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Cataloguing of Infrared Spectra[1]

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A brief method for matching an infrared spectrum, in the 2–16-μ region, of an unknown sample to a large number of known spectra has proved satisfactory for nucleic acid derivatives, and may well be an aid in many of the empirical uses of infrared data. The method is analogous to the ASTM–AXRED X-Ray Powder Pattern Card File as devised by Hanawalt and co-workers (*1*).

For the infrared spectra, the cataloguing procedure is as follows: A smooth base line is drawn on the high transmission side of the absorption bands. This affords a scattering correction. (See Fig. 1 for a solid sample

λ(μ)	9.15	9.48	11.97b
E/E₁	1.0	0.7	0.5

1-D arabinosyl uracil
(from Dr. Irving Goodman)
Capillary mineral oil mull

Curve 81198

λ(μ)	E/E₁	λ(μ)	E/E₁
2.83	0.4	9.15	1.0
3.01	0.5	9.48	0.7
5.84	0.3	9.81	0.1
5.98	0.6	10.57	0.3
6.99	0.3	11.04	0.2
7.67	0.3	11.97b	0.5
7.87	0.5	12.28	0.1
8.11	0.5	13.24	0.1
8.30	0.3	15.2bb	0.1
8.65	0.4		
8.98	0.7		

FIG. 2. The infrared file card for 1-D arabinosyl uracil.

bands (excepting those of mineral oil for mulled samples), chemistry, method of preparation, etc., are recorded on the card (see Fig. 2); the spectrum may be printed on the back. The cards are then filed according to wavelength of the strongest band.

To identify an unknown, its spectrum is run and its card tabulated, then the file is searched at the wave-

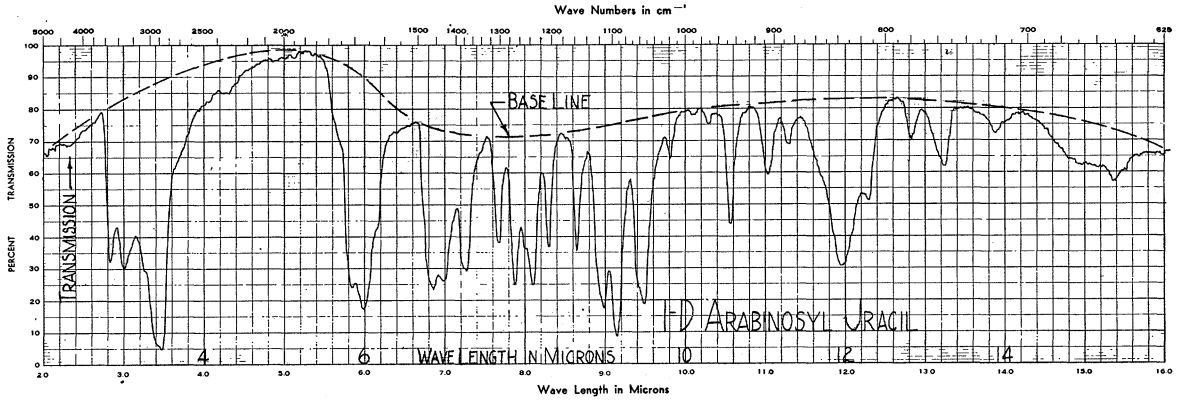

FIG. 1. The infrared absorption spectrum of 1-D arabinosyl uracil, with base line drawn in.

mulled in mineral oil. Visibly transparent samples have a flatter base line.) The extinctions, log ($T_{\text{base line}}/T_{\text{absorption peak}}$), are then determined for the three strongest bands in the region 9.00–15.00 μ. This is primarily the "backbone" vibration region, bands here being more characteristic of the whole molecule than the side group bands of shorter wavelength. The extinction of a band overlapped by other bands is calculated by subtracting from the observed extinction the extinctions of the overlap bands extrapolated. These three bands are then tabulated as to wavelength in order of decreasing extinction, and the ratios of the extinctions of the bands to the extinction of the strongest are tabulated. If two have the same extinction, the one of shorter wavelength is tabulated first. The data for each molecule are put on a 3 in.×5 in. card, the wavelengths of the three strongest bands being prominently placed in the upper left of the card, as with the x-ray cards. Other strong length of the strongest band (in the 9–15-μ region), plus or minus 0.04 μ to allow for wavelength errors. The inclusion of a secondary set of cards, on which the second strongest band is listed first, and filed accordingly, also allows for intensity errors from uncertainties of base line or from instruments of markedly different slit widths. When the three strongest bands are matched, other tabulated bands of the known and unknown are compared and then the spectra are directly matched and possibly rerun under identical conditions. Bands of widths greater than 0.1 μ within 2% of the absorption maxima are designated with a "b," greater than 0.2 μ with a "bb." Improvement in purity or sample preparation may permit resolution of several bands here, so that these values should be considered with caution. Simple mixtures may be identified by successive elimination of bands, though adjacent bands of the components may be unresolved. Chemical compounds, however, have bands in the 9–15-μ region quite different from those of the free components.

