# Acetaminophen Prodrugs I

## Synthesis, Physicochemical Properties, and Analgesic Activity

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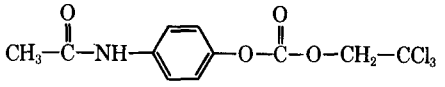
**Carbonate and carboxylic acid ester prodrugs of acetaminophen were prepared. As expected, they had lower water solubilities and higher oil/water partition coefficients than acetaminophen itself. At pH 7.4 the prodrugs were slowly hydrolyzed in phosphate buffer, but human plasma enzymes markedly accelerated the hydrolyses. The $LD_{50}$'s of some of the compounds in mice suggest that, following oral administration, their solubility properties play a greater part in controlling the availability of acetaminophen than do their hydrolysis rates. Several of the compounds were found to have weak analgesic activity orally in rats supporting the theory that their analgesic activities and toxicities are due to release of acetaminophen. The results show how the carbonate linkage may be employed to create prodrugs and how it is possible to obtain a series of pharmacologically similar compounds possessing vastly different physicochemical properties.**

THE SYNTHESIS, physical properties, and *in vitro* hydrolysis behavior of 4-acetamidophenyl 2,2,2-trichloroethyl carbonate (ATC)



ATC

have been reported previously (1). This compound was found to have the same spectrum of pharmacologic activity as a physical mixture of trichloroethanol and acetaminophen, but its analgesic and sedative properties were less intense and persisted for a longer period of time than those of the physical mixture (2). These results suggested that the pharmaceutical properties and dose-time-action profiles of trichloroethanol and acetaminophen can be modified, without modifying their qualitative pharmacologic activity, by combining them in a readily cleavable prodrug carbonate ester. The prodrug ester has physicochemical properties different from those of the parent compounds and may be absorbed at a different rate following oral administration.

It was the purpose of the present study to pursue further the principles of chemical synthesis and physicochemical and biologic evaluation of carbonate prodrugs. The authors wished to determine: (*a*) whether carbonate esters of acetaminophen, other than the trichloroethanol carbonate, would behave as prodrugs; (*b*) whether carboxylic acid esters of acetaminophen would behave as prodrugs; (*c*) which physicochemical properties are important in determining the *in vivo* activities of these compounds; (*d*) to what extent the physical properties of the prodrugs vary with structure; and (*e*) to what degree their *in vitro* cleavage rates vary with structure.

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## EXPERIMENTAL

### Chemical Synthesis

Melting points were determined in capillary tubes on a Thomas-Hoover apparatus and are uncorrected. Most of the reactions were run only once. Reported yields are of pure compound.

***p*-Acetamidophenyl Butyrate–Procedure A**—A mixture of 30.2 Gm. (0.2 mole) of 4′-hydroxyacetanilide, 32.4 ml. (0.2 mole) of butyric anhydride, 1 ml. of pyridine, and 50 ml. of tetrahydrofuran was heated on a steam bath for 2.5 hr. The solvent was removed *in vacuo*, and the residue was recrystallized from benzene and washed with ether to give the pure compound in 87% yield.

***p*-Acetamidophenyl Chloroacetate–Procedure B**—To a solution containing 30.2 Gm. (0.2 mole) of 4′-hydroxyacetanilide and 16.2 ml. (0.2 mole) of pyridine in 100 ml. of tetrahydrofuran was added with stirring 15.2 ml. (0.2 mole) of chloroacetyl chloride in 30 min. The mixture was stirred at ambient temperature for 2 hr. The solid was collected and recrystallized twice from ethanol to yield 21% of pure compound.

***p*-Acetamidophenyl 4-Morpholinoacetate Hydrochloride–Procedure C**—A mixture of *p*-acetamidophenyl chloroacetate, morpholine, and triethylamine (0.05 mole of each) in 800 ml. of benzene was heated at reflux for 1 hr. to give an oily precipitate. This precipitate was decolorized with activated carbon in acetone. The hydrochloride salt was then prepared and recrystallized from dilute alcohol.

**Bis (*p*-Acetamidophenyl) Succinate–Procedure D**—To a chilled solution containing 7.8 Gm. (0.05 mole) of succinoyl chloride and 50 ml. of toluene was added slowly with stirring, a mixture of 4 Gm.

(0.1 mole) of sodium hydroxide and 15.1 Gm. (0.1 mole) of 4′-hydroxyacetanilide in 100 ml. of water. The addition required 15 min., and the reaction was stirred for an additional 1.5 hr. at room temperature. The product was filtered off and washed with water. Two recrystallizations from ethanol gave 33% of a white solid.

