angiotensin since its replacement by a phenyl ring ([Val^5,Phe^6]-angiotensinamide[8]) or the destruction by photolysis (Paiva and Paiva[7]) of the imidazole portion of the histidine residue results in an almost complete loss of potency. The pharmacological nonequivalence of [Val^5]-angiotensin II and [Val^5,Pyr(3)Ala^6]-angiotensin II may be attributable to many factors. These may include different stability to tissue enzymes (angiotensinases), weaker binding to the receptor, and possibly others. Insight into the molecular events which underlie the biological function of angiotensin, particularly information regarding the chemical nature of the cell receptors, will be necessary before these aspects of the problem can be understood.

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Chemical Transformation of 4-Thiouracil Nucleosides to Uracil and Cytosine Counterparts[1]

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Abstract: Periodate oxidation at pH 7 and 35° of low concentrations of 4-thiouracil nucleosides with suitably protected pentose moieties yields the corresponding 2-oxypyrimidine-4-sulfonate nucleosides. Subsequent H^+ or OH^- catalyzed hydrolysis yields uracil nucleosides, while ammonolysis with NH_3 or methylamine yields cytosine and N^4-methylcytosine nucleosides, respectively. It is suggested that the mild conditions required for these reactions are suitable for specific modification of 4-thiouridylate residues in transfer RNA.

Recent attention has been drawn to the presence of 4-thiouridylate in tRNA[3] of *E. coli*.[4] Apart from the special chemical properties of greater acidity, ease of oxidation, and disulfide bond formation,[4,5] this residue would appear to have structural and hydrogen-bonding characteristics like those of uridylate.[6] Thus, the reason for its occurrence in tRNA presents an intriguing problem. A preliminary study[7] showed that susceptibility of 4-thiouridylate in tRNA to oxidation by sodium periodate depended on the conformational state of the tRNA, and that oxidation was accompanied by a decrease in the capacity of such modified tRNA's to be enzymatically deacylated. A quantitative interpretation of the results, however, was precluded by the absence of information regarding the chemical transformations undergone by the sensitive residue. This prompted an investigation of the periodate oxidation of appropriate 4-thiouridine model compounds. The present report describes reactions in which suitably protected nucleoside derivatives of 4-thiouracil are converted by periodate oxidation and subsequent nucleophilic attack to the corresponding uracil, cytosine, or N^4-methylcytosine derivatives. The conditions employed appear suitable for similar conversion of the residue in a tRNA molecule.

(1) This investigation was supported by grants from the National Institutes of Health (GM-07654) and the National Science Foundation (GB-6664).

(2) U. S. Public Health Service Predoctoral Fellow, 1964–1968.

(3) tRNA = transfer ribonucleic acid.

(4) M. N. Lipsett, *J. Biol. Chem.*, **240**, 3975 (1965).

(5) (a) M. N. Lipsett, *ibid.*, **242**, 4067 (1967); (b) M. N. Lipsett and B. P. Doctor, *ibid.*, **242**, 4072 (1967).

(6) (a) M. N. Lipsett, *Biochem. Biophys. Res. Commun.*, **20**, 224 (1965); (b) Y. Kyogoku, R. C. Lord, and A. Rich, *Proc. Natl. Acad. Sci. U. S.*, **57**, 250 (1967); (c) K. H. Scheit, *Biochim. Biophys. Acta*, **145**, 535 (1967).

