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Note

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Hortonones A to C, Hydroazulenones from the Genus *Hortonia*

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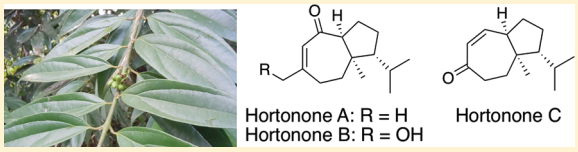
Supporting Information

ABSTRACT: The new hexahydroazulenones hortonones A (**1**) to C (**3**) were isolated from the leaves of three representative species of the endemic Sri Lankan genus *Hortonia* that belongs to the family Monimiaceae. Hortonones A (**1**) and B (**2**) have the unprecedented rearranged hortonane sesquiterpenoid carbon skeleton, and hortonone C (**3**) has the unprecedented rearranged and degraded 13-norhortonane skeleton. Hortonone C (**3**) exhibited in vitro cytotoxicity against human breast cancer MCF-7 cells at 5 μg/mL.

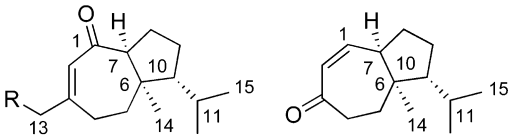


Sri Lanka has a high rate of biodiversity and biological endemism, which is impressive considering its proximity to the mainland. This has led to the proposal that during the continental drift of the Indian plate that separated the island, Sri Lanka may have experienced a higher degree of species extinction and that this may have created ecological opportunities for speciation by the remaining taxa.[1]

Hortonia is an endemic Sri Lankan plant genus belonging to the family Monimiaceae Juss, which is believed to have originated in Gondwanaland 100−120 million years ago.[2] The latest revision of the family Monimiaceae by Dassanayake lists three distinct species of *Hortonia* (*H. angustifolia* Trimen, *H. floribunda* Wight ex Arn., and *H. ovalifolia* Wight).[3] *H. angustifolia* is found in moist lowlands at elevations of about 700 m, *H. floribunda* is found in the montane forests above 1300 m, and *H. ovalifolia* is found at elevations above 1600 m. Differences in leaf morphology were taken as the key factor in the taxonomic differentiation of the three species.

Intrigued by the similar TLC patterns and mosquito larvicidal activity of the leaf extracts of the three species,[4] we investigated the phytochemistry of the three plants. Previously, we have reported the isolation of several new butenolides,[5,6] some with mosquito larvicidal activity, from the leaves of all three species. In our continued investigation of the phytochemistry of these plants, we have isolated and characterized three new rearranged hexahydroazulenones, hortonones A (**1**) to C (**3**), from each of the three species, which provides additional evidence for the phytochemical similarity of these plants. Details of the isolation, structure elucidation, and biological activities of **1**−**3** are described.

Specimens of *Hortonia* spp. were collected from the foothills of Adam's Peak in Sri Lanka. Air-dried, powdered leaves were repeatedly extracted with CH_2Cl_2 and dried in vacuo to obtain a black oil. This crude extract was subjected to repeated rounds of Si gel chromatography to yield pure samples of hortonones A (**1**) to C (**3**).



Hortonone A (**1**), obtained as an optically active, colorless oil, gave a $[M + Na]^+$ ion in the HRESIMS at m/z 243.1728, consistent with the molecular formula $C_{15}H_{24}O$, requiring four sites of unsaturation. The ^{13}C NMR data obtained for **1** (Table 1) identified resonances that could be assigned to 15 carbon atoms, in agreement with the HRESIMS data. The ^1H NMR spectrum of **1** contained one downfield methyl singlet (δ 1.91), one upfield methyl singlet (δ 1.05), and two methyl doublets [δ 0.87 (J = 6.7 Hz), 0.90 (J = 6.6 Hz)]. Analysis of the $^1H/^{13}C$/gCOSY/gHSQC/gHMBC NMR data (Table SI-1) obtained for **1** revealed the presence of an α,β-unsaturated carbonyl [δ 203.4 (C-1); 5.75 (H-2), 129.6 (C-2); 158.7 (C-3)] bearing the downfield olefinic methyl group [δ 1.91 (H-13), 26.4 (C-13)] on the β-carbon, along with four methylenes [δ 2.29/2.62 (H-4), 32.9 (C-4); 1.43/2.00 (H-5), 41.9 (C-5); 1.53/2.00 (H-8), 23.1 (C-8); 1.57/1.93 (H-9), 30.1 (C-9)], three additional methines [δ 2.75 (H-7), 65.1 (C-7); 1.12 (H-10), 55.9 (C-10); 1.56 (H-11), 30.1 (C-11)], and one saturated quaternary carbon [δ 48.5 (C-6)]. The α,β-unsaturated carbonyl moiety, the presence of which was supported by a UV absorption at λ_{max} 242 nm, accounted for two of the sites of unsaturation. A

