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SYNTHESIS AND GROWTH REGULATOR ACTIVITY OF FATTY ACYL DERIVATIVES OF D-GLUCOSE AND D-GALACTOSE

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Abstract—In an attempt to gain information about one or more components of the brassin complex, fatty acid esters of D-glucose and D-galactose were prepared and tested for growth regulator activity in a bean hypocotyl bioassay. 4-*O*-Acyl-D-glucoses and, perhaps, 1-*O*-acyl-D-galactoses had a similar qualitative activity to that of the brassin complex. 3-*O*-Acyl-D-galactoses inhibited elongation of bean hypocotyls and stimulated rooting. 3- And 6-*O*-acyl-D-glucoses both stimulated and inhibited elongation, depending on the source of fatty acids; in both cases, stimulation was observed when safflower oil was used as the source of fatty acids and inhibition was observed when peanut oil was used as the source of fatty acids. Fatty alkyl β-D-galactopyranosides were inactive.

INTRODUCTION

In 1970, Mitchell *et al.* [1] reported the isolation of a powerful, ether-soluble, growth regulator from *Brassica napus* pollen. This new growth regulator which has only been isolated as a complex and not as a pure substance, exhibited remarkable biological properties [1–6] and was termed brassin. The purified complex has been analysed by spectroscopic methods [3]. Its ^{1}H NMR spectrum exhibited a pattern characteristic of fatty acids and its IR absorptions were characteristic of long-chain carbon compounds and esters. Analysis of brassins by GC–MS showed that the product contained several components with MWs ranging from 250 to 580. UV absorptions suggested unsaturation. Together, these results pointed to the existence of a fatty acid ester in the chemical structure of brassins [3, 6]. Furthermore, the brassin complex was saponified with alcoholic potassium hydroxide to yield products identified by GC–MS as fatty acids varying in chain length from C_{16} to C_{24}.

Further characterization of the brassin complex revealed 5 active components, all fatty materials. Saponification of the fraction with the greatest biological activity, isolation of the neutral water-soluble component and pertrimethylsilylation gave a substance with a MS identical to that of penta-*O*-trimethylsilyl-D-glucose [7]. Detailed analysis showed that the fatty acid components of this fraction were linolenic acid (28.7%), palmitic acid (22.6%), stearic acid (14.4%), oleic acid (14.4%), linoleic acid (8.2%), myristic acid (10.3%), and a small percentage of C_{20}-C_{22} acids (Mandava, N., unpublished data). The ratio of carbohydrate units to fatty acids was 1:1.

The biological activity of lipid-soluble compounds has become evident by the work of Vlitos and Crosby [8], Stowe and Dotts [9] and Mitchell *et al.* [1]. Interest in this activity has been heightened by the recent report of the powerful growth regulating effect of 1-triacontanol [10].

The present project was designed to synthesize monosaccharide monoesters of fatty acids and to bioassay them for brassin activity. It was hoped that synthesis would produce a component or components of the brassin complex and, hence, reveal the structure of brassin and point to a chemical synthesis that would allow brassin to be produced in quantity.

RESULTS AND DISCUSSION

Results of the bioassays are given in Table 1. Two systems were used for weighing the brassin complex and the test compounds. At the early stage of this investigation, system A was used, in which test compounds or brassin complex were dissolved in ether before transfer to a slide. The ether solution was dropped on a slide and the ether was evaporated rapidly. Thus, test compounds or brassin complex would be left on the slide so that their weights could be measured before they were dissolved in the assay solution. In system B, methylene chloride was used as the solvent. Experimental test results from systems A and B proved that system B was superior in the bioassay. Elongation of hypocotyls showed a great difference when treated by brassin complex in systems A and B. It is thought that system B is superior because ether promoted the formation of peroxides that destroyed growth regulator activity.

For several years, it was believed that the brassin complex was composed of D-glucose esterified at *O*-1. However, Pfeffer *et al.* prepared 1-*O*-hexadecanoyl- [11] and 1-*O*-octadecanoyl- [12] α- and β-D-glucopyranoses, none of which showed any significant biological activity in the bean second-internode assay [12]. We also prepared 1-*O*-fatty acyl-α,β-D-glucopyranose and -D-galactopyranose by alternative routes (unpublished) and observed a slight growth inhibitory effect in the bean hypocotyl bioassay (unpublished data).

