procedure has hydrodynamic properties and approximate molecular weights similar to RNA prepared by the phenol methods (Petermann, 1964). This new method, however, is much simpler than the phenol methods, and the yields of RNA are comparable.

Some exploratory experiments concerned with the mechanism of the sulfonated polystyrene and ribosome reaction have been conducted. *p*-Toluenesulfonate (sodium salt) at a concentration of 0.5–1% had no apparent effect on the sedimentation pattern of purified 50S subunits. Higher levels of the sulfonated toluene (*i.e.*, 2–4%) led to changes that were probably the result of the increase in the Na^+ concentration (Elson, 1961), rather than the toluenesulfonate anion. Although this compound is not the true monomer of the sulfonated polystyrene, the results suggest the reaction is due to the unique structure of the polymer. Clearly, the reaction is not solely due to the sulfonated aromatic residues.

The other major reaction product of sulfonated polystyrene and ribosomes (*i.e.*, in addition to RNA) is the ribosomal protein. Chemical methods of purifying the protein from the contaminating sulfonated polystyrene reagent are being explored. Hopefully, the protein can be isolated free of the reagent in a state suitable for physical and chemical studies.

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The Conformation of Ribonucleosides in Solution. The Effect of Structure on the Orientation of the Base*

Werner A. Klee and S. Harvey Mudd

ABSTRACT: The conformations of a number of adenosine derivatives in dilute aqueous solution have been investigated with the aid of measurements of the optical rotatory dispersion, infrared and ultraviolet spectra, and dissociation constants. The compounds studied include adenosine, N^1-methyladenosine, 2′-adenylic acid, 3′,5′-cyclic adenylic acid, 2′-*O*-methyladenosine, 2′,3′-isopropylideneadenosine, 5′-methylthioadenosine, *S*-adenosylmethionine, and *S*-adenosylhomocysteine, as well as other adenosine derivatives containing sulfur substituents on the 5′-carbon. The introduction of a sulfur atom, of either the thioether or sulfonium type, results in change in sign of the Cotton effect centered around 260 mμ. It is proposed that this change is due to an alteration in the conformation of the nucleoside. The relevance of these observations to the more general question of the conformation of nucleosides in solution is discussed.

The present studies were initiated as the result of calorimetric investigations of the enthalpy changes during methyl transfer from a number of sulfonium compounds to the common acceptor, homocysteine (Mudd *et al.*, 1966). It was found that transmethylation from (−)-*S*-adenosyl-L-methionine, the most important biological methyl donor, is accompanied by an unusually high enthalpy change, the basis of which was not clear. To gain some further understanding of this question, *S*-adenosylmethionine and the product formed from it during methyl transfer, *S*-adenosylhomocysteine, have now been studied by a variety of techniques which give some indication of the conformations assumed by these compounds in dilute aqueous solution.

The results of these studies enable us to exclude a number of sterically plausible conformations of *S*-

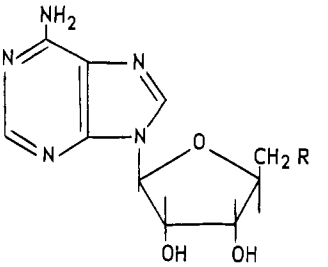
* From the Laboratory of General and Comparative Biochemistry, National Institute of Mental Health, U. S. Department of Health, Education, and Welfare, U. S. Public Health Service, National Institutes of Health, Bethesda, Maryland 20014. *Received July 11, 1966.*

adenosylmethionine and *S*-adenosylhomocysteine. The optical rotatory dispersion of these compounds indicates that they differ slightly from one another and that they differ markedly from most other adenine nucleosides.

Examination of the optical rotatory dispersion of a number of adenosine derivatives has led to the finding that, whereas the sign of the Cotton effect of purine nucleosides centered near 260 mμ is generally negative, all adenosine derivatives substituted with a sulfur atom at the 5′ position have positive Cotton effects in this region. These observations, among others, lead us to suggest a correlation of the rotatory properties of ribonucleosides with the conformation of these compounds in solution. It is proposed that 5′-sulfur-substituted adenine nucleosides may differ from other purine nucleosides in solution in the orientation of the base with respect to the ribose moiety.

Materials

The structures of many of the compounds used are shown in Figure 1. (−)-*S*-Adenosyl-L-methionine was prepared as previously described with inclusion of the preparative paper chromatographic step (Mudd *et al.*, 1966). (±)-*S*-Adenosyl-DL-methionine was prepared by mixing equimolar amounts of (±)-*S*-adenosyl-L-methionine and (±)-*S*-adenosyl-D-methionine (de la Haba *et al.*, 1959). *S*-Adenosyl-DL-homocysteine was prepared from the 2′,3′-isopropylidene derivative, a kind gift from Dr. G. A. Jamieson. The isopropylidene residue was removed by treatment with dilute sulfuric acid (22 hr, 23°) (Baddiley and Jamieson, 1955). The resulting nucleoside was adsorbed on a column of Dowex 50 (H^+) which was washed with water and then eluted with NH_4OH (0.6 N). The ammoniacal eluate was lyophilized to dryness and the nucleoside in the residue was recrystallized from water (de la Haba and Cantoni, 1959). 5′-Methylthioadenosine was prepared by thermal decomposition of *S*-adenosylmethionine (pH 5–6, 100°, 20 min). The final product, recrystallized from water, had mp 205–206°. Adenosyldimethylsulfonium salt was prepared by treatment of 5′-methylthioadenosine with methyl iodide in 38% formic acid (5 days, 23°). The solution was lyophilized to dryness, the residue was dissolved in water and extracted repeatedly with ether, and the aqueous phase was lyophilized again to dryness. The residue was dissolved in potassium phosphate buffer (pH 7, 0.01 M), and the solution was passed over a column of IRC-50 XE-64 to separate unreacted thioether from the sulfonium product essentially as outlined previously (de la Haba *et al.*, 1959). The fraction eluted from the column with 4 N acetic acid was concentrated by lyophilization. The final product yielded a single ultraviolet-absorbing, ninhydrin-negative spot upon paper chromatography and was free of contaminating 5′-methylthioadenosine. (±)-*S*-Adenosyl-(3-aminopropyl)methylsulfonium salt (decarboxylated (±)-*S*-adenosylmethionine) was a gift from Dr. G. A. Jamieson. 2′-*O*-Methyladenosine was generously donated by Dr. R. K. Robins. Adenosine, adenosine 2′-phosphate, adenosine 3′,5′-cyclic phosphate, 2′,3′-isopropylidineadenosine, dimethylpropiothetin, *S*-methyl-L-methionine, and L-methionine were all obtained commercially (Calbiochem and Sigma) and used without further purification. N^1-Methyladenosine was supplied by the Cyclo Chemical Co. as the tosylate salt and was converted to the chloride by passage through a Dowex 1 (Cl^-) column. The spectrum of the resulting product corresponded well to that of N^1-methyladenosine (Jones and Robins, 1963). Dimethylacetothetin was a synthetic product (Crum-Brown and Letts, 1874).



