

Redetermination of the Crystal and Molecular Structure of Pteridine

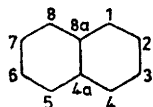
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The crystal structure of pteridine has been redetermined from three-dimensional X-ray diffraction data and refined to a final weighted R of 0.053 for 416 independent reflections measured on a diffractometer. A statistical test with the new data indicated that the previously reported molecular orientation (T. A. Hamor and J. M. Robertson, *J. Chem. Soc.*, 1956, 3586) can be rejected at the 0.995 level. The new molecular orientation, also confirmed by least-squares refinement of atomic occupancy factors, has led to significant shifts in observed bond distances and angles, e.g. C(4)–N(3) is increased from 1.28 to 1.35 Å, and C(2)–N(3)–C(4) decreased from 123 to 116°. The present molecular geometry is more consistent with expected values for bond distances and angles, and with the results of an MO calculation.

PTERIDINE is the parent structure of a large number of biologically important compounds. The pteridine ring system is structurally similar to that of naphthalene, except that it contains nitrogen atoms at the 1-, 3-, 5-, and 8-positions. It can also be considered as being composed of two fused one-ring nitrogen heterocycles: pyrazine and pyrimidine.

The recognition that the pteridines bear a close relationship to hematopoiesis and the demonstration that folic acid, a member of the vitamin B complex, contains a pteridine nucleus, have served to stimulate chemical investigations of the pteridine ring system compounds. Methotrexate, which contains a pteridine nucleus, is being investigated as a possible cancer chemotherapeutic agent.¹ For a recent review of the chemistry of the pteridines, see ref. 2.

The crystal and molecular structure of pteridine was first examined by Hamor and Robertson,³ subsequently referred to as HR. Because of the symmetry of the molecule, there can be four possible orientations [(A)–(D)] of the molecule in the crystal, which can be described by appropriately numbering the nitrogen sites of a naphthalene molecule.



- (A) N(1), N(3), N(5), N(8)
 (B) N(1), N(4), N(5), N(7)
 (C) N(2), N(4), N(5), N(8)
 (D) N(1), N(4), N(6), N(8)

In connection with another project, we routinely attempted to refine all four possible orientational

¹ G. M. dePalo, E. Bajetta, L. Luciani, R. Musumeci, F. D. Re, and G. Bonadonna, *Cancer Chemotherapy Report*, 1973, **57**, 429.

² R. C. Elderfield and A. C. Mehta, *Heterocyclic Compounds*, 1967, **9**, 1.

structures, using the observed structure factors reported by HR. To our surprise, we found that orientation (B) gave better agreement with their observed data than did their reported structure, which has orientation (A). However, the limited amount of data available did not allow good confidence in the correctness of structure (B). We therefore undertook collection of a new set of X-ray diffraction data from crystalline pteridine.

EXPERIMENTAL

Pteridine was synthesized⁴ from 4,5-diaminopyridine and purified by triple sublimation; its identity was confirmed by m.p. and n.m.r. spectrum, and by the subsequent X-ray investigation. None of the sublimed crystals were found suitable for taking X-ray data. Satisfactory crystals were obtained by slow evaporation of an ethyl acetate solution maintained at -16°C . The crystal used was a pale yellow rectangular parallelepiped of dimensions ca. $0.40 \times 0.35 \times 0.20$ mm. Accurate lattice constants (Table 1) were obtained by least-squares extrapolation⁵ of back-reflection

TABLE I
Lattice constants

Cell edge	HR	This work
a	24.70(5)	24.741(2)
b	3.79(1)	3.779(1)
c	6.32(1)	6.300(2)

data taken using Cr- K_{α} radiation ($\lambda = 2.2916$ Å). The observed systematically absent reflections were consistent with the space group $Pna2_1$ as reported by HR.

The intensities of 416 independent reflections (up to $2\theta = 45^{\circ}$) were measured on a Siemens manual diffractometer with zirconium-filtered Mo- K_{α} radiation ($\lambda = 0.7107$ Å). The diffraction unit was equipped with a

³ T. A. Hamor and J. M. Robertson, *J. Chem. Soc.*, 1956, 3586.

⁴ A. Albert and H. Yamamoto, *J. Chem. Soc. (C)*, 1968, 2289.

