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A Novel Synthesis of β-Aminoalkylnitroamines. *rac*-β-Nitroaminoalanine and *N*-Nitroethylenediamine, Two Reported Metabolites from *Agaricus silvaticus*

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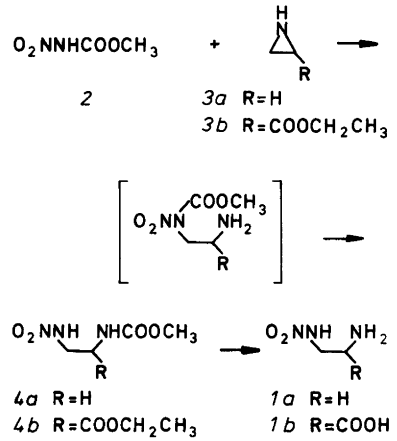
N-Nitroethylenediamine and *rac*-β-nitroaminoalanine have been prepared in good yields by the same general method, involving addition of methyl *N*-nitrocarbamate to aziridine or ethyl aziridine-2-carboxylate, respectively. Additive ^{13}C NMR shift parameters for the nitroamino group have been estimated.

In the course of an ongoing screening program for mutagens in larger fungi,[1] we noted a recent report[2,3] on the occurrence of *N*-nitroethylenediamine (*1a*) and β-nitroaminoalanine (*1b*) in two collects of *Agaricus silvaticus* Vit. ex Fr. from North Carolina and Puget Sound. The optical activity of the isolated sample of *1b* was not reported. The possible mutagenic or carcinogenic properties of monoalkylnitroamines themselves are little known, but they may undergo a chemical reduction, ultimately leading to diazoalkanes.[4] If a similar reduction were to occur under physiological conditions, alkylation of DNA might follow and result in gene damage. It should be noted that some tertiary nitroamines have recently been shown to be mutagenic to *Salmonella typhimurium*.[5] Since *A. silvaticus* is considered edible and palatable in many regions, we judged it desirable to subject its nitroamines to mutagenicity tests. However, we were unable to detect nitroamines in *A. silvaticus* from the Lund region, and we therefore had to obtain the pertinent compounds by synthesis.

N-Nitroethylenediamine (*1a*) has been prepared earlier,[6,7] *i.e. via* nitration of ethyl *N*-(2-aminoethyl) carbamate,[6] but the overall yield was very low and the general method seemed unsuitable for preparing β-nitroaminoalanine. In order to prepare the latter, we tried a Michael addition of methyl *N*-nitrocarbamate (*2*)[8,9] to ethyl-2-acetamidoacrylate, but the reaction failed, presumably due to the low nucleophilicity of the nitrocarbamic ester anion. However, an aziridinium ion was anticipated to be a sufficiently powerful electrophile to effect the desired aminoethylation, and we found that aziridine (*3a*) reacted cleanly with *2* to afford methyl *N*-(2-nitroaminoethyl) carbamate (*4a*) after initial salt formation and migration of the methoxycarbonyl group to the more basic α-amino group.



Scheme 1.

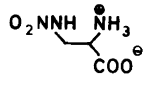
0302-4369/83 $2.50

Alkaline hydrolysis of *4a* afforded *N*-nitroethylenediamine (*1a*) in a good yield. β-Nitroaminoalanine (*1b*) was obtained similarly in a 48 % overall yield from the reaction of ethyl 2-aziridinecarboxylate (*3b*) with *2* and subsequent alkaline hydrolysis of the intermediate carbamate *4b*. The product was identical (chromatography, electrophoresis and MS) with an authentic sample of β-nitroaminoalanine from *A. silvaticus*.* We found that in these reactions methyl *N*-nitrocarbamate (*2*) is to be preferred over the corresponding ethyl carbamate as starting material, since the former yields the intermediates *4* which are more readily hydrolysed.

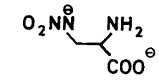
Nucleophilic ring-openings of 2-aziridinecarboxylic esters are ambiguous, since the nucleophile may enter either the α- or β-position with respect to the carboxylic group. It has been shown, however, that good nucleophiles like thiolate anions preferentially attack the α-position, and that weaker nucleophiles like water or chloride ion tend to enter the β-position.[10] The alkyl *N*-nitrocarbamate anions are apparently poor nucleophiles, as is indicated by their rather sluggish reaction with the aziridinium salt, and should consequently open the aziridine ring of *3b* to give the desired β-nitroamino intermediate *4b*. Further support for the correctness of the assigned structures *1b* and *4b* was obtained from a study of the acid catalysed decomposition of *1b* and from ^{13}C NMR data.

