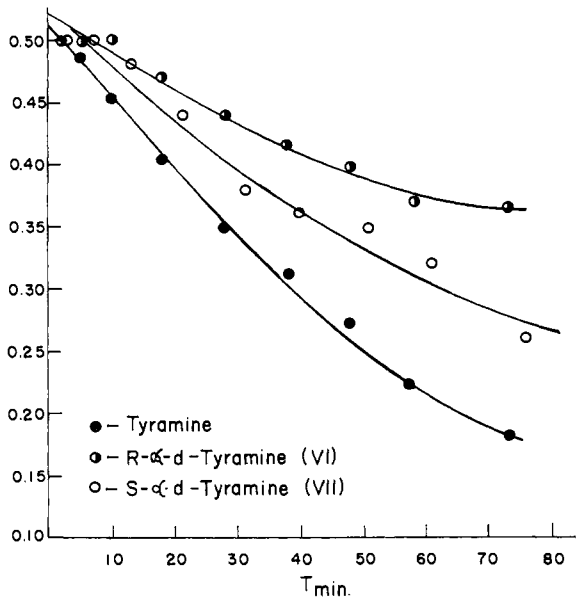


Fig. 1.—Relative rates of oxidation by monoamine oxidase of synthetic R-α-*d*-tyramine and S-α-*d*-tyramine; values are an average of three separate runs.

cording to Streitwieser's interpretation,[15] this alcohol should be of near optical purity. The well-established mechanism for the Meerwein–Ponndorf reduction[16] allows the assignment of absolute configuration (S)[15] shown in (V) (R = *p*-methoxybenzyl). The alcohol (V) was converted to the tosylate,[16,17] m.p. 58°, which was converted to the corresponding azide by reaction with excess sodium azide. This type of displacement by azide is known to proceed with complete inversion of configuration.[18] Reduction of the crude azide with lithium aluminum hydride in ether and hydrolysis with hydrobromic acid gave (R)-1-*d*-tyramine (VI) isolated as the crystalline hydrochloride. It follows that its deuterium content must be the same as that of the starting deuterioisoborneol. The preparation of (S)-1-*d*-tyramine (VII) was accomplished by first treating the alcohol (V) with phosphorus tribromide in the presence of collidine. Under these conditions (presence of collidine), virtually complete inversion of configuration should occur.[19] The bromide thus obtained (b.p. 140°(13 mm.)[13] 35% yield) was submitted to the same reaction sequence already applied to the above tosylate to give the desired (S)-1-*d*-tyramine (VII).

The rates of oxidation by rat liver monoamine oxidase of these two synthetic substrates were measured using tyramine as a standard.[20] The results are shown in Fig. 1 where it can be seen that the slope ratio (at initial velocities) is 2.00 when (VI) is compared with tyramine and 1.25 when (VII) is compared. Keeping in mind that slope ratios of 2.3 and 1.0 have been observed with the enzymically prepared enantiomers of α-*d*-tyramine,[2] it follows that the α-*d*-tyramine obtained by decarboxylation of L-tyrosine in $D_2O$ as the solvent has the same absolute configuration (VI) as the synthetic sample, giving rise to an isotope effect of 2.0. The smaller isotope effect obtained with (VI) indicates that this preparation is optically impure. On the basis of these experiments, it is clear that the enzymic decarboxylation of tyrosine and presumably of other amino acids proceeds with retention of configuration.

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active deuterio alcohols of absolute configuration (V) are dextrorotatory. In view of the existence of π-hydrogen bonding in our alcohol (see I. M. Goldman and R. O. Crisler, *J. Org. Chem.*, **23**, 751 (1958)), the sign of the rotation may lose significance.

(15) A. Streitwieser, Jr., W. D. Schaeffer and J. R. Wolfe, *Tetrahedron*, **6**, 338 (1959).

(16) W. E. Doering and R. W. Young, *ibid.*, **72**, 631 (1950); H. S. Mosher and E. LaCombe, *ibid.*, **72**, 3994 (1950).