**p-Acetamidophenyl Hydrogen Succinate–Procedure E**—To a mixture of 20 Gm. (0.2 mole) of succinic anhydride in 75 ml. of toluene was added with stirring and cooling under nitrogen a solution of 30.2 Gm. (0.2 mole) of 4′-hydroxyacetanilide and 8 Gm. (0.2 mole) of sodium hydroxide in 100 ml. of water. The addition required 15 min. and the solution was stirred for 1.5 hr. with cooling. The mixture was filtered and the aqueous layer was acidified to give the desired product. The product was dissolved in sodium bicarbonate solution and precipitated with HCl. Finally it was dissolved in tetrahydrofuran, dried over anhydrous magnesium sulfate, diluted with 3 volumes of warm benzene, and cooled to give 28% of pure compound.

**Bis (p-Acetamidophenyl) Carbonate–Procedure F**—Phosgene was added with stirring under nitrogen to a solution containing 1 mole of 4′-hydroxyacetanilide and 1 mole of sodium hydroxide in 2 L. of water until the reaction mixture was neutral. The product was collected and recrystallized from dilute alcohol containing activated carbon and sodium sulfite to give 50% of the hydrate, m.p. 207–208°.

*Anal.*—Calcd. for $C_{17}H_{16}N_2O_5 \cdot H_2O$: C, 58.96; H, 5.24; N, 8.09. Found: C, 59.37; H, 5.25; N, 8.16.

An additional recrystallization from absolute alcohol gave the desired product.

**p-Acetamidophenyl Hexylcarbonate–Procedure G**—To 4′-hydroxyacetanilide (0.2 mole) and pyridine (0.22 mole) in 150 ml. of methylene chloride was added with cooling and stirring hexyl chloroformate (0.2 mole) in 75 ml. of methylene chloride. The resulting mixture was stirred for 2 hr., washed with water, and dried over anhydrous magnesium sulfate. The solvent was removed and the product was recrystallized from dilute alcohol.

### Solubilities

The saturation solubilities of the compounds at 37° were determined in water and spectral grade cyclohexane (Fisher) in rotating bottles utilizing an assembly that has been described previously (3). To minimize hydrolysis, the aqueous samples were rotated for only 6 hr. The cyclohexane samples were rotated for 16 hr. (overnight). Samples of the clear supernatant saturated solutions were withdrawn into hypodermic syringes through Millipore filters held in Swinney filter adapters (Millipore Filter Corporation). The samples were analyzed spectrophotometrically.

### Partition Coefficients

The partition coefficients of the compounds between cyclohexane and water at 25° were determined using a method and apparatus previously described (4).

### *In Vitro* Hydrolysis Rates

*M*Half-lives for hydrolysis of the compounds in 0.1 pH 7.4 phosphate buffer with and without 2% human plasma at 37° were determined by a spectrophotometric method previously described (1). Frozen human blood plasma (Type $O^+$) was obtained in approximately 100-ml. quantities from single donors through the Philadelphia Serum Exchange.

### Particle Size Reduction

Before *in vivo* testing, all compounds were micronized by one or more passes through a fluid energy mill (Trost Jet Mill, Helme Products, Inc., Helmetta, N. J.). This procedure produced particles in the 2–20 $\mu$ size range.

### Oral Toxicity in Mice

Male Carworth Farms mice, weighing between 12–18 Gm. were randomly divided into groups of 10 and dosed orally with drug suspended in 0.5% tragacanth. Concentrations were calculated so that each animal received a dose volume of 20 ml./Kg. Control animals received 20 ml./Kg. of 0.5% tragacanth. After dosing, the animals were housed 10 per cage and observed daily for 5 days; if no deaths occurred during the subsequent 48 hr. (a total of 7 days), the study was terminated. If any deaths occurred during this 48-hr. period, the study was continued until no further deaths occurred for 2 consecutive days.

### Analgesic Activity in Rats

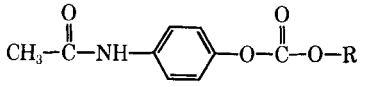
The method of Randall and Selitto was employed (5). Male Dierolf rats (100–148 Gm.) were used after fasting 16–18 hr. Inflammation was produced by the injection of 0.1 ml. of a 20% suspension of brewer's yeast into the plantar surface of the rat's left hind foot. Pressure was applied to the foot at a constant rate of 20 mm. Hg/sec. by a glass cone attached to the plunger of a 10-ml. syringe connected to an air pressure line and pressure gauge. The pain threshold was determined as the amount of pressure in mm. Hg required to induce struggle. Control threshold measurements (referred to as the Yeast Control) were obtained for both the inflamed foot and normal foot 2 hr. after injection of the yeast suspension, immediately before administration of the drug. All changes in pain threshold within each control and each treated group are reported as: mm. Hg pressure at time "$t$" after treatment minus; mm. Hg pressure at time "$t_0$" immediately preceding administration of the drug (Yeast Control). Subsequent threshold determinations were made 30, 60, 120, 180, and 240 min. after administration of the test compounds.

