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Hookerianolides A–C: three novel casbane-type diterpenoid lactones from *Mallotus hookerianus*

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Abstract—Three highly oxidized casbane-type diterpenoids with unique α,β-unsaturated γ-lactones, named Hookerianolides A (**1**), B (**2**), and C (**3**), were isolated from the methylene chloride extracts of *Mallotus Hookerianus*. Their structures were elucidated based on NMR spectroscopic data and chemical conversions. The stereochemistry was confirmed by combination of ROESY correlations and CD analyses.

Mallotus hookerianus (seem.) Muell. Arg. (Euphorbiaceae) is distributed from Southern Guangxi and Guangdong to Hainan Island.[1] To our best knowledge, only a few phytochemical studies have been published on its chemical constituents to date.[2] Chemical investigation, as part of our current interest in the *Mallotus* genera, has resulted in the isolation of three novel casbane-type diterpenoids, named Hookerianolides A (**1**), B (**2**), and C (**3**). In this letter, we describe the isolation and structural elucidation of the novel compounds based on spectroscopic analyses and chemical conversions.

A small group of casbane-type diterpenes have so far been found only in seven plants of the family Euphorbiaceae.[3–9] Compounds **1**–**3** are structurally highly oxidized casbane-type diterpenoids and the first examples having a trisubstituted epoxide and a α,β-unsaturated γ-lactone. Most notably, there is a hydroxyl at γ position of the lactone in Hookerianolide A, which formed a unique hemiketal unit.

The plant material was collected from the Hainan Province in China. The air-dried ground plant powder (14 kg) of *Mallotus Hookerianus* was percolated with 95% ethanol, and the crude ethanolic extract partitioned with CH_2Cl_2, EtOAc, and n-C_4H_9OH successively. The CH_2Cl_2 extract (100 g) was subjected to repeated CC over silica gel, using petrol/Me_2CO and $CHCl_3$/CH_3OH as eluents (increasing polarity). Compound **1** (25 mg) was obtained in fractions eluted with $CHCl_3$/CH_3OH (9:1). The $CHCl_3$/CH_3OH (20:1) fractions were further fractionated by MCI gel column chromatography, using 30% Me_2CO–H_2O as eluents, to yield two major subfractions, which were further purified by a column chromatography of Sephadex LH-20 to afford **2** (4 mg) and **3** (12 mg).

Hookerianolide A (**1**)[10] was obtained as an optically active ($[\alpha]_D^{20}$ −28.1 in methanol) white amorphous powder. The molecular formula of **1** was established as $C_{20}H_{28}O_6$ by HR-ESIMS ($[M+Na]^+$ m/z: found 387.1779, calcd 387.1784). Compound **1** also exhibited a deprotonated molecular ion at m/z 363 $[M-H]^-$ in the negative mode of its low resolution ESIMS, confirming the assignment for the suggested molecular formula, requiring 7° of unsaturation. The ^{13}C NMR spectrum showed 20 carbon signals, including one carboxylic, four olefinic (two methine and two quaternary), three oxygenated methine and two oxygenated quaternary carbons together with ten aliphatic carbons (four methyl, three methylene, two methine, and one quaternary). Two vinyl proton signals at δ_H 7.12 (H-11), 5.55 (H-3) were shown in its ^1H NMR spectrum, which equated with four olefinic carbons attributing to two trisubstituted double bonds in its ^{13}C NMR. With all the unsaturated function groups known [COOR, (C=CH) × 2], the four remaining degrees of unsaturation were ascribed to four

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ring systems. The chemical shifts at δ_C 28.7 (C-2), 32.4 (C-1), 23.3 (C-15), 16.3 (C-16), 29.7 (C-17) in the ^{13}C NMR spectrum together with *gem*-dimethyl at δ_H 1.53 and 0.76 in the ^1H NMR spectrum indicated the presence of cyclopropyl ring bearing the geminal methyl groups, which were typical signals for the casbane-type diterpenoid containing a 14 membered macrocyclic ring.

