

hydrochloric acid decomposes it rapidly, giving liquid hydrocarbons and a zirconium oxychloride solution. These hydrocarbons consisted of benzene, *t*-butylbenzene and some partly unsaturated, in all probability polynuclear, hydrocarbons.

(3) Reaction of 2,2,4-Trimethylpentane with Benzene in the Presence of (a) Magnesium Chloride ($MgCl_2$) and (b) Boron Fluoride (BF_3)

There was no reaction in the presence of magnesium chloride and hydrogen chloride at 200° and a pressure of 20 atmospheres during twenty hours. Boron fluoride,

promoted by nickel and water, effected no reaction at 55° during four hours in a nickel-lined autoclave.

Summary

A new reaction between paraffins and aromatic hydrocarbons in the presence of catalysts has been described. It consists in the formation of a lower molecular weight paraffin and an alkylated aromatic hydrocarbon, for instance, 2,2,4-trimethylpentane reacts with benzene, giving isobutane and mono- and di-*t*-butylbenzenes.

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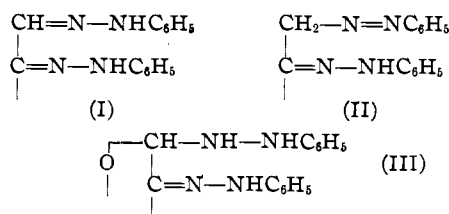
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[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, COLUMBIA UNIVERSITY]

The Constitution of Osazones¹

BY LEWIS L. ENGEL²

The first suggestion that the Fischer formula for the osazones³ does not give an adequate explanation of their behavior was made by Zerner and Waltuch,⁴ who observed a small but unmistakable mutarotation of the phenylosazones of *l*-arabinose and *l*-xylose in alcoholic pyridine solution. Later, Levene and LaForge⁵ confirmed these findings and extended them to the phenylosazones of *d*-glucose, *d*-altrose, *d*-galactose and *d*-gulose. The first observation of the mutarotation of an osazone formed from a secondary hydrazine was made by Votoček and Valentin,⁶ who reported that the methylphenylosazone of fructose shows a large mutarotation in methyl alcoholic solution. As an explanation for mutarotation, Zerner and Waltuch suggested that the classical osazone structure (I) was present in equilibrium with the isomeric azo-form (II). The finding of Votoček and Valentin, however, seems to exclude this explanation, since fructose methylphenylosazone possesses no hydrogen capable of migrating in this manner. The present author has been unable to confirm this result of Votoček and Valentin, but has found other evidence which conflicts with the hypothesis of Zerner and Waltuch.



Haworth⁷ has suggested that if one of the phenylhydrazine groups in an osazone be assumed to exist as a phenylhydrazino group attached to a cyclic sugar (III), mutarotation could be explained as being due to a shift from the α -form to the β -form. It is also possible that the mutarotation of osazones may be due to partial hydrolysis in solution. In order to test these views it is necessary to ascertain, first, whether the osazones are cyclic compounds; second, whether the nature of the attachment of the sugar to phenylhydrazine is such that hydrolysis will take place under the conditions of the mutarotation experiments; and third, whether the mutarotation is affected by factors which should affect the equilibrium.

It was hoped that the question of the ring could be settled by a study of the methylation products of glucose phenylosazone, but unexpected difficulties were encountered. Methylation by a number of methods was attempted but in no case was it possible to obtain homogeneous crystalline products. Methylation in dioxane with dimethyl sulfate and alkali yielded sirupy products with methoxyl contents ranging from 16.3

(7) Haworth, "Constitution of Sugars," Edward Arnold, London, 1929, p. 7

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(2) William J. Gies Fellow, 1933-34; Columbia University Fellow, 1934-35. This report is from a thesis submitted by L. L. Engel in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University.

(3) Fischer, *Ber.*, **20**, 821 (1887).

(4) Zerner and Waltuch, *Monatsh.*, **35**, 1025 (1914).

(5) Levene and LaForge, *J. Biol. Chem.*, **20**, 429 (1915).

