Dysiherbaine: A New Neurotoxic Amino Acid from the Micronesian Marine Sponge *Dysidea herbacea*

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Abstract: A new amino acid, dysiherbaine (**1**), was isolated from a Micronesian sponge *Dysidea herbacea.* The structure was determined by using FABMS, ESIMS, FABMS/CID/MS, and one- and two-dimensional NMR experiments of **1** and its dimethyl derivative **3** to be a novel diamino dicarboxylic acid, which consisted of a *cis*-fused hexahydrofuro[3,2-*b*]pyran ring substituted with a 3-[2-aminopropanoic acid] side chain. The relative configuration of the bicyclic portion of **1** was determined by $^{3}J_{H,H}$ analysis and difference NOE experiments, and that of the acyclic side chain was assigned by additional $^{2,3}J_{C,H}$ analysis, measured by hetero half-filtered TOCSY (HETLOC) and phase sensitive HMBC experiments. Systemic administration of **1** induced neurotoxic symptoms in mice which were reminiscent of neuroexcitatory amino acids such as domoic acid. Dysiherbaine inhibited bindings of [^{3}H]-kainic acid (KA) and [^{3}H]-1-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), but not [^{3}H]-CGS-19755, an *N*-methyl-D-asparatic acid (NMDA) receptor antagonist, on rat brain synaptic membranes, suggesting that **1** is a selective agonist of non-NMDA type glutamate receptors in the central nervous system.

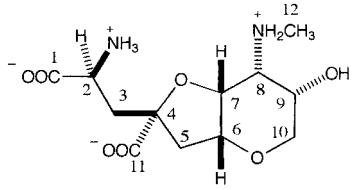
Introduction

Several important classes of neurotoxic secondary metabolites are known from marine organisms.[1] Among them, the algal and microalgal metabolites kainic acid and domoic acid—classified as neuroexcitotoxins—cause neurological disorders in mammals.[2] For example, domoic acid has been identified as a causative agent for seafood poisoning occurring in Canada in 1987. Patients showed pronounced neurological symptoms such as confusion, memory loss, disorientation, and, in some cases, death.[3] These toxins also affect laboratory animals, causing significant neuron damage leading to ischemia or epilepsy.[4] These compounds are known to act on ionotropic glutamate receptors in the mammalian central nervous system (CNS), and thus they are widely used as selective and powerful tools in neuropharmacology.[5] Nevertheless, neuroexcitotoxins with entirely novel structures are in high demand in order to investigate the relationship between glutamate receptors and brain diseases and to design potent glutamate receptor antagonists which may have therapeutic values as neuroprotective agents.[6]

While screening marine organisms collected at Yap State, Micronesia, for neuroexcitotoxins, we found that the water extract of the common Micronesian sponge *Dysidea herbacea* induced in mice severe tonic−chronic convulsions, unusual behaviors in lower doses, and death in higher doses upon intraperitoneal injection which resemble the symptoms of kainic acid and domoic acid.[4]

Here we report the isolation and structure determination of a novel neuroexcitotoxin, dysiherbaine (**1**) (Chart 1).

Chart 1



1: dysiherbaine
3: dimethyl ester of two carboxyl groups C1 and C11

Results

The active principal dysiherbaine (**1**) was isolated from the sponge as described in the Experimental section. The molecular formula of **1** ($C_{12}H_{20}N_2O_7$) was established on the basis of mass spectral and NMR data. Dysiherbaine gave peaks for the molecular ion at *m*/*z* 305 $(M + H)^+$ and 303 $(M - H)^-$ on positive-ion ESI and FAB, and negative-ion ESI mass spectra, respectively. HRFAB mass analysis for the molecular ion $(M + H)^+$ secured the above formula. ^{13}C and HMQC NMR data of **1** showed a total of 12 carbons comprising three quaternary, five methine, three methylenes, and one methyl agreed with

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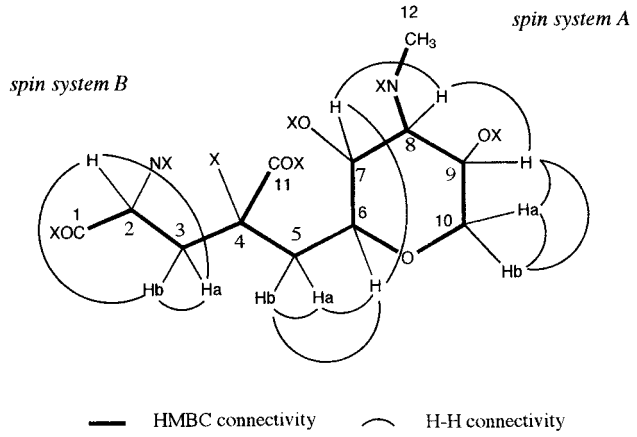
Table 1. NMR Data for **1**

