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Enzymic synthesis of indol-3-ylacetyl-*myo*-inositol galactoside

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Extracts of immature kernels of *Zea mays* catalysed the synthesis of indol-3-ylacetyl-*myo*-inositol galactoside from indol-3-ylacetyl-*myo*-inositol and UDP-galactose. Addition of 2-mercaptoethanol was required for stability of the catalytic activity during dialysis. The enzyme could be fractionated wtih $(NH_4)_2SO_4$, and 55% of the activity was recovered in the 30–60%-saturation fraction. The product of the reaction contained radioactivity from UDP-[U-^{14}C]galactose and was identified as indol-3-ylacetyl-*myo*-inositol galactoside by gas chromatography–mass spectrometry. Therefore a UDP-galactose:indol-3-ylacetyl-*myo*-inositol galactosyltransferase (indol-3-ylacetyl-*myo*-inositol galactoside synthase) is present in developing kernels of *Zea mays*. The description of this enzyme, together with the enzymes described in the accompanying paper [Michalczuk & Bandurski (1982) *Biochem. J.* **207**, 273–281] for the synthesis of indol-3-ylacetylglucose and indol-3-ylacetyl-*myo*-inositol, now provides mechanisms for the biosynthesis of one-half of the low-molecular-weight esters of indol-3-ylacetic acid in *Zea mays*.

Indol-3-ylacetic acid (IAA-OH) is a growth hormone found in plants. Free IAA-OH accounts for only 0.8% of the total IAA-OH present in *Zea mays* L. kernels (cf. Bandurski, 1980), whereas IAA-OH esterified with *myo*-inositol, *myo*-inositol galactoside and *myo*-inositol arabinoside (Labarca *et al.*, 1965; Ueda & Bandurski, 1974) accounts for nearly 50%. The other 50% of IAA-OH in *Z. mays* kernels is in the form of an indol-3-ylacetylglucan (Piskornik & Bandurski, 1972). The esters of IAA-OH are formed during development of the kernel, reaching a maximum before harvest (Corcuera, 1967). Esters of IAA-OH are hydrolysed to IAA-OH during germination (Epstein *et al.*, 1980), and the resultant IAA-OH is further metabolized to oxindol-3-ylacetic acid (Reinecke & Bandurski, 1981). In addition, IAA-OH and its esters are transported into the shoot (Nowacki & Bandurski, 1980), and IAA-*myo*-inositol can then be hydrolysed to IAA-OH by shoot extracts of *Z. mays* (Hall & Bandurski, 1981). Thus esters of IAA-OH from the kernels serve as a source of IAA-OH for the shoots during germination.

It has been proposed that esterification protects IAA-OH against peroxidative attack (Cohen & Bandurski, 1978) and that reversible synthesis and hydrolysis of esters of IAA-OH provides a hormonal homoeostatic control system (Bandurski *et al.*, 1977). Therefore, to understand the physiological role of esters of IAA-OH, it is desirable to study the synthesis and hydrolysis of these compounds by the plant. The enzymic synthesis of IAA-glucose and IAA-*myo*-inositol is described in the accompanying paper (Michalczuk & Bandurski, 1980, 1982). In the present paper we report the enzymic synthesis of IAA-*myo*-inositol galactoside.

Abbreviations used: IAA, indol-3-ylacetyl; h.p.l.c., high-pressure liquid chromatography; g.c.–m.s., gas chromatography–mass spectrometry; *m/z*, mass per unit charge: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

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Experimental

Materials

UDP-galactose and Hepes were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). UDP-[U-^{14}C]galactose (200mCi/mmol) was from Amersham International (Arlington Heights, IL, U.S.A.). Inorganic salts were from Fisher Scientific Co. (Fair Lawn, NJ, U.S.A.). $(NH_4)_2SO_4$ (enzyme grade) was from Mann Research Laboratories (New York, NY, U.S.A.). Regisil [*NO*-bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane] was

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from Regis Chemical Co. (Morton Grove, IL, U.S.A.). Mixed isomeric IAA-*myo*-inositols synthesized by Nowacki *et al.* (1978) were generously supplied by Dr. J. D. Cohen (U.S. Department of Agriculture, Beltsville, MD, U.S.A.); this sample contained the four resolvable isomers of IAA-*myo*-inositol as shown by g.c.–m.s. with molecular ions at *m/z* 769 and with the correct fragment ions (Ehmann & Bandurski, 1974; Nowacki *et al.*, 1978). Sweet corn (*Zea mays*) at the table-ready state was purchased from local greengrocers.

