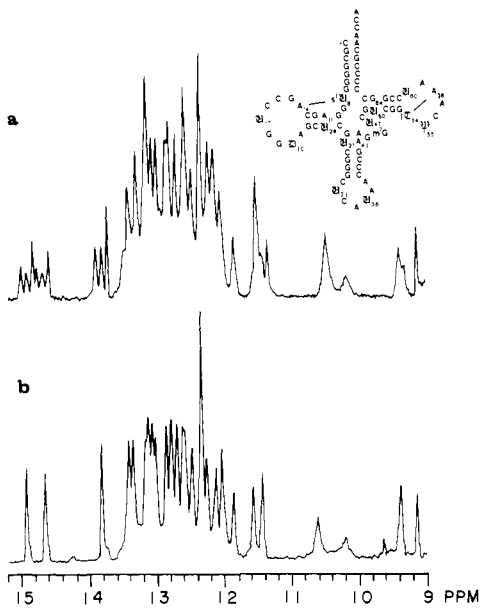

Figure 1. ^1H 500-MHz NMR spectra of *E. coli* $tRNA_f^{Met}$: (a) ^{15}N-labeled $tRNA_f^{met}$ at 30 °C; (b) unlabeled $tRNA_f^{met}$ at 35 °C.

were found in U (60%), D (64%), and Ψ (61%), and no label was detected in G (the only purine base with an imino proton).[16] Material obtained by this procedure had a specific activity of 1.5 nmol/A_{260} unit.

E. coli $tRNA_f^{Met}$ has 12 uridine-related bases that should contain ^{15}N. From the X-ray structure of the molecule[17] and previous NMR studies, ^1H–^{15}N couplings are expected for at least three secondary interactions involving U24, U27, and U50 (a wobble interaction with G64) and perhaps as many as three couplings for tertiary interactions involving s^4U8, rT54, and Ψ55. Additional ^1H–^{15}N pairs may also arise from "free" imino protons that are buried in the interior of the molecule and exchange slowly with water.

Samples for NMR spectra were dialysed against 0.1 mM sodium thiosulfate, lyophilized, and dissolved in 10 mM sodium cacodylate buffer containing 4% deuterium oxide, 50 mM sodium chloride, 10 mM magnesium chloride, and 1 mM EDTA at pH 7.0. Spectra were obtained on a Nicolet 500-MHz NMR spectrometer using a modified Redfield pulse sequence to minimize the signal from water.[18] ^1H NMR spectra of labeled and natural $tRNA_f^{Met}$ are shown in Figure 1, parts a and b, respectively. It is immediately apparent that the well-resolved 1b peaks at 13.82, 14.68, and 14.90 ppm in Figure 1b are approximately 1:1:1 trios[19] in Figure 1a. This is the pattern expected with a central peak due to ^1H–$^{14}N(3)$ flanked by the ^1H–$^{15}N(3)$ doublet ($^1J_{H-N} \sim 90$ Hz)[20] when the level of incorporation of label is 60–65%.

The resonance at 14.90 ppm has been attributed to the imino proton in the s^4U8-A11 tertiary pair in *E. coli* $tRNA_f^{Met}$ by several groups[21-25] and is regarded as one of the least controversial assignments in the region between 11 and 15 ppm.[7] As expected, that peak appears as a trio in Figure 1a. The assignment of the resonance at 14.68 ppm to the imino proton of m^7G in the m^7G46-C13-G22 triple on the basis of chemical shift comparisons among several tRNAs and chemical modification experiments[26] is also generally regarded as reliable.[7] It is clear, however, from the trio seen in Figure 1a that this is incorrect. A logical alternative more consistent with chemical shift patterns reported for other tRNAs is the rT54 imino proton in the rT54-A58 double,[24] although it should be noted that this is not a firm assignment since chemical shifts of imino protons in secondary A-U pairs have been reported as high as 14.6 ppm.[9] At present we cannot reliably choose among the imino protons of U24, U27, or rT54 for the peaks at 14.68 and 13.90 ppm, although NOE experiments planned for the future may permit a distinction.

