ETHENO DERIVATIVES OF ADENINE AND CYTOSINE (REVIEW)

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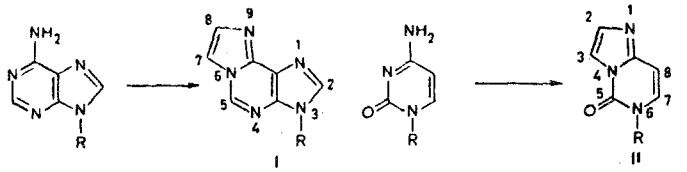
The method of modification of heterocyclic bases of nucleic acids with chloroacetaldehyde and other α-halo carbonyl compounds is examined in this review. The mechanism, kinetics, and scope of the reaction, the chemical properties of modified derivatives of adenine and cytosine, and the possibilities of the application of etheno derivatives of adenine and cytosine in biochemistry and molecular biology are discussed.

Chemical modification of nucleic acids and their components is widely used in the study of the structures and functions of these most important biopolymers. Nevertheless, the search for new reagents that lead to the formation of modified components of nucleic acids that have physicochemical properties that are convenient to use still remains an urgent task. Reagents that modify heterocyclic nucleic acids are of greatest interest, since the latter bear the chief functional burden.

We will examine one of the methods that make it possible to obtain fluorescent derivatives of adenine and cytosine in the present review.

Modification of Heterocyclic Bases of Nucleic Acids with Chloroacetaldehyde

Essential Features of the Method. In 1971 [1] Kochetkov, Shibaev, and Kost proposed a method for the modification of the components of nucleic acids with chloroacetaldehyde (CAA). It was shown that adenine and cytosine derivatives are capable of reacting with CAA to give imidazo[2,1-i]purine (I) and imidazo[1,2-c]pyrimidine (II) derivatives, respectively.



The method proved to be convenient in that it proceeds in aqueous solutions at pH values close to the neutral point and takes place rather rapidly and quantitatively [2]. Modification of the heterocyclic base is virtually unaccompanied by side reactions involving the sugar residue of the nucleoside and the phosphate group of the nucleotide [3]. The method quickly found wide application in various fields of biochemistry and molecular biology [4, 5]. This is due to the fact that, in contrast to natural adenine- and cytosine-containing compounds, imidazo derivatives II and particularly I have fluorescent properties. It is important that the fluorescent analogs of adenine and cytosine derivatives retain to a considerable extent their functional similarity to the starting compounds [7, 8]. The presence of intense (quantum yield 0.6) fluorescence in the long-wave UV region of the spectrum (emission λ_{max} 410 nm) is characteristic for virtually all compounds of the I type that do not contain groupings in R that are capable of quenching the fluorescence [9]. The intensity of the fluorescence of I depends on the ionic form of the molecule, and the neutral molecule is the principal fluorophore [10]. Compounds of type II have much weaker fluorescence (quantum yield 0.003), which is displayed in acidic media; this limits the possibilities of their application [11]. However, their fluorescent properties can be improved substantially by the introduction of substituents in the imidazole ring of the molecule [12, 13].

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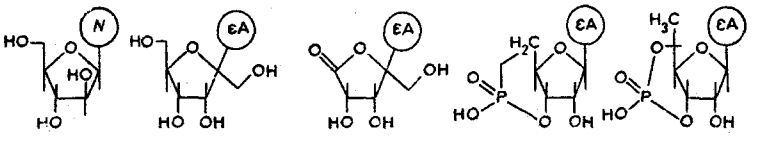
The virtues of imidazo derivatives I and II enumerated above ensured that the proposed method would have wide application in the preparation of fluorescent analogs of natural compounds used as labels or probes (see, e.g., [14]). The heterocyclic I and II systems have been called ethenoadenine (I) and ethenocytosine (II) derivatives [15], i.e., the newly formed imidazole ring is regarded as an "ethylene bridge" that links the ring nitrogen atom with the exocyclic amino group. The names 1,N^6-ethenoadenosine and 3,N^4-ethenocytidine are used in a number of studies to pinpoint the position of the "bridge." The symbols εA and εC, respectively, are used for the abbreviated designation of these compounds; in this case the numbering of the atoms in the I and II molecules is the same as for adenine and cytosine, respectively, while the carbon atoms of the "ethylene bridge" are designated by the letters α and β. In the following account we will also use the term "etheno derivatives," but we will retain the numbering of the atoms of imidazopyrimidine and imidazopurine (structures II and I) that is recommended by IUPAC.

Scope of the Method. The initially proposed conditions for the modification [1, 2] of derivatives of adenine (pH 4.5) and cytosine (pH 3.5) with CAA are suitable for the successful modification of the overwhelming majority of compounds that contain these heterocyclic bases, and virtually no changes were therefore made in them. We therefore will limit ourselves only to listing the compounds to which this method is applicable.