In this study, triglycerides were used as the fatty acid source since isolated brassin complex contains a mixture

Table 1. Bean hypocotyl bioassay results for fatty acid esters of D-glucose and D-galactose

Compound	Source of fatty acid	Bioassay system	% of control*	% of brassin response†
1-*O*-Acyl-α,β-D-Glc	safflower oil	A	84	—‡
	linseed oil	A	89	—
3-*O*-Acyl-D-Glc	peanut oil	B	67	10
	safflower oil	B	130	7
4-*O*-Acyl-D-Glc	peanut oil	B	174	—
	safflower oil	B	317	13
	linolenic acid	B	124	—
	erucic acid	B	143	—
6-*O*-Acyl-D-Glc	peanut oil	B	51	8
	safflower oil	B	105	15
Randomly acylated D-Glc	safflower oil	B	88	12
1-*O*-Acyl-α,β-D-Gal	peanut oil	A	120	9
	safflower oil	B	196	11
Alkyl β-D-galacto-pyranosides	safflower oil§	B	66	8
Methyl 2- and 3-*O*-acyl-β-D-galacto-pyranosides	peanut oil	A	94	—
	safflower oil	A	94	—
3-*O*-Acyl-D-Gal	peanut oil	A	37	—
	peanut oil	B	28	3
	safflower oil	A	73	25

* % of control = $\frac{\text{Elongation of test hypocotyls}}{\text{Elongation of control hypocotyls}} \times 100.$

† % of brassin response = $\frac{\text{Elongation of test hypocotyls}}{\text{Elongation of brassin-treated hypocotyls}} \times 100.$

‡ Not determined.

§ Fatty alcohols made by reduction of the fatty acids of safflower oil.

of fatty acids. Because analysis of the brassin complex had shown that it contains 0.51 % of 20:2 and 1.02 % of 22:1 fatty acids (Mandava, N., personal communication), peanut oil, cod liver oil and menhaden oil were chosen because they contain fatty acids with 20 or more carbon atoms. Also, because the brassin complex has high contents of linolenic acid (29 %), linoleic acid (8 %) and oleic acid (14 %), linseed and safflower oils were chosen. Peanut and safflower oils yielded products with the most biological activity and these were therefore used in subsequent investigations.

1-*O*-Fatty acyl-α,β-D-galactopyranoses were prepared in addition to the 1-*O*-fatty acyl-α,β-D-glucopyranoses because there was so much uncertainty about the nature of the brassin complex. Because the former gave greater activity (Table 1), initial investigations focused on derivatives of D-galactose.

Galactosides of fatty alcohols are analogs of 1-*O*-acyl esters with much greater stability. Fatty alkyl β-D-galactopyranosides were prepared. They inhibited elongation slightly (Table 1). Also, because 1-*O*-acyl esters might be considered to be analogs of the polyprenol phosphate sugars, fatty alkyl α-D-galactopyranosyl phosphates were made. These compounds could not be dissolved in the assay system. Since they obviously differed in physical properties from the brassins, bioassay was not performed.

Methyl 2- and 3-*O*-fatty acyl-β-D-galactopyranosides were prepared by monoacylation of methyl 6-*O*-triphenylmethyl-β-D-galactopyranoside, taking advantage of the fact that the axial hydroxyl group at C-4 is much less reactive than the equatorial hydroxyl groups at C-2 and C-3 [13, 14]. This product had no effect on the bean hypocotyls (Table 1). It has been previously reported that 2-*O*-hexadecanoyl-D-glucose failed to exhibit brassin-type activity in the bean second-internode bioassay [12].

3-*O*-Acyl-D-galactose was prepared by monoacylation of 1,2-*O*-isopropylidene-6-*O*-triphenylmethyl-α-D-galactopyranose, again taking advantage of the hydroxyl group at C-3 being more reactive than the hydroxyl group at C-4. When hypocotyls were treated (system A) with 3-*O*-acyl-D-galactose prepared with either fatty acids from peanut oil or safflower oil, elongation was inhibited. Hypocotyls treated by these derivatives elongated only 37 and 73 %, respectively, compared to the controls (Table 1). These derivatives gave the same bioassay results in system B, that is, hypocotyls elongated only 28 % compared to the control. It was assumed that the inhibitory effect shown by these compounds probably meant that either the wrong monosaccharide was used or that some of the fatty acids were the incorrect ones and that 3-*O*-acyl-D-galactose might be, therefore, a competitive inhibitor.

