Biochemical Pharmacology, Vol. 22, pp. 2267–2275. Pergamon Press, 1973. Printed in Great Britain.

DECARBOXYLASE INHIBITION AND STRUCTURE–ACTIVITY RELATIONSHIP STUDIES WITH SOME NEWLY SYNTHETIZED BENZYLOXYAMINE AND PYRIDYLMETHOXYAMINE DERIVATIVES*

Z. HUSZTI, E. KASZTREINER, G. SZILÁGYI, J. KOSÁRY and J. BORSY
Research Institute for Pharmaceutical Chemistry, Budapest, Hungary

(*Received* 27 *September* 1972; *accepted* 2 *March* 1973)

Abstract—Decarboxylase inhibiting properties and the structure–activity relationship have been studied with a number of newly synthetized benzyloxyamines and pyridylmethoxyamines. In the case of benzyloxyamines, the 3-hydroxyl group, and in that of pyridylmethoxyamines, the position of methoxyamine group in the molecule, appear to be of special importance for inhibition of aromatic L-amino acid decarboxylase. We did not find any close relationship between specific histidine decarboxylase inhibiting effect and chemical structure. 3-Hydroxy-4-nitrobenzyloxyamine and pyridyl-3-methoxyamine were the most active compounds in these series. The former compound affected markedly both aromatic L-amino acid decarboxylase and histidine decarboxylase *in vitro* and *in vivo* and produced a significant decrease in the levels of histamine in the stomach and in the lungs. The latter compound proved to be a specific inhibitor of histidine decarboxylase and had a more pronounced effect on the tissue histamine levels than most of new and old benzyloxyamine derivatives.

SINCE the discovery of dopa decarboxylase[1] the role and properties of this enzyme has been studied by several authors.[2–7] Aromatic L-amino decarboxylase (EC 4.1.1.26), as denoted later, plays an important role in the synthesis of dopamine and serotonin; while the synthesis of histamine is catalysed primarily by the specific histidine decarboxylase (EC 4.1.1.22).[8–10]

According to previous studies, benzyloxyamines are powerful inhibitors of both decarboxylases,[11–15] and have pharmacological[16–17] and therapeutic effects.[18–19] Synthesis of a number of benzyloxyamines and pyridylmethoxyamines enabled us to correlate decarboxylase inhibiting potency with chemical structure.

MATERIALS AND METHODS

Reagents. DL-5-Hydroxy-tryptophan and putrescine were obtained from Fluka, L-histidine and pyridoxal phosphate from Koch–Light and Serva respectively. All other chemicals used were analytical grade reagents. The new, selective inhibitor of

* Parts of this communication were presented at the Symposium on "Mechanism of Regulation of the Biogenic Amines Level in the Tissues with Special Reference to Histamine", Lodz, Poland, 2–8, August, 1971.

Abbreviations used: HD, histidine decarboxylase; DC, aromatic L-amino acid decarboxylase; DAO, diamine oxidase; MAO, monoamine oxidase; 5-HTP, 5-hydroxy-tryptophan; INH, isonicotinic acid hydrazine; AB-15, 1-meta-aminophenyl-2-cyclo-propylamino-ethanol-dihydrochloride PALP, pyridoxal phosphate.

MAO, 1-meta-aminophenyl-2-cyclopropylamino-ethanol (AB-15), was synthetized by Dr. Hajós in this Institute. The DAO inhibitor, isonicotinic acid hydrazine (Isonicid), was obtained from Richter Co., Budapest, and NSD-1055 (Brocresin) was kindly supplied by Smith and Nephew Research Ltd.

The compounds used in inhibition studies were synthetized by some of the authors,[20–21] and used as water soluble hydrochloride acids.

Enzyme preparations. For the enzyme inhibition studies partially purified rat stomach pyrolic HD was obtained by the Hakanson method,[22] and DAO was prepared from hog kidney, according to Okuyama and Kobayashi.[23]

Enzyme activity measurements. Aromatic L-amino acid decarboxylase activity was measured with 5-HTP as substrate and with guinea-pig liver or guinea-pig brain homogenate as enzyme source, using the method of Udenfriend *et al.*[24] The serotonin formed during the reaction was determined colorimetrically, or by the fluorimetric method of Bogdansky *et al.*[25]

Histidine decarboxylase activity was measured according to Hakanson and Owman,[22] using L-histidine as substrate and partially purified rat stomach (pyloric) histidine decarboxylase. The histamine formed during the reaction was determined using the Shore method.[26]

Diamine oxidase activity was determined according to Holmstedt and Tham,[27] with putrescine as substrate and with partially purified hog kidney DAO as enzyme.

