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Revisionof Structure of (-)-Hortoisoishwarane from *Hortonia angustifolia* via Density Functional Theory Calculations†

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†Dedicated to Professor Dr. Sukh Dev on his 100th Birthday.

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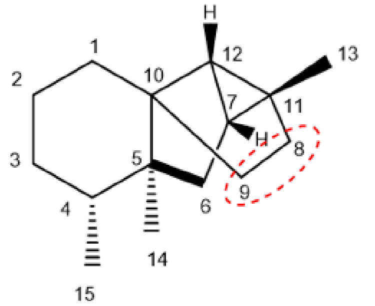
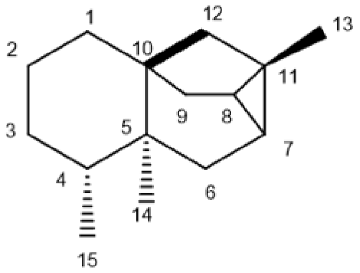
ABSTRACT

The structure of 2, a tetracyclic sesquiterpene hydrocarbon isolated from the methylene chloride extract of *Hortonia angustifolia* has been revised based on the results from Density Functional Theory (DFT) calculations and is proved to be identical with (-)-ishwarane 1.



2a, (-)-Hortoisoishwarane



1, (-)-Ishwarane

Note: The DFT study has been proved to be an extremely useful tool in revision of structure of a natural product isolated from *H. angustifolia* with the assigned structure hortoisoishwarane 2a to the correct structure ishwarane 1. It was observed that the two consecutive methylene groups, C-8 and C-9, attached to quaternary carbons C-11 and C-10 respectively in 2a were missing and hence was an erroneous assignment. The evolution of the process leading to the correction has been described in the present article.

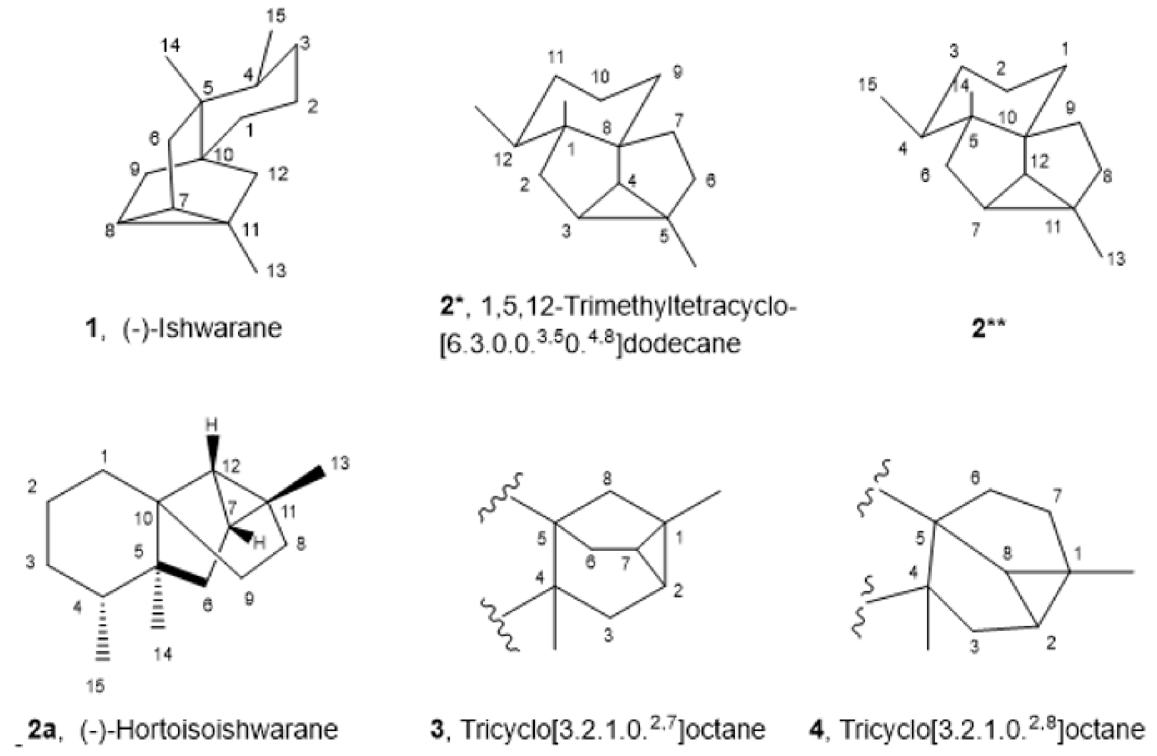
Graphical Abstract.

