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A New Bis-*seco*-abietane Diterpenoid from *Hyptis crenata* Pohl ex Benth

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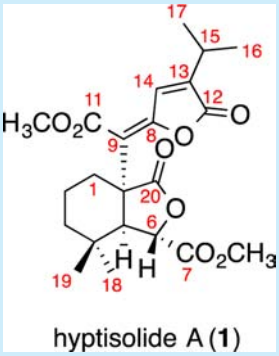
Supporting Information

ABSTRACT: A new, highly oxidized, bis-*seco*-abietane diterpenoid named hyptisolide A (**1**) was isolated from *Hyptis crenata* Pohl ex Benth. Its structure and stereochemistry were elucidated on the basis of data obtained by HRESIMS, NMR, and X-ray diffraction analyses, and its absolute configuration was determined with vibrational circular dichroism spectroscopy. By reporter gene assay, **1** was demonstrated to induce cAMP-responsive element-dependent transcription in Neuro2A cells.



hyptisolide A (**1**)

Hyptis crenata Pohl ex Benth. (Lamiaceae) is an herbaceous plant that grows in Brazil. The genus *Hyptis* is mainly known for the essential oils isolated from the aerial part, from which many phytochemicals have been identified. Besides those in the essential oils, various compounds including abietane-type diterpenoids have been isolated from several *Hyptis* species.[1] For *H. crenata*, however, phytochemicals reported so far are limited to those in the essential oil,[2−4] and no abietane-type diterpenoid has been reported. In this paper, we report the isolation, structural determination including absolute configuration, and in vitro bioactivity of a new bis-*seco*-abietane diterpenoid, named hyptisolide A from a methanolic extract of the aerial parts of this plant.

The methanolic extract (73.4 g) from the aerial parts (dry weight 430 g) of *H. crenata* was chromatographed on a silica gel column and eluted with hexane/ethyl acetate and then ethyl acetate/methanol solvent systems to yield fractions 1−6. Fraction 4 was separated on a silica gel column by elution with a $CHCl_3$/MeOH (100:0, 20:1, and 10:1) solvent system to afford ten subfractions (F.4−1−10). Further purification of subfraction F.4−6 by HPLC (65% aqueous CH_3CN) gave hyptisolode A (**1**) (12 mg).

Recrystallization of **1** from methanol gave colorless prisms that showed an optical rotation of $[\alpha]_D^{25}$ −39.4 (*c* 0.14, MeOH). The molecular formula of **1** was established as $C_{22}H_{28}O_8$ by HRESIMS measurements ($[M + H]^+$ *m*/*z* 421.1864, calcd. for $C_{22}H_{29}O_8$ 421.1862). The ^1H NMR spectrum of **1** (Table 1) exhibited signals for two secondary methyl groups at δ_H 1.23 (6H, d, J = 6.8 Hz) both coupled with a methine proton signal at δ_H 2.79 (1H, sept d, J = 6.8, 1.3 Hz), two tertiary methyl groups (δ_H 0.82 and 0.95, each 3H, s), an olefinic methine proton (δ_H 7.66, 1H, d, J = 1.3 Hz), two methoxy protons (δ_H 3.82 and 3.87, each 3H, s), and an oxygen-bearing methine proton (δ_H 4.92, 1H, d, J = 9.7 Hz). The ^{13}C NMR (Table 1) and HMQC spectra of **1** showed 22 carbon signals, consisting of four carbonyl carbons (δ_C 166.7, 168.2, 170.4, and 176.5), four olefinic carbons (δ_C 116.7, 136.4, 142.9, and 154.6), an oxymethine carbon (δ_C 77.0), two aliphatic quaternary carbons (δ_C 31.2, and 50.6), two aliphatic methines (δ_C 26.8 and 53.6), three aliphatic methylenes (δ_C 19.5, 29.2, and 34.8), four methyl carbons (δ_C 20.9 × 2, 28.5, and 28.6), and two methoxy carbons (δ_C 53.1 and 53.3).

