Carbonate Ester Prodrugs of Salicylic Acid

Synthesis, Solubility Characteristics, *In Vitro* Enzymatic Hydrolysis Rates, and Blood Levels of Total Salicylate Following Oral Administration to Dogs

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The methods of synthesis, solubilities, and partition coefficients for the ethyl-, butyl-, hexyl-, and 2,2,2-trichloroethylcarbonate esters of salicylic acid are reported. The solubility and partitioning characteristics of the ethyl- and trichloroethylcarbonates were similar to those of aspirin, whereas the butyl- and hexylcarbonates were more lipid soluble than aspirin. The *in vitro* hydrolysis rates of the compounds were also determined. The hydrolysis of the carbonate esters of salicylic acid were only slightly accelerated by 2 percent human plasma, and aspirin hydrolysis was not accelerated at all by this enzyme system. In the pseudocholinesterase and α-chymotrypsin systems, the hydrolysis of the butyl- and hexylcarbonates were accelerated to a much greater degree than those of the ethylcarbonate and aspirin. The results suggest that carbonates with 4 or 6 carbon alkyl chains fit the active sites of these esterolytic enzymes better than a carbonate with a 2 carbon alkyl chain or an acetate ester. Aspirin and the butyl-, hexyl-, and trichloroethylcarbonates were administered orally to dogs and the plasma levels of total salicylate were followed for 8 hr. The resulting blood level curves were virtually superimposable; all peaked at about 2 hr. and fell off at about the same rate. These results suggest that the prodrug carbonate esters are as readily absorbed as aspirin despite their different aqueous and lipid solubilities and that all the drugs including aspirin are converted to a common form, *i.e.*, free salicylate, within 2–3 hr. after oral administration.

FECAL BLOOD loss studies (1, 2) have shown that approximately 70% of persons taking aspirin experienced occult gastric bleeding averaging 5 ml. of blood per day, and Kelly (3) has reported that patients taking aspirin for long periods of time may develop severe iron deficiency anemia due to fecal blood loss. The mechanism by which aspirin causes gastric hemorrhage is a matter of considerable controversy (4). There is little doubt, however, that at least part of the problem is due to local irritation of gastric mucosa (5).

It was the purpose of our studies to seek prodrug derivatives of salicylic acid which would be nonirritant, would be readily absorbed, and which would be rapidly hydrolyzed in blood and other tissues to release free salicylate in the human blood stream. It was reasoned that carbonate esters of salicylic acid which are readily hydrolyzed by dilute human plasma, but which are less soluble in water and more soluble in lipids than aspirin, might be distributed and absorbed over a broader area of the gastrointestinal tract and might be less irritating to the gastric mucosa than aspirin.

This paper presents the synthesis, solubility, and partitioning properties, and *in vitro* hydrolysis rates of four carbonate ester prodrugs of salicylic acid. It also presents the blood levels of total salicylate following oral administration to dogs of three of the carbonate esters compared with aspirin.

EXPERIMENTAL

Chemical Synthesis—Melting points were determined in capillary tubes on a Thomas-Hoover apparatus and are uncorrected. Reported yields are of pure compound.

The following general procedure for synthesizing the compounds was adapted from the method of preparation of the methylcarbonate reported by Fischer (6). To an ice-cooled mixture of 85 Gm. (0.61 mole) of salicylic acid and 155 ml. (1.22 moles) of dimethylaniline in 500 ml. of dry benzene, the appropriate chloroformate (0.61 mole) was added over a period of 15 min. The ice bath was removed, and the mixture was stirred at room temperature for 2 hr. and washed with six 100-ml. portions of 10% HCl and three 100-ml. portions of water. The benzene solution was dried over anhydrous magnesium sulfate, and the solvent was removed. The remaining material was purified by crystallization from the appropriate solvent system (see Table II) after treatment with activated charcoal.

Particle Size Reduction—The purified compounds were micronized by one or more passes

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through a fluid energy mill (Trost Jet Mill, Helme Products, Inc., Helmetta, N. J.). This procedure produced particles in the 2–20 μ size range.

Solubilities—The saturation solubilities of the compounds at 37° were determined in 0.1 *N* HCl and spectral grade cyclohexane (Fischer) in rotating bottles utilizing an assembly that has been described previously (7). HCl (0.1 *N*) was used to assure that the compounds were completely unionized and to reduce the degree of hydrolysis of the compounds during equilibration. The aqueous samples were rotated for 6 hr., and 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 Corp.). The samples were analyzed spectrophotometrically.

