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Synthesis of nitroxide derivatives of alkylnitrosourea

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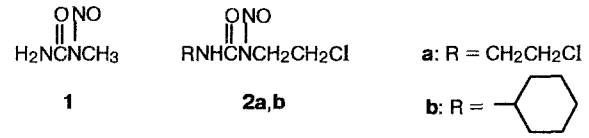
Nitroxide derivatives of alkylnitrosoureas were prepared by the reaction of amino nitroxide radicals with activated N-alkyl-N-nitrosocarbamates or by the selective reduction of oxoammonium derivatives of alkylnitrosoureas.

Key words: alkylnitrosourea nitroxides, amino nitroxides, activated N-alkyl-N-nitrosocarbamates, akylnitrsoureidooxoammonium salts.

The employmet of alkylnitrosoureas (**1**, **2a,b**) in the chemotherapy of tumors has stimulated the synthesis and investigation of compounds of this type.[1] On the other hand, improvement in the therapeutic properties of known antitumor agents as a result of the introduction of nitroxide substituents therein has been demonstrated in many examples[2].



1 **2a,b**

Here we shall describe methods for the synthesis of nitroxide derivatives of alkylnitrosoureas, which were patented earlier.[3,4] Some of the substances reported here were prepared independently by two other groups.[5,6]

According to the first method (method A), nitroxide derivatives of alkylnitrosoureas (**5—10**) (Scheme 1, Table 1) were prepared by reacting amino radicals (**3a—e**) with activated esters (**4a,b**) in DMF. This method was initially suggested[7] for the regioselective synthesis of asymmetrically substituted dialkylnitrosoureas.

According to the second method (method B), alkylnitrosoureas **5**, **6**, **9**, and **13** were prepared by

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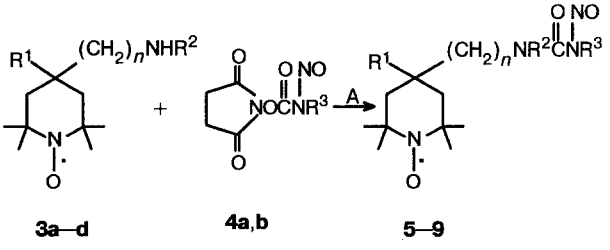
Table 1. Characteristics of alkylnitrosolurea nitroxides **5—10, 12, 7 + 19, and** and **8 + 20**

Compound	R^1	R^2	R^3	n	Method	Yield (%)	Mp (°C) (dec.)	Solvent for recrystallization	Molecular formula (M.w.)	Found / Calculated (%) C	H	N
5	H	H	Me	0	A B	72 90	133—135	$CHCl_3$—hexane	$C_{11}H_{21}N_4O_3$ (257.31)	51.60 51.35	8.43 8.23	21.40 21.77
6	H	H	CH_2CH_2Cl	0	A B	69 91	92—94*	EtOH—H_2O	$C_{12}H_{22}ClN_4O_3$ (305.78)	47.00 47.13	7.24 7.25	18.30 18.32
7	H	H	CH_2CH_2Cl	1	A	81	115—117.5	$CHCl_3$—hexane	$C_{13}H_{24}ClN_4O_3$ (319.81)	48.30 48.82	7.52 7.56	17.60 17.52
8	H	H	CH_2CH_2Cl	2	A	74	65—71	CC***	$C_{14}H_{26}ClN_4O_3$ (333.84)	50.10 50.37	7.67 7.85	17.00 16.78
9	H	Me	CH_2CH_2Cl	0	A B	57 68	Oil**	CC***	$C_{13}H_{24}ClN_4O_3$ (319.81)	48.00 48.82	7.54 7.56	17.30 17.52
10	—	—	—	—	A	75	118—120	$CHCl_3$—hexane	$C_{11}H_{20}ClN_4O_3$ (291.76)	45.30 45.28	6.75 6.91	19.30 19.20
13	COOH	H	CH_2CH_2Cl	0	B	89	129—132	MeOH—H_2O	$C_{13}H_{22}ClN_4O_5$ (349.79)	44.50 44.64	6.27 6.34	16.20 16.02
7+19	—	—	—	1	B	88	92—105	$CHCl_3$—hexane	$C_{13}H_{24}ClN_4O_3$ (319.81)	48.50 48.82	7.51 7.56	17.50 17.52
8+20	—	—	—	2	B	86	Oil	CC***	—	—	—	—

