Magnetic Circular Dichroism (MCD) of the *N*-Methyl Derivatives of Purine (1)

Leroy B. Townsend, Daniel W. Miles, Steven J. Manning, and Henry Eyring

Department of Chemistry and Department of Biopharmaceutical Sciences
University of Utah, Salt Lake City, Utah 84112

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Sir:

The isolation and characterization of several antibiotics (2) and minor components (3) of specific t-RNA's as nucleosides has generated a significant interest in the direct glycosylation and alkylation of purines and certain closely related derivatives. This has resulted in the use of instrumental methods to establish the actual site of glycosylation and to obtain other supplementary information concerning the fundamental nature and structure of these compounds. In theory, magnetic circular dichrosim (MCD) should possess certain advantages over ordinary spectrophotometric techniques due to the occurrence of negative bands and the acute sensitivity of MCD spectra to changes in electronic structure. This sensitivity of MCD spectra to the precise chromophoric structure should provide valuable information in regards to structural differences between similar compounds for structural determination, *per se*.

This prompted us to initiate a MCD study using the *N*-methyl purines since there does appear to be a significant change in electronic structure for this series of compounds. The observed MCD according to the quantum theory (4) is due to a combination of three contributions characterized by the frequency independent factors "A", "B" and "C" of Faraday rotation. For low symmetry compounds only the "B" term of Equation 33 of ref. 1 is non-zero. This term depends on the lengths and relative polarizations of the transition moments connecting various lower and higher energy states of the molecule as well as the energy differences between states. These quantities are sensitive to changes in electronic structure which may arise from changes in chromophoric structure.

The MCD and absorption spectra of 1-, 3-, 7- and 9-methylpurine (5-7) were determined (Figure 1) with the essential information from the MCD spectra being summarized in Table I. While differences in sign pattern are not observed in the MCD spectra, large differences are found in band position, band intensity and the shifts and intensity changes induced by *p*H and solvent. Introduction of a methyl group at N1 or N3 of purine causes a bathochromic shift of the entire MCD curve of about 30 nm relative to the MCD spectra of purine. The band intensitites are also lower for 1- and 3-methylpurine with a four-fold decrease being noted for 1-methylpurine relative to 7-methylpurine. Thus, 1-methylpurine and 3-methylpurine, for which considerable bond fixation was expected, are characterized in their MCD spectra by displaying MCD bands of lower intensities at lower wavelengths relative to 7- and 9-methylpurine. These differences in band position and intensity are larger than the corresponding differences in the position and intensity of the absorption bands. Both 1- and 3-methylpurine display absorption maxima near 275-nm. The ϵ max values of 1- and 3-methylpurine relative to those of 7- and 9-methylpurine are less than 25% smaller.

The MCD spectra of the cationic forms of methylpurines are of considerable interest since they appear to

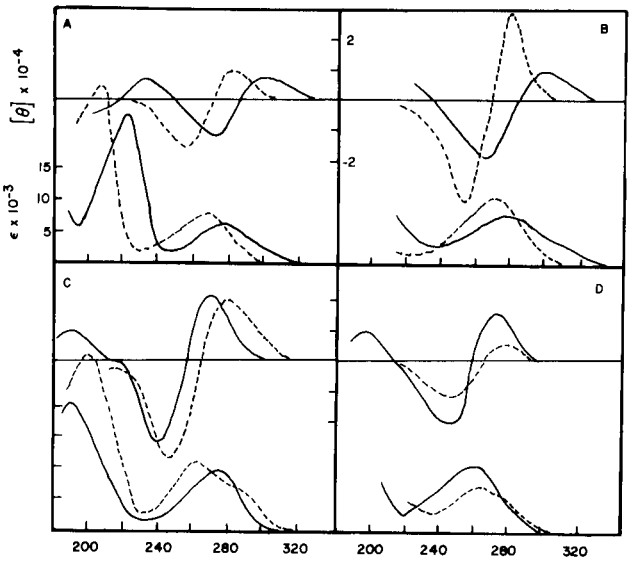


Figure 1. The MCD and absorption spectra determined in water at *p*H 7 (—) and *p*H 1 (---). (A) 1-methylpurine; (B) 3-methylpurine; (C) 7-methylpurine; (D) 9-methylpurine.

