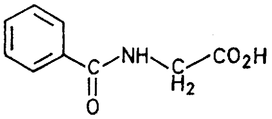
Hippuric Acid: a Neutron Diffraction Analysis

By **Murdoch Currie,*** Chemistry Department, The University, Glasgow G12 8QQ
Alistair L. Macdonald, Chemistry Department, University of British Columbia, Vancouver 8, British Columbia, Canada

Crystals of hippuric acid (*N*-benzoylglycine) are orthorhombic, $a = 10{\cdot}586$, $b = 9{\cdot}123$, $c = 8{\cdot}880$ Å, $Z = 4$ in space group $P2_12_12_1$. The structure has been refined by full-matrix, least-squares methods to R 0·056 for 727 neutron diffraction data. There is good agreement with two previous *X*-ray diffraction analyses and the hydrogen atom parameters have been more precisely determined. The two intermolecular hydrogen bonds have O ··· O 2·679(10), O–H 1·000(15) Å, O–H ··· O 167·9(12)° and N ··· O 3·018(8), N–H 0·997(13) Å, N–H ··· O 160·4(11)°.

If benzoic acid is ingested or produced metabolically it is rendered harmless by conjugation with glycine to give hippuric acid (I) which is excreted; this reaction takes place in the liver and may be used as a diagnostic test of liver function.[1] Hippuric acid is, therefore, a naturally occurring amino-acid found in the urine of most mammals.



(I)

Recently hippuric acid has been the subject of two *X*-ray investigations,[2,3] one of which was carried out in conjunction with e.s.r. studies.[4] In the crystal structure the molecules are linked by a three-dimensional network of intermolecular O–H ··· O and N–H ··· O hydrogen bonds; the present neutron diffraction analysis was undertaken mainly in order to gain more information about this bonding system.

EXPERIMENTAL

Crystal Data.[3]—$C_9H_9NO_3$, $M = 179{\cdot}18$. Orthorhombic, $a = 10{\cdot}586(1)$, $b = 9{\cdot}123(5)$, $c = 8{\cdot}880(2)$ Å, $U = 857{\cdot}6(5)$ Å^3, $D_m = 1{\cdot}371$, $Z = 4$, $D_c = 1{\cdot}388(1)$. Space group $P2_12_12_1$ (D_2^4); no molecular symmetry required.

---

[1] G. H. Bell, J. N. Davidson, and H. Scarborough, 'Textbook of Physiology and Biochemistry,' Livingston, Edinburgh and London, 1956, 3rd edn., p. 347.

[2] H. Ringertz, *Acta Cryst.*, 1971, **B27**, 285.

[3] W. Harrison, S. Rettig, and J. Trotter, *J.C.S. Perkin II*, 1972, 1036.

[4] C. A. McDowell and J. M. Park, unpublished work.

Data Collection.—A crystal, of dimensions 0·73 × 0·29 × 0·25 cm elongated in the c direction and with {011} prominent, was selected and mounted on a Mark VI four-circle diffractometer (controlled by a PDP 8 computer using the ' Andromache ' system) in the DIDO reactor at A.E.R.E., Harwell.

The intensities of *ca.* 1200 reflexions with $\theta \leqslant 45°$ were measured using neutrons monochromated to a wavelength of 1·181 Å (1 Å = 100 pm). An ω—2θ scan was carried out for each reflexion in 40 steps of 0·04° (peak) and 2 × 10 steps of 0·04° (background), with a monitor count (of neutrons) of 25,000 per step.

Corrections for the Lorentz factor, and also for the small errors due to absorption (μ 1·47 cm^{-1}), were applied to the intensities.

Structure Refinement.—Only reflexions with $|F_o| > 3\sigma(F_o)$ were included in the full-matrix least-squares refinement, whose starting point was the set of fractional co-ordinates found from one of the X-ray studies.[3] Four cycles of refinement with isotropic vibrational parameters (R 0·099) were followed by two cycles with anisotropic vibrational parameters (R 0·064). At this point a weighting scheme was introduced, such that $\sqrt{w} = |F_o|/p$ for $|F_o| \leqslant p$, and $\sqrt{w} = p/|F_o|$ for $|F_o| > p$, the value of p being 4. After a further four cycles refinement converged with R 0·056 and R' 0·049 [$R' = (\Sigma w\Delta^2/\Sigma w|F_o|^2)^{\frac{1}{2}}$] for 727 data (*cf.* 750 X-ray data for 54 fewer parameters). Neutron scattering lengths used were: O 0·575, N 0·940, C 0·663, and H −0·372 × 10^{-12} cm. Observed and calculated structure amplitudes with phases are listed in Supplementary Publication No. SUP 20972 (4 pp.).*

Atomic co-ordinates are listed in Table 1, and vibrational parameters in Table 2, with estimated standard deviations derived from the inverse of the least-squares matrix.

