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1*H*-Benzo[*g*]pteridine-2,4-dione

Rao M. Uppu^{a*} and Frank R. Fronczek^b^aDepartment of Environmental Toxicology, Southern University and A&M College, Baton Rouge, LA 70813, USA, and^bDepartment of Chemistry, Louisiana State University, Baton Rouge, LA 70803, USA. *Correspondence e-mail:

rao_uppu@subr.edu

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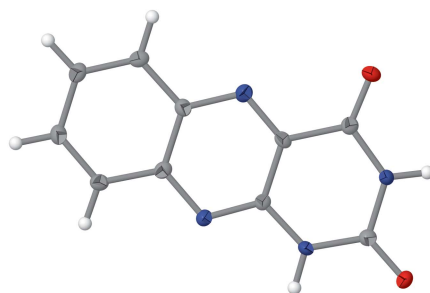
Keywords: alloxazine; Isoalloxazine; crystal structure; tautomerism; hydrogen bonding.

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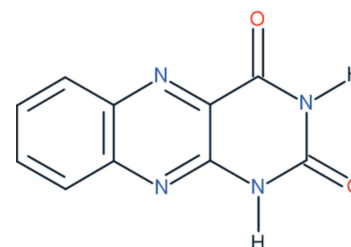
Structural data: full structural data are available from iucrdata.iucr.org

The structure of the title compound, C₁₀H₆N₄O₂, reported by Smalley *et al.* [(2021). *Cryst. Growth Des.* **22**, 524–534] from powder diffraction data and ¹⁵N NMR spectroscopy, is confirmed using low-temperature data from a twinned crystal. The tautomer in the solid state is alloxazine (1*H*-benzo[*g*]pteridine-2,4-dione) rather than isoalloxazine (10*H*-benzo[*g*]pteridine-2,4-dione). In the extended structure, the molecules form hydrogen-bonded chains propagating in the [011] direction through alternating centrosymmetric *R*₂²(8) rings with pairwise N—H···O interactions and centrosymmetric *R*₂²(8) rings with pairwise N—H···N interactions. The crystal chosen for data collection was found to be a non-merohedral twin (180° rotation about [001]) in a 0.446 (4):0.554 (6) domain ratio.

3D view



Chemical scheme



Structure description

1*H*-Benzo[*g*]pteridine-2,4-dione, popularly known as alloxazine, is a tautomer of isoalloxazine (10*H*-benzo[*g*]pteridine-2,4-dione), the same ring system that is present in riboflavin, flavin nucleotides (FMN and FAD), and flavoproteins. Unlike nicotinamide coenzymes, NAD(P)⁺ and NAD(P)H, flavin nucleotides serve in both one-electron and two-electron transfer reactions because the isoalloxazine ring can exist in several different ionization and/or redox states (Massey & Hemmerich, 1980). Further, the strong but mostly non-covalent interactions within the flavoprotein binding site allow the fine-tuning of the redox chemistry of the isoalloxazine ring system (Ghisla *et al.*, 1974; Hu *et al.*, 2015; van den Heuvel *et al.*, 2002) which, among many things, helps in minimizing the 1-electron reduction of molecular oxygen to the superoxide anion radical. It is believed that the spatial arrangement of the reacting oxygen molecule may have a direct bearing on the outcome of a flavoprotein serving as an oxidase or dehydrogenase function (Chaiyen *et al.*, 2012), a process that can be mimicked in simple chemical model



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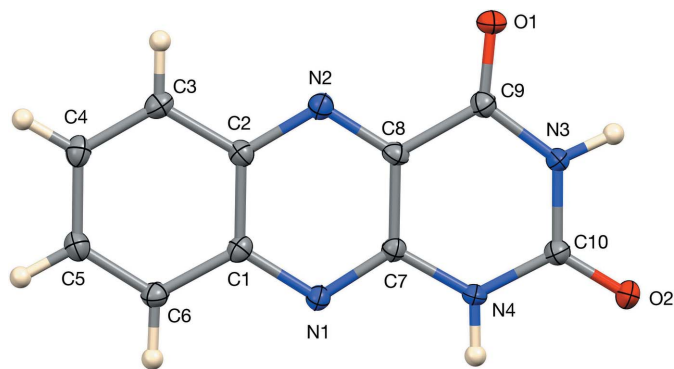


