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.

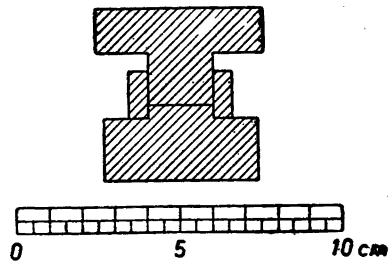

Fig. 1. Pressing tools.

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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-

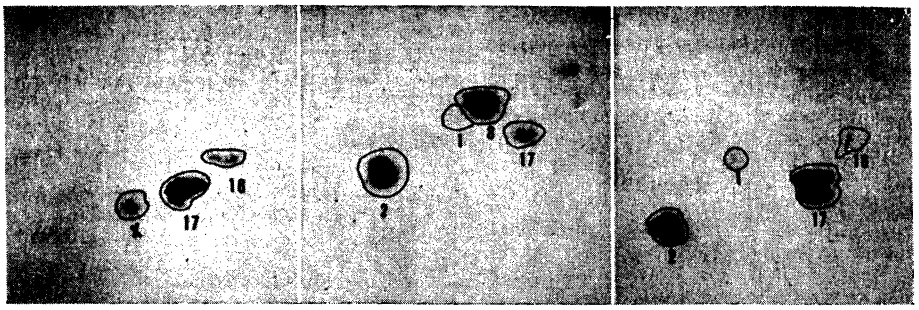

Fig. 1. Unhydrolyzed extract of spot "X". Aspartic acid = 16 and glutamic acid = 17 were added to the extract.

Fig. 2. With 1 N HCl hydrolyzed extract of spot "X". Alanine = 2 and serine = 8 were added to the extract. 1 = Gly.

Fig. 3. With 1 N HCl hydrolyzed extract of spot "X". Aspartic acid and glutamic acid were added to the extract.

per were throughly extracted with water. The extract was concentrated. The major part was hydrolyzed with 1 *N* hydrochloric acid at 108° C for 20 hours. The hydrochloric acid was carefully removed after hydrolysis. Chromatograms were prepared from the extracts by using the following additions:

1. unhydrolyzed extract ("X") + glutamic acid + aspartic acid
2. hydrolyzed extract + alanine + serine
3. hydrolyzed extract + glutamic acid + aspartic acid

1. gave a chromatogram showing, in addition to spot "X", the spots for glutamic acid and aspartic acid (Fig. 1).

2. gave a chromatogram showing clear and intense spots of glutamic acid, alanine, and serine (Fig. 2). The spot of alanine was much more intense than could have been expected on the basis of the amount of alanine added. Glutamic acid and alanine had thus been formed on the hydrolysis of spot "X". The origin of a very faint spot of glycine observed on the chromatogram is unknown. The possibility that spot "X", in addition to γ-glutamyl-alanine, should contain a small amount of γ-glutamyl-glycine cannot be overlooked. On the basis of our experimental material we cannot, however, say anything with certainty.

3. gave a chromatogram showing spots of alanine, glutamic acid and aspartic acid, and in addition a very faint spot of glycine (Fig. 3). The spot of glutamic acid was much more intense than in case 1, even if the same amount of glutamic acid had been added to the extract in both cases. This chromatogram too confirmed that only alanine and glutamic acid (and possibly a very small amount of glycine) are formed from the compound giving spot "X".

When the unhydrolyzed alcohol extract was run through Amberlite IR 4B column, we found that "X" travels together with acid amino acids. This is what could be expected from the acid nature of γ-glutamyl-alanine.

For the examination of the γ-glutamyl-alanine synthesis, we also made an experiment with an extract obtained by crushing peas which had germinated for 4 days. This extract did not give the spot "X" on paper chromatograms. When either 15 mg of alanine or 15 mg of glutathione were added to 1 ml of the extract a clear, even if faint, spot "X" appeared.

The results suggest that the same reaction which Hird and Springell [2] discovered with kidney homogenate between glutathione and alanine:

γ-glutamyl-cysteine-glycine + L-alanine

↓ γ-glutamyl transferase

γ-glutamyl-L-alanine + cysteinyl-glycine

also occurs in germinated peas.

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