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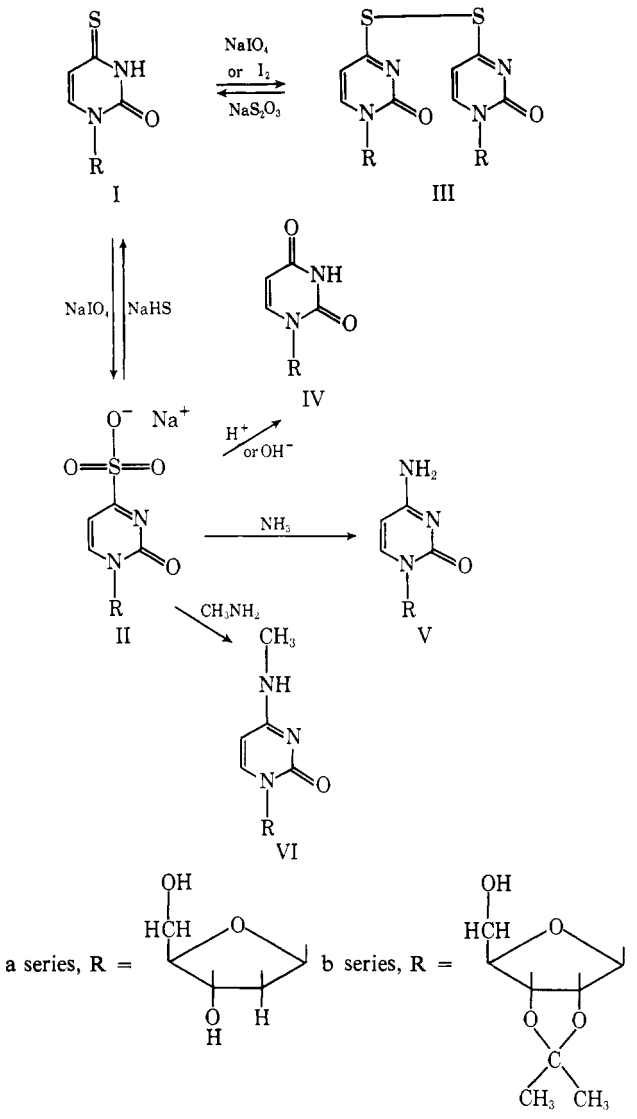
Results

Periodate Oxidation of 4-Thiouracil Nucleosides. Starting compounds for this study were either 2′-deoxy-4-thiouridine (Ia) or 2′,3′-O-isopropylidene-4-thiouridine (Ib)—see Chart I. In tRNA the ribose of 4-thiouridylate is protected from periodate oxidation by phosphate ester linkage at the 3′ position; the sugar moieties of these model compounds are similarly not reactive.

The oxidation of Ia (5×10^{-5} *M*) by sodium periodate (0.01 *M*) in aqueous solution was observed at 330 mμ (near its λ_{max}, *cf.* Figure 1a) to proceed with pseudo-first-order kinetics ($t_{1/2}$ = 15 sec at 35°, pH 7.0). The ultraviolet absorption spectrum of the final reaction mixture revealed a nucleoside product (II) with λ_{max} near 316 mμ, and greatly diminished absorption at 330 mμ. Upon addition of acid, base, or amines, a reaction of this product (II) was observed, as evidenced by irreversible loss of the absorption maximum at 316 mμ. A compound with such spectral and reaction properties (IIa or b) (Figure 1) was isolated when such oxidation mixtures were fractionated by sodium chloride gradient elution from Dowex 1 (Cl^-) columns. Attempts to isolate II free from sodium chloride were impeded by its instability. However, IIb was partially desalted by ethanol extraction of the lyophilized product.

Purified IIb was shown to contain sulfur by the nitroferricyanide, sodium azide–iodine, and lead acetate paper tests after sodium fusion. Upon acid hydrolysis or ammonolysis of IIb (see below), the sulfur was liberated as sulfite ion. The observation that synthesis of II proceeds readily at low concentrations of I (see below) indicated that II is not a dimeric compound such as a disulfide or thiosulfate. Thus, the liberation of sulfite suggested that II is the salt of a sulfinic or sul-

Chart I



fonic acid. The sulfur substituent of II was identified as a sulfonic acid moiety on the basis of the infrared spectrum of IIb. That spectrum, which has many bands in common with that of 2,3′-isopropylidineuridine (IVb), also contains two strong systems of bands between 600–700 cm^{-1} (maxima *ca.* 622, 690, and 700 cm^{-1}) and 1170–1270 cm^{-1} (maxima *ca.* 1205, 1215, and 1235 cm^{-1}) that are absent from the spectrum of IVb but are comparable to those characteristic of the S—O and S=O stretches, respectively, of sulfonic acids, and different from the S=O stretch of sulfinic acids (maxima *ca.* 1090 cm^{-1}).[8] Consistent with the conclusion that II is a sulfonic rather than a sulfinic acid is its stability in the presence of large excesses of periodate; sulfinic acids are known to be readily oxidized to sulfonic acids by a wide variety of oxidizing conditions.[9] The fact that reactions of II (see below) give rise to uracil or cytosine nucleosides, which differ from I only at C-4 of the pyrimidine ring, suggests that reaction with periodate is confined to that position.

Thus, II has been identified as a 2-oxypyrimidine-4-sulfonate nucleoside.

