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

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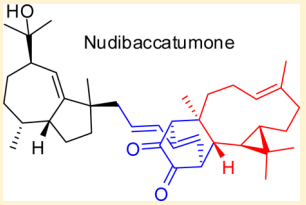
Supporting Information

ABSTRACT: A new complex natural product with a C_{39} skeleton, named nudibaccatumone, and the known sesquiterpenes (+)-spathulenol, (−)-4β,10α-aromadendranediol, and *ent*-T-muurolol, 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, kawapyrones, 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-muurolol,[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 $C_{39}H_{56}O_3$ 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 ^{13}C NMR and DEPT spectra exhibited 39 resonances, consisting of two ketocarbonyls, 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′, H_3-12′ to C-6, H_3-13′ to C-7′, and H_3-15′ to C-1′ and C-9′, the presence of a bicyclogermacrene fragment (**B**, Figure 1) was confirmed. In addition, the HMBC correlation of H_3-12″ and H_3-13″ to C-7″, H_3-15″ 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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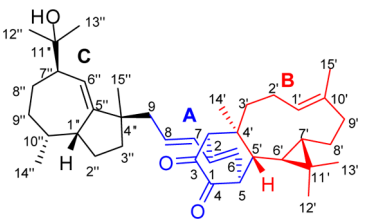
Table 1. ^{1}H (500 MHz) and ^{13}C (100 MHz) NMR Data of 1 in $CDCl_3$ (δ in ppm, J in Hz)

no.	δ_C	δ_H
1	141.2, C	
2	65.1, CH	3.28, br s
3	191.6, C	
4	189.9, C	
5	53.9, CH	3.40, dd (6.5, 3.2)
6	123.4, CH	6.03, d (6.5)
7	129.7, CH	6.16, d (15.7)
8	130.8, CH	5.94, ddd (15.7, 7.4, 7.4)
9	45.6, CH_2	2.20, m
1′	123.2, CH	5.07, d (11.3)
2′a	24.5, CH_2	1.99, m
2′b		2.35, m
3′a	42.6, CH_2	1.77, m
3′b		1.43, dt (13.8, 3.5)
4′	41.3, C	
5′	43.2, CH	1.78, m
6′	27.8, CH	0.16, t (9.7)
7′	28.7, CH	0.36, t (9.7)
8′a	24.9, CH_2	1.76, m
8′b		1.15, m
9′a	35.0, CH_2	2.34, m
9′b		1.61, m
10′	133.8, C	
11′	16.5, C	
12′	29.8, CH_3	0.98, s
13′	17.3, CH_3	0.99, s
14′	21.7, CH_3	1.01, s
15′	20.7, CH_3	1.71, s
1″	44.4, CH	2.96, m
2″β	27.7, CH_2	1.66, m
2″α		1.51, m
3″β	37.5, CH_2	1.53, m
3″α		1.35, dd (11.2, 5.8)
4″	47.5, C	
5″	152.2, C	
6″	120.8, CH	5.42, br s
7″	51.8, CH	2.15, m
8″α	24.8, CH_2	1.76, m
8″β		1.62, m
9″β	33.8, CH_2	1.97, m
9″α		1.22, m
10″	34.0, CH	1.97, m
11″	74.0, C	
12″	28.0, CH_3	1.19, s
13″	26.9, CH_3	1.18, s
14″	17.4, CH_3	0.84, d (6.2)
15″	26.9, CH_3	0.98, s

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



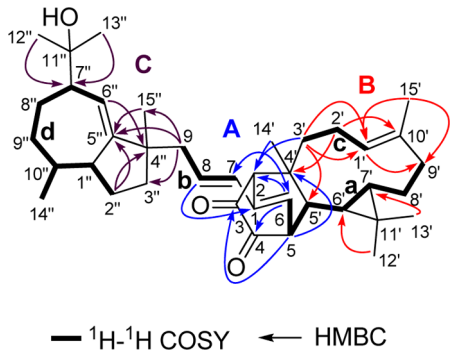


Figure 1. Key ^{1}H–^{1}H COSY and HMBC (H→C) correlations of **1**.

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

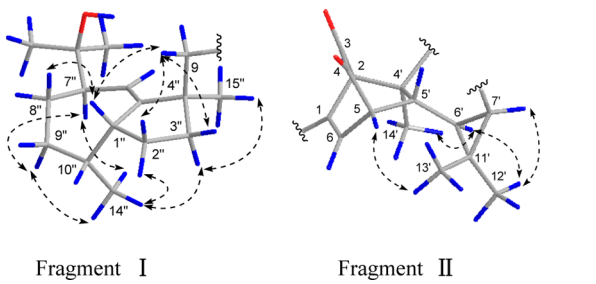


Figure 2. Key ROESY correlations of **1**.

