Chemistry of Heterocyclic Compounds, Volume 6
Klaus Hofmann

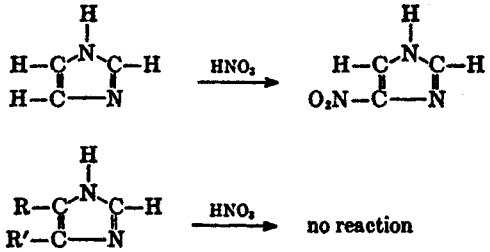
CHAPTER V

The Nitro-, Arylazo-, and Aminoimidazoles

A. Nitroimidazoles

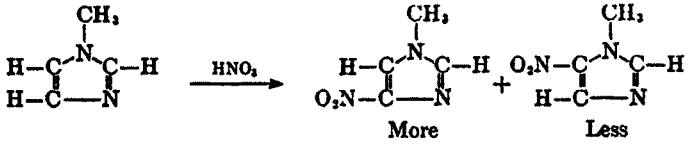
1. Synthetic Methods, and Orientation of the Nitro Group

The treatment of an imidazole with a mixture of fuming nitric and sulfuric acids or the exposure of the nitrate of an imidazole to the action of hot concentrated sulfuric acid are practical procedures for the preparation of mononitroimidazoles. Polynitroimidazoles are unknown. The entering nitro group occupies, without exception, the 4- or 5-position, and imidazoles in which these positions are substituted, such as 4,5-dimethyl- (1) and 4,5-dibromoimidazole (2), or 4,5-imidazoledicarboxylic acid (1) fail to undergo nitration. Imidazole is nitrated with the formation of 4(or 5)-nitroimidazole (1,3,4). 2-Methylimidazole affords 2-methyl-4(or



5)-nitroimidazole (5), while 4(or 5)-methylimidazole is converted into 4(or 5)-methyl-5(or 4)-nitroimidazole (1,6). 2,4(or 2,5)-Dimethylimidazole is readily nitrated to give 2,4(or 2,5)-dimethyl-5(or 4)-nitroimidazole (6). Substitution of the imino hydrogen by a methyl group does not interfere with the ability of an imidazole to undergo nitration. It must be kept in mind, however, that nitration of a *N*-methylimidazole may lead to the formation of a 1,4- or a 1,5-isomer; both these possibilities are usually realized, with the 1,4-isomer predominating. 1-Methylimidazole

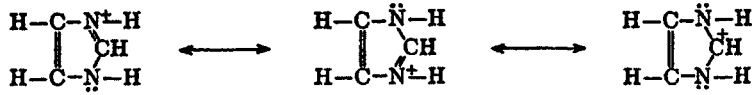
is nitrated with the formation of a mixture composed mainly of 1-methyl-4-nitroimidazole and a smaller quantity of the 1,5-isomeride (5).



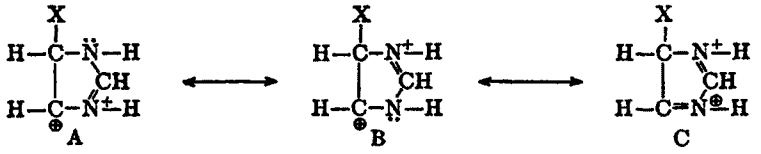
The nitration of 1,2-dimethylimidazole yields a large amount of 1,2-dimethyl-4-nitroimidazole and a smaller quantity of 1,2-dimethyl-5-nitroimidazole (7). 1,4-Dimethylimidazole is nitrated to give 1,4-dimethyl-5-nitroimidazole, while 1,5-dimethyl-4-nitroimidazole ensues from 1,5-dimethylimidazole (8). Halogenated imidazoles are also capable of undergoing nitration. 4(or 5)-Bromoimidazole is converted into 4(or 5)-bromo-5(or 4)-nitroimidazole and 2,4(or 2,5)-dibromoimidazole affords 2,4(or 2,5)-dibromo-5(or 4)-nitroimidazole (2). Both 1-methyl-5-chloroimidazole and 1-ethyl-2-methyl-5-chloroimidazole are converted into the corresponding 4-nitro derivatives (9,10). 2-Methyl-4(or 5)-bromoimidazole is nitrated with the formation of 2-methyl-4(or 5)-bromo-5(or 4)-nitroimidazole (11). Both 1-methyl-5-bromoimidazole and 1-methyl-4-bromoimidazole are readily nitrated giving 1-methyl-5-bromo-4-nitro- and 1-methyl-4-bromo-5-nitroimidazole, respectively (2).

Imidazolecarboxylic acids fail to be nitrated, and the nitroimidazolecarboxylic acids must be prepared by other methods. Certain imidazoles undergo oxidation when subjected to the action of concentrated nitric acid. 4(or 5)-Hydroxymethylimidazole, for example, is converted into a mixture of 4(or 5)-imidazolecarboxaldehyde and 4(or 5)-imidazolecarboxylic acid (12) (see Chapter III, Section A-1).

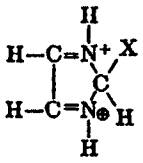
The exclusive 4(or 5)-nitration of imidazole and its derivatives may be due to: (*1*) the electron distribution in the imidazolium ion, and (*2*) a higher stability of the transition state for 4(or 5)-substitution. The 2-position, because of its location between two powerful electron-attracting nitrogen atoms is, most likely, more electron-deficient than the 4(or 5)-position. Such a charge-distribution would be expected to facilitate the attack of the NO_2^+ ion at the 4(or 5)-position. An inspection of the



transition states for 2- or 4-substitution suggests a higher degree of stabilization for the latter state, since it receives contributions from the amidine resonance (structures A, B, and C). The 2-transition state lacks this stabilization. These views are in disagreement with those expressed



4-Transition state



2-Transition state

by Dewar (13) who maintains that imidazoles are substituted *predominantly* at the 2-position because "in the transition state for 2-substitution the nitrogen atoms are at the ends of the mesomeric cation, while in the 4-transition state one nitrogen occupies a central position."

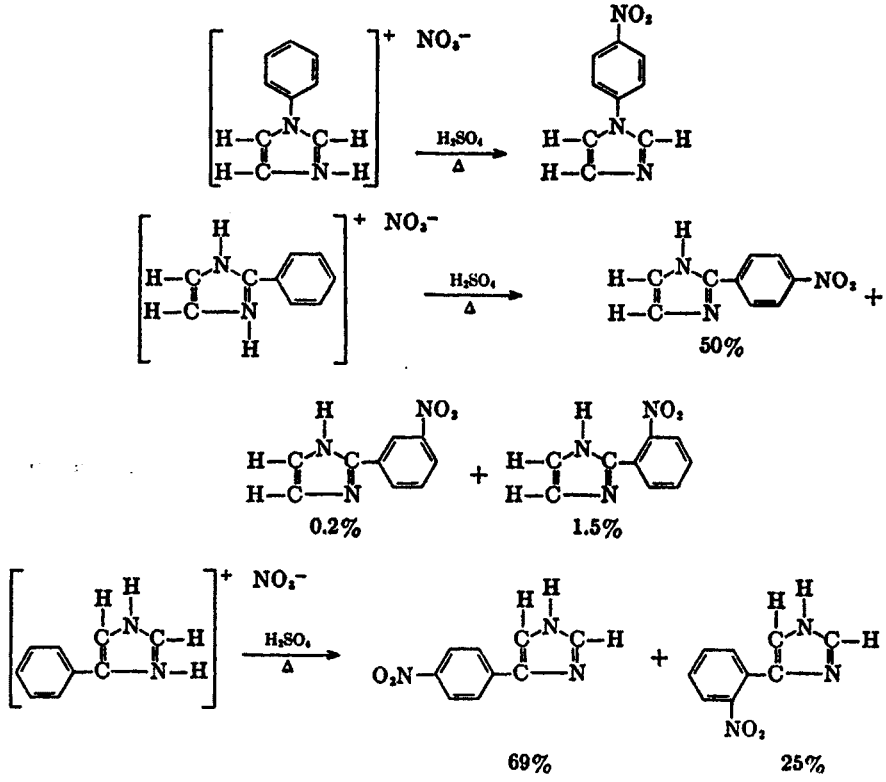
2-Transition state 4-Transition state

Transition states for 2- or 4-substitution in imidazoles, according to Dewar

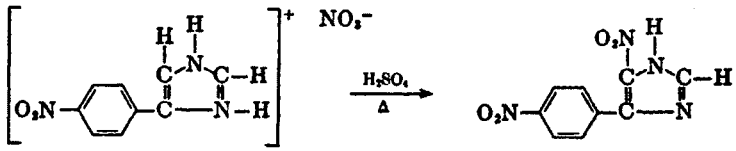
An inspection of the rather extensive material on the substitution behavior of the imidazoles, as presented in these pages, demonstrates that substitution takes place equally readily at the 4(or 5)- or the 2-position. Consequently, Dewar's "theoretical" treatment is of little significance.

The nitrates of the phenyl-substituted imidazoles undergo nitration in the phenyl nucleus upon exposure to the action of concentrated sulfuric acid at 100°. The nitro group enters predominantly the para position of the benzene ring. A number of examples may serve to illustrate this point. 1-Phenylimidazole affords 1-(*p*-nitrophenyl)imidazole in 58% yield (14), while 2-phenylimidazole is converted into a mixture of 2-(*p*-

nitrophenyl)-, 50%, 2-(*o*-nitrophenyl)-, 1.5%, and 2-(*m*-nitrophenyl)-imidazole, 0.2% respectively (15). 4(or 5)-Phenylimidazole affords 69% of 4(or 5)-(*p*-nitrophenyl)- and 25% of 4(or 5)-(*o*-nitrophenyl)-imidazole (16).



Fuming nitric acid converts 2-phenyl-4(or 5)-methylimidazole into 2-(*p*-nitrophenyl)-4(or 5)-methyl-5(or 4)-nitroimidazole (17). The nitrates of the nitrophenylimidazoles undergo further nitration when they are treated with hot, concentrated sulfuric acid, the second nitro group entering the 4(or 5)-position of the imidazole nucleus. The conversion of the nitrate of 4(or 5)-(*p*-nitrophenyl)imidazole into 4(or 5)-(*p*-nitrophenyl)-5(or 4)-nitroimidazole (16) and the transformation of the nitrate of 4-(*p*-nitrophenyl)-1-methylimidazole into 4-(*p*-nitrophenyl)-5-nitro-1-methylimidazole (5) may serve as illustrations.



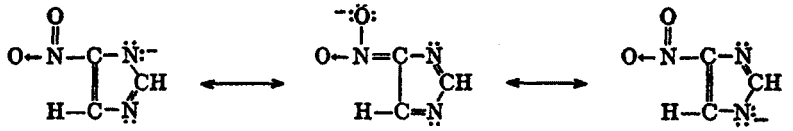
The substitution behavior of the phenylimidazoles demonstrates that the imidazolium ion is nitrated at a lower rate than the phenyl nucleus, and that the imidazolium group directs the entering nitro group predominantly into the para position. The incorporation of carboxyl groups into the 4,5-position of the imidazole moiety of 2-phenylimidazole brings about a change from a predominantly para to a predominantly meta nitrated compound (see Table XVIII). The 2-imidazolinium group is also a powerful meta director.

TABLE XVIII. Orientation of the Nitro Group in Certain Nitrated 2-Phenylimidazoles (15,18)

Compound nitrated	Composition of nitro products (%)	
	Para	Meta
2-Phenylimidazole	50	0.2
2-Phenyl-2-imidazoline	—	84.4
2-Phenyl-4(or 5)-imidazolecarboxylic acid	52	19
2-Phenyl-4,5-imidazoledicarboxylic acid	19	52

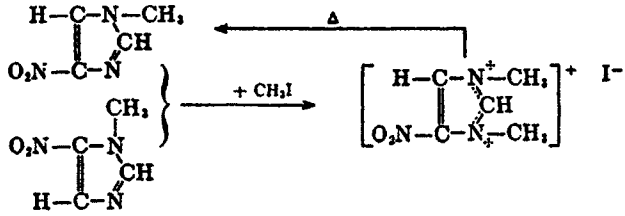
2. Properties

The nitroimidazoles are high-melting, colorless, crystalline compounds. Those possessing a free imino group dissolve in alkali hydroxides, alkali carbonates, and ammonia to form yellow solutions containing the respective nitroimidazole anion. The sodium salt of 4(or 5)-nitroimidazole is obtained in crystalline form when nitroimidazole is dissolved in hot 20% sodium hydroxide and the solution is allowed to cool (19). The yellow nitroimidazole anion may be represented as a hybrid having the major contributions shown below.

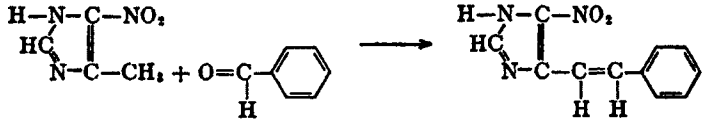


The introduction into the imidazole ring of the electronegative nitro group markedly decreases the basic strength (see Chapter I, Section C-1).