With these questions in mind, a further analysis of the brassin complex seemed necessary. Brassins were saponified with 0.1 M sodium methoxide in methanol. The water-soluble part was reduced to alditol(s), acetylated and analysed by GLC. This showed that the major carbohydrate product of this hydrolysis was glucose. Next most abundant was mannose, followed by galactose. (It is also possible that glucitol and mannitol were formed by reduction of fructose.)

After this discovery, derivatives of glucose were also made, viz. 3-*O*-acyl-D-glucose esterified with fatty acids from peanut oil and safflower oil, 6-*O*-acyl-D-glucose esterified with fatty acids from peanut oil and safflower oil, 4-*O*-acyl-D-glucose esterified with linoleic acid, linolenic acid, erucic acid (a constituent of the oil from *Brassica napus* seed), and fatty acids from peanut oil and safflower oil.

3-*O*-Acyl-D-glucoses showed both inhibitory and stimulatory effects depending on the fatty acid sources. D-Glucose esterified with fatty acids from peanut oil at *O*-3 inhibited elongation. Treated hypocotyls elongated 67 % compared to the control (Table 1). When D-glucose was esterified with fatty acids from safflower oil, a stimulatory effect was observed. Elongation of hypocotyls was 130 % compared to that of the control. Though the stimulatory effect was low compared to that of the brassin complex, it did indicate that the nature of the fatty acids is important in the biological activity.

6-*O*-Acyl-D-glucose was made by two different methods. One product was called 6-*O*-acyl-D-glucose (random) in which 6-*O*-acyl-D-glucose was believed to be the major product; the other one was 6-*O*-acyl-D-glucose (specific) in which the ester group was located only at *O*-6. Bioassay results for 6-*O*-acyl-D-glucose (random) were similar to those of 3-*O*-acyl-D-glucose. Treated hypocotyls elongated 51 and 105 % compared to the controls when the compounds were made with fatty acids from peanut oil and safflower oil respectively (Table 1). 6-*O*-Acyl-D-glucose (specific) inhibited the elongation slightly. Treated hypocotyls elongated 88 % when compared to the control (Table 1). According to a previous report, *O*-6 esters of D-glucose and palmitic, oleic, linoleic and linolenic acids do not exhibit brassin-type activity in the bean second-internode bioassay [12].

Compounds which inhibited elongation generally stimulated rooting. When hypocotyls were treated with brassin complex or other stimulatory compounds, no

root formation was observed. Hypocotyls elongated under the control system developed very few roots. When hypocotyls were treated with 3-*O*-acyl-D-glucose, long roots developed at the base of the hypocotyls. Many short roots were observed when 3-*O*-acyl-D-galactose was the test compound. Random 6-*O*-acyl-D-glucose-treated hypocotyls developed many long roots. This effect deserves further study.

In addition, a compound in which D-glucose was esterified with fatty acids at *O*-4 was prepared. 4-*O*-Acyl-D-glucose treated hypocotyls elongated 174 and 317% compared to the control when fatty acids were supplied by peanut and safflower oils, respectively (Table 1). Although the activity was only 13% of that of the authentic brassin complex, the elongation observed was similar to that produced by the brassin complex.

Finally, specific fatty acids were investigated. Accordingly, linolenic acid and erucic acid were used in the preparation. Both products had less activity than the 4-*O*-acyl-D-glucoses made with peanut oil and safflower oil.

The structure of brassin is still unknown. Instrumental methods provide some information. However, the growth regulator is a complex and it is not known whether only a part of the complex is active and other parts serve a supporting role (e.g. as emulsifiers or antioxidants) or whether the whole complex is the growth regulator. Spectroscopic methods have revealed that the brassins contain monosaccharide and fatty acids with an ester linkage on a one to one basis, even though the position of esterification is unknown. Our approach was to use known products in an attempt to identify the unknown. Based on the available information, a series of compounds was prepared. Bioassay provided the tool to identify the activity of these compounds. 4-*O*-Acyl-D-glucose and, perhaps, 1-*O*-acyl-D-galactose seemed to possess activity at least similar to that of the brassin complex. Galactose, mannose and, perhaps, fructose are also present in the complex. Whether these monosaccharides also play an active role is not known. It is possible that each of them has a different role and that together they contribute to the total activity of brassins. The brassin complex also contains a mixture of fatty acids. Again, whether a single fatty acid or a mixture of the fatty acids is responsible for growth regulator activity is not known. Plant oils provide a mixture of fatty acids; different plant oils provide different mixtures. Fatty acids from different oils did show different effects in the bioassays, and single fatty acids failed to stimulate elongation.

