81571-35-9; **17**, 81600-06-8; **18**, 96806-27-8; **19**, 81571-34-8; **20**, 96806-28-9; **21**, 81571-36-0; **22**, 81600-05-7; **23**, 81571-31-5; **24**, 81571-33-7; **25**, 96806-29-0; **26**, 81571-32-6; **27**, 81525-80-6; VLB, 865-21-4; VCR, 57-22-7; VDS, 53643-48-4; L-Leu-OMe, 2666-93-5; L-Leu-OEt, 2743-60-4; D-Leu-OEt, 37763-22-7; L-Leu-OBu, 2885-08-7; L-Leu-O-*n*-octyl, 51181-88-5; L-Leu-amide, 687-51-4; L-Ile-OMe, 2577-46-0; L-Ile-OEt, 921-74-4; L-Trp-OMe, 4299-70-1; L-Trp-OEt, 7479-05-2; L-Trp-OBu, 31338-08-6; D-Trp-OEt, 74126-25-3; L-Ala-OEt, 3082-75-5; L-Val-OEt, 17431-03-7; L-Phe-OEt, 3081-24-1; L-Ser-OEt, 4117-31-1; L-Tyr-OEt, 949-67-7; L-ε-(trifluoroacetyl)-Lys-OEt, 96806-30-3; L-Glu-OEt, 52454-78-1; Val-Trp-OEt, 81525-83-9.

Decomposition Reactions of (Hydroxyalkyl)nitrosoureas and Related Compounds: Possible Relationship to Carcinogenicity

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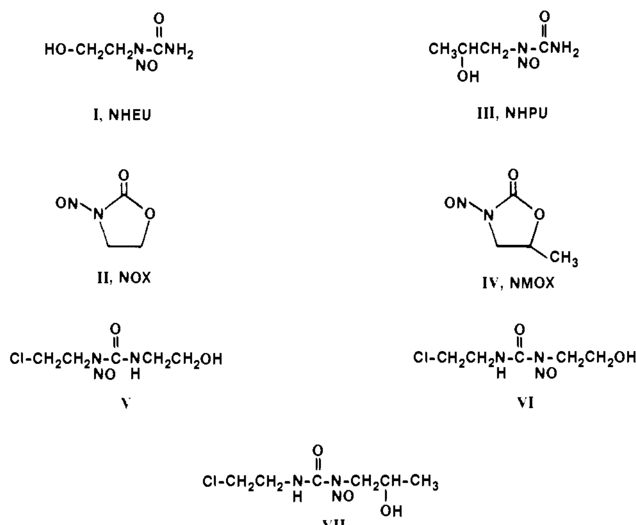
(Hydroxyalkyl)nitrosoureas and the related cyclic carbamates *N*-nitrosooxazolidones are potent carcinogens.[1] The decompositions of four such compounds, 1-nitroso-1-(2-hydroxyethyl)urea (I), 3-nitrosooxazolid-2-one (II), 1-nitroso-1-(2-hydroxypropyl)urea (III), and 5-methyl-3-nitrosooxazolid-2-one (IV), in aqueous buffers at physiological pH were studied to determine if any obvious differences in decomposition pathways could account for the variety of tumors obtained from these four compounds. The products predicted by the literature mechanisms for nitrosourea and nitrosooxazolidone decompositions (which were derived from experiments at pH 10–12) were indeed the products formed, including glycols, active carbonyl compounds, epoxides, and, from the oxazolidones, cyclic carbonates. Furthermore, it was shown that in pH 6.4–7.4 buffer epoxides were stable reaction products. However, in the presence of hepatocytes, most of the epoxide was converted to glycol. The analytical methods developed were then applied to the analysis of the decomposition products of some related dialkylnitrosoureas, and similar results were obtained. The formation of chemically reactive secondary products and the possible relevance of these results to carcinogenesis studies are discussed.

Nitrosoureas have long been of interest both as chemical carcinogens and also as cancer chemotherapeutic agents. Various nitrosoureas have been shown to cause a wide variety of tumors in test animals. Lijinsky and Reuber[1] recently reported the results of carcinogenicity studies on a group of four related nitroso compounds, 1-nitroso-1-(2-hydroxyethyl)urea (I, NHEU), 3-nitrosooxazolid-2-one (II, NOX), 1-nitroso-1-(2-hydroxypropyl)urea (III, NHPU), and 5-methyl-3-nitrosooxazolid-2-one (IV, NMOX). Compound I caused tumors in a wide variety of organs, with the most frequent tumors occurring in the lung and forestomach, although several other organs were affected. The other nitrosourea (III) caused mainly lymphomas and leukemias and forestomach tumors with a scattering of tumors in other organs. The nitrosooxazolidones, in contrast, caused forestomach tumors almost exclusively. We have undertaken a study of the decomposition reactions of these four compounds in aqueous buffers at physiological pH, seeking patterns of product formation that might be related to carcinogenesis. These reactions are also compared with those of some dialkylnitrosoureas that are chemically closely related to I and III and are currently being tested for carcinogenicity.