The reaction with CAA was used for the modification of adenine and cytosine derivatives in the composition of nucleosides [2, 6], nucleotides [2, 7], oligonucleotides [2, 16], and polynucleotides [17, 18] of both the ribo and deoxyribo series [3], RNA [6], and DNA [19]. Etheno analogs of AMP [2, 6], ADP [7, 20], ATP [7, 21], NAD [7, 22], FAD [23], coenzyme A [24], 5'-deoxyadenosylcobalamine [25], and a number of different 3',5'-cyclophosphates [26-29] were obtained. Virtually the only complication was noted in the modification of adenosine 2',3'-cyclophosphate [30], which undergoes a considerable amount of destruction to a mixture of 2'- and 3'-phosphates. It is therefore more expedient to synthesize ethenoadenosine 2'(3')-phosphate, the cyclization of which does not give rise to any complications [30]. A number of etheno analogs of adenosine with an anomalous sugar residue, viz., arabinosylethenoadenine (III), arabinosylethenocytosine (IV), and etheno derivatives of psychofuranine (V) and decoinine (VI), were obtained.



Etheno derivatives of the 3'-cyclic ester of 9-(5,6-dideoxy)-6-dihydroxyphosphinyl-β-D-ribo-hexofuranosyl)adenine (VII) and 9-(6-deoxy-β-D-allo-furanosyl)adenine 3',5'-cyclophosphate (VIII) have been synthesized [31].

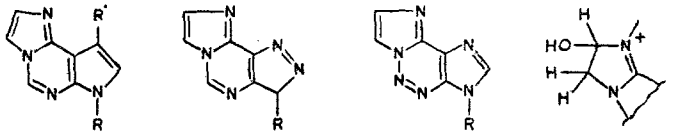
Ethenocytosine derivatives have been investigated much less intensively, and, in addition to the components of nucleic acids listed above, CDP [32] and CTP [32, 33] have been subjected to modification. The ethenocytosine analog of NAD was synthesized by the reaction of nicotinamide mononucleotide with etheno-CMP [34].

It should be noted that all of the previously examined reactions of adenine and cytosine derivatives were carried out with nucleosides, nucleotides, or the corresponding N-alkylated bases, and this excluded the possibility of attack at the glycoside nitrogen atom. However, the reaction of adenine itself with CAA leads to ethenoadenine in good yield [10], the structure of which was confirmed by alternative synthesis. Ethenocytosine is similarly formed, although in low yield (25%), in the reaction of CAA with cytosine [35]. Barrio and coworkers do not indicate the presence or absence of any side products in the reaction mixtures, although one should have expected complications precisely in these cases.

Thus, as a result of modification with CAA, a rather large number of adenine- and cytosine-containing compounds can be converted to fluorescent analogs smoothly and in high yields. Variations in the sugar residue, the presence of a pyrophosphate bond, and the presence of nicotinamide and flavin residues in the molecule do not interfere with the modification and do not require prior protection of the functional groups of the molecule being subjected to modification.

Specificity of the Reaction. As we have already noted, of the natural components of nucleic acids, adenine and cytosine derivatives have been subjected to modification with CAA in weakly acidic aqueous solutions (pH 3-5). Under these conditions, uracil, thymine, and inosine derivatives do not react with CAA. Of the anomalous nucleosides, tubercidin [36], toyocamycin [39], sangivamycin [39], formicin [37], and 2-azaadenosine [38] have been subjected to modification, as a result of which etheno derivatives IX-XI, respectively, were obtained.

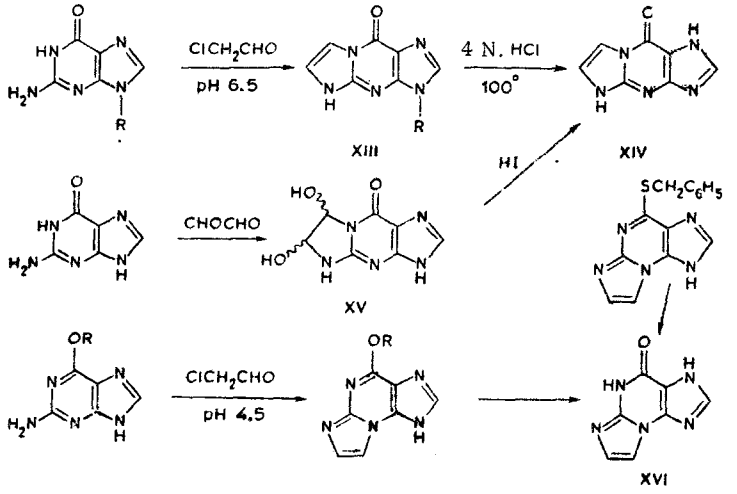
The introduction of substituents (bromo, methylthio, and benzylthio groups) in the 8 position of the adenine ring does not affect the way in which the reaction proceeds [40]. Ring-substituted cytosine derivatives have not been subjected to reaction with CAA, but, in analogy with the behavior of adenine derivatives and with the data in [41] on the reactions of aminopyrimidines with α-halo carbonyl compounds, it may be assumed that the introduction of substituents in the pyrimidine ring that do not involve the amidine fragment of the molecule and do not lead to substantial changes in its reactivity will not hinder the reaction under consideration. At the same time, the presence of an amidine grouping in the molecule is absolutely necessary. Thus 3-methylcytidine and 1-methyladenosine do not react with CAA [6], whereas N^6-methyladenosine [42], N^4-methylcytidine [42], and N^6-(Δ^2-isopentenyl)adenosine [6] form products of the XII type on reaction with CAA. However, because of the fact that the resulting imidazole ring does not have aromatic character, such compounds virtually do not have fluorescent properties.



