Mono- and Di-sesquiterpenoids from *Chloranthus spicatus*

Yong-Jiang Xu,[†] Chun-Ping Tang,[†] Chang-Qiang Ke,[†] Ji-Bao Zhang,[†] Hans-Christoph Weiss,[‡] Ernst-Rudolf Gesing,[§] and Yang Ye*,[†]

State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu-Chong-Zhi Road, Zhangjiang Hi-Tech Park, Shanghai 201203, People's Republic of China, Bayer Industry Services GmbH & Co. OHG, SUA-SPA, 51368 Leverkusen, Germany, and Bayer CropScience AG, R & D, Research Insecticides, Chemistry Insecticides, Agricultural Center Monheim, D-40789 Monheim, Germany

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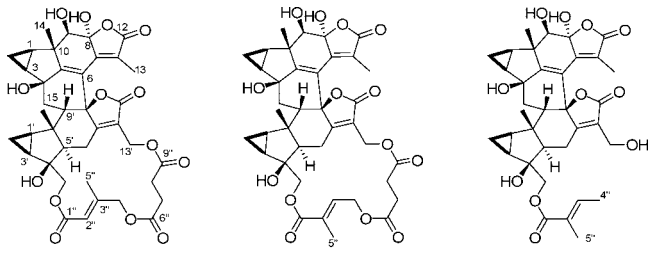
Three new dimeric sesquiterpenoids, chloramultilides B−D (**1**−**3**), along with 10 known sesquiterpenoids, were isolated from the whole plant of *Chloranthus spicatus*. Their structures were established by physical data (1D and 2D NMR, MS). The structure and absolute configuration of **1** was confirmed by X-ray crystallography. Compound **1** exhibited moderate *in vitro* antifungal activity.

Chloranthus spicatus (Thunb.) Makino (Chloranthaceae), distributed mainly in southern China, has long been regarded as a medicinal material to treat aches, trauma, and bleeding. Its roots, known as "zhu-lan-gen" in China, were used externally to cure carbuncle, furuncle, tumefaction, and ringworm.[1] So far, sesquiterpenoids[2–4] and dimeric sesquiterpenoids[5–11] were isolated from the genus *Chloranthus* as their major secondary metabolites. Diterpenoids were reported also from this genus.[12] Previous pharmacological studies revealed that sesquiterpenoid monomers exhibit antifungal activities,[13] and sesquiterpenoid dimers show tumor growth inhibitory activities.[14] Yang et al. reported that sesquiterpenoid dimers exhibit potent and selective inhibition on the delayed rectifier (I_K) K^+current.[15] In our investigation on the whole plant of *C. spicatus*, three new dimeric sesquiterpenoids (**1**–**3**) were isolated and identified, along with 10 known compounds. The new structures were established on the basis of 1D and 2D NMR data, as well as other spectroscopic analyses. The structure and absolute configuration of **1** were confirmed by X-ray crystallography. The structures of known compounds were elucidated by comparison with reported data.



1 **2** **3**

Structures of chloramultilides B-D (**1**-**3**).

Results and Discussion

Chloramultilide B (**1**) was obtained as an amorphous powder. The molecular formula was assigned as $C_{39}H_{42}O_{14}$ by HRESIMS. The IR spectrum displayed absorption bands for hydroxy (3450 cm^{-1}) and ester (1762 cm^{-1}) groups. The ^{13}C NMR spectrum displayed 39 carbon resonances, which were ascribed to five carbonyl, eight olefinic, four methyl, nine methylene, seven methine, and six quaternary carbons (Table 2). The ^1H NMR spectrum exhibited characteristic resonances of one vinylic proton (δ_H 6.09, br s) and four methyl singlets (δ_H 1.09, 1.15, 1.90, and 2.01). The above data revealed that **1** might be a sesquiterpenoid dimer. Analysis of the ^1H and ^{13}C NMR spectra indicated that the NMR data of **1** (Tables 1 and 2) strongly resembled those of chloramultilide A.[11] One major difference between these two compounds was the chemical shift of C-8. In comparison with the corresponding resonance (δ_C 199.3) in chloramultilide A, C-8 in compound **1** shifted upfield to δ_C 104.9, which was indicative of a hemiacetal atom, not a ketone carbon. The C-8 was furthermore connected to C-12 via an oxygen atom to form a five-membered α,β-unsaturated lactone ring fused at C-7 and C-8 by the key HMBC correlations between the allylic methyl (δ_H 2.01) and C-6 (δ_C 122.5), C-7 (δ_C 155.0), C-8, C-11 (δ_C 122.0), and C-12 (δ_C 172.9). The other differences, in comparison with the corresponding resonances of chloramultilide A, were the upfield shift of the olefinic carbon C-2″ (δ_C 114.0) and downfield shift of the olefinic carbon C-3″ (δ_C 152.1), which might be elucidated by the location of Me-5″ at C-3″ instead of C-2″. Thus the planar structure of **1** was established.

