[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

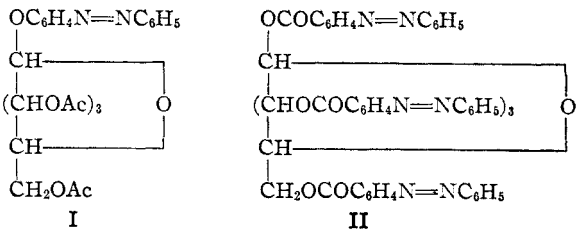
Preparation and Chromatographic Separation of *p*-Phenylazophenyl Polyacetylglycosides

BY CHARLES D. HURD AND ROBERT P. ZELINSKI[1]

One of the methods useful in separating monosaccharides from disaccharides involves propionylation and subsequent distillation.[2] The method, however, is not well adapted for the further separation of hexose pentapropionates from each other, or of disaccharide octapropionates from each other. Development of a plan for such separations was in mind in the present investigation, and chromatographic adsorption seemed promising as an approach.

Coleman's studies on the adsorbability of *p*-phenylazobenzoic esters of sugars[3] have revealed the possibility of separations of differing carbohydrate types such as monosaccharide, disaccharide; or aldose, ketose; or reducing disaccharide and non-reducing disaccharide. He also achieved separation of closer mixtures by way of the *p*-phenylazobenzoic esters in a few instances: α-D-galactose from β-D-galactose (but not β-D-arabinose from β-L-arabinose), α,α-trehalose from β,β-trehalose, β-D-glucose from α-D-xylose or β-L-arabinose. Mention is made, however, that several carbohydrate pairs have resisted separation. It is interesting to note that no separations are listed for a pair of aldohexoses or a pair of reducing disaccharides, but it has recently been shown[4] that such separations are possible in some instances by passing solutions of the sugars themselves through a clay column.

The direct visibility of colored compounds is an advantage in chromatographic separations, and azoaryl glycosides were selected in the present study for several reasons, chief of which was the fact that no more than one azo group would be involved per sugar molecule. It was planned to prepare these compounds by interaction of *p*-hydroxyazobenzene with either polyacetylglycosyl acetate or chloride, and the availability of *p*-hydroxyazobenzene was an important consideration. Although an azo group is present in the proposed compounds (such as I), attention should be drawn to the structural differences from the previously employed azo derivatives (such as II), for which the relatively inaccessible *p*-phenylazobenzoyl chloride is the reagent required. Also, the azoaryl glycosides represent a new family for adsorption studies. As a matter of fact, they represent practically a new type of sugar derivative, only the *p*-phenylazophenyl and *p*-(3-nitrophenylazo)-phenyl tetraacetyl-β-D-glucopyranosides having been reported previously.[5] Three related phenolic azoaryl glycosides,[6] made by coupling diazo compounds with *o*-hydroxyphenyl tetraacetyl-β-D-glucoside, have been reported also.



For the preparation of the *p*-phenylazophenyl polyacetylglycosides, two methods were investigated. The first was based on the acid catalyzed fusion[6,7] of phenols with fully acetylated sugars, and the second on the Koenigs–Knorr reaction of phenols with acetylated glycosyl chlorides.[8,9] Investigation of the first method with *p*-phenylazophenol (*p*-hydroxyazobenzene) showed that for each sugar there exists a set of optimum conditions, particularly the fusion temperature and the mole ratio of *p*-toluenesulfonic acid catalyst to the acetylated sugar, and that these conditions do not usually coincide within a single type of sugar such as the monosaccharides or disaccharides. Thus, temperatures and ratios which were satisfactory for the synthesis of *p*-phenylazophenyl tetraacetyl-β-D-glucoside were conditions which failed to give any yield of the corresponding derivatives of D-galactose or D-xylose. It is evident, therefore, that this method was not well adapted for the reliable conversion of a mixture of sugars to the azoaryl derivatives.

