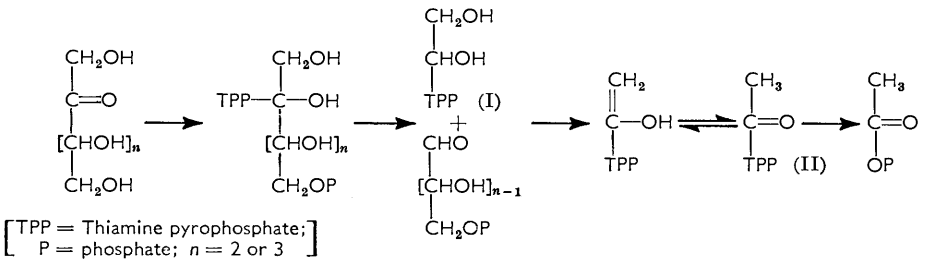
983. *Reactions of* NN'-*Dimethyl*-o-*phenylenediamine with* α-*Aldols and* α-*Ketols*

By A. B. Turner and H. C. S. Wood

Condensation of *NN'*-dimethyl-*o*-phenylenediamine with glycolaldehyde gives the monoacetyl derivative of the diamine.* The direction of dehydration in the primary adduct is thought to be controlled by the neighbouring methylamino-group. *N*-Acetyl-*NN'*-dimethyl-*o*-phenylenediamine is also formed by reaction of the diamine with acetoin. In this case, the primary adduct undergoes aerial oxidative cleavage.

The breakdown of ketose phosphates into acetyl phosphate and the corresponding aldose phosphate is catalysed by phosphoketolase, an enzyme found in several pentose-fermenting organisms. The essential co-factor in the transfer of the acetyl group in this reaction, as in the transfer of acyl groups from other substrates, is thiamine pyrophosphate. (1,2-Dihydroxyethyl)thiamine pyrophosphate (I) and acetylthiamine pyrophosphate (II) have been identified as intermediates in the reaction, and the enzyme also converts glycolaldehyde into acetyl phosphate.[1] Thus, the overall process is formulated as follows:



The direction of dehydration in the intermediate (I) appears to be under enzymic control and this Paper records an analogy for the transformation (I) ⟶ (II).

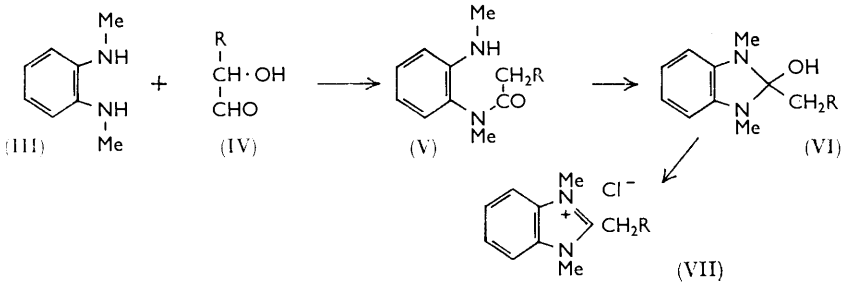
Condensation of glycolaldehyde (IV; R = H) with *NN'*-dimethyl-*o*-phenylenediamine (III), under very mild conditions, gave *N*-acetyl-*NN'*-dimethyl-*o*-phenylenediamine (V; R = H) in 35—40% yield. The identity of the product was clear from its n.m.r. spectrum (see Table), which showed three distinct methyl groups, two as singlets and the third as a doublet. Addition of deuterium oxide to the sample caused both the collapse of this doublet to a singlet and the disappearance of the broad quartet due to the aminomethyl proton. Its structure was confirmed by comparison with an authentic sample prepared by conventional monoacetylation of the diamine (III).[2] When the reaction was carried out at 100°, the major product was a low-melting solid, which was very soluble in water.

* Preliminary Communication, *Proc. Chem. Soc.*, 1964, 61.

[1] W. Schroter and H. Holzer, *Biochim. Biophys. Acta*, 1963, **77**, 474; R. Votaw, W. T. Williamson, L. O. Krampitz, and W. A. Wood, *Biochem. Z.*, 1963, **338**, 756; M. L. Goldberg and E. Racker, *J. Biol. Chem.*, 1962, **237**, PC 3841.