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(9) C. M. Suter, "The Organic Chemistry of Sulfur," John Wiley & Sons, Inc., New York, N. Y., 1944, p 365 ff.

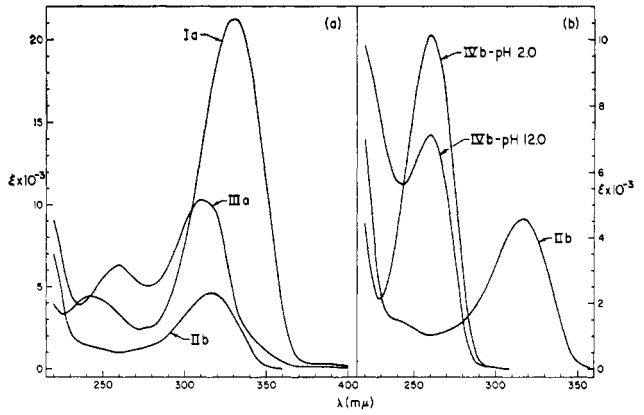

Figure 1. (a) Absorption spectra of pyrimidine nucleosides (per mole of ribose) at pH 7.0: Ia, 2′-deoxy-4-thiouridine; IIb, 1-(2,3-isopropylidene-β-D-ribofuranosyl)-2-oxypyrimidine-4-sulfonate; IIIa, bis(4,4′-dithiouracil 2′-deoxyriboside). Note that IIIa is represented on a half-molar basis since it contains two ribose moieties. (b) Spectra of IIb at pH 7, and of its hydrolysis product, IVb (2′,3′-isopropylideneuridine), recorded directly at the pH's of hydrolysis. The spectra of these products agree with those of authentic uridine at the indicated pH values, and correspond to equal yields of IVb.

Oxidation of high concentrations of Ia (4×10^{-3} *M*) by periodate yielded, in addition to IIa, a second product, which on purification displayed the spectral and chromatographic properties of bis(4,4′-dithiouracil 2′-deoxyriboside) (IIIa) (Figure 1a). This product was readily distinguished from IIa by its rapid quantitative reduction to Ia by 0.01 *M* sodium thiosulfate; IIa could not be so reduced.

The possibility that IIa is a further oxidation product of IIIa was examined. IIIa (prepared by iodine oxidation of Ia) was treated with 0.01 *M* $NaIO_4$ at 35° at neutrality for 15 min. No significant alteration of the ultraviolet spectrum of IIIa was noted, and it remained readily reducible to Ia by thiosulfate. This suggests that the formation of II is alternative to the formation of disulfide, rather than a consequence of further oxidation of the disulfide. This does not discount the possibility that the disulfide may be reactive under more vigorous oxidizing conditions.

Reactions of 2-Oxypyrimidine-4-sulfonate Nucleosides with Nucleophiles. Purified 1-(2-deoxy-β-D-ribofuranosyl)-2-oxypyrimidine-4-sulfonate (IIa) and 1-(2,3-isopropylidene-β-D-ribofuranosyl)-2-oxypyrimidine-4-sulfonate (IIb) were allowed to react with a variety of oxygen, nitrogen, and sulfur nucleophiles. Solutions of IIb adjusted to pH 2 with HCl or pH 12 with NaOH yielded a product (IV) with the spectral properties of uridine (Figure 1b). At pH 4.25 (0.02 *M* sodium acetate, 35°) the reaction, followed at 316 mμ, was pseudo first order with $t_{1/2}$ = 22 min; reaction was more rapid at lower pH's. In 0.1 *M* NH_4Cl (pH 8.5, 35°) a first-order decrease of A_{316} with $t_{1/2}$ = 6 min was observed; after complete reaction the solution exhibited ultraviolet absorption characteristic of cytidine, *i.e.*, like V.

IIa was allowed to react with tris(hydroxymethyl)-aminomethane (0.1 *M*, pH 7.5, 35°, pseudo first order, $t_{1/2}$ = 300 min) to yield a product with ultraviolet spectral and titration properties similar to Va; with NaHS (0.001 *M*, pH 8.85, 35°, pseudo first order, $t_{1/2}$ = 4 min) to yield Ia; and with cysteine (0.001 *M*, pH 8.9, 35°) and glutathione (0.001 *M*, pH 9.0, 35°). The course of re-

action with these latter, bifunctional nucleophiles, followed at 300 mμ, suggests a complex reaction mechanism. In both cases a clear indication of two-step kinetics was observed, which is presumed to reflect initial attack of IIa by the sulfur anion, followed by intramolecular displacement of the sulfur by the amino function, in the manner of a Smiles rearrangement.[10] Consistent with this interpretation, an absorption spectrum taken early in the reaction with 0.01 *M* glutathione, such that the first product was predominant, resembled that of 4-methylthiouridine;[11] furthermore, the final product with cysteine displayed spectra in acid and base similar to those of cytidine.