Other components of the brassin complex may also play a role in its action. The lipoidal *Brassica napus* pollen extract furnished to us by Dr. N. Mandava contains a steroidal-like constitutent, active in the bean second-internode bioassay (Mandava, N., personal communication). All available information points to the likelihood that the unique activity of brassin is a result of the synergistic effect of several components of the complex, two of which may be a 4-*O*-acyl-D-glucose and a steroid.

EXPERIMENTAL

Column chromatograqhy. Silicic acid (325 mesh, 120 g) was slurried in C_6H_6–MeOH (45:8). The slurry was then poured into a 2.5 × 50 cm glass tube. The column was washed with 200 ml of solvent and allowed to stand for at least 3 hr before use. A soln (2 ml) of crude product was applied to the column slowly. The column was eluted with C_6H_6–MeOH (45:8); 10 ml fractions were collected. TLC on Si gel H was used to detect components. Plates were developed with C_6H_6–MeOH–HOAc (45:8:4) (solvent A).

Preparation of fatty acid chlorides. Safflower oil, linseed oil or peanut oil (20 g), NaOH (10 g) and 95% EtOH (100 ml) were heated to 75–80° for 20 min with occasional shaking. H_2O (50 ml) was then added and thoroughly mixed with the contents. 20% H_2SO_4 (90 ml) was added to the soln, and the whole boiled for 2 min. The mixture was then transferred to a separatory funnel and the aq. layer removed. The fatty acids were washed ×3 with hot H_2O (until free of H_2SO_4). Fatty acids (10 ml) were added dropwise to $SOCl_2$ (10 ml) with stirring. The soln was heated gently for 30 min after the addition. Excess $SOCl_2$ was then removed by distillation at 76°. The fatty acid chloride was stored in the dark and protected from moisture.

Preparation of fatty alcohols. $LiAlH_4$ (3.8 g, 0.1 mol) was suspended in 110 ml of dry Et_2O. Peanut oil (25 ml) was added dropwise and when addition was complete, the mixture was gently refluxed for 1.5 hr with stirring. H_2O (60 ml) was cautiously added dropwise followed by 200 ml of 20% H_2SO_4. Et_2O (100 ml) was added, the layers separated and the aq. portion extracted twice with 25 ml portions of Et_2O. The Et_2O solns were combined, washed with 50 ml H_2O and dried (Na_2SO_4). Et_2O was evapd under red. pres. at 30°; yield of fatty alcohols, 23 ml. The IR spectrum showed a strong OH absorption at 3400 cm^{-1}.

Preparation of methyl 2- and 3-O-acyl-β-D-galactopyranoside. Me β-D-galactopyranoside (1 g, 5.1 mmol) was stirred in 8 ml of dry C_5H_5N. Triphenylmethyl chloride (1.5 g, 5.3 mmol) was then added and the contents stirred for 5 hr under dry conditions at room temp. The tritylated product had a R_f value of 0.35 (solvent A). Fatty acid chloride (2 ml, 5.5 mmol) and C_5H_5N (8 ml) were then added and the mixture refluxed for a further 10 hr. C_5H_5N was evapd under red. pres. at 55° and the syrupy residue dissolved in 10 ml of HOAc and cooled at 0°. HBr in HOAc (50% w/v, 2.5 ml) was added and the soln kept cold for 5 min. After filtration, the filtrate was poured into 50 ml of ice—H_2O. The resulting soln was extracted with $CHCl_3$ (3 × 25 ml). The extracts were combined, washed with H_2O (2 × 20 ml) and dried (Na_2SO_4). The crude product was purified by silicic acid chromatography. The pure product had a R_f value of 0.4 (solvent A). The final product was soluble in CH_2Cl_2.

