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1,N^6-Etheno-Bridged Adenines and Adenosines. Alkyl Substitution, Fluorescence Properties, and Synthetic Applications

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Abstract: It has been shown that the reaction of chloroacetaldehyde with adenosine at pH 4.5 and 37 °C that produces the fluorescent ϵ-adenosine species will not develop interfering fluorescence with N^6-alkyladenosines. The preferred site of methylation and benzylation of ϵ-adenosine and ϵ-adenine was established as N(9) (a) by acidic ring opening of the products to substituted aminobiimidazoles in which the two etheno protons were nonequivalent; (b) by reaction of N^6-substituted adenines with chloroacetaldehyde followed by polyphosphoric acid to dehydrate the intermediate to an N(9)-substituted ϵ-adenine for an unequivocal synthesis. The fluorescence of the ϵ-adenosine and ϵ-adenine species at pH 7.0 has again been confirmed, and the fluorescence properties of their N(9)-alkylated derivatives under neutral and acidic conditions have been determined. It has been shown possible, earlier reports to the contrary, to prepare N^6-substituted adenosines through Schiff-base formation on the 6-NH_2. The general method involves the use of sodium cyanohydridoborate to bring about reductive amination of aldehydes and ketones at acidic pH and is exemplified by the synthesis of N^6-ethyladenosine, N^6-benzyladenosine, and N^6-furfuryladenosine (kinetin riboside), using large excesses of aldehyde and reducing agent.

Current interest in the tertiary structure[1-7] and solution properties of tRNA has included the development of reagents capable of causing specific modification at the exposed sites.[2] Chloroacetaldehyde, an important reagent of this class, has been used to modify adenine and cytosine residues[8,9] at the nucleoside and nucleotide levels[10-12] and in tRNA.[13,14] More recently, it has been shown that at pH ~6.4 there is a slow reaction of chloroacetaldehyde with guanosine.[15] In all these reactions an etheno bridge is introduced between the exocyclic amino group of the base and a ring nitrogen to form the corresponding etheno (or ϵ) compounds.[16] Since methylated and isopentenylated derivatives of adenosine are found in various tRNAs,[17,18] it is desirable to learn which of these react with chloroacetaldehyde and which furnish fluorescent products as in the case of adenosine.

Among the methyladenosines found in tRNA, 1-methyladenosine (m^1Ado)[19] would not be expected to and did not produce an etheno-bridged product,[10] and N^6,N^6-dimethyladenosine (m_2^6Ado),[20] a questionable tRNA component, also would not be able to form a bridged compound. The reactions of chloroacetaldehyde with the remaining methyl components N^6-methyladenosine (m^6Ado) (**1a**)[21,22] and 2-methyladenosine (m^2Ado) (**2a**)[20,21,23] (as 2-methyladenine (**2c**)) were examined. We had observed earlier that N^6-(Δ^2-isopentenyl)adenosine (**1b**) reacted quantitatively with chloroacetaldehyde to yield 7,8-dihydro-8-hydroxy-9-(Δ^2-isopentenyl)3-β-D-ribofuranosylimidazo[2,1-*i*]purinium chloride (**3b**).[9] Although the position of the hydroxyl group was initially not fully confirmed, the X-ray determination of the structure of the reaction product of α-chloro-*n*-butyraldehyde and adenosine[24] left no doubt that the direction of attachment of the aldehydic carbon was to the exocyclic nitrogen. N^6-Methyladenosine (**1a**) reacted with chloroacetaldehyde to give the compound analogous to **3b**, namely, 7,8-dihydro-8-hydroxy-9-methyl-3-β-D-ribofuranosylimidazo[2,1-*i*]purinium chloride (**3a**). Similarly, N^6-methyladenine (**1c**) gave the corresponding compound **3c**. None of the "hydrated" forms (**3a–c**) obtained from chloroacetaldehyde and members of the N^6-substituted series was appreciably fluorescent. Accordingly, N^6-substituted adenosines will not interfere with the reaction of chloroacetaldehyde with adenosine at pH 4.5 and 37 °C that develops fluorescence from the neutral ϵ-adenosine species (**4a**).[25]

