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# Stability of the experimental anticancer agent [[(4-methoxyphenyl) sulfonyl]hydrazono]acetic acid (NSC-267213). II. Effect of structural modification on stability and mechanism of degradation

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## Summary

The stability and mechanism of degradation of selected derivatives of [[(4-methoxyphenyl)sulfonyl]hydrazono]acetic acid (*I*) were studied in order to evaluate the structural features contributing to its aqueous instability and the possibility of stabilizing this agent by prodrug derivatization. *II*, [[(4-methylphenyl)sulfonyl]hydrazono]acetic acid ethyl ester, the ethyl ester of a compound closely related to *I*, was found to degrade more rapidly than the parent acid via unimolecular elimination of the corresponding sulfinic acid. Electron delocalization to the α-carbonyl group was proposed to contribute to the instability of the ester. The substitution of a methyl group for a methoxy group on the benzene ring had no significant effect on the kinetics and mechanism of degradation for the parent hydrazone. The *N*-methyl derivative, [[(4-methoxyphenyl)sulfonyl]-*N*-methylhydrazono]acetic acid (*III*), demonstrated much greater stability than *I* and appeared to degrade primarily by hydrolysis. These results indicated that without the possible ionization of the NH group, the unimolecular elimination was not an important degradation route. Consideration of these structural features and the degree of instability of *I* led to a proposal to stabilize this agent by formation of an *N*-substituted bioreversible derivative.

## Introduction

The investigational anticancer agent [[(4-methoxyphenyl)sulfonyl]hydrazono]acetic acid (NSC-267213, *I*) has shown promising activity in model tumor systems; however, its instability in aqueous solution has largely precluded development for further evaluation or clinical use (Cradock, personal communication). An understanding of the degradative mechanism of *I* was necessary in order to propose a method of stabilization. A previous paper (Pretzer and Repta, 1987) described the mechanism of degradation of *I* in aqueous media. To obtain more information about factors affecting degradation of *I*, the stability and mechanism of degradation of arylsulfonylhydrazones similar to *I* were studied. The effect of carboxylic acid and NH ionizations on arylsulfonylhydrazone stability, key structural fea-

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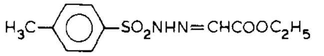
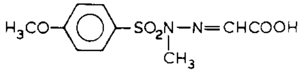
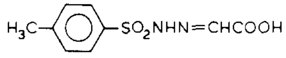
tures contributing to instability and the feasibility of prodrug formation for stabilization of *I* were evaluated by comparing the stability and degradation mechanisms of *II, III* and *IV* (structures shown below) to *I*.


*II*

[[(4–Methylphenyl)sulfonyl] hydrazono]acetic acid ethyl ester


*III*

[[(4-Methoxyphenyl) sulfonyl ]–*N*–methylhydrazono]acetic acid


*IV*

[[(4–Methylphenyl)sulfonyl]hydrazono]acetic acid

Because of synthetic considerations, the ethyl ester of *I* could not be obtained. However, *IV* exhibited degradative behavior very similar to *I*, indicating that *II* should be a good model for the ethyl ester of *I*. The derivatives *II* and *III* replaced ionizable protons of *IV* and *I*, respectively, with unionizable substituents. Since only one ionization occurred in each derivative, it was possible to assess separately the effect of the carboxylic acid and NH ionizations on arylsulfonylhydrazone degradation. Key structural features contributing to the instability of *I* could also be assessed by evaluating the effect of structural modifications on degradation rate and pathway. In addition, *II* and *III* can be regarded as models of the ester and *N*-substituted prodrugs, respectively. Thus, the stability of the derivatives was useful in evaluating the feasibility of prodrug formation for stabilization of *I*.

## Materials and Methods

### *Chemicals*

All chemicals were analytical grade and used as received from the commercial suppliers unless noted otherwise. Ethyl glyoxylate (95%) was obtained from Hoechst (France). Methanol was Fisher HPLC grade. Water for buffers and HPLC mobile phase was deionized and distilled from glass.

### *Synthesis*

*[[(4-Methylphenyl)sulfonyl]hydrazono]acetic acid ethyl ester (II).* This compound was synthesized by condensation of 4-toluenesulfonylhydrazide and ethyl glyoxylate (Blankley et al., 1969). The reaction product was a white oil which was triturated with a small amount of water to give a solid. Recrystallization from diethyl ether resulted in a white solid which melted at 104–106 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 7.0–7.5 (5H, multiplet, aromatic and =CH–), 3.6–4.0 (2H, quartet, –$CH_2$), 2.1 (3H, singlet, Ar–$CH_3$), 1.0 (3H, triplet, –$CH_3$). Infrared (KBr): 1734 $cm^{-1}$ (C=O), 1595 $cm^{-1}$ (C=N). Anal. Calculated for $C_{11}H_{14}N_2O_4S$: C, 48.87; H, 5.23; N, 10.36. Found: C, 48.91; H, 5.19; N, 10.28.

