# Acid-Labile Derivatives of Chloramphenicol as Potential Latentiation Forms

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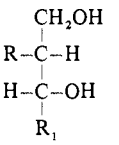
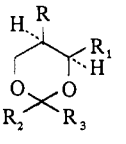
The half-lives for the hydrolysis of 2,2-dimethyl-4-*p*-nitrophenyl-5-dichloroacetamido-1,3-dioxane (**3**) at 37° in 0.1 *N* HCl in acetone–water, and dioxane–water, to afford chloramphenicol (**1**), were found to be 10.3 and 13.2 min, respectively. Compound **3** was only partially soluble in 0.1 *N* HCl in water and its low rate of hydrolysis in this solvent was attributed to its low rate of dissolution. Since **3** is tasteless, the present findings warrant further studies to determine if it could be used as a latentiation form of 1, which is suitable for oral dosage forms, and which could hydrolyze in gastric secretion to afford 1. 2-Methyl-4-*p*-nitrophenyl-5-dichloroacetamido-1,3-dioxane (**2**) was hydrolyzed very slowly indicating that acetals of this type are not suitable as latentiation forms. Maximum blood levels were observed 2 hr after oral administration of **3** in an anesthetized rat and then declined slowly. The mean blood levels of 1 and **3** at 2 hr after oral administration of equal doses (100 mg/kg) in rats were 12.75 (±1.31) and 2.48 (±0.31) μg/ml, respectively. The fivefold higher levels of 1 were probably due to a much slower rate of absorption of **3** from the gastrointestinal tract. No chloramphenicol, or only a trace of it, was found in rat blood after oral administration of **3**.

Numerous drug latentiation forms have been developed in the past to modify the duration of action of a drug, to aid in its localization in a specific tissue, to reduce its toxicity, or to improve its physical properties. The present study describes an acetal and a ketal as potential latentiation forms which might possess superior pharmaceutical or pharmacological properties. These are acid-labile derivatives of drugs which, after oral administration, could hydrolyze in the acidic gastric secretion to provide the parent compound. Although some previously reported latentiation forms were acid-labile compounds, they were not intended for hydrolysis in gastric secretion.[1,2] To demonstrate the applicability of this approach in the development of potential therapeutic agents, we undertook these studies using chloramphenicol (**1**) as a model compound for hydroxyl-containing drugs.

Chloramphenicol (**1**) has been extensively used for the treatment of acute typhoid fever, paratyphoid A fever, bacteremia, and localized infections caused by other Salmonellae.[3] The bitter taste of **1** makes it unsuitable for oral liquid preparations and numerous latentiation forms, mainly esters, have been synthesized and evaluated. Examples of these are chloramphenicol palmitate and stearate.[4] Alternative latentiation forms of chloramphenicol that would be suitable for oral liquid dosage forms were investigated. These derivatives would have to be tasteless, nontoxic, and afford chloramphenicol *in vivo*. Two previously reported chloramphenicol derivatives were chosen. These were 2-methyl-4-*p*-nitrophenyl-5-dichloracetamido-1,3-dioxane[5] (**2**) and 2,2-dimethyl-4-*p*-nitrophenyl-5-dichloracetamido-1,3-dioxane[6] (**3**). Compounds **2** and **3** were found to be tasteless, nontoxic, sparingly soluble in water, and devoid of antibacterial activity; *cf.* ref 7. These would be expected to be hydrolyzed in the acidic gastric secretion to afford **1** and acetone or acetaldehyde; the latter two substances would be nontoxic in the doses used. The present study describes the acid-catalyzed hydrolysis of **2** and **3** *in vitro*. It also reports on the blood levels of **1** and **3** after oral administration in rats.



1



**2**, $R_2$ = H; $R_3$ = Me
**3**, $R_2$ = $R_3$ = Me
**4**, $R_2$ = Me; $R_3$ = $CH_2N(CH_3)_2$

R = $NHCOCHCl_2$; $R_1$ = $-C_6H_4$-*p*-$NO_2$

## Results and Discussion

Both **2** and **3** were prepared by the condensation of chloramphenicol with acetaldehyde and acetone, respectively, using dry HCl as a catalyst. The addition of anhydrous $CaSO_4$ to the reaction mixture resulted in improved yields of **3**. However, highest yields of **3** were obtained when the method described by Stenberg, *et al.*,[8] was used with minor modifications.