We hoped to relate the site of alkylation of ϵ-adenosine (**4a**) to the N^6-substituted compounds (**3**) in a manner parallel to our assignment of structure of the alkylated ϵ-cytidines.[26] Alkylation of **4a** could conceivably take place at several of the nitrogens in ϵ-adenosine, although N(9) (see **3** for numbering system) as the nucleophilic center for attack on an alkyl halide would permit the most favorable delocalization of positive charge in the transition state. ϵ-Adenosine (**4a**) was converted efficiently to its monomethyl or monobenzyl derivative by alkylation with methyl iodide or benzyl bromide, respectively, in dimethylacetamide and metathesis to the corresponding chloride. The elemental analyses and mass spectra of the major product in each case were indicative of the introduction of one such group, as were the NMR spectra, i.e., a singlet at δ 4.38 ppm for the methyl compound or a singlet at δ 5.97 and a multiplet at δ 7.3–7.5 for the benzyl compound. Compounds of the imidazo[2,1-*i*]purine type (as in postulated **5**) are known to undergo ring opening to aminobiimidazoles readily on treatment with 2 M HCl at 100 °C.[27] Only an N(9)-substituted tricyclic compound (**5**) would generate a biimidazole (**6**) in which the two etheno protons would be nonequivalent and thus should exhibit a pair of AB doublets in the NMR spectrum. When the major product of methylation of ϵ-adenosine (**4a**) was hydrolyzed with HCl, the initial NMR finding was that the methyl-substituted aminobiimidazole in DCl or D_2O showed only a two-proton singlet (100 MHz) at δ 7.62 or 7.45, respectively. However, when the solvent was changed to $CDCl_3$ for the free base, a pair of etheno doublets was observed, δ 6.86 and 7.08, J = 1.8 Hz, demanding the N-substitution shown in **6a**. Benzylation of ϵ-adenosine followed by HCl hydrolysis produced a benzyl-substituted aminobiimidazole for which

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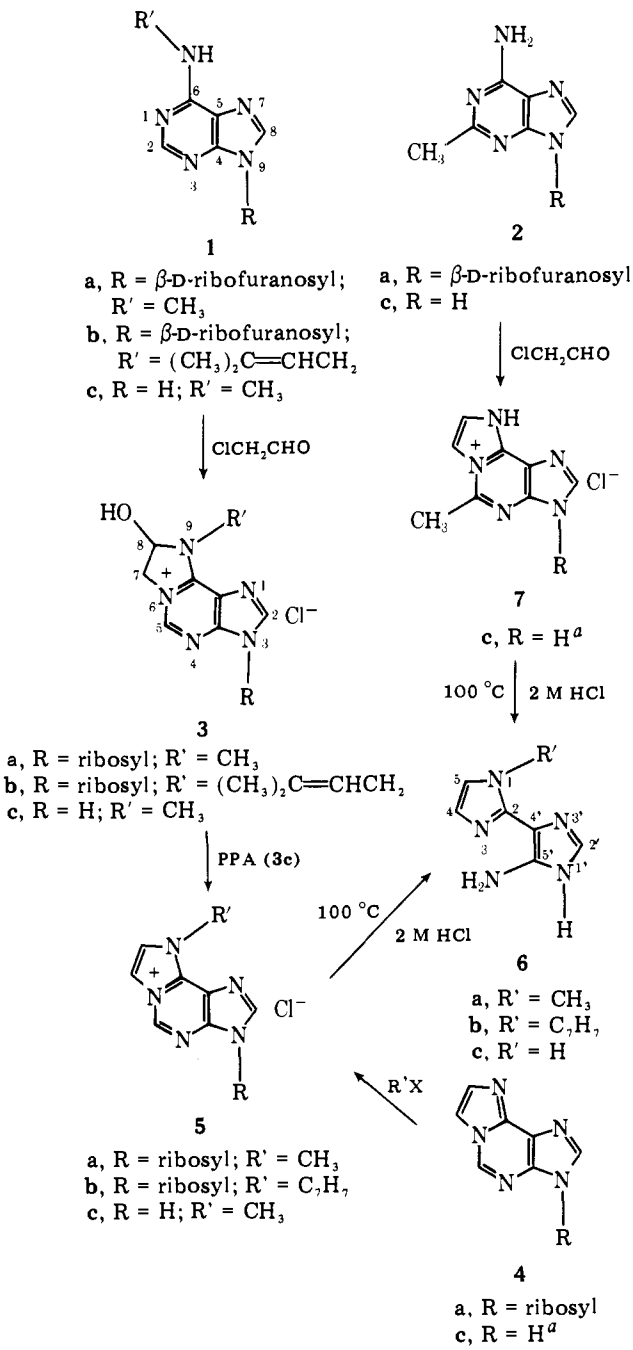

[a] Other tautomeric forms are not excluded.

etheno doublets were observed at δ 7.70 and 7.77, J = 2.1 Hz, even in DCl, and which therefore had structure **6b**.