Isolation of IAA-myo-inositol glycosides from maize kernels

The solution containing IAA-*myo*-inositol glycosides, used as a standard, was prepared from an acetone/water (7:3, v/v) extract from 300g of dry kernels. The acetone extract (2600ml) was evaporated to 20% of its volume and left overnight in a cold-room. After filtration, the filtrate was evaporated to dryness, dissolved in propan-2-ol/water (1:1, v/v) and chromatographed on a column (4.5cm × 4.0cm) of Dowex-50 eluted with propan-2-ol/water (1:1, v/v). Fractions of volume 100ml were collected and their content of IAA-*myo*-inositol glycosides was monitored by t.l.c. Fractions 4–10, which contained most of the glycosides, were pooled, evaporated to about 2.5ml and chromatographed on a PA-28 h.p.l.c. column as described below. The collected fractions containing most of the IAA-*myo*-inositol glycosides were pooled and chromatographed on a Partisil-10 h.p.l.c. column as described below. The IAA-*myo*-inositol glycoside-containing fractions from the Partisil-10 column were pooled, evaporated to dryness and dissolved in propan-2-ol/water (1:1, v/v). This solution contained IAA-*myo*-inositol galactosides and arabinosides, with an indolylic u.v.-absorption spectra and with chromatographic properties identical with those previously characterized by their 70eV mass-spectral fragmentation pattern (Ueda & Bandurski, 1974).

Preparation of enzyme

Immature kernels of *Z. mays* (100g) were homogenized in 1 litre of acetone at temperatures not higher than −5°C. The particulate material collected by filtration was washed with 1 litre of cold acetone and then with 1 litre of cold diethyl ether. The solvents were evaporated off *in vacuo* overnight and the powder was stored at −20°C. For extraction, 1g of the powder was suspended in 10ml of 25mM-Hepes/NaOH buffer, pH7.6, containing 5mM-mercaptoethanol and 5mM-$CaCl_2$. The suspension was centrifuged at 10000*g* for 10min and the supernatant fluid was then dialysed overnight against the same buffer, This preparation is referred to below as dialysed acetone-dried-powder extract. $(NH_4)_2SO_4$ fractions were prepared from the supernatant fluid and dialysed overnight. The 30–60%-satn.-$(NH_4)_2SO_4$ fraction was taken as the enzyme preparation (see below). Protein was determined with Coomassie Brilliant Blue G-250 (Bradford, 1976), and the amount of protein was calculated from a standard curve made with bovine γ-globulin.

Assay of enzyme activity

The enzyme preparation was in 25mM-Hepes buffer, pH7.6, containing 5mM-2-mercaptoethanol and 5mM-$CaCl_2$. The enzyme prepared from the acetone-dried powder contained 15mg of protein/ml, and the enzyme prepared from the 30–60%-satn.-$(NH_4)_2SO_4$-precipitable fraction contained 4.5mg of protein/ml. Unless otherwise specified, 0.5ml of enzyme solution was added to a tube containing the substrates, the mixture was incubated for 1h at 37°C and the reaction was terminated by the addition of 0.5ml of propan-2-ol. The mixture was centrifuged at 1300*g* for 10min, the supernatant fluid evaporated to dryness and the residue dissolved in 0.5ml of propan-2-ol/water (1:1, v/v). The sample was applied to a PA-28 resin (sulphonated polystyrene–divinylbenzene; Beckman Instruments, Palo Alto, CA, U.S.A.) column (bed volume 0.9cm × 17cm) and eluted with the same solvent. Fractions of volume 1ml were collected, and fractions 25–55, which contained IAA-*myo*-inositol and its galactosides, were pooled and evaporated to dryness. The residue was dissolved and applied to a Partisil-10 h.p.l.c. column and eluted with ethyl acetate/acetonitrile/ethanol/water (65:21:7:7, by vol.) (Fig. 1). Fractions of volume 5ml containing IAA-*myo*-inositol/galactoside (peaks 2 and 3) were collected and used for radioactivity measurements with a Beckman LS 7000 liquid-scintillation system. This assay, although time-consuming and expensive, has the advantage that product formation may be followed both by the u.v. elution profile and the radioactivity collected. Product yields reported were not corrected for losses and are based on radioactivity measurements or on area under peaks 2 and 3, and these agreed within 10%. Recoveries of sample were approx. 80%.