Compound	R
S-adenosylmethionine	$-\overset{\oplus}{S}(CH_3)-CH_2CH_2CH(NH_3^{\oplus})COO^{\ominus}$
S-adenosylhomocysteine	$-S-CH_2-CH_2-CH(NH_3^{\oplus})COO^{\ominus}$
5′-methylthioadenosine	$-S-CH_3$
adenosyldimethylsulfonium	$-\overset{\oplus}{S}(CH_3)-CH_3$
S-adenosyl-(3-aminopropyl)-methylsulfonium	$-\overset{\oplus}{S}(CH_3)-CH_2-CH_2-CH_2-NH_3^{\oplus}$
adenosine	$-OH$

FIGURE 1: General structure for many of the compounds discussed under Materials.

Methods

Spectrophotometric Titrations in the Infrared Region. Ehrlich and Sutherland (1954) have shown that both the COO^- and COOD groups of carboxylic acids give rise to easily measurable absorption bands in the infrared region which may conveniently be observed in D_2O solutions. Since the low pK' value of the carboxyl group of many of the compounds examined in this study makes potentiometric titrations of limited quantities difficult, we have measured the pK' of these compounds by infrared spectroscopy in D_2O solution as a function of pD. The samples were twice equilibrated with 99.7% D_2O and lyophilized and then dissolved in 99.7% D_2O at a concentration of about 5%. Spectra were recorded at room temperature in the 5.5–8-μ range with a Perkin-Elmer Model 21 infrared spectrom-

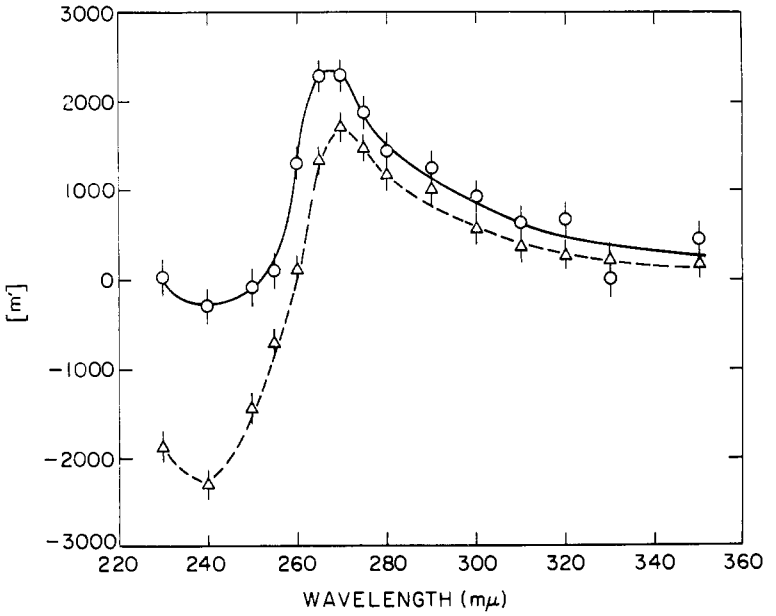


FIGURE 2: The optical rotatory dispersion of (±)-*S*-adenosyl-DL-methionine and *S*-adenosyl-DL-homocysteine in 0.1 M potassium acetate buffer at pH 5.6. The vertical bars through the points indicate the estimated experimental uncertainty. ○, (±)-*S*-adenosyl-DL-methionine. △, *S*-adenosyl-DL-homocysteine.

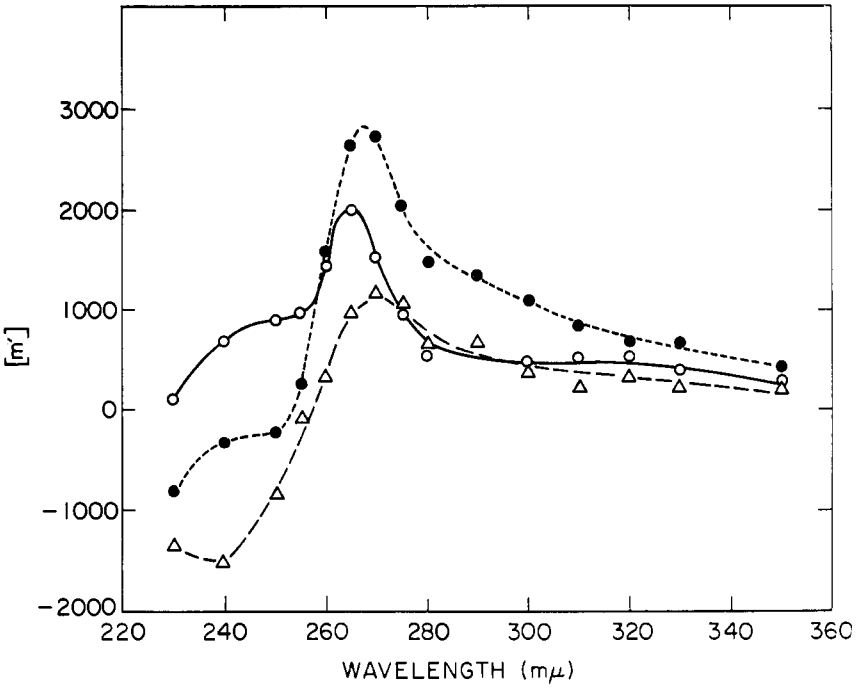


FIGURE 3: The optical rotatory dispersion of three adenine nucleosides substituted with sulfur at C'-5. The conditions of the measurements were as in Figure 2. ●, (±)-*S*-adenosyl-(3-aminopropyl)methylsulfonium; ○, adenosyldimethylsulfonium; △, methylthioadenosine.

