Reaction of Tryptophan Derivatives with Nitrite

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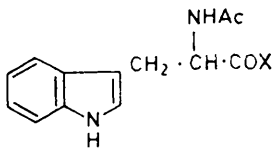
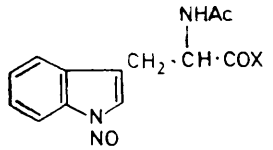
Treatment of *N*-acyltryptophan derivatives with sodium nitrite under mildly acidic conditions furnishes the nitrosamine with the nitroso-group located at the indolic nitrogen.

SINCE certain nitrosamines are carcinogenic,[1] it is pertinent to enquire to what extent such compounds can arise by the interaction of nitrite and organic nitrogenous compounds which may be ingested together.[2] The interaction with proteins and peptides is of especial interest: *N*-terminal prolyl peptide derivatives are known to be nitrosated at the secondary amine function.[3] However, although the reaction of nitrous acid with peptides has been extensively used analytically (van Slyke estimation) the nature of the organic products has often been overlooked. This appears to be the case for tryptophan derivatives. Philpot and Small[4] observed a rapid reaction between tryptophan and nitrous acid but were not able to detect a nitroso-derivative such as they had observed with tyrosine. Demyanov and Putokhin[5] reported the formation of a brick-red powder which they regarded as nitroso-β-indolylacrylic acid.

In order to avoid trivial deamination of the α-amino-function, we have examined the *N*-acetyl derivatives (I)—(III) (which are, incidentally, more satisfactory models for the peptide situation) and have shown that N^1-nitroso-compounds (IV)—(VI) are readily formed under mild conditions.



(I) X = OMe
(II) X = OH
(III) X = NH·CH_2CO_2Et



(IV) X = OMe
(V) X = OH
(VI) X = NH·CH_2CO_2Et

Thus treatment of *N*-acetyltryptophan methyl ester (I) with sodium nitrite in aqueous acetic acid at room temperature gave yellow crystals. A similar reaction occurred with the peptide ester (III): with (II) the reaction occurred in water, *i.e.* in the absence of added acid. Nitrite under acidic conditions is expected to yield an electrophilic nitrosating species (NO^+, or its equivalent, *e.g.* $H_2NO_2^+$, N_2O_3) and attack at the five-membered ring (at positions **1**, **2**, or **3**) is likely, although

[1] J. M. Barnes and P. N. Magee, *Brit. J. Ind. Med.*, 1954, **11**, 167; P. N. Magee, *Biochem. J.*, 1956, **64**, 676.

[2] For recent reviews see D. H. K. Lee, *Environ. Res.*, 1970, **3**, 484; J. Sander and F. Schweinsberg, *Zent. Bakt. Hyg. I Abt. Orig. B*, 1972, **156**, 299.

[3] F. H. C. Stewart, *Austral. J. Chem.*, 1969, **22**, 2451.

[4] J. S. L. Philpot and P. A. Small, *Biochem. J.*, 1938, **32**, 534, 542.

[5] N. Y. Demyanov and N. I. Putokhin, *Compt. rend. Acad. Sci. U.R.S.S.*, 1935, **2**, 390 (*Chem. Abs.*, 1935, **29**, 6889).

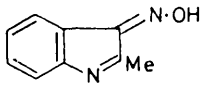
reaction in the benzenoid ring or at the amide function may also need to be considered.

The assignment of the structures of the isolated yellow derivatives may be illustrated by reference to the product from (I). Elemental analysis and mass spectrometry of this product accorded with the molecular formula ($C_{14}H_{15}N_3O_4$) of a mono-nitroso-derivative. That the nitroso-group was located at N-1 was inferred from the following evidence: (i) The i.r. spectrum of (I) possessed a sharp absorption at 3380 cm^{-1} attributed to the indole N–H stretching mode. This band was missing in the spectrum of the nitroso-derivative. A band at 1480 cm^{-1} appeared in the latter and is assigned to the N–N=O function (1430—1500 cm^{-1}).[6]

(ii) The aromatic region of the n.m.r. spectrum of the nitroso-compound integrated for five protons: one proton (presumably that at C-7) was considerably deshielded (δ 8·1).