In preliminary experiments, not described in the present paper, a rapid and sensitive detection of enzyme activity was provided by t.l.c. The enzyme reaction was stopped with propan-2-ol, the proteins were removed by centrifugation and the supernatant was subjected to t.l.c. T.l.c. of the products was on silica-gel plates developed with ethyl acetate/butan-2-one/ethanol/water (5:3:3:1, by vol.). Indoles were detected on the plates by means of Ehmann's reagent (Ehmann, 1977). After chromatography, the plate was scraped (R_F 0.14–0.35) into scintillation fluid and radioactivity measured.

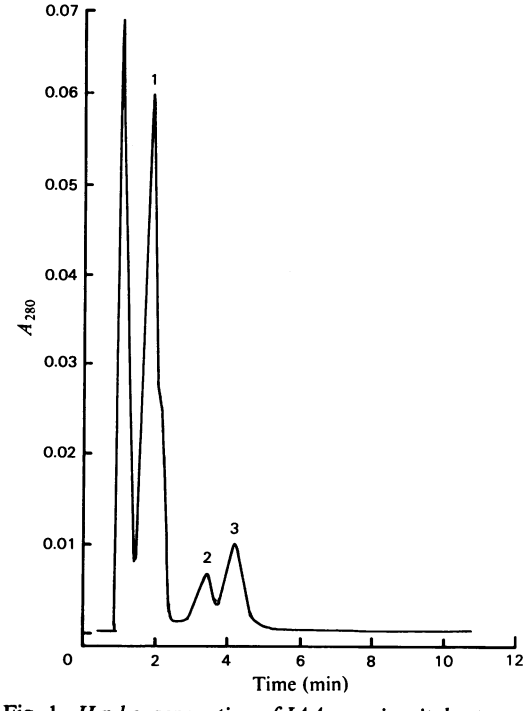

Fig. 1. *H.p.l.c. separation of IAA-myo-inositol esters and their galactosides*

IAA-*myo*-inositol and UDP-galactose were incubated in 0.5 ml of dialysed acetone-dried-powder extract of *Z. Mays* kernels as described in the Experimental section. Effluent fractions from a PA-28 h.p.l.c. column were pooled and injected into a Partisil-10 h.p.l.c. column at a solvent flow rate of 5 ml/min. The solvent was ethyl acetate/acetonitrile/water/ethanol (65:21:7:7, by vol.). A 100 μl injection loop was used. Peak 1 corresponds to the isomeric mixture of IAA-*myo*-inositols. Peaks 2 and 3 correspond to IAA-*myo*-inositol galactoside.

G.c–m.s.

Trimethylsilyl derivatives of the samples were prepared by adding 10 μl of Regisil and 10 μl of pyridine and keeping the mixtures at 50°C for 30 min before chromatography. Gas-chromatographic analyses were performed with a Varian Aerograph 2700 series apparatus equipped with a flame ionization detector. Mass spectrometry was at 70 eV and performed with an LKB-9000 instrument coupled to a 50 cm × 2 mm column of 3% OV-7 on Gas-Chrom Q at 250°C. Mass calibration was with tris(pentadecafluoroheptyl)-sym.-triazine (mol.wt. 1185) as a standard.

Results

Synthesis of IAA-myo-inositol galactoside

IAA-*myo*-inositol and UDP-[U-^{14}C]galactose were incubated together with enzyme as shown in Table 1. When both substrates were present about 19% of the IAA-*myo*-inositol added was converted into IAA-*myo*-inositol galactoside, as shown by the radioactivity found in the products. Omission of IAA-*myo*-inositol from the reaction mixture or use of boiled enzyme resulted in little IAA-*myo*-inositol galactoside being formed, showing that this is an enzyme reaction that requires IAA-*myo*-inositol as one of the substrates. The radioactivity in the products showed the participation of UDP-galactose in the reaction. Incubation of IAA-*myo*-inositol, UDP-glucose and enzyme did not result in detectable product. Thus it is unlikely that an inversion of galactose to glucose occurred during the reaction.

