

STABILITY OF MICROBIAL-PRODUCED AUXINS DERIVED FROM L-TRYPTOPHAN ADDED TO SOIL

D. A. MARTENS AND W. T. FRANKENBERGER, JR.¹

Soil microorganisms are capable of producing secondary metabolites such as auxins upon the addition of tryptophan (L-TRP) that may significantly influence plant growth and development. This study was conducted to determine the stability and availability of indole-3-acetic acid (IAA) and proposed intermediates in the production of auxins from the addition of L-TRP to soil. L-TRP metabolism was not observed with the addition of L-TRP to a steam-sterilized soil when incubated for up to 7 days, indicating a biotic mechanism in the production of soil auxins. Incubation of 3'-¹⁴C-L-TRP in non-sterile soil resulted in the conversion of the L-TRP label into indole-3-acetamide, indole-3-lactic acid, indole-3-acetic acid, indole-3-ethanol, and indole-3-aldehyde by the soil microbiota. The production of indole derivatives was dependent on the amount of L-TRP added to the soil. Adsorption-desorption isotherms showed a low affinity of auxin derivatives (5-hydroxy-indole-3-acetic acid, indole-3-acetamide, indole-3-lactic acid, indole-3-acetic acid, indole-3-ethanol, and indole-3-aldehyde) for the soil colloids. The persistence of L-TRP in five soils, measured in half-life ($t_{1/2}$), ranged from 22.8 to 28.7 h. The $t_{1/2}$ measured for the intermediates of auxin production indicated that several auxin derivatives were stable in soil and may have a greater effect on plant growth and yield when compared with auxins of lower soil stability.

Soils are known to contain compounds that exhibit strong auxin-like activity (Sheldrake 1971; Whitehead 1963). Indole-3-acetic acid (IAA) is considered to be one of the major auxin-like products of the soil microflora. Previous studies have revealed that soil fertility status and organic matter content may regulate the microbial formation of IAA (Chandramohan and Mahadevan 1968; Hamence 1946; Stewart and Anderson 1942). Also, higher auxin produc-

tion has been reported in nutrient-rich rhizosphere soils compared with root-free soils (Narayananaswami and Veerajaru 1969). Production of microbial auxin-like compounds in soil is often linked directly to substrate availability (Arshad and Frankenberger 1990; Lynch 1985). L-Tryptophan (L-TRP) has been reported to serve as an active physiological precursor for the microbial formation of IAA in soil (Purushothaman et al. 1973, 1974; Frankenberger and Brunner 1983; Frankenberger and Poth 1987a and b). The importance of understanding the conversion of L-TRP in soil has been demonstrated by Frankenberger and his co-workers who have reported that the addition of L-TRP as a soil drench to developing plant seedlings may result in increased plant yield (Frankenberger et al. 1990; Frankenberger and Arshad 1991a and b).

The pathway of IAA formation in soils has not been conclusively demonstrated. Previous work has suggested the presence of several pathways of IAA formation in soil and pure cultures. Frankenberger and Poth (1988) isolated a rhizosphere bacterium from *Festuca octoflora* that produced an aminotransferase that converted L-TRP into indole-3-pyruvic acid (IPyA). IPyA is further oxidized into indole-3-acetaldehyde (IAAID) and then converted into IAA. A second identified pathway involves the transformation of L-TRP into indole-3-acetamide (IAM) by a monooxygenase reaction and then conversion to IAAID by a hydrolase with subsequent oxidation into IAA (Frankenberger and Brunner 1983). This pathway is the route of IAA formation by the phytopathogenic bacteria, *Agrobacterium tumefaciens* and *Pseudomonas syringae* pv. *savastanoi*, which are responsible for crown gall disease in plants (Magie et al. 1963). In addition to soil microorganisms, Chalvignac and Mayaudon (1971) extracted an extracellular enzyme complex from soil that converted L-TRP into IAM and IAA.

A third pathway utilized by soil microorganisms involves the conversion of L-TRP to tryptamine (TAM) by a TRP decarboxylase which is then converted to IAAID by a monoamine oxidase reaction (Hartmann et al. 1983). Al-

¹ Dept. of Soil and Environmental Science, University of California, Riverside, CA 92521.

Received March 12, 1992; accepted Aug. 10, 1992.

though TAM is commonly found in plant tissues, its importance as a microbial intermediate of IAA formation is not known. It is likely that in some soils, different pathways may be utilized in the conversion of L-TRP to IAA. Frankenberg and Brunner (1983) found evidence supporting the first two previously described pathways of L-TRP conversion to IAA occurring in a Tollhouse soil. Martens and Frankenberg (1991) also reported that a soil-derived *Pseudomonas* sp. produced IAA, IAM, and IPyA when incubated in an L-TRP-enriched medium.

