An Unusual Stress Metabolite Induced by $CuCl_2$ and Other Constituents from the Leaves of *Chloranthus anhuiensis*

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A 6-eremophilene derivative **2** with an unusual pendant was produced as phytoalexin in the fresh leaves of *Chloranthus anhuiensis* K. F. Wu in response to abiotic stress elicitation by $CuCl_2$. The phytoalexin was characterized as a sesquiterpene with a (2,6-dimethoxy-4-hydroxy)benzyl moiety pendant. Two new sesquiterpenes, (3*R*,4*S*,5*R*,10*S*,11*S*)-3-hydroxy-8-oxo-6-eremophilen-12-oic acid (**1**) and (3*R*,4*S*,5*R*,6*R*,8*R*,10*S*)-3,6,8-trihydroxy-7(11)-eremophilen-12,8-olide (**3**), one new sesquiterpene glycoside, anhuienoside A (**4**), one new caffeoyl phenylethanoid diglycoside with an unusual cyclic structure, anhuienoside B (**5**), and one new tyramine derivative, *N*-acetyltyramine 1-*O*-*β*-D-glucoside (**6**), and three known compounds were also isolated. Their structures and relative configurations were established by spectroscopic means. The absolute configurations of **1**−**3** were defined mainly by comparison of quantum chemical TDDFT calculated and experimental ECD spectra.

Species of the genus *Chloranthus* are known to be rich in sesquiterpenes of the lindenane, germacrane, eudesmane-type including sesquiterpene dimers, trimers, and diterpenes.[1−20] Recently, we found a new sesquiterpene skeleton named chloranthane and several new sesquiterpenoid monomers and dimers from this genus.[1−6] Previous phytochemical investigations of *Chloranthus anhuiensis* K. F. Wu have shown the presence of sesquiterpenes and diterpenes.[21] In this study, a 6-eremophilene derivative with an unusual pendant was produced as phytoalexin in the fresh leaves of *C. anhuiensis* K. F. Wu in response to abiotic stress elicitation by $CuCl_2$.

Application of the abiotic stress agent $CuCl_2$ to the leaves of *C. anhuiensis* resulted in the production of an additional spot in the extract of the treated plants in comparison with that of the corresponding control extract on the TLC plates. The new compound, anhuienol (**2**), produced in response to abiotic stress treatment, and five new compounds (3*R*,4*S*,5*R*,10*S*,11*S*)-3-hydroxy-8-oxo-6-eremophilen-12-oic acid (**1**), (3*R*,4*S*,5*R*,6R,8*R*,10*S*)-3,6,8-trihydroxy-7(11)-eremophilen-12,8-olide (**3**), anhuienoside A (**4**), anhuienoside B (**5**), and *N*-acetyltyramine 1-*O*-*β*-D-glucoside (**6**), and known compounds 3α,6α-dihydroxy-8α*H*-7(11)-eremophilen-12,8-olide (**7**),[22] 6αH,8αH-7(11)-eremophilen-12,8:15,6-diolide (**8**),[23] and 3,4,5-trimethoxybenzaldehyde (**9**) were separated by preparative TLC and purified by Sephadex LH-20 column chromatography (Chart 1).

Results and Discussion. Compound **1** was isolated as a yellow oil. The molecular formula was determined to be $C_{15}H_{22}O_4$ by analysis of the HR-FTICRMS ion peak at *m*/*z* 265.1445 $[M - H]^-$ (calcd 265.1433). The IR spectrum suggested the presence of a hydroxy (3435 cm^{-1}) and a saturated carboxylic acid (1718 cm^{-1}) group. ^1H and ^{13}C NMR spectra showed signals in close agreement with those of the known sesquiterpene, 3α-hydroxy-8-oxo-6-eremophilen-12-oic acid methyl ester,[24] except that the methoxycarbonyl group was replaced by the carboxylic acid group. Analysis of the 1D and 2D NMR data and comparing with those of 3α-hydroxy-8-oxo-6-eremophilen-12-oic acid methyl ester led to identification of the structure of **1** as 3α-hydroxy-8-oxo-6-eremophilen-12-oic acid.[24] The relative configuration of **1** was proved to be the same as 3α-hydroxy-8-oxo-6-eremophilen-12-oic acid methyl ester after detailed analysis of the NOESY spectrum of **1**.[24] The assignment of NMR signals of **1** was listed in Table 1.