(6) Votoček and Valentin, *Coll. Czech. Chem. Comm.*, **3**, 432 (1931).

to 22.6% and crystalline mixtures having methoxyl contents ranging from 14.1 to 20.7%. Basic products, comprising N-methylated osazones and methylphenylhydrazine, were always formed. Methylation of partially methylated material with methyl iodide and silver oxide or carbonate led to slight increase in methoxyl content, but considerable decomposition occurred. Neither methylation of partially acetylated glucose phenylosazone with dimethyl sulfate and alkali, nor treatment

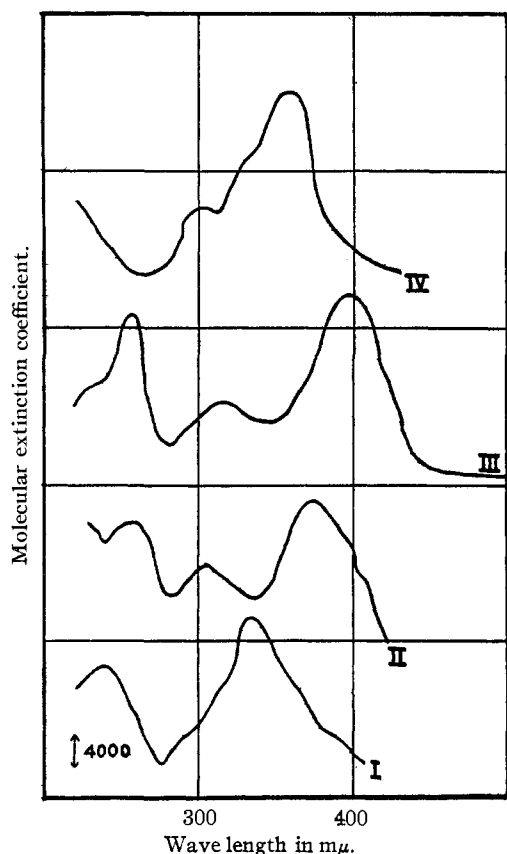
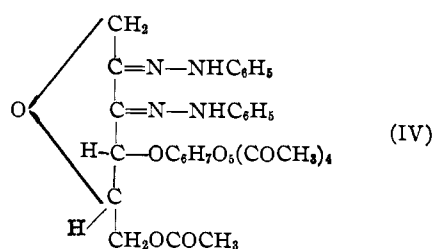


Fig. 1.—Absorption spectra of osazones. Curve I. Fructose methylphenylosazone. Curve II. Glucose phenylmethylphenylosazone. Curve III. 3, 4, 6-Trimethylglucose phenylosazone. Curve IV. Dimethylglyoxal phenylosazone. Each curve is displaced vertically by 20,000 units to avoid confusion of lines.

of a partially methylated sirup with sodium-potassium alloy followed by treatment with dimethyl sulfate, led to satisfactory results. Methylation of fructose methylphenylosazone by the liquid ammonia technique of Muskat⁸ led to cleavage of the N-N linkages instead of methylation

(8) Muskat, *THIS JOURNAL*, **56**, 693, 2449 (1934). The author wishes to express thanks to Dr. Muskat for his kindness in making available and demonstrating the use of his apparatus.

of the hydroxyl groups. The only crystalline product isolated was trimethylphenylammonium iodide. The question of ring structure could thus not be solved by methylation experiments. However, light has recently been thrown on it by the observation of Bergmann and Grafe⁹ that the compound IV displays a large and rapid mutarotation in pyridine, the rotation changing from +226 to +311° in three hours. The possibility that this change may be due to deacetylation is excluded by the fact that the deacetylated compound has a lower initial rotation and mutarotates downward.



Attention was turned to the study of the absorption spectra of osazones and their derivatives. The earliest measurements on this class of compounds were made by Baly, Tuck, Marsden and Gazdar.¹⁰ The results (Table I, Fig. 1) obtained by the present author, which are in fairly good

TABLE I
ABSORPTION OF PHENYLOSAZONES

Compound	Maxima			Minima	
Glycerose phenylosazone	250	318	381	281	335
Lactose phenylosazone	253	310	392	281	342
Lactose phenylosazone anhydride	256	310	398	282	342
Galactose phenylosazone	254	312	390	280	344
Monoacetonegalactose phenylosazone	256	313	390	280	348
3,4,6-Trimethylglucose phenylosazone	256	315	396	281	344
Tetraacetylglucose phenylosazone	256	312	392	280	327
Tetrabenzoylglucose phenylosazone			388		322
Xylose phenylosazone	256	315	390	282	332
Methylglyoxal phenylosazone	300	361		273	310
Dimethylglyoxal phenylosazone	302	357		266	310
Glucose phenylmethylphenylosazone	258	305	374	239	282
Methylated glucose phenylosazone	258	304	376	240	282
Fructose methylphenylosazone	238	335		276	340
Tetraacetylfructose methylphenylosazone	244	337		277	

