In Vitro and In Vivo Hydrolysis of 4-Benzoylphenyl N-Methylcarbamate

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Abstract ☐ The kinetics of hydrolysis of the carbamoyl group of 4-benzoylphenyl N-methylcarbamate in 50% aqueous ethanol showed that the reaction was first order with respect to both hydroxide ion and carbamate. The calculated half-life at pH 7.3, 37°, was 73 min. Following oral administration in the dog, urinary excretion products in separate experiments were (percent of the dose): the intact compound (<0.1%), 4-benzoylphenol (0.3; 0.4%), and conjugated 4-benzoylphenol (17.0; 8.3%). Taken together, these results are consistent with considerable hydrolysis of the drug in the intestine, erratic absorption, and rapid hydrolysis following absorption.

Keyphrases ☐ 4-Benzoylphenyl N-methylcarbamate—*in vitro* and *in vivo* hydrolysis, half-life, urinary excretion products in dog ☐ N-Methylcarbamate, 4-benzoylphenyl—*in vitro* and *in vivo* hydrolysis, half-life, urinary excretion products in dog ☐ Hydrolysis, *in vitro* and *in vivo*—4-benzoylphenyl N-methylcarbamate

A number of aryl N-methylcarbamates have shown anti-inflammatory activity (1, 2). 4-Benzoylphenyl N-methylcarbamate (I) possesses similar biological activity (3). Earlier investigations with salicylanilide N-methylcarbamate and 4-biphenylyl N-methylcarbamate demonstrated marked differences in hydrolytic stability and indicated that carbamate hydrolysis at pH's encountered in the intestine could have a significant effect on absorption and resultant activity (2). Accordingly, the hydrolytic stability of I was determined and is the subject of this report. Included also are data on the oral absorption and *in vivo* hydrolysis of I in the dog based on the urinary excretion of I and its metabolites.

EXPERIMENTAL

In Vitro **Hydrolysis Studies**—Solutions of I ($5.98 \times 10^{-5}\,M$) were prepared in 50% aqueous ethanolic phosphate (0.025 M) buffers at the apparent pH values shown in Table I. Absorbances (A_t) were determined at appropriate times at 295 nm., the absorption maximum of 4-benzoylphenol (II), at 37° 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**—In examining the absorption and excretion of I, a fasting 10-kg. beagle dog received 200 mg. of I orally, followed 14 days later by 220 mg. orally. A 24-hr. urine sample (blank) was collected prior to drug administration, and urine samples (acidified on collection) were obtained following drug administration (Table II).

Table I—Kinetic Constants for 4-Benzoylphenyl N-Methylcarbamate Hydrolysis in 50% Ethanol at 37°

Apparent pH	k', min.$^{-1}$	$t_{1/2}$, min.	k_{OH^-}, l. moles^{-1} min.$^{-1}$
7.4	1.14×10^{-2}	61	1.90×10^4
8.0	5.30×10^{-2}	13	2.22×10^4
8.6	1.84×10^{-1}	4	1.89×10^4
		Mean	2.00×10^4

Table II—Urinary Excretion of Free and Conjugated 4-Benzoylphenol (II) in the Dog following Oral Administration of 4-Benzoylphenyl N-Methylcarbamate

| Dose, mg.	Interval, hr.	Percent of Dose Excreted — II	Conjugated II
200	0–24	0.27	16.3
	24–55	0.05	0.7
220	0–8	0.20	5.8
	8–24	0.20	0.6
	24–48	0.04	1.9

Free and conjugated drug-related materials were determined by a quantitative TLC procedure. For free materials, 10 ml. of urine, adjusted to pH 2, was extracted with 25 ml. of chloroform. A 20-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 saturated with 0.1 N HCl. The resulting solution was quantitatively transferred to a silica gel G TLC plate containing phosphors and developed with chloroform–ethanol–formic acid (98:3:2) in parallel with standard I (R_f 0.7) and II (R_f 0.5). Zones corresponding to I and II were detected with a UV lamp, scraped from the plate, and eluted with 5 ml. of 0.1 N NaOH. Compound II was determined from the absorbance of the basic eluate at 345 nm. Interfering materials from the urine absorbing at 340 nm. made estimation of I at 345 nm., based on its hydrolysis to II in the eluting solvent, impossible. Therefore, the basic eluate of zone I was acidified and extracted with three 5-ml. portions of chloroform, and the extract was analyzed by the quantitative TLC procedure for II.

To determine conjugated materials, 15 ml. of urine was adjusted to pH 5.0, mixed with 3 ml. of 0.5 M pH 5.0 acetate buffer and 0.3 ml. of an enzyme solution[1] containing 30,000 units of β-glucuronidase and 15,000 units of sulfatase, and incubated at 37° for 48 hr. The sample was then acidified and carried through the extraction and TLC determination for II. All calculations were based on the responses (A_{345}) obtained for standard samples of I or II added to urine and carried through the same procedures, with appropriate corrections for urine blanks.

