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A Crystallographic and Molecular Mechanics Study of Inhibitors of Dihydroorotase

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Abstract

(I) Methyl L-dihydroorotate, $C_6H_8N_2O_4$, M_r = 172.14, orthorhombic, $P2_12_12_1$, a = 6.941 (2), b = 9.708 (2), c = 23.329 (5) Å, V = 1572 Å^3, Z = 8, D_x = 1.455 g cm^{-3}, Mo $K\alpha$, λ = 0.71069 Å, μ = 0.81 cm^{-1}, $F(000)$ = 720, T = 294 K, final R = 0.036 for 793 reflections. (II) Methyl L-6-thiodihydroorotate, $C_6H_8N_2O_3S$, M_r = 188.21, monoclinic, $P2_1$, a = 6.235 (2), b = 20.821 (4), c = 6.882 (1) Å, β = 110.82 (2)°, V = 835.0 Å^3, Z = 4, D_x = 1.497 g cm^{-3}, Mo $K\alpha$, λ = 0.71069 Å, μ = 3.02 cm^{-1}, $F(000)$ =

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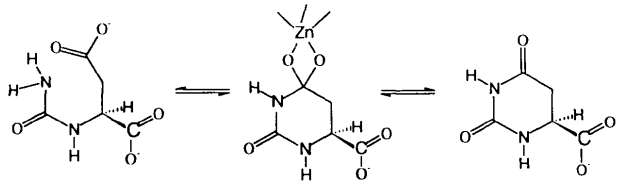
392, $T = 294$ K, final $R = 0.037$ for 1404 reflections. (III) Dimethyl *trans*-2-oxohexahydropyrimidine-4,6-dicarboxylate, $C_8H_{12}N_2O_5$, $M_r = 216.20$, triclinic, $P\bar{1}$, $a = 7.3977$ (5), $b = 8.4149$ (8), $c = 9.314$ (1) Å, $\alpha = 74.65$ (1), $\beta = 68.08$ (1), $\gamma = 98.77$ (1)°, $V = 502.80$ Å^3, $Z = 2$, $D_x = 1.428$ g cm^{-3}, Mo $K\alpha$, $\lambda = 0.71069$ Å, $\mu = 0.78$ cm^{-1}, $F(000) = 228$, $T = 294$ K, final $R = 0.040$ for 1468 reflections. (IV) Dimethyl 2-oxo-1,2,3,6-tetrahydropyrimidine-4,6-dicarboxylate, $C_8H_{10}N_2O_5$, $M_r = 214.18$, triclinic, $P\bar{1}$, $a = 7.481$ (3), $b = 8.344$ (3), $c = 9.042$ (5) Å, $\alpha = 95.05$ (3), $\beta = 111.02$ (3), $\gamma = 108.31$ (3)°, $V = 487.18$ Å^3, $Z = 2$, $D_x = 1.460$ g cm^{-3}, Mo $K\alpha$, $\lambda = 0.71069$ Å, $\mu = 0.78$ cm^{-1}, $F(000) = 224$, $T = 294$ K, final $R = 0.040$ for 1253 reflections. The three-dimensional structures of the methyl esters of dihydroorotate and three potential inhibitors of the enzyme, dihydroorotase, have been determined. Correlations between the structures of these compounds and their inhibitory activities are discussed. It is postulated that for strong binding to dihydroorotase to occur, a pyrimidine ring with three groups capable of forming strong interactions is required; two of these groups must be coplanar with the ring or equatorially disposed, and the third group must be axially disposed. Molecular mechanics modeling has been used to investigate the conformational isomerism of the compounds and the role it plays in determining binding and consequent inhibition of dihydroorotase.

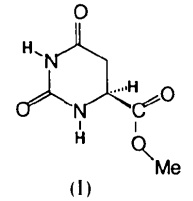
Introduction

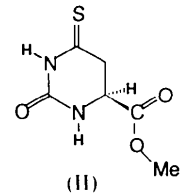
The enzyme dihydroorotase catalyzes the conversion of *N*-carbamyl-L-aspartate to L-dihydroorotate (see scheme below) the third reaction of the *de novo* pathway for pyrimidine nucleotide biosynthesis. It has been shown that the active site of dihydroorotase contains a bound zinc atom (Kelly, Mally & Evans, 1986) and a transition state of the form shown below has been proposed (Christopherson & Jones, 1980; Christopherson & Lyons, 1990).

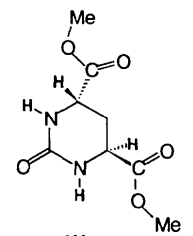


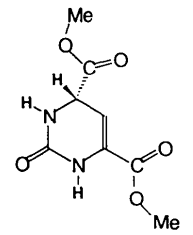
N-Carbamyl-L-aspartate Transition state L-Dihyroorotate

