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Abietane Diterpenes from *Clerodendron cyrtophyllum*

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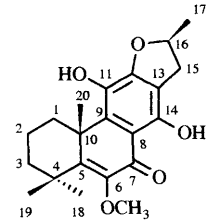
Two novel abietane derivatives, cyrtophyllones A (1) and B (2), together with six known compounds, teuvincenone F (3), uncinatone (4), sugiol (5), friedelin (6), clerodolone (7), stigmasta-5,22,25-trien-3β-ol (8) and clerosterol (9), were isolated from the stem of *Clerodendron cyrtophyllum*. One of the abietane diterpenes, cyrtophyllone A, possesses a rearranged-abietane skeleton which contains a 17(15-16)-abeo-abietane framework. The stereo-structure proposed for 1 was characterized as 16(*S*)-12,16-epoxy-11,14-dihydroxy-6-methoxy-17(15-16)-abeo-abieta-5,8,11,13-tetraen-7-one on the basis of spectral analysis (UV, IR, MS, two dimensional (2D) NMR and CD) and X-ray diffraction. The structure of 2 was also characterized as (+)-11,12,16-trihydroxy-abieta-8,11,13-trien-7-one by a combination of spectroscopic comparison with sugiol.

Keywords *Clerodendron cyrtophyllum*; rearranged-abietane; Verbenaceae; cyrtophyllone A; cyrtophyllone B

Clerodendron cyrtophyllum TURZ. (Verbenaceae) is a famous folk remedy which is called "Da qing" (大青) in traditional Chinese medicine.[1] It possesses a good reputation in the treatment of various human disorders such as the common cold, malaria and various infections. Previous chemical research on this plant has resulted in the separation of cyrtophyllin[2] and β-sitosterol.[3] In the present paper, the structures of two novel abietane diterpenes isolated from the stem of *C. cyrtophyllum*, cyrtophyllones A (1) and B (2), are described.



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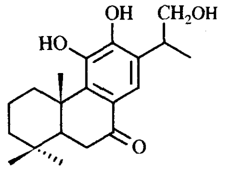

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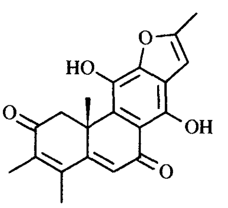

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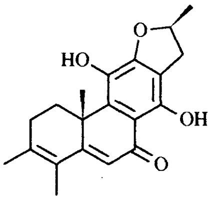

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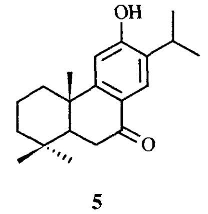

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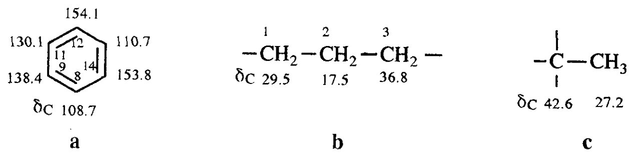
Results and Discussion

Cyrtophyllone A (1) was obtained as yellowish rectangles, mp 204—206 °C, from $CHCl_3$. The molecular formula $C_{21}H_{26}O_5$ (M^+ m/z: 358.1782, Calcd 358.1781) was determined by high-resolution mass spectrometry (HRMS). The absorption bands in ultra-violet (UV) spectrum (218, 267, 320 nm) exhibited the presence of a benzene ring and unsaturated ketone, and the infrared (IR) spectrum showed absorption bands (3300, 1680, 1670, 1597 cm^{-1}) which correspond to phenolic hydroxyl groups, α,β-unsaturated ketone and benzene moieties, respectively. In the proton nuclear magnetic resonance (^1H-NMR) spectrum including H–H shift correlated spectrometry (COSY), the presence of a methoxyl group [δ 3.83] (s)], three tertiary methyl groups [δ 1.67, 1.41, and 1.40 (each in singlet)] and a CH_2–$CH(CH_3)$–O moiety [δ 5.12 (ddq), 3.38 (dd), 2.87 (dd), and 1.51 (d)] was supported. Taking into account the carbon nuclear magnetic resonance (^{13}C-NMR) spectral data (Table II), some partial structures such as **a**, **b** and **c** (Fig. 1) were predicated. With the correlation spectroscopy *via* long-range coupling (COLOC) spectrum of 1 (Table III), a connection among the partial structures was established. The geminal methyl singlets (H-18 and H-19) were correlated with C-3, C-4 and C-5, and the methyl proton signal at 1.67 (H-20) was correlated with C-1, C-5 and C-9, respectively, which indicated the presence of a decaline structure in 1. With the correlations between a hydroxyl group at C-14 and C-8, C-13, C-14, between the methylene protons (H-15) (δ 2.87 and 3.38) and C-13, and between a secondary methyl proton (H-17) (δ 1.51) and C-15, a rearranged-abietane skeleton was characterized, which was supported by the COLOC spectrum. No aromatic proton signal can be observed in the ^1H-NMR spectrum, which means the benzene ring was fully substituted. Considering that the singlet at δ 13.41 disappeared after the addition of D_2O,



