

## Crystal structure of hirudonine sulphate – a rare polyamine<sup>†</sup>

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**Abstract.** Hirudonine sulphate ( $C_9H_{23}N_7 \cdot 1.5 H_2SO_4 \cdot 2.5 H_2O$ ) is triclinic in  $P1$  space group with cell constants  $a = 7.168(9)$ ,  $b = 14.534(6)$ ,  $c = 11.918(5) \text{ \AA}$ ,  $\alpha = 110.50(3)$ ,  $\beta = 108.75(6)$  and  $\gamma = 79.16(6)^\circ$ ,  $V = 1097(2) \text{ \AA}^3$ ,  $M_r = 421.4$ ,  $Z = 2$ ,  $d_x = 1.358(2) \text{ g cm}^{-3}$ ,  $d_o = 1.276 \text{ g cm}^{-3}$ .  $MoK_\alpha$  ( $\lambda = 0.7903 \text{ \AA}$ ),  $\mu = 1.94 \text{ cm}^{-1}$ ,  $F(000) = 436$ ,  $T = 295 \text{ K}$ ,  $R(F) = 0.144$ . The structure was solved by direct methods and refined to a final R factor of 0.144 for 1036 unique reflections. One of the sulphur atoms is in special position and is disordered. The amine molecule is hydrogen-bonded to the sulphate oxygen through water molecules. Water channels are formed at unique places involving water oxygens, amine and sulphate oxygens along the  $a$  axis.

**Keywords.** Polyamines; hirudonine sulphate; water channels.

### 1. Introduction

Polyamines are low molecular weight, aliphatic nitrogenous bases. Polyamines produced from amino acids by bacteria have pharmacological activity in animals (Fruton and Simmonds 1965). They are present in all cells in large amounts and appear to possess the potentiality of substituting for cations such as  $K^+$  or  $Mg^{++}$  that may be in short supply. They are highly regulated and are essential for cell growth and differentiation (Scalabrino and Ferioli 1981, 1982). Polyamines stabilize DNA by bridging the polynucleotide strands of the double helix through hydrogen bonds with phosphates across the grooves (Tsuboi 1964; Liquori *et al* 1967; Stevens 1967; Suevalsky *et al* 1969; Cohen 1971). It has been reported that the polyamine content of the urine of cancer patients is higher than that of healthy persons (Cohen 1971). Several models for binding of polyamines to DNA have been proposed in the past (Tsuboi 1964; Liquori *et al* 1967; Woo *et al* 1979; Vasantha Pattabhi and Chandrasekar 1982). Hirudonine sulphate is a rare polyamine and in view of its importance, the crystal structure analysis of the compound was undertaken as part of the project on studies on polyamines.

### 2. Experimental

Colourless, thin rectangular, transparent crystals of  $0.1 \times 0.2 \times 0.45 \text{ mm}$  were obtained by slow evaporation from a mixture of water and chloroform.

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Three-dimensional intensity data were collected on a Nonius CAD-4 automatic diffractometer using monochromated  $\text{MoK}\alpha$  radiation. Due to the poor quality of the crystal, out of 2108 unique reflections collected, 1036 reflections were considered as observed with  $I \geq 2\sigma(I)$ . One standard reflection (003) was checked for every 100 reflections. Lattice parameters were refined with 17 reflections for the measured  $2\theta$  values in the range  $3 \leq \theta \leq 27^\circ$ . Max  $h = 6$ , max  $k = 13$ , max  $l = 13$ , min  $h = 0$ , min  $k = -16$ , min  $l = -11$ . The data were corrected for polarisation and Lorentz effects but not for absorption.

The structure was solved in the space group  $P1$  by direct methods using the program MULTAN80 (Main *et al* 1980). It was noticed that the coordinates of the two amine molecules were related by a centre of inversion and hence the origin was shifted to the centre of symmetry, changing the space group to  $P\bar{1}$ . A few more cycles of refinement in  $P\bar{1}$  using the program SHELX76 (Sheldrick 1976) reduced the R index to 0.20. The special position sulphate oxygens were assigned an occupancy factor of 0.5 and were refined. The site occupancy and thermal parameters were refined alternately. However, the sulphate geometry thus obtained was not satisfactory and the final  $\Delta F$  map had a residual peak  $0.7 \text{ e}\text{\AA}^{-3}$ . The R index at this stage is 0.122.

