

# Vibrallactone: A Lipase Inhibitor with an Unusual Fused $\beta$ -Lactone Produced by Cultures of the Basidiomycete *Boreostereum vibrans*

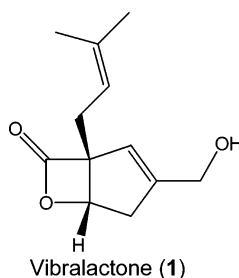
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## ABSTRACT



The structure and absolute configuration of vibrallactone (1) from the cultures of the Basidiomycete *Boreostereum vibrans* were established by spectroscopic methods and computational methods. Vibrallactone, an unusual fused  $\beta$ -lactone-type metabolite, was found to inhibit pancreatic lipase with an  $IC_{50}$  of 0.4  $\mu\text{g/mL}$ .

Lipid metabolism normally keeps a delicate balance between synthesis and degradation. When the balance is upset, hyperlipidemia may occur, which in turn can cause atherosclerosis, hypertension, diabetes, etc.<sup>1</sup> Modulators of lipid metabolism are expected to be useful in controlling these disorders. Obesity and hypercholesterolemia are to a relevant degree related to high nutritional fat intake. The key enzyme of dietary triglyceride absorption is pancreatic lipase. Inhibition of pancreatic lipase may therefore result in inhibition

of fat absorption. Orlistat, a specific pancreatic lipase, is clinically used for preventing obesity and hyperlipidemia.<sup>2</sup> Because of the biodiversity, chemodiversity, and unexplored resources of the fungal kingdom, secondary metabolites from macrofungi in China were investigated,<sup>3–6</sup> and a compound called vibrallactone was isolated from the culture broth of the polypore *Boreostereum vibrans* (Berk & M. A. Curtis; Davydkina & Bondartseva (Aphyllphorales)). It shows inhibition of pancreatic lipase with an  $IC_{50}$  of 0.4  $\mu\text{g/mL}$ . In

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previous investigations, a series of sterpurane-type sesquiterpenoids have been isolated from the closely related genus *Stereum*, and these compounds exhibited broad biological activities, including phytotoxic, antibiotic, and causative activities of the silver leaf disease.<sup>7–10</sup> In this communication, we report on the structural elucidation and assignment of the absolute configuration of vibrallactone, an unusual  $\beta$ -lactone-type metabolite.

Vibrallactone was obtained as a colorless oil,  $[\alpha]_D^{26} = -135.1$  ( $c = 0.517$ ,  $\text{CHCl}_3$ ). It exhibited a quasimolecular ion peak at  $m/z$  231 corresponding to  $[\text{M} + \text{Na}]^+$  in the positive ESI-MS, and HR-ESI-MS analysis provided the molecular formula  $\text{C}_{12}\text{H}_{16}\text{O}_3$  (calcd for  $[\text{M} + \text{Na}]^+$   $m/z$  231.1004, found 231.0997). The IR spectrum of vibrallactone exhibited carbonyl and hydroxyl group absorptions at 1816 and  $3423\text{ cm}^{-1}$ , respectively. Its  $^{13}\text{C}$  NMR spectrum showed 12 resolved peaks corresponding to 12 C-atoms, which were classified into 2 Me, 2  $\text{CH}_2$ , 1  $\text{OCH}_2$ , 1  $\text{OCH}$ , 2  $\text{sp}^2\text{ CH}$ , 2  $\text{sp}^2\text{ C}$ , 1  $\text{sp}^3\text{ C}$ , and 1  $\text{C}=\text{O}$  groups by analysis of the DEPT spectra. An analysis of the  $^1\text{H}$  NMR spectrum indicated the presence of two olefinic protons at  $\delta = 5.60$  (1H, s) and 5.11 ppm (1H, t,  $J = 7.3$  Hz), in addition to two isolated methyl groups at  $\delta = 1.71$  (3H, s) and 1.62 ppm (3H, s). The  $^{13}\text{C}$  NMR spectrum showed four olefinic carbon resonances at  $\delta = 146.6$  (s), 136.0 (s), 122.4 (d), and 117.2 ppm (d) as well as a carbonyl signal at  $\delta = 173.0$  ppm (s).

