The Infrared Examination of Compounds Related to Ascorbic Acid

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It is now realized that infrared absorption spectra can frequently be used with marked success in the differentiation of organic substances for the analysis of mixtures and for structural diagnosis. The present paper indicates briefly how it may be applied in one problem of interest in biochemistry.

Recent studies on vitamin C in plant materials have shown that its occurrence and/or functions may be better understood by reference to other closely related dienols, such as reductone (1), reductic acid (2), hydroxymaleic acid (3) and hydroxytetronic acid (4). These dienols, although possessing no anti-scorbutic activity, all react with the indophenol dye normally used to estimate ascorbic acid (5). The first three, which are known to occur naturally, can be differentiated from ascorbic acid by various modifications of Lugg's (1942) formaldehyde method (cf. Mapson, 1943 *a, b*; Wokes, Organ & Jacoby, 1943). Hydroxytetronic acid cannot be so differentiated, neither can *iso*ascorbic acid (6), which is known to occur in certain commercial fruit juices. Moreover, the formaldehyde method does not differentiate between 1, 2, 3 and 4.

The infrared absorption spectra of these substances have therefore been measured in the hope of finding characteristic differences which may be used for analysis. The results given below show that such distinguishing features occur, and these may not only enable the analyses to be carried out, but may

also provide a method which may help in following the course of plant processes.

EXPERIMENTAL

The spectra were measured between 2–14 μ . with an automatically recording single beam spectrometer, with rock salt prism (Whiffen & Thompson, 1945). A few measurements were also made in the region 5–7 μ . with a double beam spectrometer having a prism of calcium fluoride.

The compounds were obtained from Roche Products Ltd., except for dihydroxymaleic acid, which was a laboratory specimen. No impurities could be detected by their ultra-violet absorption spectra. A specially purified sample of reductic acid supplied by Prof. Reichstein was also used. The spectra of the solid powders were measured by grinding to a paste with a little paraffin in the standard manner. A satisfactory spectrum can be obtained with a few mg. of material. The paste was pressed between two small rock salt plates. Measurement of the spectrum of the solids had the advantage over solutions that the whole spectrum could be surveyed without serious disturbance by absorption bands of the solvent. For analytical work, it would be necessary to work with dilute solutions in some solvent chosen so as to have clear transmission in the region of the key absorption bands concerned. Over small regions of the spectrum shown in Fig. 1, paraffin has its own absorption bands, which may obscure those of the substance, but these are regions where the vibrations due to C—H bonds occur and it is certain that, from an analytical standpoint, they are unimportant. These spectral regions are marked *N* in Fig. 1.

RESULTS AND DISCUSSION

Fig. 1 shows the absorption spectra of the six compounds. The positions of the bands are shown on a wave-length scale (μ .), and also on a frequency scale, the customary units of reciprocal wave lengths, or waves/cm. (cm^{-1}) being used. It is seen that, whilst in several cases there are absorption bands at the same positions, each substance has a different spectrum as a whole. Each compound has a band near 1650 cm^{-1} , presumably due to the stretching vibration of the C=C bond. Some of the bands in the region $1100\text{--}1250 \text{ cm}^{-1}$ may be connected with bending vibrations of the hydroxyl groups. The three compounds having a lactone ring show a band at about 1750 cm^{-1} , which has previously been found common to many lactones (Richards & Thompson, 1947). In the region $2400\text{--}3600$ there is interesting and varied absorption due to the stretching vibration of bonded-hydroxyl groups. It is noteworthy that the different compounds show

marked differences in this respect, as has also been found in measurements on a series of sugars and their derivatives (Trotter & Thompson, 1947).

The spectra are unfortunately so complex that the analysis of a mixture of compounds 1, 2, 3, 4 and 5, if all were present together, would be very difficult.

of ascorbic acid and *iso*ascorbic acid the bands at 991 and 941 cm^{-1} respectively seem very suitable.

As with all other analyses in the infrared, the sensitivity and accuracy will depend upon the precise mixtures to be analyzed, and will probably vary from case to case. We propose to use the

Fig. 1. Infrared absorption curves of compounds related to ascorbic acid. Region marked *N* obscured by bands of paraffin. Percentage absorption covers the range 100 %.

However, if only two or three components were present, it should be possible. For example, reductone could be determined in ascorbic acid by its band at 923 cm^{-1} , or at 1580 cm^{-1} , or ascorbic acid in reductone by its bands at 1030, 1120 or 1755 cm^{-1} . Reductone could be differentiated from reductic acid by its bands at 777 or 923 cm^{-1} , and reductic acid in reductone by its band at 1126 cm^{-1} . A key band for dihydroxymaleic acid may be that at 950 cm^{-1} . Differentiation of hydroxytetrionic acid from reductic acid could be carried out with the bands between 750–900 cm^{-1} . For the differentiation

method in studying the growth processes of certain plants, but it seems desirable to draw attention now to the possibilities of this type of measurement.

SUMMARY

The infrared absorption spectra of some compounds related to ascorbic acid have been measured between 2–14 μ . There are differences between the spectra which should make analyses of mixtures possible and more satisfactory than by existing chemical methods.

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