NOTES.

132. *The Formation of Osazones. Part II. The Formation of Glucosazone.*

By G. J. Bloink and K. H. Pausacker.

The following work was undertaken in order to determine the effect of a number of compounds in catalysing the reaction between glucose and phenylhydrazine to form glucosazone.

Stempel (*J. Amer. Chem. Soc.*, 1934, **56**, 1352) has found that in an atmosphere of nitrogen glucose is not converted into the osazone in dilute mineral acid although, " contrary to general belief," glucose phenylhydrazone is formed. When he added an equivalent amount of acetic acid to the solution, the theoretical yield of osazone was rapidly obtained. We have verified Stempel's results but find that, in dilute mineral acid, the osazone is formed if the reaction vessel is filled with oxygen.

Accordingly, the catalytic effect of various other substances was investigated. Glucose (4·91 g.) was dissolved in an aqueous solution (15 ml.) of the catalyst, and phenylhydrazine (2·4 g.) in ethyl alcohol (15 ml.) added. The reaction vessel was stoppered after the air had been displaced by nitrogen. The precipitated osazone was filtered off at specified times. The pH of the solution was also determined (using a glass electrode) and was found to alter only very slightly during the reaction. The table summarises the results obtained.

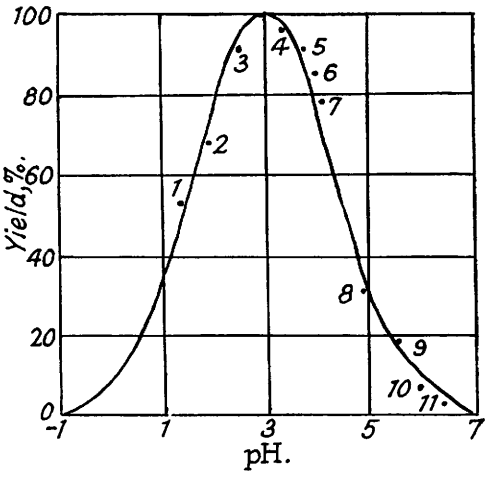
No. of expt.	Catalyst.	Normality.	Initial pH.	Yield, %. 4 days.	Yield, %. 3 weeks.
1	Oxalic acid	5·78	1·4	53	57
2	Citric acid–sodium phosphate buffer	—	1·90	68	—
3	Phosphoric acid	5·78	2·6	91	charred
4	Chloroacetic acid	5·78	3·2	98	100
5	Citric acid	5·78	3·8	91	100
6	Lactic acid	5·78	4·1	85	100
7	Acetic acid	5·78	4·2	79	99
8	,,	1·93	4·9	32	81
9	,,	0·64	5·4	19	48
10	,,	0·21	5·9	6	19
11	,,	0·075	6·3	3	—
	,, [a]	0·025	—	—	6
	Boric acid	5·78	6·4	3	charred
	Hydrochloric acid [b]	0·025	6·7	—	24
	,,	0·025	6·7	—	—
	Phthalic acid	0·025	6·9	—	3

[a] 15 Ml. of hydrochloric acid (0·075N.) were also added. [b] In an atmosphere of oxygen.

Zero yields were obtained when phenol (0·025 or 5·78N.), citric, succinic, or trichloroacetic acid (0·025N.), ammonium chloride (0·025N.), sodium acetate (0·025 or 5N.), piperidine (0·025N.), ammonia (0·025N.), or a saturated solution of sulphur dioxide, carbon dioxide, or hydrogen sulphide was used as catalyst.

It is thus seen that the yield of osazone is highly dependent on the pH of the solution, the maximum occurring at *ca.* pH 3. This is in contrast to the work of Garrard and Sherman (*J. Amer. Chem. Soc.*, 1918, **40**, 995), who claim that the optimum pH is 4·7.

