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# Massadine, a Novel Geranylgeranyltransferase Type I Inhibitor from the Marine Sponge *Stylissa* aff. *massa*

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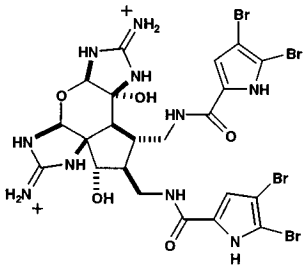
## ABSTRACT



**Massadine, a highly oxygenated alkaloid, was isolated from the marine sponge *Stylissa* aff. *massa* as an inhibitor of geranylgeranyltransferase type I (GGTase I). The structure of massadine has been deduced from spectral data. Massidine inhibited GGTase I from *Candida albicans* with an $IC_{50}$ value of 3.9 $\mu$M.**

Protein prenylation is involved in a wide range of cellular functions, e.g., the control of cell growth, differentiation, cytokinesis, membrane trafficking, and signal transduction. Prenylation is catalyzed with the $Zn^{2+}$ metalloenzymes, farnesyl transferase (FTPase), and geranylgeranyltransferase (GGTase).[1] Proteins that are prenylated include small GTP-binding proteins, of which Rho1 is an essential regulatory subunit of 1,3-$\beta$-glucan synthase.[2,3] Since GGTase I from the pathogenic fungus *Candida albicans* shares only 30% amino acid sequence homology with the human GGTase,[4] inhibitors of GGTase I are expected to be selective antifungal agents.[5] In the course of our continuing search for drug leads

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from Japanese marine invertebrates, we found *Candida* GGTase I inhibitory activity[6] in the organic extract of the marine sponge *Stylissa* aff. *massa* collected in the Gulf of Sagami. Bioassay-guided fractionation afforded a novel alkaloid named massadine. In this paper, we report the isolation, structure elucidation, and biological activity of this compound.

The MeOH extract of the frozen sponge (0.9 kg) was partitioned between water and ether, and the ether layer was further partitioned between 90% MeOH and hexane. The aqueous and 90% MeOH layers were combined and fractionated by ODS chromatography followed by gel filtration on Sephadex LH-20. Final purification by reversed-phase HPLC furnished massadine ($6.3 \times 10^{-3}$ % yield based on wet weight) as a yellow powder.

Massadine (**1**) (Figure 1) was determined to have a molecular formula of $C_{22}H_{26}Br_4N_{10}O_5 \cdot 2CF_3CO_2H$ on the basis of HR-FABMS and $^{13}C$ NMR data.[7] The $^1H$ NMR spectrum of massadine ($CD_3OH$) exhibited three aliphatic methines ($\delta$ 2.11, 2.16, and 2.41), two nitrogenous methylenes ($\delta$ 3.37/3.53 and 3.48/3.92), three oxymethines ($\delta$ 3.70, 5.41, and 5.65), two aromatic singlets ($\delta$ 6.84 and 6.90), and nine distinct exchangeable proton signals. In addition, $^{13}C$ NMR data exhibited two quaternary carbons ($\delta$ 72.0 and 89.3) and two shielded signals ($\delta$ 158.8 and 159.3) together with five coincident sets of resonances attributable to 4,5-dibromopyrrole-2-carboxyamide units. These features suggested that massadine was a highly oxygenated congener of dimeric oroidin derivatives.[8] Interpretation of COSY data in conjunction with HMQC data led to units **a**−**c**. Unit **a** contained four contiguous methines, among which both C-1 and C-15 were linked to nitrogenous methylenes attached to the dibromopyrrole carboxyamide units, while C-14 was oxygenated as determined on the basis of the carbon chemical shift of $\delta$ 79.5. Units **b** and **c** had the same framework, in which a highly deshielded methine was coupled to an NH, which was in turn long-range coupled to another NH.[9] It was not possible to assign the substituent **Y** in units **b** and **c**, because the chemical shift values of the shielded carbons were consistent with either guanidine or urea.

These substructures were assembled by interpretation of HMBC data (Figure 2a). The quaternary carbon at $\delta$ 72.0 (C-13) could be accommodated between C-2 and C-14 of unit **a** as determined on the basis of cross-peaks H-2/C-13, H-14/C-13, and H-14/C-2,[10] while it was also placed between C-9 and N-12 in unit **c** as determined using HMBC correlations H-9/C-14, H-2/C-9, NH-12/C-9, and NH-12/C-13. Another quaternary carbon at $\delta$ 89.3 (C-3) was placed adjacent to C-2 of unit **a** as well as C-7 and N-4 of unit **b** as determined using HMBC cross-peaks H-1/C-3, H-7/C-3, H-6/C-3, and H-4/C-3. The chemical shifts for C-7 ($\delta$ 92.4) and C-9 ($\delta$ 84.3) and the $^1J_{CH}$ values of 180 and 175 Hz for C-7 and C-9, respectively, indicated that these carbons were flanked by two heteroatoms (a nitrogen and oxygen atom as indicated by HMBC cross-peaks H-7/C-9 and H-9/C-7). One hydroxyl group could be placed on C-14 by NMR data in DMSO-$d_6$ (Table S2, Supporting Information). ROESY data in DMSO-$d_6$ allowed the accommodation of an additional hydroxyl group on C-3, while the absence of a scalar coupling between H-7 and H-9 in the same solvent connected C-7 and C-9 through an oxygen atom. Both **Y** substituents

