Isolation and Identification of the Hydrolytic Degradation Products of Ranitidine Hydrochloride

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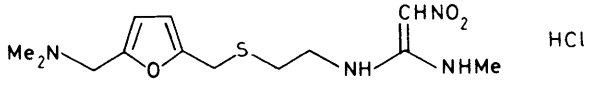
Hydrolytic degradative studies on ranitidine hydrochloride (**1**) have shown that two different pathways are operative under strongly acid and strongly alkaline conditions. At intermediate pH values both pathways are operative whilst at very low pH values ranitidine hydrochloride is resistant to hydrolytic cleavage. This resistance to hydrolysis may be ascribed to *C*-protonation of the enediamine.

Chromatographic procedures, by virtue of their potential selectivity, are used extensively to assure the identity, strength, purity, quality and stability of medicinal preparations. Identification of the degradation products capable of formation during the storage of drug substances and their formulated pharmaceutical presentations is fundamental to the acquisition of proof that, were degradation to occur, the analytical methods applied to assure the efficacy and safety of the medicine, would reveal it. Since, under normal storage conditions, the only reactive influences to which a medicinal preparation is likely to be exposed include moisture, heat, light and the atmospheric gases, hydrolytic studies represent particularly pertinent artificial degradative investigations.

This paper describes the results of the hydrolytic studies conducted on ranitidine hydrochloride, the British Approved Name for *N*,*N*-dimethyl-5-[2-(1-methylamino-2-nitrovinylamino)ethylthiomethyl]furfurylamine hydrochloride (**1**), a selective histamine H_2 antagonist used to treat peptic ulcers and related disorders.[1–4]



(**1**)

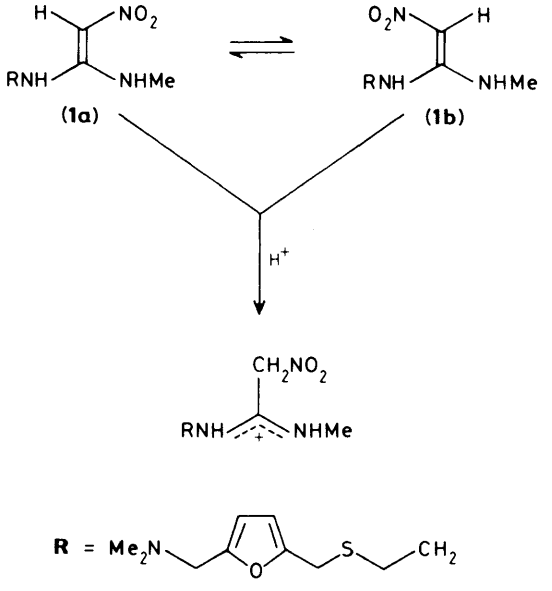
The reactive properties[5] of the substituted 2-nitro-1,1-vinyldiamino entity allow different hydrolytic behaviour of the ranitidine molecule under acidic and alkaline conditions.

At neutral pH, however, the 2-nitro-1,1-vinyldiamino entity is stable and, as found during carefully controlled stability tests with solutions for injection buffered to pH 7, hydrolysis of ranitidine occurs to an extent of $<5\%$ after 2 years at 30 °C.

Results and Discussion

Hydrolysis at Low pH (<1).—At very low pH values (<1) ranitidine hydrochloride is resistant to hydrolysis even on prolonged heating. This behaviour is attributed to the nucleophilicity[5,6] of the nitro group-bearing carbon atom which is protonated. This is borne out by u.v. spectroscopic studies which reveal a hypochromic effect at a pH value of <4.5 for the chromophore assignable to the substituted conjugated 2-nitro-1,1-vinyldiamino entity. Thus, whereas in the pH range 4.5—11 aqueous ranitidine exhibits a $\lambda_{max.}$ 315 nm with ε 15 400, at pH 2.26 the ε value at 315 nm is 7 700 and at pH *ca.* 0.75 the ε value at 315 nm is *ca.* 350 $dm^3\ mol^{-1}\ cm^{-1}$.

