

The Structure of the Monoammonium Salt of DL- γ -Carboxyglutamic Acid

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Abstract. $\text{NH}_4^+ \cdot \text{C}_6\text{H}_8\text{NO}_6^-$, orthorhombic, $Pna2_1$, $a = 9.880$ (2), $b = 11.447$ (3), $c = 7.619$ (2) Å, $V = 861.8$ Å³, $Z = 4$, $D_m = 1.60$, $D_x = 1.60$ Mg m⁻³, $\mu(\text{Cu } K\alpha) = 1.27$ mm⁻¹, $\lambda = 1.5418$ Å. The C_α carboxyl group and the neutral C_γ carboxyl group are disordered and are located at positions of unequal occupancy on either side of the mean molecular plane. The major conformational angles are $\chi^1 = -176$ (1)°, $\chi^{2,1} = -68$ (1)°, and $\chi^{3,2,1} = 160$ (1)°.

Introduction. The novel amino acid γ -carboxyglutamic acid occurs in the *N*-terminal portion of the blood-clotting enzymes by a post-ribosomal, vitamin-K mediated carboxylation of glutamic acid residues (Suttie & Jackson, 1977; Stenflo, 1977). These residues are responsible for the calcium-facilitated binding of the blood-clotting proteins to phospholipid surfaces, subsequently allowing conversion from zymogen to active enzyme in a cascade series of reactions.

Colorless, slightly translucent, thin, needle-shaped crystals were grown from a water:ethanol solution of the monoammonium salt of γ -carboxyglutamic acid at pH 2.8. A crystal of dimensions $0.05 \times 0.05 \times 0.10$ mm was cut and used in the analysis. Preliminary photographs revealed systematic absences $0kl$, $k + l = 2n + 1$ and $h0l$, $h = 2n + 1$. These absences are consistent with the noncentrosymmetric space group $Pna2_1$ ($Z = 4$) and the centrosymmetric space group $Pnma$ ($Z = 8$). The density measured by flotation in a chloroform–bromoform mixture was 1.60 Mg m⁻³, corresponding to four molecules in the unit cell and favoring the space group $Pna2_1$, since the molecule does not have a mirror plane of symmetry. The refinement confirmed this assignment of the space group. The cell constants were determined from a least-squares analysis of 15 medium-angle reflections measured on the Picker FACS-I diffractometer.

Intensity data were collected on this diffractometer using Ni-filtered Cu $K\alpha$ radiation and a θ - 2θ scan mode. A total of 1426 reflections of the type hkl , $h\bar{k}l$

with $2\theta \leq 128^\circ$ were scanned. The standard deviation in the intensities $\sigma(I)$ was computed using counting statistics and an electronic instability factor of 0.02 (Stout & Jensen, 1968). After correction for Lorentz and polarization effects, the equivalent reflections were averaged into a data set of 747 independent reflections. The agreement between the F 's of the equivalent reflections ($= \sum |F_i - \bar{F}_i| / \sum \bar{F}_i$) was 0.03. A total of 483 reflections were considered observed based on the criterion $F > 3.0\sigma(F)$. No absorption corrections were applied.

The structure was solved by direct methods with the program *MULTAN* (Main, Germain & Woolfson, 1970) using 180 reflections with E values greater than 1.1. An E map calculated for the best solution revealed 11 of the 14 non-hydrogen atoms in the structure. An electron density map calculated using the phases from these 11 atoms indicated that the four O atoms belonging to the C_α and one of the C_γ carboxyl groups were disordered. Several cycles of full-matrix least-squares refinement followed by anisotropic refinement confirmed the presence of disordered O atoms. The occupancy of the disordered O atoms was found to be approximately 2:1. A difference electron density map indicated the possible positions of some of the H atoms and the rest were geometrically fixed. The H atoms were given the isotropic thermal parameters of the heavy atoms to which they were bonded, and were not refined.

The following weighting scheme was used: $w = 1/\sigma^2$, $\sigma(F_o) = 0.0$ for $F_o < 13$, $\sigma(F_o) = 5.33 - 0.7368F_o$ for $13 \leq F_o < 29$, $\sigma(F_o) = 0.4 + 0.0973F_o$ for $29 \leq F_o \leq 52.5$, and $\sigma(F_o) = 4.5 + 0.01882F_o$ for $F_o > 52.5$. Two reflections considered to be suffering from secondary extinction were removed and further refinements on the non-hydrogen atoms (isotropic thermal parameters for the disordered O atoms) were performed to convergence. The scattering-factor tables were from Cromer & Waber (1965) for the non-hydrogen atoms, and from Stewart, Davidson & Simpson (1965) for the H atoms. The average shift to sigma ratio in the final cycle was 0.13 and the maximum value was 0.44. The final conventional R value was 0.066 for the 481 observed reflections and 0.119 for the entire data set.

