Studies on the Mechanism of Decomposition and Structural Factors Affecting the Aqueous Stability of 1,2-Bis(sulfonyl)-1-alkylhydrazines

Philip G. Penketh, Krishnamurthy Shyam, and Alan C. Sartorelli*

Department of Pharmacology and Developmental Therapeutics Program, Comprehensive Cancer Center, Yale University School of Medicine, New Haven, Connecticut 06520

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1,2-Bis(sulfonyl)-1-alkylhydrazines are highly active experimental antineoplastic agents which decompose with first-order kinetics in neutral aqueous solutions. These agents generate approximately 2 mol of the corresponding sulfinate, 1 mol of nitrogen, and 1 mol of the appropriate alcohol, produced as a consequence of the alkylation of water. Increasing the leaving-group ability of the sulfonyl moiety on N-1 shortens the half-life, while the converse happens with N-2 substitutions. Linear Hammett relationships are found for both types of substitutions. The predictable kinetics of decomposition makes these agents potential candidates for use in regional chemotherapy, where compounds with tunable short half-lives may offer some advantage. Prodrugs of extremely short-lived derivatives of this class may also have utility as targeted alkylating agents.

Introduction

Our laboratory has synthesized a series of 1,2-bis(sulfonyl)-1-alkylhydrazines, as well as a number of prodrugs of these compounds.[1-3] The prodrugs include 1-alkyl-1,2,2-tris(sulfonyl)hydrazines, which have been hypothesized to undergo spontaneous hydrolysis at N-2, and 1-acyl-1,2-bis(methylsulfonyl)-2-alkylhydrazines, which can lose the acyl group by spontaneous hydrolysis, nucleophilic attack at the carbonyl carbon, and enzymatic cleavage. Several of these compounds are highly active antineoplastic agents in murine model systems, producing 60-day "cures" of mice bearing leukemia L1210, leukemia P388, and sarcoma 180.[2,3] Pronounced activity was also found against several solid tumors, including the B16F10 melanoma, the M5076 reticulum cell sarcoma, and the M109 lung carcinoma.

Studies on the interrelationships between the physicochemical properties and the biological activities of 1-(2-chloroethyl)-1-nitrosoureas revealed that agents with relatively short half-lives generally had the greatest alkylating activity, weakest carbamoylating activity, and the highest therapeutic indices.[4] Extremely short-lived drugs would be expected to have minimal time for host distribution and, therefore, would generate a high alkylation stress close to the site of administration. In contrast, extremely long-lived agents may be extensively metabolized and/or excreted without generating the alkylating species which produces an anticancer action. Thus, the chemical half-life of alkylating agents appears to be an important parameter in the biological activity of these agents, as well as an important consideration in the formulation and storage of such drugs.

The decomposition of the 1,2-bis(sulfonyl)-1-alkylhydrazines can be prevented by the substitution of the N-2 hydrogen, which occurs in the two classes of prodrugs indicated above.[2,3] The generation of a prodrug of this class by substitution of a group which would be specifically cleaved at a selected target site would in theory result in the effective targeting of the alkylation stress only if the active 1,2-bis(sulfonyl)-1-alkylhydrazine that is generated had a sufficiently short $T_{1/2}$ to limit its diffusional radius. Thus, in this kind of therapeutic application, a prodrug of a very short-lived 1,2-bis(sulfonyl)-1-alkylhydrazine would be optimal.

There is significant interest in the study of regional chemotherapy, in which a limb or an organ is selectively treated with a chemotherapeutic regimen, in part to decrease systemic toxicity; this approach generally requires venous isolation.[5-7] Theoretically, very short-lived agents with tunable half-lives should be suitable for this application by arterial perfusion only, without the requirement for venous isolation. For similar reasons, these kinds of agents may also be suitable for cavitorial treatment of ovarian or lung carcinoma.

Therefore, we have decided to investigate the effects of structural factors on the aqueous stability of 1,2-bis(sulfonyl)-1-alkylhydrazines, which were synthesized as previously described by this laboratory,[2,8] in an effort to delineate agents of this class with clinical potential in these situations.

