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Chemical and Biochemical Studies on Carbohydrate Esters. VII.[1] Plant Growth Inhibition by an Anomeric Mixture of Synthetic 1-O-Lauroyl-D-glucose

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Anomeric mixtures of synthetic 1-O-lauroyl-D-glucose showed a marked plant growth-inhibiting effect in the Avena coleoptile straight growth test; α-anomer-rich mixture and β-anomer-rich mixture were equally effective. The chain length and location of the acyl function were clearly critical for this biological effect, since the 1-O-glucosyl esters of caprylic, myristic, and stearic acids, and 3-O- and 6-O-lauroyl-D-glucoses were ineffective. The following disaccharide esters were also ineffective in this bioassay: sucrose-monoesters of lauric, palmitic, and stearic acids, trehalose-monoester of lauric acid, and sucrose esters of hydrogenated beef tallow fatty acids. None of the samples tested showed IAA or cytokinin activity.

Keywords——1-O-lauroyl-D-glucose; D-glucosyl monoesters of fatty acids; sucrose esters of fatty acids; trehalose monoester of lauric acid; plant growth inhibitory effect; Avena coleoptile straight growth test; radish cotyledon test

Recently, we have found that various carbohydrate esters of fatty acids can inhibit the growth of certain tumor cells.[1,4] This suggests that such compounds may also exert some inhibitory effect on the growth of plant tissues. In the present study, various fatty acyl derivatives of mono- and disaccharides were examined for their plant growth-regulating abilities.

As shown in Table I, the test samples used can be classified into three groups. Group A consists of ten mono-O-acyl-D-glucose compounds. 1-Glucosyl esters of caprylic, lauric, myristic, and stearic acids were prepared as described in our preceding paper[5,6]; the ratio of α-anomer to β-anomer in each sample was determined by gas liquid chromatography (GLC). For the preparation of 3-O- and 6-O-lauroyl-α,β-D-glucoses, Hori's method[7] and Reinefeld's process[8] were employed, respectively.

Group B consists of twelve complex mixtures of partial esters of a non-reducing disaccharide. Sucrose-monoester preparations of lauric, palmitic, and stearic acids, and a trehalose monoester preparation of lauric acid were obtained as described previously.[4d] It was

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5) Y. Nishikawa and K. Yoshimoto, *Chem. Pharm. Bull.* (Tokyo), **25**, 624 (1977).
6) Two other synthetic methods for 1-O-acylglucose have also been reported: *a)* K. Ramaswarmy, B.R. Bhattacharyya, and R.K. Crane, *Biochem. Biophys. Acta*, **443**, 284 (1976); *b)* P.E. Pfeffer, E.S. Rothman, and G.G. Moore, *J. Org. Chem.*, **41**, 2925 (1976).
7) *a)* R. Hori and T. Koizumi, *Yakugaku Zasshi*, **78**, 1003 (1958); *b)* K. Yoshimoto, S. Hamada, Y. Nishikawa, and T. Ikekawa, *Seikagaku*, **48**, 709 (1976).
8) E. Reinefeld and H.F. Korn, *Stärke*, **20**, 181 (1968).

confirmed by GLC analysis that the ester compositions of these samples were identical with those of the corresponding preparations used previously for antitumor tests: the largest peak in each gas chromatogram was assigned tentatively to the sucrose- or trehalose-6-monoester.[4d] Eight types of DK-Ester used were industrial products derived from sucrose and hydrogenated beef tallow fatty acids.[9] They contain mono-, di-, tri-, and poly-esters of stearic and palmitic acids in different ratios.[1]

The three agents belonging to group C were tested as reference compounds.

