fluorine strongly prefers to be axial rather than equatorial[27-29] and whatever factors are responsible for this preference may also be at work in the five-membered rings discussed here.

Acknowledgments. Work in this system was begun as the result of a suggestion from Dr. K. N. Fang.

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Nucleoside Conformations. XI. Solvent Effects on Optical Properties of Guanosine and Its Derivatives in Dilute Solutions[1a]

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Abstract: Precise attributions of the B_{2u}, B_{1u}, and E_{1ua} bands of guanosine and several derivatives by MCD in water, propanol, and dioxane in conjunction with CD spectra have demonstrated that for a given compound the bands B_{2u} and B_{1u} are differently influenced by solvent changes or by substitutions. An attempt was made to correlate the sign of the Cotton effect with a simple sectional rule (octant rule) taking into account the flexibility of the molecules. It was concluded that small conformational changes of Guo and its analogs could be the source for relatively large changes in the Cotton effects (band inversions).

Many conformational problems of nucleic acids cannot be solved without information about all the allowed states of the monomers. This explains the many studies[2-10] on their optical properties which are supposedly linked to their conformation. Crystallographic studies[11] have demonstrated the presence of two conformations in the single crystal of guanosine and many others in its derivatives.[12,13] Nmr studies and particularly those by nuclear Overhauser effect[14-17]

have shown that there is a multitude of populations with different angles Φ_{CN} for a given nucleoside in solution, which for unhindered compounds is never all *syn* or all *anti*.

We report here the effects of substituents and solvents on the CD spectra of guanosine and its derivatives. We have assigned precisely the transitions of the bands of the CD spectra with the help of MCD and absorption spectra. B_{1u} and B_{2u} transitions are sensitive to the environment of the molecule but not in the same way; their variations and the weakness of the signals indicate that the CD spectra are an average of different conformations in solution. It appears that either of the two bands B_{2u} and B_{1u} can, but need not to be, more sensitive to solvent effects and conformational changes than the other.

Methods and Materials

Guanosine and compounds A, B, C, and M (Figure 1) were purchased from P. L. Laboratories, Milwaukee, Wis.; m^{7}Guo (L)[18] was purchased from Cyclo-Chemicals, Los Angeles, Calif.

Compounds D, E, F, G, H, and K were gifts of Dr. A. Holy (Praha). Compound J was given by Dr.

(1) (a) Part X of this series: P. Tougard, J. F. Chantot, and W. Guschlbauer, *Biochim. Biophys. Acta*, **308**, 9 (1973); (b) Boursier de these du CEA (1970–1975). Part of the Doctoral Thesis of J. M. Delabar, to be presented to the Faculty of the University, Paris VI.
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(13) W. Guschlbauer, *Jerusalem Symp. Quant. Chem. Biochem.*, **4**, 297 (1972).
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(17) S. Tran-Dinh, W. Guschlbauer, and M. Guéron, *J. Amer. Chem. Soc.*, **94**, 7903 (1972).
(18) Abbreviations used: nucleosides are abbreviated according to IUB-IUPAC convention [(*Progr. Nucl. Acid Res.*, **11** (1971) IX; nmr, nuclear magnetic resonance; CD, circular dichroism; MCD, magnetic circular dichroism.
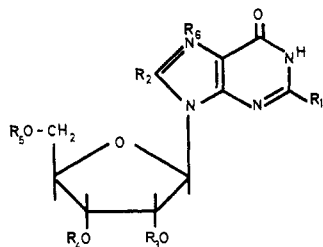
Figure 1. Compounds studied (R_1, R_2, R_3, R_4, R_5, R_6): (A) NH_2, H, OH, OH, OH, ···; (B) NH_2, H, R_3,R_4 = isopropyl, OH, ···; (C) NH_2, H, acetyl, acetyl, OH, ···; (D) NH_2, H, OH, OH, acetyl, ···; (E) NH_2, H, acetyl, acetyl, acetyl, ···; (F) NH_2, H, H, $PO_4{}^{2-}$, OH, ···; (G) NH_2, H, R_3R_4 = phosphate, acetyl, ···; (H) $N(CH_3)_2$, H, OH, OH, OH, ···; (I) NH_2, Br, OH, OH, OH, ···; (J) NH_2, Br, R_3,R_4 = isopropyl, OH, ···; (K) NH_2, OH, R_3,R_4 = isopropyl, acetyl, ···; (L) NH_2, H, OH, OH, OH, CH_3; (M) H, H, OH, OH, OH, ··· .
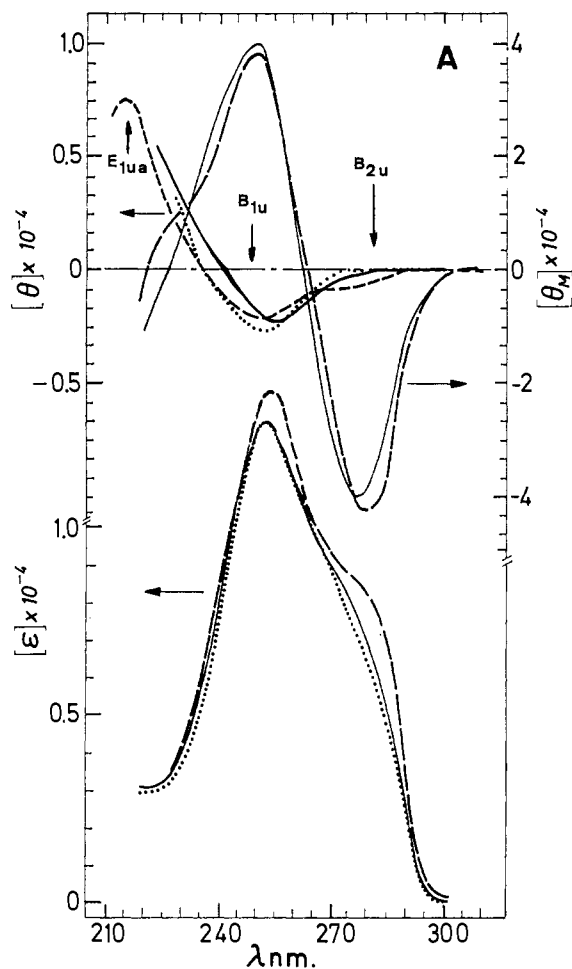