Figure 1
The title molecule with 50% ellipsoids.

systems of phenazine reacting with NAD(P)H in micelle forming surfactant solutions (Nishikimi *et al.*, 1972; Rao, 1989*a,b*; Uppu, 1995). While there have been several efforts to define flavin–protein interactions that have mainly capitalized on differences in the chemical reactivity of the protein-bound flavin, we were surprised to note that, except for one recent study by Smalley *et al.* (2022), there are hardly any studies of the crystal structure of alloxazine itself.

In view of the above and since two-thirds of flavoprotein allelic variants are linked to human diseases (Lienhart *et al.* 2013), we determined the crystal structure of alloxazine using a Bruker Kappa APEXII DUO diffractometer. Using low-temperature (90 K) data from twinned crystals, our results confirm the observations of Smalley *et al.* (2022), who used powder diffraction data along with ¹⁵N NMR spectroscopy. The tautomer in the solid state is alloxazine rather than isoalloxazine. The N-bound hydrogen atoms were located and their positions were refined in order to confirm the tautomer. The molecule, shown in Fig. 1 is nearly planar, with an r.m.s deviation for 16 non-hydrogen atoms of 0.015 Å and a maximum deviation of 0.025 (3) Å for C5.

The intermolecular hydrogen bonding (Table 1) is shown in Fig. 2. Atom N4 donates a hydrogen bond to N1, forming an $R_2^2(8)$ ring (Etter *et al.*, 1990) about the inversion center at $0, 1, \frac{1}{2}$. Similarly, N3 donates a hydrogen bond to O2, forming a centrosymmetric $R_2^2(8)$ ring about $0, \frac{1}{2}, 1$. Thus, these two pairs of interactions combine to form a hydrogen-bonded chain propagating in the $[0\bar{1}1]$ direction. The planes of the N,N

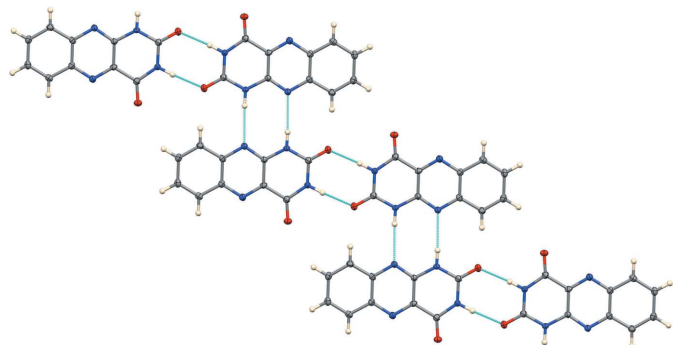


Figure 2
The hydrogen-bonded chain.

Table 1
Hydrogen-bond geometry (Å, °).

| $D-H\cdots A$ | $D-H$ | $H\cdots A$ | $D\cdots A$ | $D-H\cdots A$ |
|------------------------|----------|-------------|-------------|---------------|
| $N3-H3N\cdots O2^i$ | 0.92 (3) | 2.00 (4) | 2.883 (3) | 162 (3) |
| $N4-H4N\cdots N1^{ii}$ | 0.90 (3) | 2.22 (3) | 3.114 (3) | 176 (3) |
| $C3-H3\cdots N2^{iii}$ | 0.95 | 2.58 | 3.520 (4) | 173 |
| $C6-H6\cdots O2^{ii}$ | 0.95 | 2.21 | 3.158 (4) | 173 |

Symmetry codes: (i) $-x, -y + 1, -z + 2$; (ii) $-x, -y + 2, -z + 1$; (iii) $-x + 2, -y + 1, -z + 1$.

