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Optical Properties of Thiopyrimidine Nucleosides[1]

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The ORD, CD and UV of various 2-thiopyrimidine nucleosides have been measured and a tentative rule pertaining to the sign of ORD is presented. A nucleoside possessing a non-conjugated 2-thiocarbonyl group exhibits a negative Cotton effect at 300 to 360 mμ in the n–π^* region, while the nucleoside with a conjugated 2-thiocarbonyl function exhibits a positive Cotton effect. Protonation or deprotonation which alters the structure of the base moiety effects the sign of ORD.

The possible usefulness of ORD measurements for the detection and identification of the thiopyrimidine nucleosides in transfer ribonucleic acids is discussed.

For several years we have been interested in the synthesis and chemistry of thio-derivatives of pyrimidine nucleosides.[3–7] It has already been pointed out[7] that the replacement of the carbonyl function in pyrimidine nucleosides with a thiono function affects the reactivity of pyrimidine moieties in various reactions, such as alkylation reactions, and in some cases also affects the tautomeric ratios as compared with the oxonucleosides. Since the biological activities of nucleic acids (and possibly nucleotides and nucleosides as well) are expressed mainly through the specific hydrogen bond formation between purine and pyrimidine bases utilizing cyclic amido-function in one base and cyclic amidino-function in the other, the transformation of the amido- to a thioamido-function in the base should affect their biological activities.

This paper is mainly concerned with the optical rotatory dispersion (ORD) studies, in addition to circular dichroism (CD) and ultraviolet (UV) absorption studies of several thionucleosides; the relation of these optical properties with the tautomerism and dissociation of the thiopyrimidine nucleosides are discussed.

It has been reported by Ulbricht and coworkers[8] that, (a) the sign of the first Cotton effect in ORD is a reflection of the anomeric configuration of the glycosylic linkage: (b) the orientation of the base moiety to the sugar residue (*i.e.* syn- or anti-form) is also the determinant of the sign and magnitude of the Cotton effect. Therefore it follows that[8d] the natural pyrimidine nucleosides, for example uridine (1-β-D-ribofuranosyluracil, I), in solution exhibits a positive Cotton effect since the compound has a *beta* configuration and exists in

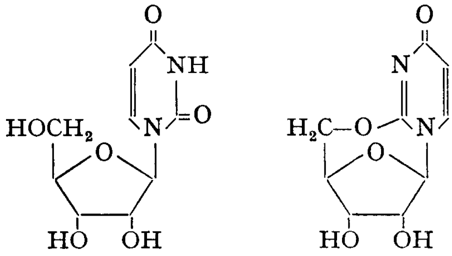
I II

Chart 1

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[1] Presented at the Meeting of Hokkaido Branch, Pharmaceutical Society of Japan, Sapporo, Feb. 1968.

[2] Location: *Kita-12, Nishi-6, Sapporo.*

[3] M. Ikahara, T. Ueda, and K. Ikeda, *Chem. Pharm. Bull.* (Tokyo), **10**, 767 (1962).

[4] T. Ueda and J.J. Fox, *J. Med. Chem.*, **6**, 679 (1963).

[5] T. Ueda, Y. Iida, K. Ikeda, and Y. Mizuno, *Chem. Pharm. Bull.* (Tokyo), **14**, 666 (1966).

[6] T. Ueda and H. Nishino, *J. Am. Chem. Soc.*, **90**, 1678 (1968).

[7] T. Ueda, Y. Iida, K. Ikeda, and Y. Mizuno, *Chem. Pharm. Bull.* (Tokyo), **16**, 1788 (1968).

[8] *a*) T.L.V. Ulbricht, J.P. Jennings, P.M. Scopes, and W. Klyne, *Tetrahedron Letters*, **1964**, 695; *b*) T.L.V. Ulbricht, T.R. Emerson, and R.J. Swan, *Biochim. Biophys. Res. Comm.*, **19**, 643 (1965); *c*) T.L.V. Ulbricht, T.R. Emerson, and R.J. Swan, *Tetrahedron Letters*, **1966**, 1561; *d*) T.R. Emerson, R.J. Swan, and T.L.V. Ulbricht, *Biochemistry*, **6**, 843 (1967).

the anti-form. Related compounds which possess the *syn*-conformation, for example O^2,5'-cyclouridine (II), has a negative Cotton effect. Some exceptions were reported and explained by the differences in the orientation (or polarization) of the planar base to the ribofuranosyl ring.[8d]

As described later it has now been observed that 2-thionopyrimidine nucleosides exhibit a Cotton effect specific for the 2-thiocarbonyl function in addition to the normal Cotton effect due to the main absorption.