Figure 2. Absorption, CD, and MCD spectra of A (Guo) in water (- - -), ethanol (————), and dioxane (···).

Ikehara (Osaka) and compound I was synthesized by Dr. Chantot in this laboratory.

The solvents used were spectroscopically pure uvasol products (Merck, Darmstadt, Germany).

The absorption spectra were recorded on a Cary 14 or Cary 15 spectrophotometer. The spectra in nonaqueous solvents were recorded in a concentrated solution in a 1-mm cuvette and then diluted 50 times in water and measured again in a 5-cm cuvette. The molar extinction coefficient of Guo in water was
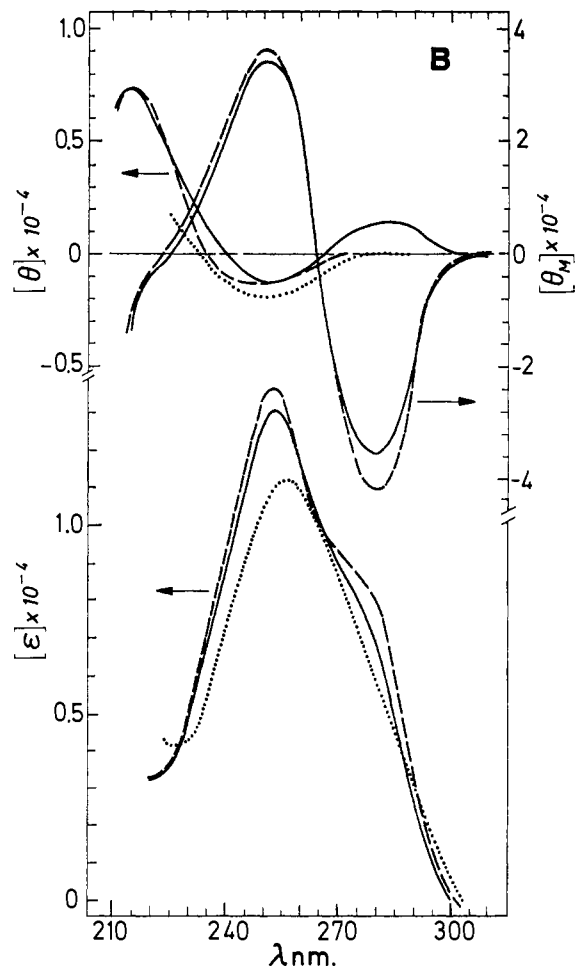

Figure 3. Absorption, CD, and MCD spectra of B ($2'3'$-O-isopropyl-Guo) in different solvents (symbols as in Figure 2).

used to calculate the corresponding extinction value in the nonaqueous solvent.

The CD spectra were recorded on a Roussel-Jouan II-185 dichrograph, flushed with dry nitrogen. MCD spectra were recorded on a modified Roussel-Jouan dichrograph, equipped with a superconducting magnet which yielded 73 kG maximally. The details of this equipment are described elsewhere.[19,20]

Results

Neither Guo nor its derivatives are soluble in hexane or cyclohexane. The Guo reference absorption spectrum in water at pH 7 shows a peak at 252 nm and a shoulder at 275 nm (Figure 2). The absorption spectra of Guo (A), $2',3'$-ip-Guo (B), $5'$-ac-Guo (D), m^7Guo (L), and of inosine (M) in Figures 2, 3, 4a, 5a, and 7 show a significant hypochromism of the band in the 252–263-nm region principally in dioxane; compared with the Guo reference spectrum this band is shifted in water to 256 nm for D, to 257 nm for L in acid buffer (the spectrum of L in acid medium and in basic medium are very different; mainly the acid form which is the most stable below the pK about 6 has been studied).

