Assignments of the Deoxyribose Vibrations: Isotopic Thymidine

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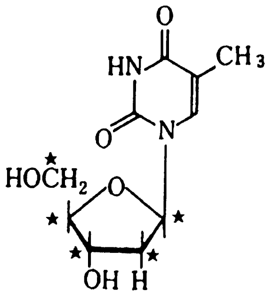
Synopsis. Raman spectrum of thymidine-$^{13}C_5$, in which five C atoms of the deoxyribose moiety are uniformly substituted with ^{13}C atoms, has been examined in the crystalline state with 488.0 nm excitation. Some of the Raman bands were observed at appreciably shifted frequencies from those of the corresponding Raman bands of the natural thymidine, and they were assigned to some vibrations involving deoxyribose motions.

Raman spectroscopy is a potentially powerful probe of nucleic acid structure. It requires, however, a detailed understanding of the vibrational modes corresponding to the Raman bands observed. On the other hand, current Raman band assignments are far from complete. Of the more than 40 prominent bands in the spectrum of DNA, less than 10 have been well-characterized by means of empirical correlations of X-ray crystallography and crystalline Raman spectroscopy. Such correlations cover some of the base vibrations and phosphate vibrations. Surprisingly, however, practically nothing is known of the ring vibrations of the deoxyribose moiety. In view of this situation, we have attempted to prepare nucleosides, in which stable isotope atoms are introduced only in the ribose or deoxyribose portions, to be subjected to Raman spectral examinations. This is the first successful result of such attempts.

D-Thymidine-1′2′3′4′5′-$^{13}C_5$ or 1-[2-deoxy-β-D-ribofuranosyl-1′,2′,3′,4′,5′-$^{13}C_5$]-5-methyluracil (**I**) has been synthesized starting from commercially available uniformly ^{13}C-labeled glucose (98% ^{13}C) (Scheme 1). A detailed description of the synthetic procedure will be given elsewhere.

The sample was a crystalline powder. It was grounded to a fine powder, and was placed on a slide glass. This was placed on the sample stage of a JASCO R-MPS-22 Raman microscope, and was irradiated by a 488.0 nm beam from an NEC GLG-3300 argon ion laser through an Olympus 50× objective. The Raman scatterings were collected with the same objective and were sent to a JASCO NR-1100 Raman spectrophotometer system. No polarizer was placed in the path of the scattering beam. The observed spectrum is shown in Fig. 1, (a). For comparison, natural D-thymidine (purchased from Yamasa Shoyu Co.) was subjected to a similar Raman spectroscopic measurement. The sample here had a form of a needle crystal, whose space group is $P2_12_12_1$ and whose a-axis is along the elongation direction.[1)] This sample was also grounded to a fine powder, which is in the same state as ^{13}C-thymidine. Its observed spectrum is shown in Fig. 1, (b).



(I) ★ = ^{13}C

Scheme 1.

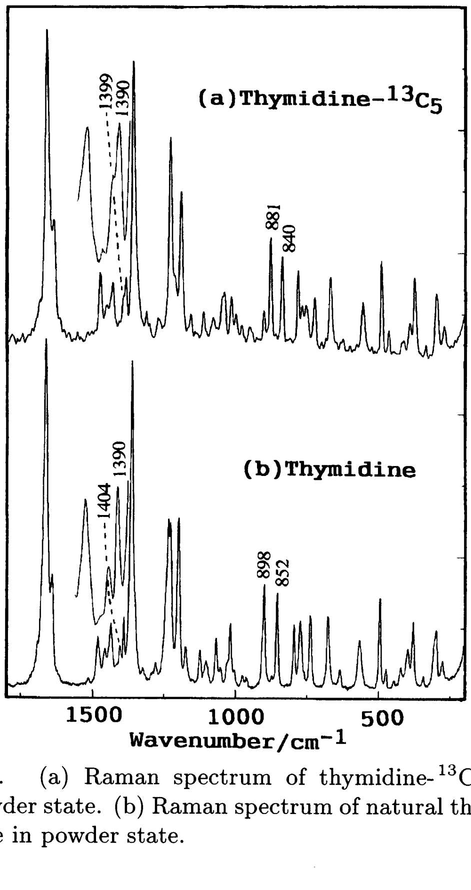


Fig. 1. (a) Raman spectrum of thymidine-$^{13}C_5$ in powder state. (b) Raman spectrum of natural thymidine in powder state.