The relative configuration of **1** was assigned by a ROESY experiment. The correlations of H-1/H-3, H-1/H-9, H-3/H-9, H-9/H-15α, H-2β/Me-14, H-1′/H-3′, H-2′β/Me-14′, H-1′/H-2′α, H-3′/H-2′α, and H-5′/H-15′α indicated the orientation of H-1 (α), H-3 (α), H-9 (α), Me-14 (β), H-1′ (α), H-3′ (α), H-5′ (α), and Me-14′ (β), respectively (Figure 1). Considering its biogenetic relationship, such elucidation was fully consistent with those of naturally occurring lindenane-type sesquiterpenoids.

An X-ray crystallographic diffraction experiment was carried out to confirm the absolute configuration of **1** (Figure 2). The crystal structure was determined with Cu Kα radiation at 100 K with 99% coverage and an averaged redundancy of 8.9. Refinement of the Flack parameter gave a value of 0.01(15) for the absolute configuration depicted. Additionally, the absolute configuration was checked by a statistical analysis of the Bijvoet pairs implemented in Platon.[16] Finally, a second crystal of the same batch was measured. In all cases the result of the assignment of the absolute configuration could be confirmed.

The formula for the contents of the asymmetric unit of the crystal structure is $C_{39}H_{42}O_{14}$ (for **1**), solvated with a molecule of dimethylformamide (C_3H_7NO) and half a molecule of water; water H atoms could not be located. Consequently, the formula of the asymmetric unit differs from the formula of the molecule. The given molecular weight of 815.82 belongs to the whole asymmetric unit.

Chloramultilide C (**2**) was obtained as an amorphous powder, whose positive ion ($[M + Na]^+$ *m*/*z* 757.2443) in the HRESIMS indicated the same molecular formula as that of **1** ($C_{39}H_{42}O_{14}$). The IR absorption bands at 3428 and 1753 cm^{-1} suggested the presence of hydroxy and ester groups. The ^1H and ^{13}C NMR data of **2** were

* To whom correspondence should be addressed. Tel: +86-21-50806726. Fax: +86-21-50807088. E-mail: yye@mail.shcnc.ac.cn.
[†] Shanghai Institute of Materia Medica, Chinese Academy of Sciences.
[‡] Bayer Industry Services GmbH & Co. OHG.
[§] Bayer CropScience AG.

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Table 1. ^{1}H NMR (300 MHz) Data of Compounds **1**−**3** (pyridine-d_5)

