Synthetic Spectroscopic Models Related to Coenzymes and Base Pairs. An "Abbreviated" Nicotinamide Adenine Dinucleotide[1]

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Abstract: An "abbreviated" model system of the coenzyme NAD^+ has been synthesized and studied by ultraviolet spectroscopy and circular dichroism, namely, 5′-(3-carbamoylpyridin-1-ylium)-5′-deoxyadenosine chloride, in which the quaternized nicotinamide is attached to the 5′-carbon of adenosine. Also required for the study were the new compounds methyl 5′-(3-carbamoylpyridin-1-ylium)-5′-deoxy-D-ribofuranoside chloride, 5′-(3-carbamoylpyridin-1-ylium)-5′-deoxy-2′,3′-*O*-isopropylideneadenosine chloride, and methyl 5′-(3-carbamoylpyridin-1-ylium)-2′,3′-*O*-isopropylidene-β-D-ribofuranoside chloride. The spectroscopic techniques verified the existence of intramolecular interaction in aqueous solution between the quaternized nicotinamide ring and the adenine ring. The ultraviolet hypochromism studies indicate that the model system, which lacks the additional ribose and diphosphate moieties present in NAD^+, shows greater inter-ring interaction than does NAD^+ itself. The interaction is greatly diminished in acidic aqueous solutions and in 95% ethanol. The circular dichroism studies indicate the presence of new bands at *ca.* 274 and 253 nm which are not present in the CD spectra of the two component "halves" of the spectroscopic model. The Cotton effects of these two bands are opposite to those observed for NAD^+ itself in the same regions. The "abbreviated" model 5′-(3-carbamoylpyridin-1-ylium)-5′-deoxyadenosine chloride, which can thus be related to a folded form of NAD^+ in aqueous solution, showed no substrate activity in a standard horse liver alcohol dehydrogenase system.

Interactions between bases in coenzymes have been detected by various spectroscopic means,[2-5] and evidence has been accumulated which indicates that coenzymes such as NAD^+ and its derivatives tend to exist in "folded" or internally complexed forms. The identification of specific conformations of the folded forms by nmr spectroscopy[6,7] is still undergoing critical analysis.[8] By contrast, it has been found by X-ray structure determination that NAD^+ bound to the enzyme dogfish lactate dehydrogenase assumes the "open" form of the coenzyme.[9,10]

Spectroscopic models have been synthesized in this laboratory in which variable-length methylene chains replace the ribose phosphates as the link between the nitrogenous bases, with the trimethylene bridge providing the best opportunity for near-parallel planar base–base interaction in aqueous solution.[2,11-13] The purpose of the present study was to provide an improved model system for examining interactions, one more closely resembling the natural system and including hydroxyl groups available for bonding. "Abbreviated" models of NAD^+ containing the elements of the two heterocyclic rings and the sugar have been synthesized. By placing a quaternized nicotinamide on the 5′-carbon of adenosine, for example, the two heterocyclic rings have the capability of existing approximately 3.4 Å apart,[14,15] in the range generally observed for interplanar distances between bases in DNA and RNA.[16] However, the conformation corresponding to maximum interaction is not necessarily dictated by this abbreviated NAD^+ model system.

At the outset we wished to make and compare spectroscopically the isopropylidene derivatives **1**, **3**, and **5** since evidence has been accumulated that the isopropylidene group holds the ribose ring conformationally less labile, with the substituent at C-1′ and the 5′-methylene group in fixed relation.[17-20] Of course, we recognized that the 5′ substituent could point away from the substituent at C-1′, but we relied upon association of the adenine and nicotinamide rings being favored in aqueous solution as in the trimethylene-bridged model.[2] The compounds necessary for the spectroscopic study were 5′-(3-carbamoylpyridin-1-ylium)-5′-deoxy-2′,3′-*O*-isopropylideneadenosine chloride (**1**) and the "half" molecules methyl 5′-(3-carbamoylpyr-

(1) (a) For the preceding paper (part VII) in this series, see H. Iwamura, N. J. Leonard, and J. Eisinger, *Proc. Nat. Acad. Sci. U. S.*, **65**, 1025 (1970). (b) The present paper may be regarded as part VIII in this series.

(2) T. G. Scott, R. D. Spencer, N. J. Leonard, and G. Weber, *J. Amer. Chem. Soc.*, **92**, 687 (1970), and references therein.

(3) J. M. Siegel, G. A. Montgomery, and R. M. Bock, *Arch. Biochem. Biophys.*, **82**, 288 (1959).

(4) D. W. Miles and D. W. Urry, *J. Biol. Chem.*, **243**, 4181 (1968).

(5) G. Weber, *Proc. Int. Congr. Pharmacol.*, **3**, 180 (1969), and references therein.

