Plant Physiol. (1967) 42, 1091–1093

Conversion of Indole-3-Ethanol to Indole-3-Acetic Acid in Cucumber Seedling Shoots[1, 2]

David L. Rayle[3] and William K. Purves

Department of Biological Sciences, University of California, Santa Barbara, California 93106

Received April 26, 1967.

Summary. Indoleethanol-^{14}C was applied to intact cucumber seedlings and to hypocotyl segments. The presence of indoleacetic acid-^{14}C in tissue extracts was demonstrated by thin layer radiochromatography. There was no evidence of conversion of indoleacetic acid to indoleethanol. It is suggested that the growth-promoting activity of indoleethanol is due to its conversion to indoleacetic acid.

The occurrence of indole-3-ethanol (IEt) in cucumber shoots was demonstrated in a previous paper (3). IEt and indole-3-acetic acid (IAA) caused equal promotions of the elongation of cucumber hypocotyl segments, and we proposed that IEt might be converted to IAA in the cucumber. Wightman has suggested that the growth-promoting activity of IEt in the wheat cylinder test is due to the conversion of IEt to IAA, although no supporting data were presented (4). In this paper, evidence is given for the rapid conversion of IEt to IAA in intact cucumber seedlings as well as in hypocotyl segments.

Materials and Methods

Preparation and purification of labeled compounds. IEt-^{14}C was prepared in a 3-step synthesis from indole as follows. One mc indole-2-^{14}C (Nuclear Chicago) was added to 15 ml ice cold anhydrous ethyl ether along with enough unlabeled indole so that the final solution was 1 mM in indole. 3-Indolylglyoxylyl chloride was obtained by the careful addition of oxalyl chloride to the indole solution (1). Ethyl-3-indolylglyoxylate was prepared from the chloride and the ester reduced with $LiAlH_4$, in dry tetrahydrofuran according to the method of Norgrady and Doyle (2). The overall yield of IEt-^{14}C was 61 % with a specific activity of 1 mc per millimole.

In the course of our experiments, 2 different methods were used to purify the crude IEt-^{14}C. Each method yielded satisfactory results. The first method utilized preparative thin layer chromatography (Eastman Chromagrams, type K 301 R2) with $CHCl_3$ as the solvent. IEt-^{14}C was isolated from the R_F zone 0.2 to 0.3 by elution from the silica gel with anhydrous ethyl ether. The ether was evaporated under nitrogen and a portion of the crystalline residue checked for purity by thin layer chromatography in isopropanol: NH_4OH:H_2O (10:1:1, v/v) and in $CHCl_3$. After chromatography, the dry chromatograms were exposed to iodine vapor or scanned for radioactivity using a Nuclear Chicago Actigraph II strip scanner (model 1025). In each solvent, only 1 spot was detected by iodine staining; and in both cases this spot corresponded to the R_F value of authentic IEt. Subsequent strip scanning indicated that most of the label was concentrated in the IEt region. The remaining label was uniformly distributed along the chromatogram (fig 1).

A second method of purification consisted of dissolving the crude IEt-^{14}C in ethyl ether and extracting this solution 7 times with 0.01 N NaOH. In each fractionation the volume of the aqueous solution was twice that of the ether. The ether solution was evaporated and the aqueous solution discarded. The IEt-^{14}C prepared by this method was checked for contaminating IAA and for possible decomposition in aqueous solution by incubating an aliquot of the purified material (approximately 20 μc) in H_2O. After 4 hours, this aqueous solution was partitioned in exactly the same manner as described below for plant extracts. The acidic fraction obtained by partitioning was checked for contaminating IAA by thin layer radiochromatography in several solvents. In no case was there any indication that the IEt-^{14}C prepared in this manner contained any contaminating IAA.

Carboxyl- and methylene-labeled IAA (New England Nuclear) were purified prior to use by dissolving them in 0.01 N NaOH and extracting these solutions 5 times with equal volumes of ethyl ether (ether fractions discarded). The aqueous fraction was adjusted to pH 2 with concentrated HCl and extracted 5 times with equal volumes of ethyl ether. These

[1] This work was supported by National Science Foundation grant GB-4922.

