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

Structure of D-Glucose Phenylosazone

BY LÁSZLÓ MESTER

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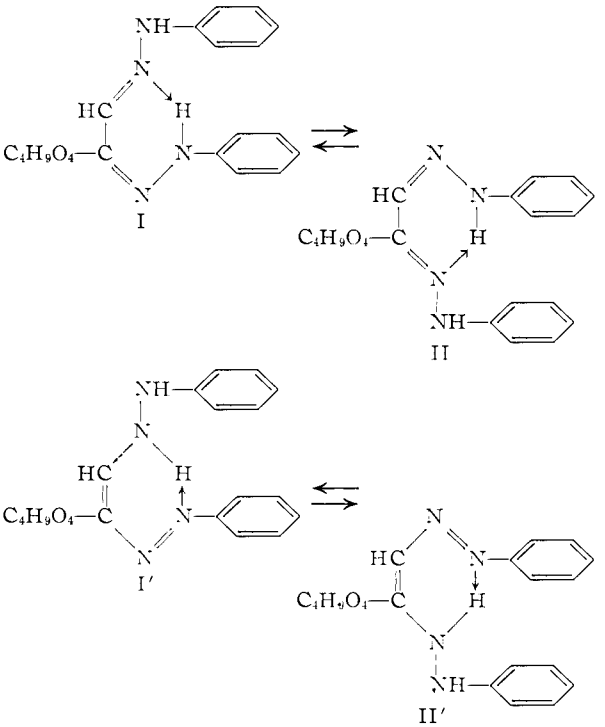
The use of the formazan reaction has confirmed the open-chain structure proposed by Fischer, and the presence of the chelate ring postulated by Fieser and Fieser, for D-glucose phenylosazone. The structure of the so-called mixed A and mixed B osazones has also been clarified. Mixed A osazone is found to be D-glucose 1-(α-methyl-α-phenyl)-2-phenylosazone, while mixed B osazone proved to be D-glucose phenylosazone contaminated with mixed A osazone.

The structure of the sugar phenosazones first prepared by Fischer[1] in 1884 has remained undefined. Besides Fischer's open-chain structure, a tautomeric azo-hydrazone[2] structure and a ring structure[3-6] have been proposed. While work in the last two decades involving ultraviolet spectra studies on the acetylated[7-9] and methylated[6,10,11] phenylosazones appears to favor the acyclic structure proposed by Fischer,[1] the problem cannot be considered as solved. The acyclic structure does not explain how the reaction stops at the second carbon atom instead of continuing to the third, and why the imino-hydrogen of only one phenylhydrazone group is methylated when each of the imino groups has the same value. In an attempt to explain the first of these facts, Baly, Tuck, Marsden and Gazdar[3] as early as 1907, advanced the assumption (open-chain form presumed) that in the sugar phenylosazones the two conjugated double bonds formed a "condensed system" due to their inner linkage which caused the reaction to stop at carbon two. Fieser and Fieser[12] proposed for the structure of the phenylosazones, the chelate tautomers I and II of the acyclic compound, these being stabilized by their ability to exist in the resonance forms I′ and II′. They considered that the formation of a stable ring on carbons one and two excluded further electron displacement necessary to osazone formation according to all modern conceptions of its mechanism. Percival,[13] in his comprehensive studies on the structure of phenylosazones, could not justify the views of the Fiesers because of lack of experimental evidence. Hardegger and Schreier,[14] the latest workers to deal with the problem, considered that any attempt to define precisely the structure of the phenylosazones, on the basis of

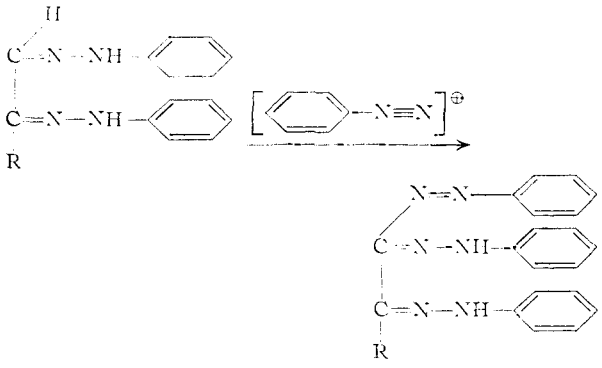


information at hand, would be immature. For this reason, insofar as possible, they used Fischer's open-chain formula for the phenylosazones and their derivatives. It would appear that at the present time the finer structure of the sugar phenylosazones and indeed, whether they are cyclic or acyclic, is uncertain.

