We are obliged to Miss Jane M. Manniello for amino acid analyses.

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Isolation and Characterization of 5′-*S*-Methyl-5′-thioadenosine from *Escherichia coli**

T. Ming Chu,† M. F. Mallette, and Ralph O. Mumma

ABSTRACT: A naturally occurring sulfur-containing nucleoside, 5′-*S*-methyl-5′-thioadenosine, was isolated from *Escherichia coli* B which was grown in a medium consisting of inorganic salts and glucose without the addition of methionine. The nucleoside was found only in the *E. coli* cells, in a concentration of 0.38 μmole/g of dry cells. The chemical and physical properties of this ^{35}S-labeled nucleoside and its derivatives were studied using gas, thin-layer, and paper chromatography, paper electrophoresis, and mass and ultraviolet spectroscopy. Microgram quantities of this nucleoside were isolated by means of thin-layer chromatography (solvent system, $CHCl_3$–CH_3OH–H_2O, 65:25:4, v/v). This adenosine derivative has its ultraviolet absorption maximum at 259.5 mμ (pH 7 and 10) and at 257 mμ (pH 2).

It possesses a pK_a' value of 3.4. 5′-*S*-Methyl-5′-thioadenosine was synthesized, and it has chemical and spectral properties identical with those of the isolated compound.

The occurrence of 5′-*S*-methyl-5′-thioadenosine (MTA)[1] in yeast extracts was first reported by Mandel and Dunham (1912), and its structure has been well established by a number of investigators (Suzuki *et al.*, 1924; Levene and Sobotka, 1925; Satoh, 1953). The addition of methionine to yeast cultures resulted in significant increases in the amount of MTA that could be isolated from the yeast. It was subsequently shown that the isolation of MTA was totally or partially the result of heat decomposition of *S*-adenosylmethionine during its extraction by boiling water (Schlenk and DePalma, 1957). However, enzymatic formation of MTA was later demonstrated by Shapiro and Mather (1958), and an enzyme was isolated from

* From the Pesticide Research Laboratory and Graduate Study Center, Departments of Biochemistry and Entomology, The Pennsylvania State University, University Park, Pennsylvania 16802. *Received November 29, 1967.* Presented in part before the Division of Biological Chemistry, 154th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1967. This work was supported in part by the U. S. Public Health Service Grant AM08481. Authorized for publication on Nov. 16, 1967, as Paper No. 3325 in the Journal Series of the Pennsylvania Agricultural Experimentation Station.

† Present address: Medical Foundation of Buffalo, Buffalo, N. Y. 14203.

[1] Abbreviation use that is not given in *Biochemistry 5*, 1445 (1966), is: MTA, 5′-*S*-methyl-5′-thioadenosine.

bakers' yeast which converts *S*-adenosylmethionine into MTA (Mudd, 1959). Yeast cultures have also been shown to assimilate MTA and convert it into *S*-adenosylmethionine (Schlenk and Ehninger, 1964).

Recently the biosynthesis of spermidine in *Escherichia coli* has been studied (Tabor and Tabor, 1964). It involves a transalkylation in which decarboxylated *S*-adenosylmethionine transfers its aliphatic side chain to putrescine to form spermidine. MTA was proposed to be one of the end products of this series of reactions. This communication reports the isolation and identification of 5′-*S*-methyl-5′-thioadenosine from *E. coli*.

Materials and Methods

Media. E. coli B was grown in a medium consisting of inorganic salts and glucose (7.36 g of K_2HPO_4, 2.96 g of KH_2PO_4, 1.0 g of NH_4Cl, 0.5 g of NaCl, 0.041 g of $MgSO_4 \cdot 7H_2O$, 0.405 g of $MgCl_2 \cdot 6H_2O$, and 4.0 g of glucose per l.). Media containing radioactive sulfate were prepared in the same manner except that the desired level of radioactivity (1 mCi/50 ml of medium) was added to the solution prior to autoclaving.

Method of Culture. Cells were grown in a sterilized 5-l. flask stoppered with a cotton plug. The growth medium (500 ml) was loop inoculated with *E. coli* B from a working slant. The cultures were incubated at 37° in a CS and E stationary incubator (Lab-Line Instruments, Inc.). Turbidity was measured with a Bausch & Lomb Spectronic 20 spectrophotometer at 650 mμ. The cells were harvested in log phase at an optical density of 0.4–0.5, which was approximately the growth level of cells reached at the end of 10–12 hr. The pH of the medium at this time was 5–6.

