Summary

We have shown:

1. That *m*-nitrodibenzoylmethane is obtained by way of a different series of reactions.

2. That *m*-nitrodibenzoylmethane reacts toward hydroxylamine, phenylhydrazine and hydrazine as 1-phenyl-3-*m*-nitrophenylpropene-one-3-ol-1, giving rise to 3-phenyl-5-*m*-nitrophenylisoxazole (V), 1,5-diphenyl-3-*m*-nitrophenylpyrazole (XI) and 3-*m*-nitrophenyl-5-phenylpyrazole (XIII), respectively.

3. That phenylhydrazine reacts by way of 1,4-addition to *m*-nitrobenzalacetophenone to yield 1,5 - diphenyl - 3 - *m* - nitrophenylpyrazoline (XII).

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Azoyl Derivatives of Sugars and Separation by Chromatographic Adsorption.[1] II

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In a previous paper[3] it was shown that the azoyl derivatives of monosaccharides could be separated from azoyl derivatives of disaccharides and trisaccharides by means of chromatographic adsorption. A ketohexose azoate, β-D-fructose tetraazoate, was separated from each of several aldohexose azoates. This type of separation had been reported previously by Reich.[4] It was also shown that in certain cases disaccharides could be partially separated.

The chromatograhic adsorption technique has been further developed and the separation of two closely related compounds, α-D-galactose pentaazoate and β-D-galactose pentaazoate, carried out. β-D-Glucose pentaazoate has been separated from both α-D-xylose tetraazoate and β-L-arabinose tetraazoate. No separation was obtained with the azoates of the enantiomorphs, β-D-arabinose and β-L-arabinose.

The method also has been applied to azoyl derivatives of methyl glycosides and to azoyl derivatives of partially acetylated sugars. The separation of methyl heptaazoyl-β-D-cellobioside and methyl tetraazoyl-α-D-glucoside was accomplished, and an unusually sharp separation made with 1-azoyltetraacetyl-β-D-glucose and 1-azoylheptaacetyl-β-D-cellobiose. The separation of azoates of the methyl glycosides was not as clean cut under the conditions used as was that of the 1-azoylacetyl sugars. The preparation and use of the latter type of derivative was undertaken with the thought of working with a derivative in which the original sugar represented a higher percentage of the molecular weight.

The chromatographic technique has been applied to the separation of a mixture of more than two sugar derivatives. A mixture of β-L-arabinose, β-D-glucose, α,α-trehalose and β-cellobiose azoates has been separated. The purity of each band is difficult to estimate on the basis of optical rotation in such a case. Several generalizations have been noted which lead us to believe that a purity of at least 90% for each compound has been obtained. In working with a mixture of two derivatives only, it was observed that the lowest band usually leaves a small amount of material behind which contaminates the higher band. Thus the principal contamination of a given band comes from the bands below. The amount of contamination decreases as the distance between the bands is increased. The higher bands are in such a case apt to be more highly contaminated than the lower.

The separation of the four azoates was made on one column. It is probable, however, that in instances where several derivatives are present the most efficient method of separation is to develop the chromatogram until it is separated into groups such as monosaccharides, disaccharides, etc., which separate quickly and then isolate each group and develop it separately. This permits maximum development for slower moving bands, diminishes contamination from previous bands and diminishes the effect of faults in the column.

In the previous work a mixture of equal volumes of commercial chloroform, benzene and ligroin was used to develop the chromatogram. It was found that if alcohol-free chloroform was used

(1) Presented at the 105th Meeting of the American Chemical Society, Division of Sugar Chemistry and Technology, April, 1943.

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(3) I, THIS JOURNAL, **64**, 1501 (1942).

(4) Reich, *Biochem. J.*, **33**, 1000 (1939).

in the mixture with magnesol as the adsorbent no development took place. Silicic acid, however, does not adsorb as strongly and some development took place with it as adsorbent. The solvent used for development of a mixture of β-D-glucose azoate and β-cellobiose azoate on silicic acid was varied from pure chloroform to chloroform containing 10% alcohol by volume. It was found that with 0.1% alcohol the cellobiose azoate band was held at the top, which is desirable. With a 0.2% alcohol developing solvent the cellobiose band moved down the column. This is illustrated in Table IV.

The polar alcohol is partially adsorbed and will displace various derivatives. This can be used to advantage if a concentration be found which will displace one azoate and not the other. This was employed very successfully in the separation of the 1-azoyl derivatives of glucose and cellobiose. An additional advantage of using alcohol in such a separation is that it cleans the chromatogram of tailings, thus reducing the contamination.

The preparation of sugar azoates from sugars and the acid chloride in pyridine solution as previously reported has been somewhat modified. The preliminary reaction is allowed to take place at 0° and then completed at 90°. After the hydroxyl on carbon atom one had been azoylated at 0° no change was observed in the configuration on heating for prolonged periods at 90°. In the preliminary reaction at 0° the low temperature minimizes the mutarotation but does not completely prevent it. A small amount of the other member of the anomeric pair is thus always obtained.

