Conformational Characteristics of Opioid κ-Receptor Agonist: Crystal Structure of (5*S*,7*S*,8*S*)-(−)-*N*-Methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]benzeneacetamide (U69,593), and Conformational Comparison with Some κ-Agonists

Mitsunobu Doi,* Toshimasa Ishida and Masatoshi Inoue

Osaka University of Pharmaceutical Sciences, 2–10–65 Kawai, Matsubara, Osaka 580, Japan. *Received December 21, 1989*

(5*S*,7*S*,8*S*)-(−)-*N*-Methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]benzeneacetamide (U69,593) is a potent agonist to opioid κ-receptor. The crystal structure of U69,593 has been analyzed by the X-ray diffraction method. The molecule, as a whole, took an open conformation, and four cyclic rings composing the main skeleton were far apart from each other. The N and O atoms substituted for the cyclohexane ring were all in the equatorial position. The best planes of two 5-membered rings were almost perpendicular to that of the cyclohexane ring, and the *N*-methylamide linkage was also orthogonal to this ring plane. The conformation of the U69,593 molecule was compared with other κ-agonists.

Keywords U69,593; κ-receptor agonist; X-ray analysis; crystal structure; opioid receptor

Introduction

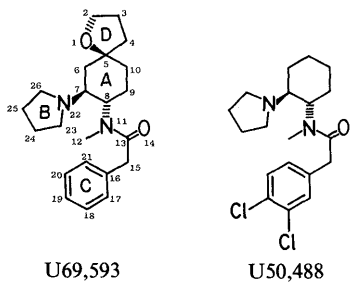
Although the use of morphine is inevitable to suppress pain, the lot of caution should be required for the method of administration to control its serious side effects such as dependence and custom. Therefore, numerous attempts have been made to develop analgesics without side effects. Recently, *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide (U50,488) was found as a potent agonist to opioid κ-receptor having high selectivity.[1,2] The research of κ-agonist was accelerated by this discovery, and several congeners have been devloped.[3–5] (5*S*,7*S*,8*S*)-(−)-*N*-Methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]benzeneacetamide (U69,593) was proved to be one of the most potent κ-agonists.[3]

We have been investigating the conformation–function relationships with respect to opioid peptides,[6–8] and the conformational information concerning the ligands binding to μ- and δ-receptors has been accumulated. The conformational specificities of κ-receptor ligands are scantly known, although they are important to consider with the analgesic mechanism induced by the opioid receptors. As a series of conformational studies of opioid ligands, the crystal structure of U69,593 has been analyzed by the X-ray diffraction method, and the conformational characteristics of the κ-agonist are discussed based on the conformational comparison with other κ-ligands.

Results and Discussion

Molecular Structure The chemical structure of U69,593 and its atomic numbering are shown in Fig. 1. The obtained fractional coordinates are listed in Table I. The bond lengths and angles are in Table II; they are in the normal range. The selected torsional angles are given in Table III. The molecular conformation of U69,593 is shown in Fig. 2, and the crystal packing is in Fig. 3. No specific electrostatic or hydrogen-bonding interaction, except for the molecular contacts, was observed in the crystal packing; the U69,593 molecule was in a neutral state.

The characteristic skeleton of the U69,593 molecule is composed of three separated cyclic rings (A, B and D-rings) and one aromatic ring (C-ring). These four rings are far



U69,593 U50,488

Fig. 1. Chemical Structures of U69,593 and U50,488

The atomic numbering used for the fractional coordinates obtained by the X-ray analysis is also shown. The characters of A, B, C and D indicate the cyclohexane, pyrrolidine, benzene and hydrofuran rings, respectively.

