Structure of the Modified Nucleoside Q Isolated from *Escherichia coli* Transfer Ribonucleic Acid. 7-(4,5-*cis*-Dihydroxy-1-cyclopenten-3-ylaminomethyl)-7-deazaguanosine†

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ABSTRACT: The structure of the unknown modified nucleoside Q, which is present in the first position of the anticodons of *Escherichia coli* $tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asn}$, $tRNA^{Asp}$, is proposed to be 7-(4,5-*cis*-dihydroxy-1-cyclopenten-3-ylaminomethyl)-7-deazaguanosine (**1**). The structure of Q was deduced by means of its uv absorption, mass spectrometry, proton magnetic resonance spectroscopy, and studies of its chemical reactivity. The structure of Q is unique since it is a derivative of 7-deazaguanosine having cyclopentenediol in the side chain at the C-7 position. This is the first example of purine skeleton modification in a nucleoside from tRNA.

An unidentified modified nucleoside, designated as Q,[1] was first discovered in the first position of the anticodon of *Escherichia coli* $tRNA^{Tyr}$ (RajBhandary et al., 1969; Goodman et al., 1968; Doctor et al., 1969). Later it was found that Q is also present in the same position in *E. coli* $tRNA^{His}$, $tRNA^{Asn}$, and $tRNA^{Asp}$ (Harada and Nishimura, 1972). All four *E. coli* tRNAs containing Q recognize the codon sequences of XAU and XAC. It has been shown that modification of G to produce Q results in the preferential recognition of codon sequences ending with U as compared with those ending with C, in vitro (Harada and Nishimura, 1972). However, it is unlikely that replacement of G by Q in the first position of the anticodons has any drastic effect on in vivo protein synthesis, since such preferential recognition of code words ending with U by tRNAs containing Q is not always absolute (Harada and Nishimura, 1972). Thus, the real function of Q in *E. coli* tRNA is not yet clearly understood. In 1973 White et al. (1973) made the interesting observation that Q or its derivative is found in *Drosophila* tRNAs corresponding to the same four amino acids, and that the amount of Q varies during the life cycle of this organism. They suggested that Q plays a role in the regulatory function of tRNA rather than in protein synthesis. It is probable that inhibition of the enzymatic activity of tryptophan pyrrolase by *Drosophila* $tRNA^{Tyr}$ from a vermilion mutant depends on whether the $tRNA^{Tyr}$ contains Q or guanosine in the first position of the anticodon (Jacobson, 1971; White et al., 1973). A Q-like nucleoside designated as N was also isolated from pancreatic RNase digest of rat liver tRNA as the dinucleotides N-Up and N-ψp (Rogg and Staehelin, 1969). The properties and structure of a nucleoside designated Q were also reported by Hayes and Lis (1973); however their data refer to a different nucleoside.

In order to understand the function of Q, it is first necessary to establish its structure. In this communication we wish to report on the elucidation of the structure of Q (structure **1**) based upon its uv absorption, mass and ^1H nuclear magnetic resonance (NMR) spectra, and chemical properties. Q has a unique structure; in addition to the presence of a cyclopentenediol moiety, it is the only nucleoside thus far isolated from ribonucleic acid in which the purine skeleton is modified to a 7-deaza structure.

Materials and Methods

Isolation of Q. Unfractionated *E. coli* tRNA was prepared from *E. coli* B cells harvested in the late-log phase of growth as described by Zubay (1962), except that treatment with alkali was omitted. Generally 100,000 A_{260} units (approximately 5 g) of unfractionated *E. coli* tRNA was extensively hydrolyzed by 100 mg of pancreatic RNase, and the digest was fractionated by DEAE-Sephadex A-25 column chromatography (5 cm × 100 cm) in the presence of 7 *M* urea. As shown in Figure 1, the dinucleotide Q-Up was eluted from the column earlier than most mononucleotides. It should be noted that all Q present in *E. coli* tRNAs was derived as Q-Up by pancreatic RNase digestion, since the tRNAs containing Q have the same sequence, U-Q-U, in the anticodon region. Separation of Q-Up from the remain-

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[1] Abbreviations used are: Q, modified nucleoside isolated from *E. coli* tRNA; Q_{Ac1} monoacetyl derivative of Q; Q_{Ac2} diacetyl derivative of Q; Q_{NaIO_4}, the modification product obtained by $NaIO_4$ oxidation; Q_{Isop}, di-*O*-isopropylidene Q; Q_{Me_3Si}, trimethylsilyl derivative of Q (all trimethylsilyl derivatives are designated as -Me_3Si, for example, the Me_3Si derivative of Q_{Ac1} is designated as $Q_{Ac1\text{-}Me_3Si}$; $Q_{Ac1\text{-}Me}$, *N,O*-permethylated derivative of Q_{Ac1}; A_{260} unit, the amount of material giving an absorbance of 1.0 at 260 nm when dissolved in 1 ml of water and measured in a cell of 1-cm light path.

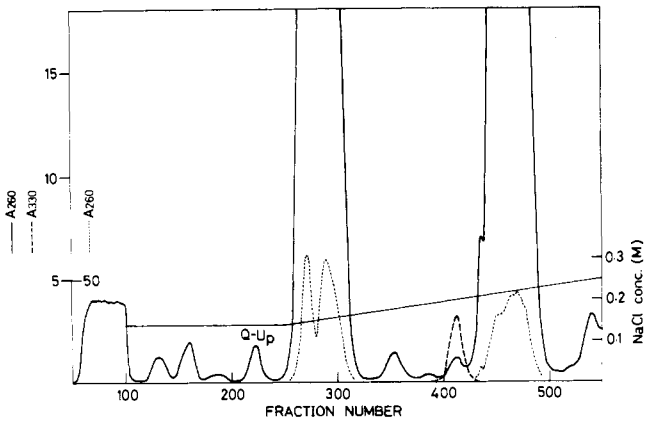


FIGURE 1: Chromatography of a pancreatic RNase digest of unfractionated *E. coli* tRNA (100,000 A_{260} units) on a column (5 × 100 cm) of DEAE-Sephadex A-25 (chloride form) in the presence of 7 *M* urea. Elution was performed with linear salt gradient obtained by placing 5 l. of 0.14 *M* NaCl containing 0.02 *M* Tris-HCl buffer (pH 7.5) and 7 *M* urea in the mixing chamber and 5 l. of 0.3 *M* NaCl containing 0.02 *M* Tris-HCl buffer (pH 7.5) and 7 *M* urea in the reservoir; 20 ml of effluent was collected in each fraction.

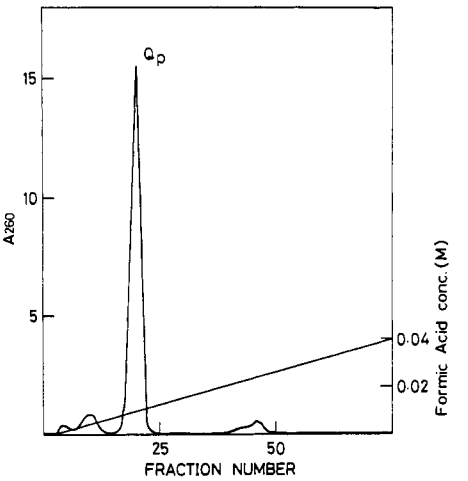


FIGURE 2: Chromatography of a RNase T_2 digest of Q-Up fraction on a column (1 × 20 cm) of Dowex 1-X2 (formate form). Elution was performed with a linear gradient obtained by placing 200 ml of water in the mixing chamber and 200 ml of 0.05 *M* formic acid in the reservoir; 5 ml of effluent was collected in each fraction.

ing dinucleotides made it possible to isolate Q relatively easily from unfractionated *E. coli* tRNA.

Hydrolysis of Q-Up by RNase T_2 gave Qp, which was isolated by Dowex-1 column chromatography (Figure 2). The nucleoside Q was then obtained from Qp by treatment with *E. coli* alkaline phosphomonoesterase. In order to obtain highly purified Q, the incubation mixture was applied to a column of Dowex-1 (OH^- form) and extensively washed with distilled water before elution of nucleoside with 1 *N* formic acid. The eluate was evaporated to dryness and passed through a Dowex-1 column (formate form) to remove contaminants. Approximately 70 A_{260} units of Q was obtained from 100,000 A_{260} units of unfractionated *E. coli* tRNA. On occasion, Q was isolated on four times this scale, fractionating 400,000 A_{260} units of tRNA hydrolysate on a 10 cm × 100 cm column.

