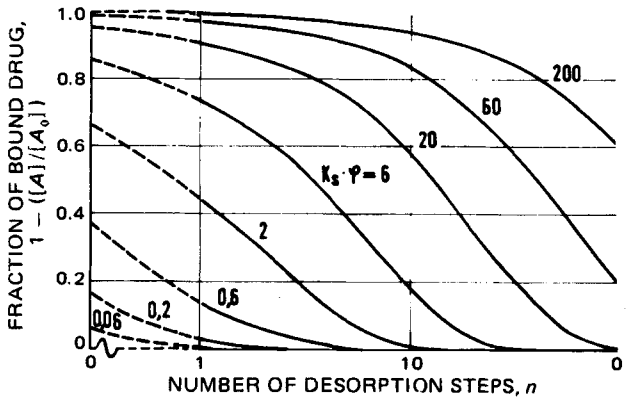

Figure 6—*Decrease of the initially sorbed amount of the drug as a function of the number of desorption steps,* n, *for various values of* $K_s\varphi$.

Finally, theoretical desorption curves are shown in Fig. 6 and were calculated according to Eq. 13. The graph depicts the decrease of the initially sorbed amount of the drug as a function of the number of desorption steps for increasing values of $K_s\varphi$. With regard to K_s values on the order of 2–10 M^{-1}, indicating moderate binding (Table I) even when considering high values of φ (*i.e.*, $\varphi = 0.02$), it follows from Fig. 6 that only a few desorption steps are necessary for complete release of the bound compound from the polymer.

Presumably, with the exception of tannic acid and closely related compounds, the presence of the disintegrant should not interfere with GI absorption of the pharmaceutical.

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Improved Delivery through Biological Membranes VIII: Design, Synthesis, and *In Vivo* Testing of True Prodrugs of Aspirin

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Received August 25, 1980, from the **Department of Pharmaceutical Chemistry, School of Pharmacy, University of Kansas, Lawrence, KS 66045*, the ‡*Inter$_x$ Research Corporation, Lawrence, KS 66044*, and the §*Department of Medicinal Chemistry, College of Pharmacy, J. Hillis Miller Health Center, University of Florida, Gainesville, FL 32610.* Accepted for publication October 16, 1980.

Abstract □ Novel activated ester-type prodrugs of aspirin were designed and synthesized. The methylthiomethyl, methylsulfinylmethyl, and methylsulfonylmethyl esters of aspirin (acetylsalicylic acid) were cleaved *in vitro* in plasma to form aspirin rather than the corresponding salicylates. *In vivo* studies using dogs indicated that at least one aspirin derivative, methylsulfinylmethyl-2-acetoxybenzoate, is a true aspirin prodrug since aspirin was detected in the blood after prodrug administration.

Keyphrases □ Aspirin—activated ester-type prodrugs of aspirin synthesized and evaluated for analgesia, *in vivo* and *in vitro* studies □ Prodrugs, aspirin—activated ester-type prodrugs synthesized and evaluated for analgesic activity, *in vitro* and *in vivo* studies □ Analgesic activity—activated ester-type prodrugs of aspirin, synthesized and evaluated for analgesia, *in vitro* and *in vivo* studies

The GI side effects of aspirin (acetylsalicylic acid, I) are well known and documented (1). These side effects are associated with the free carboxylic group; thus, transient derivatives (prodrugs) and various formulations of I were prepared and tested in which the free carboxyl group was derivatized or bound. The results obtained were unsatisfactory due to the specific properties of I. The *o*-acetyloxyl group is rather labile. Extensive studies of the chemical hydrolysis (2–8) and *in vivo* hydrolysis (9) demonstrated the facile conversion of I to salicylic acid (II). Although II is also a potent anti-inflammatory agent, it was shown (10) that I is a far more potent analgesic and, thus, delivery to the bloodstream in the intact form is desirable. To achieve this, the carboxyl-protecting function must be cleaved

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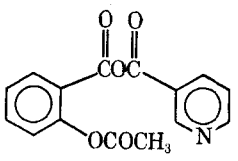

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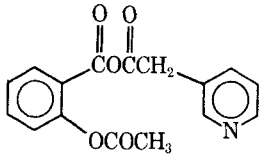
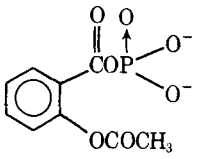


IV

prior to the labile *o*-acetyl one, which is difficult since the prodrug must at the same time be stable enough in the neat form and in formulation.

BACKGROUND

An early recommendation for an aspirin prodrug with decreased gastric irritation was aspirin anhydride (11). However, its low solubility in water, slow dissolution rate, and immunogenicity (12, 13) prevented further development. Attempts to increase the solubility were made by preparing mixed anhydrides[1] such as the pyridine-containing derivatives III and IV. However, these mixed anhydrides cannot be synthesized in a 100% pure form since some disproportionation always occurs and aspirin anhydride is formed as an impurity, even when using the clean acyl chloride–thallium carboxylate reaction (14). Mixed anhydrides probably also have immunogenic–allergenic properties similar to aspirin anhydride. A phosphoric acid mixed anhydride[2] (V) was considered, but all attempts at preparation failed, even when the stable ester (VI) was used, due to facile intramolecular reactions leading to the stable cyclic salicylphosphate (VII).



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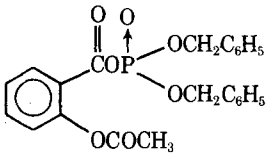
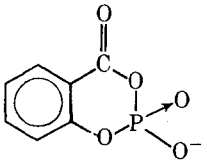


VI



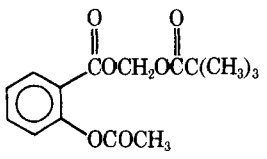
VII

Stable acyloxyalkyl derivatives of I such as the pivalyloxymethyl[1], benzoyloxymethyl, and analogous esters were synthesized, but they also showed low aqueous solubility and dissolution rates. More recently, the 4-acetamidophenyl ester (IX) (15, 16) and the triglycerides (X) (17, 18) were prepared.

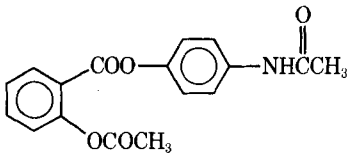
Although decreased GI irritation is claimed with both IX and X, there is no proof that these derivatives are true prodrugs of I and not of salicylic acid. Recent work (18) on X showed only the blood salicylate levels and the pharmacological testing involved only anti-inflammatory studies, but X still was indicated (without any proof) to be an aspirin prodrug.

