Synthesis of 5-Carboxymethyluridine. A Nucleoside from Transfer Ribonucleic Acid*

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ABSTRACT: The synthesis of 5-carboxymethyluridine, a new nucleoside recently discovered in tRNA, is described. Its anomeric configuration (β) is assigned and data regarding ionization and nuclear magnetic resonance, circular dichroism, and other spectrophotometric properties are presented.

The presence of the 5-carboxymethyl group diminishes the acidity constant related to the first ionization of the pyrimidine ring, a property which may be of significance in its role in tRNA.

Transfer ribonucleic acid contains a variety of rare nucleotides. The list of these trace components has been recently expanded by the isolation of two nucleosides structurally related to uridine. These are 2-thiouridineacetic acid methyl ester from yeast tRNA (Baczynskyj *et al.*, 1968) and 5-carboxymethyluridine from yeast and wheat embryo transfer ribonucleates (Gray and Lane, 1967, 1968). Although the latter has not been isolated in crystalline form, it has been characterized by its chromatographic and spectrophotometric properties, and its hydrolysis to the known 5-carboxymethyluracil (Johnson and Speh, 1907). However, the nucleoside is not available for studies of its chemical and biological properties. We undertook the total synthesis of 5-carboxymethyluridine and some of its derivatives to establish the proposed structure and to obtain complete physical and spectral data.

Materials and Methods

Chemicals were commerical samples and were used without further purification except as specified.

Melting points were determined with a Mel-Temp apparatus calibrated to 235° and are uncorrected above that temperature.

Paper Chromatography. Ascending technique on Whatman No. 1 paper was used with the solvents (A) *n*-BuOH–HOAc–H_2O (60:15:25, v/v), (B) *n*-BuOH–H_2O (85:15, v/v), and (C) *i*-PrOH–H_2O–NH_4OH (70:20:10, v/v).

Nuclear magnetic resonance spectra were measured with a Varian A-60 spectrometer, with dimethyl sulfoxide-d_6 as solvent, unless otherwise indicated, and $(CH_3)_4Si$ as the internal standard.

Ultraviolet absorption spectra were recorded with a Unicam SP800 spectrophotometer and extinction coefficients were measured with a Beckman DU spectrophotometer.

Infrared spectra were recorded with a Perkin-Elmer Infracord spectrophotometer using KBr disks.

pK's were determined by methods described (Albert and Sergeant, 1962) spectrophotometrically with a Beckman DU spectrophotometer or electrometrically with 0.01 M solutions.

Circular dichroism measurements were performed with the Cary 60 CD spectropolarimeter, using 0.1-mg/ml solutions in a 10-mm cell. The cell compartment was flushed continually with dry nitrogen to eliminate absorption bands in the far-ultraviolet region.

Results

The formylation of dimethyl succinate was effected with methyl formate in the presence of $NaOCH_3$ and gave the α-formylsuccinate (**4**) (Figure 1), in higher yield (62%) than previously reported (Payot and Grob, 1954; Jones and Kornfeld, 1956). Condensation of it with thiourea provided the methyl ester of carboxymethyl-2-thiouracil (**7**). In neutral or acid solution, **4** shows a weak absorption at 236 nm, while a strong absorption at 270 nm appears at ~pH 12. The nuclear magnetic resonance spectrum (given in τ values) of **4** showed in dimethyl sulfoxide-d_6 CH_2CO (singlet, 6.8) and vinyl H (singlet, 2.3). In $CDCl_3$ both tautomers of the formylsuccinate are present. In that solvent a relatively large coupling, J = 13 Hz, is observed between enolic (1.5) and vinyl (2.86) protons of the vinyl tautomer **4a** (see Discussion). The methylene protons of the aldehyde tautomer, **4**, appear as a doublet (7.05, J = 6.5 Hz) and the aldehydic proton as a singlet (0.05).

Methyl α-carbamyliminomethylenesuccinate (**5**) was prepared from **4** and urea. The vinyl proton (doublet, 2.05) is coupled to the adjacent NH (doublet, 0.98, J = 12.5 Hz) and the primary amide group gives rise to a singlet at τ 3.64. None of the desired pyrimidine was obtained from the attempted cyclization of **5** in the presence of $NaOCH_3$.

Cyclization of the formylsuccinate **4** with thiourea in the presence of $NaOC_2H_5$ gave the 5-carbethoxymethyl-2-thiouracil **8** and a small quantity of the corresponding acid **9**. The ultraviolet absorption and other properties of the pyrimidines reported here are shown in Table I. The nuclear magnetic resonance spectra of the thiouracil derivatives **7**, **8**, and **9** were consistent with the structures shown. Hydrolysis of the ester **8** with aqueous NaOH gave the acid **9**, while with concentrated aqueous ammonia solution the amide **6** was obtained. Etha-

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TABLE I: Spectrophotometric, Ionization, and Chromatographic Data on Various Pyrimidine Derivatives Shown on Figure 1.

