Low-Temperature Spectroscopy of Biological Compounds[1]

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It is well substantiated that the absorption spectra of complex molecules may be sharpened, and in many cases the vibrational and rotational details brought out, by refrigeration of the absorbing material to the temperature of liquid nitrogen or lower (*1, 3, 5–7*). This principle seems of great potential utility for the characterization and differentiation of compounds of biological interest heretofore indistinguishable by their absorption characteristics, and conceivably for the solution of cytochemical problems. However, the techniques that have been employed have only limited application to polar compounds. We have therefore developed new techniques with which we have undertaken a study of the absorption spectra of a number of purines, pyrimidines, amino acids, proteins, etc. at the temperature of liquid nitrogen (77° K).

Many polar compounds including amino acids (*2*) will sublime at temperatures well below their melting points

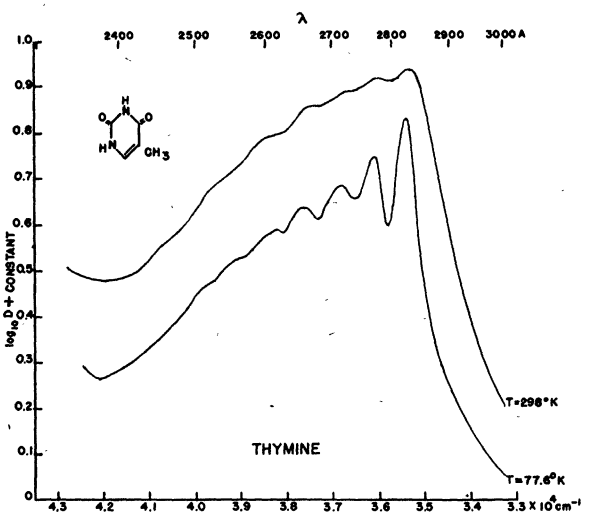

FIG. 1

at reduced pressures. This principle has been used to prepare thin films of purines, pyrimidines, and amino acids, by sublimation onto quartz slides in a molecular still. Thin films of nucleotides and proteins, which could not be sublimed in this manner, have been prepared by drying water solutions on a clean, hydrophilic quartz surface. Details of the techniques will be given in a later paper.

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Absorption spectra of the thin films were obtained at room temperature and with the slides immersed in pure liquid nitrogen contained in an all-quartz Dewar flask (*4*), designed with four plane quartz windows for passage of the ultraviolet radiation. An Hanovia hydrogen discharge tube and a small Hilger quartz spectrograph were used. The graphs were obtained from the Sinclair Smith recording microdensitometer traces of the spectrum plate density.

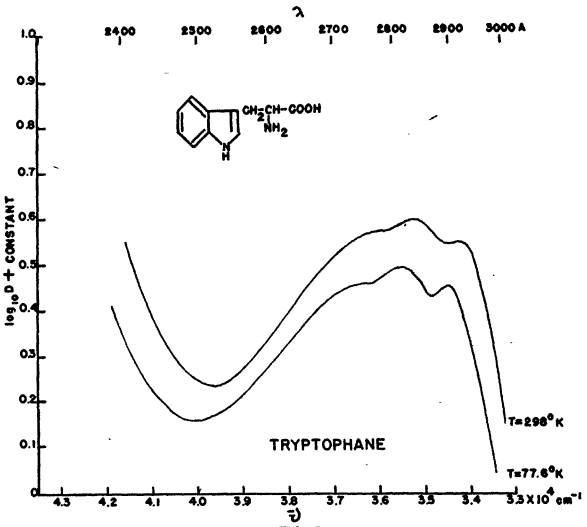

FIG. 2

The absorption spectra of such sublimed films of thymine and of tryptophane at room temperature and at 77° K are presented in Figs. 1 and 2. The spectra are plotted as log optical density (which is independent of film thickness) versus wave number. The increase in spectral detail is evidently far greater for thymine than for tryptophane. Reasonable proof that the materials on the film have not been altered by sublimation was obtained in each case by subliming a relatively large quantity (1–2 mg), dissolving the sublimate in buffer, and obtaining ultraviolet absorption curves and nitrogen analyses from these solutions. In both cases such ultraviolet absorption curves were identical with those of the original material, and the ratio of ultraviolet absorption to nitrogen content was in satisfactory agreement with similar ratios obtained upon the original material.

This is a preliminary report on a series of such studies to be extended to lower temperatures and to other regions of the spectrum.

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