The selective incorporation of ^{15}N into the uridine-derived bases of $tRNA_f^{Met}$ permits us to unambiguously locate resonances for three of the six potential hydrogen bonds involving s^4U8, U24, U27, U50, rT54, and Ψ55. In the following communication we describe variable-temperature and NOE studies that allow us to uncover trio patterns in more congested regions of the spectrum.

Acknowledgment. We thank the National Science Foundation (PCM 7916861 to C.D.P.) for financial support. Potassium [^{15}N]cyanide was provided by the Stable Isotopes Resource Center at Los Alamos Scientific Laboratories, jointly supported by the National Institutes of Health and the Department of Energy (P 41 RR 00962). The *E. coli* SΦ-187 mutant was a gift from Professor Jan Neuhard, and we gratefully acknowledge the assistance of Drs. Henrianna Pang and James A. McCloskey with the tRNA hydrolysis experiments.

Registry No. Uridine, 58-96-8; 4-thiouridine, 13957-31-8; ribothymidine, 1463-10-1; pseudouridine, 1445-07-4.

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^1H NMR Studies of ^{15}N-Labeled *Escherichia coli* $tRNA_f^{Met}$. An Unambiguous Assignment for the G-U Pair and Detection of a Uridine Resonance at 11.4 ppm

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The secondary G-U[1] wobble interaction is found in the stems of several tRNAs and may perturb the normal helical pattern in adjacent base pairs.[2] In a series of elegant NOE experiments, Johnston and Redfield[3,4] located resonances for two spatially

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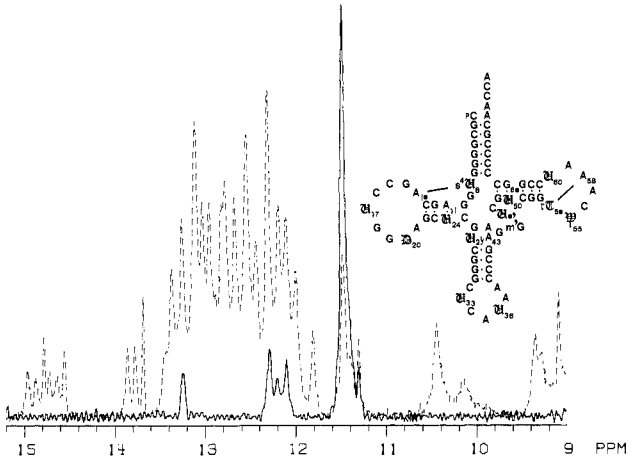

Figure 1. Normal (---) and NOE difference spectra (—) for *E. coli* $tRNA_f^{Met}$ in the 10 mM sodium cacodylate buffer containing 4% deuterium oxide, 50 mM sodium chloride, 10 mM magnesium chloride, and 1 mM EDTA at pH 7.0, 35 °C; preirradiation at 11.50 or 19.50 ppm for 0.5 s. An NOE run of the quality shown required 8 h of accumulation time with 4 mg of sample dissolved in 150 μL of buffer (~0.7 mM).

adjacent protons in *E. coli* $tRNA_f^{Met}$, *E. coli* $tRNA^{Val}$, and yeast $tRNA^{Phe}$ and attributed the paired peaks to a G-U wobble interaction in each molecule. Although ring-current calculations suggest that the U imino proton resonanates at lower field in each pair,[5] unambiguous assignments have not been made. In theory, the spectrum of labeled *E. coli* $tRNA_f^{Met}$ presented in the previous communication[6] could be used to verify the G-U assignments. However, the peak at 12.4 ppm attributed to U50 is located in a congested region of the spectrum where the presence of a $^1H-^{15}N$ doublet could not be easily discerned. Furthermore, inspection of the region near 11.5 ppm suggested that one of the two nearby peaks was split by ^{15}N.[7] We now report assignment of the individual peaks in the G-U wobble pair.