Preparation of 3-O-acyl-D-galactose. 1,2-*O*-Isopropylidene-α-D-galactopyranose [15–17] (1 g, 4.5 mmol) was dissolved in 8 ml of dry C_5H_5N with stirring. Triphenylmethyl chloride (1.5 g, 5.3 mmol) was then added to the soln and the mixture stirred at room temp. for 5 hr. Fatty acid chlorides (2 ml, 5 mmol) and C_5H_5N (8 ml) were added to the soln and the mixture refluxed gently for 12 hr. The C_5H_5N was evapd under red. pres., leaving a syrup which was dissolved in 15 ml HOAc. The soln was cooled at 0°, HBr in HOAc (50% w/v, 3 ml) was added and the contents were kept cold for 5 min. The soln was filtered and the filtrate was poured into 50 ml of cold H_2O. The product was extracted with $CHCl_3$ (3 × 25 ml), the extracts combined and dried (Na_2SO_4). The $CHCl_3$ was removed by evapn under red. pres. The product was purified by silicic acid chromatography. The pure product had a R_f value of 0.40 (solvent A).

Preparation of fatty alkyl α-D-galactopyranosyl phosphates. 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl phosphate pyridinium salt [18–20] (1 g, 2 mmol), fatty alcohols obtained by reduction of oils (0.8 g, 3 mmol) and dicyclohexylcarbodiimide (DCC) (4 g, 19 mmol) were mixed. H_2O was removed by the addition of toluene, followed by evapn under red. pres. Dry

C_5H_5N (16 ml) was added to the mixture with stirring and the mixture stirred at room temp. for an additional 20 hr. The reaction mixture was diluted to 50 ml with C_5H_5N and after addition of 20 ml of H_2O, the ppt. of DCC and *N,N'*-dicyclohexylurea was removed by filtration. Evapn, followed by addition and evapn of toluene, gave a crude product which was dissolved in a small vol. of $CHCl_3$ and applied to a Si gel column (325 mesh). The column was first eluted with $CHCl_3$, then with $CHCl_3$–MeOH (50:1), and finally with $CHCl_3$–MeOH (20:1). By using these solvents, all the DCC, *N,N'*-dicyclohexylurea and fatty alcohols were removed from the column. The desired product was eluted with $CHCl_3$–MeOH (5:1); yield 0.88 g. The above product (fatty alkyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl phosphates) was dissolved in 15 ml 1% NaOMe in MeOH. The soln was kept at room temp. for 30 min, then diluted to 60 ml with MeOH. The MeOH soln was treated with Amberlite IR-120 (H^+) cation-exchange resin to bring the pH to 7. The resin was then removed by filtration. The filtrate was evapd under red. pres., giving an amorphous product; yield 0.27 g. The product was not purified further.

Preparation of fatty alkyl β-D-galactopyranosides. Ag_2O (2.5 g, freshly prepared), dry $CHCl_3$ (10 ml), fatty alcohol (2.7 ml, 8 mmol) and Drierite (10 g, dried by heating to 150° for 24 hr) were mixed and stirred for 1 hr. I_2 (0.5 g) was added, followed by addition of tetra-*O*-acetyl-α-D-galactopyranosyl bromide [21] (2.75 g, 7 mmol) in 10 ml dry $CHCl_3$. The contents were stirred at room temp. for 24 hr, then filtered through glass filter paper. The filtrate was evapd under red. pres. to a syrup which was dissolved in 20 ml of 0.1 M NaOMe in MeOH. After standing at room temp. for 45 min, Amberlite IR-120(H^+) cation-exchange resin was added to neutralize the soln. After filtration, the filtrate was evapd under red. pres. Purification of this compound was performed by silicic acid column chromatography. The syrupy product was dissolved in 3 ml C_6H_6–MeOH (45:12) before application to the column and the column was then eluted with the same solvent. The purified product had a R_f value of 0.39 (solvent A).

Fatty alkyl β-D-galactopyranoside was dissolved in 5% 2-PrOH to give a soln of 4.5 mg/ml; 1 ml of this soln was incubated with 0.1 ml of β-galactosidase (from bovine liver, Sigma) soln (2 mg/ml) in 0.1 M Pi buffer, pH 7, at room temp. for 4 hr; 1 ml of substrate incubated with 0.2 ml of 0.1 M Pi buffer (pH 7) served as control. At the end of the incubation, a reducing sugar test was positive.