a b c

Fig. 1

Table I. ^{1}H-NMR Spectral Data of Compounds **1**, **2**, **4** and **5**

H	**1**	**2**	**4**	**5**
Chemical shift				
1β-H	3.08 (m)	3.23 (dt)	3.23 (m)	3.21 (dt)
1α-H	1.5—1.7$^{a)}$	1.43 (dt)	1.57 (m)	1.4—1.8$^{a)}$
2β-H	1.85 (m)	1.70 (m)	2.51 (m)$^{b)}$	1.4—1.8$^{a)}$
2α-H	1.5—1.7$^{a)}$	1.52 (m)	2.21 (m)$^{b)}$	1.4—1.8$^{a)}$
3β-H	2.02 (m)	1.36 (td)		1.4—1.8$^{a)}$
3α-H	1.3—1.5$^{a)}$	1.21 (td)		1.4—1.8$^{a)}$
5α-H		1.80 (dd)	6.22 (s)	1.85 (dd)
6β-H		2.48 (dd)		2.59 (dd)
6α-H		2.56 (dd)		2.68 (dd)
11-H				6.96 (s)
14-H		7.43 (s)		7.91 (s)
15β-H	2.87 (dd)		2.89 (dd)	
15α-H	3.38 (dd)	3.12 (m)	3.40 (dd)	3.13 (sept)
16β-H		4.00 (dd)		
16α-H	5.12 (ddq)	3.74 (dd)	5.12 (ddq)	
16-H_3				1.27 (d)
17-H_3	1.51 (d)	1.30 (d)	1.52 (d)	1.25 (d)
18-H_3	1.41 (s)	0.88 (s)	1.91 (s)	0.92 (s)
19-H_3	1.40 (s)	0.91 (s)	1.88 (s)	0.99 (s)
20-H_3	1.67 (s)	1.33 (s)	1.50 (s)	1.22 (s)
6-OCH_3	3.83 (s)			
11-OH	4.74 (sh)		4.88 (sh)	
12-OH		6.18 (sh)		5.33 (br s)
14-OH	13.41 (sh)		13.73 (sh)	
Coupling constant (*J*)				
1α,1β		13.4	13.2	13.2
3α,3β		13.4		
5α,6α		2.0—2.4		3.6—4.1
5α,6β		14.4—14.8		13.3—14.0
6α,6β		14.5		18.03
15α,18β	15.2		15.15	
15α,16α	8.8	7.4	8.8	
15β,16α	7.3		7.4	
16β,17	5.8		6.4	
15α,16β		2.2		
16α,16β		9.4		
15α,17		7.3		7.0

Tetramethylsilane (TMS) as internal standard, ppm, *J* is shown in Hz. *a*) Overlapping, *b*) assignment may be interchanged.

Table II. ^{13}C-NMR Spectral Data of Compounds **1**, **2** and **4**

C	**1**	**2**	**4**
1	29.5	35.9	29.2
2	17.5	18.4	30.3
3	36.8	40.7	140.4
4	37.1	32.7	125.3
5	159.0	50.5	165.3
6	147.7	34.9	118.5
7	185.6	196.9	190.1
8	108.7	123.8	109.3
9	138.4	138.9	136.2
10	42.6	39.6	39.6
11	130.1	142.7	131.0
12	154.1	148.7	153.8
13	110.7	129.4	111.0
14	153.8	117.7	154.2
15	34.4	35.6	34.5
16	83.1	66.1	83.0
17	22.0	16.3	22.1
18	29.3	32.8	20.7
19	29.0	21.2	15.0
20	27.2	17.5	22.0
21	60.0		

Assignment was confirmed by the aid of DEPT (distortionless enhancement by polarization transfer), ^{1}H–^{13}C COSY and COLOC. TMS as internal standard.

