Four new butanolide derivatives from *Hortonia*, a genus endemic to Sri Lanka

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Three new butanolides, (2*E*,3*R*,4*R*,9'*Z*)-2-(dodec-9'-en-11'-ynylidene)-3-hydroxy-4-methylbutanolide **(1)**, (2*E*,3*R*,4*R*)-2-(dodeca-9',11'-diynylidene)-3-hydroxy-4-methylbutanolide**(2)**,(2*E*,3*R*,4*R*,9'*E*)-2-(dodeca-9',11'-dienylidene)-3-hydroxy-4-methylbutanolide **(3)** and one new ring-opened butanolide, methyl (2*Z*,11*Z*,1'*R*,2'*R*)-2-(1',2'-dihydroxypropyl) tetradeca-2,11-dien-13-ynoate **(4)** were isolated from the leaves of the three representative species of the endemic primitive genus *Hortonia* (Monimiaceae), namely *H. angustifolia*, *H. floribunda* and *H. ovalifolia*. Their structures were elucidated on the basis of spectroscopic evidence.

Keywords: *Hortonia angustifolia, Hortonia floribunda, Hortonia ovalifolia,* Monimiaceae, new butanolide derivatives, chemotaxonomic significance

Hortonia is a genus endemic to Sri Lanka, and which is considered to have originated in Gondwanaland about 100–120 million years ago.[1] It is regarded as a surviving representative of the ancestral stock from which members of the Monimiaceae alliance have evolved. It is included in the Monimiaceae because it resembles the remaining family members more than the Monimiaceae alliance.[2] Members of this family are trees or shrubs and rarely climbers. The family comprises about 39 genera, of which 440 species are widely spread in the Southern Hemisphere, mainly tropical and subtropical regions of the Americas.[3] This family occurs in Sri Lanka, Oceania, Polynesia, Australia, Malaysia, Madagascar and South America.[3,4] They are very rare in Africa and absent in India. *Siparuna* and *Mollinedia* comprising 150 and 94 species respectively are the two major genera of Monimiacea.[4]

Wight described three species of *Hortonia* (*H. floribunda*, *H. ovalifolia,* and *H. acuminata*) from the wet zone of Sri Lanka.[5] Thawaites considered all three species to be varieties of *H. floribunda* having numerous intermediate forms.[6] Trimen, on the other hand, considered *H. floribunda* and *H. angustifolia* as two different species.[7] An oval-leaved species collected at Adam's Peak, in central Sri Lanka, was classified as a variety of *H. floribunda.* The latest revision of the family Monimiaceae by Dassanayake lists only three distinct species (*H. floribunda* Wight ex Arn., *H. angustifolia* (Thw.) Trimen, and *H. ovalifolia* Wight) in Sri Lanka.[8] Having observed the identity in the TLC profile of the CH_2Cl_2 extracts of the leaves of all three species, a phytochemical study was undertaken to resolve the speciation of *Hortonia*. Previously, we have reported the isolation of two new butenolides from the leaves of all three species (*H. angustifolia, H. floribunda* and *H. ovalifolia*).[9] In the course of further investigation of the leaves of these three plants, we have isolated three new butanolides, **1–3**, and a new ring-opened butanolide, **4**, whose structures are reported here (Fig. 1).