The success of the formazan reaction in establishing the structure of the phenylhydrazones[15] suggested its use in the study of the phenylosazones. It is known that the formazan reaction depends upon two conditions.[16-20] The first is the presence of a Schiff base, (—CH=N—) which is fulfilled in *aldehydo*-phenylhydrazones, but not in *keto*-phenylhydrazones nor in phenylhydrazones derived from the cyclic hemiacetal form of the aldoses. The second condition is the presence of free imino-hydrogen in the phenylhydrazone group. If Fischer's open-chain structure is correct, the phenylhydrazone group on the first carbon satisfies both

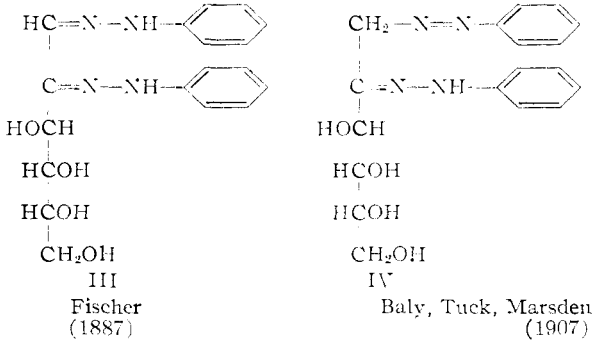
(1) E. Fischer, *Ber.*, **17**, 579 (1884); **20**, 821 (1887).
(2) E. Zerner and R. Waltuch, *Monatsh.*, **35**, 1025 (1914).
(3) E. C. C. Baly, W. B. Tuck, E. G. Marsden and M. Gazdar, *J. Chem. Soc.*, **109**, 1572 (1907).
(4) R. Behrend and F. Lohr, *Ann.*, **362**, 78 (1908).
(5) W. N. Haworth, "The Constitution of Sugars," Edward Arnold and Co., London, 1929, p. 7.
(6) E. Elizabeth Percival and E. G. V. Percival, *J. Chem. Soc.*, **137**, 1398 (1935).
(7) L. L. Engel, THIS JOURNAL, **57**, 2419 (1935).
(8) M. L. Wolfrom, M. Konigsberg and S. Soltzberg, *ibid.*, **58**, 490 (1936).
(9) E. Elizabeth Percival and E. G. V. Percival, *J. Chem. Soc.*, **139**, 1320 (1937).
(10) E. G. V. Percival, *ibid.*, **138**, 1770 (1936).
(11) S. Akiya and S. Tejima, *J. Pharm. Soc. Japan*, **72**, 894, 1574 (1952); *C. A.*, **47**, 6351, 9275 (1953).
(12) L. F. Fieser and Mary Fieser, "Organic Chemistry," D. C. Heath and Co., Boston, 1944, p. 353.
(13) E. G. V. Percival, *Advances in Carbohydrate Chem.*, **3**, 23 (1948).
(14) E. Hardegger and E. Schreier, *Helv. Chim. Acta*, **35**, 232 (1952).
(15) L. Mester and A. Major, THIS JOURNAL, **77**, 4297 (1955).
(16) M. Busch and H. Pfeiffer, *Ber.*, **59**, 1162 (1926).
(17) M. Busch and R. Schmidt, *J. prakt. Chem.*, **239**, 182 (1931).
(18) G. Zemplén and L. Mester, *Magyar Tudományos Akad. Kém. Tudományok Osztályának Közleményei*, **1**, 73 (1952).
(19) G. Zemplén, L. Mester, A. Messmer and Ede Eckhart, *Acta Chim. Acad. Sci. Hung.*, **2**, 25 (1952).
(20) S. Hünig and O. Boes, *Ann.*, **579**, 28 (1953).

