pH-Dependent Hydrolysis of 4,4′-Diformamidodiphenylsulfone

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Abstract □ The hydrolysis of the antimalarial agent, 4,4′-diformamidodiphenylsulfone (I), was studied at five hydrogen-ion concentrations and four different temperatures. Under all conditions, 4,4′-diformamidodiphenylsulfone was first hydrolyzed to 4-amino-4′-formamidodiphenylsulfone (II) and then to 4,4′-diaminodiphenylsulfone (III) according to the reaction: $I \xrightarrow{k_1} II \xrightarrow{k_2} III$. The k_1 rate constants were greater than k_2. Both k_1 and k_2 followed apparent first-order kinetics with respect to 4,4′-diformamidodiphenylsulfone and 4-amino-4′-formamidodiphenylsulfone concentrations, respectively, and were dependent on pH in the order of $k_{pH\,2} >>> k_{pH\,8} \simeq k_{pH\,10} > k_{pH\,4} \simeq k_{pH\,6}$. Thus, at the stomach pH, extremely rapid nonenzymatic hydrolysis is to be expected. It is, however, limited by the solubility of the drug which is extremely low. 4,4′-Diformamidodiphenylsulfone was found to be most stable at pH ~6.

Keyphrases □ 4,4′-Diformamidodiphenylsulfone—pH-dependent hydrolysis □ Hydrolysis, pH dependent—4,4′-diformamidodiphenylsulfone □ TLC—reaction monitoring

4,4′-Diformamidodiphenylsulfone has a suppressive action against *Plasmodium berghei* infections in mice (1) and shows a modest repository activity (2, 3). It has an antimalarial activity similar to, but more prolonged than, 4,4′-diaminodiphenylsulfone against chloroquine-resistant strains of *Plasmodium falciparum* infections in man (4). The observed reversal of antimalarial action of 4,4′-diaminodiphenylsulfone against *P. falciparum* infections in man (5) and of 4,4′-diformamidodiphenylsulfone against *P. berghei* infections in mice (6) by *p*-aminobenzoic acid suggests that 4,4′-diformamidodiphenylsulfone acts as an antimalarial by hydrolysis to the 4,4′-diaminodiphenylsulfone or 4-amino-4′-formamidodiphenylsulfone. Therefore, a knowledge of the hydrolysis of 4,4′-diformamidodiphenylsulfone in solutions of various pH's at various temperatures seemed vital before reaching conclusions about the mechanism of its antimalarial activity. This report presents the rates of hydrolysis of 4,4′-diformamidodiphenylsulfone at several pH's and temperatures.

METHODS

Because of its low solubility in water (~10 mcg./ml.), a stock solution was prepared by dissolving 4,4′-diformamidodiphenylsulfone in dimethyl sulfoxide to make a concentration of 5 mg./ml. One- or two-milliliter portions of this solution were added to 2 or 3 ml. of buffer at pH 1.7, 4.05, 5.95, 7.85, and 9.65 (buffer compositions given in Table I), which had been kept at the relevant temperature. The ionic strength of all the buffers was kept constant by adding KCl. The final concentration of 4,4′-diformamidodiphenylsulfone was 2500 or 1250 mcg./ml. A few experiments were done starting with 4-amino-4′-formamidodiphenylsulfone in concentrations of 2500 mcg./ml. In all cases, the reactions were followed either to completion or through one half-life.

At various time intervals, 0.1-ml. aliquots were withdrawn and analyzed for free arylamine by the method of Bratton and Marshall (7) as modified in this laboratory. The method permitted the determination of 4,4′-diaminodiphenylsulfone or 4-amino-4′-formamidodiphenylsulfone in presence of 4,4′-diformamidodiphenylsulfone without any hydrolysis of 4,4′-diformamidodiphenylsulfone during analytical procedures. The method involved addition of 4.5 ml. of trichloroacetic acid–sodium citrate buffer[1] to a final 0.5-ml. volume of sample plus water.