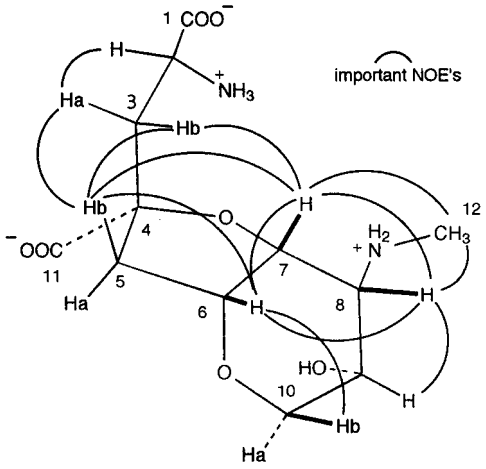
C no.	^{1}H δ, mult, J (Hz)[a]	^{13}C[b]	$\Delta\delta^{13}C_H-^{13}C_D$[c]	NOE[d]	HMBC (H no.)
1		174.6	0.033		3a, 3b, 2
2	3.47, dd, 11.5, 2.5	54.4	0.131	NE[e]	3a, 3b
3	a: 2.59, dd, 15.0, 2.5	40.1	0.050	a: 3b (s), 2 (m)	5b, 2
	b: 1.93, dd, 15.0, 11.5			b: 7 (m), 5b (m), 3a (s), 2 (m)	
4		89.4	0.016		6, 5b, 3a, 3b, 2
5	a: 2.58, dd, 14.0, 0.5	45.2	0	a: NE	3b
	b: 2.15, dd, 14.0, 3.5			b: 7 (m), 6 (s), 5a (s), 3b (m)	
6	4.15, br s	77.0	0	10b (s), 8 (m), 7 (s), 5a (m), 5b (m), 3b (vw), 10a (n), 9 (n)	10a, 10b, 7, 5a
7	4.30, br m	75.7	0.041	8 (s), 6 (s) CH_3 (m), 5b (m), 3b (w), 9 (n), 5a (n)	6, 5a
8	3.55, dd, 3.5, 3.5	57.3	0.074	NE	10a, 9, 7, 5a, 12
9	3.85, m	63.0	0.074	8(s)	10a, 10b, 7
10	a: 3.88, dd, 13.0, 2.5	69.5	0.025	a: 10b (s), 6 (n, vw)	
	b: 3.54, dd, 13.0, 1.0			b: NE	
11		181.0	0		5a, 5b, 3b
12	2.75, s	30.4	0.107	9 (m), 8 (m), 7 (m)	8

[a] In D_2O (*t*-BuOH = 1.13 ppm as an internal standard). [b] In D_2O with addition of methanol-d_4 as an internal standard (49.9 ppm). [c] The spectrum was taken in D_2O first then with added H_2O (1:1). [d] Difference NOE experiment. Protons enhanced by an irradiation at the proton attached to the carbon. s = strong, m = moderate, w = weak, vw = very weak, n = negative NOE. [e] NE = not evaluated due to signal overlap.

Scheme 1



Scheme 2



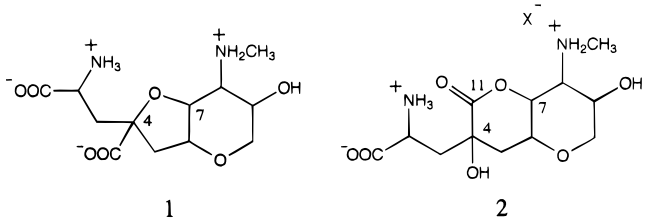
the number of carbons deduced from the mass spectral data. On the other hand, only 14 hydrogen atoms, out of 20, were counted from the above NMR data taken in D_2O, suggesting the presence of exchangeable hydrogen atoms. The molecular formula of **1** corresponded to four degrees of unsaturation, two of which were assignable to two carbonyl carbons shown by ^{13}C NMR signals at δ 174.6 and 181.0. Since no other sp^2 carbon signals were observed in the NMR spectra, the remaining two degrees of unsaturation were attributed to two rings.

