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N-Acyl-N-Nitrosoamino Acids and Peptides

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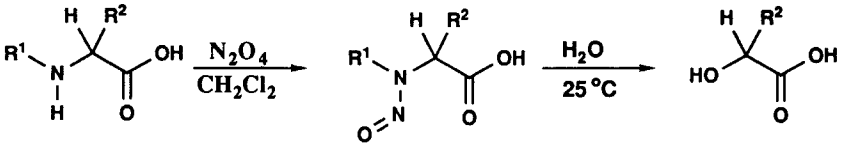
Abstract: *N-Acylamino acids and peptides are readily nitrosated by N_2O_4 and the products can be readily isolated. Hydrolysis of the N-nitrosoacylamino acids and polynitrosoacylpeptides in aqueous media (pH 1-8) occurs preferentially from the C-terminus.*

There has been much recent interest in N-nitrosopeptides since certain N-nitroso compounds are known to be potent carcinogens;[1,2] nitrosopeptides could in principle arise naturally from the reaction of a peptide with nitrous acid in animal digestive tracts, Esters of N-protected α-amino acids and peptides readily yield stable N-nitroso products, which have been examined with respect to deamination and mutagenesis.[3,4] On the other hand, nitrosopeptides bearing a free carboxyl group were reported to be impossible to isolate from attempted nitrosation in the usual aqueous media; low conversions, however, were detected by spectroscopic methods.[5] For the preparation of several members of this class of compounds in organic solvents, several methods have been reported: hydrogenolysis of benzyl esters of N-nitrosopeptides,[6] the use of nitrosonium tetrafluoroborate in the presence of pyridine[2] and the use of N_2O_4 in the presence of sodium acetate.[7] To our knowledge, however, N-acetyl-N-nitrosoglycine (**2a**) has been reported only as a transient species,[8] and N-acetyl-N-nitrosoalanine (**2b**) has not been reported in the literature.

We report here a simple method for the synthesis of N-acetyl-N-nitrosoamino acids and polynitrosopeptides and an outline of their decomposition in aqueous media. N-Acetyl-N-nitrosoglycine (**2a**), N-acetyl-N-nitrosoalanine (**2b**), N-acetyl-N-nitrosophenylalanine (**2c**),[7] dinitrosoacetylglycylglycine (**2d**) and trinitrosoacetylglycylglycylglycine (**2e**) were readily obtained in almost quantitative yields through the use of excess dinitrogen tetraoxide alone in methylene chloride (eq.1).[9] This method avoids exposure to water, which is known to destroy these compounds;[5] further, all the by-products are volatile and readily removed. Approximately 5 molar equivalents of liquid N_2O_4 was added to a suspension of N-acetylglycine (**1a**) in methylene chloride at 0° C; a homogeneous solution was formed in ~ 3 h. Evaporation under high vacuum yielded practically pure nitroso product **2a** in quantitative yield. Similarly, the treatment of acetylalanine (**1b**) with N_2O_4 produced N-nitrosoalanine **2b**, which was practically pure; recrystallization from ether and petroleum ether gave a yellow solid, mp 37-39° C. In a similar manner, dinitrosoglycylglycine **2d** and trinitrosoglycylglycylglycine **2e** were obtained in the form of yellowish solids after the treatment of the corresponding acetylglycine derivatives with an excess of N_2O_4.

Nitrosoacyl derivatives of amino acids rapidly decompose in aqueous solution; half-lives for

the decomposition of **2a** and **2b** in D_2O at 25° C were 20 h and 1.5 h, respectively. The half-life of **2a** in D_2O at pD = 1.6 (25° C) was surprisingly long (19 h), indicating a lack of pronounced acid catalysis in the decomposition. The half-life of **2b** in a pH 7.8 phosphate buffer was *ca* 1.5 h at 25° C. The products from decompositions of these nitroso compounds in aqueous solution (D_2O) are as



(1)

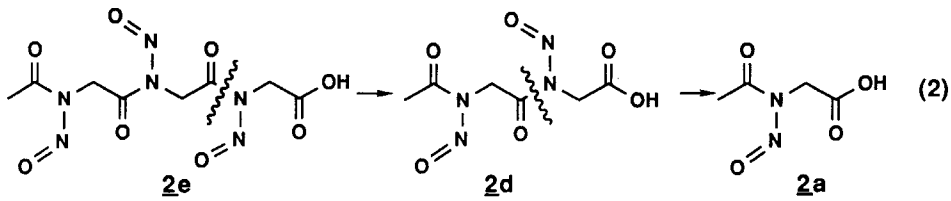
1a R^1 = Ac, R^2 = H
1b R^1 = Ac, R^2 = Me
1c R^1 = Ac, R^2 = Benzyl
1d R^1 = Ac-Gly, R^2 = H
1e R^1 = Ac-Gly-Gly, R^2 = H

