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The Structure of L-Tyrosyl-L-prolyl-L-asparaginyl-L-glycine, the 23–26 Fragment of Human ACTH

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Abstract

The synthetic tetrapeptide L-tyrosyl-L-prolyl-L-asparaginyl-L-glycine, $C_{20}H_{27}N_5O_7$, $M_r = 449{\cdot}5$, the 23–26 fragment of human ACTH, crystallized by free diffusion between a concentrated peptide solution in methanol–water and chloroform. The crystal is orthorhombic, $P2_12_12_1$, with $a = 8{\cdot}896$ (2), $b = 12{\cdot}858$ (3), $c = 18{\cdot}146$ (4) Å, $Z = 4$, $D_x = 1{\cdot}44$ Mg m^{-3}. Data were collected on a Nonius CAD-4 diffractometer and the structure was solved by direct methods. The final R value was 0·033 for 1240 independent reflexions. The molecule exists in the crystal as a zwitterion and the rather high density (1·44 Mg m^{-3}) can be explained by extensive intermolecular hydrogen bonding. There is no intramolecular hydrogen bond and the peptide chain is in an extended configuration.

Introduction

Adrenocorticotropic hormone (ACTH), a linear polypeptide (39 residues), is one of the pituitary hormones. Among a variety of biological properties, the stimulation and regulation of steroidogenesis (Ramachandran, 1973) and the formation and maintenance of learned behaviour (De Wied, Witter & Greven, 1975; De Wied, 1977) are certainly the most extensively investigated in terms of structure–activity relationships.

Among the numerous types of investigations, several results from prediction methods (Chou & Fasman, 1977; Burgess & Scheraga, 1975; Garnier, Osguthorpe & Robson, 1978), proton NMR studies (Toma, Fermandjian, Löw & Kisfaludy, 1978) or circular dichroism studies (Jibson & Li, 1979) indicate a most probable conformation (β turn) for the 23–26 fragment of human ACTH. The present paper reports the X-ray study of this important peptide situated between the N-terminal part, essential for the biological effects, and the C-terminal part, which probably only increases the stability of the hormone.

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Experimental

Synthesis and purification

The title compound, Tyr-Pro-Asn-Gly, was synthesized according to the Merrifield solid-phase procedure with automatic equipment monitored by a microprocessor. After gel filtration in biogel P2 the peptide was purified using high-pressure liquid chromatography, with a C_{18} reversed phase.

Crystallization

Among the several trials using different solvents or techniques only one gave crystals sufficiently large for X-ray work. This method uses a concentration by slow evaporation and free-liquid diffusion. A saturated peptide solution in methanol–water (1:1) was slowly set in a beaker of approximately the same volume of chloroform. The top was sealed with Parafilm pierced with a few holes in order to allow slow evaporation. The mixture having been allowed to stand for a week at room temperature, one colourless crystal appeared at the liquid interface (0·40 × 0·15 × 0·03 mm) and was used for data collection.

Crystal data and determination of the structure

X-ray intensity data were measured on a Nonius CAD-4 automatic diffractometer using monochromated Cu $K\alpha$ radiation within the Cu sphere of $\theta \leq 60°$. The two reference reflexions monitored every 50 reflexions did not show a significant variation in intensity over the data-collection period. The intensity data were corrected for Lorentz–polarization effects. No absorption correction was applied. Of the 1774 observed reflexions, 1240 independent reflexions with $I > 3\sigma(I)$ were selected and used in the structure analysis.

The cell parameters (see *Abstract*) were determined by a least-squares fit of 22 reflexions.

The structure was solved by direct methods using the *MULTAN* system of computer programs (Main, Woolfson, Lessinger, Germain & Declercq, 1978). *E*