The normal ^1H spectrum of *E. coli* $tRNA_f^{Met}$ and an NOE difference spectrum with preirradiation of the resonance at 11.50 ppm are shown in Figure 1. It is immediately obvious that transfer of saturation has occurred to resonances in two regions of the spectrum, an intense (37%) trio pattern[8] at 12.20 ppm indicative of a labeled uridine $^1H-^{15}N$ pair and a single peak (10%) at 13.34 ppm.[9] In control experiments, preirradiation of the resonance at 11.54 ppm in unlabeled *E. coli* $tRNA_f^{Met}$ gave a difference spectrum with single peaks at 12.31 and 13.32 ppm, while preirradiation of labeled *E. coli* $tRNA_f^{Met}$ at 12.20 ppm produced a negative NOE to a single peak at 11.5 ppm. These experiments provide incontrovertible evidence for a stable G-U wobble interaction in the molecule that gives the peaks at 11.50 (G64) and 12.20 (U50) ppm. In addition, the large NOE (10%) to the peak at 13.20 ppm requires assignment of that resonance to an imino proton in one of the flanking G–C pairs. Previous suggestions for the origin of this resonance include rT54,[10] G15,[11] and G53.[12] Additional NOE studies will be needed to completely unravel these assignments.

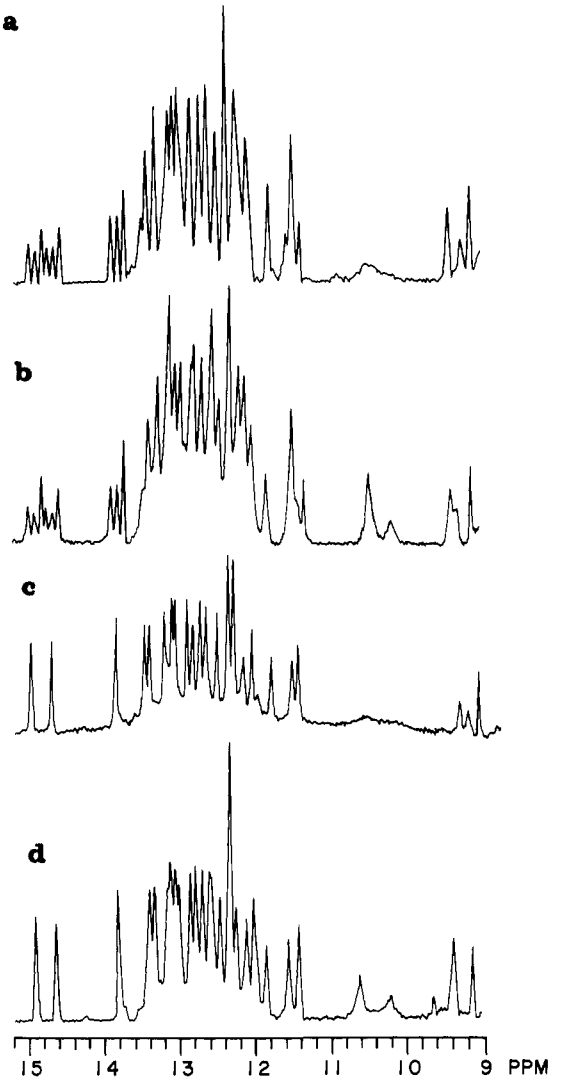

Figure 2. NMR spectra of *E. coli* $tRNA_f^{Met}$ at 500 MHz in 10 mM sodium cacodylate buffer containing 4% deuterium oxide, 50 mM sodium chloride, 10 mM magnesium chloride, and 1 mM EDTA at pH 7.0, ^{15}N-Labeled tRNA at 54 °C (a) and 30 °C (b); unlabeled tRNA at 45 °C (c) and 35 °C (d).

The peaks at 11.54 and 11.40 ppm in normal $tRNA_f^{Met}$ (Figure 2b) are not as well-resolved for the labeled material Figure 2d). As mentioned previously, the peaks overlap, and the resonance at higher field appears to be a trio pattern indicative of a labeled uridine. As the temperature of the labeled sample is raised to 54 °C (Figure 2a), the relative positions of the trio and the single peak at 11.50 ppm shift so that both halves of the $^1H-^{15}N$ doublet are clearly visible.[13] The chemical shift of the trio is further upfield than would be expected for a uridine imino proton hydrogen bonded to a ring nitrogen of another base. A likely assignment is Ψ55 in the G18-Ψ55-P58 triple. Model studies indicate that hydrogen bonding to a phosphate moiety shifts the imino resonance in "free" uridine from 9.5 to 12.0 ppm.[14] A less appealing possibility for the resonance at 11.40 ppm is U60. The X-ray structure of $tRNA_f^{Met}$[15] indicates that the base is located at a sharp turn in the phosphodiester backbone and may be thrust into the interior of the molecule. The other bases (U17a, D20,

