The diacetate XXb, recrystallized from methanol–ether, exhibited m.p. 210–212°, $[\alpha]^{29}\mathrm{D}$ −46°.

Anal. Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 71.92; H, 9.40.

Chromium trioxide oxidation of 0.1 g. of the diol XXa and recrystallization of the acid fraction from acetone produced 0.08 g. of **3,4-*seco*-22a,25a-spirostane-3,4-dioic acid (XXIa)**, m.p. 264–266°, $[\alpha]^{25}\mathrm{D}$ −19° (pyridine).

Anal. Calcd. for $C_{27}H_{42}O_6$: C, 70.10; H, 9.15. Found: C, 69.90; H, 8.92.

The dimethyl ester XXIb was recrystallized from methanol; m.p. 195–197°, $[\alpha]^{25}\mathrm{D}$ −47°.

Anal. Calcd. for $C_{23}H_{46}O_6$: C, 70.98; H, 9.45. Found: C, 70.80; H, 9.31.

3α,4α-Oxido-22a,25a-spirostan (XXVII).—A solution of 4.14 g. of 22a,25a-spirostan-3-one (IV)[10] in 250 cc. of anhydrous ether was treated dropwise over a period of 24 hours with a solution of 1.6 g. of bromine in 50 cc. of chloroform at 5°. Removal of the solvent left a residue, which by analysis (Calcd. for $C_{27}H_{41}BrO_3$: C, 65.86; H, 8.39. Found: C, 66.27; H, 8.18) represented chiefly monobrominated material but which appeared to be approximately a 60–40 mixture of the 4-bromoketone XXIV ($\lambda_{\max}^{\mathrm{CHCl_3}}$ 5.80 μ) and 23-bromoketone ($\lambda_{\max.}^{\mathrm{CHCl_3}}$ 5.87 μ). The presence of the 4-bromo-3-ketone XXIV was established by dehydrobromination with dinitrophenylhydrazine in glacial acetic acid[35] which yielded the **2,4-dinitrophenylhydrazone XXV**, m.p. 272–274° (after recrystallization from ethanol–chloroform), identical with a sample prepared from Δ^4-diosgenone (IX).

Anal. Calcd. for $C_{33}H_{44}N_4O_6$: C, 66.87; H, 7.48. Found: C, 66.36; H, 7.29.

The bromination product (4.8 g.) was suspended in 300 cc. of ethanol and stirred for 24 hours with 2.5 g. of sodium borohydride. Dilution with water and collection of the precipitate gave 4.85 g. of product which did not exhibit any carbonyl absorption in the infrared (presumably mixture of epimeric bromohydrins and some side-chain brominated epismilagenin). The crude material (4.3 g.) was refluxed for 2.5 hours with 20 g. of potassium hydroxide in 500 cc. of ethanol and the product was chromatographed on 150 g. of neutral alumina. Elution with hexane–benzene (9:1) and recrystallization from acetone gave 0.96 g. of the desired **epoxide XXVII**, m.p. 177–179°, $[\alpha]^{25}\mathrm{D}$ −103°; admixture with the epimeric epoxide XXII depressed the melting point by 15°.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.03; H, 10.07.

The hexane–benzene (1:1) eluates yielded 0.4 g. of smilagenone (IV), arising from a 3β,4β-bromohydrin (epimer of XXVI), while subsequently eluted (benzene and ether mixtures) material (m.p. 205–225°, 0.65 g.) contained bromine, presumably in the side chain.

22a,25a-Spirostan-4α-ol (XXVIIIa).—A sample (150 mg.) of the epoxide XXVII was reduced with lithium aluminum hydride and the product recrystallized from acetone; yield 100 mg., m.p. 170–171°, depressed to 140° upon admixture with the starting material, $[\alpha]^{25}\mathrm{D}$ −70°.

Anal. Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65. Found: C, 77.66; H, 10.58.

The acetate XXVIIIb crystallized as needles from ethanol; m.p. 166–168°, $\lambda_{\max.}^{\mathrm{CHCl}}$ 5.78 μ and type B band at 8.0 μ.