(6) R. H. Sarma and N. O. Kaplan, *Biochemistry*, **9**, 539 (1970).

(7) R. H. Sarma, M. Moore, and N. O. Kaplan, *ibid.*, **9**, 549 (1970).

(8) (a) J. Jacobus, *ibid.*, **10**, 161 (1971); (b) N. J. Oppenheimer, L. J. Arnold, and N. O. Kaplan, *Proc. Nat. Acad. Sci. U. S.*, **68**, 3200 (1971).

(9) M. J. Adams, A. McPherson, Jr., M. G. Rossmann, R. W. Schevitz, and A. J. Wonacott, *J. Mol. Biol.*, **51**, 31 (1970).

(10) A. McPherson, Jr., *ibid.*, **51**, 39 (1970).

(11) (a) N. J. Leonard and R. F. Lambert, *J. Org. Chem.*, **34**, 3240 (1969); (b) R. F. Lambert, Ph.D. Thesis, University of Illinois, 1967.

(12) R. F. Lambert, G. Weber, and N. J. Leonard, 7th International Congress of Biochemistry, Tokyo, 1967, Paper F-8:7.

(13) D. T. Browne, J. Eisinger, and N. J. Leonard, *J. Amer. Chem. Soc.*, **90**, 7302 (1968).

(14) For a similar system, not a quaternized nicotinamide but adenosine 5′-mononicotinate, see D. W. Miles and D. W. Urry, *J. Phys. Chem.*, **71**, 4448 (1967).

(15) "Double-headed" deoxyribonucleosides have been made in the Merck Sharp and Dohme Research Laboratories bearing adenine and/or thymine moieties at the 1′ and 5′ positions: R. Fecher, K. H. Boswell, J. J. Wittick, and T. Y. Shen, *J. Amer. Chem. Soc.*, **92**, 1400 (1970). The authors are grateful to Dr. Shen for providing a preprint of their communication while our work was in progress. For a review, see T. Y. Shen, *Angew. Chem. Int. Ed. Engl.*, **9**, 678 (1970). The present authors prefer the designation "abbreviated" in referring to coenzyme or dinucleoside models of this type.

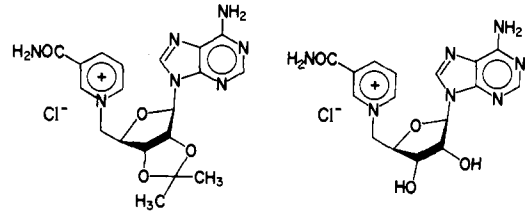
(16) M. Sundaralingam, *Biopolymers*, **7**, 821 (1969).

(17) B. A. Otter, E. A. Falco, and J. J. Fox, *J. Org. Chem.*, **34**, 1390 (1969).

(18) E. J. Reist, A. Benitez, and L. Goodman, *ibid.*, **29**, 554 (1964).

(19) B. Bannister and F. Kagan, *J. Amer. Chem. Soc.*, **82**, 3363 (1960).

(20) R. W. Chambers and V. Kurkov, *ibid.*, **85**, 2160 (1963).



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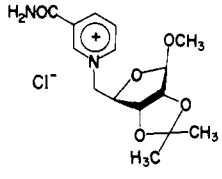

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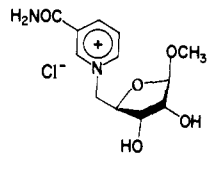

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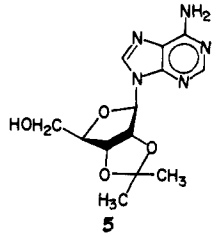

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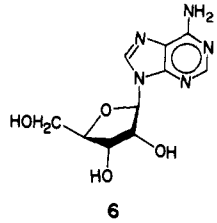

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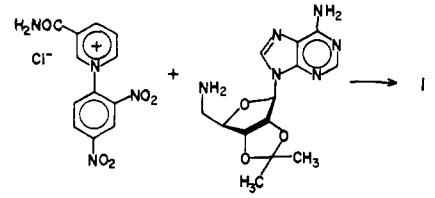
idin-1-ylium)-2′,3′-*O*-isopropylidene-β-D-ribofuranoside chloride (**3**) and 2′,3′-*O*-isopropylideneadenosine (**5**), as well as the corresponding unprotected ribonucleosides **2**, **4**, and **6**. The synthesis of 5′-(3-carbamoylpyridin-1-ylium)-5′-deoxy-2′,3′-*O*-isopropylideneadenosine chloride (**1**) was achieved by reaction of 5′-amino-5′-deoxy-2′,3′-*O*-isopropylideneadenosine (**8**)[21,22] with 3-carbamoyl-1-(2,4-dinitrophenyl)pyridinium chloride (**7**).[23] The "half" molecule methyl 5′-(3-carbamoylpyridin-1-ylium)-5′-deoxy-2′,3′-isopropylidene-β-D-ribofuranoside chloride (**3**) was obtained similarly by reaction of methyl 5-amino-5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranoside (**9**)[24] with **7**. The formulas as drawn (**1**–**6**) are not intended necessarily to represent preferred conformations but are constructed for convenience in comparison of the "half" molecules (**3**–**6**) with the coenzyme models (**1**, **2**).