Some aspirin salts, such as lysine acetylsalicylate (XI), were reported (19) to produce three times less GI bleeding than I.

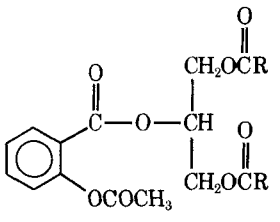
Other intended aspirin prodrugs include the 1′-ethoxyethyl ester (20), *N*-hydroxyethyl nicotinamide ester (21), and salicylamide ester (22). The patent literature also includes various derivatives but without much specific information.



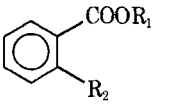
VIII



IX



X: R = $(CH_2)_nCH_3$



XI: $R_1 = CH_2SCH_3$, $R_2 = H$
XII: $R_1 = CH_2SOCH_3$, $R_2 = H$
XIII: $R_1 = CH_2SO_2CH_3$, $R_2 = H$
XIV: $R_1 = CH_2SCH_3$, $R_2 = OH$
XV: $R_1 = CH_2SOCH_3$, $R_2 = OH$
XVI: $R_1 = CH_2SO_2CH_3$, $R_2 = OH$
XVII: $R_1 = CH_2SCH_3$, $R_2 = OCOCH_3$
XVIII: $R_1 = CH_2SOCH_3$, $R_2 = OCOCH_3$
XIX: $R_1 = CH_2SO_2CH_3$, $R_2 = OCOCH_3$

None of the chemical derivatives of aspirin was reported to form aspirin in the body after oral or intravenous administration.

The present study reports novel derivatives of aspirin, at least one of which resulted in aspirin *in vivo*. These new prodrugs are activated esters that undergo further activation *in vivo*. The overall result is delivery of aspirin (I) rather than of salicylic acid.

Protective groups capable of being removed under mild conditions are of great value in peptide synthesis. Methylthiomethyl and β-ethylthiomethyl esters are used as protective groups for carboxylic acids for this purpose (23, 24). They are somewhat stable under normal conditions but can be made more labile by conversion into a sulfonium salt or by oxidation to the sulfone, which then is cleaved readily under neutral or mild alkaline conditions at room temperature to regenerate the acid. Therefore, similar groups might be suitable as temporary transport moieties for aspirin and other carboxylic acids.

Sulindac, a new anti-inflammatory agent containing a sulfinyl group, undergoes reversible reduction–oxidation biotransformation to the sulfide and an irreversible oxidation to the sulfone in several animal species including humans (25). The methylthiomethyl ester thus should be susceptible to enzymatic oxidation and subsequent cleavage in the body. Benzoic acid was used first as a model compound, and the corresponding methylthiomethyl (XI), methylsulfinylmethyl (XII), and methylsulfonylmethyl (XIII) esters were synthesized. Analogous derivatives of salicylic acid (XIV–XVI) and of aspirin (XVII–XIX) then were prepared.

Detailed studies as to the physicochemical properties and chemical and enzymic hydrolysis rates were carried out on XI–XIX. The results, including the complex hydrolysis characteristics of XVII–XIX, will be reported in separate papers. This report describes the enzymic hydrolysis studies on XIV–XIX; the data also show that at least one aspirin derivative is an effective true prodrug of I, resulting in aspirin *in vivo*.

EXPERIMENTAL

Methylthiomethyl Benzoate (XI)—The compound was prepared by reacting benzoic acid with chloromethyl methyl sulfide in the presence of triethylamine as described earlier (23). The obtained ester was distilled under reduced pressure, bp 93–95°/0.3 mm [lit. (23) bp 106–108°/2 mm]; mol. wt. 182.24; IR (neat): 3060, 2920, 1710, 1595, 1450, 1330, 1310, 1250, 1100, 1070, 1025, 930, 750, and 710 cm^{-1}; NMR ($CDCl_3$): δ 7.2–8.2 (m, 5H), 5.4 (s, 2H), and 2.3 (s, 3H) ppm; mass spectrum (70 ev): *m/z* (relative intensity) 182 (9), 105 (100), and 77 (41).

Methylsulfinylmethyl Benzoate (XII)—To a chloroform solution containing 0.91 g (0.005 mole) of XI was added, in portions over 15 min with stirring, 1.0 g (0.005 mole) of *m*-chloroperbenzoic acid (85% solution). The resultant solution was stirred at room temperature overnight, washed twice with saturated aqueous sodium bicarbonate solution, and dried over anhydrous sodium sulfate. The reaction mixture was filtered, and the filtrate was evaporated under reduced pressure to afford a pale-yellow liquid, which crystallized on standing several hours at room temperature. Trituration of the residue in petroleum ether (bp 30–60°) gave 0.7 g (0.0035 mole) of the desired product (70% yield) as a white solid, mp 43–45°; IR (neat, melt): 3060, 3000, 2920, 1710, 1590, 1445, 1310, 1240, 1100, 1050, 800, and 710 cm^{-1}; NMR ($CDCl_3$): δ 7.3–8.3 (m, 5H), 5.2 (*AB*, 2H), and 2.7 (s, 3H) ppm; mass spectrum (70 ev): *m/z* (relative intensity) 168 (2), 135 (4), 105 (100), and 77 (42).

Anal.—Calc. for $C_9H_{10}O_3S$: C, 54.52; H, 5.08; S, 16.18. Found: C, 54.22; H, 5.22; S, 16.40.

Methylsulfonylmethyl Benzoate (XIII)—Use of the method described for XII, but with 2 equivalents of *m*-chloroperbenzoic acid, afforded the desired product, mp 116–118°; IR (KBr): 3060, 3000, 2920, 1730, 1590, 1545, 1420, 1300, 1250, 1095, 950, 800, and 720 cm^{-1}; NMR

[1] N. Bodor, unpublished work.

[2] N. Bodor, unpublished work. The pivalyloxymethyl ester (VIII) is a solid which has a low melting point (36–38°) and which is soluble to the extent of 25% in oil. The solution did not show any decomposition for 2 years at room temperature.

($CDCl_3$): δ 7.3–8.4 (m, 5H), 5.3 (s, 2H), and 3.0 (s, 3H) ppm; mass spectrum (70 ev): *m/z* (relative intensity) 184 (4), 135 (2), 105 (100), and 77 (36).

Anal.—Calc. for $C_9H_{10}O_4S$: C, 50.45; H, 4.71; S, 14.97. Found: C, 50.61; H, 5.00; S, 15.18.

