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Oxidation of Indole-3-acetic Acid and Oxindole-3-acetic Acid to 2,3-Dihydro-7-hydroxy-2-oxo-1*H* Indole-3-acetic Acid-7′-*O*-β-D-Glucopyranoside in *Zea mays* Seedlings[1]

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ABSTRACT

Radiolabeled oxindole-3-acetic acid was metabolized by roots, shoots, and caryopses of dark grown *Zea mays* seedlings to 2,3-dihydro-7-hydroxy-2-oxo-1*H* indole-3-acetic acid-7′-*O*-β-D-glycopyranoside with the simpler name of 7-hydroxyoxindole-3-acetic acid-glucoside. This compound was also formed from labeled indole-3-acetic acid supplied to intact seedlings and root segments. The glucoside of 7-hydroxyoxindole-3-acetic acid was also isolated as an endogenous compound in the caryopses and shoots of 4-day-old seedlings. It accumulates to a level of 4.8 nanomoles per plant in the kernel, more than 10 times the amount of oxindole-3-acetic acid. In the shoot it is present at levels comparable to that of oxindole-3-acetic acid and indole-3-acetic acid (62 picomoles per shoot). We conclude that 7-hydroxyoxindole-3-acetic acid-glucoside is a natural metabolite of indole-3-acetic acid in *Z. mays* seedlings. From the data presented in this paper and in previous work, we propose the following route as the principal catabolic pathway for indole-3-acetic acid in *Zea* seedlings: Indole-3-acetic acid → Oxindole-3-acetic acid → 7-Hydroxyoxindole-3-acetic acid → 7-Hydroxyoxindole-3-acetic acid-glucoside.

Research on the catabolism of the plant growth substance IAA has been mainly concerned with its decarboxylation as catalyzed by IAA-oxidase. Recently, however, work in two laboratories, on the *in vivo* metabolism of IAA in *Zea mays* seedlings, has shown that the loss of CO_2 accounts for only a minor portion of IAA catabolism in this plant (4, 13). Instead, OxIAA[3] has been identified as the major oxidation product formed when radiolabeled IAA is supplied to the roots, shoots, and endosperm of dark-grown maize seedlings (12, 15). OxIAA has also been extracted and identified as an endogenous component of maize caryopses and shoots (15, 16).

In this paper we report on the further metabolism of OxIAA. Radiolabeled OxIAA was synthesized and supplied to intact *Z. mays* seedlings and to root and coleoptile segments. The OxIAA was metabolized more slowly than IAA to a single major metabolite identified as 2,3-dihydro-7-hydroxy-2-oxo-1*H* indole-3-acetic acid-7′-*O*-β-D-glucopyranoside, more simply called, 7-hydroxyoxindole-3-acetic acid-glucoside (7-OH-OxIAA-glc). The identity of this compound was established by MS and by comparison of the putative 7-OH-OxIAA formed by hydrolysis of the plant glucoside with the authentic synthetic compound. 7-OH-OxIAA-glc was also formed following application of [5-^{3}H]IAA to plant tissue. The endogenous level of 7-OH-OxIAA-glc in the shoots and caryopses was measured by a GC double-standard isotope dilution method (1, 3) and by its UV absorbance. The identity and purity of the GC peak was confirmed by MS.

MATERIALS AND METHODS

Instrumentation. A Varian model 5000 gradient liquid chromatograph with a Rheodyne high-pressure loop injector and a UV-5 selectable wavelength detector was used for HPLC analyses. Reverse-phase columns (250 × 5 mm i.d.) were packed with either Partisil 10-ODS (Whatman) or a polystyrene-divinylbenzene adsorbent PRP-1 (Hamilton). A solvent gradient of 10% (v/v) to 60% ethanol in 1% aqueous acetic acid was normally employed and the column washed with ethanol after each sample. The ion-exchange column was packed with Partisil-10 SAX (Whatman) and eluted with a gradient of 0 to 10% acetic acid in 50% aqueous ethanol.

