A PARTIAL CHARACTERIZATION OF INDOLEACETYLINOSITOLS FROM *ZEA MAYS*[1]

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This is a report of the isolation from corn kernels, of four esters of indole-3-acetic acid (IA) and myo-inositol. Two of the esters, B_1 and B_2, contain IA and inositol and two, B_3 and B_4, contain IA, inositol and arabinose. The conjugation of IA with inositol is of possible physiological significance, since IA is a plant growth hormone and inositol is a growth factor (Pollard *et al*, 1961, Letham, 1963). Other reports have indicated the existence of IA conjugates in plant tissues (cf. Andreae and Good, 1955, Hamilton *et al*., 1961, Zenk, 1964).

Methods and Results

Preparative procedure: Fifteen kg of ground corn kernels (*Zea mays*, var. Stowells Evergreen Hybrid) were extracted twice with 30 liters of 50 per cent aqueous acetone (Berger and Avery, 1944). The combined extracts were saturated with NaCl and the acetone phase and acetone washings of the aqueous phase saved. Upon concentration, *in vacuo*, a precipitate and an aqueous supernatant fluid were obtained. A limited study of the precipitate, the "bound auxin" fraction of Berger and Avery, has been made (Labarca, 1965). Forty g aliquots of the solid obtained by lyophilization of the supernatant fluid were subjected to adsorption chromatography on 1 liter bed volume columns of Sephadex G-25, using water as eluant. The IA containing fractions were lyophilized, redissolved in water, and chromatographed in aliquots of 20 mg

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on 57 x 46 cm sheets of Whatman No. 1 paper (solvent 1)[4]. After 20 hours of irrigation four Ehrlich positive, Salkowski negative bands were observed at 44, 41, 37 and 31 cm from the origin. They were designated, according to decreasing mobility, as B_1, B_2, B_3 and B_4. After elution, lyophilization, and rechromatography on sephadex G-25, a total of 56 mg of B_1 (containing 34.0% IA), 50 mg B_2 (26.5% IA), 42 mg B_3 (20.5% IA) and 36 mg B_4 (22.2% IA) were obtained. These fractions were free from unesterified IA, inositol and reducing sugars but were contaminated with varying amounts of glucosides which could be removed by thin layer chromatography (TLC) on silica gel H (solvent 10). The TLC purified fractions, B_1 and B_2, yielded only IA and inositol on alkaline hydrolysis while alkaline hydrolysis of fractions B_3 and B_4 yielded IA and a non-reducing moiety of low chromatographic mobility. This moiety in turn yielded inositol and arabinose on acid hydrolysis.

Identification and estimation of IA: IA was identified as one moiety of B_1, B_2, B_3 and B_4 by paper chromatography of the products of alkaline hydrolysis

4. Ammonolysis-Concentrated NH_4OH at room temperature for 16 hours.
Alkaline hydrolysis-pH 12 for 5 to 10 minutes at 60°.
Estimation of IA-After alkaline hydrolysis, solutions were acidified to pH 2.5, extracted with peroxide-free ether, and IA estimated spectrophotometrically or, after drying, by the Salkowski reagent.
Acid hydrolysis-After removal of IA by alkaline hydrolysis and ether extraction, the conjugates were hydrolysed in 1.5 N H_2SO_4 for 1 hr at 120°.
Enzymatic hydrolysis-Snail gut juice (Suc d'*Helix pomatia*, Industrie Biol. France S.A., Gennevilliers, France) was diluted with 2 volumes of 0.2 M phosphate, pH 6.0 and clarified by centrifugation. For 1 ml of a 1 mg/ml solution of the compounds, 0.02 ml of the enzyme preparation was used. Incubation was for 48 hours at 30° and the reaction terminated by boiling.
Chromatographic procedures-See (Block *et al.*, 1958, Stahl, 1965). Reference by number to the solvent system used is made in the text. For descending chromatography on Whatman No. 1 paper: 1. n-butanol:acetic acid:water, 5:1:2.2; 2. n-butanol:acetic acid:water, 5:1.5:3.5; 3. ethyl acetate: pyridine:water, 8:2:1; 4. isopropanol:acetic acid:water, 5:1:7.5; 5. phenol:water, 4:1. TLC was on 250μ layers of silica gel G or H or on Kieselguhr G with the following solvents: 6. as for paper but double development; 7. ethyl acetate:methyl ethyl ketone:formic acid:water, 5:3:1:1; 8. chloroform:ethyl acetate:formic acid, 5:4:1; 9. n-butanol: pyridine:water, 81:10:9; 10. as 7 except ethanol substituted for formic acid.
Spray reagents-for indolic compounds were Ehrlich's or Salkowski's reagent on paper and p-dimethylaminocinnamaldehyde (DMAC) on TLC (Durkee and Sirois, 1964), and for carbohydrates, silver nitrate dip reagent, aniline phthalate or p-anisidine on paper and aniline phthalate or benzidine-periodate on TLC.