no.	1	2	3
1	2.21, m	2.20, m	1.81, m
2	0.98, m 1.45, m	0.95, m 1.42, m	0.79, m 1.03, m
3	2.05, m	2.00, m	1.79, m
9	4.48, s	4.48, s	3.67, s
13	2.01, s	2.02, s	1.52, s
14	1.09, s	1.09, s	0.80, s
15	2.20, m 3.03, m	2.10, m 3.04, m	1.82, m 2.67, m
1′	1.72, m	1.70, m	1.61, m
2′	0.63, m 1.60, m	0.63, m 1.62, m	0.65, m 1.19, m
3′	1.59, m	1.58, m	1.65, m
5′	2.81, m	2.90, m	2.39, m
6′	3.60, m 3.68, m	3.42, m	2.41, m 2.89, m
9′	3.25, m	3.20, m	2.68, m
13′	4.91, d (11.9) 5.32, d (11.9)	4.93, d (12.0) 5.30, d (12.0)	4.25, m
14′	1.15, s	1.13, s	0.96, s
15′	4.21, d (15.4) 5.10, d (15.4)	4.18, d (11.4) 5.20, d (11.4)	3.98, d (11.1) 4.02, d (11.1)
2″	6.09, br s		
3″		7.02, br s	6.97, m
4″	4.21, d (9.6) 4.81, d (9.6)	4.20, d (7.4) 4.82, d (7.4)	1.89, d*
5″	1.90, s	1.52, s	1.78, s
7″	2.59, m	2.60, m	
8″	2.61, m	2.70, m	

* Overlapped by other resonances.

Table 2. ^{13}C NMR (100 MHz) Data of Compounds **1**−**3** (pyridine-d_5)

no.	1	2	3
1	29.6, d	29.9, d	29.7, d
2	9.9, t	9.9, t	9.9, t
3	31.5, d	31.5, d	31.5, d
4	77.3, s	77.4, s	77.5, s
5	164.2, s	164.3, s	164.4, s
6	122.5, s	122.5, s	123.7, s
7	155.0, s	154.7, s	155.1, s
8	104.9, s	104.7, s	104.7, s
9	79.8, d	80.5, d	86.0, d
10	49.6, s	51.0, s	50.6, *s*
11	122.0, s	122.0, s	124.1, s
12	172.9, s	172.9, s	172.7, s
13	10.8, q	10.9, q	10.8, q
14	14.4, q	12.4, q	14.1, q
15	41.7, t	41.7, t	41.8, t
1′	27.5, d	27.3, d	27.5, d
2′	11.0, t	10.7, t	11.0, t
3′	29.3, d	29.6, d	29.8, d
4′	77.2, s	77.0, s	77.3, s
5′	56.1, d	55.9, d	52.8, d
6′	25.6, t	25.0, t	22.0, t
7′	177.3, s	176.8, s	169.8, s
8′	86.5, s	86.6, s	86.0, s
9′	50.9, d	51.4, d	51.5, d
10′	45.3, s	45.7, s	45.0, s
11′	121.7, s	121.7, s	128.6, s
12′	173.3, s	173.3, s	173.7, s
13′	55.1, t	54.7, t	54.4, t
14′	24.0, q	24.1, q	24.4, q
15′	73.2, t	73.9, t	69.7, t
1″	166.2, s	171.8, s	167.9, s
2″	114.0, d	129.1, s	129.2, s
3″	152.1, s	136.9, d	137.0, d
4″	66.4, t	61.6, t	12.2, q
5″	14.9, q	14.5, q	14.5, q
6″	172.2, s	172.3, s	
7″	29.0, t	29.3, t	
8″	29.3, t	29.5, t	
9″	172.0, s	171.8, s	

similar to those of **1**, except for the upfield shifts of C-3″ ($\Delta\delta_C$ −15.2) and Me-5″ ($\Delta\delta_H$ −0.38), as well as the downfield shifts of C-2″ ($\Delta\delta_C$ 15.1) and the vinylic proton ($\Delta\delta_H$ 0.93) in comparison with compound **1** (Tables 1 and 2). Therefore, Me-5″ was supposed to be located at C-2″ instead of C-3″. This substitution pattern was further supported by the NMR similarity at positions 2″, 3″, and 5″ of **2** and chloramultilide A. Its relative configuration was also assigned by the ROESY spectrum. Accordingly the structure of **2** was established.