*Ref.:[5] mp 92°C, Ref.:[6] mp 80—83°C.
**Ref.:[6] mp 68—70°C.
***Isolated by column chromotography on silica gel, eluent $CHCl_3$—acetone—MeOH (10:1:1).

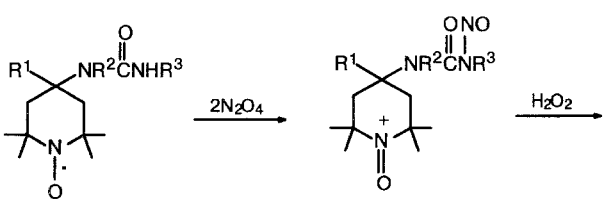
Scheme 1



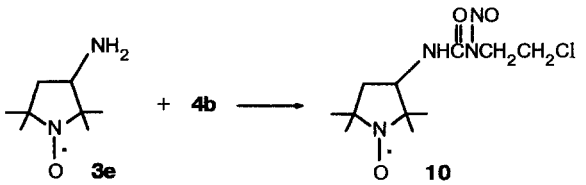
a: $R^1 = R^2 = H$, $n = 0$
b: $R^1 = R^2 = H$, $n = 1$
c: $R^1 = R^2 = H$, $n = 2$
d: $R^1 = H$, $R^2 = Me$, $n = 0$

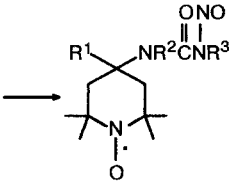
a: $R^3 = Me$
b: $R^3 = CH_2CH_2Cl$

Scheme 2



a: $R^1 = R^2 = H$, $R^3 = Me$
b: $R^1 = R^2 = H$, $R^3 = CH_2CH_2Cl$
c: $R^1 = H$, $R^2 = Me$, $R^3 = CH_2CH_2Cl$
d: $R^1 = COOH$, $R^2 = H$, $R^3 = CH_2CH_2Cl$



