Acknowledgment.—The author wishes to thank Professor G. Zemplén for his advice; A. Major for assisting in the experiments; Dr. V. Cieleszky and the Institute of Food Research, Budapest, for the spectrophotometric measurements; and Miss Ilona Batta and Mrs. Ede Eckhart for the microanalyses.

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%).

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.

BUDAPEST, HUNGARY

---

[30] All melting points are capillary and are uncorrected.

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

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

The Structure of Diels' Anhydro-D-glucose Phenylosazone

BY LÁSZLÓ MESTER AND ÁDÁM MAJOR

RECEIVED AUGUST 9, 1954

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

---

[1] L. Mester, THIS JOURNAL, **77**, 4301 (1955).

[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.

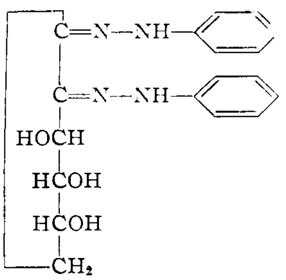
[4] G. Zemplén and L. Mester, *Magyar Tudományos Akad. Kém. Tudományok Osztályének Közleményei*, **1**, 1 (1951); **7**, 73 (1952); **3**, 7 (1953).

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

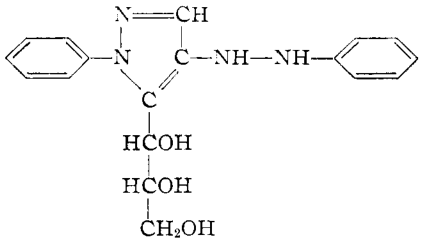
[6] G. Zemplén, L. Mester and A. Messmer, *Ber.*, **86**, 697 (1953).

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

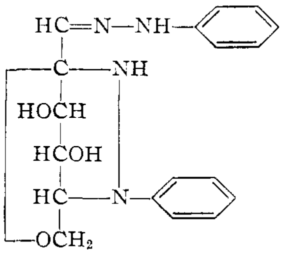
[8] L. Mester and A. Major, THIS JOURNAL, **77**, 4297 (1955).


I

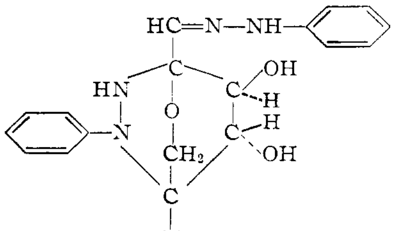
Diels and Meyer
(1935)


II

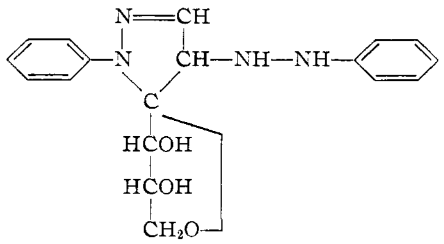
Diels, Meyer and Onnen
(1936)


III

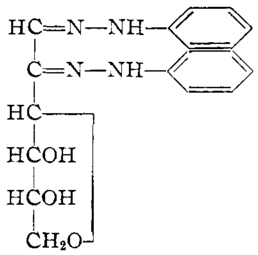
Percival
(1945)


IV

Percival
(1945)


V

Hardegger and Schreier
(1952)