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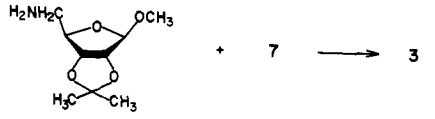

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In order to examine the unprotected model system, it was only necessary to remove the isopropylidene group from the 2′- and 3′-hydroxyls. In the case of **1** this was accomplished with 50% aqueous formic acid at 25°, yielding 5′-(3-carbamoylpyridin-1-ylium)-5′-deoxyadenosine chloride (**2**). In the case of **3**, methanolic hydrogen chloride at 25° rapidly removed the isopropylidene group, while retaining the 1-methoxyl group, but preservation of the β configuration in the product, methyl 5′-(3-carbamoylpyridin-1-ylium)-5′-deoxy-"β"-D-ribofuranoside chloride (**4**), was not guaranteed.

In this regard, it is interesting to note that in all the compounds with a quaternary nitrogen on the 5′ position the ribose ring protons are shifted downfield in such a manner that they overlap each other, whereas ordinarily they are very well separated. Though this is valuable in recognizing the quaternized compound, it makes interpretation of the spectra extremely difficult. In fact, this shift is the reason that the anomeric configuration cannot readily be confirmed for the ribofuranoside **4**, since the 1′-H signal overlaps with those of the other ribose protons. Quaternization also markedly affects the nicotinamide protons, with chemical shifts extending nearly to δ 10.

The models were then examined for interaction between the quaternized nicotinamide and purine rings by means of ultraviolet spectroscopy and by circular dichroism at concentrations low enough so that intramolecular rather than intermolecular phenomena were being observed. In previous studies involving base pairs connected by alkylene chains,[1,2,11–13,25] a meaningful criterion for assessing interaction has been shown to be the per cent hypochromism, H.[26] Hypochromism is defined in terms of the oscillator strengths f, which in our study have been determined by computer integration of the absorption curves, using intervals of 2.5 nm, beginning in the vicinity of the absorption minimum. Calculation of the per cent hypochromism is then carried out by substitution of the oscillator strengths in the equation $H = [1 - f_{AB}/f_A + f_B]100$, where f_A and f_B are the oscillator strengths of the half molecules and f_{AB} is the oscillator strength of the coenzyme model.

Alternatively, hypochromicity[3,27–29] has been used to evaluate base–base interaction. This refers to a decrease in the absorbance at a particular wavelength, usually the absorbance maximum, and does not encompass the entire band. However, in the study of trimethylene-bridged base pairs,[13] the maximum hypochromicity did not occur at the absorbance maximum of the base pair but was in general shifted to longer wavelengths to varying degrees. In addition, the values obtained for the hypochromicity showed only small changes with respect to base variation in the dinucleotide analogs. On the other hand, a definite trend was observed in the values recorded for the per cent hypochromisms over the spectrum of analogs examined. It was therefore concluded that per cent hypochromism afforded more meaningful results with

(21) W. Jahn, *Chem. Ber.*, **98**, 1705 (1965).
(22) M. G. Stout, M. J. Robins, R. K. Olsen, and R. K. Robins, *J. Med. Chem.*, 12, 658 (1969).
(23) H. Lettré, W. Haede, and E. Ruhbaum, *Justus Liebigs Ann. Chem.*, **579**, 123 (1953).
(24) N. J. Leonard and K. L. Carraway, *J. Heterocycl. Chem.*, **3**, 485 (1966).
(25) N. J. Leonard, H. Iwamura, and J. Eisinger, *Proc. Nat. Acad. Sci. U. S.*, **64**, 352 (1969).
(26) (a) I. Tinoco, Jr., *J. Amer. Chem. Soc.*, **82**, 4785 (1960); (b) *ibid.*, **83**, 5047 (1961); (c) *J. Chem. Phys.*, **33**, 1332 (1960); (d) *ibid.*, **34**, 1067 (1961); (e) W. Rhodes, *J. Amer. Chem. Soc.*, **83**, 3609 (1961).
(27) L. G. Whitby, *Biochem. J.*, **54**, 437 (1953).
(28) S. Aoyagi and Y. Inoue, *J. Biol. Chem.*, **243**, 514 (1968).
(29) T. Hill, *J. Chem. Phys.*, **30**, 383 (1959).