It was found necessary to add a little water to the pyridine solution to decompose the azoylpyridinium complex before precipitating the azoate by addition of the solution to a larger volume of water. If this was not done considerable azoic anhydride was formed and this complicated the purification. The formation of acid anhydrides by the addition of a pyridine solution of acid chloride to cold water has been known for some time. Minunni[5] first reported the reaction in the preparation of benzoic anhydride. Fischer[6] mentions its formation in connection with the preparation of the *p*-bromobenzoates of sugars. This method of preparing anhydrides of the higher acids is apparently very general and excellent yields are obtained. Azoic anhydride was prepared by this method in 98% yields.

The separation of the azoates from the azoi acid was effected by dissolving in chloroform and precipitating them by the addition of alcohol. This is very effective since azoic acid is but slightly soluble in chloroform and relatively soluble in alcohol.

In determining certain physical properties of the azoates it was observed that the derivatives obtained from sugars in which the hydroxyl groups on carbon atoms one and two had a *cis* relationship usually were more soluble than the corresponding derivatives obtained from sugars in which a *trans* relation existed. While the number of compounds studied is at present limited, this generalization applies to the azoates of several monosaccharides and disaccharides. For example, α-D-galactose pentaazoate is far more soluble in chloroform and similar solvents than is β-D-galactose pentaazoate. The azoate of α,α-trehalose is far more soluble than is that of β,β-trehalose. This solubility relationship is not independent of the rest of the molecule. In general the larger the number of groups having the *cis* relationship the higher the solubility. β-Maltose azoate seems to present something of an exception as it is less soluble than β-cellobiose azoate. The higher solubility of the compounds having the *cis* relationship in general makes their purification more difficult since some of the anomeric partner is usually present. The purification of the azoates is somewhat analogous to that of the benzoates[7] and as with them a relatively large number of crystallizations is often required to obtain pure derivatives.

With a limited number of exceptions all of the azoates prepared had sharp melting points. However, only derivatives containing four or less azoyl groups have as yet been obtained with easily discernible crystalline form.

The analysis of the azoates for the percentage of azoyl by a modified Zemplén hydrolysis as previously reported has been simplified. The results obtained with the method are consistently low by 0.3–0.6% depending on the compound. To determine whether the method was reliable, *p'*-iodoazoic acid (*p*-(*p*-iodophenylazo)-benzoic acid) was synthesized and from it a D-glucose

(5) Minunni, *Gazz. chim. ital.*, **22, II**, 213 (1892).
(6) Fischer and Noth, *Ber.*, **45**, 2724 (1912).
(7) Fischer and Freudenberg, *ibid.*, **45**, 2724 (1912); Levene and Meyer, *J. Biol. Chem.*, **76**, 513 (1928).

derivative prepared. This derivative was analyzed by the sodium peroxide bomb method for the per cent. of iodine and for the per cent. of *p*-iodoazoyl by hydrolysis. The results of the analyses by the two methods agreed fairly well. The comparison showed that the percentage of *p'*-iodoazoyl was 0.2% lower when determined by hydrolysis than that by determination for iodine. The hydrolysis method is regarded as reasonably reliable with the results slightly lower than calculated.

The azoates of several diacetone sugars have been prepared and all are crystalline compounds. The azoate of diacetoneglucose had previously been prepared by Freudenberg[8] using a slightly different procedure. The melting points of the two compounds are in close agreement. The sugar azoates and the azoates of the sugar derivatives which have been prepared are listed in Tables II and III.

The sugars used in the preparation of the azoyl derivatives were carefully checked for purity by measurement of their initial specific rotation at 0°. β-D-Arabinose was found to have an initial rotation (Fig. 1) which agreed favorably with that of Montgomery and Hudson $[\alpha]^{20}D$ 202° for β-L-arabinose.[9]

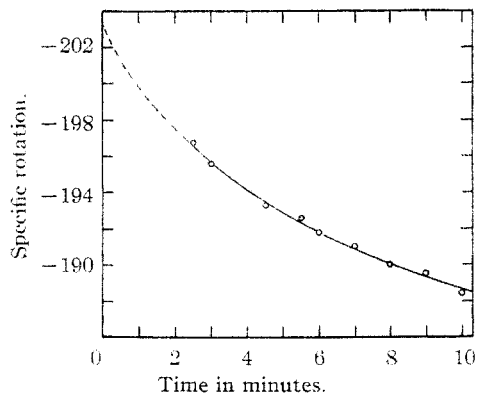


Fig. 1.—Mutarotation of β-D-arabinose in water at 0° with sodium D line.

