Ceylon Journal of Science 46(3) 2017: 115-118
DOI: http://doi.org/10.4038/cjs.v46i3.7448

RESEARCH NOTE

A novel Tetracyclic Sesquiterpene from the Genus *Hortonia*

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Received: 30/05/2017; Accepted: 07/08/2017

Abstract: A new tetracyclic sesquiterpene, 1, 5, 12 trimethyltetracyclic [6, 3, 0, 0, $0^{3, 4, 8}$] dodecane was isolated from the dichloromethane extract of *Hortonia angustifolia*. It was characterized by means of Mass and NMR spectroscopic analysis. It was found to be biologically inactive in the mosquitolarvicidal assay and moderately active against the fungus *Cladosporium cladosprioides*.

Keywords: *Hortonia agnustifolia*, endemic plant, dichloromethane extract novel tetracyclic sesquiterpene.

INTRODUCTION

Sri Lanka is a biodiversity hot spot with 25 % of its flowering plants being endemic. The 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. Among the lower plants such as lichens, the recent reports of new species being discovered frequently indicate that their diversity may be as high as the higher plants (Orange *et al*., 2001). Sri Lankan plants have been tested for biological activity with promising results (Hewage *et al*., 1998). In addition, the structural diversity among Sri Lankan higher and lower plants are typified by the discovery of, alkaloids, (Puvenendran *et al*., 2008), compounds with iron chelating function (Sahib *et al*., 2008; Kathirgmanathar *et al*., 2006; Karunaratne *et al*., 1992; Kumar *et al*., 1989), and phenolic acids and ketones (Kumar *et al*., 1990), all possessing a variety of bioactivities.

The genus *Hortonia* is endemic to Sri Lanka and Dassanayake (1996) lists three distinct species (*H. floribunda* Wight ex Arn., *H. angustifolia* (Thw.) Trimen and *H. ovalifolia* Wight). Some phytogeographers consider the genus *Hortonia* to have originated in Gondwanaland about 100-200 million years ago (Jayasekara, 1997). We have previously reported the isolation of several biologically active and unique 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). As part of our continuing research to find biologically active compounds from this plant, we isolated one tetracyclic sesquiterpene.

MATERIALS AND METHODS

The plant material

H. angustifolia was collected from Kanneliya and was identified with the help of voucher specimens at the Department of Botanic Gardens, Peradeniya, Sri Lanka.

Isolation of 1, 5, 12 trimethyltetracyclic [6, 3, 0, 0, $0^{3, 4, 8}$] dodecane (figure 1)

The first fraction, which was inactive in the mosquito larvicidal assay, obtained from the MPLC fractionation of CH_2Cl_2 extract of leaves of *H. angustifolia* was subjected to flash chromatography with CH_2Cl_2-hexane (1:1) as the eluent to yield the tetracyclic sesquiterpene (figure **1**) (yield 2.3% with respect to leaf extract) as a colourless oil. Compound 1 was subjected to the mosquitolarvicidal assay using the 2nd instar larvae of *Aedes aegypti* (Bandara *et al.*, 2000) and antifungal assay with the fungus *Cladosporium cladosporioides* using the TLC bioassay technique (Klarman and Sanford, 1968).

RESULTS AND DISCUSSION

The tetracyclic sesquiterpene **1** gave a molecular ion [M^+] at 204.1878 in its HREIMS consistent with the molecular formula $C_{15}H_{24}$ requiring four sites of unsaturation. The IR spectrum of compound **1** did not show any diagnostic

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stretching frequencies for functional groups including 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. Resonances detected in the APT spectrum indicated the presence of three quaternary carbons (δ 43.91 (C-8), 35.64 (C-1) and 22.44 (C-5)), three methines (δ 38.63 (C-12), 22.97 (C-4) and 19.81 (C-3)), six methylenes (δ 39.24 (C-7), 35.83 (C-6), 34.63 (C-2), 33.58 (C-9), 30.83 (C-11) and 23.97 (C-10)) and three methyl groups (δ 20.30 (C-15), 16.72 (C-14) and 16.58 (C-13)).

Analysis of $^{1}H/^{13}C$/COSY/HMBC NMR data (Table 1) of 1 showed two singlets [δ 0.79, H-14, 16.72, C-14, 1.14, H-15, 20.30, C-15 for the methyl groups correlating with quaternary carbons (δ 35.64, C-1, 22.44, C-5) and one doublet [δ 0.74 (*J* = 6.7 Hz), H-13, 16.58 (C-13) for a secondary methyl group. Two methylene doublets [δ 2.09, 1H, *J* = 11.9 Hz, H-7, 1.00, 1H, br d, *J* = 2.8 d, H-7, 39.24, C-7; 1.87, 1H, *J* =11.9 Hz, H-6, 1.16, 1H, br d, *J* = 2.8, 35.83, C-6] showed that they are attached to adjacent carbon atoms. One cyclopropyl proton each appeared as multiplets [δ 0.47, H-3, 19.81, C-3; 0.85, H-4, 22.97, C-4] which indicated that the third carbon in the three-membered ring was quaternary (22.44, C-5). The protons appearing under the multiplets [δ 0.97, 1.58 1H each, H-9, 33.58, C-9; 1.02, 1.32 1H each, H-11, 30.83, C-11; 1.36, 1.52 (1H each, H-10, 23.97, C-10; 1.51, 1.68 1H each, H-2, 34.63, C-2; 1.64, 1H, H-12, 38.63, C-12) were assigned to a methine (C-12) and four methylenes (C-11, 10, 9 and 2).

