solvents, was recrystd from C_6H_6 and melted at 273–274°. *Anal.* ($C_{20}H_{10}N_2$) C, H, N.

Acknowledgments.—We are indebted to Professor M. S. Newman, Ohio State University, for generous donation of samples of several rare hydrocarbons, including 1-, 3-, and 11-methylbenz[*a*]anthracene, and for detailed description of the synthesis of 1-bromo-3-methylnaphthalene. We are also grateful to Professor Charles B. Huggins, The University of Chicago, for permission to cite the biological test data obtained in his laboratory.

Specificity in Enzyme Inhibition. 1. Synthesis of 4-(4-Imidazolyl)-3-amino-2-butanone, 4-(4-Imidazolyl)-3-acetamido-2-butanone, and 4-(4-Imidazolylmethyl)-2,5-dimethyloxazole for Assay as Inhibitors of Histidine Decarboxylase

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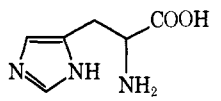
Received December 10, 1970

A general approach to specific enzyme inhibition is discussed. The synthesis and results of assay of 4-(4-imidazolyl)-3-amino-2-butanone (**2**), 4-(4-imidazolyl)-3-acetamido-2-butanone (**4**), and 4-(4-imidazolylmethyl)-2,5-dimethyloxazole are described.

The receptor sites of many metabolic enzymes which utilize amino acids as substrates can be depicted as having 2 binding sites and 1 active site. The binding sites can be designated as specific and nonspecific. For example, if one views a model for the receptor site of a specific amino acid decarboxylase (Figure 1), the specific binding would be to the R group and would act to differentiate the amino acid to be used as a substrate. A nonspecific site would bind the amino group; the latter would act as the orienting function and would place the carboxyl group in juxtaposition to the site of chemical change, the active site.

On the basis of this model, an active-site-directed inhibitor of a decarboxylase enzyme should be capable of binding with the specific as well as the nonspecific sites of the enzyme but it should be incapable of undergoing the required chemical transformation, decarboxylation, at the active site. The same argument should apply to transaminases, aminopeptidases, aminesynthetases, certain oxidases, etc.

In order to investigate the applicability of this hypothesis, histidine was chosen as the substrate to be modified. L-Histidine decarboxylase is specific for the biosynthesis of histamine,[2] and a specific inhibitor of this enzyme should possess an imidazole ring which would approach and bind to the specific binding site, a basic N for binding to the nonspecific site, and a function incapable of decarboxylation to approach the active site. This phase of the investigation considered only reversible endo binding to the receptor.[3] In the initial study of the requirements of histidine analogs for decarboxylase inhibitor activity 3 compds were prepared. The α-amino ketone **2** would be expected to have the specific and nonspecific binding



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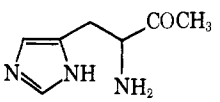

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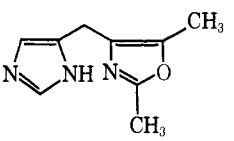

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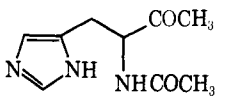

4

functions of histidine (**1**). The oxazole **3** would possess the basic nonspecific function and the specific imidazole ring but sterically might be less capable of endo binding to the receptor site. The *N*-acetyl-α-amino ketone **4** would have the specific binding function and the active-site-directed function which is incapable of decarboxylation, but it does not have the nonspecific binding group required for orientation of the Ac group to the active site.

It could be predicted that **2** would be an excellent and specific inhibitor, **3** would be specific but less active, and **4** would possess little or no activity as an inhibitor.

These compds were prepared from histidine·HCl (**1**) which underwent decarboxylative acetylation in pyridine and Ac_2O soln to provide 4-(4-imidazolyl)-3-acetamido-2-butanone·HCl (**4**) in 64% yield. Dakin and West[4] were unable to characterize this material, since they failed to obtain a crystalline product. In this study a crystalline product was obtained and spectral data support the proposed structure. The maximum yield was secured with mild reaction conditions.

