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Conformational Feature of Neuroactive Domoic Acid: X-Ray Structural Comparison with Isodomoic Acid A and α-Kainic Acid

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SUMMARY: As an aid for developing a new type of potent insecticide acting on the neuromuscular junction, conformational characteristics of domoic acid and isodomoic acid A, the naturally occuring glutamate agonists, were investigated by X-ray crystal analyses. Conformational comparison with a neuroactive α-kainic acid provides information concerning the stereochemical feature responsible for the biological activity.

Domoic acid (**1**) was first isolated from the red alga *Chondria armata* as an anthelmintic (1) and recently found in the diatom *Nitzschia pungens* Grun. f. *multiseries* Hasle as amnesic shellfish poison (2). Its absolute structure was confirmed by a total synthesis to be (2*S*,3*S*,4*S*)-2-carboxyl-4-[1-methyl-5(*R*)-carboxyl-1(*Z*),3(*E*)-hexadienyl]pyrrolidine-3-acetic acid (3). α-Kainic acid (**2**), isolated from the red alga *Digenea simplex*, is well known as a glutamate agonist (4-7). It has the structural feature similar to glutamic acid that functions as the natural excitatory neurotransmitter.

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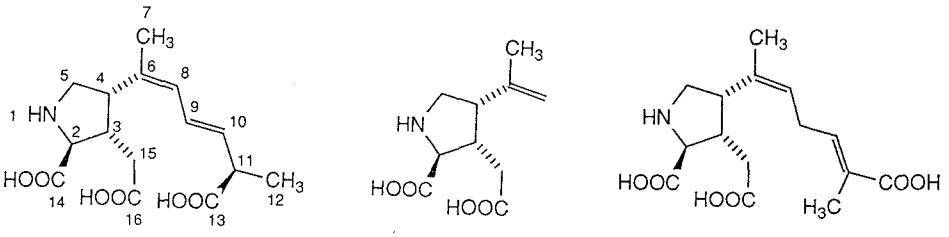

1 2 3

Fig.1. Chemical structures of domoic acid (**1**), α-kainic acid (**2**) and isodomoic acid (**3**). Atomic numbering used is also given for **1**.

1, which has similar structure to **2**, is also a glutamate agonist. These two naturally occurring glutamate agonists are valuable pharmacological tools for the study of neurochemical pathway in the brain (8,9). In addition to their neuroexcitatory functions, **1** and **2** display the extremely strong insecticidal activities, in which their insecticidal action sites have been proposed to be the neuromuscular junction in insects (10-12). In recent years, there is increasing request to develop new type insecticide with a different mode of action from the pyrethroids acting on central nerve system. As an aid for achieving this end, it is important to know the conformational features of **1**, **2** and their related compounds, which are responsible for the activity. Thus, we investigated the molecular conformations of **1** and isodomoic acid A (**3**) by X-ray single crystal analyses. Present paper deals with their conformational features, together with that of **2** (13); the insecticidal effects of **2** and **3** are about 1/150 and 1/10 as active as that of **1**, respectively (12). The chemical structures of **1**, **2** and **3**, together with the atomic numbering used, are shown in Fig.1.

MATERIALS AND METHODS

Transparent needle crystals of **1** and **3** were obtained from aqueous solution by slow evaporation at room temperature, and their crystal structures were determined by the X-ray diffraction method. Crystal data: **1**, $C_{15}H_{21}NO_6.2H_2O$, M=347.37, monoclinic, space group $P2_1$, a=11.833(2), b=8.585(2), c=9.391(2) Å, β=112.71(1)°, V=880.0(3) Å^3,

