Phytochemistry, 1967, Vol. 6, pp. 313 to 315. Pergamon Press Ltd. Printed in England

SHORT COMMUNICATION

INDOLE AUXINS IN BARLEY SEEDLINGS

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(*Received* 27 *April* 1966)

Abstract—Germinated barley of varying ages has been examined for indole auxins. Tryptophan and indole-3-carboxylic acid (ICA) were positively identified. A compound similar to IAA was also found but its identity was not elucidated. The chemical composition of seedlings was similar whether the barley was germinated for a long or a short time. The variation in the content of ICA and possible interrelationship between kinetin and ICA are discussed.

INTRODUCTION

THE presence of IAA and the other indole auxins in plants has been based mainly on chromatographic evidence, yet it would be desirable to confirm these claims by isolation of the compounds in adequate quantities. However, such isolation requires large amounts of plant material.

Countercurrent distribution (ccd) has proved to be an especially valuable tool for separating substances of biological origin. In most cases, the best results have been obtained by a combination of ccd with some other fractionating procedure, such as chromatography. Holley *et al.*[1] used ccd in auxin analysis of cabbage; and Jones and Taylor[2] isolated ICA from cabbage by its use.

The purpose of this work was to isolate indole auxins, and to confirm their chromatographic and spectrophometric characteristics. Barley was a suitable material because it has been extensively analysed and several indole derivatives have been detected chromatographically. The availability of germinated barley on an industrial scale is another advantage.

RESULTS

Extracts obtained from barley germinated for varying times were analysed for indole compounds using ccd, *Avena* curvature test, thin-layer chromatography (TLC) and spectrometry. Active auxins could be detected in the acidic ethanol-soluble fractions when they were subjected to ccd using cyclohexane/butanol/water (92:8:100).

One peak of very large activity ($K \sim 1$, 0·95 g) was detected in tubes 50–80, and another small but definite peak in tubes 10–20 ($K \sim 0{\cdot}4$, 0·90 g). The two fractions were then separately purified by ccd in butanol/water. The second ccd of the high activity fraction in this mixture gave a peak in tubes 80–100, but the low activity fraction showed no evident growth activity or u.v. absorption. The progress of each ccd run was followed by TLC.

The high activity fraction was further purified by elution from a cellulose column with distilled water.[3] A total of forty-eight fractions, each of 2–3 ml, were collected and the u.v. spectra of tubes 20–45 showed indole-like characteristics (λ_{max} 285–290 nm, λ_{min} 255 nm). The fractions were chromatographed in silica gel plates with butanol saturated with water.

[1] R. W. HOLLEY, F. P. BOYLE, H. K. DURFEE and A. D. HOLLEY, *Arch. Biochem.* **32**, 192 (1951).
[2] E. R. H. JONES and W. C. TAYLOR, *Nature* **179**, 1138 (1957).
[3] J. ELEMA, Ph.D. Thesis, University of Utrecht (1960).

The chromatograms showed a spot (unknown I) with chromogenic reactions identical to ICA, the R_f values in four solvents were the same (Table 1) and the u.v. spectrum was similar to that of authentic ICA. The intensity of the colour reactions with Ehrlich reagent and the u.v. spectrum indicated large amounts of this indole compound in the eluate. As expected[4] no curvature was observed in the biotest.

The nature of the second compound (unknown II) detected in the acid–ether fraction is unknown. This substance showed IAA-like properties; it had high growth activity in the *Avena* curvature test, similar R_f values in TLC with two solvents, and similar partition coefficients in two ccd systems (cyclohexane/butanol/water (92:8:100); butanol/water), but from the spectrophotometric evidence and from its colour with Ehrlich reagent it would not appear to be identical to IAA.

The presence of a compound (unknown III) identified as tryptophan was demonstrated in the original aqueous extract by means of TLC in three different solvents and its colour reactions with three reagents.

TABLE 1. R_f VALUES OF THE UNKNOWN SUBSTANCES* FROM BARLEY

Solvent	Unknown I*	ICA*	Unknown III†	Tryptophan†
Butanol/water	0·85	0·86	0·23	0·24
Benzene/dioxan/acetic acid (90:25:4)	0·70	0·72	—	—
Isopropanol/ammonia/water (8:1:1)	0·89	0·91	0·47	0·47
n-Butyl acetate/water	0·95	—	0·95	0·00

* Both give purple with Ehrlich reagent.
† Both give blue with Ehrlich, light yellow with ninhydrin and yellow with Prochazka.