The second method, however, was more general in its application. The polyacetyl-α-glycosyl chlorides related to D-glucose, D-galactose, D-xylose, maltose and lactose all reacted smoothly with *p*-phenylazophenol in the presence of dry quinoline and silver oxide. The *p*-phenylazophenyl polyacetylglycosides prepared were yellow or orange solids identical with those obtained by the fusion reaction.

Tentatively, the phenylazophenyl glycosides have been assigned the beta configuration, since this is the configuration quite generally reported for aryl glycosides prepared from other phenols, using the same two methods. In no instance, however, has it been possible to prepare the cor-

(1) Pabst Brewing Company Fellow, 1942–1945.

(2) Hurd and collaborators, THIS JOURNAL, **63**, 2656, 2657, 2659 (1941); **66**, 2015 (1944).

(3) Coleman, *et al.*, *ibid.*, **64**, 1501 (1942); **65**, 1588 (1943); **67**, 381 (1945).

(4) Lew, Wolfrom and Goepp, *ibid.*, **68**, 1449 (1946).

(5) Hurd and Bonner, *J. Org. Chem.*, **11**, 50 (1946).

(6) Helferich, Lang and Schmitz-Hillebrecht, *J. prakt. Chem.*, **138**, 275 (1933).

(7) Helferich and Schmitz-Hillebrecht, *Ber.*, **66**, 378 (1933).

(8) Zemplén and Müller, *ibid.*, **62**, 2107 (1929).

(9) Robertson and Waters, *J. Chem. Soc.*, 2729 (1930).

responding phenylazophenyl α-D-glycosides. All attempts to prepare the latter by fusion in the presence of zinc chloride yielded the same beta product as that obtained by the acid catalyzed fusion.

Only one other aglucone, *p*-anisylazophenol, was tested, but this yielded a *p*-anisylazophenyl tetraacetyl-β-D-glucoside of such light color that it was rejected for chromatographic purposes.

The *p*-phenylazophenyl polyacetylglycosides were readily deacetylated by hydrolysis with sodium methoxide in methanol or by aqueous sodium hydroxide in acetone to yield the *p*-phenylazophenyl glycosides. Such derivatives from the disaccharides proved difficult to crystallize. These in turn were propionylated to form crystalline *p*-phenylazophenyl polypropionylglycosides, but it should be noted that these propionates were more difficult to crystallize than the corresponding acetates.

Chromatographic separation of mixtures of the *p*-phenylazophenyl polyacetylglycosides was tested on columns of floridin, pumice and silica gel. Although the first two adsorbed the glycosides, silica gel behaved best of the three and possessed advantages of being white and forming an opalescent column which facilitated visual inspection of the progress of chromatography. Two and three component mixtures were separated by development with benzene or chloroform or a solution of equal parts by volume of benzene, chloroform and petroleum ether. After separation had been effected the colored bands were removed and the glycosides recovered by extraction with acetone and evaporation. The components were identified by melting points and specific rotations of the recrystallized residues.

As shown in Table I, mixtures of the acetylated *p*-phenylazophenyl glycosides of the following sugars were separated in this manner: D-xylose and D-glucose; D-glucose and D-galactose; D-glucose and lactose; D-glucose and maltose; lactose and maltose; D-xylose, D-glucose and lactose. The disaccharide remained at the top of the column, followed by the hexose and finally the pentose. Mixtures containing all three or any two components of the above classes of carbohydrates were usually readily and distinctly separated. Galactose appeared above glucose, and lactose above maltose, but in these instances visually clear separation was not evident even after development with large volumes. Even though a continuous zone appeared to be formed, the pure components were readily isolated from the two ends. Although the method was primarily developed to afford a qualitative analysis, rough material balances, based on the amounts recovered after evaporation of the acetone solution, indicated good recovery. Occasional increases in weight were probably due to the presence of traces of solvent. Rotations were not attempted on the recovered material because of the small quantities involved. That the recovered material was essentially pure before crystallization was witnessed by the fact that the yield of each fraction after crystallization was about the same as the yield obtained from the same weight of pure material, similarly recrystallized.

