The Position of the Dinitrophenyl Group in *im*-Dinitrophenylhistidine Derivatives

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Reinvestigation of the ring-opening reaction of *N*-(2,4-dinitrophenyl)imidazole with benzyl chloroformate in ethyl acetate–aqueous sodium hydrogen carbonate has shown the product to be benzyl *N*-[*cis*-2-(2,4-dinitroanilino)vinyl]-*N*-formylcarbamate (2), which undergoes deformylation on further treatment with alkali. Spectroscopic examination of corresponding products obtained from the 2,4-dinitrophenyl derivatives of 4(5)-methylimidazole and N^{α}-benzyloxycarbonyl-L-histidine has proved that 2,4-dinitrophenylation of these compounds occurs exclusively at the less hindered heterocyclic nitrogen atom, giving 1-(2,4-dinitrophenyl)-4-methylimidazole (1) and N^{α}-benzyloxycarbonyl-N^{τ}-(2,4-dinitrophenyl)-L-histidine (8), respectively.

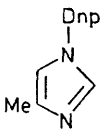
In the course of work involving the use of *im*-dinitrophenylhistidine in peptide synthesis [1,2] it became of interest to know the position † of the dinitrophenyl substituent. There is considerable confusion in the literature on this point, the dinitrophenyl group having been shown as attached to N^{τ} on some occasions [4,5] but, more recently, to N^{π} on others.[1,2] The only relevant investigation is that by Henkart,[6] who subjected N^{α}-acetylhistidine to dinitrophenylation followed by carboxymethylation, removed the dinitrophenyl group by thiolysis, and hydrolysed the product; amino-acid analysis then showed only one major peak 'at a position corresponding to 1'-carboxymethylhistidine.' It was therefore deduced that dinitrophenylation had occurred 'predominantly at the 3'-nitrogen.' Un-

† The system used here for distinguishing between the heterocyclic nitrogen atoms in histidine is that recommended in ref. 3: N^{π} refers to the nitrogen atom nearer the CH_2 group, and N^{τ} to the one further away.

[1] S. Shaltiel and M. Fridkin, *Biochemistry*, 1970, **9**, 5122.
[2] M. Fridkin and S. Shaltiel, *Arch. Biochem. Biophys.*, 1971, **147**, 767.
[3] I.U.P.A.C. Information Bulletin, Appendix No. 23 on Tentative Nomenclature, Symbols and Standards, June 1972, p. 3, footnote 5.
[4] E. Siepmann and H. Zahn, *Biochim. Biophys. Acta*, 1964, **82**, 412.
[5] H. Zahn and H. Pfannmüller, *Biochem. Z.*, 1958, **330**, 97.

fortunately this statement is ambiguous and only compounds the confusion, as Henkart does not indicate which of the two numbering systems [3] for positions in the heterocyclic ring of histidine he is using. Because of this, and because Henkart's result is in any case not as secure as is desirable, since it is wholly dependent on a single chromatographic identification, we decided to re-examine the question by using a different approach.

The differentiation of 1,4- and 1,5-disubstituted imidazoles is a long-standing problem which has been tackled in many ways.[7] In reactions of 4(5)-alkyl- and -aryl-imidazoles as nucleophiles, the major product is generally formed by attack of the less hindered heteroatom, although both possible products can result.[7-10] This suggests that in simple low molecular weight histidine derivatives, dinitrophenylation of N^{τ} should predominate. In fact it appears that such reactions have invariably led to dinitrophenylation of a single position: [1,4-6,11] the conclusion that this position is N^{τ} is consistent with the structure (1) which has been assigned [12] to the single product obtained on dinitrophenylation of 4(5)-methylimidazole, but this assignment [12] is also incompletely supported, being based entirely on measurement of the chemical shift of the methyl protons and comparison with the slightly different chemical shifts of the methyl protons in a series of related compounds. Matthews and



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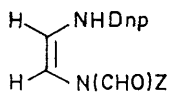

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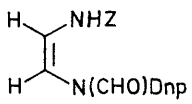

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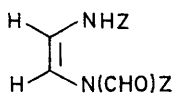

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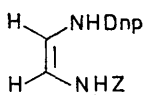

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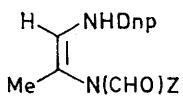