Two spin systems A and B separated by a quaternary carbon (C4) were revealed by ^{1}H−^{1}H COSY, TOCSY, and HMQC−TOCSY spectra of **1** in addition to the above 1D data (Scheme 1). Of the four remaining carbons, two were assigned as carbonyls and the other two as quaternary and methyl carbons. The chemical shifts for C6, C7, C9, and C10 suggested these were oxygen-bearing carbons, and those of C8 and C12 were typical for nitrogen-substituted methine and methyl carbons, respectively. Mutual HMBC correlation between C12 and C8 methine further indicated that they resided on the same nitrogen atom. Chemical shifts for C6 and C10, HMBC crosspeaks between C6 and H10a, and C6 and H10b revealed connectivity between C6 and C10 through an ether bond to form a substituted tetrahydropyran ring structure.

HMBC correlations around C4 connected three fragments: spin system B, spin system A, and a carbonyl C11 (δ 181.0). C4 should also be connected to an oxygen atom because of its ^{13}C NMR shift at δ 89.4. The second carbonyl group at C1 was connected to C2 by an HMBC correlation from H3a or H2 to C1. The remaining nitrogen was situated at C2 because a ^{13}C chemical shift of δ 54.4 is typical for the α-carbon of α-amino acids. All the atoms assigned so far accounted for a formula of $C_{12}H_{14}N_2O_5$, leaving two oxygen and six hydrogen atoms to be assigned.

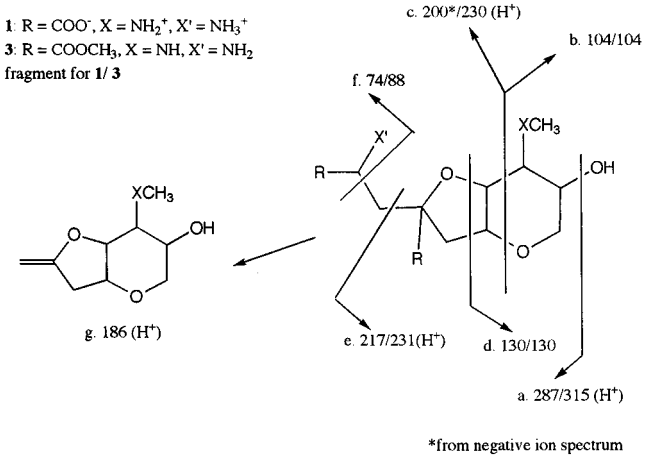
A deuterium-induced ^{13}C NMR isotope shift experiment suggested that the oxygen atom on C7 forms an ether or ester linkage. Several ^{13}C NMR signals in 1:1 D_2O-H_2O shifted downfield from those in D_2O (Table 1). Of the carbons bearing heteroatoms, relatively larger downfield shifts resulting from deuterium replacements with H were observed on C2 > C12 > C8 = C9, listed in order of magnitude, while a shift less than 0.05 ppm (C10, C7, or C4) could be attributed to long-range isotope shifts.[7] Since an additional ring structure is necessary to assemble a planar structure for dysiherbaine, two possible structures were proposed: (**1**) oxygen atom on C7 connected by an ether bond to the C4 to form tetrahydrofuran ring and (**2**) the oxygen atom and the C11 connected to form a δ-lactone and a free hydroxyl group attached to C4 (Chart 2). Unfortunately, any definite HMBC correlation to specify the linkage between C4 and H7 or C11 and H7 were not observed,[8] although difference NOE experiments for **1**, showing NOE's between H3b and H7, H3b and H5b, and H7 and H5b, suggested the proximity of C7 to C4 (Scheme 2).

