[Chem. Pharm. Bull. 22(4) 930—937 (1974)]

UDC 547.854.4.04 : 547.963.32.04

Nucleosides and Nucleotides. IX. Synthesis of Sulfur-bridged Cyclonucleosides: (S)-2,2′-, 2,3′-, and 2,5′-Cyclo-2-thiouridines[1]

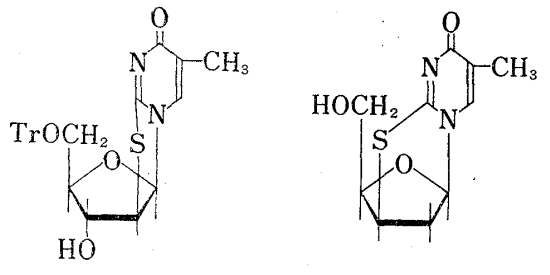
Tohru Ueda and Susumu Shibuya

Faculty of Pharmaceutical Sciences, Hokkaido University[2]

(Received September 14, 1973)

Three sulfur-bridged cyclonucleosides, (S)-2,2′-, 2,3′- and 2,5′-cyclo-2-thiouridines (VII, XV, and XIV) have been synthesized. The present synthetic method involves the use of uridine as the starting material through 2-thiouridines as the intermediates. The ultraviolet, optical rotatory dispersion (ORD), circular dichroism (CD), and nuclear magnetic resonance data of these (S)-cyclo-2-thiouridines are presented. (S)-2,5′-Cyclonucleoside (XIV) was thought to be present as an "endo" conformation in terms of bridging sulfur. The limitation of the applicability of Ulbricht's rule on the correlation of the sign of ORD (CD) and torsion angle around the glycosidic bond was discussed.

In the field of synthetic chemistry of nucleosides cyclo(or anhydro)nucleosides occupy an unique situation in that both glycosyl and aglycone moieties are involved in the reaction. Therefore the cyclonucleosides play a key role as the intermediate for the derivatization of base and/or glycosyl moiety. For instance, O^2, 2′-cyclouridine[3] was transformed into such various nucleosides as arabinofuranosyluracil,[3] 2′-deoxy-2′-halogenouridines,[4] 3′-deoxy-3′-ethylthio-xylofuranosyluracil,[5] arabinofuranosylisocytosine,[5] arabinofuranosyl-2-thiouracil,[6] 2′-deoxy-2′-mercaptouridine,[7] and 2′-amino-2′-deoxyuridine[8] by the alkyl-O or aryl-O bond fission of the cyclo-linkage with nucleophiles.



Tr=trityl

Chart 1

Most of the cyclonucleosides so far studied have oxygen as the bridge atom. It can be also expected that the cyclonucleosides possessing sulfur or nitrogen as the bridge atom will serve as the interesting starting material for various bridge-opening reactions. This report describes the synthesis of sulfur-bridged cyclonucleosides derived from uridine. Preliminary account of some of the results has appeared.[9] As to the sulfur-bridged pyrimidine cyclonucleosides two compounds have been prepared. Shaw and Warrener reported[10] the synthesis of a (S)-2,2′-cyclonucleoside (I) derived from 1-β-D-ribofuranosyl-2-thiothymine, as an intermediate leading to the synthesis of thymidine. More recently Wempen and Fox prepared[11] (S)-2,3′-cyclo-2-thiothymidine (II) starting from a 2,3′-cyclothymidine.

1) Part VIII: K. Miura, M. Shiga, and T. Ueda, *Chem. Pharm. Bull.* (Tokyo), **21**, 1613 (1973).
2) Location: *Kita-12, Nishi-6, Kita-ku, Sapporo.*
3) D.M. Brown, A.R. Todd, and S. Varadarajan, *J, Chem. Soc.*, **1956**, 2388.
4) J.F. Codington, I.L. Doerr, and J.J. Fox, *J. Org. Chem.*, **29**, 558, 564 (1964).
5) D.M. Brown, D.B. Parihar, A.R. Todd, and S. Varadarajan, *J. Chem. Soc.*, **1958**, 3028.
6) *a*) T. Sekiya and T. Ukita, *Chem. Pharm. Bull.* (Tokyo), **15**, 1497 (1967); *b*) W.V. Ruyle and T.Y. Shen, *J. Med. Chem.*, **10**, 331 (1967).
7) M. Imazawa, T. Ueda, and T. Ukita, *Tetrahedron Letters*, **1970**, 4807.
8) J.P.H. Verheyden, D. Wagner, and J.G. Moffatt, *J. Org. Chem.*, **36**, 250 (1971).
9) T. Ueda and S. Shibuya, *Chem. Pharm. Bull.* (Tokyo), **18**, 1076 (1970).
10) G. Shaw and R.N. Warrener, *J. Chem. Soc.*, **1959**, 50.
11) I. Wempen and J.J. Fox, *J. Org. Chem.*, **34**, 1021 (1969).