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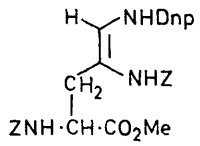

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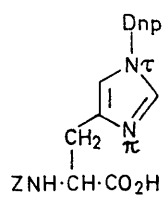

(8)

Dnp = 2,4-$(NO_2)_2C_6H_3$; Z = $PhCH_2$·O·CO

Rapoport [7] have examined the cross-ring proton coupling constants for a number of 1,4- and 1,5-disubstituted imidazoles of established structure and have concluded that this coupling constant is a reliable criterion for distinguishing between 1,4- and 1,5-structures: values were always in the range 0·9—1·0 Hz for the 1,5-disubstituted compounds but between 1·0 and 1·5 Hz for the 1,4-series. We find the coupling constant for the dinitrophenyl derivative of 4(5)-methylimidazole to be 1·3 Hz, which provides further support for structure (1) if the criterion of Matthews and Rapoport [7] is applicable to this type of disubstituted imidazole; but it is uncertain that such an extrapolation is permissible since the examples upon which they base their generalisation do not comprise any *N*-aryl derivatives.

The report [13] that *N*-dinitrophenylimidazole undergoes a Bamberger-type ring-opening reaction when treated with benzyl chloroformate and alkali in a two-phase system prompted us to attempt to use this reaction for distinguishing between the N^{τ}- and N^{π}-dinitrophenylhistidine structures, because it seemed likely that the product from ring-opening would be amenable to unambiguous structure determination by spectroscopic examination. As a prelude to this attempt the reaction of *N*-dinitrophenylimidazole itself with benzyl chloroformate and alkali in a two-phase system was re-investigated. The product of this essentially quantitative reaction has the structure (2) rather than the isomeric structure (3) previously drawn [13] for it. This is clear from a comparison of its spectroscopic properties with those of compound (4): [13] the product from *N*-dinitrophenylimidazole and compound (4) have similar bands in the carbonyl regions of their i.r. spectra and the chemical shifts of their formyl protons are identical, suggesting that the $PhCH_2$·O·CO·N(CHO)·CH= system of (4) is also present in the product. In contrast the N*H* signal of (4) is observed as a broad band centred at τ 3·4 whereas that of the product is found well downfield at τ 0·12; this is clearly inconsistent with the formulation (3). Further treatment of the product, which can now be assigned the revised structure (2), with alkali gave compound (5), which shows a new N*H* signal with the same chemical shift as that in compound (4). Treatment of the dinitrophenyl derivative of 4(5)-methylimidazole with benzyl chloroformate in aqueous sodium hydrogen carbonate–ethyl acetate gave a product which could be identified as (6) by spectroscopic comparison with (2): the methyl group must be located as shown since the $(NO_2)_2$-C_6H_3·N*H* signal at τ 0·22 exhibits coupling, as shown by deuterium-exchange and double-resonance experiments, to the vinylic proton. It follows that the structure of the dinitrophenyl derivative of 4(5)-methyl imidazole is indeed (1), as earlier [12] proposed.

[6] P. Henkart, *J. Biol. Chem.*, 1971, **246**, 2711.
[7] H. R. Matthews and H. Rapoport, *J. Amer. Chem. Soc.*, 1973, **95**, 2297.
[8] J. B. Jones and D. W. Hysert, *Canad. J. Chem.*, 1971, **49**, 3012.
[9] R. M. Acheson, M. W. Foxton, P. J. Abbot, and K. R. Mills, *J. Chem. Soc.* (*C*), 1967, 882.
[10] R. A. Olofson and R. V. Kendall, *J. Org. Chem.*, 1970, **35**, 2246.
[11] F. Chillemi and R. B. Merrifield, *Biochemistry*, 1969, **8**, 4345.
[12] J.-L. Imbach and R. Jacquier, *Compt. rend.*, 1963, **257**, 2683.
[13] E. Babad and D. Ben-Ishai, *J. Heterocyclic Chem.*, 1969, **6**, 235.