Table 1. Raman Frequencies Observed for Thymidine and Thymidine-1′2′3′4′5′-$^{13}C_5$

Thymidine cm^{-1}	Thymidine-$^{13}C_5$ cm^{-1}	Intensity	Shift cm^{-1}
1665	1665	s	0
1642	1642	m	0
1482	1481	w	−1
1436	1436	m	0
1404	1399	w	−5
1390	1390	w	0
1364	1363	s	−1
1234	1232	s	−2
1198	1195	s	−3
1174	1161	w	−13
1124	1118	w	−6
898	881	m	−17
852	840	m	−12
795	785	m	−10
771	770	m	−1
737	727	m	−10
675	671	m	−4
632	625	w	−7
563	558	m	−5
494	493	m	−1
472	468	w	−4
396	393	w	−3
378	377	m	−1
302	300	m	−2
275	274	w	−1

As may be seen in Fig. 1, the spectrum (a) is quite similar to the spectrum (b) except some frequency differences of the peaks. Therefore, two samples are considered to be practically the same crystalline thymidine, and the only difference must be the ^{13}C substitutions. The observed frequency differences are listed in Table 1.

Four strong Raman bands at 1665, 1364, 1234, and 1198 cm^{-1} are shifted only slightly, 0, −1, −2, and −3 cm^{-1}, respectively, on ^{13}C substitution. These bands are all assignable primarily to the thymine ring vibrations. The observed slight isotope shifts, however, indicate that the vibrational couplings of the deoxyribose motions with these base vibrations are not totally absent. It is interesting to see that such a small vibrational coupling increases as the frequency of the base vibration decreases. In Fig. 1 (a), a weak band at 1399 cm^{-1} is found as a shoulder of a stronger band at 1390 cm^{-1}, whereas the corresponding bands in Fig. 1 (b) appear well resolved from each other. Therefore, it is clear that there must be an isotope shift here. When the spectra were observed with an expanded wavenumber scale, the weak band was found at 1404 cm^{-1} for normal species and at 1399 cm^{-1} for thymidine-$^{13}C_5$. The observed frequency shift −5 cm^{-1} is approximately equal to what is expected for the ^{13}C isotope shift of CH_2 scissoring mode.

A medium-intensity band at 898 cm^{-1} showed the greatest frequency shift, −17 cm^{-1}. This band is now assignable to the ring breathing vibration of the deoxyribose residue on the following bases: (1) If it is assumed that in the ring breathing vibration all the atoms, C(1′)H.N, C(2′)H_2, C(3′)H.OH, C(4′)H.C(5′), and O(ring), displace evenly along radial lines away (and then towards) the center of the five-membered ring, then the vibrational frequency ratio is calculated to be $\nu(^{12}C)/\nu(^{13}C)=\sqrt{1.045}=1.022\fallingdotseq 898/881$, which is almost exactly equal to what was actually observed. (2) This Raman band gives almost equal intensities for different orientation of the single crystal (aa and cc polarization spectra, our unpublished work). This fact suggests that the local Raman tensor for this vibration may be rather isotropic. This is expected in general for a Raman band of ring breathing vibration. (3) In the Raman spectrum of cyclopentane, the ring breathing band was located at 886 cm^{-1}, and of tetrahydrofuran at 910 cm^{-1}.[2)]

It should be pointed out here that, in the Raman specrum of 5′-thymidylic acid, only weak bands were found in the 850—950 cm^{-1} range.[3)] Therefore, 5′-phosphorylation is considered to cause a considerable perturbation on this ring breathing vibration.

Another medium-intensity band at 852 cm^{-1} showed rather great isotope shift, −12 cm^{-1}, and may be assigned to another ring vibration of the deoxyribose ring. In the 800—200 cm^{-1} region, a number of prominent bands (at 795, 737, 675, 632, and 563 cm^{-1}) show appreciable isotope shifts. These must be assigned to some complicated modes in which base and deoxyribose are involved in some vibrational couplings. On the other hand, the bands at 494, 378, 302, and 275 cm^{-1} show almost no isotope shifts. They must be assigned to pure base vibrations. The vibrational modes in this frequency region are to be clarified by taking other data into account, including polarized Raman spectra for the crystal, Raman spectra of other isotope species, and the result of a normal coordinate treatment.

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