Results

According to the literature decomposition pathways (determined at pH 10–12) for nitrosoureas[2] and nitrosooxazolidones,[3] one may anticipate the formation of certain products (Schemes I and II). NHEU (I) could give acetaldehyde, ethylene glycol, and ethylene oxide, II could give these compounds and ethylene carbonate, III should give acetone, propylene glycol, propionaldehyde and propylene oxide, while IV would be expected to give these same products plus propylene carbonate. Different methods of analysis were required for the different classes of compounds. Glycols are particularly difficult to analyze and are not readily extracted from aqueous solutions. Direct GC analysis of glycols is possible, but quantitation is difficult since the glycols are apt to adhere to the column packing and elute as "ghost peaks" in later injections particularly when aqueous solutions are employed.[4] Direct GC analysis of the cyclic carbonates is, however, readily achieved.

The test compounds were dissolved in pH 7.4, 0.067 or 0.1 M phosphate buffer at 37 °C. Several different methods of analysis were employed. The reactions were monitored for disappearance of starting material by HPLC,

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(1) Lijinsky, W.; Reuber, M. D. *Cancer Res.* **1982**, *43*, 214–221.
(2) Hecht, S. M.; Kozarich, J. W. *J. Org. Chem.* **1973**, *38*, 1821–1824.
(3) Hassner, A.; Reuss, R. H. *J. Org. Chem.* **1974**, *39*, 553–560.
(4) Porter, W. A.; Auansakul, A. *Clin. Chem.* **1982**, *28*, 75–78.

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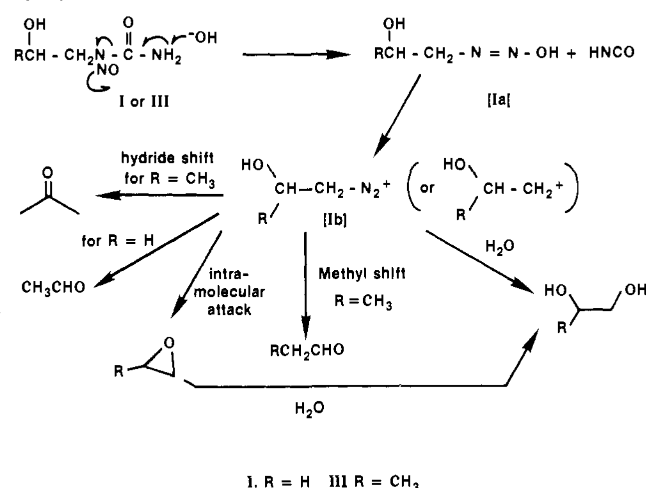
Table I. Decomposition Products from Compounds I–IV and Percent Yields

compd	half-life, min	as DNP: CH_3CHO	GC analysis: RCHO	GC analysis: epoxide $\overline{OCH_2}CHR$	GC analysis: $(CH_3)_2CO$	GC analysis: N_2	glycol[b]	cyclic carbonate
NHEU (I)	14	38				105	50	
NHEU (I) by NMR[c]		33		8			50	
NOX (II)	50	5				92	30	50
NHPU (III)								
buffer	15		8	17	53	100	24	
media			6	18	57		20	
media and hepatocytes			0	2.5	52		44	
NMOX (IV)	99		3	trace	18	100	33	50

[a] Super Q, 1.8 m × 2 mm, 60 mL/mm of He, 110 °C (5-min hold), 5 °C/min to 210 °C; direct injection of aqueous reaction mixture. [b] Analyzed as phenylboronate ester. [c] pH 6.4 in D_2O 0.067 M phosphate buffer at 25 °C; 8% NHEU remaining at 96 h; percentages based on total integratable protons.

