In Vitro and *In Vivo* Hydrolysis of Salicylanilide *N*-Methylcarbamate and 4-Biphenylyl *N*-Methylcarbamate

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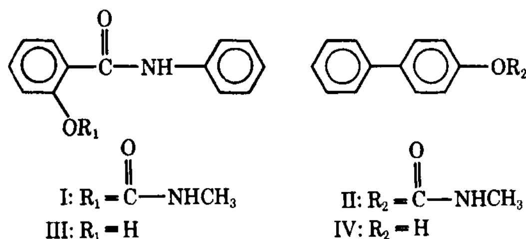
Abstract □ The kinetics of hydrolysis of the carbamoyl group in salicylanilide *N*-methylcarbamate (I) and 4-biphenylyl *N*-methylcarbamate (II) showed that the reaction was first order with respect to both hydroxide ion and carbamate. At 37°, the hydroxide-ion-catalyzed hydrolysis of I to yield salicylanilide (III) proceeded at a rate over 200 times that for the hydrolysis of II to produce 4-biphenylol (IV). Half-lives of I and II at pH 7.3 were 11 min. and 38.2 hr., respectively. Following oral administration of I to human subjects, only conjugated III, corresponding to 10% of the dose, was detected in urine. In the case of II, urinary excretion products were II (0.40%), IV (1.14%), and conjugated IV (40%). Taken together, these results are consistent with extensive, if not complete, hydrolysis of I in the intestine to yield relatively poorly absorbed III compared to a considerably more efficient absorption of the neutral Compound II followed by its nearly complete hydrolysis and conjugation.

Keyphrases □ Salicylanilide *N*-methylcarbamate—hydrolysis, *in vitro* kinetics, *in vivo* urinary excretion, metabolites □ 4-Biphenylyl *N*-methylcarbamate—hydrolysis, *in vitro* kinetics, *in vivo* urinary excretion, metabolites □ Urinary excretion—hydrolysis of salicylanilide and 4-biphenylyl *N*-methylcarbamates, metabolites □ Hydrolysis, *in vitro*, *in vivo*—salicylanilide and 4-biphenylyl *N*-methylcarbamates

Both salicylanilide *N*-methylcarbamate (I) and 4-biphenylyl *N*-methylcarbamate (II) were shown to inhibit carrageenin-induced edema in the rat when administered parenterally, whereas only II was active following oral administration[1]. Possible explanations for this phenomenon were poor absorption of I or its rapid hydrolysis in the GI tract prior to absorption. Although both compounds have low aqueous solubilities, I was over 1.7 times as soluble as II in 0.05 *N* HCl (pH 1.3) at 37° and displayed an *in vitro* dissolution rate over 1.4 times that of II under the same conditions (1). These results suggested that the lack of absorption of intact I was not a likely explanation for its oral inactivity. Accordingly, the relative hydrolytic stabilities of I and II were determined and are the subject of this report. Included also are data on the oral absorption and *in vivo* hydrolysis of I and II in man based on the urinary excretion of the administered compounds and their metabolites.



EXPERIMENTAL

***In Vitro* Hydrolysis Studies**—Samples of I or II were dissolved in diglyme and diluted with 0.1 *M* phosphate buffers or 0.1 *M* borate buffers, preequilibrated at 37°, to yield the final concentrations and pH values shown in Table I. Similar samples were diluted with 0.1 *N* HCl to a final pH of 1.1. The final water–diglyme ratio (v/v) was 97.6:2.4. Each solution was placed in a UV spectrophotometer equipped with a constant-temperature cell compartment maintained at 37°. Absorbances (A_t) were measured at appropriate times at the wavelength maximum of the product [salicylanilide (III) or 4-biphenylol (IV)], previously determined for each reaction medium (Table I). Reactions were followed until no further absorbance increases occurred (A_∞). Pseudo-first-order reaction rate constants, k', were obtained from the slopes of plots of log ($A_\infty - A_t$) *versus* time.

***In Vivo* Studies with I**—To examine the absorption and excretion of I and its metabolites, a single normal male subject received 500 mg. and a second subject received 1500 mg. orally. In each case, 24-hr. urine samples were collected prior to (blank) and immediately following drug administration. For the subject receiving 1500 mg., serum samples were also collected at 0, 0.5, 1, 2, 4, 6, and 8 hr. All samples were held frozen until analyzed.