Solvent Systems for Paper and Thin-Layer Chromatography. The solvent systems used for paper and thin-layer chromatography were (v/v): solvent A, isobutyric acid–0.5 *M* NH_4OH (5:3); solvent B, 2-propanol–concentrated HCl–water (70:15:15); solvent C, 1-butanol–acetic acid–water (4:1:2); solvent D, 2-propanol–concentrated NH_4OH–water (7:1:2); solvent E, 2-propanol–water (1:1); solvent F, 2-propanol–water (2:1); solvent G, 1 *M* sodium acetate (pH 7.5)–ethanol (3:7).

Mass Spectrometry of Trimethylsilyl or Permethyl Derivatives. Conversion of Q and several derivatives of Q was made to trimethylsilyl derivatives by variation of an earlier procedure (McCloskey et al., 1968a): a dried sample of nucleoside was dissolved in pyridine–*N,O*-bis(trimethylsilyl)-trifluoroacetamide–trimethylchlorosilane (10:100:1), then heated for 1 hr at 100°. Permethyl derivatives were formed by the method of von Minden and McCloskey (1973), using methylsulfinyl carbanion and methyl iodide. Mass spectra were recorded using an LKB 9000 or 9000S instrument (Figures 4, 5, 7, and 8) or Varian CH7 (Figure 6) with ionizing electron energies of 70 or 20 eV and ion source temperature 250°. The sample was introduced by direct probe after removal of silylation or permethylation reagents under vacuum. The amount of nucleoside used for each experiment was approximately 0.2 A_{260} unit. The field desorption mass spectrum of Q_{Isop} was recorded through the courtesy of Mr. Carter Cook, University of Illinois, using a Varian CH5-DF instrument. High resolution mass spectra were acquired using a CEC 21-110B instrument with photographic recording. Computer-derived elemental compositions from high resolution mass spectra, and determination of the numbers of Me_3Si or methyl groups in various ion species, were based on mass shifts in the spectra of Q following derivatization with perdeuterated reagents (McCloskey et al., 1968b; von Minden and McCloskey, 1973).

Nuclear Magnetic Resonance Spectroscopy. The proton NMR spectra of Q and Qp were obtained on a Varian Associates field sweep HR-220 proton magnetic resonance spectrometer with the signal enhanced using a Nicolet 1074 computer. The nucleoside or nucleotide, 100 A_{260} units, was lyophilized twice from 99.8% D_2O and then dissolved in 100% D_2O. A Radiometer Model 25 pH meter equipped with a combination glass electrode was used to measure the pD of the solution using the standard electrode correction pD = meter reading + 0.4 (Glasoe and Long, 1960). Chemical shifts were measured relative to internal tetramethylammonium chloride and reported relative to 3-trimethylsilylpropionate-*2,2,3,3-d*$_4$ where the chemical shift of tetramethylammonium chloride is taken as 3.20 ppm. The sample temperature was 22 ± 1° as measured by a methanol temperature calibration sample.

Methods of Chemical Modification. For the acetylation of Q, 5 A_{260} units was reacted with a mixture of 0.4 ml of methanol and 0.1 ml of acetic anhydride at room temperature for 3 hr. The reaction mixture was dried, then purified by two-dimensional paper chromatography (first dimension, solvent system C; second dimension, solvent system F). Two products Q_{Ac1} and Q_{Ac2} were obtained. R_f values of Q_{Ac1} and Q_{Ac2} were 0.38 and 0.49 for solvent system C, and 0.53 and 0.68 for solvent system F, respectively.

To obtain the *O*-isopropylidene derivative of Q, 2 A_{260} units were dissolved in a mixture of 0.6 μl of concentrated HCl, 40 μl of acetone, and 10 μl of 2,2-diethoxypropane, and the mixture was allowed to stand at room temperature for 2 hr. R_f values for Q_{Isop} were 0.87 for solvent system E and 0.89 for solvent system G. Sodium periodate oxidation of Qp was carried out under the same conditions as described by Harada and Nishimura (1972). The product was

Table I: Thin-Layer Electrophoretic Mobilities of Q and Qp (cm).

	Mobility to Anode				Mobility to Cathode		
pH	G	Q	7MeG	1MeA	G	Qp	Gp
6.0	0.0	1.5	2.1	2.4	0.0	0.7	1.7
7.5	0.0	1.2	1.5	1.9	0.0	1.0	2.4
8.0	0.0	0.9	0.7	1.9	0.0	1.2	2.6

further purified for mass spectrometry by paper chromatography using solvent systems C and F.

Attempts to incorporate deuterium by exchange at position 8 of the purine skeleton of adenosine, guanosine, and Q were made by placing 0.4 A_{260} unit (approximately 20 μg) in a glass capillary with 20 μl of D_2O, which then was sealed. The tube was heated at 85° for 90 min, opened, and evaporated to dryness over P_2O_5. The residues from guanosine and Q were acetylated and permethylated as described above. The adenosine residue was redissolved in cold H_2O, then reevaporated to dryness. All three samples were submitted to mass spectrometry, using direct probe introduction.

Materials. Bovine pancreatic RNase (RNase A) (five-times recrystallized), RNase T_2, and *E. coli* alkaline phosphomonoesterase were obtained from Sigma Chemical Co., Sankyo Co. Ltd., Tokyo, and Boehringer Mannheim, respectively. *N,O*-Bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane used for trimethylsilylation was purchased from Regis Chemical Co., Morton Grove, Ill. Deuterated reagents, i.e., *N,O*-bis(trimethylsilyl)acetamide-d_{18}, trimethylchlorosilane-d_9, and methyl-d_3 iodide were purchased from Merck Sharp & Dohme of Canada, Pointe Claire, Quebec. Thin-layer glass plates coated with Avicel SF cellulose were purchased from Funakoshi Pharmaceutical Co., Tokyo. Authentic 7-deazaguanine was a gift from Dr. R. E. Bowman of Parke-Davis & Co., Pontypool, Mon.

Results

General Properties of Q. The phosphodiester bond of Q is not split by RNase T_1 and pancreatic RNase, but is cleaved by RNase T_2. The dinucleotide Q-Up elutes just before the main mononucleotide fraction, when the pancreatic RNase digest of unfractionated *E. coli* tRNA is subjected to DEAE-Sephadex A-25 column chromatography (pH 7.5) in the presence of 7 *M* urea (Figure 1). This suggests that the nucleoside Q has one positive charge at this pH. In addition, Q and Qp have electrophoretic mobilities which indicate that Q has one positive charge at pH 7.5 (Table I). Uv absorption spectra of Q (Figure 3) are similar to G, but λ_{max} is shifted to longer wavelength (262 nm) and there is a second peak at 220 nm. It is noteworthy that the uv absorption spectrum of Q is similar to that of 7-deazaguanine at neutral pH (Davoll, 1960), and to that of 7-deazaguanosine found by Tolman (1969). Based on the spectral changes, Q appears to have ionizing groups with pK_a values of 1.1, 7.7, and 10.4. Attempts to obtain the free Q base from the nucleoside or nucleotide by acid hydrolysis were unsuccessful. Under the usual conditions for hydrolyzing purine nucleosides (i.e., 1 *N* HCl at 100° for 1 hr), Q is not converted to the corresponding base, which we attribute to altered reactivity associated with the 7-deazapurine structure. The stability of Q under acid (as described above) or alkaline treatment (0.3 *N* KOH at 37° for 15 hr) was confirmed by taking mass spectra of the treated Q or by checking its mobility on thin-layer chromatography. Microscale deuterium exchange reactions were carried out using Q and two models, adenosine and guanosine, under conditions which normally exchange the labile hydrogen at C-8. Mass spectrometrically measured deuterium contents were as follows: Q, 0% d_1, 100% d_0; guanosine, 70% d_1, 30% d_0; adenosine, 80% d_1, 20% d_0.

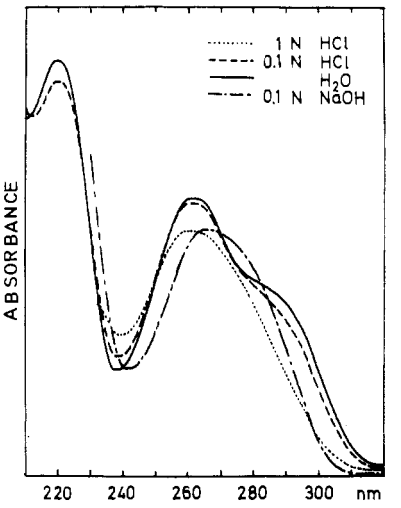


FIGURE 3: Uv absorption spectra of nucleoside Q.