As compared to the various punch card systems, such

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as that described by Wright (*2*), this card file has the advantages of lower cost and greater simplicity of matching spectra. It is not readily searched for subject, author, functional groups, and other detailed information possible to a punch card system, but it is recommended for chemical studies paralleled by infrared identification on a limited class of chemicals, most of which may be identified simply by the three strongest bands in the 9–15-μ region. Such a card file has proved valuable in the rapid identification of over 75 purines, pyrimidines, nucleosides, nucleotides, and nucleic acids, using the mineral oil mull method of sample preparation. Infrared identification of nucleic acid hydrolyzates by this method is now being attempted.

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Colloidal Dispersion of Chloroplast Material[1]

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Chloroplasts, isolated from leaves and placed in a suitable medium, are able when illuminated to liberate oxygen from water (*1*). This photochemical activity is little diminished when chloroplasts are broken into fragments barely visible under a microscope. In order to observe the properties of chloroplast material having submicroscopic particle size, it is necessary to prepare colloidal dispersions of the material. These dispersions are consistently and easily prepared by use of the apparatus described here. It is anticipated that other small particles, such as blood cells, unicellular organisms, and homogenates of animal tissue, for example, can be disintegrated in the same apparatus.

It was found impractical by blending, grinding with sand in a mortar, or using a colloid mill to disperse an appreciable fraction of chloroplast material. Exposure of a suspension of chloroplasts to ultrasonic energy resulted in the disintegration of part of the material to colloidal dimensions. Dispersion was much more conveniently accomplished by using high pressure to force a suspension of chloroplasts through a small orifice. Fig. 1 illustrates the device which was used.

It is made as follows: A 1-in. hole is bored 4 in. deep in the center of a 3-in. steel bar 5 in. long. A smaller hole is bored the rest of the way through the bar and is threaded to take a Hoke steel needle valve. A steel piston slightly less than 1 in. in diam has attached to it a leather washer which fits snugly in the bore of the cylinder. The top ¼ in. of a No. 5 rubber stopper makes a watertight seal between the piston and the cylinder. The leather washer is necessary to prevent the rubber from becoming wedged between the piston and cylinder wall. The top of the piston is drilled and tapped to take a ¼-in. bolt. This provides means for attaching a crosspiece to the piston, in order to withdraw it from the cylinder.

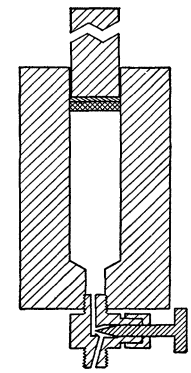

FIG. 1. Dispersion unit.

In operation, 40 ml of a filtered suspension containing 20–30 mg chloroplasts/ml is placed in the dispersion unit. The rubber washer is inserted, broader side down, followed by the piston. The chamber is inverted, the valve opened, and the air forced out. With the valve closed again, the chamber is placed on its base (not shown in the figure) and is put under a 60-ton hydraulic press. After the desired pressure is built up in the chamber, the needle valve is barely opened, so that the liquid runs through at the rate of a few ml/min. During passage of the material through the valve, the pressure is maintained by use of the pump on the hydraulic press. A unit with a smaller bore and with a built-in needle valve for use with laboratory presses has been designed but not tested.

Following passage through the needle valve, the liquid is diluted to contain about 0.5 mg chlorophyll/ml, then it is centrifuged 1 hr at 12,000 × gravity. This empirically determined dilution gives the best yield of material in dispersion at nearly the highest concentration. We consider the chloroplast material which is not sedimented in 1 hr at 12,000 × gravity to be in colloidal dispersion. After separation from the sediment, the dispersion of chloroplast material is dark green, appearing very clear with the light behind it but almost opaque when illuminated obliquely.

Using a pressure of 20,000 psi to force a suspension through the valve, ⅔–¾ of the chloroplast material is colloidally dispersed. In order to do this in one passage through the valve, it was found advisable to freeze the leaves just before isolation of the chloroplasts. Otherwise two passages through the valve were required to attain the same degree of dispersion.

The photochemical activity (*2*) per unit of chlorophyll is about a fourth as great in such dispersions as the activity of intact chloroplasts. Several tests were made to see whether the large loss of activity might be due to the effect of high pressure alone, or to passage of the material through the valve at high pressure. Chloroplast suspensions held 30 min in the chamber at pressures of 5,000, 10,000, 15,000, and 20,000 psi, without passing

[1] The progress of this work was greatly facilitated by a grant from the Research Corporation.