Two New Lactones with Mosquito Larvicidal Activity from Three *Hortonia* Species

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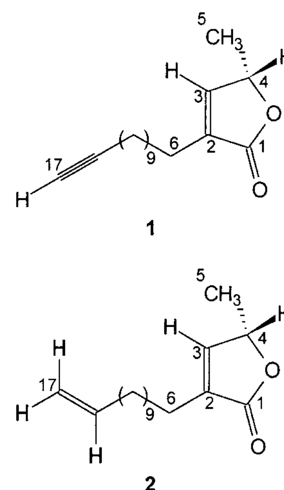
The structures of the butenolides **1** and **2**, isolated from the endemic plants *Hortonia floribunda*, *H. angustifolia, and H. ovalifolia,* collected in Sri Lanka, have been elucidated by spectroscopic analysis. These compounds exhibited potent mosquito larvicidal activity against the second instar larvae of *Aedes aegypti*.

Hortonia (family Monimiaceae), a genus endemic to Sri Lanka, is considered to have originated in Gondwanaland about 100−120 million years ago.[1] Wight in 1853 described three species of *Hortonia* (*H. floribunda*, *H. ovalifolia,* and *H. acuminata*) from the wet zone of Sri Lanka.[2] Thawaites in 1864 considered all three species to be varieties of *H. floribunda* having numerous intermediate forms.[3] Trimen in 1895 considered *H. floribunda* and *H. angustifolia* as two different species.[4] An oval-leaved species collected at Adam's Peak, in central Sri Lanka, was classified as a variety under *H. floribunda.* The latest revision of the family Monimiaceae by Dassanayake (1996) lists three distinct species (*H. floribunda* Wight ex Arn., *H. angustifolia* (Thw.) Trimen, and *H. ovalifolia* Wight) in Sri Lanka.[5] The present study was initiated with a view to isolating the bioactive compounds from the genus *Hortonia* in Sri Lanka.

Although three species of *Hortonia* are found in a range of environments from sea level (*H. angustifolia*) to the montane (*H. floribunda* and *H. ovalifolia*) regions, there have been no reports of the medicinal use of these plant in Sri Lanka. Furthermore, there have been no reports on either the chemistry or the biological activity of these species. In this first report on the chemistry and the biological activity of the genus *Hortonia*, the structures of two new mosquito larvicidal butenolides (**1** and **2**) from the leaves of all three species (*H. angustifolia*, *H. floribunda,* and *H. ovalifolia*) are described. Both compounds exhibited potent mosquito larvicidal activity.

The dichloromethane extracts of the leaves of the three species exhibited significant mosquito larvicidal activity against the second instar larvae of *Aedes aegypti* (100% mortality at 62.5 ppm). Bioassay-guided fractionation of the dichloromethane extracts yielded the butenolides **1** and **2**.



Butenolide **1**, obtained as a colorless oil, showed a self-protonated molecular ion (MH^+) at *m*/*z* 263 in its EIMS. Accurate mass measurement gave a value of *m*/*z* 263.2011, corresponding to a molecular formula of $C_{17}H_{26}O_2$. The ^1H/^{13}C/APT/GHMQC NMR data obtained for **1** showed 17 carbon resonances (1 × CH_3, 10 × CH_2, 3 × CH, 3 × C). The IR spectrum of compound **1** exhibited absorptions at 3000 and 2140, and 1755 cm^{-1}, corresponding to an ethynyl moiety and a carbonyl group, respectively. The latter, when taken together with the UV absorption of compound **1** at λ_{max} 232 nm, indicated that an α,β-unsaturated γ-lactone moiety was present. The downfield ^1H and ^{13}C NMR resonances were assigned to a double bond [δ 6.95, d, J = 1.5 Hz (δ 148.9, 133.8)], an aliphatic methine residue attached to an oxygen atom [δ 4.92, dq, J = 7.0, 1.5 Hz (δ 77.2)], and a γ-lactone carbonyl (δ 173.7) (see Table 1). Additional ^1H and ^{13}C NMR resonances could be assigned to a terminal ethynyl group [δ 1.86, t, J = 2.6 Hz (δ 84.4, 67.8)] and a secondary methyl group [δ 1.32, d, J = 7.0 Hz (δ 18.9)]. The remaining NMR resonances were assigned to 10 aliphatic methylenes. All five sites of unsaturation required by the molecular formula were accounted for by the acetylenic group and the α,β-unsaturated γ-lactone ring.

