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ABSORPTION, CIRCULAR DICHROISM AND MAGNETIC CIRCULAR DICHROISM STUDIES ON 2-THIOURACILS

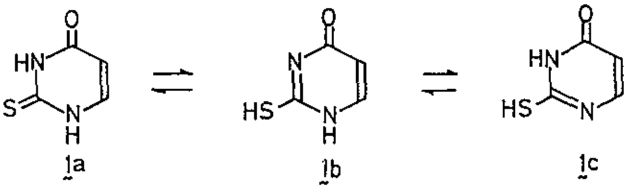
Akio Tajiri[a*], Nobuko Yamamoto[a], Masahiro Hatano[a*],
and Tohru Ueda[b]

a) Chemical Research Institute of Non-aqueous Solutions,
Tohoku University, Katahira 2-1-1, Sendai 980

b) Faculty of Pharmaceutical Sciences, Hokkaido University,
Kita-ku 12, Nishi 6, Sapporo 060

Abstract —— The longest wavelength absorption band of 2-thiouracil in solution has been clarified to be superposition of two $\pi \rightarrow \pi^*$ electronic transitions taking place in a thioketone form of the three possible tautomers of 2-thiouracil.

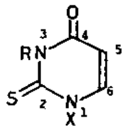
The thio derivatives of pyrimidine and purine bases and nucleosides found in some tRNAs as minor constituents have been a subject of appreciable interest in recent years both from synthetic and physico-chemical points of view[1 - 10]. Above all, the possible tautomerism present in solution of nucleic acid bases and nucleosides is one of the most interesting phenomena, which has been so far subjected to spectroscopic investigation and interpretation in connection with their biochemical functions and behaviors *in vivo.*



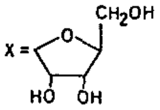
1a **1b** **1c**

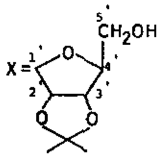
On various evidence, 2-thiouracil (**1**) is suggested to be a thione as early as in 1961[11], although the spectroscopic assignment remains still uncertain. Herein we report magnetic circular dichroism (MCD) spectra of **1**, 2-thiouridine (**2**) and 2',3'-O-isopropylidene-3-methyl-2-thiouridine (**3**)[12], which exhibits a direct evidence of little contribution of thiol forms of **1** in solution. This work is on the line of our application of the MCD technique for clarifying spectroscopic assignment and, in turn, molecular structures of interesting heterocycles.

All measurements were carried out at room temperature using spectrograde methanol and acetonitrile as solvents. Circular dichroism (CD) and MCD spectra were measured with a JASCO J500C recording circular dichrometer equipped with a 1.25 T electromagnet for the MCD run. Absorption spectra were recorded on a JASCO UVIDEC 510 recording spectrophotometer. Molar ellipticities, $[\theta]$ and $[\theta]_M$, are expressed in $deg\ M^{-1}cm^{-1}$ and $deg\ M^{-1}cm^{-1}T^{-1}$, respectively. An *ab initio* MO calculation was carried out by the use of IMSPACK and an NEAC ACOS1000 computer at the computer center of Tohoku University.



1 R = H , X = H

2 R = H , X =


3 R = CH$_3$, X =


Absorption, CD and MCD spectra of **1** and **2** are shown in Fig. 1 and Fig. 2, respectively. In aqueous solution a single broad absorption band is observed in the wavenumber region 32000 ∿ 40000 cm^{-1} both for **1** and **2**. An additional shoulder appears at the longer wavelength side of absorption in methanol, which becomes intense on changing solvent from methanol to acetonitrile. The molecule **2** exhibits positive CD extrema in the wavenumber region of absorption at around 37000 cm^{-1}. A negative CD extreme at the lowest wavenumber region in each spectrum of **2** is considered to arise from the $n \rightarrow \pi^*$ transition within the thiouracil moiety.

On the other hand, both **1** and **2** give rise to two distinct MCD extrema, clearly indicating two electronic origins in the wavenumber region of interest. The MCD spectral profile of **1** looks very similar with that of **2** ; a sharp negative extreme at around 35000 cm^{-1} and a sharp positive peak at around 38000 cm^{-1}, which are almost equal in magnitude. The $n \rightarrow \pi^*$ transition is detected as a tail in the MCD spectrum of **2**, but not in the spectrum of **1**.