Primary alkylnitroamines readily eliminate dinitrogen oxide on warming with dilute mineral acids, forming products which would be expected from an alkyl cation intermediate.[11] When synthetic β-nitroaminoalanine was warmed with dilute hydrochloric acid, DL-serine was the main product, accompanied by traces of 2-amino-3-chloropropanoic acid. Neither isoserine nor 3-amino-2-chloropropanoic acid, the expected products from 3-amino-2-nitroaminopropanoic acid, were detected. The latter might conceivably decompose *via* intermediate formation of aziridine-2-carboxylic acid. but then a mixture of both aminochloropropanoic acids would be the expected products.[10]

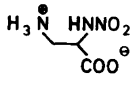
The ^{13}C NMR shifts of β-nitroaminoalanine are strongly influenced by the pH of the solution. The interpretation of the spectra in structual terms requires information on the additive shift parameters for the nitroamino group and its conjugate base, and such data seem not to have been reported in the literature so far. Most published ^{13}C NMR spectra concern open chain and cyclic compounds containing at least two nitroamino groups, all of which are undissociated[12] and there is just one report on an alkylnitroamine, methylnitroamine, (in 1, 2-dimethoxyethane) and its sodium salt (in D_2O).[13] Using the ^{13}C shift parameter set of Rabenstein and Sayer[14] for calculating carbon shifts of amines, carboxylic acids and amino acids in D_2O, shift increments of 11.3 and 17.3 ppm for methylnitroamine and its conjugate base, respectively, are obtained. This implies that deprotonation of the nitroamino group results in downfield shift of *ca.* 6 ppm for the attached carbon atom, making allowance for the different solvents used. Provisional α, β, and γ shift parameters for the nitroamino substituent in D_2O solution were determined using propylnitroamine as a model compund (Table 1). In conformity with the results of the previous investigators, a significant downfield shift (5.93 ppm) was observed for the α-carbon upon deprotonation of the nitroamino group, while the shift effects on the β and γ carbons were rather small, both for the nitroamine itself and its sodium salt. A solution of β-nitroaminoalanine in water had pH 3.8, with the solute probably best represented by formula *6a*. Titration with dilute sodium hydroxide indicated two dissociation steps with pK_a-values of 5.0 and 9.8. Since titration of propylnitroamine similarly gave pK_a 6.4, it may be assumed that the first dissociation step above primarily involves deprotonation of the nitroamino group of *6a*. The reported[15] pK_a-value for methylnitroamine in 50 % aqueous ethanol is 7.08.



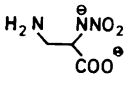
6a



6b



7a



7b

* The authentic sample was a kind gift from Prof. W.S. Chilton, University of Washington, Seattle, U.S.A.

Table 1. ^{13}C NMR (90.52 MHz) shifts for β-nitroaminoalanine (*1b*) and propylnitroamine (*5*) and their sodium salts in D_2O with sodium 3-(trimethylsilyl) propanesulfonate as internal standard. Calculated shifts for *1b* and 2-amino-3-nitroaminopropanoic acid, in different states of protonation (*6a,b* and *7a,b*, resp.).

	pD 3.9 Found		Calc.[a]		pD 12.8 Found		Calc.[a]	
	5	*1b*	*6a*	*7a*	*5*	*1b*	*6b*	*7b*
CO_2		174.4	175.3	181.5		183.7	184.2	185.0
C_α	50.2	57.1	54.2	67.7	56.2	57.1	56.1	78.2
C_β	22.1	48.8	50.2	42.6	23.1	58.8	60.7	44.5
C_δ	13.3				14.3			

[a] Using the parameter set of Ref. 14, the shift parameters for carbon atoms 1, 2 and 3 bonds removed from the nitroamino group as tentatively derived from observed shifts in *5* become: At pH 3.9, $\delta_{1bond}=14.15$, $\delta_{2bond}=-1.23$ and $\delta_{3bond}=-1.58$, at pH 12.5, $\delta_{1bond}=20.08$, $\delta_{2bond}=-0.21$ and $\delta_{3bond}=-0.59$.

On titration of β-nitroaminoalanine in D_2O solution from pD 3.8 to pD 5.7, the β-carbon triplet in the ^{13}C NMR spectrum shifted downfield 5.3 ppm, with much smaller increments of the α-carbon and carboxyl carbon shifts. This is in accord with the expected effects of a deprotonation of a nitroamino substituent in the β-position. Increasing the pD to 12.8 caused a further strong downfield shift of the β-carbon, as well as that of the carboxyl carbon, which is in conformity with the proposed structure and the known strong effects on the carboxyl and β-carbon shifts but small effect on the α-carbon shift upon deprotonation of an α-ammonium group of amino acids.[14] Using known shift parameters for amino acids[14] and tentative parameters derived from the propylnitroamine ^{13}C NMR spectrum, we have calculated ^{13}C NMR shifts for β-nitroaminoalanine (*6a,b*) as well as for the 2-amino-3-nitroaminopropanoic acid isomer (*7a,b*) at pD 3.9 and 12.5 (Table 1). These calculations show good agreement between found and calculated shifts for structure *6* and would seem to rule out the isomer *7*. However, interactions may occur between the rather congested and strongly polar substituents of these molecules, and thus care must be exercised in interpretations until more ^{13}C NMR shift data have been collected for nitroamines.

