IMIDAZOLE NUCLEOSIDES AND NUCLEOTIDES

LEROY B. TOWNSEND

Department of Chemistry, University of Utah, Salt Lake City, Utah 84112

Received January 23, 1967

CONTENTS

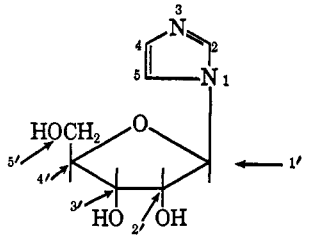
I. INTRODUCTION, SCOPE, AND NOMENCLATURE

This review covers the area of synthetic and naturally occurring imidazole nucleosides, nucleotides, and various related derivatives. Certain biochemical aspects of these compounds are also reviewed briefly.

This ring system has been designated as glyoxaline, iminazole, 1,3-diazole, and imidazole. The first preparation of the parent ring system was accomplished (57) from ammonia and glyoxal; hence the origin of the term glyoxaline is self-evident and is still used extensively in some areas. The terms iminazole and 1,3-diazole are very rarely used in the recent literature. Imidazole, which is the term (83) applied most frequently to this ring system, indicates a five-membered heterocyclic ring system containing an imino group and a tertiary nitrogen and will be used throughout this review.

It was originally proposed (108) that the term nucleoside be used only for carbohydrate derivatives of purines and pyrimidines isolated from the alkaline hydrolyzates of yeast nucleic acid. This was found to limit the use of this term since the major carbohydrate constituents of yeast nucleic acid are either D-ribose or 2-deoxy-D-*erythro*-pentose (2-deoxy-D-ribose). It has now been generally accepted that the term purine nucleoside refers to all glycosyl derivatives of purines, both synthetic and natural, and it seems logical at this time to extend this concept of terminology to encompass the entire field of nitrogen heterocycles. Therefore, in this review all glycosyl derivatives (133) of imidazoles, regardless of the nature of the carbohydrate moiety, will be referred to as imidazole nucleosides. The term imidazole nucleotide will be used when a phosphate ester has been formed on a hydroxy group of the carbohydrate moiety of an imidazole nucleoside.

The numbering system will use numerals to designate positions on the aglycon and primed numerals for positions on the carbohydrate moiety. Numbering of the aglycon always begins at the substituted nitrogen and proceeds so that the second nitrogen in the ring is at position 3. Numbering of the carbohydrate moiety originates at the anomeric carbon which is the carbon involved in the glycosidic linkage.



A number of reviews have (26, 47, 79, 85, 125, 149) presented some imidazole nucleoside and nucleotide chemistry or biochemistry but only in connection with another subject. It is the object of this review to summarize and complement these previous reports and present a complete and comprehensive review on imidazole nucleosides and nucleotides. The generally accepted nomenclature (68, 133) has been used in this review. The literature survey pertaining to this re-

view was essentially concluded in June 1966 although a few more recent references have been included.

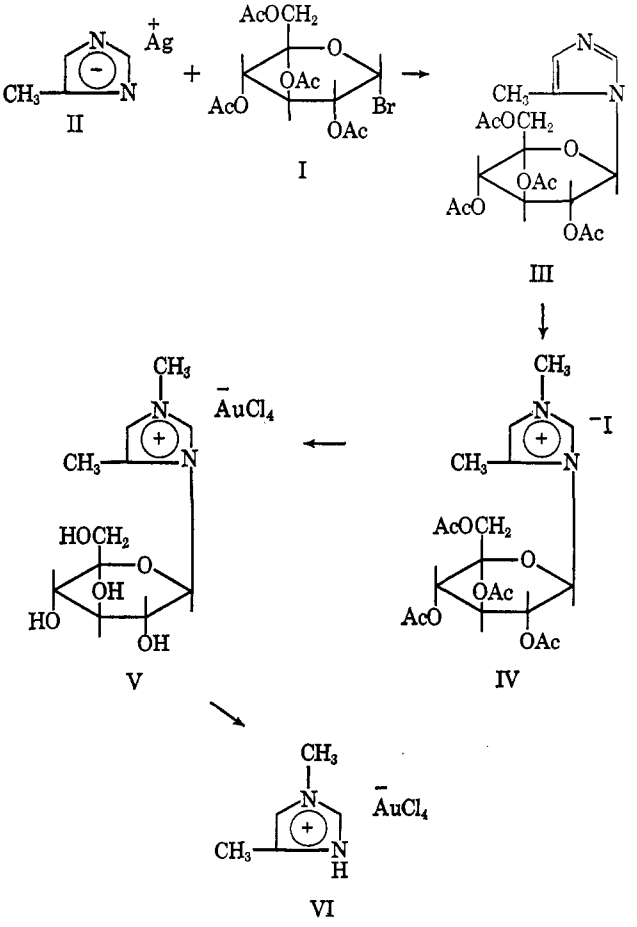
II. CHEMICAL SYNTHESIS OF IMIDAZOLE NUCLEOSIDES AND NUCLEOTIDES

The chemical preparation of imidazole nucleosides is a relatively new area in comparison to the related areas of purine and pyrimidine nucleosides. The discovery that nucleic acids contained purine and pyrimidine nucleotides generated an early interest in the chemical synthesis of analogs of these naturally occurring compounds. Interest in the area of imidazole nucleosides and nucleotides developed rather slowly but has recently escalated rapidly. The major factor involved in this increased interest can probably be attributed to the fact that the major purine nucleotides found in nucleic acids depend on imidazole nucleotides as precursors.

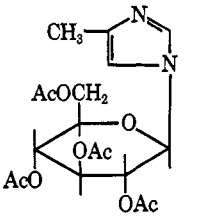
A. CONDENSATIONS OF HEAVY METAL SALTS OF PREFORMED IMIDAZOLES WITH ACYLGLYCOSYL HALIDES

The first reported chemical synthesis of an imidazole nucleoside was accomplished in connection with the elucidation of the site of glycosidic attachment of purine nucleosides isolated from nucleic acid.

The condensation of α-acetobromoglucose (I) with the silver salt of 5(4)-methylimidazole (II) in a non-

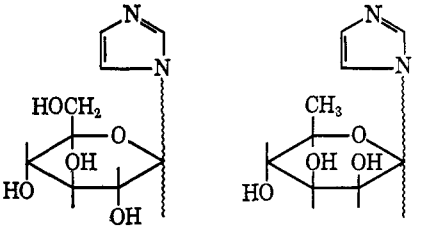


polar solvent produced (80) the first crystalline synthetic imidazole nucleoside, 5-methyl-1-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)imidazole (III). Elucidation of the site of glycosidation was accomplished by alkylation of III with methyl iodide to afford a methiodide derivative of IV. Conversion of IV to the methochloroaurate derivative (V) followed by acid hydrolysis resulted in the isolation of 1,4-dimethylimidazole chloroaurate (VI). The structure of VI was established by a comparison of its solubility and melting point with the previously prepared (143) 1,4- and 1,5-dimethylimidazole chloroaurates and corroborated the initially assigned site of glycosidation. The stability of imidazole nucleosides was firmly established when V was recovered unchanged after refluxing in 21% hydrochloric acid for 1.5 hr; 38% hydrochloric acid at 150° for 2 hr was finally required to cleave the glycosidic bond. Although III was the only crystalline nucleoside isolated, it was suggested that the isomeric compound, 4-methyl-1-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl)imidazole (VII), was present in the mother liquor of the original reaction mixture. This type of condensation with subsequent deacetyla-



VII

tion was used (41) to synthesize 1-(D-glucopyranosyl)-imidazole (VIII) and 1-(D-rhamnopyranosyl)imidazole (IX) from the silver salt of imidazole and the appropriate 1-bromo peracetylated carbohydrate. Preparations of the corresponding peracetylated galactosido and lactosido derivatives of imidazole were reported

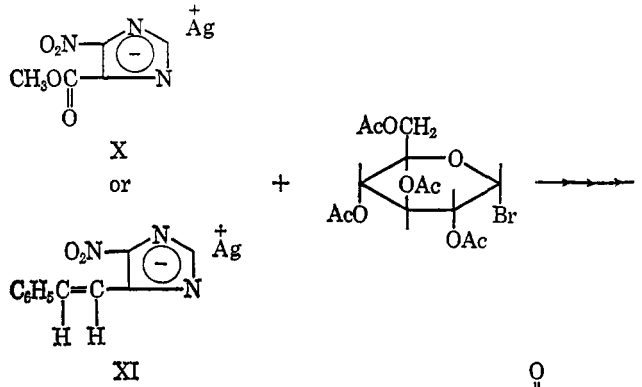


VIII IX

(41) to occur as syrupy masses but were unsubstantiated by experimental details or physical data.

An early preparation of a 9-D-glycosylxanthine (XII) was envisaged as proceeding from imidazole nucleoside precursors. This route was found (22) to be unrewarding when the silver salt of methyl 4(5)-nitroimidazole-5(4)-carboxylate (X) or 4(5)-nitro-5(4)-styrylimidazole (XI) failed to condense with α-aceto-

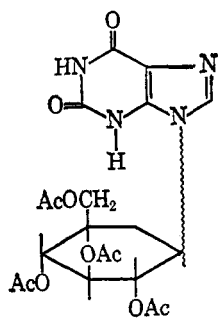
bromoglucose to produce a suitable imidazole nucleoside for ring closure to the glycosylpurine (XII). If glycosidation had occurred at the same site as



X

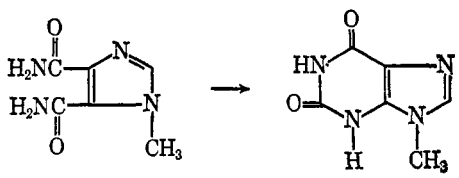
or

XI



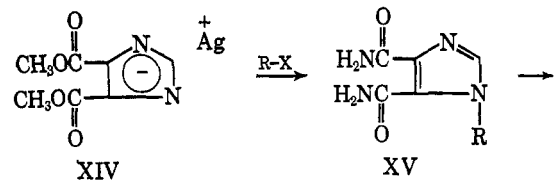
XII

methylation in this investigation, a 7-glycosylxanthine would have been obtained instead of the desired 9-glycosylxanthine. However, a successful (38) preparation of 9-methylxanthine (XIII) from 4,5-dicarboxamido-1-methylimidazole opened a new route for the preparation of 9-glycosylxanthines. The condensation of various peracetylated glycosyl halides with the



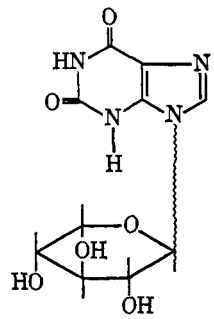
XIII

silver salt of XIV followed by treatment of the product with alcoholic ammonia proceeded smoothly to produce (39) the corresponding 1-(D-glucopyranosyl)-, 1-(L-arabinopyranosyl)-, 1-(D-arabinopyranosyl)-, and 4,5-dicarboxamido-1-(D-xylopyranosyl)imidazoles (XV). Treatment of XV (R = D-xylopyranosyl) with alkaline potassium hypobromite furnished 9-(D-xylopyranosyl)-xanthine (XVI). The pyranose form of XV (R = D-xylopyranosyl) and XVI was established by periodate studies, and the pyranose form was then assigned the remaining compounds prepared in this investigation on that basis with no attempt at establishing their anomeric configuration. That ring closure with alkaline potassium hypobromite had produced the desired 9-glycosylxanthine rather than the isomeric 7-glycosyl-



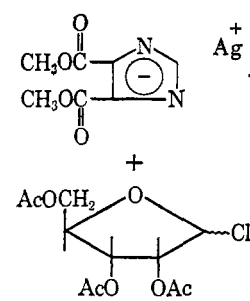
XIV XV

R = glycosyl moiety

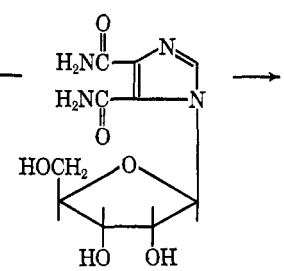


XVI

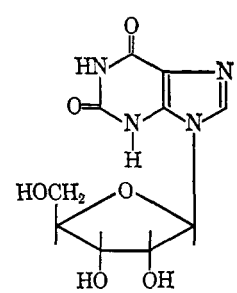
xanthine was determined by a comparison of the ultraviolet absorption spectra of XVI with the spectra of 7- and 9-methylxanthine prepared by unambiguous routes. The subsequent preparation (37) of 4,5-dicarboxamido-1-(D-ribopyranosyl)imidazole and the corresponding 1-(D-mannopyranosyl) derivative was accomplished using the now familiar condensation of a silver salt and the appropriate glycosyl halide. These two compounds were subsequently converted to the corresponding 9-glycosylxanthines using the alkaline potassium hypobromite ring closure, and the pyranose form of both compounds was determined by periodate studies. This synthetic route for the preparation of 9-glycosylxanthines was applied (91) to the synthesis of xanthosine, a naturally occurring purine nucleoside, after the successful preparation of the appropriate carbohydrate precursor was reported (90). The condensation of methyl 4,5-imidazoledicarboxylate (silver



XVII



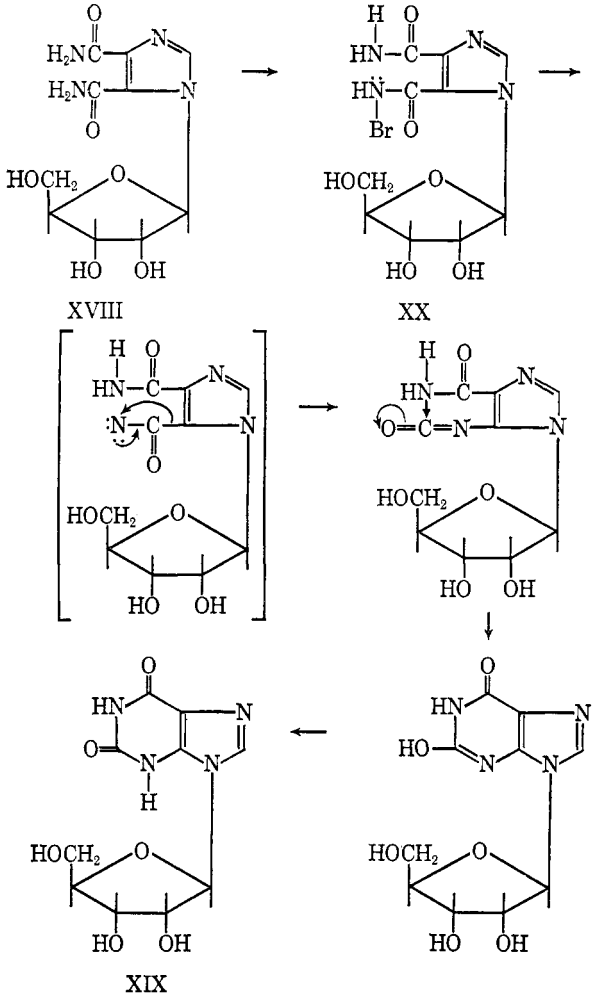
XVIII



XIX

salt) and 1-chloro-2,3,5,-tri-*O*-acetyl-D-ribofuranoside (XVII) and subsequent treatment with alcoholic ammonia produced 4,5-dicarboxamido-1-(β-D-ribofuranosyl)imidazole (XVIII). Treatment of XVIII with alkaline potassium hypobromite furnished a product identical in all respects with xanthosine and therefore established the anomeric configuration of XVIII. This ring closure of the diamide using Hofmann reaction conditions apparently goes through the series of reactions shown in Scheme I.

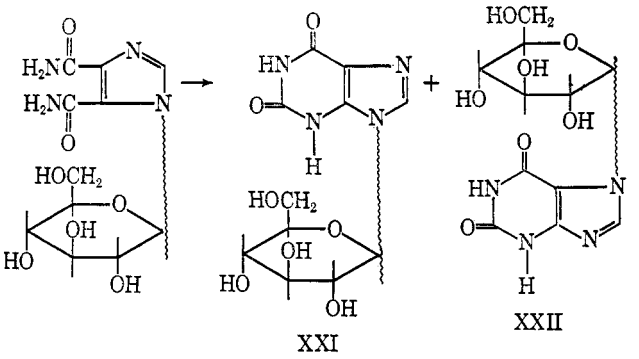
SCHEME I



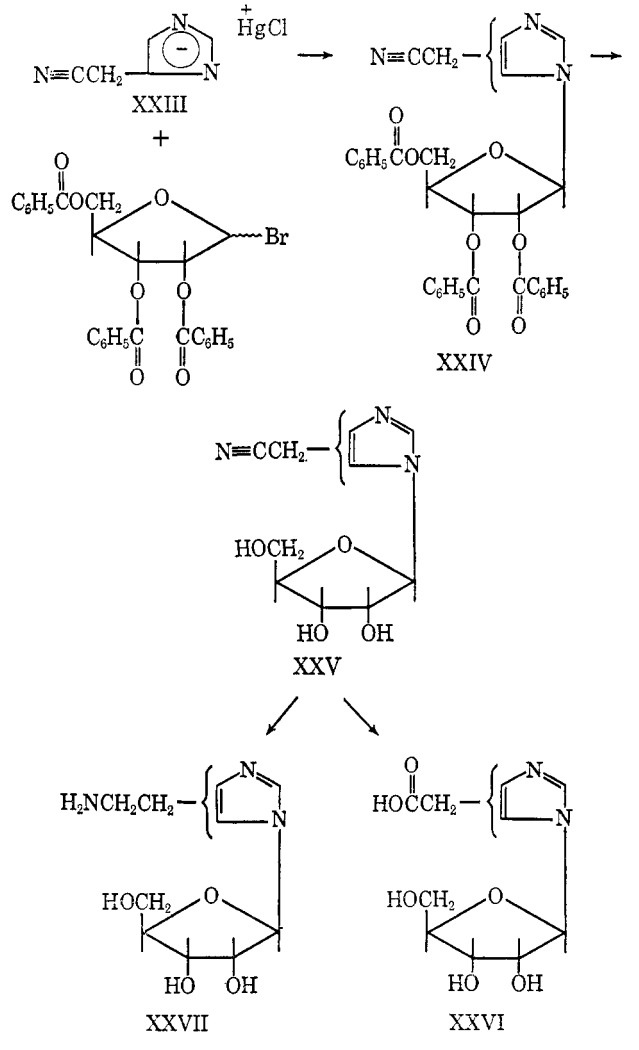
XVIII XX XIX

A reinvestigation (31) of the previous (39) unsuccessful ring closure of 4,5-dicarboxamido-1-(D-glucopyranosyl)imidazole to 9-D-glucosylxanthine produced not only 9-D-glucosylxanthine (XXI), but spectroscopic (ultraviolet) evidence was also obtained for a small amount of the 7 isomer (XXII) in the reaction mixture.