Experimental Method

ORD and CD curves were measured by JASCO Model ORD/UV-5 Spectropolarimeter at room temperature using 10 mm cell or 20 mm cell depending upon the concentration of the sample. The measurements were repeated several times if necessary, in which case the curves given indicating average values. UV absorption spectra were measured by Hitachi ESP-3T recording spectrophotometer and most of the data are reported in the previous paper.[7]

2,4-Dithiouridine,[5,7] N^4-methyl- and N^4-dimethyl-2-thiocytidine,[7] and 2-thiocytidine[5,6,7] were prepared in this laboratory.

2-Thiouridine——This was prepared by the reported method[9] from O^2,5'-cyclo-2',3'-O-isopropylideneuridine *via* 2',3'-O-isopropylidene-2-thiouridine which was prepared by the modified method described below: O^2,5'-cyclo-2',3'-O-isopropylideneuridine,[9] 400 mg, was dissolved in 37 ml of pyridine and hydrogen sulfide was absorbed until its saturation under cooling with dry ice-acetone and set aside for 4 days at room temperature in a sealed tube. After evaporation of pyridine under reduced pressure with several co-distillation with EtOH, the residue was treated with MeOH to afford crystals of 5'-deoxy-5,6-dihydro-5',6-epithio-2',3'-O-isopropylideneuridine (107 mg). The mother liquor was concentrated and taken up in a boiling $CHCl_3$ from which crystals of 2',3'-O-isopropylidene-2-thiouridine (191 mg, 41%) were separated. More crystals can be obtained from the mother liquor.

2-Methylthiouridine——2-Thiouridine, 35 mg, was dissolved in 10 ml of 50% EtOH and to which was added CH_3I (0.012 ml) and triethylamine (0.025 ml) and stirred for one hr. On evaporation and twice co-evaporation with EtOH crystals were obtained (25 mg, 65%), mp 171—173° (decomp.), *Anal.* Calcd. for $C_{10}H_{14}O_5N_2S$: C, 43.78; H, 5.11; N, 10.10. Found: C, 43.61; H, 5.18; N, 9.91. UV $\lambda_{max}^{H_2O}$, mμ (ε): 236.5 (26500), 265 shoulder (9000). Treatment of 2-methylthiouridine with 1.0N HCl afforded uridine.

Results and Discussions

The ORD, CD and UV curves of the compounds in neutral and ionic species are given in Fig. 1 to 6 and extrema and amplitude of ORD are given in Table I. As can be seen in Fig. 1 to 6 compounds having 2-thiono function exhibit the first Cotton effect of either positive or negative sign at longer wavelength region compared with that of the normal pyrimidine nucleosides where the extinction coefficient of that region is quite low in each case. The nature of these Cotton effects can be explained by the presence of the 2-thiocarbonyl group which is non-conjugated or conjugated with the N^3-C^4 double bond as will be described in detail.

2-Thiouridine (Fig. 1)

As a neutral species 2-thiouridine (III) exhibits a strong positive Cotton effect at 303 mμ (cross-over point at 283 mμ) which is comparable to that of uridine and an additional negative Cotton effect at 343 mμ (Fig. 1a). The CD curve shows two positive components at the main absorption region, the additional CD curve being located at a wavelength region (320 mμ) where the UV absorption is almost negligible. Since 2-thiouridine is known to exist in the 2-thiono-4-keto form in neutral solution[10,11] the additional negative CD (and hence negative ORD) must be derived from an $n-\pi^*$ transition of the 2-thiono function of (III). In general,

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[9] D.M. Brown, D.B. Parihar, Sir A. Todd, and S. Varadarajan, *J. Chem. Soc.*, **1958**, 3028.

[10] D. Shugar and J.J. Fox, *Bull. Soc. Chim. Belg.*, **61**, 293 (1952).

[11] M. Sano, *Chem. Pharm. Bull.* (Tokyo), **10**, 320 (1962).