The percentage yield of osazone (after 4 days) is approximately $100e^{-0.120(pH-3)^2}$, the theoretical curve and certain of the results (indicated by their experimental numbers given in the table) being shown in the figure. However, it may be noted that whenever high yields were obtained the catalysing acid was weak. When (as suggested by a Referee) sufficient hydrochloric acid was used to bring the pH to 3 the yield of osazone (after 4 days) was 4%. This result may be readily explained, for calculation, using the standard salt hydrolysis equations, shows that phenylhydrazine is almost completely converted by hydrochloric acid into the phenylhydrazinium ion whereas with 5·78N-acetic acid the conversion is *ca.* 90%. Conant and Bartlett (*J. Amer. Chem. Soc.*, 1932, **54**, 2881) have found that semicarbazone formation is acid-catalysed but proceeds *via* the free semicarbazide. If hydrazone formation, which is involved in osazone formation, follows the same mechanism, then the difference between hydrochloric and acetic acids at the same pH may be explained by their differing salt-forming capacities. Thus, though the above equation is applicable when weak acids are employed, it cannot be used for predicting the yield with strong acids.



CHEMISTRY DEPARTMENT, UNIVERSITY OF MELBOURNE,
VICTORIA, AUSTRALIA.

[*Received, October 2nd,* 1950.]

---

133. *The Determination of Phosphorus in Deoxypentose Nucleic Acids.*

By A. S. JONES, W. A. LEE, and A. R. PEACOCKE.

SMALL amounts of organic phosphorus are frequently determined by Allen's method (*Biochem. J.*, 1940, **34**, 858) in which 60% perchloric acid is used for the conversion into orthophosphate in place of the sulphuric acid used by earlier workers (*e.g.*, Fiske and Subbarow, *J. Biol. Chem.*, 1925, **66**, 375). It has now been found that the latter reagent, with suitable additions, is more satisfactory for the determination of phosphorus in deoxypentose nucleic acids.

When perchloric acid was used for the digestion of these acids, the mixture required about 7 hours' heating to ensure release of all the phosphorus (see table; 2 ml. of nucleic acid solution were heated in micro-Kjeldahl flasks with 2·2 ml. of 60% perchloric acid, experiments being in triplicate). This long digestion time renders the method laborious and inaccurate and it is difficult to obtain consistent results with these substances.

Time of heating (mins.)	40	85	180	300	360	420	495
Phosphorus released (mg.)	0·027	0·032	0·037	0·045	0·046	0·046	0·046

A mixture of concentrated sulphuric acid, copper sulphate, potassium hydrogen sulphate, and a little powdered selenium had previously (Ma and Zuazaga, *Ind. Eng. Chem., Anal.*, 1942, **14**, 280) been found to release all the nitrogen from nucleic acids in about 2 hours. With such a mixture we find that phosphorus is completely released from deoxypentose nucleic acids in 45 minutes (subsequent to removal of water).

The procedure is as follows. To the solution (1—2 ml., containing 0·01—0·10 mg. of phosphorus), in a micro-Kjeldahl flask, concentrated sulphuric acid (0·3 ml.), a solution (0·5 ml.) containing copper sulphate (10 g./l.) and potassium hydrogen sulphate (120 g./l.), together with a few grains of selenium powder, are added. The water is removed by boiling and after a further 45—60 minutes' heating, the solution is allowed to cool and transferred to a 25-ml. flask. Thereafter, Allen's technique is employed,

the optical density being determined by means of a " Spekker'" absorptiometer (Ilford spectrum red filter No. 608).

The following experiments were carried out in order to validate the method.

Orthophosphates. Known amounts of potassium dihydrogen orthophosphate were treated as described above and the usual linear calibration curve of " Spekker " drum reading against the quantity of phosphorus present was obtained. The increase in drum reading for an increase of 0·01 mg. in the amount of phosphorus present was 0·046. It was necessary to heat the phosphate solution with the digestion mixture under conditions comparable with those of the determinations to obtain a consistent calibration curve. Determination of pure orthophosphate is therefore possible in the presence of this mixture. A blank determination on all the reagents was made.

Pyrophosphate. Small quantities of sodium pyrophosphate were treated as previously described. The following figures show that release of orthophosphate was quantitative within 1—2%. Therefore hydrolysis of a complex phosphorus compound will not stop at the pyrophosphate stage. However, only about 15% of the pyrophosphate was transformed into orthophosphate if heating was omitted.