4(or 5)-Nitroimidazole fails to form a stable hydrochloride or picrate. It dissolves in concentrated hydrochloric acid, but is regenerated unchanged when the solution is diluted with water (1). Similar behavior is observed with 4(or 5)-bromo-5(or 4)-nitroimidazole (2). 1-Methyl-5-nitroimidazole is a stronger base than 1-methyl-4-nitroimidazole as evidenced by the finding that the former, but not the latter, forms a stable hydrochloride and picrate. A possible explanation for this difference in basicity is found in Chapter I, Section C-1. It is of interest that the 1,4-isomeride exhibits a greater chemical stability than does the 1,5-compound. This is shown by the fact that heating of 1,3-dimethyl-4(or 5)-nitroimidazolium iodide (resulting from the reaction of methyl iodide with either 1-methyl-4-nitro- or 1-methyl-5-nitroimidazole) causes elimination of methyl iodide to give 1-methyl-4-nitroimidazole (5).



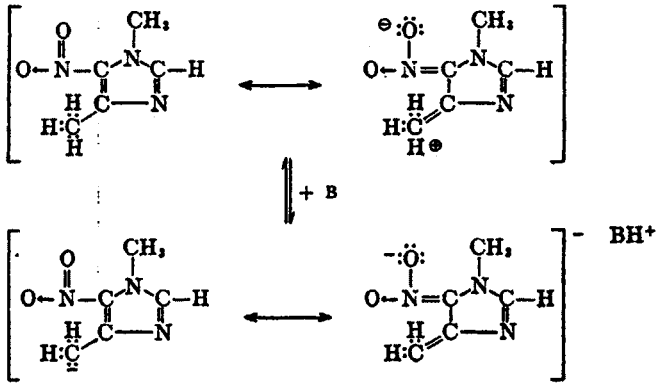
In Chapter IV, Section A-2, mention was made of the fact that an adjacent nitro group facilitates the displacement by sulfite ion of an otherwise inert halogen in the 4(or 5)-position on the imidazole ring. A methyl group in the 4(or 5)-position is also activated by a neighboring nitro group, as evidenced by the ability of this methyl group to condense with benzaldehyde to form a styrylnitroimidazole. The simplest example of this reaction is the formation of 4(or 5)-styryl-5(or 4)-nitroimidazole from 4(or 5)-methyl-5(or 4)-nitroimidazole and benzaldehyde (20). The condensation proceeds with optimal yields at temperatures of 150–160° in the presence of piperidine (21). *N*-Methylimidazoles such as 1,4-



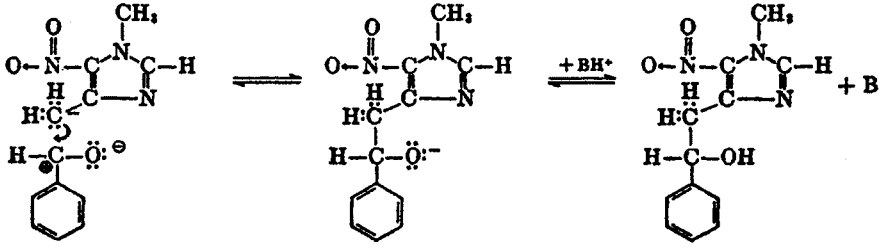
dimethyl-5-nitro- or 1,5-dimethyl-4-nitroimidazole also condense with benzaldehyde to give the respective styrylimidazoles (21).

The former compound may serve as the example for a discussion of plausible electronic explanations for both the activation of the methyl

group and the base-catalyzed condensation with benzaldehyde. The first step in the condensation reaction may involve the formation of the 1,4-dimethyl-5-nitroimidazole anion by the removal of a proton from the 4-methyl group by the base B (piperidine). The nitro group, because of its tendency to remove electrons from the methyl group (hyperconjugation), facilitates this process:

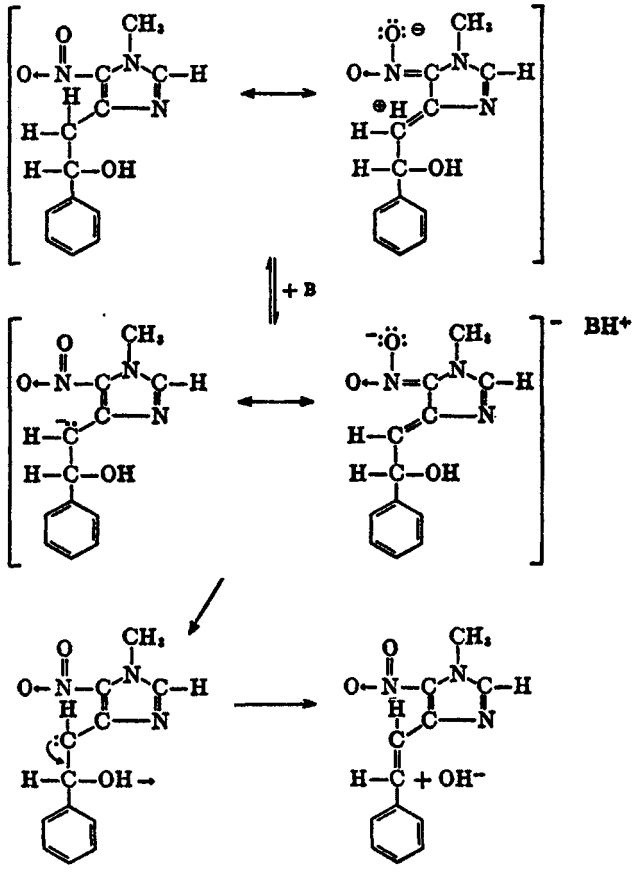


The condensation of the 1,4-dimethyl-5-nitroimidazole anion with benzaldehyde to give the intermediate carbinol may proceed through the following intermediate steps:



The base-catalyzed elimination of water from the carbinol may involve the initial removal of a proton from the "activated" methylene group followed by the expulsion of a hydroxide ion. See equation (1), page 134. It should be noted that the behavior of the methyl group in the nitromethylimidazoles closely parallels that of the methyl group in α-picoline and *N*-methyl-α-picolinium ion, where similar mechanisms are indicated (22–24).

As has been mentioned in Chapter IV, Section A-2, the bromine atom in 5-nitro-1,4-dimethyl-2-bromoimidazole is readily displaceable by sul-

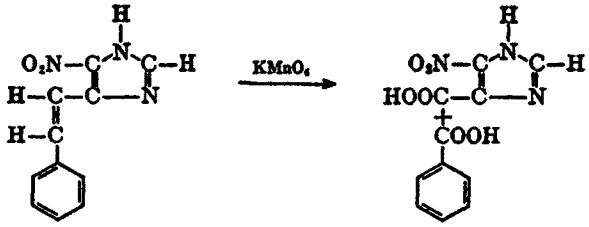


(1)

fite ion to give the corresponding 2-sulfonic acid. This suggests the possibility that the 2-methyl group in 1,2-dimethyl-5-nitroimidazole may be capable of undergoing a base-catalyzed condensation with benzaldehyde. This, however, is not the case, as both 5-nitro-1,2-dimethylimidazole and 4-nitro-1,2-dimethylimidazole fail to react with benzaldehyde (7).

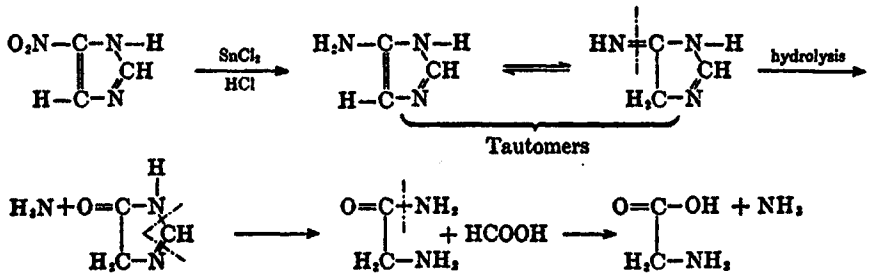
Oxidation with potassium permanganate in acetone solution converts the yellow, high-melting styrylimidazoles into the corresponding nitroimidazolecarboxylic acids and benzoic acid. Compounds such as 4(or 5)-nitro-5(or 4)-imidazolecarboxylic acid, 1-methyl-5-nitro-4-imidazolecarboxylic acid, or 1-methyl-4-nitro-5-imidazolecarboxylic acid are readily available through this route (20,21). See equation (1), page 135.

The constitution of the nitroimidazoles is determined from their behavior on reduction with stannous chloride in the presence of hydrochloric



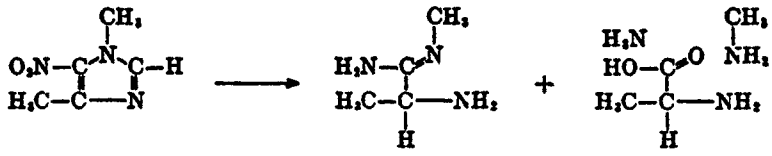
(1)

acid. In some instances this treatment results in the formation of an aminoimidazole; in most cases, however, it brings about a complete fragmentation of the imidazole nucleus into ammonia and other nitrogenous degradation products (25). The location of the nitro group in the original compound follows from the nature of these fragments. The reduction by this procedure of 4(or 5)-nitroimidazole results in the formation of glycine, formic acid, and ammonia (26). The existing evidence suggests that this fragmentation proceeds in the manner illustrated, with 4(5*H*) (or 5(4*H*))-imidazolone as an intermediate (27). The location of the



nitro group in 1-methyl-5-nitroimidazole follows from its conversion into glycine, methylamine, and ammonia by treatment with stannous chloride and hydrochloric acid (5).

A different mode of fission is observed with 2-methyl-4(or 5)-nitroimidazole and 1,4-dimethyl-5-nitroimidazole. The former compound affords acetamidine (26), while the latter is converted into a mixture of DL-N-methyl-2-aminopropamidine, DL-alanine, methylamine, and ammonia (8).

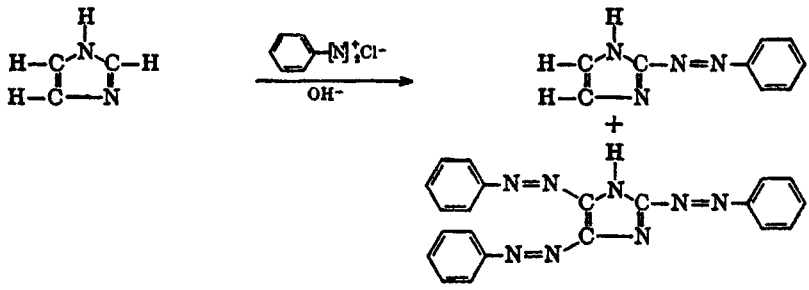


B. Arylazoimidazoles

1. Formation and Orientation of the Arylazo Group

Most imidazoles possessing a free imino group and a hydrogen atom or a free carboxyl group in positions 2, 4, or 5 couple with diazotized aromatic amines with the formation of deep-colored azo-dyes (Pauly diazo reaction). Coupling occurs in alkaline milieu only and is usually carried out by adding a solution of a diazonium salt to a solution of an imidazole in sodium carbonate. Although discovered earlier (3,28), the diazo reaction was extensively investigated for the first time by Burian (29) who formulated the coupling products as *N*-azo derivatives. The instability of these products toward mineral acid and the fact that only imidazoles possessing a free imino group have the ability to couple led him to this conclusion. Pauly (30), and especially Pyman and his group, elucidated the structure of the coupling products as *C*-azo derivatives and established the orientation of the arylazo group in a great variety of arylazoimidazoles.

Imidazole couples with phenyldiazonium chloride with the formation of a large amount of 2-phenylazoimidazole and a small quantity of 2,4,5-tris-(phenylazo)imidazole (1). Other aryldiazonium chlorides also



attack imidazole with the predominant formation of the respective 2-arylazoimidazoles. The nature of the azo component exerts a distinct influence upon the yield, as illustrated in Table XIX. Imidazoles in which the 2-position is blocked by an alkyl or aryl group couple into the 4(or 5)-position. Examples are 2-methyl- and 2-phenylimidazole, both of which undergo coupling with the formation of 4(or 5)-arylazo derivatives (1). 4(or 5)-Methylimidazole reacts with diazotized aniline to give approximately equal amounts of 2-phenylazo-4(or 5)-methyl-, 5(or 4)-phenylazo-4(or 5)-methyl-, and 2,4(or 2,5)-bis(phenylazo)-5(or 4)-

methylimidazole (1). Halogenoimidazoles such as 2-bromo-4(or 5)-methyl- or 4(or 5)-methyl-5(or 4)-bromoimidazole afford the expected arylazo derivatives (32).

