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REVIEW ARTICLE

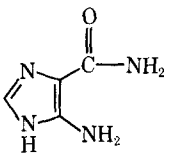
Syntheses and Biological Activity of 5-Aminoimidazoles and 5-Triazenoimidazoles

Y. FULMER SHEALY

Keyphrases ☐ 5-Aminoimidazoles—methods of synthesis ☐ Triazenoimidazoles—synthesis, stability ☐ Antineoplastic activity—triazenoimidazoles ☐ Microbiological activity—triazenoimidazoles ☐ Clinical studies—5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide, 5-[3,3-bis(2-chloroethyl)-1-triazeno]imidazole-4-carboxamide

I. INTRODUCTION

In 1945, Stetten and Fox (1) isolated a heterocyclic amine that accumulated (1, 2) during sulfonamide bacteriostasis of *Escherichia coli* and also detected this compound in sulfonamide-inhibited cultures of other bacteria. Shive *et al.* (3), proceeding from the hypothesis that purine biosynthesis had been affected, showed that the heterocyclic amine was 5-aminoimidazole-4-carboxamide[1] (I) (AIC[2]), an imidazole that had been synthesized more than 20 years earlier by Windaus and Langenbeck (4) as a potential intermediate for the chemical synthesis of purines (5). Shive *et al.* (3) correctly postulated that AIC "functions as a precursor of purine bases or is formed from a precursor of purines" and that a *p*-aminobenzoic acid "coenzyme [formyltetrahydrofolic acid] functions in combining a single carbon unit into the pyrimidine ring." Subsequent studies of purine biosynthesis revealed that the 1-(β-D-ribofuranosyl) 5′-phosphate derivatives of AIC and of four other imidazoles constitute the middle group of steps in the biosynthetic pathway to purine ribonucleotides. All imidazole moieties of these compounds have an amino group (or its formyl derivative) at the 5-position of the imidazole ring; all, save the first, have a carboxyl or a carboxamide group at the 4-position.



I
AIC

Other imidazole derivatives possess various types and varying degrees of biological significance. The physiological and pharmacological importance of histidine and histamine is well known, and the literature relating to these compounds, their derivatives, and their analogs is extensive. Certain imidazole structures are reported to display antiprotozoal, antifungal, antibacterial, monoamine oxidase-inhibitory, her-

[1] When a nitrogen atom of the imidazole ring has an exocyclic substituent, that nitrogen atom is assigned position 1. Prior to 1967, the subject indexes of *Chemical Abstracts* listed Compound I as 5(or 4)-aminoimidazole-4(or 5)-carboxamide, because the position (which should be number 1) of the labile ring proton cannot be fixed. This nomenclature convention, in which the number of the alternative numbering sequence is included in parentheses, had been employed for similar imidazoles that have a proton on a ring nitrogen and substituents at positions 4 and 5. In recent subject indexes, Compound I is named 5-aminoimidazole-4-carboxamide, and this simplified nomenclature is also employed for similar imidazoles. In agreement with later *Chemical Abstracts* usage and for the sake of simplicity, a single number, without the parenthetical alternative numbering system, will be used thoughout this review to designate positions 4 and 5. In the structures of compounds, such as AIC, having the labile proton instead of another substituent (alkyl, ribofuranosyl, *etc.*) on a nitrogen atom, the proton is generally placed on the nitrogen atom that corresponds to the point of attachment of the ribofuranosyl group of the biosynthetic imidazoles.

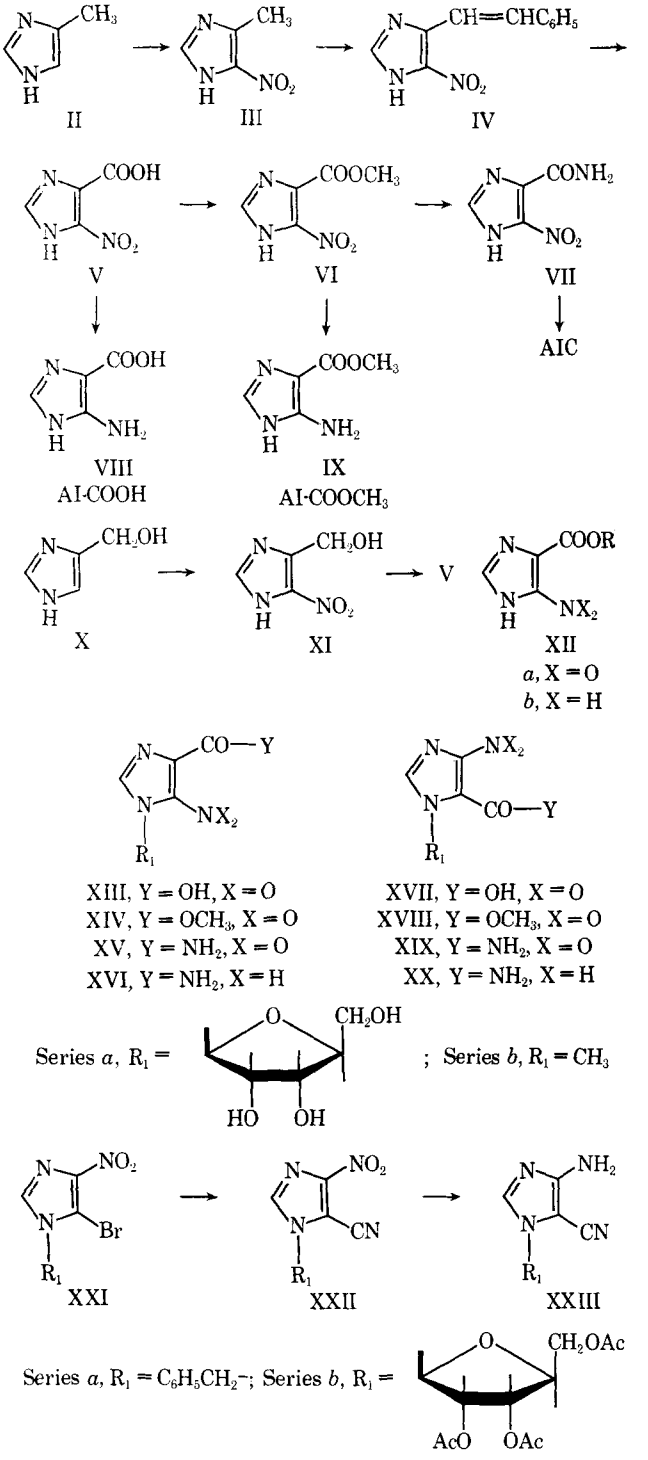
[2] The letter designations AIC and AICA are both employed in the literature for Compound I. Since either abbreviation could also be logically used for 5-aminoimidazole-4-carboxylic acid, the ribonucleotide of which is a precursor of the ribonucleotide of I, neither is specific. The shorter abbreviation will, therefore, be used in this article.

bicidal, and other types of activity. The following examples are illustrative: the activity of imidazole-4,5-dicarboxamide and some of its derivatives against coccidiosis in poultry (6, 7); the activity of 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole (metronidazole) (8–10) and other nitroimidazoles (*e.g.*, 10–15) against *Trichomonas vaginalis*, other *Trichomonas* species, *Entamoeba histolytica*, and other protozoa; and the broad-spectrum antibacterial and antiprotozoal activity of 2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole and related structures (16). The antiprotozoal activity of metronidazole is clinically important in the oral treatment of trichomoniasis (14, 17–19) and amoebiasis (14, 20, 21).

However, to limit the scope of this review, it deals, first, with chemical syntheses and biological properties of 5-aminoimidazoles related to those in the biosynthetic pathway to purine ribonucleotides, the principal emphasis being on imidazoles bearing a member of the carboxylic acid family of groups (carboxyl, ester, amide, thioamide, amidine, nitrile, *etc.*) *ortho* to the amino group. Secondly, it summarizes the chemical and biological properties of triazenoimidazoles related to the imidazoles mentioned.[3] An excellent review of the entire imidazole field covers the period from 1919 to 1950 (22). Recent reviews (23, 24) differ from the present review in that: (*a*) they were limited to nucleosides and nucleotides of imidazoles, and (*b*) consideration of these types of derivatives was not confined to 5-aminoimidazoles.

II. SYNTHESES OF 5-AMINOIMIDAZOLES[4, 5]

Chemical syntheses of 5-aminoimidazole derivatives are presented by describing the basic methods for forming the imidazole ring with an amino group (or a precursor group) at position 5 rather than by structural type (amide, ester, nitrile, *etc.*). To preserve the continuity of certain synthetic sequences, further transformations of the structures obtained from a basic route are also frequently included in the section in which the basic method is described.



Scheme I

The synthetic methods of Sections B, C, and D are related and, in some of their variations, may include similar or common intermediates. However, they were developed at different times by different investigators; to simplify the presentation, they are treated separately.

A. Reduction of the Nitro Group (Windaus–Langenbeck and Sarasin–Wegmann Routes)—Because of its importance in aromatic chemistry, reduction of a nitro group (or an arylazo group) naturally received initial attention as a method for preparing 5-aminoimidazoles. These early efforts to synthesize 5-aminoimidazole (AI) and its methyl derivatives met with difficulties

[3] Triazenoimidazoles and aminoimidazoles are included in the same review because of the derivation, structures, and biological activity of the former. There is no intention to imply that inhibition of biochemical reactions involving aminoimidazoles is the primary mechanism of action of the triazeno derivatives (Part IV).

[4] To minimize both the number of letter designations and the number of long systematic names, three abbreviations will be employed in Parts II and III, with some exceptions in Part III where commonly accepted letter designations are shown with the structures. The three are AI for 5-aminoimidazole, AIC for Compound I, and AICAR for the ribonucleotide of Compound I. Admittedly, the latter two are not consistent; but there is no reason to change the generally accepted AICAR, and the reasons for choosing AIC are explained in *Footnote 2*. These three letter designations are employed in combination with the formulas of functional or protecting groups and in combination with names of groups or substituents to designate certain derivatives. For brevity, the terms ribofuranoside or ribonucleoside and ribonucleotide will be used for imidazoles bearing the β-D-ribofuranosyl and the β-D-ribofuranosyl 5′-phosphate groups at the position of these groups in the biosynthetic imidazoles (*i.e.*, adjacent to the amino group). This shorthand nomenclature method is illustrated by the following examples: 2-methyl-AIC ribonucleotide for 5-amino-2-methyl-1-(β-D-ribofuranosyl)imidazole-4-carboxamide 5′-(dihydrogen phosphate) (LX*d*); 1-cyclohexyl-AI-COOH for 5-amino-1-cyclohexylimidazole-4-carboxylic acid (XXXVII; R′ = R₂ = H, R₁ = cyclohexyl); and AI-COOCH₃ for 5-aminoimidazole-4-carboxylic acid methyl ester (IX).

[5] For convenience, carboxyl, amino, and phosphoric acid groups are depicted in their unionized forms throughout this review. However, ionic species are present in the biochemical environments in which imidazole derivatives having these groups are biosynthesized and transformed, and they are usually the forms isolated from chemical reactions.

owing to the instability of the products under the conditions of the chemical reductions (25–27, *cf.*, 28). Yields were low or nil because of degradation by ring-opening, but both AI (29) and its 4-methyl derivative (30) were subsequently obtained (as dihydrochlorides) in moderate yields by chemical reduction of the corresponding nitro compounds. The examples of prime interest in the present context have a carboxamide, a carboxyl, or similar group at position 4. During the period that difficulties were being experienced in preparing the simple aminoimidazoles, Windaus and Langenbeck (4) succeeded in synthesizing AIC and AI-$COOCH_3$[4] (IX) by a route that began with 4-methylimidazole (II) and terminated in the reduction of a nitro group (Scheme I). A nitration (25), an aldol-type condensation, and oxidation of the resulting styryl derivative (IV) yielded the key intermediate, 5-nitroimidazole-4-carboxylic acid (V), which had been obtained earlier by a less productive method (31). The nitro amide (VII) (4, 32) was prepared from the ester (VI) and reduced catalytically to AIC (4). Further studies and modifications of the route to V and IX were made by Allsebrook *et al.* (33), who also used the method to prepare the two nitro acids (XIII*b* and XVII*b*) having a methyl group on a ring nitrogen atom. The route was employed by Shive *et al.* (3) to prove the structure of AIC isolated from bacterial cultures; by Rabinowitz (34) to prepare (in weakly basic solution) 5-aminoimidazole-4-carboxylate (VIII), a catabolite of xanthine formed by extracts of *Clostridium cylindrosporum;* by Allsebrook *et al.* (33) to prepare 5-ureidoimidazole-4-carboxylic acid and its methyl ester for purine synthesis; by Taylor *et al.* (35) to prepare 5-aminoimidazole-4-carbohydroxamic acid; and by Robinson and Shepherd (36) to prepare 5-nitroimidazole-4-carboxylic acid hydrazide.

Kulev and Gireva (37) and Gireva and Dobychina (38) described an improved synthesis of the nitro acid (V); 4-(hydroxymethyl)imidazole (X), prepared from invert sugar, was nitrated and 4-(hydroxymethyl)-5-nitroimidazole (XI) was oxidized in high yield to V in the nitration mixture. These authors and others have reported the preparation of several esters (XII*a*) of V (38–42), the reduction of some of the nitro esters to esters (XII*b*) of 5-aminoimidazole-4-carboxylic acid (38, 40, 41), the preparation of acyl and sulfonyl [including bis(2-chloroethyl)aminobenzoyl and bis-(2-chloroethyl)aminobenzenesulfonyl] derivatives of the amino group of XII*b* ($R = C_2H_5$) (43), and the synthesis of *N*-aryl carboxamide analogs of VII (44, 45).

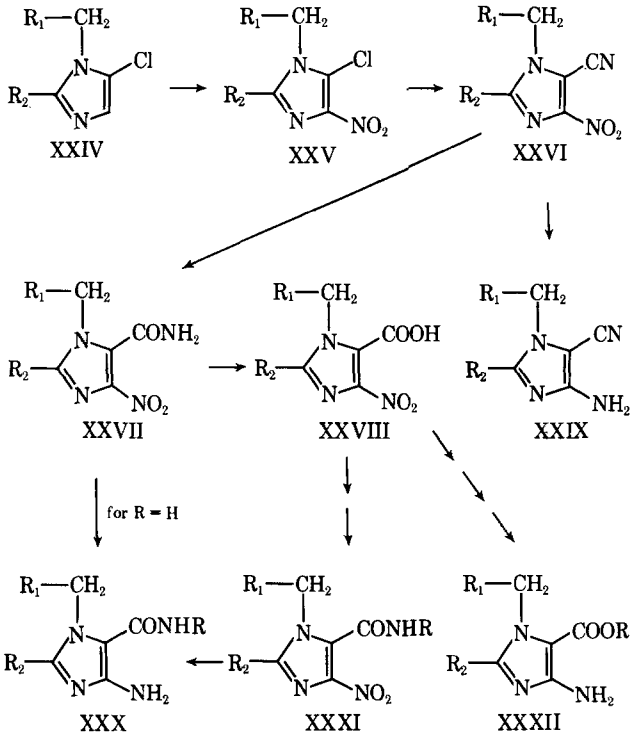
A synthesis (*cf.*, Parts IIB and IIE) of AIC ribonucleoside (XVI*a*) based on the nitro acid (V) was accomplished by Baddiley *et al.* (32, 46). Glycosidation of the silver or chloromercury salt of methyl 5-nitroimidazole-4-carboxylate (VI) with 2,3,5-tri-*O*-benzoylribofuranosyl chloride evidently gave a mixture of ribofuranosides (XIV*a* and XVIII*a* tribenzoates) that was transformed by ammonia to a mixture of the nitro amides (XV*a*, XIX*a*), which were separated. Catalytic reduction of each isomer gave AIC ribonucleoside (XVI*a*) and its isomer, 4-amino-1-(β-D-ribofuranosyl)imidazole-5-carboxamide (XX*a*). Methylation of the silver salt of the nitro amide (VII) gave the 1-methyl derivative (XV*b*) (32, 33) and its isomer (XIX*b*), which was also prepared from XVIII*b* (32); catalytic reduction of XV*b* afforded 1-methyl-AIC (XVI*b*).

Several findings on alkylation of these nitroimidazoles are germane to aminoimidazoles because of their potential or actual precursor role. Methyl 1-methyl-4-nitroimidazole-5-carboxylate (XVIII*b*) was the only reported product of methylation of the silver salt of VI (32, 33); the nitro styryl derivative (IV), like VII, gave a mixture of *N*-methyl isomers (32), and alkylation of the sodium salts of IV and its 2-methyl derivative gave mixtures of isomers (15). Ikehara *et al.* (47) isolated XVIII*a* tribenzoate as the main product of glycosidation of the chloromercury derivative of VI with 2,3,5-tri-*O*-benzoylribofuranosyl chloride and, in contrast, 4-methyl-5-nitro-1-β-D-ribofuranosylimidazole tribenzoate as the main product (44%) of glycosidation of 4-methyl-5-nitroimidazole (III). These results suggest either that the position of attack varies with the type of nitroimidazole substrate, the alkylating agent, and the conditions or that the methods of isolation or detection sometimes failed to reveal a second isomer.