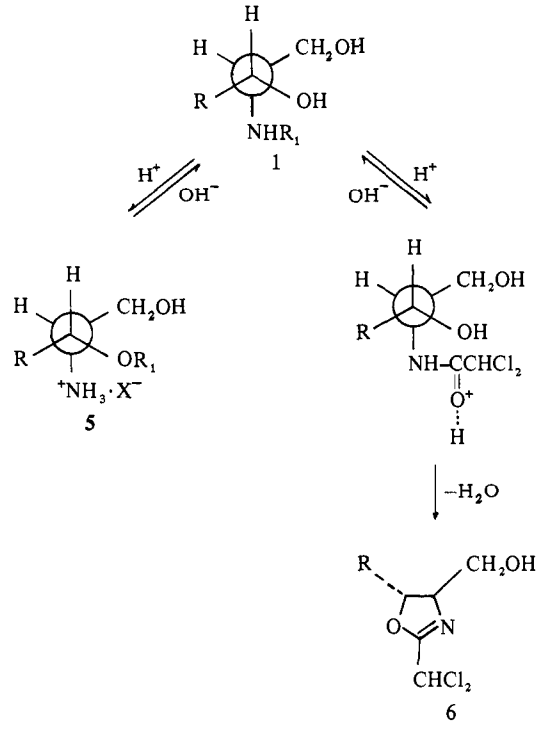
In an effort to prepare a new latentiation form of **1** that would have appreciable solubility in aqueous acids, we also attempted the synthesis of 2-dimethylaminomethyl-2-methyl-4-*p*-nitrophenyl-5-dichloroacetamido-1,3-dioxane (**4**). Condensation of **1** with the dimethylaminoacetone in the presence of *p*-toluenesulfonic acid in boiling benzene or 1,2-dichloroethane, with concurrent removal of water, failed to afford **4**. However, two products were believed to be produced by the action of *p*-toluenesulfonic acid on **1**. This was later confirmed when the same products were obtained when the amino ketone was excluded from the reaction mixture.

The first of these two products was obtained as a white crystalline material on cooling the reaction mixture. It was soluble in $H_2O$ and gave **1** on treatment with aqueous $Na_2CO_3$ solution. This compound was identified as the *p*-toluenesulfonic acid salt of *threo*-2-amino-1- (or 3-) dichloroacetoxy-1-*p*-nitrophenyl-1,3-propanediol (**5**). Compound **5** would be formed by the acid-catalyzed N → O acyl migration of the dichloroacetyl group, a reaction which has been previously reported for similar compounds.[9] In basic media, **5** would afford **1** by O → N acyl migration (Scheme I).

Concentration of the mother liquor after separation of **5** afforded the second product. The mass spectrum of this product showed that it was derived from **1** by the loss of one molecule of $H_2O$. Its physical and spectral properties were identical with those reported for (−)-*threo*-2-dichloromethyl-4-hydroxymethyl-5-*p*-nitrophenyloxazoline (**6**). The latter has been previously prepared by a different method.[10] A plausible mechanism for the formation of **6** from **1**, with retention of configuration at $C_1$, involves the protonation of the amide carbonyl to induce sufficient polarization for hydroxyl attack. A similar mechanism has been recently proposed for the formation of oxazolines from *N*-acyl-β-hydroxyamines.[11]

The acid-catalyzed hydrolysis of **3** would be expected to

Scheme I



R = $-C_6H_4$-*p*-$NO_2$; $R_1$ = $COCHCl_2$; X = *p*-toluenesulfonate

involve preequilibrium protonation of the substrate followed by unimolecular decomposition of the conjugate acid to afford an alcohol and resonance stabilized carbonium ion.[12] Further protonation and cleavage would afford **1** and acetone. The alternative pathway, in which the decomposition of the conjugate acid occurs *via* the cleavage of the alcohol carbon-oxygen bond, would result in epimerization at the $C_1$ center and afford a mixture of **1** and the L-erythro isomer. The latter is devoid of antibacterial activity. However, numerous studies on the hydrolysis of acetals and ketals of optically active alcohols showed that in the majority of cases the reaction occurs *via* cleavage of the carbonyl carbon-oxygen bond, with retention of configuration of the alcohol.[13] Treatment of **3** with acid produced **1** which was identified by its behavior on tlc, melting point, specific rotation, and melting point of its mixture with an authentic sample of **1**.