Table 2
Experimental details.

| | |
|----------------------------------------------------------------------------|------------------------------------------------------------------------|
| Crystal data | $C_{10}H_6N_4O_2$ |
| Chemical formula | 214.19 |
| M_r | Triclinic, $P\bar{1}$ |
| Crystal system, space group | 90 |
| Temperature (K) | 5.8027 (2), 7.5404 (3), 10.1345 (4) |
| a, b, c (Å) | 70.483 (2), 84.150 (2), 84.208 (3) |
| α, β, γ (°) | 414.72 (3) |
| V (Å ³) | 2 |
| Z | Cu $K\alpha$ |
| Radiation type | 1.06 |
| μ (mm ⁻¹) | $0.11 \times 0.06 \times 0.02$ |
| Crystal size (mm) | |
| Data collection | |
| Diffractometer | Bruker Kappa APEXII DUO CCD |
| Absorption correction | Multi-scan (<i>TWINABS</i> ; Bruker, 2001) |
| T_{min}, T_{max} | 0.774, 0.979 |
| No. of measured, independent and observed [$I > 2\sigma(I)$] reflections | 6802, 6802, 4832 |
| R_{int} | ? |
| $(\sin \theta/\lambda)_{max}$ (Å ⁻¹) | 0.607 |
| Refinement | |
| $R[F^2 > 2\sigma(F^2)], wR(F^2), S$ | 0.048, 0.144, 1.07 |
| No. of reflections | 6802 |
| No. of parameters | 152 |
| H-atom treatment | H atoms treated by a mixture of independent and constrained refinement |
| $\Delta\rho_{max}, \Delta\rho_{min}$ (e Å ⁻³) | 0.26, -0.28 |

Computer programs: *APEX2* (Bruker, 2016), *SAINT* (Bruker, 2016), *SHELXT2014/5* (Sheldrick, 2015*a*), *SHELXL2017/1* (Sheldrick, 2015*b*), *Mercury* (Macrae *et al.*, 2020), and *publCIF* (Westrip, 2010).

dimers related by the $N-H\cdots O$ hydrogen bonds are offset by 0.915 (2) Å, as illustrated in Fig. 3.

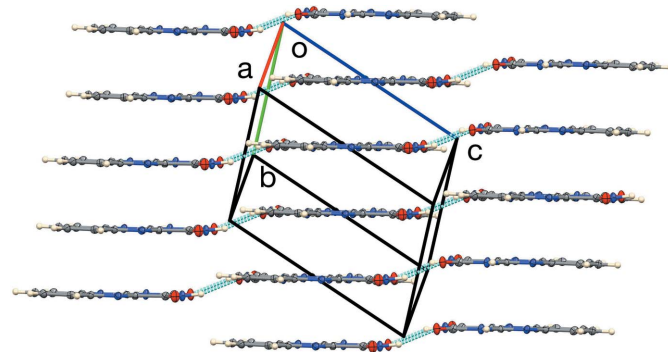


Figure 3
The unit cell, showing the offsets of hydrogen-bonded dimers.

Synthesis and crystallization

The title compound, C₁₀H₆N₄O₂ (alloxazine) was obtained from Sigma-Aldrich, St. Louis, Missouri, USA and was used without further purification. Single crystals in the form of pale yellow plates were prepared by slow cooling of a nearly saturated solution of alloxazine in dimethyl formamide at 135 ± 2°C

Refinement

Crystal data, data collection and structure refinement details are summarized in Table 2. The crystal chosen for data collection was refined as a two-component non-merohedral twin, by 180° rotation about the reciprocal [001] direction. Both twin components were integrated. Refinement was against an HKLF 5 file prepared using *TWINABS*. The refined BASF parameter is 0.446 (4). Seven outlier reflections were omitted from the refinement.