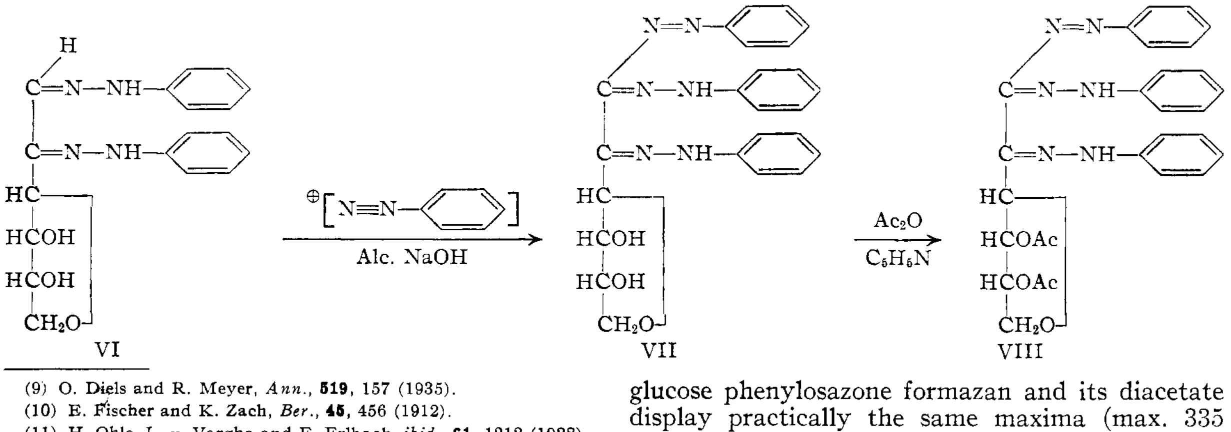

VI

Hardegger and Schreier
(1952)

was isolated by Diels and Meyer[9] on boiling D-glucose phenylosazone (D-*arabino*-hexose phenylosazone) in methanol containing traces of sulfuric acid. They believed it to be identical with 3,6-anhydro-D-glucose phenylosazone which had been previously prepared by other means.[10,11] After observing differences in the physical properties of the two compounds they suggested formula I[9] which they modified a year later by proposing the pyrazole structure II.[12] This was found to be inadequate for several reasons and the bicyclic formula III or IV was put forward by Percival.[13] After additional experimental work Hardegger and Schreier[14] proposed formula VI, observing that Fischer's original structure for the osazones was retained in it, since exact structures of osazones had not yet been unequivocally established. They also pointed out that there was only indirect evidence against Diels' formula II and the spirocyclic formula V, which they themselves had proposed, and that neither could yet be discarded.

The presence of the azomethine group (—CH=N–) and of free imino-hydrogen in phenylhydrazones is known to be a criterion for formazan formation. Diels' anhydro-D-glucose phenylosazone, like D-glucose phenylosazone, did not react in pyridine solution while in an alkaline ethanol medium (with pyridine added to improve the solubility) an osazone formazan was obtained which crystallized as black needles. Mild acetylation of this compound yielded a black diacetate. Analysis showed both acetyl groups to be oxygen-linked.

The fact that a formazan is formed eliminates structures I, II and V, which do not meet the conditions for this reaction. Hardegger and Schreier[14] had disregarded II and V only on indirect evidence. The bicyclic structures III (or IV) proposed by Percival[13] can be dismissed on spectrophotometric grounds (Fig. 1). This structure, after coupling, should contain two chromophoric groups of the simple formazans, whereas osazone formazans contain three of them. Anhydro-D-glucose phenylosazone formazan and its diacetate display practically the same maxima (max. 335 and 405 $m\mu$, 340 and 405 $m\mu$, respectively) as the known acylic methylglyoxal phenylosazone formazan, and differ substantially from the values for



VI VII VIII

---

[9] O. Diels and R. Meyer, *Ann.*, **519**, 157 (1935).

[10] E. Fischer and K. Zach, *Ber.*, **45**, 456 (1912).

[11] H. Ohle, L. v. Vargha and E. Erlbach, *ibid.*, **61**, 1212 (1928).

[12] O. Diels and R. Meyer, *Ann.*, **525**, 94 (1936).

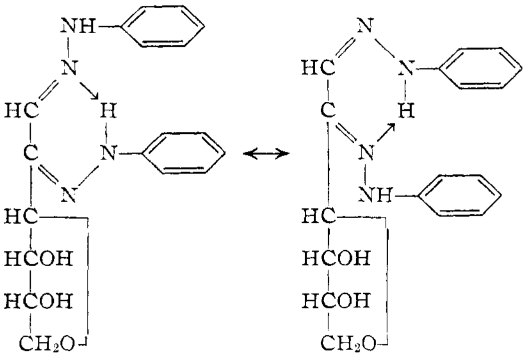
[13] E. G. V. Percival, *J. Chem. Soc.*, **142**, 783 (1945).

[14] E. Hardegger and E. Schreier, *Helv. Chim. Acta*, **35**, 232 (1952).

the simple D-glucose diphenyl formazan (max. 255 and 425 mμ).

Formazan formation (VII), the presence of two *O*-acetyl groups in the acetylation product VIII of the osazone formazan obtained, and the comparative study of the ultraviolet spectra, are all in harmony with formula VI proposed by Hardegger and Schreier[14] and afford evidence of its correctness.