*[[(4-Methoxyphenyl)sulfonyl]-N-methylhydrazono]acetic acid (III).* A two-step method for synthesis of *N*-substituted sulfonylhydrazones was used to synthesize *III* (Nurrenbach and Pommer, 1969). 4-Methoxybenzenesulfonyl-*N*-methylhydrazide was obtained by reaction of 4-methoxybenzenesulfonyl chloride and *N*-methylhydrazine (Nurrenbach and Pommer, 1969).

The *N*-methyl hydrazide (3.92 g, 0.02 mol) dissolved in absolute ethanol (50 ml) was added to a glyoxylic acid solution (1.34 g, 0.02 mol in 10 ml absolute ethanol) with stirring. A trace of *p*-toluenesulfonic acid was added and the mixture was stirred at room temperature. After 24 h a white precipitate had formed which was removed by filtration and washed with cold ethanol. The solid was dried under reduced pressure; mp 149–150 °C. The yield was 1.2 g (23% of theoretical). $^1$H NMR (DMSO-$d_6$): $\delta$ 7.0–8.0 (5H, multiplet, aromatic and =CH–), 3.80 (3H, singlet, –$OCH_3$), 3.2 (3H, singlet, –$NCH_3$). Anal. Calculated for $C_{10}H_{12}N_2O_5S$: C, 44.11; H, 4.45; N, 10.29. Found: C, 44.30; H, 4.60; N, 10.19.

*[[(4-Methylphenyl)sulfonyl]hydrazono]acetic acid (IV).* This compound was synthesized by con-

densation of 4-toluenesulfonylhydrazide and glyoxylic acid according to the method of Blankley et al. (1969). The product was a white solid; mp 146–147 °C (literature 148–154 °C). Anal. Calculated for $C_9H_{10}N_2O_4S$: C, 44.61; H, 4.17; N, 11.57. Found: C, 44.33; H, 4.30; N, 11.40.

### *Analysis*

Elemental analyses were performed by Mr. T.N. Nguyen of the Medicinal Chemistry Department, University of Kansas, using a Hewlett-Packard Model 195-B CHN Analyzer or by Galbraith Laboratories, Inc., Knoxville, TN. Uncorrected melting points were determined using an Electrothermal melting point apparatus. Nuclear magnetic resonance spectra ($^1H$ NMR) were obtained using either a Varian Model FT-80A or T60 spectrophotometer. Infrared spectra were obtained using either a Perkin-Elmer Model 727 or a Beckman Model 33 or an IBM FTIR-32 and solids were analyzed as 1–3% dispersions in potassium bromide pellets. UV and visible absorbance measurements, high pressure liquid chromatography (HPLC) analyses and pH measurements were done as described previously (Pretzer and Repta, 1987).

### *$pK_a$ Determinations*

The $pK_a$ values for the NH group of the ester (*II*) and the carboxylic acid of the N-methyl derivative (*III*) were determined using the following procedure. The spectrophotometer initially was zeroed to cuvettes (fitted with teflon stoppers) containing 2.0 ml of the appropriate buffer in the sample and reference cells (pH 1.0, 0.01 M HCl; pH 2.0–3.1, phosphate (0.05 M); pH 3.3–6.0, acetate (0.05 M); pH 5.5–7.4, phosphate (0.05 M); $\mu = 0.2$, adjusted with NaCl). An aliquot of known volume of a concentrated stock solution in methanol was added to the buffer in the sample cuvette. The same volume of methanol was added to the reference cuvette. Both cuvettes were quickly inverted several times to assure mixing of the solutions, then scanned over the appropriate range (usually 300–200 nm). The temperature in the cells was controlled at 25.0 °C. Absorbance data were analyzed according to equations described by Albert and Serjeant (1971).

### *Kinetic studies*

Degradation of *II* was monitored by UV spectroscopy at 290 nm and formation of the degradation product ethyl diazoacetate (*V*) was followed at 248 nm. For each reaction the spectrophotometer was zeroed to cuvettes containing 2.0 ml of the appropriate buffer (buffer composition listed above for $pK_a$ determinations) in the sample and reference cells. An aliquot (15 $\mu$l) of a stock solution of *II* in methanol was added to the sample cuvette (resulting concentration = $4.2 \times 10^{-5}$ M) and an equal volume of methanol was added to the reference cuvette. Both were inverted several times to mix, then the absorbance was read at 290 nm and 248 nm. The temperature of the cell holder was maintained at 25.0 °C unless noted otherwise. Rate constants for loss of *II* and appearance of *V* were determined from plots of log ($\Delta$ absorbance) vs time which were linear at pH $\geq$ 4.2.

