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Parenteral formulation development for the positive inotropic agent RS-82856. Hydrolysis and oxidation kinetics, solubility and i.v. formulation considerations

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Summary

The degradation kinetics and solubility of *N*-cyclohexyl-*N*-methyl-4-(1,2,3,5-tetrahydroimidazo[2,1-b]quinazolin-2-one-7-yloxy)butyramide (**1**, RS-82856) hydrogen sulfate in aqueous and organic solutions were investigated. It was found that **1** reached a ~ 50/50 equilibrium in water with its imidazole-ring opened product **2** (RS-31621), which then further degraded to give several secondary products. The rate constants for the acid catalyzed (k_H), spontaneous or water-catalyzed (k_0) and base-catalyzed (k_{OH}) reactions for both the forward and reverse reactions were determined at 40 °C, 60 °C, and 80 °C. The reacting species responsible for each reaction were proposed. Biphasic kinetics were also observed for the autoxidation of RS-82856 hydrogen sulfate in organic solvents; the 5-oxo analog (RS-82890) was the only product detected. The t_{90}s in propylene glycol, dimethylacetamide and dimethyl sulfoxide for the hydrogen sulfate salt at 25 °C are less than 4 weeks. These stability results and various solubility data were combined to evaluate possible intravenous formulations for toxicological and clinical studies. It was concluded that all solution formulations (aqueous, aqueous-organic or organic) are unsuitable for RS-82856 hydrogen sulfate and alternatives should be sought.

Introduction

N-Cyclohexyl-*N*-methyl-4-(1,2,3,5-tetrahydroimidazo[2,1-b]quinazolin-2-one-7-yloxy)butyramide (**1**, RS-82856) was shown to be a potent and selective inhibitor of Type IV cyclic AMP phosphodiesterase in human platelets (Alvarez et al., 1984a and b, 1986; Jones et al., 1987). In a number of cardiovascular model studies, RS-82856 has also been shown to possess significant inotropic activity when administered to both anesthetized and conscious dogs, with a long duration of action (≥ 8 h) (Alvarez et al., 1986). RS-82856 is structurally analogous to cilostamide (Endoh et al., 1980) and anagrelide (Tang and Frojmovic, 1980).

The initial preformulation studies revealed that **1** has an intrinsic aqueous solubility of ~ 5.0 μg/ml and two pK_as of 3.5 and 11.3. The drug was unstable in basic solutions. The hydrogen sulfate salt of **1** was selected for formulation development for reasons of higher aqueous solubility (0.21 mg/ml) and bioavailability (Gu et al., 1987).

In view of the fact that many compounds with the general structure of 2-oxo-imidazo[2,1-b]

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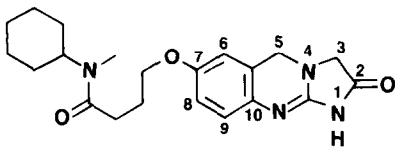
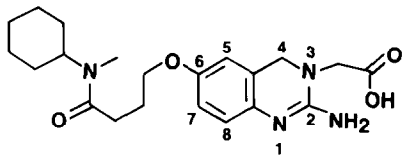
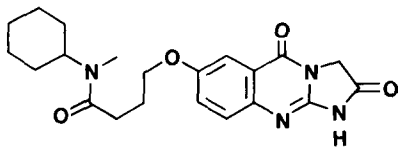


1, RS-82856



2 = RS-31621



3, RS-82890

quinazoline have demonstrated biological activities (Fleming et al., 1975; Tang and Frajmovic, 1980; Alvarez et al., 1984b), it is surprising that no literature data are available on the facile chemical reactivity of this type of compound. In this paper, we report the detailed hydrolysis kinetics of **1** in aqueous solution as well as the solubility and the stability of the hydrogen sulfate salt in commonly used organic solvents. The mechanisms for the observed reactions are proposed and the implication of the results to the design of intravenous (i.v.) dosage forms of RS-82856 hydrogen sulfate is discussed.

Materials and Methods

Chemicals

RS-82856 (**1**), 2-[2-amino-3,4-dihydro-6-(*N*-cyclohexyl-*N*-methylbutyramidyl-4-oxy)-quinazolin-3-yl]acetic acid (RS-31621, **2**), and *N*-cyclohexyl-*N*-methyl-4-(1,2,3,5-tetrahydroimidazo[2,1-b]-quinazolin-2,5-dione-7-yloxy)butyramide (RS-82890, **3**) were prepared according to the methods reported elsewhere (Venuti et al., 1987; Jones et al., 1987) by the Institute of Bioorganic Chemistry, Syntex Research. Nanopure water and high-performance liquid chromatography (HPLC)-grade acetonitrile were used for the preparation of mobile phase. All other chemicals and solvents were reagent grade and were used as received.