$F(000)$=372, Dm=1.305(3) (flotation in C_6H_6-CCl_4 mixture), Dc=1.311g.cm^{-3}, Z=2, μ(Cu Kα)=8.61 cm^{-1}; **3**, $C_{15}H_{21}NO_6$, M=311.34, orthorhombic, space group $P2_12_12_1$, a=7.262(1), b=8.589(2), c=25.337(4) Å, V=1580(1) Å^3, $F(000)$=664, Dm=1.305(2) (flotation in C_6H_6-CCl_4 mixture), Dx=1.309 g.cm^{-3}, Z=4, μ(Cu Kα)=8.10 cm^{-1}. Independent reflections of $2^o<2\theta<130^o$ were collected using an ω-2θ scan mode on a Rigaku AFC-5 diffractometer using the graphite-monochromated Cu Kα radiation (λ=1.5418 Å). Intensities were corrected for Lorentz and polarization factors, but not for absorption. The structures were solved by direct and successive Fourier methods. Refinements with anisotropic thermal parameters for non-H atoms and isotropic ones for H atoms converged to R=0.041 and Rw=0.069 using 1585 reflections for **1** and R=0.065 and Rw=0.075 using 1576 reflections for **3**. The final atomic coordinates are listed in Table 1. The anisotropic thermal parameters, and bond lengths and angles have been deposited at the Cambridge Crystallographic Data Centre.

RESULTS AND DISCUSSION

The molecular conformations of **1** and **3** are shown in Figure 2, where that of **2** is also given for the comparison. Selected torsion angles are given in Table 2. All of (a) the electron densities found in the difference Fourier map, (b) the bond lengths and angles concerning the pyrrolidine N atom and the carboxyl groups (14), and (c) the hydrogen bonding modes observed in the crystal structures indicate clearly that both of **1** and **3**, as in the case of **2**, take a zwitterionic structure in which the C(2) carboxyl group is in an anionic state and the ring N atom is in a cationic one.

As for the stereochemical relation of **1** pyrrolidine substituents, the C(2) carboxyl and C(3) carboxymethyl groups are axial positioned to the ring, respectively, and are *trans* oriented to each other, while the positioning of C(4) substituent is equatorial. This is in contrast with that of **3**, although the absolute configurations of respective substituents are both identical. The orientations of **3** substituents relative to the ring are C(2)-equatorial, C(3)-equatorial and C(4)-axial, respectively. The difference of the substituent positioning between **1** and **3** is mainly due to