Effect of cations on enzyme activity

It was observed that EDTA decreased the activity of the enzyme extracted from the acetone-dried powder. Since this could be due to removal of cations necessary for enzyme activity, the effect of several cations (5 mM) was tested. Salts of various cations were separately added to 0.5 ml of medium containing dialysed enzyme prepared from an acetone-dried powder, 0.024 μmol of IAA-*myo*-inositol and 0.8 μmol of UDP-[U-^{14}C]galactose. The samples were incubated and purified as described in the Experimental section (PA-28 and Partisil-10 h.p.l.c.). Yields of IAA-*myo*-inositol galactoside were calculated from the radioactivity found in the products and are expressed as percentages of IAA-*myo*-inositol converted into product. The product yield of the control with no cations added was 17% (or 2140 d.p.m.), with $CaCl_2$ 19%, with $MgCl_2$ 14%, with $MnCl_2$ 12%, with $ZnCl_2$ 4% and with EDTA 10%. Thus the activity of the enzyme was slightly increased by additions of $CaCl_2$, but all other cations tested and EDTA decreased the activity of the enzyme, with $ZnCl_2$ being the most inhibitory.

Fractionation of the enzyme extract

The enzyme was partially purified by $(NH_3)_2SO_4$ precipitation and dialysis, with 55% of the activity being recovered from the 30–60-satn.-$(NH_4)_2SO_4$-precipitable fraction, with a 2-fold increase in specific activity. Addition of 2-mercaptoethanol was required to preserve activity during $(NH_4)_2SO_4$ fractionation and dialysis. A time course of the reaction comparing the active $(NH_4)_2SO_4$-precipitable fraction and the dialysed extract from the acetone-dried powder is shown in Fig. 2. Although the reaction was virtually completed after 60 min, about 50–70% of the IAA-*myo*-inositol remained and product yields were usually less than 30% of the IAA-*myo*-inositol initially added. Factors that could account for the low product yield are the presence of glycosidases in the extract and preferential utilization of one of isomeric IAA-*myo*-inositols by the

Table 1. *Enzymic synthesis of IAA-myo-inositol galactoside*

IAA-*myo*-inositol and UDP-[U-^{14}C]galactose (0.8 μmol; 419000 d.p.m.) were incubated for 1 h at 37°C together with 0.5 ml of an acetone-dried-powder extract of *Z. mays* kernels (15 mg of protein/ml) in 25 mM-Hepes buffer, pH 7.6, containing 5 mM-2-mercaptoethanol and 5 mM-$CaCl_2$. The enzyme assay was as described in the Experimental section. Product yields are expressed as percentages of the IAA-*myo*-inositol added.

Treatment	IAA-*myo*-inositol added (nmol)	Product formed (d.p.m.)	Product formed (nmol)	Yield (%)
Enzyme	24	2370	4.5	19
Enzyme	0	265	0.5	2
Boiled enzyme	24	63	0.1	0.5

enzyme, since the synthetic IAA-*myo*-inositol added as substrate would include the 2-*O*, DL-1-*O*, DL-4-*O*- and 5-*O*-IAA-*myo*-inositols.

Presence of glycosidases in the extract

Glycosidase activity could be demonstrated in the crude extract as well as in the three $(NH_4)_2SO_4$-precipitable fractions, since *p*-nitrophenyl α-galactopyranoside and *p*-nitrophenyl β-galactopyranoside were hydrolysed. These *p*-nitrophenyl galactosides were also used to determine if the galactosidase could transfer a galactose residue to IAA-*myo*-inositol, but no product was detected.

It thus is possible that glycosidases present in the extract hydrolysed some of the IAA-*myo*-inositol galactoside formed. To test this, IAA-*myo*-inositol glycosides were incubated together with a dialysed acetone-dried-powder extract for 1 h. About 20% of the glycosides were hydrolysed to IAA-*myo*-inositol. Thus glycosidases present in the extract would decrease the amount of product accumulated in the reaction by hydrolysing IAA-*myo*-inositol galactoside back to IAA-*myo*inositol.

