cluding the cuvette, to be washed free from air by means of nitrogen. The first 15 ml of the solution were used to rinse away remaining traces of oxygen. The solutions were stable in the cuvette for at least two hours. Fresh solutions were used for the determination of the base ferri porphyrin spectra, since reoxidation with air caused deterioration. We have found this arrangement – a number have been tried – to be most convenient for the handling of solutions of this kind.

The base ferro porphyrins (haemochromogens) gave one band within the range 315–330 mμ. The substitution of the hydrogen atoms at the β-positions 2 and 4 in deuterohaemin by two vinyl or two ethyl groups to give protohaemin and mesohaemin caused shifts in wavelength of the band analogous to what is found in visible light. We conclude that the 316 mμ-band in ferrocytochrome c is to be attributed to its nature of being a haemochromogen. Notably the spectra of oxy- and carboxyhaemoglobin [6] and of ferrocytochrome b_3 [7], which contain protohaemin, possess a band at 330–340 mμ.

The ferricytochrome c spectrum shows an absorption band at 364 mμ[1]. It is present also in base ferri porphyrin and free ferri porphyrin spectra, and its position varies as above with the substitution at the positions 2 and 4. Most, possibly all, haemproteids in the threevalent state show a peak at 350–370 mμ or a shoulder adjacent to the Soret band. The "sharpening" of the Soret band upon reduction is partly caused by its migration 10–15 mμ towards red and the vanishing of the band or shoulder at 350–370 mμ.

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Note on the Potassium Bromide Disk Technique for Measurements of Infrared Spectra

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Attempts to find a general method for quantitative measurements of infrared absorption of solids have led to the development of the potassium bromide disk technique (Stimson [1], Schiedt [2,3] and their associates, *cf.* also [4]). A few milligrams of the sample are mixed with some hundred milligrams of potassium bromide powder, the mixture pressed to a disk and the absorption of the disk measured. It is evident that the sample must be uniformly distributed throughout the disk in order to obtain quantitative results, and that the particles of the sample must be so small that further dispersion does not increase the absorption. The technique has been developed by Andersen and Woodall [5] for samples as small as 0.01 mg by using a beam-condensing system of silver chloride lenses to reduce the area of the sample beam.

We have used the potassium bromide technique in an investigation of the infrared absorption of furan compounds. Our disks were prepared essentially after the directions of Stimson [1] and of Schiedt [2,3], but the mixture of sample and potassium bromide was not evacuated during the pressing (*cf.*[5]). The pressing tools described by Schiedt were simplified. The potassium bromide powder was prepared by precipitation of an aqueous solution of the salt with acetone. This way of preparing powder, which gives transparent disks, is simpler than grinding. The technique was not only used for solids but also for viscous liquids.

At first we found it difficult to distribute the sample uniformly and in sufficient dispersion throughout the disk. Grinding the sample in a mortar [1,3] or in a small ball

mill [3] with potassium bromide usually did not give sufficient dispersion, in particular when the sample consisted of soft crystals or was liquid. Evaporation of a solution of the sample on the potassium bromide [2] gave good results with some compounds, but (1) could not be used for volatile compounds and (2) was not reliable, as solid samples occasionally crystallized in too large crystals during the evaporation. We have found that *grinding the sample with potassium bromide followed by pressing, regrinding of the disk, and pressing once more consistently gave reproduceable results.*

The experimental part only contains directions for the preparation of the potassium bromide and of the disks. No examples of the use of the method for quantitative measurements can be reported as a systematic investigation on a large number of compounds lies outside the scope of our laboratory program. However, judging from experiments carried out so far, we believe that the method is of general applicability.

Experimental. Preparation of the potassium bromide. Potassium bromide (analytical grade, 335 g) was dissolved in water (purified by heating under reflux with potassium permanganate and distilling, 500 ml) and the solution precipitated with acetone (analytical grade, heated under reflux with potassium permanganate and distilled, 500 ml). After about one minute the resulting precipitate was removed by filtration and dried at 110° C, with occasional grinding of the large lumps. The product was stored in the dark. The transmission of disks prepared from this product is 85—88 %.

The pressing tools, shown in Figure 1, were made of Sverker 3 (Uddeholm A/S, Copenhagen), hardened to 64° Rockwell C. The pressing surfaces were polished as recommended by Stimson [1]. The difference between the diameters of the plunger and the anvil and of the die shell is about 0.03 mm.

Preparation of the disks. The sample is mixed for 3—4 minutes in a small mortar of stainless steel with 500 mg of potassium bromide. The mixture is placed in the die and pressed for 30 seconds at 90 kg/mm². The disk is ground finely in the mortar and the resulting powder pressed again. The die shell holding the new disk is then placed in the spectrophotometer.

Liquid samples or samples of very soft crystals are weighed directly into the potassium bromide powder and the mixture pressed without prior grinding. The sample is then ground, pressed, ground and pressed again. Only rather viscous liquids, which are not squeezed out of the potassium bromide under the pressing may be measured in this way.

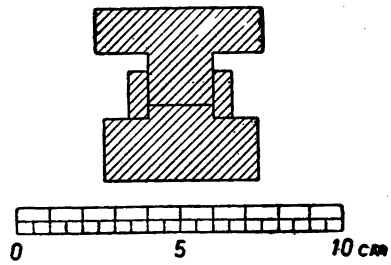

Fig. 1. Pressing tools.

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γ-Glutamyl-Alanine in Pea Seedlings

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In an earlier paper [1] from this laboratory, it was reported that during germination of pea seeds a ninhydrin positive substance is formed, which on a two-dimensional chromatogram (solvents: butanol + acetic acid and phenol + NH_3), gives the spot "X" below serine. We have now investigated this substance more closely, and found it to be γ-glutamyl-alanine.

After 5 days of germination, pea seeds contain a comparatively great amount of the compound forming spot "X", and an extract was made from them with 70 % alcohol. Following a phenol run, a two-dimensional paper chromatogram was dried at 110° C, after which the spots formed were examined in UV-light. Spot "X" was cut off from many paper chromatograms, and the detached pieces of pa-