When Q is acetylated with methanol-acetic anhydride, two products, mono- and diacetyl derivatives, are formed. Both compounds have lost the positive charge which is exhibited by Q at neutral pH. Mass spectrometry shows that the first reactive site for acetylation is the secondary amine group in the side chain and the second site is the amino group at C-2 of the 7-deazaguanosine nucleus, as discussed later.

Experiments employing isopropylidene formation and periodate oxidation were carried out to test for the presence of cis diol groups in the molecule. Mass spectrometry (discussed in the following section) revealed that the diisopropylidene derivative was formed, which is strong and direct evidence for presence of a diol moiety in the Q side chain as well as in the ribose moiety. Qp was oxidized by periodate, and the phosphate group then removed by treatment with *E. coli* alkaline phosphomonoesterase. Q_{NaIO_4} thus obtained was subjected to mass spectrometry.

Mass Spectrometry. The most valuable information concerning the structure of Q was obtained by mass spectrometry, in particular from high resolution data. Overall structural features were established from the mixed derivatives $Q_{Ac1\text{-}Me}$ and $Q_{Ac1\text{-}Me_3Si}$ (compounds **2** and **3**). The mass spectra of these two derivatives, shown in Figures 4 and 5, were used to corroborate the identities of ions by their shifts in mass and in particular to aid in the assignment of elemental composition from experimentally determined exact mass values.

High resolution data from $Q_{Ac1\text{-}Me}$, corresponding to Figure 4, are given in Table II, which also lists the number of methyl and acetyl groups present in each ion. The molecular composition $C_{27}H_{41}N_5O_8$, when corrected for presence of 1 acetyl and 8 methyl groups, leads to a molecular weight of 409 or $C_{17}H_{23}N_5O_7$ for Q, compound **1**. Application of the standard formula for calculation of the total number of rings plus double bonds in a molecule or ion from its ele-

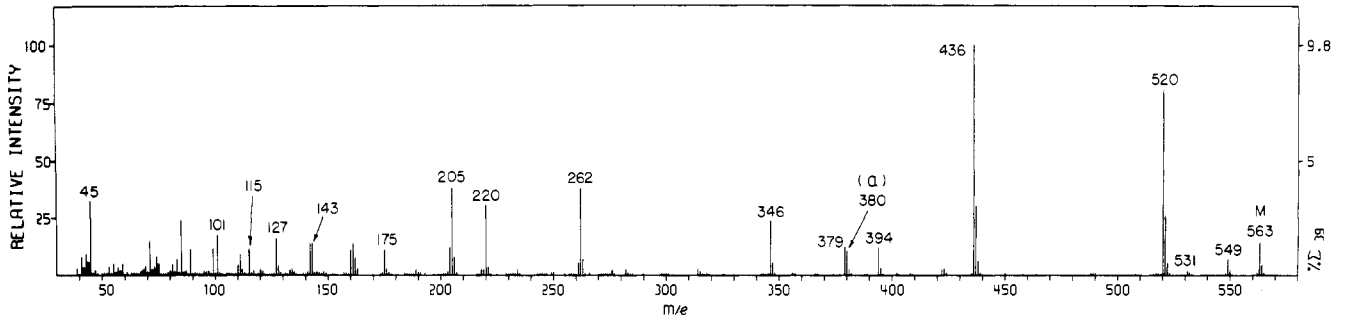
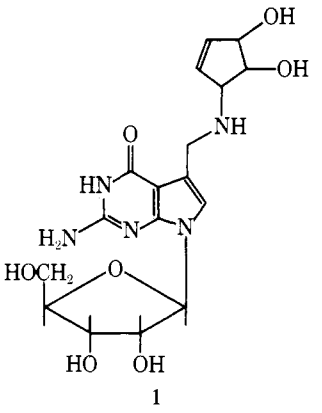


FIGURE 4: Mass spectrum of $Q_{Ac1\text{-}Me}$ (70 eV).

Table II: High Resolution Data from the Principal Ions from the Mass Spectrum of $Q_{Ac1\text{-}Me}$ (2).

Mass	(error)[a]	Composition	CH_3/Ac[b]
563.2978	(2.2)	$C_{27}H_{41}N_5O_8$	8/1
531.2724	(3.2)	$C_{26}H_{37}N_5O_7$	7/1
520.2767	(−0.5)	$C_{25}H_{38}N_5O_7$	8/0
436.2204	(0.8)	$C_{20}H_{30}N_5O_6$	6/1
380.2043	(−1.6)	$C_{18}H_{28}N_4O_4$	6/0
379.1964	(−1.7)	$C_{18}H_{27}N_4O_5$	6/0
346.1904	(2.5)	$C_{17}H_{24}N_5O_3$	5/0
262.1327	(2.3)	$C_{12}H_{16}N_5O_2$	3/1
220.1226	(2.8)	$C_{10}H_{14}N_5O$	3/0
205.1090	(0.1)	$C_{10}H_{13}N_4O$	3/0
175.0985	(1.4)	$C_8H_{15}O_4$	3/0
143.0708	(0.0)	$C_7H_{11}O_3$	2/0
127.0766	(0.1)	$C_7H_{11}O_2$	2/0

[a] Found minus theoretical value in millimass units. [b] Number of methyl and acetyl groups established from separate deuterium labeling experiments.



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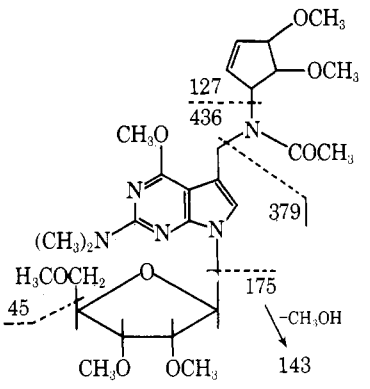
mental composition (McLafferty, 1973) shows 10 in the derivative $Q_{Ac1\text{-}Me}$ and 9 (one acetyl less) in compound **1**. These values, as well as the elemental composition, show Q to be a very highly modified nucleoside. Principal structure assignments are shown below, and are based on the general fragmentation behavior of nucleosides (McCloskey, 1974), and of *O,N*-permethyl derivatives in particular (von Minden and McCloskey, 1973). Metastable transitions support the sequences *m/e* 563 → 520 and 436, 520 → 346, 436 → 262, and 262 → 220.

The ions of highest mass in Figure 4 are represented by the molecular ion (M) *m/e* 563, the molecular ion of the lower derivative *m/e* 549 (heptamethylmonoacetyl), and the fragment *m/e* 531 resulting from loss of CH_3OH. In terms of the proposed structure **2**, cleavage of the three amine bonds as shown leads to *m/e* 127 or 436, *m/e* 520 (M − CH_3CO) and *m/e* 379. Composition of the *m/e* 127 frag-



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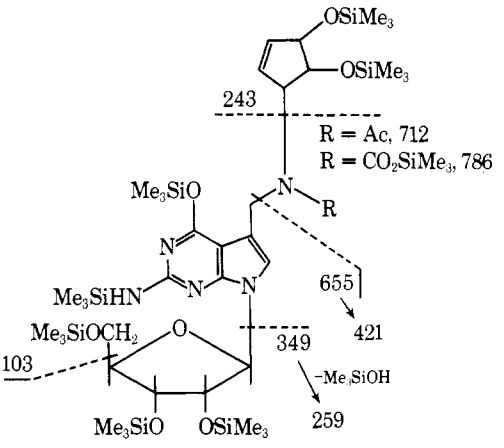
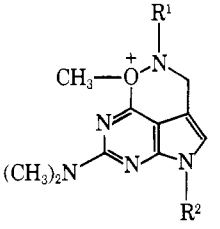


3, R = $COCH_3$
4, R = CO_2Me_3Si

ment ($C_7H_{11}O_2$) and its counterpart *m/e* 436 indicates a side chain in the Q molecule of composition $C_5H_7O_2$, having two rings plus double bonds. The high abundance of *m/e* 436 and 520 ions, which is unexpected if the charge resides solely on nitrogen, is accounted for by direct stabilization from O^6 by formation of a six-membered ring as shown. A diagnostic ion (designated ion a) arising by loss of the C_5 side chain moiety and nitrogen, is present in all mass spec-



m/e 436, $R^1 = CH_3CO$, R^2 = ribosyl $(CH_3)_3$
m/e 262, $R^1 = CH_3CO$, $R^2 = H$
m/e 520, $R^1 = C_7H_{11}O_2$, R^2 = ribosyl $(CH_3)_3$
m/e 346, $R^1 = C_7H_{11}O_2$, $R^2 = H$

