

RESEARCH NOTE

Novel Epoxy Butenolides from the Genus *Hortonia*

Rukmal Ratnayake¹, Shanal Gunasekera², David Williams³, Raymond Andersen³ and Veranja Karunaratne^{2,1,*}

¹Department of Chemistry, Faculty of Natural Sciences, Open University of Sri Lanka, Sri Lanka

²Department of Chemistry, University of Peradeniya, Peradeniya, Sri Lanka

³Departments of Chemistry and Earth & Ocean Sciences, University of British Columbia, Vancouver, B.C., Canada, V6T 1Z1

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Abstract: Two new epoxy butenolides, (4*S*)-4-methyl-2-(9-epoxy-11-dodecynyl)-2-butenolide and (4*S*)-4-methyl-2-((9*Z*)-11-epoxy-9-dodecynyl)-2-butenolide were isolated from the dichloromethane extract of *Hortonia angustifolia*. They were characterized by means of Mass and NMR spectroscopic analysis. The latter compound showed moderate mosquito larvicidal activity against *Aedes aegypti*.

Keywords: *Hortonia agnustifolia*, endemic plant, dichloromethane extract, novel epoxy butenolides

INTRODUCTION

Sri Lanka is a plant biodiversity hot spot with 25 % of its flowering plants being endemic. The independent diversity of Sri Lankan flora in comparison to peninsular India has led to speculation that during the continental drift, Sri Lanka may have experienced a higher degree of impoverishment, which would have contributed to the facilitation of speciation of new taxa on the other hand. Among the lower plants particularly among lichens, the recent reports of new species being discovered indicate that their diversity may be as prominent as the higher plants (Orange *et al.*, 2001). Sri Lankan plants have been tested for biological activity with promising results (Hewage *et al.*, 1997). In addition, the structural diversity among Sri Lankan higher and lower plants are typified by the discovery of, antioxidant alkaloids, (Puvendran *et al.*, 2008), compounds with iron sequestering ability (Kathirgmanathar *et al.*, 2006; Karunaratne *et al.*, 1992; Karunaratne *et al.*, 2005; Tezuka *et al.*, 1994; Bandara *et al.*, 1990), and aromatic acids and ketones (Thadhani *et al.*, 2012), all exhibiting a variety of biological activities.

The genus *Hortonia* is endemic to Sri Lanka and Dassanayake (1996) records three distinct species (*H. floribunda* Wight ex Arn., *H. angustifolia* (Thw.) Trimen and *H. ovalifolia* Wight). Some phytogeographers assume the genus *Hortonia* to have begun in Gondwanaland about 100-200 million years ago (Jayasekara, 1994). A noteworthy aspect of the chemistry of the genus *Hortonia* is the presence of compounds with five-membered rings

highlighting their importance in natural products in nature (Piers and Karunaratne, 1984). We have previously reported the isolation of several biologically active and unique butenolide and hydrazulenone natural products from the three species of genus *Hortonia* (Ratnayake *et al.*, 2001; Ratnayake *et al.*, 2008a; Ratnayake *et al.*, 2008b; Ratnayake *et al.*, 2008c; Carr *et al.*, 2012).

MATERIALS AND METHODS

The plant material

H. angustifolia was collected from Kanneliya and was identified by Dr. Siril Wijesundara, former Director Department of Botanic Gardens.

Isolation of (4*S*)-4-methyl-2-(9-epoxy-11-dodecynyl)-2-butenolide 1 and (4*S*)-4-methyl-2-((9*Z*)-11-epoxy-9-dodecynyl)-2-butenolide 2

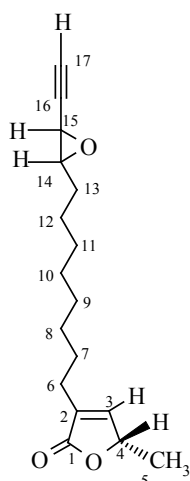
Air-dried, powdered leaves were repeatedly extracted with CH₂Cl₂ and dried *in vacuo* to obtain black oil. This crude extract was then subjected to repeated rounds of silica gel chromatography using MeOH-CH₂Cl₂ (1:99) as the eluent in a gradient of increasing polarity up to MeOH-CH₂Cl₂ (1:1), gave a fraction which was purified by flash chromatography with CH₂Cl₂-hexane (4:1) and C₁₈ reversed-phase HPLC to yield compound 1 and 2 as colourless oils, (0.06% and 0.87%, respectively, with respect to leaf extract).