Table 1. *Final atomic parameters* ($\times 10^4$; $\times 10^3$ *for hydrogen atoms*)

$$B_{eq} = \tfrac{4}{3} \sum_i \sum_j \beta_{ij} \mathbf{a}_i . \mathbf{a}_j .$$

	x	y	z	B_{eq} (Å^2)		x	y	z	B_i (Å^2)
Tyrosyl									
N	7656 (5)	−2828 (3)	0538 (3)	2·5 (2)	H1(N)	682 (8)	−301 (5)	025 (4)	7·2 (8)
C^{α}	7141 (6)	−2344 (5)	1252 (3)	2·6 (3)	H2(N)	828 (8)	−228 (5)	029 (3)	6·5 (6)
C′	5878 (7)	−1589 (4)	1051 (3)	2·3 (2)	H3(N)	827 (7)	−335 (5)	069 (3)	5·1 (5)
O	5699 (5)	−1295 (3)	0417 (2)	2·8 (3)	H(C^{α})	677 (6)	−291 (4)	156 (3)	2·6 (3)
C^{β}	8451 (7)	−1765 (5)	1634 (3)	2·8 (3)	H1(C^{β})	806 (6)	−146 (4)	212 (3)	3·6 (4)
C^{γ}	9716 (6)	−2481 (4)	1864 (3)	2·4 (2)	H2(C^{β})	898 (6)	−119 (4)	127 (3)	3·4 (3)
$C^{\delta 1}$	9699 (7)	−2989 (5)	2541 (3)	2·9 (3)	H($C^{\delta 1}$)	884 (8)	−287 (5)	287 (4)	7·2 (7)
$C^{\delta 2}$	10928 (7)	−2612 (5)	1398 (3)	2·9 (3)	H($C^{\delta 2}$)	1095 (7)	−229 (4)	089 (3)	4·0 (4)
$C^{\varepsilon 1}$	10885 (8)	−3642 (5)	2740 (3)	3·3 (3)	H($C^{\varepsilon 1}$)	1088 (6)	−400 (4)	321 (3)	2·9 (3)
$C^{\varepsilon 2}$	12117 (7)	−3273 (5)	1591 (3)	3·0 (3)	H($C^{\varepsilon 2}$)	1296 (6)	−338 (4)	126 (3)	2·8 (3)
C^{ζ}	12086 (7)	−3763 (4)	2270 (3)	3·0 (3)	H(O^{η})	1352 (9)	−451 (6)	294 (4)	9·1 (9)
O^{η}	13269 (6)	−4410 (4)	2431 (3)	4·7 (5)					
Prolyl									
N	5039 (5)	−1244 (3)	1613 (2)	2·2 (2)	H(C^{α})	313 (6)	−073 (4)	118 (3)	3·0 (3)
C^{α}	3866 (6)	−0464 (4)	1472 (3)	2·1 (2)	H1(C^{β})	214 (7)	−000 (5)	218 (3)	4·7 (5)
C′	4507 (6)	0472 (4)	1071 (3)	2·4 (2)	H2(C^{β})	389 (7)	050 (5)	245 (3)	5·5 (5)
O	5753 (5)	0849 (3)	1215 (2)	3·5 (3)	H1(C^{γ})	274 (5)	−165 (4)	264 (3)	2·6 (3)
C^{β}	3264 (7)	−0188 (5)	2243 (3)	3·2 (3)	H2(C^{γ})	361 (5)	−096 (4)	322 (2)	2·2 (2)
C^{γ}	3587 (7)	−1165 (5)	2696 (3)	3·3 (3)	H1(C^{δ})	502 (6)	−239 (4)	243 (3)	3·0 (3)
C^{δ}	5013 (7)	−1616 (5)	2385 (3)	3·1 (3)	H2(C^{δ})	602 (9)	−147 (6)	277 (4)	9·0 (9)
Asparaginyl									
N	3599 (5)	0873 (3)	0562 (2)	2·2 (2)	H(N)	264 (7)	055 (4)	045 (3)	6·0 (5)
C^{α}	4053 (6)	1750 (4)	0111 (3)	2·3 (2)	H(C^{α})	522 (6)	178 (4)	013 (3)	2·7 (3)
C′	3470 (7)	2784 (4)	0434 (3)	2·8 (3)	H1(C^{β})	230 (6)	162 (4)	−065 (3)	3·1 (3)
O	2380 (5)	2799 (3)	0854 (2)	4·1 (4)	H2(C^{β})	381 (6)	217 (4)	−103 (3)	3·5 (4)
C^{β}	3453 (7)	1625 (4)	−0676 (3)	2·8 (3)	H1($N^{\delta 2}$)	563 (9)	−007 (6)	−151 (4)	8·2 (8)
C^{γ}	3923 (7)	0584 (4)	−0997 (3)	3·0 (3)	H2($N^{\delta 2}$)	607 (9)	106 (6)	−133 (4)	7·8 (8)
$N^{\delta 2}$	5309 (6)	0544 (4)	−1260 (3)	4·4 (4)					
$O^{\delta 1}$	3059 (5)	−0159 (3)	−1001 (3)	4·0 (4)					
Glycine									
N	4202 (6)	3612 (4)	0198 (3)	3·1 (3)	H(N)	501 (7)	352 (5)	−022 (3)	4·7 (5)
C^{α}	3773 (7)	4688 (4)	0388 (4)	3·2 (3)	H1(C^{α})	264 (8)	486 (5)	015 (3)	5·3 (5)
C′	4874 (7)	5437 (5)	0015 (3)	2·6 (3)	H2(C^{α})	382 (6)	473 (4)	094 (3)	2·9 (4)
O_1	4737 (5)	6380 (3)	0197 (2)	2·9 (3)					
O_2	5743 (5)	5079 (3)	−0456 (3)	3·9 (3)					