eter in calcium fluoride cells of 0.05-mm path length with D_2O in a matched cell in the reference beam. The wavelength calibration of the instrument was routinely checked by recording the CO_2 internal standard on each chart. The pD of the sample was adjusted with DCl or NaOD and was measured with a Radiometer TTT1c titrator standardized with buffers in H_2O. pD values were estimated by adding 0.40 to

the pH meter reading (Mikkelsen and Nielsen, 1960; Lumry *et al.*, 1951). The pK' values for carboxylate groups as estimated in D_2O were corrected to the corresponding value in water by subtracting 0.5 (LaMer and Chittum, 1936; Lumry *et al.*, 1961; Li *et al.*, 1961). The reliability of this method of determining dissociation constants was tested with methionine whose pK' value is well known. The pK' of 2.2 ± 0.1 obtained by spectrophotometric titration is in good agreement with the literature value of 2.28 (Emerson *et al.*, 1931).

Optical Rotatory Dispersion (ORD). ORD measurements were made at 27° using a Cary Model 60 spectropolarimeter. Solutions were generally made up to a maximum absorbancy of 1.5 and run in a 1-cm pathlength cell. In a few cases, solutions were used with a maximum absorbancy of 2.2. The solvent used in these experiments was generally 0.1 M potassium acetate buffer, pH 5.6. This pH was chosen to minimize variations between compounds due to different states of ionization. The two ORD spectra which were taken down to a limit of 200 mμ were performed with solutions of ten times the usual concentration in cells of 0.1-cm path length. In this instance, the solvent was 0.04 M potassium phosphate, pH 6.0.

The small rotations of many of the samples examined led us to take a number of special precautions in making measurements. The reproducibility of cell positioning was checked before most runs by repeatedly replacing the cell, with the instrument set at 270 mμ, and taking 20 readings at 5-sec intervals at each positioning. It was found that the cell could readily be placed so that six such trials gave average values within ±0.0002° if suitable care were exercised in manipulating the sample compartment and if the neck of the cell were placed upright rather than in the slot machined in the side of the cell carriage. In our later runs, these positioning trials were dispensed with as being unnecessary. Since our data were obtained with the most sensitive setting of the instrument (full scale = 0.02°) noise was a serious problem in regions of absorption. We therefore adopted a procedure which allowed suitable averaging of points over time to minimize the influence of machine noise. The instrument was set to a particular wavelength and the pen reading at 5-sec intervals (with a pen period of 10 sec for 98% of full response) was recorded manually. At least 20 such readings were made at each wavelength and 40 or more consecutive measurements were made in regions of considerable noise. Averaging these readings reduces the noise interference to negligibly low levels in all but the most extreme cases (*i.e.*, 210 mμ and below, when in addition a pen period of 30 sec was found necessary and 100 readings were taken). Solvent readings were taken before and after each run at one or more wavelengths. In general, the instrument showed negligible drift during a run by this test.

We have expressed our data as reduced molecular rotations, defined as $[m']\lambda = (3/(n^2 + 2))(MW/100)\cdot[\alpha]\lambda$, and have used the refractive index dispersion of pure water (Fasman, 1963) in making our calculations.

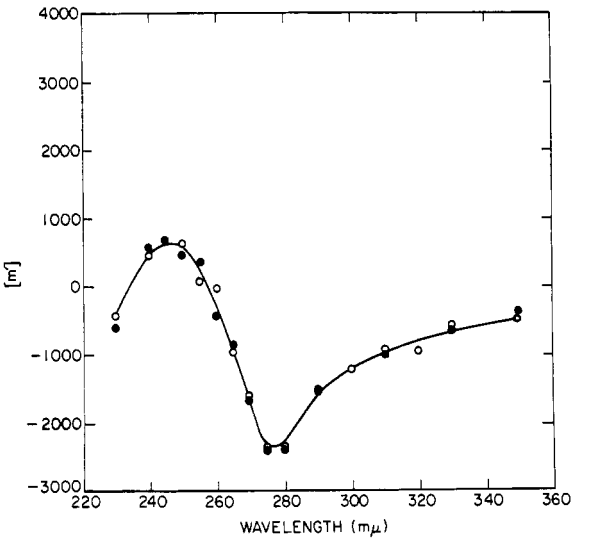


FIGURE 4: The optical rotatory dispersion of adenosine and 2′-adenylic acid. The conditions of the measurements were as in Figure 2. O, adenosine; ●, adenosine 2′-phosphate.

Concentrations were determined spectrophotometrically from absorption at the peak near 259 mμ for adenosine derivatives. The extinction coefficient used was 15,400 for all compounds except N^1-methyladenosine, for which the value 14,600 (at 257 mμ) (Jones and Robins, 1963) was employed. The molar extinction coefficients of many of the compounds used in this study are not precisely known. They are, in general, however, close enough to 15,400 so that any deviations will not contribute greatly to our experimental uncertainty.

S-Adenosylmethionine has two potentially asymmetric centers in addition to those of the ribose. *S*-Adenosylhomocysteine has a single extra asymmetric center. In order to simplify the comparison of the ORD curves of these two compounds with one another and with other adenosine derivatives, the samples used were racemic about all asymmetric centers other than those of the ribose. Thus, (±)-*S*-adenosyl-DL-methionine and *S*-adenosyl-DL-homocysteine were studied rather than the naturally occurring compounds.

Spectroscopy. Ultraviolet spectra were taken with a Cary Model 14 spectrophotometer, which instrument was also used in the spectrophotometric titration of the 6-amino (or N^1) (Tsuboi *et al.*, 1962) group of the adenosine moiety of some of the compounds examined. These studies were performed by titration of samples in the pH meter to suitable pH values with 6 N HCl. The change in absorbancy at 285 mμ was followed as a function of pH and the points were fitted to theoretical titration curves. The data are considered to be reliable to about ±0.1 pK unit. Potentiometric titrations were performed using the Radiometer TTT1c titrator along with a Radiometer SBR2 titrigraph.