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Table 1. *Positional and thermal parameters of atoms in the monoammonium salt of DL- γ -carboxyglutamic acid*

Positional parameters for non-hydrogen atoms are $\times 10^4$ and for H atoms $\times 10^3$. Anisotropic thermal parameters are $\times 10^4$. Atom designation is in accordance with the IUPAC-IUB Commission on Biochemical Nomenclature (1970). E.s.d.'s are given in parentheses and refer to the least significant digit. The anisotropic temperature factors are of the form $\exp[-(\beta_{11}h^2 + \dots + \beta_{12}hk + \dots)]$.

| | <i>x</i> | <i>y</i> | <i>z</i> | β_{11} | β_{22} | β_{33} | β_{12} | β_{13} | β_{23} |
|---------------------------------------------|-----------|-----------|-----------|--------------|--------------|--------------|--------------|--------------|--------------|
| O(3)—O _{ϵ} 2,1 | -297 (5) | 1927 (4) | 3687 (-) | 41 (4) | 35 (3) | 454 (17) | 23 (7) | -19 (21) | -4 (18) |
| O(4)—O _{ϵ} 2,2 | -1267 (4) | 203 (4) | 3634 (12) | 18 (4) | 47 (3) | 328 (13) | 9 (6) | 46 (18) | 27 (18) |
| N(1) | 2079 (6) | -2962 (4) | 3730 (15) | 42 (5) | 25 (3) | 361 (18) | -18 (8) | 16 (21) | -52 (20) |
| N(2) | 984 (6) | 4121 (4) | 3510 (16) | 47 (5) | 35 (3) | 376 (20) | -2 (8) | 20 (22) | -24 (18) |
| C(1) | 3664 (7) | -1357 (6) | 3408 (21) | 50 (6) | 20 (4) | 530 (31) | -11 (9) | 103 (29) | -22 (26) |
| C(2)—C _{α} | 2178 (7) | -1743 (5) | 3177 (21) | 30 (6) | 17 (4) | 690 (45) | 14 (10) | 112 (30) | -22 (25) |
| C(3)—C _{β} | 1218 (7) | -959 (6) | 3934 (18) | 52 (7) | 33 (4) | 396 (28) | 15 (9) | 20 (29) | -61 (19) |
| C(4)—C _{γ} | 1136 (6) | 283 (5) | 3403 (18) | 27 (5) | 29 (4) | 379 (23) | 13 (8) | 34 (23) | 43 (22) |
| C(5)—C _{δ} 2 | -254 (5) | 844 (5) | 3620 (13) | 34 (5) | 39 (4) | 135 (12) | -3 (8) | 3 (20) | 24 (16) |
| C(6)—C _{δ} 1 | 2261 (6) | 1071 (4) | 3843 (15) | 40 (5) | 21 (4) | 287 (19) | -2 (9) | -11 (24) | 7 (18) |

Table 1 (cont.)

| | <i>x</i> | <i>y</i> | <i>z</i> | <i>B</i> (Å ²) |
|----------------------------------------------|-----------|------------|-----------|----------------------------|
| O(1)* | 4030 (9) | -634 (7) | 2109 (12) | 2.7 (13) |
| O(2)* | 4361 (8) | -1753 (6) | 4472 (11) | 2.45 (13) |
| O(1)' | 4302 (19) | -1713 (15) | 2852 (22) | 4.12 (36) |
| O(2)' | 4151 (26) | -593 (2) | 4957 (36) | 6.84 (54) |
| O(5)*—O _{ϵ} 1,1 | 2612 (7) | 1901 (5) | 2773 (11) | 1.45 (11) |
| O(6)*—O _{ϵ} 1,2 | 2781 (8) | 964 (6) | 5360 (11) | 2.12 (13) |
| O(5)' | 2736 (25) | 1053 (18) | 1989 (31) | 7.33 (53) |
| O(6)' | 2638 (20) | 1881 (14) | 4251 (27) | 5.67 (40) |
| H(1) | 250 | -292 | 467 | 2.9 |
| H(2)† | 253 | -353 | 263 | 2.9 |
| H(3)† | 111 | -319 | 360 | 2.9 |
| H(4) | 250 | -217 | 175 | 3.6 |
| H(5) | 158 | -83 | 517 | 3.8 |
| H(6) | -8 | -125 | 325 | 3.8 |
| H(7) | 108 | 25 | 167 | 2.6 |
| H(8) | 342 | 233 | 317 | 2.2 |
| H(9) | 175 | 458 | 333 | 3.8 |
| H(10) | 100 | 342 | 333 | 3.8 |
| H(11)† | 26 | 437 | 407 | 3.8 |
| H(12)† | 59 | 421 | 199 | 3.8 |

† Hydrogen atoms with positions geometrically fixed.