An interperitoneal injection of histamine produced a number of metabolites in the urine of rats and mice. One of these metabolic products has been shown (98, 99, 184) by degradative studies to be a ribofuranosyl derivative of imidazole-4(5)-acetic acid. However, these degradative studies failed to establish the total structure of this compound since the anomeric configuration and actual site of the ribosyl group in relation to the acetic acid side chain were undetermined. The anomeric configuration was later established as β (based entirely upon the *trans* rule) by two independent laboratories. Condensation of the mercuric chloride salt of methylimidazole-4(5)-acetic acid and the appropriate glycosyl halide produced (29) a compound (XXVI) identical with the urinary product in all respects. XXVI was also obtained (34, 35) when the mercuric chloride salt of 4(5)-cyanomethylimidazole (XXIII) was condensed with the appropriate



XXI XXII

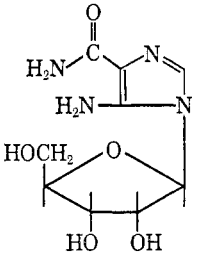


XXIII XXIV XXV XXVII XXVI

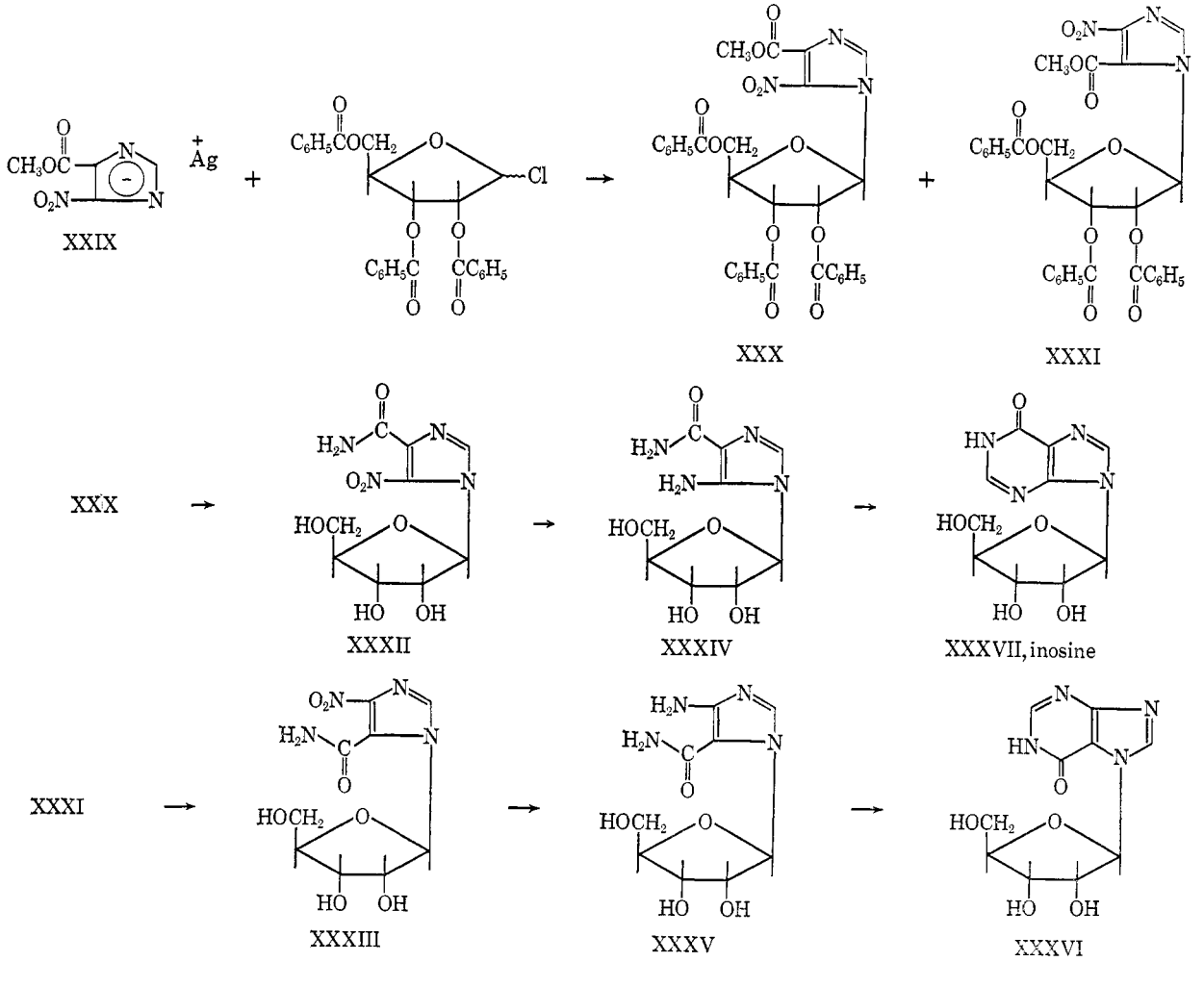
glycosyl halide to afford 4(5)-cyanomethyl-1-(β-D-ribofuranosyl)imidazole (XXV). Subsequent hydrolysis of the cyano group produced XXVI which was also identical with the urinary product isolated previously (98, 99, 184).

A very interesting compound, 4(5)-β-aminoethyl-1-(β-D-ribofuranosyl)imidazole (XXVII, histamine riboside), was produced by the catalytic reduction of the cyano group. The total structure of the naturally occurring riboside XXVI is still unknown since the actual site of glycosidation has not yet been established.

Another naturally occurring imidazole nucleoside of considerable interest is 5-amino-4-carboxamido-1-(β-D-ribofuranosyl)imidazole (XXVIII), the chemical synthesis of which has been accomplished (27, 28) using the heavy metal salt condensation method. The condensation of tri-*O*-acetyl-D-xylosyl bromide with the silver salt of 4(5)-nitro-5(4)-styrylimidazole occurred readily to afford, after deacetylation, 5-nitro-4-styryl-1-(β-D-xylopyranosyl)imidazole. However, the condensation of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride with the same silver salt was entirely unsuccessful but did proceed when the mercury chloride salt was used instead of the silver salt. This approach was abandoned when oxidation of the styryl group to the corresponding carboxylic acid proved to be unfruitful. The condensation of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride with the silver salt of methyl 4(5)-nitroimidazole-5(4)-carboxylate (XXIX) produced a mixture of the isomeric glycosides XXX and XXXI. Treatment of this isomeric mixture with methanolic ammonia produced a mixture of the carboxamido derivatives (XXXII and XXXIII) which were separated chromatographically; the nitro groups were then reduced to afford the corresponding amines. Ring closure of these isomeric compounds to the corresponding purine ribosides established the actual site of glycosidation by a comparison of ultraviolet absorption spectra. In



XXVIII



XXIX

XXX

XXXI

XXXII

XXXIII

XXXIV

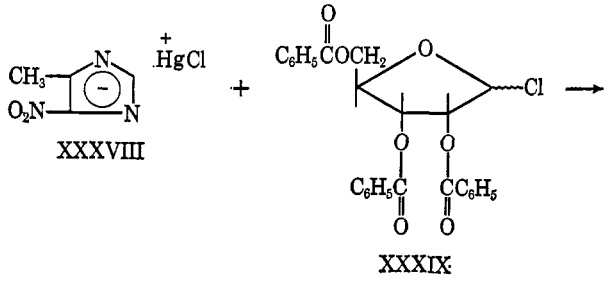
XXXV

XXXVII, inosine

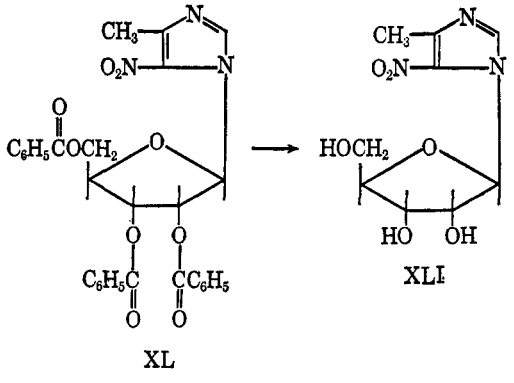
XXXVI

fact, the existence of XXXVI was based entirely on an ultraviolet spectrum of the reaction mixture since the isolation and characterization of XXXVI was precluded by the small amount of precursor (XXXV) available for the reaction.

The tremendous interest in XXXIV prompted a subsequent investigation (95) on the chemical synthesis of various analogs. Condensation of the chloromercury salt of 4(5)-methyl-5(4)-nitroimidazole (XXXVIII) (196) with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride (XXXIX) (96) produced a solid compound which was purified by column chromatography. Although both N-substituted isomers were expected,



XXXVIII + XXXIX



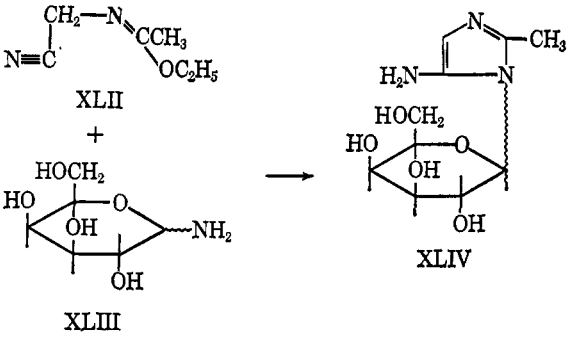
XL → XLI

only one isomer was isolated which was assigned the structure 4-methyl-5-nitro-1-(β-D-ribofuranosyl)imidazole (XLI) after deblocking. Condensation of the chloromercury salt of methyl 4(5)-nitroimidazole-5-(4)-carboxylate with XXXIX furnished, after deblocking the carbohydrate moiety, 5-carboxamido-4-nitro-1-(β-D-ribofuranosyl)imidazole (XXXIII).

B. RING CLOSURE OF GLYCOSYLAMINES

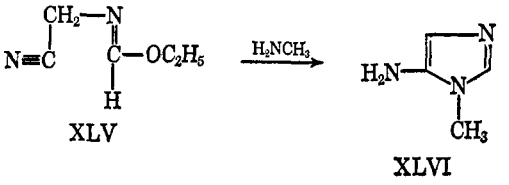
The successful (68, 146) ring closure of linear glycosylamines to pyrimidine nucleosides revealed an alternate route for the preparation of imidazole nucleosides. Initial exploration (164, 165) in this area proved most rewarding when the reaction of N-cyanomethylacetimidate (XLII) with D-galactosylamine (XLIII) produced 5-amino-2-methyl-1-(D-galactopyranosyl)imidazole (XLIV).

It is of interest that use of ethyl N-cyanomethylformimidate (XLV) in the ring closure reaction with



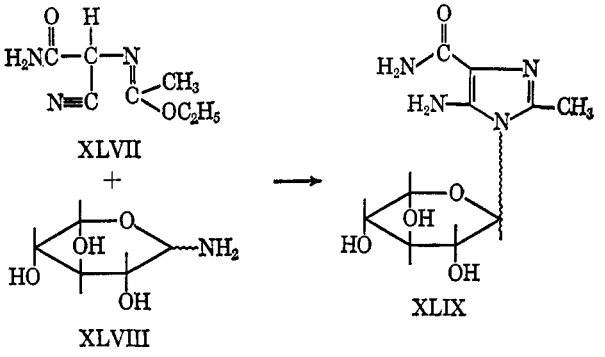
XLII + XLIII → XLIV

methylamine produced 5-amino-1-methylimidazole (XLVI) while the same reaction conditions using 2,3,5-tri-*O*-benzoyl-D-ribofuranosylamine instead of methylamine produced an unstable compound which could not be isolated but was assumed to be 5-amino-1-(D-ribofuranosyl)imidazole on the basis of an ultraviolet spectrum of the reaction mixture. Extension of this ring closure was examined using ethyl N-(carbamoylcyanomethyl)acetimidate (XLVII) prepared from ethyl acetimidate hydrochloride and α-amino-α-cyanoacetamide (179). Treatment of XLVII with



XLV → XLVI

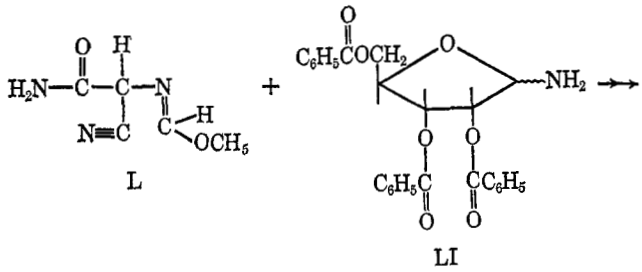
D-xylopyranosylamine (XLVIII) produced a compound which was shown to be 5-amino-4-carboxamido-2-methyl-1-(D-xylopyranosyl)imidazole (XLIX).



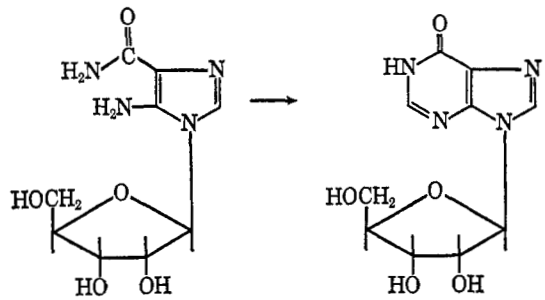
XLVII + XLVIII → XLIX

The importance of this synthetic route for the preparation of imidazole nucleosides was demonstrated (165) by the successful synthesis of 5-amino-4-carboxamido-1-(β-D-ribofuranosyl)imidazole (LII) from the reaction of ethyl N-(carbamoylcyanomethyl)formimidate (L) with 2,3,5-tri-*O*-benzoyl-D-ribofuranosylamine (LI, prepared *in situ* from 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl azide) (30) followed by deblocking of the carbohydrate moiety. The structure of LII was established unequivocally by a rigorous comparison with the naturally occurring compound (78), and the anomeric configuration was firmly established as β by subsequent ring

closure of LII to inosine. This configurational assignment is in complete agreement with the finding (166) that ring closure of a linear glycosylamine to a pyrimidine nucleoside produced only the β nucleoside. One

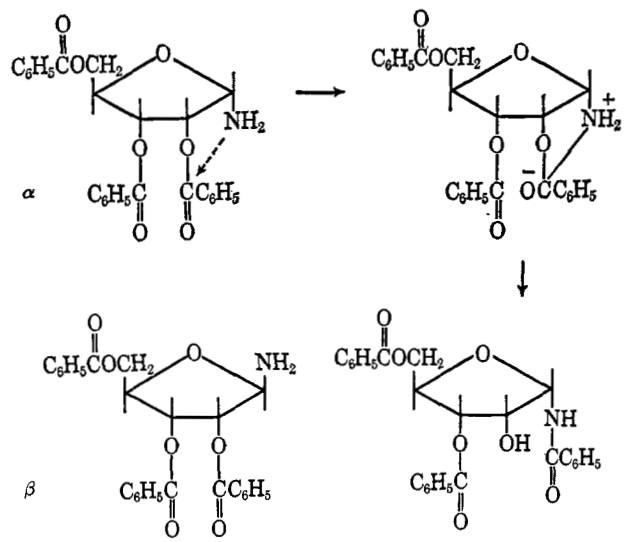


L LI



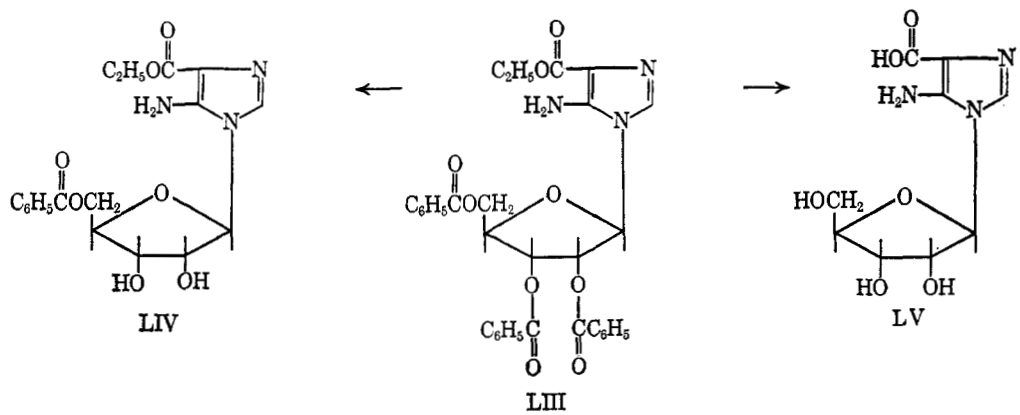
LII inosine

explanation for this has been that steric hindrance caused by the 2-*O*-benzoyl group reduced the accessibility of the amino group in the α anomer. Another explanation is the intermediate formation of an oxazoline with the possibility of an O to N migration of the 2-*O*-benzoyl group in the case of the α anomer, whereas this migration is precluded in the β anomer and therefore leaves the β-amino group available for ring closure.

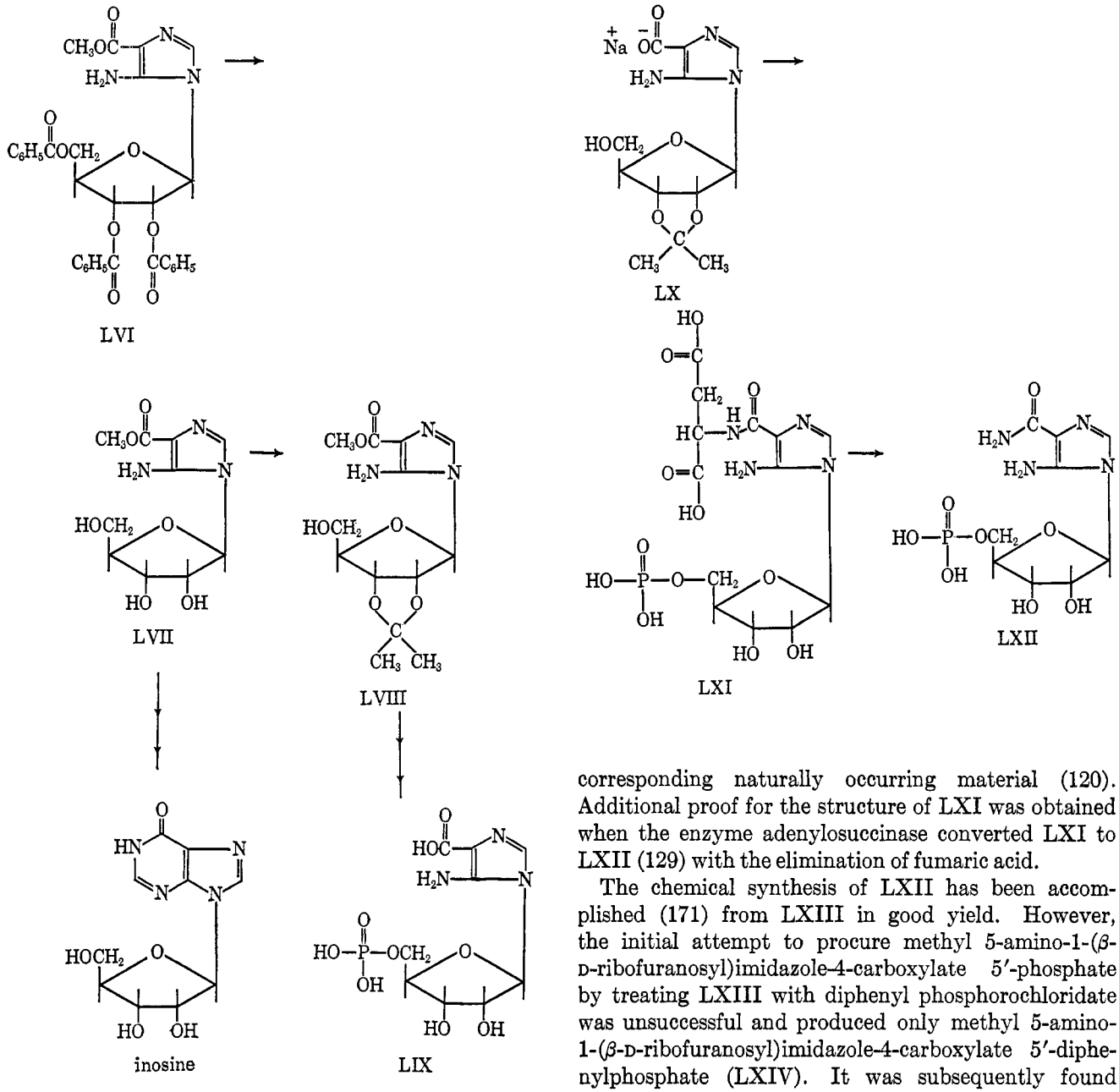


Extension of this type of ring closure depends to a large extent on the precursors available for condensation with glycosylamines. Treatment of 2,3,5-tri-*O*-benzoyl-D-ribofuranosylamine with ethyl N-cyano-N-(ethoxycarbonylmethyl)formimidate produced (167, 168) ethyl 5-amino-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazole-4-carboxylate (LIII). Deblocking of the carbohydrate moiety with triethylamine in absolute ethanol produced a monobenzoyl derivative of LIII, presumably LIV. Complete debenzoylation of LIII was effected by refluxing alkali; however, this treatment was accompanied by saponification which furnished 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxylic acid (LV). These difficulties were solved by using the methyl imidate in the ring closure with the glycosylamine to produce methyl 5-amino-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazole-4-carboxylate (LVI) which was deblocked readily to produce methyl 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxylate (LVII). Subsequent formation of the isopropylidene derivative (LVIII) proceeded smoothly from LVII. Conversion of LVIII to the 5′-phosphate derivative (LIX) was accomplished using 2-cyanoethyl phosphate and dicyclohexylcarbodiimide in pyridine (185) to phosphorylate, followed by acidic hydrolysis of the isopropylidene group. It was shown that LIX possessed physical constants in excellent agreement with those reported for the naturally occurring compound (119, 121). The anomeric configuration was unequivocally established as β when LVII was converted to inosine *via* the 4-carboxamido derivative.