Preferential utilization of IAA-myo-inositol isomers

An additional factor that could contribute to incomplete conversion of IAA-*myo*-inositol into IAA-*myo*-inositol galactoside would be selective utilization of the six possible IAA-*myo*-inositol isomers by the enzyme. Although some 50–70% of the IAA-*myo*-inositol added remained after the reaction was completed, as shown for example in Fig. 2, addition of twice the amount of IAA-*myo*-inositol produced nearly 50% more product (Table 2). However, addition of fresh enzyme or more UDP-galactose did not increase the amount of IAA-*myo*-inositol galactoside formed. Thus it is possible that the reaction was limited by the concentration of some of the six isomeric IAA-*myo*-inositols. The composition of the isomeric mixture of IAA-*myo*-inositols, before and after incubation with enzyme, was examined by h.p.l.c. (Fig. 3). The relative amounts of the four chemically resolvable isomers changed during the reaction, with

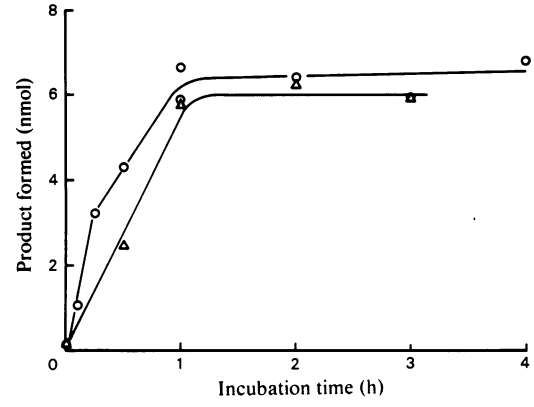

Fig. 2. *Time course of the enzymic synthesis of IAA-myo-inositol galactosides*

IAA-*myo*-inositol (0.024 μmol) and UDP-[U-^{14}C]-galactose (0.8 μmol; 419000 d.p.m.) were incubated at 37°C in 0.5 ml of dialysed acetone-dried-powder extract of *Z. mays* kernels (○) or a 30–60%-satn.-$(NH_4)_2SO_4$ fraction (△) for various times. For this experiment 1 g of acetone-dried powder was suspended in 10 ml of buffer, purified and assayed as described in the text. The buffer was 25 mM-Hepes buffer, pH 7.6, containing 2-mercaptoethanol and 5 mM-$CaCl_2$. The amount of protein in the acetone-dried-powder extract was 6.5 mg/ml, and was 3.2 mg/ml in the 30–60%-satn.-$(NH_4)_2SO_4$ fraction.

a decrease of isomers b, c and d relative to a. Thus the isomers were differentially used by the enzyme.

Reaction product formed as a function of time (Fig. 2) showed that the amount of product did not decrease for several hours after apparent completion of the reaction, even though there are glycosidases that hydrolysed the IAA-*myo*-inositol glycosides in the enzyme preparation. Since hydrolysis of IAA-*myo*-inositol galactoside could yield the IAA-*myo*-inositol of the correct configuration, it could be again converted into the galactoside, thus producing an apparent steady state.

Table 2. *Requirements for IAA-myo-inositol, UDP-galactose and enzyme in IAA-myo-inositol galactoside synthesis*
All samples contained IAA-*myo*-inositol as indicated, 0.8 μmol of UDP-[U-^{14}C]galactose (340000 d.p.m.) and 0.5 ml of *Z. mays* enzyme prepared from a 30–60%-satn.-$(NH_4)_2SO_4$ fraction containing 4.5 mg of protein/ml. Samples were incubated at 37°C for 1 h; then to some samples an additional 1 ml of enzyme or buffer and 0.8 μmol of UDP-[U-^{14}C]galactose were added as indicated and the samples were incubated an additional 1 h. The reaction was stopped after incubation for a total of 2 h and assayed as described in the Experimental section.

