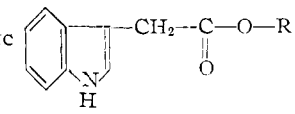
TABLE I

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, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Parthenocarpic activity[b] % acid equivalent 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]

BY CHEN-CHUAN TU AND KYLE WARD, JR.

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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

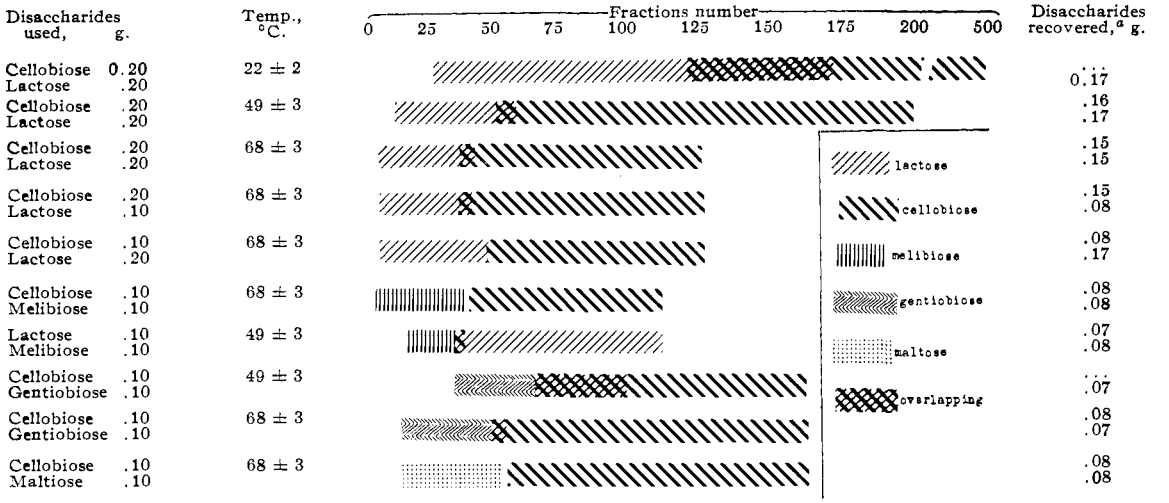
Construction of the Column.—The thermocolumn is constructed from four tubes of different diameter. The innermost tube, 20 by 810 mm., is later to be filled with charcoal. Around this tube is placed a heater made by winding 95 turns of nichrome wire (2.7 ohm per inch) on a glass tube, 34 by 620 mm. A 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

(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).

CHART I, SEPARATION OF DISACCHARIDES ON A THERMOCOLUMN



[a] The discrepancy between the amount of each disaccharide used and that recovered is due to the loss during spot-checking, concentration, precipitation and drying, as well as to overlapping.

thermoregulator is placed inside this tube. A small window in the asbestos tape permits observation of the thermometer.

Preparation of the Thermocolumn.—The thermocolumn, 20 by 420 mm., is prepared from a mixture of charcoal and Celite (1:1) according to Whistler and Durso.[4] After washing with water, the column is heated to the temperature at which the chromatographic separation is to be carried out. The thermocolumn then is washed with 4 l. of 3% aqueous ethanol. Fractions are collected automatically.

Separation of Sugars.—A pair of disaccharides, dissolved in a small amount of 3% aqueous ethanol was transferred onto the top of the column. As the solution moved into the column, about 5 ml. of 3% aqueous ethanol was added at the top of the column. This was repeated three times. Then the column was eluted continuously with 3% aqueous ethanol by gravity. Each fraction collected was about 20 ml. at 50° (23 ml. at 70°). Each fifth fraction was spot-checked by paper chromatography using *n*-butyl alcohol–pyridine–water (6:4:3) as the solvent. The fractions containing the same sugar were combined, concentrated, and dried in the usual manner. The recovered sugars were weighed and spot-checked again, separately.

Results.—A series of five pairs of disaccharides, lactose and cellobiose, melibiose and lactose, melibiose and cellobiose, gentiobiose and cellobiose, and maltose and cellobiose was selected for chromatographic separation in the thermocolumn. The amount of the sugar in each pair being separated, the temperature used for the chromatography, and the results are shown in Chart I. The procedure described above was used for the separation of each of the pairs of the disaccharides.

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(4) R. L. Whistler and D. F. Durso, THIS JOURNAL, **72**, 677 (1950).

COMMUNICATIONS TO THE EDITOR

CYCLIC POLYOLEFINS. XXXVIII. POSITION ISOMERS OF DISUBSTITUTED CYCLOÖCTATETRAENES

Sir:

We wish to report separation of the four possible position isomers of diphenylcycloöctatetraene (1,2-, 1,3-, 1,4-, 1,5-) and the identification of each.

The reaction of phenyllithium with cyclooctatetraene has been reported previously to form phenylcycloöctatetraene.[1] Phenyllithium and phenylcycloöctatetraene have been found to react in a similar manner, forming a mixture of hydrocarbons containing the diphenylcycloöctatetraenes. After a preliminary short-path distillation under reduced pressure to separate most of the phenylcycloöctatetraene as a low-boiling fraction, and a high-boiling residue, the fraction of intermediate boiling point was subjected to countercurrent distribution in a 200-tube automatic instrument[2] in which the volume of each phase is 10 ml. The first distribution employed as solvents (in the following ratios) cyclohexane (166 ml.)–ethanol (100 ml.) and water (20 ml.) containing silver nitrate (13.1 g.). Hydrocarbons less soluble in the aqueous alcoholic silver nitrate phase ($K \sim 6.5$)[3] than the diphenylcycloöctatetraenes, and the remaining phenylcycloöctatetraene ($K \sim 0.17$), which was more soluble in that phase, were readily separated. After 658 fundamental transfers, the upper phase was removed by single withdrawal

(1) A. C. Cope and M. R. Kinter, THIS JOURNAL, **73**, 3424 (1951).

(2) Manufactured by the H. O. Post Scientific Instrument Co., based on the design described by L. C. Craig, W. Hausmann, E. H. Ahrens, Jr., and E. J. Harfenist, *Anal. Chem.*, **23**, 1236 (1951).

(3) The partition ratio K is defined as the concentration of the material in the upper phase (in this case principally cyclohexane) divided by the concentration in the lower phase.