The proof of structures **6a** and **6b** dictated the precursor structures **5a** and **5b** and thus demonstrated the preferred site of alkylation of ε-adenosine (**4a**). It was also possible to dehydrate the hydroxyamino compounds (**3**), as exemplified by the conversion of 7,8-dihydro-8-hydroxy-9-methylimidazo[2,1-*i*]purinium chloride (**3c**) to 9-methylimidazo[2,1-*i*]purinium chloride (**5c**) with polyphosphoric acid in over 90% yield. The product, characterized by elemental analysis, mass spectrum, and in the NMR spectrum by a pair of etheno doublets at δ 7.93 and 8.29, J = 2.5 Hz, was also obtained by direct methylation of ε-adenine (**4c**).[25] Compound **5c** prepared by either route was hydrolyzed to the same biimidazole (**6a**) described earlier. The reaction of 2-methyladenine (**2c**) with chloroacetaldehyde at pH 4.5 and 37 °C gave the expected 1,N^6-etheno-2-methyladenine (5-methylimidazo[2,1-*i*]purine) as the hydrochloride (**7c**). Hydrolysis with 2 M HCl at 100 °C opened the pyrimidine ring to yield 5'-amino-2,4'-biimidazole (**6c**).[27]

The biological activity of N^6-substituted adenosines makes these compounds of continuing interest.[28,29] Two major routes of synthesis have been used in the past. One involves quaternization of N(1) of adenosine with an appropriate halide followed by a Dimroth rearrangement.[29,30] The other involves nucleophilic substitution at the 6 position of 6-chloro-β-D-ribofuranosylpurine with an appropriate amine.[31,32] Since direct alkylations in aqueous media usually give a mixture of products, [33,34] they are not of preparative value for N^6-alkyladenosines. While aromatic amines undergo reductive alkylation when refluxed with an appropriate alcohol in the presence of Raney nickel, 2-aminopyridine was reportedly unreactive[35] and in our hands adenosine could not be N^6-alkylated by this procedure.[36] Attempts to prepare a Schiff base as a route to N^6-furfuryladenine (kinetin) were reported as unsuccessful.[37,38] The only reported Schiff base of adenosine is that formed from dimethylformamide dimethyl acetal,[39] suggested to participate as the ionic structure;[40] nevertheless, a fluorogenic reaction of α-hydroxy aldehydes with adenine has been ascribed to Schiff-base formation.[41]

On further consideration of the sequence of events involved in the cyclization reaction of chloroacetaldehyde with adenosine, we posed the hypothesis that initial reversible aldehyde–amine formation might facilitate intramolecular displacement at N(1).[42] By utilizing the ability of sodium cyanohydridoborate to bring about reductive amination of aldehydes and ketones at acidic pH,[43,44] we have developed another synthesis of N^6-substituted adenosines. For example, treatment of an aqueous solution of adenosine with seven successive hourly batches of acetaldehyde (tenfold excess in all) and sodium cyanohydridoborate ($NaBH_3CN$) (20-fold excess) at 37 °C and pH 4.5 produced N^6-ethyladenosine. The compound could be isolated in about 80% yield by concentration followed by adjustment of the pH to ~7. The reaction did not proceed to any appreciable extent above pH 5, probably owing to the absence of an intermediate positively charged, readily reducible[44] iminium salt.[45] Below pH 4.0, reduction of the aldehyde predominated. N^6-Ethyladenosine so obtained had spectroscopic characteristics similar to those reported.[34] and the elemental analyses and mass spectrum were indicative of the introduction of one ethyl group. Worthy of note is our finding that in the mass spectrum the base peak at *m/e* 192 corresponds to B + 30, instead of the usual B + 1 as observed for the fragmentation of N^6-methyladenosine and N^6,N^6-dimethyladenosine.[46] A direct analysis of daughter ions (DADI) supported the fragmentation pattern as 295 (M^+) → 192 (B + 30) → 164 (B + 2), which can then fragment in the usual manner.[46] While ions at B + 30 are prevalent in nucleoside fragmentation,[47-49] they are usually not the base peak. The atypical behavior of N^6-ethyladenosine in the mass spectrometer was confirmed with a check sample of the compound prepared from ethylamine and 6-chloro-β-D-ribofuranosylpurine.

The reductive amination method using sodium cyanohydridoborate was extended to the synthesis of representative N^6-substituted adenosines, N^6-furfuryladenosine (kinetin riboside) and N^6-benzyladenosine, and to the synthesis of N^6-furfuryladenine (kinetin). The method can be extended to other N^6-substituted adenosines and adenines but suffers from the requirement of large excesses of aldehyde and reducing agent. It is chiefly of interest because it shows that adenine is indeed capable of forming Schiff-base type intermediates with aldehydes, notwithstanding earlier reports.[37,38]

Fluorescence Properties. Analysis of the data in Table I shows that N(9)-substituted derivatives of ε-Ado or ε-Ade (**5a–c**) are weakly fluorescent, i.e., that they emit at an intensity about one-tenth that of ε-Ado and have a much shorter