The GHMBC spectrum of compound **1** showed a correlation between H-3 and carbon C-6, indicating that they are attached to adjacent carbon atoms of the lactone ring. The connectivities in the ring were clearly established by the GHMBC data. The H-3 proton (δ 6.95) showed a two-bond correlation to C-4 (δ 77.2) and a three-bond correlation via the ring double bond to C-1 (δ 173.7). A correlation was observed between Me-4 (δ 1.32) and C-4 (δ 77.2). Furthermore, the GHMBC correlation observed between H-4 (δ 4.92) and the quaternary carbon (C-2; δ 148.9), together with that between the C-6 methylene in the side chain at δ 2.17 and C-1 (δ 173.7), established that a C_{12} side chain

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Table 1. ^{1}H (300 Mz) and ^{13}C (75 Mz) NMR Data for Butenolides **1** and **2** Recorded in $CDCl_3$

position	(1) δ_H	(1) δ_C	(2) δ_H	(2) δ_C
1		173.7		173.8
2		148.9		148.8
3	6.95 d (1.5)	133.8	6.98 dd (3.3, 1.4)	134.2
4	4.92 dq (7.0, 1.5)	77.2	4.99 m	77.4
5	1.32 d (7.0)	18.9	1.40 d (6.6)	19.1
6	2.17 t (7.1)	24.8	2.26 m	25.1
7	1.45 m	27.1	1.54 m	27.3
8	1.26 m	28.4	not resolved	not resolved
(8) 9,10,11,12,13	1.2 brs	28.7, 28.8, 28.9, 29.1, 29.1	1.2–1.4 m	29.0, 29.1, 29.2, 29.4, 29.4, 29.5
14	1.4 m	28.1	1.36 m	28.8
15	2.08 td (7.1, 2.6)	18.1	2.04 m	33.7
16		67.8	5.80 dd (17.0, 11.0)	139.2
17	1.86 t (2.6)	84.4	4.96 dd (11.0, 2.0), 4.98 dd (17.0, 2.0)	114.0

was situated at C-2. Previous chemical studies on *Litsea japonica* (Lauraceae) have resulted in the isolation of two related butenolides, hamabiwalactones A and B, which also possess a side-chain at C-2 and a methyl group at C-4, differing from compounds **1** and **2** only by the presence of a double bond between C-6 and C-7.[6] In addition, plants of the family Annonaceae elaborate a wide variety of butenolides of biological importance.[7] The stereochemistry at C-4 in compound **1** was assigned *S* configuration on the basis of biogenetic considerations.[8]

Butenolide **2**, obtained as a colorless oil, gave an M^+ ion at m/z 264.2089 in its HREIMS, corresponding to a molecular formula of $C_{17}H_{28}O_2$. The ^{1}H NMR spectrum of **2** exhibited similarities with that of compound **1**, but the former had additional peaks in the olefinic region. That the IR spectrum of compound **2** also showed a peak at 1755 cm^{-1} and an UV absorption at λ_{max} 228 nm indicated the presence of an α,β-unsaturated γ-lactone moiety. The ^{1}H and ^{13}C NMR data were assigned unambiguously by COSY, GHMQC, and GHMBC experiments, confirming compound **2** possesses a terminal double bond instead of an acetylenic group on the C_{12} side-chain as in **1**. The three protons of the terminal double bond in compound **2** appeared at δ 4.96 (dd,1H, J = 11.0, 2.0 Hz), 4.98 (dd, 1H, J = 17.0, 2.0 Hz), and 5.80 (dd,1H, 17.0, 11.0 Hz), and the two carbons at δ 114.0 and 139.2. As in the case of butenolide **1**, the stereochemistry at C-4 in compound **2** was assigned *S* configuration.[8]

The extracts of the three *Hortonia* species showing mosquito larvicidal activity had similar TLC patterns under identical elution conditions. Both butenolides **1** and **2** exhibited mosquito larvicidal activity with LC_{50} values of 0.41and 0.47 ppm, respectively.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Bellingham + Stanley ADP 220 polarimeter using $CHCl_3$ as solvent. UV spectra were obtained in CH_2Cl_2 using a Shimadzu (UV-160) spectrometer. IR spectra were recorded on a Shimadzu (IR-408) spectrometer as a film on NaCl. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 300 and 75 MHz, respectively, on a Varian VXR300S spectrometer and were referenced to the solvent (^{1}H 7.26 ppm; ^{13}C 77.0 ppm). GHMQC and GHMBC experiments were carried out at 500 MHz on a Varian Inova NMR spectrometer. All two-dimensional NMR experiments were carried out using standard and in-house modified Varian pulse sequences. EIMS were recorded on a Fisons VG Autospec mass spectrometer operating at 70 eV (direct insertion). Perfluorokerosine was 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 Merck Si gel 60 F_{254} plates using CH_2Cl_2 as eluent.