(iii) The mass spectrum showed a weak molecular ion (*m/e* 289, 0·5%) together with strong fragments corresponding to losses of NO and (NO + CO_2Me). The base peak at *m/e* 130 corresponded to the β-indolyl methyl carbonium ion (or its isomer).

(iv) The electronic spectrum showed two maxima at 267 and 274, and a broad but less intense band at 335 nm. This spectrum is not that expected for a simple 3*H*-indole chromophore (such as might result from attack at C-3): however, it does resemble the spectra reported for *N*-nitrososkatole [$\lambda_{max.}$ (hexane) 264 and 329—334 nm][7] and for the nitroso-derivative of 2-methylindole,[8] which exists predominantly as the hydroxyimino-tautomer (VII) and has $\lambda_{max.}$(EtOH) 242, 255, and 315 nm. Such a 3-hydroxyimino-structure might arise during the nitrosation of (I) if an acid-catalysed C-3–C-2 rearrangement occurred. The electronic spectrum of the 3-hydroxyimino-compound (VII) underwent a profound change (to $\lambda_{max.}$ 245, 285, and



(VII)

345 nm) when the mesomeric anion was formed by adding dilute sodium hydroxide to the solution. Similar basification of the solution of the nitroso-derivative from (I) caused no change in the spectrum, and hence the possibility of a 3-hydroxyimino-structure is eliminated.

It is concluded that the nitrosation product of *N*-acetyltryptophan methyl ester (I) is the nitrosamine derivative (IV). The products from (II) and (III) are formulated analogously as (V) and (VI). These conclusions are supported by ^{15}N n.m.r. observations.[9]

The N^1-nitrosamine bond in the tryptophan derivatives is cleaved with mineral acids. Thus after brief treatment of the N^1-nitroso-carboxylic acid (V) with HBr–HOAc the acid (II) was detected by paper chromatography as the major product. The acid (V) was also photolabile, so much so that the electronic spectrum could not be accurately measured. Thus irradiation of an ethanolic solution with white light (100 W tungsten lamp at 15 cm for 30 min) caused complete loss of the 335 nm peak: under these conditions the corresponding ester (IV) was little affected.

These results show that under mild conditions of temperature and pH sodium nitrite reacts with simple tryptophan derivatives, including an *N*-acetyl dipeptide, to give nitroso-derivatives in which the nitroso-group is substituted at N-1 of the indole system. Such substitution at tryptophan units might occur when proteins are treated with nitrite, although it would be expected to be accompanied by reaction at other sites (*e.g.*, histidine, tyrosine). These reactions are under active investigation.

EXPERIMENTAL

The following spectroscopic facilities were employed: electronic spectra, Unicam SP 800B, calibrated with holmium glass and reported as λ/nm (ε) for maxima; i.r. spectra, Perkin-Elmer 225, KBr disc, reported as $\nu_{max.}/cm^{-1}$; n.m.r. spectra, Varian A60, with tetramethylsilane as internal reference, reported as δ values; mass spectra, A.E.I. MS902, ionising voltage 70 eV, direct insertion, probe temperature indicated, reported as *m/e* values with relative abundance in parentheses (%). Petroleum refers to that fraction of light petroleum with b.p. 60—80°. Solvent ratios refer to volumes.

DL-N-*Acetyl*-N^1-*nitrosotryptophan Methyl Ester*.—DL-*N*-Acetyltryptophan methyl ester (280 mg; powder; from the corresponding acid and diazomethane), sodium nitrite (160 mg), diethyl ether (5 ml), water (1 ml), and acetic acid (0·2 ml) were shaken together (3 h). The yellow crystalline solid which formed was filtered off and washed with water. Crystallisation from ethyl acetate gave bright yellow rods (180 mg, 55%) of DL-N-*acetyl*-N^1-*nitrosotryptophan methyl ester*, m.p. 145—149° (decomp.) (Found: C, 58·05; H, 5·3; N, 14·7. $C_{14}H_{15}N_3O_4$ requires C, 58·15; H, 5·25; N, 14·55%), λ (EtOH) 267 (14,000), 274 (11,500), and 335 (7300), ν 3330, 1750, 1640, 1520, 1480, 1435, 1270, 1115, 765, 755, and 750, δ ($CDCl_3$) 8·1 (1H, m, 7-H), 7·4 (4H, m, Ar*H*), 6·2br (1H, NH, exchangeable slowly), 5·0 (1H, m, >CH–), 3·69 (3H, s, OMe), 3·25 (2H, m, $-CH_2-$), and 1·98 (3H, s, MeCO), mass spectrum (96°) 289 (M^+, 0·5), 260 (17), 259 *M* − NO, 28), 201 (37), 200 (*M* − NO − CO_2Me, 21), 157 (21), 131 (28), 130 (β-indolyl-CH_2^+, 100), 129 (42), 103 (18), and 102 (23).