	Spectral Data							
	0.1 N HCl		0.1 N NaOH			R_F in Solvents		
Compd	nm	$\epsilon \times 10^{-3}$	nm	$\epsilon \times 10^{-3}$	pK's	A	B	C
								0.37
6	274 max	14.9						
	240 min	3.1						
	214 max	13.9						
7	276 max	16.6				0.76	0.66	0.57
	240 min	3.6						
	214 max	15.6						
8	276 max	16.3				0.81	0.79	0.67
	241 max	3.6						
	213 max	15.2						
9	276 max	15.6	313 max	8.3	4.15 ± 0.06	0.55		0.23
	242 min	3.7	288 min	5.9	8.44 ± 0.05			
			259 max	11.1	~13.5			
			244 min	9.1				
			236 max	9.9				
11	263 max	8.1	290 max	5.5	4.31 ± 0.06	0.37		0.20
	232 min	1.4	246 min	2.5	9.97 ± 0.04			
					~14.2			
12	262 max	8.2				0.64	0.50	0.52
	232 min	1.9						
13	262 max	8.2				0.75	0.67	0.65
	232 min	1.9						
16	265 max	5.2				0.51	0.37	
	232 min	1.1						
17[a,b]	265 max	9.7	265 max	7.1	4.23 ± 0.1	0.30		0.17
	232 min	1.7	244 min	4.7	9.83 ± 0.07			
18	265 max	10.0	267 max	7.0		0.21	0.06	0.23
	242 min	5.8	244 min	4.8				

[a] In the systems 1 and 2 of Gray and Lane (1968), the chromatographic mobilities of **17** are R_{Up} 1.17 and R_F 0.57, respectively. The corresponding values for "5-carboxymethyluridine" from tRNA (Gray and Lane, 1968) are R_{Up} 1.18 and R_F 0.56, 0.57. [b] The following spectrophotometric data for "5-carboxymethyluridine" isolated from brewer's yeast tRNA have been reported: $\lambda_{max}^{pH\ 1}$ 265 nm, $\lambda_{min}^{pH\ 1}$ 234 nm; $\lambda_{max}^{pH\ 7}$ 266.5 nm, $\lambda_{min}^{pH\ 7}$ 236 nm; $\lambda_{max}^{pH\ 13}$ 266.5, $\lambda_{min}^{pH\ 13}$ 245.5 (Gray and Lane, 1968).

nolic ammonia at 120° converted **8** into the isocytosine derivative **3**. The first pK_a = 4.15 ± 0.06 of **9** is due to the ionization of the carboxyl group. The second pK_a = 8.44 ± 0.05 corresponds to the formation of the monoanion of the pyrimidine moiety, while the third pK_a observed at ~pH 13.5 is due to the complete ionization of the pyrimidine ring.

When 5-carbethoxymethyl-2-thiouracil (**8**) was treated with CH_3I in the presence of base, the methylmercapto derivative **10** was obtained which could be hydrolyzed to 5-carboxymethyluracil (**11**). Alternatively, **11** could be prepared quantitatively in one step by the sequential actions of chloroacetic and hydrochloric acids upon **8** (Brown, 1952). The nuclear magnetic resonance spectrum of **11** shows that H-6 (doublet, 2.64, J = 5.5 Hz) is coupled with H-1 (doublet, −0.64, J = 5.5 Hz), as evidenced by collapse to a singlet of the H-6 signal when D_2O is added (the signal due to H-1 at −0.64 disappears). Other signals are: CH_2CO (singlet, 6.84), H-3 (singlet, −0.98), and OH (broad signal centered at about −2.08). As is expected **11** exhibits three pK_a's, one at pH 4.8 ± 0.06 related to the ionization of the carboxyl group, a second at 9.97 ± 0.04, and a third at about 14.2.

The esters **12** and **13** were obtained by refluxing 5-carboxymethyluracil (**11**) with the appropriate alcohol in the presence of concentrated H_2SO_4. The bistrimethylsilyl derivative **14** was treated with 2′,3′,5′-tri-*O*-benzoyl-D-ribofuranosyl chloride (prepared in a manner analogous to that reported by Stevens *et al.*, 1967), to give a product containing one component (by thin-layer chromatography and nuclear magnetic resonance). This proved to be nucleoside **15**. Under the same experimental conditions, the β anomer of 1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)thymine was obtained exclusively (Wittenberg, 1968). The ultraviolet spectrum of **15** in ethanol was similar to that of 1-(2′,3′,5′-*O*-tribenzoyl-β-D-ribofuranosyl)-uracil. Compound **15** was debenzoylated with a catalytic amount of $NaOCH_3$ in CH_3OH to give the methyl ester of 5-carboxymethyluridine (**16**), the nuclear magnetic resonance

TABLE II: Relative Electrophoretic Mobilities of Synthetic 5-Carboxymethyluridine (**17**).[a]

Compound	Buffer pH				
	1.8	3.5	5.0	9.2 (Formate)	9.2 (Borate)
17	−0.23	+0.26	+0.57	+0.57	+1.17
Cm^5U (yeast tRNA)[b]	−0.08	+0.25	+0.51	+0.53	+1.02
Cm^5U (wheat embryo tRNA)[b]	−0.05	+0.22	+0.52	+0.52	+1.04