Chart 2



1 2

Scheme 3



Of the possible structures, **2** was ruled out for several reasons. In the IR spectrum of **1**, a strong absorption was observed at 1625 cm^{-1}, indicating the presence of carboxylate, whereas absorption around the 1730−1750 cm^{-1} region for the lactone carbonyl for the possible structure **2** was not observed. In addition, a small shift (0.016 ppm) of C4 in the above deuterium-induced ^{13}C NMR isotope shift experiment suggested the absence of the free hydroxyl group on C4.[7] Positive FABMS/CID/MS analysis of the molecular ion of **1** (*m*/*z* 305, $M + H^+$ as a precursor ion) revealed the fragment ions as shown in Scheme 3. The product ions were mostly consistent with both possible structures **1** and **2**, but ion g can be better explained by structure **1**, which presumably formed upon losses of [NH_2-CHCOOH] and CO_2. Treatment of **1** with methanol and thionyl chloride yielded a dimethyl ester **3**, whose structure was secured by FABMS, ^1H NMR, and HMBC data.[9] HMBC correlations from two methyl group resonances in the δ 3.85 region to carbon signals at δ 170.5 (C1) and 176.5 (C11) established the presence of two methyl esters.[10] A positive-ion FABMS/CID/MS spectrum of **3** gave intact product ions b−d and g in Scheme 3, whereas ions corresponding to fragment ions a, e, and f were shifted by 28, 14, and 14 amu, which were parallel to incorporation of two, one, and one methyl group to the carboxyl groups of these fragments, respectively. Although the product ion c for **1** was not observed in the positive-ion spectra, a strong signal at *m*/*z* 200, which can be assigned as ion c, was observed in a negative-ion FABMS/CID/MS spectra of **1** [*m*/*z* 303 $(M - H)^-$ as a precursor ion]. This suggested that the ions c from **1** carried negatively charged species whereas the ion c from the methyl ester **3** can readily be positively charged. All above observations supported **1** as a planar structure of dysiherbaine.

Stereochemistry. The relative configuration of the bicyclic portion of **1** was elucidated by analyses of $^3J_{H,H}$ and NOE data (Table 1), as shown in Scheme 2.

In the tetrahydropyran ring, NOE's observed between H6−H8 and H6−H10b, indicated that these protons are 1,3-diaxial, and thus the tetrahydropyran ring is in a chair conformation with the NCH_3 group on C8 extending equatorially. Small $^3J_{H,H}$ values for H10a−H9 (2.5 Hz), H10b−H9 (∼1.0 Hz), and H8−H9 (3.5 Hz) suggested H9 to be equatorial so that the hydroxyl group stands axially toward the α-face of the ring. In the tetrahydrofuran ring, on the other hand, an NOE between H7 and H5b indicated that these protons were positioned pseudo-axially. H7 also had a long-range NOE to H3b. These and an NOE between H3b and H5b were particularly informative in assigning the relative configuration of the quaternary C4, which has the β-attached side chain and α−carboxyl group. These data, especially a strong NOE between H7 and H5b, revealed that the five-membered ring is in a twisted envelope conformation. A prominent NOE between H7 and H6 and their small $^3J_{H,H}$ indicated a *cis*-fused hexahydrofuro[3,2-*b*]pyran ring system.

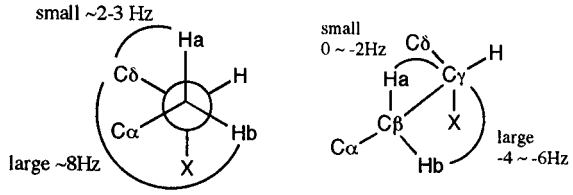


Figure 1. Relationships between the torsion angle and $^{2,3}J_{C,H}$ in the staggered system. $^3J_{C,H}$ with respect to methine−methylene bond (left). A large $^3J(C\delta-Hb)$ and a small $^3J(C\delta-Ha)$ can be distinguished by comparing intensities of crosspeaks in the PS-HMBC spectrum. When X is electron negative atom such as oxygen, usually $^2J(C\gamma,Ha) = 0$ to −2 Hz ("small"), and $^2J(C\gamma,Hb) = -4$ to −6 Hz ("large") (right).[9]

The relative stereochemistry at C2, the stereogenic center on the acyclic side chain of **1**, was determined by difference NOE experiments together with conformation analyses based on $^3J_{H,H}$ and $^{2,3}J_{C,H}$ values for the C3−C4 and C2−C3 bonds.[11] It is well established that $^3J_{C,H}$ values, as is the case with $^3J_{H,H}$, depend on the dihedral angle between the carbon and proton in the vicinal position.[12] It is also known that $^2J_{C,H}$ values depend on the torsion angle between X and the proton in an [*H*−C−*C*−X] system when X is an electronegative atom.[11] Precise $^{2,3}J_{C,H}$ values can be obtained by hetero half-filtered TOCSY experiments (HETLOC) when all carbon atoms in the system bare proton(s) whose magnetization are relayed to the long-range-coupled protons on the relevant carbon by TOCSY coherence transfer.[13] When a spin system is separated by a quaternary carbon or weakly coupled spin system HETLOC is not applicable, however, $^{2,3}J_{C,H}$ can be determined by the relative intensity of the crosspeaks in a phase sensitive (PS)-HMBC spectrum.[11a,14]