Kinetic studies of *III* were done in a manner similar to those of *I* (Pretzer and Repta, 1987). Resulting data were plotted as log molar concentration (determined from standard curve) vs time and rate constants were determined from the slope of such plots. The rate constants for appearance of the degradation product 4-methoxybenzenesulfinic acid (*VI*) were determined from plots of log[(molar concentration at $t_\infty$) − (molar concentration at any time $t$)] vs time. Molar concentration of *VI* was determined from standard curves. Analysis of glyoxylic acid (*VII*) and glycolic acid (*VIII*) was done as described previously (Pretzer and Repta, 1987).

Kinetic studies of *IV* were conducted similarly to studies of *I* (Pretzer and Repta, 1987). Rate constants for the loss of *IV* were determined from the linear portion of plots of log peak areas vs time. The following degradation products were analyzed during degradation of *IV*: 4-toluenesulfinic acid (*IX*), 4-toluenesulfonic acid and 4-toluenesulfonylhydrazide.

### *Determination of activation parameters*

To determine activation parameters for *II* and *III*, kinetic reactions were done at different temperatures (controlled by the temperature of the water bath or of the cell holder in the spectrophotometer) and the data were evaluated according to the Eyring equation. Reactions of *II* ($C_0 = 4.2 \times 10^{-5}$ M) and *III* ($C_0 \simeq 6 \times 10^{-5}$ M) were done at pH 7.0 (0.05 M phosphate, $\mu = 0.2$, buffer pre-equilibrated to the reaction temperature) over the temperature ranges 15.5–35.0 °C for *II* and 34.0–60.0 °C for *III*.

## Results and Discussion

### *Ester derivative (II)*

The ethyl ester derivative containing a *p*-methyl group on the benzene ring (*II*) was chosen for stability studies because attempts to synthesize the ethyl ester of *I* (*p*-methoxy group on the benzene ring) were unsuccessful. Condensation of 4-methoxybenzenesulfonylhydrazide and ethyl glyoxylate under a variety of conditions resulted in an oily residue which degraded with gas evolution (presumably nitrogen). In contrast, *II* was observed as a stable solid from condensation of 4-methylbenzenesulfonylhydrazide and ethyl glyoxylate. The inability to isolate the methoxy-substituted ester was probably due to a difference in crystal lattice structure rather than an inherent difference in its chemical stability compared to that of *II*. This proposed difference in crystal structure did not allow facile formation of a solid in the case of the methoxy ester and degradation occurred due to the residual water in the oily product.

To determine whether or not methyl vs methoxy substitution on the benzene ring had any significant effect on the stability of *I*, [[(4-methylphenyl)sulfonyl]hydrazono]acetic acid, *IV* (the methyl analog of *I*) was studied. Stability studies of *IV* at pH 4.5 and 7.4 demonstrated no significant difference compared to *I* in kinetic behavior. The products observed by HPLC upon degradation of *IV* were the *p*-methyl analogs of the products resulting from decomposition of *I*, indicating similar degradative mechanisms for *I* and *IV*. It was therefore concluded that derivatives of *IV* would accurately reflect the chemical behavior of similar derivatives of *I*.

Stability studies of *II* (monitored by UV absorbance changes) at pH 4.2–7.0 showed that *II* degraded rapidly in an apparent first order manner (see Table 1 for rate constants). Two degradation products were observed by HPLC after complete loss of *II* at pH 7.0. The products were identified by coelution with authentic samples as 4-toluenesulfinic acid (*IX*) and ethyl diazoacetate (*V*). The UV absorbance of an authentic sample of ethyl diazoacetate (248 nm) corresponded to the absorbance maximum of the degradation product observed during loss of *II* at pH 4.2–7.0. The NMR spectrum of an equimolar mixture of *V* and *IX* did not differ from that of a degraded solution of *II* (in $D_2O$/NaOD).

Both *V* and *IX* were produced quantitatively from degradation of *II*. The rate of appearance of *IX* was determined from absorbance data at pH 5.2, 6.2 and 7.0. These rate constants did not differ significantly from the rate constants for loss of *II* (see Table 1). Rate constants for the appearance of *V* were not determined.