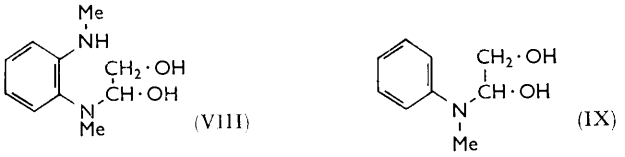
[2] C. W. Smith, R. S. Rasmussen, and S. A. Ballard, *J. Amer. Chem. Soc.*, 1949, **71**, 1082.

The absence of amide carbonyl absorption from its infrared spectrum, together with the symmetry revealed by its n.m.r. spectrum (the protons of the two *N*-methyl groups appearing as a singlet at $\tau = 5{\cdot}98$), indicated that it was the carbinol (VI; R = H).



This was confirmed when the acetyl compound (V; R = H) was found to cyclise to the same product at 100°.

In this reaction, the first step is assumed to be formation of the adduct (VIII). Compounds analogous to this adduct, but lacking the methylamino-group in the *ortho*-position, undergo the Amadori rearrangement, which is catalysed by a second molecule of amine.[3] It was hoped that Amadori rearrangement of the primary adduct (VIII) would be subject to intramolecular catalysis by the neighbouring methylamino-group, in a manner similar to that suggested for the rearrangement of pyrimidine glycosylamines during the biosynthesis of pteridines from purine nucleosides.[4]



In fact, dehydration of the intermediate (VIII) takes place in the direction opposite to that in Amadori rearrangements, and it is the apparently unactivated β-hydroxyl group which is eliminated. This suggests that the methylamino-group aids in the displacement of the primary hydroxyl group. Support for this hypothesis is available from the condensation of *N*-methylaniline with glycolaldehyde under similar conditions. In this case there is no neighbouring group in the primary adduct (IX), and the *N*-acetyl derivative of the amine is not obtained. Instead, more complex products are formed,* in low yield.

The operation of a similar neighbouring-group effect may underlie the distinction between phosphoketolase and the closely related transketolase, which also utilises the intermediate (I) formed from thiamine pyrophosphate and ketoses. In reactions catalysed by the latter enzyme, this intermediate does not undergo dehydration, but instead transfers the two carbon fragment to aldoses.[5]

Similar condensation of the diamine (III) with 2-hydroxypropanal (IV; R = Me) gave the high-melting benzimidazolium chloride (VII; R = Me) together with the carbinol (VI; R = Me). The yield of benzimidazolium salt corresponded to the amount of ammonium chloride present in the mixture (0·25 equivalent). In this case, no open-chain amide form was obtained directly from the reaction mixture, but the cyclised product (VI; R = Me) was converted into the monopropionyl derivative (V; R = Me) of the diamine by heating with alkali. This reaction thus follows a similar course to that of the simpler analogue.

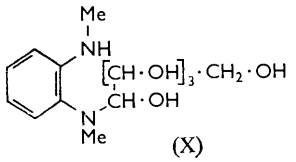
* The structures of these will be reported separately.

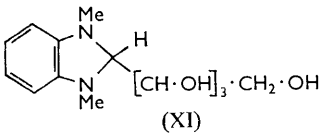
[3] F. Micheel and I. Dijong, *Annalen*, 1962, **658**, 120.

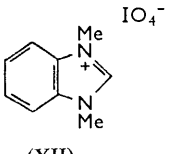
[4] H. C. S. Wood, T. Rowan, and A. Stuart, in " Pteridine Chemistry," eds. W. Pfleiderer and E. C. Taylor, Pergamon Press, Oxford, 1964, p. 129.

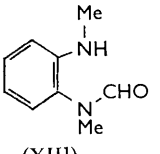
[5] B. Horecker, *J. Cell. Comp. Physiol.*, 1959, **54**, Suppl. 1, 89.

The diamine (III) condensed with arabinose under the same mild conditions, with the elimination of a molecule of water. The product ($C_{13}H_{20}N_2O_4$) was not very soluble in water and its ultraviolet spectrum was not characteristic of pseudo-bases of type (VI), but neither did it show carbonyl absorption in the infrared. Its nuclear magnetic resonance (n.m.r.) spectrum (see Table) suggested the cyclic structure (XI). Periodate oxidation of this product gave the salt (XII), the structure of which was confirmed by its conversion into *N*-formyl-*NN'*-dimethyl-*o*-phenylenediamine (XIII) with alkali. The production of formic acid during the oxidation may effect ring-opening, so that the hydrated form (X) is the species undergoing cleavage by periodate. It thus appears that arabinose gives a different type of product from those formed in the previous condensations, as it is the α-hydroxyl group which is displaced in the primary adduct (X).


