

CHEMISTRY OF THE BIOLOGICALLY IMPORTANT IMIDAZOLES¹

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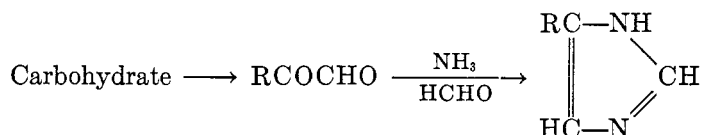
I. INTRODUCTION

The imidazoles include many substances of both biological and chemical interest. Of special significance are the purines, proteins, and other imidazoles discussed in this review. Inasmuch as the imidazole moiety is part of a fused-ring system in the purines, these compounds have been excluded from consideration here. The imidazole content of protein is due chiefly to histidine, which is separately discussed. The isolation, structure determination, and synthesis of biological imidazoles are reviewed in the following sections. Only occasional references to important physiological, biochemical, and pharmacological attributes are made, since these latter are thoroughly treated in Guggenheim's *Die biogenen Amine* (45a).

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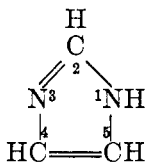
Speculation on the biogenesis of the imidazoles would lead one to believe that the simpler metabolic intermediates arise from natural sugars and ammonia. This type of synthesis has its laboratory counterpart (29, 80, 103, 108), and is a kind of process for which the intermediates should be readily available phytochemically. For instance, known sugar metabolites might react as follows:



After conversion to histidine, the imidazole is incorporated into protein. Alkaloids containing the imidazole nucleus may also arise as conversion products of histidine, as well as of other amino acids, such as arginine (compare page 57). The structure of the purines is sufficiently complex to suggest that they result from elaboration of the imidazole molecule from ingested histidine.

The prototype of the group, imidazole, was first observed by Debus (29) in reacting ammonia and glyoxal. This reaction was responsible for the alternative name for imidazole, i.e., glyoxaline. Hantzsch (49) later suggested the use of the name imidazole. Both names are very common in the literature, but imidazole has been given official recognition (81).

The numbering of the imidazole ring begins with the nitrogen atom bearing a hydrogen atom.



In the case of some imidazoles monosubstituted on either the 4- or the 5-carbon atom, the designation 4 (or 5) is employed, since decision as to where the group is attached has not been made. A mathematical treatment of the corresponding resonance forms of histidine has been published (58).

II. BETTER-KNOWN NATURALLY OCCURRING IMIDAZOLES

A. Anserine

In 1929 Ackermann and coworkers (6) announced the discovery of a new, nitrogenous component of goose muscle. This compound proved to be basic, possessed the empirical formula $\text{C}_{10}\text{H}_{16}\text{N}_4\text{O}_3$, and was given the name anserine. Its aqueous solution was alkaline; anserine could be precipitated from it by picric acid, by alcoholic mercuric chloride, and as the chloroaurate. Its solubilities in alcohol and water resembled those of most peptides. It could best be purified as the copper salt, of which these workers obtained 8.4 g. from 5.7 kg. of fresh goose muscle. Color tests for histidine, tyrosine, tryptophan, and arginine, as well as a biuret test, were negative. The ninhydrin reaction was positive.

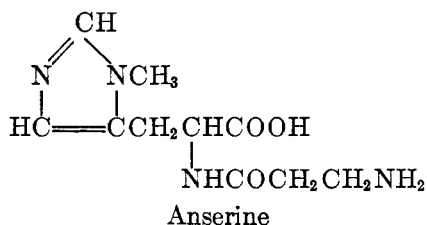
In the same year Linneweh, Keil, and Hoppe-Seyler (75) solved most of the problem of the structure of anserine by means of several experiments and some intuition. The reaction with nitrous acid indicated that one of the four nitrogens was present in a primary amino group. Failure of a solution of the material to decolorize permanganate eliminated the possibility of an aliphatic double bond. Oxidation with chromic acid, chlorine water, permanganate, nitric acid, or hydrogen peroxide, reduction with hydriodic acid, and dry distillation all failed to yield further clues. These investigators therefore resorted to a procedure which had been adapted by Jowett (64e) to pilocarpine, i.e., heating with soda lime in a stream of hydrogen. As a result of this treatment it was possible to isolate 1,5-dimethylimidazole as the chloroaurate, possessing the properties recorded by Jowett for this compound.

This reaction established the presence of an imidazole ring and the locus of the $-\text{NCH}_3$ group, and also diminished the number of possible points of attachment of the rest of the molecule. These facts further checked the previous notion that anserine was simply monomethylated carnosine with similar properties. The Pauly diazo color test for the imidazole nucleus, which was positive in the case of carnosine, was negative for anserine. This diazo color evidently, then, required an unsubstituted ring $-\text{NH}-$ for its appearance, in agreement with observations of Burian (24).

With the above information as a background it was possible to continue in the same fashion employed for carnosine, as described in the section on that compound. Hydrolysis with barium hydroxide solution gave *dl*-methylhistidine, separated as the phosphotungstate and isolated and identified as the picrolonate and nitrate. β -Alanine was also isolated from the phosphotungstate filtrate, as the naphthylurea, and it checked in nitrogen content and melting point with a synthetic preparation.

The order of attachment of the two amino acids was not settled by the above work, but the authors suggested that the same order known to exist in carnosine was likely. The validity of this surmise was substantiated by Keil (68a), who employed the device of condensing the peptide with trinitrotoluene (17) and hydrolyzing the resultant compound with dilute sulfuric acid. β -(Dinitrotoluy)-alanine was isolated.

Keil synthesized both the 1,4- and 1,5-dimethylimidazoles and separated them by the method of Pyman (87f). The chloroaurate of the dimethylimidazole from anserine had a melting point of 224°C ., and a mixed melting point with 1,5-dimethylimidazole chloroaurate of 224°C . The structure of anserine was therefore established as



Keil (68b) subsequently attempted to synthesize anserine by methylation of carnosine with dimethyl sulfate in soda solution, but a polymethyl product resulted. The partial synthesis of anserine was accomplished by Behrens and du Vigneaud (20), who employed a supply of 1-*N*-methylhistidine furnished by Ackermann. This compound was converted to the β -alanyl peptide by the method used for carnosine by Sifferd and du Vigneaud (94). The product was identical with natural anserine.

An excellent review on the chemistry and physiology of carnosine and anserine has been published by du Vigneaud and Behrens (100). Anserine has, since 1929, been found in the muscles of many species (113). The possible importance of this compound and of carnosine as natural buffers in muscle systems has been suggested by Smith (95).