Table III. Long Range ^{1}H–^{13}C COSY of Compounds **1** and **2**

	1	**2**
1	2, 20	2
2		3
3	18, 19	
4	18, 19	2, 5, 18, 19
5	18, 19, 20	1, 3, 6, 18, 19, 20
6	6-OMe	5
7		5, 6, 14
8	14-OH	6
9	11-OH, 20	1, 14, 20
10	20	2, 5, 6, 20
11	11-OH	5
12		14
13	14-OH, 15	17
14	14-OH	
15	17	14
16	17	
18		3, 5
19		3, 5
20	1	1, 5

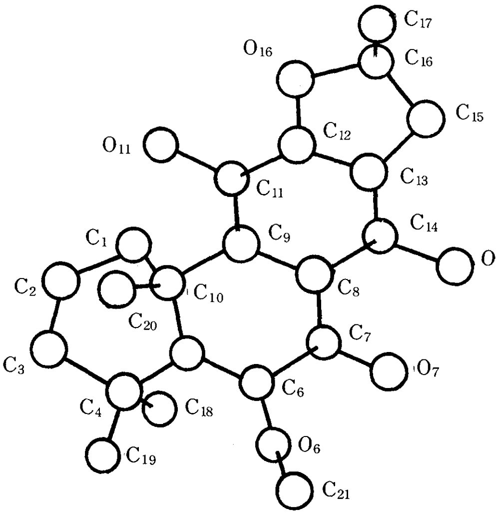


Fig. 2. The Computer Drawing of Compound **1**

the formation of an intermolecular hydrogen bond between the carbonyl at C-7 and the phenolic hydroxyl group at C-14 was proposed. The position of a methoxyl group was characterized at C-6 by a nuclear Overhauser effect (NOE) experiment. On irradiation of the singlet at δ 3.81, a 4.8% NOE enhancement of the 18-methyl group at δ 1.41 (s) was observed. Therefore, the structure of cyrtophyllone A was deduced to be 12,16-epoxy-11,14-dihydroxy-6-methoxy-17(15-16)-abeo-abieta-5,8,11,13-tetraen-7-one. The X-ray crystallographic analysis of cyrtophyllone A provided the stereochemistry of the structure of **1** as well as its structural confirmation. A computer generated drawing and the related data are shown in Fig. 2 and Tables IV, V.

As the circular dichroism (CD) curve of cyrtophyllone A showed a negative absorption at 318 nm (the n–π* transition of a carbonyl group), the β-orientation of the methyl group at C-10 was indicated. The positive Cotton signal at 264 nm also supported the above indication according to the rule for π–π* transition of an α,β-unsaturated ketone. Thus, the absolute structure of **1** was established to be 16(*S*)-12,16-epoxy-11,14-dihydroxy-6-methoxy-17(15-16)-abeo-abieta-5,8,11,13-tetraen-7-

Table IV. Bond Length (Å) of Cyrtophyllone A (**1**)

$C_{(1)}$–$C_{(2)}$	1.508	$C_{(2)}$–$C_{(3)}$	1.495
$C_{(3)}$–$C_{(4)}$	1.545	$C_{(4)}$–$C_{(19)}$	1.534
$C_{(4)}$–$C_{(18)}$	1.533	$C_{(5)}$–$C_{(6)}$	1.349
$C_{(5)}$–$C_{(10)}$	1.527	$C_{(6)}$–$C_{(7)}$	1.463
$C_{(6)}$–$O_{(6)}$	1.385	$C_{(7)}$–$C_{(8)}$	1.459
$C_{(7)}$–$O_{(7)}$	1.258	$C_{(8)}$–$C_{(9)}$	1.415
$C_{(8)}$–$C_{(14)}$	1.410	$C_{(9)}$–$C_{(10)}$	1.535
$C_{(9)}$–$C_{(11)}$	1.397	$C_{(10)}$–$C_{(20)}$	1.547
$C_{(14)}$–$C_{(13)}$	1.364	$C_{(14)}$–$O_{(14)}$	1.375
$C_{(13)}$–$C_{(12)}$	1.384	$C_{(13)}$–$C_{(15)}$	1.497
$C_{(12)}$–$C_{(11)}$	1.383	$C_{(12)}$–$O_{(16)}$	1.356
$C_{(11)}$–$O_{(11)}$	1.380	$C_{(15)}$–$C_{(16)}$	1.486
$C_{(16)}$–$C_{(17)}$	1.378	$C_{(16)}$–$O_{(16)}$	1.432
$C_{(21)}$–$O_{(6)}$	1.442		

Table V. Bond Angles (°) of Cyrtophyllone A (**1**)