GC analyses were on a Varian model 2740 gas chromatograph with a 1.83 m × 3 mm i.d. column packed with 3% OV-17, using nitrogen carrier gas with a flow rate of 30 ml/min and a flame-ionization detector. Trimethylsilyl (TMS) derivatives were prepared by dissolving the compounds in 20 μl of pyridine and heating with 30 μl BSTFA containing 1% trimethylchlorosilane at 60°C for 2 h.

Mass spectra were obtained at 70 ev using a Hewlett-Packard 5985 quadrapole combined GC-MS with a 1.8 m × 3 mm i.d. 3% SP2250 column. UV spectra were obtained with a Cary model 15 scanning spectrophotometer.

Radioactivity was determined by counting in a Beckman LS 7000 liquid scintillation spectrometer. Values were corrected for background radiation and counting efficiency.

Chemical Syntheses. Unlabeled OxIAA was synthesized using the method of Hinman and Bauman (6), in which IAA, dissolved in dry *t*-butanol is oxidized by an equimolar amount of *N*-bromosuccinimide. The product was purified by partitioning into ethyl acetate from an aqueous solution, and recrystallized from acetone-benzene: Tm 141 to 143°C; UV absorbance λ_{max}

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[3] Abbreviations: OxIAA, oxindole-3-acetic acid; 7-OH-OxIAA-glc, 2,3-dihydro-7-hydroxy-2-oxo-1*H* indole-3-acetic acid-7′-*O*-β-D-glucopyranoside; BSTFA, *N*,*O*-bis(trimethylsilyl)trifluoroacetamide; TMS, trimethylsilyl.

nm (ε) 280 sh (1251), 249 (7686), 206 (22,371) in 95% ethanol; MS of methyl ester m/z (%) M^+ 205 (23), 172 (48), 146 (40), 145 (100), 144 (72), 132 (16), 117 (53), 116 (47) (see Kinashi *et al.* [7]).

.[^{3}H]OxIAA was synthesized in the same manner on a smaller scale. [5-^{3}H]IAA (1.4 μg, specific activity 1×10^6 Bq·nmol^{-1} [10]) was dissolved in 30 μl of dry *t*-butanol. To this was added, in 5-μl aliquots with mixing, *N*-bromosuccinimide (1.42 μg) dissolved in 20 μl of *t*-butanol. After 2 h incubation at room temperature, with intermittent mixing, the reaction mixture was diluted with 1 ml 50% propan-2-ol, loaded onto a 170- × 7-mm column of Sephadex LH-20, and eluted with 50% aqueous propan-2-ol. [5-^{3}H]OxIAA, eluting after 8 to 9 ml was obtained in 34% yield. Half of the [^{3}H]OxIAA was diluted with unlabeled OxIAA to a specific activity of 3.7×10^4 Bq·nmol^{-1}; the remainder had a specific activity of 1×10^6 Bq·nmol^{-1}. The identity of the labeled product was confirmed by cochromatography with authentic material on two HPLC columns: Partisil 10-ODS and PRP-1, eluted with (a) 20% aqueous ethanol plus 1% acetic acid or (b) 20% aqueous acetonitrile plus 1% acetic acid.

The [5-^{3}H]IAA was synthesized from [5-^{3}H]tryptophan by Michalczuk and Chisnell (10). 7-Hydroxyoxindole-3-acetic acid was synthesized by L. Kruse, using a method described elsewhere (14).

Samples of OxIAA and 7-hydroxyOxIAA were converted to their respective quinolone derivatives (Fig. 1) by dissolving in 2 N HCl and heating in a boiling water bath for 1.5 h (18). Ring expansion was quantitative. Quinolone samples formed in this way were purified using the PRP-1 HPLC column: 1,2,3,4-tetrahydro-2-oxo-quinoline-4-carboxylic acid: Tm 217°C; UV absorbance λ_{max} (ε) 280 sh (1853), 251 (8365), 206 (21,524); MS of TMS derivative m/z (%) 335 (M^+, 1.4), 320 (7), 218 (57), 202 (60). 1,2,3,4-Tetrahydro-8-hydroxy-2-oxo-quinoline-4-carboxylic acid: UV absorbance, λ_{max} 212, 250, and 294; MS of TMS derivative m/z (%) M^+ 423 (6.8), 408 (87), 351 (16), 306 (65), 290 (64), 218 (13), 202 (10).