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Table 1. ^{1}H and ^{13}C NMR Data for Hortonones A (1) to C (3)

	1[a]		2[b]		3[c]	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	203.4		204.5		152.4	6.38, dd (12.0, 4.9)
2	129.6	5.75, s	126.1	6.03, s	131.6	5.79, dt (12.0, 1.7)
3	158.7		159.9		204.2	
4	32.9	2.62, dd (18.5, 10.8)	27.7	2.45, dd (18.6, 11.1)	40.4	2.49, ddd (17.4, 11.0, 1.6)
		2.29, dd (18.5, 7.2)		2.20, dd (18.6, 7.2)		2.45, ddt (17.4, 7.3, 1.7)
5	41.9	2.00, m	40.9	2.03, m	35.9	1.87, ddd (14.4, 7.3, 1.6)
		1.43, m		1.43, m		1.71, m
6	48.5		48.3		48.8	
7	65.1	2.75, dd (7.6, 2.4)	65.0	2.76, dd (7.6, 2.4)	51.8	2.64, m
8	23.1	2.00, m	22.7	1.98, m	30.8	2.04, m
		1.53, m		1.56, m		
9	30.1	1.93, m	30.0	1.98, m	29.9	1.56, m
		1.57, m		1.54, m		1.46, m
10	55.9	1.12, m	55.1	1.16, dt (10.6, 8.4)	57.3	1.33, m
11	30.1	1.56, m	29.8	1.55, m	30.9	1.60, m
12	23.5	0.90, d (6.6)	23.1	0.89, d (6.6)	23.4	0.96, d (6.7)
13	26.4	1.91, s	66.4	4.17, d (15.8)		
				4.13, d (15.8)		
14	21.2	1.05, s	20.9	1.04, s	21.6	1.03, s
15	23.4	0.87, d (6.7)	23.1	0.87, d (6.6)	23.3	0.90, d (6.6)

[a]Spectra collected in acetone-d_6 at 400 MHz. [b]Spectra collected in CD_2Cl_2 at 600 MHz. [c]Spectra collected in acetone-d_6 at 600 MHz.

lack of ^{13}C NMR evidence for further unsaturated functionality required that hortonone A (**1**) must contain two rings.

The olefinic proton of the α,β-unsaturated ketone carbonyl (H-2, δ 5.75) showed an HMBC correlation to a methylene carbon at δ 32.9 (C-4) that correlated in the HSQC spectrum to proton resonances at δ 2.29/2.62 (H-4). The H-4 resonances in turn were shown to couple to the H-5 protons resonating at δ 1.43/2.00 (HSQC correlations to δ 41.9) in the COSY spectrum. A methyl singlet resonating at δ 1.05 (Me-14) correlated to the C-5 methylene resonance (δ 41.9), the quaternary C-6 resonance (δ 48.5), and the C-7 (δ 65.1) and C-10 (δ 55.9) methine resonances. Since the H-7 methine proton (δ 2.75) showed an HMBC correlation to the unsaturated carbonyl resonance at δ 203.4 (C-1), the presence of a 3-methyl-6,7-disubstituted-hepten-1-one ring was established. COSY correlations between H-7 (δ 2.75) and H-8 (δ 2.00/1.53), and additional HMBC correlations from H-7 to C-9 (δ 30.1) and C-10 (δ 55.9), and COSY correlations from the two H-9 resonances (δ 1.57/1.93) to the H-10 methine resonance (δ 1.12) established that a five-membered ring was fused to the seven-membered ring at C-6 and C-7. Finally, the two methyl doublets (Me-12: δ 0.90; Me-15: δ 0.87), which each showed COSY correlations to the H-11 methine resonance (δ 1.56), both correlated in the HMBC to the C-10 methine carbon (δ 55.9), placing an isopropyl moiety at C-10.