conditions and the formazan reaction would be expected to proceed according to the equation



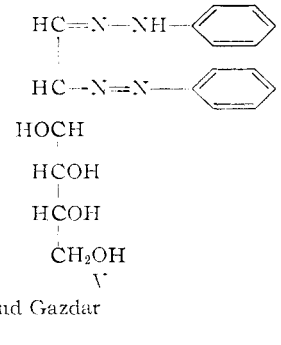
In harmony with the existing nomenclature, the author wishes to suggest the name phenylosazone formazan for this group of compounds; thus the formazan derivative of D-glucose phenylosazone would be D-glucose phenylosazone formazan. A few simple derivatives of this group are known, such as those where R in the above formula is methyl or carboxyl; these were not made from osazones, but by treatment of the corresponding α-keto-formazans with phenylhydrazine. Methylglyoxal phenylosazone formazan (N,N'-diphenylformazyl methyl ketone phenylhydrazone) was prepared by Bamberger and Lorenzen[21] by condensation of acetylformazan with phenylhydrazine. The violet or brownish-black color characteristic of all known compounds of this group can be attributed to the three chromophoric groups present in the structure.

In the present investigation, all attempts to prepare the formazan from D-glucose phenylosazone (D-*arabino*-hexose phenylosazone) have failed, which may be due to one or both of the following reasons. First, carbon one of D-glucose phenylosazone may have a ring or azo structure unsuitable for formazan reaction. In this connection, Hardegger and Schreier[14] have stated that if all structural possibilities are taken into account, the number may be as high as one hundred. In the case of D-glucose phenosazone, these may be represented by nine basic compounds of which the formulas IV, VI, VII, X and XI and all of the isomers (*syn*, *anti*, α, β) derivable from them are unsuitable for formazan reaction. Only the basic compounds

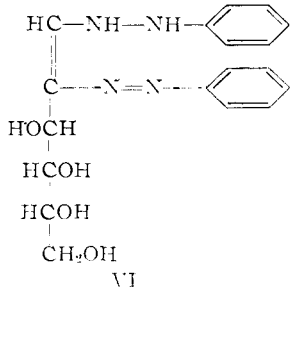


III
Fischer
(1887)

IV

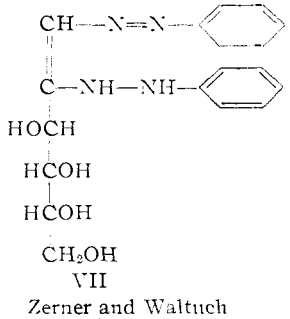


V

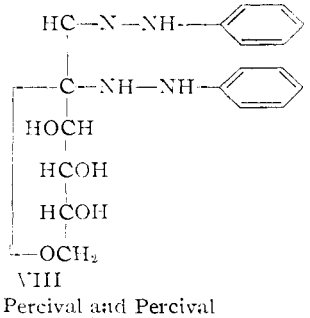


VI

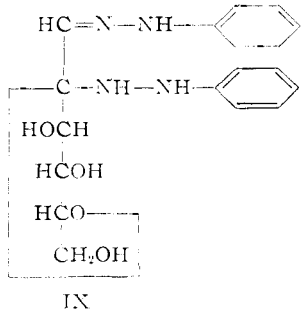
Baly, Tuck, Marsden and Gazdar
(1907)



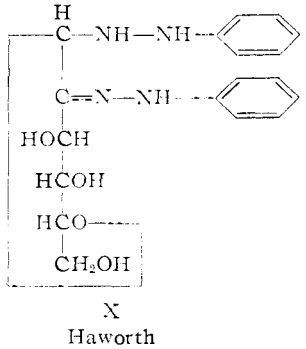
VII
Zerner and Waltuch
(1914)



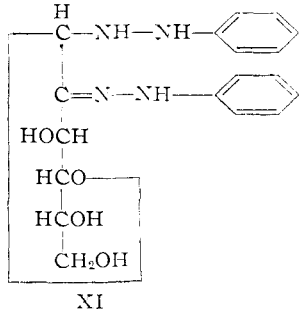
VIII
Percival and Percival
(1935)