Two main routes can be designed for the synthesis of (S)-cyclonucleosides. One is the synthesis of a compound containing properly oriented SH group in a sugar portion with appropriate leaving group on C-2 of the pyrimidine portion. The other is the reversed case of the arrangement. We adopted the latter path since the properly oriented SH group in a sugar portion tends to add to the 5,6-double bond of the pyrimidine moiety as reported in the case of 5′-deoxy-5′-mercaptouridines by Bannister and Kagan[12] and others.[13,14]

We have chosen a O²,2′-cyclouridine as a starting material for the synthesis of (S)-2,2′-cyclo-2-thiouridine((S)-2,2′-cyclo-1-(2-deoxy-β-D-arabinofuranosyl)-2-thiouracil).

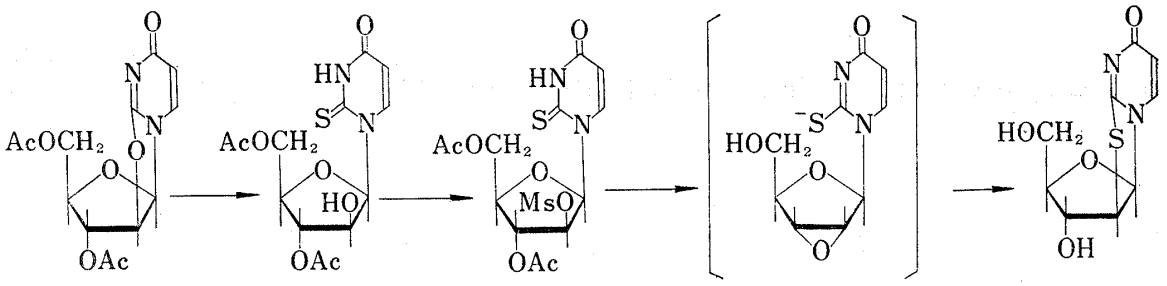


Chart 2

Treatment of 3′,5′-di-O-acetyl-O²,2′-cyclouridine (III)[15] with saturated hydrogen sulfide in pyridine at 70° afforded 3′,5′-di-O-acetyl-1-β-D-arabinofuranosyl-2-thiouracil (IV), in high yield, without the loss of acetyl groups. Deacetylation of IV gave known arabinofuranosyl-2-thiouracil.[6] Compound IV was then mesylated to give the 2′-O-mesylate (V) which was treated with sodium methoxide in methanol. The reaction proceeded rapidly (completed within one hour) to yield (S)-2,2′-cyclo-2-thiouridine (VII) in almost quantitative yield. During the course of the reaction two spots other than V and VII on thin-layer chromatography appeared whose ultraviolet (UV) spectra are those of 2-thiouridines. Therefore the reaction should have proceeded by the initial deacetylation and the attack of 3′-hydroxyl to the neighboring 2′-carbon to form an epoxide (VI) which was rapidly cleaved by the attack of thiolate ion on C-2 of the pyrimidine portion to furnish VII. Similar sequence of the reaction has been advanced in the formation of a purine(S)-8,2′-cyclo-nucleoside[16] starting from a xylofuranosylpurine. The structure of VII was confirmed by spectral analysis. The UV spectra of VII were closely resembled to those of 1,2-dialkyl-2-thiouracil,[17] especially of 2-methylthiouridine.[18] The nuclear magnetic resonance (NMR) spectra are in good agreement with the structure VII (see Table I). The signal of H-2′ was shifted to higher magnetic field by —1 ppm as compared with that of O²,2′-cyclouridine,[19] due to the lower electronegativity of the bridged sulfur atom than that of oxygen. The preferred formation of 2,2′-cyclo over 2,3′-cyclonucleoside(such as VII from VI) is well documented in accumulated examples.[20] Some chemical evidences for the structure of VII are as follows: treatment of VII with Ni catalyst afforded a compound (VIII), exhibiting UV spectra closely resembled

12) B. Bannister and F. Kagan, *J. Am. Chem. Soc.*, **82**, 3363 (1960).
13) R.W. Chambers and V. Kurkov, *J. Am. Chem. Soc.*, **85**, 2160 (1963).
14) E.J. Reist, A. Benitz, and L. Goodman, *J. Org. Chem.*, **29**, 554 (1964).
15) Y. Furukawa and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), **16**, 2286 (1968).
16) M. Ikehara and H. Tada, *J. Am. Chem. Soc.*, **87**, 606 (1965).
17) D. Shugar and J.J. Fox, *Bull. Soc. Chim. Belg.*, **61**, 293 (1952).
18) T. Ueda and H. Nishino, *Chem. Pharm. Bull.* (Tokyo), **17**, 920 (1969).
19) M. Honjo, Y. Furukawa, M. Nishikawa, K. Kamiya, and Y. Yoshioka, *Chem. Pharm. Bull.* (Tokyo), **15**, 1076 (1967).
20) For a review of the chemistry of cyclonucleosides see: J.J. Fox and I. Wempen, "Pure and Applied Chemistry," Vol. 18, Butterworth, London, 1969, p. 223.