Instrumentation

Ultraviolet (UV) spectra were taken on a Hewlett Packard 8450A spectrophotometer. A Radiometer Model PHM64 Research pH meter equipped with a Radiometer Model GK2410C combination electrode was used to measure the pH. HPLC was performed using a Spectra-Physics Model 8700 instrument equipped with a Kratos 757 variable wavelength UV detector, a Micromeritics 728 autosampler and a Spectra-Physics 4100 computing integrator. An HP 1090 chromatographic system equipped with a photodiode array UV detector was used to obtain the UV spectra of the HPLC peaks.

HPLC method

The reverse-phase HPLC method employed an Ultrasphere I.P. C18 (Altex) 5 μ column (4.6 mm $\times$ 250 mm), a mobile phase of phosphate buffer (0.01 M, pH 6.5)/acetonitrile/tetrahydrofuran (70/25/5). The detection wavelength was 280 nm and the detector sensitivity was 0.05 AUFS. The linearity of the method was demonstrated in the range of 0.053–1.32 μg compound injected using either peak area or peak height measurement ($\gamma = 0.999$). The stability specificity of the method was supported by obtaining the identical UV spectra at the upslope, apex and downslope of the parent peak when degraded samples of **1** were injected. Also, complete disappearance of **1** was observed when a totally degraded sample was injected.

Solubility measurements

To a number of 2.5 ml colorless glass vials was added 0.5–2 ml of the appropriate solvent and sufficient amount of drug such that an excess of solid drug always remained. The vials were sealed with Teflon-lined squeeze caps and were equilibrated by tumbling (25 rpm) at 25 °C. At appropriate timepoints vials were removed from the

tube, the contents filtered through a 0.45 μm Millipore-HV disposable filter, and then diluted with the mobile phase to afford a concentration of 2–10 μg/ml. The diluted solutions were assayed by HPLC.

Stability studies

Buffer solutions were prepared at 0.01 ~ 0.20 M total buffer concentration using acetate (pH 3–5), phosphate (pH 6–8), borate (pH 8–9) and carbonate (pH 9–10). NaCl was added to adjust the ionic strength to 0.20 and the pH of each buffer solution was measured at the reaction temperature. For very acidic (pH 0–2) and basic (pH 10–13) solutions, aqueous HCl and KOH solutions were used to obtain the desired pH. Typically, 1.0 ml of a 1.0 mg/ml methanolic stock solution was added to 100 ml of freshly prepared buffer solution and then 5 ml aliquots of this solution were transferred to several 5 ml clear-glass ampoules. The ampoules were then flame-sealed and stored at constant temperature until predetermined time periods where the samples were removed, quenched to 0 °C using an ice bath, and warmed to room temperature immediately before assaying by HPLC. The stability of hydrogen sulfate salt of **1** in organic solvents was determined using a similar procedure for the sample storage and analysis.

pK_a Determination at 40, 60 and 80 °C

A 100 ml stock solution of 0.05 M KCl in H_2O was prepared and equilibrated in a water bath at the desired temperature. The pH of the solution was measured and adjusted by adding 0.10 N KOH or 0.10 N HCl. An aliquot (3 ml) of this solution and a 50 μl aliquot of a stock solution of drug in methanol were transferred into a 4 ml quartz cuvette and equilibrated in the water bath for an additional 5 min to ensure that the desired temperature was obtained. The pH change of the solution due to the addition of the drug was negligible and the variation in temperature was found to be ±1.5 °C. The cuvette was then removed from the bath and the UV spectrum was taken immediately. Due to the rapid degradation at elevated temperatures, the mixture in the cuvette was discarded after each measurement. The procedure was repeated until sufficient UV spectra at the desired pHs (from pH 1.5 to pH 12.4) were obtained. The representative UV spectra of **1** at various pH values are shown in Fig. 1. The absorbance difference at 242 nm and 300 nm were used to determine the first and second pK_as of **1** respectively. For compound **2**, the absorbance difference at 283 nm was used to determine pK_{a_2}.