Results

The complete decomposition of 1,2-bis(phenylsulfonyl)-1-methylhydrazine in 200 mM phosphate buffer, pH 7.4, resulted in a solution with spectral characteristics essentially identical to that of benzenesulfinic acid (Figure 1). The yield of benzenesulfinic acid, calculated by comparison with authentic material, was 1.96 mol of sulfinic acid per mole of drug, a finding that agrees well with the theoretical value of 2.0. Similar experiments utilizing 1,2-bis(methylsulfonyl)-1-methylhydrazine suggest that this compound also generates approximately 2 mol of sulfinic acid per mole of drug. We have therefore assumed that the generation of approximately 2 mol of sulfinic acid per mole of 1,2-bis(sulfonyl)-1-alkylhydrazine is common to all of the derivatives included in this study. In agreement with the above results, the total yield of H^+ per mole of drug, as determined by an acidification assay, was almost exactly 2.0 for all of the agents studied. Furthermore, nitrogen (241 mL at 294 K) corresponding to a molar yield of 0.98 evolved from 0.01 mol of 1,2-bis(methylsulfonyl)-1-methylhydrazine. In previous studies, we

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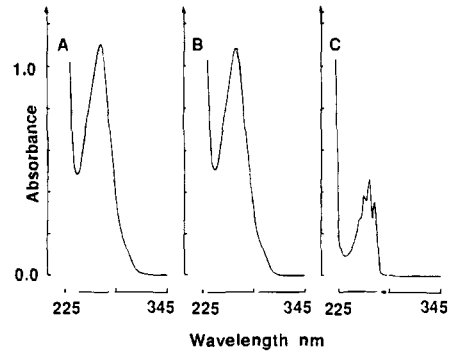
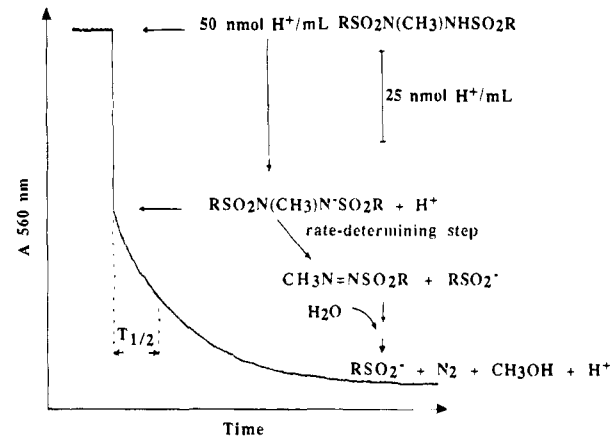


Figure 1. UV spectra of 1,2-bis(phenylsulfonyl)-1-methylhydrazine decomposition products and comparison with authentic materials. (A) UV spectral scan of 1 mM benzenesulfinic acid (sodium salt) in buffer vs buffer alone. (B) UV spectral scan of fully decomposed 0.5 mM 1,2-bis(phenylsulfonyl)-1-methylhydrazine (added as 5 μL/mL of a 100 mM solution in DMSO) in buffer vs buffer containing 5 μL/mL of DMSO. (C) UV spectral scan of 1 mM benzenesulfonic acid (sodium salt) in buffer vs buffer alone.