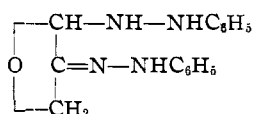
agreement with some of the findings of these investigators, indicate that the absorption spectra of the osazones studied can be divided very sharply into two classes: one class consisting of the sugar osazones and their derivatives, and the other, the osazones of simple α -keto-aldehydes and α -diketones. The compounds of the second class studied in the present investigation were the phenylosa-

(9) Bergmann and Grafe, *J. Biol. Chem.*, **110**, 173 (1935).

(10) Baly, Tuck, Marsden and Gazdar, *J. Chem. Soc.*, 1572 (1907).

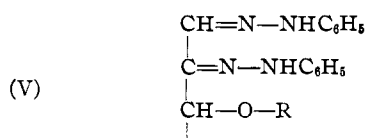
zones of methylglyoxal and dimethylglyoxal (diacetyl), which have almost identical absorption spectra. Sugar osazones show among themselves an equally marked resemblance in absorption characteristics. In the case of tetrabenzoylglucose phenylosazone the four benzoyl groups are undoubtedly responsible for the intense absorption in the region of shorter wave length.

The substantial coincidence of the absorption spectra of all the sugar osazones, including glycerose phenylosazone, indicates their structural similarity. In the cyclic formula of Haworth the conjugated system $\text{—N}=\text{C}=\text{C}=\text{N}—$, presumably strongly chromophoric, is replaced by the grouping $\text{—N}—\text{C}—\text{C}=\text{N}$, which would be expected to absorb far less strongly. Application of the Haworth formula to glycerosazone would lead to the inherently improbable structure



This four-membered ring cannot exist in 3,4,6-trimethylglucose phenylosazone, the absorption characteristics of which coincide with those of the phenylosazones of glucose and glycerose.

The grouping responsible for the specific absorption of the sugar osazones may therefore be represented by V, in which R may be hydrogen,



alkyl or acyl. The difference between the absorption spectra of sugar osazones on the one hand, and the osazones of simple α -diketones on the other, is ascribable to the presence on carbon atom 3 of the oxygen atom which functions as an auxochrome.

It is interesting to note the coincidence of the absorption spectrum of one of the crystalline methylation products of glucose phenylosazone (m.p. 130° (corr.), OCH_3 , 20.4%) with that of the mixed osazone, glucose phenylmethylphenylosazone (Fig. 1). The absorption characteristics of these compounds differ quite markedly from those of fructose methylphenylosazone (Fig. 1). Acetylation of the latter compound leads to a tetraacetate, the absorption spectrum of which is closely similar to that of the parent compound.

Evidence that the attachment of the hydrazine group in osazones is of such a nature that it may be split off under the conditions which obtain during mutarotation has been accumulating for some time. In an extended study, Votoček and Vondracek¹¹ found that the phenylhydrazine group in the sugar phenylhydrazones and osazones readily could be replaced by another hydrazine group. In the case of the osazones, they found that either one or both of the hydrazine groups could be replaced by the entering hydrazine. In the case of the condensation products of glucose with phenylhydrazine and methylphenylhydrazine, they prepared both isomeric mixed osazones in pure form. They carried out the reactions at water-bath temperature; the present author has found that certain of them can be conducted at room temperature. If fructose methylphenylosazone is allowed to stand in methyl alcoholic solution for two weeks with two moles of *p*-nitrophenylhydrazine, the mixed osazone, glucose *p*-nitrophenyl-methylphenylosazone crystallizes out. Similarly, fructose methylphenylosazone in contact with phenylhydrazine yields glucose phenylmethylphenylosazone.

This type of reaction is characteristic of the condensation products of carbonyl compounds with nitrogenous bases in general. Phenylhydrazones can be reversibly converted to oximes,^{12,13,14} semicarbazones¹⁵ and azines.¹⁶

The carbohydrate residue in an osazone or hydrazone may similarly be replaced by another carbonyl compound, as in the most commonly employed method of preparing a free sugar from a hydrazone, or an osone from an osazone.

All of these reactions suggest that hydrolysis may occur in solution even when the compound is not subjected to the influence of a competing hydrazine or carbonyl compound.