Methylthiomethyl 2-Hydroxybenzoate (XIV)—To an acetonitrile solution containing 13.8 g (0.1 mole) of salicylic acid and 10.1 g (0.1 mole) of triethylamine at 0° was added dropwise, with stirring, 9.6 g (0.1 mole) of chloromethyl methyl sulfide. The solution was heated under reflux for 24 hr. The solvent was then removed under reduced pressure, and the residue was suspended in benzene. The formed triethylamine hydrochloride was removed by filtration, and the filtrate was washed twice with saturated aqueous sodium bicarbonate solution, dried over anhydrous sodium sulfate, and filtered. Removal of benzene by evaporation under reduced pressure afforded a liquid. Vacuum distillation gave 15.8 g (0.08 mole) of the desired product (80% yield) as a clear, colorless liquid, bp 107–109°/0.8 mm; IR (neat): 3200, 2920, 1670, 1600, 1580, 1480, 1415, 1350, 1280, 1200, 1170, 1140, 1080, 920, 840, 760, and 700 cm^{-1}; NMR ($CDCl_3$): δ 10.5 (broad s, 1H), 6.6–8.0 (m, 4H), 5.4 (s, 2H), and 2.3 (s, 3H) ppm; mass spectrum (70 ev): *m/z* (relative intensity) 198 (7), 121 (15), and 61 (100).

Anal.—Calc. for $C_9H_{10}O_3S$: C, 54.52; H, 5.08; S, 16.18. Found: C, 54.70; H, 5.26; S, 16.01.

Methylsulfinylmethyl 2-Hydroxybenzoate (XV)—Substitution of an equivalent quantity of XIV for the benzoic acid methylthiomethyl ester (XI) used for the synthesis of XII and repetition of the described procedure afforded the desired product, mp 110–112° (ethanol); IR (KBr): 3200, 3000, 1680, 1600, 1580, 1480, 1345, 1280, 1130, 1090, 940, and 770 cm^{-1}; NMR ($CDCl_3$): δ 10.2 (s, 1H), 6.7–8.1 (m, 4H), 5.3 (*AB*, 2H), and 2.8 (s, 3H) ppm; mass spectrum (70 ev): *m/z* (relative intensity) 214 (1), 121 (100), 93 (13), and 65 (10).

Anal.—Calc. for $C_9H_{10}O_4S \cdot 1/2H_2O$: C, 48.42; H, 4.97; S, 14.36. Found: C, 48.14; H, 4.51; S, 14.94.

Methylsulfonymethyl 2-Hydroxybenzoate (XVI)—By using the method described for the preparation of XIII, the ester XIV was transformed into the desired product in an 85% yield, mp 124–126° (ethanol); IR (KBr): 3200, 3000, 1680, 1600, 1580, 1480, 1420, 1345, 1320, 1280, 1240, 1190, 1130, 1090, 940, 770, and 710 cm^{-1}; NMR ($CDCl_3$): δ 10.0 (s, 1H), 6.8–8.1 (m, 4H), 5.3 (s, 2H), and 3.1 (s, 3H) ppm; mass spectrum (70 ev): *m/z* (relative intensity) 230 (10), 121 (100), 93 (10), and 65 (12).

Anal.—Calc. for $C_9H_{10}O_5S$: C, 46.95; H, 4.38; S, 13.93. Found: C, 47.14; H, 4.52; S, 14.52.

Methylthiomethyl 2-Acetoxybenzoate (XVII)—Substitution of an equivalent quantity of aspirin for the salicylic acid used in the preparation of XIV and repetition of that described procedure afforded the desired product, bp 148–150°/1 mm; IR (neat): 3060, 2920, 1755, 1715, 1600, 1480, 1450, 1365, 1280, 1200, 1060, 920, 750, and 705 cm^{-1}; NMR ($CDCl_3$): δ 6.9–8.2 (m, 4H), 5.3 (s, 2H), 2.3 (s, 3H), and 2.2 (s, 3H) ppm; mass spectrum (70 ev): *m/z* (relative intensity) 240 (2), 198 (10), 163 (9), 121 (41), and 61 (100).

Anal.—Calc. for $C_{11}H_{12}O_4S$: C, 54.98; H, 5.04; S, 13.35. Found: C, 55.17; H, 5.15; S, 13.69.

Methylsulfinylmethyl 2-Acetoxybenzoate (XVIII)—The method described for the synthesis of XV was used, starting with 7.2 g (0.03 mole) of the methylthiomethyl ester, XVII. Recrystallization from tetrahydrofuran of the crude product resulted in pure XVIII in an 80% yield (6.1 g), mp 118–122°; IR (KBr): 3000, 1750, 1720, 1600, 1480, 1370, 1300, 1180, 1080, 920, 830, 755, and 705 cm^{-1}; NMR ($CDCl_3$): δ 7.0–8.2 (m, 4H), 5.2 (*AB*, 2H), 2.6 (s, 3H), and 2.4 (s, 3H) ppm; mass spectrum (70 ev): *m/z* (relative intensity) 214 (2), 163 (23), 121 (100), 93 (7), 92 (7), and 65 (7).

Anal.—Calc. for $C_{11}H_{12}O_5S \cdot 1/2H_2O$: C, 49.80; H, 4.94; S, 12.09. Found: C, 49.49; H, 4.59; S, 12.69.

Methylsulfonylmethyl 2-Acetoxybenzoate (XIX)—To a stirred chloroform solution of 7.2 g (0.03 mole) of methylthiomethyl 2-acetoxybenzoate (XVII) at 0° was added 12.0 g (0.06 mole) of *m*-chloroperbenzoic acid. The reaction mixture was worked up as for XVI as a white crystalline product, mp 151–153°; IR (KBr): 3010, 1750, 1725, 1600, 1480, 1300, 1245, 1185, 1120, 1090, 1000, 920, 760, and 710 cm^{-1}; NMR ($CDCl_3$): δ 7.0–8.2 (m, 4H), 5.3 (s, 2H), 3.0 (s, 3H), and 2.4 (s, 3H) ppm; mass spectrum (70 ev): *m/z* (relative intensity) 272 (<1), 230 (21), 163 (11), 121 (100), 93 (10), 92 (10), and 65 (11).

Anal.—Calc. for $C_{11}H_{12}O_6S$: C, 48.52, H, 4.44; S, 11.78. Found: C, 48.48; H, 4.43; S, 11.76.

