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Dysibetaine: a new α,α-disubstituted α-amino acid derivative from the marine sponge *Dysidea herbacea*

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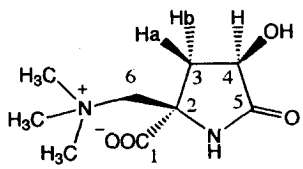
Abstract

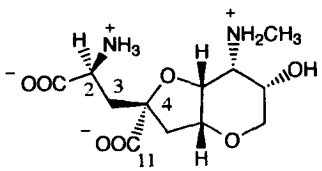
A new α,α-disubstituted α-amino acid derivative, dysibetaine (**1**), was isolated from an aqueous extract of the marine sponge *Dysidea herbacea* collected in Yap, Micronesia. The structure of **1**, (2*R**,4*R**)-2-(trimethylammonium)methyl-4-hydroxy-5-oxoprolinate, was determined by spectral methods and X-ray crystallography.

Keywords: amino acid; amino acid metabolites; marine metabolites; natural products.

α,α-Disubstituted α-amino acids have generated considerable interest due to their significant biological activities. For example, lactacystin was isolated from a cultured *Streptomyces* sp. as a neurotrophic agent[1] and later found to be a selective inhibitor of proteasome.[2] More recently, kaitocephalin, a novel kainate/α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-type glutamate receptor (GluR) antagonist was isolated from a culture of *Eupenicillium shearii*.[3] Synthetic compounds, such as 1-aminocyclopentane-1,3-dicarboxylic acid and derivatives, have been designed as conformationally restricted analogues of glutamate which possess selective agonist or antagonist activity to GluRs.[4]



1: dysibetaine



2: dysiherbaine

We have previously reported the isolation of a non-NMDA (*N*-methyl-D-aspartic acid) type glutamate receptor agonist dysiherbaine (**2**) from an aqueous extract of the marine sponge *Dysidea herbacea*

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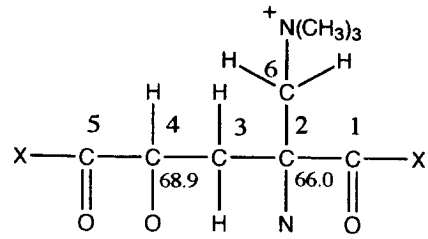

Figure 1. C-H connectivity of **1** established by COSY, HMQC and HMBC NMR. X represents N or O

collected in Yap, Micronesia.[5] Since **2** possesses a unique skeletal structure, we searched for compounds structurally related to **2** which would not only provide clues to the biosynthesis of **2** but also potentially exhibit interesting biological activities. Herein we report the structure of a new α,α-disubstituted α-amino acid derivative from *D. herbacea*, dysibetaine (**1**), containing a novel γ-lactam structure with a (trimethylammonium)methyl group.

During the fractionation of the water extract of the sponge, we noticed that a fraction that eluted after **2** contained ^{1}H NMR signals reminiscent of a portion of the glutamate substructure, H2–H3 system, of **2**. Since the compound responsible for those resonances was presumably structurally related to **2**, we conducted further separation of the extract using this ^{1}H NMR pattern as a guide and isolated **1**.

D. herbacea (200 g wet) was homogenized with water and centrifuged to give an aqueous extract and residual tissue. The tissue was re-extracted twice with water. 2-Propanol (250 mL) was added to the combined extracts (250 mL), and after centrifugation (10000 rpm, 10 min), the solvents were removed and the extract residue (9.33 g) was fractionated on a Sephadex LH 20 column (5×66 cm, eluent: water, flow rate: 4 mL/min). Fractions eluting between 560–1010 mL were combined (1.26 g) and further chromatographed successively on Bio Gel P2 (5×69 cm, 2 mL/min), Toyopearl HW-40 (2.5×116 cm, 0.5 mL/min) and then purified by packed column (Superdex peptide HR, Pharmacia) to give pure **1** [3.1 mg, $[\alpha]_D^{20}$ −7.3 (*c* 0.26, H_2O)].† FABMS analysis of **1** [*m/z* 217 $(M+H)^+$, HRFABMS (*m/z* 217.1191, calcd for $C_9H_{17}N_2O_4$, Δ 0.3 mmu)] suggested a formula of $C_9H_{16}N_2O_4$, which requires three degrees of unsaturation.

One (^{1}H and ^{13}C) and two-dimensional (COSY and HMQC) NMR data showed seven structural units; two carbonyls, three equivalent methyl groups, an isolated methylene, a quaternary carbon, and an R-(X-)CH-CH_2- system. The signals resonating at δ_H 3.09 (3H×3) and at δ_C 55.5 were assigned to be a trimethylammonium group.[6] HMBC data connected these partial structures to construct the carbon framework of **1** (Fig. 1). The NMR chemical shifts for the quaternary C2 (δ 66.0) and a methine C4 (δ 68.9) carbons were suitable for placement of a nitrogen and oxygen atoms, respectively, leaving an oxygen and two hydrogen atoms to be assigned (Fig. 1).