Plant Material. Specimens of *H. angustifolia* were collected from Kanneliya (Southern Province), *H. floribunda* from Hakgala (Central Province), and *H. ovalifolia* from the foothills of Adam's Peak (Central Province), Sri Lanka, in September 1998. The plants were identified by Dr. Siril Wijesundara, Royal Botanic Gardens, Peradeniya, Sri Lanka, and 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. Air-dried, powdered leaves of *H. angustifolia* were extracted repeatedly into CH_2Cl_2 (3×500 mL) at 27 °C. The combined CH_2Cl_2 extract was concentrated in vacuo, to obtain a brown oil (25 g). The extract was subjected to MPLC on Si gel [step gradient hexane to hexane/methanol (95:5)] to give 2.16 g of a mosquito larvicidal (against second instar larvae of *Aedes aegypti*) fraction. This material was further fractionated using Si gel flash chromatography (CH_2Cl_2) to provide 1.39 g of a fraction that elicited mosquito larvicidal activity. Pure butenolides **1** (328 mg) and **2** (78 mg) were obtained via flash chromatography on Si gel [eluent: step gradient from hexane to CH_2Cl_2/hexane (4:1)]. Compounds **1** and **2** were similarly isolated from leaves of *H. floribunda* and *H. ovalifolia* in comparable yields using identical chromatographic conditions.

Compound 1: colorless oil (328 mg); $[\alpha]^{22}_D$ +38° (c 0.0026, $CHCl_3$); IR $_{\nu max}$ 1755 (lactone C=O), 3000 and 2140 (ethynyl group), 2950 and 2860 (CH), 1655 (C=C) cm^{-1}; UV (CH_2Cl_2) λ_{max} 232 nm; ^{1}H and ^{13}C NMR data, see Table 1; EIMS m/z $[MH]^+$ 263 (5), 247 (3), 233 (6), 217 (15), 149 (34), 135 (33), 112 (62), 95 (71), 81 (69), 67 (67), 55 (72), 43 (100); HREIMS m/z $[MH]^+$ 263.2011 (cacld for $C_{17}H_{27}O_2$, 263.2010).

Compound 2: colorless oil (78 mg); $[\alpha]^{22}_D$ +80° (c 0.0028, $CHCl_3$); IR $_{\nu max}$ 1755 (lactone C=O), 2925 and 2850 (CH), 1640 (C=C) cm^{-1}; UV (CH_2Cl_2) λ_{max} 228 nm; ^{1}H and ^{13}C NMR data, see Table 1; EIMS m/z $[M]^+$ 264 (3), 246 (1), 235 (2), 219 (5), 207 (4), 193 (5), 207 (4), 179 (9), 165 (8), 152 (14), 137 (13), 123 (16), 112 (62), 109 (24), 95 (54), 81 (50), 67 (73), 55 (74), 53 (15), 43 (77), 41 (100); HREIMS m/z $[M]^+$ 264.2089 (cacld for $C_{17}H_{28}O_2$, 264.2089).

Mosquito Larvicidal Assay.[9] Initially, solutions of 500 ppm for the plant extracts and 20 ppm for the butenolides **1** and **2** were prepared by dissolving the appropriate weight in acetone (1 mL) and poly(ethylene glycol) (24 μL/4 mg) and making up the solution to 100 mL with distilled water. To 40 mL of this solution contained in a glass beaker was introduced 10 second instar larvae of *A. aegypti* mosquito larvae. Each concentration was replicated into four. Similar solutions of acetone and poly(ethylene glycol) were used as control. Percentage mortality was determined after 24 and 48 h. For active extracts, fractions, and compounds, serial dilutions gave solutions for testing at lower concentrations. The median 50% lethal (LC_{50}) concentrations were determined using probit analysis (MSTAT package).

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