RESULTS AND DISCUSSION

Compound 1 gave [M⁺] at 276.1730 in its HREIMS suggesting that molecular formula of the compound 1 was C₁₇H₂₄O₃. The IR spectrum of compound 1 showed an absorption at 2110 cm⁻¹ indicating the presence of an ethynyl moiety, an α,β-unsaturated γ-lactone carbonyl at 1755cm⁻¹ and unsaturation at 1680 cm⁻¹. The absorption at 1270 cm⁻¹ and 950 cm⁻¹ indicated the presence of an epoxide moiety. The UV spectrum of compound 1 showed absorption at λ_{max} 231 nm indicating that an α,β-unsaturated γ-lactone moiety was present.

*Corresponding Author's Email: veranjak@slintec.lk





Compound 1

The UV absorption band at 231 nm, the mass fragment peaks at m/z 67 (100%), 81 (65%) and 95 (88%) and the ^1H NMR signals at d 4.99 (H-4), 1.40 (H-5) and 6.99 (H-3), confirmed the presence of an α,β -unsaturated γ -lactone moiety. The ^1H and ^{13}C NMR data obtained for compound **1** (Table 1) showed 17 carbon resonances with 24 attached hydrogen atoms (1 x CH_3 , 8 x CH_2 , 5 x CH, 3 x C). The resonances detected in ^{13}C NMR and APT spectrum indicated the presence of one ester carbonyl (δ 173.9), two sp^2 carbons (δ 134.2 and 148.9), two sp carbons (δ 71.7 and 80.5), three oxygen substituted methines (δ 44.8, 57.9 and 60.3), eight methylenes (δ 31.6, 29.1, 29.1, 27.3, 25.8, 25.5, 25.1, and 25.1) and one methyl (δ 19.2). The presence of a carbonyl group (δ 173.9) and oxygenated methine carbon (δ 60.3) suggested an ester function. The molecular formula of $\text{C}_{17}\text{H}_{24}\text{O}_3$ was established by HRMS and was fully consistent with the number of carbon centers detected in the ^{13}C NMR spectrum.

Five of the six degrees of unsaturation present were

Table 1: NMR spectroscopic data of epoxy butenolides **1** and **2**.

Carbon	(1) ^{13}C	(1) ^1H	(1) ^1H - ^1H	(2) ^{13}C	(2) ^1H	(2) ^1H - ^1H
1	173.9			169.1		
2	134.2			129.7		
3	148.9	6.99 d (1.7)	4, 6	150.1	6.62 dt (7.7, 1.2)	6
4	60.3	4.99 dq (6.8, 1.7)	3, 5	77.7	4.60 m	5
5	19.2	1.40 d (6.8)	4	14.5	1.43 d (6.6)	4
6	25.1	2.25 br t (7.3)	3, 7	28.3	2.80 m	3
7	25.1	1.53 m	6	29.0	1.38 br s	
8	25.5	1.31 br s		29.2		
9	25.8			29.4		
10	27.3			29.6		
11	29.1			29.6		
12	29.1			30.1	13	
13	31.6		14	30.6	2.38 tdd (7.5, 7.5, 1.2)	12, 14, 15
14	44.8	2.33 dt (14.5, 1.5)	13, 15, 17	146.6	6.05 dt (10.8, 7.5)	13, 15
15	57.9	3.08dd	14, 17	108.4	5.50 dq (10.8, 1.2)	13, 14, 17
16	71.7			71.8	4.72 m	
17	80.5	1.68br s	14, 15	81.2	3.17 d (2.2)	15

accounted for the ester carbonyl, a triple bond and an α,β -unsaturated γ -lactone ring. The remaining degree of unsaturation was assigned to an epoxide ring.