The hydrolysis of the acetamido ketone **4** to produce 4-(4-imidazolyl)-3-amino-2-butanone·2HCl (**2**) was accomplished in 82% yield with 4 *N* HCl.

4-(4-Imidazolylmethyl)-2,5-dimethyloxazole·HCl (**3**) was obtained in 40% yield by refluxing the acet-

(1) Taken in part from the dissertation presented by J. A. Weis, Sept 1968, to the Graduate School of the University of Kansas, Lawrence, Kan., in partial fulfillment of the requirements for the Doctor of Philosophy Degree.

(2) G. Kahlson, E. Rosengren, and R. Thunberg, *J. Physiol. (London)*, **169**, 467 (1963).

(3) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," Wiley, New York, N. Y., 1967.

(4) H. D. Dakin and R. West, *J. Biol. Chem.*, **78**, 745, 757 (1928).

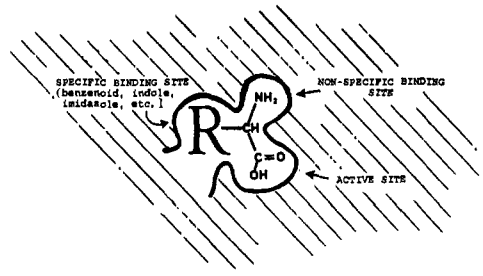

Figure 1.

amido ketone **4** in Ac_2O. This material had been obtained previously only as the $AuCl_2$ complex.[5]

Compds **2**, **3**, and **4** were assayed as inhibitors against histidine decarboxylase and dopa decarboxylase. The results are shown in Table I.

TABLE I

	Concn, m*M*	% inhibition
	Histidine Decarboxylase[a]	
2	1.0	87
3	1.0	56
4	1.0	10
	Dopa Decarboxylase	
2	10.0	30
2	7.5	33
2	2.5	10
3	2.0	6
3	0.2	7
4	2.0	18

[a] After the submission of this work in thesis form (ref 1) a report by S. R. Mardashev, N. A. Gouchar, and N. S. Dabagov, *Dokl. Acad. Nauk SSR*, **189** (4), 895 (1969), appeared.

As is evident from the results, **2** exhibits unique specificity as an inhibitor, being an excellent inhibitor of histidine decarboxylase and a poor inhibitor of dopa decarboxylase. Furthermore, the rationale for the preparation of **2**, **3**, and **4** is substantiated.

This approach has been utilized with other amino acids, and the results will be the subject of future reports.

Experimental Section[6]

4-(4-Imidazolyl)-3-acetamido-2-butanone·HCl (4).—Ac_2O (141 g, 1.43 moles) and pyridine (94.5 g, 1.19 moles) were added to L-histidine·HCl·H_2O (**1**) (50 g, 0.24 mole). While stirring, the mixt was heated until gas evoln commenced (70°). External heating was stopped as the temp rose spontaneously to 100°. After 5 min, gentle heating was applied to maintain the reaction temp at 90° for 15 min. The mixt was allowed to cool over a 1-hr period. Excess volatile reactants were removed by distn *in vacuo*. Trace amts of volatile reactants were removed by steam distn *in vacuo*. The resulting aq soln was decolorized with activated charcoal, and H_2O was removed by distn *in vacuo*. The resulting orange gum was cryst from hot *i*-PrOH. The crystd mass was broken up, and addl *i*-PrOH was added to make a filterable slurry. The crystals were collected and recrystd (*i*-PrOH) affording 35.4 g (64%): mp 165–168°; ir (Nujol) 1540 (amide II band), 1635 (amide C=O), and 1729 cm^{-1} (C=O); nmr (D_2O) 8.82 (d, 1, J = 1.5 Hz, N=CHN), 7.46 (s, 1, NCH=C), 4.83 (m, 1, CHC=O), 3.33 (m, 2, CH_2), 2.38 (s, 3, $COCH_3$), and 2.10 ppm (s, 3, acetamide CH_3). *Anal.* ($C_9H_{12}ClN_3O_2$) C, H, N.