In another synthesis of aminoimidazole nucleosides *via* glycosidation of nitro derivatives, more conclusive evidence of the position of predominant attack was obtained. Rousseau *et al.* (48, 49) employed an acid-catalyzed fusion procedure to glycosidate VI and 4-bromo-5-nitroimidazole with tetra-*O*-acetyl-β-D-ribofuranose. The *O*-triacetyl derivative of XVIII*a* was obtained in 83% yield, and the isomer could not be detected chromatographically. Although 4-bromo-5-nitroimidazole is not a product of the Windaus–Langenbeck route, it may be appropriate at this point to note that benzylation of this compound in solution (DMF) afforded a 78% yield of the derivative (XXI*a*) with the alkyl group adjacent to the bromo group[6] (50) and that 5-bromo-4-nitro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole (XXI*b*) was isolated in 72% yield from the acid-catalyzed fusion procedure (49), whereas less than a 5% yield of the isomeric ribofuranoside could be isolated. The amino derivatives, XXIII*a* (50) and XXIII*b* (48, 49), were obtained in yields of 99 and 87%, respectively, by catalytic hydrogenation of the nitro derivatives (XXII); similarly, XX*a* was obtained in high yield from XVIII*a* triacetate by the sequence consisting of amination of the ester group, deacetylation, and catalytic hydrogenation (49).

Another synthesis of aminoimidazolecarboxylic acid derivatives originated soon after the Windaus–Langenbeck route and produces structures related to those just discussed. In a sequence (Scheme II) of high-yielding steps (XXIV–XXVII, XXX), Sarasin and Wegmann (51) converted 5-chloro-1-methylimidazole (XXIV, $R_1 = R_2 = H$) to 4-amino-1-methylimidazole-5-carboxamide (XXX, $R = R_1 = R_2 = H$). The total synthetic route begins with the Wallach imidazole-ring formation (52, 53) from an *N,N'*-dialkyloxamide (XXXIII) and results in the introduction of a substituent on the imidazole-ring nitrogen adjacent to the car-

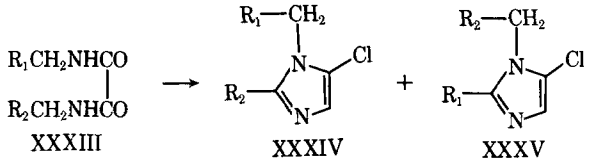
[6] Benzylation of the sodium salt gave a mixture of approximately equal amounts of the isomers.



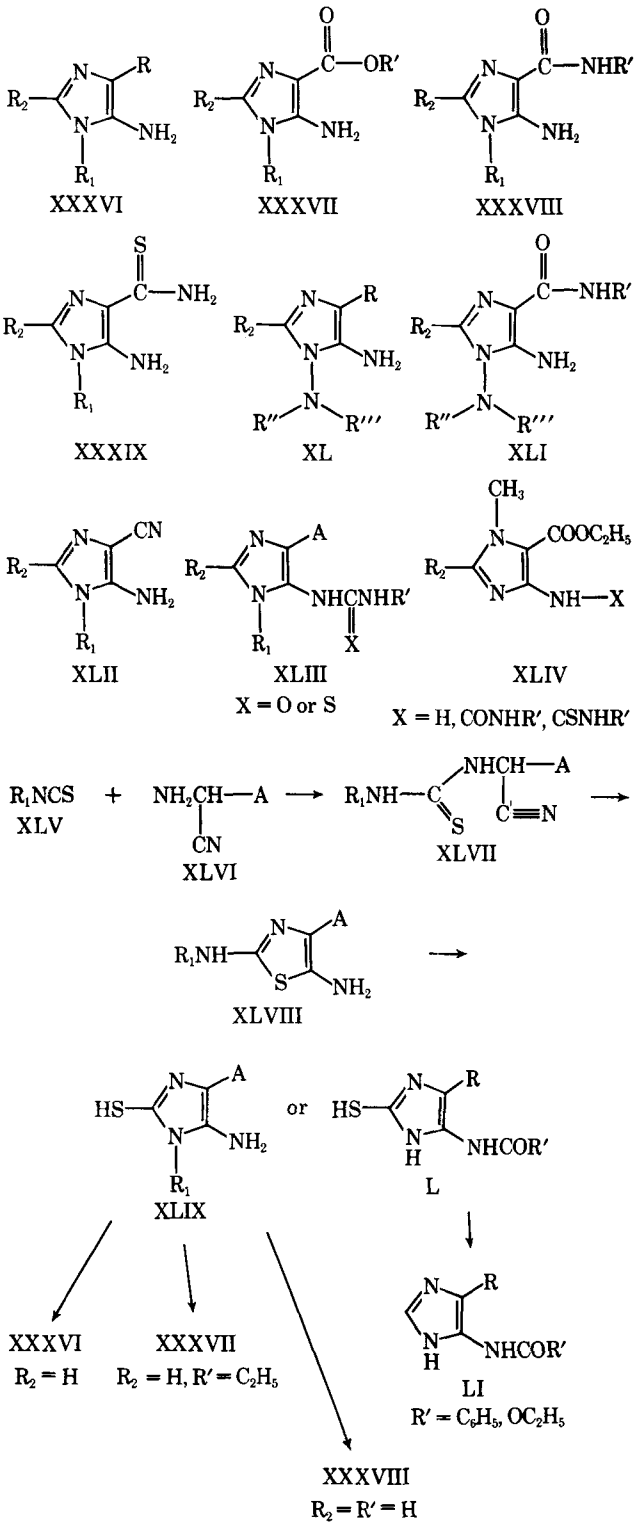
Scheme II

boxyl-type group (*cf.*, XVII–XX, XXII, XXIII) rather than at the position of the ribofuranosyl group in the biosynthetic imidazoles. In its most general form, the route is represented by XXIV–XXXII (Scheme II). Although hydrolysis of the amides (XXVII) to acids (XXVIII) is difficult, Mann and Porter (54) introduced a modification that gave high yields of the carboxylic acids (XXVIII) (*cf.*, 55). From the acids, *N*-substituted amides (XXXI, R_1 and R_2 = H or CH_3) (54–56), nitro esters (54), and a hydroxamic acid (57) have been prepared *via* acid chlorides. Originally, the nitro group of XXVI and XXVII was reduced chemically (tin and hydrochloric acid) (51, 55, 58, 59), but catalytic reduction to amino amides (XXX) (54, 56, 60–62), esters (XXXII, $R_1 = R_2 = H$) (54), and carbonitriles (XXIX) (60) is superior. However, incomplete reduction of the nitro group of 1-methyl-4-nitroimidazole-5-carbonitrile (XXVI, $R_1 = R_2 = H$) to an hydroxylamino group has been observed (63). The aminoimidazole derivatives resulting from most of these investigations (51, 54, 56, 58–64) were employed as intermediates for the synthesis of *N*-alkylpurines.

Recent investigations (61, 62, 65, 66) have extended the scope of and have clarified the reaction of oxamides with phosphorus pentachloride, the initial step of the overall synthesis of the amino amides (XXX). The isomers isolated have always been the 5-chloroimidazoles (51, 58, 61, 62, 67). In the recent study of several products by Trout and Levy (62), vapor-phase chroma-



Scheme III



In XLV–XLIX, A = H, CH_3, C_6H_5, $—COOC_2H_5$, or $—CONH_2$ and R_1 = H, CH_3, C_6H_5, $—COC_6H_5$, $—COOC_2H_5$, but not in all possible combinations.

Scheme IV

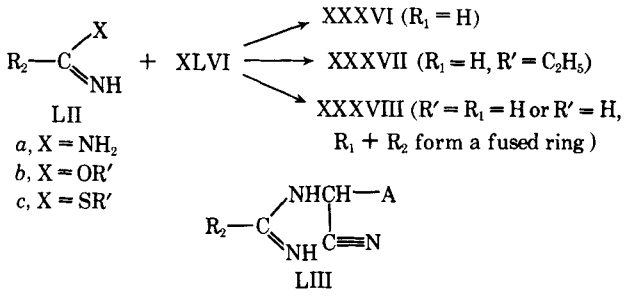
tography and TLC failed to reveal any of the 4-chloro derivatives. An unsymmetrical oxamide (XXXIII) (Scheme III) gave two separable 5-chloro-1,2-dialkylimidazoles (XXXIV and XXXV) (61). Several 4-amino-1,2-dialkylimidazole-5-carboxamides (XXX) were prepared by the Sarasin–Wegmann route (XXIV–XXVII, XXX)

in the course of these studies (61, 62) and used for the synthesis of purines (64).

5-Chloro-1-methyl-4-nitroimidazole (XXV, R_1 = R_2 = H) (51, 67) has also served as a starting point for the synthesis of some 4-amino-1-methylimidazole-5-sulfonamides (68, 69).

B. From α-Aminonitriles (Cook–Heilbron and G. Shaw Methods)—Synthetic routes that begin with α-aminonitriles were pioneered by Cook, Heilbron, G. Shaw, and coworkers and have proved to be exceptionally important in syntheses of 5-aminoimidazoles (XXXVI–XLII), including the nucleotides of the biosynthetic pathway (Part III). In the first route (Scheme IV) (70–75), reactions of isothiocyanates (XLV) with α-aminonitriles (XLVI) produce 2,5-diaminothiazoles (XLVIII) *via* thioureas (XLVII), which may or may not be isolated. Under the influence of mild aqueous base, these 5-aminothiazoles rearrange to 5-amino-2-mercaptoimidazoles (XLIX, or the tautomeric 5-amino-2-thioxoimidazolines). The group (R_1) at position 1 is derived from the isothiocyanate, whereas the substituent (A) at position 4 is that present on the α-carbon atom of the aminonitrile. When an acylisothiocyanate is one of the reactants, the acyl group appears on the 5-amino group of the imidazole derivative (L). Presumably, the 1-acylimidazole is first formed, and then acyl migration occurs under the influence of the basic conditions of the thiazole → imidazole rearrangement (70). The mercapto (or thioxo) substituent at position 2 may be removed by Raney nickel desulfurization (71–73), or it may be alkylated to afford compounds such as 2-(methylthio) and 2-(benzylthio) derivatives (75, 76). This synthetic route has produced 5-aminoimidazoles devoid of a functional substituent at position 4 (71–73), 5-aminoimidazole-4-carboxylic acid esters (73), or 5-aminoimidazole-4-carboxamides (73–75) with (XLIX, L) or without (LI, XXXVI–XXXVIII with R_2 = H) a sulfur function at position 2. Treatment of a thiazole ester (XLVIII; A = $COOC_2H_5$, R_1 = CH_3) with ammonia and subsequent desulfurization also gave amides, 1-methyl-2-mercapto-AIC and 1-methyl-AIC (XVI*b* or XXXVIII; R_1 = CH_3, R_2 = R′ = H) (73). Recently, 2-mercapto-AIC was prepared by the route XLV–XLIX (77) (see last paragraph of this section).

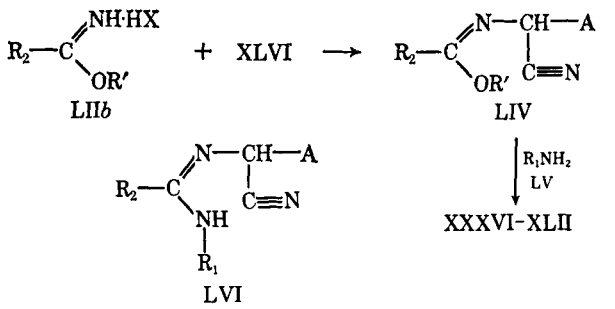
In a second route (Scheme V) (70, 74, 78, 79) based on α-



Scheme V

aminonitriles, the imidazole ring is formed by interaction of an amidine (LII*a*), an imidic acid ester (imidate) (LII*b*), or an imidic acid thioester (thioimidate) (LII*c*) with an appropriate α-aminonitrile (XLVI). This type of reaction produces 2-substituted derivatives and may be visualized as proceeding through an intermediate amidine (LIII). Again, the 5-amino group and the 4-substituent are furnished by the α-aminonitrile; the substituent at the 2-position is determined by the structure (LII) of the amidine or imidic acid derivative. This method was responsible for an early synthesis (74) of AIC from formamidine (LII*a*, R_2 = H). Soon after its role in purine biosynthesis had been discovered, AIC labeled at the 5-position with ^{14}C was prepared (80, 81) (*cf.*, Part IIC) from ethyl formimidate (LII*b*, R′ = C_2H_5) and labeled 2-aminocyanoacetamide (XLVI, A = $CONH_2$). Thioimidate (LII*c*) hydrochlorides were considered (79) to give more satisfactory results in this type of synthesis, and they were utilized in the preparation of a considerable number of 5-aminoimidazoles (XXXVI; R_1 = H, R = hydrogen, alkyl, or aryl) (79, 82, 83), 5-aminoimidazolecarboxylates (XXXVII; R_1 = H, R′ = C_2H_5) (79, 82, 83), and 5-aminoimidazole-4-carboxamides (XXXVIII; R_1 = R′ = H) (74, 84) with several different substituents at position 2. Several reported observations are of interest in the light of subsequent syntheses of this type: (*a*) the imidates (LII*b*) were used as free bases (79–81); (*b*) the thioimidate (LII*c*) hydrochloride sometimes failed to give imidazoles (or gave low yields) owing, apparently, to replacement by the α-aminonitrile of the imino group rather than the thioether group (82, 83); and (*c*) an intermediate imidate (LIV; R_2 = $C_6H_5CH_2$—, A = —$COOC_2H_5$), rather than an imidazole, was obtained when an equivalent of acid was present during the reaction of ethyl aminocyanoacetate with a starting imidate (LII*b;* R_2 = $C_6H_5CH_2$—, R′ = C_2H_5).[7] More recently, bicyclic compounds that are 1,2-disubstituted AIC derivatives by virtue of ring fusion at positions 1 and 2 of the imidazole ring have been synthesized from cyclic imidates and aminocyanoacetamide (85–90). These reactions of *N*-substituted imidates were usually performed in the presence of a catalytic amount of acid.

By preparing intermediate linear imidates (LIV), either in the isolated form or in solution, G. Shaw and coworkers (91–95) added an extra dimension of versatility to this method of synthesis. The general route (Scheme VI) is shown by Structures LII*b*, XLVI,



Scheme VI

LIV, and XXXVI–XLII. As before, the starting imidate supplies the carbon atom at position 2 with its sub-

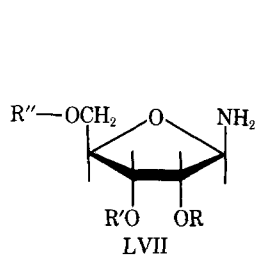
[7] Observations by Abraham, Baker, Barltrop, Chain, Waley, and Robinson cited in *Reference 79*.

stituent, but a primary amine (LV), or ammonia, then completes the imidazole ring by furnishing the nitrogen atom at position 1 with its substituent. The amidine (LVI) may be assumed to be an intermediate in this process (Scheme VI). Thus, appropriate choices of α-aminonitriles, imidates, and primary amines permit, in the absence of complications in specific cases consequent to the use of such sensitive intermediates, the introduction of various substituents at positions 1, 2, and 4 of 5-aminoimidazoles. From such linear imidates (LIV) and primary amines or ammonia, 1-alkyl and 1,2-dialkyl 5-aminoimidazoles (XXXVI, R = H) (92), 1-substituted and 1,2-disubstituted derivatives of AIC (XXXVIII, R′ = H) and of its *N*-methylamide analog (XXXVIII, R′ = CH_3) (91, 92, 96, 97), and 1-substituted 5-aminoimidazole-4-carboxylic acid esters (XXXVII, R_2 = H) (95) have been synthesized. Included among the AIC derivatives prepared by the Shaw method were 1,1′-bis derivatives (97) joined by an alkylene chain. When the primary amines were replaced in the synthetic sequence by hydrazine, a substituted hydrazine (LV, R_1 = R″R‴N—), or a semicarbazide, then 1,5-diaminoimidazoles (XL, XLI) were obtained (94, 97). Moreover, beginning the sequence with α-aminocyanothioacetamide (XLVI, A = —$CSNH_2$) produced thioamide analogs of AIC (XXXIX) (93), which have also been prepared by thiation of amides or addition of hydrogen sulfide to nitriles (Part IIF). The synthesis of the thioamides was sometimes frustrated by thiazole formation and other complications, but these difficulties could be circumvented by choosing specific esters of imidic acid hydrochlorides as reactants with aminocyanothioacetamide. Thus, benzyl acetimidate hydrochloride produced 2-methyl derivatives (XXXIX, R_2 = CH_3), whereas isopentyl formimidate (but not certain other formimidic acid esters) gave thioamides unsubstituted (R_2 = H) at position 2. (However, in a nonaqueous solvent the isopentyl ester gave a thiazole.) The formation of 5-amino-2-methylimidazole-4-thiocarboxamide (XXXIX; R_1 = H, R_2 = CH_3) from ethyl acetimidate free base is consistent with earlier observations (79) that imidate free bases and α-aminonitriles form 5-aminoimidazoles without a requirement for added ammonia. Only recently, after aminomalononitrile (XLVI, A = CN) became available as a result of studies related to prebiological syntheses (Part IID), has a carbonitrile (XLII) been synthesized by this route (96).