TABLE I

CHROMATOGRAPHIC SEPARATION OF *p*-PHENYLAZOPHENYL POLYACETYL-β-D-GLYCOSIDES ON SILICA GEL

Components[a]	Mixture M. p., °C.	Wt., g.	Developing agent Type[b]	Vol., ml.	Recovered materials Wt., g. before recryst.	M. p. (°C.) after recryst.
Glucoside	172	0.10	C	300	0.09	169–170
Xyloside	139.5	.10			.08	133–136
Glucoside	172	.20	B	3500	.22	169–170
Xyloside	139.5	.20			.16	138–139
Glycoside	172	.10	B, C, P	1000	.09	172
Xyloside	139.5	.10			.09	138–139
Galactoside	134–135	.10	B, C, P	3500	.04	133–134
Glucoside	172	.10			.02	169–170
Lactoside	137–138	.10	C	150	..	138–139
Glucoside	172	.10			..	171
Lactoside	137–138	.20	B	1000	.17	137–138
Glucoside	172	.20			.14	171–172
Maltoside	171–172	.10	B	3500	.12	170–171[c]
Glucoside	172	.10			.12	171–172[c]
Lactoside	137–138	.10	B, C, P	7500	.09	137–138
Maltoside	171–172	.10			.06	172
Lactoside	137–138	.10			.10	132–133
Glucoside	172	.10	B	450	.09	169–170
Xyloside	139.5	.10			.12	136–137

[a] The first component in each mixture was found in the upper zone. [b] B signifies benzene, C means chloroform, and B, C, P indicates a solution of benzene, chloroform and petroleum ether (Skellysolve B, b. p. 60–70°) in a 1:1:1 volume ratio. [c] Identity confirmed by measurement of specific rotation.

Experimental

Preparation of *p*-Phenylazophenyl Polyacetylglycosides. (a) The Fusion Method with *p*-Toluenesulfonic Acid and β-Sugar Acetates.—A mixture of the β-D-glycose polyacetate (0.0128 mole), *p*-phenylazophenol (*p*-hydroxyazobenzene) (0.0505 mole), and *p*-toluenesulfonic acid monohydrate was heated under water pump vacuum for one hour by an oil-bath to temperatures of 140–175°. The residue was extracted three or four times with benzene (60-ml. portions) and the combined extract was washed twice with 10% aqueous sodium hydroxide solution (500 ml. total). It was usually necessary to filter the first wash mixture through Celite to break up the emulsion which formed. Washing with water removed the last of the alkaline solution and left clear red solutions from which the benzene was removed by distillation from a steam-bath. Crystallization of the residue was effected from *i*-propyl alcohol.

In a search for optimum conditions about fifteen trials were performed on each of five sugars. The fusion time was held constant at one hour, the variables being the temperature (145 to 175°) and the mole ratio of *p*-toluenesulfonic acid catalyst to sugar acetate (0.09 to 1.00). Details of some representative experiments are presented in Table II. Zero yields were formed in all instances when the mole ratio of catalyst was as low as 0.10.

(b) Experiment with α-D-Glucose Pentaacetate.—The optimum conditions found in (a) for β-D-glucose pentaacetate were applied to the alpha isomer. The only isolable product was *p*-phenylazophenyl acetate, m. p. 85–86°. A sample of this substance synthesized from

TABLE II

FUSION SYNTHESES USING *p*-TOLUENESULFONIC ACID AS CATALYST

Temp., °C.	Mole ratio[a]	Yields,[b] % X	Ga	G	L	M
145	0.18	0	..	29	0	..
	.37	0	..	32	0	..
	.55	28	..	0	7	..
	.77	23	..	..	21	..
155	.18	0	0	35	0	0
	.37	0	..	22	0	0
	.55	21	31	0	19	[d]
	.77	12	23	..	..	..
	.80	..	..	..	37	[d]
160	.15	..	..	..	..	26[e]
165	.18	0	0	34	0	[d]
	.37	0	4	23	0	[d]
	.55	21	44	0	22	[d]
	.77	11	22	..	[d]	[d]
175	.18	..	..	26	..	..
	.37	..	..	6	..	.