[a] Picric acid used as a marker was assigned a mobility of +1.00 at each pH value. The buffers and technique are those of Gray and Lane (1968). [b] Mobilities of 5-carboxymethyluridine from natural sources reported by Gray and Lane (1968).

spectrum of which showed H-6 (singlet, 2.18), CH_2CO (singlet, 6.71), OCH_3 (singlet, 6.40), and H-2′ (doublet, 4.22), $J_{1',2'}$ = 4.75 Hz. Saponification of the methyl ester **16** gave 5-carboxymethyluridine (**17**) which exhibits two ionization pK's, one of the acetate group at pH 4.23 ± 0.1, and a second of the pyrimidine ring at 9.83 ± 0.07. The nuclear magnetic resonance spectrum of **17** showed H-6 (singlet, 2.20), CH_2CO (singlet, 6.84), COOH (broad peak centered at about −1.33), and H-1′ (doublet, 4.20), $J_{1',2'}$ = 4.75 Hz. The relative electrophoretic mobilites of the synthetic 5-carboxymethyluridine (**17**) are given in Table II.

Concentrated aqueous ammonia converted the ester **16** to the amide **18** in good yield. The nuclear magnetic resonance spectrum of **18** showed H-6 (singlet, 2.19), $CONH_2$ (two broad peaks centered at 2.70 and 3.14), CH_2CO (singlet, 6.91), and

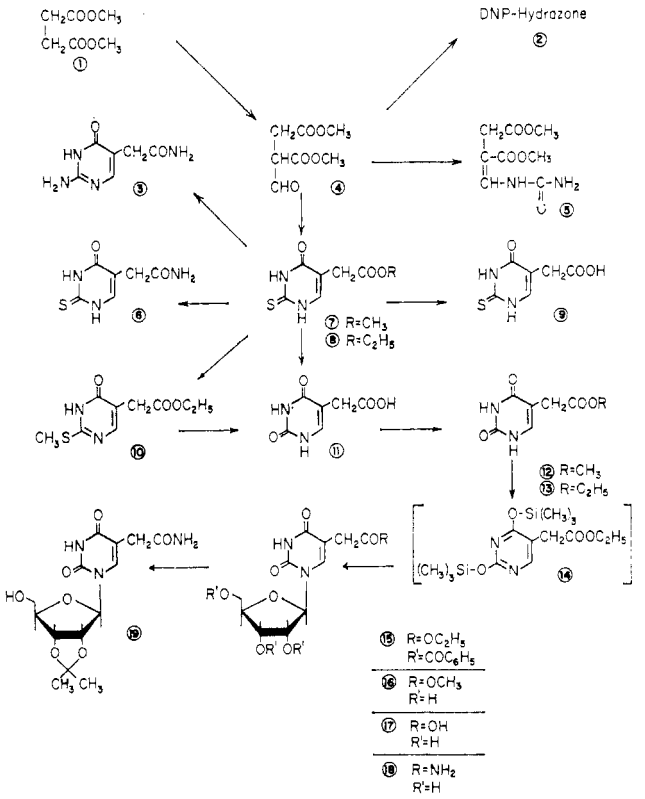


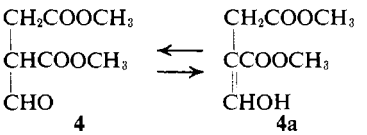
FIGURE 1: Synthetic route to 5-carboxymethyluridine.

H-1′ (doublet, 4.15), $J_{1',2'}$ = 4.5 Hz. The method employed to prepare 2′,3′-*O*-isopropylideneuridine (Fromageot *et al.*, 1967) was used for the synthesis of the 2′,3′-*O*-isopropylidene derivative of **16**. However, this compound proved to be very hygroscopic and could not be obtained in crystalline form. Conversion of it into the corresponding amide gave two components, one of mp 120–123° and the other of mp 176–180°. In the nuclear magnetic resonance spectra of both samples, the coupling constant observed in the signal due to the anomeric proton was $J_{1',2'}$ = 2.3 Hz.

Discussion

The preparation of 5-substituted uracils from the condensation of α-formyl lactones or α-formyl esters and ureas in acid solutions (Fissekis and Markert, 1966; Fissekis and Markert Creegan, 1967; F. Sweet, 1969, unpublished data) and subsequent cyclization of the α-carbamyliminomethylene derivatives has proven to be a convenient procedure in our laboratory for the preparation of 5-substituted uracils (Fissekis and Markert, 1966; Fissekis and Markert Creegan, 1967; Chkhikvadze and Magidson, 1964).