values were calculated with the assumption of randomly distributed known groups of atoms in the unit cell. The 227 E_{max} were used to determine the phases, and the best figure of merit, obtained with the help of a magic integer, revealed all heavy atoms of the molecule. The atomic positions were refined by block-diagonal least squares, starting with an isotropic temperature factor of 3·0 Å^2 for all the atoms. H atoms were introduced in theoretical positions or were found on the difference map. The refinement with anisotropic temperature factors for only non-hydrogen atoms and isotropic for H atoms gave a final reliability index R = 0·033. Atomic scattering factors were taken from *International Tables for X-ray Crystallography* (1974) for heavy atoms and from Stewart, Davidson & Simpson (1965) for H. The weighting scheme used was $\sqrt{w} = 1$ if $|F_o| < p$ and $\sqrt{w} = p/F_o$ if $|F_o| > p$ with $p = |F_o^2(\text{max})/10|^{1/2}$. The final atomic coordinates are listed in Table 1.*

Results and discussion

Peptide conformation

Bond lengths and angles in the peptide molecule are shown in Fig. 1. These values agree reasonably well with those found for other peptides from X-ray analysis. The average peptide bond length is 1·33 (1) Å.

* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 36783 (13 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

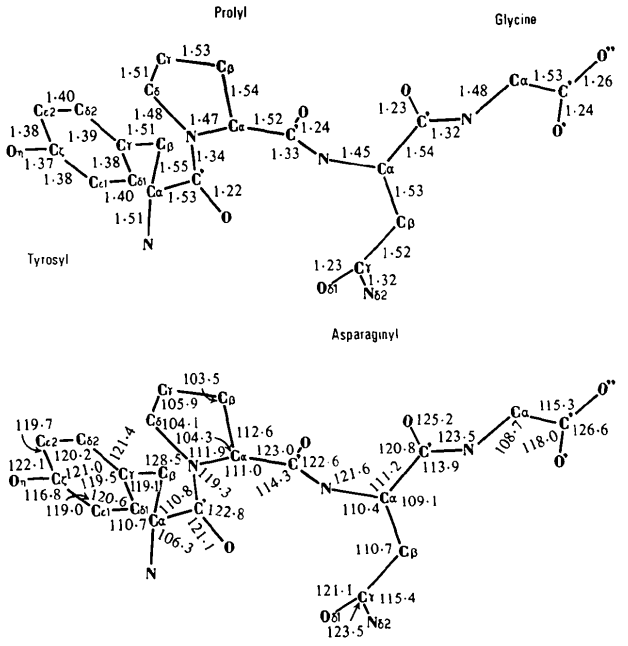

Fig. 1. Bond distances (Å) and bond angles (°). Average standard deviations are 0·01 Å and 0·1° respectively. [C^{α}–C′–N = 116·0, C^{α}–C^{β}–C^{γ} = 113·0°.]

The tyrosyl ring is planar, with all C atoms deviating less than 0·01 Å from their mean plane.