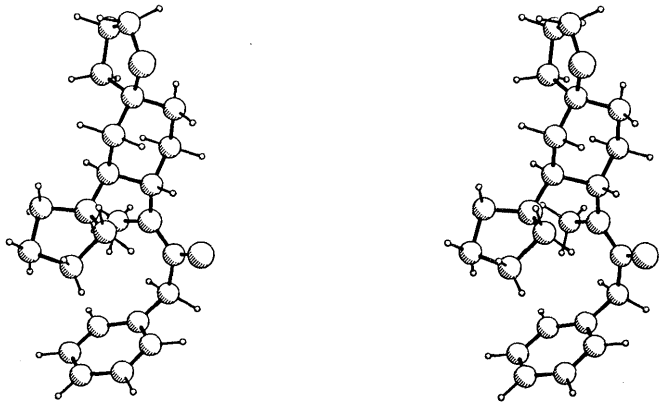


Fig. 2. Stereoscopic Drawing of the Molecular Conformation

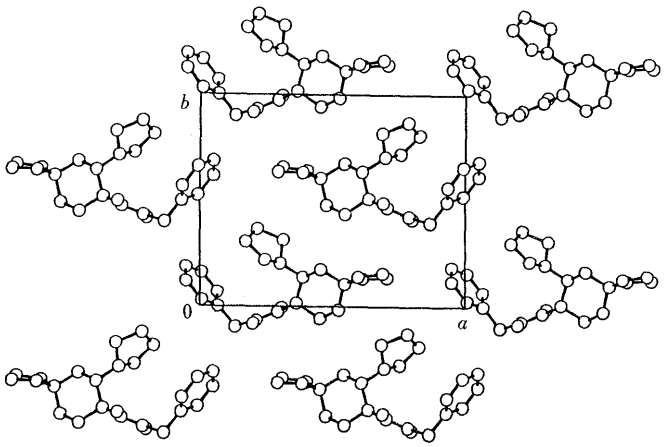


Fig. 3. Crystal Packing of U69,593 Molecule

apart from each other in the crystal structure, and the U69,593 molecule, as a whole, took an open conformation as shown in Fig. 2.

The 7- and 8-positions of the cyclohexane A-ring showing a chair form are both *S*-configuration, and the N(11) and N(22) atoms are equatorially connected to the A-ring: N(11)–C(8)–C(7)–N(22) = 47.9(2)°. The best plane of the pyrrolidine B-ring is at an almost right angle to the A-ring plane: the dihedral angle = 122.3°. The conformation of the B-ring and its orientation with respect to the A-ring appear to be affected by the *N*-methylamide group, because both atomic groups are close to each other. The *N*-methylamide linkage takes a *trans* conformation (C(8)–N(11)–C(13)–C(15) = 172.0(3)°), and is also vertical to the A-ring plane. The benzene C-ring is at an extended location from the A-ring as a result of the *trans* conformation of the amide linkage. The hydrofuran D-ring is connected to the shared C(5) atom of the A-ring with right angles, and its planarity is high compared with the B-ring: the torsional angles are within −21—21° for the D-ring and −41—40° for the

TABLE I. Fractional Coordinates and Equivalent Thermal Factors with Estimated Standard Deviations in Parentheses

Atom	*x*	*y*	*z*	B_{eq} (Å^2)
O(1)	0.4038 (1)	0.6438 (3)	0.3911 (3)	5.8 (1)
C(2)	0.3108 (2)	0.6604 (5)	0.3972 (7)	7.5 (2)
C(3)	0.2943 (3)	0.6393 (5)	0.6292 (8)	8.3 (3)
C(4)	0.3876 (2)	0.6351 (4)	0.7782 (6)	5.3 (2)
C(5)	0.4551 (2)	0.6096 (3)	0.6119 (5)	4.1 (1)
C(6)	0.5440 (2)	0.6789 (3)	0.6512 (5)	4.1 (1)
C(7)	0.6119 (2)	0.6408 (3)	0.8664 (4)	3.4 (1)
C(8)	0.6363 (2)	0.5153 (3)	0.8364 (4)	3.2 (1)
C(9)	0.5470 (2)	0.4431 (3)	0.8067 (5)	3.9 (1)
C(10)	0.4773 (2)	0.4838 (3)	0.6018 (5)	4.0 (1)
N(11)	0.7071 (2)	0.4730 (2)	1.0235 (4)	3.4 (1)
C(12)	0.6918 (2)	0.4949 (3)	1.2547 (5)	5.1 (2)
C(13)	0.7880 (2)	0.4314 (3)	0.9753 (5)	3.4 (1)
O(14)	0.8003 (1)	0.4179 (2)	0.7791 (3)	4.33 (9)
C(15)	0.8668 (2)	0.4017 (3)	1.1687 (5)	4.3 (1)
C(16)	0.9323 (2)	0.5021 (3)	1.2171 (5)	3.9 (1)
C(17)	0.9344 (2)	0.5687 (4)	1.4102 (5)	5.8 (2)
C(18)	0.9932 (3)	0.6630 (4)	1.4466 (6)	7.0 (2)
C(19)	1.0510 (2)	0.6893 (4)	1.2984 (7)	6.1 (2)
C(20)	1.0518 (2)	0.6207 (4)	1.1098 (6)	5.5 (2)
C(21)	0.9921 (2)	0.5290 (3)	1.0688 (6)	4.6 (2)
N(22)	0.6972 (2)	0.7088 (2)	0.9241 (4)	4.0 (1)
C(23)	0.7556 (2)	0.7242 (3)	0.7516 (6)	4.9 (2)
C(24)	0.8273 (2)	0.8107 (4)	0.8588 (7)	6.4 (2)
C(25)	0.7842 (3)	0.8712 (4)	1.0370 (8)	7.3 (2)
C(26)	0.6855 (3)	0.8205 (3)	1.0176 (7)	5.6 (2)