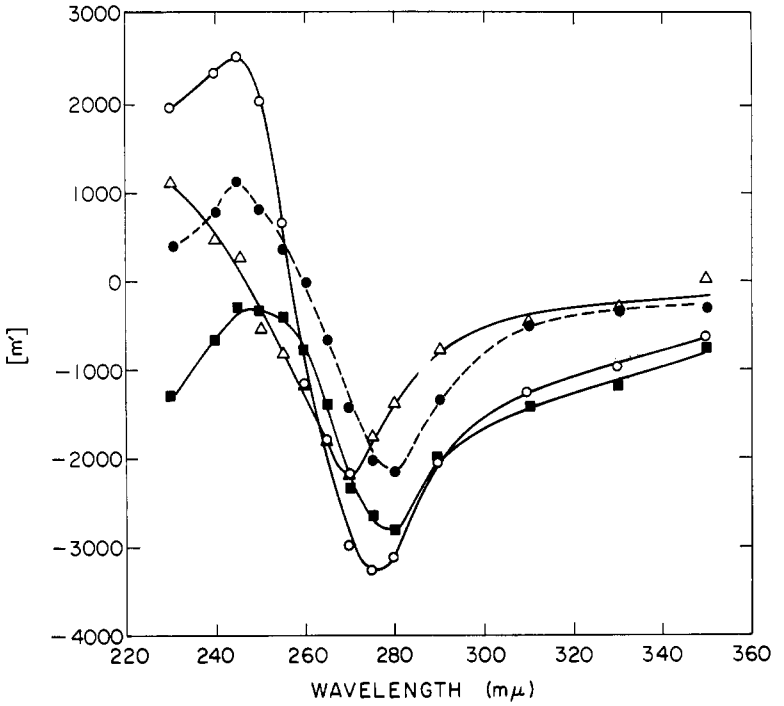


FIGURE 5: The optical rotatory dispersion of four nonsulfur-containing adenosine derivatives. The conditions of the measurements were as in Figure 2. O, adenosine 3′,5′-cyclic phosphate; Δ, N^1-methyladenosine; ●,2′-*O*-methyladenosine; ■ 2′,3′-isopropylideneadenosine.

Results

Preliminary model building studies of *S*-adenosylmethionine and *S*-adenosylhomocysteine led to the conclusion that there are a large number of conformations which can be assumed by both compounds. A number of conformations of *S*-adenosylmethionine result in the charged sulfonium center or the adenine ring being in close proximity to either the positive or the negative charge of the aminobutyric acid side chain. We have sought evidence for the occurrence of such proximity by measurements of (a) ionization constants, (b) infrared spectra, and (c) ultraviolet spectra. These measurements have produced evidence against the predominant existence of conformations which involve such interactions.

(a) pK' values of the ionizable groups in *S*-adenosylmethionine, *S*-adenosylhomocysteine, and a number of suitable model compounds are recorded in Table I. The pK' values of *S*-adenosylmethionine and *S*-adenosylhomocysteine are the same within our experimental error. It is thus clear that there are no strong coulombic interactions which exist in the one, but not the other molecule. The magnitude of the pK' shifts which can occur on close approximation of two charged groups may be seen by comparison of the pK' values of dimethylacetothetin and dimethylpropiothetin where the interposition of a second methylene group between a positively charged sulfonium group and a carboxylate group increases the carboxyl pK' by almost 2 pH units. Adenosine and its derivatives contain a group which ionizes near pH 3.5. For the purposes of this discussion, we will refer to this ionization as that of the 6-amino group (Edsall and Wyman, 1958) even though there is some evidence which suggests that it is the N^1 atom of the purine which may actually be protonated at this pH (Tsuboi *et al.*, 1962). The constancy of this pK' value in the various adenosine derivatives examined indicates that there are no strong interactions between this center and any substituent groups of the 5′-carbon, sulfonium or otherwise.

(b) Table II summarizes the results of our infrared studies. The adenine frequencies of *S*-adenosylmethionine and *S*-adenosylhomocysteine in acid solution are identical with one another and with those found for adenosine (Tsuboi *et al.*, 1962) under similar conditions. The absorption bands due to the COO^- and COOD groups of *S*-adenosylmethionine and of *S*-adenosylhomocysteine are also very close to one another. The small shift in the COO^- band may reflect a slight perturbation by a relatively far removed sulfonium center (compare methionine and *S*-methylmethionine sulfonium salt). Closely spaced groups lead to a larger change in frequency (dimethylacetothetin *vs.* dimethylpropiothetin).

(c) As seen in Table III, the ultraviolet absorption maxima of *S*-adenosylmethionine and *S*-adenosylhomocysteine are close to one another and to those of most other adenosine derivatives, indicating that there are no strong ring interactions in any of these com-

TABLE 1: pK' Values for *S*-Adenosylmethionine and Some Related Compounds.

	pK'		
	α-COOH	α-NH_2	Adenine
(−)-*S*-Adenosyl-L-methionine	1.8	7.8	3.4[a]
S-Adenosyl-DL-homocysteine	1.95	*b*	3.5
(±)-*S*-Adenosyl-(3-aminopropyl)methylsulfonium salt	—	*b*	3.5
Adenosyldimethylsulfonium salt	—	—	3.6
5′-Methylthioadenosine	—	—	3.6
Adenosine	—	—	3.5
Dimethylacetothetin	1.5	—	—
Dimethylpropiothetin	3.35	—	—
L-Methionine	2.2[c]	9.2	—
S-Methyl-L-methionine	1.9	7.9[d]	—

[a] (±)-*S*-Adenosyl-DL-methionine was employed in this measurement. All of these values are ±0.1. [b] Not measured. [c] DL-Methionine was employed in this measurement. [d] Durell *et al.* (1962).

pounds. It was noted that all of the 5′-sulfonium derivatives of adenosine have absorption maxima which are shifted to lower wavelengths by somewhat less than 0.5 mμ. These shifts are presumably the results of general charge effects. The absorption maximum of N^1-methyladenosine is shifted appreciably, reflecting significant changes in the electronic structure of the adenine ring of this compound.