B. Carnosine

Carnosine, one of the so-called muscle extractives, was first isolated from "Liebig's meat extract" as the phosphotungstate, silver salt, and nitrate by Gulewitsch and Amiradzibi in 1900 (47). The nitrate was found to exhibit optical activity: $[\alpha]_D^{20} = +22.3^\circ$. A melting range of 211–212°C. was recorded. Analysis of the nitrate and silver salts in both cases corresponded to an empirical formula of $C_9H_{14}N_4O_3$ for the carnosine base. In 1906, Gulewitsch (46a) showed that carnosine was identical with Kutscher's base (74a) "ignotin", as shown by its melting point and the mixed melting point of the nitrates.

The similarity in behavior of carnosine and arginine first suggested to Gulewitsch that the new compound contained a guanidine group. In order to check his belief, the compound was hydrolyzed with barium hydroxide solution, but none of the expected urea was obtained. Instead, there was isolated a base which precipitated with silver nitrate and barium hydroxide. When the argentous precipitate was decomposed with hydrogen sulfide, a base was liberated, the analysis and decomposition point of which corresponded to those of histidine. Its precipitant properties with phosphotungstic acid and with mercuric chloride also agreed with the properties of histidine. From the equation

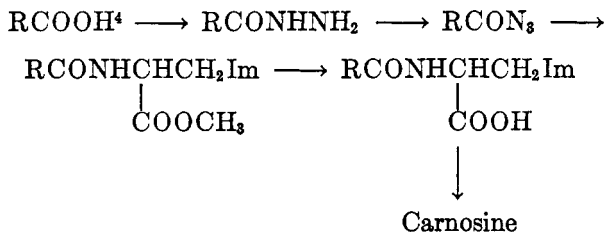


Gulewitsch concluded that the other product was probably alanine.

Subsequently (46b) the hydrolysis was repeated. On this occasion the histidine was completely removed and the residue was treated with α -naphthyl isocyanate. Analysis of the product again indicated $C_3H_7NO_2$. The melting point of the urea was 230–232°C., whereas 198°C. was found to be the melting point for the *dl*- α -alanine derivative.

Gulewitsch therefore concluded that the compound was β -alanine. This substance was therefore prepared from β -iodopropionic acid and converted into the naphthylureide. The melting point of this compound was 231–233°C. These experiments constituted the first record of the existence of β -alanine in nature.

Siffert and du Vigneaud in 1935 (94), employing the Curtius and Bergmann reactions:



The over-all yield from histidine was 65 per cent.

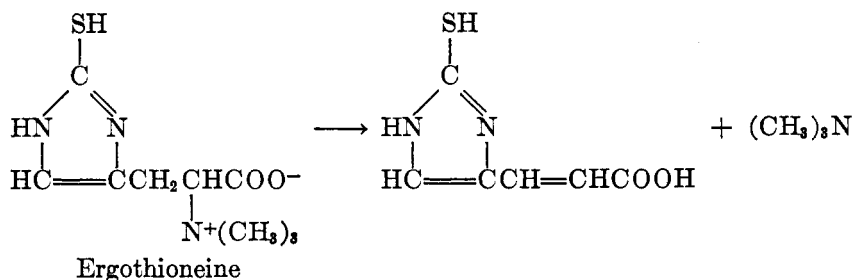
Methyl histidinate was successfully condensed with β -alanyl chloride in another method (55). Abderhalden (1) also successfully concluded Barger's attempted synthesis, by employing the methyl ester of histidine.

du Vigneaud and Hunt (101) prepared *d*-carnosine and found that this gave no blood pressure lowering in twenty times the dose adequate to give a pronounced effect with *l*-carnosine. The short-lived depressor effect of *l*-carnosine is not of pharmacological importance, although it may be important physiologically.

C. Ergothioneine

Ergothioneine (thioneine, thiasine) derived its name from its original isolation from an alcoholic extract of ergot by Tanret (98). It has been isolated from other sources, such as mammalian blood (61). Tanret found the substance to be optically active with $[\alpha]_D = +110^\circ$ (no temperature was given). The compound proved to be a weak base and formed salts with mineral acids and mercuric chloride. It had the empirical formula $\text{C}_9\text{H}_{15}\text{O}_2\text{N}_3\text{S}$. Practically all of the structural work published on ergothioneine is contained in the paper of Barger and Ewins (16). From the facts that it formed a silver precipitate as histidine does, and had a large nitrogen content these workers suspected the presence of an imidazole ring. The carbon and hydrogen content also suggested a betaine group to them. Confirmation of the first guess was furnished by the red color obtained with *p*-diazobenzenesulfonic acid, a color reaction typical of imidazoles (61).

Heating with concentrated potash cleaved ergothioneine to trimethylamine and a yellow acid having the composition shown by the formula $\text{C}_6\text{H}_8\text{O}_2\text{N}_2\text{S}$.

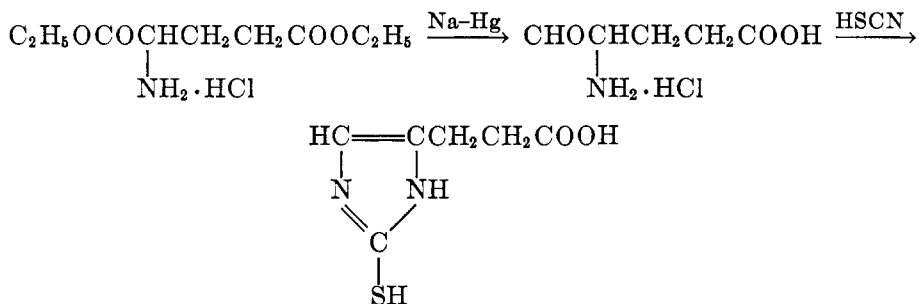


⁴ R = $\text{C}_6\text{H}_5\text{CH}_2\text{OCONHCH}_2\text{CH}_2-$.

When the sulfur of the acid was oxidized away with nitric acid and the resultant compound was reduced with sodium and ethanol, there resulted a substance which, by melting point and mixed melting point, was shown to be identical with an authentic specimen of β -imidazolypropionic acid.

Oxidation of the original compound with iodine gave a disulfide. The substance thus showed a similarity in reaction to cysteine, in contrast to its stability toward hot alkali, which would not obtain for cysteine. From these results Barger and Ewins concluded that the sulfur was present in thiolimidazole form, although they did not rule out as possible an attachment of sulfur to the beta carbon atom.

The position of the sulfur in the ring was confirmed synthetically twenty-two years later by Akabori. Akabori (9) prepared thiolimidazolypropionic acid from glutamic acid, by a ring closure known to give thiolimidazoles; the same compound was obtained by reduction of Barger and Ewins' thiolimidazolyacrylic acid with sodium amalgam.