A number of potentially potent 'transition state' inhibitors of this enzyme have been designed on the basis of this proposed mechanism of catalysis and some are potent inhibitors of the enzyme (Christopherson, Schmalzl, Szabados, Goodridge, Harsanyi, Sant, Algar, Anderson, Armstrong, Sharma, Bubb & Lyons, 1989). The reaction catalysed by dihydroorotase is freely reversible and the dissociation constants for interaction of the substrate and the product *N*-carbamyl-L-aspartate and L-dihydroorotate with the enzyme are 420 and 20 μM respectively (Christopherson *et al.*, 1989). L-6-Thiodihydroorotate (II) is a potent inhibitor with a dissociation constant of 0.85 μM which decreases to 0.17 μM in the presence of excess Zn^{2+} ion. 2-Oxo-1,2,3,6-tetrahydropyrimidine-4,6-dicarboxylate (IV) was synthesized as a 'transition-state analogue' for the reaction and has a dissociation constant of 0.74 μM, and the further reduced form *trans*-2-oxohexahydropyrimidine-4,6-dicarboxylate (III) has a dissociation constant of approximately 12 μM (Christopherson *et al.*, 1989). To establish what structural features these inhibitors might have in common we have determined the crystal structures of compounds (II), (III) and (IV) (see scheme below). In addition, the structure of the natural substrate, L-dihydroorotate [DHO, (I)] has been determined. Crystallization of the free acids proved to be difficult and therefore all structures were determined as the methyl esters.


(I)


(II)


(III)


(IV)

Experimental

The preparation and the measurement of cytotoxic activity of compounds (II)–(IV) against leukaemia cells growing in culture has been described elsewhere (Christopherson *et al.*, 1989; Brooke, Szabados, Lyons & Christopherson, 1990). Crystals were obtained from non-aqueous solvents by either slow evaporation or cooling.

Structure determination and refinement

Crystals were mounted on glass fibres with cyanoacrylate resin. Data were collected on an

Table 1. *Summary of data-collection and processing parameters*

	(I)	(II)	(III)	(IV)
Data-collection range (°)	$2 < 2\theta < 45$	$2 < 2\theta < 50$	$2 < 2\theta < 50$	$2 < 2\theta < 50$
Scan width ($+0.34\tan\theta$) (°)	1.00	1.00	1.00	1.50
Horizontal counter aperture ($+1.05\tan\theta$) (mm)	2.70	2.70	2.70	2.70
Scan type	ω–θ	ω–θ	ω–θ	ω–0.67θ
Range of hkl	$+h, +k, +l$	$\pm h, +k, +l$	$\pm h, \pm k, +l$	$\pm h, \pm k, +l$
R_{int}	–	0.010	0.007	0.010
Total data collected	1240	1649	1900	1814
Data with $I > 2.5\sigma(I)$	793	1404	1468	1253
Total variables	229	227	185	177
$R = \sum(\lvert F_o\rvert - \lvert F_c\rvert)/\sum F_o$	0.036	0.037	0.040	0.040
$wR = \sum(w^{1/2}F_o - F_c)/w^{1/2}\sum(F_o)$	0.039	0.043	0.047	0.048
Weighting constants*	$k = 1.43$	$k = 1.08$	$k = 4.64$	$k = 2.29$
	$g = 0.00033$	$g = 0.0012$	$g = 0.00010$	$g = 0.00021$

*Weight $w = k/[\sigma^2(F_o) + gF_o^2]$, g and k refined.

Enraf–Nonius CAD-4 automatic diffractometer using graphite-monochromated Mo $K\alpha$ radiation. 25 independent reflections with $20 < 2\theta < 30°$ were used for least-squares determination of cell constants. Intensities of three reflections were monitored, and indicated less than 2.5% decomposition for all structures. The structures were solved by direct methods using *SHELXS*86 (Sheldrick, 1985). Non-hydrogen atoms were refined with anisotropic thermal parameters. The positions of the hydrogen atoms were determined from difference density maps and were refined with isotropic thermal parameters. Full-matrix least-squares refinement converged with all shifts less than 0.01σ. Maximum excursions in final difference maps for (I) were 0.2 and -0.2 e Å^{-3} and for (II), (III) and (IV) were 0.3 and -0.2 e Å^{-3}. The carboxylate group in molecule 2 of structure (I) was found to be disordered by rotation by 180° about the C(14)—C(15) bond. As a consequence two sites for C(16) were observed and refined; at convergence the ratio of the two sites was 77 (2): 23 (2). Data reduction and application of Lorentz and polarization corrections were carried out using the *Enraf–Nonius Structure Determination Package* (Frenz, 1985). Absorption corrections were not applied. All other calculations were performed using the *SHELX*76 system of programs (Sheldrick, 1976). Drawings were produced using program *ORTEP* (Johnson, 1965). Scattering factors used were those supplied in *SHELX*76. Data collection and refinement parameters are collected in Table 1. Final positional parameters and bond lengths and angles are listed in Tables 2–9.*

* Lists of structure amplitudes, anisotropic thermal parameters of non-hydrogen atoms, positional and thermal parameters of hydrogen atoms, least-planes calculations, and close contacts have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 55507 (51 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 2. *Final atomic coordinates for* (I) *with e.s.d.'s in parentheses, and equivalent isotropic thermal parameters* B_{eq} (Å^2)

The prime indicates the minor site of a disordered atom; occupancy C(16):C(16′) 77 (2):23 (2).