TABLE XIX. Yields of 2-Arylazoimidazoles on Coupling of Imidazole with Various Aryldiazonium Chlorides (31)

Compound coupled	Yield (%)
2-Phenylazoimidazole	74
2-(*o*-Tolylazo)-	26
2-(*p*-Tolylazo)-	84
2-(*p*-Bromophenylazo)-	85
2-(*o*-Methoxyphenylazo)-	10
2-(*p*-Ethoxyphenylazo)-	64
2-(*p*-Sulfophenylazo)-	52

The behavior of the imidazolecarboxylic acids toward diazotized aromatic amines deserves special mention. A number of these compounds couple normally, while their esters or anilides fail to give a positive diazo test. Table XX summarizes a series of acids exhibiting this abnormal behavior. Acids in which the carboxyl group is not directly attached to the imidazole ring, such as 4(or 5)-imidazoleacetic acid or

TABLE XX. Imidazolecarboxylic Acids Exhibiting Abnormal Behavior toward Aryldiazonium Salts (33)

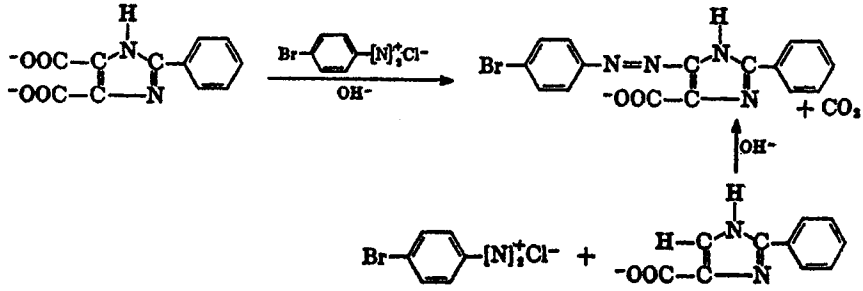
2-Methyl-4(or 5)-imidazolecarboxylic acid	2-Phenyl-4(or 5)-
2-Ethyl-4(or 5)-	4(or 5)-Methyl-5(or 4)-
	4(or 5)-Imidazolecarboxylic acid

4(or 5)-imidazolepropionic acid, produce colors regardless of whether the free acids or the esters are used (12,33). 4,5-Imidazoledicarboxylic acid couples readily with diazotized sulfanilic acid with the formation of 2-(*p*-sulfophenylazo)-4,5-imidazoledicarboxylic acid. The reaction is accompanied by some decarboxylation (1,29). 2-Phenyl-4,5-imidazoledicarboxylic acid reacts readily with *p*-bromophenyldiazonium chloride with the elimination of one of the carboxyl groups to give 2-phenyl-4(or 5)-(*p*-bromophenylazo)-5(or 4)-imidazolecarboxylic acid. The same coupling product ensues from the reaction of *p*-bromophenyldiazonium chloride and 2-phenyl-4(or 5)-imidazolecarboxylic acid. 2-Methyl-4,5-imidazoledicarboxylic acid exhibits similar behavior (34).

TABLE XXI. R_f Values and Diazo Colors on Filter Paper of a Number of Imidazoles (37,38)

Compound	Solvent System *a*	Solvent System *b*	Solvent System *c*	Diazo Color
Imidazole	0.88	0.58	0.68	Orange[d]
2-Methyl-	—	—	0.70	Yellow[d]
4(or 5)-Hydroxymethyl-	0.75	0.56	—	Red[e]
4(or 5)-(2-Hydroxyethyl)-	—	—	0.55	Red[d]
			0.50	Orange[d]
Histamine; 4(or 5)-(2-Aminoethyl)-	0.65	0.10–0.28	0.30	Red[d]
4(or 5)-(2-Ethylaminoethyl)-	—	—	0.60	Red[d]
4(or 5)-(2-Dimethylaminomethyl)-	—	—	0.75	Red[d]
4(or 5)-(2-*n*-Propylaminomethyl)-	—	—	0.76	Red[d]
4(or 5)-(2-Dimethylaminoethyl)-	—	—	0.79	Red[d]
4(or 5)-(2-Diethylaminomethyl)-	—	—	0.82	Red[d]
4(or 5)-(2-Piperidinomethyl)-	—	—	0.86	Red[d]
4(or 5)-(2-Piperidinoethyl)-	—	—	0.90	Red[d]
4(or 5)-(2-Benzylmethylaminoethyl)-	—	—	0.94	Red[d]
4(or 5)-Imidazolecarboxylic acid	0.23	0.29	—	Yellow[e]
4(or 5)-Amino-5(or 4)-imidazolecarboxamide	0.52	0.51	—	Blue[e]
4(or 5)-Imidazolelactic acid	0.26	0.27	—	Red[e]
4(or 5)-Imidazolepyruvic acid	0.31	0.31	—	Red[e]
4(or 5)-Imidazoleacrylic acid	0.34	0.69	—	Red[e]
Histidine	0.22	0.12	—	Red[e]
Histidine methyl ester	0.75	0.45	—	Red[e]
Carnosine	0.24	0.06	—	Red[e]
Histidinol	0.65	0.10–0.28	—	Red[e]
Ergothioneine	0.27	0.26	—	Red
2-Mercaptohistidine	0.25	0.15	—	Red[e]
2(3*H*)-Imidazolethione	—	—	0.56	Orange[d]
2-Hydroxyethyl-4-phenyl-2(3*H*)-imidazolethione	—	—	0.83	Yellow[d]

[a] 3:1 *n*-propanol–0.2 *N* ammonia. [b] 3:1 *n*-propanol–1 *N* acetic acid. [c] *n*-Butanol saturated with 20% aqueous pyridine. [d] With diazotized *p*-bromoaniline. [e] With diazotized sulfanilic acid.



The nitroimidazoles fail to give a positive Pauly test.

2. Application of the Diazo Test to the Identification and Estimation of Imidazoles

The Pauly diazo test provides a basis for the identification and in some instances the quantitative estimation of imidazoles. The non-specificity of the test and the fact, apparent from the preceding discussion, that many imidazoles fail to couple with diazotized aromatic amines limit the usefulness of the method. The application of the diazo reaction to the quantitative estimation of histamine and histidine will be discussed in Section D-3-(b) and Chapter VI, Section A-6-d-(2).

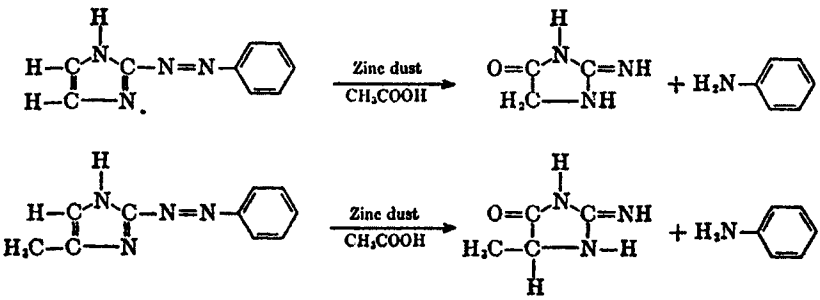
The Pauly test provides a convenient method for localizing imidazole spots on a paper chromatogram. Imidazoles are readily separable by paper chromatography, and spraying of the developed chromatogram with a solution of a diazotized aromatic amine followed by sodium carbonate results in the development of colored imidazole spots (35–38). The R_f values, *i.e.*, the ratio of the distance travelled by the substance to that travelled by the solvent front, of a number of imidazoles and the color characteristics of the resulting diazo spots are summarized in Table XXI. As little as 0.3 microgram of an imidazole is readily detectable by this technique.

3. Properties

The arylazoimidazoles are orange to brown colored, crystalline, high-melting compounds. They are sparingly soluble in water, dilute aqueous ammonia, or sodium carbonate, but dissolve to some extent in sodium hydroxide. They dissolve readily in hydrochloric acid, and evaporation of such solutions affords the crystalline hydrochlorides. The stability of the arylazoimidazoles toward boiling, dilute hydrochloric acid varies considerably. 2-Phenylazoimidazole, for example, is rather resistant

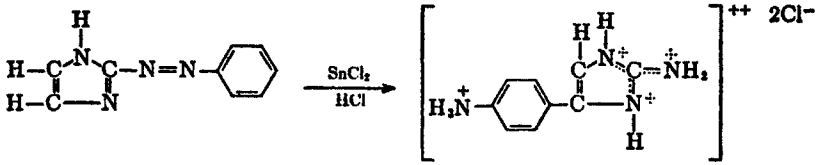
toward boiling 10% hydrochloric acid, while 4(or 5)-methyl-5(or 4)-phenylazoimidazole is rapidly destroyed. The arylazoimidazoles dissolve in concentrated sulfuric acid to give brightly colored solutions. The solutions of the monoarylazo compounds are orange to magenta in color, while those of the bis- and tris-(arylazo)imidazoles are green and of higher color intensity.

The orientation of the arylazo group in the 2-arylazoimidazoles follows from the nature of their reduction products. The reduction of 2-phenylazoimidazole with zinc dust and acetic acid leads to the formation of 2-imino-4-imidazolidone (glycocyamidine) and aniline. The same treatment converts 2-phenylazo-4(or 5)-methylimidazole or 2-(*p*-

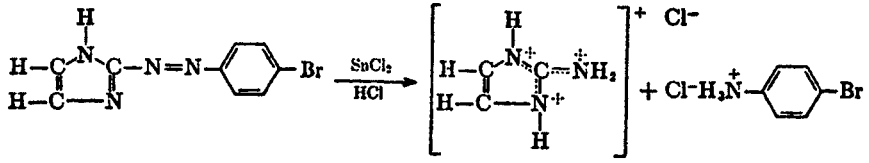


bromophenylazo)-4(or 5)-methylimidazole into 2-imino-5-methyl-4-imidazolidone (alacreatinine) (1,39,40).

Reduction of arylazoimidazoles with stannous chloride in hydrochloric acid takes an entirely different course from that observed in the zinc-dust reduction. Arylazoimidazoles in which the para position on the benzene ring is unsubstituted undergo a benzidine-type rearrangement and are converted into 2-amino-4(or 5)-(*p*-aminophenyl)imidazoles.



2-Aminoimidazole becomes the major reaction product when *p*-substituted 2-arylazoimidazoles are reduced by stannous chloride in hydrochloric acid. The behavior of 2-phenylazoimidazole and of *p*-bromophenylazoimidazole serve as illustrations. The former compound



undergoes the benzidine-type rearrangement to give 2-amino-4(or 5)-(*p*-aminophenyl)imidazole in the form of its dihydrochloride in 65% yield, while the latter is converted into the monohydrochloride of 2-aminoimidazole in 56% yield (1,31).

C. Aminoimidazoles

1. 2-Aminoimidazoles

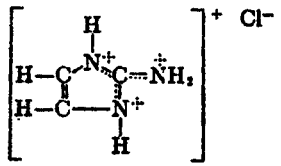
As has been mentioned in the previous section, the 2-aminoimidazoles are readily obtained by the reduction of para-substituted 2-phenylazoimidazoles with stannous chloride in the presence of hydrochloric acid (1,31). The yields which are realized with a variety of arylazoimidazoles are summarized in Table XXII. 4,5-Dimethyl-2-aminoimidazole results when 4,5-dimethyl-2-(*p*-bromophenylazo)imidazole is similarly reduced (39).

TABLE XXII. Yield of 2-Aminoimidazole from the Stannous Chloride Reduction of a Number of para-Substituted 2-Phenylazoimidazoles (31)

Arylazo derivative	2-Aminoimidazole Yield (%)
2-(*p*-Bromophenylazo)imidazole	56
2-(*p*-Tolylazo)-	15
2-(*p*-Ethoxyphenylazo)-	26
2-(*p*-Sulfophenylazo)-	43

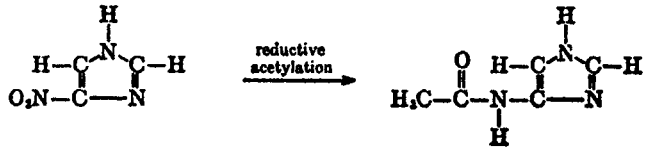
2-Aminoimidazole is a monoacidic base exhibiting pronounced chemical stability. It remains unchanged when refluxed with acids or alkalis and forms well-crystallized salts with acids. The free base has not been obtained in crystalline form. Acetylation and benzoylation convert 2-aminoimidazole into the respective monoacyl derivatives, which exhibit a positive Pauly test. 2-Aminoimidazole fails to undergo diazotization when treated with nitrous acid, but is converted into a nitroso derivative. These properties demonstrate that 2-aminoimidazole fails to behave like an "aromatic" amine, and that it is best regarded as a guanidine derivative, the 2-aminoimidazolium ion receiving major contributions from the

structures shown below, with the positive charge evenly distributed among all three nitrogens.

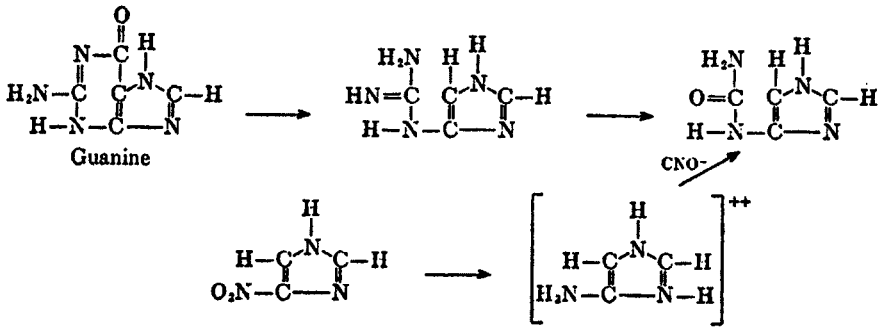


2. 4(or 5)-Aminoimidazoles

The free 4(or 5)-aminoimidazole is a highly unstable compound which has thus far not been isolated in pure form. The reduction of 4(or 5)-nitroimidazole with sodium amalgam in methanol at low temperature provides a method for the preparation of a methanol solution of the base. The addition of mercuric acetate to such a solution brings about the precipitation of the mercury salt of 4(or 5)-aminoimidazole, which may be converted into the crystalline dihydrochloride by treatment with hydrogen sulfide and hydrochloric acid (41). The reduction of 4(or 5)-nitroimidazole with stannous chloride in the presence of hydrochloric acid and acetic anhydride (reductive acetylation) results in the formation of the crystalline acetyl derivative of 4(or 5)-aminoimidazole (41).