P used as $Na_4P_2O_7$, mg.	0·020	0·040	0·060
P found as PO_4''', mg.	0·0198	0·0408	0·0608

Deoxypentose nucleic acids. Equal volumes of the same solution of a deoxypentose nucleic acid, isolated from herring sperm, were digested (in triplicate) for different times, and the orthophosphate released was determined, with the following results :

Time (mins.).*	Drum readings.	Mg. of P obtained as PO_4'''.
10	0·225, 0·224, 0·224	0·042
45	0·231, 0·229, 0·227	0·043
120	0·227, 0·227, 0·230	0·043

* After removal of water vapour, *i.e.*, after boiling for 10—20 minutes.

Heating for 45 minutes subsequent to removal of water was sufficient. The addition of 0·02 mg. of phosphorus, as orthophosphate, to the same amount of this same nucleic acid solution caused increases in the " Spekker " drum reading of 0·087, 0·090 and 0·093 in different experiments, the calibration curve predicting a value of 0·092. Hence within the limits of accuracy of the instrument, the determination of orthophosphate by this method is not vitiated by the presence of nucleic acid decomposition products.

The authors thank Professor M. Stacey, F.R.S. for his interest in this work and acknowledge gratefully receipt of a Beit Memorial Fellowship by one of them (A. S. J.).

DEPARTMENT OF CHEMISTRY, THE UNIVERSITY,
EDGBASTON, BIRMINGHAM, 15.

[*Received, October 3rd,* 1950.]

---

134. *The Vapour Pressure of Diphenyl, Dibenzyl, and Diphenylmethane.*

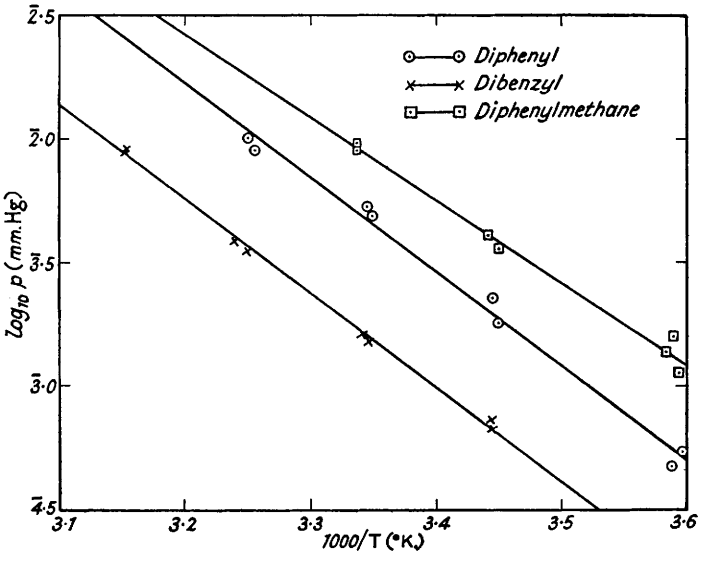
By NORMAN F. H. BRIGHT.

THE vapour pressures of diphenyl, dibenzyl, and diphenylmethane have been measured over the temperature range 5—45°, by the effusion method which was recently used for *cis*- and *trans*-azobenzene (Bright, Carson, and Dyson, *Research*, 1950, **3**, 185). This method is valid for pressures at which the mean free path of the molecules in the molecular beam is long in comparison with the dimensions of the effusion orifice. In the present work, this upper limit is of the order of 1—2 $\times$ 10^{-2} mm. of mercury. The lower limit of applicability is fixed by the vacuum attainable in the evacuated space and by the convenience of experimental times involved; vapour pressures down to 10^{-5} mm. of mercury can be measured without great difficulty. In the present work, the lowest pressures encountered were well in excess of this limit.

Experimental.—The very pure hydrocarbons, provided by Miss M. F. Penney, of the Physical Chemistry Laboratory, Oxford, were characterised as follows : Diphenyl, m. p. 68·8—68·9° (Found : C, 93·3, 93·5; H, 6·6, 6·8. Calc. for $C_{12}H_{10}$: C, 93·5; H, 6·5%). Dibenzyl, m. p. 51·6—51·8° (Found : C, 92·3, 92·5; H, 7·8, 7·8. Calc. for $C_{14}H_{14}$: C, 92·25; H, 7·75%). Diphenylmethane, m. p. 25·0° (Found : C, 92·8, 93·0; H, 7·0, 6·8. Calc. for $C_{13}H_{12}$: C, 92·8; H, 7·2%).