(X)


(XI)


(XII)


(XIII)

Breslow [6] has suggested that the enzyme phosphoketolase acts by bringing about the concerted dehydration and cleavage of the co-enzyme adduct (I), resulting in direct formation of acetylthiamine pyrophosphate (II) and aldose phosphate. Although, with the demonstration that the diol (I) is formed and utilised in the phosphoketolase reaction,[1] this concerted mechanism is no longer thought to operate, we have sought to extend the above diamine condensation to ketoses, to see whether concerted dehydration and fragmentation could be achieved.

Attempts to condense the diamine (III) with fructose under the conditions of the reaction with glycolaldehyde were unsuccessful, as were others under more vigorous conditions. The reactions of the diamine (III) with two simpler, and more reactive, α-ketols were then examined.

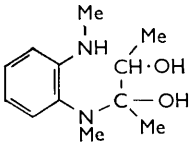
Under the original mild conditions, condensation with acetoin gave a yellow oil, which appeared to be the primary adduct (XIV). This product oxidised slowly in benzene solution exposed to the atmosphere. After a few hours the solution had become deep green, and some days later a sticky, crystalline precipitate had formed. This was insoluble in organic solvents, although very soluble in water, and could not be recrystallised. Its n.m.r. spectrum suggested the carbinol structure (VI; R = H), and this was confirmed by conversion into *N*-acetyl-*NN'*-dimethyl-*o*-phenylenediamine (V; R = H) in good yield, by the action of alkali. Attempts to establish the nature of the second fragment formed in the cleavage of the adduct (XIV) by vapour-phase chromatography were inconclusive.

Finally, the reaction between the diamine (III) and dihydroxyacetone was examined. As in the case of acetoin, condensation was complete after 24 hr., and an oil was obtained. This partly decomposed during distillation, the distillate consisting of a complex mixture of products. The major component was the benzimidazolone (XV), and this compound was also obtained when the crude condensation product was treated with base. A concerted dehydration and cleavage of the type postulated by Breslow did not occur, as no trace of the acetyl derivative (V; R = H) was found.

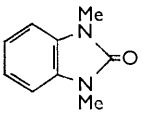
The structures of the *N*-acyl-*NN'*-dialkyl-*o*-phenylenediamines described in this work have been the subject of much debate.[7] They were long thought to exist only in the cyclic carbinol forms, and came to be known as " pseudo-bases." The work of Smith, Rasmussen, and Ballard [2] established the open-chain amide structures, and it now appears that both forms can be isolated. The marked differences in the n.m.r. spectra of the two

[6] R. Breslow, in ref. 5, p. 100.
[7] J. B. Wright, *Chem. Rev.*, 1951, **48**, 397.

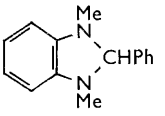
types are clear from the Table. The aromatic protons of the symmetrical benzimidazolium cations resonate at apparently lower field than those of the open-chain forms, and in addition, appear as simple rather than complex bands.



(XIV)



(XV)



(XVI)

N.m.r. spectral data *

Compound	Solvent	Types of protons: Aromatic	N–H	*N*-Me secondary	*N*-Me tertiary	Others
(XIII)	CCl_4	3·17 (4; m)	4·83 (1; u)	7·16 (3; 2, J = 5)	6·92 (3; 1)	2·06 (1; 1)
(V; R = H) ...	$CDCl_3$	3·00 (4; m)	5·71 (1; u)	7·12 (3; 2, J = 5)	6·84 (3; 1)	8·18 (3; 1)
	$CDCl_3$ + D_2O	3·00 (4; m)	—	7·12 (3; 1)	6·84 (3; 1)	8·18 (3; 1)
(V; R = Me) ...	$CDCl_3$	3·00 (4; m)	5·90 (1; u)	7·11 (3; 2, J = 5)	6·81 (3; 1)	7·91 (2; 4, J = 7)
	$CDCl_3$ + D_2O	3·00 (4; m)	—	7·12 (3; 1)	6·83 (3; 1)	8·97 (3; 3. J = 7)
(VI; R = H) ...	D_2O	2·30 (4; u)	—	—	6·01 (6; 1)	7·10 (3; 1)
(VII; R = Me)	D_2O	2·30 (4; u)	—	—	5·94 (6; 1)	6·65 (2; 4, J = 7·5) 8·54 (3; 3, J = 7·5)
(XV)	$CDCl_3$	2·96 (4; u)	—	—	6·59 (6; 1)	—
(XVI) [8]	$CDCl_3$	2·50 (5; m), 3·40 (4; m)	—	—	7·45 (6; 1)	5·11 (1; 1)
(XI)	Deuterated dimethyl sulphoxide	3·46 (4; m)	—	—	7·20 (6; 1)	

Chemical shifts in τ units (followed by intensity and multiplicity); J values in c./sec.; m = multiplet; u = unresolved.