Representatives and derivatives of the general structures XXXVI–XLIV were synthesized during the course of these studies from aminoimidazoles obtained from the basic routes. Thus, carbonitriles (XLII) were also prepared by dehydration of some 1-alkyl- and 1,2-dialkyl-AIC derivatives with pyrophosphoryl chloride (96). Earlier, representatives of XLII had been obtained by treating thioamides (XXXIX) with mercuric chloride and methylamine (93). 1-Cyclohexyl derivatives of XXXVIII (1-cyclohexyl-AIC and some of its *N*-alkyl derivatives), as well as an analogous *N,N*-disubstituted amide, were synthesized from 1-cyclohexyl-AI-COOH by the active ester, mixed anhydride, or dicyclohexylcarbodiimide methods of peptide synthesis (98, 99). Since the ultimate aim of much of the earlier work on the routes from α-aminonitriles was the synthesis of purines, further transformations of the initially obtained aminoimidazole derivatives were directed toward that end. Conversion of the amino group to ureido and to thioureido groups with isocyanates and isothiocyanates, respectively, afforded urea and thiourea derivatives (XLIII, XLIV) (70, 73, 75, 78, 79, 82, 83, 100–102) for cyclization to purines. Alkylation of the imidazole ring with diazomethane, methyl iodide, or methyl sulfate gave some 1-methyl-4-aminoimidazoles (XLIV, X = H) (101, 102) for the preparation of *N*-alkylpurines *via* urea or thiourea derivatives.

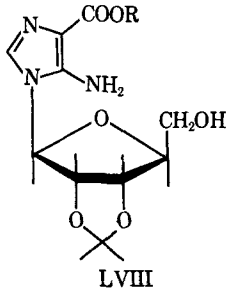
The linear imidates (LIV, A = $COOCH_3$ or $CONH_2$) derived from methyl α-aminocyanoacetate or α-aminocyanoacetamide served not only as sources of alkyl and aryl derivatives but also as precursors of the biosynthetic ribonucleotides (Part III) and the corresponding ribonucleosides. Interaction of the appropriate linear imidate and 2,3,5-tri-*O*-benzoylribofuranosylamine (LVII*a*) (103) gave, after removal of protecting groups, AIC ribonucleoside (XVI*a*) (92) and the methyl ester of AI-COOH ribonucleoside (95, 104). Although 5-aminoimidazole-4-carboxylic acids readily undergo decarboxylation in the acid form, AI-COOH ribonucleoside could be obtained as its calcium salt (95) or the pyridine salt of its isopropylidene derivative (LVIII*a*) (98) after alkaline hydrolysis of derivatives of the ethyl or methyl esters. AIC ribonucleoside was also prepared (105) by amidation of the isopropylidene derivative (LVIII*b*) (95, 104, 105) of AI-$COOCH_3$. The ribofuranoside analogs, 1-galactosyl-2-methyl-AI and 2-methyl-1-xylopyranosyl-AIC, were synthesized from glycosylamines and linear imidates (LIV) (92).

The ribonucleotides were first synthesized *via* the linear imidate method (*cf.*, Part IIE) by phosphorylating the 5′-hydroxyl group of isopropylidene derivatives of the nucleosides with 2-cyanoethylphosphate and dicyclohexylcarbodiimide (106) or with pyrophosphoryl chloride (107). Thus, phosphorylation of the isopropylidene derivative (LVIII*b*) of AI-$COOCH_3$ ribonucleoside and subsequent removal of protecting groups led to the isolation of AI-COOH ribonucleotide (Part III) (95, 104) and its methyl ester (105) in the form of their barium salts. Succino-AICAR (Part III) was then synthesized in 15% yield from LVIII*b* by a sequence of operations consisting of coupling the pyridine salt of LVIII*a* with dimethyl aspartate in the presence of dicyclohexylcarbodiimide, phosphorylation, removal of protecting groups, and purification by ion-exchange methods (98, 108). Subsequently, this sequence of steps was employed for the preparation of analogs of succino-AICAR in which the aspartyl moiety is replaced by other amino acid residues (LIX) (109); a similar sequence of steps that began with the pyridine salt of LVIII*a* and proceeded *via* active esters—the 2,4-dinitrophenyl and the pentachlorophenyl esters (LVIII*c*, LVIII*d*)—also produced succino-AICAR (99). From nucleosides obtained by the linear imidate method, AICAR (Part III) was prepared by the following methods (99, 105) (including the usual deblocking and purification operations): (*a*) phosphorylation of



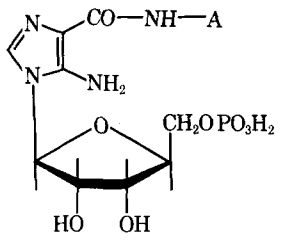
LVII

a, R = R′ = R″ = C_6H_5CO—
b, R + R′ = isopropylidene, R″ = H_2O_3P—



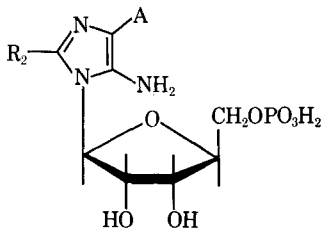
LVIII

a, R = H
b, R = CH_3
c, R = 2,4-dinitrophenyl
d, R = pentachlorophenyl



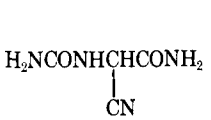
LIX

—NH—A derived from the following amino acids: D-aspartic; L-, D-, and DL-*threo*-β-methylaspartic; DL-*erythro*-β-methylaspartic; DL-β,β-dimethylaspartic; L-glutamic; and glycine.

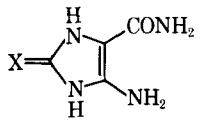


LX

a, R_2 = H, A = CN
b, R_2 = H, A = $CONHCH_3$
c, R_2 = H, A = $CON(C_2H_5)_2$
d, R_2 = CH_3, A = $CONH_2$



LXI



LXII

a, X = O
b, X = S

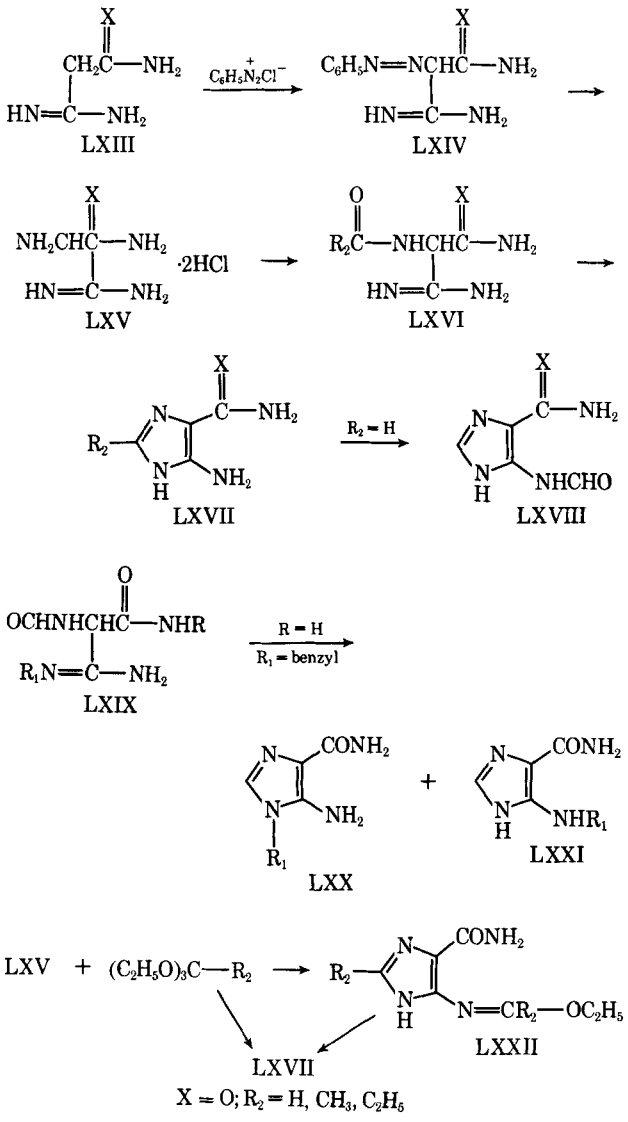
LVIII*b* followed by amidation (40–50% yield) of the ester group with ammonia; (*b*) phosphorylation of the 2′,3′-*O*-isopropylidene derivative of AIC ribonucleoside; (*c*) amidation of the pyridine salt of LVIII*a* with ammonia in the presence of dicyclohexylcarbodiimide; and (*d*) phosphorylation of the active ester LVIII*c* followed by amidation. Method *a* is apparently superior to the other three methods. Subsequently, it was found (96) that the major product of phosphorylation of isopropylidene AIC ribonucleoside with pyrophosphoryl chloride is, after deblocking, AI-CN ribonucleotide (LX*a*) formed by concomitant dehydration of the amide group. As mentioned, alkyl derivatives of AIC were similarly dehydrated to nitriles. The last method (*d*) applied in the reverse order, *i.e.*, amidation followed by phosphorylation, also yielded the *N*-methyl (LX*b*) and *N*,*N*-diethyl (LX*c*) analogs of AICAR (99).

After some of these syntheses of ribonucleotides from ribofuranosides had been performed, 2′,3′-*O*-isopropylideneribosylamine 5′-phosphate (LVII*b*) was synthesized (110, 111) and used for syntheses of imidazole ribonucleotides directly from the linear imidate intermediates. The ribonucleotides of AIC and AI-$COOCH_3$ were obtained from reactions of LVII*b* with LIV (A = —$CONH_2$ or —$COOCH_3$) (110, 111), and 2-methyl-AIC ribonucleotide (LX*d*) was similarly formed in very low yield (111).

A synthesis of 2-hydroxy-AIC (or the tautomeric 5-amino-2-oxoimidazoline-4-carboxamide, LXII*a*) and of ^{13}C-labeled LXII*a* is similar to the thiourea–thiazole method of Cook, Heilbron, and coworkers. The urea (LXI) obtained from aminocyanoacetamide (XLVI, A = —$CONH_2$) cyclized in aqueous base to LXII*a* (112). The analogous 2-mercapto-AIC (LXII*b*), mentioned previously, was formed from XLVI and potassium thiocyanate in acidic solution without identification of intermediates (77).

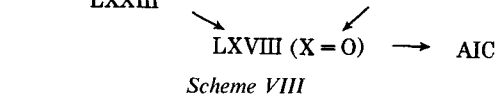
C. From Aminomalonic Acid Derivatives (E. Shaw Method)—Soon after AIC had been identified as a biologically significant compound, E. Shaw and Woolley (113) devised a new synthesis (Scheme VII) that is also applicable to similar aminoimidazoles. The basic intermediate is a three-carbon unit, typified by malonamamidine (LXIII, X = O), obtainable from nitriles such as ethyl cyanoacetate and malononitrile. The 2-amino group is introduced by coupling an aryldiazonium salt at the active methylene group of LXIII and reducing the resulting azo derivative (LXIV). Reduction with zinc in formic acid affords a formamide derivative (LXVI, R_2 = H) that can be thermally cyclized to the imidazole (LXVII). In this way, AIC was synthesized (Scheme VII) from malonamamidine (LXIII, X = O) (113), and the analogous carboxamidine (LXVII; R_2 = H, X = NH) was prepared from malonodiamidine (LXIII, X = NH) (114). Both gave formyl derivatives (LXVIII) on treatment with formic acid and acetic anhydride (114). Similarly, from ethyl chloroformate and aminomalonodiamidine (LXV, X = NH), which was isolated by substituting hydrochloric acid for formic acid in the reduction of the phenylazo derivative (LXIV), 5-amino-2-hydroxyimidazole-4-carboxamidine (LXVII; R_2 = OH, X = NH) was obtained (Scheme VII) (115), and radioactive AIC labeled with ^{14}C at position 5 was synthesized (116) by the E. Shaw route (see Part IIB for labeled AIC). The phenylazo derivative (LXIV) can also be reduced catalytically (117), and the cyclization of LXVI (R_2 = H, X = O) is more conveniently effected in refluxing formic acid (117) or with triethyl orthoformate (118, 119). 5-Formamidoimidazole-4-carboxamide (LXVIII, X = O) obtained by the first modification of the procedure is easily hydrolyzed to AIC.

The adaptability of the method for the introduction of other substituents was demonstrated with syntheses of *N*-benzyl-AIC from *N*-benzyl cyanoacetamide *via* LXIX with R_1 = H and R = benzyl (120); 2-methyl-AIC *via* LXVI (R_2 = CH_3) by substituting acetic acid for formic acid in the reduction (121); and the



Scheme VII

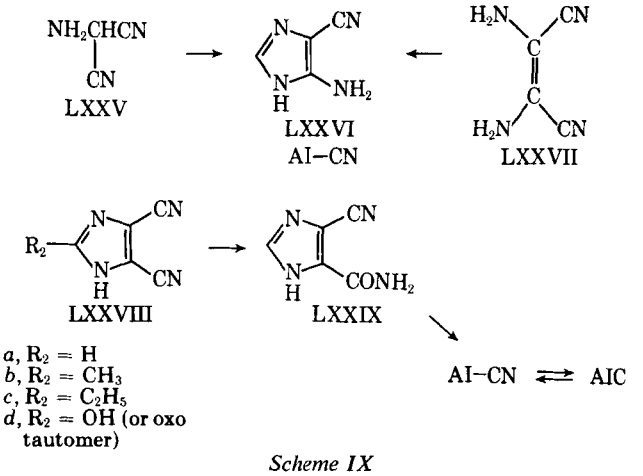
N-benzylamidine (LXIX; R = H, R_1 = benzyl) analog of LXVI. Intermediates of the latter type can cyclize to either a 1-substituted AIC (LXX) or to an AIC substituted on the amino group (LXXI). Either of the two benzyl AIC derivatives could be obtained as the major product by properly choosing the reaction conditions (122). A further modification by Richter *et al.* (118) yields 2-substituted AIC derivatives. The reaction of aminomalonamamidine dihydrochloride (LXV, X = O) (112, 113) with a molar equivalent of an orthoester gives a 2-substituted AIC derivative (LXVII); an excess of the orthoester produces an alkoxy alkyl (or aryl) methyleneamino derivative (LXXII), which can be cyclized to a purine or hydrolyzed to the AIC derivative (LXVII).



Scheme VIII

Another route (Scheme VIII) (123, 124) to AIC that begins with malononitrile and proceeds through oximinomalonodiamidoxime (LXXIII) (125) is essentially a variation of the E. Shaw method. Nitrosation, rather than azo coupling, provides the precursor group at the 2-position, and reaction of hydroxylamine at the nitrile groups then gives the three-carbon unit (LXXIII) similar to LXIV. Reduction of LXXIII with zinc and formic acid gives formyl-AIC (LXVIII). Alternatively, the furazan (1,2,5-oxadiazole) (LXXIV) (125) may be prepared from LXXIII and aqueous base (or from malononitrile without isolating LXXIII) and converted to formyl-AIC by treatment with formic acid and reduction with zinc–formic acid (123). Reduction of other furazans or furazanopyrimidines obtained from LXXIV also gave 5-formamidoimidazole-4-carboxamide (LXVIII) (123).

D. From Hydrogen Cyanide—Interest in prebiotic synthesis of purines stimulated investigations of simple precursor molecules that give rise to adenine (126, 127). Oró and Kimball (128) proved that AIC and 5-aminoimidazole-4-carboxamidine (LXVII; X = NH, R_2 = H) (AI-amidine), as well as formamidine, were among products formed from hydrogen cyanide and aqueous ammonia. They proposed (128, 129) a multistep route to these compounds which included formamidine and 2-aminomalononitrile, a hydrogen cyanide trimer. Ferris and Orgel (130, 131) succeeded in preparing the long-sought (*e.g.*, 75, 132) aminomalononitrile (LXXV) and showed that interaction of this compound with formamidine in ethanol did, indeed, give an imidazole, 5-aminoimidazole-4-carbonitrile (LXXVI, AI-CN), which was isolated in 35% yield[8] (Scheme IX). This imidazole can be hydrolyzed to AIC



Scheme IX

(131, 133, 134) or prepared from it by dehydration with thionyl chloride in pyridine (130, 131) or with phosphorus oxychloride (135).

However, aminomalononitrile also reacted with cyanide in aqueous solution (130, 131) to give the more stable hydrogen cyanide tetramer, diaminomaleonitrile

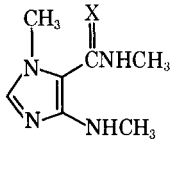
[8] This reaction is the same type as the Cook–Heilbron synthesis of AIC (74) (Part IIB).