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(7) We noticed small differences in chemical shifts as a function of temperature and for tRNA isolated from different batches. Occasionally it was not possible to distinguish between closely spaced peaks when comparing spectra of different samples of tRNA, especially when the temperature was varied.
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(13) A shoulder at 13.45 ppm is also more clearly resolved at 54 °C. A similar but smaller shoulder is seen in the spectrum of unlabeled *E. coli* $tRNA_f^{Met}$. Given the resolution of spectrum a in Figure 2, it is unlikely that the shoulder is part of a trio and may result from an impurity, probably *E. coli* $tRNA_2^{Met}$, in the sample. Additional experiments will be needed to verify this supposition.
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U33, U36, and U47) are all exposed, and their imino hydrogens should exchange with water rapidly. One might argue that crystalline $tRNA_f^{Met}$ changes conformation in solution to shield U33, the "swivel base". It is unlikely, however, that the barrier to conformational interchange would be high enough to explain the persistence of the peak at 54 °C.

The number of imino resonances between 11 and 15 ppm for *E. coli* $tRNA_f^{Met}$ can be estimated from Figure 2d. The highly resolved spectrum has 22 distinct peaks in the low-field region, and only the peak at 12.35 ppm clearly represents more than one hydrogen. Warming the sample to 45 °C produces substantial changes in the spectrum, as shown in Figure 2c. The peaks at 12.55 and 13.10 ppm disappear, and a peak in the envelope at 12.35 ppm moves upfield to combine with the peak at 12.30 ppm, producing new two-proton peaks at 12.30 and 12.35 ppm. It follows that the envelope at 12.35 ppm in Figure 2a contains peaks for three protons. The peaks at 11.95 and 12.10 ppm shift, and a new, broad signal appears at 12.05 ppm. Assignment of this peak to an imino proton in *E. coli* $tRNA_f^{Met}$ is, however, tenuous. There is no distinct two-proton envelope in the region near 12.0 ppm in Figure 2d. Although less than integral values for some peaks in tRNAs are expected,[4] the small peaks at 13.55, 13.75, and 14.25 ppm raise the possibility of a minor contaminant in the sample. Nevertheless, it is evident that signals for only 24–25 imino protons are clearly seen for *E. coli* $tRNA_f^{Met}$ at 35 °C. Since 21 imino hydrogens participate in presumably more stable secondary interactions, only 3 or perhaps 4 peaks for tertiary hydrogen bonds are visible. Our results with ^{15}N-labeled material indicate that uridine imino hydrogens in s^4U8-A14, rT54-A58, and G18-Ψ55-P58 (or U60) account for three of these tertiary signals.

Acknowledgment. We thank Susan Ribiero for a sample of unlabeled *E. coli* $tRNA_f^{Met}$ and Dr. Paul Johnston for several helpful discussions. Financial support from the National Science Foundation (PCM 7916861 to CDP) and the Los Alamos Stable Isotopes Resource, jointly supported by the Department of Energy and the National Institutes of Health (P 41 RR 00962), is gratefully acknowledged.

Registry No. Uridine, 58-96-8; guanosine, 118-00-3; pseudouridine, 1445-07-4.

Reaction of Cumene with Ozone. Formation of a Charge-Transfer Complex, Its Thermal and Photochemical Conversion to Cumyl Hydrotrioxide, and the Mechanism of Decomposition of the Hydrotrioxide

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The reaction of cumene with ozone is of interest from two perspectives. First, the mechanism for the reaction of hydrocarbons with ozone is under intense scrutiny,[1,2] and cumene, with its unique and reactive hydrogen, is an attractive substrate. Second, ozone reacts with olefins to produce small yields of radicals,[3-5] in addition to the normal Criegee products, and this radical production appears responsible for a large fraction of the biological damage that occurs when lung tissue in vivo or polyunsaturated fatty acids in vitro are exposed to polluted air containing ozone.[6-10] We have suggested that the mechanism of radical production involves the formation of an allylic hydrotrioxide,[4] and cumene represents a more easily studied model for this process than does a typical olefin.