Extraction and Isolation Procedures. All the cultures were harvested in the same manner. When the optical density of the cell culture reached 0.4–0.5, the cell suspension was centrifuged at 0–3° at 4500*g* for 5 min in a Sorvall RC-2 centrifuge. After decanting the medium, cells were washed twice with distilled water and immediately extracted by a slightly modified procedure of Kates and Volcani (1966). This method involves the extraction of cells and the concentration of extracts at room temperature. The packed cells (*ca.* 1 ml) were resuspended in water (4.8 ml) and to this suspension were added 12 ml of methanol and 6 ml of chloroform; the mixture was shaken and stored overnight at room temperature. The next morning 6 ml of chloroform and 6 ml of water were added to the extract; the mixture was then shaken briefly and centrifuged. The water and the chloroform phases were carefully separated, evaporated to dryness in a stream of nitrogen, and the residues immediately dissolved in 1 ml of either water or chloroform. An aliquot of the medium was also concentrated and later analyzed.

Aliquots of the above extracts were separated by paper or thin-layer chromatography. Labeled compounds, responsible for the radioactive areas observed by radioautography, were eluted from the adsorbent. All isolated compounds were rechromatographed to check their purity. These eluates were used for subsequent investigation.

Chromatography. All paper chromatograms were run two dimensionally on Whatman No. 4 filter paper (464 × 571 mm). The developing solvent for the first (long) direction was phenol–water (100:40, w/w) and for the second direction was *n*-butyl alcohol–propionic acid–water (200:100:140, v/v). Thin-layer chromatography with silica gel (Adsorbosil-2) as the adsorbent at thicknesses of 0.30 and 0.50 mm was used extensively. The solvent system chloroform–methanol–water (65:25:4, v/v) proved to be the most effective. Other solvent systems used in thin-layer chromatography were ethanol–water (70:30, v/v), *n*-butyl alcohol–water (70:30, v/v), *n*-butyl alcohol–acetic acid–water (80:20:20, v/v), and *n*-butyl alcohol–methyl ethyl ketone–formaldehyde–water (40:30:15:15, v/v). All reaction products were analyzed by paper and thin-layer chromatography and paper electrophoresis.

Gas chromatography of the trimethyl silyl ethers of the nucleosides, prepared with bis(trimethylsilyl)-acetamide (Sasaki and Hashizume, 1966), was conducted on a Barber-Coleman Model 5000 gas chromatograph with a 3% OV-1 (dimethylsilicone) column at 232°. Retention times were based upon both the appearance of peaks and upon the radioactivity of the trapped samples.

Electrophoresis. The electrophoretic properties of the compounds were studied at different pH values with a Reco-Electrophoresis Migration Chamber, Model E800-2. The following buffers were used: 0.1 and 0.01 M hydrochloric acid for pH 1 and 2, hydrochloric acid–glycine for pH 3, citric acid–sodium citrate for pH 4–5, sodium dihydrogen phosphate–disodium hydrogen phosphate for pH 6 and 7, and sodium carbonate–sodium bicarbonate for pH 10. A potential of 400 V for a period of 2 hr was used.

Spectrometry. Ultraviolet spectra were obtained with a Cary Model 14 spectrophotometer. Dilute hydrochloric acid (0.01 M), ethanol, and 0.05 M sodium carbonate–sodium bicarbonate were used as the solvents.

Mass spectra were obtained with a Nuclide Associates Model 1290-G mass spectrophotometer. A source temperature of 200–270° and ionization voltages of 20 and 70 eV were used.

Determination of Radioactivity. Radioactivity (counts per minute) was determined either by liquid scintillation counting (Packard Tri-Carb liquid scintillation spectrometer, Model 526) or by the use of a pancake Anton counter tube. Radioautograms were obtained with Kodak Single Coated Medical X-Ray film. Exposure times of 1–10 days were used.

Acid Hydrolysis. The desired compounds plus 0.20 ml of 1.0 N hydrochloric acid were heated in a sealed ampule for 2 hr at 100°.

Base Hydrolysis. The desired compounds were dissolved in 1 ml of 0.1 N KOH in methanol for 30 min at 37°.

Acetylation. The sample was dissolved in 0.30 ml of acetic anhydride and 0.10 ml of pyridine. The resulting solutions were allowed to stand at 37° for 12 hr.

Reaction with Sodium p-Hydroxymercuribenzoate. The samples were dissolved in 0.20 ml of 0.1 M phosphate buffer (pH 7.0), and to this solution was added 0.20 ml of 0.01 M sodium *p*-hydroxymercuribenzoate solution. The mixture was allowed to stand at room temperature for 1 hr.

Reaction with Iodomethane. The samples were dissolved in 0.5 ml of methanol, to which was added 1 ml of redistilled iodomethane. The resulting solution was shaken in the dark at room temperature for 20 hr.

Test for Aldehyde and Keto Groups. The samples were dissolved in 0.20 ml of 2,4-dinitrophenylhydrazine reagent and stored at room temperature for 6 hr. The reagent was prepared by dissolving 1.5 g of 2,4-dinitrophenylhydrazine in 7.5 ml of concentrated sulfuric acid. This solution was then added with stirring to 10 ml of water and 35 ml of 95% ethanol.

Reaction with Ninhydrin. The samples were dissolved in 0.2 ml of 0.15% ninhydrin in ethanol and allowed to stand at room temperature for 1 hr.

