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Table I—Possible Changes in Physical Properties of Drugs with Free OH Groups

$\begin{array}{c} \text{O} \quad \text{R} \\ \parallel \quad \\ \text{D}-\text{O}-\text{C}-\text{CH}-\text{NHR}' \\ \text{drug} \quad \text{amino acid} \end{array}$			
Drug	Amino Acid	R'	Change in Physical Properties
Nonpolar	Polar-acidic-basic	H, COCH ₃	More polar-ionic
Polar	Nonpolar	COCH ₃	Less polar
Liquid at room temperature	Polar-nonpolar	H	To a salt
Low aqueous solubility	Acidic-basic	HCOCH ₃	To acid or base (salt)

This study attempted to make aspirin derivatives such that a stable suspension formulation could be made. A secondary goal was to reduce the local gastric irritation and bleeding associated with aspirin therapy.

This report describes the synthesis and some pharmaceutical properties of aspirin phenylalanine ethyl ester (I), aspirin phenylalanine amide (II), and aspirin phenyllactic ethyl ester (III).

THEORY

Prodrug Considerations—On the basis of the known specificities of the enzymes reviewed (1), a general rationale for modification of a drug's physical properties can be developed. Given that a drug has a free carboxyl, amino, or hydroxyl group, corresponding esters or amides of amino acids can be made so as to alter the physical properties in almost any desired direction from that of the parent drug, with one or more of the hydrolase enzymes serving as the *in vivo* reconversion site(s).

The diverse properties of the various amino acid residues, combined with the fact that they are usually nontoxic, gives this approach wide applicability. Classifying the amino acids as nonpolar, polar, acidic, and basic, a given drug molecule may be made more or less polar or more or less soluble in a given solvent. Its acid/base properties may be altered or completely changed (*i.e.*, converted from acidic to basic drug or vice versa), or it may be converted from a neutral compound to an ionic (acidic or basic) compound or from an ionic to a neutral compound. Thus, great flexibility is afforded the pharmaceutical scientist in modifying physical properties of drugs using this rationale.

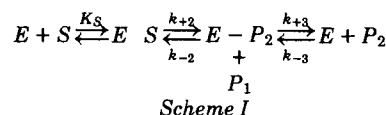
Tables I–III briefly summarize some of these possibilities. In general, for compounds in Tables I and II, α -chymotrypsin, trypsin, elastase, or an aminopeptidase could serve as a reconversion site. For compounds in Table III, a carboxypeptidase or an initial cleavage of the ester group by α -chymotrypsin–trypsin–elastase followed by a carboxypeptidase would effect the reconversion.

A recent example of a prodrug approach utilizing lipases as target sites for reconversion is the study by Baugess *et al.* (2). The acetate and dodecanoate esters of acetaminophen were shown to be substrates for lipases. The acetate ester was completely hydrolyzed in 15 min *in vitro*, and its pharmacokinetics in dogs were indistinguishable from those of the parent drug. Since the proteolytic enzymes α -chymotrypsin and

carboxypeptidase A are the target reconversion sites, their properties are briefly reviewed, followed by the aspirin derivatives selected.

α -Chymotrypsin— α -Chymotrypsin, an endopeptidase involved in protein digestion, has a molecular weight of ~25,000 g/mole. α -Chymotrypsin catalyzes the hydrolysis of a broad spectrum of peptides, amides, and esters. An absolute requirement of the substrate candidate is that the amino acid residue be of L-configuration. Substrates having an aromatic residue (*e.g.*, phenylalanine, tryptophan, and tyrosine) are hydrolyzed at appreciable rates (3), but the hydrolysis of substrates having hydrophobic residues (*e.g.*, leucine and methionine) is also catalyzed (4).

Kinetic studies have revealed a bell-shaped relationship between the overall reaction rate and pH, with maximum activity occurring near pH 8. The kinetic scheme shown in Scheme I seems to apply to an α -chymotrypsin-catalyzed hydrolysis.



The three reaction steps represent the formation of the noncovalent (Michaelis) enzyme–substrate complex, the formation of an acyl enzyme with the loss of the leaving group, and the deacylation step. For non-specific substrates with good leaving groups and for specific ester substrates, the deacylation step is rate limiting; for specific amides, the rate is determined by the acylation step.