The analysis of ^1H–^1H COSY clearly outlined two groups of protons bearing spin coupling units as drawn with bold bonds (Fig. 2). The ^1H and ^{13}C NMR spectra with HMQC analysis indicated the presence of a trisubstituted epoxide[11] (δ_H 2.64, d, $J = 8.8$ Hz; δ_C 65.0 and 62.1 ppm). The scaffold of **1** was figured out by HMBC experiments (Table 1 and Fig. 2), which allowed for the connection of the protons bearing spin coupled units. The HMBC correlations could link most of the bonds together, except for the bond between C-10 and C-20. It was confirmed that **1** owned three free hydroxyl groups, due to the appearance of three methyl signals at δ_H 2.09, 2.07, 2.04 in the ^1H NMR spectrum of its triacetate derivative (**4**).[4] The remaining ring was assigned to a α,β-unsaturated γ-lactone which formed the quaternary hemiketal carbon δ_C 103.7 (C-10). Thus, the planar structure of **1** was elucidated.

Attempts to grow crystals suitable for single-crystal X-ray diffraction analysis failed. The relative stereochemistry of **1** was obtained from analysis of ROESY spectra. The ROESY correlations for H-6/CH_3-19 and H-9β indicated that CH_3-19, H-6, and H-9β were β-oriented. The H-7 correlated to H-9β and H-5, suggesting that they were β-oriented. The CH_3-16 correlated to H-2, H-1, and H-13β indicated that they were also β-oriented. In the ROESY spectrum of **4**, CH_3-19 correlated to 10-OCOCH_3, H-7 correlated to H-14β, suggesting that they were β-oriented. Thus OH-10, CH_3-19, CH_3-16, H-14β, H-13β, H-9β, H-7, H-6, H-5, H-2, and H-1 of **1** were all in β-orientations. Therefore, the structure of **1** was unambiguously elucidated as shown in Figure 1.

The absolute stereochemistry of **1** was determined on the basis of its CD spectrum. The stereochemistry at C-10 was proposed to be *R*, according to a negative Cotton effect at 218 nm (π–π^*) and a positive Cotton effect at 252 nm (n–π^*), which were identical to those of the known *epi*-Sarcotin A and reversed to those of the known Pukalide[12,13] (Figs. 3 and 4).

HR-ESIMS analysis of Hookerianolide B (**2**)[14] indicated the molecular formula $C_{20}H_{28}O_5$ (seven unsaturations), differing from **1** by the loss of an oxygen atom. The

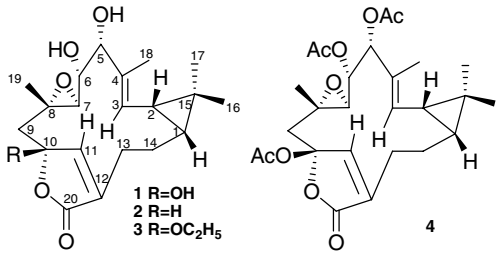


Figure 1. Structures of Hookerianolides A (**1**), B (**2**), C (**3**) and Hookerianolide A triacetate (**4**).

Table 1. ^1H NMR Data of Hookerianolides A (**1**), B (**2**), C (**3**), and Hookerianolide A triacetate (**4**)

