1745 (s, ester C=O), 1730 (s, ester C=O, 1670 (s, C=O), 1615 (m, C=C), 1240 cm^{-1} (s, CO); ^1H NMR δ 0.83 (s, 3, CH_3), 2.07 (s, 3, $OCOCH_3$ C-17), 2.20 (s, 3, $OCOCH_3$ C-3), 4.73 (m, 1, CHOAc C-17), 5.35 (dd,1, J = 7, 13 Hz, CHOAc, C-3); UV λ_{max} 249 nm (ϵ 13 800); R_f 0.61. Anal. Calcd for $C_{22}H_{30}O_5$: C, 70.56; H, 8.08. Found: C, 70.50; H, 8.20.

3α,17β-Diacetoxy-5β,10β-epoxyestran-4-one (17). Enone **16** (425 mg, 1.13 mmol) was converted into epoxy ketone **17** in 99% yield using the procedure described earlier for the preparation of epoxy ketone **4**. Compound **17** was recrystallized from MeOH and had: mp 213-215 °C; IR 1740 (s, ester C=O), 1720 (s, C=O), 1230 cm^{-1} (s, CO; ^1H NMR δ 0.83 (s, 3, CH_3), 2.07 (s, 3, $OCOCH_3$ C-17), 2.15 (s, 3, $OCOCH_3$ C-3), 4.67 (m, 1, CHOAc C-17), 5.73 (dd, 1, J = 6, 10 Hz, CHOAc C-3); R_f 0.65. Anal. Calcd for $C_{22}H_{30}O_6$: C, 67.67; H, 7.74. Found: C, 67.80; H, 7.91.

3α,17β-Diacetoxy-5,10-secoestr-4-yn-10-one (18). Epoxy ketone **17** (446 mg, 1.14 mmol) was converted into seco steroid **18** using the method described earlier for the preparation of compound **5**. Seco steroid **18** was isolated from the reaction by ether extraction and purified by column chromatography on silica gel (2.0 cm × 44 cm column eluted with CH_2Cl_2). Recovered seco steroid **18** (171 mg, 40%) was recrystallized from $MeOH/H_2O$: mp 151-153 °C; IR 1740 (s, ester C=O), 1700 (m, C=O), 1236 cm^{-1} (s, CO); ^1H NMR δ 0.83 (s, 3, CH_3), 2.03 (s, 3, $OCOCH_3$ C-17), 2.08 (s, 3, $OCOCH_3$ C-3), 4.68 (m, 1, CHOAc C-17), 5.28 (m, 1, CHOAc C-3); R_f 0.61. Anal. Calcd for $C_{22}H_{30}O_5$: C, 70.56; H, 8.08. Found: C, 70.37; H, 8.01.

3α,17β-Dihydroxy-5,10-secoestr-4-yn-10-one (19). Seco steroid **18** (170 mg, 0.45 mmol) was converted into diol **19** using the method described earlier for the preparation of compound **6**. The product was isolated by EtOAc extraction and recovered as a solid (87 mg, 67%) which was recrystallized from hexane/acetone: mp 186-188 °C; IR 3425 (s, br, OH), 1705 cm^{-1} (s, C=O); ^1H NMR δ 0.78 (s, 3, CH_3), 3.68 (m, 1, CHOH C-17), 4.37 (m, 1, CHOH C-3); R_f 0.16 (1:1 benzene/EtOAc). Anal. Calcd for $C_{18}H_{26}O_3$: C, 74.45; H, 9.02. Found: C, 74.59; H, 9.41.

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Registry No. **1**, 76563-77-4; **2**, 83747-34-6; **3**, 83747-35-7; **4**, 83747-36-8; **5**, 83747-37-9; **6**, 77407-26-2; **7**, 77407-25-1; **8**, 77407-24-0; **9**, 76563-80-9; **10**, 83747-38-0; **11**, 83747-39-1; **12a**, 83747-40-4; **12b**, 83747-41-5; **13a**, 83747-42-6; **13b**, 83747-43-7; **14a**, 83747-44-8; **14b**, 83762-90-7; **15**, 83747-45-9; **16**, 83747-46-0; **17**, 83829-14-5; **18**, 83829-15-6; **19**, 83461-51-2.