Scheme I



with time points taken every 6 min and samples were analyzed immediately. Bubble formation in the reaction mixture made accurate sampling difficult, but half-lives were established from a first-order semilog plot of concentration vs. time. Results are shown in Table I. The glycols were quantified by derivatization with phenylboronic acid.[4] The resultant cyclic ester is readily analyzed by GC on a polar stationary phase (3% SP 2310 on Supelcoport). The cyclic carbonates in the same reaction mixture can also be analyzed on this column. The carbonates do slowly hydrolyze to the glycols, ethylene carbonate giving a 30% yield of ethylene glycol in 18 h in buffer at 37 ° C, but if the analyses were run within a few hours of the derivatization, no increase in glycol duue to carbonate hydrolysis was seen. In another experiment, NOX (II) was decomposed in 0.067 M phosphate buffer in D_2O (pH 6.4), at 25 °C, and the decomposition was followed by NMR. Ethylene glycol and ethylene carbonate were both observed early in the decomposition, and the ratio of carbonate to glycol remained constant (2/1) over the first 16 h of the reaction (ca. 1 half-life under these conditions). After 41 h and again after 5 days, the ratio was 1.6/1. When ethylene carbonate was placed in buffer under these conditions (4 mg of substrate to 1 mL of 0.067 M buffer), no decomposition occurred in 24 h. It therefore seems likely that the glycol and carbonate are formed via different pathways from the decomposition of the intermediate IIa (Scheme II) and that hydrolysis of the carbonate is not the principal source of the glycol.

The active carbonyl products were first analyzed by formation of the dinitrophenylhydrazones (DNP's) extraction into isooctane and back-extraction into acetonitrile followed by HPLC analysis on a reversed-phase column (Econosphere C_{18}, 5 μm, Alltech).[5] This method proved appropriate for acetaldehyde but was not suitable for quantitation of acetone. The best method of obtaining final product yields for all the volatile products except acetaldehyde was direct GC injection of the aqueous reaction mixture onto a column packed with Super Q. Propylene oxide, propionaldehyde, and acetone were resolved and quantified under these conditions, with retention times of 8.9, 9.9, and 10.9 min, respectively. Results are presented in Table I. Sizable yields of propylene oxide (18%) were obtained from NHPU (III), but ethylene oxide as a product from NHEU (I) could not be determined by this method since ethylene oxide and acetaldehyde coelute on the Super Q column at temperatures as low as 90 °C, with a retention time of 9.0 min.

In another experiment, NHEU was allowed to decompose in an NMR tube in pH 6.4, 0.067 M phosphate buffer in deuterium oxide, with sodium 3-(trimethylsilyl)-propionate-*2,2,3,3-d*$_4$ (TSP) as internal standard. The sample was monitored several times during the course of the decomposition, and peaks corresponding to ethylene oxide (δ 2.80), acetaldehyde (δ 1.30 and 2.23), and ethylene glycol (δ 3.70) appeared, while the starting material gave two triplets centered at δ 3.60 and 4.00. Acetaldehyde shows one methyl peak in organic solvents but two methyl peaks in water, probably due to the presence of free and hydrated species. The reaction was run slowly (at pH 6.4 and low buffer/substrate concentration) so that bubble formation would not interfere with the spectral resolution. The NMR spectra obtained at t = 0, 20, and 96 h are shown in Figure 1. From the integrations obtained from the 96-h trace, yields of each product could be estimated: acetaldehyde, 33%; ethylene oxide, 8%; NHEU, 8%; and ethylene glycol, 50%. These values compare favorably with yields obtained for acetaldehyde by HPLC (34–40%) and ethylene glycol by GC (45–50%). There is no evidence that the acetaldehyde formed is undergoing exchange with the solvent, since the NMR spectra are not dissimilar at 29 and 96 h, and the amount of acetaldehyde relative to the other products as determined by NMR integration is the same as the yield determined by derivitization and quantitation.

A series of dialkylnitrosoureas that are structurally related to compounds I and III were also subjected to decompositions in buffer followed by product analysis. These compounds include both nitroso derivatives of 1-(2-chloroethyl)-3-(2-hydroxyethyl)urea (V and VI), and 3-(2-chloroethyl)-1-(2-hydroxypropyl)-1-nitrosourea (VII). Results are given in Table II, along with results from lit-

(5) Farrelly, J. G.; Stewart, M. L.; Hecker, L. I. *Chem.-Biol. Interact.* 1982, *41*, 341–351.

Scheme II

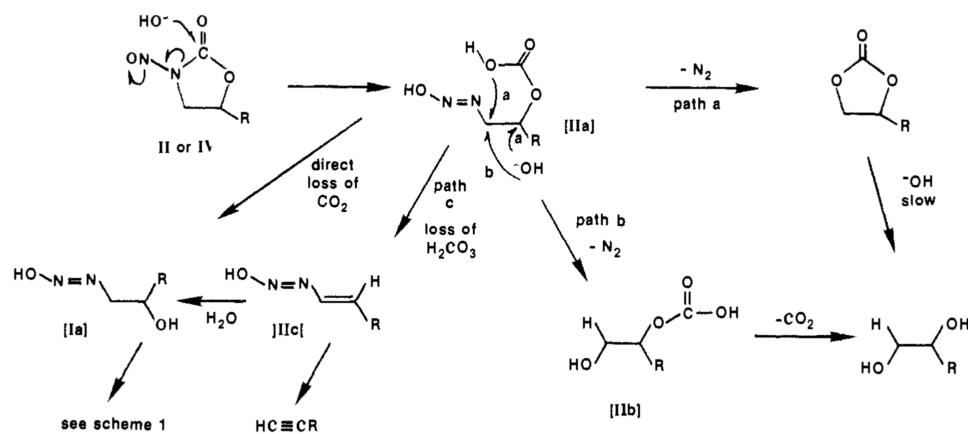


Table II. Decomposition of Dialkylnitrosoureas at pH 7.4[a]

