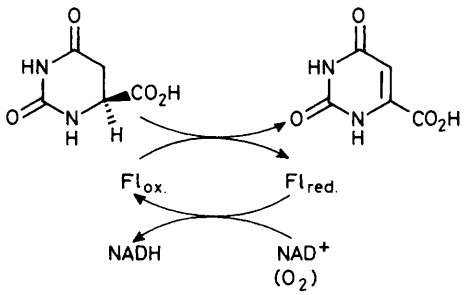
Irreversible Inhibition of Dihydro-orotate Dehydrogenase by Hydantoins Derived from Amino Acids

Ian G. Buntain, Colin J. Suckling, and Hamish C. S. Wood

Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow G1 1XL, Scotland

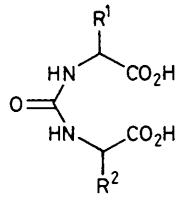
3-(1-Carboxy-2-phenylethyl)-5-benzylhydantoin is a time-dependent, irreversible inhibitor of dihydro-orotate dehydrogenase from *Zymobacterium* (*Clostridium*) *oroticum*.

Recently, there has been an increasing awareness of the potential of inhibitors of *de novo* pyrimidine biosynthesis for the chemotherapy of parasitic and neoplastic diseases.[1—3] Based upon our considerable experience of pyrimidine chemistry in relation to pteridine biosynthesis[4—6] we selected dihydro-orotase and dihydro-orotate dehydrogenase as targets for inhibition. Dihydro-orotate dehydrogenase catalyses the reversible dehydrogenation of dihydro-orotic acid to orotic acid; it is a flavoprotein in which hydrogen transfer to flavin is believed to occur as part of the catalytic cycle (Scheme 1). In the *Zymobacterium oroticum* enzyme, oxidising equivalents are provided by NAD, although more usually an electron-transport chain is involved. Our initial plans focused upon the preceding enzyme in the pathway, dihydro-orotase, but we have found that a series of hydantoins comprises a number of inhibitors of dihydro-orotate dehydrogenase and that some hydantoins derived from phenylalanine are time-dependent, irreversible inhibitors of the enzyme.

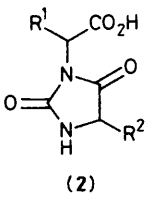


Scheme 1

The potential inhibitors were prepared by modifications of standard procedures;[7] compounds derived both from symmetrically and unsymmetrically substituted ureas were obtained (Table 1). Initial assessments of the inhibitory



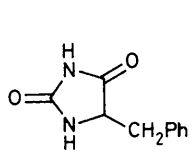
(1)



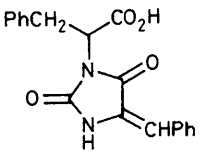
(2)

Table 1

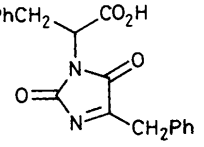
Compound	R^1	R^2	K_i/mM
(**1a**)	H	H	2.6
(**1b**)	H	(*S*)-CH_2Ph	0.7
(**1c**)	(*S,S*)-CH_2Ph		0.4
(**2a**)	H	H	4.5
(**2b**)	H	(*S*)-CH_2Ph	2.3
(**2c**)	(*S,S*)-CH_2Ph		0.7
(**2d**)	(*R,R*)-CH_2Ph		0.9
(**2e**)	(*S,S*)-CH_2Pr^i		3.3
(**2f**), N^1-Me	CH_2Ph	CH_2Ph	0.6



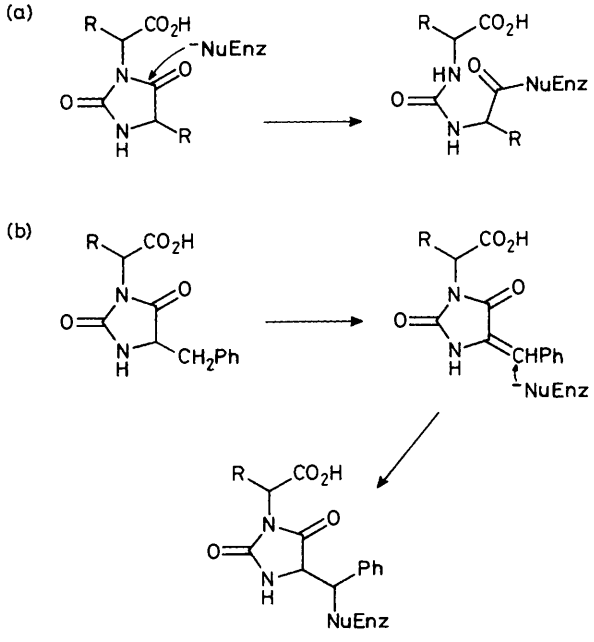
(3)