Table I. Optical Rotatory Dispersion of Thiopyrimidine Nucleosides

Compound	Solvent	Extremum		Amplitude
		$[\phi]\times 10^3$	λ (mμ)	
2-Thiouridine	H_2O	−10.3	348	−272
		+16.9	303	
		−36.6	250	
	1 N NaOH	+15.4	290	+472
		−31.8	254	
2,4-Dithiouridine	EtOH	−9.1	380	−240
		+14.9	344	
		+7.6	313	
		+18.1	290	
		−22.9	260	
	1 N NaOH	+7.8	381	+140
		−6.2	338	
		+1.5	317	
		−2.4	210	
		+18.1	293	
		−21.4	257.5	
2-Thiocytidine	EtOH	+15.3	291	+445
		−29.2	258	
	1 N HCl	−5.7	360	−332
		+27.4	321	
		+20.5	297	
		+21.4	289	
		−25.2	250	
N^4-Methyl-2-thiocytidine	H_2O	+18.6	300	+408
		−22.2	258	
	1 N HCl	−4.4	362	−309
		+26.5	325	
		+13.8	294	
		+17.7	285	
		−30.2	253	
N^4-Dimethyl-2-thiocytidine	H_2O	+7.7	309	+65
		+1.2	295	
		+1.7	286	
		−17.2	260	
		−14.9	245	
		−16.2	231	
	1 N HCl	−5.7	370	−297
		+24.0	330	
		+8.7	298	
		+11.1	288.5	
		−26.2	258	
2-Methylthiouridine	H_2O	+7.0	278	+243
		−17.3	250	
4-Methylthiouridine	H_2O	+15.5	323	+271
		−11.6	282	
		+7.0	254	
		+8.8	238	

substituted thioamide derivatives absorb at 300—400 mμ region with a low extinction coefficient due to the n—π^* transiton of the thiocarbonyl group.[12]

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[12] C. Djerassi, K. Undheim, R.C. Sheppard, W.G. Terry, and B. Sjoberg, *Acta Chem. Scand.*, **15**, 903 (1961).

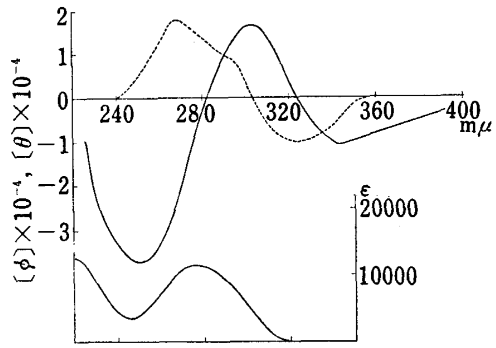


Fig. 1a. 2-Thiouridine in H_2O

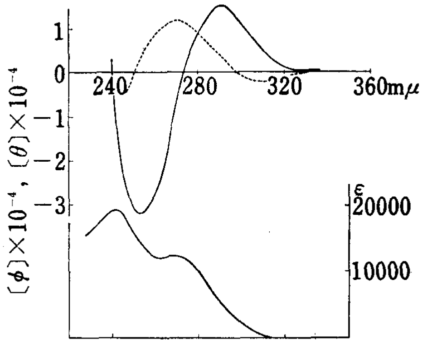
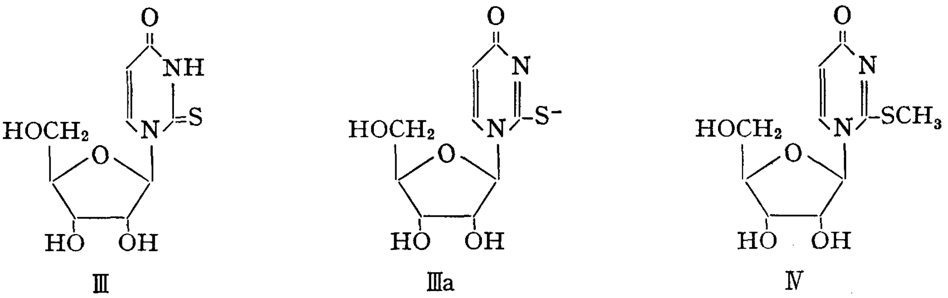


Fig. 1b. 2-Thiouridine in 1N NaOH



III IIIa IV

Chart 2

In alkaline solution the extra negative Cotton effect disappeared and 2-thiouridine showed the usual ORD pattern (Fig. 1b) found in pyrimidine nucleosides. This phenomenon can be correlated with the dissociation of III to IIIa, as a predominant mesomeric form, resulting in the disappearance of the 2-thiocarbonyl group. It is to be noted that the UV and ORD curves of 2-thiouridine anion (IIIa) closely resembles those of 2-methylthiouridine (IV, Fig. 2), which was prepared by the methylation of III in the presence of alkali and methyl iodide.