Although L-TRP is believed to be the primary precursor of IAA in plants and microorganisms, the stability of applied L-TRP and the derived intermediates of auxin transformations in soil are not well understood. Since the production of auxins is regulated by the availability of L-TRP, factors such as adsorption of L-TRP or the auxin intermediates with soil constituents will significantly influence the supply of exogenous sources of auxins available for plant uptake (Müller et al. 1989).

The objectives of this study were to identify auxin intermediates produced during L-TRP metabolism in various California soils and to determine their stability based upon calculated half-life ($t_{1/2}$) and soil adsorption (K_d values) data which would influence the availability of these auxins for plant assimilation and use.

MATERIALS AND METHODS

Reagents

The auxins were obtained from Sigma Chemical Co. (St. Louis, MO) except for 3-indoleacetyl-aspartic acid and 3-indoleacetyl-glycine which were obtained from Research Organics (Cleveland, OH). Labeled TRP [L-(3'-¹⁴C)tryptophan] was obtained from Amersham Corporation (Arlington Heights, IL).

Procedure

Surface samples of five California soils (0–25 cm) were selected to obtain a diverse range in chemical and physical properties (Table 1) and maintained in a moist condition (–33 kPa). The soil characterization methods are described by Martens and Frankenberg (1991). The production of soil auxins upon addition of L-TRP or auxin intermediates to sterile and non-sterile soils was monitored as follows: L-TRP or an auxin derivative (560 µg) in 1 ml H₂O was added to 2.5 g of soil in a 50-ml Erlenmeyer flask and incubated at 30°C for various times. Stock solutions of the auxin derivatives were made by dissolving the compound in 45% methanol, and an aliquot (110 µl) was added to 0.89 µl of H₂O and then applied to the soil sample. Because of the insolubility of IPyA in water, this compound was added directly to the soil, and the soil moisture was adjusted with 1 ml of water; or 110 µl of IPyA (560 µg) in 100% methanol was added to 0.89 ml of water and then added to the soil. The L-TRP remaining after incubation and the auxin derivatives were extracted with 4 ml 0.1 M KH₂PO₄ (pH 7.0) and shaken on a rotary shaker (200 rev min⁻¹; 4°C; 10 min), and an aliquot was filtered through a 0.22 µm Millipore GS filter (Bedford, MA).

In place of liquid-liquid partitioning of the soil auxins with ethyl acetate, an on-line HPLC solid phase extraction system was employed as described by Martens and Frankenberg (1991). Briefly this involves adding a calibrated aliquot (5–40 µl) of the filtered soil extract to 0.4 ml of water and injection onto a 5-µm O.D.S. guard column (30 × 4.6 mm). Rinses with water and mobile phase removed ionic interferences, and the auxins were eluted with the mobile phase onto a separator column (R-Sil C₁₈) for subsequent UV detection (280 nm). The auxins

TABLE 1
Properties of soils used

Soil	pH	Organic C	Total N	Clay	Sand	CEC ^a
				g kg ⁻¹		
Sheephead	6.87	7.9	2.0	260	560	13.9
Altamont	6.45	12.3	2.6	460	210	26.8
Domino	8.55	7.4	1.1	290	430	16.8
Redding	6.56	2.0	1.2	640	250	25.4
Hesperia	7.22	8.6	2.9	180	740	6.6

^a CEC, cation exchange capacity (cm⁺ kg⁻¹ soil).

were identified by co-chromatography and UV spectral confirmation with authentic standards. Under sterile conditions, the soils were steam sterilized (121°C; 0.104 MPa; 2 h), and the L-TRP solutions were filter-sterilized (0.22 μm filter).

The conversion of [$3\text{'-}^{14}\text{C}$]-L-TRP (specific activity 53.5 mCi mM^{-1}) into soil auxins was determined by treating 2.5 g of soil with 500 μg L-TRP plus 1.85 kBq of [$3\text{'-}^{14}\text{C}$]-L-TRP. The treated soil sample was incubated for 48 h at 30°C. Labeled auxins were separated by HPLC as described above, and 0.25-ml fractions were collected with an ISCO Retriever II fraction collector (Lincoln, NE) in 4.5-ml scintillation cocktail (Complete Counting Cocktail, Research Products, Mt. Prospect, IL). Radioactivity in each of the fractions was determined on a Beckman Model 5000 TD liquid scintillation counter (Beckman Instruments, Inc., Fullerton, CA).