Its ^1H NMR spectrum showed a broad singlet for twelve aliphatic methylene protons at δ 1.31 (H-8, 9, 10, 11, 12, 13). The multiplet at δ 1.53 and the broad triplet at δ 2.25 ($J = 7.3$ Hz) were assigned to H-7 and H-6, respectively. The down field ^1H and ^{13}C NMR resonances were assigned to the conjugated double bond in the γ -lactone ring [δ_{H} 6.99, 1H, d, $J = 1.7$ Hz, H-3, (δ_{C} 148.9 and 134.2)], an aliphatic methine residue attached to an oxygen atom [δ_{H} 4.99, H-4, dq, $J = 6.8, 1.7$ Hz, (δ_{C} 60.3)] and a γ -lactone carbonyl (δ 173.7). Additional ^1H and ^{13}C NMR resonances could be assigned to a terminal ethynyl group [δ_{H} 1.68, H-17, broad s, (δ_{C} 80.5 and 71.7)], a secondary methyl group [δ_{H} 1.40, 3H, d, $J = 6.8$ Hz, H-5, (δ_{C} 19.2)] and an epoxide moiety [δ_{H} 2.33, dt, $J = 14.5, 1.5$ Hz, H-14, (δ_{C} 44.8), δ_{H} 3.08, dd, H-15, (δ_{C} 57.9)]. The large coupling of 14.5 Hz between H-14 and one of the protons attached to H-13 illustrated the rigidity in that area of the molecule introduced by the presence of the epoxide group.

The H-H COSY spectrum of compound **1** showed coupling between δ 3.08 (H-15) and (H-14) of the epoxide ring. In addition the H-H COSY spectrum showed both H-15 (δ 3.08, dd) and H-14 (δ 2.33, dt) HMBC correlation with H-17 (δ 1.68, broad s) indicating that epoxide moiety was attached to the ethynyl group. The coupling observed between C-5 methyl doublet (δ 1.40, d, $J = 6.8$ Hz) and H-4 (δ 4.99, dq, $J = 6.8, 1.7$ Hz) established that C-5 methyl is attached to C-4 of the γ -lactone ring. The C-6 methylene protons which coupled to H-3 (δ 6.99, d, $J = 1.7$ Hz) also correlated to H-7 (δ 1.53, m) suggesting that 12 C atoms containing side chain was situated at C-2. With a negative optical rotation literature precedent indicated that the absolute configuration of compound **1** at C-4 was *S* (Martinez *et al.*, 1981).

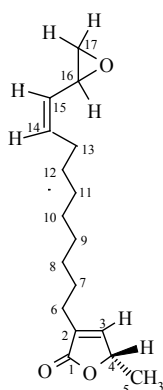
Compound 1 was found to be inactive against the 2nd instar larvae of *Aedes aegypti* at 10 ppm (Bandara et al., 2000).

(4S)-4-methyl-2-((9Z)-11-epoxy-9-dodeceny)-2-butenolide

Compound 2 showed a molecular ion [M⁺] at 278.0456 in its HREIMS suggesting that the molecular formula of compound 2 is C₁₇H₂₆O₃. The IR spectrum of compound 2 exhibited frequencies corresponding to α , β -unsaturated- γ -lactone carbonyl at 1730 cm⁻¹ and unsaturation at 1670 cm⁻¹. The absorption at 1175 cm⁻¹ and 1040 cm⁻¹ indicated the presence of an epoxide moiety⁵². The UV spectrum of compound 2 showed an absorption at λ_{\max} 244 nm indicating that an α , β -unsaturated- γ -lactone moiety was present. Furthermore, the fragments which appeared in the low resolution mass spectrum of compound 2 [*m/z* 67 (66%), 81 (100%) and 95 (71%)] along with the three characteristic ¹H NMR signals at δ 4.60 (H-4), 1.43 (H-5) and 6.62 (H-3), also suggested the presence of an α , β -unsaturated- γ -lactone moiety.