***In Vivo* Studies**—Four purebred female beagle dogs, 1.5 years old, were used to determine the blood levels of various metabolites after oral and intravenous administration of aspirin (I) and the three aspirin derivatives (XVII–XIX). For oral administration, the compounds were dissolved in polyethylene glycol 400 and placed in gelatin capsules. Dimethyl sulfoxide solutions were used for the intravenous dosing. The doses were adjusted so that the dogs received equimolar doses of aspirin and the aspirin derivatives, both intravenously and orally. Each experiment was repeated at least once. Each dog was allowed to rest for a minimum of 1 week before it was used again.

Blood (2 ml) was withdrawn from each dog and acidified immediately by mixing with 2 ml of chilled 10% aqueous potassium bisulfate and 1 drop of potassium fluoride (~25 mg) in a 15-ml centrifuge tube. This process lowers the sample pH to ~1 and quenches enzymatic ester hydrolysis. Ether (7.0 ml) was added to each tube, and the samples were mixed on a mechanical shaker for 30 min and then centrifuged. A 5.0-ml portion of the supernate was removed and evaporated to dryness, and the residue was dissolved in 1.0 ml of acetonitrile. This solution then was analyzed by high-pressure liquid chromatography (HPLC). The recoveries (±*SE*) of aspirin (I) and salicylic acid (II) from whole blood were 97.8 ± 3.2 and 98.3 ± 2.7%, respectively. Aspirin hydrolysis during the entire workup was 5.4 ± 0.5% (*SE*).

Enzymatic Hydrolytic Cleavage of XIV–XIX and Aspirin in Human Plasma—The plasma[3] was stored in a refrigerator and used within 1 week from the date it was collected. A portion of 100 μl of a standard solution of the compound in acetonitrile was added to 10 ml of plasma at 37°. The plasma samples were extracted according to a literature method (26). Thus, 0.50-ml samples of plasma were withdrawn from the test medium, placed in 15-ml stoppered centrifuge tubes, and acidified with 0.50 ml of 10% potassium bisulfate and 1 drop of potassium fluoride (~25 mg). Chloroform (3 ml) was added to each tube and mixed on a mechanical shaker for 15 min.

The resulting emulsions were centrifuged, and 2.0 ml of the chloroform solution was recovered. These extracts were evaporated to dryness under continuous nitrogen flow, and the residue was dissolved in 0.50 ml of methanol. The methanolic solution was analyzed by HPLC. Plasma samples were also analyzed separately by injecting 5–10 μl of plasma directly into the chromatograph. Both methods gave the same results. In the plasma studies, the substrate concentration was kept low ($<10^{-3}$ *M*), and the rate constants were calculated as first order by following the disappearance of the compound with time.

Analytical Methods—An HPLC method was developed for studies of the complex degradation of 2-acetoxybenzoates because hydrolysis of XVII–XIX can lead to at least three possible hydrolytic products for each compound. The chromatographic analysis was performed on a component system[4]. A 30-cm × 3.9-mm i.d. column[5], operated at ambient temperature, was used for all separations. The mobile phase used for the separation of methylthiomethyl 2-acetoxybenzoate (XVII) and its degradation products consisted of acetonitrile–acetic acid/sodium acetate–water (450:10:540). The pH was adjusted to 3.86 with 25% aqueous NaOH. At a flow rate of 2.0 ml/min, the retention times of XVII, XIV, aspirin (I), and salicylic acid (II) were 6.2, 9.2, 2.2, and 2.0 min, respectively.

The mobile phase used for separation of methylsulfinylmethyl (XVIII) and methylsulfonylmethyl (XIX) 2-acetoxybenzoates and their degradation products consisted of acetonitrile–methanol–acetic acid/sodium acetate–water (140:115:5:740). The pH was adjusted to 3.86 with 25% aqueous NaOH. At a flow rate of 2.0 ml/min, the retention times of XVIII, XIX, XV, XVI, I, and II were 6.9, 9.5, 7.1, 10.1, 5.2 and 3.6 min, respectively. For the separation of the benzoate derivatives, 70% methanol in water was used as the mobile phase. The plot of the peak heights *versus* the various amounts of each derivative studied was linear in the concentration range studied. In the reaction mixture, the concentration of the compounds was determined by comparison of the peak heights for the compounds to the corresponding standard curves. When the biological extracts or fluids were analyzed, the column was protected by a 5-cm × 2-mm i.d. precolumn packed with CO:Pell ODS[6].

RESULTS AND DISCUSSION

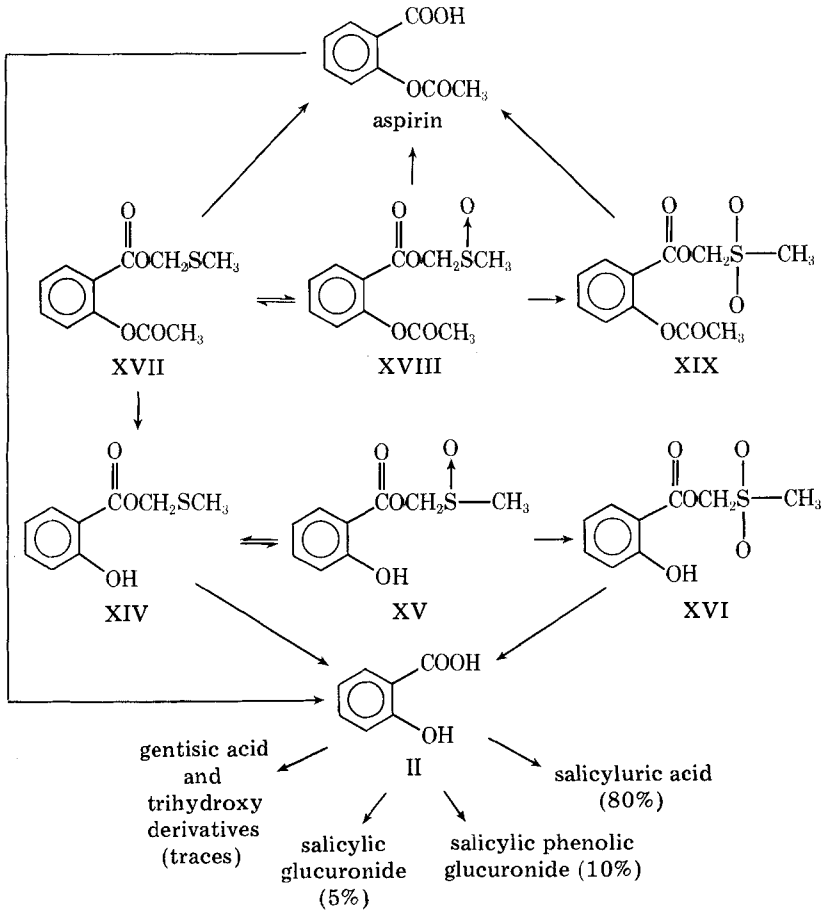
The prodrugs of aspirin (XVII–XIX) and those of salicylic acid (XIV–XVI) were well-defined, stable compounds and are easy to synthesize. As expected, the melting point of the compounds increases in the

[3] The human plasma was obtained from the Community Blood Center of Greater Kansas City and contained ~80% plasma diluted with anticoagulant citrate phosphate dextrose solution USP.