(solvent 1); by TLC of the same products on silica gel G (solvent 8); by the color reactions of the appropriate spots with Salkowski's and Ehrlich's reagents and DMAC; and by the UV absorption spectrum of ether extracts of the alkaline hydrolysates (λmax. 291, 281, and 274 mμ). The IA content of the ether extracts (Table I) was estimated by the Salkowski reagent (Tang and Bonner, 1947) and by UV spectrophotometry (molar absorbancy index, 5.6×10^{-3} at 281 mμ). The two methods agreed within 10%.

Table I

*Molar Ratios of IA, Inositol and Arabinose**

Compound	IA	Inositol	Arabinose
B_1	1.00	1.08	0.00
B_2	1.00	0.91	0.00
B_3	1.00	1.01	0.74
B_4	1.00	not determined	0.88

*Samples of 0.6 mg of each compound in 0.3 ml of water were hydrolyzed in alkali, extracted with ether to estimate IA and then hydrolyzed in acid. The resultant solutions were deionized by treatment with Dowex 3 (HCO_3^- form) and IRC 50 (H^+ form), concentrated and chromatographed on silica gel G (solvent 6). Appropriate sectors of the plate, as determined by authentic markers, were eluted with 80 per cent ethanol, the eluates evaporated to dryness, dissolved in water and analyzed for reducing sugar (Nelson, 1944) or inositol (Saio, 1964).

Identification and estimation of inositol: Inositol was identified by chromatography on paper (solvents 2, 3, 4 and 5), by TLC on silica gel (solvent 6) and kieselguhr (solvents 7 and 9), and by electrophoretic mobility on paper in borate buffer 0.05 M, pH 9.2. In addition a 10 mg sample of B_1 was hydrolyzed with Permutit SKF resin. The hydrolysate gave a positive Scherer test (Feigl, 1960). Following lyophilization of the hydrolysate, 1.1 mg of a white powder which melted at 221-223° was obtained. The mixed melting point with authentic myo-inositol was 221-225°, while the melting point of authentic myo-inositol was 224-225°. The amount of inositol in hydrolysates was determined as described in Table I.

Identification and estimation of arabinose: Arabinose in hydrolysates of B_3 and B_4 migrated coincidently with authentic arabinose on paper (solvents 1 and 3), on silica gel G (solvent 6) and silica gel H (solvent 10). It was visualized on paper with silver nitrate or by its color reaction with aniline phthalate or p-anisidine. Detection on TLC was with aniline phthalate or benzidine periodate. Quantitative data are shown on Table I.

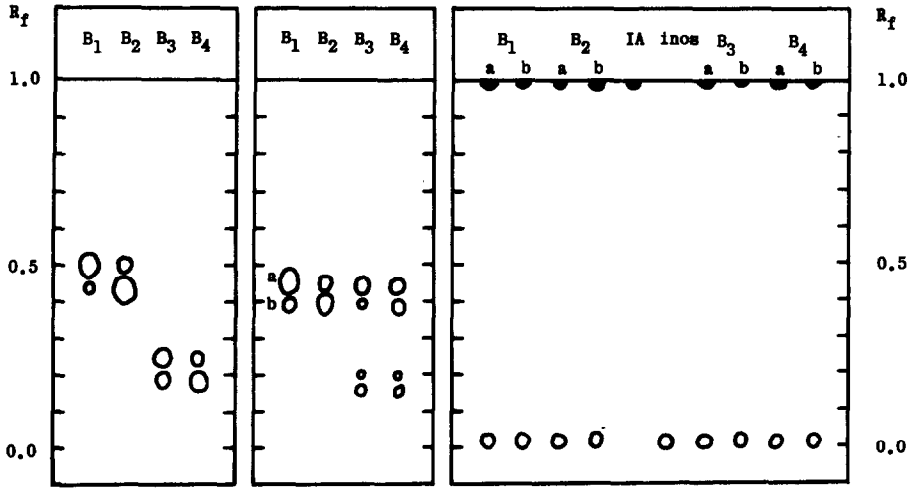
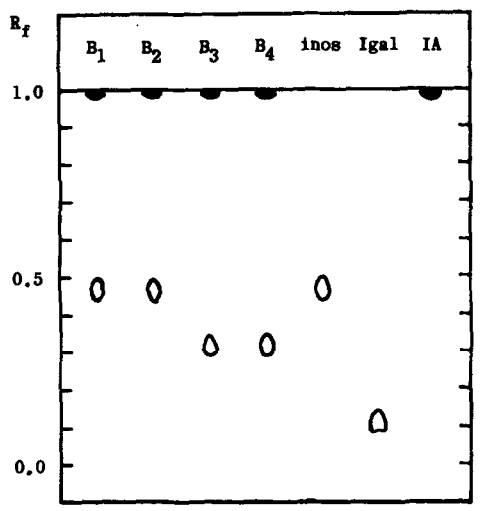


