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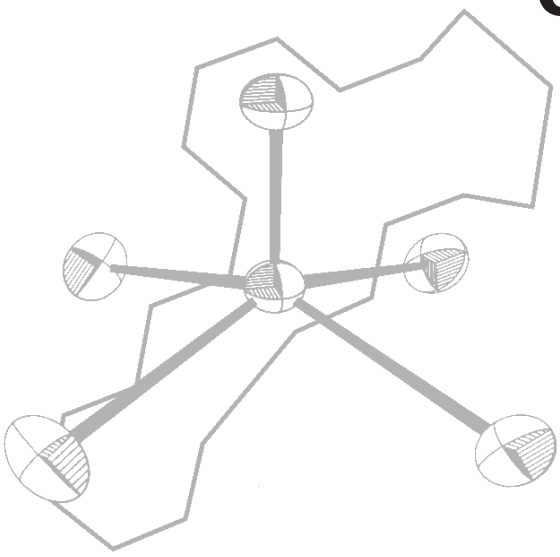

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Synthesis and Structural Characterization of *N*-Methyl-DL-glutamic Acid

Andrew B. Hughes, Maureen F. Mackay and Luigi Aurelio

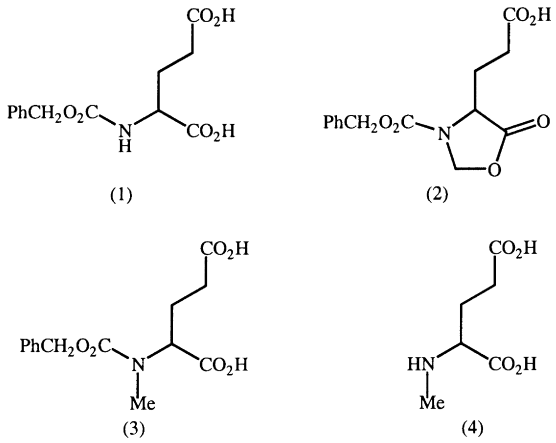
Department of Chemistry, La Trobe University, Bundoora, Vic. 3083.

The solid-state conformation of racemic *N*-methylglutamic acid has been defined by single-crystal X-ray crystallography. Orthorhombic crystals belong to the space group *P bca* with *a* 15.219(2), *b* 10.583(1), *c* 9.595(1) Å and *Z* 8. The structure was refined to a final *R* value of 0.049 for the 1285 measured data. In the crystal the molecules adopt a zwitterionic form with protonation having occurred at the amino nitrogen atom. The α-carboxyl is unprotonated with the δ-carboxy group retaining a proton. The i.r. spectrum shows absorptions which also are indicative of the amino acid being in the zwitterionic form. Intermolecular H-bonds involving the carboxylate proton and the two protons on the N-atom link the molecules into a three-dimensional network in the crystal.

Keywords. X-Ray crystallography; zwitterion; glutamic acid; peptide.

Introduction

As part of a project concerning the syntheses of heavily *N*-methylated peptides, the syntheses of a number of *N*-methyl amino acids were undertaken. One of these amino acids was *N*-methylglutamic acid. Racemic *N*-methylglutamic acid was prepared from *N*-benzyloxycarbonylglutamic acid (1) by formation of the oxazolidin-5-one (2) (86%).[1] The oxazolidinone (2) was then reductively cleaved to give the *N*-methyl compound (3) (63%) by using triethylsilane and trifluoroacetic acid. Finally, the benzyloxycarbonyl group was removed by hydrogenolysis over palladium catalyst to give the expected *N*-methyl-DL-glutamic acid (4) (63%). Crystals of *N*-methyl-DL-glutamic acid (4) suitable for an X-ray structure analysis precipitated from an n.m.r. sample in D_2O at 5°C; consequently the amino and carboxylic protons have been replaced by deuterium atoms. An X-ray structure analysis was undertaken to define the conformational detail in the molecule. A search of the Cambridge Crystallographic Database[2] revealed that the only crystal structures of isolated and neutral *N*-methylated α-amino acids reported to date are those of *N*-methyl-L-tryptophan (abrine)[3] and *N*-methyl-D-aspartic acid monohydrate.[4] The crystal structure of the title compound therefore provides a third example in the series.



