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Vibsatins A and B, Two New Tetranorvibsane-Type Diterpenoids from *Viburnum tinus* cv. variegatus

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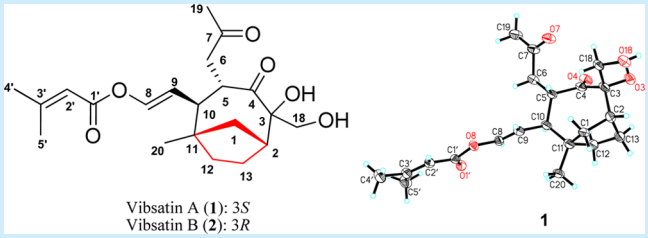
Supporting Information

ABSTRACT: Vibsatins A (**1**) and B (**2**), a pair of 14,15,16,17-tetranorvibsane-type diterpenoids that feature a bicyclo[4.2.1]nonane moiety formed by a new C-13/C-2 bond, were isolated from the twigs and leaves of *Viburnum tinus* cv. variegatus. The structures and absolute configurations were elucidated by a combination of NMR spectra, optical rotation, and X-ray diffraction experiments. A possible biogenetic pathway is also proposed. Moreover, vibsatin A (**1**) enhanced the neurite outgrowth of NGF-mediated PC12 cells at a concentration of 10 μM.



Vibsane-type diterpenoids are uniquely natural products, and their occurrence has been limited to five species of *Viburnum* (Caprifoliaceae): *V. awabuki*,[1,2] *V. odoratissimum*,[3,4] *V. suspensum*,[5] *V. sieboldii*,[6] and *V. chingii*.[7] Until now, more than 80 vibsane-type diterpenoids have been reported.[8,9] According to the carbon skeletons, these diterpenoids were further classified into three subtypes: 11-membered ring type, seven-membered ring type, and the rearranged type, with vibsanin B,[10] vibsanin C,[10] and neovibsanin A[11] as the represented examples, respectively. The plausible biosynthesis pathway between those three subtypes has been established on the basis of the thermal and photochemical reactions among vibsanin B, vibsanin C, and neovibsanin A.[11,12] In recent years, some vibsane-type diterpenoids have attracted increasing attention and have become the challenging targets of total synthesis.[13−15] Among them, neovibsanins showed prominent neurite outgrowth promoting activity.[6,16−18]

V. tinus cv. variegatus, a cultivated specie of *V. tinus*,[19] has become a widely used landscape plant in China. No chemical constituents have been reported from this plant. Previous phytochemical investigations of *V. tinus* have resulted in the isolation of iridoids, coumarins, saponins, and flavonoids, which are associated with neuroprotective, hepatoprotective, sedative, and spasmolytic activities.[20,21] As part of an ongoing program aimed at discovering structurally novel and bioactive compounds from this genus,[7,22,23] two novel vibsane-type diterpenoids, vibsatins A (**1**) and B (**2**), with the known vibsanins B (**3**), C (**4**), and K (**5**) were isolated from *V. tinus* cv. variegatus. Spectroscopic and X-ray diffraction techniques suggested vibsatins A (**1**) and B (**2**) were a pair of 14,15,16,17-tetranorvibsane-type diterpenoids that featured a core bicyclo[4.2.1]nonane moiety formed by a new C-13/C-2 bond (Figure 1). The degradation of C-14,15,16,17 and the formation of a unique C-13/C-2 bond in **1** and **2** constructed a new subtype of vibsane-type diterpenoids.

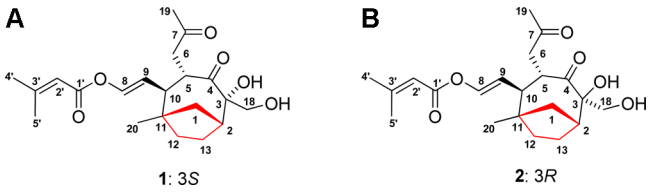


Figure 1. Structures for vibsatins A (**1**) and B (**2**).