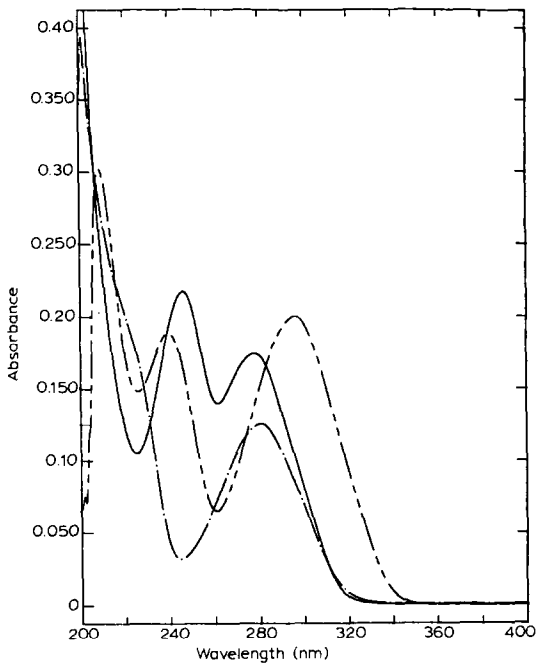


Fig. 1. UV spectra of **1** (3.77 μg/ml) in pH 2.1 (·–·–·), pH 8.0 (———) and pH 12.2 (—·—·—) aqueous solutions.

Results and Discussion

*Hydrolysis of **1** in aqueous solution*

The hydrolysis of RS-82856 hydrogen sulfate was studied in buffer solutions of pH 1 to pH 13 at 40, 60 and 80 °C. The extent of reaction was followed using a stability-specific HPLC method. Buffer concentrations were varied from 0.010 M to 0.20 M and were found to have no effect on the rate of degradation. Biphasic reaction kinetics, i.e. fast degradation to ~ 50% remaining followed by a slower process, were observed throughout the acidity range studied. HPLC analyses of the degradation samples revealed that only one product,

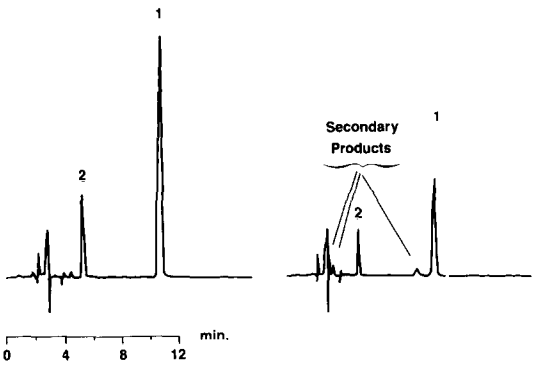
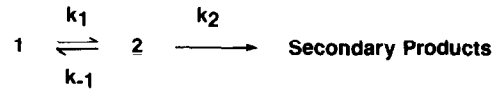


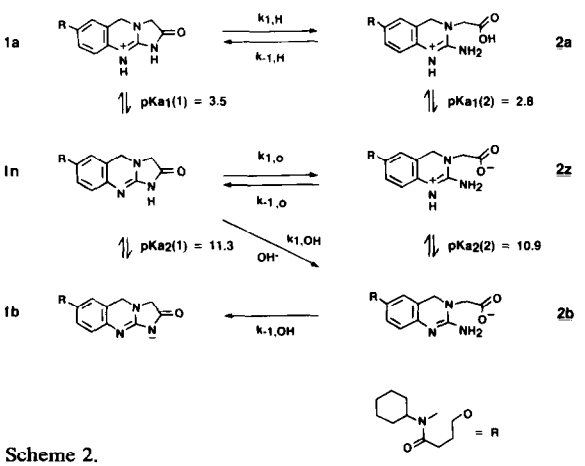
Fig. 2. HPLC analyses of the degraded samples of RS-82856 in pH 8.8 buffer at (a) 50% remaining, and (b) 25% remaining.

RS-31621 (**2**), was formed initially (Fig. 2a) which then degraded further to give at least 3 secondary products (Fig. 2b). Also, the ratio of [**1**]/[**2**] appeared to reach a constant value at ~50% remaining, even though both compounds continued to degrade. The simplest kinetic scheme explaining these observations is shown in Scheme 1,



Scheme 1.

where k_1 and k_{-1} are the observed pseudo-first-order rate constants for the forward and reverse reaction respectively, and k_2 is the sum of observed secondary reactions. The concentrations of



Scheme 2.

1 and **2** at a given time can be expressed as (Capellos and Bielski, 1972)

$$[\mathbf{1}] = [\mathbf{1}]_0\left(\left(\frac{k_{-1}+k_2-\gamma_1}{\gamma_2-\gamma_1}\right)e^{-\gamma_1 t} + \left(\frac{k_{-1}+k_2-\gamma_2}{\gamma_1-\gamma_2}\right)e^{-\gamma_2 t}\right) \quad (1)$$

$$[\mathbf{2}] = k_1[\mathbf{1}]_0\left(\left(\frac{1}{\gamma_2-\gamma_1}\right)e^{-\gamma_1 t} + \left(\frac{1}{\gamma_1-\gamma_2}\right)e^{-\gamma_2 t}\right) \quad (2)$$

where $[\mathbf{1}]_0$ is the initial concentration of **1**, and the roots γ_1 and γ_2 are defined as

$$\gamma_1,\gamma_2 = \frac{(k_1+k_{-1}+k_2) \pm \sqrt{(k_1+k_{-1}+k_2)^2 - 4k_1k_2}}{2} \quad (3)$$