4-(4-Imidazolyl)-3-amino-2-butanone·2HCl (2).—Compd **4** (12 g, 52 mmoles) was dissolved in 120 ml of 4 *N* HCl. The soln was stirred while refluxing for 3 hr. H_2O was removed by distn *in vacuo*. Trace amts of H_2O were removed by codistn with *i*-PrOH *in vacuo*. The cryst residue was collected and washed with *i*-PrOH. Recrystn (MeOH–*i*-PrOH) provided yellow crystals weighing 9.69 g (82%): mp 212–215° (lit.[4] 205–206°); ir (Nujol) 1518 ($^+$NH), 1585 ($^+$NH), and 1725 cm^{-1} (C=O); nmr (D_2O) 8.86 (d, 1, J = 1.5 Hz, N=CHN), 7.60 (s, 1, NCH=C), 4.72 (q, 1, CHCO), 3.37–3.73 (m, 2, CH_2), and 2.45 ppm (s, 3, $COCH_3$).

4-(4-Imidazolylmethyl)-2,5-dimethyloxazole·HCl (3).—Compd **4** (12.3 g, 53 mmoles) was refluxed with stirring in Ac_2O (100 ml) for 50 min. When the mixt cooled, crystals formed. The product was collected and washed with Et_2O. The solid was dried *in vacuo* (60°) overnight. The dry material was dissolved in 95% EtOH, and the soln was decolorized with activated charcoal. The EtOH was removed by distn *in vacuo*, and the resulting thick gum spontaneously crystd. The solid mass was triturated with Me_2CO and collected and washed with Me_2CO. Recrystn from an abs EtOH–Me_2CO mixt furnished 4.5 g (40%) of product, mp 161–163°. An anal. sample of mp 163–165° was obtained by recrystn from $CHCl_3$–cyclohexane; ir ($CHCl_3$) 1585 ($^+$NH), 1616 ($^+$NH), and 1655 cm^{-1} (oxazole C=N); nmr ($CDCl_3$) 14.41 (s, 2, $^+$NH), 9.05 (d, 1, J = 1.5 Hz, N=CHN), 7.21 (s, 1, NCH=C), 3.97 (s, 2, CH_2), 2.36 (s, 3, N=CCH_3), and 2.28 ppm (s, 3, C=CCH_3). *Anal.* ($C_9H_{12}ClO$) C, H, N.

Purification of Enzymes.—Histidine decarboxylase (EC 4.1.1.22) was prepd by a modification of methods described by Hakanson[7] and Levine and Watts.[8] Whole rat fetuses (19–20 days gestation) obtd from Sprague–Dawley rats (Carworth Farms) were homogenized in 2 vol of 0.1 *M* NaOAc, pH 5.5. After centrifugation for 45 min at 90,000*g*, the supernatant was fractionated using $(NH_4)_2SO_4$. The protein pptng between 25 and 45% satn was dissolved in 0.1 *M* K_3PO_4, pH 7.0, and dialyzed overnight at 5° against H_2O. The dialyzed fraction was dild with 0.05 K_3PO_4, pH 7.0, to a concn of 40 mg of protein per ml, and could be stored at −15° for several months with no loss of activity.

Aromatic L-amino acid decarboxylase (EC 4.1.1.26) was prepd from guinea pig kidneys by the method of Clark, *et al.*[9] The kidneys were homogenized in 4 vol of H_2O and were centrifuged at 20,000*g* for 30 min. The protein fraction pptd from the supernatant by $(NH_4)_2SO_4$ between 37 and 55% satn was dissolved in 0.05 *M* K_3PO_4, pH 7.0, and dialyzed overnight against H_2O at 5°. The vol after dialysis was adjusted to a concn of 65 mg of protein per ml and the enzyme was stored at −15°.

Assay of Histidine Decarboxylase.—Histidine decarboxylase activity was determined by measuring the CO_2-^{14}C produced from L-histidine-*carboxyl*-^{14}C as previously described.[10] The standard reaction mixt contd 100 μmoles of K_3PO_4 buffer (pH 6.8), 0.25 μmole of L-histidine contg 0.25 μCi of L-histidine-*carboxyl*-^{14}C, 0.01 μmole of pyridoxal 5-phosphate, and 2 mg 0.05 mole) of the histidine decarboxylase prepn. Inhibitors were added at various concns in 0.1 ml of H_2O. The final vol was made to 1.0 ml with H_2O. The mixt was incubated for 90 min at 37°. Controls were included to correct for nonenzymatic decarboxylation.