IX



X
Haworth
(1929)



XI
(Haworth 1929)

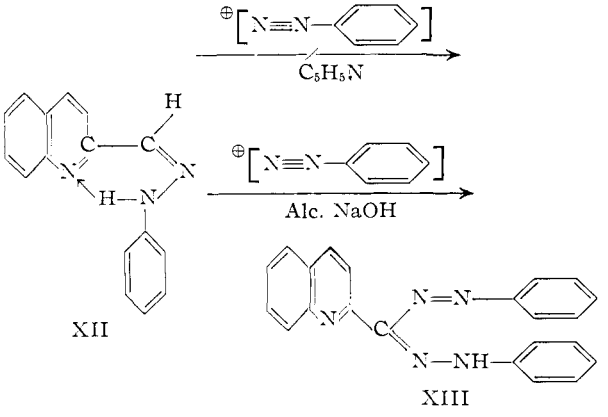
III, proposed by Fischer, and V, VIII and IX can undergo formazan formation. Second, the D-glucose phenylosazone structure may lend itself to coupling (III, V, VIII and IX) but may contain no free imino-hydrogen. It has long been established that if the free imino-hydrogen of the phenylhydrazone is replaced by an alkyl or aryl group, no formazan reaction will occur.[16–20] Our most recent investigation[22] reveals that chelation of the imino-hydrogen produces a similar result. It is

(21) E. Bamberger and J. Lorenzen, *Ber.*, **25**, 3542 (1892).

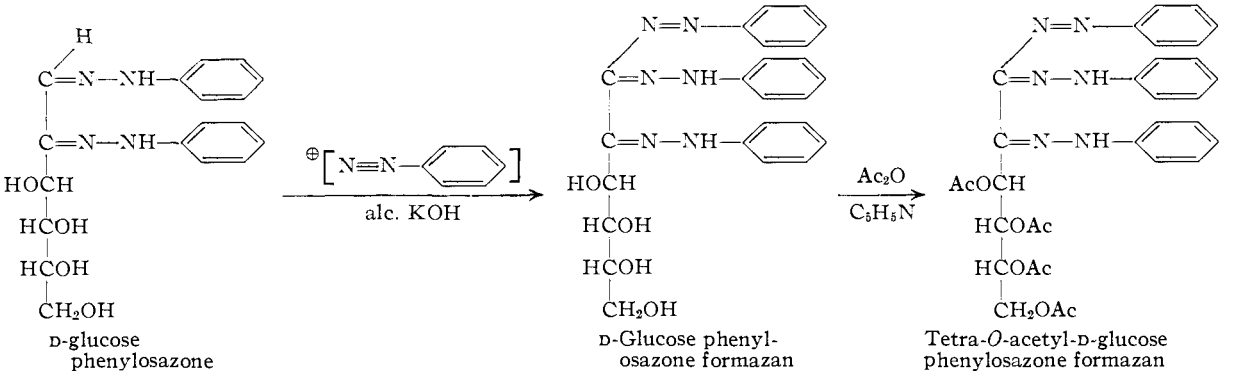
(22) G. Zemplén, L. Mester, A. Messmer and A. Major, *Acta Chim. Acad. Sci. Hung.*, in press.

possible that the structural formula proposed by Fieser and Fieser,[12] in which the imino-hydrogen of the hydrazone on the carbon atom one takes part in chelation, would explain the lack for formazan reaction.

To aid in finding the reason for failure of formazan reaction, a model experiment was set up using 2-formylquinoline phenylhydrazone (XII)[23] whose structure, if the nitrogen of the quinoline be considered, is like that of a phenylosazone with the six-membered chelate ring in it corresponding to the chelate ring structure proposed by Fieser and Fieser[12] for D-glucose phenylosazone. It was found in the model reaction that the chelate N→H–N inhibited formazan reaction in pyridine solution, which indicates an explanation for a similar failure in D-glucose phenylosazone. It was found that on replacing the pyridine with alkaline ethanol (often used to catalyze the formazan reaction) the chelate ring was loosened and coupling occurred to give N,N′-diphenyl-C-(2-quinolyl)-formazan (XIII). In work to be reported elsewhere,[22] it was found that the *o*-nitrophenylhydrazone of benzaldehyde, because of chelation (O→H–N) between the nitro and imino groups, does not couple with diazotized aniline in pyridine solution, while in alkaline ethanol the reaction takes place readily.