A non-linear least-squares regression computer program was used to fit the observed kinetic data at different acidities to Eqn. 1. A plot demonstrating the excellent correlation between the experimentally obtained and the theoretically calculated concentrations of **1** and **2** according to Eqns. 1 and 2 is given in Fig. 3. The values for the pseudo-first-order rate constants k_1, k_{-1} and k_2 thus obtained are plotted as a function of pH in Fig. 4.

From the shape of the log(rate)-pH profiles displayed in Fig. 4, both the hydrolysis of **1** (k_1) and the cyclization of **2** (k_{-1}) can be described in terms of acid catalyzed (k_H), spontaneous or neutral catalyzed (k_0) and base-catalyzed (k_{OH}) reactions. The proposed major species involved in the equilibrium process between **1** and **2** are summarized in Scheme 2. As suggested by the plateau region for k_1 at pH < pK_{a_1}(1) (= 3.5), the cation **1a** is probably responsible for the acid-catalyzed hydrolysis. The unionized form, **1n**, is also involved but to a much lesser extent and only at the intermediate pHs (5–7). At pH > pK_{a_2}(**1**) (= 11.3), **1** exists mainly as the non-electrophilic anion (**1b**).

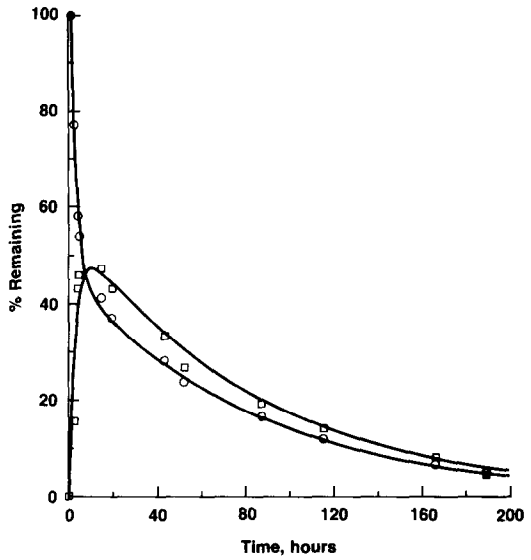


Fig. 3. Hydrolysis kinetics of (**1**) showing the % remaining of RS-82856 (○, **1**) and RS-31621 (□, **2**) at 80 °C in pH 8.8 buffer. The solid lines are the theoretical curve fit of the experimental data using Eqns. 1–3 and the rate constants given in Fig. 4.

Because water is not a good nucleophile, the reaction between **1b** and water is improbable and so a more logical (and kinetically equivalent) process involving reaction of OH^- with **1n** is proposed to account for the base-catalyzed hydrolysis reaction. The plateau observed at $pH > pK_{a_2}$(**1**) is due to the compensating effects of the increase in $[OH^-]$ and the decrease in [**1n**] by ionization.

The cyclization of **2** showed a similar log (rate)–pH profile. Because of the striking rate resemblance to enzymatic reactions this intramolecular type of aminolysis reaction involving amino acids or amino esters has been the subject of a number of studies (Camilleri et al., 1979, Abdallah and Moodie, 1983, Fife and Duddy, 1983 and refs. cited therein). In theory, all 4 possible forms of an amino acid (Scheme 3) are potential reactants. The most basic site in **2** is the aniline nitrogen (N_1) which is, however, not structurally oriented for a possible intramolecular reaction (Scheme 1). The most likely reaction site is therefore the terminal primary amine group (2-amino) which is in resonance with the aniline nitrogen. Since the two pK_as of **2** are very different (see below), the neutral form of **2** is not expected to build up significantly at any pH, and therefore, the reactions involving the neutral **2** may not be important. In similarity to the hydrolysis mechanism of **1**, the cation (**2a**) and the zwitterion of **2** (**2z**) are proposed to be the major species for the cyclization at $pH < pK_{a_2}$ (**2**). The base catalyzed cyclization process, on the other hand, is probably between the carboxylate anion of **2** (**2b**) and water because the rate reached a plateau in the very basic region. For the kinetic process outlined in Scheme 2, the observed pseudo first-order rate