(4a)



(4b)



Scheme 2

activities of the ureas (**1a—c**) and the hydantoins (**2a—e**) were made by incubating the inhibitor at 25 °C with dihydro-orotate dehydrogenase (0.02 μM), orotic acid (0.67 mM), and NADH (0.13 M) in sodium phosphate buffer at pH 6.5 containing dithiothreitol to stabilise the enzyme. As shown in Table 1, all the compounds were modest inhibitors (*cf.* orotic acid, K_m 22 μM). However those hydantoins derived either from *S*-phenylalanine (**2c**) or *R*-phenylalanine (**2d**) were also time-dependent inhibitors. Further studies of the properties of these two compounds were carried out by incubating the enzyme (0.7 μM) with the inhibitor (1—13 mM) alone and subsequently initiating reaction in samples taken at suitable time intervals by the addition of NADH and orotic acid. Under these conditions, good first-order kinetics were observed for both stereoisomers, the half-life of the enzyme in the presence of the *S,S*- and *R,R*-isomers (**2c, d**; 13 mM) being 40 and 105 min respectively. Inhibition was not reversed by gel filtration. The presence of either NAD or orotic acid or both in the reaction mixture partially protected the enzyme from inhibition. Such properties are characteristic of irreversible inhibition at the active site.

In order to throw light upon the mechanism of inhibition, the structural features required for inhibition were investigated. The carboxylate in the N-3 side chain was found to be essential. Also no time-dependent inhibition was observed with the monobenzyl hydantoin (**2b**) or with the derivative (**3**). However the N-1 proton is not essential; methylation of (**2c**) with dimethyl sulphate and subsequent hydrolysis of the methyl ester afforded the *N*-methyl derivative (**2f**) in a partially epimerised form at both centres. This compound retained the ability to inhibit dihydro-orotate dehydrogenase in a time-dependent manner. It is possible that a major contributor to the inhibitory potency of (**2c**) is the hydrophobicity of the phenyl groups. However the fact that the isopropyl analogue (**2e**) derived from *S*-leucine was one of the most weakly binding compounds suggested that the electronic properties of at least one of the phenyl groups are critical. This argues against inhibition by acylation by the anhydride equivalent (C-2—C-4) of the hydantoins (Scheme 2a). We therefore postulated that (**2c**) might be oxidised by the enzyme to one of the tautomeric derivatives (**4a, b**) both of which contain an electrophilic α,β-unsaturated system that could cause inhibition. The hydantoin (**2c**) was dehydrogenated readily in nonenzymic experiments using chloranil[8] or sodium hypochlorite to afford the exocyclically oxidised tautomer (**4a**) only. Unfortunately, the u.v. absorption of this compound in the 340 nm region made assay of its inhibitory properties at concentrations greater than 2 mM difficult but at 1.9 mM, the rate of inhibition of dihyro-orotate dehydrogenase by (**4a**) was comparable to that by (**2c**) under similar conditions [$t_{\frac{1}{2}}$ (**4a**), 1.9 mM = 135 min; $t_{\frac{1}{2}}$ (**2c**), 1.6 mM = 120 min]. The above considerations lead us to propose the tentative mechanism for inhibition as shown in Scheme 2b.

An unusual feature of these results is that the enantiomers (**2c**) and (**2d**) are both time-dependent inhibitors of this enzyme. Using the hypothesis above and the fact that hydrogen transfer to flavin occurs, it was possible to rationalise the inhibitory ability of both enantiomers with the aid of computer graphics techniques. Thus superimposition of the dehydrogenation site of the substrate with the hypothetical dehydrogenation site of the inhibitors and adjustment of the conformation of each inhibitor revealed that it is possible to place the dehydrogenation site, a negative charge, and the