ROESY correlations between H-7″/H_3-14″, H_3-14″/H-3″α, and H-3″α/H_3-15″ showed that the four protons were cofacial and were arbitrarily assigned an α-orientation. The correlations of H-3″β/H_2-9 and H_2-9/H-1″ indicated that H-1″ was in a β-orientation. Likewise, in fragment II, the ROESY cross-peaks of H-6′/H_3-14′, H-6′/H_3-12′, and H_3-12′/H-7′ showed that they were cofacial and were arbitrarily assigned in an α-orientation. On the basis of the ROESY correlations of H-6′/H_3-14′ and the $J_{5',6'}$ = 9.7 Hz coupling constant,[8] H-5′ must be in a β-orientation. The ROESY cross-peak between H_3-15′ and H-2a′ indicated an *E*-geometry for the $\Delta^{1'(10')}$ double bond (Supporting Information). In addition, the Δ^7 double bond was also assigned an *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 mirrored, only **1b**, **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 searches[9] and truncated Newton conjugate gradient (TNCG) optimizations were carried out using the OPLS_2005 force field[10] 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 G09.[11] For the geometries

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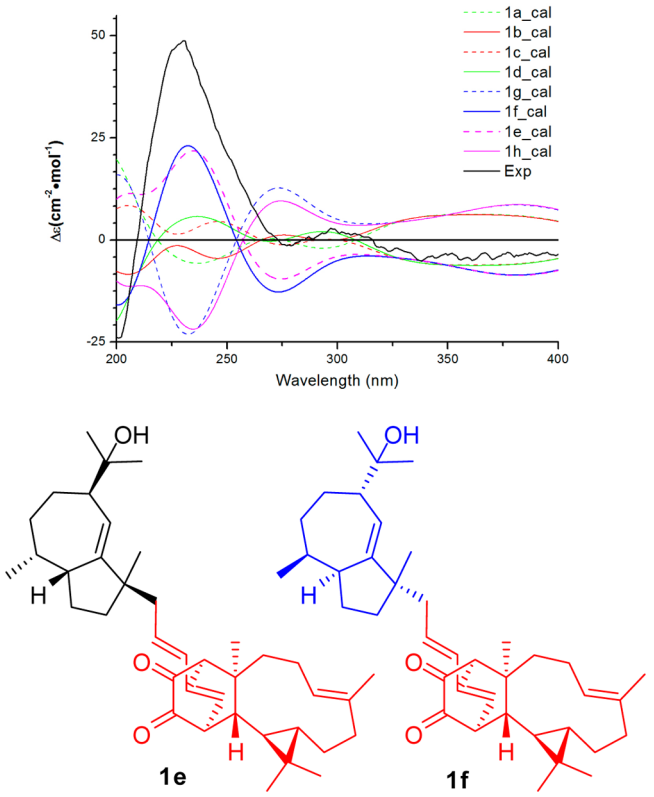

Figure 3. Experimental ECD spectrum of **1** (black) and conformationally averaged calculated ECD spectra of all possible configurations.[14]

of each configuration in an energy window of 3 kcal/mol relative to the lowest energy conformer, the electronic transitions and rotational strength were determined by time-dependent density functional theory (TDDFT). The solvent effect in MeOH solution was included using a universal continuum solvation model developed by Marenich and coworkers (called SMD in G09).[12] The Boltzmann-population-weighted calculated ECD curves were generated via SpecDis.[13] As depicted in Figure 3, the calculated ECD spectra of **1f** and **1e** (the enantiomer of **1h**) are similar to the experimental ECD spectrum of the natural product **1**, with a positive Cotton effect around 230 nm and a negative Cotton effect around 280 nm. Therefore, the absolute configuration of **1** is more likely as in **1e** or **1f** rather than any other possible configuration.