The spectral changes due to the decrease of the dielectric constant permit us to define two groups of Guo

(19) C. H. Schneider, DEA Université Paris VI.
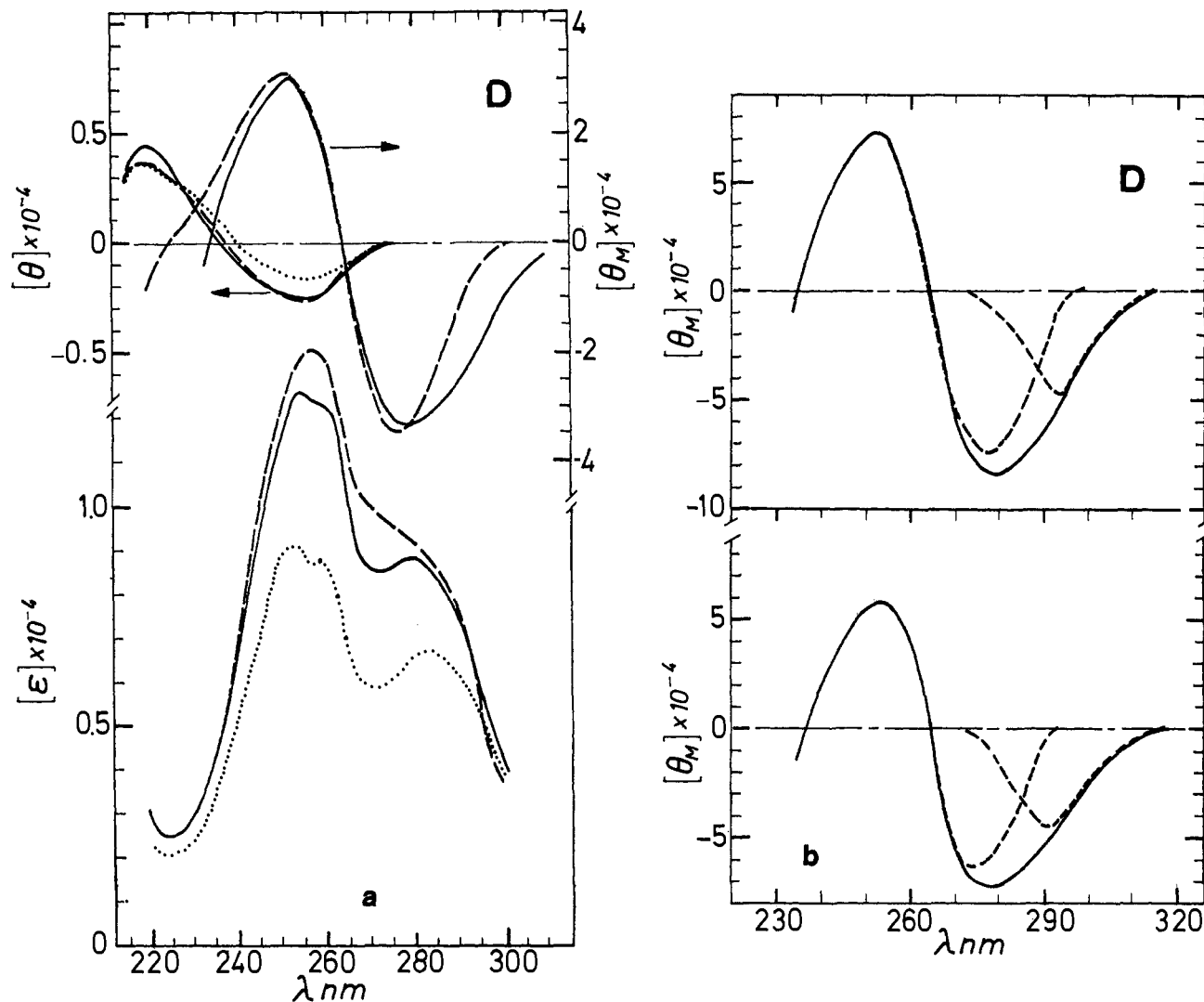(20) R. Blondet, D. Rappanello, and Ch. Schneider, Proc. Int. Cryog. Eng. Conf., 3rd, 238 (1970).

Figure 4. (a) Absorption, CD, and MCD spectra of D (5'-*O*-acetyl-Guo) in different solvents (symbols as in Figure 2); (b) attempt of decomposition of MCD spectrum of D in ethanol (top) and dioxane (bottom).

derivatives: on one hand, A, B, E, L (Figures 2, 3, 6, 7) where the shoulder at 275 nm disappeared in dioxane and which show weak hypochromism at 250 nm; on the other hand, D and M where the shoulder is moving to the red to 265 nm for M and even becomes a peak at 280 nm for D. To explain the hypochromism the same reasoning as for Ado derivatives[10] can be applied: aggregation, but the Lambert-Beer law is followed until 0.002 M in water and 0.001 M in ethanol.[21] Moreover, the CD signal does not increase in dioxane and even decreases; the hypochromism observed is not due to a shift in the absorption bands because the location of the B_{1u} and B_{2u} bands in MCD does not change significantly in different solvents (Figures 2, 3, 6, 7). Probably a purely electronic solvent and/or conformational effect act on the intensity of the two transitions at 252 and 275 nm and possibly in a different way.