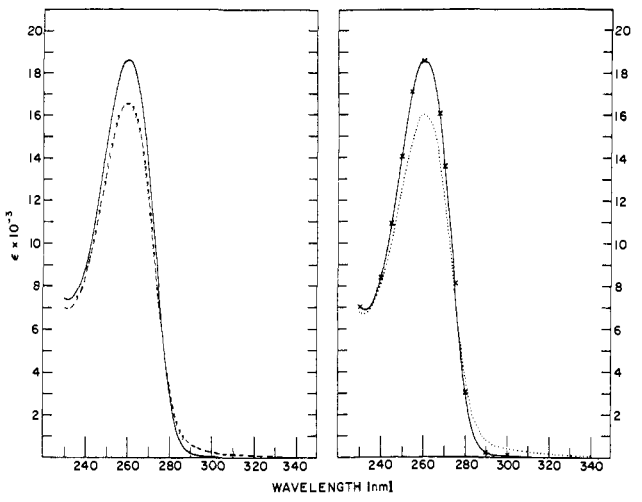

Figure 1. (Left) Ultraviolet spectra of 5′-(3-carbamoylpyridin-1-ylium)-5′-deoxy-2′,3′-*O*-isopropylideneadenosine chloride (**1**) (——) *vs.* the sum of 2′,3′-*O*-isopropylideneadenosine (**5**) and methyl 5′-(3-carbamoylpyridin-1-ylium)-5′-deoxy-2′,3′-*O*-isopropylidene-β-D-ribofuranoside chloride (**3**) (– – –). (Right) Ultraviolet spectra of 5′-(3-carbamoylpyridin-1-ylium)-5′-deoxyadenosine chloride (**2**) (— x — x —) *vs.* the sum of adenosine (**6**) and methyl 5′-(3-carbamoylpyridin-1-ylium)-5′-deoxy-D-ribofuranoside chloride (**4**) (· · · · ·).

respect to base stacking than did the per cent hypochromicity (*h*) for those systems.

The values obtained for the *h* at the absorbance maximum and for the *H* for the coenzyme model *vs.* the corresponding component molecules are presented in Table I. Also included are the values for NAD$^+$ with respect to its components, adenosine 5′-monophosphate (AMP) and nicotinamide mononucleotide (NMN).[30] Looking first at the data for the coenzyme analogs with respect to NAD$^+$ itself, the higher values for both *H* and *h* in aqueous solution at pH 7 indicate that the coenzyme models spend a greater portion of their time in stacked conformation than does NAD$^+$. It is interesting to note that while the *H* values of **1** and **2** are within experimental error of each other, the *h* values are significantly different. Examination of Figure 1, showing the sums of the two component halves (**3** + **5** and **4** + **6**) plotted with the coenzyme model in each case (**1** and **2**), shows the basis of the disparity. The hypochromic effect in the two systems is not distributed evenly over the entire band but is concentrated in the region of the absorption maximum. In the case of the unprotected nucleoside **2** there is a larger hypochromic effect in the region of the absorbance maximum than with **1**, but this is balanced by a correspondingly larger hyperchromic effect[31] at longer wavelength. Hence the *H* values tend to be equalized, while the *h* values are not subject to this balancing effect. Perhaps the most striking feature of the curves is the tail extending out to approximately 340 nm exhibited by the coenzyme models. This tail, which is not present in any of the half molecules, contributes a significant hyperchromic effect, causing a reduction in the observed *H* values. This tail absorption is also prominent in the ultraviolet spectrum of NAD$^+$.[3]

Table I. Computed Per Cent Hypochromism and Hypochromicity for Coenzyme Analogs *vs.* NAD$^+$

Compd	pH[a]	*H*,[b] %	*h*,[b] %
NAD$^+$[28]	7	3.7	7.6
1	7	6.6	9.8
2	7	7.3	14.2
NAD$^+$[28]	1	~0	~0
1	1	2.9	5.2
2	1	3.5	9.9
1	95% EtOH	1.9	4.5

[a] Aqueous solution. [b] Values of *H* and *h* are reproducible to 1–2%.

A clearer picture of the effects over the entire curve can be obtained through examination of the difference spectra, a plot made by subtracting the sum of the spectra for the half molecules from that of the coenzyme analog. The difference spectra of **1** with respect to **3** and **5** are shown in Figure 2, in which negative values of $\Delta\epsilon$ correspond to a hypochromic effect and positive values to a hyperchromic effect. At pH 7 (A), the region of the curve exhibiting a hyperchromic effect, beginning at about 277.5 nm, causes a reduction in the overall *H* of about 1.8. That is, if we calculate *H* from the absorption minimum of the coenzyme model to 277.5 nm the value obtained is 8.4%.