Solid-State Carbon-13 Nuclear Magnetic Resonance Study of Ribonucleosides and Ribonucleic Acid

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Natural-abundance ^{13}C NMR spectra of cytidine, uridine, adenosine, guanosine, and Torula yeast ribonucleic acid in the solid state could be easily measured by using the combined techniques of high-power proton decoupling, cross-polarization, and magic angle spinning. A proton flip-back pulse sequence was employed to overcome the sensitivity problem caused by the long proton spin-lattice relaxation and yet retain the resolution. The solid-state ^{13}C spectra of uridine and guanosine reveal two distinct conformations of the ribose which could not be detected in the solution state.

Nuclear magnetic resonance spectroscopy has been routinely used in the configurational and conformational analysis of nucleosides, nucleotides, and nucleic acids in solutions.[1,2] However, there exist few solid-state carbon-13,[3,4] phosphorus-31,[5,6] and nitrogen-15[7] NMR studies that have been performed by using the combined techniques of high-power proton decoupling, cross-polarization (CP),[8] and magic angle spinning (MAS) to enhance resolution and sensitivity. The applicability of solid-state NMR is also hampered in part by the lack of a direct spectral correlation between solid and liquid samples. The additional spectral complications involving solid-state spectra may result from the subtle intramolecular and intermolecular interactions inherent in microcrystalline powders and the second-order splittings cause by quadrapolar nuclei. In the present study, we report the solid-state ^{13}C NMR spectra analysis of ribonucleosides and Torula yeast RNA and briefly discuss the approaches employed to resolve the

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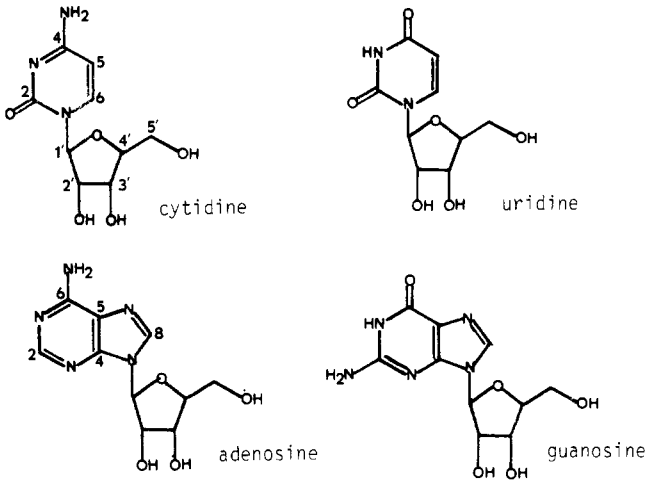
Table I. Carbon-13 Chemical Shifts of Ribonucleosides[a]

compd	state	carbon									
		2	4	5	6	8	1′	2′	3′	4′	5′
cytidine	solid	157.8	168.5	96.0	142.0		92.7	67.0	76.2	84.5	61.1
	solution[b]	157.7	166.2	96.3	141.8		90.6	69.3	74.3	84.0	61.0
uridine	solid	151.5	167.4	100.8	142.2		92.1	67.0[d]	75.4	82.6	58.9[d]
								69.3			62.4
	solution[b]	151.7	166.2	102.4	142.0		89.6	69.7	74.0	84.4	61.0
adenosine	solid	155.6	148.9	120.4	155.6	138.6	92.7	72.1	75.8	85.6	63.6
	solution[c]	152.6	149.3	119.6	156.3	140.2	88.2	70.9	73.8	86.2	61.9
guanosine	solid	151.0	151.0	116.0	157.6	138.2	87.0	72.9[d]	76.7[d]	86.4	63.5
								76.7	79.2		
	solution[c]	154.6	152.3	117.5	157.8	136.9	87.3	71.5	74.9	86.4	62.2

[a] Chemical shift expressed in parts per million from Me_4Si. [b] In D_2O solution. [c] In Me_2SO-d_6. [d] A doublet was observed.

complex spectra data and their potential application in conformational analysis.