Another technique used to search for conformational differences was optical rotatory dispersion. The ORD curves of (±)-*S*-adenosyl-DL-methionine and of *S*-adenosyl-DL-homocysteine are shown in Figure 2. The points are drawn so as to indicate the probable error of our measurements. The two curves are qualitatively similar and differ primarily in the amplitude ([m'] peak − [m'] trough) of the Cotton effect, that of *S*-adenosylhomocysteine being almost twice the magnitude of that of *S*-adenosylmethionine. An unexpected result of these measurements is the positive sign of the Cotton effect, since purine riboside derivatives generally exhibit negative Cotton effects (Yang and Samejima, 1963; Ulbricht *et al.*, 1965; Lamborg *et al.*, 1965; Sarkar and Yang, 1965; Holcomb and Tinoco, 1965; Yang *et al.*, 1966).[1]

A positive Cotton effect was found for all the other adenine nucleosides examined which carry a sulfur substituent on the 5′-carbon atom (Figure 3). Note that the sign of the Cotton effect is independent of whether the sulfur is a positively charged sulfonium atom or is simply part of a thioether.

The expected negative Cotton effects were found in the ORD curve of adenosine as well as in the curves for a number of adenosine derivatives which do not contain a sulfur substituent on the 5′-carbon (Figures 4 and 5). The data for adenosine are in good quantitative agreement with those of adenosine 2′-phosphate and with literature values for similar compounds (see, for example, Holcomb and Tinoco, 1965). The derivatives whose ORD curves are recorded in Figures 4 and 5 were chosen to examine the influence of changes in the electronic structure of the adenine ring (N^1-methyladenosine) or changes in the ribose part of the molecule.

The ORD data discussed so far have been concerned only with the Cotton effect associated with the major electronic transition near 260 mμ. Adenosine derivatives are known, however, to undergo other transitions near 207 and 185 mμ. The first of these transitions is, at least in part, within the usable range of our instrument. Measurements made with adenosine and 5′-methylthioadenosine down to 200 mμ are shown in Figure 6. Although the Cotton effects at low wavelength appear to be complex, it is evident that the 207-mμ transition gives rise to Cotton effects of opposite sign in adenosine and in 5′-methylthioadenosine.

Discussion

The Orientation of the Base in Nucleosides. With only one exception, all structures of purine and pyrimidine nucleosides and nucleotides which have been formulated on the basis of crystallographic studies show a similar orientation of the base with respect to the furanose ring of the ribose (Kraut, 1965). In these structures, carbon 2 of the base is directed away from carbon 5′ of the ribose (Figure 7a) in a conformation called *anti* (Donohue and Trueblood, 1960). The opposite conformation, the *syn* (Figure 7b), has so far been observed only in a mixed crystal of deoxyguanosine and 5-bromodeoxycytidine (Haschemeyer and Sobell, 1964), and only the purine exhibits this conformation. There is, therefore, little doubt that in most crystals, as well as in all of the stereochemically reasonable nucleic acid helices discovered to date, the orientation of the base is generally *anti* even though there may be considerable variation in the degree to which the bases approximate a completely *anti* conformation (Kraut, 1965).

In a preliminary report Emerson *et al.* (1966) have proposed that the usual negative Cotton effect found for purine β-ribosides indicates that these compounds exhibit a predominantly *anti* conformation in solution. Their assignment is based upon the rotatory properties of the model compounds 2′,3′-isopropylidene, N^3,-5′-cycloadenosine, and C^8,5′-cycloadenosine, which are locked into *syn* and *anti* conformations, respectively, by ring formation. There is some question of the usefulness of the former compound as a model

[1] Pyrimidine nucleosides, however, exhibit positive Cotton effects.

because of its great instability.[2] Furthermore, model building shows that the glycosidic bonds in both N^3,-5′-cycloadenosine and C^8,5′-cycloadenosine are under considerable torsional strain. It is difficult to predict the effect of such strain on the rotatory properties of these substances. Although we shall offer no compelling evidence against the assignment made by Emerson *et al.*, we suggest that the question of *syn vs. anti* conformation of purine ribosides in solution be left open since the following observations are more easily explicable if the preferred orientation of these compounds were closer to a *syn* than to an *anti* conformation.

(*a*) *The Differences between Purine and Pyrimidine Nucleosides.* A number of recent measurements have shown that the sign of the 260-mμ Cotton effect of purine ribosides, ribotides, and deoxyribotides is negative, while that of the corresponding pyrimidine derivatives is positive (Yang and Samejima, 1963; Ulbricht *et al.*, 1964; Fasman *et al.*, 1964; Lamborg *et al.*, 1965; Sarkar and Yang, 1965; Holcomb and Tinoco, 1965). This Cotton effect is a function of the asymmetry of the environment of the base or, more precisely, of the orientation of the transition dipole of the base with respect to the asymmetric centers of the neighboring ribose moiety. Since there is evidence that the direction of the transition dipoles of the ordinary purines and pyrimidines is not very different (Stewart and Davidson, 1964; Stewart and Jensen, 1964; Clark and Tinoco, 1965), it is reasonable to expect that the sign of the Cotton effect at 260 mμ may reflect primarily the orientation of the base with respect to the ribose.[3] Similar reasoning has led to the recent suggestion that the optical rotatory properties of a series of 1-substituted indans and of the corresponding α-phenylethyl compounds may be manifestations of the orientation of the benzene ring with respect to the asymmetric carbon (Brewster and Buta, 1966).

The adenosine nucleosides which we have studied fall into two classes, those with positive Cotton effects, and those with negative Cotton effects. It is notable that only one kind of structural modification of adenosine resulted in a change from a negative to a positive Cotton effect, namely, the introduction of a substituted sulfur atom directly onto the 5′-carbon. Most adenosine nucleosides, and indeed all other β-purine ribosides so far studied, have negative Cotton effects. The negative sign has been found to persist even after alkylation of the ring (N^1-methyladenosine) or of the ribose (2′-*O*-methyladenosine and 2′,3′-isopropylideneadenosine) or after phosphorylation of the sugar in any of a number of positions. The fact that all of these types of modification do not alter the sign of the Cotton effect makes it unlikely that this parameter is a reflection primarily of the covalent or electronic structure of the molecules.