have determined the yield of alcohol, which is generated as a consequence of the alkylation of water, from the decomposition of 1,2-bis(methylsulfonyl)-1-methylhydrazine and 1,2-bis(phenylsulfonyl)-1-methylhydrazine in 200 mM sodium phosphate, pH 7.4, and found decomposition to yield as high as 0.8 mol of methanol per mole of drug.[2] Similar results were obtained with 1,2-bis(methylsulfonyl)-1-(2-chloroethyl)hydrazine, a representative chloroethylating agent, which produced close to a molar yield (0.88) of chloroethanol when allowed to completely decompose in 100 mM Tris-HCl buffer, pH 8.8 (data not shown). When the kinetics of acidification were measured using a spectroscopic acidification assay with more than 20 1,2-bis(sulfonyl)-1-alkylhydrazines at a pH of approximately 7.4, all of the agents tested were found to release approximately 2 mol of proton per mole of drug in a biphasic manner. One mole was released essentially instantaneously, and the second mole was released with first-order kinetics (Figure 2). The addition of thioglycerol, a strong nucleophile, to the reaction mixture at concentrations up to 50 mM (a 2000-fold molar excess over the concentration of drug) did not measurably affect the half-life of the drugs but significantly decreased the alkylation of 4-(4′-nitrobenzyl)-pyridine (NBP) by the chloroethylating agents, but did not measurably decrease the relatively small degree of alkylation of NBP by their methylating counterparts (Table 1). The pH dependence of the decomposition kinetics was also studied for 1,2-bis(methylsulfonyl)-1-methylhydrazine. At low pH values of 4–5, decomposition was prevented or greatly reduced and the initial instantaneous release of 1 mol of protons was not observed. At pH 6.5, the half-life of 1,2-bis(methylsulfonyl)-1-methylhydrazine was approximately double that at pH 7.4 (6.1 ± 0.4 vs 2.8 ± 0.2 min) and the initial instantaneous release of protons was reduced to about 0.5 mol, while the total proton generation for complete decomposition remained at 2 mol of H^+/mol of drug. For the highest pH values studied (pH 7.0–7.8), the proton generation kinetics were similar to those described earlier for pH 7.4. The decomposition of these agents was also highly temperature dependent as evidenced by the observation that a 7 °C increase in temperature from 30 °C to 37 °C halved the $T_{1/2}$ for decomposition (Table 2). Another major factor affecting the first-order rate constant for the decay of these drugs in aqueous solution is the electron affinity of the alkyl group on N-1. As shown in Table 3, the $T_{1/2}$ values of various chloroethylating agents are approximately one-fifth of the values for their methyl-substituted counterparts.



$XSO_2N(CH_3)NHSO_2Y$

X	Y	$T_{1/2}$ (min)
CH_3	CH_3	2.80 ± 0.08
4-$CH_3OC_6H_4$-	CH_3	0.96 ± 0.01
4-$CH_3C_6H_4$-	CH_3	0.54 ± 0.02
C_6H_5-	CH_3	0.34 ± 0.03
4-ClC_6H_4-	CH_3	0.25 ± 0.01
4-$NO_2C_6H_4$-	CH_3	0.08 ± 0.01
CH_3	4-$CH_3OC_6H_4$-	2.30 ± 0.09
CH_3	4-$CH_3C_6H_4$-	2.75 ± 0.21
CH_3	C_6H_5-	3.03 ± 0.61
CH_3	4-BrC_6H_4-	4.65 ± 0.11

Figure 2. Decomposition of 1,2-bis(sulfonyl)-1-methylhydrazines as measured by medium acidification in 1 mM potassium phosphate, pH 7.3–7.4.

Table 1. Alkylation of 4-(4′-Nitrobenzyl)pyridine by 1,2-Bis(methylsulfonyl)-1-methylhydrazine (A) and 1,2-Bis(methylsulfonyl)-1-(2-chloroethyl)hydrazine (B) in the Presence and Absence of 10 mM Monothioglycerol (MTG)

		absorbance at 545 nm	
drug	concn, mM	no MTG	plus MTG
A	5	0.057 ± 0.010	0.056 ± 0.011
A	10	0.106 ± 0.004	0.114 ± 0.006
B	0.1	0.236 ± 0.022	0.000
B	1	1.298 ± 0.052	0.021 ± 0.002

Table 2. Effect of Temperature on the Relative Half-Lives ($T_{1/2}$) of 1,2-Bis(sulfonyl)-1-alkylhydrazines