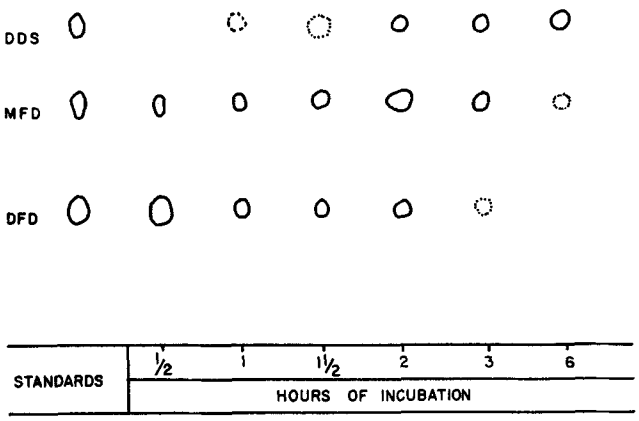

Figure 1—*TLC of 4,4′-diformamidodiphenylsulfone (DFD) (after hydrolysis in buffer of pH 1.7 at 37°) on silica gel, developed twice in ethyl acetate and seen under shortwave UV light. DDS = 4,4′-diaminodiphenylsulfone; MFD = 4-amino-4′-formamidodiphenylsulfone.*

Duplicate 2-ml. aliquots were then diazotized and coupled by adding 0.3 ml. each of 0.1% sodium nitrite, 0.5% ammonium sulfamate, and 0.1% *N*-(1-naphthyl)ethylenediamine dihydrochloride (coupling reagent) in the order mentioned, mixing, and waiting for 1 min. after addition of each reagent. The reaction mixtures were then allowed to stand at room temperature for 10 min. One milliliter of 100% ethanol was added, and the absorbance was measured at 540 nm., using 1-cm. lightpath. The method was sensitive enough to detect 1.5 mcg. of 4,4′-diaminodiphenylsulfone per sample. Total arylamines were determined after boiling for 45 min. 0.1-ml. aliquots of the incubation mixtures in 0.2 ml. of 5 *N* HCl, 1 ml. of 15% trichloroacetic acid, and 3.7 ml. water. This procedure hydrolyzed all the 4,4′-diformamidodiphenylsulfone and 4-amino-4′-formamidodiphenylsulfone to 4,4′-diaminodiphenylsulfone. Duplicate 2-ml. aliquots of the hydrolyzed samples were diazotized and coupled, and the A_{540} was determined as previously described. The detailed discussion of the methods as applied to the mixtures of 4,4′-diformamidodiphenylsulfone 4-amino-4′-formamidodiphenylsulfone, and 4,4′-diaminodiphenylsulfone in aqueous solutions, urine, or plasma will be published (8, 9).

The hydrolysis of 4,4′-diformamidodiphenylsulfone was monitored by TLC using silica gel-coated glass plates (F254). After spotting the samples, the plates were run twice to the same mark in ethyl acetate with intermittent drying. This gave a better separation of hydrolytic products. The resulting separation of hydrolytic products was observed under shortwave UV light. The dried plates were then sprayed with Ehrlich's reagent (*p*-dimethylaminobenzaldehyde, HCl) which produced yellow spots, reacting with free amine of 4,4′-diaminodiphenylsulfone and 4-amino-4′-formamidodiphenylsulfone. A light-yellow color was also seen at 4,4′-diformamidodiphenylsulfone spots, probably due to deformylation of tiny amounts of 4,4′-diformamidodiphenylsulfone releasing free amine with the Ehrlich reagent spray. Standard solutions of 4,4′-diaminodiphenylsulfone, 4-amino-4′-formamidodiphenylsulfone, and 4,4′-diformamidodiphenylsulfone were similarly treated along with test samples for the purpose of identification of hydrolytic products of 4,4′-diformamidodiphenylsulfone.

[1] Trichloroacetic acid, 245 g., and sodium citrate, 163 g., dissolved in distilled water and the volume adjusted to 500 ml.; 40 ml. of 1% polysorbate 80 (Tween 80) added and pH adjusted, if necessary, to 2.35 with 60% trichloroacetic acid.

