CONVERSION OF TRYPTOPHAN-2-C^{14} TO INDOLEACETIC ACID BY WATERMELON TISSUE SLICES[1,2]

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It was suggested some years ago that the plant growth hormone, IAA[4] arises from TTP through a series of reactions involving IPyA or TNH_2 and IAc (13, 21). Subsequently, when IAN was isolated from natural sources, it was proposed that IAN and IAM (14) were likely intermediates in the conversion of TTP to IAA (11). This latter view received additional support with the demonstration that IAN can be converted to IAA (19). Recently, however, IAM was demonstrated in the presence of respiring tissue when exogenous IAA was added and this suggests that IAM arises from IAA rather than being an intermediate in the conversion of TTP to IAA (7). Still other investigators present evidence suggesting that IPyA and TNH_2 are not intermediates in the conversion of TTP and IAA by the mung bean (8, 22).

Experiments carried out in this laboratory in which DL-TTP-2-C^{14} was incubated with watermelon tissue slices indicate that TTP can serve as a precursor of IAA in this tissue and furthermore the data suggest that this pathway is through IPyA.

MATERIALS AND METHODS

The watermelon seed used in these experiments were of the New Hampshire Midget variety No. 879 obtained from the Farmer Seed and Nursery Company, Faribault, Minnesota. The seeds were germinated and the plants were grown under artificial light in sand culture watered with nutrient solution (17). Approximately 7 to 10 days after hand pollination, when the melons were approximately 3 cm long, the outer covering (rind) was removed, and the remaining tissue sliced into thin sections. These sections were randomized and slices equivalent to two melons (10 to 12 gm) were added to a 125-ml Erlenmeyer flask containing 9 ml of M/15 KH_2PO_4. Each ml of buffer contained 300 units of penicillin G and 5 mg of DL-TTP (specific activity equal to 1,032,000 cpm/mg). The medium was made 1×10^{-3} M with respect to ascorbic acid to inhibit the destruction of IAA (2, 23). The final pH was 4.5 ± 0.1.

The optimum pH for the conversion of TTP to IAA by spinach tissues (25) has been reported to be 7.5, but it is also at this pH that IAA is most rapidly destroyed (20). It was decided therefore to use pH 4.5 to retard this enzymatic destruction, and even though the amounts of TTP converted would probably be decreased, the radioactive label would enable detection of the indole derivatives. In addition, the acid pH would discourage bacterial growth and enhance the stability of IPyA (1). The flasks were plugged with cotton, wrapped in black, light-proof paper and placed on a shaker at room temperature ($25 \pm 2°$ C) for 20 hours.

In more recent experiments, where TTP-2-C^{14} was incubated with Avena coleoptile tissue for 20 hours, compounds apparently identical to those found in watermelon were observed. Further experiments with acetone dried powder of Avena showed these same compounds to be formed after a 30-minute incubation period (unpublished, Dannenburg and Liverman). It appears then that the conversion of TTP to IAA in these experiments with watermelon tissue constitutes a normal biochemical conversion. After incubation, the flasks were placed in boiling water for 10 minutes and then cooled. The contents and wash water from each flask were placed in a mortar and triturated with washed, ignited sand. The resulting brei was centrifuged, the supernatant was decanted, and then partitioned into anhydrous, peroxide-free ether at pH 8.1 and 2.8, respectively.

PRELIMINARY EXTRACTION EXPERIMENTS: A clear chromatographic separation of the indole compounds was not possible without previous treatment because of the proximity of the neutral and basic substances in our solvent system. Success with a preliminary solvent extraction procedure for separating TNH_2, IAM and IAN was so promising that a mixture of several know acidic, basic, and neutral indole compounds were partitioned as discussed below prior to attempting a separation of the products of TTP metabolism by paper chromatography (fig 1). The chemical separation was done to obtain information as to the type of compounds present as far as solubility is related to structure and to separate the basic substances from the neutral substances. Table I shows that this preliminary partitioning with solvents results in a clean separation of all compound except the IAM which is found in both the neutral and the basic fractions. The presence of IAM in both the basic and neutral fraction was not expected since amides are generally insoluble in 5 % HCl (16).

