686. *Imidazolic Compounds from the Reaction of Pyruvaldehyde with Ammonia*

By M. R. Grimmett and E. L. Richards

Pyruvaldehyde reacts with ammonia in solution to form equimolecular quantities of 4(5)-methylimidazole and 2,4(5)-dimethylimidazole. The previously undescribed 2-acetyl-4(5)-methylimidazole is also formed.

A number of imidazoles have been isolated from the interaction of carbohydrates and ammonia.[1-3] The formation of 4(5)-methylimidazole (II) from a hexose and ammonia is believed [2] to involve an initial alkaline degradation of the hexose to a pentose plus formaldehyde and to the trioses, glyceraldehyde and dihydroxyacetone. Dehydration of either triose to pyruvaldehyde (I), followed by its condensation with a molecule of formaldehyde and two molecules of ammonia produces 4(5)-methylimidazole. Bernhauer [1] was unable to detect 4(5)-methylimidazole in a mixture of pyruvaldehyde, ammonia, and zinc hydroxide and hence deduced that pyruvaldehyde could not act as a source of formaldehyde. As it was considered that pyruvaldehyde should undergo alkaline fission of the bond linking the carbonyl functions (cf. fission of 2,3-butanedione in ammoniacal solution leading to the formation of 2,4,5-trimethylimidazole [4]) it was decided that the reaction merited further investigation.

It was immediately apparent from chromatography of the pyruvaldehyde–ammonia system that at least four imidazolic compounds were present. The two main components, 4(5)-methylimidazole and 2,4(5)-dimethylimidazole were present in approximately equimolecular proportions. Such a result provides strong evidence for the fission of pyruvaldehyde to produce equal amounts of formaldehyde and acetaldehyde. Whether this reaction involves a mechanism similar to that described by Davidson *et al.*[5] for the interaction of benzil and ammonia, or whether under alkaline conditions pyruvaldehyde reacts with water by two routes in about equal amounts to form acetaldehyde and formic acid or acetic acid and formaldehyde is uncertain. Both pathways may operate simultaneously. A similar doubt exists for the mechanism of the transformation of 2,3-butanedione in ammoniacal medium.[6]

Whereas in hexose–ammonia systems most of the formaldehyde required for 4(5)-methylimidazole formation is undoubtedly formed by direct degradation of the hexose molecule,[7] our work indicates that a portion may result from fission of pyruvaldehyde.

[1] K. Bernhauer, *Z. physiol. Chem.*, 1929, **183**, 67.

[2] J. Parrod, *Ann. Chim. (France)*, 1933, **19**, 205.

[3] Masahiko Komoto, *J. Agric. Chem. Soc. Japan*, 1962, **36**, 546.

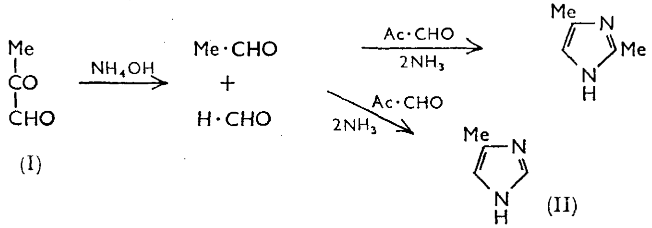
[4] H. von Pechmann, *Ber.*, 1888, **21**, 1411.

[5] D. Davidson, M. Weiss, and M. Jelling, *J. Org. Chem.*, 1937, **2**, 319.

[6] K. Hofmann, " The Chemistry of Heterocyclic Compounds, Imidazole and its Derivatives, Part I," Interscience Publishers Inc., New York, 1953, p. 36.

[7] R. L. Whistler and J. N. BeMiller, *Adv. Carbohydrate Chem.*, 1958, **13**, 329.

