sirupy sugar preparations used by these workers were obtained as acid "reversion" mixtures from D-glucose or as various types of starch hydrolyzates, all of which are known to be complex mixtures of not readily separable carbohydrate materials. Although it appears that some of the preparations previously described may have been essentially isomaltose (6-α-D-glucopyranosyl-D-glucose) phenylosazone, they are almost certain to have been impure. Fischer[2] records melting points of 150–153° and 158°, with the other workers[3] inclined to agree, although Aso[3] records 206–208°.

We wish to describe herein the phenylosazone of isomaltose (6-α-D-glucopyranosyl-D-glucose) prepared from amorphous isomaltose which had been purified through its crystalline β-D-octaacetate.[4] The properties of this substance appear to be much like those reported by the previous workers except that we find a melting point of 177–179° (cor.). The phenylosazone is soluble in ethanol and in hot water. It separates as yellow needle-like crystals which have a tendency to darken and change to amorphous material upon drying in the open air. It will retain its color and crystalline character if properly purified and dried in a vacuum over phosphorus pentoxide at room temperature. The optical rotation was determined in methyl cellosolve,[5] a solvent recommended by Hudson[6] for osazones.

The optical rotation of gentiobiose (6-β-D-glucopyranosyl-D-glucose) phenylosazone in this solvent and its X-ray powder diffraction data are reported herein for comparative purposes. We also record the preparation and description of crystalline isomaltose phenylosotriazole. The comparative specific rotations of the phenylosazones (+33° for the isomaltose derivative and −67° for the gentiobiose derivative) and phenylosotriazoles (+42.5 and −34°,[7] respectively) reflect the structural differences between these disaccharides, which differences should lie only in the opposed configurations of their glycosidic linkages.

Experimental

Isomaltose Phenylosazone.—Sodium acetate (8 g.) and phenylhydrazine (5 g.) were dissolved in 50 ml. of water and filtered (decolorizing carbon). To the filtrate was added 2.5 g. of amorphous isomaltose (prepared from pure, crystalline β-isomaltose octaacetate[4]) and heated for 2.5 hr. in a boiling water-bath. The solution was cooled and diluted with 30 ml. of water. The crystalline material which separated was filtered and, without drying, was immediately recrystallized twice from hot water. The product was dried over phosphorus pentoxide at room temperature and under reduced pressure; yield 2.2 g., m.p. 176–178° (cor.). A portion of this material (0.5 g.) was treated with decolorizing carbon in hot water, filtered and allowed to crystallize. The bright yellow crystalline product was dried as above; m.p. 177–179° (cor.), $[\alpha]^{23}D$ +32.6° (initial) → +46° (24 hr., with deepening of color; *c* 2, methyl cellosolve[5]); X-ray powder diffraction data: 14.60[8]–20,[9] 8.34–30, 7.69–10, 7.04–5, 4.39–25, 4.12–10, 3.82–100, 3.59–5, 324–70.

Anal. Calcd. for $C_{24}H_{32}O_9N_4$: C, 55.37; H, 6.19; N, 10.76. Found: C, 55.26; H, 6.28; N, 10.92.

Isomaltose Phenylosotriazole.—Following the general procedure of Haskins, Hann and Hudson[10] for preparing the phenylosotriazoles of disaccharides, isomaltose phenylosazone (1.0 g.) was suspended in 100 ml. of water containing 0.53 g. of cupric sulfate pentahydrate. The mixture was boiled for 30 min. The copper ions were removed by precipitation with hydrogen sulfide and filtration. The filtrate was neutralized with powdered calcium carbonate, filtered and evaporated to a sirup under reduced pressure. The material crystallized from ethanol; yield 0.4 g., m.p. 176–178° (cor.). Pure material was obtained by recrystallization from ethanol; m.p. 177–178° (cor.), $[\alpha]^{25}D$ +42.5° (*c* 3.4, water); X-ray powder diffraction data: 11.68[8]–35,[9] 8.69–5, 7.27–5, 6.57–5, 6.32–25, 5.96–5, 5.72–100, 5.00–25, 4.68–5, 4.52–10, 4.33–100, 4.12–50, 3.99–5, 3.88–5, 3.79–5, 3.64–30, 3.54–30, 3.37–20, 3.27–15, 3.08–20, 3.00–2, 2.91–2, 2.82–20, 2.74–20.

Anal. Calcd. for $C_{18}H_{25}O_9N_3$: C, 50.58; H, 5.90; N, 9.83. Found: C, 50.83; H, 5.91; N, 10.00.