For compounds like D and M two facts indicate that the hypochromism observed may be due to other effects: first, the 256-nm band of D and the 248-nm band of M are split into two bands with a gap of 7-8 nm; the protonation of D is excluded because the CD signal of this band is negative in water while it is positive in L (Figure 7) which carries a positive charge and remains negative in other solvents. It is possible to follow solvent effects on the absorption spectra of M by adding increasing amounts of water to a solution of inosine in pure ethanol (Figure 5b): the absorbance increases progressively, the 265-nm shoulder and the split of the 248-nm band disappear; also the width of the highest wavelength MCD band of D (Figure 4a) and M (Figure 5a) broadens with decreasing dielectric constant. Assuming that the B_{1u} and B_{2u} MCD bands are approximately equal,[24a] one can conclude that another transition near 285-290 nm for D and around 265 nm for M appears which could possibly be a n-π^* transition. One can explain these changes of the spectra by differ-

(21) Guanosine aggregation has been studied extensively in this laboratory[1a,22,23] and deviation from the Lambert-Beer law was observed only above 0.002 M in aqueous salt solutions for Guo and most of its derivatives.

(22) J. F. Chantot, M. Th. Sarocchi, and W. Guschlbauer, *Biochimie*, 53, 347 (1971).

(23) J. F. Chantot and W. Guschlbauer, *Jerusalem Symp. Quant. Chem. Biochem.*, 4, 205 (1972); J. F. Chantot, *Arch. Biochem. Biophys.*, 153, 347 (1972).

(24) (a) W. Voelter, R. Records, E. Bunnenberg, and C. Djerassi, J. *Amer. Chem. Soc.*, 90 6163 (1968); (b) unpublished MCD experiments in this laboratory on 1-methylguanine and 7-methylguanine in water, ethanol, and dioxane have shown that the wavelength positions of the peaks and troughs are unchanged.
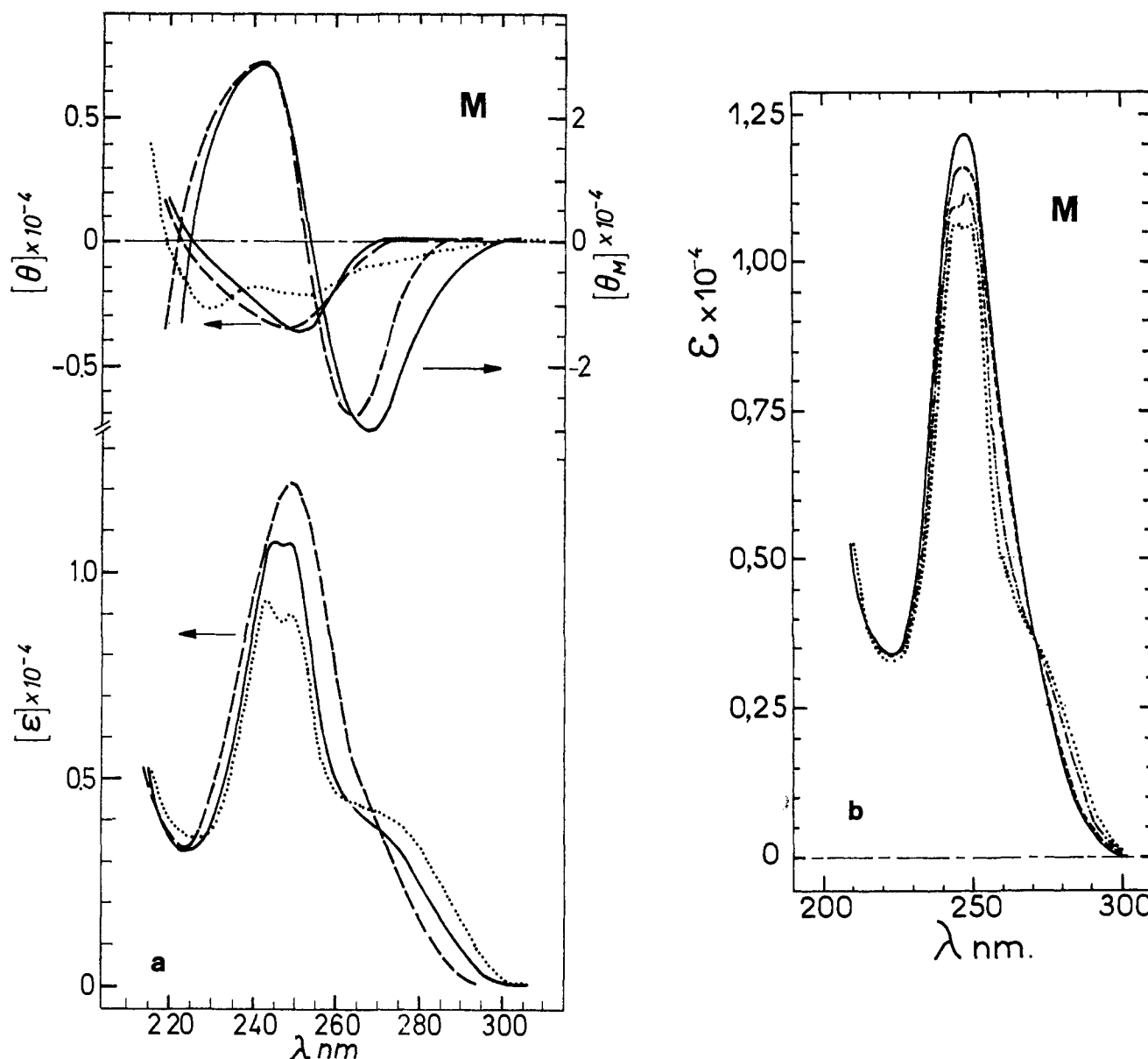
Figure 5. (a) Absorption, CD, and MCD spectra of M (inosine) in different solvents (symbols as in Figure 2); (b) absorption spectra of M: in 100% EtOH (. . . . . .), in 75% EtOH–25% water (– – –), 25% EtOH–75% water (-·-·-·-), 100% water (—).

ent effects on the two kinds of transitions, n–π* and π–π*.