For the preparation of N^{α}-benzyloxycarbonyl-N^{im}-dinitrophenylhistidine, the procedure of Shaltiel and Fridkin [1] (who draw their product as the N^{π} derivative) was carefully followed, but the material which separated on acidification as detailed by them proved to be not the ethyl acetate-soluble unprotonated compound they reported but the poorly soluble corresponding hydrochloride previously described [4] by Siepmann and Zahn. Treatment of this material with benzyl chloroformate in chloroform–aqueous sodium hydrogen carbonate gave a complex mixture from which we were unable to isolate any pure compounds, but we succeeded in isolating the major product of the exposure of the corresponding methyl ester to the same conditions. The n.m.r. spectrum proved the structure of this product to be (7): the $(NO_2)_2C_6H_3{\cdot}NH$ signal at τ −0·17 showed coupling to the vinylic proton (this was confirmed by double-resonance and deuterium-exchange investigation) whereas the $PhCH_2{\cdot}CO{\cdot}NH$ signal at τ 3·74 was a broad singlet. This is consistent only with the location of the dinitrophenyl group of N^{α}-benzyloxycarbonyl-N^{im}-dinitrophenylhistidine at N^{τ} as shown in (8), since the N^{π} isomer would give a ring-opening product with the dinitroanilino-group attached to a fully substituted carbon atom. We therefore conclude that the dinitrophenylation of N^{α}-benzyloxycarbonylhistidine gives the product (8) of attack by the less hindered heterocyclic nitrogen atom, *i.e.* N^{τ}. The same position is presumably substituted in the dinitrophenylation of other simple low molecular weight histidine derivatives.

EXPERIMENTAL

M.p.s were determined on a Kofler hot-stage apparatus. I.r. spectra were recorded on a Perkin-Elmer 257 spectrometer. U.v. spectra were recorded with dioxan as solvent on a Cary 14M spectrometer. N.m.r. spectra were recorded by Mrs. E. Richards and her staff on a Perkin-Elmer R32 spectrometer operating at 90 MHz with deuteriochloroform as solvent and tetramethylsilane as internal standard. Deuterium exchange is described as 'slow' if it was incomplete after 1 h at room temperature and 'very slow' if it was incomplete overnight. The optical rotation was recorded on a Perkin-Elmer 141 automatic polarimeter using a 1 dm cell. Light petroleum was of boiling range 40—60°.

N-(2,4-*Dinitrophenyl*)*imidazole*.—Prepared by the method of Wiltshire,[14] this compound had m.p. 142—145° (from methanol) (lit.,[14] 146—148°; lit.,[5] 144°).

Benzyl N-[cis-2-(2,4-*Dinitroanilino*)*vinyl*]-N-*formylcarbamate* (2).—*N*-(2,4-Dinitrophenyl)imidazole was treated with benzyl chloroformate in ethyl acetate–aqueous sodium hydrogen carbonate as described by Babad and Ben-Ishai.[13] The *product* had m.p. 160—162° (lit.,[13] 155—156°); i.r. spectrum as in the literature; [13] $\lambda_{max.}$ 233 and 358 nm (ε 19,200 and 19,200); τ 0·12br (1H, d, *J* 10 Hz, exchanges very slowly with D_2O, s on decoupling from band at 3·45, NH), 0·69 (1H, s, CHO), 0·92 (1H, d, *J* 3 Hz, H-3 of Dnp), 1·69 (1H, dd *J* 9 and 3 Hz, H-5 of Dnp), 2·63 (5H, s, Ph), 2·85 (1H, d, *J* 9 Hz, H-6 of Dnp), 3·45 (1H, dd, *J* 10 and 7 Hz, DnpNH·C*H*=), 4·15 (1H, d, *J* 7 Hz, NH·CH=C*H*), and 4·63 (2H, s, $PhCH_2$).

