Supplementary Material Available: Selected ^1H and ^{13}C NMR and DEPT spectra of key intermediates and final TAME salicylideneamine products (61 pages). Ordering information is given on any current masthead page.

Decomposition of 1-(Nitrosoalkyl)-3-(2-hydroxyalkyl)ureas

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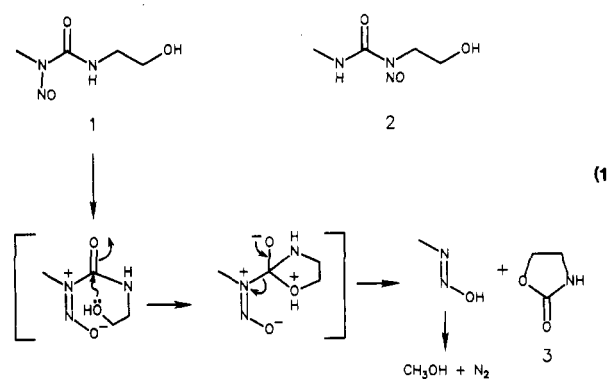
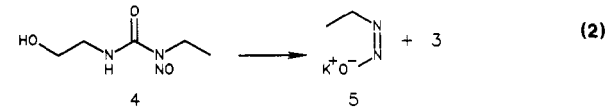
Nitrosoureas are compounds of increasing interest due to their carcinogenic and mutagenic activities[1] and for their therapeutic properties in the treatment of cancer.[2] For example, two commonly used compounds in cancer chemotherapy, presumably because of their alkylating properties,[3] are nitrosobis(2-chloroethyl)urea and nitroso(2-chloroethyl)cyclohexylurea. The study of unsymmetrical nitrosodialkylureas is of interest because of the insight it can give into the role of the nitroso group in the formation of alkylating agents. Lijinsky et al.[4] have looked into the carcinogenic effects of isomeric dialkylureas in relation to the monoalkylated analogues. During the course of these studies, it was necessary to synthesize 1-nitroso-1-methyl-3-(2-hydroxyethyl)urea (**1**) and 1-nitroso-1-(2-hydroxyethyl)-3-methylurea (**2**). Nitrosation of 1-methyl-3-(2-hydroxyethyl)urea with nitrous acid gave a mixture of **1** and **2** in a 9:1 ratio. Although the mixture decomposed slowly as a neat oil, the pure major isomer **1** was stable in acetone, or ethyl acetate, solution. However, this compound decomposed explosively when it was handled in its crystalline form, with evolution of nitrogen. Analysis of the decomposition products revealed that **1** breaks down into and 2-oxazolidone (**3**) and very likely into nitrogen and methanol, probably through the reaction pathway in eq 1.



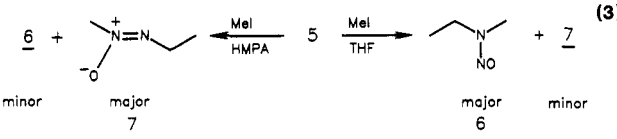
(1)

Further studies of **1** were abandoned due to its explosive nature; however, the compound's unusual decomposition prompted us to look at one of its stable analogues, 1-nitroso-1-ethyl-3-(2-hydroxyethyl)urea (**4**).[4a] Unlike **1**, the ethyl congener **4** was stable in the crystalline form, and no spontaneous decomposition was observed even after standing at room temperature for several hours. The half-life of this compound had been measured to be 40 h at pH 7 and 2.4 h at pH 8; however, the nature of the decomposition product was not identified.[5] We now know that in aqueous solution at pH 7, **4** is converted quantitatively into the oxazolidone **3** and ethanol. At pH 4, 1-nitroso-1-ethyl-3-(2-hydroxyethyl)urea remained unchanged even after 19 h at 37 °C. When a solution of **4** in ethyl acetate was stirred with solid anhydrous potassium carbonate, a 96% yield of 2-oxazolidone (**3**) was obtained. This suggested that the hydroxy group is participating in the decomposition of the nitrosourea and that potassium ethanediazoate (**5**) is a likely intermediate in the reaction, eq 2. Similar results were obtained when a THF solution of **4** was stirred with either sodium ethoxide or triethylamine.



