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Structure of *N*-Methyl-D-aspartic Acid Monohydrate

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Abstract. $C_5H_9NO_4.H_2O$, $M_r = 165{\cdot}17$, monoclinic, $P2_1$, $a = 12{\cdot}697$ (3), $b = 5{\cdot}478$ (2), $c = 11{\cdot}175$ (3) Å, $\beta = 103{\cdot}75$ (3)°, $V = 755{\cdot}0$ Å^3, $Z = 4$, $D_m = 1{\cdot}45$ (1), $D_x = 1{\cdot}45$ Mg m^{-3}, λ(Mo $K\alpha$) = 0·71069 Å, $\mu = 0{\cdot}14$ mm^{-1}, $F(000) = 352$, $T = 292$ K, $R = 0{\cdot}046$ for 1773 observed reflexions. There are two crystallographically independent molecules (I) and (II) in the asymmetric unit. Both molecules exist as zwitterions. The conformation about the $C^{\alpha}(2)$—$C^{\beta}(3)$ bond is *gauche–gauche*, angles χ^1 [N(1)—C(2)—C(3)—C(4)] and χ^2 [C(1)—C(2)—C(3)—C(4)] are −67·3 (3), 55·3 (4)° in (I) and −70·9 (4), 53·1 (4)° in (II). An NMR study in aqueous solution also shows the *gauche–gauche* conformation to be very stable. Two intramolecular N—H···O hydrogen bonds stabilize the conformation. The crystal structure is stablized by eight intermolecular N—H···O and O—H···O hydrogen bonds involving amino groups, carboxyl groups and water molecules.

Introduction. Excitation of neurons in the mammalian central nervous system is mediated principally by various dicarboxylic amino acids acting upon specific protein receptors. It has been known since 1965 that very powerful excitation is induced by *N*-methyl-D-aspartic acid (NMDA) in micromolar concentrations (Curtis & Watkins, 1965). Consequently, one of the receptors participating in the transmission of nerve impulses has been classified as the NMDA receptor, and NMDA itself is being extensively used in studies of neuron excitation (for recent reviews see Curtis & Watkins, 1965; Watkins & Olverman, 1987; Maldrum, 1987; Mayer & Westbrook, 1987).

The NMDA receptor has not yet been structurally characterized and the only information available is based on structures of amino acids which bind at the active site and exert agonist or antagonist action (Watkins & Olverman, 1987). Clearly, more precise information about the receptor-site structure may be inferred from a detailed knowledge of the geometry of NMDA itself, including its conformation in solution. In this study we describe the X-ray structure of NMDA and report on conformational studies by NMR methods.

Experimental. Clear colourless crystals grown from water at room temperature, dimensions 0·39 × 0·45 × 0·45 mm; D_m by flotation in carbon tetrachloride/ethylene bromide; monoclinic $P2_1/m$ or $P2_1$ from Weissenberg photographs, $P2_1/m$ excluded owing to known optical activity; Syntex $P2_1$ computer-controlled four-circle diffractometer, scintillation counter, graphite monochromator; cell parameters by least squares from setting angles of 15 reflexions with $18 \le 2\theta \le 25°$ measured on diffractometer; 2280 independent reflexions; $2\theta_{max} = 55{\cdot}0°$; variable θ–2θ scans, scan rate 2·0–29·3° min^{-1}, depending on intensity; two standards (313, 43$\bar{1}$) every 50 reflexions, variation in intensities ±2%; correction for Lorentz and polarization factors, but not for absorption; 1773 with $I \ge 3\sigma(I)$ used for structure determination; index range h 0 to 16, k 0 to 7, l −14 to 13; neutral-atom scattering factors and anomalous-dispersion corrections from *International Tables for X-ray Crystallography* (1974); direct methods, *SHELXS*86 (Sheldrick, 1986); block-diagonal least squares [Syntex (1976) *XTL*/*XTLE* system], minimizing $\sum w(|F_o| - |F_c|)^2$, $w = 1/\sigma^2(F)$; difference synthesis revealed H atoms; non-H atoms refined with anisotropic thermal parameters and H atoms isotropically; max. $\Delta/\sigma = 0{\cdot}01$, $\Delta\rho$ within +0·19 and −0·18 e Å^{-3}, $R = 0{\cdot}046$, $wR = 0{\cdot}051$, $S = 4{\cdot}705$.

The absolute configuration was assigned as D with reference to the known D configuration of *N*-methyl-D-aspartic acid.