The lack of chromophore, attributable to loss of conjugation by protonation of the nitro group-bearing carbon atom (pK_a of this process is 2.3) in aqueous solution and which is complete in M-hydrochloric acid, can be interpreted as shown in Scheme 1. Here the solute species are shown as a mixture of *Z* and *E* isomers (**1a**) and (**1b**) in accordance with the ^1H n.m.r. evidence obtained from spectral determinations in D_2O and CD_3OD at various temperatures.[7]

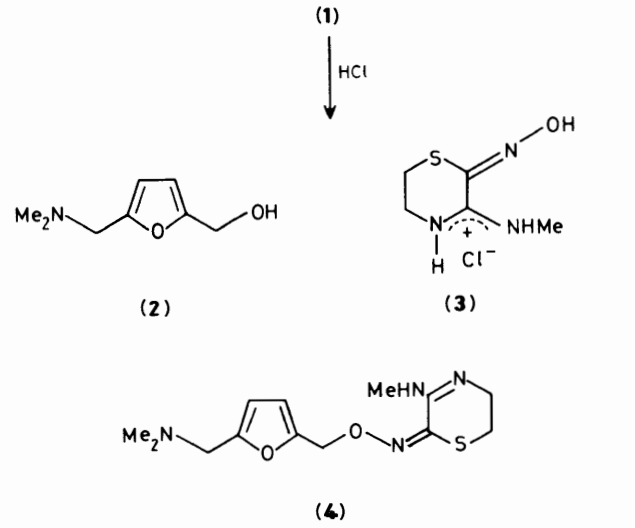


Scheme 1. Protonation of the 2-nitro-1,1-vinyldiamino moiety

Hydrolysis Between pH 2 *and* 4.—In the pH range *ca.* 2—4 hydrolytic cleavage of ranitidine is rapid at elevated temperatures, hydrolysis being complete in *ca.* 6 h at reflux temperature. A mixture of products is formed, from which four compounds, (**2**)—(**5**) (see Scheme 2), have been isolated. The products and their relative proportions appeared to be independent of the acid employed and, by t.l.c., not to be artefacts of the work-up procedure. Unequivocal identification was provided by n.m.r. and/or mass spectrometry.

Of the two major products, (**2**) and (**3**), the structure of the latter, was first deduced from ^1H and ^{13}C n.m.r. studies and later confirmed by *X*-ray studies.[8]

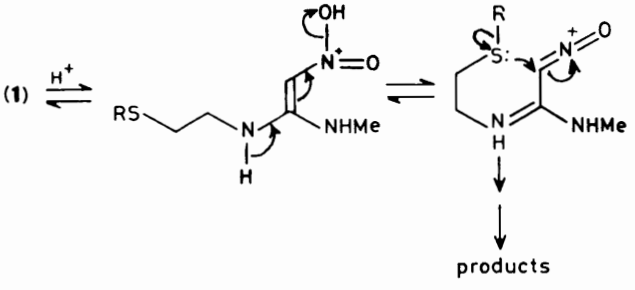
The nature of the products suggests a proton-induced degradation involving the nitro group and a double bond shift (see Scheme 3). Nucleophilic attack followed by ring closure between the sulphur atom and the carbon atom bearing the



Scheme 2.

original nitro group would lead to the substituted dihydrothiazin-2-one oxime hydrochloride (**3**).