IA-*myo*-inositol (nmol)	Additions after 1 h of incubation	Product (d.p.m.)	Product (nmol)
24	None	3115	7.3
48	None	4674	11.0
24	Additional enzyme	3368	7.9
24	Buffer + UDP-galactose	3245	7.6
24	Additional enzyme + UDP-galactose	3355	7.9

Identification of product

UDP-galactose (10 μmol) was incubated as described with 3 μmol of the mixed isomeric IAA-*myo*-inositols (Ehmann & Bandurski, 1974) and 2.5 ml of the 30–60%-satn.-$(NH_4)_2SO_4$ fraction. After termination of the reaction and removal of proteins by centrifugation, the supernatant fluid was passed through a column (bed volume 1 ml of DEAE-Sephadex (acetate form) (Sigma Chemical Co.) and the deionized solution was concentrated to 0.3 ml and applied to a PA-28 h.p.l.c. column. The presence of indolylic compounds in the collected fractions was detected by t.l.c. as Ehmann-positive substrates (Ehmann, 1977). Fractions containing compounds with R_F values similar to that of the IAA-*myo*-inositol galactosides (0.16–0.2) were evaporated to dryness and, after treatment with Regisil, were analysed by g.l.c. (Fig. 4). The peaks near the solvent front correspond to the four isomers of IAA-*myo*-inositol. Peaks I, II and III co-chromatographed with standards of IAA-*myo*-inositol glycosides isolated from *Z. mays* kernels. These three peaks were collected. Their u.v.-absorption spectrum was that of an indole having maximum at 290, 280, 272 and 222 nm. The samples used for g.l.c. analysis were also used for mass-spectral characterization. The three g.l.c. peaks each had a molecular ion at $m/z = 1147$, which corresponds to the fully silylated IAA-*myo*-inositol galactoside (Table 3). The main ion fragments are identical with those reported previously for IAA-*myo*-inositol galactoside (Ueda & Bandurski, 1974) and for IAA-*myo*-inositol (Hall, 1980). Ions at $m/z = 769$ and 229 correspond to the fully silylated IAA-*myo*-inositol and the base peak of trimethylsilyl-IAA-*myo*-inositol respectively (Hall, 1980). Since m/z at 361 is a typical fragment of sugars (Ehmann, 1974), it may have originated from galactose. The ion at $m/z = 204$ is the base peak of galactinol (1-*O*-β-D-galactopyranosyl-*myo*-inositol; Ueda & Bandurski, 1974). Evidence that the product was IAA-*myo*-inositol galactoside and not a glucoside was as follows: (1) incubation of IAA-*myo*-inositol with UDP-glucose in place of UDP-galactose did not lead to measurable product formation; (2) the g.l.c. and h.p.l.c. retention times of

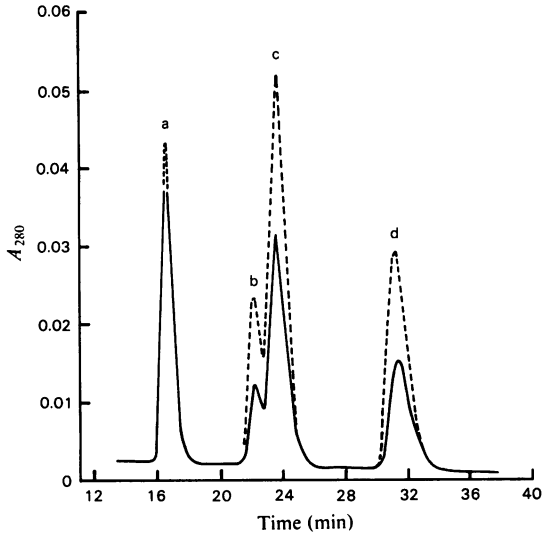

Fig. 3. *Differential use of IAA-myo-inositol isomers as substrates*
IAA-*myo*-inositol (48 μmol) and UDP-galactose (0.8 mmol) were added to the enzyme [0.5 ml of the 30–60%-satn.$(NH_4)_2SO_4$ fraction. One sample was incubated for 2 h at 37°C (——), and the other (– – – –) was a zero-time sample with no incubation. The reaction was stopped with 0.5 ml of propan-2-ol, and the mixture was centrifuged and subjected to Partisil-10 h.p.l.c. as described for Fig. 1. The resultant partially purified IAA-*myo*-inositol was collected and re-chromatographed on a reverse-phase Partisil-10–ODS-3 h.p.l.c. column, as shown in this Figure, with ethanol/water (1:19, v/v) as eluent with a 1 ml/min flow rate. Peaks a, b, c and d corresponded to isomers of IAA-*myo*-inositol.