Reaction with Nitrous Acid. To the unknown compounds were added 1 drop of concentrated hydrochloric acid, 1 drop of water, and 0.25 ml of sodium nitrite solution (1 g of sodium nitrite in 5 ml of water) in an ice bath. After 1 min the reaction mixture was evaporated under nitrogen, and the residue was taken up with 0.1 ml of water and analyzed.

Reaction with 2,4-Dinitrofluorobenzene. The samples were dissolved in 0.1 ml of 10% sodium bicarbonate solution and shaken at room temperature in the dark for 4 hr with 0.1 ml of a 10% ethanolic solution of 2,4-dinitrofluorobenzene. The unreacted dinitrofluorobenzene was removed with ether. The reaction mixture was acidified with 1 drop of 0.1 N hydrochloric acid, evaporated to dryness under nitrogen, and the residue was dissolved in 0.1 ml of 95% ethanol and analyzed by chromatography.

Oxidation. The compounds were oxidized either by exposure to air or by 30% hydrogen peroxide at room temperature for 2 hr.

Reaction with Periodate. The sample was dissolved in 0.1 ml of 0.05 M potassium periodate–0.3 N H_2SO_4 and allowed to stand at room temperature for 6 hr. To this mixture was added 1 drop of bromine water in order to oxidize any aldehydes produced in the reaction. The resulting solution was evaporated, and the residue was redissolved in 0.10 ml of water and analyzed. No attempt was made to follow this reaction quantitatively.

Synthesis of 5′-S-Methyl-5′-thioadenosine. 5′-*S*-Methyl-5′-thioadenosine was synthesized by preparing the 5′-toluene-*p*-sulfonate of 2′:3′-isopropylideneadenosine followed by replacement of the toluene-*p*-sulfonate group with methylthio- and hydrolysis of the isopropylidene residue (Baddiley, 1951). The crystalline product (mp 202°) was obtained in 25% yield. The ^{35}S-labeled product was synthesized using [^{35}S]sodium methyl sulfide.

Results

Extraction and Characterization. The *E. coli* cells were extracted with chloroform–methanol at room temperature according to the method of Kates and Volcani (1966). Chloroform and water extracts of the cells and of the medium were prepared.

Among the numerous spots appearing on a radioautogram of a two-dimensional paper chromatogram of the water extract of ^{35}S-labeled *E. coli* were I and II, the compounds identified in this report. Compounds I and II were the most intense spots on the radioautogram, representing 15% of the total radioactivity in the water extract. They possess R_F values of 0.93 in phenol–water and 0.64 and 0.43 in *n*-butyl alcohol–propionic acid–water, respectively. Compound II was not present in freshly prepared extracts but was chiefly an artifact of paper chromatography. Compound I was partially oxidized to II following the phenol–water direction. Only one intense spot was found on the radioautogram if the directions were reversed. This same effect has been observed by other investigators (Edwards *et al.*, 1959; Fisher and Mallette, 1961; Faith and Mallette, 1966) for other sulfur compounds.

Compound I was mainly concentrated in the water extract of the cells and completely absent in the medium. The lipid solvent system, chloroform–methanol–water (65:25:4, v/v), proved to be very useful on thin-layer chromatography of I. Compound I migrates just above the phospholipid, phosphatidylethanolamine, and thus behaves somewhat unexpectedly like a lipid on thin-layer chromatography; it is, however, water soluble. Since most of the sulfur-labeled compounds in the water extract do not move in this thin-layer chromatography solvent system, compound I was separated discretely and could be easily isolated pure in microgram quantities for ultraviolet and mass spectroscopic analysis.

Methionine, ethionine, and 3-ethylthiopropionic acid have been shown to be oxidized partially to their sulfoxide derivatives following the phenol–water direction on paper chromatography (Edwards *et al.*, 1959; Fisher and Mallette, 1961; Faith and Mallette, 1966). Thus the sulfoxide derivative formed following chromatography is always found on the two-dimensional paper chromatogram immediately beneath the parent molecule containing a sulfide linkage. If one reverses the solvent order, then only one spot is observed. The paper chromatographic properties of compounds I (R_F 0.93 and 0.64) and II (R_F 0.93 and 0.43) show positional patterns similar to those of methionine (R_F 0.78 and 0.49), methionine sulfoxide (R_F 0.79 and 0.26), ethionine (R_F 0.81 and 0.58), ethionine sulfoxide (R_F 0.81 and 0.30), 3-ethylthiopropionic acid (R_F 0.54 and 0.63), and 3-ethylthiopropionic acid sulfoxide (R_F 0.54 and 0.29) in phenol–water and in *n*-butyl alcohol–propionic acid–water. These results thus suggested that I and II were sulfide and sulfoxide derivatives, respectively. Compounds I and II were soluble in water, methanol, ethanol, and even chloroform. The partition coefficient between water and chloroform was approximately 50:1.