Keywords: Genus *Hortonia*; Tetracyclic Sesquiterpene; DFT Calculations; Structure Revision; Identity; (-)–Ishwarane

Introduction

The genus *Hortonia* (family *Monimiaceae*) is endemic to Sri Lanka and only three species *H. angustifolia* (Trimen), *H. floribuda* (Wight ex Arn) and *H. ovalifolia* (Wight) are found at different geographical locations and have been found to exhibit mosquito larvicidal as well as antifungal activities [1]. During the past fifteen years all three species have been subjected to phytochemical investigation and observed that while butenolides are responsible for mosquito larvicidal activity [2] the sesquiterpene fraction exhibits antifungal activity [1]. The previously known teracyclic sesqiterpene hydrocarbon, ishwarane **1**, [3] was isolated from the dichloromethane extract of all the species of the genus *Hortonia* and shown to have antifungal activity against *Clodosporium codosporiods* [4]. Ishwarane **1** has been isolated from different sources apparently unrelated to the family Monimiaceae [4]. As a part of the investigation on the bioactive compounds from the genus *Hortonia*, another tetracyclic sesquiterpene hydrocarbon was isolated from the dichloromethane root extract of Hortonia *angustifolia*, $[\alpha]_D$ -64.41° ($CHCl_3$) [5]. The assignment of structure including relative stereochemistry was based on extensive spectral analysis (1D, 2D NMR, HR-ESI-MS) and was assigned the 1,5,12-trimethyltetracyclo- [6.3.0.0.^{3,5}0^{4,8}] dodecane skeleton **2 (Figure 1)**. Since (-)-ishwarane 1 was isolated from the same source, the newly isolated hydrocarbon 2 looked in all probability structurally related to **1**. Based on the known chemical interrelationship among **1** and the other ishwarane based sesquiterpenes [6] a putative structure **2a** was considered for *Hortonia* tetracyclic sesquiterpene. (-)-Ishwarane **1** contains a tricyclo- [3.2.1.0^{2,7}] octane system **3** and the putative structure **2a** possesses tricyclo [3.2.1.0^{2,8}] octane part structure as shown in **4 (Figure 1)**. A close examination of molecular models of **1** and **2a** revealed that ring-A is common in both the structures. Comparison of the chemical shifts of the ring A protons (at C1, C2 and C3 methylene, C4 methine and C14, C15 methyl protons) and the corresponding ^{13}C NMR chemical shifts (of C1, C2, C3, C4, C5, C10, C14 and C15 carbons) were found to be nearly identical [4].

The difference lies in the arrangement of a rings B, C and D. The tricyclo [3.2.1.0^{2,7}] octane ring system **3** of (-)-ishwarane **1** has undergone an acid catalysed molecular rearrangement to produce tricyclo [3.2.1.0^{2,8}] octane system 4 present in hortoisoisharane **2a (Figure 1)**. In recent years, comparison of ^{13}C-NMR chemical shifts experimentally observed on natural product and those calculated by density functional theory (DFT) [7-11] has become an unambiguous method for checking the correctness of structure and stereochemical assignments of natural products [12] based on 1D and 2D NMR spectral analysis and HR-ESI-MS data. In an attempt to determine the stereochemistry of structure **2a** by Density Functional Theory (DFT) calculations, some problems surfaced and a reassignment of the structure became essential. The results of the study are presented here.



