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Structure of Guanidinium Bicarbonate: A Model for the Bicarbonate Anion Binding Site of the Transferrins

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Abstract. Guanidinium bicarbonate (aminomethanamidinium hydrogencarbonate), $C_2H_7N_3O_3$, $M_r = 121{\cdot}10$, monoclinic, $P2_1/n$, $a = 3{\cdot}7608\ (8)$, $b = 7{\cdot}6783\ (8)$, $c = 18{\cdot}056\ (2)$ Å, $\beta = 99{\cdot}59\ (1)°$, $V = 514{\cdot}10$ Å^3, $Z = 4$, $D_m = 1{\cdot}54$, $D_x = 1{\cdot}56$ g cm^{-3}, Mo $K\alpha$, $\lambda = 0{\cdot}71073$ Å, $\mu = 0{\cdot}98$ cm^{-1}, $F(000) = 256$, $T = 293$ K, final $R = 0{\cdot}0452$ for 1051 unique reflections. The structure is proposed as a simple model for the binding of the synergistic anion, HCO_3^-, to an arginine in apotransferrins. Two of the oxygens of the planar bicarbonate form hydrogen bonds (2·820 and 2·912 Å) with different nitrogens on a guanidinium cation with a dihedral angle of 15·8°.

Introduction. The requirement of a synergistic anion, which is physiologically bicarbonate or possibly carbonate, for strong binding of Fe^{3+} or other metal ions to the transferrins is apparently unique in metalloprotein chemistry (Chasteen, 1977; Aisen, 1980). The anion which is bound directly to the metal ion (Chasteen, 1977; Aisen, 1980; Zweier, Peisach & Mims, 1982; Harris, Gray & Aisen, 1974) is almost certainly hydrogen-bonded to an essential arginine residue (Schlabach & Bates, 1975; Feeney, Osuga, Meares, Babin & Penner, 1983) although NMR studies on ovotransferrin have suggested that a histidine may be involved (Alsaadi, Williams & Woodworth, 1981). A comparison of the amino acid sequences of human serum transferrin (Yang *et al.*, 1984), lactoferrin (Metz-Boutique *et al.*, 1984) and ovotransferrin (Jeltsh & Champon, 1982) shows that there are three fully conserved arginines and two fully conserved histidines in each of the two metal binding domains. The two histidine residues are directly bound to the metal ion (Feeney *et al.*, 1983; Alsaadi *et al.*, 1981; Pecoraro, Harris, Carrano & Raymond, 1981; Gaber, Miskowski & Spiro, 1974) and are thus not available for anion binding. The guanidinium group of arginine has on the other hand been shown to act as an anion recognition site in many metalloenzymes (Riordan, 1979).

As part of a programme to study the anion binding site of the transferrins we have determined the structure of guanidinium bicarbonate as a simple model system. The structure of bis(guanidinium) carbonate has been reported previously by Adams & Small (1974).

Experimental. Guanidinium bicarbonate, $C(NH_2)_3HCO_3$, was prepared by bubbling CO_2 gas through a 1·0 *M* solution of guanidinium carbonate (BDH, Analar), until the pH stabilized at 7·10. Crystals were grown from an aqueous solution under a CO_2 atmosphere. The title compound crystallizes as colourless, needle-shaped crystals. A crystal of dimensions 0·44 × 0·39 × 0·28 mm was chosen and used for preliminary X-ray investigations using the Weissenberg technique, and for data collection; D_m by flotation in $CCl_4/CHCl_3$; Enraf–Nonius CAD-4 diffractometer, graphite-monochromated Mo $K\alpha$ radiation ($\lambda = 0{\cdot}71073$ Å) at room temperature, ω–2θ scan. Lattice parameters from least-squares refinement of 23 reflections, $16 \le \theta \le 18°$. Three standard reflections. ($2\bar{2}9$, $3\bar{1}1$, $\bar{2}5\bar{1}$) monitored after every 104 reflections, showed no more than 0·6% intensity variation throughout the data collection. All (1232) reflections (h: −4 to 4, k: 0 to 9, l: 0 to 9) in the range $3 < \theta < 27°$ were measured. Max. $\sin\theta/\lambda = 0{\cdot}64$ Å^{-1}. 1051 unique reflections with $I > 2\sigma(I)$ used in the analysis; $R_{int} = 0{\cdot}0246$; empirical absorption correction factors (max. and min. values 1·000, 0·9929) and LPF corrections. Structure solved by direct methods using *SHELX*76 (Sheldrick, 1976). Least-squares refinement of positional and thermal parameters and scale factor {all H atoms were located from a difference Fourier map and were allowed to refine freely except H(2) and H(7) which were fixed, d[N(1)–H(2)] = 1·00 Å and d[O(3)–H(7)] = 1·00 Å} gave $R = 0{\cdot}0452$, $wR = 0{\cdot}0396$; $w = 1/\sigma^2(F)$. 100 parameters refined including anisotropic thermal parameters for non-H atoms and isotropic thermal parameters for H atoms. $(\Delta/\sigma)_{max}$ for non-H atoms 0·009, for H 0·024. Max. and min. residual electron density on final difference Fourier synthesis 0·244, −0·285 e Å^{-3}. Atomic scattering factors for non-H atoms from Cromer & Mann (1968); anomalous-dispersion correction factors from Cromer & Liberman (1970); H-atom scattering factors from Stewart, Davidson & Simpson (1965).