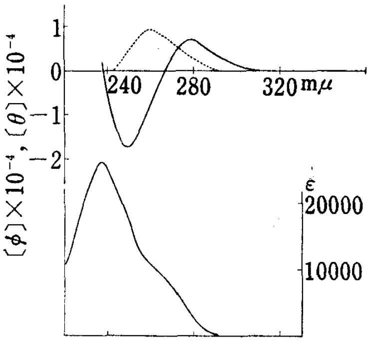
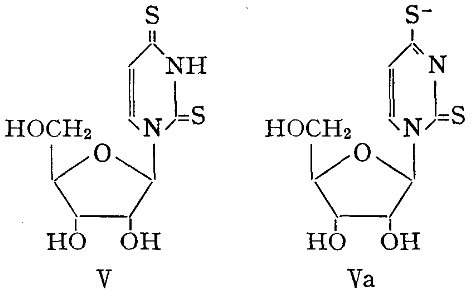


Fig. 2. 2-Methylthiouridine in H_2O



V Va

Chart 3

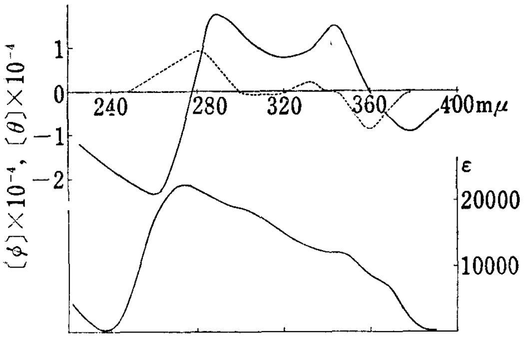


Fig. 3a. 2,4-Dithiouridine in EtOH

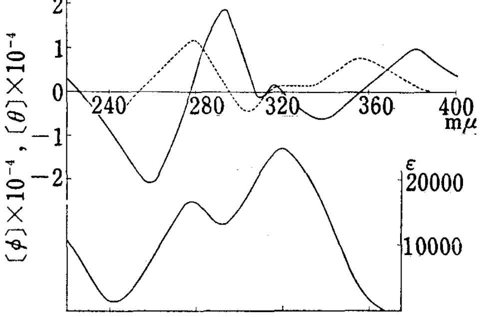


Fig. 3b. 2,4-Dithiouridine in 1N NaOH

There is a weak negative CD at 310 mμ, the effect of which was apparently cancelled by the second strong positive CD at 270 mμ in 2-thiouridine anion (IIIa). This phenomenon remains unexplained in the present stage.

2,4-Dithiouridine (Fig. 3)

In neutral species 2,4-dithiouridine has been known to exist in the 2,4-dithiono form V by UV spectral studies.[7] Although the CD curve shows some irregularities in the 300—340 mμ region, the main CD curve consists of two Cotton effects, one positive (280 mμ) and the other negative (360 mμ). The latter CD can be attributed to the UV of the 2-thiocarbonyl group. In alkali, in which V exists in the anionic form (Fig. 3b), the CD at 280 mμ region remained unchanged and in fact it is almost superimposable on that of V in ethanol (Fig. 3a). However, the sign of CD (and ORD) at 360 mμ is completely reversed. In this case the UV spectra clearly shows that the UV absorption around 360 mμ is very weak, thus suggesting the $n-\pi^*$ transition of 2-thiocarbonyl function. The methylation of V with methyl iodide in alkali gives 4-methylthio-2-thiouridine,[7] and therefore the anionic species of V can be described as Va among other canonical structures.

2-Thiocytidines (Fig. 4—6)

2-Thiocytidine (VI: $R_1=R_2=H$) and N^4-methyl-2-thiocytidine (VI: $R_1=H$, $R_2=CH_3$) behave similarly in neutral and protonated forms in terms of the ORD and CD curves (Fig. 4 and 5). The first positive Cotton effect is obviously not simple and a small but distinct negative CD curve was observed in each case. N^4-Dimethyl-2-thiocytidine (VI: $R_1=R_2=CH_3$), however, shows a rather different pattern in the ORD curve compared to the former two and this apparent irregular curve can be explained by the presence of two appropriately close (or separate, compared to the overlapping CD at 275 mμ of 2-thiocytidine and N^4-methyl-2-thiocytidine, Fig. 4a and 5a) CD curve, thus resulting in the cancellation of ORD in the area of 280 to 295 mμ (Fig. 6a). Since compound (VI: $R_1=R_2=CH_3$) is fixed in the 2-thiono-4-