EXPERIMENTAL

Infrared spectra were recorded on a Perkin Elmer model 257 instrument. ^1H NMR spectra were recorded at 60 MHz on a Jeol JNM-PMX 60 or at 360 MHz on a Nicolet WB 360 instrument and ^{13}C spectra at 15.09 MHz on a Jeol FX-60 or at 90.52 MHz on a Nicolet WB 360 instrument. Mass spectra were recorded on a Finnegan 4000 instrument, using chemical ionisation with methane as the reactant gas. Merck Kieselgel 60 (0.063–0.200 mm) was used for column chromatography and Merck Alufolie Kieselgel F_{254} or Cellulose F_{254} for TLC. Melting points were taken on a micro hot stage (Reichert) and are uncorrected.

Methyl N-*(2-nitroaminoethyl) carbamate* (4a). Aziridine (0.96 g, 22 mmol) was bubbled as a saturated vapor in nitrogen into a suspension of methyl *N*-nitrocarbamate[8,9] (2.76 g, 23 mmol) in chloroform (50 ml). After the addition was complete (4 h), the mixture was heated under reflux for 1 h. Evaporation of the solvent gave a solid residue which was purified on a silica gel column (120 ml) using 3 % ethanol in ether as eluant. Concentration of the eluate and trituration of the residue with a small volume of cold ether afforded chromatographically pure *4* (2.78 g, 73 %, m.p. 83–86 °C), which was used for hydrolysis to *1a*, m.p. 85–88 °C (from methanol). Anal. $C_4H_9N_3O_4$: C, H, N, O. ^1H NMR (60 MHz, deuterioacetone): δ 3.3–3.8 (4 H, m), 3.6 (3 H, s). ^{13}C NMR (15.09 MHz, deuterioacetone): δ 39.1 ($-CH_2-N-CO$), 46.6 ($N-N-CH_2$), 52.3 ($O-CH_3$), 158.3 (*CO*).

N-*Nitroethylenediamine* (1a). Methyl *N*-(2-nitroaminoethyl) carbamate (750 mg, 4.6 mmol) dissolved in 2.5 M sodium hydroxide (5 ml) was heated under reflux for 2 h and the reaction mixture was then neutralised with 2 M HCl and added to a Dowex 50WX4 (H form) column (38 ml). Elution with water afforded unreacted

carbamate (160 mg) and subsequent elution with 0.6 M aqueous ammonium hydroxide afforded chromatographically pure *N*-nitroethylenediamine, which was recrystallised from a small volume of water: Yield 350 mg (73 %, uncorrected for recovered starting material), m.p. 240–241 °C (lit.[6] 240 °C). IR and ^{1}H NMR were in agreement with published spectra:[2] TLC (Cellulose, BuOH-HOAc-H_2O 12:3:5) R_{Leu} 0.59 (lit.[2] 0.52).

Ethyl 2-(methoxycarbonylamino)-3-nitroaminopropionate (4b). A solution of ethyl aziridine-2-carboxylate[16] (*3b*) (500 mg, 4.35 mmol) in anhydrous dichloromethane (5 ml) was added slowly and with efficient stirring to a suspension of an excess of methyl *N*-nitrocarbamate[8,9] (*2*) (1.044 g, 8.70 mmol) in anhydrous dichloromethane (45 ml). The reaction mixture was heated under reflux until NMR indicated the absence of *3b* (16 h), and was then concentrated to give a solid mixture of crude *4b* and excess *2*, which was used as such for hydrolysis to *1b*.

A sample of crude *4b* dissolved in ethanol-ether (2:1) was purified by filtration through a short column of silica gel, concentration of the filtrate and two recrystallisations of the residue from water–ethanol (9:1) to afford pure *4b*, m.p. 84.0–84.5 °C. Anal. $C_7H_{13}N_3O_6$: C, H, N, O. ^{1}H NMR (360 MHz, $CDCl_3$): δ 1.31 (3 H, t*),3.72 (3 H, s), 3.95 (1 H, dd broad, *J* 14.1 Hz and 6.2 Hz), 4.07 (1 H, dd, *J* 14.5 Hz and 5.1 Hz), 4.25 (2 H, m*), 4.56 (1 H, m), 5.87 (1 H, d, *J* 6.2 Hz), 9.80 (1 H, s). ^{13}C NMR (15.09 MHz, deuterioacetone): δ 14.3 (C–CH_3), 47.1 (C3), 52.4 (O–CH_3), 52.9 (C2), 62.2 (O–CH_2–), 157.5 (N–C=O), 170.4 (C1). IR (KBr): 3300 (s, NH), 1760 (s, O–C=O), 1700 (s, N–C=O), 1584 (m), 1450 (m), 1413 (m), 1380 (s), 1345 (s), 1260 (s, $N–NO_2$), 1208 (s), 1078 (s).