In a staggered rotamer shown in Figure 1, ^3J(*anti* C,H) is much larger (∼8 Hz) than ^3J(*gauche* C,H, 2−3 Hz). In the same system, $^2J(C\gamma,Hb)$ for *gauche* Hb-X is usually −4 to approximately −6 Hz, whereas $^2J(C\gamma,Ha)$ for *anti* Ha-X is usually 0 to approximately −2 Hz if X is oxygen or another electronegative atom.[11a] These significant differences in $^{2,3}J_{C,H}$ between these staggered rotamers allow qualitative elaboration of $^{2,3}J_{C,H}$ ("large" or "small") simply by comparing intensities

(7) Note that a shift of 0.05 ppm was observed on C3, a methylene carbon. See: Jeremic, D.; Milosavljevic, M. Lj. *Tetrahedron* **1982**, *38*, 3325−3328.

(8) HMBC experiments for J_{CH} of 10 and 5 Hz were performed.

(9) Treatment of **1** with diazomethane gave a complicated mixture. Acetylations of **1** with pyridine−acetic anhydride or acetic acid−acetic anhydride were both unsuccessful.

(10) The carboxy methyl groups appeared as split singlets in the ^1H NMR spectrum in the δ 3.85 region. H2 and H3b was also separated into two sets. This may be due to partial racemization at C2 during the reaction.

(11) Determination of relative configurations for complex oxygenated acyclic spin systems by analyses of $^3J_{H,H}$ and $^{2,3}J_{C,H}$ were successfully demonstrated by the following: (a) Matsumori, N.; Murata, M.; Tachibana, K. *Tetrahedron* **1995**, *51*, 12229−12238. (b) Matsumori, N.; Nonomura, T.; Sasaki, M.; Tachibana, K.; Satake, M.; Yasumoto, T. *Tetrahedron Lett.* **1996**, *37*, 1269−1272.

(12) (a) Lemieux, R. U.; Nagabhushan, T. L.; Paul, B. *Can. J. Chem.* **1972**, *50*, 773. (b) Schwarcz, J. A.; Perlin, A. S. *Can. J. Chem.* **1972**, *50*, 3667−3676. (c) Wasylishen, R.; Schaefer, T. *Can. J. Chem.* **1973**, *51*, 961−973.

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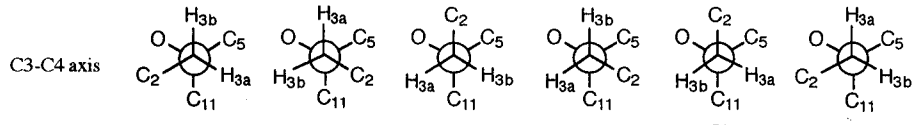
(14) Quantitative $^{2,3}J_{C,H}$ can be calculated from the relative intensity of a crosspeak in the PS-HMBC spectrum and that in the reference spectrum; see ref 11a and the following: (a) Zhu, G.; Bax, A. *J. Magn. Reson.* **1993**, *104A*, 353−357. (b) Zhu, G.; Live, D.; Bax, A. *J. Am. Chem. Soc.* **1994**, *116*, 8370−8371.



	(A)	(A')	(A*)	(B)	(B')	(B*)
^3J(H3b-C11)	large	small	small	large	small	small
^3J(H3a-C11)	small	large	small	small	small	large
^2J(H3a-C4)	small	large	large	large	small	large
^2J(H3b-C4)	large	large	small	large	large	small
^3J(H3a-C5)	small	small	large	large	small	small
^3J(H3b-C5)	small	large	small	large	large	small

Figure 2. Three possible C3−C4 axis rotamers for each of two possible prochiralities at C3 of **1**. The expected $^{2,3}J_{C,H}$ for each case is given (see Figure 1). Only A had the coupling pattern similar to that of **1**.