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 75.84; H, 10.28.

22a,25a-Spirostan-3β,4α-diol (XXIII).—Acetolysis of either isomeric epoxide (XXII or XXVII) and direct saponification of the reaction product in the manner described above (XIV → XVIa) produced in *ca.* 50% yield the identical diol XXIII which was recrystallized from acetone; m.p. 181–184°, $[\alpha]^{25}\mathrm{D}$ −74°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 75.08; H, 10.23.

Conversion of Mexogenin to Samogenin.—Mexogenin (100 mg., m.p. 237–238°, $[\alpha]^{25}\mathrm{D}$ −6°, $\lambda_{\max.}^{\mathrm{CHCl_3}}$ 5.88 μ) was reduced by the Huang-Minlon procedure exactly as described above (IV → III) to yield 85 mg. of samogenin (Ia) (m.p. 206–208°), identified with authentic material by mixture melting point determination and infrared comparison.

Oxidation of Isorhodeasapogenin.—Chromium trioxide oxidation of a sample of the sapogenin (m.p. 245–248°) kindly furnished by Dr. H. Nawa[31] led to colorless needles (from acetone) of the derived *seco*-acid, m.p. 246–248°; mixture melting point determinations showed depressions (indicated after each acid) with the following acids: gitogenic acid (225–235°), samogenic acid (235–245°), 3,4-*seco*-acid XVII (230–240°). The dimethyl ester after two recrystallizations from methanol exhibited m.p. 155–157°, depressed to 138–145° upon admixture with the dimethyl ester XXIb.

Anal. Calcd. for $C_{29}H_{46}O_6$: C, 70.98; H, 9.45. Found: C, 70.86; H, 9.49.

DETROIT, MICHIGAN

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(35) C. Djerassi, THIS JOURNAL, **71**, 1003 (1949).

[CONTRIBUTION FROM THE INSTITUTE OF ORGANIC CHEMISTRY, TECHNICAL UNIVERSITY OF BUDAPEST]

Structural Proof of Sugar Phenylhydrazones

BY LÁSZLÓ MESTER AND ÁDÁM MAJOR

RECEIVED AUGUST 9, 1954

The controversial question as to whether sugar phenylhydrazones are cyclic or acyclic can be settled by the formazan reaction in which only open-chain (*aldehydo-*) structures participate. The structures of several sugar phenylhydrazones, including the three D-glucose phenylhydrazones, have been established by this reaction. In addition, the proportion of open-chain material in solution of a mixture of sugar phenylhydrazones can be determined, making it possible to follow and interpret structurally the optical changes in a mutarotating phenylhydrazone solution. The formazan reaction is also applicable to acetylated *aldehydo*-phenylhydrazones.

When Emil Fischer,[1] almost 70 years ago, prepared sugar phenylhydrazones for the first time, he represented them as open-chain compounds. The conception of ring structure[2–5] arose later to account for mutarotational effects in solution. The question is still moot, although many workers have attempted to clarify the point. Frèrejacque[6] found that definitive evidence could not be obtained from a study of hydrolysis rates and rotational values of the hydrolyzates. Later, Stempel[7] on the basis of similar experiments came to the same conclusion. In earlier work, attempts[2] have been made to elucidate the structure of the sugar phenylhydrazones on the basis of their mutarotations, but the later work of Butler and Cretcher[8] and of Stempel[7] revealed that while mutarotational studies permitted many interesting observations,

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(1) E. Fischer, *Ber.*, **20**, 821 (1887).

(2) H. Jacobi, *Ann.*, **272**, 170 (1893).

(3) R. Behrend, *ibid.*, **353**, 106 (1907).

(4) R. Behrend and F. Lohr, *ibid.*, **362**, 78 (1908).

(5) A. Hofmann, *ibid.*, **366**, 277 (1909).

(6) M. Frérejacque, *Compt. rend.*, **180**, 1210 (1925).

(7) G. H. Stempel, THIS JOURNAL, **56**, 1351 (1934).

