On the 2′-*O*-Methylribonucleoside Content of Ribonucleic Acids*

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Received February 17, 1964

A procedure for isolating 2′-*O*-methylribonucleosides from ribonucleic acid (RNA) is reported. RNA is hydrolyzed by means of whole snake venom and bacterial alkaline phosphatase to its constituent nucleosides. The mixture of nucleosides is fractionated on a partition column of diatomaceous earth using a solvent system consisting of 1-butanol, ammonium hydroxide, and sodium borate solution. Under these conditions the 2′-*O*-methylribonucleosides precede the ribonucleosides from the column, which facilitates isolation of even small amounts of these nucleosides. Thirteen samples of ribosomal and soluble RNA and total RNA from yeast, bacterial, and mammalian tissue were analyzed. Twelve of these thirteen samples contained the 2′-*O*-methylribonucleosides of the four common bases. The total amount of the 2′-*O*-methylribonucleosides in most of the samples analyzed is of the order of 1% of the total nucleosides. In addition, a fifth 2′-*O*-methylribonucleoside, tentatively identified as 2′-*O*-methylpseudouridine, was isolated from three of the samples. The amount of this compound isolated is about 1% of the pseudouridine content of s-RNA.

The biological significance of the methylated nucleosides present in small amounts in RNA has been receiving increasing attention. Work originated by Mandel and Borek (1961, 1963) and Srinivasan and Borek (1963) and confirmed by others (Starr, 1963; Gold *et al.*, 1963) has demonstrated that in *E. coli* and in mammalian tissues methylation of the heterocyclic bases of soluble RNA occurs at the polymer level. These studies further indicate that the RNA of each species has a definite quota of methylated nucleosides which presumably are arranged in each RNA molecule in a specific pattern. This specificity suggests that the methylated components of RNA play an important role in the determination of the nature of the biological activity of the s-RNA molecules.

In addition to the large group of minor nucleosides of RNA in which methyl groups are attached to the heterocyclic base, another group of minor RNA nucleosides exists in which methyl groups are attached to the 2-*O* position of ribose. Smith and Dunn (1959) first isolated 2′(3′)-*O*-methyladenosine from the RNA of wheat germ and rat liver. This nucleoside was identified in yeast s-RNA as the 2′ isomer by Hall (1963b) who also isolated 2′-*O*-methylguanosine, 2′-*O*-methyluridine, and 2′-*O*-methylcytidine. The presence of this class of trace components in RNA raises additional questions concerning the role played by minor components in RNA. For example, an *O*-methyl group located at the 2′ position of a ribonucleoside will prevent this particular hydroxyl group from participating in any chemical reactions, a situation analogous to that of deoxyribonucleotides. Thus a considerable change in the characteristics of the internucleotide bond with respect to enzymic hydrolysis would be expected. An enzyme with the properties of pancreatic RNase would not hydrolyze bonds formed between 2′-*O*-methyluridine-3′-phosphate or 2′-*O*-methylcytidine-3′-phosphate and the 5′-hydroxyl of an adjacent nucleotide. These nucleosides could also modify the physical properties of the RNA molecule.

In order to provide more information about the distribution of the 2′-*O*-methylribonucleosides in the various RNA species, improved techniques of detection are required. Isolation of the 2′-*O*-methylribonucleosides solely by means of paper chromatography remains very difficult by virtue of the small amounts present in RNA. These nucleosides, however, can be isolated conveniently from enzymic digests of RNA by a column technique which makes use of the property that they do not form complexes with sodium borate while ribonucleosides will form such complexes. A preliminary communication describing this method has appeared (Hall, 1963a). The present paper presents details of this rapid analytical technique and records the amounts of the 2′-*O*-methylribonucleosides isolated from a variety of tissues.

* This work was partially supported by grants (CA-04640 and CA-05697) from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

EXPERIMENTAL

Yeast s-RNA was prepared according to the method of Holley *et al.* (1961) and dialyzed against three batches of distilled water at 5°, 8 hours each time. The sedimentation coefficient as determined by the sucrose-gradient-centrifugation technique of Britten and Roberts (1960) was 4 S. Calf liver s-RNA was a commercial sample obtained from the International Chemical and Nuclear Corp. *E. coli* s-RNA was a commercial sample obtained from General Biochemicals, Chagrin Falls, Ohio. These commercial samples were dialyzed as described above before hydrolysis. The phenol used in all the RNA extractions was freshly distilled before use and contained 10% water by weight.