Although attempts to synthesize ergothioneine have been made, this goal has not yet been attained. Jackson and Marvel (62) attempted the preparation of a possibly useful intermediate from 2-thiol-4(or 5)-hydroxymethylimidazole. Experiments designed to convert the hydroxyl group to chloride by thionyl chloride and by hydrochloric acid were, however, unsuccessful because of polymer formation.

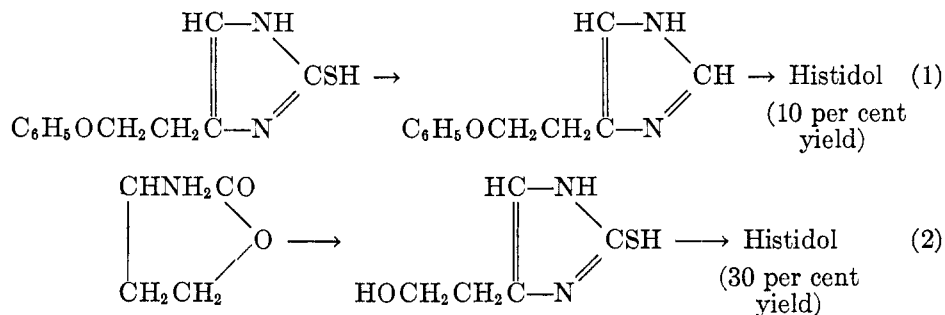
At about the same time several attempts at the same preparation were recorded by Harington and Overhoff (53). Their paper also includes a critical analysis of the synthetic problem and the various possible approaches. These investigators concluded that the most likely mode of synthesis will involve the action of trimethylamine on an appropriate halogen compound. The problem of synthesis therefore remains to be solved. The physiological importance of ergothioneine, if any, has yet to be established also.

Ergothioneine is one of the non-sugar-reducing substances in blood and its value may be related to this fact (18). Extensive tests emphasizing its negative pharmacological action have been reported by Tainter (97).

D. Histamine

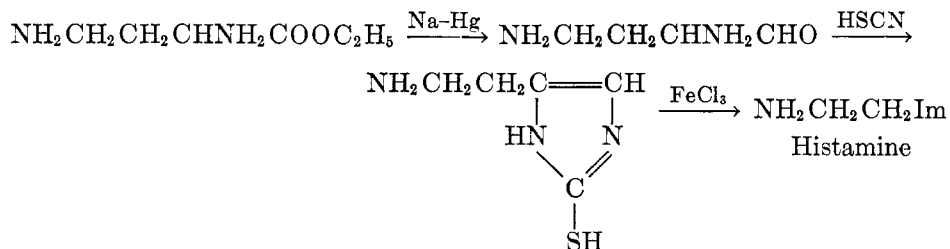
This physiologically important amine is probably the best known of the imidazole group. Before it was recognized as a naturally occurring substance,

converts hydroxyethylimidazole (histidol) to the chloride, and thence to the amine (80 per cent) with alcoholic ammonia.



Besides these syntheses, Ewins and Pyman (36) developed the chemical decarboxylation of histidine to histamine. This involved reaction periods of a few hours, instead of the weeks employed in Ackermann's bacterial process. This method is the principal one used for the commercial preparation of histamine. Ewins and Pyman found that heating with 20 per cent sulfuric acid, concentrated hydrochloric acid, or potassium acid sulfate gave no decarboxylation in 3 hr. at 240°C. At 265–270°C., however, yields up to 25 per cent were obtained. Histidine, benzoylated on the α -amino group, could be heated at 240°C. in a vacuum, alone, and the resultant benzoylhistamine hydrolyzed to give yields of 10 to 20 per cent.

The most recent synthesis of histamine was reported by Akabori and Numano (11c). Glutamic acid was converted in six steps to α , γ -diaminobutyric acid (also prepared (8) by the action of hydrazoic acid on glutamic acid), from which the synthesis proceeded as follows:



The medical utility of histamine includes its use as a gastric stimulant (it is probably a natural gastric hormone (90)), and in ointments for the treatment of rheumatism. An excellent review of the physiological literature to 1931 has been published by Best and McHenry (21a). Histamine is believed to be liberated as part of the mechanism of allergic manifestations in shock, urticaria, asthma, sneezing, etc. For such maladies it has been recommended as a desensitizing agent (37a, 105).

In a recent paper, Kapeller-Adler (66) describes an isolation of histamine. The base is finally separated as the diflavinate, and a few milligrams are re-

coverable. By this method it was possible to demonstrate that, although histidine is excreted by normal pregnant women, histamine, instead, is excreted by those suffering from toxemia.

E. Histidine

Kossel (72) is credited with the original isolation of histidine, from the protamine sturine. Kossel gave the base its present name. The compound was isolated by sulfuric acid hydrolysis and separation as the mercury salt. After removal of mercury, the base was neutralized with hydrochloric acid, and the hydrochloride was isolated. Analysis indicated either $C_{12}H_{20}N_6O_4 \cdot 2HCl + 2H_2O$ or $C_6H_9N_3O_2 \cdot HCl + H_2O$. A small amount of histidine was prepared by removing chloride ion with silver and precipitating from the concentrated aqueous solution with alcohol and ether. The amount of material obtained permitted three analyses, two of which corresponded most closely to the C_6 formula. On the other hand, the boiling-point elevation in phenol indicated a molecular weight of 296, whereas the C_{12} formula requires 312.

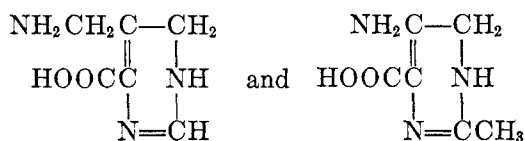
In the same number of "Hoppe-Seyler" (56), Hedin reported further on the chemistry of histidine isolated from blood serum and from casein. The material was prepared from the arginine filtrate with silver, giving $C_6H_{12}N_3O_3Cl$. The base analyzed as $C_6H_9N_3O_2$. A determination of molecular weight by freezing-point lowering, more dependable than Kossel's ebullioscopic method, indicated 155.4 as against a theoretical value of 155.1. The C_6 formula was thus firmly established.

Hedin found that histidine could be precipitated from its aqueous solution by careful addition of ammonia. This precipitate dissolved in excess ammonia. Attempts to obtain a crystalline sulfate or nitrate were unsuccessful. Identity of the substance with Kossel's histidine was proven by crystallographic comparison. Kossel and Kutscher reported later (73), in contrast to Hedin, that natural histidine is optically active: $[\alpha]_D = -39.7^\circ$. Kossel and Kutscher showed that histidine dihydrochloride, melting point $231-233^\circ C.$, could be obtained from the monohydrochloride by evaporation with hydrochloric acid.