Experimental

Preparation of Sugars.—The sugars were in general prepared by slow crystallization from a suitable solvent and kept in contact with an alcoholic solution for a period of at least a month. The initial rotation as measured at 0° corresponded favorably with previous values except in the case of β-D-arabinose (Fig. 1 and Table I). α-D-Glucose was prepared by the slow crystallization from glacial acetic acid and β-D-glucose by the rapid crystallization from glacial acetic acid as described by Hudson and Dale.[10] β-D-Galactose was prepared by rapid crystallization from alcohol at 0° by the method of Hudson and Yanovsky[11] and β-lactose from hot aqueous solution by the method of Hudson and Brown.[12] α-Lactose, β-cellobiose, β-melibiose, α-D-xylose, β-D-fructose and α-D-galactose were crystallized from aqueous alcohol. β-L-Arabinose was crystallized from water and β-gentiobiose dimethyl alcoholate from anhydrous methyl alcohol.

β-D-Arabinose was crystallized slowly from water or aqueous alcohol and the crystals very finely powdered and suspended in 80% alcohol for one month. The sugar was isolated and its specific rotation in water determined. Results are shown in Table I and Fig. 1.

TABLE I

MUTAROTATION OF β-D-ARABINOSE IN WATER AT 0°

4-dm. tube, sample 0.8684 g., vol. 34.60 ml.

Time, min.	Observed rotation	$[\alpha]^{5}D$
2.5	19.74	196.7
3.0	19.65	195.5
4.5	19.40	193.2
5.5	19.33	192.5
6.0	19.25	191.7
7.0	19.20	191.0
8.0	19.07	190.0
9.0	19.02	189.5
10.0	18.92	188.5

β,β-Trehalose was prepared by the condensation of tetraacetyl-α-D-glucosyl bromide and tetraacetyl-β-D-glucose in anhydrous chloroform solution. Its preparation will be described in detail in a later paper.

Preparation of Azoyl Derivatives of Sugars.—The finely powdered sugar (1 g.) was thoroughly mixed with 10 g. of azoyl chloride, 100 ml. of anhydrous pyridine cooled to 0° was added, and the mixture shaken well for thirty minutes. The solution was placed in the refrigerator at 0° for seven days during which time it was shaken several times a day. The solution, which in many cases solidifies, was removed from the refrigerator and heated for four days at 90° to complete reaction. To the heated solution upon removal from the oven was added 5 ml. of water to prevent anhydride formation. After five minutes the pyridine solution was poured into 1–1.5 liters of cold water with good stirring. The precipitate was filtered off and washed with a little alcohol. Much of the azoic acid may be recovered by acidifying the filtrate.

The dried precipitate was ground and refluxed with 50–150 ml. of chloroform and filtered hot. If there was considerable undissolved material, the residue was refluxed again and washed with hot chloroform until the washings had only a golden color. The residues are largely acid and acid decomposition products. The chloroform solution was added to six times its volume of alcohol with good stirring. In general the precipitate coagulates and is readily filtered from the solution. Certain azoates tend to form colloidal suspensions and are boiled a short time, then set aside overnight in order to induce coagulation.

In order to ensure more complete azoylation, the dry

(8) Freudenberg and Plankenhorn, *Ber.*, **73**, 621 (1940).

(9) Montgomery and Hudson, THIS JOURNAL, **56**, 2074 (1934).

(10) Hudson and Dale, *ibid.*, **39**, 324 (1917).

(11) Hudson and Yanovsky, *ibid.*, **39**, 1013 (1917).

(12) Hudson and Brown, *ibid.*, **30**, 984 (1908).

azoate was dissolved in the minimum amount of hot anhydrous pyridine and one-half its weight of azoyl chloride added. The solution was heated for two days at 90°. Purification was effected as before with two precipitations from the chloroform to remove the last traces of acid. With very few exceptions the yields of crude material were above 90%.

With β-D-fructose and β-D-maltose azoates a modified procedure was used as heating caused decomposition. After seven days at 0° the reaction mixture was left at room temperature for four days. It was necessary to use aqueous alcohol in the precipitation of β-D-fructose from chloroform due to its higher solubility in alcohol.

TABLE II

SUGAR AZOATES

s. = soluble, s. s. = slightly soluble, v. s. s. = very slightly soluble, v. s. = very soluble.