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Discussion. Final atomic positional parameters and bond distances and angles are presented in Tables 1 and 2.* The structure of guanidinium bicarbonate (Fig. 1) shows that as anticipated the guanidinium cation can form two hydrogen bonds [average N–H···O distance 2·87 (5) Å] with the same bicarbonate anion, and is thus well suited for an anion binding role. The guanidinium cation is essentially planar with an average C–N bond length of 1·324 (3) Å, compared with 1·342 Å in bis(guanidinium) carbonate (Adams & Small, 1974), and forms a dihedral angle of 15 (2)° with the planar bicarbonate anion. The substitution of a hydrogen on one of the nitrogens by a $-CH_2-$ as in arginine is expected to have no significant effect on the C–N bond length (Cotton, Hazen, Day, Larsen, Norman, Wong & Johnson, 1973; Curtis & Pasternak, 1955).

Alternating guanidinium cations and bicarbonate anions form infinite hydrogen-bonded chains in the crystal (Fig. 2). The structure is, however, much simpler than that of bis(guanidinium) carbonate (Adams & Small, 1974), where each carbonate is hydrogen-bonded to six guanidinium cations.

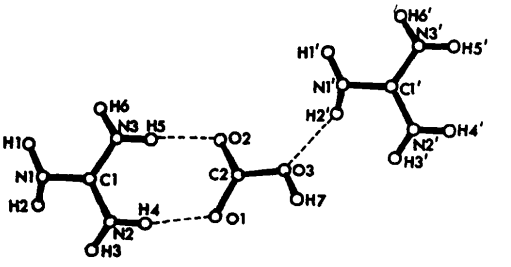

Fig. 1. Structure of guanidinium bicarbonate, showing the immediate environment of a bicarbonate ion hydrogen-bonded to two neighbouring guanidium cations.

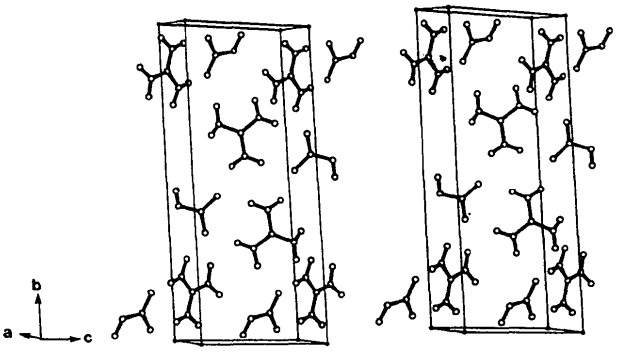

Fig. 2. Packing of guanidinium bicarbonate in the unit cell.

Studies on the mechanism of Fe^{3+} uptake by transferrin (Bates, 1981) show that the bicarbonate synergistic anion binds in a pre-equilibrium step to the apoprotein prior to binding of the metal ion. The structure of guanidinium bicarbonate is probably a good model for the apotransferrin-bicarbonate adduct. However, binding on an Fe^{3+} to say O(3) in Fig. 1 would almost certainly result in displacement of the proton from O(3). Since the bicarbonate proton is not released to the medium (Gelb & Harris, 1980) it must bind to one of the other carbonate oxygens, to the OH^- ligand on the Fe^{3+} (Carrano, Spartalian, Appa Rao, Pecoraro & Sundaralingam, 1985) or a basic residue on the protein.