Table I. Test Samples and Their ABA Activity

No.	Test sample: Constituent	Final concentration (ppm)	Growth inhibition ratio (%)
Group A: Mono-O-acyl-D-glucose compounds			
1	Mixture of 1-O-capryloyl-α- and -β-D-glucoses (62 : 38)	500/3	−3.2
2	Mixture of 1-O-lauroyl-α- and -β-D-glucoses (60 : 40)	500/3	95.4
	Mixture of 1-O-lauroyl-α- and -β-D-glucoses (60 : 40)	500/3	87.2
3	Mixture of 1-O-lauroyl-α- and -β-D-glucoses (21 : 79)	500/3	97.3
	Mixture of 1-O-lauroyl-α- and -β-D-glucoses (21 : 79)	500/3	97.2
	Mixture of 1-O-lauroyl-α- and -β-D-glucoses (21 : 79)	100/3	18.3
	Mixture of 1-O-lauroyl-α- and -β-D-glucoses (21 : 79)	20/3	5.6
4	Mixture of 1-O-myristoyl-α- and -β-D-glucoses (60 : 40)	500/3	11.0
5	Mixture of 1-O-myristoyl-α- and -β-D-glucoses (42 : 58)	500/3	8.6
6	Mixture of 1-O-myristoyl-α- and -β-D-glucoses (37 : 63)	500/3	0.5
7	1-O-Stearoyl-α-D-glucose	250/3	5.5
8	Mixture of 1-O-stearoyl-α- and -β-D-glucoses (87 : 13)	250/3	0.8
9	3-O-Lauroyl-α,β-D-glucose	500/3	−18.1
10	6-O-Lauroyl-α,β-D-glucose	500/3	0.6
Group B: Partially acylated disaccharide compounds			
i) Monoester mixture			
11	Sucrose-monolaurate preparation	500/3	−6.4
12	Sucrose-monopalmitate preparation	500/3	23.0
13	Sucrose-monostearate preparation	500/3	−6.2
14	Trehalose-monolaurate preparation	500/3	9.3
ii) Sucrose ester of hydrogenated beef tallow fatty acids			
15	DK-Ester F-160	500/3	−9.6
16	DK-Ester F-140	500/3	5.5
17	DK-Ester F-110	500/3	0.2
18	DK-Ester F-90	500/3	9.6
19	DK-Ester F-70	500/3	−4.1
20	DK-Ester F-50	500/3	2.7
21	DK-Ester F-20	500/3	2.7
22	DK-Ester F-10	500/3	0.5
Group C: Reference compounds			
23	Caprylic acid	500/3	11.7
24	Lauric acid	500/3	22.8
25	Methyl laurate	500/3	8.4

The plant growth-inhibiting effect (abscisic acid (ABA) activity) of the samples was evaluated by means of Nitsch's straight growth assay of Avena coleoptile, modified by the addition of indol-3-ylacetic acid (IAA);[10] the addition of IAA increases the differences among the inhibitions.[11] Test samples were administered at a final concentration of 500/3 ppm, though smaller doses were adopted in some experiments. The results obtained are presented in Table I.

9) Dai-ichi Kogyo Seiyaku Co. Ltd., Kyoto, Japan.
10) *a*) J.P. Nitsch and C. Nitsch, *Plant Physiol.*, **31**, 94 (1956); *b*) Y. Ohwaki, *Syokubutsu No Kagakuchosetsu*, **5**, 172 (1970).
11) Y. Tsukamoto and T. Ando, *Proc. Jpn. Acad.*, **49**, 627 (1973).

Samples **2** and **3**, which consisted of anomeric mixtures of 1-O-lauroyl-D-glucose, exhibited strong growth-inhibitory effects at the standard dosage. Their activities were satisfactorily reproducible. However, decrease in the dose of sample **3** to 100/3 and 20/3 ppm resulted in considerably smaller effects. Sample **2** contained predominantly α-anomer, while sample **3** consisted mainly of β-anomer. Since both samples were equally effective, it appears that the biological activity of 1-O-lauroylglucose is not significantly affected by the stereochemistry at the C-1 position. Owing to the unavailability of anomerically pure samples, the possibility of synergistic action of the two anomers cannot be definitely excluded, however.