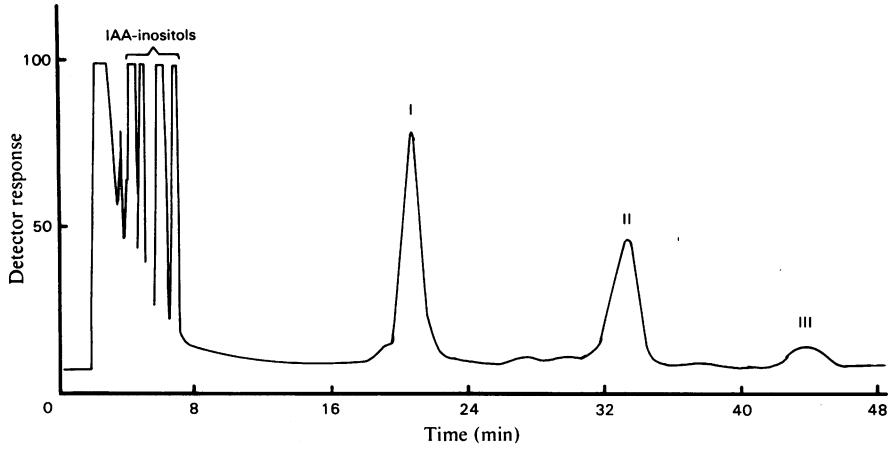

Fig. 4. *G.l.c. analysis of IAA-myo-inositol galactosides*
IAA-*myo*-inositol and UDP-galactose were incubated in 0.5 ml of the 30–60%-satn.-$(NH_4)_2SO_4$ fraction from *Z. mays* kernels. See the text for details. The purified products were treated with Regisil for g.l.c. Chromatographic conditions were: air, H_2 and N_2 flow rates were 300, 27 and 30 ml/min respectively; detector and injector temperatures were 300°C. The samples were chromatographed isothermally at 250°C. The column (1.8 m × 2 mm) was 3% OV-17 on Gas-Chrom G. Full scale represents 3.2×10^{-11} A·mV. Peaks I, II and III correspond to IAA-*myo*-inositol galactosides.

Table 3. *Mass spectra of trimethylsilyl ethers of enzymically synthesized IAA-myo-inositol galactosides*
IAA-*myo*-inositol and UDP-galactose were incubated with an enzyme fraction from *Z. mays*. See the text for details. Mass spectra of the g.l.c. peaks I, II and III (see Fig. 3) of the products of the enzyme-catalysed reaction are shown, including all fragments of *m/z* above 400 with relative abundance greater than 10%. In the range *m/z* 200–400 fragment ions with relative abundance greater than 70% in one of the peaks are mentioned. The molecular ion at *m/z* = 1147 corresponds to the fully silylated nonakis(trimethylsilyl)-IAA-*myo*-inositol galactoside. The molecular ion *m/z* = 1075, previously reported by Ueda & Bandurski (1974), is the octakis(trimethylsilyl) compound, which lacks a trimethylsilyl group on the indole nitrogen atom, as it was produced by silylation with the reagent trimethylsilylimidazole. Similarly the peak at *m/z* = 769 is the hexakis(trimethylsilyl)-IAA-*myo*-inositol produced by fragmentation of the ion at *m/z* = 1147, whereas the ion at *m/z* = 697 is the pentakis(trimethylsilyl) compound, with a trimethylsilyl group lost from the indole nitrogen atom.

Fragment (*m/z*)	Relative abundance			Possible origin	Reference
	Peak I	Peak II	Peak III		
1147	2.4	0.3	0.3	IAA-inositol galactoside	—
1075	0.3	1.7	1.5	IAA-inositol galactoside	Ueda & Bandurski (1974)
769	21	2	1	IAA-inositol	Hall (1980)
697	14	30	5	IAA-inositol	Hall (1980)
434	10	11	2	—	—
433	22	26	3	IAA-inositol	Hall (1980)
429	13	12	15	—	—
361	73	76	43	Galactose	Ehmann (1974)
229	99	24	18	IAA-inositol	Hall (1980)
217	91	99	96	Inositol	Sherman *et al.* (1970)
204	99	99	99	Inositol	Sherman *et al.*(1970)
202	99	12	9	IAA-inositol	Hall (1980)

the products of the reaction are identical with those of the authentic IAA-*myo*-inositol galactoside isolated and characterized from *Z. mays* kernels, and inositol glucosides have different g.l.c. retention times from those of inositol galactoside (Ueda & Bandurski, 1970); (3) despite careful examination (Ueda & Bandurski, 1970), IAA-*myo*-inositol glucosides have never been found to occur in kernels of *Z. mays*.