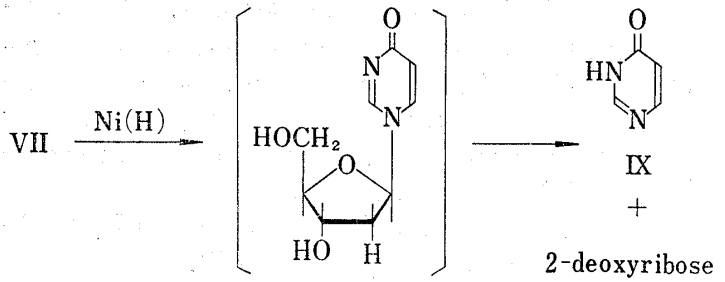


Chart 3

to those of 1-methyl-4-pyrimidinone,[21] and successive acid hydrolysis of VIII gave 4-pyrimidinone (IX) and 2-deoxyribose. Compound VII was fairly stable in acidic solution but labile in alkaline solution in which it yielded a uridine type compound suggestive of aryl-S bond cleavage in VII.[22] Another approach to the synthesis of VII has been accomplished more recently in our hands,[23] in which the treatment of 2-thiouridine with diphenyl carbonate was involved.[24]

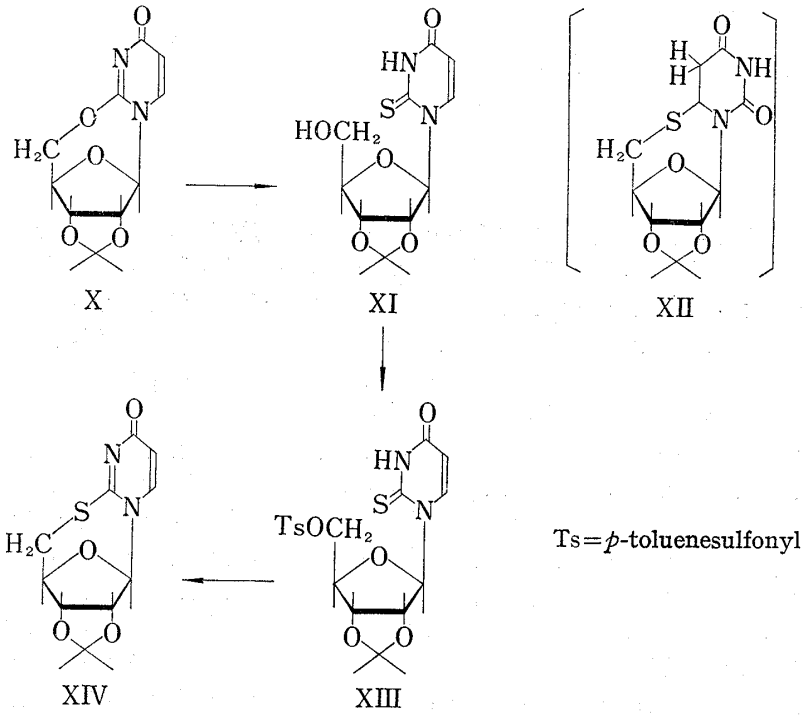


Chart 4

The reaction sequences selected in the synthesis of (S)-2,5′-cyclo derivative was similar to those of the synthesis of O²,5′-cyclouridine derivative,[25] except the starting nucleoside. The main problem, therefore, seemed to establish the efficient synthesis of starting nucleoside, 2′,3′-O-isopropylidene-2-thiouridine. It has been reported that the cleavage of the cyclo-linkage of 2′,3′-O-isopropylidene-O²,5′-cyclouridine (X) with hydrogen sulfide and triethylamine in dimethylformamide afforded 2′,3′-O-isopropylidene-2-thiouridine (XI) along with a 5′-deoxy-5′-mercaptouridine (which was isolated as a 6,5′-epithio form (XII)).[5,13] The treatment of X with saturated hydrogen sulfide in pyridine improved the yield of XI in some

21) D.J. Brown, E. Hoeger, and S.F. Mason, *J. Chem. Soc.*, **1955**, 211.
22) Formation of 2′-deoxy-2′-mercapto-arabinofuranosyluracil, in an 6,2′-epithio form, was recently confirmed: M. Imazawa and T. Ueda, to be published.
23) T. Ueda and H. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **18**, 1491 (1970).
24) A. Hampton and A.W. Nichol, *Biochemistry*, **5**, 2076 (1966).
25) *a*) D.M. Brown, A.R. Todd, and S. Varadarajan, *J. Chem. Soc.*, **1957**, 868; *b*) J. Nagyváry, *Biochemistry*, **5**, 1316 (1966).