In order to improve the geometry of the sulphate group at special position, bond length- bond angle-constrained refinement was carried out with 0.5 occupancy for sulphate oxygens. The sulphate geometries for both constrained and unconstrained refinements are shown in figure 1. Four water molecules were located from difference Fourier out of which three were disordered with occupancies 0.5, 0.6 and 0.4. It is interesting to note here that the observed density accounts for four water molecules, but in this case their occupancies add up to 2.5 only. Thus the difference in calculated and observed density may be due to the disorder in the molecule which could not be accounted for completely. The amine, sulphate S(2), special position sulphur and one of the water molecules were refined anisotropically and the rest isotropically. The hydrogens were located from  $\Delta F$  map and checked against stereochemically fixed positions. Out of 31 hydrogens present in the molecule, two hydrogens attached to N(1) and N(15), water hydrogens, and the

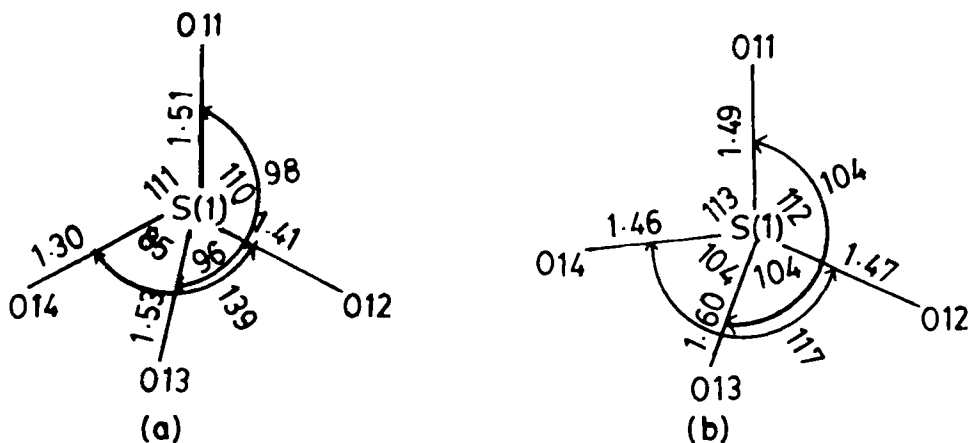


Figure 1. Sulphate geometry (a) constrained and (b) unconstrained.

Table 1. Final fractional positional parameters and equivalent temperature factor ( $\text{\AA}^2$ ) for non-hydrogen atoms with esd's in parentheses.