[a] Of *p*-toluenesulfonic acid to acetylated sugar. [b] X, D-xylose; Ga, D-galactose; G, D-glucose; L, lactose; M, maltose. [c] Could not be duplicated. [d] Product would not crystallize.

acetic anhydride and *p*-hydroxyazobenzene melted[10] at 86–87°. When zinc chloride, dissolved in acetic acid, was tested as catalyst in place of toluenesulfonic acid, the α-D-glucose pentaacetate was recovered unchanged after fusion for one hour at 145°. The molar ratio of catalyst to acetylated sugar was 0.9.

(c) The Fusion Method with Zinc Chloride.—Experiments with β-D-glucose pentaacetate were performed as in (a), except for replacement of the sulfonic acid catalyst by zinc chloride in acetic acid solution. Mole ratios (M. R.) of zinc chloride to sugar acetate of 0.04, 0.54 and 0.85 promoted yields of 7, 19 and 17%, respectively. β-D-Galactose pentaacetate (M. R. 0.85), β-lactose octaacetate (M. R. 0.85), and β-D-xylose tetraacetate (M. R. 0.08, 0.43) gave these yields, respectively: 26, 16, 30, 43%. Changing the catalyst did not affect the configuration of the azo glycosides obtained, since all were beta as in (a).

(d) The Koenigs-Knorr Reaction.—The polyacetylglycosyl acetate (0.0100 mole) was dissolved in absolute chloroform (19 ml.) and refluxed for three hours on a steam-bath with titanium tetrachloride (0.0100 mole) in chloroform (10 ml.). The clear solution was washed with water, filtered through Celite and dried over sodium sulfate. The residue obtained from vacuum distillation of the chloroform at 50–55° was dissolved in anhydrous quinoline (10 ml.) and stirred with silver oxide (0.0100 mole) and *p*-phenylazophenol (0.0300 mole) for fifteen minutes. After another hour the thick paste was shaken with hot benzene (200 ml.) and filtered. The silver oxide precipitate was washed with more benzene, and the combined benzene solutions were washed successively with 10% hydrochloric acid, 10% sodium hydroxide, and finally with water. The residues left by distillation of the benzene were then recrystallized from *i*-propyl alcohol. By comparisons of melting points and specific rotations the products were shown to be identical with those obtained by the fusion method. The yields obtained for *p*-phenylazophenyl tetraacetyl-β-D-glucoside and -β-D-galactoside were 30 and 22%, respectively; and for *p*-phenylazophenyl heptaacetyl-β-maltoside and -β-lactoside, 31 and 29%. The -β-cellobioside was prepared in 44% yield from heptaacetyl-α-cellobiosyl bromide instead of the chloride. The bromide was selected, since two attempts to prepare the chloride using the titanium chloride procedure on α-cellobiose octaacetate resulted in substantial recovery of unreacted octaacetate.

(10) Wallach and Kiepenhauer, *Ber.*, **14**, 2617 (1881), reported m. p. 84–85°.

Deacetylation to *p*-Phenylazophenyl Glycosides.—The *p*-phenylazophenyl polyacetylglycoside was dissolved in hot methanol, and a small piece of sodium was added. After several hours at room temperature, the methanol was distilled off leaving a solid yellow residue. As an alternative, aqueous sodium hydroxide could be added to an acetone solution of the acetylated compound. After two days of standing at 5°, dilution with water precipitated the deacetylated product.

Recrystallization from boiling water gave 84–97% yields of the *p*-phenylazophenyl glycosides except with the maltoside. This was soluble in water and could be contained in a solid form only by evaporation to dryness of a solution in methanol. On standing in air, it reverted to a gum.

