TABLE I

COMPARISON OF LADINO SAPOGENINS WITH THE CORRESPONDING SOYASAPOGENOLS

		Ochiai, *et al.*	Meyer, *et al.*	Ladino sapogenins
Soy. A	M.p., °C.	311	321	318–320
	[α]D	+102	+103	. . .
Soy. B	M.p., °C.	260	259–260	260
	[α]D	+92	+90	+91
Soy. C	M.p., °C.	238–239	239–240	240–241
	[α]D	+70.7	+65	+63
B-triAC	M.p., °C.	175–176	179–180	180–181
	[α]D	. . .	+78	+78
C-diAC	M.p., °C.	198	199–200	205–207
	[α]D	. . .	+59	+58

Soyasapogenol B.—The eluates melting at 258–260° were combined and recrystallized from methanol–chloroform yielding material of m.p. 260°, $[\alpha]^{25}\text{D}$ +91°.

Anal. Calcd. for $C_{30}H_{50}O_3$: C, 78.55; H, 10.99. Found: C, 78.20; H, 10.91.

A sample was acetylated by refluxing with acetic anhydride for 2 hours. Recrystallization from chloroform–methanol yielded the **triacetate** as laths, m.p. 180–181°, undepressed upon admixture with an authentic specimen,[10] $[\alpha]^{24}\text{D}$ +78°; identity was confirmed by comparison of the infrared spectra.

Anal. Calcd. for $C_{36}H_{56}O_6$: C, 73.93; H, 9.65. Found: C, 73.80; H, 9.61.

Soyasapogenol C.—The eluates melting at 239–241° were combined and recrystallized from methanol–chloroform whereupon they exhibited m.p. 240–241°, $[\alpha]^{25}\text{D}$ +63°. The melting point was not depressed on admixture with an authentic sample.[10]

Anal. Calcd. for $C_{30}H_{48}O_2$: C, 81.76; H, 10.98. Found: C, 82.05; H, 11.02.

Acetylation with acetic anhydride–pyridine (room temperature, 20 hours) followed by crystallization from methanol–chloroform furnished the **diacetate**, m.p. 205–207°, $[\alpha]^{27}\text{D}$ +58°, which also was obtained by direct chromatography (elution with 7:3 hexane–benzene) of the acetylated sapogenin mixture.

Anal. Calcd. for $C_{34}H_{52}O_4$: C, 77.82; H, 9.99. Found: C, 78.01; H, 9.92.

Oxidation of the diacetate (160 mg.) with an equal amount of selenium dioxide in acetic acid solution in the standard manner[11] and recrystallization from methanol yielded the **Δ11,13(18),(?)15-triene** as blades, m.p. 208–211°, $\lambda_{\max}^{\text{EtOH}}$ 241, 249 and 259 mμ, log ε 4.20, 4.23 and 4.17; lit.,[8] m.p. 208–209°.

Anal. Calcd. for $C_{34}H_{50}O_4$: C, 78.12; H, 9.64. Found: C, 78.13; H, 9.78.

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[11] *Cf.* L. Ruzicka, G. Müller and H. Schellenberg, *Helv. Chim. Acta*, **22**, 767 (1939).

Aliphatic Esters of 3-Indoleacetic Acid. Preparation and Activity in Parthenocarpic Fruit Induction[1,2]

BY LOWELL E. WELLER, SYLVAN H. WITTWER AND HAROLD M. SELL

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The isolation and discovery of the unique plant growth regulating properties of heteroauxin soon led to various methods of biological assay. Such tests made it possible to compare other compounds, possessing biological properties similar to 3-indoleacetic acid. Kögl and Kostermans,[3] using the *Avena* curvature test, reported that 3-indoleacetic acid was more active than the methyl, ethyl, propyl or isopropyl esters. The biological action of the esters decreased as the size of the ester radical increased. This activity order has been attributed to a parallel decrease in the hydrolyzability of the ester and assumes that the esters exhibit activity only indirectly by giving rise to 3-indoleacetic acid upon hydrolysis *in vivo*.[3,4] Although logical, it has not been substantiated by experimental evidence. An equally plausible explanation[5] is the impeded transport of the ester *per se*.