No.	**1**[a]	**2**[b]	**3**[c]	**4**[d]
1	0.76 (t, 8.7)	0.73 (t, 8.8)	0.78 (t, 8.8)	0.78 (t, 8.6)
2	1.53 (dd, 11.7, 8.7)[e]	1.53 (dd, 11.0, 8.8)	1.55 (dd, 11.2, 8.8)	1.39 (dd, 11.3, 8.6)
3	5.55 (d, 11.7)	5.59 (d, 11.0)	5.60 (d, 11.2)	5.67 (d, 11.3)
5	4.07 (d, 8.8)	4.06 (d, 8.9)	4.07 (d, 8.9)	5.54 (d, 9.5)
6	3.57 (t, 8.8)	3.51 (t, 8.9)	3.54 (t, 8.9)	5.26 (t, 9.5)
7	2.64 (d, 8.8)	2.61 (d, 8.9)	2.63 (d, 8.9)	2.69 (d, 9.5)
9α	2.78 (d, 14.1)	2.67 (dd, 12.5, 4.7)	2.74 (d, 13.8)	3.17 (d, 14.3)
9β	1.24 (d, 14.1)	0.88 (t, 12.5)	1.24 (d, 13.8)	1.18 (d, 14.3)
10		5.05 (d, 12.5)		
11	7.12 (t, 2.0)	7.52 (d, 1.3)	7.12 (t, 2.0)	7.15 (t, 2.1)
13α	2.22–2.32 (m)	2.15–2.27 (m)	2.26–2.36 (m)	2.33–2.42 (m)
13β	2.49–2.55 (m)	2.42–2.48 (m)	2.45–2.51 (m)	2.53–2.58 (m)
14α	2.00 (dt, 14.7, 3.8)	1.97 (dt, 14.7, 3.9)	1.92 (dt, 14.6, 3.8)	2.00–2.10 (m)
14β	1.44–1.50 (m)	1.50–1.56 (m)	1.50–1.60 (m)	1.31–1.35 (m)
16	1.14 (s)	1.12 (s)	1.12 (s)	1.14 (s)
17	1.08 (s)	1.02 (s)	1.04 (s)	1.05 (s)
18	1.78 (s)	1.76 (s)	1.76 (s)	1.77 (s)
19	1.53 (s)	1.46 (s)	1.48 (s)	1.61 (s)
10-O$CH_2$$CH_3$			3.41 (q, 7.0)	
10-OCH$_2$$CH_3$			1.15 (t, 7.0)	
10-OCOCH_3				2.09 (s)
6-OCOCH$_3$				2.04 (s)
5-OCOCH_3				2.07 (s)

[a] Spectra recorded at 300 MHz for ^1H and 100 MHz for ^{13}C in CD_3OD.
[b,c] Spectra recorded at 300 MHz for ^1H and 100 MHz for ^{13}C in DMCO-d_6.
[d] Spectra recorded at 300 MHz for ^1H and 100 MHz for ^{13}C in $CDCl_3$.
[e] The *J*-values (Hz) are shown in parentheses.

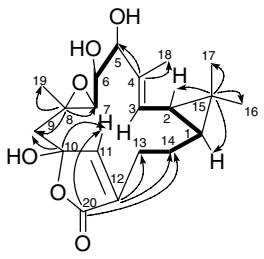


Figure 2. ^{1}H–^{1}H COSY correlations and key HMBC correlations of compound **1**.

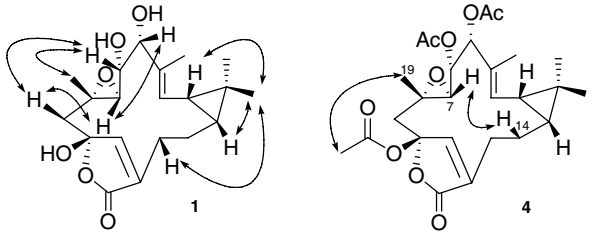


Figure 3. Key ROESY correlations of compounds **1** and **4**.

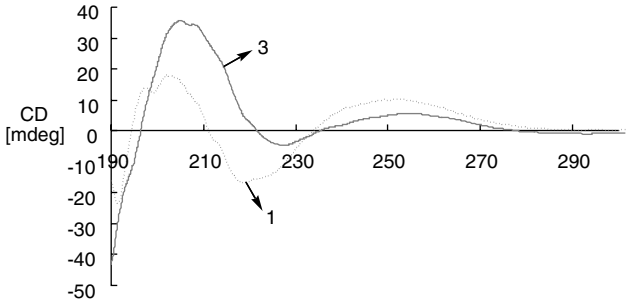
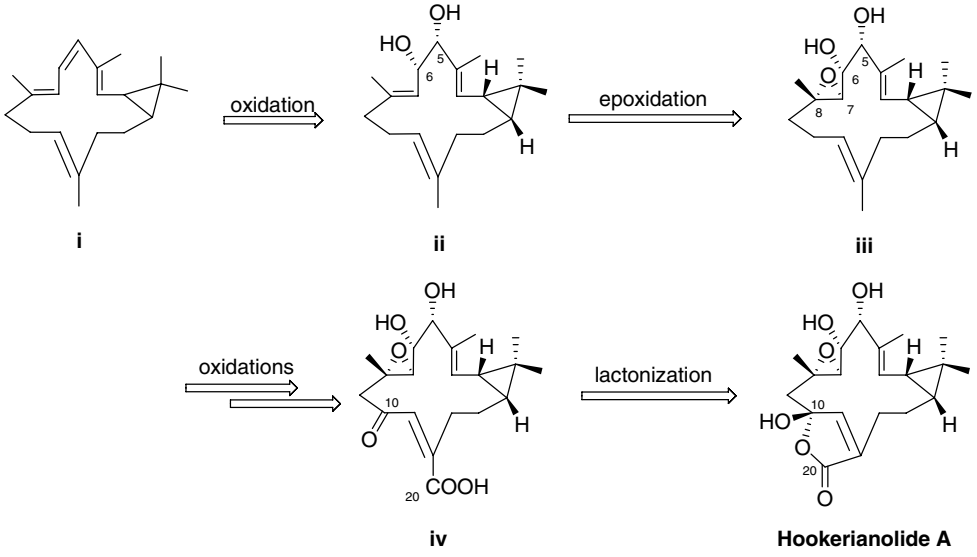


