*CIRCULAR DICHROISM OF NUCLEOSIDE DERIVATIVES, VI. THE OPTICALLY ACTIVE BANDS OF ADENINE NUCLEOSIDE DERIVATIVES**

By Daniel W. Miles, Morris J. Robins, Roland K. Robins, and Henry Eyring

DEPARTMENT OF CHEMISTRY AND INSTITUTE FOR THE STUDY OF RATE PROCESSES, UNIVERSITY OF UTAH, SALT LAKE CITY

Communicated September 19, 1968

Abstract and Summary.—The circular dichroism data on 17 adenine nucleosides show that the 260- and 207-mμ absorption systems of the adenine chromophore each contain at least two electronic transitions. The CD maxima are commonly found at around 260, 220, and 200 mμ, and occasionally at 240 mμ. Solvent studies suggest that these CD bands arise from pi-pi* transitions. A weak absorption band that obeys the McConnell criteria of an *n*-pi* band is resolved at about 290 mμ in hydrocarbon solvents. This band exhibits very little rotatory power.

Extensive circular dichroism[1, 2] and other spectroscopic studies[3–14] on the naturally occurring purine and pyrimidine nucleosides and their derivatives have given important information that is necessary for the interpretation of the electronic spectra and optical rotatory dispersion (ORD) and circular dichroism (CD) curves of the more complex DNA and RNA fragments, such as the dimers and oligomers of known nucleoside composition. In such systems, the wave functions and excited states are derived from those of the component base chromophores, such as is done in the spectral theory of crystals. From this, it is clear that if a band has been missed in the nucleoside monomer spectrum, then the current interpretation of the spectra of the dimers and oligomers of nucleic acids may well be in error. Recent circular dichroism studies on uridine and cytidine derivatives[1, 2] have revealed electronic transitions not previously discovered experimentally nor predicted theoretically.[15, 16] The CD results, including the present study of the CD properties of 45 adenine nucleosides and preliminary results on some 30 guanine nucleoside derivatives, show that four CD bands are found between 190 and 300 mμ. These bands, which are found at about 260, 240, 220, and 200 mμ, appear to be related in the five major base constituents of nucleic acids and their derivatives. The present communication discusses the optically active transitions at 260, 240, 220, and 200 mμ found in the CD spectra of adenine nucleoside derivatives. In addition, a very weak CD band is found near 290 mμ in hydrocarbon solvents. Previous studies[3, 4, 7, 12–14] have suggested that the 260-mμ absorption system of adenine is composite in nature. In addition, several workers[3, 10, 11] have pointed out absorption bands near 207, 185, and 165 mμ in the adenine chromophore. However, the nature and relative positions of the component bands of the 260-mμ absorption system have not been clearly defined nor has any previous spectroscopic study indicated the presence of a 220-mμ electronic transition in the adenine chromophore.

Experimental.—The experimental procedures are given in reference 1. References describing the preparation and characterization of these nucleosides or their commercial source are given in Figure 1.

Results and Discussion.—The systematization of the absorption spectra of nucleic acid bases introduced by Clark and Tinoco[3] and extended by Berthod *et al.*[16] has led to the designation of the longest wavelength of absorption as the B_{2u} band, and the two strong maxima, one around 197–207 mμ and another toward 180 mμ, as related to the benzene E_{1u} bands. In between these two extremes, a fourth absorption band is sometimes found and has been designated the B_{1u} band. Attempts to classify and systematize the spectra of the purines and pyrimidines are chiefly aimed at establishing that general correlations involving the spectral properties of these compounds do exist. Correlations with the spectrum of benzene, while not critical for these considerations, provide a convenient and well-known nomenclature.

