Nuclear Spin-Labeled Nucleic Acids. 1. ^{19}F Nuclear Magnetic Resonance of *Escherichia coli* 5-Fluorouracil-5S-RNA

Sir:

While unpaired *electron* (usually artificial nitroxide) spin-labels have been used for more than 10 years as probes of macromolecular structure, the introduction of artificial *nuclear* spin-labels has so far been limited to ^{13}C enrichment of specific carbons in proteins[2] and nucleic acids,[3,4] and to fluorine-labeling of a number of enzymes.[5,6] In this communication, we report the first ^{19}F nuclear magnetic resonance study of fluorine-labeled 5S-ribonucleic acid (FU-5S-RNA).[1]

The specific advantages of fluorine as a nuclear spin-label in RNA are manifold. First, of the four major bases, only uracil is replaced by 5-fluorouracil (FU):[1] thus only 20 of the 120 5S-RNA bases (or about 11 of the 80-odd bases of transfer-RNA) contribute to the ^{19}F NMR spectrum, providing very great simplification in spectral assignment and analysis. Second, the replacement of uracil by FU does not significantly alter the function of the 5S-RNA (as judged by binding to ribosomes)[7] or the transfer-RNA (as judged by amino acid acceptor function,[8] optical spectrum,[8] or heat-denaturation profile[9]) from *E. coli*. Third, since ^{19}F NMR chemical shifts are much more widely dispersed than proton shifts, one can expect to monitor relatively small changes in the environments of various individual fluorouridylates simultaneously.[5] Fourth, compared to ^{13}C (even when nonselectively[3] or selectively[4] enriched) or ^{15}N or ^{31}P NMR from native transfer-RNA,[10,11] the fluorine label gives much stronger NMR signals—the spectra in Figure 1 were obtained from about 10 000 to 30 000 transients for a FU-5S-RNA concentration of less than 3×10^{-4} M, while comparable ^{13}C or ^{31}P spectra would require of the order of 1 000 000 transients using either much higher RNA concentration or much larger sample volume. Finally, the FU label is cheaper than commercially available ^{13}C-enriched nucleic acid bases by a factor of about 1000.

The FU-5S-RNA isolated from *E. coli* B strain exhibits extensive (up to about 80%) replacement of uridine by 5-fluorouridine.[12] The present FU-5S-RNA samples were obtained using the methods described in ref 12, from cells of *E. coli* B which had been grown on a medium to which FU was added during early exponential growth. These cells were kindly provided by Professor I. I. Kaiser. The lyophilized product (from which contaminating ribosomal-RNA and transfer-RNA had been removed) was lyophilized twice more against D_2O, to give an NMR sample of concentration, 30 mg/ml, as determined by optical density (260 nm) measurements.

^{19}F FT-NMR spectra of FU-5S-RNA at 35 °C (middle spectrum) and 72 °C (bottom spectrum) are shown in Figure 1. These spectra were phased by reference to a sample of 5-fluoro-deoxy-UMP and 5-fluorouracil (top spectrum). Each of the FU-5S-RNA spectra represents a superposition of the ^{19}F NMR signals from about 20 labeled bases. Nevertheless, at least four distinct groups of peaks are resolved at 35 °C, and the heat-denatured species (72 °C) is sufficiently unfolded such that virtually all the fluorine nuclei have a common chemical shift (sharp signal at 5 ppm downfield from free FU), pointing to a common chemical environment for most of the fluorouracils in denatured FU-5S-RNA. Moreover, the denatured spectrum is centered near the resonant frequency of free 5F-deoxy-UMP, indicating that this chemical shift corresponds to an environment in which the fluorine-label is exposed to solution. Comparing the relative intensity of the FU-5S-RNA signal at this frequency at 35 and 72 °C, it is possible to estimate that approximately 25–35% of the fluorouracil residues in the native FU-5S-RNA structure are exposed to solution. From the relative signal-to-noise ratios for spectra obtained using varying delay times between successive transient acquisitions, the spin-lattice relaxation time(s) for the FU-5S-RNA peak(s) must be short (less than 0.4 s), corresponding to a relatively rigid solution structure. Finally, and in contrast to prior gel filtration chromatographic results for normal 5S-RNA,[13] the heat denaturation of FU-5S-RNA was reversible, as judged by the appearance of the low-temperature (18 °C) ^{19}F NMR spectrum before and after heating to 72 °C.