Further studies (169, 170) in this area were prompted by the isolation and characterization (120) of N-(5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxyl)-L-aspartic acid 5′-phosphate (LXI) as an intermediate in

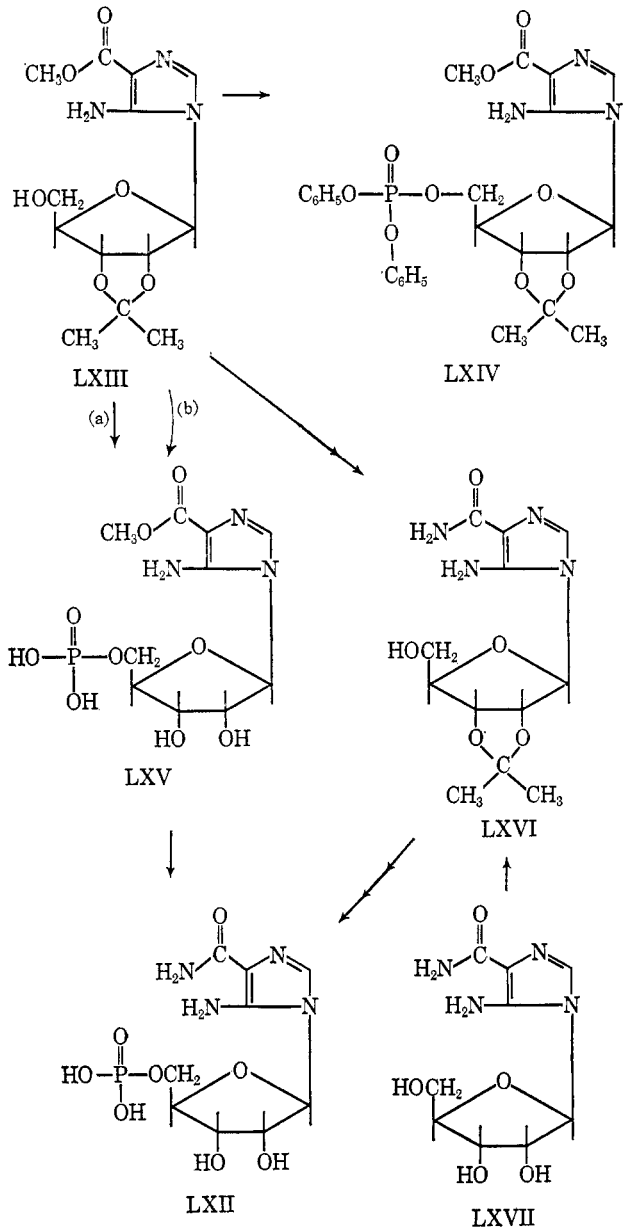


LIV LIII LV

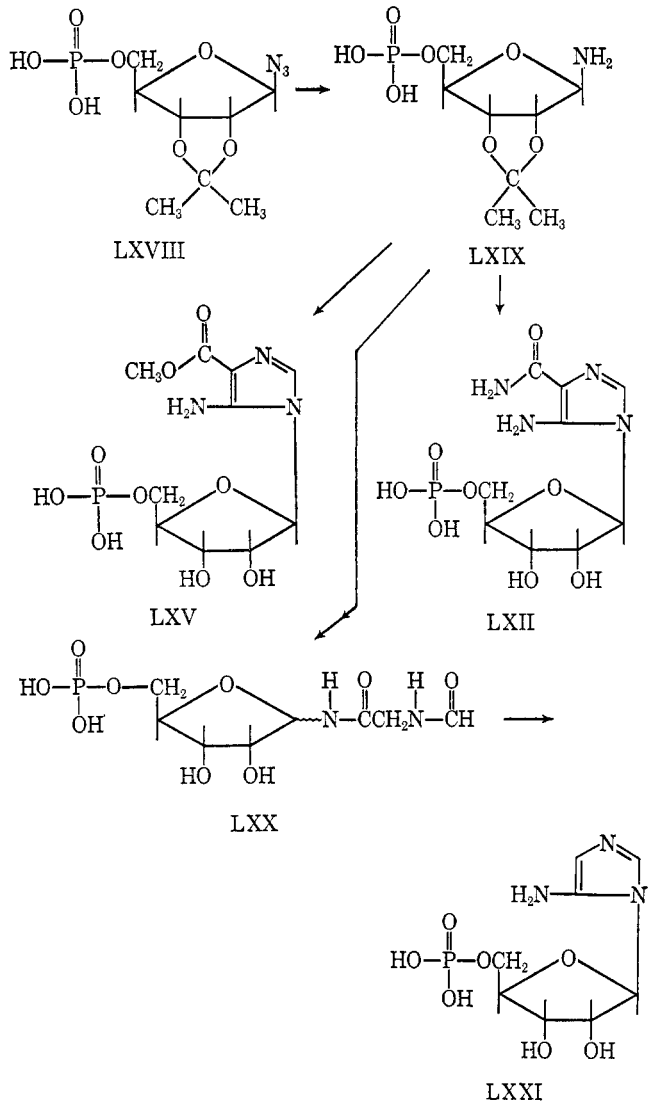


the *de novo* biosynthetic pathway of purine nucleotides. Hydrolysis of LVIII produced the sodium salt of 5-amino-1-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)-imidazole-4-carboxylic acid (LX) which was converted to the pyridine salt with an ion-exchange resin. Treatment of LX (pyridine salt) with dicyclohexylcarbodiimide and dimethyl L-aspartate followed by 2-cyanoethyl phosphate produced a product which was not fully characterized. This product was heated with acetic acid and then lithium hydroxide to furnish a compound which was purified by ion-exchange resin chromatography to afford N-[5-amino-1-(β-D-ribofuranosyl)imidazole-4-carbonyl]-L-aspartic acid 5′-phosphate (LXI) as the barium salt. The physical constants for LXI agreed with those published for the corresponding naturally occurring material (120). Additional proof for the structure of LXI was obtained when the enzyme adenylosuccinase converted LXI to LXII (129) with the elimination of fumaric acid.

The chemical synthesis of LXII has been accomplished (171) from LXIII in good yield. However, the initial attempt to procure methyl 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxylate 5′-phosphate by treating LXIII with diphenyl phosphorochloridate was unsuccessful and produced only methyl 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxylate 5′-diphenylphosphate (LXIV). It was subsequently found that either mono-2-cyanoethyl phosphate and dicyclohexylcarbodiimide or pyrophosphoryl chloride followed by removal of the isopropylidene group produced the desired compound LXV. Treatment of LXV with aqueous ammonia furnished 5-amino-4-carboxamido-1-(β-D-ribofuranosyl)imidazole 5′-phosphate (LXII, AICAR). Another route used by these same investigators involved the formation of the carboxamide from LXIII to afford LXVI which was also obtained from LXVII. Phosphorylation of LXVI followed by removal of the isopropylidene group gave LXII in a low yield. Alkaline hydrolysis of LXIII followed by amination and phosphorylation also gave LXII but in an extremely low yield. Although this investigation provided three routes for the preparation of LXII, only the first route would appear to be of preparative value.



Since the manipulations required to convert a free riboside to a ribotide (5′-phosphate) are inherently difficult, it seemed of interest to prepare the ribotides directly. This required the precursor 2,3-*O*-isopropylidene-5-phospho-D-ribofuranosylamine (LXIX), which was prepared *in situ* (50, 51) by hydrogenation of 2,3-*O*-isopropylidene-5-phospho-D-ribofuranosyl azide (LXVIII). Reaction of LXIX, prepared *in situ*, with the appropriate imidate followed by removal of the isopropylidene group produced methyl 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxylate 5′-phosphate (LXV). Treatment of LXIX with formylglycyl chloride produced N-(N-formylglycyl)-D-ribofuranosylamine 5-phosphate (LXX) which was converted enzymatically to 5-amino-1-(β-D-ribofuranosyl)imidazole 5′-phosphate (LXXI, AIR). There is as yet no chemical synthesis of LXXI or the corresponding riboside.

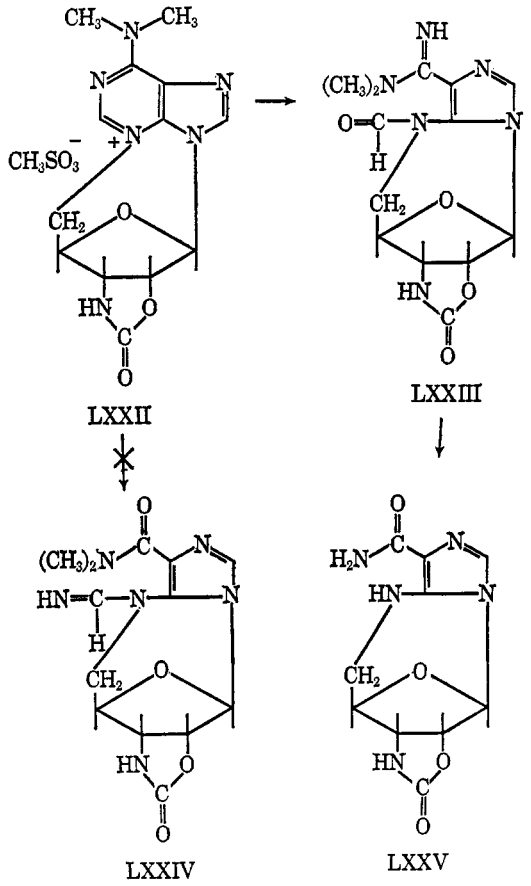


C. DEGRADATION OR RING OPENING OF PURINE NUCLEOSIDES AND NUCLEOTIDES

The tremendous interest in nucleic acids has provided sufficient motivation for the development of a number of versatile methods for the synthetic preparation of purine nucleosides and nucleotides. In addition there are a number of purine nucleosides and nucleotides which are naturally occurring (nucleic acids, antibiotics, etc.). It was inevitable that this abundance would suggest that degradation of a purine nucleoside or nucleotide might afford a facile preparation of either imidazole nucleosides or nucleotides. It has been demonstrated (189) that methylation at N-7 of guanosine and xanthosine conveys a certain degree of instability to the imidazole ring of these nucleosides since ring opening at the 8 position occurs in the presence of a very weak base. Therefore, alkylation of the pyrimidine ring of purine nucleosides or nucleotides should, by analogy, induce ring opening of the pyrimidine ring to afford imidazole nucleosides or nucleo-

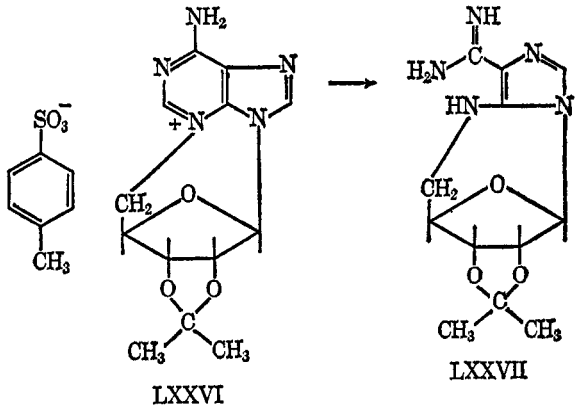
tides. This labilization of the pyrimidine ring is presumably due to the presence (188, 190) of a charged species with the pyrimidine ring possessing a partial or incipient positive charge which would favor potential ring opening by nucleophilic attack. The major objective in the preparation of imidazole nucleosides and nucleotides by degradation of a purine nucleoside or nucleotide is labilization of the normally stable pyrimidine ring whereby ring opening may be accomplished without affecting the carbohydrate moiety.

It is interesting that the first imidazole nucleoside prepared *via* this degradative route occurred by chance, and it is a tribute to these investigators (32) that they possessed the thoroughness and tenacity to pursue the structure elucidation of an unexpected product. A routine ultraviolet absorption spectra of 3,5′-cyclo-6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)purine-2′,3′-carbonate (LXXII) revealed an instability when exposed to a pH 14 solution for 5 min or longer. Treatment of LXXII with barium hydroxide

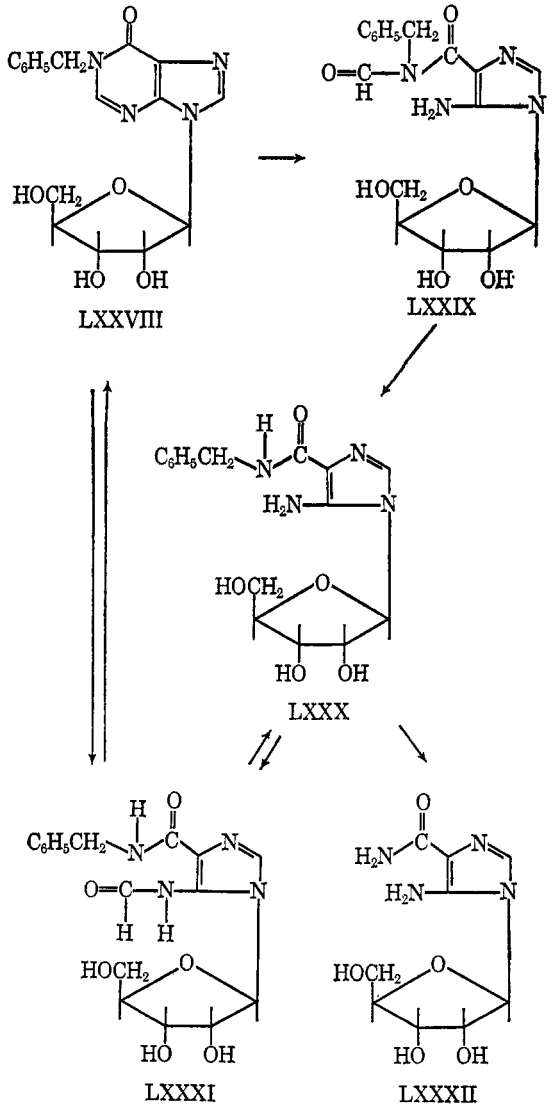


LXXII LXXIII LXXIV LXXV

produced an imidazole nucleoside which could have possessed either of two structures, LXXIII or LXXIV, depending on the site of ring cleavage (1,2 or 1,6 bond). Subsequent hydrolysis of this nucleoside produced 5′,N⁵-cyclo-1-(2′,3′-carbonyl-3′-amino-3′-deoxy-β-D-ribofuranosyl)-5-aminoimidazole-4-carboxamide (LXXV). This indirectly established the initial degradation product as 5′,N⁵-cyclo-1-(2′,3′-carbonyl-3′-amino-3′-deoxy-β-D-ribofuranosyl)-5-formamidoimidazole-4-(N,N-dimethyl)carboxamidine (LXXIII) since hydrolysis of LXXIV could not produce LXXV. Further corroboration for the structure assignment of LXXV was obtained from ultraviolet and infrared



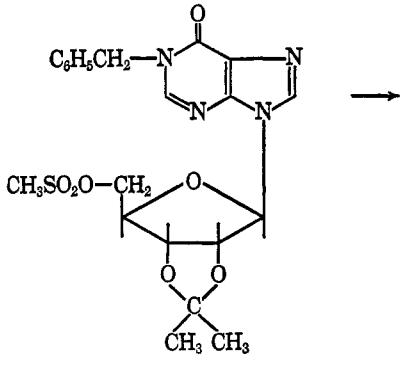
LXXVI LXXVII



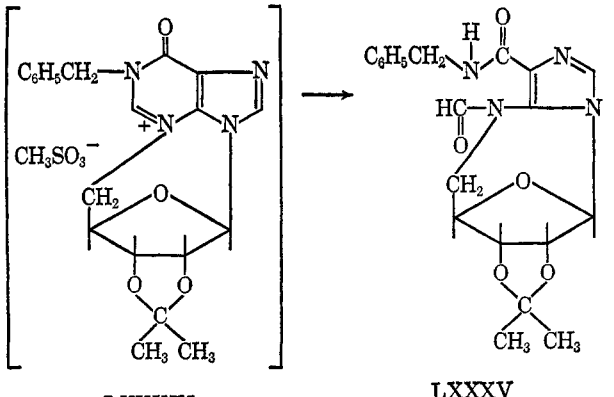
LXXVIII LXXIX LXXX LXXXI LXXXII

spectral data. A very similar reaction was observed (126) when basic hydrolysis of 3,5′-cyclo-6-amino-9-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)purine (LXXVI) produced the imidazole nucleoside LXXVII. This ring opening was successful primarily because of intramolecular alkylation which labilized the pyrimidine ring.

A subsequent series of experiments were designed specifically to labilize the pyrimidine ring by direct alkylation. Treatment of inosine with benzyl chloride in N,N-dimethylformamide produced (161) 1-benzylinosine (LXXVIII) which was found to be very susceptible to ring opening. Ring opening of LXXVIII under basic conditions was conveniently followed using the Bratton–Marshall (43) test. Ring opening produced initially either LXXIX or LXXXI which under the reaction conditions was readily converted *in situ* to 5-amino-4-(N-benzylcarboxamido)-1-(β-D-ribofuranosyl)imidazole (LXXX). A refluxing mixture of acetic anhydride and formic acid converted LXXX to LXXXI which on subsequent treatment with alkali produced 1-benzylinosine (LXXVIII). Debenzylation of LXXX was accomplished with sodium in liquid ammonia at −60° after it was found that debenzylation with palladium and other similar catalysts resulted in a fission of the glycosidic bond. However, this preparation of 5-amino-4-carboxamido-1-(β-D-ribofuranosyl)imidazole (LXXXII) was found to proceed with the concomitant formation of other products, presumably dihydroimidazole derivatives.



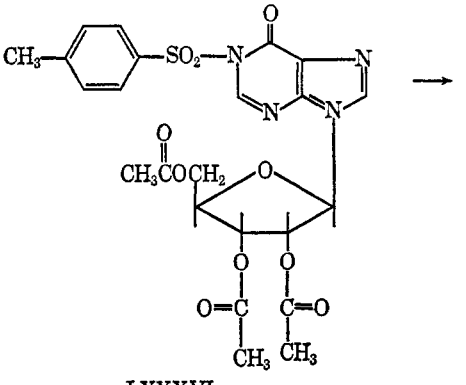
LXXXIII



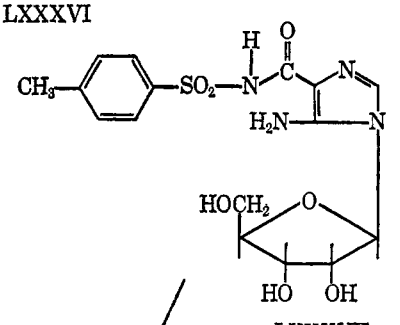
LXXXIV LXXXV

A recent attempt (132) to effect a nucleophilic displacement of the 5′-mesyloxy group of 1-benzyl-9-(2′,3′-*O*-isopropylidene-5′-*O*-mesyl-β-D-ribofuranosyl)-6-purinone (LXXXIII) resulted in the formation of the anhydronucleoside of 4-(N-benzylcarboxamido)-5-formamido-1-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)imidazole (LXXXV), presumably *via* the anhydronucleoside LXXXIV.