The torsion angles of the molecule given in accordance with the IUPAC–IUB Commission on Biochemical Nomenclature (1970) recommendations are listed in Table 2. All the ω_i values are in the neighbourhood of 180°, which is generally the case in experimentally determined structures. Of the other principal angles φ_i and ψ_i, describing rotations about N–C^{α} and C^{α}–C′, three have values very different from −150 or 150° and the peptide main chain cannot be described as a fully extended conformation. The proline angles [φ = −53·2 (8), ψ = 141·8 (8), ω = 182·0 (8)°] are not far from those of poly(L-proline)II (φ = −78, ψ = 149, ω = 180°) (Arnott & Dover, 1968) or those found for the tetrapeptide L-Pro-L-Tyr-L-Ile-L-Leu (φ = −54, ψ = 169, ω = 170°) (Cotrait, Geoffre, Hospital & Précigoux, 1979). Because of the value φ = −96·2 (8)° for its main chain, the asparaginyl side chain can be folded down towards the O atom of the tyrosyl–prolyl peptide bond. However, the intramolecular distance O(Tyr)–NH_2(Asn) is longer and there is no hydrogen bond, as shown in Fig. 2.

Table 2. *Torsion angles* (°) *in accordance with the IUPAC–IUB Commission on Biochemical Nomenclature* (1970) (σ = 0·8°)

Peptide backbone angles	Side-chain angles
Tyrosyl	
ψ = 166·0	N–C^{α}–C^{β}–C^{γ} = −63·9
ω = 176·0	C^{α}–C^{β}–C^{γ}–$C^{\delta 1}$ = −86·9
Prolyl	
φ = −53·2	N–C^{α}–C^{β}–C^{γ} = −26·2
ψ = 141·8	C^{α}–C^{β}–C^{γ}–C^{δ} = 32·8
ω = 182·0	C^{β}–C^{γ}–C^{δ}–N = −26·2
	C^{γ}–C^{δ}–N–C^{α} = 9·4
	C^{γ}–N–C^{α}–C^{β} = 10·8
Asparaginyl	
φ = −96·2	N–C^{α}–C^{β}–C^{γ} = −54·1
ψ = 160·1	C^{α}–C^{β}–C^{γ}–$N^{\delta 2}$ = −80·7
ω = 175·3	
Glycine	
φ = 180·1	
N–C^{α}–C′–O′ = 187·5	

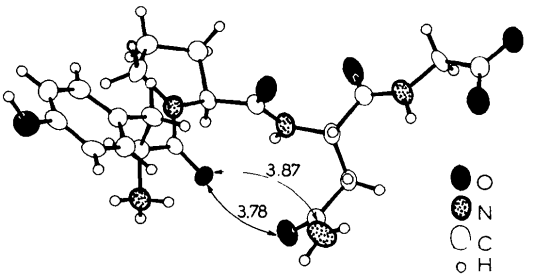

Fig. 2. Intramolecular distances between O(Tyr) and O,N(Asn) side chain (σ = 0·01 Å).

Crystal structure and intermolecular hydrogen bonds

Viewed down the *x* axis (Fig. 3), the molecule is seen to be elongated along the *y* axis. It crystallizes without

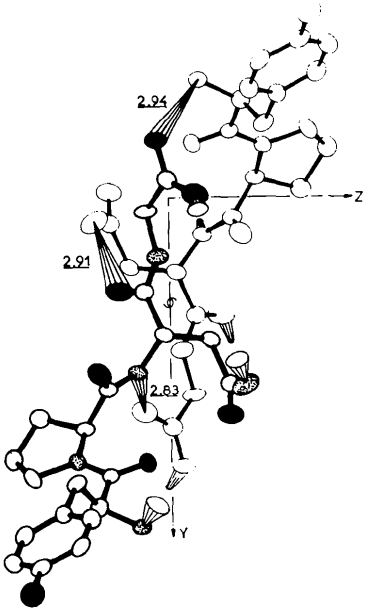

Fig. 3. Projection of the structure along *x* showing all the hydrogen bonds (σ = 0·1 Å).