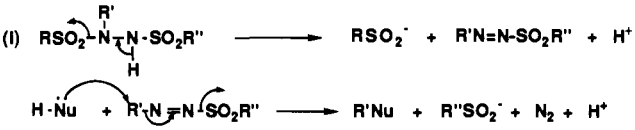
X	R	Y	$T_{1/2}$ at 30 °C/$T_{1/2}$ at 37 °C
4-$NO_2C_6H_4$	CH_3	CH_3	2.2
4-ClC_6H_4	CH_3	C_6H_5	2.0
4-ClC_6H_4	$ClCH_2CH_2$	C_6H_5	2.2
CH_3	$ClCH_2CH_2$	CH_3	2.2
CH_3	CH_3	CH_3	2.0
C_8H_5	CH_3	C_6H_5	2.1

Table 3. Effect of the Alkyl Substituent on the Relative $T_{1/2}$ at 37 °C of 1,2-Bis(sulfonyl)-1-alkylhydrazines

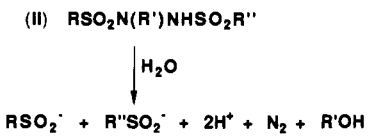
compound	$T_{1/2}$ (R = CH_3)/$T_{1/2}$ (R = CH_2CH_2Cl)
4-$ClC_6H_4SO_2N(R)NHSO_2C_6H_5$	6.0
$CH_3SO_2N(R)NHSO_2CH_3$	5.1
4-$CH_3C_6H_4SO_2N(R)NHSO_2C_6H_5$	6.5

Discussion

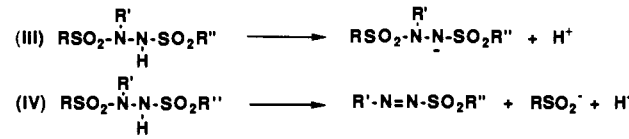
The 1,2-bis(sulfonyl)-1-alkylhydrazines were conceived as antineoplastic agents that functioned to produce cytotoxicity through alkylation. They were hypothesized to decompose to form the active species by the following mechanism.[1]



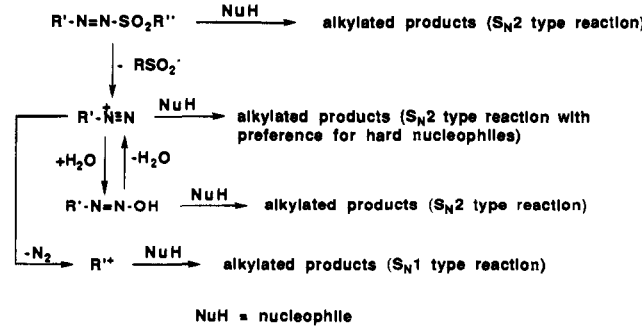
Therefore, the overall stoichiometry if water was the predominant nucleophile would be as follows.