extent.[18,26] We have found that the treatment of X with large excess of liquid hydrogen sulfide in pyridine (1: 1, by volume) in a sealed tube at room temperature for 4 days resulted in almost exclusive aryl-O fission to give XI in a excellent yield, which made the subsequent isolation procedure quite easier. It is to be noted here that the reaction of X with sodium ethylmercaptide gave 5′-deoxy-5′-ethylthiouridine derivative exclusively by the alkyl-O fission.[5]

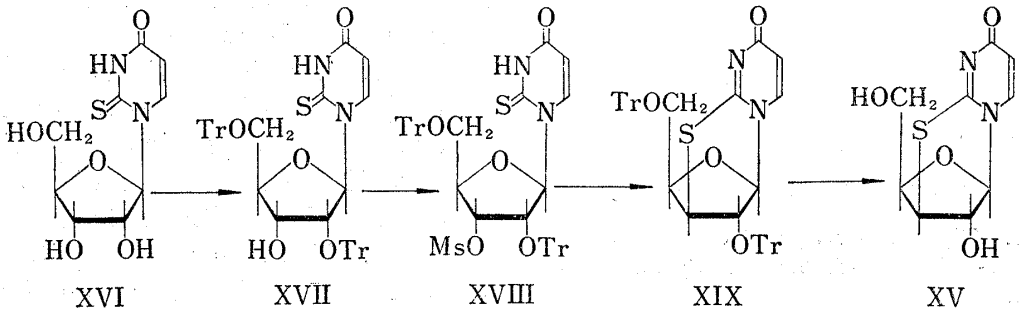
Treatment of XI with *p*-toluenesulfonyl chloride in pyridine afforded the 5′-O-tosylate (XIII). Compound XIII was rapidly cyclized to (S)-2,5′-cyclo-2′,3′-O-isopropylidene-2-thiouridine (XIV) by treatment with triethylamine in dioxane. This high reactivity is the reflection of strong nucleophilic nature of 2-thiono group in XIII. The NMR spectra of XIV are well agreeable with the 2,5′-cyclo structure (Table I). The signals of H-5′ protons of

Table I. The NMR Data (δ) of Sulfur-bridged 2-Thiouridine Cyclonucleosides Taken at 60 MHz in d_6-DMSO

Proton (s) of	Compound VII			Compound XV			Compound XIV		
	ppm	J (Hz)		ppm	J (Hz)		ppm	J (Hz)	
C-6	7.82 d[a]	$J_{5,6}$	8	7.76	$J_{5,6}$	8	8.05 d	$J_{5,6}$	8
C-5	5.88 d	$J_{5,6}$	8	5.80 d	$J_{5,6}$	8	5.92 d	$J_{5,6}$	8
C-1′	6.38 bd	$J_{1',2'}$	7	5.44 s			5.83 s		
C-2′	4.28 m			4.85 s			5.34 d	$J_{2',3'}$	6
C-3′	4.34 m	$J_{3',4'}$	3	3.70 d	$J_{3',4'}$	3	4.95 d	$J_{2',3'}$	6
C-4′	3.98 pd	$J_{3',4'}$	3	4.56 dt	$J_{3',4'}$	3	4.92 q	$J_{4',5'a}$	2
		$J_{4',5'}$	5		$J_{4',5'}$	6		$J_{4',5'b}$	4
C-5′	3.44 d	$J_{4',5'}$	5	3.78 d	$J_{4',5'}$	6	3.50 q(Ha)	J_{ab}	14
							3.16 q(Hb)	$J_{4',a}$	2
								$J_{4',b}$	4
Methyl (isopropylidene)							1.46 s		
							1.32 s		

a) Following abbreviations were used for splittings; d, doublet; t, triplet; q, quartet; m, multiplet; s, singlet.

XIV are located at higher magnetic field by —1 ppm as compared with those of X.[27] From the coupling constants between H-4′ and H-5′ a, b (2 and 4 Hz, respectively) it can be assumed that the conformation of C-4′ and C-5′ substituents in XIV is staggered, or "endo", rather than eclipsed, or "exo". This point will be discussed in a later part of this paper.



XVI XVII XVIII XIX XV

Tr=trityl Ms=methanesulfonyl

Chart 5

26) K.K. Kotchetkov, E.I. Budowsky, and V.N. Shibaev, "Synthetic Procedures in Nucleic Acid Chemistry," Vol. 1 (W.W. Zorbach and R.S. Tipson eds.), Interscience Publishers, New York, 1968, p. 500.
27) J. Zemlicka and F. Sorm, *Collection Czech. Chem. Commun.*, 32, 576 (1967).