(8) C. L. Butler and L. H. Cretcher, *ibid.*, **53**, 4356 (1931).

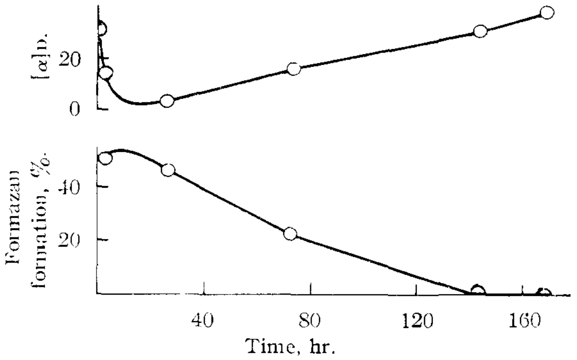


Fig. 1.—Upper curve, mutarotation of D-mannose phenylhydrazone (c 0.9, pyridine–ethanol (9:1 by vol.)); lower curve, D-mannose diphenylformazan formation.

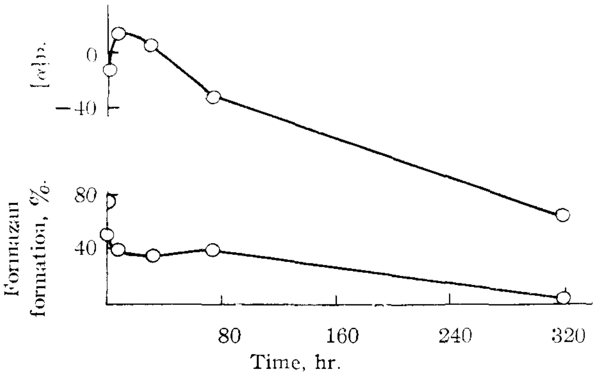


Fig. 2.—Upper curve, mutarotation of L-rhamnose phenylhydrazone (c 1.27, pyridine–ethanol (1:1 by vol.)); lower curve, L-rhamnose diphenylformazan formation.

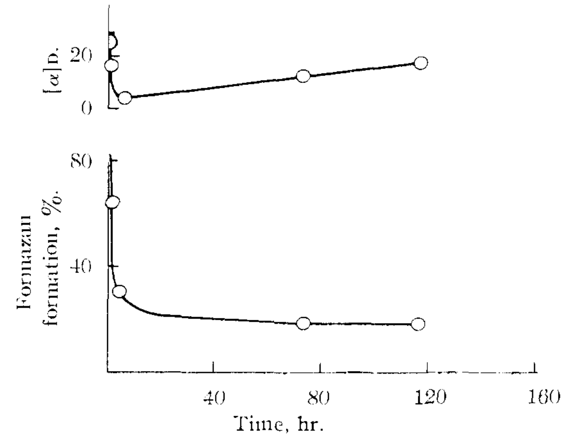


Fig. 3.—Upper curve, mutarotation of D-galactose phenylhydrazone (c 5.4, pyridine); lower curve, D-galactose diphenylformazan formation.

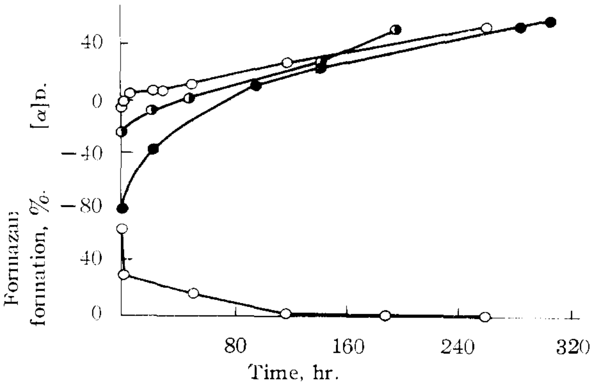
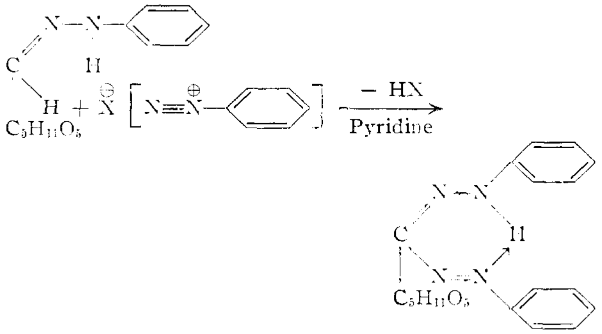