IX a-c X XI XII

IX a R'=H ; b R'=CN ; c R'=$CONH_2$

In the light of the information stated above, the fact that guanine derivatives, which react via the amidine fragment with glyoxal, ketoxal [43], and even bromoacetone [44], do not react under the indicated conditions with CAA was perplexing. Special studies [42, 45] showed that CAA reacts with guanosine but in more alkaline media than with adenine and cytosine derivatives. The optimum pH of the reaction is 6.5. At higher pH values the rate of decomposition of the reagent becomes very high, while at lower pH values the rate of reaction with guanosine is slowed down markedly. The principal reaction product is 5,9-dihydro-9-oxo-3-β-D-ribofuranosylimidazo[1,2-a]purine (XIII). Under the same conditions guanine itself does not react with CAA, and the corresponding base XIV can be obtained either by acid hydrolysis of nucleoside XIII or from the adduct of guanine with glyoxal (XV). An increase in the nucleophilicity of the guanine system by conversion of guanine to O^6-methyl-, O^6-benzyl-, or O^6-benzylthioguanine facilitates the reaction of CAA at the N^3 atom, which leads to the formation of an angular ethenoguanine (XVI).



XIII XIV XV XVI

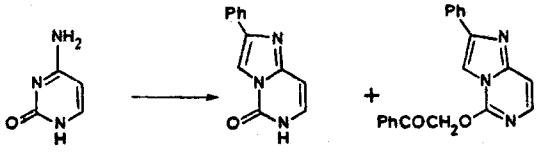
The reaction of guanine derivatives with CAA proceeds relatively slowly and gives the product in low yield. Sattsangi and co-workers [45] established by PMR spectroscopy that at the instant the reaction ceased 45% of etheno derivative XIII was present but could be isolated from the reaction mixture in only 17% yield. The formation of side products, the

structures of which were not investigated, was noted; the principal reaction product (XIII) does not have fluorescence. It is important that guanosine undergoes virtually no modification at pH values below 4.5, and this makes it possible to modify adenine and cytosine derivatives without involving guanine. The conversion of guanosine to XIII may prove to be useful in structural studies of nucleic acids. Since the XV in the composition of RNA is not split out by the phosphodiesterase of snake venom [46], it might be expected that XIII would prove to be a "brake" for this enzyme. For work of this sort completeness of the modification is not essential, and the use of XIII, because of its greater stability as compared with XV, may prove to be preferable.

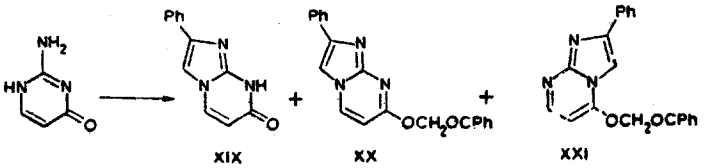
Use of Other Reagents for the Synthesis of Etheno Derivatives

The reaction of CAA with adenine and cytosine derivatives is a special case of the Chichibabin reaction [47]. One should therefore expect that various α-halo carbonyl compounds in addition to CAA could be used in the condensation. In fact, α-chloropropionaldehyde [2], α-chlorobutyraldehyde [2, 48], and α-bromovaleraldehyde [31], as well as chloroacetone [49], bromoacetone [49], α-bromoacetophenone [2, 31, 49, 50], and a number of p-substituted phenacyl bromides [51, 52], have been used to obtain "ethene bridge"-substituted ethenoadenine and ethenocytosine derivatives. All of these reagents react with adenine and cytosine derivatives under more severe conditions and at lower rates than in the case of CAA, and the yields are, as a rule, not quantitative. Their solubilities in water are limited. All of this hinders their use for the modification of oligo- and polynucleotides by restricting the range of application of the modification of nucleosides and mononucleotides. Some α-hydroxy carbonyl compounds can also be used as reagents in place of α-halo carbonyl compounds. Thus tosyloxyacetone reacts with N^1-methylcytosine and 5'-cytidylic acid in the same way as chloro- and bromoacetone; in this case the reaction rates and the yields of the modification products are virtually identical for these three reagents [53]. It has been reported that fluorescent derivatives with unelucidated structures are formed when adenine derivatives are allowed to stand with α-hydroxy aldehydes in acetic acid [54]. Perk and co-workers [55] explain the development of fluorescence by means of the formation of Schiff bases. However, all of the experimental data presented can be successfully explained if one assumes the formation of ethenoadenine derivatives in this case.

In the cases presented above the authors did not note any ambiguity in the condensation and the formation of side products. However, it has been shown [55] that the condensation of cytosine with phenacyl bromide under rather severe conditions [refluxing in dimethylformamide (DMF)] leads to the formation of two reaction products, viz., 2-phenylimidazo[1,2-c]pyrimidin-5(6H)-one (XVII) and 2-phenyl-5-phenacyloxyimidazo[1,2-c]pyrimidine (XVIII), in 36 and 21% yields, respectively.