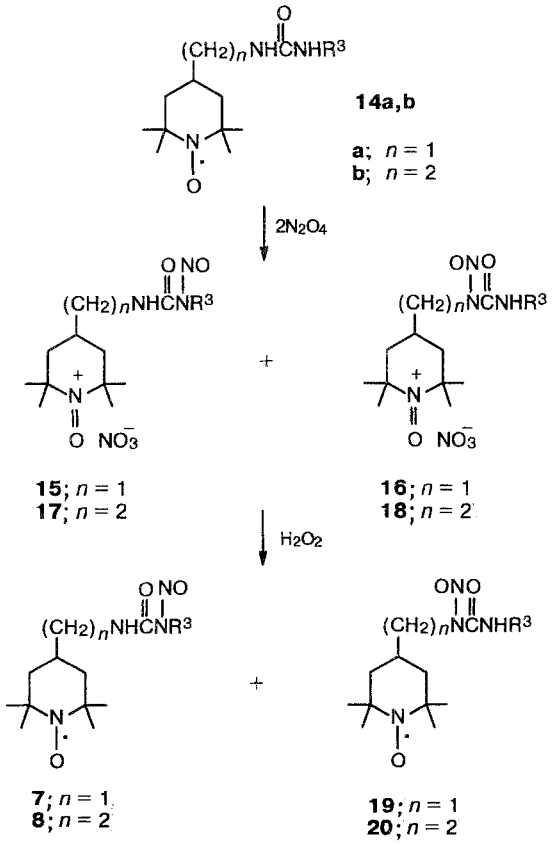


5, 6, 9, 13

selective reduction of the corresponding oxoammonium derivatives (**12a—d**) (see Ref.[8]) under the action of hydrogen peroxide in a neutral or weakly acidic medium. The optimal reduction conditions were found on the basis of data on the kinetics of the reaction of oxoammonium salts with H_2O_2.[9] At 0°C and pH 7—8 the reaction goes practically to completion during the period of mixing of the reactants, and the target products precipitated from the solution as crystals or oils.

The samples of compounds **5** and **6** obtained by the two methods were identical and consisted of the 3-nitroso isomers. According to HPLC, radicals **5**, **6**, and **13** prepared by the reduction of salts **12a**, **b**, and **d** did not contain any admixture of 1-nitroso isomers. The regioselectivity in the synthesis of akylnitrosoureidooxoammonium salts **12a**, **b**, and **d** from the corresponding substituted ureas (**11**)[8] is evidently caused by the steric hindrance of the piperidyl substituent. The nitrosation of ureas (**14a**, **b**), in which the bulky piperidyl and ureido groups are separated by one or two methylene fragments, yields pairs of isomers: (**15**, **16**) and (**17**, **18**) respectively. Reduction of these pairs gave mixtures of radicals, in which the ratios between of the regioisomers were equal to 2 (**7** : **19**) and 9 (**20** : **8**).

Scheme 3



In the case of **14b**, the shielding effect of the piperidyl substituent is too weak and the direction of nitrosation is governed mainly by the inductive $-I$ effect of the chloroethyl group.

The advantage of method A lies in the possibility of the selective introduction of a nitroso group in only one of the two possible positions in the asymmetrically substituted urea. Method B is more practicable and provides a better yield of the products. Besides, this method is of interest for synthesizing new nitroxide radicals by means of the intermediate formation of oxoammonium salts. In this case, it is possible to realize electrophilic substitution in radicals **11** by protecting the $>N^{\cdot}—O$ group which is unstable toward electrophiles by oxidizing it to $>N^{+}=O$. The synthesis according to method B involves "removal of the protection" followed by electrophilic substitution and the formation of radicals **5—9**, **13**, **19**, and **20**.

Yellow or pink crystalline alkylnitrosoureas **5—8**, **10**, **13**, and **7** + **19** are storage-stable and decompose when heated to their melting points with the evolution of gas. Compounds **9** and **8** + **20** are red oils and decompose with the evolution of gas at >70° C. The stability of the products is nearly the same as that of the known 1,3-dialkylnitrosoureas. The β-chloroethyl derivatives are more stable in unbuffered hydroalcoholic solutions. For example, the half-lives of compounds **5** and **6** are 9 and 70 h, respectively, in H_2O—EtOH (3 : 1) at 20°C. The higher stability of **6** may be explained by the stabilizing effect of an acidic medium due to the partial hydrolysis of the β-chloroethyl group.

The structure of the compounds obtained was confirmed by elemental analysis and spectral data (Tables 1, 2). The IR spectra of the nitrosoureas (except for **9**) contained only one band of moderate intensity at 3270—3340 cm^{-1}, which corresponds to stretching vibrations of the N—H bond. The presence of the electronegative N-nitroso group is manifested by displacement of the band of the carbonyl group to a higher frequency (1690—1730 cm^{-1}) and the amide II band (CONH) to a lower frequency (1520—1540 cm^{-1}) in comparison to the corresponding ureas **11** and **14**.[8]

The electronic spectra of the alkylnitrosoureas in the visible region displayed a band of the nitrosoureido group with a maximum at about 400 nm, which partially (for **5—9**) and completely (for **10**, **13**) overlaps a weaker band of the nitroxide group. In the UV region of the spectra there was a broad band with a maximum at about 235 nm, which is attributed to the overlap of the absorption of the nitrosoureido and nitroxide groups.