Butanolide **1**, obtained as a colourless oil, was shown by HRESIMS (299.1631, $[M + Na]^+$) to possess a molecular formula of $C_{17}H_{24}O_3$. The $^1H/^{13}C$/DEPT/GHMQC/HMBC NMR data obtained for **1** showed 17 carbon resonances (1 × CH_3, 7 × CH_2, 6 × CH, 3 × C). The downfield $^1H/^{13}C$ NMR resonances (Table 1) were assigned to an exocyclic double bond which was conjugated to a γ-lactone moiety (δ_H 6.80, H-1'; δ_C 145.9, C-1'; δ_C 130.9, C-2; δ_C 169.1, C-1). A methine carbon bearing a methyl group was linked to the γ-lactone oxygen (δ_H 3.80, H-4; δ_C 77.9, C-4; δ_H 1.08, H-5; δ_C 13.6, C-5), and it was bonded to a second methine carbon bearing a hydroxyl group (δ_H 4.05, H-3; δ_C 67.2, C-3). The coupling constants between H-3 and H-4 (J = 5.2 Hz), and a NOESY correlation between H-3 and H-4, indicated a *cis*-relationship between the substituents at C-3 (OH) and C-4 (Me) of the butanolide **1**.[10] Based on literature precedent, the positive optical rotation of compound **1** indicated that the absolute stereochemistry at C-3 and C-4 were *R*.[10] The configuration of the tri-substituted double bond in butanolide **1** was shown to be *E* from the chemical shift value of H-1' (δ_H 6.80) in the ^1H NMR spectrum and the correlation between H-3 and H-2' observed in the NOESY spectrum.[11] The substituent attached to C-1' was assigned to an 11 carbon chain with

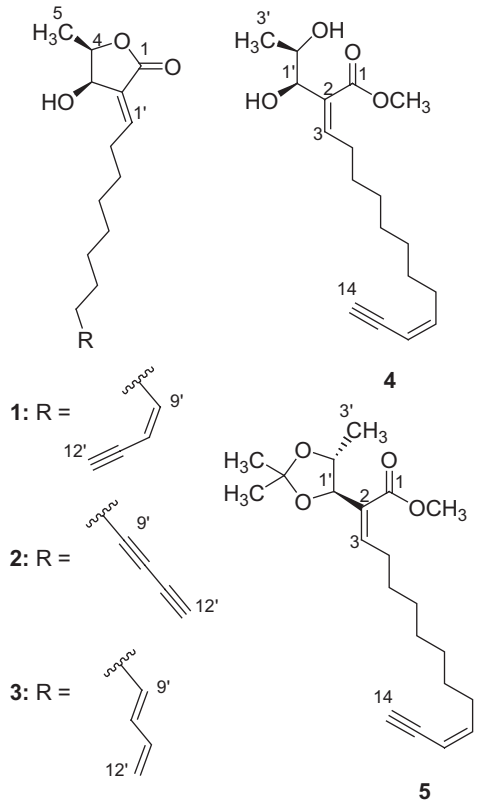

Fig. 1 Structures of compounds **1–5.**

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Table 1 ^{1}H and ^{13}C NMR data for **1**, **2** and **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	169.1		169.1		170.2	
2	130.9		130.1		130.4	
3	67.2	4.05 br, s (3.9)	67.1	4.82 br d (4.8)	67.6	4.78 br d (5.5)
4	77.9	3.80 dq (4.8, 3.9)	78.2	4.52 m	78.9	4.48 dq (6.6, 5.5)
5	13.6	1.08 d (4.8)	13.3	1.43 d (6.0)	13.9	1.42 d (6.6)
1'	145.9	6.80 td (5.7, 1.2)	146.6	6.90 td (8.4, 1.2)	147.6	6.89 t (7.9)
2'	29.3	1.95 m	29.2	2.40 m	29.7	2.36 m
3'	29.0	1.10–1.26 m	28.2	1.53 m	28.3	1.48 m
4'–7'	28.0–30.0[d]	1.10–1.50 m	28.0–30.0[d]	1.30–1.39 m	28.0–30.0[d]	1.25–1.35 m
8'	30.0	2.27 td (5.6)	18.4	2.28 t (7.2)	32.4	2.03 m
9'	145.2	5.66 td (10.8, 5.7)	78.1		135.3	5.66 td (15.1, 7.0)
10'	108.4	5.35 ddt (10.8, 5.7, 2.7)	63.9		130.9	6.01 dd (15.1,10.3)
11'	80.6		67.7		137.2	6.27 td (17.0,10.3)
12'	81.6	2.80 s	64.0	2.04 s	114.6	5.05 br d (17.0) 4.92 br d (10.3)

[a]Spectra collected in C_6D_6 at 400 MHz.
[b]Spectra collected in CD_2Cl_2 at 600 MHz.
[c]Spectra collected in $CDCl_3$ at 400 MHz.
[d]Signals are interchangeable.

a *Z*-double bond (δ_H 5.66, H-9'; δ_C 145.2, C-9'; δ_H 5.35, H-10'; δ_C 108.4, C-10') conjugated to a terminal ethynyl group (δ_H 2.80, H-12'; δ_C 81.6, C-12'; δ_C 80.6, C-11'). Based on these data, compound **1** was (2*E*,3*R*,4*R*,9'*Z*)-2-(dodec-9'-en-11'-ynylidene)-3-hydroxy-4-methylbutanolide.