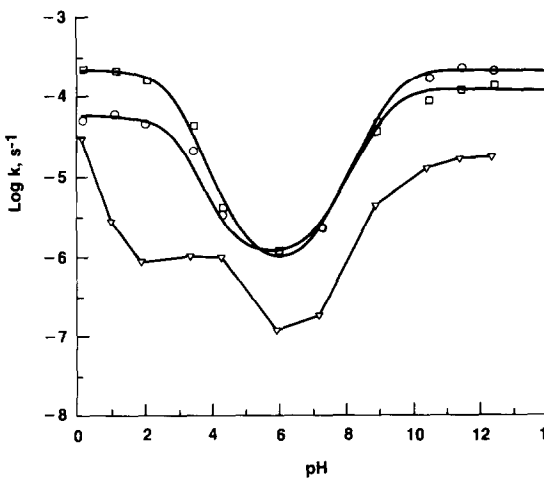
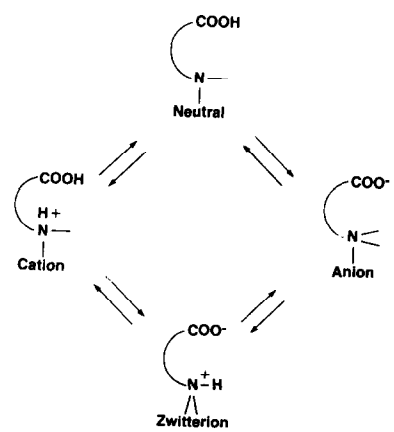


Fig. 4. Log(rate)–pH constant profile for the degradation of RS-82856 in aqueous solution at 80 °C. ○, k_1; □, k_{-1}; ▽, k_2. The lines drawn for k_1 and k_{-1} are from the best fit values of k_H, k_0, k_{OH} obtained by non-linear least-squares regression analysis using Eqn. 4.



Scheme 3.

constants k_1 and k_{-1} can be expressed in the general form

$$k_{obs} = (k_H a_H + k_0 + k_{OH} a_{OH}) \times \left(\frac{1}{1 + a_H/K_{a_1} + K_{a_2}/a_H} \right) \quad (4)$$

where a_H is the hydrogen ion activity, a_{OH} is the hydroxide ion activity and K_{a_1} and K_{a_2} are the dissociation constants of **1** and **2**, respectively. Values for the various rate constants and pK_as at 80 °C were determined according to Eqn. 4 using the non-linear regression analysis method. The rate constants from the various fits are listed in Table 1 and the solid curves drawn for k_1 and k_{-1} in Fig. 4 were constructed from these constants. The excellent agreement between the experimental results and those calculated according to Eqn. 4 indicates that Eqn. 4 adequately describes the observed pH effect on the rate constants of k_1 and k_{-1}. The pK_a values of **1** and the pK_{a_2} value of **2** at 80 °C obtained by this kinetic determination are in satisfactory agreement with those determined directly by the spectrophotometric method. Although no comparison can be made for pK_{a_1} of **2** (because it lacks the absorption difference in the carboxylic group necessary for spectrophotometric determination), the kinetically obtained value of 2.88 is consistent with that of a typical compound having an amino acid structure.

The hydrolysis–cyclization kinetics were subsequently studied at 40 °C and 60 °C. Since only 8 different buffer solutions (pH 2–12) were used at these two temperatures, the respective rate constants were calculated using Eqn. 4 and the spectrometrically measured values of pK_{a_1} and pK_{a_2} of **1** and pK_{a_2} of **2**. Eq. 4 is therefore simplified to a 3 parameter fit for solving k_{-1} and a 4-parameter fit for solving k_{-1}. The values obtained for the rate constants at pK_{a_1} of **2** at 60 °C and 40 °C are also listed in Table 1. All rate constants that involved the reversible reaction between **1** and **2** were found to obey the Arrhenius equation. The corresponding activation energies, entropies, and enthalpies derived are summarized in Table 2 and the calculated rate constants are 25 °C are included in Table 1.

Thus, the rate constants for both the acid- and base-catalyzed ring-opening hydrolysis reactions of **1** at 25 °C are much larger (> 100 fold) than those of simple 5- or 6-membered ring lactams (Wan et al., 1980; Holley and Holley, 1949). This is easily rationalized inasmuch as the two unpaired electrons at the N_1 position of **1** are in resonance with the aromatic ring and thus diminish the electron-donating ability to the carbonyl group compared with simple lactams.