On the basis of preliminary trials, a dose of 400 mg./Kg. of acetaminophen was selected, and the prodrugs were administered in molar equivalent doses. Because of its low oral toxicity, the 4-acetaminophenyl ester was administered in twice the molar equivalent dose, *i.e.*, equivalent to 800 mg./Kg. of acetaminophen. The drugs were administered orally as suspensions in 0.5% tragacanth, and concentrations were adjusted so that each animal received 20 ml./Kg. The control animals received 20 ml./Kg. of 0.5% tragacanth. There were 5 animals per treated group and 5 per control group for the acetaminophen carbonates; there were 11 treated animals and 11 controls for acetaminophen. All testing was done on a "blind" basis.

## RESULTS AND DISCUSSION

**Synthesis and Physical Properties**—Synthesis of the compounds, some of which are new composi-

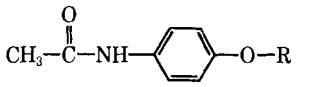
TABLE I—SYNTHESIS PROCEDURES AND PHYSICAL PROPERTIES FOR CARBONATE ESTERS OF ACETAMINOPHEN



| R | Formula | Mol. Wt. | Yield, % | Synthesis Procedure | M.p.°C.[a] | Carbon, % Calcd. | Carbon, % Found | Hydrogen, % Calcd. | Hydrogen, % Found | Nitrogen, % Calcd. | Nitrogen, % Found |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Methyl[b] | $C_{10}H_{11}NO_4$ | 209.2 | 54 | D | 115.5–116.5 | 57.41 | 57.51 | 5.30 | 5.36 | 6.70 | 6.76 |
| Ethyl | $C_{11}H_{13}NO_4$ | 223.2 | 71 | D | 121–122[c] | ... | ... | ... | ... | ... | ... |
| Isopropyl[b] | $C_{12}H_{15}NO_4$ | 237.3 | 45 | D | 131.5–132 | 60.75 | 60.62 | 6.37 | 6.06 | 5.90 | 5.87 |
| Butyl | $C_{13}H_{17}NO_4$ | 251.3 | 46 | D | 119.5–120[d] | 62.14 | 61.95 | 6.82 | 6.69 | 5.57 | 5.59 |
| Isobutyl[b] | $C_{13}H_{17}NO_4$ | 251.3 | 24 | D | 119–121 | 62.14 | 62.12 | 6.82 | 6.77 | 5.57 | 5.54 |
| Hexyl[b] | $C_{15}H_{21}NO_4$ | 279.3 | 68 | G | 112.5–113.5 | 64.50 | 64.26 | 7.58 | 7.34 | 5.01 | 4.99 |
| Octyl[b] | $C_{17}H_{25}NO_4$ | 307.4 | 68 | G | 82.5–83 | 66.42 | 66.15 | 8.20 | 8.30 | 4.56 | 4.53 |
| Chloroethyl[b] | $C_{11}H_{12}ClNO_4$ | 257.7 | 89 | D | 122.5–123 | 51.27 | 51.45 | 4.69 | 4.79 | 5.44 | 5.48 |
| Phenyl[b] | $C_{15}H_{13}NO_4$ | 271.3 | 59 | D | 139–140.5 | 66.41 | 66.16 | 4.83 | 4.64 | 5.16 | 5.19 |
| *p*-4-Acetaminophenyl | $C_{17}H_{16}N_2O_5$ | 328.3 | 50 | F | 219.5–220[e] | 62.19 | 62.16 | 4.91 | 4.75 | 8.53 | 8.56 |

[a] Recrystallization solvent: ethanol–water. [b] These represent new compositions of matter. [c] Literature m.p. 120° (6). [d] Literature m.p. 117–120°(6). [e] Literature m.p. 200° (6).