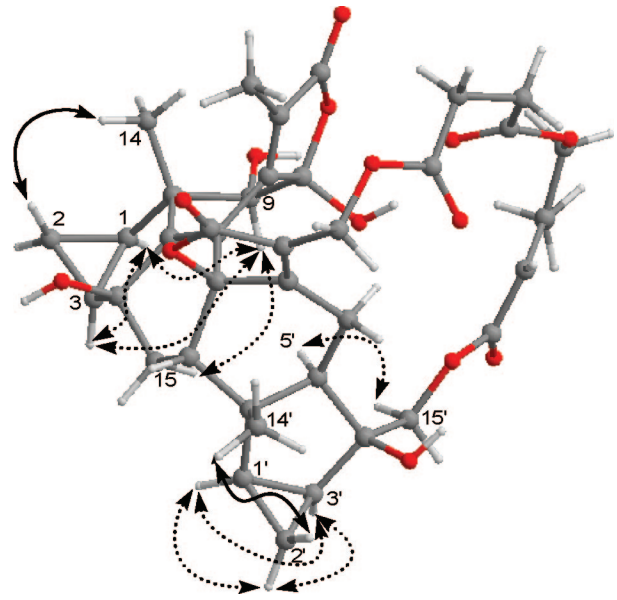

Figure 1. Key ROESY correlations for chloramultilide B (**1**).

Chloramultilide D (**3**) was obtained as an amorphous powder. The molecular formula $C_{35}H_{40}O_{11}$ was established by the HRES-IMS. The IR spectrum similarity of **3** and **1** indicated a close relationship between these two compounds. Compound **3** resembled most NMR resonances of **1**, except for the absence of those of positions 6″, 7″, 8″, and 9″ (Tables 1 and 2). The lack of this succinyl moiety ($C_4H_2O_3$) in **3** was not only suggested by the NMR data but also reflected by its molecular formula. The chemical shift changes at C-4″ (δ_H 1.89, d, 3H; δ_C 12.2) also supported this conclusion. The low-field chemical shift of the vinylic proton (δ_H 6.97) indicated the location of Me-5″ at C-2″, the same substitution pattern as in **2**. The relative configuration of **3** was also elucidated by the ROESY experiment.

Comparison of NMR and MS data with literature values showed that the known compounds were shizukaol E,[8] chloramultilide A,[11] chlorahololide B (**4**),[15] 8-epiasterolid,[17] hydroxyisogermafureno-

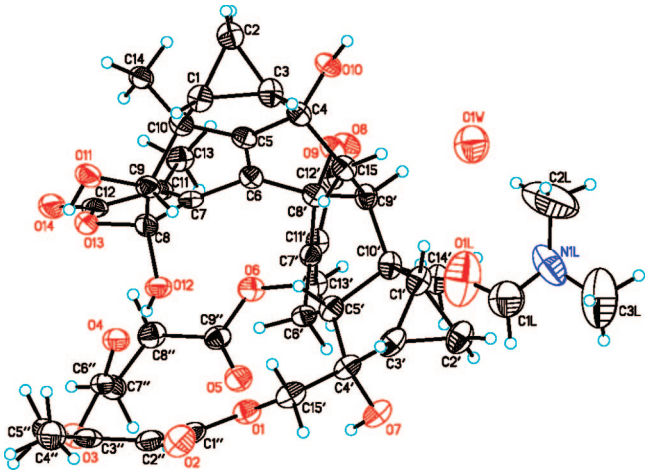

Figure 2. Perspective ORTEP drawing for chloramultilide B (**1**). This drawing contains one molecule of chloramulilide B, one molecule of dimethylformamide solvent, and half a molecule of water.

lide,[18] shizukanolide F,[3] shizukanolide C,[3] chloranoside A,[19] sarcaglaboside A,[20] and sarcaglaboside B.[20]

