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The Chemical Synthesis of 1-*O*-(Indol-3′-ylacetyl)-β-D-glucopyranose

THE HIGHER ACTIVITY OF THE GLUCOSIDE IN COMPARISON WITH EXOGENOUS INDOL-3-YLACETIC ACID IN PLANT-SECTION ELONGATION TESTS

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1. The synthesis of 1-*O*-(indol-3′-ylacetyl)-β-D-glucopyranose via the fully benzylated 1-*O*-(indol-3′-ylacetyl)-D-glucopyranose is described. The configuration of the free ester glucoside was confirmed by complete hydrolysis with β-glucosidase and by the n.m.r. spectrum of the tetra-acetyl derivative. 2. The growth-promoting effect of the glucoside in *Avena* coleoptile- and pea stem-section tests distinctly exceeds the responses stimulated by equimolar amounts of indol-3-ylacetic acid or equimolar mixtures of indol-3-ylacetic acid and glucose at all concentrations investigated. Time-sequence experiments revealed that the sections stimulated by the glucoside exhibit a markedly greater rate of elongation than those promoted by indol-3-ylacetic acid. 3. 1-*O*-(Indol-3′-ylacetyl)-β-D-glucopyranose was isolated from intact *Avena* coleoptiles. 4. According to the results, the conjugation of indol-3-ylacetic acid with glucose could not be considered merely as a detoxication mechanism for indol-3-ylacetic acid in plant tissues.

Although numerous studies have been concerned with the plant hormone IAA*, surprisingly little is known about the metabolism of the acid and its mode of action in plant tissues. In recent years several indolic compounds exerting auxin-like stimulations have been detected in plants; however, their activity could be interpreted as being due to IAA itself because in each case a conversion *in situ* into IAA could be presumed (Zenk, 1961*a*). Experiments performed in plant tissues with exogenously supplied IAA revealed that the acid can be converted into some complexes with amino acids, sugars, or both. Thus, *N*-(indol-3-ylacetyl)-aspartic acid was recognized as a metabolite of added IAA in a variety of plant tissues (Andreae & Good, 1955; Good, Andreae & Ysselstein, 1956); it has since been found to occur naturally in plants (Klämbt, 1960). Zenk (1961*b*) found that the leaves of the monocotyledonous plant *Colchicum neapolitanum* Ten. converted about 65% of exogenously supplied IAA into an IAA–glucose conjugate; the purified non-crystalline preparation showed properties characteristic of an ester linkage in the C-1 position of the β-glucose. Consequently, Zenk (1961*b*) assigned the 1-*O*-(indol-3′-ylacetyl)-β-D-glucose structure to the metabolite, and reported it to be active in the *Avena* cylinder test. Similar results were reported by Klämbt (1961) who studied the metabolism of exogenous IAA in wheat coleoptile tissues. Both authors considered the conjugation with glucose as a detoxication pathway of IAA in the plant, although Klämbt (1961) suggested that such compounds might also play a key role in the synthesis of cellulose.

Some evidence for the natural occurrence of IAA-monosaccharide complexes has been obtained by Shantz & Steward (1957) and Steward & Shantz (1959) who isolated an IAA–arabinose complex from corn. In addition, Srivastava (1963, 1964) separated from maize kernels a water-soluble fraction containing several indolic compounds, all of which were auxin-active and yielded free IAA upon hydrolysis with alkali; one of them gave arabinose as a product of hydrolysis.

Studies on the biological role of the above IAA–monosaccharide complexes are seriously hampered by the fact that our knowledge of the chemical and physical properties of these compounds is very small. After our work on the synthesis of 1-*O*-acyl-D-glucopyranuronic acids via the fully benzylated 1-*O*-acyl-D-glucopyranuronates (Keglević, Pravdić & Tomašić, 1968) it was decided to determine whether the same synthetic pathways could lead to the IAA ester glycosides. In the present paper the preparation of 1-*O*-(indol-3′-ylacetyl)-β-D-glucopyranose is described, and results pointing

* Abbreviation: IAA, indol-3-ylacetic acid.

to its high stimulatory effect in plant-cell-extension assays are reported.