Electrophoretic Properties. The ionic natures of I and II were established by studying their electrophoretic mobilities at different pH values. It was found that both

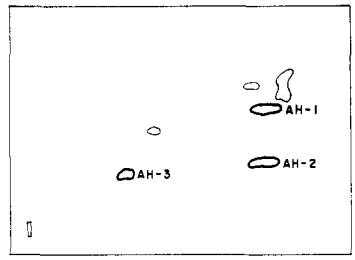


FIGURE 1: Tracing of radioautogram of a paper chromatogram of the acid hydrolysis products of I. The solvent system in the horizontal direction is phenol–water (100:40, w/w) and in the vertical direction is *n*-butyl alcohol–propionic acid–water (200:100:140, v/v).

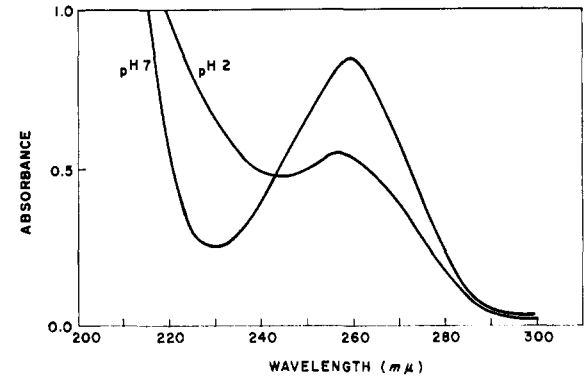


FIGURE 2: Ultraviolet absorbance of I at pH 2 and 7.

I and II migrated as cations at low pH but were neutral at pH 4.5 or higher. This established the pK_a' value of 3.4 for I and II. These results indicated that I and II contained a weakly basic group.

Chemical Characterization of I and II. Acid hydrolysis of both I and II resulted in the same hydrolytic products. At least six radioactive areas were observed on the radioautograms and none of these spots corresponded to the position of the original starting material. Three of the radioactive areas were intense and were major products as shown in Figure 1. The two most intense areas, AH-1 and AH-2, had R_F values of 0.90 in phenol–water and 0.61 and 0.40 in *n*-butyl alcohol–propionic acid–water, respectively. These two products behaved on paper chromatography as if they were sulfide and sulfoxide derivatives. The third major product, AH-3, was located at R_F values of 0.35 in phenol–water and 0.34 in *n*-butyl alcohol–propionic acid–water. The hydrolysis was also studied using different concentrations of hydrochloric acid and reaction times. The products of hydrolysis were always the same, but AH-3 predominated at longer reaction times. Compounds I and II were resistant to base hydrolysis and, therefore, devoid of the ester linkage.

Compounds I and II reacted with acetic anhydride which indicated the presence, in both I and II, of at least one easily acetylated group, *i.e.*, OH, SH, or NH. However, they failed to react with *p*-hydroxymercuribenzoate, thus a sulfhydryl group was absent. Compound I reacted with iodomethane, and the product possessed properties of a methylsulfonium iodide derivative. The product contained a single positive charge at neutral conditions and two positive charges under acidic conditions as evidenced by paper electrophoresis and chromatography. It possessed R_F values of 0.85 and 0.10 in phenol–water and *n*-butyl alcohol–propionic acid–water, respectively. This result confirmed the presence of the suspected sulfide linkage. Compounds I and II failed to react with 2,4-dinitrophenylhydrazine, indicating the absence of aldehyde or keto functions. Compounds I and II reacted with periodate suggesting the presence of a 1,2-glycol system or potential glycol system.

The electrophoresis data of I and II suggested the presence of a weakly basic function. To investigate further this possibility, three reagents were used; ninhydrin, nitrous acid, and DNFB. Compounds I and II formed derivatives with nitrous acid and DNFB but did not react with ninhydrin. The derivatives produced by the action of nitrous acid on I and II were analyzed by electrophoresis and found to be neutral. Thus, nitrous acid removed the basic group which was presumably an aromatic amino group. DNFB reacted with I to produce a single derivative having R_F values of almost 1.0 in both directions. The radioactive spot was also fluorescent under ultraviolet light. Since ninhydrin did not react with I or II, this behavior suggested that the amino group was substituted on an aromatic ring.

Hydrogen peroxide (30%) oxidation of I and II produced only one major degradation product, HP-1. The original radioactivity could be recovered (90–95%) from this major degradation product. HP-1 possessed R_F values of 0.35 in phenol–water and 0.34 in *n*-butyl alcohol–propionic acid–water and had properties identical with those of AH-3, the major product of prolonged acid hydrolysis. To check this identity further, HP-1 and AH-3 were mixed and chromatographed together on paper and on thin-layer chromatog-

TABLE I: Results of Functional Group Tests on Compound I.