Azoate	Sinters, °C.	M. p., cor., °C.	$[\alpha]^{25}_{5438}$ ($CHCl_3$)	Per cent. azoyl	Sol. in $CHCl_3$
α-D-Glucose		265–266	223	84.94	s.
β-D-Glucose		252–253	− 50	84.90	s. s.
α-D-Galactose		275–276	436	84.36	v. s.
β-D-Galactose		255–255.5	170	84.92	s.
β-D-Fructose		124.5–125.5	−440	82.16	v. s.
α-D-Xylose		156–157	244	82.20	v. s.
β-D-Arabinose		261.5–262	−755	84.50	s. s.
β-L-Arabinose		262–262.5	755	84.52	s. s.
Sucrose		125–125.5	35	83.06	v. s.
α,α-Trehalose		134–134.5	210	82.74	v. s.
β,β-Trehalose		328–329	17	82.76	s. s.
α-Lactose	265	287–288	320	82.30	v. s. s.
β-Lactose		199–204	167	82.44	s.
α-Gentiobiose		232–233	62	82.36	s. s.
β-Maltose		274–275	2	82.36	v. s. s.
β-Cellobiose	268	272–273	105	82.62	s. s.
β-Melibiose		279.5–280	172	83.10	s. s.
Melezitose	127-130		188	81.74	v. s.
Raffinose		143–145	146	82.16	v. s.

Calculated per cent. azoyl:

Hexose pentaazoate	85.66	Pentose triazoate	81.01
Hexose tetraazoate	82.61	Disaccharide octaazoate	83.35
Pentose tetraazoate	85.13	Disaccharide heptaazoate	81.37

TABLE III

AZOATES OF SUGAR DERIVATIVES

Azoate	M. p., cor., °C.	$[\alpha]^{22}_{5438}$	Per cent. azoyl	Calcd.
Diacetoneglucose	111–112	− 81.5	44.27	44.65
Diacetonegalactose	124.5–126	− 57	44.06	44.65
Diacetonemannose	190.5–191	19	44.20	44.65
2,3,4,6-Tetraacetyl-β-D-glucose	182–183	− 63	37.09	37.59
Heptaacetyl-β-cellobiose	234–234.5	− 54.5	24.19	24.76
Monoacetoneglucose	166–166.5	−352	74.20	74.40
Methyl α-D-glucoside	214–215	74	80.02	81.59
Methyl β-cellobioside	282–284	209	79.38	80.74

Melting points were taken by the capillary method. Specific rotations were all taken in alcohol-free chloroform at 25° (*c*, 0.5). The light source was a mercury cadmium light and Wratten F filter. The zero setting was determined with a methyl azoate solution (*c*, 0.5).

α-D-Glucose azoate was crystallized[13] from dioxane six times and β-D-glucose azoate from dioxane four times. α-D-Galactose azoate was crystallized by dissolving in chloroform, adding four times the volume of carbon tetrachloride and evaporating to the original volume. The first crop of crystals, largely β-D-galactose azoate, was removed and the filtrate concentrated nearly to dryness. The entire process was repeated to remove more of the β-compound. The residue from the second evaporation was recrystallized from chloroform and carbon tetrachloride in the normal manner. The azoates of β-D-galactose, β-D-fructose, α-D-xylose, β-L-arabinose and β-D-arabinose were all crystallized three times by dissolving in chloroform, diluting with carbon tetrachloride and concentrating. The azoates of β-L-arabinose and β-D-arabinose formed red-orange platelets and the azoate of β-D-fructose orange needles. The azoates of sucrose, α,α-trehalose, β-lactose and raffinose were crystallized three times from cyclohexanol. Melezitose azoate was crystallized first from cyclohexanol, then a mixture of dioxane and alcohol and finally from cyclohexanol. α-Gentiobiose, β-maltose, β-cellobiose, and β-melibiose azoates were crystallized from a mixture of dioxane and *n*-butyl alcohol. α-Lactose was crystallized once from cyclohexanol and twice from dioxane and *n*-butyl alcohol. β-Maltose azoate and α-D-lactose azoate behaved peculiarly on repeated crystallization and the properties and physical constants varied considerably. The constants chosen were those of the products which gave the correct analysis and higher melting points.

The samples before analysis were dried at 80° and 10 mm. over sulfuric acid for twenty-four hours. The azoates with the exception of β-D-fructose azoate, are in general soluble in dioxane, pyridine and chloroform, only slightly soluble in most other common organic solvents and insoluble in alcohol and ligroin.

The azoates of sucrose, α,α-trehalose, melezitose and raffinose were precipitated from alcohol as a colloidal suspension and were dried to a fine golden powder. α-Lactose and β-lactose azoates tend to form an oil at first which soon solidifies. β-Melibiose azoate is also of this type but coagulates usually somewhat solvated. All of the others prepared were precipitated highly solvated. β-D-Fructose azoate dries to a fluffy precipitate. The azoates of α-D-xylose and β-arabinose dry to a pink voluminous solid. The azoates of glucose, galactose, β,β-trehalose, α-gentiobiose, β-maltose and β-cellobiose shrink in size enormously during drying to hard brittle deep red solids which in some cases are almost black.