Table 1. Atomic coordinates of non-H atoms of domoic acid and isodomoic acid A

$$B_{eq} = 4/3\ \Sigma_i\Sigma_j B_{ij}\mathbf{a}_i\mathbf{a}_j$$

Atom	x	y	z	βeq
Domoic acid				
N(1)	0.0692(2)	-0.3553*	0.2328(3)	3.09(9)
C(2)	0.1188(2)	-0.2810(3)	0.1235(3)	2.37(9)
C(3)	0.2417(2)	-0.2109(3)	0.2256(3)	2.15(9)
C(4)	0.2884(2)	-0.3324(3)	0.3584(3)	2.30(9)
C(5)	0.1721(3)	-0.3666(4)	0.3894(4)	3.2(1)
C(6)	0.3993(2)	-0.2853(4)	0.4983(3)	2.7(1)
C(7M)	0.3834(3)	-0.2475(5)	0.6460(4)	4.1(1)
C(8)	0.5096(3)	-0.2759(4)	0.4919(4)	3.0(1)
C(9)	0.5395(3)	-0.3090(4)	0.3593(4)	3.2(1)
C(10)	0.6519(3)	-0.3013(5)	0.3600(4)	3.4(1)
C(11)	0.6888(3)	-0.3366(5)	0.2260(4)	3.6(1)
C(12M)	0.5807(4)	-0.3762(6)	0.0745(5)	4.8(2)
C(13)	0.7641(3)	-0.2007(4)	0.2131(4)	3.3(1)
O(13')	0.8748(2)	-0.1973(3)	0.2791(3)	3.64(8)
O(13")	0.7007(2)	-0.0860(4)	0.1288(4)	5.6(1)
C(14)	0.1364(3)	-0.4089(3)	0.0207(3)	2.51(9)
O(14')	0.1091(2)	-0.5428(3)	0.0380(3)	3.65(8)
O(14")	0.1834(2)	-0.3641(3)	-0.0726(3)	4.21(9)
C(15)	0.2328(3)	-0.0471(4)	0.2843(4)	2.9(1)
C(16)	0.1959(3)	0.0759(4)	0.1596(3)	2.63(9)
O(16')	0.1674(2)	0.0532(3)	0.0244(3)	3.90(9)
O(16")	0.1962(3)	0.2156(3)	0.2175(3)	4.8(1)
solvent				
O(1W)	0.9508(3)	0.3659(4)	0.3037(4)	5.7(1)
O(2W)	0.0232(3)	0.0609(4)	0.4285(4)	5.8(1)
Isodomoic acid A				
N(1)	0.0983(5)	1.1883(4)	0.4388(1)	3.2(1)
C(2)	0.1118(5)	1.0457(4)	0.4733(1)	2.5(1)
C(3)	0.1986(5)	0.9201(4)	0.4388(1)	2.4(1)
C(4)	0.1466(5)	0.9658(4)	0.3815(1)	2.7(1)
C(5)	0.1558(6)	1.1443(4)	0.3838(2)	3.3(2)
C(6)	-0.0380(6)	0.9026(4)	0.3617(1)	2.8(1)
C(7M)	-0.2125(6)	0.9640(6)	0.3854(2)	4.2(2)
C(8)	-0.0485(7)	0.7938(5)	0.3242(2)	3.4(2)
C(9)	0.1072(8)	0.7143(5)	0.2955(2)	4.0(2)
C(10)	0.1472(7)	0.7902(5)	0.2432(2)	3.6(2)
C(11)	0.3111(7)	0.8176(6)	0.2228(2)	3.9(2)
C(12M)	0.491(1)	0.779(1)	0.2485(3)	7.7(4)
C(13)	0.3293(6)	0.8963(5)	0.1708(2)	3.7(2)
O(13')	0.4816(5)	0.9299(5)	0.1540(2)	6.0(2)
O(13")	0.1774(4)	0.9228(4)	0.1455(1)	4.1(1)
C(14)	0.2190(5)	1.0845(5)	0.5233(2)	3.1(1)
O(14')	0.2665(6)	1.2219(4)	0.5295(1)	4.8(1)
O(14")	0.2462(5)	0.9735(3)	0.5546(1)	3.6(1)
C(15)	0.1446(6)	0.7580(4)	0.4574(1)	2.9(1)
C(16)	0.2546(7)	0.6284(4)	0.4345(2)	3.2(2)
O(16')	0.2262(7)	0.4945(4)	0.4461(2)	6.0(2)
O(16")	0.3893(5)	0.6681(4)	0.4028(2)	4.6(1)

* Fixed.

the ring puckering, *i.e.*, C(3)-*exo*–C(4)-*endo* puckering for **1** and C(3)-*endo*–C(4)-*exo* puckering for **3**, when a plane consisting of N(1), C(2) and C(5) atoms is considered. These two puckering modes would belong

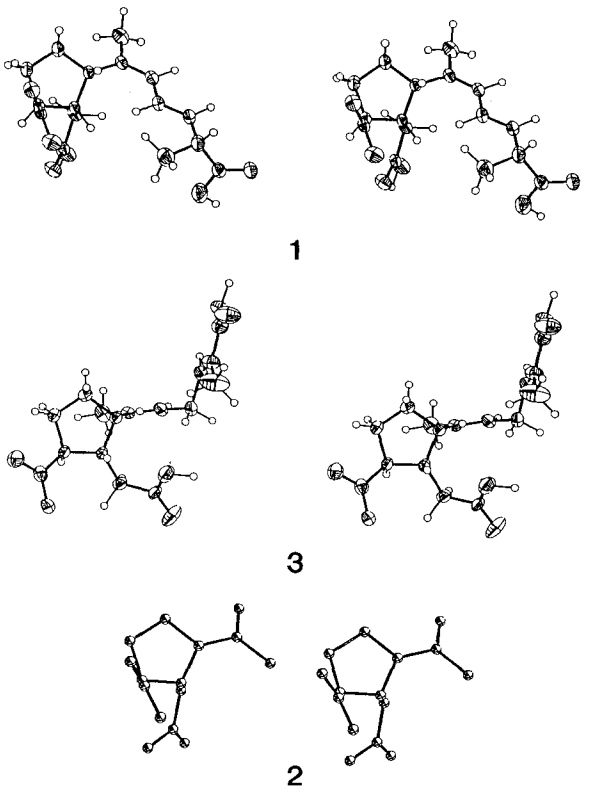

Fig.2. Stereoscopic molecular conformations of **1** and **3** observed in their crystal structures, together with that of **2** for the comparison.