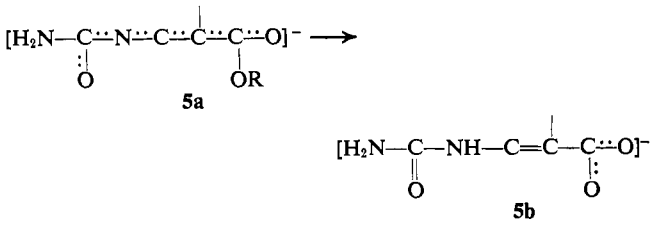
Formylation of dimethyl succinate gave **4**, which in alkaline solution possesses a strong ultraviolet absorption in the region characteristic of α,β-enolic esters **4a**. This absorption is suppressed upon neutralization; therefore in neutral aqueous solution **4** is the prevalent tautomeric form. In contrast the



nuclear magnetic resonance spectrum of this compound in dimethyl sulfoxide-d_6 solution indicates that **4a** is the predominant tautomer, while in $CDCl_3$ both species **4** and **4a** are present in approximately equal amounts.

The tautomeric form of the ureide **5** was assigned on the basis of nuclear magnetic resonance data. As in similar cases previously reported (Fissekis and Markert Creegan, 1967; J. D. Fissekis, 1969, unpublished data), the J value of the signal due to the vinylic proton adjacent to the NH (J = 12.5 Hz) is characteristic of this structure. The ultraviolet spectrum of **5** in acid or neutral solutions shows a single absorption at 263 nm. An absorption above 300 nm is observed at ~pH 12

(conditions under which the compound is hydrolyzed) due to the formation of the monoanion **5a**. Disappearance of this 300-nm peak is associated with hydrolysis of the esters and is accompanied by a shift to anion **5b** which absorbs at 263 nm.



5a

5b

The condensation of the formylsuccinate with thiourea in CH_3OH–$NaOCH_3$ gave **7** (Vorbrüggen *et al.*, 1969; Baczynskyj *et al.*, 1969), and in C_2H_5OH–$NaOC_2H_5$ gave 5-carbethoxymethyl-2-thiouracil (**8**) and a small amount of the acid **9** (the latter derived from the water generated in the reaction). The reaction of 5-carbethoxymethyl-2-mercaptouracil (**8**) with ammonia gave, at 22°, the corresponding amide **6** or, at 120°, the isocytosine derivative **3**. The latter was identified by comparison of its ultraviolet spectrum with those of known compounds (*e.g.*, 2-amino-4-hydroxy-5-(2-hydroxyethyl)pyrimidine, Fissekis *et al.*, 1964), elemental analysis, and relevant nuclear magnetic resonance data. It is well documented that 2-mercapto groups in pyrimidines are not usually aminated unless a 5-nitro group is present, with only two known instances of direct amination of a 2-mercapto group, both under forcing conditions (Brown, 1962). Although **3** was previously prepared by heating a solution of the corresponding 2-ethylthiopyrimidine in alcoholic ammonia at mp 170–180° (Johnson and Speh, 1907), its formation from **8** was unexpected.

Comparison of the pK_a's of 5-carboxymethyluracil (**11**) with those of 5-(2′-hydroxyethyl)uracil shows the extent to which ionization of the carboxyl group weakens the acidic character of the pyrimidine moiety. The second ionization of **11** (which is the first ionization of its pyrimidine ring) shows a $pK_a = 9.97 \pm 0.04$, while the first pK_a of 5-(2′-hydroxyethyl)uracil is 9.68 ± 0.05 (Fissekis *et al.*, 1964), *i.e.*, the acidity constant of the latter is almost twice that of **11**. Also the second ionization of its nucleoside **17** shows a pK_a of 9.83 ± 0.07 while the pK_a of the corresponding ionization of uridine is 9.2 (Symons, 1969). The diminished acidity of the uracil moiety of **17** caused by the ionization of the carboxyl group may have biological significance in terms of hydrogen-bonding capacity of this nucleoside in a tRNA, since the 5 substituent, $pK_a = 4.8$, of **17** is highly ionized at physiological pH.

Assignment of configuration of ribofuranosyl nucleosides cannot be readily made on the basis of the $J_{1',2'}$ coupling constant alone since the flexibility of the furanose ring gives $J_{1',2'}$ values of 3.5–8 Hz for *cis* hydrogens and 0–8 Hz for *trans*. Only values of $J_{1',2'}$ smaller than 1 Hz allow assignments of the anomeric configuration to be made (Lemieux and Lineback, 1963). However, it has been shown that for 2′,3′-*O*-isopropylidene derivatives, in which the ribofuranose ring is rigid due to fusion with a second ring, the $J_{1',2'}$ value of β-nucleosides is reduced (Nishimura *et al.*, 1964). Unfortunately preparation of the 2′,3′-*O*-isopropylidene derivative of the ester **16** gave a product which could not be crystallized and the nuclear magnetic resonance spectrum of the impure material was poorly resolved.

