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EBC-342: A Novel Tetrahydrofuran Moiety Containing Casbane from the Australian Rainforest[‡]

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Abstract: The Australian rainforest plant, *Croton insularis*, has provided a further casbane diterpene family member in the form of EBC-342, which contains a novel tetrahydrofuran ring. A combination of DP4 and TD-DFT CD methods were required to confirm both structure and absolute stereochemistry.

Introduction

Croton insularis, an Australian rainforest plant, continues to provide a surprisingly diverse range of casbane natural products [e.g. EBC-324 (**1**)].[1–3d] At the present time, although casbanes have been known for a considerable period, they are nevertheless still viewed as rarely occurring.[4,5] On this occasion, however, we report herein an additional family member to this class, EBC-342 (**2**), being clearly defined from previous cases by the presence of a tetrahydrofuran moiety (Figure 1).
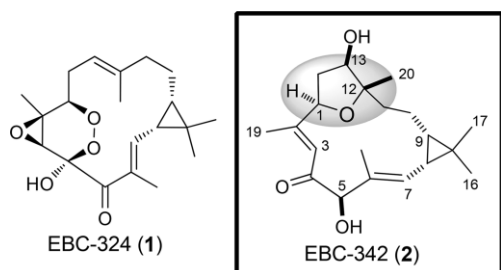

Figure 1. Casbanes EBC-324 (**1**) and EBC-342 (**2**); casbanes **2** contains an unprecedented tetrahydrofuran ring.

Results and Discussion

EBC-342 (**2**) was isolated as a minor component of *Croton insularis*. The elucidation was initiated with the observation of two

higher field shifted methyl groups (δ_H = 1.44, 2.09 ppm) fitting that previously observed for casbane diterpenes.[1] Three extended connectivities in the molecule, namely C13–C14–C1, Me-19 via a quaternary methine with C3, and C18 via a quaternary methine with fragment C7–C9 and C10–C11, were determined from analysis of the COSY spectrum (Figure 2, see bold bonds in structure). The ^{13}C and HSQC NMR spectra analysis revealed two trisubstituted double bonds and five oxygenated carbons, four monoxygenated with rather low field chemical shifts (78.5, 80.8, 84.4, 87.1 ppm) and one carbonyl (199.8 ppm). HMBC cross peaks for Me-16 (1.07 ppm) and Me-17 (1.14 ppm) with C8 (25.0 ppm), C9 (31.6 ppm) and C15 (20.4 ppm) revealed a *gem*-dimethyl group and built the cyclopropane ring. HMBC between Me-18 (1.44 ppm), C5 (84.4 ppm), C6 (133.9 ppm) and C7 (129.5 ppm) attached C18 (10.7 ppm) and C5 to C6 to provide the combined fragment C5–C9. HMBC correlations for Me-19 (2.09 ppm) with C1 (80.8 ppm), C2 (162.2 ppm) and C3 (113.2 ppm) connected C19 (16.2 ppm) to C2. Me-20 (1.24 ppm) HMBC interactions with C11 (41.1 ppm), C12 (87.1 ppm) and C13 (78.5 ppm) joined the two carbon chains C10–C11 and C13–C14–C1–C3. The extended fragments C5–C9 and C10–C14–C1–C3 were connected on the basis of 9-H (0.61 ppm) and 10-H (1.29/1.75 ppm) correlations with C11 and C8 respectively. The analysis of the C4 (199.8 ppm) interaction with 5-H
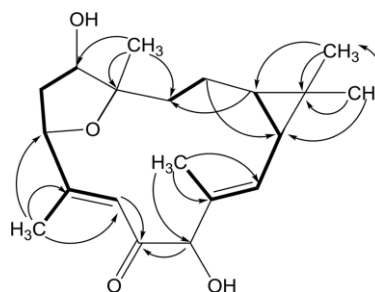
Figure 2. COSY (bold bonds) and selected HMBC (curved arrows) correlations for EBC-342 (**2**).

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(4.56 ppm) and 3-H (6.90 ppm), completed the casbane skeleton by connecting C3 to C5 via carbonyl C4 (Figure 2).