Since the indole nucleus is known to be labile in the presence of strong mineral acids (18), an experiment was set up to determine the rate of destruc-

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[4] IAA (indoleacetic acid); IPyA (indolepyruvic acid); IGA (indoleglycolic acid); IAH (indolealdehyde); IAM (indoleacetamide); IAN (indoleacetonitrile); IAAP (indoleacetylaspartic acid); TTP (tryptophan); TNH_2 (tryptamine); IAc (indoleacetaldehyde); IOA (indoleglyoxylic acid); TOH (tryptophol); 5-OH TTP (5-hydroxytryptophan).

tion of the indole ring by 5 % HCl. The indole ring is more stable towards mineral acid than the pyrrole ring to which the reaction is attributed (12). One mg of indole was dissolved in 20 ml of 5 % HCl with the aid of ethanol and placed on a shaker. Aliquots were taken at intervals and the amount of indole determined (5). Table II shows there is a rapid destruction of indole with time, but the amount of destruction is small after the first 15 minutes which approximates the time the indole compounds are in contact with the 5 % HCl. This destruction is approximately 40 %. Though this amount of destruction does not interfere with a qualitative test for the compounds, it would discourage the quantitative aspects of the extraction procedure.

Basic and Neutral Fractions: As shown in figure 1, the aqueous solution (approx. 50 ml) was adjusted to pH 8.1 with Na_2CO_3 and partitioned three

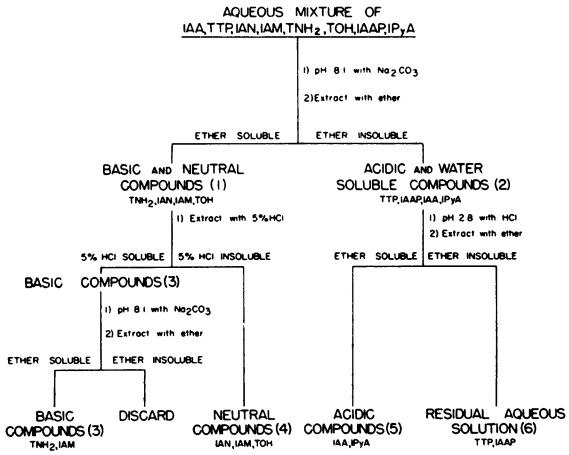

Fig. 1. Partitioning of an aqueous solution of known indole compounds into the residual aqueous solution, neutral, basic and acidic fractions.

times into 20-ml portions of ether. The ether extracts (I) containing the basic and neutral compounds were combined and then further resolved by partitioning the basic compounds into 5 % HCl, leaving the neutral compounds in the ether fraction (IV). The pH of the aqueous 5 % HCl extract was adjusted to 8.1 with Na_2CO_3 and the basic components were extracted back into ether with three 20-ml portions (III). The aqueous portion contained no Ehrlich positive material and in experiments with the tissue it was discarded, since it had no radioactivity. The two ether extracts containing the neutral and basic substances were washed with a saturated solution of NaCl, dried with anhydrous Na_2SO_4, filtered, and evaporated to dryness under reduced pressure without heat. The residue of each fraction was resuspended in 1 ml of anhydrous peroxide-free ether.

Acidic Fraction: The aqueous solution (II) remaining after removal of the basic and neutral substances was adjusted to pH 2.8 with HCl and extracted with three 20-ml portions of ether. The ether extracts (V) were combined, washed with a saturated solution of NaCl, dried with anhydrous Na_2SO_4, filtered and taken to dryness under reduced pressure without heat. This residue was then resuspended in 1 ml of ether.

Residual Aqueous Solution: The residual aqueous solution (VI) remaining after the removal of the basic, neutral, and acidic compounds was lyophilized and resuspended in one ml of water.

Chromatography: The acidic, basic, and neutral fractions were streaked separately along a one-inch line of Whatman 3 MM paper, with the exception of the aqueous sample where only 0.5 ml was applied to the paper. The chromatograms were equilibrated for 12 hours in the chromatographic chamber before being placed in the solvent of isopropanol : NH_4OH : H_2O (8 : 1 : 1, v/v) for ascending development. In addition to making these one dimensional chromatograms two dimensional chromatograms were made on all fractions except the residual aqueous solution. The developing solvent for the acidic and basic frac-

Table I

The Distribution of IAA, TTP, IAN, IAM, TNH_2, IPyA, TOH, IAAP When Partitioned According to the Described System

Compound in mixture	Fraction indole compound found			
	Acidic	Basic	Neutral	Residual aqueous
Indoleacetylaspartic acid (IAAP)*	...	...	...	+++
Indoleacetic acid (IAA)	+++	...	...	...
Indolepyruvic acid (IPyA)*	+++	...	...	...
Tryptophan (TTP)	...	...	...	+++
Tryptophol (TOH)*	...	...	+++	...
Indoleacetonitrile (IAN)	...	...	+++	...
Indoleacetamide (IAM)	...	+	++	...
Tryptamine (TNH_2)	...	+++	...	...