$B_{eq} = (8\pi^2/3)\sum_i\sum_j U_{ij}a_i^*a_j^*\mathbf{a}_i.\mathbf{a}_j$.

	x	y	z	B_{eq}
O(1)	−0.0145 (6)	0.3702 (4)	0.1594 (2)	3.52
O(2)	−0.3512 (7)	0.2737 (6)	0.3189 (2)	5.30
O(3)	0.2549 (8)	0.4226 (5)	0.3772 (2)	5.38
O(4)	0.1913 (11)	0.5340 (6)	0.2964 (2)	8.13
N(1)	−0.1750 (7)	0.3336 (5)	0.2418 (2)	3.07
N(2)	0.1544 (7)	0.2943 (6)	0.2361 (2)	3.41
C(1)	−0.0080 (10)	0.3344 (6)	0.2101 (2)	2.85
C(2)	−0.1958 (10)	0.2795 (7)	0.2959 (3)	3.47
C(3)	−0.0149 (10)	0.2241 (7)	0.3221 (3)	4.10
C(4)	0.1673 (10)	0.2876 (8)	0.2974 (3)	3.66
C(5)	0.2066 (10)	0.4284 (8)	0.3222 (3)	4.23
C(6)	0.2990 (17)	0.5524 (11)	0.4057 (4)	9.18
O(11)	−0.4919 (6)	−0.1245 (5)	0.3276 (2)	4.49
O(12)	0.1350 (7)	0.1042 (5)	0.4566 (2)	4.48
O(13)	−0.6974 (9)	0.0038 (7)	0.5448 (2)	7.38
O(14)	−0.6602 (13)	−0.1671 (8)	0.4834 (3)	9.10
N(11)	−0.3190 (7)	−0.0161 (6)	0.3959 (2)	3.38
N(12)	−0.6528 (7)	0.0045 (6)	0.3922 (2)	3.40
C(11)	−0.4917 (10)	−0.0496 (6)	0.3701 (2)	3.25
C(12)	−0.2957 (10)	0.0795 (7)	0.4380 (3)	3.10
C(13)	−0.4727 (9)	0.1519 (7)	0.4562 (3)	4.07
C(14)	−0.6527 (9)	0.0658 (7)	0.4486 (3)	3.89
C(15)	−0.6699 (11)	−0.0481 (12)	0.4941 (3)	5.70
C(16)	−0.7101 (22)	−0.1164 (17)	0.5898 (6)	11.39
C(16′)	−0.6967 (51)	−0.2274 (34)	0.5349 (13)	4.71

Table 3. *Final atomic coordinates for* (II) *with e.s.d.'s in parentheses, and equivalent isotropic thermal parameters* B_{eq} (Å^2)

$B_{eq} = (8\pi^2/3)\sum_i\sum_j U_{ij}a_i^*a_j^*\mathbf{a}_i.\mathbf{a}_j$.

	x	y	z	B_{eq}
S(1)	0.3756 (2)	0.7773 (1)	0.1413 (2)	4.05
C(1)	0.0412 (7)	0.9364 (2)	0.1682 (6)	3.31
N(1)	0.1746 (6)	0.8889 (2)	0.1222 (6)	3.40
C(2)	0.2293 (6)	0.8311 (2)	0.2159 (6)	3.14
O(1)	0.0307 (6)	0.9901 (2)	0.0949 (5)	4.54
N(2)	−0.0699 (6)	0.9188 (2)	0.2935 (6)	3.66
C(3)	0.1498 (6)	0.8208 (2)	0.3957 (6)	3.32
C(4)	−0.0811 (6)	0.8522 (2)	0.3513 (6)	3.00
C(5)	−0.1728 (6)	0.8486 (2)	0.5283 (6)	3.47
O(2)	−0.1428 (5)	0.7911 (2)	0.6101 (4)	3.79
O(3)	−0.2654 (8)	0.8917 (2)	0.5766 (7)	5.96
C(6)	−0.2509 (7)	0.7774 (3)	0.7620 (6)	4.53
S(11)	0.0883 (2)	1.1501 (1)	−0.1600 (2)	4.51
C(11)	0.3528 (8)	0.9847 (2)	−0.2496 (7)	3.93
N(11)	0.2322 (6)	1.0339 (2)	−0.1923 (6)	3.84
C(12)	0.2388 (7)	1.0964 (2)	−0.2327 (6)	3.27
O(11)	0.3643 (7)	0.9314 (2)	−0.1738 (7)	5.65
N(12)	0.4511 (7)	1.0010 (2)	−0.3859 (6)	4.14
C(13)	0.3969 (7)	1.1152 (2)	−0.3478 (7)	4.76
C(14)	0.4072 (6)	1.0621 (2)	−0.4957 (6)	3.25
C(15)	0.5896 (8)	1.0726 (2)	−0.5864 (6)	3.69
O(12)	0.5864 (5)	1.1313 (2)	−0.6573 (5)	4.25
O(13)	0.7267 (9)	1.0329 (2)	−0.5935 (8)	7.14
C(16)	0.7509 (9)	1.1456 (3)	−0.7573 (8)	5.36

Molecular mechanics

The parameters for modeling the pyrimidine ring were taken from the *AMBER* force field and were modified along the lines outlined previously (Hambley, 1988). A list of force field parameters has been deposited. Energy minimization was performed using a local program, *MOMEC*87 (Hambley, 1987*b*). The barrier to conformational intercon-