Degradation of *II* was attributed to the unimolecular elimination of sulfinate (*IX*) and associated production of ethyl diazoacetate (*V*) (see Scheme 1). The identification of *V* and *IX* as degradation products in equimolar quantities was consistent with the proposed degradation mechanism which is analogous to the unimolecular elimination reaction of *I* (Pretzer and Repta, 1987). There was no evidence of ester hydrolysis to form the acid, *IV*.

The entropy of activation ($\Delta S^{\ddagger}$) for the degradation reaction of *II* was evaluated for rate

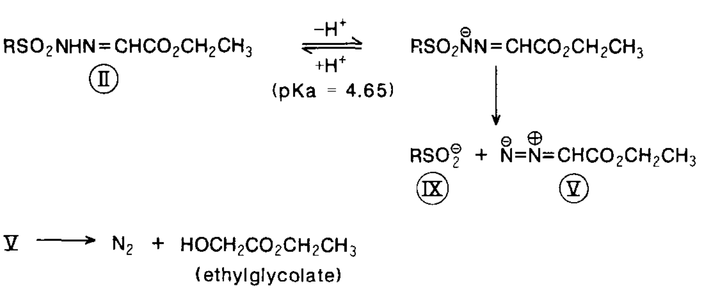
TABLE 1

*Observed first-order rate constants for the loss of II and formation of V in aqueous solution at pH 4.2 to 7.0*

| pH | *II* [a] $k_{obs}$ ($min^{-1}$) | *V* [b] $k_{obs}$ ($min^{-1}$) | Number of determinations |
|---|---|---|---|
| 4.2 | 0.042 | – | 1 |
| 5.2 | 0.183 | 0.192 | 1 |
| 6.2 | 0.258 | 0.286 | 1 |
| 7.0 | 0.275 | 0.290 | 2 |

[a] The $k_{obs}$ was determined from a plot of log ($\Delta$ absorbance) vs time for data at 290 nm.

[b] The $k_{obs}$ was determined from a plot of log ($\Delta$ absorbance) vs time for data at 248 nm.



Scheme 1. Proposed mechanism for degradation of *II*. (R = 4-methylphenyl).

data at pH 7.0 over the temperature range 15.5–35.0 °C. Analysis of the data by the Eyring equation resulted in $\Delta S^{\ddagger} = 1.1 \pm 3.0$ eu at 25 °C, consistent with the unimolecular reaction mechanism (Jencks, 1969) proposed in Scheme 1.

A pH-rate profile for the loss of *II* is shown in Fig. 1. At pH values greater than the $pK_a$ of *II* (4.65), the rate of loss of *II* appeared to reach a maximum value. This indicated that the ionization of the NH group was important in the degradation reaction as previously discussed for the degradation of *I* (Pretzer and Repta, 1986). The rate of loss of *II* appeared to decrease with decreasing pH, indicating that the unionized form was more stable. Rate constants at pH < 4.2 were not obtained. At these lower pH values, absorbance at 290 nm (attributed to ionized *II*) was inadequate for determination of rate constants. The instability of the product *V* at acidic pH values precluded the determination of rate constants by following product formation. In addition, attempts to determine rate constants by following absorbance at 248 nm ($\lambda_{max}$ for *V*) and fitting to an equation for a consecutive reaction scheme (i.e., $II \rightarrow V \rightarrow$ products) in order to account for subsequent degradation of *V*, resulted in poor fit of the data, indicating that other reactions in addition to the elimination might be occurring under these conditions. Attempts to follow the loss of *II* by HPLC at pH < 4.2 were not successful due to inability to obtain adequate chromatographic separation and peak shape for *II* even though a variety of mobile phase compositions were evaluated. The experimental difficulty in determining $k_{obs}$ for loss of *II* at lower pH values may be due to other degradation routes in addition to elimination.

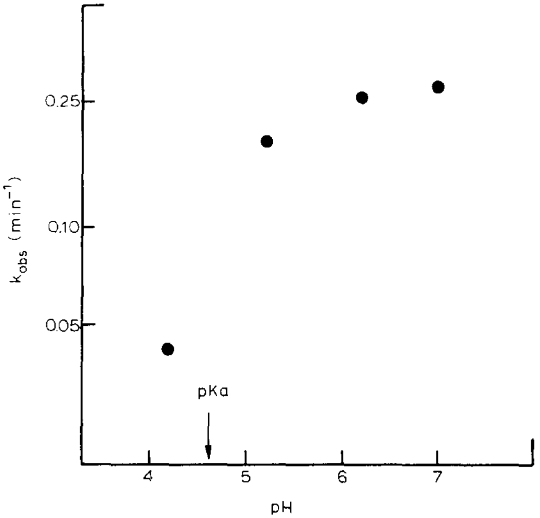
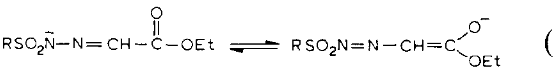


Fig. 1. Plot of $k_{obs}$ vs pH for loss of *II* at pH 4.2–7.0 at 25 °C. Reaction conditions are described in Materials and Methods and rate constants are listed in Table 1. (Only the rate constants calculated from absorbance data at 290 nm were included.)

