1H, dd, 17.5, 12.5)
	2.40 (1H, dd, 17.9, 6.5)	2.62 (1H, dd, 18.8, 6.4)	2.28 (1H, dd, 17.8, 6.6)	2.45 (1H, dd, 17.5, 6.9)
9′	2.57 (1H, m)	2.54 (1H, dd, 10.0. 7.2)	2.61 (1H, dd, 10.2, 7.2)	2.65 (1H, dd, 10.1, 7.2)
13′	5.25 (1H, d, 12.2)	4.95 (1H, d, 13.0)	4.86 (1H, d, 13.0)	4.44 (1H, d, 13.6)
	4.56 (1H, d, 12.2)	4.82 (1H, d, 13.0)	4.82 (1H, d, 13.0)	4.37 (1H, d, 13.6)
14′	0.93 (3H, s)	0.90 (3H, s)	0.96 (3H, s)	0.85 (3H, s)
15′	4.41 (1H, d, 11.7)	4.86 (1H, d, 11.7)	4.22 (1H, d, 11.4)	4.00 (2H, d, 5.9)
	3.86 (1H, d, 11.7)	3.52 (1H, d, 11.7)	3.92 (1H, d, 11.4)	
2″		5.93 (1H, d, 1.1)		2.06 (3H, s)
3″	6.57 (1H, m)		6.86 (1H, m)	
4″	5.12 (1H, dd, 14.5, 4.9)	5.07 (1H, d, 16.5)	1.85 (3H, d, 1.0)	
	4.63 (1H, dd, 14.5, 7.0)	4.36 (1H, d, 16.5)		
5″	1.94 (3H, s)	2.12 (3H, s)	1.86 (3H, s)	
2‴	2.81 (1H, m)[a]	2.76 (1H, m)[a]	2.08 (3H, s)	
	2.64 (1H, m)[a]	2.68 (1H, m)[a]		
3‴	2.86 (1H, m)[a]	2.95 (1H, m)[a]		
	2.54 (1H, m)[a]	2.76 (1H, m)[a]		
OMe	3.74 (3H, s)	3.82 (3H, s)	3.78 (3H, s)	3.80 (3H, s)
OOH	8.23 (1H, s)	8.41 (1H, s)	8.42 (1H, s)	8.42 (1H, s)

[a] Assignment may be interchanged in each column.

Spicachlorantin D (**2**)[11] had a molecular formula of $C_{40}H_{44}O_{15}$, established by HRESIMS, which was the same as that of **1**. The ^{1}H and ^{13}C NMR spectra were closely correlated with those of **1**, showing signals arising from two lindenane units, a hydroperoxy group, and a macrocyclic trilactone ring (Tables 1 and 2). However, the olefinic proton resonance as well as the five ^{13}C resonances due to the 4-hydroxy-2-methylbut-2-enoyl group seen in **1** was slightly different from those found in **2**. The HMBC correlations of Me-5″ with C-2″, C-3″, and C-4″ indicated the presence of a 4-hydroxy-3-methylbut-2-enoyl group in **2** (Fig. 4). Therefore planar structure of compound **2** was concluded as shown in Figure 4. The relative configuration for **2** was also elucidated from ROESY correlations, which was quite similar to those seen in **1** (Fig. 5).

The molecular formula of spicachlorantin E (**3**),[12] determined by HRESIMS at *m/z* 731.2670 $[M+Na]^+$, was $C_{38}H_{44}O_{13}$. A characteristic ^{1}H resonance at δ_H 8.42 as seen in **1** and **2** ascribable to a hydroperoxy group was also observed in **3**. The ^{1}H and ^{13}C NMR spectra were closely correlated with those of **1** and **2**, showing signals due to two lindenane units with structures similar to **1** and **2** (Tables 1 and 2). In addition, they also exhibited the presence of an acetyl and a 2-methylbut-2-enoyl group instead of a macrocyclic trilactone ring. The olefinic proton resonance at δ_H 6.86 was indicative of *E*-geometry of the 2-methylbut-2-enoyl group to be a tigloyl group.[6] In addition, the HMBC correlations observed in **3** were coincided with the presence of the same lindenane sesquiterpene dimer units as **1** and **2**. The locations of the acetyl and tigloyl groups were concluded to be attached to C-13′ and C-15′, respectively, from the HMBC correlations of H_2-13′ with C-1‴ and of H_2-15′ with C-1″ (Fig. 4). The relative configurations were elucidated as shown in Figure 5 from the following ROESY correlations:

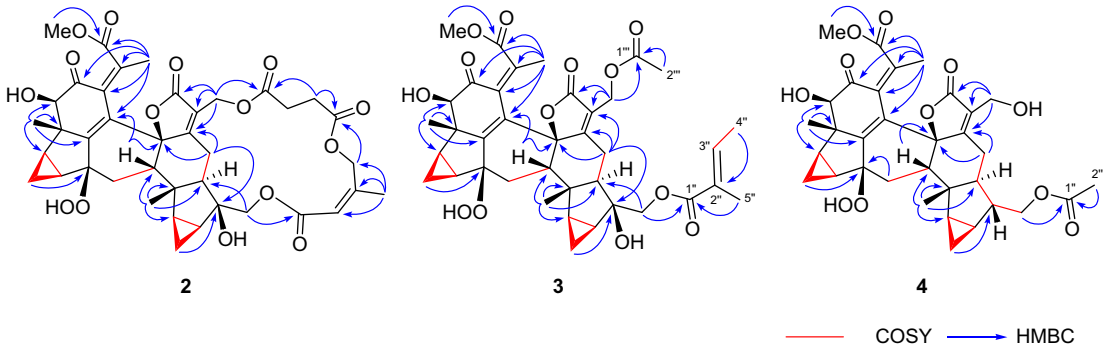

Figure 4. Selected COSY and HMBC correlations of **2**–**4**.

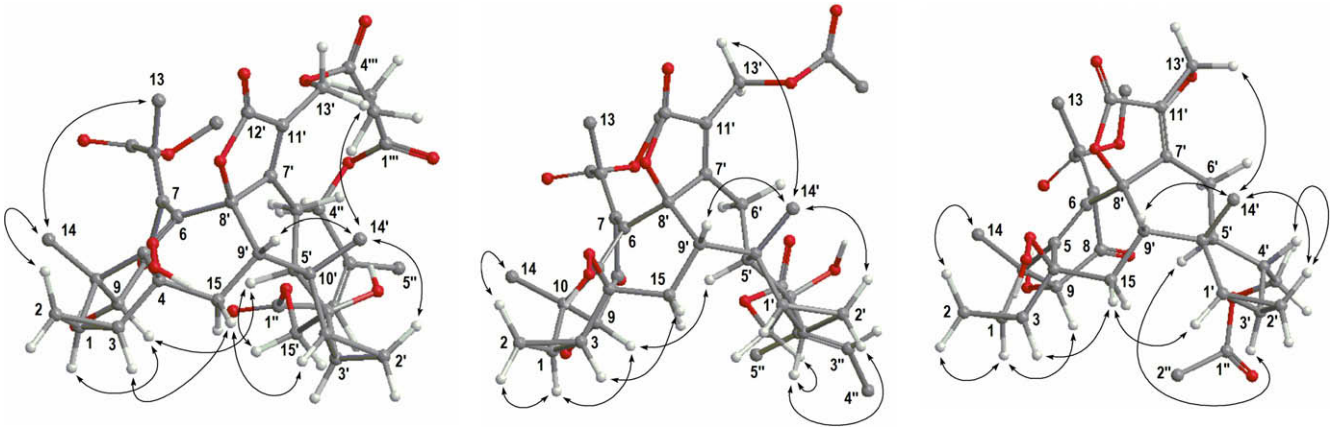

Figure 5. Key ROESY correlations of **2**–**4**.

H-1/H-2α and 9, H-2α/H-3, H-2β/Me-14, H-3/H-15β, H-9/H-5′, H-1′/H-2′α, H-2′α/H-3′, H-2′β/Me-14′, H-3′/H_2-15′, H-9′/Me-14′, and Me-13′/Me-14′.