Carboxypeptidase A—The primary action of carboxypeptidase A is that of a C-terminal exopeptidase. The enzyme is also a good esterase. Having a molecular weight of ~34,600 g/mole, the active enzyme consists of a single chain of 307 amino acid residues and one zinc ion. The amino acid sequence is known completely (5). The zinc ion has been shown to be essential for catalytic activity, even though its removal does not greatly affect the enzyme structure (6). There are two absolute requirements for potential carboxypeptidase A substrates (7, 8); the terminal carboxyl group must be free and the C-terminal residue must be of the L-configuration. The hydrolysis of substrates wherein R is a branched aliphatic or, optimally, an aromatic group is significantly favored.

Aspirin Derivatives—The chemical instability of aspirin was studied extensively (9–12). For present purposes, the pH of maximum stability is 2.5. The pH is below that usually observed for esters due to the presence of the carboxyl group, which functions as a general base catalyst and increases the reaction rate at pH 2.5–8. Aspirin, being a weak acid, also shows minimum solubility in acid. However, even suspensions of aspirin are not satisfactorily stable (12).

One approach to stabilizing aspirin is to esterify the carboxyl group. While this approach does improve stability, the esters (prodrugs) typically do not revert back to aspirin because the acetyl group is more labile than the ester group, with the resultant formation of salicylic acid esters (13). If the ester group were chemically more labile than the acetyl group, formulation instability would likely be a problem.

The aspirin derivatives in the present study (I–III) were chosen such that rapid biochemical reconversion in the intestinal tract would be affected. In each case, improved chemical stability is expected since the carboxyl group is masked. The esters and amides were chosen such that prodrug solubility would be somewhat less than that of the aspirin-free acid and further increase the stability of aspirin in suspension form. Other amino acid and ester groups could be chosen to obtain somewhat different physical properties if desired.

The biochemical reconversion of I–III is expected to be *via* an initial α -chymotrypsin hydrolysis of the ester or amide group followed by a

Table II—Possible Changes in Physical Properties of Drugs with Free NH₂ Groups

$\begin{array}{c} \text{O} \quad \text{R} \\ \parallel \quad \\ \text{D}-\text{NH}-\text{C}-\text{CH}-\text{NHR}' \\ \text{drug} \quad \text{amino acid} \end{array}$			
Drug	Amino Acid	R'	Change in Physical Properties
Weak base	Nonpolar	COCH ₃	To a neutral compound
Weak base	Acidic	COCH ₃	To an acidic compound
Zwitterionic	Polar-nonpolar	COCH ₃	To an ionic (acidic) compound
Weak base	Polar-nonpolar	H	Change in pKa

Table III—Possible Changes in Physical Properties of Drugs with Free COOH Groups

$\begin{array}{c} \text{O} \quad \text{R} \quad \text{O} \\ \parallel \quad \quad \parallel \\ \text{D}-\text{C}-\text{NH}-\text{CH}-\text{C}-\text{OR}' \\ \text{drug} \quad \text{amino acid} \end{array}$			
Drug	Amino Acid	R'	Change in Physical Properties
Weak acid	Polar-nonpolar	H	change in pKa
Weak acid	Basic	H	Zwitterionic compound
Weak acid	Basic	OC ₂ H ₅	To a basic compound
Zwitterionic	Polar/nonpolar	OC ₂ H ₅	To a basic compound

carboxypeptidase cleavage of the amide-releasing aspirin in the intestinal lumen or at the membrane wall (14). To the extent that the gastric irritation associated with oral dosing of aspirin is a local phenomenon (15–17), this approach may also reduce or eliminate the problem since aspirin would not be formed until the prodrug reached the intestine.

EXPERIMENTAL

Phenylalanine Ethyl Ester, Hydrochloride—Thionyl chloride (10 ml) was added to ethanol (40 ml), portionwise, in cold. Phenylalanine (2.3 g) was added to the mixture and refluxed for 2 hr. The reaction mixture was cooled, and the solvent was evaporated under reduced pressure. The flaky solid residue was washed with cold anhydrous ether and was then purified by selective precipitation from the ethanol–ether mixture, mp 154–156°, yield 2.9 g.

Aspirin Phenylalanine Ethyl Ester (I)—Aspirin¹ (1.8 g) was dissolved in 35 ml of dry methylene chloride and dicyclohexylcarbodiimide² (1 g) was added to it at room temperature. The mixture was stirred for 2 hr, and the precipitated dicyclohexylurea was filtered off.