Adsorption or partition isotherms of auxins were constructed for five soils. The auxins were applied in 1 ml of water at 5, 15, 25, 45, and 60 $\mu\text{g g}^{-1}$ soil in 50 ml Erlenmeyer flasks, allowed to equilibrate for 10 min, and then equilibrated with the addition of 4 ml of phosphate extraction solution (0.1 M KH_2PO_4 , pH 7.0). The flask was shaken on a rotary shaker as previously described (10 min). Longer equilibration times were not used to limit microbial degradation of the auxins in soil. An aliquot was filtered through a 0.22- μm Millipore GS filter, and the auxins remaining in the soil solution were analyzed by the IS-HPLC method described. The quantities sorbed by the soil were determined by an indirect sorption method with the difference between the amounts added and that in soil solution assumed to be the amount sorbed.

Soil respiration (carbon dioxide evolution) upon addition of L-TRP or auxin derivatives (0 or 2500 μg) was monitored by incubating 10-g soil samples with 1 ml H_2O for 0, 1, 2, or 5 days at 30°C in a 125-ml Erlenmeyer screw-cap flask equipped with a Mininert® gas-sampling valve (Dynatech, Baton Rouge, LA). A 1-ml headspace sample was separated on a Porapak Q column (Alltech Assoc., Inc., Deerfield, IL). The constituent peaks were detected by thermal conductivity on a gas chromatograph (Varian Associates, Inc., Model 3700) with a He flow rate of 30 ml min^{-1} , a column temperature of 70°C, an injector temperature of 70°C, and a detector temperature of 110°C. Constituent peaks (CO_2)

were confirmed through use of authentic external standards.

Correlation coefficients were determined in accordance with the SAS procedure (SAS Institute, Inc. 1985).

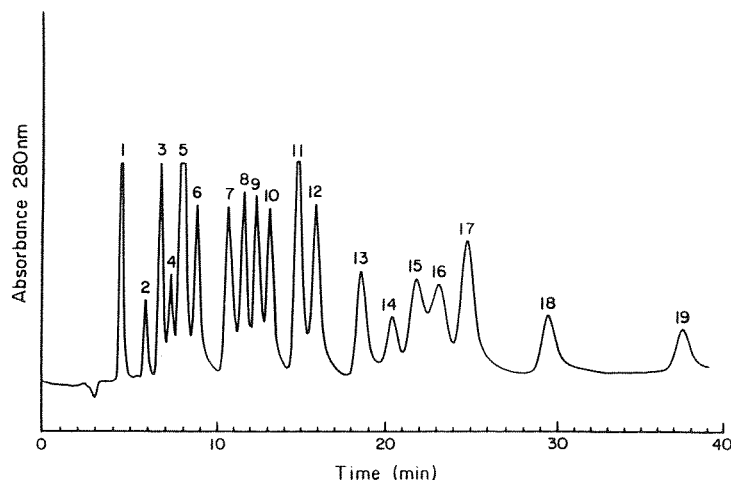
RESULTS AND DISCUSSION

The necessity for liquid-liquid partitioning with ethyl acetate to extract and purify the auxins produced in soil and the complications involved with this extraction procedure have limited research investigating the pathways and soil factors influencing microbial formation of IAA from L-TRP. To eliminate the need for liquid-liquid partitioning, Martens and Frankenberger (1991) developed an on-line, solid phase partitioning IS-HPLC method for the determination of the substrate (L-TRP), acidic, neutral, and basic auxins, and auxin conjugates in soil extracts and bacteria broths. The potential of this on-line extraction methodology for purification of 18 standard auxin derivatives and the substrate, L-TRP, is shown in Fig. 1.

Soil bacteria have been isolated that produce IAA in L-TRP amended minimal media (Martens and Frankenberger 1991; Müller et al. 1989; Frankenberger and Poth 1987b; Erntsen et al. 1987; Prikryl et al. 1985; Hartmann et al. 1983). In addition, a soil enzyme complex has been extracted from soil which converts L-TRP to IAA (Chalvignac and Mayandon 1971). To determine if this transformation is a biotic reaction, a steam-sterilized soil (Hesperia soil) was monitored for auxin formation. Although IAA was not detected, low levels of 5-hydroxyindole-3-acetic acid (5-OH-IAA) and 5-hydroxytryptophan (5-OH-TRP) were found upon the amendment of L-TRP, suggesting a nonbiological hydroxylation reaction of L-TRP occurring in soil (Fig. 2).