Gentiobiose Phenylosazone.—The constants of an authentic sample of gentiobiose phenylosazone were determined: m.p. 184–186° (cor.), $[\alpha]^{23}D$ −66.6° (initial) → −58° (7 hr., with coloration preventing further readings, *c* 1 in methyl cellosolve[5]); X-ray powder diffraction data: 13.81[8]–10,[9] 9.51–10, 8.40–15, 7.05–15, 5.58–15, 5.15–30, 4.92–5, 4.82–5, 4.60–90, 4.17–70, 3.91–80, 3.61–25, 3.44–5, 3.31–100, 3.15–5, 3.05–10.

(4) M. L. Wolfrom, L. W. Georges and I. L. Miller, THIS JOURNAL, **69**, 473 (1947); **71**, 125 (1949).

(5) Ethylene glycol monomethyl ether.

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(7) W. T. Haskins, R. M. Hann and C. S. Hudson, THIS JOURNAL, **70**, 2288 (1948).

(8) Interplanar spacing, Å.; CuKα radiation.

(9) Relative intensity as percentage of strongest line, estimated visually.

(10) W. T. Haskins, R. M. Hann and C. S. Hudson, THIS JOURNAL, **67**, 939 (1945).

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Chromatography of I^{131}-Labeled Esters[1]

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Mixtures of colorless compounds have been chromatographed after conversion to derivatives which are colored[2,3] or labeled with radioactive atoms.[4,5] Klyne's report[6] on the advantages arising from the chromatography of steroids as benzoates suggested to us that benzoates substituted with I^{131} might be better adapted to chromatography than the more strongly adsorbed, colored *p*-phenylazobenzoates which others have employed.

We have found that chromatography of the labeled *p*-iodobenzoates of the sterols permits improved separations to be made, the esters of cholestanol, cholesterol and 7-dehydrocholesterol having been separated on a 60-cm. column in approximately 16 hours. Since esters of high specific activity can be prepared, the method allows the detection of any weighable component. Quantitative estimation of the content of a zone is simultaneous with its localization, permitting the analysis of any alcohol mixtures whose esters can be formed in high yields. The method can, of course, be extended to other mixtures of compounds

(1) This work was supported by grants-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council and from the American Heart Association.

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(4) A. S. Keston, S. Udenfriend and M. Levy, *ibid.*, **72**, 748 (1950).

(5) W. S. Ruliffson, H. M. Lang and J. P. Hummel, *J. Biol. Chem.*, **201**, 839 (1953).

(6) R. V. Brooks, W. Klyne and E. Miller, *Biochem. J.*, **54**, 212 (1953).

possessing common functional groups capable of forming labeled derivatives. Perhaps the greatest advantage of the method lies in the fact that repeated surveys permit continuous quantitative estimation of the development of the column.

Experimental

p-**Iodobenzoyl Chloride-I^{131}.**—*p*-Iodobenzoic acid-I^{131} was first prepared according to a modification of the method of Meyer.[7] Subsequently Whitmore and Woodward's synthesis[8] was employed at 1/100th scale. The 5 mc. of NaI^{131} in approximately 2 ml. of H_2O was added with the first portion of I_2. The product, carefully dried in a vacuum oven at 60°, was refluxed for 2 hours with 10 ml. of thionyl chloride. The excess thionyl chloride was distilled off under vacuum. The *p*-iodobenzoyl chloride was sublimed under vacuum. The product melted at 65°.

Steryl *p*-Iodobenzoates.—The labeled esters were prepared by dissolving approximately 100 mg. of sterol with a 0.3 molar excess of the labeled acid chloride in sufficient dry pyridine to effect solution. The mixture was kept at 5° for 12 hours and worked up in the usual way. The weight yields of crude esters were 90–96%. The recrystallized esters had the following properties.

Cholestanyl *p*-iodobenzoate: m.p. 186° to a cloudy melt clearing at 230°. *Anal.* Calcd. for $C_{34}H_{51}IO_2$: C, 65.90; H, 8.30; I, 20.48. Found: C, 66.02; H, 8.44; I, 20.58.

Cholesteryl *p*-iodobenzoate: m.p. 184.5° to a brilliantly colored mesomorphic melt clearing at 242°. *Anal.* Calcd. for $C_{34}H_{49}IO_2$: C, 66.11; H, 7.99; I, 20.55. Found: C, 66.00; H, 8.06; I, 20.85.

7-Dehydrocholesteryl *p*-iodobenzoate: m.p. 178.5° (unsharp) to a cloudy melt which decomposes. *Anal.* Calcd. for $C_{34}H_{47}IO_2$: C, 66.33; H, 7.69; I, 20.62. Found: C, 66.54; H, 7.71; I, 20.65.