$$Beq = \frac{8\pi^2}{3} \sum \sum U_{ij} a_i^* a_j^* a_i \cdot a_j$$

| Atom                                   | x         | y         | z          | Beq    | SOF* |
|--|-----------|-----------|------------|--------|------|
| S( 2)                                  | 0.454(1)  | 0.0995(5) | 0.2805(7)  | 4.0(3) |      |
| O(21)                                  | 0.431(4)  | 0.204 (1) | 0.298 (2)  | 9 (1)  |      |
| O(22)                                  | 0.482(5)  | 0.080 (2) | 0.387 (2)  | 14 (1) |      |
| O(23)                                  | 0.628(5)  | 0.056 (2) | 0.243 (3)  | 14 (2) |      |
| O(24)                                  | 0.305(4)  | 0.050 (2) | 0.183 (3)  | 17 (2) |      |
| N( 1)                                  | 0.259(3)  | 0.266 (2) | 1.080 (2)  | 4 (1)  |      |
| C( 2)                                  | 0.252(3)  | 0.199 (2) | 0.966 (2)  | 3 (1)  |      |
| N( 3)                                  | 0.302(3)  | 0.104 (2) | 0.951 (2)  | 5 (1)  |      |
| N( 4)                                  | 0.193(3)  | 0.233 (1) | 0.867 (2)  | 4 (1)  |      |
| C( 5)                                  | 0.156(4)  | 0.170 (2) | 0.741 (2)  | 4 (1)  |      |
| C( 6)                                  | 0.094(4)  | 0.231 (2) | 0.655 (3)  | 5 (1)  |      |
| C( 7)                                  | 0.043(4)  | 0.168 (2) | 0.519 (2)  | 5 (1)  |      |
| C( 8)                                  | -0.014(4) | 0.229 (2) | 0.431 (2)  | 4 (1)  |      |
| N( 9)                                  | -0.061(3) | 0.171 (1) | 0.299 (2)  | 5 (1)  |      |
| C(10)                                  | -0.127(4) | 0.230 (2) | 0.209 (2)  | 4 (1)  |      |
| C(11)                                  | -0.171(4) | 0.164 (2) | 0.077 (2)  | 4 (1)  |      |
| C(12)                                  | -0.247(4) | 0.222 (2) | -0.015 (2) | 4 (1)  |      |
| N(13)                                  | -0.288(3) | 0.156 (1) | -0.140 (2) | 4 (1)  |      |
| C(14)                                  | -0.346(4) | 0.182 (2) | -0.241 (3) | 4 (1)  |      |
| N(15)                                  | -0.386(3) | 0.115 (2) | -0.354 (2) | 5 (1)  |      |
| N(16)                                  | -0.362(3) | 0.278 (2) | -0.234 (2) | 6 (1)  |      |
| <i>Water oxygens</i>                   |           |           |            |        |      |
| Ow(1)                                  | 0.501(5)  | 0.324 (2) | 0.537 (2)  | 11 (1) | 1.0  |
| Ow(2)                                  | 0.63 (1)  | 0.476 (5) | 0.196 (6)  | 13 (2) | 0.5  |
| Ow(3)                                  | -0.18 (1) | 0.494 (6) | -0.348 (9) | 19 (1) | 0.6  |
| Ow(4)                                  | 0.23 (1)  | 0.512 (6) | 0.598 (7)  | 14 (1) | 0.4  |
| S( 1)                                  | 0.0       | 0.5       | 0.0        | 13 (1) |      |
| <i>Sulphate oxygens, unconstrained</i> |           |           |            |        |      |
| O(11)                                  | -0.184(6) | 0.457(5)  | -0.011(6)  | 25(1)  |      |
| O(12)                                  | 0.095(6)  | 0.435(5)  | -0.089(3)  | 7(1)   |      |
| O(13)                                  | 0.136(8)  | 0.470(5)  | 0.112(4)   | 16(2)  |      |
| O(14)                                  | 0.011(8)  | 0.589(2)  | 0.078(3)   | 6(1)   |      |
| <i>Sulphate oxygens, constrained</i>   |           |           |            |        |      |
| O(11)                                  | -0.205(4) | 0.475(4)  | 0.025(5)   | 18(1)  |      |
| O(12)                                  | 0.089(6)  | 0.442(3)  | -0.101(3)  | 12(1)  |      |
| O(13)                                  | 0.126(6)  | 0.462(3)  | 0.115(3)   | 15(2)  |      |
| O(14)                                  | 0.021(7)  | 0.606(1)  | 0.048(4)   | 15(2)  |      |

\* Site occupancy factor.

ones belonging to the sulphate groups could not be located. Hydrogens were not refined and were included in the structure factor calculation only. As the quality of the data was very poor, the accuracy of the results obtained is restricted. Unit weighting scheme was applied as the individual weighting scheme based on counting statistics did not show any improvement. The final R index is 0.144. Final

difference Fourier map had no peaks greater than  $0.6 \text{ e}\text{\AA}^{-3}$ . Final (shift/e.s.d.) mean = 0.3. Scattering factors are as given in SHELX76. The fractional positional parameters with equivalent isotropic temperature factors are given in table 1 and anisotropic thermal parameters in table 2.

### 3. Discussion

The bond lengths and angles are given in figure 2. Figure 3 gives the ORTEP diagram (Johnson 1965) for the thermal ellipsoids drawn at 50% probability level. The bond lengths and bond angles in the putrescine moiety are in good agreement with the values reported for agmatine and arcaine. The average values C–C 1.51(4), C–N 1.44(3) Å and C–C–C 112(2), N–C–C 112(2)° are in good agreement with the values 1.48(2), 1.51(2) Å and 113(2), 112(2)° in arcaine sulphate (Thailambal *et al* 1985) and 1.491(6), 1.525(6) Å and 109.7(5), 110.5(5)° in agmatine sulphate (Chandrasekar *et al* 1982). The average C–N distance 1.34(4) Å in the guanidyl group compares well with the value 1.35(2) Å in arcaine sulphate and 1.333(3) Å in agmatine sulphate. The guanidyl groups and the butylamine segment are planar. The dihedral angle between the guanidyl groups and the