Two derivatives of 4(or 5)-aminoimidazole, namely 4(or 5)-guanidoimidazole and 4(or 5)-ureidoimidazole, are of importance as hydrolytic products of the purine base guanine (42). The 4(or 5)-ureidoimidazole



is readily obtained when the crude methanol solution of 4(or 5)-aminoimidazole, resulting from the sodium amalgam reduction of 4(or 5)-nitroimidazole, is treated with potassium cyanate and acetic acid (43).

4(or 5)-Carbomethoxyaminoimidazole and 4(or 5)-carbethoxyaminoimidazole, two well-crystallized, high-melting, stable derivatives of 4(or 5)-aminoimidazole, result from the Curtius degradation of 4(or 5)- imidazolecarboxylic acid (44). Their hydrolysis to 4(or 5)-aminoimidazole has not yet proved feasible. 4(or 5)-Methyl-5(or 4)-aminoimidazole exhibits a greater stability than the parent compound. Stannous chloride reduction of 4(or 5)-methyl-5(or 4)-nitroimidazole provides a convenient method for the preparation of its crystalline dihydrochloride (25,26). Small amounts of the hydrochlorides of 4-amino-1,5-dimethyl- and 5-amino-1,4-dimethylimidazole are obtained, in addition to ring fission products, when the corresponding nitrodimethylimidazoles are reduced with stannous chloride (8).

The properties of the 4(or 5)-aminoimidazoles differ markedly from those of the isomeric 2-aminoimidazoles. They are highly unstable, diacidic bases forming crystalline dihydrochlorides and picrates. Short exposure to room temperature of an aqueous solution of the dihydrochloride of 4(or 5)-aminoimidazole leads to its decomposition with the formation of black pigments. The dihydrochloride of 4(or 5)-methyl-5(or 4)-aminoimidazole exhibits higher stability. An aqueous solution of this salt can be evaporated to dryness *in vacuo* without causing destruction of the material. 4(or 5)-Aminoimidazole undergoes deamination and ring fission under the conditions of the Van Slyke amino-nitrogen determination (41).

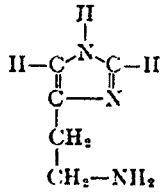
The behavior of 4(or 5)-methyl-5(or 4)-aminoimidazole toward nitrous acid differs markedly from that of the parent compound. Here normal diazotization takes place, and the resulting diazonium salt couples with α-naphthol or resorcinol (25). 4(or 5)-Methyl-5(or 4)-aminoimidazole is readily acetylated and benzoylated, and forms a benzylidene derivative on treatment with benzaldehyde (25,26). Acid hydrolysis causes fragmentation of 4(or 5)-acetamidoimidazole with the formation of glycine, ammonia, formic and acetic acids (41). The close parallelism between this process and that involved in the reductive fragmentation of 4(or 5)-nitroimidazole (see Section A-2) is apparent. 4(or 5)-Aminoimidazole and 4(or 5)-guanidoimidazole produce blue colors with diazotized sulfanilic acid.

D. Histamine

1. Discovery, Distribution in Nature, and Pharmacological Effects

The most important representative of the aminoalkylimidazoles is the substance histamine. Histamine was discovered in 1907 by Win-

daus and Vogt (45), who prepared it synthetically by the Curtius degradation of 4(or 5)-imidazolepropionic acid. This classical synthesis of histamine established its chemical structure as 4(or 5)-(2-aminoethyl)-imidazole. Its discoverers were not aware of the powerful physiological



Histamine

effects of the base, and the compound aroused little interest until 1910, when histamine was shown to be a constituent of ergot (46,47). In the same year it was demonstrated by Ackermann (48) that putrefactive bacteria could produce histamine from histidine. Shortly thereafter, Mellanby and Twort (49) reported that organisms usually present in the intestinal tract of man and animals could effect the decarboxylation of histidine and that histamine was a normal constituent of the intestinal contents. Histamine is usually found when histidine-containing materials are attacked by bacteria. In 1911 Barger and Dale (50) isolated histamine from the intestinal mucosa, and in 1919 Abel and Kubota (51) reported its occurrence in the pituitary gland. The classical investigation of Best, Dale, Dudley, and Thorpe (52) established beyond question that histamine is a normal constituent of many tissues. Its distribution is not uniform throughout various tissues of a given animal species, nor is it uniform for a given tissue in various species. Representative figures for the histamine content of various tissues and body fluids are found in Guggenheim's excellent treatise (53).

The various physiological actions of histamine are: (*1*) its stimulating action upon smooth muscles; (*2*) its dilating effect upon the capillaries; (*3*) its stimulation of glands. The histamine sensitivity of various organs and of the same organ in different species differs greatly. Thus the rabbit ileum responds *in vitro* to a dose only several hundred times that needed to cause an excellent contraction of the ileum of the guinea pig. The uterus of all species studied is contracted by histamine, except that of the rat which is relaxed (54).

Numerous pathological conditions such as peptone shock, experimental anaphylaxis, allergic diseases in man, surgical shock, toxemia and shock in severe burns, toxemia in pregnancy, and inflammation have

been linked to histamine. It is far beyond the scope of this monograph to discuss these various biological and medical aspects; the reader is referred to a number of excellent reviews on the subject (53,55,56).

2. Isolation from Natural Materials

Histamine, as has been mentioned previously, is a normal constituent of animal tissues, and several isolations of the base from animal sources are on record (50,51,57–59). Best, Dale, Dudley, and Thorpe (52) established in a most convincing manner the presence of histamine in alcoholic extracts from ox liver and ox lung in amounts sufficient to account for the vasodilator activity of these extracts. Pure samples of histamine dipicrate were isolated, and the progress of the purification was followed by biological assay. The isolation scheme was based on the Kossel-Kutscher silver method. The fresh tissue is minced and immediately extracted with alcohol. The alcohol extracts are evaporated to a small volume, and fat is removed by ether extraction. The basic constituents are then precipitated with phosphotungstic acid and are regenerated from the phosphotungstates by treatment with barium hydroxide. This is followed by a silver-barium hydroxide precipitation, and the silver precipitate, containing the histamine, is decomposed with hydrogen sulfide. The silver sulfide is removed by filtration, and solid barium hydroxide is added to the filtrate, which is then evaporated. The dried residues are extracted with ethanol, and the histamine is precipitated as the dipicrate from the ethanol extract. Several modifications of this general scheme have been used to isolate histamine. It seems advantageous, for example, to separate the initial phosphotungstic acid precipitate into an acetone-soluble and an acetone-insoluble fraction prior to its decomposition. The histamine phosphotungstate is acetone-soluble and is thus separated from basic constituents forming acetone-insoluble phosphotungstates (60–62).

Hot water extracts of tissues have been employed as the starting point for the isolation of histamine. The extracts are clarified by tannic acid precipitation and are then carried through the phosphotungstic acid and silver precipitations. The filtrates from the decomposition of the silver precipitates are directly treated with flavianic acid to give a crude histamine diflavianate which is purified by recrystallization (63–65). The amount of histamine actually isolated is usually small compared to the amount originally present in the tissue extracts, as determined by biological assay.

Toxemic pregnancy urine contains large amounts of histamine, and several isolations of the base from this source have been recorded. The

isolation from these urines involves an initial precipitation with zinc hydroxide. The zinc hydroxide precipitate is decomposed with hydrogen sulfide, and the ensuing alkaline solution is extracted with amyl alcohol. The histamine passes into the amyl alcohol layer, from which it is recovered by extraction with dilute sulfuric acid. Precipitation as the diflavianate and recrystallization complete the process (66). In two isolations amounts of 34.6 and 62.5 mg. of histamine diflavianate were obtained from 7.7 and 8.5 liters, respectively, of toxemic pregnancy urine.

3. Quantitative Estimation of Histamine

(*a*) *Biological Methods*

The spasmogenic effect of histamine on smooth-muscle preparations and its lowering effect on the blood pressure of the anesthetized cat provide the basis for the quantitative biological estimation of histamine (56,67,68). The biological assays are carried out on tissue extracts, and a variety of procedures have been suggested for the purification of histamine extracts for this purpose. Alcohol extracts, which were freed of fat by ether extraction, were originally used. Such crude tissue extracts contain substances which interfere with the biological assay. Acid hydrolysis destroys the biological activity of the interfering substances without seriously affecting the biological activity of histamine. Consequently, hydrolysis of tissues with hydrochloric acid has been used in the preparation of extracts for the quantitative determination (69).

The Code method (70) for the biological estimation of histamine in blood involves the preparation of a trichloroacetic acid filtrate and hydrolysis with hydrochloric acid. The hydrochloric acid is removed by repeated evaporation with alcohol, and the resulting dry residue is extracted with small amounts of water. The water extracts are filtered and assayed.

Rather advantageous is the application of electrodialysis to the extraction of histamine. A three-cell electrodialyzer, patterned after the apparatus described by Foster and Schmidt (71), is employed. The middle compartment is separated by parchment or collodion membranes from the cathode and anode cells. The tissue mince is placed in the center compartment, and the other cells are filled with distilled water. Under the influence of a direct current the histamine passes quantitatively into the cathode chamber, while histidine and other compounds which interfere with the biological assay remain in the center compartment (72–74).

Another micro-method for the extraction of histamine from blood is based on extraction and adsorption procedures. This method offers

distinct advantages as far as speed and accuracy are concerned, when the estimation of histamine in amounts of less than two micrograms is desired. An aqueous histamine-containing extract from tissues is shaken with *n*-butanol under conditions which assure a quantitative extraction, *i.e.*, at a pH of 12.5–13 in the presence of sodium sulfate and trisodium phosphate. The histamine is then selectively removed from the *n*-butanol extract by a cation-exchange medium and is eluted from the adsorbent by extraction with 0.4 *N* hydrochloric acid. A cellulose acid succinate, prepared by treatment of cotton with succinic anhydride, sodium acetate, and glacial acetic acid, serves as the cation-exchange medium. The final eluate is neutralized with sodium hydroxide and is then ready for histamine determination either by biological assay or by paper chromatography (75). A modification of this method especially adapted for the determination of histamine in whole blood uses Amberlite IRC-50 as a selective adsorbing agent. (76).

(*b*) *Colorimetric Methods*

The colorimetric estimation of histamine is based on the Pauly diazo reaction (30), *i.e.*, the capacity of imidazoles to form azo-dyes upon exposure to diazotized aromatic amines in the presence of sodium carbonate. The reaction is not specific for histamine. Numerous imidazoles and a great variety of other organic compounds, such as phenols, aromatic amines, tyrosine, tyramine, pyrroles, and indoles, which may be present in natural extracts, form colors with the Pauly reagent. In order to adapt the diazo reaction to the quantitative estimation of histamine in natural extracts, it is essential to remove these interfering compounds quantitatively prior to the colorimetric determination. Such a purification is rather difficult to achieve without incurring serious losses of histamine. Despite these limitations the method has been used in certain instances (69).

The determination of histamine in bacterial cultures and its estimation in blood will be described to illustrate the principle of the procedure. The methods are based on the observation that histamine can be quantitatively extracted from alkaline solution by such organic solvents as *n*-butanol, isobutyl alcohol, or *n*-amyl alcohol. Histidine is not extracted under these conditions (77).

A bacterial culture, to be analyzed for histamine, is alkalized by the addition of sodium carbonate, and the histamine is extracted with a mixture of chloroform and amyl alcohol. Back-extraction with dilute sulfuric acid affords a solution containing histamine, ammonia, and volatile amines. These latter compounds have to be removed, since they interfere with the colorimetric determination. To this end the sulfuric

acid extract is neutralized with sodium carbonate and is rendered alkaline by the addition of borax. Boiling for five to eight minutes dispels the ammonia and the volatile bases, and the solution is then ready for the colorimetric estimation (78).

The colorimetric histamine determination in blood involves a similar scheme. The blood samples are deproteinized by the addition of trichloroacetic acid, and the filtrates are alkalized by the addition of sodium hydroxide. The alkaline solutions are then extracted with ether, which removes interfering bases but fails to extract the histamine. The ethereal extracts are discarded, and the histamine is extracted from the aqueous phase with amyl alcohol. Back-extraction with dilute hydrochloric acid affords a solution containing histamine hydrochloride. Following concentration and neutralization, the solution is ready for the colorimetric determination (79).

Small amounts of histamine can be readily identified by paper chromatography. This technique is well suited for the selective separation of the base from closely related imidazoles and impurities. The identification of histamine by this method rests on the development on a paper strip of a colored band of established R_f value (see Section B-2). The R_f values of histamine, acetylhistamine, and histidine differ widely, and the substances are readily separable in a unidimensional chromatogram.

