Anal. Calcd. for $C_{23}H_{32}O_6$: C, 68.29; H, 7.98. Found: C, 68.06; H, 7.70.

There was a strong depression in m.p. on admixture with the corresponding 11-ketone IV, and the infrared spectra were different.

Oxidation of VI to IV.—The oxidation of 100 mg. of the hydrocortisone acetate rearrangement product VI was carried out with 50 mg. of chromium trioxide in 10 cc. of acetic acid for 10 minutes at room temperature. Isolation with chloroform and crystallization from methanol yielded the triketone IV, m.p. 196–200°, identified with the material (m.p. 199–201°) obtained from cortisone acetate by mixture m.p. determination and infrared comparison.

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Derivatives of 2-Phenylbenzimidazole. II

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As a continuation of work reported earlier[2] all twelve of the 5-nitro-2-monohalophenylbenzimidazoles have been prepared. The data for the derivatives are given in Table I. The method used in the preparation of these compounds was essentially that of Walther and v. Pulawski.[3] The *o*- and *p*-chloro derivatives were prepared also by the method of Weidenhagen[4] using the appropriate halobenzaldehyde, cupric acetate and 4-nitro-*o*-phenylenediamine. The yields were 17 and 45%, respectively.

In addition the three 2-monofluorophenylbenzimidazoles were prepared using the same method as previously reported.[1] The data for these compounds are given in Table II.

Experimental

4-Nitro-*o*-phenylenediamine (0.013 mole) and the appropriate halobenzoic acid (0.013 mole) were heated in a Pyrex tube at 210–220° in an oil-bath for one hour. The cooled mass was pulverized, triturated with a saturated solution of sodium bicarbonate, filtered and the residue extracted with hot ethanol. The product was obtained from the alcohol solution by the addition of water. Repeated crystallization from aqueous ethanol using charcoal gave analytically pure samples in the yields indicated in Table I.

The *o*-fluoro-, *o*-chloro- and *o*-bromonitro derivatives were white crystalline substances, while the *o*-iodonitro compound was light yellow. All of the other halonitro derivatives were yellow crystalline substances. The *p*-fluoro- and *o*-chloronitro compounds turned yellow on heating and melted to give a yellow liquid. The *o*-bromo isomer melted to give a yellow liquid while the *o*-iodo isomer turned white on heating but melted to give a yellow liquid. The three 2-fluorophenylbenzimidazoles were white crystalline substances. All of the derivatives were insoluble in water but soluble in acetone, ether, dioxane and alcohol.

TABLE I

4-Nitro-*o*-phenylenediamine condensed with, acid	Yield, %	M.p., °C.[a]	Formula	Nitrogen,[b] % Calcd.	Found	Halide,[c] % Calcd.	Found
o-Fluorobenzoic	21	189	$C_{13}H_8FN_3O_2$	16.33	16.58	7.4	7.4
m-Fluorobenzoic	18	208			16.21		7.2
p-Fluorobenzoic	9	260			16.55		7.7
o-Chlorobenzoic	11	181	$C_{13}H_8ClN_3O_2$	15.38	15.35	13.0	12.7
m-Chlorobenzoic	13	223			15.76		12.5
p-Chlorobenzoic	10	308			15.14		12.7
o-Bromobenzoic	5	173	$C_{13}H_8BrN_3O_2$	13.20	13.55	25.2	24.6
m-Bromobenzoic	10	226			13.62		24.7
p-Bromobenzoic	7	294			13.58		24.7
o-Iodobenzoic	4	208	$C_{13}H_8IN_3O_2$	11.50	11.32	34.8	35.1
m-Iodobenzoic	11	230			11.23		34.9
p-Iodobenzoic	10	264			11.78		34.9

[a] All melting points were determined by means of a Fisher–Johns hot-stage, melting point block. [b] Micro-Dumas nitrogen analyses by C. F. Geiger, 312 Yale St., Ontario, California. [c] Halogen analyses, except fluorine, after fusion in a micro-peroxide bomb were by Volhard titration. Fluorine analyses after fusion in a peroxide bomb were by the method of Nichols and Olsen.[5]

TABLE II

o-Phenylenediamine condensed with, acid	Yield, %	M.p., °C.[a]	Formula	Nitrogen[a] % Calcd.	Found	Halide,[a] % Calcd.	Found
o-Fluorobenzoic	26	207	$C_{13}H_9FN_2$	13.21	13.14	9.0	9.3
m-Fluorobenzoic	46	258			13.57		9.6
p-Fluorobenzoic	39	257			13.94		8.5

[a] See notes to Table I.

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Isomaltose Phenylosazone and Phenylosotriazole

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The phenylosazone of a crude "isomaltose" has been described by Fischer[2] and others.[3] The

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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.

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

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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

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