The chemical and physical properties of the five soils used in this study are shown in Table 1. The low molar phosphate extraction solution (0.1 M) utilized in this investigation accounts only for auxin products released into the soil environment by the soil microbiota and not for the intracellular intermediates of L-TRP metabolism. When L-TRP was added to soils at a rate of 80, 160, 320, 640, and 1280 $\mu\text{g L-TRP g}^{-1}$ soil, the two lowest rates resulted in rapid metabolism of the applied L-TRP and very low levels of IAA production ($<3 \mu\text{g IAA g}^{-1}$ soil) (Table 2). Higher levels of L-TRP addition resulted in

FIG. 1. IS-HPLC chromatogram of 18 auxin derivatives and tryptophan. 1 = 5-OH-IAA; 2 = IAA-Asp; 3 = IAA-Gln; 4 = 5-OH-TRP; 5 = IAM; 6 = ILA; 7 = 5-OH-TAM; 8 = IAA; 9 = IAAID; 10 = TOL; 11 = IAID; 12 = IAN; 13 = IPA; 14 = IPyA; 15 = TRP; 16 = IM; 17 = IAcry; 18 = IBA; 19 = TAM.



a greater rate of IAA production in as little as 1 day of incubation (Table 2). The formation of IAA in soil is highly dependent on the availability of L-TRP for the soil microbiota (Arshad and Frankenberger 1990; Lynch 1985). Factors such as adsorption (partitioning of L-TRP) and competing metabolic pathways for L-TRP will limit the availability of this substrate for IAA production. The formation of IAA from L-TRP (average of five soils) followed first order kinetics ($R^2 = 0.99$).

The pathway of IAA formation and the importance of auxin intermediates in soil has not been conclusively shown. To establish which auxin intermediates are present in soils, $3'$ - ^{14}C -L-TRP (1.85 kBq) was incubated with 200 μg

L-TRP g^{-1} soil. The extracts were separated by HPLC, fractionated and the disintegrations per minute (dpm) in the collected fractions were compared with resulting UV chromatograms. The peaks of activity aligned with the retention times for 5-OH-IAA, 5-OH-TRP, 5-OH-TAM, IAM, ILA, TOL, IAID, and IAA. Ernsten et al. (1987) reported that pure cultures of *Rhizobium phaseoli* synthesized labeled IAA, TOL, and indole-3-methanol when ^3H -, ^{14}C - and ^2H -labeled L-TRP was used as a substrate. The detection of elevated levels of 5-OH indole compounds indicates the presence of a tryptophan-5-hydroxylase in soil (Joseph 1989). This pathway is important for the synthesis of the biogenic amine, serotonin (5-OH-TAM) in mammals, but its importance in the metabolism of L-TRP in soil is not known.

The influence of auxins on plant growth and development may be affected by the availability of these applied auxins in soil. The adsorption of nonionic organic compounds by soil from aqueous systems is controlled mainly by the organic matter content of the soil (Chiou 1989). In previous work, we have noted that the application of L-TRP to a sterilized standard U.C. mix (33% sand; 33% peat; 33% soil) did not result in increased plant growth or yield promotion. The high percentage of peat in this mixture may have partitioned much of the L-TRP or auxin derivatives out of the soil solution, making it unavailable for plant uptake. The extent of auxin partitioning from the soil solution was measured by use of adsorption-desorption isotherms of five soils for six auxin com-

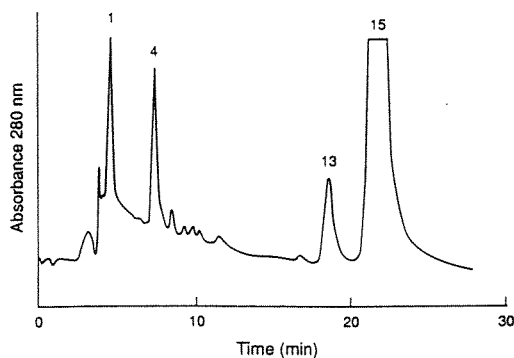


FIG. 2. IS-HPLC chromatogram of an extract of a steam sterilized soil incubated for 7 days after addition of filter sterile L-TRP. 1 = 5-OH-IAA; 4 = 5-OH-TRP; 13 = TPA (internal standard); 15 = L-TRP.

TABLE 2
IAA production in soil upon L-TRP addition^a

L-TRP addition	Soil					Average
	Sheephead	Altamont	Domino	Redding	Hesperia	
$\mu\text{g g}^{-1}$ soil						
0	ND ^b	ND	ND	ND	ND	ND
80	ND	ND	1.5	ND	1.1	0.5
160	2.9	1.8	6.2	ND	3.5	2.9
225	8.3	5.6	12.3	0.8	12.1	7.8
320	12.2	6.2	18.3	2.3	14.6	10.7
640	21.0	24.3	38.6	12.2	48.3	29.9
1280	72.0	100.8	129.6	42.6	138.3	96.7

^a Soil samples (2.5 g) were treated with the specified level of L-TRP and incubated at 30°C for 5 days.