Nudibaccatumone is the first natural product that combines a quinone methide and two sesquiterpene moieties. It has a unique fused bicyclo[2.2.2]octenedione skeleton with 39 carbons. Notably, this complex natural product might originate from hydroxychavicol, (−)-α-gurjunene,[15] and (+)-bicyclogermacrene.[16] A plausible biogenetic pathway for **1** is shown in Scheme 1. (−)-α-Gurjunene, which might be derived from farnesyl diphosphate through a series of complicated reactions,[15] could react with the quinone methide from oxidation of the hydroxychavicol[17] to provide intermediate **i** through a 1,8-Michael addition reaction. Further oxidation of **i** would generate 1,2-benzoquinone intermediate **ii**. Finally, **1** would be produced through an intermolecular, inverse electron demand Diels−Alder reaction between **ii** and the (+)-bicyclogermacrene.

In the biosynthesis pathway, compound **1**, as well as (+)-spathulenol and (−)-4β,10α-aromadendranediol, which were also obtained from this plant, might share the same precursors (Scheme 1).[18] Therefore, the three-dimensional structure of **1** was suggested as **1e**. Owing to its unique molecular structure, it may provide a challenge for chemists to confirm this assignment by synthesis from these simple natural products.

Compound **1** was evaluated for activity against the bacteria *Escherichia coli* and *Staphylococcus aureus*, as well as the fungus *Candida albicans*. The cytotoxic activity of **1** to human myeloid leukemia (K562) and human lung adenocarcinoma (A549) cell lines was also tested. However, compound **1** had no significant effect on any of these organisms or types of cells at a concentration of 100 μM.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using a Horiba SEPA-300 polarimeter. UV spectra were determined by a Shimadzu double-beam 210A spectrometer. IR spectra were measured using a Bio-Rad FTS-135 infrared spectrophotometer with KBr disks. ECD spectra were obtained from an Applied Photophysics spectropolarimeter. 1D and 2D NMR spectra were obtained using a Bruker DRX-500 spectrometer with TMS as internal standard. MS analyses were performed on a VG Auto Spec-3000 mass spectrometer. Silica gel G (80−100 and 300−400 mesh, Qingdao Makall Group Co., Ltd.), C_{18} silica gel (40−75 μm, Fuji Silysia Chemical Ltd.), silica gel H (10−40 μm), and Sephadex LH-20 (GE Healthcare Bio-Xciences AB) were used for column chromatography, and silica gel GF_{254} (Qingdao) was used for preparative TLC as precoated plates. The TLC spots were visualized under UV light and by dipping into 5% H_2SO_4 in EtOH, followed by heating.

Plant Material. The aerial parts of *P. nudibaccatum* (13 kg) were collected from Xishuangbanna of Yunnan Province, People's Republic of China, in May 2011, and identified by one of the authors (C.-L.L.). A voucher specimen (No. HGW-00705) was deposited at the Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany.

Extraction and Isolation. The air-dried powder of the plant material (13 kg) was exhaustively extracted with MeOH (3 × 20 L). The resulting MeOH extract (2.3 kg) was suspended in H_2O and further partitioned into three fractions, petroleum ether (A, 358 g), $CHCl_3$ (B, 300 g), and H_2O (C). Subsequently, fractions A and B were combined and subjected to column chromatography (silica gel; petroleum ether/EtOAc, 100:5 → 0:100, v/v) to afford Frs. A_1−A_6. Fr. A_3 was separated on RP-18 silica gel eluting with MeOH/H_2O (70−100%) to yield three fractions (Frs. A_{31}−A_{33}). Fr. A_{31} was purified by silica gel eluted with petroleum ether/EtOAc (15:1) to give 4-allylbenzene-1,2-diol (30 g). Fr. A_{33} was purified by Sephadex LH-20 eluted with MeOH and silica gel eluted with petroleum ether/EtOAc (40:1) to give (+)-spathulenol (11 mg) and *ent*-T-muurolol (66 mg). Fr. A_4 (petroleum ether-/EtOAc, 5:1, v/v) was further purified by RP-18 silica gel (MeOH/H_2O, 95:5, v/v) and Sephadex LH-20 (MeOH) column chromatography and preparative TLC ($CHCl_3$/MeOH, 100:1, v/v), to afford compound **1** (35 mg, 0.00026% yield). Fr. A_6 (petroleum ether/EtOAc, 0:1, v/v) was further purified by RP-18 silica gel (MeOH/H_2O, 75:25, v/v) and Sephadex LH-20 (MeOH) column chromatography and recrystallization (MeOH) to afford compound (−)-4β,10α-aromadendranediol (100 mg).

