

Acta Cryst. (1996). **C52**, 1687–1688**HEPES**

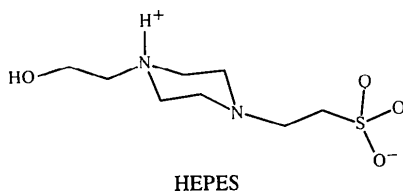
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*Institut für Anorganische Chemie der Universität Göttingen, Tammannstrasse 4, D-37077 Göttingen, Germany. E-mail: jwouters@shelx.uni-ac.gwdg.de**(Received 30 August 1995; accepted 25 January 1996)***Abstract**

The crystal structure of HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, $C_8H_{18}N_2O_4S$] is presented. This widely used buffer crystallizes as a zwitterion, the N10 atom of the piperazine ring being the protonation site. The molecules form a network of hydrogen bonds involving both N atoms of the piperazine moiety. The piperazine ring adopts a chair conformation.

Comment

HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] is a widely used biological buffer presenting a useful pH range between 6.8 and 8.2 ($pK_a = 7.5$).



In the solid state, HEPES adopts an extended conformation with the piperazine ring in a chair conformation (Fig. 1). The S4—O bond lengths are 1.441 (2), 1.433 (2) and 1.439 (3) Å for O1, O2 and O3, respectively. Slight deviations from an ideal tetrahedral configuration around S4 are also observed: the largest deviations from an ideal tetrahedral angle are O2—S4—O3 112.9 (2) and C5—S4—O1 106.6 (1)°.

HEPES crystallized as a zwitterion, the proton of the sulfonic acid group being transferred to the N10 atom of the piperazine ring. This preferred site of protonation was unambiguously determined from difference Fourier maps. No residual electron density remains near the O atoms of the sulfonic acid group after anisotropic refinement.

There is probably little difference in the pK_b values of the two N atoms; possibly protonation of N10 rather than N7 gives a more stable hydrogen-bonding pattern.

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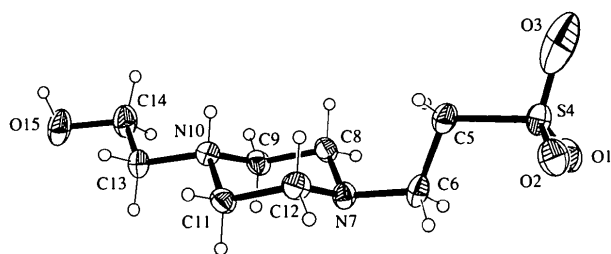


Fig. 1. Molecular structure and conformation of HEPES. Non-H atoms are represented by displacement ellipsoids at the 50% probability level. The atomic numbering scheme used in the paper is shown. Note the appreciable thermal motion of the O atoms of the sulfonate group.

The crystal packing is determined by hydrogen bonding (Table 3); the zwitterions are linked into dimers by a hydrogen bond from N10 (piperazine) to O3 (sulfonic acid), and these are further linked into corrugated sheets perpendicular to the y axis (Fig. 2) by hydrogen bonds from O15 (terminal hydroxyl) to N7 (piperazine).

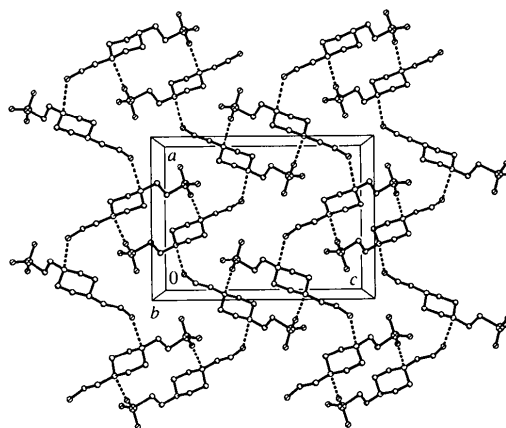


Fig. 2. A packing diagram viewed parallel to the y axis. Hydrogen bonds are indicated by broken lines. H atoms are omitted for clarity.

Experimental

Crystals were obtained by cooling a saturated solution of HEPES in methanol.