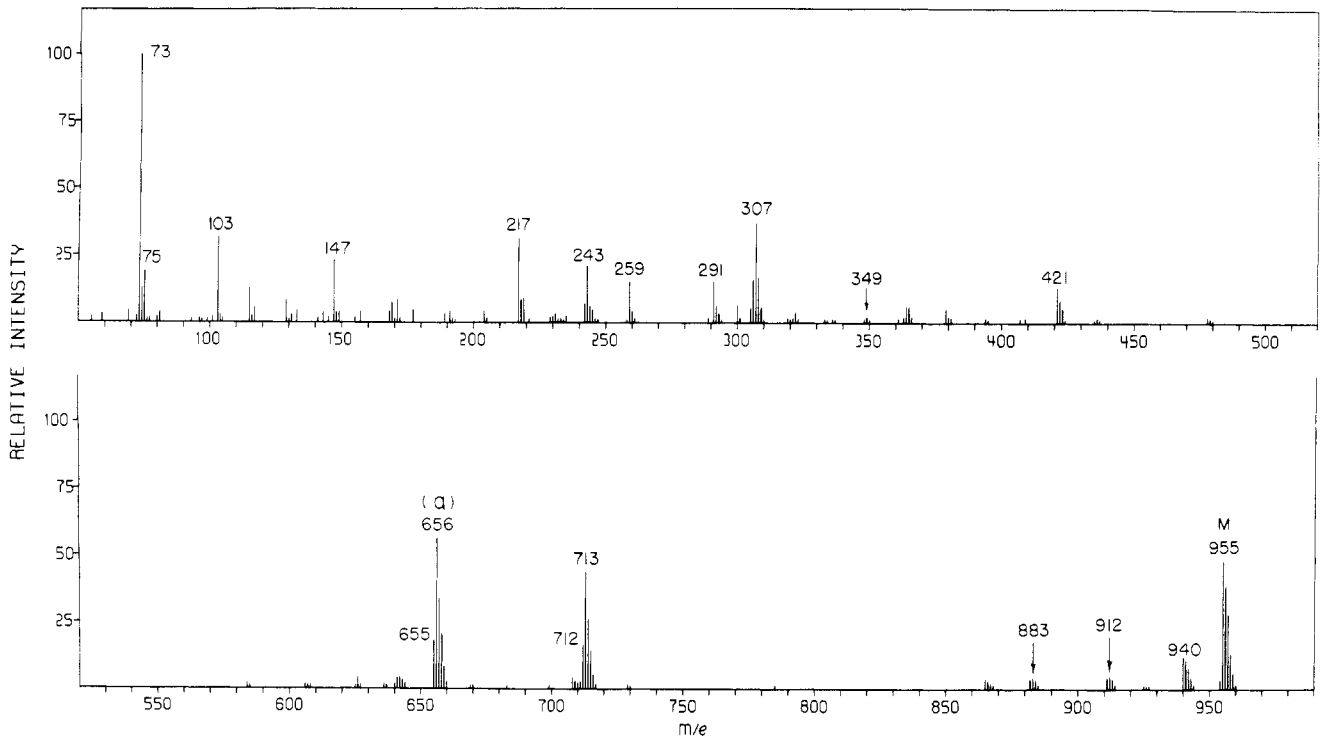
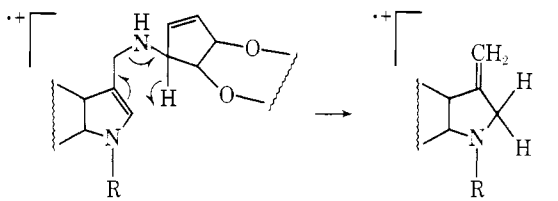


FIGURE 5: Mass spectrum of $Q_{Ac1\text{-}TMS}$ (70 eV).

tra of Q derivatives. Its composition (*m/e* 380; Table II) requires the presence of rearranged hydrogen, which we view as likely occurring via the well-known McLafferty rearrangement (Budzikiewicz et al., 1967):



ion a

R = H or ribose derivative

Ions *m/e* 379, 380, and others listed in Table II having four nitrogens are required to have six (ribose moiety absent) or seven (ribose present) rings plus double bonds. Further, the nitrogen which is lost in forming these ions cannot include an exocyclic amino group such as N^2 of guanosine, because such cleavages are energetically unfavorable and thus occur in very low abundance in nucleoside spectra. These factors strongly suggest a deazapurine nucleus. The absence of modification of ribose is shown in Figure 4 by the normal sugar ions *m/e* 175, 143, 115, 101, and 45 (von Minden and McCloskey, 1973). Reactions to form ions of the base + H type (McCloskey, 1974) in which ribose is lost with transfer of hydrogen to the base are represented by *m/e* 520 → 346, 379 → 205, and 436 → 262, which further support the identities of base-containing ions and excludes a C-nucleoside glycosidic linkage (Hecht et al., 1969; Rice and Dudek, 1969; Townsend and Robins, 1969; Crain et al., 1973). Loss of ketene from *m/e* 436, a common reaction of acetates, yields *m/e* 394.

The mass spectrum of $Q_{Ac1\text{-}Me_3Si}$ (Figure 5) exhibits peaks whose mass values corroborate the structural assignments made for **2**, as indicated in structure **3**. Use of perdeuterated derivatizing reagents shows incorporation of one acetyl and seven Me_3Si groups. The molecular ion value of 955 therefore represents a molecular weight of 409 for Q, in support of the permethylation data. The upper mass range is marked by characteristic loss of a methyl radical from a trimethylsilyl group (*m/e* 940) (McCloskey et al., 1968a) or of CH_3CO (*m/e* 912), and the molecular ion of the hexasilyl derivative, *m/e* 883. The intensity pattern $M > M - CH_3$ is relatively uncommon, and in the case of nucleosides is characteristic of analogs of guanosine (Lyman et al., 1974). Ion a (*m/e* 656) becomes the dominant fragment ion other than $+Si(CH_3)_3$, *m/e* 73. The related simple cleavage product *m/e* 655 and its base + H type analog *m/e* 307 (McCloskey, 1974), supported by measurement of exact mass, confirm the structure assignment for ion a gained from the permethyl derivative. Unlike the permethyl derivative, cleavage adjacent to the side chain nitrogen does not lead to intense peaks, possibly because steric hindrance from Me_3Si would prevent cyclization of O^6. As a result, hydrogen rearrangement becomes competitive and *m/e* 713 is formed in preference to *m/e* 712. The high resolution mass spectrum of **3** shows the *m/e* 243 species to be a doublet, approximately 30% representing the side chain ($C_{11}H_{23}O_2Si_2$) and the remainder a common sugar ion, $C_{10}H_{19}O_3Si_2$ (McCloskey et al., 1968a). The heterocyclic moiety has incorporated two silyl groups as opposed to three methyl groups in the case of $Q_{Ac1\text{-}Me}$, which indicates presence of an exocyclic amino group on a pyrimidine or purine ring (White et al., 1972; von Minden and McCloskey, 1973), such as N^2 of guanosine. Trimethylsilyl derivatives of Q exhibit a peak of *m/e* 421, which from its elemental composition appears to be derived from the even-electron *m/e* 655 ion, and has direct analogy in the even-electron base + 116 fragment ion from silylated nucleosides (McCloskey et al., 1968a). With the exception of *m/e* 291 (307 − CH_4), most other peaks in the lower mass range (*m/e* 349, 259, 217, 147, 103, part of 243) are derived from the ribose moiety (McCloskey et al., 1968a). The peak rep-