Azoates of Methyl Glycosides.—The derivatives prepared were not stable to heat in pyridine solution and thus were prepared at room temperature. The amounts of reagents are the same as for the sugars, but the reaction time was cut to two days. Purification is similar to that of fully azoylated sugars. Methyl tetraazoyl-α-D-glucoside was crystallized twice from dioxane and *n*-butyl alcohol and once from carbon tetrachloride and chloroform. It is precipitated from chloroform by alcohol in a solvated form and dries to a brittle orange solid with the loss of solvent. Methyl heptaazoyl-β-cellobioside[14] was crystallized three times from nitrobenzene. It is precipitated highly solvated from chloroform and dries to a black brittle

(13) The word crystallized as used in this paper refers to a crystallization procedure and does not imply that the substance is crystalline. All crystalline compounds are described as such.

(14) Methyl β-cellobioside was prepared by the method of Pacsu, THIS JOURNAL, **52**, 2571 (1930).

solid. The glucoside is soluble in chloroform and the cellobioside is very slightly soluble.

Azoates of Acetone sugars.—Diacetonehexoses (4.5 g.) were treated with 8 g. of azoyl chloride and 50 ml. of dry pyridine. With diacetonemannose the reaction was carried out at 0° for two days and then ten hours at 90°. Diacetoneglucose was left at room temperature for two days and then heated for ten hours at 90°. Diacetonegalactose was left at room temperature for three days. Diacetonegalactose azoate is not stable to heat in pyridine solution, diacetoneglucose azoate is stable for short periods and diacetonemannose azoate is stable for prolonged periods.

The purification of diacetonehexose azoates is identical to that described under the sugar azoates. To the chloroform extract was added four times its volume of alcohol and this was concentrated to its original volume. On cooling the solutions to room temperature, diacetonegalactose and diacetonemannose azoates crystallized out while that of diacetoneglucose did so on cooling to 0°. The crystals were filtered off and recrystallized. Diacetonemannose azoate crystallized from alcohol containing one-fourth volume of chloroform as light reddish-golden rectangular platelets. Diacetonegalactose azoate crystallized from alcohol containing only a small amount of chloroform as light reddish-golden needles. Diacetoneglucose azoate crystallized from methyl alcohol containing a little water as small pink needles. Yields obtained were diacetonemannose azoate 88%, diacetonegalactose azoate 89%, and diacetoneglucose azoate 78%. They are in general soluble in chloroform and common organic solvents, slightly soluble in alcohol and hot ligroin, and insoluble in water. The solubilities decrease from diacetoneglucose to diacetonemannose.

Monoacetoneglucose triazoate was prepared from 2.1 g. of monoacetoneglucose, 10 g. of azoyl chloride and 100 ml. of pyridine according to directions for sugars. It precipitated from chloroform on the addition of alcohol in a highly solvated form and dried to a bulky light orange solid. It was crystallized once from ethyl acetate and ligroin, once from carbon tetrachloride, and twice from glacial acetic acid, yield of crude product 98%; solubility is comparable to that of fully azoylated sugars but slightly greater.

1-Azoyl-2,3,4,6-tetraacetyl-β-D-glucose.—A solution of 20 g. of tetraacetylglucosyl bromide in 400 ml. of anhydrous benzene was refluxed with 30 g. of finely powdered silver azoate for three hours. The silver salts were filtered off and washed with chloroform. The filtrate was evaporated to dryness, taken up in a small amount of hot glacial acetic acid and diluted well with water. The precipitate was filtered, dried and crystallized as fine golden needles from methyl alcohol. By slow crystallization from ethyl alcohol large red plates were obtained. Solubility corresponds somewhat to that of the pentaacetate; yield 79%.

1-Azoylheptaacetyl-β-cellobiose.—A solution of 20 g. of heptaacetylcellobiosyl bromide in 300 ml. of benzene was treated with 20 g. of silver azoate as before. The product crystallized from glacial acetic acid as fine pink needles. Solubility conforms to that of cellobiose octaacetate; yield 30%.

Analysis for Percentage Azoyl.—The per cent. azoyl was determined by saponification of the sugar esters and recovery of the azoic acid. The powdered sugar azoate (0.3–0.4 g.) was placed in a large Kjeldahl flask and dissolved by warming in 10 ml. (or the minimum amount) of dioxane. A solution of 15 mg. of sodium in 15 ml. of methyl alcohol was added slowly and the solution refluxed on the water-bath for thirty minutes. To the hot solution was added 30 ml. of 0.6 *N* sodium hydroxide while twirling the flask to ensure thorough mixing. The precipitate of methyl azoate which first formed soon saponified and after fifteen minutes on the steam-bath the clear solution was transferred to a 600-ml. beaker and diluted with 400 ml. of water. The solution was placed in an ice-bath and after ten minutes was slightly acidified with 6 *N* hydrochloric acid. The resulting precipitate was stirred to coagulate it and left in the ice-bath for four hours to complete precipitation and coagulation. The solution was finally filtered through a sintered glass crucible. The precipitate was washed well with water and dried to constant weight at 105°. The acid was extracted from the crucible by washing with pyridine and the weight of the acid determined by difference.