compd	RCH_2CHO	$RCH(OH)CH_2OH$	R-oxirane	$(CH_3)_2C{=}O$	$ClCH_2CH_2OH$
BCNU[b]	20	2	0		52
$HOCH_2CH_2N(NO)C(O)NHCH_2CH_2OH$ [b]	33	23			
$ClCH_2CH_2N(NO)C(O)N(CH_3)_2$ [b]	31	14			5
$HOCH_2CH_2N(NO)C(O)NHC_6H_{11}$ [c]	25-35	20-25			8-10
$ClCH_2CH_2N(NO)C(O)NHC_6H_{11}$ [c]	5-10	nd			18-25
$HOCH_2CD_2N(NO)C(O)NHC_6H_{11}$ [c]	MeCHO-*d*	*d**	D_2-oxirane		
$HOCH_2CH_2N(NO)C(O)NHCH_2CH_2Cl$	60-70	25-30	*d**		0
$ClCH_2CH_2N(NO)C(O)NHCH_2CH_2OH$	25	0			*d*
$CH_3CH(OH)CH_2N(NO)C(O)NHCH_2CH_2Cl$	5-6	17	18	50-60	nd

[a] R = CH_3 or H, as appropriate. [b] Reference 6, bis(2-chloroethyl)nitrosourea. [c] Reference 7. [d] Demonstrated by NMR. * yield ~10%.

erature studies on similar compounds.[6,7] those compounds in which the nitroso group is on the nitrogen bearing a hydroxyalkyl group gave product yields and distributions virtually identical with the analogous monoalkylnitrosoureas. When the nitroso was α to the chloroethyl group, acetaldehyde and chloroethanol were the major products, as expected from the literature (see Table II).

When the decomposition of VI was monitored by NMR, a peak corresponding to ethylene oxide was observed. Acetaldehyde could not be seen, possibly because it gave a Schiff base by reaction with the 2-chloro-1-ethylamine formed. When the NMR spectra of separate solutions of 2-chloroethylamine and acetaldehyde in D_2O/phosphate buffer were compared with a spectrum of the mixture of the two solutions, the acetaldehyde peaks showed considerable broadening after mixing, which suggests some reaction was occurring with preformed materials. Reaction might occur more readily in a decomposition reaction where it would be limited by the slow rate-determining formation of acetaldehyde and 2-chloroethylamine in a solvent cage.

A pH profile on the rate of decomposition of NHPU from pH 3.0 to 8.0 (see Table III) gave the anticipated result, the reaction being fastest at pH 8 and slowest at pH 3.0. In all cases, analysis of product distribution when

(6) Brundrett, R. B. *J. Med. Chem.* **1980**, *23*, 1245–1247.
(7) (a) Lown, J. W.; Chauhan, S. M. S. *J. Org. Chem.* 1981, *46*, 2480–2489. (b) Lown, J. W.; Chauhan, S. M. S. *J. Med. Chem.* 1981, *24*, 270–279.