Diels' anhydro-D-glucose phenylosazone, like D-glucose phenylosazone, was found to couple in alcoholic sodium hydroxide solution to form formazan but no coupling occurred in pyridine solution. For reasons discussed in connection with D-glucose phenylosazone,[1] it is necessary to assign to the finer structure of Diels' anhydro-D-glucose phenylosazone the chelate ring structure IX postulated by Fieser and Fieser without definitive experimental evidence.



IX

Acknowledgment.—Our sincere thanks are due to Professor G. Zemplén for his valuable advice; to Miss Ilona Batta and Mrs. Ede Eckhart for making the microanalyses; and to Mr. V. Cieleszky for the spectrophotometric work.

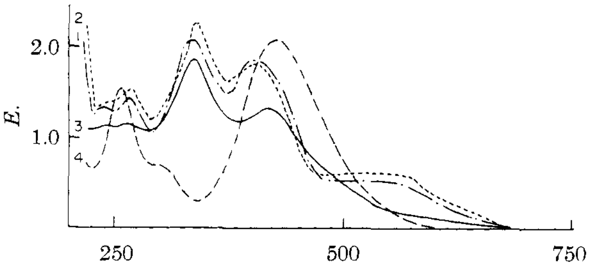


Fig. 1.—1, Diels' anhydro-D-glucose phenylosazone formazan; 2, Diels' di-*O*-acetyl-anhydro-D-glucose phenylosazone formazan; 3, methylglyoxal phenylosazone formazan; 4, D-glucose diphenylformazan.

Experimental

3,6-Anhydro-D-glucose (D-*arabino*-Hexose) Phenylosazone Formazan.—Anhydro-D-glucose phenylosazone,[9] m.p. 174°, was dissolved in 68 ml. of pyridine–ethanol mixture (1:1 by vol.) and 68 ml. of 8% sodium hydroxide by shaking (two phases appeared), and, after cooling to −5°, was coupled with 15 ml. of a solution of diazotized aniline. (This solution had been prepared by dissolving 9.3 g. of aniline in 50 ml. of 18% hydrochloric acid and diazotizing it at a temperature of 0 to 5° with a solution of 7.5 g. of sodium nitrite in 15 ml. of water. This solution was then diluted to 93 ml. to contain the diazo compound from 0.1 g. of aniline per ml.) After 15 min., the mixture was poured into five times its volume of ice and water and a black precipitate was formed. The next day the product was filtered with suction and dried; yield 4.1 g. Recrystallization from 36.9 ml. of 1-butanol and 4.1 ml. of pyridine resulted in a crop of black crystalline needles; yield 1.2 g. (27%), m.p. 179–180°. Recrystallization in the same manner gave a product of m.p. 182–183°.

Anal. Calcd. for $C_{24}H_{24}O_3N_6$: C, 64.85; H, 5.44; N, 18.91. Found: C, 64.81, 64.48; H, 5.28, 5.61; N, 18.75, 18.62.

An attempt to couple anhydro-D-glucose phenylosazone in pyridine solution resulted in no reaction. The starting material was recovered unchanged.

A blank run made in the same manner but omitting the addition of diazotized aniline solution, to determine whether alkali treatment was decomposing the anhydro-D-glucose phenylosazone, resulted in the recovery of unchanged starting material, which, after recrystallization, was identified by melting point and mixed melting point. Alkaline treatment lasting 3 hr. produced the same results.

Di-*O*-acetyl-3,6-anhydro-D-*arabino*-hexose Phenylosazone Formazan.—A solution of 0.4 g. of anhydro-D-glucose phenylosazone formazan in 4 ml. of pyridine and 4 ml. of acetic anhydride was allowed to stand at room temperature. The next day it was evaporated to dryness under reduced pressure and the crude product was crystallized from 8 ml. of 1-butanol; yield 0.3 g. of black needles, m.p. 165–166°. Recrystallization from butanol resulted in material of m.p. 169–170°.

Anal. Calcd. for $C_{28}H_{28}O_5N_6$: C, 63.62; H, 5.34; N, 15.90; acetyl groups, 2.00; CH_3CO, 16.3. Found: C, 63.44, 64.03; H, 5.37, 5.19; N, 15.91, 15.97; acetyl groups, 1.95, 1.94; CH_3CO, 15.9, 15.8; *O*-acetyl groups 2.17, 1.71; *O*-CH_3CO, 17.7, 14.8.

BUDAPEST, HUNGARY