Herzog (57) reported several chemical tests run on a sample of histidine. The tests chosen indicated that Herzog was unaware of the type of structure present in his compound. He noted a positive biuret reaction, which was probably due to the presence of peptide impurities. Methoxyl and methylimide tests were negative. It was reported that hydroxylamine hydrochloride reacted with histidine to give a crystalline substance. Oxidation with barium permanganate in neutral solution evolved hydrocyanic acid. Histidine also reacted as a saturated substance when tested with bromine in acetic acid. From these tests, Herzog concluded that the substance was neither dimethyl malonylguanide nor malonylguanide, which conclusion was correct as far as it went.

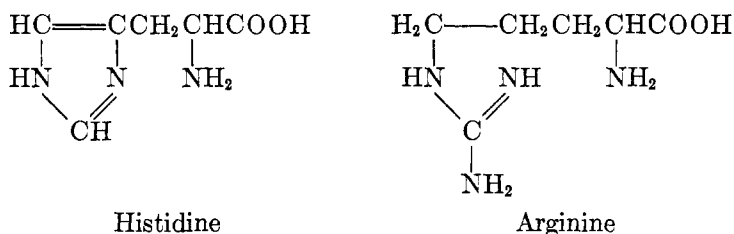
Fraenkel (40a) heated histidinium chloride above its melting point and found that carbon dioxide was evolved. This reaction indicated the presence of a carboxyl group, and histidine could be formulated as $C_6H_9N_3COOH$. Sodium

hypobromite was found to remove one nitrogen; therefore one amino group was present. Fraenkel, however, reported a positive Wiedel reaction, which incorrectly led to the suggestion of the two pyrimidine structures



By a critical review of the published observations and by means of a few well-chosen experiments, Pauly (82) arrived at the now-accepted structure of histidine. He failed to get a definite compound from histidine by the action of benzoyl chloride and alkali, but succeeded with benzenesulfonyl chloride. Two molecules reacted for each molecule of histidine. Since one nitrogen had already been shown to be a primary amino nitrogen, there was thus evidently a secondary amino nitrogen also, and the third was correctly concluded to be tertiary nitrogen. From the analysis, Pauly concluded that there must be two double bonds or a triple bond. Only one could be between nitrogen and carbon, since one nitrogen bore a hydrogen atom. If a $=\text{C}=\text{NH}$ group were present, barium hydroxide treatment, in contrast to the experimental finding, would hydrolyze it. The other double bond must be between two carbon atoms. Stability to acid permanganate indicated that the double bonds must be in the ring.

Two two-nitrogen rings were known at the time—pyrazole and imidazole. Pauly considered that the former did not merit consideration as a physiological component. Because of the close physiological and chemical similarity to arginine, Pauly suggested the correct formula for histidine:

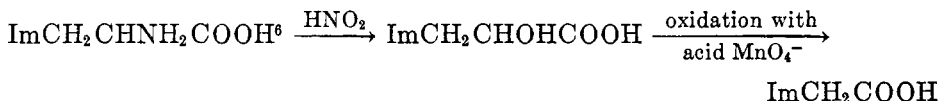


The imidazole authorities, Knoop and Windaus (70), reported experiments designed to settle whether the histidine ring was a derived pyrimidine structure of the type suggested by Fraenkel, or was Pauly's imidazole. Knoop and Windaus treated histidine with sodium and alcohol, without change. A reaction would not be expected with an imidazole, whereas one would be with a pyrimidine (26). Further, by reducing Fraenkel's desaminohistidine with hydriodic acid and phosphorus, they obtained an acid of melting point 208–209°C. This substance was shown to be identical with imidazolylpropionic acid, an intermediate in their histamine synthesis described above.

Fraenkel, however, refused to accept the accumulated evidence for the imidazole structure. In a short paper (40c) he pointed out that pyrimidine

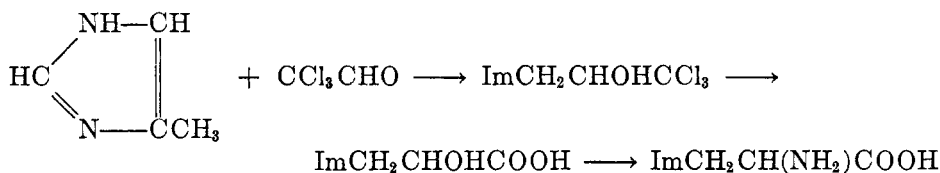
derivatives with no oxygen in the ring give a color with diazobenzenesulfonic acid as does histidine. A more logical argument was the statement that the Bamberger reaction (fission of the imidazole ring by acid chloride in alkali) did not occur with histidine when it was treated with benzenesulfonyl chloride. This argument is of little significance however, since, even to the present date, the specificity of the Bamberger reaction has not been thoroughly elucidated, and it is clear that the reaction is not a general one.

Knoop (69) also settled the position of the amino group by the following reaction:

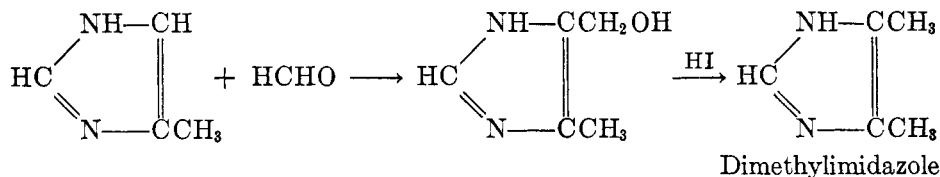


An acid of the correct analysis for imidazolylacetic acid was obtained.

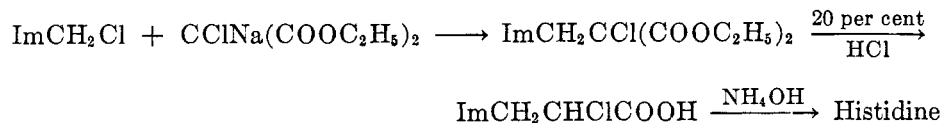
The synthesis of histidine did not occasion as many false starts as did the determination of its structure, but it also was not without an unfruitful attempt. Gerngross (42a) first attempted a synthesis from methylimidazole as follows:



Gerngross thought the first step went as expected, but it was later shown that condensation occurred on the unsubstituted 4(or 5)-carbon atom (106). This was established by hydriodic acid reduction of the formaldehyde condensation product to dimethylimidazole:

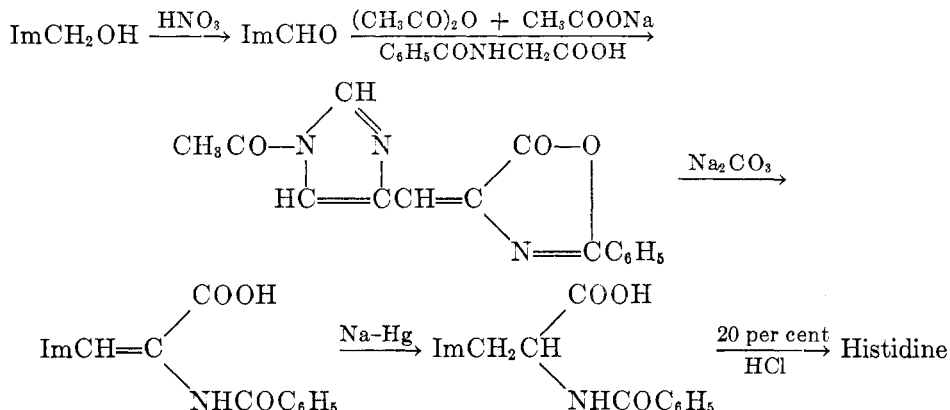


The only recorded syntheses of histidine are those due to Pyman (87b, 87e). Pyman employed imidazolylmethyl chloride, an intermediate in his histamine synthesis. He reacted this with the novel reagent, sodiochloromalonic ester:



⁶ Im = imidazolyl.

In Pyman's later preparation an Erlenmeyer synthesis was employed:



Abderhalden and Weil (3) worked out a resolution of histidine with *d*-tartaric acid.

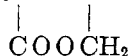
Histidine is an "essential" amino acid (7). It has achieved some recognition as a medicament in the treatment of ulcers. Commercially it is made by isolation from blood paste and from other protein sources. The uncoupled imidazole radical of histidine contributes to the basic properties of proteins. A large part of the ability of the proteins to form azo dyes can be attributed to histidine also. That it is the 1-nitrogen atom and not the 4- or 5-carbon atom which couples was demonstrated by Burian (24), who found that *N*-methylimidazole did not react, whereas *N*-unsubstituted imidazoles, including purines, did.

F. *Pilocarpine and related alkaloids*

Several alkaloids have been isolated from the leaves of *Pilocarpus jaborandi*. These include pilocarpine (the one known to medicine), isopilocarpine, pilocarpidine, and pilosine.

Pilocarpine was discovered independently by Hardy (51) and Gerrard (43) in 1875. Harnack and Meyer (54) established the empirical formula of $\text{C}_{11}\text{H}_{16}\text{O}_2\text{N}_2$. Hardy and Calmels (50) presented a volume of evidence that the alkaloid was a derivative of β -pyridyllactic acid and trimethylamine. This was shown by others, such as Merck (77), to be an incorrect formulation.

Elucidation of the structure of pilocarpine was the result of the labors of Jowett (64a, c, d, e, f) and of Pinner and Schwarz (83). Jowett first found that pilocarpine was converted to isopilocarpine by heat. It was convenient to conduct the structural studies on the latter isomer. By oxidation with permanganate, Jowett obtained a new acid, $\text{C}_7\text{H}_{10}\text{O}_4$, which he named pilopic acid. Titration of pilopic acid showed it to be lactonic, and since further oxidation resulted in butyric acid, the formula $\text{C}_2\text{H}_5\text{CH}-\text{CHCOOH}$ was suggested.



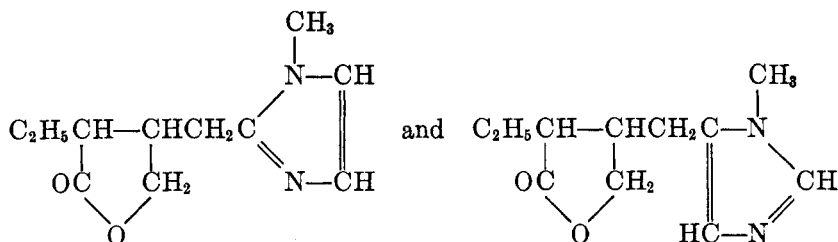
The silver and barium salts, the ester, and the diamide of the dicarboxylic acid were prepared. The homolog, homopilopic acid, $C_2H_5CH-CHCH_2COOH$,



was also obtained by oxidation of isopilocarpine. Alkaline fusion of the latter led to the compound $C_2H_5CH(COOH)CH(COOH)CH_2COOH$, previously known.

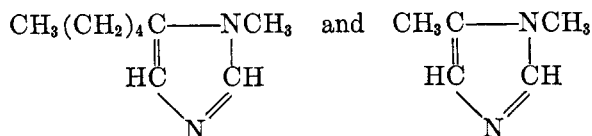
The suggestion that the nitrogenous moiety was an imidazole came from Pinner and Schwarz. These investigators found a small quantity of methylurea after oxidizing the alkaloid. From this information, from the fact that substitution rather than addition of active reagents occurred, and with the empirical formula as a background, the imidazole structure was deduced.

To confirm this deduction partially, a test developed by Rung and Behrend (89) was applied. The original imidazole did not evolve an amine on alkaline treatment, but the ethyl iodide addition product did. From such evidence, the two formulas



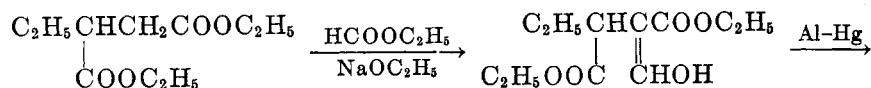
were proposed. The second structure, later shown to be correct, was favored because it was more likely to yield methylurea on oxidation.

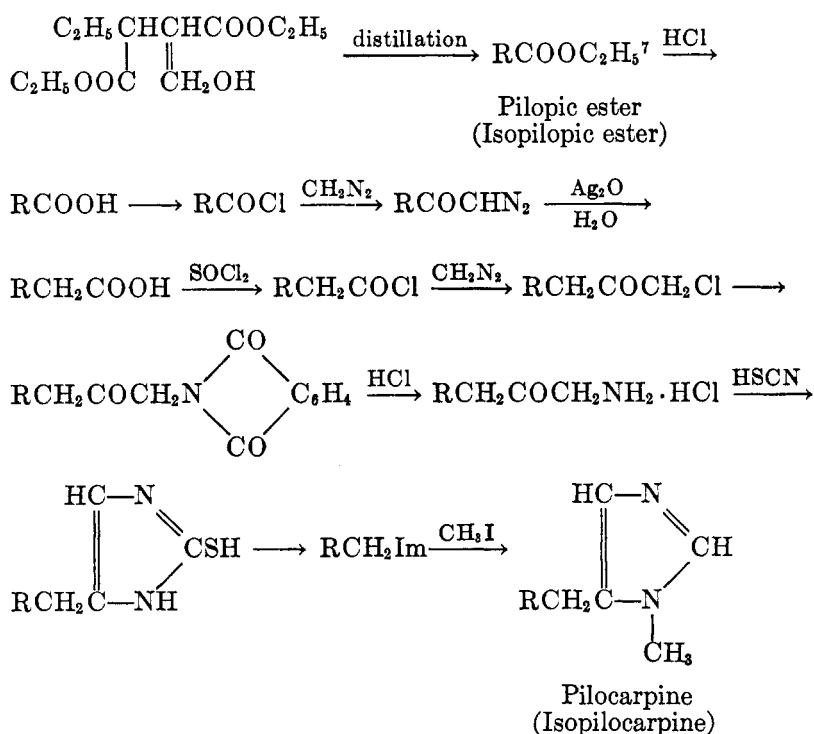
Since the above evidence was not conclusive, Jowett studied the products of alkaline pyrogenesis of isopilocarpine. He found



The latter was established by comparison with the synthetic 1,5-dimethylimidazole (64f, 87f).