[4] Waters Associates model 600-A solvent delivery system, model U-6K injector, and model 440 dual-channel detector operated at 280 nm.

[5] Waters Associates μBondpak C_{18}.

[6] Whatman.



Scheme I—Possible metabolic routes of methylthiomethyl (XVII), methylsulfinylmethyl (XVIII), and methylsulfonylmethyl (XIX) 2-acetoxybenzoates (based on Refs. 25, 27, and 28 and results obtained in this work).

order of level of oxidation stage. Thus, while the methylthiomethyl derivative XVII is a liquid, XVIII has a melting point of 120° and XIX melts at 153°. The solubilities follow the reverse order since XVII is freely soluble in acetonitrile, methanol, and polyethylene glycol 400 (0.1 g/ml) while XIX is only sparingly soluble in methanol and acetonitrile and almost insoluble in polyethylene glycol.

Detailed physicochemical studies of the new esters, XII–XIX, were carried out after it was shown that enzymic cleavage of the aspirin prodrugs XVII–XIX results in significant amounts of aspirin.

The possible routes for the complex interconversion–metabolism of the prodrugs are shown in Scheme I. Thus, XVII, XVIII, and XIX theoretically could cleave to provide aspirin or the corresponding salicylates (XIV, XV, and XVI, respectively). Everything would finally result in salicylic acid, which undergoes the usual metabolism (25, 27, 28).

Alternatively, the thiomethyl ester(s) could undergo reversible oxidation to the sulfinyl and then to the corresponding sulfonyl esters.

Aspirin is hydrolyzed rapidly at 37° in human whole blood and plasma, with an average half-life of ~30 min and 1 hr, respectively (29). In the human body, aspirin is hydrolyzed even faster, with a half-life of 13–20 min (9) to form salicylic acid, which then is conjugated in part with glycine and glucuronic acid to form various acyl and phenolic conjugates (30). Several other minor metabolites also are formed. Free salicylic acid and its metabolites are eliminated from the body by renal excretion. For any derivative to be called a true aspirin prodrug, it has to be hydrolyzed enzymatically or nonenzymatically within the body to form aspirin. The rate of conversion must ensure buildup of the drug to a level capable of eliciting a pharmacological effect. Aspirin is much more active as an analgesic agent than salicylic acid, and aspirin formation in the body could be important for the analgesic activity of the compound (18).

Human plasma was used to determine the hydrolysis routes and rates of the compounds of main interest (XVII–XIX), as compared to the salicylates (XIV–XVI) and aspirin.

The hydrolysis rates in plasma of the 2-acetoxybenzoate esters (XVII–XIX) measured by following the disappearance of the compound with time and that of aspirin are listed in Table I. All three aryl esters have much shorter half-lives than that obtained for aspirin (~2 hr). Rowland *et al.* (9) studied aspirin hydrolysis in 90% volume fresh human blood and plasma at 37° and found the average half-life to be ~30 min in whole blood and slightly over 1 hr in plasma. In the present experiment,

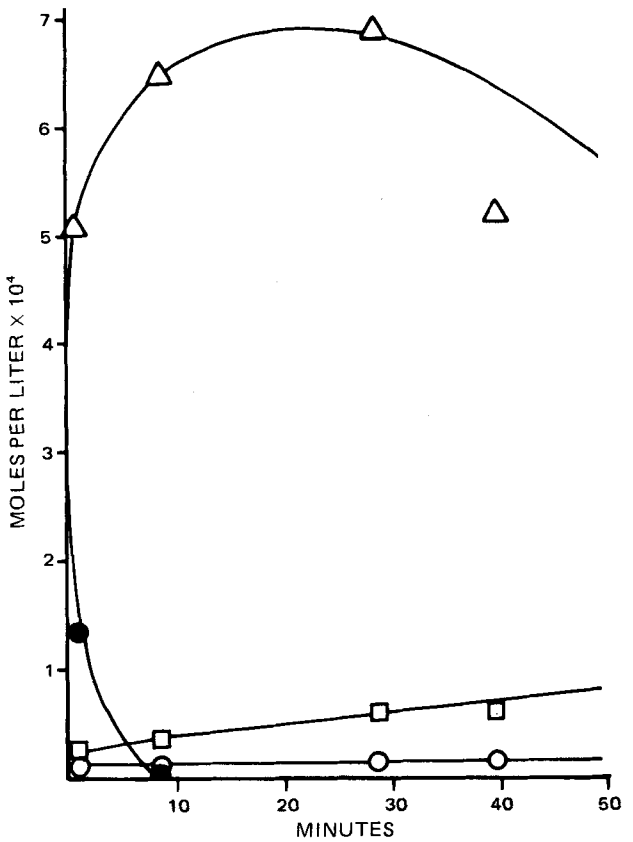


Figure 1—*Concentration–time profile for the hydrolytic cleavage of the aspirin derivative XVII (●) in human plasma* in vitro *at 37° and its hydrolytic products, the salicylate ester XIV (△), aspirin (□), and salicylic acid (○).*