Urine from the subject receiving 500 mg. of I was analyzed for free and conjugated drug-related materials by a UV spectrophotometric procedure. In the extraction of free materials, 5 ml. of urine was mixed with 5 ml. of water and 1 ml. of 0.1 *N* HCl and extracted with 25 ml. of chloroform. A 20-ml. portion of the chloroform phase was extracted with 5 ml. of 0.1 *N* NaOH. This step hydrolyzed any intact I in the extract to III (yield of ~94%) and transferred it, along with any III in the extract, to the aqueous base. The UV spectrum of the aqueous base (diluted as necessary) was determined over the 250–400-nm. range and compared with the spectrum obtained in the same way for pretreatment (blank) urine. To determine conjugated materials, 10 ml. of urine, 2 ml. of 0.5 *M* pH 5.0 acetate buffer, and 0.2 ml. of an enzyme solution[2], containing 20,000 units of β-glucuronidase and 10,000 units of sulfatase, were incubated at 37° for 60 hr. A 5-ml. portion of the hydrolysis mixture was carried through the extraction and UV analysis already described. In each case, calculations were based on the response (A_{335}) obtained for I added to urine and carried through the same procedures.

Urine from the subject receiving 1500 mg. of I was analyzed for free and conjugated drug-related materials by a quantitative TLC procedure. For free materials, 10 ml. of urine was mixed with 0.5 ml. of 6 *N* HCl and extracted with 25 ml. of chloroform. A 10-ml. portion of the chloroform phase was concentrated to dryness in a nitrogen stream, and the residue was dissolved in a few drops of chloroform. The resulting solution was quantitatively transferred to a silica gel G TLC plate containing phosphors and developed, in parallel with standards I and III, with chloroform–formic acid (98:2). To determine conjugated materials, buffered urine was incubated in β-glucuronidase–sulfatase as described previously and then carried through the extraction and TLC. The only drug-related material detected on the TLC plate with a UV lamp was III in the conjugated fraction. Accordingly, that zone was scraped from the plate and eluted with 5 ml. of 0.1 *N* NaOH. The UV spectrum of the basic eluate was determined as already described. Calculations were based on the response (A_{335}) obtained for III carried through the same procedure.

Five-milliliter serum samples from the subject receiving 1500 mg. of I were mixed with 0.5 ml. of 2 *N* HCl and extracted with 25 ml. of

[1] E. M. Glenn, Hypersensitivity Diseases Research, The Upjohn Co., personal communication.

[2] Glusulase, Endo Laboratories, Richmond Hill, N. Y.

Table I—Reaction Conditions and Kinetic Constants for Salicylanilide N-Methylcarbamate (I) and 4-Biphenylyl N-Methylcarbamate (II) Hydrolysis at 37°

Compound	Concentration, $M \times 10^4$	pH	λ, nm.	k', min.$^{-1}$	$t_{1/2}$, min.	k_{OH^-}, l. mole^{-1} min.$^{-1}$
I	1.62	6.03	300	3.52×10^{-3}	197	
	1.62	7.00	305	2.50×10^{-2}	28	
	1.62	7.68	335	1.77×10^{-1}	4	1.30×10^5
II	1.10	7.72	275	6.50×10^{-4}	1068	
	1.15	7.99	275	1.79×10^{-3}	388	
	1.15	8.67	275	6.97×10^{-3}	99	
	1.15	9.42	275	4.08×10^{-2}	17	6.17×10^2

chloroform. The extracts were then analyzed by the quantitative TLC method. Standards of I and III were run in parallel with the samples; recoveries were ~95% for each compound, and at least 8 mcg. of I/ml. of serum could be detected and estimated.

***In Vivo* Studies with II**—To examine the absorption and excretion of II and its metabolites, three normal male subjects each received three doses of 120 mg. orally over a 12-hr. period; three additional subjects served as controls. In each case, 24-hr. urine samples were collected and frozen until analyzed.

Urines were analyzed for free IV, for the sum of free II and IV (and thereby II by difference), and for conjugated IV by quantitative TLC. The determination of II by the difference method was necessitated by the inability to separate II from urinary interferences on TLC.

In the determination of free and conjugated IV, the extraction–TLC procedures described for free and conjugated III were followed except for the use of benzene–formic acid (98:2) as the developing solvent. Calculations were based on the response (A_{287}) obtained for IV carried through the same procedures; recoveries were quantitative.

In the determination of the sum of free II and IV, 10 ml. of urine was mixed with 0.5 ml. of 6 N NaOH and allowed to stand 1 hr. to convert all free II in the sample to IV. The sample was acidified with 1 ml. of 6 N HCl, and the extraction–TLC analysis was completed. Intact II was determined by the difference between this result and that for free IV. In each case, results were corrected for the corresponding blank values obtained on control urines.