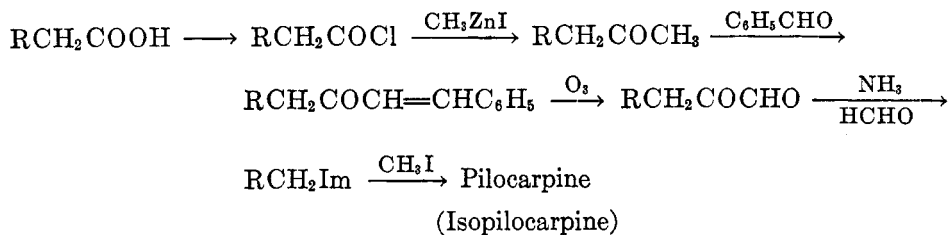
Although the formula was thus established and Jowett attempted the synthesis by various approaches, it was not until 1933 that a synthesis was announced from Moscow (85, 86, 99).



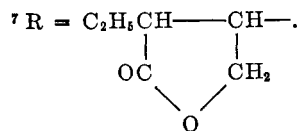


There were obtained both pilocarpine and isopilocarpine by the use of stereoisomeric homopilopic acids separated with strychnine. The yield of pilocarpine nitrate was 29 mg.

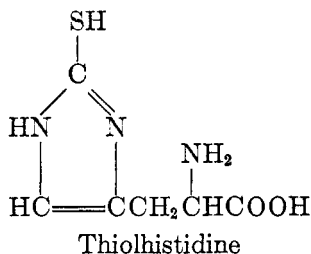
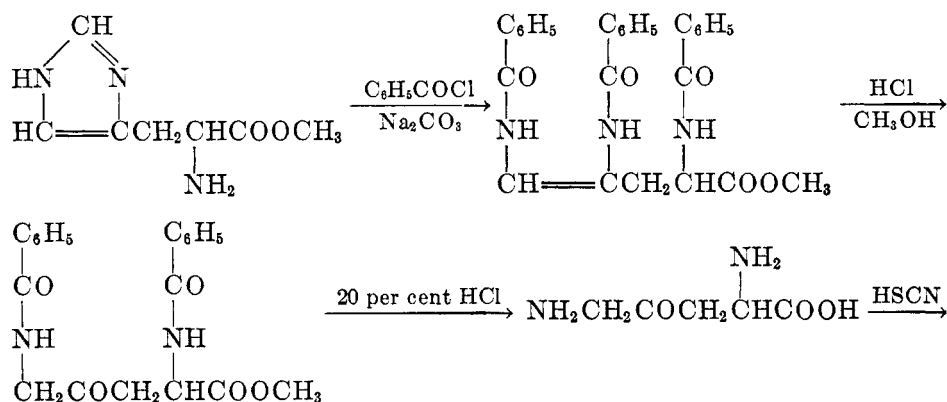
Dey (30a) has developed an alternative synthesis for the homopilopic acids. These were converted to the alkaloids as follows:



Pilocarpine is widely used in medicine as a diaphoretic and myopic.

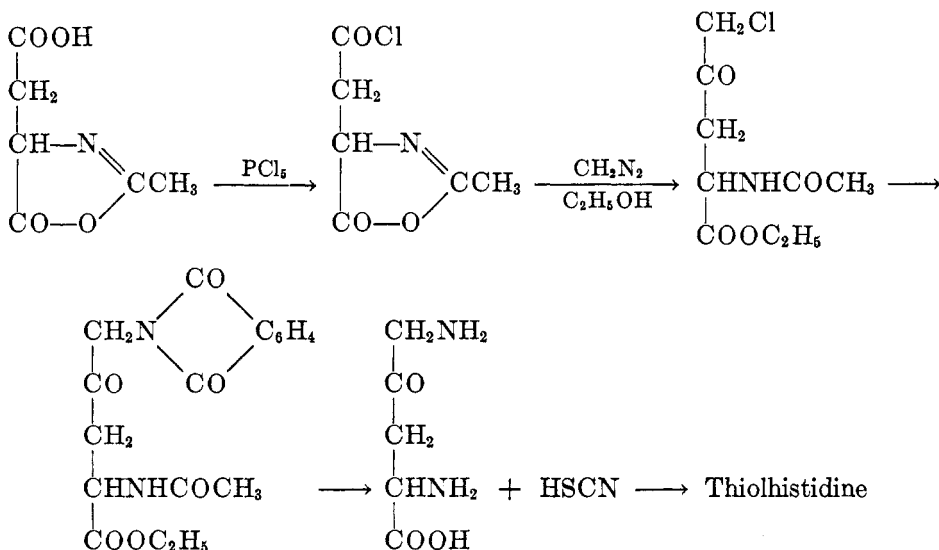


In the first synthesis (14), histidine serves as an initial material which is converted to a desired intermediate by a Bamberger reaction:

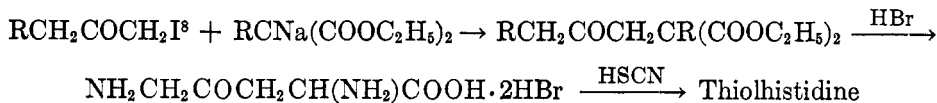


The nature of the product was proved by almost quantitative conversion to histidine with ferric sulfate. Both the starting and final products were optically active.

The second synthesis was developed during attempts to prepare ergothioneine (53):



Dey (30b) has also recorded two syntheses, which are similar. His products were racemic. In the first synthesis:



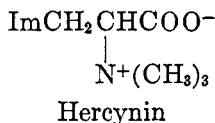
III. OTHER NATURALLY OCCURRING IMIDAZOLES

A. Dimethylhistamine

Ackermann and coworkers (5) have isolated as the picrate a dimethylhistamine from *Geodia gigas*. The structure was determined by a positive diazo reaction and analysis. Although the structure is probably correct, synthetic confirmation is lacking, and the positions of the methyl groups have not been fixed.