2a R^1 = Ac, R^2 = H
2b R^1 = Ac, R^2 = Me
2c R^1 = Ac, R^2 = Benzyl
2d R^1 = Ac-NO-Gly, R^2 = H
2e R^1 = Ac-NO-Gly-NO-Gly, R^2 = H

3a R^2 = H
3b R^2 = Me

follows: from **2a**, glycolic acid (**3a**, 100%) and acetic acid (100%); from **2b** lactic acid (**3b**, 100%) and acetic acid (100%). The decompositions of compound **2a** and **2b** in aqueous solution were first order:[10] for **2a** in D_2O, k_{obs} = 5.8 x 10^{-4} min^{-1} and at pD 1.6, k_{obs} = 6.3 x 10^{-4} min^{-1}; for **2b**, k_{obs} = 7.2 x 10^{-3} min^{-1} at pH 7.8 in a 50 mM phosphate buffer (H_2O) at 25° C. The nitrosoacylamino acids are soluble in both chloroform and in aqueous solution, and although they decompose in the latter, in non-polar aprotic organic solvents such as chloroform and ether they are relatively stable: in $CDCl_3$, only *ca.* 10% of **2a** decomposed in 5 days at room temperature (to produce N-acetylglycine). The decomposition of dinitrosoglycylglycine **2d** in D_2O (25° C, 144 h) gave 2 mol of glycolic acid and 1 mol of acetic acid, as expected. An accumulation of nitrosoacetylglycine **2a** was observed during the decomposition; thus the central amide bond of **2d** (estimated half-life of hydrolysis ~ 9 h) was hydrolyzed almost twice as fast as the nitrosoacetylamide bond of **2a**. NMR analysis[11] detected only **2d**, **2a**, **3a** and acetic acid, and no other intermediates, in the course of decomposition of **2d** in D_2O at 25° C.

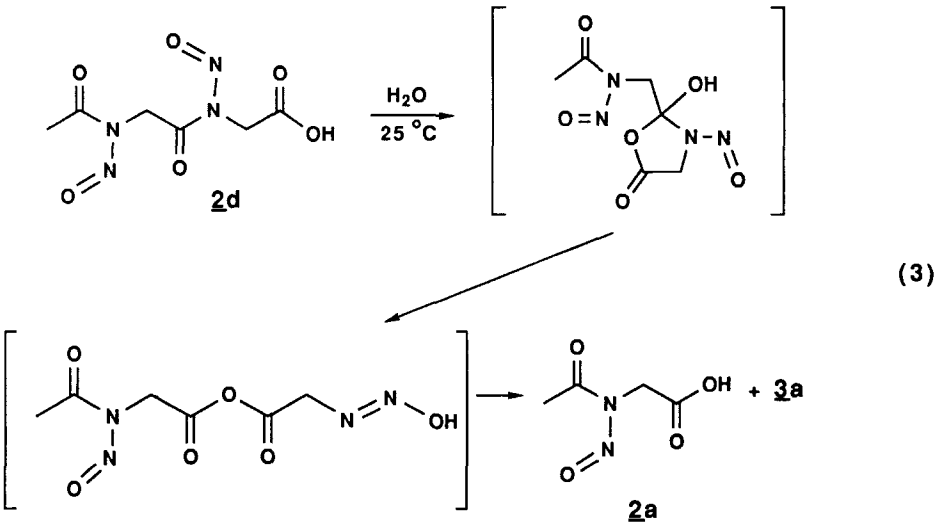
NMR analysis of the decomposition[11] of trinitrosoglycylglycylglycine **2e** in D_2O (25° C) revealed the starting compound **2e**, dinitrosoglycylglycine **2d**, nitrosoglycine **2a**, glycolic acid **3a**



(2)

and acetic acid in a molar ratio of 66:25:7:23:2 after 2.5 h; after 18 h, the ratio was 6:36:43:154:15 (eq. 2). The decomposition was complete after 6 days, and glycolic acid (3 mol) and acetic acid (1 mol) were the only products. Thus, hydrolysis of polynitrosoglycine derivatives in aqueous solution

occurs preferentially at the C-terminal amino acid residue, suggesting a possible use in sequencing. These results suggest that hydrolysis involves participation of the carboxyl group. A likely mechanism consists of addition of a carboxyl oxygen to the proximal carbonyl group and an elimination from the tetrahedral intermediate to form an anhydride-diazotate[12] (eq. 3); further hydrolytic reactions produce the final products. The formation of a ring in the rate determining step is consistent with the fact referred to earlier that methyl substituents accelerate the hydrolysis rate



(3)

(**2b** > **2a**). The mechanism of eq. 3 is consistent with the pH near independence of the hydrolysis rate observed for compound **2a** in the pH range of 1-8.