Three lines of equal intensity were observed in the ESR spectra of dilute solutions of the alkylnitrosoureas. The hyperfine coupling constants are characteristic of piperidyl and pyrrolidine nitroxide radicals.

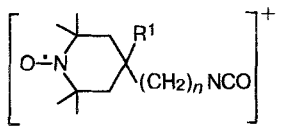
There were peaks of the molecular ions in the mass spectra of **5—8**, **10**, and **13**. The chloroethyl derivatives produced doublet peaks for the molecular ions, which corresponded to the natural abundance of the chlorine

Table 2. Spectral data of alkylnitrosolurea nitroxides **5—10, 12, 7 + 19,** and **8 + 20**

Compound	IR spectrum*		UV spectrum (in EtOH)		ESR spectrum (benzene, 25°C)	Mass spectrum**, m/z (I_{rel}, %)
	ν (cm^{-1})	Group	λ_{max} (nm)	ε l(mol cm)$^{-1}$	a_N (mT) (±0.01)	
5	3292	N—H	448	11.1	1.55	257 $[M]^+$ (6),
	1725	C=O	415	92		197 $[M-60]^+$ (27)
	1532	C(O)NH	397	111		154 $[M-103]^+$ (18)
			384 sh	84		141 $[M-116]^+$ (32)
			368 sh	53		84 $[M-173]^+$ (100)
			233	8200		
6	3272	N—H	449	11.3	1.55	307 $[M]^+$ (1.6)
	1725	C=O	418	92		305 $[M]^+$ (4.7)
	1532	C(O)NH	400	105		197 $[M-108]^+$ (10)
			387 sh	74		191 $[M-116]^+$ (3.1)
			373 sh	48		189 $[M-116]^+$ (9)
			234	8100		154 $[M-151]^+$ (13)
						84 $[M-221]^+$ (100)
7	3328	N—H	451 sh	10.9	1.56	321 $[M]^+$ (0.9)
	1727	C=O	418	87		319 $[M]^+$ (2.7)
	1525	C(O)NH	399	100		211 $[M-108]^+$ (33)
			385 sh	73		125 $[M-194]^+$ (53)
			370 sh	45		69 $[M-250]^+$ (100)
			234	7700		
8	3318	N—H	450 sh	10.4	1.55	335 $[M]^+$ (0.7)
	1728	C=O	418	91		333 $[M]^+$ (2.3)
	1528	C(O)NH	399	104		225 $[M-108]^+$ (41)
			385 sh	74		138 $[M-195]^+$ (58)
			370 sh	45		41 $[M-292]^+$ (100)
			234	8100		
9	1693	C=O	449 sh	8.9	1.56	—
			415 sh	59		
			392	195		
			375 sh	75		
			242	6950		
10	3334	N—H	418	85	1.40	293 $[M]^+$ (4.3)
	1730	C=O	400	101		291 $[M]^+$ (12)
	1539	C(O)NH	387 sh	73		183 $[M-108]^+$ (78)
			371 sh	46		177 $[M-116]^+$ (17)
			234	8000		175 $[M-116]^+$ (51)
						97 $[M-196]^+$ (100)
13	3340	N—H	418	105	1.50	351 $[M]^+$ (0.1)
	2560	COOH	400	125		349 $[M]^+$ (0.3)
	1686, 1722, 1732	C=O	386	97		241 $[M-108]^+$ (15)
	1523, 1542	C(O)NH	372 sh	72		155 $[M-194]^+$ (36)
			236	8300		41 $[M-308]^+$ (100)
7+19 (2:1)	3335	N—H	450 sh	11.0	1.56	—
	1727	C=O	418	88		
	1525	C(O)NH	400	102		
			386 sh	70		
			371 sh	44		
			234	7800		
8+20 (1:9)	3325	N—H	450 sh	10.6	1.55	—
	1725	C=O	418	90		
	1530	C(O)NH	400	102		
			385 sh	76		
			369 sh	43		
			235	7900		

*For **9** and **8+20**, neat; for other compounds, in vaseline oil.
For **5, **6**, **8**, and **10** at 70 eV, for **7** and **13** at 13 eV.

isotopes. The most typical path for fragmentation of the molecular ions is the elimination of R^3 N_2OH to form, in the case of **5**—**8** and **13**, fragment ions having general formula **21**.