Figure 4. CD spectra of compounds **1** and **3**.

^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1**, except for the presence of an additional hydrogen atom at δ_H 5.05 (H-10) and an oxygenated methine at δ_C 78.5 (C-10) instead of an hemiketal moiety in **1**, indicating that the hydroxyl of C-10 in **1** was replaced by a hydrogen in **2**. This conclusion was further confirmed by the ^{1}H–^{1}H COSY, HSQC, and HMBC correlations. The relative stereochemistry of **2** was in agreement with that of **1** due to the similar ROESY correlations and by comparison of their NMR data. The H-10 correlated to CH_3-19 in the ROESY spectrum, suggesting that H-10 was β-oriented. **2** was considered to be 10-deoxy Hookerianolide A.

Table 2. ^{13}C NMR Data of Hookerianolides A (**1**), B (**2**), C (**3**) and Hookerianolide A triacetate (**4**)

No.	1	2	3	4
1	32.4 (d)	30.9 (d)	30.5 (d)	30.9 (d)
2	28.7 (d)	28.8 (d)	26.9 (d)	26.8 (d)
3	130.6 (d)	128.4 (d)	128.7 (d)	133.8 (d)
4	136.4 (s)	133.9 (s)	135.5 (s)	128.7 (s)
5	83.3 (d)	81.0 (d)	81.9 (d)	79.6 (d)
6	72.5 (d)	71.2 (d)	71.0 (d)	69.4 (d)
7	65.0 (d)	64.8 (d)	63.3 (d)	60.4 (d)
8	62.1 (s)	59.8 (s)	60.0 (s)	59.9 (s)
9	49.5 (t)	44.5 (t)	48.6 (t)	47.8 (t)
10	103.7 (s)	78.5 (d)	107.7 (s)	104.2 (s)
11	148.7 (d)	148.6 (d)	145.7 (d)	143.6 (d)
12	137.0 (s)	135.5 (s)	137.8 (s)	136.9 (s)
13	28.0 (t)	27.2 (t)	26.8 (t)	26.5 (t)
14	21.6 (t)	20.4 (t)	21.8 (t)	20.0 (t)
15	23.3 (s)	21.7 (s)	21.8 (s)	22.6 (s)
16	16.3 (q)	15.5 (q)	15.5 (q)	15.5 (q)
17	29.7 (q)	28.8 (q)	28.6 (q)	28.6 (q)
18	12.4 (q)	11.8 (q)	11.4 (q)	11.7 (q)
19	20.3 (q)	17.9 (q)	19.7 (q)	19.1 (q)
20	173.9 (s)	173.5 (s)	170.7 (s)	170.0 (s)
10-O*CH*$_2$CH$_3$			59.6 (t)	
10-OCH$_2$*CH*$_3$			15.2 (q)	
10-O*CO*CH$_3$				167.8 (s)
10-OCO*CH*$_3$				21.8 (q)
6-O*CO*CH$_3$				169.6 (s)
6-OCO*CH*$_3$				21.1 (q)
5-O*CO*CH$_3$				169.9 (s)
5-OCO*CH*$_3$				20.7 (q)



Scheme 1. Biogenetic pathway proposed for Hookerianolide A.