The pyruvaldehyde–ammonia mixture did not produce hexose or pentose sugars in detectable quantities and thus it may be assumed that all the formaldehyde is in this case derived directly from the pyruvaldehyde. If fission of pyruvaldehyde does occur to any extent in hexose–ammonia mixtures, 2,4(5)-dimethylimidazole would be expected among the reaction products. In general this compound has not been detected in such mixtures although it has been isolated from the interaction of sucrose and ammonia at 200°.[8] Its presence in this case may be due to thermal effects on the sidechains of more complex compounds. Windaus and Ullrich [9] have also reported the isolation of 2,4(5)-dimethylimidazole from rhamnose and ammonia, but this is a system in which acetaldehyde would be expected as an initial degradation product.



A small quantity of a further compound, $C_6H_8N_2O$, which gave a positive Pauly [10] test (formation of an azo-dye with alkaline-diazotised sulphanilic acid) for the imidazole nucleus was isolated. Infrared analysis and a positive iodoform reaction indicated an aromatic methyl ketone. The 60 Mc./sec. proton magnetic resonance spectrum was run in deuterochloroform solution. A comparatively broad singlet at 790 c./sec. can be assigned to an imidazole NH proton. It is very similar to that shown by imidazole itself [11] (803 c./sec.) and was removed by addition of a drop of deuterium oxide. Comparison with the n.m.r. spectrum of 4(5)-methylimidazole [4(5)-H, 410 c./sec.; 5(4)-CH_3, 133 c./sec.] showed that the singlet at 424 c./sec. must be due to the 4(5)-proton and the three-proton singlet at 142 c./sec. must be due to the 4(5)-methyl. The remaining three proton singlet at 160 c./sec. is assigned to an acetyl group at $C_{(2)}$ (cf. acetophenone, 157 c./sec.[12]). There was no band similar to that of the 4(5)-methylimidazole 2-H at 477 c./sec. and thus the compound was assigned a structure corresponding to 2-acetyl-4(5)-methylimidazole (IV). A Wolff–Kischner reduction produced 2-ethyl-4(5)-methylimidazole.

The Table demonstrates that yields of 4(5)-methylimidazole formed from pyruvaldehyde and ammonia under a series of reaction conditions show only minor differences. This is in contrast to the behaviour of hexoses in ammoniacal solution where æration decreases the yield of 4(5)-methylimidazole and increases formation of 4(5)-hydroxymethylimidazole.[2] The yield of 4(5)-methylimidazole is also reported to be increased when the reaction is carried out in glacial acetic acid with ammonium acetate as the source of ammonia.[5]

Yields (g.) of 4(5)-methylimidazole per g. of pyruvaldehyde under various reaction conditions

Pyruvaldehyde–ammonia in stoppered flask	0·124
Pyruvaldehyde–ammonia ærated continuously	0·121
Pyruvaldehyde–ammonia nitrogenated continuously	0·110
Pyruvaldehyde–ammonium acetate–glacial acetic acid	0·124

A further fraction from the reaction mixture contained traces of an imidazolic substance which was not identified.

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[8] I. Jezo, *Chem. Zvesti*, 1963, **17**(2), 126.

[9] A. Windaus and A. Ullrich, *Z. physiol. Chem.*, 1914, **92**, 276.

[10] H. Pauly, *Z. physiol. Chem.*, 1904, **42**, 508.

[11] Varian Associates, " High Resolution N.M.R. Spectra Catalogue," Palo Alto, California, 1962, No. 20.

[12] R. M. Silverstein and G. C. Bassler, " Spectrometric Identification of Organic Compounds," Wiley and Sons Inc., New York, 1963, p. 84.

EXPERIMENTAL

Analyses were carried out by Dr. A. D. Campbell and infrared spectra determined as paraffin mulls on a Perkin-Elmer model 421 infrared spectrometer by Mr. S. F. Downes at the University of Otago. Proton magnetic resonance spectra were obtained by Dr. R. M. Golding of Dominion Chemical Laboratories, Lower Hutt, on a Varian DP 60 spectrometer in deuterochloroform solution. Chemical shifts are given in c./sec. from tetramethylsilane as internal reference. All m. p.s were determined on a Gallenkamp electrical apparatus. Unless stated, chromatographic data refer to ascending development on aluminium oxide G chromatoplates (unactivated) using ethyl acetate–toluene–28% ammonia solution (3 : 1 : 0·1) as irrigant. R_{Im} refers to the ratio (distance travelled by compound) : (distance travelled by imidazole). All evaporations were carried out at low temperature under vacuum.