TABLE II—SYNTHESIS PROCEDURES AND PHYSICAL PROPERTIES FOR CARBOXYLATE ESTERS OF ACETAMINOPHEN



| R | Formula | Mol. Wt. | Yield, % | Synthesis Procedure | M.p.°C. | Recryst. Solvent[a] | Carbon, % Calcd. | Carbon, % Found | Hydrogen, % Calcd. | Hydrogen, % Found | Nitrogen, % Calcd. | Nitrogen, % Found |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Acetyl | $C_{10}H_{11}NO_3$ | 193.2 | 88 | A | 154–155[b] | A | 62.17 | 61.98 | 5.74 | 5.78 | 7.25 | 7.40 |
| Butyryl[c] | $C_{12}H_{15}NO_3$ | 221.3 | 87 | A | 139.5–141.5 | D | 65.14 | 64.93 | 6.83 | 6.73 | 6.33 | 6.61 |
| Hexanoyl[c] | $C_{14}H_{19}NO_3$ | 249.3 | 36 | B | 108–109 | A | 67.45 | 67.75 | 7.68 | 7.68 | 5.62 | 5.79 |
| Stearoyl[c] | $C_{26}H_{43}NO_3$ | 417.6 | 89 | B | 117–118 | A | 74.77 | 74.55 | 10.38 | 10.32 | 3.35 | 3.34 |
| Pivalyl[c] | $C_{13}H_{17}NO_3$ | 235.3 | 34 | D | 162.5–163 | C | 66.36 | 65.92 | 7.28 | 7.09 | 5.95 | 6.11 |
| Crotonyl[c] | $C_{12}H_{13}NO_3$ | 219.2 | 7 | B | 146–147 | C | 65.74 | 65.82 | 5.98 | 6.01 | 6.39 | 6.44 |
| Fumaryl[c] | $C_{20}H_{18}N_2O_6$ | 382.4 | 5 | D | 278–279 | G | 62.82 | 63.09 | 4.74 | 4.78 | 7.33 | 7.36 |
| Acid succinyl | $C_{12}H_{13}NO_5$ | 251.2 | 28 | E | 145.5–146.5[d] | F | 57.37 | 57.39 | 5.22 | 5.10 | 5.58 | 5.50 |
| Succinyl[c] | $C_{20}H_{20}N_2O_5$ | 384.4 | 33 | D | 229–230 | B | 62.49 | 62.41 | 5.24 | 5.22 | 7.29 | 7.28 |
| Benzoyl | $C_{15}H_{13}NO_3$ | 255.3 | 61 | D | 170.5–171.5[e] | E | 70.58 | 70.38 | 5.13 | 5.08 | 5.49 | 5.50 |
| Cinnamyl | $C_{17}H_{15}NO_3$ | 281.3 | 62 | B | 200–201[f] | B | 72.58 | 72.69 | 5.37 | 5.45 | 4.98 | 4.96 |
| Chloroacetyl[c] | $C_{10}H_{10}ClNO_3$ | 227.7 | 21 | B | 184.5–185 | B | 52.76 | 52.92 | 4.43 | 4.41 | 6.15 | 6.18 |
| Morpholinoacetyl·HCl | $C_{14}H_{18}N_2O_4\cdot HCl$ | 314.8 | 29 | C | 258–259 | C | 53.42 | 53.61 | 6.08 | 6.14 | 8.90 | 8.80 |

[a] Recrystallization solvents are: A, toluene; B, ethanol; C, ethanol–water; D, benzene; E, ethanol–hexane; F, tetrahydrofuran–benzene; G, acetic acid–water. [b] Literature m.p. 150° (7). [c] These represent new compositions of matter. [d] Literature m.p. 220° (8). [e] Literature m.p. 136–137° (9). [f] Literature m.p. is 171° (7).

tions of matter, did not present unusual problems. The molecular weights, yields, melting points, recrystallization solvents, and elemental analyses of the compounds are shown in Tables I and II.

The solubilities of acetaminophen and the various esters in water and cyclohexane at 37° and the cyclohexane/water partition coefficients for the compounds at 25° are shown in Table III. As expected, the prodrug esters of acetaminophen, in most cases, were much less soluble in water and much more soluble in cyclohexane than acetaminophen. The aqueous solubilities of the carbonate esters decreased rapidly as the chain length of the alcohol moiety increased.

Branching in the alcohol chain tended to increase both aqueous and cyclohexane solubilities as illustrated by the solubilities of the butylcarbonate compared with those of the isobutylcarbonate. This effect could be attributed to loss of symmetry in the molecules which reduces crystal lattice energy. As the alcohol chain length increased, the cyclohexane solubilities and cyclohexane/water partition coefficients of the carbonate esters also increased. Although chain branching tended to increase the cyclohexane solubility, it did not increase the partition coefficient because of the concomitant increase in aqueous solubility. It would seem, however, that branching would tend to increase the oral availability of these compounds, since both the aqueous and lipid solubilities were increased. Chlorine substitution in the alcohol moiety decreased aqueous solubility and increased cyclohexane solubility. Thus, the chloroethylcarbonate had the highest partition coefficient of all the carbonate esters. The acetaminophencarbonate esters of phenol and of 4-acetaminophenol (bisacetaminophenyl carbonate) were relatively high melting compounds and, consequently, were not very soluble in either water or cyclohexane. The discrepancies between the measured partition coefficients shown in Table III, and the partition coefficients that could be calculated for these two compounds from the solubility data, are due to ex-