RNA of Sheep Heart and Liver.—Total RNA was extracted by a method based on that of Kirby (1962). To 200 g of freshly thawed and chopped tissue was added 800 cc of ice-cold 0.015 M 1,5-naphthalene disulphonate, *p*H 6.8. This mixture was blended in a Waring Blendor for 3 minutes, then mixed with 800 cc of phenol containing 0.1% 8-hydroxyquinoline. The mixture was stirred vigorously with a paddle stirrer at room temperature for 20 minutes after which it was centrifuged at 4000 rpm for 10 minutes. The phenol layer was stirred 5 minutes with 100 ml of fresh buffer and then centrifuged as above. The combined aqueous layers were diluted with two volumes of ethanol. After 1 hour the precipitated RNA was recovered by low-speed centrifugation and washed with a 3:1 mixture of ethanol-water. The RNA was dissolved in 250 cc of ice-cold distilled water and centrifuged at 30,000 × *g* for 1 hour. To the supernatant was added 5.0 g potassium acetate followed by two volumes of ethanol. The solution was allowed to stand at 4° for 16 hours, after which the RNA was recovered by low-speed centrifugation. The dissolution and pre-

cipitation step was repeated twice more. The air-dried product obtained from the liver weighed 1.6 g, of which 563 mg was RNA as determined by spectrophotometric analysis (ϵ at 260 mμ = 7.9). The product obtained from heart weighed 375 mg and contained 80 mg of RNA. These RNA samples, which contained 0.85% DNA, were used without further purification. The 2′-*O*-methyl derivatives of uridine and cytidine were not detected in the heart RNA, but in view of the small size of the sample this circumstance was not unexpected.

Microsomal and Soluble RNA from Sheep and Normal Human Liver.[1] Each liver was treated by a method based on that of Hall and Doty (1959) as follows: The chopped liver (115-g portion) was homogenized with 300 cc of buffer solution (0.25 M sucrose, 0.035 M Tris, 0.002 M EDTA, *p*H 7.4) in a Waring Blendor for 5 minutes at 4°. The homogenate was centrifuged at 20,000 × *g* for 10 minutes. The precipitate was resuspended in 100 cc of the buffer solution and recentrifuged at 20,000 × *g* for 10 minutes. The combined supernatant fractions were centrifuged at 30,000 rpm in the number 30 rotor of the Spinco Model L centrifuge. The supernatant from this centrifugation was set aside for subsequent extraction of the s-RNA. The pellet was homogenized in 150 cc of the buffer solution and centrifuged at 20,000 × *g* for 10 minutes after which the supernatant was centrifuged at 30,000 rpm for 4 hours. The pellet from this centrifugation was washed once more as above. These preparations were examined in a Spinco Model E ultracentrifuge at 20° with schlieren optics. The ribosomes were suspended in a buffer consisting of 0.1 M sodium chloride, 0.01 M sodium phosphate, 0.01 M sodium citrate, *p*H 6.8, to give a concentration of approximately 2 mg/cc. Under these conditions the sedimentation coefficients of the chief components of both samples were 15 S and 37 S. The combined pellets from 400 g of liver were homogenized in 310 cc of 0.02 M sodium phosphate buffer (*p*H 7.0) and 1.9 g of sodium lauryl sulfate was added. The solution was stirred at 0° for 20 minutes, then shaken vigorously with 400 cc of phenol for 20 minutes. The phases were separated by centrifugation at 8000 rpm for 10 minutes. The aqueous phase was re-extracted with 250 cc of fresh phenol as above. The aqueous layer was extracted with ether three times; then the solution was dialyzed against three changes of distilled water for a total of 36 hours. The solution obtained from sheep liver contained 650 mg of RNA as determined spectrophotometrically and the corresponding solution from human liver contained 720 mg. The solutions were concentrated to 250 cc and the enzymic hydrolysis was carried out directly according to the procedure outlined.