A strong NOESY correlation between H-7 (δ 2.75) and Me-14 (δ 1.05) established that the C-6/C-7 ring junction was *cis*, and a NOESY correlation between Me-14 (δ 1.05) and H-11 (δ 1.56) established that the C-6 methyl group and the C-10 isopropyl group were *cis* to each other. Thus, hortonone A (**1**) contains a *cis*-hydroazulenone ring system with methyl substituents at C-3 and C-6 and an isopropyl substituent at C-10. The relative configuration of **1** is 6*R**,7*S**,10*R**.

Hortonone B (**2**), obtained as an optically active, colorless oil, gave an $[M + Na]^+$ ion in the HRESIMS at m/z 259.1664, appropriate for a molecular formula of $C_{15}H_{24}O_2$, which differed from the molecular formula of **1** by the addition of an oxygen atom. The ^{1}H NMR spectrum of **2** was markedly similar to that of **1**, the major difference being the absence of the olefinic methyl singlet assigned to Me-13 (δ 1.91). Instead, the ^{1}H NMR spectrum of **2** contained downfield methylene resonances at δ 4.13 and 4.17 (H-13) that showed HSQC correlations to a carbinol carbon at δ 66.4 (C-15) and HMBC correlations to two olefinic carbons (C-2 and C-3; δ 126.1 and 159.0, respectively) and a methylene carbon (C-4; δ 27.7). This set of HSQC and HMBC correlations indicated that **2** was the C-13 hydroxy analogue of **1**. All other structural features of **1** and **2** were identical.

Hortonone C (**3**) gave an $[M]^+$ ion in the HREIMS at m/z 206.1663, consistent with the molecular formula $C_{14}H_{22}O$, which differed from that of **1** by the loss of CH_2 but still required four sites of unsaturation. The ^{1}H NMR spectrum of **3** was similar to that of **1**, but as with **2**, the olefinic methyl singlet assigned to Me-13 (δ 1.91) was absent. In the NMR data for **3**, there was no evidence for an alkyl substituent of any kind at C-3. Analysis of the UV (λ_{max} 244 nm) and 1D and 2D NMR data (Tables 1 and SI-3) indicated that **3** contained an α,β-unsaturated carbonyl moiety [δ 6.38 (H-1), 152.4 (C-1); 5.79 (H-2), 131.6 (C-2); 204.2 (C-3)] but lacked the 3-methyl or 3-hydroxymethyl group found in **1** and **2**, respectively. In addition to coupling, as expected, to the α olefinic proton (H-2, δ 5.79) the β olefinic proton resonating at δ 6.38 (H-1; dd, J = 12.0, 4.9 Hz) also showed a COSY correlation to the resonance at δ 2.64, assigned to H-7. H-7 (δ 2.64) in turn correlated to H-8 (δ 2.04) in the COSY spectrum, and it showed HMBC correlations to the olefinic carbons C-1 (δ 152.4) and C-2 (δ 131.6) as well as to carbons in the five-membered ring (δ 29.9 (C-9); 57.3 (C-10)) and the C-6 bridgehead methyl carbon (δ 21.6 (C-14)). These correlations suggested that the α,β-unsaturated carbonyl in **3** was transposed relative to **1** and **2** with no alkyl substituent on the β carbon. This was confirmed by the observation that the methylene resonances at δ 2.45 and 2.49, assigned to H-4, were α to the carbonyl carbon [δ 204.2

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(C-3)] since they exhibited HMBC correlations to both C-2 (δ 131.6) and C-3 (δ 204.2). The H-5 methylene protons (δ 1.71/1.87) were correlated to the H-4 protons (δ 2.45/2.49) in the COSY spectrum, and since the bridgehead Me-14 resonance (δ 1.03) correlated to C-5, C-6, C-7, and C-10 (δ 35.9, 48.8, 51.8, and 57.3, respectively) in the HMBC spectrum, the structural assignment of **3** was confirmed. NOESY correlations established the relative configuration of hortonone C (**3**) to be 6*R**,7*R**,10*R** as in **1** and **2**.

Hortonones A (**1**), B (**2**), and C (**3**) were evaluated for in vitro cytotoxicity. Hortonone C (**3**) was cytotoxic toward human breast cancer MCF-7 cells at 5 μg/mL, while hortonones A (**1**) and B (**2**) were not active at concentrations as high as 100 μg/mL.