Analysis of $^1H-^1H$ COSY and HMQC spectra indicated the presence of three carbon-chain fragments in the molecule: a trimethylene group (C-1−C-2−C-3), a dimethine group (C-5−C-6), and an isopropyl group (C-16−C-15−C-17). HMBC data revealed the relationships between those carbon-chain fragments and the nature of the other skeleton atoms involved. HMBC correlations from H-1a (δ_H 2.73) to C-5 (δ_C 53.6) and C-10 (δ_C 50.6), from H_2-2 (δ_H 1.65) to C-10, from H-3b (δ_H 1.39) to C-4 (δ_C 31.2) and C-5, and from H-5 (δ_H 3.00) to C-1 (δ_C 29.2), C-3 (δ_C 34.8), C-4, and C-10 indicated that the trimethylene group, C-1−C-2−C-3, one of the methine carbon (C-5) of the dimethine group, and two quaternary carbons, C-4 and C-10, formed a cyclohexane ring. The correlations from H-3a (δ_H 1.65), H-3b, and H-5 to C-18 (δ_C 28.5) and C-19 (δ_C 28.8), and from H_3-18 (δ_H 0.95) and H_3-19 (δ_H 0.82) to C-3, C-4, and C-5 showed that two methyl carbons, C-18 and C-19, were connected to C-4. HMBC correlations from H-1b (δ_H

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Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectral data of 1 in acetone-d_6 at 300 K

	δ_H [a]	δ_C
1a	2.73 (1H, brd, 14.9)	29.2
1b	1.59 (1H[b])	
2	1.65 (2H[b])	19.5
3a	1.65 (1H[b])	34.8
3b	1.39 (1H, brd)	
4		31.2
5	3.00 (1H, d, 9.7)	53.6
6	4.92 (1H, d, 9.7)	77.0
7		170.4
8		154.6
9		116.7
10		50.6
11		166.7
12		168.2
13		142.9
14	7.66 (1H, d, 1.3)	136.4
15	2.79 (1H, sept d, 6.8, 1.3)	26.8
16	1.23 (3H, d, 6.8)	20.9
17	1.23 (3H, d, 6.8)	20.9
18	0.95 (3H, s)	28.5
19	0.82 (3H, s)	28.8
20		176.5
7-OMe	3.82 (3H, s)	53.1
11-OMe	3.87 (3H, s)	53.3

[a]J-values are given in Hz in parentheses. [b]Multiplicity was not determined due to overlapping and/or broadening of the signals.

1.59) and H-6 (δ_H 4.92) to the carbonyl carbon C-20 (δ_C 176.5) indicated that C-5, C-6, C-10, C-20, and an oxygen atom formed a γ-lactone ring. The correlations from H-5, H-6, and the methoxy protons at δ_H 3.82 to the carbonyl carbon C-7 (δ_C 170.4) suggested that a carbomethoxy group was connected to the methine carbon C-6.

HMBC correlations from H-1a, H-1b, and H-5 to the olefinic quaternary carbon C-9 (δ_C 116.7) showed that C-9 was connected to C-10. The methoxy proton signals at δ_H 3.87 showed an HMBC correlation to the C-11 carbonyl carbon signal at δ_C 166.7, revealing the presence of another carbomethoxy group in the molecule of **1**. These methoxy protons also displayed long-range couplings with C-9 and the olefinic quaternary carbon C-8 (δ_C 154.6). These long-range $^4J_{CH}$ and $^5J_{CH}$ couplings[5] indicated that the C-11 carbonyl carbon was connected to C-9, and C-8 and C-9 formed a double bond. From the observation of the HMBC correlations from H-14 (δ_H 7.66) to C-8, C-12 (δ_C 168.2), and C-13 (δ_C 142.9), we considered that C-8, C-12, C-13, C-14, and an oxygen atom formed an α,β-unsaturated-γ-lactone ring. Thus, compound **1** had three rings and six double bonds in the structure that accounted for the nine degrees of unsaturation calculated from the molecular formula. The location of the isopropyl group was determined to be at C-13 from the HMBC correlations from H-14 to C-15 (δ_C 26.8), from H-15 (δ_H 2.79) to C-12, C-13, and C-14, and from H_3-16 (δ_H 1.23) and H_3-17 (δ_H 1.23) to C-13. Accordingly, the planar structure of **1** was established as shown in Figure 1.