It would be expected that the solvent water acting as a nucleophile would yield compound (**2**), any nitroso form of the

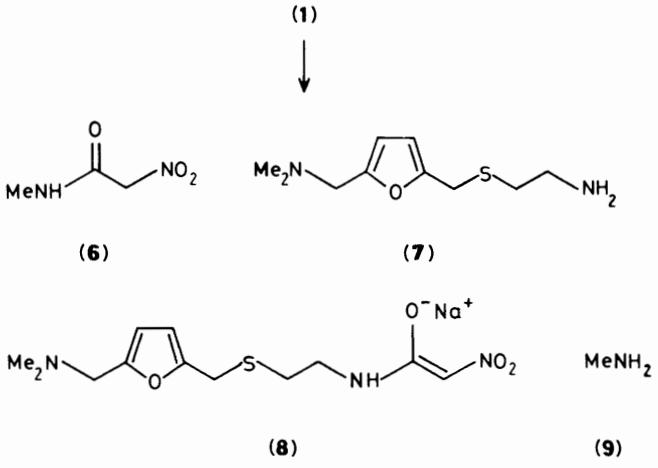


Scheme 3.

free base of compound (**3**) as nucleophile would yield compound (**4**), and ranitidine (**1**) itself acting as the nucleophile would yield compound (**5**). Independent experiments revealed that under acid conditions there is no reaction between compounds (**2**) and (**1**) or between compounds (**2**) and (**3**) to yield compounds (**5**) and (**4**) respectively.

Hydrolysis at >pH 9.—At pH values >9 the hydrolysis of ranitidine at elevated temperatures is again rapid, complete degradation occurring in *ca.* 4 h at reflux temperature. Hydrolysis under strongly alkaline conditions proceeds *via* a different path to give different products from those formed by the acid-catalysed path at pH 2—4.

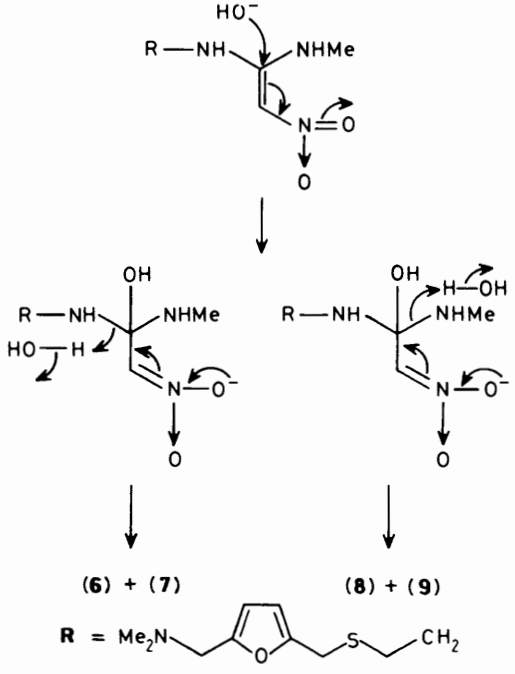
The four products (**6**)—(**9**) (see Scheme 4) were isolated and their structures unequivocally determined from ^{1}H n.m.r. spectral evidence and independent synthesis. In the case of



Scheme 4.

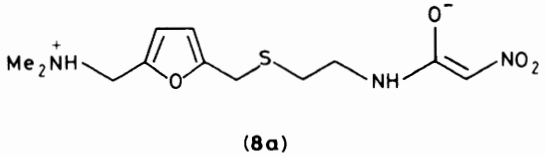
methylamine [compound (**9**) as hydrochloride] comparison was made with commercially available material.

The structures of compounds (**6**)—(**9**) suggest that hydrolytic cleavage of ranitidine under strongly basic conditions occurs *via* hydroxy ion attack on the β-carbon atom of the nitrovinyl group followed by alternative eliminations from the diamino-alcohol intermediate (Scheme 5).



Scheme 5.

Compound (**8**) when isolated as its sodium salt at high pH was shown by ^{1}H n.m.r. and i.r. studies to exist in the nitrovinyl form [as $-NHC(=CHNO_2)O^-Na^+$ salt]. At neutral pH in D_2O, the compound exists as a zwitterion (**8a**), protonation of



the NMe_2 group being as indicated by the lowfield signal at 2.72 p.p.m. In strongly acidic solution (H_2O–HCl, pH <1), compound (**8**) exists in the nitromethylene form as indicated by a methylene signal at δ_H 5.42 (*cf.* $MeNHCOCH_2NO_2$ at δ_H 5.16 in $CDCl_3$).