Spicachlorantin F (**4**),[13] whose molecular formula of $C_{33}H_{38}O_{11}$ as determined by HRESIMS (*m/z* 633.2309 $[M+Na]^+$), also had a hydroperoxy group as shown by the ^1H NMR spectroscopy. The ^1H and ^{13}C NMR spectral data implied that **4** also possesses a lindenane sesquiterpene dimer moiety closely related to **3**, together with an acetyl group. The presence of an sp^3 methine [δ_H 1.54 (1H, m); δ_C 44.4] instead of an oxygen-bearing quaternary carbon ascribable to C-4′ was revealed by the ^1H and ^{13}C spectroscopies (Tables 1 and 2). The assignment of this methine to C-4′ was established by the ^1H–^1H COSY correlations of H-4 with H-5 and H_2-15. The location of the acetyl group was concluded to be C-15″ from the HMBC correlation of H_2-15 with C-1′ (Fig. 4), and thus establish the planar structure of **4**. The relative configurations were elucidated from the ROESY correlations as shown in Figure 5.

The absolute configurations for **1**, **2**, and **4** were elucidated by the CD spectral analyses, in which the first positive cotton effects [Δε CD +24.3 (259 nm), +18.7 (261 nm), and +27.2 (260 nm), respectively] were similar to those of the related compounds, chloraholides A and B.[5] On the basis of the spectral examinations described above, the absolute stereostructures of **1**, **2**, and **4** were established. However, the absolute configuration for compound **3** still remains to be determined owing to the loss of the sample, since the stability of this compound was lower than the other compounds.

Spicachlorantins C–F were presumed to be derived by the following pathway. Thus, cycloaddition of two lindenane sesquiterpene by Diels–Alder yields a dimeric lindenane sesquiterpene with $\Delta^{4(5)}$ double bond. Reaction of this double bond with O_2 yields a hydroperoxide at 4 position accompanying a migration of $\Delta^{4(5)}$ double bond to $\Delta^{5(6)}$. The hydroperoxy group is then cleaved by a peroxydase to give a dimeric lindenane sesquiterpene with a hydroxy group at C-4.[14,15] Thus, spicachlorantins C–F were considered to be biogenetic precursors of sesquiterpene dimers having a hydroxy group at C-4, and appear to be a new series of lindenane sesquiterpene dimer in *Chloranths* plants.

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9. Spicachlorantin C (**1**) amorphous powder; $[\alpha]_D$ −76.7 (*c* 0.2, MeOH); HRESIMS *m/z* 787.2588, $[M+Na]^+$ (calcd for $C_{40}H_{44}O_{15}Na$, 787.2578); ^1H and ^{13}C NMR: Tables 1 and 2; CD (MeOH; 2.0 × 10^{-5} M, Δε) λ_{max} 330 (−11.7), 259 (+24.3), 224 (−11.4).
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11. Spicachlorantin D (**2**) amorphous powder; $[\alpha]_D$ −46.6 (*c* 0.7, $CHCl_3$); HRESIMS: *m/z* 787.2583, $[M+Na]^+$ (calcd for $C_{40}H_{44}O_{14}Na$, 787.2578); ^1H and ^{13}C NMR: Tables 1 and 2; CD (MeOH; 2.0 × 10^{-5} M, Δε) λ_{max} 335 (−10.7), 261 (+18.7), 228 (−9.1).
12. Spicachlorantin E (**3**) amorphous powder; $[\alpha]_D$ −38.1 (*c* 0.5, MeOH); HRESIMS: *m/z* 731.2670, $[M+Na]^+$ (calcd for $C_{38}H_{44}O_{13}Na$, 731.2680); ^1H and ^{13}C NMR: Tables 1 and 2.
13. Spicachlorantin F (**4**) amorphous powder; $[\alpha]_D$ −103.6 (*c* 0.1, MeOH); HRESIMS: *m/z* 633.2309, $[M+Na]^+$ (calcd for $C_{33}H_{38}O_{11}Na$, 633.2312); ^1H and ^{13}C NMR: Tables 1 and 2; CD (MeOH; 2.0 × 10^{-5} M, Δε) λ_{max} 341 (−4.5), 260 (+27.2), 227 (−1.5).
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