There are also several other possible schemes by which these compounds could decompose in neutral aqueous solutions. Schemes involving the hydrolysis of an S—N bond would result in the formation of a sulfonic rather than a sulfinic acid. We, therefore, examined the decomposition products of some 1,2-bis(sulfonyl)-1-alkylhydrazines to test the scheme proposed above. The decomposition of 1,2-bis(methylsulfonyl)-1-methylhydrazine was found to yield approximately a 0.98 molar yield of N_2 gas. The methanol yield in the case of 1,2-bis(methylsulfonyl)-1-methylhydrazine was as much as 0.8 mol per mole of drug. Similar results were obtained with other derivatives of this class, including 1,2-bis(methylsulfonyl)-1-(2-chloroethyl)hydrazine, a representative chloroethylating agent, which produced a 0.88 molar yield of 2-chloroethanol when allowed to completely decompose in 100 mM Tris-HCl buffer, pH 8.8. The observed 0.1—0.2 mol deficit of alcohol may in part be due to the alkylation of buffer components. Since N_2, 2-chloroethanol, and methanol have no significant UV absorption above 200 nm in the 1 mM concentration range, unlike sulfonic and sulfinic acids which have strong and characteristic UV spectra, a UV scan of the decomposition products was used to distinguish between these decomposition products. Close to 2 mol of sulfinic acid were generated per mole of drug, with very little or no sulfonic acid being produced. These findings, therefore, support the overall stoichiometry of decomposition given in eq II. The kinetics of decomposition of a series of 1,2-bis(sulfonyl)-1-alkylhydrazines was studied using a spectroscopic acidification assay. Because sulfinic acids are typically strong acids, and therefore, are 100% ionized (e.g., pK_a of benzenesulfinic acid = 1.5) at near neutral pH values, 2 mol of protons should be released per mole of drug. This was confirmed in these experiments. At pH 7.4, 1 mol of H^+ was released instantaneously, with a second mole of protons being released with first-order kinetics. Substitution of better leaving groups on N-1 shortened the $T_{1/2}$ of this second phase of H^+ liberation. Conversely, the opposite effect occurred when more electron-withdrawing groups were placed onto N-2, but the magnitude of the effects of substituents on N-2 was less than that observed for N-1 substitutions. The addition of relatively high concentrations of thioglycerol, a strong nucleophile, did not alter the $T_{1/2}$ of this second phase of proton release. The only scheme which is consistent with these observations is shown in Figure 2. The instantaneous release of 1 mol of protons at pH 7.4 cannot reflect the initial elimination step, since the insertion of a better leaving group on N-1 affects the kinetics of the second phase of H^+ liberation, and this phenomenon could not occur if this group was lost in the liberation of the first mole of H^+. Therefore, the instantaneous release of protons reflects the ionization of the drug. Moreover, the lack of appreciable decomposition and initial instantaneous release of protons at low pH values and the approximate doubling of the $T_{1/2}$ and the halving of the magnitude of the initial instantaneous release of protons at pH 6.5 support this hypothesis. Furthermore, these results suggest that only the anionic forms of these drugs decompose at significant rates in the pH range studied and that the pK_a of 1,2-bis(methylsulfonyl)-1-methylhydrazine is about 6.5. Due to the electron-releasing inductive effect of the methyl group, 1,2-bis(methylsulfonyl)-1-methylhydrazine would be expected to be the least acidic drug in the spectrum of agents studied. Therefore, reaction III rather than reaction IV is represented by the first phase of H^+ release.



The absence of any effect of the strong nucleophile thioglycerol on the rate of the second phase of H^+ liberation strongly suggests that the elimination of the sulfonyl moiety on N-1 is the rate-limiting reaction in the subsequent sequence of events involved in the release of the final proton and not the reaction of $RSO_2N{=}NR$ with a nucleophile. The rate of the second phase of proton liberation is, however, strongly dependent upon the leaving group ability of the N-1 substituent. On the basis of the above evidence, we propose the scheme depicted in Figure 2. There are several possible species, or a combination thereof, which could be the ultimate electrophile(s), as indicated below.



These alkylating species vary in character from hard electrophiles, which favor S_N1 types of alkylation reactions with hard nucleophiles, to very soft electrophiles which undergo S_N2 type reactions with soft nucleophiles like thiols. A similar state of affairs occurs with the nitrosoureas.[9-11] In the case of the (chloroethyl)hydrazine derivatives a chloronium ion could also be generated. Chloronium ions have been proposed to account