The ^{1}H NMR spectra of aqueous (D_2O) solutions of *N*-methyl-D-aspartic acid (concentration 0·15 mol dm^{-3}) were recorded on a 100 MHz Tesla BS 5671 spectrometer at 298 K. ^{1}H NMR spectra of the Asp(CH_2—CH) unit were analyzed as *ABC* spectra. The rotamer population (Table 3, Fig. 2) was calculated according to the approach proposed by Martin (1979).

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Discussion. Final atomic coordinates are given in Table 1.* The molecular structure of the two independent molecules (I) and (II) and the atom numbering are shown in Fig. 1. Table 2 gives the interatomic distances and angles in both molecules. These distances are quite similar internally and are consistent with those found in L-aspartic acid (Derissen, Endeman & Perdeman, 1968) and DL-aspartic acid (Rao, 1973). There are larger differences among the angles and the reason for this can be seen in Table 2 which summarizes the torsion angles.

Fig. 1 shows that the α-carboxyl groups of both molecules (I) and (II) are ionized, the proton being transferred to the amino group forming a zwitterion. The C—O and C—OH bond lengths in the α- and β-carboxyl groups compare well with those usually found in carboxylic acid structures (Sundaralingam & Putkey, 1970). The α- and β-carboxyl groups are essentially planar. The maximum deviations from the least-squares planes through the α-carboxyl groups, O(1), O(2), C(1) and C(2) are 0·012 (3) for (I) and 0·017 (3) Å for (II). The distances of the N atom from these planes are −0·402 (3) and 0·394 (3) Å, respectively. The four atoms O(3), O(4), C(4), C(5) of the β-carboxyl groups show maximum deviations of 0·002 (3) in (I) and 0·003 (3) Å in (II). The β-carboxyl group is twisted in opposite directions about C(3)—C(4) in the two molecules. The carbonyl O(3) atom is synperiplanar to the carbon backbone C(2)—C(3)—C(4)—O(3) [7·2 (4)°] in (I), in contrast to the antiperiplanar orientation [−162·9 (4)°] in (II). The C(2)—C(3)—C(4) angle is 116·1 (3)° in (II) considerably larger than the 112·2 (3)° observed in (I).

* Lists of structure factors, anisotropic thermal parameters and H-atom parameters have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 52941 (14 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

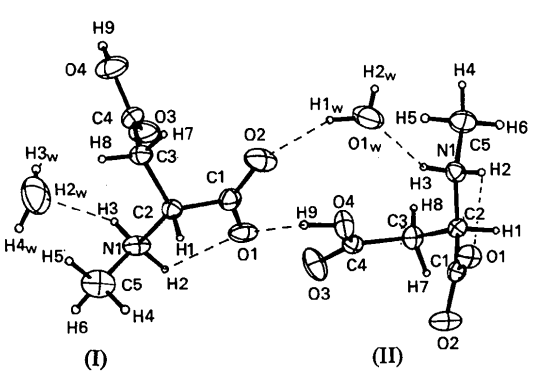


Fig. 1. An *ORTEP* (Johnson, 1976) drawing of the title compound with the atom-numbering scheme. Thermal ellipsoids have been drawn at the 50% probability level.

Table 1. *Positional parameters and equivalent isotropic temperature factors* (Å²) *with e.s.d.'s in parentheses*