Table 4. *Final atomic coordinates for* (III) *with e.s.d.'s in parentheses, and equivalent isotropic thermal parameters* B_{eq} (Å^2)

$B_{eq} = (8\pi^2/3)\sum_i\sum_j U_{ij}a_i^*a_j^*\mathbf{a}_i.\mathbf{a}_j$.

	x	y	z	B_{eq}
O(1)	0.7605 (2)	−0.0028 (2)	0.4708 (2)	3.81
O(2)	0.1089 (2)	0.5053 (2)	0.8045 (2)	5.01
O(3)	0.1438 (2)	0.4039 (2)	0.5961 (2)	5.26
O(4)	0.6961 (3)	0.2559 (2)	0.9902 (2)	5.73
O(5)	0.7429 (3)	0.0084 (2)	0.9246 (2)	6.01
N(1)	0.4891 (2)	0.1765 (2)	0.6082 (2)	3.34
N(2)	0.8074 (2)	0.1530 (2)	0.6114 (2)	3.60
C(1)	0.6888 (2)	0.1032 (2)	0.5611 (2)	2.91
C(2)	0.3915 (3)	0.2830 (2)	0.7282 (2)	2.86
C(3)	0.5334 (3)	0.3814 (2)	0.7255 (2)	3.04
C(4)	0.7361 (3)	0.2612 (3)	0.7282 (2)	3.18
C(5)	0.2015 (3)	0.4021 (2)	0.7000 (2)	3.10
C(6)	−0.0761 (4)	0.6273 (4)	0.7904 (4)	5.67
C(7)	0.7261 (3)	0.1578 (2)	0.8901 (2)	3.40
C(8)	0.6868 (7)	0.1728 (4)	1.1493 (3)	6.83

Table 5. *Final atomic coordinates for* (IV) *with e.s.d.'s in parentheses, and equivalent isotropic thermal parameters* B_{eq} (Å^2)

$B_{eq} = (8\pi^2/3)\sum_i\sum_j U_{ij}a_i^*a_j^*\mathbf{a}_i.\mathbf{a}_j$.

	x	y	z	B_{eq}
O(1)	0.2625 (2)	0.4927 (2)	0.0232 (2)	4.22
O(2)	0.6078 (3)	1.0324 (2)	−0.2708 (2)	4.87
O(3)	0.7655 (3)	0.8838 (3)	−0.1150 (3)	5.93
O(4)	−0.1656 (3)	0.7579 (2)	−0.4836 (2)	5.28
O(5)	−0.1873 (3)	0.5027 (3)	−0.4249 (3)	7.26
N(1)	0.4182 (3)	0.6917 (2)	−0.0903 (2)	3.56
N(2)	0.0941 (3)	0.6533 (3)	−0.1094 (2)	4.20
C(1)	0.2564 (3)	0.6050 (3)	−0.0559 (2)	3.15
C(2)	0.4166 (3)	0.8219 (3)	−0.1759 (3)	3.16
C(3)	0.2505 (3)	0.8603 (3)	−0.2393 (3)	3.65
C(4)	0.0571 (3)	0.7619 (3)	−0.2237 (3)	3.56
C(5)	0.6141 (3)	0.9136 (3)	−0.1845 (3)	3.55
C(6)	0.7948 (5)	1.1273 (4)	−0.2869 (5)	5.39
C(7)	−0.1122 (3)	0.6551 (3)	−0.3879 (3)	3.79
C(8)	−0.3331 (7)	0.6752 (5)	−0.6434 (5)	7.08

Table 6. *Bond lengths* (Å) *and angles* (°) *for* (I)

C(1)—O(1)	1.233 (6)	C(2)—O(2)	1.206 (8)
C(5)—O(4)	1.194 (8)	C(5)—O(3)	1.328 (7)
C(6)—O(3)	1.458 (10)	C(1)—N(1)	1.375 (7)
C(2)—N(1)	1.376 (8)	C(1)—N(2)	1.338 (8)
C(4)—N(2)	1.435 (7)	C(3)—C(2)	1.496 (9)
C(4)—C(3)	1.520 (9)	C(5)—C(4)	1.509 (9)
C(11)—O(11)	1.229 (6)	C(12)—O(12)	1.221 (7)
C(15)—O(14)	1.184 (10)	C(15)—O(13)	1.299 (9)
C(16)—O(13)	1.573 (15)	C(11)—N(11)	1.380 (8)
C(12)—N(11)	1.361 (8)	C(11)—N(12)	1.339 (8)
C(14)—N(12)	1.443 (8)	C(13)—C(12)	1.477 (9)
C(14)—C(13)	1.513 (9)	C(15)—C(14)	1.538 (11)
C(6)—O(3)—C(5)	117.2 (6)	C(2)—N(1)—C(1)	125.8 (5)
C(4)—N(2)—C(1)	121.2 (5)	N(1)—C(1)—O(1)	119.1 (6)
N(2)—C(1)—O(1)	123.2 (6)	N(2)—C(1)—N(1)	117.6 (4)
N(1)—C(2)—O(2)	121.4 (6)	C(3)—C(2)—O(2)	123.6 (6)
C(3)—C(2)—N(1)	115.0 (6)	C(4)—C(3)—C(2)	113.5 (5)
C(3)—C(4)—N(2)	110.1 (6)	C(5)—C(4)—N(2)	110.6 (6)
C(5)—C(4)—C(3)	111.9 (6)	O(4)—C(5)—O(3)	123.1 (7)
C(4)—C(5)—O(3)	112.2 (6)	C(4)—C(5)—O(4)	124.7 (6)
C(16)—O(13)—C(15)	109.2 (9)	C(12)—N(11)—C(11)	125.4 (6)
C(14)—N(12)—C(11)	120.9 (5)	N(11)—C(11)—O(11)	119.5 (6)
N(12)—C(11)—O(11)	122.9 (6)	N(12)—C(11)—N(11)	117.7 (5)
N(11)—C(12)—O(12)	120.0 (6)	C(13)—C(12)—O(12)	124.3 (5)
C(13)—C(12)—N(11)	115.6 (6)	C(14)—C(13)—C(12)	113.0 (5)
C(13)—C(14)—N(12)	109.5 (5)	C(15)—C(14)—N(12)	109.4 (6)
C(15)—C(14)—C(13)	112.4 (6)	O(14)—C(15)—O(13)	125.4 (8)
C(14)—C(15)—O(13)	111.1 (9)	C(14)—C(15)—O(14)	123.5 (8)