Reagent	Functional Group Test	Results
Ninhydrin	Aliphatic NH_2	−
2,4-Dinitrofluorobenzene	NH_2	+
Nitrous acid	NH_2	+
Acetic anhydride	NH, SH, OH	+
Sodium *p*-hydroxymercuribenzoate	SH	−
Iodomethane	RSR′	+
2,4-Dinitrophenylhydrazine	CHO, >C=O	−
Periodate	COHCOH, $COHCNH_2$	+
Base	C(=O)OR	−
Acid	Acid-labile linkage	+
Air	Ease of oxidation	+
H_2O_2	Ease of oxidation	+

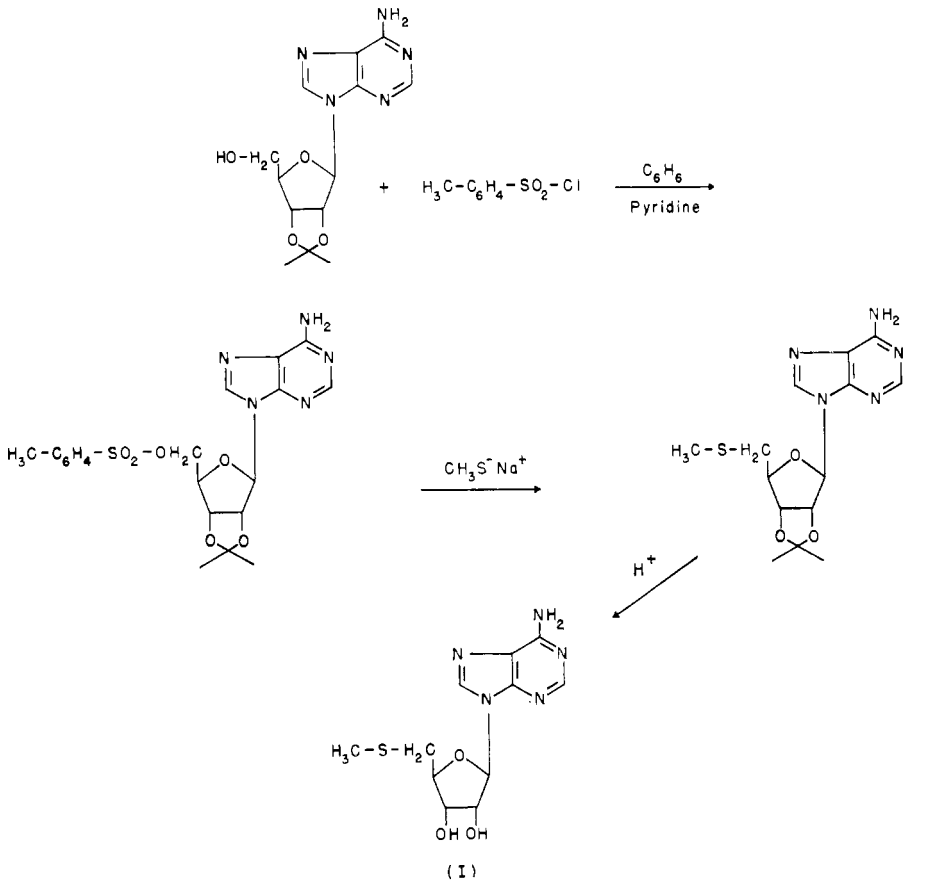


FIGURE 3: Reaction scheme for the synthesis of 5′-*S*-methyl-5′-thioadenosine.

raphy in seven different solvent systems. It was found that they were indistinguishable and, therefore, it was assumed that HP-1 possessed the same structure as AH-3. These data indicated that one side of the sulfide linkage must be strongly resistant to oxidative cleavage with hydrogen peroxide. Methylthio compounds have been shown to possess this property (Schlenk, 1965). Table I summarizes the results of the chemical tests on I.

Characterization of the Acid Hydrolysis Products AH-1 and AH-2. The acid hydrolysis products AH-1 and AH-2 behaved as neutral compounds on paper electrophoresis. AH-2 was shown to arise by oxidation of AH-1 and thus presumably was the sulfoxide derivative. AH-1 formed derivatives with acetic anhydride, iodomethane, periodate, and most importantly with 2,4-DNPH. These significant results indicated that AH-1 contained a carbonyl group which was probably in an acetal structure in I and also that AH-1 possessed a sulfide linkage and probably was a sugar derivative. The aglycone thus contained the weakly basic amino group and was presumably an aromatic derivative.

Characterization of the Oxidation Product HP-1. The hydrogen peroxide oxidation product HP-1, which was indistinguishable from AH-3, possessed a net charge of −1 at pH values 1–10, thus indicating the presence of a sulfonic acid. Presumably HP-1 was methanesulfonic acid. Three methionine molecules labeled at different positions ($^{14}CH_3SCH_2CH_2CHNH_3^+$-$COO^-$, $CH_3{}^{35}SCH_2CH_2CHNH_3^+COO^-$, and CH_3-$SCH_2CH_2CHNH_3^+{}^{14}COO^-$) were oxidized with hydrogen peroxide in the same manner as I. The chromatographed oxidation products of only $^{14}CH_3SCH_2CH_2$-$CHNH_3^+COO$ and $CH_3{}^{35}SCH_2CH_2CHNH_3^+COO^-$ showed one common degradation product. This product had R_F values equal to those of HP-1 and AH-3 in seven different solvent systems as mentioned before. Therefore, methanesulfonic acid, the only possible labeled common degradation product for both $^{14}CH_3$-$SCH_2CH_2CHNH_3^+COO^-$ and $CH_3{}^{35}SCH_2CH_2CHNH_3^+$-$COO^-$ possessing a sulfonic acid group, was proposed for the structure of HP-1.