Funding information

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full crystallographic data

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1H-Benzo[g]pteridine-2,4-dione

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1H-Benzo[g]pteridine-2,4-dione*Crystal data*

$C_{10}H_6N_4O_2$

$M_r = 214.19$

Triclinic, $P\bar{1}$

$a = 5.8027$ (2) Å

$b = 7.5404$ (3) Å

$c = 10.1345$ (4) Å

$\alpha = 70.483$ (2)°

$\beta = 84.150$ (2)°

$\gamma = 84.208$ (3)°

$V = 414.72$ (3) Å³

$Z = 2$

$F(000) = 220$

$D_x = 1.715$ Mg m⁻³

Cu $K\alpha$ radiation, $\lambda = 1.54184$ Å

Cell parameters from 4433 reflections

$\theta = 4.6$ – 69.1 °

$\mu = 1.06$ mm⁻¹

$T = 90$ K

Plate, pale yellow

$0.11 \times 0.06 \times 0.02$ mm

Data collection

Bruker Kappa APEXII DUO CCD
diffractometer

Radiation source: $I\mu S$ microfocus

QUAZAR multilayer optics monochromator

φ and ω scans

Absorption correction: multi-scan
(*TWINABS*; Bruker, 2001)

$T_{\min} = 0.774$, $T_{\max} = 0.979$

6802 measured reflections

6802 independent reflections

4832 reflections with $I > 2\sigma(I)$

$\theta_{\max} = 69.4$ °, $\theta_{\min} = 4.6$ °

$h = -7 \rightarrow 7$

$k = -9 \rightarrow 9$

$l = -12 \rightarrow 12$

Refinement

Refinement on F^2

Least-squares matrix: full

$R[F^2 > 2\sigma(F^2)] = 0.048$

$wR(F^2) = 0.144$

$S = 1.07$

6802 reflections

152 parameters

0 restraints

Primary atom site location: dual

Hydrogen site location: mixed

H atoms treated by a mixture of independent
and constrained refinement

$w = 1/[\sigma^2(F_o^2) + (0.0713P)^2 + 0.0047P]$

where $P = (F_o^2 + 2F_c^2)/3$

$(\Delta/\sigma)_{\max} < 0.001$

$\Delta\rho_{\max} = 0.26$ e Å⁻³

$\Delta\rho_{\min} = -0.28$ e Å⁻³

Special details

Geometry. All esds (except the esd in the dihedral angle between two l.s. planes) are estimated using the full covariance matrix. The cell esds are taken into account individually in the estimation of esds in distances, angles and torsion angles; correlations between esds in cell parameters are only used when they are defined by crystal symmetry. An approximate (isotropic) treatment of cell esds is used for estimating esds involving l.s. planes.

Refinement. Refined as a 2-component twin, by 180 deg. rotation about reciprocal 0 0 1. Refinement was vs. an HKLF 5 file prepared using TWINABS. The refined BASF parameter is 0.446 (4). Seven outlier reflections were omitted from the refinement.

All H atoms were located in difference maps and those on C were thereafter treated as riding in geometrically idealized positions with C—H distances 0.95 Å. Coordinates of the N—H hydrogen atom were refined. $U_{\text{iso}}(\text{H})$ values were assigned as $1.2U_{\text{eq}}$ of the attached atom.

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

| | <i>x</i> | <i>y</i> | <i>z</i> | $U_{\text{iso}}^*/U_{\text{eq}}$ |
|-----|-------------|------------|------------|----------------------------------|
| O1 | 0.6141 (4) | 0.4107 (3) | 0.8404 (2) | 0.0215 (6) |
| O2 | −0.0946 (3) | 0.7091 (3) | 0.8834 (2) | 0.0213 (6) |
| N1 | 0.2942 (4) | 0.8937 (4) | 0.4485 (2) | 0.0150 (6) |
| N2 | 0.6687 (4) | 0.6283 (4) | 0.5564 (2) | 0.0160 (6) |
| N3 | 0.2614 (4) | 0.5646 (4) | 0.8587 (3) | 0.0166 (6) |
| H3N | 0.231 (5) | 0.487 (5) | 0.949 (4) | 0.020* |
| N4 | 0.1014 (4) | 0.7992 (4) | 0.6692 (2) | 0.0160 (6) |
| H4N | −0.018 (6) | 0.884 (5) | 0.638 (3) | 0.019* |
| C1 | 0.4878 (5) | 0.8700 (4) | 0.3647 (3) | 0.0154 (7) |
| C2 | 0.6759 (5) | 0.7382 (4) | 0.4193 (3) | 0.0154 (7) |
| C3 | 0.8747 (5) | 0.7183 (4) | 0.3300 (3) | 0.0165 (7) |
| H3 | 1.001181 | 0.631113 | 0.366387 | 0.020* |
| C4 | 0.8827 (5) | 0.8255 (5) | 0.1911 (3) | 0.0181 (7) |
| H4 | 1.014200 | 0.811737 | 0.130392 | 0.022* |
| C5 | 0.6963 (5) | 0.9562 (5) | 0.1379 (3) | 0.0179 (7) |
| H5 | 0.704994 | 1.030108 | 0.041346 | 0.022* |
| C6 | 0.5034 (5) | 0.9801 (5) | 0.2209 (3) | 0.0171 (7) |
| H6 | 0.380553 | 1.069981 | 0.182484 | 0.020* |
| C7 | 0.2926 (5) | 0.7827 (4) | 0.5806 (3) | 0.0135 (6) |
| C8 | 0.4803 (5) | 0.6503 (4) | 0.6354 (3) | 0.0147 (7) |
| C9 | 0.4648 (5) | 0.5301 (5) | 0.7850 (3) | 0.0163 (7) |
| C10 | 0.0784 (5) | 0.6913 (5) | 0.8079 (3) | 0.0164 (7) |