Critical to the calculation of *H* is the wavelength at which the calculation is started. Since the 260-nm band overlaps with the higher energy band, a cutoff point must be selected to balance out this overlap. This point has been previously chosen in the vicinity of the λ_{min} of the dinucleotide analog,[13,32] and the present calculations have also been carried out in this manner.[33] See Table II for a summary of the ultraviolet data.

Table II. Comparative Ultraviolet Absorption Data

Compd	pH[a]	λ_{max}, nm	ϵ ($\times 10^{-3}$)	λ_{min}, nm	ϵ ($\times 10^{-3}$)
5	7	259	15.1	226	2.3
5	1	257	14.9	228	3.2
3	7	265.5	4.2	247	2.3
3	1	265.5	4.2	247	2.3
1	7	260	16.6	231.5	6.9
1	1	258	17.2	232	7.3
6	7	259	15.1	226.5	2.5
6	1	257	14.7	230	3.5
4	7	265.5	4.2	245.5	2.0
4	1	265.5	4.3	246	2.1
2	7	260	16.0	232	6.6
2	1	258.5	16.5	232.5	7.1

[a] Aqueous solution.

The comparison of curves in Figure 1 affords additional information. Contrary to the previously mentioned dinucleotide analogs,[13] there is no band shift of the λ_{min} of the coenzyme analog with respect to the sum of the half molecules. Also, the greatest value of $\Delta\epsilon$

(30) Values for NAD$^+$ have been determined by Dr. J. H. Craig, University of Illinois. They will be included in part X of this series (in preparation).

(31) A hyperchromic effect refers to an increase in the absorbance for the coenzyme model with respect to the two half molecules, in contrast to a hypochromic effect, which refers to a corresponding decrease.

(32) M. M. Warshaw and I. Tinoco, Jr., *J. Mol. Biol.*, 20, 29 (1966).

(33) This system necessitates the inclusion of a portion of the higher energy band of the quaternized nicotinamide moiety and the exclusion of a portion of the 260-nm band of the adenosine moiety due to the wide separation of the λ_{min} values (Table II) for the separate half molecules.

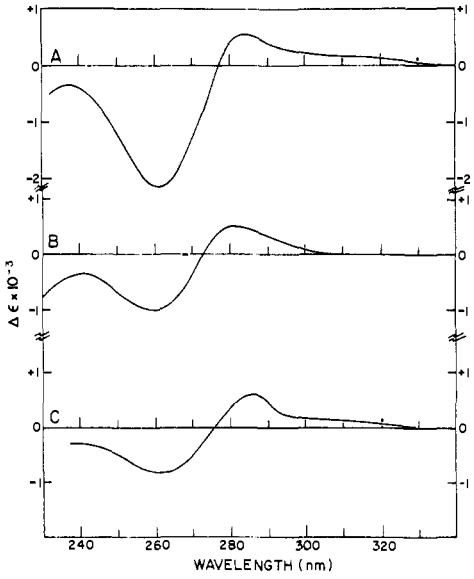

Figure 2. Difference spectra of 2′,3′-*O*-isopropylideneadenosine (**5**) and methyl 5′-(3-carbamoylpyridin-1-ylium)-5′-deoxy-2′,3′-*O*-isopropylidene-β-D-ribofuranoside chloride (**3**) *vs.* 5′-(3-carbamoylpyridin-1-ylium)-5′-deoxy-2′,3′-*O*-isopropylideneadenosine chloride (**1**): (A) pH 7 in H_2O, (B) pH 1 in H_2O, (C) 95% ethanol.

