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INHIBITION OF AMINE OXIDASE BY *ISO*THIOUREA DERIVATIVES

BY

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It is known that many compounds which contain the amidine group —C(:NH)NH_2 inhibit amine oxidase. Blaschko and Duthie (1945a), using a preparation from rabbit's liver, studied a number of homologous series of amidines and of amidine derivatives. They found that with increasing number of methylene groups there occurred at first a gradual increase in inhibitory activity. Thus with the mono-amidines of the series $CH_3(CH_2)_nC(:NH)NH_2$ inhibition was at a maximum at about $n = 10$, but with longer chains inhibitory activity fell off; it seems likely that this was due to the relative insolubility of the higher members, as suggested by Trim and Alexander (1949), for, in this series at least, surface activity and inhibitory potency reach a maximum at about the same member.

Some di*iso*thiourea derivatives were found by Blasckho and Duthie to be strong inhibitors of amine oxidase, but their study did not include any mono*iso*thioureas. We have now examined some forty amidine derivatives of the *iso*thiourea type, including thirteen members of the homologous series $CH_3(CH_2)_nS.C(:NH)NH_2$, as inhibitors of amine oxidase. At the same time we have studied the reversibility of the enzymic inhibition.

MATERIAL AND METHODS

The preparation of amine oxidase used was an acetone-dried powder of rabbit's liver suspended in phosphate buffer. The enzyme suspension contained 28.5 mg. of powder in 1 ml. 0.067 M-sodium phosphate buffer of *p*H 7.4. Soluble material was removed by centrifugation: after each of three washings fresh phosphate buffer was added to the sediment to bring the suspension up to its original volume. This procedure ensures a specimen of high enzymic activity and low enzyme blank (Blaschko and Hawkins, 1950).

Open manometers and conical flasks fitted with a side-bulb and potash tube were employed. The gas phase was oxygen and the bath temperature 37.5°. The main compartment of each flask contained 1.4 ml. of the liver suspension and 0.2 ml. of neutralized 0.1 M-semicarbazide hydrochloride; the potash tube 0.3 ml. of N-KOH; and the side-bulb 0.2 ml. of 0.1 M-tyramine hydrochloride and 0.2 ml. of either water or an aqueous solution of an *iso*thiourea salt.

The percentage inhibition of amine oxidase activity produced by an *iso*thiourea derivative was calculated from the amount of oxygen consumed during the first fifteen minutes after tipping in the flask containing enzyme and substrate (tyramine) only and the corresponding amount for the flask containing inhibitor also, corrections being made for the enzyme blank.

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Most of the *iso*thioureas examined had been synthesized for earlier studies (Fastier and Smirk, 1943; Fastier, 1948). We are grateful to Abbott Laboratories and to Dr. Harold King for some of the compounds used.

RESULTS

Inhibitory action of isothiourea derivatives on the oxidation of tyramine

(*i*) *Mono-iso*thioureas *of general formula* $CH_3(CH_2)_nS.C(:\overset{+}{N}H_2)\overset{-}{N}H_2\}X$

Thirteen members of this series were examined, viz., the first ten (n = 0–9), the twelfth, fourteenth, and sixteenth (n = 11, 13, 15); X = Br except for S-methyl *iso*thiourea, the hydrochloride of which was used. Each salt was tested, if its solubility permitted, at a concentration of 10^{-3}M.

The percentage inhibitions produced by these *iso*thioureas are shown in Fig. 1, which illustrates the influence of chain-length on activity. It can be seen that

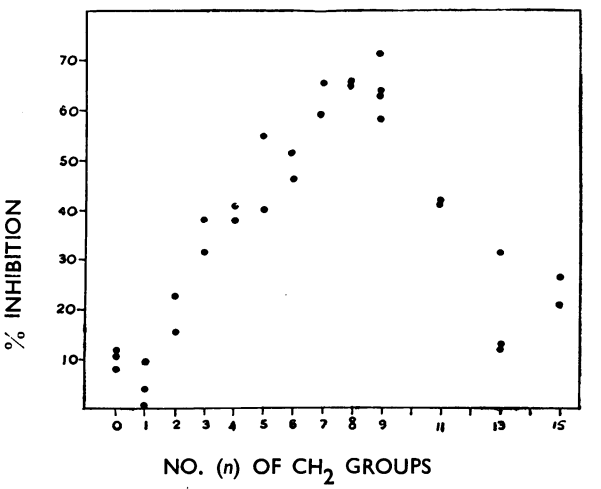

FIG. 1.—Inhibition of amine oxidase of rabbit's liver by S-alkyl *iso*thioureas of formula $CH_3(CH_2)_nS.C(:NH)NH_2$. Each dot represents one experiment. Inhibitor concentration of 10^{-3}M.

activity reaches a peak at about the tenth member (n = 9). The subsequent abrupt fall is probably determined by the sparing solubility of higher homologues in the aqueous phase. Even with the *n*-decyl derivative, it was noticed that some precipitation occurred when a 10^{-2}M aqueous solution, placed in the side-arm of the manometer flask, was diluted ten times with phosphate buffer.