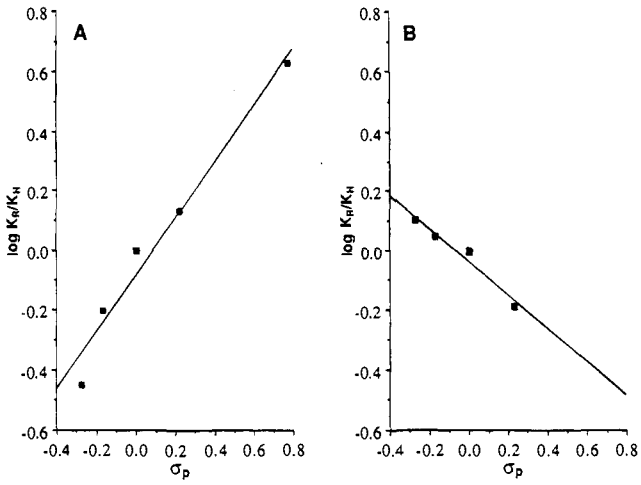


Figure 3. Hammett analysis of the kinetic data presented in Figure 2 for the decomposition of 1,2-bis(sulfonyl)-1-methylhydrazines. (A) Hammett plot for various 1-(arylsulfonyl)-2-(methylsulfonyl)-1-methylhydrazines. (B) Hammett plot for various 2-(arylsulfonyl)-1-(methylsulfonyl)-1-methylhydazines.

for a minor portion of the alkylation reactions produced by 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU).[11] The greater alkylation of NBP by the chloroethyl derivatives of 1,2-bis(sulfonyl)-1-alkylhydrazines compared to corresponding methyl-substituted agents and the greater susceptibility of the alkylation of NBP to competitive inhibition by thioglycerol support the idea that the predominant electrophile is of a softer nature and favors a more S_N2 type mechanism than the predominant electrophile produced by the methylating derivatives. If a single electrophilic species involving the same leaving group was generated by both the methylating and chloroethylating derivatives, one would have expected a greater S_N2 component in the reactions of the methylating agents than of the chloroethylating derivatives, since the reactivity of substrates in S_N2 reactions is $CH_3W > 1°$ and the reverse for S_N1 reactions. The observation that the chloroethylating agents in this series of drugs appear to show a greater S_N2 component than the methylating agents suggests therefore that the predominant electrophiles involved in these reactions employ different leaving groups, hence distorting the expected nucleophile preferences. This would be expected because the electron-withdrawing effect of the chloroethyl group in the alkyl diazosulfinate intermediate would impede the elimination of the sulfinate and also destabilize the diazonium ion if formed, while the inductive electron-releasing effect of the methyl group would have the opposite effects. We would therefore expect the hard alkyldiazonium electrophile to play a greater role in the alkylation reactions of the methylating agents than of the chloroethylating derivatives.

Hammett analysis (σ_p values taken from ref 12) of the effects of various para substituents in the benzenesulfonyl moieties in the N-1 and N-2 positions on the first-order rate constants gave linear plots (Figure 3). The reaction constant, ϱ, for N-1 substituents was found to be +0.95, while for N-2 substituents ϱ was found to be −0.55. The larger positive value for ϱ in the case of various para substituents in the benzenesulfonyl moiety attached to N-1 indicates that the development of a negative charge in the arenesulfonyl moiety attached to N-1 is the rate-limiting step in this reaction and that the overall reaction is greatly accelerated by electron-withdrawing and retarded by electron-donating substituents, which respectively improve and impair the ability of the arenesulfonyl moiety on N-1 to act as a leaving-group. The smaller negative value of ϱ for para substituents in the benzenesulfonyl moiety attached to N-2 indicates that the effects of electron-withdrawing and -donating groups in this position will produce results of smaller magnitude and opposite in direction to N-1 substitutions. This phenomenon is almost certainly due to electron-withdrawing and electron-donating groups acting to stabilize and destabilize, respectively, the delocalization of the negative charge of the anion on the oxygens of the arenesulfonyl moiety in the N-2 position, which would tend to inhibit the rate-limiting elimination reaction. The nature of the alkyl group on N-1 would also be expected to have an effect on the "electronic tug of war" between the arylsulfonyl moieties on N-1 and N-2. The electron-withdrawing chloroethyl group on N-1 should assist the arylsulfonyl moiety on N-1 in favoring elimination, while the electron-donating methyl substituent should have the opposite effect. This prediction is in fact observed, since the chloroethyl derivatives, in general, have half-lives that are 5−6-fold shorter than their methyl counterparts as can be seen in Table 3.