Table I. Fluorescence Characteristics of ϵ-Adenosine and Related Derivatives

compd	pH	fluorescence emission max, nm (cor)	Φ^a
5a	7.0	358[b]	0.06[c]
5a	0.1 M HCl	366	0.02[c]
5b	7.0	363	0.08[c]
5b	0.1 M HCl	366	0.02[c]
5c	7.0	370	0.05[c]
5c	0.1 M HCl	363	0.02[c]
4c	7.0	414	0.21[d]
4c	0.1 M HCl	445	0.03[c]
7c, free base	7.0	403	0.14
7c	0.1 M HCl	455	0.08

[a] Quantum yields at 25 °C based on ϵ-Ado in 0.025 M phosphate buffer at pH 7.0, $\Phi = 0.56$.[10] [b] Reported, 365 nm.[50b] [c] Fluorescence lifetime ca. 1 ns as determined by phase measurement. [d] Fluorescence lifetime 12 ± 1 ns as determined by phase and modulation measurements.

fluorescence lifetime, ca. 1 ns. Acid, i.e., 0.1 M HCl, produces an additional quenching effect, lowering the intensity to about 1/30 of the original for ϵ-Ado (**4a**). The emission maxima of **5a–c** in aqueous solution at room temperature are centered at about 360 nm, different from that of ϵ-Ado. ϵ-Adenine (**4c**) and the 1,N^6-etheno derivative of 2-methyladenine (**2c**) at neutral pH show fluorescence emission characteristics similar to those of ϵ-Ado (λ_{em} 412 nm) at the same pH, but with a lower quantum yield[25] and a fluorescence lifetime of 12 ± 1 ns. Under acidic conditions (0.1 M HCl), ϵ-Ade and ϵ-m^{2}Ade show very weak emission maxima centered at 450 nm, rather than at 360 nm observed for **5a–c**. This is an indication of emission from a fluorescent species protonated on a nitrogen other than N(9), probably N(1) (corresponding to the N(7) of adenine), but N(4) has not been ruled out. N(1)-Methylated ϵ-Ado, the structure of which was based on ^{1}H NMR evidence, shows a fluorescence maximum in water at room temperature, pH 7.0, at 410 nm.[50b] The fluorescence lifetime has not been reported.

These results on the fluorescence emission characteristics of substituted ϵ-Ado (and ϵ-Ade) derivatives need not bring confusion to the interpretation of the fluorescence data obtained with the neutral species of ϵ-adenosine.[50] This is not a matter of controversy. The spectral similarity and comparable fluorescence yields of ϵ-9-propyladenine (3-propylimidazo[2,1-*i*]purine) in anhydrous dioxane,[25] where it cannot acquire a proton in the excited state, with different ϵ-adenine derivatives in aqueous solution[10,11,25] provide direct and positive evidence that the unprotonated excited state of ϵ-adenosine is fluorescent. Furthermore, the fluorescence properties of $N(1)$-deaza-ϵ-Ado,[50c] which obviously cannot protonate at position 1 (see **3** for numbering), are similar to those of ϵ-adenosine.

Experimental Section

Melting points were recorded on a Büchi melting point apparatus and are uncorrected. The NMR spectra were recorded by Mr. Steven Silber on a Varian Associates A-60 (indicated where used) or HA-100 spectrometer using tetramethylsilane or the methyl signals of sodium 2,2-dimethyl-2-silapentane-5-sulfonate as internal standard. The ultraviolet spectra were obtained on a Beckman Acta MVI spectrophotometer. Corrected fluorescence emission and fluorescence excitation spectra were acquired on a Spex Fluorolog spectrofluorometer. All fluorescence measurements were made at room temperature (22 °C). The fluorescence measured for each solution was normalized for differences among the samples in optical density at the exciting wavelength, and it is therefore a quantitative representation of quantum efficiencies relative to quinine taken as 0.7.[51] The mass spectra were obtained by Mr. J. Wrona on a Varian-MAT CH-5 spectrometer coupled with a 620i computer and STATOS recorder. Direct analysis of daughter ions (DADI), metastable ion scans, were also done on the CH-5 spectrometer.