Table I—Conditions, Observed[a] First-Order Rate Constants, and Energy of Activation for the Hydrolysis of 4,4′-Diformamidodiphenylsulfone[b] in Various Buffers at Different Temperatures

Buffer Composition[c]	Buffer pH	$10^5 k$ in min.$^{-1}$ at Temperatures 25° k_1	25° k_2	37° k_1	37° k_2	60° k_2	70° k_2	80° k_2	ΔH_a for k_2, kcal. mole^{-1}
25 ml. of 0.2 *M* KCl 6.5 ml. 0.2 *M* HCl	1.7	920	460	—	1160 1200[e]	6000	12,000	25,000	14.186
10 ml. of 0.5 *M* CH_3COONa 10 ml. of 2 *M* CH_3COOH	4.05	—	1.3[d]	6.2	3	13	24	40	12.886
25 ml. of 0.1 *M* KH_2PO_4 2.8 ml. of 0.1 *M* NaOH	5.95	—	1.1[d]	4.7	2.4	10	17	27	12.039
25 ml. of 0.1 *M* KH_2PO_4 23 ml. of 0.1 *M* NaOH	7.85	—	9.9[d]	37	18 16[f]	69	116	190	11.669
25 ml. of 0.05 *M* $NaHCO_3$ 5.3 ml. of 0.1 *M* NaOH	9.65	—	5.1[d]	23	11	42	73	120	12.035

[a] Unless otherwise stated. [b] 2.5 mg./ml. in 50% dimethyl sulfoxide unless otherwise stated. [c] Ionic strength μ adjusted to 1.4 with KCl. [d] Calculated from ΔH_a values. [e] Hydrolysis was started with 4-amino-4′-formamidodiphenylsulfone. [f] Using 1250 mcg./ml. of 4,4′-diformamidodiphenylsulfone in 25% dimethyl sulfoxide.

RESULTS AND DISCUSSION

TLC analysis of the reaction mixture at various time intervals showed that 4,4′-diformamidodiphenylsulfone (I) is initially deformylated to 4-amino-4′-formamidodiphenylsulfone (II), which is then deformylated to 4,4′-diaminodiphenylsulfone (III) according to the reaction: $\text{I} \xrightarrow{k_1} \text{II} \xrightarrow{k_2} \text{III}$.

A typical TLC tracing is shown in Fig. 1. R_f values of 4,4′-diformamidodiphenylsulfone, 4-amino-4′-formamidodiphenylsulfone, and 4,4′-diaminodiphenylsulfone were found to be 0.29, 0.53, and 0.7, respectively, under these conditions.

The logarithm of the difference in A_{540} for free amine at time t (A_t) and at time of complete hydrolysis (A_∞) was plotted against time From the equation:

$$\log (A_\infty - A_t) = \frac{-k \cdot t}{2.303} + \log A_\infty \qquad \text{(Eq. 1)}$$

k_1 was calculated taking the data of the time period before appearance of any 4,4′-diaminodiphenylsulfone (as seen by TLC). Similarly, k_2 was calculated using the data of the time period after disappearance of 4,4′-diformamidodiphenylsulfone from the incubation mixture (as seen by TLC). A_∞ for k_2 was the A_{540} due to the complete hydrolysis of the reaction mixture starting with 4,4′-diformamidodiphenylsulfone. A_∞ for k_1 was taken as half of A_∞ for k_2, because only one end group is removed during the initial reaction period. In some cases, the rate constants k_2 were also obtained in reaction mixtures starting with 4-amino-4′-formamidodiphenylsulfone.