Hookerianolide C (**3**)[15] was isolated as a white powder. HR-ESIMS spectrum provided the molecular formula $C_{22}H_{32}O_6$. The ^1H and ^{13}C NMR spectral data of **3** showed close similarity to those of **1**, except for the presence of an additional oxygenated ethyl at δ_H 3.41 (q, $J = 7.0$ Hz) and δ_H 1.15 (t, $J = 7.0$ Hz). The HMBC spectrum of **3** yielded correlations between δ_H 3.41 (10-OCH_2CH$_3$) and both the δ_C 107.7 (C-10) and δ_C 15.2 (10-OCOCH_3), indicating that **3** was an 10-ethyl ether of **1**. The relative stereochemistry of **3** was identical to that of **1** as confirmed by ROESY experiments. The absolute configuration at C-10 was also proposed to be *R*, in accordance with the CD spectrum. Compound **3** is proposed to be an artifact, which may result from etherization of Hookerianolide A with ethanol during the extraction process (Table 2).

The biogenetic origin of Hookerianolide A is proposed in Scheme 1. The oxidation of C-5, C-6 in **i** was subsequently followed by epoxidation of C-7, C-8 in intermediate **ii**, and then oxidation of C-10 and C-20 in intermediate **iii** gave the key intermediate **iv**. The intermediate **iv** was further lactonized to afford Hookerianolide A.

Hookerianolides A–C are a new class of casbanoid-derived diterpenes and are the first examples of multiple oxidations and cyclization modes of casbanoids within the family Euphorbiaceae with the α,β-unsaturated γ-lactone functionality.

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10. Hookerianolide A: white amorphous powder; mp 174–176 °C; $[\alpha]_D^{20}$ −28.1 (*c* 0.31, CH_3OH); CD (*c* 1.7×10^{-3} M, MeOH) $\Delta\varepsilon + 3.63$ (252.8), 0 (233.7), −5.93 (218.6), 0 (211.1), +6.43 (202.0); UV (MeOH) λ_{max} (log ε) 202 (3.55) nm; IR (KBr) ν_{max} 3502, 3388, 2935, 1758, 1730, 1431, 1061, 953, 824 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive ESIMS *m/z* 387.2 $[M+Na]^+$; negative ESIMS *m/z* 363.3 $[M-H]^-$; HR-ESIMS (positive) $[M+Na]^+$, *m/z* 387.1779 $[C_{20}H_{28}O_6Na]^+$ (calcd 387.1784).
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14. Hookerianolide B: white amorphous powder; mp 88–90 °C; $[\alpha]_D^{20}$ −21.5 (*c* 0.34, CH_3COCH_3); UV (MeOH) λ_{max} (log ε) 213 (3.55), 253 (3.02), 312 (2.63) nm; IR (KBr) ν_{max} 3423, 2956, 1759, 1635, 1597, 1439, 1371, 1254, 1219, 1105, 980, 783, 704 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive ESIMS *m/z* 719.5 $[2M+Na]^+$; negative ESIMS *m/z* 347.2 $[M-H]^-$; HR-ESIMS (positive) $[2M+Na]^+$, *m/z* 719.3741 $[C_{40}H_{56}O_{10}Na]^+$ (calcd 719.3771).
15. Hookerianolide C: white amorphous powder; mp 71–73 °C; $[\alpha]_D^{20}$ −38.7 (*c* 0.60, CH_3COCH_3); CD (*c* 1.8×10^{-3} M, MeOH) $\Delta\varepsilon + 1.84$ (255.1), 0 (235.2), −1.53 (227.5), 0 (221.5), +11.7 (205.2); UV (MeOH) λ_{max} (log ε) 202 (3.59), 273.5 (2.57) nm; IR (KBr) ν_{max} 3448, 3105, 2926, 1770, 1649, 1288, 1200, 1068, 955, 775 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive ESIMS *m/z* 415.4 $[M+Na]^+$; negative ESIMS *m/z* 391.1 $[M-H]^-$; HR-ESIMS (positive) $[M+Na]^+$, *m/z* 415.2106 $[C_{22}H_{32}O_6Na]^+$ (calcd 415.2097).