For *in vivo* measurements of aromatic L-amino acid decarboxylase activity, male guinea-pigs (350–400 g) were treated with various doses of the compounds and 1 hr after the treatment animals were sacrified, livers or brains removed, homogenized in 0·1 M phosphate buffer pH 8·1, and the enzyme activity of the homogenates was determined according to Udenfriend *et al.*[24]

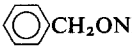
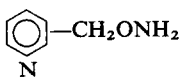
Histamine assay. The tissue histamine determinations were carried out according to Shore *et al.*[26] Groups of five male rats (170–180 g) were treated with the compounds, once or three times in 24 hr, and 3 hr after the last treatment animals were sacrificed, organs were removed, homogenized in 0·4 M $HClO_4$ and the histamine content measured. Control animals received 0·9% NaCl in these experiments.

Calculations. The *in vitro* inhibiting potencies of compounds were expressed as I_{50} values and obtained from the percentage of inhibition–log inhibitor concentration plots. The *in vivo* inhibiting potencies of compounds were expressed as ED_{50} values and obtained from the percentage of inhibition–log inhibitor dose plots. The percentage inhibition was obtained from the average values of homogenates from treated and untreated animals.

RESULTS

The inhibiting potencies of new and old compounds on aromatic L-amino acid decarboxylase and histidine decarboxylase are shown in Table 1. All of the new compounds exerted some inhibitory action on both decarboxylases; the most active compound was 3-hydroxy-4-nitrobenzyloxyamine, which gave I_{50} values of 3×10^{-7} M and 6×10^{-7} M for guinea-pig liver CD and for rat stomach HD, respectively. These values are in agreement with those of the most active inhibitors of specific and non-specific decarboxylases. Pyridylemthoxyamines seem to be less inhibitory than benzyloxyamines *in vitro*, especially to aromatic L-amino acid decarboxylase. In this case, the inhibiting potency of the compounds relates mainly to the position of

TABLE 1. THE AROMATIC L-AMINO ACID DECARBOXYLASE AND HISTIDINE DECARBOXYLASE INHIBITING POTENCY OF BENZYLOXYAMINE AND PYRIDYLMETHOXYAMINE DERIVATIVES

Chemical name	Structure	Derivatives	I_{50} aromatic L-amino acid DC	Histidine DC
Benzyloxyamines		3-Hydroxy-4-bromo- (NSD-1055)	3×10^{-7}	1×10^{-7}*
		3-Hydroxy-4-nitro-	3×10^{-7}	6×10^{-7}
		3-Hydroxy-(NSD-1024)	5×10^{-7}	6×10^{-7}*
		3-Nitro-4-chloro-	7×10^{-6}	3×10^{-6}
		3-Nitro-4-bromo-	3×10^{-5}	1×10^{-6}
		3-Nitro-	1×10^{-4}	5×10^{-6}
		3-Nitro-4-hydroxy-	2×10^{-4}	1×10^{-4}
Pyridylmeth-oxyamines		Pyridyl-2-methoxyamine	5×10^{-6}	1×10^{-6}
		Pyridyl-3-methoxyamine	1×10^{-4}	5×10^{-6}

* Data from Shepherd and Mackay.[28]

Aromatic L-amino acid decarboxylase activity measurements were carried out according to Udenfriend,[24] using 5-HTP as substrate in a final concentration of 5×10^{-3} M, and guinea-pig liver homogenate as enzyme source. Reaction mixtures were incubated for 15 min without substrate and for 60 min with substrate. The specific activity of homogenates was: 17·5 × 0·8 nmoles/mg protein/60 min.

Histidine decarboxylase activity measurements were made with partially purified rat stomach (pyloric) histidine decarboxylase and with histidine, in a final concentration of 3×10^{-3} M. The specific activity of enzyme preparations was 2·8 ± 0·15 nmoles/mg protein/60 min.