to one of the most energetically stable regions, as usually observed in the ribose puckering of nucleic aicd. It is noteworthy that the stereochemical relation of **1** is in good agreement with that of **2**. Thus, it may be reasonable to consider the C(3)-*exo*–C(4)-*endo* puckering of pyrrolidine ring as a favorable form to reveal the insecticidal activity of **1** or **2**, although this is not conclusive because of a still high activity of **3** and of similar ring conformations of **1** and **3** in solution (12).

On the other hand, the orientation of the C(4) substituent relative to the ring could also be closely related to the activity. The hexadienyl side chain of **1** is nearly planar because of the conjugated diene structure, and makes a triangle of its terminal carboxyl group with respect to the C(2) carboxyl and C(3) carboxymethyl groups with dimensions of C(14)-C(16)=4.337 Å, C(13)-C(14)=7.160 Å and C(13)-C(16)=6.963 Å, while that of **3** takes a folded conformation, and a triangle consisting of

Table 2. Comparison of selected torsion angles (°). Their e.s.d's are given in parentheses.

	1	3	2
C(2)-C(3)-C(4)-C(5):φ_0	-42.7(2)	36.2(3)	-39.3
N(1)-C(2)-C(3)-C(4):φ_1	35.2(2)	-26.4(3)	37.4
C(5)-N(1)-C(2)-C(3):φ_2	-14.3(2)	6.0(3)	-21.1
C(2)-N(1)-C(5)-C(4):φ_3	-12.8(2)	17.1(3)	-3.4
N(1)-C(5)-C(4)-C(3):φ_4	34.4(2)	-32.5(3)	26.3
C(14)-C(2)-C(3)-C(15)	157.6(3)	84.7(3)	160.6
C(15)-C(3)-C(4)-C(6)	-46.8(3)	36.4(3)	-50.3
N(1)-C(2)-C(14)-O(14')	0.7(3)	4.4(4)	12.5
C(2)-C(3)-C(15)-C(16)	-69.3(3)	-166.6(4)	-76.9
C(3)-C(15)-C(16)-O(16')	4.8(3)	2.6(3)	35.6
C(3)-C(4)-C(6)-C(8)	-71.0(3)	-111.0(4)	-54.3
C(4)-C(6)-C(8)-C(9)	-0.8(3)	0.4(4)	
C(6)-C(8)-C(9)-C(10)	-178.4(4)	-96.4(4)	
C(8)-C(9)-C(10)-C(11)	179.1(4)	139.1(5)	
C(9)-C(10)-C(11)-C(13)	129.9(4)	-179.0(6)	
C(10)-C(11)-C(13)-O(13')	-86.9(3)	-5.8(4)	

its terminal carboxyl, C(2) carboxyl and (3) carboxymethyl groups has the dimensions of C(14)-C(16)=4.525 Å, C(13)-C(14)=9.112 Å and C(13)-C(16)=7.087 Å. The largest difference is observed at the spatial disposition between C(13) and C(14) carboxyl groups, and this may be in part responsible for the difference of insecticidal activities of **1** and **3**.

When the structure-activity relationship of domoic acid is considered, the present data provide some useful informations for considering novel insecticides acting on the neuromuscular junction, that is, (a) the puckering mode of pyrrolidine ring, (b) the conformation and bulkyness of C(4)-substituted lipophilic side chain and its spatial orientation relative to the ring, and (c) the spatial disposition among three carboxyl groups.

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