(LXXVII) (136–138 and references cited), and both the tetramer and AI-CN were stated to be among the products of Oró's hydrogen cyanide–aqueous ammonia reactions (130, 131). Further studies (133, 139) showed that the yields of AI-CN were 5–50%, depending on reaction conditions, from formamidine and aminomalononitrile in *aqueous* solutions; that reaction of the latter compound with cyanide to the tetramer (LXXVII), however, was faster and proceeded in yields of 60–80%; that the tetramer also produced very low yields of AI-CN, AIC, and AI-amidine by reaction with formamidine; and that formation of the tetramer and its subsequent reaction with formamidine could be responsible for AI-CN, AIC, and the amidine formed in solutions of HCN and aqueous ammonia. In dilute aqueous solutions, the tetramer was converted photochemically *via* its *trans*-isomer (139, 140) to AI-CN in yields as high as 80% (139). (However, AI-CN is subject to photochemical degradation, and the yields quoted were determined by analytical methods.) As a result of these studies, the proposed steps in the pathway from hydrogen cyanide and ammonia to imidazoles and purines were revised to place greater emphasis on the role of the tetramer (LXXVII) (139, 141, 142).

Since the aim of these studies was to demonstrate the formation of purines under postulated primitive earth conditions, much of the work was performed with dilute aqueous solutions; yields were only of secondary importance. Wakamatsu *et al.* (143) and Yamada *et al.* (144, 145) then found, however, that anhydrous solutions of ammonia and hydrogen cyanide (or sodium cyanide and ammonium chloride) yield adenine and imidazole-4,5-dicarbonitrile (LXXVIII*a*) in addition to the tetramer (144) and a pyrimidopyrimidine (146). Yields of LXXVIII*a*, determined analytically, under optimal conditions were only about 20% (144), but the starting materials are simple. In anhydrous ammonia, the formation of LXXVIII*a* from the tetramer was attributed to reversion of the tetramer to aminomalononitrile (146).

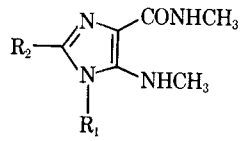
Prior to these studies of either the aqueous or the anhydrous ammonia–hydrogen cyanide systems, the tetramer (LXXVII) had been converted to imidazole-4,5-dicarbonitriles (LXXVIII*a–c*) with triethyl orthoesters (147), to the 2-oxoimidazoline (LXXVII*d*, OH-tautomer) with phosgene (148), and to LXXVIII*b* with ethyl acetimidate (149). The relevance of the formation of dinitriles from hydrogen cyanide or its oligomers to aminoimidazoles is that AI-CN and AIC were prepared from LXXVIII*a* in good yields by the following sequence of steps: partial hydrolysis to the monoamide (LXXIX) (150, 151), a Hofmann hypobromite reaction to AI-CN (150, 152), and alkaline hydrolysis to AIC (150 and references already cited). AI-CN, LXXVIII*a* and its 1-substituted derivatives, and other dicarbonitriles derivable from hydrogen cyanide or its oligomers are potential precursors of other aminoimidazole derivatives (153–155).

E. By Ring Cleavage of Purines—Much of the early work on 5-amino-4-substituted imidazoles was motivated by plans to form a pyrimidine ring from the substituents and, thereby, to produce purines. The reverse process, cleavage of the pyrimidine ring of purines, has



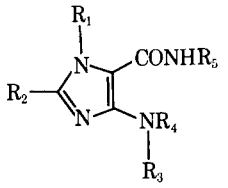
LXXX

a, X = O
b, X = S



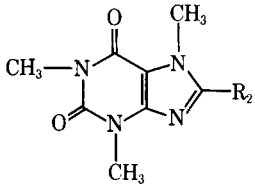
LXXXI

a, $R_1 = R_2 = H$
b, $R_1 = CH_3$; R_2 = H, CH_3, $NH_2CH_2CH_2$—
c, R_1 = H; $R_2 = NO_2$
d, R_1 = H; $R_2 = NH_2$, Cl, Br

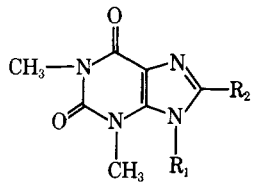


LXXXII

a, R_3 = COOH; $R_1 = R_4 = CH_3$;
R_2 = H or COOH; $R_5 = CH_3$ or C_2H_5
b, $R_2 = R_3$ = H; $R_4 = R_5 = CH_3$;
R_1 = OH or 2,3-dihydroxypropyl
c, $R_1 = R_4 = R_5 = CH_3$; R_2 = H; $R_3 = CH_3$ or C_2H_5
d, $R_1 = R_5 = C_6H_5CH_2$—; $R_2 = R_3 = R_4$ = H
e, R_1 = glucopyranosyl; $R_2 = R_3 = R_4$ = H;
R_5 = H or glucopyranosyl
f, $R_1 = R_2 = R_3 = R_5$ = H; $R_4 = CH_3$
g, $R_1 = R_3 = R_5 = C_6H_5CH_2$—; R_2 = H; R_4 = CHO



LXXXIII

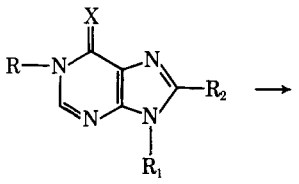


LXXXIV

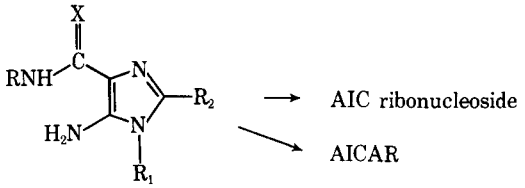
furnished 5-aminoimidazole-4-carboxylic acid derivatives and has become increasingly important as a route to these compounds. Imidazoles obtained by this method have sometimes been formed inadvertently during studies of purines, sometimes as a means of establishing the structure of certain purines, and sometimes by design. The formation of imidazoles from purines is part of the larger area of pyrimidine-ring cleavage and rearrangement reactions of fused pyrimidine heterocycles such as pteridines, quinazolines, 8-azapurines, and thiadiazolopyrimidines. The products of cleavage of these ring systems are usually similar in structure to the aminoimidazolecarboxylic acid derivatives under consideration here. Correlation of findings from these areas is obviously beyond the scope of this review. The following discussion simply narrates the historical highlights and attempts to record most of the known examples of formation of derivatives of the aminoimidazolecarboxylic acid-type from purines.[9]

The action of barium, sodium, or potassium hydroxide solutions on the methylated purines (caffeine, theophylline, and theobromine) provided the first

[9] Demonstrations of the formation of imidazoles by ring opening of purines have not always included isolation, nor have the yields always been of practical significance.



LXXXV



LXXXVI

LXXXVI*a–k*

a, R = $C_6H_5CH_2$—; *b*, R = *p*-$CH_3C_6H_4SO_2$—; *c*, R = NH_2—; *d*, R = CH_3OCH_2—
for LXXXVI*a–d*: X = O, R_2 = H, R_1 = β-D-ribofuranosyl
e, R = CH_3OCH_2—; *f*, R = $HOOCCH_2CH_2$—
for LXXXVI*e–f*: X = O, R_2 = H, R_1 = β-D-ribofuranosyl 5′-(dihydrogen phosphate)
g, X = O, R = R_1 = $C_6H_5CH_2$—, R_2 = H
h, X = O, R = CH_3, R_2 = H, R_1 = 2′,3′-*O*-ethoxymethylene-β-D-ribofuranosyl
i, X = O, R = glucopyranosyl, R_1 = H or glucopyranosyl, R_2 = H
j, X = S, R = benzyl, R_1 = β-D-ribofuranosyl, R_2 = H
k, formyl derivative of LXXXVI with X = S, R = R_2 = H, R_1 = β-D-ribofuranosyl 5′-(dihydrogen phosphate)

Scheme X

examples of this method of synthesis. Caffeidine [1, *N* - dimethyl - 4 - (methylamino)imidazole - 5 - carboxamide] (LXXX*a*) was obtained from caffeine (LXXXIII, R_2 = H) (156–158 and earlier references cited in these publications) and from 8-carbamoylcaffeine (LXXXIII, R_2 = $CONH_2$) (159). The initial products resulting from alkaline degradation of caffeine, 8-carbamoylcaffeine, and 1-ethyltheobromine (160) were believed to be the carbamic acids (LXXXII*a*) (158, 161). Later, thiocaffeine and theophylline (LXXXIV, R_1 = R_2 = H) were degraded by alkaline conditions to thiocaffeidine (LXXX*b*) (162) and to theophyllidine (LXXXI*a*) (163), respectively. More recently, some isocaffeines (LXXXIV, R_1 = CH_3) were similarly cleaved to 1,*N*-dimethyl-5-(methylamino)imidazole-4-carboxamides (LXXXI*b*) (164), and some 7- and 8-substituted theophyllines produced (165) 1-substituted *N*-methyl-4 - (methylamino)imidazole - 5 - carboxamides (LXXXII*b*) and a 2-substituted theophyllidine (LXXXI*c*), respectively (165). Certain 2-substituted theophyllidines (LXXXI*d*) (165) were also obtained from 2-nitrotheophyllidine. Methylation (55, 158, 166) and ethylation (156) of caffeidine gave dialkylamino derivatives (LXXXII*c*), and the methylamino group of LXXX*a* was converted to benzoyl, urea, and nitroso derivatives (158). Caffeidine has also been transformed to its methyl ester analog *via* the formyl derivative of its methylamino group and the nitroso derivative of its amide group (167).

Purines not substituted on the ring-nitrogen atoms are ionizable in alkaline media. The anion formed is then stabilized to attack by nucleophiles. In the methylated derivatives just discussed, the two carbonyl groups in the pyrimidine ring, as well as the *N*-alkyl substituents, contribute to labilization of the purine system. During the year that the degradation of theophylline was recorded, the initial reports (120, 168) of the extensive investigations of purine *N*-oxides by Brown (169) and coworkers and of the purposeful labilization of the purine ring by E. Shaw appeared. These series of publications served to emphasize the potential utility of purines substituted on ring-nitrogen atoms for imidazole formation. Prior evidence, to be presented, by Baker and Joseph (170) of the increased susceptibility of nitrogen-substituted purines to ring opening may have gone unrecognized because of the specialized (cyclonucleoside) structure.

E. Shaw synthesized (Scheme X) AIC ribonucleoside (*cf.*, Parts IIA and IIB) from inosine (LXXXV; R = R_2 = H, R_1 = β-D-ribofuranosyl) or its triacetate by first labilizing the pyrimidine ring by placing a substituent (R) at position 1. The first method (120) consisted of alkaline ring cleavage of 1-benzylinosine and removal of the benzyl group of *N*-benzyl-AIC ribonucleoside (LXXXVI*a*) with sodium and liquid ammonia. The overall yield suffered because of the difficulty of the debenzylation step. The second method (171) consisted of tosylation of inosine triacetate, alkaline ring opening of 1-(*p*-toluenesulfonyl)inosine triacetate, hydrazinolysis of the resulting *N*-(*p*-toluenesulfonyl)-AIC ribonucleoside (LXXXVI*b*), and reductive fission of the acid hydrazide (LXXXVI*c*) to the amide (AIC ribonucleoside). A third method (172) gave AIC ribonucleoside from inosine triacetate and AICAR from the di(*p*-nitrophenyl) ester of the 2′,3′-*O*-isopropylidene derivative of inosinic acid [inosine 5′-(dihydrogen phosphate) or IMP, Part III] as follows: the methoxymethyl group was introduced at position 1, the pyrimidine ring was again opened under alkaline conditions, the labilizing methoxymethyl group was simultaneously removed hydrolytically from the carboxamide group of LXXXVI*d* and of the dinitrophenyl isopropylidene derivative of LXXXVI*e*, and the protecting groups were removed. This method constituted the first chemical synthesis of AICAR. The β-alanine analog (LXXXVI*f*) of succino-AIC ribonucleotide was similarly prepared by employing the principle of ring labilization (173). Inosinic acid was alkylated with propiolactone at position 1 by careful control of the pH; alkaline ring opening of 1-(2-carboxyethyl)inosinic acid then gave LXXXVI*f*.

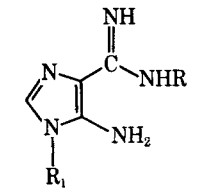
Other aminoimidazolecarboxamides have been formed by alkaline cleavage of substituted hypoxanthines—mostly 1-substituted derivatives—as follows: 1,*N*-dibenzyl-AIC (LXXXVI*g*) (174) from 1,9-dibenzylhypoxanthine (LXXXV; X = O, R = R_1 = benzyl, R_2 = H); 4-amino-1,*N*-dibenzylimidazole-5-carboxamide (LXXXII*d*) from 1,7-dibenzylhypoxanthine (175); the ethoxymethylene derivative of *N*-methyl-AIC ribonucleoside (LXXXVI*h*) (176) from the corresponding 1-methylinosine; several glucopyranosyl-AIC derivatives (LXXXVI*i*, LXXXII*e*) (175) from various glucopyranosylhypoxanthines; and 5-(methylamino)imidazole - 4 - carboxamide (LXXXII*f*) (177) from 3-methylhypoxanthine, 3-methyladenine, or 6-(dimethylamino)-3-methylpurine. The last two purines may have been converted to 3-methylhypoxan-

thine prior to ring opening. Cleavage of hypoxanthinium salts is outlined near the end of this section (IIE).

In addition, ring opening of certain purine-6(1*H*)-thiones (LXXXV, X = S) (6-mercaptopurines) has resulted in imidazole formation. 1-Benzyl-9-β-D-ribofuranosylpurine-6(1*H*)-thione, the sulfur analog of 1-benzylinosine, was cleaved to AI-CSNHCH$_2$C$_6$H$_5$ ribonucleoside (LXXXVI*j*) (178); 1-methylpurine-6-(1*H*)-thione (LXXXV; R = CH$_3$, R$_1$ = R$_2$ = H) gave AIC on treatment with aqueous ammonia (179); and 5-formamido-1-β-D-ribofuranosylimidazole-4-thiocarboxamide 5′-(dihydrogen phosphate) (formyl-thio-AIC ribonucleotide, LXXXVI*k*) was reported (180) to be formed from 6-mercaptopurine ribonucleotide pyridinium salt.

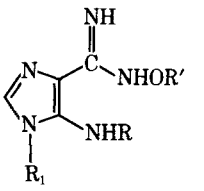
As mentioned, initial investigations of purine *N*-oxides and 1-substituted hypoxanthines were reported almost simultaneously. Although the isolation of 4-guanidinoimidazole after vigorous acidic hydrolysis of guanine (181) and of AIC after treatment of hypoxanthine with zinc and sulfuric acid (182) has been reported, acidic ring cleavage of the common purines unsubstituted on ring-nitrogen atoms usually results in extensive degradation (183, 184 and references cited). However, under milder, but still strenuous, conditions, it was possible to isolate 5-aminoimidazole-4-carboxamidine (LXXXVII*a*) in 10% yield by acidic hydrolysis of adenine (183). In contrast, the oxide of adenine prepared by Stevens and Brown (168) was cleaved under much less vigorous conditions to 5-aminoimidazole-4-carboxamidoxime (LXXXVIII*a*) in yields in excess of 75% (168, 185). This reaction established the structure of the oxide as adenine 1-*N*-oxide (LXXXIX*a*); catalytic reduction of the carboxamidoxime produced the amidine (LXXXVII*a*) (*cf.*, Parts IIC and IID), and hydrolysis gave AIC. Formation of the amidoxime by acidic ring opening of 2-methyladenine 1-*N*-oxide (LXXXIX*b*) (186) and of the 2′-, 3′-, and 5′-phosphates of adenosine 1-*N*-oxide (LXXXIX*c*) (with the expected concomitant loss of the ribofuranosyl group) was also demonstrated (187). Ring cleavage of adenosine 1-*N*-oxide and its 2′-, 3′-, and 5′-phosphates by aqueous sodium hydroxide produced, respectively, the ribonucleoside (LXXXVIII*b*) and the 2′-, 3′-, and 5′-phosphates of LXXXVIII*b* (187).