No direct comparison between the cyclization rate of **2** and simpler systems can be made due to lack of literature data on the latter. The observed rate constant for the acid catalysed cyclization of **2** at low pH values (pH ≤ 2) and 40 °C (6.1×10^{-6} s^{-1}) however, is much smaller than that of 3-(2-

TABLE 1

Rate constants and the ionization constants for the hydrolysis of RS-82856 (1) and the cyclization of RS-31621 (2)

Reaction	Temp. (°C)	$k_H \times 10^2$ (M^{-1} s^{-1})	$k_0 \times 10^7$ (s^{-1})	$k_{OH} \times 10^2$ (M^{-1} s^{-1})	pK_{a_1} [a]	pK_{a_2} [a]
Hydrolysis of **1**	80	5.6	12	34	3.0 (3.1)	9.6 (9.8)
	60	1.5	2.7	6.2	(3.2)	(10.2)
	40	0.24	0.57	0.86	(3.3)	(10.6)
	25 [b]	0.062	0.15	0.17	(3.5)	(11.3)
Cyclization of **2**	80	14	9.7	33	2.8	9.3 (9.5)
	60	2.7	1.9	5.6	2.7	(9.9)
	40	0.38	0.28	0.60	2.8	(10.5)
	25 [b]	0.079	0.059	0.10	2.9	(10.9)

[a] Values in parentheses represent spectrometrically measured pK_as.

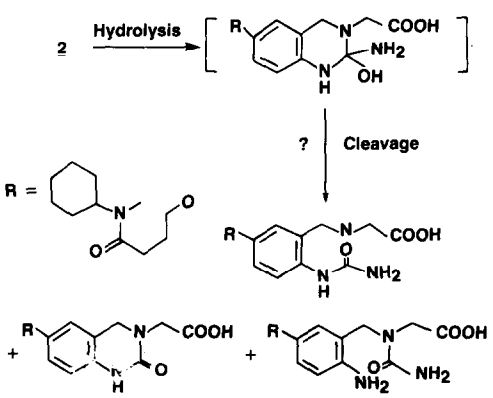
[b] Extrapolated from E_a and log A given in Table 2.

TABLE 2

Summary of activation parameters for the degradation of RS-82856 (1) in aqueous solution

Rate Constant	E_a (kcal · mol^{-1})	log A	$\Delta H^{\neq}$ (kcal · mol^{-1})	$\Delta S^{\neq}$ (cal · mol^{-1} · K^{-1})
Hydrolysis				
k_H	17.3 ± 1.0	9.5 ± 6.8	16.7 ± 1.0	−17.2 ± 1.8
k_0	16.7 ± 0.38	4.4 ± 0.25	16.1 ± 0.37	−40.4 ± 6.7
k_{OH}	20.2 ± 0.17	12.0 ± 0.11	19.5 ± 0.18	−5.6 ± 0.07
Cyclization				
k_H	19.8 ± 0.31	11.4 ± 0.21	19.2 ± 0.32	−8.4 ± 0.21
k_0	19.5 ± 0.23	6.0 ± 0.15	18.8 ± 0.24	−33.0 ± 1.7
k_{OH}	22.0 ± 0.69	13.2 ± 0.46	21.4 ± 0.71	−0.37 ± 0.017

aminophenyl) propionic acid at 40 °C (3.6×10^{-3} s^{-1}). This could be due to the different ring size (e.g. 5 vs 6) and/or the significantly different basicity of the terminal amino groups of these two compounds (p*K*a of 11.0 vs 4.3). For the base-catalyzed cyclization reaction of **2**, the anion (**2b**) is proposed to be the reacting species. This is in contradiction to the anion of 3-(2-aminophenyl) propionic acid which is virtually inert (Camilleri et al., 1979). Finally, the nature of the secondary degradation (k_2) reaction shown in Scheme 1 can not be examined closely at this time (due to the lack of structural identification of the secondary degradation products) but it is likely that hydrolysis occurs at the C=N$^+$ bond of **2** to yield several products as shown in Scheme 4. Hydrolysis at the N_{10} position is probably not important for **1**, especially at pH ≥ 3.5, due to the absence of a driving force (the C=N bond in **1** is not charged) for the nucleophilic attack by water.



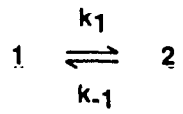
Scheme 4.

I.v. formulation considerations, solubility and stability in organic solvents

I.v. formulations are typically comprised of an aqueous solution of the active substance and are usually prepared at a specific pH for optimal solubility and chemical stability. In some instances a cosolvent is introduced to enhance drug solubility and, in extreme cases, a concentrated solution can be prepared in a totally organic system. Despite often dramatically different requirements, both the solubility and the stability of a drug play important roles in designing successful parenteral formulations for toxicological or clinical studies.