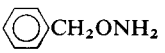
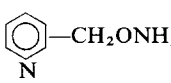
methoxyamine group in the molecule. Pyridyl-2-methoxyamine acts on both enzymes appropriximately to the same extent, while pyridyl-3-methoxyamine selectively inhibits histidine decarboxylase to a strong degree, but has a rather weak effect on aromatic L-amino acid decarboxylase. An interesting correlation was found among benzyloxyamine derivatives. The inhibition of aromatic L-amino acid decarboxylase was substantially stronger with the hydroxyl in the meta position than that in para position. The importance of the 3-hydroxyl group for inhibitory activity of aromatic L-amino acid decarboxylase is well demonstrated by the fact that the I_{50} of 3-hydroxy-4-nitrobenzyloxyamine was 3×10^{-7} M, while that of 3-nitro-4-hydroxy-benzyloxyamine was 2×10^{-4} M.

Table 2 shows the diamine oxidase inhibiting effect of these compounds. Most of benzyloxyamines which strongly inhibited both decarboxylases had a weaker inhibitory effect on diamine oxidase. 3-Hydroxy-4-nitrobenzyloxyamine had little activity against this enzyme, while pyridyl-3-methoxyamine is a relatively potent inhibitor also of the hog kidney DAO.

Results of *in vivo* inhibition studies on aromatic L-amino acid decarboxylase are summarized in Table 3. Data from a study using NSD-1055, NSD-1026 and SNR-1531[15] are also included in Table 3 to demonstrate the *in vivo* effectiveness of these compounds.

Rats were used in those experiments, and the values are therefore not comparable with ours. They clearly show, however, that NSD-1055 was less effective than SNR-1531 or NSD-1024. The new compound 3-hydroxy-4-nitrobenzyl-oxyamine produced

TABLE 2. THE DIAMINE OXIDASE INHIBITING POTENCY OF BENZYLOXYAMINES AND PYRIDYLMETHOXYAMINES

Chemical name	Structure	Derivatives	I_{50} (M)
Benzyloxyamines		3-Nitro-4-chloro-	5×10^{-6}
		3-Nitro-4-bromo-	1×10^{-5}
		3-Hydroxy-4-bromo- (NSD 1055)	1×10^{-5}
		3-Nitro-	4×10^{-5}
		3-Hydroxy-4-nitro-	6×10^{-5}
Pyridylmethoxyamines		Pyridyl-3-methoxyamine	4×10^{-6}
		Pyridyl-2-methoxyamine	1×10^{-4}

Enzyme activity measurements were carried out with partially purified hog kidney DAO and with putrescine as substrate, using the method of Holmstedt and Tham.[27] The composition of reaction mixture was: putrescine, 5 μmoles; *o*-aminobenzaldehyde 12 μmoles; partially purified hog kidney DAO, (2 mg protein) inhibitor, 0·001–1 μmoles and phosphate buffer, pH 7·6, 100 μmoles, in a final volume of 3 ml. Reaction mixtures were incubated for 15 min. without substrate, and for 30 min with substrate at 38°. Specific activity of enzyme preparations was: 1·58 μmoles/mg protein/60 min.

TABLE 3. *In vivo* AROMATIC L-AMINO ACID DECARBOXYLASE INHIBITING EFFECT OF SOME BENZYLOXYAMINE DERIVATIVES

	Enzyme inhibition (%)		
	Doses (mg/kg i.p.)		
Benzyloxyamines	10	20	40
3,4-Dihydroxy (SNR-1531)	77*	—	—
3-Hydroxy-(NSD-1024)	64*	—	—
3-hydroxy-4-bromo-(NSD-1055)	49*	—	—
3-Hydroxy-4-nitro-	45	63	82
3-Nitro-4-chloro-	—	18	42
3-Nitro-4-bromo-	—	—	15
3-Nitro-4-hydroxy-	—	—	15

* Data from Lazare and Watson.[15]

Groups of five guinea-pigs were sacrificed 1 hr after intraperitoneal administration of inhibitors. Livers were removed, homogenized in phosphate buffer pH 8·1, and incubated with 5-HTP as substrate. The enzyme activity measurements and the composition of the reaction mixtures were the same as described in Table 1.

Per cent inhibition was calculated from the average values of the specific activities of homogenates obtained from treated and untreated animals. Specific activity of liver homogenates obtained from control animals was: 3·5 ± 0·2 μmoles/g tissue/60 min. Standard errors of measurements were within ±10 per cent.

in guinea-pigs rather strong inhibition of aromatic L-amino acid decarboxylase; ED_{50} was 14 mg/kg after intraperitoneal treatments. Other new benzyloxyamines were less effective. Pyridylmethoxyamines showed no significant effect *in vivo*, pyridyl-2-methoxyamine had a slight effect, while pyridyl-3-methoxyamine showed no ability to inhibit decarboxylation of serotonin and dopamine *in vivo*.