Hydrolysis in the Range pH 5—8.—In the pH range *ca.* 5—8 and in particular around neutrality, hydrolysis of ranitidine is slow, having gone only 20—50% to completion (dependent upon actual pH) after 5—8 days under reflux. Under these conditions the presence of compounds (**2**), (**3**), (**6**), (**7**), and (**8**), as shown by t.l.c. of the reaction mixtures, indicated hydrolytic degradation by the simultaneous operation of the paths shown in Schemes 2 and 4 or of Scheme 2 and a modification of Scheme 4 involving intercession of protons in a push-pull variation. The products were not, however, isolated. Hydrolysis of solutions buffered to neutral pH is particularly slow having proceeded to <5% after 2 years at 30 °C, with the basic pathway predominating over the acid pathway.

Experimental

Materials.—Ranitidine hydrochloride was synthesized by the Chemical Development Department, Glaxo Group Research Ltd., Ware, Hertfordshire.

U.v. spectra were recorded on a Cecil 595 spectrophotometer and i.r. spectra on Perkin-Elmer 357 or 377 spectrophotometers. ^1H And ^{13}C n.m.r. spectra were obtained on a Varian EM390 and JEOL FX100 (25.1 MHz) spectrometer respectively. Mass spectra were obtained on an AEI MS30 instrument.

Behaviour of Ranitidine Hydrochloride (**1**) *at* pH 0.75.—A solution of ranitidine hydrochloride (500 mg) in water (10 ml), was adjusted to pH 0.75 with hydrochloric acid and heated on a steam-bath under reflux for 48 h. The pH was monitored on cooled samples and adjusted as required. T.l.c. [silica gel, ethyl acetate–isopropyl alcohol–ammonia (*d*, 0.88)–water (25:15:4:2)] at intervals revealed unchanged ranitidine with only traces (<5% total) of other compounds. Aqueous ranitidine hydrochloride at pH 6.5 revealed $\lambda_{max.}$ 315 (ε 15 400) for the diaminonitrovinyl entity (pK_a 2.26). Below pH 4.5 the absorption exhibited a hypochromic effect with $\lambda_{max.}$ 315 (ε 7 700) at pH 2.3 and $\lambda_{max.}$ 315 (ε 350) at pH ≈0.75. In M-hydrochloric acid this band disappears completely.

Hydrolysis of Ranitidine Hydrochloride at pH 3.—A solution of ranitidine hydrochloride (1.0 g) in water (30 ml) was adjusted to pH 3 with citric acid and heated on a steam-bath under reflux for 6 h. The solution was cooled to room temperature, made alkaline with aqueous sodium hydroxide [20% w/v; 3 ml] and extracted with ether (2 × 50 ml). The basic aqueous layer was retained and the ether extracts were combined, dried ($CaSO_4$), and evaporated to dryness. T.l.c. [silica gel, ethyl acetate–isopropyl alcohol–ammonia (*d*, 88)–water (25:15:4:2)] revealed two components. The extract was redissolved in water and again extracted with ether (2 × 50 ml). The ether extracts were dried ($CaSO_4$) and evaporated to dryness to yield a yellow oil (10 mg) identified as 3-methylamino-5,6-dihydro-2*H*-thiazin-2-one *O*-5-dimethylaminomethylfurfuryloxime (**4**). δ_H($CDCl_3$; Me_3Si), 2.26 (6 H, s, NMe_2), 2.75 (2 H, m, SCH_2), 2.80 (3 H, s, NH*Me*), 3.45 (2 H, s, NCH_2), 3.75 (2 H, m, ring NCH_2), 5.11 (2 H, s, OCH_2), 4.7—5.5 (1 H, br s, NH), 6.20 (1 H, d), and 6.35 (1 H, d); *m/z* 296.1282 (M^+, 5%) ($C_{13}H_{20}N_4O_2S$ requires 296.1307), 253 (20), 154 (30), 138 (100), and 58 (50).