TABLE II. Bond Lengths (Å) and Angles (°)

O(1)–C(2)	1.374 (5)	C(13)–O(14)	1.225 (4)
O(1)–C(5)	1.453 (4)	C(13)–C(15)	1.519 (4)
C(2)–C(3)	1.466 (7)	C(15)–C(16)	1.513 (4)
C(3)–C(4)	1.489 (6)	C(16)–C(17)	1.389 (5)
C(4)–C(5)	1.542 (5)	C(16)–C(21)	1.381 (5)
C(5)–C(6)	1.512 (4)	C(17)–C(18)	1.395 (6)
C(5)–C(10)	1.518 (4)	C(18)–C(19)	1.357 (6)
C(6)–C(7)	1.543 (4)	C(19)–C(20)	1.386 (5)
C(7)–C(8)	1.536 (4)	C(20)–C(21)	1.379 (5)
C(7)–N(22)	1.466 (4)	N(22)–C(23)	1.455 (4)
C(8)–C(9)	1.537 (4)	N(22)–C(26)	1.448 (5)
C(8)–N(11)	1.468 (4)	C(23)–C(24)	1.517 (5)
C(9)–C(10)	1.523 (4)	C(24)–C(25)	1.504 (6)
N(11)–C(12)	1.457 (4)	C(25)–C(26)	1.540 (6)
N(11)–C(13)	1.350 (4)		
C(2)–O(1)–C(5)	111.7 (2)	C(12)–N(11)–C(13)	123.2 (3)
O(1)–C(2)–C(3)	108.9 (2)	N(11)–C(13)–O(14)	121.9 (2)
C(2)–C(3)–C(4)	106.6 (3)	N(11)–C(13)–C(15)	119.5 (2)
C(3)–C(4)–C(5)	103.9 (2)	O(14)–C(13)–C(15)	118.6 (2)
O(1)–C(5)–C(4)	104.1 (2)	C(13)–C(15)–C(16)	109.4 (2)
O(1)–C(5)–C(6)	106.4 (2)	C(15)–C(16)–C(17)	121.4 (2)
O(1)–C(5)–C(10)	108.3 (2)	C(15)–C(16)–C(21)	120.2 (3)
C(4)–C(5)–C(6)	114.7 (2)	C(17)–C(16)–C(21)	118.4 (2)
C(4)–C(5)–C(10)	112.3 (2)	C(16)–C(17)–C(18)	120.2 (2)
C(6)–C(5)–C(10)	110.5 (2)	C(17)–C(18)–C(19)	120.8 (3)
C(5)–C(6)–C(7)	112.2 (2)	C(18)–C(19)–C(20)	119.3 (3)
C(6)–C(7)–C(8)	108.2 (2)	C(19)–C(20)–C(21)	120.4 (2)
C(6)–C(7)–N(22)	115.4 (2)	C(16)–C(21)–C(20)	120.8 (2)
C(8)–C(7)–N(22)	110.4 (2)	C(7)–N(22)–C(23)	118.4 (2)
C(7)–C(8)–C(9)	109.7 (2)	C(7)–N(22)–C(26)	115.8 (2)
C(7)–C(8)–N(11)	112.5 (2)	C(23)–N(22)–C(26)	106.3 (2)
C(9)–C(8)–N(11)	111.5 (2)	N(22)–C(23)–C(24)	103.2 (2)
C(8)–C(9)–C(10)	110.5 (2)	C(23)–C(24)–C(25)	105.9 (2)
C(5)–C(10)–C(9)	113.0 (2)	C(24)–C(25)–C(26)	105.1 (2)
C(8)–N(11)–C(12)	117.2 (2)	N(22)–C(26)–C(25)	102.2 (2)
C(8)–N(11)–C(13)	118.9 (2)		