The experimental results, shown in the figure, were treated by least square methods, to give equations of the form $\log_{10} p$ (mm. Hg.) $= -(A/T) + B$. Values of A and B, and the standard deviation of the determined vapour pressures, are given in the following table :

	Diphenyl.	Dibenzyl.	Diphenylmethane.
Temp. range	4·9—34·5°	17·1—44·2°	5·1—26·5°
A	3799	3783	3341
B	10·38	9·86	9·12
Standard deviation (%) of a determination of v. p. ...	±12·8	±4·8	±9·7



Discussion.—Previously published data for these hydrocarbons are confined largely to boiling points at higher pressure ranges (Garrick, *Trans. Faraday Soc.*, 1927, **23**, 561; Montillon, Rohrbach, and Badger, *Ind. Eng. Chem.*, 1931, **23**, 764). Our measurements, while not being in very good accord with the published data for the lower ranges, give quite good agreement for higher temperatures. Our values for latent heat are in reasonable accord with those obtained by Wolf and Weghofer (*Z. physikal. Chem.* 1938, *B*, **39**, 198). The comparatively low value for diphenylmethane is in accord with its surprisingly low melting point.

	Latent heat of sublimation (kcals./mol.).	
	Present work.	Wolf and Weghofer.
Diphenyl	17·4 ± 0·7	16·4 ± 0·2 at 22°
Dibenzyl	17·3 ± 0·3	17·5 ± 0·2 at 25°
Diphenylmethane	15·3 ± 0·7	17·2 ± 0·2 at 24°

IMPERIAL CHEMICAL INDUSTRIES LIMITED,
NOBEL DIVISION, RESEARCH DEPARTMENT,
STEVENSTON, AYRSHIRE.

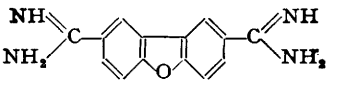
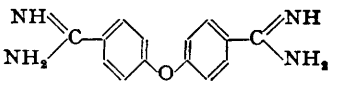
[*Received, November 3rd*, 1950.]

135. 3 : 6-*Diamidinodibenzofuran.*

By J. S. MOFFATT.

3 : 6-DIAMIDINODIBENZOFURAN has been prepared because of its structural resemblance to di-(*p*-amidinophenyl) ether (phenamidine) (II) which is one of a series of aromatic diamidines prepared by Ashley, Barber, Ewins, Newbery, and Self (*J.*, 1942, 103) and shown to be active against *Trypanosoma rhodesiense* infections in mice (Lourie and Yorke, *Ann. Trop. Med. Parasit.*, 1939, **33**, 289). 3 : 6-Diamidinophenanthrene, a similar modification of di-4-amidinostilbene (stilbamidine), has been described by Barber and Stickings (*J.*, 1945, 167) as being only

slightly active as a trypanocide. The dibenzofuran derivative (I) was examined by Professor C. H. Browning, F.R.S., in the Bacteriology Department of this University. Administered

(I.)   (II.)

subcutaneously in doses approaching the maximum tolerated, it cured infections of *T. congolense* and *T. brucei* in mice.

Experimental.—3 : 6-*Dicyanodibenzofuran.* A mixture of 3 : 6-dibromodibenzofuran (5·5 g.) (Hoffmeister, *Annalen*, 1871, **159**, 215) and cuprous cyanide (6·5 g.) was added, in small portions, to boiling, purified quinoline (20 c.c.). The mixture was boiled under reflux for 30 minutes, allowed to cool, and then added to concentrated hydrochloric acid (200 c.c.). The mixture was boiled for a short time and then filtered. The residue was washed with dilute hydrochloric acid and water, and then sublimed at 220—230°/0·5 mm. The sublimate, on recrystallisation from acetic acid, gave needles (2·9 g.), m. p. 299°, of the *dicyanide* (Found : C, 76·9; H, 2·8; N, 12·8. $C_{14}H_6ON_2$ requires C, 77·1; H, 2·8; N, 12·8%).