9-Methyl-β-D-ribofuranosylimidazo[2,1-*i*]purinium Chloride (5a). Triethylamine (0.28 mL, 2 mmol) was added with stirring to a solution of 1,N^6-ethenoadenosine hydrochloride (328 mg, 1 mmol) in dimethylacetamide (10 mL) at 40 °C. To the resulting solution of 1,N^6-ethenoadenosine (**4a**) was added methyl iodide (0.25 mL, 4 mmol) and stirring was continued overnight. The product (90% yield) was isolated by precipitation with ether and recrystallization from absolute ethanol. The corresponding chloride was obtained by application of the product to a column of Dowex 1-X2 (chloride form) and elution with water. Combination of the first few fractions from the column, evaporation, and recrystallization of the residue from absolute ethanol furnished **5a**: mp 193–195 °C; NMR (D_2O) δ 4.43 (s, 3, CH_3), 6.37 (d, 1, J = 5 Hz, 1′-H), 8.00 (d, 1, J_{78} = 2.5 Hz, 8-H), 8.38 (d, 1, J_{78} = 2.5 Hz, 7-H), 8.86 (s, 1, 2-H), 9.46 (s, 1, 5-H); λ_{max} (pH 7.0 or 0.1 M HCl) 276 nm (ϵ 10 000); mass spectrum (70 eV) *m/e* 305 ($M^+ - HCl$), 173 ($C_8H_7N_5^+$).

Anal. Calcd for $C_{13}H_{16}ClN_5O_4$: C, 45.69; H, 4.72; N, 20.49. Found: C, 45.83; H, 4.74; N, 20.29.

5′-Amino-1-methyl-2,4′-biimidazole (6a). Compound **5a** was treated with 2 M HCl at 100 °C for 30 min, evaporated to small volume, and precipitated with acetone. The precipitate was washed with ether, dried, and dissolved in D_2O to record the NMR (D_2O, pD 5.5, 60 MHz): δ 3.88 (s, 3, CH_3), 7.52 (s, 2, 4,5-H), 7.67 (s, 1, 2′-H); (D_2O, pD 2.5, 60 MHz) δ 3.88 (s, 3, CH_3), 7.68 (s, 2, 4,5-H), 8.57 (s, 1, 2′-H); (D_2O, pD 5.5, 100 MHz) δ 3.88 (s, 3, CH_3), 7.45 (s, 2, 4,5-H; on expansion an unresolved AB pair of doublets can be detected), 7.62 (s, 1, 2′-H). The pD of the NMR sample was adjusted to 6–7, and the sample was evaporated to dryness and taken up in $CDCl_3$: NMR ($CDCl_3$) δ 3.88 (s, 3, CH_3), 6.86 (d, 1, J_{45} = 1.8 Hz, 4- or 5-H), 7.08 (d, 1, J_{45} = 1.8 Hz, 4- or 5-H), 7.32 (s, 1, 2′-H), 8.80 (br, NH_2 and NH, disappears on D_2O shake); mass spectrum (70 eV) *m/e* 163 (M^+).

9-Benzyl-3-β-D-ribofuranosylimidazo[2,1-*i*]purinium Chloride (5b). 1,N^6-Ethenoadenosine (**4a**) was treated with benzyl bromide as described for the methyl compound **5a**, and the bromide was converted to the chloride (80% yield): mp 212°; NMR (D_2O) δ 5.97 (s, 2, CH_2), 6.37 (d, 1, J = 5 Hz, 1′-H), 7.3–7.5 (m, 5, C_6H_5), 8.01 (d, 1, J_{78} = 2.5 Hz, 8-H), 8.39 (d, 1, J_{78} = 2.5 Hz, 7-H), 8.87 (s, 1, 2-H), 9.47 (s, 1, 5-H); λ_{max} (pH 7.0 or 0.1 M HCl) 277 nm (ϵ 13 000); mass spectrum (70 eV) *m/e* (rel abundance) 381 (M^+), 248 (19, $M^+ - 133$), and 91 (100, $C_7H_7^+$).

Anal. Calcd for $C_{19}H_{20}ClN_5O_4$: C, 54.62; H, 4.82; N, 16.76. Found: C, 54.69; H, 4.80; N, 16.67.

5′-Amino-1-benzyl-2,4′-biimidazole (6b). This compound was obtained by treatment of compound **5b** with 2 M HCl at 100 °C for 1 h: NMR (DCl) δ 5.19 (s, 2, CH_2), 7.1–7.5 (m, 5, C_6H_5), 7.70 (d, 1, J_{45} = 2.1 Hz, 4- or 5-H), 7.77 (d, 1, J_{45} = 2.1 Hz, 4- or 5-H), 8.40 (s, 1, 2′-H); mass spectrum (10 eV) *m/e* (rel abundance) 239 (M^+, base peak), 149 (33, $M^+ - 91 + 1$), 92 (72, $C_7H_8^+$), 91 (42, $C_7H_7^+$); (70 eV with HCl salt) 239 (M^+), 91 (9), 36 (100, HCl^+).