As for the absorption spectra, the CD spectrum of Guo (A) (Figure 2) in water at pH 7 is taken as reference: it shows three bands above 210 nm: one positive at 215 nm and the two others negative at 252 and 275 nm. The 275-nm band decreases in propanol and disappears in dioxane as in the absorption spectrum; in the same time the MCD spectrum does not vary significantly;[24b] this permits us to attribute the three Cotton effects (215, 252, 275 nm) in water to the E_{1ua}, B_{1u}, and B_{2u} transitions, respectively. But it is the unique case where the changes vary in the same way in CD and absorption spectra. For compounds like C or D we have a positive Cotton effect below 220 nm and one negative around 250 nm which does not vary with a change of solvent (Figures 8 and 4a); moreover, the B_{2u} band does not appear in the CD spectrum in any of the solvents used. With an isopropyl group on the sugar (B) the B_{2u} band is visible in CD and gives a

positive Cotton effect around 280 nm but only in alcoholic solvents.

The behavior of E (Figure 6) is quite different: in water B_{2u} has a negative Cotton effect and the B_{1u} band is not visible; in propanol the B_{2u} Cotton effect is zero and the B_{1u} negative. Among the compounds unsubstituted on the base, F in water at pH 6.6 (Figure 9) shows a negative Cotton effect for B_{1u} and a positive one for E_{1u}. Guo-2',3'>P (G) shows three positive bands in water while the B_{1u} band is zero in propanol (Figure 9).

Among the compounds substituted on the base the two 8-Br analogs I and J, both obligatorily in the syn conformation, which have little rotational freedom around the glycosidic linkage, have the same behavior; from the three positive bands in water only the B_{1u} band becomes zero in propanol. Another compound, K, substituted on C-8, shows also three positive bands in water (Figure 10), but shows only one positive below 220 nm in propanol; here the B_{1u} and B_{2u} bands are
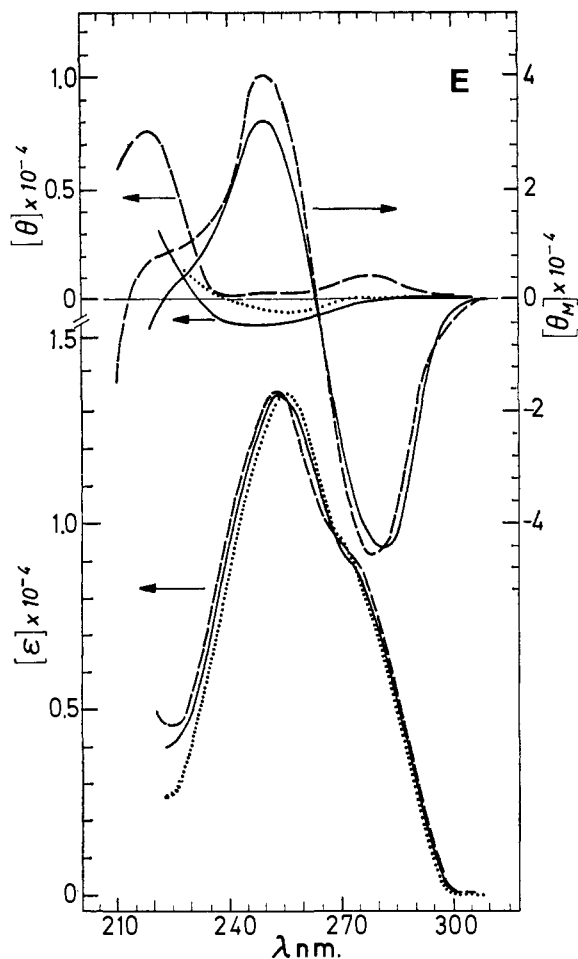
Figure 6. Absorption, CD, and MCD spectra of E (2',3',5',-tri-O-acetyl-Guo) in different solvents (symbols as in Figure 2).

Figure 7. Absorption, CD, and MCD spectra of L (7-CH₃-Guo). in different solvents (symbols as in Figure 2).

zero. Compound L (m⁷Guo) is also substituted on the imidazole part of the base but the B_{2u} and E_{1uA} bands are negative and B_{1u} positive in the three solvents studied: water, propanol, and dioxane. The compounds H and M are modified on C-2 of the base. They differ from A and between each other by the B_{2u} band, which is negative for H (Figure 11) and not visible for M in water. In dioxane the compound M shows three negative bands at 275 nm for the B_{2u} Cotton effect, at 250 nm for the B_{1u}, at 230 nm for E_{1ua}, and one positive below 220 nm for E_{1ub}.