The positional and thermal parameters for the atoms are given in Table 1.* Among the disordered O atoms, the starred O atoms are of higher occupancy while the primed O atoms are of lower occupancy. The atom-numbering scheme is presented in an ORTEP plot (Fig. 1) (Johnson, 1965).

Discussion. The C _{α} carboxyl group [O(1)—C(1)—O(2)] and one of the two C _{γ} carboxyl groups [O(3)—C(5)—O(4)] are ionized while the amino group is protonated. The net charge of the molecule is balanced by the positively charged ammonium ion.

A prominent feature of this structure is the presence of disorder in the molecule. The C _{α} carboxyl group and

* A list of structure factors has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 34195 (5 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

the neutral C _{γ} carboxyl group are in positions of unequal occupancy on either side of the molecular skeleton formed by the backbone atoms N, C _{α} , C _{β} , C _{γ} , C _{δ} 2, O _{ϵ} 2,1, O _{ϵ} 2,2. This disorder can be roughly visualized as two different rotations of the carboxyl groups around the C(2)—C(1) and the C(4)—C(6) bonds respectively. A molecule in the crystal lattice can contain a C _{α} carboxyl group and a neutral C _{γ} carboxyl group with either a conformation described by the type 1 (with *) O atoms or a conformation described by the type 2 (primed) O atoms. If a molecule contains a mixture of the two types of O atoms, the O(1)*—O(5)' distance is 2.40 Å and O(6)*—O(2)' is 2.19 Å. These contacts are considerably shorter than the allowed van der Waals distances and therefore a molecule of mixed type cannot exist in the lattice.

In addition to the disorder of the carboxyl groups, the thermal ellipsoids of all atoms of the molecule have a considerable elongation along a direction approximately normal to the mean plane of the backbone atoms.

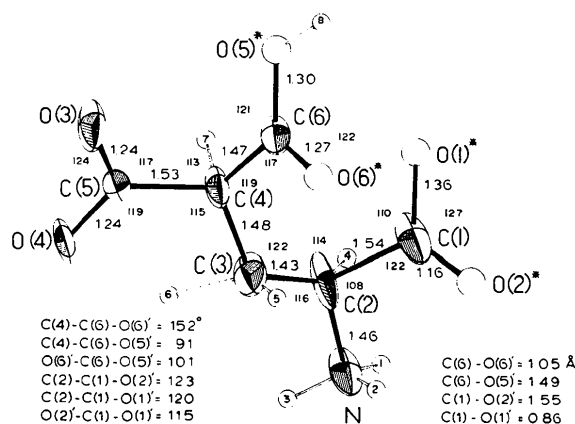


Fig. 1. An ORTEP plot of the molecule showing the atom-numbering scheme, the bond lengths (Å), and the bond angles (°). The average standard deviation of the bond lengths is 0.01 Å and that of the bond angles is 1°. The disordered O atoms with higher occupancy are indicated by asterisks. The anisotropic ellipsoids are drawn at the 50% probability level.

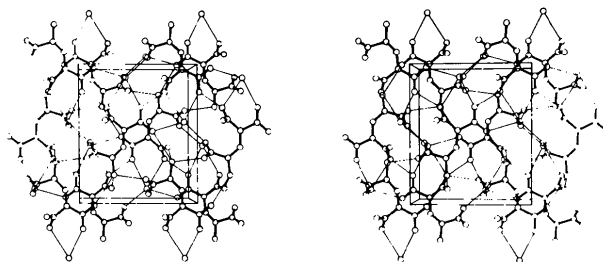


Fig. 2. Stereopacking diagram viewed down the c axis.

The bond lengths and angles are shown in Fig. 1. The effect of disorder appears to result in a general shortening of the bond lengths from the normal values and an opening of the tetrahedral angles.