^b ND, not detected.

pounds. The adsorption-desorption of many organic compounds applied at low concentrations is frequently represented by a linear adsorption isotherm. The results showed a linear relationship ($R^2 \geq 0.98$) between the equilibrium concentrations of the indole derivatives applied and the amounts not recovered. The K_d values were calculated from a modified Freundlich equation of $S = K_d C$, where S = amount sorbed ($\mu\text{g g}^{-1}$ soil) and C = equilibrium concentration ($\mu\text{g mL}^{-1}$). K_{oc} values were calculated as the ratio of the calculated K_d values to the organic C content. The resulting slopes or K_d and the K_{oc} values for partitioning of the auxin derivatives are given in Table 3. The measured K_d values suggest limited soil adsorption and are comparable to K_d values reported for ionic carboxylic herbicides such as chloramben and picloram, with little to no adsorption to soil colloids (Hamaker and Tompson 1972). The measured partitioning or K_d values of the aux-

ins, 5-OH-IAA, IAM, IAA, ILA, TOL, and IAID were not significantly correlated with the organic C content of the soils used in this study. This may be due in part to the short equilibration times used in establishing the adsorption isotherms which may not have allowed all of the soils to approach the same level of equilibrium with the auxins. However, longer equilibration times would have introduced errors due to auxin transformations by the soil microbiota. Also, the lack of significant correlation with the organic C content of these soils may be due to a limited range of organic C content in the soils used or due to the ionic nature of the auxin derivatives at the measured soil pH values (Table 1) limiting partition uptake with the soil organic C. The low partitioning coefficients suggest that the auxin derivatives are not adsorbed to a great extent by soil organic matter.

Carbon dioxide evolution studies indicated that most (avg. 80%) of the applied L-TRP-C

TABLE 3
The distribution coefficient, K_d , and sorption coefficient, K_{oc} , of five California soils treated with auxin derivatives^a

Auxin	Domino		Sheephead		Altamont		Redding		Hesperia	
	K_d	K_{oc}	K_d	K_{oc}	K_d	K_{oc}	K_d	K_{oc}	K_d	K_{oc}
5-OH-indole-3-acetic acid	0.20	27	0.40	50	0.35	28	0.43	215	0.87	101
Indole-3-acetamide	0.14	19	0.25	32	0.50	40	0.38	190	0.37	43
Indole-3-lactic acid	0.20	27	0.33	42	0.50	41	0.32	160	0.47	55
Indole-3-acetic acid	0.28	37	0.36	46	0.48	39	0.43	215	0.50	58
Indole-3-ethanol	0.27	36	0.34	43	0.30	24	0.35	175	0.61	71
Indole-3-aldehyde	0.35	47	0.60	76	0.63	51	0.48	240	0.56	65

^a 2.5 g of field-moist soil were treated with 1 ml water containing 5–60 μg of auxin derivative g^{-1} soil, allowed to react for 10 min and then equilibrated with 4 ml 0.1 M KH_2PO_4 (pH 7.0) for 10 min, filtered and analyzed by IS-HPLC.

was mineralized into CO₂-C after 5 days of incubation (Table 4). Mineralization of the auxin derivatives in soil resulted in a more rapid breakdown in the Altamont soil when compared with the other four soils. Considerably less CO₂-C was evolved upon mineralization of TOL (avg. 29%), IAA (avg. 40%), IPyA (avg. 45%), and IAM (avg. 43%) after 5 days of incubation (Table 4). IS-HPLC analysis indicated that the L-TRP and auxin derivatives not recovered in soils were accounted for by CO₂-C analyzed in the flask headspace.

The persistence of auxins produced in soil upon L-TRP applications will no doubt influence the extent to which auxin derivatives could possibly affect plant growth and development. Auxins and L-TRP recovery studies were conducted from 0 to 7 days. Decomposition of L-TRP and auxin derivatives in soil followed first-order kinetics. The measured half-lives ranged from an average of 25 h for L-TRP to over 127

h for IAM with the five soils tested (Table 5). IPyA and IAAID were also evaluated for their persistence, but neither was extracted from soils. IPyA and IAAID have been reported to be very unstable indole compounds (Frankenberger and Poth 1987b). Frankenberger et al. (1990) reported that IPyA was less effective than IAA, IAM, and ILA additions in stimulating the growth of *Raphanus sativus* (radish). The diminished growth promotion effectiveness of IPyA may be related to the instability of this compound. IAM addition was found to be the most effective among the auxin derivatives tested in growth promotion of radish, and the effectiveness of IAM may be related to its stability in soil.