⁵ D. E. Williams, 'LCR-2, A Fortran Lattice Constant Refinement Program,' U.S. At. Energy Comm., Report IS 1052.

single-crystal orienter, scintillation counter, and a pulse-height analyser. Intensities were measured by use of the θ - 2θ scan technique, at a scan rate of 2° min^{-1} . Pteridine is known to decompose upon exposure to light and in order to minimize the exposure time the background was determined graphically. The observed intensity was set equal to total counts (C_T) minus estimated background (C_B). The estimated standard deviation was obtained from the equation: $\sigma^2(I) = C_T + C_B + (0.05 \cdot C_T)^2 + (0.05 \cdot C_B)^2$. A total of 44 reflections were observed as having zero intensity ($C_T \leq C_B$), and were included in the least-squares refinement. Weighting factors were set equal to the reciprocal of the estimated variance of F_o . The usual Lorentz and polarization corrections were made.

The data were refined⁶ using the initial atomic locations found by HR. All four possibilities for the nitrogen atom locations were considered concurrently. Table 2 summarizes the results of the refinements. Observed and calculated structure factors are listed in Supplementary Publication No. SUP 21156 (2 pp.).*

TABLE 2
Summary of weighted R factor results

	(A)	(B)	(C)	(D)	No. of variables
(1) All non-H atoms; isotropic temperature factors	0.131	0.126	0.148	0.141	40
(2) All non-H atoms; anisotropic temperature factors	0.098	0.087	0.106	0.107	90
(3) All atoms; anisotropic temperature factors; vary H positional parameters only	0.069	0.053			102

DISCUSSION

As with the data of HR, our data also gave the best agreement for orientation (B). The weighted R factors obtained corresponding to the alternative orientations were tested for the hypotheses that structures (A), (C), and (D) can be rejected⁷ in favour of structure (B). For refinements (1) it was found that orientations (C) and (D) could be rejected at the 0.995 significance level; this was also true for refinements (2). However, neither (1) nor (2) could distinguish between orientations (A) and (B).

The location of the hydrogen atoms is important in making a distinction between the orientations. When these hydrogens were included, in refinements (3), it was found that orientation (A) could be rejected at the 0.995 significance level. Additional attempts to pinpoint the hydrogen locations by means of difference-Fourier maps did not lead to conclusive results. Also, the hydrogens in refinement (3) for (A) converged to reasonable positions.

To further establish the correctness of orientation (B), we undertook a least-squares refinement of the occupancy factors of atomic sites (1)–(8). In this refinement all heavy-atom sites were assigned carbon scattering-factors

* See Notice to Authors No. 7 in *J.C.S. Perkin II*, 1973, Index issue.

⁶ W. R. Busing, K. O. Martin, and H. A. Levy, ORFLS, Oak Ridge National Laboratory, Oak Ridge, Tennessee, 1962, Report ORNL TM 305.

and initial occupancy factors of 1.00 so as not to bias any particular site in favour of being that of a nitrogen atom. Except for atoms (4a) and (8a), which were definitely known to be carbon, all heavy-atom sites were assigned variable occupancy factors.

Table 3 shows the results of these refinements. Two

TABLE 3
Occupancy factors refinement

Atom	No H	All atoms
(1)	1.34(3)	1.33(3)
(2)	0.98(3)	0.99(3)
(3)	1.04(4)	1.04(4)
(4)	1.31(3)	1.30(3)
(4a)	1.00	1.00
(5)	1.33(3)	1.32(3)
(6)	1.00(3)	0.99(3)
(7)	1.27(3)	1.27(3)
(8)	1.00(3)	1.00(3)
(8a)	1.00	1.00
R	0.068	0.051

series were calculated, first without hydrogen atoms, and then with these included at the indicated sites

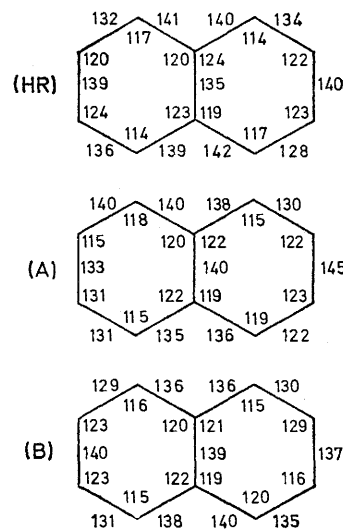


FIGURE 1 The observed structure reported by HR, and our results for orientations (A) and (B). For the purpose of comparing molecular structures, the nitrogen positions here are always those of orientation (A). Distances ($\text{\AA} \times 10^2$) and angles (deg.) are given

2, 3, 6, and 8. Table 3 clearly shows, even without the possible bias resulting from the inclusion of hydrogen, that orientation (B) is correct. The difference between carbon and nitrogen exhibited by the occupancy factors is *ca.* 10σ .

We therefore conclude, not only that orientation (B) is correct, but also that it is unlikely that even a fraction of orientation (A) is contained in our crystal. The data of HR were taken from a crystal prepared by sublimation whereas our crystal was obtained by low-temperature crystallization from solution. Thus, it is possible that our crystal was more ordered than theirs because of our lower temperature of crystallization.