**Table 2.** Anisotropic thermal coefficients ( $\times 10^3$ ) for non-hydrogen atoms with esd's in parentheses.

$$\text{Temperature} = \exp[-2\pi^2(U_{11}h^2a^{*2} + U_{22}k^2b^{*2} + U_{33}l^2c^{*2} + 2U_{12}hka^*b^* + 2U_{13}hla^*c^* + 2U_{23}klb^*c^*)]$$

| Atom  | $U_{11}$ | $U_{22}$ | $U_{33}$ | $U_{12}$ | $U_{13}$ | $U_{23}$ |
|-------|----------|----------|----------|----------|----------|----------|
| S( 1) | 157(20)  | 157(10)  | 157(10)  | 13(10)   | 44(10)   | 49(10)   |
| S( 2) | 80( 6)   | 31( 4)   | 32( 4)   | 10( 4)   | 12( 4)   | 8( 3)    |
| O(21) | 198(28)  | 33(12)   | 87(16)   | 30(14)   | 13(16)   | 22(11)   |
| O(22) | 369(47)  | 78(18)   | 61(15)   | 57(22)   | 104(21)  | 7(13)    |
| O(23) | 257(36)  | 49(16)   | 253(35)  | 15(19)   | 209(32)  | -17(18)  |
| O(24) | 138(27)  | 49(15)   | 265(35)  | 30(16)   | -153(27) | 22(19)   |
| N( 1) | 35(15)   | 65(15)   | 52(14)   | 3(11)    | 17(11)   | 4(12)    |
| C( 2) | 26(17)   | 52(18)   | 52(19)   | 4(14)    | 12(14)   | 19(16)   |
| N( 3) | 69(19)   | 48(16)   | 87(17)   | 15(13)   | 43(14)   | 29(13)   |
| N( 4) | 60(17)   | 33(12)   | 47(14)   | 8(11)    | 11(11)   | 8(11)    |
| C( 5) | 52(20)   | 54(18)   | 44(17)   | 7(15)    | 19(14)   | 7(15)    |
| C( 6) | 58(22)   | 45(17)   | 70(21)   | 13(15)   | 26(16)   | 18(15)   |
| C( 7) | 83(22)   | 60(19)   | 52(19)   | 9(16)    | 32(15)   | 15(16)   |
| C( 8) | 50(19)   | 78(21)   | 37(17)   | 4(16)    | 25(13)   | 6(15)    |
| N( 9) | 86(19)   | 38(13)   | 56(15)   | 21(12)   | 31(12)   | 18(11)   |
| C(10) | 48(19)   | 48(16)   | 65(19)   | -17(14)  | 17(14)   | 21(15)   |
| C(11) | 47(19)   | 49(16)   | 52(18)   | 17(14)   | 13(14)   | 16(14)   |
| C(12) | 22(17)   | 71(19)   | 59(18)   | 5(14)    | 16(13)   | 19(16)   |
| N(13) | 56(16)   | 41(12)   | 48(15)   | 15(11)   | 25(12)   | -1(12)   |
| C(14) | 34(19)   | 74(23)   | 49(21)   | -2(16)   | 21(15)   | 23(19)   |
| N(15) | 67(18)   | 77(17)   | 45(14)   | 7(14)    | 23(12)   | 23(13)   |
| N(16) | 64(19)   | 61(17)   | 91(19)   | 11(14)   | 22(14)   | 29(15)   |
| Ow(1) | 221(33)  | 85(18)   | 135(23)  | 5(19)    | 72(22)   | 22(16)   |