DL-N-*Acetyl*-N^1-*nitrosotryptophan*.— DL-*N*-Acetyltryptophan (264 mg) and sodium nitrite (162 mg) were shaken in water (10 ml) for 2 h. A yellow precipitate formed. After acidification (N-HCl, 5 ml) the organic product was extracted without delay into ethyl acetate (30 ml). The organic layer was separated, dried (Na_2SO_4), and taken to dryness. Crystallisation of the residue from ethyl acetate gave

[6] L. J. Bellamy, 'The Infra-red Spectra of Complex Molecules,' Methuen, London, 1958, p. 306.

[7] H. F. Hodson and G. F. Smith, *J. Chem. Soc.*, 1957, 3546; *cf.* E. Fischer, *Annalen*, 1886, **236**, 126.

[8] W. E. Noland, L. R. Smith, and K. R. Rush, *J. Org. Chem.*, 1965, **30**, 3457.

[9] R. Bonnett, R. Holleyhead, B. L. Johnson, and E. W. Randall, unpublished work.

DL-N-*acetyl*-N^1-*nitrosotryptophan* (159 mg, 53%) as yellow plates, m.p. 137—139° (Found: C, 56·4; H, 4·6; N, 14·95. $C_{13}H_{13}N_3O_4$ requires C, 56·75; H, 4·75; N, 15·3%), λ (EtOH) 267 (*ca.* 11,000), 274 (*ca.* 8900), and 335 (*ca.* 6100), the spectrum changing with time, ν 3310, 1740, 1720, 1600, 1540, 1490, 1440, 1275, 1180, 1120, 755, and 750.

The product gave a positive Liebermann reaction. Brief treatment with 48% HBr–HOAc regenerated the starting material [paper chromatography, Whatman No. 1, irrigated with Bu^nOH–H_2O–0·880-NH_3 (270 : 30 : 3) and visualised with Erhlich reagent].

DL-N-*Acetyl*-N^1-*nitrosotryptophylglycine Ethyl Ester*.—DL-*N*-Acetyltryptophylglycine ethyl ester (760 mg),[10] sodium nitrite (597 mg), ethyl acetate (30 ml), water (10 ml), and acetic acid (1 ml) were shaken together (3 h). The ethyl acetate layer was separated, the aqueous layer being extracted with further portions of ethyl acetate (2 × 25 ml). The organic extracts were dried and concentrated, the residue being crystallised from ethyl acetate–petroleum to give yellow rods (431 mg, 52%) of DL-N-*acetyl*-N^1-*nitrosotryptophylglycine ethyl ester*, m.p. 147—150° (Found: C, 56·8; H, 5·55; N, 15·45. $C_{17}H_{20}N_4O_5$ requires C, 56·65; H, 5·6; N, 15·55%), λ (EtOH) 267 (11,000), 274 (8400), and 335 (6300), ν 3240, 1745, 1665, 1625, 1540, 1480, 1425, 1275, 1205, 1120, 770, 750, 735, and 710, δ (C_5D_5N) 7—8·5 (m, ArH), 5·5 (1H, m, >CH–), *ca.* 4·25 (2H, m, –NHCH_2CO–), 4·08 (2H, q, –CH_2O–), 3·44 (2H, m, β-indolyl-CH_2), 2·02 (3H, s, MeCO–), and 1·07 (3H, t, C$H_3$$CH_2$–).

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[10] A. Previero, E. Scoffone, and C. A. Benassi, *Gazzetta*, 1963, **93**, 849.