Assay of Aromatic L-Amino Acid Decarboxylase.—Aromatic L-amino acid decarboxylase activity was determined using DL-dopa-*carboxyl*-^{14}C as the substrate and measuring the CO_2-^{14}C in the same manner as with histidine decarboxylase.[10] The std reaction mixt contd 50 μmoles of K_3PO_4 buffer (pH 6.8), 0.5 μmole of DL-dopa contg 0.11 μCi of DL-dopa-*carboxyl*-^{14}C, 0.033 μmole of pyridoxal 5-phosphate, and 0.13 mg of the decarboxylase prep. Inhibitors were added in 0.1 ml of H_2O, and the final vol

(5) F. Wrede and W. Keil, *Hoppe-Seyler's Z. Physiol. Chem.*, **203**, 279 (1931).

(6) Melting points were obtd on a calibrated Thomas-Hoover Uni-Melt and are cor. Ir data were recorded on IR-8 and IR-10 spectrophotometers and nmr data on Varian Associates Model A-60 and A-60-A spectrometers, using Me_4Si or Na 3-(trimethylsilyl)-1-propanesulfonate as internal standards and are reported as δ (ppm). Microanalyses were performed on an F and M CHN analyzer Model 185 in this department and by Midwest Microlab, Inc., Indianapolis, Ill. Where anal. are indicated only by symbols of the elements, anal. results obtained for those elements were within ±0.4% of the theor values.

(7) R. Hakanson, *Biochem. Pharmacol.*, **12**, 1289 (1963).

(8) R. J. Levine and D. E. Watts, *ibid.*, **15**, 841 (1966).

(9) C. T. Clark, H. Weissbach, and S. Udenfrend, *J. Biol. Chem.*, **210**, 139 (1954).

(10) F. J. Leinweber and L. A. Walker, *Anal. Biochem.*, **21**, 131 (1967).

was made to 0.5 ml with H_2O. The mixt was incubated for 5 min at 37°. Controls were included to correct for nonenzymatic decarboxylation.

Acknowledgment.—The authors gratefully acknowledge the support of this project by the National Institutes of Health Grants GM-09254 and GM-01341. We wish to thank Dr. R. J. Taylor, Jr., and F. J. Leinweber, McNeil Laboratories, Fort Washington, Pa., for the histidine decarboxylase and dopa decarboxylase assays.

5-Homopyridoxals, 5-Thiopyridoxal, and Related Compounds. Synthesis, Tautomerism, and Biological Properties[1]

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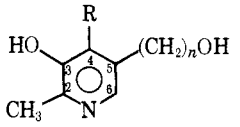
Received January 19, 1971

Homopyridoxals with 1 or 2 additional CH_2 groups in the 5 position have been obtained by controlled oxidn of the corresponding homopyridoxols with MnO_2. More vigorous oxidn yielded the corresponding 4-homopyridoxic acids. 4-Deoxyhomopyridoxols have also been prepd from homopyridoxols by hydrogenolysis with hydrazine. Reaction of hydrazine-d_4 with pyridoxol gave 4-deoxypyridoxol in which the α^2 and α^4 Me groups and the 6 position were deuterated. These deuterations as well as the formation of 4-deoxypyridoxol have been rationalized assuming the formation of quinone methide intermediates. 5-Thiopyridoxal was prepd and was found to be a hemiacetal in the narrow pH range in which it was stable. Likewise, the two homopyridoxals exist in a cyclic (hemiacetal) form in acid and neutral soln, whereas in an alkaline medium a marked tendency to revert to the aldehyde form, particularly in the 2 C homolog, has been observed. Derivatives of both the aldehyde and hemiacetal forms of these pyridoxal analogs have been obtained. Pyridoxal was found to undergo a Cannizzaro reaction when treated with alkali. The oximes of homopyridoxals and the 4-deoxyhomopyridoxols are inhibitors of pyridoxal phosphokinase. The effect of some of these compds on *Saccharomyces carlsbergensis*, tissue culture cells, and certain enzymes *in vivo* has also been determined.