Anhuienol (**2**) was obtained as a yellow gum. The HR-FTICRMS exhibited an ion peak at *m*/*z* 439.2100 $[M + Na]^+$ (calcd 439.2091), indicating that the molecular formula was $C_{24}H_{32}O_6$ with nine degrees of unsaturation. The IR spectrum revealed the presence of hydroxy groups, a saturated ketone, a conjugated ketone, and an aromatic ring characterized by absorptions at ν_{max} 3420, 1707, 1665, and 1612 cm^{-1}, respectively. The phenolic nature of the compound was indicated by its characteristic color reactions ($FeCl_3$, purple; phosphomolybdic acid reagent, deep blue). The NMR data (Table 2) in the upfield region of **2** were similar to those of the eremophilane-type sesquiterpene, 3α-hydroxy-8-oxo-6-eremophilen-12-oic acid (**1**), isolated from the same plant, except that deshielded methylene protons [δ_H 3.60 (d, $J = 16.5$ Hz) and 3.52 (d, $J = 16.5$ Hz), δ_C 47.6 (t, C-1′)] were added in **2**. In the downfield region of the NMR spectra of **2**, two equivalent aromatic protons at δ_H 6.36 (s, H-3′, H-7′), four aromatic carbon at δ_C 125.2 (s, C-2′), 107.5 (d, C-3′, C-7′), 148.2 (s, C-4′, C-6′), and 136.6 (s, C-5′), and a carbonyl carbon at δ_C 208.3 (s, C-13) were added, whereas the C-13 carboxylic acid carbon signal at δ_C 175.8 (s) was missing (Table 1) when compared with those of compound **1**. These differences suggested that C-13 was substituted in **2**. The carbonyl carbon at δ_C 208.3 (s) was attributed to C-13 from the observation of long-range correlations from the proton signals at δ_H 3.73 (m, H-11) and 1.04 (d, $J = 7.0$ Hz, H-12) to the carbon signal at δ_C 208.3 (s). The structure of the aromatic unit at C-13 was proved to be a 4-hydroxy-3,5-dimethoxyphenyl moiety, attached to C-13 via a methylene bridge from analysis of the HMBC cross peaks from H_2-1′ to C-2′, C-3′, C-7′, and carbonyl C-13, and HMBC cross peaks from OCH_3 to C-4′, C-6′. The relative configuration of **2** was deduced to be the same as in **1** and the known compound, 3α-hydroxy-8-oxo-6-eremophilen-12-oic acid methyl ester.[24] The NOESY cross peak of Me-14α/H-9α, Me-14α/H-10, H-6 and H-3*β*, and H-6 and H-4*β* (Figure 1) implied a *cis*-eremophilane.[24] The hydroxy group at C-3 was α-oriented as shown by the NOESY cross peak between H-6 and H-3*β* observed in the NOESY experiment.[24] The new 6-eremophilene derivative was given the common name anhuienol.

Compound **3** was obtained as a yellowish oil. The HR-FTICRMS exhibited a molecular ion peak at *m*/*z* 281.1383 $[M - H]^-$ (calcd 281.1394), corresponding to the molecular formula $C_{15}H_{22}O_5$. The IR spectrum exhibited a broad OH absorption band at 3416 and an

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Chart 1

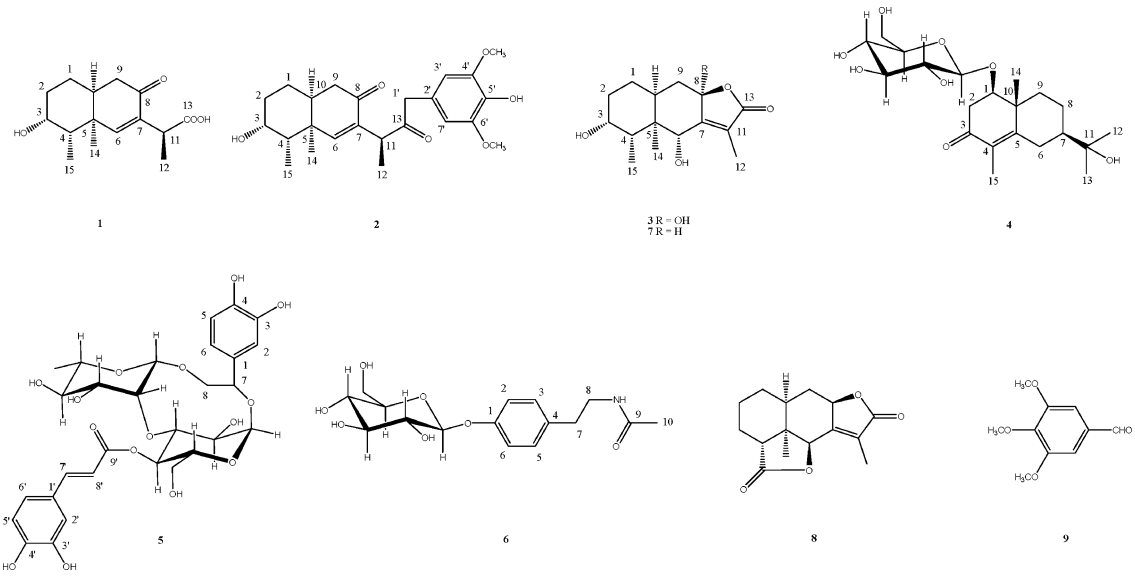

Table 1. NMR Data (500 MHz) for Compounds **1**, **3**, and **4** in DMSO-d_6

	1		**3**		**4**	
position	δ_C,[a,b] mult	δ_H,[c] mult (*J* in Hz)	δ_C,[a,b] mult	δ_H,[c] mult (*J* in Hz)	δ_C,[a,b] mult	δ_H,[c] mult (*J* in Hz)
1	26.9, CH_2	1,50, m, 1.27, m	27.4, CH_2	2.04, m, 1.36, overlap	79.1, CH	3.78, dd (13.0, 4.5)
2	28.1, CH_2	1.36, m	29.3, CH_2	1.47, m, 1.36, overlap	39.5, CH_2	2.61, dd (16.5, 4.5), 2.46, d (16.5)
3	69.1, CH	3.47, m	66.7, CH	3.83, m, 4.40 br, s, OH	197.0, C	
4	44.9, CH	1.77, m	37.7, CH	2.19, m	128.3, C	
5	40.0, C		46.9, C		163.7, C	
6	154.7, CH	6.48, s	68.8, CH	4.82, s	28.7, CH_2	2.83, d (14.0), 1.90, t (13.5)
7	136.5, C		162.6, C		49.1, CH	1.34, m
8	197.6, C		104.8, C		22.3, CH_2	1.62, m, 1.31, m
9	40.0, CH_2	2.64, d (16.5), 2.10, d (16.5)	38.7, CH_2	1.95, m, 1.74, m	37.8, CH_2	2.10, d (8.5), 1.28, m
10	35.6, CH	1.93, m	35.1, CH	1.67, m	40.5, C	
11	38.2, CH	3.36, q (7.0)	122.8, C		70.9, C	
12	16.4, CH_3	1.15, d (7.0)	9.0, CH_3	1.88, s	26.6, CH_3	1.08, s
13	175.8, C		172.2, C		28.0, CH_3	1.11, s
14	25.3, CH_3	1.15, s	19.0, CH_3	0.70, s	17.3, CH_3	1.1, s
15	7.7, CH_3	0.88, d (7.0)	8.0, CH_3	0.81, d (7.0)	11.1, CH_3	1.67, s
			Glucose			
1					100.8, CH	4.19, d (7.5)
2					73.9, CH	2.93, m
3					77.3,[d] CH	3.14, m
4					70.8, CH	3.03, m
5					77.4,[d] CH	3.08, m
6					61.8, CH_2	3.64, 3.28, 2 m