The ester derivative (*II*) was less stable in aqueous solution than *I*. At pH 7.0, *II* degraded ~ 20 times faster than *I* ($k_I = 1.6 \times 10^{-2}$ min$^{-1}$, $k_{II} = 2.8 \times 10^{-1}$ min$^{-1}$). The greater instability of the ester probably was due to the more effective delocalization of the negative charge of the ionized species (Eqn. 1).



$$RSO_2\bar{N}-N=CH-\overset{\overset{O}{\|}}{C}-OEt \rightleftharpoons RSO_2N=N-CH=C\begin{matrix}O^-\\ OEt\end{matrix} \qquad (1)$$

The delocalization of the negative charge is favorable for the ester of *II* compared to the acid of *I* where electrostatic repulsion of the ionized carboxylic acid would preclude formation of an azo resonance form. Such delocalization was proposed to be a destabilizing factor towards the elimination reaction by weakening the $SO_2$–N bond (Pretzer and Repta, 1987) thereby enhancing the rate of loss of the ester.

*N-methyl derivative (III)*

Kinetic studies to evaluate the stability of *III* were done at pH 4.0, 4.5, 7.0 and 7.4. A semilog plot for loss of *III* at pH 4.5 and 7.4 is shown in Fig. 2. The degradation of *III* occurred in an apparent first order manner and the observed rate constants are reported in Table 2.

A major degradation product of the decomposition of *III* was 4-methoxybenzenesulfinic acid, *VI*. Quantitative production of *VI* from *III* occurred for reactions at pH 2.0, 4.0 and 4.5. (For reactions at pH 7.0 and 7.4, standard curves for *III* and *VI* on the particular HPLC column used were not obtained.) The rate constant for appearance of *VI* was 0.041 $h^{-1}$ at pH 4.5 (for one reaction) and 0.124 $h^{-1}$ at pH 4.0 (average of two determinations). These values were approximately equal to the rate constants for loss of *III* (0.043 $h^{-1}$ for the particular reaction at pH 4.5 and 0.124 $h^{-1}$ for the two determinations at pH 4.0). Rate constants for the formation of *VI* were not calculated at other pH values. Further degradation of *VI* to the corresponding sulfonic acid occurred during reactions at all pH values studied.

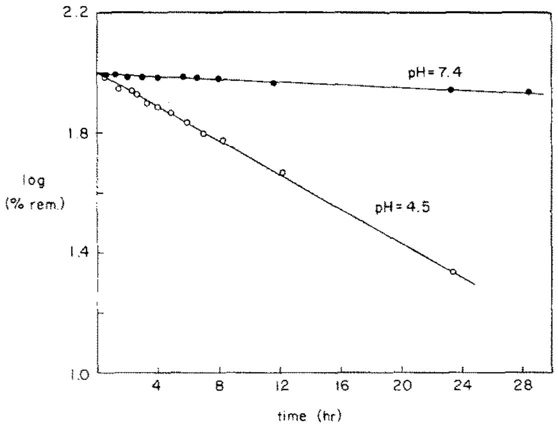


Fig. 2. Semilog plot for loss of *III* as a function of time at pH 4.5 and 7.4. Reaction conditions are described in Materials and Methods and rate constants are reported in Table 2.

TABLE 2

*Observed first order rate constants for the loss of III at pH 2.0 to 7.4* [a]

| pH | $k \times 10^2$ ($h^{-1}$) | $T$ (°C) |
|---|---|---|
| 2.0 | > 12 [b] | ~ 22 |
| 4.0 | 12.40 [c] | 30.0 |
| 4.5 | 5.35 [d] | ~ 22 |
| 7.0 | 0.88 [e] | ~ 22 |
| 7.4 | 0.45 [f] | ~ 22 |

[a] Reaction conditions: $C_0 \simeq 4–8.5 \times 10^{-5}$ M, 0.05 M buffer (buffer species listed in Materials and Methods), $\mu = 0.2$ or uncontrolled. The degradation of *III* was monitored by HPLC and rate constants determined as described previously (see Materials and Methods).

[b] Estimated from one reaction where less than 1% of the initial concentration of *III* remained after ~ 48 h.

[c] Average of two determinations over > 4 half-lives.