There seems to exist two possibilities to elucidate the observed MCD spectrum of **1** and **2**. One is based on an assumption that the negative and positive bands are ascribed to electronic transitions within the rigid framework of **1**a, the thioketone form of **1**. In this case, the two MCD extrema are reasonably considered to be due to the local $\pi \rightarrow \pi^*$ excitations in the thiourea and acrolein moieties. The other possibility lies in a consideration of the tautomeric equilibrium of 2-thiouracil in solution, such as **1**a $\rightleftharpoons$ **1**b[13].

A brief answer is illustrated in Fig. 3 which represents absorption, CD and MCD

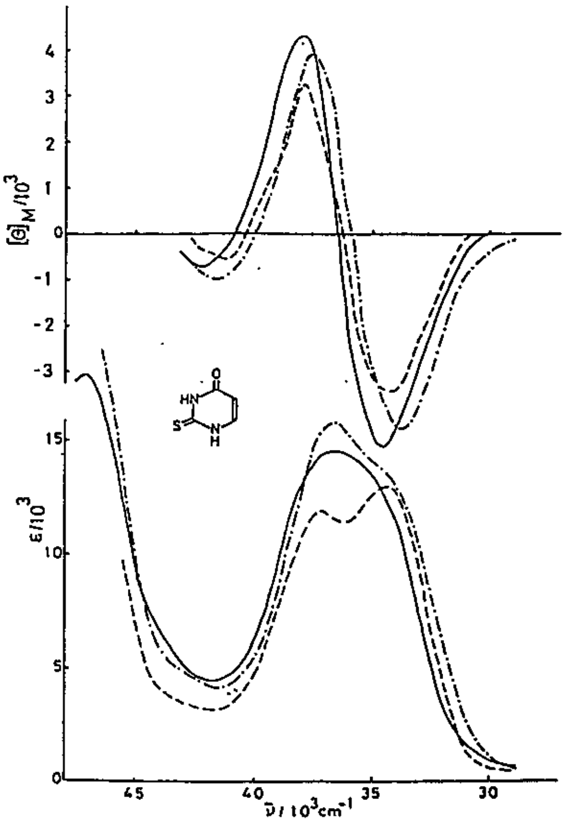


Fig. 1 Absorption (bottom) and MCD (top) spectra of 1 in H_2O (———), CH_3OH (-·-·-) and CH_3CN (-----) at room temperature.

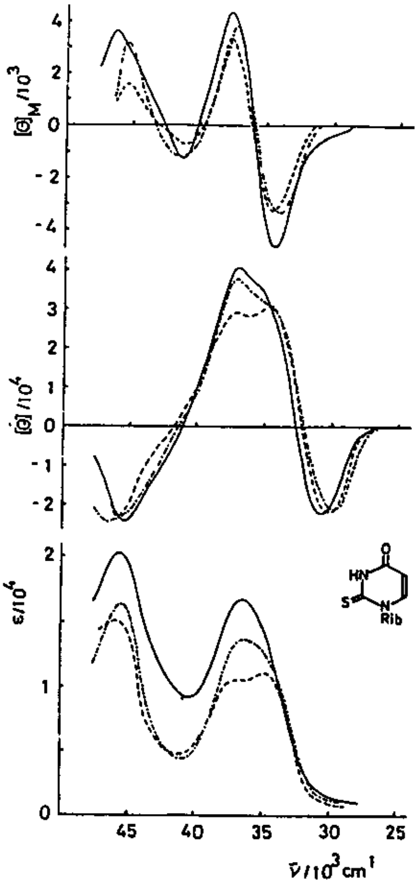


Fig. 2 Absorption (bottom), CD (middle) and MCD (top) spectra of 2 in H_2O (———), CH_3OH (-·-·-) and CH_3CN (-----) at room temperature.