The production of IAA from the auxin intermediates, ILA and TOL, resulted in an average (five soils) of 1% and <1% conversion to IAA, respectively, after 5 days of incubation at 30°C compared with a 5% rate for L-TRP (Figs. 3a and b and 4a). Figure 3b also shows the detection of L-TRP upon addition of ILA to soil suggesting a reversible reaction of the IPyA pathway. However, the addition of the proposed direct precursor, IAAID, resulted in little or no IAA formation in the five soils tested (Fig. 4b). The addition of IAM or TAM resulted in larger percentages of conversion to IAA (20 and 18%, respectively) (Figs. 4c and 5). The longer stability and relatively high percentage of substrate conversion into IAA suggests that IAM and TAM may hold promise as a soil additive in affecting plant growth and yield.

In summary, the formation of IAA from L-TRP was a biological transformation and dependent on the level of substrate available for microbial conversion. L-TRP and the interme-

TABLE 4
Recovery of CO₂-C from five soils after treatment with 225 µg of L-TRP or auxin derivative g⁻¹ soil after 5 days of incubation

Soil	Compound				
	L-TRP	TOL	IAA	IPyA	IAM
	CO ₂ -C recovery (%)*				
Sheephead	80	33	51	73	40
Altamont	85	83	84	85	86
Domino	86	<1	10	13	18
Redding	78	8	21	18	26
Hesperia	72	20	35	37	43
Average	80	29	40	45	43

* Values are corrected for CO₂-C evolved from the control soil (no compound added).

TABLE 5
Half-life (t_{1/2}) of L-TRP, IAA, and intermediates of IAA formation in soil^a

Compound	Soil					Average
	Altamont	Domino	Redding	Sheephead	Hesperia	
	t _{1/2} (h)					
Tryptophan	23.7	24.9	28.7	22.8	24.8	25.0
Indole-3-acetic acid	34.4	58.7	45.6	30.7	19.5	37.8
Indole-3-acetamide	47.7	292.4	121.8	114.6	60.9	127.5
Indole-3-ethanol	51.6	147.6	135.8	53.6	39.2	85.6
Indole-3-lactic acid	50.9	59.0	65.7	54.0	36.1	53.1
Tryptamine	40.8	230.6	110.4	105.7	57.3	109.0

^a Soil samples (2.5 g) were treated with 225 µg compound g⁻¹ soil and incubated at 30°C for 0, 1, 3, 5 and 7 days.

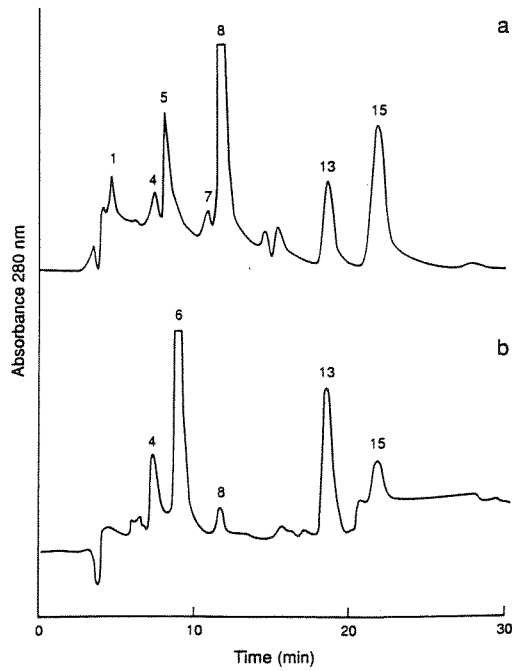


FIG. 3. IS-HPLC chromatogram of an extract of a) an Hesperia soil incubated for 2 days after treatment with L-TRP and b) a Domino soil incubated for 5 days after treatment with ILA. 1 = 5-OH-IAA; 4 = 5-OH-TRP; 5 = IAM; 6 = ILA; 7 = 5-OH-TAM; 8 = IAA; 13 = IPA (internal standard); 15 = TRP.