TABLE 1

Fractional co-ordinates (×10^5) with standard deviations in parentheses

	x	y	z
O(1)	85163(59)	37067(83)	12642(85)
O(2)	66433(63)	43286(95)	3686(89)
O(3)	52480(58)	52716(65)	38502(76)
N	54572(32)	30063(48)	28666(52)
C(1)	72922(43)	38029(57)	13428(60)
C(2)	68183(47)	31071(59)	27726(65)
C(3)	47621(47)	41106(56)	34222(54)
C(4)	33609(46)	39063(54)	35476(61)
C(5)	26921(49)	49103(67)	44125(63)
C(6)	13855(51)	48241(75)	45126(78)
C(7)	7447(53)	37106(67)	37863(65)
C(8)	14003(46)	26830(66)	29310(83)
C(9)	27179(49)	27736(68)	28057(79)
H(1)	88821(112)	41804(135)	3468(145)
H(2)	72056(111)	20246(143)	28656(178)
H(3)	71917(103)	37881(161)	37021(141)
H(4)	50493(114)	21069(135)	24527(164)
H(5)	32199(138)	57978(146)	49805(178)
H(6)	8417(146)	56071(167)	51721(163)
H(7)	−2926(109)	36296(172)	38457(175)
H(8)	8867(144)	18067(181)	23275(197)
H(9)	32079(109)	19647(154)	21445(182)

DISCUSSION

Hippuric acid is unusual among those amino-acids whose structures have been determined by diffraction methods in that like N-acetylglycine,[5] it does not exist in the crystal as a zwitterion. This was deduced from the inequality of the C–O bond lengths in the carboxylic acid group as found from the X-ray studies and is now confirmed by the precise location of the hydrogen atoms associated with the carboxy and amide groups. Proton transfer to the nitrogen atom is inhibited as a consequence of resonance stabilisation in the amide group. For the same reason, acetylation of the α-amino-group in histidine causes proton transfer to a different site in the molecule which retains its zwitterionic form.[6]

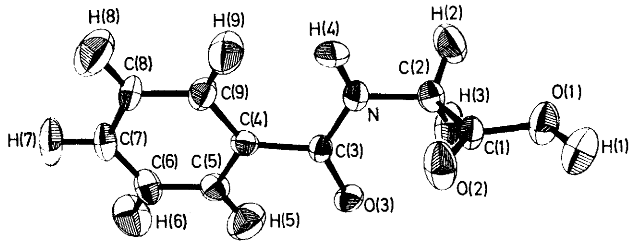
TABLE 2

Anisotropic vibrational parameters (Å^2 × 10^3) * with standard deviations in parentheses

	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O(1)	28(4)	63(5)	43(4)	−4(3)	3(3)	18(4)
O(2)	31(3)	76(5)	44(4)	6(3)	−5(3)	24(4)
O(3)	33(3)	31(3)	43(3)	−6(2)	−9(3)	−4(3)
N	25(2)	34(2)	40(2)	−1(2)	−2(2)	−2(2)
C(1)	27(3)	36(3)	29(2)	−2(2)	0(2)	5(3)
C(2)	27(3)	38(3)	27(3)	3(2)	0(2)	1(3)
C(3)	25(2)	31(3)	22(2)	−1(2)	−2(2)	5(2)
C(4)	28(3)	25(2)	33(2)	0(2)	1(2)	1(2)
C(5)	33(3)	34(3)	36(3)	3(2)	6(2)	−7(3)
C(6)	31(3)	55(4)	44(3)	9(3)	7(3)	−6(3)
C(7)	23(3)	59(4)	37(3)	4(2)	5(2)	2(3)
C(8)	25(3)	46(3)	57(4)	−9(3)	−7(3)	−1(3)
C(9)	29(3)	40(3)	44(3)	−3(2)	−1(3)	−7(3)
H(1)	46(6)	64(7)	44(7)	−18(6)	6(6)	2(6)
H(2)	45(6)	58(7)	77(8)	15(6)	13(7)	23(7)
H(3)	32(6)	90(8)	47(6)	−15(6)	−10(6)	−18(7)
H(4)	46(6)	28(5)	86(10)	1(5)	−1(6)	−16(7)
H(5)	65(8)	54(7)	81(10)	−10(7)	11(7)	−20(7)
H(6)	66(9)	82(9)	67(8)	27(8)	16(7)	−24(8)
H(7)	21(7)	93(10)	83(9)	−4(5)	−6(6)	7(8)
H(8)	61(8)	82(9)	102(11)	−24(8)	−6(9)	−37(9)
H(9)	45(6)	69(8)	77(8)	−6(6)	3(6)	−40(8)