spectra of 3 being an alternative of 1a. In the wavenumber region 32000 ∿ 40000cm^{-1} there is no appreciable difference between the absorption, CD and MCD spectra of 3 and those of 2 with respect to their intensities and sign. A comparison of Fig. 3 with Fig. 1 or Fig. 2 leads to a conclusion that the spectral profile of 1 is almost the same as those of 2 and 3 and, in turn, that there is little contribution of thiol tautomers to the observed absorption, CD and MCD spectra of 1 in solution, in agreement with the result from ^{13}C-NMR experiment[5]. The assumption that the $\pi \rightarrow \pi^*$ transitions in the local thiourea and acrolein chromophores give rise to two MCD extrema is partially justified from an *ab initio* MO calculation based on the STO-3G basis set. In carrying out calculation, the molecular geometry was taken from the x-ray diffraction data of 1[14].

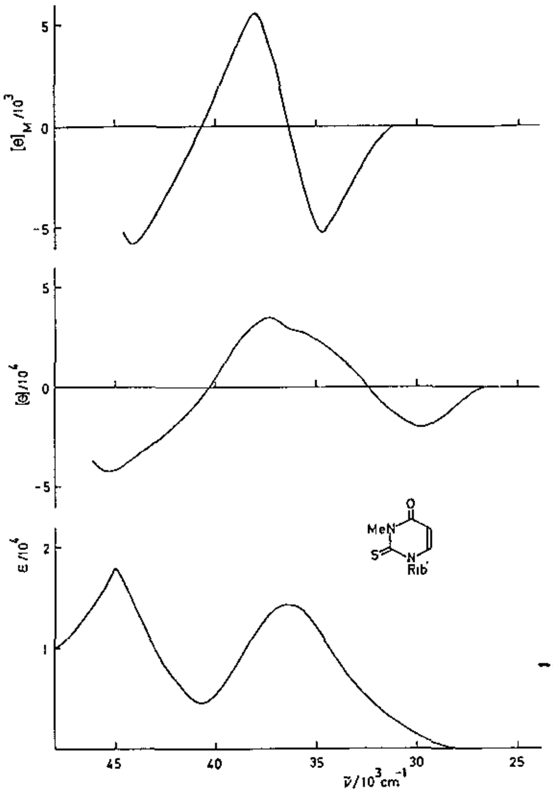
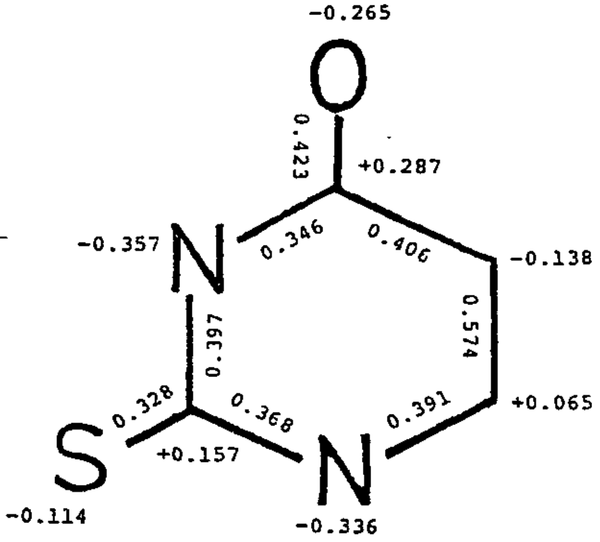


Fig. 3 Absorption (bottom), CD (middle) and MCD (top) spectra of 3 in CH_3OH at room temperature, where Rib' stands for the 2',3'-O-isopropylidene group.



1a

The π-MOs of 1a are illustrated in Fig. 4. The HOMO ϕ_{33} extends over the thiourea moiety, while the π-MO ϕ_{31} comprises a network over acrolein. The MO ϕ_{32} not represented in Fig. 4 is a lone pair orbital.