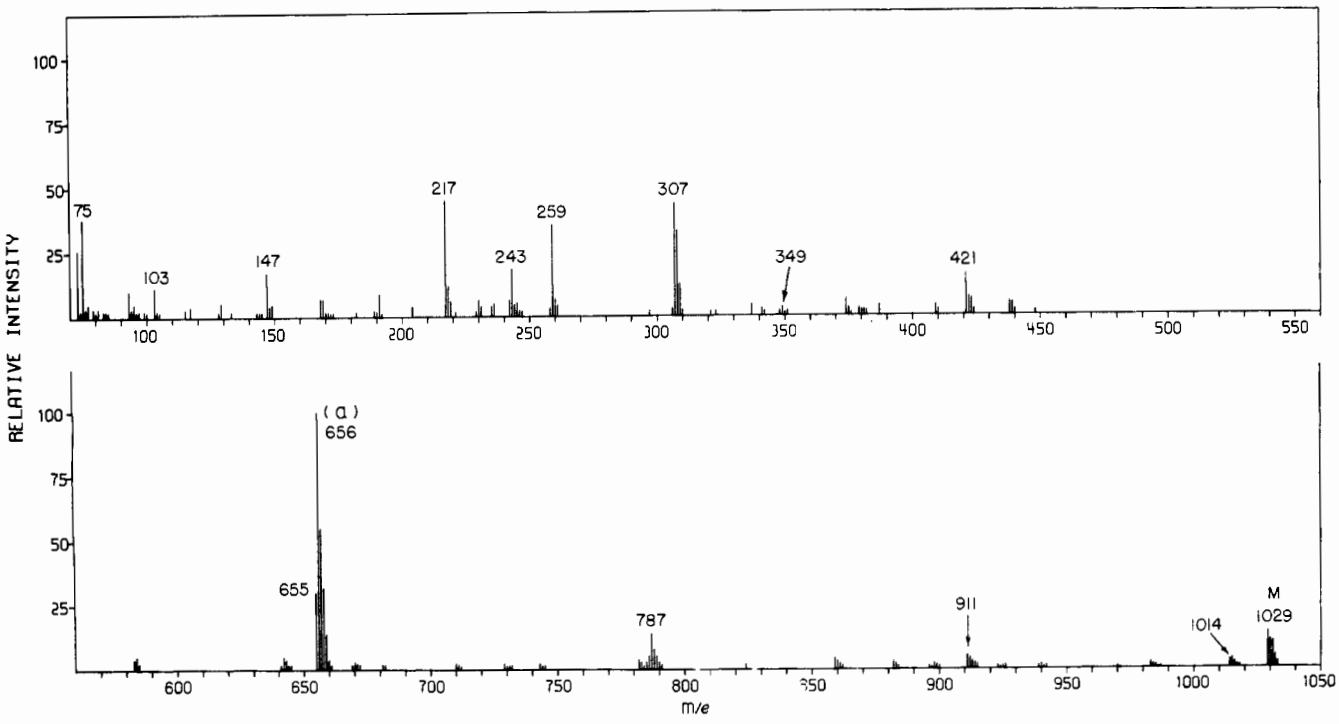
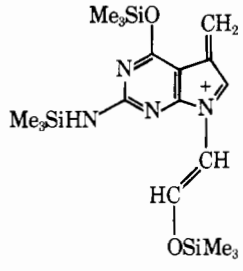


FIGURE 6: Mass spectrum of Q_{TMS} (30 eV).



m/e 421

resenting *m/e* 75 is a common silyl ion and bears no structural information. The low and high resolution mass spectra of $Q_{Ac2\text{-}Me_3Si}$ (M = 925) show that the hexasilyl-diacetyl derivative was formed. The fragmentation pattern is similar to that of $Q_{Ac1\text{-}Me_3Si}$, and shows that the second acetyl group is bound to the heterocyclic moiety, presumably at N^2. Trimethylsilylation of Q in the presence of pyridine led to products whose molecular weight was 116 mass units higher than expected from other derivatives which were examined. The mass spectrum of Q_{Me_3Si} (Figure 6) exhibits a molcular ion of *m/e* 1029 which corresponds to incorporation of CO_2 into structure **1**. The anomalous mass increase (H vs. CO_2Me_3Si = 116) was judged to be an artifact of derivatization or sample handling, based on the following observations. (1) The relative abundances of *m/e* 1029 and *m/e* 913, the molecular ion of the heptasilyl derivative expected from structure **1**, varied with silylation conditions, with the intensity of *m/e* 1029 in Figure 6 representing the maximum observed. Use of freshly distilled pyridine or use of less pyridine greatly reduced the intensity of *m/e* 1029 nearly to the vanishing point. (2) Hydrolysis of Q_{Me_3Si} by warm ethanol–water (1:1) produced a product having a different mobility than Q on thin layer chromatography and which streaked slightly, presumably due to presence of an N-CO_2H group. (3) The field desorption mass spectrum of Q_{Isop} exhibited peaks of *m/e* 490, and less abundant *m/e* 512. These correspond to the protonated molecule MH and the adduct MNa typically produced from traces of salt (Shulten and Beckey, 1973). The molecular weight of Q is therefore 409 in agreement with structure **1**. No peaks corresponding to a carboxylated form of Q were observed.

Recognition of this unusual artifact is important in view of the widespread use of mass spectrometry and trimethylsilylation in structural problems. Further, the product derived from silylation of the isopropylidene derivative of Q provided valuable evidence concerning presence of cis diol groups in the molecule. Location of the carboxyl group was rationalized by comparison of spectra representing compounds **3** and **4**, which differ only by presence of an acetyl group in the side chain. The carboxyl group does not reside in either the 243 or 655 amu moieties, leaving only the side chain as a point of attachment. Otherwise the principal peaks in the spectrum of **4** are analogous or identical with those from **3**. The small series of peaks between 913 and 1029 correspond to simple elimination reactions which are of no diagnostic value.

The mass spectrum of Q_{Isop} after trimethylsilylation is shown in Figure 7. The molecular ion *m/e* 821 corresponds to the diisopropylidene derivative and provides firm evidence for the presence of two cis diol groups in the molecule, in support of structure **5**. The spectrum is dominated by ion a (*m/e* 552) and the simple cleavage product *m/e* 551 and its base + H analog (*m/e* 307), whose masses require that the second isopropylidene function not reside in the nucleoside portion of the molecule. A small amount of monoisopropylidene contaminant is represented by *m/e* 656. Other less important fragment ions include *m/e* 763, which represents expulsion of acetone from the isopropylidene group (DeJongh and Biemann, 1964), and *m/e* 705 which can be either M − 2 × acetone or M of the noncarboxylated analog of **5**.

Additional evidence for presence of a vicinal diol group in Q and of the proposed side chain structure was obtained by periodate oxidation of Qp. The mass spectrum of the dephosphorylated and silylated product (Figure 8) is domi-

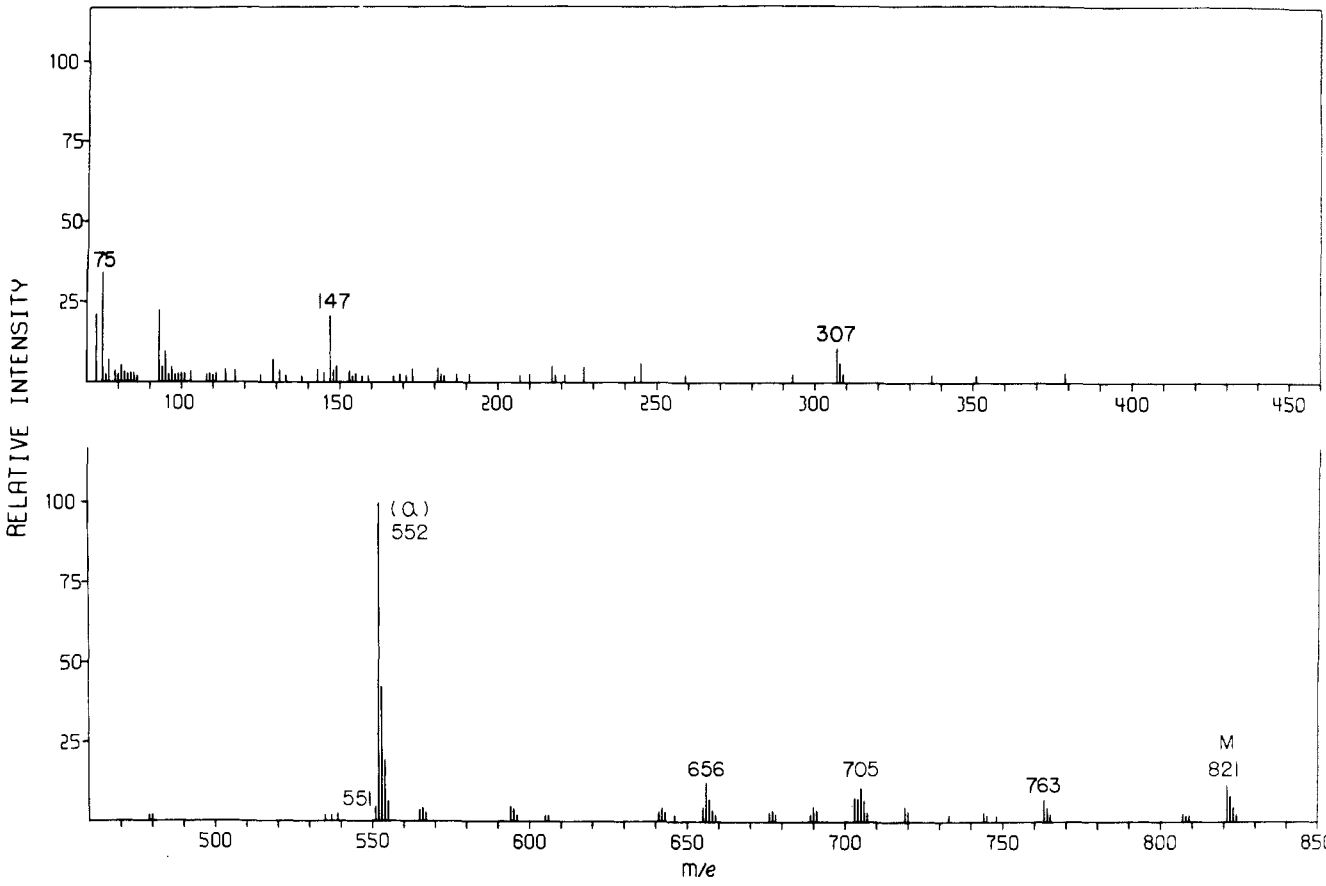
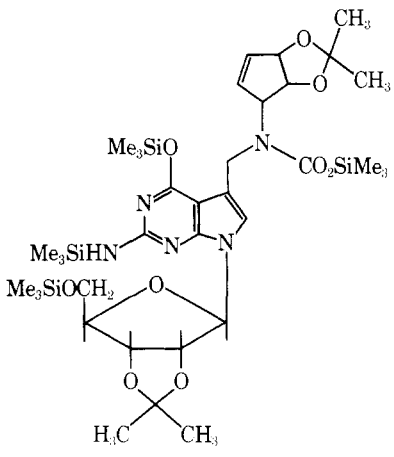