Preparation of 3-O-acyl-D-glucopyranose. 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose [22] (1.2 g, 4 mmol) was dissolved in 10 ml of dry C_5H_5N. Fatty acid chloride (2 ml, 5.5 mmol) was added to the C_5H_5N soln and mixture refluxed for 14 hr. C_5H_5N was evapd under red. pres. below 60°. The resulting brown syrup was dissolved in 10 ml HOAc and 3 ml 50% w/v HBr in HOAc was added. After 5 min at room temp., the HOAc soln was poured into 50 ml of cold H_2O. The aq. soln was extracted with $CHCl_3$ (3 × 20 ml), the extracts washed with H_2O (2 × 20 ml), dried (Na_2SO_4) and evapd under red. pres. Before hydrolysis, the product had a R_f value of 0.7 (solvent A); the final product had a R_f value of 0.39. Purification of the product was done by silicic acid column chromatography. A reducing sugar test was positive.

Preparation of 6-O-acyl-D-glucose. 1,2:3,5-Di-*O*-benzylidene-α-D-glucofuranose [23] (1.2 g, 3 mmol) was dissolved in 8 ml of dry C_5H_5N and fatty acid chloride (from safflower oil, 1.5 ml, 5 mmol) was added. The mixture was refluxed for 8 hr with stirring. C_5H_5N was then removed by evapn under red. pres., giving a crude syrupy product. The syrup was dissolved in 50 ml 0.1 M HCl in Me_2CO and the mixture refluxed for 3 hr. The soln was neutralized with Amberlite IR-45(OH^-) anion-exchange resin and Me_2CO was then removed by evapn. TLC showed that the major product after acid-catalysed hydrolysis had a R_f value of 0.42 (solvent A). Purification of this compound was done by silicic acid column chromatography.

Preparation of randomly acylated D-glucose. Allyl α-D-glucopyranoside [24] (14 g, 63 mmol), 9 g of freshly fused $ZnCl_2$ and 50 ml of freshly distilled benzaldehyde were stirred under dry conditions for 24 hr. H_2O (100 ml, 0°) was added to the mixture with vigorous stirring, followed by the addition of petrol (50 ml). At this point, a solid formed. The mixture was kept cold for 15 min before filtration. The isolated product was washed with cold H_2O, then with petrol.

Allyl 4,6-*O*-benzylidene-α-D-glucopyranoside was recrystallized by dissolving it in 150 ml of hot H_2O. NH_4OH was added dropwise until the pH reached 7 and the soln filtered through a thin layer of Darco G-60 carbon. Crystallization took place as soon as the soln cooled to room temp. The crystals were collected by filtration and air-dried; yield 5.2 g (16 mmol); mp 114–116°. The product had R_f values of 0.94 (solvent B) and 0.64 (solvent A). (Found: C, 61.59; H, 6.50. Calc. for $C_{16}H_{20}O_6$: C, 62.34; H, 6.49%).

NaH (12 g, 0.5 mol), allyl bromide (15 ml, 0.18 mol), dry C_6H_6 (20 ml) were mixed and allyl 4,6-*O*-benzylidene-α-D-glucopyranoside (4 g, 12 mmol) dissolved in 80 ml of dry C_6H_6, slowly added to the mixture at 60°. The mixture was stirred at 60° for 4 hr. At the end of this time MeOH was cautiously added in order to destroy excess NaH. The C_6H_6 soln was washed with H_2O and dried (K_2CO_3). C_6H_6 was removed by evapn under red. pres. Allyl 2,3-di-*O*-allyl-4,6-*O*-benzylidene-α-D-glucopyranoside crystallized after standing 18 hr at room temp. The product had R_f values of 1 (solvent B) and 0.71 (solvent A); yield 4.7 g (12 mmol). (Found: C, 68.97; H, 7.22. Calc. for $C_{15}H_{24}O_6$: C, 68.04; H, 7.22%).