Partition Coefficients—The partition coefficients of the compounds between cyclohexane and 0.1 *N* HCl at 25° were determined using a method and apparatus previously described (8). Since 0.1 *N* HCl was used as the aqueous phase in these experiments, the partition coefficients shown in Table III are "true partition coefficients" of the unionized compounds.

In Vitro Hydrolysis Rates—Half-lives for hydrolysis of the compounds at 37° in 0.1 *M* phosphate buffer with and without enzymes were determined by a spectrophotometric method previously described (9). Frozen human blood plasma (Type O^+) was obtained in approximately 100-ml. quantities from single donors through the Philadelphia Serum Exchange. Purified human pseudocholinesterase was obtained from Sigma Chemical Co., and α-chymotrypsin (salt free from EtOH) was obtained from Nutritional Biochemicals Co.

Blood Levels of Total Salicylate in Dogs—Purebred beagle dogs were used in a 4 × 4 crossover study with aspirin and the butyl- and trichloroethylcarbonates of salicylic acid. Each dog received an equimolar dose of drug per Kg. body weight according to Table I.

The drugs were administered *via* a stomach tube in single doses of finely ground powder suspended in 20 ml. of 0.5% tragacanth. Blood specimens were collected at 0, 15, 30, 60, 120, 240, 360, and 480 min. following drug administration, and total salicylate in the plasma was determined by the method of Cosmides, Stemler, and Miya (10).

Table I—Dosing Schedule

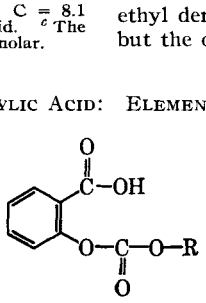
Compound	Mol. Wt.	Dose, mg./Kg.	Dosing Schedule[a] Day 1	4	8	11
Aspirin	180.16	75	A	B	C	D
Trichloroethyl[b]	313.54	130	B	C	D	A
Butyl[b]	238.24	90[c]	C	D	A	B
Hexyl[b]	266.30	111	D	A	B	C

[a] Dog weights: A = 12.8 Kg.; B = 8.6 Kg.; C = 8.1 Kg.; D = 12.0 Kg. [b] Carbonate of salicylic acid. [c] The dose of the butyl carbonate is 10% less than equimolar.

RESULTS AND DISCUSSION

The molecular weight, yields, melting points, elemental analyses, and recrystallization solvents for the four carbonate ester prodrugs of salicylic acid used in this study are shown in Table II. The ethyl derivative has been reported previously (11) but the others are new compounds. All are white

Table II—Carbonate Esters of Salicylic Acid: Elemental Analyses and Physical Properties



R	Formula	Mol. Wt.	Yield, %	M.p., °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Recrystallization Solvent
Ethyl (C_2H_5—)	$C_{10}H_{10}O_5$	210.190	40	93.5–94.5[a]	57.14	57.21	4.80	4.77	Chloroform-hexane
n-Butyl (C_4H_9—)	$C_{12}H_{14}O_5$	238.244	36	79.5–80.5	60.50	60.36	5.92	5.83	Carbon tetrachloride
n-Hexyl (C_6H_{13}—)	$C_{14}H_{18}O_5$	266.298	54	79.5–80.5	63.15	63.20	6.81	6.73	Carbon tetrachloride
Trichloroethyl (Cl_3C—CH_2—)	$C_{10}H_7Cl_3O_5$	313.537	42	126–128	38.31	38.37	2.25	2.29	Isopropanol-hexane

[a] Literature melting point is 95° (11).

Table III—Aspirin and Carbonate Esters of Salicylic Acid: Solubilities, Partition Coefficients, and Hydrolysis Rates

Derivative	Solubilities in mg./ml., 37° 0.1 *N* HCl[a]	Solubilities in mg./ml., 37° Cyclohexane	Cyclohexane/0.1 *N* HCl Partition Coefficient, 25°[a]	Half-Lives for Hydrolysis, 37° Phosphate Buffer (0.1*M*) pH 7.4, hr.	Phosphate Buffer (0.1*M*) pH 12, min.	Enzyme in Phosphate Buffer (pH 7.4, 0.1*M*)[b] 2% Human Plasma, hr.	0.05% Human Pseudocholinesterase, hr.	0.05% α-Chymotrypsin, hr.
Ethyl	6.7	1.1	0.17	41	3.8	13.3 (3.1)	7.4 (5.5)	53 (—)
n-Butyl	2.8	2.7	1.0	29	4.5	11.7 (2.5)	1.23 (24)	1.38 (21)
n-Hexyl	0.28	5.6	20	15	4.8	8.2 (1.8)	1.33 (11)	0.25 (58)
Trichloroethyl	1.1	0.24	0.22	0.53	1.1	0.35 (1.5)	...	...
Aspirin	5.3	0.06	0.05	10	1.5	21 (—)	3.3 (3)	6.3 (1.6)