Synthesis of uracil, cytosine, or N^4-alkylcytosine nucleosides from I could be achieved under mild reaction conditions appropriate for the specific modification of tRNA. In these syntheses it was not necessary to isolate the sulfonic acid intermediate II prior to its further transformation. To obtain high yields, however, it was necessary to avoid formation of the disulfide III by maintaining minimal concentrations of I during the course of periodate oxidation. Thus, IIa was prepared by dropwise addition of Ia to 0.01 *M* sodium periodate at 35° and pH 7. After oxidation was completed, acidification to pH 4 for 100 min (or to pH 2 for 10 min) yielded 2′-deoxyuridine (IVa). Alternatively, addition of NH_4Cl to a final concentration of 0.14 *M* at pH 8.5 and incubation for 60 min, or of methylamine to 0.1 *M* at pH 9.5 and incubation for 15 min, yielded respectively 2′-deoxycytidine (Va) and 2′-deoxy-N^4-methylcytidine (VIa). These products were isolated by chromatography on Dowex columns and identified by their ultraviolet spectra in acid and base and by thin layer chromatography (Table I).

Table I. Identification of Products by Thin Layer Chromatography

Nucleoside	R_f, solvent A[a] Authentic	Product	R_f, solvent B[b] Authentic	Product
2′-Deoxyuridine	0.55	0.54	0.70	0.69
2′-Deoxycytidine	0.22	0.22	0.76	0.76
2′-Deoxy-N^4-methylcytidine		0.19		0.81

[a] Solvent A = 1-butanol–acetic acid–H_2O, 90:10:25. [b] Solvent B = 2-propanol–NH_4OH–H_2O, 60:30:10. Each product chromatographed as a single spot. When standard and sample were applied as a single spot, no separation was observed.

Discussion

Oxidative conversions of thiopyrimidines to the corresponding pyrimidinones are well documented. In some cases these derivatives have been obtained directly after oxidation (*e.g.*, with H_2O_2, HNO_3). The hydrolysis of an unstable sulfinic or sulfonic acid has been proposed to explain the elimination of sulfur in these reactions.[12] In other cases, the sulfinate or sulfonate is sufficiently stable to allow its isolation. Subsequent hydrolysis has yielded the hydroxyl counterparts,[13–15] while ammonolysis has allowed the introduction of a variety of amino substituents.[15,16] Of particular interest are the oxidations of 6-mercaptopurine and 6-thioguanine by excesses of $KMnO_4$ in alkaline solution to yield the corresponding 6-sulfonates.[17] These sulfonates were found to be labile in acid solution yielding the corresponding 6-hydroxy compounds, hypoxanthine and guanine. A similar series of reactions has been utilized with pyrimidine thioethers. Their oxidation to sulfoxides or sulfones, followed by hydrolysis, has yielded hydroxyl derivatives.[18] Of note are the hydrolytic and ammonolytic reactions of 4-methylsulfinylpyrimidine and 4-methylsulfonylpyrimidine,[19] which are analogous to the transformations described in the present work.

Sodium periodate has been utilized as an oxidant of aromatic thioethers, for example, in the preparation of phenyl sulfoxide, methyl phenyl sulfoxide, and phenylsulfinylacetic acid from the corresponding sulfides[20] and in the synthesis of 2-methylsulfinylpyrimidine from 2-methylthiopyrimidine.[19] A mechanism for the periodate oxidation of sulfides has been proposed.[21] Mention has also been made that periodate oxidation of 4-thiouridine, which contains a sensitive ribose moiety, yields the free base uracil, but a detailed study of the reaction was not reported.[4] In the present work, the oxidation by periodate of 4-thiouracil nucleosides to 2-oxypyrimidine-4-sulfonate nucleosides has been established.