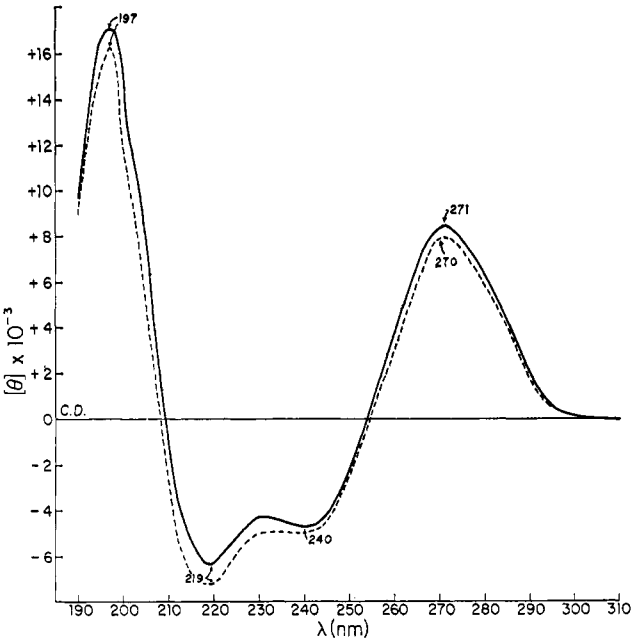


FIGURE 2: Circular dichroism curves of 5-carbomethoxymethyluridine (**16**, - - -) and 5-carboxymethyluridine (**17**, —) in water.

The amide **18**, prepared by the action of ammonia on **16**, gave a crystalline product. The nuclear magnetic resonance spectrum of **18** contained the H-1′ signal (doublet, 4.15) with $J_{1',2'} = 4.5$ Hz. The nuclear magnetic resonance spectrum of the 2′,3′-*O*-isopropylidene compound derived from it, **19**, showed the signal due to the anomeric proton (doublet, 4.23) with $J_{1',2'} = 2.3$ Hz. This reduction in the magnitude of $J_{1',2'}$ from 4.5 to 2.3 Hz when **18** is converted into **19** is consistent with the β-anomeric configuration for this series of nucleosides (Nishimura *et al.*, 1964).

It has been previously reported that configurational assignments (at the anomeric center) of pyrimidine nucleosides can be made on the basis of circular dichroism curves. Such assignments are valid provided the nucleoside under study has a similar electronic environment in the chromophore and that the conformation of the sugar component is reasonably similar to the reference nucleosides (Miles *et al.*, 1969b; Fric̆ *et al.*, 1966). Most uracil nucleosides satisfy these conditions (Miles *et al.*, 1969b). It was therefore expected that 5-carboxymethyluridine, and its methyl ester, could be analyzed by circular dichroism to establish firmly its configuration at C-1′.

Figure 2 shows tracings of the circular dichroism spectra of the nucleosides **16** and **17** and Table III contains the circular dichroism spectroscopic data of these compounds. Similar data for uracil and thymine ribonucleosides (Miles *et al.*, 1969a) are included for comparison. The terminology used to describe the transitions in the pyrimidine bases (*e.g.*, B_{2u}, E_{1ua}, and E_{1ub}) is that adopted by a number of authors (Miles *et al.*, 1969a,b, and references therein). Four Cotton effects are observed in the region 300–185 nm (Table II). All these Cotton effects have the same signs and are of approximately the same magnitude as the corresponding bands in uridine and

TABLE III:[a] Circular Dichroism Data on Some Uridine Derivatives.

Compound	Solvent	B_{2u}	B_{1u}	E_{1ua}	E_{1ub}	λ_1	λ_2
Uridine[b]	Water	267 (8,500)	240 (−3,700)	215 (−4,400)	196 (7,600)	262	205
5-Methyluridine[b]	Water	272 (5,500)	242 (−4,000)	217 (−4,400)	196 (11,000)	267	205
16	Water	271 (8,470)	240 (−4,750)	219 (−6,300)	197 (17,100)	265	209
17	Water	270 (7,980)	240 (−5,070)	219 (−7,250)	197 (16,200)	265	209

[a] The maxima in circular dichroism and absorption spectra are given in nanometers. The molar ellipticities are in parentheses. [b] From Miles *et al.* (1968).

thymine ribonucleoside. It has been well documented that β-D- and α-L-pentofuranosylpyrimidines and, more specifically, uracils lacking C-6 substituents give positive (B_{2u}) Cotton effects, whereas α-D and β-L derivatives give negative effects provided the foregoing conditions are met (Nishimura *et al.*, 1968; Yang and Samejima, 1969; Miles *et al.*, 1967, 1969a,b; Frič *et al.*, 1966). Therefore both nuclear magnetic resonance and circular dichroism data indicate that nucleosides **16** and **17** possess the β-anomeric configuration.

The comparisons of the chromatographic, electrophoretic, and ultraviolet properties of the synthetic 5-carboxymethyluridine with those reported for the naturally occurring nucleoside support the structure assigned to the latter. Unequivocal proof of structure could be derived only by direct comparison of "fingerprint" properties such as infrared, nuclear magnetic resonance, and mass spectra if the natural product becomes available in crystalline form.