7,8-Dihydro-8-hydroxy-9-methylimidazo[2,1-*i*]purinium Chloride (3c). A suspension of N^6-methyladenine (2.23 g, 15 mmol) in water (500 mL) at 37 °C was treated with 11 mL of 2 M aqueous chloroacetaldehyde solution at pH 4.0–4.5, followed by three subsequent 4-mL portions at 6-h intervals. After 4 days, the reaction mixture was extracted three times with ether to remove polymeric material and was then concentrated to small volume until crystals started appearing. Ethanol was added, and the product was recrystallized from aqueous ethanol: yield 2.59 g (90%) of almost colorless crystals, mp 300 °C dec; NMR (D_2O) δ 3.72 (s, 3, CH_3), 4.70 (dd, 1, J_{gem} = 13, J_{vic} = 3.5 Hz, 7-H), 5.06 (dd, 1, J_{gem} = 13, J_{vic} = 7.5 Hz, 7-H), 6.02 (dd, 1, J_{vic} = 3.5, 7.5 Hz, 8-H), 8.55 (s, 1, 2-H), 8.75 (s, 1, 5-H); λ_{max} (0.1 M HCl) 212 nm (ϵ 21 900), 262 (12 400); λ_{max} (pH 7.0) 228 (20 300), 274 (10 550); λ_{max} (0.1 M NaOH) 228 (20 400), 274 (11 800); mass spectrum (10 and 70 eV) *m/e* 191 ($M^+ - HCl$), 173 ($M^+ - HCl - H_2O$, base peak).

Anal. Calcd for $C_8H_{10}ClN_5O \cdot H_2O$: C, 39.11; H, 4.92; N, 28.51. Found: C, 39.00; H, 5.00; N, 28.68.

7,8-Dihydro-8-hydroxy-9-methyl-3-β-D-ribofuranosylimidazo-[2,1-*i*]purinium Chloride (3a). N^6-Methyladenosine (**1a**) [NMR ($(CD_3)_2SO$) δ 3.07 (d, 3, $J_{N^6\text{-}H,CH_3}$ = 4.8 Hz, CH_3), 5.92 (d, 1, J = 6 Hz, 1′-H), 7.55 (br d, 1, $J_{N^6\text{-}H,CH_3}$ = 4.8 Hz, N^6-H), 8.25 (s, 1,8-H), 8.33 (s, 1, 2-H)] was treated with chloroacetaldehyde as described

for the free base **1c**. The residue, after ether extraction and evaporation of the solvent, was passed through a cellulose column using butanol–H_2O (86:14) as eluent. The fractions containing the desired compound were pooled and concentrated to small volume, and the product was recrystallized from water–butanol (yield 70%): mp 160 °C (slow decomposition); NMR (D_2O) δ 3.68 (s, 3, CH_3), 3.90–5.10 (br m, 7-H and ribosyl protons), 5.92 (m, 1, 8-H), 6.10 (d, 1, J = 5 Hz, 1′-H), 8.65 (s, 2, 2-H and 5-H); λ_{max} (0.1 M HCl and pH 7.0) 215 nm (ϵ 22 200), 262 (13 700); λ_{max} (0.1 M NaOH) 263 (10 700).

Anal. Calcd for $C_{13}H_{18}ClN_5O_5$: C, 43.40; H, 5.04; N, 19.46. Found: C, 43.49; H, 5.37; N, 18.68.

Dehydration of 7,8-Dihydro-8-hydroxy-9-methylimidazo[2,1-*i*]purinium Chloride (3c) to 9-Methylimidazo[2,1-*i*]purinium Chloride (5c). A viscous solution of compound **3c** (228 mg, 1 mmol) and 3 g of polyphosphoric acid (Practical Grade, Matheson Coleman and Bell) was stirred with a glass rod and heated at 120 °C for 15 min, when the initial effervescence subsided. After an additional 1 h of heating, it was poured over crushed ice and quickly neutralized with saturated aqueous $NaHCO_3$. The mass was evaporated to dryness after addition of 1-propanol and then extracted three times with 50-mL portions of hot 1-propanol. The combined extracts were evaporated to dryness, dissolved in $CHCl_3$–CH_3OH (7:3), filtered, and evaporated to dryness again. The residue was dissolved in water, the pH was adjusted carefully to 3.0 using 0.1 N HCl, and the chloride was recrystallized from aqueous acetone: yield 218 mg (98%) of colorless crystals, mp 300 °C; NMR (D_2O) δ 4.34 (s, 3, CH_3), 7.93 (d, 1, J_{78} = 2.5 Hz, 8-H), 8.29 (d, 1, J_{78} = 2.5 Hz, 7-H), 8.50 (s, 1, 2-H), 9.30 (s, 1, 5-H); λ_{max} (0.1 M HCl) 275 nm (ϵ 12 000); λ_{max} (pH 7.0) 280 (12 500), λ_{max} (0.1 M NaOH) 285 (13 100); mass spectrum (10 and 70 eV) *m/e* 173 (M^+ − HCl, base peak).

Anal. Calcd for $C_8H_8ClN_5$: C, 45.83; H, 3.85; N, 33.41. Found: C, 45.76; H, 4.10; N, 33.09.