MATERIALS AND METHODS

Indol-3-ylacetic acid, from British Drug Houses Ltd. (Poole, Dorset), was recrystallized from benzene before use. The silver salt of indol-3-ylacetic acid was prepared by the general procedure of Wilson (1957). 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranose was a gift from Dr H. G. Fletcher, jun. Drierite (Koch–Light Laboratories Ltd., Colnbrook, Bucks.) was dried at 110° and powdered before use. β-Glucosidase (from sweet-almond meal, 400 units/mg.) was supplied by British Drug Houses Ltd. The n.m.r. spectra were obtained in deuterochloroform solution by using a Varian A-60*A* spectrometer and tetramethylsilane as an internal standard.

Chromatographic methods. T.l.c. was carried out on microscope slides and 20cm.×5cm. glass slides coated with Kieselgel G (E. Merck A.-G., Darmstadt, Germany). The solvent systems were as follows: solvent 1, light petroleum–ether (2:1, v/v); solvent 2, ether–light petroleum (3:2, v/v); solvent 3, ether–light petroleum–methanol (6:2:1, v/v); solvent 4, propan-2-ol–light petroleum–water (55:30:11, v/v), (Zenk, 1961*b*); solvent 5, butan-1-ol–acetic acid–water (12:3:5, v/v); solvent 6, propan-2-ol–aq. NH_3 (sp.gr. 0·88)–water (10:1:1, v/v); solvent 7, methanol–water (1:1, v/v). Light petroleum is the fraction of b.p. 60–90°.

Paper chromatograms on Whatman no. 1 paper were developed by downward elution in solvents 5, 6 and 7.

Column chromatography was performed on silica gel, particle size 0·2–0·5mm. (E. Merck), alumina, standardized, acidic (E. Merck), or cellulose powder, standard grade (Whatman), packed as a slurry by using a plunger.

Detection methods used were: charring with 10% aq. (v/v) H_2SO_4 on t.l.c. plates (giving intensive red-violet spots with indolic compounds); spraying with Ehrlich's reagent [2% (w/v) *p*-dimethylaminobenzaldehyde in 2M-HCl] on thin-layer and paper chromatograms; exposure to iodine vapour.

Enzymic hydrolysis. Incubations with β-glucosidase were carried out in 0·1M citric acid–sodium-citrate buffer, pH 5, at 35° as described by Baruch & Swain (1957) for 1hr. The products were examined by t.l.c. in solvents 4, 5 and 7.

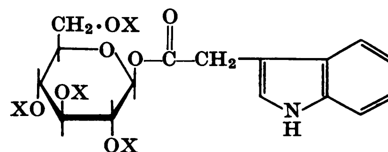
Plant material and cell extension assays. Oat (*Avena sativa* var. Golden Rain) and pea (*Pisum sativum* var. Express) seeds were soaked in tap water at room temperature for 24hr. and then germinated in prewashed water-moistened sand at 25–26° in darkness with daily exposure to red light for 20min. *Avena* coleoptiles (1·8–2·0cm. long) were selected, sections (5mm.) were cut off, kept 2hr. in twice-distilled water, and then incubated with various concentrations of substrate in twice-distilled water at 25° in the dark as described by Bentley (1950). Pea epicotyls (15–18cm. long) were selected, segments (10mm.) were excised from the basis of the fourth internode and treated as described above (Kent & Gortner, 1951). Length measurements were determined to the nearest 0·1mm. by use of a dissection microscope equipped with an ocular micrometer. For the slit-pea-stem curvature test, segments (3·6cm.) from the upper part of the third internode were selected, a median slit reaching 3cm. downward was made, and the sections were incubated as described by Kent & Gortner (1951).