Allyl 2,3-di-*O*-allyl-4,6-*O*-benzylidene-α-D-glucopyranoside (4.7 g, 12 mmol) was dissolved in 30 ml HOAc; the resulting soln was then diluted quickly with 75 ml of H_2O, constantly stirring the mixture during the process. The mixture was refluxed for 1 hr with stirring and the soln then cooled at 0°. The product was removed by extraction with Et_2O (4 × 30 ml), the Et_2O extracts combined, washed with H_2O, aq. Na_2CO_3 soln, and again with H_2O, dried (Na_2SO_4) and evapd at 30° under red. pres. The syrupy residue separated into two layers, with the lower layer containing the major product. Allyl 2,3-di-*O*-allyl-α-D-glucopyranoside had R_f values of 0.48 (solvent A) and 0.89 (solvent B); yield 3.2 g (11 mmol).

Allyl 2,3-di-*O*-allyl-α-D-glucopyranoside (3.2 g, 11 mmol) was dissolved in DMSO (40 ml) containing K *t*-butoxide (3 g) and the soln kept at 100° for 30 min. After this period, a major product was prop-1′-enyl 2,3-di-*O*-(prop-1′-enyl)-α-D-glucopyranoside; R_f value of 0.60 (solvent A). The soln was poured into 100 ml of ice–H_2O and the product removed by extraction with Et_2O (5 × 30 ml). The Et_2O extracts were combined, washed with H_2O and dried (Na_2SO_4). Darco G-60 carbon (1 g) was added for decolorization. The Et_2O was evapd under red. pres. to give the product; yield of syrup, 3.1 g (11 mmol). The product had a R_f value of 0.60 (solvent A).

Prop-1′-enyl 2,3-di-*O*-(prop-1′-enyl)-α-D-glucopyranoside (1 g, 3.6 mmol) was dissolved in 8 ml of dry C_5H_5N. Triphenylmethyl chloride (1 g, 3.6 mmol) was added and the mixture stirred at room temp. for 5 hr. The product prop-1′-enyl 2,3-di-*O*-(prop-1′-enyl)-6-*O*-triphenylmethyl-α-D-glucopyranoside was used for the next reaction without isolation. Fatty acid chlorides (1.2 ml, 3.6 mmol) were added to the soln with 8 ml of dry C_5H_5N. The mixture was refluxed for 12 hr with stirring. C_5H_5N was then removed by evapn under red. pres. The brown syrupy residue was dissolved in 20 ml HOAc and the soln

cooled at 0°. HBr in HOAc (50% w/v, 3 ml) was added to the soln. The mixture was kept in ice for 5 min, then quickly filtered. The soln and the filtrate were poured into 50 ml of ice–H_2O and the product extracted with $CHCl_3$ (3 × 25 ml). The extracts were combined, washed with H_2O (2 × 20 ml), and dried (Na_2SO_4). Further purification was carried out by silicic acid chromatography. The purified product had a R_f value of 0.45 (solvent A); yield 0.05–0.12 g.

Bioassay methods. Hooked, etiolated bush bean seedlings were obtained from seeds of *Phaseolus vulgaris* L. (cv Executive) planted at a depth of 3 cm in moist Vermiculite and grown in the dark at 22 ± 2°. The seedlings were used within 3 days of emergence. Hooked hypocotyls were excised immediately below the cotyledons to give a shank length of 3.5 cm. These 3.5 cm hooks were placed lengthwise in Petri dishes (group of 5) containing 10 ml of either dist. H_2O, an emulsion of the brassin-complex or a soln of the emulsifying agent alone. Stable emulsions of the brassin-complex were achieved by using the stabilizer Colloidal SG (Colloidal Products Corp., Petaluma, California) at a 1:1 ratio. The emulsion stabilizer was always included in the controls of assays involving the brassin-complex. Treated hypocotyls were placed under fluorescent lights (Itt CW F/40). Brassin complex and all synthetic compounds were assayed at a concn of 54 μg/ml. Separate controls with and without added brassin were run, for each assay; therefore, only data (Table 1) within a single bioassay can be compared. Measurements were taken after 4 days.

Lanolin pastes of the brassin complex (5% w/w) were prepared as described in ref. [25] and applied by means of a small glass applicator in a thin (1 mm) ring around the designated hypocotyl zone. Again, tested hypocotyls were placed under fluorescent lights (Itt CW F/40). Measurements were taken after 4 days. This bioassay has been shown to be extremely sensitive to the brassin complex [26].

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