COMMUNICATION TO THE EDITOR

The Mechanism of Action of the 2-Phenylcyclopropylamine Type of Monoamine Oxidase Inhibitors[1]

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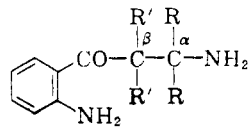
In continuation of our studies of the mechanism of action of monoamine oxidase (MAO) using deuterium isotope effects as a tool,[2-5] we have examined the effect of deuterium substitution on the carbons *alpha* and *beta* to the amine function of the substrate kynuramine (I and II) on the kinetics of oxidation by rat liver MAO. The α-bis-



(I, R = R = D, R′ = R′ = H)
(II, R = R = H, R′ = R′ = D)

deuteriokynuramine (I) was prepared by lithium aluminum deuteride reduction of indoleacetonitrile,[6] then ozonolysis according to the procedure of Weissbach *et al.*[7] The β-bisdeuteriokynuramine (II) was obtained by prolonged treatment of kynuramine dihydrobromide with excess deuterium oxide at 100°. Both substrates were labeled to the extent of at least 90% as determined by n.m.r. analysis. The MAO was prepared according to Hawkins[8] and the rates of oxidation measured spectrophotometrically following the procedure of Weissbach *et al.*[7] The results are shown in Fig. 1, where it can be seen that the ratio of the slopes of the initial velocities $v_H/v_{\alpha\text{-}D}$ is 2.1 when (I) is compared with kynuramine. The magnitude of this primary

(1) This investigation represents a portion of the thesis submitted by J. M. in partial fulfillment of the requirements for the M.Sc. degree in Biochemistry.

(2) B. Belleau, M. Fang, J. Burba, and J. Moran, *J. Am. Chem. Soc.*, **82**, 5752 (1960).

(3) B. Belleau and J. Burba, *ibid.*, **82**, 5751 (1960).

(4) B. Belleau, J. Burba, M. Pindell, and J. Reiffenstein, *Science*, **133**, 102 (1961).

(5) B. Belleau and J. Moran, Abstracts, Meeting American Chemical Society, New York, N. Y., Sept. 1960, p. 26-C.

(6) T. Nógrádi, *Monatsh.*, **88**, 768 (1957).

(7) H. Weissbach, T. E. Smith, J. W. Daly, B. Witkop, and S. Udenfriend, *J. Biol. Chem.*, **235**, 1160 (1960).

(8) J. Hawkins, *Biochem. J.*, **50**, 577 (1952).

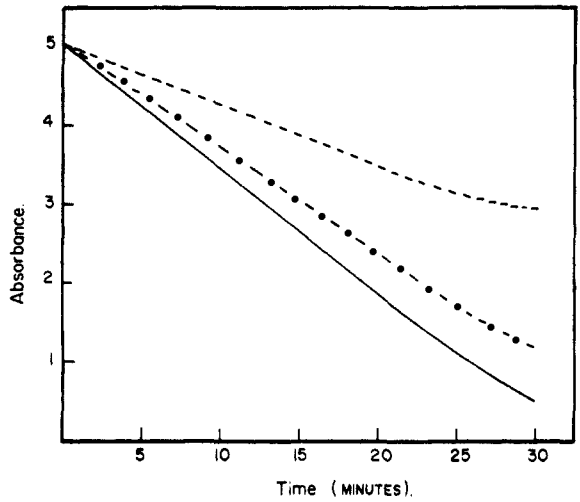


Fig. 1.—Rates of oxidation of kynuramine (——), β-D_2-kynuramine (— —), and α-D_2-kynuramine (- - - -) followed *in situ* with a recording Beckman DK-2 spectrophotometer with time drive attachment. In all cases the substrate concentration was 1.2×10^{-4} mole/liter.

isotope effect is comparable to that already reported for α-bis-deuteriotyramine.[2] A significant novelty is the observation that the presence of deuterium on the carbon *beta* to the amino group (II) also leads to retardation of the rate of oxidation by MAO (Fig. 1), the ratio $v_H/v_{\beta\text{-}D}$ being 1.18. The Michaelis constants for each substrate also were measured in the usual manner and whereas (I) forms a notably looser complex with MAO[5] ($K_{mH}/K_{mD} = 0.3$), II is bound to the same extent as kynuramine by the enzyme.

The significance of these results rests in the conclusions that can be drawn regarding the properties of the transition state for oxidation and in the striking analogy between these properties and those of the 2-phenylcyclopropylamine inhibitors.[9] This correlation which is novel in the field of inhibitor–enzyme relationships becomes discernible easily after these considerations.