We found, in an analogous manner, that D-glucose phenylosazone underwent reaction, in alkaline ethanol, with diazotized aniline below −5° to give a product consisting of dark violet needles. Mild acetylation produced a black tetraacetate derivative.



(23) V. V. Miller and J. Spady, *Ber.*, **18**, 3405 (1885).

We found by control experiments that D-glucose phenylosazone is resistant to alkali treatment under the conditions of the experiment and could be recovered as unchanged material, which is in agreement with the data in the literature.[24]

From the above discussion, it is obvious that the breaking of the chelation produces, on the first carbon atom, a Schiff base structure which is suitable for the formazan reaction. Such a structure can only be possible in formulas III, V, VIII and IX. Structure V must be discarded for lack of stability, as it contains isolated double bonds. Systems containing conjugated double bonds are known to be more stable and all modern studies on the mechanism of osazone formation show that it is the formation of this stable conjugated system which is the driving force[25] behind the formation of osazones. Moreover, structure V would yield on formazan reaction a product containing an azo group, which is definitely excluded by the ultraviolet spectra studies to be discussed below. The studies of Engle[7] on D-glucose phenylosazone, those of Wolfrom and co-workers[8] on tetra-*O*-acetyl-D-glucose phenylosazone and of Akiya and Tejima[11] on methylated D-glucose phenylosazone are all in agreement with the acyclic structure. This would appear to exclude the ring structure VIII, proposed by Percival and Percival,[6] and the ring structure IX. Hardegger and Schreier[14] make no use of them in their work. In structures VIII and IX, the compound produced upon coupling should contain two chromophoric groups of the simple formazans (CH=N, N=N), which should result in the usual red color. The dark-violet color of the product obtained is similar to that of methylglyoxal phenylosazone formazan which contains three chromophoric groups. This view is supported by a comparison of the ultraviolet spectra of D-glucose phenylosazone formazan (max. 335, 410 mμ) which coincides with the acyclic methylglyoxal derivative (max. 335, 410 mμ) and differs substantially from the simple D-glucose diphenyl formazan (max. 255, 425 mμ; Fig. 1). The product obtained upon mild acetylation of D-glucose phenylosazone formazan contains four *O*-acetyl groups[8] which is consistent only with an acyclic structure.

The possibility that a ring structure may have been

(24) O. Diels, R. Meyer and O. Onnent, *Ann.*, **525**, 102 (1936); **525**, 113 (1936).

(25) C. R. Noller, "Chemistry of Organic Compounds," W. B. Saunders, Co., Philadelphia, Pa., 1952, p. 359.

opened during the mild acetylation is refuted by the fact that the ultraviolet spectra of the free and acetylated D-glucose phenylosazone formazan are almost identical (max. 335, 410 mμ). Such a ring opening would result in the disappearance of one chromophoric group (Fig. 1). Formulas V, VIII and IX are thus eliminated, leaving only Fischer's acyclic structure III, in agreement with all of the available evidence.

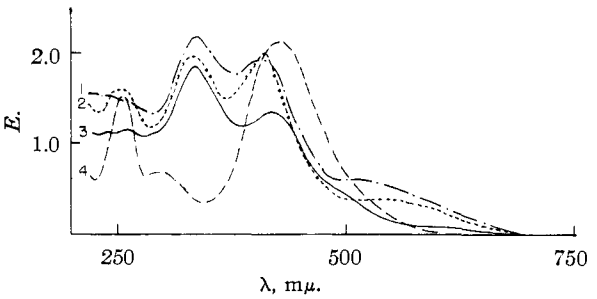
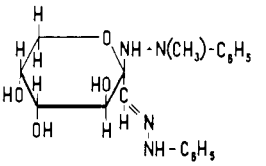


Fig. 1.—1, D-glucose diphenylformazan; 2, D-glucose phenylosazone formazan tetraacetate; 3, methylglyoxal phenylosazone formazan; 4, D-glucose diphenylformazan.