(17) R. S. Tipson, *J. Org. Chem.*, **9**, 235 (1944).

(18) A. Streitwieser, Jr., and W. D. Schaeffer, THIS JOURNAL, **78**, 5597 (1956).

(19) A. Streitwieser, Jr., *ibid.*, **75**, 5014 (1953).

(20) S. Udenfriend and J. R. Cooper, *J. Biol. Chem.*, **196**, 227 (1952).

## THE ABSOLUTE OPTICAL SPECIFICITY OF MONOAMINE OXIDASE

*Sir:*

The widely distributed enzyme monoamine oxidase has been shown in recent years to play an important role in adrenergic mechanisms especially in the inactivation of catecholamines and serotonin in the central nervous system.[1] The subject of the occurrence, nature and role of monoamine oxidase has been reviewed[2] and it is apparent that little is known about its mechanism of action although Zeller and his group[3] have offered some interesting speculations concerning the nature of the active sites.

A most striking feature of monoamine oxidase is the relative lack of optical specificity[4] in addition to the well-known lack of substrate specificity.[2] Moreover, the enzyme does not distinguish between the geometrical isomers (*cis*- and *trans*-) of phenylcyclopropylamine as judged from their virtual equipotency as inhibitors.[5] In contrast with these results is our recent discovery[6] of a marked increase in the potency of sympathomimetic amines produced by stereospecific deuterium substitution, an observation which led us to postulate that monoamine oxidase may be the

(1) P. A. Shore, J. A. R. Mead, R. Kuntzman, S. Spector and B. B. Brodie, *Science*, **126**, 1063 (1957); S. Spector, D. Prockop, P. A. Shore and B. B. Brodie, *ibid.*, **127**, 704 (1957); "Symposium on Monoamine Oxidase Inhibitors," *Ann. N. Y. Acad. Science*, **80**, 568 (1959).

(2) A. N. Davison, *Physiol. Rev.*, **38**, 729 (1958); H. Blaschko, *Pharmacol. Rev.*, **4**, 415 (1952).

(3) E. A. Zeller, J. Barsky, L. A. Blanksma and J. C. Lazanas, *Fed. Proc.*, **16**, 276 (1957); J. Barsky, W. L. Pacha, S. Sarkar and E. A. Zeller, *J. Biol. Chem.*, **234**, 389 (1959).

(4) P. Pratesi and H. Blaschko, *Brit. J. Pharmacol. Chemotherapy*, **14**, 256 (1959); J. H. Biel, A. C. Conway, F. Diperro, A. E. Drukker and P. A. Nuhfer, THIS JOURNAL, **81**, 4995 (1959); H. Blaschko, D. Richter and H. Schlossmann, *J. Physiol. (Lond.)*, **31**, 2187 (1937).

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

(6) B. Belleau, J. Burba, M. Pindell and J. Reiffenstein, *Science*, forthcoming publication.

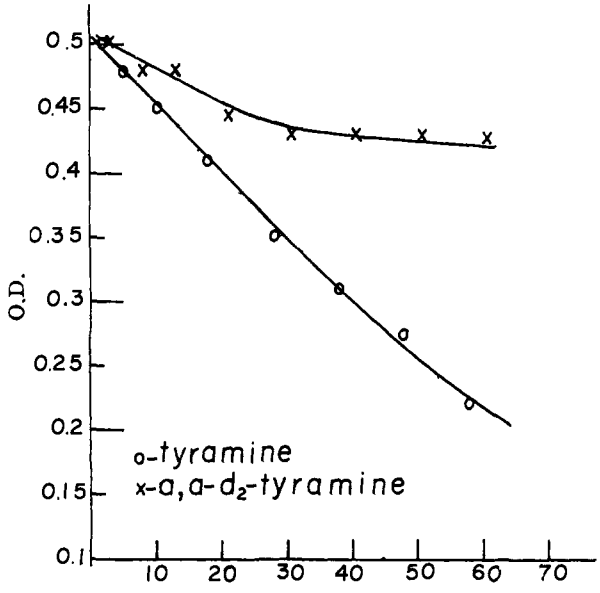



Fig. 1.—Relative rates of oxidation by monoamine oxidase of tyramine and $\alpha,\alpha$-$d_2$-tyramine: optical densities corresponding to unchanged tyramine are plotted against time. Assay procedure was according to ref. 9. Values are an average of five separate runs.