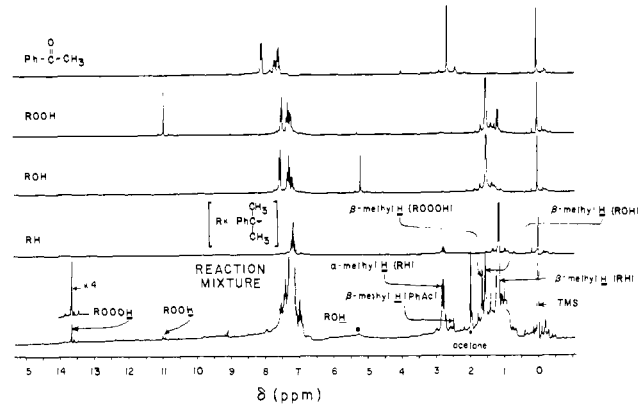

Figure 1. 200-MHz ^1H NMR spectra of the cumene–ozone reaction mixture, cumene, cumyl alcohol, cumyl hydroperoxide, and acetophenone in acetone-d_6 at −73 °C. Abbreviations: ROOOH = cumyl hydrotrioxide, RH = cumene, ROH = cumyl alcohol, ROOH = cumyl hydroperoxide, AP = acetophenone, TMS = tetramethylsilane.

The reaction of ozone with C–H bonds has been studied extensively.[1] The ultimate products from such reactions suggest that a hydrotrioxide is an intermediate, but hydrotrioxides have been identified only from the reaction of aldehydes, acetals, and similar substrates, and not for hydrocarbons.[1] The mechanism that has been accepted, eq 1, involves abstraction of a hydrogen atom in

$$RH + O_3 \rightarrow [R\cdot \cdot O_3H \leftrightarrow R^+ \ ^-O_3H] \rightarrow ROOOH \quad (1)$$

a transition state with appreciable dipolar character.[1,11] Recently, however, Benson[2] has suggested the mechanism involves hydride abstraction, eq 2.

$$RH + O_3 \rightarrow [R^+ \ ^-O_3H] \rightarrow ROOH \quad (2)$$

We here report the production of cumyl hydrotrioxide both by a thermal process and by photolysis of the charge-transfer (CT) complex of cumene and ozone. We also describe a kinetic study of the decomposition of the hydrotrioxide.

We find that ozonation of cumene in acetone at −78 °C produces a CT complex with absorption at 360 nm.[12] Formation of the CT complex is reversible: blowing out the ozone with nitrogen causes the absorbance to disappear.

Cumyl hydrotrioxide could be prepared by bubbling ozone (0.1 mmol/min) in oxygen through 3.58 M cumene in 4 mL of acetone-d_6 at −40 °C for 3 h in the dark or by irradiating the solution with light of 340–390 nm while ozonating for 15 h at −75 °C. The thermal preparation gave ca. 0.07 M solutions (ca. 2% yield) of ROOOH (where R is $PhCMe_2$), contaminated with 0.22 M cumyl alcohol, 0.14 M cumyl hydroperoxide, and 0.07 M ace-

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(12) (a) Charge-transfer complexes also were observed in Freon-11, $CFCl_3$ (380 nm) and pentane (382 nm). The absorption in pentane solvent agrees with that reported[12b] for toluene and ethylbenzene in a pentane glass at −195 °C. (b) Bailey, P. S.; Ward, J. W.; Carter, T. P.; Nich, E.; Fischer, C. M.; Khashab, A. Y. *J. Am. Chem. Soc.* **1974**, *96*, 6136. (c) Varkony, T. H.; Pass, S.; Mazur, Y. *J. Chem. Soc., Chem. Commun.* **1975**, 709. (d) Charge-transfer complexes of O_2 and hydrocarbons are well-known; for references see: Pryor, W. A.; Patsiga, R. A. *Spectrosc. Lett.* **1969**, *2*, 61, 353. (e) Full details of our experiments will be published: Pryor, W. A.; Ohto, N.; Church, D. F., to be submitted for publication.

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