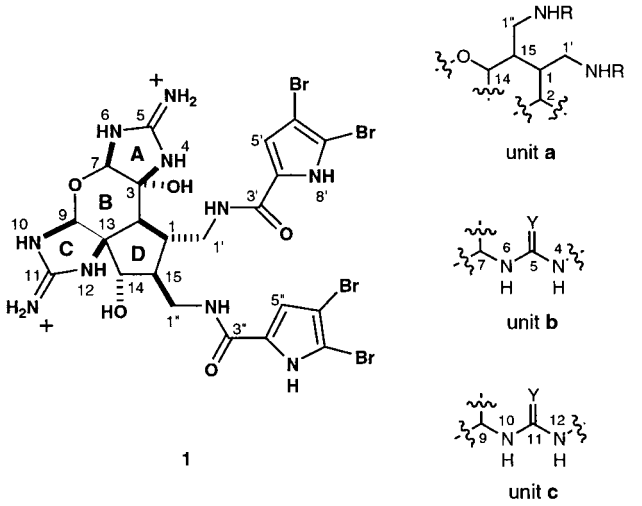


**Figure 1.** Structure of massadine (**1**) and units **a**−**c**.

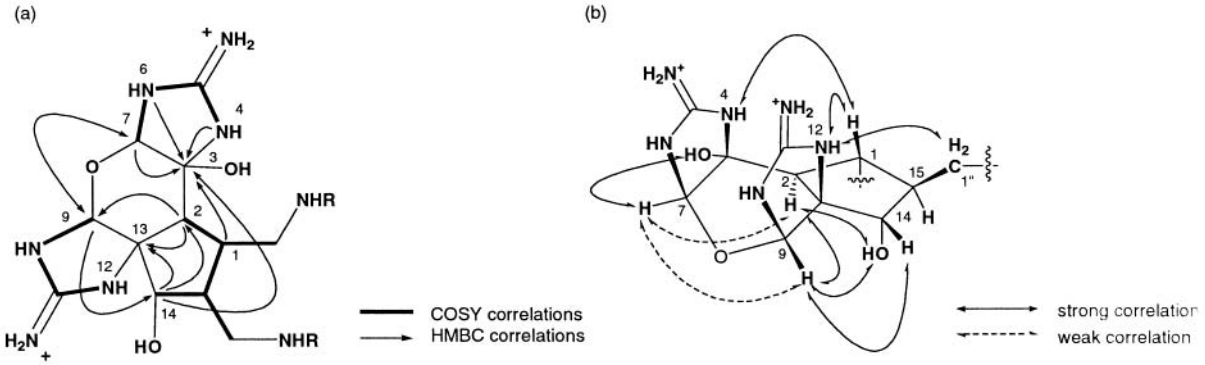


**Figure 2.** (a) COSY and key HMBC correlations and (b) NOESY correlations for massadine.

**Table 1.** $^{1}H$, $^{13}C$, and $^{15}N$ NMR Spectral Data for **1** in $CD_3OH$[a]

| position | $^{1}H$ (mult, $J$ in Hz) | $^{13}C$ (mult) | $^{15}N$[b] |
|---|---|---|---|
| 1 | 2.11 (m) | 42.1 (t) | |
| 2 | 2.41 (d, 12.3) | 44.2 (d) | |
| 3 | | 89.3 (s) | |
| 4 | 9.60 (br s) | | 104.1 |
| 5 | | 158.8 (s) | |
| 6 | 8.95 (br s) | | 94.1 |
| 7 | 5.41 (s) | 92.4 (d) | |
| 9 | 5.65 (s) | 84.3 (d) | |
| 10 | 9.19 (br s) | | 99.7 |
| 11 | | 159.3 (s) | |
| 12 | 9.20 (br s) | | 91.2 |
| 13 | | 72.0 (s) | |
| 14 | 3.70 (s) | 79.5 (d) | |
| 15 | 2.16 (m) | 52.9 (d) | |
| 1′a | 3.48 (ddd, 14.1, 5.8, 5.6) | 43.0 (t) | |
| 1′b | 3.92 (ddd, 14.1, 5.8, 3.4) | | |
| 2′ | 8.33 (br t, 5.8) | | 105.4 |
| 3′ | | 162.2 (s) | |
| 4′ | | 128.5 (s) | |
| 5′ | 6.90 (s) | 114.9 (d) | |
| 6′ | | 100.0 (s) | |
| 7′ | | 106.74 (s) | |
| 8′ | 12.1 (br s) | | 112.0 |
| 1″a | 3.37 (ddd, 13.9, 10.4, 5.9) | 43.0 (t) | |
| 1″b | 3.53 (ddd, 13.9, 5.9, 5.4) | | |
| 2″ | 8.43 (br t, 5.9) | | 107.2 |
| 3″ | | 162.2 (s) | |
| 4″ | | 128.8 (s) | |
| 5″ | 6.84 (s) | 114.6 (d) | |
| 6″ | | 100.0 (s) | |
| 7″ | | 106.5 (s) | |
| 8″ | 12.2 (br s) | | 112.3 |