3 : 6-*Diamidinodibenzofuran.* A suspension of the dicyanide (2·8 g.) in anhydrous ethanol (45 c.c.) was cooled at 0° and then saturated with dry hydrogen chloride. After 14 days at room temperature the mixture was filtered. The residue was washed with absolute ether and then kept for several hours in a vacuum desiccator containing sodium hydroxide. It was heated with saturated ethanolic ammonia (45 c.c.) at 45—50° for 6 hours. The mixture was cooled and then filtered. The residue (3·6 g.) was separated, by extraction with hot 2N-hydrochloric acid, into unchanged dicyanide (0·9 g.), and the *diamidine dihydrochloride dihydrate* which crystallised from 2N-hydrochloric acid in long needles (2·8 g.), m. p. >320° (Found : C, 46·7; H, 4·9; N, 15·2. $C_{14}H_{12}ON_4,2HCl,2H_2O$ requires C, 46·5; H, 5·0; N, 15·5%).

Grateful acknowledgment is made to Professor J. W. Cook, F.R.S., for his interest in this work, to the Medical Research Council for a Grant, and to Mr. J. M. L. Cameron for micro-analyses.

UNIVERSITY OF GLASGOW. [*Received, November 14th*, 1950.]

---

136. *Difficulties in the Alkylation of* 2 : 6-*Di-iodo*-4-*nitrophenol.*

By J. H. WILKINSON.

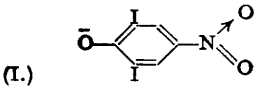
2 : 6-DI-IODO-4-NITROPHENOL cannot be alkylated in the conditions normal for the alkylation of phenols. Kalb, Schweizer, Zellner, and Barthold (*Ber.*, 1926, **59**, 1869), however, succeeded in preparing 2 : 6-di-iodo-4-nitroanisole by using a large excess of hot methyl sulphate and sodium hydroxide, and suggested that the difficulty was due to steric hindrance. That this cannot be the complete explanation is shown by the fact that several other 2 : 6-di-iodophenols have been alkylated in reasonably good yields under much milder conditions. Wheeler and Liddle (*Amer. Chem. J.*, 1910, **42**, 441) have described the methylation of 4-hydroxy-3 : 5-di-iodobenzoic acid with methyl iodide and alkali in methanolic solution (method I), and 3 : 5-di-iodoanisaldehyde dimethyl acetal was obtained in 50% yield by treatment of the hydroxy-aldehyde with methyl sulphate and alkali in methanol, initially at 0° (method II) (Wilkinson, *J.*, 1949, 2370). During the course of work on thyroxine analogues, it became necessary to alkylate a number of 2 : 6-di-iodophenols, and some of the results are shown in the accompanying table.

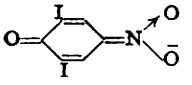
Phenol.	Alkylating agent.	Method.	Yield of product.
2 : 6-Di-iodo-*p*-cresol	Me_2SO_4	II	63%
4-Hydroxy-3 : 5-di-iodobenzaldehyde	Et_2SO_4	II	60%
p-Hydroxybenzaldehyde	Et_2SO_4	II	73%
2 : 6-Di-iodo-4-nitrophenol	Me_2SO_4	II	0—5%
p-Nitrophenol	Me_2SO_4	II	27%
Phenol	Me_2SO_4	II	70%
4-Hydroxy-3 : 5-di-iodobenzonitrile	EtI	I	60%

Iodine substitution in the *ortho*-position produces only a slight falling off in yield, whilst a nitro-group in the *para*-position to the hydroxyl group causes a marked reduction. When the two effects are combined, the yield by method II falls practically to zero. It is therefore concluded that the effect of the nitro-group on the ratio of the resonance forms (I and II) of the ion is the dominating factor, and that the iodine atoms may either enhance this or exert slight steric hindrance. The contribution of the normal phenoxide ion (I) is diminished to such an extent

that alkylation is negligible under mild conditions, whilst the ion (II) becomes the major component.