Fig. 4.—Upper curves, mutarotation of D-glucose phenylhydrazones (c 5.4, pyridine–ethanol (1:1 by vol.)); ●, "α"-D-glucose phenylhydrazone; ◑, Skraup's D-glucose phenylhydrazone; ○, "β"-D-glucose phenylhydrazone Lower curve, D-glucose diphenylformazan formation in a mutarotating solution of *aldehydo*-D-glucose phenylhydrazone. No formazan was produced by the cyclic D-glucose phenylhydrazones during mutarotation.

they were of no use in correlating structure to mutarotation. There were a few isolated cases in which the products, obtained by mild acetylation of sugar phenylhydrazones, were compared successfully with acetates obtained by other means. Early in the century, Behrend and Reinsberg,[9] using this method, found evidence of ring structure in the so-called "α"-D-glucose phenylhydrazone, the first sugar phenylhydrazone, in which the cyclic structure was definitely established. Again, by the same method, Wolfrom and Christman[10] proved the *aldehydo* structure of D-galactose phenylhydrazone.

In our attempts to solve this problem we employed the formazan reaction, in which only real Schiff systems, (*aldehydo* or acyclic) phenylhydrazones participate.[11-18]



Our investigations indicate that the structures of the phenylhydrazones do not undergo any change when subjected to the mild conditions of the formazan reaction (coupling in pyridine with a solution of diazotized aniline at a temperature below −5°). This is borne out by the fact that known *aldehydo*-D-galactose phenylhydrazone produced D-galactose diphenylformazan in 86% yield, while "α"-D-glucose phenylhydrazone of recognized ring structure did not react at all.[12] Zemplén, Mester and

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(9) R. Behrend and W. Reinsberg, *Ann.*, **377**, 189 (1910).

(10) M. L. Wolfrom and C. C. Christman, THIS JOURNAL, **53**, 3413 (1931).

(11) G. Zemplén and L. Mester, *Magyar Tudomanyos, Akad. III, és VI Osztályának Közleményei*, 1, 1 (1951).

(12) G. Zemplén and L. Mester, *Acta Chim. Acad. Sci. Hung.*, **2**, 9 (1952).

(13) G. Zemplén and L. Mester, *Magyar Tudományos Akad. Kém. Tudományok Osztályának Közleményei*, **1**, 73 (1952).

(14) G. Zemplén, L. Mester, A. Messmer and Ede Eckhart, *Acta Chim. Acad. Sci. Hung.*, **2**, 25 (1952).

(15) M. Busch and H. Pfeiffer, *Ber.*, **59**, 1162 (1926).

(16) M. Busch and R. Schmidt, *J. prakt. Chem.*, **239**, 182 (1931).

(17) M. Busch and R. Schmidt, *Ber.*, **63**, 1950 (1930).

(18) S. Hünig and O. Boes, *Ann.*, **579**, 28 (1953).