(2)

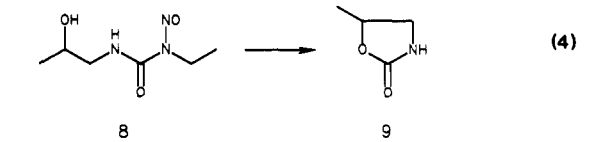
It is well established that the alkylation of metal alkanediazoates gives nitrosamines and/or azoxyalkanes, depending on the solvent systems used.[6,7] This compound was treated with potassium ethoxide in THF followed by alkylation with methyl iodide in order to demonstrate that the ethanediazoate **5** was an intermediate in the decomposition of **4**. A mixture of *N*-nitrosomethylethylamine (**6**) and (*Z*)-(methyl-*O*,*N*,*N*-azoxy)ethane (**7**) in a 1.8:1 ratio was obtained (eq 3). The major product, **6**, was isolated



(3)

in 22% yield by vacuum distillation. When the reaction was carried out in *N*,*N*-dimethylformamide, a 1.4:1 ratio of **7** to **6** was measured by GLC. In hexamethylphosphoramide, the alkylation strongly favored formation of the azoxyalkane, giving a 4:1 ratio of **7** and **6**. These results clearly indicate that the (*Z*)-ethanediazoate **5** is an intermediate in the decomposition of the hydroxyurea **4**.

N-(Nitrosoethyl)-*N'*-(2-hydroxypropyl)urea (**8**)[8] which contains a secondary hydroxyl group, was expected to undergo a similar type of decomposition to **4**, that is, the participation of the hydroxyl group in the formation of the corresponding oxazolidone. Compound **8** in pH 7 buffer was stirred at 25 °C for 1 week to give a yield of 96% ethanol and 75% 5-methyl-3-oxazolidone (**9**) eq 4.



(4)

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In summary, 1-(nitrosoalkyl)-3-(2-hydroxyalkyl)ureas break down under neutral conditions to the corresponding 2-oxazolidone and the alkyldiazonium ion. These reactions take place via a nucleophilic attack by the hydroxyl group on the electrophilic urea carbonyl to give a hemiacetal intermediate (eq 1). The formation of such intermediate and its subsequent antiperiplanar collapse to products[9] parallels hydrolysis mechanisms proposed by Stock et al.[10] and Lown et al.[11] for various nitrosoureas. In their studies, they proposed the formation of a *gem*-diol tetrahedral intermediate by direct addition of water, followed by formation of an imidourea,[9] which finally collapses to the alkyldiazonium ion and other observed products. Because DNA alkylation by certain nitrosoureas has been found to be site- and sequence-specific,[3a] these data point to the importance of the electrophilic nature of the urea carbonyl (or imidourea).[9] This type of sequence-specificity may be possible through a regioselective mechanism involving the entire nitrosourea molecule in a major groove of DNA.[3a] However, a mechanism involving a lone alkyldiazonium ion may still be in operation.[12]

Experimental Section

Gas chromatographic analyses were carried out on 10% Carbowax 20M (2% KOH) on GaschromQ and Tenax GC 80/100 columns. Dr. W. Lijinsky, from this institution, provided us with a sample of an isomeric mixture of 1-nitroso-1-methyl-3-(2-hydroxyethyl)urea (1) and 1-nitroso-1-(hydroxyethyl)-3-methylurea (2).

Purification and Decomposition of 1-Nitroso-1-methyl-3-(2-hydroxyethyl)urea (1). A 9:1 mixture (22 g) of 1-nitroso-1-methyl-3-(2-hydroxyethyl)urea (1) and 1- nitroso-1-(2-hydroxyethyl)-3-methylurea (2) was chromatographed through dry-packed silica gel, using 20:1 ethyl acetate/hexane as the eluant. A fraction with an R_f corresponding to the nitrosoureas was collected. The solution was evaporated to dryness and the residual oil suspended in ether–petroleum ether and allowed to crystallize at 0 °C. The crystalline solid was collected by filtration, giving 9.38 g of material, out of which an NMR sample was rapidly prepared in $CDCl_3$. A 19-mg portion of the crystalline material was separated and stored at 0 °C overnight. The bulk of the product was stored at 4 °C in a closed container. It then exploded within 15 min of containment. The residue from the explosion was analyzed by NMR spectroscopy and was identical with 2-oxazolidone (3).[8] The crystalline material (19 mg) which was stored at 0 °C, gave a proton and carbon NMR identical with that of 3 as well. However, the original crystalline product which had been dissolved in chloroform-d_3 gave a NMR spectrum consistent with the structure of 1: mp 39–41 °C; ^{1}H NMR ($CDCl_3$) δ 2.90 (s, 1 H), 3.189 (s, 3 H), 3.65 (m, 2 H), 3.84 (t, 2 H), 7.53 (b, 1 H); ^{13}C NMR 26.65 ppm, 43.02, 61.37, 157; MS (9:1 mixture of 1 and 2) *m/z* 148 ($M^+ + 1$, 2), 117 (1), 102 (5), 99 (1), 90 (6), 88 (100), 87 (46), 61 (7), 60 (47), 59 (57), 58 (49), 57 (9), 56 (15), 45 (39), 44 (11), 43 (42), 42 (54), 41 (7); exact mass (chemical ionization), calcd for $C_4H_{13}N_4O_3$, $[M^+NH_4]^+$ 165.0987, found $[M^+NH_4]^+$ 165.0935; exact mass (electron impact) calcd for $C_4H_{10}N_3O_3$ ($M^+ + 1$) 148.0721, found ($M^+ + 1$) 148.041.

