ISOLATION OF 2'-O-METHYLRIBONUCLEOSIDES FROM THE RNA OF MAMMALIAN TISSUES AND FROM E. COLI

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In addition to the minor components of RNA which contain methyl groups attached to the heterocyclic base, another series of minor components of RNA exist in which methyl groups are attached to the 2 position of ribose. Smith and Dunn (1959) first reported the occurrence of 2(3)-O-methylribose in the RNA of rat liver and plants and identified one of the nucleosides containing the sugar as 2'(3')-O-methyladenosine. This nucleoside was found in yeast S-RNA by Hall (1963) as the 2' isomer, and 2'-O-methylguanosine, 2'-O-methyluridine and 2'-O-methylcytidine were also isolated from this source. The purpose of this communication is to report isolation of all four of the 2'-O-methylribonucleosides from the microsomal and soluble fractions of RNA of mammalian tissues and from soluble RNA of E. coli.

Calf liver S-RNA was obtained from the International Chemical and Nuclear Corporation and E. coli S-RNA was obtained from General Biochemicals Incorporated. The microsomal fraction of sheep liver was prepared by conventional differential centrifugation of a tissue homogenate in a buffer of 0.25 M sucrose, 0.035 M tris, 0.002 M EDTA; pH 7.4. RNA was extracted from this fraction by the method used by Hall and Doty (1959). The RNA samples were hydrolyzed to their constituent nucleosides by means of snake venom and bacterial alkaline phosphatase as previously described (Hall, 1963). The nucleoside digest was fractionated on a partition column containing sodium borate. The solvent system for the column was prepared by vigorously shaking

three liters of n-butanol with one liter of water containing 38 g. of $Na_2B_4O_7 \cdot 10H_2O$ and 50 cc of concentrated ammonium hydroxide. The column (size; 2.54 cm. x 82 cm.) was packed with 140 g. of Celite-545 and 65 cc of lower phase according to the general procedure of Hall (1962). The sample (1 g.) was introduced onto the column by the technique described in this reference. The column was developed with 900 cc. of upper phase which eluted all four 2'-O-methylribonucleosides as well as contaminating deoxyribonucleosides. The effluent containing these products was concentrated to a small volume and streaked on Whatman 3 MM paper which was then developed for 36 hours in n-butanol saturated with 5% aqueous ammonium hydroxide. The separated ultraviolet absorbing bands were eluted and the products identified by comparison with authentic samples by means of paper chromatography (see reference (Hall, 1963) for table of Rf values). Each of the ultraviolet absorbing fractions so obtained which contained a 2'-O-methylribonucleoside was concentrated and restreaked on Whatman 3 MM paper. The second chromatograms were developed in the upper phase of the solvent system, ethylacetate : n-propanol : water (4 : 1 : 2) for 15 hours (descending manner) in the case of 2'-O-methylcytidine and eight hours in the case of the other three nucleosides. The amounts of the 2'-O-methylribonucleosides thus obtained were estimated spectrophotometrically using ϵ values of the parent ribonucleosides, and are expressed in the table as the number of milligrams isolated from 1,000 mg. of the mixed nucleoside digest.

	2'-O-Methyl Derivatives of			
RNA Sample	A	U	G	C
Sheep Liver Microsomes	2.4	1.9	2.1	2.5
E. coli S-RNA	0.05	0.6	1.1	0.6
Calf Liver S-RNA	2.0	2.0	1.7	1.1

Unlike the methylated base nucleosides which seem to occur only in S-RNA (Berquist, 1962), the methylated sugar nucleosides occur in both the soluble and microsomal fractions of mammalian RNA. The amount of the methylated sugar nucleosides in E. coli S-RNA is considerably less than in mammalian tissue.

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