Synthesis of Nitrosothioureas. ^{15}N N.M.R. Evidence for the Formation of Thionitrosyl Compounds in the Nitrosation of Thioureas

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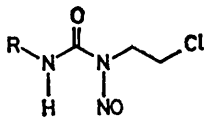
Summary Nitrosothioureas may be prepared by treatment of thioureas with $NaNO_2$ in 0·1 N HCl at −5 °C by direct N-nitrosation whereas ^{15}N n.m.r. studies at −10 °C which employed specifically ^{15}N-enriched compounds revealed the intermediacy of a thionitrosyl compound under more acidic conditions which gave the urea by hydrolysis.

2-CHLOROETHYLNITROSOUREAS [CENUs, *e.g.* (**1a**)—(**1c**)] are clinically useful in the treatment of a range of neoplasms and decompose in aqueous solution to give electrophiles which include 2-chloroethyldiazohydroxide and an isocyanate.[1] While the *in vivo* activity appears to be due



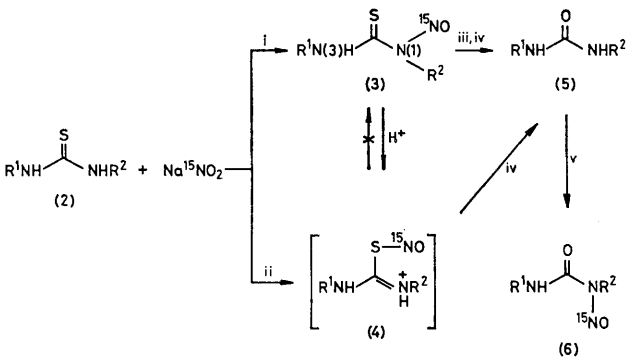
(1)

a; R = C_6H_{11}
b; R = $C_6H_{10}Me$-4
c; R = CH_2CH_2Cl

largely to the alkylation of biological macromolecules (principally DNA) additional toxicity may arise from the carbamoylation of protein-NH_2 groups by the isocyanate.[1,2] CENUs have been synthesized which were designed to trap the isocyanate intramolecularly[3,4] in attempts to reduce toxicity. Since released isothiocyanate may be less reactive than isocyanates[5] we are exploring the alternative approach of preparing nitrosothioureas as potential anti-neoplastic agents.

While various attempts have been made to prepare thionitrosoureas only one such compound has been fully characterized.[6] The reactive species generated from nitrous acid during normal nitrosation may cause difficulty in selective nitrosation at either S or N in thioureas.[7,8] We report an investigation of the reaction conditions necessary to favour N-nitrosation, and include a study by ^{15}N n.m.r. spectroscopy using both natural abundance and specifically ^{15}N-enriched compounds of a key thionitrite-reaction intermediate.

A suspension of the thiourea [(**2a**)—(**2e**)] and $NaNO_2$ (1 equiv.) in CH_2Cl_2 at −5 °C was treated with 0·1 N HCl (1 equiv.), added as drops, and the mixture was slowly warmed to 10 °C to give the corresponding nitrosothioureas (**3a**)—(**3e**) as single regioisomers in 60—90% yields. These all exhibit bands at ν 1460—1435 (NO) and 1220—1180 cm^{-1} (C=S) and were further characterized by ^1H n.m.r. and mass spectra, and elemental analysis.† The position of N-nitrosation, *i.e.* next to R^2, was confirmed in each case by



a; $R^1 = R^2 = Me$
b; $R^1 = C_6H_{11}$, $R^2 = Me$
c; $R^1 = R^2 = Et$
d; $R^1 = C_6H_{11}$, $R^2 = Et$
e; $R^1 = C_6H_{11}$, $R^2 = CH_2CH_2F$

SCHEME. *Reagents:* 0·1 N HCl; ii, 1·0 N HCl or HCOOH; iii, anhydrous HCl in ether; iv, H_2O; v, an excess of $Na^{15}NO_2$.

the characteristic ^1H n.m.r. chemical shifts of the adjacent alkyl groups.[6a] This group of compounds includes the first reported example, compound (**3e**), of a thio-analogue of a clinically active 2-halogenoethylnitrosourea.

TABLE. ^{15}N Chemical shifts of nitrosothioureas.[a,b]

Compound	Solvent (conc./M)	δ_N/p.p.m. N(3)	>N(1)	N=O
(**3a**)	$CHCl_3$ (1)	98·5	273·7	556·0
	aqueous HCl (0·01)	c	c	560·8
	MeCN (0·01)	c	c	559·0
	EtOH (0·01)	c	c	560·9
	EtOH (1)	121·1	273·9	560·8
(**3b**)	$CHCl_3$ (1)	141·9	273·6	555·5
	EtOH (0·01)	c	c	560·8
	EtOH (1)	147·5	274·1	560·1
(**3c**)	$CHCl_3$ (1)	130·5	285·3	556·1
(**3d**)	$CHCl_3$ (1)	141·1	285·2	552·2
(**3e**)	$CHCl_3$ (1)	142·6	275·8	557·5

[a] Proton-decoupled spectra were taken using NH_3 as standard and were measured to an accuracy of ±0·05 p.p.m. *Ca.* 85 000 scans were required for natural abundance and approximately 1000 scans for ^{15}N-enriched compounds. [b] The relaxing agent Cr(acetylacetone)$_3$ was used at 0·05 M. [c] Owing to enrichment in N=O the unenriched N(1) and N(3) atoms were not observed for the concentration and number of scans used.