* Spectra were determined on a Perkin-Elmer instrument operating at 40 mc./sec., using tetramethylsilane as the internal reference.

Experimental

Condensations of NN′-*Dimethyl-o-phenylenediamine.*[9]—(a) *With glycolaldehyde.* The diamine (1·00 g.) in ethanol (5 ml.) was added dropwise to the aldehyde (0·44 g., 1·0 equiv.) and ammonium chloride (0·05 g., 0·2 equiv.) in ethanol (3 ml.) and water (2 ml.). The resulting colourless solution was kept at 37° for 3 days. Evaporation to half-volume at room temperature, followed by refrigeration, gave *N*-acetyl-*NN*′-dimethyl-*o*-phenylenediamine as colourless needles) (0·48 g., 37%), m. p. 162·5—164° (from benzene). Admixture with an authentic sample (m. p. 163—164°), prepared by acetylation of the diamine with acetic anhydride,[2] showed no depression, and the infrared spectra of the two products were identical.

When the condensation was carried out on the same scale by heating the mixture for 8 hr. at 100°, no crystalline material was obtained by the above procedure. Extraction with ether yielded a yellow oil (0·6 g.) shown by thin-layer chromatography on silica gel to consist mainly of starting diamine, while evaporation of the aqueous layer gave a gum (0·7 g.), which was very soluble in water or ethanol but insoluble in benzene. Chromatography of this material on silica gel yielded 1,2,3-trimethylbenzimidazolium hydroxide (eluted with 5% ethanol in ethyl acetate) as sticky crystals (0·3 g., m. p. 55—65°).

(b) *With 2-hydroxypropanal.*[10] A solution of the aldehyde (0·37 g.) in ethanol (1·5 ml.) and water (2 ml.) was added dropwise to the diamine (0·68 g., 1·0 equiv.) in ethanol (5 ml.). Ammonium chloride (0·04 g., 0·2 equiv.) was added and the mixture was left at 37° for 3 days. No crystals were deposited after evaporation to half-volume at room temperature, followed by

[8] O. Fischer and M. Rigaud, *Ber.*, 1901, **34**, 4203.
[9] G. W. H. Cheeseman, *J.*, 1955, 3308.
[10] L. Hough and J. K. N. Jones, *J.*, 1952, 4052.

prolonged refrigeration. Thin-layer chromatography on silica gel showed that the diamine had reacted completely to give a very polar product. Evaporation of the solvent yielded an oil, which partly dissolved in ethanol, leaving a white precipitate of 1,3-dimethyl-2-ethylbenzimidazolium chloride. This was collected (0·20 g., m. p. 252—255°) and heated with 4N-sodium hydroxide (2 ml.) for a few minutes. Extraction with ether gave *NN′*-dimethyl-*N*-propionyl-*o*-phenylenediamine [2] as a colourless oil (0·12 g.), which solidified after several hours. It was recrystallised from benzene (m. p. 158—159·5°). A mixture of this material with an authentic sample (m. p. 160—161·5°, see below) had m. p. 159—160°. The residual oil left upon evaporation of the original ethanolic solution gave a further 0·12 g. of this product (m. p. 158—159°) after treatment with alkali.

(c) *With arabinose.* The diamine (5·2 g.) in ethanol (25 ml.) was added to a solution of L(+)-arabinose (5·7 g., 1 equiv.) in water (25 ml.) containing ammonium chloride (0·25 g.), and the mixture was kept at 37° under nitrogen for 3 days. On cooling the mixture to 0°, a heavy, white precipitate (7·6 g.) was formed. This was collected and recrystallised from ethanol to m. p. 127—131° (decomp.) (Found: C, 58·35; H, 7·3; N, 10·4. $C_{13}H_{20}N_2O_4$ requires C, 58·2; H, 7·5; N, 10·45%). The product was not very stable, slowly turning brown on keeping. It was thought to be 2,3-*dihydro*-1,3-*dimethyl*-2-(1,2,3,4-*tetrahydroxy*-n-*butylbenzimidazole* on the basis of spectral data and the following periodate oxidation. The product (1·2 g.), suspended in water (25 ml.), was treated dropwise with sodium periodate (5·35 g.) in water (75 ml.). The resulting solution was left at 4° overnight. After being washed with ether, the aqueous solution was evaporated to dryness, and the white, solid residue was extracted with hot ethanol. On cooling, the extracts deposited colourless needles of NN′-*dimethylbenzimidazolium iodate* (0·55 g.), m. p. 171—172·5° (efferv.) (from ethanol) (Found: C, 32·4; H, 4·0; N, 8·1. $C_9H_{11}N_2IO_4$ requires C, 31·95; H, 3·25; N, 8·3%).