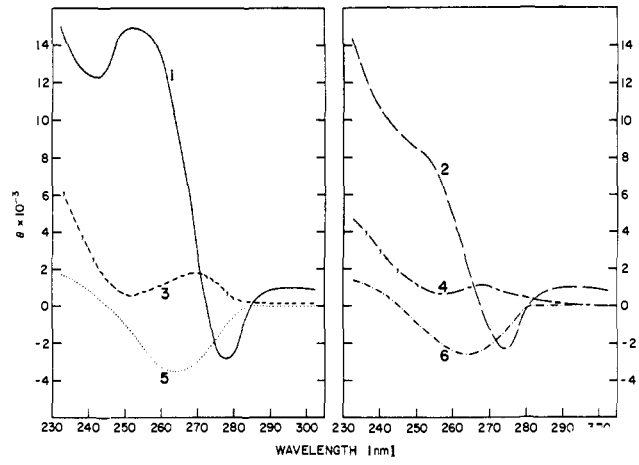

Figure 3. (Left) Circular dichroism spectra of 2′,3′-*O*-isopropylideneadenosine (**5**) (·····), methyl 5′-(3-carbamoylpyridin-1-ylium)-5′-deoxy-2′,3′-*O*-isopropylidene-β-D-ribofuranoside chloride (**3**) (– – –), and 5′-(3-carbamoylpyridin-1-ylium)-5′-deoxy-2′,3′-*O*-isopropylideneadenosine chloride (**1**) (———) in aqueous solution at pH 7. (Right) Circular dichroism spectra of adenosine (**6**)[15] (–·–·–), methyl 5′-(3-carbamoylpyridin-1-ylium)-5′-deoxy-β-D-ribofuranoside chloride (**4**) (— — —), and methyl 5′-(3-carbamoylpyridin-1-ylium)-5′-deoxy-2′,3′-*O*-isopropylideneadenosine chloride (**2**) (— —) in aqueous solution at pH 7. All concentrations about 1×10^{-4} *M* to diminish intermolecular interactions.

is coincident with the λ_{max}. This lends more significance to the values obtained for *h*. This fact, along with the aforementioned complications, appears to have made *h* a more sensitive measure of interaction than *H* in the case of **1** and **2**.

Studies were also run in aqueous solution at pH 1 and in 95% ethanol, which has been shown to be a good denaturing solvent for DNA.[34] In both cases the hypochromic effect is considerably reduced, but definitely not eliminated. The difference spectra (Figure 2, pH 1 (B) and 95% EtOH (C)) indicate that the greatest reduction occurs in the hypochromic 260-nm band and with only slight reduction in the long-wavelength hyperchromic effect. These data indicate that some available conformations still contribute to the hypochromicity, even with the repulsive interaction caused by protonation of the adenine ring (B). Again it is worthwhile noting that in aqueous solution at pH 1 and in 95% ethanol the *H* values are within experimental error of each other, while the *h* values appear more sensitive, indicating a difference in either electrostatic interaction or degree of stacking.

Additional support for the stacking interaction in the NAD^+ analogs is found from examination of the circular dichroism spectra (Figure 3). Numerous circular dichroism studies on the conformation of dinucleotides and oligonucleotides[35-40] have shown that the spectrum of the dinucleotide or oligonucleotide cannot be explained simply through a summation of the monomeric species. Hence the new band must have resulted from an interaction between the adjacent bases. Figure 3 shows the circular dichroism spectra of **1**, **3**, and **5**. It is obvious that the sum of the spectra of **3** and **5** would not at all resemble the spectrum of **1**. The new negative CD band at 278 nm and positive CD band at 253 nm for the coenzyme model **1** are strong evidence for the interaction between the quaternized nicotinamide ring and the adenine ring. It is worth noting that both NAD^+[4] and adenosine 5-mononicotinate[14] have circular dichroism curves which also bear no simple relation to the curves for their component parts. However, NAD^+ shows a positive CD band at long wavelength while the abbreviated model **1** shows a negative CD band in the same wavelength range. In fact, the circular dichroism curves for **1** and NAD^+[4] have the same shape in the 240–290-nm region but are opposite in the sign of the molar ellipticities. A full explanation is lacking because of the many variables in structure and conformation. Nevertheless, it is tempting to conclude that the transition moments for the two halves (**3**, **5**) of model **1** bear similar relation to those for the NMN and AMP halves of NAD^+, that in the average folded conformations the two rings in the model and in NAD^+ are facing each other in similar fashion (*i.e.*, they do not have a diastereomeric relationship), and that the average conformations for the reversibly folded forms of **1** and of NAD^+ are close to being enantiomeric (*i.e.*, this might be uniquely defined, for example, by a conformational rotation about the 9–1′ bond of the adenosine moiety in the model of *ca.* 180° in comparison with NAD^+).[41]

The negative Cotton effect at *ca.* 278 nm and positive Cotton effect at *ca.* 253 nm for the isopropylidene compound **1** are similar but more pronounced than for

(34) (a) T. T. Herskovits, S. J. Singer, and E. P. Geiduschek, *Arch. Biochem. Biophys.*, **94**, 99 (1961); (b) T. T. Herskovits, *ibid.*, **97**, 474 (1962).

(35) C. R. Cantor, M. M. Warshaw, and H. Shapiro, *Biopolymers*, **9**, 1059 (1970).

(36) M. M. Warshaw and C. R. Cantor, *ibid.*, **9**, 1079 (1970).

(37) J. Brahms, J. C. Maurizot, and A. M. Michelson, *J. Mol. Biol.*, **25**, 465 (1967).

(38) J. Brahms, J. C. Maurizot, and A. M. Michelson, *ibid.*, **25**, 481 (1967).

(39) J. Brahms, A. M. Michelson, and K. E. Van Holde, *ibid.*, **15**, 467 (1965).

