

Nudibaccatumone, a Trimer Comprising a Phenylpropanoid and Two Sesquiterpene Moieties from *Piper nudibaccatum*

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S Supporting Information

ABSTRACT: A new complex natural product with a C₃₉ skeleton, named nudibaccatumone, and the known sesquiterpenes (+)-spathulenol, (−)-4β,10α-aromadendranediol, and *ent*-T-muurolool, as well as the phenylpropanoid hydroxychavicol, were isolated from the aerial parts of *Piper nudibaccatum*. The structure and absolute configuration of nudibaccatumone were elucidated using spectroscopic methods and ECD calculations. A 1,8-Michael addition reaction and an intermolecular, inverse electron demand Diels–Alder reaction are proposed as the key steps in the biosynthesis of nudibaccatumone.

The genus *Piper* is a medicinally important member of the family Piperaceae, consisting of approximately 2000 species. There are approximately 60 species distributed in the tropical areas of China.¹ Many species in this genus have been used to alleviate pain and for the treatment of rheumatoid arthritis in traditional Chinese medicine.² Previous phytochemical investigations of *Piper* species have revealed the occurrence of amides, propenylphenols, lignans, neolignans, terpenes, steroids, kawapyrone, piperolides, and flavonoids.³ During our field research of medicinal plants used by the Jinuo people of Xishuangbanna Dai Autonomous Prefecture in the Yunnan Province of China during 2010 and 2011, we found that *Piper nudibaccatum* Tseng, a climbing liana found only in the Yunnan Province of China,¹ has been used as one of the ingredients in areca quid chewing by the local people, who believed that this plant protects teeth. In our continuing research on bioactive constituents of *Piper*,⁴ nudibaccatumone (**1**), a trimer comprising a phenylpropanoid and two sesquiterpene moieties, and the known sesquiterpenes (+)-spathulenol,⁵ (−)-4β,10α-aromadendranediol,⁵ and *ent*-T-muurolool,⁶ along with 4-allylbenzene-1,2-diol (hydroxychavicol),⁷ were isolated from the aerial parts of *P. nudibaccatum*. Herein, we report the isolation and structural elucidation of **1** and the results of antimicrobial and cytotoxicity bioassays.

Nudibaccatumone (**1**) was obtained as a yellowish oil. The molecular formula of **1** was established as C₃₉H₅₆O₃ by HRESIMS (*m/z* 595.4143 [M + Na]⁺, calcd 595.4127), requiring 12 indices of hydrogen deficiency. The IR spectrum showed absorption peaks for hydroxy (3432 cm^{−1}) and

carbonyl (1732 cm^{−1}) groups. Its ¹H NMR spectrum (Table 1) indicated a cyclopropyl ring (δ_H 0.16, t, *J* = 9.7 Hz; δ_H 0.36, t, *J* = 9.7 Hz) and eight methyl groups, at δ_H 1.71, 1.19, 1.18, 1.01, 0.99, 0.98, 0.98, and 0.84 ppm. The ¹³C NMR and DEPT spectra exhibited 39 resonances, consisting of two ketocarbons, three trisubstituted double bonds, one disubstituted double bond, nine aliphatic methines, eight aliphatic methylenes, four aliphatic quaternary carbons, and eight methyl groups. Since these functional groups accounted for six indices of hydrogen deficiency, the remaining six indices suggested the presence of a hexacyclic system in the structure of **1**.

The ¹H–¹H COSY spectrum revealed the presence of four fragments (Figure 1): a (C-5/C-6/C-5'/C-6'/C-7'/C-8'/C-9'), b (C-7/C-8/C-9), c (C-1'/C-2'/C-3'), and d (C-6"/C-7"/C-8"/C-9"/C-10"/C-1"/C-2" and C-14"/C-10"). On the basis of the existence of fragments a and c and the HMBC cross-peaks (Figure 1) of H-2'a to C-4' and C-10', H-3'a to C-1' and C-5', H₃-12' to C-6, H₃-13' to C-7', and H₃-15' to C-1' and C-9', the presence of a bicyclogermacrene fragment (**B**, Figure 1) was confirmed. In addition, the HMBC correlation of H₃-12" and H₃-13" to C-7", H₃-15" to C-5", H-2"α to C-4" and C-5", and H-6" to C-4", along with the fragment d, indicated that there was a gurjuene substructure (**C**, Figure 1). Meanwhile, the HMBC correlations of H-5 to C-3 and C-4', H-6 to C-7, C-2, and C-4, H-7 to C-6 and C-2, and H-8 to C-1, together with fragment b, established the remaining substructure (**A**, Figure

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Table 1. ^1H (500 MHz) and ^{13}C (100 MHz) NMR Data of 1
in CDCl_3 (δ in ppm, J in Hz). 4.74666 472.0818 53.1779 Tm (11)T23pmostBi9 0 DCICDCI

The relative configuration of 1 was deduced by the analysis of ROESY correlations (Figure 2). In fragment the key

ROESY correlations between H_3 -14, H_3 -14/ H_3 -15, and H_3 -14/ H_3 -15 showed that the four protons were cofacial and were arbitrarily assigned orientation. The correlations of H_2 -9/ H_2 -9 and H_2 -9/ H_1 -1 indicated that H_1 -1 was in a α -orientation. Likewise, in fragment II, the ROESY cross-peaks of H_6 -14/ H_3 -14, H_6 -14/ H_3 -12, and H_3 -12/ H_7 -7 showed that they were cofacial and were arbitrarily assigned orientation. On the basis of the ROESY correlations of H_3 -6 and the $J_{5,6} = 9.7$ Hz coupling constant, H_5 -5 must be in a α -orientation. The ROESY cross-peak between H_5 -5 and H_2 -2a indicated an E-geometry for the 10 double bond (Supporting Information). In addition, the 7 double bond was also assigned E-geometry based on the $J_{7,8} = 15.7$ Hz coupling constant.

Since the relative spatial relationships of the fragments were established, eight possible absolute configurations were proposed by combining different fragment configurations (Figure S1 in the Supporting Information). To determine the absolute configuration of nudibaccatumone, computational studies of electron circular dichroism (ECD) were carried out. As the ECD spectra of enantiomers are mirror images, only 1d, 1f, and 1h, which are not mirror images of each other, were calculated (solid lines in Figure 3), while the other spectra were deduced as mirror images.

Conformational analysis of these four configurations was performed to locate the low-energy conformers and their Boltzmann-weighted distributions. Mixed torsional/low-mode conformational search and truncated Newton conjugate gradient (TNCG) optimizations were carried out using the OPLS_2005 force field¹⁰ as implemented in MacroModel 2010. All conformations within the range of 10 kcal/mol above the most stable minimum were further optimized by using the B3LYP/6-31+G level of theory in Gaussian 09. For the geometries

1). The substructures A-C were joined on the basis of the following HMBCs: H_9 -9 to C-3, C-5, and C-15; H_3 -3 and H_3 -14 to C-2; and H_5 -5 to C-4