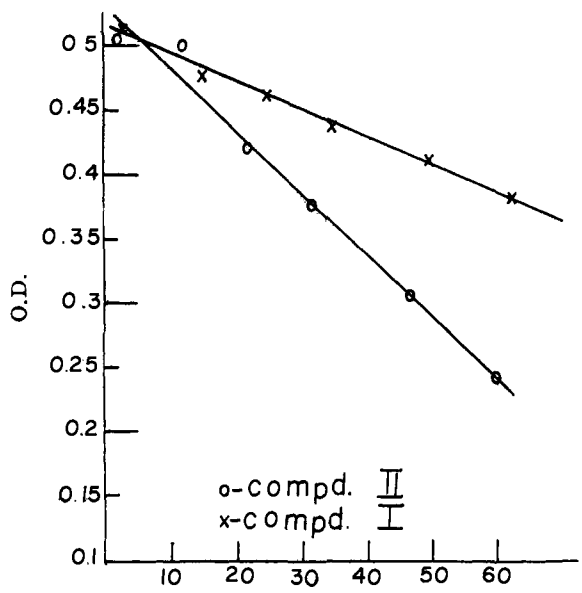
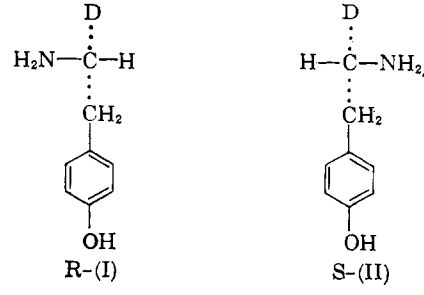



Fig. 2.—Relative rates of oxidation by monoamine oxidase of (R)-$\alpha$-$d$-tyramine and (S)-$\alpha$-$d$-tyramine: values are an average of three separate runs.

enzyme involved in these isotope effects. We now wish to report the discovery of a configuration dependent deuterium isotope effect in the enzymatic oxidation of asymmetrically labeled tyramine, thus revealing monoamine oxidase as an enzyme exhibiting a degree of stereospecificity paralleling that of alcohol dehydrogenase.[7] This result establishes the operation of a three point contact between enzyme and substrate and allows some deductions about the nature of the groups interacting with the active sites of the enzyme.

Rat liver monoamine oxidase was purified as described by Hawkins[8] and assayed according to Udenfriend and Cooper.[9] The deuterated tyramine was prepared by reduction of homoanisonitrile with lithium aluminum deuteride. The isotopic purity of the resulting $\alpha,\alpha$-$d_2$-tyramine was estimated to be at least 95%.[10] The asymmetrically labeled tyramine was prepared by enzymic decarboxylation of L-tyrosine in $D_2O$ according to Mandeles, *et al.*,[11] and isolated according to Davis and Awapara.[12] The absolute configuration of this $\alpha$-$d$-tyramine was established as described in another communication[13] and is shown in formula I. The enantiomer (II) was obtained by enzymic decarboxylation in water of DL-$\alpha$-$d$-tyrosine which was prepared according to well-established synthetic procedures. Both enantiomers had an isotopic purity exceeding 90%. Incubations were carried out at 37° following a standard procedure and the rate of tyramine oxidation measured according to the method of Udenfriend and Cooper.[9] It can be seen in Fig. 1 that $\alpha,\alpha$-$d_2$-tyramine is oxidized by monoamine oxidase at a much slower rate than tyramine. The ratio of the slopes of the initial velocities is 2.3. Accordingly, it would appear that the rate-controlling step in the oxidation of tyramine involves breaking of a carbon–hydrogen bond and that there should be appreciable bond breaking in the transition state, a deduction which we believe to be especially pertinent to the mode of action of certain inhibitors (to be discussed in a full paper).