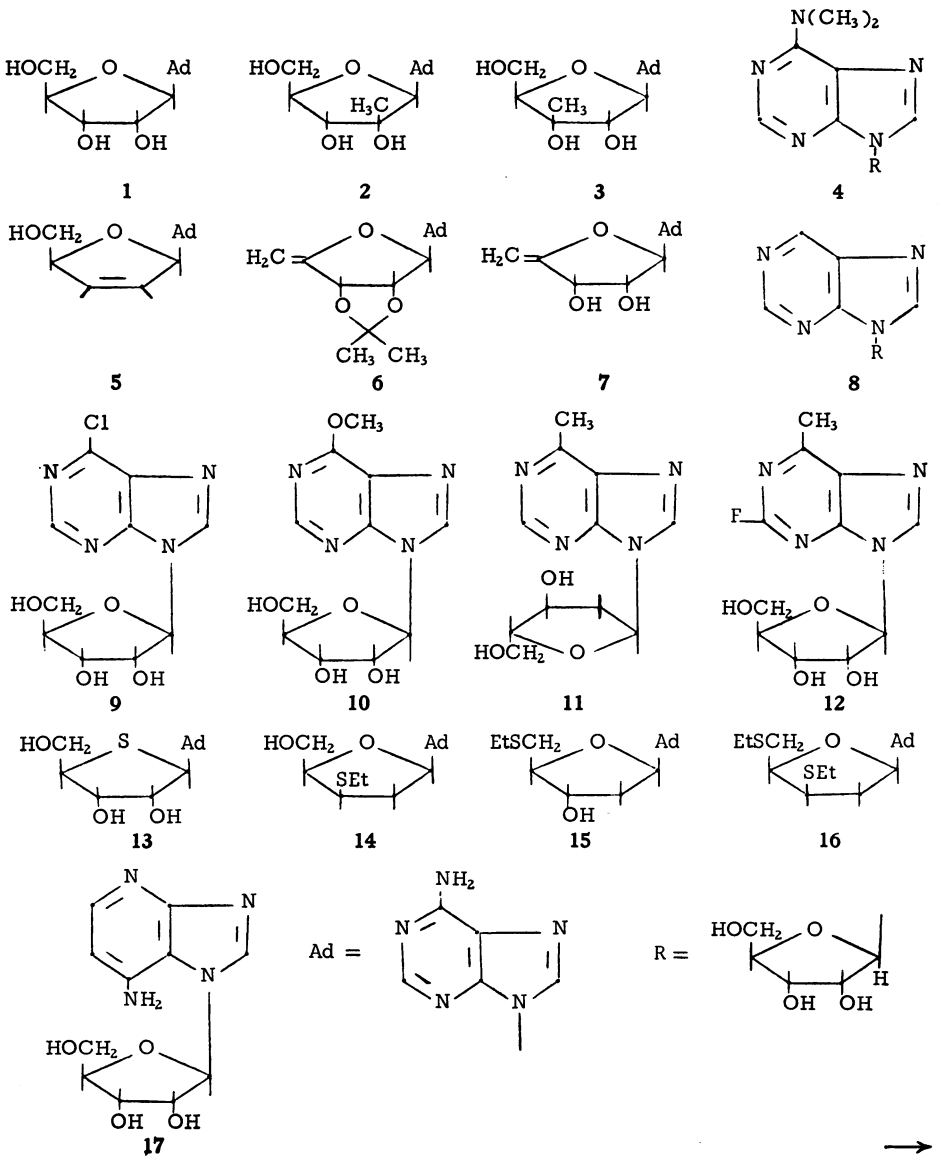
That important general correlations do exist in these compounds is supported by recent CD studies on uracil,[1] cytosine,[2] and now adenine nucleoside derivatives that show that CD bands are found at very nearly the same wavelengths in most nucleoside derivatives containing the uracil, cytosine, or adenine chromophore, or their closely related analogs featuring weakly perturbing ring substituents.

In Figures 2–6, we present the CD curves (and several characteristic absorption curves) of 17 purine nucleoside derivatives (see Fig. 1 for sturctures) that are quite representative of the 45 adenine nucleoside derivatives studied in our laboratories. An important discovery is the general occurrence of CD bands of opposite sign at about 220 and 200 mμ in the majority of adenine nucleoside derivatives. Clark and Tinoco[3] have correlated the 207- and 186-mμ absorption bands with the E_{1u} band of benzene. The present study demonstrates that there are at least two electronic transitions[17] contained in the 206-mμ band of adenine,[18] which has, in the past, been tacitly assumed to contain a single electronic band. For the sake of convenience, the benzene nomenclature will be retained and the two Cotton effects at 220 and 200 mμ will be referred to as the E_{1ua} and E_{1ub} Cotton effects, respectively.

