THE ACTION OF WEAK ALKALIES UPON GLUCOSE. I[1].

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Received January 25th, 1936. Published June 25th, 1936.

It has been frequently recorded in the literature that by the action of various alkaline reagents, glucose and certain other hexoses can be decomposed into methylglyoxal. Even in the distillation of the sugars with dilute soda solution, A. Fernbach and M. Schoen[2], and especially F. Fischler[3] have postulated the appearance of methylglyoxal in the distillate. In analogous experiments, however, O. Baudisch and H. J. Deuel[4] have made plausible the presence of acetol, instead of methylglyoxal, in the distillate, by converting it into 3-hydroxyquinaldine which was recognized by its intense colouration. Such a divergency in the results may be due to a slight difference in the experimental conditions. This divergency may, however, also be due to the formation of methylglyoxalphenylosazone (on whose formation the argumentation of most of the previous investigators seems to be mainly based), which can be formed from acetol as well as from methylglyoxal.

It was found in our previous work[5] that when acetol and methylglyoxal coexist in a solution, they can be respectively identified by converting them into their well known semicarbazones which have quite different solubilities in water. In the present investigation, this principle was applied to the distillate which had been obtained, following Fischler, from a mixture of glucose and $^1/_{25}$ mol solution of sodium carbonate (alone or with sodium sulphite). This distillate was warmed on a water bath with an excess of semicarbazide hydrochloride and sodium acetate. And from the reaction product, acetolsemicarbazone in the main and a little diacetyl-bis-semicarbazone were isolated, but no methylglyoxal-bis-semicarbazone was obtained though carefully searched for. The distillate naturally gave methylglyoxalpheylosazone and diacetylphenylosazone when it was treated with phenylhydrazine.

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(1) A paper under the same title was read before the 57th annual meeting of the Chemical Society of Japan (April 1935).

(2) A. Fernbach and M. Schoen, *Compt. rend.*, **158** (1914), 976.

(3) F. Fischler, *Z. physiol. Chem.*, **157** (1926), 1; **165** (1927), 53; **175** (1928), 237.

(4) O. Baudisch, *Biochem., Z.*, **89** (1918), 279; O. Baudisch and H. J. Deuel, *J. Am. Chem. Soc.*, **44** (1922), 1585.

(5) R. Nodzu, this Bulletin, **10** (1935), 122.

On the other hand, L. Marchlewski and his co-workers[6] have found that the absorption spectrum of Fischler's distillate (with Na_2SO_3) is quite different from that of a solution of synthetic methylglyoxal. The cause of this disagreement has been left unexplained.

Now we traced Marchlewski's spectrographic experiments and arrived at results identical with his. As seen in the diagram (Fig. 1) which is prepared in the ordinary way, the absorption curve (Curve I) for Fischler's distillate (0.036% as methylglyoxal, 0.03% as acetol) has its maximum at 2673Å and minimum at 2301Å, figures that agree well with the values (max. 2665Å, min. 2260Å) of Marchlewski. For a solution (0.014%) of synthetic methylglyoxal, however, we found (Curve III_1 for *p*H 2.7 and III_2 for *p*H 7.0) the maxima at 2778Å (*p*H 2.7) and 2832Å (*p*H 7.0), which is in good agreement with the data of C. Neuberg and Schou (2780Å)[7], Marchlewski (2765Å, *p*H 2.7; 2870Å, *p*H 11), and F. Fischler (2842Å for a neutral solution)[8].

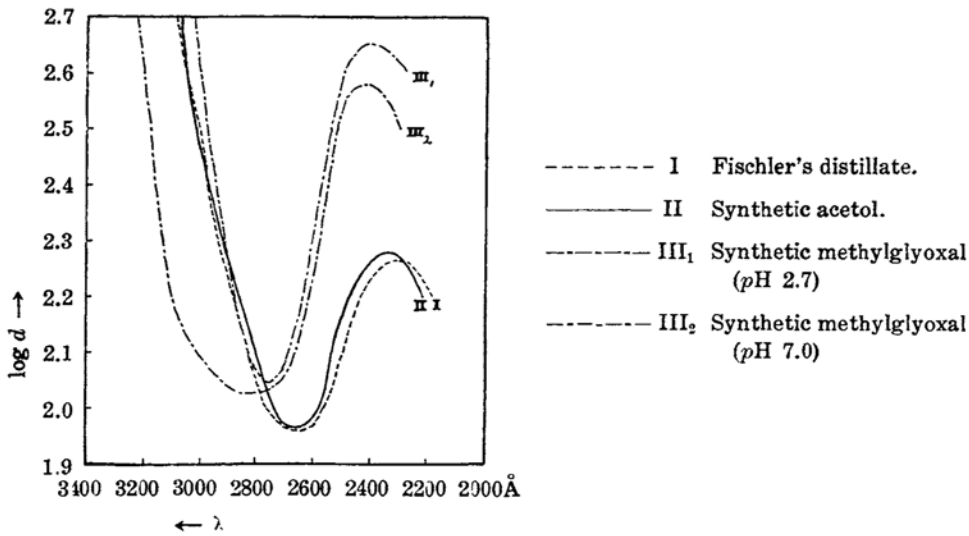

Fig. 1.