In efforts to develop more selective antagonists of vitamin B_6 that might be active as anticancer agents[2] we previously synthesized a series of homologs of pyridoxol (I, R = CH_2OH) by extension and branching of the 4- and 5-hydroxymethyl side chains.[3,4] Compds obtained by extension of the 5 position (I, R = CH_2OH; n = 2–4) were found to be inhibitors of *Saccharomyces carlsbergensis*[4] but were ineffective in inhibiting mammalian systems.[5]



I

It was hoped that by modifying the 4-hydroxymethyl to a formyl (I, R = CHO; n = 2,3) or Me (I, R = CH_3; n = 2,3), improved inhibitors could be obtained, since they would more closely resemble the biologically more active form of vitamin B_6 or the well-known antimetabolite 4-deoxypyridoxol (I, R = CH_3; n = 1), resp. (It has also been found that when 5-deoxypyridoxol was converted to the corresponding 4-aldehyde, toxicity was increased markedly.[6])

In addn to compds of the types already mentioned, we have synthesized 5-thiopyridoxal (XVIII) and two 5-homopyridoxic acids (VIII and XIII). Chem properties of these compds, particularly ring–chain tautomerism, have been studied, and have been compared with those of pyridoxal. Some of these compds have been evaluated for their biol and enzymatic activity in several systems.

Chemistry. Synthesis.—Scheme I depicts the synthesis of the homopyridoxals (III and X), homopyridoxic acid (VIII and XIII), their derivs, and 4-deoxyhomopyridoxols (VI and XII); and Scheme II that of 5-thiopyridoxal (XVIII) and its ethyl acetal deriv.

Oxidn of the 4-CH_2OH group to the CHO and COOH groups has been carried out with MnO_2 as shown in Scheme I. Conditions for this oxidn had to be varied for each compd. Probably because of the ring–chain tautomerism of these compds (see below), the length of the side chain had a profound effect on the oxidizability of the 4-CH_2OH group. Thus conditions[7] that had been worked out earlier for the oxidn of pyridoxol to pyridoxal and 4-pyridoxic acid could not be applied.

In the synthesis of 5-thiopyridoxal (XVIII, Scheme II), it was necessary to block the SH group of 5-thiopyridoxol by benzoylation, as in XVI. This was accomplished in a more direct manner and in better yield (from the blocked chloro derivative XIV) than has been reported previously.[8] The most crucial step in this synthesis was the deblocking step to yield 5-thiopyridoxal from XVII. Both acid and alkaline hydrolysis of the thiobenzoate XVII gave a mixt of products, but a base-catalyzed transesterification with

(1) (a) Pyridoxine Chemistry. 26. Preceding paper in this series: W. Korytnyk and B. Lachmann, *J. Med. Chem.*, **14**, 641 (1971). (b) A brief report of this study has appeared: Abstracts of the 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969, MEDI 48.

(2) For a review of the syn and biol activity of vitamin B_6 analogs see W. Korytnyk and M. Ikawa, *Methods Enzymol.*, **18A**, 524 (1970).

(3) W. Korytnyk and B. Paul, *J. Med. Chem.*, **13**, 187 (1970).

(4) W. Korytnyk, B. Paul, A. Bloch, and C. A. Nichol, *ibid.*, **10**, 345 (1967).

(5) Compd IX was tested against S-180 in Swiss mice fed complete or vitamin B_6 deficient diets. It was found to be inactive at doses up to 400 mg/kg per day x 7 ip or 0.025% in diets (Dr. E. Mihich, personal communication).

(6) F. Rosen, E. Mihich, and C. A. Nichol, *Vitam. Horm. (New York)*, **22**, 609 (1964).

(7) H. Ahrens and W. Korytnyk, *J. Heterocycl. Chem.*, **4**, 625 (1967).

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