* In the form: $\exp[-2\pi^2(U_{11}h^2a^{*2} + U_{22}k^2b^{*2} + U_{33}l^2c^{*2} + 2U_{12}hka^*b^*\cos\gamma^* + 2U_{13}hla^*c^*\cos\beta^* + 2U_{23}klb^*c^*\cos\alpha^*)]$.

The structure of hippuric acid with anisotropic vibrational ellipsoids for all atoms as derived from the present neutron analysis is shown in the Figure, the atom numbering scheme being that of Harrison *et al.*[3] Bond distances and angles for the non-hydrogen atoms as found from the three analyses are compared in Table 3 and those involving hydrogen atoms, from the neutron analysis only, are given in Table 4.



The hippuric acid molecule viewed down c. The ellipsoids of vibration include 50% probability

The ' heavy ' atom geometry agrees well with that found in both X-ray analyses (Table 3) although certain dimensions of the carboxy-group were erroneously reported by Harrison *et al.*[3] Of all the bond lengths and

---

* For details see Notice to Authors No. 7 in *J.C.S. Perkin II*, 1973, Index issue (items less than 10 pp. are sent as full-size copies).

[5] G. B. Carpenter and J. Donohue, *J. Amer. Chem. Soc.*, 1950, **72**, 2315.

[6] T. J. Kistenmacher, D. J. Hunt, and R. E. Marsh, *Acta Cryst.*, 1972, **B28**, 3352.

TABLE 3

Interatomic distances (Å) and bond angles (degrees) between the non-hydrogen atoms, with standard deviations in parentheses

	Present work (neutron)	Previous work (*X*-ray) Ref. 2	Ref. 3
C(1)–O(1)	1·301(8)	1·328(5)	1·321(3)
C(1)–O(2)	1·204(9)	1·196(6)	1·192(5)
C(1)–C(2)	1·506(8)	1·513(7)	1·506(4)
C(2)–N	1·446(6)	1·454(6)	1·447(4)
N–C(3)	1·342(6)	1·336(6)	1·331(4)
C(3)–O(3)	1·237(8)	1·238(5)	1·250(3)
C(3)–C(4)	1·499(7)	1·496(6)	1·495(4)
C(4)–C(5)	1·389(8)	1·405(6)	1·392(4)
C(5)–C(6)	1·388(8)	1·396(7)	1·386(5)
C(6)–C(7)	1·381(9)	1·391(8)	1·373(5)
C(7)–C(8)	1·392(9)	1·400(8)	1·377(5)
C(8)–C(9)	1·402(7)	1·384(6)	1·389(4)
C(9)–C(4)	1·402(8)	1·407(7)	1·397(4)
O(1)–C(1)–O(2)	123·8(6)	124·1(4)	124·9(3)
O(1)–C(1)–C(2)	110·4(5)	108·9(4)	109·6(3) *
O(2)–C(1)–C(2)	125·7(5)	127·0(4)	125·6(2) *
C(1)–C(2)–N	114·0(5)	112·5(4)	114·0(2)
C(2)–N–C(3)	121·3(4)	121·2(4)	121·7(3)
N–C(3)–C(4)	118·5(4)	117·9(4)	118·8(2) †
N–C(3)–O(3)	121·9(5)	121·5(4)	121·0(3)
O(3)–C(3)–C(4)	119·7(5)	120·6(4)	120·2(3) †
C(3)–C(4)–C(9)	122·5(5)	123·3(4)	123·2(3)
C(3)–C(4)–C(5)	117·6(5)	116·6(4)	117·3(3)
C(4)–C(5)–C(6)	120·4(5)	119·7(4)	119·9(3)
C(5)–C(6)–C(7)	120·1(6)	120·1(5)	120·5(3)
C(6)–C(7)–C(8)	120·4(5)	120·1(4)	120·3(3)
C(7)–C(8)–C(9)	120·0(6)	120·6(5)	120·3(3)
C(8)–C(9)–C(4)	119·3(6)	119·5(5)	119·7(3)
C(9)–C(4)–C(5)	119·9(5)	120·1(4)	119·5(3)

*,† These angles were accidentally transposed in the report of the *X*-ray work.

