Brit. J. Pharmacol. (1955), **10**, 314.

ENZYMIC OXIDATION OF DERIVATIVES OF ETHYLENEDIAMINE

BY

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(RECEIVED MARCH 24, 1955)

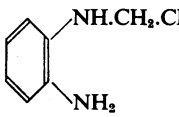
A number of derivatives of ethylenediamine, prepared recently in Dr. H. R. Ing's laboratory, have been examined as possible substrates of amine oxidases, and the results of this study are described in this paper.

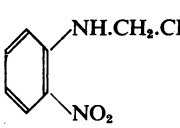
Ethylenediamine is a poor substrate of histaminase; it is oxidized neither by amine oxidase nor by spermine oxidase, two enzymes that will act upon diamines in which the two amino groups are more widely separated. The lack of affinity of ethylenediamine and other short-chain diamines, e.g., putrescine or cadaverine, for these enzymes must be due to a disturbing effect exerted by one amino group upon the reaction of the other amino group with the enzyme.

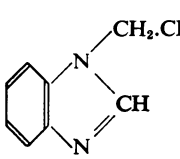
The action of amine oxidase, spermine oxidase, and histaminase on the compounds described below has been examined in this study.

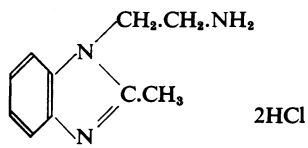
MATERIAL AND METHODS

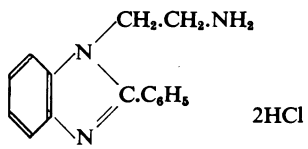
(a) *Compounds Studied.*—These were:

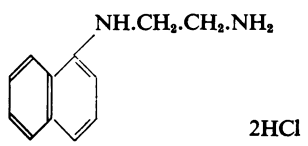
I  3HCl — *N-o*-aminophenylethylenediamine trihydrochloride

II  HCl — *N-o*-nitrophenylethylenediamine hydrochloride

III  2HCl — 1-2′-aminoethylbenziminazole dihydrochloride

IV  2HCl — 1-2′-aminoethyl-2-methylbenziminazole dihydrochloride

V  2HCl — 1-2′-aminoethyl-2-phenylbenziminazole dihydrochloride

VI  2HCl — α-naphthylethylenediamine dihydrochloride

For compounds I to V we are grateful to Drs. E. F. Rogers and R. Foster and to Mr. F. Jenkins; compound VI was a commercial product, obtained from Messrs. Roche.

(b) *Enzyme Preparations.*—These were similar to preparations commonly used in this laboratory. A fully dialysed preparation of either guinea-pig or rabbit liver (1 g. fresh tissue in a total volume of 2 ml.) in 0.02M-sodium phosphate buffer of *p*H 7.4 served as a source of amine oxidase. The histaminase preparation was an extract, in 0.067M-sodium phosphate buffer of *p*H 7.4, of an acetone-dried powder of pig kidney. Ox serum, fully dialysed against the same 0.067M-phosphate buffer, was used in the experiments on spermine oxidase.

RESULTS

Amine Oxidase.—All the compounds were tested with the preparations of both rabbit and guinea-pig liver. Compounds I, II, and III were found to be oxidized. That the oxidation was due to

amine oxidase is strongly supported by the finding that with each of these compounds the rate in the presence of sympatol was intermediate between the rate with the compound alone and sympatol alone.

Oxygen uptake, in competition with sympatol, also occurred with compound VI in the presence of rabbit liver extract, but no oxygen uptake occurred in the presence of guinea-pig liver extract.

Compounds IV and V were oxidized neither by rabbit liver nor by guinea-pig liver.

These results are summarized in Table I.

TABLE I

OXIDATION OF ETHYLENEDIAMINE DERIVATIVES BY RABBIT AND GUINEA-PIG LIVER

Substrate concentrations: 5×10^{-3}M.

The figures represent $\mu l.O_2$ consumed in the first 15 min. of incubation.

Compound	Rabbit Liver			Guinea-pig Liver		
	With Ethylenediamine Derivative	With Sympatol	With Both Amines	With Ethylenediamine Derivative	With Sympatol	With Both Amines
I	94	29	87	100	73	96
II	41	3	7	5	62	13
III	29	18	23	19	50	34
IV	0	39	26	0	52	32
V	0	39	17	0	59	46
VI	25	24	26	0	30	—

Spermine Oxidase (Table II).—Ox serum rapidly oxidized compounds I, II, and VI. The oxygen uptake in the presence of spermine and one of these three compounds was competitive and not additive, suggesting that one and the same catalyst was responsible for the oxidation of spermine and of compounds I, II, and VI.

TABLE II

OXIDATION OF ETHYLENEDIAMINE DERIVATIVES BY SPERMINE OXIDASE OF OX SERUM

Substrate concentrations: 5×10^{-3}M.