Inspection of Figures 2 through 6 shows that in certain compounds (notably compounds **1–4, 8–12, 14,** and **16**) the E_{1u} Cotton effects not only carry opposite signs but are nearly equal in intensities. It is significant to note that little noticeable distortion in the positions of the adenine 220- and 200-mμ CD bands is found in measurements on compounds **5–7** and **13–16** (Figs. 3 and 6) despite the presence of additional chromophoric elements. The weak absorption developed by the olefinic bonds and the dialkyl sulfide group[19] *above* 200 mμ is unlikely to develop observable rotatory-strength "couplets" with the adenine transitions, at least by the coupled-oscillator mechanism.[20]

Mason[4] proposed in 1954 that the 260-mμ band of adenine is composite in nature and consists of two pi-pi* transitions in the 265–240-mμ region. His theory was based largely on the observation of two bands in the purine spectrum in methylcyclohexane, i.e., a strong band at 265 mμ which he labeled x_1 and a shoulder at approximately 240 mμ which he labeled x_2. The absorption curves of **11** and **12** given in Figure 4 illustrate this presence of two absorption bands in the 240–265-mμ region in 6-methylpurine nucleosides. In 6-aminopurine

(adenine), one strong absorption band is observed at 260 mμ, with indications of a shoulder at 267 mμ. This shoulder is observed in the adenine nucleoside derivatives **6** and **16** in methylcyclohexane at about 273 mμ but not in water. Several investigators have disagreed with Mason's proposal that the more intense component of the 260-mμ adenine band lies at the lower energy. Nagata *et al.*,[18] Tanaka and Nagakura,[21] and Devoe and Tinoco,[22] for example, have assigned the 267-mμ shoulder to the 6 → 7 molecular orbital promotion and credit the 6 → 8 promotion as being responsible for the main band at 260 mμ. Kwiatkowski[15] also disagrees with Mason and correlates the 260-mμ band of



adenine with the x_2 band of purine. By contrast, Hoffman and Ladik[23] have assigned the 267-mμ shoulder to an n-pi* transition, whereas Kleinwachter *et al.*[9] attribute it to a vibrational component of the 260-mμ main band. Callis, Rosa, and Simpson,[12] and Stewart and Davidson[13] have concluded from polarized fluorescence and polarized absorption studies on adenine that two pi-pi* transitions lie within the envelope of the 260-mμ absorption of adenine, with the strong component lying at longer wavelength. The ratio of their intensities appears in these studies to be about 1:10.

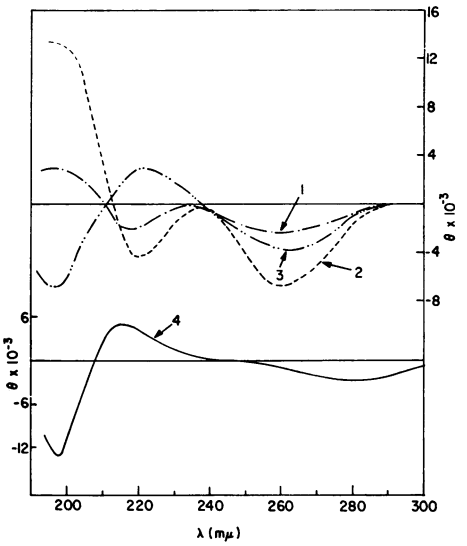
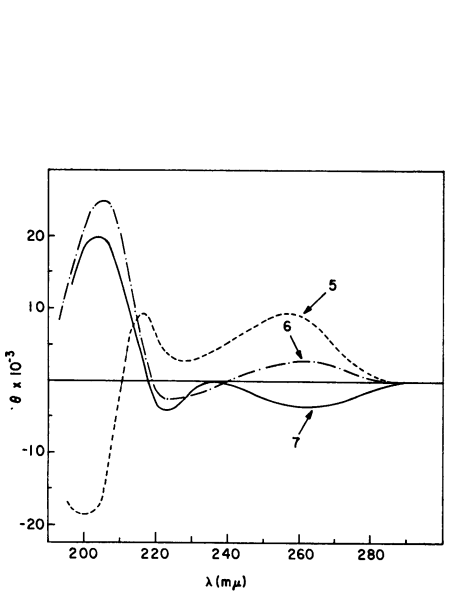