Ultraviolet Spectra. Compound I possessed strong ultraviolet absorption with a single maximum of 259.5 mμ at pH 7 and 10 or 257 mμ at pH 2 (Figure 2). These data resembled the absorption characteristics of known nucleosides and nucleotides. Only adenosine, of the more common nucleosides, has ultraviolet absorption characteristics identical with those of I; however, adenosine is easily separated from I by thin-layer chromatography under the conditions used in this study.

From the summation of chemical and spectral evidence, the structure of I was postulated to be MTA. Therefore, MTA was independently synthesized according to the scheme shown in Figure 3, and synthesis of radiolabeled MTA was accomplished by using $CH_3{}^{35}S^-Na^+$. Thece llular concentration of MTA

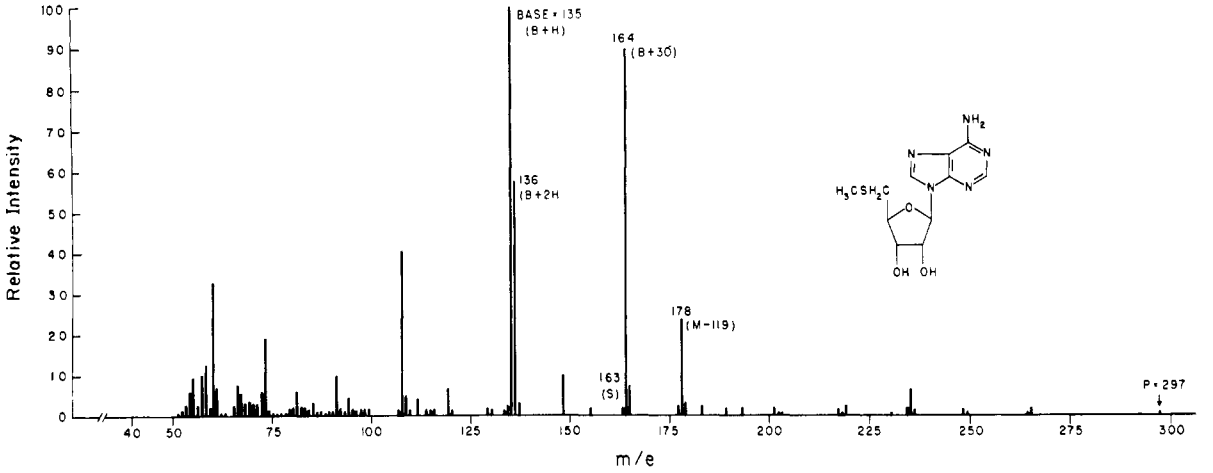
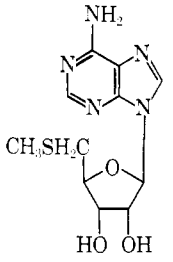


FIGURE 4: Mass spectrum of 5′-*S*-methyl-5′-thioadenosine (I).

was calculated to be 0.38 μmole/g of dry cells from the ultraviolet absorption data.

Gas Chromatography. The trimethylsilyl (TMS) derivatives of the isolated I, synthetic MTA, and related compounds were prepared with bis(trimethylsilyl)-acetamide. The retention times of the silylated derivatives of synthetic MTA and of isolated I were identical (Table II), based upon both the recovery of the radioactivity (Mumma and Kantner, 1966) and upon the appearance of peaks.



I

Mass Spectra. Mass spectra were obtained for adenosine, synthetic MTA, and the isolated compound I. The mass spectra of I (Figure 4) and synthetic MTA were identical and were similar to that of adenosine.

Nucleosides typically show molecular ion peaks of low intensity and fragment into two major portions, the nitrogen base (B) and the sugar residue (S). MTA gave a molecular ion of low intensity at *m/e* 297. Also, MTA gave rise to prominent, expected peaks at *m/e* 135 (B + H) ($C_5H_5N_5^+$) and 136 (B + 2H) ($C_5H_6N_5^+$). The sugar fragment (S) is of low intensity at *m/e* 163. Prominent fragments at *m/e* 164 (B + 30 or S + 1) and 178 (M − 119) can be interpreted as shown in Chart I. These data correlated nicely with those reported for the mass spectrum of adenosine (Biemann and McCloskey, 1962). Peaks occurring at *m/e* 100 or lower are due mainly to fragments of the sugar. The mass spectrum of compound I did not contain a fragment at 267, which is the molecular ion characteristic of adenosine, and thus adenosine was not a contaminant of either the synthetic or isolated preparations.