TABLE III—SOLUBILITIES, PARTITION COEFFICIENTS, AND AVAILABILITY FACTORS FOR ACETAMINOPHEN PRODRUGS

| Derivative | Solubilities (37°) Water mg./ml. | Solubilities (37°) Cyclohexane mg./ml. | Cyclohexane/Water Partition Coefficients 25° | f.d.[a] | Availability Factor[b] |
|---|---|---|---|---|---|
| Acetaminophen | 20 | 0.0015 | 0.000075 | 0.000075 | 0.10 |
| **Carbonate Esters** | | | | | |
| Methyl | 6.0 | 0.048 | 0.008 | 0.0079 | 2.3 |
| Ethyl | 1.1 | 0.036 | 0.03 | 0.032 | 1.6 |
| Isopropyl | 1.1 | 0.05 | 0.09 | 0.043 | 2.1 |
| Butyl | 0.16 | 0.081 | 0.48 | 0.34 | 2.2 |
| Isobutyl | 0.38 | 0.13 | 0.50 | 0.25 | 3.8 |
| Hexyl | 0.037 | 0.12 | 0.84 | 0.76 | 1.0 |
| Octyl | 0.0044 | 0.14 | 0.94 | 0.97 | 0.14 |
| Chloroethyl | 0.39 | 0.55 | 1.2 | 0.59 | 8.9 |
| Phenyl | 0.063 | 0.02 | 0.32 | 0.24 | 0.56 |
| 4-Acetaminophenyl | 0.060 | 0.001 | 0.015 | 0.016 | 0.03 |
| **Carboxylate Esters** | | | | | |
| Acetyl | 3.4 | 0.019 | 0.0043 | 0.0056 | 1.0 |
| Butyryl | 0.54 | 0.019 | 0.053 | 0.034 | 0.83 |
| Hexanoyl | 0.057 | 0.083 | 0.85 | 0.59 | 1.3 |
| Stearoyl | 0.015 | 0.018 | 1.0 | 0.55 | 0.2 |
| Pivalyl | 0.11 | 0.042 | 0.3 | 0.28 | 1.3 |
| Crotonyl | 0.43 | 0.015 | ... | 0.034 | 0.67 |
| Fumaryl | ... | ... | ... | ... | ... |
| Acid succinyl | 6.5 | 0.0007 | 0.0009 | 0.0001 | 0.03 |
| Succinyl | 0.0068 | <0.0001 | <0.0001 | ... | ... |
| Benzoyl | 0.017 | 0.012 | 0.55 | 0.41 | 0.3 |
| Cinnamoyl | 0.0014 | 0.0013 | 1.3 | 0.48 | 0.023 |
| Chloroacetyl | 0.28 | 0.11 | 0.18 | 0.28 | 3.4 |
| Morpholinoacetyl·HCl | 102 | 0.0087 | 0.019 | 0.00008 | 0.26 |

[a] *f.d.* = fractional distribution of the compound in a cyclohexane/water system calculated by dividing the cyclohexane solubility by the sum of the cyclohexane solubility and the water solubility. [b] Availability factor = *f.d.* × molar aqueous solubility × $10^4$.

perimental analytical error resulting from the low concentrations measured in the solubility studies. Nonetheless, it would seem that addition of the polar acetamido group significantly reduces lipid solubility and consequently the cyclohexane/water partition coefficient is reduced.

The solubility behavior of the carboxylate esters was essentially the same as that of the carbonate esters. Increasing chain length in the acid moiety decreased aqueous solubility and increased lipid solubility, although the cyclohexane solubilities of the carboxylate esters were not as high as their analogous carbonate esters. Apparently, carboxylates are inherently more polar than carbonates. Branching in the acid moiety seemed to have less effect on the solubilities of the carboxylates than it did on the solubilities of the carbonates (compare butyryl with pivalyl and butylcarbonate with isobutylcarbonate) and unsaturation in the acid moiety also seemed to have little effect on the solubility (compare butyryl with crotonyl). The high melting fumaryl ester was extremely insoluble in both water and cyclohexane so that no measurement of its solubilities could be made by our methods. The succinyl ester, also a high melting compound, was also very insoluble in both water and cyclohexane. The acid succinyl ester was quite soluble in water due to its free carboxyl group, but its lipid solubility was very low. As with the carbonate esters, aromatic groups in the carboxylate esters tended to decrease both aqueous and lipid solubilities, and chlorine tended to decrease aqueous, but increase lipid, solubilities. Because it is a salt, the morpholinoacetyl·HCl derivative was very soluble in water and very insoluble in cyclohexane.

**Availability Factors**—In evaluating the availability of a relatively insoluble compound for oral absorption, one must take into consideration both the aqueous solubility, which is directly related to the rate at which the compound dissolves in the gut lumen, and the lipid solubility, which is directly related to the rate at which the compound partitions into the lipid gut barrier. In order to compare compounds, it would be convenient to express both these factors in one number. The "availability factors" listed in Table III are an attempt to do this. They are products of the molar aqueous solubilities and a factor related to the lipid/water distribution characteristics of the compounds. Because partition coefficients can be extremely low or extremely high and thus offset significant changes in aqueous solubilities, the lipid/water distribution characteristics of the compounds were expressed in terms of "fractional distributions" (*f.d.*). These values were calculated using the following empirical equation:

$$f.d. = \frac{\text{cyclohexane solubility}}{\text{cyclohexane solubility} + \text{aqueous solubility}}$$

Whereas partition coefficients may vary from 0 to infinity, fractional distributions, as defined above, vary from 0 to 1.