+++ Compound only in this fraction.
++ Majority of compound in this fraction.
+ Indicates trace amount found.
* Not partitioned as mixture, but separately.

Table II

The Effect of 5 % Hydrochloric Acid on the Destruction of the Indole Nucleus

Min	% Indole recovered
3	76.5
6	68.3
9	62.2
15	59.7
20	54.6
25	54.6
35	53.8
45	58.0
60	52.7

TABLE III

R_f VALUES OF INDOLE DERIVATIVES OBTAINED BY INCUBATION OF WATERMELON TISSUE SLICES WITH TRYPTOPHAN-2-C^{14} AND DEVELOPED ON WHATMAN 3 MM PAPER WITH ISOPROPANOL : NH_4OH : H_2O AS THE DEVELOPING SOLVENT

FRACTION OR COMPOUND **	R_f VALUES (AV) *	R_f VALUES CALCULATED FROM		COLOR WITH EHRLICH'S REAGENT	R_f VALUE OF UNKNOWN OVER-SPOTTED WITH KNOWN COMPOUND	PROBABLE COMPOUND **
		RADIOACTIVITY	COLOR			
Acidic	0.17	+	...	.....	...	IPyA
	0.38	+	...	.....	...	Unidentified
	0.49	+	+	Pink	0.49	IAA
	0.65	+	+	Pink	...	IGA †
	0.81	...	+	Pink	0.84	IAH
Basic	0.81	+	...	.....	...	Unidentified
	0.89	+	...	.....	0.85	TNH_2
Neutral	0.81	+	+	Blue	...	Unidentified
	0.96	+	...	.....	0.95	IAN
	0.99	+	+	Purple	0.99	Unidentified
Residual aqueous solution	0.07	+	...	.....	...	IAAP
	0.15	+	...	.....	...	5-OH TTP
	0.40	+	+	Purple	0.40	TTP
	0.50	+	+	Light blue	...	Unidentified
	0.71	+	+	Light blue	...	Unidentified
IAA	0.48	...	+	Pink	...	IAA
IPyA	0.17	...	+	Yellow-green	...	IPyA
IGA	0.61 (0.64) †	...	+	Pink	...	IGA
IAH	0.84	...	+	Pink	...	IAH
IAM	0.79	...	+	Purple	...	IAM
TNH_2	0.85	...	+	Blue	...	TNH_2
IAN	0.95	...	+	Purple	...	IAN
IAAP	0.073	...	+	Purple	...	IAAP
TTP	0.40	...	+	Purple	...	TTP
5-OH TTP	0.17	...	+	Purple	...	5-OH TTP

* These values represent the averages of several determinations.
** See text for abbreviations.
† R_f value taken from Fischer, Planta 43: 288–314. 1954. Identification is tentative.

tion in the first direction was isopropanol : acetic acid : H_2O (6 ml acetic acid/100 ml of 80 % isopropanol); in the second direction the above isopropanol : NH_4OH : H_2O solvent was used. The neutral fraction was developed with distilled water in the first direction, while isopropanol : NH_4OH : H_2O was used in the second direction. The R_f values obtained with the known indole compounds for both one and two dimensional chromatography are shown in tables III and IV.

RADIOACTIVITY MEASUREMENTS: In experiments with tissue incubated in the presence of TTP-2-C^{14}, the same procedure was used as for the separation of known compounds, with the exception that soluble proteins which still remained were precipitated with boiling ethanol. After drying the paper chromatograms and spraying with Ehrlich's reagent, they were strip counted on a Nuclear D-47 gas flow counter and subsequently were used for the preparation of radioautograms. Two dimensional chromatograms were not strip counted, but only subjected to radioautography.