When the two enantiomers (I) and (II) were incubated under identical conditions the results shown in Fig. 2 were obtained. The ratio $k_{II}/k_I$ was 2.3 as in the preceding experiment, thus clearly establishing that the two $\alpha$-hydrogens are not equivalent for the enzyme. This result can only be rationalized by assuming a three-point contact between substrate and enzyme. Obviously, the amino group and one $\alpha$-hydrogen (the one corresponding to the $\alpha$-deuterium of R-$\alpha$-$d$-tyramine) must be involved in this three-point contact but it does not appear possible at the present time to decide in favor of either the second

(7) F. A. Loewus, F. H. Westheimer and B. Vennesland, THIS JOURNAL, **75**, 5018 (1953); D. E. Koshland, Jr., in "Mechanism of Enzyme Action," John Hopkins Press, Baltimore, Md., 1954, p. 357.

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

(9) S. Udenfriend and J. R. Cooper, *J. Biol. Chem.*, **196**, 227 (1952).

(10) We are grateful to Dr. Robert Fraser for some of the n.m.r. analyses for deuterium and to Dr. Norman Jones of the National Research Council of Canada for some of the combustion analyses.

(11) S. Mandeles, R. Koppelman and M. E. Hanke, *ibid.*, **209**, 327 (1954).

(12) V. E. Davis and J. Awapara, *ibid.*, **235**, 124 (1960).

(13) B. Belleau and J. Burba, THIS JOURNAL, **82**, 5751 (1960).

α-hydrogen or the benzilic methylene as the third group completing the contact. The determination of Michaelis constants using the deuterium labeled substrates might be expected to provide an answer to this problem and such experiments are under way.[14] It is of interest to note that the optical stereospecificity of monoamine oxidase parallels that of D-amino acid oxidase but the significance of this correlation remains to be ascertained.

**Acknowledgments.**—The authors are grateful to the National Research Council of Canada for the financial support of this work.

(14) See B. Belleau and J. Moran, Abstracts of the American Chemical Society Meeting, September, 1960, New York, N. Y., page 26-C, paper 71, for a preliminary account. In this report, the word "labeled" on line 6 from the bottom (third paragraph) should read "unlabeled."

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## OPTICAL ROTATORY DISPERSION OF PHTHALIMIDES OF *p*-SUBSTITUTED α-PHENYLETHYLAMINES

*Sir:*

The rotatory dispersion curves of phthalimides of *para*-substituted (−)(S) α-phenylethylamines (I) show positive Cotton effects superimposed on strong negative backgrounds[1] (Figs. 1 and 2).