TABLE III. Selected Torsional Angles (°) with Estimated Standard Deviations in Parentheses

Cyclohexane A-ring	
C(10)–C(5)–C(6)–C(7)	−55.4 (3)
C(5)–C(6)–C(7)–C(8)	59.7 (2)
C(6)–C(7)–C(8)–C(9)	−60.2 (2)
C(7)–C(8)–C(9)–C(10)	58.4 (2)
C(8)–C(9)–C(10)–C(5)	−54.6 (3)
C(6)–C(5)–C(10)–C(9)	52.6 (3)
Pyrrolidine B-ring	
C(26)–N(22)–C(23)–C(24)	39.9 (3)
C(23)–N(22)–C(26)–C(25)	−41.2 (3)
N(22)–C(23)–C(24)–C(25)	−21.7 (3)
C(23)–C(24)–C(25)–C(26)	−2.7 (3)
C(24)–C(25)–C(26)–N(22)	26.1 (3)
Hydrofuran D-ring	
C(5)–O(1)–C(2)–C(3)	0.8 (3)
C(2)–O(1)–C(5)–C(4)	−13.8 (3)
O(1)–C(2)–C(3)–C(4)	13.2 (3)
C(2)–C(3)–C(4)–C(5)	−20.7 (3)
C(3)–C(4)–C(5)–O(1)	20.8 (3)
A...B-rings	
C(6)–C(7)–N(22)–C(23)	−54.5 (3)
C(6)–C(7)–N(22)–C(26)	73.5 (3)
C(8)–C(7)–N(22)–C(23)	68.3 (3)
C(8)–C(7)–N(22)–C(26)	−163.6 (3)
A...D-rings	
C(2)–O(1)–C(5)–C(6)	−135.4 (3)
C(2)–O(1)–C(5)–C(10)	105.9 (3)
C(3)–C(4)–C(5)–C(6)	136.6 (3)
C(3)–C(4)–C(5)–C(10)	−96.1 (3)
A-ring...amide linkage	
C(7)–C(8)–N(11)–C(13)	−120.9 (3)
C(9)–C(8)–N(11)–C(13)	115.3 (3)
Benzeneacetamide moiety	
C(13)–C(15)–C(16)–C(17)	106.7 (3)
N(11)–C(13)–C(15)–C(16)	−93.9 (3)

B-ring (Table III).

Thus, it could be described as conformational characteristics of the U69,593 molecule that the B-ring and the amide linkage are equatorially connected to the A-ring, and their respective planes are orientated at a right angle to each other. It seems that the chair conformation observed for the cyclohexane ring is advantageous to induce the substituted groups into such conformation.

Conformational Comparisons between κ-Agonists As was stated in the introduction, U50,488 is also a potent κ-agonist,[1,2] and the crystal structure has been reported as a methanesulfonate salt.[9] The U50,488 is almost the same as the U69,593, except for a D-ring (Fig. 1). Therefore, the cyclohexane ring and the nitrogen atoms could be used for molecular fitting as the common structure of both compounds. The result is shown in Fig. 4. The root mean square of shifts, r and d (see the experimental section) between the corresponding atoms were 0.397 and 0.189 Å, respectively.