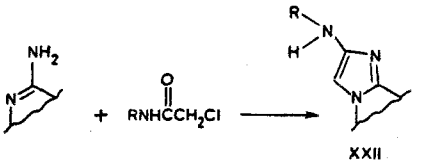
A mixture of 2-phenylimidazo[1,2-a]pyrimidin-7(8H)-one (XIX) and 2-phenyl-7-phenacyloxyimidazo[1,2-a]pyrimidine (XX) and its 5-phenacyloxy analog (XXI) in 19, 5, and 10% yields, respectively, is formed in the reaction of isocytosine with phenacyl bromide under similar conditions [55].



Thus various α-halo-substituted aldehydes and ketones can be made to condense with adenine and cytosine derivatives. Chloro and bromo derivatives have been used, but on the basis of the literature analogies [56], it may be assumed that iodo ketones will also react similarly.

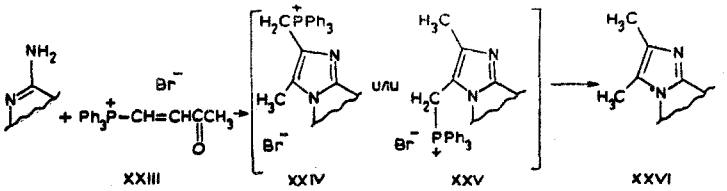
It was demonstrated relatively recently that acetylchloroacetamide can be made to react with cytidine [35]; an acetamido group is incorporated in the imidazole ring in this

case. This substituent makes it possible to obtain compounds of the XXII type that have intense fluorescence. This synthesis demonstrates the possibility of the use of amides of monochloroacetic acid in the reaction under consideration and opens up prospects for the preparation of a large number of ethenocytosine derivatives that have good fluorescence characteristics.



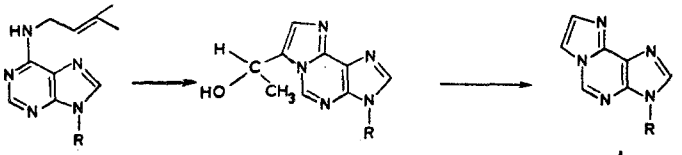
XXII

Of the other reagents that lead to the formation of etheno derivatives, one should note α,β-dibromoethyl acetate, which undergoes hydrolysis to bromoacetaldehyde under the reaction conditions. This reagent has been used successfully along with CAA for the synthesis of 5'-deoxy-1,N^6-ethenoadenosylcobalamin, the fluorescent analog of a cobamide coenzyme [57]. The reaction of adenine, cytosine, and guanine with β-acylvinylphosphonium salts (XXIII) is worthy of mention [58]. The direction of attack of the reagent and, correspondingly, the



XXIII XXIV XXV XXVI

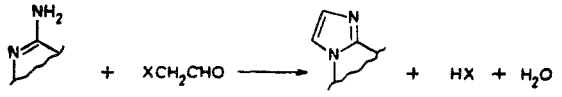
structure of the intermediate phosphonium salt (XXIV or XXV) have not been established. However, subsequent alkaline treatment leads to XXVI, the structure of which is unambiguous, regardless of the direction of attack of the reagent. Reagent XXIII has not been so widely accepted, since the modification takes place under relatively severe conditions, although the yields of the reaction products are quite high (60-70%). In addition, prior protection of the hydroxy groups of the ribose is required in this case to avoid side processes. It is curious to note that N^6-(Δ^2-isopentenyl)adenosine (XXVII) undergoes cyclization during oxidation to ethenoadenosine derivative XXVIII, which is capable of oxidative cleavage of the side chain and conversion to an ethenoadenosine (I) [59].



XXVII XXVIII I

Kinetics and Mechanism of the Reaction

The reaction under consideration can be described by the formal scheme

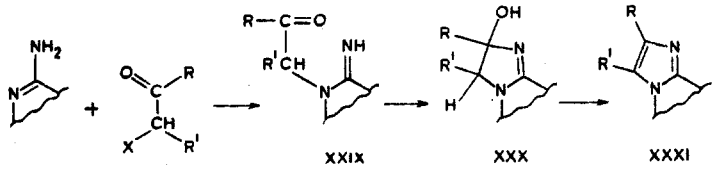


X = Cl, Br

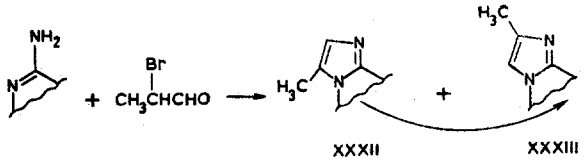
It is apparent that the course of the reaction can be monitored by potentiometric titration (under pH-steadying conditions), spectrophotometry, or fluorimetry. Because of the intensive decomposition of CAA in alkaline media, which is accompanied by HCl evolution, the reaction with adenine and cytosine derivatives can be investigated by titrimetry only at pH 2.0-5.0. It has been shown by this method that the dependence of the reaction rate on the pH has a bell-shaped character with a maximum at pH 3.5 for the cytosine derivatives and at pH 4.5 for the adenine derivatives [2]. However, these pH values are too low for the modification of some biological subjects. This reaction at pH 6.0-8.0 was therefore studied by spectrophotometry and fluorimetry [42, 60, 67]. It was found that adenine derivatives are modified at pH 4.5-6.5 at virtually the same rate, whereas the rate of modification of cytosine derivatives is maximal at pH 3.5-5.5. The reaction rate constants determined by various authors by means of various methods differ so markedly that there is

no sense in discussing them without serious additional studies. The fact that the studies thus far carried out demonstrate the possibility of the use of CAA over a broader range of pH values than previously assumed is important. However, it should be noted that the three heterocyclic bases, viz., adenine, cytosine, and guanine, will undergo modification in the case of modification of nucleic acids in the neutral pH range.