If mutarotation is partly or wholly ascribable to the establishment of an equilibrium between the osazone and its hydrolysis products, it should be possible to influence its course by the addition of one of the components of the system. Experimentally, it is easiest to measure the effect of phenylhydrazine since it is optically inactive and readily obtainable in the pure state. The rota-

(11) Votoček and Vondracek, *Ber.*, **37**, 3848 (1904).

(12) Just, *ibid.*, **19**, 1205 (1886).

(13) Pechmann, *ibid.*, **20**, 2539 (1887).

(14) Fulda, *Monatsh.*, **23**, 907 (1902).

(15) Knöpfer, *ibid.*, **31**, 87 (1910).

(16) Knöpfer, *ibid.*, **30**, 29 (1909).

tion of 3,4,6-trimethylglucose phenylosazone in alcoholic pyridine was found to change from -48.8 to $+61.5^\circ$ in one hundred and eight hours. In the presence of phenylhydrazine (16.3 moles of phenylhydrazine per mole of osazone) the rotation

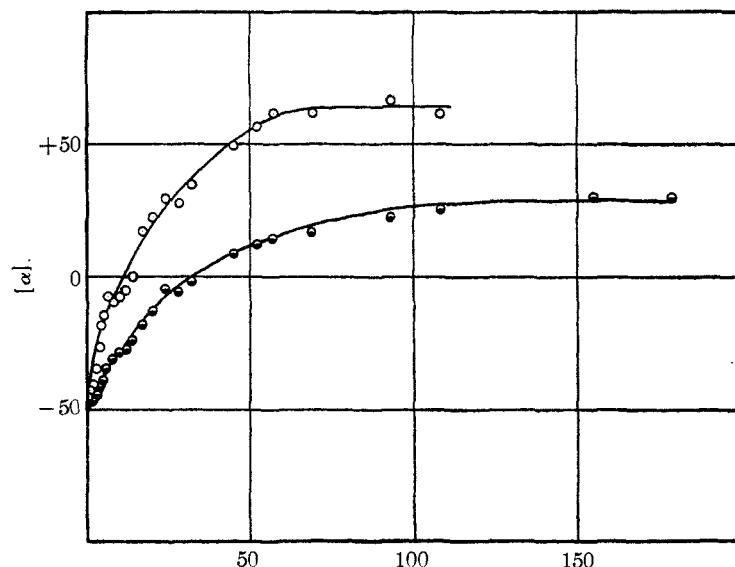


Fig. 2.—Mutarotation of 3,4,6-trimethylglucose phenylosazone. O—3,4,6-Trimethylglucose phenylosazone in 1:1 95% alcohol-pyridine. ●—3,4,6-Trimethylglucose phenylosazone and phenylhydrazine in 1:1 95% alcohol-pyridine (16.3 moles of phenylhydrazine per mole of osazone).

changed from -50.6 to $+30.0^\circ$ in one hundred and fifty-five hours (Fig. 2). The addition of phenylhydrazine, therefore, has the effect which it would be expected to have if it were one of the components of a system approaching equilibrium.

Experimental

Methylation of Glucose Phenylosazone.—Of many experiments, only one will be described in detail. To a suspension of 50 g. of the osazone in 250 cc. of dioxane were added, in twenty portions of each, 500 cc. of methyl sulfate and 1500 cc. of 30% sodium hydroxide at $50-55^\circ$. Stirring was continuous throughout the addition, which occupied five hours. The temperature was then raised to 65° for half an hour. The dioxane layer, in which all of the methylated osazone had dissolved, was evaporated to dryness under reduced pressure; the residue dissolved in 50% alcohol was held at 0° for a week. The solid which separated was fractionally crystallized from mixtures of ethyl acetate and petroleum ether and from aqueous alcohol of various concentrations. Four crystalline but apparently inhomogeneous, fractions were obtained:

- (1) M. p. $102-105^\circ$ (corr.); CH_3O , 14.24%
- (2) M. p. 110° (corr.); CH_3O , 15.3%
- (3) M. p. $106-108^\circ$ (corr.); CH_3O , 18.68%
- (4) M. p. $110-116^\circ$ (corr.); CH_3O , 20.67%

In another similar experiment, the crude product was dissolved in ether and shaken repeatedly with 0.1 *N*

hydrochloric acid. The neutral fraction, which formed the principal product, contained 16.3% of methoxyl. The basic fraction was separated into two fractions by shaking an ethereal solution with 5% acetic acid. That extracted by acetic acid consisted mainly of methylphenylhydrazine; the *p*-nitrobenzal derivative had a m. p. 135.5° (corr.), unaltered on admixture of an authentic sample. The fraction soluble in hydrochloric acid but insoluble in acetic acid was a yellow sirup which possessed an absorption spectrum identical with that of fructose methylphenylosazone. An authentic sample of the latter was also found to be soluble in 0.1 *N* hydrochloric acid.