The rates of hydrolysis of **2** and **3** were studied at 37° in 0.1 *N* HCl in water, water-acetone (1:1), or water-dioxane (1:1) mixture. The amount of unhydrolyzed substrate was determined at different time intervals using gas chromatographic methods which were designed to permit the determination of **2** or **3** without interference of **1**. An internal standard was added to all samples and the reliability of the method was checked by running control samples concurrently with each experiment. Linear relationships were observed between ln *C* of unhydrolyzed substrate and time, and regression analysis was used to determine the line of best fit for the data points. When a solution of **2** in 0.1 *N* HCl in acetone-water (1:1) was incubated at 37° for 7 hr, only 6% of the incubated amount was hydrolyzed. This finding indicated that the hydrolysis of **2** under these conditions was extremely slow[14] and, consequently, no further studies were carried out on this compound. The observed rate constants ($k_{obsd}$) and half-lives ($t_{1/2}$) for the hydrolysis of **3** in 0.1 *N* HCl were $5.26 \times 10^{-2}$ $min^{-1}$ and 13.2 min in dioxane-water (1:1) and $6.74 \times 10^{-2}$ $min^{-1}$ and 10.3 min in acetone-water (1:1). The slower rate of hydrolysis of **3** in dioxane-water than in acetone-water could be attributed to the higher polarity and lower basicity of acetone.[12] In both acetone-water and dioxane-water mixtures, **3** was completely soluble, but it was only partially soluble in water. Therefore, the rate of hydrolysis of **3** in 0.1 *N* HCl in water would be influenced by its rate of dissolution and the rate of hydrolysis of the substrate in solution. If the rate of dissolution of **3** is relatively fast, the rate of hydrolysis would be rate limiting, and the disappearance of **3** would follow zero-order kinetics, reflecting the presence of a constant concentration of **3** in solution, until insoluble **3** has disappeared. This was not observed, and a linear relationship was observed between ln of unhydrolyzed **3** and time ($t_{1/2}$ 63.6 min). Also, previous studies had indicated that the rates of acid-catalyzed hydrolysis of acetals and ketals in mixed solvents (organic solvent-water) decreased as the ratio of organic solvent component was increased. Therefore, the much lower rate of hydrolysis of **3** in 0.1 *N* HCl in water could be attributed to the rate-determining, slow dissolution of **3** in this solvent.

The time courses of blood levels of **3** after oral administration in anesthetized rats were studied. Blood samples were collected through a cannula inserted in the femoral artery. The amount of **3** in blood was determined by a gas chromatographic method similar to that described for the hydrolytic studies. Maximum blood levels of **3** were observed 2 hr after oral administration and then declined very slowly (Table I). Rat 2 required an additional anesthetic shortly before the collection of the third-hour sample and was administered an iv dose of pentobarbital (10 mg/kg). This might explain the low levels of **3** at 3 hr for this rat. To examine the possible effects of the anesthetic on the absorption of **3** from the rat gastrointestinal tract, the following study was conducted. Rats under light ether anesthesia were given oral doses of either **3** or **1**. The rats recovered from anesthesia within 5 min. They were sacrificed at 2 hr and the blood levels of **3** or **1** were determined. The method described by Margosis[15] was used with minor modifications for the determination of **1**. The mean blood level of chloramphenicol was 12.75 (±1.31) μg/ml; this is in agreement with the value observed in rabbits.[16] The mean blood level of **3** at 2 hr was 2.48 (±0.31) μg/ml, in contrast to the value of 0.69 (±0.04) μg/ml obtained with anesthesized rats. The 3.6-fold higher levels of **3** in unanesthesized rats were not unexpected and are probably due to much lower rate of absorption of the drug from the gastrointestinal tract in these rats. Also the levels of chloramphenicol were about fivefold higher than the levels of **3** at 2 hr after po administration of equal doses. This probably indicates the much slower rate of absorption of **3** from the gastrointestinal tract.