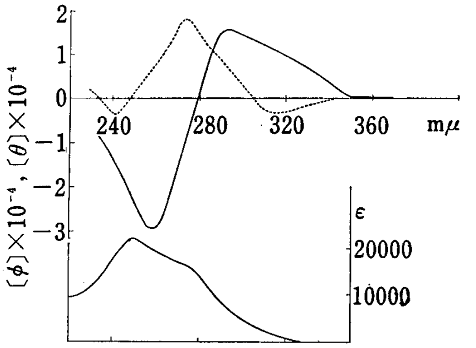


Fig. 4a. 2-Thiocytidine in EtOH

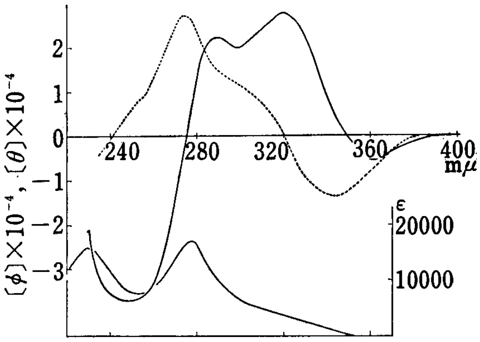


Fig. 4b. 2-Thiocytidine in 1N HCl

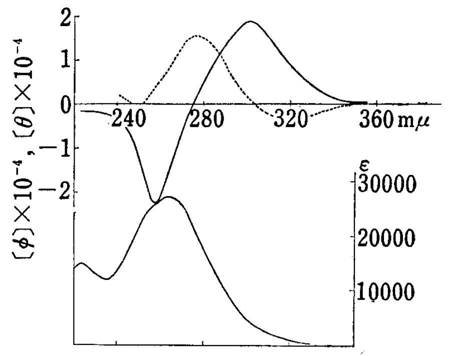


Fig. 5a. N^4-Methyl-2-thiocytidine in H_2O

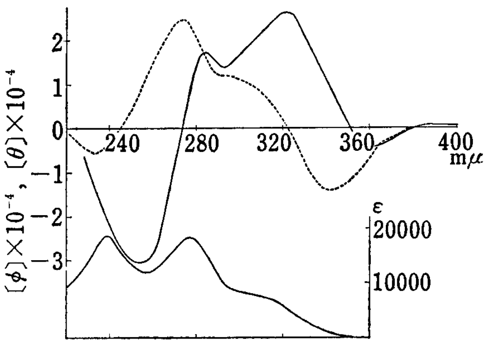


Fig. 5b. N^4-Methyl-2-thiocytidine in 1N HCl

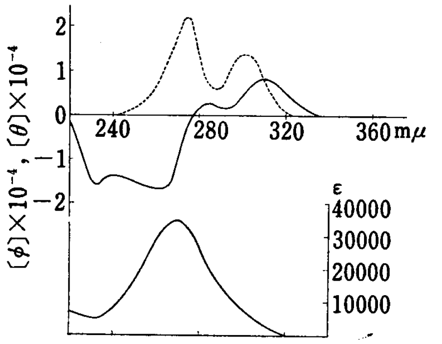


Fig. 6a. N^4-Dimethyl-2-thiocytidine in H_2O

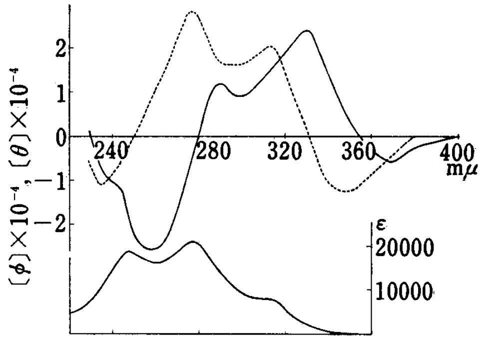
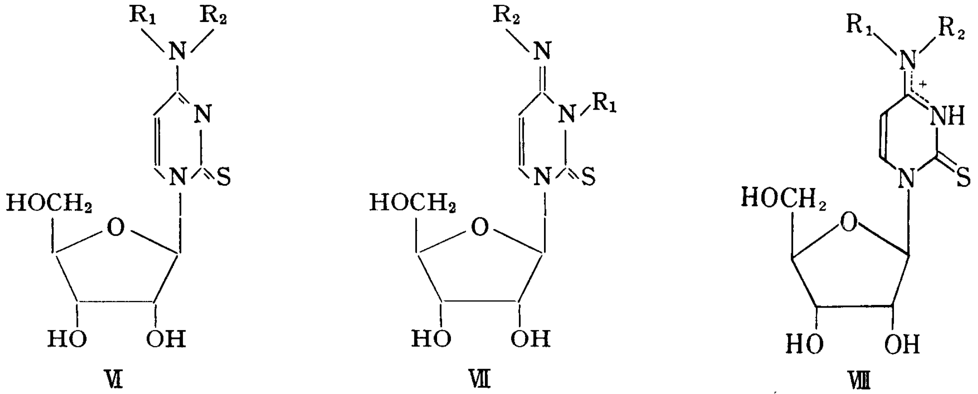


Fig. 6b. N^4-Dimethyl-2-thiocytidine in 1N HCl

amino form, and the UV absorptions where the first positive CD appear is relatively low, the CD in VI: $R_1=R_2=CH_3$ can be assigned to the $n-\pi^*$ transition of the conjugated 2-thiocarbonyl group. The presence of a weak negative CD curve in VI: $R_1=R_2=H$ and VI: $R_1=H$, $R_2=CH_3$ may be the reflection of the minor contribution of the tautomeric structure (VII) in addition to VI. This has been pointed out earlier from UV spectral comparisons in the 2-thiocytidine series.[7] The UV, CD and ORD curves of the protonated form of the three compounds are all similar, now exhibiting a negative Cotton effect at 360 mμ (Fig. 4b, 5b, and 6b). This can be assigned to the 2-thiocarbonyl group in the protonated species such as depicted in structure (VIII).

General Considerations

From the above results some general conclusions can be tentatively drawn: the orientation of the base moiety to the sugar ring in 2-thiopyrimidine nucleosides should be all in the anti form irrespective of neutral, protonated or deprotonated species since the ORD derived from the major absorption region are all positive, this being in accordance with that reported by Ulbricht and co-workers.[8] Secondly, the ORD appearing above 300 mμ can be assigned to the $n-\pi^*$ transition of the 2-thiocarbonyl function, and these are further divided into positive and negative Cotton effects. The thiocarbonyl group not conjugated with the N^3–C^4 double bond tends to exhibit a negative Cotton effect while the conjugated group exhibits a positive Cotton effect. The reversal of the sign (*i.e.* VI to VIII and V to Va) or disappearance of the Cotton effect (III to IIIa) at this absorption region by protonation or deprotonation can also be explained by the presence or absence of the thiocarbonyl group which conjugated or non-conjugated.