Butanolide **2**, which was obtained as a colourless oil, was shown by HRESIMS (297.1469, $[M + Na]^+$) to possess a molecular formula of $C_{17}H_{22}O_3$. The $^{1}H/^{13}C$/DEPT/HMQC/HMBC NMR data (Table 1) obtained for **2** showed 17 carbon resonances (1 × CH_3, 7 × CH_2, 4 × CH, 5 × C). From an analysis of the $^{1}H/^{13}C$ NMR and the optical rotation data, it was apparent that compound **2** had the same *cis*-(3*R*,4*R*)-3-hydroxy-4-methyl–γ-lactone structure with an *E*-alkylidene group (δ_H 6.90, H-1') as that of butanolide **1**.[11] The group attached to C-2 was also composed of a 12 carbon fragment, with a terminal butadiyne (δ_C 78.1, C-9'; δ_C 63.9, C-10'; δ_C 67.7, C-11'; δ_H 2.04, H-12'; δ_C 64.0, C-12'). Thus, compound **2** was (2*E*,3*R*,4*R*)-2-(dodeca-9',11'-diynylidene)-3-hydroxy-4-methylbutanolide.

Butanolide **3**, obtained as a colourless oil, was shown by HRESIMS (301.1911, $[M + Na]^+$) to possess a molecular formula of $C_{17}H_{26}O_3$. The $^{1}H/^{13}C$/DEPT/HMQC/HMBC NMR data (Table 1) showed 17 carbon resonances (1 × CH_3, 8 × CH_2, 6 × CH, 2 × C). From an analysis of $^{1}H/^{13}C$ NMR and optical rotation data, it was apparent that compound **3** was related to compounds **1** and **2**. It too had the same *cis*-(3*R*,4*R*)-3-hydroxy-4-methyl–γ-lactone structure with an *E*-alkylidene group (δ_H 6.89, H-1') found in **1** and **2**. The only difference in **3** was that the side chain attached to C-2 ended in a butadiene (δ_H 5.66, H-9'; δ_C 135.3, C-9'; δ_H 6.01, H-10'; δ_C 130.9, C-10'; δ_H 6.27, H-11'; δ_C 137.2, C-11'; δ_H 4.92, H-12'; δ_H 5.05, H-12'; δ_C 114.6, C-12'). These data showed compound **3** to be (2*E*,3*R*,4*R*,9'*E*)-2-(dodeca-9',11'-dienylidene)-3-hydroxy-4-methylbutanolide.