The alkaline solution retained from above was then extracted with chloroform (5 × 50 ml) and again retained; the chloroform extracts were combined, dried ($CaSO_4$), and evaporated to dryness.

T.l.c. revealed two components. Preparative t.l.c. on silica gel and elution with ethyl acetate–isopropyl alcohol–ammonia (*d*, 0.88)–water (25:15:4:2) afforded a high running band identified as 5-*N*,*N*-dimethylaminomethyl-2-furylmethanol (**2**) (200 mg), b.p. 124—128 °C at 15 mmHg (lit.,[9] b.p. 125—131 °C at 15 mmHg); $\nu_{max.}$($CHBr_3$) 3 600 (OH) 2 820, 2 780, 1 012, and 791 cm^{-1}; δ_H($CDCl_3$) 2.25 (6 H, s, NMe_2), 3.45 (2 H, s, NCH_2), 4.27 (1 H, br s, OH), 4.55 (2 H, s, OCH_2), and 6.1—6.25 (2 H, AB, furan H). The lower running band afforded an orange solid (10 mg) identified as *N*,*N*-dimethyl-5-{2-[1-methylamino-2-(5-dimethylaminomethyl-2-furfuryl)-2-nitrovinylamino]ethylthiomethyl}furfurylamine (**5**); δ_H($CDCl_3$) 2.24 (12 H, s, NMe_2), 2.75 (2 H, t, SCH_2CH_2), 3.03 (3 H, s, NH*Me*), 3.40 and 3.42 (6 H, s and t, NCH_2 and NCH_2CH_2), 3.70 (2 H, s, SCH_2), 4.00 (2 H, s, CH_2C=), 6.10 (2 H, s, furan H), and 6.13 (2 H, s, furan H); *m/z* 452.2321 (M^+ + H, 20%) ($C_{21}H_{34}N_5O_4S$ requires 452.2332) 436 (10), 405 (30), 172 (70), 138 (40), and 58 (100).

The alkaline solution retained from the chloroform extraction was neutralized with hydrochloric acid and evaporated to dryness to yield an orange solid. Recrystallization from ethanol yielded colourless granular crystals of 3-methylamino-5,6-dihydro-2*H*-1,4-thiazin-2-one oxime hydrochloride (**3**) (210 mg), m.p. 250 °C (decomp.) (Found: C, 30.7; H, 5.15; N, 21.2. $C_5H_9N_3OS$·HCl requires C, 30.70; H, 5.16; N, 21.5%); $\nu_{max.}$(Nujol) 3 350—2 500 (br s, NH, OH), 1 670 cm^{-1} (s, C=N^+); δ_H([$^2H_6$]–DMSO) 2.97 (3 H, d, NH*Me*), 3.18 (2 H, m, SCH_2), 3.70 (2 H, m, NCH_2), 9.55 (1 H, q, N*H*Me), 10.50 (1 H, t, N*H*CH_2) and 13.50 (1 H, s, OH); δ_C([$^2H_6$]–DMSO, Me_4Si), 26.4 (t, C-6), 31.3 (q, $NHCH_3$), 42.7 (t, C-5), 140.3 (s, C-2), and 153.2 (s, C-3); *m/z* 161.0417 (M^+, 1%) ($C_5H_9N_3O^{34}S$ requires 161.0424), 159.0472 (M^+, 30) ($C_5H_9N_3O^{32}S$ requires 159.0466), 142 (100), 114 (20), 96 (30), 83 (30), and 67 (80).

Alkaline Hydrolysis of Ranitidine.—A solution of ranitidine hydrochloride (3.5 g) in water (20 ml) containing sodium hydroxide (0.4 g) was heated on a steam-bath for 4 h as evolved gases were passed into methanol–dilute hydrochloric acid (1:1, v/v). Evaporation to dryness of this mixture gave methylamine hydrochloride (**9**) (100 mg), m.p. 226—230 °C identical (mixed m.p., i.r., and ^1H n.m.r.) with an authentic sample.

