ISOLATION OF 1-METHYLINOSINE AND INOSINE FROM YEAST SOLUBLE RIBONUCLEIC ACID

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Soluble RNA (S-RNA) contains several methylated derivatives of the major purine components adenosine and guanosine. This communication describes the isolation of another purine derivative, 1-methylinosine, as well as the parent nucleoside, inosine, from S-RNA. The free base, 1-methylhypoxanthine, has been found in human urine (Weissmann, 1957) but not previously in RNA, and hypoxanthine has been isolated from the ribosomes of E. coli (Szafranski, 1962).

METHODS: A five-gram sample of yeast S-RNA purified, and hydrolyzed enzymatically as previously described (Hall, 1963) was fractionated by partition chromatography on a column of Celite-545* (column 1) to yield six fractions (see figure in the above reference). The fraction which contained guanosine was concentrated to dryness in vacuo and the residue was dissolved in 15 ml. of hot water. The sample was kept in the refrigerator for several days during which time most of the guanosine crystallized. The supernatant from this solution was lyophilized and the residue was fractionated on a partition column (150 g. of Celtie-545*, size 2.54 cm. x 80 cm. (column 2), according to the general procedure of Hall (1962a), using the solvent system, n-butanol-water-concentrated ammonium hydroxide (15:5:2). Two ultraviolet absorb-

* Johns-Manville Company, 3101 Euclid Avenue, Cleveland 15, Ohio.

ing peaks were obtained. The material in the first peak was contained in the 150 ml. to 450 ml. fraction, while that of the second was contained in the 800 ml. to 1200 ml. fraction. The middle third of fraction 1 was concentrated to a small volume and streaked across a sheet of Whatman 3 MM paper. This sheet was developed 24 hours (descending) in solvent D (see Table 1). Examination of the developed chromatogram under ultraviolet light revealed three bands. The band nearest the origin was identified as 5-methylcytidine and the fastest moving band as 2'-O-methylcytidine. The middle band consisted of a compound subsequently identified as 1-methylinosine.

An additional amount of 1-methylinosine was isolated from the fraction labelled B of column 1. The mixture of nucleosides in this fraction was partially resolved on a 150 gram column of Celite-545* (column 3), using the solvent system, ethyl acetate-n-propanol-water (4:1:2). The first portion of solvent (1100 cc.) to pass through the column eluted a mixture of methylated guanosines. The second portion (600 ml.) eluted a small quantity of ultraviolet absorbing material which was chromatographed on Whatman 3 MM paper for 24 hours in solvent D. Examination of the chromatogram revealed four distinct bands. The compound in band 1 was identified as guanosine, that in band 2 as 1-methylguanosine, and that in band 4 as 2'-O-methylcytidine. Band 3 consisted of two nucleosides which were resolved by further paper chromatography on Whatman 3 MM paper in solvent system A for 30 hours. These two compounds were identified as N^2-methylguanosine and 1-methylinosine.

Inosine was isolated from the second fraction of column two. This fraction also contained guanosine and the mixture of the two nucleosides was easily resolved by means of paper chromatography for 24 hours in solvent E.

The isolated samples of inosine and 1-methylinosine were identified by comparison of their properties with those of authentic samples as shown in

TABLE 1

PAPER CHROMATOGRAPHIC IDENTIFICATION

	Rf Values in Different Solvent Systems				
Compound	A	B	C	D*	E
Inosine	0.07	0.60	0.30	1.00	0.36
Inosine (isolated)	0.07	0.60	0.29	1.00	0.36
O^6-Methylinosine (syn.)	0.59	0.80	0.59	4.80	0.70
1-Methylinosine (syn.)	0.25	0.68	0.50	1.30	0.55
1-Methylinosine (isolated)	0.25	0.68	0.50	1.30	0.55
Hypoxanthine	0.11	--	0.30	2.00	0.39
Hypoxanthine (from isolated compound)	0.11	--	0.30	2.00	0.39
1-Methylhypoxanthine (syn.)	0.21	--	0.41	3.10	0.52
1-Methylhypoxanthine (from isolated compound)	0.21	--	0.40	3.10	0.53

Solvent Systems: A, n-butanol-H_2O-conc. NH_4OH (86:14:5); B, i-propanol-1% $(NH_4)_2SO_4$ Soln. (2:1); C, i-propanol-conc. HCl - H_2O (680:170:144); D, ethyl acetate-n-propanol-H_2O (4:1:2); E, i-propanol-5% NH_4OH Soln. (2:1).