The high temperature sensitivity of decomposition, a 7 °C increase in temperature more than halving the $T_{1/2}$ of most of these agents as shown in Table 2, indicates that the Arrhenius activation energy for the rate-limiting elimination reaction is very high and corresponds to a range of 75.0−87.4 kJ/mol. The Arrhenius activation energy equals the enthalpy of activation when there is insignificant pressure/volume work done on or by the surrounding system as a result of volume changes in the attainment of the transition state.[13] Therefore, we would expect the enthalpy of activation to be approximately 80 kJ/mol for these agents. The only way these first-order decomposition reactions can proceed at such high rates at 37 °C, despite a high enthalpy of activation, would be if a relatively large positive entropy of activation (about 20 J K^{-1} mol^{-1} for the shortest lived agents) occurred. A positive entropy of activation indicates that the transition state is less ordered than the reactants, a situation that is typical for unimolecular decompositions.[13] It is highly likely that because of conjugation and the possible partial double bond character of the N—N bond the anionic forms of the 1,2-bis-(sulfonyl)-1-alkylhydrazines are planar molecules. Elimination of the sulfonyl moiety on N-1 results in the breaking of this ordered structure and the generation of two molecular species from one, a process that may be responsible for the large positive entropy of activation that is observed. The predictable kinetics of decomposition of the 1,2-bis(sulfonyl)-1-alkylhydrazines, which are highly active antineoplastic agents in experimental systems, makes these agents potential candidates for use in regional chemotherapy, where compounds with tunable short half-lives may have advantages. Moreover, since the decomposition of these compounds can be prevented by substituents on N-2, the use of a chemically or enzymatically cleavable group on N-2 may make these agents suitable for drug targeting strategies.

The importance of the half-life on the biological activity of alkylating agents and the rate of delivery of

an alkylating stress also may be investigated using these agents, since a wide range of chemically similar drugs can be produced, which generate the same or similar alkylating species, while having a wide range of half-lives. It is interesting to note that in a study of a relatively large number of (chloroethyl)nitrosoureas, whose half-lives spanned a range of 19 to 68 min, the therapeutic indices generally increased with decreasing half-lives,[4] while the general trend with the bis(sulfonyl)methyl- and (chloroethyl)hydrazine derivatives tested so far for antineoplastic activity in tumor-bearing mice, whose half-lives spanned from several seconds to almost 5 min, was the converse. This finding implies that the optimum half-life for the expression of maximum therapeutic indices in mice for alkylating agents of these types is between 5 and 19 min. This phenomenon may also account for a related observational difference between the nitrosoureas and the sulfonylhydrazines, in that the therapeutic index of the nitrosoureas generally decreases as the toxicity decreases,[14] whereas with the sulfonylhydrazines the therapeutic index tends to rise with decreasing toxicity (unpublished data from this laboratory). The half-life value range is probably the result of the interaction of a large number of pharmacokinetic factors affected by the half-life and the rate of delivery of the alkylation stress.

Experimental Section

Decomposition Kinetics. Decomposition of the 1,2-bis(sulfonyl)-1-alkylhydrazines was followed using a previously described spectrophotometric acidification assay developed in this laboratory.[3] Briefly, the acidification of a weakly buffered, 1 mM potassium phosphate solution of phenol red (20 mg/L) was followed spectrophotometrically at 560 nm. All mixtures were adjusted to a pH of 7.2–7.3 at room temperature (23 °C); this corresponded to a pH of 7.3–7.4 at 37 °C due to the temperature dependence of the dissociation constants of the buffer constituents. The mixtures were then sealed with parafilm to prevent pH changes due to CO_2 exchange and brought to 37 °C prior to addition of drug. The decomposition kinetics of some of these drugs were also studied at temperatures lower than 37 °C. The assay was calibrated using HCl standards. Calibrations were carried out for all of the reaction conditions tested to allow for changes in sensitivity resulting from differences in buffering capacity due to other additives such as thioglycerol or to other pH ranges. Reactions were initiated by the addition of a 10 mM solution of drug in a 5-μL volume of DMSO to 0.99 mL of assay mixture. Typical pH changes in the assay solution upon complete decomposition were less than 0.1 pH unit. This assay was also used to follow the kinetics of drug decomposition in the pH 6.6–7.8 range. To study decomposition at more acidic pH values, other very similar assays were used. For the pH range 5.8–6.5, a 1 mM potassium maleate buffer containing 20 mg/L of bromphenol red was used and the reaction was monitored at 574 nm. For the pH range 5.4–5.7, the potassium maleate buffer was replaced with a 1 mM potassium acetate/1 mM potassium phosphate buffer. For the pH range 5.0–5.3, a 1 mM potassium acetate buffer containing 20 mg/L of chlorophenol red was used, with monitoring at 575 nm. For pH values of 4.0–5.0, 20 mg/L of bromphenol blue in 2 mM potassium formate buffer was utilized, with measurements being conducted at 590 nm.