Experimental Section[1]

Methyl α-Formylsuccinate (**4**). To a cooled stirred suspension of 13.5 g (0.25 mole) of $NaOCH_3$ in 200 ml of dry ether, a mixture of 36.5 g (0.25 mole) of dimethyl succinate and 30 g (0.50 mole) of methyl formate was added dropwise. Stirring was continued for 2–3 hr with cooling, and then overnight at room temperature. After decanting the solvent the viscous residue was washed with petroleum ether (bp 30–60°), and finally dissolved in ~80 ml of cold 3 N HCl. The solution was made slightly acidic with a few drops of concentrated HCl and extracted continuously with ether, and the extract dried (Na_2SO_4), filtered, and concentrated. The oily residue was distilled under reduced pressure (bp 104–105.5° (6–5.5 mm) and 97–99° (4 mm)) to give 26.83 g (62%) (lit. (Payot and Grob, 1954) bp 112–116° (10 mm)). The 2,4-dinitrophenylhydrazone of **4** (yellow small crystals from ethanol) melted at130–132°.

Anal. Calcd for $C_{13}H_{14}N_4O_8$: N, 15.81. Found: N, 15.70.

Methyl α-Carbamyliminomethylenesuccinate (**5**). A solution of 870 mg (5 mmoles) of **4** and 600 mg (10 mmoles) of urea in 10 ml of 1 N HCl was stirred overnight at room temperature. After cooling for several hours, the product was filtered, washed with water, and dried (68% yield), mp 170–180° dec (MeOH).

Anal. Calcd for $C_8H_{18}N_2O_5$: C, 44.28; H, 5.95; N, 12.91. Found: C, 44.52; H, 5.60; N, 12.90.

5-Carbethoxymethyl-2-thiouracil (**8**). Thiourea (8.36 g, 0.11 mole) was added to a solution of 2.53 g (0.11 g-atom) of sodium dissolved in 250 ml of ethanol, then 17.4 g (0.1 mole) of methyl α-formylsuccinate was added. After refluxing for 6 hr, the mixture was evaporated to dryness and the residue was treated with 100 ml of cold 15% aqueous acetic acid. After cooling the solution for several hours, the precipitated solid was collected, dried, and recrystallized from water to give 10.34 g of product, mp 185–186° (lit. (Johnson, 1911) mp 178–180°). The filtrate from the reaction mixture was evaporated to dryness and the residue fractionally crystallized from water to give an additional 630 mg of **8** and 2.72 g of 5-carboxymethyl-2-thiouracil (**9**), total yield of 66%. The nuclear magnetic resonance spectrum of **8** showed H-6 (singlet, 2.51), CH_2CO (singlet, 6.68), OCH_2C (quartet, 5.90), and C-CH_3 (triplet, 8.82, J = 7 Hz).

Anal. Calcd for $C_8H_{10}N_2O_3S$: C, 44.85; H, 4.70; N, 13.07; S, 14.97. Found: C, 44.78; H, 4.55; N, 13.17; S, 14.78.

5-Carbomethoxymethyl-2-thiouracil (**7**). The procedure for **8** was followed on one-tenth the scale and with methanol as solvent. The product was recrystallized from 40 ml of water to give 860 mg of small needles, softening at 205° and melting at 211°. The nuclear magnetic resonance spectrum showed H-6 (singlet, 2.52), CH_2CO (singlet, 6.67), and OCH_3 (singlet, 6.38).

Anal. Calcd for $C_7H_8N_2O_3S$: C, 41.99; H, 4.03; N, 13.99; S, 16.07. Found: C, 41.85; H, 3.89; N, 14.08; S, 15.86.

Amination of 5-Carbethoxymethyl-2-thiouracil. 2-Amino-5-carbamylmethyl-4-hydroxypyrimidine (**3**). A suspension of **8** (428 mg, 0.002 mole) in 10 ml of dry ethanol was saturated with ammonia at 0°, sealed, and heated at 120° overnight. A crystalline precipitate formed. The solvent was removed with a stream of air and the residue washed with a little cold water and recrystallized from water to give 180 mg (53.5%) of product, mp 295° dec (lit. (Johnson and Speh, 1907) mp ~280°).

Anal. Calcd for $C_6H_8N_4O_2$: C, 42.86; H, 4.79; N, 33.32. Found: C, 42.88; H, 4.83; N, 33.11.

5-Carbamylmethyl-2-thiouracil (**6**). A solution of the ester **8** (428 mg) in concentrated ammonia was stirred overnight at room temperature. After removing the solvent the residue was recrystallized from hot acetic acid containing a few drops (minimum volume required to dissolve residue) of water. The product was washed twice each with a small volume of cold water, then with ether, and dried, giving 330 mg (89%) of pure product, mp 269–270° dec.

[1] All solvents were removed in a Büchler flash evaporator under reduced pressure, unless otherwise indicated. Drying of all solids was accomplished under reduced pressure over P_2O_5 at suitable temperatures.

Anal. Calcd for $C_6H_7N_3O_2S$: C, 38.91; H, 3.81; N, 22.69; S, 17.31. Found: C, 38.92; H, 3.71; N, 22.70; S, 17.29.

5-Carboxymethyl-2-thiouracil (**9**). A solution of 428 mg (0.002 mole) of **8** in 8 ml of 0.5 N NaOH was refluxed gently for 2 hr. After removing the solvent the solid residue was crystallized from a small volume of hot acetic acid containing a few drops of water. White crystals were collected, washed with a small volume of ice-cold water, then with ether, and dried (290 mg, 78%), mp 275–279° dec. The nuclear magnetic resonance spectrum showed H-6 (singlet, 2.55) and CH_2CO (singlet, 6.75).