Crystal data $C_8H_{18}N_2O_4S$ $M_r = 238.30$

Orthorhombic

Pbca $a = 11.804 (3) \text{ \AA}$ $b = 11.392 (3) \text{ \AA}$ $c = 16.256 (4) \text{ \AA}$ Cu $K\alpha$ radiation $\lambda = 1.54178 \text{ \AA}$

Cell parameters from 19 reflections

 $\theta = 19.629\text{--}44.421^\circ$ $\mu = 2.658 \text{ mm}^{-1}$ $T = 293 (2) \text{ K}$

$V = 2186.0 (10) \text{ \AA}^3$
 $Z = 8$
 $D_x = 1.448 \text{ Mg m}^{-3}$
 D_m not measured

Prism
 $0.4 \times 0.4 \times 0.3 \text{ mm}$
 Colourless

Data collection

Siemens P4 diffractometer $\theta_{\max} = 53.74^\circ$
 $2\theta/\omega$ scans $h = -1 \rightarrow 12$
 Absorption correction: $k = -1 \rightarrow 11$
 none $l = -16 \rightarrow 1$
 1700 measured reflections 3 standard reflections
 1274 independent reflections monitored every 97
 1234 observed reflections reflections
 $[I > 2\sigma(I)]$ intensity decay: 3.7%
 $R_{\text{int}} = 0.0388$

Refinement

Refinement on F^2 $(\Delta/\sigma)_{\max} = 0.107$
 $R[F^2 > 2\sigma(F^2)] = 0.0487$ $\Delta\rho_{\max} = 0.426 \text{ e \AA}^{-3}$
 $wR(F^2) = 0.1362$ $\Delta\rho_{\min} = -0.463 \text{ e \AA}^{-3}$
 $S = 1.103$ Extinction correction: none
 1273 reflections Atomic scattering factors
 141 parameters from *International Tables*
 $w = 1/[\sigma^2(F_o^2) + (0.0893P)^2$ for *Crystallography* (1992),
 $+ 1.7382P]$ Vol. C, Tables 4.2.6.8 and
 where $P = (F_o^2 + 2F_c^2)/3$ 6.1.1.4)

Table 1. Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters (\AA^2)

U_{iso} for H atoms, $U_{\text{eq}} = (1/3)\sum_i \sum_j U_{ij} a_i^* a_j^* \mathbf{a}_i \cdot \mathbf{a}_j$ for all others.

	x	y	z	$U_{\text{iso}}/U_{\text{eq}}$
S4	0.72554 (6)	0.01943 (6)	0.62902 (4)	0.0252 (3)
O1	0.8386 (2)	0.0508 (2)	0.6036 (1)	0.0393 (7)
O2	0.7251 (2)	-0.0680 (2)	0.6924 (2)	0.0570 (10)
O3	0.6590 (2)	0.1211 (3)	0.6497 (2)	0.0737 (11)
O15	0.3827 (2)	-0.3220 (2)	0.1049 (1)	0.0399 (8)
N7	0.6809 (2)	-0.1990 (2)	0.4303 (1)	0.0248 (7)
N10	0.5160 (2)	-0.2614 (2)	0.3073 (1)	0.0218 (7)
C5	0.6576 (3)	-0.0429 (3)	0.5414 (2)	0.0283 (9)
C6	0.7300 (2)	-0.1389 (3)	0.5026 (2)	0.0293 (10)
C8	0.6509 (2)	-0.1184 (2)	0.3635 (2)	0.0269 (9)
C9	0.6152 (2)	-0.1854 (2)	0.2876 (2)	0.0262 (9)
C11	0.5462 (3)	-0.3418 (3)	0.3767 (2)	0.0272 (9)
C12	0.5825 (2)	-0.2713 (2)	0.4509 (2)	0.0285 (9)
C13	0.4713 (2)	-0.3292 (2)	0.2352 (2)	0.0264 (10)
C14	0.4391 (3)	-0.2514 (3)	0.1641 (2)	0.0355 (10)
H10	0.463 (2)	-0.214 (2)	0.321 (2)	0.020 (7)
H15	0.321 (1)	-0.293 (2)	0.094 (2)	0.0598