Our results are consistent with those of Challis,[6] namely that the nitrosopeptides are rather stable in the pH range of 1-8. Therefore, other factors such as unfavorable equilibria for a nitrosation step or lack of effective nitrosating agents probably account for the low yields obtained in attempted syntheses of these compounds in aqueous media. With a relatively long half-life of 19 h at pD = 1.6, the water-soluble N-nitrosoglycine derivatives, through their potential for alkylation, would appear to be candidate mutagenic agents in cells of the animal body.

Finally, the N-nitroso-N-toluenesulfonyl derivatives of glycine and alanine were prepared by the techniques described above and characterized; they appear to be somewhat more stable than the amino acid based analogs.[13]

Acknowledgement

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9. **2a**: ^{1}H NMR ($CDCl_3$) δ 4.53 (s, 2H), 2.85 (s, 3H); IR ($CDCl_3$) 3400-2500, 1736, 1521 cm^{-1}; UV (Et_2O) λ_{max} 385, 402, 422 nm. **2b**: mp 37-39° C; ^{1}H NMR ($CDCl_3$) δ 5.36 (q, *J* = 7.1 Hz, 3H), 2.82 (s, 3H), 1.35 (d, *J* = 7.1 Hz, 3H); IR (KBr) 3650-2300, 1735, 1517 cm^{-1} ; UV (Et_2O) λ_{max} 258 (ε = 3.92 x 10^{-3}), 385, 402, 422 nm. **2c**[7]: ^{1}H NMR ($CDCl_3$) δ 7.25 (m, 3H), 6.99 (m, 2H), 5.61 (AB quartet, *J* = 10.5, 5.6 Hz, 1H), 3.40 (dd, *J* = 11.5, 5.6 Hz, 1H), 3.08 (dd, *J* = 14.2, 10.56 Hz, 1H), 2.61 (s, 3H); IR ($CHCl_3$) 1720, 1640 cm^{-1}. UV (Et_2O) λ_{max} 387, 402, 422 nm. **2d**: mp 91° C (dec); ^{1}H NMR ($CDCl_3$) δ 5.41 (s, 2H), 4.51 (s, 2H), 2.94 (s, 3H); IR (KBr) 3436-2473, 1753, 1731, 1715, 1528, 1518 cm^{-1}. Anal. calcd. for $C_6H_8N_4O_6$: C, 31.04; H, 3.47; N, 24.13. Found: C, 31.34; H, 3.52; N, 23.58. **2e**: mp 43° C (dec); ^{1}H NMR (D_2O) δ 5.66 (s, 2H), 5.56 (s, 2H), 4.47 (s, 2H), 2.95 (s, 3H); ^{1}H NMR ($CDCl_3$) δ 5.50 (s, 2H), 5.37 (s, 2H), 4.49 (s, 2H), 2.95 (s, 3H); IR (KBr) 1740, 1528 cm^{-1}.
10. The first-order decomposition was also noted by Challis *et al.*[6] and Chow *et al.*[2]
11. Products were identified by direct comparisons with authentic samples (*via* 300 MHz NMR).
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13. **N-Nitroso-N-4-toluenesulfonylglycine.** Recrystallized from ethanol (46% yield of a yellow solid): mp 140.5° C (lit.[14] 140° C); ^{1}H NMR (acetone-d_6) d 7.8 (d, *J* = 8 Hz, 2H), 7.3 (d, *J* = 8 Hz, 2H), 4.2 (s, 2H), 2.45 (s, 3H); IR (KBr) 1710, 1590, 1510, 1380, 1165 cm^{-1}; UV (CH_2Cl_2) λ_{max} 390, 404 nm. **N-Nitroso-N-4-toluenesulfonyl-DL-alanine.** Recrystallized from *tert*-butanol and pentane (46% yield) to give a yellow solid: mp 114° C; ^{1}H NMR (D_2O) δ 7.5 (m, 4H), 5.0 (q, 1H), 2.45 (s, 3H), 1.4 (d, 3H); IR (KBr) 1710, 1595, 1500, 1388, 1255 cm^{-1}; UV (CH_2Cl_2) λ_{max} 390 (ε = 90), 403 nm. Anal. calcd. for $C_{10}H_{12}N_2O_5S$: C, 44.11; H, 4.44. Found: C, 44.24; H, 4.25. Stability in H_2O at pH 7 or in H_2O + EtOH (1/1): ~ 3% decomposed per hour at 25° C for both cases.
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