^{15}N N.m.r. chemical shifts are strongly dependent on electronic, steric, and solvent effects and are therefore useful in identifying N-containing reaction intermediates.[9] The ^{15}N n.m.r. chemical shifts of the nitrosothioureas (**3**) in $CHCl_3$ and EtOH are given in the Table. The N(3) signals

† All new compounds cited in this communication had ^1H n.m.r., ^{13}C n.m.r., i.r., and mass spectra, together with elemental analyses (± 0·3%), in accord with the assigned structures.

appear in the range δ_N 98·5—147·5 p.p.m., and the N(1) signals in the range δ_N 273·6—285·3 p.p.m. The assignments of the $^{15}N{=}O$ signals, which appear in the range δ_N 556·0—560·9 p.p.m., were confirmed by specific labelling using $Na^{15}NO_2$ (95% enrichment).

Treatment of the thioureas (**2a**) or (**2c**) (0·1 mmol) with $Na^{15}NO_2$ (1 equiv., 95% enrichment) in 0·1 N HCl (10 ml) generated a compound with a yellow colour. Simultaneous accumulation of ^{15}N resonances revealed three peaks at δ_N 354·0 ($H_2{}^{15}NO_2^+$), 560·7 ($N{-}^{15}N{=}O$), and 588·1 p.p.m. ($Na^{15}NO_2$).[10] The peak at δ_N 560·7 p.p.m. slowly increased at the expense of the δ_N 354·0 and 588·1 p.p.m. peaks which disappeared after 1 h. The nitrosation of the thioureas (**2a**) or (**2c**) was repeated using 1·0 N HCl in EtOH (to reduce the nucleophilicity of the solvent) and at lower temperature (−10 °C) (to increase the lifetime of intermediates). Under these conditions the solution immediately turned transient light red and a new ^{15}N n.m.r. peak appeared at δ_N 762·9 p.p.m. which was ascribed to the thionitrosyl compounds (**4**).‡ ^{15}N N.m.r. studies have provided chemical shifts for the thionitrosyl compounds (**4**) in the same region (*e.g.* EtSNO, δ_N 764 p.p.m.[10]). The δ_N 762·9 p.p.m. peak disappeared after 30 min at −10 °C, which showed that under these conditions the nitrosothioureas were not formed (the δ_N 560·8 p.p.m. peak was not detected) and that the ureas (**5**) were the only products.

In contrast, similar treatment of the thiourea (**2c**) with $NaNO_2$ (1 mol equiv.) in HCOOH afforded elemental sulphur together with the unchanged thiourea (**2c**), the urea (**5c**), and the nitrosourea (**6c**).

A thionitrosyl intermediate similar to (**4**) has been proposed to form at 0 °C during the reaction of NOCl with thioamides.[7,11] In one instance, reaction with HCl formed HNSO which decomposed to give products which included S and NO.[12] More stable thionitrosyl compounds than (**4**) have been further characterized by u.v. and i.r. spectra.[13] The transient nature of the compounds (**4**) precludes such spectral examination and dictates that ^{15}N n.m.r. at low temperature is ideally suited for their detection.

Treatment of the nitrosothioureas (**3a**) with anhydrous HCl in ether followed by addition of water afforded the urea (**5a**) quantitatively. Reaction of the nitrosothiourea (**3a**) with excess of $NaNO_2$ in HCl gave the nitrosourea (**6a**) in high yield. These data are in accord with the Scheme and suggest that the ureas (**5**) have been formed *via* the thionitrosyl intermediate (**4**) followed by attack of water on the amide carbon with displacement of the SNO group.

An analogous conversion of thiocarbonyl groups into carbonyl groups *via* S-protonation followed by nucleophilic attack at the adjacent carbon has been proposed.[7d,14]

It may be concluded that when thioureas are treated with $NaNO_2$ and 0·1 N HCl (1 equiv.) the nitrosothioureas are probably formed by direct N-nitrosation and not *via* a thionitrosyl compound,§ contrary to the suggestions of S → N transfer of an N=O group in the thiourea case.[7] The results should open the way to the synthesis of a variety of 2-halogenoethylnitrosothioureas as potential anticancer agents.

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‡ Owing to the specific enrichment in the N=O group and the transient nature of compounds (**4**) the unenriched N(1) and N(3) signals were not observed for the number of scans permitted by the lifetime of (**4**).

§ One of the referees has suggested that under conditions of mild acidity a low concentration of thionitrosyl compound may form. We consider that the thionitrosyl compound would rapidly hydrolyse to urea under these conditions.

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