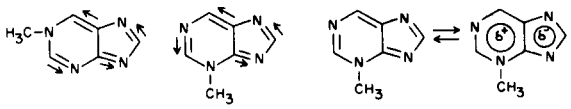
TABLE I

The Wavelength and Intensity of the B_{2u} MCD Band of the Neutral and Cationic Forms

Compound	λ max neutral (a)	λ max cation (b)	Δ λ max (b-a)	θ max x 10^{-3} neutral (a)	θ max x 10^{-3} cation (b)	Δ θ max (b-a)
9-methylpurine	269	275	+6	16.5	5.5	-11.0
7-methylpurine	268	278	+10	23.5	20.5	-3.0
3-methylpurine	298	279	-19	10.0	30.0	+20.0
1-methylpurine	295	280	-15	6.7	9.5	+2.8
purine	269	275	+6	10.2	5.8	-4.4

provide additional information which would be useful in differentiating amongst these compounds. Cation formation leads to a 6- and 10-nm red displacement of the first MCD band of 9- and 7-methylpurine and a 2- and 9-nm blue displacement of the first absorption maximum. In direct contrast, protonation of both 1- and 3-methylpurine is characterized by a blue shift in the first band in the MCD spectra of 15- and 20-nm and a blue shift of 7- and 2-nm in the absorption spectra, respectively. Protonation affords a means of sharply distinguishing whether or not the sample is 1- or 3-methylpurine. Formation of the cation of 3-methylpurine is accompanied by a 3-fold *increase* in intensity whereas only a quarter-fold *increase* in intensity is observed for 1-methylpurine. Protonation of 9-methylpurine is accompanied by a 3-fold *decrease* in intensity whereas only a quarter fold *decrease* in intensity is observed for 7-methylpurine. The Δ λ min values obtained from the absorption spectra of certain adenine derivatives can be used (8) for assigning the site of alkylation and are very similar to the Δ λ max values (Table I) and Δ λ min values (Figure I) obtained from the MCD spectra of the *N*-methylpurines (*vide infra*).

One salient feature of the absorption spectra of 1- and 3-methylpurine would suggest that delocalization of excitation may be of relatively more importance than electron delocalization (mesomerism) in these derivatives. The absorption spectra of 1- and 3-methylpurine exhibit a long tail extending to 320-nm which is reminiscent of the overtone bands of linear polyenes (9). The MCD band near 300-nm in the MCD spectra of these compounds are, therefore, associated with regions of low intensity in the absorption spectra. A qualitative treatment using a simple dipole model based on adding up ethylenic transition moments predicts low intensity for the long wavelength absorption bands. In this model the ethylenic transition dipoles are lined up in a minimum energy configuration, head to tail, along the perimeter of the system. This affords a weak intensity for the lowest energy transition of 1- and 3-methylpurine, since the



moments in both rings largely cancel one another. This would suggest that a theoretical treatment formulated in terms of weakly coupled ethylene groups may be superior to a delocalized orbital treatment.

Protonation sharpens the absorption bands by removing the long red tail and, in addition, increases the intensity of the corresponding MCD bands. This may suggest a possible confirmation of a qualitative resonance argument used to explain the exceptional reactivity of 3-methylpurine which requires the invocation of a zwitterionic structure of the type shown. The zwitterionic structure would display more aromatic character than the fixed bond structure. Protonation would stabilize the aromatic structure shifting the equilibrium to the right. In the simple bond dipole model the Faraday rotation would be zero unless the exciton states of the system included the doubly excited configurations. The coefficients of these configurations is always small in the lower energy states so one can anticipate that small Faraday rotations will be exhibited by systems for which the exciton theory is applicable. The simple bond dipole theory thus predicts low intensity in the absorption and MCD spectra but is not applicable to the aromatic forms of 3-methyl- or 1-methylpurine. For these forms one would expect the absorption and MCD spectra to be quite similar to that of 9-methylpurine, as observed. Therefore, MCD is potentially capable of providing new evidence in this area about problems such as tautomeric isomerism, protonation sites, the extent of π-electron delocalization (aromaticity and "double bond fixation", *etc.*) and in investigating substituent and solvent effects on electronic structure.

Another area under investigation in our laboratory involves a correlation between the MCD spectra of a nucleoside and the MCD spectra of the corresponding model methyl compound. The MCD spectra of 7-methyl-

purine (*vide infra*) and the MCD spectra of 7-(β-D-ribofuranosyl)purine (10) are essentially superimposable and would indicate that this comparison may be valid although additional corroboration must be obtained prior to the general use of this technique.

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