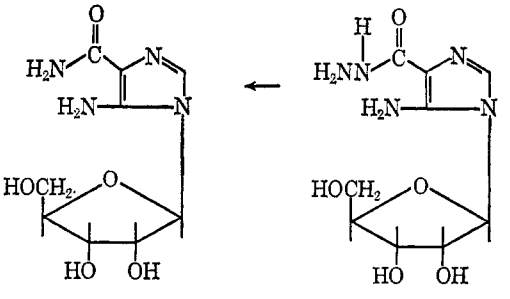
The difficulty experienced in debenzylation of LXXX prompted a search (162) for a labilizing group which could be more readily removed. Alkylation of the sodium salt of 9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-6-purinone with *p*-toluenesulfonyl chloride produced 1-(*p*-toluenesulfonyl)-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-6-purinone (LXXXVI). Exposure of LXXXVI to basic conditions resulted in a facile ring opening of the pyrimidine ring to afford 5-amino-4-[N-(*p*-toluenesulfonyl)carboxamido]-1-(β-D-ribofuranosyl)imidazole (LXXXVII). Attempts to remove the labilizing group from LXXXVII to pro-



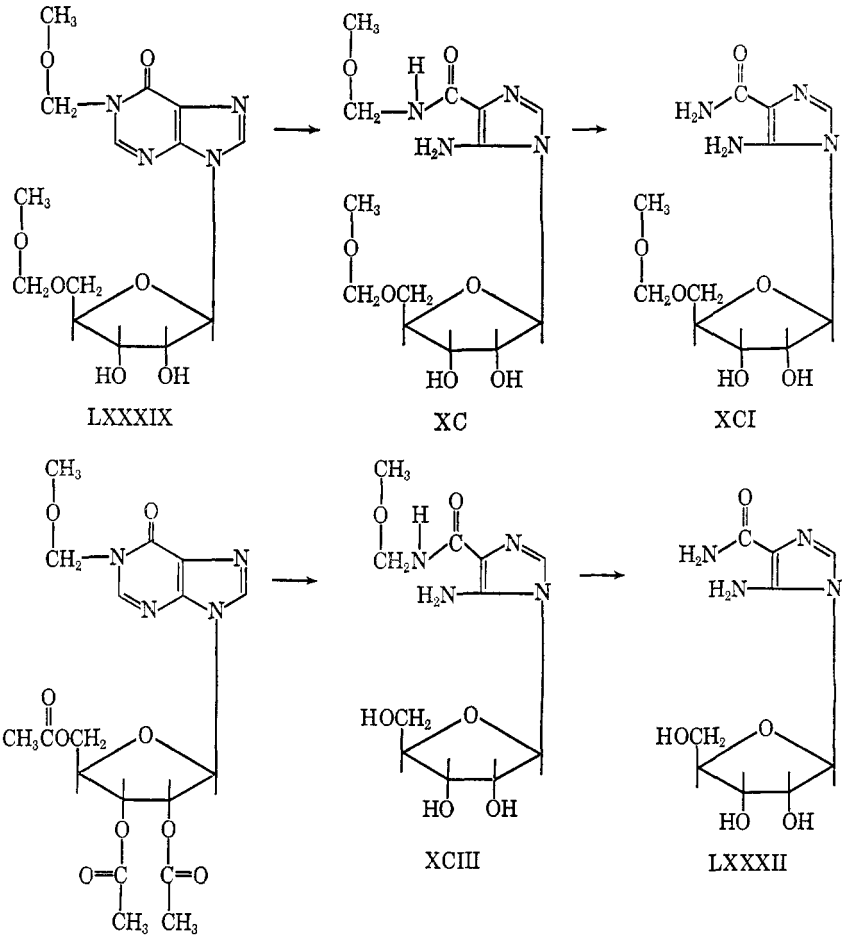
LXXXVI



LXXXVII



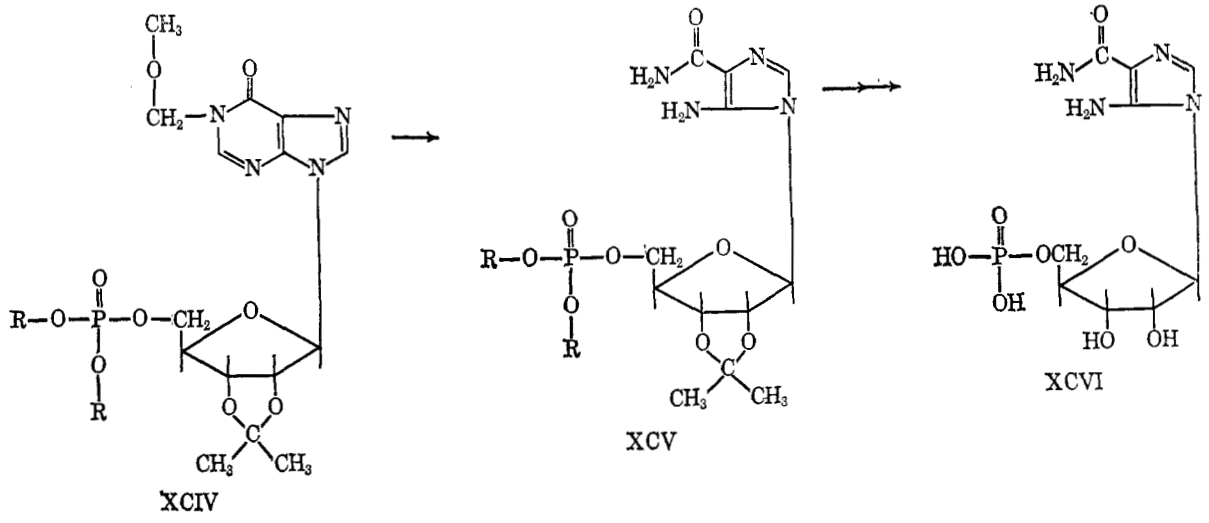
LXXXII LXXXVIII



LXXXIX XC XCI

XCII XCIII LXXXII

duce LXXXII was accomplished only at elevated temperatures with concentrated ammonium hydroxide and was accompanied by considerable decomposition. Removal of the sulfonamide group was achieved with hydrazine to furnish the hydrazide (LXXXVIII) which was smoothly reduced to LXXXII with Raney nickel in ethanol. Preparation of the 5′-phosphate of LXXXII, which is the actual intermediate in the *de novo* pathway of purine biosynthesis, required a new labilizing group. A methoxymethyl group was selected (163) as the most logical candidate, and it was assumed that alkylation of a nucleoside with subsequent ring opening, following reaction conditions previously reported, would successfully demonstrate the ability of the methoxymethyl group to serve as a labilizing group for the preparation of imidazole nucleotides. Treatment of inosine with chloromethyl methyl ether produced a dialkylated compound, presumably LXXXIX, instead of a monoalkylated compound. Alkaline hydrolysis of LXXXIX produced XC only as a transient compound, detected by paper chromatography, which was rapidly hydrolyzed to 5-amino-4-carboxamido-1-(5′-methoxymethyl-β-D-ribofuranosyl)-imidazole (XCI). This hydrolysis of the methoxymethyl group was interpreted as depending on the removal of a proton from the amide nitrogen to form an anion which was followed by elimination of a methoxide group and subsequent formation of formaldehyde and XCI. The 5′-methoxymethyl group was found to be stable under alkaline conditions but very labile when subjected to acid hydrolysis. The use of inosine triacetate, to prevent 5′-alkylation, resulted in the formation of 1-methoxymethyl-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-6-purinone (XCII) which when exposed to basic hydrolysis furnished LXXXII *via* the transitory intermediate XCIII. These same reaction conditions, *i.e.*, alkylation and basic hydrolysis, were used to obtain 5-amino-4-carboxamido-1-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)imidazole 5′-di-(*p*-nitrophenyl)phosphate (XCV). Enzymatic removal of the diester groups with *Crotalus adamanteus* venom and acidic hydrolysis of the isopropylidene group produced a compound which possessed physical properties in complete accord with the naturally occurring 5-amino-4-carboxamido-1-(β-D-ribofuranosyl)imidazole 5′-phosphate (XCVI). Additional corroboration of this structural assignment was provided when the compound was accepted as a substrate by the yeast enzyme adenylosuccinase (129) and was converted to the N-succino derivative of XCVI. It is of interest that XCVI

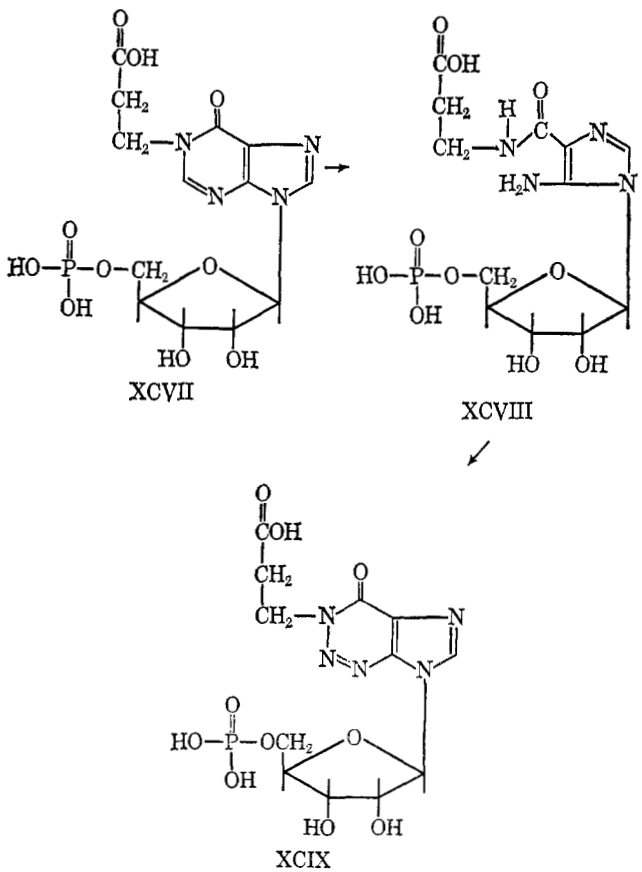


XCIV XCV XCVI

R = *p*-$NO_2C_6H_4$

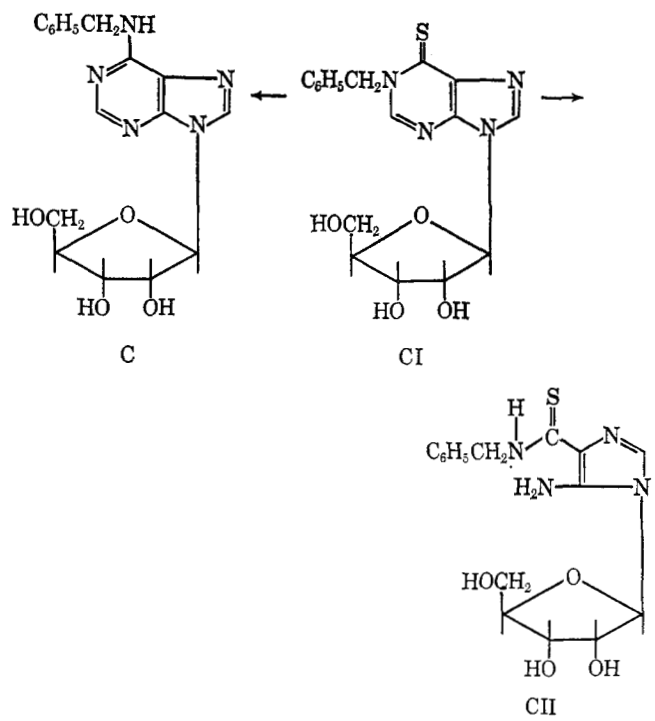
has been prepared (127) by heating inosinic acid with hydrogen chloride, ammonium chloride, and zinc dust and also by enzymatic degradation (64, 67, 193) of inosinic acid.

The alkylation of inosinic acid with β-propiolactone to furnish 1-(2-carboxyethyl)-9-(β-D-ribofuranosyl)-6-purinone 5′-phosphate (XCVII) has been recently reported (36) in connection with an investigation on the specificity of adenylosuccinase. The preparation of 5-amino-4-[N-(2-carboxyethyl)carboxamido]-1-(β-D-ribofuranosyl)imidazole 5′-phosphate (XCVIII) was accomplished smoothly by treating XCVII with boiling aqueous alkali. Cleavage of the 5′-phosphate with alkaline phosphatase furnished the corresponding nucleoside of XCVIII, and diazotization of XCVIII produced the 2-aza derivative (XCIX) of XCVII *via* an intramolecular ring closure. The 5′-sulfate derivatives of all compounds prepared in this investigation (36) were supposedly prepared from inosine 5′-sulfate, but complete details and characterization of products were not presented.



XCVII XCVIII XCIX

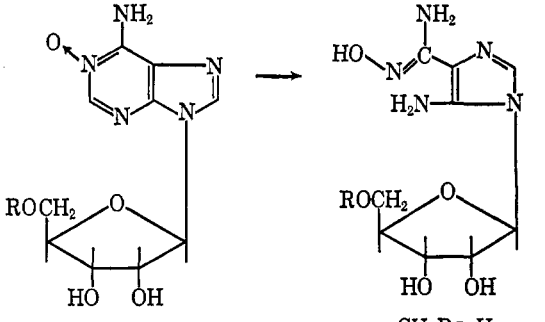
The products obtained from the ring opening of a compound such as 1-benzyl-9-(β-D-ribofuranosyl)-purine-6-thione (CI) may be entirely dependent on the reaction conditions. Treatment of CI with alcoholic



C CI CII

ammonia resulted (134) in 6-benzylamino-9-(β-D-ribofuranosyl)purine (C) *via* a ring opening to produce an imidazole nucleoside intermediate which subsequently reclosed *in situ* to C. However, when dilute sodium hydroxide was used, a facile ring opening without subsequent reclosure occurred and produced 5-amino-4-(N-benzylthiocarboxamido)-1-(β-D-ribofuranosyl)imidazole (CII).

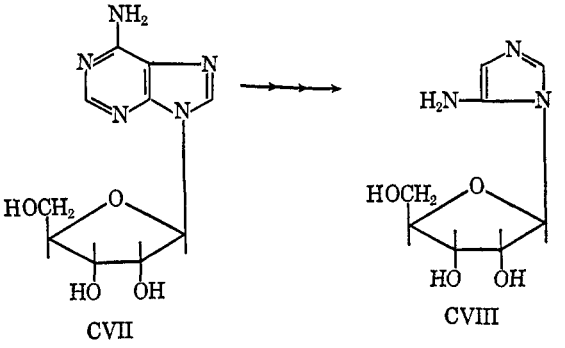
Adenosine 1-N-oxide (CIII) and adenylic acid 1-N-oxide (CIV) are quite labile to both acidic and basic hydrolysis (183). Acidic hydrolysis produced ring opening of the pyrimidine ring but also effected, either simultaneously or most probably prior to ring opening, complete cleavage of the glycosidic bond. However, basic hydrolysis produced ring opening of the pyrimidine ring while leaving the glycosidic bond intact. The products obtained from the basic hydrolysis of CIII and CIV were CV and CVI, respectively, which although not isolated in sufficient quantity for analysis were thoroughly characterized by the Bratton–Marshall (43) and Pauly (140) ferric chloride (84), *cis*-hydroxyl (46), and phosphate (82) tests. The 2′- and 3′-phosphate derivatives of adenosine 1-N-oxide were also exposed to similar hydrolytic conditions and furnished the expected imidazole nucleotide derivatives.



CIII, R = H
CIV, R = PO_3H_2

CV, R = H
CVI, R = PO_3H_2

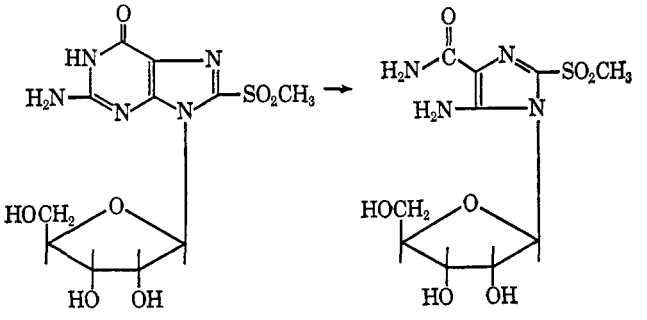
Nonenzymatic cleavage, without prior labilization of the pyrimidine ring, of adenosine (CVII) on treatment with fructose, cupric chloride, and pyrophosphate produced (100) three compounds, one of which (produced in less than 1% yield) was tentatively identified as 5-amino-1-(β-D-ribofuranosyl)imidazole (CVIII) on the basis of ultraviolet absorption spectra, Bratton–Marshall (43) test, modifications of the Pauly test (24, 102), orcinol test, and chromatographic data. The small amount of material isolated precluded further characterization or elemental analysis. Similar reaction conditions, except that pyrophosphate was unessential, on adenosine-5′-tri-phosphate presumably produced the 5′-triphosphate derivative of CVIII which although not completely characterized was assigned this structure by analogy.



CVII

CVIII

Cleavage of the pyrimidine ring of 2-amino-8-methylsulfonyl-9-(β-D-ribofuranosyl)-6-purinone (CIX) with sodium *t*-butoxide in dimethyl sulfoxide has been reported (94) to furnish 5-amino-4-carboxamido-2-methylsulfonyl-1-(β-D-ribofuranosyl)imidazole (CX). The basis for this structural assignment was on ultraviolet absorption spectra, paper chromatographic mobility, and the presence of both sulfur and carbohydrate material. Although in all probability this



CIX

CX

structural assignment is correct, it was proposed as a tentative assignment since the scale of the reaction precluded the isolation and complete characterization of the degradation product.

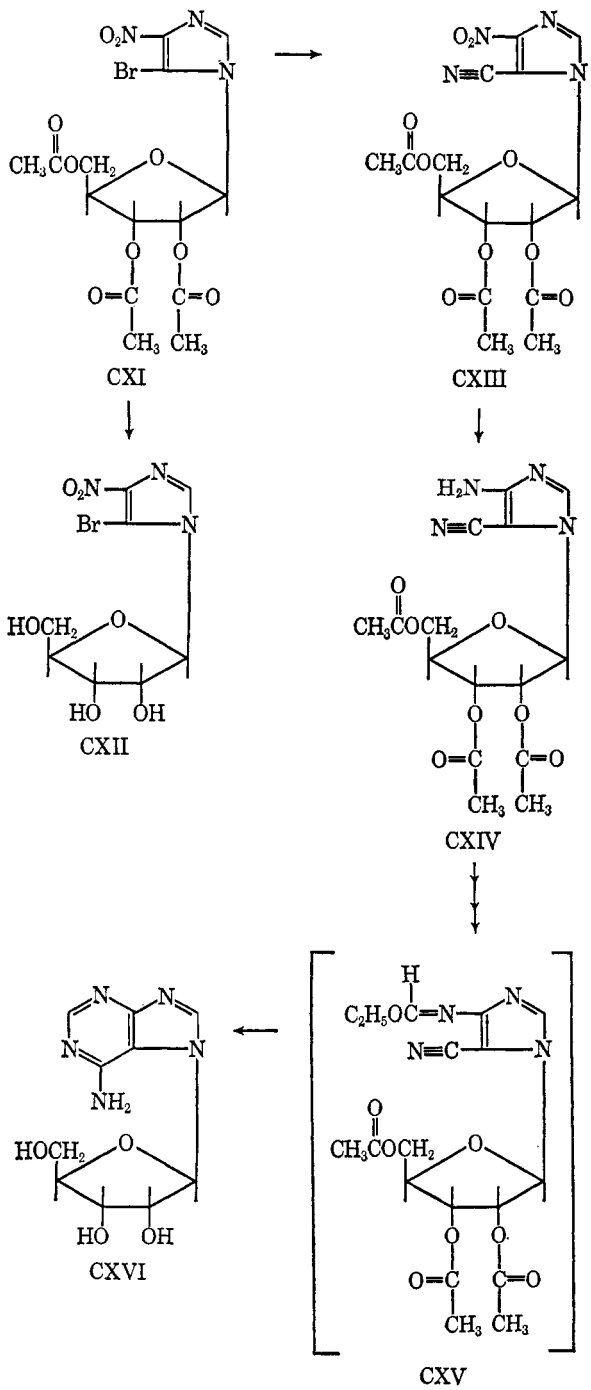
The abundance of purine nucleosides and nucleotides should keep this route for the preparation of imidazole nucleosides and nucleotides very active in the future since it appears that there may be a number of ways to labilize the pyrimidine ring which are yet to be discovered.

D. ACID-CATALYZED FUSION

As with any field, organic synthesis is continually changing as better or more facile procedures are discovered, and the area of imidazole nucleosides and nucleotides is no exception. A recent and perhaps the most significant advance in the field of nucleoside synthesis was the advent of acid-catalyzed fusions. This technique was first used for nucleoside synthesis with purines (156) to produce N-glycosylpurines and has subsequently been applied to other heterocyclic systems, *e.g.*, imidazo[4,5-*c*]pyridine (153).