The conditions and the first-order rate constants, k_1 and k_2, in buffers of various pH's at various temperatures are given in Table I. The rate constants k_1 were found to be two times k_2 (Table I). The overall rate constant, k', for conversion of 4,4′-diformamidodiphenylsulfone $\xrightarrow{k'}$ 4,4′-diaminodiphenylsulfone could not be determined accurately due to the limitation of the analytical method. By using this method, 4-amino-4′-formamidodiphenylsulfone concentration could be determined in the presence of 4,4′-diformamidodiphenylsulfone. Similarly, 4,4′-diaminodiphenylsul-

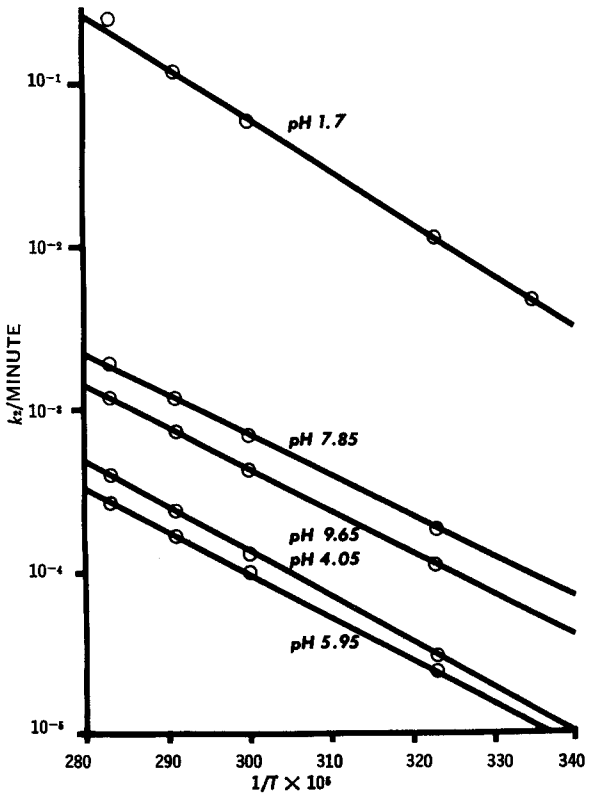

Figure 2—*Arrhenius plots;* k_2 *for 4,4′-diformamidodiphenylsulfone → 4,4′-diaminodiphenylsulfone as a function of pH.*

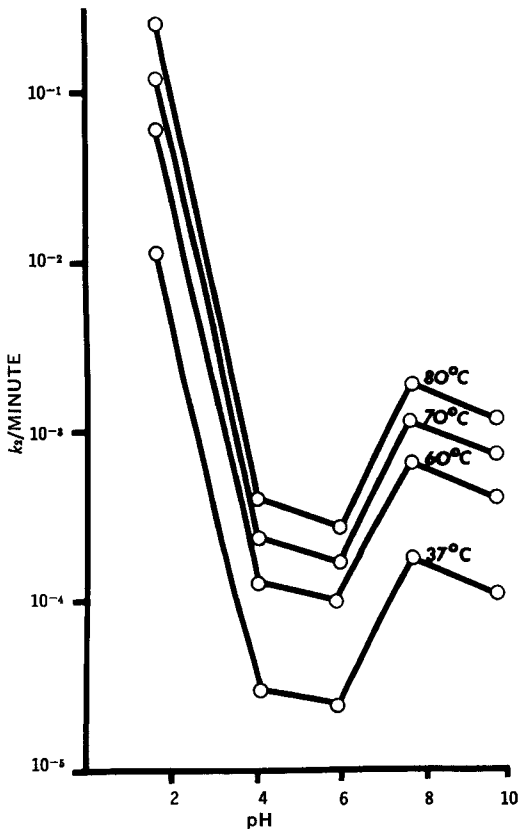

Figure 3—*Log* k_2*-pH profiles for the hydrolysis of 4,4′-diformamidodiphenylsulfone at various temperatures.*

fone concentration could be determined in the presence of 4-amino-4′-formamidodiphenylsulfone or 4,4′-diformamidodiphenylsulfone. However, the concentrations of 4,4′-diaminodiphenylsulfone, 4-amino-4′-formamidodiphenylsulfone, and 4,4′-diformamidodiphenylsulfone cannot be determined in a mixture of all three. As $k_1 > k_2$, then k' would be practically the same as the slower rate constant k_2.