(*ii*) *Di-iso*thioureas *of general formula* $\overset{-}{B}r\{NH_2(\overset{+}{N}H_2:)C.S(CH_2)_nS.C(:\overset{+}{N}H_2)\overset{-}{N}H_2\}Br$

Eight members of this series were examined, viz., the first six (n = 1–6), tenth, and twelfth (n = 10, 12). The two latter were not sufficiently soluble in the aqueous phase to provide a concentration of 10^{-3}M. Nevertheless, enough dissolved to produce almost complete inhibition of amine oxidase activity. The lower homologues tested produced far feebler inhibition at a concentration of 10^{-3}M.

Our results with this series confirm and extend those obtained by Blaschko and Duthie (1945a). The contrast between the relatively weak inhibitory activity of the

short-chain compounds and the much more pronounced inhibition caused by the two long-chain members is clearly shown in Fig. 2.

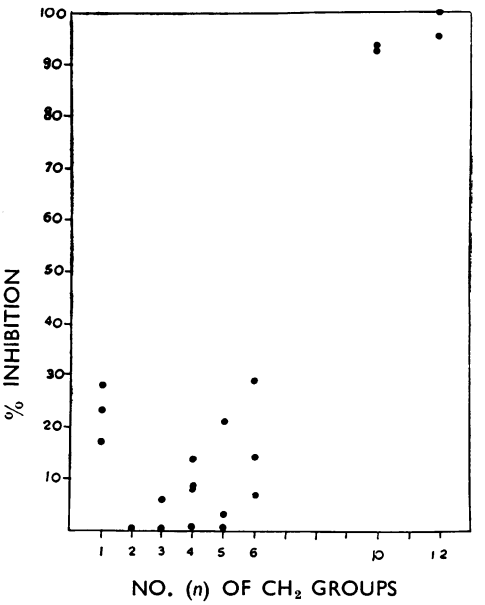

FIG. 2.—Inhibition of amine oxidase by SS′-alkylene di*iso*thioureas of formula $NH_2(NH:)C.S(CH_2)_nS.C(:NH)NH_2$. Each dot represents one experiment with an inhibitor concentration of 10^{-3}M.

(*iii*) *Other* iso*thioureas*

The introduction of an aromatic or alicyclic group into the side-chain of an S-alkyl *iso*thiourea may increase inhibitory activity considerably, as can be seen when the percentage inhibitions produced by the compounds listed in Table I are compared with those produced under the same conditions by S-methyl *iso*thiourea and its near homologues. Even the presence of substituents in the amidine group itself does not necessarily result in the loss of inhibitory activity. This is well shown when the percentage inhibitions produced by the three chlorobenzyl and the 2-pyridyl *iso*thioureas (Table I) are compared with those produced by the corresponding four derivatives of *NN′*-ethylene *iso*thiourea (2-thiol-4: 5-dihydroglyoxaline) (Table II). Four other N-substituted *iso*thioureas were tested, viz., S-*m*-xylyl-NN′-dimethyl *iso*thiourea hydrobromide, S-methyl-NN′-diphenyl *iso*thiourea hydrochloride, SN-trimethylene *iso*thiourea hydrobromide, and SN-(2-bromo-)trimethylene *iso*thiourea hydrobromide. The average percentage inhibitions which they produced were 96, 13, 5, and 0 respectively.

Reversal of inhibition of amine oxidase by washing

Using a different preparation of amine oxidase, Blaschko and Duthie (1945a) howed that the inhibition of the enzyme by pentamidine was not easily reversible.

