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INDOLE-3-ACETYL-*MYO*-INOSITOL IN KERNELS OF *ORYZA SATIVA*

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Key Word Index—*Oryza sativa*; Gramineae; rice; seed; IAA-ester; IAA-*myo*-inositol; GC–MS.

Abstract—IAA-*myo*-inositol was isolated from kernels of *Oryza sativa* and characterized by its chromatographic properties and its mass spectral fragmentation pattern. This is the first demonstration of the occurrence of a *myo*-inositol ester of IAA in a plant other than *Zea mays*.

INTRODUCTION

Kernels of rice (*Oryza sativa* L.) contain *ca* 4.5 mg/kg of IAA, 30% occurring as the free acid and the remaining 70% as esters [1–3]. Measurements, by bioassay, of the amount of IAA in *O. sativa* have been reported by others [4, 5]. The present work constitutes the first attempt to chemically characterize the IAA esters of rice. We wish to report that *ca* 10% of the IAA ester fraction consists of esters of IAA and *myo*-inositol. This finding is significant since IAA-*myo*-inositol has hitherto been found only in the taxonomically, distantly-related *Zea mays* and its close relatives. Thus, possibly there is some unique biological significance to *myo*-inositol as an esterifying alcohol for IAA.

RESULTS AND DISCUSSION

IAA-*myo*-inositol was purified from acetone extracts of *O. sativa* kernels by chromatography over Dowex-50, Beckman PA 28 and Sephadex LH-20. The product was characterized by Si gel TLC, from its ammonolysis products and by GC and MS comparison with the authentic compound.

The ester from *O. sativa* migrates with the same R_f as authentic IAA-*myo*-inositol on TLC. Owing to acyl migration, the isolated ester consists of a mixture of the four possible, chemically distinguishable isomers [6]. Since TLC resolves the axial 2-*O* ester from the equatorial esters, a characteristic two-spot migration pattern is observed upon chromatography in solvent A. R_f values of 0.34 and 0.39 are observed for the equatorial and axial esters respectively [6].

Temperature program and isothermal GLC of the TMSi derivative of the ester gives peaks at RR_t values identical to those of authentic hexakis-TMS-IAA-*myo*-inositol.

Ammonolysis of an IAA ester yields the free acid, the amide and the alcohol moiety. Two Ehmann-positive products with R_fs corresponding to those of IAA and indoleacetamide are observed on TLC. GLC of the silylated products gives peaks with RR_ts corresponding to the TMSi derivatives of IAA, indoleacetamide and *myo*-inositol (Table 1).

Final characterization of the rice product was accomplished using GC–MS. Hexakis-TMSi-IAA-*myo*-inositol and the ester from *O. sativa* both have an M^+ at *m/e* 769. Fragment ions characteristic of the inositol moiety are seen at *m/e* 507 ($M^+ - R - 15$) and 433 ($M^+ - 90 - 70 - R$) while ions characteristic of the indole ring occur at *m/e* 229 (which is base peak and consists of ($M^+ - I$), 202 ($M^+ - I - 44$) and 130 ($M^+ - I - 72 - 44$) (Table 2).

Mechanisms that account for the major fragment ions from the two isomers have already been discussed [7]. MS reported here indicate that both axial and equatorial esters are present in purified extracts of *O. sativa*.

Table 1. GC retention times of TMSi derivatives of the products of ammonolysis of some indolylic compounds

Compound	RR_t			
Indoleacetamide*†	0.9	—	2.1	—
IAA*	—	1.5	—	—
Myo-inositol*	—		—	3.4
IAA-*myo*-inositol* NH_4OH treated)				
Preparation 1	1.0	1.5	2.3	3.3
Preparation 2	1.0	1.4	2.3	3.3
Rice ester (NH_4OH treated)				
Preparation 1	—	1.4	2.2	3.3
Preparation 2	—	1.4	2.2	3.3

* These compounds are authentic standards and may be compared to the rice ester sample.

† Indoleacetamide may be singly or doubly silylated on the amide nitrogen depending upon the ratio of derivatizing reagent (BSTFA) to indoleacetamide.