In the solvent mixture, *n*-butanol saturated with 10% aqueous ammonia, the R_f value of histamine is 0.56, while that of *N*-acetylhistamine is 0.71. The histidine band remains practically stationary in this system. The developed chromatograms are dried and are then drawn through a solution of diazotized *p*-bromoaniline for the development of the colored bands. Since small variations of the experimental conditions may affect the R_f values, it is desirable to run strips of pure histamine simultaneously with the chromatograms of the unknowns. The quantitative estimation of histamine by this method involves the comparison of the color intensity of the histamine band in an unknown sample with the color intensities of bands derived from graded amounts of histamine. The results obtained by this method agree well with those of biological determinations on identical samples. Amounts of histamine in excess of one millimicrogram are readily detected (36, 80).

Various aryldiazonium salts may be employed in the colorimetric estimation of histamine. The original Koessler and Hanke method (81) uses diazotized sulfanilic acid. The color is first yellow, then red; it reaches its maximum intensity in four to five minutes, and then begins to fade. The quantitative evaluation is based on a comparison with color standards, prepared from mixtures of methyl orange and congo red

indicator solutions (78). Amounts of histamine as small as ten millimicrograms can be determined. The instability of the sulfanilic acid dye represents a serious drawback. This difficulty is overcome by the use of diazotized *p*-nitroaniline or *p*-bromoaniline. The dyes which result when these diazo components are coupled with histamine are soluble in *n*-butanol or isobutyl alcohol. The color is stabilized in these organic solutions and can be measured with a photoelectric colorimeter (79,82). Traces of copper, cobalt, or nickel interfere with the Pauly reaction. These metals form complexes with the histamine and thus prevent the coupling reaction (83).

4. Formation by Microorganisms

The ability of microorganisms to bring about the conversion of histidine into histamine was first observed by Ackermann (48). He was able to demonstrate a 50% conversion of histidine to histamine by a culture isolated from a piece of contaminated pancreas. The decarboxylation of histidine by microorganisms has since been studied by numerous investigators (84–92).

A large number of coliform organisms have the ability to decarboxylate histidine with the formation of histamine in high yield. The activity of such cultures increases with age and reaches a maximum between the fourteenth and sixteenth hours of incubation, after which it falls steadily to the forty-eighth hour. The ability of the organisms to decarboxylate histidine is increased by the presence in the medium of fermentable carbohydrates. The decarboxylase activity of cultures grown in the absence of carbohydrate is rather low. Cultures which are grown in the absence of amino acids also fail to develop the ability to decarboxylate histidine. The decarboxylase activity has a sharp maximum at pH 4. Organisms grown at pH 7 are low in decarboxylase activity, while those grown at pH 4–5 or in the presence of D-glucose are highly active. The increased activity of cultures grown in the presence of D-glucose is the result of the fall in pH caused by the formation of acids during the fermentation. Washed cell suspensions of coliform organisms decarboxylate histidine with practically quantitative yields under anaerobic as well as aerobic conditions. The addition of D-glucose to such preparations has no effect on the rate of decarboxylation.

In addition to the coliform organisms, numerous other microbial forms such as those of the *Proteus* and *Clostridium* groups have the ability to decarboxylate histidine. Here the pH maximum for decarboxylation lies at 2.5–3.0, and the above-mentioned dependence upon the presence in the medium of fermentable carbohydrate for maximum activity is also observed. These organisms are capable of bringing about the

decarboxylation of histidine under both aerobic and anaerobic conditions. The activity to decarboxylate histidine is due to a specific histidine decarboxylase. The histidine decarboxylase of a strain of *Clostridium welchii* (Type A) has been partially purified (93).

Animal tissues also have the ability to decarboxylate histidine with the formation of histamine (94–97).

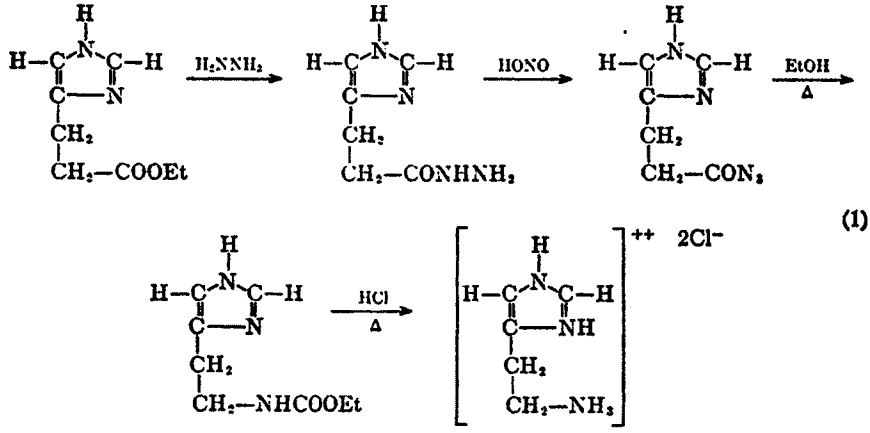
5. Preparative Methods

Histamine is prepared commercially by the bacterial decarboxylation of histidine. The base is formed in high yields and is usually isolated in the form of its dipicrate. This salt is then converted into the dihydrochloride or diacid phosphate for marketing purposes. The picrate method has certain disadvantages. Histamine dipicrate is sparingly soluble, and large volumes of solvents have to be used in its purification and conversion to other salts. This difficulty is avoided by the use of 3,4-dichlorobenzenesulfonic acid (98) as the precipitating reagent. Histamine bis-3,4-dichlorobenzenesulfonate is precipitated in practically quantitative yields when the sulfonic acid is added to a histidine fermentation mixture. The salt is readily purified by recrystallization and serves as a convenient starting material for the preparation of other histamine salts (99).

In addition to its formation by the biological decarboxylation of histidine, histamine is also formed when histidine is treated with acids at elevated temperatures. For example, heating of histidine in concentrated hydrochloric or 20% sulfuric acid at temperatures of 265–270° affords histamine in yields of 20–25%. Similarly, heating of histidine hydrochloride with potassium hydrogen sulfate at 265–270°, or heating of *N*-benzoylhistidine in the dry state at a temperature of 240° *in vacuo* followed by hydrolysis of the ensuing *N*-benzoylhistamine (100), lead to histamine formation.

Besides these methods, a number of synthetic procedures for the preparation of the base may be mentioned. The classical Windaus synthesis, which led to the discovery of histamine, involves the conversion of ethyl 4(or 5)-imidazolepropionate into its hydrazide by treatment with hydrazine, and reaction of the hydrazide with ethanolic hydrogen chloride and amyl nitrite to give the corresponding azide. Decomposition of the azide by refluxing with ethanol affords *N*-carbethoxyhistamine, which is converted into histamine hydrochloride by hydrolysis with concentrated hydrochloric acid (46). See equation (1), page 151.

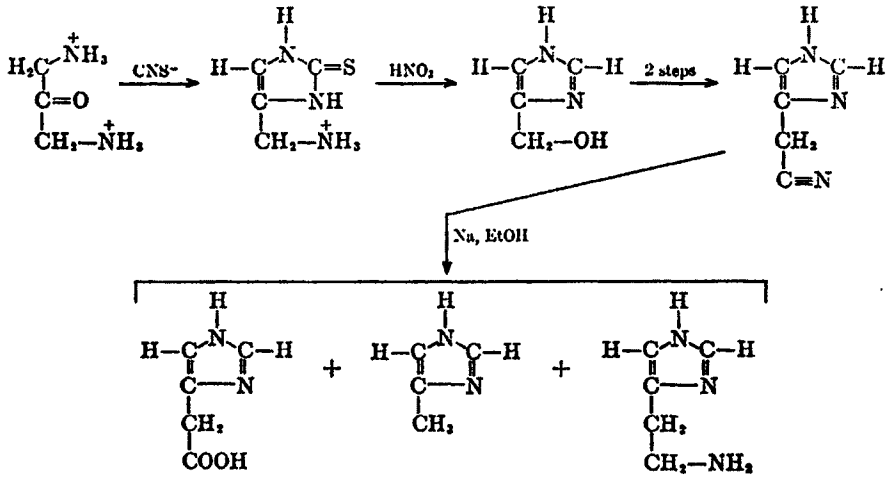
Diaminoacetone, available from citric acid, served as the starting material for the first Pyman synthesis of histamine (101). Its dihydrochloride, on treatment with an equivalent amount of ammonium or po-



(1)

Histamine dihydrochloride

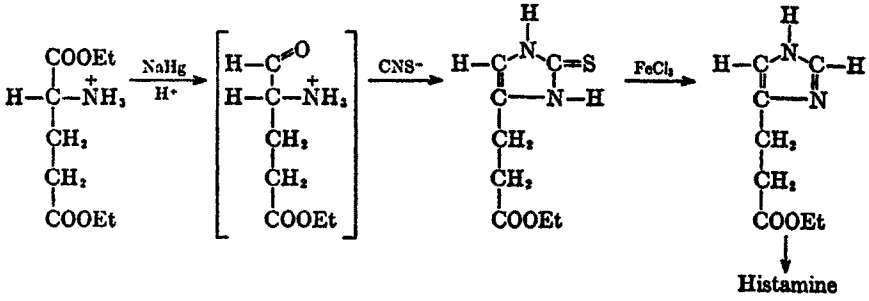
tassium thiocyanate, is converted into 4(or 5)-aminomethyl-2(3*H*)-imidazolethione. Oxidation of this imidazole with dilute nitric acid leads to the formation of 4(or 5)-hydroxymethylimidazole, which is converted into 4(or 5)-cyanomethylimidazole through the chloromethyl derivative. The cyanomethylimidazole, upon reduction with sodium in ethanol, affords histamine. 4(or 5)-Imidazoleacetic acid and 4(or 5)-methylimidazole are the by-products in the process. Under optimal conditions (102) it is possible to obtain 165 g. of histamine dihydrochloride from 4530 g. of



citric acid by this process. In the last analysis this method involves the conversion of 4(or 5)-hydroxymethylimidazole into histamine; this intermediate is more readily available from D-fructose (103) (see Chapter III, Section B-1-a).

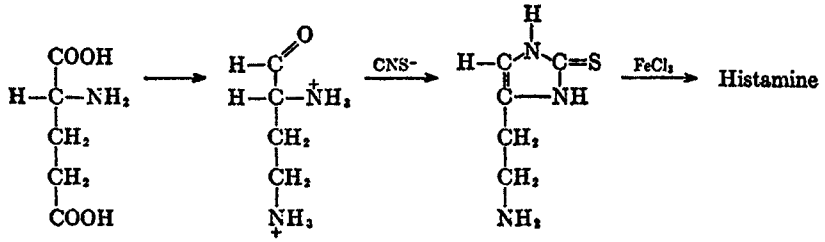
Closely related to this conversion of hydroxymethylimidazole into histamine is the preparation of the base by direct ammonolysis of 4(or 5)-(2-chloroethyl)imidazole. This reaction proceeds in high yields (104).

Essentially two routes for the conversion of L-glutamic acid into histamine are known. The first of these involves the transformation of diethyl glutamate into α-amino-γ-carbethoxybutyraldehyde hydrochloride and its conversion with ammonium thiocyanate into ethyl 2(3*H*)-imidazolethione-4(or 5)-propionate. This 2(3*H*)-imidazolethione is in turn transformed into ethyl 4(or 5)-imidazolepropionate by oxidation with ferric chloride.

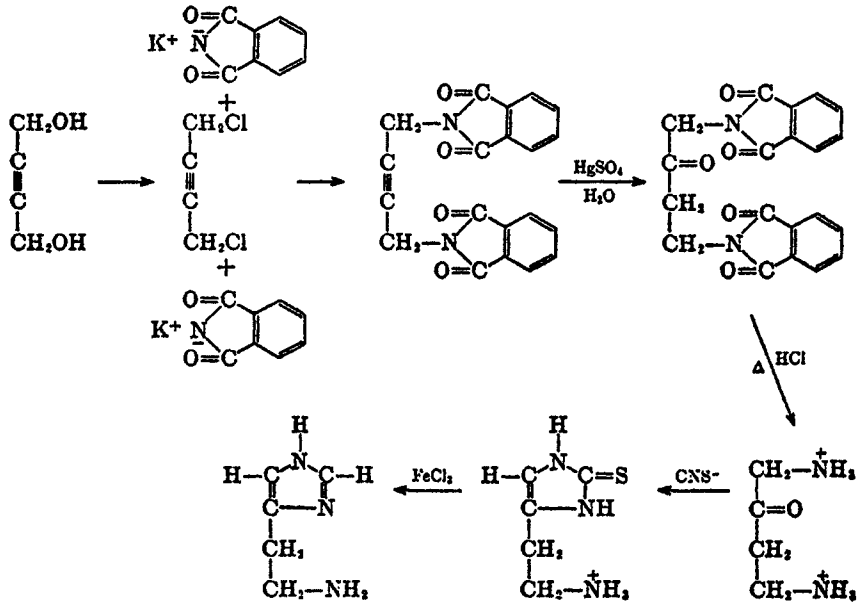


The exchange of the carbethoxy group of this ester for an amino group may be accomplished according to the Windaus scheme (45,105).

In the second procedure L-glutamic acid is converted into ethyl α,γ-diaminobutyrate which is reduced to give α,γ-diaminobutyraldehyde. Exposure of the dihydrochloride of this aldehyde to one equivalent of ammonium thiocyanate leads to the formation of 4(or 5)-(2-aminoethyl)-2(3*H*)-imidazolethione (2-mercaptohistamine), and by oxidative desulfurization to histamine (106,107).



