bination are responsible for the main part of the 7α-hydroxylating capacity. Only a minor part is found in the supernatant alone in the presence of pure ATP.

Impure ATP preparations contain a factor causing the formation of relatively large amounts of other polar products from taurodesoxycholic acid *in vitro*. In *in vivo* experiment with bile fistula rats practically the only product formed from desoxycholic acid is cholic acid.

Further details will be published shortly.

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Received October 29, 1955.

Infrared Absorption Spectra of some Salts of DL-2-Phosphoglyceric Acid

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In a previous study [1], the strength of interaction between D,L-2-phosphoglyceric acid (PGA), the substrate of the enzyme enolase, and metal ions activating this enzyme was determined. Analogously to the concept of metal-ion activation of peptidases developed by Smith and his associates [2], it was suggested that the metal ion furnishes one of the points of interaction between enzyme and substrate. Thus, knowledge of the nature of the complexes between PGA and activating ions is important in interpreting the mechanism of the enzymic reaction. Studies by Rosenberg [3] have shown that the formation of metal chelates of amino acids results in characteristic changes in infrared absorption which can be correlated with the type of bond involved. In attempts to obtain similiar information about some metal-ion complexes of PGA, their infrared absorption spectra have been recorded.

PGA was synthesized according to Kiessling [4] and purified as described by Warburg and Christian [5]. To form a metal complex, a 0.02 M solution of the neutral Na^+ salt of PGA, containing an equimolar amount of metal chloride, was allowed to stand for about 1 h at room temperature. Then, absolute ethanol was added until a turbidity formed. (With the pure Na^+ salt, 64 vol. % ethanol was required, while the Mn^{++}, Zn^{++} and Ni^{++} complexes precipitated at concentrations of 13, 11 and 17 %, respectively.) After the addition of ethanol, the sample was placed in a refrigerator (4°) for 12 h. The precipitate formed was centrifuged off and dried in a vacuum dessicator over P_2O_5. Analysis showed the metal and acid to be present in a 1:1 ratio, as in solution [1]. A Perkin-Elmer model 21 recording spectrophotometer equipped with a NaCl prism was used for the absorption measurements. The substances were examined as pressed KBr discs prepared according to Schiedt and Reinwein [6].

Some typical results are shown in Figs. 1–3, which give the spectra of the Na^+, Zn^{++} and Ni^{++} salts of PGA. The absorption spectrum of each substance was recorded twice; the second time the sample was cooled to −170° by the use of a cell described by Rosenberg [3]. As seen in the figures, the use of the low temperature resulted in considerably better resolution as compared to room temperature.

The infrared absorption spectra of PGA and its salts are dominated by bands arising from the absorption by two highly polar groups, namely carboxyl and phosphate. The carboxyl group is characterized by its antisymmetrical vibration (ν|C(=O)O). It has been established by numerous investigations summarized by Bellamy [7] that this vibration of the ionized carboxyl group has a frequency of 1 600–1 590 cm^{-1} (about 6.3 μ). The Na^+ salt of PGA can be regarded as a normal salt, and it exhibits, as expected, a broad and intense band at approximately 1 590 cm^{-1}. If the carboxyl group were to form a bond of more covalent character with Zn^{++} and Ni^{++}, a shift of this band to higher wave num-

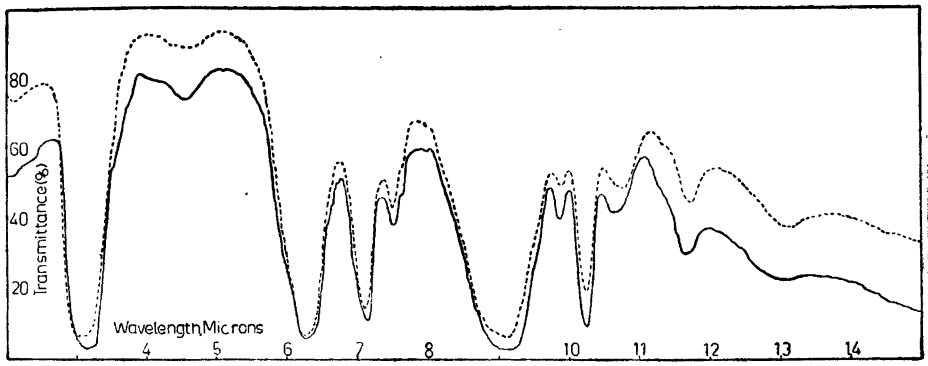

Fig. 1. The infrared absorption spectrum of the Na^+ salt of PGA. In this and the following figures, the dashed curve was recorded at room temperature and the solid curve at — 170°.