Fig. 1. The linkage of IA to inositol. Fig. 1a (left) The mobility of the isomeric pairs B_1-B_2 and B_3-B_4. Fig. 1b (center) The mobility of the compounds after treatment with snail gut juice. In this experiment incubation was stopped before B_3 and B_4 were completely hydrolyzed. Fig. 1c (right) By use of DMAC reactive guide spots, eight sectors of plate 1b (B_1 a and b, B_2 a and b, B_3 a and b, B_4 a and b) were eluted, hydrolyzed with alkali and rechromatographed on replicate plates. IA was located with DMAC and inositol with benzidine-periodate. Chromatography was on silica gel (solvent 7).

Isomerization of B_1-B_2 and B_3-B_4: The double spots shown in Fig. 1 a and b arose from originally chromatographically homogeneous compounds. Storage of solutions of B_3 and B_4 resulted in partial conversion of B_3 to B_4 and B_4 to B_3. These compounds were originally widely separated on a paper chromatogram and could not have been cross-contaminated. A similar observation has been made for B_1 and B_2. This interconversion is understandable since acyl migration is expected in inositol esters (Lemieux, 1964).

Linkage of IA to inositol: We conclude that IA is esterified through its



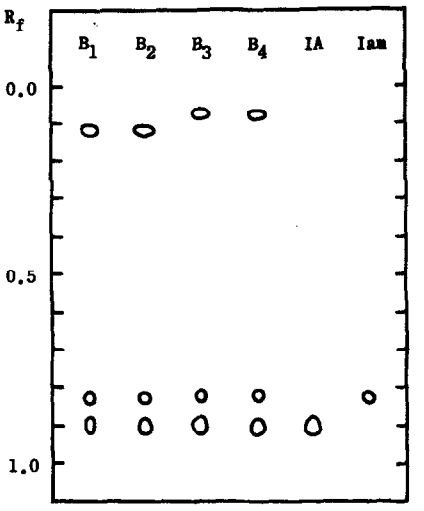


Fig. 2. (left) TLC on kieselguhr G (solvent 7) of the products of alkaline hydrolysis of B_1, B_2, B_3 and B_4 and the following authentic compounds: myo-inositol (inos), D-l-myo-inositol-α-D-galactopyranoside (Igal) and IA.
Fig. 3. (right) Paper chromatography (solvent 1) of the products of ammonolysis of B_1, B_2, B_3, B_4 and authentic IA and indoleacetamide (Iam).

carboxyl to inositol in all four compounds for the following reasons. Snail gut juice (mixed glycosidases) converts B_3 and B_4 into B_1 and B_2 (Fig. 1b). B_1 and B_2 contain only IA and inositol. Mild alkaline hydrolysis liberates IA (Fig. 2) while ammonolysis yields IA and indoleacetamide (Fig. 3). The latter reaction could only occur if the IA carboxyl was in a reactive bond, such as an ester bond. Inositol is esterified since free inositol reacts with alkaline silver nitrate while the esters B_1 and B_2 are very faintly reactive, as expected for an inositol ester (Angyal, 1957).

Linkage of inositol to arabinose: Alkaline hydrolysis or ammonolysis of B_3 and B_4 yield, in addition to IA or indoleacetamide, a non-reducing substance of low chromatographic mobility (Figs. 2 and 3) which, like inositol, reacts with silver nitrate and benzidine periodate. On silica gel H (solvent 10) using double development, this substance moves to hR_f 0.2 while free inositol moves to 0.4. After elution, acid hydrolysis and rechromatography, inositol at hR_f 0.4 and arabinose at hR_f 3.0 are obtained. B_3 and B_4 therefore contained an inositol arabinoside.

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