Because of its strong *in vivo* effect, 3-hydroxy-4-nitrobenzyloxyamine was selected for further study, and tested on cerebral decarboxylase. 20 mg/kg of the compound was administered to guinea-pigs intraperitoneally and orally and the aromatic L-amino acid decarboxylase activity of brain from treated and untreated animals was measured at various times after the treatments (Table 4). This relatively small dose of the compound produced marked inhibition of brain enzyme in both treatments. The effect was of a long duration; no significant diminution of the inhibition could be observed 6 hr after drug administration. The enzyme activity, however, returned to normal within 24 hr.

TABLE 4. INHIBITION OF GUINEA-PIG BRAIN DECARBOXYLASE MEASURED AFTER ORAL AND INTRAPERITONEAL ADMINISTRATIONS OF 3-HYDROXY-4-NITRO-BENZYLOXYAMINE

Treatment	Dose (mg/kg)	Inhibition at various times after 20 mg/kg 3-hydroxy-4-nitro-benzyloxyamine (%)		
		3 hr	6 hr	24 hr
Intraperitoneal	20	82	79	5
Oral	20	60	58	0

Groups of five animals were sacrificed at various times after administration of inhibitor. Brains were removed, homogenized in phosphate buffer pH 8·1, and inbucated with 5-HTP as substrate in a final concn. of 5×10^{-3} M.

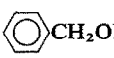
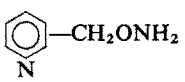
Serotonin formed during the reaction was measured by the fluorometric method of Bogdansky *et al.*[25] Per cent inhibition was calculated from the average values of specific activities of homogenates obtained from treated and untreated animals. Specific activity of brain homogenates from control animals was 0·110 ± 0·010/g tissue/60 min.

Standard errors of measurements were within ±10 per cent.

The histamine lowering effect of these compounds was investigated in rats. Inhibitors were injected subcutaneously, three times in 24 hr, at a dose of 30 mg/kg. The animals were sacrificed 3 hr after the last treatment, and the histamine in the stomach and the lungs was measured. The results are presented in Table 5. Most of the compounds altered the histamine levels *in vivo*. Administration of 3-hydroxy-4-nitrobenzyloxyamine to rats resulted in markedly decreased levels of histamine in stomach (45 per cent) and in lungs (30 per cent). 3-Nitrobenzyloxyamine also had an effect on the stomach. In these experiments NSD-1055 was less effective, producing about 30 per cent decrease in the histamine levels of both organs. Pyridyl-3-methoxyamine lowered the level of histamine in lungs to a greater extent than that in stomach, and this effect was more pronounced (42 per cent decrease in the level of histamine) than that of benzyloxyamines.

Further studies were carried out with this compound. The activity of pyridyl-3-methoxyamine was also studied after single treatment. Various doses of the inhibitor were administered to rats and 3 hr after the treatment the levels of histamine in stomach and lungs measured (Table 6). Less decrease in levels of histamine was achieved by this method than by repeated administrations. The compound showed no activity in the lungs at high doses, 5 and 15 mg/kg doses significantly decreased the levels of histamine in both tissues, and the level of histamine in stomach could be lowered further by treatment with 45 and 135 mg/kg of inhibitor. The duration of its

TABLE 5. THE HISTAMINE LOWERING EFFECT OF BENZYLOXYAMINE AND PYRIDYLMETHOXYAMINE DERIVATIVES IN RATS

Chemical name	Structure	Derivatives	Histamine μg/g tissue	
			Stomach	Lungs
		Control	20·75 ± 1·44	6·60 ± 0·15
Benzyloxy-amines		3-Hydroxy-4-nitro-	11·70 ± 1·12*	4·60 ± 0·80*
		3-Hydroxy-4-bromo- (NSD-1055)	14·80 ± 1·08*	4·30 ± 0·36*
		3-Nitro-	12·54 ± 0·83*	6·00 ± 0·69
		3-Nitro-4-chloro-	17·90 ± 1·12	5·10 ± 0·99*
		3-Nitro-4-bromo-	18·90 ± 1·59	6·70 ± 0·93
Pyridylmethoxy-amines		Pyridyl-3-methoxyamine	15·80 ± 0·58*	3·80 ± 0·31
		Pyridyl-2-methoxyamine	16·10 ± 0·40*	5·50 ± 0·58

* $P < 0.10$.