Based on accumulated pharmacological data for RS-82856 (Alvarez et al., 1986), concentrations of 0.25 mg/ml or greater may be expected for clinical evaluations. To achieve such dosage levels in aqueous solution, the pH must be adjusted to pH ≤ 2 or pH ≥ 12.5, as based on the values of intrinsic solubility and the two pK_as of the drug. Aqueous solutions with such acidities are in fact not uncommon in commercially available drugs (Wang and Kowal, 1980). To determine if an aqueous solution of **1** exhibits adequate chemical stability ($t_{90} \geq 2$ years) for i.v. formulation at these pHs, the effect of pH on aqueous shelf-life is estimated from the kinetic data. The relationship of [**1**] vs time (t) given in Eqn. 1 indicates that t is a complex function of [**1**], $[\mathbf{1}]_0$, k_1, k_{-1}, and k_2 such that there is no simple solution for t_{90} (time for 90% remaining). Fortunately, from the k_1, k_{-1} and k_2 values shown in Fig. 4, $k_2[\mathbf{2}]$ is found to

be negligible compared with $k_1[\mathbf{1}]$ and $k_{-1}[\mathbf{2}]$ at greater than 90% remaining of the drug. Therefore, at $t \leq t_{90}$, Scheme 1 can be approximated by a simple reversible reaction between **1** and **2** as shown in Scheme 5.



Scheme 5.

An estimate of the shelf-life of **1** at 25 °C, according to Scheme 5 and Eqn. 3 using the appropriate rate constants in Table 1 is plotted as a function of pH in Fig. 5. The estimated t_{90} of **1** at 25 °C is ~ 4 days at pH ≤ 2 and ~ 5 h at pH 12.5, both of which are obviously too short for clinical formulation of **1**. The estimated t_{90} of **1** at the pH of maximum stability (pH 6–7) is ~ 3 months. This suggests that a cosolvent system would also be unlikely for a clinical formulation because the rate of hydrolysis is often not significantly affected by the organic content except at very high levels (Irwin et al., 1984; Mojaverian and Repta, 1984). Although the stability requirement is often much less stringent for short-term toxicological studies, the possible use of an aqueous formulation can also be ruled out because the ~ 3 mg/ml drug concentration required for such studies can only be achieved at pH < 1 or > 13, and neither is acceptable for apparent tolerance reasons.

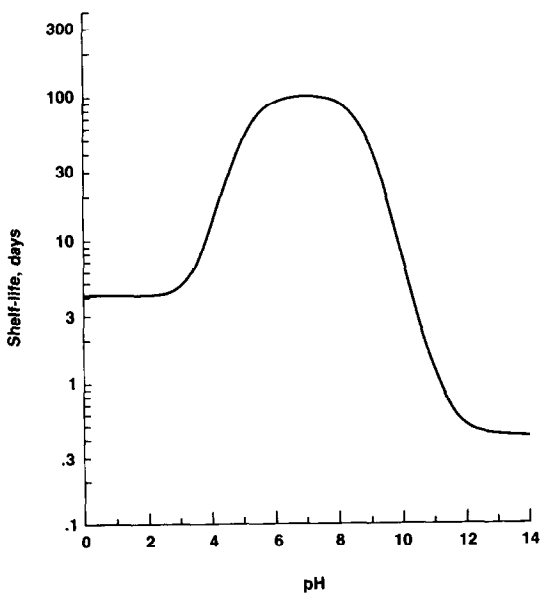


Fig. 5. Estimated aqueous shelf-life (90% remaining) of RS-82856 at 25 °C as a function of pH. The solid line is obtained using Scheme 5, Eqn. 4 and the appropriate rate constants listed in Table 1.

TABLE 3

Solubility and stability of RS-82856 hydrogen sulfate in organic solvents

Solvent	Solubility (mg/ml)	t_{90}, days	
		25 °C	40 °C
Acetone	0.013	–	–
CH_3CN	0.027	–	–
EtOH	2.5	–	–
PG	6.7	18	3.3
DMA	15	19	2.6
DMSO	250	68	29

The use of a concentrated organic formulation (e.g. diluted prior to administration) can be an effective way to increase drug solubility. For this reason, the solubility of the hydrogen sulfate, in various commonly used organic solvents was evaluated; Table 3 summarizes the results. In general, the solubility of the hydrogen sulfate salt was found to increase with the polarity of the solvent and propylene glycol (PG), dimethyl acetamide