The ¹H/¹³C/APT spectra obtained for compound 2 showed 17 carbons resonance with 26 attached hydrogen atoms (1 x CH₃, 9 x CH₂, 5 x CH, 2 x C). The resonances detected in ¹³C NMR and APT spectra indicated the presence of one ester carbonyl (δ 169.1), four sp² carbons (δ 150.1, 146.6, 129.7 and 108.4), two oxygen substituted methines and one oxygen substituted methylene (δ 77.7 and 71.8 and 81.2), eight methylenes (28.3, 29.0, 29.2, 29.4, 29.6, 29.6, 30.1 and 30.6) and one methyl group (δ 14.5). The presence of a carbonyl group at (δ 169.1) and oxygenated methine carbon (δ 77.7) suggested an ester function.

Molecular formula of C₁₇H₂₆O₃ was established by HREIMS and was fully consistent with the number of carbon centers detected in the ¹³C NMR spectrum. Four of the five degrees of unsaturation present were accounted for by the ester carbonyl, a double bond, an α , β -unsaturated γ -lactone ring. The remaining degree of unsaturation was assigned to an epoxide ring.



Compound 2

Its ¹H NMR spectrum showed broad singlet for twelve aliphatic methylene protons at δ ~1.38 (H-7, 8, 9, 10, 11 and 12). A doublet of a triplet at δ 2.38 was assigned to H-13 (2H, *J* = 7.5, 1.2 Hz) and the HMBC spectrum showed coupling to the adjacent methylene group H-12 (*J* = 7.5 Hz), and allylic coupling to the olefinic protons H-14 (*J* = 7.5 Hz) and H-15 (*J* = 1.2 Hz). The appearance of a multiplet at δ 2.80 was due to H-6. ¹H and ¹³C NMR resonances could be assigned to a terminal epoxide moiety [δ_{H} 3.17, 2H, d, *J* = 2.2 Hz; 4.72, 1H, m, (δ_{C} 81.2 and 71.8)]. This epoxide in turn was attached to a *cis*-double bond (C-14, 15) [δ_{H} 5.50, dq, *J* = 10.8, 1.2 Hz, H-15; δ_{H} 6.05, dt, *J* = 10.8, 7.5 Hz, H-14 (δ_{C} 146.6 and 108.4) and secondary methyl group [δ_{H} 1.43, d, *J* = 6.6 Hz, (δ_{C} 14.5)]. The presence of a *cis*-double bond in compound 2 was further substantiated by the absence of a 700-900 cm⁻¹ IR absorption that is characteristic of *trans* double bonds. There is ample literature precedent for the occurrence of *cis*-double bonds in acetogenin side chains. Similar to previously reported butenolides (Ratnayake et al., 2001) in 2 the down field ¹H and ¹³C NMR resonances were assigned to a double bond [δ_{H} 6.62, dt, *J* = 7.7, 1.2 Hz, H-3, (δ_{C} 129.7 and 150.1)], an aliphatic methine residue attached to an oxygen atom [δ_{H} 4.60, m, H-4 (δ_{C} 77.7)] and a γ -lactone carbonyl (δ 169.1).

The H-H COSY spectrum of compound 2 showed coupling between δ 3.17 (H-17) and δ 5.50 (H-15) in addition to correlation between δ 5.50 (H-15) and δ 6.05 (H-14) indicating that epoxide moiety was attached to a double bond moiety. The presence of only two olefinic protons along with three epoxy protons confirmed that it was the epoxide group which was terminal and not the double bond. The coupling observed between H-15 (δ 5.50) and H-13 (δ 2.38), H-14 (δ 6.05) and H-13 (δ 2.38) indicated that the *cis*-double bond was attached to C-13. The correlation between C-5 methyl doublet (δ 1.43) and H-4 (δ 4.60, m) established that C-5 methyl is attached to C-4 of the γ -lactone ring. The COSY spectrum further showed that C-6 methylene protons (δ 2.80, m) were coupled to H-3 (δ 6.62) suggesting that C₁₂ side chain was situated at C-2.

With a negative optical rotation literature precedent indicated that the absolute configuration of compound 2 at C-4 was *S* (Martinez et al., 1981). Compound 2 exhibited moderate mosquito larvicidal activity at 10 ppm (LC₅₀ = 7.87).

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