EXPERIMENTAL

*2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl chloride* (I). This was synthesized from 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose and thionyl chloride by the method of Austin, Hardy, Buchanan & Baddiley (1964) except that the heating was omitted, and the mixture was kept at 0° for 48hr. After the removal of thionyl chloride, the material was rapidly passed through a silica gel column with solvent 1; longer contact with silica gel caused decomposition of the product into tetrabenzylglucose. By this means, 70–75% of chromatographically pure chloride (I) was obtained as a light-yellow oil showing $[\alpha]_D + 123 \pm 2°$ (*c* 3·2 in benzene); Austin *et al.* (1964) quote $[\alpha]_D + 95°$ in the same solvent.

*2,3,4,6-Tetra-*O*-benzyl-1-*O*-(indol-3′-ylacetyl)- β- and α-D-glucopyranose* (II). The chloride (I) (5·14g., 9·22m-moles), silver salt of IAA (2·59g., 9·22m-moles), and 1·5g. of powdered Drierite were refluxed in 100ml. of dry benzene for 6hr. with vigorous mechanical stirring and exclusion of moisture; the reaction was monitored by t.l.c. in solvent 2. The precipitate was collected by centrifugation, washed with dry benzene and the combined supernatants were concentrated *in vacuo* to a syrup which was chromatographed on a silica gel column (67cm.×1·8cm.) with solvent 2; fractions (3ml.) of the eluate were collected. After displacement of the unchanged chloride (I), the ester product (II) (4·53g.) emerged from the column contaminated with some coloured material; further elution of the column with chloroform displaced tetrabenzylglucose. The anomeric composition of the product was followed by t.l.c. in solvent 2 on large Chromatoplates; charring with 10% H_2SO_4 gave a violet fused double spot. Fractions in which the slower-moving part of the spot corresponding to the β-anomer, prevailed, were pooled, evaporated to dryness, and the residual oil was dissolved in dry benzene and subsequently precipitated with light petroleum. After several hours at 0°, the β-anomer of product (II) deposited as a crystalline solid (2·06g., 32%, m.p. 89–95°); two further crystallizations from benzene–light petroleum gave the pure β-anomer of 2,3,4,6-tetra-*O*-benzyl-1-*O*-(indol-3′-ylacetyl)-D-glucopyranose (II), m.p. 99–100°, $[\alpha]_D - 1·7 \pm 1°$ (*c* 1 in $CHCl_3$). (Found: C, 75·69; H, 6·38; N, 2·09. $C_{44}H_{43}NO_7$ requires C, 75·73; H, 6·21; N, 2·01%). $\nu_{max.}$ 3300 (sharp; indolic NH), 1750 (strong; ester C:O) $cm.^{-1}$. τ 4·32 (d, $J_{1,2}$ 7·5Hz, H-1, β-anomer). Re-fractionation of the remaining anomeric mixture raised the yield on the crystalline β-anomer of compound (II) to 46%.

Fractions giving the predominantly faster moving part of the double spot corresponding to the α-anomer of compound (II) were pooled and evaporated to dryness, and the



(II-β); $X = C_6H_5 \cdot CH_2$
(III) ; $X = H$
(IV) ; $X = CH_3 \cdot CO$

residual oil was fractionated on an acidic alumina column (15g.) with solvent 2. Fractions containing highly enriched α-anomer of compound (II) were then rechromatographed on a silica gel column. By this means, chromatographically homogeneous 2,3,4,6-tetra-*O*-benzyl-1-*O*-(indol-3′-ylacetyl)-α-D-glucopyranose (II-α) was obtained as a viscous oil (128mg., 2%) showing $[\alpha]_D + 55{\cdot}8 \pm 1°$ (*c* 1 in $CHCl_3$). Found: C, 75·61; H, 6·07; N, 1·98. $C_{44}H_{43}NO_7$ requires C, 75·73; H, 6·21; N, 2·01%). τ 3·58 (d, $J_{1,2}$ 2·2Hz, H-1, α-anomer).