The present study has also shown that 2-oxypyrimidine-4-sulfonate nucleosides can serve as common starting compounds for the synthesis of uracil, cytosine, and N^4-methylcytosine derivatives. These transformations are comparable to the nucleophilic displacement of chloride from 6-chloropurine riboside in aqueous solution by a series of nitrogen, sulfur, and oxygen compounds.[22] As in the case of 6-chloropurine riboside,[22] the effectiveness of the nucleophiles utilized in the present study were observed to be related to the pH-dependent proportion of their free base form. Such nucleophilic attack at the 4 position of pyrimidines is presumed to be activated by the electron-withdrawing effect of the *ortho* and *para* ring nitrogens.[23] An analogy can be drawn with the activated sulfonic acid substituent of 2,4-dinitrobenzenesulfonic acid, which is readily replaced by nitrogen and sulfur nucleophiles.[24,25] A mechanism in which water is the attacking nucleophile has been proposed for the acid hydrolysis of the sulfonic acid moiety of 2,4-dimethoxy-6-pyrimidinesulfonic acid.[26] This reaction is similar to the hydrolysis of II to IV at low pH.

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(18) Reference 12, p 236.
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(21) C. A. Bunton in "Oxidation in Organic Chemistry," Vol. 5-A, K. B. Wiberg, Ed., Academic Press, New York, N. Y., 1965, p 390.
(22) B. T. Walsh and R. Wolfenden, *J. Am. Chem. Soc.*, **89**, 6221 (1967).
(23) R. G. Shepherd and J. L. Fedrick, *Advan. Heterocyclic Chem.*, **4**, 150 (1965).
(24) J. F. Bunnett and R. E. Zahler, *Chem. Rev.*, **49**, 289 (1951).
(25) Reference 9, p 436.
(26) S. B. Greenbaum and W. L. Holmes, *J. Am. Chem. Soc.*, **76**, 2899 (1954).

While conversions of 4-thiouracil nucleosides to uracil, cytosine, or N^4-methylcytosine counterparts have been reported earlier,[27] the reaction conditions employed are too vigorous for application to tRNA. Although reactivity of 4-thiouridylate residues of tRNA with periodate has been demonstrated,[7,28,29] the reaction products have not been established. The reactions described in this report are of particular interest because of the possibility that they may be applied to 4-thiouridylate residues in tRNA to achieve definable transformations.

Experimental Section

Methods. Ultraviolet absorbance measurements were made with either a Cary 14 recording spectrophotometer for spectra and kinetics, a Gilford Model 220 optical density converter with a Beckman DU monochrometer for concentration determinations, or a Gilford multisample absorbance recorder, Model 2000, for kinetics. Each instrument was equipped with a thermostated cell compartment controlled to ±0.1°.

The concentration of nucleoside solutions was determined using the following molar extinction coefficients: uracil nucleosides at pH 7, ϵ_{260} 10.1×10^3;[30] cytosine nucleosides at pH 2, ϵ_{280} 13.2×10^3;[30] 2′-deoxy-N^4-methylcytosine nucleosides at pH 2, ϵ_{280} 14.8×10^3;[31] 4-thiouracil nucleosides at pH 7, ϵ_{331} 21.2×10^3;[32] bis-(4,4′-dithiouracil 2′-deoxyriboside) at pH 7, ϵ_{310} 20.7×10^3;[33] 2-oxypyrimidine-4-sulfonate nucleosides at pH 7, ϵ_{316} 4.6×10^3.[34]

Reaction kinetics were followed spectrophotometrically in stoppered quartz cuvettes. Reactions were initiated by the introduction of periodate, buffer, or nucleoside in less than 5% of the total reaction volume. Suitable blanks and dilution corrections were always employed.

Because of the high absorbance of periodate, spectra of solutions containing this reagent were sometimes taken after ethylene glycol had been added to destroy it. This could not be done, however, when cytosine or N^4-methylcytosine nucleosides were present because of their reactivity with the formaldehyde arising from the periodate oxidation of ethylene glycol.

Infrared spectra were obtained on a Perkin-Elmer 421 grating spectrophotometer. Samples were evaporated on KBr wafers from methanol solution.

Purity and retardation factors (R_f's) of nucleoside compounds were assessed by ascending thin layer chromatography on 0.2-mm layers of silica gel GF_{254} (Brinkmann Instruments Inc.) on glass plates at 22° except as noted. Compounds were visualized with a Mineralight short-wave ultraviolet lamp. 4-Thiouracil nucleosides could also be visualized as white spots against a brown background after spraying plates with sodium azide–iodine solution.[4] All column chromatography was carried out at 22°.