Atomic displacement parameters (\AA^2)

| | U^{11} | U^{22} | U^{33} | U^{12} | U^{13} | U^{23} |
|----|-------------|-------------|-------------|--------------|--------------|--------------|
| O1 | 0.0173 (11) | 0.0228 (13) | 0.0192 (11) | 0.0062 (9) | −0.0022 (9) | −0.0018 (9) |
| O2 | 0.0187 (11) | 0.0237 (13) | 0.0164 (10) | 0.0027 (9) | 0.0043 (9) | −0.0024 (9) |
| N1 | 0.0137 (12) | 0.0175 (15) | 0.0133 (13) | −0.0003 (10) | −0.0001 (10) | −0.0047 (11) |
| N2 | 0.0168 (12) | 0.0151 (14) | 0.0160 (13) | −0.0017 (10) | 0.0009 (10) | −0.0055 (11) |
| N3 | 0.0150 (13) | 0.0179 (14) | 0.0124 (12) | 0.0033 (10) | 0.0007 (10) | −0.0008 (10) |
| N4 | 0.0141 (13) | 0.0180 (14) | 0.0125 (12) | 0.0046 (11) | −0.0020 (10) | −0.0016 (10) |
| C1 | 0.0145 (14) | 0.0168 (16) | 0.0163 (16) | −0.0015 (12) | −0.0001 (12) | −0.0074 (13) |
| C2 | 0.0159 (14) | 0.0156 (16) | 0.0143 (15) | −0.0015 (12) | −0.0017 (12) | −0.0040 (13) |
| C3 | 0.0139 (14) | 0.0174 (17) | 0.0199 (15) | −0.0003 (12) | −0.0012 (12) | −0.0085 (13) |
| C4 | 0.0164 (15) | 0.0220 (17) | 0.0167 (15) | −0.0043 (12) | 0.0015 (12) | −0.0071 (12) |
| C5 | 0.0197 (15) | 0.0195 (17) | 0.0140 (15) | −0.0025 (12) | −0.0010 (12) | −0.0044 (12) |
| C6 | 0.0151 (14) | 0.0195 (17) | 0.0170 (15) | 0.0018 (12) | −0.0030 (12) | −0.0068 (13) |
| C7 | 0.0140 (14) | 0.0127 (16) | 0.0139 (14) | −0.0014 (11) | 0.0000 (12) | −0.0048 (12) |

| | | | | | | |
|-----|-------------|-------------|-------------|--------------|--------------|--------------|
| C8 | 0.0159 (15) | 0.0149 (16) | 0.0133 (16) | 0.0006 (12) | -0.0048 (12) | -0.0039 (13) |
| C9 | 0.0162 (15) | 0.0176 (17) | 0.0152 (16) | -0.0007 (12) | -0.0014 (12) | -0.0056 (13) |
| C10 | 0.0155 (15) | 0.0176 (17) | 0.0158 (15) | 0.0010 (12) | -0.0013 (12) | -0.0056 (12) |