Another histamine synthesis uses 2-butyne-1,4-diol as the starting material. This compound is converted into 1,4-dichloro-2-butyne by treatment with thionyl chloride in pyridine. The replacement of the chlorine atoms by phthalimido groups leads to the formation of 1,4-diphthalimido-2-butyne, which is readily hydrated to give 1,4-diphthalimido-2-butanone. The dihydrochloride of 1,4-diamino-2-butanone, resulting from the acid hydrolysis of 1,4-diphthalimido-2-butanone, reacts with one equivalent of potassium thiocyanate to give 4(or 5)-(2-aminoethyl)-2(3*H*)-imidazolethione. The final step involves the oxidative desulfurization of the 4(or 5)-(2-aminoethyl)-2(3*H*)-imidazolethione by means of ferric chloride to give histamine (108).



From the practical point of view these methods are of little importance, since they are unable to compete successfully with the much more economical decarboxylation of the readily accessible histidine.

6. Physical and Chemical Properties*

Free histamine was obtained for the first time by Pyman, who treated an aqueous solution of histamine dihydrobromide with an excess of sodium

* In order to differentiate the primary amino group from the ring nitrogens, it is customary to designate this nitrogen as the *N*-position.

carbonate, evaporated the solution to dryness, and extracted the base from the inorganic salts with chloroform. The base is much more conveniently prepared by passing a solution of its dihydrochloride through a column of the ion-exchange resin, Amberlite IRA-400 (109,110).

Histamine crystallizes in clear, wedge-shaped plates, melts at 83–84°, and distils at 209–210° at 18 mm. pressure. It is highly deliquescent, very soluble in water and ethanol, readily soluble in hot chloroform, but sparingly soluble in cold chloroform, and insoluble in ether. As a diacidic base it has the ability to form well crystallized salts, the most important ones being listed in Table XXIII. Histamine exhibits no characteristic absorption maximum in the ultraviolet region, in accord with the general absorption behavior of imidazoles not possessing a carbonyl function in conjugation with the imidazole ring (111).

TABLE XXIII. Melting Points of a Number of Histamine Salts

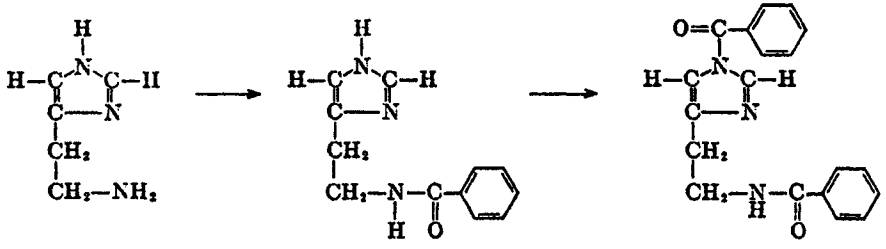
Salt	M.p., °C.	References
Monohydrochloride	193	(112)
Dihydrochloride	244–246	(45,102,105,112)
Monohydrobromide	182–183	(109)
Dihydrobromide	284	(101)
Di(acid phosphate)	132–133	(109)
Dinitrate	149–150	(112)
Chloroaurate[a]	200–215	(48,113,114)
Iodobismuthate	—	(115)
Chloroplatinate	dec. 200	(45)
Iodoplatinate	—	(115)
Phosphotungstate	—	(115)
Silicotungstate	—	(115)
Monopicrate	233–234	(100)
Dipicrate[b]	238–242	(45,100–102)
Dipicrolonate	266	(45)
Diliturate	—	(116)
Monoflavianate[c]	260	(63,117)
Diflavianate	262–263	(63,66)
Disozoidolate[d]	241	(113)
Bis-2-nitroindane-1,3-dionate	—	(118)
Bis-3,4-dichlorobenzenesulfonate	225–227	(99)
Diaminoquinonedisulfonate	—	(119)

[a] For the behavior of this compound on treatment with water, see Strack and Schwaneberg (120).
[b] For crystallographic data on this compound, see Takahashi, Yaginuma, and Hayakawa (121) and Yaginuma and Hayakawa (122).
[c] For crystallographic data, consult Wanag and Dombrowski (118).
[d] Sozoidolic acid is 2,6-diiodophenol-4-sulfonic acid.

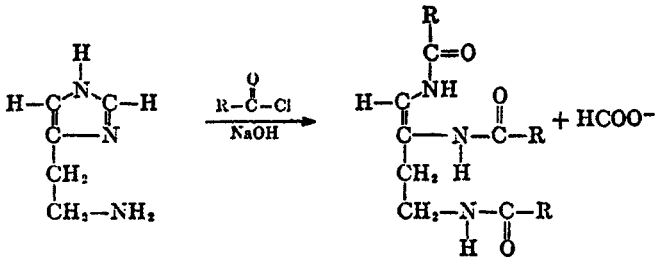
The addition of copper sulfate to a water solution of the base causes the formation of a dark blue complex (123).. The addition of alkali to a water solution of a histamine salt containing cobaltus nitrate results in the formation of a dark blue precipitate and in high dilution of a violet color. The color is sensitive to oxidizing agents and to air. Other imidazole derivatives such as pilocarpine or anserine fail to produce a color. Carnosine and histidine form yellow solutions when tested in the presence of air but fail to develop color when tested under anaerobic conditions. 4,5-Imidazoledicarboxylic acid produces a pink precipitate, while imidazole forms a blue precipitate which is not decolorized on exposure to air. The identification of small amounts of histamine by this Zimmermann test (124) is best carried out in evacuated Thunberg tubes. The reaction is not very sensitive; less than 0.5 mg. of histamine will not respond. Nickel ions also complex with histamine (83). Histamine forms a brownish red color with bromine (Knoop test) (125–130).

Refluxing of histamine with acetic anhydride or with mixtures of acetic anhydride and sodium acetate leads to the formation of *N*-acetylhistamine.

Depending upon the conditions which are employed, the benzoylation of histamine may result in the formation of different products. Benzoylation by the Schotten-Baumann procedure under carefully controlled conditions or by treatment of histamine base in chloroform with somewhat less than one equivalent of benzoyl chloride leads to the formation of *N*-benzoylhistamine. This monobenzoyl derivative couples normally with diazotized aromatic amines to form monoazo-dyes. Treatment of one mole of the *N*-benzoyl derivative with an additional mole of benzoyl chloride in benzene results in the formation of a dibenzoyl derivative, the second benzoyl group substituting the imino hydrogen of the imidazole nucleus. As is to be expected, this compound fails to couple with aryldiazonium salts (112,132).

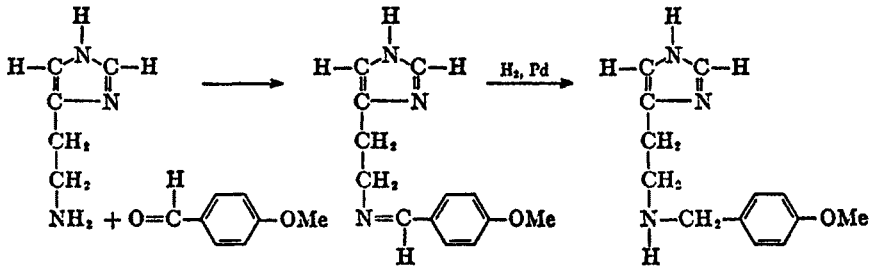


The reaction between an excess of benzoyl chloride and histamine in the presence of strong alkali affords 1,2,4-tribenzamidobutene-1, the imidazole ring undergoing fission during the process. Isovaleryl chloride under similar conditions converts histamine into 1,2,4-triisovalerylamidobutene-1 (133,134).



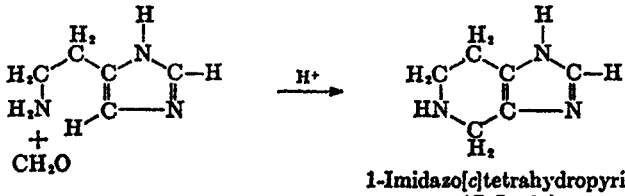
Histamine reacts readily with such reagents as potassium cyanate, phenyl isocyanate, or cyanamide to form *N*-carbamyl-, *N*-phenylcarbamyl-, and *N*-guanylhistamines, respectively (131).

Two types of reaction products may arise from the interaction of histamine with aldehydes. The primary reaction products resulting from the normal interaction of the primary amino group with the aldehyde are of the Schiff-base type. They are yellow to orange colored compounds which undergo catalytic hydrogenation in the presence of noble metal catalysts to form *N*-alkylated histamines. Anisaldehyde, for example, reacts readily with histamine to form the yellow colored *N*-(p-methoxybenzylidene)histamine, which is smoothly converted into *N*-(p-methoxybenzyl)histamine when hydrogenated over a palladium catalyst (131).



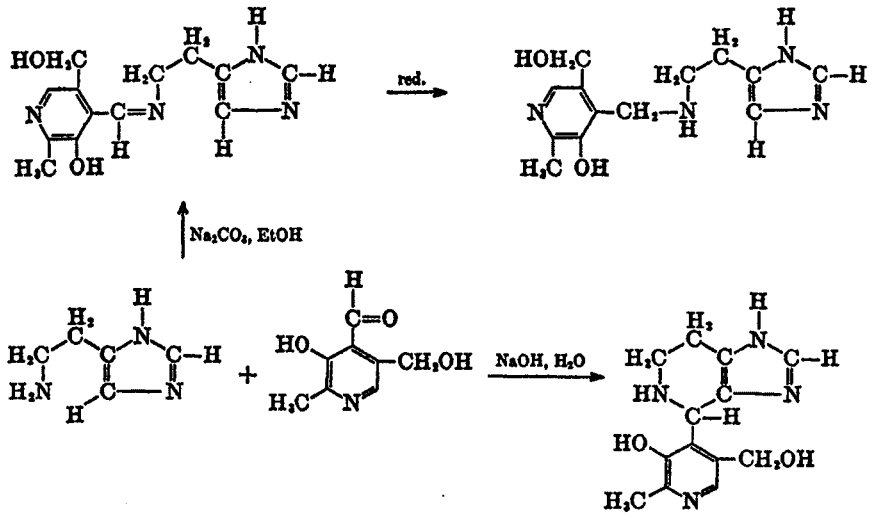
The reaction between histamine and an aldehyde may, under certain conditions, lead to the formation of bicyclic compounds. The simplest example of this type of reaction involves the condensation of histamine with formaldehyde in the presence of hydrochloric acid. The only isolable

product of this reaction is the compound 1-imidazo[*c*]tetrahydropyridine (*R.I.* 764) (135).



1-Imidazo[*c*]tetrahydropyridine (*R.I.* 764)

The reaction between histamine and pyridoxal may be influenced by varying the reaction conditions to give either a Schiff base or a bicyclic condensation product. *N*-Pyridoxylidene histamine results in ethanol solution in the presence of sodium carbonate, while condensation in the presence of sodium hydroxide affords 4-(2-methyl-3-hydroxy-5-hydroxymethyl-4-pyridyl)-1-imidazo[*c*]tetrahydropyridine. The two products are readily distinguishable. The former substance is bright yellow and undergoes hydrogenation with the formation of *N*-pyridoxylhistamine, whereas the latter is colorless and fails to absorb hydrogen (136).



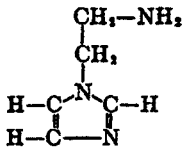
Histamine is smoothly deaminated with the formation of 4(or 5)-(2-hydroxyethyl)imidazole when treated with barium nitrite and mineral acid (137,138).

7. Structural Analogues of Histamine

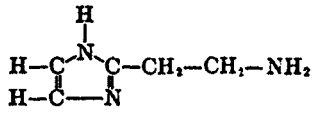
The pronounced physiological activity of histamine has provided the stimulus for chemical and pharmacological investigations on its structural analogues. These studies have to a certain degree clarified the specific structural requirements endowing a compound with histamine-like activity. During the classical era of histamine research which had its origin with the discovery of the physiological activity of the base, it was mainly the pioneering work of Pyman and his collaborators that provided the synthetic methods for the preparation of histamine analogues for pharmacological testing. These earlier investigations were disappointing from a practical standpoint, since it was soon discovered that modification of the histamine molecule invariably led to compounds with inferior physiological activity or substances that were completely inactive. As a consequence, there was little active interest in histamine analogues for many years. The discovery of the antihistaminics, *i.e.*, compounds having the ability to antagonize histamine both *in vitro* and *in vivo*, and the recognition of their important clinical applications in numerous allergic manifestations resulted in a renewed interest in histamine analogues. The metabolite-antimetabolite concept, it was reasoned, might be applied to histamine, and histamine analogues by virtue of their structural relationship to histamine might exhibit antihistaminic properties. Indeed, a few of the structural analogues were found to possess weak antihistaminic action. However, none of the analogues has thus far become of practical importance as an antihistaminic agent. A more detailed account of the preparative methods employed and of the relationships between chemical structure and histamine-like activity will be given in the following sections.