Results and Discussion

One of the main reasons for employing the cross-polarization method emerges from the fact that rapid carbon signal accumulation is possible due to the fast proton spin–lattice relaxation. Thus the cycle time of the experiment can be fairly short (0.5–3 s). Yet by using a standard single-contact pulse sequence with a 1-s cycle the ^{13}C natural-abundance spectrum of cytidine (Figure 1A) produced a spectrum with a very poor signal-to-noise ratio. The low sensitivity of this experiment may be attributed to the unique long proton spin–lattice relaxation time, which results in a substantial fraction of proton polarization remaining transverse at the end of the observation period. Induction of a paramagnetic impurity was recently shown by Ganapathy et al.[10] to be an effective method to reduce the relaxation time. Carbon-13 spectra of cytosine, uracil, and thymine were thus easily obtained when doped with Cu^{2+} as a paramagentic impurity.[10] Induced after doping with $CuCl_2$ at a molar concentration of 0.001%, a prominent signal enhancement was achieved as shown in the spectrum of cytidine (Figure 1B). However, the sensitivity was gained at the expense of resolution. Figure 1B displays very little spectral specificity especially in the region of ribose carbons. Therefore, it appears that this doping method may not be an appropriate method of acquiring the complicated spectra of nucleosides.

Another technique has recently been devised by Tegenfeldt and Haeberlen[11] to improve upon the efficiency of

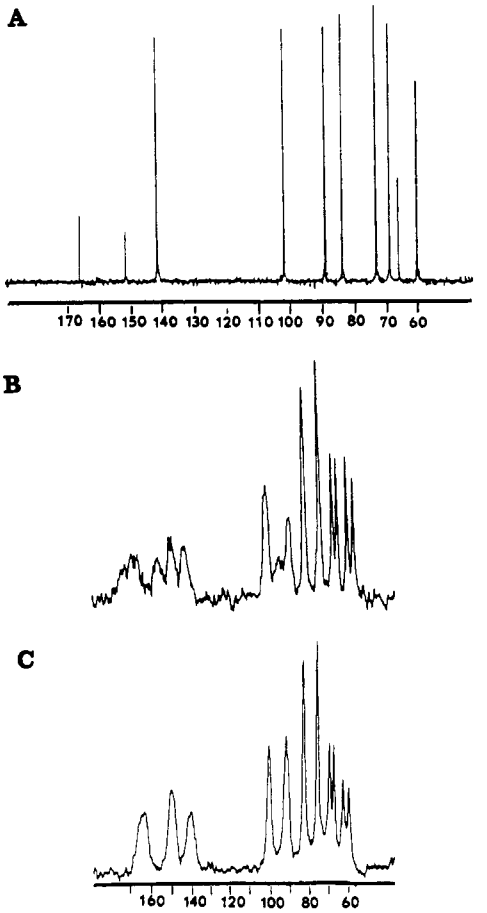


Figure 1. Solid-state ^{13}C NMR spectra of uridine: (A) D_2O solution liquid NMR at 25 MHz; (B) solid state at 25 MHz; (C) solid state at 50 MHz. Reference: hexamethylbenzene.

the standard CP/MAS pulse sequence. The difference between this new flip-back experiment and the standard experiment is the extra 90° pulse at the end of the acquisition. This reverse-phase 90° pulse flips back the remaining proton magnetization at the end of the observation period. By use of this flip-back pulse sequence, the sensitivity was significantly enhanced while the resolution was still retained (Figure 1C).