Experimental

All melting points are uncorrected and were recorded on a Reichert 'Thermopan' microscope hot-stage apparatus. Infrared spectra were recorded on a Perkin–Elmer 1720X Fourier-transform i.r. spectrometer, using a diffuse reflectance accessory with KBr background. N.m.r. spectra including DEPT experiments were recorded in (D)chloroform solution unless otherwise stated on a Brüker AM-300 spectrometer (^{1}H at 300.13 MHz and ^{13}C at 75.47 MHz) or on a Brüker DRX 400 MHz machine. Chemical shifts are reported as δ values and coupling constants (*J*) in Hz relative to residual solvent. Electrospray (e.s.) mass spectra were obtained on a VG Bio-Q triple quadrupole mass spectrometer by using water/methanol/acetic acid (0:99:1 or 50:50:1) mixtures as the mobile phase. Low-resolution mass spectra (e.i.) were performed at La Trobe University by Dr John Traeger, on a Shimadzu GCMS-QP5050A mass spectrometer fitted with a direct insertion probe at 70 eV, with a transfer line temperature of 250°C. Other low- and high-resolution mass spectra were measured at the University of Tasmania by Dr Noel Davies and coworkers, on a Kratos concept mass spectrometer at 70 eV with a source temperature of 200°C. Optical rotations were obtained on a Perkin–Elmer 141 polarimeter. 'Flash column chromatography' was carried out using silica gel (silica gel 60, 230–400 mesh ASTM) supplied by Merck Chemicals (Darmstadt). Ethyl acetate and hexane used for chromatography were distilled prior to use. All solvents were purified by distillation. For dry solvents, procedures from Perrin and Armarego[5] were followed. Dry dichloromethane was distilled and stored over Linde-type 4 Å molecular sieves. All other reagents and solvents were purified or dried as described by Perrin and Armarego.[5]

N-*Benzyloxycarbonyl*-N-*methyl*-DL-*glutamic Acid (3)*[6]

The oxazolidinone (2) (1.74 g, 5.9 mmol) was dissolved in chloroform (30 ml) in a round-bottom flask followed by addition of trifluoroacetic acid (30 ml) and triethylsilane (2.8 ml, 17.5 mmol) and the mixture was left to stir for 5 days. After being stirred, the solution was concentrated to give an oily residue. The residue was taken up in ethanol and reconcentrated under reduced pressure from that solvent three times. The residue was taken up in a mixture of ether (100 ml) and

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5% sodium bicarbonate solution (60 ml). The ethereal solution was extracted with 5% sodium bicarbonate solution (2×30 ml). The combined aqueous layers were washed with ether (2×30 ml) and the aqueous layer was then acidified with 5 mol dm^{-3} HCl to pH 2 and extracted with ethyl acetate (3×60 ml). The combined ethyl acetate layers were dried ($MgSO_4$), filtered and concentrated to give an oil. The oil was further purified by silica gel chromatography, with $CHCl_3$/MeOH/acetic acid (90 : 9.9 : 0.1) as eluent, to give the *N*-methyl amino acid (3) as an oil (1.1 g, 63%). ν_{max}/cm^{-1} (NaCl) 3500–2750 (CO_2H), 3093, 3066 and 3035 (CH, aromatic), 3000–2900 (CH, saturated), 1710 (C=O), 1486, 1453, 1405, 1322, 1189, 1143, 770, 698. *m/z* (e.s. mass spectrum) 296 (M+1, 100%), 277 (23), 252 (41), 204 (37), 160 (26). δ_H (300 MHz, $CDCl_3$) (rotamers) 10.42, s, 2H, 2×CO_2H; 7.20, s, 5H, ArH; 4.99, s, 2H, $ArCH_2$; 4.65–4.61 and 4.52–4.49, 2m, 1H, NCHCO; 2.74, s, 3H, NCH_3; 2.24–2.17 and 1.92–1.86, 2m, 4H, C**H**$_2$C**H**$_2$$CO_2$H. δ_C (75 MHz, $CDCl_3$) (rotamers) 175.13, 174.98 and 172.85 (2×CO_2H), 156.60 and 156.00 (CO carbamate); 136.06 (quaternary Ar C); 128.01, 127.49 and 127.13 (5×Ar C), 66.96 ($ArCH_2$), 57.78 (N**C**HCO), 30.95, 30.41 and 30.24 (NCH_3), 23.75 and 23.45 (**C**H_2**C**H_2CO_2H).