$C_{(1)}$–$C_{(2)}$–$C_{(3)}$	115.2	$C_{(2)}$–$C_{(3)}$–$C_{(4)}$	115.5
$C_{(3)}$–$C_{(4)}$–$C_{(19)}$	107.9	$C_{(3)}$–$C_{(4)}$–$C_{(18)}$	108.0
$C_{(19)}$–$C_{(4)}$–$C_{(18)}$	109.5	$C_{(6)}$–$C_{(5)}$–$C_{(10)}$	120.9
$C_{(5)}$–$C_{(6)}$–$C_{(7)}$	123.1	$C_{(5)}$–$C_{(6)}$–$O_{(6)}$	121.1
$C_{(7)}$–$C_{(6)}$–$O_{(6)}$	115.1	$C_{(6)}$–$C_{(7)}$–$C_{(8)}$	119.4
$C_{(6)}$–$C_{(7)}$–$O_{(7)}$	120.0	$C_{(8)}$–$C_{(7)}$–$O_{(7)}$	120.5
$C_{(7)}$–$C_{(8)}$–$C_{(9)}$	119.6	$C_{(7)}$–$C_{(8)}$–$C_{(14)}$	119.2
$C_{(9)}$–$C_{(8)}$–$C_{(14)}$	121.2	$C_{(8)}$–$C_{(9)}$–$C_{(10)}$	121.6
$C_{(8)}$–$C_{(9)}$–$C_{(11)}$	118.3	$C_{(10)}$–$C_{(9)}$–$C_{(11)}$	120.1
$C_{(5)}$–$C_{(10)}$–$C_{(9)}$	114.5	$C_{(5)}$–$C_{(10)}$–$C_{(20)}$	107.5
$C_{(9)}$–$C_{(10)}$–$C_{(20)}$	105.8	$C_{(8)}$–$C_{(14)}$–$C_{(13)}$	119.8
$C_{(8)}$–$C_{(14)}$–$O_{(14)}$	120.9	$C_{(13)}$–$C_{(14)}$–$C_{(13)}$	119.3
$C_{(14)}$–$C_{(13)}$–$C_{(12)}$	118.3	$C_{(14)}$–$C_{(13)}$–$C_{(15)}$	133.7
$C_{(12)}$–$C_{(13)}$–$C_{(15)}$	107.9	$C_{(13)}$–$C_{(12)}$–$C_{(11)}$	124.1
$C_{(13)}$–$C_{(12)}$–$O_{(16)}$	112.3	$C_{(11)}$–$C_{(12)}$–$O_{(16)}$	123.1
$C_{(9)}$–$C_{(11)}$–$C_{(12)}$	118.2	$C_{(9)}$–$C_{(11)}$–$O_{(11)}$	122.5
$C_{(12)}$–$C_{(11)}$–$O_{(11)}$	119.2	$C_{(13)}$–$C_{(15)}$–$O_{(16)}$	102.8
$C_{(15)}$–$C_{(16)}$–$C_{(17)}$	118.9	$C_{(15)}$–$C_{(16)}$–$O_{(16)}$	109.0
$C_{(17)}$–$C_{(16)}$–$O_{(16)}$	111.1	$C_{(6)}$–$O_{(6)}$–$C_{(21)}$	114.6
$C_{(12)}$–$O_{(16)}$–$C_{(16)}$	107.7		

one.

Cyrtophyllone B (**2**), colorless crystals from $CHCl_3$ (mp 249—251 °C, $C_{20}H_{28}O_4$). IR spectrum (3500, 3200, 1660, 1600, 1560 cm^{-1}) showed the presence of a hydroxyl group, a phenolic hydroxyl group on unsaturated carbonyl, and a benzene ring. The UV data (218, 238, 285 nm) also supported the above elucidation. From the ^1H-NMR spectrum, three tertiary methyl groups (δ 1.33, 0.91 and 0.88) and a $CH(CH_3)$–CH_2–OH structure [δ 4.00 (dd), 3.74 (dd), 3.12 (m), 1.30 (d)] were proposed. The ^1H- and ^{13}C-NMR spectral data (Tables I and II) revealed that cyrtophyllone B possessed an abietane skeleton. An ABX system [δ 2.48 (dd), 2.56 (dd), 1.80 (dd)] in the ^1H-NMR spectrum exhibited a carbonyl group at C-7. The downfield shift of an aromatic proton signal at δ 7.43 (s) showed that an aromatic proton was located at C-14, which indicated that phenolic hydroxyl groups were located at both C-11 and C-12, respectively. The COLOC data (Table III) showed that H-5 correlated with C-4, C-6, C-7, C-10 and H-6 with C-5, C-7, C-8, which supported the presence of a decaline moiety and a carbonyl group at C-7. Taking into account the correlation between H-16, H-17 and C-13, the abietane skeleton and a hydroxyl group at C-16 were determined. As the CD curve of **2** was identical with that of sugiol (**5**)[4,5] the configurations of C-5 and C-10 were determined to be *S* and *S*, respectively. The structure of cyrtophyllone B was then concluded to be (+)-11,12,16-trihydroxy-abieta-8,11,13-trien-7-one. The stereochemistry at C-15 remained unclarified.