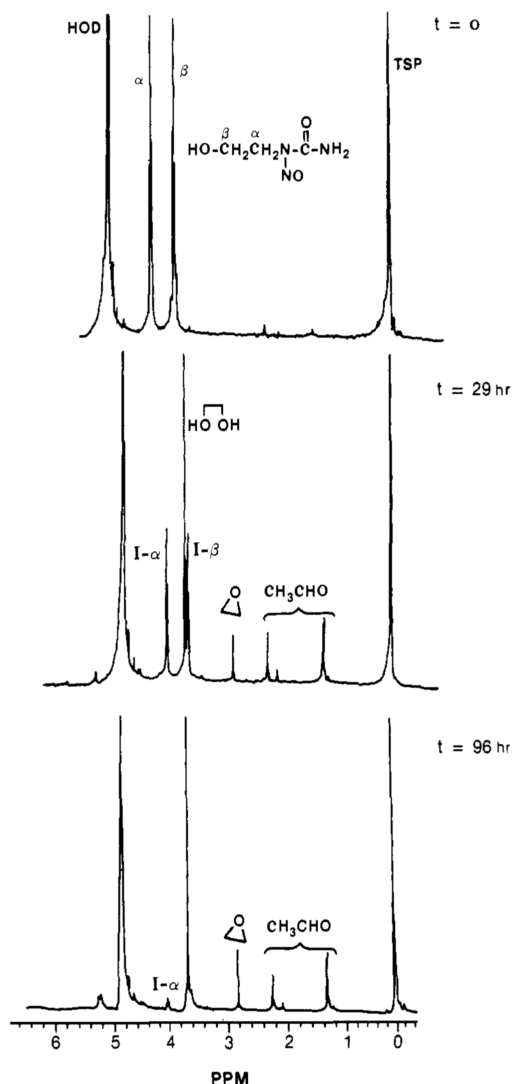


Figure 1. Decomposition of NHEU followed by NMR. A peak corresponding to ethylene oxide (δ 2.80) grew throughout the course of the reaction.

Table III. Decomposition of NHPU (III) at Different pH Values

[buffer], M	pH	half-life, min	Mb/Ms[a]
0.067	8	6.5	4
0.067	7.4	14.5	10
0.067	7.4	9.5	4
0.067 (D_2O)	7.4	70	10
0.067 (D_2O)	6.5	26	10
0.067 (D_2O)	6.0	120	10
0.067 (D_2O)	5.5	410	10
0.10 M	7.4	20	33
0.10 M D_2O	7.4	37.2	33
$HClO_4$, 0.01 M	3.0	600–780	

[a] Ratio of moles of buffer to moles of substrate.

no starting material was remaining gave the same results. Acetone, propylene oxide, propionaldehyde, and propylene glycol were all present and in the same quantities at each pH. Results are also given in Table III showing the change in decomposition rate at higher buffer to substrate ratios and the decrease in reaction rate (isotope effect) in D_2O buffer. Good linear first-order plots of ln concentration vs. time can only be obtained at the higher buffer/substrate ratio ($\geq$10/1).

The decomposition of NHPU (III) in Hank's balanced salt solution with and without hepatocytes was also examined. With medium alone, the products were the same as those found in decompositions in phosphate buffer. When noninduced Fischer 344 rat hepatocytes[5] were present, the reaction products changed dramatically, apparently from the action of cellular enzymes such as epoxidases and aldehyde reductase. Acetone was present in a slightly reduced amount, propylene oxide and propionaldehyde were absent, and the yield of propylene glycol increased by the amount of the missing propylene oxide (see Table I). A small amount of 2-propanol was observed (less than 1%). If 1-propanol was formed, it could not be determined because it would coelute with the acetone.

Discussion

It has long been assumed that chemical carcinogenesis involves alkylation of DNA by some active molecule derived from the chemical carcinogen. Since nitrosoureas can be made to form an alkylating moiety simply by placing them in aqueous alkaline solution and thus do not require metabolic activation, they are considered to be "direct-acting" mutagens and presumably direct-acting carcinogens. They can be expected to decompose on exposure to physiological fluids, and a study of their decomposition reactions at physiological pH's could shed some light on their mechanism of action. The two primary nitrosoureas studied here (I and III) present a synthetic problem, because the standard synthesis of the urea can also lead to the corresponding oxazolidone (II and IV, respectively), and care must be taken to assure that each urea is free of the oxazolidone.[1] However, it is apparent that the nitrosoureas do not convert to the nitroso-oxazolidones under the decomposition conditions used, and at no time was a cyclic carbonate, which is unique to nitroso oxazolidone decomposition, obtained from the nitrosoureas.

The most widely accepted mechanism for decomposition of nitrosoureas is that of Hecht and Kozarich[2] in which decomposition is initiated by abstraction of a proton from the primary NH_2 (See Scheme I). Other mechanisms proposed include nucleophilic attack at the carbonyl[8] or at the nitroso amine nitrogen.[9] Whichever mechanism prevails, the ultimate products are HNCO or its hydrolysis products and a diazohydroxide, the latter either leading to a carbonium ion that will react with water, for example, to give an alcohol or being subject to direct displacement by water to give the alcohol. In the case of I, where a hydroxyl group is already present as a substituent, three possibilities exist: trapping the reactive intermediate from [Ib] with water to give the glycol, intramolecular attack by the hydroxyl to give ethylene oxide, or rearrangement (hydride shift) to give acetaldehyde (Scheme I). The presence of acetaldehyde was confirmed by derivatization as the (dinitrophenyl)hydrazone (DNP) with subsequent HPLC analysis or by direct GC analysis of the reaction mixture. A solution of ethylene oxide in buffer did not react at all with DNPH reagent, even on standing for several days. It could not be proven, however, that ethylene oxide did not form acetaldehyde under GC conditions. Ethylene oxide coeluted with acetaldehyde on GC, but the reaction could be followed in the NMR (Figure 1) and each of the three products could be monitored. Ethylene oxide can be hydrolyzed to ethylene glycol under acidic or basic hydrolysis, but standards were stable under these reaction conditions, and the amount of ethylene oxide increased steadily during the course of the reaction. Thus, epoxide hydrolysis is probably not an important source of glycol.