The observed isotope effect with I is due in part to a decrease in the Michaelis complex, of the vibrational frequencies of the α-car-

(9) S. Sarkar, R. Banerjee, M. S. Ise, and E. A. Zeller, *Helv. Chim. Acta*, **43**, 439 (1960).

bon–hydrogen bond to be broken in the transition state. This does not apply to II since no isotope effect is operative at the Michaelis complex level. It follows that the effect with II must reflect the weakening of a β-carbon-hydrogen bond in the transition state, a conclusion which remains valid regardless of the mechanism (*cf.* the hyperconjugation[10] and non-bonded repulsions theories[11]) responsible for the bond-weakening effect. It is clear on that basis, that in the transition state for oxidation, there is a simultaneous weakening of an α- and β-carbon–hydrogen bond and, consequently, the α,β-carbon–carbon bond must acquire double bond (sp^2) character in the transition state. This is schematized in Fig. 2, where it should be noted

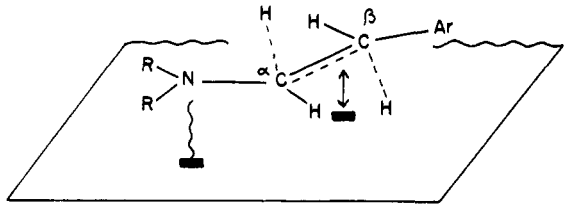


Fig. 2.—Interactions in the transition state for substrate oxidation by MAO.

that the α- and β-carbons approach the trigonal state. These features of the transition state bear a striking analogy to the potent inhibitor 2-phenylcyclopropylamine[9] (PCA) first synthesized by Burger and Yost.[12] In the formal conversion of the weak inhibitor 1-amino-2-phenylpropane to PCA, two outstanding properties are changed: (1) in PCA, the α- and β-carbons approach the trigonal state in contrast to the tetrahedral geometry of the corresponding carbons in 1-amino-2-phenylpropane. Hence, PCA reproduces the geometrical features of the transition state for oxidation (Fig. 3); (2) it is well-known that the cyclopropane bonds have sp^2 character *in the ground state*,[13] another feature now known to be a characteristic of the transition state for oxidation. Since steric and electronic com-

(10) V. J. Shiner, H. R. Mahler, R. H. Baker, and R. R. Hiatt, *Annals N. Y. Acad. Sci.*, **84**, 583 (1960).

(11) L. S. Bartell, *J. Am. Chem. Soc.*, **83**, 3567 (1961).

(12) A. Burger and W. L. Yost, *ibid.*, **70**, 2198 (1948).

(13) L. L. Ingraham, in "Steric Effects in Organic Chemistry," M. S. Newman, Editor, John Wiley and Sons, New York, N. Y., 1956, p. 519.

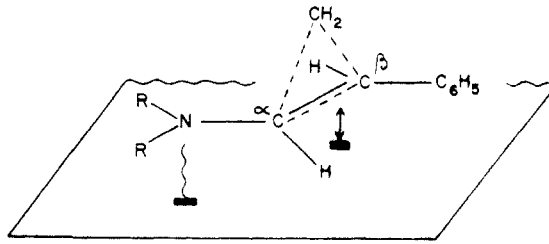


Fig. 3.—Mode of interaction between 2-phenylcyclopropylamine and MAO.

plementation between substrate and enzyme must increase as the transition state is approached, it follows that PCA should be tightly bound by MAO, both the ground state steric and electronic properties of this inhibitor reproducing key features of the transition state for oxidation. It can further be inferred that the π-electrons generated between the α,β-carbon–carbon bond of substrates contribute largely together with the amino group to the binding energy in the transition state since at that stage the bond-breaking process at the α-carbon of substrates already is advanced and makes the departing hydrogen a poor anchoring group. If this is true, the high affinity of PCA for the enzyme should be ascribed to the high ground state electron density between the α,β-carbon–carbon bond (Fig. 3) rather than to direct interactions of the α-substituents (excepting the amino group) with the enzyme. These considerations cannot be reconciled with the speculations of Zeller *et al.*,[9] who ascribe a critical role to the α-hydrogen of PCA in the binding process. The recent interesting finding of Burger *et al.*[14] on the high inhibitory potency of 1-methyl-2-phenylcyclopropylamine strongly supports our interpretation of the factors contributing to the binding energy in the transition state.

Using kynuramine as the substrate we have established that PCA acts competitively as evidenced by conventional Lineweaver–Burk plots. The drug also is recovered unchanged after a 24 hr. incubation with the enzyme. These experiments show that the intact molecule of PCA, not a metabolite, is the active species (at least *in vitro*), as required by our interpretation.

The results reported offer a new perspective in the search for en-

(14) A. Burger, C. S. Davis, H. Green, D. H. Tedeschi, and C. L. Zirkle, *J. Med. Pharm. Chem.*, **4**, 571 (1961).

zyme inhibitors and it should be noted that a somewhat analogous interpretation has been offered by Bernhard and Orgel[15] in connection with the affinity of organophosphorus drugs for hydrolytic enzymes. A comprehensive paper covering our work with MAO will be published in the near future.

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(15) S. A. Bernhard and L. E. Orgel, *Science*, **130**, 625 (1959).