Vibsatin A (**1**), colorless needles (from CH_3OH), had the molecular formula $C_{21}H_{30}O_6$ as determined by the HR-EI-MS at *m/z* 378.2041 (calcd 378.2042), corresponding to seven degrees of unsaturation. The IR spectrum showed absorptions attributable to hydroxyl (3441 cm^{-1}) and carbonyl groups (1726 and 1705 cm^{-1}). Analysis of the ^1H and ^{13}C NMR spectra of **1** (Table 1) showed the presence of a β,β-dimethyl acrylate group [δ_H 1.99 (s), 2.24 (s), and 5.76 (s), δ_C 164.9], a disubstituted olefin [δ_H 7.18 (d, J = 12.3 Hz) and 5.33 (dd, J = 12.3, 11.0 Hz)], an oxymethylene [δ_H 4.29 (d, J = 12.0 Hz) and 3.74 (d, J = 12.0 Hz); δ_C 68.9], two methyl groups [δ_H 2.15 (s) and 1.01 (s)], and two carbonyl groups (δ_C 215.1 and 211.6). Among them, the β,β-dimethyl acrylate and the two carbonyl groups were the characteristic chemical signals for seven-membered ring vibsane-type diterpenoids.[24] However, compared with the common vibsane-type diterpeniods, **1** missed

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Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data of 1 and 2 in CD_3OD (δ in ppm, J in Hz)

	1		2	
no.	δ_H	δ_C	δ_H	δ_C
1a	2.30 (dd, 14.2, 2.3)	38.2	2.56 (dd, 13.8, 1.7)	37.4
1b	1.48 (m)		1.34 (dd, 13.8, 6.5)	
2	2.44 (t, 6.2)	47.1	2.40 (t, 6.6)	46.7
3		85.4		82.5
4		215.1		216.4
5	3.17 (td, 9.7, 2.5)	46.6	3.78 (td, 11.1, 3.0)	43.8
6a	3.25 (dd 18.2, 9.7)	46.4	3.10 (dd, 18.4, 11.1)	46.6
6b	2.78 (dd, 18.2, 2.5)		2.74 (dd, 18.4, 3.0)	
7		211.6		210.8
8	7.18 (d, 12.3)	138.5	7.15 (d, 12.3)	137.8
9	5.33 (dd, 12.3, 11.0)	117.3	5.26 (dd, 12.3, 10.9)	117.5
10	1.89 (dd, 11.0, 9.7)	54.5	1.73 (dd, 11.1, 10.9)	52.7
11		44.4		44.5
12a	1.46 (m)	41.3	1.50 (m)	41.8
12b	1.12 (m)		1.30 (m)	
13a	1.97 (m)	27.2	1.67 (m)	27.2
13b	1.51 (m)		1.53 (m)	
18a	4.29 (d, 12.0)	68.9	3.99 (d, 11.6)	64.9
18b	3.74 (d, 12.0)		3.63 (d, 11.6)	
19	2.15 (s)	30.0	2.11 (s)	29.6
20	1.01 (s)	32.2	1.01 (s)	32.5
1′		164.9		164.8
2′	5.76 (s)	115.5	5.77 (s)	115.5
3′		162.1		161.8
4′	2.24 (s)	20.7	2.24 (s)	20.6
5′	1.99 (s)	27.8	1.99 (s)	27.6

four carbon signals in the ^{13}C NMR spectrum. The molecular formula also indicated that the carbon number for **1** was four less than that of normal vibsane-type diterpeniods.

Extensive analysis of HSQC and ^{1}H−^{1}H COSY gave two fragments (**a** and **b**). In order to determine the connectivity between the two partial structures, HMBC experiments were carried out (Figure 2). The HMBC correlations of H_2-18 with C-2 (δ_C 47.1), C-3 (δ_C 85.4), and C-4 (δ_C 215.1) indicated the connections of C-3 with C-2, C-4, and C-18. And the HMBC correlations of H-5 with C-4 established the connection of C-4

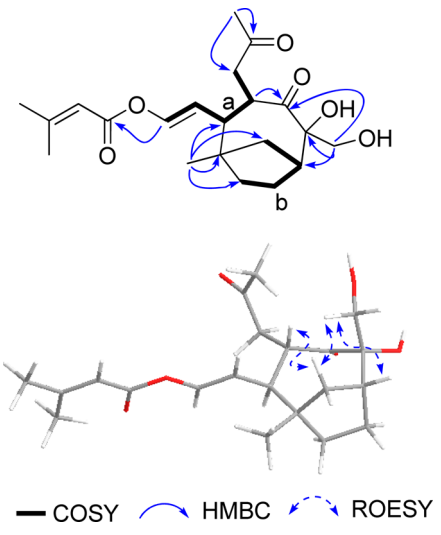

Figure 2. Key 2D correlations of compound **1**.