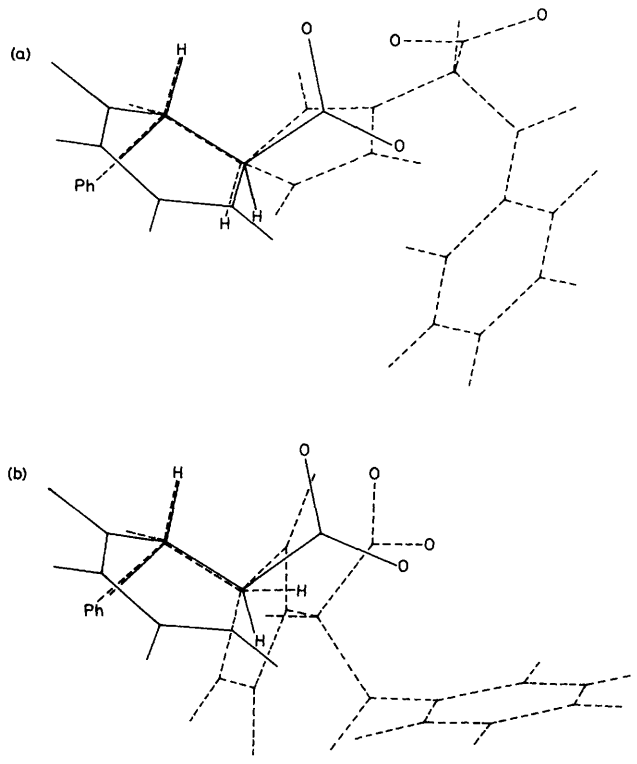


Figure 1. Computer-generated superimpositions of (a) the *R,R*-hydantoin (**2d**) and (b) the *S,S*-hydantoin (**2c**) (dashed line) onto *S*-dihydro-orotic acid (full line).

bulk of the aromatic rings of both inhibitors in the same relative positions (Figure 1). Thus each compound presents a similar electron distribution to the active site and all three can undergo dehydrogenation.

The synthesis of one potential suicide inhibitor of dihydro-orotate dehydrogenase has been reported to date,[9] the 5-ethynyl derivative of orotic acid; however inhibition data were not given. Our compounds therefore are the first irreversible inhibitors of this important enzyme and have the bonus of potential for wide structural variation for the elucidation of the mechanism of action of the enzyme and for the design of further inhibitors. We are currently pursuing these ends.

We thank the S.E.R.C. for a reseaich studentship (to I. G. B.), the Smith and Nephew Foundation for the provision of a Royal Society Senior Research Fellowship (to C. J. S.), and Drs. P. Bladon and R. Breckenridge for the development of the Strathclyde computer graphics system.

Received, 3rd December 1984; Com. 1696

References

1 T. W. Kensler and D. A. Cooney, *Adv. Pharmacol. Chemother.* 1981, **18**, 273.
2 R. A. Pascal, Jr., and C. T. Walsh, *Biochemistry,* 1984, **23**, 2745.
3 C. H. Levenson and R. B. Meyer, Jr., *J. Med. Chem.,* 1984, **27**, 228.
4 H. C. S. Wood, in 'Chemistry and Biology of Pteridines,' ed. W. Pleiderer, de Gruyter, Berlin, 1975, p. 27.
5 R. Cameron, W. J. S. Lyall, S. H. Nicholson, D. R. Robinson, C. J. Suckling, and H. C. S. Wood, in 'Chemistry and Biology of Pteridines,' ed. J. Blair, de Gruyter, Berlin, 1983, p. 79.
6 C. D. Ginger, R. Wrigglesworth, W. D. Inglis, R. J. Kulick, C. J. Suckling, and H. C. S. Wood, *J. Chem. Soc., Perkin Trans. 1,* 1984, 953.
7 F. Wessely and M. John, *Hoppe-Seyler's Z. Physiol. Chem.,* 1927, **170**, 167; F. Wessely and J. Mayer, *Monatsh.,* 1928, **50**, 444; H. Finkbeiner, *J. Org. Chem.,* 1965, **30**, 3414; T. B. Johnson and J. S. Bates, *J. Am. Chem. Soc.,* 1916, **38**, 1087; Y. Ishizuka, *Nippon Kagaku Zasshi,* 1955, **76**, 802; E. Ware, *Chem. Rev.,* 1950, **46**, 403; H. C. Wheeler and C. Hoffman, *Am. Chem. J.,* 1911, **45**, 368.
8 T. E. Young, L. J. Heitz, and D. J. Steklenski, *J. Org. Chem.,* 1973, **38**, 1567.
9 R. Bhatt, N. Kundu, T. L. Chwang, and C. Heidleberger, *J. Heterocycl. Chem.,* 1981, **18**, 771.