Note: *Carbon numbering used by Ratnayake [5]. **Carbon numbers in 2** corresponds to the original ones based on ishwarane 1 numbering [17].
Figure 1: (-)-Ishwarane and (-)- hortoisoishwarane.

Results and Discussion

The isolation of a tetracyclic sesquiterpene hydrocarbon from *H. angustifolia* was reported and was assigned structure 2 based on an extensive 1D and 2D NMR spectral analysis together with HR-ESI-MS. One important feature of natural products belonging to isoprenoids is the stereochemistry. ^{13}C NMR has recently been shown to be a useful tool in establishing this [13]. Analysis of compound *2* (**Figure 1**) turned out to be difficult as it is a hydrocarbon, so the spread in both the ^{1}H and the ^{13}C chemical shifts is narrow even after changing the NMR solvent to C_6D_6 from $CDCl_3$, making the analysis of even 2D NMR spectra complicated. While most of the structural assignments were the same as reported previously [5], a reassignment of the resonances based on ^{1}H/^{13}C/COSY/ HMBC correlations revealed that besides from the cyclohexane ring, two CH_2 groups were not neighbours **(Table 1**). In addition, an important feature was the coupling constant of 11.9 Hz observed as a geminal coupling according to the COSY spectrum indicating that both H-9 and H-12 should be a part of the proposed five-membered rings. The full analysis led to the assignment as included in **Table 1**. An attempt to determine the absolute structure of the molecule from *H. angustifolia* by ^{13}C NMR showed that a comparison of experimental and calculated nuclear shielding by Density Functional Theory (DFT) calculations (GIAO approach) gave a very poor fit, indicating that the assigned structure probably needed revision (**Figure 2**). However, plotting the experimental reassigned ^{13}C chemical shifts from **Table 2** were very close to those of (-)-ishwarane vs. calculated nuclear shielding, and gave a very good correlation (**Figure 3**). Therefore, it was concluded that the compound isolated from *H. angustifolia* was as a matter of fact (-)-ishwarane **1**. The stereochemistry is confirmed by the ^{13}C NMR calculations.

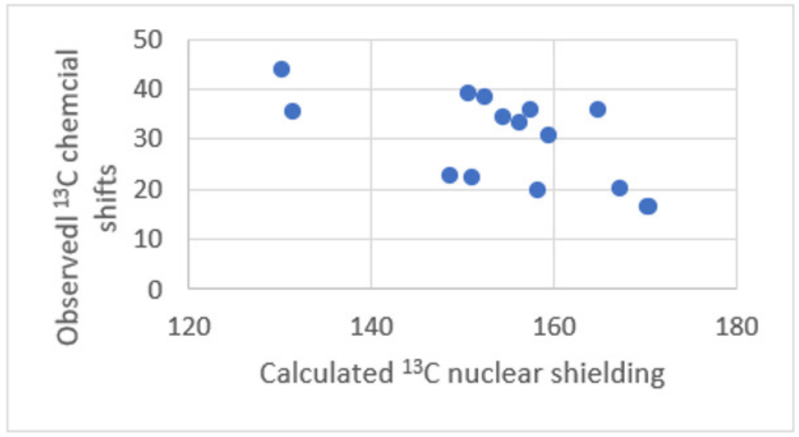


Figure 2: Plot of the observed chemical shifts for Hortoishiwarane [5] vs. calculated nuclear shielding (B3LYP/6-31G(d)).