An antifungal assay of compounds **1**−**4** was conducted with the NCCLS M27-A method.[21] The results revealed that compound **1** showed inhibitory activities against *Candida albicans* and *C. parapsilosis* with MIC values of 0.068 μM. The MIC values of compounds **2**–**4** were more than 0.157 μM. Compounds **1**, **2**, and **3**were also tested for the *in vitro* growth inhibition against human tumor cells P-388 and A-549. These compounds showed no significant inhibitory activity.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241MC polarimeter or a Perkin-Elmer 341 polarimeter. IR spectra were recorded by using a Perkin-Elmer 577 spectrometer. LRESIMS were measured by using a Finnigan LCQ-DECA mass spectrometer. HRESIMS were obtained on a Q-TOF Micro LC-MS-MS spectrometer. NMR spectra were run on a Bruker AM-400 spectrometer or a Bruker AM-600 spectrometer with TMS as internal standard. Column chromatographic separations were carried out by using Si gel H60 (300–400 mesh, Qingdao Haiyang Chemical Group Corporation, People's Republic of China), MCI GEL CHP20P (75–150 μm, Mitsubishi Chemical Industries), and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden) as packing material. HSGF254 Si gel TLC plates (Yantai Chemical Industrial Institute, People's Republic of China) were used for analytical TLC. The analytical HPLC system was composed of a Waters 2690 separations module and a Waters 996 diode array detector (all from Waters, Milford, MA). Preparative HPLC was performed on a Varian SD1 instrument with a 320 single-wave detector. Chromatographic separation was carried out on a C18 column (220 × 25 mm, 10 μm, Merck).

Plant Material. The whole plants of *C. spicatus* were collected in Anshun County, Guizhou Province, People's Republic of China, in October 2004, and identified by Jin-Gui Shen of Shanghai Institute of Materia Medica. A voucher specimen (20041012) was deposited at the herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and Isolation. Air-dried whole plant of *C. spicatus* (10 kg) were ground into powder and extracted with 95% EtOH (20 L × 3) at room temperature, three days each time. After filtration and evaporation of percolate under reduced pressure, the combined EtOH extract (475 g) was suspended in H_2O (1 L), then partitioned successively with EtOAc (1 L × 3) and *n*-BuOH (1 L × 3), affording EtOAc (300 g) and *n*-BuOH (50 g) extracts.

The EtOAc extract was subjected to column chromatography over Si gel (3.0 kg, 120 × 12 cm) with petroleum ether−acetone (from 20:1 to 1:2, then pure acetone, each 5 L) to give 11 fractions, A−K. Fraction B (23 g) was chromatographed on an MCI column (300 g, 80 × 5 cm) and eluted with MeOH−H_2O (MeOH from 30% to 100%, each 1 L) to give nine subfractions (B1−B9). Subfraction B5 (457 mg) was separated by preparative HPLC (RP18 column; CH_3CN−H_2O, from 5% to 95% in 30 min; flow 3 mL/min; 254 nm) to afford 8-epiasterolid (29 mg) and hydroxyisogermafurenolide (38 mg). Subfraction B6 afforded shizukanolide C (66 mg). Fraction C (20 g) was chromatographed over an MCI column (300 g, 80 × 5 cm) and eluted with MeOH−H_2O (from 30% to 100%, each 1 L) to give five subfractions (C1−C5). Fraction C3 (1.7 g) was separated by preparative HPLC (RP18 column; CH_3CN−H_2O, from 15% to 85% in 30 min; flow 3 mL/min; 254 nm) followed by preparative TLC to afford shizukaol E (50 mg). From subfraction C5, shizukanolide F (44 mg) was isolated by preparative TLC. Fraction H (40 g) was subjected to Si gel CC (1.0 kg, 100 × 12 cm) and eluted with $CHCl_3$−MeOH (100:1–10:1, each 2 L) to give eight subfractions (H1−H8). Subfraction H2 (4.4 g) was rechromatographed on Si gel (80 g, 50 × 4 cm) and eluted with $CHCl_3$−MeOH (60:1–10:1, each 500 mL) to give four fractions (H2A−H2D). Chlorahololide B (392 mg) was purified by CC over Sephadex LH-20 (150 × 2 cm) from fraction H2A. Chloramultilide A was purified by CC over Sephadex LH-20 (150 × 2 cm) from fraction H2B. A mixed crystal (2.0 g) was obtained from subfraction H4 (15 g) in petroleum ether−acetone. The mixed crystal was then separated by preparative HPLC (RP18 column; CH_3CN−H_2O, from 25% to 80% in 30 min; flow 3 mL/min; 254 nm), yielding compounds **1** and **2**. Fraction H7 (4.5 g) was subjected to Si gel CC (80 g, 50 × 5 cm) using $CHCl_3$−MeOH (50:1, 500 mL) as eluent to give **3** (852 mg). The *n*-BuOH extract was submitted to CC over macroporous resin (2.0 kg, 120 × 12 cm) using MeOH−H_2O (0%, 30%, 60%, and 90%, each 2 L) as eluent to obtain four fractions. The 60% MeOH fraction (2.53 g) was again subjected to an MCI column (80 g, 50 × 4 cm), eluting with MeOH−H_2O (20%, 40%, 60%, 80%, and 100%, each 500 mL) to yield five subfractions. By preparative HPLC (RP18 column; CH_3CN−H_2O, from 10/ to 60% in 30 min; flow 3 mL/min; 254 nm), sarcaglabosides A (121 mg) and B (87 mg) were separated from the 40% subfraction, and chloranoside A (31 mg) was obtained from the 60% subfraction.