FIGURE 7: Mass spectrum of $Q_{Isop\text{-}Me_3Si}$ (20 eV).



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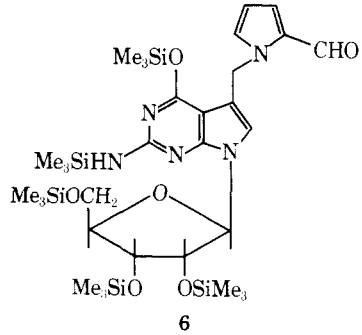


6

nated by the molecular ion, *m/e* 749, and in addition exhibits two peaks of unknown origin at higher mass (*m/e* 801, 5%; *m/e* 873, 3%). The molecular ion of the lower (tetrasilyl) derivative is observed at *m/e* 677. The molecular mass corresponds to dehydration; its odd (vs. even) character implies retention of all five nitrogen atoms. The most striking feature is the absence of ion a, indicating side chain modification and suggesting the absence of hydrogen γ to the C-7,8 double bond of the deazapurine ring. The presence of the base + H species at *m/e* 401 confirms (by difference) that the sugar has not undergone modification. These data in addition to the uv spectrum of Q_{NaIO_4} (discussed in the following section) support the pyrrole structure **6** as the final product.

Confirmation of the Structure of Q by Proton NMR Spectroscopy. The proton NMR spectrum of Q shown in Figure 9 indicates resonances from 14 nonexchangeable protons. The assignment of resonances is shown in Figure 10 and the coupling constants of the cyclopentenediol protons of Q and Qp are listed in Table III.

The presence of an unmodified ribose was deduced from the resonances at 5.982 ppm (1 H, double, 1′ H, $J_{1'-2'}$ = 6.4 Hz), 4.605 ppm (1 H, triplet, 2′ H, $J_{2'-3'}$ = 5.4 Hz) 4.350 ppm (1 H, triplet, 3′ H, $J_{3'-4'}$ = 3.5 Hz), 4.191 ppm (1 H, quartet, 4′ H, $J_{4'-5'}$ = 3.5, 4.1 Hz), 3.850 and 3.807 ppm (2 H, A-B pattern, 5′ H, $J_{5'-5'}$ = −13 Hz). The relative chemical shifts and coupling constants are typical of those found in purine nucleosides and nucleotides (Altona and Sundaralingam, 1973). The assignments of 1′ H, 2′ H, 4′ H, and 5′ H were confirmed by appropriate homonuclear spin decoupling. The assignment of 3′ H was confirmed from the spectrum at high pD where the cyclopentenediol ring protons shift upfield allowing an accurate measurement of $J_{2'-3'}$ and $J_{3'-4'}$.

There are four resonances not assigned to the ribose moiety in the region immediately upfield from the residual proton peak of water: two multiplet signals at 4.695 and 4.291

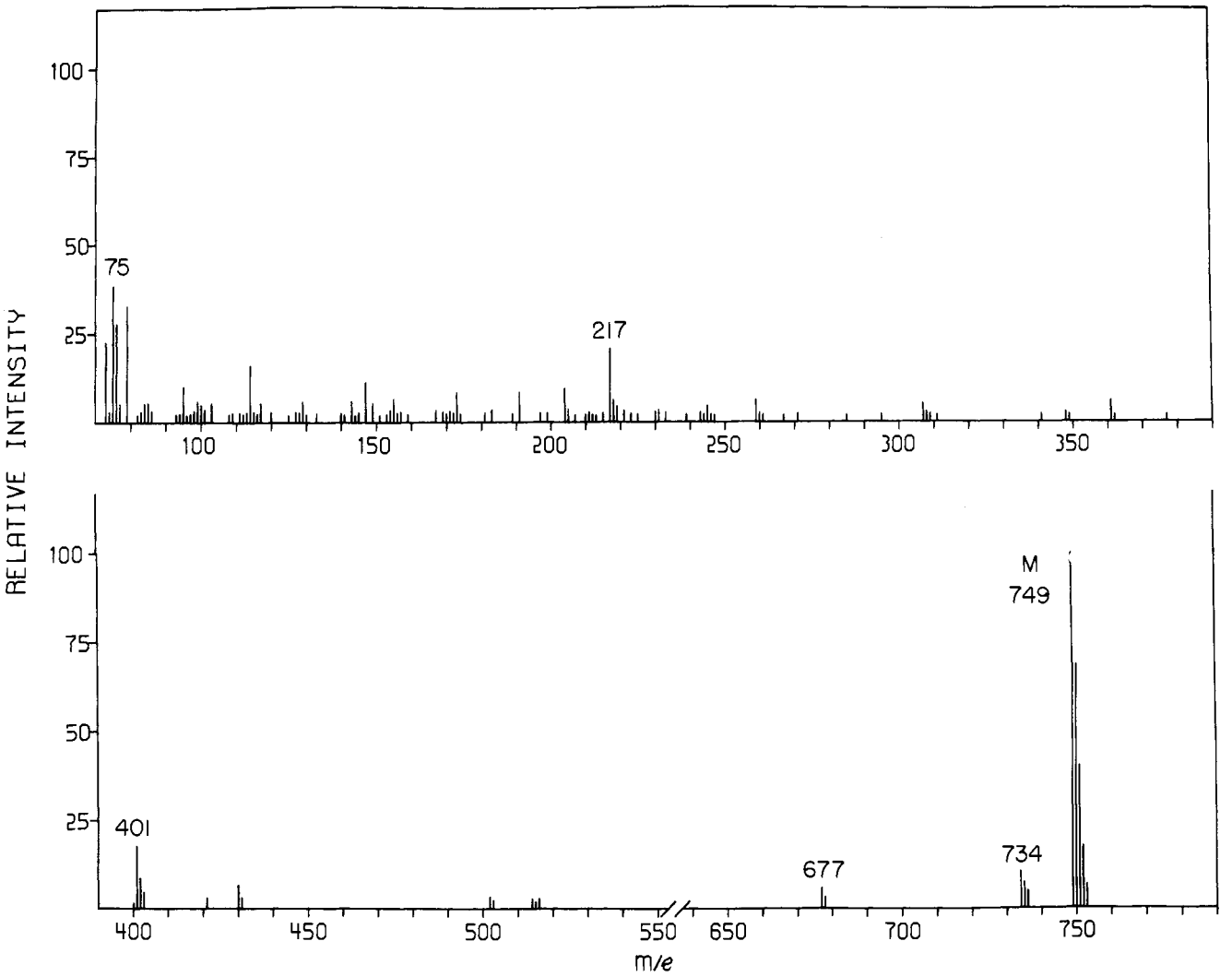


FIGURE 8: Mass spectrum of $Q_{NaIO_4\text{-}Me_3Si}$ (20 eV).

