Nucleoproteins, which seem to be present in viruses, bacteria, animal tissue, and plant tissue and partake in many vital processes (3), are conjugates of various proteins with nucleic acids, and as such may vary in many ways depending on the specific chemical, and perhaps spatial, composition of the whole structure. The brilliant work of Levene (4) and many subsequent investigators (5, 6) has shown that the nucleic acid moieties are polymers of nucleotides (purine or pyrimidine-sugar-phosphates), but little is known concerning the actual structural details.

The two presently recognized types of nucleic acids are that in which the sugar residue is pentose (D-ribose) and that in which the sugar residue is desoxypentose (2-desoxyribose); the first type is exemplified by the nucleic acid which may be prepared from yeast; the second type has been obtained from thymus glands, rat livers, etc. Another difference between the ribose and desoxyribonucleic acids occurs in the pyrimidine portions of the molecules; ribonucleic acid contains cytosine and uracil, whereas the desoxyribonucleic acid contains cytosine and thymine (5-methyluracil) (4), but both materials seem to contain the same purines, namely adenine and guanine. There is also some evidence that the two types of nucleic acids may differ markedly in molecular weight (6, 7), but such work must be closely scrutinized because of the varying methods of extraction and treatment of the materials. The ultraviolet absorption of both types of nucleic acid is essentially similar (8), and it is doubtful whether one can differentiate between them in this region of the spectrum by usual techniques. Our investigation was undertaken with the purpose of attempting to differentiate different types of nucleic acids and their components by means of their infra-red spectra.

It is well known that absorption in the infra-red region of the spectrum is characteristic of particular molecular groupings (9) and relates to the vibrational-rotational motions of the atoms. Thus two closely related
substances may be easily differentiated by minor chemical differences, such as the replacement of a hydroxyl group by a hydrogen; even in large molecules and polymers, it is possible to detect small differences in composition (10).

The infra-red spectra of ribonucleic acid (from yeast) and desoxyribonucleic (from thymus) are shown in Fig. 1 for the region of 2 to 15 μ (670 to 5000 cm⁻¹). Certain similarities between the two spectra appear obvious; for example, the strong absorptions around 3 μ (which are probably associated with hydroxyl and amino stretching vibrations), the bands at 6 μ (C=C, C=N, and C=O stretching), the bands at 8 μ, and the strong bands at 9.2 μ. The differences between the spectra of the two materials seem to lie mostly at wave-lengths longer than 9 μ; in particular the desoxy compound shows a band at 9.8 μ, not shown in ribonucleic acid in which the 9.2 μ band is symmetrical, and the ribonucleate has a band at 11.7 μ which is not present in the several samples of desoxyribonucleic acid that we have examined. We have not attempted to give any assignment to these absorptions.

1 It should be noted that because of the very slight solubility of the nucleic acids, nucleotides, and nucleosides in any but aqueous solvents and the rather strong absorption of infra-red by water in the region 2 to 15 μ except in very thin layers it is necessary to measure these materials in the solid state. We have used the following techniques: (a) "casting" of a concentrated aqueous solution on silver chloride disks, followed by removal of the water, leaving a continuous film; (b) evaporation of the material in high vacuum upon sodium chloride disks; (c) finely divided powders on sodium chloride disks; and (d) powders muled into mineral oil. These methods
The spectra of three nucleotides (yeast adenylic, cytidylic, and guanylic acids) have also been determined and are shown in Fig. 2. Guanylic acid gave a satisfactory film upon casting from aqueous solution, but since adenylic and cytidylic acid did not, they were measured as powders, which explains the sloping absorption cut-offs shown by these materials at wavelengths shorter than 5.5 μ. For a comparison of the two techniques we also measured the same sample of guanylic acid as a powder and reproduced its spectral curve in Fig. 2. It is obvious from this and other materials measured in our laboratory that powder films generally give less clearly defined bands than cast or evaporated films, probably due to the scattering of the radiation even at these relatively long wavelengths (11). These nucleotides are characterized by the strong band or group of bands around 6 μ similar to those seen in the nucleic acids. In addition all the

\[\text{YEAST ADENYLC ACID}\]

\[\text{POWDER}\]

\[\text{CYTIDYLC ACID}\]

\[\text{POWDER}\]

\[\text{GUANYLC ACID}\]

\[\text{CAST FILM}\]

\[\text{POWDER}\]

Fig. 2. Infra-red spectra of yeast adenylic, cytidylic, and guanylic acids

\[\text{PRELIMINARY measurements on a sample of adenosine-5-phosphate (kindly supplied by Dr. Fritz Lipmann) have been made in an attempt to differentiate it from the isomeric 3-phosphate (yeast adenylic acid).}\]
compounds show a band in the region of 9.2 to 9.6 μ. However, from the differences in the spectra of the nucleotides at wave-lengths in the region of 7 to 15 μ, it is obviously possible to differentiate samples of these materials by such measurements.