Chloramultilide B (1): amorphous powder; $[\alpha]^{20}_D$ −91.9 (*c* 0.037, MeOH); IR (KBr) ν_{max} 3450, 2939, 1762, 1403, 1228, 1162, 1012, 968 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; ESIMS *m/z* 733 [M − H]$^+$, 757 [M + Na]$^+$; HRESIMS *m/z* 757.2510 (calcd for $C_{39}H_{42}O_{14}Na$, 757.2472).

Chloramultilide C (2): amorphous powder; $[\alpha]^{20}_D$ −31 (*c* 0.087, MeOH); IR (KBr) ν_{max} 3428, 2937, 1753, 1384, 1263, 1151, 1008, 972 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; ESIMS *m/z* 733 [M − H]$^+$, 757 [M + Na]$^+$; HRESIMS *m/z* 757.2443 (calcd for $C_{39}H_{42}O_{14}Na$, 757.2472).

Chloramultilide D (3): amorphous powder; $[\alpha]^{20}_D$ −24 (*c* 0.054, MeOH); IR (KBr) ν_{max} 3426, 2939, 1751, 1646, 1382, 1269, 1149, 1269, 1012, 746 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; ESIMS *m/z* 635 [M − H]$^+$, 659 [M + Na]$^+$; HRESIMS *m/z* 659.2482 (calcd for $C_{35}H_{40}O_{11}Na$, 659.2468).

X-Ray Crystallographic Data for Compound 1. $C_{39}H_{42}O_{14}\cdot C_3H_7NO\cdot 0.5H_2O$, MW 815.82, orthorhombic space group $P2_12_12_1$, a = 9.4871(4) Å, b = 17.1663(8) Å, c = 23.9638(12) Å, V = 3902.7(3) Å^3, Z = 4, d = 1.388 g/cm^3. $F(000)$ = 1728, μ = 0.891 mm^{-1}. A single crystal of dimensions 0.30 × 0.10 × 0.08 mm was used for X-ray measurements. The data collection was performed on a Gemini R Ultra diffractometer using Cu Kα radiation. Data were collected up to θ = 65.62° at 100 K. A total of 33 916 reflections, of which 6562 independent reflections were measured having an R_{int} of 0.036. Programs used: Data collection and reduction Crysalis Version 1.171.35 (Oxford Diffraction 2006). Crystal structure solution and refinement were achieved using direct methods as implemented in SHELXTL Version 6.12 (Sheldrick, University of Gottingen (Germany), 2000) and visualized using XP program. A total of 539 parameters were refined using 5897 reflections with $F_o > 4\sigma(F_o)$ giving R1 = 0.0393, wR2 = 0.0953, goodness of fit 1.057, remaining difference electron density 0.324 and −0.249 e$^-$ Å^{-3}. The absolute structure could be determined properly giving a Flack parameter 0.01 (15). CCDC 631433 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road; Cambridge CB2 1EZ, UK; fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk).

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Supporting Information Available: ^{13}C and ^{1}H NMR and ROESY spectra for chloramultilides B−D (**1**−**3**) are available free of charge via the Internet at http://pubs.acs.org.

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