buoyant density and G + C content. The order of elution from PLK columns appeared to be the same as that from methylated albumin – kieselguhr columns (9). Mazen and Champagne (10) recently reported similar results using histone–kieselguhr columns, but these authors failed to fractionate DNA on PLK columns.

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Synthesis of 2-thio-5-carboxymethyluridine methyl ester: a component of transfer RNA

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2-Thio-5-carboxymethyluridine methyl ester was synthesized by condensing the mercuric salt of 2-thio-5-carboxymethyluracil methyl ester with 2,3,5-tri-*O*-benzoyl-D-ribosyl chloride. The product is identical to a nucleoside isolated from yeast tRNA

2-Thio-5-carboxymethyluridine methyl ester has been isolated from yeast tRNA (1). This communication reports the chemical synthesis of this nucleoside. The properties of both the synthetic material and of the naturally occurring product are identical.

2-Thio-5-carboxymethyluracil methyl ester was synthesized by treating a solution of dimethylformylsuccinate (17.4 g, 0.1 mole) (2) with thiourea (7.6 g, 0.1 mole) and sodium methoxide (5.4 g, 0.1 mole) in 300 ml of methanol at 0°. The reaction mixture was left at room temperature for 5 days and then refluxed for 3 h. Acetic acid (5.7 ml) was added and the solution was evaporated to about 200 ml. On addition of cold water (200 ml), a white precipitate appeared (4.65 g). An additional 2.92 g of product was obtained from the mother liquor: total yield 7.57 g (38%). The thio compound was recrystallized from absolute ethanol to give colorless needles: m.p. 218–220°.

The ultraviolet absorption spectra are: $\lambda_{max}^{pH\,1}$ 275, 214 mμ; $\lambda_{max}^{pH\,7}$ 275, 215 mμ; $\lambda_{max}^{pH\,12}$ 313, 260, 234 mμ. The high-resolution mass

PLATE I

FIG. 4. Isopycnic equilibrium centrifugation patterns of four samples from the elution pattern of Fig. 3. Each sample was mixed with *M. lysodeikticus* DNA as a marker of buoyant density (1.731 g/cm^3 in buffered CsCl of density about 1.70 g/cm^3). The samples were centrifuged for 18 h at 44 770 r.p.m. in a four-cell rotor of a Spinco model E analytical ultracentrifuge. The marker DNA appears as the band on the left.

TABLE I

Nuclear magnetic resonance spectrum of 2-thio-5-carboxymethyluridine methyl ester

Chemical shift (p.p.m., δ scale)	Multiplicity	Assignment
3.30 (2H)	Singlet	$-CH_2-$ attached to heterocycle
3.62 (3H)	Singlet	$-OCH_3$
3.82–4.10 (4H)	Broad band	Eight protons due to the ribose
4.92–5.42 (4H)	Broad band	Part
6.58 (1H)	Doublet	Anomeric hydrogen
8.16 (1H)	Singlet	C-6 hydrogen
12.64 (1H)	Broad singlet	N-3 hydrogen

spectrum of this sample showed a molecular ion at 200.0248 corresponding to the molecular composition $C_7H_8N_2O_3S$ (calcd. 200.0254).

The nucleoside was prepared by condensing 2,3,5-tri-*O*-benzoyl-D-ribosyl chloride with the mercuric adduct of the free base (3). 2-Thio-5-carboxymethyluracil methyl ester (2.5 g, 12.5 mmoles) was dissolved in 75 ml of water and 25 ml of *N,N*-dimethylacetamide; the solution was heated to 100°, and mercuric acetate (4.0 g, 12.5 mmoles) was added. The reaction mixture was refluxed for 35 min and the precipitate was recovered by filtration: yield 4.9 g (98%). This mercuric derivative (12.3 mmoles) was condensed with 2,3,5-tri-*O*-benzoyl-D-ribosyl chloride (18.5 mmoles) in refluxing xylene (75 ml) for 1½ h. The intermediate was worked up according to Fox *et al.* (3) and the benzoyl groups were removed by refluxing a methanolic solution of the compound in the presence of a catalytic amount of sodium methoxide. The product was recovered and crystallized from ethanol: yield 0.45 g (11%), m.p. 199°, $[\alpha]_D^{25}$ +9° (*c* 0.20, 95% ethanol). The natural product melts at 198–200°.

Anal. Calcd. for $C_{12}H_{16}O_7N_2S$: C, 43.37; H, 4.82; N, 8.43; S, 9.64. Found: C, 43.32; H, 5.04; N, 8.22; S, 9.77.

Ultraviolet absorption spectra: $\lambda_{max}^{pH\,1}$ 277 mμ (ξ 15 600), $\lambda_{max}^{pH\,7}$ 277 mμ (ξ 15 800), $\lambda_{max}^{pH\,12}$ 242 mμ (ξ 22 400), shoulder 271 mμ (ξ 15 800). These ultraviolet absorption spectra are identical to those of the natural compound (1).

The nuclear magnetic resonance (n.m.r.) spectrum was obtained on a 60 Mhz instrument, in DMSO-d_6 solution using tetramethylsilane as an internal standard (Table I).

The high-resolution mass spectrum of the synthetic sample was obtained under conditions identical to those used for obtaining the spectrum of the naturally occurring nucleoside. Characteristic ions appeared at *m/e* 301.0498 ($C_{11}H_{13}N_2O_6S$), 298.0000 ($C_{12}H_{14}N_2O_7$), 201.0305 ($C_7H_9N_2O_3S$), 200.0251 ($C_7H_8N_2O_3S$), and 133.0496 ($C_5H_9O_4$). These ions correspond to M–OCH_3, M–H_2S, B+2, B+1, and ribose fragments, respectively. This high-resolution mass spectrum is identical to that obtained from the naturally occurring nucleoside.

The similarity of the natural nucleoside with the synthetic product was further confirmed by paper chromatography in two solvent systems: 1-butanol – ammonium hydroxide – water (86:5:14), and 2-propanol – ammonium hydroxide – water (7:1:2).

In summary, four points of similarity exist between the properties of the naturally occurring nucleoside and those of the synthetic product: (*1*) melting point, (*2*) mass spectrum, (*3*) ultraviolet absorption spectra, and (*4*) paper chromatography.

On the basis of these data, we conclude that the nucleoside isolated from yeast tRNA is 2-thio-5-carboxymethyluridine methyl ester.

Dr. Helmut Vorbrüggen of the Schering A.G., Berlin, has informed us that he has also synthesized this nucleoside.

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