TABLE I

INHIBITION OF AMINE OXIDASE ACTIVITY OF RABBIT'S LIVER POWDER BY VARIOUS *iso*THIOUREA DERIVATIVES OF FORMULA $R.S.C(:NH_2)NH_2\}A^-$

The percentage inhibitions given are those obtained in individual experiments with an inhibitor concentration of 10^{-3}M

Structure of *iso*thiourea salt		Percentage inhibition
Radical	*Anion*	
benzyl	Cl	64, 55
β-phenyl-ethyl	Cl	67, 52
γ-phenyl-*n*-propyl	Br	77, 76
o-chlorobenzyl	Cl	89, 82
m-chlorobenzyl	Br	67, 52
p-chlorobenzyl	Cl	76, 64
o-bromobenzyl	Cl	99, 76
m-bromobenzyl	Br	75, 60
p-bromobenzyl	Br	74, 59
β-*cyclo*hexyl-ethyl	Br	81, 68
ε-*cyclo*hexyl-*n*-amyl	Br	75, 69
3-bromo*cyclo*hexyl	Br	47, 45
2-pyridyl	Br	30, 22
2-thienyl-methyl	Cl	50, 47

Since the preparation used in our own work was completely insoluble, it was easy to follow the removal of an inhibitor from the enzyme by repeated washings.

The method adopted was essentially the following: the enzyme was first treated three times with a concentration of inhibitor sufficient to cause a marked inhibition (60–90 per cent) of the enzymic activity. The enzyme was then washed three times with phosphate buffer and the inhibition remaining was determined; finally, the inhibition was again determined after another three washings.

The experimental details were as follows: In each experiment 350 mg. of rabbit's liver powder were suspended in 14 ml. phosphate buffer containing the inhibitor. The suspension was centrifuged at 740 *g* for 3 min. The sediment was re-suspended in another 14 ml. of the same solution, and the resulting suspension was centrifuged. The sediment from the second centrifugation was once again re-suspended and centrifuged, and the resulting sediment was again re-suspended in a fresh portion of the original inhibitor solution.

TABLE II

INHIBITION OF AMINE OXIDASE ACTIVITY OF RABBIT'S LIVER BY DERIVATIVES OF *NN'*-ETHYLENE *iso*THIOUREA

The percentage inhibitions given are those obtained in individual experiments with an inhibitor concentration of 10^{-3}M

S-substituent	Salt	Percentage inhibition
o-chlorobenzyl	Cl	95, 66
m-chlorobenzyl	Cl	66, 59
p-chlorobenzyl	Cl	78, 59
p-methoxybenzyl	Cl	63, 62
2-pyridyl	Br	90, 86

Another 350 mg. of the powder was treated in exactly the same way but with plain phosphate buffer. This specimen served as a control.

One-third of each specimen was retained after this treatment for the estimation of the initial enzymic activities. The remaining portions were centrifuged and each sediment was washed three times with plain phosphate buffer. After each centrifugation, phosphate buffer was added to bring the suspension up to the initial volume. Two suspensions were thus obtained, one which had originally been treated with inhibitor, the other a control which had been washed with plain phosphate buffer throughout. One-half of each suspension was kept for estimation of enzymic activity; the two remaining portions were subjected to three further washings in exactly the same way as before. One of the two suspensions finally obtained, that originally treated with inhibitor, had been washed six times in phosphate buffer before its enzymic activity was examined.

The amine oxidase activity of all six samples was estimated manometrically, either 0.4 ml. 0.05 M-tyramine or 0.4 ml. water being added to 1.4 ml. of the suspensions plus 0.2 ml. 0.1 M-semicarbazide.

Four members of the mono-*iso*thiourea series with differing inhibitory activities were examined first (n = 3, 5, 7, and 9). Suitable inhibitor concentrations were first found by trial; these were:

S-*n*-butyl *iso*thiourea ..	25×10^{-3}M	S-*n*-octyl *iso*thiourea ..	1.2×10^{-3}M
S-*n*-hexyl *iso*thiourea ..	6×10^{-3}M	S-*n*-decyl *iso*thiourea ..	0.2×10^{-3}M

The results illustrated in Fig. 3 show that the inhibitory effects of the four S-alkyl *iso*thioureas tested were always decreased by repeated washing of the treated powder,