Finally, the possible *in vivo* hydrolysis of **3** to **1** was investigated. Blood samples were obtained at 2 hr after oral administration of **3** to three rats. The samples were analyzed using a glc method that was capable of the detection and

**Table I.** Time-Course Blood Levels of **3** in Rats after Oral Administration

| | Blood level of **3**, μg/ml | | |
|---|---|---|---|
| Time, min | Rat 1 | Rat 2 | Rat 3 |
| 30 | 0.36 | 0.30 | 0.32 |
| 60 | 0.62 | 0.38 | 0.31 |
| 120 | 0.74 | 0.71 | 0.62 |
| 180 | 0.67 | 0.27 | 0.57 |
| 240 | 0.71 | 0.62 | 0.61 |

quantitation of as low as 0.2 μg/ml of **1**. No chloramphenicol was detected in the blood of two rats, and only a trace (<0.1 μg/ml) was detected in the blood of the third rat. These results indicate that the *in vivo* hydrolysis of **3** to provide chloramphenicol took place to a very small extent, if at all, in the rat.

## Conclusions

The hydrolysis of **3** in aqueous media containing HCl in concentrations comparable to those found in gastric secretions took place readily with half-lives of about 64 min. Since the average stomach emptying time in humans varies from 75 to 210 min, depending on the nature of the food ingested,[17] administration of **3**, immediately after meals, would result in 55 (in 75 min) to 90% (in 210 min) hydrolysis to provide **1**. The hydrolysis of **3** in acetone–water or dioxane–water mixtures, in which it was completely soluble, was much faster than it was in water. Modifications of the physical properties of **3**, which would enhance its rate of dissolution, would therefore increase the rate of its hydrolysis in the gastric secretions. Since **3** was found nontoxic and tasteless, the findings presented in this report suggest that **3** could be used as a latentiation form of **1** that is suitable for oral dosage forms. The low rate of hydrolysis of **2** indicates that acetals of this type cannot be used as latentiation forms.

The higher blood levels of **1** compared to those of **3** after oral administration, in rat, of equal doses probably is due to the slower rate of absorption of **3** from the gastrointestinal tract. This could be attributed to the slow rate of dissolution of **3**. The rat gastric secretion was found to contain 0.06–0.1 *N* HCl.[18] Therefore, the presence of only a trace amount of **1**, if any at all, in rat plasma after oral administration of **3** was unexpected.

## Experimental Section

The melting points were determined in open capillary tubes with a Thomas-Hoover apparatus and are uncorrected. The ir spectra were obtained with a Perkin-Elmer 237B spectrophotometer in KBr disks. The nmr spectra were obtained in $CDCl_3$ with a Varian A-60D spectrometer, using TMS as internal standard. The uv spectra were taken with a Beckman DB-GT spectrophotometer. The mass spectra were obtained with a Hitachi Perkin-Elmer RMU-6D mass spectrometer. Microanalyses were performed by M-H-W Laboratories, Garden City, Mich. Glc analyses were carried out on a Perkin-Elmer 900 instrument equipped with a flame ionization detector and a 0.625 × 180 cm column packed with 3% OV-17 on Chromosorb W (80–100 mesh). The rate of flow of carrier gas ($N_2$) was 30 ml/min. The temperatures of the column oven, injector, and detector were 240, 300, and 330°, respectively. The detector output was recorded on a Honeywell Electronik 194 strip chart recorder. A 1-μl portion of the sample to be analyzed was injected in the gas chromatograph. Controls with different known concentrations of the compound to be analyzed were run concurrently with the experiment to construct correlation curves and to serve as a check on the overall technique. For the correlation curve, the ratios of peak height of the substances to that of the internal standard were determined and linear relationships were obtained between these ratios and concentrations. *m*-Phenylene dibenzoate was used as the internal standard. Regression analysis was used to determine the line of best fit for the data points. Calculations were performed on a Hewlett-Packard 9100A calculator.

**2-Methyl-4-*p*-nitrophenyl-5-dichloroacetamido-1,3-dioxane (2).** In a dry stoppered conical flask, paraldehyde (20 ml) was saturated with dry HCl. Chloramphenicol (1.0 g, 3.1 mmol) was added and the solution was allowed to stand at room temperature for 4 hr. This solution was poured into a stirred solution of $Na_2CO_3$ (10 g) in $H_2O$ (800 ml) and the mixture was extracted with three successive portions of $CHCl_3$. The combined $CHCl_3$ extracts were evaporated to dryness. The residue was crystallized from EtOH–$H_2O$ to afford 0.88 g of **2** (81%): mp 134–134.5° (lit.[6] 129–130°); [α]D −15.48° [*c* 2, EtOH (lit.[6] −15°)].