$B_{eq} = (1/3)\Sigma_i\Sigma_j B_{ij} a_i^* a_j^* \mathbf{a}_i.\mathbf{a}_j$

	x	y	z	B_{eq}
$O(1)_w$	0·4772 (2)	0·1187 (1)	0·1069 (3)	5·3 (3)
$O(2)_w$	0·0289 (2)	0·0529 (9)	0·4376 (3)	5·6 (3)
(I)				
O(1)	−0·1748 (2)	−0·1071†	0·0043 (2)	2·8 (2)
O(2)	−0·3394 (2)	−0·0334 (6)	0·0345 (2)	3·6 (2)
O(3)	−0·1793 (2)	−0·1700 (5)	0·3012 (2)	3·6 (2)
O(4)	−0·2827 (3)	0·0055 (6)	0·4118 (3)	4·4 (2)
N(1)	−0·0719 (2)	0·1886 (6)	0·1770 (3)	2·3 (2)
C(1)	−0·2402 (2)	0·0055 (7)	0·0530 (3)	2·1 (2)
C(2)	−0·1929 (2)	0·2170 (7)	0·1400 (3)	1·8 (2)
C(3)	−0·2412 (2)	0·2353 (7)	0·2521 (3)	2·0 (2)
C(4)	−0·2304 (3)	0·0036 (7)	0·3229 (3)	2·0 (2)
C(5)	−0·0107 (3)	0·4225 (8)	0·1964 (4)	3·5 (3)
(II)				
O(1)	0·4235 (2)	0·2235 (5)	0·4508 (2)	2·4 (2)
O(2)	0·2557 (2)	0·1192 (6)	0·4603 (2)	3·2 (2)
O(3)	0·1023 (2)	−0·0951 (6)	0·1279 (3)	3·2 (2)
O(4)	0·2605 (2)	0·1005 (6)	0·1628 (2)	2·7 (2)
N(1)	0·4503 (2)	−0·1428 (5)	0·3104 (2)	1·8 (2)
C(1)	0·3448 (2)	0·0826 (7)	0·4300 (3)	1·8 (2)
C(2)	0·3554 (2)	−0·1630 (6)	0·3684 (3)	1·6 (2)
C(3)	0·2510 (2)	−0·2504 (7)	0·2819 (3)	1·9 (2)
C(4)	0·1966 (2)	−0·0732 (7)	0·1842 (3)	1·8 (2)
C(5)	0·4841 (3)	−0·3726 (8)	0·2576 (3)	3·1 (3)

† Fixed parameter.

Important differences exist between the torsion angles of (I) and (II) and between these molecules and the L- and DL-aspartic acids. Molecules (I) and (II) show similar conformations of their α-amino moieties, but there are some differences in the side chain (Table 2). In both (I) and (II) the C(4) atom is *gauche* to N and C(1), and the torsion angle ψ^1 [O(1)—C(1)—C(2)—N(1)] = −17·1 (4) (I) and −17·9 (4)° (II). In L- and DL-aspartic acid C(4) is *trans* to C(1) and ψ^1 = −37·8 (L) and −7·3° (DL).

Excluding metal complexes in which coordination requirements introduce severe steric constraints, such a *gauche–gauche* conformation for the aspartic acid side chain has so far been observed only in the crystal structure of histidine–aspartic acid (Bhat & Vijayan, 1978) and in *N*-carbamoyl-DL-aspartic acid (Rao, Murthy, Rao & Vijayan, 1982). Furthermore, the C(methyl)—N(1)—C(2)—C(3) torsion angle of −87·9 (3)° in (I) is considerably greater than the −59·7 (4)° observed on (II).

The molecular and crystal structure is highly stabilized by a network of O—H···O and N—H···O hydrogen bonds (Table 2). The molecular conformations of (I) and (II) are both stabilized by the formation of intramolecular hydrogen bonds between the amino group and O(1) of the α-carboxyl group. The amino groups of (I) and (II) also form intermolecular hydrogen bonds with the O atoms of the water molecules. In (II) the parameters of a third

Table 2. *Molecular geometry* (Å,°) *with e.s.d.'s in parentheses*

	(I)	(II)
O(1)—C(1)	1·258 (4)	1·240 (4)
O(2)—C(1)	1·245 (4)	1·272 (4)
O(3)—C(4)	1·207 (5)	1·219 (4)
O(4)—C(4)	1·319 (4)	1·308 (4)
C(1)—C(2)	1·540 (5)	1·532 (5)
C(2)—N(1)	1·501 (4)	1·502 (4)
C(2)—C(3)	1·523 (4)	1·521 (4)
C(3)—C(4)	1·485 (5)	1·501 (4)
C(5)—N(1)	1·488 (5)	1·496 (5)
O(2)—C(1)—O(1)	126·2 (3)	125·5 (3)
C(2)—C(1)—O(1)	116·2 (3)	118·6 (3)
C(2)—C(1)—O(2)	117·4 (3)	115·9 (3)
C(2)—N(1)—C(5)	114·6 (3)	116·3 (3)
C(1)—C(2)—N(1)	108·4 (3)	107·1 (2)
C(3)—C(2)—N(1)	111·5 (3)	114·6 (3)
C(1)—C(2)—C(3)	113·3 (3)	113·9 (3)
C(2)—C(3)—C(4)	112·2 (3)	116·1 (3)
O(4)—C(4)—O(3)	123·2 (3)	123·6 (3)
C(3)—C(4)—O(3)	123·6 (3)	122·5 (3)
C(3)—C(4)—O(4)	113·3 (3)	113·9 (3)
ψ^1 O(1)—C(1)—C(2)—N(1)	−17·7 (4)	−17·9 (4)
ψ^2 O(2)—C(1)—C(2)—N(1)	164·1 (4)	165·0 (4)
χ^1 N(1)—C(2)—C(3)—C(4)	−67·3 (3)	−70·9 (4)
C(2)—C(3)—C(4)—O(3)	7·2 (4)	−162·9 (4)
C(2)—C(3)—C(4)—O(4)	−172·5 (3)	19·1 (4)
χ^2 C(1)—C(2)—C(3)—C(4)	55·3 (4)	53·1 (4)
C(5)—N(1)—C(2)—C(1)	146·7 (4)	172·9 (4)
C(5)—N(1)—C(2)—C(3)	−87·9 (3)	−59·7 (4)