1-O-*(Indol-3′-ylacetyl)-β-D-glucopyranose* (III). To the β-anomer of compound (II) (500mg., 0·717m-moles, m.p. 99–100°) in 2-methoxyethanol (15ml.), 10% palladium on charcoal (500mg., Fluka, puriss.) and acetic acid (0·4ml.) were added, and the mixture was shaken in the presence of H_2 at room temperature and pressure. Samples were taken at 4–5hr. intervals and subjected to t.l.c. in solvent 3. The reaction was stopped when, at the start of the Chromatoplate charred with 10% H_2SO_4, a dark-grey spot began to appear; at this stage ($\sim$24hr. of hydrogenation) the red-violet spot corresponding to the fully debenzylated glucoside ($R_F \sim 0{\cdot}1$) has already reached the greatest intensity of all the other faster-moving indolic spots. The catalyst was removed by centrifugation and washed with 2-methoxyethanol, the combined supernatants were evaporated *in vacuo* (0·1mm. Hg, water bath at 35°) to dryness, and the remaining light-violet oil (286mg.) was dissolved in solvent 4 (1·5ml.) and subjected to chromatography on a cellulose column (25g., 73cm. × 1cm.) with the same solvent. Fractions (1ml./0·5hr.) were examined by t.l.c. in solvent 3 and those (nos. 31–51) containing the free glucoside (III) were pooled, evaporated *in vacuo*, and the oily residue was extracted three times with portions (15ml.) of ethyl acetate with mechanical shaking. The combined extracts were concentrated to about one-third of the volume; light petroleum was added gradually at 0° until no more turbidity was formed, and the mixture was left overnight at 0°. After removal of the supernatant, the residual glassy film was dried *in vacuo* over conc. H_2SO_4 whereupon it solidified and could be scratched to a pink-coloured powder (102mg., 42%). It was extracted with ethyl acetate–propan-2-ol (20:1, v/v), and some drops of light petroleum were added to the filtered solution at 0°; this precipitated the remaining coloured material. The almost colourless supernatant was transferred into a second centrifuge tube where the precipitation with light petroleum was accomplished. After a second identical treatment, 1-*O*-(indol-3′-ylacetyl)-β-D-glucopyranose (III) was obtained as clusters of minute pinkish-white needles (58mg., 24%), m.p. 172–173°, $[\alpha]_D$ 0° (*c* 1 in water), $[\alpha]_D$ $+5 \pm 2°$ (*c* 1 in methanol). (Found: C, 57·21; H, 5·84; N, 4·04. $C_{16}H_{19}NO_7$ requires C, 56·97; H, 5·68; N, 4·15%). $\nu_{max.}$ 3370 (very strong; OH, indolic NH), 1770 (ester C:O) cm.$^{-1}$. $\lambda_{max.}^{EtOH}$ 280 (ϵ 6000), $\lambda_{max.}^{Etoh}$ 290nm. (ϵ 5000). R_F (paper chromatography): 0·70 (solvent 5); 0·0–0·1, 0·40 (IAA), 0·74, 0·83 (indoleacetamide) (solvent 6); 0·72 (solvent 7). The compound was highly soluble in water, methanol and propan-2-ol, very sparingly soluble in ethyl acetate and insoluble in ether, chloroform and benzene.

2,3,4,6-Tetra-O-acetyl-1-O-(indol-3′-ylacetyl)-β-D-glucopyranose (IV). Method *A*. The glucoside (III) (20mg.) was dissolved in a precooled mixture of acetic anhydride–dry pyridine (1:1, v/v; 2ml.); the solution was kept overnight at 0°, then thrown on ice. The product was extracted with ether and washed consecutively with cold water, 3% H_2SO_4 water, $NaHCO_3$ solution and water. The ethereal solution was dried (over Na_2SO_4), evaported to dryness, and the residue was fractionated on a silica gel column (8g., 43·5cm. × 0·9cm.) with benzene–ethyl acetate (1:1, v/v). The chromatographically homogeneous syrup solidified upon drying *in vacuo* over conc. H_2SO_4; dissolution in dry ether and addition of light petroleum precipitated white needles of 2,3,4,6-tetra-*O*-acetyl-1-*O*-(indol-3′-ylacetyl)-β-D-glucopyranose (IV) (21mg., 70%), m.p. 121–122°, $[\alpha]_D$ $-26{\cdot}0 \pm 2°$ (*c* 1 in $CHCl_3$). (Found: C, 57·17; H, 5·37; N, 2·87. $C_{24}H_{27}NO_{11}$ requires C, 57·03; H, 5·38; N, 2·77%). $\nu_{max.}$ 3330 (indolic NH), 1750 (C:O) cm.$^{-1}$. τ 1·7 (s, indolic NH), 4·28 (d, $J_{1,2}$ 7·8Hz, H-1, β-anomer, 7·93, 7·99, 8·07, and 8·41 ($CH_3{\cdot}CO{\cdot}O$, 12H).