For the synthesis of (S)-2,3′-cyclo-1-(3-deoxy-β-D-xylofuranosyl)-2-thiouracil (XV) we have followed the reaction path reported by Yung and Fox[28] in a synthesis of O²,3′-cyclouridine, using 2-thiouridine in place of uridine. Treatment of 2-thiouridine (XVI)[5] with three equivalent molar trityl chloride in pyridine afforded 2′,5′-di-O-trityl-2-thiouridine (XVII) in 36% yield. Treatment of XVII with methanesulfonyl chloride gave the 3′-O-mesylate (XVIII) in a good yield. Heating of XVIII with triethylamine in dimethylformamide at 100° resulted in a separation of crystals in the reaction mixture which was pure ditritylated (S)-cyclonucleoside (XIX). Detritylation of XIX with ethanolic hydrogen chloride afforded the desired (S)-2,3′-cyclo-2-thiouridine (XV) as white needles in a yield of 54%. The structure of XV was confirmed by UV, Mass and NMR spectral measurements. In NMR spectra (Table I) the H-3′ proton signal was shifted to higher magnetic field as expected. Other signals are also quite distinguishable from those of VII. Compound XV is rather stable in acidic solution. In alkaline medium aryl-S fission occurred to give a uridine-type compound, as judged by UV spectral behaviors.

The cleavage reactions of these (S)-cyclonucleosides with a number of reagents are in progress and will be reported separately.

Extensive studies on the optical properties of nucleosides have been accomplished recently since measurements of optical rotatory dispersion (ORD) and circular dichroism (CD) are useful tool for analyses of anomeric configuration as well as the conformation about the glycosidic bond in nucleosides. For the conformational studies the cyclonucleosides offer informations on the orientation of the base-ring with respect to the sugar-ring since these are "fixed" nucleosides. In recent reports Rogers and Ulbricht have presented the experimental rule from the accumulated examples, which can be summarized as follows:[29]

1. The sign of the long wave-length (B_{2u}) Cotton effect is determined by the anomeric configuration at C-1′ (simple pyrimidine β-D-nucleosides found in RNA or DNA give positive Cotton effect).

2. The sign and magnitude of the Cotton effect is also a function of the nucleoside conformations. A positive Cotton effect is associated with the con-

Table II. ORD Data of Sulfur-bridged 2-Thiouridine Cyclonucleosides

Compound	First extremum		Second extremum		UV	
	$[\phi]$	λ (nm)	$[\phi]$	λ (nm)	λ_{max} (nm)	ε
VII	$+3.38\times10^3$	267	-2.53×10^4	238	230	27600
XV	$+3.50\times10^3$	273	-1.05×10^4	249	236	27300
XIV	-2.65×10^4	260	$+1.11\times10^5$	234	244	18600

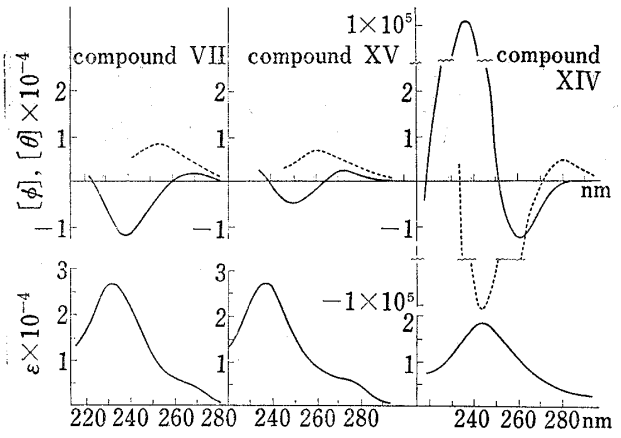


Fig. 1. The UV, ORD, and CD Spectra of Sulfur-Bridged 2-Thiouridine Cyclonucleosides

-------: CD ———: ORD

28) N.C. Yung and J.J. Fox, *J. Am. Chem. Soc.*, **83**, 3060 (1961).

29) *a*) T.R. Emerson, R.J. Swan, and T.L.V. Ulbricht, *Biochemistry*, **6**, 843 (1967); *b*) G.T. Rogers and T.L.V. Ulbricht. *Biochem. Biophys. Res. Commun.*, **414**, 419 (1970); *c*) G.T. Rogers and T.L.V. Ulbricht, *Eur. J. Biochem.*, **22**, 457 (1971).

formation which possesses sugar-base torsion angle (ϕ_{CN})[30] in the range of −60° through 0° to +120°.[29c]

The UV, ORD, and CD spectra of the (S)-cyclonucleosides prepared are shown in Figure 1 and Table II. The ORD and CD spectra of VII and XV in aqueous solution showed positive Cotton effect, which are similar with those of 2-methylthiouridine.[18] Compound XIV exhibits negative Cotton effect with stronger amplitude as compared with VII or XV. These features are also similar to those of (O)-cyclouridines and -thymidines.[29] A small positive CD band of XIV at —280 nm is assumed to be from n—π^* transition.[29b] Rogers and Ulbricht reported that the reversal of the sign of O²,5′-cyclothymidine is accounted for by the change of the torsion angle (+160°) which falls in the negative region (see Fig. 1 of ref. 28*c*). Apparently the same situation should hold in a (S)-2,5′-cyclonucleoside (XIV).