Aida and Nagata[15] have carried out *ab initio* MO calculations of 2-thioribosylthymine (s^2T) and ribosylthimine (T) using the 4-31G basis set. They have pointed out that the higher lying HOMO and the lower lying LUMO in s^2T play an important role in preferable stacking of the s^2T : G pair as compared with that of T : G pair. The present calculation puts the HOMO at -0.197 au (-5.34 eV) and the LUMO at 0.223 au (6.07 eV), while in s^2T the HOMO and LUMO lie at around -7.0 eV and 2.5 eV respectively. Alkyl substituents in s^2T lower the LUMO and HOMO of the 2-thiouracil skeleton in accordance with the result from a perturbation treatment for the interaction between 2-thiouracil and, for example, the methyl group.

It seems not meaningless to give a tentative spectroscopic assignment to the observed spectra of 1 using the SCF MOs calculated, which, however, should be further confirmed quantitatively by taking the electron correlation *among excited* states into account. As the MOs ϕ_{33} and ϕ_{31} bear the appreciable contribution from the thiourea and acrolein moieties, electronic transitions $\phi_{33} \rightarrow \phi_{34}$ and $\phi_{31} \rightarrow \phi_{34}$ are those characteristic of the constituent chromophores, and these two transitions are considered to be a plausible theoretical interpretation of the observed two MCD extrema in the wavenumber region 32000 ∿ 40000 cm^{-1}. Calculated net charges and π-bond-orders are represented in the molecular diagram above. Negative charge is calculated to be higher at N_3 than at N_1. The oxygen atom carries higher negative charge than the sulfur atom. The positive charge is calculated on C_6 in contrast to the negative charge on C_5, indicating a down-field shift of the H_6 proton in the ^{1}H-NMR spectrum. The H_6 proton is known to appear at lower field than the H_5 proton from ^{1}H-NMR experiments of 2-thiouracil and uracil, although the olefinic protons exhibit always a larger down-field shift in the former than in the latter. The present theoretical result is in agreement with NMR data.

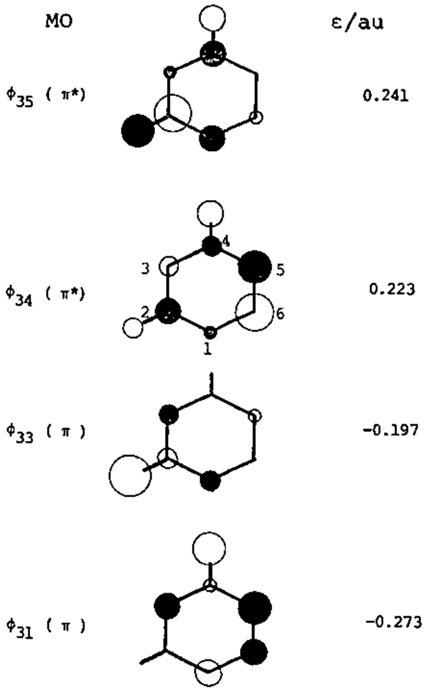


Fig. 4 π-MOs of 1a. Bonding π-MOs ϕ_{28}, ϕ_{27}, ϕ_{23} and anti-bonding π-MO ϕ_{36} are neglected.

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12. The 3-methyl derivative **3** was prepared by treatment of 2',3'-O-isopropylidene-2-thiouridine with diazomethane in methanol. The major product was the 2-methyl derivative, and **3** was obtained as a minor product. Rf values on TLC (Merck $60F_{254}$, $CHCl_3$: CH_3OH = 20 : 1) : isopropylidene-2-thiouridine 0.22, isopropylidene-2-methylthiouridine 0.13, and **3** 0.37. ^{1}H-NMR ($CDCl_3$) 1.38 and 1.62 (s, 3H, 3H, isopropylidene), 2.10 (br. t, 1H, OH), 3.73 (s, 3H, N^3-CH_3), 4.0 (m, 2H, H-5'), 4.30 (m, 1H, H-4'), 4.65 (d,d, J = 6.4, 2.2, 1H, H-2'), 4.83 (d,d, J = 6.4, 6.0, 1H, H-3'), 6.01 (d, J = 8.0, 1H, H-5), 7.00 (d, J = 2.2, 1H, H-1'), 8.03 (d, J = 8.0, 1H, H-6).

13. The form **1**c is excluded because **1** gives a similar spectrum with that of **2** where the tautomeric form **2**c is absent.

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