EXPERIMENTAL RESULTS

Two types of controls were used with the incubation mixture, one was the tissue mixture without added TTP, and the second was with a heated tissue incubation mixture containing TTP. Chromatograms of extracts of the first control did not contain any detectable Ehrlich positive compounds. Strip counting or radioautograms of extracts of the heated tissue were negative except for the TTP. This shows that the tissue without added TTP or heat inactivated tissue with TTP does not produce indole compounds in amounts detectable by Ehrlich's reagent or radioactivity. In addition, the solution mixtures in the flasks after incubation were clear and the tissue was essentially the same color as it was when placed into the flasks. Since the controls did not show any of the indole compounds detected in experimental flasks, it is assumed that the conversion of TTP to IAA or its intermediates were due to the tissue per se and not due to contamination by microorganisms or to non-enzymatic conversion.

The results of these experiments using one and two dimensional chromatograms are summarized in tables III and IV while the radioautograms for one dimensional chromatograms are shown in figure 2.

ACIDIC COMPOUNDS: Five compounds were detected in the acidic fraction by strip counting, by radioautography, and by the use of Ehrlich's reagent. Two of the compounds have been tentatively identi-

fied as IAA and IPyA. The R_f values of the compounds suspected of being IAA and IPyA were calculated from the radioactive peak of the strip count and from the radioautograms. The calculated R_f values agreed with those determined with known IAA and IPyA. Additional evidence that one of the radioactive spots was IAA was obtained by overspotting an aliquot of the unknown fraction with known IAA and chromatographing. The new spot after movement, as detected by Ehrlich's reagent, coincided with the radioactivity and agreed with the R_f value found for IAA chromatographed alone. The identification of IPyA was made by comparing the R_f value of the unknown with synthetic IPyA (3). This unknown acidic compound and IPyA gave a yellow-green color with Ehrlich's reagent and showed a yellow fluorescence with a dark purple background when viewed under ultraviolet light after spraying with 1,2-diamino-4-nitrobenzene (10). This reagent is relatively specific for keto groups. The spot identified as IAA did not react with this reagent.

It has been reported that IPyA is unstable (19, 25) and more recently Bentley and co-workers reported that it is completely destroyed when chromatographed in basic solvent systems (1). The results of experiments reported here confirm these results in all respects except that our experiments show that there is not a complete destruction of IPyA chromatographed in a basic buffered solvent system. Several spots were found upon chromatographing the synthetic IPyA, but the amount of decomposition could be decreased to only three spots by chromatographing and drying in an atmosphere of nitrogen. In addition to the spot identified as IPyA, one spot was identified as IAA while a third spot which migrated below IAA was not identified. The R_f values of these compounds were 0.17, 0.45, and 0.36, respectively.

The acidic compounds moving ahead of IAA (R_f 0.65 and 0.81) are probably decomposition products of IAA. An explanation for these observations may be that IAA arises from exposure to normal room light and air during the 12-hour equilibration and 5- to 6-hour developing time since IAA is photolabile and decomposes when chromatographed on paper (15). The light mediated decomposition apparently results in the formation of IGA and IAH (4). Although our developing solvent is different with regard to the amount of NH_4OH present, the R_f values of the unknown compounds found in our experiments agree with the R_f values reported for IGA and IAH (4). Experiments have demonstrated (19) that the minimum concentration of NH_4OH in this solvent system is critical in order to obtain comparable R_f values of indole compounds. The concentration of

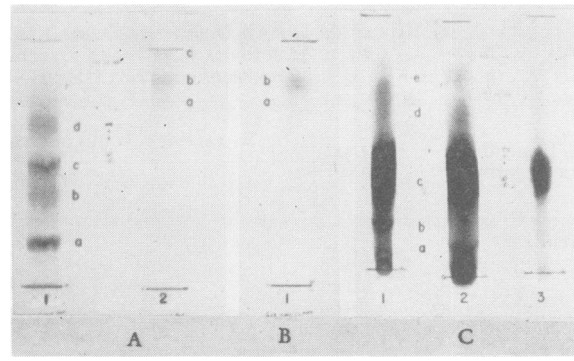

FIG. 2. Radioautograms of the acidic, neutral, basic, residual aqueous solution, and extract of brei, obtained by incubating watermelon tissue slices in tryptophan-2-C^{14} and chromatographed on Whatman 3 MM paper using isopropanol : NH_4OH : H_2O (8 : 1 : 1) as the developing solvent.