B. Hercynin

Hercynin was discovered in mushrooms (74c) and molds (88, 111). The facts that it gave a Pauly reaction for the imidazole nucleus and that it analyzed as the betaine of histidine led Kutscher to deduce its structure correctly:



The partial synthesis of hercynin was first reported by Barger and Ewins (16), who oxidized ergothioneine with ferric chloride. Another synthesis (35) depends upon the reaction of the reaction product of histidine and nitrous acid with alcoholic trimethylamine. The intermediate was not isolated, but it was undoubtedly imidazolylactic acid, and the reactivity of the hydroxyl group with trimethylamine is somewhat surprising in an aliphatic side chain.

C. Histidol

Histidol, 4(or 5)-hydroxyethylimidazole, $\text{ImCH}_2\text{CH}_2\text{OH}$, was discovered by Ehrlich (32) as a product of yeast fermentation of histidine. Three syntheses have been recorded (41, 109). The simplest of these involves the treatment of histamine with nitrous acid. Pyman and Garforth (41) synthesized histidol by two methods which use it as an intermediate for histamine (see page 54).

D. Imidazolylglycine

A small amount of imidazolylglycine, $\text{ImCH}(\text{NH}_2)\text{COOH}$, was isolated from normal human urine as the picrolonate, by Engeland (34). The compound was synthesized by Stewart (96) from imidazole aldehyde by the Strecker reaction. The melting point of the picrolonate of Stewart's base was 243°C ., 1°C . lower than that reported by Engeland. Since the range of melting points of compounds of this type is not large, however, a mixed melting point with an isolated specimen is to be desired.

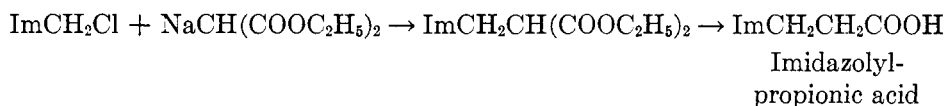
[§] R = $\text{C}_6\text{H}_4(\text{CO})_2\text{N}-$.

E. Imidazolylactic acid

Imidazolylactic acid, $\text{ImCH}_2\text{CHOHCOOH}$, was found as a bacterial product (59). Hirai obtained it in 11 per cent yield from histidine by the action of *Proteus vulgaris*. Prior to this discovery, two syntheses were recorded. Fraenkel (40b) isolated the compound as a reaction product of histidine hydrochloride and silver nitrite. Pyman prepared it from the chloro analog that he used in the synthesis of histidine (87b). Conversion was brought about by moist silver oxide.

F. Imidazolylpropionic acid

This compound represents another bacterial conversion product of histidine (4b). Prior to its discovery as a natural product, Knoop and Windaus (70) prepared it by the hydriodic acid reduction of imidazolylactic acid, and by the condensation of glyoxylpropionic acid with ammonia and formaldehyde as on page 54. The compound was also prepared by Pyman (87b) from (imidazolylmethyl)malonic acid.



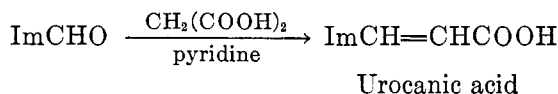
Barger and Ewins (16) reduced imidazolylacrylic acid to this compound with sodium and alcohol.

Two patents describe the synthesis of imidazolylpropionic acid from glutamic acid by the Akabori method (11a, 93). Glutamic acid ester is reduced to the aminoaldehyde, which is condensed with thiocyanic acid and the product is then oxidized with ferric chloride in the usual fashion.

G. Urocanic acid

Urocanic acid, imidazolylacrylic acid, was discovered in 1874 by Jaffe (63) in the urine of dogs and coyotes. It is also formed in pancreatic protein digests (2, 60).

The first synthesis started from the alkaline treatment of ergothioneine (*q. v.*). The imidazole sulfur of the resultant acrylic acid (16) was oxidized away with nitric acid. Urocanic acid was also obtained in small yield by the action of trimethylamine on $\text{ImCH}_2\text{CHClCOOH}$. Akabori and coworkers (12) developed the following method:



IV. OTHER IMIDAZOLES

A. Histamine analogs

This subject has been well reviewed by Guggenheim (45b) in his chapter on "Imidazolverbindungen."

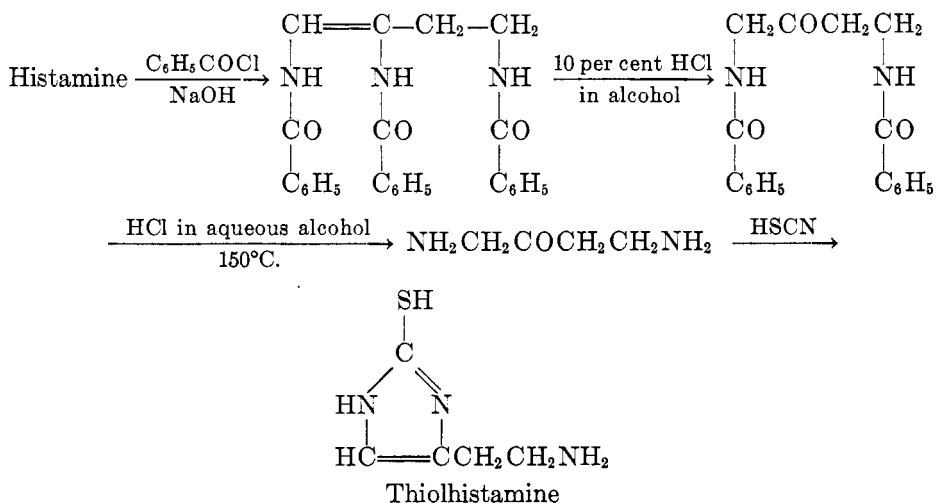
Analogs of histamine are of interest principally because of their pharmacological possibilities. The difference in pharmacological activity between an amino acid and the amine resulting from decarboxylation is especially emphasized in the case of histidine and histamine. One would expect that derivatives of the carboxyl group of histidine might resemble histamine in their action. This expectation has been tested by Arai (13), who prepared histidine methyl ester and found a similarity to histamine in effects on blood pressure and smooth musculature. The action was, however, less marked.

In the homologous series of imidazolyl alkylamines, $\text{Im}[(\text{CH}_2)_n\text{NH}_2]$ (where $n = 1$ to 4), the greatest activity is found when $n = 2$. The only homolog which shows appreciable relative activity is the propyl compound ($n = 3$).

Pyman (87c) prepared the methyl homolog from his own supply of 2-thiolimidazolyl-4(or 5)-methylamine by oxidation. It was not possible to desulfurize with nitric acid, because the resultant nitrous acid would react upon the primary amino group. Ferric chloride was accordingly employed instead. Pyman prepared the butyl analog, but not the propyl, and concluded that imidazolyl-ethylamine was the only active compound in the group.