Treatment of Plant Material. Caryopses of *Zea mays* cv Stowells Evergeeen (W. Atlee Burpee Co.) were surface sterilized for 10 min in a 1% NaOCl solution, soaked overnight under running water, then rolled in paper towels and germinated in darkness at 25°C.

Root segments, 2 cm in length, were cut from 1 mm behind the root tip of plants, 3 d after planting. At this stage, plants were approximately 12 cm in length. Coleoptile segments were obtained from plants 4 d after planting (approximately 20 cm in length): 1-cm sections were excised from 2 mm behind the apex. All sections were incubated in groups of 50 in glass Petri dishes, containing 5 ml of an aqueous solution of OxIAA or IAA.

Four-d-old seedlings were used for the study of OxIAA metabolism in intact plants. A 2-μl aliquot, containing OxIAA dissolved in 50% propan-2-ol, was placed in a 1- × 3-mm hole in the endosperm.

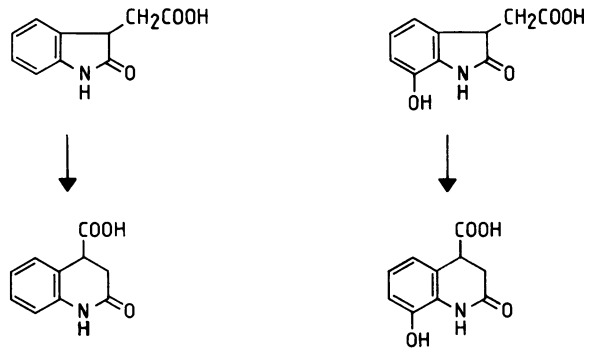

FIG. 1. Ring expansion of 2-oxindole-3-acetic acid and 7-hydroxy-2-oxindole-3-acetic acid on heating for 1 h at 100°C in 2 N HCl.

Extraction and Purification Procedure. After incubation, segments were washed with water five times, for 1 min each wash. Shoot and root sections were then extracted overnight at 4°C in methanol. The roots and shoots were excised from intact plants. Endosperms were homogenized in 80% methanol in a Waring Blendor for 2 min and extracted overnight at 4°C. Shoots were washed with water and extracted with methanol. Extracts were filtered, concentrated *in vacuo* to an aqueous phase, then diluted to 5× the original volume with distilled H_2O. The aqueous extracts were partitioned 3× against equal volumes of freshly distilled diethyl ether. Ether distillation was over potassium metal in a nitrogen atmosphere. The ether fractions were pooled and washed with a small volume of water and then discarded. The aqueous fractions were combined and concentrated *in vacuo* to remove residual ether. Samples were loaded, in 5-ml aliquots, onto C_{18} Sep-pak cartridges (Waters Associates). Each aliquot was eluted with 2 ml of 50% aqueous ethanol and the cartridge washed with ethanol before reuse.

Samples dissolved in 50% ethanol were loaded onto a DEAE-Sephadex column (185 × 28 mm) in the acetate form. The solvent gradient was as follows: solvent A, 100 ml 50% ethanol; solvent B, 100 ml 1% acetic acid in 50% ethanol; solvent C, 250 ml 10% acetic acid in 50% ethanol. OxIAA and its metabolites eluted between 100 and 140 ml of solvent C. This fraction was further purified on a Sephadex LH-20 column (240 × 22 mm), eluted with 50% propan-2-ol. The OxIAA metabolite, eluting in 80 to 110 ml was collected for HPLC. Two HPLC columns were used: first, the Partisil-10 SAX column; and second, the Partisil-10 ODS column. Samples were injected in 200 μl of the eluting solvent. The eluant was collected in 0.5-ml fractions and aliquots were taken for assay of radioactivity. Radioactive compounds eluting from the column were reduced to dryness under a stream of N_2.

RESULTS

Formation of [5-^{3}H]7-OH-OxIAA-glc from [5-^{3}H]IAA. When radiolabeled IAA is supplied to *Z. mays* seedlings up to 10 labeled products can be extracted (12, 13) of which the most prominent is OxIAA. In a separate paper, we describe the identification of 7-OH-OxIAA-glc as the major metabolite formed from [5-^{3}H]OxIAA supplied to *Z. mays* seedlings. The following experiment demonstrated that 7-OH-OxIAA-glc is an IAA metabolite.