[a] 0.1 *N* HCl was used in these experiments to assure that the compounds were completely unionized in the aqueous phases and to reduce the degree of hydrolysis in the aqueous phase during equilibration. [b] The numbers in parentheses are $t_{1/2}$ buffer (pH 7.4)/$t_{1/2}$ enzyme ratios which represent the degree of enzymatic catalysis.

crystalline, somewhat waxy, solids in their pure forms.

The solubilities of the carbonate esters and aspirin in 0.1 *N* HCl and in cyclohexane at 37° are shown in Table III along with the corresponding cyclohexane–0.1 *N* HCl partition coefficients at 25°. In these studies, 0.1 *N* HCl was used in place of water to assure that the compounds would not hydrolyze appreciably during equilibration and that they were completely unionized in the aqueous phases. Thus, the solubilities shown in Table III are those of the unionized compounds and the partition coefficients are "true partition coefficients."

As expected, the solubilities of the aliphatic carbonate esters decrease in 0.1 *N* HCl and increase in cyclohexane with increasing chain length. The measured partition coefficients show the expected increase with increasing chain length. The trichloroethyl derivative is less soluble in cyclohexane and much less soluble in water than the ethyl derivative. It might have been expected that the cyclohexane solubility of the trichloroethyl derivative would be greater than it is, since trichloroethyl groups tend to be more lipophilic than ordinary ethyl groups, but the trichloroethylcarbonate of salicylic acid has a higher melting point than the other derivatives indicating a stronger crystal lattice which tends to reduce solubility in all solvents.

Predictions of the relative availabilities of the compounds for oral absorption based on the solubilities and partition coefficients shown in Table III must take into account the fact that they probably do not have precisely equivalent pKa values; therefore, at pH's near their pKa's, some of the compounds might show relatively higher or lower apparent partition coefficients than would be expected on the basis of the true partition coefficients shown in Table III. Also, because the compounds are weak acids, the aqueous solubilities will increase with increasing pH. However, the fact that solubilities shown in Table III are of a reasonable order of magnitude suggests that if the drugs are finely ground and properly wetted by water, they should all be readily absorbed following oral administration.

If the solubilities and partition coefficient of aspirin are compared with those of the carbonates, it might be predicted that the ethyl derivative would have gastrointestinal absorption characteristics similar to those of aspirin. In subsequent tests (12) it was found that the ethyl derivative was almost as irritating to the gastric mucosa of rats as aspirin itself. It would seem that salicylate derivatives with relatively high aqueous solubilities and relatively low lipid solubilities are more irritating to the gastric mucosa than those which are less soluble in water and more soluble in lipids.

Table III also shows enzymatic and nonenzymatic hydrolysis data for the four carbonate esters and aspirin. The aliphatic carbonates are more stable toward hydrolysis in buffer than aspirin. The trichloroethylcarbonate is less stable, probably because the chlorine atoms act as good "electron sinks" drawing electrons away from the carbonyl carbon and making it susceptible to nucleophilic attack.

In 2% human plasma in pH 7.4 buffer, the aliphatic carbonates and aspirin are very slowly hydrolyzed. The values for $t_{1/2}$ buffer/$t_{1/2}$ enzyme, shown in parentheses in Table III, represent the degree of enzymatic catalysis and show that the esterases of human plasma have very little catalytic effect on the hydrolysis of these compounds. Even though the hydrolysis of the trichloroethylcarbonate derivative is relatively rapid, the degree of enzymatic catalysis is practically nil. In 0.05% "purified" human pseudocholinesterase and 0.05% α-chymotrypsin (from bovine pancreas) the ethylcarbonate derivative and aspirin are hydrolyzed relatively slowly and the degree of enzymatic catalysis in each case is relatively small. On the other hand, the butyl- and hexylcarbonates are hydrolyzed relatively rapidly by these enzymes and the degree of enzymatic catalysis is considerably larger. These prodrugs, however, are not nearly as susceptible to enzymatic hydrolysis by these enzymes as is 4-acetamidophenyl 2,2,2-trichloroethyl carbonate. For this carbonate ester prodrug of acetaminophen, the $t_{1/2}$ buffer/$t_{1/2}$ enzyme values were 1680 for 0.05% human pseudocholinesterase and 646 for 0.05% α-chymotrypsin (12). Of all the enzymes studied, these two were the most powerful catalysts for the hydrolysis of carbonate esters of acetaminophen, but they are apparently much weaker catalysts for the hydrolysis of carbonate esters of salicylic acid.