VI VII VIII

Chart 4

It is to be noted that among the natural nucleosides, protonation or deprotonation do not effect the sign of ORD.[8] Only two cases have been reported so far with respect to reversal of the sign, *i.e.* N^3,5'-cycloguanosine and N^3,5'-cycloinosine.[13,14] Therefore it is worthy to emphasize that the reversal of the sign of ORD in this particular series is explained in terms of the tautomerism and degree of the contribution of the resonant ionic species: accordingly ORD measurements as well as UV measurements afford effective methods for studying the structure and tautomerism of such heterocyclic compounds.

The ORD of other sulfur containing pyrimidine nucleosides, namely 4-thiouridine has been reported recently.[15] 4-Thiono group apparently has no effect on the assymmetric center of the nucleoside. 4-Methylthiouridine[4] also exhibits a normal positive Cotton effect (Table I), the pattern and amplitude being comparable to those of 4-thiouridine disulfide reported.[15] The ORD measurements may have some advantages for the detection of 2-thiopyrimidine nucleosides which are now known to be present in some transfer ribonucleic acids.[16-19] The main UV absorption of 2-thiouridine or 2-thiocytidine falls into the usual absorption region of major nucleosides, 260—280 mμ, thus rendering UV measurements ineffective for their detection in the mixture. The ORD of the 2-thiopyrimidine nucleosides are relatively isolated from those of the usual nucleosides having a stronger amplitude, thus enabling the minor components to be detected easily.

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19) The presence of 2-thiocytidine has been observed recently: private communication from Dr. S.Nishimura and Dr. A. Carbon.