The aqueous reaction solution from above was cooled to room temperature, extracted with chloroform (2 × 25 ml) and retained.

The chloroform extracts were bulked and evaporated to dryness to yield an orange gum (400 mg) identified as *N*,*N*-dimethyl-5-(2-aminoethylthiomethyl)furfurylamine (**7**), b.p. 103—106 °C at 0.1 mmHg (lit.,[10] 104—106 °C at 1.4 mmHg); $\nu_{max.}$($CHBr_3$), 3 380, 3 310w, 2 820, 2 780, 1 015, and 790 cm^{-1}; δ_H($CDCl_3$) 1.63 (2 H, br s, NH_2), 2.30 (6 H, s, NMe_2), 2.5—3.0 (4 H, m, SCH_2CH_2N), 3.48 (2 H, s, NCH_2), 3.72 (2 H, s, SCH_2), and 6.17 (2 H, s, furan H).

The aqueous solution retained from above was evaporated to dryness and the residue was recrystallized from ethanol–ethyl acetate (50:50) to yield sodium 1-[2-(5-dimethylaminomethylfuryl)ethylamino]-2-nitroethenoxide (**8**) (840 mg) as pale yellow crystals, m.p. 155—158 °C (Found: C, 44.3; H, 5.4; N, 12.6. $C_{12}H_{18}N_3NaO_4S$ requires C, 44.45; H, 5.50; N, 12.8%); $\nu_{max.}$(Nujol), 3 700—3 200 (br m, NH), 3 120 (s, =CH str), 1 590, 1 545, and 760 (nitrovinyl), 1 015 and 788 cm^{-1} (2,5-disubst. furan); δ_H([$^2H_6$]–DMSO) 2.15 (6 H, s, NMe_2), 2.53 (2 H, t, SCH_2CH_2), 3.33 (2 H, q, $NHCH_2CH_2$), 3.38 (2 H, s, $NHCH_2$), 3.77 (2 H, s, SCH_2), 6.00 (1 H, s, =CH), 6.15—6.3 (2 H, AB, furan), and 9.70 (1 H, t, CH_2NH).

The ethanol–ethyl acetate mother liquors from above were acidified with dilute hydrochloric acid and chromatographed on silica gel. Elution with ethyl acetate–isopropyl alcohol–ammonia (*d*, 0.88)–water (25:15:6:2) afforded a low running

band identified as *N*-methyl-2-nitroacetamide (**6**) (270 mg), m.p. 74 °C (lit.,[11] 75—76 °C) (Found: C, 30.6; H, 5.1; N, 23.4. Calc. for $C_3H_6N_2O_3$: C, 30.51; H, 5.12; N, 23.7%); $\nu_{max.}$(CHBr$_3$) 3 430 (NH), 1 690 (CO), 1 560 (NH), 1 535 and 1 375 cm^{-1} (NO_2); δ_H(CDCl$_3$ 2.90 (3 H, d, NH*Me*), 5.16 (2 H, s, CH_2NO_2), and 6.9 (1 H, br, s, NH).

Preparation of Authentic Samples.—5-N,N-*Dimethylaminomethyl-2-furylmethanol* (**2**). A mixture of furfuryl alcohol (1 ml) and dimethylamine hydrochloride (0.95 g) was stirred at 37 °C and aqueous formaldehyde solution (39%, w/v; 1 ml) was added over 2 h while the temperature was maintained at 37 °C. The mixture was stirred for 20 h at 37 °C then cooled to *ca.* 10 °C. The mixture was basified (pH 11.5) with 1.0M-sodium hydroxide and extracted with toluene (3 × 30 ml). The combined extracts were evaporated under reduced pressure and the residue purified by distillation *in vacuo* to yield compound (**2**) (500 mg), b.p. 126—130 °C at 15 mmHg (lit.,[9] b.p. 125—131 °C at 15 mmHg) (Found: C, 61.8; H, 8.5; N, 8.9. Calc. for $C_8H_{13}NO_2$: C, 61.9; H, 8.44; N, 9.0%); identical (n.m.r. and i.r.) with hydrolysis product.