One hundred root segments were incubated in an aqueous solution of [5-^{3}H]IAA (2.1×10^{-8} M, 1×10^6 Bq·nmol^{-1}) for 24 h. The tissue was extracted with methanol and the labeled metabolite purified. At each step, the radioactive fraction coeluting with 7-OH-OxIAA-glc was collected. A compound was obtained which co-chromatographed with 7-OH-OxIAA-glc on all three HPLC columns. Ring expansion on heating in acid or base, to give the respective quinolone derivative is a characteristic reaction of oxindoles (*e.g.* 18). Acid hydrolysis of the IAA metabolite yielded a product which coeluted with 1,2,3,4-tetrahydro-8-hydroxy-2-oxo-quinoline-4-carboxylic acid, confirming that the metabolite was 7-OH-OxIAA-glc. A compound coeluting with 7-OH-OxIAA-glc on HPLC was also extracted from the shoots and caryopses of intact plants incubated with [^{3}H]IAA for 24 h.

HPLC Analysis of Extracts from Roots, Shoots, and Caryopses Supplied with [5-^{3}H]OxIAA. The metabolism of [5-^{3}H]OxIAA supplied to intact *Z. mays* seedlings, and to root and coleoptile sections was studied. Segments were incubated in aqueous solutions of [^{3}H]OxIAA (3.7×10^4 Bq·nmol^{-1}, 5×10^{-8} M, or 1×10^{-7} M) for 24 h. Intact plants were supplied with a 2-μl drop containing 1.0 to 1.3 pmol [^{3}H]OxIAA (1×10^6 Bq·nmol^{-1}) by injection into a hole in the endosperm. Plant material

was extracted with methanol and the extracts analyzed by HPLC with a minimum of purification to avoid selective loss of metabolites. Extracts of root and coleoptile segments were purified using C_{18} Sep-pak cartridges (12). Samples from the caryopses and intact shoots were also passed through a ephadex LH-20 column and all radioactive fractions collected. This step did not appear to result in selective loss of OxIAA or its metabolites since HPLC analysis of aliquots from root extracts before and after column chromatography showed the same profile of radioactive components.

Analysis of all extracts by reverse-phase C_{18} HPLC revealed one major metabolite of [^{3}H]OxIAA, eluting after approximately 7 min (Table I). This compound was not present in significant amounts in control extracts of boiled tissues. The compound was more polar than OxIAA and was shown to have an acidic function by its retention on a DEAE-Sephadex column. Detailed chemical characterization of the metabolite as 7-OH-OxIAA-glc (Fig. 2) is published separately. Identification of the hydroxylated oxindole-3-acetic acid moiety was by HPLC comparison of the product of enzyme hydrolysis with synthetic 7-OH-OxIAA and by GC-MS comparison of the quinolone derivative with synthetic 1,2,3,4-tetrahydro-8-hydroxy-2-oxo-quinoline-4-carboxylic acid. The position of the hydroxyl group was determined by UV spectroscopy. The mass spectrum of the TMS derivative of the intact metabolite showed ions at m/z (%) 801 (0.97), 450 (2.6), 423 (11), 361 (31), 351 (37), 271 (7.9), 234 (8.1), 217 (19), 204 (2.9), and 191 (3.1), indicating a conjugate of 7-hydroxyoxindole-3-acetic acid with a hexose sugar. The identity of the hexose moiety as glucose was determined by GC-MS after acid hydrolysis and by an enzymic glucose assay based on hexokinase and glucose-6-phosphate dehydrogenase. Finally, the position of the glucose was determined by comparison of the UV spectra at neutral and alkaline pH.

Metabolism in the roots was more rapid than in the coleoptiles with 31% and 72% of the radioactivity in respective extracts remaining as OxIAA. In the shoots and caryopses of intact plants, 60% and 52% of the label remained in OxIAA, respectively. Metabolism of OxIAA in both roots and coleoptiles was slower than that for IAA: after 24 h incubation of root and shoot segments with [^{14}C]IAA less than 1% of the radioactivity remains in IAA (12).