Compound **4**, obtained as a colourless oil, was shown by HRESIMS (331.1895, $[M + Na]^+$) to possess a molecular formula of $C_{18}H_{28}O_4$. The $^{1}H/^{13}C$/DEPT/HMQC/HMBC NMR data (Table 2) showed 18 carbon resonances (2 × CH_3, 7 × CH_2, 6 × CH, 3 × C). From this spectroscopic data it was clear that the 3-hydroxy-4-methyl butanolide ring found in compounds **1**–**3** was opened in compound **4**. The chemical shift values of the two methine residues in compound **4** had shifted (δ_H 3.78, H-2'; δ_C 69.8, C-2'; δ_H 3.92, H-1'; δ_C 79.1, C-1'). In an attempt to determine the relative stereochemistry of H-1' and H-2', compound **4** was converted to its acetonide, **5**. The coupling constant between H-1' and H-2' in compound **5** was 8.3 Hz. A comparison of the coupling constants of similar systems in the literature indicates a *trans*-configuration in compound **5**, corresponding to an *erythro*-relationship between the substituents at C-1' (OH) and C-2' (Me) in compound **4,** as was observed in compounds **1**–**3**.[12] Furthermore, the NOESY spectrum showed that the two singlets of the gem-dimethyl groups of the acetonide at δ_H 1.38 and δ_H 1.41 correlated to H-2' and H-1', respectively, confirming the above assignment. The presence of a methyl ester (δ_H 3.75, OMe; δ_C 51.3, OMe; δ_C 168.0, C-1) conjugated to a *Z*-double bond (δ_H 6.16, H-3; δ_C 144.7, C-3; δ_C 131.2, C-2), instead of a lactone moiety, suggested that compound **4** had a ring-opened structure. Significantly, the upfield nature of H-3 (δ_H 6.16) compared to H-1' in compounds **1**–**3**, and the NOESY correlations between H-3 and H-1', proved that the conjugated double bond had a *Z*-geometry. The substituent attached to C-3 was found to be identical to compound **1**, where the 11 carbon chain had a double bond (δ_H 6.01, H-11; δ_C 146.1, C-11; δ_H 5.44, H-12; δ_C 107.6, C-12) conjugated to a terminal ethynyl group (δ_H 3.11, H-14; δ_C 80.8, C-14; δ_C 80.3, C-13). Based on the above evidence, the ring-opened butanolide **4** was methyl (2*Z*,11*Z*,1'*R*,2'*R*)-2-(1',2'-dihydroxypropyl)tetradeca-2,11-dien-13-ynoate.

Table 2 ^{1}H and ^{13}C NMR data for **4**

	4[a]	
Position	δ_C	δ_H (*J* in Hz)
1	168.0	
2	131.2	
3	144.7	6.16 t (7.6)
4	29.4	2.40 td (7.2, 7.6)
5	28.5	1.41 m
6–9	28.0–30.0[d]	1.31 m
10	30.1	2.31 ddt (7.2, 7.6,1.6)
11	146.1	6.01 td (7.6, 10.8)
12	107.6	5.44 br d (10.8)
13	80.3	
14	80.8	3.11 d (1.6)
1'	79.1	3.92 d (6.0)
2'	69.8	3.78 br q (6.0)
3'	18.7	1.08 d (6.0)
OMe	51.3	3.75 s

[a]Spectra collected in CD_2Cl_2 at 400 MHz.
[d]Signals are interchangeable.

Interestingly, several analogous butanolides isolated from the plant *Lindera obtusiloba* Blume have exhibited cytotoxicity against human tumour cell lines.[13] Isolation of the same compounds from all three species is of chemotaxonomic significance.

Experimental

Optical rotations were measured with a JASCO P-1010 polarimeter using CH_2Cl_2 as solvent. The ^1H, ^{13}C NMR, HMQC and HMBC spectra were recorded in C_6D_6 (compound **1**), CD_2Cl_2 (compound **2** and **4**) and $CDCl_3$ (compound **3**) at 400 (compounds **1**, **3** and **4**) and 600 (compound **2**) MHz on a Bruker spectrometer and were referenced to the solvent. All two-dimensional NMR experiments were carried out using standard and in-house modified Bruker pulse sequences. ESIMS were recorded on a Fisions VG Autospec mass spectrometer operating at 70 eV (direct insertion). HRESIMS were recorded on a Micromass LCT spectrometer. Arg-Lys (compound **1** and **2**), perflurokerosene (compound **3**) and Arg-Phe (compound **4**) were used as the internal reference for HRMS measurements. Medium pressure liquid chromatography (MPLC) and flash chromatography were performed on Merck Si gel 60 (230-400 mesh). Thin-layer chromatography (TLC) was performed on Merk Si gel 60 F_{254} plates using EtOAc/hexane as eluent.