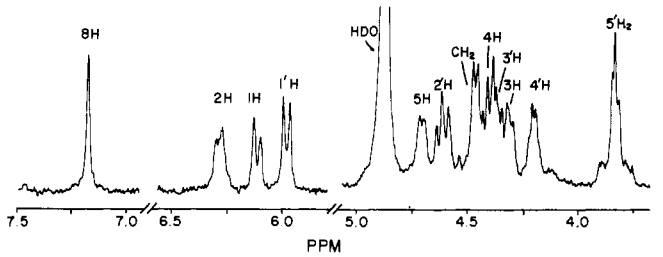


FIGURE 9: ^{1}H NMR spectrum of Q at 220 MHz, 22°, and pD 7.0.

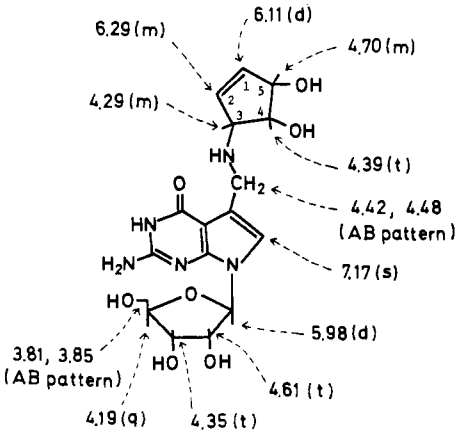


FIGURE 10: Chemical shifts (ppm) in the proton NMR spectrum of Q.

Table III: Coupling Constants (Hz) between Protons in the Cyclopentenediol Ring

	J_{1-2}	J_{2-3}	J_{2-5}	J_{1-5}	J_{1-3}	J_{3-4}	J_{4-5}
Q	6.5	2.5	1.7	1.5	~0.6	5.2	5.2
Qp	6.3	2.7	1.7	1.4	~0.6	5.2	5.2

ppm, a triplet at 4.393 ppm, and a two proton doublet centered at 4.45 ppm with a small peak at 4.52 ppm, just downfield from the doublet. The presence of the small peak indicates that the two proton doublet is not caused by scalar coupling to a third proton but instead represents the two central peaks of a strongly coupled A–B pattern where the geminal coupling constant is −14 Hz and the nonequivalence is 10 Hz. This interpretation is confirmed by lower magnetic fields which cause a coalescence of the A–B pattern into a singlet as the nonequivalence in Hz becomes smaller. Thus NMR spectrum taken at 100 MHz shows a sharp singlet in the region where at 220 MHz the two proton doublet appears, indicating the presence of an isolated, strongly deshielded diastereomeric methylene in Q.

Of the remaining six protons, the two olefinic protons at 6.286 and 6.111 ppm, and the three protons at 4.695, 4.393, and 4.291 ppm are assigned to the five protons of the cyclopentenediol moiety (Figure 10). The second-order effects on

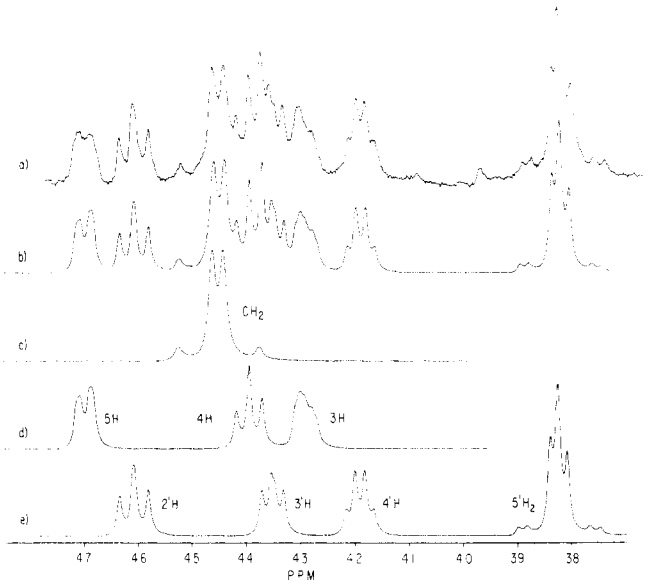


FIGURE 11: Computer simulation of the ribose region of the spectrum of Q. (a) The spectrum of Q at 220 MHz, 22°, and pD 7.0. (b) The complete simulated spectrum made by summing the subspectra c, d, and e. (c) The methylene protons. (d) The 5 H, 4 H, and 3 H protons of the cyclopentenediol ring. (e) The ribose proton absorptions. The chemical shifts and coupling constants used are within 0.002 ppm and 0.2 Hz, respectively, of the observed spectrum.

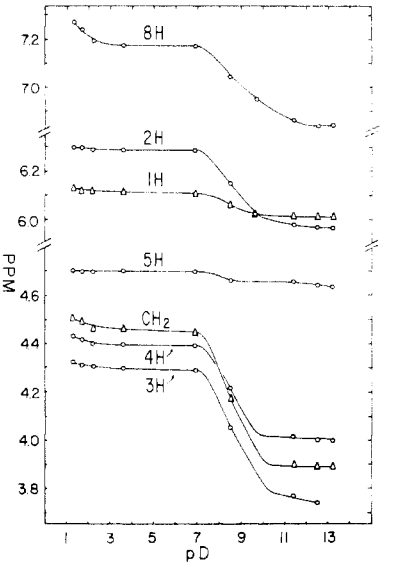


FIGURE 12: Change in chemical shift of cyclopentenediol protons with pD.

the peak shape of the olefinic protons as well as their coalescence to a sharp singlet at pD 9.7 as the chemical shifts become identical confirms that they are on adjacent carbons and have a mutual coupling constant of 6.5 Hz. This value is consistent with the coupling expected for a cyclopentene ring; a cyclohexene ring would have a value of about 8.5 Hz and a trans olefin between 10 to 14 Hz (Bothner-By, 1965). Homonuclear spin decoupling experiments show that the downfield olefinic proton is coupled to the resonances at 4.695 and 4.291 ppm while the upfield olefinic proton is coupled to the peak at 4.291 ppm. The magnitude of the coupling indicates that these protons are adjacent to the olefinic protons. Both protons at 4.695 and 4.291 ppm show a further coupling of about 5 Hz; however, spin decoupling experiments have failed to show any evidence for coupling between these protons. This coupling can, however, be accounted for by the triplet at 4.393 ppm which has two equal coupling constants of 5.2 Hz. Furthermore, the distortion in the shape of the peaks at 4.393 and 4.291 ppm indicates that these protons are mutually coupled. This coupling could not be confirmed by homonuclear decoupling because of the close proximity of the coupled resonances to the irradiating signal of the decoupler. The fit of the computer-simulated spectrum (Figure 11) strongly supports the assignment of the resonance at 4.393 ppm as the middle proton of a series of three methyne protons. The magnitude of the couplings suggest that the three substituents on the cyclopentene ring are all cis to each other, although coupling constants for saturated carbons in five-membered rings cannot be used to assign the conformation unambiguously (Bothner-By, 1965). The observation of a single, unique set of resonances indicates that the cyclopentenediol ring is a pure stereoisomer and is strong evidence for its biological origin.

The specific assignments of the cyclopentenediol ring protons are based on the relative magnitude of their changes in chemical shift with increasing pD. The dependence of the chemical shifts on pD shown in Figure 12 indicates the presence of a titratable group with a pK of between 8.5 and 9.0 attached to the methylene and the cyclopentenediol ring. The large shift of the C-3 proton of 0.52 ppm with increasing pD and its upfield position assign the C-3 as the site of attachment of the cyclopentenediol ring. The larger shift of the olefin proton at 6.286 ppm assigns this absorption as the C-2 proton, adjacent to the C-3. The remaining assignments follow from the scalar coupling analysis. The titration effects may be more complex than they appear since both the secondary amine and the 7-deazaguanosine base would be expected to have pK's of between pD 8 and 10. Thus there may be a zwitterionic interaction between the deprotonated base and the protonated amine in this pH range. The observed large shifts with increasing pD are consistent with the deprotonation of a secondary amine which connects the cyclopentenediol ring to the methylene group as shown in Figure 10.

The remaining resonance, the downfield singlet at 7.170 ppm, corresponds to an isolated unsaturated or aromatic proton. This absorption cannot be attributed to a typical purine since it is shifted upfield about 1 ppm from where the protons of adenosine or guanosine absorb (Schweizer et al., 1968). The upfield shift for the proton in Q is, however, consistent with a C-8 proton in a 7-deazapurine nucleoside such as 7-deazaadenosine (tubercidin) or 7-deazainosine (Pike et al., 1964). This result strongly supports the 7-deazaguanine base for Q with a side chain at C-7.