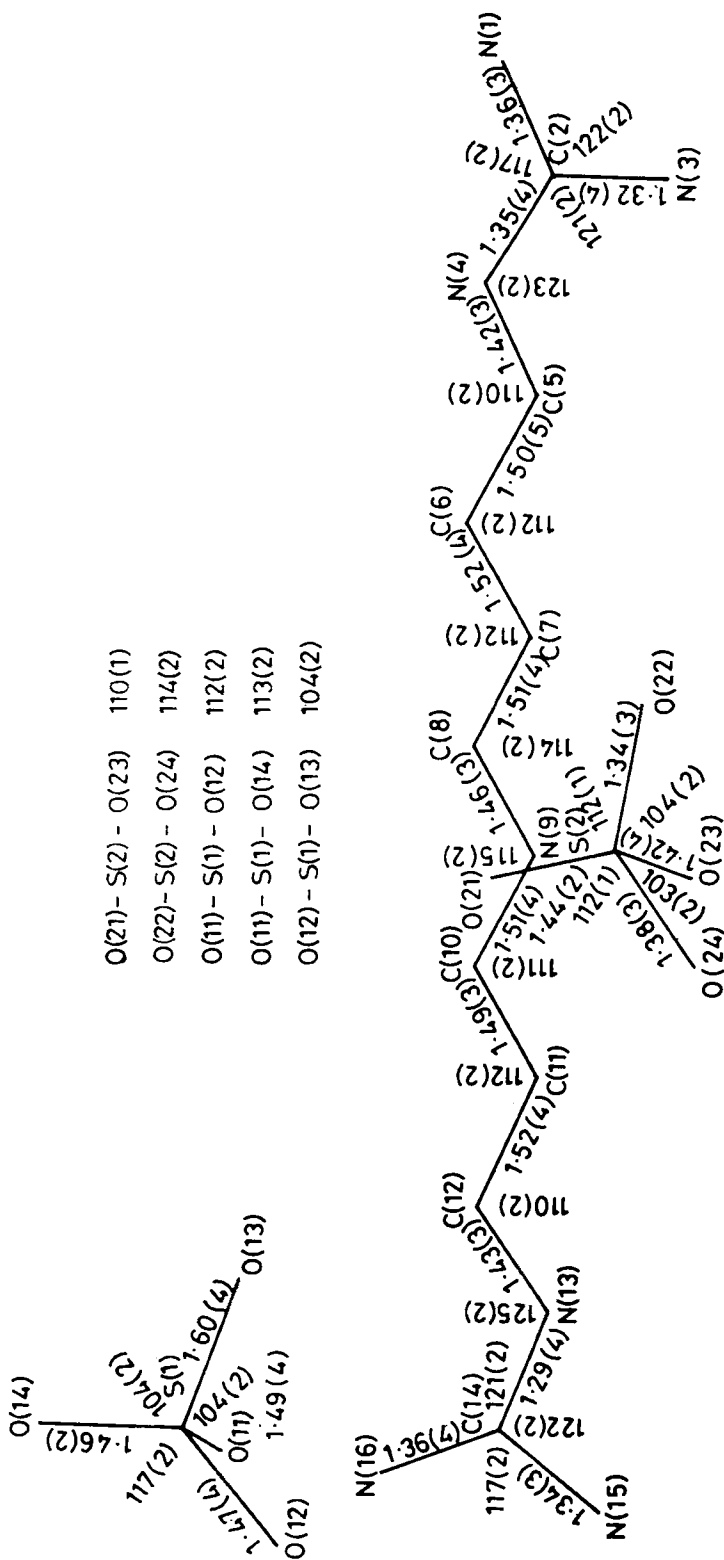


Figure 2. Bond lengths and bond angles involving non-hydrogen atoms.

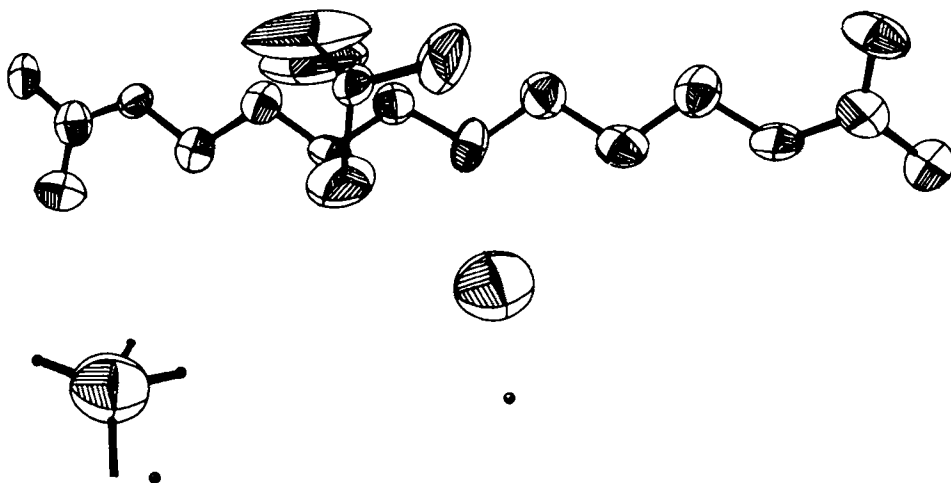


Figure 3. ORTEP diagram for the thermal ellipsoids drawn at 50% probability level.