Propionylation.—The *p*-phenylazophenyl β-D-glycoside was dissolved in pyridine, mixed with an excess of propionic anhydride, and allowed to stand at room temperature for three days before being hydrolyzed with cold water. The hydrolysis mixture was extracted with benzene and the benzene was washed successively with 10% hydrochloric acid, water, 10% sodium hydroxide and finally with water. Evaporation and recrystallization from *i*-propyl alcohol gave 73–89% yields of orange, fine needles for the monosaccharides. Yields of the corresponding disaccharide derivatives were lower (*e. g.*, 45% from maltose) primarily because of the greater difficulty in crystallization.

Table III lists the constants and analyses of the seventeen new compounds.

TABLE III

PHYSICAL CONSTANTS AND ANALYSES OF THE *p*-PHENYLAZOPHENYL β-GLYCOSIDES AND DERIVATIVES

p-Phenylazophenyl glycoside	M. p., °C.	$[\alpha]^{25}D$ in chloroform[c]	Nitrogen, % Obs.[d]	Calcd.
Tetraacetyl-β-D-glucoside	172	− 3.2	5.40	5.30
β-D-Glucoside	188.5–189	−96.6[a]	7.43	7.77
Tetrapropionyl-β-D-glucoside	93–94	2.7	4.79	4.79
Tetraacetyl-β-D-galactoside	134–135	30.0	4.96	5.30
β-D-Galactoside	179–179.5	−96.1[a]	7.52	7.77
Tetrapropionyl-β-D-galactoside	81–82	25.8	4.98	4.79
Triacetyl-β-D-xyloside	139.5	−39.9	6.38	6.11
β-D-Xyloside	203–204	−44.1[a]	8.30	8.36
Tripropionyl-β-D-xyloside	119–120	−32.9	5.64	5.62
Heptaacetyl-β-lactoside	137–138	− 8.2	3.59	3.42
β-Lactoside	202.5–203.5	−41.7[b]	5.63	5.36
Heptapropionyl-β-lactoside	126–127	− 5.1	3.08	3.06
Heptaacetyl-β-maltoside	171.5–172	56.2	3.70	3.42
β-Maltoside	140–142	−15.1[b]	4.41	5.36
Heptapropionyl-β-maltoside	140–141	52.4	2.79	3.06
Heptaacetyl-β-cellobioside	228.5–229	−14.1	3.86	3.42
p-Anisylazophenyl tetracetyl-β-D-glucoside	174.5–175	5.6	5.00	5.02

[a] In dioxane. [b] In pyridine. [c] Concn. of 1.0–1.6 g./100 ml. [d] Analyses (micro Dumas) no. 2, 3, 11, 12, 14, 16 by Winifred Brandt; no. 15 by Margaret Ledyard; others by T. S. Ma.

Preparation of Silica Gel.—A solution of 3 *N* hydrochloric acid (200 ml.) was added slowly with stirring to a solution of water-glass (100 g.) in water (200 ml.). The

gel which formed was broken up and heated on a steam-bath in an air stream to evaporate most of the excess water and acid. It was crushed, washed with water until no longer acid to litmus, and then dried at 70° for three or four days. The product was pure white.

Chromatographic Separations.—Columns were prepared by slowly pouring the gel (40–60 mesh) into a glass tube (20 × 500 mm.) and with rapid tapping of the sides to pack the adsorbent to a depth of 300 mm. or more. After it was wetted with the developing solution, there was added a solution of the *p*-phenylazophenyl polyacetyl-β-glycosides dissolved in a minimum volume of the developing agent. Separation was effected by further addition of the developing agent. The amount and composition of the latter is shown in Table I. The colored zones were removed and extracted with acetone in a Soxhlet extractor. Filtration and evaporation at reduced pressure left sirupy residues which were then weighed and recrystallized from *i*-propyl alcohol. Identity was determined from melting points and specific rotation.

The separation of a D-glucoside, D-xyloside, lactoside mixture offers typical results. After development with 450 ml. of benzene, the lactoside was present in a 25-mm. orange band at the top and was followed by a 35-mm. colorless band, an 80-mm. orange zone containing the glucoside, an 80-mm. colorless band, and finally a 175-mm. light orange band containing the xyloside.