Model building studies show that, in the case of purine nucleosides, neither the *syn* nor the *anti* con-

[2] We have examined a number of samples of N^3,5′-cycloadenosine and of its 2′,3′-isopropylidene derivative and have consistently found large *negative* Cotton effects. These samples were generously provided by Dr. G. A. Jamieson and had been synthesized a number of years ago. They have the properties expected for N^3,5′-cycloadenosine (λ_{max} in H_2O at pH 5, 271–272 mμ; $pK_a = 1.5$; nuclear magnetic resonance (in dimethyl sulfoxide with a tetramethylsilane internal standard) shows the expected 12 hydrogens with the C_8 and C_2 protons shifted downfield to τ 1.25 and 1.50 and the CH_2 multiplet shifted to τ 5.70). Dr. T. L. V. Ulbricht kindly provided us with a sample of his 2′,3′-isopropylidenecycloadenosine, which is perhaps of more recent origin and which we found to have a small positive Cotton effect, in qualitative agreement with that reported by Emerson *et al.* (1966). During paper chromatography in isopropyl alcohol–formic acid–water (70:10:20) all samples migrated as single spots with the same R_F, but Dr. Ulbricht's sample was converted to a material with the rotatory properties of our own samples (that is, with a large negative Cotton effect). Furthermore, all samples are extremely labile. For example, in sodium acetate, 0.1 M at pH of 7.6, all samples undergo a characteristic series of spectral changes. (Transient peak at 328 mμ disappearing within 3 min at room temperature to give a final peak at 270–272 mμ with an extinction coefficient less than that of the original compound). The ORD of the product after 30 min at pH 7.6 showed a strong positive Cotton effect: $[m']_{290} +15 \times 10^3$ (peak), $[m']_{250} -15 \times 10^3$ (trough); crossover at 270–271 mμ. We do not understand the explanation of these observations and for this reason feel that N^3,5′-cycloadenosine or its isopropylidene derivative are not suitable models for nucleoside conformation studies.

[3] The finding that α- and β-anomeric nucleosides have Cotton effects of opposite sign (Ulbricht *et al.*, 1964) is consistent with this expectation. All compounds discussed in this paper are of the β-D configuration.

An alternate explanation of the differences in sign of optical rotation of purine and pyrimidine nucleosides has been advanced by the Jardetzkys. These authors studied the nuclear magnetic resonance spectra of a series of purine and pyrimidine ribosides and observed that the purine nucleosides have higher C_1' coupling constants than do the pyrimidine nucleosides (Jardetzky and Jardetzky, 1960; Jardetzky, 1962). These data were interpreted as being due to systematic differences in the manner in which the ribose ring of these compounds is puckered (*i.e.*, displaced from a planar conformation) (Jardetzky, 1960). It was further suggested that such differences in puckering could explain the differences in the sign of the optical rotations (at 589 mμ) observed for purine and pyrimidine ribosides (Jardetzky, 1962).

To test the validity of ascribing the optical rotatory differences to differences in ribose puckering, we have now examined the optical rotatory dispersion of adenosine 3′,5′-cyclic phosphate. This compound was exceptional in the nuclear magnetic resonance studies in that its C_1' coupling constant was much lower than that found for other purine nucleosides and indeed was lower than any found for the pyrimidine nucleosides. The conformation which was proposed for the ribose of adenosine 3′,5′-cyclic phosphate as a result of these data (Jardetzky, 1962) resembles the one proposed for the ribose of pyrimidine nucleosides (Jardetzky, 1960). We reasoned that if these differences in the puckering of the ribose do indeed lead to differences in the sign of the optical rotation, then adenosine 3′,5′-cyclic phosphate should have a Cotton effect opposite in sign to that of adenosine 2′-phosphate (the compound with the highest reported C_1' coupling constant (Jardetzky, 1962). This is clearly not the case (Table III). Thus, differences in the puckering of the ribose, while they certainly exist (Kraut, 1965), do not account for the differences in sign between purine and pyrimidine Cotton effects. Furthermore, alkylation of the 2′- and 3′-oxygen atoms of adenosine has no influence on the sign of the Cotton effect (Figure 5), a result which agrees with the thesis that minor changes in ribose structure are not the basis of the differences in the ORD phenomena.

TABLE II: Infrared Absorption Bands of *S*-Adenosylmethionine and of Some Related Compounds in D_2O Solution.

	Wavelength (μ)					
	COO^-		COOD	Adenine		
(−)-*S*-Adenosyl-L-methionine	6.15	7.10	5.78	6.00	6.32	6.62
S-Adenosyl-DL-homocysteine	6.18	7.08	5.74	6.00	6.32	6.64
DL-Methionine	6.19	7.12	5.80	—	—	—
S-Methyl-L-methionine	6.17	7.12	5.79	—	—	—
Dimethylacetothetin	6.17	7.25	5.82	—	—	—
Dimethylpropiothetin	6.28	7.16	5.83	—	—	—

TABLE III: Ultraviolet Optical Rotatory Dispersion Parameters of Adenosine Derivatives at pH 5.6.

	Ultraviolet Absorption Max (mμ)	260-mμ Cotton Effect					
		Sign	Amplitude	Peak		Trough	
				λ (mμ)	[*m'*]	λ (mμ)	[*m'*]
Adenosine	259	−	3000	248	+600	278	−2350
(±)-*S*-Adenosyl-DL-methionine	259	+	2600	268	+2300	240	−300
S-Adenosyl-DL-homocysteine	259	+	4000	270	+1700	240	−2300
(±)-*S*-Adenosyl-(3-aminopropyl)-methylsulfonium salt	259	+	2900	268	+2700	245	−200
Adenosyldimethylsulfonium salt	259	+	1200	265	+2000	245	+800
5′-Methylthioadenosine	259	+	2600	270	+1100	240	−1500
Adenosine 3′,5′-cyclic phosphate	259	−	5700	245	+2500	275	−3200
Adenosine 2′-phosphate	259	−	3000	245	+600	278	−2400
N^1-Methyladenosine	257	−	3200	230	+1100	270	−2100
2′-*O*-Methyladenosine	259	−	3200	245	+1100	280	−2100
2′,3′-Isopropylideneadenosine	259	−	3500	245	+700	280	−2800

formations necessitate very close interatomic distances while pyrimidine nucleosides are clearly hindered in the *syn* conformation (see also Donohue and Trueblood, 1960). Thus, on this basis alone purine nucleosides would be more likely to exhibit the *syn* orientation than would their pyrimidine analogs. [4]Indeed, studies of the optical rotatory dispersion of pyrimidine nucleosides and of some relatively unstrained pyrimidine cyclonucleosides led Ulbricht *et al.* (1965, 1966) to conclude that pyrimidine nucleosides retain the *anti* conformation in solution. It would be most consistent with the experimental transition moments to argue that the conformation of purine nucleosides should be opposite to that of the pyrimidines in solution since the optical rotations are of opposite sign. These considerations would thus support the *syn* conformation for the ordinary purine nucleosides.[5]

(*b*) *The Effect of Sulfur Substitution at the 5′-Carbon of Adenosine.* Substitution of a sulfur atom on the 5′-carbon of adenosine results in an inversion of the sign of the Cotton effect. If adenosine exists in a *syn* conformation, a bulky sulfur atom on the 5′-carbon would lead to steric interference and drive the molecule more into the *anti* conformation, affording a

[4] However, even pyrimidine nucleosides are capable of assuming a *syn* conformation as evidenced by model building and by the fact that a number of O^2,5′-cyclopyrimidine nucleosides can be prepared (Michelson, 1963).