![Infra-red spectra of yeast adenosine, guanosine, and xanthosine](image)

Fig. 3. Infra-red spectra of yeast adenosine, guanosine, and xanthosine

The infra-red spectra of the natural purine ribosides, adenosine and guanosine, as well as the analogous compound, xanthosine, have also been measured and the spectral curves are reproduced in Fig. 3. All the curves are characterized by at least two intense absorption bands in the 3 μ region.

*Measurements on too few materials have been made to locate any bands associated with the phosphoric ester portion of the molecules with certainty, although unpublished observations of H. W. Thompson (12) indicate that phosphites and phosphonates absorb in the region of 10.4 to 11.8 μ.*
(presumably N—H and O—H stretching) and two intense bands in the 6 μ region (C=C, C=N, or C=O stretching). Numerous other bands (possibly useful for analytical purposes) are observed at longer wave-lengths, including a seemingly characteristic strong absorption in the region 9.2 to 9.6 μ. When adenosine was measured both as an evaporated film and as a powder, good agreement was obtained between the spectra (Fig. 3). Comparison of the spectral curve of xanthosine, as a powder in mineral oil, with that obtained on an evaporated sample indicates, however, that changes in this material must have occurred during the sublimation, since the spectra beyond 8 μ do not agree either in location or intensity of many of the important bands. Perhaps this difference in behavior between adenosine and xanthosine is associated with the free hydroxyl groups on the purine portion of the xanthosine which are not present in adenosine.

Finally we have determined the infra-red spectrum of D-ribose (Fig. 4). This material, determined as a powder film, shows very sharp absorption bands beyond 6.5 μ. This is perhaps due to a more uniform particle size distribution because of the crystalline nature of the material. The strongest band in the spectrum is that at 9.3 μ (O—H bending) and seems to be correlatable with strong bands in the 9.2 to 9.6 μ region observed in the more complicated ribose-containing compounds described above.

We wish to acknowledge the assistance of Miss P. L. Snow and Miss A. P. Sutton, who gave invaluable technical aid in this work.

**EXPERIMENTAL**

**Materials**—All of the materials were obtained from commercial sources and purified when necessary as shown by ultraviolet absorption measurement or elementary analysis. The data are shown in Table I. The ribose

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4 We are particularly indebted to Dr. Earl D. Stewart of the Schwarz Laboratories for supplying us with samples of some materials not commercially available and with some of the elementary analysis data.
<table>
<thead>
<tr>
<th>Material</th>
<th>M.p.</th>
<th>Ultraviolet spectral data</th>
<th>Elementary analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reported</td>
<td>Found</td>
<td>Calculated</td>
</tr>
<tr>
<td></td>
<td>$^\circ$C.</td>
<td>$^\circ$C.</td>
<td>N</td>
</tr>
<tr>
<td>Sodium yeast ribonucleate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;tymus deoxyribonucleate†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenylic acid                                          194-195 (4)</td>
<td>195$^\S$</td>
<td>260*</td>
<td>260 32,500</td>
</tr>
<tr>
<td>Cytidylic &quot;                                             227 (4)</td>
<td>226-228§</td>
<td>260 (4)</td>
<td>14,000</td>
</tr>
<tr>
<td>Guanylic &quot;                                             180 (4)$^\S$</td>
<td>174§</td>
<td>252</td>
<td>6,250</td>
</tr>
<tr>
<td>Adenosine                                              220-230 (4)</td>
<td>225-229</td>
<td>260 (15)</td>
<td>14,500</td>
</tr>
<tr>
<td>Guanosine                                              237 (1)</td>
<td>&gt;250</td>
<td>250 (16)</td>
<td>12,000</td>
</tr>
<tr>
<td>Xanthosine                                             &gt;250</td>
<td>240 (16)</td>
<td>8,600</td>
<td>253 8,790</td>
</tr>
</tbody>
</table>

Water was used as a solvent unless otherwise noted. The values for the samples under "Found" were made on samples used for infra-red measurements. Under "Reported" the figures in parentheses refer to the bibliography.

* See Stimson and Reuter (8). Both measurements were made in 0.1 N NaOH.
† The sample from the Dougherty Chemical Company, which was reported to have N 15.3 per cent and P 8.9 per cent, was purified according to the method of Gulland, Jordan, and Threlfall (13).
‡ Calculated for $C_{19}H_{19}N_{19}O_{26}P_{2}Na_{4}$, since the Gulland method (see above) was used in purification. The N:P ratio was the same as the calculated (1.69).
§ With decomposition.
|| With 2H$_2$O.
used had a melting point of 82-83° and $[\alpha]_{D}^{23} = -19.60^\circ$ (reported by Levene and Jacobs (17), m.p. 86-87° and $\alpha_{D} = -19.5^\circ$).