To the filtrate was added a mixture of phenylalanine ethyl ester hydrochloride (1.15 g, 5 mmoles) and triethylamine (0.5 g, 5 mmoles) dissolved in 10 ml of methylene chloride. The mixture was stirred overnight at room temperature and the solvent was removed under reduced pressure. Dry ethyl acetate (20 ml) was added to the residue. A white precipitate appeared, which was filtered and found to be dicyclohexylurea. The filtrate was washed three times with 10% sodium bicarbonate and three times with water to make it free from alkali and then dried over anhydrous magnesium sulfate. The dried extract was filtered, and the solvent was removed under reduced pressure.

The residue was dissolved in ethanol, and water was added until turbidity appeared. It was then kept in the freezer overnight. The oily precipitate solidified and was filtered and further purified by selective precipitation from ethanol–water mixtures, mp 72–74°, yield 1.6 g. The product gave a single spot on TLC. Structure was confirmed by NMR³ and mass spectrum ($M^+ = 356$); NMR (90 MHz, $CDCl_3$): δ 1.35 (t, 3H, CH_3 of ethyl ester), 2.1 (s, 3H, $OCOCH_3$ of aspirin), 3.27 (d, 2H, aromatic CH_2 of phenylalanine), 4.2 (q, 2H, $COOCH_2$ of ethyl ester), 5.05 (m, 1H, CH of phenylalanine group), 7.1–7.65 (m, 7H, aromatic H), and 8.05 (d, 1H, aromatic H proton next to CONH group of aspirin nucleus).

Phenylalanine Amide—To a solution of L-phenylalanine (0.83 g) in water–dioxane (1:1, 8 ml) and triethylamine (1.05 ml, 7.5 mM) was added 2-*tert*-butoxycarbonyloxyamino-2-phenylacetone nitrile¹ (1.36 g) at room temperature. The solution was stirred overnight at room temperature. After 15 ml of water was added to the reaction mixture, it was washed twice with 15-ml portions of ethyl acetate; the ethyl acetate washings were discarded. The aqueous layer was acidified to pH 2.0 by 5% citric acid solution and saturated with sodium chloride. It was then extracted with three 20-ml portions of ethyl acetate, and the combined ethyl acetate extracts were washed three times with saturated sodium chloride solution to make the organic extract acid free. The ethyl acetate extract was dried over anhydrous magnesium sulfate and filtered, and the solvent was removed under reduced pressure to obtain an oily residue of *tert*-butoxycarbonylphenylalanine, yield 1.2 g.

Ethyl chloroformate was added dropwise over 10 min at -10° to a well-stirred solution of *tert*-butoxycarbonylphenylalanine and triethylamine in dry tetrahydrofuran. The mixture was stirred for 30 min at -10° and then saturated with dry ammonia. The reaction mixture was stirred overnight at room temperature, the solvent was evaporated under reduced pressure, and the residue was stirred with 20 ml of ice-cold water, filtered, and dried at 40–60°. It was then treated with 4 *N* hydrochloric acid–dioxane for 30 min at room temperature. The solvent was removed to obtain a solid residue of L-phenylalanine amide hydrochloride.

Aspirin Phenylalanine Amide (II)—To a solution of 1.8 g of aspirin in 35 ml of dry methylene chloride was added 1.1 g of dicyclohexylcarbodiimide. The reaction mixture was stirred for 2 hr at room temperature, and a white precipitate of dicyclohexylurea was formed and removed by filtration. To the filtrate was added a mixture of 5 mM L-phenylalanine amide (1.1 g) and 5 mM triethylamine (0.7 ml) in 15 ml of methylene chloride. The reaction mixture was stirred overnight at room temperature, and the solvent was removed under reduced pressure. The residue was dissolved in chloroform and washed once with water, three times with

15-ml portions of 10% sodium bicarbonate, and three times with 10-ml portions of 2 *N* HCl, followed by a final washing with water to make it acid free.