Low resolution ESI mass spectroscopy suggested a number of high oxygen content possibilities, which unfortunately prevented sensible HRESI data being obtained. However, a molecular formula consisting $C_{20}H_{30}O_4$ was deducted based on a combination of LRMS and ^{13}C NMR data. A subsequent challenge was the placement and connectivity of the oxygen atoms as the molecular formula corresponded to 6 ring double bond equivalents. The presence of a carbonyl at C4 (199.8 ppm) and hydroxyl functionality at C13 (78.49 ppm) was obvious from the ^{13}C NMR chemical shifts, leaving three remaining oxygenated carbons to distribute. It was found, however, that the oxygenated methine carbons, C1 (80.8 ppm) and C12 (87.1 ppm), possessed similar chemical shifts to that reported for a 1-methyltetrahydrofuran unit buried within the macrocyclic skeletons of lobocrasols,[6] latheranes,[7] flexibilanes,[8] and cembranes.[9] Therefore, connection of C1, via a one oxygen bridge to C12, provided a tetrahydrofuran ring. The ^{13}C NMR chemical shift of C5 (84.4 ppm) was very close to that reported for casbane EBC-182,[1e] suggesting a C5 hydroxyl and thus completing the flat structure. The relative stereochemistry of **2** was determined from NOE analysis (Figure 3).
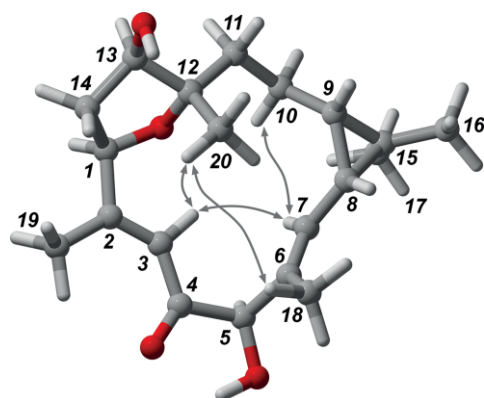

Figure 3. Geometry of the lowest-energy conformer of EBC-342 (**2**) with arrows indicating key NOE correlations.

Considering, the molecular formula determination difficulties, and that both an endoperoxide casbane[1a] (e.g., **1**. Figure 1) and hydroperoxy casbanes[1c] have been previously isolated from *Croton insularis*; a DP4 chemical shift analysis was employed to compare the assignment of EBC-342 (**2**) against a hypothetical endoperoxy-hydroperoxy casbane **3** (Figure 4).
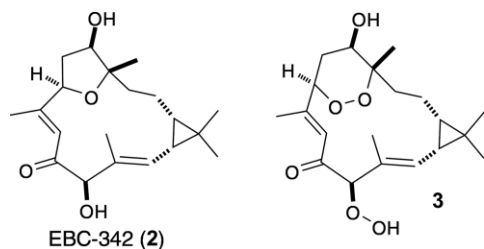

Figure 4. EBC-342 (**2**) and hypothetical **3**.

The DP4 probabilities associated with these two assignments were calculated by the method of Goodman.[10] This involved:

(i) MMFF (MMFF94)[11] conformational searches with the Monte Carlo Multiple Minimum (MCMM) algorithm as implemented in MacroModel 10.6,[12] (ii) DFT reoptimization of the low-lying conformers (≤ 10 kJ mol^{-1}) with B3LYP/6-31G(d,p) in Gaussian 09,[13] (iii) computation of GIAO shielding constants at the same DFT level, (iv) Boltzmann weighting according to the B3LYP potential energies, and (v) DP4 probability calculations. Considering structures **2** and **3** as the two candidates, the DP4 calculation including all ^{13}C and ^1H shifts assigned EBC-342 (**2**) with 100.0 % probability. The statistical analyses in Table 1 show that the most diagnostic nucleus in the ^{13}C spectrum is C-5, which gave an error of 16.7 ppm when the structure is assigned as **3**, compared to 2.7 ppm when assigned as **2**. This carbon atom is the hydroperoxide-bearing carbon of **3**, and a HO-bearing carbon in **2**. Conversely, carbons C-1 and C-12, which are bound either to endoperoxide (in **3**) or ether oxygens (in **2**) revealed greater errors in **2**, although these were much smaller ($\Delta\delta \approx$ 4 ppm) than the error for C-5 in **2**. The mean unsigned error of the ^{13}C chemical shifts for **3** is 1 ppm larger than that for **2**.

Table 1. Experimental and calculated ^{13}C NMR chemical shifts for **3** and EBC-342 (**2**).

Position	^{13}C δ /ppm			Unsigned error ($\|\delta_{calc} - \delta_{expt}\|$) /ppm	
	Expt	Calc for **3**[a]	Calc for **2**[a]	**3**	**2**
1	80.8	82.3	76.7	1.5	4.1
2	162.2	152.4	159.8	9.8	2.4
3	113.2	119.1	114.2	5.9	0.9
4	199.8	196.5	195.1	3.3	4.7
5	84.4	101.2	87.2	16.7	2.7
6	133.9	127.8	137.5	6.1	3.6
7	129.5	129.4	123.9	0.1	5.6
8	25.0	32.2	32.3	7.3	7.3
9	31.6	37.1	37.1	5.5	5.4
10	19.4	24.7	23.2	5.3	3.8
11	41.1	40.3	41.8	0.8	0.7
12	87.1	86.6	82.8	0.5	4.3
13	78.5	75.6	81.0	2.9	2.5
14	40.3	35.4	38.6	4.8	1.6
15	20.3	27.2	26.7	6.9	6.3
16	28.8	29.8	29.5	1.0	0.7
17	15.6	16.9	17.0	1.3	1.4
18	10.7	13.6	12.9	2.9	2.2
19	16.2	13.6	14.0	2.5	2.2
20	18.0	18.2	17.9	0.2	0.1
Max. unsigned error:				16.7	7.3
Mean unsigned error:				4.3	3.1

[a] B3LYP/6-31G(d,p).