TABLE 4

Interatomic distances (Å) and bond angles (degrees) involving the hydrogen atoms, with standard deviations in parentheses (neutron analysis only)

C(2)–H(2)	1·073(14)	Mean C(sp^3)–H	1·090(14)
C(2)–H(3)	1·106(14)		
C(5)–H(5)	1·106(15)	Mean C(Ar)–H	1·095(16)
C(6)–H(6)	1·088(16)		
C(7)–H(7)	1·102(13)		
C(8)–H(8)	1·105(18)		
C(9)–H(9)	1·076(16)		
N–H(4)	0·997(13)	O(1)–H(1)	1·000(15)
C(1)–C(2)–H(2)	109·0(9)	C(6)–C(7)–H(7)	121·1(10)
C(1)–C(2)–H(3)	105·9(8)	H(7)–C(7)–C(8)	118·6(10)
H(2)–C(2)–H(3)	108·9(11)	C(7)–C(8)–H(8)	120·4(9)
N–C(2)–H(2)	108·5(8)	H(8)–C(8)–C(9)	119·6(10)
N–C(2)–H(3)	110·4(7)	C(8)–C(9)–H(9)	118·9(9)
C(4)–C(5)–H(5)	118·5(9)	H(9)–C(9)–C(4)	121·9(8)
H(5)–C(5)–C(6)	121·5(9)	H(4)–N–C(2)	117·5(8)
C(5)–C(6)–H(6)	121·6(10)	H(4)–N–C(3)	121·1(8)
H(6)–C(6)–C(7)	118·3(10)	C(1)–O(1)–H(1)	113·6(9)

angles only C(1)–O(1)[2] is more than 3 estimated standard deviations different from the present results. Within the carboxy-group the sum of the C–O distances is 2·505 Å and their difference 0·097 Å. This difference, the fact that the longer C–O bond subtends the smaller angle with the C–C bond, is typical of a normal un-ionised carboxy-group.

The atoms of the molecule are arranged in three planes containing the carboxy, amide, and phenyl groups; details of the planes, given in Table 5, show good agreement with the *X*-ray results and the hydrogen atoms can now be given equal weight. The phenyl and carboxy-groups are planar within experimental error. The amide group is only slightly non-planar with a maximum atomic displacement from the mean plane of 0·04 Å; this is in agreement with previous results,[7] planarity being required for effective resonance contributions to the stability of the group.

TABLE 5

Equations of the best least-squares planes through the molecule in the form $lX + mY + nZ = p$ where X, Y, and Z are co-ordinates in Å. Displacements (Å) of individual atoms from relevant planes are given in square brackets

	l	m	n	p
Plane (1) O(1), O(2), C(1), C(2), H(1) [O(1) 0·0186, O(2) 0·0071, C(1) −0·0104, C(2) −0·0030, H(1) −0·0123, N 0·1299]	−0·0624	−0·8850	−0·4613	−4·0922
Plane (2) C(2)—C(4), N, O(3), H(4) [C(2) −0·0135, C(3) 0·0052, C(4) −0·0328, N −0·0106, O(3) 0·0290, H(4) 0·0408, C(1) 1·3332, C(5) −0·3068, C(9) 0·2236]	−0·0927	0·3955	−0·9138	−1·7664
Plane (3) C(4)—C(9), H(5)—H(9) [C(4) 0·0099, C(5) −0·0093, C(6) 0·0011, C(7) −0·0090, C(8) −0·0080, C(9) 0·0009, H(5) 0·0049, H(6) −0·0009, H(7) 0·0070, H(8) 0·0078, H(9) −0·0044, O(3) 0·3354, N −0·1872, C(3) 0·0712, H(4) −0·3299]	−0·0926	0·5839	−0·8066	−0·7994

Angles between planes: (1)–(2) 85·6°, (2)–(3) 12·4°.

In the phenyl group the mean C–C and C–H distances are 1·392 and 1·095 Å respectively [*cf.* the normal values of 1·395(3)[8] and 1·096[9]]. The mean of the angles in the group is, of course, 120° but the H–C–H angles differ more from this value than the C–C–C angles. Elsewhere, the geometry involving hydrogen atoms is normal with the exception of the C(1)–C(2)–H(3) angle which is 3·5° less than tetrahedral. This may be because atom H(3) is on the same side of the molecule as the peptide oxygen atom and is only 2·47 Å displaced from it. While this is a normal van der Waals contact ($r_{\rm H}$ 1·0 Å[10]) it would be shortened were the C(1)–C(2)–H(3) angle increased.