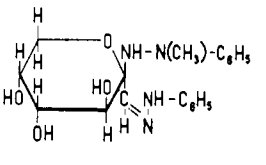
The ultraviolet spectra of D-glucose phenylosazone (max. 256, 310, 396 mμ) and of D-glucose (α-methyl-α-phenyl)-phenylosazone (mixed osazone A) (max. 258, 305, 390 mμ) which also has a structure capable of chelate ring formation, are very similar but differ markedly from that of D-glucose α-methyl-α-phenylosazone[7] (max. 264, 355 mμ) in which there is no possibility of chelate ring formation. This absence of the chelate structure and not the presence of the methyl group is evidently responsible for the spectral difference, since in mixed osazone A, a structure capable of chelate formation, the introduction of a methyl group made no spectral difference. Some of the earlier investigators, in evaluating ultraviolet spectra, favored an azo[3] and others a hydrazo[26] structure. This apparent contradiction is removed if one considers that the phenylosazones have a chelate structure, in which, because of its quasi-aromatic character, definite double bonds do not interfere and sharply defined azo and hydroazo structures disappear.

The theory of the chelate structure would also explain the fact that only one of the two imino-hydrogens of the phenylosazones is methylated even upon treatment rigorous enough to methylate the hydroxyl groups.[11]

The structure of the mixed A and mixed B osazones can be clarified by means of the formazan reaction. Mixed A was described by Votoček and Vondraček[27] as acyclic D-glucose 1-phenyl-2-(α-methyl-α-phenyl)-osazone and mixed B as acyclic D-glucose 1-(α-methyl-α-phenyl)-2-phenylosazone. Percival and Percival[28] assigned the same D-fructopyranose 1-phenyl-2-(α-methyl-α-phenyl)-osazone structure to the compounds but differentiated them as probable *syn* (XIV) and *anti* (XV) forms. Of these proposed structures, only that of the D-glucose 1-(α-methyl-α-phenyl)-

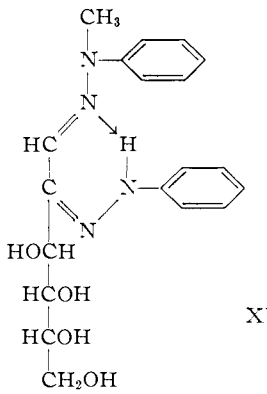


XIV



XV

2-phenylosazone, suggested by Votoček and Vondraček[27] for mixed osazone B, is theoretically unsuited for formazan formation since the imino-hydrogen of the phenylhydrazone group on carbon one is replaced by a methyl group. Since mixed osazone A failed to undergo formazan reaction even in alkaline solution, it would appear that the D-glucose 1-(α-methyl-α-phenyl)-2-phenylosazone structure should be assigned to this substance instead of to the mixed osazone B. This view is fully confirmed by recently published work of Henseke and Hautschel.[29] Akiya and Tejima[11] found that mixed osazone A is identical with D-glucose mono-N-methylphenylosazone obtained by mild methylation of D-glucose phenylosazone and that even vigorous treatment would not replace the second imino-hydrogen with a methyl group. On the basis of these facts the present author wishes to propose the Fieser and Fieser[13] chelate ring form of D-*arabino*-hexose 1-(α-methyl-α-phenyl)-2-phenylosazone for the structure XVI of the mixed osazone A. It is to be noted that the placement of the disubstituted hydrazine on carbon one is that suggested by Votoček and Vondraček[27] for mixed osazone B.



XVI—Mixed osazone A

Mixed osazone B underwent formazan reaction with ease to give the same product as D-glucose phenylosazone. Further investigation showed that mixed osazone B was D-glucose phenylosazone contaminated with mixed osazone A and that careful purification always yielded D-glucose phenylosazone.