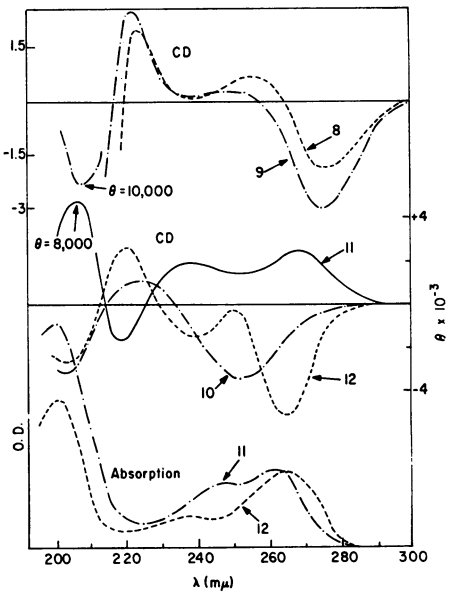
FIG. 2.—The circular dichroism spectra of compounds **1–4** measured in water at pH 7.

Purine ribonucleoside (**8**) and its 6-substituted derivatives, as well as all adenine nucleoside derivatives (such as compounds **2** and **3**), which contain no sulfur or double bond in the sugar residue, exhibit but a single negative CD band in the 260–270-mμ region with molecular ellipticities in the range $-1{,}000$ to $-7{,}000$.

When substantial changes in ellipticity are found in adenine nucleoside derivatives, as in compounds **5–7** and **13–16,** they are explicable on the basis of the dominant interactions introduced by the presence of sulfur or pi-electrons. It was hoped that these new and stronger interactions might selectively enhance the intensity of the B_{1u} band relative to the B_{2u} band and resolve the B_{1u} band. This would require a rather fortuitous base-sugar geometry in which the B_{2u}-sugar interactions were minimal and the B_{1u}-sugar interactions were maximal,

FIG. 1.—The structural formulas of the nucleoside derivatives (with source). **1,** Adenosine (Cyclo Chemical Corporation); **2,** 2′-*C*-methyladenosine (Walton, E., S. R. Jenkins, R. F. Nutt, M. Zimmerman, and F. W. Holly, *J. Am. Chem. Soc.*, **88,** 4524 (1966)); **3,** 3′-*C*-methyladenosine (same source as **2**); **4,** 6-*N*,*N*-dimethylamino-9-β-D-ribofuranosylpurine (same source as **1**); **5,** 2′,3′-didehydro-2′,3′-dideoxyadenosine (McCarthy, J. R., Jr., M. J. Robins, L. B. Townsend, and R. K. Robins, *J. Am. Chem. Soc.*, **88,** 1549 (1966)); **6,** 6-amino-9-(2′3,′-*O*-isopropylidine-5′-deoxy-β-D-*erythro*pent-4′-enofuranosyl)purine (McCarthy, J. R., Jr., M. J. Robins, and R. K. Robins, *Chem. Commun.* (1967), p. 536); **7,** 6-amino-9-(5′-deoxy-β-D-*erythro*pent-4′-enofuranosyl)purine (same source as **6**); **8,** 9-β-D-ribofuranosylpurine (same source as **1**); **9,** 6-chloro-9-β-D-ribofuranosylpurine (same source as **1**); **10,** 6-methoxy-9-β-D-ribofuranosylpurine (same source as **1**); **11,** 6-methyl-9-(2′-deoxy-α-D-*erythro*-pentofuranosyl)-purine (Robins, M. J., and R. K. Robins, *J. Am. Chem. Soc.*, **87,** 4934 (1965)); **12,** 2-fluoro-6-methyl-9-β-D-ribofuranosylpurine (Montgomery, J. A., and K. Hewson, *J. Med. Chem.*, **11,** 48 (1968)); **13,** 4′-thioadenosine (Reist, E. J., D. E. Gueffory, and L. Goodman, *J. Am. Chem. Soc.*, **86,** 5668 (1964)); **14,** 6-amino-9-(3′-*S*-ethyl-3′-thio-2′,3′-dideoxy-β-D-*threo*-pentofuranosyl)-purine (Robins, M. J., J. R. McCarthy, Jr., and R. K. Robins, *Biochemistry*, **5,** 224 (1966)); **15,** 5′-*S*-ethyl-5′-thio-2′,5′-dideoxyadenosine (same source as **14**); **16,** 6-amino-9-(3′,5′-di-*S*-ethyl-3′,5′-dithio-2′,3′,5′-trideoxy-β-D-*threo*-pentofuranosyl)purine (same source as **14**); **16,** 6-amino-7-β-D-ribofuranosylpurine (Rousseau, R. J., R. K. Robins, and L. B. Townsend, *J. Am. Chem. Soc.*, **90,** 2661 (1968)).