Method *B*. 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (1·23g.) (Redemann & Niemann, 1955) was dissolved in dry benzene (25ml.); the silver salt of IAA (843mg.) was then added and the mixture was shaken at room temperature for 24hr. in the dark. The precipitate was collected by centrifugation and washed with benzene; the combined supernatants were evaporated to dryness, and the residue was chromatographed on a silica gel column as described above. The syrup thus obtained gave on recrystallization from dry ether–light petroleum 2,3,4,6-tetra-*O*-acetyl-1-*O*-(indol-3′-ylacetyl)-β-D-glucopyranose (IV) (0·64g., 42%), m.p. 121–122°, $[\alpha]_D$ $-27{\cdot}0 \pm 2°$ (*c* 1·1 in $CHCl_3$). (Found: C, 57·39; H, 5·52; N, 2·90. Calc. for $C_{24}H_{27}NO_{11}$: C, 57·03; H, 5·38; N, 2·77%). Infrared and n.m.r. spectra corresponded exactly to those of (IV) prepared from the glucoside (III).

Isolation of the glucoside (III) *from intact* Avena *coleoptiles*. A batch (7g.) of etiolated *Avena* coleoptiles (2–3cm.) was homogenized in 50% aq. (v/v) methanol (40ml.), the precipitate was collected by centrifugation, the supernatant evaporated *in vacuo* at 25° to about ¼ of the volume, and to the concentrate, methanol (30ml.) was added, by means of which a second precipitation took place; this process was repeated four times. The final supernatant was evaporated *in vacuo*, the residual oil dissolved in solvent 4 (0·5ml.) and chromatographed on a cellulose column (65cm. × 1·1cm.) with solvent 4 as described for the glucoside (III). Fractions containing the indole-positive spot with R_F values that coincided to those of the glucoside (III) were pooled, concentrated *in vacuo* and rechromatographed on a second cellulose column (40cm. × 0·4cm.) with the same solvent. Fractions containing the chromatographically homogeneous indolic compound were pooled, evaporated to dryness, the residue was extracted with ethyl acetate, the extracts were filtered and evaporated *in vacuo*. The amount of the residual glassy film (12–15μg.) was estimated by co-chromatography on thin-layer plates with a series of samples of synthetically prepared glucoside (III).

RESULTS AND DISCUSSION

In order to obtain the fully benzylated 1-*O*-(indol-3′-ylacetyl)-D-glucopyranose (II) a number of preliminary experiments were carried out. 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranose and IAA in the presence of dicyclohexylcarbodi-imide gave only negligible amounts of the desired product and from the reaction mixture, appreciable quantities

of N-indol-3′-ylacetylurea were isolated. The reaction of 2,3,4,6-tetra-O-benzyl-α-D-glucosyl bromide (Weygand & Ziemann, 1962) with the silver salt of IAA failed because even under very mild conditions, most of the bromide decomposed before the reaction. However, 2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl chloride (I) (Austin *et al.* 1964) reacted smoothly in boiling benzene with the silver salt of IAA, yielding the fully benzylated ester glucoside (II) as an anomeric mixture. After silica-gel column chromatography and repeated crystallization, the pure β-anomer of 2,3,4,6-tetra-O-benzyl-1-O-(indol-3′-ylacetyl)-D-glucopyranose (II) was obtained in 30–35% yield. The α-anomer, which was formed in a considerably lower yield, was separated after extensive column chromatography as a viscous oil.