version was estimated by fixing torsion angles at intermediate values at 10° intervals using the method of Lagrangian multipliers (Hambley, 1987*a*).

Table 7. *Bond lengths* (Å) *and angles* (°) *for* (II)

C(2)—S(1)	1.638 (4)	N(1)—C(2)	1.353 (5)
C(3)—C(2)	1.504 (5)	C(1)—N(1)	1.400 (5)
O(1)—C(1)	1.219 (5)	N(2)—C(1)	1.336 (5)
C(4)—N(2)	1.452 (5)	C(4)—C(3)	1.511 (5)
C(5)—C(4)	1.521 (5)	O(3)—C(5)	1.178 (5)
O(2)—C(5)	1.310 (5)	C(6)—O(2)	1.460 (5)
C(12)—S(11)	1.649 (4)	N(11)—C(12)	1.334 (5)
C(13)—C(12)	1.519 (5)	C(11)—N(11)	1.409 (5)
O(11)—C(11)	1.220 (5)	N(12)—C(11)	1.334 (5)
C(14)—N(12)	1.456 (5)	C(14)—C(13)	1.521 (6)
C(15)—C(14)	1.495 (5)	O(13)—C(15)	1.203 (6)
O(12)—C(15)	1.315 (5)	C(16)—O(12)	1.455 (5)
N(1)—C(2)—S(1)	122.3 (3)	C(3)—C(2)—S(1)	122.9 (3)
C(4)—C(2)—N(1)	114.8 (3)	C(2)—N(1)—C(1)	126.3 (3)
O(1)—C(1)—N(1)	120.3 (3)	N(2)—C(1)—N(1)	116.0 (3)
N(2)—C(1)—O(1)	123.8 (4)	C(4)—N(2)—C(1)	121.8 (3)
C(3)—C(4)—N(2)	109.5 (3)	C(5)—C(4)—N(2)	109.5 (3)
C(5)—C(3)—C(4)	114.8 (3)	C(4)—C(3)—C(2)	109.9 (3)
O(2)—C(5)—C(3)	123.4 (4)	O(2)—C(5)—C(4)	110.6 (4)
O(3)—C(5)—O(2)	125.9 (4)	C(6)—O(2)—C(5)	117.1 (4)
N(11)—C(12)—S(11)	122.4 (3)	C(13)—C(12)—S(11)	121.9 (3)
C(13)—C(12)—N(11)	115.7 (4)	C(12)—N(11)—C(11)	126.3 (4)
O(11)—C(11)—N(11)	120.0 (4)	N(12)—C(11)—N(11)	115.9 (4)
N(12)—C(11)—O(11)	124.1 (4)	C(14)—N(12)—C(11)	122.6 (3)
C(13)—C(14)—N(12)	109.4 (3)	C(15)—C(14)—N(12)	108.3 (3)
C(15)—C(14)—C(13)	113.1 (3)	C(14)—C(13)—C(12)	110.6 (3)
O(13)—C(15)—C(14)	125.0 (4)	O(12)—C(15)—C(14)	112.2 (3)
O(13)—C(15)—O(12)	122.8 (4)	C(16)—O(12)—C(15)	116.5 (4)

Table 8. *Bond lengths* (Å) *and angles* (°) *for* (III)