(*Left*) FIG. 3.—The circular dichroism spectra of compounds **5**–**7** measured in water at pH 7.
(*Right*) FIG. 4.—(*Top*) The circular dichroism spectra of compounds **8** and **9**. (*Middle*) The circular dichroism spectra of compounds **10**–**12**. (*Bottom*) The absorption spectra of compounds **11** and **12**. All measurements were conducted in water at pH 7.

especially if the B_{1u} transition were small relative to the B_{2u} moment. Such expectations appear to be realized in the CD spectrum of 4′-thioadenosine (**13**), the spectra of which exhibit a 240-mμ negative Cotton effect in ethanol and water. The CD spectra of **13** are given in Figure 3 (*top*). It was noted, however, that the spectrum of **13** in water has a complex CD behavior at about 260 mμ. Figure 4 contains the circular dichroism and absorption spectra of **17** in water and its circular dichroism behavior in ethanol as a function of temperature (*top*). Compound **17** is identical in structure to adenosine (**1**) except that the ribose ring is attached to the 7 position. A molecular model of **17** shows that this structure is capable of hydrogen bonding between the 6-amino group and the ribose hydroxyl groups.

Despite the clear indications of a 243-mμ band in the absorption spectrum, a 240-mμ Cotton effect is not noted in aqueous solution. In ethanol, a 240-mμ band is resolved and changes in intensities of the other bands are noted. The temperature study rules out any equilibrium effects, since both the 240- and 260-mμ Cotton effects increase in intensity as the temperature is lowered from 50° to −50°. Three bands are resolved in the absorption spectrum of **17**: at 273, 245, and 210 mμ.

A 240-mμ Cotton effect is also noted in purine ribonucleoside (**8**) and some of its 6-substituted derivatives (see Fig. 3). The second Cotton effect (B_{1u}) is red-shifted on going from **11** or **12** to **9** and is missing in **10** and **1**. The presence of an amino group at position 6 of the purine ring is known to have but a small hypsochromic effect on the long-wavelength band, but it was suggested by Mason[4]

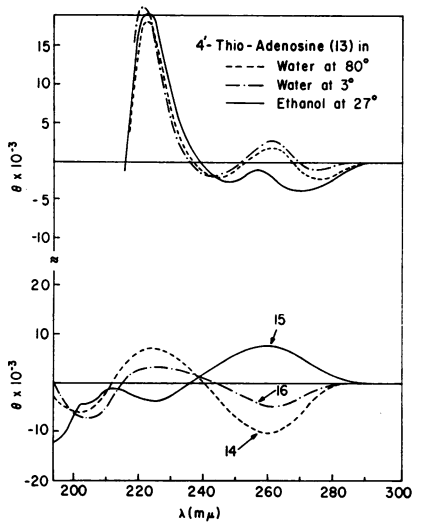


FIG. 5.—(*Top*) The circular dichroism spectra of 4'-thioadenosine (**13**) measured in water at 80° and at 3°, and in ethanol at 27°.

(*Bottom*) The circular dichroism spectra of **14**, **15**, and **16** measured in water at pH 7.