Groups of five rats were injected subcutaneously with 30 mg/kg of inhibitors, three times in 24 hr and sacrificed 3 hr after the last treatment. Stomach and lungs were removed, homogenized in 0·4 M $HClO_4$ and histamine levels determined using the Shore method.[26]

TABLE 6. HISTAMINE LOWERING EFFECT OF PYRIDYL-3-METHOXYAMINE IN RATS BY SINGLE ORAL ADMINISTRATION

Treatment	Dose (mg/kg)	Histamine (μg/g tissue)	
		Stomach	Lungs
Control	—	21·30 ± 1·48	7·01 ± 0·55
Treated	5	16·5 ± 1·32*	5·27 ± 0·62*
	15	15·6 ± 0·96*	4·72 ± 0·60*
	45	15·0 ± 1·12*	6·35 ± 0·55*
	135	14·3 ± 0·98*	6·80 ± 0·58*

* $P < 0.10$.

Groups of five rats were treated orally with various doses of pyridyl-3-methoxyamine and 3 hr after the last treatment animals were sacrificed, organs removed, homogenized in 0·4 M $HClO_4$ and histamine levels determined according to Shore *et al.*[26]

effect is shown in Table 7. Administration of 15 mg/kg to the animals resulted in maximally decreased levels of histamine in stomach at 4 hr, and in lungs at 5 hr after the treatment. At 16 hr only slight diminutions were observed, and at 24 hr the histamine levels returned to normal.

In prolonged treatments animals received the substance once daily for 28 days, and histamine measurements were carried out at 24 hr after the last treatment (Table 7). The markedly decreased levels of histamine in stomach and especially in lungs obtained at 48 hr after the last administration suggest a much longer duration of its effect than after the single treatment.

TABLE 7. DURATION OF THE HISTAMINE LOWERING EFFECT OF PYRIDYL-3-METHOXYAMINE IN RATS BY SINGLE ORAL ADMINISTRATION

Treatment	Time after treatment (hr)	Histamine (μg/g tissue)	
		Stomach	Lungs
Control	0	20·40 ± 0·91	6·96 ± 0·88
Treated	1	17·22 ± 0·98	7·27 ± 0·82
	3	16·07 ± 1·29*	5·74 ± 0·99*
	5	16·74 ± 0·58*	4·41 ± 0·65*
	16	17·33 ± 1·15	5·52 ± 0·83
	24	19·73 ± 0·53	5·62 ± 0·62

* $P < 0·10$.

Groups of five rats were treated orally with 15 mg/kg of pyridyl-3-methoxyamine and sacrificed at various times after treatment. Stomach and lungs were removed, homogenized in 0·4 $HClO_4$, and histamine content determined according to Shore *et al.*[26]

TABLE 8. HISTAMINE LOWERING EFFECT OF PYRIDYL-3-METHOXYAMINE IN RATS BY PROLONGED TREATMENTS

Treatment	Dose (mg/kg)	Histamine (μg/g tissue)	
		Stomach	Lungs
Control	—	21·20 ± 1·96	6·17 ± 0·66
Treated	50	17·20 ± 1·12	3·27 ± 0·18
	100	15·87 ± 0·72*	3·80 ± 0·56*

* $P < 0·10$.

Groups of seven rats were treated orally with 50 mg/kg and 100 mg/kg doses of the compound once daily for 28 days. Animals were killed 48 hr after the last treatment, organs removed, homogenized in 0·4 M $HClO_4$ and histamine content determined according to Shore *et al.*[26]

DISCUSSION

Recent studies on the inhibition of decarboxylases confirmed previous findings that the 3-hydroxyl group is important for high activity of inhibitors against aromatic L-amino acid decarboxylase.[29–30] Among the newly synthetized benzyloxyamines 3-hydroxy-4-nitrobenzyloxyamine showed the most potent effect on both aromatic L-amino acid and histidine decarboxylases. 3-Nitro-4-hydroxybenzyloxyamine had only slight activity against both enzymes. 3-Nitrobenzyloxyamine was a powerful inhibitor of histidine decarboxylase, but it had no remarkable effect on aromatic L-amino acid decarboxylase. In general, the 3-nitro- derivatives of benzyloxyamine showed considerably lower activity than the 3-hydroxy derivatives of this compound.