NOESY experiments established the stereochemistry of **1** (Figure 1). NOESY correlations between H-3a/H-6, H-5/H_3-18, H-5/OCH_3-11, and H-6/H_3-19 suggested that H-3a, Me-19, and the C-6 methine carbon were on the same face of the

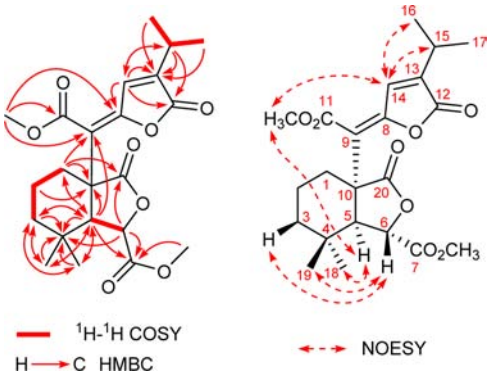

Figure 1. Key ^{1}H−^{1}H COSY, HMBC, and NOESY correlations of **1**.

cyclohexane ring, whereas H-5 and Me-18 were on the opposite face, H-5 and the carbomethoxy group at C-6 were on the same face of the γ-lactone ring, and the cyclohexane ring and the γ-lactone ring were *cis* fused at C-5/C-10. The correlation between OCH_3-11/H-14 indicated that the C-8/C-9 double bond had *E* configuration. From these observations, the structure of **1** was determined as shown in Figure 1.

The structure including relative configuration was confirmed by single-crystal X-ray diffraction analysis of **1** recrystallized from MeOH, as shown in Figure 2.

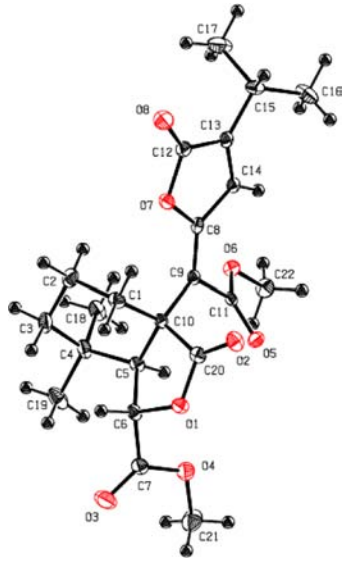

Figure 2. ORTEP drawing of the crystal structure of **1**.

The absolute configuration of **1** was determined by comparing the experimental vibrational circular dichroism (VCD) spectrum of **1** with the theoretically calculated spectra of both enantiomers of **1** using crystal diffraction data. As shown in Figure 3, the experimental VCD spectrum was very similar to the calculated spectrum of (5*S*,6*S*,10*R*)-**1**. Thus, the 5*S*, 6*S*, 10*R* configuration was assigned to **1**.

There are 58 naturally occurring *seco*-abietane diterpenoids that have been reported so far.[6] Of the 7,8-*seco*-abietane-type, however, only two compounds are known: 7,8-*seco*-*para*-ferruginone from *Salvia prionitis*[7] and 7,8-*seco*-7(20),11(20)-diepoxy-7,14-dihydroxyabieta-8,11,13-triene from *Hyptis martiusii*.[8] In comparison with these compounds, hyptisolide A (**1**), is unique in that in addition to the C-7/C-8 bond, the C-11/C-12 bond of the abietane skeleton has also been oxidatively

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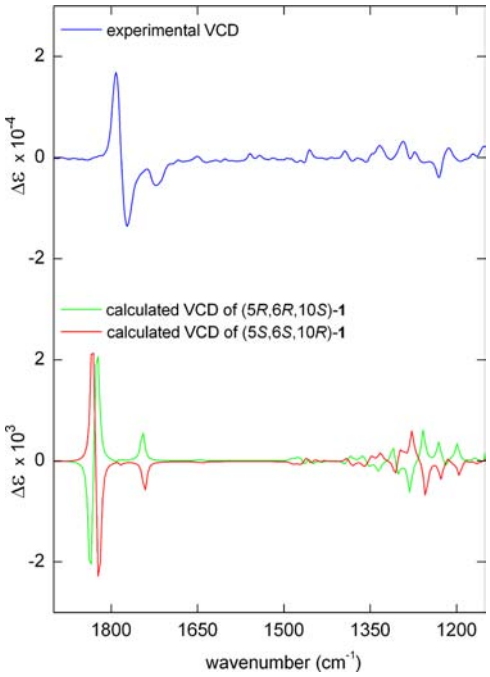