The first glycosylimidazole prepared by the acid-catalyzed fusion method was recently reported (154) in connection with the synthesis of 7-glycosylpurines.

Fusion of 4(5)-bromo-5(4)-nitroimidazole with tetra-*O*-acetyl-β-D-ribofuranoside produced a good yield of nucleoside material. Theoretically the possibility existed of condensation at either of the two ring nitrogens of the imidazole precursor to produce an isomeric mixture. However, this nucleoside material was subsequently established as being one isomer, 5-bromo-4-nitro-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole (CXI), by a comparison of ultraviolet spectra with model compounds (70). Low-temperature deacetylation of CXI with methanolic ammonia produced CXII without replacement of the bromo group by an amino group. However, a facile displacement of the bromo group was observed when treatment of CXI with potassium cyanide in anhydrous dimethyl sulfoxide furnished 5-cyano-4-nitro-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole (CXIII). This facile displacement corroborated the initial glycosidation assignment since bromine is readily displaced by cyanide only (28, 31) when the N substituent resides adjacent to the bromo group. Reduction of the 4-nitro group with Raney nickel provided a very versatile imidazole nucleoside, 4-amino-5-cyano-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole (CXIV). The site of glycosidation and anomeric configuration of this entire series of imidazole nucleosides were unequivocally established when treatment of CXIV with a mixture of acetic anhydride and ethyl orthoformate followed by methanolic ammonia produced a compound of known (135) structure, 6-amino-7-(β-D-ribofuranosyl)purine (CXVI).

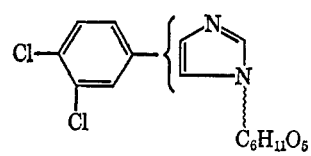


Since the initial finding (191) that a number of reported 7-glycosylpurines were in actuality 3-glycosylpurines, it has been shown (56, 104) that, when a preformed purine is used for direct glycosidation, the N-3 position must be blocked or the secondary site of glycosidation is generally N-3. Use of the directive influence (44, 136) exerted by 3-substituted purines has produced (134, 135) several 7-glycosylpurines even though this approach possesses some serious inherent disadvantages. Therefore, it appears that the most desirable and facile procedure available for the preparation of 7-glycosylpurines is from imidazole nucleosides.

The fusion technique, although still new, possesses the potential (192) to become the most versatile method presented in this review (*vide supra*) for the chemical preparation of imidazole nucleosides.

E. MISCELLANEOUS

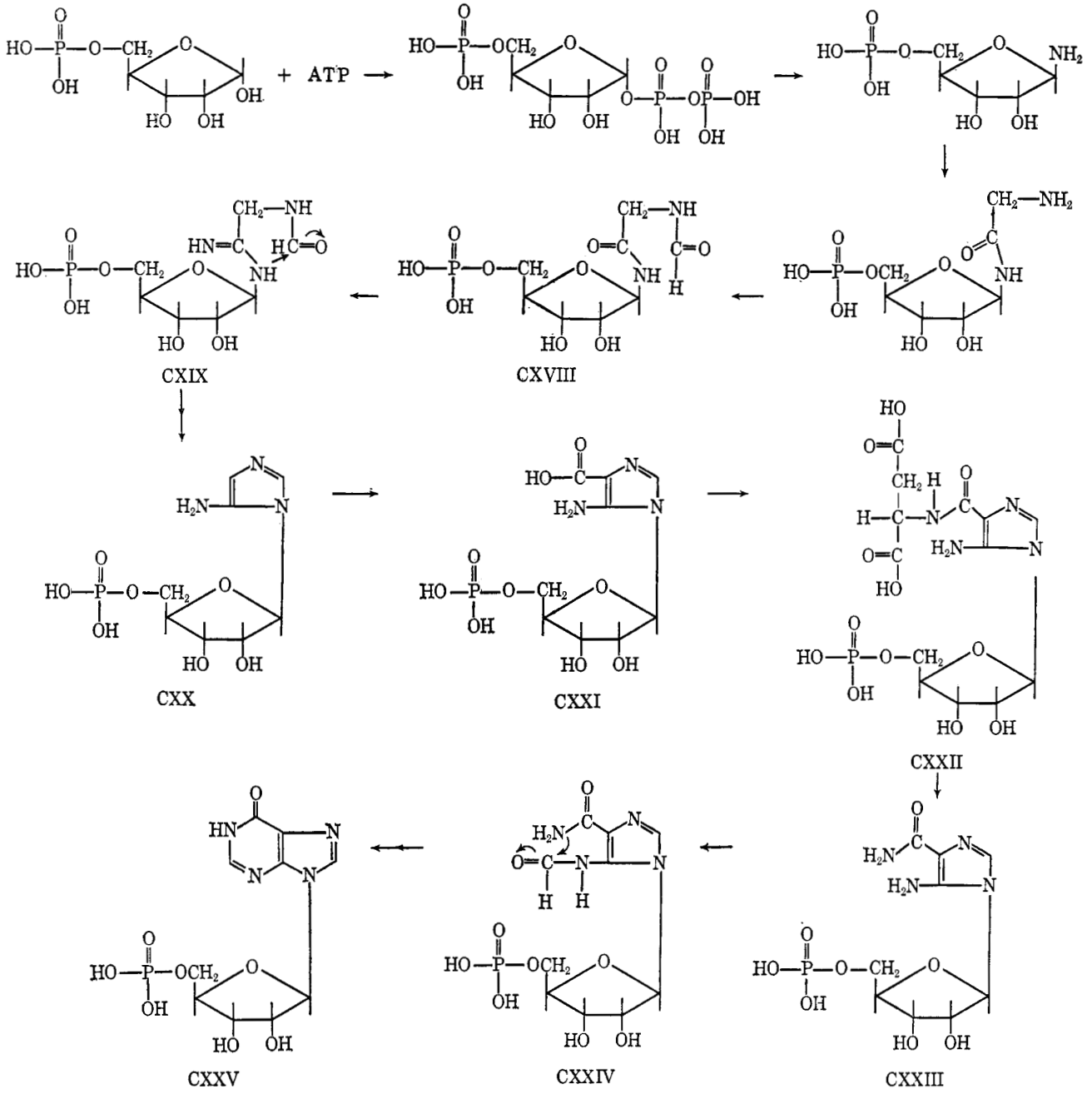
An imidazole nucleoside, 4(5)-(3,4-dichlorophenyl)-1-(D-glycosyl)imidazole (CXVII), of uncertain structure was reported (61) in a recent investigation on the antifungal activity of a series of imidazole derivatives. No attempt was made to establish the anomeric configuration, ring size (furanose or pyranose), or actual site of glycosidation.



III. IMIDAZOLE NUCLEOSIDES AND NUCLEOTIDES INVOLVED IN PURINE NUCLEOTIDE BIOSYNTHESIS

The universal presence of nucleic acids has generated enormous interest in the elucidation of the metabolic

SCHEME II

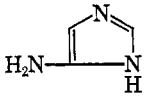


pathways involved in the biosynthesis of these macromolecules. Another interesting facet of this problem was the determination of their composition by degradation. Degradation studies revealed that the common nucleic acids contain purine and pyrimidine nucleotides in a linear polymeric form with two polymeric strands hydrogen bonded together in a helical spiral. The purine and pyrimidine nucleotides are also components of several biologically important coenzymes and the corresponding nucleosides have been covered recently in several comprehensive reviews (68, 133). Interest in the area of imidazole nucleosides and nucleotides is more recent since it has only lately been unequivocally established that imidazole nucleotides are precursors of purine nucleotides in the *de novo* biosynthetic pathway, illustrated in Scheme II.

The nonaromatic compounds will not be discussed in this review but they have been definitely established (71, 87, 88, 107) as intermediates in this biosynthetic pathway.

The enzymatic conversion of CXVIII to 5-amino-1-(β-D-ribofuranosyl)imidazole 5′-phosphate (CXX) *via* the intermediate CXIX provided the first aromatic compound in the *de novo* pathway of purine biosynthesis in avian liver (105, 106), yeast (69, 111, 113, 114, 131), and *Escherichia coli* (113). This compound was iso-

lated as the corresponding riboside from a purine requiring mutant of *E. coli* (113, 114), yeast (52, 53), and a culture filtrate (197) of *Saccharomyces cerevisia* strain (47) which were deficient in the coenzyme biotin. The aglycon, 5-aminoimidazole, has been reported (144, 145) to be involved in the catabolism of purines by microorganisms and was characterized by a comparison with CXXVI prepared (93) chemically from 5-nitroimidazole. It has been established (49) that inhibition of purine nucleotide biosynthesis in yeast by sonifica-



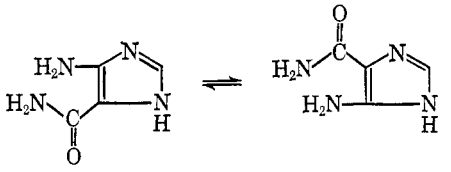
CXXVI

tion is due to the inhibition of 5-amino-1-(β-D-ribofuranosyl)imidazole 5′-phosphate (CXX) formation.

The fixation of CO_2 to CXX by a carboxylase enzyme to produce (121) 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxylic acid 5′-phosphate (CXXI) was postulated to be biotin dependent, and this postulation received considerable support when CXX was found to accumulate in several systems deficient in biotin (52, 53, 197). However, it has been recently established (4) that this CO_2 fixation is not biotin dependent but is most likely an enzyme-mediated reaction. The enzymatic formation of CXXI from CXX is a reversible reaction, and a recent investigation (110) demonstrated that decarboxylation of CXXI to produce CXX can be inhibited in the presence of nickel and other transition metal ions. It was also found that CXXI is decarboxylated much faster than the corresponding nucleoside which prompted the supposition that this enchanced rate of decarboxylation is an intramolecular reaction involving the 5′-phosphate group. It has been reported (195) that aminopterin inhibition of *Aerobacter aerogenes* creates an accumulation of CXXI.

It was first postulated that (118, 128) N-[5-amino-1-(β-D-ribofuranosyl)-4-imidazolecarbonyl]-L-aspartic acid 5′-phosphate (CXXII) occurred only as an intermediate in the *de novo* pathway in extracts of avian liver. However, the enzymatic preparation of CXXII was subsequently reported (120) to occur from CXX, ATP, and CO_2 *via* CXXI. Treatment of 5-amino-4-carboxamido-1-(β-D-ribofuranosyl)imidazole 5′-phosphate (CXXIII) with excess fumarate in the presence of adenylosuccinase is also a very convenient method for the enzymatic preparation (129) of CXXII. A similar method has been used (92) for the preparation of CXXII in preparative quantities from the riboside of CXXIII by phosphorylation of the riboside with actively fermenting yeast followed by treatment with excess fumarate. The accumulation of CXXII has been observed in culture broths of adenine requiring mutants of *E. coli* (73) and *Neurospora crassa* (42).

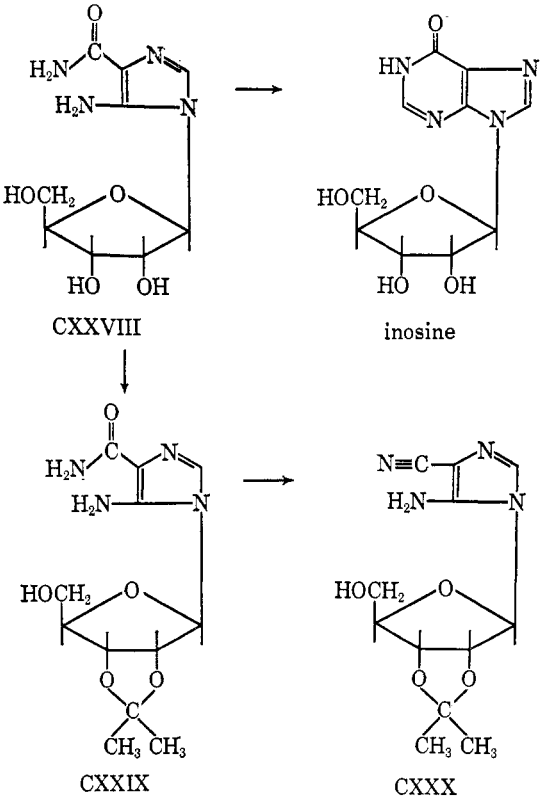
Treatment of *E. coli* cultures with sulfonamides produced a compound which was isolated (182) and subsequently characterized (175) as 4(5)-amino-5(4)-imidazolecarboxamide (CXXVII). This was one of the first indications that imidazole nucleosides or



CXXVII

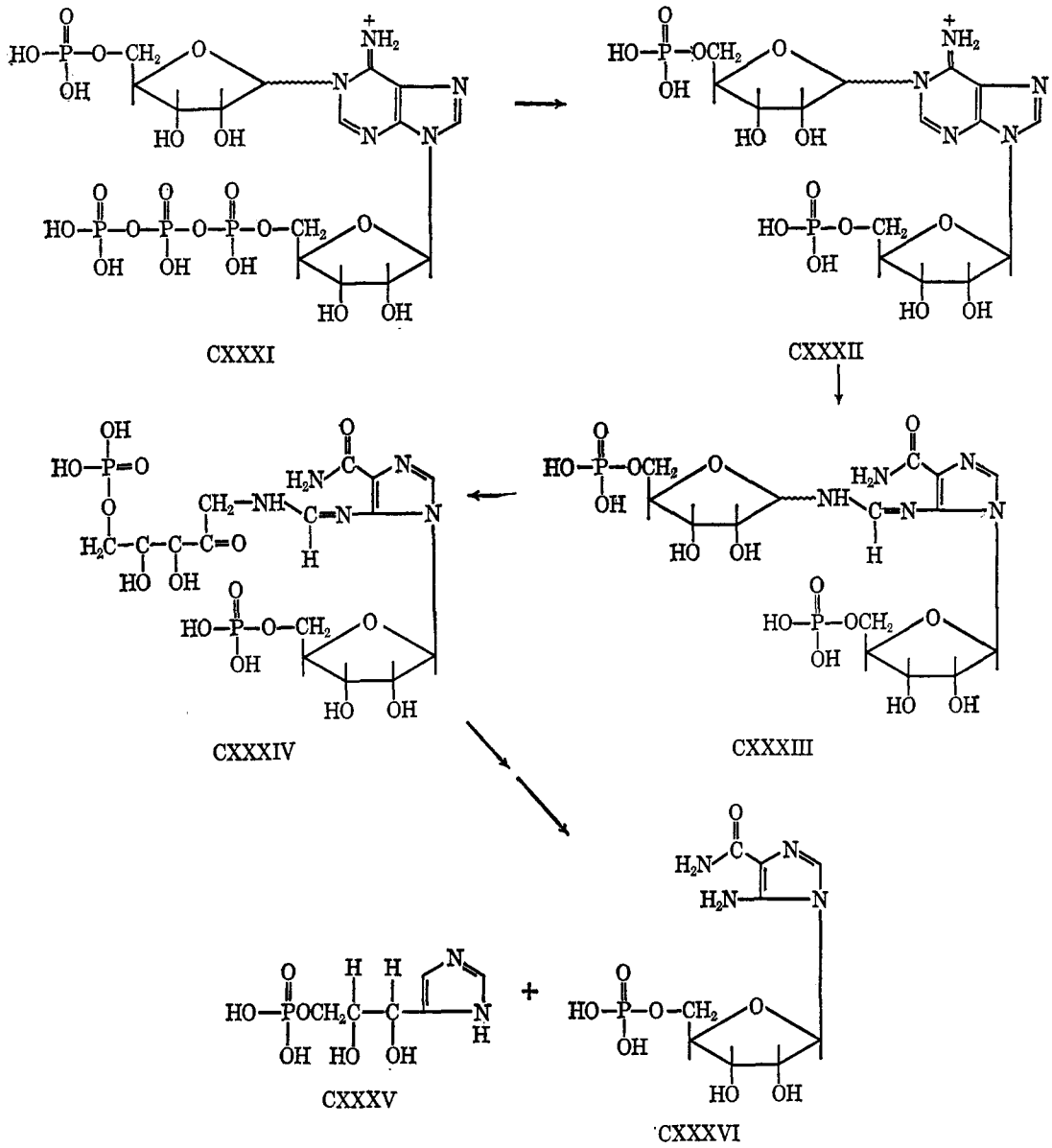
nucleotides were possible intermediates in the *de novo* biosynthetic formation of purine nucleotides since CXXVII can be chemically converted to a number of purines. These inhibition studies eventually led to the isolation of not only CXXVII but also the corresponding riboside (72, 76, 78, 194), 5-amino-4-carboxamido-1-(β-D-ribofuranosyl)imidazole (CXXVIII). This structural assignment was established unequivocally by ring closure of CXXVIII to 9-(β-D-ribofuranosyl)-6-purinone (inosine).

The enzymatic formation of CXXVIII has been subsequently observed (5, 6, 103, 152, 173, 174) in a number of systems and in some instances in sufficient quantity to be considered of preparative value. This ready availability of CXXVIII has prompted a few simple chemical transformations (7, 8); *e.g.*, formation of the 2′,3′-*O*-isopropylidene derivative CXXIX has been followed by dehydration to furnish CXXX. It has been demonstrated (81, 116, 176, 186) that the addi-



CXXVIII inosine

CXXIX CXXX



CXXXI CXXXII CXXXIII CXXXIV CXXXV CXXXVI

tion of CXXVIII to a number of enzymatic systems greatly enhances the rate of nucleic acid synthesis and therefore presumably serves as a substrate for a kinase prior to incorporation. The postulation (48, 64, 75, 76, 159) that the 5′-phosphate derivative of CXXVIII was the actual intermediate in the biosynthesis of inosinic acid (CXXV) prompted (77) the enzymatic preparation of 5-amino-4-carboxamido-1-(β-D-ribofuranosyl)imidazole 5′-phosphate (CXXIII). This central intermediate in the *de novo* pathway has been prepared enzymatically by a number of investigators (65, 92, 122, 129, 130) and shown (74, 112, 187) to accumulate by inhibition of several enzyme systems.

The enzymatic formation of inosinic acid (CXXV) from CXXIII proceeds *via* the intermediate 5-formamido-4-carboxamido-1-(β-D-ribofuranosyl)imidazole 5′-phosphate (CXXIV). The enzyme 5-amino-4-carboxamido-1-(β-D-ribofuranosyl)imidazole 5′-phosphate transformylase catalyzes the formylation of CXXIII and N^{10}-formyltetrahydrofolic acid has been established (67, 86) as the formyl donor. The formation of inosinic acid (CXXV) from CXXIII has also been demonstrated to occur enzymatically (inosinicase) in rabbit erythrocytes (115) and human erythrocytes (117) *in vitro* as well as in several other enzymatic systems (66, 160). While this is a comprehensive presentation of the role imidazole nucleosides and nucleotides occupy in the *de novo* pathway, it is definitely not a *complete* survey or review of this area.

IV. Other Naturally Occurring Imidazole Nucleosides and Nucleotides

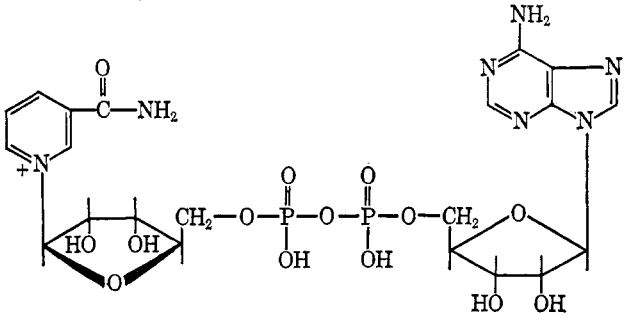
A. Histidine Biosynthesis

The present biosynthetic pathway of histidine had its inception when a bacterial extract was incubated

with adenosine triphosphate, ribose 5-phosphate, and glutamine to produce (137, 172) CXXXVI and D-*erythro*-imidazole glycerol phosphate ester (CXXXV). It was reported (101) almost simultaneously that this conversion was more than a one-step reaction which immediately prompted other investigations for the isolation and characterization of the intermediates. The initial intermediate was subsequently isolated and shown (23, 124) to be CXXXI which can be visualized as a simple condensation of adenosine triphosphate and PRPP. The isolation and characterization of CXXXII as the second intermediate in this biosynthetic pathway has only recently been reported (178). The triphosphate derivative of CXXXIII was isolated, characterized, and originally postulated (177) to be a precursor of CXXXIII; however, it now appears to occur only as a by-product with CXXXIII being the actual intermediate. A presumed Amadori rearrangement of CXXXIII provides (177) CXXXIV which in the presence of glutamine or ammonia has furnished the end products CXXV and CXXXVI. There is at least one other imidazole nucleotide intermediate between CXXXIV and CXXXVI which has at the present time evaded isolation and characterization. It is of considerable interest that of all the imidazole nucleotide intermediates between adenosine triphosphate and CXXXVI which have been isolated and characterized none have been synthetically (chemically) prepared.