A methanolic solution of the salt (0·4 g.) was percolated through a column of Amberlite CG400, (OH^-). Evaporation of the resulting solution gave *N*-formyl-*NN′*-dimethyl-*o*-phenylenediamine as a pale brown gum (0·17 g.) which ultimately crystallised (m. p. 50—55°). Recrystallised from 2 : 1 benzene–ethanol to m. p. 58—62°, the compound had an infrared spectrum which was identical with that of an authentic specimen (m. p. 68—69°), prepared by the action of alkali on *NN′*-dimethylbenzimidazolium iodide.[11]

(d) *With acetoin.* A solution of the diamine (1·89 g.), acetoin (1·23 g., 1 equiv.), and ammonium chloride (0·05 g.) in ethanol (12 ml.) was kept at 37° for 18 hr. Evaporation of the solvent gave a yellow oil (3·2 g.), which almost completely dissolved in benzene, leaving a colourless precipitate of 1,2,3-trimethylbenzimidazolium chloride (0·16 g., m. p. 215—228°). This yielded *N*-acetyl-*NN′*-dimethyl-*o*-phenylenediamine (0·10 g., m. p. 161—163°) after treatment with alkali. The benzene solution, containing the main product, was initially yellow, but slowly turned green. It was left exposed to the atmosphere for 10 days, during which time sticky, green crystals were deposited. The supernatant solution was decanted, and the precipitate (1·7 g., m. p. 50—63°) was washed with benzene. It was taken up in 2N-sodium hydroxide (30 ml.), heated on a steam-bath for 30 min., and evaporated to half-volume. Extraction with ether (3 × 30 ml.), followed by evaporation of the dried extracts, gave a brown oil, which soon crystallised. The crystals consisted mainly of *N*-acetyl-*NN′*-dimethyl-*o*-phenylenediamine (0·60 g., m. p., 162—164°), together with colourless needles (0·12 g., m. p. 183—184·5°) which were much less soluble in ether. This second product was recrystallised from ethanol (m. p. 185—186°), but its structure was not elucidated.

(e) *With dihydroxyacetone.* A solution of the diamine (1·94 g.), dihydroxyacetone (1·28 g.) and ammonium chloride (0·14 g.) in ethanol (12 ml.) and water (3 ml.) was kept at 37° for 48 hr. Removal of the solvent gave a reddish-brown oil, which contained a polar product and no starting diamine. (Attempts to distil this material under reduced pressure caused decomposition.) It was redissolved in aqueous ethanol and left exposed to the atmosphere for 6 days. The ethanol was distilled off, and the aqueous residue diluted with an equal volume of 4N-sodium hydroxide. Extraction with ether yielded a colourless oil (0·45 g.) which was crystallised from light petroleum (m. p. 57—59°, raised to 103—106° upon drying *in vacuo*). Chromatography on silica gel gave 2,3-dihydro-1,3-dimethylbenzimidazol-2-one, eluted with benzene, as colourless needles (0·27 g., m. p. 105—107°). A mixture of this material and an authentic sample [12] (m. p. 103—105°) had m. p. 103—105°.

[11] O. Fischer and M. Rigaud, *Ber.*, 1902, **35**, 1258.
[12] J. Pinnow and C. Samann, *Ber.*, 1899, **32**, 2189.

NN'-*Dimethyl*-N-*propionyl*-o-*phenylenediamine*.—Propionic anhydride (1·30 g.) was added dropwise to a stirred solution of *NN'*-dimethyl-*o*-phenylenediamine (1·36 g., 1 equiv.) in dry pyridine (10 ml.). The reaction was exothermic, and, on cooling, colourless needles were formed. These were collected and combined with a second crop obtained from the concentrated mother-liquor. Three recrystallisations from benzene gave the monopropionyl derivative of the diamine as colourless needles, m. p. 160—161·5° (lit.,[4] 161—162·2°) (Found: C, 69·0; H, 8·5; N, 14·9. Calc. for $C_{11}H_{16}N_2O_2$: C, 68·75; H, 8·3; N, 14·6%).

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