Discussion

As for Ado,[10] the solvent induced optical changes exclude a change of the direction of the transition moment of the base. Guanine, like all aromatic bases, possesses the C_s symmetry[25] defined by the plane of the base. From data in the literature[6-10] and in this paper it can be shown that a simple C_s symmetry rule for the optical activity of nucleosides is unsatisfactory. A plane perpendicular to the base plane along the transition moment studied would permit us to define a sectional, probably a quadrant, rule. Alternatively, an octant rule[26] could be envisaged with another plane perpendicular to the two previous ones. This is illustrated in Figure 12 where two Guo molecules are aligned along the B_{2u} transition.[27, 28] The difference between Figures
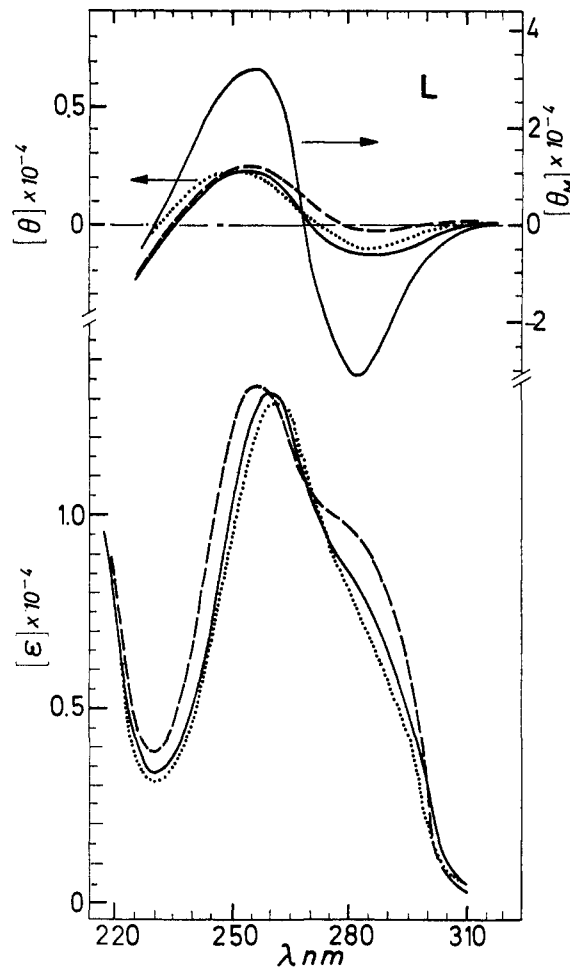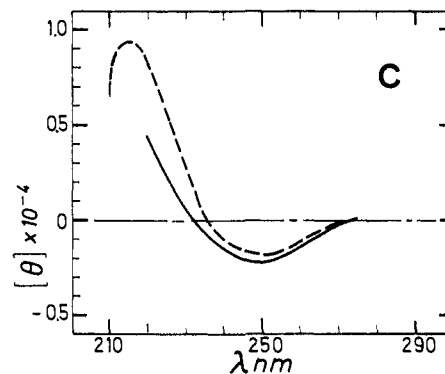

Figure 8. CD spectra of C (2'3'-di-O-acetyl-Guo) in different solvents (symbols as in Figure 2).

12a and 12b is the glycosidic torsion angle Φ_{CN} which differs by about 60°, but is anti in both cases.

Therefore a change of the sign of the Cotton effect may result from a small move of some of the atoms from one octant to another due to the flexibility of the sugar and its substituents. Also small differences in the glycosidic linkage as depicted in Figures 12a and b would move the entire sugar moiety into a different

(25) J. A. Schellmann, *Accounts Chem. Res.*, 1, 144 (1968).
(26) W. Moffitt, R. B. Woodward, A. Moscowitz, W. Klyne, and C. Djerassi, *J. Amer. Chem. Soc.*, 83, 4013 (1961).

(27) P. R. Callis, B. Fanconi, and W. J. Simpson, *J. Amer. Chem. Soc.*, 93, 6679 (1971).
(28) A. Fucarolo and L. S. Forster, *J. Amer. Chem. Soc.*, 93, 6443 (1971).
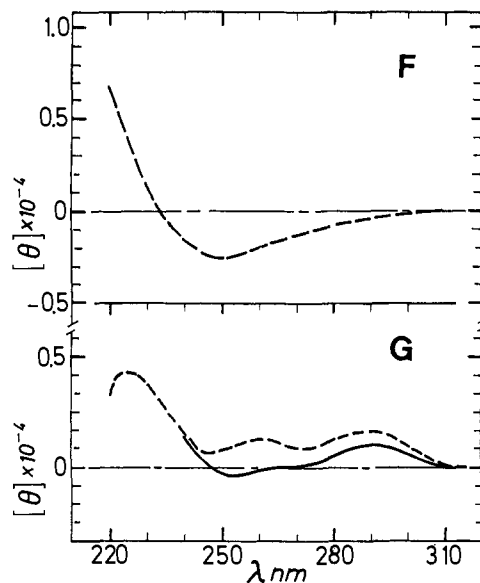
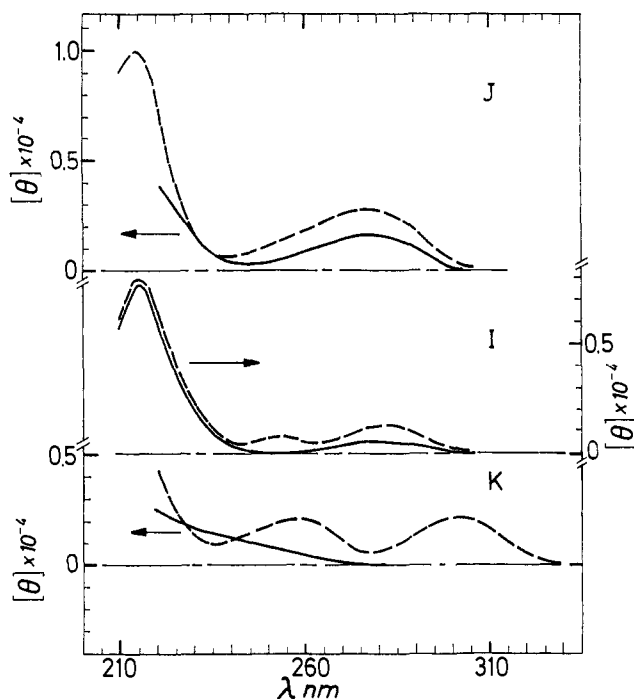Figure 9. Top, CD spectrum of F (d Guo-3'-P) in water, pH 6.6; bottom, CD spectra of G (Guo-2',3' > P).