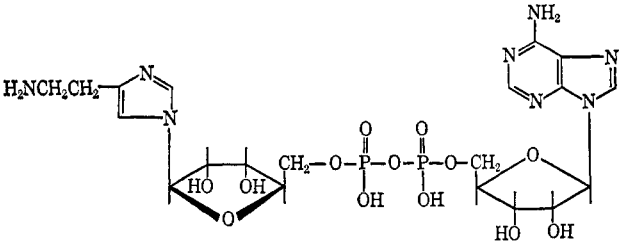
B. SUBSTITUTED IMIDAZOLE–ADENINE DINUCLEOSIDE PYROPHOSPHATES (COENZYME ANALOGS)

The enzymatic hydrolysis (irreversible) of the nicotinamide–ribose bond present in nicotinamide–adenine dinucleotide pyrophosphate (CXXXVII, coenzyme I, NAD) by water has been well-documented. This enzymatic hydrolysis has been postulated to be a direct nucleophilic displacement of the nicotinamide moiety. On this *a priori* principle, certain investigations were performed, and it was demonstrated that certain imidazoles can successfully compete with water in this enzymatic displacement of nicotinamide *in vitro* with proper maintenance of pH. The enzymatic



CXXXVII

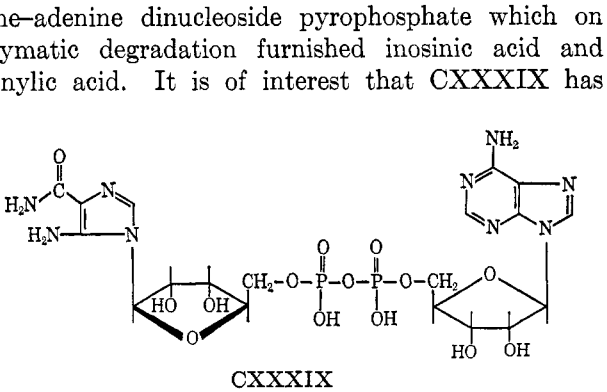
replacement (irreversible) of nicotinamide in CXXXVII by histamine has been accomplished (1, 9–12, 18) *in vitro* in the presence of enzymes (NADases) from such varied sources as beef spleen, guinea pig lungs, and bull semen. The structure proposed for this enzymatic interaction is histamine–adenine dinucleoside pyrophosphate (CXXXVIII, HAD). The site of attachment of ribose to histamine in HAD was determined (14) to be at a ring nitrogen on the basis of its remarkable stability toward acidic hydrolysis. However,



CXXXVIII

the actual site of glycosidic attachment to histamine (which ring nitrogen) or anomeric configuration (α or β) has not been unequivocally established. The formation of HAD *in vivo* has been reported (12) to occur only in trace amounts, although this may possibly be the result of a rapid *in vivo* degradation of HAD analogous to the already established (18) *in vitro* pathway. This would suggest that any of the naturally occurring imidazoles should possess the ability to replace histamine in this exchange reaction. However, it has been shown (15), *e.g.*, that L-histidine, the *in vivo* precursor of histamine, and imidazole-4(5)-acetic acid, one of the catabolic products from histamine, are both unsuitable replacements for histamine.

The ability of 5(4)-amino-4(5)-carboxamidoimidazole to function as a substrate for this enzymatic reaction was demonstrated (20, 21) by the formation of (5-amino-4-carboxamidoimidazole)adenine dinucleoside pyrophosphate (CXXXIX, IAD). The site of glycosidic attachment and anomeric configuration were both unequivocally established when treatment of CXXXIX with certain liver extracts produced (16, 17) hypoxanthine–adenine dinucleoside pyrophosphate which on enzymatic degradation furnished inosinic acid and adenylic acid. It is of interest that CXXXIX has



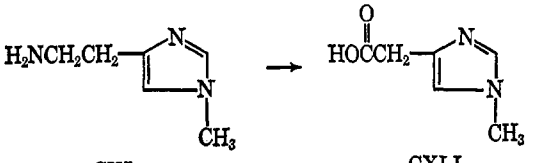
CXXXIX

demonstrated (109) the ability to be incorporated into both RNA and DNA of ascites cells and into RNA of liver cells. This incorporation was demonstrated

using the radioactivity from (5-amino-4-carboxamido-2-^{14}C-imidazole)adenine dinucleoside pyrophosphate which had been injected interperitoneally in tumor-bearing mice.

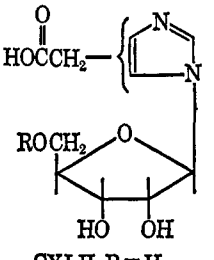
C. HISTAMINE BIOSYNTHESIS AND METABOLISM

The metabolism of histamine has been demonstrated (157) to occur *via* two completely different pathways. One pathway proceeds by enzymatic methylation of histamine with histamine N-methyltransferase (45) to produce 1-methyl-4-(β-aminoethyl)imidazole (CXL) which is subsequently converted to 1-methylimidazole-4-acetic acid (CXLI). The other metabolic pathway is initiated by the formation of imidazole-4(5)-acetic



CXL CXLI

acid followed by the formation of 1-(β-D-ribofuranosyl)-imidazole-4(5)-acetic acid (CXLII) presumably *via* the corresponding ribotide (CXLIII). The enzymatic condensation of imidazole-4(5)-acetic acid with PRPP to produce CXLIII has been accomplished (54, 55, 62, 63) and would tend to support the assumption that CXLII is formed by dephosphorylation of CXLIII with a phosphatase. Investigations which elucidated the latter pathway were initiated by the isolation and characterization (89, 98, 99, 158, 184) of CXLII from the urine of rats, mice, and men after an interperitoneal or intradermal injection of histamine. Both CXLII and CXLIII have been found to occur not only in the urine but also in various tissues (150, 151, 180, 181) after administration of histamine. In patients suf-

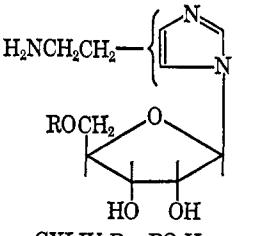


CXLII, R = H
CXLIII, R = PO_3H

fering from gout the level of CXLIII in the urine has been found (198) to increase as the level of uric acid increases.

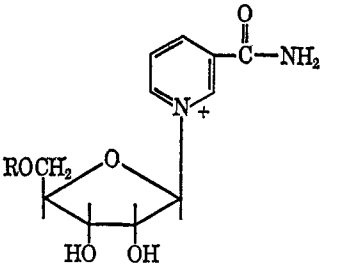
The chemical preparation of CXLII has been achieved (29, 34, 35); however, the actual site of glycosidation is as yet undetermined even though most investigators tend to assign the structure 1-(β-D-ribofuranosyl)imidazole-4-acetic acid to the naturally occurring material.

This still leaves unanswered the question of the origin of histamine riboside or ribotide since the only glycoside which has been isolated and characterized as a product from histamine catabolism is 1-(β-D-ribofuranosyl)imidazole-4(5)-acetic acid or the corresponding ribotide. The probability of a histamine glycoside being formed initially followed by a conversion to the glycoside of imidazole-4(5)-acetic acid has been eliminated. The enzymatic condensation of histamine with PRPP to produce histamine ribotide has provided inconsistent results since some investigators (13) report no condensation while other investigators (54, 62) have enjoyed limited success. It has been recently stated that CXLV originates from HAD. Cleavage of the pyrophosphate linkage of HAD with a phosphodiesterase from snake venom has produced (138) adenylic acid and 4(5)-(β-aminoethyl)-1-(β-D-ribofuranosyl)imidazole 5′-phosphate (CXLIV, histamine ribotide). Subsequent treatment of CXLIV with human prostatic acid phosphatase resulted in the formation (139) of histamine ribonucleoside (CLXV). The enzymatic preparation of CXLIV and CXLV



CXLIV, R = PO_3H
CXLV, R = H

has also been reported (2) to occur by the direct interaction of histamine with CXLVI and CXLVII, respectively. Continued interest in this area is apparent from the recent report (19) that CXLV and HAD are probably either incorporated or attached to



CXLVI, R = PO_3H
CXLVII, R = H

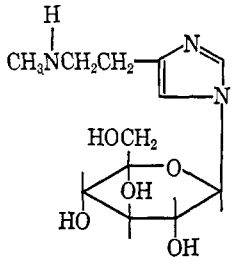
soluble proteins *in vivo* and that their biological function in this respect is still obscure.

D. ALKALOIDS

The term alkaloid, according to Websters' dictionary, is defined as "an organic substance of alkaline properties especially one occurring naturally in plants and animals; an organic base, specifically, a plant base" and can be

seen to encompass an enormous area. One segment of this area is composed (33) of a number of imidazoles and related derivatives. The imidazole nucleosides occupy a salient position in this area simply because of their paucity and in most reviews their apparent nonexistence.

The fruit (*Zapote blanco*) of the tree *Casimiroa edulis*, which is found throughout Mexico and Central America, as the name implies is edible and is used extensively for this purpose. However, it is general knowledge that the seeds possess hypnotic, sedative, and hypotensive properties and can be extremely harmful if ingested. A crude crystalline substance termed alkaloidal was isolated from the seeds of *Casimiroa edulis* and published in a thesis of Jose Sanchez in 1893. However, a later and more refined investigation reported (142) the isolation of a number of constituents from the seeds of *Casimiroa edulis*. One of these was accorded the empirical formula of $C_{17}H_{24}O_5N_2$ and given the name casimiroedine. A reinvestigation (60) of this constituent revealed an error in the previously assigned empirical formula, and the correct empirical formula of $C_{12}H_{27}N_3O_6$ was confirmed by another laboratory (3). Hydrogenation of casimiroedine in the presence of 10% palladized-charcoal catalyst produced dihydrocasimiroedine which indicated the presence of one reducible double bond. The acidic and basic hydrolysis of casimiroedine produced cinnamic acid and a new base (casimidine, $C_{12}H_{21}N_3O_5$). Degradation studies (59) on casimidine firmly established the presence of N-methylhistamine which left one unidentified fragment ($C_6H_{10}O_5$). Previous chemical studies suggested that the remaining fragment was carbohydrate in nature. The chemical elucidation of this fragment as D-glucose still left unsolved the assignment of anomeric configuration and site of glycosidation and whether it existed as the furanoside or pyranoside. The total structure of casimidine was unequivocally established (147, 148) by X-ray diffraction studies as CXLVIII. Therefore, this established the total structure of casimiroedine as either

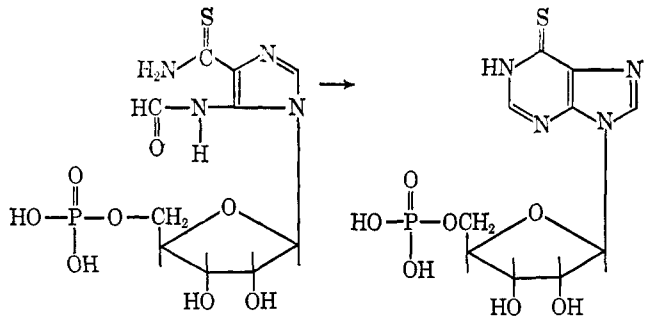


CXLVIII

the *cis*- or *trans*-cinnamic acid amide of CXLVIII. Although at the present time this appears to be the only imidazole nucleoside in the area of alkaloids, it is of interest that N,N-dimethylhistamine has also been isolated from *Casimiroa edulis*.

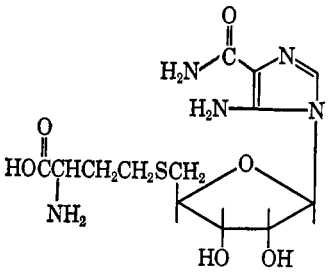
E. MISCELLANEOUS

In a study (25) on the synthesis of 6-mercaptopurine–nicotinamide dinucleoside pyrophosphate, an unknown by-product was formed during the preparation of one of the precursors. This by-product has been tentatively assigned the structure 4-thiocarboxamido-5-formylamino-1-(β-D-ribofuranosyl)imidazole 5′-phosphate (CXLIX) since a 5′-nucleotidase hydrolyzed CXLIX to the corresponding nucleoside and CXLIX was converted smoothly to 6-mercapto-9-(β-D-ribofuranosyl)purine 5′-phosphate (CL) with dilute base.



CXLIX CL

Homocystinurics possess a deficiency of the enzyme cystathionine synthase, and this deficiency creates an increase in the concentration of methionine and homocystine in the blood. Since the major degradative pathway of methionine metabolism is blocked by this deficiency, it was very interesting that only a small portion of the daily dietary intake of methionine could be accounted for by urinary excretion. A recent study (141) of this problem has resulted in the isolation of a new sulfur-containing compound from the urine of homocystinurics. Mild acid hydrolysis of this compound produced homocystine, 5(4)-amino-4(5)-carboxamidoimidazole, and another compound which was found to be identical with S-ribosylhomocysteine obtained from the acid hydrolysis of S-adenosylhomocysteine. The ultraviolet absorption spectrum of the urinary compound was similar to the ultraviolet spectrum of 5-amino-4-carboxamido-1-(β-D-ribofuranosyl)-imidazole, and the presence of phosphate was eliminated by a negative ammonium molybdate test. This compound was tentatively assigned the structure, 5-amino-4-carboxamido-1-(5′-deoxy-5′-S-homocysteinyl-β-D-ribofuranosyl)imidazole (CLI, AICHR), although no unequivocal justification for the anomeric assignment as β or the D configuration of ribose was given. Two plausible routes have been proposed for the metabolic origin of CLI: the enzymatic condensation of homocysteine with 5-amino-4-carboxamido-1-(β-D-ribofuranosyl)imidazole analogous to the enzymatic condensation of homocysteine with adenosine to produce (58) S-adenosylhomocysteine being one possibility and the other possibility being an enzymatic degradation



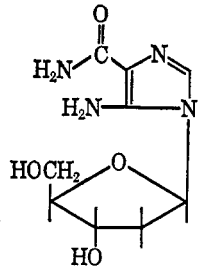
CLI

of S-adenosylhomocysteine. Elucidation of this biosynthetic pathway will provide another fertile field of study for biochemists while the synthetic preparation of CLI should prove to be of considerable interest to the synthetic organic chemist.

V. 2′-DEOXYRIBOFURANOSYLIMIDAZOLES

The synthetic preparation of purine and pyrimidine 2′-deoxyribosides has recently been one of the most active areas of nucleoside synthesis. Therefore, it was rather surprising to find that the organic chemist has never synthetically (chemically) prepared any imidazole 2′-deoxynucleosides or nucleotides.

The only imidazole 2′-deoxyglycoside ever reported (97, 123) was prepared enzymatically using a *trans*-N-glycosidase. Incubation of 5(4)-amino-4(5)-carboxamidoimidazole with thymidine in the presence of a dialyzed enzyme preparation from *Lactobacillus helveticus* has "presumably" produced a 5(4)-amino-4(5)-carboxamidoimidazole 2′-deoxyriboside. Although not assigned, it would be assumed to possess the structure 5-amino-4-carboxamido-1-(2′-deoxy-β-D-ribofuranosyl)imidazole (CLII) since most N-glycosyl transferase reactions maintain the original anomeric configuration. This same 2′-deoxyriboside has also been presumably (40) formed in a cell suspension of *E. coli*



CLII

in a phosphate buffer. A very interesting supposition is that CLII might serve as a substrate in the *de novo* purine biosynthetic pathway to produce purine 2′-deoxyribotides rather than the normal purine ribotides.

VI. TABLES

Table I lists the ultraviolet spectra at different pH values and maximum absorption for a few representative imidazole nucleosides and nucleotides.

Table II contains various physical properties and the methods of preparation for a number of imidazole nucleosides and nucleotides. Tables I and II are both alphabetically arranged by the substituents on the imidazole moiety.

TABLE I

ULTRAVIOLET ABSORPTION SPECTRA OF CERTAIN IMIDAZOLE NUCLEOSIDES AND NUCLEOTIDES

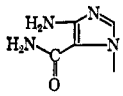
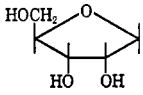
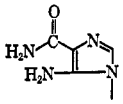
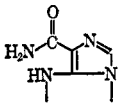
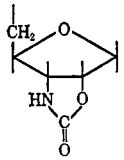
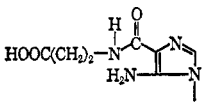
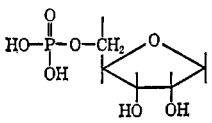
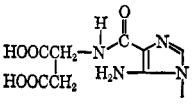
Imidazole moiety	Glycosyl moiety	λ_{max}, mμ (pH) Acidic	Neutral	Basic	Ref
		266 (<1) 241	273 (7)	274 (>10)	28
	As above	267 (<1) 247	266 (7)	267 (>10)	28
		259 (1)	271 (H_2O)	272 (14)	32
		267 (0.1 *N* HCl) 246	268 (7)	268 (0.1 *N* NaOH)	36
	As above	269–270 (1) 244	269 (7)		170

TABLE I (*Continued*)

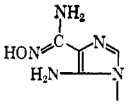
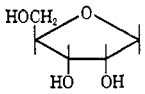
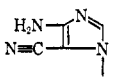
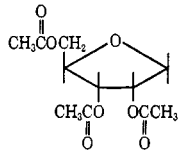
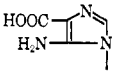
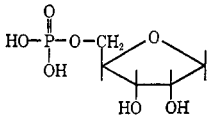
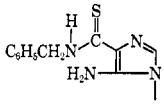
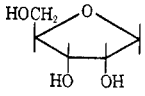
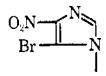
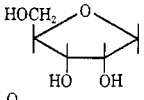
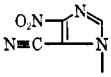
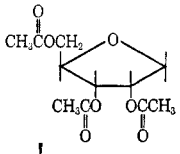
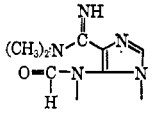
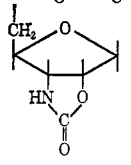
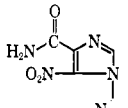
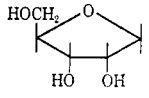
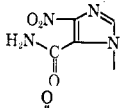
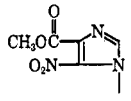
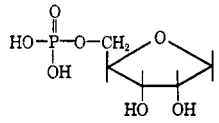
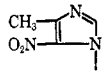
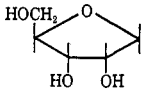
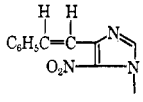
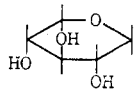
Imidazole moiety	Glycosyl moiety	λ_{max}, mμ (pH) Acidic	Neutral	Basic	Ref
		257 (3) 210		260 (13) 228	183
		264 (1) 233		263 (11) 230	155
		264 (0.25 *N* H_2SO_4) 243	249 (8.2)	249 (12)	121
		321 (1) 286	327 (7) 275	327 (13) 274	134
		307 (1)		307 (11) 236	155
		292 (1)		292 (11) 238	155
		278 (1)	288 (H_2O)	235 (14)	32
		304 (<1)	301 (7)	301 (>10)	28
	As above	295 (<1)	294 (7)	295.5 (>10)	28
		268 (0.1 *N* HCl) 245–253 (sh)	271 (H_2O)	271 (9)	171
		303 (1)	304 (7)	309 (10)	95
		372 (<1) 270		385 (>10) 273.5	28