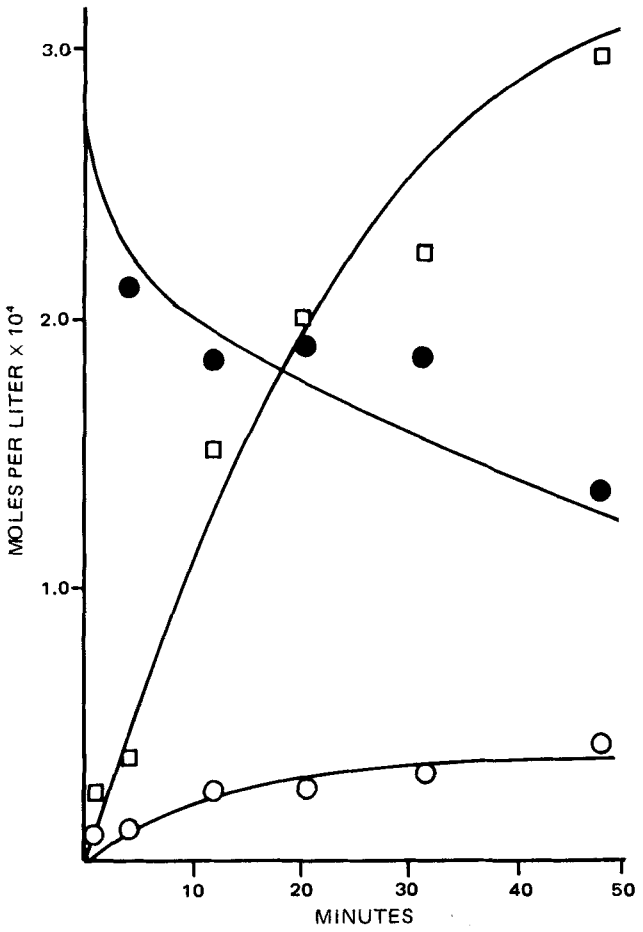


Figure 2—*Concentration–time profile for the hydrolytic cleavage of the ester XVIII (●) in human plasma* in vitro *at 37°. Aspirin (□) and salicylic acid (○) were forming.*

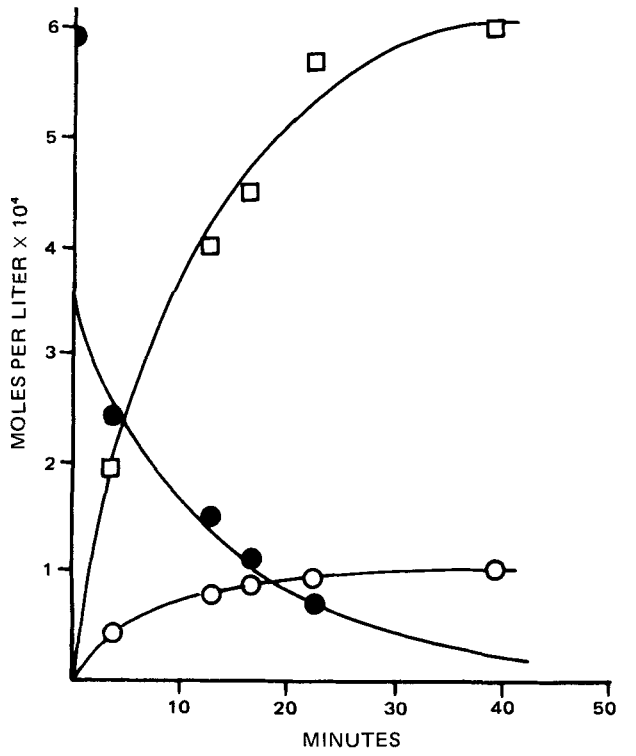


Figure 3—*Concentration–time profile for the hydrolysis of the aspirin derivative XIX (●) in human plasma* in vitro *at 37°. Aspirin (□) and salicylic acid (○) were the hydrolysis products.*

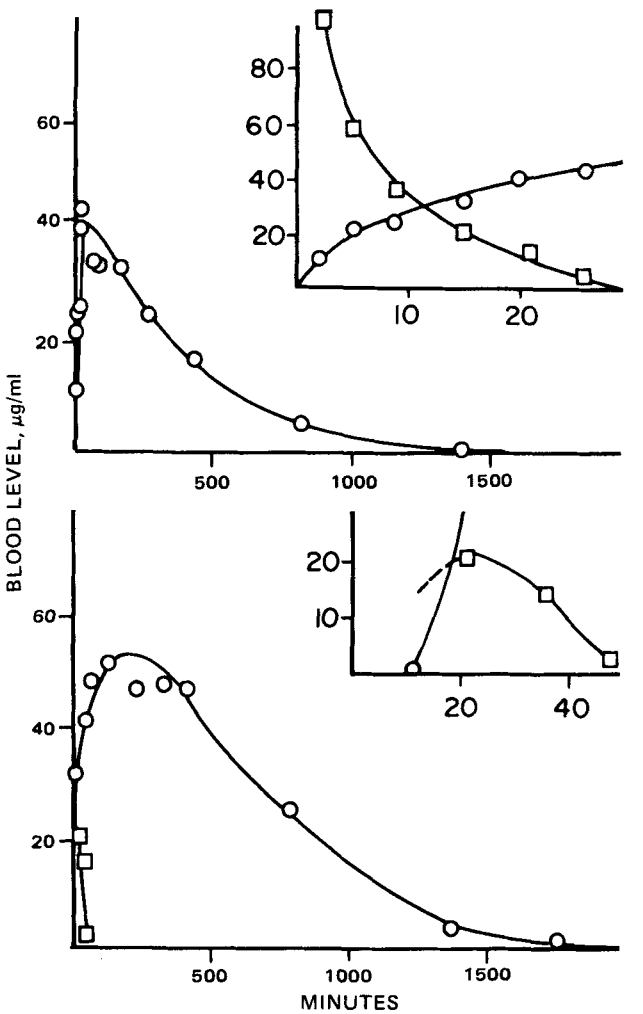


Figure 4—*Blood levels of aspirin (□) and salicylic acid (○) following an intravenous dose of 250 mg of aspirin (I) to an 8.4-kg dog (top) and an oral dose of 397 mg of I to an 11.6-kg dog (bottom).*

80% volume plasma was used under different conditions and the aspirin half-life was somewhat longer. A wide variation in hydrolysis rates of aspirin was seen in plasma from different individuals (31). The hydrolysis rates of the 2-hydroxybenzoates (XIV–XVI) are compared to that of methyl salicylate (XX) in Table II. The ester, XX, was selected as a model

Table I—Hydrolysis of Methylthiomethyl (XVII), Methylsulfinylmethyl (XVIII), and Methylsulfonylmethyl (XIX) 2-Acetoxybenzoates and Aspirin (I) in Two Batches of Human Plasma at 37.0°

Compound	k_{obs}[a], min^{-1}			$t_{1/2}$, min
	Batch I	Batch II	Average	
XVII	0.45	0.73	0.59	1.2
XVIII	5.2×10^{-2}	4.8×10^{-2}	5.0×10^{-2}	14
XIX	6.6×10^{-2}	8.1×10^{-2}	7.4×10^{-2}	9.4
I	—	6.0×10^{-3}	6.0×10^{-3}	1.2×10^{2}

[a] Based on disappearance of starting compound.