Thus we find a wide difference in the characteristics of the absorption curves between the distillate and a methylglyoxal solution.

On the other hand, a solution (0.04%) of synthetic acetol gave an absorption curve (Curve II) which has its maximum at 2680Å and minimum at

(6) L. Kwiecinski and L. Marchlewski, *Bull. Soc. Chim.*, **45** (1929), 610; L. Marchlewski, J. Pizlo, and W. Urbańczyk, *Biochem. Z.*, **264** (1933), 437.

(7) C. Neuberg and S. A. Schou, *Biochem. Z.*, **191** (1927), 469.

(8) F. Fischler, H. Hauss, and K. Täufel, *Biochem. Z.*, **227**, (1930), 161.

2344Å, and closely resembles that of Fischler's distillate (Curve I). A certain quantitative disagreement between the two curves may be partly due to an inaccuracy in the iodometric estimation of acetol[9] and partly to the presence of some slight impurities, e.g. diacetyl, in the distillate.

From the above evidence, it can no longer be doubted that it is not methylglyoxal but acetol that gave the methylglyoxalosazone in the distillate. This conclusion is also supported by the fact, which will be reported in a subsequent paper, that synthetic methylglyoxal did not distil over as such in a similar distillation experiment.

Moreover, glucose gives acetol on distillation with solid potassium hydroxide,[10] alkali phosphates (M_2HPO_4) solution[11] or zinc carbonate[12] in water. It seems quite likely, therefore, that acetol rather than methylglyoxal distils over when glucose is distilled with different alkaline reagents.

Experimental Part.

Distillation. $^1/_{25}$ Mol sodium carbonate solution was gently distilled, alone or with sodium sulphite, a glucose solution, and after its exhaustion distilled water being supplied drop by drop. The distillate was neutral to litmus paper and reduced Fehling's solution in the cold and gave a marked iodoform reaction.

Estimation of Methylglyoxal and Acetol.[13] The content of the iodine-consuming substance in the distillate was determined as methylglyoxal by the iodometry of Fischler[14]. The value for acetol was obtained from that for methylglyoxal by computation, assuming the equation (1) for acetol instead of Fischler's (2) for methylglyoxal.

$$CH_3COCH_2OH + 5\,I_2 + 9\,KOH = CHI_3 + C_2O_4K_2 + 7\,KI + 7\,H_2O \quad (1)$$

$$CH_3COCHO + 4\,I_2 + 7\,KOH = CHI_3 + C_2O_4K_2 + 5\,KI + 5\,H_2O \quad (2)$$

Semicarbazones. The distillate was warmed on a boiling water-bath for half an hour with a quantity of semicarbazide hydrochloride and sodium acetate equal to five equivalents to the methylglyoxal content. After letting the reaction product stand overnight, the white precipitate deposited was filtered. In the course of evaporation of the filtrate under reduced pressure to a small volume, a further precipitation occurred and the precipitate was filtered. These precipitates melted above 275°. The filtrate was concentrated over concentrated sulphuric acid in a vacuum desiccator to a syrup from which a brownish yellow solid mass was isolated. This mass was fractionally crystallized from water into a less soluble part (m.p. 245–257°), and a more soluble part (m.p. 194–196°) in major quantity. After recrystallization of these three substances from hot or

(9) See the experimental part.
(10) A. Emmerling and G. Loges, *Ber.*, **14** (1881), 1005; **16** (1883), 837.
(11) Recorded in a hitherto unpublished paper of ours.
(12) W. Löb, *Biochem. Z.*, **12** (1908), 78.
(13) See also this Bulletin, **10** (1935), 124.
(14) F. Fischler and R. Boettner, *Z. anal. Chem.*, **74** (1928), 28.