***p'*-Iodoazoic Acid (*p*-(*p*-Iodophenylazo)-benzoic Acid).**—*p*-Aminobenzoic acid (8 g.) was dissolved in 100 ml. of hot absolute alcohol and 15 ml. of glacial acetic acid was added. To this solution was added 15 g. of powdered *p*-iodonitrosobenzene[15] and the mixture allowed to stand for three days at room temperature with frequent shaking. The solution from which the *p*-iodonitrosobenzene had disappeared was filled with flaky crystals. It was placed in the ice-box for a few hours, filtered, and the crystals washed well with alcohol. The yield was about 70%. The acid crystallized from boiling nitrobenzene in orange-red flakes, m. p. 332–334°. The acid is nearly insoluble in many organic liquids but fairly soluble in dioxane, hot cyclohexanol and hot nitrobenzene.

Anal. Calcd. for $C_{13}H_9O_2N_2I$: I, 36.08. Found: I, 36.05.

***p'*-Iodoazoyl Chloride.**—*p'*-Iodoazoic acid (10 g.) was mixed with an equal weight of anhydrous sodium carbonate and refluxed with 150 ml. of thionyl chloride for three hours. The acid was entirely in solution by this time and the excess thionyl chloride was distilled off. The residue was extracted with ligroin which was filtered hot, concentrated, and on cooling the acid chloride crystallized out in reddish-brown needles, m. p. 170–171°. The yield was from 90–95%.

Methyl *p'*-Iodoazoate.—An excess of methyl alcohol was added to a solution of *p'*-iodoazoyl chloride in pyridine and the solution warmed for ten hours. The solution was poured into ice-water to precipitate the ester which was filtered from the solution, dried and crystallized twice from *n*-butyl alcohol. The methyl ester sintered sharply at 213° but did not melt sharply, some solid being still apparent at 235°.

Anal. Calcd. for $C_{14}H_{11}O_2N_2I$: I, 34.68. Found: I, 34.66.

D-Glucose *p'*-Iodoazoate.—*p'*-Iodoazoyl chloride (2.3 g.) was mixed well with 0.160 g. of finely powdered anhydrous D-glucose. Pyridine (11 ml.) was added and the mixture shaken until the material solidified. About 2 ml.

(15) Bamberger, *Ber.*, **28**, 249 (1895).

of dry dioxane was added and the shaking continued for thirty minutes. The solution was finally heated in the oven at 90° for several hours. The solution was poured into water and the precipitate was treated in a Soxhlet extractor with chloroform to remove the glucose azoate from the acid. Alcohol was added to the chloroform extract to precipitate the azoate, which was filtered from the solution and dried; yield 1.3 g., 76%. The azoate was recrystallized twice from dioxane and dried over sulfuric acid for twenty-four hours at 70° and 3 cm. It is very slightly soluble in carbon tetrachloride, slightly soluble in chloroform and hot cyclohexanol and fairly soluble in pyridine and dioxane.

Anal. Volhard method for halogen: calcd. for pentaazoate I, 34.29; tetraazoate I, 29.43. Found: I, 33.62, 33.70. *p'*-Iodoazoyl determination by hydrolysis: calcd. for pentaazoate, 90.55; tetraazoate, 77.72. Found: 88.28. This percentage of *p'*-iodoazoyl corresponds to 33.45% I.

Azoic Anhydride (*p*-Phenylazobenzoic Anhydride).[16]—Azoyl chloride (20 g.) was dissolved in 100 ml. of pyridine. The solution was brought to boiling and poured with good stirring into 4 liters of ice water. The precipitate was filtered off and washed with a little 95% alcohol to facilitate drying. A small amount of acid was recovered by acidifying the filtrate. The yield was 19.3 g., 98%; m. p. 199–200° cor. The anhydride crystallizes in red platelets from anhydrous dioxane; m. p. 199–200°. It is readily hydrolyzed to the acid by alcoholic sodium hydroxide.

Anal. Calcd. per cent. azoyl: 96.30. Found: 94.80.

Chromatographic Adsorption.—Three sizes of columns were used. For test purposes a column 9 mm. in diameter and 45 cm. long proved satisfactory with 10 mg. of each component. Where quantitative separations were to be made on a mixture of two components a column 25 mm. by 45 cm. was used with 75 mg. of each. For separations involving several azoates a column 40 mm. by 70 cm. was used with 150 mg. of each component. The adsorbent was packed in the column by filling it several times with a benzene suspension of the adsorbent and filtering under pressure of 20 cm. of mercury. This pressure of nitrogen gas was also used throughout all separations.