RESULTS AND DISCUSSION

***In Vitro* Hydrolysis Studies**—The method utilized for the determination of the kinetics of hydrolysis of I and II is based on the differences in UV spectra of the carbamates and their product phenols, III and IV, respectively. In the case of I, interference due to hydrolysis of the anilide group was shown to be absent by the observations that the final UV spectrum obtained was stable, was identical to that of III in the same medium, and was markedly different from that of a salicylic acid–aniline mixture. These results are consistent with the half-life of 38 hr. for III in 1 N $HClO_4$ at 90° calculated from the data of Koshy (2).

Both I and II were totally stable at pH 1.1, 37°, for at least 6 days, indicating that significant hydrolytic degradation would not occur in the acidic environment of the stomach. These observations agree with those reported for the acid stability of other carbamates (3, 4).

Dittert and Higuchi (5) showed that hydrolysis of certain carbamates is first order in both hydroxide ion and carbamate (C). At constant hydroxide-ion concentration in the buffered solutions employed in the present studies, the integrated form of the rate expression is:

$$\ln C_t/C_0 = -k_{OH^-}[OH^-]t = -k't \quad \text{(Eq. 1)}$$

Substitution of $(A_\infty - A_t)$ for C_t and conversion to common logarithms give the working equations:

$$\log (A_\infty - A_t) = \frac{-k_{OH^-}}{2.303}[OH^-]t + \log (A_\infty - A_0) \quad \text{(Eq. 2)}$$

$$= \frac{-k't}{2.303} + \log (A_\infty - A_0) \quad \text{(Eq. 3)}$$

where $(A_\infty - A_0)$ is a constant.

A plot of $\log (A_\infty - A_t)$ *versus* time (t) at each pH for I and II was linear, confirming the first-order dependence on carbamate. Slopes of these curves provided pseudo-first-order reaction rate constants, k' (Table I).

From the relationship between pH and $[OH^-]$ and from Eq. 1, Eq. 4 may be derived:

$$\log k' = \log k_{OH^-} + \text{pH} - \text{pKw} \quad \text{(Eq. 4)}$$

where K_w is the ion product for water (pKw 13.61 at 37°). For both I and II, a plot of $\log k'$ *versus* pH was linear with a slope of unity, establishing first-order dependence on hydroxide ion and permitting calculation of the specific reaction rate constant, k_{OH^-}, from Eq. 4. Results are summarized in Table I and indicate that the rate of hydroxide-ion-catalyzed hydrolysis of I is over 200 times that of II at 37° over the pH range that would be encountered in the small intestine and the bloodstream. Calculated half-lives for I and II at pH 7.3 are 11 min. and 38.2 hr., respectively. Accordingly, the facile hydrolysis of I compared to II provides a reasonable explanation for the oral inactivity of I. Elucidation of the mechanism of hydrolysis of I and related compounds will be the subject of a separate report.

***In Vivo* Studies with I**—UV analysis of urine from the subject who received a 500-mg. oral dose of I showed no detectable drug-related substances in the extract of free materials. The extract of β-glucuronidase–sulfatase-hydrolyzed urine exhibited an absorption spectrum (corrected for the control urine blank) identical to that of III and indicated that 10% of the administered drug was excreted as a glucuronide and/or sulfate conjugate, presumably at the hydroxyl group of III, over 24 hr. The more descriptive TLC analysis of urine from the subject who received 1500 mg. of I also failed to detect any I, III, or other drug-related materials in the free extract. Only III was detected in the conjugated fraction, again corresponding to 10% of the administered dose. Equivalent absorption of I and/or III was indicated over a threefold range of doses. Failure to detect excretion of I was consistent with predicted extensive hydrolysis based on the *in vitro* kinetics study. Although no estimate of the relative contributions of hydroxide-ion-catalyzed and enzymatic hydrolyses can be made, it is doubtful that the latter was significant.

Excretion of III as glucuronide and/or sulfate conjugates is qualitatively similar to results reported for salicylamide (6). In the latter case, drug was excreted in the urine only in conjugated form (glucuronide and sulfate) and as small amounts of gentisamide glucuronide. However, excretion of these salicylamide metabolites over 24 hr. ranged from 44 to 62% for a relatively slowly dissolving form (1-g. pellets) to from 84 to 101% of the dose for solutions (150 mg.–1 g.). Excretion of only 10% of administered I as conjugated III may reflect relatively poorer absorption of III produced in the

Table II—Urinary Excretion of 4-Biphenylyl N-Methylcarbamate (II) and Its Metabolites in Man following Oral Administration of 360 mg.

