ISOLATION OF 3-METHYLURIDINE AND 3-METHYLCYTIDINE FROM SOLUBLE RIBONUCLEIC ACID

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A number of methylated bases have been found in ribonucleic acid including 5-methyluridine and 5-methylcytidine. This communication describes isolation of two additional methylated pyrimidine nucleosides from soluble RNA, 3-methyluridine and 3-methylcytidine. Five grams of yeast soluble RNA prepared according to the method of Holley et al. (1961) was dialyzed against three changes of distilled water (12 hours each change). The RNA was hydrolyzed enzymatically to its constituent nucleosides as previously described (Hall, 1963). The digest was divided into four equal portions and each portion was fractionated on a partition column using the basic procedure of Hall (1962) as illustrated in Figure 1. The fractions corresponding to peak A were pooled and concentrated to a gum. This material was refractionated in the above manner on a second partition column containing 50 g. of Celite-545. (Solvent system, n-butanol : water : conc. ammonium hydroxide, 15 : 5 : 2). The first 100 cc of

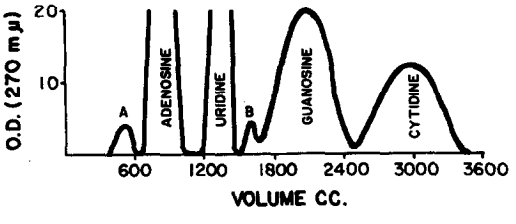

Fig. 1. Fractionation of the mixed nucleosides obtained from enzymic hydrolysis of 1.25 grams of yeast S-RNA. Column size 2.54 cm. x 80 cm., packed with 150 g. of a mixture of Celite-545 : Microcel-E, 9 : 1. Solvent 1; ethylacetate : 2-ethoxyethanol : 2% formic acid, 4 : 1 : 2. At point between uridine peak and peak B, solvent was changed to ethylacetate : n-butanol : water, 1 : 1 : 1.

solvent eluted 2'-O-methyladenosine and the second 100 cc of solvent eluted a second ultraviolet absorbing peak. The second fraction was concentrated and streaked on Whatman #3 MM paper which was then developed in solvent A for 24 hours. The major band contained 2'-O-methyluridine. A faint band running just ahead of the 2'-O-methyluridine was eluted and rechromatographed in solvent D. Re-elution of the resulting band gave 200 μg of a nucleoside identified as 3-methyluridine. The isolated compound exhibits the same ultraviolet spectra and paper chromatographic properties as a synthetic sample prepared according to the procedure of Miles (1956).

3-Methylcytidine was isolated from the last peak (labelled cytidine) of the column separation shown in Figure 1. The fractions containing cytidine were rechromatographed on a partition column packed with 150 g of Celite-545 according to the basic procedure of Hall (1962). (Solvent system, ethylacetate: n-butanol: water, 1:1:1). A small peak was eluted first, followed by a major peak consisting mostly of cytidine. The fractions corresponding to the leading edge of this large peak were concentrated to a small volume which was then streaked on a sheet of Whatman #3 MM paper, 6 inches wide. After development for 24 hours in solvent E, a faint band was observed just ahead of a heavy band of cytidine. This faint band contained 500 μg of a compound which chromatographically and spectroscopically was identified with 3-methylcytidine synthesized according to the method of Brookes and Lawley (1962). When the isolated compound was boiled in N/10 sodium hydroxide for 30 min. it was converted into a new compound identical with 3-methyluridine. This inherent susceptibility to deamination raises the possibility that 3-methyluridine could arise from 3-methylcytidine during the isolation procedure. 3-Methyluridine (230 μg) was isolated in the manner described above from a two-gram sample of soluble RNA prepared from human liver according to the

method of Brunngraber (1962). This RNA sample was dialyzed for 18 hours against running tap water and then for several hours against two changes of distilled water. 3-Methylcytidine was not detected in this sample but in view of the small amounts of material involved, this negative result is not considered to be significant.

Poly-3-methyluridylic acid, synthesized by Szer and Shugar (1961), exhibited physical properties which were markedly different from those of polyuridylic acid. In contrast to 5-methyluridylic acid, 3-methyluridylic acid in place of uridylic acid residues of synthetic polymers does not encode phenylalanine (Wahba, 1963). It appears that the presence of 3-methyluridine and presumably 3-methylcytidine at selected sites in the RNA molecule significantly alters the properties of the RNA, perhaps more so than some of the other methylated bases found in RNA.

TABLE I

PAPER CHROMATOGRAPHY

	Rf Values				
	A	B	C	D	E
1-Ribosylthymine	0.21	0.55	0.71	0.34	0.52
3-Methyluridine (Syn.)	0.45	0.63	0.88	0.50	0.72
3-Methyluridine (Isol.)	0.45	0.63	0.88	0.50	0.72
Uridine	0.06	0.46	0.65	0.25	0.42
3-Methylcytidine (Syn.)	0.27	0.64			0.66
3-Methylcytidine (Isol.)	0.27	0.64			0.66

Solvent Systems: A - n-Butanol saturated with H_2O containing 5% concentrated NH_4OH; B - Isobutyric acid 400 cc, H_2O 208 cc, conc. NH_4OH 0.4 cc; C - Isopropanol 170 cc, conc. HCl 41 cc, H_2O to make 250 cc; D - Ethyl acetate: n-propanol: H_2O (4:1:2); E - Isopropanol: 5% NH_4OH solution (2:1).

TABLE II

ULTRAVIOLET ABSORPTION SPECTRA

	λ_{max} (pH 2.0)	Relative O.D.	λ_{max} (pH 11.0)	Relative O
3-Methyluridine (Syn.)	260	1.0	260	1.0
" (Isolated)	260	1.0	260	1.0
Alkali treatment of 3-methylcytidine	260	1.0	260	1.0
3-Methylcytidine (Syn.)	276	1.0	266	0.76
" (Isolated)	276	1.0	266	0.76

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