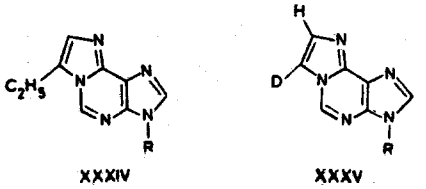
A large amount of experimental data on the reaction of α-halo carbonyl compounds with α-aminoheterocycles has been accumulated [41]. The structures of the condensation products and the effect of the reaction conditions on the composition of the reaction mixture have been studied in detail, but virtually no research on the kinetics and mechanism of reactions of this type has been done. Chichibabin, who discovered this reaction, also proposed a possible mechanism for it [47]. The endocyclic nitrogen atom of the heterobase initially undergoes alkylation, after which the ring is closed as a result of reaction of the exocyclic amino group with the carbonyl group of the reagent. Subsequent dehydration of intermediate XXX leads to the final product (XXXI).



The direction of addition of the reagent has been confirmed repeatedly in the most diverse examples [41]. Intermediate structures XXIX were isolated in a number of cases in the reactions of α-halo ketones (less reactive than the corresponding aldehydes). Thus the mechanism of this condensation has raised no doubts among researchers, although some exceptions have been noted. Thus, for example, both possible isomeric reaction products, viz., XXXII and XXXIII, were isolated from the reaction of 4-aminopyrimidine with α-bromopropionaldehyde.



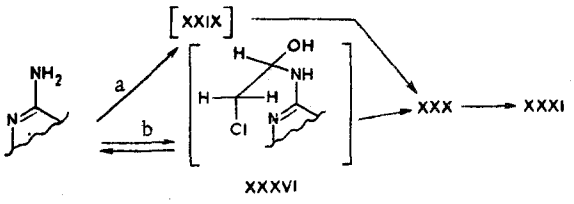
However, the result was explained reasonably by Guerret and co-workers [62] by rearrangement of primary product XXXII to the more stable XXXIII (the Dimroth rearrangement [63]). The possibility of this rearrangement was demonstrated by the conversion of XXXII to XXXIII under the reaction conditions [62]. Adenine and cytosine derivatives also do not display deviations from the proposed reaction scheme. As we noted above, only a single reaction product, the structure of which corresponds to the expected direction of addition of the reagent, is formed in all cases [7, 48, 49]. Thus, for example, the data from x-ray diffraction analysis of the product of the reaction of α-chlorobutyraldehyde with adenosine unambiguously confirm the structure of the product as 7-ethyl-3-β-D-ribofuranosylimidazo-[2,1-i]-purine (XXXIV).



Deuterium-labeled $ClCD_2CHO$ was used to solve the problem of the direction of addition of CAA to adenosine. The PMR spectrum of the reaction product does not contain the signal of a proton attached to C_7, and the signal of the 8-H proton is converted to a singlet, in agreement with structure XXXV.

Thus, in this case also, the direction of addition of the reagent and the structures of the final products are in agreement with the generally accepted ideas regarding the reaction mechanism and do not raise any doubts. However, the sequence of addition of the reagent to the heterobase is not apparent, especially since special studies of the relative reactivities of the functional groups of CAA in aqueous solutions showed higher re-

activity of the aldehyde group than of the halo group at low (20-50°C) temperatures [64]. In addition to the generally accepted reaction scheme (pathway a), one should also examine an alternative possibility (pathway b):



In the latter case the first step in the reaction is the reversible reaction of the carbonyl group of the reagent with the exocyclic amino group of the heterobase, which determines the direction of the subsequent alkylation. The intermediate formation of hydrated ethenoadenine and ethenocytosine derivatives of the XXX type was proved by their isolation from the reaction mixtures and the spectral and chemical confirmation of their structures [42, 65]. In addition, when high concentrations of the starting components (adenosine or cytidine and CAA), the presence of another intermediate, attempts to isolate which lead to its decomposition to the starting nucleoside, can be detected in the reaction mixture by thin-layer chromatography (TLC) [42]. The authors logically assume that this compound has the XXXVI structure rather than the XXIX structure, since the well-studied products of N-alkylation of adenosine and cytidine of the XXIX type are extremely stable, and no difficulties are encountered in their isolation, whereas the products of the reaction of adenosine and cytidine with aldehydes are very labile. These data, in our opinion, constitute indirect evidence for the possibility of reaction via pathway b. In addition, it is well known that the alkylation of adenosine virtually always proceeds ambiguously and leads to a mixture of monoalkyl derivatives [46], but this is not observed in this case.

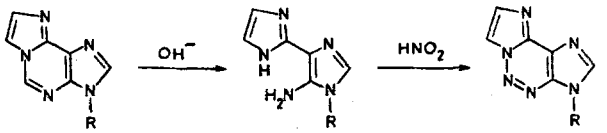
It might be assumed that the reaction of adenine and cytosine derivatives with α-halo carbonyl compounds can, in the general case, proceed via both pathway a and pathway b. However, the choice between these two possible reaction pathways will be determined by the relative reactivity of the carbonyl group of the reagent. It is possible that reagents that have an active carbonyl group (particularly CAA) react preferably via pathway b, while reagents with a less reactive carbonyl group (ketones and derivatives of α-halo acids) react mainly via pathway a. In favor of this assumption one can cite the fact that intermediates of the XXIX type were detected only in the reaction of halo ketones but not in the reaction of aldehydes [41].