Cleavage of 1-*N*-oxides by acetic anhydride apparently proceeds through the initial acetylation of the *N*-oxide group (186). Adenine 1-*N*-oxide and 2,6-diaminopurine 1-*N*-oxide were cleaved by refluxing acetic anhydride, and an oxadiazole ring was formed by the reagent; the product was a 3-(5-acetamidoimidazol-4-yl)oxadiazole (XC*a*), which was obtained also from LXXXVIII*a* and acetic anhydride. The 2-acetoxy derivative (XC*b*) was likewise formed from 8-hydroxyadenine 1-*N*-oxide. Under milder conditions the product obtained from adenine 1-*N*-oxide was the formamide (XC*c*), and XC*a* was evidently formed from it by an acyl-exchange reaction. Both XC*a* and XC*b* were hydrolyzed under acidic conditions to the 5-aminoimidazole derivatives (XC*d* and XC*e*) (186). In other studies of purine oxides, 4-acetyl-5-aminoimidazole was obtained by acid hydrolysis of 6-methylpurine



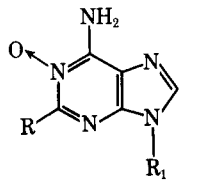
LXXXVII

a, R$_1$ = R = H
b, R = H, R$_1$ = β-D-ribofuranosyl
c, R = CH$_3$, R$_1$ = H
d, R = benzyl, R$_1$ = H
e, R = —CH$_2$COOH, R$_1$ = H



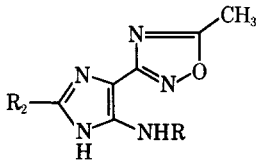
LXXXVIII

a, R$_1$ = R = R′ = H
b, R = R′ = H, R$_1$ = β-D-ribofuranosyl
c, R = CHO, R$_1$ = R′ = C$_2$H$_5$
d, R = H, R′ = benzyl, R$_1$ = β-D-ribofuranosyl



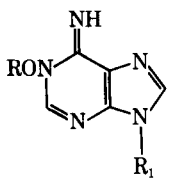
LXXXIX

a, R = R$_1$ = H
b, R = CH$_3$, R$_1$ = H
c, R = H, R$_1$ = β-D-ribofuranosyl



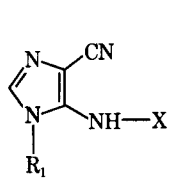
XC

a, R = —COCH$_3$, R$_2$ = H
b, R = —COCH$_3$, R$_2$ = —OCOCH$_3$
c, R = —CHO, R$_2$ = H
d, R = R$_2$ = H
e, R = H, R$_2$ = OH



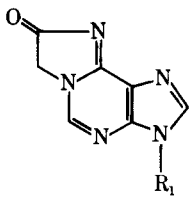
XCI

a, R = R$_1$ = C$_2$H$_5$
b, R = benzyl, R$_1$ = β-D-ribofuranosyl



XCII

a, X = —CONH$_2$, R$_1$ = β-D-ribofuranosyl
b, R$_1$ = X = CH$_3$

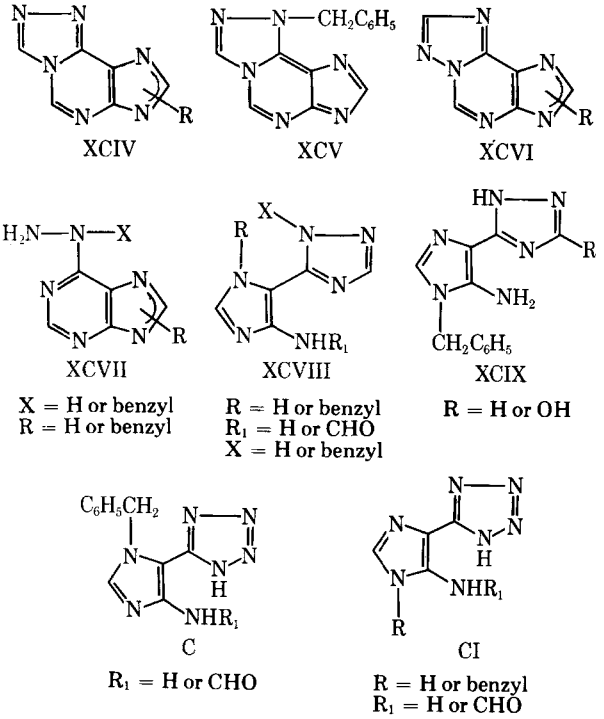


XCIII

a, R$_1$ = H
b, R$_1$ = CH$_3$

1-*N*-oxide (188); the amidoxime (LXXXVIII*a*) and AIC were minor components of the acid hydrolysate of 1-hydroxyisoguanine (189); 5-chloro-AIC was one of several products of the action of hydrochloric acid on hypoxanthine 3-*N*-oxide (190); and 1-β-D-ribofuranosyl - 5 - ureidoimidazole - 4 - carbonitrile (XCII*a*) was one of the products isolated, in low yield, after photolysis of adenine 1-*N*-oxide (191).

Syntheses of imidazoles from purine *N*-oxides and from purines having an alkyl group on a ring-nitrogen atom converged when the *N*-oxide group was alkylated to 1-alkoxy derivatives (XCI) (192, 193). As 1-substituted adenine derivatives, such compounds are highly susceptible to the well-known rearrangement, generally believed to involve ring opening and reclosure, that results in placing the 1-substituent on the 6-amino group. This reaction was, indeed, observed to occur with 1-alkoxyadenines (192). However, the substituted imidazolecarboxamidoxime (LXXXVIII*c*) could be isolated in high yield by cleavage of XC*a* in neutral solution at low temperature (192). Likewise, 1-benzyloxyadenosine (XCI*b*) was cleaved in methanolic ammonia to a substituted imidazolecarboxamidoxime (LXXXVIII*d*) (193). Reductive removal of the benzyloxy group gave the amidine analog (LXXXVII*b*) of AIC ribonucleoside, and diazotization of LXXXVII*b*

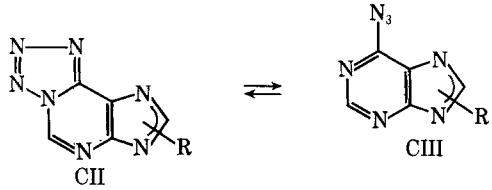


provided a convenient synthesis of 2-azaadenosine. [2-Azaadenine 1-*N*-oxide and 2-azaadenosine 1-*N*-oxide had also been prepared (194) by diazotizing the amidoximes LXXXVIII*a*, LXXXVIII*b*.] At this point it may be recalled that ring fission of adenine 1-*N*-oxide by acetic anhydride, discussed previously, evidently proceeds *via* a 1-acyloxy derivative (186) analogous to the 1-alkoxyadenines.

Although 1-alkyladenines readily rearrange in basic media to 6-(alkylamino)purines, they may undergo cleavage to carboxamidines in acidic solutions. Brookes and Lawley showed that 5-amino-*N*-methylimidazole-4-carboxamidine (LXXXVII*c*) (195) and the *N*-benzyl derivative (LXXXVII*d*) (196) are formed by acidic cleavage of 1-methyladenine and 1-benzyladenine, respectively. Acidic degradation of 1-methyladenosine and 1-benzyladenosine removed the ribofuranosyl group and gave the same carboxamidines (LXXXVII*c*, LXXXVII*d*) (196).

Cleavage of the pyrimidine ring of a cyclic adenine derivative (XCIII*a*), formed from N^6-glycyladenine, also occurs under acidic conditions (197). The ring-cleavage products isolated—and probably formed *via* 1-carboxymethyladenine (197, 198)—were the *N*-substituted amidine LXXXVII*e* and AIC. Acidic hydrolysis of the related cyclic intermediate (XCIII*b*) from N^6-glycyl-9-methyladenine produced, among other products, a small amount of 1-methyl-AIC (XVI*b*) (199). An earlier acidic cleavage of *N*-(purin-6-yl)aspartic acid, the aglycone of adenylosuccinic acid (Part III), resulted in the formation of AIC and was postulated to involve a cyclic intermediate (200).

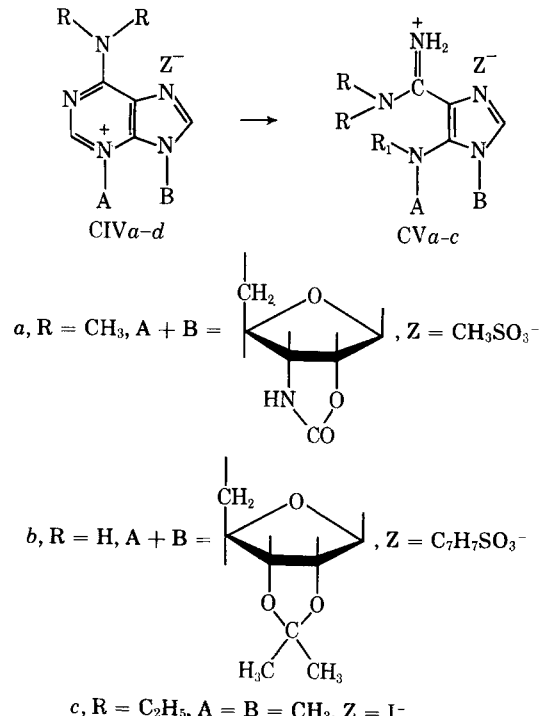
5-Aminoimidazol-4-yl heterocycles that are structurally similar to the imidazolyloxadiazoles (XC) already described have been obtained from purines to which a heterocyclic ring is fused at positions 1 and 6 (tricyclic heterocycles) (XCIV–XCVI, CII) or from



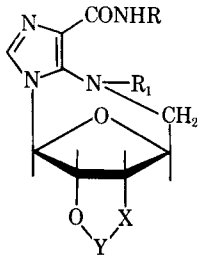
Scheme XI

purines that can form such tricyclic compounds (XCVII, CIII). Temple *et al.* (201–203), in a series of publications, described the synthesis of 4-aminoimidazol-5-yl-*s*-triazoles (XCVIII), 5-aminoimidazol-4-yl-*s*-triazoles (XCIX), and aminoimidazolyltetrazoles (C and CI) by acid- or base-catalyzed ring cleavage. The presence of a benzyl group on one of the imidazole ring-nitrogen atoms may aid, but is not necessary for, opening of the pyrimidine ring. The aminoimidazoles represented by XCVIII and XCIX (201, 203) were obtained from appropriately substituted *s*-triazolo[3,4-*i*]purines (XCIV, XCV) or from the isomeric *s*-triazolo[5,1-*i*]purines (XCVI). Furthermore, the 6-hydrazinopurines from which the *s*-triazolo[3,4-*i*]purines were prepared also underwent ring cleavage to derivatives of type XCVIII or XCIX. When formic acid, diethoxymethyl acetate, or phosgene was the reactant, cyclization to an *s*-triazolo[3,4-*i*]purine may have preceded ring opening. But, when 7-benzyl-6-hydrazinopurine was converted to XCVIII (R = benzyl, R_1 = X = H) with mineral acid, prior formation of a tricyclic system was not possible. Similarly, the 5-[amino (or formamido)imidazolyl]tetrazoles (C and CI) (202) were isolated in good yields by acidic or basic cleavage of the tetrazolopurines (CII), which are in tautomeric equilibrium with the 6-azidopurines (CIII) (Scheme XI).

Quaternized purines, positively charged in the purine ring, may be expected to be even more susceptible

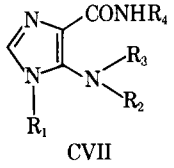


Scheme XII



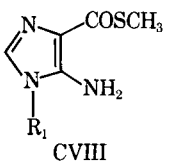
CVI

a, R = R_1 = H, X = NH, Y = CO
b, R = $C_6H_5CH_2$—, R_1 = CHO, X = O, Y = $C(CH_3)_2$
c, R = R_1 = H, X = O, Y = $C(CH_3)_2$



CVII

a, R_1 = H, R_2 = R_4 = benzyl, R_3 = CHO
b, R_1 = R_3 = H, R_2 = R_4 = benzyl
c, R_1 = R_2 = benzyl, R_3 = R_4 = H
d, R_1 = R_2 = benzyl, R_3 = CHO, R_4 = H
e, R_1 = R_2 = CH_3, R_3 = R_4 = H



CVIII

R_1 = H or CH_3

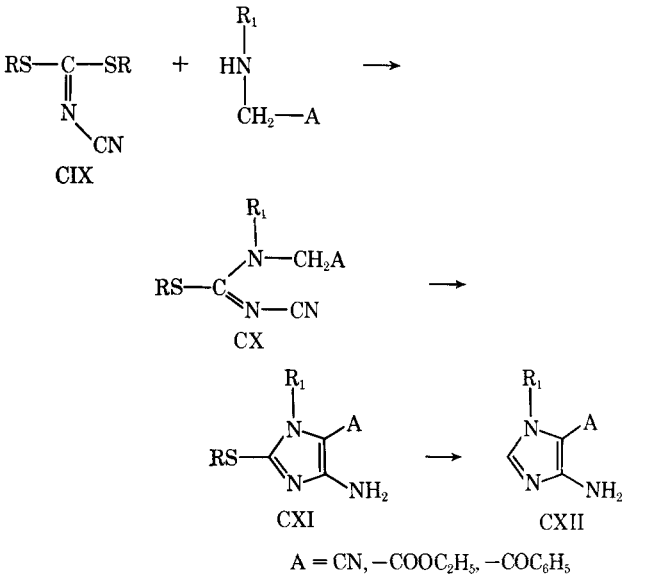
to nucleophilic opening of either the pyrimidine or the imidazole ring than are uncharged species. The studies of puromycin provided an example of pyrimidine-ring opening of a quaternary derivative. As mentioned, Baker and Joseph (170) found that the cyclonucleoside (CIV*a*) (Scheme XII) was easily cleaved by dilute base to CV*a* (R_1 = CHO). Further, mild basic treatment hydrolyzed the amidine and the formamide groups, yielding an AIC ribonucleoside analog (CVI*a*). Michelson (204) mentioned the formation of a similar amidine (CV*b*, R_1 = H) from the cyclonucleoside of 2′,3′-isopropylideneadenosine (CIV*b*); Montgomery *et al.* (174) observed the formation of an *N*-benzyl-AIC ribonucleoside analog (CVI*b*) by facile ring cleavage of the isopropylidene-protected cyclonucleoside of 1-benzylinosine, a hypoxanthinium salt. Kusashio and Yoshikawa (205) isolated small amounts of CVI*c* and carbonitrile analogs of CVI*c* (R_1 = CHO and H) during studies of the phosphorylation of 2′,3′-*O*-isopropylideneinosine with phosphorus oxychloride containing a small quantity of water or with pyrophosphoryl chloride. These imidazoles were presumably formed from the cyclonucleoside of inosine. Montgomery and his coworkers (174, 206) also found that derivatives of aminoimidazolecarboxamides were formed from quaternary salts under mild conditions as follows: CVII*a* and LXXXII*g* from 1,3-dibenzyl- and 1,3,7-tribenzylhypoxanthinium bromides, respectively; and CVII*c* from 3,9-dibenzyl-6-(dimethylamino)-purinium bromide (CIV*d*). Compounds CVII*b* and CVII*d* were prepared from CVII*a* and CVII*c*, respectively. Cleavage of the dimethylaminopurinium derivative (CIV*d*) might have proceeded by initial hydrolysis at position 6 to 3,9-dibenzylhypoxanthine; however, its similarity to cyclonucleosides (CIV*a*, CIV*b*) (Scheme XII), which first yielded the amidines (CV*a*, CV*b*), is evident, and ring opening could have preceded hydrolytic removal of the dimethylamino group. Furthermore, Marsico and Goldman (207) found that alkaline degradation of 6-(diethylamino)-3,9-dimethylpurinium iodide (CIV*c*) produced the amidine CV*c* (R_1 = H); further degradation yielded the nitrile (XCII*b*) and finally 1-methyl-5-(methylamino)imidazole-4-carboxamide (CVII*e*), which was independently synthesized by lithium aluminum hydride reduction of formyl 1-methyl-AIC. Although the foregoing studies were not designed to determine relative facility of ring opening, it appears that, in general, the quaternized derivatives yielded imidazoles under milder conditions than uncharged, ring-nitrogen-substituted derivatives.

Other reported examples of imidazole formation by ring opening include the interception of 1-methyl-4-ureidoimidazole-5-carbonitrile during the alkaline hydrolysis of 2-chloro-7-methyladenine (208); isolation of 2-(methylsulfonyl)AIC ribonucleoside (or a derivative) after treatment of 8-(methylsulfonyl)guanosine with *tert*-butoxide ion in dimethyl sulfoxide (209); and the nonenzymatic formation of AI ribonucleoside from adenosine, a ketopentose, cupric ions, and pyrophosphate (210).

Finally, an interesting acid-catalyzed ring opening of 6-(methylthio)purines was recently reported by Albert (211) and afforded the first imidazole thiol esters (CVIII), one of which was converted by ammonia to AIC.

At the beginning of this section, it was stated that ring-opening and rearrangement reactions of purines are related, but the transitory formation of imidazole derivatives during rearrangement reactions has not been treated here. During such reactions, facile reclosure of a pyrimidine ring to a more stable purine may preclude ring opening as a source of imidazoles. Likewise, the fact that the imidazole, rather than the pyrimidine, ring of purines is sometimes cleaved has not been discussed, but this fact is relevant to ring opening as a method of imidazole synthesis.

F. Miscellaneous Methods—1-Substituted-4-aminoimidazoles, which belong to the structural type furnished by the Sarasin–Wegmann method, are formed by a route (Scheme XIII) that begins with *N*-cyanoiminodithiocarbonic acid esters (CIX) (212). The imidazole



A = CN, $-COOC_2H_5$, $-COC_6H_5$

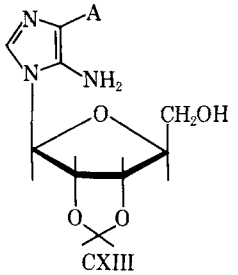
Scheme XIII

ring is formed (CXI) by base-catalyzed cyclization of the intermediate isothioureas (CX). The thioether group at position 2 may be removed with Raney nickel (CXII).

Approaches to 1-substituted derivatives of AI and AI-COOH (VIII) similar to the steps in the biosynthesis of inosinic acid have been studied by Shaw and coworkers (*cf.*, Part III). The 1-cyclohexyl and ribonucleotide derivatives of AI, the latter in admixture with formylglycinamidine ribonucleotide (FGAM), were obtained by decarboxylation of the amino acids under careful control to minimize ring opening (213). The influence of metal ions, pH, and other factors on the decarboxylation has been studied (214). Conversely, carboxylation of these two AI derivatives with bicarbonate gave the amino acids (215), and cyclization of the *N*-cyclohexyl derivatives of glycinamidine (with ethyl formimidate) and formylglycinamidine gave small amounts of 1-cyclohexyl-AI (213, 215).