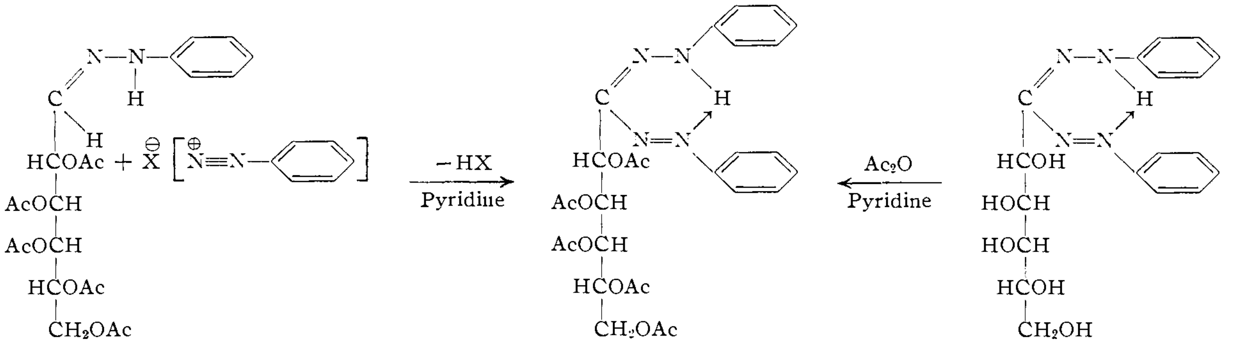
TABLE I

Modification	M.p., °C.	$[\alpha]_D$ in water	Formazan %
"α"-D-Glucose phenylhydrazone	159–160	$-87° \rightarrow -50°$	0
"β"-D-Glucose phenylhydrazone	140–141	$-2° \rightarrow -50°$	67
Skraup's D-glucose phenylhydrazone	115–116	$-7° \rightarrow -49°$	0

Eckhart[19] discuss the nomenclature of the formazans and suggest the form used above. In the case of sugar phenylhydrazones of unknown or questionable structure, the formazan reaction not only enabled us to distinguish if they were cyclic or acyclic, but also to determine quantitatively the presence of the *aldehydo* form. Specifically, two of the three D-glucose phenylhydrazone modifications[2–4,7,20,21] did not undergo formazan reaction while the third did react,[12] indicating the former two were cyclic while the latter was acyclic. The yield of D-glucose diphenylformazan, indicated the presence of 67% of the acyclic form (Table I).

We then proceeded to interpret the mutarotation curves of the sugar phenylhydrazones on the basis of the formazan reaction. A repetition of the measurements of earlier workers[5,8] on the mutarotation of D-mannose, L-rhamnose and D-galactose phenylhydrazones in pyridine and in pyridine–ethanol solutions produced results similar to those previously found (Figs. 1–3). The mutarotation curves of the three D-glucose phenylhydrazones were found to approach a common point of equilibrium (Fig. 4). Earlier workers[4,7–9] had concluded from the nature of the curves that there were at least three modifications participating in the mutarotation, yet it remained undetermined until the present whether they were all cyclic, or whether the *aldehyo* form was represented among them, and if so in what proportion. With the object of clarifying this point, the extent of formazan formation was measured at certain critical points during mutarotation by cooling aliquots of the pyridine solutions below $-5°$, and coupling them with the corresponding amount of diazotized aniline solution. The yields of formazan at the different rotational values indicated the proportion of phenylhydrazones present in the *aldehydo* form. These data are presented in the lower curves in the figures. It thus appears that the formazan reaction offers a method of tracing and interpreting optically observable structural changes. The formazan values observed during mutarotation clearly show, as indicated in part in Table I, that the so-called "β"-form reacts and therefore has an *aldehydo* structure, whereas the other two do not react, and therefore are two phenylhydrazones having ring structures. It might henceforth be expedient if, "α"-D-glucose phenylhydrazone having a more negative rotation were designated "β" and Skraup's hydrazone, which rotates to a lesser degree in the negative direction, be called "α."

The formazan reaction has proved to be equally useful in disclosing the structure of acetylated sugar phenylhydrazones.[12,14] D-Galactose phenylhydrazone pentaacetate coupled with diazotized aniline solution produced the same D-galactose diphenylformazan pentaacetate as was obtained by acetylation of D-galactose diphenylformazan, which is in agreement with the findings of Wolfrom and Christman.[10]