(8) Snyder, J. K.; Stock, L. M. *J. Org. Chem.* **1980**, *45*, 1990–1999.
(9) Muck, D. L.; Jones, W. M. *J. Am. Chem. Soc.* **1966**, *88*, 3798–3804.

The products expected from III are analogous to those from I, except that more possibilities exist. Acetone was the principal carbonyl compound produced, and a small amount of propionaldehyde also occurred. Propylene oxide is the other major product and can readily be quantified by GC.

The decompositions of nitrosooxazolidones have been studied at some length in the past[3,10] but always under strongly alkaline conditions. Hassner and Reuss[3] elucidated the mechanism of decompositions with a careful product study of the decomposition of various substituted nitrosooxazolidones in methanol/methoxide and an adaptation of their mechanism is shown in Scheme II. Initial attack of hydroxide on the nitrosooxazolidone carbonyl leads to formation of the intermediate β-(hydroxydiazo)ethyl carbonic acid [IIa], which can decompose via three paths: (a) internal attack of the carbonate hydroxide with elimination of N_2 to give the cyclic carbonate, (b) attack by hydroxide at C–N to give the β-hydroxyethyl carbonic acid [IIb], which is unstable and would eliminate CO_2 to give the glycol (it is also possible for the glycol to arise from hydrolysis of the carbonate), and (c) loss of CO_2 to give the vinyl diazohydroxide [IIc], which would, on addition of water, give the same diazohydroxide as the nitrosoureas [Ia] and thus the same products as the nitrosoureas. (It is also possible that the unsaturated carbonium ion (vinyl from NHEU, allyl from NHPU) is the reactive species, as it would lead to the same products).

Hassner and Reuss were able to trap the "vinyl diazohydroxide" as the vinyl ether. In our aqueous conditions such products would be unstable, but the existence of a vinyl diazohydroxide (or carbonium ion) as an intermediate cannot be totally discounted.

The principal products obtained from the decompositions of NOX and NMOX were the cyclic carbonates and glycols. Small amounts of the products from path c (the "nitrosourea products") were obtained, but clearly this is a very minor path. The NMR study of NOX clearly indicated that path a, internal attack to form the cyclic carbonate, was favored, the carbonate to glycol ratio remaining constant (2:1) for 1 half-life (16 h) and then dropping to 1.6 after 41 h, by which time some hydrolysis of carbonate should have occurred. When ethylene carbonate was dissolved in D_2O buffer and monitored in the NMR, no decomposition to glycol was seen in 24 h. Therefore, we can assume that all early glycol formation occurs via path b, attack of hydroxide on the first intermediate to give a β-hydroxyethyl carbonic acid, which then loses CO_2 to give the glycol.

Compounds I–IV are all carcinogens on chronic administration to F344 rats.[1] The nitrosooxazolidones both cause forestomach tumors almost exclusively; the nitrosoureas both cause a variety of tumors, with NHEU (I) giving a wide tumor spectrum, with the principal sites being lung, forestomach, and colon, while NHPU (III) causes tumors of the thymus, leukemias, and forestomach tumors. It was tempting to speculate that forestomach tumors are caused by direct action of the carcinogen at the site of application (the compounds are given by gavage in a corn oil/ethyl acetate solution), but a pH profile (Table III) clearly shows that NHPU is very stable at pH 3 (which would approximate stomach pH), with only an 8% decrease in 2 h, and passage time through the stomach would be much less than that. At pH 7.4 NHEU and NHPU exhibit half-lives of 11 and 35 min, respectively, while the half-lives of the oxazolidones II and IV are 50 and 90 min, respectively. Thus, the compounds that cause predominantly forestomach tumors are the most stable ones in this group. (A reviewer has suggested that the lack of carcinogenicity of the nitrosooxizolidones at sites other than forestomach might be due to the sensitivity of such compounds to esterases. In a study in which the chemical and biological activities of *N*,*N'*-bis(2-chloroethyl)-*N*-nitrosourea, *N*-nitroso-*N*-(2-chloroethyl)acetamide, and *N*-nitroso-*N*-(2-chloroethyl)urethane were compared with and without the presence of esterase, the biological activity of the acetamide and the urethane were strongly affected by the esterase, while the BCNU was not affected at all.[14] The cyclic carbamates III and IV would also be sensitive to esterase and might thus be rapidly decomposed. It may also be relevant to note that other *N*-nitroso carbamates that have been tested for carcinogenicity in rats also give forestomach tumors exclusively.[15]