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* Lists of structure factors, anisotropic thermal parameters and deviations of atoms from mean planes have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 42965 (8 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 1. *Fractional coordinates* (C, N, O $\times 10^4$, H $\times 10^3$) *and equivalent isotropic temperature factors* (Å^2 $\times 10^3$)

$$U_{eq} = \tfrac{1}{3}\sum_i\sum_j U_{ij} a_i^* a_j^* \mathbf{a}_i.\mathbf{a}_j.$$

	x	y	z	U_{eq}
O(1)	650 (4)	1966 (2)	5427 (1)	37 (1)
O(2)	3753 (4)	1551 (2)	6577 (1)	41 (1)
O(3)	3094 (4)	−617 (2)	5773 (1)	39 (1)
N(1)	7893 (5)	−2731 (2)	6820 (1)	39 (1)
N(2)	8355 (5)	−4586 (3)	5835 (1)	42 (1)
N(3)	10560 (5)	−5399 (2)	7046 (1)	36 (1)
C(1)	8932 (5)	−4236 (3)	6565 (1)	31 (1)
C(2)	2485 (5)	1033 (3)	5937 (1)	33 (1)
H(1)	827 (6)	−255 (3)	738 (1)	47 (6)
H(2)	830 (7)	301 (3)	852 (1)	58 (7)
H(3)	224 (7)	364 (3)	448 (2)	72 (9)
H(4)	−82 (8)	432 (4)	565 (2)	90 (10)
H(5)	153 (7)	358 (3)	688 (1)	61 (7)
H(6)	1114 (8)	−502 (4)	758 (1)	90 (7)
H(7)	189 (7)	−101 (3)	526 (1)	90 (7)

Table 2. *Bond lengths* (Å) *and angles* (°)

C(1)–C(2)	1·273 (2)	O(2)–C(2)	1·238 (2)
O(3)–C(2)	1·327 (2)	N(1)–C(1)	1·325 (2)
N(2)–C(1)	1·325 (3)	N(3)–C(1)	1·322 (2)
O(3)–H(7)	1·000 (1)	N(1)–H(1)	1·00 (2)
N(2)–H(4)	0·97 (3)	N(3)–H(5)	0·94 (3)
N(3)–H(6)	1·000 (1)		

O(1)–C(2)–O(2)	124·9 (2)	O(1)–C(2)–O(3)	117·8 (2)
O(2)–C(2)–O(3)	117·3 (2)	N(1)–C(1)–N(2)	120·8 (2)
N(1)–C(1)–N(3)	119·4 (2)	N(2)–C(1)–N(3)	119·8 (2)

References

ADAMS, J. M. & SMALL, R. W. H. (1974). *Acta Cryst.* **B30**, 2191–2193.

AISEN, P. (1980). *Annv. Rev. Biochem.* **49**, 357–393.

ALSAADI, B. M., WILLIAMS, R. J. P. & WOODWORTH, R. C. (1981). *J. Inorg. Biochem.* **15**, 1–10.
BATES, G. W. (1981). *The Biochemistry and Physiology of Iron*, edited by P. SALTMAN & J. HEGENAUER, pp. 3–18. New York: Elsevier.
CARRANO, C. J., SPARTALIAN, K., APPA RAO, G. V. N., PECORARO, V. L. & SUNDARALINGAM, M. (1985). *J. Am. Chem. Soc.* **107**, 1651–1658.
CHASTEEN, N. D. (1977). *Coord. Chem. Rev.* **22**, 1–36.
COTTON, F. A., HAZEN, E. E., DAY, V. W., LARSEN, S., NORMAN, J. G., WONG, S. T. K. & JOHNSON, K. H. (1973). *J. Am. Chem. Soc.* **95**, 2367–2369.
CROMER, D. T. & LIBERMAN, D. (1970). *J. Chem. Phys.* **53**, 1891–1898.
CROMER, D. T. & MANN, J. B. (1968). *Acta Cryst.* A**24**, 321–324.
CURTIS, R. M. & PASTERNAK, R. A. (1955). *Acta Cryst.* **8**, 675–681.
FEENEY, R. E., OSUGA, D. T., MEARES, C. F., BABIN, D. R. & PENNER, M. H. (1983). *Structure and Function of Iron Storage and Transport Proteins*, edited by I. URUSHIZAKI, P. AISEN & I. LISTOWSKY, pp. 231–240. New York: Elsevier.
GABER, B. P., MISKOWSKI, V. & SPIRO, T. (1974). *J. Am. Chem. Soc.* **96**, 6868–6873.
GELB, M. H. & HARRIS, D. C. (1980). *Arch. Biochem. Biophys.* **200**, 93–98.
HARRIS, D. C., GRAY, G. A. & AISEN, P. (1974). *J. Biol. Chem.* **249**, 5261–5264.
JELTSH, J. M. & CHAMPON, P. (1982). *Eur. J. Biochem.* **122**, 291–295.
METZ-BOUTIQUE, M. H., JOLLES, J., MAZURIER, J., SCHOENTGEN, F., LEGRAND, D., SPIK, G., MONTREUIL, J. & JOLLES, P. (1984). *Eur. J. Biochem.* **145**, 659–676.
PECORARO, V. L., HARRIS, W. R., CARRANO, C. J. & RAYMOND, K. N. (1981). *Biochemistry*, **20**, 7033–7039.
RIORDAN, J. F. (1979). *Mol. Cell. Biochem.* **26**, 71–92.
SCHLABACH, M. R. & BATES, G. W. (1975). *J. Biol. Chem.* **250**, 2182–2188.
SHELDRICK, G. M. (1976). *SHELX76*. Program for crystal structure determination. Univ. of Cambridge, England.
STEWART, R. F., DAVIDSON, E. R. & SIMPSON, W. T. (1965). *J. Chem. Phys.* **42**, 3175–3187.
YANG, F., LUM, J. B., MCGILL, J. R., MOORE, C. M., NAYLOR, S. L., VAN BRAGT, P. H., BALDWIN, W. D. & BOWMAN, B. H. (1984). *Proc. Natl Acad. Sci. USA*, **81**, 2752–2756.
ZWEIER, J. L., PEISACH, J. & MIMS, W. B. (1982). *J. Biol. Chem.* **257**, 10314–10316.