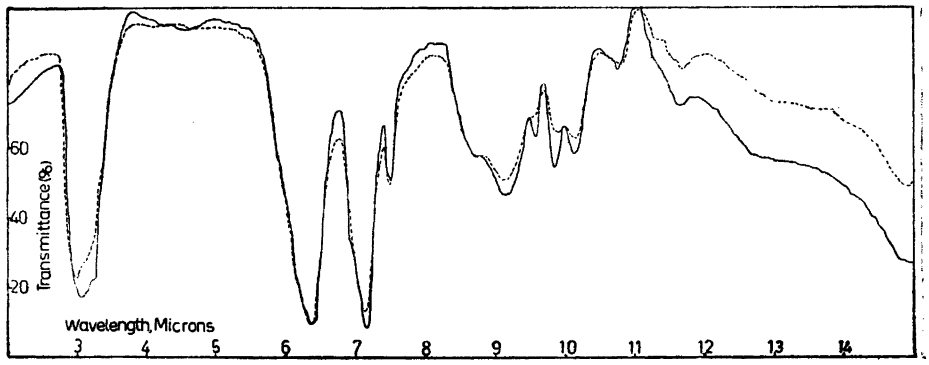

Fig. 2. The infrared absorption spectrum of the Zn^{++} complex of PGA.

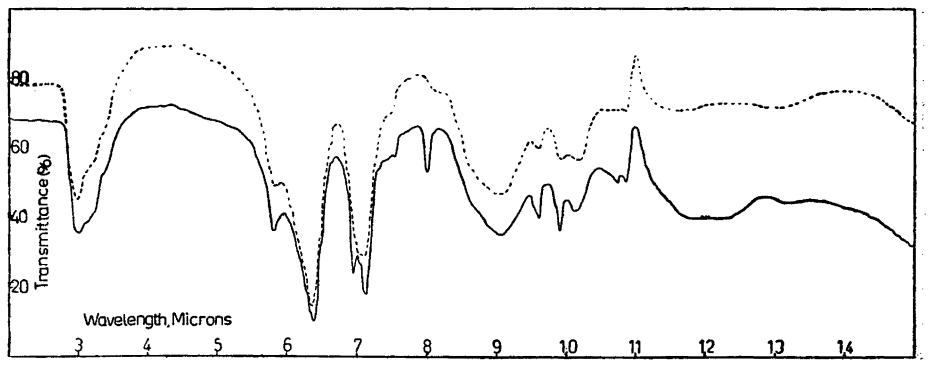

Fig. 3. The infrared absorption spectrum of the Ni^{++} complex of PGA.

bers would be expected. Such a behavior has been observed by Rosenberg [3] for different metal chelates of amino acids, where, for example, the carboxyl frequency at 1 590 cm^{-1} of the Na^+ salt showed a shift of 29 cm^{-1} in Cu^{++} chelates and of 55 cm^{-1} in Pt^{++} compounds. The carboxyl frequency in the Zn^{++} and Ni^{++} complexes of PGA shows no such trend. On the contrary, a small shift towards lower wave numbers can be observed. If this shift has any significance of its own is difficult to decide as the position of the band for the Na^+ salt is very approximative owing to its broadness; but it is clear, anyhow, that the carboxyl group shows no evidence for covalent bond formation with Zn^{++} and Ni^{++}.

The absorption due to the other dominant group, the ionized phosphate, exhibits remarkable changes when Na^+ is substituted by Ni^{++} or Zn^{++}. Unfortunately, there is very little information available on the infrared absorption of ionized phosphate groups [7], which makes it difficult to interpret the changes in the spectra. The intense absorption band between 1 150 and 1 050 cm^{-1} (8.7—9.5 μ) (probably due to the ionized group and P—O—C stretchings) loses much of its intensity in the Zn^{++} and Ni^{++} complexes. In the Zn^{++} complex, the absorption peak shows a tendency to split into two bands and at the same time small changes in the position of the maximum absorption can be detected. The absorption bands in the 1 050—950 cm^{-1} (9.5—10.5 μ) region, also characteristic of the phosphate group, show a more complex picture in the Zn^{++} and Ni^{++} compounds compared to the Na^+ salt.

Zn^{++} activates enolase while the Ni^{++} enzyme is inactive [8]. As an explanation for this, it has been suggested, on the basis of ultraviolet absorption measurements, that the Ni^{++} complex with the substrate is of a different type than the complexes with the activating ions [8]. The infrared absorption data reported here show that such a difference, if it exists, cannot be the amount of covalent character of the interaction with the carboxyl group, since this is completely ionic in both complexes. On the other hand, distinct differences exist between the phosphate absorption bands of the Zn^{++} and Ni^{++} complexes, but unfortunately these cannot be properly evaluated at present.

This investigation is part of a program on the infrared absorption of compounds of biochemical interest supported by the *Nobel Fund*.

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Received November 8, 1955

Free Amino Acids in Pollen

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In the summer 1954 we studied the composition of the free amino acid fraction in the pollen especially of wind-pollinated plants. The method used was that generally employed in this laboratory for the identification of free amino acids in green plants, *i. e.* homogenisation of the plant material, extraction with 70 % alcohol, separation of amino acids with Amberlite IR-120, elution with 1 N ammonia, and subjection of the evaporated extract to two-dimensional paper chromatography with butanol-acetic acid and phenol-ammonia.

The wind-pollinated plants investigated are: *Alnus incana, Betula alba, Corylus avellana, Quercus robur, Pinus silvestris, Populus balsamifera, Populus tremula, Secale cereale, Salix caprea.* For the identification of the spots of hydroxyproline and citrulline a special colour reaction was used: the spot of hydroxyproline turns red[1], and that of citrulline yellow [2], when treated with isatin + *p*-dimethylaminobenzaldehyde (PDB). The amounts of the amino acids were roughly estimated on the basis of the intensity of the spots.