C(1)—O(1)	1.244 (2)	C(5)—O(2)	1.320 (2)
C(6)—O(2)	1.453 (3)	C(5)—O(3)	1.189 (2)
C(7)—O(4)	1.318 (2)	C(8)—O(4)	1.448 (3)
C(7)—O(5)	1.190 (2)	C(1)—N(1)	1.354 (2)
C(2)—N(1)	1.446 (2)	C(1)—N(2)	1.345 (2)
C(4)—N(2)	1.442 (2)	C(3)—C(2)	1.520 (2)
C(5)—C(2)	1.513 (2)	C(4)—C(3)	1.523 (2)
C(7)—C(4)	1.517 (3)		
C(6)—O(2)—C(5)	116.9 (2)	C(8)—O(4)—C(7)	116.7 (2)
C(2)—N(1)—C(1)	124.1 (2)	C(4)—N(2)—C(1)	124.9 (1)
N(1)—C(1)—O(1)	121.0 (2)	N(2)—C(1)—O(1)	121.2 (1)
N(2)—C(1)—N(1)	117.7 (1)	C(3)—C(2)—N(1)	110.0 (1)
C(5)—C(2)—N(1)	108.8 (1)	C(5)—C(2)—C(3)	111.2 (1)
C(4)—C(3)—C(2)	110.2 (1)	C(3)—C(4)—N(2)	110.3 (1)
C(7)—C(4)—N(2)	111.4 (1)	C(7)—C(4)—C(3)	111.6 (1)
O(3)—C(5)—O(2)	124.3 (2)	C(2)—C(5)—O(2)	111.2 (2)
C(2)—C(5)—O(3)	124.5 (2)	O(5)—C(7)—O(4)	123.6 (2)
C(4)—C(7)—O(4)	110.8 (2)	C(4)—C(7)—O(5)	125.6 (2)

Table 9. *Bond lengths* (Å) *and angles* (°) *for* (IV)

C(1)—O(1)	1.230 (2)	C(5)—O(2)	1.316 (3)
C(6)—O(2)	1.441 (3)	C(5)—O(3)	1.198 (3)
C(7)—O(4)	1.320 (3)	C(8)—O(4)	1.455 (4)
C(7)—O(5)	1.178 (3)	C(1)—N(1)	1.359 (3)
C(2)—N(1)	1.388 (3)	C(1)—N(2)	1.338 (3)
C(4)—N(2)	1.443 (3)	C(3)—C(2)	1.322 (3)
C(5)—C(2)	1.469 (3)	C(4)—C(3)	1.486 (3)
C(7)—C(4)	1.520 (3)		
C(6)—O(2)—C(5)	117.2 (2)	C(8)—O(4)—C(7)	117.0 (2)
C(2)—N(1)—C(1)	122.6 (2)	C(4)—N(2)—C(1)	126.9 (2)
N(1)—C(1)—O(1)	121.6 (2)	N(2)—C(1)—O(1)	122.7 (2)
N(2)—C(1)—N(1)	115.7 (2)	C(3)—C(2)—N(1)	121.6 (2)
C(5)—C(2)—N(1)	114.4 (2)	C(5)—C(2)—C(3)	124.0 (2)
C(4)—C(3)—C(2)	120.6 (2)	C(3)—C(4)—N(2)	110.6 (2)
C(7)—C(4)—N(2)	110.8 (2)	C(7)—C(4)—C(3)	111.3 (2)
O(3)—C(5)—O(2)	124.4 (2)	C(2)—C(5)—O(2)	113.6 (2)
C(2)—C(5)—O(3)	122.0 (2)	O(5)—C(7)—O(4)	124.5 (2)
C(4)—C(7)—O(4)	110.2 (2)	C(4)—C(7)—O(5)	125.3 (2)

Description of the structures

(I) The structure consists of two independent L-dihydroorotate methyl ester molecules linked by hydrogen bonds between the H(amine) atoms and an exocyclic O atom. There are only small conformational differences between the independent mol-

ecules. A plot of one of these molecules is shown in Fig. 1. The conformation of the dihydroorotate ring approximates an envelope; the largest deviations from the least-squares plane through atoms N(1), N(2), C(1), C(2) and C(3) is 0.05 Å. C(4), which defines the peak, lies 0.54 Å out of the same plane. The carboxylate group is axially disposed with respect to the dihydroorotate ring. Bond lengths in the pyrimidine ring show there is delocalization of electrons from the carbonyl C=O bond into the adjacent C—N bonds. These bonds, C(1)—N(1) [1.375 (7) Å], C(2)—N(1) [1.376 (8) Å] and C(1)—N(2) [1.338 (8) Å] are substantially shorter than the other C—N bond N(2)—C(4) [1.435 (7) Å]. The C(1)—O(1) bond [1.233 (6) Å] is longer than a true double bond such as in the carboxylate groups (1.19 Å). Bonds to the amine N atom bonded to the two carbonyl C atoms are longer [1.375 (7) and 1.376 (8) Å] than the other C(carbonyl)—N(amine) bond in this structure.

(II) The structure of the 6-L-thiodihydroorotate methyl ester molecules is analogous to that of (I) in that it consists of two independent molecules linked by hydrogen bonds between the H(amine) atoms and exocyclic and carboxylate O(carbonyl) atoms of the independent molecules. The molecules are indentical apart from small differences in the conformation of the carboxylate groups. Discussion hereafter is limited to molecule 1, an *ORTEP* plot of which is shown in Fig. 2. The conformation of the dihydroorotate ring is best described as ruffled; C(1), N(1), C(2) and C(3) are close to being coplanar, N(2) lies slightly to one side of this plane and C(4) lies further toward the same side. The carboxylate group, in contrast to that in (I), is equatorial with respect to the dihydroorotate ring. Bond lengths in the ring are generally similar to those in (I); a significant difference is in the C(1)—N(1) and C(2)—N(1) bond lengths [1.400 (5) and 1.353 (5) Å] which are longer and shorter respectively than the equivalent bonds in (I). The difference in these bonds indicates that the presence of the S atom results in more electron delocalization from the adjacent N—C bond than is the case in the oxo compound.