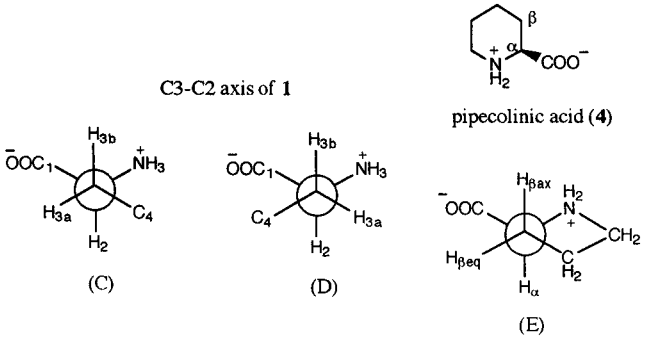


Figure 3. Newman projections along the C3−C2 axis of the major rotamer of **1**, showing two possible configurations at prochiral center C3 (C, D) and a Newman projection along the Cβ−Cα axis of **4** (E).

of the crosspeaks in the PS-HMBC spectrum. Should the free-rotating C3−C4 axis of **1** exist in one major staggered conformer, one could assign the position of enantiotopic protons H3a and H3b relative to the bicyclic portion based on the above $^{2,3}J_{C,H}$ analyses. This stereochemical information can then be transferred to the stereogenic center at C2, eventually resulting in the stereochemical assignment between C4 and C2.

The major rotamer around the C3−C4 axis appeared to be predominant over the other possible rotamers and to have the staggered conformation.[15] Figure 2 shows the expected $^{2,3}J_{C,H}$ values for the three possible rotamers for each of two possible prochiral centers at C3. Of these possible structures, only structure A was consistent with the following observations: (i) In the PS-HMBC spectrum of **1**, an intense crosspeak for the H3b−C11 correlation was observed, while that for H3a−C11 did not appear, indicating large ^3J(H3b−C11, 5 Hz) and small ^3J(H3a−C11, 1−2 Hz) values; (ii) NOE's between H3b and H5b, H3b and H7, and H3a and H5b were observed (Scheme 2); (iii) an intense H3b−C4 crosspeak and a weak H3a−C4 crosspeak were observed in the PS-HMBC spectrum, indicating large ^2J(H3b−C4, −4 Hz) and small ^2J(H3a−C4, −2 Hz) values; and (iv) PS-HMBC crosspeaks for H3b−C5 and H3a−C5 which were about the same weak intensity, indicating small ^3J(H3a−C5, 2 Hz) and ^3J(H3b−C5, 2 Hz) values.[16]

The relative configuration at C2 was determined by correlating that of the enantiotopic proton pair, H3a and H3b, to H2 and to C1 based on $^3J_{H,H}$ and $^{2,3}J_{C,H}$ on [C3−C2−C1] spin system (Figure 3). Since $^2J_{C,H}$ data for zwitterionic system such as C and D were not available, we took pipecolinic acid (**4**) as a model. Large ^3J (H3b−H2, 11.5 Hz) and a small ^3J(H3a−H2, 2.5 Hz) values in **1** are similar to those of **4**, ^3J(Hα−Hax, 11.6 Hz) and ^3J(Hα−Heq, 3.7 Hz) values, respectively. Since the dominant rotamer in **4** has the staggered Cα−Cβ axis with an equatorial carboxylate group, the above data indicated that the predominant conformation in **1** around the C3−C2 axis has the staggered system with H3b *anti* to H2. Newman projections, C and D in Figure 3 for C3−C2 axis of **1**, show two possible prochiralities for a methylene group at C3 (a Cβ−Cα projection of **4** (*E*) is also shown). The HETLOC experiments revealed ^2J(H3a−C2, −2.5 Hz) and ^2J(H3b−C2, −4.5 Hz) for **1**, and ^2J(Hβeq−Cα, −1.8 Hz) and (Hβax−Cα, −4.5 Hz) for **4**. The fact that the above $^2J_{C,H}$ values for **1** were very similar to those for **4** strongly suggested that **1** has the same relative configuration (C) as that of pipecolinic acid, with respect to the enantiotopic protons.

In the PS-HMBC spectra of **1**, correlation between H3a and C1 was observed as a very small crosspeak and that for H3b−C1 was missing. In **3**, the same experiment showed a similar result where no crosspeaks between β-protons and COO$^-$ were observed, indicating their small $^3J_{C,H}$'s. Those observations were consistent with the idea that both H3a and H3b were *gauche* to carboxylate C1 in **1**.