However, if one assumed that this explanation is correct, it follows that the preferred conformation of XIV must be "exo" form. As stated before the plausible conformation of XIV resulted from the NMR analysis is "endo" rather than "exo", and the torsion angle should be around +110°, which falls in a positive region. Although this discrepancy should be solved by further studies, one point could be raised in the present stage. The UV spectra of XIV are rather different from those of VII, XV or 2-methylthiouridine. Whereas the latter three compounds exhibit partially separated spectra having absorption maxima at 230—236 nm and a shoulder at 260—270 nm (probably of B_{2u}), respectively, XIV showed longer wave-length of absorption maximum (244 nm) with no shoulder. Therefore the negative ORD and CD curve of XIV are probably caused not by the B_{2u} transition alone but by a sum of nearly coalescent B_{2u}, B_{1u}, and E_{1u} transitions, assuming that the small positive CD band is from n—π^* transition. Similar phenomena have been observed in pyrimidine N-cyclonucleosides.[31] It is to be noted here that the good correlation between sign and magnitude of Cotton effect and sugar-base torsion angle has been observed in a series of O- and S-bridged purine cyclonucleosides.[32]

Experimental

NMR spectra were determined on a Hitachi H-60 or R-20b spectrometer. ORD and CD measurements were performed with JASCO-ORD/UV-5 spectropolarimeter at 18—22°, using H_2O as a solvent. Molar eclipsities (θ) are presented with multiplying the determined values by 1.5 as a factor of the instrument.

1-(3,5-Di-O-acetyl-β-D-arabinofuranosyl)-2-thiouracil (IV)——3′,5′-Di-O-acetyl-O²,5′-cyclouridine (III, 1 g) was dissolved in 100 ml of pyridine, previously saturated with H_2S under ice-cooling, and kept at 60—70° in a stainless steel tube overnight. After the removal of the solvent *in vacuo* the residue was taken up in EtOH and concentrated to leave a mass. On repetition of the process the residue solidified which was recrystallized from C_6H_6 to yield 0.8 g (73%) of IV, mp 149—151°. *Anal.* Calcd. for $C_{13}H_{16}O_7N_2S$: C, 45.39; H, 4.69; N, 8.14; S, 9.32. Found: C, 45.42; H, 4.65; N, 8.11; S, 9.48. UV λ_{max}^{EtOH} nm (ε): 276 (13000), 219 (16900). NMR (d_6-DMSO), ppm: 2.18, 2.22 (6H, s, AcO), 6.25 (1H, d, J=10 Hz, C_5-H), 7.12 (1H, d, J=4 Hz, C_1-H), 8.18 (1H, d., J=10 Hz, C_6-H). Treatment of IV with methanolic ammonia yielded 1-β-D-arabinofuranosyl-2-thiouracil.[6]

1-(2-O-Methanesulfonyl-3,5-di-O-acetyl-β-D-arabinofuranosyl)-2-thiouracil (V)——To a solution of IV (2.0 g, 5.6 mmol) in 40 ml of pyridine was added dropwisely methanesulfonyl chloride (0.88 ml, 11.2 mmol) in an ice-water bath and the solution kept overnight at 0—5°. The reaction mixture was poured into ice-water under stirring and the resultant suspension was concentrated to *ca.* 10 ml and left to effect precipitation. The precipitate was collected, washed with water and recrystallized from EtOH to give 1.6 g (67%) of V, mp 181—182°. *Anal.* Calcd. for $C_{14}H_{18}O_9N_2S_2$: C, 39.84; H, 4.30; N, 6.64; S, 15.20. Found: C, 39.84; H, 4.35; N, 6.86; S, 15.36. UV λ_{max}^{EtOH} nm (ε): 276 (14000), 219 (17300).

30) J. Donohue and K.N. Trueblood, *J. Mol. Biol.*, **2**, 363 (1960).
31) J. Yamashita, H. Inoue, K. Muneyama, and T. Ueda, presented at the Local Meeting of the Pharmaceutical Society of Japan, Sapporo, July, 1973.
32) *a*) M. Ikehara, *Accounts Chem. Res.*, **2**, 47 (1969); *b*) M. Ikehara, M. Kaneko, Y. Nakahara, S. Yamada, and S. Uesugi, *Chem. Pharm. Bull.* (Tokyo), **19**, 1381 (1971); *c*) M. Ikehara and M. Muraoka, *Chem. Pharm. Bull.* (Tokyo), **20**, 550 (1972).