Sodium fusion and subsequent sodium azide–iodine and nitroprusside paper tests for sulfur were carried out according to Feigl.[35] Sulfur was also demonstrated after sodium fusion by the lead acetate paper test. The distinction between sulfite and sulfate ion, described in Table II, was made upon the difference in solubility properties of $BaSO_4$ and $BaSO_3$. Both salts are insoluble near neutral pH, whereas only $BaSO_4$ is insoluble at acid pH. Furthermore, in acid, soluble $BaSO_3$ may be oxidized by H_2O_2 to insoluble $BaSO_4$.[36]

Table II. Identification of Sulfite Released from 1-(2,3-Isopropylidene-β-D-ribofuranosyl)-2′-oxypyrimidine-4-sulfonate (IIb) upon Acid Hydrolysis or Ammonolysis

Reactant	Precipitate formation after reagent addition[a]: $BaCl_2$	HCl + $BaCl_2$	HCl + $BaCl_2$ + H_2O_2
IIb	−	−	+
IIb + NH_4Cl	+	−	+

[a] The reagents, 0.1 *M* $BaCl_2$, 1 *M* HCl, 3% H_2O_2 were added alone or *in the sequence indicated* to 5×10^{-3} *M* IIb, or to a mixture of IIb and 0.1 *M* NH_4Cl, pH 8.5, preincubated for 5 min; all reactants were added in equal volume.

Materials. 2′-Deoxy-4-thiouridine (Ia) was the generous gift of Dr. Gertrude Elion. It chromatographed on thin layer as a single spot (R_f 0.69, 1-butanol–H_2O, 86:14; R_f 0.72, 2-propanol–NH_4OH–H_2O, 60:10:30). 2′,3′-O-isopropylidene-4-thiouridine (Ib) was prepared from 2′,3′,5′-tri-O-benzoyl-4-thiouridine (the generous gift of Dr. J. J. Fox) according to the procedure of Kotchetkov, *et al.*[32] The product chromatographed as a single spot on thin layer (R_f 0.27, benzene–ethanol, 90:10). Sodium periodate (Fisher reagent grade) solutions were prepared just prior to use. Aqueous methylamine was obtained from Matheson Coleman and Bell. 2′-Deoxyuridine, 2′-deoxycytidine, L-cysteine·HCl, 2-mercaptoethanol, and reduced glutathione, all A grade, were obtained from the California Corporation for Biochemical Research. Tris-(hydroxymethyl)aminomethane, reagent grade, was obtained from the Sigma Chemical Co. Analytical grade Dowex 1 and 50 ion-exchange resins, 200–400 mesh and 4% cross-linked, were obtained from Bio-Rad Laboratories.

Synthesis of 2-Oxypyrimidine-4-sulfonate Nucleosides II. In a typical experiment, 23.4 μmol of Ib in 5 ml of ethanol was added dropwise with stirring over 45 min to 50 ml of 0.01 *M* $NaIO_4$, 0.01 *M* $PO_4(Na^+)$, pH 7.0, at 35°. The solution was incubated an additional 20 min. Similar conditions were employed for synthesis of IIa, but starting from Ia in 0.01 *M* $PO_4(Na^+)$, pH 7. Similar reaction mixtures containing II served as the starting point for the syntheses of IV, V, and VI described below.

Isolation of 2-Oxypyrimidine-4-sulfonate Nucleosides. To the above reaction mixture containing IIb was added 0.2 ml of ethylene glycol to destroy excess periodate. (Omission of the glycol did not affect the nature of the product but resulted in oxidation of the Dowex resin used in the next step, with release of ultraviolet absorbing material.) The reaction mixture was then applied to a Dowex 1 (Cl^-) column (6.5 × 1 cm) equilibrated with H_2O at pH 7. A linear gradient from 0.0 to 0.25 *M* NaCl,[37] pH 7 (400 ml), was used to elute the column at 1 ml/min. The starting compound and nucleoside by-products were not retained by the column, and IO_3^- eluted early. The product, identified spectrally, was eluted as a completely separated symmetrical peak at approximately 0.17 *M* NaCl in 60 ml (65% yield); this eluate was lyophilized to a white powder (mostly NaCl). When necessary, partial desalting was accomplished by ethanol extraction, but this step always resulted in some loss of product, presumably due to adsorption on the insoluble NaCl. Upon elution from the Dowex column, IIb could be stored indefinitely at −20° so long as the pH was maintained at 7.0.