Two parts of silicic acid, Mallinckrodt, 100 mesh, "specially prepared for chromatographic analysis by the method of Ramsey and Patterson" and one part of Celite 535[9] were mixed in a ball mill and heated for four hours in an oven at 180°. A tube, 14-mm. internal diameter and 80 cm. long, was treated with Silicone Resin Solution[10] as recommended by Idler and Baumann.[11] The adsorbent was poured into a large funnel attached to the upper end of the tube by a rubber sleeve, drawn into the tube and packed by suction, and further settled by gentle tapping. A perforated porcelain disc was placed on top of the column to protect the adsorbent and the column was prewashed with one volume of 10:1 ligroin[12]–benzene. The esters (15 to 75 mg. of each) were applied and washed into the column with the same solvent mixture and the chromatogram was developed with ligroin flowing under suction at the rate of 2 to 3 ml. per minute.

The column was scanned at hourly intervals by a collimated scintillation counter employing a $1^3/_4$ inch diameter by 2 inch high sodium iodide thallium activated crystal and an RCA # 6199 photomultiplier tube. The counter assembly was mounted in a lead shield providing a minimum of 1.5 inches of lead in all directions except for the "viewing" slit. Interchangeable lead plugs with various slit widths were used depending on the level of the activity. The counter and shield were mounted on a vertical steel shaft by means of a counterpoised sliding bracket. The chromatographic column was supported by the same steel shaft. Usually one minute counts were taken at each cm. of length.

In a typical run with a mixture of 25 mg. of each of the esters, the 7-dehydrocholesteryl ester zone was apparent after one-half hour and was completely separated in 7 hours. The cholesteryl–cholestanyl ester zone began to resolve in 5 hours and the two were virtually separate in 16 hours. At this time the column was allowed to run dry and a careful survey taken. A plot of net counts per minute against distance from the top of the column showed bell-shaped curves, with some tailing, corresponding to the zones. The maxima of the three esters appeared at the following distances: cholestanol, 46.5 cm.; cholesterol, 34 cm.; 7-dehydrocholesterol, 16 cm. Appropriate zones were cut from the column and the esters eluted and identified by melting points and mixed melting points. The area under the curve for each zone was proportional to the millimoles of unrecrystallized ester recovered with an average deviation of 9.6%.

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(8) F. C. Whitmore and G. E. Woodward, "Organic Syntheses," Coll. Vol. I, 2nd Ed., John Wiley and Sons, Inc., New York, N. Y., 1941, pp. 159, 325.

(9) In subsequent runs Celite 545 was substituted, allowing a more rapid development of the column.

(10) Silicone Resin Solution, S R-53, General Electric Co., Waterford, N. Y.

(11) D. R. Idler and C. A. Baumann, *J. Biol. Chem.*, **195**, 624 (1952).

(12) "Skellysolve C," *n*-heptane, boiling range 86–100°.

Oxidation of Aromatic Alcohols with Manganese Dioxide

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Manganese dioxide has been used to oxidize polyene alcohols[1] and allyl alcohols[2,3] to the corresponding carbonyl compounds. Benzyl alcohol resisted oxidation.[1] A naturally occurring alcohol, lymphokentric acid,[4] has been found to be oxidized by manganese dioxide at room temperature as detected by the biological test used for examining the oxidation products of reference 4. Because this material appeared to have no reducible double bonds, we have re-examined the effect of aromatic rings in activating the oxidation of alcohols with manganese dioxide.

The following alcohols showed no oxidation: furfuryl alcohol, benzyl alcohol, *p*-anisyl alcohol, ethyl β-phenyl-β-hydroxypropionate and methyl mandelate. Oxidation to the corresponding ketones was successful with secondary alcohols, as shown in Table I.

Experimental

Oxidation Method.—The alcohols, in 2-g. samples, were treated with equal weights of manganese dioxide (prepared according to reference 2) at room temperature for twelve days (method A), or by refluxing for one hour (method B). The solvent was dry benzene (20 ml.), except in the case of furoin, where 1:1 chloroform–benzene (40 ml.), was used.

γ-(5,6,7,8-Tetrahydro-1-naphthyl)-butyric Acid.—β-(1-Naphthoyl)-propionic acid, prepared by the method of Lontz,[5] was reduced to γ-(1-naphthyl)-butyric acid by the method of Huang-Minlon[6,7] in 80% yield. This product was then reduced by the Raney alloy procedure,[8] which

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