Figure 1 shows the bond distances and angles for ⁷ W. C. Hamilton, 'Statistics in Physical Science,' Ronald Press, New York, 1964.

HR's structure [orientation (A)] and for our refinements of orientations (A) and (B) using the new data. We obtained some changes from the HR structure for orientation (A). Bond length C(7)-N(8) increased from 1.32 to 1.40 Å and other shifts of ≥ 0.05 Å were observed. The angles at C(6) and C(7) became more distorted from 120°, but C(2)-N(3)-C(4) remained 123°. The observed structure for orientation (B) shows changes which will be discussed. The exact bond distances and bond angles and their standard deviations are given in Table 4.

TABLE 4

Bond distances (Å) and bond angles (°), and their standard deviations as obtained from the final refinement of orientation (B)

(a) Distances			
C(8a)-N(1)	1.361(10)	C(4a)-N(5)	1.384(10)
N(1)-C(2)	1.304(7)	N(5)-C(6)	1.308(7)
C(2)-N(3)	1.365(8)	C(6)-C(7)	1.400(10)
N(3)-C(4)	1.350(10)	C(7)-N(8)	1.286(10)
C(4)-C(4a)	1.399(9)	N(8)-C(8a)	1.359(8)
C(4a)-C(8a)	1.392(7)		
(b) Angles			
N(8)-C(8a)-N(1)	117.9(6)	C(4)-C(4a)-C(8a)	119.1(6)
N(8)-C(8a)-C(4a)	120.9(5)	C(4)-C(4a)-N(5)	119.1(6)
C(4a)-C(8a)-N(1)	121.2(6)	C(8a)-C(4a)-N(5)	121.8(5)
C(8a)-N(1)-C(2)	115.2(5)	C(4a)-N(5)-C(6)	114.5(5)
N(1)-C(2)-N(3)	129.1(6)	N(5)-C(6)-C(7)	122.9(6)
C(2)-N(3)-C(4)	115.6(6)	C(6)-C(7)-N(8)	123.4(7)
N(3)-C(4)-C(4a)	119.8(7)	C(7)-N(8)-C(8a)	116.4(6)

The observed structures of *s*-triazine,⁸ pyrazine,⁹ and pyrimidine¹⁰ may be used to obtain reference distances

is unexpectedly short. The angle C(2)-N(3)-C(4) is also unexpectedly large.

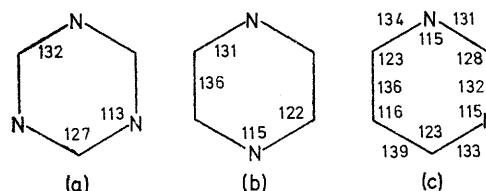


FIGURE 2 Observed distances and angles in (a) *s*-triazine, (b) pyrazine, and (c) pyrimidine

When orientation (B) is used for the refinement of the structure, the values of C(8a)-N(1), C(2)-N(3), and C(8a)-N(8) are shifted towards those expected; distance C(7)-N(8) changes from being 0.07 Å longer than expected to become 0.04 Å smaller than expected. Distance C(4)-N(3) is much improved, so that the C(4)-N(3) and C(2)-N(3) distances are nearly equal, in marked contrast to the situation in orientation (A).

The observed bond angles in orientation (B) are markedly improved. Figure 2 shows that the C-N-C angles in *s*-triazine, pyrazine, and pyrimidine are all less than 120°, with a mean of 114°. In view of the lone pair of electrons on the nitrogen atom, VSEPR rules¹¹ predict that this angle should be less than 120°. Many other examples of aromatic C-N-C angles less than 120° are known; when this angle is greater than 120°, some other effect, such as steric strain, is the likely cause.

TABLE 5

Observed atomic co-ordinates, and temperature factors * ($\times 10^4$)