* These values are relative to the movement of inosine.

Tables 1, 2 and 3. The authentic samples were obtained as follows: O^6-methylinosine was synthesized according to the method of Johnson and Schaeffer (1958), 1-methylinosine was prepared according to the method of Jones and Robins (1963), and the 1-methylhypoxanthine was a synthetic sample obtained from Dr. G. Elion, which had been prepared by an unambiguous route (Elion, 1962). The sugar of the isolated compounds was identified as ribose on the basis of the

following criteria: The electrophoretic mobility of both nucleosides was increased by addition of borate to the glycine buffer (Table 2). The sugar obtained by acid hydrolysis of each nucleoside had an Rf value identical with that of ribose when subjected to paper chromatography in system D (see Hall, 1962b, for table of Rf values of various sugars in this system).

TABLE 2

ELECTROPHORETIC IDENTIFICATION

Compound	Distance moved from origin (cm) 0.05 M glycine pH 9.2	0.01 M glycine 0.01 M borate pH 9.2
Inosine	+ 7.0	+ 17
Inosine (isolated)	+ 7.0	+ 17
1-Methylinosine (syn.)	- 3.5	+ 8
1-Methylinosine (isolated)	- 3.5	+ 8

Electrophoresis was conducted for four hours at 20 volts per centimeter in an apparatus made by the E. C. Apparatus Company, Hatsboro, Pennsylvania.

DISCUSSION: From five grams of yeast soluble RNA were obtained 0.8 mg. of 1-methylinosine and 6 mg. of inosine. The possibility that either or both of the substances arose by means of deamination of 1-methyladenosine or adenosine during the isolation procedure cannot be entirely discounted. In order to eliminate this possibility, adenosine and 1-methyladenosine were incubated with venom (Crotalus adamanteus) and bacterial alkaline phosphatase at pH 8.6 for 24 hours, which were the conditions used in the hydrolysis of the RNA sample. Paper chromatography conducted in a manner that would have permitted the detection of about 0.1% of the corresponding deaminated products revealed neither the presence of inosine nor 1-methylinosine. Furthermore, prolonged dialysis of the RNA prior to work-up, precludes these nucleosides as contamin-

TABLE 3

ULTRAVIOLET ABSORPTION SPECTRA

Compound	pH 2.0 max	pH 7.0 max	pH 11.5 max
Inosine	249	249	253
Inosine (isolated)	249	249	249
1-Methylinosine (syn.)	250	249	249
1-Methylinosine (isolated)	250	249	249
O^6-Methylinosine	250	250	250
Hypoxanthine	247	—	259
Hypoxanthine (from isolated compound)	247	—	259
1-Methylhypoxanthine (syn.)	249	—	260
1-Methylhypoxanthine (from isolated compound)	250	—	261

ants. Therefore these two nucleosides appear to be genuine constituents of yeast S-RNA.

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REFERENCES

Elion, G. B., J. Org. Chem., 27, 2478 (1962).
Hall, R. H., J. Biol. Chem., 237, 2283 (1962a).
Hall, R. H., Analytical Biochem., 4, 395 (1962b).
Hall, R. H., Biochem. Biophys. Res. Comm. 12, 361 (1963).
Johnson, J. A., Thomas, H. J., and Schaeffer, H. J., J. Am. Chem. Soc., 80, 699 (1958).
Jones, J. W., and Robins, R. K., J. Am. Chem. Soc., 85, 193 (1963).
Szafranski, P., and Lane, B. G., Biochim. Biophys. Acta, 61, 141 (1962).
Weissmann, B., Bromberg, P. A., and Gutman, A. B., J. Biol. Chem. 224, 407 (1957).