The availability factors for nearly all the acetaminophen carbonate esters are of the same order of magnitude and are larger than that for acetaminophen itself. The isobutylcarbonate derivative is more soluble in both water and cyclohexane than the other carbonates; and, consequently, its availability factor is somewhat higher than that of the other aliphatic carbonates. How-

ever, the chloroethylcarbonate has an even higher factor which is primarily due to its high cyclohexane solubility. The factor for the octylcarbonate is low due to its low aqueous solubility, while the factors for the phenyl- and 4-acetaminophenylcarbonates are low because both their aqueous and cyclohexane solubilities are low.

The factors for the carboxylate esters show a similar degree of variability and are generally lower than those of the carbonates. Factors for some of the carboxylates could not be calculated because their solubilities in water and cyclohexane are too low. The factor for the acid succinyl derivative is notably low because of its low cyclohexane solubility and in spite of its relatively high aqueous solubility. This is also true of the morpholinoacetyl·HCl derivative, although this compound would probably have a higher factor in its free base form than in its salt form. The benzoyl and cinnamoyl derivatives have low factors due to low aqueous and cyclohexane solubilities.

It should be mentioned that conjecture based on availability factors, such as those shown in Table III, must be restricted to compounds of closely similar structure. Cyclohexane and most other organic solvents probably have solvent properties which are markedly different from those of the G.I. barrier lipids. However, cyclohexane was chosen for these solubility and partitioning studies because it is one of the most nonpolar pure solvents available commercially. Moderate solubilities in cyclohexane should reflect a high degree of lipophilic character in the drugs. However, the G.I. barrier is not pure hydrocarbon, and drugs which have a relatively low solubility in cyclohexane may have a much greater solubility in G.I. barrier lipids due to polar interactions. As long as one is dealing with drugs of similar enough structure that the polar interactions are relatively equal and the structural differences result in changes in the nonpolar portions of the molecule, it is relatively safe to make comparative predictions of oral absorbability. However, when the structures differ as much as the difference between a carbonate ester and a carboxylate ester of equal chain length, or even as little as the difference between an aliphatic carbonate and a chlorine-substituted aliphatic carbonate ester, such predictions must be made with great caution. Therefore, it is dangerous to say that acetaminophen itself is less "available" than the prodrugs; but it is reltively safe to say that the aliphatic carbonates are all about equally available, with the isobutyl derivative being perhaps slightly more rapidly available for absorption than the rest.

**In Vitro Hydrolysis Behavior**—It was expected that the prodrug carbonate and carboxylate esters of acetaminophen would be hydrolyzed *in vivo* before, during, or after absorption to release free acetaminophen which would then exert its characteristic pharmacologic actions. It was, therefore, desirable to determine the relative susceptibility of these compounds to both nonenzymatic and enzymatic hydrolysis *in vitro*. It was of particular interest to look for relationships between the hydrolysis rates and the pharmacologic activities of the compounds.

Table IV shows half-lives for the hydrolyses of the prodrugs at 37° in pH 7.4 phosphate buffer with and without 2% human plasma. In general, all the compounds hydrolyzed slowly in pH 7.4 buffer; and most exhibited half-lives in excess of 24 hr. (1440 min.). The carbonate and carboxylate esters behaved very similarly in this regard, but the cinnamoyl and chloroacetyl (carboxylate ester) derivatives were unusually labile to hydrolysis exhibiting half-lives of 1.2 min. and 12 min., respectively, in buffer.

Table IV—Half-Lives for the Hydrolysis of Acetaminophen Prodrugs in pH 7.4 Phosphate Buffer (0.1 *M*) With and Without 2% v/v Human Plasma (37°)

| Compd. | 2% Human Plasma (min.) | Buffer (min.) | $t_{1/2}$ Buffer/ $t_{1/2}$ Plasma |
|---|---|---|---|
| **Carbonate Esters** | | | |
| Methyl | 180 | 9,000 | 50 |
| Ethyl | 45 | 12,000 | 267 |
| Isopropyl | 55 | 18,000 | 327 |
| Butyl | 15 | 18,000 | 1200 |
| Isobutyl | 19 | 21,000 | 1105 |
| Hexyl | 11 | 22,800 | 2073 |
| Octyl | 14 | 19,800 | 1414 |
| Chloroethyl | 25 | 1,320 | 53 |
| Phenyl | 25 | 240 | 9.6 |
| 4-Acetaminophenyl | 130 | 420 | 3.2 |
| **Carboxylate Esters** | | | |
| Acetyl | 38 | >1,440 | ... |
| Butyryl | 34 | >1,440 | ... |
| Hexanoyl | 30 | >1,440 | ... |
| Stearoyl | ... | ... | ... |
| Pivalyl | 462 | ... | ... |
| Crotonyl | 258 | >1,440 | ... |
| Fumaryl | 58 | 138 | 2.4 |
| Acid succinyl | 1.1 | 660 | 60 |
| Succinyl | 38 | 390 | 10 |
| Benzoyl | 98 | >1,440 | ... |
| Cinnamoyl | 2.8 | 1.2 | 0.43 |
| Chloroacetyl | 4.6 | 12 | 2.6 |
| Morpholinoacetyl · HCl | 138 | 438 | 3.2 |