**2,2-Dimethyl-4-*p*-nitrophenyl-5-dichloroacetamido-1,3-dioxane (3).** In a dry stoppered conical flask, chloramphenicol (2.0 g, 6.2 mmol) was dissolved in acetone (15 ml). To this solution were successively added 0.20 g of Amberlite IR-120 (previously dried at 100° for 24 hr) and 3.0 g of Drierite (anhydrous $CaSO_4$, Hammond). The mixture was stirred for 4 hr at room temperature and filtered under reduced pressure and the filtrate was evaporated to dryness. The residue was crystallized from EtOH–$H_2O$ to afford 1.8 g of **3** (80%): mp 149–150° (lit.[6] 149°); [α]D −8.32° [*c* 2, EtOH (lit.[6] −8°)].

***threo*-2-Amino-1- (or 3-) dichloroacetoxy-1-*p*-nitrophenyl-1,3-propanediol *p*-Toluenesulfonate (5) and 2-Dichloromethyl-4-hydroxymethyl-5-*p*-nitrophenyloxazoline (6).** Chloramphenicol (1.0 g, 3 mmol) was dissolved in boiling benzene (1.5 l.) in a flask connected to a Dean and Stark distillation receiver. *p*-Toluenesulfonic acid (0.172 g, 1 mmol) was added. A precipitate appeared 1 hr after the distillation had started. Heating was continued for 4 hr, and the reaction mixture was cooled and filtered. The precipitate (482 mg) was identified as **5**, mp 191.5–193.5°. *Anal.* ($C_{18}H_{20}Cl_2N_2O_8S$) C, H, N.

The filtrate was evaporated to dryness; the residue was dissolved in ethyl acetate (20 ml) and shaken with 10% aqueous $Na_2CO_3$ (20 ml). The combined ethyl acetate extracts were evaporated. The residue showed two components on silica gel tlc ($CHCl_3$–EtOAc, 100:5), the slower migrating component having the same $R_f$ value as chloramphenicol. The two compounds were separated by column chromatography on basic alumina ($CHCl_3$). The first compound eluted (65 mg) was crystallized from $CHCl_3$–petroleum ether to afford **6** (35 mg): mp 135–136° (lit.[10] 132°); [α]D −12.25° [*c* 0.7, EtOAc, (lit.[10] −13.65°)].

**Identification of the Product of Hydrolysis of 3.** **3** (1.0 g) was dissolved in acetone (3 ml), 0.2 *N* HCl (3 ml) was added, and the solution was allowed to stand at room temperature for 4 hr. NaOH, 0.1 *N* (6.1 ml), was added, and the mixture was extracted with $CHCl_3$ (3 ml). The $CHCl_3$ solution was discarded and the aqueous solution was extracted with EtOAc. The extracts were evaporated to dryness and the residue was crystallized from EtOH–$H_2O$ to afford 0.12 g of **1**: mp 150–151° (lit.[19] 150.5°); [α]D −24.8°. The product gave no depression of melting point when mixed with authentic **1**.

**Hydrolysis of 2 in 0.1 *N* HCl in Acetone–Water (1:1).** In each of two centrifuge tubes, 1.0 ml of the standard solution of **2** (1.0 mg/ml of acetone) and 1 ml of 0.2 *N* HCl were added. The tubes were placed in a water-bath shaker at 37° (±0.1) for 7 hr. The reaction was terminated by the addition of 2.1 ml of 0.1 *N* NaOH. To each tube, for experiment and correlation curve, 0.8 ml of the internal standard solution (10 mg/ml in acetone) and 3.0 ml of ethyl acetate were added. The tubes were stoppered, shaken mechanically (5 min), and centrifuged (5 min). The upper phase was separated and 1 μl of each was injected in the gas chromatograph.