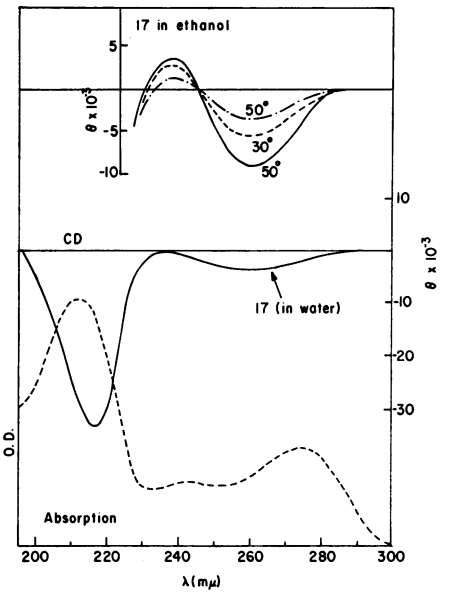


FIG. 6.—(*Top*) The circular dichroism spectra of **17** in ethanol at various temperatures.

(*Middle*) The circular dichroism spectra of **17** in water at pH 7.

(*Bottom*) The absorption spectra of **17** measured in water at pH 7.

that this substituent has a large bathochromic effect on the B_{1u} component. Clark and Tinoco[3] also concluded that the B_{1u} band is displaced toward longer wavelengths with progression through the series (—H, —Cl, —OCH_3, and —NH_3). However, the absorption data presented by them[3] show only that the 6-chloro substituent red shifts the B_{1u} band relative to the purine spectrum. The direction of the shift of the B_{1u} band when the 6-hydrogen of purine is exchanged for amino, keto, or methoxy is not demonstrated by the absorption data of reference 3.

The following data support a new proposition that the energy of *both* the B_{2u} and B_{1u} bands or purine is relatively unaffected by a 6-amino substituent:

(1) There are CD bands in the spectra of compounds **13** and **17** with positions of maxima quite comparable to the 240-mμ Cotton effects found in **11** and **12**.

(2) In Figure 6, which gives the absorption spectrum of compound **6**, no band or shoulder is resolved in either water or cyclohexane, but the absorption in the 240-mμ region is significantly enhanced by methylcyclohexane. The B_{1u} component is also missing in the absorption spectrum of aqueous solutions of purine and 9-methyl-purine but is resolved when examined in nonpolar solvents.[3] It is also interesting to note that the B_{1u} band is absent in aqueous purine solutions but is resolved in aqueous solutions of purine ribonucleoside. Apparently the intensity, but not the position, of the B_{1u} band is very sensitive to structural and solvent changes.

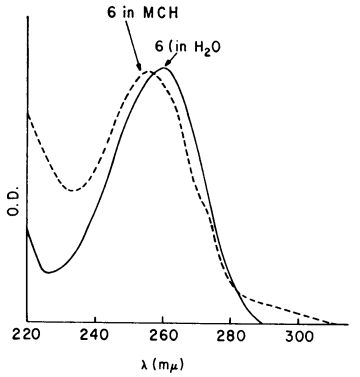


FIG. 7.—The absorption spectra of **6** in methylcyclohexane (MCH) and in water at pH 7.

(3) A 240-mμ absorption band is resolved by exchanging the position of ribose attachment from position 9 to position 7 of adenine.

(4) The polarized fluorescence studies of Callis, Rosa, and Simpson[12] indicate that the B_{1u} band is near 240 mμ.

Thus, these experiments are in accord with the hypothesis that a 6-amino substituent has little effect on the positions of the B_{2u} and B_{1u} bands.

In addition, solvent studies on **6** indicate, in agreement with the conclusion of Kleinwachter *et al.*,[9] that the 267-mμ shoulder in the absorption spectra of adenine arises from a vibrational component of the B_{2u} band. The shoulder is found at 273 mμ in the absorption spectra of **6** in methylcyclohexane, dioxane, and ethanol but is missing in water. The position of this shoulder does not change in spectra determined in ethanol or methylcyclohexane but the intensity of the long-wavelength tail is greatly enhanced. Solvent shifts of the shoulder would be expected if this absorption were of *n*-pi* origin. The absorption that does occur at long wavelengths in hydrocarbon solvents gradually disappears as ethanol is added to the solution, but the 273-mμ shoulder is unaffected. The long-wavelength absorption and not the 273-mμ shoulder, therefore, obeys the McConnell blue-shift criteria[24] for an *n*-pi* band in hydrogen-bonding solvents. The circular dichroism of **6** is relatively insensitive to solvents, i.e, little noticeable change in ellipticity is observed in the 280–300-mμ region or in the intensity of the B_{2u} Cotton effect.