DISCUSSION

As judged by u.v. absorption at 285 nm the highest content of ICA was found in 80 hr germinated barley seedlings, and it seems that there may be a correlation between the time of germination and the ICA content. It is noteworthy that ICA has always been found in young plants, as is the case here. The kinetin activity is also at a maximum at 80 hr,[5] and the synchronous disappearance of ICA and kinetin during subsequent growth suggest that some interrelationship may exist between both substances. The physiological significance of ICA is unknown, but its presence might be explained as an end-product in the α-oxidation of indole nitriles.[6] The intermediate products formed when IAN is so oxidized, however, were not identified, because of their neutral character.

EXPERIMENTAL

Material. For each experiment 100 kg of germinated barley seedling of varying ages were used. The seeds were germinated under standard conditions of light and temperature for various times.

Extraction. The plant material was homogenized in ethanol (1 g/1·5 ml) and was then allowed to stand for 24 hr at room temperature in the dark.

[4] P. E. PILET, *Les Phytohormones de croissance*, p. 188. Masson, Paris (1961).
[5] G. J. M. VAN DER KERK, G. W. VAN EIJK and J. A. WEBER, *Chem. Weekblad* **60**, 185 (1964).
[6] P. M. CARTWRIGHT, J. T. SYKES and R. L. WAIN, In *The Chemistry and Mode of Action of Plant Growth Substances* (Edited by R. L. WAIN and F. WIGHTMAN), pp. 32–39. Butterworth, London (1956).

Fractionation. The pH of the filtrate was adjusted to pH 2·9 with 6 N HCl and shaken with peroxide-free ether. The ether layer was shaken with a 1·0 M Na_2CO_3 solution. The acidic auxins were recovered from the carbonate solution, following acidification and re-extraction. The acid fraction was evaporated to dryness *in vacuo* and the residue was dissolved to 100 ml 60% methanol. The methanolic solution was extracted with light petroleum (40–60°), followed by CCl_4 and evaporated to dryness under reduced pressure. Neither the petroleum nor the CCl_4 removed active substances. The neutral fraction was evaporated to a viscous sirup. Attempts to purify the auxins, were unsuccessful due to obstinate emulsion formation.

Countercurrent distribution. All the ccd were carried out in a fully automatic instrument with 100 units of 2 × 25 ml capacity each. The residue from each of the acid fractions was divided between 50 ml each of the upper and lower phases of the system cyclohexane/butanol/water (92:8:100) and the process was run for 100 cycles, at constant temperature (21°) in the dark. After locating the major components with growth activity, the contents of tubes having similar components were pooled and evaporated to dryness under reduced pressure. These fractions were purified by a second ccd with butanol/water as solvent mixture. The tubes were again combined, concentrated to dryness *in vacuo* and dissolved in 5 ml ethanol. A 1 ml aliquot from each was tested by the *Avena* biotest.

Column elution. The active fractions were collected, concentrated to small volume under reduced pressure, and then added to cellulose powder, which had been previously washed successively with 50% ethanol, 98% ethanol and ether and filtered. Alcohol was removed *in vacuo*, the dry powder stood overnight in a desiccator, and then pressed on the top of a cellulose powder column (13 cm × 4 cm). The column was eluted at a rate of 6 ml per hour with distilled water.

Bioassay. The *Avena* coleoptile curvature test, using Wageningen variety, was used as described by Post.[7] Standards of IAA were included with each run. All assays of unknowns were carried out at 2–4 dilutions. Six replicates were used for each concentration of extract and for controls.

Spectroscopy. Measurements were made on a Zeiss M4QII spectrophotometer in the range 220–325 nm.

Chromatography. This was carried out by TLC on silica gel using water-saturated butanol as solvent, and spraying with Ehrlich reagent.

Acknowledgements—This work was carried out at the Organisch Chemisch Laboratorium der Rijksuniversiteit, Utrecht (Holland), during tenure of a postdoctoral fellowship. We thank Professor G. J. M. van der Kerk for the facilities and Drs. J. A. Weber and G. M. van Eijk for their kind advice in the course of this work. The author is grateful to CSIC and to Rijksuniversiteit te Utrecht for the receipt of a postdoctoral fellowship. We are indebted to Dr. T. Swain, Dr. B. Truelove and Professor B. B. Stowe for correcting the English manuscript. The Amstel Bierbrouwerij, Amsterdam, is acknowledged for providing germinated barley.

[7] L. C. Post, Ph.D. Thesis, University of Utrecht (1960).