Anal. Calcd for $C_6H_6N_2O_3$: C, 38.71; H, 3.25; N, 15.04; S, 17.22. Found: C, 38.81; H, 3.11; N, 14.89; S, 17.19.

5-Carbethoxymethyl-2-methylmercapto-4-hydroxypyrimidine (**10**). A solution of 1.071 g (0.005 mole) of **8** in 10 ml of 0.5 N NaOH was stirred at 0–5° overnight with 0.35 ml of CH_3I. The solid was washed with water and dried (1.03 g, 90%). Recrystallization of this product from water gave small white needles melting at 180–181°.

Anal. Calcd for $C_9H_{12}N_2O_3S$: C, 47.19; H, 5.63; N, 12.23; S, 14.00. Found: C, 47.00; H, 5.60; N, 12.24; S, 14.06.

5-Carboxymethyluracil (**11**). (a) A mixture of 4.28 g (0.02 mole) of **8**, 1.05 g (0.022 mole) of chloracetic acid, and 25 ml of water was refluxed for 2 hr. To the clear solution 12 ml of concentrated HCl was added, refluxing was continued for 7 hr, and the solution was concentrated. The residual solid, still containing some starting material, was again subjected to the foregoing procedure to ensure complete hydrolysis. Upon cooling crystals accumulated and were collected, washed with cold water, and dried (3.33 g, 98.5%, mp 315–318°). This product was used for the synthesis of the nucleoside. (b) A solution of 2-methylmercapto-4-hydroxy-5-carbethoxymethylpyrimidine (**10**, 1.3 g, 0.0045 mole) was refluxed for 7 hr with 30 ml of 6 N HCl. The solution was evaporated to dryness, the hydrolysis was repeated, reevaporation took place, and the solid residue was recrystallized from water. The product (715 mg, 93.5%) melted at 316–318°.

Anal. Calcd for $C_6H_6N_2O_4$: C, 42.36; H, 3.55; N, 16.47. Found: C, 42.20; H, 3.55; N, 16.35.

5-Carbomethoxymethyl- and 5-Carbethoxymethyluracil (**12**, **13**). The acid **11** was esterified with large excess of dry methanol (or ethanol) containing 2 or 3 drops of concentrated H_2SO_4. After refluxing for ~20 hr, the solvent was partially removed by boiling until a solid began to separate. The resultant mixture was chilled for several hours and the product collected, washed with cold water, and dried. Recrystallization from MeOH gave **12** (73%), melting at 236–237°.

Anal. Calcd for $C_7H_8N_2O_4$: C, 45.66; H, 4.38; N, 15.21. Found: C, 45.63; H, 4.37; N, 15.22.

Compound **13** was obtained from **11** and ethanol in 96% yield and melted at 205–207°. The melting point remained unchanged after recrystallization from ethanol.

Anal. Calcd for $C_8H_{10}N_2O_4$: C, 48.48; H, 5.08; N, 14.13. Found: C, 48.32; H, 4.99; N, 14.03.

5-Carbethoxymethyl-2,4-bis-O-trimethylsilyluracil (**14**). A suspension of 1.98 g (0.01 mole) of **13** in a mixture of 8 ml of hexamethyldisilazane and 0.12 ml of trimethylchlorosilane was stirred at 150–160° for 12 hr. After ~10 min of heating, a clear solution resulted. The reaction mixture was fractionated in a short path still. The viscous oily product, **14**, boiled at 85–87° (23×10^{-3}) and crystallized upon standing (3.185 g, 91%).

1-(2′,3′,5′-Tri-O-benzoyl-β-D-ribofuranosyl)-5-carbethoxymethyluracil (**15**). Dry hydrogen chloride was bubbled into an ice-cold solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (5.55 g, 0.011 mole) in 30 ml of dry dichloromethane for 30 min. After 1.5 hr at 0° and 15 min at room temperature, it was concentrated to a syrupy residue. Dry dichloromethane and dry benzene were successively evaporated from the residue, the syrup was immediately dissolved in dry benzene (~40 ml), and 3.185 g of **14** (from 0.01 mole of **13**) was added. After 2.5 g of $HgBr_2$ and 2.5 g of HgO were added to the benzene solution, the mixture was refluxed for 6 hr, and allowed to stand at room temperature overnight. After filtration the solids were well washed with chloroform. The volume of the combined filtrates was adjusted to ~100 ml by adding chloroform. The chloroform solution was successively extracted with 50 ml of saturated NaCl, twice with 50-ml portions of 10% KI, with 30 ml of saturated aqueous $NaHCO_3$, and with 30 ml of water. The chloroform was dried (Na_2SO_4), filtered and concentrated to a viscous oil which solidified after trituration with benzene. The crude product was purified on a silica gel column (4 × 25 cm) which was first eluted with 500 ml of benzene, then with 1000 ml of benzene–ethyl acetate (9:1, v/v). These eluates were discarded. The column was then eluted with 1000 ml of benzene–methanol (9:1, v/v). The fractions that contained ultraviolet-absorbing material were combined and evaporated. Trituration of the residue with a small volume of methanol afforded crystals, from which the solvent was removed with a capillary pipet. The crystalline material was washed with petroleum ether, yield 5.2 g (89%). Recrystallization of the product from methanol gave white needles, mp 151–152.5°.