3-*Methylamino-5,6-dihydro-*2H-1,4-*thiazin-2-one oxime hydrochloride* (**3**). 1,1-Bis(methylthio)-2-nitroethene was prepared by the method of Gompper and Schaeffer.[12] A solution of methylamine (33%, w/w in MeOH, 0.1 g) was added dropwise with stirring over 2 h to a solution of 1,1-bis-(methylthio)-2-nitroethene (0.17 g) in ethanol (30 ml) at 50 °C. To the resultant mixture a solution of 2-aminoethanethiol hydrochloride (0.1 g) in ethanol (30 ml) was added dropwise over 3 h while the temperature was maintained at 50 °C. The mixture was then cooled, evaporated to dryness, and the residue shaken with water (30 ml) and 1.0M-hydrochloric acid (3 ml). The mixture was heated on a steam-bath for 2 h, cooled to room temperature, and evaporated to dryness to yield a pale orange crystalline solid which upon recrystallization from ethanol gave compound (**3**) (80 mg) as white crystals, m.p. 250 °C (decomp.) (Found: C, 31.1; H, 5.25; N, 21.8. $C_5H_9N_3OS$·HCl requires C, 30.7; H, 5.16; N, 21.5%); identical (n.m.r. and i.r.) with hydrolysis product.

N-*Methyl-2-nitroacetamide* (**6**). 1,1-Bis(methylthio)-2-nitroethene was prepared as reported in the literature.[12] A solution of 1,1-bis(methylthio)-2-nitroethene (1 g) in ethanol (50 ml) at 40 °C was treated with an excess of methylamine. The solution was stirred at 40 °C for 2 h, cooled, evaporated to dryness, and the residue dissolved in 1.0M-sodium hydroxide (35 ml). The solution was heated on a steam-bath for 2 h cooled to room temperature, neutralized with concentrated hydrochloric acid and evaporated to dryness. The residue was dissolved in ethanol and filtered to give a solid which, on crystallization, afforded compound (**6**) (300 mg) as white crystals, m.p. 73 °C (lit.,[11] 75—76 °C) (Found: C, 30.25; H, 5.25; N, 23.4. Calc. for $C_3H_6N_2O_3$: C, 30.51; H, 5.12; N, 23.7%); identical (^{1}H n.m.r. and i.r.) with hydrolysis product.

N,N-*Dimethyl-5-(2-aminoethylthiomethyl)furfurylamine* (**7**). Compound (**7**) was prepared as described in the literature,[10] b.p. 104—107 °C at 0.1 mmHg (lit.,[10] 104—106 °C at 0.1 mmHg) (Found: C, 55.8; H, 8.3; N, 13.1. Calc. for $C_{10}H_{18}N_2OS$: C, 56.0; H, 8.47; N, 13.0%); identical (n.m.r. and i.r.) with hydrolysis product.

Sodium 1-[2-(5-*dimethylaminomethylfurfurylthio)ethylamino*]-2-*nitroethenoxide* (**8**). 1,1-Bis(methylthio)-2-nitroethene was prepared by the method of Gompper and Schaeffer.[12] A solution of compound (**7**) (2 g) in ethanol (10 ml) containing 1,1-bis(methylthio)-2-nitroethene (1.6 g) was heated at 40 °C for 1 h. The solution was evaporated to dryness and the residue dissolved in water (30 ml) containing sodium hydroxide (0.4 g). The solution was heated on a steam-bath for 2 h, cooled to room temperature, and evaporated to dryness. The residue was recrystallized from ether–ethanol (1:1) to yield compound (**8**) (1.4 g) as pale yellow crystals, m.p. 155—158 °C (Found: C, 44.9; H, 5.45; N, 12.6. $C_{12}H_{18}N_3O_4S$·Na requires C, 44.67; H, 5.61; N, 12.8%); identical (^{1}H n.m.r. and i.r.) with hydrolysis product.

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