(*a*) *Position Isomers*

The two position-isomers of histamine, namely 1-(2-aminoethyl)imidazole (I) and 2-(2-aminoethyl)imidazole (II), are known. The synthesis of the physiologically inactive 1-(2-aminoethyl)imidazole involves the

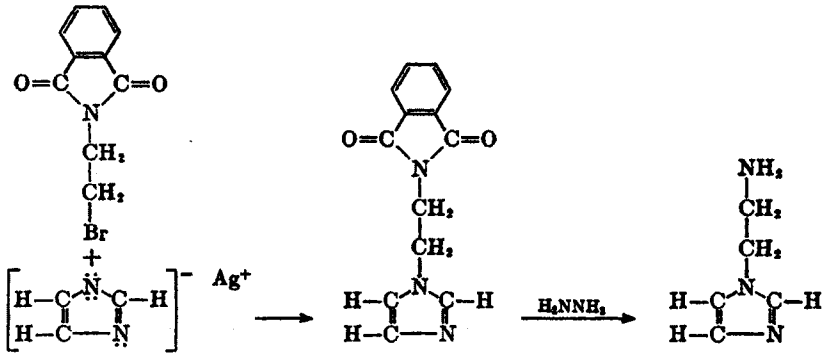


(I)

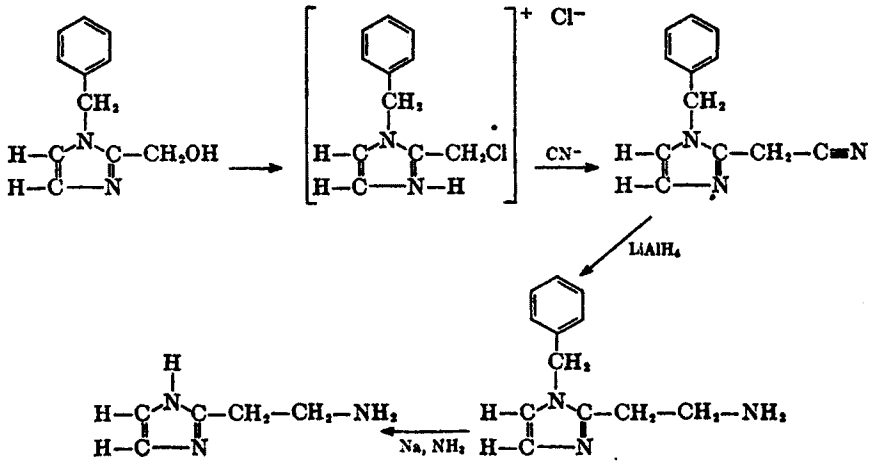


(II)

alkylation of imidazole silver with 2-bromoethylphthalimide in boiling xylene, followed by removal of the phthalyl group from the ensuing 1-(2-phthalimidoethyl)imidazole with hydrazine (139).

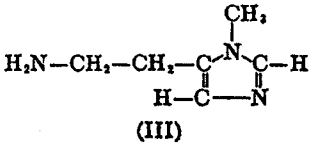


2-(2-Aminoethyl)imidazole is readily obtainable from 1-benzylimidazole. Hydroxymethylation of 1-benzylimidazole leads to the formation of 1-benzyl-2-hydroxymethylimidazole, and through the 1-benzyl-2-chloromethylimidazole hydrochloride this compound is converted into 1-benzyl-2-cyanomethylimidazole. Reduction of the nitrile with lithium aluminum hydride affords 1-benzyl-2-(2-aminoethyl)imidazole and on debenzylation with sodium in liquid ammonia 2-(2-aminoethyl)imidazole (140).

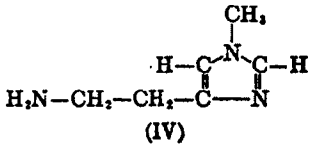


(*b*) *Ring-Substitution Products*

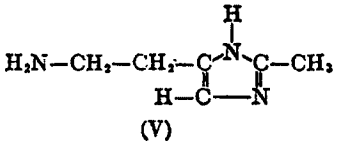
The four ring-methylated histamines, 1-methyl-5-(2-aminoethyl)imidazole (III), 1-methyl-4-(2-aminoethyl)imidazole (IV), 2-methyl-4(or 5)-(2-aminoethyl)imidazole (V), and 4(or 5)-methyl-5(or 4)-(2-aminoethyl)imidazole (VI), have been synthesized. The Pyman histamine



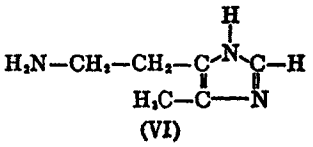
(III)



(IV)

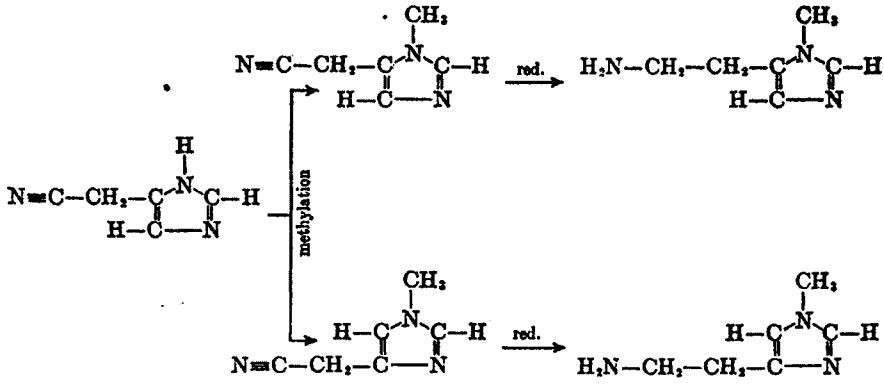


(V)

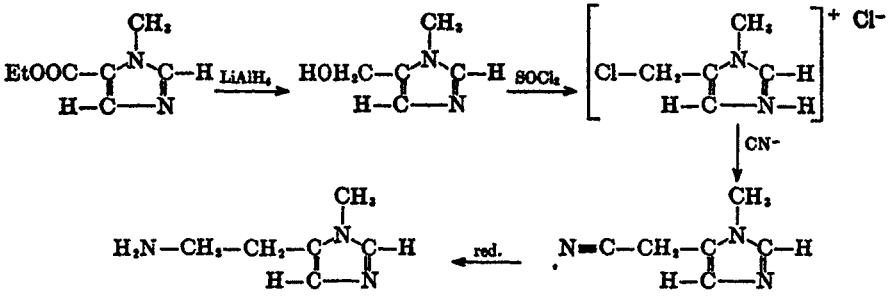


(VI)

synthesis provides the basis for the preparation of the two ring-nitrogen methylated compounds. The methylation of 4(or 5)-cyanomethylimidazole affords a mixture from which it is possible to isolate 1-methyl-4-cyanomethylimidazole in 43% yield and 1-methyl-5-cyanomethylimidazole in 15% yield. Sodium ethanol reduction of these isomeric nitriles affords 1-methyl-5-(2-aminoethyl)- and 1-methyl-4-(2-aminoethyl)imidazole, respectively. The corresponding dimethylimidazoles, *i.e.*, 1,4- and 1,5-dimethylimidazole, and the respective methylimidazoleacetic acids, *i.e.*, 1-methyl-4- and 1-methyl-5-imidazoleacetic acid, are the by-products of the reaction (141).

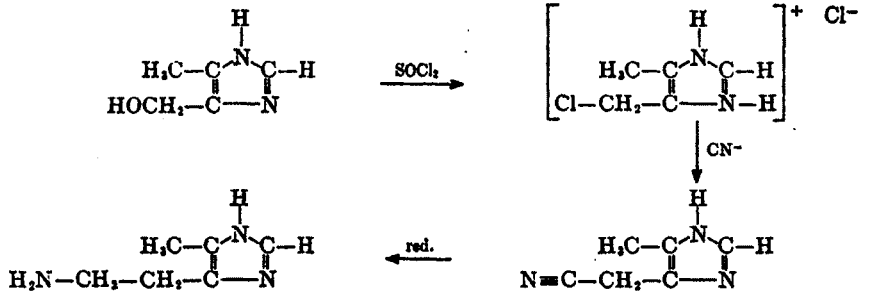


Although adequate for the preparation of 1-methyl-4-(2-aminoethyl) imidazole, this method is not satisfactory when the preparation of 1-methyl-5-(2-aminoethyl)imidazole is desired. The isolation of the 1-methyl-5-cyanomethylimidazole from the methylation mixture is rather tedious, and the yields reported by Pyman have not been substantiated. This difficulty is avoided by preparing 1-methyl-5-cyanomethylimidazole from ethyl 1-methyl-5-imidazolecarboxylate. Reduction of this ester with lithium aluminum hydride affords 1-methyl-5-hydroxymethylimidazole, which is readily converted into the desired nitrile through 1-methyl-5-chloromethylimidazole hydrochloride. The nitrile is identical with the



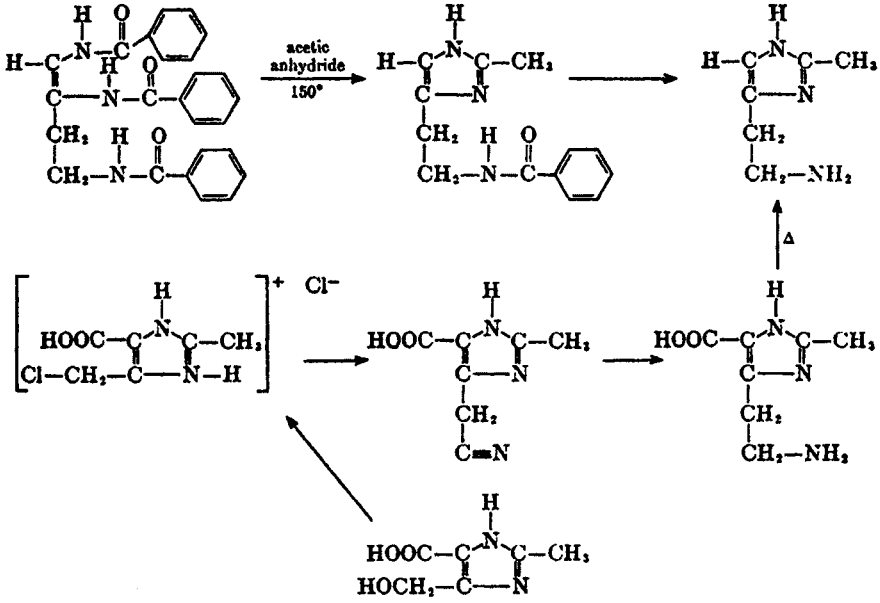
compound prepared according to Pyman. The ring nitrogen-substituted histamines fail to give the Pauly reaction (142).

4(or 5)-Methyl-5(or 4)-(2-aminoethyl)imidazole is readily available from 4(or 5)-methyl-5(or 4)-hydroxymethylimidazole via 4(or 5)-methyl-5(or 4)-chloromethylimidazole hydrochloride and 4(or 5)-methyl-5(or 4)-cyanomethylimidazole (62,143).

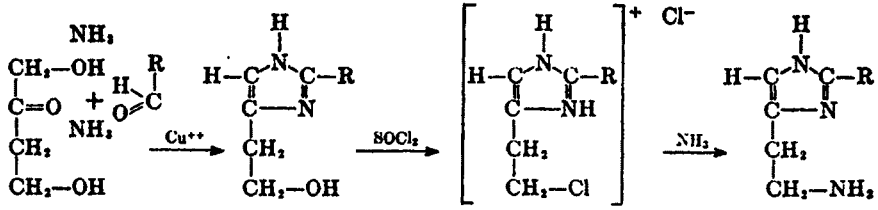


1,2,4-Tribenzamidobutene-1, the product of the Schotten-Baumann benzoylation of histamine, may serve as the starting material for the

preparation of 2-methyl-4(or 5)-(2-aminoethyl)imidazole. Treatment with acetic anhydride at 150° converts the compound into 2-methyl-*N*-benzoylhistamine, which is converted into 2-methyl-4(or 5)-(2-aminoethyl)imidazole by acid hydrolysis (131). The compound has also been prepared from 2-methyl-4(or 5)-hydroxymethyl-5(or 4)-imidazolecarboxylic acid in the manner shown below (144). The reported properties of 2-methylhistamine dihydrochloride prepared by both methods agree.

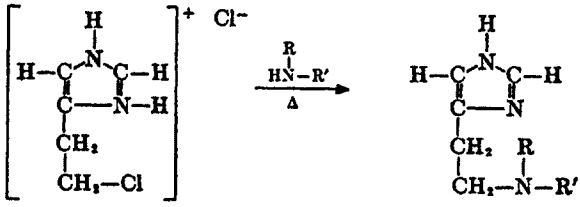


A more generally applicable method for the preparation of 2-alkyl- or 2-aryl-substituted histamine derivatives involves the interaction of suitably 2-substituted 4(or 5)-(2-chloroethyl)imidazole hydrochlorides with ammonia. The required halides are readily available from 2-alkyl- or 2-aryl-substituted 4(or 5)-(2-hydroxyethyl)imidazoles resulting from the reaction of an aldehyde with 1,4-dihydroxybutanone-2 in the presence of ammonia and cupric acetate (37).