All data are consistent with the relative stereostructure of dysiherbaine (**1**) being that as depicted in Chart 1. The HMBC spectrum of **1** showed an unusual $^4J_{C,H}$ correlation between H5a and C8, which presumably due to *w*-coupling. On the other hand, a $^3J_{C,H}$ correlation between H7 and C4 was missing. This maybe due mainly to the dihedral angle of 90° between H7 and C4 in the tetrahydrofuran ring which, by theory, must show minimum coupling between these atoms.[12]

Dysiherbaine (**1**) represents an entirely new class of amino acids with the unique skeletal structure of *cis*-fused tetrasubstituted hexahydrofuro[3,2-*b*]pyran. This ring system is particularly unusual and only found in a few compounds, e.g. halichondrins.[17] Yet, **1** is different from them in substitution pattern and functionalities, thus posing an intriguing question as to its biosynthesis. It is noteworthy that **1** contains a glutamate structural moiety, C1−C2−C3−C4−C11, in the molecule which may play an important role in its neurological action.

Biological Activity. Administration of **1** by intraperitoneal injection in mice (20 μg/kg) induced "scratching" behavior; i.e., mice uncontrollably scratch their side shoulder with their hindlimbs 10−20 min after administration. The same behavior was observed by injecting domoic acid but at much higher doses (4 mg/kg). An injection of **1** in higher doses (1.3 mg/kg) induced severe tonic−chronic convulsions and seizure, although the mice usually recovered from these symptoms on the next day. Further increased doses (6.5 mg/kg) resulted in death after severe convulsions lasting for about 40 min. The radioligand binding assay of **1** toward three ionotropic glutamate receptors

(15) The rotational conformer around the C3−C4 axis turned out to be dominated by a single staggered rotamer since all $^{2,3}J_{C,H}$ values carrying the dihedral information were typical for either *gauche* or *anti* interaction. If either multiple rotamers predominantly occur or the rotation deviates significantly from the staggered position, some of the $^{2,3}J_{C,H}$ values should have an intermediate value.

(16) Approximate $^{2,3}J_{C,H}$ values (in parentheses) were calculated based on the intensities of the crosspeaks in PS-HMBC spectra using ^2J(H3−C2) as a reference value.

(17) Uemura, D.; Takahashi, K.; Yamamoto, T.; Katayama, C.; Tanaka, J.; Okumura, Y.; Hirata, Y. *J. Am. Chem. Soc.* **1985**, *107*, 4796−4798.

(KA, AMPA, and NMDA subtypes) showed that **1** inhibited, on rat brain synaptic membranes, bindings of [^{3}H]KA (IC_{50} = 59 ± 7.8 nM) and [^{3}H]AMPA (IC_{50} = 224 ± 22 nM), but not [^{3}H]CGS-19755, an NMDA antagonist (IC_{50} >10 000 nM).[18] These results indicated that **1** is a selective agonist of non-NMDA type glutamate receptors in CNS.

Dysiherbaine showed no *in vitro* cytotoxicity against L1210 murine leukemia cells (5 μg/mL) or antimicrobial activity (*Bacillus subtilus*, 200 μg/disk). More detailed physiological evaluations of **1** are now in progress.

Experimental Section

General. NMR spectra were obtained on JEOL α500 spectrometers (500 MHz). A sample of **1** (4 mg) was dissolved in 0.5 mL of D_2O as a solvent. Initial NMR experiments, 1D ^{1}H and ^{13}C and 2D COSY, TOCSY, HMQC, HMQC−TOCSY, and HMBC, were performed in Professor Higa's laboratory at the University of the Ryukyus. Difference NOE, 2D HETLOC, and PS-HMBC experiments were performed at the University of Tokyo at 300 K. The hetero half-filtered TOCSY (HETLOC) spectrum was obtained with the pulse sequence proposed by Wollborn and Leibfritz.[13c] The duration of the trim pulse was 2.5 ms, and the MLEV 17 spin−lock period was set to 30 ms. Twofold zero filling in *F1* yielded the final data matrix of 2K(t_2) × 512(t_1). The pulse sequence of phase sensitive HMBC is essentially the same as that reported by Davis.[19] The delay time was set at 50 ms to optimize for 10 Hz couplings. Twofold zero filling was carried out in *F1* to give a data set of 2K(t_2) × 512(t_1). FABMS and HRFABMS were recorded on a VG 70SE-4F using magic bullet matrix.[20] FAB/CID/FAB mass data were obtained on 70SE-4F four-sector tandem mass spectrometer using He as the collision gas. All FABMS experiments were conducted at University of Illinois. ESIMS data were obtained with a Finnigan Matt TSQ-700 spectrometer at Tohoku University. The optical rotation was measured with DIP 370 digital polarimeter with an Na lamp (589 nm) using a 5 × 0.35 cm (1.0 mL) cell. Circular dichromism was measured with a JASCO J-720 spectropolarimeter. Mouse assays were performed using male DDY mice (15 g, Owada experimental animals). Each fraction (100 μL) was diluted with water (900 μL) and was injected intraperitoneally. Fractions which elicited scratching behavior or convulsion within 0.5 h after injection were regarded as those containing **1**. The synaptic membrane preparation and the binding assay were performed as described previously.[21] The L1210 assay was performed by Dr. G. W. Wilson at University of Illinois. Antimicrobial assay against Gram-positive bacteria *Bacillus subtilis* was done using paper disk diffusion method.

