α-Oxidation of Indoleacetonitrile

It is known that certain plant tissues can convert 3-indolylacetonitrile to the highly active plant growth substance 3-indolylacetic acid[1-3] and we have shown that other plant tissues are able to bring about an α-oxidation of 3-indolylacetonitrile to yield 3-indolylcarboxylic acid which is inactive[3,4]. A recent publication by Thimann and Mahadevan[5] describing the extraction of what is believed to be a hydrolytic enzyme from the stems and leaves of certain monocotyledons (Gramineae), which is capable of converting 3-indolylacetonitrile to 3-indolylacetic acid, prompts us to report upon experiments using a cell-free extract of etiolated stems of a dicotyledon (*Pisum*). A clear demonstration of the α-oxidation of 3-indolylacetonitrile to 3-indolylcarboxylic acid is given by this extract.

Pea seedlings (var. Alaska) were grown at 25°C. in red light and harvested when 12 cm. in height. About 100 gm. of stems from which the terminal 5 mm. had been removed, were frozen at −15°C. and then ground at this temperature. The tissue was allowed to thaw, 25 ml. of phosphate buffer (*p*H 7·0, 0·025 *M*) added, and the crude extract strained. This extract was centrifuged in nylon tubes for 25 min. at 11,000 r.p.m. (approx. 14,000 *g*) in a refrigerated centrifuge at 2°C. The cell-free supernatant liquid was pipetted from the centrifuge tubes and used immediately for the metabolic studies. 3-indolylacetonitrile was dissolved in 0·2 per cent aqueous acetone to give a 20 p.p.m. solution. A 25 ml. quantity of this nitrile solution was mixed with 25 ml. of the cell-free extract in a 200 ml. glass stoppered tube and incubated for 12 hr. at 25°C. in darkness. The contents of the tube were then acidified to *p*H 2·8–3·1 and extracted with peroxide-free ether. The presence of 3-indolylcarboxylic acid in this extract was shown on a two-dimensional paper chromatogram developed first in *iso*propanol/ammonia (0·880)/water (10:1:1) and then in *iso*propanol/acetic acid (glacial)/water (4:1:1). After spraying the chromatogram with Ehrlich reagent, a pink spot which gave a characteristic red fluorescence in ultraviolet light slowly appeared. This chromatogram was compared with one bearing synthetic 3-indolylcarboxylic acid developed simultaneously. A comparison of the 3-indolylcarboxylic acid content of different extracts was made possible by applying the extracts to the starting line of a chromatogram which was then developed once in the ammoniacal solvent and sprayed with Ehrlich reagent to give pink spots (R_F 0·18).

In addition to showing the degradation of 3-indolylacetonitrile to 3-indolylcarboxylic acid evidence was obtained of the presence of an aldehyde (R_F 0·79) on chromatograms developed in *iso*propanol/ammonia/water and sprayed with a solution of 2:4-dinitrophenylhydrazine hydrochloride. This aldehyde was inseparable from synthetic 3-indolealdehyde by two-dimensional chromatography and it is likely that this compound, which was also found in our earlier metabolic studies[6], is an intermediate product in the conversion of 3-indolylacetonitrile to 3-indolylcarboxylic acid.

The α-oxidation of 3-indolylacetonitrile was prevented by boiling the cell-free extract for a period of 1–2 min. prior to the addition of the nitrile solution; the amount of 3-indolylcarboxylic acid found in the ether extract was then no greater than the trace normally found in extracts of pea tissue. The natural occurrence of ether extractable 3-indolylcarboxylic acid in pea tissue has previously been reported[7]. Considerably reduced amounts of it were produced from 3-indolylacetonitrile when the enzyme inhibitors, iodoacetate and phenyl mercuric nitrate, were added to the solutions before incubation, and these indications that sulphydryl groups may be involved in the α-oxidation are being further investigated.

By subjecting the cell-free extract to increasing concentrations of ammonium sulphate at *p*H 7·0, a series of precipitates was obtained, one of which contained most of the enzyme activity. This active fraction, which was precipitated when the ammonium sulphate concentration of the extract was raised from forty per cent to sixty per cent saturated, was readily redissolved in phosphate buffer for metabolic studies. Since a quantity of material was precipitated at ammonium sulphate concentrations below forty per cent saturated, this procedure proved to be a useful purification method.

Whilst the efficiency of conversion of 3-indolylacetonitrile to 3-indolylcarboxylic acid was greatly increased by using this purified preparation, there was still no evidence on the chromatograms for the production of 3-indolylacetic acid. This confirms the work of Thimann[1] and Seeley *et al.*[3] and is in marked contrast to the behaviour of 3-indolylacetonitrile in wheat and maize coleoptiles[3] and with enzyme extracts of *Avena* and *Hordeum* tissue[5] where conversion to 3-indolylacetic acid readily occurs.

All these results correlate well with those of biological tests; thus, for example, 3-indolylacetonitrile is highly active at low concentrations as a plant growth substance in tests using the coleoptiles of Gramineae, but at these concentrations is completely inactive in tests using pea tissue[1,3,8].

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Reaction of Formyl Porphyrins with Acetone-Hydrochloric Acid

Acetone-hydrochloric acid has been widely used to split hæmoproteins, including cytochromes of the *a* type which contain formyl substituents in their prosthetic groups. Results reported below show that formyl porphyrins and hæmins react with acetone-hydrochloric acid, and hence it is necessary to exercise caution in the use of this reagent. Fortunately the reaction between formyl porphyrins