Evidence for the identity of the enzyme products

may be summarized as follows: (*a*) the appearance of the products during the enzyme reaction depended on the addition of IAA-*myo*-inositol as one of the substrates; (*b*) the glucose moiety from UDP-glucose was not transferred to IAA-*myo*-inositol, but with UDP-[U-^{14}C]galactose as substrate a radioactive product resulted, showing the presence of galactose in the products; (*c*) the products co-chromatographed with authentic samples of IAA-*myo*-inositol galactoside on PA-28 h.p.l.c., Partisil 10 h.p.l.c., silica-gel t.l.c. and OV-17 g.l.c.; (*d*) the products reacted with Ehmann's reagent, showing the presence of an indole; (*e*) the u.v.-absorption spectra of the products corresponded to that of an indole; (*f*) mass-spectral analysis showed that the products had a molecular weight that corresponded to that of IAA-*myo*-inositol galactoside, and the main fragments corresponded to those previously observed for IAA-*myo*-inositol galactoside. We conclude that IAA-*myo*-inositol galactoside is formed by enzyme extracts from immature kernels of *Z. mays*.

Discussion

Michalczuk & Bandurski (1980, 1982) studied the enzymic synthesis of IAA-*myo*-inositol and showed the following reaction sequence:

$$\text{IAA-OH} + \text{UDP-glucose} \rightarrow \text{IAA-glucose} + \text{UDP} \quad (1)$$

$$\text{IAA-glucose} + \textit{myo}\text{-inositol} \rightarrow \text{IAA-}\textit{myo}\text{-inositol} + \text{glucose} \quad (2)$$

Now, with the additional enzyme described in the present paper, we can account for the synthesis of most of the esters of IAA-OH found in *Zea mays*:

$$\text{IAA-}\textit{myo}\text{-inositol} + \text{UDP-galactose} \rightarrow \text{IAA-}\textit{myo}\text{-inositol galactoside} + \text{UDP} \quad (3)$$

Naccarato *et al.* (1975) isolated 6-*O*-β-D-galactopyranosyl-*myo*-inositol (6-β-galactinol) from rat mammary gland and from rat milk. This compound is made by a β-galactosidase that catalyses the transfer of a galactose residue from lactose to *myo*-inositol. The enzyme could also transfer a galactose residue from *o*- or *p*-nitrophenyl β-galactopyranoside to *myo*-inositol (Kuo & Wells, 1978). By contrast, the *Z. mays* enzyme that transfers galactose to IAA-*myo*-inositol does not use *p*-nitrophenyl α- or β-galactopyranoside as substrate, but uses UDP-galactose among the substrates tested. The biosynthesis of IAA-*myo*-inositol galactoside more closely resembles that of galactinol (1-*O*-α-D-galactopyranosyl-*myo*-inositol) which involves the transfer of a galactosyl residue from UDP-galactose to free inositol (Frydman & Neufeld, 1963).

Galactinol is important in the biosynthesis of oligosaccharides in plants (Tanner & Kandler, 1966). Enzymes from several plant sources catalyse transfer of the galactosyl moiety of galactinol to form oligosaccharides and free inositol. Inositol glucosides were also involved in the synthesis of callose, a β-1,3-glucan synthesized by *Phaseolus vulgaris* (Kemp *et al.*, 1978). Thus there is precedent that inositol glycosides may act as glycosyl-transfer reagents.

The physiological role of IAA-*myo*-inositol glycosides is not known, although it has been proposed that these compounds can be hydrolysed to yield IAA-*myo*-inositol in the germinating kernel. The enzymic experiments described in the present paper demonstrate that indeed IAA-*myo*-inositol glycosides can be hydrolysed to yield IAA-*myo*-inositol.

The reaction described in the present paper now permits the synthesis of radioactively labelled IAA-*myo*-inositol galactoside. It should now be possible to study the rate of hydrolysis of this compound in the germinating seed, as well as to determine its products. It should also be possible to determine whether IAA-*myo*-inositol galactoside is transported to the shoot during germination and whether it plays a role similar to that of galactinol as a galactosyl-transfer reagent.

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