TABLE II: METHOD OF PREPARATION AND PHYSICAL PROPERTIES

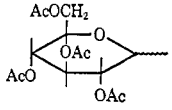
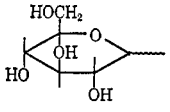
Imidazole moiety[a]	Glycosyl moiety[b]	Mp, °C	Recryst solvent	[α]D, deg (solvent)[c]	Spectra	Misc[d]	Method of prep[e]	Ref
		205–208	1-Propanol				I	41
As above		217–218	1-Propanol, also ethanol–water (1:1)	0.062, 3.02 (i)			VI	41

TABLE II (*Continued*)

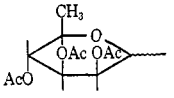
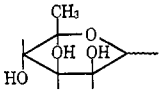
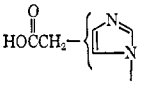
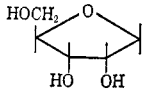
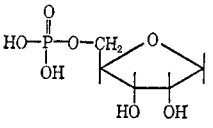
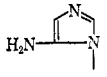
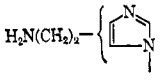
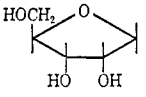
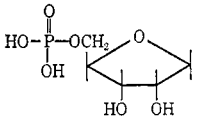
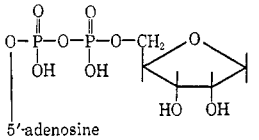
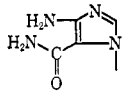
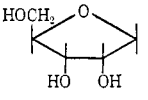
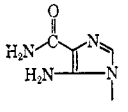
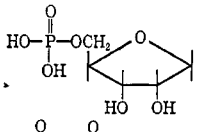
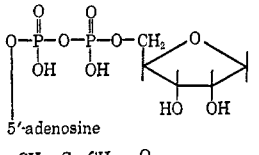
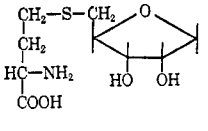
Imidazole moiety[a]	Glycosyl moiety[b]	Mp, °C	Recryst solvent	[α]D, deg (solvent)[c]	Spectra	Misc[d]	Method of prep[e]	Ref
As above		177–179	Benzene				I	41
As above		153–156	Ethyl propionate				VI	41
		185	Water–acetone	−37.0 (a)	IR	HCl salt	VI	34
		177–179	Dilute HCl–acetone	−52.6 (b)		HCl salt	I	29
		185	Water–acetone	−49.7 (b)	IR	HCl salt	VI	35
						Paper chromatography	V	150
						Paper chromatography	V	181
						Paper chromatography	V	180
As above					UV	Paper chromatography	V	150
	As above				UV	UV spectra of diazo-coupled product	V	106
			Ethanol–diethyl ether		UV	Paper chromatography	V	139
			Water–acetone		UV	Paper chromatography	V	139
		174–175	Water–ethanol			HCl salt	VI	35
As above		176–180 dec	Water–acetone		UV	Paper chromatography	V	138
		176–180	Water–acetone		UV	Paper chromatography	V	139
			Water–acetone		UV	Paper chromatography	V	139
As above					UV	Paper chromatography	V	138
					UV	Paper chromatography	V	10
					UV		V	18
		187–189	Methanol		UV	Paper chromatography	VI	28
		213–214	Ethanol–water (4:1)	−50.3 (a)	UV	Paper chromatography	VI	28
		213–214 dec	Water		UV	Paper chromatography	V	78
	As above	210–211	Water	−63.0 (a)		Paper chromatography	V	92
			Ethanol–water				VI	162
		164 dec	Water			Picrate	II	165
					UV	Paper chromatography	V	65
						Paper chromatography	II	51
As above			Repptd	−67.6 (c)	UV	Paper chromatography	V	92
					UV	Paper chromatography	V	65
					UV	Barium salt	V	77
As above					UV	Paper chromatography	V	21
As above					UV	Paper chromatography	V	141

TABLE II (*Continued*)

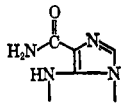
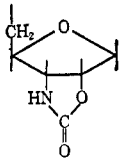
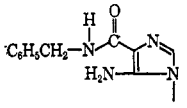
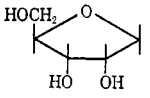
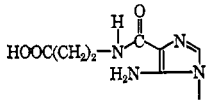
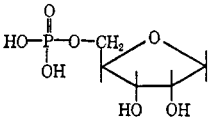
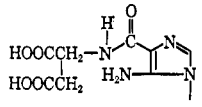
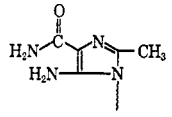
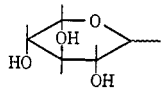
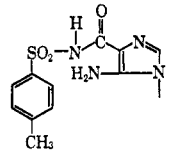
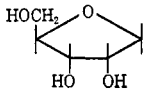
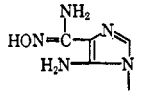
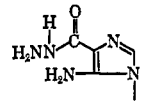
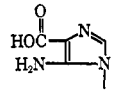
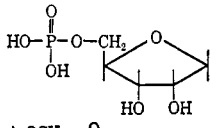
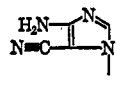
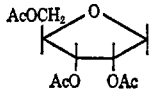
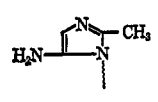
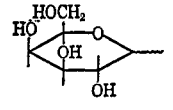
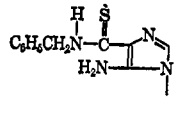
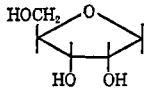
Imidazole moiety[a]	Glycosyl moiety[b]	Mp, °C	Recryst solvent	[α]D, deg (solvent)[c]	Spectra	Misc[d]	Method of prep[e]	Ref
		>300	Triturated with ethanol	+78 (d)	UV IR		VI	32
		171–172	Ethanol–water				III	161
	As above					Paper chromatography	VI	36
As above					UV	Paper chromatography	III	36
					UV		V	120
	As above		Water–acetone Pptd out of water with ethanol	−26.3 (c)	UV UV	Paper chromatography Barium salt	V VI	92 170
		186–187 dec	Water				II	165
		207–209	Aqueous (pH 5)			pK_a	III	162
	As above				UV	Paper chromatography	III	183
	2′-Phosphate				UV	Paper chromatography	III	183
	3′-Phosphate				UV	Paper chromatography	III	183
	5′-Phosphate				UV	Paper chromatography	III	183
	As above	186–187	Ethanol–water				VI	162
			Water, pptd with ethanol Water, pptd with ethanol		UV UV	Barium salt Barium salt	VI V	168 121
		58–60			IR PMR		VI	154
		172 dec	Water			Picrate	II	165
		113	Ethanol		UV	Paper chromatography	III	134

TABLE II (*Continued*)

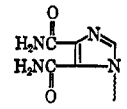
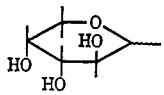
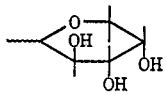
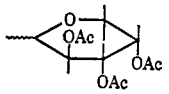
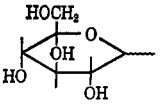
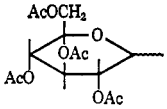
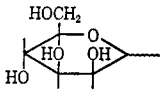
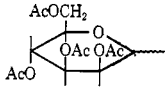
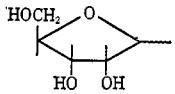
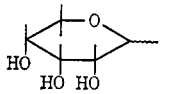
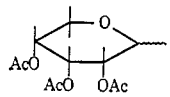
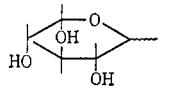
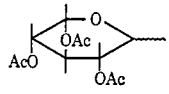
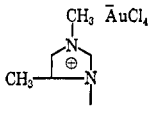
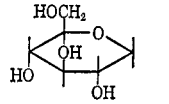
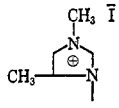
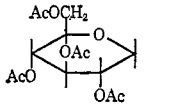
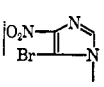
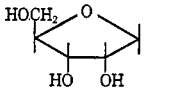
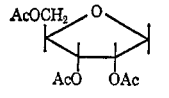
Imidazole moiety[a]	Glycosyl moiety[b]	Mp, °C	Recryst solvent	[α]D, deg (solvent)[c]	Spectra	Misc[d]	Method of prep[e]	Ref
		Sintering 175–180, and melting 216	Ethanol–water	−40 (e)			I	39
As above		Sintering at 175–180, melting at 215–216 dec	Ethanol–water	+44 (e)			I	39
As above		132–133	Ethanol, then Et_2O and petr ether (40–60°)				VI	39
As above		232–233 146–148	Ethanol–pyridine Aqueous ethanol as monohydrate				VI VI	39 39
As above		162–163	Methanol–water				VI	39
As above		208–210 dec	Methanol–water	−4 (a)			I	37
As above		184–185	Ethanol–water				VI	37
As above		218–220 (rapid heating)	Water	−16 (±5° (e)			I	91
As above		215–216	Ethanol–water	+17 (a)			VI	37
As above		246–248	Ethanol	+55 (f)			VI	37
As above		Sintering at 165–170 and melting at 224–225 dec	Ethanol–water	+34 (a)			VI	39
As above		158–159	Ethanol				VI	39
		144–145					VI	80
		232 dec	Ethanol				VI	80
		180–182		−8.54 (a)			VI	154
As above		93–95		+1.71 (g)			IV	154

TABLE II (*Continued*)

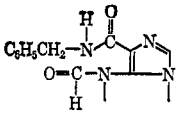
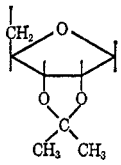
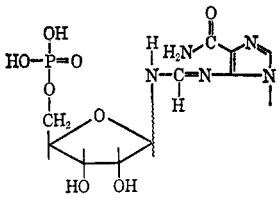
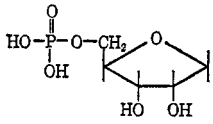
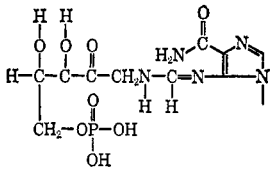
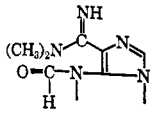
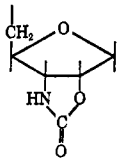
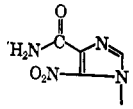
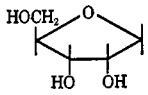
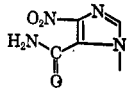
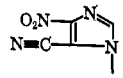
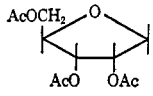
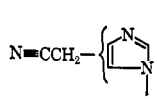
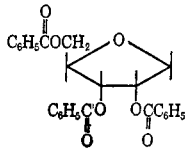
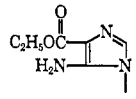
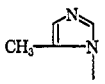
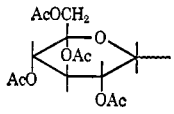
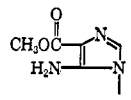
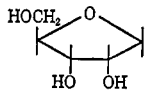
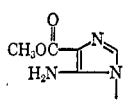
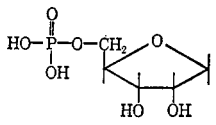
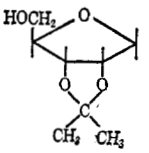
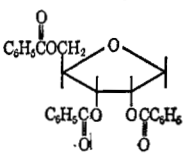
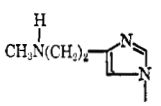
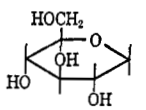
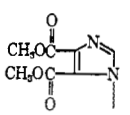
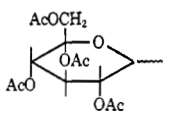
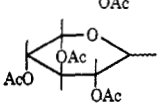
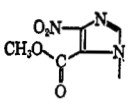
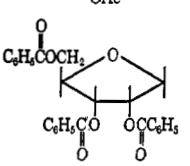
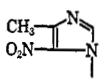
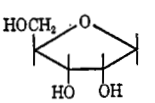
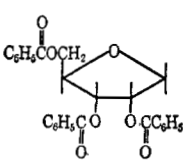
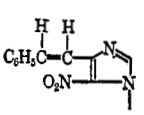
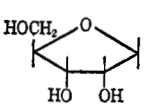
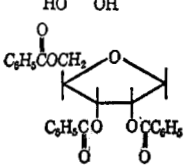
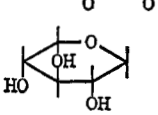
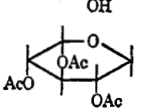
Imidazole moiety[a]	Glycosyl moiety[b]	Mp, °C	Recryst solvent	[α]D, deg (solvent)[c]	Spectra	Misc[d]	Method of prep[e]	Ref
		204	Ethanol		IR, UV		III	132
					UV	pK_a	V	177
	As above				UV	pK_a	V	177
		243–244 dec	Methanol		UV IR		III	32
		Syrup			UV	Paper chromatography	I	28
	As above	175–177	Methanol	−0.6 (a)	UV	Paper chromatography	I	28
		148–150	Ethanol		UV		VI	95
		99–100		+11.2 (f)	IR		VI	154
			CCl_4 and petr ether			HCl salt	I	34
						Paper chromatography	I	35
	As above	206–207	Ethanol				II	168
		176	Diethyl ether and water				I	80
		152	Residue triturated with ethyl acetate				VI	168
			Pptd out of water with ethanol		UV	Barium salt and paper chromatography	VI II	171 51

TABLE II (*Continued*)

Imidazole moiety[a]	Glycosyl moiety[b]	Mp, °C	Recryst solvent	$[\alpha]_D$, deg (solvent)[c]	Spectra	Misc[d]	Method of prep[e]	Ref
As above		161–162	Ethyl acetate				VI	168
As above		223–225 dec	Methanol				II	168
		207–209 198–200 130, solidify then melting at 174–176	Methanol Methanol Dilute ethanol	+11 (h)		Neut equiv Neut equiv Dipicrate	VI VI VI	60 60 60
		123	Methanol	+16 (f)			I	39
As above		166–167	Methanol	+22.6 (f)			I	39
		116–118	Methanol		UV		I	95
		Glass			UV	Paper chromatography	VI	95
As above		164–165	Methanol				I	95
		255–256	Methanol		UV		VI	28
As above		115–118	Ethanol		UV		I	28
As above		185–187	Methanol		UV		VI	28
As above		149–150	Methanol				I	28

[a] The position of glycosidic attachment to the imidazole moiety can be easily determined by visual inspection, and all compounds are of the β configuration unless they possess a wavy line at the anomeric center which denotes either an anomeric mixture or a compound of unknown or undetermined anomeric configuration. [b] The abbreviation –OAc denotes $-OCOCH_3$. [c] Solvents used in the determination of optical rotations appear as letters in parentheses: (a) water, (b) methanol, (c) 0.1 *M* sodium bicarbonate, (d) 4:1 water–pyridine, (e) pyridine, (f) chloroform, (g) ethanol, (h) 80% ethanol, (i) 24% sulfuric acid. [d] IR = infrared; UV = ultraviolet; PMR = proton magnetic resonance. [e] Key to the method of preparation: I, condensation of heavy metal salt of preformed imidazole with acylglycosyl halide; II, ring closure of glycosylamine; III, degradation or ring opening of purine nucleoside or nucleotide; IV, acid-catalyzed fusion; V, naturally occurring or enzymatic synthesis; VI, transformation of a preformed imidazole nucleoside or nucleotide.

ACKNOWLEDGMENTS.—The author wishes to express his sincere appreciation to Professor Roland K. Robins for encouragement, for a critical reading of the manuscript, and for many valuable suggestions. The author is grateful to Dr. Ralph L. Shriner for the opportunity to prepare this manuscript and appreciates the cooperation of a number of colleagues, especially R. J. Rousseau and A. F. Lewis. He also expresses his gratitude for the continuous encouragement and assistance of his wife, Sammy, and to Miss Dorlene Kearl for preparation of the manuscript. The author is indebted to the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, for financial support on Research Contract PH 43-65-1041.

VII. REFERENCES

(1) Abdel-Latif, A. A., and Alivisatos, S. G. A., *J. Biol. Chem.*, **236**, 2710 (1961).
(2) Abdel-Latif, A. A., and Alivisatos, S. G. A., *Biochim. Biophys. Acta*, **51**, 398 (1961).
(3) Aebi, A., *Helv. Chim. Acta*, **39**, 1495 (1956).
(4) Ahmad, F., Missimer, P., and Moat, A. G., *Can. J. Biochem.*, **43**, 1723 (1965).
(5) Ajinomoto Co. Inc., Japanese Patent 3550 (1962); *Chem. Abstr.*, **58**, 5012*f* (1963).
(6) Ajinomoto Co. Inc., French Patent 1,375,784 (Oct 23, 1964); *Chem. Abstr.*, **63**, 18999*g* (1965).
(7) Ajinomoto Co. Inc., Dutch Patent 6,409,142 (Feb 17, 1965); *Chem. Abstr.*, **63**, 5731*b* (1965); Dutch Patent 6,409,133 (Feb 8, 1965); *Chem. Abstr.*, **63**, 3029*a* (1965); Dutch Patent 6,409,806 (March 1, 1956); *Chem. Abstr.*, **63**, 10055*c* (1965).
(8) Ajinomoto Co. Inc., Japanese Patent 1534 (Feb 4, 1966); *Chem. Abstr.*, **64**, 12781*e* (1966).
(9) Alivisatos, S. G. A., *Federation Proc.*, **17**, 180 (1958).
(10) Alivisatos, S. G. A., *Nature*, **181**, 271 (1958).
(11) Alivisatos, S. G. A., *Nature*, **183**, 1034 (1959).
(12) Alivisatos, S. G. A., *Federation Proc.*, **24**, 769 (1965).
(13) Alivisatos, S. G. A., Abdel-Latif, A. A., Ungar, F., and Mourkides, G. A., *Nature*, **199**, 907 (1963).
(14) Alivisatos, S. G. A., and LaMantia, L., *Biochem. Biophys. Res. Commun.*, **2**, 164 (1960).
(15) Alivisatos, S. G. A., LaMantia, L., and Matijevitch, B. L., *Biochim. Biophys. Acta*, **58**, 201 (1962).
(16) Alivisatos, S. G. A., LaMantia, L., Ungar, F., and Matijevitch, B. L., *J. Biol. Chem.*, **237**, 1212 (1962).
(17) Alivisatos, S. G. A., LaMantia, L., Ungar, F., and Savich, B., *Biochim. Biophys. Acta*, **30**, 660 (1958).
(18) Alivisatos, S. G. A., Ungar, F., Lukacs, L., and LaMantia, L., *J. Biol. Chem.*, **235**, 1742 (1960).
(19) Alivisatos, S. G. A., Ungar, F., and Mourkides, G. A., *Nature*, **211**, 187 (1966).
(20) Alivisatos, S. G. A., and Woolley, D. W., *J. Am. Chem. Soc.*, **77**, 1065 (1955).
(21) Alivisatos, S. G. A., and Woolley, D. W., *J. Biol. Chem.*, **221**, 651 (1956).
(22) Allesbrook, W. E., Gulland, J. M., and Story, L. F., *J. Chem. Soc.*, 232 (1942).
(23) Ames, B. N., Martin, R. G., and Garry, B. J., *J. Biol. Chem.*, **236**, 2019 (1961).
(24) Ames, B. N., and Mitchell, H. K., *J. Am. Chem. Soc.*, **74**, 252 (1952).
(25) Atkinson, M. R., Jackson, J. F., Morton, R. K., and Murray, A. W., *Nature*, **196**, 35 (1962).
(26) Baddiley, J., and Buchanan, J. G., *Ann. Rept. Chem. Soc.*, **54**, 329 (1957).
(27) Baddiley, J., Buchanan, J. G., Hardy, F. E., and Stewart, J., *Proc. Chem. Soc.*, 149 (1957).
(28) Baddiley, J., Buchanan, J. G., Hardy, F. E., and Stewart, J., *J. Chem. Soc.*, 2893 (1959).
(29) Baddiley, J., Buchanan, J. G., Hayes, D. H., and Smith, P. A., *J. Chem. Soc.*, 3743 (1958).
(30) Baddiley, J., Buchanan, J. G., Hodges, R., and Prescott, J. F., *J. Chem. Soc.*, 4769 (1957).
(31) Baddiley, J., Buchanan, J. G., and Osbourne, G. O., *J. Chem. Soc.*, 3606 (1958).
(32) Baker, B. R., and Joseph, J. P., *J. Am. Chem. Soc.*, **77**, 15 (1955).
(33) Battersby, A. R., and Openshaw, H. T., "The Alkaloids," Vol. III, R. H. F. Manske and H. L. Holmes, Ed., Academic Press, New York, N. Y., 1953, Chapter 20.
(34) Bauer, H., *Biochim. Biophys. Acta*, **30**, 219 (1958).
(35) Bauer, H., *J. Org. Chem.*, **27**, 167 (1962).
(36) Baugh, C. M., and Shaw, E. N., *Biochim. Biophys. Acta*, **114**, 213 (1966).
(37) Baxter, R. A., McLean, A. C., and Spring, F. S., *J. Chem. Soc.*, 523 (1948).
(38) Baxter, R. A., and Spring, F. S., *J. Chem. Soc.*, 232 (1945).
(39) Baxter, R. A., and Spring, F. S., *J. Chem. Soc.*, 378 (1947).
(40) Ben-Ishai, R., Bergmann, E. D., and Volcani, B. E., *Nature*, **168**, 1124 (1951).
(41) Bergmann, E., and Heimhold, H., *J. Chem. Soc.*, 505 (1936).
(42) Bernstein, H., *J. Gen. Microbiol.*, **25**, 41 (1961).
(43) Bratton, A. C., and Marshall, E. K., *J. Biol. Chem.*, **128**, 537 (1939).
(44) Broom, A. D., Townsend, L. B., Jones, J. W., and Robins, R. K., *Biochemistry*, **3**, 494 (1964).
(45) Brown, D. D., Tomchick, R., and Axelrod, J., *J. Biol. Chem.*, **234**, 2948 (1959).
(46) Buchanan, J. G., Dekker, C. A., and Long, A. G., *J. Chem. Soc.*, 3162 (1950).
(47) Buchanan, J. M., and Hartman, S. C., *Advan. Enzymol.*, **21**, 199 (1959).
(48) Buchanan, J. M., and Schulman, M. P., *J. Biol. Chem.*, **202**, 241 (1953).
(49) Burns, V. W., *Science*, **146**, 1056 (1964).
(50) Carrington, R., Shaw, G., and Wilson, D. V., *Tetrahedron Letters*, 2861 (1964).
(51) Carrington, R., Shaw, G., and Wilson, D. V., *J. Chem. Soc.*, 6864 (1965).
(52) Chamberlain, N., Cutts, N. S., and Rainbow, C., *J. Gen. Microbiol.*, **7**, 54 (1952).
(53) Chamberlain, N., and Rainbow, C., *J. Gen. Microbiol.*, **11**, 180 (1954).
(54) Crowley, G. M., *Federation Proc.*, **19**, 309 (1960).
(55) Crowley, G. M., *J. Biol. Chem.*, **239**, 2593 (1964).
(56) Darnall, K. R., and Townsend, L. B., *J. Heterocyclic Chem.*, **3**, 371 (1966).
(57) Debus, H., *Ann.*, **107**, 199 (1858).
(58) de la Haba, G., and Cantoni, G. L., *J. Biol. Chem.*, **234**, 603 (1959).
(59) Djerassi, C., Bankiewicz, C., Kapoor, A. L., and Riniker, B., *Tetrahedron*, **2**, 168a (1958).
(60) Djerassi, C., Herran, J., Khastjir, H. N., Riniker, B., and Roma, J., *J. Org. Chem.*, **21**, 1510 (1956).
(61) Ellis, G. P., Epstein, C., Fitzmaurice, C., Golberg, L., and Lord, G. H., *J. Pharm. Pharmacol.*, **16**, 400 (1964).