[a] Recorded at 125 MHz. [b] Multiplicities inferred from DEPT and HMQC experiments. [c] Recorded at 500 MHz. [d] Interchangeable.

α,*β*-unsaturated lactone band at 1740 cm^{-1}. When comparing the NMR data of **3** (Table 1) with those of the known sesquiterpene, 3α,6α-dihydroxy-8α*H*-7(11)-eremophilen-12,8-olide (**7**), isolated from the same plant,[22] compound **3** was deduced to be the 8α-hydroxy derivative of **7**. Compound **3** might be the precursor of several known sesquiterpenes, such as eremopetasitasitenins C1 and C2.[25] The relative configuration of **3** and **7** were confirmed by the NOESY cross peaks of H-8α/Me-14α and H-3*β*/H-6*β* in the NOESY spectrum of **7** and NOESY cross peaks of OH-3α/OH-6α/OH-8α in the NOESY spectrum of **3**. Thus, compound **3** was identified as a new sesquiterpene, 3α,6α,8α-trihydroxy-7(11)-eremophilen-12,8-olide.

Anhuienoside A (**4**) was obtained as a yellowish oil. The HR-FTICRMS exhibited an ion peak at *m/z* 437.2139 $[M + Na]^+$ (calcd 437.2146), corresponding to the molecular formula, $C_{21}H_{34}O_8$. The ^{13}C NMR spectrum showed the presence of six signals for a glucopyranose moiety, with the remaining 15 resonances corresponding to a sesquiterpene skeleton. The ^1H and ^{13}C NMR spectra indicated compound **4** to be a glycosylated dihydroxy-oxo-eudesmane derivative (Table 1). The 15 signals for the aglycone comprised four methyls (δ_C 26.6, 28.0, 17.3, and 11.1), four methylenes (δ_C 39.5, 28.7, 22.3, and 37.8), a methine (δ_C 49.1), an oxymethine (δ_C 79.1), a quaternary carbon (δ_C 40.5), an oxygenated quaternary carbon (δ_C 70.9), a carbonyl carbon (δ_C 197.0), and a tetrasubstituted double bond (δ_C128.3 and 163.7). Two of the four methyls (δ_C 26.6 and 28.0) were assigned to an oxygenated isopropyl group (carbinol signal at δ_C 70.9) and one (δ_C 11.1) to a double bond unit, with the fourth (δ_C 17.3) being Me-14. In the COSY spectrum of **4**, the oxymethine proton at δ_H 3.78 (dd, J = 13.0, 4.5 Hz, H-1) was coupled with the methylene protons at δ_H 2.61 (dd, J = 16.5, 4.5 Hz, H-2) and 2.46 (d, J = 16.5 Hz, H-2). Another sequence of H-6/H-7/H-8/H-9 was also observed in the COSY spectrum. The terminal glucopyranose moiety was assigned at C-1 from the observation of HMBC correlations from the oxymethine proton at δ_H 3.78 (dd, J = 13.0, 4.5 Hz, H-1) to the anomeric carbon resonance at δ_C 100.8 (d, C-1′) and Me-14 at δ_C 17.3 (q, C-14). The HMBC peaks from two methyl groups at δ_H 1.11 (s, Me-14) and 1.67 (s, Me-15) to the carbon at δ_C 163.7 (s, C-5) assigned the double bond to C-4 and C-5. The α,*β*-unsaturated keto group was assigned to C-3 from analysis of the HMBC cross

Table 2. NMR Data (500 MHz) for Compound **2** in DMSO-d_6

position	δ_C,[a,b] mult	δ_H,[c] mult (*J* in Hz)
1	26.8, CH_2	1,57, m, 1.18, m
2	27.9, CH_2	1.40, m
3	69.3, CH	3.48, m
4	44.3, CH	1.75, m
5	44.1, C	
6	156.2, CH	6.42, s
7	134.7, C	
8	197.9, C	
9	40.0, CH_2	2.65, d (14.5), 2.18, d (14.5)
10	35.9, CH	1.95, m
11	44.1, CH	3.73, m
12	15.9, CH_3	1.04, d (7.0)
13	208.3, C	
14	25.1, CH_3	1.14, s
15	8.0, CH_3	0.87, d (7.0)
1′	47.6, CH_2	3.60, d (16.5), 3.52, d (16.5)
2′	125.2, C	
3′/7′	107.5, CH	6.36, s
4′/6′	148.2, C	
5′	136.6, C	
OCH_3	56.4, CH_3	3.72, s

[a] Recorded at 125 MHz. [b] Multiplicities inferred from DEPT and HMQC experiments. [c] Recorded at 500 MHz. [d] Interchangeable.