The use of the formazan reaction confirms the acyclic structure of D-glucose phenylosazone and eliminates other proposed formulas. This reaction also furnishes sound experimental evidence for the chelate ring structures in the osazones as proposed by Fieser and Fieser,[12] it being understood that the six-membered chelate ring containing conjugated double bonds is to be regarded as a quasi-aromatic system.

Further experiments are in progress on the mutarotation of D-glucose phenylosazone.

(26) P. Grammaticakis, *Compt. rend.*, **223**, 1139 (1946).

(27) E. Votoček and R. Vondraček, *Ber.*, **37**, 3848 (1904).

(28) E. Elizabeth Percival and F. G. V. Percival, *J. Chem. Soc.*, **143**, 750 (1941).

(29) G. Henseke and H. Hautschel, *Ber.*, **87**, 477 (1954).

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Experimental

Aniline Diazonium Chloride Reagent.—Aniline (9.3 g.) was dissolved in 50 ml. of 18% hydrochloric acid, cooled to 0–5° and diazotized with 7.5 g. of sodium nitrite in 15 ml. of water. This solution was diluted to 93 ml., the concentration then being that of the diazonium salt from 0.1 g. of aniline per ml.

N,N'-Diphenyl-C-(2-quinolyl)-formazan.—To a solution of 2-formylquinoline phenylhydrazone,[23] m.p. 204° (0.19 g.) in 50 ml. of methanol containing 0.2 g. of sodium hydroxide, was added dropwise 11 ml. of diazotized aniline solution at −5°. After 15 min., the reaction mixture was poured into five times its volume of ice and water. A red product separated which was removed by filtration after standing overnight; yield 0.17 g. Upon treatment with 4 ml. of warm 1-butanol, the unchanged phenylhydrazone remained undissolved, while a dark red formazan separated from the solution; yield 0.05 g. (18.5%), m.p. 148–149°.[30] Pure material was obtained on recrystallization from 1-butanol; m.p. 150–151°.

Anal. Calcd. for $C_{22}H_{17}N_5$: N, 19.93. Found: N, 19.85, 20.2.

No observable coupling took place when the above reaction was carried out in pyridine solution and the starting material was recovered.

D-*arabino*-Hexose Phenylosazone Formazan.—D-Glucose (D-*arabino*-hexose) phenylosazone (7.2 g.) was dissolved in 216 ml. of 2 *N* potassium hydroxide in ethanol, cooled to −5° and treated by the dropwise addition of 30 ml. (1.5 mol. + 7.5%) of the diazotized aniline reagent. After 5 min. the reaction mixture was poured into five times its volume of ice and water. The violet-brown precipitate which separated was filtered to yield, on drying, a brownish-black solid; yield 9.92 g. This material was crystallized from 40 ml. of pyridine and 100 ml. of ethanol; yield 1.85 g. (20%), violet black needles, m.p. 187–188°. Pure material was obtained on further recrystallization from pyridine–ethanol and from hot dioxane by the addition of hot isopropyl alcohol; m.p. 204–205°. Starting material was recovered on evaporation of the mother liquor from the first recrystallization; recovery 2.6 g. (36%).

(30) All melting points are capillary and are uncorrected.

Anal. Calcd. for $C_{24}H_{26}O_4N_6$: C, 62.32, H, 5.67; N, 18.17. Found: C, 62.09, 62.48; H, 5.63, 5.66; N, 18.30, 18.06.

A blank with D-glucose phenylosazone (1 g.), m.p. 210°, $[\alpha]_D$ −67.8° (*c* 0.5, pyridine–ethanol, 1:1), was run for 15 min. (and 3 hr.) in 30 ml. of cold 2 *N* potassium hydroxide in ethanol, but omitting the diazotized aniline solution. Starting material was recovered unchanged; yield 1 g., m.p. 205°. The product was recrystallized from 60% ethanol; m.p. 210° $[\alpha]_D$ −65.4° (*c* 0.5, pyridine–ethanol, 1:1).