The method was also applied to the two D-mannose phenylhydrazone acetates described in the literature. The fact that anhydro-D-mannose phenylhydrazone tetraacetate[22] does not undergo formazan reaction supports the pyrazoline structure for this substance proposed by Wolfrom and Blair. This structure is an *aldehydo* form but has no imino-hydrogen; it therefore contains only one of the two prerequisites for formazan reaction.[13–18] On the other hand, the amorphous D-mannose phenylhydrazone acetate described by Hofmann,[23] and more recently by Stepanenko and co-worker,[24] underwent reaction but failed to produce the readily crystalline D-mannose diphenylformazan pentaacetate obtained by the acetylation of D-mannose diphenylformazan. The amorphous, acetylated D-mannose phenylhydrazone has a wide melting point range and appears to be a mixture of partially acetylated phenylhydrazones in which the presence of the *aldehydo* form can be readily demonstrated by our formazan reaction.

The experimental results indicate that the formazan reaction can be used to distinguish the open-chain from the ring structure in the free sugar phenylhydrazones and also in their acetates. The reaction can also be used to estimate the rela-

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(19) G. Zemplén, L. Mester and Ede Eckhart, *Ber.*, **86**, 472 (1953).

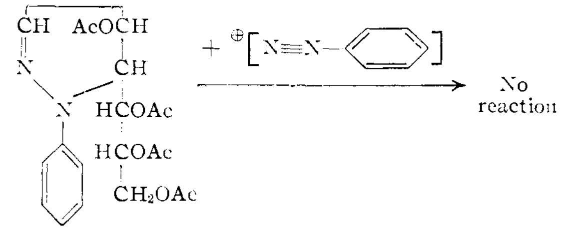
(20) H. Skraup, *Monatsh.*, **10**, 406 (1889).

(21) C. L. Butler and L. H. Cretcher, THIS JOURNAL, **51**, 3163 (1929).

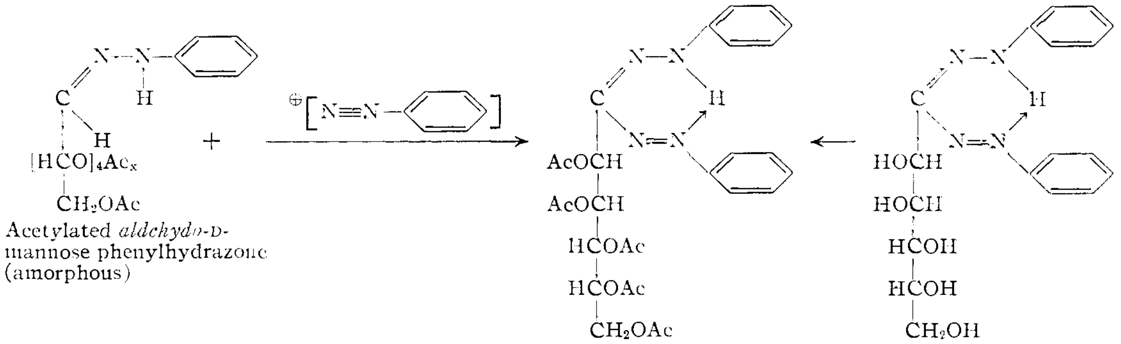
(22) M. L. Wolfrom and M. Grace Blair, *ibid.*, **68**, 2110 (1946).

(23) A. Hofmann, *Ann.*, **366**, 315 (1909).

(24) B. N. Stepanenko and V. A. Ignatyuk-Maĭstrenko, *Doklady Akad. Nauk S.S.S.R.*, **73**, 1251 (1950); *C. A.*, **45**, 2877 (1951).



Anhydro-D-mannose phenylhydrazone tetraacetate


Acetylated *aldehydo*-D-mannose phenylhydrazone (amorphous)

D-Mannose diphenylformazan pentaacetate

D-Mannose diphenylformazan

tive quantities of *aldehydo* and ring forms present at any time during the mutarotation of sugar phenylhydrazones.

Acknowledgment.—We wish to express our thanks to Professor G. Zemplén for his valuable advice, and to Miss Ilona Batta and Mrs. Ede Eckhart, for the analyses. Mrs. Eckhart also collaborated in the acetylation of D-mannose diphenylformazan.