Table II—Hydrolysis Rates of the Salicylate Esters XIV, XV, and XVI and Methyl Salicylate (XX) in Human Plasma at 37°

Compound	k_{obs}, min^{-1}	$t_{1/2}$, min	k/k_m[a]
XX	1.4×10^{-3}	5.0×10^{2}	1.0
XIV	8.8×10^{-2}	79	6.3
XV	4.5×10^{-2}	15	32
XVI	9.4×10^{-2}	7.4	67

[a] Represents the relative hydrolysis rates of XIV, XV, and XVI compared to XX.

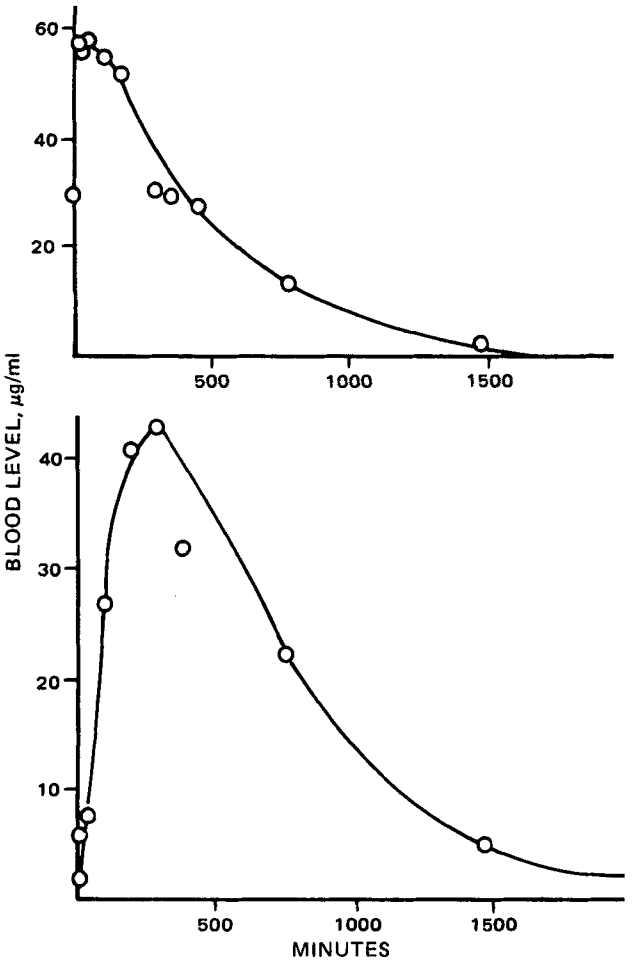


Figure 5—*Blood levels of salicylic acid (○) after administration of an intravenous dose of 302 mg of methylthiomethyl 2-acetoxybenzoate (XVII) to an 8.6-kg dog (top) and an oral dose of 570 mg of XVII to an 8.4-kg dog (bottom).*

for a salicylate. The k_{obs} values were determined following the disappearance of the starting materials.

All sulfur-containing "activated" esters (XIV–XVI) are hydrolyzed faster in plasma than the methyl 2-hydroxybenzoate (XX). Thus, the methylsulfonylmethyl ester (XVI) is hydrolyzed 67 times faster than the methyl ester.

The disappearance of the aspirin prodrugs and appearance of aspirin, salicylic acid, and the corresponding salicylates are shown in Figs. 1–3. The prodrugs XVIII and XIX result in significant aspirin concentrations, while XVII cleaves according to both pathways, one leading to the salicylate (XIV) and the other to aspirin. These results imply that XVIII and XIX are promising true prodrugs of aspirin since no formation of the salicylates XV and XVI, respectively, was observed, and the main hydrolysis product was aspirin. The delivery characteristics of XVII–XIX thus were determined *in vivo* using beagle dogs.

After oral administration of XVII, XVIII, XIX, or aspirin equivalent to 50 mg of aspirin/kg and intravenous administration of XVII, XVIII, XIX, or aspirin equivalent to 25 mg of aspirin/kg to dogs, the appearance in the blood of the compound and its possible metabolites was followed as a function of time. With XIX, only 8 mg/kg was administered intravenously to the dog because of its low solubility in the vehicle dimethyl sulfoxide. The appearance and disappearance of salicylic acid in the blood were fitted to an exponential equation $C_p = Ne^{-k_a t} + Le^{-\alpha t} + Me^{-\beta t}$ (32, 33). The blood aspirin concentration after oral and intravenous administration of aspirin also was fit to the exponential equation, where k_a was set equal to infinity after intravenous administration (34). The disappearance of XVIII in the blood after intravenous administration was fit to a simple first-order equation. The results are shown in Figs. 4–7.

The methylthiomethyl (XVII) and methylsulfonylmethyl (XIX) 2-acetoxybenzoates were metabolized rapidly in the body, resulting in peak blood salicylic acid levels within the first 10 min after an intravenous dose (Figs. 5 and 7). The first samples were withdrawn at 2 min after the intravenous injection. No aspirin, starting 2-acetoxybenzoate, or the possible intermediate 2-hydroxybenzoate esters was detected in these samples. After oral administration of XVII and XIX, only salicylic acid was detected. Methylsulfinylmethyl 2-acetoxybenzoate (XVIII) was metabolized more slowly, with a peak blood salicylic acid level at 30 min after an intravenous dose (Fig. 6), which is about the same time as obtained after intravenous aspirin (Fig. 5). Within 3–5 min after the intravenous administration, the aspirin formation rate was equal to the disappearance rate of XVII. However, after 7 min, no XVIII or aspirin was detected in the blood. After oral administration of XVIII, only salicylic acid was detected with a peak blood level at 300 min (Fig. 5).

All aspirin derivatives (XVII–XIX) apparently are metabolized mainly to salicylic acid.