	<i>x</i>	<i>y</i>	<i>z</i>	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
N(1)	1344(2)	617(14)	-2576(9)	23(1)	723(45)	205(18)	1(5)	12(4)	-74(25)
C(2)	830(2)	528(17)	-3054(13)	18(1)	829(58)	206(19)	-1(7)	-4(5)	3(30)
N(3)	399(2)	1722(14)	-1907(14)	20(1)	884(44)	254(21)	3(6)	-6(4)	-20(32)
C(4)	519(3)	3178(16)	-5(16)	23(1)	721(56)	245(33)	15(7)	14(6)	-16(32)
C(4a)	1059(2)	3475(15)	633(17)	12(1)	579(43)	225(22)	-4(6)	5(4)	37(31)
N(5)	1174(2)	4999(14)	2580	23(1)	878(48)	165(14)	-17(6)	10(3)	-6(22)
C(6)	1689(3)	5168(17)	3033(13)	23(1)	854(57)	243(21)	22(7)	-2(5)	-51(32)
C(7)	2091(3)	3817(23)	1698(15)	16(1)	1141(71)	295(24)	-6(8)	-11(6)	7(39)
N(8)	1992(2)	2387(15)	-112(15)	11(1)	1036(51)	323(28)	3(5)	-6(5)	-67(28)
C(8a)	1464(3)	2175(16)	-687(16)	24(2)	547(49)	140(19)	27(7)	7(5)	71(26)
H(2) †	789(21)	-764(155)	-4449(110)						
H(4) †	176(19)	4286(147)	889(91)						
H(6) †	1851(24)	6513(158)	4431(107)						
H(7) †	2432(23)	3732(183)	2036(101)						

* In the form: $\exp(-\beta_{11}h^2 - \beta_{22}k^2 - \beta_{33}l^2 - 2\beta_{12}hk - 2\beta_{13}hl - 2\beta_{23}kl)$. † These atoms were assigned the same temperature factors as the atoms to which they are bonded.

and angles for comparison, and are shown in Figure 2. The C-N distances range from 1.314 to 1.343 Å, with a weighted mean of 1.332 Å. The C-N-C angles range from 113.2 to 115.2°, with a weighted mean of 114.6°. Conjugation of the fused ring system in pteridine complicates the use of these reference values, but an MO calculation (see later) takes this conjugation effect into account.

The pteridine structure corresponding to orientation (A) has unexpectedly long C-N distances C(8a)-N(1), C(2)-N(3), C(7)-N(8), and C(8a)-N(8), although C(4)-N(3)

The observed C(2)-N(3)-C(4) angle of 116° for orientation (B) is therefore strong evidence for this structure, as compared with the value of 123° for both the HR structure and our refinement of orientation (A). It may be further noted that the N(5)-C(6)-C(7) and C(6)-C(7)-N(8) angles appear to be improved in orientation (B), as contrasted with orientation (A).

Bond C(4)-N(3) is known to be highly reactive in pteridine; orientation (B) depicts it as being slightly longer than expected, in contrast to the very short distance for orientation (A). Since chemical reactivity

⁸ P. J. Wheatley, *Acta Cryst.*, 1955, **8**, 224.

⁹ P. J. Wheatley, *Acta Cryst.*, 1957, **10**, 182.

¹⁰ P. J. Wheatley, *Acta Cryst.*, 1960, **13**, 80.

¹¹ R. J. Gillespie, *Angew. Chem. Internat. Edn.*, 1967, **6**, 819.

involves consideration of the geometry and electronic structure of the transition state, it is not advisable to predict reactivity from the ground-state structure alone.

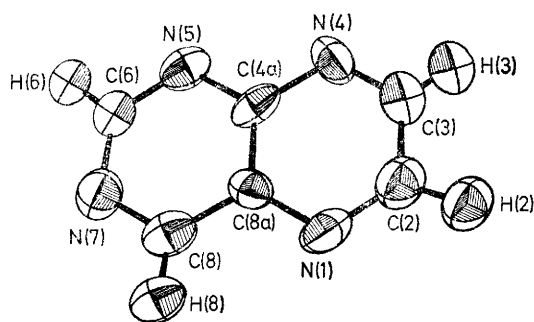


FIGURE 3 The observed thermal motion

A recent SCF-MO calculation¹² for pteridine predicted C(4)-N(3) 1.31 Å, in reasonable agreement with HR's value of 1.28 Å, but in poor agreement with our

results for orientation (A) (1.22 Å). Our result (1.35 Å) for orientation (B) agrees much better with the SCF-MO result. We calculated the root-mean-square deviations of the HR structure, orientation (A), and orientation (B) from the SCF-MO results as 0.042, 0.059, and 0.026 Å. Thus, orientation (B) also yields the best agreement with this theoretical prediction.

In conclusion, the combination of the statistical test, occupancy factor test, agreement with observed distances and angles in similar molecules, and agreement with the SCF-MO calculation supports the correctness of structure (B) for the pteridine molecule and orientation (B) in the crystal. Table 5 shows the final refined atomic co-ordinates for structure (B). Figure 3 shows the observed anisotropic vibrational ellipsoids.¹³

We thank the National Institutes of Health for financial support of this work.

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¹² B. Tinland, *Theor. Chim. Acta*, 1967, **8**, 361.

¹³ C. K. Johnson, ORTEP, Oak Ridge National Laboratory, Oak Ridge, Tennessee, 1965, Report ORNL 3794.