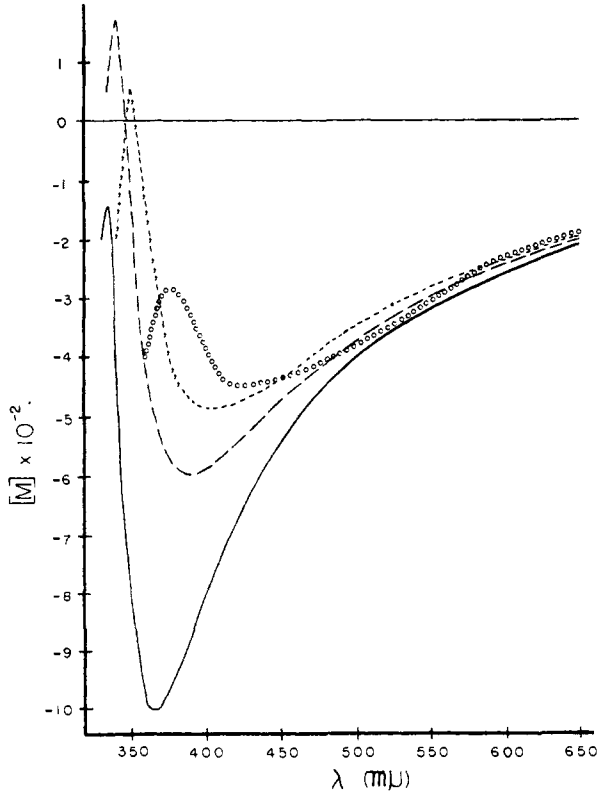


Fig. 1.—The optical rotatory dispersion [M] of phthalimides of *p*-substituted (−)(S)α-phenylethylamines (II) in chloroform solution; *para* substituents (Z): $NO_2$, —; Br, – –; $OCH_3$, - - -; $NH_2$, oooo.

(1) C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill Book Co., New York, N. Y., 1960, pp. 11–17.

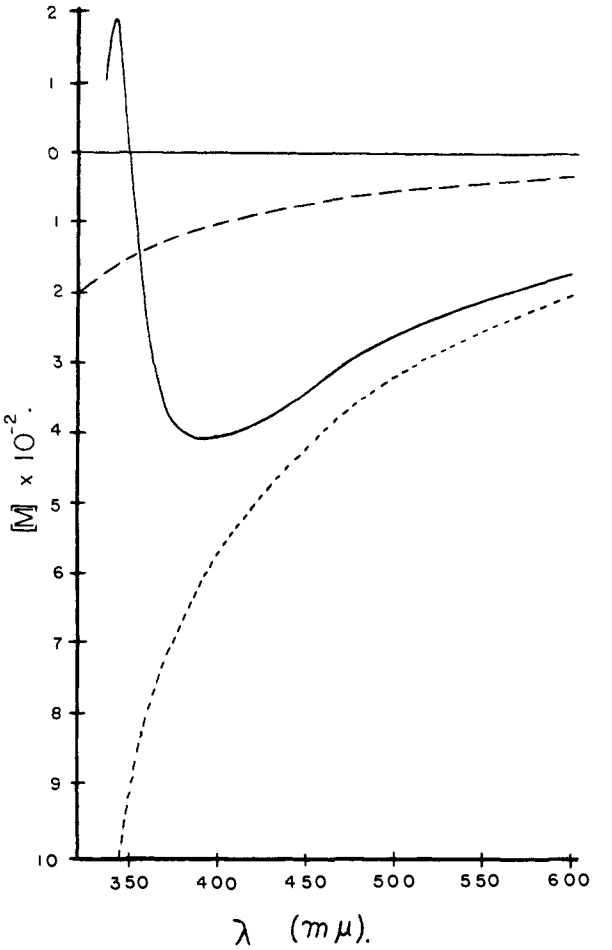


Fig. 2.—The optical rotatory dispersion [M] in chloroform of (−)(S)α-phenylethylamine, – –; its phthalimide, —; its succinimide, - - -.

These curves can be summarized, for the region of transparency (350–700 mμ), by two-term Drude expressions

$$[M] = -\frac{A}{\lambda^2 - x} + \frac{B}{\lambda^2 - y}$$

(see Table I). The positive Cotton effect (first extremum,[1] 335–380 mμ; $\lambda_0$, from the Drude constants, about 322 mμ) occurs in the same region of the spectrum as a broad absorption band at 275–350 mμ which is shown by phthalimide and its N-alkyl derivatives but not by α-phenylethylamine or its succinimide; these last compounds show only plain dispersion (Fig. 2). These facts indicate that the phthalimido chromophore produces this Cotton effect.

The position of the first extremum[1] appears to be influenced by the ability of the substituent Z (I) to attract or release electrons. Measurements of the electrical properties of substituents on aromatic nuclei might, thus, be made in this and related systems by use of optical rotatory dispersion methods. Since it is the *position* rather than the magnitude of the Cotton effect which is important, such measurements could be made on substances of unknown optical purity and absolute configuration and would require neither the precise data nor