**Hydrolysis of 3 in 0.1 *N* HCl in Acetone–Water (1:1).** In each of 12 centrifuge tubes, 1.0 ml of the standard solution of **3** (1.0 mg/ml of acetone) was added and the tubes were placed in a water-bath shaker at 37°. HCl (1 ml, 0.2 *N*) was added to each tube at zero time and the solutions were shaken. The reaction was terminated in three of the tubes by the addition of 2.1 ml of 0.1 *N* NaOH at 5, 10, 15, and 20 min. The internal standard solution (0.8 ml, 10 μg of *m*-phenylene dibenzoate/ml of acetone) and 3.0 ml of ethyl acetate were added to each tube. The tubes were stoppered, shaken (5 min), and centrifuged (5 min). The upper layer was removed and 1 μl of each solution was used for glc analysis.

**Hydrolysis of 3 in 0.1 *N* HCl in Dioxane–Water (1:1).** The procedure used was essentially the same as described above except that the standard solution of **3** in acetone was replaced by the standard solution of **3** in dioxane.

**Correlation Curve.** Concurrently, with each of the previous experiments controls were prepared as follows. In each of eight centrifuge tubes, 2.1 ml of 0.1 *N* NaOH and 1.0 ml of 0.2 *N* HCl were added. The tubes were then divided into four pairs. To both tubes in each pair were added either 1.0, 0.8, 0.6, 0.4, or 0.2 ml of the standard solution of either **2** or **3** and, in the same order, either 0.0, 0.2, 0.4, 0.6, or 0.8 ml of the solvent (acetone or dioxane). Then, 0.8 ml of the internal standard solution and 3.0 ml of ethyl acetate were added to each tube. The tubes were stoppered, shaken (5 min), and centrifuged (5 min). The upper organic layer was removed and 1 μl of each solution was injected in the gas chromatograph.

**Hydrolysis of 3 in a Suspension in 0.1 *N* HCl.** In each of 12 conical flasks (25 ml) was introduced 10 mg of **3**, the flasks were placed in a water-bath shaker at 37°, 2.0 ml of 0.1 *N* HCl was added, and the suspensions were shaken. The reaction was terminated in

three of the flasks by the addition of 2.1 ml of 0.1 *N* NaOH at 60, 120, 180, and 240 min. Controls for the correlation curve were prepared as follows. In each of eight conical flasks (25 ml), 2.1 ml of 0.1 *N* NaOH and 2.0 ml of 0.1 *N* HCl were added. The flasks were then divided into four pairs. To both flasks in each pair were added 10.0, 8.0, 6.0, or 4.0 mg of **3**, respectively. To each flask, both for the correlation curve and experiment, 0.8 ml of the internal standard solution (10.0 mg of *m*-phenylene dibenzoate/ml of acetone) and 3.0 ml of ethyl acetate were added. The flasks were stoppered, shaken mechanically (5 min), and allowed to stand for 30 min. The upper phases were separated and 1 μl of each was injected in the gas chromatograph.

Animal Studies. Adult male Sprague–Dawley rats (400–450 g) were used. The animals were deprived of food for about 12 hr prior to the experiment. Chloramphenicol and **3** were administered po as 10% suspensions in 1% carboxymethylcellulose in water using a 10-cm blunt end no. 18 hypodermic needle. Blood samples were collected in tubes containing heparin sodium powder and were analyzed immediately.

Time-Course Blood Levels of **3** in Rats after Oral Administration. Rats were anesthesized with pentobarbital (50 mg/kg ip[20]) and a heparinized polyethylene cannula (i.d. 0.011 × o.d. 0.024 in.) was inserted in the femoral artery. A blood sample was collected and designated as control. The anesthesized rats were given single oral doses of 100 mg/kg of **3** and blood samples (0.3 ml) were collected at 30, 60, 120, 180, and 240 min. Additional anesthetic was administered to the rats (5 mg/kg iv) during the experiment, whenever necessary. Known volumes of each of the collected blood samples, including the control, were transferred into centrifuge tubes. Concurrently, controls for the correlation curve were prepared as follows. In four centrifuge tubes 0.3 ml of heparinized blood, either 0.1, 0.2, 0.3, or 0.4 ml of the standard solution of **3** (0.8 μg/ml of $H_2O$), and, in the same order, either 0.3, 0.2, 0.1, or 0.0 ml of water were added. To each tube, experiment and controls, 0.2 ml of saponin solution (1% in $H_2O$), 1 ml of pH 7.0 phosphate buffer (0.05 *M*), and 2.5 ml of internal standard solution (4 μg of *m*-phenylene dibenzoate/ml in ethyl acetate) were added. The tubes were shaken (10 min) and centrifuged (10 min). The upper layers were removed and evaporated to dryness, the residues were dissolved in 20 μl of EtOAc, and 1 μl of each solution was injected in the gas chromatograph.