Ammonia solution (AnalaR, 25%; 140 ml.) was added carefully with cooling to a 25% aqueous solution of pyruvaldehyde (95 ml.) prepared by purification [13] of a similar solution obtained from L. Light and Co. The mixture was stored at 19° in a stoppered flask for five weeks. The solution rapidly darkened as melanoidin formation proceeded and within 24 hr. chromatography showed the presence of four imidazolic compounds. At the end of the reaction period the ammonia and water were evaporated and the resultant dark brown product (30·6 g.) added to a column containing Amberlite IRC-50 resin (H-form; 400 g.) and washed with distilled water (5 l.). On concentration the eluate and washings gave a polymeric fraction (5·3 g.) which was found to contain neither imidazolic material nor aldose and ketose components. The basic material was eluted from the resin with 4N-ammonium hydroxide and concentrated to yield a product (20·2 g.) again containing a large proportion of brown material. Thin-layer chromatography showed four compounds giving dyes with Pauly reagent and appearing at R_{Im} 2·55 (orange-yellow), 2·00 (red), 1·45 (lemon-yellow), and 1·09 (red). A quantity (1 g.) of the brown material was separated on a cellulose column using butan-1-ol half-saturated with water and containing 0·25% glacial acetic acid as mobile phase. Three main fractions were collected and evaporated to dryness:

Fraction 1; 2-*Acetyl*-4(5)-*methylimidazole*.—This fraction (0·04 g.) contained a compound (R_{Im} 2·55) identified as 2-*acetyl*-4(5)-*methylimidazole*. The syrup was purified by chloroform extraction and then by passage in the same solvent through a column containing alumina. Evaporation of the chloroform gave white crystals (0·03 g.), m. p. 109° (Found: C, 57·9; H, 6·7; N, 22·75. $C_6H_8N_2O$ requires C, 58·05; H, 6·5; N, 22·6%); $\nu_{\text{max.}}$ 3160 (aromatic CH), 1678 (aryl C:O), 1397, 1370 (methyl ketone CH), and 1170 cm.$^{-1}$ (aryl ketone); *picrate*, m. p. 157° (Found: N, 20·2. $C_{12}H_{11}N_5O_8$ requires N, 19·85%); 2,4-dinitrophenylhydrazone, m. p. 275° (decomp.); positive iodoform test. Proton magnetic resonance spectra were determined in deuterochloroform and deuterium oxide. For comparison similar spectra were determined for 4(5)-methyimidazole and 2-methylimidazole. A Wolff–Kischner reduction of 2-acetyl-4(5)-methylimidazole gave a compound chromatographically identical to an authentic sample of 2-ethyl-4(5)-methylimidazole obtained by purification of a product from L. Light and Co. using adsorption chromatography in chloroform–pyridine (3 : 1) on an alumina column. Pure 2-ethyl-4(5)-methylimidazole gave a lemon-yellow colour with Pauly reagent spray at R_{Im} 1·91 on a thin-layer chromatoplate; picrate, m. p. 131° (lit.,[14] 131°) (Found: C, 42·3; H, 4·3; N, 20·2. Calc. for $C_{12}H_{13}N_5O_7$: C, 42·5; H, 3·9; N, 20·6%).

Fraction 2.—This fraction (0·07 g.) contained two imidazolic compounds, one of which chromatographically resembled 2-acetyl-4(5)-methylimidazole. The fraction was not further investigated.

Fraction 3; 4(5)-*Methylimidazole and* 2,4(5)-*Dimethylimidazole*.—The final fraction (0·56 g.) contained the imidazoles with R_{Im} values 1·45 and 1·09 as well as a quantity of polymeric material. The imidazoles were distilled at 120—200°2 mm. and separated on alumina using chloroform–pyridine (4 : 1) as irrigant.