Replacement of Methylphenylhydrazine by Phenylhydrazine in Fructose Methylphenylosazone.—Two-tenth gram (0.52 millimole) of fructose methylphenylosazone and 0.2 cc. (1.95 millimoles) of phenylhydrazine were dissolved in methyl alcohol and the solution made up to 25 cc. After standing at room temperature for a week, pale yellow needles separated out. After recrystallization from 50% alcohol they melted at $192-193^\circ$ (corr.). Glucose methylphenyl-phenylosazone is reported to melt at 192° .¹¹

Anal. Calcd. for $\text{C}_{19}\text{H}_{24}\text{N}_4\text{O}_4$: C, 61.3; H, 6.45. Found: C, 60.82; H, 6.50.

Replacement of Methylphenylhydrazine by *p*-Nitrophenylhydrazine in Fructose Methylphenylosazone.—A mixture of 0.5 g. (1.3 millimoles) of fructose methylphenyl-

osazone and 0.4 g. (2.6 millimoles) of *p*-nitrophenylhydrazine was dissolved in methyl alcohol and diluted to 25 cc. After standing for two weeks, the orange needles were filtered off, washed with methyl alcohol and recrystallized from dilute ethyl alcohol. The product was glucose methylphenyl-*p*-nitrophenylosazone, m. p. $223.5-224.5^\circ$ (corr.).

Anal. Calcd. for $\text{C}_{19}\text{H}_{22}\text{N}_6\text{O}_6$: N, 16.79. Found: N, 16.86.

Monoacetone Galactose Phenylosazone.—To a suspension of 5 g. of galactose phenylosazone in 200 cc. of acetone 10 g. of phosphorus pentoxide¹⁷ was added in small portions with stirring over a period of one hour, keeping the temperature at 0° . Stirring was continued for two hours more and the solution was filtered and washed with saturated potassium carbonate. The acetone was evaporated under reduced pressure and the residue taken up in warm ethyl acetate. The product crystallized on the addition of ligroin to the dried solution. After recrystallization, once from 95% alcohol and once from ether and petroleum ether, monoacetone galactose phenylosazone was obtained as yellow needles, m. p. $183.5-184.5^\circ$ (corr.).

Anal. Calcd. for $\text{C}_{21}\text{H}_{28}\text{N}_4\text{O}_4$: N, 14.08. Found: N, 14.14.

3,4,6-Trimethylglucose Phenylosazone Hydrate.—Trimethyl inulin was prepared by the method of Haworth

(17) Smith and Lindberg, *Ber.*, **64**, 505 (1931).

and Streight.¹⁸ Acid hydrolysis¹⁹ yielded 3,4,6-trimethylfructofuranose which was converted to the osazone.²⁰ For the mutarotation and absorption spectrum measurements, the osazone was recrystallized three times from petroleum ether containing a trace of ether and three times from dilute alcohol; m. p. 81–82° (corr.).

Glycerose Phenylsazone.—To a mixture of 264 g. of diacetin, 149.4 g. of $\text{Na}_2\text{Cr}_2\text{O}_7 \cdot 2\text{H}_2\text{O}$ and 450 cc. of glacial acetic acid, a solution of 227.4 g. of sulfuric acid in 375 cc. of acetic acid was added with stirring over a period of eight hours at 20–25°. Then 55.5 g. of hydrated sodium acetate was added and the solution stirred for two hours. The precipitate was filtered off and washed with acetic acid. The filtrate and washings were evaporated to dryness under reduced pressure, the residue was taken up in ether, washed thoroughly with saturated sodium chloride, dried over sodium sulfate and distilled under reduced pressure. The fraction boiling at 130–150° (8 mm.) was redistilled, yielding 111.3 g. (b. p. 134–136° at 8 mm.) of a liquid.