In pH 7.4 buffer containing 28% human plasma, the hydrolysis rates were much more rapid. The degree of acceleration is illustrated in the column headed "$t_{1/2}$ buffer/$t_{1/2}$ plasma." This column shows that some of the compounds hydrolyzed as much as 2,000 times faster in the presence of 2% human plasma than in plain pH 7.4 buffer. This accelerated hydrolysis rate is attributed to the esterases present in the plasma. In other experiments, it was observed that as plasma concentrations increased, hydrolysis rates likewise increased.

**Toxicity and Analgesic Activity**—On the basis of the hydrolysis data, it might be concluded that all of the carbonate and carboxylate compounds should behave as prodrugs of acetaminophen, hydrolyzing to release free acetaminophen in the tissues of humans and animals. On the basis of the availability factors shown in Table III for the carbonate esters, for example, it might be concluded that these compounds should not differ appreciably in their rates of dissolution and absorption from the gastrointestinal tract. To test the validity of these conclusions *in vivo*, the oral $LD_{50}$'s were determined of some of the compounds in mice and the analgesic

TABLE V—HALF-LIVES FOR HYDROLYSIS IN HUMAN PLASMA, AVAILABILITY FACTORS, AND ORAL $LD_{50}$'s IN MICE FOR PRODRUG CARBONATE ESTERS OF ACETAMINOPHEN

| Derivative | Availability Factor[a] | $LD_{50}$ (Limits) moles/Kg. | $LD_{50}$ (Limits)[b] mg./Kg. |
|---|---|---|---|
| Acetaminophen | 0.10 | 7.1 (5.4–9.4) | 1080 (824–1415) |
| **Carbonate Esters** | | | |
| Methyl | 2.3 | 8.4 (6.7–10.5) | 1750 (1400–2188) |
| Ethyl | 1.6 | 6.5 (5.3–7.9) | 1450 (1190–1770) |
| Isopropyl | 2.1 | 14.8 (10.5–16.9) | 3500 (2500–4000) |
| Butyl | 2.2 | 6.4 (4.4–9.8) | 1614 (1093–2449) |
| Isobutyl | 3.8 | 8.6 (6.7–10.9) | 2150 (1693–2731) |
| Phenyl | 0.56 | 12.5 (8.9–17.7) | 3400 (2411–4794) |

[a] See Table III for definition. [b] $LD_{50}$'s and 95% confidence limits were calculated by the method of Litchfield and Wilcoxon (12).

activities of some of the compounds in rats using the Randall-Selitto technique.

Table V shows half-lives for enzymatic hydrolysis, availability factors, and oral $LD_{50}$'s in mice for some of the carbonate esters. The $LD_{50}$'s are given in moles/Kg. since 1 mole of each prodrug would release 1 mole of acetaminophen regardless of the molecular weight of the prodrug. Thus, comparison of toxicities on a molar basis gives a more precise evaluation of *in vivo* availability than comparison on a mg./Kg. basis. The molar $LD_{50}$'s of the carbonate prodrugs are virtually identical, and their confidence limits overlap each other and those of acetaminophen in almost all cases. In those cases in which the confidence limits do not overlap, the lack of agreement does not appear to be the result of either availability or slow enzyme hydrolysis. For example, the isobutyl derivative has the highest availability factor; but its toxicity overlaps that of the phenyl compound which has the lowest factor. The methyl compound is the slowest hydrolyzing compound, but it is equitoxic to the isobutyl compound, which is relatively rapidly hydrolyzed. It would seem that normal biological variation or some physical factor, such as poor wetability, may have caused the variations in the toxicities of these compounds. All of the compounds were micronized to reduce the particles to the 2–20 $\mu$ size range before biological testing.

The oral $LD_{50}$'s of the acetyl, butyryl, hexanoyl, and stearoyl carboxylate esters in mice were found to be in excess of 12 moles/Kg. The stearoyl derivative was so insoluble that no deaths could be produced at any dose. Thus, it would appear that the generally lower availability factors given for these compounds in Tabe III indicate that they are less available than the carbonates. However, the $LD_{50}$'s were so high that meaningful comparisons could not be made.