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Pentacyclo[19.3.1.1^{2,6}.1^{9,13}.1^{14,18}]octacosa-1(25),2,4,6(28),9,11,13(27),14,16,18(26),21,23-dodecaene ([2.0.2.0]-*m*-Cyclophane)

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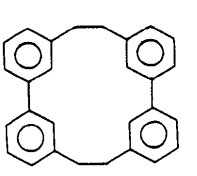
Abstract. $C_{28}H_{24}$, $M_r = 360{\cdot}5$, orthorhombic, *Pbca*, $a = 8{\cdot}968\ (4)$, $b = 12{\cdot}337\ (3)$, $c = 36{\cdot}75\ (1)$ Å, $V = 4066\ (3)$ Å^3, $Z = 8$, $D_x = 1{\cdot}18$ g cm^{-3}, D_m not measured, Mo $K\alpha$, $\lambda = 0{\cdot}71073$ Å, $\mu = 0{\cdot}62$ cm^{-1}, $F(000) = 1536$, $T = 295$ K, final $R = 0{\cdot}044$ for 2175 observed reflections. The conformation of the title compound in the crystalline state is established by an X-ray study as being one which approaches D_2 symmetry, proposed to predominate for the compound in solution on the basis of NMR studies. The angles of twist between aromatic rings in the biphenyl systems are 46·8 (2) and 52·1 (2)° rather than ~30° as proposed for the molecule in solution or 89·9° as observed in an X-ray study of a related compound.

Introduction. The title compound (1), first prepared by Vögtle (1969), was noted by Leach & Reiss (1978) to have aromatic protons absorbing unusually far upfield in the NMR, and an '*anti*' conformation (1*a*) was proposed to predominate in solution to fit this finding. In 1981 (Olsson, Tanner, Thulin, Wennerström & Liljefors, 1981), the NMR spectrum was reinterpreted as showing a twisted D_2 conformation (1*b*) to predominate, and variable-temperature NMR behavior was noted and rationalized in terms of equilibrating enantiomeric D_2 forms. The angle of twist between adjacent aromatic rings was estimated to be about 30°. An X-ray study on the corresponding molecule with methoxyl groups in the 2 positions and methyl groups in the 5 positions found D_2 symmetry with twist angles of 89·9° between adjacent aromatic rings (Kaneda *et al.*, 1985). We recently found a better synthetic route to (1) (and many other cyclophanes) (Bates, White, Kane & Mishra, 1986), and when this compound was found to crystallize well, we performed an X-ray study on it to determine its conformation in the solid state.



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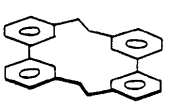
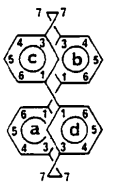


(1*a*)



(1*b*)

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