The formation of aldehydes and epoxides by the nitrosoureas is of considerable interest, since these compounds are highly reactive and could be expected to readily interact with cellular constituents. Thus one might expect these nitrosoureas could exhibit activity by two routes—on arising from the alkylating ability of the diazohydroxide formed initially, and the other activity arising from the secondary decomposition products formed by interaction of the diazohydroxide with water, i.e., acetaldehyde and ethylene oxide from I and propylene oxide and propionaldehyde from III. Acetaldehyde, propylene oxide, and ethylene oxide have been tested for carcinogenicity and mutagenicity. The two epoxides both produce forestomach tumors when given intragastrically to Sprague–Dawley rats.[11] Acetaldehyde has only been administered to rats by inhalation but did cause tumors in the upper respiratory tract.[12] One may speculate that NHEU would be distributed in the body rapidly after administration and that decomposition could then occur in a variety of target organs, where either the diazohydroxide, acetaldehyde, or ethylene oxide might react with a cellular constituent to cause damage that would eventually lead to a tumor. NHPU has different target organs, which could be a result of molecular size, liposolubility, or the fact that the yield of active compounds from NHPU is very different than that from NHEU, since acetone, which is generally regarded as innocuous, is the principal product, with propylene oxide being formed in 18% yield and propionaldehyde in 4–6% yield. It should be noted that in one in vitro experiment, NHPU was decomposed in Hank's balanced salt solution in the presence of rat hepatocytes, virtually all of the propylene oxide formed was converted to the glycol, and 2-propanol was identified as a reaction product for the first time.

A group of dialkylnitrosoureas (V–VII) related to *N*,*N'*-bis(2-chloroethyl)-*N*-nitrosourea (BCNU) were synthesized. CHNU-I (V) and CPNU-I (VII) are closely related to NHEU (I) and NHPU (III), but being dialkylnitrosoureas, they are much more stable in base than monoalkylnitrosoureas.[8] However, when the compounds are allowed to decompose to completion in pH 7.4 buffer,

(10) Newman, M. S.; Kutner, A. *J. Am. Chem. Soc.* **1951**, *73*, 4199–4204.

(11) Dunkelberg, H. *Br. J. Cancer* **1982**, *46*, 924–933.
(12) Wouterson, A.; et al. *Toxicology*, in press.
(13) Lijinsky, W.; Singer, G. M.; Reuber, M. *Carcinogenesis*, in press.
(14) Aukerman, S. L.; Brundrett, R. B.; Hilton, J.; Hartman, P. E. *Cancer Res.* **1982**, *43*, 214–221.
(15) (a) Lijinsky, W.; Taylor, H. W. *Cancer Lett.* (*Shanon, Irel.*) **1976**, *1*, 275–279. (b) Lijinsky, W.; Schmähl, D., IARC Scientific Publications, 1978, No. 19, pp 495–501.

the products and yields closely matched those obtained from the analogous monoalkylnitrosoureas. We might predict then that compounds V and VII would give tumor spectra similar to I and III. The difference in stability and the possible differences in transport of these larger molecules may greatly affect their effectiveness as carcinogens. Table II gives a compilation of results of decomposition reaction product studies, including, for comparison, results from the literature on related compounds.

Conclusions

A product study for several mono- and dialkylnitrosoureas and two nitrosooxazolidones revealed no clear-cut pattern of product formation that could be directly related to carcinogenesis by the test compounds. However, it could readily be shown that several of the products obtained were highly active compounds which have been shown to be carcinogens in their own right. It is possible that these products are responsible for some or all of the carcinogenic activity of the test compounds, if they are formed by decomposition of the test compound at the target organ, although other modes of action can be postulated. The results of carcinogenicity testing of the dialkylnitrosoureas and further chemical studies on these compounds may shed more light on the question.

Experimental Section

Materials and Instrumentation. Organic chemicals were obtained from Aldrich and Eastman and generally were not purified further. Inorganic chemicals were Fisher ACS reagent grade. Solvents for HPLC were Burdick and Jackson "distilled in glass". HPLC was carried out on a Waters Associates chromatograph equipped with a Model 440 absorbance detector (254 nm). GC analysis was performed on a Tracor Model MT220 with flame ionization detectors. Quantitation was accomplished with the aid of Nelson Analytical Intelligent Interfaces and software and a Hewlett-Packard 9816 computer.

Mass spectra (GC/MS) were obtained on a Finnegan 1015C mass spectrometer and NMR spectra were run on a Nicolet GE magnetic NT-300 wide-bore spectrometer.