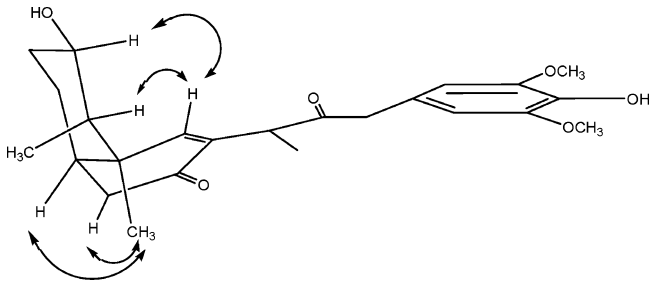

Figure 1. Key NOESY correlations of compound **2**.

peaks of H-1/C-3 and H-2/C-3. The sugar obtained by the acid hydrolysis of **4** was identified by co-TLC and GC analysis and were confirmed as D-glucose. The configuration of the glycosidic linkage of the glucopyranoside moiety in **4** was determined to be β based on the coupling constant of the anomeric proton at δ_H 4.19 (d, J = 7.5 Hz). H-1 in **4** was axially oriented to exhibit its proton signal as a double doublet at δ_H 3.78 with 13.0 and 4.5 Hz coupling constants. The anomeric proton of the glucopyranose moiety at δ_H 4.22 (s) showed NOESY correlation to the Me-14 proton at δ_H 1.11 (s, Me-14) contributing a β-oriented glucopyranose unit in **4** as drawn. The carbon signal at δ_C 49.1 (d) is close to that reported for the H-7α configuration, which downshifted about 5 ppm in contrast with their H-7β epimers.[26,27] The inference of the 7β-isopropanol moiety was further confirmed by the 13.5 Hz coupling constant between H-6β and H-7α in agreement with their axial positions.[26] Therefore, the structure of this isolate was elucidated as 1β-(β-D-glucopyranosyloxy)-3-oxo-7α*H*-4-eudesmen-11-ol and given the trivial name anhuienoside A.

Anhuienoside B (**5**) was obtained as a yellowish gum. The HR-FTICRMS of **5** exhibited a molecular ion at *m*/*z* 645.1804 [M + Na]$^+$, which was in accordance with the mass calcd for $C_{29}H_{34}O_{15}$ (calcd 645.1790). ^{1}H and ^{13}C NMR data (Table 3) indicated the presence of glucosyl and rhamnosyl residues. After acid hydrolysis, the carbohydrate moieties were further determined as D-glucose and L-rhamnose by TLC and GC analysis. The ^{1}H NMR spectrum displayed two aromatic ABX-type coupling patterns, indicating the presence of two 1,3,4-trisubstituted phenyl groups [δ_H 6.75 (s, H-2), 6.70 (d, J = 8.0 Hz, H-5), 6.62 (d, J = 8.0 Hz, H-6)] and [δ_H 7.03 (s, H-2′), 6.77 (d, J = 8.0 Hz, H-5′), 6.98 (d, J = 8.0 Hz, H-6′)] aided by the analysis of COSY and HMBC spectra. One of these moieties was part of the 4-*O*-caffeoyl-β-glucopyranosyloxy unit, and another was attributed to the aglycone unit. The aglycone was

Table 3. NMR Data (500 MHz) for Compounds **5** and **6** in DMSO-d_6

	5		**6**	
position	δ_C,[a,b] mult	δ_H,[c] mult (*J* in Hz)	δ_C,[a,b] mult	δ_H,[c] mult (*J* in Hz)
1	128.5, C		155.8, C	
2	114.0, CH	6.75, s	116.0, CH	6.94, d (8.3)
3	145.6, C		129.3, CH	7.10, d (8.3)
4	145.6, C		132.6, C	
5	115.8, CH	6.70, d (8.0)	129.3, CH	7.10, d (8.3)
6	117.6, CH	6.62, d (8.0)	116.0, CH	6.94, d (8.3)
7	76.5, CH	6.53, overlap	34.2, CH_2	2.63, t (4.5)
8	71.4, CH_2	3.94, d (10.0), 3.50, m	40.3, CH_2	3.29, t (4.5)
9			169.0, C	
10			22.5, CH_3	1.77, s
1′	126.0, C			
2′	115.2, CH	7.03, s		
3′	146.0, C			
4′	149.0, C			
5′	116.3, CH	6.77, d (8.0)		
6′	122.0, CH	6.98, d (8.0)		
7′	146.4, CH	7.49, d (16.0)		
8′	113.8, CH	6.19, d (16.0)		
9′	165.9, C			
	Glucose			
1	100.8, CH	4.98, s	100.4, CH	4.80, d (7.6)
2	70.7, CH[d]	3.55, m	73.1, CH	3.26, m
3	74.8, CH	4.04, t (9.5)	76.9, CH	3.30, m
4	69.3, CH[e]	4.90, t (9.5)	69.6, CH	3.17, m
5	76.5, CH	3.73, m	76.5, CH	3.30, m
6	60.9, CH_2	3.45, 3.38, 2 m	60.6, CH_2	3.69, 3.46, 2 m
	Rhamnose			
1	97.4, CH	4.55, d (8.0)		
2	80.9, CH	3.36, m		
3	70.9, CH[d]	3.24, d (9.5)		
4	71.9, CH	3.50, m		
5	69.2, CH[e]	3.42, m		
6	18.4, CH_3	1.01, d (6.0)		

[a] Recorded at 125 MHz. [b] Multiplicities inferred from DEPT and HMQC experiments. [c] Recorded at 500 MHz. [d] Interchangeable. [e] Interchangeable.