Section A-1, Acidic Fraction: spot a, indolepyruvic acid; b, unidentified; c, indoleacetic acid; d, indoleglycolic acid (tentative identification).

Section A-2, Neutral Fraction: spot a, unidentified; b, indoleacetonitrile; c, unidentified.

Section B-1, Basic Fraction: spot a, unidentified; b, tryptamine.

Section C-1, and C-2, Residual Aqueous Fraction: spot a, indoleacetylaspartic acid (see text); b, 5-OH TTP; c, tryptophan; d, unidentified; e, unidentified.

Section C-3, Extract of Brei: spot c, tryptophan.

TABLE IV

R_f VALUES OBTAINED ON TWO DIMENSIONAL CHROMATOGRAPHY OF INDOLE COMPOUNDS ON WHATMAN 3 MM PAPER

FRACTION OR COMPOUND *	R_f VALUE SOLVENT **			R_f VALUES CALCULATED FROM		PROBABLE COMPOUND
	A	B	C	RADIOACTIVITY	COLOR	
Acidic	0.76	0.18	...	+	...	IPyA
	0.67	0.39	...	+	...	Unidentified
	0.82	0.49	...	+	...	IAA
	0.84	0.68	...	+	...	IGA †
	0.78	0.80	...	+	+	IAH
Basic	0.78	0.79	...	+	...	TNH_2
	0.88	0.79	...	+	...	Unidentified
Neutral	...	0.92	0.43	+	...	IAN
	...	0.95	0	+	...	Unidentified
	...	0.93	0.54	+	...	Unidentified
IPyA	0.79	0.18	...	...	+	IPyA
	0.81	0.37	...	...	+	Unidentified
	0.87	0.50	...	...	+	IAA
IAA	0.84	0.48	...	...	+	IAA
	0.82	0.63	...	...	+	IGA †
	0.83	0.81	...	...	+	IAH
IAN	...	0.91	0.44	...	+	IAN
IAM	0.80	0.78	0.58	...	+	IAM
TNH_2	0.76	0.80	...	...	+	TNH_2
TTP	0.37	0.38	...	...	+	TTP

* For abbreviations, see text.

** Solvent A, isopropanol : HOAc : H_2O. Solvent B, isopropanol : NH_4OH : H_2O. Solvent C, distilled water.

† Identification of this compound is tentative.

NH_4OH in the solvent system of isopropanol : NH_4OH : H_2O (80 : 5 : 15, v/v) is above this critical concentration, and consequently, the R_f values obtained in the two systems are comparable. The presence of more than one spot due to ionization was eliminated by using an adequately buffered solvent system (19). These results do not agree with those of Bentley et al (1) and since our identification of IGA is indirect depending upon R_f values obtained by other workers (4), this identification can only be tentative. When high concentrations of IAA (0.1 to 0.2 mg) were chromatographed under the conditions of our experiments, these same spots are detected. The spots are not obtained when lower concentrations of IAA (0.001 to 0.01 mg) are used.

The identity of the spot with an R_f value of 0.38 which travels below IAA is unknown. It was detected by its radioactivity. It is not TTP as shown by the results of two dimensional chromatography (table IV). This evidence coupled with the fact that TTP has never been observed in the acidic fraction when using the described method of solvent partition is additional evidence that the compound is not TTP. A compound with a similar R_f value has been reported and it was observed in our experiments that synthetic IPyA decomposed to give a spot with a R_f value similar to that of the reported compound (7). The results obtained with a two dimensional chromatogram agreed with those found on a one dimensional chromatogram as shown in table IV. Recent evidence is not in agreement with this observation, and the data obtained by other workers (1) indicate that the spot arising from IPyA with R_f 0.38 is IGA.

Basic Compounds: Two compounds are chromatographically detected in the basic fraction (fig 2 B). One of these compounds has been identified as TNH_2, while the other remains unidentified. The R_f value of the unknown agrees well with known TNH_2 in one and two dimensional chromatography (tables III and IV). When the basic fraction was overspotted with known TNH_2 and chromatographed in one dimension, the known as detected with Ehrlich's reagent coincided with the radioactivity. The unidentified compound has the same R_f as IAM when developed in isopropanol : NH_4OH : H_2O, but two dimensional chromatography using isopropanol : HOAc : H_2O in addition to the basic buffered solvent shows that this compound cannot be IAM. Its identity is unknown.