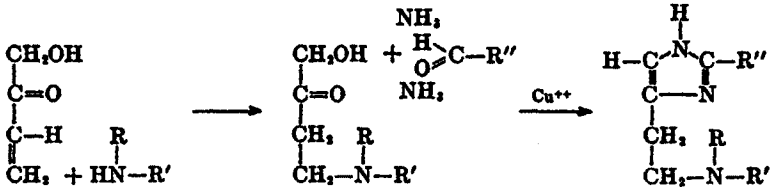


(c) *N-Monoalkyl- and N,N-Dialkylhistamine Derivatives*

The hydrochloride of 4(or 5)-(2-chloroethyl)imidazole serves as the key intermediate for the preparation of representatives of this class of compounds. As has been stated previously (see Chapter IV, Section C), the covalently bound chlorine in this substance is highly reactive and is readily replaced by ammonia, primary or secondary amines. In practice, an ethanolic solution of the halide is treated with an excess of an amine in a sealed tube at a temperature of 100°, and after removal of the unreacted amine the alkylaminoethylimidazole is precipitated as the dipicrate. Refluxing of the halide with an excess of the amine in *n*-propanol may also be used in order to avoid the use of pressure tubes (104,145).



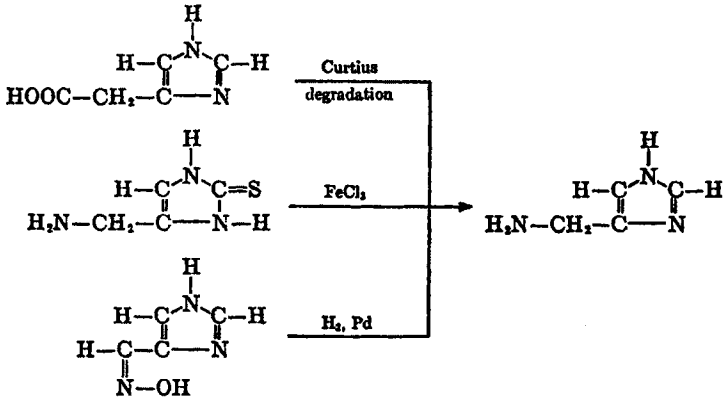
An alternate route to the *N*-alkylated histamines, which is especially useful when the preparation of 2-alkyl- or 2-aryl-substituted 4(or 5)-(2-alkylaminoethyl)imidazoles is desired, employs hydroxymethyl vinyl ketone as the starting material. This ketone adds secondary amines with the formation of aminohydroxy ketones. These are not isolated, but are allowed to react directly with an aldehyde and ammonia according to the Weidenhagen process (see Chapter II, Section A-3) to give the desired imidazole. Such compounds as 4(or 5)-(2-piperidinoethyl)imidazole, 2-ethyl-4(or 5)-(2-piperidinoethyl)imidazole, or 2-phenyl-4(or 5)-(2-piperidinoethyl)imidazole are readily prepared in this manner (37).



The aminoalkylimidazoles are diacidic bases forming characteristic dipicrates and dihydrochlorides, which are employed in their isolation and characterization. With the exception of the ring nitrogen-substituted compounds, they give a positive Pauly reaction.

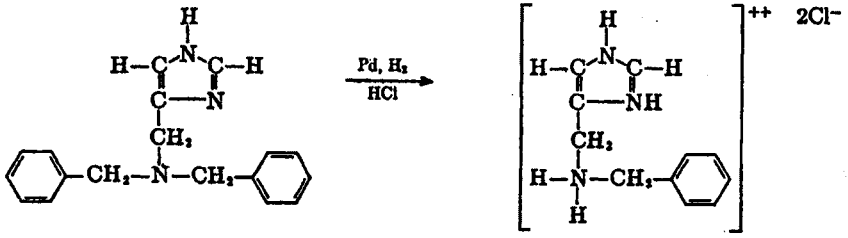
(*d*) *Histamine Analogues Possessing Longer or Shorter Aliphatic Side Chains*

4(or 5)-Aminomethylimidazole, the simplest representative of this class of imidazoles, was obtained for the first time by Windaus and Opitz (137) by the Curtius degradation of 4(or 5)-imidazoleacetic acid. The substance also results from the oxidation of 4(or 5)-aminomethyl-2(3*H*)-imidazolethione with ferric chloride or from the treatment of 4(or 5)-chloromethylimidazole hydrochloride with ammonia (141). A superior method for its preparation involves the catalytic hydrogenation of the oxime of 4(or 5)-imidazolecarboxaldehyde in the presence of two equivalents of hydrogen chloride, the dihydrochloride being obtained under these conditions (146).

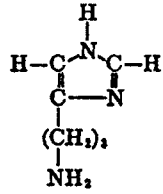


4(or 5)-(Dialkylaminomethyl)imidazoles are obtained from 4(or 5)-chloromethylimidazole hydrochloride and secondary amines. The reaction proceeds in good yields, and the resulting tertiary amines are isolated in the form of their dipicrates or dihydrochlorides.

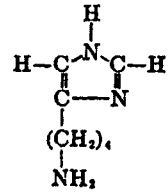
The 4(or 5)-(moncalkylaminomethyl)imidazoles are not readily obtained in this manner. A practical procedure for their preparation involves the catalytic debenzylation of 4(or 5)-(benzylalkylaminomethyl) imidazoles. The dihydrochlorides of compounds such as 4(or 5)-(methylaminomethyl)-, or 4(or 5)-(ethylaminomethyl)imidazole are readily obtained by catalytic hydrogenation of 4(or 5)-(benzylmethylaminomethyl)- and 4(or 5)-(benzylethylaminomethyl)imidazole, respectively, in the presence of a palladium catalyst and hydrochloric acid. Hydrogenolysis of 4(or 5)-(dibenzylaminomethyl)imidazole under the above-mentioned conditions affords 4(or 5)-(benzylaminomethyl)imidazole (146, 147).



Among the various known histamine derivatives containing additional methylene groups in the aliphatic side chain, the compounds 4(or 5)-(3-aminopropyl)- and 4(or 5)-(4-aminobutyl)imidazole may be mentioned. These may be obtained from arginine and lysine, respectively (148,149).



4(or 5)-(3-Aminopropyl)imidazole



4(or 5)-(4-Aminobutyl)imidazole

8. Pharmacological Specificity

Three physiological effects of histamine provide the biological basis for studies on the relationships between chemical structure and physiological activity in the histamine series: (*1*) its spasmogenic effect upon smooth muscle preparations; (*2*) its depression of blood pressure in the anesthetized cat; (*3*) its stimulatory action on certain glands. A given structural alteration of the histamine molecule will not necessarily elicit a comparable effect in all these tests, and a histamine analogue may have little effect upon the blood pressure of the cat, but may be highly active when assayed on the guinea pig uterus. Also the response of the guinea pig uterus and the guinea pig ileum to a given compound may vary significantly.

N-Methyl- and *N*-ethylhistamine provide good examples to illustrate this point. *N*-Methylhistamine is half as active as histamine when assayed on the cat, but exhibits twice its activity as far as its effect on the guinea pig uterus is concerned. Its stimulating effect on gastric secretion in the human parallels that of histamine. *N*-Ethylhistamine, on the other hand, has only 5% of the activity of histamine on the cat and the guinea pig uterus, but is 75% as active when tested on the guinea pig ileum (146,150–153).

It is thus important to compare the biological activity of a series of histamine analogues in terms of one and the same test system. The pharmacological testing of numerous histamine derivatives and analogues has led to the recognition of certain critical structural elements. The presence of a free primary, secondary, or tertiary amino group in the side chain is essential for histamine activity. Acylation of the primary amino group destroys the biological activity. Compounds such as *N*-acetylhistamine, *N*-benzoylhistamine, or substances in which histamine is linked to peptide structures through its primary amino group are devoid of histamine activity (154,155). *N*-Acetylhistamine is of special interest in this connection since it represents a metabolic product of histamine. The compound is present in the urine of the dog and man following the oral administration of histamine and thus seems to be a physiologically important "detoxified" form of histamine (36,110,131).

Aldehydes bring about a complete inactivation of histamine. Their effect is pH dependent and must be attributed to either the formation of Schiff bases or of 1-imidazo-[*c*]-tetrahydropyridine derivatives (see Section D-6) (135,156–158).

A number of *N*-alkyl and *N,N*-dialkyl derivatives of histamine are biologically active. Using the contracting effect on the guinea pig ileum as the criterion for physiological activity, it may be generally stated that the activity of these compounds is inversely proportional to the size of the alkyl group. *N*-Methylhistamine is highly active, *N*-ethylhistamine possesses 75% of the activity of histamine, while the N-propyl derivative is only 5% as active. *N,N*-Dibenzylhistamine possesses no histamine activity. This compound is weakly antihistaminic in its action, *i.e.*, it has the capacity to suppress the action of histamine on the guinea pig ileum (145).

N,N-Dimethylhistamine exhibits approximately 20% the activity of histamine when assayed on the cat, while its effect upon the guinea pig uterus is approximately 30% that of histamine. The substance possesses 75% of the activity of histamine as far as its effect on the guinea pig ileum is concerned. *N*-Trimethylhistamine has no histamine activity (145,150).

A high order of histamine activity depends upon the presence of the 2-aminoethyl side chain; shortening or elongation of the side chain markedly decreases the biological activity. The next lower homologue of histamine, 4(or 5)-aminomethylimidazole, is inactive (62). Its *N*-mono and *N,N*-dialkyl derivatives show a variable physiological response. Some of the compounds such as 4(or 5)-methylaminomethyl-, 4(or 5)-dimethylaminomethyl-, and 4(or 5)-ethylaminomethylimidazole

possess a low order of histamine activity, while others such as 4(or 5)-dibenzylaminomethylimidazole have the ability to antagonize histamine. Their antihistaminic properties are not very pronounced (146, 153).

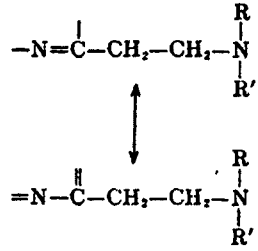
Substitution of the ring hydrogens in histamine by methyl groups exerts a pronounced effect upon the physiological activity. 2-Methylhistamine is the most active of these methylated products. Its effect upon the guinea pig ileum is 30% that of histamine, while it is only 15% as effective when assayed on the cat (152). 4(or 5)-Methyl-5(or 4)-(2-aminoethyl)imidazole is a compound of low activity. It is only 0.5% as active as histamine when tested on the guinea pig uterus (62).

It is of interest to note that the two ring-nitrogen methylated histamines differ in their biological activity. 1-Methyl-4-(2-aminoethyl)imidazole possesses 6% the activity of histamine on the guinea pig ileum and is 2% as active in depressing the blood pressure in the cat, while its isomer, 1-methyl-5-(2-aminoethyl)imidazole, is completely devoid of histamine activity.

The substitution of the imidazole ring by such other groups as in 4(or 5)-(2-aminoethyl)-2(3*H*)-imidazolethione leads to inactive compounds (159,160). The two position-isomers of histamine, 1-(2-aminoethyl)imidazole (53) and 2-(2-aminoethyl)imidazole (152), are completely inactive.

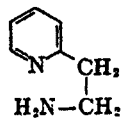
The presence of the imidazole ring is not essential for histamine activity. Several compounds in which the 2-aminoethyl side chain is attached to other heterocyclic ring systems possessing aromatic character exhibit histamine-like activity (152, 161). All the highly active compounds possess the structural element I, in which the portions

are part of the resonance-stabilized aromatic system. The presence within a molecule of this fragment does not imply that the compound must possess

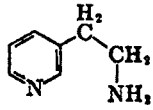


(I)

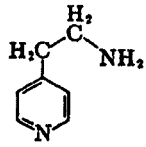
histamine activity. Other structural requirements also have to be satisfied. In addition to the presence of the fragment I, the size and shape of the aromatic nucleus seems to play an important part. The most highly active compounds are those possessing a small, unsubstituted nucleus. The observation that 2-(2-aminoethyl)pyridine possesses histamine activity in contrast to 3-(2-aminoethyl)pyridine and 4-(2-aminoethyl) pyridine, which are physiologically inert, led to the postulation that intramolecular hydrogen bonding between the primary amino group and



2-(2-Aminoethyl)pyridine



3-(2-Aminoethyl)pyridine

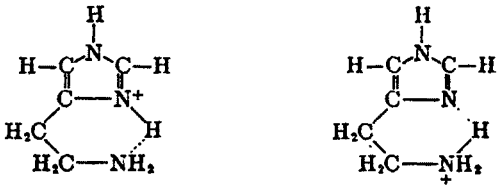


4-(2-Aminoethyl)pyridine

the pyridine nitrogen in the cation is an essential prerequisite for histamine activity (162,163). Such hydrogen-bonded structures may be written for the 2-(2-aminoethyl)pyridine, but not for the other two compounds. The physiologically active forms of the histamine cation, according to

2-(2-Aminoethyl)pyridine cation

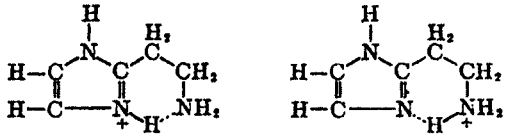
this view, are to be represented by the following hydrogen-bonded structures.



Histamine cation, according to Niemann and Hays

The compound 2-(2-aminoethyl)imidazole provides just as favorable a structure for hydrogen bonding as does the histamine itself. However,

the compound is completely devoid of histamine activity. The occurrence under physiological conditions of hydrogen bonding between two basic



2-(2-Aminoethyl)imidazole cation

centers is highly improbable, and there seems to be little basis for assuming the existence of a relationship between hydrogen bonding and histamine activity.

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