Nudibaccatumone (1): yellowish oil; $[\alpha]^{23}_{D}$ −85.4 (*c* 0.28, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 245.5 (4.54) nm; ECD $\Delta\varepsilon$ (*c* 0.025, MeOH) −0.06 (279), +2.13 (231), 0 (209), −1.04 (201), −0.06 (279); IR (KBr) ν_{max} 3432, 2928, 2869, 1732, 1684, 1634 cm^{-1}; ^1H and ^{13}C NMR see Table 1; ESIMS (pos.) *m*/*z* 595 ([M + Na]$^+$); HRESIMS (pos.) *m*/*z* 595.4134 [M + Na]$^+$ (for $C_{39}H_{56}O_3Na$, calcd 595.4127).

Bioassay Testing. The antimicrobial activity of **1** was measured by the microdilution assay.[19] The MIC values of the positive control gentamicin against *E. coli* and *Staph. aureus* were 1.2 and 0.05 μg/mL, respectively. Nystatin was the positive control against *C. albicans*, with

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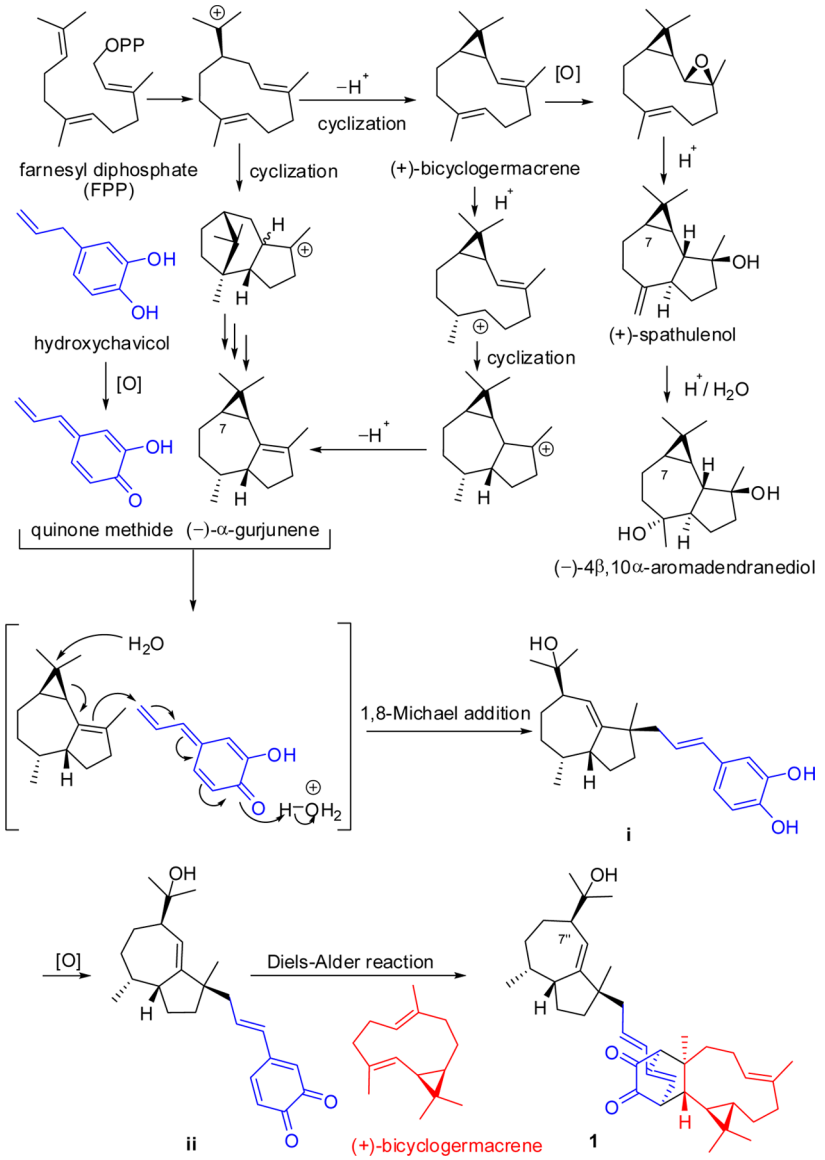
Scheme 1. Hypothesis for the Biosynthesis of 1



an MIC value of 1.0 μg/mL. The cytotoxicity of **1** against the K562 cell line (doxorubicin as positive control, IC_{50} = 0.28 μM) was measured by the MTT method,[20] while that of the A549 cell line (doxorubicin as positive control, IC_{50} = 0.049 μM) by the SRB method,[21] respectively.

■ ASSOCIATED CONTENT

Supporting Information

1D and 2D NMR, HRESIMS, IR, UV, and ECD spectra of **1**. 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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