Neutral Compounds: Three components were found in the neutral fraction (fig 2 A). One of the compounds was identified as IAN by one and two dimensional chromatography (tables III and IV), and overspotting with known IAN and developing in one dimension demonstrated that the spot as detected with Ehrlich's reagent coincided with the radioactivity. As in the basic fraction, one of the compounds had the same R_f as IAM when developed in a basic solvent system, but two dimensional development employing water in addition to the basic solvent system indicated that this compound was not IAM. On one dimensional chromatograms, it was not thought odd that a compound found in both basic and neutral fractions should have the same R_f value since known IAM partitioned in a similar manner.

In addition to the IAN and the unidentified spot of the neutral fraction, an Ehrlich positive radioactive spot was found which travelled with the solvent front when using isopropanol : NH_4OH : H_2O. A two dimensional chromatogram using water as the solvent in the first direction showed this compound to remain at the origin (table IV). A compound has been reported previously (4) which behaves similar to this compound, but it was not identified.

Residual Aqueous Compounds: The residual aqueous solution contained TTP, possibly indoleacetylaspartic acid (IAAP) (6), 5-OH TTP, and two other unidentified compounds (table III and fig 2 C). The unidentified compounds with R_f values of 0.50 and 0.71 colored a light blue with Ehrlich's reagent, while those with R_f values of 0.073 and 0.15 which showed no color were detected by their radioactivity. The compound identified as IAAP agrees with data obtained by other workers (6), but is contrary to their later publication (7) which stated that at pH 3.0, the IAAP was also found in the acid-ether extract. In an attempt to resolve this contradiction, an aqueous solution of known IAA and IAAP was partitioned into ether at pH 2.8. The results showed that the IAAP remains in the aqueous fraction, although if the water is not completely removed from the extracting ether with anhydrous Na_2SO_4 before paper chromatography IAAP will be found in the ether layer. These results would seem to reconcile the apparent discrepancy reported above. The identification of IAAP was based on published reports (6, 7), but recent evidence (communication, Norman Good) indicates that this compound is not a conjugate of IAA and aspartic acid, rather one of TTP and malonic acid.

The compound in the residual aqueous solution with an R_f of 0.15 has the same R_f value as known 5-OH TTP. Since this spot is ninhydrin positive, it has been tentatively identified as 5-OH TTP.

Since it is desirable to know if the aqueous extraction of the tissue was complete, the brei remaining after the described treatment was extracted with 95 % ethanol over a period of three days. Radioautograms indicated that the only radioactive compound remaining was TTP (fig 2 C).

Discussion

Numerous indole compounds have been detected by the techniques employed in the present study, but the sequence in the formation of IAA and TTP is still only speculative. The evidence presented in these experiments shows that IAA is formed from TTP and that during this biosynthesis several indole compounds are formed. Indole compounds are not detected with Ehrlich's reagent in the absence of added TTP, nor can they be detected by either radioactivity or Ehr-

lich's reagent in the presence of heat inactivated tissue. This confirms previous observations (9, 24, 25) that TTP acts as a precursor of IAA and that the process is enzymatic.

Since each fraction except the residual aqueous solution represented the same amount of tissue, the amount of radioactivity as seen by the darkening of the x-ray film should give an indication as to which compounds are most abundant in the formation of IAA, and consequently those which are involved in the biosynthetic pathway. Of all the compounds detected, IPyA contained the greatest radioactivity relative to the spot identified as IAA which showed only slightly less radioactivity. This was indicated by the relative degree of darkening of the x-ray film, and by the activity detected by strip counting. The results suggest that IPyA is probably one of the principal intermediates in the conversion of TTP to IAA and that the pathway previously postulated (21) is probably correct.

The data do not offer definite proof that the spot identified as IPyA is the principal intermediate, but all the data is certainly compatible with this view. It appears to contain the most radioactivity with a single exception. This apparent exception is found in the compound in the aqueous fraction identified as 5-OH TTP (fig 2 C, spot b). This fraction represents only one-half of the tissue, and the spot with an R_f of 0.15 appears to contain more radioactivity than that present in the IPyA of the acid fraction. The formation of a hydroxy derivative of TTP would not be inconsistent with the pathway going through either IPyA or TNH_2 as this could represent the first step in the oxidation of TTP. Since this compound does contain more radioactivity than IPyA or TNH_2 and since IPyA is more radioactive than TNH_2 it is reasonable to suspect it of being the precursor of both TNH_2 and IPyA. This finding gives additional evidence of IAA biosynthesis from TTP with IPyA being the principal intermediate.