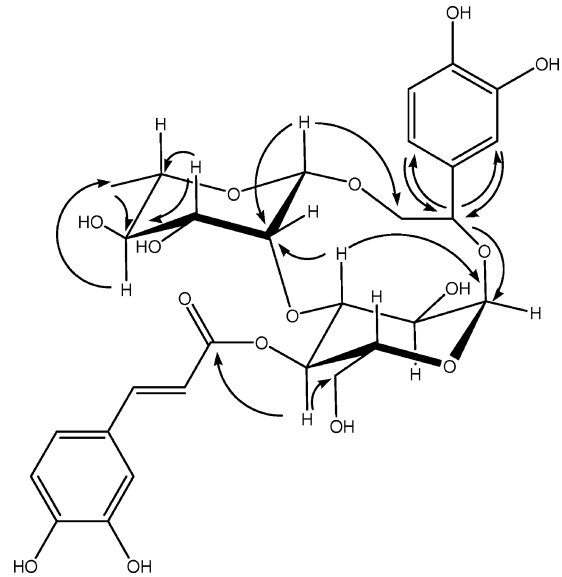

Figure 2. Key HMBC correlations of compound **5**.

deduced to be 4-(1,2-dihydroxyethyl)benzene-1,2-diol from the observation of the HMBC cross peaks of H-8/C-1, H-7/C-2 and H-7/C-6 (Figure 2). The HMBC cross peaks from the methylene protons at δ_H 3.94 (d, J = 10.0 Hz, H-8a), 3.50 (m, H-8b) to the carbon at δ_C 97.4 (d, Rha-1), and the rhamnosyl anomeric proton at δ_H 4.55 (d, J = 8.0 Hz, Rha-1) to the carbon at δ_C 71.4 (t, C-8), indicating that the glucosyloxy unit was attached at C-7. This was

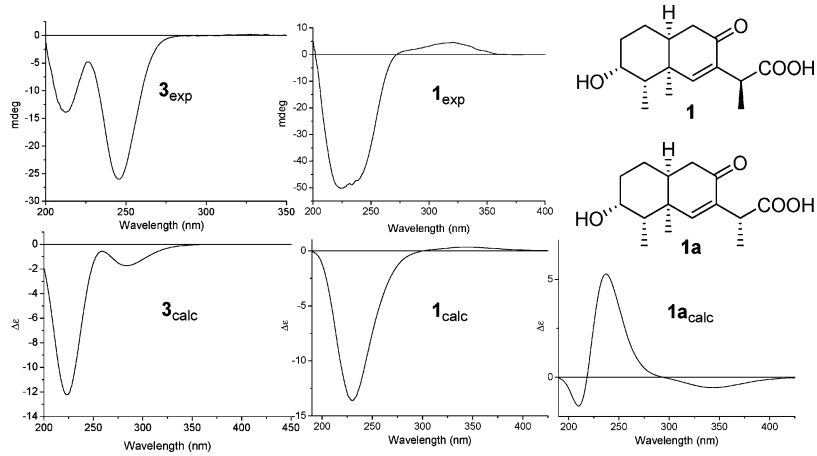

Figure 3. Experimental ECD spectra (upper) and velocity representation of B3LYP/aug-cc-pVDZ//B3LYP/6-311++G(2d,2p) calculated ECD spectra (lower, conformationally averaged by relative Gibbs free energy, ΔG; $\sigma = 0.25$ for compound **3** and 0.2 eV for compounds **1** and **1a**) of compounds **3**, **1**, and **1a**.

confirmed by the HMBC cross peak of H-7/C-1-Glu. A rare 10-membered diglycosidic ring was present between the Rha-2 and Glu-3 positions via an oxygen bridge based on the long-range correlation from the glucosyl proton at δ_H 4.04 (t, $J = 9.5$ Hz, H-3-Glu) to the downshifted rhamnosyl carbon at δ_C 80.9 (d, C-2-Rha). The configuration of glycosidic linkages in **5** was determined to be β for rhamnosyl and α for glucosyl units on the basis of the J values [δ_H 4.55 (d, $J = 8.0$ Hz, H-1-Rha) and 4.98 (s, H-1-Glu)] of the two anomeric protons. The unusual cyclic caffeoylphenylethanoid diglycoside was named as anhuienoside B.

Compound **6**, obtained as a yellowish oil, had the molecular formula $C_{17}H_{27}NO_7$, determined by HR-FTICRMS (*m/z* 364.1360 $[M + Na]^+$, calcd 364.1367). IR bands at 3402 and 1648 cm^{-1} and a signal appearing at δ_C169.0 (s) in the ^{13}C NMR spectrum suggested that hydroxy groups and an amide group might be present. A typical AABB system at δ_H 6.94 (d, $J = 8.3$ Hz, 2H) and 7.10 (d, $J = 8.3$ Hz, 2H) for H-2/6 and H-3/5 and two coupled methylene proton triplets at δ_H 2.63 (t, $J = 4.5$ Hz, 2H) and 3.29 (t, $J = 4.5$ Hz, 2H) for H-7 and H-8 of the tyramine moiety were observed in the ^1H NMR spectrum (Table 3). The ^{13}C NMR spectrum of **6** showed the presence of six signals for a terminal glucopyranose moiety, with the remaining eight signals representing a tyramine moiety, an amide carbonyl group, and a methyl group. The HMBC correlations from the C-8 methylene proton, and C-10 methyl protons to the C-9 carbonyl carbon, suggested that compound **6** was a glycoside of *N*-acetyltyramine, with the glucose moiety located at C-1. The D-glucose obtained by the acid hydrolysis of **6** was identified by co-TLC and GC analysis. The configuration of the glycosidic linkage was determined to be β on the basis of the J value [δ_H 4.80 (d, $J = 7.6$ Hz)] of the anomeric proton. Therefore, compound **6** was elucidated as *N*-acetyltyramine 1-*O*-β-D-glucoside.