The structures of other known compounds isolated in the present experiment were charactrized as teuvincenone F (**3**),[6] uncinatone (**4**),[7] sugiol (**5**),[4,5] friedelin (**6**),[8] clerodolone (**7**),[9,10] stigmasta-5,22,25-trien-3β-ol (**8**)[11] and clerosterol (**9**),[9,10] respecively, by means of spectral data.

Experimental

Plant Material *Clerodendron cyrtophyllum* was collected in Jiangxi province, China. The voucher speciemen was deposited in the Herbarium of China Pharmaceutical University.

Extraction and Isolation The naturally dried and pulverized whole plant mateial of *C. cyrtophyllum* (10 kg) was extracted with hot EtOH three times. The solvent was recovered *in vacuo* to yield a crude extract (580 g). A certain volume of water was added to the crude extract and the mixture was agitated thoroughly to form a suspension, which was extracted with $CHCl_3$. After evaporation, the $CHCl_3$ extract (100 g) was subjected to column chromatography on slica gel eluted with an EtOAc–petroleum ether gradient to give cyrtophyllones A (**1**) (11 mg) and B (**2**) (800 mg), teuvincenone F (**3**)[6] (39 mg), uncinatone (**4**)[7] (49 mg), sugiol (**5**)[4,5] (39 mg), friedelin (**6**)[8] (66 mg), clerodolone (**7**)[9,10] (68 mg), stigma-5,22,25-trien-3β-ol (**8**)[11] (92 mg), and clerosterol (**9**)[9,10] (18 mg), respectively.

Cyrtophyllone A (1) Yellowish rectangles, mp 204—206 °C ($CHCl_3$). HRMS M^+ *m/z*: 358.1782 (Calcd 358.1781 for $C_{21}H_{26}O_5$). CD (MeOH, $c=0.000165$) nm ($\Delta\varepsilon$): 205 (0), 215 (+6.28), 234 (+2.20), 264 (+7.56), 296 (0), 318 (−1.50), 355 (0). UV λ_{max} (log ε): 218 (3.83), 267 (3.78), 320 (3.47). IR ν_{max} cm^{-1}: 3300, 2910, 1680, 1670, 1597, 1590, 1460, 1395, 1328, 1225. EIMS (direct inlet) *m/z* (rel. int.): 358 [M^+] (54), 343 [$M-CH_3$] (100), 289 (27), 288 (56), 276 (38), 275 (33), 273 (12), 83 (10).

X-Ray Structure Determination of 1 An orthorhombic system was determined in space group $P2_1\ 2_1\ 2_1$, with $a=9.877$ (4), $b=13.327$ (5), $c=14.096(7)$ Å, $V=1883.82(1.38)$ Å^3, $Z=4$, and $D_C=1.270$ g/cm^{-3}. Intensity data were collected in the range of $2° < 2\theta < 45°$ by an *R* 3 m/*E* four-circle diffractometer. MoK_α radiation (graphite-monochromated) was performed, and 1433 independent reflections were recorded; 1244 with [$I > 30\sigma(I)$] were considered from among those observed and were used for the structure determination and refinement. The structure was solved by direct methods with the SHELXS-86 program; 20 atoms were located from the coordinates of all carbon and oxygen atoms. The hydrogen atoms were included at calculated positions, $R=0.0604$.

Cyrtophyllone B (2) Colorless needles, mp 249—251 °C ($CHCl_3$). $[\alpha]_D=17.1°$ ($c=1.46$). CD (MeOH, $c=0.000140$) nm ($\Delta\varepsilon$): 212 (+9.3), 227 (0), 237 (−3.2), 255 (−0.4), 290 (−2.2), 304 (0), 318 (+2.7), 358 (0). EIMS (direct inlet) *m/z* (rel. int.): 322 [M^+] (100), 317 [$M-CH_3$] (17), 314 ($M-H_2O$] (27), 301 (80), 257 (45), 231 (27), 229 (37), 203 (24), 179 (51), 177 (32). UV λ_{max} nm (log ε): 218 (3.86), 238 (3.78), 285 (3.67). IR ν_{max} cm^{-1}: 3500, 3200, 2910, 2850, 1660, 1600, 1560, 1458, 1360, 1305, 1280.

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