Blood Levels of 1 at 2 hr after Oral Administration in Rats. Three rats were put under light ether anesthesia and were administered single oral doses of 100 mg/kg of 1. Each rat was decapitated and exsanguinated at 2 hr and blood was collected. Blood samples (5 ml) were transferred into centrifuge tubes. Controls for the correlation curve were prepared as follows. Heparinized blood (5 ml), either 0.1, 0.2, 0.3, 0.4, or 0.8 ml of the standard solution of 1 (5 μg/ml in $H_2O$), and, in the same order, either 0.7, 0.6, 0.5, 0.4, or 0.0 ml of water were added. To each tube, experiment and control, 1.3 ml of the internal standard solution (60 μg of *m*-phenylene dibenzoate/ml in ethyl acetate) and 3.7 ml of ethyl acetate were added. The tubes were shaken (15 min) and centrifuged (15 min). The upper layers were transferred into centrifuge tubes containing saturated NaCl solution (2.5 ml). The tubes were shaken (5 min) and centrifuged (5 min) and the upper layers were transferred into centrifuge tubes containing anhydrous $Na_2SO_4$ (about 0.1 g). The mixtures were allowed to stand for 1 hr and were centrifuged (5 min). The solutions were removed, poured into different centrifuge tubes, and evaporated to dryness and the residue was redissolved in $CH_3CN$ (0.3 ml). The solutions after centrifugation were evaporated to dryness and the residues were treated with 0.1 ml of *N,O*-bis(trimethylsilyl)acetamide solution in $CH_3CN$. The tubes were heated at 45° for 15 min and 1 μl of each solution was injected in the gas chromatograph.

Blood Levels of **3** at 2 hr after Oral Administration. Three rats were put under light ether anesthesia and were administered single oral doses of 100 mg/kg of **3**. Each rat was decapitated and exsanguinated at 2 hr and blood was collected. Blood samples (5 ml) were transferred into centrifuge tubes. The controls for the correlation curve were prepared as follows. In five centrifuge tubes, 5 ml of heparinized blood, either 0.1, 0.2, 0.3, 0.4, or 0.5 ml of the standard solution of **3** (20 μg/ml in $H_2O$), and, in the same order, either 0.4, 0.3, 0.2, 0.1, or 0.0 ml of $H_2O$ were added. To all tubes, experiment and control, 5 ml of the internal standard solution (3 μg of *m*-phenylene dibenzoate/ml in ethyl acetate) was added. The tubes were shaken and centrifuged and the upper layers were removed and poured into centrifuge tubes containing saturated NaCl solution (2.5 ml). They were shaken and centrifuged and the upper layers were removed and evaporated to dryness. To each tube, 0.1 ml of EtOAc was added and 1 μl injected in the gas chromatograph.

Blood Levels of 1 at 2 hr after Oral Administration of **3** in Rats. Three rats were put under light ether anesthesia and were administered single oral doses of 100 mg/kg of **3**. Each rat was decapitated and exsanguinated at 2 hr and blood was collected. Blood samples (5 ml) were transferred into centrifuge tubes and 5 ml of the internal standard solution (3 μg of *m*-phenylene dibenzoate/ml in ethyl acetate) was added. The tubes were shaken and centrifuged and the upper phases were transferred into centrifuge tubes containing saturated NaCl solution (2.5 ml). The tubes were shaken and centrifuged and the upper layers were removed and poured into centrifuge tubes containing anhydrous $Na_2SO_4$ (1 g). The solutions were transferred into centrifuge tubes and evaporated to dryness, and the residue was dissolved in $CH_3CN$ (0.3 ml). The tubes were centrifuged and the solutions were transferred into centrifuge tubes and evaporated to dryness. To each tube, 0.05 ml of *N,O*-bis(trimethylsilyl)-acetamide solution was added. The tubes were heated at 45° for 15 min and 1 μl of each solution was injected in the gas chromatograph.

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