Computational calculation of spectroscopic properties of organic molecules by quantum chemical methods, especially the density functional theory, have been proved to be a powerful tool for the determination of their structures and absolute configurations.[28−30] The absolute configurations of compounds **1** and **3** were determined by TD-DFT calculations of their ECD spectra, in comparison with the corresponding experimental ones. Following similar procedure as previously described,[30] lowest energy conformations were identified by a conformational searching at AM1 level with Spartan 04 software package.[31] The resulting conformations were reoptimized using DFT at the B3LYP/6-311++G (2d, 2p) level in the GAUSSIAN 09 program.[32] The B3LYP/6-311++G (2d, 2p) harmonic vibrational frequencies were further calculated to confirm their stability. The energies, oscillator strengths, and rotational strengths of the electronic excitations of all the conformers were calculated using the TD-DFT method at the B3LYP/aug-cc-pVDZ level, and the ECD spectra were then simulated by the overlapping Gaussian function. To generate the final spectrum of a molecule, all the simulated spectra of the lowest energy conformations were averaged according to the Boltzmann distribution theory in which their Gibbs free energy (G) was adopted.

Conformational searching for compound **3** at the AM1 level resulted in identification of the four lowest energy conformations, which were considered for further calculation and simulation of its ECD spectrum (see Supporting Information). In the 200−400 nm region, compared to the experimental negative Cotton effects at 246 and 212 nm, the calculated one showed the same pattern with two negative Cotton effects at 283 nm (+37 nm) and 222 nm (+10 nm), respectively. (Figure 3) Therefore, qualitative analysis of the result allowed the assignment of the absolute configuration of **3** as 3*R*,4*S*,5*R*,6R,8*R*,10*S*.

The configuration at C-11 remains unassigned in previous studies.[24] In the current study, the absolute configuration at C-11 for compound **1** was determined on the basis of the TD-DFT method and biogenetic considerations. First, from the above results of compound **3**, 3*R*,4*S*,5*R*,10*S* absolute configuration was adopted for compound **1** because they have the same biological origin. Second, the configuration at C-11 may influence the ECD spectrum because of the neighboring α,β-unsaturated carbonyl chromophore. Therefore, the C-11 epimers **1** and **1a** were subjected to TD-DFT studies. Compared to the experimental ECD spectrum of **1**, the calculated ECD spectrum for **1** showed a similar positive Cotton effect at 343 nm and a negative Cotton effect at 229 nm, while the calculated ECD spectrum for **1a** showed a negative Cotton effect at 343 nm and positive Cotton effect at 237 nm (Figure 3). The above results allowed the determination of 11*S* configuration. Finally, the absolute configuration of **1** was determined as 3*R*,4*S*,5*R*,10*S*,11*S*. The absolute configuration of compound **2** was also assigned as 3*R*,4*S*,5*R*,10*S*,11*S* based on the results of compound **1** and their similar biological origin and specific rotations.

Recently, several eudesmane-type sesquiterpenes biogenetically related to compound **4** and diterpenes had been isolated from the same species.[21] However, this is the first report of eremophilane-type sesquiterpenes in *C. anhuiensis*. Except the new phytoalexin **2**, no obvious changes of the amounts of other compounds were observed after abiotic stress treatment.

Experimental Section. General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer-341 polarimeter. The IR spectra ($CHCl_3$) were run on a NicoletAvatar-360FT-IR spectrometer. ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were measured at 25 °C on a Bruker AVANCE DMX 500 NMR spectrometer with TMS as internal standard. HR-FTICRMS