TABLE II: Gas Chromatographic Data of TMS Derivatives of Nucleosides.

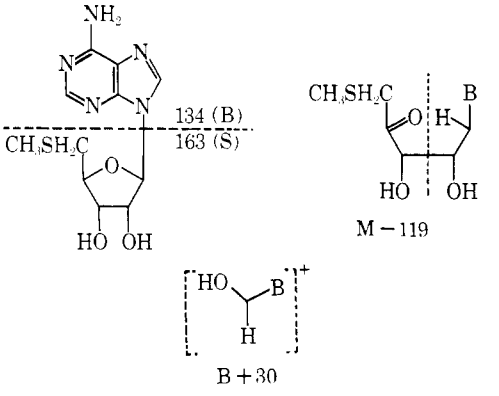
Compound	Retention Time (min)	
Adenosine	12.5	14.5[a]
Isopropylideneadenosine	10.25[a]	13.25
Synthetic MTA	11.12[a]	12.25
Compound I	11.12[a]	12.25

[a] Represents major peak. All showed double peaks.

Discussion

The chemical and physical evidence clearly indicated that I was MTA. The synthetic MTA, both the non-labeled and labeled, was identical with I in all respects. Methods used for comparison were paper, gas, and thin-layer chromatography, paper electrophoresis, ultraviolet, and mass spectrometry. Figure 5 shows a summation of the chemical reactions of I.

CHART I



Thin-layer chromatography (chloroform–methanol–water) was used to isolate microgram quantities of I for ultraviolet, mass spectrometry, and gas–liquid partition chromatography analysis. Compound I was radiochemically pure on paper and thin-layer chromatography (five different solvent systems) and homogeneous on gas–liquid partition chromatography (by radiochemical techniques and by response to the detector). Although contaminating adenosine cannot be detected by ultraviolet absorption measurements, adenosine is easily separated from MTA on thin-layer chromatography.

From the results of chemical analyses and by analogy with the findings of other investigators (Edwards *et al.*, 1959; Fisher and Mallette, 1961; Faith and Mallette, 1966) II is proposed to be the sulfoxide derivative of I and was present only in extracts that had been exposed to air. Also I was oxidized partially to II during either paper or thin-layer chromatography.

Mild acid hydrolysis of I produced the ribose derivative AH-1 (5-*S*-methyl-5-thioribose). Mild air oxidation of AH-1 produced the sulfoxide AH-2, which was also a product of acid hydrolysis of I. Prolonged acid treatment of I, II, AH-1, or AH-2 at 100° produced methanesulfonic acid (HP-1 or AH-3). This unusual reaction was not completely unexpected since a similar reaction has been shown to occur when *S*-methyl-L-cysteine sulfoxide is treated with hydrochloric acid (Ostermayer and Tarbell, 1960).

MTA was found only in the cells of *E. coli* at a concentration of 0.38 μmole/g of dry cells or about 0.01% of the dry weight when the cells were harvested during the log phase. Also, the radioactivity of this nucleoside was 15% of the total activity of ^{35}S-labeled compounds in the water extract. It was the most intense spot found on the radioautogram and, therefore, the most concentrated water-soluble, sulfur-containing molecule present in *E. coli* when grown under these conditions. In contrast to *E. coli* most of the more common free nucleosides isolated from fungi and bacteria are not located in the cells, but are found in the medium (Suhadolnik, 1967). It has been reported (Schlenk *et al.*, 1965) that the concentration of *S*-adenosylmethionine in yeast depended upon the nutrition of the cultures, and significant amounts of methionine were required to produce microgram quantities of *S*-adenosylmethionine. Since only glucose and inorganic salts were used in the growth medium of *E. coli*, the concentration of MTA found in the cells does not reflect any contribution from external methionine. Evidence has been presented (Svihla and Schlenk, 1959) that *S*-adenosylmethionine was located chiefly in the vacuoles in yeast. Therefore, it would be of interest to determine the location of MTA in the *E. coli* cell.

MTA has been shown to be a product of spermidine biosynthesis in purified enzyme preparations from *E. coli*. However, in crude enzyme preparations, MTA was never isolated because it was rapidly metabolized (Tabor and Tabor, 1964). Previously it had been demonstrated that spermidine biosynthesis was dependent on the pH of the medium, negligible at pH 5.1, and increased to pH 8.4 (Tabor *et al.*, 1958). MTA also