The s-RNA fraction was obtained from the microsomal supernatant fraction by extraction with an equal volume of phenol for 20 minutes. After the phases were separated the aqueous layer was extracted three times with ether and dialyzed against distilled water overnight. The solution was evaporated *in vacuo* to 20% of the original volume and the RNA was precipitated by addition of three parts of ethanol in the presence of 2% sodium acetate. The precipitated RNA was collected by centrifugation and redissolved in 100 cc of water. These solutions were directly used in the subsequent hydrolysis procedure. The yield of RNA was: human liver, 600 mg; sheep liver, 300 mg (spectrophotometric estimation ϵ at 260 mμ = 7.9). The sedimentation coefficient (Britten and Roberts, 1960) of these samples was 4 S.

Extraction of Total RNA from Tumor Tissue.—Method A: Use of buffer containing 1,5-naphthalene disulfonate (Kirby, 1962).—The total RNA fractions of two human tumors were extracted by the method described above for the total RNA of sheep liver and heart. The first tumor, a rhabdomyosarcoma (584 g), was removed from a patient during an operation and frozen within 30 minutes. This tissue yielded 115 mg of RNA which was contaminated with 4.5% DNA (the amount of DNA was calculated from the amount of deoxyribonucleosides subsequently isolated). The second sample was obtained from a liver which was riddled with metastatic tumor (obtained at autopsy 2.5 hours following death). The primary lesion was in the lung. The large metastases were dissected away from the normal tissue and frozen immediately (weight 500 g). This sample yielded 340 mg of RNA which contained 8% DNA. In our hands this method was not at all satisfactory for tissues with a relatively high ratio of DNA to RNA, and we therefore developed the following method based on the observation (Brawerman *et al.*, 1962) that the *p*H of the buffer is critical to the extraction of DNA.

Method B: Use of buffer at *p*H 6.0.—This method was applied to two tumors, the Murphy-Sturm lymphosarcoma of the rat and Ehrlich ascites tumor of the mouse. The freshly thawed solid tumor (100 g) was homogenized with four volumes of buffer, 0.01 M NaCl, and 0.01 M NaH_2PO_4, *p*H 6.0. The *p*H of the homogenate was readjusted to 6.0 at this point if necessary. In a like manner 300 cc of frozen and thawed ascites fluid was homogenized with three volumes of the buffer and the *p*H was adjusted to 6.0. The remainder of the procedure was identical for both tissues.

The homogenate was shaken with an equal volume of phenol on a reciprocating shaker after which the aqueous layer was re-extracted with one-half volume of phenol. The aqueous layer was concentrated in a rotating evaporator to a volume of 50 cc. One g of sodium acetate was dissolved in this solution and 100 cc of ethanol was added. The solution was allowed to stand for 24 hours at −10° and the RNA was recovered by low-speed centrifugation. The precipitate was dissolved in 50 cc of water and centrifuged at 33,000 × *g* for 1 hour. The RNA was precipitated from the supernatant fluid in the presence of sodium acetate as above. The RNA was redissolved in water and centrifuged at 33,000 × *g* for 1 hour; the supernatant fluid was used directly for the enzymic hydrolysis step. The yield of RNA was estimated spectrophotometrically and the amount of DNA was calculated from the amount of thymidine subsequently isolated; 200 g of Murphy-Sturm lymphosarcoma yielded 450 mg RNA (0.07% DNA) and 1200 cc of Ehrlich ascites fluid yielded 550 mg RNA (0.05% DNA).

Enzymic Hydrolysis of RNA.—All the above samples of RNA were hydrolyzed according to the following procedure described for yeast s-RNA. A sample (1.25 g) which contained 608 mg of RNA was dissolved in 100 cc of water to which was added 0.1 cc of 1 M magnesium sulfate solution, 100 mg of lyophilized snake venom (*Crotalus adamanteus*), and 2.5 mg of purified bacterial alkaline phosphatase (Worthington Biochemical Corp.). The *p*H of the solution was immediately adjusted to 8.8 with 0.5 N sodium hydroxide and it was gently stirred with a paddle stirrer at 37°. The *p*H was maintained at 8.8 with further additions of 0.5 N sodium hydroxide. The theoretical amount of alkali was usually consumed within 8 hours; however, incubation was continued for a total of 24

[1] The sheep liver was removed and frozen immediately after slaughter of the animal. The human liver was removed at autopsy 4 hours following death.