Augustinsson and Nachmansohn (13) reported the presence of an aspirin esterase in human blood which is clearly different from the pseudocholinesterase of serum. Morgan and Truitt (14) studied the *in vitro* hydrolysis of aspirin in serum from various species of laboratory animals and humans, and they reported that the aspirin esterase activity of human blood is very weak. The esterases of human blood, having a somewhat greater affinity for carbonate esters, than for aspirin, hasten the hydrolysis of these esters.

The *in vitro* enzyme hydrolysis studies suggested that free salicylate might be released at different rates following oral administration of aspirin and the carbonate prodrugs, but there are many esterolytic enzymes and many sites in the body other than the blood stream where hydrolysis of these compounds might occur. To investigate the release rates and sites of hydrolysis and to study the influence of the physical properties of the carbonate prodrugs on their *in vivo* performance, the prodrugs and aspirin were administered orally to dogs, and the blood levels of salicylate were followed. The analytical method employed (10) converts the carbonate esters and aspirin to free salicylate, therefore, the blood levels were obtained in terms of total salicylate.

The average total salicylate plasma levels in dogs following oral administration of aspirin and the butyl-, hexyl-, and trichloroethylcarbonates of salicylic acid are shown in Fig. 1. The curves in Fig. 1 show that the drugs are nearly identical with respect to the plasma salicylate levels they produce in dogs. All curves reach a peak near 20 mg. % at 2 hr. and then fall off at about the same rate. The minimum and maximum individual blood levels for all drugs overlap at all time points.

These results suggest that the solubility and partitioning properties of these prodrug carbonate esters did not grossly affect their gastrointestinal absorption rates. A possible exception is the hexyl derivative which appears to have a somewhat slower absorption rate than the others. However, the absorption rate of this compound is apparently fast.

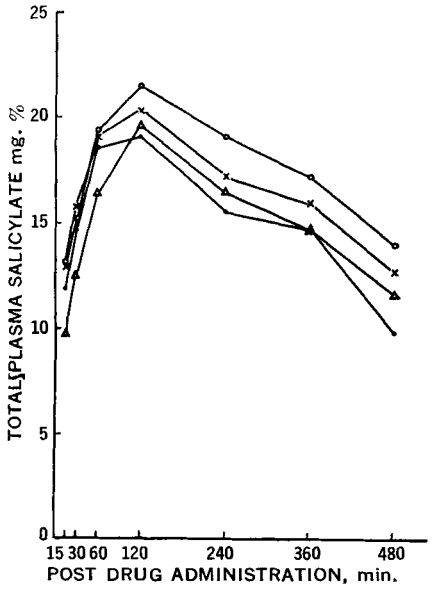


Fig. 1—Plot showing plasma levels of total salicylate in purebred beagle dogs following oral administration of equimolar doses of aspirin (.—., 75 mg./Kg.); trichloroethylcarbonate of salicylic acid (○—○, 130 mg./Kg.); butylcarbonate of salicylic acid (×—×, 90 mg./Kg.); and hexylcarbonate of salicylic acid (Δ—Δ, 111 mg./Kg.). The study was carried out according to a 4×4 crossover plan, and each point represents the average of four plasma levels. The minimum and maximum individual plasma levels overlapped for all drugs at all time points.

enough to catch up with the other carbonates and aspirin at the 2-hr. point. The fact that all the blood levels follow the same pattern and fall off at about the same rate suggests that beyond 2 or 3 hr., aspirin and the carbonate prodrugs of salicylic acid are all in the same form, that is, they have been completely converted to free salicylate. Thus, it would appear that aspirin and the three carbonates are rapidly converted to free salicylate *in vivo*, and it might be expected that the pharmacologic activities of the three carbonates would be very similar to those of aspirin.

Based on these findings, extensive pharmacologic evaluations of the butyl-, hexyl-, and trichloroethylcarbonates were undertaken. Future publications in this series will deal with their analgesic, antipyretic, and anti-inflammatory activities and their gastrointestinal irritation liabilities compared with aspirin.

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Keyphrases

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