The other samples tested were nearly or completely ineffective. Since none of the 1-O-acylglucose analogs (**1**, **4**—**8**) except for laurate were active, and neither of the positional isomers, *i.e.*, 3- and 6-esters (**9** and **10**), of 1-laurate was active, the importance of chain length and location of the acyl function for the plant growth-inhibiting effect of glucosyl monoester is clear.

As reported previously, the disaccharide-ester preparations (**11**—**22**) in group B show growth-inhibitory action against Ehrlich ascites carcinoma, sarcoma 180 ascites tumor, and a mouse leukemia cell line, L-5178Y,[1,4] in spite of their inactivity in the present bioassay with plant materials. It thus appears that there is no direct relation between the antitumor effect and the plant growth-inhibiting activity. The inactivity of the disaccharide esters tested here may be attributed to inappropriate location of their acyl functions, in view of the structure-activity relationships observed with glucosyl esters. Further studies on 1-O-acyl derivatives of reducing disaccharides are in progress in our laboratory.

It is known that certain fatty acids and related substances have plant growth-regulating abilities.[11,12] According to Tsukamoto and Ando, fatty acids and their methyl esters with 8,

Table II. IAA and Cytokinin Activity

Test sample No.[c]	IAA activity[a] Increase in length Δ (mm)	Cytokinin activity[b] Increase in fresh wt. Δ (mm)
3	0.2±0.2	32.3±4.2
4	3.6±0.3	35.6±5.2
7	3.6±0.5	36.8±3.2
8	3.3±0.5	39.5±4.8
15	3.5±0.4	34.2±2.5
16	3.7±0.3	38.4±4.1
17	3.1±0.4	33.2±3.7
18	3.3±0.4	32.9±5.0
19	3.9±0.1	42.9±6.1
20	3.9±0.4	32.2±2.4
21	3.7±0.4	35.3±2.1
22	3.5±0.8	28.3±2.9
Control[d]	2.7±0.4[e]	41.4±4.5

a) Final concentration of test-sample, 500/3 ppm.
b) Final concentration of test-sample, 500/2 ppm.
c) Compound numbers are the same as in Table I.
d) Buffer solution.
e) Buffer solution containing 1 ppm of IAA gave a value of 6.7±0.5.

12) *a*) D. Gross, *Phytochemistry*, **14**, 2105 (1975); *b*) N. Le Poidevin, *ibid.*, **4**, 525 (1965); *c*) T. Ando and Y. Tsukamoto, *ibid.*, **13**, 1031 (1974); *d*) S. Bittner, S. Gazit, and A. Blumenfeld, *ibid.*, **10**, 1417 (1971); *e*) T.C. Tso, *Nature* (London), **202**, 511 (1964); *f*) T.C. Tso, G.L. Steffens, and M. Engelhaupt, *J. Agr. Food Chem.*, **13**, 78 (1965); *g*) G.L. Steffens, T.C. Tso, and D.W. Spaulding, *ibid.*, **15**, 972 (1967); *h*) H.M. Cathey, G.L. Steffens, N.W. Stuart, and R.H. Zimmerman, *Science*, **153**, 1382 (1966); *i*) Y. Mikami, H. Takahara, H. Imura, A. Suzuki, and S. Tamura, *Agr. Biol. Chem.* (Tokyo), **34**, 977 (1970); *j*) N. Komoto, M. Noma, S. Ikegami, and S. Tamura, *ibid.*, **36**, 2547, 2555 (1972); *k*) B.B. Stowe, "Plant Growth Substances 1973," (Proc. 8th Int. Conf. Plant Growth Substances), Hirokawa, Tokyo, 1974, p. 997; *l*) T.C. Tso and J.E. McMurtrey, Jr., *Tob. Sci.*, **7**, 101 (1963); *m*) F. Hayashi and L. Rappaport, *Plant Physiol.*, **41**, 53 (1966); *n*) B.B. Stowe and V.W. Hudson, *ibid.*, **44**, 1051 (1969).