In all experiments, the extraction efficiencies were at least 90%.

Effect of OxIAA Concentration on its Metabolism. To determine whether the metabolism of exogenous OxIAA was dependent on its concentration, groups of 50 root segments were incubated in aqueous solutions of 10^{-5} and 10^{-6} M OxIAA. During the 20-h incubation, 65 to 75% of the radioactivity was metabolized to 7-OH-OxIAA-glc at either concentration. Thus, the proportion of OxIAA metabolized remains constant over three orders of magnitude.

Identification and Measurement of Endogenous 7-Hydroxyoxindole-3-acetic Acid Glucoside. Measurement of the endogenous content of 7-OH-OxIAA-glc was made using the isotope-dilution method of Rittenberg and Foster (17). Extracts were prepared separately from the shoots and caryopses of two batches of 1600 and 1250 4-d-old plants. As labeled 7-OH-OxIAA-glc was not available for use as the internal standard, 100 plants in each batch were preincubated with its labeled precursor, [^{3}H]OxIAA (0.8 pmol·plant^{-1}, 1×10^6 Bq·nmol^{-1}), which was supplied in a 2-μl drop to a 1-mm hole in the endosperms of 3-d-old plants. The amount added to each plant was equivalent to approximately 0.2% of the endogenous OxIAA (14). On average, 1% of the radioactivity moved into the shoots, and in both shoots and kernels 24% of the radioactivity was metabolized to 7-OH-OxIAA-glc in 24 h (Table II).

The metabolite from shoot and kernel extracts was purified. Aliquots from the kernel extracts were silylated and analyzed by GC-MS. The spectrum showed ions at m/z 801, 450, 423, 361, 351, 271, 234, 217, 204, and 191, confirming its identity as 7-OH-OxIAA-glc.

Aliquots from each extract were hydrolyzed in 2 N HCl at 100°C for 1 h, giving a product which co-chromatographed with synthetic 1,2,3,4-tetrahydro-8-hydroxy-2-oxo-quinoline-4-carboxylic acid on all three HPLC columns. The identity and purity of the compound in the caryopsis extracts was confirmed by GC-MS of the TMS derivatives. Plant samples and the synthetic quinolone derivative both gave a molecular ion at m/z 423 and major fragment ions at m/z 408, 351, 306, 290, 218, and 202.

The specific activity of 7-OH-OxIAA-glc from caryopsis extracts was determined using a GC double-standard isotope-dilution method (1, 3). Aliquots from both extracts were hydrolyzed in 2 N HCl to yield the quinolone as this compound could be derivatized for GC more reproducibly than the glucoside. The products were purified on the PRP-1 column, and an approximately equal amount of [6-^{3}H]-1,2,3,4-tetrahydro-2-oxo-quinoline-4-carboxylic acid of known specific activity added as the second internal standard. The mixture was derivatized using BSTFA and chromatographed on a 1.8-m 3% OV-17 column. Peak areas and radioactivity were measured on parallel injections and the specific activity calculated with reference to the second internal standard.

$$\frac{\text{standard peak area}}{\text{metabolite peak area}} \times \frac{\text{metabolite Bq}}{\text{standard Bq}} \times \text{specific activity of standard} = \text{specific activity of 7-OH-OxIAA-glc}$$

The amount of endogenous 7-OH-OxIAA-glc was calculated using the isotope dilution equation of Rittenberg and Foster (16). As labeled 7-OH-OxIAA-glc was not available for use as the

Table I. *Metabolism of [5-^{3}H]OxIAA by Root and Coleoptile Segments and Intact Z. mays Seedlings (24 Hours Incubation)*

The experiment with segments was done in duplicate; that with intact plants was repeated four times.

	Segments		Intact Plants	
	Roots	Coleoptiles	Caryopses	Shoots
Radioactivity in methanol extract (Bq)	5,093	13,600	76,111	1,519
	8,273	5,548	61,725	1,013
			83,015	1,088
			76,756	533
% of radioactivity remaining as OxIAA	32	71	53	46
	31	74	60	46
			63	58
			63	56
% of radioactivity as 7-OH-OxIAA-glc	63	24	33	34
	63	21	11	15
			26	25
			21	22

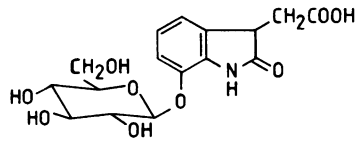

FIG. 2. Molecular structure of 7-hydroxy-2-oxindole-3-acetic acid-7′-*O*-β-D-glucoside.