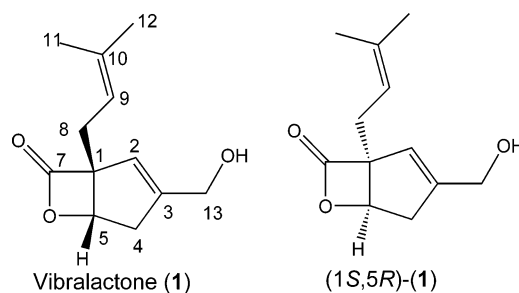
The connectivity of the protons and C-atoms was established by the  $^1\text{H},^{13}\text{C}$  HSQC spectrum. The cross-peaks between H-2 and H-4/H-13, H-9 and H-8/H-11/H-12, H-5 and H-4, and H-13 and H-4 were observed in the  $^1\text{H},^1\text{H}$  COSY spectrum. It allowed establishment of two H-atom systems, one at C-8 through C-11 and C-12 and the other at C-2 through C-13 and C-5.  $^{13}\text{C},^1\text{H}$  long-range couplings ( $^3J$ ) observed in the HMBC experiments gave the following correlations from H-2 to C-4, C-5, and C-13, from H-9 to C-1, C-11, and C-12, from H-5 to C-2, C-3, C-7, and C-8, from H-13 to C-2 and C-4, from H-4 to C-2, from H-8 to C-2, C-5, and C-10, from H-12 to C-9 and C-11, and from H-11 to C-9 and C-12. In addition, the HMBC spectrum showed unusual correlations across five bonds between H-13 and C-7 as well as between H-13 and C-8. It also happened in the same compound, and we observed the correlations across five bonds between H-11, H-12, and C-1. The presence of the substructure from C-1 through C-8, C-9, C-10, C-11, and C-12 in vibrallactone is doubtless. The presence of a double bond in the five bonds of this unique structure could be the reason. By combining all this evidence and data, we were able to assign planar structure **1** to vibrallactone (Table 1, Figure 1). It explains the characteristic IR absorption at  $1816\text{ cm}^{-1}$  for the  $\beta$ -lactone as well as the singlet for the  $\text{CH}_2\text{OH}$  group at  $\delta_{\text{H}} = 4.22$  ppm ( $\delta_{\text{C}} = 61.3$  ppm) in the  $^1\text{H}$  NMR spectrum.

The sharp singlet for an olefinic proton at  $\delta_{\text{H}} = 5.60$  ppm attributed to H-2 in the  $^1\text{H}$  NMR spectrum suggested that

**Table 1.** NMR Spectral Data for Vibrallactone (**1**) in  $\text{CDCl}_3$

	$^{13}\text{C}$	$^1\text{H}$	$^1\text{H},^1\text{H}$ COSY	HMBC
1	75.1			
2	122.4	5.60 (1H, s)	H-4, 13	C-1, 3, 4, 5, 13
3	146.6			
4	37.3	2.77 (1H, dd, 18.8, 4.3) 2.71 (1H, d, 18.8)	H-2, 5, 13	C-2, 3, 5
5	78.5	4.79 (1H, d, 4.3)	H-4	C-2, 3, 4, 7, 8
7	173.0			
8	27.6	2.60 (1H, dd, 15.1, 7.3) 2.41 (1H, dd, 15.1, 7.3)	H-8b, 9 H-8a, 9	C-1, 2, 5, 9, 10
9	117.2	5.11 (1H, t, 7.3)	H-8, 11, 12	C-1, 11, 12
10	136.0			
11	18.0	1.62 (3H, s)	H-9	C-1, 9, 10, 12
12	25.8	1.71 (3H, s)	H-9	C-1, 9, 10, 11
13	61.3	4.22 (2H, s)	H-4	C-1, 2, 3, 4, 7, 8

the  $\text{CH}_2\text{OH}$  group was attached to C-3. This was confirmed by the correlations between H-4 and H-13 in the  $^1\text{H},^1\text{H}$  COSY spectrum and between H-2 and H-8 in the HMBC. Further evidence for this is the presence of cross-peaks between H-2 and H-8 and H-13 in the ROESY.