[a] NMR experiments were carried out in $CD_3OH$/TFA (370:2). [b] Chemical shift values were determined by $^{1}H/^{15}N$ HSQC and $^{1}H/^{15}N$ HMBC spectra.

in units **b** and **c** must be NH (or $NH_2$ in an ionized form) by a process of elimination.[11] This was in agreement with a deuterium-induced $^{13}C$ isotope shift experiment (Figure S15, Supporting Information).[12,13]

The relative stereochemistry of **1** was assigned on the basis of coupling constants and ROESY (DMSO-$d_6$) and NOESY ($CD_3OH$) data (Figure 2b). A small coupling constant ($J$ < 1 Hz) between H-14 and H-15 indicated their trans relationships,[14] whereas a large coupling constant ($J$ = 12.3 Hz) between H-1 and H-2 implied their trans-diaxial relationships, which was confirmed by NOESY correlations H-2/$H_2$-1′ and H-14/$H_2$-1″. NOESY, cross-peaks, H-15/$H_2$-1′ and H-1/$H_2$-1″, showed that H-1 and H-15 were trans. Further NOESY cross-peaks, H-1/NH-4 and H-1/NH-12, not only established the trans fusion of the B/D rings but also placed N-12 and N-4 on the same face of ring B. ROESY cross-peaks, H-2/H-7 and H-2/H-9, indicated cis fusion of both rings A/B and rings B/C, thus establishing the relative stereochemistry of massadine.

In the proposed structure, two cyclic guanidines (rings A and C) faced each other in the endo positions of ring B, which adopted a boat conformation (Figure 2b). This was supported by long-range $^{1}H/^{13}C$ coupling constants obtained by the $J$-resolved HMBC spectrum;[15] $^{3}J_{H-7/C-9} = 9.1$ Hz and $^{3}J_{H-9/C-7} = 7.9$ Hz were in accordance with the equatorial orientation of both H-7 and H-9. The CD spectrum of massadine showed a positive exciton split [294 nm ($\Delta\epsilon$ +0.75) and 271 nm ($\Delta\epsilon$ −0.52)] due to 4,5-dibromopyrrole-2-carboxyamide side chains and were thus assigned 1(*R*),15(*S*) stereochemistry.[16]

Massadine inhibited GGTase I from *C. albicans* with an $IC_{50}$ value of 3.9 $\mu$M. Though massadine inhibited the growth of *Cryptococcus neoformans* with an MIC value of 32 $\mu$M, it did not inhibit the growth of *C. albicans* at a concentration of 64 $\mu$M.

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**Supporting Information Available:** Experimental section, FABMS spectrum, CD spectrum, all NMR spectra ($^{1}H$ NMR, $^{1}H-^{1}H$ COSY, $^{13}C$ NMR, $^{1}H-^{13}C$ HMQC and HMBC, $^{1}H-^{15}N$ HSQC and HMBC, NOESY, ROESY, INADEQUATE, and $J$-resolved HMBC) for massadine, and CD data for reference compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Assignments for the NH signals was secured by $^{1}H/^{15}N$ HSQC data (Figure S11, Supporting Information).

(10) There was one HMBC cross-peak (H-14/C-3) arising from four-bond coupling, which was eventually assigned as a W-type coupling. To confirm the carbon skeleton of **1**, the INADEQUATE spectrum was measured (Figure S13, Supporting Information).

(11) Protons attached to nitrogen atoms on C-5 and C-11 were both observed as part of a broad signal in the two solvents.

(12) Proposed gross structure was in agreement with the $^{1}H/^{15}N$ HMBC data.

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(16) Absolute stereochemistry of massadine was assigned on the basis of exciton chirality method in CD spectrometry.[17] The conformations of the two pyrrolecarboxyamide termini, as shown by NOESY cross-peaks H-2′/H-5′ and H-2″/H-5″, were identical with those reported for septrin and oroidin in the solid state,[18] while the orientations of the side chains with respect to the tetracyclic portion were determined on the basis of NOESY peaks H-2′/H-1, H-2′/H-15, H-2”/H-14, and H-2″/H-15 and coupling constants: $J_{H-1/H-1'a} = 5.6$ Hz, $J_{H-1/H-1'b} = 3.4$ Hz, $J_{H-15/H-1''a} = 10.4$ Hz, and $J_{H-15/H-1''b} = 5.4$ Hz (Figure S16, Supporting Information). Two model compounds for bis-pyrrole carboxylate derivatives demonstrated that the exciton chirality method is applicable to this system (Figures S17, S18, Supporting Information). Attempts at introducing an MTPA group at the 14-hydroxyl group were unsuccessful.

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