Chemical Properties of the Etheno Derivatives

Quantum-chemical calculations of the distribution of the charges and orders of the bonds of ethenoadenosine and ethenocytidine in the ground and lower excited states within the Pariser–Parr–Pople (PPP) and complete neglect of differential overlap (CNDO/S) approximations show [52, 66] that the creation of an "etheno bridge" should not have a substantial effect on the reactivity of the molecule as a whole. One should expect that the properties of etheno derivatives will not differ substantially from the properties of their unmodified analogs. However, the formation of a new imidazole ring opens up interesting possibilities for its use in synthesis. The chemical properties of etheno derivatives have not been studied systematically, but a series of data that are worthy of discussion have been accumulated.

It has been shown that the conversion of deoxyadenosine to the etheno analog does not affect the strength of the N-glycoside bond (the rate constants for acid hydrolysis of deoxyadenosine 5'-phosphate and its etheno analog are virtually identical [3]). Although no quantitative data on the rate of acid hydrolysis are available for ethenocytosine derivatives, they do not differ qualitatively with respect to their hydrolytic stability from pyrimidine derivatives [67]. Ethenoadenine and ethenocytosine bases are stable in acidic media and can be subjected to acid treatment under the standard conditions of hydrolysis of nucleic acids (0.1 N HCl or $HClO_4$, 100°C, 1-3 h) without destruction of the heterobase [3]. However, an increase in the acidity (3 N HCl, 100°C) leads to cleavage of the pyrimidine ring of ethenoadenine and to the formation of diimidazolyl XXXVII [10]. On the other hand, the etheno derivatives are much less stable under alkaline conditions than their natural analogs. The structures of the products of alkaline degradation of ethenocytosine have not been investigated. It is known only that substantial and irreversible changes in its UV spectrum that

attest to disruption of the aromatic character of the heterocyclic system occur when a solution of ethenocytidine in 1 N KOH is allowed to stand at 20°C for several hours [67]. Ethenoadenine undergoes alkaline destruction relatively easily (0.1 N KOH, 25°C, 18 h), as

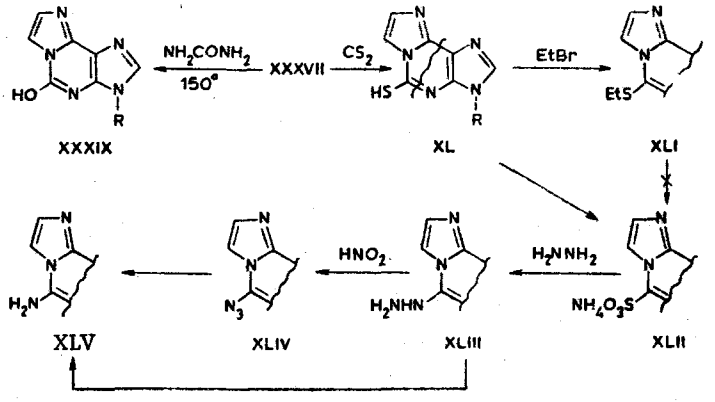


I XXXVII XXXVIII

a result of which, as in the case of acid hydrolysis, the pyrimidine ring is opened, and I is converted quantitatively to 4-amino-5-(2-imidazolyl)imidazole derivatives (XXXVII) [68]. Subsequent treatment of diimidazolyl XXXVII with sodium nitrite in acetic acid gives the aza analog (XXXVIII) of ethenoadenine [68].

This approach is applicable for the preparation of aza analogs of ethenoadenosine mono-, di-, and triphosphates and 3',5'-cyclophosphate. Yip and Tsou [68] have named XXXVIII 2-azaethenoadenosine using the numbering of the adenine ring, although it is more correct to call it 5-azaethenoadenosine (which we will also hold to in what follows). The proposed approach is synthetically simple and convenient. 5-Azaetheno derivatives are of interest as fluorescent labels and probes, since, while retaining a rather high fluorescence intensity (the quantum yield is ∿0.2), they fluoresce at 480-500 nm, which is a convenient region for biochemical studies. A disadvantage of the proposed method is the necessity for the use of alkaline and acidic treatments, which in the case of ADP and ATP leads to partial dephosphorylation [68, 69], whereas attempts to synthesize azaetheno analogs of FAD and NADH were generally unsuccessful [68].

The ability of ethenoadenine compounds to rather easily undergo opening of the pyrimidine ring makes it possible to introduce various substituents in the 5 position of the ethenoadenine system. The promising character of the use of this approach is illustrated in the scheme [70].