**Preparation of Samples**—Four techniques were employed in the preparation of samples for infra-red measurements; viz., (a) powder films on sodium chloride disks, (b) powders mulled in mineral oil, then spread on sodium chloride disks, (c) high vacuum sublimation (at $\sim 10^{-5}$ mm. of Hg) onto sodium chloride disks, and (d) casting of aqueous solutions to give continuous films on silver chloride disks. The particular method used for each compound is noted on the absorption curve.

*Powder Films (11)*—To prepare a sample by this method, a small quantity of the compound is ground as finely as possible in an agate mortar; a thin layer of the powder is then spread as evenly as possible over the face of a rock salt disk. A second rock salt disk is then placed on top of the first and rotated slightly so as to produce a more uniform layer. Uniform distribution of the powder over the rock salt disk is facilitated by sifting the material through a fine mesh screen.

*Mineral Oil Suspension*—When the preparation of satisfactory powder specimens failed, a sample of the material was ground in mineral oil and the suspension placed between rock salt disks for measurement. This technique has the advantage of reducing scattering and of yielding a more uniform sample. On the other hand, the use of mineral oil suffers from the disadvantage that it introduces absorption bands due to the oil. These bands, which occur around 3.4 $\mu$ (C—H stretching) and at 6.8 and 7.2 $\mu$ (CH$_2$ and CH$_3$ deformation), must be corrected for by measuring the sample against an identical cell filled only with mineral oil. This procedure was employed for guanosine and xanthosine.

*Vacuum Sublimation*—An apparatus was used in which the sample was sublimed (e.g., adenosine sublimed at 170-190° at approximately $10^{-5}$ mm. of Hg pressure) directly onto a clean rock salt disk held about 3 inches away from the material. In general films obtained by this method are very uniform and often are completely transparent in the visible region. No attempt was made to measure the thickness of the material sublimed, but simply sufficient sublimate was allowed to collect so that the transmission in the 6 $\mu$ region was between 10 and 20 per cent. As a check against decomposition during sublimation, samples of a compound before and after sublimation were submitted to spectral measurements in the vicinity of its ultraviolet absorption maximum. In general, the position of the absorption band was unaltered and the $\epsilon$ value unchanged ($\pm 5$ per cent) by this treatment. In some cases, the rate of evaporation was so slow that it was not practicable to collect sufficient material for this purpose.

As noted above (Fig. 3) the spectrum of the evaporated sample of adenosine agreed well with that obtained with a powder of the material be-
tween sodium chloride disks. In the case of xanthosine, however, some
darkening of the residual material in the sublimer was noted, and on pro-
longed heating, decomposition occurred. The spectrum of the evaporated
sample did indeed agree with that of the mineral oil suspension of xantho-
sine at wave-lengths shorter than 8 μ, but at longer wave-lengths obvious
differences appeared.

Guanosine and adenyllic acid charred so readily under conditions re-
quired for their sublimation that no attempt was made to evaporate samples
of these materials or of the other nucleotides for infra-red measurement.

The simple purines and pyrimidines, on the other hand, showed no ap-
parent evidence of decomposition under the conditions used for subli-
mation. The infra-red spectral studies on these compounds will be re-
ported shortly.

Cast Films—The technique used can perhaps best be illustrated by the
following example. A cast film of sodium ribonucleate was prepared by
dissolving 54 mg. of the salt in 0.4 cc. of distilled water and spreading 1
drop of this solution evenly over a 1 inch silver chloride disk. The disk
was then placed in a desiccator, stored for at least 24 hours in the dark
over phosphorus pentoxide, and then dried for at least 40 hours over this
material at room temperature and 1 mm. pressure. The resultant film
was continuous, non-crystalline, and satisfactory for infra-red measure-
ments. Films were prepared from thymus deoxyribonucleic acid in a
similar manner.

Instrumentation and Measurements—The spectral measurements were
made on a Perkin-Elmer infra-red spectrometer, model 12A, with a ten
cycle chopper, a Strong bolometer, an alternating current amplifier, and
a Brown Instrument Company recording potentiometer. For initial ob-
servation, a complete spectrum from 1 to 15 μ was recorded continuously.
For drawing the final curves in Figs. 1 to 4, the data were obtained on a
point to point basis, the points being taken from 5 to 10 cm.⁻¹ apart at
frequencies up to 1900 cm.⁻¹ (5.2 μ) and at larger intervals at higher fre-
quencies.

SUMMARY

The infra-red spectrum from 2 to 15 μ has been measured for samples
of the sodium salts of yeast ribonucleic acid and thymus deoxyribonucleic
acid. These compounds exhibit different infra-red spectra, especially at
wave-lengths longer than 9 μ.

The infra-red spectra of several nucleotides and nucleosides have also
been determined. By use of the infra-red spectra of these compounds,
differentiation and identification can be made of pure samples.
BIBLIOGRAPHY

ABSORPTION SPECTRA: VII. THE INFRA-RED SPECTRA OF SOME NUCLEIC ACIDS, NUCLEOTIDES, AND NUCLEOSIDES
Elkan R. Blout and Melvin Fields


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