with C-5 in the fragment **a**. While, the H_3-20 showed HMBC cross-peaks with C-1 (δ_C 38.2), C-10 (δ_C 54.5), C-11 (δ_C 44.4), and C-12 (δ_C 41.3) that suggested the quaternary carbon C-11 was connected to C-1 and C-12 in fragment **b**, C-10 in fragment **a**, and C-20. Here, a novel bicyclo[4.2.1]nonane moiety was established. Furthermore, the HMBC correlations of H_3-19 with C-6 (δ_C 46.6) and C-7 (δ_C 211.6) indicated that a methyl ketone was bonded to C-6 in fragment **a**. Finally, the connectivity between the β,β-dimethyl acrylate group and C-8 in fragment **a** was constructed by a cross-peak of H-8 with C-1′. Therefore, the gross structure of **1** was established as 14,15,16,17-tetranorvibsane-type diterpenoid, which featured a unique bicyclo[4.2.1]nonane moiety formed by a new C-13/C-2 bond.

The relative stereochemistry of **1** was elucidated by ROESY experiment. The ROESY correlations of H-1a/H-5, H-1a/H-18, and H-18/H-2 indicated that H-5 was β-oriented and OH-3 was α-oriented. The α-orientation of H-10 was inferred from the ROESY cross-peak of H-9/H-12 (Figure 2). Additionally, the coupling constant (J = 12.3 Hz) indicated the double bond at C-8/C-9 should take *E*-geometry. This deduction was further confirmed by an X-ray diffraction experiment using Cu Kα radiation (Figure 3). Thus, the structure of **1** was identified as a

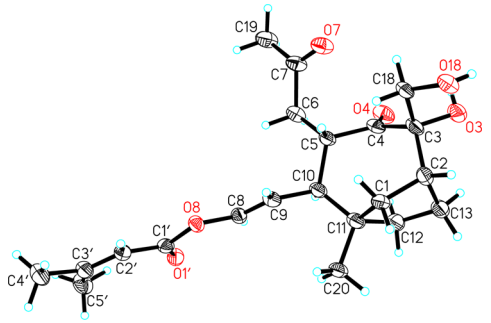

Figure 3. X-ray crystal structure of **1**.

unique 14,15,16,17-tetranorvibsane-type diterpenoid, featured with a unique bicyclo[4.2.1]nonane moiety. And the absolute configuration was determined as 2*R*,3*S*,5*S*,10*S*,11*S*.

The absolute configuration of vibsanin C was reported as 5*S*,10*R*,11*S*.[12] However, according to the Cahn−Ingold−Prelog (CIP) system,[25,26] the absolute configuration of C-10 in vibsanin C should be *S* rather than *R* (see the Supporting Information).

Vibsatin B (**2**) showed the same molecular formula, $C_{21}H_{30}O_6$, as that of **1** by analysis of the HR-EI-MS. The ^{1}H and ^{13}C NMR spectra of **2** (Table 1) revealed 21 carbon signals, due to four methyls, five methylenes, three methines, and five quaternary carbons, which was similar with that of **1**. However, the different ^{13}C NMR chemical shifts of C-3 and C-18 in CD_3OD (Table 1) suggested the opposite configuration of the hydroxyl group attached at C-3, which was further confirmed by the ROESY correlation of H_2-18/H-13b (Figure 4). Detailed 2D NMR data analysis indicated that the other parts of **2** were the same as those of **1**. Thus, the structure of **2** was established as a C-3 epimer of **1**. Based on the biosynthesis point of view, the absolute configuration for **2** was 2*R*,3*R*,5*S*,10*S*,11*S*.