Table 2. Characteristic ions of the 70 eV mass spectra of the TMSi derivatives of authentic and rice IAA-*myo*-inositol

	Abundance (%)			
	Equatorial ester		Axial ester	
m/e	Authentic	Isolated	Authentic	Isolated
130	4.0	3.0	3.0	2.0
202	30.0	24.0	33.0	24.5
229	100.0	100.0	100.0	100.0
318	24.0	24.5	24.0	47.0
433	0.6	1.0	1.0	13.5
507	1.25	2.0	2.0	13.0
574		—	0.75	2.5
607	0.25	0.5		—
679	2.0	0.5		—
697	–	—	0.25	1.0
769	5.0	2.3	42.0	79.0

IAA-*myo*-inositol is a major low MW ester of IAA present in kernels of *Z. mays* [8]. Ester and amide conjugates of IAA are found in all plants examined, but esterified IAA is the predominant conjugate in seeds of cereal plants, and is present in greater amounts than free IAA [1]. The chemical nature of these conjugates is known only for *Z. mays* [7–10] and *Avena sativa* [11] (Table 3). The importance of IAA esters in plants is suggested by several experiments: the relative concentration of free and esterified hormone changes in response to external stimuli such as light [12]; esterified IAA is protected from oxidative destruction by plant peroxidases [13]. In *Z. mays*, IAA-*myo*-inositol serves as a seed auxin-precursor and appears to move from seed to growing shoot, thus playing a transport role [14].

This work is the first demonstration of IAA-*myo*-inositol in a plant other than *Z. mays* and its close relatives. The occurrence of IAA-*myo*-inositol in two widely separated graminaceous plants is striking and of possible importance in seed auxin physiology.

EXPERIMENTAL

Seeds of *O. sativa* cv Calrose were obtained from Sutter County, CA, 1976 harvest. Dehusked kernels (1 kg) were ground and extracted as described previously [1]. The IAA-*myo*-inositol standard, synthesized on a preparative scale by the method of ref. [15], was a gift of Dr. J. D. Cohen.

The product was purified by three column chromatographic steps: (1) Dowex 50X2-400 (Sigma), Na^+ form in 50% *iso*-PrOH, in a 6.5 × 5 cm fritted disc Buchner funnel with an elution vol. for IAA esters between 300 and 600 ml with 50% *iso*-PrOH; (2) Beckman PA 28 in 50% *iso*-PrOH in an HPLC system described in ref. [16] using a 0.9 × 17 cm column at 8.6 atm pres., elution vol. from 22 to 50 ml with 50% *iso*-PrOH; (3) Sephadex LH-20 in 50% *iso*-PrOH in a 2 × 35 cm column, elution vol. from 80 to 90 ml with 50% *iso*-PrOH.

TLC on Si gel were developed in solvent A: MeCOEt–EtOAc–H_2O–EtOH, (3:5:1:1). Indole compounds were visualized by spraying with Ehmann's reagent [17]. The product was ammonolysed by adding 2x (vol.) 58% NH_4OH to the sample in a glass vial, then heating the capped vial for 45 min at 45°. Samples were then dried under N_2 and dissolved in 50% *iso*-PrOH.

Samples for GC and GC–MS were silylated with BSTFA + 1% TMCS at 45° for 45 min. GC samples were chromatographed on a 1.2 m × 3 mm i.d. 2% OV-1 column programmed from 220 to 280° at 6°/min, or on a 1.8 m × 3 mm i.d. 1% OV-1 column isothermally at 240°. Products of ammonolysis were chromatographed on a 1.8 m × 3 mm i.d. 1% OV-1 column isothermally at 160°. The carrier gas was N_2 with a flow rate of 120 ml/min and peaks were detected with an FID. GC–MS analyses were performed with a 70 eV accelerating voltage. Samples of 2 μl were introduced through a 10 m OV-101 WCOT capillary column operating in a split/splitless mode, with a 50:1 split ratio, from 130 to 300° using He carrier gas.

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Table 3. A comparison of the indolylic esters of 3 cereal plants

Plant	High MW		Low MW	
	Nature	Quantity (%)	Nature	Quantity (%)
Avena sativa	IAA-glucoprotein*	80	Unknown	20
Oryza sativa	Unknown	90 (estimated)	IAA-*myo*-inositol	10
Zea mays	IAA-cellulosicglucan†	50	IAA-*myo*-inositol and IAA-*myo*-inositol glycosides	50

*IAA linked to a glucoprotein containing β-D-(1→4)- and β-D-(1→3)-linked D-glucose residues.
†IAA linked to a glucan containing β-D-(1→4)-linked D-glucose residues.

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