N-*Methyl*-DL-*glutamic Acid (4)*[7,8]

To a mixture of the *N*-benzyloxycarbonyl-*N*-methyl amino acid (3) (880 mg, 2.98 mmol) and ethanol (80 ml) in a round-bottom flask was added 10% Pd-on-C catalyst (50 mg). The mixture was stirred at room temperature in an atmosphere of H_2 until the required amount of hydrogen gas was absorbed. The greyish mixture was then concentrated under reduced pressure. The residue was taken up in hot distilled water (10 ml) and filtered through Celite. The filter cake was washed with hot water (2×2 ml) and the combined filtrates were evaporated under vacuum to afford a white solid. The solid was then dissolved in a minimum of hot distilled water and crystallized by addition of ethanol to afford the pure *N*-methyl amino acid (4) (302 mg, 63%), m.p. 172–176°C. ν_{max}/cm^{-1} (KBr) 3600–3250 (CO_2H), 3300–3000, 2970, 2950, 2853, 2710 (m, $CH_3NH_2^+$), 2571, 2535, 2507 and 2459, 1723 (CO_2H), 1618, 1571 (CO_2^-), 1504, 1480, 1450, 1392, 1330, 1301, 1257, 1218, 1198, 1058, 1000, 846, 653, 532. *m/z* (e.i. mass spectrum) 176 (100%), 162 (M+1, 18), 144 (12), 130 (9). δ_H (300 MHz, D_2O) 3.47, t, *J* 6.0 Hz, 1H, NCHCO; 2.56, s, 3H, NCH_3; 2.36, t, *J* 7.3 Hz, 2H, C**H**$_2$$CO_2$H; 2.07–1.89, m, 2H, C**H**$_2$$CH_2CO_2H$. δ_C (75 MHz, D_2O) 176.88 and 172.75 (2×CO_2H), 62.53 (N**C**HCO), 31.65 (NCH_3), 29.79 (**C**H_2CO_2H), 24.25 (**C**$H_2CH_2CO_2H$).

Crystallography

Crystal data. $C_6H_8D_3NO_4$, *M* 164.1, orthorhombic, space group *P bca*, *a* 15.219(2), *b* 10.583(1), *c* 9.595(1) Å, *V* 1545.4(3) $Å^3$, D_m 1.40(1), D_c 1.411 (*Z* = 8) g cm^{-3}, *F*(000) 688, μ(Cu Kα) 1.00 cm^{-1}.

Structure determination. Intensity data were measured with Cu Kα radiation from a cleaved specimen of dimensions *c*. 0.41 by 0.26 by 0.10 mm aligned on a Rigaku-AFC four-circle diffractometer, recorded by an ω–2θ scan with 2θ scan rate of 2.0° min^{-1}, and 10 s stationary background counts. Three reference reflections monitored every 100 reflections indicated no decay. Data to a $2\theta_{max}$ 130° yielded a total of 1599 terms (R_{merge} 0.013); corrections for Lorentz and polarization effects were applied. Analytical absorption corrections were made with SHELX-76[9] (transmission factors 0.758–0.920). The structure was solved by direct methods with SHELX-76[9] and least-squares refinements were carried out with SHELXS-93[10] on a VAX8800 computer with 1285 unique terms. Full-matrix least-squares refinements [on $(F_o)^2$], with anisotropic factors given to the non-hydrogen atoms and isotropic factors given to the hydrogen atoms, gave residuals* for the 1285 data of *R* 0.049 and *wR* 0.120 with *S* 1.005 (145 variables). The function minimized in the refinements was $\Sigma w[(F_o)^2-(F_c)^2]^2$ with $w = [\sigma^2(F) + (0.0587P)^2+1.8008P]^{-1}$, where $P = [\max(F_o^2, 0)+2F_c^2/3]$. The maximum and minimum residual electron-density peak heights were +0.29 and –0.23 e $Å^{-3}$. An extinction parameter was applied to the F_c terms with SHELXS-93;[10] the extinction coefficient was 0.017(1)×10^{-6}.

Results

The X-ray results are presented in Tables 1–3 and Figs 1 and 2. The latter were prepared from the output of ORTEPII.[11] Material deposited† includes anisotropic thermal parameters, hydrogen atom parameters, and observed and calculated structure amplitudes.

Discussion

As the *N*-methyl-DL-glutamic acid molecule (4) was recrystallized from D_2O it is partially deuterated, the amino and carboxylate protons having been replaced by deuterium atoms. A perspective view of the molecule given in Fig. 1 illustrates that it is in the zwitterionic form in the crystal as are the solid-state structures of *N*-methyl-L-tryptophan,[3] *N*-methyl-D-aspartic acid[4] and, in general, neutral α-amino acids. The δ-carboxy group is protonated whereas the α-carboxyl is unprotonated. This is in accord with the C(5)–O(5) and C(5)–O(5′) bond lengths of 1.324(3) and 1.204(3) Å

Table 1. Fractional atomic coordinates and equivalent isotropic temperature factors of the non-hydrogen atoms