It is relevant to this explanation that a quinonoid substance is produced when 2 : 4 : 6-tri-iodophenol is oxidised under very mild conditions (cf. Woollett, Davis, Jones, and Neill, *J. Amer. Chem. Soc.*, 1937, **59**, 861). Also the intense orange colour of the sodium salt of 2 : 6-di-iodo-4-nitrophenol suggests the presence of (II).


(I.)


(II.)

Experimental (m. p.s are uncorrected).—*Method I.* 4-*Ethoxy*-3 : 5-*di-iodobenzonitrile.* 4-Hydroxy-3 : 5-di-iodobenzonitrile (von Auwers and Reis, *Ber.*, 1896, **29**, 2359) (9·3 g.) was dissolved in ethanol (100 c.c.), aqueous sodium hydroxide (40%; 5 c.c.) and ethyl iodide (15·6 g.) were added, and the mixture was heated under reflux for 2 hours. After evaporation to *ca.* 30 c.c., the mixture was filtered and cooled; 4-*ethoxy*-3 : 5-*di-iodobenzonitrile* (6·0 g., 60%) was precipitated. Recrystallisation from alcohol gave large needles, m. p. 110—111° (Found: I, 64·4. $C_9H_7ONI_2$ requires I, 63·8%). When heated under reflux with alcohol (3 c.c.) and 5N-sodium hydroxide (5 c.c.) for 1 hour, the nitrile (0·5 g.) was hydrolysed to 4-*ethoxy*-3 : 5-*di-iodobenzoic acid* (0·42 g.) which was obtained by pouring the mixture into water (50 c.c.) and acidifying it with acetic acid. The acid crystallised from 60% alcohol in needles, m. p. 208—209° (Found: C, 25·9; H, 2·2; I, 60·9. $C_9H_8O_3I_2$ requires C, 25·8; H, 1·9; I, 60·8%).

Method II. The phenol (0·1 g.-mol.) was dissolved in methanol (100 c.c.) and treated with methyl sulphate (0·1 g.-mol.). The mixture was cooled to 0—10° and treated with 40% sodium hydroxide (10 c.c.). In each case the temperature rose to *ca.* 70°. The mixture was set aside for 30 minutes, made alkaline (phenolphthalein), and diluted with water (500 c.c.). The product was collected either by filtration or extraction with ether. Ethylations were carried out similarly, except that ethanol (100 c.c.), ethyl sulphate (0·3 g.-mol.), and 40% sodium hydroxide (30 c.c.) were used, and the mixture was gently boiled for 10 minutes before being cooled and diluted with water. The following were among the alkyl phenyl ethers prepared by this method:

3 : 5-*Di-iodo*-4-*methoxytoluene* distilled at 106—108° at 10^{-5} mm., and was obtained as an almost colourless oil, which on cooling crystallised in waxy needles, m. p. 25° (Found: C, 26·5; H, 2·15; I, 67·5. $C_8H_8OI_2$ requires C, 25·7; H, 2·15; I, 67·8%).

4-*Ethoxy*-3 : 5-*di-iodobenzaldehyde* crystallised from light petroleum (b. p. 40—60°) in needles, m. p. 84° (Found: C, 27·0; H, 2·2; I, 63·0. $C_9H_8O_2I_2$ requires C, 26·9; H, 2·0; I, 63·2%). The *oxime* formed needles, m. p. 155°, from benzene (Found: C, 26·2; H, 2·15; N, 3·4. $C_9H_9O_2NI_2$ requires C, 25·9; H, 2·15; N, 3·4%). The aldehyde (1 g.) was heated under reflux with 1% methanolic hydrochloric acid (7 c.c.) for 1 hour and cooled, and the solution poured into excess of sodium carbonate solution and ice, giving the *dimethyl acetal* (1·03 g.), which crystallised from light petroleum (b. p. 40—60°) in needles, m. p. 47—48° (Found: C, 29·5; H, 3·1. $C_{11}H_{14}O_3I_2$ requires C, 29·5; H, 3·1%).

The microanalyses were by Drs. Weiler and Strauss, Oxford.

Department of Chemical Pathology,
Westminster Medical School (University of London),
London, S.W.1.

[*Received, November 25th*, 1950.]