**Figure 1.** Structure of vibrallactone (**1**).

A literature search indicated that all 6-oxy-bicyclo[3.2.0]-hept-2-en-7-ones reported so far are cis-fused. The trans-fused species can be excluded due to the instability of the lactone group caused by the high strain energy of this system.<sup>11–14</sup> The cis stereochemistry of vibrallactone is supported by computation of the  $^{13}\text{C}$  NMR shifts for the cis and trans stereoisomers (see Supporting Information).

For the assignment of absolute configuration, the optical rotation values for vibrallactone (**1**) and its enantiomer (1S,5R)-**1** were calculated. The first use of modern HF calculations for optical rotations was reported by Polavarapu

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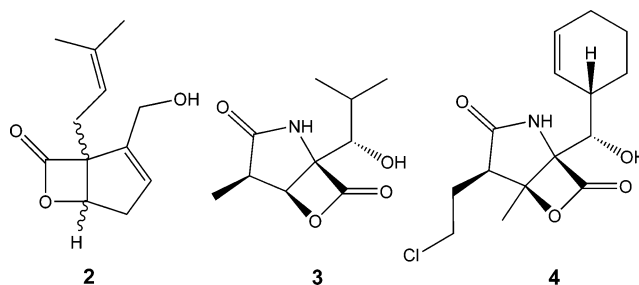
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in 1997,<sup>15</sup> using the Rosenfeld method developed in the CADPAC program by Amos.<sup>16</sup> Then, scientists reported various improved methods of optical rotation calculations.<sup>17</sup> Now, it is popular to use B3LYP/aug-cc-pVDZ//B3LYP/6-31G(d) for prediction of the optical rotation in chiral rigid compounds. The computed optical rotation values for each enantiomer are summarized in Tables 2 and 3 of the Supporting Information. The sum of optical rotations for vibrallactone (**1**) is  $-127.4^\circ$  and for its enantiomer is  $+127.4^\circ$ . The first value is very close to the experimental value of  $-135.1^\circ$ , which strongly suggests the configuration of vibrallactone given in structure **1**.

Surprisingly, initial attempts to proof the cis junction between the lactone and the cyclopentene ring in **1** by ROESY experiments showed no correlations between the protons at C5 and C8. From the calculations given in the Supporting Information, the distances between 5-H and the methylene protons 8a-H and 8b-H in the most stable cis-conformer **1e** are 3.1 and 3.9 Å, respectively. These distances decrease to 2.6 and 2.8 Å in the third most stable conformation **1b** (relative energy of 0.322 kcal/mol) and the second most stable conformation **1c** (0.241 kcal/mol), respectively.

Interestingly, a structurally closely related metabolite, percyquinnin (**2**), of undetermined relative and absolute configuration has recently been isolated from cultures of *Stereum complicatum*.<sup>18</sup> **2** displays significant lipase-inhibiting properties. After completion of our work, Dr. M. Brönstrup, Aventis-Sanofi, kindly informed us that plane structure **2** for percyquinnin is wrong and has to be changed into that of vibrallactone **1**. The chiroptical properties of percyquinnin are still unknown.

Other bioactive  $\beta$ -lactones with a fused 4/5 ring system are salinosporamide A (**3**) and omuralide (**4**) (Figure 2).<sup>19–22</sup> Salinosporamide A (**3**) and omuralide (**4**) are potent naturally derived substances which inhibit proteasome function with very high selectivity. Proteasome inhibition offers considerable promise in the therapy of a number of types of cancer and is already used for multiple myeloma.<sup>23</sup> A test on the potential proteasome activity of vibrallactone (**1**) against chymotrypsin was planned.