The very same procedures were employed, with similar yields, to isolate IIa. However, no attempt was made to desalt this product.

Ultraviolet spectral measurements (spectra and kinetics) on IIa and IIb were performed on peak fractions of the Dowex effluent. Wet chemistry and infrared mass spectra[38] were performed on desalted samples.

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(31) Reported for N^4-methylcytidine 5′-phosphate by R. L. C. Brimacombe and C. B. Reese, *J. Chem. Soc.*, 588 (1966).

(32) This is the value reported for 4-thiouridine by N. K. Kochetkov, E. I. Budowsky, V. N. Shibaev, G. I. Yeliseeva, M. A. Grachev, and V. P. Demushkin, *Tetrahedron*, **19**, 1207 (1963). A value of 17.0×10^3 has been reported for 4-thiouridylate.[4] A use of this latter value would lead to correspondingly lower yields than those reported here.

(33) This value was determined on the basis of the spectrophotometric yield of 2′-deoxy-4-thiouridine after 2-mercaptoethanol reduction. Consequently, any inaccuracy in ϵ_{331} for the latter nucleoside would be reflected in this value as well.

(34) This value was determined from the spectrophotometric yield of IV upon acid hydrolysis of II.

(35) F. Feigl, "Spot Tests," Elsevier Publishing Co., Amsterdam, 1954: Vol. II, p 70; Vol. I, p 279.

(36) C. H. Sorum, "Introduction to Semimicro Qualitative Analysis," 3rd ed, Prentice-Hall, Inc., Englewood Cliffs, N. J., 1960, pp 202–203.

(37) Compound II is reactive with amine-containing volatile buffers.

(38) High-resolution mass spectra of IIb, kindly performed by Dr. F. Vane on a CEC21-110 mass spectrometer (70 eV; source temperature = 190–200°; sample temperature ~300°), failed to yield a parent ion peak, although compatible degradation products including SO, SO_2, and fragments of the pyrimidine and ribose moieties were observed.

Bis(4,4′-dithiouracil nucleosides) III. IIIa was prepared by I_2 oxidation, as has been reported previously for the disulfide of 4-thiouracil[39] and its nucleoside.[4] Ia (6.5 μmol), 8 μmol of KI, and 4 μmol of I_2 in 0.8 ml of 0.004 *M* $PO_4(Na^+)$, pH 7.1, were incubated for 10 min at 22°. The solution was extracted five times with 0.4 ml of CCl_4 and chromatographed on a Sephadex G-10 column (103 × 1 cm) equilibrated and eluted with H_2O. The product eluted as a well-separated peak, in an amount corresponding to 80% yield. Aliquots concentrated by flash evaporation revealed on thin layer chromatography (butanol–H_2O, 86:14) a major spot (R_f 0.24) and traces of starting compound and a second unidentified contaminant (presumably formed during concentration).

IIIa was also obtained as a major product of the $NaIO_4$ oxidation of Ia when the latter was present in relatively high concentration (0.004 *M*). Thus, 4 μmol of Ia and 50 μmol of $NaIO_4$ were incubated in 1.0 ml of 0.3 *M* $PO_4(Na^+)$, pH 7.1, at 22° for 20 min. Ethylene glycol (50 μl) was then added and the reaction mixture chromatographed on Sephadex G-10 as described above. A peak eluting at the same volume as IIIa obtained by the iodine procedure, and corresponding to 40% yield, was spectrally and chromatographically indistinguishable from IIIa. In addition, both products were readily reduced to Ia by 0.01 *M* sodium thiosulfate (determined spectrophotometrically). Other products eluted from the Sephadex column included IIa, 2′-deoxyuridine, and IO_3^-.

IIIb was detected spectrophotometrically as a significant product upon oxidation of Ib (10^{-4} *M*) for 30 min with $NaIO_4$ (10^{-3} *M*) at pH 6.5 at 35°. After addition to this reaction mixture of ethylene glycol to 1% concentration and sodium thiosulfate to 0.005 *M*, an increase in A_{330} was observed corresponding to the reduction of IIIb to Ib. When higher concentrations of Ib (3×10^{-3} *M*) were incubated at 22° with 5×10^{-3} *M* $NaIO_4$ in 50% ethanol for 60 min, a white product, insoluble in water and ethanol, resulted. A similarly insoluble precipitate was also observed when concentrated ethanolic solutions of Ib were stored for several days at 4°. These precipitates dissolved upon addition of reducing agents (*e.g.*, thiosulfate or mercaptoethanol), suggesting that they were IIIb.