COSY/HMBC correlations taken together indicated that the spin system extended from C-13 to C-9, C-7 to C-6 and C-4 to C-2. Since there were no inter-correlations between the above carbon fragments, it was concluded that C-12, C-9, C-7, C-4 and C-2 were attached to quaternary carbons (Figure 2) with C-3, C-4 and C-5 forming the cyclopropyl ring. Thus, in order to complete the structure, the remaining two methyl groups (attached to quaternary carbons) were placed at C-1 and C-5. The resulting structure which fitted the data was concluded to be 1, 5, 12-trimethyltetracyclic [6, 3, 0, 0, $0^{3, 4, 8}$] dodecane (1) (Ratnayake *et al*., 2008b).

The tetracyclic sesquiterpene was examined for its biological activity using the mosquito larvicidal and antifungal assay. It was found to be inactive (after 48 hrs) against the 2nd instar larvae of *Aedes aegypti* at 10 ppm. Interestingly, in the antifungal assay 1 showed a moderate activity against *Cladosporium cladosporioides* in the TLC bioassay technique (Table 2).

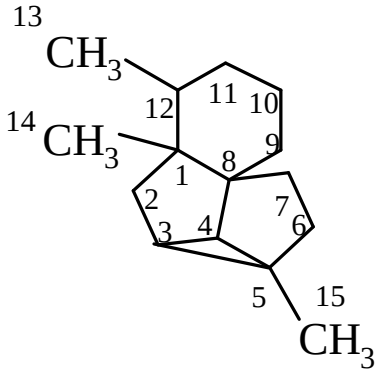

Figure 1: Tetracyclic sesquiterpene, 1, 5, 12 trimethyltetracyclic [6, 3, 0, 0, $0^{3, 4, 8}$] dodecane

Table 1: 1D and 2D NMR spectroscopic data of compound **1.**

Carbon	**^{13}C**	**DEPT**	**^{1}H**	**multiplicity (*J*, Hz)**	**^{1}H-^{1}H**	**HMBC**
1	35.64	C	-		-	
2	34.63	CH_2	a 1.51 b 1.68	m ($W_{1/2}$ 6.15) m ($W_{1/2}$ 6.9)	2b, 3 2a, 3	1, 4, 8, 14, 15 1, 3, 4, 12, 14
3	19.81	CH	0.47	m	2a, 2b, 4	
4	22.97	CH	0.85	m (W1/2 12)	3	4,12
5	22.44	C	-		-	-
6	35.83	CH_2	a 1.16 b 1.87	br d (2.8) br d (11.9)	6b 6a, 7b	4, 5, 7, 15 5, 6, 7, 8
7	39.24	CH_2	a 1.0 b 2.09	br d (2.8) br d (11.9)	7b 7a, 6b	8 4, 6, 8
8	43.91	C	-		-	-
9	33.58	CH_2	a 0.97 b 1.58	m ($W_{1/2}$ 5.8) m ($W_{1/2}$ 5.8)	9b 9a, 10a	
10	23.97	CH_2	a 1.36 b 1.52	m ($W_{1/2}$ 5.8) m ($W_{1/2}$ 6.15)	9b 11a	11, 12 1, 7, 8, 13
11	30.83	CH_2	a 1.02 b 1.32	m m	10b 12	1, 8, 9
12	38.63	CH	1.64	m ($W_{1/2}$ 6.7)	13, 11b	
13	16.58	CH_3	0.74	d (6.7)	12	1, 12
14	16.72	CH_3	0.79	s	-	1, 8, 12
15	20.30	CH_3	1.14	s	-	4, 7, 15

Table 2: Antifungal activity of compound 1.

Fungus	Diameter (mm) of the inhibition zone	
	Compound 1	Control (Benor)[a]
Cladosporium cladosporioides	22	37

[a] Benor (benomyl) {methyl-1-(butylcarbonyl)-2-benzimidazolecarbamate}

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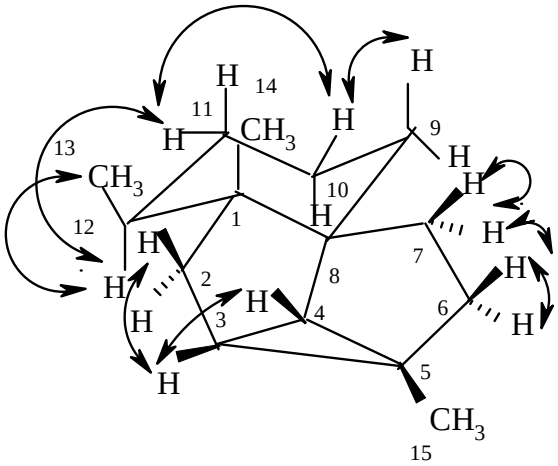

Figure 2: COSY correlations of the compound 1

ACKNOWLEDGEMENTS

The authors thank Dr. Siril Wijesundara for helping with the identification of the plant species.

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