[2] This material was included in a doctoral thesis submitted by D. L. Rayle to the Graduate Division of the University of California, Santa Barbara.

[3] National Defence Education Act predoctoral fellow. Present address: MSU/AEC Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48823.

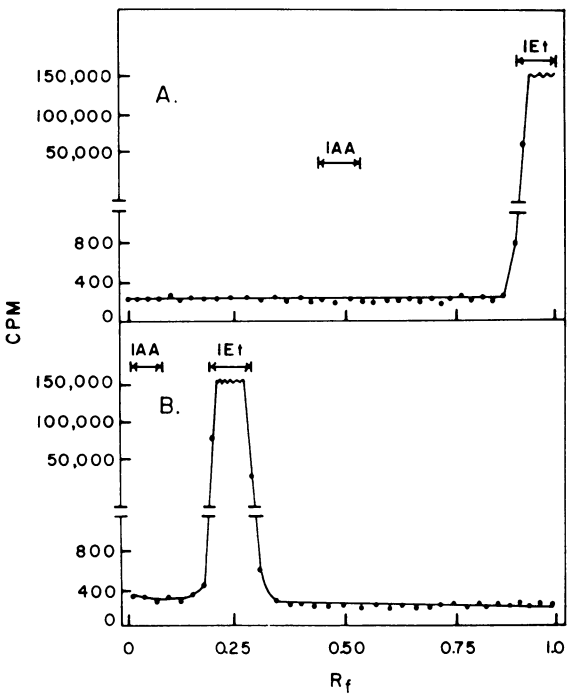

FIG. 1. Thin layer radiochromatograms: purified indole-3-ethanol-^{14}C. Peak readings beyond capacity of counter, i.e., greater than 10^6 cpm. R_F values of indoleacetic acid and indoleethanol indicated. Solvents: A) isopropanol : NH_4OH : H_2O (10:1:1, v/v); B) $CHCl_3$.

ether fractions were combined and evaporated, and the crystalline residue was dissolved in anhydrous acetonitrile and stored in the dark at 4° until use.

Application and Extraction of Labeled Compounds. Cucumber seedlings were grown as previously described (3) and treated with labeled compounds in 2 different ways. In 1 series of experiments, 50 μc purified IEt-^{14}C or IAA-^{14}C was dissolved in 5 ml of an aqueous solution containing acetonitrile and Tween 80 (both 1 %, v/v) and applied to the cotyledons of approximately 200 intact cucumber seedlings. In another series of experiments, 400 2.0-cm hypocotyl segments were incubated in an aqueous solution containing 25 μc of purified IEt-^{14}C or IAA-^{14}C. Similar results were obtained with both methods of application. However, the second method (incubation of segments) is preferable, since it resulted in greater uptake of labeled compounds and since it facilitated measurement of the treated plant material.

After the application of labeled compounds, the segments or seedlings were grown for 4 hours in the light (640 ft-c) at 26°. After incubation, the plant tissue was rinsed with approximately 1500 ml H_2O, ground in a mortar, and extracted 3 times with approximately 100 ml ethyl ether each time. The combined ether fractions were extracted 5 times with 0.01 N NaOH. The ether fraction, containing neutral and basic indoles, was evaporated to dryness and saved for chromatographic analysis. The combined aqueous fractions were adjusted to pH 2 with concentrated HCl and extracted 5 times with ethyl ether. These ether fractions, containing the acidic indoles, were combined, evaporated, and saved for analysis. The acidic aqueous phase was discarded.

The residues to be analyzed were suspended in 10 ml ethyl ether, and 20 μl aliquots were applied to Eastman Chromagram sheets. After development in the appropriate solvent, the radioactive areas were detected by strip scanning. Authentic samples of various indoles were run simultaneously. The R_F values for the known samples were calculated after exposing the dry chromatograms to iodine vapor and compared with the radioactive peaks obtained from the unknown samples.