Interconversions of carboxamide and carbonitrile and of thiocarboxamide and carbonitrile groups are potential sources of derivatives of all three types. Dehydration of AIC to AI-CN (LXXVI) and hydrolysis of the latter compound to AIC were described in Part IID; hydrolysis of nitrile products of the Sarasin–Wegmann method to amides was depicted in Part IIA; and conversion of some 1-alkyl-5-aminoimidazole-4-carboxamides and -thiocarboxamides to the corresponding nitriles was mentioned in Part IIB. The thiocarboxamide analog (AI-$CSNH_2$) (XXXIX, R_1 = R_2 = H) of AIC was first prepared by thiation of AIC with phosphorus pentasulfide (47, 216) and by the linear imidate method of Shaw and Butler (93) (Part IIB). Recently, thiation of AIC has been improved (217, 218), but conversion of the nitrile to AI-$CSNH_2$ with a methanolic alkaline solution of hydrogen sulfide proved to be superior (217, 219). Treatment of AI-$CSNH_2$ with formic acid and sodium formate yielded the 5-formamidoimidazole-4-thiocarboxamide (LXVIII, X = S).

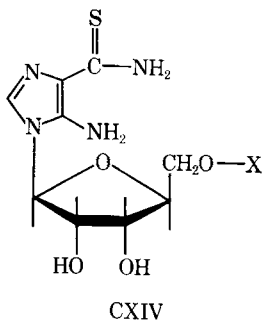
These reactions have been extended to include the syntheses of the thioamide analogs of AIC ribonucleoside and ribonucleotide (217, 219). 5-Amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carbonitrile (CXIII*a*), obtained by dehydrating AIC



CXIII

a, A = CN

b, A = $-CSNH_2$



CXIV

a, X = H

b, X = PO_3H_2

ribonucleoside with *p*-toluenesulfonylchloride in pyridine (220), was transformed by the hydrogen sulfide procedure to isopropylidene AI-$CSNH_2$ ribonucleoside (CXIII*b*). The usual mildly acidic removal of the isopropylidene group furnished the thioamide analog of AIC ribonucleoside (CXIV*a*), which reverted to the nitrile upon treatment with methyl iodide and base. Phosphorylation of CXIII*b* by the phosphorus oxychloride–trimethyl phosphate method, removal of the isopropylidene group, and application of the usual ion-exchange purification procedures yielded the thiocarboxamide analog (CXIV*b*) of AICAR.

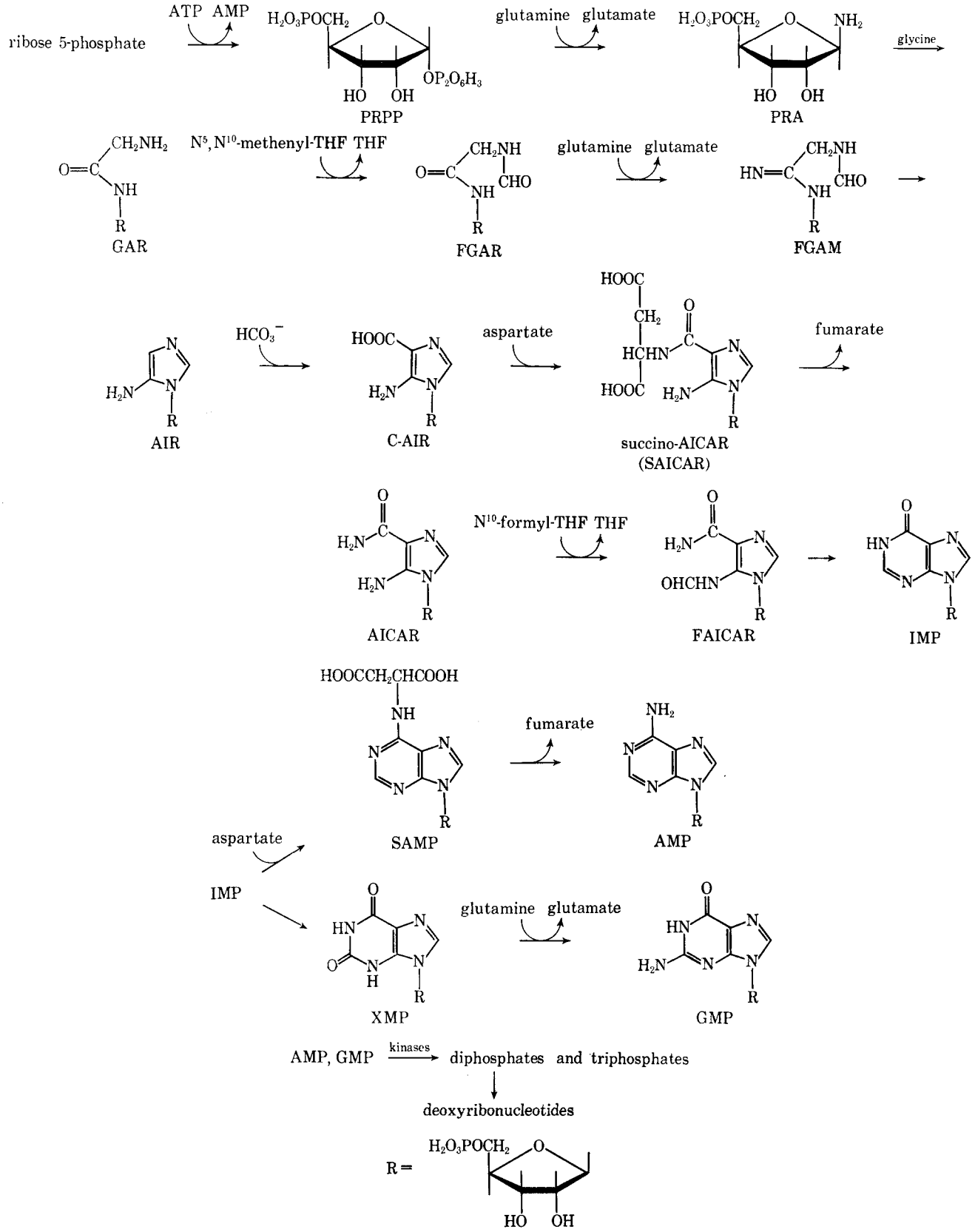
Other interconversions and further transformations of functional groups, many of which have been mentioned in the preceding sections, provide access to desired derivatives. Some examples involving the amino group are its conversion by orthoesters to alkoxyanils (50, 93, 94, 221, 222) exemplified by LXXII; the preparation of 5-formamido derivatives (93–95, 114, 117, 217), which are of potential interest because a derivative of this type is the immediate biosynthetic precursor of inosinic acid; and the formation of ureido, thioureido, and guanidino derivatives (167, 212, 223, 224, and references cited in Parts IIA and IIB).

Investigations of the biosynthesis of aminoimidazole ribonucleotides by bacteria were an integral part of fundamental studies of purine biosynthesis. Microbiological synthesis has become an important source of AIC ribonucleoside (225–232), AIC ribonucleotide (227, 233–235), and succino-AIC ribonucleotide or the analogous ribonucleoside (227, 236) as a result of the use of bacterial species, mutant strains, or yeasts that accumulate these compounds. Microbiological synthesis has provided these compounds in quantities sufficient for the employment of AIC ribonucleoside as a starting material for the synthesis of purine ribonucleosides (224, 237) and for consideration of AICAR and succino-AICAR as food-seasoning agents (Part III).

III. BIOCHEMISTRY AND BIOLOGICAL ACTIVITY OF 5-AMINOIMIDAZOLES[4]

AIC and its ribofuranosyl derivatives, alone or in combination with other metabolites or antimetabolites, have been the subjects of many biochemical, microbiological, pharmacological, and other biological investigations. These studies are too numerous to be recounted here; rather, the purpose of items 1–7 of Part III is to point out several known biological roles of aminoimidazoles in order to emphasize their importance in metabolic processes.

1. The prominent role of ribonucleotides of aminoimidazoles in the biosynthesis of purines is now well known. The postulated (3) association of AIC, or a derivative, with purine biosynthesis came during a period when studies designed to identify the ultimate sources (*e.g.*, glycine and formate) of the individual atoms of the purine ring and when investigations probing the role of folic acid in one-carbon metabolism were being intensively pursued. The identification of AICAR, rather than AIC itself, as a true precursor of purines resulted from the discoveries, along with other findings, of the precursor role of inosinic acid (IMP) for hypoxanthine (238) and of the accumulation of AIC ribonucleoside (XVI*a*) (239–243) and AICAR (240, 244) during sulfonamide bacteriostasis. The convergence and integration of these studies of simple



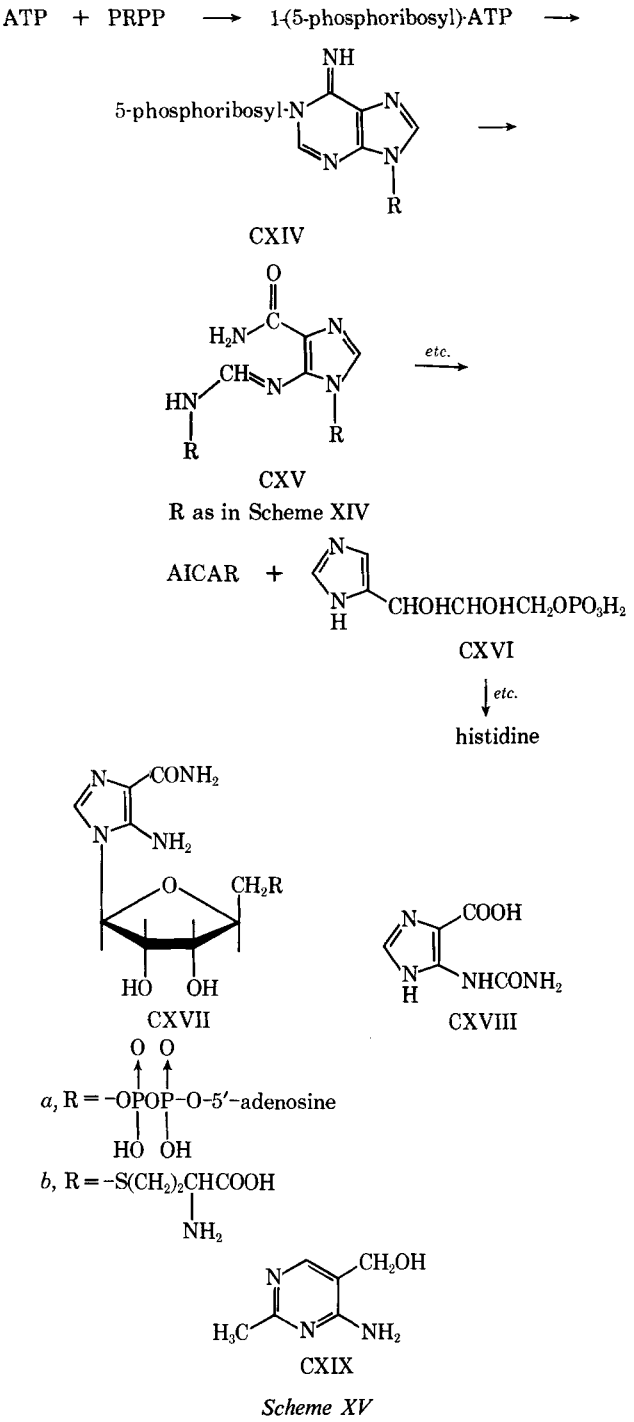
Scheme XIV—Biosynthesis of purine ribonucleotides

purine precursors, aminoimidazole derivatives, and folic acid coenzymes led to the delineation of the complete sequence of steps in the synthesis *de novo* of inosinic acid (Scheme XIV). The chronology of these events and the detailed biochemical processes of the individual steps have been reviewed (245-248) and will not be reexamined here. More recent reviews (249–252) have dealt with the effects of inhibitors of some of the

reactions of Scheme XIV,[5, 10, 11] the principal emphasis being, of necessity, on the actions of purines and purine analogs on the initial and the late steps.

The prominent role of aminoimidazole ribonucleotides in purine synthesis *de novo* is evident from Scheme XIV. Only certain salient aspects (arbitrarily selected) of the synthesis *de novo* of inosinic acid will be mentioned. (*a*) Glutamine plays a donor role in two steps (PRPP → PRA and FGAR → FGAM), and tetrahydrofolate derivatives insert a one-carbon unit in two of the steps (GAR → FGAR and AICAR → FAICAR). (Glutamine is not the only nitrogen source for PRPP → PRA. Interference with N^{10}-formyltetrahydrofolate metabolism was responsible for blocking the formylation of AIC ribonucleotide and its resulting accumulation during sulfonamide bacteriostasis.) (*b*) The formation of PRA is subject to feedback inhibition by purine derivatives and purine analogs. (*c*) Salvage pathways for the utilization of preformed purines, AIC, AIC ribonucleoside, and purine ribonucleosides are available to certain cells and organisms. (*d*) The transformations of succino-AICAR to AICAR and of adenylosuccinic acid to AMP are apparently effected by the same enzyme, adenylosuccinase (253, 254). (*e*) Biotin apparently does not participate directly in the carboxylation of AIR; impairment of aspartate synthesis was probably responsible for evidence of biotin-deficiency inhibition of this step (255).

In addition to these selected fragments of biochemical information, the following chemical findings are relevant to the role of aminoimidazoles in purine biosynthesis. (*a*) Evidence for the nonenzymatic formation of PRA from ribose 5-phosphate and aqueous ammonia has been presented (256), and derivatives of PRA have been employed in chemical syntheses of imidazole nucleotides (Part IIB). (*b*) 5-Aminoimidazole-4-carboxylic acids are easily decarboxylated to the corresponding aminoimidazoles, which are likewise unstable. Decarboxylations of AI-COOH (VIII) (34), its ribonucleoside (214), its ribonucleotide (C-AIR) (213,



Scheme XV

214, 257), and its 1-cyclohexyl derivative (XXXVII; R_1 = C_6H_{11}, R_2 = R′ = H) (95, 213, 214) occur in mildly acidic or neutral media; certain metal ions have a stabilizing influence (214). (*c*) Using AI-COOH ribonucleotide and 1-cyclohexyl AI-COOH (as a model compound), Franks *et al.* (213) have further shown that the steps from GAR to C-AIR may be caused to proceed nonenzymatically in the reverse direction simply by varying the pH and by heating. (*d*) By these degradative methods and by synthetic procedures, the open-chain precursors (GAR, FGAR, and FGAM) and the analogous cyclohexyl derivatives have been formed by purely chemical means (110, 111, 213, 258). (*e*) Nonenzymatic carboxylation of AIR with bicar-

[10] Abbreviations used in Scheme XIV are: ATP = adenosine triphosphate = adenosine 5′-(tetrahydrogen triphosphate)*; AMP = adenosine monophosphate = 5′-adenylic acid*; THF = tetrahydrofolate; PRPP = 5-phosphoribosylpyrophosphate = ribofuranose 5-(dihydrogen phosphate) 1-(trihydrogen pyrophosphate)*; PRA = 5-phosphoribosylamine = 2-amino-2-deoxyribose 5-(dihydrogen phosphate)*; GAR = glycinamide ribonucleotide = 2-amino-*N*-β-D-ribofuranosylacetamide 5′-(dihydrogen phosphate)*; FGAR = formylglycinamide ribonucleotide = 2-formamido-*N*-β-D-ribofuranosylacetamide 5′-(dihydrogen phosphate)*; FGAM = formylglycinamidine ribonucleotide = 2-formamido-*N*-β-D-ribofuranosylacetamidine 5′-(dihydrogen phosphate)*; AIR = 5-aminoimidazole ribonucleotide = 5-amino-1-β-D-ribofuranosylimidazole 5′-(dihydrogen phosphate)*; C-AIR = 5-amino-4-carboxyimidazole ribonucleotide = 5-amino-1-β-D-ribofuranosylimidazole-4-carboxylic acid 5′-(dihydrogen phosphate)*; succino-AICAR or SAICAR = 5-amino-4-imidazole-*N*-succinocarboxamide ribonucleotide = *N*-[(5-amino-1-β-D-ribofuranosylimidazol-4-yl)carbonyl]aspartic acid 5′-(dihydrogen phosphate)*; AICAR = 5-aminoimidazole-4-carboxamide ribonucleotide = 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide 5′-(dihydrogen phosphate)*; FAICAR = 5-formamidoimidazole-4-carboxamide ribonucleotide = 5-formamido-1-β-D-ribofuranosylimidazole-4-carboxamide 5′-(dihydrogen phosphate)*; IMP = inosine monophosphate = 5′-inosinic acid*; SAMP = adenylosuccinic acid = *N*-(9-β-D-ribofuranosyl-9*H*-purin-6-yl)aspartic acid 5′-(dihydrogen phosphate); XMP = 5′-xanthylic acid*; GMP = 5′-guanylic acid*. (**Chemical Abstracts* names.)