Summary

p-Phenylazophenyl polyacetyl-β-glycosides related to D-xylose, D-glucose, D-galactose, lactose, maltose, and cellobiose have been prepared. From these the corresponding *p*-phenylazophenyl β-D-glycosides and *p*-phenylazophenyl polypropionyl-β-D-glycosides have been made.

Chromatographic separations of *p*-phenylazophenyl polyacetylglycosides have been carried out with mixtures of several carbohydrate types including pairs of reducing monosaccharides and a pair of reducing disaccharides.

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5-Desoxy-L-sorbose[1]

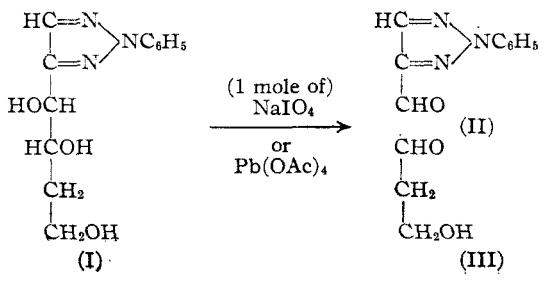
BY PETER P. REGNA

A new crystalline desoxy sugar, 5-desoxy-L-sorbose (IV) (synonym 5-desoxy-D-fructose), was isolated from the mother liquors of the large-scale production of L-sorbose which is obtained from sorbitol by submerged growths of *Acetobacter suboxydans*. The commercial sorbitol used in this particular fermentation was not prepared by the catalytic hydrogenation of D-glucose, but by the electro-reduction of the sugar under mild alkaline conditions. The desoxysorbose undoubtedly arises from the oxidative fermentation of 2-desoxy-D-sorbitol which has been recently demonstrated by Wolfrom, Konigsberg, Moody and Goepp[2] to be present in small amounts in the electro-reduction product.

The desoxy-L-sorbose crystallizes in needles from absolute ethanol, gives a Rosenthaler test similar to a methyl pentose and forms several well-defined derivatives: the tetraacetate and phenylosazone. The desoxysorbose tetraacetate gives a positive Pacsu keto-acetate test indicating the presence of a free ketone group in the molecule. The phenylosazone was converted to phenyl-5-desoxy-L-sorbosotriazole and from this was prepared the tribenzoate derivative.

The desoxysorbose was catalytically hydrogenated under high pressure to a mixture of 2-desoxy-D-sorbitol and 2-desoxy-L-iditol. The solution containing the mixed sugar alcohols was subjected to fermentation with a suspension of cells of *Acetobacter suboxydans* from which the desoxy sugar was isolated as phenyl-5-desoxy-L-sorbosazone. The fermentation of the alcohols produced about 65% of the desoxyketose and shows that the hydrogenation favors, in almost 2:1 ratio, the formation of the D-sorbitol isomer. The preparation of the sugar by oxidative fermentation indicates that the structure of the starting alcohol satisfies Bertrand's rule in that the two hydroxy groups, adjacent to the terminal primary alcohol group, are in *cis*-position; this establishes the position of the hydroxyl on C_3 of 5-desoxy-L-sorbose (IV).

In a preliminary experiment, it was found that with an excess of sodium periodate the methyl desoxysorboside consumed less than 1.5 equivalents of oxygen suggesting that a single glycol grouping existed in the molecule. Further evidence of the structure of the desoxy sugar was obtained when phenyl-5-desoxy-L-sorbosotriazole (I) was oxidized by lead tetraacetate or sodium periodate. With one molecular equivalent of either reagent the osotriazole readily consumes two equivalents of oxygen and splits smoothly into two aldehydes which have been identified as 2-phenyl-4-formylosotriazole (II) and β-hy-



(1) The material in this paper was presented before the Division of Sugar Chemistry and Technology at the Chicago Meeting of the American Chemical Society in September, 1946.

(2) M. L. Wolfrom, M. Konigsberg, F. B. Moody and R. M. Goepp, Jr., THIS JOURNAL, **68**, 122 (1946).