On the basis of the previously reported chemical shifts of cytidine in solution,[12] the chemical shift of all ribose carbons except C-1′ could be directly assigned (Table I). The C-1′ and most carbons of cytosine are shown as complex multiplets (Figure 1C). The spectral perturbation is mainly caused by the dipolar coupling with quadrupolar

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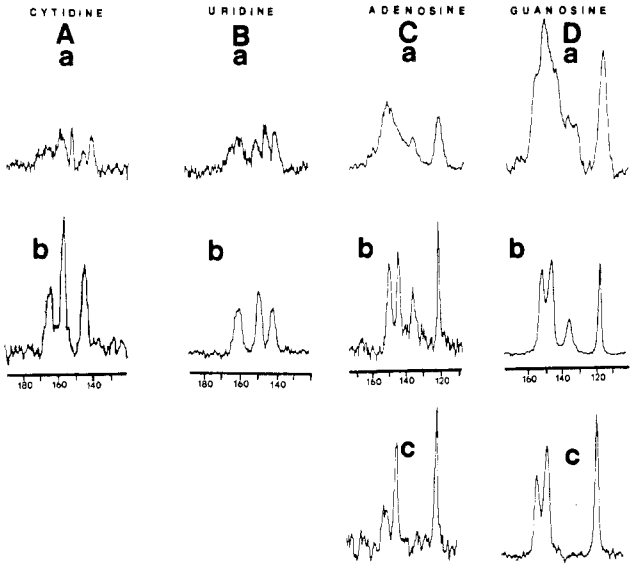

Figure 2. Solid-state ^{13}C NMR spectra of the base portion of ribonucleosides (a) at 25 MHz, (b) at 50 MHz, (c) at 50 MHz with a dephasing time of 50 μs before the acquisition; line broadening of 10 Hz at 25 MHz and of 20 Hz at 50 MHz. A = cytidine, B = uridine, C = adenosine, and D = guanosine.

nuclei. Although magic angle spinning is able to average out chemical shift anisotropy and ^1H–^{13}C dipolar interactions, it is unable to completely suppress the effect of ^{13}C–^{14}N dipolar interaction because of the quadrupolar moment of ^{14}N.[3,13] It has been established that this dipolar splitting is inversely proportional to the ratio of the ^{14}N Zeeman frequency to the ^{13}C–^{14}N quadrupolar coupling constant.[13] This is clearly demonstrated in Figure 2A. At higher field strengths the spectral pattern is simplified, which permits direct measurement of the chemical shifts. The complex spectral pattern near the C-1′ signal at 25 MHz is obscured due to its overlapping with the C-5 signal. However, it is revealed as a doublet in the spectrum of uridine (Figure 3B), reminiscent of the splitting pattern of the α-carbon of amino acids.[3]

The most distinct spectral difference between the spectrum of uridine in the D_2O solution (Figure 3A) and that in the solid state (Figure 3B) is the splittings of the C-2′ (67.6 and 69.3 ppm) and C-5′ (58.9 and 62.4 ppm) signals, suggestive of two different conformers in a unit cell of uridine crystals. The X-ray spectrum of uridine[14] indicates that it consists of two molecules in an asymmetric unit. These two independent molecules (A and B) have similar gross conformations; the conformation about the glycosyl bond is anti, the sugar ring is puckered with C(3′) endo, and the conformation about C(4′)–C(5′) is gauche–gauche. However, a number of the bond angles and the torsional angles of the ribose ring are significantly different. In molecule A, the 5′-OH forms one intermolecular hydrogen bond with the 5′-oxygen of molecule B, and the 2′-OH forms one H bond with the 3′-oxygen of molecule B. On the other hand, in molecule B, the 5′-OH makes two H-bond with two different molecules of A, and the 2′-OH makes one H-bond with the 2-oxygen of molecule A. Therefore, the difference in intermolecular H bonding and/or the difference in angles might account for the splittings of the C-2′ and C-5′ resonances. Further studies are necessary for unraveling the exact physical origin of the chemical shift anisotropy.