Uracil Nucleosides IV. Ia (11.6 μmol) was converted to IIa as described above. The reaction mixture was acidified with acetic acid to pH 4.0, incubated 100 min longer at 35°, and passed through a Dowex 1 (acetate) column (9 × 1 cm) under pressure. At this pH, periodate and phosphate are bound to the resin, while IVa is not. The column effluent and a subsequent water wash were combined (86 ml), adjusted to pH 11.6 with NaOH, and passed through a second Dowex 1 (acetate) column (6 × 1 cm) that retained the nucleoside anion. After washing the column with H_2O, 10.7 μmol of product (92% yield) was eluted with dilute acetic acid and taken to dryness several times by flash evaporation.

The feasibility of similar preparation of IVb, either directly from Ib, or starting with IIb, was demonstrated on a small scale (*e.g.*, Figure 1b). It should be noted, however, that the acid lability of the isopropylidene blocking group makes the displacement of the sulfonic acid moiety of IIb by hydroxide ion preferable to acid hydrolysis.

Cytosine Nucleosides V. To prepare Va, NH_4Cl was added to a reaction mixture containing IIa (prepared as described above from 7.5 μmol of Ia) to 0.14 *M*, and the pH was adjusted to 8.5 with KOH. The reaction mixture was then incubated at 35° for 60 min, brought to pH 2 with HCl, and passed through a Dowex 50 (H^+) column (6 × 1 cm). The column was washed with several column volumes of H_2O to remove anionic contaminants. Va was then eluted with 1 *M* NH_4OH in 88% yield. A similar reaction of IIb to yield Vb was also demonstrated on a small scale.

N^4-Methylcytosine Nucleosides VI. To a reaction mixture containing IIa (prepared as described above from 4.7 μmol of Ia) concentrated aqueous methylamine was added dropwise with stirring to 0.1 *M*. During this addition pH was maintained between 7.5 and 10.0 by titration with HCl, the final pH being 9.5. After incubation for 15 min at 35°, the solution was acidified with HCl to pH 2.4 and passed through a Dowex 50 column (8 × 1 cm). The column was washed with H_2O, after which VIa was eluted with 1 *M* NH_4OH (90% yield). Voltaile amines were removed by flash evaporation.

VIb was prepared during the course of synthesizing N^4-methylcytidine by a modified procedure in which oxidant and nucleophile were simultaneously present. Ib (6.2 μmol) in 3 ml of ethanol was added dropwise with stirring to 50 ml of 0.01 *M* methylamine, 0.01 *M* $NaIO_4$, pH 10.4, over 15 min at 35°. After incubating 60 min longer at 35°, periodate was removed by passing the reaction mixture through a Dowex 1 (Cl^-) column (6 × 1 cm). The effluent and water wash were combined (72 ml) and acidified to pH 2 with HCl. At this point, the solution had the spectral characteristics of N^4-methylcytidine. To ensure removal of the isopropylidene blocking group, the solution was incubated for 60 hr at 22°, and then was passed through a Dowex 50 (H^+) column (4 × 1 cm). After washing the column with water, N^4-methylcytidine was eluted with 1 *M* NH_4OH in 91% yield. On descending paper chromatography (Whatman No. 1) developed with saturated $(NH_4)_2SO_4$–2-propanol–1 *M* sodium acetate (pH 6.0), 80:2:18, the product appeared as a single component with R_f 0.46, whereas a cytidine standard displayed R_f 0.56.[40]

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(39) W. H. Miller, R. O. Roblin, Jr., and E. B. Astwood, *J. Am. Chem. Soc.*, **67**, 2201 (1945).

(40) NOTE ADDED IN PROOF. Recently, we have become aware of a report that the oxidation of 4-thiouridine by OsO_4 in the presence of NH_3 yields cytidine as a major product [K. Burton, *Biochem. J.*, **104**, 686 (1967)]. This conversion presumably involves mechanisms similar to those reported here. In recent experiments the periodate reactions that we have described for model nucleosides have been applied successfully to tRNA for the conversion of 4-thiouridylate residues to N^4-methylcytidylate.