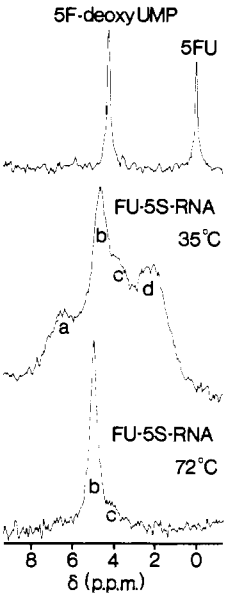

Figure 1. ^{19}F NMR spectra of 5-fluorouracil (FU) in various molecules: (Top) Approximately equimolar mixture (each 0.0075 M) of FU and 5-fluoro-deoxy-UMP in 0.01 M Cacodylate D_2O buffer, pH 7. This sample was used to determine correct phase adjustment for this spectral range. (Middle) 3×10^{-4} M FU-5S-RNA at 35 °C, 30 000 transients, 0.01 M Cacodylate buffer, pH meter reading 7.6 in D_2O. The four resolved groups of peaks are labeled from a to d. (Bottom) 3×10^{-4} M FU-5S-RNA at 72 °C, 10 000 transients, 0.01 M Cacodylate buffer, pH meter reading 7.6 in D_2O. The dominant signal, labeled "b", corresponds to FU residues which are exposed to the external solution (see text). No proton decoupling was employed in any of the spectra.

The present results confirm the potential advantages of ^{19}F as a nuclear spin-label for study of the chemical and motional environment at several specific sites on fluorine-labeled RNA, based on ^{19}F NMR chemical shifts and relaxation times. Further study of the dependence of both normal and partly relaxed ^{19}F NMR spectra on temperature, pH, and presence of metal ions using purified FU-5S-RNA or specific FU-transfer-RNA's promises to provide some of the most simple and direct information yet obtained about the structure and flexibility of these RNA species in solution.

Acknowledgments. The authors wish to acknowledge the continuing advice of Dr. D. C. Roe in the formulation and development of the present work. We also thank Professor I. I. Kaiser for his kind provision of 1 kg of cells of *E. coli* B grown on a medium to which FU had been added. Professor G. Tener is thanked for helpful discussions on RNA fractionation and isolation. Work was supported by grants (to A.G.M.) from the Research Corporation, from the National Research Council of Canada (A-6178), and from the University of British Columbia Research Committee (21-9659).

References and Notes

(1) Abbreviations: FU, 5-fluorouracil; FT-NMR, Fourier transform nuclear magnetic resonance.
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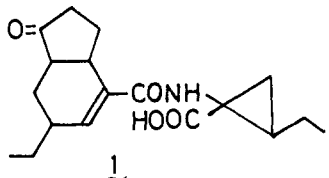
The Structure of Coronatine

Sir:

Coronatine (**1**), produced by *Pseudomonas coronafacience var. atropurpurea*, is a toxin which induces chlorosis on the leaves of Italian ryegrass; it also expands potato cells at concentrations of 1×10^{-7} mol/l.[1] In this communication, we forward structure **1** for coronatine on the basis of spectroscopic data of the derivatives and x-ray crystallographic analysis of coronafacic acid (**2b**).



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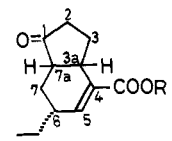
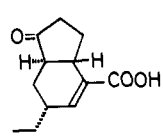
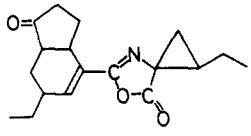
Coronatine (**1**), $[\alpha]^{20}_D$ +68.4° (*c* 2.2, CH_3OH), mp 151–153 °C was formulated as $C_{18}H_{25}O_4N$ (*m/e* M^+ found, 319.1753; calcd, 319.1731) and shows the following spectral data, UV λ_{max}^{EtOH} 208 nm (ϵ 8378); IR ν_{max}^{KBr} 1740 (five-membered ring C=O), 1620 (C=C), 3270, 1645, 1525 cm^{-1} (-CONH-); NMR (90 MHz) $\delta_{Me_4Si}^{CD_3COCD_3}$ 0.94 (6 H, t, *J* = 7 Hz, CH_2CH_3), 3.15 (1 H, br. q, -CHCO), 6.50 (1 H, s, =CH). The high resolution mass spectrum of **1** indicates that coronatine consists of two fragments, $C_{12}H_{15}O_2$ (*m/e* found, 191.1075; calcd, 191.1071) and $C_6H_{10}O_2N$ (*m/e* found, 128.0682; calcd 128.0710), which are bonded to each other by an amide linkage. Other ion peaks below *m/e* 191 are quite similar to those of coronafacic acids, **2a** and **2b**, which were isolated directly from the culture broth. In fact, hydrolysis of coronatine gave an acid, whose R_f value on TLC is identical with that of **2a**, and an α-amino acid which is identified as coronamic acid (**4**).