Methylation of 1,N^6-Ethenoadenine (4c) to 9-Methylimidazo[2,1-*i*]purinium Chloride (5c). 1,N^6-Ethenoadenine (**4c**) was methylated as described earlier for compound **4a**, and the iodide (80% yield) was converted to the chloride, mp 300 °C. The chloride was identical with a sample of **5c** obtained by dehydration of compound **3c**. On treatment with 2 M HCl at 100 °C it gave the same 5′-amino-1-methyl-2,4′-biimidazole (**6a**) characterized earlier.

5-Methylimidazo[2,1-*i*]purine Hydrochloride (1,N^6-Etheno-2-methyladenine Hydrochloride, 7c). A solution of 90 mg (0.4 mmol) of 2-methyladenine hemisulfate in 20 mL of water and 4 mL of 2 M aqueous chloroacetaldehyde solution at pH 4.0–4.5 was stirred at 37 °C for 24 h and concentrated to a syrup in vacuo, and the product was precipitated using ethanol–ether. Recrystallization of the residue from aqueous ethanol with decolorization furnished 80 mg (96%) of colorless crystals of **7c**: mp 241–242 °C; NMR (DCl) δ 3.07 (s, 3, CH_3), 8.01 (d, 1, J_{78} = 2.8 Hz, 8-H), 8.24 (d, 1, J_{78} = 2.2 Hz, 7-H), 8.60 (s, 1, 2-H); λ_{max} (0.1 M HCl) 275 nm (ϵ 12 700); λ_{max} (pH 7.0) 264 sh (7300), 274 (9200), 281 sh (7900), 296 sh (5050); λ_{max} (0.1 M NaOH) 224 (26 300), 232 sh (22 100), 272 (7150), 280 (8900), 297 (6700); mass spectrum (10 and 70 eV) *m/e* 173 (M^+ − HCl, base peak).

Anal. Calcd for $C_8H_8ClN_5 \cdot H_2O$: C, 42.21; H, 4.43; N, 30.76. Found: C, 42.12; H, 4.28; N, 30.37.

5′-Amino-2,4′-biimidazole (6c). Treatment of 1,N^6-etheno-2-methyladenine (**7c**) with 2 M HCl for 1.5 h on a steam bath produced 5′-amino-2,4′-biimidazole (**6c**): NMR (DCl) δ 7.68 (s, 2, 4,5-H) and 8.57 (s, 1, 2′-H); mass spectrum (70 eV) *m/e* 149 (M^+). The UV spectra [λ_{max} (0.1 M HCl) 282 nm and λ_{max} (0.1 M NaOH) 278 nm] approximated literature values[27] (pH 1, 287 nm; pH 7, 275 nm; pH 13, 282 nm), but 5′-amino-2,4′-biimidazole (**6c**) obtained from HCl cleavage of 1,N^6-ethenoadenine and from 1,N^6-ethenoadenosine was identical with the sample produced from **7c**.

N^6-Ethyladenosine (1, R = H; R′ = Et). A solution of adenosine (520 mg, 2 mmol) in 25 mL of water at pH 3.3 was treated with 5 mL (10 mmol) of an aqueous solution of sodium cyanohydridoborate ($NaBH_3CN$) and 10 mL (20 mmol) of an aqueous solution of acetaldehyde under nitrogen at 37 °C and pH 4.4 ± 0.05, maintained by using 0.2 M HCl solution in the reservoir of a pH stat. After seven portions of the latter two reactants were added successively each hour, the reaction was complete. After another 2 h, the reaction mixture was cooled to 5 °C and the pH was adjusted to 3.0 to decompose excess $NaBH_3CN$. After 15 min the reaction mixture was brought to pH ~ 7 at 40 °C and treated with Darco for 30 min. Filtration and concentration deposited 450 mg (80%) of a white powder which was recrystallized from water as white needles: mp 198–200 °C; NMR [$(CD_3)_2SO$] δ 1.17 (t, 3, $J_{\alpha,\beta}$ = 7 Hz, CH_3, collapsed to a singlet on irradiation at 3.6 ppm), 3.6 (m, 2, 5′-CH_2, OH (pattern simplified on D_2O Shake) and CH_2N (irradiation of the signal collapsed the triplet at δ 1.17), 5.90 (d, 1, $J_{1'}$ = 6 Hz), 7.8 (t, 1, J = 6 Hz, N^6H, disappears on D_2O shake), 8.20 (s, 1, 2-H or 8-H) 8.32 (s, 1, 2-H or 8-H); λ_{max} (0.1 M HCl) 263 nm (ϵ 17 750); λ_{max} (pH 7.0) 267 (16 650); λ_{max} (0.1 M NaOH) 267 (17 000); mass spectrum (70 and 10 eV) *m/e* 295 (M^+), 192 (B + 30, base peak), 164 (B + 3).