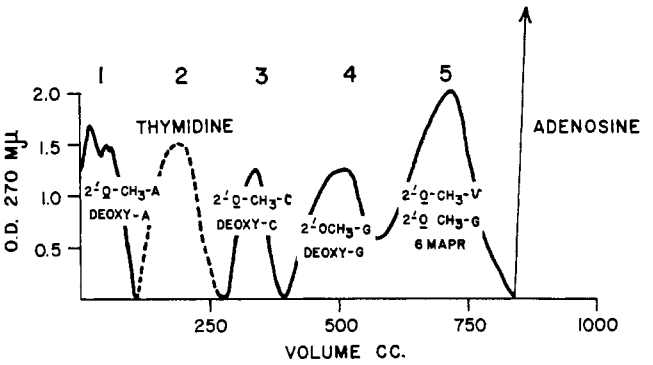

Fig. 1.—Enzymatic hydrolysate of a sample of yeast s-RNA (total weight 1.25 g, containing 450 mg of mixed nucleosides) fractionated on a partition column containing sodium borate. The elution pattern of the 2′-*O*-methylribonucleosides, deoxyribonucleosides, and 6-methylamino-9-(β-D-ribofuranosyl)purine (6-MAPR) is shown.

hours. Toluene was added to the solution during the incubation period to prevent bacterial contamination. At the conclusion of the incubation period, the solution was adjusted to *p*H 8.2 and heated at 60° for 30 minutes, and left at 4° for several hours. The solution was centrifuged at 30,000 × *g* for 1 hour and the clear supernatant was lyophilized. The lyophilized sample was the starting material for the column separation.

Partition Chromatography of the Nucleoside Digest.—Hydrated sodium borate ($Na_2B_4O_7 \cdot 10\ H_2O$; 38 g) was dissolved in 1 liter of warm water. After standing overnight at room temperature, the solution was filtered and 5 cc of concentrated ammonium hydroxide and 3 liters of 1-butanol were added.[2] The biphasic solution was shaken on a reciprocating shaker for 30 minutes, after which the phases were permitted to separate. A glass precision-bore tube 2.54 × 92 cm was dry-packed with 140 g of Celite-545[3] which had been mixed thoroughly with 65.33 cc of lower phase. (See previous paper for complete details on this method of packing partition columns [Hall, 1962].) The lyophilized sample of RNA (total weight 1–2 g) was dissolved in 10–15 cc of lower solvent phase (the *p*H was adjusted to a value between 8.2 and 8.6) and this solution was mixed thoroughly with exactly 2.15 g of Celite-545 for each cc of solution. The free-flowing mixture was packed on top of the column. The column was eluted with the upper phase of the solvent system at a flow rate of 150 cc/hour. (If the solvent was not clear, it was filtered by gravity through Whatman No. 1 paper.) The optical density of the effluent was monitored continuously, using a flow cell with a 5-mm path length. The elution patterns for each run were very similar; about 650–850 cc of solvent eluted all the 2′-*O*-methylribonucleosides.

The elution pattern for a sample of yeast s-RNA is shown in Figure 1. This material contained a negligible amount of thymidine, but in those samples which contained larger amounts of thymidine an additional peak appeared at the place indicated by the dotted line in Figure 1. The fraction corresponding to the fifth peak contained 6-methylamino-9-(β-D-ribofuranosyl)-purine in addition to 2′-*O*-methylguanosine and 2′-*O*-methyluridine. A large peak corresponding to adenosine next appeared, and if elution of the column was continued the other ribonucleosides were eventually removed. Recovery of the ribonucleosides from the effluent of this type of column is not very practical because of the sodium borate content.

Chromatographic Separation.—Fractions corresponding to each of the peaks of Figure 1 were concentrated in a rotating evaporator (bath temperature 30°) and the entire concentrate of each fraction was streaked across a strip of Whatman 3 MM paper (16.5 cm wide). The paper strips were developed in a solvent system according to the protocol in Table II. The desired bands were cut out and eluted with distilled water. The amount of each nucleoside was estimated spectrophotometrically (ε values used were the same as for the parent ribonucleoside) and the results are shown in Table I. In some experiments peaks 3 and 4 were incompletely separated. The substances corresponding to these peaks were first chromatographed in solvent system B for 16 hours which separated the deoxyribonucleosides from the 2′-*O*-methylribonucleosides. 2′-*O*-Methylcytidine and 2′-*O*-methylguanosine were obtained in the same band and they were separated by

[2] The amount of ammonia was reduced from that previously reported (Hall, 1963a) without affecting the performance of the column.