Benzyl N-[cis-2-(2,4-*Dinitroanilino*)*vinyl*]*carbamate* (5).—The *N*-formylcarbamate (2) (0·50 g) was dissolved in chloroform (50 ml) and N-sodium hydroxide (25 ml) was added. After stirring at room temperature overnight, the organic layer was separated, washed with water, and dried. Removal of solvent and recrystallisation of the residue from ethyl acetate gave the *carbamate* (5) as maroon crystals (0·30 g, 64%), m.p. 159—161°; $\nu_{max.}$ (Nujol) 1700br cm^{-1}; $\lambda_{max.}$ 240 and 375nm (ε 23,000 and 17,000); τ 0·55br (1H, d, *J* 8 Hz, exchanges slowly with D_2O, DnpN*H*), 0·90 (1H, d, *J* 3 Hz, H-3 of Dnp), 1·75 (1H, dd, *J* 9 and 3 Hz, H-5 of Dnp), 2·67 (5H, s, Ph), 2·94 (1H, d, *J* 9 Hz, H-6 of Dnp), 3·3—3·7 (2H, complex, CO·N*H*·CH=C*H*·NHDnp; 1H, d, *J* 7 Hz at 3·52 after D_2O exchange), 4·33 [1H, m (d, *J* 7 Hz after D_2O exchange), CO·NH·C*H*=CH·NHDnp], and 4·82 (2H, s, $PhCH_2$) (Found: C, 53·5; H, 3·8; N, 15·7. $C_{16}H_{14}N_4O_6$ requires C, 53·6; H, 3·9, N, 15·6%).

Benzyl N-(cis-2-*Benzyloxycarbonylaminovinyl*)-N-*formylcarbamate* (4).—This compound, prepared by the method of Babad and Ben-Ishai,[13] crystallised when set aside at room temperature with petroleum for several weeks; m.p. 98—100° (lit.,[13] 103°); i.r. spectrum as in the literature; [13] τ 0·82 (1H, s, CHO), 2·67 and 2·71 (10H, both s, both Ph), 3·3—3·6 (2H, complex, N*H*·C*H*=CH; 1H, d, *J* 8 Hz at 3·43 after D_2O exchange which was slow), 4·56 (1H, d, *J* 8 Hz, NH·CH=C*H*), and 4·74 and 4·91 (both 2H, both s, both $PhCH_2$).

1-(2,4-*Dinitrophenyl*)-4-*methylimidazole*.—Prepared by the general method of Wiltshire,[14] this compound had m.p. 130—133° (from ethanol) (lit.,[5] 133°; lit.,[12] 133—134°); τ 1·22 (1H, d, *J* 3 Hz, H-3 of Dnp), 1·46 (1H, dd, *J* 9 and 3 Hz, H-5 of Dnp), 2·33 (1H, d, *J* 9 Hz, H-6 of Dnp), 2·43 (1H, d, *J* 1·3 Hz, H-2 of imidazole), 3·20 (1H, m, collapsing to q, *J* 0·9 Hz on decoupling from band at 2·43, H-5 of imidazole), and 7·73 (3H, d, *J* 0·9 Hz, CH_3).

Reaction of 1-(2,4-*Dinitrophenyl*)-4-*methylimidazole with Benzyl Chloroformate and Sodium Hydrogen Carbonate*.—1-(2,4-Dinitrophenyl)-4-methylimidazole (0·94 g) was treated with benzyl chloroformate in ethyl acetate–aqueous sodium hydrogen carbonate as described by Babad and Ben-Ishai [13] for *N*-(2,4-dinitrophenyl)imidazole. Removal of the ethyl acetate and trituration with ether gave a powder, which crystallised from ethyl acetate–ether to give *benzyl* N-[cis-2-(2,4-*dinitroanilino*)-1-*methylvinyl*]-N-*formylcarbamate* (6) as scarlet crystals which retained ethyl acetate of solvation even after extensive drying; m.p. 92—94°; $\nu_{max.}$ (Nujol) 1690, 1710, 1740sh, and 1750 cm^{-1}; $\lambda_{max.}$ 233 and 364 nm (ε 17,100 and 18,900); τ 0·22br (1H, d, *J* 10 Hz, exchanges very slowly with D_2O, s on decoupling from band at 3·37, NH), 0·72 (1H, s, CHO), 0·95 (1H, d, *J* 3 Hz, H-3 of Dnp), 1·73 (1H, dd, *J* 9 and 3 Hz, H-5 of Dnp), 2·70 (5H, s, Ph), 2·89 (1H, d, *J* 9 Hz, H-6 of Dnp), 3·37 [1H, dd, *J* 10 and 1 Hz (d, *J* 1 Hz after D_2O exchange, d, *J* 10 Hz on decoupling from the band at 8·00), CH=], 4·69 (2H, s, $PhCH_2$), and 8·00 (3H, d, *J* 1 Hz, CH_3); the n.m.r. spectrum also contained bands corresponding to ethyl acetate (0·5 mol. equiv.) (Found: C, 54·1; H, 4·5; N, 12·5. $C_{18}H_{16}N_4O_7$,0·5EtOAc requires C, 54·0; H, 4·5; N, 12·6%).