The molecule contains two hydrogen atoms which are covalently bonded to nitrogen or oxygen and both are involved in the formation of intermolecular hydrogen bonds, the amide oxygen being the acceptor atom in each case. Each molecule is hydrogen-bonded to four other molecules with the result that they are linked into a three-dimensional network in which they are arranged in rows parallel to *a* and stacked in layers along *c*. Details of the two independent hydrogen bonds are given in Table 6 with the *X*-ray results for comparison.

The bond lengths ($D \cdots A$) agree well for all three

---

[7] R. E. Marsh and J. Donohue, *Adv. Protein Chem.*, 1967, **22**, 235.

[8] 'International Tables for *X*-Ray Crystallography,' Kynoch Press, Birmingham, 1962, vol. III, p. 276.

[9] L. E. Sutton, 'Tables of Interatomic Distances and Configuration in Molecules and Ions,' *Chem. Soc. Special Publ.*, No. 18, 1965.

[10] W. H. Baur, *Acta Cryst.*, 1972, **B28**, 1456.

TABLE 6

Hydrogen bond data (distances in Å, angles in degrees)

	C–*D*–H · · · *A*	*D* · · · *A*	*D*–H	H · · · *A*	*D*–H · · · *A*	C–*D* · · · *A*	C–*D*–H
X [a]		2·680(6)	0·74(7)	1·95(6)	166(6)		113(5)
X [b]	C(1)–O(1)–H(1) · · · O(3^{I})	2·667(3)	0·80(5)	1·88(5)	171(5)	119·3(2)	114(3)
N		2·679(10)	1·000(15)	1·692(14)	167·9(12)	120·4(5)	113·6(9)
X [a]		3·011(6)	0·89(6)	2·30(6)	137(5)		103(4) 130(4)
X [b]	C(2), C(3) >N–H(4) · · · O(3^{II})	3·022(3)	0·85(4)	2·19(4)	166(4)	105·6(2) 131·8(2)	115(3) 123(3)
N		3·018(8)	0·997(13)	2·059(15)	160·4(11)	105·7(3) 132·1(3)	117·5(8) 121·1(8)

Roman numerals as superscripts denote the following symmetry related positions relative to atoms x, y, z:

I $3/2 - x, 1 - y, -\frac{1}{2} + z$; II $1 - x, -\frac{1}{2} + y, \frac{1}{2} - z$

[a] Ref. 2. [b] Ref. 3.

sets of results but for both bonds the *D*–H distances, as derived from the *X*-ray analyses are considerably shortened; the electron density of the hydrogen atom is smeared out in the direction of the electronegative atom. An inverse correlation has been observed between the H · · · O and N–H distances in N–H · · · O hydrogen bonds [11] and the trend is confirmed by the present results with the long, weak N–H · · · O bond (3·02 Å) having a relatively short N–H distance (1·00 Å); a similar trend is discernible in O–H · · · O bonds, which tend to be stronger than N–H · · · O bonds.

The angles at O(1) and N involving the hydrogen atoms are significantly distorted from their normal values thus allowing the hydrogen bonds more closely to approach linearity. The angle at O(3) between the hydrogen bonds is 106·5(6): for H(1) · · · O(3) · · · H(4) and 103·7(3)° for O(1) · · · O(3) · · · N.

We thank Professor C. A. McDowell and J. M. Park of the Chemistry Department, University of British Columbia, for providing excellent crystals. We are also indebted to the S.R.C. and the United Kingdom Atomic Energy Authority for making neutron diffraction facilities available and to Mr. D. H. C. Harris and other members of the Materials Physics Division at A.E.R.E., Harwell for their help. Most of the calculations were carried out using the '*X*-Ray' system of programs [12] on the I.C.L. 1906A computer at the Atlas Computer Laboratory, Chilton, where we are grateful to Mrs. C. Y. Hirst for assistance.

[3/2190 *Received*, 24*th October*, 1973]

---

[11] *E.g.* M. N. Frey, M. Lehmann, T. F. Koetzle, and W. C. Hamilton, *Acta Cryst.*, 1973, **B29**, 876.

[12] J. M. Stewart, '*X*-Ray' System of Crystallographic Programs, Computer Science Center, University of Maryland, 1970.