Table 3. *Hydrogen bonds* (Å) *between a molecule and its neighbours*

O^{η}(Tyr)–O(Pro) $(2-x, \frac{1}{2}-y, \frac{1}{2}-z)$	2·63 (1)
N(Tyr)–$O^{\delta 1}$(Asn) $(\frac{1}{2}+x, -\frac{1}{2}-y, -z)$	2·75 (1)
N(Tyr)–O″(Gly) $(\frac{1}{2}+x, \frac{1}{2}-y, -z)$	2·94 (1)
N(Tyr)–O″(Gly) $(x, y-1, z)$	2·86 (1)
O(Tyr)–O″(Gly) $(x, y-1, z)$	3·14 (1)
O(Pro)–O^{η}(Tyr) $(2-x, \frac{1}{2}+y, \frac{1}{2}-z)$	2·63 (1)
$O^{\delta 1}$(Asn)–N(Tyr) $(\frac{1}{2}+x, -\frac{1}{2}-y, -z)$	2·75 (1)
$N^{\delta 2}$(Asn)–O(Asn) $(\frac{1}{2}+x, \frac{1}{2}-y, -z)$	2·91 (1)
O(Asn)–$N^{\delta 2}$(Asn) $(-\frac{1}{2}+x, \frac{1}{2}-y, -z)$	2·91 (1)
N(Asn)–O′(Gly) $(-\frac{1}{2}+x, \frac{1}{2}-y, -z)$	2·83 (1)
O″(Gly)–N(Tyr) $(x, 1+y, z)$	2·86 (1)
O″(Gly)–N(Tyr) $(-\frac{1}{2}+x, \frac{1}{2}-y, -z)$	2·94 (1)
O″(Gly)–O(Tyr) $(x, 1+y, z)$	3·14 (1)
O′(Gly)–N(Asn) $(\frac{1}{2}+x, \frac{1}{2}-y, -z)$	2·83 (1)

included water molecules. All O atoms of the peptide are involved in hydrogen bonding and these numerous interactions (14 for each molecule), listed in Table 3, explain the high value found for the crystal density ($D_x = 1{\cdot}44$ Mg m^{-3}).

Around the twofold axis parallel to the x direction the crystal packing can be described as made up of infinite chains in which the molecules lie side by side, with hydrogen bonds from all the amino acids (Fig. 4). In the crystal, the tetrapeptide exists as a zwitterion and the COO^- and NH_3^+ terminal groups take part in hydrogen-bond interactions. These bonds connect one chain to another to give sheets perpendicular to the c axis.

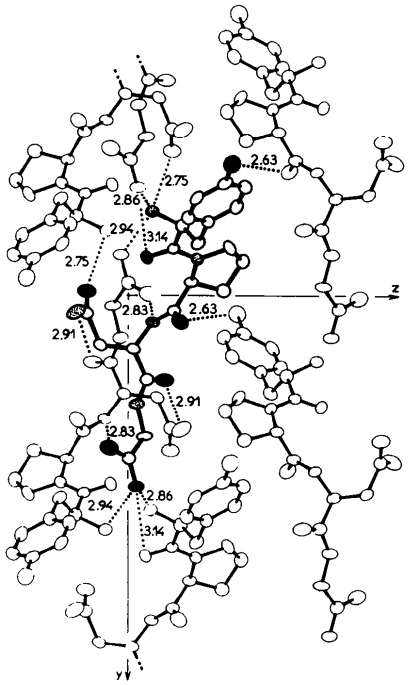

Fig. 4. Projection of the crystal packing in the plane $y0z$. The molecules lie side by side, with hydrogen bonds from all the amino acids ($\bar{\sigma} = 0{\cdot}1$ Å).

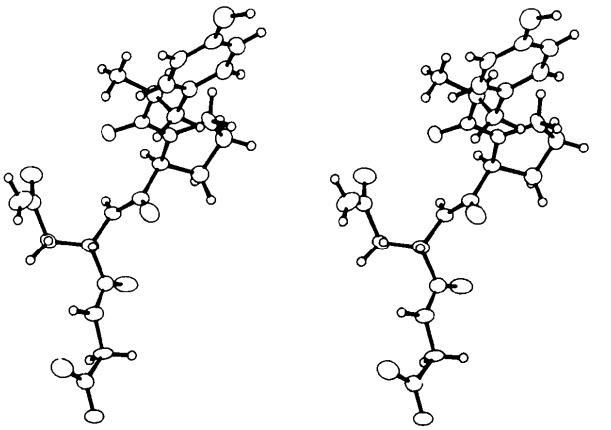

Fig. 5. A stereoview of L-Tyr-L-Pro-L-Asn-L-Gly. Thermal ellipsoids of 50% probability are shown.

The surfaces of the sheets so formed are covered by hydrophobic and hydrophilic groups and the stacking in the **c** direction is the result of both van der Waals forces and hydrogen bonds between OH of the Tyr side chain and O of Pro [2·63 (1) Å]. The shorter contacts between the molecules are between Pro and Asn residues of two different sheets: C^{γ}(Pro)–C^{β}(Asn) = 3·52 (1), C^{γ}(Pro)–C^{γ}(Asn) = 3·34 (1) Å. A stereoview of the molecular conformation is shown in Fig. 5.