Figure 3. Comparison of the experimental VCD spectrum of **1** in chloroform-*d* (top) with the calculated VCD spectra of (5*R*,6*R*,10*S*)-**1** and (5*S*,6*S*,10*R*)-**1** (bottom).

cleaved. As far as we know, hyptisolide A (**1**) is the first naturally occurring diterpenoid that has the 7,8;11,12-bis-*seco*-abietane skeleton. A possible biogenetic pathway for **1** is shown in Figure 4. Rosmanol, an abietane diterpenoid that was also

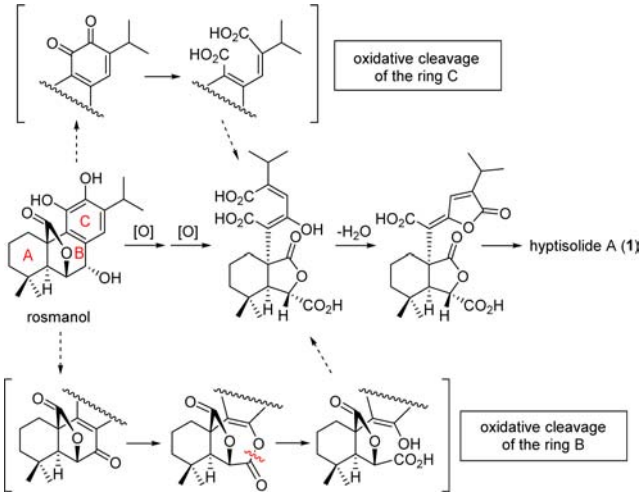

Figure 4. A possible biosynthetic pathway from rosmanol to hyptisolide A (**1**).

isolated from this plant in the present study, is a possible precursor. Oxidative cleavage and successive esterification in rings B and C of rosmanol may lead to generation of **1**. Ring B might be cleaved via oxidation to a ketone, followed by conversion into a lactone by Baeyer–Villiger-type oxidation, and hydrolysis. Baeyer–Villiger-type oxidation can be mediated by cytochrome P450.[9] Oxidative cleavage of ring C might occur through oxygenation of the catechol moiety to the dicarboxylic acid form, although catechol 1,2-dioxygenase from plants has not been identified.

The bioactivity of **1** was examined in vitro using a reporter gene assay system for cAMP response element (CRE)-dependent transcriptional induction. Mouse neural crest-derived cells (Neuro2A) were transfected with an expression plasmid, pGL4.29 [luc2P/CRE/Hygro], which contains a CRE to control transcription of the luciferase reporter gene in response to cAMP. The addition of **1** (50 μM) to pGL4.29-transfected cells increased luciferase activity about 3-fold after 4 h, suggesting that **1** has the potential to enhance CRE-dependent transcription in Neuro2A cells. Although some flavonoids,[10−12] secoiridoid glucosides,[13] and a chalcone derivative[14] enhance CRE binding protein (CREB)-dependent transcriptional induction, forskolin is the only diterpenoid known to induce CREB-dependent transcription in neuronal cells.[15] Previously, we reported that three abietane-type diterpenes from rosemary suppress cAMP responsiveness of CRE and of gluconeogenic gene promoters in human hepatoma HepG2 cells.[16] In contrast, the *seco*-abietane-type diterpenoid isolated in this study induced CRE-dependent transcription in Neuro2A cells. Because activation of CREB induces expression of brain-derived neurotrophic factor, tyrosine hydroxylase (which functions in synthesis of the neurotransmitter dopamine), and a corticotrophin-releasing factor in nerve cells,[17] hyptisolide A (**1**) might exhibit neuroprotective effects or affect some neuronal functions.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, UV, IR, and NMR spectra, crystallographic data, and details of VCD calculations, HRESIMS, and luciferase assay. 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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