[11] See *References 247–249* for the enzyme, metal-ion, and ATP or GTP requirements for these steps and for other species (phosphate, ADP, and water) either required for or produced in the various steps. Systematic enzyme names are given in *Report of the Commission on Enzymes of the International Union of Biochemistry*, Pergamon Press, New York, N. Y., 1961.

bonate at 85° has been demonstrated (215). (*f*) Cusack *et al.* (215) have pointed out that most of the forward reactions to inosinic acid and most of those that are enzymatically reversible have been performed chemically under relatively mild conditions, and they have suggested a possible relevance of these findings to primeval synthesis.

2. AICAR is a coproduct of an intermediate in histidine biosynthesis (*e.g.*, 259, 260 and references cited). Adenosine triphosphate is glycosidated at position 1 by PRPP; a ring opening, analogous to one of the chemical methods of synthesis previously described, then produces an AIC ribonucleotide derivative (CXV) (Scheme XV). In several steps, AICAR and 4-imidazolylglycerol phosphate (CXVI) are formed, the latter arising from the ribofuranosyl group of PRPP, the nitrogen atom at position 1 of ATP, and nitrogen donated by glutamine. Histidine is formed from CXVI in several additional steps. It is interesting to note certain biochemical interrelationships: (*a*) AICAR may be recycled to ATP *via* IMP and AMP, and (*b*) biodegradation of histidine in several steps *via* urocanic acid yields formiminoglutamic acid, a one-carbon donor for tetrahydrofolate (261).

3. The pyrophosphate (CXVII*a*) of AICAR and AMP is formed from AIC and nicotinamide adenine dinucleotide (NAD) in the presence of NAD glycohydrolase from beef spleen (262–265). Enzymatic conversion of the dinucleotide (CXVII*a*) to the pyrophosphate of IMP and AMP and incorporation of the imidazole moiety into nucleic acids have been demonstrated. Evidence has also been presented for the transfer of a deoxyribofuranosyl group from pyrimidine deoxyribofuranosides to AIC by bacterial enzymes (266, 267).

4. Xanthine is degraded by extracts of certain species of *Clostridia* to 5-aminoimidazole-4-carboxylic acid (VIII) (34). The amino acid is formed *via* 4-ureidoimidazole-5-carboxylic acid (CXVIII) (268) and undergoes further degradation to 5-aminoimidazole (34). Again, it is interesting for correlative purposes to note that the latter compound is further degraded to formiminoglycine, which is a one-carbon source for tetrahydrofolic acid (THF). The sequential formation of 5-formimino-THF, 5,10-methenyl-THF, and 10-formyl-THF is effected by enzymes from *C. cylindrosporum* extracts (269). Thus, interrelationships among purines, aminoimidazole derivatives, and folic acid coenzymes may be found in catabolic, as well as anabolic (Scheme XIV), processes.

5. AIC has been identified as a urinary excretion product of healthy human beings and of animals (270–275). It is normally excreted by adult human beings in quantities of about 1 mg. per day (271, 273, 276, 277), and for most individuals it is comparable to creatinine in the constancy of its excretion (273, 276). The excretion levels may be elevated in abnormal circumstances such as during acute leukemia (276, 278); after oral administration of adenine, glycine, or AIC (279); or during folic acid deficiency (280). More recently, AIC ribonucleoside has been identified as one of several ribonucleosides excreted by normal subjects and by patients with leukemia or gout (281).

6. A compound believed to be 5-aminoimidazole-4-carboxamide 5′-*S*-homocysteinylriboside {5-amino-1-[5-*S*-(3-amino-3-carboxypropyl)-5-thio-β-D-ribofuranosyl]imidazole-4-carboxamide} (CXVII*b*) has been isolated from the urine of patients with homocystinuria (282, 283).

7. In studies with mutants of *Salmonella typhimurium*, Newell and Tucker (284, 285) showed that purines and the pyrimidine moiety (CXIX) of thiamine share the early parts of their biosynthetic pathways. The evidence indicates that this pyrimidine is formed from AIR. The transformation of AIR to the pyrimidine requires the loss of the ribofuranosyl group and the addition of one carbon atom in the ring and the two carbon atoms in the exocyclic groups.

In addition to their involvement in these metabolic processes, aminoimidazolecarboxylic acid derivatives have displayed certain types of biological activity. 5-Amino-2-thioxoimidazoline-4-carboxamide (LXII*b* or, in the thiol tautomeric form, 2-mercapto-AIC) was reported to be moderately inhibitory to Ehrlich ascites carcinoma in mice (77). However, imidazoline-2-thione (2-mercaptoimidazole) and several of its derivatives that do not have the functional groups of the biosynthetic imidazoles appear to be more active against this neoplasm; some of these same compounds—LXII*b* was not included—are also effective in inhibiting solid rodent neoplasms (77, 286). Imidazoline-2-thione and some related compounds inhibited thymidine uptake by ascites cells (77). The activity of LXII*b* in the Ehrlich ascites test may, therefore, be unrelated to its formal resemblance to AIC.

Among 15 analogs or derivatives of AIC and of AIC ribonucleoside tested against Nakahara-Fukuoka sarcoma (a tumor especially sensitive to 6-mercaptopurine and related compounds), only 5-formamidoimidazole-4-thiocarboxamide (LXVIII, X = S) and 5-amino-1-β-D-ribofuranosylimidazole-4-thiocarboxamide (CXIV*a*) were active (287). Further evaluation (288) of these two compounds against seven mouse tumors and leukemias showed that they are inhibitory to adenocarcinoma 755, Ehrlich carcinoma (ascites), sarcoma 180 (ascites), leukemia L-1210, C1498 leukemia, SR-61 leukemia, and Nakahara-Fukuoka sarcoma. 6-Mercaptopurine and its ribonucleoside, employed as reference compounds in these tests, were also active against the same mouse neoplasms. A subline of SR-61 leukemia resistant to these two purine anticancer agents was also resistant to the two imidazole derivatives. These results, the fact that the structures of the two imidazoles suggest that they are potential precursors of 6-mercaptopurine, and the observation (289) that 6-mercaptopurine is excreted after administration of the two compounds to mice suggest that the observed activity may be due to 6-mercaptopurine formed *in vivo* rather than to the two imidazoles *per se*. The thiocarboxamide analog (XXXIX, $R_1 = R_2 = H$) of AIC was reported to be weakly active against the Ehrlich ascites carcinoma and adenocarcinoma 755 (288); however, in an earlier test, it did not significantly increase the lifespan of animals with Ehrlich ascites carcinoma (290).

Inhibition of the growth of a rat sarcoma by ethyl 5-aminoimidazole-4-carboxylate (XII*b*, R = C_2H_5) and its *p*-[bis(2-chloroethyl)amino]phenylsulfonyl derivative has also been reported (43).

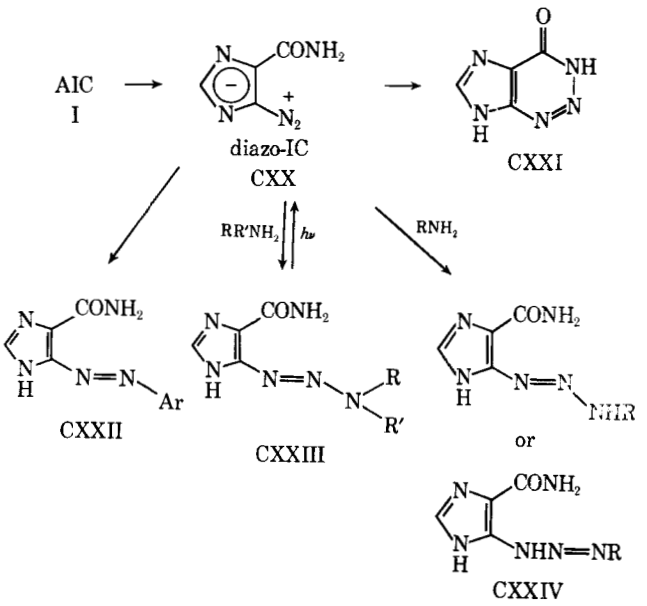
The activity of 8-azaguanine against mouse leukemia L-1210 was found to be potentiated by AIC (291). This effect resulted from inhibition by AIC of the enzyme guanine deaminase (292). Inhibition of adenylosuccinase by an analog of succino-AICAR also represents activity at the enzyme level and deserves mention because of its potential importance for further investigation. Burrows *et al.* (109) found that the L-*threo*-β-methylaspartic acid analog of succino-AICAR is a competitive inhibitor of the enzyme.

AICAR is reported to possess potent activity in enhancing the flavor of a wide variety of food products (227, 233, 293). It is also claimed that succino-AICAR has flavor-enhancing activity (227).

IV. TRIAZENOIMIDAZOLES AND RELATED COMPOUNDS

Interest in triazenoimidazoles began with the prototype, 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388, DIC), which was synthesized by Shealy *et al.* (294) for evaluation as a potential anticancer agent. Initial biological evaluation revealed activity in prolonging the survival time of mice with lymphoid leukemia L-1210 and in inhibiting the growth of the solid tumors adenocarcinoma 755 (Ca755) and sarcoma 180 (S180) in mice (295). This evidence of activity against more than one type of neoplasm led to pharmacological studies and clinical trials under the auspices of the Cancer Chemotherapy National Service Center (CCNSC). The chemistry and the biological properties of this compound and of related triazenoimidazoles are discussed in this section. There is no intention to imply that biological activities of the types to be mentioned are confined to imidazole triazenes.

Diazotization of 5-aminoimidazoles, followed by the addition of a coupling agent, such as 1- or 2-naphthol or the Bratton–Marshall reagent [*N*-(1-naphthyl)ethylenediamine] (296), usually produces intensely colored solutions. This property has frequently been exploited in characterizing 5-aminoimidazoles (*e.g.*, 26, 27, 30, 73, 82, 92) and is the basis for an assay procedure for excreted AIC (273). Diazotization of AIC (Scheme XVI) initially gave 2-azahypoxanthine[12] (CXXI) (1, 297), the product of intramolecular coupling of the diazo group with the adjacent amide nitrogen atom. Later, an intermediate in the formation of 2-azahypoxanthine was isolated and characterized as 5-diazoimidxazole-4-carboxamide (CXX) (298). [The substance that initially precipitated from one of the earlier diazotizations (297) might have been CXX.] This compound readily cyclizes to 2-azahypoxanthine over a wide range of pH values, particularly at pH 7 and in basic solutions. Despite this propensity for intramolecular coupling, which is amply precedented in the formation of benzo-*v*-triazines (299), intermolecular



Scheme XVI

coupling with aromatic compounds and with amines was sufficiently competitive to permit isolation (usually in good yields) of the resulting arylazo (CXXII) (294), dialkyl- or arylalkyl-triazeno (CXXIII) (294, 300), and monoalkyl- or monoaryl-triazeno (CXXIV) (301, 302) derivatives.

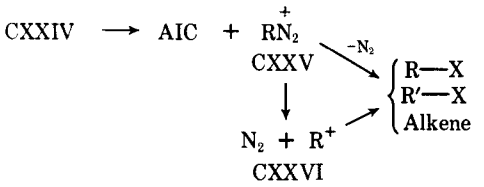
In the initial studies (294), representative disubstituted-triazeno derivatives (CXXIII) (including DIC) in phosphate buffer (pH 7), as well as DIC in 0.1 *N* hydrochloric acid, were shown by UV spectroscopy to be essentially unchanged in the dark during the periods of observation (limited to 1–2 days). Exposure of these solutions to ambient light resulted in the formation of 2-azahypoxanthine (CXXI). The disubstituted-triazeno derivatives (CXXIII) were evidently dissociated by certain wavelengths of light to CXX and the secondary amine (RR'NH), and CXX then cyclized to CXXI. Further observations (300) by TLC of the dimethyltriazeno (DIC) and the butylmethyltriazeno (CXXIII; R = CH_3, R' = C_4H_9) derivatives in 50% aqueous methanol or ethanol showed that the formation of 2-azahypoxanthine is rapid in sunlight or in 365-mμ light (*cf.*, 303), slow under fluorescent lighting, and very slow or negligible under incandescent lighting.[13]

[12] *Chemical Abstracts* names: 4*H*-imidazo[4,5-*d*]-*v*-triazin-4-one, 3,7-dihydro-; 7*H*-imidazo[4,5-*d*]-*v*-triazin-4-ol.

[13] These *qualitative* observations simply indicate a trend and should not be interpreted to be applicable to all lighting situations involving sunlight and artificial lighting. They indicate that suitable precautions should be exercised in handling solutions of triazenoimidazoles. It cannot be emphasized too strongly that the behavior of solutions of these and similar compounds will depend on the wavelengths of light to which they are exposed and on the intensity of the sensitizing light that reaches these solutions. Since the intensity of light decreases rapidly with distance at rates that depend on the geometrical arrangement of the solution and the light source, relatively small differences in the distance of a solution from a light source (*e.g.*, a 365-mμ lamp) catalyzing decomposition can make considerable differences in the apparent rate of decomposition. [The intensity of light from a germicidal lamp was found to be inversely proportional to distance at short distances and roughly inversely proportional to the *square* of the distance at greater distances (304). The Inverse Square Law applies to a point source of light.] For these reasons, exact comparisons of the rates of change of a compound in different solutions or of the rates of change of different compounds cannot be made unless the solutions are exposed under comparable conditions to light of the same wavelength and intensity. Comparisons of stability of different compounds might also be made at the light wavelengths that are optimal for the decomposition of each compound. Obviously, much effort would be needed to acquire the data necessary for precise comparisons.

Loo and Stasswender (303, *cf.*, 305, 306) developed a colorimetric method for determining DIC in biological material, based on the deliberate dissociation of DIC, by exposing solutions containing both this compound and the Bratton–Marshall reagent to UV light in the 365-mμ range. Taken together, these studies indicated that DIC is stable in the dark for at least 24 hr. in phosphate buffer (294), for at least 4–7 days in 50% alcohol (300), and for at least 1 month in refrigerated 0.1 *N* hydrochloric acid solutions (303). Considerable (and various degrees of) dissociation of several disubstituted-triazeno derivatives (CXXIII) in Krebs–Ringer phosphate buffer (pH 7.4) within 20 hr. has been reported (307) but protection from light was not mentioned. In agreement with the observations at pH 7, Skibba *et al.* (306) recently reported DIC to be stable for at least 24 hr. in the dark in lactated Ringer's solution (pH 6.5).[14]

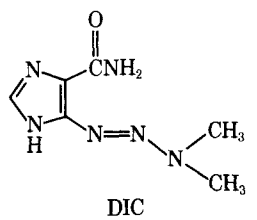
In contrast, it was found (Scheme XVII) that simple



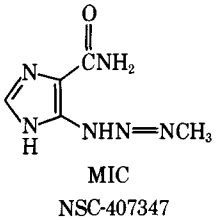
Scheme XVII

monoalkyltriazeno derivatives (CXXIV) are unstable in aqueous and alcoholic solutions even in the dark, that AIC is formed during the decomposition of these derivatives, and that the decomposition follows first-order kinetics (302). Within the series consisting of CXXIV with R = methyl, ethyl, *n*-butyl, cyclohexyl, and *tert*-butyl, stability appeared to decrease in the order in which these compounds are listed. This decomposition was assumed to be similar to deamination of primary amines by nitrosation. Deamination *via* decomposition of triazenes of the benzenoid series is known to occur in organic solvents (308, 309) or in aqueous acids (310–312). Although the mechanistic details of deamination reactions are complex (313–315), they suggested that the simple monoalkyltriazenoimidazoles dissociate, in essence, in accordance with the equations shown (302), producing the unstable alkyldiazonium ion (CXXV) in addition to AIC. The alkyldiazonium ion, in turn, evolves nitrogen and forms a carbonium ion (CXXVI). Some form[15] of the carbonium ion or the alkyldiazonium ion may rearrange (R′), form an alkene, or react with nucleophiles available *in vitro* or *in vivo* (water; hydroxyl, amino, or thiol groups; electron-rich centers of heterocycles; *etc.*). Certain monosubstituted-triazeno derivatives (CXXIV), in which the substituent is an aryl group or a more complex alkyl moiety, are more stable (302); the mode of decomposition may be different or may yield a more stable diazo derivative.