The catalytic hydrogenation of the fully benzylated β-anomer of compound (II) in 2-methoxyethanol proceeded extremely slowly; after 4–5 days of shaking, the hydrogenation mixtures gave up to nine indolic spots on Chromatoplates, the one corresponding to the free ester glucoside not being among the strongest. The addition of small amounts of acetic acid substantially accelerated the uptake of hydrogen; however, under these conditions the absorption of hydrogen continued beyond the values required for the removal of the four benzyl groups. Thin-layer Chromatoplates of such hydrogenation mixtures revealed, besides the free ester glucoside and traces of partially debenzylated intermediates, several indole-negative spots; these increased with an increase in the amount of acetic acid added.

It has been reported (Kuhn & Butula, 1968) that the addition of acetic acid enhances the catalytic reduction of the indole nucleus. Hence, it appears that in the presence of acetic acid the catalytic debenzylation of the glucoside (II) is closely followed by the reduction of the indole ring. In fact the two reactions partly overlap and therefore the measured amount of hydrogen absorbed is not a true indicator of the completeness of the reaction. In addition, the intensity of the spots on Chromatoplates charred with sulphuric acid does not reflect the true ratio of the components of the hydrogenation mixture because the sensitivity of the indolic compounds exceeds by several times the sensitivity of non-indolic components. It should be emphasized that in a number of experiments performed, there was never a notable amount (more often not even a trace) of IAA to be detected, this indicating the stability of the C-1 ester bond under hydrogenation conditions.

Purification of the free glucoside was achieved by cellulose-column chromatography, followed by fractional crystallization. The pure 1-O-(indol-3′-ylacetyl)-β-D-glucopyranose (III) was surprisingly stable both in crystalline form and in aqueous solution, but in alkaline media, a rapid cleavage of

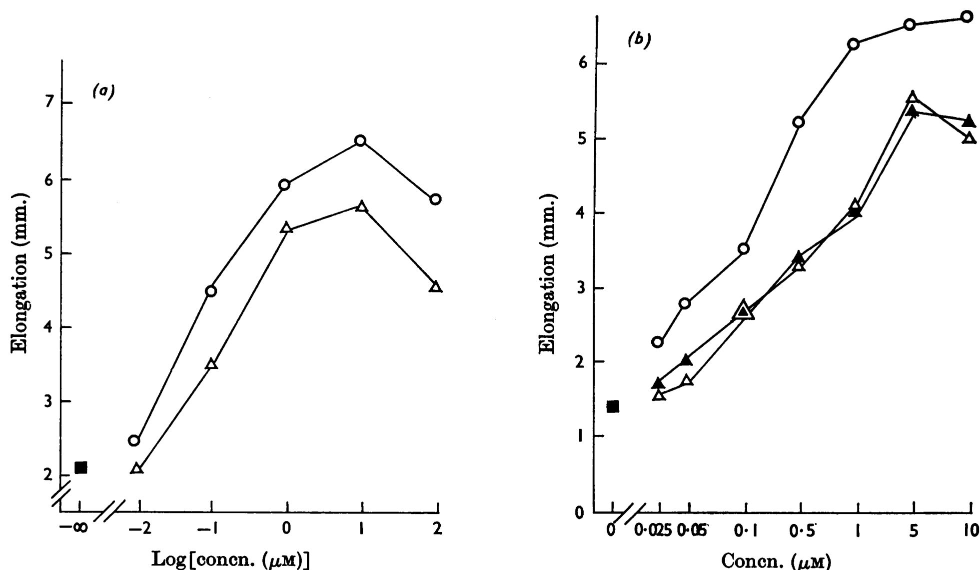


Fig. 1(*a*) and (*b*). Increase in length of *Avena* coleoptile sections as a function of substrate concentration. Batches of 20 stem sections were incubated with the substrate dissolved in 10 ml. of twice-distilled water at 25° in darkness for 24 hr.; each point represents the mean for 20 sections. ○, 1-O-(Indol-3′-ylacetyl)-β-D-glucopyranose (III); △, IAA; ▲, equimolar amounts of IAA and D-glucose; ■, control.