(III) The structure consists of neutral molecules of *trans*-HTDP, *trans*-2-oxo-hexahydropyrimidine-4,6-dimethoxycarboxyate, with hydrogen bonds between the H(amine) atoms and the exocyclic O atom. An *ORTEP* plot of the molecule is shown in Fig. 3. The reduced pyrimidine ring has an envelope conformation; deviations from the least-squares plane through N(1), N(2), C(1), C(2) and C(4) are all less than 0.05 Å. C(3), which defines the peak of the envelope, is 0.60 Å out of the plane and the exocyclic O atom, O(1), lies 0.14 Å toward the opposite side of the plane. The methoxycarboxylate groups bonded to C(2) and C(4) are respectively equatorial and axial with respect to the pyrimidine ring. The two N—C(carbonyl) bond lengths are almost identical [1.345 (2) and 1.354 (2) Å].

(IV) The structure consists of neutral HDDP methyl ester molecules, dimethyl 2-oxo-1,2,3,6-tetrahydropyrimidine-4,6-dicarboxylate, with hydrogen bonds between each of the H(amine) atoms and

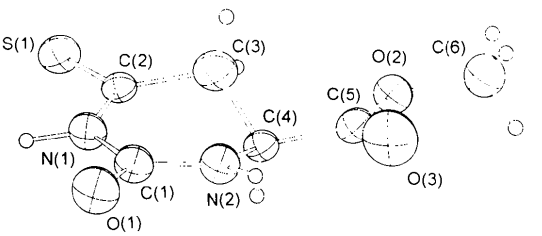


Fig. 2. An *ORTEP* plot of (II) giving the crystallographic atom numbering. Thermal ellipsoids are shown at the 30% probability level.

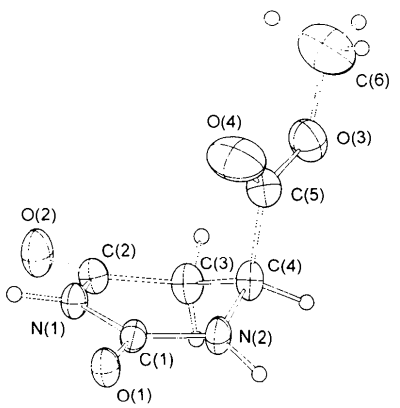


Fig. 1. An *ORTEP* plot of (I) giving the crystallographic atom numbering. Thermal ellipsoids are shown at the 30% probability level.

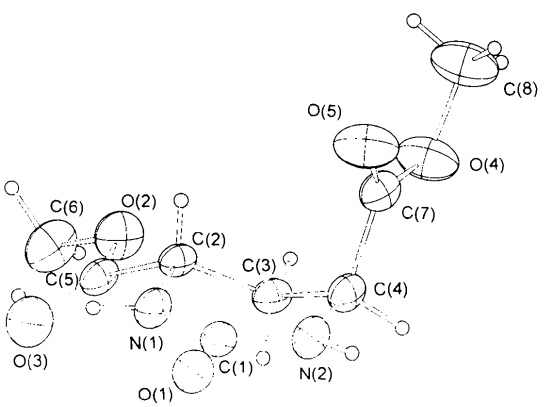


Fig. 3. An *ORTEP* plot of (III) giving the crystallographic atom numbering. Thermal ellipsoids are shown at the 30% probability level.

the exocyclic O(carbonyl) atom. An *ORTEP* plot of the molecule is shown in Fig. 4. The ring in the HDDP structure contains only one reduced C atom [C(4)] and is therefore approximately planar. The largest deviation from the least-squares plane through the six ring atoms is 0.081 Å [C(4)]. An alternative description of the conformation is an envelope with C(4) as the peak; the largest deviation from the plane through the other five atoms is 0.034 Å and C(4) lies 0.176 Å out of this plane. The exocyclic O(carbonyl) atom lies 0.027 Å out of the pyrimidine plane and the C atom of the 'equatorial' carboxylate lies 0.192 Å out of the same plane. The C—N bond adjacent to the C—C double bond in the ring [1.388 (3) Å] is substantially shorter than N to saturated C bonds and is longer than most N—C(carbonyl) bonds. All other bond lengths in the ring are not significantly affected by the presence of the double bond.

Discussion of the molecular mechanics

The carboxylate groups at C(4) in the closely related structures of (I) and (II) adopt axial and equatorial dispositions respectively. This group is believed to be involved in substrate/enzyme binding and its orientation is therefore of significance. Thus, molecular mechanics calculations were performed in order to determine the relative stabilities of the axial and equatorial conformers of DHO and to determine the barrier to interconversion between these two conformers. As a first test of the force field, the ability to reproduce the crystal structures of DHO and *trans*-HTDP was assessed. The geometry of each of the molecules was well reproduced, deviations in bond angles being less than 3° in each case. The total strain energy of the equatorial conformer was 26.8 kJ mol^{-1} and that of the axial conformer, 27.0 kJ mol^{-1}. Given the approximations inherent in the molecular mechanics method the conclusion is that there is little or no difference in the stabilities of these two conformers and in solution a nearly equal mixture of the two would occur. The barrier to interconversion was estimated to be 11.5 kJ mol^{-1} and therefore it can also be concluded that the two conformers will interconvert at a very rapid rate. These results are consistent with the observation of the axial conformer in the structure of (I) and the equatorial conformer in the structure of (II). Evidently the conformation adopted is determined by crystal packing effects.