The arrangement of substituents around the $C_\alpha-C_\beta$ and $C_\beta-C_\gamma$ bonds is staggered. The N atom is *trans* to C_γ and the C_α atom is *trans* to C_δ [$\chi^1 = -176(1)^\circ$ and $\chi^{2,2} = 153(1)^\circ$]. The $\chi^{3,2,2}$ angle is $-24(1)^\circ$, placing $O_\epsilon 2,1$ in a closely eclipsed position to C_β . Thus, the backbone of the molecule is in an extended conformation similar to that found in the structure of the free acid of γ -carboxyglutamic acid but differs from the conformation found in the structure of the calcium salt by rotation about $C_\beta-C_\gamma$ (Satyshur, 1978). The normal to the molecular plane formed by the seven atoms N, C_α , C_β , C_γ , $C_\delta 2$, $O_\epsilon 2,1$, and $O_\epsilon 2,2$ is nearly parallel to the c axis. The remaining six atoms belong to the disordered carboxyl groups and have the following torsional angles: ψ^{1*} [$N(1)-C(2)-C(1)-O(2)^*$] = $-30(1)^\circ$, $\psi^{1'}$ [$N(1)-C(2)-C(1)-O(1)'$] = $60(1)^\circ$, $\chi^{3,1,1*}$ [$C(3)-C(4)-C(6)-O(5)'$] = $144(1)^\circ$, and $\chi^{3,1,1'}$ [$C(3)-C(4)-C(6)-O(5)'$] = $104(1)^\circ$. The mean molecular plane formed by the seven backbone atoms has an r.m.s. deviation of 0.156 \AA .

Fig. 2 illustrates the general features of the packing of γ -carboxyglutamic acid in the unit cell. The

molecules lie on planes that are almost perpendicular to the c axis. Each layer of γ -carboxyglutamic acid molecules interacts with the layer above or below through hydrogen bonds between the type starred or the type primed O atoms and the N atoms. These hydrogen bonds form a spiral around each of the twofold screw axes.

The ammonium ion forms five hydrogen bonds (Table 2) which include one bifurcated bond between H(10) and the two acceptor atoms O(3) and O(5)*. This H atom is in the plane of the donor and acceptor atoms (displacement = 0.07 \AA), and is characteristic of bifurcated hydrogen bonds (Koetzle, Hamilton & Parthasarathy, 1972).

The amino group interacts with five neighboring atoms. They are: O(4) of the charged C_γ carboxyl group, O(2)* [or O(1)'] of the C_α carboxyl group, and a pair of atoms from the neutral C_γ carboxyl group O(5)-C(6)-O(6). The H(1) atom is donated to O(5)* or O(5)' of the molecule *b*. The atom H(2) is involved in what appears to be a bifurcated hydrogen bond with atoms O(4) and O(6)* of different molecules. The deviation from the plane of the atoms N(1), O(4) and O(6)* is 0.80 \AA . The third atom H(3) is donated to O(2)* [or O(1)'] of a fourth molecule.

The hydrogen-bonding scheme is completed with the formation of a short hydrogen bond from O(5)* [or O(6)'] to O(3) of 2.56 \AA (2.49 \AA). This distance is shorter than the commonly found $O \cdots O$ distance in $O-H \cdots O$ type hydrogen bonds of 2.7 \AA (Hamilton & Ibers, 1968). The H(8) atom in the same position forms a hydrogen bond to either O(5)* or O(6)'.

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Table 2. *Hydrogen-bonding distances (Å) and angles (°)*

The e.s.d.'s in the distances are $0.01-0.02 \text{ \AA}$.

| $A-H \cdots B$ | Symmetry code | $A \cdots B$ | $A-H \cdots B$ | Symmetry code | $A \cdots B$ |
|-----------------------------------|------------------------------|--------------|---------------------------|------------------------------|--------------|
| N(1)-H(1) \cdots O(5)' | <i>b</i> | 2.73 | N(2)-H(9) \cdots O(4) | <i>f</i> | 2.83 |
| N(1)-H(1) \cdots O(5)* | <i>b</i> | 3.10 | N(2)-H(10) \cdots O(3) | <i>a</i> | 2.82 |
| N(1)-H(2) \cdots O(4) | <i>c</i> | 3.04 | N(2)-H(10) \cdots O(5)* | <i>a</i> | 3.06 |
| N(1)-H(2) \cdots O(6)* | <i>d</i> | 2.85 | N(2)-H(11) \cdots O(2)' | <i>g</i> | 2.71 |
| N(1)-H(3) \cdots O(2)* | <i>e</i> | 2.76 | N(2)-H(11) \cdots O(1)* | <i>h</i> | 2.76 |
| N(1)-H(3) \cdots O(1)' | <i>e</i> | 2.85 | N(2)-H(12) \cdots O(1)* | <i>g</i> | 2.80 |
| O(5)*-H(8) \cdots O(3) | <i>f</i> | 2.56 | N(2)-H(12) \cdots O(2)' | <i>i</i> | 2.73 |
| O(6)' \cdots H(8) \cdots O(3) | <i>f</i> | 2.49 | | | |
| Symmetry code | | | | | |
| (a) | x, y, z | | (f) | $0.5 + x, 0.5 - y, z$ | |
| (b) | $0.5 - x, -0.5 + y, 0.5 + z$ | | (g) | $-0.5 + x, 0.5 - y, z$ | |
| (c) | $0.5 + x, -0.5 - y, z$ | | (h) | $0.5 - x, 0.5 + y, 0.5 + z$ | |
| (d) | $0.5 - x, -0.5 + y, z$ | | (i) | $0.5 - x, 0.5 + y, -0.5 + z$ | |
| (e) | $-0.5 + x, -0.5 - y, z$ | | | | |