2,4(5)-*Dimethylimidazole*. The faster-moving compound (0·091 g.) was chromatographically identical to 2,4(5)-dimethylimidazole giving, with alkaline-diazotised sulphanilic acid spray, the yellow colour often characteristic of 2-substituted imidazoles.[15] It crystallised from chloroform as white needles m. p. 88° (lit.,[1] 92°), and readily formed a picrate, m. p. and mixed m. p. 142° (Found: N, 21·3. Calc. for $C_{11}H_{11}N_5O_7$: N, 21·5%). The infrared spectrum was identical

[13] H. Lento, J. Underwood, and C. Willits, *Food Res.*, 1960, **757.**

[14] A. Windaus and W. Langenbeck, *Ber.*, 1922, **55***B*, 3706.

[15] C. F. Huebner, *J. Amer. Chem. Soc.*, 1951, **73**, 4667.

with that of a sample of 2,4(5)-dimethylimidazole picrate prepared by the method of Windaus and Langenbeck.[14]

4(5)-*Methylimidazole*. The remaining compound (0·077 g.) gave a red colour with Pauly reagent at R_{Im} 1·09 and appeared to be identical to 4(5)-methylimidazole. The syrup obtained from the alumina column failed to crystallise but formed a picrate, m. p. 159°, undepressed on admixture with 4(5)-methylimidazole picrate [16] (Found: C, 38·9; H, 3·0; N, 22·5. Calc. for $C_{10}H_9N_5O_7$: C, 38·6; H, 2·9; N, 22·5%). An infrared spectrum was identical to a similar spectrum determined for 4(5)-methylimidazole picrate.

Comparison of Yields of 4(5)-*Methylimidazole under Different Reaction Conditions*.—The following reaction mixtures were stored at room temperature: (*a*) pyruvaldehyde (10 ml. of 25% solution) and 25% ammonia solution (15 ml.) in a stoppered flask; (*b*) as (*a*) but with air continuously bubbled through the solution; (*c*) as (*a*) but with nitrogen continuously bubbled through the solution; (*d*) pyruvaldehyde (10 ml. of 25% solution), ammonium acetate (9·25 g.), and glacial acetic acid to make 25 ml. After a week 0·05 ml. aliquot portions of the solution were taken, the 4(5)-methylimidazole separated by chromatograph on Whatman No. 3MM paper using butan-1-ol–glacial acetic acid–water (4 : 1 : 1), and determined spectrophotometrically using the method of Grimmett and Richards.[17] It was not possible to estimate 2,4(5)-dimethylimidazole by this method because an azo-dye of low colour intensity was formed and even slight contamination with 4(5)-methylimidazole gave erroneous values. Results are in the Table. All mixtures (*a*) to (*d*) contained the same component imidazolic compounds.

Quantitative Gravimetric Separation of 4(5)-*Methylimidazole and* 2,4(5)-*Dimethylimidazole*.—Aliquot portions (1·196 g.; 1·761 g.) of the bases from the pyruvaldehyde–ammonia mixture were adsorbed on a column packed with aluminium oxide (AnalaR; 150 g.). Irrigation with chloroform removed the fast-moving imidazoles (0·078 g.; 0·112 g.). Further passage of chloroform–pyridine (3 : 1) and collection of 10 ml. fractions allowed separation of 2,4(5)-dimethylimidazole and 4(5)-methylimidazole. Finally, irrigation with pyridine removed the remaining 4(5)-methylimidazole from the adsorbent. The appropriate fractions were combined, evaporated, dried *in vacuo*, and weighed giving 2,4(5)-dimethylimidazole (0·3335 g.; 0·555 g.) and 4(5)-methylimidazole (0·312 g.; 0·458 g.). These results gave molar ratios for 2,4(5)-dimethylimidazole : 4(5)-methylimidazole of 1 : 1·09 and 1 : 0·97.

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[16] K. K. Koessler and M. T. Hanke, *J. Biol. Chem.*, 1919, 503.

[17] M. R. Grimmett and E. L. Richards, *Austral. J. Chem.*, 1964, **17**, 1379.