Table II. *Quantitative Determination of the Amount of 7-OH-OxIAA-glc in Shoots and Caryopses of Dark-Grown Z. mays Seedlings Using the Isotope Dilution Method (14)*

The final specific activity of 7-OH-OxIAA-glc was calculated by comparison with labeled 1,2,3,4-tetrahydro-2-oxo-quinoline-4-carboxylic acid of known specific activity.

Experiment and Tissue	Total Radioactivity in Crude Extract	Radioactivity Metabolized to 7-OH-OxIAA-glc	Amount of 7-OH-OxIAA-glc Derived from Exogenous OxIAA	Final Specific Activity of 7-OH-OxIAA-glc	Amount of Endogenous 7-OH-OxIAA-glc	
	Bq	%	*pmol*	*Bq·nmol*$^{-1}$	*pmol·plant*$^{-1}$	*nmol·g*$^{-1}$ *fresh wt*
1. Caryopses	83,015	26	21.5	3.16	4300	10.6
2. Caryopses	76,756	21	16.1	2.46	5300	14.3
1. Shoots	1,088	25	0.274	2.55	67	0.48
2. Shoots	553	22	0.122	1.74	56	0.51

internal standard, however, the equation was modified.

$$\left(\frac{C_i}{C_f} - 1\right)X = Y \quad (1)$$

can be rearranged to

$$\frac{C_i X}{C_f} - X = Y$$

which is equivalent to

$$\frac{\text{bq of 7-OH-OxIAA-glc}}{C_f} - X = Y \quad (2)$$

where C_i is the initial specific activity of an added internal standard; C_f is its final specific activity in the plant extract; X is the number of mol of internal standard added, in this case the number of mol of 7-OH-OxIAA-glc derived from exogenous OxIAA; Y is the number of mol of 7-OH-OxIAA-glc present in the extract and as extraction efficiencies were always greater than 90%, Y is equivalent to the amount of endogenous 7-OH-OxIAA-glc.

The percentage of [^{3}H]OxIAA which had been metabolized to 7-OH-OxIAA was determined by reverse-phase HPLC analysis of aliquots of each extract prior to purification, combined with assay of radioactivity eluting from the column. From measurements of the total radioactivity in the crude extracts, the amount of radioactivity present as 7-OH-OxIAA-glc was calculated. Then knowing the original specific activity of the OxIAA added, the number of mol of 7-OH-OxIAA-glc derived from exogenous OxIAA (X) was found (Table II).

Thus, it was possible to add labeled OxIAA, permit the plant to convert it to 7-OH-OxIAA-glc and to calculate Y from equation 2 above. The caryopses contained an average of 4.8 nmol·kernel^{-1} (Table II), which is approximately 10 times the amounts of IAA and OxIAA in seedlings of the same variety and similar size (Table III).

The amount of radioactivity in shoot extracts was too small to allow measurement of specific activity by GC. This was calculated by comparison of the UV absorbance and radioactivity of the purified quinolone with an aliquot of the quinolone from the endosperm extract. The shoots contained an average of 62 pmol·plant^{-1}, the same order of magnitude as levels of IAA and OxIAA (Table III).

Table III. *Comparison of Levels of 7-Hydroxyoxindole-3-acetic Acid-Glucoside with OxIAA and IAA in Z. mays Seedlings*

	7-OH-OxIAA-glc	OxIAA[a]	IAA[b]
	pmol/plant		
Caryopsis	4800	357	308
Shoot	62	49	27

[a] From Reinecke and Bandurski (15).
[b] From Epstein *et al.* (4).