The biogenetic origin of **1** and **2** could plausibly be traced back to geranylgeraniol (GGPP, **i**) (Scheme 1). Geranylgeraniol underwent cyclization to produce the humulane carbon

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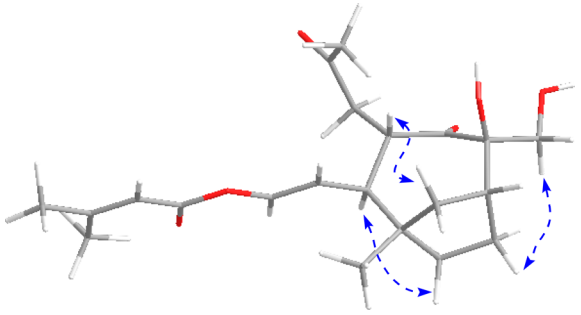
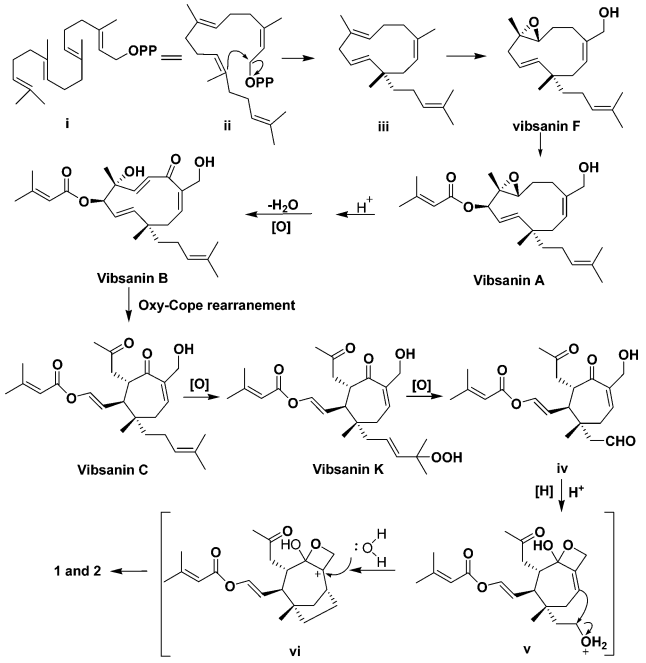


Figure 4. Key ROESY correlations of **2**.

Scheme 1. Plausible Biogenetic Pathway of 1 and 2



skeleton **iii**. Intermediate **iii** was oxidized to vibsanin F, which was further oxidized to generate vibsanin A. Vibsanin A underwent protonation, dehydration, and oxidation steps to obtain vibsanin B, which went through oxy-Cope rearrangement, oxidation, and successive oxidative degradation to yield vibsanin C, vibsanin K, and intermediate **iv**, respectively. Lastly, intermediate **iv** was reduced, protonated, and cyclized to form intermediate **vi**, which was hydrolyzed to give **1** and **2**.

In view of the prominent biological activities of vibsane-type diterpenoids,[16−18,27] all of the isolates examined their ability to stimulate nerve growth factor (NGF) mediated neurite outgrowth on PC12 cells.[28] The results showed vibsanins C, K, and vibsatin A had obviously increasing activity of neuronal differentiation after 72h at the concentration of 10 μM. The differentiation rates are 22.76%, 24.88%, and 13.61%, respectively, compared with 8.48% of the negative control.

Up to now, only two norvibsane-type diterpenoids were reported, both of which were one-carbon norvibsane-type diterpenoids.[6,29] It should be emphasized that vibsatins A (**1**) and B (**2**) are the first examples of 14,15,16,17-tetranorvibsane diterpenoids containing a core bicyclo[4.2.1]nonane moiety, which represented a new subtype of vibsane-type diterpenoids. This type of natural products has not been documented so far. The biological activity for vibsatin A, vibsanin K, and vibsanin C suggested, in addition to the neovibsanins, another type of vibsane-type diterpenoid that also possessed NGF-potentiating effects on PC12 cells.[9] And the discovery of vibsatins A and B and vibsanins B, C, and K from the same plant sheds new insights into the biosynthesis of vibsane-type diterpenoids for the first time.

■ ASSOCIATED CONTENT

Supporting Information

1D, 2D NMR, HR-EI-MS, IR, and UV spectra and optical rotation data of **1** and **2**; detailed experimental procedures and X-ray data for **1** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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