[5] A number of adenine nucleosides, whose conformations are of the *syn* variety due to steric constraints, have been synthesized. Although the ORD of these substances has not in general been studied, optical rotation at the sodium D line had generally been reported and is invariably negative. Since there seems to be an excellent correlation between the sign of the D line rotation with that of the 260-mμ Cotton effect for both purine and pyrimidine nucleosides it may be presumed that these materials will also exhibit negative Cotton effects. The compounds which fall into this class are: (1) 3,5′-cyclo-6-dimethylamino-9-(3′-deoxy-β-D-ribofuranosyl)purine 2′,3′-carbonate (Baker and Joseph, 1955); (2) 8,2′-cyclo-2-chloro-8-mercapto-9-β-D-arabinofuranosyladenine (Ikehara and Tada, 1965); (3) 8,2′-cyclo-8-hydroxy-9-β-D-arabinofuranosyladenine (Ikehara *et al.*, 1966). Although two of these compounds are arabinose derivatives, it has been shown that the sign of the Cotton band is the same in arabinosides as in the corresponding ribosides (Fric *et al.*, 1966).

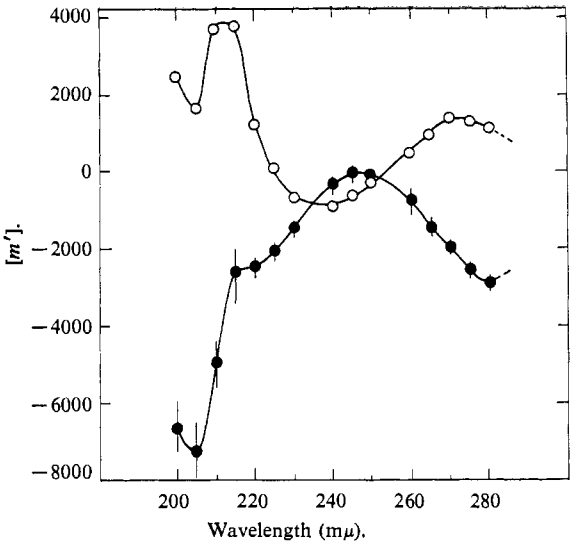


FIGURE 6: Optical rotatory dispersion of adenosine and 5′-methylthioadenosine extended into the far-ultraviolet region. The vertical bars indicate the experimental uncertainties of the measurements. The measurements were performed in potassium phosphate, 0.04 M, pH 6.0. O, methylthioadenosine; ●, adenosine.

ready explanation of the change in the sign of the Cotton effect. No such ready explanation is available if adenosine usually exists in the *anti* conformation. Other theories which might account for an inversion in the sign of the 260-mμ Cotton effect in a pair of compounds which are as similar as adenosine and 5′-methylthioadenosine, for example, appear to be less likely.[6] With this pair of compounds, the signs of not only the 260-mμ Cotton effect but also those of the Cotton effects related to the 207-mμ transition are opposite (Figure 6).

(*c*) *The ORD of Adenosine 3′,5′-Cyclic Phosphate.* The amplitude of the 260-mμ Cotton effect of adenosine 3′,5′-cyclic phosphate is unusually large (Table III). Model building shows that in this compound the 5′-carbon atom is constrained, along with its substituents, to occupy a position far removed from the adenine ring. Thus, one factor which ordinarily tends to decrease the likelihood of the *syn* conformation, namely, steric interference of some rotational isomers at the 5′-carbon, is removed and the molecule may spend more, indeed possibly most, of the time in the *syn* conformation. It is more difficult to see how this large Cotton effect can be explained if the conformation were actually *anti* since in that case no change in amplitude should be observed on going from adenosine to the 3′,5′-cyclic phosphate.

(*d*) *Difference in Rate of Acid Hydrolysis of 5′-Methylthioadenosine and Adenosine.* One factor which is thought to affect the rate of acid hydrolysis of ribosides is the ease with which protons may be transferred from the base to the ring oxygen (Kenner, 1957). Such proton transfer is possible only in *syn* conformations. We therefore compared the rate of acid hydrolysis of adenosine with that of 5′-methylthioadenosine over the temperature range between 24 and 40° and found that adenosine is hydrolyzed at a rate which is 1.2 to 1.4 times that of 5′-methylthioadenosine (see Figure 8). Although other factors may be affecting these rates, the differences found are compatible both qualitatively and quantitatively with the difference in conformation we propose. Allowance must be made for the fact that both compounds spend part of the time in the *syn* and part in the *anti* conformation.

The observations discussed above are not amenable to unambiguous interpretation and cannot therefore provide a firm answer to the question of nucleoside conformation in solution. However, the combined weight of the evidence is, to our view, such that the question of the *syn vs. anti* preference of β-purine ribosides in solution be left open to await the results of further experimentation.

The Conformations of S-Adenosylmethionine and S-Adenosylhomocysteine. The data shown in Table I and II serve to rule out a number of possible conformations of *S*-adenosylmethionine which might have been proposed. For example, there are ample possibilities for hydrogen-bond formation between the ribose oxygens and the carboxylate group of *S*-adenosylmethionine. Such hydrogen bonds would have the effect of bringing the negatively charged COO^- group close to the positively charged sulfonium center. However, all structures involving strong coulombic interactions are considered to be highly unlikely because of the relatively normal p*K′* values found for *S*-adenosylmethionine. In addition the infrared and ultraviolet spectral data do not indicate the existence of any strong side-chain–ring interactions.