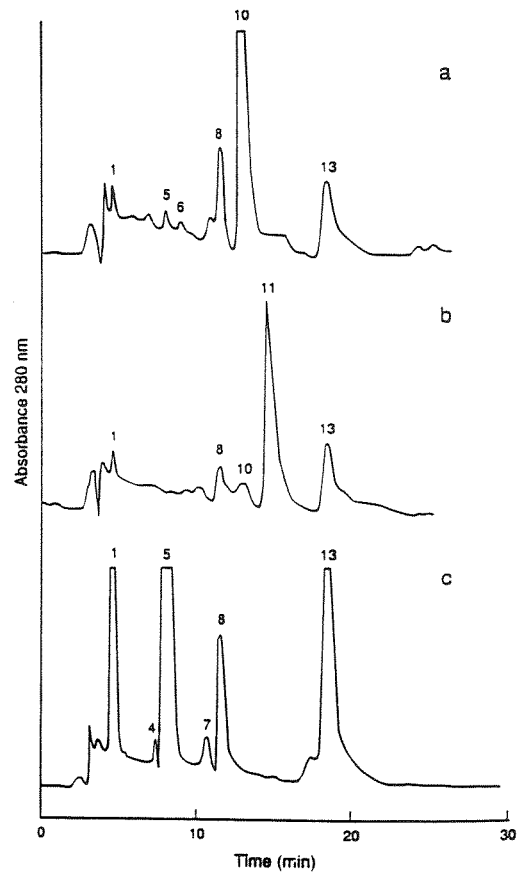
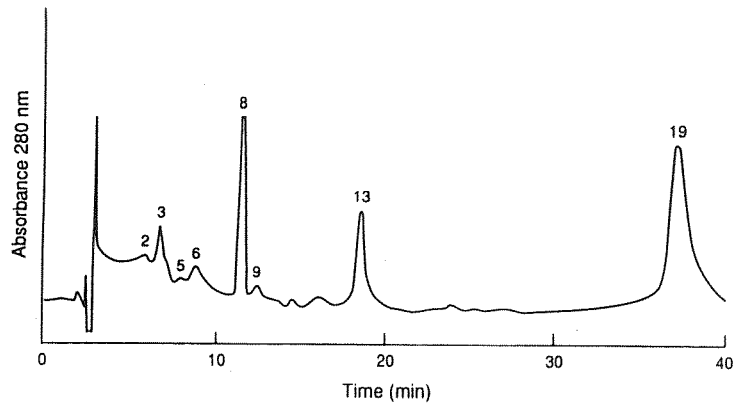


FIG. 4. IS-HPLC chromatogram of an extract of a) an Altamont soil incubated for 5 days after treatment with TOL, b) an Hesperia soil incubated for 5 days after treatment with IAAID; and c) a Domino soil incubated for 5 days after treatment with IAM. 1 = 5-OH-IAA; 4 = 5-OH-TRP; 5 = IAM; 6 = ILA; 7 = 5-OH-TAM; 8 = IAA; 10 = TOL; 11 = IAAID; 13 = IPA (internal standard).

FIG. 5. IS-HPLC chromatogram of an extract of a Redding soil incubated for 5 days after addition of TAM. 2 = IAA-Asp; 3 = IAA-Gln; 5 = IAM; 6 = ILA; 8 = IAA; 9 = IAAID; 13 = IPA (internal standard); 19 = TAM.



diates of L-TRP metabolism to IAA were not readily adsorbed to the soil organic matter. IAM and TAM show promise as stable auxin compounds for promotion of plant growth and yield.

ABBREVIATIONS USED

IAA	indole 3-acetic acid
IS-HPLC	ion suppression reverse phase high performance liquid chromatography
TAM	tryptamine
IAAID	indole-3-acetaldehyde
IAM	indole-3-acetamide
IAN	indole-3-acetonitrile
IAcry	3- β -indoleacrylic acid
IAID	indole-3-aldehyde
IBA	indole-3-butyric acid
TOL	indole-3-ethanol
ILA	indole-3-lactic acid
IM	indole-3-methanol
IPA	indole-3-propionic acid
IPyA	indole-3-pyruvic acid
IAA-Asp	3-indoleacetyl-aspartic acid
IAA-Gln	3-indoleacetyl-glycine
5-OH-IAA	5-hydroxyindole-3-acetic acid
5-OH-TAM	5-hydroxytryptamine
5-OH-TRP	5-hydroxytryptophan
TRP	tryptophan