were recorded on a Bruker Apex III spectrometer. ESIMS were recorded on a Bruker Esquire-3000^{plus} spectrometer. GC was performed on a Varian 3300 apparatus using a HP-innowax capillary column (30 m × 0.25 mm i.d.). TLC was performed using Merck precoated plates (Si gel 60 F254) of 0.25 mm thickness. Sephadex LH-20 (Amersham) was used for column chromatography.Plant Material and Stress Applications. *C. anhuiensis* were collected in Jixi County, Anhui Province, People's Republic of China, in June 2008 and identified by Prof. Changxi Zhang (Jinhua Medical College, Jinhua, People's Republic of China.). A voucher specimen (Sd1034) is maintained at the Jinhua Medical College, Jinhua, People's Republic of China. The stress experiments were carried out in pots. Forty plants were cultivated. All the plants were separated into control (10 plants) and stressed groups (30 plants). To elicit the stress, plants were sprayed with 2% aq solution of $CuCl_2$. After 48 h, leaves of the control and sprayed plants were collected and dried at 60 °C, and finely powdered in an electronic blender and kept in separate containers for extraction.Extraction and Isolation. The dried, powdered $CuCl_2$ treated leaves (405 g) of *C. anhuiensis* and untreated leaves (111 g) were extracted at room temperature with MeOH (3 × 1 L), respectively. The extracts were evaporated in vacuo to afford a gummy residue (38 g) for treated and a gummy residue (12 g) for the corresponding control. The residues were partitioned in H_2O (500 mL) and extracted with EtOAc (4 × 500 mL) and *n*-butanol (4 × 500 mL), successively. The EtOAc and *n*-butanol extracts of treated and the corresponding control were subjected to TLC examination on aluminum sheets precoated with Si gel 60 F 254 (Merck). The spots were applied in as equal amounts as possible. The plates were developed in the following developing solvent systems: benzene−acetone (6:1), benzene−EtOAc (5:1), petroleum ether−EtOAc (5:1) for the EtOAc extract; $CHCl_3$−MeOH (3:1), CH_2Cl_2−MeOH (4:1), and benzene−$CHCl_3$−MeOH (1:3:1) for the *n*-butanol extract. After development, the plates were examined under UV light (250 nm) to locate any additional spots in the different extracts of the treatments in comparison with that of the corresponding control extracts. The spots on the plates were also visualized by spraying with an EtOH−H_2SO_4 solution. Several prep-TLC plates were prepared and the compounds were separated by preparative TLC in different solvent systems. The crude compounds were applied to a Sephadex LH-20 column (1 cm × 80 cm, 38 g, Amersham), and eluted with MeOH to yield pure compounds **1** (2.5 mg), **2** (4.8 mg), **3** (2.2 mg), **4** (3.5 mg), **5** (2.8 mg), **6** (2.3 mg), **7** (2.2 mg), **8** (3.3 mg), and **9** (10.7 mg). The extract of untreated leaves were separated by the same methods to afford **1** (1.0 mg), **3** (1.1 mg), **4** (1.2 mg), **5** (1.0 mg), **6** (1.0 mg), **7** (1.2 mg), **8** (1.0 mg), and **9** (4.3 mg).(3*R*,4*S*,5*R*,10*S*,11*S*)-3-Hydroxy-8-oxo-6-eremophilen-12-oic Acid (**1**). Yellow oil; $[\alpha]^{24}_D$ −15 (*c* 0.001, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 232 (3.97) nm; IR ν_{max} 3435, 2925, 1718, 1667, 1459, 1384, 1208, 1083, 1027, 1004, 826, 767, 562 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; ESIMS *m/z* 265 $[M - H]^-$; HR-FTICRMS *m/z* 265.1445 $[M - H]^-$ (calcd for $C_{15}H_{21}O_4$, 265.1433).Anhuienol (**2**). Yellow gum; $[\alpha]^{24}_D$ −10 (*c* 0.001, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 230 (4.28), 254 (4.03), 280 (4.35) nm; IR ν_{max} 3420, 2926, 2855, 1707, 1665, 1612, 1517, 1460, 1428, 1384, 1331, 1216, 1116, 1031, 1005, 913, 876, 713, 542, 474 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; ESIMS *m/z* 439 $[M + Na]^+$; HR-FTICRMS *m/z* 439.2100 $[M + Na]^+$ (calcd for $C_{24}H_{32}O_6Na$, 439.2091). (3*R*,4*S*,5*R*,6*R*,8*R*,10*S*)-3,6,8-Trihydroxy-7(11)-eremophilen-12,8-olide (**3**). Yellow oil; $[\alpha]^{24}_D$ −25 (*c* 0.001, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 225 (4.22) nm; IR ν_{max} 3416, 2930, 1740, 1686, 1638, 1438, 1384, 1344, 1296, 1198, 1136, 1088, 1047, 1022, 1009, 939, 912, 883, 829, 762, 581 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; ESIMS *m/z* 281 $[M - H]^-$; HR-FTICRMS *m/z* 281.1383 $[M - H]^-$ (calcd for $C_{15}H_{21}O_5$, 281.1394).Anhuienoside A (**4**). Yellow oil; $[\alpha]^{24}_D$ +60 (*c* 0.001, MeOH); UV (MeOH) λ_{max} (log ϵ) 230 (3.95) nm; IR ν_{max} 3408, 2933, 1648, 1607, 1459, 1366, 1329, 1156, 1078, 1027, 915, 822, 634 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; ESIMS *m/z* 437 $[M + Na]^+$; HR-FTICRMS *m/z* 437.2139 $[M + Na]^+$ (calcd for $C_{21}H_{34}O_8Na$, 437.2146).Anhuienoside B (**5**). Yellow gum; $[\alpha]^{24}_D$ −35 (*c* 0.001, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (4.11), 250 (3.93), 280 (4.23) nm; IR ν_{max} 3403, 2926, 1706, 1630, 1605, 1523, 1448, 1382, 1263, 1159, 1132, 1042, 1023, 815, 777, 595 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 2; ESIMS *m/z* 645 $[M + Na]^+$; HR-FTICRMS *m/z* 645.1804 $[M + Na]^+$ (calcd for $C_{29}H_{34}O_{15}Na$, 645.1790).*N*-Acetyltyramine 1-*O*-β-Glucoside (**6**). Yellow oil; $[\alpha]^{24}_D$ +30 (*c* 0.001, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (4.24), 254 (3.98), 282 (4.10) nm; IR ν_{max} 3402, 3291, 2927, 1647, 1542, 1512, 1364, 1233, 1075, 1045, 835, 719, 636, 599 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 2; ESIMS *m/z* 364 $[M + Na]^+$; HR-FTICRMS *m/z* 364.1360 $[M + Na]^+$ (calcd for $C_{17}H_{27}NO_7Na$, 364.1367).Acid Hydrolysis of **4**−**6.** Each compound (2.0 mg) in 10% HCl was stirred at 90 °C for 4 h. The reaction mixture was filtered and examined by TLC together with authentic D-glucose and L-rhamnose. The dried filtrate was dissolved in dry pyridine,[33] to which was added L-cysteine methyl ester hydrochloride. The mixture was stirred at 60 °C for 1.5 h, then hexamethyldisilazane-trimethylchlorosilane (2:1) was added and stirred for 0.5 h.[34] After centrifugation, the supernatant was directly subjected to GC analysis. The sugar derivatives obtained from **4**−**6** were detected in each case by coinjection of the D-glucose and L-rhamnose derivatives.