Indolepyruvic acid chromatographed on Whatman 3 MM paper in isopropanol : NH_4OH : H_2O was detected with Ehrlich's reagent under the conditions of the present experiments. Our results confirm those of other workers (19, 25) which show that IPyA is unstable, although we find that the IPyA is not completely destroyed in basic solvent systems as reported by Bentley (1). At present the discrepancy cannot be explained unless possibly the solvent extraction removes impurities resulting in greater stability of the IPyA or there is greater stability due to chromatographing in a nitrogen atmosphere. The agreement of the R_f value obtained by color reaction and radioactivity seems to confirm the view (19) that isopropanol : NH_4OH : H_2O can be used as a developing solvent for IPyA, although an acidic buffered solvent system appears to result in greater stability of the IPyA.

It has been reported that the decomposition of IAA results in the formation of IGA and IAH (4). In our experiments TTP labeled in the 2 position of the side chain gives rise to radioactive IAA and nonradioactive IAH; and in the presence of TTP-3-C^{14}, both IAA and IAH are radioactive (unpublished, Dannenburg and Liverman). This would tend to confirm the suggestion that the TTP molecule remains intact during the conversion of TTP to IAA.

The R_f values obtained for two dimensional chromatography of one of the unknown acidic compounds agrees with those obtained for known IAA. Since the spot identified as IAA does not react with 1,2-diamino-4-nitrobenzene, a keto reagent it appears unlikely that the spot is IOA which has been reported to have the same R_f value as IAA (4).

The role of TNH_2 and IAN in the conversion of TTP to IAA is not well understood. It has been demonstrated that TNH_2 and IAN can be converted to IAA (19) and Gordon has shown that when the enzyme system capable of converting IAc to IAA is inactivated by x-irradiation that an alternate pathway does exist (8). Since both TNH_2 and IAN can apparently be formed in the presence of TTP as shown in these experiments, they may function as alternate pathways of IAA synthesis from TTP when the pathway through IAc is blocked.

The presence of a transoximase in plants has been demonstrated (26) and it is possible that IAN may be formed by the condensation of hydroxylamine with IAc forming an oxime which is then dehydrated to IAN. This seems particularly plausible in view of the recent evidence showing IAM formation in the presence of exogenous IAA (7).

Summary

1. A mixture of known indole compounds which may be intermediates in the conversion of TTP to IAA have been separated by solvent partition and paper chromatographic methods.

2. Evidence as to the conversion of labelled TTP to IAA by watermelon tissue slices has been presented with the detection of several radioactive indole compounds. Results indicate it to be a potentially useful tool in determining the biosynthetic pathway of IAA from TTP.

3. The main pathway in the conversion of TTP to IAA using watermelon tissue slices seems to be through IPyA based on the radioactivity of IPyA in relation to IAA.

4. Both TNH_2 and IAN are formed from TTP and it is possible that they function as alternate pathways for IAA formation when the pathway through IAc is blocked.

5. Indoleacetonitrile formation may be by the condensation of IAc with hydroxylamine to form an oxime with subsequent dehydration to IAN.

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Norman Good of Science Service Laboratory, London, Ontario. D,L-Tryptophan-2-C^{14} (0.00233 mc/mg) obtained from Tracerlab, Inc., Boston, Massachusetts.

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SEDOHEPTULOSE IN COLEUS[1]

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Large amounts of the seven-carbon sugar, sedoheptulose, are found in Crassulacean plants, particularly in Sedum (8, 9). A role for sedoheptulose phosphate has been demonstrated in the photosynthetic carbon cycle by Benson et al (2) and in the pentose phosphate cycle by Horecker and Mehler (5). A function for the free sugar remains obscure (11, 12). This paper presents data indicating that varieties of Coleus contain large amounts of free sedoheptulose and sedoheptulose phosphate, comparable to the amount in Sedum. In Coleus, the reservoir of sedoheptulose, to-

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