10, and 12 carbon atoms inhibit the elongation of Avena coleoptiles.[11,12c] Under the present bioassay conditions, lauric acid (**24**) produced only a slight growth inhibition: caprylic acid and methyl laurate (**23** and **25**) were also almost ineffective. Therefore, the high activity of 1-O-lauroylglucose cannot be explained in terms of the liberation of lauric acid.

For evaluation of the plant growth-promoting effect (IAA activity), we next carried out Avena coleoptile straight growth tests in the absence of IAA, using the twelve samples listed in Table II. As anticipated, sample **3** again showed a strong inhibiting effect, while the others appeared to accelerate the elongation of coleoptiles, though the effects were not significant. These samples were also tested in the radish cotyledon bioassay,[13] but none of them showed cytokinin activity (Table II).

So far, no carbohydrate ester of a fatty acid has been reported to show an inhibiting effect; 1-O-lauroyl-D-glucose is thus a new synthetic plant growth inhibitor. In this connection, the preparation called "brassins" seems interesting.[14] This preparation was originally extracted from *Brassica napus* (rape) pollen by Mandava and co-workers. When bioassayed by the bean second-internode method, it caused both elongation and thickening of the internode. On the basis of spectroscopic evidence, the components contained in the preparation were identified tentatively as 1-O-β-D-glucosyl esters of linolenic, palmitic, stearic, oleic, and linoleic acids. The individual esters have not been separated, and hence, as stated by Milborrow, brassin activity cannot yet be attributed to any specific substance.[15] Subsequently, a series of new 6-glucosyl esters (palmitate, oleate, linoleate, and lonolenate) were isolated from the rape pollen, but they were inactive as plant growth promoters.[16] The following synthetic glucosyl esters were also tested in the bean second-internode bioassay, and all failed to exhibit brassin-type activity[16]: 1-α- and 1-β-palmitate; 1-α-stearate; 2-α- and 2-β-palmitate; 6-α-palmitate, oleate, linoleate, and linolenate.

The plant growth inhibition caused by 1-O-lauroylglucose may be due to physical effects resulting from changes in the surface tension of the membrane, or to penetration of the compound into the plant tissue, affecting the metabolic system. Further investigations are in progress to elucidate the exact mechanism involved in this biological action.

Experimental

Test Samples——(i) 1-O-Acyl-D-glucoses (Samples **1**—**8**): Selective acylation of the anomeric hydroxy group of D-glucose was carried out as described in our preceding paper.[5] The resulting product consisted mainly of the α-anomer. Pure α-anomer was obtained by repeated recrystallization. Evaporation of the mother liquor yielded a sample containing β-anomer as a predominant constituent. The ratio of α- to β-anomers in each sample was determined by GLC (for GLC conditions, see ref. 5), and the results are shown in Table I.

(ii) 3-O- and 6-O-Lauroyl-α, β-D-glucoses (Samples **9** and **10**): These were synthesized by using Hori's method and Reinefeld's process, respectively.[7,8]

(iii) Sucrose- and Trehalose-monoester Preparations (Samples **11**—**14**): These were prepared in the same mannner as before.[4d] Partial acylation of the disaccharide was conducted by the original procedure of Osipow, and the resulting products were fractionated by column chromatography to furnish a monoester mixture. The gas chromatograms of these samples were identical with those of the corresponding preparations reported earlier.[4d]

13) D.S. Lethman, "Biochemistry and Physiology of Plant Growth Substances," ed. by F. Wightman and G. Setterfield, Runge Press Ltd., Ottawa, 1973, p. 19.