Confirmation of Sulfinic Acids as Major Decomposition Products. The decomposition of various 1,2-bis(sulfonyl)-1-alkylhydrazines (0.5 mM final concentration added as 5 μL/mL of a 100 mM solution in DMSO) was followed in 200 mM sodium phosphate buffer, pH 7.4, at 37 °C by monitoring the increase in absorption due to the liberation of sulfinic acids at 225 nm. When decomposition was complete, the solutions were degassed to prevent problems due to bubble formation from N_2 gas and UV spectral scans from 225–345 nm were recorded vs buffer containing 0.5% DMSO and compared with that of authentic materials under identical conditions. Neither methanol nor chloroethanol had any significant absorbance between these wavelengths at a concentration of 1 mM, the maximum theoretical concentration of these alcohols produced upon complete decomposition of the drugs.

Quantification of Gas Production. A saturated solution of disodium hydrogen phosphate (200 mL) was added to 2.02 g of 1,2-bis(methylsulfonyl)-1-methylhydrazine. The reaction was carried out in a vessel using a minimum of head space, and the gas evolved was passed through a narrow-gauge, low-volume tube and collected over water in a graduated column. The gas was analyzed by the Baron Consulting Analytical Services Co. of Orange, CT.

Determination of Methanol. The amount of methanol generated during decomposition in 200 mM sodium phosphate buffer, pH 7.4, was measured using alcohol oxidase by methodology previously described by our laboratory and a Gilson oxygraph, *Pichia pastoris* alcohol oxidase and methanol standards.[3]

Determination of 2-Chloroethanol. The amount of 2-chloroethanol generated during decomposition in 100 mM Tris-HCl buffer, pH 8.8, was assayed as previously described using bakers' yeast alcohol dehydrogenase (EC 1.1.1.1).[3] This enzyme was found to be capable of using 2-chloroethanol as a substrate. The initial rate of reduction of NAD^+ was found to be proportional to the concentration of 2-chloroethanol in the 0–2 mM concentration range.

Alkylation of 4-(4'-Nitrobenzyl)pyridine (NBP). The ability of 1,2-bis(sulfonyl)-1-alkylhydrazines to alkylate the model nucleophile NBP in aqueous media was determined using a modified version of the assay of Wheeler and Chumley.[15] To 0.5 mL of 100 mM sodium phosphate buffer, pH 7.4, was added 5 μL of NBP (80 mg/mL) in DMSO. The alkylating agent to be tested was added to this mixture as a concentrated solution dissolved in 5 μL of DMSO to give a final concentration of 0.1–10.0 mM. The mixture was incubated at 37 °C for 30 min. The extent of alkylation of NBP was determined spectroscopically by the addition of 1 mL of 1-octanol, followed by 0.1 mL of 10 M NaOH, to the reaction mixture. The mixture was shaken immediately and centrifuged at 10000*g* for 1 min. The absorbancy of the upper octanol layer was then recorded at 545 nm against an equivalent treated sample not containing alkylating agent. Assays were also conducted with 10 mM monothioglycerol added to the 100 mM sodium phosphate buffer as a competing nucleophile. The use of 1-octanol in place of ethyl acetate results in a very stable solution of colored product in contrast to that of the previously reported solvent.

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