One difference between *S*-adenosylmethionine and *S*-adenosylhomocysteine is in the amplitude of the 260-mμ Cotton effect, that of *S*-adenosylhomocysteine being somewhat larger than that of *S*-adenosylmethionine. A similar relationship exists between 5′-methylthioadenosine and adenosyldimethylsulfonium salt. In this case also, conversion of a thioether to the corresponding methylsulfonium salt decreases the amplitude of the Cotton effect by a comparable amount. The effect of the amino acid side chain is indicated by a comparison of *S*-adenosylmethionine with adenosyldimethylsulfonium salt and of *S*-adenosylhomocysteine with 5′-methylthioadenosine. In both cases, replacement of a methyl group by the four-carbon amino acid side chain leads to an increase in the amplitude of the Cotton effect of the attached adenosine chromophore. This increase is noted whether the compound is a thioether or a sulfonium nucleoside.

[6] An alternative explanation is that the changed sign of the Cotton effect reflects a transition due to the sulfur atom. Such an explanation is unlikely in view of the facts that the spectra of adenosine and 5′-methylthioadenosine are closely similar and that no Cotton effects are observed in the 260-mμ spectral region with methionine, ethionine, and *S*-ethyl- or *S*-methylcysteine (Schellman, 1960).

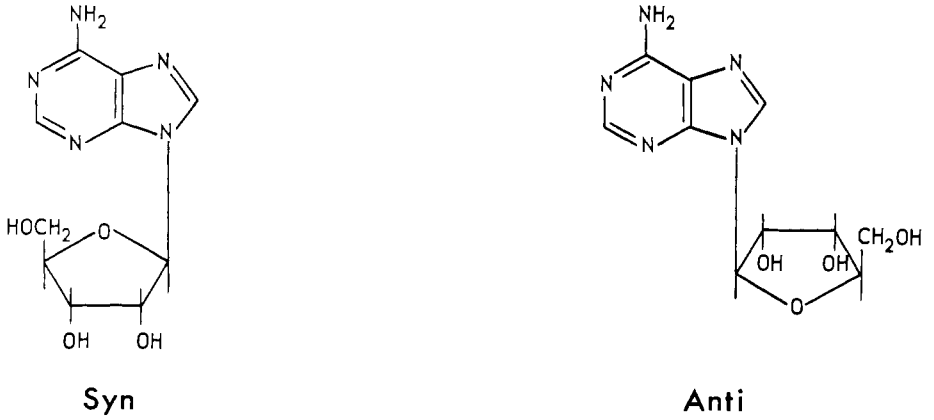


FIGURE 7: Diagramatic illustrations of the *syn* and *anti* conformations of adenosine.

Interpretation of these quantitative differences must be tentative. In these closely related compounds a decrease in the amplitude of the Cotton effect may be due to increased mobility of the purine base with respect to the ribose. The positively charged sulfonium atom with its tendency to be hydrated might be expected to increase mobility by reducing intramolecular interactions. The four-carbon amino acid side chain, on the other hand, might decrease mobility by providing more opportunity for intramolecular interactions.[7]

These arguments lead to the following picture of the conformation of *S*-adenosylmethionine: that of a reasonably, but not completely, extended molecule which is highly hydrated due to many charged and other polar groups. The adenine ring may be *anti* more often than *syn*, but not for as much of the time as is the adenine of *S*-adenosylhomocysteine, which molecule has more opportunities for intramolecular bonding due to the absence of a charged sulfur atom.

To return to the question posed in the introduction. Why is the enthalpy of transmethylation from *S*-adenosylmethionine higher than that of transmethylation from a series of other sulfur compounds? If the conformations of *S*-adenosylmethionine and *S*-adenosylhomocysteine are as we propose then it is reasonable that the extra intramolecular interactions which are seen in *S*-adenosylhomocysteine and not in *S*-adenosylmethionine will contribute toward a negative enthalpy change of transmethylation. Whether such conformation differences can account for the entire enthalpy difference found is not clear. It is possible that other factors such as a shielding by the adenosine moiety to hinder the full hydration of the sulfonium center of *S*-adenosylmethionine may contribute to the enthalpy change. Such interference with hydration of charged groups by nucleosides has been postulated in connection with the ionization constants of nucleoside di- and triphosphates (Phillips *et al.*, 1965). Hindered hydration of the sulfonium center of *S*-adenosylmethionine will result in an enhancement of the enthalpy change during transmethylation in a manner analogous to that described for the simple thetins in our previous communication (Mudd *et al.*, 1966).

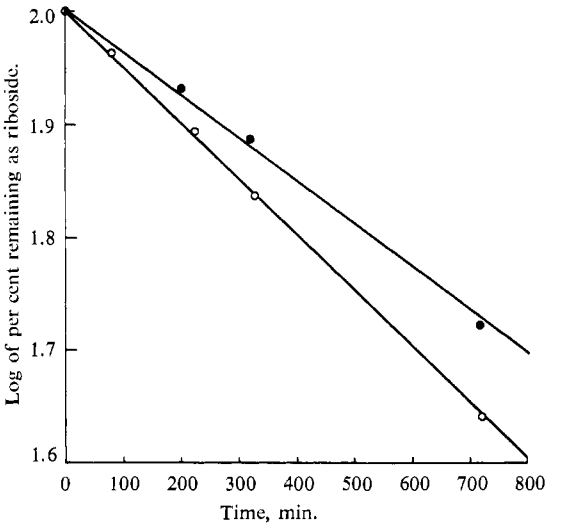


FIGURE 8: The rate of hydrolysis of adenosine (lower curve) and of 5′-methylthioadenosine (upper curve) in 1 N HCl at 37°. Timed aliquots of 0.05 ml of the incubation mixture were added to 3.0 ml of 0.1 N KOH and absorbance was read at 252 and 253 mμ. The change in absorbance with time of incubation in acid provided a measure of the rate of the hydrolysis. These absorbancy values were corrected for small concentration differences by also monitoring the absorbance at an isobestic point near 267 mμ.

[7] Aggregation of one or the other nucleoside under the conditions of our experiments seems highly unlikely in view of the very small association constants found for nucleosides in aqueous solution (T'so and Chan, 1964). The ORD curves of adenosine and of 5′-methylthioadenosine show no concentration dependence up to concentrations at least ten times that which we generally employed.

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