Geometric parameters (Å, °)

| | | | |
|------------|-----------|-----------|-----------|
| O1—C9 | 1.217 (3) | C1—C6 | 1.414 (4) |
| O2—C10 | 1.224 (4) | C1—C2 | 1.422 (4) |
| N1—C7 | 1.321 (4) | C2—C3 | 1.420 (4) |
| N1—C1 | 1.372 (4) | C3—C4 | 1.368 (4) |
| N2—C8 | 1.318 (4) | C3—H3 | 0.9500 |
| N2—C2 | 1.358 (4) | C4—C5 | 1.407 (4) |
| N3—C10 | 1.372 (4) | C4—H4 | 0.9500 |
| N3—C9 | 1.381 (4) | C5—C6 | 1.364 (4) |
| N3—H3N | 0.92 (3) | C5—H5 | 0.9500 |
| N4—C10 | 1.369 (4) | C6—H6 | 0.9500 |
| N4—C7 | 1.377 (4) | C7—C8 | 1.423 (4) |
| N4—H4N | 0.90 (3) | C8—C9 | 1.483 (4) |
| C7—N1—C1 | 115.5 (3) | C5—C4—H4 | 120.0 |
| C8—N2—C2 | 116.9 (3) | C6—C5—C4 | 122.0 (3) |
| C10—N3—C9 | 127.3 (3) | C6—C5—H5 | 119.0 |
| C10—N3—H3N | 113 (2) | C4—C5—H5 | 119.0 |
| C9—N3—H3N | 119 (2) | C5—C6—C1 | 119.5 (3) |
| C10—N4—C7 | 123.5 (3) | C5—C6—H6 | 120.2 |
| C10—N4—H4N | 116 (2) | C1—C6—H6 | 120.2 |
| C7—N4—H4N | 121 (2) | N1—C7—N4 | 118.0 (3) |
| N1—C1—C6 | 119.7 (3) | N1—C7—C8 | 123.0 (3) |
| N1—C1—C2 | 121.5 (3) | N4—C7—C8 | 119.0 (3) |
| C6—C1—C2 | 118.8 (3) | N2—C8—C7 | 122.0 (3) |
| N2—C2—C3 | 118.8 (3) | N2—C8—C9 | 118.3 (3) |
| N2—C2—C1 | 121.1 (3) | C7—C8—C9 | 119.8 (3) |
| C3—C2—C1 | 120.1 (3) | O1—C9—N3 | 121.7 (3) |
| C4—C3—C2 | 119.5 (3) | O1—C9—C8 | 124.5 (3) |
| C4—C3—H3 | 120.3 | N3—C9—C8 | 113.8 (3) |
| C2—C3—H3 | 120.3 | O2—C10—N4 | 121.8 (3) |
| C3—C4—C5 | 120.1 (3) | O2—C10—N3 | 121.6 (3) |
| C3—C4—H4 | 120.0 | N4—C10—N3 | 116.6 (3) |

Hydrogen-bond geometry (Å, °)

| <i>D</i> —H... <i>A</i> | <i>D</i> —H | H... <i>A</i> | <i>D</i> ... <i>A</i> | <i>D</i> —H... <i>A</i> |
|---------------------------|-------------|---------------|-----------------------|-------------------------|
| N3—H3N...O2 ⁱ | 0.92 (3) | 2.00 (4) | 2.883 (3) | 162 (3) |
| N4—H4N...N1 ⁱⁱ | 0.90 (3) | 2.22 (3) | 3.114 (3) | 176 (3) |
| C3—H3...N2 ⁱⁱⁱ | 0.95 | 2.58 | 3.520 (4) | 173 |
| C6—H6...O2 ⁱⁱ | 0.95 | 2.21 | 3.158 (4) | 173 |

Symmetry codes: (i) $-x, -y+1, -z+2$; (ii) $-x, -y+2, -z+1$; (iii) $-x+2, -y+1, -z+1$.