No such correlation was found between the histidine decarboxylase inhibiting potency and chemical structure of the compounds.

Pyridylmethoxyamines represent a new class of decarboxylase inhibitors. The *in vitro* inhibiting effect of pyridyl-2-methoxyamine on both aromatic L-amino acid and histidine decarboxylases, and the selective effect of pyridyl-3-methoxyamine on histidine decarboxylase and on the level of histamine of various tissues, were remarkable. The position of the methoxyamine group seems to greatly influence the *in vitro* aromatic L-amino acid decarboxylase inhibiting effect of these compounds.

Among the heterocyclic methoxyamines, imidazol-4,5-yl-methoxyamine and thiazol-4-yl-methoxyamine are known inhibitors of histidine decarboxylase.[28–30,31] Pyridil-3-methoxyamine proved to be a more potent and selective inhibitor of this enzyme than the above compounds. The minimal dose of this inhibitor required to lower the level of histamine in the lungs was 5 mg/kg by single oral treatments, and this was much lower than that of NSD-1055, α-methylhistidine or thiazol-4-yl-methoxyamine.[13,30]

Results obtained with 3-hydroxy-4-nitrobenzyloxyamine on guinea-pig brain decarboxylase *in vivo* showed a marked inhibition of this enzyme and suggest a complete passage of the compound across the blood-brain barrier. In spite of this, the dopamine level in the brains of rats receiving L-dopa was significantly higher when animals were pretreated with the inhibitor than when no inhibitor was given to the animals.* These results could only be explained if we assume that the decarboxylation of dopa proceeds mainly in the liver and the inhibition of the liver enzyme is exclusively controlling the formation of dopamine level in the tissues after the L-dopa administration.

The therapeutic application of α-methyl-dopa as an adjunct to the treatment of Parkinson's disease,[32] as a potent inhibitor of aromatic L-amino acid decarboxylase both in brain and liver,[29,33] confirms this assumption.

The histamine lowering effect of some of these compounds was also remarkable. 3-Hydroxy-4-nitrobenzyloxyamine and 3-nitrobenzyloxyamine had a marked effect after repeated treatments; the former compound produced decreased levels of histamine both in the stomach and in the lungs, while the latter lowered the level of histamine in the lungs selectively. These effects were less pronounced, however, than those obtained with pyridyl-3-methoxyamine on the level of histamine in the lungs.

NSD-1055 decreased the levels of histamine in the stomach and in the lungs to a lesser extent than in the experiments of Levine *et al.*[13] and of Radwan and West.[34] According to the data of Michaelson[35] the histamine lowering effect of NSD-1055 found by different authors showed a great variation.[36–38] This could presumably be explained by the different sensitivities of rats of different strains.[39] The other problem arising from the measurement of the histamine lowering effect of HD inhibitors is probably the simultaneous effect of the compounds on diamine oxidase. According to Kobayashi,[40] NSD-1055 produces a marked effect also on diamine oxidase and this effect works against the histamine lowering activity of the compound. This was confirmed by the results of our studies, in the case of pyridyl-3-methoxyamine; the diamine oxidase inhibiting effect appeared besides its strong activity on histidine decarboxylase and a close correlation has to be assumed between this effect and the reduced activity of the compound on tissue histamine levels at high doses.

* M. Fekete, personal communication.

Finally, present results suggest that the most active compounds of these series are valuable research tools, and will be useful in further studies on histamine.