N^{α}-*Benzyloxycarbonyl*-N^{im}-(2,4-*dinitrophenyl*)-L-*histidine Hydrochloride*.—This compound was prepared according to the procedure described by Shaltiel and Fridkin [1] for

[14] J. F. K. Wiltshire, *Austral. J. Chem.*, 1966, **19**, 1935.

the corresponding base, but when the reaction mixture was acidified with hydrochloric acid and extracted with ethyl acetate, a lemon-yellow solid separated from the aqueous phase. Filtration and washing with hydrochloric acid and ethyl acetate gave the hydrochloride previously reported by Siepmann and Zahn [4] in 60—70% yield; m.p. 203—205° (lit.,[4] 193—195°), $[\alpha]_D^{20}$ −7·8° (*c* 1 in MeOH) (Found: C, 48·6; H, 3·7; Cl, 7·5; N, 14·1. Calc. for $C_{20}H_{18}ClN_5O_8$: C, 48·8; H, 3·7; Cl, 7·2; N, 14·2%).

Reaction of N$^{\alpha}$-*Benzyloxycarbonyl*-N^{im}-(2,4-*dinitrophenyl*)-L-*histidine Methyl Ester with Benzyl Chloroformate and Sodium Hydrogen Carbonate.*—A solution of *N*$^{\alpha}$-benzyloxycarbonyl-*N*^{im}-(2,4-dinitrophenyl)-L-histidine hydrochloride in methanol was treated with sufficient ethereal diazomethane for the intense yellow colour to persist. The intense yellow colour was discharged with glacial acetic acid and the solvents were removed. Trituration with petroleum gave a red powder which was shown by n.m.r. to be a mixture of which the required methyl ester was the major component. This mixture (445 mg) was dissolved in chloroform (4 ml) and a solution of sodium hydrogen carbonate (370 mg) in water (4 ml) was added. After cooling to 0°, benzyl chloroformate (226 mg) dissolved in chloroform (3 ml) was added dropwise with vigorous stirring. After 1 h at 0° and 2 h at room temperature, the organic phase was separated, washed with water, and dried. Removal of solvent gave a red oil which was shown by t.l.c. to be a complex mixture with a major yellow component at R_F 0·79 on unbaked Kieselgel $HF_{254/366}$ plates eluted with 9 : 1 v/v chloroform–methanol. A chromatographically homogeneous sample of this component was isolated by preparative layer chromatography in the same system and obtained after trituration with petroleum as an amorphous orange powder of principal m.p. *ca.* 170°. This product *methyl* 2,4-*bis*(*benzyloxycarbonylamino*)-5-(2,4-*dinitroanilino*)*pent*-4-*enoate* (7), had $\nu_{max.}$ ($CHCl_3$) 1720br cm^{-1}; $\lambda_{max.}$ 242 and 375 nm (ε 18,000 and 17,500); τ −0·17br (1H, d, *J* 9 Hz, s on decoupling from band at 3·86, exchanges slowly with D_2O, DnpN*H*), 0·94 (1H, d, *J* 3 Hz, H-3 of Dnp), 1·86 (1H, dd, *J* 9 and 3 Hz, H-5 of Dnp), 2·69 and 2·80 (10H, both s, both Ph), 3·09 (1H, d, *J* 9 Hz, H-6 of Dnp), 3·74br (1H, s, exchanges slowly with D_2O, $PhCH_2{\cdot}O{\cdot}CO{\cdot}NH{=}$), 3·86 (1H, d, *J* 9 Hz, =CH), 4·50br (1H, d, *J* 7 Hz, exchanges slowly with D_2O, $PhCH_2{\cdot}O{\cdot}CO{\cdot}NH{\cdot}CH$), 4·84 and 4·97 (4H, both s, both $PhCH_2$), 5·3—5·8 (1H, complex, NH·C*H*·CO), 6·33 (3H, s, OCH_3), and 7·1—7·5 (2H, complex, $CH_2{\cdot}C{=}$); the n.m.r. spectrum also revealed the presence of traces of contaminants which were shown by a control experiment to be derived from the chromatographic plate employed.

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