That dihydroxyacetone diacetate was the principal constituent was demonstrated by the preparation of the 2,4-dinitrophenylsazone of monoacetylaldehyde by warming 0.5 g. with 0.6 g. of 2,4-dinitrophenylhydrazine in 25 cc. of hot glacial acetic acid on the steam-bath for a half hour. The product, crystallized once from absolute alcohol and once from glacial acetic acid, formed yellow needles, m. p. 196–197° (corr.).

Anal. Calcd. for $\text{C}_{17}\text{H}_{14}\text{N}_8\text{O}_{10}$: N, 22.86. Found: N, 22.93.

Three and one-half grams of crude dihydroxyacetone diacetate was dissolved in 15 cc. of water and 5 cc. of 28% ammonia by warming on the steam-bath. The solution was cooled, treated with 6 cc. of glacial acetic acid and 6.5 g. of phenylhydrazine, and then warmed on the steam-bath for fifteen minutes, after which it was diluted with water and chilled. The product was filtered off and recrystallized once from 50% alcohol and once from benzene; m. p. 130° (corr.).

Tetrabenzoylglucose Phenylsazone.—This compound was prepared by the method of Fischer and Freudenberg²¹ and was obtained as an amorphous solid, m. p. 100–110°, which decomposed at 125° (corr.).

Anal. Calcd. for $\text{C}_{46}\text{H}_{38}\text{N}_4\text{O}_8$: N, 7.24. Found: N, 7.20. $[\alpha]^{24}_D$ –16.5°, for $c = 0.606$ in *s*-tetrachloroethane.

$[\alpha]^{24}_D$ +20.7°, for $c = 0.435$ in a mixture of 1.5 parts 95% ethyl alcohol and 1 part pyridine. After forty-eight hours this value fell to +17.2°. Fischer and Freu-

denberg found $[\alpha]^{20} -12.16^\circ$ in *s*-tetrachloroethane (Welsbach light).

Acetylation of Glucose Phenylsazone.—Two grams of glucose phenylsazone was suspended in a mixture of 8 cc. of acetic anhydride and 20 cc. of pyridine. On heating to 50° for one hour the osazone dissolved, giving a dark orange solution which was poured into ice-water and allowed to stand overnight. The brown gum crystallized partly after standing for several weeks. It was washed repeatedly with 50% alcohol on a centrifugal filter until microscopic examination showed it to consist entirely of yellow needles. The product was dried *in vacuo*; m. p. 102–104° (corr.); $[\alpha]^{25}_D -58.5^\circ$, for $c = 0.431$ in 95% alcohol.

Anal. Calcd. for tetraacetylglucose phenylsazone, $\text{C}_{26}\text{H}_{30}\text{N}_4\text{O}_8$: C, 59.25; H, 5.86; N, 10.64. Found: C, 59.33; H, 5.60; N, 10.73.

Acetylation of Fructose Methylphenylsazone.—Fructose methylphenylsazone was acetylated as in the preceding description. The product, after one recrystallization from 95% alcohol, was obtained in orange-yellow needles, m. p. 126–127° (corr.); $[\alpha]^{27}_D -184.8^\circ$, for $c = 0.403$ in 95% alcohol.

Anal. Calcd. for tetraacetylfructose methylphenylsazone, $\text{C}_{28}\text{H}_{34}\text{N}_4\text{O}_8$: C, 60.62; H, 6.18; N, 10.11. Found: C, 60.62; H, 7.24; N, 10.09.

The author wishes to express his gratitude to Professor Hans T. Clarke for the guidance and encouragement he has given throughout the course of this research.

Conclusions

1. The spectral absorption characteristics of the sugar osazones are practically identical and in concord with the structure originally suggested by Fischer.

2. The bis-phenylhydrazones of methylglyoxal and dimethylglyoxal have practically coincident absorption curves; the differences from those of the sugar osazones are ascribable to the presence in the latter of an oxygen atom on the neighboring carbon atom.

3. Osazones are readily susceptible to hydrolysis under mild conditions; the mutarotation of sugar osazones appears to depend upon the establishment of an equilibrium between them and their hydrolysis products.

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RECEIVED JULY 27, 1935

(18) Haworth and Streight, *Helv. Chim. Acta*, **15**, 809 (1932).

(19) Haworth, Hirst and Percival, *J. Chem. Soc.*, 2384 (1932).

(20) Haworth and Learner, *ibid.*, 619 (1928).

(21) Fischer and Freudenberg, *Ber.*, **46**, 1116 (1913).