14) *a*) N. Mandava and J.W. Mitchell, *Chem. Ind.* (London), **1972**, 930; *b*) J.W. Mitchell, N. Mandava, J.F. Worley, J.R. Plimmer, and M.V. Smith, *Nature* (London), **225**, 1065 (1970); *c*) J.W. Mitchell, N. Mandava, J.F. Worley, and M.E. Drowne, *J. Agr. Food Chem.*, **19**, 391 (1971); *d*) J.F. Worley and J.W. Mitchell, *J. Amer. Soc. Hort. Sci.*, **96**, 270 (1971); *e*) J.W. Mitchell and L.E. Gregory, *Nature New Biology*, **239**, 259 (1972); *f*) N. Mandava and J.W. Mitchell, *Ind. Agric.*, **15**, 19 (1971); *g*) N. Mandava, *Sci. Reporter*, **8**, 1 (1971).

15) B.V. Milborrow and R.J. Pryce, *Nature* (London), **243**, 46 (1973).

16) M.D. Grove, G.F. Spencer, P.E. Pfeffer, N. Mandava, J.D. Warthen, Jr., and J.F. Worley, *Phytochemistry*, **17**, 1187 (1978).

(iv) DK-Ester (Samples **15**—**22**): The eight types of DK-Ester (sucrose esters of hydrogenated beef tallow fatty acids produced industrially by an improved Osipow process) were supplied by the manufacturing company,[9] and were used without further treatment. Their ester compositions are shown in Table III.

(v) Caprylic and Lauric Acids, and Methyl Laurate (Samples **23**—**25**): These chemicals were commercial products of the highest purity available.

Table III. Composition of DK-Ester[a]

DK-Ester	Ester composition (%)		Monoester composition (mg in 100 mg of DK-Ester)			
	Mono-	Di-, tri-, and poly-	Stearate	Palmitate	Myristate	Total
F-160	*ca.* 70	*ca.* 30	45.7	28.1	1.4	75.2
F-140	60	40	43.5	20.4	1.2	65.1
F-110	50	50	34.9	15.0	1.0	50.9
F- 90	45	55	28.7	11.2	0.9	40.8
F- 70	40	60	26.6	9.5	0.7	36.8
F- 50	30	70	24.7	6.2	0.4	31.3
F- 20	10	90	8.1	2.2	0.1	10.4
F- 10	0	100	Trace	Trace	Trace	Trace

a) The data are cited from ref. 1.

Methods——(i) ABA Activity: This activity was evaluated by the Avena coleoptile straight growth test in the presence of IAA.[10,11] Oat seeds (*Avena sativa* L., cv. Victory) were grown for 3.5 days at 25° in darkness. The test sample was dissolved or suspended in water containing one drop of Tween 20. The following solutions were added successively to a test tube: 1 ml each of buffer solution (10 mM potassium phosphate–sodium phosphate system, pH 5.2), test solution containing 500 ppm of the sample, and an aqueous solution of IAA (3 ppm). A section 6 mm in length was cut from the coleoptile about 2—3 mm below the tip, and 15 sections per tube were floated. After growth for 18 hr at 25° in the dark, the section length was measured, and the growth inhibition ratio was calculated from the following equation: Growth inhibition ratio (%) $=100-(\Delta T/\Delta C\times 100)$; where ΔT is the average final length (mm) of the treated group minus the initial length (6 mm), and ΔC is the corresponding value for the control (buffer solution containing 1 ppm of IAA). The results obtained are shown in Table I.

(ii) IAA Activity: This activity was assayed by the Avena coleoptile straight growth test in the absence of IAA. The general experimental conditions employed were as above, but water was used instead of IAA solution, and during the course of growth for 18 hr the test tube was exposed to red light occasionally. The results are presented in Table II.

(iii) Cytokinin Activity: This activity was evaluated by the radish cotyledon bioassay.[13] After radish seeds (*Raphanus sativus* L., cv. Risou Daikon) had germinated for 1.5 days at 28° in the dark, the cotyledon was excised from each seedling. Filter paper circles in Petri dishes (diam., 9 cm) were wetted with 1.5 ml of the test solution (or suspension) and 1.5 ml of 4 mM potassium phosphate buffer, pH 5.8, then 15 cotyledons were placed in each dish. After 3 days at 28° under continuous fluorescent lighting, the fresh cotyledons were weighed. The results are shown in Table II.

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