Tetra-*O*-acetyl-D-*arabino*-hexose Phenylosazone Formazan.—D-Glucose (D-*arabino*-hexose) phenylosazone formazan (1 g.) was dissolved in a mixture of 10 ml. of pyridine and 10 ml. of acetic anhydride and allowed to stand for 24 hr. at room temperature. The solid obtained on evaporation to dryness under reduced pressure was recrystallized from ethanol, yield 0.7 g. of black prisms, m.p. 158–160°. Pure material was obtained on further crystallization from ethanol; m.p. 160–161°.

Anal. Calcd. for $C_{32}H_{34}O_8N_6$: C, 60.94; H, 5.43; N, 13.33; CH_3CO, 27.3; acetyl groups, 4.00. Found: C, 61.04, 61.14; H, 5.27, 5.20; N, 13.56, 13.50; CH_3CO[31] 26.61, 26.66; acetyl groups, 3.9, 3.9; *O*-acetyl, 28.1, 28.8; *O*-acetyl groups, 4.1, 4.2.

Coupling Mixed Osazone A with Diazotized Aniline.—Mixed osazone A,[11,27] m.p. 193°, dissolved in 10 ml. of 2 *N* potassium hydroxide in ethanol and treated with 1 ml. of diazotized aniline solution in the above described manner gave no reaction at all, and the starting material was recovered unchanged.

Coupling Mixed Osazone B with Diazotized Aniline.—Mixed osazone B[27] (0.56 g.) m.p. 205°, dissolved in 16.8 ml. of 2 *N* potassium hydroxide in ethanol, was treated at −5°, in the above described manner, with 2.25 ml. of diazotized aniline solution. A marked reaction occurred. After 10 min. the mixture was poured into ice and water and allowed to stand overnight; yield 0.6 g. After purification by recrystallization from pyridine–ethanol (1:3) violet needles were obtained; m.p. 198° undepressed on admixture with D-glucose phenylosazone formazan.

After two recrystallizations from 60% ethanol a sample of the mixed osazone B used in the experiment gave a product, m.p. 210°, which showed no depression on admixture with D-glucose phenylosazone.

Mixed osazone B prepared according to Percival and Percival[9] produced identical results.

(31) E. P. Clark, *Ind. Eng. Chem., Anal. Ed.*, **8**, 487 (1936); **9**, 539 (1936).

BUDAPEST, HUNGARY

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

The Structure of Diels' Anhydro-D-glucose Phenylosazone

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The formazan reaction confirms the structural formula proposed by Hardegger and Schreier for Diels' anhydro-D-glucose (D-*arabino*-hexose) phenylosazone, and demonstrates that the formulas suggested by earlier workers are incorrect. Evidence is given that, as in the case of D-glucose (D-*arabino*-hexose) phenylosazone, the chelate ring structure proposed by Fieser and Fieser is present.

One of us has previously reported[1] that by means of the formazan reaction, the acyclic structure assigned to D-glucose (D-*arabino*-hexose) phenylosazone by E. Fischer[2] was confirmed and that the molecule contains a chelate ring as postulated by Fieser and Fieser.[3] We now wish to report the applicaton of the formazan reaction[4–8] to the definition of the equally controversial structure of Diels' anhydro-D-glucose phenylosazone. This compound

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(2) E. Fischer, *Ber.*, **17**, 579 (1884); **20**, 221 (1886).

(3) L. F. Fieser and Mary Fieser, "Organic Chemistry," D. C. Heath and Co., Boston, Mass., 1944, p. 353.

(4) G. Zemplén and L. Mester, *Magyar Tudományos Akad. Kém. Tudományok Osztályénok Közleményei*, **1**, 1 (1951); **7**, 73 (1952); **8**, 7 (1953).

(5) G. Zemplén and L. Mester, *Acta Chim. Acad. Sci. Hung.*, **2**, 9, 25 (1952).

(6) G. Zemplén, L. Mester and A. Messmer, *Ber.*, **86**, 697 (1953).

(7) G. Zemplén, L. Mester and Ede Eckhart, *ibid.*, **86**, 472 (1953).

(8) L. Mester and A. Major, THIS JOURNAL, **77**, 4297 (1955).