**Figure 2.** Structure of percyquinnin (**2**), salinosporamide A (**3**), and omuralide (**4**).

The structure of vibrallactone (**1**) is interesting in connection with tetrahydrolipstatin (orlistat), a  $\beta$ -lactone-type natural lipase inhibitor. The pancreatic lipase activity of vibrallactone (**1**) was measured using 4-methylumbelliferyl oleate (4-MU oleate) as a substrate. It showed pancreatic lipase inhibitory activity with  $IC_{50}$  of 0.4  $\mu\text{g/mL}$ . The details are given in the Supporting Information. Tetrahydrolipstatin (orlistat), a reduced form of the natural product lipstatin, is an antiobesity agent marketed under the tradename Xenical that was recently approved by the FDA as the first over-the-counter weight-loss medication.<sup>24</sup> The  $\beta$ -lactone-containing natural products inhibit gastric and pancreatic lipase by blocking hydrolysis of triglycerides and thus the uptake of fatty acids from diet.<sup>25</sup> The mechanism of inhibition involves covalent but reversible modification of the active site serine via acylation by the  $\beta$ -lactone. The pancreatic lipase inhibitory activity of vibrallactone (**1**) is probably due to the same mechanism.

**Vibrallactone (1):** The culture broth was filtered to remove the mycelium. The filtrate (12 L) was then successively extracted twice with ethyl acetate, and the crude extract (1.75 g) was chromatographed on a silica gel column using a  $\text{CHCl}_3/\text{MeOH}$  gradient. Several fractions of increasing polarity were collected. Fraction II (120 mg) eluted with  $\text{CHCl}_3/\text{MeOH}$  (98:2, v/v) was further subjected to column chromatography over silica gel and Sephadex LH-20, using a petroleum ether/ethyl acetate (8:1, v/v) and  $\text{CHCl}_3/\text{MeOH}$  (1:1, v/v), respectively, to yield 65 mg of **1** as a colorless oil:  $[\alpha]_D^{26} = -135.1$  ( $c = 0.52$  in  $\text{CHCl}_3$ ); IR (neat)  $\nu = 3423$  (OH), 2971, 2915, 2860, 1816 ( $\beta$ -lactone), 1112, 1000, 836  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ , reference,  $\delta = 7.26$  ppm)  $\delta = 1.62$  (s, 3 H, 11-H), 1.71 (s, 3 H, 12-H), 2.41 (dd,  $J = 15.1, 7.3$  Hz, 1 H, 8b-H), 2.60 (dd,  $J = 15.1, 7.3$  Hz, 1 H, 8a-H), 2.71 (d, 1 H,  $J = 18.8$  Hz, 4 $\alpha$ -H), 2.77 (dd,  $J = 18.8, 4.3$  Hz, 4 $\beta$ -H), 4.22 (s, 2 H, 13-H), 4.79 (d,  $J = 4.3$  Hz, 1 H, 5-H), 5.11 (t,  $J = 7.3$  Hz, 1 H, 9-H), 5.60 ppm (s, 1 H, 2-H);  $^{13}\text{C NMR}$  (125 MHz,  $\text{CDCl}_3$ , reference,  $\delta = 77.0$  ppm)  $\delta = 18.0$  (C11), 25.8 (C12), 27.6 (C8), 37.3 (C4), 61.3 (C13), 75.1 (C1), 78.5 (C5), 117.2 (C9), 122.4 (C2), 136.0

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(C10), 146.6 (C3), 173.0 ppm (C7); MS (ESI<sup>+</sup>) *m/z* (%) 231 (100) [M + Na]<sup>+</sup>, 439 (85) [2M + Na]<sup>+</sup>; HR-ESI-MS *m/z* calcd for C<sub>12</sub>H<sub>16</sub>O<sub>3</sub>Na [M + Na]<sup>+</sup> 231.1004, found 231.0997.

In total, 24 conformations of **1** were obtained in the gas phase at the B3LYP/6-31G(d) level of theory. The obtained magnetic shielding magnitudes of <sup>13</sup>C NMR were recorded at the B3LYP/6-311+G(2d,p) level and were corrected using the slope and intercept of the least-squares correlation line. All of the shieldings were finally converted into chemical shifts. The optical rotation values were calculated using B3LYP/aug-cc-pVDZ theory. The Boltzmann formula was used to produce the sum of six different conformational

optical rotations. The details are given in the Supporting Information.

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**Supporting Information Available:** NMR data of **1**, computations, and bioassay experiment (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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