dicarboxylic acids. This was best resolved for *m*-phthalate at both pH values, as indicated by biphasic plots, and was characterized by K_d approx. 1 mM.

Significantly different values for the absorption at the long-wavelength maximum of the enzyme at infinite ligand concentration are obtained from the intercepts of the plots. Thus at pH6.5, relative to glutarate as 1.0, the values are 0.85 for α-oxoglutarate and 0.8 for *o*-, *m*- and *p*-phthalate; at pH7.5 these values are 0.73 for α-oxoglutarate and 0.2, 0.35 and 0.4 for *o*-, *m*- and *p*-phthalate respectively. These differences may be in part due to differences in protonic dissociation constant of the protonated enzyme–ligand complex, $E\text{–}H\text{–}L^+$, since conversion of all the enzyme into this form does not necessarily occur. In addition, contributions to these differences from conformational or environmental factors in the region of the pyridoxal coenzyme may be inferred from the fine differences in the characteristic points of the spectrum of the $E\text{–}H\text{–}L^+$ complex.

The affinity for a given ligand, the change in apparent pK of the enzyme, the relative disposition of the binding sites for the two carboxyl groups and the conformational adaptability of the enzyme are evidently interrelated properties. The analogy between these properties and those of the enzyme–substrate complex provides the basis for obtaining structural information about the active site.

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Inhibitors of Shikimate Dehydrogenase as Potential Herbicides

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The shikimate biosynthetic pathway, which occurs in higher plants but not in animals, leads to several essential amino acids and also to a variety of other metabolites. It was hoped therefore that an inhibitor of the pathway might have a damaging effect on plant metabolism and possibly be a useful commercial herbicide with low mammalian toxicity. Shikimate dehydrogenase (EC 1.1.1.25), the enzyme that catalyses the conversion of dehydroshikimate (I) into shikimate (II), was chosen as the target for inhibition because it occurs early in the pathway and because of the accessibility of synthetic substrate analogues.

It was our intention to prepare active-site-directed irreversible enzyme inhibitors for the enzyme, and as a starting point for this we prepared the novel dehydroshikimate analogue 1,6-dihydroxy-2-oxoisonicotinic acid (III). When tested against shikimate dehydrogenase from pea (assayed in the direction shikimate → dehydroshikimate) this had K_i 0.13 mM (K_m 0.17 mM). A hydrophobic area exists near the active site of the enzyme, since a series of 6-*O*-alkyl derivatives of compound (III) bound as well as or better than the parent compound. Maximum binding was obtained with the isopropyl ether (K_i 0.07 mM). We think this area may also have been instrumental in helping to complex the 6-*O*-phenyl analogue (K_i 0.12 mM) and the related potential active-site-directed inhibitors.

It is of interest that these compounds, all of which are more like dehydroshikimate than shikimate, are very weak inhibitors when the enzyme is assayed in the direction dehydroshikimate → shikimate (Dowsett *et al.* 1971).

Although chemical synthesis was difficult, we have prepared some iodoacetamidophenoxy derivatives of compound (III) that appear to cause progressive irreversible inhibition directed at the active site. The most powerful of these caused 50% inhibition after 48 min when tested at 1 mM.

The compounds were inactive as herbicides *in vivo* when screened on a range of representative plants, and possible reasons for this will be discussed.

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Metabolism and some Biochemical Effects of *N*-Nitrosomorpholine

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The hypothesis that the carcinogenic and other biological activities of *N*-nitroso compounds could be mediated by their conversion *in vivo* into alkylating agents has received support from studies on structure–activity relationships by Druckrey *et al.* (1967) and from direct evidence of alkylation of nucleic acids or proteins *in vivo* by several nitrosamines and nitrosamides (see Magee, 1969). However, Argus *et al.*