Phytochemistry 1974 Vol 13, pp 239 to 241 Pergamon Press. Printed in England.

INDOL-3YL-ACETIC ACID IN ROOTS OF *ZEA MAYS*

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(*Received* 19 *September* 1973 *Accepted* 1 *October* 1973)

Key Word Index—*Zea mays*, Gramineae, maize roots, identification, indol-3yl-acetic acid, auxin

Abstract—Indol-3yl-acetic acid was identified in extracts of sterile roots of *Zea mays* seedlings by means of TLC, chromogenic reactions, GLC and GC–MS

INTRODUCTION

IN PREVIOUS papers we, and others[1–5], have discussed the controversy regarding the presence and role of indol-3yl-acetic acid (IAA) in roots The controversy has existed because attempts to characterize the root auxins have yielded results which were chemically inconclusive or ambiguous and because there have been doubts about the extent to which the compounds examined were actually produced by epiphytic bacteria on the non-sterile plant tissues used [6] The use of MS in combination with various chromatographic techniques permits conclusive identification of indolic compounds from plant tissues,[2,7,8] Here we have used TLC, GLC and GC–MS to provide unequivocal evidence that the auxin previously examined by Greenwood *et al* [3] is IAA and that it may be extracted from roots of sterile *Zea mays* seedlings This is the first time that GC–MS has been successfully applied to the identification of an auxin from vegetative plant material, the techniques described should be applicable to a wide range of other materials

RESULTS AND DISCUSSION

The putative IAA was purified in the manner described in the Experimental The eluate from the ethyl acetate–2-propranol–water chromatogram contained 188 μg of IAA (esti-

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mated by the method of Knegt and Bruinsma [9] A portion of the methanol solution containing *ca* 10 μg IAA was used for the determination of TLC R_f values and for chromogenic tests As shown in Table 1 there was precise correspondence between the properties of the root auxin and those of IAA chromatographed in parallel with the root auxin

TABLE 1 COMPARISON OF CHROMATOGRAPHIC (TLC) AND CHROMOGENIC PROPERTIES OF THE ROOT AUXIN WITH THOSE OF INDOL-3YL-ACETIC ACID

Chromatographic solvent	Root auxin (R_fs)	IAA
AcOMe–*iso*PrOH–NH_4OH (9 7 4)	0 31	0 32
$CHCl_3$ (1% EtOH) HOAc (19 1)	0 29	0 29
$CHCl_3$ HOAc MeOH (19 1 4)	0 79	0 80
EtOAc *iso*PrOH–H_2O (65 24 11)	0 66	0 66
$CHCl_3$ CCl_4 MeOH (2 1 1)	0 28	0 29
$CHCl_3$ CCl_4 MeOH (1 2 2)	0 33	0 34
Chromogenic test	**Colour**	
Ehrlich reagent	Purple	Purple
p-Dimethylaminocinnamaldehyde reagent	Red purple	Red purple
Prochazka reagent (UV)	Yellow fluorescence	Yellow fluorescence

The data of Table 1 provide strong circumstantial evidence of the identity of the root auxin with IAA In order to provide confirmation of this identification the techniques of GLC and GC–MS were used The auxin in the portion of the eluate remaining after the TLC studies was methylated [10] The methylated auxin yielded peaks with retention characteristics identical to those of authentic methyl indolyl acetate when subjected to GLC on 5% Versamid 900 and 2 5% OV17 columns Finally, a GC–MS examination of the methylated root auxin yielded a MS identical to that of authentic methyl indolyl acetate having the molecular ion at *m/e* 189 and major fragmentation peaks at *m/e* 130 (corresponding to loss of CO_2Me) and *m/e* 103 (corresponding to a loss of HCN from the *m/e* 130 fragment)

Thus identity of the root auxin of *Zea mays* with IAA is established on the basis of bioassay activity,[3] chromogenic reactions and TLC R_f values of the free acid, and GLC and GC–MS data for the methylated acid The physiological significance of IAA in regulation of growth and differentiation in the maize root remains a matter of dispute it is hoped that experiments currently in progress will solve some of the problems