Zimmerman, *et al.*,[6] employing tomato stem and petiole elongation as a criterion for biological activity, reported that methyl 3-indoleacetate as well as the methyl esters of other indole acids were more active than the corresponding free acids. Similar results have been obtained in the stimulation of parthenocarpy in the tomato,[7,8] the lower molecular weight aliphatic esters all being more active than 3-indoleacetic acid. These results have led to a study of other esters of 3-indoleacetic acid. The synthesis and properties of these new esters are described in this report.

Experimental

The esters of 3-indoleacetic acid were prepared from the acid and the appropriate alcohol, with hydrogen chloride catalyst, similar to the procedure described by Jackson[9] for the methyl and ethyl esters. The esterifications usually were carried out at room temperature except in those cases where elevated temperatures were necessary to effect solution of the reaction mixture. After esterification, the excess alcohol was removed by vacuum distillation and the residue ultimately vacuum distilled. The esters were purified further by repeated recrystallization from petroleum ether (b.p. 62–67°). Esterification with *n*-dodecyl, *n*-tetradecyl, *n*-hexadecyl and *n*-octadecyl alcohol required warming to affect solution and subsequent reaction. These esters were not vacuum distilled but were recrystallized from petroleum ether. All esters were recrystallized to a constant melting point and subjected to elemental analysis. The chemical and physical properties are given in Table I.

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[1] Journal Article No. 1721 from the Michigan Agricultural Experiment Station, East Lansing.

[2] This research was supported by the Horace H. Rackham Research Endowment.

[3] F. Kögl and D. G. Kostermans, *Z. physiol. Chem.*, **235**, 201 (1935).

[4] F. W. Went and K. V. Thimann, "Phytohormones," The Macmillan Co., New York, N. Y., 1937, p. 294.

[5] S. A. Gordon, *Ann. Rev. Plant Physiol.*, **5**, 357 (1954).

[6] P. W. Zimmerman, A. E. Hitchcock and F. Wilcoxon, *Contrib. Boyce Thompson Inst.*, **8**, 105 (1936).

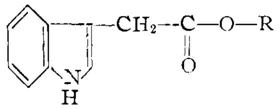
[7] C. T. Redemann, S. H. Wittwer and H. M. Sell, *Archiv. Biochem. Biophys.*, **32**, 80 (1951).

[8] H. M. Sell, S. H. Wittwer, T. L. Rebstock and C. T. Redemann, *Plant Physiol.*, **28**, 481 (1953).

[9] R. W. Jackson, *J. Biol. Chem.*, **88**, 659 (1930).

TABLE I

CHEMICAL AND PHYSICAL PROPERTIES AND ACTIVITY IN THE INDUCTION OF PARTHENOCARPIC FRUIT DEVELOPMENT OF ALIPHATIC ESTERS OF 3-INDOLEACETIC ACID[a]



R	M.p., °C.	Empirical formula	Carbon, %		Hydrogen, %		Nitrogen, %		Parthenocarpic activity[b] % acid equivalent		
			Calcd.	Found	Calcd.	Found	Calcd.	Found	1.0	0.1	0.01
H (control)									+++	+++	+
n-Hexyl	31–32	$C_{16}H_{21}NO_2$	74.10	74.35	8.16	8.40	5.40	5.24	+++	+++	+++
n-Heptyl	26.5–27.5	$C_{17}H_{23}NO_2$	74.69	74.85	8.48	8.46	5.12	5.35	+++	+++	+++
n-Octyl	27–28	$C_{18}H_{25}NO_2$	75.22	75.13	8.77	8.81	4.87	5.04	+++	+++	+++
n-Nonyl	29.5–30.5	$C_{19}H_{27}NO_2$	75.71	75.71	9.03	9.32	4.65	4.77	+++	+++	++
n-Decyl	38.5–39.5	$C_{20}H_{29}NO_2$	76.15	76.34	9.27	9.21	4.44	4.47	+++	+++	+
n-Undecyl	39.5	$C_{21}H_{31}NO_2$	76.55	76.81	9.48	9.38	4.25	4.41	+++	+++	Inact.
n-Dodecyl	48–49	$C_{22}H_{33}NO_2$	76.92	77.28	9.68	9.80	4.08	4.33	+++	+++	Inact.
n-Tetradecyl	78–79	$C_{24}H_{37}NO_2$	77.58	77.85	10.04	10.28	3.77	3.82	+++	Inact.	Inact.
n-Hexadecyl	60–62	$C_{26}H_{41}NO_2$	78.14	78.14	10.34	10.11	3.51	3.73	+++	Inact.	Inact.
n-Octadecyl	52	$C_{28}H_{45}NO_2$	78.63	78.85	10.61	10.60	3.28	3.48	+++	Inact.	Inact.