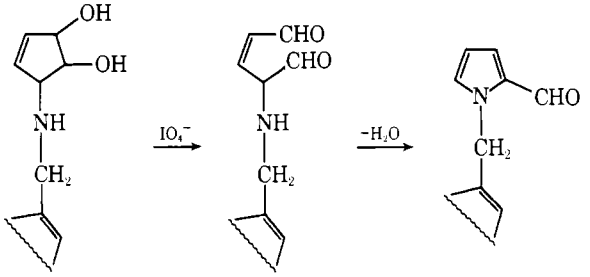
Discussion

Preceding results indicate that the modified nucleoside Q is a derivative of 7-deazaguanosine having a side chain at C-7 which contains a cyclopentenediol group linked to the base through an aminomethyl group.

The 7-deazaguanine nucleus was identified by the following results. (1) High resolution mass spectra of several different derivatives of Q suggest that the heterocyclic nucleus of Q contains only four nitrogen atoms. (2) In the ^{1}H NMR spectrum of Q, the signal corresponding to the purine C-8 proton was shifted to considerably higher field. (3) The uv absorption spectrum of Q is similar to that of 7-deazaguanine at neutral pH, and that of 7-deazaguanosine. (4) The glycosidic bond in Q is resistant to acid hydrolysis,

while mass spectral and ^1H NMR data strongly support the presence of a normal (N-C) glycosidic bond. (5) Unlike normal purines, the hydrogen at C-8 is resistant to exchange by deuterium.

The presence of a cyclopentenediol moiety in the side chain was deduced from high resolution mass spectra which showed a C_5O_2 group having two rings plus double bonds and two active hydrogens, and from the ability to form an *O*-isopropylidene derivative. Also, important in the ^1H NMR study, five protons of the cyclopentenediol group were identified by spin decoupling experiments. Periodate oxidation, also consistent with a diol function, leads to pyrrole formation by condensation between the side chain nitrogen and aldehyde group for which there is ample precedent (e.g., Brown and Jacobson, 1961). The uv absorption spectrum of Q_{NaIO_4} (Figure 13) is considerably different from that of Q (Figure 3). Appearance of two λ_{max} is accounted for as the sum of the spectra of Q and *N*-methyl-2-pyrrolecarboxaldehyde, which has λ_{max} (ϵ) at 253 nm (7500) and 280 nm (16,000) (Jones and Wright, 1968). The formation of a pyrrole ring is thought to proceed according to the reaction scheme as shown below. The cis diol in the cyclopentene group is first oxidized to form two aldehyde groups, one of which reacts with the secondary amine function followed by dehydration to form a stable pyrrole ring.



When the data from mass spectrometry and ^1H NMR alone are considered for the structural assignment of Q, a structure having a 3-deazaguanine nucleus and a side chain at the C-8 position cannot be ruled out. However, such a structure is unlikely since 3-deazapurine nucleosides should be easily hydrolyzed to the corresponding base by acid treatment (Rousseau et al., 1966) while the glycosidic bond of Q is stable under the same conditions.

In order to unambiguously establish the structure of Q, its synthesis or that of closely related models, such as 7-alkyl-7-deazaguanosine, is desirable. Alternatively, X-ray crystallographic studies of Q should be highly informative. Work along both of these lines is now in progress.

Modification of the purine nucleus is thus far unique in nucleosides from tRNA. It is interesting to note that Q or a Q-like nucleoside has also been isolated from *Drosophila* tRNA (White et al., 1973). A mass spectrum of trimethylsilylated Q of *Drosophila* was essentially the same as that of Q_{Me_3Si} from *E. coli* tRNA (H. Kasai and S. Nishimura, unpublished results). This indicates that at least one of the Q-like compounds in *Drosophila* tRNA has a structure identical with Q from *E. coli*. It is of interest that the chromatographic profile of tRNA isolated from cancer cells frequently differs from that of normal tRNA in case of tRNA for tyrosine, histidine, asparagine, and aspartic acid (Baliga et al., 1969; Sekiya and Oda, 1972). These results have been further confirmed recently by Katze (1974). This observation, together with the finding of a Q-like compound from rat liver tRNA (Rogg and Staehelin, 1969), suggests

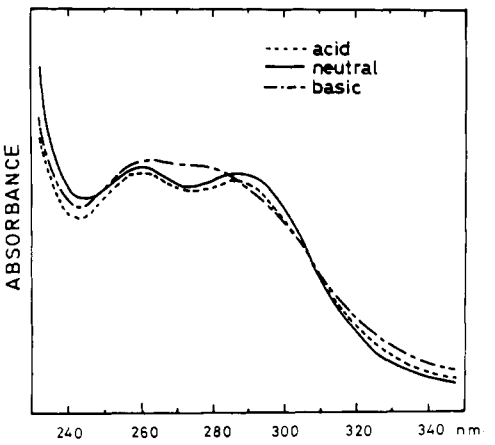


FIGURE 13: Uv absorption spectra of Q_{NaIO_4} (acid, 0.1 *N* HCl; basic, 0.1 *N* NaOH).

that the Q nucleoside is also present in mammalian tRNA, and that the extent of modification of G to Q is related to growth conditions and stage of cells. Thus, it seems that Q occurs widely in tRNA from prokaryotes to eukaryotes such as mammalian cells.

Elucidation of the structure of Q raises an interesting question as to its biosynthesis. Goodman et al. (1970) were able to isolate $tRNA^{Tyr}$ containing normal guanosine instead of Q by infecting *E. coli* with ϕ80 su3$^-$ which carries a wild type of $tRNA^{Tyr}$ gene. It is likely that modification of guanylate residue to produce Q in the $tRNA^{Tyr}$ molecule does not proceed completely due to the synthesis of $tRNA^{Tyr}$ in large excess amounts in cells, thereby resulting in the formation of unmodified $tRNA^{Tyr}$. Thus, it is reasonable to conclude that the precursor of Q is guanosine in the case of *E. coli*. A mechanism for replacement of N-7 of guanosine by carbon, without disintegration of the polynucleotide linkage, is difficult to imagine. Biosynthetic mechanisms involved in the synthesis of pyrrolopyrimidine nucleosides such as tubercidin (Smulson and Suhadolnik, 1967) and toyocamycin (Suhadolnik and Uematsu, 1970; Uematsu and Suhadolnik, 1970) and that of pterdine (Burg and Brown, 1966; Levenberg and Kaczmarek, 1966; Krumdieck et al., 1966) might be also possible in the synthesis of Q.

White et al. (1973) reported that amounts of tRNAs containing Q decreased, with concomitant increase of the corresponding tRNAs containing normal guanosine, during the change of life cycle from egg to the third instar in *Drosophilia*. The possibility that Q is replaced by guanosine in the tRNA molecule without transcription of the polynucleotide chain cannot be excluded. In this respect, it should be noted that Farkas et al. (1973) reported that guanine was incorporated into the polynucleotide chain of one of two isoaccepting species of $tRNA^{His}$ in reticulocytes of rabbit, sheep, and man after completion of transcription of the polynucleotide chain. There might be a specific mechanism for replacement of guanosine residue by Q or vice versa. These problems require further study.

The function of Q in *E. coli* tRNAs is not yet understood. Q has a greater affinity for U than C in codon-anticodon base pairing, since among the trinucleotides corresponding to code words, those ending with U always cause the greatest stimulation of binding of tRNAs containing Q to ribosomes (Harada and Nishimura, 1972). However, it is not clear whether such modification is absolutely necessary for protein synthesis in cells in vivo, since the extent of pref-

erential recognition of code words ending with U is not particularly great. Another possible explanation of the function of Q is that it prevents any miscoding which might occur if G is present in the first position and U in the second position of the anticodon of *E. coli* tRNAs. Isolation of an *E. coli* mutant, in which the enzymes involved in the biosynthesis of Q are temperature sensitive, should be very useful for understanding the function of Q as well as for elucidation of its biosynthetic pathway.

Pseudouridine in $tRNA^{His}$ of *Salmonella* was found to be important in the regulatory function of tRNA in expression of the histidine operon (Singer et al., 1972). It is possible that other modifed nucleosides may be involved in similar regulatory functions. As discussed in the introduction section, the modified nucleoside Q in *Drosophila* tRNA may be related to differentiation of cells (White et al., 1973) or to specific inhibition of enzymatic activity (Jacobson, 1971). The bulky side chain present in Q may facilitate specific interaction with ehzymes or protein components that are related to regulation. These problems as to the function of Q in eukaryotic tRNA warrant further study.

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