The techniques described here should permit an early resolution of the controversial data regarding the presence of IAA in roots of other species [1–5] The purification technique is critical Direct determination of the MS of IAA in the eluate of the ethyl acetate–2-propanol–water chromatogram using a direct insertion probe should be possible when a comparatively rich source of IAA like the *Zea mays* root is used, but the GC–MS technique is certain to be required for sterile root material of most other plants

EXPERIMENTAL

Plant material Seeds of *Zea mays* L cv Giant White Horsetooth were sterilized by rinsing in 5% Teepol for 1 min and soaking in 0 1% $HgCl_2$ for 20 min then germinated on sterilized trays on Whatman 3MM chromat-

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ography paper soaked in dist H_2O After 3 5 days at 25° samples were taken from each tray and tested for sterility [6 11] The primary roots (5–15 cm long) of the remaining seedlings were harvested under dim green light into ice-cold dist H_2O, rinsed several times, rapidly surface dried and then frozen by placing in a container immersed in a dry ice–MeOH bath The frozen material was freeze dried Material from separate trays was not combined until the results of the sterility tests were known

Extraction and purification As far as possible all manipulations were carried out in darkness or dim green light and the extracts were kept cold All solvents were redistilled before use and the Et_2O freed of peroxides 141 6 g of freeze dried material (equivalent to 2000 g fr wt) was suspended in 7000 ml MeOH for 20 hr at 4° After filtration the residue was re-extracted (× 2) with 3500 ml 80% MeOH for 5 hr at 4° The filtrates were combined The IAA content was estimated[9] at 246 μg The MeOH was removed under reduced pressure at 35° then the aq residue was acidified to pH 3 0 with 6N HCl and partitioned × 4 against equal vols of Et_2O The combined Et_2O layers were evaporated to dryness under reduced pressure at 30°, the residue redissolved in 10 ml 50% MeOH and the soln divided equally between 5 columns each containing the equivalent of 10 g dry wt of DEAE-cellulose Each column was eluted with 200 ml dist H_2O to remove low MW neutral compounds, then with 250 ml 0 05 M Na_2SO_4 The Na_2SO_4 eluate was acidified to pH 3 0 with 6N HCl and partitioned 4 × against equal vols of Et_2O The combined ether layers were evaporated to dryness under reduced pressure at 30° The residue was dissolved in 80% MeOH and applied as streaks (dried in N_2) to thin-layers (0 25 mm) of Sil G (Machery-Nagel Co) which were developed in iso Bu–MeOH–H_2O (16 1 3) The developed chromatograms were dried in N_2 The band corresponding to the IAA marker was cut out and eluted with MeOH The MeOH soln was concentrated under reduced pressure at 30° and applied to thin-layers of Sil G (dried in N_2) which were developed in EtOAc–*iso*PrOH–H_2O (65 24 11) The developed chromatograms were dried in N_2 then the band corresponding to the IAA marker was cut out and eluted with MeOH and used for further study

Analytical TLC and chromogenic tests TLC was carried out on 0 25 mm layers of Sil G Ehrlichs reagent is 2% *p*-dimethylaminobenzaldehyde in a mixture of conc HCl and acetone (1 4). *p*-Dimethylaminocinnamaldehyde reagent is 10% (w/v) in a mixture of equal vols conc HCl and EtOH Prochazka reagent prepared as in Randerath (1963) [12] *GLC* carried out as previously described[13] on columns of 5% Versamid 900 or 2 5% OV17 *GC–MS* The low resolution MS of the methylated root auxin was determined using a 2 5% OV17 GLC sample system under the following conditions ion source 220° electron energy 70 eV trap current, 100 μA

Acknowledgements—We wish to thank Professor H E Street for his kindness in permitting us to use his GLC equipment and we thank the S R C Physico-Chemical Measurements Unit for the GC–MS

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