The logarithmic version of the Arrhenius expression is:

$$\log k_2 = \log P - \frac{\Delta H_a}{2.303R} \cdot \frac{1}{T} \qquad \text{(Eq. 2)}$$

where ΔH_a is the energy of activation in cal. mole^{-1}, R is 1.987 cal. deg.$^{-1}$ mole^{-1}, T is the absolute temperature, and log P is the intercept of the plot of log k_2 against the reciprocal of the absolute temperature. The observed k_2 rate constants are given in Table I. The Arrhenius plots for the k_2 rate constants at various pH's are shown in Fig. 2. The ΔH_a values obtained from the slopes of Arrhenius plots for k_2 rate constants are listed in Table I. The k_2 rate constants at 25° at all pH's except pH 1.7 were calculated from the Arrhenius expression and are also included in Table I.

The log k_2–pH profiles for the hydrolysis of 4,4′-diformamidodiphenylsulfone at several temperatures are given in Fig. 3. At low pH (1.7), which is comparable to stomach pH, 4,4′-diformamidodiphenylsulfone is very rapidly hydrolyzed. At pH 4–6 the hydrolysis of 4,4′-diformamidodiphenylsulfone is much slower than at pH 1.7 (about 1/500). The hydrolysis is, however, enhanced at pH 7.85 and is about 5 times faster than at pH 4–6. For unknown reasons, at pH 9.65 the hydrolysis appeared to slightly decrease again. The results indicate that the hydrolysis of 4,4′-diformamidodiphenylsulfone is affected by pH and is first order with respect to 4,4′-diformamidodiphenylsulfone concentration. It may also be first order with respect to H^+-ion concentrations. 4,4′-Diformamidodiphenylsulfone is most stable at pH $\sim$ 6. Another arylamine substituted compound, *N*-acetyl-*p*-aminophenol, shows a similar log *k*–pH profile with a most stable pH $\sim$ 6 (10).

The present results agree with the *in vitro* and *in vivo* results reported by Gleason and Vogh (11) on the enzymic hydrolysis of 4,4′-diformamidodiphenylsulfone in mouse plasma. In their experiments, analysis of plasma showed a 4,4′-diformamidodiphenylsulfone undergoing a stepwise hydrolysis to 4,4′-diaminodiphenylsulfone through 4-amino-4′-formamidodiphenylsulfone as an intermediate.

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Drug Biotransformation Interactions in Man V: Acetaminophen and Salicylic Acid

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Abstract □ Orally administered salicylic acid (1 g. at −2 hr. and 0.5 g. at 4 hr.) had no significant effect on the formation of acetaminophen glucuronide and acetaminophen sulfate, or on the biologic half-life of acetaminophen in healthy adult volunteers who received a 1-g. oral dose of this drug. Acetaminophen did not inhibit the formation of salicylic glucuronides and salicylurate from salicylate. These results indicate that salicylamide is the major determinant in the previously reported biotransformation interactions between acetaminophen and salicylamide and between salicylate and salicylamide.

Keyphrases □ Salicylic acid—effect on acetaminophen, man □ Acetaminophen—influence of salicylic acid, man □ Biotransformations, man—acetaminophen–salicylic acid interaction

Previous studies in this series revealed mutual inhibitory effects in the biotransformation of acetaminophen and salicylamide to their respective glucuronides and sulfates (1), and of salicylic acid and salicylamide to their glucuronides (2). There was also some indication of an inhibitory effect of salicylate on the formation of salicylamide sulfate (2). To elucidate further the biotransformation interactions of these widely used analgesics and antipyretics, the effect of salicylate on the pharmacokinetics of acetaminophen was studied in normal adults. The results of this investigation will help to identify the major determinant in the previously described interactions, since each of the three interacting drugs has now been studied in the presence of the two others.

In addition to these mechanistic considerations, there has been some concern about a trend among pediatricians to prescribe full doses of aspirin and acetaminophen every 4 hr. so that the patient receives one or the other drug every 2 hr. (3). Acetaminophen is eliminated mainly by the formation of its glucuronide and sulfate (1). Salicylic acid is also subject to glucuronidation, and it inhibits the formation of at least some types of sulfates (2, 4, 5). Concern about the possible consequences of the