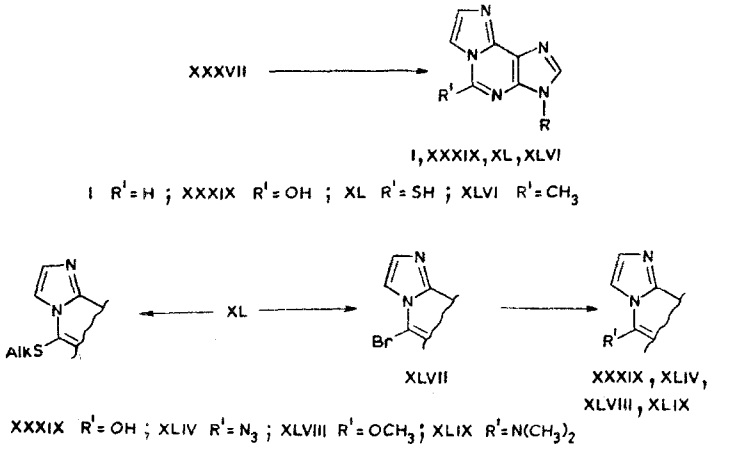


XXXIX XL XLI XLII XLIII XLIV XLV

The reaction of diimidazolyl XXXVII with urea proceeds ambiguously under severe conditions, although the chief product is XXXIX. The formation of a pyrimidine ring in aminodiimidazolyl XXXVII with carbon disulfide leads to 5-thioethenoadenine derivative XL, which, judging from the long-wave shift of the absorption spectrum as compared with alkylthio derivatives XLI, exists in the thione form (like 6-mercaptopurine) rather than in the thiol form. At the same time, hydroxy derivative XXXIX probably exists in the enol form, since its IR spectrum does not contain the absorption bands of a carbonyl group. Compound XXXIX fluoresces only in the neutral form (2.4 < pH < 6.7) and may be useful as a fluorescent pH indicator. Like 2-alkylthiopurine ribosides, ethylthio derivative XLI does not undergo amination and is stable in alkaline solutions. Attempts to oxidize it with hydrogen peroxide and N-chlorosuccinimide were unsuccessful. On the other hand, thio derivative XL is oxidized rapidly by N-chlorosuccinimide to sulfonate XLII. Sulfonate XLII is stable in acid solutions but decomposes with the loss of fluorescence at alkaline pH values and is resistant to alkylation but is readily converted to hydrazine XLIII. The latter can be converted to azide XLIV, which, however, does not undergo cyclization to the corresponding tetrazole, as is characteristic for many purines [71]. Azide XLIV is interesting as a potentially photoreactive molecule, although the possibilities for its use in biological systems are limited by its lack of fluorescence. It is difficult to expect that its photoaddition to a protein

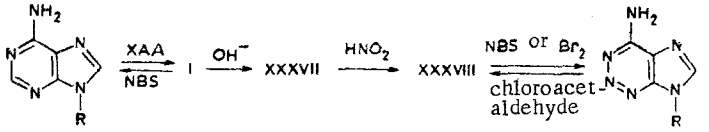
or nucleic acid would lead to "regeneration" of its fluorescence properties, since 5-amino-ethenoadenosine XLV also does not fluoresce.

A similar chain of transformations has been realized by another group of researchers [72].



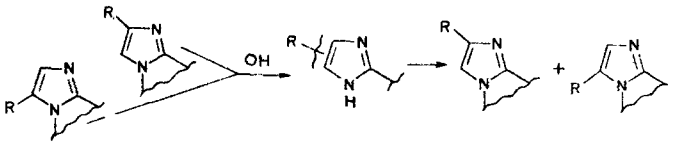
The formation of a ring by the reaction of diimidazolyl XXXVII with cyanogen bromide leads to starting I, while the use of triethyl orthoacetate makes it possible to obtain 5-methyl-substituted XLVI. However, 5-hydroxy derivative XXXIX is obtained when carbonyldiimidazole is used. A comparison of these reactions with similar ring formation to give 5-amino-1-β-D-ribopyranos-4-yl carboxamide 3',5'-cyclophosphate led the authors to the conclusion that the cyclization proceeds more smoothly and with fewer complications in this case, i.e., an "etheno bridge" promotes regiospecificity of the reaction. The reaction of 5-thio derivative XL with bromine in hydrobromic acid gives (in high yield) bromide XLVII, in which the bromine atom is rather active, and this makes it possible to obtain a number of 5-substituted ethenoadenosines from it. It should be noted that the chain of transformations presented above was realized for ethenoadenosine 3',5'-cyclophosphate, which is a biologically important and rather labile molecule.

Also of interest is the research by Yamaji and Kato [38], who showed that treatment of ethenoadenine compounds with bromine at pH 3.0-5.0 or with N-bromosuccinimide leads to the starting adenine derivative in high yield.



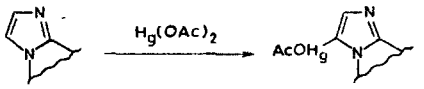
This method was successfully used to obtain 2-azaadenosine 3',5'-cyclophosphate from adenosine 3',5'-cyclophosphate. In this case the production of the etheno derivative was accomplished for protection of the amidine fragment of adenine. This approach makes it possible to not only create fluorescent adenine derivatives but also, where necessary, to reconvert them to the starting compounds. A disadvantage of this method is the fact that it is inapplicable to compounds that are capable of undergoing bromination under the conditions of removal of the "etheno bridge." Thus, the analogous conversion of ethenocytidine to cytidine can hardly be accomplished by this method because of the high reactivity of the $C_5=C_6$ bond of pyrimidines [46].