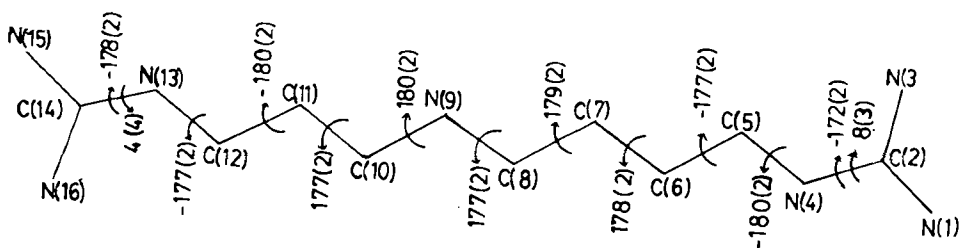


Figure 4. Important torsion angles observed in the amine molecule.

butylamine segment are  $9(2)^\circ$  and  $127(2)^\circ$ . The amine molecule as a whole is in all-*trans* conformation (figure 4). The sulphate S(2) is in a regular tetrahedral geometry with an average S–O distance of  $1.39(2)$  Å and O–S–O of  $109(1)^\circ$ . The oxygens of the sulphate group S(1) are highly disordered with an average distance of  $1.44(4)$  Å and O–S–O of  $107(2)^\circ$  in the unconstrained refinement; in the constrained refinement the bond lengths vary between  $1.46(4)$  and  $1.60(4)$  Å and the bond angles between  $104(2)$  and  $117(2)^\circ$ .

The structure exhibits a layerwise packing with the zig-zag amine molecules arranged in planes parallel to the *bc* plane. The water sulphate hydrogen bond network is interleaved between the symmetry-related amine molecules along the *b* axis. All the nine protons available in the amine molecule are involved in hydrogen bonding. The sulphate oxygen O(21) is linked to the water oxygen Ow(1) through a hydrogen bond and Ow(1) is in turn hydrogen-bonded to Ow(4). Though the structure is well stabilized by hydrogen bond network, it shows holes in unique positions, e.g. a water channel involving N(1)...O(21), O(21)...Ow(1), Ow(1)...Ow(4), Ow(4)...Ow(3), Ow(3)...O(13), O(13)...N(1) is formed along the *a* axis. The possible hydrogen bonds are given in table 3. The molecular packing is shown in figure 5.