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Supporting Information Available: 1D NMR spectra for compounds **1**−**9**, COCY, HMQC and HMBC spectra for compounds **1**−**8**, NOESY spectra for compounds **1**, **2**, **3**, **4**, **7**, and **8**, IR and HR-FTICRMS spectra for compounds **1**−**6**, and computational calculation data of compounds **1** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Wu, B.; He, S.; Pan, Y. *Tetrahedron Lett.* **2007**, *48*, 453–456.
(2) Wu, B.; Chen, J.; Qu, H.; Cheng, Y. *J. Nat. Prod.* **2008**, *71*, 877–880.
(3) Wu, B.; He, S.; Pan, Y. *Planta Med.* **2006**, *72*, 1334–1338.
(4) Wu, B.; He, S.; Wu, X.; Wu, D.; Pan, Y. *Helv. Chim. Acta* **2007**, *90*, 1586–1592.
(5) Wu, B.; Qu, H.; Cheng, Y. *Helv. Chim. Acta* **2008**, *91*, 725–733.
(6) Wu, B.; He, S.; Wu, X.; Pan, Y. *Chem. Biodiversity* **2008**, *5*, 1298–1303.
(7) Kawabata, J.; Tahara, S.; Mizutani, J. *Agric. Biol. Chem.* **1981**, *46*, 1447–1453.
(8) Kawabata, J.; Mizutani, J. *Agric. Biol. Chem.* **1989**, *53*, 203–207.
(9) Li, Y.; Zhang, D.-M.; Li, J.-B.; Yu, S.-S.; Li, Y.; Luo, Y.-M. *J. Nat. Prod.* **2006**, *69*, 616–620.
(10) Tsui, W.-Y.; Brown, G. D. *Phytochemistry* **1996**, *43*, 819–821.
(11) Takeda, Y.; Yamashita, H.; Matsumoto, T.; Terao, H. *Phytochemistry* **1993**, *33*, 713–715.
(12) Kusano, G.; Abe, M.; Koike, Y.; Uchida, M.; Nozoe, S.; Taira, Z. *Yakugaku Zasshi* **1991**, *111*, 756–764.
(13) Tahara, S.; Fukushi, Y.; Kawabata, J.; Mizutani, J. *Agric. Biol. Chem.* **1981**, *45*, 1511–1512.
(14) Takemoto, T.; Uchida, M.; Kusano, G. *Chem. Pharm. Bull.* **1976**, *24*, 531–533.
(15) Kawabata, J.; Fukushi, Y.; Tahara, S.; Mizutani, J. *Phytochemistry* **1990**, *29*, 2332–2334.
(16) Kawabata, J.; Fukushi, E.; Mizutani, J. *Phytochemistry* **1995**, *39*, 121–125.
(17) Yang, S.-P.; Yue, J.-M. *Tetrahedron Lett.* **2006**, *47*, 1129–1132.
(18) Kawabata, J.; Fukushi, E.; Mizutani, J. *Phytochemistry* **1993**, *32*, 1347–1349.
(19) Kawabata, J.; Fukushi, E.; Mizutani, J. *Phytochemistry* **1998**, *47*, 231–235.
(20) Kawabata, J.; Mizutani, J. *Phytochemistry* **1992**, *31*, 1293–1296.

(21) Xu, Y.-J.; Tang, C.-P.; Tan, M.-J.; Ke, C.-Q.; Wu, T.; Ye, Y. *Chem. Biodiversity* **2010**, *7*, 151–157.
(22) Sugama, K.; Hayashi, K.; Mitsuhashi, H. *Phytochemistry* **1985**, *24*, 1531–1535.
(23) Zhao, Y.; Jia, Z.; Peng, H. *J. Nat. Prod.* **1995**, *58*, 1358–1364.
(24) Yaoita, Y.; Kikuchi, M. *Chem. Pharm. Bull.* **1995**, *43*, 1738–1743.
(25) Tori, M.; Kawahara, M.; Sono, M. *Phytochemistry* **1998**, *47*, 401–409.
(26) Su, W.-C.; Fang, J.-M.; Cheng, Y.-S. *Phytochemistry* **1995**, *39*, 603–607.
(27) Van Beak, T. A.; Kleis, R.; Posthumus, M. A.; Van Veldhuizen, A. *Phytochemistry* **1989**, *28*, 1909–1911.
(28) McCann, D. M.; Stephens, P. J. *J. Org. Chem.* **2006**, *71*, 6074–6098.
(29) Ding, Y.; Li, X. C.; Ferreirra, D. *J. Org. Chem.* **2007**, *72*, 9010–9017.
(30) Gan, L.-S.; Zheng, Y.-L.; Mo, J.-X.; Liu, X.; Li, X.-H.; Zhou, C.-X. *J. Nat. Prod.* **2009**, *72*, 1497–1501.
(31) *Spartan 04*; Wavefunction Inc.: Irvine, CA.
(32) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J. Fox, D. J. *Gaussian 09, revision A.1*; Gaussian, Inc.: Wallingford CT, 2009.
(33) Hara, S.; Okabe, H.; Mihashi, K. *Chem. Pharm. Bull.* **1986**, *34*, 1843–1845.
(34) Fang, W.; Ruan, J.; Wang, Z.; Zhao, Z.; Zou, J.; Zhou, D.; Cai, Y. *J. Nat. Prod.* **2006**, *69*, 1641–1644.

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