the C-1 ester bond took place. The configuration of the glucoside (III) was established by enzymic and chemical methods. Incubation with β-glucosidase resulted in the complete hydrolysis of the compound into IAA and glucose. Acetylation of compound (III) in acetic anhydride–pyridine led to the crystalline β-anomer of 2,3,4,6-tetra-*O*-acetyl-1-*O*-(indol-3′-ylacetyl)-β-D-glucopyranose (IV), the n.m.r. spectrum of which revealed the *trans*-diaxial arrangement of hydrogen substituents at C-1 and C-2. In addition, the reaction product obtained from 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide and the silver salt of IAA proved to be identical in every way with the acetylated glucoside (IV), prepared from the free ester glucoside (III).

Biological auxin tests

The growth-promoting effect of 1-*O*-(indol-3′-ylacetyl)-β-D-glucopyranose (III) in *Avena* coleoptile section tests was studied in parallel experiments with equimolar amounts of IAA and compared with untreated controls. In Fig. 1(*a*) the effects of the glucoside (III) and of IAA are presented over a wide range of concentrations. Compared with the typical 'bell-shaped' curve obtained with IAA, the concentration-versus-growth curve of the glucoside (III) is qualitatively similar, but shows greater growth at all concentrations investigated. Fig. 1(*b*) illustrates the stimulation by glucoside (III), IAA and equimolar mixtures of IAA and glucose at concentrations that are suboptimal and optimal for exogenously supplied IAA. Although the curves for IAA and the mixture IAA and glucose are practically identical, the curve for glucoside (III) is steeper at low concentrations and has a definitely higher and broader maximum at concentrations of 5–10 μM. Thus, it follows that the stimulatory effect of the glucoside (III) starts at lower concentrations than that of IAA (the effect promoted by 0·1 μM-compound (III) is comparable with that of 0·5 μM-IAA), and that at optimum concentrations, which are very similar for both compounds, the growth responses stimulated by the glucoside (III) exceed those promoted by IAA.

Time-sequence investigations (Fig. 2) performed on *Avena* coleoptile sections at concentrations optimum for both substrates revealed that the sections stimulated by the glucoside (III) exhibit a markedly greater rate of elongation than those promoted by IAA. The difference in responses between the two compounds is already seen ½ hr. after incubation, this indicating that there is a shorter lag period of response with the glucoside (III) than with the auxin. After 24 hr. both compounds exhibit maximal but not equal stimulation; the effect of the glucoside is distinctly greater than that of IAA.

In Table 1 the growth-response of pea epicotyls induced by the glucoside (III) and IAA are compared. Under identical conditions, the glucoside

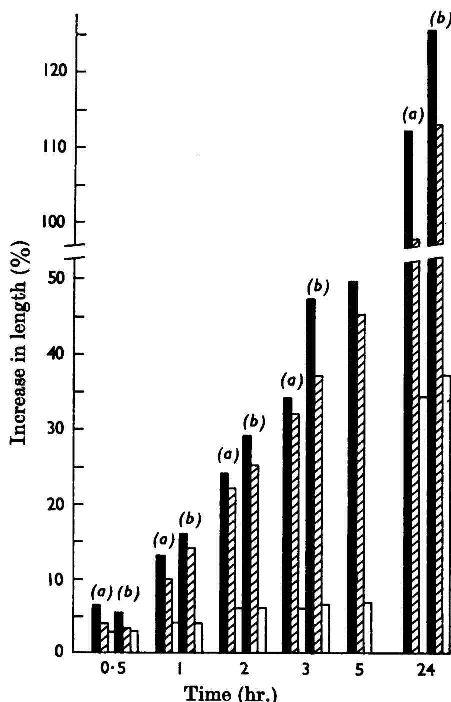


Fig. 2. Increase in length of *Avena* coleoptile sections as a function of time when the concentration of the substrate was 10 μM. Conditions were identical with those in Fig. 1. Each bar represents the mean for 20 sections; two separate experiments (*a*) and (*b*) are illustrated. ■, 1-*O*-(Indol-3′-ylacetyl)-β-D-glucopyranose; ▨, IAA; □, control.