Table 2. Selected geometric parameters (\AA , $^\circ$)

S4—O1	1.441 (2)	N10—C9	1.491 (3)
S4—O2	1.433 (2)	N10—C11	1.496 (4)
S4—O3	1.439 (3)	N10—C13	1.500 (3)
S4—C5	1.783 (3)	C5—C6	1.524 (5)
O15—C14	1.421 (4)	C8—C9	1.510 (4)
N7—C6	1.478 (4)	C11—C12	1.511 (4)
N7—C8	1.466 (3)	C13—C14	1.505 (4)
N7—C12	1.463 (3)		
O1—S4—O2	112.4 (1)	C9—N10—C13	114.0 (2)
O1—S4—O3	111.9 (2)	C11—N10—C13	111.0 (2)
O1—S4—C5	106.6 (1)	S4—C5—C6	111.3 (2)

O2—S4—O3	112.9 (2)	N7—C6—C5	116.2 (2)
O2—S4—C5	107.2 (2)	N7—C8—C9	110.9 (2)
O3—S4—C5	105.2 (2)	N10—C9—C8	109.7 (2)
C6—N7—C8	113.2 (2)	N10—C11—C12	110.1 (2)
C6—N7—C12	113.0 (2)	N7—C12—C11	110.0 (2)
C8—N7—C12	109.3 (2)	N10—C13—C14	112.7 (2)
C9—N10—C11	109.3 (2)	O15—C14—C13	107.8 (2)
O1—S4—C5—C6	50.8 (2)	C11—N10—C9—C8	-57.0 (3)
O2—S4—C5—C6	-69.8 (2)	C11—N10—C13—C14	179.6 (2)
O3—S4—C5—C6	169.8 (2)	C13—N10—C11—C12	-175.7 (2)
C6—N7—C8—C9	172.8 (2)	C9—N10—C13—C14	-56.5 (3)
C8—N7—C6—C5	56.8 (3)	C9—N10—C11—C12	57.7 (3)
C12—N7—C6—C5	-68.2 (3)	S4—C5—C6—N7	178.6 (2)
C12—N7—C8—C9	-60.3 (3)	N7—C8—C9—N10	59.0 (2)
C8—N7—C12—C11	60.2 (3)	N10—C11—C12—N7	-59.7 (3)
C6—N7—C12—C11	-172.8 (2)	N10—C13—C14—O15	-171.4 (2)
C13—N10—C9—C8	178.2 (2)		

Table 3. Hydrogen-bonding geometry (\AA , $^\circ$)

$D-H \cdots A$	$D-H$	$H \cdots A$	$D \cdots A$	$D-H \cdots A$
N10—H10 \cdots O3 ⁱ	0.86 (2)	1.85 (2)	2.703 (4)	175.7
O15—H15 \cdots N7 ⁱⁱ	0.82 (2)	2.01 (2)	2.822 (3)	171.9

Symmetry codes: (i) $1-x, -y, 1-z$; (ii) $x - \frac{1}{2}, y, \frac{1}{2} - z$.

A rotating anode X-ray generator was used. Corrections for background, decay, Lorentz and polarization factors, but not for absorption effects (an attempted absorption correction based on ψ scans was unsuccessful), were included in the data reduction using XSCANS (Fait, 1991). The structure was solved by direct methods using the program SHELXS86 (Sheldrick, 1990) and refined anisotropically by least-squares techniques using SHELXL93 (Sheldrick, 1993). The H atoms required for hydrogen bonding (on the hydroxyl O atom and on the N atom) were located from difference Fourier maps and refined freely. Methylene H atoms were calculated and refined using a riding model.

Data collection: XSCANS (Fait, 1991). Cell refinement: XSCANS. Molecular graphics: SHELXTL/PC XP. Software used to prepare material for publication: SHELXTL/PC, PLATON (Spek, 1995).

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Lists of structure factors, anisotropic displacement parameters, H-atom coordinates and complete geometry have been deposited with the IUCr (Reference: JZ1093). Copies may be obtained through The Managing Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

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