Conclusion

Most physicochemical studies or conformational predictions lead to a β-turn conformation for the 23–26 fragment of ACTH; however, a few of the studies are in favour of a random structure (Mutter, Mutter & Bayer, 1979). In the crystal, the observed conformation does not show any turn and can be described as extended. Nevertheless the remarkable $\varphi = -53°$ value must be noted, which is in the prolyl range and is often encountered for the β-turn conformation (Karle, 1978, 1979; Kostansek, Thiessen, Schomburg & Lipscomb, 1979; Cook, Einspahr, Trapane, Urry & Bugg, 1980).

In the present case, it is very difficult to correlate our X-ray crystallographic results with the conformation proposed for the peptide in solution or the most probable structure obtained from conformational predictions.

On the one hand, in the crystal, the tetrapeptide exists as a zwitterion forming many intermolecular hydrogen bonds. On the other, it is included in a larger sequence in which the conformation is independent of the end charge effects. To try to simulate Tyr-Pro-Asn-Gly in a larger peptide chain, the protected analogs Acetyl-Tyr-Pro-Asn-Gly and Tyr-Pro-Asn-Gly-*O*-methyl were synthesized. The crystallization assays are currently in progress.

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Structures of the Nitrosamides: (I) *N*-Methyl-*N*-nitrosourea, (II) *N*,*N'*-Dimethyl-*N*-nitrosourea, (III) 2-Nitroso-2-azabicyclo[2.2.2]octan-3-one and (IV) *N*-Methyl-*N*-nitroso-*p*-nitrobenzamide

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Abstract

The crystal and molecular structures of the title compounds have been determined by X-ray methods. (I) $C_2H_5N_3O_2$, $M_r = 103{\cdot}08$, is monoclinic, with $a = 5{\cdot}302\,(1)$, $b = 5{\cdot}617\,(1)$, $c = 15{\cdot}442\,(2)$ Å, $\beta = 90{\cdot}09\,(1)°$, $U = 459{\cdot}9$ Å^3, $Z = 4$, $D_c = 1{\cdot}49$ Mg m^{-3}, space group $P2_1/n$; 725 reflections, $R_w = 0{\cdot}057$; (II) $C_3H_7N_3O_2$, $M_r = 117{\cdot}11$, is monoclinic, with $a = 8{\cdot}412\,(1)$, $b = 9{\cdot}953\,(1)$, $c = 7{\cdot}435\,(1)$ Å, $\beta = 115{\cdot}09\,(1)°$, $U = 563{\cdot}8$ Å^3, $Z = 4$, $D_c = 1{\cdot}37$ Mg m^{-3}, space group $P2_1/a$; 809 reflections, $R_w = 0{\cdot}057$; (III) $C_7H_{10}N_2O_2$, $M_r = 154{\cdot}17$, is orthorhombic, with $a = 15{\cdot}634\,(2)$, $b = 6{\cdot}966\,(2)$, $c = 6{\cdot}698\,(6)$ Å, $U = 729{\cdot}5$ Å^3, $Z = 4$, $D_c = 1{\cdot}40$ Mg m^{-3}, space group *Pnam*; 540 reflections, $R_w = 0{\cdot}064$; (IV) $C_8H_7N_3O_4$, $M_r = 209{\cdot}16$, is triclinic, with $a = 7{\cdot}116\,(3)$, $b = 8{\cdot}414\,(2)$, $c = 8{\cdot}462\,(4)$ Å, $\alpha = 103{\cdot}24\,(3)$, $\beta = 103{\cdot}11\,(6)$, $\gamma = 97{\cdot}98\,(4)°$, $U = 470{\cdot}5$ Å^3, $Z = 2$, $D_c = 1{\cdot}48$ Mg m^{-3}, space group $P\bar{1}$; 679 reflections, $R_w = 0{\cdot}052$. The nitrosamide residue R–C(O)–N(NO)–R' is best regarded as a planar group in all four compounds. Cohesion of the crystal structures is effected by hydrogen-bonding networks in (I) and (II) and van der Waals packing forces in (III) and (IV).

Introduction

N-Nitroso compounds are an important group of chemical carcinogens which may represent a con-

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