Hortonones A (**1**) and B (**2**) represent the first examples of the rearranged "hortonane" sesquiterpenoid skeleton, which likely arises from cyclization of *trans,cis*-farnesol pyrophosphate followed by methyl migration from C-7 to C-6. Hortonone C (**3**), missing the C-3 methyl substituent, appears to be the first example of the rearranged and degraded "13-norhortonane" terpenoid skeleton.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using a Jasco P-1010 polarimeter with sodium light (589 nm). The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-600 spectrometer with a 5 mm CPTCI cryoprobe or on a Bruker Avance 400 spectrometer. ^{1}H chemical shifts are referenced to the residual acetone-d_6 or CD_2Cl_2 signal (δ 2.05 and 5.32, respectively), and ^{13}C chemical shifts are referenced to the acetone-d_6 or CD_2Cl_2 solvent peak (δ 29.92 and 53.8 ppm, respectively). Low- and high-resolution ESI-QIT-MS were recorded on a Bruker-Hewlett-Packard 1100 Esquire-LC system mass spectrometer. Low- and high-resolution EIMS were recorded on a Kratos AEI MS-50 mass spectrometer. MPLC and Si gel flash chromatography were performed using Merck Si gel 60 (230−400 mesh). Merck type 5554 silica gel plates and Whatman MKC18F plates were used for analytical thin-layer chromatography.

Plant Material. Specimens of *H. angustifolia*, *H. floribunda*, and *H. ovalifolia* were collected from Kanneliya Hakgala, in the foothills of Adam's Peak, Sri Lanka, in September 2005. *H. angustifolia* was collected at 700 m, *H. floribunda* was collected in the montane forests above 1300 m, and *H. ovalifolia* was collected at elevations above 1600 m. The plants were identified by one of the authors (S.W.). Voucher specimens (*H. angustifolia* PDA 526; *H. floribunda* PDA 24083; *H. ovalifolia* PDA 522) were deposited at the National Herbarium, Peradeniya, Sri Lanka.

Extraction and Isolation of Compounds. Air-dried, powdered leaves of *H. angustifolia* (650 g) were extracted with CH_2Cl_2 (3 × 700 mL) at 27 °C for 24 h. The combined CH_2Cl_2 extracts were concentrated in vacuo to obtain a black oil (37.4 g), which was subjected to MPLC on Si gel (eluent: step gradient from hexanes to EtOAc) to provide six fractions. The second fraction (5.85 g) was subjected to MPLC on silica gel using a gradient from hexanes to CH_2Cl_2 followed by isocratic MPLC on silica gel (2:3 CH_2Cl_2/hexane) and a final isocratic MPLC purification on Si gel (8:92 EtOAc/hexanes) to give hortonones A (**1**) (15 mg) and C (**3**) (30 mg). The fourth fraction from the first MPLC column (22.86 g) was subjected to MPLC on Si gel using a gradient from hexanes to MeOH, followed by isocratic Si gel flash chromatography (3:7 EtOAc/hexanes) to give hortonone B (**2**) (5.3 mg). Hortonones A (**1**) to C (**3**) were all isolated using the same chromatographic conditions and in similar yields from the leaves of all three species of *Hortonia*.

*Hortonone A (**1**):* colorless oil; $[\alpha]^{20}_D$ +24 (*c* 0.47, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 242 (3.34) nm; ^{1}H and ^{13}C NMR data see Table 1; (+)-HRESIMS *m/z* $[M + Na]^+$ 243.1728 (calcd for $C_{15}H_{24}ONa$, 243.1725).

*Hortonone B (**2**):* colorless oil; $[\alpha]^{20}_D$ +24 (*c* 0.27, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 241 (3.38) nm; ^{1}H and ^{13}C NMR data see Table 1; (+)-HRESIMS *m/z* $[M + Na]^+$ 259.1664 (calcd for $C_{15}H_{24}O_2Na$, 259.1674).

*Hortonone C (**3**):* colorless oil; $[\alpha]^{20}_D$ +74 (*c* 4.4, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 244 (3.38) nm; ^{1}H and ^{13}C NMR data see Table 1; HREIMS *m/z* $[M]^+$ 206.1663 (calcd for $C_{14}H_{22}O$, 206.1671).

Cytotoxicity Assay. MCF-7 mp-53 cells were maintained in RPMI-1640 medium with 10% (v/v) fetal bovine serum; 10 000 cells were seeded per well in a 96-well plate and grown for 18 h. Cells were treated with various concentrations of drugs for 48 h, and cell viability was measured at the end of the incubation period using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay (M2128, Sigma) as described.[7]

ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR NMR spectra and tables of 2D NMR data for compounds **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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