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Triflocine: Acide [*m*-(Trifluorométhyl)phénylamino]-4 Nicotinique

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Abstract. C₁₃H₉F₃N₂O₂, monoclinic, $P2_1/n$, $a = 11.110$ (6), $b = 13.964$ (3), $c = 9.400$ (3) Å, $\beta = 125.39$ (4)°, $V = 1188.98$ Å³, $Z = 4$, $D_c = 1.576$ Mg m⁻³, $\mu(\text{Cu } K\alpha) = 1.248$ mm⁻¹. A least-squares refinement of 1780 reflections collected on a Hilger & Watts diffractometer yielded a final R of 0.064. The crystal structure consists of infinite chains of molecules, held together by hydrogen bonds between carboxyl groups and pyridyl rings.

Introduction. Nous avons déterminé la structure cristalline de la triflocine (Fig. 1), dans le cadre de nos recherches sur le mode d'action des diurétiques. Les cristaux ont été obtenus par évaporation lente d'une solution dans l'éthanol. Les données, 2015 réflexions mesurées sur un diffractomètre Hilger & Watts à quatre cercles, dont 1838 sont considérées comme observées, ont été corrigées des facteurs de Lorentz et de polarisation, mais pas de l'absorption. Le programme *MULTAN* (Germain, Main & Woolfson, 1971) a donné huit solutions. La carte des E_h relative à la meilleure figure de mérite combinée a révélé l'ensemble des atomes non-hydrogène. Les positions des atomes H ont été calculées. La structure a été affinée par moindres carrés, à l'aide des programmes de Ahmed, Hall, Pippy & Huber (1966). Le schéma de pondération est celui de Cruickshank (1961). Le

Tableau 1. Coordonnées atomiques ($\times 10^4$; pour les H $\times 10^3$)

| | <i>x</i> | <i>y</i> | <i>z</i> |
|-------|-----------|-----------|-----------|
| N(1) | 9502 (2) | 9298 (1) | 1951 (2) |
| N(2) | 12002 (2) | 11594 (1) | 2373 (2) |
| O(8) | 11715 (2) | 8221 (1) | 2673 (2) |
| O(9) | 13849 (2) | 8921 (1) | 3604 (2) |
| F(1) | 4297 (2) | 7915 (1) | -2041 (2) |
| F(2) | 5229 (2) | 6971 (1) | 112 (2) |
| F(3) | 3490 (2) | 7906 (1) | -452 (2) |
| C(1) | 10301 (2) | 10053 (1) | 2076 (2) |
| C(2) | 9725 (2) | 10991 (1) | 1650 (3) |
| C(3) | 10587 (2) | 11732 (1) | 1786 (2) |
| C(4) | 12593 (2) | 10707 (1) | 2770 (2) |
| C(5) | 11785 (2) | 9917 (1) | 2602 (2) |
| C(7) | 12535 (2) | 8943 (1) | 3015 (2) |
| C(10) | 8179 (2) | 9321 (1) | 1834 (2) |
| C(11) | 7083 (2) | 8651 (1) | 756 (2) |
| C(12) | 5828 (2) | 8596 (1) | 746 (2) |
| C(13) | 5626 (2) | 9193 (1) | 1772 (3) |
| C(14) | 6726 (2) | 9861 (1) | 2834 (2) |
| C(15) | 7987 (2) | 9925 (1) | 2872 (2) |
| C(16) | 4709 (2) | 7862 (1) | -406 (3) |
| H(N1) | 992 | 859 | 194 |
| H(2) | 858 | 1112 | 118 |
| H(3) | 1009 | 1246 | 140 |
| H(4) | 1375 | 1062 | 326 |
| H(O8) | 1223 | 757 | 265 |
| H(11) | 721 | 819 | -9 |
| H(13) | 464 | 912 | 177 |
| H(14) | 659 | 1034 | 366 |
| H(15) | 885 | 1045 | 371 |