[a] For comparative activity of the lower molecular weight esters, *cf.* ref. 8. [b] The number of plus signs indicates the relative magnitude of activity of each substance in stimulating parthenocarpy in the tomato.

Biological Activity

The esters of 3-indoleacetic acid were assayed for their ability to induce parthenocarpic fruit development in the tomato according to the method described by Sell, *et al.*[8] The biological activities, summarized in Table I, show that all esters were active at a concentration of 1.0%. Previously, Redemann, *et al.*,[7] and Sell, *et al.*,[8] have demonstrated that the methyl or ethyl ester of 3-indoleacetic acid was approximately 100 times more effective than the free acid in stimulating parthenocarpic fruit development in the tomato. The homologous series of esters from propyl through decyl possess nearly equivalent activities, all being about 10 times more active than the free acid. The undecyl and dodecyl esters were equally as active as 3-indoleacetic acid while the esters containing 14 or more carbon atoms in the ester moiety were all less active than the parent acid.

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Chromatography of Disaccharides on a Thermocolumn[1]

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Separation of a series of homologous polymers of xylose, as reported by Whistler and Tu,[2] can be applied to other homologous series, but the separation of mixtures of disaccharides has not been examined fully. However, the separation of two disaccharides, 4-(β-mannopyranosyl)-β-D-mannopyranose and 6-(2-D-galactopyranosyl)-β-D-mannopyranose, on a charcoal column was reported by Whistler and Durso.[3] This work was undertaken to develop an adequate method for the separation of disaccharides.

The authors have found that the rate of desorption of disaccharides on carbon increases with increase of temperature, but the increase is greater with some disaccharides than with others. This magnifies the differences in sorption of the disaccharides at higher temperature and makes possible the ready separation of certain hexose disaccharides.

This work demonstrates the separation on a thermocolumn, a heated charcoal column, of a series of pairs of disaccharides, differing either in the component sugars or in the linkage between sugar units. The disaccharides used contained only hexose units. Five pairs were selected for study: lactose and cellobiose, melibiose and cellobiose, lactose and melibiose, cellobiose and gentiobiose, and maltose and cellobiose.

The temperature of the column is regulated to the point at which the separation is considered to be optimum. The disaccharides on the column can be removed completely with 3% aqueous ethanol by merely increasing the temperature. Thus, the column can be used repeatedly.

Paper chromatograms of the separated sugars indicated that they were undamaged on the column at 71°, the highest temperature employed in this work. However, in most cases 50° is more favorable. If the sugar is very strongly adsorbed, or if the separation at 50° is not satisfactory, a higher temperature can be used. In some cases 40° or higher can be used at the beginning to facilitate the removal of one sugar, and then 60° or higher for the other.

Because the sorption isotherm of each sugar is usually not ideal, the amount of sugar being separated on the column at a given temperature must be regulated to avoid overlapping and to afford a sharp separation. When overlapping can be reduced to a negligible amount, chromatographic separation on a thermocolumn may be made quantitative by careful control.

Experimental

Construction of the Column.—The thermocolumn is constructed from four tubes of different diameter. The innermost tube, 20 by 810 mm., is later to be filled with charcoal. Around this tube is placed a heater made by winding 95 turns of nichrome wire (2.7 ohm per inch) on a glass tube, 34 by 620 mm. A thermometer is inserted in the space between these two tubes. Another tube, 42 by 620 mm., is placed outside the heater to protect it. To prevent the loss of heat there is an outermost tube, 66 by 530 mm., wrapped with two layers of asbestos tape, $^1/_{16}$ by $^1/_2$ inch, and a

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[1] Presented before the Division of Carbohydrate Chemistry at the 127th Meeting of the American Chemical Society, Cincinnati, Ohio, March 29 to April 7, 1955.

[2] R. L. Whistler and C. C. Tu, THIS JOURNAL, **74**, 3609 (1952).

[3] R. L. Whistler and D. F. Durso, *ibid.*, **73**, 4189 (1951).