Figure 10. CD spectra of I (Br⁸-Guo), J (2'3'-O-isopropyl-Guo), K (OH⁸-Guo) in different solvents (symbols as in Figure 2).

octant. Similarly, substitution of acetate in 3', for instance, would have an opposite effect in the case of Figure 12a of that in Figure 12b. On the other hand, in the two conformations substitution on the 5' position would have little effect in either case, since in both cases it would fall close to a nodal plane. Turning the sugar moiety in Figure 12a around the glycosidic bond for 180° (into an extreme syn conformation) should yield a Cotton effect for this band of the same sign. For the B_{1u} band which is perpendicular to the B_{2u} transition[29] a similar, but perpendicular, octant system can be con-

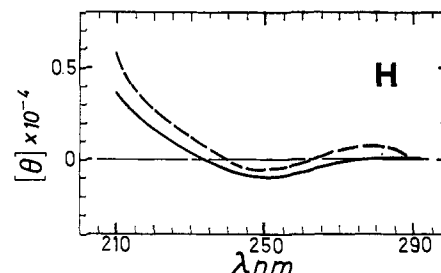(29) H. Berthod, C. Giessner-Prettre, and A. Pullmann, *Int. J. Quantum Chem.*, 1, 123 (1967).

Figure 11. CD spectra of H (N²(CH₃)₂-Guo) in different solvents (symbols as in Figure 2).

structed. Here only four octants will be implicated, thus changing the symmetry system considerably.

Therefore the response of the B_{1u} band to changes in Φ_{CN} will be very different from that of the B_{2u} band. The CD of the B_{1u} band should show a very large increase in amplitude in going from the conformation in Figure 12a to that in Figure 12b. On the other hand, a change of 180° in Φ_{CN} of Figure 12a should yield an opposite Cotton effect in the case of the B_{1u} band. Similar reasoning can be applied to the E_{1ua} band which seems to be parallel to the B_{1u} band, but of opposite polarity.[29]

For the study of the influence of substituents, the compounds A, C, D, E, all substituted with acetyl groups on different parts of the ribose, are very useful. All show an E_{1ua} band with a positive Cotton effect in all the solvents studied, but A, C and D, E have a different response to solvent changes for the B_{1u} and B_{2u} transitions; this shows also that the 5' substituent is not an important factor. Very similar results on the uridine and cytidine series have been obtained by Rabczenko and Shugar[30] which would be perfectly compatible with such a sectional rule.[31] In the same way, compounds I and J which are sterically restricted to a rather narrow syn range are interesting, because they do not show a change of sign for the B_{1u} and B_{2u} band but a difference in intensity between each other, both in propanol and water. According to the octant rule this is expected for compounds with large substituents on C-8 which restrict the rotation around the glycosidic linkage while substitution on the 2' and 3' positions on such hindered compounds should increase the intensity, but could not shift the atoms into another octant.

Some authors[9] have tried to establish a rule for the behavior of the derivatives of guanine when the solvent is changed; they proposed that the Cotton effects become more "syn-like" in propanol than in water. It is quite clear from the present work that these formulations are oversimplified. Moreover, there is no obvious general behavior: if the B_{2u} band of A, B becomes more positive, for C, G, I, J, L, M it does not change significantly, and for E, H, K it becomes more negative; the B_{1u} band of A, B, C, D, H, L, M does not change, but for E, G, I, J, K it becomes more negative.