A specimen of *D. herbacea* (200 g wet wt) collected in shallow water (3−5 m) in Yap Island, Micronesia, in July 1995 was homogenized with water (20 mL) and then centrifuged (10 000 rpm at 4 °C) to give an extract (bright pink liquid, 200 mL). 2-Propanol (100 mL) was added to the extract to precipitate macromolecules. The supernatant collected after centrifugation was evaporated to an aqueous suspension, lyophilized, and dissolved in water (50 mL) to apply to a Sephadex LH 20 (3.5 × 100 cm) column. Each 10 mL of the extract was loaded per run using water as an eluent. The bioactive fraction was separated by medium-pressure ODS (WAKO 60, H_2O) column chromatography. Fractions which showed the scratching activity were further separated by repeating the Sephadex LH 20 column chromatography. Dysiherbaine was finally purified by RP18 HPLC (Bio Rad, 1 × 25 cm, 10 μm particle, 1 mL/min) using water as a mobile phase. The eluate was monitored with UV detection at λ 210 nm. Dysiherbaine **1**: 4 mg, white amorphous solid; t_R = 14.6 min; R_f = 0.7 (C-18, H_2O), 0.4 (C-18, MeOH−0.5 M NaCl, 5:1, ninhydrin orange spot); $[\alpha]^{26}_D$ = −3.5° (*c* 0.4, H_2O); IR (KBr) 3384, 3400−2400 br, 1625, 1493, 1110 cm^{-1}; UV (H_2O) end absorption; CD (H_2O) λ_{ext} 223 nm, $\Delta\epsilon$ −1.4; λ_{ext} 203 nm, $\Delta\epsilon$ 11.6.; NMR (500 MHz, D_2O, see Table 1); ESIMS *m/z* 305 (M + H), 327 (M + Na), 303 (M − H); (HRFABMS) calcd for $C_{12}H_{21}N_2O_7$ 305.1349, found 305.1348.

Dimethyl Ester 3. Thionyl chloride (100 μL) was added to MeOH (1 mL) at 0 °C. A portion of this mixture (100 μL) was added to a stirred suspension of **1** (2.2 mg) in MeOH (0.9 mL) at room temperature. Solvent was removed after 24 h by N_2, and H_2O (1mL) was added. The solution was lyophilized to give dimethyl product **3** (single spot on TLC R_f = 0.7, MeOH−0.5 M NaCl, 5:1): ^{1}H NMR (CD_3OD) δ 4.55 (br m, H7), 4.22 (br s, H6), 4.07 (m, H2), 3.95 (dd, *J* = 5, 13 Hz, H10a), 3.89 (br s, H9), 3.85−3.88 (split methyls), 3.65 (br m, H8), 3.62 (d, *J* = 13 Hz, H10b), 2.87 (s, NCH_3), 2.82 (br m, H3), 2.73 (d, *J* = 13.5 Hz, 5a), 2.37 (m, H5b), 2.25−2.35 (m, H3b); IR (KBr) 3380, 3300, 1743, 1643, 1219, 1000, 770 cm^{-1}; FABMS *m/z* 333 (M + H); HRFABMS calcd for $C_{14}H_{25}N_2O_7$ 333.1662, found 333.1671.

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Supporting Information Available: FABMS, FABMS/CID/MS, ^{1}H NMR, COSY, HMBC, and IR spectra for **1** and **3** and ESIMS, CD, ^{13}C NMR, HMQC, PSHMBC, and HETLOC spectra for **1** (22 pages). See any current masthead page for ordering and Internet access instructions.

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(18) In the same assay, binding of [^{3}H]kinate was inhibited by KA (IC_{50} = 4.3 ± 0.5 nM) or glutamate (Glu, 110 ± 18), and that of [^{3}H]AMPA was inhibited by AMPA (5.6 ± 1.1) or Glu (124 ± 41).

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