[d] Mean of 4 determinations over > 2 half-lives, S.D. = $0.71 \times 10^{-2}$.

[e] One determination over > 1 half-life.

[f] One determination over < 1 half-life.

Analysis for glyoxylic acid, *VII*, and glycolic acid, *VIII*, was done in solutions of degraded *III* (after complete loss of *III* as evidenced by HPLC). The results are shown in Table 3. At pH 2.0, *VII* was a major degradation product although *VIII* was also observed. In each case, the sum of the acid species, *VII* and *VIII*, was greater than 100%, which may have been due to interference by other components produced in the reaction. For reactions allowed to go to completion at pH 4.0 and 4.5 and room temperature, quantitative production of *VII* was found (*VIII* was not determined in these solutions). For reactions which were heated to increase the rate of degradation, the percentage of *VII* found decreased and a substantial percentage of *VIII* was also detected. At pH 7.0, relatively low levels (< 10%) of *VII* and *VIII* were found. These results suggested that elevated temperatures or high starting concentration of *III* (greater than the solubility, resulting in a suspension) might alter the course of the degradation reaction. Analysis of *VII* was also done in samples from a solution of *III* undergoing degrada-

TABLE 3

*Levels of glyoxylic acid (VII) and glycolic acid (VIII) after degradation of III* [a] *at various pH values (as % of initial concentration of III)*

| pH | *VII* | *VIII* | Reaction conditions |
|---|---|---|---|
| 2.0 | 122 | – [b] | $C_0 \simeq 4 \times 10^{-5}$ M, RT [c] (one determination) |
| | 76 | 39 | $C_0 \simeq 1 \times 10^{-3}$ M (suspension), RT [c] (one determination) |
| | 130 | 62 | $C_0 \simeq 3.5 \times 10^{-4}$ M (suspension), 60 °C (avg. of 3 determinations) |
| 4.0 | 100 | – [b] | $C_0 \simeq 8 \times 10^{-5}$ M, 30 °C (avg. of two determinations) |
| 4.5 | 100 | – [b] | $C_0 \simeq 4 \times 10^{-5}$ M, RT [c] (one determination) |
| | 62 | 34 | $C_0 \simeq 3.5 \times 10^{-4}$ M (suspension), 60 °C (avg. of two determinations) |
| 7.0 | 8 | – [b] | $C_0 \simeq 4 \times 10^{-5}$ M, 50 °C (one determination) |
| | 9 | 18 | $C_0 \simeq 4 \times 10^{-4}$ M (suspension), 60 °C (avg. of two determinations) |

[a] The degradation of *III* was monitored by HPLC and *VII* and *VIII* analyzed when $<1\%$ of the initial concentration of *III* remained.

[b] Not determined.

[c] Room temperature at the time of the study ( ~ 23 °C).

tion at pH 4.5 (absorbance values were corrected for interference from *III*). The rate constant for production of *VII* (0.046 $h^{-1}$) was approximately equal to the rate constants for loss of *III* (0.043 $h^{-1}$) and appearance of *VI* (0.041 $h^{-1}$). Quantitative production of *VII* was observed in this case (determination of *VIII* was not done).

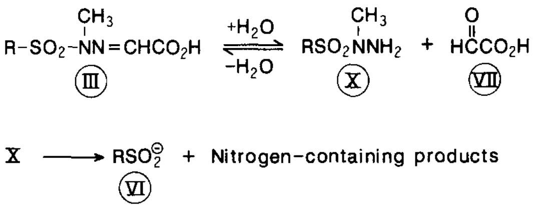
The degradation of *III* was proposed to occur by the hydrolytic mechanism shown in Scheme 2. Hydrolysis of *III* would result in formation of the *N*-methylhydrazide, *X* and glyoxylic acid, *VII*. Rapid degradation of *X* ($t_{1/2}$ ~ 30 min at pH 2–9 (Pretzer, 1986)) produced *VI* and nitrogen-containing products (which were not identified).

The hydrolysis of hydrazones is an equilibrium reaction as seen for *I* (Cordes and Jencks, 1962; Lowry and Richardson, 1981; Smith, 1983). However, the loss of *III* occurred in an apparent first-order manner and did not exhibit equilibrium behavior (initial rapid loss, subsequent stable phase). The apparent first-order loss was due to the instability of *X* which did not allow its accumulation. Only the forward reaction of the equilibrium (hydrolysis) was significant in the degradation of *III*. Because *X* degraded rapidly, the rate constant for appearance of *VI* was approximately equal to the rate of loss of *III*. The observed quantitative production of *VI* would be expected from the mechanism shown in Scheme 2.