Anal. Calcd for $C_{12}H_{17}N_5O_4$: C, 48.80; H, 5.80; N, 23.72. Found: C, 48.58; H, 5.82; N, 24.00.

N^6-Furfuryladenosine (Kinetin Riboside) (1, R = ribosyl; R′ = C_5H_5O). This compound was prepared following a procedure similar to that described for N^6-ethyladenosine with the modification that the 2-furaldehyde solution was prepared in methanol and 14 additions of reagents at 2-h intervals were required for completion. The product was isolated by removing methanol, extracting with ether, and discarding the extract. The aqueous layer was then cooled in the ice bath, the pH was adjusted to 3.0, and the compound was isolated as described above as light brown material which was recrystallized from methanol to obtain a cream-colored compound characterized by MS, UV, and cochromatography with an authentic sample. The same procedure with benzaldehyde produced N^6-benzyladenosine.

N^6-Furfuryladenine (kinetin) (1, R = H; R′ = C_5H_5O) was prepared as described for the riboside. The reaction temperature was raised to 55 °C to improve the solubility of adenine in water. The crude compound obtained in the isolation was purified by column chromatography on silica gel using chloroform–methanol and was recrystallized from absolute ethanol to yield 85% of the product characterized by UV and cochromatography with an authentic sample.

Fluorescence Lifetimes. These were determined using a cross-correlation fluorometer[52] interfaced to a Monroe programmable calculator. Samples were excited at 300 nm. The fluorescence emission was filtered with a Corning filter 0-54. Compound **4c** in 0.050 M phosphate buffer at pH 7.0 and room temperature was found to have a fluorescence lifetime of 12 ± 1 ns by phase and modulation. The same compound in 0.1 N HCl and compounds **5a–c** at pH 7.0 or in 0.1 N HCl have fluorescence lifetimes of ca. 1 ns as determined by phase measurement.

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Stereochemical Study of the [3,3] Sigmatropic Rearrangement of 1,5-Diene-3-alkoxides. Application to the Stereoselective Synthesis of (±)-Juvabione

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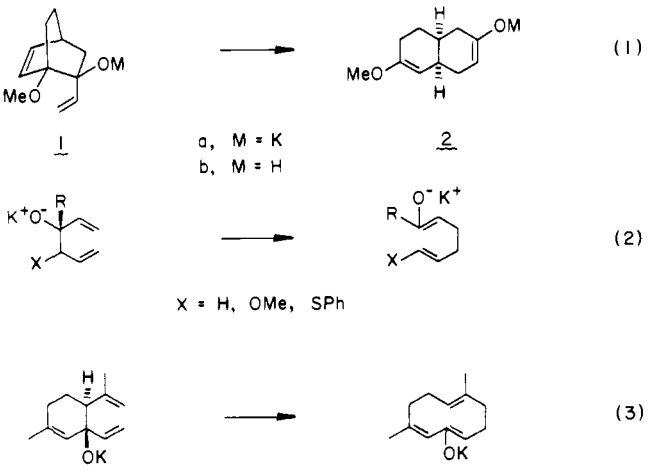
Abstract: A study was carried out on the [3,3] sigmatropic rearrangement of the potassium salts of the individual dienols **6a**, **6b**, **7a**, and **7b**. It has been found that these rearrangements proceed in a concerted fashion predominately via chair transition states to give the diastereoisomeric ketones **8** and **9**. The application of these modified oxy-Cope rearrangements to the synthesis of (±)-*erythro*-juvabione (**15a**) is reported.

Introduction

In 1975 we reported the observation that the oxy-Cope rearrangement of diene alkoxide **1a** proceeded at approximately 10^{12} times the rate of the corresponding alcohol **1b** (eq 1).[2] In subsequent investigations, we[3] and others[4] have established that these initial alkoxide-promoted rate accelerations are generalizable to other systems (eq 2 and 3).

In spite of the apparently "concerted" nature of these rearrangements, only one published experiment bears on this important issue.[2] In this regard, we found that, under conditions in which **1a** underwent rearrangement with a half-life of 1.4 min (THF, 60 °C), the diastereoisomeric alkoxide **3** was stable for ca. 24 h. While these experiments contribute permissive evidence for the concerted nature of the transformation of **1a** → **2a**, it does *not* rule out a nonsynchronous mechanism. The observations could be equally well explained by assuming that single-bond cleavage occurs in both substrates via either a homolytic or heterolytic pathway[5] but that the rotational barrier for the interconversion of these intermediates (cf. **4** and **5**) is large relative to the recombination barrier **5** → **3** (Scheme I, homolysis).



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