Anal. Calcd for $C_{34}H_{30}N_2O_{11}$: C, 63.55; H, 4.70; N, 4.36. Found: C, 63.08; H, 4.72; N, 4.36.

5-Carbomethoxymethyluridine (**16**). The benzoylated nucleoside **15** (2.12 g, 0.00333 mole) was dissolved in a solution of 115 mg of sodium in 60 ml of dry methanol. The mixture was refluxed for 6 hr. After standing overnight at room temperature, it was neutralized with 4 ml of Dowex 50 (H^+, 200–400 mesh) and filtered, and the resin was washed well with methanol. The filtrate and washings were evaporated to dryness and the residue was washed several times with petroleum ether and then ether (until no odor of methyl benzoate could be detected), and dried. The crude material (1.010 g, 96%) was purified on a silica gel column (4 × 26 cm) which was eluted with acetone. The solvent was evaporated from the fractions containing the product; the solid residue was triturated with ether, and the solid was collected and dried (860 mg). Recrystallization from methanol gave crystals, mp 163–165°, when dried at bp 111° (0.030).

Anal. Calcd for $C_{12}H_{16}N_2O_8$: C, 45.57; H, 5.10; N, 8.86. Found: C, 45.68; H, 5.05; N, 8.93.

5-Carboxymethyluridine (**17**). A small sample (316 mg, 1 mmole) of carbomethoxymethyluridine (**16**) obtained as described above was hydrolyzed by heating in 6 ml of water containing 1.0 mmole of NaOH on a steam bath. Hydrolysis was monitored by thin-layer chromatography (Chromagram, silica gel, acetone–H_2O, 95:5, v/v) to completion (4 hr), then the solution was neutralized with IRC-50 (H^+), the resin was washed well with water, and the combined eluates were concentrated to a small volume and filtered. The filtrate was concentrated further to a few milliliters, acidified with a few drops of 1 N HCl to ~pH 3, and cooled. The solid product

was collected, washed twice with a small volume of cold water, and dried, to yield 220 mg (73%), mp 238–240°. The infrared spectrum shows four bands in the carbonyl region at 1718, 1701, 1676, and 1656 cm^{-1}. Other major bands appear at 1479, 1423, 1329, 1215, 1086, 889, and 806 cm^{-1}.

Anal. Calcd for $C_{11}H_{14}N_2O_8$: C, 43.71; H, 4.67; N, 9.27. Found: C, 43.55; H, 4.63; N, 9.20.

5-Carbamylmethyluridine (**18**). A quantity (240 mg) of **16** in 20 ml of concentrated aqueous ammonia was left at room temperature overnight, and then the solution was evaporated. The residue was dissolved in methanol, the solution was again evaporated, and the crude product recrystallized from methanol (containing the minimum volume of water necessary to dissolve the material) to give 200 mg (85%) of product, mp 227–230°.

Anal. Calcd for $C_{11}H_{15}N_3O_7$: C, 43.86; H, 5.02; N, 13.95. Found: C, 43.65; H, 5.20; N, 13.82.

5-Carbamylmethyl-2′,3′-isopropylideneuridine (**19**). A mixture of 3.6 mg of **16**, 30 mg of *p*-toluenesulfonic acid monohydrate, 1.0 ml of 2,2-dimethoxypropane, and 4 ml of dry acetone was stirred at room temperature overnight. The resulting solution was neutralized with IR-45 (OH^-) and filtered, and the resin was washed well with acetone and then water. The filtrate and washings were evaporated to dryness. Often the crude product was sufficiently pure (by thin-layer chromatography, Chromagram silica gel, benzene–acetone, 1:1, v/v) to be converted into the corresponding amide. However, when the glassy residue was found to be highly impure, it was chromatographed on a column of silica gel G (2.5 × 18 cm), which was first eluted with 100 ml of benzene–acetone (3:1, v/v), and then with 250 ml of benzene–acetone (1:1, v/v). The elution was monitored by thin-layer chromatography.

The crude material was dissolved in ~40 ml of concentrated aqueous ammonia, and after standing at room temperature overnight the solution was heated on a steam bath for 1 hr. Then it was concentrated to dryness and the solid residue was washed with ether and recrystallized from ethanol, to give a first crop of crystals (240 mg), mp 121–123°. The ethanolic filtrate was evaporated to dryness and the residue recrystallized from acetone to give a second crop of crystals (150 mg), mp 176–180°. Both the first and second crops were chromatographically identical (thin-layer chromatography) and free of 2′,3′-isopropylidene-5-carboxymethyluracil and **18**. Additional recrystallizations from EtOH did not give satisfactory elemental analyses for either sample.

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