Estimated standard deviations are in parentheses; U_{eq} ($Å^2$) were calculated from the refined anisotropic temperature parameters $U_{eq} = 1/3(U_{11}+U_{22}+U_{33})$

Atom	*x*	*y*	*z*	U_{eq}
O(1)	0.18985(10)	0.12830(13)	0.32912(14)	0.0301(4)
O(1′)	0.20851(11)	0.12470(13)	0.09805(15)	0.0352(5)
O(5)	–0.07033(10)	0.45288(15)	0.1445(2)	0.0369(5)
O(5′)	0.02318(12)	0.5797(2)	0.2545(3)	0.0635(7)
N(1)	0.27539(11)	0.3612(2)	0.0872(2)	0.0252(5)
C(1)	0.20967(13)	0.1775(2)	0.2131(2)	0.0249(5)
C(2)	0.23538(13)	0.3176(2)	0.2212(2)	0.0239(5)
C(3)	0.15652(14)	0.3991(2)	0.2608(2)	0.0292(5)
C(4)	0.07523(14)	0.3798(2)	0.1711(2)	0.0312(5)
C(5)	0.00790(15)	0.4816(2)	0.1959(2)	0.0319(5)
C(6)	0.3633(2)	0.3065(2)	0.0546(3)	0.0369(6)

Table 2. Bond lengths (Å) involving the non-hydrogen atoms

Estimated standard deviations are in parentheses

Atoms	Length	Atoms	Length
O(1)–C(1)	1.265(2)	N(1)–C(2)	1.496(3)
O(1′)–C(1)	1.237(2)	C(1)–C(2)	1.535(3)
O(5)–C(5)	1.324(3)	C(2)–C(3)	1.526(3)
O(5′)–C(5)	1.204(3)	C(3)–C(4)	1.521(3)
N(1)–C(6)	1.492(3)	C(4)–C(5)	1.506(3)

Table 3. Bond angles (degrees) involving the non-hydrogen atoms

Estimated standard deviations are in parentheses

Atoms	Angles	Atoms	Angles
C(2)–N(1)–C(6)	115.2(2)	C(1)–C(2)–C(3)	111.0(2)
O(1)–C(1)–O(1′)	126.6(2)	C(2)–C(3)–C(4)	115.0(2)
O(1)–C(1)–C(2)	114.4(2)	C(3)–C(4)–C(5)	111.6(2)
O(1′)–C(1)–C(2)	119.0(2)	O(5)–C(5)–O(5′)	123.1(2)
N(1)–C(2)–C(1)	111.0(2)	O(5)–C(5)–C(4)	112.9(2)
N(1)–C(2)–C(3)	111.1(2)	O(5′)–C(5)–C(4)	124.0(2)

* $R = \Sigma||F_o|-|F_c||/\Sigma|F_o|$ and $wR = [\Sigma[w(F_o)^2-(F_c)^2]/\Sigma[w(F_o)^2]]^{½}$.

† Copies are available, until 31 December 2005, from the Australian Journal of Chemistry, P.O. Box 1139, Collingwood, Vic. 3066.

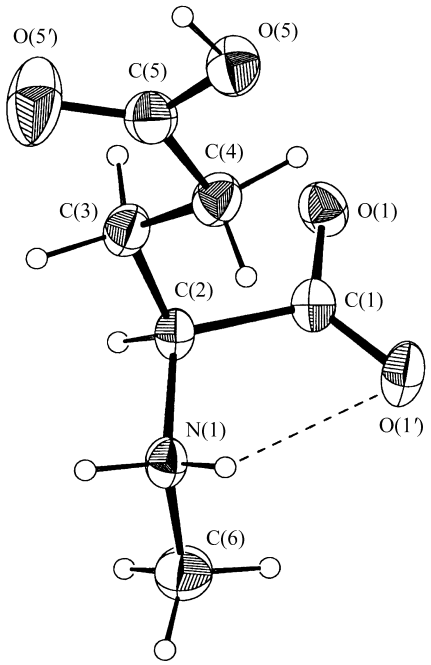

Fig. 1. Perspective view of *N*-methylglutamic acid (4) with thermal ellipsoids drawn at the 50% probability level. Hydrogen and deteurium atoms are represented by spheres of arbitrary radius.