Akabori was, however, able to prepare the propyl, as well as the butyl, analog by his aminoaldehyde method (page 55), and found the former to be fairly active (10, 11b). The starting materials for these syntheses were arginine and lysine. This is of incidental interest as an *in vitro* example of the suggested physiological convertibility of arginine to imidazole bodies, especially purines (7).

The two monomethyl derivatives of histamine (87c) are almost inactive. The same statement applies to 2-thiolhistamine. The latter compound is also an analog of thiolhistidine. Its synthesis (87g, 107) illustrates an utilization of the Bamberger reaction:



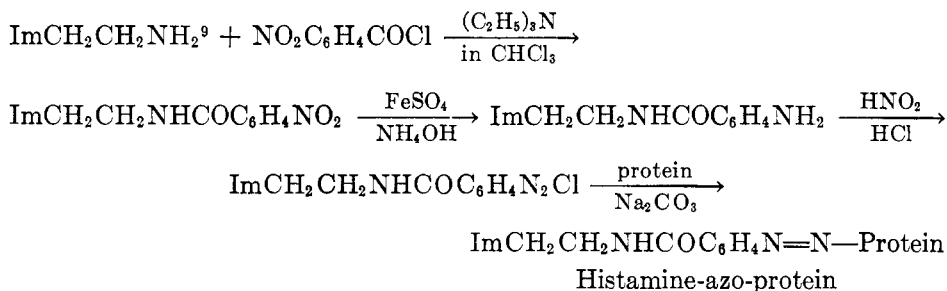
The histamine-like activity of the three isomeric β -pyridylethylamines has been studied (78, 102). Of these only the β -(2-pyridyl)ethylamine exhibited an

effect. The molecular fragment $-\text{CH}=\text{N}-\text{C}(\text{CH}_2\text{CH}_2\text{NH}_2)=\text{CH}-$, common to both this amine and histamine, has accordingly been postulated as the essential structure for the pharmacological activity.

B. Histamine-azo-protein

Since the release of histamine in the tissues is believed to be responsible for allergic manifestations (37b), attempts have been made to provide counteracting treatments as relief for asthma, hay-fever, etc. Desensitization by graded doses of injected histamine and the employment of histaminase have not (21b) provided a complete answer to this problem.

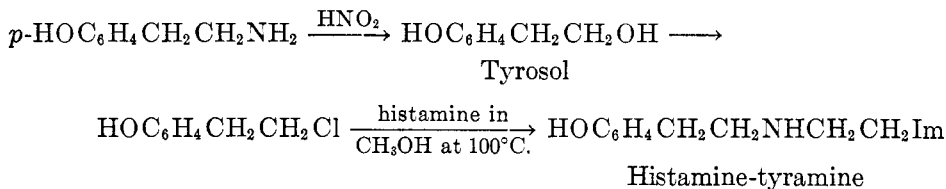
More encouraging results have been obtained by the provision of an immunity to histamine by the administration of histamine, as a hapten, coupled to protein (92). The rationale of this attack is based on the fact that proteins participate in almost all studied cases of antigenicity, and upon the pioneering work of Harington (52), who was able to elicit active antisera to thyroxine and aspirin by similar means. Of a number of coupling methods tried, the most practical seemed to be the following:



The use of this compound is indeed a hopeful example in the fast-growing field of drug antisera production.

C. Histamine-tyramine

This interesting compound was prepared since it combines in one molecule two pharmacologically active compounds found together in ergot. No pharmacological data were reported, but the synthesis followed the plan below (33, 42b).



D. Imidazolylacetic acid

This compound, ImCH_2COOH , has been suggested as a probable metabolic intermediate (44); thus it bears an analogy to indoleacetic acid, which is found

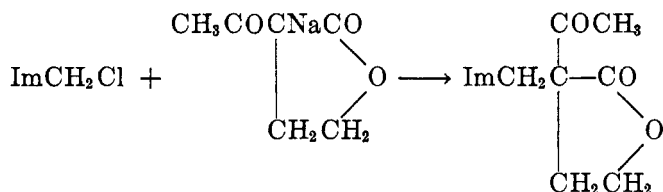
⁹ Im = imidazolyl.

in urine and may arise entirely or in part from tryptophan. Imidazolylacetic acid was first synthesized by oxidation of imidazolylactic acid (69) and later was obtained as a by-product in one of Pyman's histamine syntheses (87a).

E. Pilocarpine analogs

It is natural that several Russian workers have prepared analogs of pilocarpine. Information on the medical value of these compounds is unfortunately not available.

One analog uses acetobutyrolactone, a commercial thiamine intermediate, and (chloromethyl)imidazole (27):



Some general syntheses of analogs have been patented (67). There is certainly considerable room for improving the medical situation regarding pilocarpine by cheapening its synthesis or by providing more readily available active analogs.

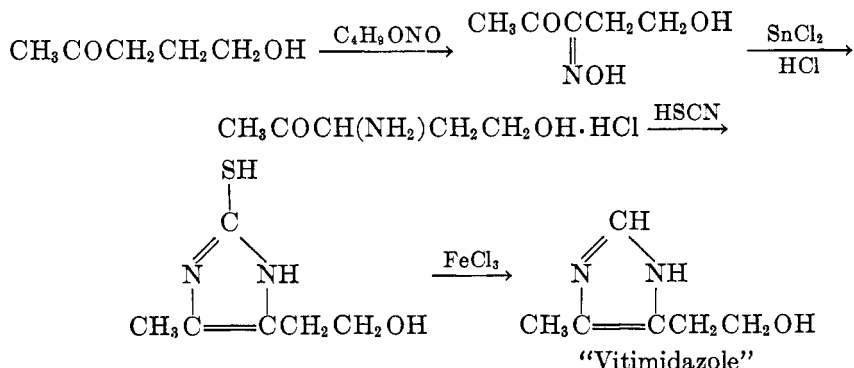
F. Sulfa imidazoles

No knowledge of useful sulfa compounds containing an imidazole nucleus has come to hand. Northey has reported negative tests (79) with sulfahistamine, and two British chemists with considerable experience in imidazoles have assigned a patent for the preparation of such derivatives (76). The process involves the conventional coupling of acylated sulfanilyl chloride and aminoimidazole, followed by deacylation.

G. "Vitimidazole"

The imidazole analog of thiamin thiazole has been synthesized in this laboratory (39). Before the structure of thiamin was elucidated, imidazolemethylethylcarbinol was actually claimed to be antineuritic (91). This was later disputed (48).

The synthetic process was carried out as follows:



When tested on *Phycomyces Blakesleeanus*, this compound lacked the characteristic activity of the analogous thiazole (22).

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