The two carbonyl functions of the substructure accounted for two of three degrees of unsaturation, with the remaining being attributed to a ring. A strong absorption at 1705 cm^{-1} in the IR spectrum of **1** suggested the presence of a γ-lactam moiety, which was assembled by connecting C2 nitrogen to C5.

The remaining oxygen atom was located on C1 to form a carboxylate, which was indicated by IR absorption at 1605 cm^{-1}. The remaining hydrogen atoms were located on the oxygen atom at C4 and

† NMR data for **1**: ^{1}H NMR [400 MHz, D_2O, HOD at 4.65 ppm at 25.6°C; (δ, multiplicity, *J* Hz, assignment)] 4.23 (dd, 8.0, 5.5, H4), 3.92 (d, 14.0, H6b), 3.62 (d, 14.0, H6a), 3.09 (s, CH_3×3), 2.55 (dd, 13.9, 8.0, H3b), 1.88 (dd, 13.9, 5.5, H3a); ^{13}C NMR [100 MHz, D_2O, CD_3OD at 49.0 ppm as internal standard; δ, (assignment by HMQC and HMBC)] 179.5 (C5), 176.8 (C1), 72.9 (C6), 68.9 (C4), 66.0 (C2), 55.5 (CH_3), 42.3 (C3); HMBC (H to C) 3a, 3b, 6a, and 6b to 1; 3a, 3b, 6a, 6b, and 4 to 2; 6a and 6b to 3; 3a and 3b to 4; 3a, 3b, and CH_3 to 6; 6a and 6b to CH_3.

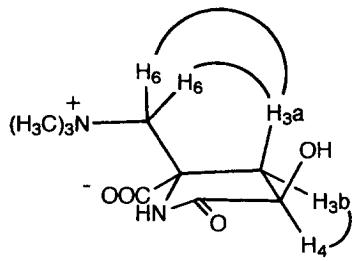

Figure 2. NOEs observed in the difference NOE spectra for **1**

the amide nitrogen atom. ^{1}H NMR spectra of **1** in DMSO-d_6 and H_2O at 245 K showed each proton as a doublet at δ 6.45 (J=4.4 Hz, coupled to H4) and a singlet at δ 8.56, respectively. The presence of an alcohol was further supported by a broad IR absorption at 3400 cm^{-1} as well as a product ion at m/z 199 in the FAB/MS/CID/MS spectrum, which can be assigned as $(M+H-H_2O)^+$. All above data were consistent with the planar structure of **1**.

In the NOE difference spectra of **1**, a strong enhancement was observed between H4 and H3b, but not between H4 and H3a, indicating H4 is *cis* to H3b (Fig. 2). Because NOEs between H3a (but not H3b) and two methylene protons H6a and H6b revealed that the (trimethylammonium)methyl group at C2 and H3a was *cis*, the relative stereochemistry of **1** was assigned as 2R^*,4R^*.

A single crystal suitable for X-ray cryastallography was grown from H_2O–DMSO. The X-ray defraction data for the crystal confirmed the structure of **1** (Fig. 3) but the absolute configuration could not be solved from the data set by the Eta method.‡ Because only a small amount of natural **1** is available, a synthetic approach is necessary to unambiguously confirm its absolute configuration.

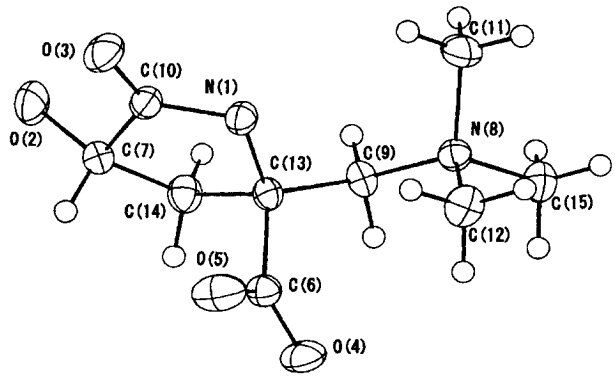

Figure 3. The ORTEP drawing of the X-ray structure for **1**

The biosynthetic relationship of dysibetaine (**1**) to dysiherbaine (**2**) is not clear, although both contain the same structural unit (C1-C5 of **1**; C1-C4 and C11 of **2**) which corresponds to 4-hydroxyglutamate.

Intracerebral injection of **1** in mice (20 μg/mouse) induced scratching behavior which can be considered as an early sign of convulsion. [7] Because compounds which induce convulsive behavior in mice may have some action to glutamate receptors in the central nervous system, [8] further pharmacological characterization of **1** is now in progress.

‡ An η value is required to be between 1.0 and −1.0 for reliable determination of the absolute configuration, whereas the value obtained in this experiment was −3.6.

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