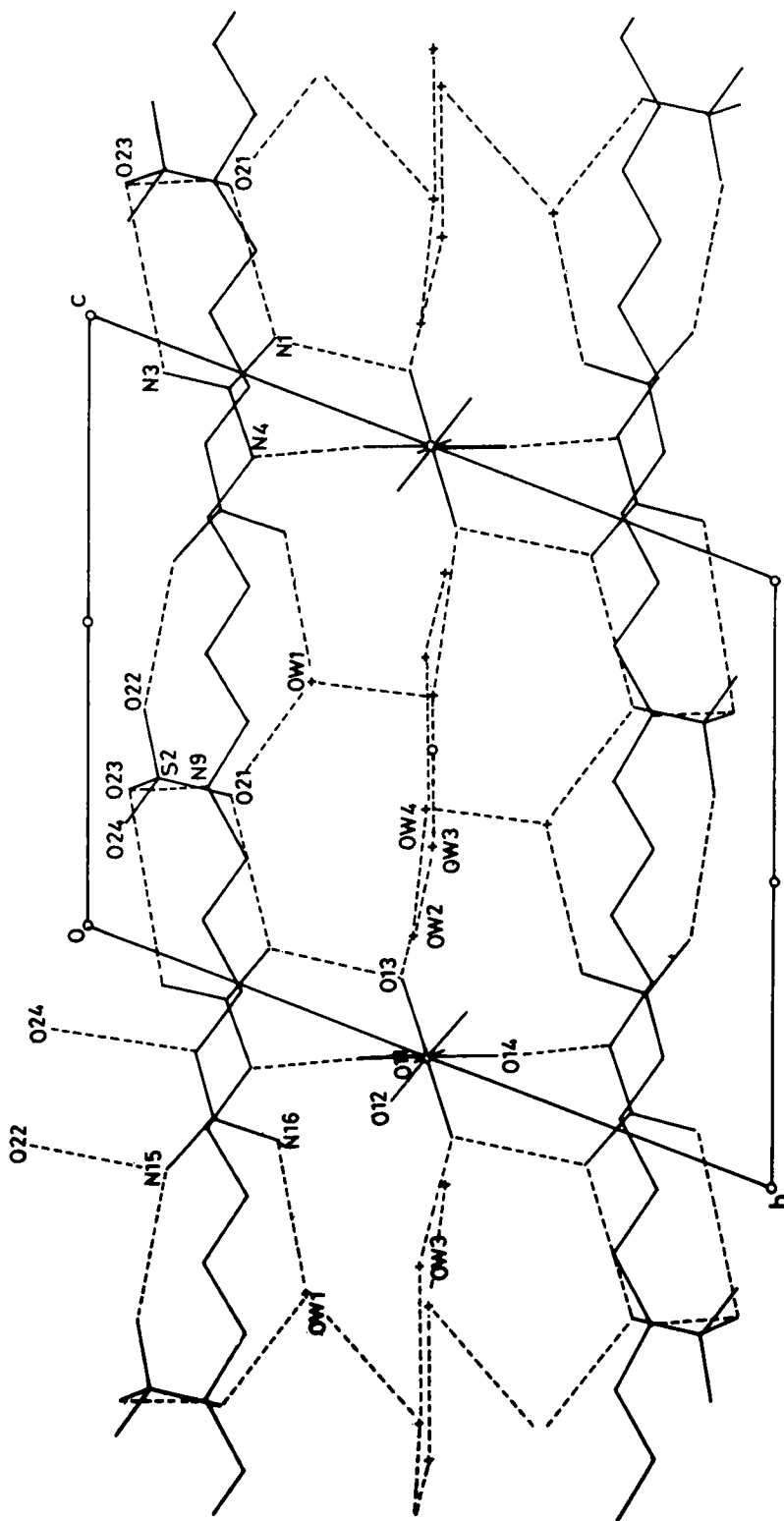


Figure 5. Packing of the molecules down *a* axis in the unit cell and hydrogen bonding scheme.

Table 3. Possible hydrogen bonds.

| Contact       | Symmetry        | Distance (Å) |
|---------------|-----------------|--------------|
| Ow(4)...Ow(1) | $x, y, z$       | 2.91(7)      |
| Ow(1)...O(21) | $x, y, z$       | 2.71(3)      |
| O(23)...N( 9) | $1+x, y, z$     | 2.81(4)      |
| N( 1)...O(13) | $x, y, 1+z$     | 2.86(7)      |
| N( 1)...O(21) | $x, y, 1+z$     | 2.87(3)      |
| N( 4)...O(12) | $x, y, 1+z$     | 2.80(4)      |
| Ow(1)...N(16) | $1+x, y, 1+z$   | 2.86(4)      |
| O(22)...N(15) | $1+x, y, 1+z$   | 2.81(3)      |
| O(13)...Ow(3) | $-x, 1-y, -z$   | 2.60(6)      |
| Ow(2)...Ow(4) | $1-x, 1-y, 1-z$ | 2.35(4)      |
| Ow(4)...Ow(3) | $-x, 1-y, -z$   | 2.80(4)      |
| O(14)...N( 4) | $-x, 1-y, 1-z$  | 2.71(3)      |
| O(22)...N(15) | $-x, -y, -z$    | 2.91(4)      |
| O(23)...N( 3) | $1-x, -y, 1-z$  | 2.75(4)      |
| O(24)...N(13) | $-x, -y, -z$    | 2.87(4)      |