Decomposition of 1-Nitroso-1-ethyl-3-(2-hydroxyethyl)-urea. (a) pH 7 Buffer. A solution of 161 mg (1 mmol) of 4 in 5 mL of pH 7 buffer was placed in a 37 °C water bath for 144 h. The reaction mixture was analyzed for ethanol formation on a Tenax GC 80/100 column. Ethanol was formed in quantitative yield based on peak areas. The reaction mixture was extracted with ethyl acetate, dried over sodium sulfate, and filtered through magnesium sulfate. The solvent was removed on a rotary evaporator and the crude product recrystallized from ether–petroleum ether to give 78 mg (90%) of 2-oxazolidone: mp 82 °C (lit. mp[13] 86–89 °C). The NMR spectrum was identical with that described in the literature.

(b) Triethylamine. To a 0.5 M solution of 4 in THF was added 1 equiv of triethylamine. The resulting solution was stirred at 25 °C for 96 h to give after workup a 79% yield of oxazolidone 3.

(c) Sodium Ethoxide. A 0.5 M solution of 4, in THF, was stirred for 6 h with 2 equiv of sodium ethoxide to give 3 in 73% yield.

(d) Potassium Carbonate. A slurry of potassium carbonate in a 0.5 M solution of 4 in ethyl acetate was stirred at room temperature for 48 h to give 3 in 96% yield.

Formation and in Situ Alkylation of (*Z*)-Potassium Ethanediazoate (5) from *N*-(Nitrosoethyl)-*N'*-(2-hydroxyethyl)urea (4). (a) Potassium Ethoxide–THF. Potassium metal (120 mg, 3.07 mmol) was dissolved in 5 mL of anhydrous ether containing 0.250 mL of absolute ethanol at 0 °C. A solution of *N*-(nitrosoethyl)-*N'*-(2-hydroxyethyl)urea (4) in 2 mL of tetrahydrofuran was added dropwise. A white precipitate formed immediately and was stirred in the cold for 1 h. The ice bath was removed, 0.311 mL (5 mmol) of iodomethane was introduced into the reaction mixture, and this was stirred overnight at room temperature under nitrogen. GLC analysis of the reaction mixture indicated a 1.8:1 ratio of *N*-nitrosomethylethylamine (6) and (methyl-*O*,*N*,*N*-azoxy)ethane (7). The mixture was evaporated to near dryness and the residue was extracted with methylene chloride. The solution was washed with water, and the organic layer was dried. The solvent was removed on a rotary evaporator and the residue was vaccum distilled to give 24 mg (22%) of *N*-nitrosomethylethylamine (6), which was identical with an authentic sample of the nitrosamine.

(b) Potassium Ethoxide–HMPA. Potassium metal (120 mg, 3.07 mmol) was dissolved in 5 mL of ether containing 0.250 mL of ethanol and treated with 4 as described above. The solvent was removed under a stream of nitrogen, the (*Z*)-potassium ethanediazoate (5) was resuspended in 2 mL of hexamethylphosphoramide, and the resulting mixture was treated with 0.311 mL (5 mmol) of iodomethane. After 20 h at 25 °C, GLC of the reaction mixture indicated a 4:1 ratio of the azoxy compound 7 and nitrosamine 6. The reaction was worked up and the product isolated and purified as described previously.[7] (*Z*)-(Methyl-*O*,-*N*,*N*-azoxy)ethane (7) was obtained in 37% yield.

(c) Potassium Ethoxide–DMF. Alkylation of the diazotate 5 with iodomethane in *N*,*N*-dimethylformamide gave a 1.4:1 ratio of 7 and 6.

Decomposition of *N*-(Nitrosoethyl)-*N'*-(2-hydroxypropyl)urea (8) in pH 7 Buffer. A solution of 161 mg (0.91 mmol) of 8 in 5 mL of pH 7 buffer, $t_{1/2}$ 23 h, was stirred at 25 °C for 1 week. Ethanol was formed in 96% yield, as measured by peak area on Tenax GC 80/100. The aqueous solution was evaporated to dryness and the residue was extracted with methylene chloride. The solution was dried and filtered and the solvent was removed on a rotary evaporator. Pot-to-pot distillation of the residue under vacuum gave 69 mg (75%) of 5-methyl-2-oxazolidone (9), which was identical with an authentic sample prepared as described.[9]

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