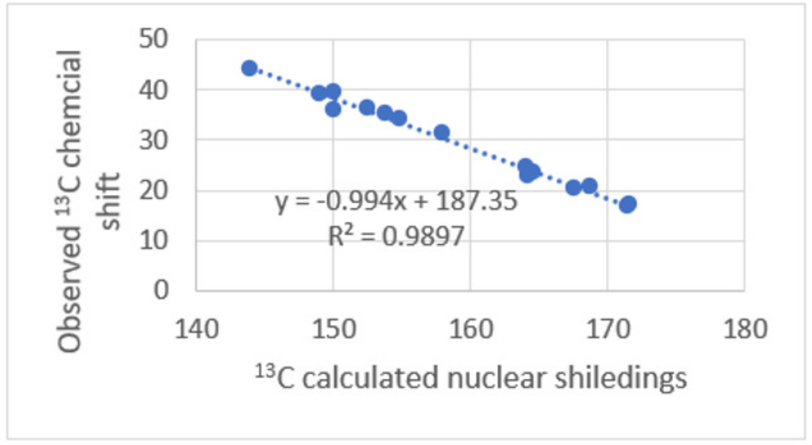


Figure 3: Reassigned observed chemical shifts for Hortoishiwarane vs. calculated nuclear shielding for (-)-Ishiwarane.

Table 1: ^{1}H, ^{13}C chemical shifts and correlations obtained from DEPT, COSY, HMQC and HMBC spectra for 2a.

C No.	δ C	DEPT	δ H	COSY	HMBC
1	33.91	2	a 0.96 b	1b,2a 1a	
2	24.31	2	a 1.34 b 1.42	1a, 2b 2a	
3	31.12	2	a 1.02 b 1.32	3b 3a	1,2,4,5
4	38.92	1	1.64	15	15
5	35.93	0	-	-	
6	34.94	2	a 1.51 b 1.68	6b 6a,7	5,7,10,14 4,5,7,8,14
7	20.26	1	0.47	6b,8	5,13
8	23.43	1	0.85	7	7
9	36.18	2^b	a 1.20 b 1.93	8,9b 9a	5,7,8,10,12 5,8,10,11,12
10	44.13	0	-	-	
11	22.69	0	-	-	
12	39.51	2^b	a 0.98 b 2.03	12b 12a	1,5,10,11,13 7,8,9,10
13	20.51	3	1.18		7,11,12
14	16.95	3	0.79		4,5,10
15	16.80	3	0.73	4	3,4,5

Note:

a. The HMQC spectrum confirmed that all CH_2 groups had two different ^{1}H chemical shifts

b. A coupling constant of 11.9 Hz could be measured.

Table 2: Calculated nuclear shielding and experimental, reassigned ^{13}C chemical shifts.

C No.	DEPT	Experimental δ ppm	Calculated ^{a} δ ppm
1	CH_2	33.91	154.9
2	CH_2	24.31	164.1
3	CH_2	31.12	158.0
4	CH	38.92	149.1
5	C	35.93	150.1
6	CH_2	34.94	153.9
7	CH	20.26	167.6
8	CH	23.43	164.6
9	CH_2	36.16	152.5
10	C	44.13	144.0
11	C	22.69	164.2
12	CH_2	39.51	150.1
13	CH_3	20.51	168.8
14	CH_3	16.95	171.6
15	CH_3	16.80	171.5

Note:

a. B3LYP/6-31G(d) (see experimental).

Conclusion

The DFT calculations have been found to be extremely helpful in conclusively showing that the laevorotatory tetracyclic sesquiterpene isolated from the methylene chloride root extract of *H. angustifolia* possesses neither structure 2 nor 2a but is identical with (-)-ishwarane 1.

Experimental

Calculations

Structures were subjected to calculation of carbon chemical shifts using the Gaussian 16 program package [14] using the B3LYP/6-31G(d) functional and Pople basis set. [15]. The nuclear shielding was calculated using the GIAO software [16,17].

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Highlights

- Isolation of natural products from *H. angustifolia*.
- Use of DFT calculations to predict correct structure of complex fused ring compounds.
- Correction of published structures.

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