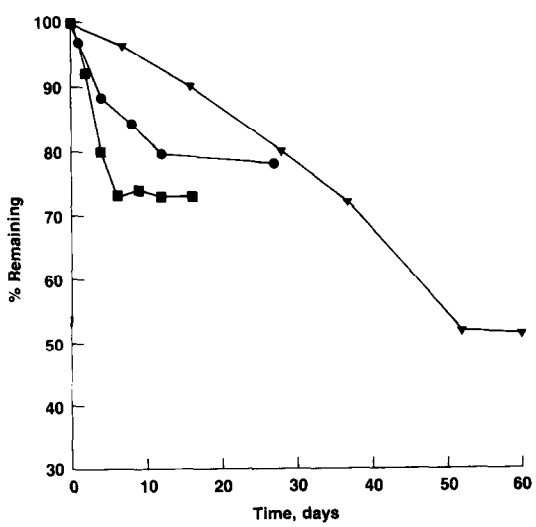


Fig. 6. Degradation kinetics of RS-82856 hydrogen sulfate salt in PG (●), DMA (■) and DMSO (▼) at 40 ° C.

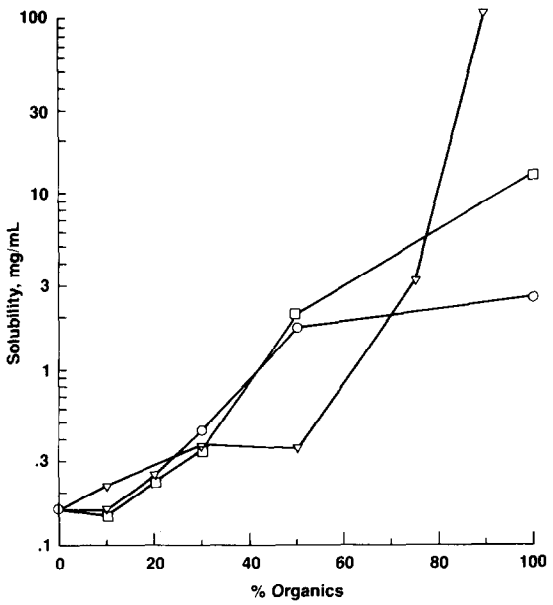


Fig. 7. Solubility of RS-82856 hydrogen sulfate salt in mixed organic-aqueous solvents. ○, PG; □, DMA; ▽, DMSO.

(DMA) or dimethyl sulfoxide (DMSO) appeared to be suitable for solubilizing the drug (solubility > 3 mg/ml). In order to test these organic solvents as candidates for formulation of **1**, the stability of the hydrogen sulfate salt in PG, DMA and DMSO was evaluated at 25 and 40 °C. The degradation observed in the 5 ~ 10 mg/ml drug solutions was again found to be biphasic which was relatively independent of the solvent used (Fig. 6). Since the major decomposition product (RS-82890, **3**) apparently results from oxidation of the parent drug, the observed biphasic kinetics were probably due to the depletion of the available oxygen in the sealed ampules. The t_{90}s of the 5 mg/ml drug in DMA and PG are only ~ 3 days at 40 °C and increased to ~ 2–3 weeks at 25 °C. DMSO appeared to retard the autoxidation process with a t_{90} of $2\frac{1}{2}$ weeks at 40 °C and 10 weeks at 25 °C (Table 3).

Finally, to screen for a possible cosolvent vehicle with drug concentration of > 3 mg/ml for toxicological studies, the solubility of the hydrogen sulfate salt as a function of % organics in PG, DMA and DMSO was determined (see Fig. 7). It was found that ≥ 60% organic content is necessary to provide a 3 mg/ml drug solution for toxicological studies. This ⩾ 60% organic content is again unsuitable to be used in animals.

Conclusion

The chemical reactivity of the 2-oxo-imidazo [2,1,-b]quinazoline ring structure using **1** as a case study is explored herein. Due to the incorporation of the two unpaired electrons of N_1 into the quinazoline system, the ring opening hydrolysis of **1** at the imidazole ring was found to be rapid, e.g. at least 100-fold faster than those of simple lactams. Interestingly, intramolecular aminolysis of the hydrolysis product, **2**, had a rate similar to the hydrolysis reaction of **1** and thus formed a ~ 50/50 equilibrium mixture with **1** after short reaction time. This ratio is likely to vary for compounds with different benzene ring substitution because the electronic requirement for the ring opening and cyclization reactions is expected to be different. Electron withdrawing substitution (at the benzene ring) should accelerate the hydrolysis by weakening the amide bond in **1**, whereas an electron-donating group should make the cyclization reaction faster by increasing the basicity of amino group in **2**.

In organic solvents, the hydrogen sulfate of **1** was found to oxidize rapidly and quantitatively to give **3**. The poor solubility and stability of **1** in aqueous and organic solvents indicates the difficulty in the development of i.v. solution formulations for both clinical and toxicological studies. Alternatives involving complexation and/or lyophilization to resolve the solubility and stability problems has been explored in a separate paper (Visor et al., 1987).

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