The test compounds I–VII were synthesized as described elsewhere.[1,13]

Decomposition Reactions. The test compound (0.25 mM) was weighed into a Wheaton reaction vial, 0.067 M phosphate buffer (0.5 mL) was added, and the vial was sealed and placed in a 37 °C bath. The vial was cooled in ice before opening, and aliquots were taken for analysis by the following methods. As DNPs: The reaction mixture (200 μL) was treated with 0.8 mL of 12.7 mM (2,4-dinitrophenyl)hydrazine (DNPH) in 2 N HCl. This mixture was then extracted with isooctane (3 × 2 mL, distilled from DNPH), the isooctane back-extracted with acetonitrile, and the acetonitrile extract diluted to volume for HPLC analysis. The latter was best accomplished with either an Altex 5-μm Ultrasphere ODS (15 cm), 65% acetonitrile/water, 1.0 mL/min or an Alltech 5-μm Econosphere ODS (25 cm) with the same solvent. Both these columns can achieve base-line separation of acetone and propionaldehyde (dinitrophenyl)hydrazones. This procedure provided good recoveries for acetaldehyde and propionaldehyde but poor recovery for acetone. The latter two compounds were readily analyzed by the GC method described below.

Direct GC Analysis of Voltile Products. Aliquots of the reaction mixture were taken directly for GC analysis on a 1.82 m × 2 mm column packed with Super Q, 80–100 mesh (Alltech Associates), program 110 °C (5 min hold) to 200 °C at 5 °C min, 60 mL/min He carrier. The following retention times were observed: acetaldehyde, 3.0 min; propylene oxide, 9.1 min; propionaldehyde, 9.9 min, acetone, 10.9 min, 2-propanol, 12.9 min; and 1-chloro-2-ethanol, 18.5 min.

GC Analysis of Glycols. Glycols were determined as their phenylboronate esters in a modification of the method of Porter and Auansakul.[4] An aliquot of reaction mixture (200 μL) was treated with phenylboronic acid (20 μL, 40 mM in 2,2-dimethoxypropane or acetone) and this mixture was then diluted to 2 mL with acetone, which was necessary for miscibility. The mixture became slightly cloudy due to some precipitation of buffer salts, but this was ignored as it did not interfere with the analysis. The boronate esters were analyzed on a 1.82 m × 2 mm column packed with 3% SP2310, on 100–120-mesh Supelcoport (Supelco, Inc.), 115 °C. When the cyclic carbonates were present (from the reactions of II and IV), a temperature program was employed: 115 (5-min hold) to 160 °C, 5 °C/min. Retention times are as follows: ethylene glycol boronate ester, 2.9 min; propylene glycol boronate ester, 2.95 min; ethylene carbonate, 7.5 min; propylene carbonate, 8.0 min.

Gas Evolution. Gas was collected as evolved in a buret inverted over water. Results are given in Table I.

pH Profile. NHPU (III) (0.02–0.03 M in 10 mL of buffer for kinetic studies and 0.05 M for product studies) was decomposed as described above in the following buffers: pH 3.0 perchloric acid and 0.067 M phosphate buffer at pH 5.5, 6.0, 6.5, and 8.0. The disapperance of NHPU was monitored by immediate direct HPLC analysis of aliquots. Half-lives were obtained from a plot of ln concentration vs. time. At completion, samples were analyzed by GC as described above for products. (The more concentrated samples were used for product analyses.) Yields of all products were constant throughout the pH range studied, approximately equal to those obtained at pH 7.4. Decompositions were also carried out in 0.1 M phosphate buffer, both protio and deuterio. Increasing the buffer capacity thus affected the reaction kinetics and isotope effect but did not affect product distribution.

NMR Analysis and Kinetics. NHEU (I, 4.9 mg) or NOX (II, 4.0 mg) was weighed into an NMR tube, and 0.067 M phosphate buffer (pH 6.4, in D_2O, 1 mL) was added. TSP-d_4 (sodium 3-(trimethylsilyl)propionate-*2,2,3,3,-d*$_4$) was added to serve as integration and chemical shift standard. The sample was maintained at 25 °C, and spectra were taken at regular intervals. The last point was taken at 96 h for NHEU, at which time 8% of the starting material remained, and 102 h for NOX, at which point 7% of the starting material remained.

Reaction of NHPU (III) with Hepatocytes. NHPU (III, 7.0 mg) was allowed to decompose in Hank's balanced salt solution at 37 °C. Direct GC analysis of the reaction mixture showed the same product distribution and yields obtained in phosphate buffer. NHPU was then decomposed in Hanks in the presence of hepatocytes, prepared as described elsewhere.[5]

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