RESULTS AND DISCUSSION

Previous reports (1, 2, 4) showed that the hydrolysis of aryl carbamates is first order in both hydroxide ion and carbamate. In the present kinetic studies, plots of log ($A_\infty - A_t$) versus time were strictly linear, confirming the first-order dependence on I. Pseudo-first-order reaction rate constants, k', are shown in Table I. Moreover, a plot of log k' versus apparent pH was linear with a slope of unity, establishing first-order dependence on hydroxide ion and permitting calculation of the specific reaction rate constants, k_{OH^-}, presented in Table I.

Use of 50% ethanol as solvent was dictated by the low solubility of I in water. As a consequence, a direct calculation of hydrolysis rates in aqueous systems cannot be made. However, earlier studies

I: R = C—NHCH₃

II: R = H
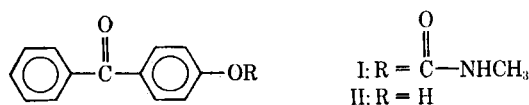
[1] Glusulase, Endo Laboratories, Richmond Hill, N. Y.

with the structurally similar 4-biphenylyl N-methylcarbamate (1, 2) indicated that k_{OH^-} in water was six times that of the value in 50% ethanol. Thus, it may reasonably be predicted from the data in Table I that considerable hydrolysis of I would occur over the pH range encountered in the small intestine and that absorption of the intact drug could be variable from dose to dose. In addition, nonenzymatic hydrolysis at the pH of the blood, following absorption, would be rapid. The half-life of I at pH 7.3, 37°, calculated from the data in Table I is 73 min. Comparison of these results with those obtained earlier with 4-biphenylyl N-methylcarbamate (1) indicates that substitution of the 4-benzoyl group for the 4-phenyl group increases the hydrolysis rate nearly 200-fold.

Following oral administration of I to the dog, traces of intact I were detected in the urine but were below the level of reasonable quantification (<0.1% of the dose). The only drug-related materials detected in the urine were the hydrolysis product, II, and glucuronide and/or sulfate conjugates of II (Table II). Overall excretion ranged from 8.7 to 17.3% of the dose in the two experiments. These results are consistent with erratic absorption and the predicted extensive hydrolysis of I based on the in vitro kinetic studies.

Urinary excretion of 47–92% of orally administered II as a glucuronide conjugate in the rabbit was reported by Robinson (5). The present results for II (produced from I) in the dog are qualitatively similar.

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Mass Fragmentographic Detection of Normorphine in Urine of Man after Codeine Intake

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Abstract ☐ The biological disposition of codeine in urine was studied after intake of a single therapeutic dose of 20 mg. of codeine phosphate. Human urine after ingestion of codeine was previously reported to contain codeine as well as two of its metabolites, morphine and norcodeine. The presence of an additional metabolite, normorphine, was detectable after acid hydrolysis of an aliquot of human urine collected for 10 hr. after intake. Analysis was performed using a combined GC–mass spectrometer, with an additional accelerating voltage alternator unit as a specific detector. Presence of normorphine was indicated by mass fragmentography. A partial mass spectrum for identification was obtained by repeated scanning across the normorphine peak area.

Keyphrases ☐ Normorphine—mass spectroscopic detection as a metabolite of codeine in human urine ☐ Codeine—mass spectroscopic detection of normorphine as a metabolite, human urine ☐ Mass spectroscopy—detection of normorphine as a metabolite of codeine in human urine

Researchers have investigated the biological disposition of codeine in man, monkey, dog, rat, mouse, rabbit, and guinea pig (1, 2). The metabolic pathway of codeine in different species includes conjugation, O- and N-dealkylation to morphine and norcodeine, and conjugation of these metabolites.

In 1962, Way and Adler (3) reported: "Thus far there has been no direct evidence to indicate that the same codeine molecule is demethylated at both the O- and the N-positions to yield normorphine." However, during the same year, Kuhn and Friebel (4) identified traces of normorphine in urine of rats after treatment with codeine. In 1970, Yeh and Woods (5) reported the detection of normorphine by paper and thin-layer chromatography in urine and bile of rats after subcutaneous injection of large amounts of codeine phosphate. In the present investigation, traces of normorphine were detected, by means of mass fragmentography, in addition to the already known metabolites in urine of man after intake of a single therapeutic dose of either 10 or 20 mg. of codeine phosphate.

MASS FRAGMENTOGRAPHY

In 1968, mass fragmentography was introduced and its application has been demonstrated for the identification of chlorpromazine and its metabolites in human blood (6). With mass fragmentography, it is possible to take advantage of certain physicochemical properties of compounds or a group of compounds with similar chemical structures. The mass spectrometer is used as a GC detector. Compounds are detected by the presence of characteristic mass numbers (their molecular ion or fragments).

The accelerating voltage alternator[1] allows the detection and simultaneous recording of up to three mass numbers of a compound by keeping the magnetic field constant while switching the accelerating voltage. With this technique, amounts as small as 5 pg. can be detected. Another advantage is the high selectivity of mass fragmentography. There is very little chance that two different compounds have the same retention time and correspond at the same time to preselected fragments with identical peak ratios. The information for identification is, however, less than that obtained when a complete mass spectrum is recorded.

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[1] LKB Produkter AB, Bromma, Sweden.