From these results it appears necessary to be careful in the assignments of the CD spectra: the B_{1u} and B_{2u} bands are always at the same wavelength in all the compounds studied, at 250 nm for the B_{1u} and 280 nm for the B_{2u} band (except for inosine), but their optical

(30) A. Rabczenko and D. Shugar, *Acta Biochim. Pol.*, in press.
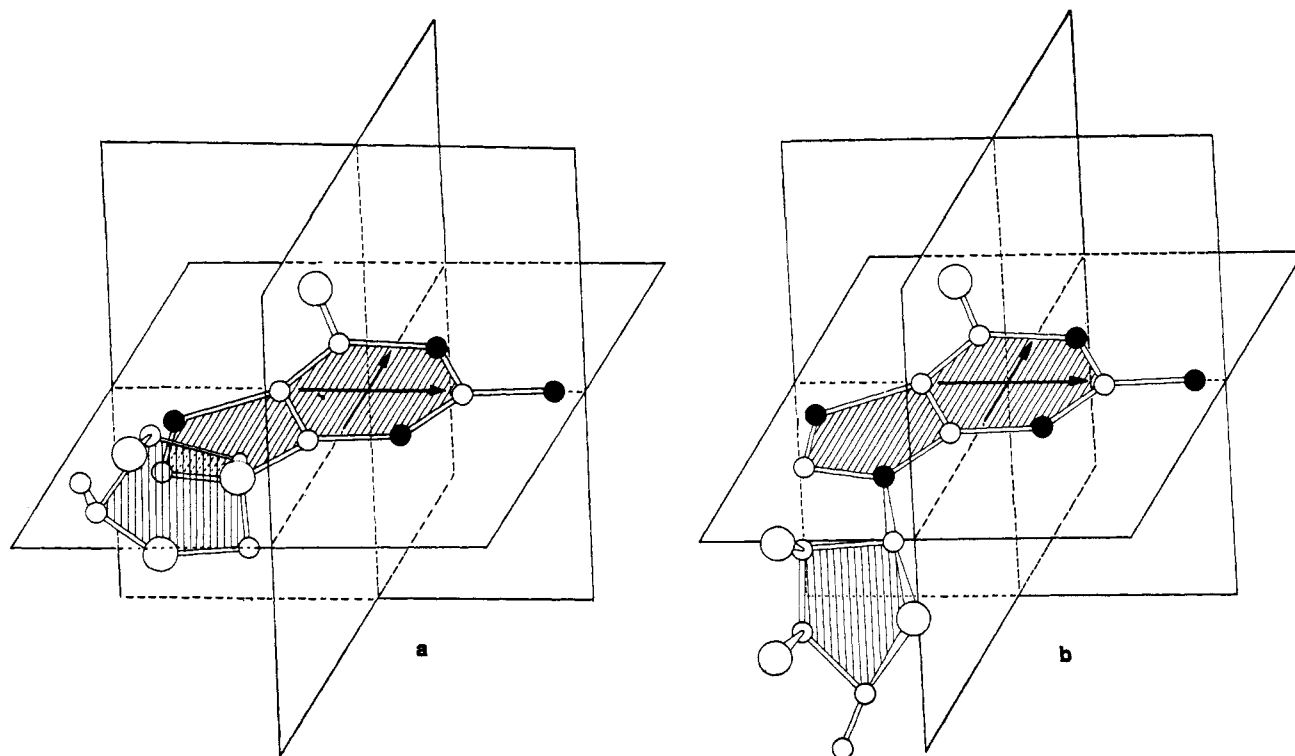(31) J. M. Delabar and W. Guschlbauer, Euchem Conference, Caen, France, May 1973.

Figure 12. Guanosine aligned along the B_{2u} transition: $\Phi_{CN} = -0°$ (A); $\Phi_{CN} = 90°$ (B).

activity need not be detectable, if the sugar residue is located near a nodal plane.

It also has been suggested[9] that a two state model with the syn and anti conformations as extrema, eliminating more than 75% of all possible conformational ranges, could explain the solvent effects on CD spectra. The conclusions were obtained by comparison with cyclonucleosides.[8,9] But there are several lines of evidence[14-17] showing that the unhindered nucleosides, which can rotate almost freely, can assume all angles Φ_{CN}. The relative amount of these populations can change with various conditions, like the pH or the substituents; solvent effects have to be added to this list of variables.

This implies that a CD band is the algebraic sum of an infinity of signals each of which corresponds to an angle Φ_{CN}; it is therefore only rarely possible to associate the sign of a Cotton effect to a given conformation, because it results from a population of different conformers; while part of the syn conformation could have a negative Cotton effect the other one could have a positive one; the sum could be negative or positive.

Transmethylation from Toxoflavines to Nucleophiles

Fumio Yoneda* and Tomohisa Nagamatsu

Contribution from the Faculty of Pharmaceutical Sciences, Kumamoto University, Oe-honmachi, Kumamoto, Japan. Received May 7, 1973

Abstract: The antibiotic toxoflavine and analogs undergo demethylation with nucleophiles to give the corresponding 1-demethyltoxoflavines (8-demethylfervenulins), while nucleophiles themselves are methylated by the methyl group eliminated. During the reactions, toxoflavine radical anions, novel radical species, were observed. A possible reaction mechanism is proposed for this transmethylation.

The structure of an antibiotic toxoflavine (identical with xanthothricin) having strong physiological activities has been established as 1,6-dimethylpyrimido-[5,4-e]-as-triazine-5,7(1H,6H)-dione by total synthesis in 1961.[1] The structural relationship of toxoflavine to riboflavine is particularly remarkable, since both compounds possess similar oxidation–reduction systems. It is also known that the peroxide-generating capacity of toxoflavine may be responsible for its poisonous character.[2] We have now found another

(1) G. D. Daves, R. K. Robins, and C. C. Cheng, *J. Amer. Chem. Soc.*, **83**, 3904 (1961).

(2) H. E. Latuasan and W. Berends, *Biochim. Biophys. Acta*, **52**, 502 (1961).