TABLE IV

EFFECT OF THE SOLVENT AND ADSORBENT ON THE SEPARATION OF β-D-GLUCOSE PENTAAZOATE AND β-CELLOBIOSE OCTAAZOATE

Adsorbent	Solvent	Development
Magnesol and dicalite	1–1–1, ligroin, benzene, chloroform	None
Magnesol and dicalite	Same with 0.4% alcohol by vol.	Two good bands
Magnesol and dicalite	Same with 1–10% alcohol	Poor to none
Silicic acid	1–1–1, as above	Two good bands, no displacement of cellobiose band
Silicic acid	Same with 0.4% alcohol	Two bands fair
Silicic acid	Same with 1–10% alcohol	Poor to none
Silicic acid	Chloroform	Two bands, no displacement of cellobiose band
Silicic acid	Same with 0.1% alcohol	
Silicic acid	Same with 0.2% alcohol	Two bands, displacement of cellobiose band
Silicic acid	Same with >0.2% alcohol	Poor

The mixture of azoates was dissolved in the smallest possible amount of chloroform[17] from which they were adsorbed on the column. The chromatogram was developed by passing the required solvent through the adsorbent. The adsorbent was pushed out and cut into bands. The adsorbed azoate was eluted with chloroform containing 5–10% of methyl alcohol. The chloroform solution was concentrated, filtered into a tared receiver, evaporated to dryness and dried at 105° for one hour. After weighing, the azoate was dissolved in chloroform, transferred to a 25-ml. volumetric flask and the optical rotation measured.

Typical Chromatographic Separations

1-Azoyltetraacetyl-β-D-glucose and 1-Azoylheptaacetyl-β-cellobiose.—Adsorbent, silicic acid; solvent, chloroform with 0.2% alcohol; column, 27 mm. diameter. From 125 mg. of each derivative there was formed a top band (not displaced) 2.5 cm. wide. From this was eluted 114.4 mg., $[\alpha]^{25}_{6438}$ 54.75°, corresponding to the pure cellobiose derivative of $[\alpha]^{25}_{6438}$ 54.5°, calculated purity 99%. The lower band was separated from the upper by 1.5 cm. The lower band was 2 cm. wide. From this was obtained 115.9 mg., $[\alpha]^{25}_{6438}$ 62.6°, corresponding to the glucose derivative $[\alpha]^{25}_{6438}$ 63°, calculated purity 99%. Both derivatives crystallized on evaporation of eluant.

α-D-Galactose and β-D-Galactose Azoates.—Adsorbent, silicic acid; solvent, chloroform with 0.2% alcohol; column, 27 mm. diameter. The chromatogram was developed well down the column. From 125 mg. of each derivative was obtained a small intensely colored top band giving by elution 140.0 mg. of derivative $[\alpha]^{25}_{6438}$ 194°, corresponding to β-D-galactose azoate $[\alpha]^{25}_{6438}$ 170°, calculated purity 91%. The lower band was wide and diffuse, giving on elution 107.0 mg., $[\alpha]^{25}_{6438}$ 434°, corresponding to α-D-galactose azoate $[\alpha]^{25}_{6438}$ 436°, calculated purity 99%.

α,α-Trehalose and β,β-Trehalose Azoates.—Adsorbent, silicic acid; solvent, chloroform with 0.5% alcohol; column, 27 mm. diameter. From 125 mg. of each derivative was obtained a more or less solid band of 5 cm. width which was split in approximately the middle. From the upper half was eluted 94.1 mg., $[\alpha]^{25}_{6438}$ 44°, corresponding to $[\alpha]^{25}_{6438}$ 17° for pure β,β-trehalose azoate, calculated purity 85%. From the lower half was eluted 143.3 mg., $[\alpha]^{25}_{6438}$ 167°, corresponding to $[\alpha]^{25}_{6438}$ 210° for pure α, ω-trehalose azoate, calculated purity 78%.

β-D-Arabinose and β-L-Arabinose Azoates.—Adsorbent, silicic acid; solvent, chloroform with 0.5% alcohol; column, 27 mm. diameter. From 75 mg. of each derivative was obtained a single band which when split in approximately the middle gave on elution for the top 59.7 mg., $[\alpha]^{25}_{6438}$ 0°, and the lower 78.9 mg., $[\alpha]^{25}_{6438}$ 0°.

(16) The work on azoic anhydride was done in conjunction with Mr. Frank Stuart of this Laboratory.

(17) Alcohol-free chloroform was used throughout the separations.

β-D-Glucose, α,α-Trehalose, and β-Cellobiose Azoates.—Adsorbent, silicic acid; solvent, chloroform with 0.2% alcohol; column, 27 mm. diameter. From 75 mg. of each derivative was obtained a top band of 81.6 mg., $[\alpha]^{25}_{6438}$ 116°, corresponding to $[\alpha]^{25}_{6438}$ 105° for β-cellobiose azoate. A second band of 63 mg., $[\alpha]^{25}_{6438}$ 178°, corresponding to $[\alpha]^{25}_{6438}$ 210° for α,α-trehalose azoate. A third band of 59.7 mg., $[\alpha]^{25}_{6438}$ −33°, corresponding to $[\alpha]^{25}_{6438}$ −50 for β-D-glucose azoate. These separations represent a purity of at least 85–90%.