[3] Celite-545 is a Johns-Manville brand of diatomaceous earth.

TABLE I

OCCURRENCE OF 2′-*O*-METHYLRIBONUCLEOSIDES IN RNA

Source	Amounts of 2′-*O*-Methylribonucleosides (moles/1000 moles total nucleosides in enzymic digest) 2′-*O*-Methyl Derivative of A	U	G	C
Sheep liver (total)	2.1	3.1	2.7	2.6
Sheep liver (microsomal)	2.3	2.5	2.8	2.6
Sheep liver (soluble)	1.1	2.2	1.3	1.2
Calf liver (soluble)[a]	1.9	2.1	1.6	1.1
E. coli (soluble)[a]	0.05	0.6	1.0	0.6
Yeast (soluble)	0.3	0.6	2.5	2.8
Human liver (soluble)	1.9	2.2	3.0	3.4
Human liver (microsomal)	0.8	0.4	1.5	0.9
Metastatic tumor in liver (total)	0.4	[b]	0.6	0.6
Rhabdomyosarcoma (total)	0.6	1.1	1.8	0.6
Mouse Ehrlich ascites (total)	1.7	2.0	2.3	1.4
Murphy-Sturm lymphosarcoma (total)	3.0	2.1	3.0	2.0
Sheep heart (total)	2.6	[c]	3.8	[c]

[a] Data taken from Hall (1963a). [b] Nucleoside was present but was not quantitatively isolated. [c] Nucleoside not detected.

TABLE II

PAPER CHROMATOGRAPHY OF THE COLUMN ELUATES

Peak	Solvent[a]	Development Time (hr)	Nucleoside	Distance from Origin (cm)
1	B	8	Deoxyadenosine	20
			2′-*O*-Methyladenosine	27.3
3	B	30	Deoxycytidine	13
			2′-*O*-Methylcytidine	22.5
4	B	16	Deoxyguanosine	5.9
			2′-*O*-Methylguanosine	10.5
5	A	40	6-Methylaminopurine-riboside	37.7
			2′-*O*-Methylguanosine	7.9
			2′-*O*-Methyluridine	13

[a] Solvent systems: A, 1-butanol–water–concd ammonium hydroxide, 86:14:5; B, ethyl acetate–1-propanol–water, 4:1:2.

TABLE III
PAPER CHROMATOGRAPHY OF 2′-O-METHYLPSEUDOURIDINE

Compound	Solvent System[a]		
	B[b]	C[b]	D[c]
2′-O-Methylpseudouridine	1.7	2.6	0.85
ψ-Uridine	1.0	1.0	0.69
Uracil	1.7	2.0	0.54
Thymine	2.0	2.7	0.62

[a] Solvent systems: B, see footnote to Table II; C, isopropanol–ammonium hydroxide–water, 7:1:2; D, isopropanol (170 cc), concd hydrochloric acid (41 cc), and water to make 250 cc. [b] The values for solvent systems B and C are movement relative to ψ-uridine. [c] The values for solvent system D are R_F.

TABLE IV
PAPER ELECTROPHORESIS[a] OF 2′-O-METHYLPSEUDOURIDINE

Compound	0.05 M Tris (pH 7.5) (cm)	0.05 M Tris, 0.05 M Borate (pH 7.5) (cm)
2′-O-Methylpseudouridine	−4.0	−3.2
ψ-Uridine	−3.0	+4.0

[a] Electrophoresis conducted for 4 hours at 22 v/cm. Values are distances moved from the origin.

rechromatographing in solvent system C for 20 hours. The identity of the four 2′-O-methylribonucleosides was established by their ultraviolet absorption spectra and a comparison of the R_F values with those of authentic compounds in the solvent systems listed in the previous paper (Hall, 1963b).