The identification of *VII* as a major degradation product of *III* would be expected only as a result of hydrolysis. The presence of *VIII* after complete degradation of *III* at higher temperatures and concentrations indicated that some fraction of *III* might degrade via an elimination mechanism analogous to the elimination reaction of *I* (Pretzer and Repta, 1987) and *II* under these more stressful conditions.

The entropy of activation ($\Delta S^{\ddagger}$) for the degradation reaction of *III* was evaluated using data obtained from kinetic studies at pH 7.0 over the temperature range 34.0–60.0 °C. The Eyring plot of the data was linear. Curvature of the Eyring plot might be expected if more than one reaction mechanism was operative, i.e., both hydrolysis and elimination. The $\Delta S^{\ddagger}$ evaluated from the data was $-36.0 \pm 1.2$ at 25 °C. This value for $\Delta S^{\ddagger}$ was in the range expected for a bimolecular reaction (Jencks, 1969), consistent with the hydrolysis reaction shown in Scheme 2.

The dependence of the rate of loss of *III* on pH also indicated that hydrolysis was the major route of degradation of *III*. The greater observed



Scheme 2. Proposed hydrolytic degradation mechanism for *III*. (R = 4-methoxyphenyl).

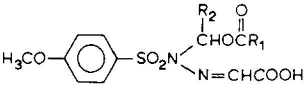
rate constants at more acidic pH values were expected since such hydrolysis reactions are known to be catalyzed by specific as well as general acids (Lowry and Richardson, 1981).

The methyl substitution on the amide nitrogen resulted in greater stability of *III* compared to *I*. At pH 4.5, $k_{\mathrm{obs}_{III}} = 5.35 \times 10^{-2}\ \mathrm{h}^{-1}$ and $k_{\mathrm{obs}_I} = 0.124\ \mathrm{h}^{-1}$. At pH 7.4, the difference in stability was greater with $k_{\mathrm{obs}_{III}} = 4.5 \times 10^{-3}\ \mathrm{h}^{-1}$ and $k_{\mathrm{obs}_I} = 1.25\ \mathrm{h}^{-1}$. The elimination of sulfinate from *III* would not be expected to be a facile reaction because the nitrogen (adjacent to the sulfonyl moiety) of *III* cannot ionize. The absence (or reduced importance) of the elimination reaction for *III* confirmed earlier speculation (Pretzer and Repta, 1987) that this ionization was an important destabilizing factor in the structure of *I*.

*Stabilization by prodrug formation*

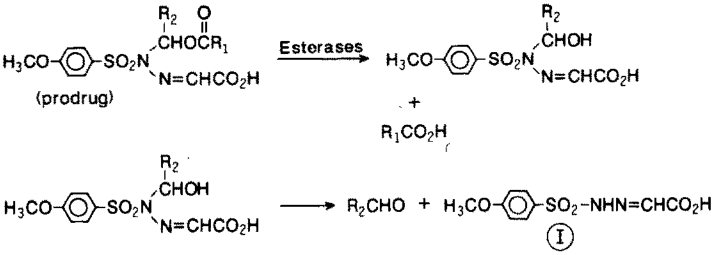
Due to the extremely short $t_{90}$ ($< 10$ min (Pretzer and Repta, 1987)) in aqueous solution, a substantial increase in the stability of *I* was needed in order to formulate *I* for use as an intravenous preparation. Although no absolute stability requirement exists for experimental formulations of anticancer agents, an increase of approximately two orders of magnitude in the stability of *I* would be necessary in order to avoid $> 10\%$ loss during formulation by lyophilization of solutions of *I* and dissolution previous to administration. From studies of derivatives *II* and *III*, it appeared that the $\alpha$-carbonyl and the NH group of *I* were important factors in the elimination degradation route. Electron delocalization to the $\alpha$-carbonyl would not be expected to occur to any significant extent when the carboxylic acid of *I* is ionized. Thus, the destabilizing effect of the $\alpha$-carbonyl group in *I* could be minimized by formulation at a pH well above the $\mathrm{p}K_a$ of the acid ($\sim 3$, estimated from the $\mathrm{p}K_a = 3.06$ for *III*).