β-L-Arabinose, β-D-Glucose, α,α-Trehalose, and β-Cellobiose Azoates.—Adsorbent, silicic acid; solvent, chloroform with 0.1% alcohol; column, 40 mm. diameter and developed for twelve hours. One hundred and fifty milligrams of each derivative was used and four bands obtained. The top band which was not displaced was 11 cm. wide from which was eluted 152.7 mg., $[\alpha]^{25}_{6438}$ 109°, corresponding to $[\alpha]^{25}_{6438}$ 105° for β-cellobiose azoate. The eluted material was sparingly soluble in chloroform as is β-cellobiose azoate. A second band 2 cm. in width was separated from the first band by 1 cm. and was intensely colored. The second band gave on elution 136.0 mg., $[\alpha]^{25}_{6438}$ 199°, corresponding to $[\alpha]^{25}_{6438}$ 210° for α,α-trehalose azoate. The eluted material was a glassy amorphous solid which is characteristic of α,α-trehalose azoate after evaporation of a chloroform solution in which it is extremely soluble. There was 15 cm. between the second and third bands. The third band was 10 cm. wide from which was eluted 138.0 mg., $[\alpha]^{25}_{6438}$ 13°, corresponding to $[\alpha]^{25}_{6438}$ −50° for β-D-glucose azoate. The eluted material was typical of β-D-glucose azoate in appearance and solubility. The fourth band was 7 cm. below the third and was 10 cm. wide. It gave on elution 130.4 mg., $[\alpha]^{25}_{6438}$ 721°, corresponding to $[\alpha]^{25}_{6438}$ 750° for β-L-arabinose azoate. The eluted material formed the golden crystals typical of β-L-arabinose azoate. The purity of band three was calculated as 92.5%, assuming β-L-arabinose azoate as the impurity. The purity of band four was calculated as 96.25%, assuming β-D-glucose azoate as the impurity.

Acknowledgment is made of funds received from the Corn Products Refining Company which made possible this investigation.

Summary

1. The method of preparation, purification and analysis of the azoyl derivatives of sugars has been modified and several new sugar azoates have been prepared.

2. Chromatographic adsorption separations have been carried out with closely related compounds as well as with several new types of azoyl derivatives. One separation involving four sugar derivatives was accomplished.

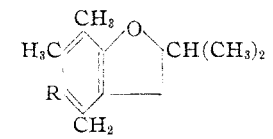
IOWA CITY, IOWA RECEIVED APRIL 13, 1943

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA]

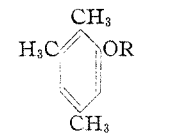
Polyalkylbenzenes. XXXIII.[1] 4,6,7-Trimethylcoumaran-3-one and its Conversion into 2-Isopropyl-4,6,7-trimethyl-5-hydroxycoumaran

BY LEE IRVIN SMITH, JOHN A. KING, WILLA IRWIN GUSS AND JOSEPH NICHOLS

In a previous paper[2] there was described a successful synthesis of 2-isopropyl-4,6,7-trimethyl-5-hydroxycoumaran (I), a substance of interest in connection with the chemistry of vitamin E. Previous to this successful synthesis, experiments had been started with a view to a synthesis of 2-isopropyl-4,6,7-trimethylcoumaran (VI) by means of a series of reactions in which, as a first step, 2,3,5-trimethylphenol (II) was to be converted into 2,3,5-trimethylphenoxyacetic acid (III). The latter was then to be cyclized to 4,6,7-trimethylcoumaran-3-one (IV). Condensation of IV with acetone would give 2-isopropylidine-4,6,7-trimethylcoumaran-3-one (V), which by reduction under the proper conditions could be transformed into VI. The reactions apparently proceeded normally, but the properties of the substance supposed to be V were so unusual that for some time it was believed the substance could not possibly have this structure. However, later work showed that the substance actually had this structure; it was possible, though difficult, to reduce V to VI. The coumaran VI was identified by transforming it into the known hydroxycoumaran I, via

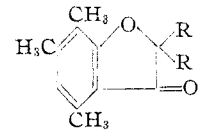


I, R = OH
VI, R = H



II, R = H
III, R = CH_2COOH

VII, R = Br
VIII, R = MgBr
XV, R = OAc



IV, R = H
V, R + R = $=C(CH_3)_2$

(1) XXXII, THIS JOURNAL, **65**, 202 (1943).
(2) Smith and King, *ibid.*, **65**, 441 (1943).