In discussing the possibilities of the production of 2-substituted adenine and 5-substituted ethenoadenine derivatives one should mention that the basis of the examined principle is the production of diimidazolyl XXXVII by alkaline treatment of the corresponding I. This method is consequently suitable only for compounds that are stable in alkaline media. In addition, the cleavage of the pyrimidine ring of I, which is a special case of the Dimroth reaction [63], retains all of its inadequacies and limitations. Since the "etheno bridge" in the examined cases does not have substituents, the pathway of cyclization of diimidazolyl XXXVII does not play a role. However, when the analogous reaction with 7- or 8-substituted ethenoadenosines is carried out, one should expect the formation of a mixture of both possible isomers with preponderance of the thermodynamically more favorable isomer.



The separation of this mixture is a task that is far from simple, and the use of a mixture of isomers in biological studies complicates the interpretation of the results substantially.

Another important property of I and II consists in their ability to undergo quantitative mercuration when they are treated with mercury acetate (pH 4.0-4.5, 37°C, 1 h) [19].



I, II

The possibility of the quantitative conversion of adenine and cytosine derivatives to I and II in conjunction with the quantitative mercuration of the latter opens up prospects for the use of etheno derivatives for the direct electron-microscope detection of adenine and cytosine bases in the composition of nucleic acids. The possibility of the realization of this approach was demonstrated in the case of modification (with CAA and mercury acetate) of DNA from salmon sperm. Rose [19] assumes that he achieved 100% modification of adenine and cytosine bases of DNA under the influence of CAA and then accomplished their quantitative and strictly selective mercuration. However, there is every reason to suppose that the results are not sufficiently reliable. First, a year after the publication of this paper it was shown that mercury acetate reacts rapidly under similar conditions (pH 5.0-8.0, 37°C, 3 h) with uridine and cytosine bases of polynucleotides to give the 5-mercury derivatives in high yields [73], and, second, CAA is sensitive to the secondary structure of nucleic acids and can modify virtually only the single-chain portions of the DNA molecule [61]. Thus, both of the theses declared in [19] must be considered doubtful.

Applications of the Etheno Derivatives

The possibilities for the application of etheno derivatives of adenine and cytosine are rather extensive. Derivatives I and II are used most intensively for the study of the structures and functions of proteins. Approximately 100 such studies are presently known. This problem is discussed in detail in a previous review [74]. We will note here only the principal trends and principles in the use of I and II.

Ethenoadenine and ethenocytosine derivatives differ favorably from most of the fluorescent labels and probes used in biochemistry and enzymology [14] with respect to their close affinity with natural coenzymes, substrates, and other biologically active compounds. Derivatives I and II are therefore often enzymatic analogs of coenzymes and substrates. Thus of the investigated enzymes, 21 are capable of using derivatives I or II as the substrate or coenzyme, and only 9 do not display activity with analogs of substrates based on εA and εC derivatives [74]. The character of the structural rearrangements of the protein molecule under the influence of natural biologically active compounds and their etheno analogs is often monotypic (which is rarely noted for other classes of fluorescent probes) and makes it possible to obtain reliable information regarding the mechanisms of the action of enzymes. The use of derivatives I and II as probes makes it possible to determine the activity of a number of enzymes [75], to ascertain the role of the structure of the heterocyclic base in processes involving the recognition of substrates and coenzymes by enzymes [30], and to ascertain the fine differences in the structures and specificities of the same enzymes isolated from different sources [76].

Etheno derivatives I and II are a virtually unique class of fluorescent probes that can be easily converted to fluorescent labels by chemical (attachment to the amino groups of the protein via the ribose residue [77], and attachment to the nucleophilic groups of the protein via phosphate [78]), photochemical [79] (using 2-azido-I derivatives), and "enzymatic" methods [80]. The use of derivatives I as labels has also proved effective for the solution of a number of problems in studies of the more highly developed structures of proteins [74].

Ethenoadenine derivatives can be used successfully for analytical purposes. Their intense fluorescence makes it possible to detect adenine derivatives with high sensitivity on paper chromatograms [81] after spraying with a solution of CAA, and the amounts of ethenoadenine derivatives in aqueous solutions can be determined quantitatively in concentrations up to 10^{-8} mole/liter [82].

The modification of adenine and cytosine links in the composition of nucleic acids may prove to be a useful instrument in the study of their secondary structures (since it is known [83] that CAA modifies primarily the exposed links of the polynucleotide chain), as well as their primary structure. In the latter case one can use the fact that pancreatic ribonuclease is not capable of using ethenocytosine residues of a polynucleotide as a substrate, and the enzyme will consequently cleave the chain only at the uridine links after treatment of RNA with CAA [2].

Synthetic polynucleotides with various percentages of ethenoadenine and ethenocytosine links have proved to be useful instruments in the study of nucleic-protein interactions [84, 85].

In conclusion, we would like to emphasize that although the examined method for the modification of adenine and cytosine derivatives with chloroacetaldehyde has already found extensive and diversified application, its possibilities are still far from having been exhausted. On the contrary, the vigorous application of this method shows how necessary it is to make a further search for new reagents that lead to the creation of fluorescent analogs of the components of nucleic acids suitable for use in molecular biology, biochemistry, and enzymology.

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