Other Minor Nucleosides.—6-Methylamino-9-(β-D-ribofuranosyl)purine (6-MAPR) was found in all the samples of RNA analyzed. It appeared regularly just ahead of adenosine in the column analyses (Fig. 1). In addition to this nucleoside, most of the samples of RNA contained trace amounts of ultraviolet-absorbing compounds whose spectra did not correspond to any of the four major nucleosides. These trace components were distributed throughout the effluent corresponding to the five peaks of Figure 1. This position in the elution pattern suggests that these compounds are nucleosides which do not contain ribose and indeed one of these trace compounds, on the basis of preliminary evidence, appears to be 2′-O-methylpseudouridine. This compound was isolated from the samples of yeast s-RNA, Murphy-Sturm lymphosarcoma total RNA, and sheep liver s-RNA.

In each experiment the fraction of the column effluent corresponding to approximately the first quarter of the adenosine peak (Fig. 1) was chromatographed on Whatman 3 MM paper for 20 hours in solvent C which resulted in the separation of the starting material into three bands. The middle band was rechromatographed in solvent system B for 10 hours. Three bands were obtained which consisted of adenosine, deoxyguanosine, and presumed 2′-O-methylpseudouridine (fastest band). The compound eluted from this band moves as a single spot upon paper chromatography in three systems (Table III). The electrophoretic mobility at pH 7.5 is not changed by the presence of borate ion as shown in Table IV. The ultraviolet-absorption spectra of the isolated compound, shown in Figure 2, resembles that of pseudouridine (Cohn, 1959) and therefore the compound correspondingly may be 2′-O-methylpseudouridine. There is

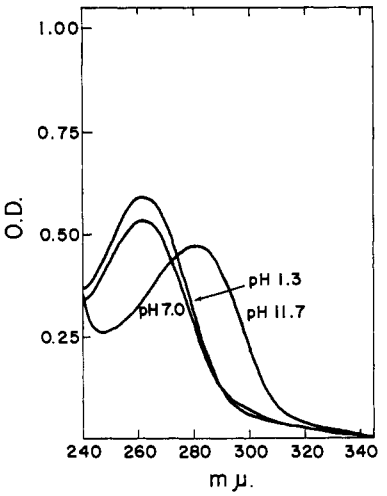

FIG. 2.—Ultraviolet absorption spectra of presumed 2′-O-methylpseudouridine.

no direct proof that this is the case but the similarity of the ultra-violet-absorption spectra and the fact that the compound moves faster than pseudouridine upon paper chromatography make this a reasonable assumption. The most reliable quantitative data were obtained from the experiment with the sheep liver s-RNA in which the yield of presumed 2′-O-methylpseudouridine was 0.2 mg per g of s-RNA, as estimated spectrophotometrically.

DISCUSSION

The column separation and recovery of the separated 2′-O-methylribonucleosides is very reproducible. In model experiments using known amounts of nucleosides, final recovery was reproducible within 5%. The main source of error in this analytical procedure could arise in the enzymatic hydrolysis of the RNA. The completion of the enzymic hydrolysis was determined on the basis of uptake of alkali and release of inorganic phosphate, but these criteria do not preclude existence of difficultly hydrolyzable RNA fragments containing a high proportion of the 2′-O-methylribonucleosides. These compounds in the form of nucleotides would not pass through the column. A comparison of the values for sheep liver total and sheep liver ribosomal RNA as well as the results of analyses of two separate samples of yeast s-RNA suggest that overall accuracy of the method is of the order of ±15%. The borate-column method represents a distinct improvement over the previously described procedure (Hall, 1963b) from the standpoint of simplicity and efficiency of recovery of the total 2′-O-methylribonucleosides. The only complicating factor is the presence of deoxyribonucleosides in the enzymic digest of the RNA. Since final isolation of the 2′-O-methylribonucleosides would be simplified if the deoxyribonucleosides were absent, elimination of DNA from the original RNA extract becomes highly desirable. In the latter part of the program this goal was most nearly achieved by extracting the tissue with phenol at pH 6.0.