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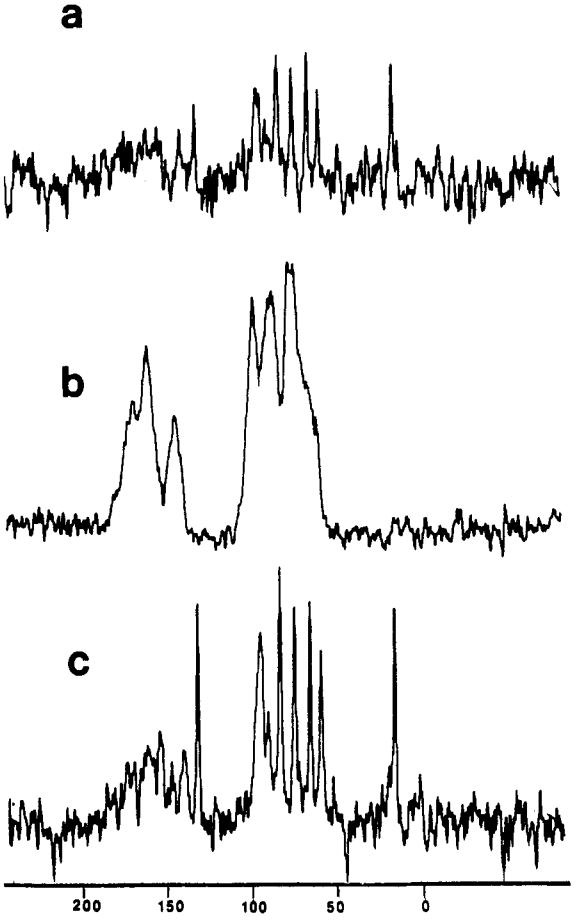

Figure 3. ^{13}C NMR spectra of cytidine at 25 MHz in the solid state with chemical shifts relative to Me_4Si (3 ms contact time, 1 s recycle time): (A) using standard CP/MAS pulse sequence, 30K scans; (B) containing 0.001% of $CuCl_2$, using standard CP/MAS pulse sequence, 720 scans; (C) using flip-back pulse sequence, 30K scans.

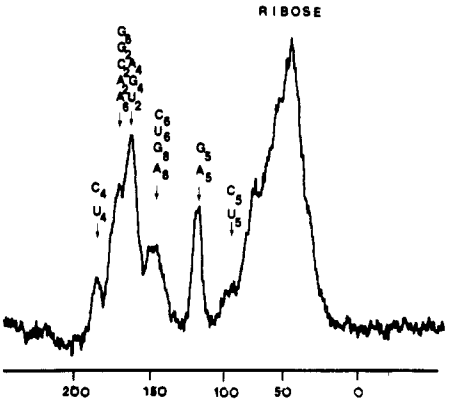

Figure 4. Solid-state ^{13}C NMR spectrum of Torula yeast RNA at 50 MHz.

The ribose carbon signals of adenosine are well-resolved and can be easily assigned (Table I). Only four signals are observed in the base region of adenosine (Figure 2Cb). In order to clarify this, we have employed a modified pulse sequence initially established by Opella and Frey[15] for solid-state NMR to detect nonprotonated carbons. This experiment was accomplished by inserting a brief delay (50 μs) without proton decoupling between the development of ^{13}C magnetization (^1H–^{13}C contact time) and the data acquisition with proton decoupling. Only three

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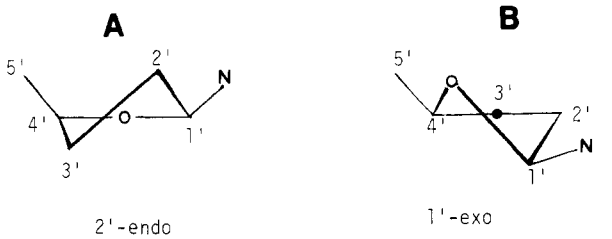
nonprotonated carbon signals are detected in this experiment as shown in Figure 2Cc. We can assign the signals at 155.6, 148.9, and 120.4 ppm to the carbon resonances of C-6, C-4, and C-5, respectively, on the basis of the NMR data in solution. The remaining peaks at 138.6 and 155.6 ppm can thus be designated to C-8 and C-2, respectively (Table I).