respectively and the C(1)–O(1) and C(1)–O(1′) lengths of 1.265(2) and 1.237(2) Å. A similar situation was noted in the neutron diffraction structure of the β-form of L-glutamic acid.[12] In the structures of the hydrochlorides of glutamic acid[13] and *N*-methylglycine,[14] as expected, the α-carboxy groups are protonated so that the molecules do not adopt zwitterionic forms. The major difference between the *N*-methylated and glutamic acid structures is the conformation of the side chain. In the former it is extended so that C(5) (or Cδ) is *trans* to C(2) (or Cα), the torsion angle C(2)–C(3)–C(4)–C(5) being –165.7(2)° (Table 4). In the hydrochloride[13] structure the side chain is also extended but in the neutral structure,[12] the side chain is buckled with Cδ gauche to Cα and the Cα–Cβ–Cγ–Cδ torsion angle –73.1(2)°. The conformation about the Cα–Cβ bond in *N*-methylglutamic acid is *gauche–gauche*, the torsion angles C(1)–C(2)–C(3)–C(4) and N(1)–C(2)–C(3)–C(4) having values of –52.3(2) and 71.7(2)° respectively. The *N*-methyl-D-aspartic acid structure[4] also has *gauche–gauche* conformers and it is claimed that the conformer is stabilized when the α-amino group is protonated, the stability deriving from the intramolecular hydrogen bond between the amino and α-carboxy groups. The *N*-methylglutamic acid structure also has a similar intramolecular hydrogen bond (N(1)·····O(1′) distance 2.704(2) Å) but this interaction is much weaker than the interaction observed in the aspartic acid structure (see Table 5).

Table 4. Torsion angles (degrees) involving the non-hydrogen atoms
Estimated standard deviations are in parentheses

Atoms	Angles	Atoms	Angles
C(6)–N(1)–C(2)–C(3)	167.5(2)	N(1)–C(2)–C(3)–C(4)	71.7(2)
C(6)–N(1)–C(2)–C(1)	–68.5(2)	C(1)–C(2)–C(3)–C(4)	–52.3(2)
O(1′)–C(1)–C(2)–N(1)	–13.0(3)	C(2)–C(3)–C(4)–C(5)	–167.7(2)
O(1)–C(1)–C(2)–N(1)	168.2(2)	C(3)–C(4)–C(5)–O(5′)	15.2(3)
O(1′)–C(1)–C(2)–C(3)	111.0(2)	C(3)–C(4)–C(5)–O(5)	–165.7(2)
O(1)–C(1)–C(2)–C(3)	–67.8(2)		

Table 5. Hydrogen-bond distances (Å) and angles (degrees)
Estimated standard deviations are given in parentheses

A–D···B[A]	A–D	D···B	A·····B	A–D···B
O(5)–D···O(1)^{I}	0.96(4)	1.68(4)	2.612(2)	166(2)
N(1)–D(NDα)···O(1)^{II}	0.90(3)	1.96(3)	2.800(2)	154(2)
N(1)–D(NDβ)···O(1′)^{III}	0.91(3)	1.89(3)	2.801(2)	178(2)
N(1)–D(NDα)···O(1′)	0.90(3)	2.50(3)	2.704(2)	87(2)

[A] Code for symmetry-related atoms: I, $-x$, $1/2+y$; $1/2-z$; II, x, $1/2-y$; $-1/2+z$; III, $1/2-x$; $1/2+y$, z.

The molecular packing is illustrated in Fig. 2. Intermolecular hydrogen bonds (see Table 5) link the molecules into a three-dimensional network. The N–D···O interactions of 2.801(2) and 2.800(2) Å link the molecules along the *b* and *c* crystal axes and thus into layers parallel to the *bc* planes in the crystal, whereas the stronger O–D···O bond of 2.612(2) Å between the carboxy groups link the molecular layers along the *a* axis.

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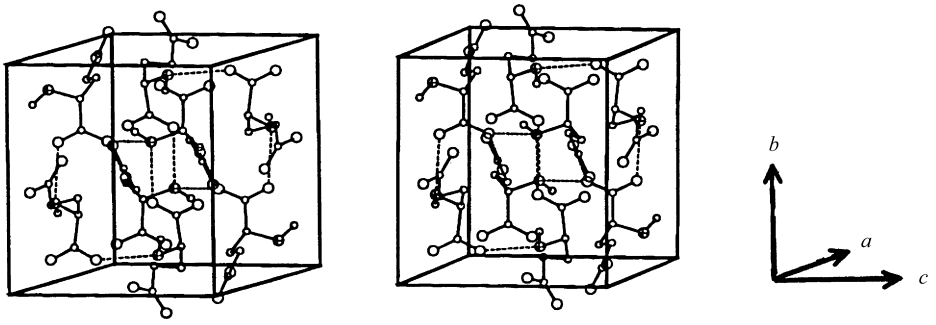

Fig. 2. A stereoscopic view of the crystal structure of *N*-methylglutamic acid. The large open circles are oxygens whilst the lined circles are nitrogen.

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