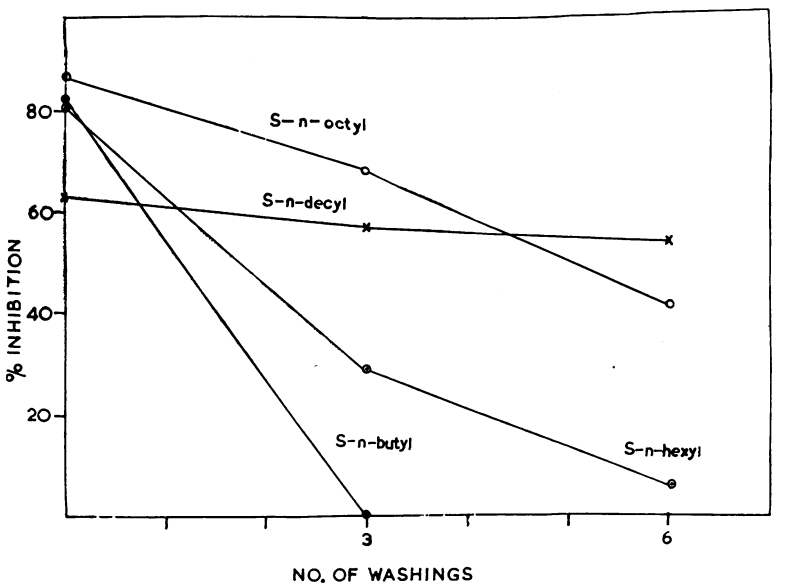

FIG. 3.—Reversal of the inhibition of amine oxidase, produced by S-alkyl *iso*thioureas, upon repeated washing with phosphate buffer.

but not equally. There is a clear gradation between S-*n*-butyl *iso*thiourea, which appears to have been completely removed from its site of action by three washings,

and S-*n*-decyl *iso*thiourea, the reversal of whose inhibitory effect was far from complete even after six washings. In this homologous series, the more tenaciously held compounds are those with the stronger inhibitory activity.

Similar experiments were also performed with S-benzyl *iso*thiourea and with pentamidine. When tested in a concentration of 1.2×10^{-3}M, S-benzyl *iso*thiourea produced 73 per cent inhibition of amine oxidase activity. The degree of inhibition was reduced to 23 per cent after three washings and to 8 per cent after six washings. Pentamidine, however, was held much more tenaciously. A concentration of 10^{-5}M in phosphate buffer sufficed to cause 92 per cent inhibition, and there was still 83 per cent inhibition after six washings.

Discussion

The experiments described in the first section of this paper show clearly that the *iso*thiourea derivatives have an affinity for amine oxidase, like so many other amidine derivatives. The experiments also show that the degree of inhibitory activity is determined not only by the presence of the basic amidine group but also by the hydrocarbon part of the inhibitor molecule.

Blaschko and Duthie (1945a) found that the diamidines included much more potent inhibitors of amine oxidase than the corresponding monoamidines. The same is true for *iso*thioureas. The mono*iso*thioureas examined by us were less active as inhibitors than the decamethylene and dodecamethylene di*iso*thioureas. The fact that compounds with two basic groups separated by a long hydrocarbon chain have a marked affinity for amine oxidase finds a parallel in the observation that the long-chain aliphatic diamines are good substrates of this enzyme (Blaschko and Duthie, 1945b; Blaschko and Hawkins, 1950).

It has already been pointed out (Fastier and Reid, 1948) with regard to certain pharmacological properties of *iso*thioureas that the increasing potency noted during the ascent of a homologous series does not necessarily mean that the molecule of a long-chain *iso*thiourea is more effective than that of a lower homologue at the site of action; the lower homologue may have a partition coefficient which is so much more unfavourable that many more molecules have to be present in the enveloping medium (in which the concentration is measured) in order to maintain the same number of molecules as the higher homologue at the " biophase " (Ferguson, 1939). If this is so, a long-chain *iso*thiourea should be washed away from its site of action less easily than a lower homologue; for supposing that a state of equilibrium is approached during each washing, the amount of *iso*thiourea removed will be determined largely by the partition coefficient. The results illustrated in Fig. 3 are therefore in accord with the view that the different degrees of inhibition of amine oxidase activity caused by a fixed dose of S-methyl *iso*thiourea and its homologues (Fig. 1) are due mainly to differences in their distribution between the aqueous " external phase " and the " biophase," in this case the enzyme.

Summary

1. Forty-four *iso*thiourea derivatives have been tested as inhibitors of the amine oxidase of rabbit's liver. Several of the compounds examined caused a marked inhibition of the enzyme. Of the S-alkyl mono*iso*thioureas examined, the decyl

member was the strongest inhibitor. Some of the long-chain alkylene di*iso*thioureas were even stronger inhibitors.

2. The reversibility of some of these inhibitions was studied by repeated washings and centrifugations; in the mono*iso*thiourea series the results show that the inhibition is most easily reversed with the low members of the series which are also the weakest inhibitors.

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