(62) Fernandes, J. F., Castellani, O., and Plese, M., *Biochem. Biophys. Res. Commun.*, **3**, 679 (1960).
(63) Fernandes, J. F., Castellani, O., and Plese, M., *Ciencia Cult.* (Sao Paulo), **13**, 87 (1961); *Chem. Abstr.*, **57**, 13071*i* (1962).
(64) Flaks, J. G., and Buchanan, J. M., *J. Am. Chem. Soc.*, **76**, 2275 (1954).
(65) Flaks, J. G., Erwin, M. J., and Buchanan, J. M., *J. Biol. Chem.*, **228**, 201 (1957).
(66) Flaks, J. G., Erwin, M. J., and Buchanan, J. M., *J. Biol. Chem.*, **229**, 603 (1957).
(67) Flaks, J. G., Warren, L., and Buchanan, J. M., *J. Biol. Chem.*, **228**, 215 (1957).
(68) Fox, J. J., and Wempen, I., *Advan. Carbohydrate Chem.*, **14**, 283 (1959).
(69) Friedman, H., and Moat, A. G., *Arch. Biochem. Biophys.*, **78**, 146 (1958).
(70) Gallo, G. C., Pasqualucci, C. R., Radaelli, P., and Lancina, G. C., *J. Org. Chem.*, **29**, 862 (1964).
(71) Goldthwait, D. A., Peabody, R. A., and Greenberg, G. R., *J. Am. Chem. Soc.*, **76**, 5258 (1954).
(72) Gots, J. S., *Nature*, **172**, 256 (1953).
(73) Gots, J. S., and Gollub, E. G., *Proc. Natl. Acad. Sci., U. S.*, **43**, 826 (1957).
(74) Gots, J. S., and Gollub, E. G., *Proc. Soc. Exptl. Biol. Med.*, **101**, 638 (1959).
(75) Greenberg, G. R., *J. Biol. Chem.*, **190**, 611 (1951).
(76) Greenberg, G. R., *J. Am. Chem. Soc.*, **74**, 6307 (1952).
(77) Greenberg, G. R., *J. Biol. Chem.*, **219**, 423 (1956).
(78) Greenberg, G. R., and Spilman, E. L., *J. Biol. Chem.*, **219**, 411 (1956).
(79) Grunberg-Manago, M., *Ann. Rev. Biochem.*, **31**, 301 (1962).
(80) Gulland, J. M., and Macrae, T. F., *J. Chem. Soc.*, 662 (1933).
(81) Hall, R., *Biochim. Biophys. Acta*, **61**, 530 (1962).
(82) Hanes, C. S., and Isherwood, F. A., *Nature*, **164**, 1107 (1949).
(83) Hantzsch, A., *Ann.*, **249**, 1 (1888).
(84) Hantzsch, A., and Besch, C. H., *Ann.*, **323**, 23 (1902).
(85) Hartman, S. C., and Buchanan, J. M., *Ann. Rev. Biochem.*, **28**, 365 (1959).
(86) Hartman, S. C., and Buchanan, J. M., *J. Biol. Chem.*, **234**, 1812 (1959).
(87) Hartman, S. C., Levenberg, B., and Buchanan, J. M., *J. Am. Chem. Soc.*, **77**, 501 (1955).
(88) Hartman, S. C., Levenberg, B., and Buchanan, J. M., *J. Biol. Chem.*, **221**, 1057 (1956).
(89) Hiatt, H. H., *J. Clin. Invest.*, **37**, 1453 (1958).
(90) Howard, G. A., Lythgoe, B., and Todd, A. R., *J. Chem. Soc.*, 1052 (1947).
(91) Howard, G. A., McLean, A. C., Newbold, G. I., Spring, F. S., and Todd, A. R., *J. Chem. Soc.*, 232 (1949).
(92) Huang, H. T., *Biochemistry*, **4**, 58 (1965).
(93) Hunter, G., and Nelson, J. A., *Can. J. Res.*, **19b**, 296 (1941).
(94) Ikehara, M., and Muneyama, K., *Chem. Pharm. Bull.* (Tokyo), **14**, 46 (1966).
(95) Ikehara, M., Nakazawa, N., and Nakayama, H., *Chem. Pharm. Bull.* (Tokyo), **10**, 660 (1962).
(96) Ishikawa, F., Nomura, A., Ueda, T., Ikehara, M., and Mizuno, Y., *Chem. Pharm. Bull* (Tokyo), **8**, 380 (1960).
(97) Kalckar, H. M., *Fortschr. Chem. Org. Naturstoffe*, **9**, 389 (1952).
(98) Karjala, S. A., *J. Am. Chem. Soc.*, **77**, 504 (1955).
(99) Karjala, S. A., Turnquest, B. W., and Schayer, R. W., *J. Biol. Chem.*, **219**, 9 (1956).
(100) King, M. E., and Carter, C. E., *Biochim. Biophys. Acta*, **44**, 232 (1960).
(101) Klopotowski, T., Luzzati, M., and Slonimski, P., *Biochem. Biophys. Res. Commun.*, **3**, 150 (1960).
(102) Koessler, K. K., and Hanke, M. T., *J. Biol. Chem.*, **39**, 497 (1919).
(103) Korn, E. D., and Buchanan, J. M., *J. Biol. Chem.*, **217**, 183 (1955).
(104) Leonard, N. J., and Laursen, R. A., *J. Am. Chem. Soc.*, **85**, 2026 (1963).
(105) Levenberg, B., and Buchanan, J. M., *J. Am. Chem. Soc.*, **78**, 504 (1956).
(106) Levenberg, B., and Buchanan, J. M., *J. Biol. Chem.*, **224**, 1005 (1957).
(107) Levenberg, B., and Buchanan, J. M., *J. Biol. Chem.*, **224**, 1019 (1957).
(108) Levene, P. A., and Jacobs, W. A., *Ber.*, **42**, 2474 (1909).
(109) Liakopoulou, A., and Alivisatos, S. G. A., *J. Biol. Chem.*, **240**, 3110 (1965).
(110) Litchfield, G. J., and Shaw, G., *Chem. Commun.*, 563 (1965).
(111) Lones, D. P., Rainbow, C., and Woodward, J. D., *J. Gen. Microbiol.*, **19**, 46 (1958).
(112) Love, S. H., *J. Bacteriol.*, **72**, 628 (1956).
(113) Love, S. H., and Gots, J. S., *J. Biol. Chem.*, **212**, 647 (1955).
(114) Love, S. H., and Levenberg, B., *Biochim. Biophys. Acta*, **35**, 367 (1959).
(115) Lowy, B. A., and Williams, M. K., *J. Biol. Chem.*, **235**, 2924 (1960).
(116) Lowy, B. A., Williams, M. K., and London, I. M., *J. Biol. Chem.*, **236**, 1442 (1961).
(117) Lowy, B. A., Williams, M. K., and London, I. M., *J. Biol. Chem.*, **237**, 1622 (1962).
(118) Lukens, L. N., and Buchanan, J. M., *Federation Proc.*, **15**, 305 (1956).
(119) Lukens, L. N., and Buchanan, J. M., *J. Am. Chem. Soc.*, **79**, 1511 (1957).
(120) Lukens, L. N., and Buchanan, J. M., *J. Biol. Chem.*, **234**, 1791 (1959).
(121) Lukens, L. N., and Buchanan, J. M., *J. Biol. Chem.*, **234**, 1799 (1959).
(122) Lukens, L. N., and Flaks, J. G., *Methods Enzymol.*, **6**, 696 (1963).
(123) MacNutt, W. S., *Biochem. J.*, **50**, 384 (1952).
(124) Martin, R. G., *J. Biol. Chem.*, **238**, 257 (1963).
(125) Michelson, A. M., "The Chemistry of Nucleosides and Nucleotides," Academic Press Inc., New York, N. Y., 1963.
(126) Michelson, A. M., "The Chemistry of Nucleosides and Nucleotides," Academic Press Inc., New York, N. Y., 1963, p 20, ref 74.
(127) Miller, R. W., and Buchanan, J. M., *J. Biol. Chem.*, **237**, 485 (1962).
(128) Miller, R. W., Lukens, L. N., and Buchanan, J. M., *J. Am. Chem. Soc.*, **79**, 1573 (1957).
(129) Miller, R. W., Lukens, L. N., and Buchanan, J. M., *J. Biol. Chem.*, **234**, 1806 (1959).
(130) Mitsugi, K., Okumura, S., Shiro, T., and Takahashi, M., *Agr. Biol. Chem.* (Tokyo), **29**, 1104 (1965).
(131) Moat, A. G., Wilkins, C. N., and Friedman, H., *J. Biol. Chem.*, **223**, 985 (1956).
(132) Montgomery, J. A., Hewson, K., Clayton, S. J., and Thomas, H. J., *J. Org. Chem.*, **31**, 2202 (1966).
(133) Montgomery, J. A., and Thomas, H. J., *Advan. Carbohydrate Chem.*, **17**, 301 (1962).
(134) Montgomery, J. A., and Thomas, H. J., *J. Org. Chem.*, **28**, 2304 (1963).
(135) Montgomery, J. A., and Thomas, H. J., *J. Am. Chem. Soc.*, **87**, 5442 (1965).

(136) Montgomery, J. A., and Thomas, H. J., *J. Org. Chem.*, **30**, 494 (1965).
(137) Moyed, H. S., and Magasanik, B., *J. Biol. Chem.*, **235**, 149 (1960).
(138) Muraoka, S., Inoue, M., and Yamasaki, H., *Nature*, **190**, 532 (1960).
(139) Muraoka, S., Sugiyama, M., and Yamasaki, H., *Nature*, **196**, 441 (1962); *Biochem. Pharmacol.*, **14**, 27 (1965).
(140) Pauly, H., *Z. Physiol. Chem.*, **42**, 508 (1904).
(141) Perry, T. L., Hansen, S., Bar, H. P., and MacDougall, L., *Science*, **152**, 776 (1966).
(142) Power, F. B., and Callan, T., *J. Chem. Soc.*, **99**, 1993 (1911).
(143) Pyman, F., *J. Chem. Soc.*, **97**, 1814 (1910).
(144) Rabinowitz, J. C., and Pricer, W. E., Jr., *Federation Proc.*, **14**, 266 (1955).
(145) Rabinowitz, J. C., and Pricer, W. E., Jr., *J. Am. Chem. Soc.*, **78**, 5702 (1956).
(146) Ralph, R. K., and Shaw, G., *J. Chem. Soc.*, 1877 (1956).
(147) Raman, S., Reddy, J., and Lipscomb, W. N., *Acta Cryst.*, **16**, 364 (1963).
(148) Raman, S., Reddy, J., Lipscomb, W. N., Kapoor, A. L., and Djerassi, C., *Tetrahedron Letters*, 357 (1962).
(149) Reichard, P., *Advan. Enzymol.*, **21**, 263 (1959).
(150) Robinson, J. D., and Green, J. P., *Nature*, **203**, 1178 (1964).
(151) Robinson, J. D., and Green, J. P., *Federation Proc.*, **24**, 777 (1965).
(152) Rogers, D., King, T. E., and Cheldelin, V. H., *J. Bacteriol.*, **80**, 787 (1960).
(153) Rousseau, R. J., Townsend, L. B., and Robins, R. K., *Biochemistry*, **5**, 756 (1966).
(154) Rousseau, R. J., Townsend, L. B., and Robins, R. K., *Chem. Commun.*, 265 (1966).
(155) Rousseau, R. J., Townsend, L. B., and Robins, R. K., unpublished observations.
(156) Sato, T., Shimidate, T., and Ishido, Y., *J. Chem. Soc. Japan, Ind. Chem. Sect.*, **81**, 1440 (1960), and subsequent papers.
(157) Schayer, R. W., *Physiol. Rev.*, **39**, 116 (1959).
(158) Schayer, R. W., and Cooper, J. A. D., *J. Appl. Physiol.*, **6**, 481 (1956).
(159) Schulmann, M. P., and Buchanan, J. M., *J. Biol. Chem.*, **196**, 573 (1952).
(160) Scott, J. L., *J. Clin. Invest.*, **41**, 67 (1962).
(161) Shaw, E., *J. Am. Chem. Soc.*, **80**, 3899 (1958).
(162) Shaw, E., *J. Am. Chem. Soc.*, **81**, 6021 (1959).
(163) Shaw, E., *J. Am. Chem. Soc.*, **83**, 4770 (1961).
(164) Shaw, G., and Warrener, R. N., *Proc. Chem. Soc.*, 193 (1958).
(165) Shaw, G., Warrener, R. N., Butler, D. N., and Ralph, R. K., *J. Chem. Soc.*, 1648 (1959).
(166) Shaw, G., Warrener, R. N., Maguire, M. H., and Ralph, R. K., *J. Chem. Soc.*, 2294 (1958).
(167) Shaw, G., and Wilson, D. V., *Proc. Chem. Soc.*, 381 (1961).
(168) Shaw, G., and Wilson, D. V., *J. Chem. Soc.*, 2937 (1962).
(169) Shaw, G., and Wilson, D. V., *Proc. Chem. Soc.*, 115 (1962).
(170) Shaw, G., and Wilson, D. V., *J. Chem. Soc.*, 1077 (1963).
(171) Shaw, G., Wilson, D. V., and Green, C. P., *J. Chem. Soc.*, 2650 (1964).
(172) Shedlovsky, A. E., and Magasanik, B., *J. Biol. Chem.*, **237**, 3725 (1962).
(173) Shiro, T., *Agr. Biol. Chem.* (Tokyo), **25**, 350 (1961).
(174) Shiro, T., Yamanoi, A., Konishi, S., Okumura, S., and Takahashi, M., *Agr. Biol. Chem.* (Tokyo), **26**, 785 (1962).
(175) Shive, W., Ackermann, W. W., Gordon, M., Getzendander, M. E., and Eakin, R., *J. Am. Chem. Soc.*, **69**, 725 (1947).
(176) Shuster, L., *J. Biol. Chem.*, **238**, 3344 (1963).
(177) Smith, D. W. E., and Ames, B. N., *J. Biol. Chem.*, **239**, 1848 (1964).
(178) Smith, D. W. E., and Ames, B. N., *J. Biol. Chem.*, **240**, 3056 (1965).
(179) Smith, L. H., Jr., and Yates, P., *J. Am. Chem. Soc.*, **76**, 6080 (1954).
(180) Snyder, S. H., and Axelrod, J., *Federation Proc.*, **24**, 774 (1965).
(181) Snyder, S. H., Axelrod, J., and Bauer, H., *J. Pharmacol. Exptl. Therap.*, **144**, 373 (1964).
(182) Stetten, M. R., and Fox, C. L., Jr., *J. Biol. Chem.*, **161**, 333 (1945).
(183) Stevens, M. A., Smith, H. W., and Brown, G. B., *J. Am. Chem. Soc.*, **81**, 1734 (1959).
(184) Tabor, H., and Hayaishi, O., *J. Am. Chem. Soc.*, **77**, 505 (1955).
(185) Tener, G. M., *J. Am. Chem. Soc.*, **83**, 159 (1961).
(186) Thompson, R. Y., Paul, J., and Davidson, J. N., *Biochem. J.*, **69**, 553 (1958).
(187) Tomisek, A. J., Kelly, H. J., Reid, M. R., and Skipper, H. E., *Arch. Biochem. Biophys.*, **76**, 45 (1958).
(188) Townsend, L. B., and Robins, R. K., *J. Am. Chem. Soc.*, **84**, 3008 (1962).
(189) Townsend, L. B., and Robins, R. K., *J. Am. Chem. Soc.*, **85**, 243 (1963).
(190) Townsend, L. B., and Robins, R. K., *J. Heterocyclic Chem.*, **3**, 241 (1966).
(191) Townsend, L. B., Robins, R. K., Loeppky, R. N., and Leonard, N. J., *J. Am. Chem. Soc.*, **86**, 5320 (1964).
(192) Townsend, L. B., Rousseau, R. J., and Robins, R. K., unpublished observations.
(193) Warren, L., Flaks, J. G., and Buchanan, J. M., *J. Biol. Chem.*, **229**, 627 (1957).
(194) Weaver, J. M., and Shive, W., *J. Am. Chem. Soc.*, **75**, 4628 (1953).
(195) Webb, M., *Biochem. J.*, **70**, 472 (1958).
(196) Windaus, A., *Ber.*, **42**, 758 (1909).
(197) Woodward, J. D., and Rainbow, C., *J. Gen. Microbiol.*, **25**, 141 (1961).
(198) Wyngaarden, J. D., Jones, O. W., and Ashton, D. M., *Atti Congr. Lega Intern. Reumatismo, 10°, Rome, 1*, 249 (1961); *Chem. Abstr.*, **61**, 9880 (1964).