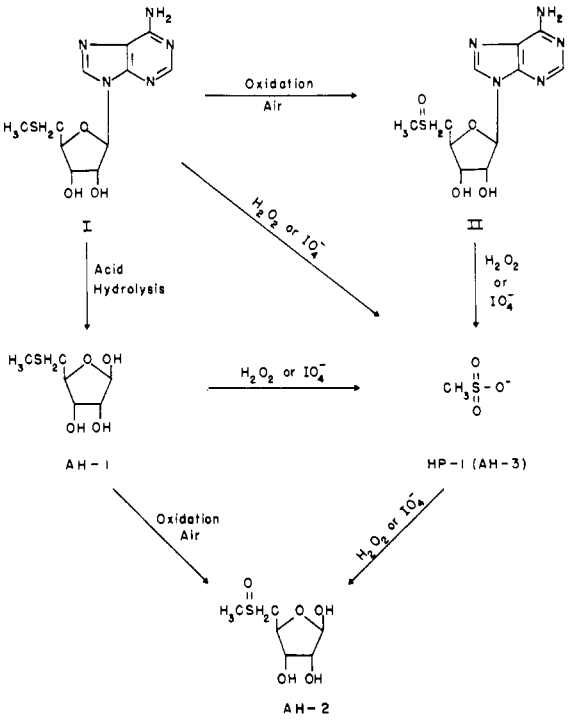


FIGURE 5: Summation of reactions of I.

has been shown to act as a methyl donor in yeast (Schlenk and Ehninger, 1964). The question arises: Can the high concentration of MTA found in *E. coli* during log-phase growth be accounted for entirely by spermidine biosynthesis? Perhaps MTA itself serves some biological function in the *E. coli* cell, such as a methyl donor, or perhaps it may be involved in a regulatory process. These questions are currently under investigation in our laboratory.

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The Action of Diazomethane on Ribonucleosides. Preparation of Ribonucleoside 2′- and 3′-Methyl Ethers*

D. M. G. Martin,† C. B. Reese,‡ and G. F. Stephenson†

ABSTRACT: Under the conditions described by Broom and Robins (Broom, A. D., and Robins, R. K. (1965), *J. Am. Chem. Soc. 87*, 1145) both adenosine and cytidine were reacted with diazomethane to give *ca.* three parts of their 2′- to one part of their 3′-*O*-methyl derivatives. 2′- and 3′-*O*-methyluridines have been prepared by deamination of the corresponding cytidine derivatives. The orientations of the 2′- and 3′-methyl ethers of all three nucleosides have been established by nuclear magnetic resonance spectroscopy; the orientations of the uridine derivatives have been confirmed by a chemical method. The stabilities of the glycosidic bonds of adenosine and its 2′- and 3′-methyl ethers are compared.

The study of the effect of methylating agents on nucleic acid components (Haines *et al.*, 1962, 1964; Brimacombe *et al.*, 1965; Griffin *et al.*, 1967) and the development of methods for the selective protection of the 2′- and 3′-hydroxyl functions of ribonucleosides (Reese and Trentham, 1965a; Griffin *et al.*, 1966, 1968; Fromageot *et al.*, 1967; Reese *et al.*, 1967) are two of the aspects of nucleic acid chemistry which have occupied our attention recently. The significance of chemical methylation studies has been considered previously (Haines *et al.*, 1962) and will not be discussed here. The development of methods whereby the secondary hydroxyl functions of a ribonucleoside *cis*-2′,3′-diol system may be differentiated is one of the fundamental problems of ribonucleoside chemistry and is of crucial importance in oligoribonucleotide synthesis (see Griffin *et al.*, 1968). This problem consists essentially of two parts: the first involves a search for reactions which occur specifically at the 2′- or 3′-hydroxyl group or, alternatively, satisfactory methods for the separation of mixtures of 2′- and 3′-isomers; the second part requires the development of a technique (or techniques) by which a pair of 2′- and 3′-isomers may be distinguished.

In connection with the second part of the problem, we have recently proposed a general method of orientation, based on nuclear magnetic resonance spectroscopy (Fromageot *et al.*, 1966), and have also devised a chemical procedure (Reese and Trentham, 1965a) for the orientation of uridine derivatives. However, it seems likely that it will, in general, be difficult to find reagents which attack ribonucleosides *exclusively* on either the 2′- or the 3′-hydroxyl functions, but that it will usually be possible to separate the mixtures of 2′- and 3′-isomers obtained. The electrophilic reagents, toluene-*p*-sulfonyl chloride (Brown *et al.*, 1958) and triphenylmethyl chloride (Reese and Trentham, 1965a), both appear to attack the 2′- approximately twice as fast as they attack the 3′-hydroxyl functions of ribonucleosides and thus, despite their presumably different steric requirements, exhibit similar and only marginal selectivity. No information relating to the behavior of acyl halides and anhydrides (derived from carboxylic acids) is available, as 2′- and 3′-*O*-acyl ribonucleoside derivatives readily isomerize (Reese and Trentham, 1965b) under the reaction conditions.

We were therefore interested in the recent reports by Robins and his coworkers (Broom and Robins, 1965;

* From the University Chemical Laboratory, Cambridge, England. *Received October 24, 1967.*

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‡ To whom enquiries should be addressed.