boiling water, they were identified as diacetyl-bis-semicarbazone (m.p. 279–280°), hydrazodicarbonamide (m.p. 257–258°) — a condensation product of semicarbazide itself — and acetolsemicarbazone (m.p. 198–200°), respectively, by determination of the mixed melting point and elementary analysis: Diacetyl-bis-semicarbazone (Found: C, 36.1; H, 5.8; N, 42. Calc. for $C_6H_{12}O_2N_6$: C, 36; H, 6; N, 42%), hydrazodicarbonamide (Found: N, 47 6. Calc. for $C_6H_6O_2N_4$: N, 47.5%), acetolsemicarbazone (Found: C, 36.6; H, 6.6; N, 31.9. Calc. for $C_4H_9O_2N_3$: C, 36.6; H, 6.9; N, 32%). In spite of careful search, methylglyoxal-bis-semicarbazone could not be isolated from the reaction product. Some experimental results are cited in Table 1.

Table 1.

No.	Glucose (g.)	Na_2CO_3 ($^1/_{25}$ mol soln.) (c.c.)	Na_2SO_3 (g.)	Distillate (c.c.)	Iodine consuming substance as acetol. (g.)	Semicarbazone (g.)		
						m.p. 275°	m p. 245°	m.p. 194°
1	5	250	—	700	0.097	0.01	0.05	0.10
2	5	250	—	700	0.097	0.01	0.04	0.10
3	5	250	15	600	0.114	0.01	0.03	0.11
4	5	250	15	850	0.167	0.02	0.04	0.16
5	5	250	15	250	0.075	0.01	0.04	0.07

Phenylosazone. When the distillate was warmed with phenylhydrazine and acetic acid on a boiling water-bath for an hour, a brownish yellow precipitate was obtained. The insoluble part in hot 60% alcohol of the precipitate was recrystallized from boiling benzene into diacetylphenylosazone; m.p. 241–242°. (Found: N, 20.6. Calc. for $C_{16}H_{18}N_4$: N, 21.1%.) By recrystallisation of the soluble part from aqueous alcohol, methylglyoxal-phenylosazone was obtained in a pure state; m.p. 149°. (Found: N, 2 .8. Calc. for $C_{15}H_{16}N_4$: N, 22.2%.) Neither of the osazones showed any lowering of melting point on admixing with its respective authentic specimen. The data of roughly quantitative experiments are cited in Table 2.

Table 2.

No.	Glucose (g.)	Na_2CO_3 ($^1/_{25}$ mol soln.) (c.c.)	Na_2SO_3 (g.)	Distillate (c.c.)	Iodine consuming substance as methylglyoxal. (g.)	Phenylosazone (g.)	
						m.p. 241°	m.p. 149°
1	5	250	15	750	0.168	0.015	0.028
2	5	250	—	750	0.150	0.008	0.024

Methylglyoxal and Acetol. A dilute methylglyoxal solution was prepared by oxidation of glycerol by means of hydrogen peroxide, following C. Neuberg and E. Hofmann's

directions[15]. It was acidic (*p*H 2.7) as already observed by Marchlewski and his collaborator. Acetol ($n_D^{20}=1.4222$, $D_{20}^{20}=1.0850$, b.p.=53-54°/18 mm.) was synthesised from monobromoacetone following A. Levene and A. Walti[16].

The semicarbazones of acetol and methylglyoxal were prepared in a mixed solution, and easily separated from each other, by taking advantage of their difference of solubility in hot water — methylglyoxal-bis-semicarbazone is sparingly soluble, while acetol-semicarbazone easily soluble. It was also observed that the iodometric titration of acetol solution gave a far lower value than the actual one, i.e., 62-65% fluctuating with its concentration (0.2-0.02%). Hence, the iodometric titration does not appear to obey equation (1) merely.

Spectroscopy. For the spectroscopic investigation, Hilger's spectrograph E_2, a Geisler hydrogen tube as the light source and Eastman's panchromatic film were used. The experimental results are graphically shown according to Hartley (Fig. 1). A distillate (0.036% as methylglyoxal, 0.03% as acetol) obtained under the same experimental conditions as No. 5, Table 1, gave an absorption spectrum which is shown as Curve I in Fig. 1. The absorption curves for 0.014% solution of methylglyoxal at *p*H. 2.7 and *p*H. 7.0 (after neutralization with Na_2CO_3) are Curves III_1 and III_2, respectively. Curve II is for an acetol solution (0.04%). The acetol and methylglyoxal contents of solutions examined were all determined by iodometry.

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(15) C. Neuberg and E. Hofmann, *Biochem. Z.*, **224** (1930), 491.
(16) A. Levene and A. Walti, "Organic Syntheses", Vol. 10, 1930, p. 1.