Ionization of the NH moiety of arylsulfonylhydrazones was previously proposed to be the necessary first step in the elimination of sulfinate (Powell and Whiting, 1959; Shapiro, 1976; Makhova et al., 1978). The stability associated with *III* is consistent with these reports. Hydrolysis appeared to be the most important degradation route for *III* compared to the elimination mechanism for *I* (Pretzer and Repta, 1987). These results indicated that a bioreversible *N*-substituted derivative might be a promising approach to stabilization of *I*. Such a derivative would be expected to have some of the stability characteristics of *III* and after bioactivation would yield *I* which has demonstrated cytotoxic activity. An *N*-acyloxyalkyl derivative of the type shown below might be a useful prodrug derivative of *I*. In the proposed prodrug, the promoiety, $-\mathrm{CHR_2OCOR_1}$, has been substituted for the amide hydrogen of *I*. This substitution should confer the stability (relative to the elimination reaction undergone by *I*) associated with *N*-methylation (as demonstrated for *III*) to the prodrug. At pH $\geqslant 7.4$ the stability would be adequate (based on values of $k_{\mathrm{obs}}$ for *III*) for formulation by lyophilization of solutions of the prodrug. Additionally, under these conditions the solubility of the prodrug would be enhanced by essentially complete ionization of the carboxylic acid. After administration of the reconstituted prodrug, bioactivation (i.e., regeneration of *I*) would be expected to occur according to Scheme 3 where carboxylesterases present in the bloodstream and/or liver would cleave the ester portion of the prodrug. The *N*-hydroxymethyl species would degrade rapidly (where $R_2 = H$ or small alkyl group), releasing *I*. Toxicity of the acid and aldehyde (containing $R_1$ and $R_2$, respectively) and physical characteristics desired for the prodrug would be necessary considerations in determining the $R_1$ and $R_2$ moieties to be incorporated into the prodrug. While limited attempts made during the course of this work to obtain the prodrug where $R_1 = -\mathrm{C(CH_3)_3}$ and $R_2 = H$ were unsuccessful, further variations of reaction conditions or use of a promoiety containing different $R_1$ and $R_2$ groups may allow the desired prodrug to be isolated.



*N*—Acyloxyalkyl Prodrug of *I*

In conclusion, the results confirmed that ionization of the NH group was a major factor contributing to the instability of *I*. Although a bioreversible *N*-substituted derivative was not obtained, the



Scheme 3. Proposed mechanism for regeneration of *I* from an *N*-acyloxyalkyl prodrug.

greater stability of *III* compared to *I* illustrates that an understanding of the mechanism of degradation can lead to a rational approach to stabilization.

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## References

Albert, A. and Serjeant, E.P., *The Determination of Ionization Constants*, 2nd edn., Chapman and Hall, London, 1971, p. 45.

Blankley, C.J., Sauter, F.J. and House, H.O., Crotyl diazoacetate (acetic acid, diazo-, trans-2-butenyl ester). *Org. Synth.*, 49 (1969) 22–27.

Cordes, E.H. and Jencks, W.P., On the mechanism of Schiff base formation and hydrolysis. *J. Am. Chem. Soc.*, 84 (1962) 832–837.

Cradock, J.C., Private Communication, National Cancer Institute, 1982.

Jencks, W.P., *Catalysis in Chemistry and Enzymology*, McGraw-Hill, New York, 1969, pp. 605–614.

Lowry, T.H. and Richardson, K.S., *Mechanism and Theory in Organic Chemistry*, 2nd edn., Harper and Row, New York, 1981, pp. 635–641.

Makhova, N.N., Mikhajluk, A.N., Karpov, G.A., Protopopova, N.V., Khasapov, B.N., Khmelnitski, L.I. and Novikov, S.S., Reaction of substituted hydrazones with bases. Synthesis of α-aminodialkyl-diazenes. *Tetrahedron*, 34 (1978) 413–418.

Nurrenbach, A. and Pommer, H., Über die Umsetzung von Nitroso-Verbindungen mit Phosphoryliden. *Liebigs Ann. Chem.*, 721 (1969) 34–42.

Powell, J.W. and Whiting, M.C., The decomposition of sulphonylhydrazone salts. I. Mechanism and stereochemistry. *Tetrahedron*, 7 (1959) 305–310.

Pretzer, D., Aspects of the stability of the experimental anticancer agent [[(4-methoxyphenyl)sulphonyl]hydrazono]-acetic acid (NSC-267213) and related arylsulfonylhydrazones, Ph.D. thesis, University of Kansas, 1986.

Pretzer, D. and Repta, A.J., Stability of the experimental anticancer agent [[(4-methoxyphenyl)sulfonyl]hydrazono] acetic acid (NSC-267213). I. Kinetics and mechanism of degradation. *Int. J. Pharm.*, 38 (1987) 147–160..

Shapiro, R.H., Alkenes from tosylhydrazones. *Org. React.*, 23 (1976) 405–507.

Smith, P.A.S., *Derivatives of Hydrazine and Other Hydronitrogens Having N–N Bonds*. Benjamin/Cummings, Reading, MA, 1983, Chs. 2 and 3.