It is obvious from Fig. 4 that the conformations of the cyclohexane A-rings are almost identical, and the spatial dispositions of the 7- and 8-substituted groups are similar, despite the protonation state of U50,488. Although the puckerings are slightly different from the B-ring, the bonds of the pyrrolidine rings are similar on the C(23)–N(22)–C(26) sequence. Therefore, the lone pair of the N(22) atom also takes the same direction to the H–N^+ bond of the protonated U50,488. Further, the *N*-methylamide linkages are lying on a common plane in spite of their slight positional shift. This is a reason for the relatively large displacement of the spatial orientation on the benzene, C-ring. Thus, it seems that the relative dispositions of the nitrogen atoms substituted for the cyclohexane A-ring are important for the κ-agonist activity. Indeed, the diastereomers of U50,488 exhibit different activity relating to the relative positions of the nitrogen atoms to the cyclohexane ring.[10]

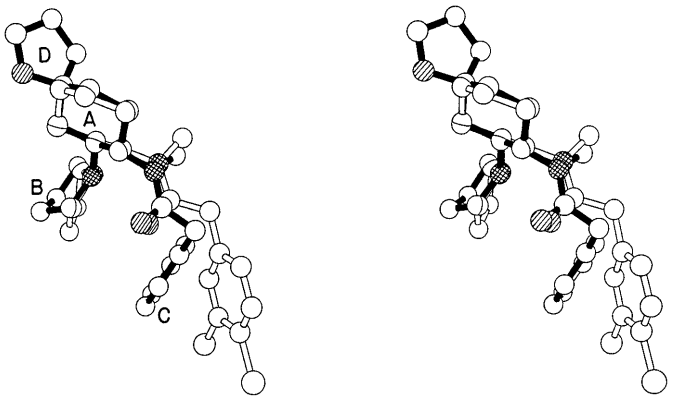


Fig. 4. Stereoscopic Drawing of Superimposition between U69,593 (Black Bond) and U50,488 (White Bond) Molecules

The striped and hatched balls indicate oxygen and nitrogen atom, respectively.

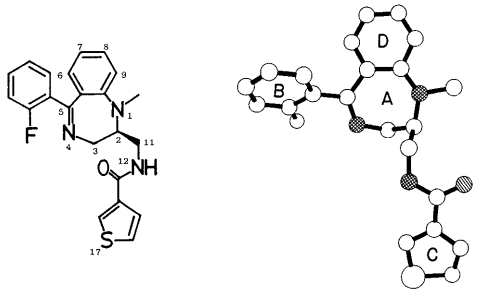


Fig. 5. Chemical Structure and Conformation of Tifluadom

The striped and hatched balls indicate oxygen and nitrogen atoms, respectively. The characters of A, B, C and D indicate the rings corresponding to U69,593.

Tifluadom is also known to be a potent κ-receptor agonist,[11,12] and has a ring system similar to U69,593, as shown in Fig. 5.[13] The model fitting with U69,593 gave no satisfactory agreement, because the 7-membered ring corresponding to the U69,593 A-ring is almost parallel with the aromatic D-ring. However, a noticeable similarity could be recognized on the relative disposition of their four rings as is conceivable from the conformational comparison of Figs. 4 and 5. Further, it seems important to note the distance between the N(1) and N(4) atoms is *ca.* 3.1 Å in tifluadom, and this would correspond to the distance between the N(11) and N(22) atoms in U69,593 (=*ca.* 2.8 Å).

Conclusion

The results of the present work are summarized as follows: [1] the planes of the atomic groups bonded to the cyclohexane ring are all in a right angle; [2] the *S*-configurations of 7- and 8-positions in the cyclohexane ring appear to be very important to form the observed conformation. This is not in conflict with the fact that the (−)-isomer of U50,488 with *S*-configuration of the substituted groups for the cyclohexane ring is the most potent κ-agonist.[10]

Experimental

X-Ray Analysis U69,593 was purchased from Sigma (U.S.A.). Crystals of U69,593 were grown from aqueous methanol solution as a needle ($0.1 \times 0.1 \times 0.6\,mm^3$). Cell dimensions were determined by a Rigaku AFC-5 diffractometer using graphite-monochromated CuK_α radiation (λ= 1.5418 Å). Crystal data are as followed: $C_{22}H_{32}N_2O_2$, M_r=356.5, monoclinic, space group $P2_1$, a=14.546(3), b=11.765(2), c=5.963(1) Å, β=100.02(2)°, V=1004.9(3) Å^3, Z=2, Dx=1.178 g·cm^{-3}, μ(CuK_α)= 5.55 cm^{-1}. A total of 1800 intensity data was measured within 2θ=120° with the 2θ–ω scan mode at 2θ-speed of 4°/min. The data were corrected for Lorentz and polarization effects and no absorption corrections were applied.