(S)-2,2′-Cyclo-1-(2-deoxy-β-D-arabinofuranosyl)-2-thiouracil ((S)-2,2′-cyclo-2-thiouridine) (VII)——Compound V (1.0 g, 2.4 mmol) was dissolved in 120 ml of abs. MeOH to which was added 5 ml of 2.5 N $NaOCH_3$ in MeOH under stirring. After 1 hr the solution was neutralized with the addition of Dowex 50 (H^+ form) resin and filtered. The resin was washed with MeOH, H_2O and the filtrate and washings were combined and concentrated to leave a solid, which was recrystallized from aqueous EtOH to give 0.53 g (93%) of VII, mp 189—191°. Mass Spectrum: m/e=242 (M^+). *Anal.* Calcd. for $C_9H_{10}O_4N_2S$: C, 44.63; H, 4.16; N, 11.57; S, 13.19. Found: C, 44.41; H, 4.19; N, 11.67; S, 13.22. UV $\lambda_{max}^{H_2O}$ nm (ε): 230 (27100), 266 infl (5300). NMR: see Table I. Compound VII was taken up in aq. EtOH and treated with Ni catalyst (about 10 fold wet wt.) under reflux for 30 min. After the removal of Ni catalyst the filtrate was concentrated to leave a gum (VIII), UV $\lambda_{max}^{H_2O}$, 241 nm, $\lambda_{max}^{0.1NHCl}$, 229, 255 (sh) nm. Paper chromatography (solvent, *n*-BuOH: H_2O, 86:14) of VIII gave a spot at *Rf* 0.20, positive for cysteine-H_2SO_4 reagent[33] and aniline-phthalate reagent.[34] Compound VIII was heated in 0.1 N HCl at 80° for 1 hr and applied to paper chromatography. Two spots (*Rf* 0.31 and 0.40) were detected. The former was identified as 2-deoxyribose with the comparison of the authentic sample by *Rf* value and color tests. The latter was identified as 4-pyrimidinone by the comparison of *Rf* value and UV spectra with the authentic material. Compound VII was stable in 1 N HCl at room temperature for 24 hr. In 1 N NaOH for 5 hr VII gave a product with uridine-type UV spectra.[22]

O^2,5′-Cyclo-2′,3′-O-isopropylideneuridine (X)——The title compound was prepared by Nagyváry's procedure[25b] with slight modification. A mixture of 2′,3′-O-isopropylidene-5′-O-tosyluridine[25b] (3.3 g) and 4-morpholino-N,N′-dicyclohexylcarboxamidine[35] (1.77 g) in DMF (6 ml) and dioxane (60 ml) was heated at 80° for 4 hr. After the reaction mixture was cooled to room temperature, the crystals of 4-morpholino-N,N′-dicyclohexylcarboxamidinium *p*-toluenesulfonate were added to induce crystallization of the salt. The separated salt was removed by filtration and the filtrate was concentrated to leave a mass which was recrystallized from EtOH. The yield of X was almost quantitative.

2′,3′-O-Isopropylidene-2-thiouridine (XI) from X——Hydrogen sulfide gas was conducted in 50 ml of pyridine in a dry-ice-acetone bath (*ca.* −70°) and condensed to yield 100 ml solution, which was poured into previously cooled stainless steel tube (200 ml inner volume) containing 1.5 g of X, and sealed. The reaction mixture was set aside for 4 days at room temperature. After evaporation of H_2S, pyridine was removed *in vacuo* and the residue was dissolved in MeOH. The solvent was removed *in vacuo* and MeOH was added and repeated the process several times. The final white fluffy residue was dissolved in 80 ml of $CHCl_3$ from which colorless needles (XI) separated out in a few min. The yield was 1.58 g (93%), mp 192—193°[5] From the mother liquor XII[5,12,13] was obtained (0.03 g, 1.8%) in a crystalline form from MeOH.

2′,3′-O-Isopropylidene-5′-O-*p*-toluenesulfonyl-2-thiouridine (XIII)——To a solution of XI (1.0 g, 3.3 mmol) in 10 ml of pyridine was added TsCl (1.26 g, 6.6 mmol) in an ice-water bath. The mixture was kept overnight at 0—5° and poured into ice-water. The precipitate was collected, dried and crystallized from dioxane–EtOH to yield 1.2 g (80%) of XIII, mp 175—177° (decomp). *Anal.* Calcd. for $C_{19}H_{22}O_7N_2S_2$: C, 50.22; H, 4.88; N, 6.17; S, 14.13. Found: C, 50.08; H, 4.83; N, 6.27; S, 13.83.

(S)-2,5′-Cyclo-1-(5-deoxy-2,3-O-isopropylidene-β-D-ribofuranosyl)-2-thiouracil ((S)-2,5′-cyclo-2′,3′-O-isopropylidene-2-thiouridine) (XIV)——The 5′-O-tosylate XIII (2.0 g, 4.4 mmol) and triethylamine (1.2 ml, 7.5 mmol) were dissolved in 60 ml of dioxane and refluxed for 1 hr. The reaction mixture was concentrated and the residue was taken up in $CHCl_3$. The $CHCl_3$ layer was washed with 5% $NaHCO_3$ solution, water, and concentrated. The residue was dissolved in C_6H_6 containing small amount of EtOH and left to stand for few days from which white crystals (XIV), 0.9 g (72.5%), separated. mp 246—247°. Mass Spectrum m/e: 282 (M^+). *Anal.* Calcd. for $C_{12}H_{14}O_4N_2S$: C, 51.06; H, 5.00; N, 9.93; S, 11.37. Found: C, 51.05; H, 4.91; N, 10.00; S, 11.29. UV λ_{max}^{EtOH} nm (ε): 243 (18600). NMR: see Table I. Compound XIV was treated with 1 N HCl overnight. The UV spectra of the solution remained unchanged. By treatment of XIV in 1 N NaOH the absorption maximum changed to a shoulder at 260 nm. This behavior is similar to that of XII.[13]