β-Nitroaminoalanine (1b). Crude ethyl 2-(methoxycarbonylamino)-3-nitroaminopropionate (*4b*) (1.71 g, 7.3 mmol) in 2 M sodium hydroxide (6 ml) was heated under reflux for 2 h. The reaction mixture was neutralised with dilute hydrochloric acid, and transferred to a Dowex 50WX4 (H form) column (38 ml), which was then eluted extensively with water until the effluent was no longer acidic. The β-nitroaminoalanine fraction, as located by TLC, was concentrated, and the residue recrystallised from dilute aqueous ethanol to afford pure *1b*: Yield 308 mg (48 % from *3b*), m.p. *ca.* 194 °C (dec.). Anal. $C_3H_7N_3O_4$: C, H, N, O. ^{1}H NMR (360 MHz, D_2O pD 3.8, $Me_3Si(CH_2)_3SO_3Na$): δ 3.90 (1 H, dd, *J* 15.5 Hz and 6.8 Hz), 4.05 (1 H, dd, *J* 6.8 Hz and 3.6 Hz), 4.15 (1 H, dd, *J* 15.5 Hz and 3.6 Hz). IR (KBr): 3345 (s), 2960 (s, broad), 1593 (s), 1413 (s), 1391 (s), 1275 (m), 1187 (m), 1145 (m), 1090 (m), 885 (m). MS CI: *m/e* (% rel. int. synth. *1b*, % rel. int. isol. *1b* from *A. silvaticus*): 70 (87, 100), 74 (22, 20), 75 (15, 20), 81 (14, 23), 87 (22, 8), 88 (100, 95), 90 (10, 48), 150 (33, 82). The identity of synthetic *1b* with an authentic sample from *A. silvaticus* was confirmed by TLC, MS and electrophoresis in pyridine–acetic acid buffer at pH 6.1.

Acid-catalysed reactions of β-nitroaminoalanine (1b). A solution of *1b* (25 mg) in 2 M hydrochloric acid (5 ml) was heated under reflux for 18 h and then passed through a Dowex 50WX4 (H form) column (10 ml). The resin was washed with water and then the amino acids were eluted with aqueous ammonia (7 %) and recovered by evaporation. TLC (cellulose, pyridine–water 85:15) indicated the presence of serine (R_f 0.53) and traces of 2-amino-3-chloropropanoic acid (R_f 0.71) but no isoserine (R_f 0.50) or 3-amino-2-chloropropanoic acid (R_f 0.66). Recrystallisation from water gave pure DL-serine (5.5 mg), identified by its IR spectrum.[17]

Propylnitroamine (5) Propylnitroamine was prepared using a modification of an early procedure.[18] Methyl propylcarbamate (14.5 g, 0.125 mol), obtained by the reaction of propylamine with methyl chloroformate in a 2:1 molar ratio in toluene, was dissolved in 95 % sulfuric acid (ice-bath) and then potassium nitrate (12.5 g, 0.125 mol) was added in portions. After 3 h at 0 °C the mixture was stirred into ice (400 g), extracted with ether and the ether solution washed with sodium sulfate solution and dried over sodium sulfate. On bubbling dry ammonia through the ether solution of methyl *N*-nitro-*N*-propylcarbamate, an oil separated which was washed with ether and then dissolved and carefully neutralised with 1 M sulfuric acid. Extraction with ether, drying (sodium sulfate) and subsequent distillation afforded colourless and spectroscopically (NMR) pure propylnitroamine; Yield 5.0 g (38 %), b.p. 47–48 °C/0.3 mmHg, n_D^{22} 1.4595 (lit.[18] b.p. 52–56 °C/0.1 mmHg, n_D^{20} 1.4610). ^{1}H NMR (60 MHz, $CDCl_3$): δ 1.0 (3 H, t, *J* 7.2 Hz), 1.6 (2 H, m), 3.6 (2 H, t, *J* 6.8 Hz), 9.3 (1 H, s). ^{13}C NMR (90.52 MHz, deuterioacetone): δ 11.8 (C3), 21.2 (C2), 47.9 (C1).

* The ethyl group gave rise to an ABX_3 spectrum, since the methylene protons are diastereotopic.

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