Experimental

Aniline Diazonium Chloride Reagent.—Freshly distilled aniline (9.3 g.) was dissolved in 50 ml. of 18% hydrochloric acid and diazotized at 0 to 5° with a solution of sodium nitrite in 15 ml. of water. After dilution of this solution to 93 ml., the concentration was the amount of diazo compound obtained from 0.1 g. of aniline, per ml.

L-Rhamnose Diphenylformazan.—To a solution of L-rhamnose phenylhydrazone,[8] m.p. 159° (2.54 g.) dissolved in 50 ml. of pyridine–ethanol (1:1 by vol.) and cooled to a temperature below −5°, was added dropwise, with stirring, 10 ml. of the diazotized aniline reagent. The solution immediately turned red, and, after 10 min., was poured into five times its volume of water. The red precipitate, which formed, was filtered the following day, yield 2.8 g. (78%). It was recrystallized from butanol, yield 2 g. (55.8%), m.p. 165°.

Further recrystallization gave pure material, m.p. 175°.

Anal. Calcd. for $C_{18}H_{22}O_4N_4$: N, 15.63. Found: N, 15.66, 15.59.

D-Mannose Diphenylformazan Pentaacetate.—D-Mannose diphenylformazan[12,14] (0.5 g.) was dissolved in a mixture of 5 ml. of pyridine and 3 ml. of acetic anhydride and allowed to stand at room temperature for 24 hr. Upon pouring the mixture into water, the product solidified; yield 0.8 g. The substance was brought to crystallization after dissolving in 5 volumes of dioxane by the addition of 3.5 volumes of water; yield 0.6 g., m.p. 80–85°. It was further recrystallized from 3 ml. of ethanol upon the addition of 3 ml. of water; m.p. 95°.

Anal. Calcd. for $C_{17}H_{18}O_5N_4(COCH_3)_5$: N, 9.59; acetyl groups, 5.00; acetyl (%), 36.85. Found: N, 9.59, 9.56; acetyl groups, 4.94, 4.98; acetyl (%), 36.4, 36.7.

The amorphous product obtained on acetylating D-mannose phenylhydrazone according to Hofmann[23] or Ignatyuk-Maistrenko,[24] m.p. 55–65° (2.9 g.), was dissolved in 18 ml. of a (1:1 by vol.) pyridine–ethanol mixture and treated with 6 ml. of diazotized aniline solution, as previously described. The solution turned red indicating that coupling had taken place. Upon pouring into water, red-colored material slowly solidified, but failed to crystallize even after nucleating with D-mannose diphenylformazan pentaacetate; yield 2 g. (57.1%). Although the product was not homogeneous, it gave a blue coloration with concd. sulfuric acid, which is characteristic of formazans.

Attempt to Couple Anhydro-D-mannose Phenylhydrazone Tetraacetate.—Anhydro-D-mannose phenylhydrazone tetraacetate,[22] m.p. 123° (0.45 g.), was dissolved in 5 ml. of pyridine and treated with 1 ml. of diazotized aniline. The original material was recovered unchanged.

Correlation of Formazan Formation with Mutarotation of Sugar Phenylhydrazones.—Stock solutions of the hydrazones of D-mannose,[5] L-rhamnose,[8] D-galactose,[1] "α"-D-glucose,[7] "β"-D-glucose[2,4] and Skraup's[2] D-glucose phenylhydrazone were prepared and rotation measurements were made. At various time intervals, aliquots containing 10^{-3} moles of phenylhydrazone were removed, quickly cooled below −5° and treated with 1 ml. (7.5% in excess of 10^{-3} mole) of diazotized aniline prepared as described above. When coupling occurred, the solution instantaneously changed to a red color. After 10 min., when it was poured into five volumes of ice and water, the sugar diphenylformazans separated as vivid red precipitates. When coupling did not occur, no red color appeared upon the addition of the diazotized aniline solution, and no precipitate was formed upon dilution with ice and water. The data obtained from these measurements are recorded and compared graphically in Figs. 1–4.

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