2′,5′-Di-O-trityl-2-thiouridine (XVII)——2-Thiouridine (XVI, prepared by the procedure reported[5] and recrystallized from H_2O, mp 207—209°, 1.0 g, 3.8 mmol) was dissolved in 10 ml of pyridine to which was added trityl chloride (3.3 g, 11.8 mmol), kept overnight at room temperature, and heated at 100° for 4 hr. After the removal of the solvent *in vacuo* EtOH and acetone were added and evaporated. This procedure was repeated several times and the residue was triturated with EtOH. The insoluble material (XVII) was collected by filtration. The yield was 1.0 g (36%). This gave single spot on TLC (silica gel, $CHCl_3$–EtOH, 8:2). A portion was recrystallized from hexane–C_6H_6–EtOH to give pure material, mp 227—228°. *Anal.* Calcd. for $C_{47}H_{40}O_5N_2S$: C, 75.87; H, 5.42; N, 3.77; S, 4.31. Found: C, 75.99; H, 5.35; N, 3.81; S, 4.28. UV λ_{max}^{EtOH} nm (ε): 278 (15000).

2′,5′-Di-O-trityl-3′-O-methanesulfonyl-2-thiouridine (XVIII)——To a solution of XVII (0.5 g, 0.68 mmol) in 6 ml of pyridine was added MsCl (0.08 ml, 1 mmol) slowly at 0—5°, and kept overnight. The solution

33) J.G. Buchanan, *Nature*, **168**, 1091 (1951).
34) S.M. Partridge, *Nature*, **164**, 443 (1949).
35) J.G. Moffatt and H.G. Khorana, *J. Am. Chem. Soc.*, **83**, 649 (1961).

was poured to ice-water and the precipitate was collected and crystallized from C_6H_6–EtOH to yield 0.45 g (82%) of XVIII, mp 195—196° (decomp.). *Anal.* Calcd. for $C_{48}H_{42}O_7N_2S_2$: C, 70.13; H, 5.15; N, 3.41; S, 7.81. Found: C, 70.79; H, 5.12; N, 3.45; S, 7.64. UV λ_{max}^{EtOH} nm (ε): 278 (15600).

(S)-2,3′-Cyclo-1-(2,3-di-O-trityl-3-deoxy-β-D-xylofuranosyl)-2-thiouracil (XIX)——The mesylate XVIII (0.5 g, 0.6 mmol) and triethylamine (1 ml) in 2 ml of DMF were heated at 100° for 2 hr. After cooling the separated crystalline XIX was collected (0.26 g), mp 285—286°. From the mother liquor on addition of ether 0.08 g of XIX was obtained (total yield, 77%). *Anal.* Calcd. for $C_{47}H_{38}O_4N_2S$: C, 77.67; H, 5.27; N, 3.85; S, 4.41. Found: C, 77.75; H, 5.42; N, 3.77; S, 4.40. UV λ_{max}^{EtOH} nm (ε): 232 (49300).

(S)-2,3′-Cyclo-1-(3-deoxy-β-D-xylofuranosyl)-2-thiouracil ((S)-2,3′-cyclo-2-thiouridine) (XV)——Compound XIX (0.3 g) was suspended in 50 ml of EtOH and HCl gas was saturated under ice-cooling, heated at 70° for 20 min, and evaporated *in vacuo*. The residue was taken up in C_6H_6–H_2O. The aqueous layer was concentrated to a small volume (—5 ml) and kept at room temperature from which white crystals separated. This was collected and recrystallized from H_2O to give 0.054 g (54%) of XV, mp 265—268°. *Anal.* Calcd. for $C_9H_{10}O_4N_2S$: C, 44.63; H, 4.16; N, 11.57; S, 13.19. Found: C, 44.54; H, 4.07; N, 11.31; S, 12.98. Mass Spectrum *m/e*: 242 (M^+). UV λ_{max}^{EtOH} nm (ε): 236 (27300), 260sh (8400). NMR: see Table I. Compound XV was stable in 1 N HCl overnight as judged by UV spectra and in 1 N NaOH the spectra has changed as in the case of VII.

Acknowledgement This work was supported, in part, by the Grant-in-Aid for Scientific Research from the Ministry of Education, which is gratefully acknowledged. The NMR, mass, and elemental analyses were performed by the members of the Central Analytical Room of this faculty to whom our thanks are due.