Results and Discussion

In the first experiment, cucumber segments were incubated in IEt-^{14}C as described in Materials and Methods. The data obtained by chromatographing the acidic indole fraction in 6 solvents are presented in figure 2. The R_F values for known IEt and IAA are shown for comparison. In each solvent, a strong

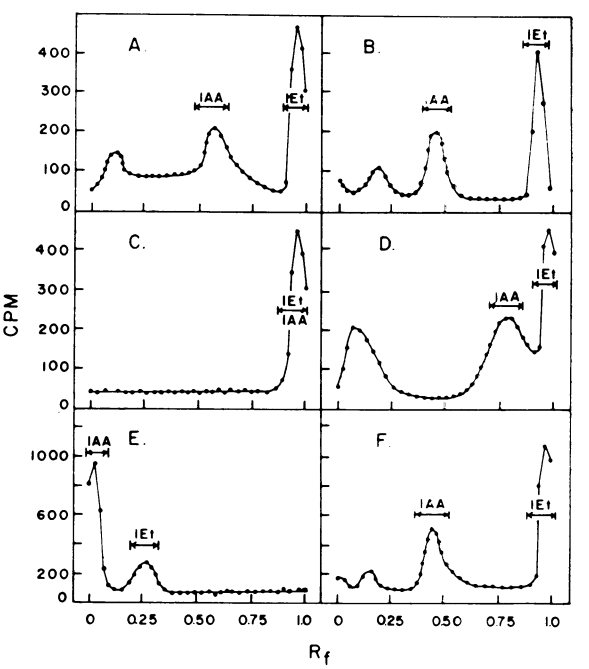

FIG. 2. Thin layer radiochromatograms: acidic fraction of plants treated with indoleethanol-^{14}C. R_F values of indoleacetic acid and indoleethanol indicated. Solvents: A) methyl ethyl ketone : pyridine : H_2O (14:1:3, v/v); B) isopropanol : NH_4OH : H_2O (10:1:1, v/v); C) isopropanol : glacial acetic acid : H_2O (50:5:1, v/v); D) isopropanol : H_2O : 50 % acetic acid (200:20:1, v/v); E) $CHCl_3$; F) ethyl acetate: NH_4OH: isopropanol (9:7:4, v/v).

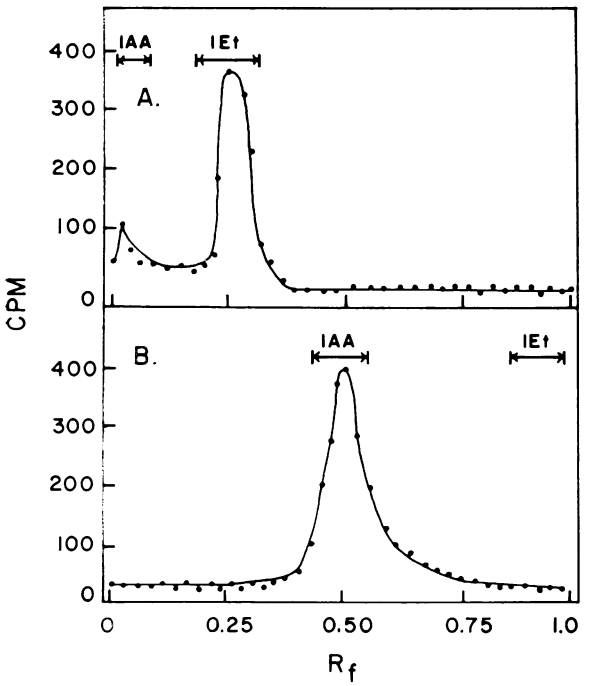

FIG. 3. Thin layer radiochromatograms. R_F values of indoleacetic acid and indoleethanol indicated. A) Neutral-basic fraction after treatment of plants with indoleethanol-^{14}C; solvent: $CHCl_3$. B) Neutral-basic fraction after treatment of plants with indoleacetic acid-^{14}C; solvent: isopropanol : NH_4OH : H_2O (10:1:1, v/v).