The electronic transitions responsible for the 240, 220, and 200-mμ CD bands are presumably pi-pi* transitions, since no solvent shifts[24] in the position of peaks in the CD spectra of **6** and **16** are observed between water and methylcyclohexane.

We wish to thank Dr. E. Walton of Merck, Sharp, and Dohme for samples of compounds **2** and **3**; Dr. J. A. Montgomery, Southern Research Institute, for **12**; and Dr. A. Bloch, Roswell Park Memorial Institute, for **13**. The excellent technical assistance of William Miles is acknowledged.

* This research was supported by grant no. GM-12862-02 from the National Institutes of Health and grant no. CA-08109-03 from the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

[1] Miles, D. W., M. J. Robins, R. K. Robins, M. W. Winkley, and H. Eyring, *J. Am. Chem. Soc.*, paper IV in this series, in press.

[2] *Ibid.*, paper V in this series, in press.

[3] Clark, L. B., and I. Tinoco, Jr., *J. Am. Chem. Soc.*, **87**, 11 (1965).

[4] Mason, S. F., *J. Chem. Soc.* (1954), p. 2071; *ibid.* (1959), p. 1240; *ibid.* (1959), p. 1253; *ibid.* (1960), p. 219.

[5] Miles, D. W., R. K. Robins, and H. Eyring, these PROCEEDINGS, **57**, 1138 (1967).

[6] Cohen, B. J., and L. Goodman, *J. Am. Chem. Soc.*, **87**, 5487 (1968).

[7] Drobnik, J., and L. G. Augenstein, *Photochem. Photobiol.*, **5**, 13 (1966); *ibid.*, **5**, 83 (1966).

[8] Drobnik, J., V. Kleinwachter, and L. G. Augenstein, *Photochem. Photobiol.*, **6**, 147 (1967).

[9] Kleinwachter, V., J. Drobnik, and L. Augenstein, *Photochem. Photobiol.*, **5**, 579 (1966); *ibid.*, **6**, 133 (1967).

[10] Brahms, J., J. Pilet, H. Damany, and V. Chandrasekharan, these PROCEEDINGS, 60, 1130 (1968).
[11] Yamada, T., and H. Fukutome, *Biopolymers*, 6, 43 (1968).
[12] Callis, P. R., R. J. Rosa, and W. T. Simpson, *J. Am. Chem. Soc.*, 86, 2292 (1964).
[13] Stewart, R. F., and N. Davidson, *J. Chem. Phys.*, 39, 255 (1963).
[14] Stewart, R. F., and L. H. Jensen, *J. Chem. Phys.*, 40, 2071 (1964).
[15] Kwiatkowski, J. S., *Theoret. Chim. Acta*, 10, 47 (1968) (and bibliography).
[16] Berthod, H., C. Giessner-Prettre, and A. Pullman, *Theoret. Chim. Acta*, 5, 53 (1966); Berthod, H., C. Giessner-Prettre, and A. Pullman, *Intern. J. Quantum Chem.*, 1, 123 (1967).
[17] Wellmen, K. M., P. H. A. Laun, W. S. Briggs, A. Moscowitz, and C. Djerassi, *J. Am. Chem. Soc.*, 87, 66 (1965).
[18] Nagata, C., A. Imamura, Y. Tagashira, and M. Kodama, *Bull. Chem. Soc. Japan*, 38, 1938 (1965).
[19] Jaffe, H. H., and M. Orchin, *Theory and Applications of Ultraviolet Spectroscopy* (New York: J. Wiley and Sons, Inc., 1962), p. 474.
[20] Schellman, J. A., *Accounts Chem. Res.*, 1, 144 (1968).
[21] Tanaka, M., and S. Nagakura, *Theoret. Chim. Acta*, 6, 320 (1966).
[22] Devoe, H., and I. Tinoco, Jr., *J. Mol. Biol.*, 4, 500 (1962).
[23] Hoffman, T. A., and J. Ladik, in *Advances in Chemical Physics*, ed. J. Dochesne (New York: Academic Press, 1964), vol. 7, p. 84.
[24] McConnell, H., *J. Chem. Phys.*, 20, 700 (1952).