REFERENCES

- Arshad, M., and W. T. Frankenberger, Jr. 1990. Microbial production of plant hormones. *Plant Soil* 133:1-8.
- Chalvignac, M. A., and J. Mayaudon. 1971. Extraction and study of soil enzymes metabolizing tryptophan. *Plant Soil* 34:25-31.
- Chandramohan, D., and J. Mahadevan. 1968. Indole acetic acid metabolism in soil. *Curr. Sci.* 37:112-113.
- Chiou, C. T. 1989. Theoretical considerations of the partition uptake of nonionic organic compounds by soil organic matter. In *Reactions and movements of organic chemicals in soils*. B. L. Sawhney and K. Brown (eds.). American Society of Agronomy, Madison, WI, pp. 1-3.
- Ernsten, A., G. Sandberg, A. Crozier, and C. T. Wheeler. 1987. Endogenous indoles and the biosynthesis and metabolism of indole-3-acetic acid in cultures of *Rhizobium phaseoli*. *Planta* 171:422-428.
- Frankenberger, W. T., Jr., and W. Brunner. 1983. Method of detection of auxin-indole-3-acetic acid in soils by high performance liquid chromatography. *Soil Sci. Soc. Am. J.* 47:237-241.
- Frankenberger, W. T., Jr., and M. Poth. 1987a. Biosynthesis of indole-3-acetic acid by the pine ectomycorrhizal fungus, *Pisolithus tinctorius*. *Appl. Environ. Microbiol.* 53:2908-2913.
- Frankenberger, W. T., Jr., and M. Poth. 1987b. Determination of substituted indole derivatives by ion suppression-reverse phase high performance liquid chromatography. *Anal. Biochem.* 165:300-308.
- Frankenberger, W. T., Jr., and M. Poth. 1988. L-Tryptophan transaminase of a bacterium isolated from the rhizosphere of *Festuca octoflora* (Graminae). *Soil Biol Biochem.* 20:299-304.
- Frankenberger, W. T., Jr., A. C. Chang, and M. Arshad. 1990. Response of *Raphanus sativus* to the auxin precursor, L-tryptophan applied to soil. *Plant Soil* 129:235-241.
- Frankenberger, W. T., Jr., and M. Arshad. 1991a. Yield response of watermelon and muskmelon to L-tryptophan applied to soil. *HortScience* 26:35-37.
- Frankenberger, W. T., Jr., and M. Arshad. 1991b. Yield response of *Capsicum annuum* to the auxin precursor, L-tryptophan applied to soil. *Plant Growth Regul. Soc. Am. Quart.* 19:231-240.
- Hamaker, J. W., and J. M. Tompson. 1972. Adsorption. In *Organic chemicals in the soil environments*. C. A. Goring and J. W. Hamaker (eds.). Marcel Dekker, Inc., New York, pp. 49-144.
- Hamence, J. H. 1946. The determination of auxins in soil including a note on synthetic substances. *Analyst* 71:111-116.
- Hartmann, A., M. Singh, and W. Klingmüller. 1983. Isolation and characterization of *Azospirillum* mutants excreting high amounts of indoleacetic acid. *Can. J. Microbiol.* 29:916-923.
- Joseph, M. H. 1989. The analysis of kynurenines and the biochemical pharmacology of kynurenines in the CNS. In *Quinolinic acid and the kynurenines*. T. W. Stone (ed.). CRC Press, Inc., Boca Raton, FL, pp. 39-52.
- Lynch, J. M. 1985. Origin, nature and biological activity of aliphatic substances and growth hormones found in soil. In *Soil organic matter and biological activity*. D. Vaughan and R. E. Malcolm (eds.). Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, The Netherlands, pp. 151-174.
- Magie, A. R., E. E. Wilson, and T. Kosuge. 1963. Indole-acetamide as an intermediate in the synthesis of indole acetic acid in *Pseudomonas savatanoi*. *Science* 141:1281-1282.
- Martens, D. A., and W. T. Frankenberger, Jr. 1991. On-line solid phase extraction of soil auxins produced from exogenously applied tryptophan with ion-suppression reverse-phase HPLC analysis. *Chromatographia* 32:417-422.
- Müller, M., C. Deigele, and H. Ziegler. 1989. Hormonal interactions in the rhizosphere of maize (*Zea mays* L.) and their effects on plant development. *Z. Pflanzenernähr. Bodenkd.* 152:247-254.
- Narayanaswami, R., and V. Veerajulu. 1969. IAA synthesis in paddy soils as influenced by ammonium sulfate fertilization. *Curr. Sci.* 38:517-518.
- Přikryl, Z., V. Vancura, and M. Wurst. 1985. Auxin formation by rhizosphere bacteria as a factor of root growth. *Biol. Plant.* 27:159-163.
- Purushothaman, D., K. Balaraman, and G. Oblisami. 1973. Indole acetic acid metabolism in soil as

- influenced by pesticide application. *Curr. Sci.* 42:365-366.
- Purushothaman, D., T. Marimuthu, C. V. Venkatar-
amahan, and R. Kesavan. 1974. Role of actino-
mycetes in the biosynthesis of indole acetic acid
in soil. *Curr. Sci.* 43:413-414.
- SAS Institute, Inc. 1985. *SAS Users' Guide: Statistics*,
Version 5 Edition. SAS Institute, Inc., Cary, NC.
- Sheldrake, A. R. 1971. The occurrence and signifi-
cance of auxin in the substrate of bryophytes.
New Phytol. 70:519-526.
- Stewart, W. S., and M. S. Anderson. 1942. Auxins in
some American soils. *Bot. Gaz.* 103:570-575.
- Whitehead, D. C. 1963. Some aspects of the influence
of organic matter on soil fertility. *Soil Fertil.*
26:217-223.