All the guanine carbons of guanosine can also be measured by the same approach (Figure 2D). The crystallographic analysis of guanosine showed the presence of two molecules (A and B) in a unit cell.[16] The ribose ring of



molecule A assumes a C-(2′) endo conformation whereas that of molecule B assumes an unusual C(1′) exo conformation. This conformational difference was disclosed by the splittings of the C-2′ (76.7 and 72.9 ppm) and C-3′ (79.2 and 76.7 ppm) signals in the solid-state NMR spectrum of guanosine. Further interpretation of the observed chemical shift change in terms of a specific variation in conformation must await the results from additional studies on the related compounds.

From our preliminary data, it appears that solid-state NMR can be an extremely useful tool to study the conformations of ribose in the solid state since the ^{13}C NMR demands on the proper size and form of crystal is much less stringent. It can at least furnish the X-ray crystallographer a very valuable starting point for the refinement of molecular structure. We are unable at present to specially correlate the solid-state NMR data with the features of base-pairing and base-stacking of nucleosides because of the limited resolution in the region of the base carbon resonances. This is in part ascribed to the residual ^{13}C–^{14}N dipolar interaction.

One of the unique advantages of solid-state NMR is that it enables one to study the molecules that are insoluble or denatured in solution. This is particularly important in studying biological macromolecules and intact tissues. We measured the ^{13}C natural-abundance spectrum of Torula yeast RNA (Figure 4). Compared with the spectrum in solution,[17] it takes less time to acquire natural-abundance carbon signals of RNA in the solid state. But the resolution is relatively too poor to provide much specific information. It may therefore be required to prepare a specific stable isotope-labeled compound by chemical or biochemical methods for obtaining more specific structure information.

Experimental Section

The high-resolution solid-state ^{13}C NMR spectra were all obtained on either a Bruker CXP-100 or a Bruker CXP-200 instrument, both equipped with Z32DR ^{13}C-MASS Supercon magnet probe heads for proton-enhanced magic angle spinning experiments (CP/MAS). Samples were carefully packed in Andrews-type rotors made from boron nitride (body material) and Kel-F (spinner head material). These rotors, constructed in this laboratory, will hold approximately 200 milligrams of material. The spinning speeds ranged from 3.2 to 3.6 KHz which was sufficient to suppress any unwanted spinning side bands found in these types of biomaterials. All chemical shifts are expressed externally referenced to the ^{13}C resonance of Me_4Si. The actual referencing material used was hexamethylbenzene, either periodically mixed with the sample or run separately in the rotor prior to running the sample alone. The high-field peak of solid hexamethylbenzene is assumed to be at 17.6 ppm downfield from Me_4Si. The cross-polarization contact times ranged from 1.25 to 3.0 ms and the recycle times from 1 to 2 s. These were determined experimentally for best sensitivity results for each sample. Each spectra resulted from averaging as little as 10 000 and as much as 60 000 scans, depending upon sample sensitivity. The dipolar-dephased data were obtained by using a 50 μs delay. This particular delay period seems to be optimal for obliterating all methine and methylene carbon resonances in the spectra of these types of compounds.

All nucleosides and the Torula yeast RNA were obtained from Sigma Chemical Co. The RNA was purified according to the procedures previously described.[17]

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Registry No. Cytidine, 65-46-3; uridine, 58-96-8; adenosine, 58-61-7; guanosine, 118-00-3.

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Synthesis and Properties of the Seven Isomeric Phenols of Dibenz[*a*,*h*]anthracene

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The seven possible monohydroxy derivatives of dibenz[*a*,*h*]anthracene (**4a–g**) were synthesized by using the reductive cyclization of *o*-naphthoylnaphthoic acids with hydroiodic acid–red phosphorus. The mass, ^1H NMR, and UV spectral properties are discussed in detail as a means of identifying metabolically formed phenols of dibenz[*a*,*h*]anthracene.

Hydroxy derivatives of polycyclic aromatic hydrocarbons (PAH) were the first observed metabolites of this class of chemical carcinogens.[1] Phenols of PAH can be metabolically formed either by direct hydroxylation or by no-

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