The 2′-O-methylribonucleosides appear to be widely distributed in nature where they are found in both the high- and low-molecular-weight fractions of the RNA of several organisms. The presence of similar amounts of the 2′-O-methylribonucleosides in both the ribosomal and s-RNA (Table I) is in contrast to the distribution of the ribonucleosides having methylated bases which are found predominantly (or even exclusively) in s-RNA (Bergquist and Matthews, 1962). The total

amount of 2-*O*-methylribose in the RNA samples falls into a range of about 0.5–1% of the ribose content. The rather uniform levels in both the microsomal and s-RNA suggest that perhaps there is a statistical distribution of the 2′-*O*-methylribose throughout the RNA. This implies that about one in every 100 ribose residues per se contains the 2-*O*-methyl group and therefore the ratio to each other of the 2′-*O*-methylribonucleosides in the total RNA sample would reflect the ratios of the parent ribonucleosides to each other. The data of Table I, taking into consideration the accuracy of the analyses, permit some general observations in this regard. In the s-RNA of several species there is near equivalence of the molar ratios of adenosine to uridine and guanosine to cytidine, with the latter pair predominating. For example, this is the case in yeast s-RNA (Monier *et al.*, 1960) and in rat liver s-RNA (Lipshitz and Chargaff, 1960). The amounts of 2′-*O*-methylguanosine and of 2′-*O*-methylcytidine are nearly equivalent in the yeast s-RNA and human liver s-RNA, and further, this pair is present in significantly larger amounts than the 2′-*O*-methyladenosine and 2′-*O*-methyluridine pair. Morisawa and Chargaff (1963) also observed a preponderance of 2′-*O*-methylguanosine and 2′-*O*-methylcytidine in the RNA of rat liver and s-RNA of yeast. A statistical distribution of 2′-*O*-methylribose should also be reflected in the occurrence of nucleosides in RNA consisting of the so-called minor bases and this sugar. The anticipated levels would be very small indeed. The isolation of a new nucleoside, tentatively identified as 2′-*O*-methylpseudouridine, lends support to this contention. The isolated amount of this compound is about 1% of the amount of pseudouridine in s-RNA.

ACKNOWLEDGMENT

The author wishes to acknowledge the skilled technical assistance of Mr. L. Stasiuk and Mr. J. Mozejko. He also wishes to acknowedge the interest and support of Dr. C. A. Nichol in this program.

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Purine *N*-Oxides. XII. Photochemical Changes Induced by Ultraviolet Radiation*

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Received February 27, 1964

Adenine 1-*N*-oxide is found to be very sensitive to ultraviolet radiation. For it, and related purine derivatives, two main pathways of decomposition are observed: direct loss of oxygen, and rearrangement of the oxygen to the adjacent carbon.

Since the correlation (Hollander and Emmons, 1941; Stadler and Uber, 1942) of the ultraviolet absorption of nucleic acids with the wavelengths most efficient in producing mutations, there have been studies of the effects of ultraviolet light (Kland and Johnson, 1957) and of other ionizing radiations (Scholes and Weiss, 1952; Barron *et al.*, 1952; Daniels *et al.*, 1955; Hems, 1958, 1960; Ponnamperuma *et al.*, 1961, 1963) on purine derivatives. With high doses, some deamination of adenine to hypoxanthine (Kland and Johnson, 1957; Ponnamperuma *et al.*, 1961), a more extensive opening of the imidazole rings (Hems, 1960; Ponnamperuma *et al.*, 1961), and some formation of 8-hydroxyadenine (Ponnamperuma *et al.*, 1963) are the major alterations observed with the purine bases.

* This investigation was supported in part by funds from the Atomic Energy Commission (Contract No. AT[30-1]-910), and from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (CA-03190-07).

Because of the ease of oxidation of adenine nucleotides to their 1-*N*-oxides by neutral hydrogen peroxide (Stevens *et al.*, 1959; Cramer and Randerath, 1958; Cramer *et al.*, 1963) and of the secondary production of peroxides by ionizing radiation (Scholes and Weiss, 1952), we first attempted the production of an adenine *N*-oxide derivative by the action of ultraviolet light or X irradiation without success, for reasons now obvious. Subsequently we found that 50% of adenine oxide in an unbuffered aqueous solution may be altered in 45 minutes by an intensity of ultraviolet light which does not measurably alter adenine in 4 days; it is altered with the remarkably high quantum efficiency of 0.1 (Levin *et al.*, 1964). It is also more sensitive to γ irradiation from ^{60}Co than is adenine (Levin *et al.*, 1964).

EXPERIMENTAL

Irradiation.—A Hanovia quartz lamp, Type S-100, with a Corning No. 9863 filter transmitting strongly