D—H···*A*	*D*—H	H···*A*	*D*···*A*	∠*D*—H···*A*
$O(1)_w$—H(1)···O(2,I)^i	0·90 (4)	2·05 (4)	2·806 (5)	142 (4)
$O(1)_w$—H(2)···O(2,I)^{ii}	0·73 (4)	2·06 (4)	2·769 (4)	166 (5)
$O(2)_w$—H(4)···O(2,II)	0·95 (5)	1·91 (4)	2·852 (4)	172 (2)
N(1,I)—H(2,I)···O(1,I)	1·00 (3)	2·05 (3)	2·618 (4)	114 (2)
N(1,I)—H(3,I)···$O(2)_w$	0·95 (4)	2·12 (4)	2·984 (4)	151 (4)
O(4,I)—H(9,I)···O(2,II)^{iii}	0·99 (5)	1·55 (5)	2·532 (3)	173 (5)
N(1,II)—H(2,II)···O(1,II)	0·86 (4)	2·29 (5)	2·619 (4)	103 (3)
N(1,II)—H(2,II)···O(1,II)^{iii}	0·86 (4)	2·28 (4)	2·862 (3)	125 (4)
N(1,II)—H(3,II)···$O(1)_w$	0·89 (4)	1·97 (4)	2·777 (4)	151 (4)
O(4,II)—H(9,II)···O(1,II)^i	0·99 (4)	1·52 (5)	2·504 (4)	172 (5)

Symmetry code: (i) $-x, \frac{1}{2}+y, -z$; (ii) $1+x, y, z$; (iii) $-x, -\frac{1}{2}+y, 1-z$.

amino hydrogen bond indicate a weak intermolecular (bifurcated) interaction N(1,II)—H(2,II)···O(1,II). The β-carboxyl O(4) atoms act as donors in the strong hydrogen bonds [2·532 (3) and 2·504 (4) Å] with O(2,II) and O(1,I) atoms of the α-carboxyl groups. Water $O(1)_w$ forms two hydrogen bonds with α-carboxyl O atoms, whilst water $O(2)_w$ is involved in one similar interaction.

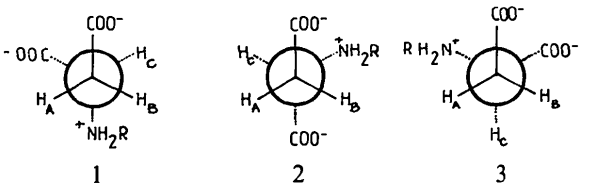
NMR results. The ^{1}H NMR analysis of the *N*-CH_3-D-Asp conformation in aqueous solution indicates that isomer 3 (Table 3, Fig. 2) is a very stable conformer at pH < 7·5, *i.e.* when the amino group remains protonated. Deprotonation of the methylated amino group distinctly decreases the population of isomer 3 and rotamer 2 becomes the most stable, with two carboxyl groups *trans* to each other. A similar conformational behaviour was also found earlier for Asp and Asn amino acids in aqueous solution (Kozłowski, Świątek & Siatecki, 1981).

Both solid-state and solution studies indicate that the *gauche–gauche* conformer is stabilized when the

Table 3. *Rotamer populations of the N-*CH_3*-*D*-Asp molecule at different* pH*'s*

Rotamer populations were calculated according to the approach proposed by Martin (1979) for the rotamer notation of Fig. 2.

pH	p_1	p_2	p_3
2·9	0·56		0·44
7·2	0·56		0·44
10·3	0·16	0·57	0·27



1 2 3

Fig. 2. Rotamer notation for the *N*-CH_3-D-Asp ($R = CH_3$) molecule.

N—CH_3 amino group is protonated. This stability derives from the intramolecular hydrogen bonds involving the secondary ammonium group. Furthermore, the carboxylates are also involved in a network of strong hydrogen bonds which determine the molecular interactions. The ability for very effective hydrogen-bond formation, besides stabilizing the molecular structure, must have a significant effect on molecular interactions at the acceptor site.

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