Plant material

Specimens of *H. angustifolia*, *H. floribunda*, *H. ovalifolia* were collected from Kanneliya, Hakgala, from the foothills of Adam's Peak, Sri Lanka, respectively in September 1998. 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 afford a black oil (30 g). This was subjected to MPLC on silica gel (eluent: step gradient from hexane to EtOAc) followed by silica gel flash chromatography (eluent: step gradient from hexane to EtOAc/hexane, 3:7) to provide pure butanolides **1** (53 mg), **2** (8 mg), **3** (28 mg) and **4** (16 mg). Compounds **1**–**4** were similarly isolated from leaves of all three species of Hortonia collected from all other locations in comparable yields using identical chromatographic conditions.

(2E,3R,4R,9'Z)-2-(dodec-9'-en-11'-ynylidene)-3-hydroxy-4-methylbutanolide (**1**)**:** Colourless oil, 53 mg; $[\alpha]_D^{25}$ + 47° (*c* 0.01, CH_2Cl_2); UV_{max} (CH_2Cl_2) : 230 nm (3.48); IR (dry film) ν_{max} 3428, 2361, 1741, 1678, 1509, 1340 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HR-ESIMS *m/z* 299.1631 (Calc. for $C_{17}H_{24}O_3Na$, 299.1623).

(2E,3R,4R)-2-(dodeca-9',11'-diynylidene)-3-hydroxy-4-methylbutanolide (**2**): Colourless oil, 8 mg; $[\alpha]_D^{25}$ + 12° (*c* 0.003, CH_2Cl_2); UV_{max} (CH_2Cl_2): 231 nm (4.60); IR (dry film) ν_{max} 3392, 2850, 1739, 1681, 1509, 1338 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HR-ESIMS *m/z* 297.1469 (Calc. for $C_{17}H_{22}O_3Na$, 297.1467).

(2E,3R,4R,9'E)-2-(dodeca-9',11'-dienylidene)-3-hydroxy-4-methylbutanolide (**3**): Colourless oil, 28 mg; $[\alpha]_D^{25}$ + 38° (*c* 0.026, $CHCl_3$); UV_{max} (CH_2Cl_2): 236 nm (4.38); IR (Nujol) ν_{max} 3430, 2094, 1724, 1673, 1512, 1368 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HR-ESIMS *m/z* 301.1911 (Calc. for $C_{17}H_{26}O_3Na$, 301.1931).

Methyl (2Z,11Z,1'R,2'R)-2-(1',2'-dihydroxypropyl)tetradeca-2,11-dien-13-ynoate (**4**): Colourless oil, 16 mg; $[\alpha]_D^{25}$ + 19° (*c* 0.018, CH_2Cl_2); UV_{max} (CH_2Cl_2): 229 nm (4.24); IR (dry film) ν_{max} 3437, 2096, 1724, 1671, 1510, 1380 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 331.1895 (Calc. for $C_{18}H_{28}O_4Na$, 331.1885).

Synthesis of acetonide **5**: To a solution of **4** (2.4 mg) in CH_2Cl_2 (1 ml) was added 2,2-dimethoxypropane (0.1 ml) followed by PPTS (1.5 mg). After stirring for 2 hours at room temperature, the crude mixture which was obtained was purified by Si gel flash chromatography (eluent: CH_2Cl_2) to give 1.4 mg of **5** (52%). ^1H NMR (400 MHz, CD_2Cl_2): δ 6.23 (t, *J* = 7.6 Hz, 1H, H-3), 6.04 (dt, *J* = 10.7, 7.5 Hz, 1H, H-11), 5.47 (d, *J* = 10.7 Hz, 1H, H-12), 4.21 (d, *J* = 8.3 Hz, 1H, H-1'), 3.98 (dq, *J* = 8.3, 6.0 Hz, 1H, H-2'), 3.75 (s, 3H, OMe), 3.14 (s, 1H, H-14), 2.31–2.43 (m, 4H, H-4 and H-10), 1.41 (s, 3H, $-C(CH_3)_2$), 1.38 (s, 3H, $-C(CH_3)_2$), 1.35–1.28 (m, 10H, H-5, H-6, H-7, H-8, H-9), 1.25 (d, *J* = 6.0 Hz, 3H, H-3'); HRESIMS *m/z* 371.2197 (calcd for $C_{21}H_{32}O_4Na$, 371.2198).

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