21

In the mass spectra of **6**, and **10** the $[M-116]^+$ ions included the chloroethyl group. Their formation may be explained by the fragmentation of the molecular ion

with the successive elimination of NO and $O^{\cdot}\overline{NCMe_2CH_2}$ (cf. Ref.[8]).

Experimental

IR spectra of liquids (**9**, **8** + **20**) or Nujol mulls were recorded on a Specord 75-IR spectrophotometer, electronic spectra of ethanolic solutions, on a Specord UV-VIS spectrophotometer, ESR spectra in benzene at 25°C, on a EPA-2A radio-frequency spectrometer, and electron-impact mass spectra, on a Hitachi M 80B mass spectrometer. HPLC was performed on a Milichrom chromatograph with a 2×64 mm column packed with Separon C_{18}, 5 μ, 50 % MeOH as the eluent, and detection at 240 nm. The molar extinction coefficients ε at 240 nm are the same (within the experimental error of ±3 %) for **7** and a **7** + **19** mixture, as well as for **8** and an **8** + **20** mixture, and the quantitative composition of mixtures was determined from the ratio between peak areas. The retention volumes for the isomers were 850 and 980 μL (for **7** and **19**) and 1300 and 1575 μL for **8** and **20**, respectively. The melting points were determined on an PHMK hot-stage apparatus. The starting compounds were prepared as described elsewhere: **3a**,[10] **3b**,[11] **3c**,[12] **3d**,[13] and **3e**.[14]

Method A. 2,2,6,6-Tetramethyl-4-[3-(2-chloroethyl)-3-nitrosoureido]piperidine-1-oxyl (6). A solution of 1.71 g (10 mmol) of **3a** in 3 mL of DMF was added over the course of 5 min to a solution of 2.74 g (11 mmol) of activated ester **4b**[7] in 5 mL of dry DMF with stirring and cooling to 0°C. After stirring for 1 h at 0°C, the reaction mixture was poured into 100 g of crushed ice. The precipitate formed was separated, washed with cold water, and dried. The yields of **6**, the solvents for recrystallization, the melting points and the data from elemental analysis are given in Table 1.

Compounds **5** and **7**—**10** are obtained in the same way. When the reaction mixture was poured onto crushed ice, compounds **8** and **9** separated as oils. They were extracted with ether (3×20 mL), dried with Na_2SO_4, and chromatographed on a silica gel column with $CHCl_3$—acetone—MeOH (10 : 1 : 1) as the eluent.

Method B. 2,2,6,6-Tetramethyl-4-(3-methyl-3-nitrosoureido)piperidine-1-oxyl (5). To a stirred and ice-cooled solution of 3.19 g (10 mmol) of **12a**[8] in 10 mL of water were simultaneously added over the course of 5 min 0.58 mL (5 mmol) of 30 % aqueous H_2O_2 and a solution of 0.84 g $NaHCO_3$ in 5 mL of H_2O. The reaction was accompanied by the evolution of gas, and **5** precipitated from the solution as pink crystals, which were separated, washed with ice water, and dried in air; the yield was 90 %.

Compounds **6**, **9**, and **13** and the **7** + **19** and **8** + **20** mixtures of isomers were prepared in the same manner. In the synthesis of acid **13**, the final pH of the reaction solution was adjusted to 2—3. Oily products **9** and **8** + **20** were extracted with ether and chromatographed on a silica gel column.

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