(40) K. E. Van Holde, J. Brahms, and A. M. Michelson, *ibid.*, 12, 726 (1965).

(41) T. R. Emerson, R. J. Swan, and T. L. V. Ulbricht, *Biochem. Biophys. Res. Commun.*, 22, 505 (1966). The presence of the isopropylidene group does not alter the sign of the Cotton effect.

the model **2** lacking the isopropylidene group (Figure 3, right panel). This CD finding is consistent with the greater conformational limitation imposed when the isopropylidene group is present. In aqueous solution the abbreviated NAD^+ model **2** also shows a *negative* Cotton effect at *ca.* 274 nm, in the range (273 nm)[4] where NAD^+ shows a positive effect, and a *positive* Cotton effect (shoulder) at *ca.* 252 nm, in the range (253 nm)[4] where NAD^+ shows a negative effect. Interring interaction in this model is thus indicated, and an enantiomeric relation of the two rings in **2** with those in the average folded conformation of NAD^+ is suggested.

Of corollary interest is the fact that the "abbreviated" model 5′-(3-carbamoylpyridin-1-ylium)-5′-deoxyadenosine chloride (**2**) showed no substrate activity in a standard horse liver alcohol dehydrogenase system.[42] Aside from other differing features between this molecule and NAD^+, the model **2** is essentially that of a folded form whereas enzyme-bound NAD^+ assumes the open form.[9,10]

Experimental Section[43]

Electronic Absorption Spectra. The measurements were obtained on a Cary Model 15 spectrophotometer as described previously, using dilute solutions of water or 95% ethanol. Average values of ϵ were used for the sum curves and the difference spectra. The electronic absorption spectra were digitized at intervals of 2.5 nm using a Benson-Lehner Corp. decimal converter, Model F. Oscillator strengths were calculated by an IBM 360 computer using a program based on Simpson's rule. Difference spectra were also calculated by computer.

5′-Amino-5′-deoxy-2′,3′-*O*-isopropylideneadenosine (8) was prepared following earlier directions.[21,22]

5′-(3-Carbamoylpyridin-1-ylium)-5′-deoxy-2′,3′-*O*-isopropylideneadenosine Chloride (1). To a solution of 153 mg (0.5 mmol) of 5′-amino-5′-deoxy-2′,3′-*O*-isopropylideneadenosine (**8**) in 40 ml of methanol was added 163 mg (0.5 mmol) of solid 3-carbamoyl-1-(2,4-dinitrophenyl)pyridinium chloride (**7**).[23] The solution immediately turned dark red and over a period of 30 min gradually turned orange. The solution was reduced to one-half volume *in vacuo*, 25 ml of water was added, and the remaining methanol was removed *in vacuo*, leaving a suspension of 2,4-dinitroaniline in water. Filtration followed by evaporation of solvent *in vacuo* left an orange solid. This solid was dissolved in isopropyl alcohol, decolorized, and allowed to cool, depositing colorless needles, mp 226–227°. Concentration of the solution furnished additional product: total yield 78 mg (35%); nmr (D_2O)[43,44] δ 1.94 and 2.16 (2, s, 6, $C(CH_3)_2$), 5.24–6.11 (m, 5, $C_{2',3',4'}$-H, $C_{5'}$-H_2), 6.75 (d, 1, J = 2 Hz, $C_{1'}$-H), 8.46 and 8.68 (2 s, 2, Ad $C_{2,8}$-H), 8.58 (m, 1, Nic C_5-H), 9.18–9.43 (m, 3, Nic $C_{2,4,6}$-H).

Anal. Calcd for $C_{19}H_{22}ClN_7O_4$: C, 50.95; H, 4.95; N, 14.29. Found: C, 50.70; H, 4.98; N, 14.15.

5′-(3-Carbamoylpyridin-1-ylium)-5′-deoxyadenosine Chloride (2). A solution of 128 mg (0.31 mmol) of 5′-(3-carbamoylpyridin-1-ylium)-5′-deoxy-2′,3′-*O*-isopropylideneadenosine chloride (**1**) in 25 ml of 50% formic acid was allowed to stand at room temperature for 24 hr. Evaporation of solvent *in vacuo* followed by addition of benzene and reevaporation gave an oil which was dissolved in methanol, decolorized, and added dropwise with stirring to diethyl ether. Filtration under nitrogen and drying gave 96 mg (83%) of a colorless, hygroscopic solid. Analytically pure material was obtained by again dissolving in methanol and adding this solution dropwise with stirring to diethyl ether, followed by filtration under nitrogen. In order to obtain anhydrous material it was necessary to dry the solid for at least 18 hr at 65° and 0.01 mm: dec pt 160.5° (start); nmr (DMSO-d_6) δ 3.34–3.80 (m, 1, $C_{4'}$-H), 4.33–4.84 (2 m, 2, $C_{2',3'}$-H), 5.14–5.34 (m, 2, $C_{5'}$-H_2), 5.66–5.90 (m, 2, $C_{2',3'}$-OH), 6.00 (d, 1, J = 4.5 Hz, $C_{1'}$-H), 7.38 (br s, 2, Ad NH_2), 8.06–8.24 (m, 1, Nic C_5-H), 8.06 and 8.44 (2s, 2, Ad $C_{2,8}$-H), 8.76 (br s, 2, Nic NH_2), 8.95–9.37 (m, 2, Nic $C_{4,6}$-H), 9.64 (s, 1, Nic C_2-H). Exchange with D_2O resulted in the disappearance of all signals assigned to OH and NH_2.