Subject	Percent of Dose Excreted, 24 hr.		
	II	IV	Conjugated IV
W	0.62	1.31	29.4
A	0.24	1.14	37.8
B	0.35	0.96	52.8
Average	0.40	1.14	40.0

GI tract, production of undetected metabolites, and/or biliary secretion of metabolites.

No intact I or free III was detectable in serum samples collected over an 8-hr. period following oral administration of 1500 mg. of I (analytical sensitivity $\geq$ 8 mcg./ml.). Extensive distribution, analogous to that reported for salicylamide in animals (7), apparently occurred.

***In Vivo* Studies with II**—Urinary excretion of II and its detected metabolites is shown in Table II. On the basis of the *in vitro* kinetics studies, absorption of intact II was predicted. This was supported by the detection of small but significant amounts of II in the urine (mean: 0.40% of the dose). Overall mean 24-hr. excretion of II, of its hydrolysis product, IV (1.14%), and of conjugated IV (40.0%) was 41.5% of the administered dose, suggesting considerably more efficient absorption of II than was found in the case of I. This observation also was consistent with the stability of II which would present a neutral molecule for diffusion across the intestinal barrier as contrasted to III, produced from I, which would exist at least partially in the anionic form at the pH of the intestine. Extensive enzymatic hydrolysis of II was indicated, in agreement with the reported *in vivo* hydrolysis of aryl carbamates (8) as opposed to the stability of aliphatic carbamates (9, 10).

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ACKNOWLEDGMENTS AND ADDRESSES

Received December 17, 1971, from the *Research Laboratories, The Upjohn Company, Kalamazoo, MI 49001*

Accepted for publication March 1, 1972.

The authors thank Dr. F. G. McMahon for assistance with the clinical portion of the study and Mr. R. C. Meeks for technical assistance.

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Anesthetic Gas Absorption Properties of Surfactant Systems

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Abstract ☐ Studies were undertaken to determine the possibility of solubilization being a method of gas transport in biological systems by aggregation through associated micelle formation. Specific gases, surfactant concentration, type of structure in surfactant systems, and biopharmaceutical and physiological aspects were considered in determining the transport mechanism of water-insoluble anesthetic gases. Gas solubilization was analyzed by GC with a modified tonometer as an absorption chamber. Halothane, ethyl ether, trichloroethylene, and nitrous oxide were used with surfactant systems of polysorbate 80, dioctyl sodium sulfosuccinate, and bovine albumin. The surfactant concentration was an important factor in anesthetic gas absorption, since increased gas absorption was observed with concentrations of surfactant above the CMC. The mechanism of absorption tends to indicate solubilization of the gases through the formation of micelles.

Keyphrases ☐ Anesthetic absorption, gaseous—effect of surfactant systems ☐ Surfactant effect—absorption of gaseous anesthetics ☐ Solubilization, micelle formation—possible mechanism of gaseous anesthetic absorption

The fact that most inhaled anesthetic gases and many physiologically important gases are nonpolar and have poor solubility in highly polar water must be considered in attempting to understand their possible transport mechanism. The generally accepted hypothesis of gases diffusing through the lung lining presents some theoretical difficulties. Not only are these gases insoluble in water, they would be diffusing through media containing carbon dioxide and carbonates which could greatly reduce the degree of permeability, solubility, and rate of diffusion. It appears unlikely that simple partition and diffusion mechanisms can account for the large quantities of nonpolar anesthetic gases transported through lung tissue to the bloodstream.

Although solubilization of micelles is a reasonably well-established concept, its application as an alternate mechanism of solubilization through the formation of micelles has now been proposed for the rapidity of gas absorption (1, 2). An important factor which has emerged from general studies is that these surfactant solutions become significant solvents at a stage where surfactant molecules begin aggregating. The surfactant molecules acting as aggregated units become excellent solvents (3).

EXPERIMENTAL

The concept of solubilization, which has been defined as "the spontaneous dissolving of a normally water-insoluble substance by an aqueous solution of surfactant" (4), was utilized in these laboratories as a means of explaining the nature of the data obtained on gas absorption. Studies were undertaken to obtain gas absorption data on several surfactant systems and to expand a previous concept by experimentally using several anesthetic gases for observation of gas absorption on different surfactant systems. The surfactants