2a R=H
5 R=CH_3



2b



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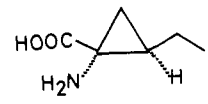

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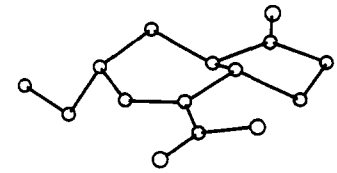

Figure 1. The molecular configuration.

Treatment of coronatine with acetic anhydride–pyridine afforded anhydrocoronatine **3**, *m/e* 301 (M^+), whose IR spectrum exhibited peaks at 1800, 1640, 1605 cm^{-1} assignable to an azlactone moiety. Since the NMR spectrum of **3** (and **1** also) shows the presence of two ethyl groups (δ 0.98, 6 H, t, *J* = 7 Hz), in which one arises from coronafacic acid part, the amino acid, coronamic acid, must be depicted as **4** (plane structure) by considering the degree of unsaturation. The structure **4** including stereochemistry (NH_2/CH_2CH_3 trans) was also confirmed by the synthesis of *dl*-coronamic acid.[2]

Coronafacic acids exist as two stereoisomers, **2a** ($C_{12}H_{16}O_3$ (*m/e* M^+ 208): mp 125–126 °C, $[\alpha]^{20}_D$ +119.1° (*c* 3.3, CH_3OH), IR ν_{max}^{KBr} 3250, 1720, 1640, 1403 cm^{-1}; NMR (90 MHz) $\delta_{Me_4Si}^{CDCl_3}$ 1.00 (3 H, t, *J* = 7 Hz, $-CH_2CH_3$), 3.15 (1 H, quintet, -CHCO-), 7.28 (1 H, br s, =CH), 11.58 (1 H, br s, -COOH)) and/or **2b** ($C_{12}H_{16}O_3$ (*m/e* M^+ 208), mp 141–142 °C, IR ν_{max}^{KBr} 3250, 1730, 1625 cm^{-1}; NMR (90 MHz) $\delta_{Me_4Si}^{CDCl_3}$ 1.00 (3 H, t, *J* = 7 Hz, $-CH_2CH_3$), 7.11 (1 H, dd, *J* = 4 Hz, 2 Hz, =CH), 11.08 (1 H, br s, -COOH), depending on the conditions of recrystallization. The isomer **2b** is easily convertible through enolisation to **2a**.[3] Esterification of each of **2a** and **2b** by methanolic HCl afforded the same methyl ester **5**: *m/e* 222 (M^+); IR ν_{max}^{film} 1740, 1715, 1645, 1405 cm^{-1}. The UV spectrum ($\lambda_{max}^{CH_3OH}$ 217 nm, ϵ 8558) of **2b** is compatible with α,β-unsaturated acid, whose double bond is located in a six-membered ring.[4] The IR spectra (1740 cm^{-1} in $CHCl_3$, five-membered ring ketone) of **2a** and **2b**, D_2O treatment of **2b** (*m/e* M^+ 211), and the NMR spectrum of **2a** (δ 3.15 quintet, *J* = 10 Hz, 7 Hz, 6 Hz, $COC(CH)H\text{-}CH_2$) suggest a 1-hydrindanone structure for coronafacic acids. Decisive structure including relative configuration of **2b** was obtained by x-ray analysis. Coronafacic acid (**2b**) crystallizes in the orthorhombic space group $P2_12_12_1$ with unit-cell dimensions a = 8.727 (4), b = 16.437 (6), and c = 7.638 (4) Å; there are four molecules in the unit cell. Intensities of 1144 independent reflections with 2θ values up to 140° were collected on an automatic, four-circle diffractometer using Cu Kα radiation monochromatized with a LiF crystal. The structure was solved by the direct method[5] on the basis of 242 $|E|$-values above 1.30, and refined by the block-diagonal-matrix least-squares method with anisotropic temperature factors for oxygen and carbon atoms. The hydrogen positions were obtained from a difference Fourier map. Further refinement of the structure including these hydrogen atoms reduced the R-value to 4.5%.[6] The molecular configuration obtained is shown in Figure 1. The molecule contains a trans-fused, bicyclic system, both rings in which are approximately in the envelope form. Thus, the structure of coronafacic acid has been established as **2b**, and plane structure of coronatine is depicted as **1**. Stereochemical and synthetic studies of coronatine are in progress.

References and Notes

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