REFERENCES

1. P. A. Holtz, R. Heise and K. Lüdtke, *Arch. exp. Path. Pharmak.* **191,** 87 (1938).
2. H. Blaschko, *Adv. Enzymol.* **5,** 67 (1945).
3. S. Udenfriend, C. T. Clark and E. Titus, *J. Am. chem. Soc.* **75,** 501 (1953).
4. C. T. Clark, H. Weissbach and S. Udenfriend, *J. biol. Chem.* **210,** 139 (1954).
5. E. Werle and D. Aures, *Hoppe–Seyl. Z.* **316,** 45 (1959).
6. W. Lovenberg, H. Weissbach and S. Udenfriend, *J. biol. Chem.* **237,** 89 (1962).
7. T. L. Sourkes, *Pharmac. Rev.* **18,** 53 (1966).
8. D. Mackay, J. F. Riley and D. M. Sheperd, *J. Pharm. Pharmac.* **13,** 257 (1961).
9. H. Weissbach, W. Lovenberg and S. Udenfriend, *Biochem. biophys. Acta,* **50,** 177 (1961).
10. P. O. Ganrot, A. M. Rosegren and E. Rosengren, *Experientia* **17,** 263 (1961).
11. J. D. Reid and D. M. Sheperd, *Life Sci.* **1,** 5 (1963).
12. E. Hansson, R. M. Fleming and W. G. Clark, *Int. J. Neuropharm.* **3,** 177 (1964).
13. R. J. Levine, T. L. Sato and A. Sjoerdsma, *Biochem. Pharmac.* **14,** 139 (1965).
14. H. Hamor, D. M. Breslow and G. W. Fisch, *J. Pharm. Sci.* **59,** 1752 (1970).
15. R. Lazare and P. A. Watson, *J. pharm. Pharmac.* **24,** 361 (1972).
16. R. J. Levine, *Fedn. Proc.* **24,** 1331 (1965).
17. A. Nikulin, P. Stern and Z. Zeger-Vidovic, *Arch. Int. Pharmacodyn* **166,** 305 (1967).
18. R. J. Levine, *Science, N.Y.* **154,** 1017 (1966).
19. J. P. Phair, R. J. Levine and F. S. Kantor, *J. Allergy* **39,** 111 (1967).
20. E. Kasztreiner, G. Szilágyi, J. Szakály-Kosáry, Z. Huszti, M. Fekete and J. Borsy, *J. chem. Soc. Perkin Trans. I. (Org. Chem.)* in press.
21. E. Kasztreiner, L. Vargha, G. Szilágyi, Z. Huszti, J. Borsy, J. Szakály–Kosáry, S. Elek and I. Polgári, *Hung. Pat. No.* 60, 1132, in press.
22. R. Hakanson and Ch. Owman, *Biochem. Pharmac.* **15,** 489 (1966).
23. T. Okuyama and Y. Kobayashi, *Arch. Biochem.* **95,** 242 (1961).
24. S. Udenfriend, H. Weissbach and C. T. Clark, *J. biol. Chem.* **215,** 337 (1955).
25. D. F. Bogdansky, A. Pletscher, B. B. Brodie and S. Udenfriend, *J. Pharmac.* **117,** 82 (1956).
26. P. A. Shore, A. Burkhalter and V. H. Cohn, *J. Pharmac. exp. Ther.* **127,** 182 (1959).
27. B. Holmstedt and R. Tham, *Acta Physiol. Scand.* **45,** 152 (1959).
28. D. M. Shepherd and D. Mackay, in *Progress in Medicinal Chemistry* (Eds. G. P. Ellis and G. B. West), Vol. 5, p. 199. Plenum Press, New York (1967).
29. T. L. Sourkes, *Arch. Biochem. Biophys.* **51,** 444 (1954).
30. D. Aures, L. E. A. Rodrigues, W. G. Clark and S. S. Laws, *Abstr. Commun. IV. Int. Cong. Pharmac.*, Basle, Switzerland, p. 179 (1967).
31. M. K. Menon, W. G. Clark and D. Aures, *Life Sci.* **10,** 1097 (1971).
32. A. T. N. Chase and A. N. Watanabe, *Neurobiology* **22,** 384 (1972).
33. D. J. Drain, M. Horlington, R. Lazare and G. A. Poulter, *Life Sci.* **1,** 93 (1962).
34. A. G. Radwan and G. B. West, *Br. J. Pharmac. Chemother.* **30,** 392 (1967).
35. A. Michaelson and H. R. Smithson, *Proc. Soc. exp. Biol. Med.* **136,** 660 (1971).
36. H. L. Johnson, *Biochem. Pharmac.* **18,** 651 (1969).
37. H. Green and R. W. Erickson, *Arch. Int. Pharmacodyn.* **166,** 127 (1967).
38. L. L. Foster, *J. Pharmac. exp. Ther.* **169,** 132 (1969).
39. R. J. Levine, *Biochem. Pharmac.* **18,** 403 (1966).
40. Y. Kobayashi, J. Kupelian and D. V. Maudsley, *Biochem. Pharmac.* **19,** 1761 (1970).