peak was observed at the R_F of IAA. A second small peak was observed near the origin in several solvents; however, the identity of this compound is unknown. The R_F values for the unknown compound did not correspond with those for indole-3-acetaldehyde, the presumed intermediate between IEt and IAA. A large peak due to residual IEt was also seen in each chromatogram. The average segment length after the 4-hour incubation period in IEt-^{14}C (approximately 0.5 mM) was 2.8 cm, while a control group of segments incubated in H_2O averaged 2.1 cm in length. The content of the neutral and basic indole fraction was also investigated by thin layer radiochromatography in 3 solvents [$CHCl_3$, aqueous NaCl (8%, w/v), and isopropanol:NH_4OH:H_2O (10:1:1, v/v)]. Even when the aliquot analyzed was increased 10-fold (to 0.2 ml), the only labeled area corresponded to the R_F of known IEt. The data for 1 solvent are presented in figure 3A as representative of the results obtained after chromatography of this fraction. Essentially identical results were obtained in 2 repeat experiments, one with intact seedlings and the other with excised segments.

Since these data indicated that intact cucumber seedlings or segments were capable of the in vivo conversion of IEt to IAA, it was of interest to learn whether the reverse reaction occurred. For these experiments, both carboxyl- and methylene-labeled IAA were used. In a typical experiment in which segments were incubated 4 hours in an aqueous medium containing 25 μc of IAA (approximately 0.1 mM), the average length of the segments after the incubation period was 2.7 cm, while control segments incubated in H_2O were only 2.1 cm in length. The neutral and basic indole fraction was isolated as described above, and aliquots were removed for analysis. Since liquid scintillation counting indicated this fraction to be labeled only slightly above background, approximately half of the initial sample was chromatographed in isopropanol:NH_4OH:H_2O (10:1:1, v/v). The data obtained from subsequent strip scanning are shown in figure 3B. No IEt-^{14}C could be detected; and it would appear that, while IEt could be converted to IAA, the reverse reaction did not occur to any significant extent. Essentially identical data were obtained in 2 repeat experiments, 1 with intact seedlings and the other with excised segments. (In 1 experiment in which the commercial IAA-^{14}C was not further purified, there appeared to be IEt-^{14}C in the tissue extract; however, no such results were obtained with purified IAA-^{14}C.)

The data presented here indicate the in vivo conversion of IEt to IAA. The presence of IAA in cucumber seedlings is substantiated by the observation that tryptophan-^{14}C and tryptamine-^{14}C can also be converted to IAA-^{14}C in these plants (J. E. Sherwin and W. K. Purves, unpublished data). It therefore seems likely that the growth response elicited by IEt in the cucumber seedling is due to its conversion to IAA. This hypothesis is further substantiated by our previously published data on growth responses to IEt and IAA (3). While cucumber segments responded as strongly to IEt as to IAA, zucchini squash segments responded to IAA but not to IEt. If IEt were itself an active auxin, it should promote growth in all systems in which IAA is active. The inability of IEt to promote growth in zucchini, in which IAA is strongly active, suggests that this species lacks the enzyme(s) responsible for the conversion of IEt to IAA. Our inability to detect IEt-^{14}C formation from IAA-^{14}C also lends support to the idea that IEt is an intermediate in the formation of IAA, and that IAA is indeed the active auxin in cucumber seedlings.

Literature Cited

1. HOCHSTEIN, F. A. AND A. M. PARADIES. 1957. Alkaloids of *Banisteria caapi* and *Prestonia amazonicum*. J. Am. Chem. Soc. 79: 5735–36.
2. NORGRADY, T. AND T. W. DOYLE. 1964. A convenient preparation of tryptophol. Can. J. Chem. 42: 485–86.
3. RAYLE, D. L. AND W. K. PURVES. 1967. Isolation and identification of indole-3-ethanol (tryptophol) from cucumber seedlings. Plant Physiol. 42: 520–24.
4. WIGHTMAN, F. 1963. Pathways of tryptophan metabolism in tomato plants. Colloq. Intern. Centre Natl. Rech. Sci. Paris 123: 191–212.