The figures represent $\mu l.O_2$ consumed in the first 30 min. of incubation

Compound	With Ethylenediamine Derivative	With Spermine	With Both Amines
I	37	97	65
II	34	97	46
III	7	97	5
IV	9	97	68
V	0	76	13
VI	27	44	32

With compounds III, IV, and V, oxygen uptake in the presence of ox serum was small and of doubtful significance, but these substances had a marked effect upon the rate of oxidation of spermine. This is clearly shown by the following experiment, in which the rate of oxidation of spermine in the presence of different concentrations of compound III was measured. In the first 30 min. of the reaction the oxygen uptakes were:

with	5×10^{-3}M	spermine alone	71 $\mu l.$ O_2
plus	5×10^{-3}M	compound III	1 „ „
„	5×10^{-4}M	„ „	13 „ „
„	5×10^{-5}M	„ „	39 „ „
„	5×10^{-6}M	„ „	70 „ „

In other words, compound III acted as an inhibitor of spermine oxidase.

Histaminase.—None of the compounds examined (I to V) was oxidized by histaminase. Added in a concentration equimolecular to that of the substrate, 5×10^{-3}M cadaverine dihydrochloride, they all depressed the rate of oxidation ; the inhibitions were between 50 and 90%.

DISCUSSION

The experiments described clearly indicate that the derivatives of ethylenediamine studied, when tested with amine oxidases, differ from the aliphatic parent compound. None of the substances was oxidized by histaminase. Several compounds were oxidized by amine oxidase, an enzyme that does not act upon ethylenediamine. The oxidation of compound I, which is a derivative also of *o*-phenylenediamine, by the liver preparations might be considered as due to cytochrome oxidase, but this is unlikely as it has been shown that the rate of oxidation in the presence of sympatol is competitive. For the other compounds tested, there is even less reason to doubt that the catalyst responsible for oxidation was amine oxidase.

The fact that the benziminazole derivatives substituted in position 2 by a methyl or a phenyl group were not oxidized by amine oxidase is possibly explained by steric factors, which prevent the attachment of the substrate essential for the occurrence of oxidation.

The specificity requirements of spermine oxidase are in many ways similar to those of amine oxidase, and it is therefore of interest that ox serum acted upon three of the ethylenediamine derivatives tested (I, II, and VI) and that the oxidation of spermine was strongly inhibited by the other compounds.

Solutions of compounds III to V were strongly acid ; these compounds are dihydrochlorides, but each requires about one equivalent of alkali for neutralization. Compound I, which is a trihydrochloride, required about two equivalents of base for neutralization.

This observation suggested that these compounds differed from ethylenediamine in that they con-

tained only one strongly basic amino group which was ionized at the *p*H at which the experiments were carried out.

A sample of the benziminazole derivative (III) was therefore sent to Professor A. Albert, who has kindly determined the ionization constants of this compound. A report on his findings appears as an Addendum to this paper. His results allow us to conclude that compound III, and probably also all the other substances examined in this study, were present as mono-cations at the *p*H at which the experiments were carried out.

The experimental evidence thus strongly suggests that the degree of ionization is one of the factors which determines whether an amine is oxidized by amine oxidase or by histaminase. The ethylenediamine derivatives examined are substrates of amine oxidase because they carry, like monoamines, only one basic group, the free amino group at the end of the side chain. They differ from the aliphatic diamines which, at the *p*H of our experiments, are present almost fully as the di-cations (Schwarzenbach, 1933).

These considerations, which help us to understand the enzymic oxidation of diamines, must be more generally applicable. Graham and Tonks (1954) have recently studied the pharmacological properties of certain ethylenediamine derivatives, in which one of the two amino nitrogen atoms is directly attached to an aromatic ring. It seems safe to predict, therefore, that the authors were investigating compounds which, at the hydrogen ion concentration of the tissue, were present as singly charged compounds.

Summary

1. A number of derivatives of ethylenediamine have been examined as substrates of amine oxidases.

2. In contrast to ethylenediamine, some of these compounds were oxidized by amine oxidase and by spermine oxidase, but not by histaminase.

3. These results are interpreted as being due to differences in the degree of ionization of ethylenediamine and its derivatives.

One of us (M. L. C.) wishes to record his deep gratitude to Professor J. H. Burn, F.R.S., for unfailing help and encouragement during his stay in this laboratory.

ADDENDUM

By A. Albert

A 0.005M-aqueous solution of 1-2′-aminoethylbenziminazole dihydrochloride was potentiometrically titrated with 0.1N-potassium hydroxide, which was added in tenth-equivalent portions (at 20°), and the antilogarithms of the pK_a values averaged. Two pK_a values were thus found, 4.32 ± 0.02 and 8.24 ± 0.02. The value 4.32 refers to the nitrogens of the benziminazole-ring, which share the positive charge between them because of a resonance effect; the higher value belongs to the more basic β-amino-group on the aliphatic chain. Thus, at *p*H 7.4, about 90% of this substance is ionized, but this is entirely a mono-cation with the positive charge on the β-amino-group. The second basic group (4.32) is about a hundred times weaker than those of ethylenediamine (6.98) and histamine (6.03). At *p*H 7.4, only 4% of histamine is present as the di-cation, which appears to be the ionic species required by histaminase. Putrescine and cadaverine are entirely di-cations at *p*H 7.4.

References

Graham, J. D. P., and Tonks, R. S. (1954). *Brit. J. Pharmacol.*, **9**, 379.

Schwarzenbach, G. (1933). *Helv. chim. Acta*, **16**, 522.