The absolute stereochemical determination of EBC-342 (**2**) was performed using quantum chemical calculations to predict the CD spectrum Gaussian 09[13] and ORCA 3.0.1.[14] The geometries of conformers of **2** and the Boltzmann weightings were the same as those employed for the NMR spectroscopic calculations described above. The conformational search identified three low-energy conformers of **2** ($\Delta E \leq 3.1$ kcal mol^{-1}). For each of these conformers, a time-dependent density functional theory calculation was performed at the TD-RI-B2PLYP/TZVP level of theory[15] using the auxiliary TZV/C correlation fitting basis set. Solvation in acetonitrile was simulated using the

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COSMO model.[16] Simulation of 50 excitations was found to allow coverage of the wavelength range of the experimental measurements. The Boltzmann-weighted CD spectra were normalized for comparison to experiment. The calculated and experimental CD spectra are plotted in Figure 5, and show good correlation as compared to previously reported casbane modelling and CD prediction.[1e]
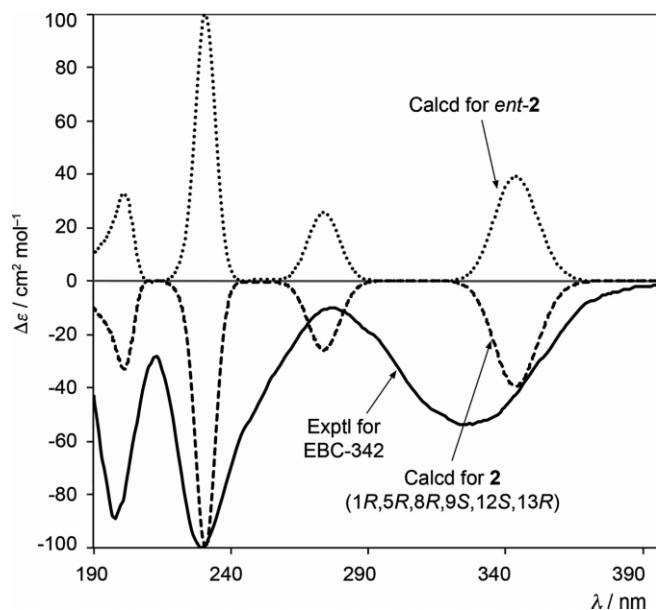

Figure 5. Comparison between experimental and calculated CD spectra of EBC-342 (**2**).

The comparison between the experimental and calculated spectra support the assignment of the absolute configuration for EBC-342 (**2**) as that shown in Figure 6, i.e. 1*R*,5*R*,8*R*,9*S*,12*S*,13*R*-**2**.
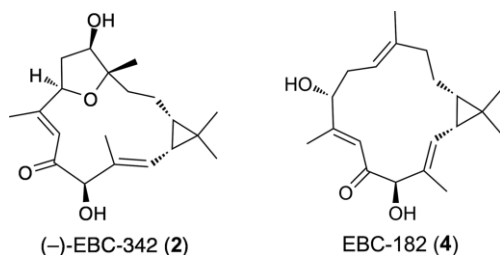

Figure 6. Absolute stereochemistry of EBC-342 (**2**) i.e. 1*R*,5*R*,8*R*,9*S*,12*S*,13*R*, and EBC-182 (**4**).

In terms of biosynthesis, numerous casbanes isolated from *C. insularis* contain a 12,13-double bond, and some of these members are hydroxylated at C1.[1] Therefore, it is conceivable that EBC-342 (**2**) is biogenetically derived from EBC-182 (**4**)[1e] following stereoselective epoxidation and intramolecular cyclisation. Epoxidation at the 12,13-position has previously been observed in this series (i.e. EBC-218[1e]).

Conclusions

A tetrahydrofuran containing casbane has been isolated from *Croton insularis* for the first time i.e. EBC-342 (**2**). Although the tetrahydrofuran moiety has been reported in other diterpene natural products, such as lobocrasols, latheranes, flexibilanes, and cembranes, this observation is unique for casbanes. DP4 was utilized to aid in assigning the chemical structure along with the deployment of TD DFT methods facilitate the determination of absolute stereochemistry of **2**.

Experimental Section

Supporting Information (see footnote on the first page of this article): Experimental details, characterization data and copies of 1D and 2D NMR spectra are provided in the supporting information.

Conflict of Interest

G.M. B. was a recipient of a fellowship co-sponsored by EcoBiotics Ltd. G.M. B., P.G. P. and C.M. W. were recipients of contract research funding from EcoBiotics Ltd.

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