Anal. Calcd for $C_{16}H_{18}ClN_7O_4$: C, 47.12; H, 4.45; N, 24.04. Found: C, 47.31; H, 4.71; N, 23.76.

Methyl 5′-(3-Carbamoylpyridin-1-ylium)-5′-deoxy-2′,3′-isopropylidene-β-D-ribofuranoside Chloride (3). To a solution of 0.59 g (2.90 mmol) of methyl 5-amino-5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranoside (**9**)[24] in 100 ml of methanol was added 0.71 g (2.2 mmol) of solid 3-carbamoyl-1-(2,4-dinitrophenyl)pyridinium chloride (**7**).[23] The solution immediately turned deep red. After 1 hr the color had gradually changed to orange. The methanol solution was reduced to one-third volume *in vacuo* and 100 ml of water was added. The remaining methanol was removed *in vacuo* leaving a suspension of 2,4-dinitroaniline in water. The suspension was extracted with three 25-ml portions of diethyl ether to remove 2,4-dinitroaniline and excess starting amine. The water was removed *in vacuo* to leave 0.92 g (crude yield 92%) of an orange oil. Trituration with acetone gave a pink solid which was filtered and recrystallized twice from isopropyl alcohol as colorless, hygroscopic needles: mp 105–107.5° dec; nmr (D_2O)[44] δ 1.90 and 2.00 (2 s, 6, $C(CH_3)_2$), 3.95 (s, 3, OCH_3), 5.13–5.65 (m, 5, $C_{2,3,4}$-H, C_5-H_2), 5.65 (s, 1, C_1-H), 8.62–8.90 (dd, 1, Nic C_5-H), 9.37–9.58 (m, 2, Nic $C_{4,6}$-H), 9.73 (s, 1, Nic C_2-H).

Anal. Calcd for $C_{15}H_{21}ClN_2O_5$: C, 52.25; H, 6.14. Found: C, 51.88; H, 6.28.

Methyl 5′-(3-Carbamoylpyridin-1-ylium)-5′-deoxy-D-ribofuranoside Chloride (4). To a solution of 200 mg (0.58 mmol) of methyl 5′-(3-carbamoylpyridin-1-ylium)-5′-deoxy-2′,3′-*O*-isopropylidene-β-D-ribofuranoside chloride (**3**) in 25 ml of methanol was added 2 ml of methanolic hydrogen chloride (saturated at 0°). The solution was allowed to stand at room temperature for 8 hr. Evaporation of solvent *in vacuo* followed by a further addition of methanol and reevaporation gave a white foam, which was dissolved in methanol and added dropwise with stirring to diethyl ether. Filtration under nitrogen gave a colorless, deliquescent solid (142 mg, 76% based on the monohydrate), which was dried for 12 hr at 65° and 0.01 mm. Slow loss of volume began at 76°.

Anal. Calcd for $C_{12}H_{17}ClN_2O_5 \cdot H_2O$: C, 44.66; H, 5.93; N, 8.68. Found: C, 44.91; H, 5.65; N, 8.45.

Acknowledgment. The authors are pleased to acknowledge the support of this work by a research grant (USPHS-GM-05829) from the National Institutes of Health, U. S. Public Health Service. One of us (J. A. S.) has been a U. S. Public Health Service predoctoral fellow since 1969.

(42) K. Dalziel, *Acta Chem. Scand.*, 11, 397 (957).

(43) All melting points are uncorrected. Nuclear magnetic resonance spectra were determined on Varian Associates A-60A or HR-100 spectrometers using tetramethylsilane as internal standard, except as noted. Electronic absorption spectra were recorded on a Cary Model 15 spectrophotometer. Microanalyses were performed by Mr. Josef Nemeth and his staff, who also weighed samples for quantitative electronic absorption studies. We are indebted to the Lilly Research Laboratories, Eli Lilly and Co., for making available the circular dichroism spectral data.

(44) TMS capillary as reference for the nmr of this compound.