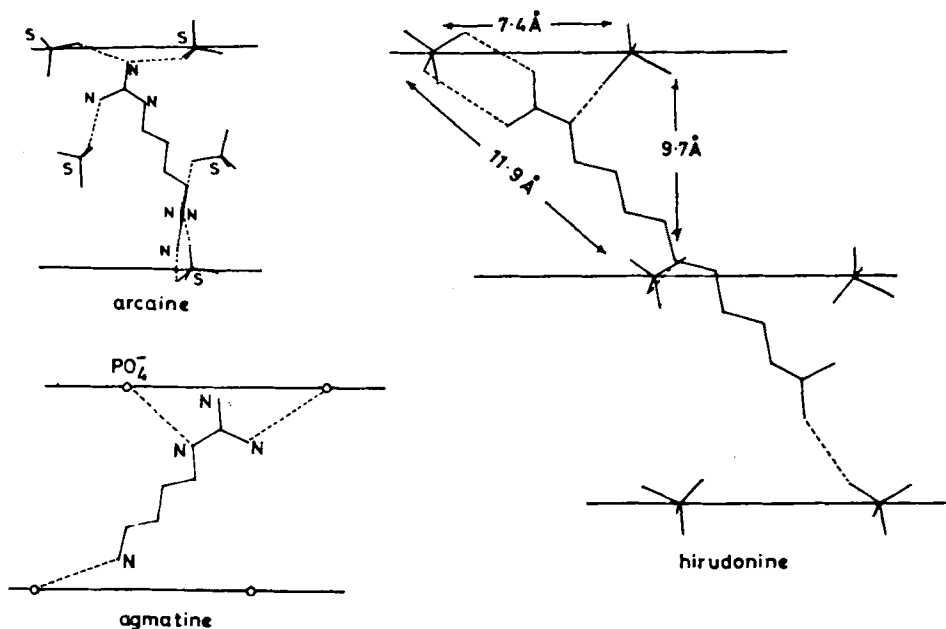


Figure 6. Possible mode of binding for hirudonine, arcaine and agmatine.

The configurational similarities between the sulphate and phosphate ions make it possible to extrapolate the observations made in hirudonine sulphate to the possible mode of binding with nucleotide. The inter-sulphate distance of 7.17 Å along the  $a$  axis and 7.43 Å between those hydrogen bonded to the same guanidyl group can be compared with the distance of 7.3 Å between the successive phosphate groups along the helix in polynucleotides (Langridge *et al* 1960). The



hirudonine molecules have a packing similar to other polyamines. This is made possible by the presence of an additional sulphate group at special position. The two halves of the molecules maintain a geometry such that they can mimic the binding of putrescine and agmatine with DNA. Hence it can be concluded that there is a possibility of hirudonine binding to DNA across the narrow groove making two hydrogen bonds with one strand and one with the other as in the case of arcaïne and agmatine (figure 6).

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### References

- Chandrasekar K, Vasantha Pattabhi and Ragunathan S 1982 *Acta Cryst.* **B38** 2538  
Cohen S S 1971 *Introduction to polyamines* (Englewood Cliffs, NJ: Prentice-Hall)  
Fru-ton J S and Simmonds S 1965 *General Biochemistry* (New Delhi: Asia Publishing House)  
Johnson C K 1965 *ORTEP: A FORTRAN thermal-ellipsoid plot program for crystal structure illustrations* Report ORNL-3794, Oakridge National Laboratory, Tennessee  
Langridge R, Marvin D A, Seeds W E, Wilson H R, Cooper C W, Wilkins M H F and Hamilton L D 1960 *J. Mol. Biol.* **2** 38  
Liquori A M, Constantino V, Crescenzi V, Elia V, Giglio E, Puliti R, De Santis Savino M and Vitageino V 1967 *J. Mol. Biol.* **24** 113  
Main P, Fiske S J, Hull S E, Lessinger L, Germain G, Declercq J P and Woolfson M M 1980 *A system of computer programs for the automatic solution of crystal structures from X-ray diffraction data* University of York, England  
Scalabrino G and Ferioli M E 1981 *Adv. Cancer Res.* **35** 151  
Scalabrino G and Ferioli M E 1982 *Adv. Cancer Res.* **36** 1  
Sheldrick G M 1976 *SHELX76: Program for crystal structure determination* University of Cambridge, England  
Stevens L 1967 *Biochem. J.* **103** 811  
Suevalsky M, Tranb W, Shmueli D and Subirana J A 1969 *J. Mol. Biol.* **42** 363  
Thailambal V G, Vasantha Pattabhi, Lee F L, Le Page Y and Gabe E J 1985 *Acta Cryst.* **C41** 105  
Tsuboi M 1964 *Bull. Chem. Soc. Jpn.* **37** 1514  
Vasantha Pattabhi and Chandrasekar K 1982 in *Conformation in biology* (eds) R Srinivasan and R H Sarma (Adenine Press) p. 291  
Woo N, Seeman N C and Rich A 1979 *Biopolymers* **18** 539