DISCUSSION

When physiological amounts of radiolabeled IAA are supplied to dark-grown *Z. mays* seedlings, several metabolites are formed, at least 10 in the roots and 5 in the coleoptiles (12, 13). The major product has been identified as OxIAA (12, 15, 16). We have now identified a further metabolite in this pathway, as 7-hydroxy-2-oxindole-3-acetic acid-7′-*O*-β-D-glucoside. This compound was produced from both IAA and OxIAA supplied, in amounts equal to less than 1% of the endogenous level, to intact plants as well as root and coleoptile sections. It is also naturally present in the caryopses and shoots at levels of 4.8 nmol·kernel^{-1} and 62 pmol·shoot^{-1} and it may therefore be concluded that this compound is a natural metabolite of IAA in maize seedlings. From the results published here and previous work, we propose the following route for IAA catabolism in *Zea* (Fig. 3): IAA → OxIAA → 7-hydroxyoxindole-3-acetic acid → 7-hydroxyoxindole-3-acetic acid glucoside.

7-OH-OxIAA-glc has not previously been identified in plant tissues, nor to our knowledge have there been any prior reports of its occurrence in any biological system. However, a related compound, zeanic acid (1,2-dihydro-8-hydroxy-2-oxo-quinoline-4-carboxylic acid) was extracted from corn steep liquor by Matsushima *et al.* (9). Treatment of the plant material during extraction of the corn steep liquor could have produced this compound from a 7-hydroxyoxindole derivative.

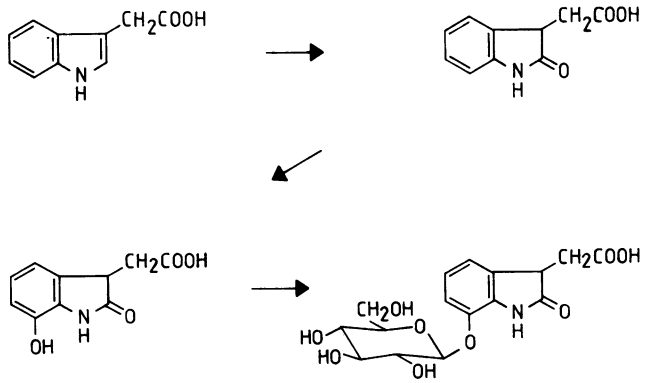

FIG. 3. Proposed route for IAA catabolism in *Z. mays*. Indole-3-acetic acid to 2-oxindole-3-acetic acid to 7-hydroxy-2-oxindole-3-acetic acid to 7-hydroxy-2-oxindole-3-acetic acid-7′-*O*-β-D-glucoside.

Although sugar and amino acid conjugates of IAA have been extracted from many species (2), 7-OH-OxIAA-glc is the first conjugate of an IAA oxidation product to be completely characterized. Klämbt (8) reported the presence of a glucoside of OxIAA in extracts of *Ribes rubrum*, *Aquiligia vulgaris*, wheat and potato; however, details of the chemical characterization were not shown. Tsurumi and Wada (19) showed that labeled IAA is metabolized by *Vicia* roots to two derivatives of dioxindole-3-acetic acid which may also be conjugates. Thus, conjugates of IAA oxidation products may have general occurrence.

This pathway for IAA metabolism shows a striking similarity with that of ABA in tomato. Milborrow and Vaughan (11) have shown that after two oxidation steps, forming phaseic acid and dihydrophaseic acid, the conjugate dihydrophaseic acid 4′-*O*-β-D-glucopyranoside is produced. A similar route is followed in gibberellin metabolism with the formation of GA_8 glucoside. At present, the reason for the conjugation step is unknown, although conjugation would make the compounds highly water soluble and perhaps favor movement into the vacuole. Indeed, this high solubility in water is probably the reason why these compounds were not isolated earlier since 7-OH-OxIAA-glc does not partition into organic solvents.

The oxidation of OxIAA to 7-OH-OxIAA-glc takes place more slowly than the catabolism of IAA. A few studies on the biological activity of OxIAA (5, 20) have indicated that OxIAA is not physiologically active. The rapid catabolism of IAA to OxIAA may be the critical deactivation step. The metabolism of OxIAA is slower in the coleoptiles than in the roots, reflecting a similar difference in the rate of IAA metabolism. 7-OH-OxIAA-glc appears to accumulate in the kernels of maize seedlings; however, levels in the shoots are similar to those of IAA and OxIAA and, thus, it is likely that the compound is further metabolized.

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