Table 1. *Increase in length of fourth internodes of* Pisum sativum

Epicotyl sections were incubated in 10 ml. of twice-distilled water containing 0·1 mM substrate for 24 hr. at 25° in the dark; each section was initially 10 mm. long. The values are the final lengths in mm. and are presented as means ± S.E.M. of twelve sections/batch. Fisher's *P* values are given in parentheses and indicate the significance of differences between values for glucoside (III) and IAA.

	Lengths (mm.)	
	Expt. I	Expt. II
Glucoside (III)	14·8 ± 0·1	13·8 ± 0·1
IAA	13·4 ± 0·4	13·1 ± 0·2
Control	10·7 ± 0·1	10·7 ± 0·3
	(<0·05)	(<0·05)

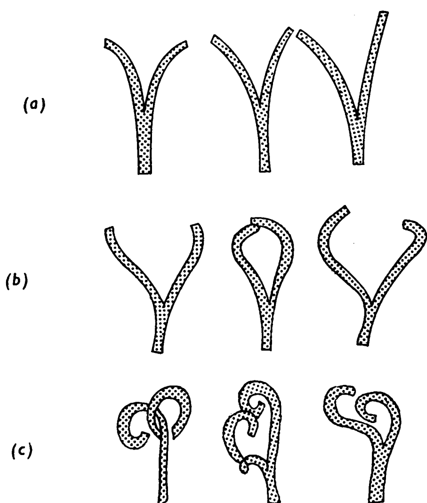


Fig. 3. Slit pea-stem curvature test. Concentration of the substrate was 0·1 mM in twice-distilled water. Conditions used were identical with those in Fig. 1. (*a*) Control; (*b*) IAA; (*c*) 1-*O*-(indol-3′-ylacetyl)-β-D-glucopyranose (III).

has a significantly greater effect than the acid. In the slit pea-stem curvature test also (Fig. 3) the conjugate proved to be superior to the free acid; with the former compound a more pronounced inward curvature was obtained.

An attempt was then made to establish whether 1-*O*-(indol-3′-ylacetyl)-β-D-glucopyranose occurs in normal plant metabolism or is only a metabolic product of exogenously supplied IAA. A large amount of intact *Avena* coleoptiles was subjected to extraction and column-chromatographic procedures, and an indole-positive compound that was chromatographically identical with the synthetically prepared glucoside (III) was isolated. The compound was hydrolysed by β-glucosidase into IAA and glucose, and furthermore in the *Avena* elongation test run in parallel with IAA and synthetically prepared 1-*O*-(indol-3′-ylacetyl)-β-D-glucopyranose, it exhibited the same activity as the conjugate.

Zenk (1964), in discussing the possible significance of the formation of IAA–aspartic acid and IAA–glucose conjugates in ripening fruits, suggested that by accumulation of these metabolites the action of IAA might be blocked at the time when growth ceases in a maturing fruit and IAA is no longer needed (Shantz, 1966). However, the results presented clearly indicate that, at least under the experimental conditions used, the IAA ester glucoside is more active biologically than the acid itself. Hence, the conjugation of IAA with glucose could not be considered as merely a detoxication process by which either exogenous or endogenous superficial IAA is rendered biologically inert. Taking into account the high growth-promoting activity of 1-*O*-(indol-3′-ylacetyl)-β-D-glucopyranose as well as its short lag period of response, one can speculate that this compound, highly soluble in water, takes an active part in the growth-induction mechanism in plant tissues.

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