Aspirin is hydrolyzed rapidly in the body to form salicylic acid. After intravenous aspirin, the plasma aspirin concentration *versus* time curve can be described by the biexponential equation $C_p = Ae^{-\alpha t} + Be^{-\beta t}$ (34). The half-life of the first exponent (α) is 2–5 min and that of β is 13–19 min. Therefore, aspirin disappears rapidly as it is formed from its prodrugs XVII–XIX and never reaches high blood levels. Salicylic acid was shown to be the only direct metabolite of aspirin (32). For the methylsulfinylmethyl 2-acetoxybenzoate (XVIII) to give such high aspirin levels in the body (Fig. 6), comparable to those obtained after an oral dose of aspirin (Fig. 4), a considerable fraction of the dose must be metabolized to form aspirin. Thus, at least XVIII is a true aspirin prodrug.

The analgesic efficacy of aspirin in the dog and the correlation between such analgesia and the plasma levels of aspirin and its primary metabolite, salicylic acid, were studied (35). It was concluded that the onset and duration of aspirin analgesia after oral and intravenous administration cannot be correlated with plasma aspirin levels and that, during analgesia, aspirin can only be detected in extremely low concentrations because of its rapid hydrolysis to salicylic acid. Therefore, although XVIII is the only

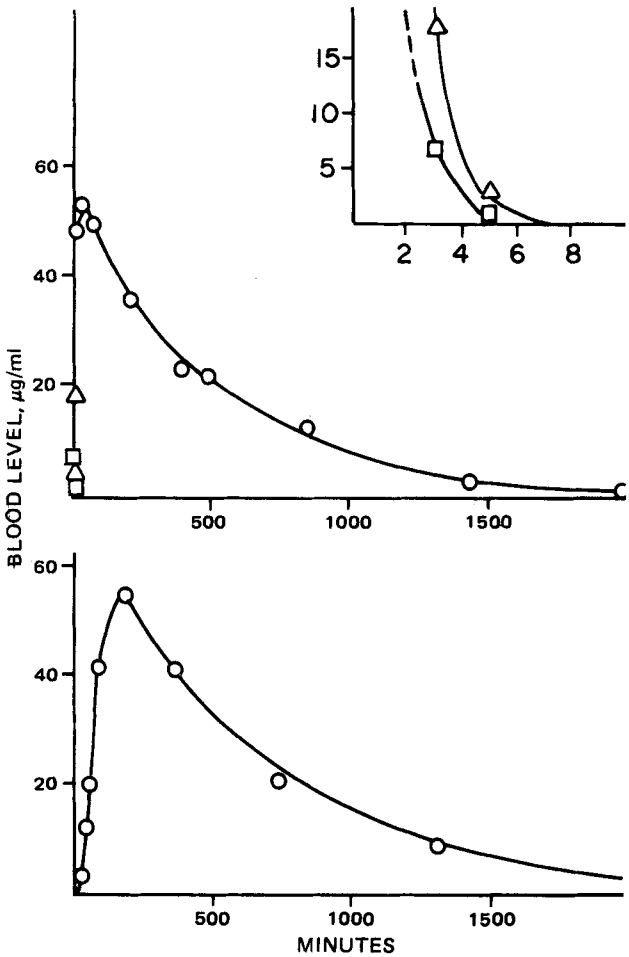


Figure 6—*Blood levels of methylsulfinylmethyl 2-acetoxybenzoate (XVIII) (△), aspirin (□), and salicylic acid (○) after administration of an intravenous dose of 370 mg of XVIII to a 9.2-kg dog (top) and an oral dose of 620 mg of XVIII to a 9.0-kg dog (bottom).*

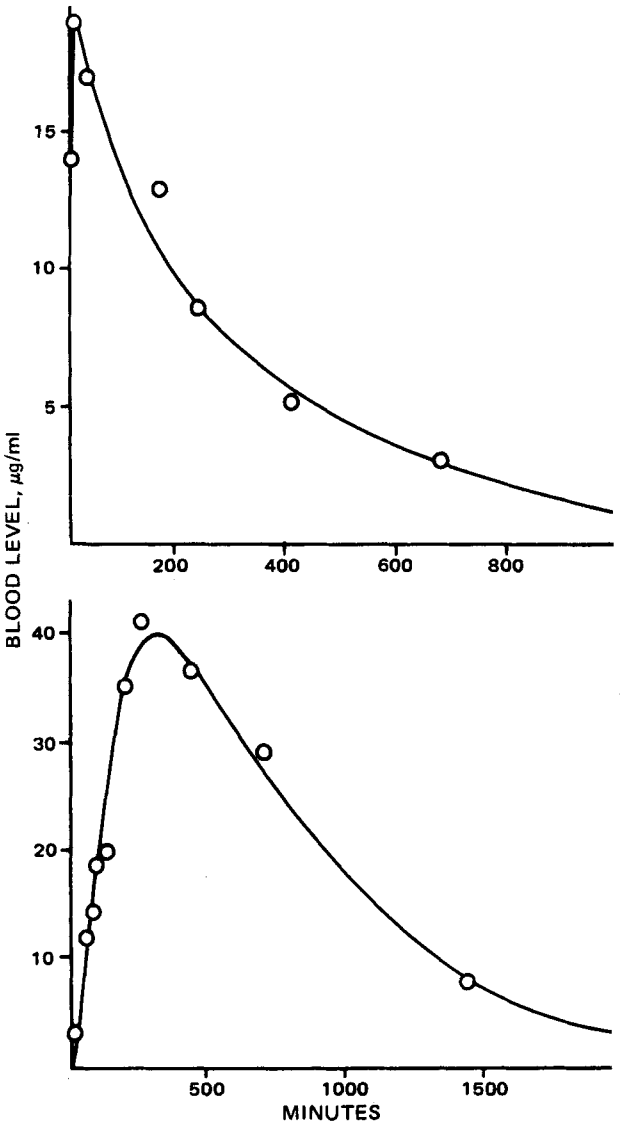


Figure 7—*Blood levels of salicylic acid (○) after administration of an intravenous dose of 115 mg of methylsulfonylmethyl 2-acetoxybenzoate (XIX) to a 9.2-kg dog (top) and an oral dose of 750 mg of XIX to a 9.2-kg dog (bottom).*

one of the three aspirin derivatives that gives detectable blood levels of aspirin in the dog, this finding does not necessarily mean that XVII and XIX are not useful prodrugs. This is particularly true since aspirin is metabolized about three times faster in dogs than in humans. Based on the human plasma cleavage data, XVII results mainly in the salicylate XIV. On the other hand, the methylsulfonylmethyl ester (XIX) has solubility problems. Thus, at least the methylsulfinylmethyl ester XVIII is a promising aspirin prodrug.

Similar prodrugs should be useful for improved delivery of a wide variety of acidic drugs, including the various known anti-inflammatory agents.

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