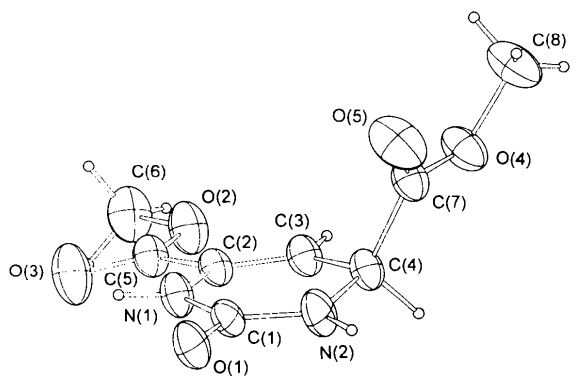


Fig. 4. An *ORTEP* plot of (IV) giving the crystallographic atom numbering. Thermal ellipsoids are shown at the 30% probability level.

Concluding remarks

The present study was undertaken in order to determine the geometric features common to those modified pyrimidines that significantly inhibit dihydroorotase. The parent compounds of (I)–(IV) (non-methylated) all bind tightly to dihydroorotase (Christopherson *et al.*, 1989); they have the following features in common.

(i) An exocyclic carbonyl group [at C(1)] bonded to two N atoms.

(ii) An exocyclic group in the C(2) position which can coordinate to the Zn^{2+} atom that is bound at the catalytic site of dihydroorotase In all four structures this group is close to being coplanar with the ring (*i.e.* is equatorial).

(iii) A carboxylate group in the C(4) position. In structures (I), (III) and (IV) this group is in an axial position. In (II) it adopts an equatorial position but we have shown that in the closely related compound, (I), there is no significant difference between the energies of the axial and equatorial conformers, and there is no significant barrier to interconversion between the two.

As a result of these observations we have adopted the working hypothesis that the presence at C(2) of an equatorial or coplanar group capable of binding Zn and at C(4) of a group capable of forming strong hydrogen bonds or electrostatic bonds to the enzyme are required for strong binding. In accordance with this hypothesis is the poor binding of the *cis* analogue of (III). In the *cis* compound, the carboxylate groups at positions C(2) and C(4) can only be either both equatorial or both axial.

It is noteworthy that in all four structures the exocyclic O(carbonyl) atom at C(1) and both H(amine) atoms are involved in hydrogen bonds, indicating that these are good hydrogen-bonding acceptors and donors, respectively. Also, it is interesting that the O and S atoms in the C(2) positions of structures (I) and (II) are not involved in hydrogen bonding. While this does not preclude their involvment in hydrogen bonds in different environments, it does suggest they are less basic than the O(carbonyl) at C(1). According to the currently accepted model,

it is this O(carbonyl) atom at C(2) that is coordinated to the Zn atom.

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Structures of Riboflavin Tetraacetate and Tetrabutyrate: Molecular Packing Mode of Riboflavin Tetracarboxylate and its Extensive Stacking and Hydrogen-Bonding Characteristics

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Abstract

Riboflavin tetraacetate (RTAc) acetone solvate monohydrate, $2C_{25}H_{28}N_4O_{10}.C_3H_6O.H_2O$, M_r = 1165.12, orthorhombic, $P2_12_12_1$, a = 61.896 (8), b = 11.424 (1), c = 8.134 (1) Å, V = 5751.5 (12) Å^3, Z = 4, D_m = 1.320 (3), D_x = 1.345 g cm^{-3}, λ(Cu $K\alpha$) = 1.5418 Å, μ = 7.84 cm^{-1}, $F(000)$ = 2456, T = 278 K, final R = 0.046 for 5060 independent observed ($F_o > 0.0$) reflections. Riboflavin tetrabutyrate (RTB), $C_{33}H_{44}N_4O_{10}$, M_r = 656.73, triclinic, $P1$, a = 19.409 (2), b = 15.332 (2), c = 11.784 (1) Å, α = 95.51 (1), β = 92.17 (1), γ = 83.59 (1)°, V = 3465.1 (6) Å^3, Z = 4, D_m = 1.250 (2), D_x = 1.259 g cm^{-3}, λ(Cu $K\alpha$) = 1.5418 Å, μ = 7.39 cm^{-1}, $F(000)$ = 1400, T = 255 K, final R = 0.094 for 6342 independent observed [$F_o > 3\sigma(F_o)$] reflections. The crystal structures of RTAc and RTB consist of two and four crystallographically independent molecules, respectively, and are stabilized by extensive stacking (aromatic spacing of 3.3–3.5 Å) and NH···O=C hydrogen-bonded dimer formation between the neighboring molecules. Each of the RTAc and RTB molecules assumes an open conformation, in order to avoid short contacts among four neighboring acetyl or butyryl groups. Although the ribityl backbones are in one of the usually observed conformations, the flexibility of the ester groups contributes to the formation of many conformers in the riboflavin derivatives. The structural characteristics inherent in the isoalloxazine ring and ribityl conformation are discussed.

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