The structure was solved by the direct method using MULTAN87 programs.[14] Block-diagonal least-squares refinements were performed for obtained non-hydrogen atoms with anisotropic thermal factors minimizing the quantity of $\sum w(|F_o|-|F_c|)^2$. Hydrogen atoms were calculated at respective ideal positions and included into the calculation of the structure factors with overall isotropic thermal factors, but not refined. The weighting scheme was finally $w=1/(\sigma^2(F_o)-0.21218F_o+0.01475F_o^2)$, where $\sigma(F_o)$ is standard deviation of F_o from counting statistics. The refinement of U69,593 converged to R=0.0496 and R_w=0.0532 for 1671 reflections with $F_o > 3\sigma(F_o)$. The atomic scattering factors are given in "International Tables for X-Ray Crystallography."[15]

Molecular Fitting The molecular fitting method was performed by monitoring the differences of (1) the quantity of vector product and (2) the distance between the corresponding atoms. The former was calculated by $r=\sum_{i,j}|[e_i \times e_j]|/n$, and the latter by $d=\sum_{i,j}((p_i-p_j)^2/n)^{0.5}$, where n was number of atoms, e was the atom vector from the molecular center, and p was position of the atom.

References

1) P. F. VonVoigtlander, R. A. Lathi and J. H. Ludens, *J. Pharmacol. Exp. Ther.*, **224**, 7 (1983).
2) T. Yamamoto, M. Ohno and S. Ueki, *Eur. J. Pharmacol.*, **156**, 173 (1988).
3) P. F. VonVoigtlander and R. A. Lewis, *J. Pharmacol. Exp. Ther.*, **246**, 259 (1988).
4) R. A. Lathi, M. M. Mickelson, J. M. McCall and P. F.

VonVoigtlander, *Eur. J. Pharmacol.*, **109**, 281 (1985).
5) C. R. Clark, B. Birchmore, N. A. Sharif, J. C. Hunter, R. G. Hill and J. Hughes, *Br. J. Pharmacol.*, **93**, 618 (1988).
6) S. Yoneda, K. Kitamura, M. Doi, M. Inoue and T. Ishida, *FEBS. Lett.*, **239**, 271 (1988).
7) M. Doi, M. Tanaka, K. Ikuma, M. Nabae, K. Kitamura, M. Inoue and T. Ishida, *Biochem. J.*, **251**, 581 (1988).
8) T. Ishida, S. Yoneda, M. Doi, M. Inoue and K. Kitamura, *Biochem. J.*, **255**, 621 (1988).
9) M. Doi, T. Ishida and M. Inoue, *Acta Crystallogr., Sect. C*, **46**, 676 (1990).
10) B. R. de Costa, W. P. Brown, S. B. Hellewell, C. George, R. B. Rothman, A. A. Reid, J. M. Walker, A. E. Jacobson and K. C. Rice, *J. Med. Chem.*, **32**, 1996 (1989).
11) D. Römer, H. H. Büscher, R. C. Hill, R. Maurer, H. Zeugner, W. Benson, E. Finner, W. Milkowski and P. W. Thies, *Nature* (London), **298**, 759 (1982).
12) D. Römer, H. H. Büscher, R. C. Hill, R. Maurer, T. J. Petcher, H. Zeugner, W. Benson, E. Finner, W. Milkowski and P. W. Thies, *Life Sci.*, **31**, 1217 (1982).
13) T. J. Petcher, A. Winder, U. Maerzel and H. Zeugner, *Acta Crystallogr., Sect. C*, **41**, 909 (1985).
14) T. Debaerdemaeker, G. Germain, P. Main, C. Tate and M. M. Woolfson, MULTAN87, "Computer Programs for the Automatic Solution of Crystal Structure from X-Ray Diffraction Data," Univ. of York, England, 1987.
15) "International Tables for X-Ray Crystallography," Vol. III, Kynoch Press, Birmingham, 1974.