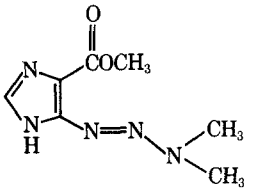
In the initial synthesis (294) of 5-(3,3-disubstituted-1-triazeno)imidazole-4-carboxamides (CXXIII), the substituents on the triazeno group included straight-chain alkyl, cyclic alkyl, aralkyl, and aryl groups, and the number of carbon atoms in the straight-chain groups was doubled [from the dimethyltriazeno (DIC) to the



DIC
NSC-45388



MIC
NSC-407347



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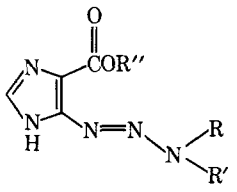
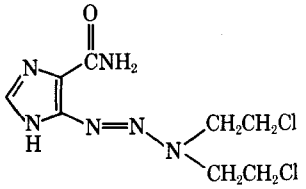
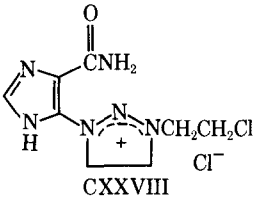


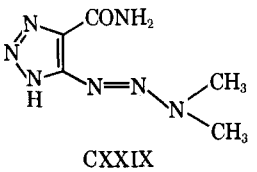
CXXVII



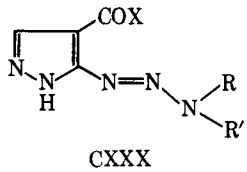
NSC-82196



CXXVIII



CXXIX



CXXX

a, X = OCH_3 or OC_2H_5
b, X = NH_2

dioctyltriazeno derivative]. Because of the antileukemic (L-1210) activity of DIC, its *v*-triazole analog (316), and some methyltriazenes of the benzenoid series (317), one of the alkyl substituents in a later group of dialkyltriazenoimidazolecarboxamides was a methyl group (300). Routine screening of the dialkyltriazeno derivatives against mouse neoplasms indicated that those derivatives in which at least one of the alkyl substituents is a methyl group are the most effective against L-1210.[16] Thus, in addition to DIC, the methyl propyl (NSC-76418), methyl butyl (NSC-70874), methyl isobutyl (NSC-83113), methyl pentyl (NSC-87981), methyl cyclohexyl (NSC-83111), and methyl 2-hydroxyethyl (NSC-83112) derivatives all caused an increase in lifespan (ILS) of treated leukemic mice, as did 5-(3-methyl-1-triazeno)imidazole-4-carboxamide (MIC) (302). The maximum values of the ILS observed in preliminary tests of these compounds fall within the range 40–70% (Reference 300 and additional tests os NSC-83112). By way of comparison, preliminary testt of DIC against L-1210 indicated the ILS to be abouf

[14] In the author's laboratories, there was no significant decrease in the absorbance of DIC in Krebs-Ringer buffer during 24 hr. in the dark and only a small decrease during 4 days.

[15] Summaries of theories on the nature of the carbonium ion (hot, nonsolvated, vibrationally excited, solvent-caged) from deamination reactions and the roles of the alkyldiazonium ion and the counterion may be found in *References 309* and *313–315*.

[16] Since doses in some of the L-1210 tests were determined by prior results from S180 and Ca755 tests, more detailed testing might reveal some activity among derivatives that failed the preliminary L-1210 tests.

50–60% (295); further, extensive evaluation has given values of the ILS of 60–90%, depending on the dose and on the route and frequency of administration (318). Activity by methyltriazeno derivatives was also found among the analogous triazenoimidazolecarboxylic acid esters; methyl 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxylate (NSC-87982) increased the lifespan of leukemic mice by 60% (319). At dose levels determined by the L-1210 primary screening protocols, other (but not all) methylalkyltriazenoimidazole esters (CXXVII) have likewise caused some increase in the lifespan of treated leukemic mice (320).

Antineoplastic activity of triazenoimidazoles is not confined to leukemia L-1210. DIC is active against other leukemias and inhibits the growth of solid rodent tumors (295, 318), and most of the aforementioned methylalkyl derivatives also inhibit Ca755 and S180 (300). Derivatives of this group selected for tests against intramuscular Walker carcinosarcoma 256 (IMW256) in rats inhibited tumor growth (300), and an initial test of MIC against the subcutaneous form of this tumor suggested modest inhibition (302). Inhibition of solid tumor growth was frequently accompanied by loss of weight by the host animals. The apparent association of L-1210 activity with at least one methyl group may not apply to other tumors. Modest inhibition of S180 by triazenoimidazolecarboxamides lacking a methyl group was observed (300). Futhermore, certain monoaryltriazeno derivatives of the imidazolecarboxylate variety (CXXVII) that did not inhibit L-1210 in primary screening did display modest inhibition of IMW256 (320). Such derivatives, of course, cannot form a methyl or alkyl carbonium ion; rather, nonenzymic dissociation should produce a diazoimidazole, an aryldiazonium ion, or both. (Aryldiazonium ions are more stable than the alkyl variety, and their coupling properties are well known.) In addition, Hano *et al.* (307) described some dialkyl- and monoalkyltriazenoimidazolecarboxamides (CXXIII and CXXIV) in addition to those mentioned and evaluated the new and some of the old derivatives against the Ehrlich carcinoma. Within the series of derivatives of CXXIII in which R and R′ were identical and comprised of one to five carbon atoms, the dipropyltriazene and DIC were considered to be the most active against the solid form of the tumor (321). The butyl methyl and the methyl propyl derivatives were subsequently shown to display similar activity (307). None of the dialkyltriazeno derivatives inhibited proliferation of the ascites form of the Ehrlich carcinoma, but two monosubstituted-triazeno derivatives (CXXIV, R = ethyl or hydroxyethyl) were reported to increase lifespan. The potentiating effect (322) of MIC on the activity of 8-azaguanine against Ehrlich ascites is probably due to AIC, which is known (301, 302, 307) to be formed, as previously explained, from the triazene and which is known to potentiate the activity of 8-azaguanine by inhibiting guanine deaminase (see Part III).

The uncertainties of the association of structural elements with activity are emphasized by the fact that the most effective derivative in the L-1210 test system is one that does not bear a methyl group; namely, 5-[3,3-bis(2-chloroethyl)-1-triazeno]imidazole-4-carboxamide (NSC-82196) (323, 324). At *certain* dose levels and dosage schedules, a majority of leukemic mice treated with this compound survived until tests were discontinued after long periods (2–8 months) (323, 325). In addition to these encouraging effects in the standard L-1210 test, Hoffman *et al.* (325) demonstrated the effectiveness of NSC-82196 in advanced L-1210 leukemia. Intraperitoneal administration produced a high proportion of 60-day survivors on certain treatment schedules; oral administration was less effective. Knowledge of the L-1210 test system gained from correlations of inoculum size to average lifespan and from cell-kill kinetic studies (326) indicates that leukemic cells had been completely eradicated from the long-term survivors of the L-1210 tests. Using spleen-colony and host-survival criteria, Wodinsky *et al.* (327) showed that the activity of both NSC-82196 and DIC against L-1210 extends also to an L-1210 line resistant to 6-mercaptopurine. This study indicated a low level of crossresistance of the 6-MP-resistant line to NSC-82196 but not to DIC. Additionally, Tyrer *et al.* (328) developed a strain of L-1210 resistant to NSC-82196 and showed that the resistant variant is crossresistant to DIC and to nitrosoureas; but, interestingly, it is sensitive to certain nitrogen mustard derivatives such as cyclophosphamide and melphalan.

Unfortunately for purposes of handling and administration, this compound is beset by problems of instability. In addition to its susceptibility to light-catalyzed dissociation, it undergoes an internal alkylation to an ionic transformation product in solution and even, slowly, in the solid state (323, 324), but it can be preserved in the solid form at low temperatures. The *v*-triazolinium salt structure (CXXVIII), the most likely among several candidate structures, was identified as the structure of the ionic isomer by an X-ray crystal structure analysis (329). The quality of specimens of NSC-82196 was originally estimated from distinctive differences in the IR spectra of the two compounds. Determinations of NSC-82196 based on a microbiological assay (330), light-catalyzed dissociation to 5-diazoimidazole-4-carboxamide (303, 305), a UV absorption method (331), and titrimetric determination of the ionic isomer (332) have been described. The latter method also provided information on the rate of change of NSC-82196 in solution. A nonaqueous titration procedure developed by Sternglanz (333) provides a direct determination of NSC-82196 and other triazenoimidazole amides and esters.

Bis[3,3-(2-fluoroethyl)-1-triazeno] derivatives of the imidazole (CXXIII and CXXVII) and pyrazole (CXXX) types have likewise demonstrated activity against L-1210, but they are, thus far, more toxic and less active than NSC-82196 (334*a*). The association of L-1210 activity with methyltriazenes also applies, as mentioned previously, to the *v*-triazole analog (CXXIX) (316) of DIC and to certain triazenopyrazolecarboxylic acid esters (CXXX*a*) (334*b*) and triazenopyrazolecarboxamides (CXXX*b*) (334*b*, 335). The triazeno-*v*-triazoles (316) and the triazenopyrazoles (334*b*, 335) appear[13] to be more stable to light than the triazenoimidazoles.

In addition to the antineoplastic effects produced by certain triazenoimidazoles, antimicrobial activity has also been demonstrated. Methyl 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxylate (NSC-87982) (319) is strongly inhibitory *in vitro* to a broad spectrum of Gram-positive and Gram-negative bacteria, mycobacteria, yeasts, filamentous fungi, and algae (319, 336, 337). Strains of *Escherichia coli* and *Streptococcus faecalis* resistant to various antibiotics and antimetabolites were not crossresistant to NSC-87982 (338). Activity *in vivo* was demonstrated against *Staphylococcus aureus* in mice (336), and significantly high blood levels and detectable concentrations in several mouse tissues were found after administration by various routes (337). It has also been shown that a number of related 5-(disubstituted-triazeno)imidazole-4-carboxylic acid esters have broad-spectrum antimicrobial activity *in vitro* (320). Additionally, from among a number of bacteria sensitive to DIC, a strain of *E. coli* resistant to glutamic acid γ-hydrazide (and sensitive to DIC at a concentration of 5 mcg./ml. in the disk-plate method) was selected for a microbiological method of assay of DIC in blood and tissues (339). Recently, inhibition of *E. coli* B (340) by DIC and several of its homologs (at concentrations of about 10 mcg./ml.) and inhibition of *Bacillus subtilis* (341) by DIC were reported. Although the antimicrobial activity of the 3,3-bis(2-chloroethyl)-1-triazeno derivative (NSC-82196) is minimal, inhibition of two species of *Mycobacteria* and of drug-resistant *E. coli* strains at high concentrations was observed (330).

Pharmacological studies of DIC have been conducted in the dog (342–344), rat (305, 345), mouse (339), monkey (305), and man (306, 342, 343, 345, 346); DIC and some of its homologs have also been studied in rabbits and cats (347). NSC-82196 has been subjected to pharmacological and clinical evaluation (305, 348). In clinical trials, DIC (NSC-45388) has brought about objective remissions of malignant melanoma in some of the treated patients (306, 349). Objective responses by several other cancers have also been observed in patients treated with DIC (349).

The discussion, thus far, has dealt with triazenoimidazoles. Although 5-diazoimidazole-4-carboxamide (CXX, diazo-IC, NSC-22420), the precursor of the carboxamides, is an unstable compound in solution, there are reports that it also has various types of biological activity; these findings have possible relevance to the question of the mechanism of action of triazenoimidazoles prepared from it. Reports of antineoplastic activity include inhibition of human epidermoid carcinoma (H.Ep.-2) cells in culture (298), subcutaneous Walker carcinoma 256 (298), and Ehrlich ascites carcinoma (298, 321). Antimicrobial activity by diazo-IC (or, because of the instability of CXX, a mixture of diazo-IC and 2-azahypoxanthine) in the form of inhibition of *Mycobacterium tuberculosis* has been reported (350). Furthermore, diazo-IC completely inhibits *E. coli in vitro* (290, 340) at low concentrations and is strongly inhibitory to *B. subtilis* (341). A third type of activity is represented by the potent inhibition of xanthine oxidase by the diazo compound and by two thioazo derivatives prepared from it (351).

At this time, there are unanswered questions concerning both the relationship of structure to activity and the mechanism of action of triazenoimidazoles and related compounds. Several potential mechanisms of action may be considered, and these are not necessarily mutually exclusive. The structural relationship to, and chemical derivation from, the imidazole moieties of the imidazole ribonucleotides suggested *a priori* that the triazenoimidazoles or diazo-IC might interfere in some way with imidazole and purine metabolism (295, 298). A second possibility that was also considered (295) is that the triazenes may be latent forms of the active agent—the corresponding diazo compound for disubstituted-triazeno derivatives or, in the case of NSC-82196, the diazo compound and bis(2-chloroethyl)amine. A third possibility is that the *monosubstituted*-triazeno derivatives are carrier forms (302) of a reactive species (an alkyldiazonium ion or a carbonium ion) and, on the basis of recent findings (345), that the disubstituted-triazeno derivatives may act similarly after *N*-dealkylation.

In what is, of necessity, a speculative vein, a number of findings may be brought to bear on the question of mechanism of action without, at this time, providing a definitive answer. Some observations tend to place the triazenoimidazoles in the class of chemically reactive anticancer drugs ("biological alkylating agents") such as alkylating agents and diazo compounds. Studies by Wilkoff *et al.* (352) of the cell-kill kinetics of DIC and NSC-82196 in cultures of leukemia L1210 cells indicate that these drugs are probably not cell-cycle-stage specific agents. Their behavior in this system, therefore, is similar to that of certain chemically reactive drugs. The crossresistance to nitrosoureas of the L-1210 variant that is resistant to NSC-82196 (328) is consistent with the reactive species idea, but the lack of crossresistance to other drugs having a bis(2-chloroethyl)amino group is not. The earlier findings of Pittillo and Hunt (338) that sulfur-containing amino acids reversed the inhibition of *E. coli* ATCC9637 and of *Saccharomyces cerevisiae* by NSC-87982 and the inhibition of another *E. coli* strain by NSC-82196 suggested (along with observations of crossresistance) involvement of a chemically reactive species as one, but not the sole, mechanism of action (330). Subsequently, Yamamoto (340) reported that cysteine abolished the inhibitory action of diazo-IC on *E. coli* B and suggested that this compound might be the active form for inhibition of this organism by dialkyltriazenoimidazolecarboxamides. More direct evidence in this connection is the discovery by Saunders and Saunders (341) that the inhibitory action of DIC on *B. subtilis* is markedly increased by exposing cultures growing in the presence of the compound to light and that a strain resistant to DIC is also resistant to the diazo compound. These studies in bacteria also indicated that RNA and protein synthetic processes are inhibited by NSC-87982 in *E. coli* (338), that DNA synthesis is inhibited in *E. coli* B by diazo-IC (340), and that DNA synthesis in *B. subtilis* is inhibited by DIC (341).

In the studies of Pittillo and Hunt (330), the partial protection to NSC-82196 inhibition of an *E. coli* strain

afforded by AIC suggested the involvement of purine metabolism. The observations in these studies of NSC-82196 and of NSC-87982 of collateral sensitivity of bacteria resistant to certain purine analogs (330, 337, 338) and of partial crossresistance to 8-azaguanine and to 2,6-diaminopurine of an *E. coli* strain resistant to NSC-82196 (330) also tended to implicate purine metabolism. In this connection, the low level of crossresistance to NSC-82196 by 6-mercaptopurine-resistant L-1210 cells (327) should be recalled. The statement made at the outset that potential mechanisms of action should not be considered mutually exclusive is illustrated by the report of Peters and McGeer (290) that diazo-IC, as well as some related compounds, showed inhibitory effects on the incorporation of glycine into hypoxanthine by pigeon liver homogenates.

A mechanism involving some form of alkyldiazonium ion or carbonium ion must be considered for the monoalkyltriazenoimidazoles because of their demonstrated behavior *in vitro*, as explained previously. Housholder and Loo (353) and Skibba *et al.* (345) reported that patients given DIC excrete large amounts of AIC; the latter investigators found that DIC is *N*-demethylated by rat liver microsomes to formate and AIC. It is appropriate to recall at this point that MIC not only forms AIC in solution but also has antineoplastic activity. If metabolic *N*-demethylation results in the formation of MIC, then a mechanism involving some form of an alkyldiazonium ion or a carbonium ion must also be considered for DIC and other disubstituted triazenoimidazoles. By analogy with organic deamination reactions, some form of these highly reactive species should be generated and should react with nucleophilic centers such as those listed earlier in this section. Recently, Preussmann *et al.* (354), in a comprehensive paper on the mechanism of carcinogenesis of phenyltriazenes, reported that formaldehyde and aniline are formed by incubation of 3,3-dimethyl-1-phenyltriazene with rat liver or lung microsomal fractions in the presence of oxygen and an NADPH-generating system, that formaldehyde or acetaldehyde are formed similarly from certain other carcinogenic dimethyl- or diethyltriazenes of the phenyl and pyridyl series, and that 3-methyl-1-phenyltriazene is a potent carcinogen. Their proposed mechanism of carcinogenesis consists of oxidative dealkylation of a 3,3-dialkyl-1-aryltriazene *in vivo* to an aryl monoalkyltriazene, decomposition of this intermediate to a carbonium ion, and alkylation by this species of "biopolymers, probably nucleic acids." (The authors mention certain, as yet, unexplained phenomena such as the failure to observe tumors in the liver or lungs.) The later stages of their mechanism are essentially those proposed (302) for the dissociation of 5-(3-alkyl-1-triazeno)imidazole-4-carboxamides (CXXIV), and their findings are similar to those of Skibba *et al.* (345), except that formate was identified in the latter study. Whether *N*-dealkylation is the basis of a mechanism of action or simply a catabolic process remains to be seen. One may speculate that the particular mechanism by which a triazene acts (such as reversion to a diazo derivative, interference in purine metabolism, oxidative *N*-dealkylation, or combinations of these processes) may depend on the biochemical environment in which it is placed or to which it is transported.[17]

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[17] *Addendum added in proof*—The following publications dealing with the mechanism of action, metabolism, and biological effects of DIC and NSC-82196 appeared since this manuscript was completed:

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