It is well known that it is difficult to quantitate the activity of mild nonnarcotic analgesics in laboratory animals (10); therefore, the authors attempted only to establish whether or not the acetaminophen carbonates possess analgesic activity in the range of acetaminophen. The method of Randall and Selitto was employed, and a control group of animals was included to show that the yeast-injected rat paw retains its increased sensitivity to pain for the duration of the study (4 hr.). The results of a statistical analysis of the postdrug pain threshold data are shown in Table VI. The isopropyl and phenyl carbonates had significant analgesic activity at doses equimolar to a 400 mg./Kg. dose of acetaminophen. These compounds have availability factors of 2.1 and 0.56, respectively (Table V); but the difference between these values is apparently not great enough to cause a significant difference in analgesic activity by this test. If prodrug derivatives had been prepared of a drug with a more potent and more easily quantitated pharmacologic activity, perhaps a difference in availability factor such as that found for these prodrugs would have made a significant difference in the pharmacologic responses. However, with drug activity as weak and difficult to measure as mild analgesia, it is impossible to tell whether or not the carbonate prodrugs are absorbed at any different rates or with greater or lesser efficiencies than acetaminophen itself.

TABLE VI—ANALGESIC ACTIVITY (RANDALL-SELITTO) OF ACETAMINOPHEN AND CARBONATE PRODRUGS OF ACETAMINOPHEN ORALLY IN RATS

| Compd. | Time Postdrug (Min.)[a] 30 | 60 | 120 | 180 | 240 |
|---|---|---|---|---|---|
| Acetaminophen | + | + | + | + | + |
| **Carbonate Esters** | | | | | |
| Isopropyl | + | 0 | + | + | 0 |
| Phenyl | 0 | 0 | + | + | + |

[a] + = Significant ($p \leq 0.05$) analgesic activity; 0 = no significant analgesic activity. Significance was determined by means of Student's $t$ test (13).

Because this study was concerned primarily with establishment of principles rather than a determination of the pharmacologic activity profiles of every compound made, a number of the acetaminophen esters were not studied *in vivo* for their activity profiles. Nevertheless, since each of the compounds that were thus studied exhibited the gross pharmacologic activities of acetaminophen, and each underwent relatively rapid hydrolysis in the presence of plasma, it seems fair to assume that most of the compounds reported here would behave as acetaminophen prodrugs.

## CONCLUSIONS

Carbonate and carboxylate esters prepared as potential prodrugs of acetaminophen were shown to have a variety of physicochemical properties, particularly lipid and water solubilities. *In vitro* enzyme-catalyzed hydrolysis studies suggested that most of the compounds would be hydrolyzed to release acetaminophen following oral administration to animals and humans. Toxicity and analgesic

activity studies in mice and rats suggested that the lipid and water solubilities of the compounds, rather than their enzyme-catalyzed hydrolysis rates, probably control the availability of acetaminophen following oral administration. These experiments provide additional evidence that the carbonate linkage may be an importantly useful one for the creation of variant physical and chemical properties in an entity with a singular pharmacologic action (11). This might then offer the opportunity to the formulator to choose among a variety of compounds with a singular pharmacologic action for the one which is: (*a*) amenable to physical formulation in a given dosage form; (*b*) stable in a given dosage form where all forms of the drug may not be equally stable; (*c*) satisfactory from the standpoint of taste or consumer acceptability; and (*d*) appropriate therapeutically from the standpoint of its time-action profile.

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*Keyphrases*

Acetaminophen prodrugs
Carbonate esters, acetaminophen–synthesis
Carboxylic acid esters, acetaminophen–synthesis
Hydrolysis rates, *in vitro*–acetaminophen prodrugs
Toxicity, oral–acetaminophen prodrugs
Analgesic activity–acetaminophen prodrugs

# Acetaminophen Prodrugs II

## Effect of Structure and Enzyme Source on Enzymatic and Nonenzymatic Hydrolysis of Carbonate Esters

By L. W. DITTERT*, G. M. IRWIN, C. W. CHONG, and J. V. SWINTOSKY*

**Hydrolysis rates are reported for acetaminophen prodrugs with the structure $CH_3CONH$-$\phi$-OCOOR at pH 7.4 in phosphate buffer alone or containing 1% human plasma or serum from several animal species. The hydrolysis rates in buffer decreased as the electrophilic character of the R group decreased. Dilute plasma or serum accelerated the hydrolysis; and the number of carbon atoms, the degree of chain branching, aromaticity, and chlorine substitution in the R group variously affected the degree of acceleration. In general, the sera of small rodents (mouse, guinea pig, and rat) were more potent catalysts of the hydrolyses of all types of acetaminophen carbonates than that of other animals (cat, dog, sheep, and rabbit) or human plasma.**

THE METHODS of preparation and the physical properties of a series of carbonate esters of acetaminophen have been previously reported (1). These compounds were found to hydrolyze at various rates in dilute (2% v/v) human plasma solutions, and some of them had analgesic activity on the order of acetaminophen in rats. It was postulated that the analgesic activity was due to free acetaminophen released in the blood streams of the rats following oral administration of the prodrugs.

This report discusses the influence of the structure of acetaminophen carbonate prodrugs on the nonenzymatic hydrolysis of the compounds at pH 7.4 and on the enzymatic hydrolysis of the compounds catalyzed by blood plasma from

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