The organic extract was evaporated to dryness under reduced pressure, and the residue was washed with ether, filtered, and recrystallized from chloroform, mp 176–178°, yield 1.4 g. The structure was identified by NMR and mass spectrum ($M^+ = 327$). The product gave a single spot on TLC; NMR (90 MHz, $CDCl_3$): δ 2.2 (s, 3H, $OCOCH_3$ of aspirin), 3.15 (d, 2H, aromatic CH_2 of phenylalanine), 4.92 (m, 1H, CH of phenylalanine group), 7.05–7.65 (m, 7H, aromatic H), and 7.85 (d, 1H, aromatic H proton next to CONH group of aspirin nucleus).

Aspirin Phenyllactic Ethyl Ester (III)—A solution of 0.4 g of aspirin chloride dissolved in 10 ml of tetrahydrofuran was chilled to 5°; to it was added a cooled solution of L-phenyllactic acid (0.33 g) in 4 ml of tetrahydrofuran. Aliquots of pyridine totaling 0.5 ml were added to the reaction mixture over 5 min, and the system was stirred at 5° for 1 hr. After an additional hour of stirring at room temperature, the solvent was removed on a rotary evaporator and the clear colorless oil was dissolved in chloroform. The chloroform solution was extracted with water, dried over anhydrous magnesium sulfate, and evaporated under reduced pressure to yield a colorless oil, which was purified by selective precipitation from chloroform by *n*-hexane. The oil did not solidify. Its purity was checked by TLC, and NMR and mass spectrometry were used to confirm its identity as L-phenyllactic acid ester of aspirin.

Aspirin phenyllactic acid (0.23 g) was dissolved in dry methylene chloride, and the solution was chilled to 0°. To it was added magnesium ethoxide–dried ethanol (0.35 ml) followed by dicyclohexylcarbodiimide (0.13 g, 0.7 mmole). After stirring at 0° for 1 hr, it was stirred for an additional 1 hr at room temperature. The solvent was removed under reduced pressure to obtain a clear colorless oil, which was further purified by selective precipitation from ethanol by water. The oil could not be solidified. TLC showed only one spot, the NMR and mass spectrometry ($M^+ = 355$) confirmed it to be III; NMR (90 MHz, $CDCl_3$): δ 1.3 (t, 3H, CH_3 of ethyl ester), 2.1 (s, 3H, $OCOCH_3$ of aspirin), 3.27 (d, 2H, aromatic CH_2 of phenylalanine), 4.2 (q, 2H, $COOCH_2$ of ethyl ester), 5.45 (m, 1H, CH of phenylalanine group), 7.05–7.65 (m, 7H, aromatic H), and 7.95 (d, 1H, aromatic H proton next to COO group of aspirin nucleus).

Aspirin Phenylalanine—Compound I (60 mg) was dissolved in 10 ml of ethanol and added to 100 ml of a pH 7.5 phosphate buffer solution containing α -chymotrypsin (100 mg). It was mixed well, allowed to stand for ~2 min, and then transferred to a 250-ml separator. It was then acidified with 2 *N* HCl to pH 1.0 and extracted with 100 ml of chloroform. A very thick emulsion resulted on shaking, and the layer had to be separated by centrifugation at 2000 rpm for 20 min.

The upper aqueous layer was aspirated out, the lower layer was dried over anhydrous magnesium sulfate, and the solvent was removed in a rotary evaporator to obtain a clear, colorless, oily residue, which was further purified by selective precipitation from ethanol with water. NMR and mass spectrometry ($M^+ = 328$) confirmed it to be aspirin phenylalanine; NMR (90 MHz, $CDCl_3$): δ 2.1 (s, 3H, $OCOCH_3$ of aspirin), 3.27 (d, 2H, aromatic CH_2 of phenylalanine), 5.05 (m, 1H, CH of phenylalanine group), 7.0–7.65 (m, 7H, aromatic H), and 7.85 (d, 1H, aromatic H proton next to CONH group of aspirin nucleus).

TLC Systems—To follow the hydrolysis of aspirin phenylalanine ethyl ester qualitatively and to identify the products, a TLC system was sought that would separate the reactants and products. Of several solvent systems tried, methanol–acetic acid–ether–benzene (1:18:60:20) was able to resolve aspirin and salicylic acid (which appears as a dense blue spot under short wavelength UV). The support was silica gel with UV₂₅₄ fluorescent background. This solvent system could not differentiate between aspirin and I. However, cyclohexane–chloroform acetic acid (4:5:1) was able to distinguish between aspirin and I but not between aspirin and salicylic acid.

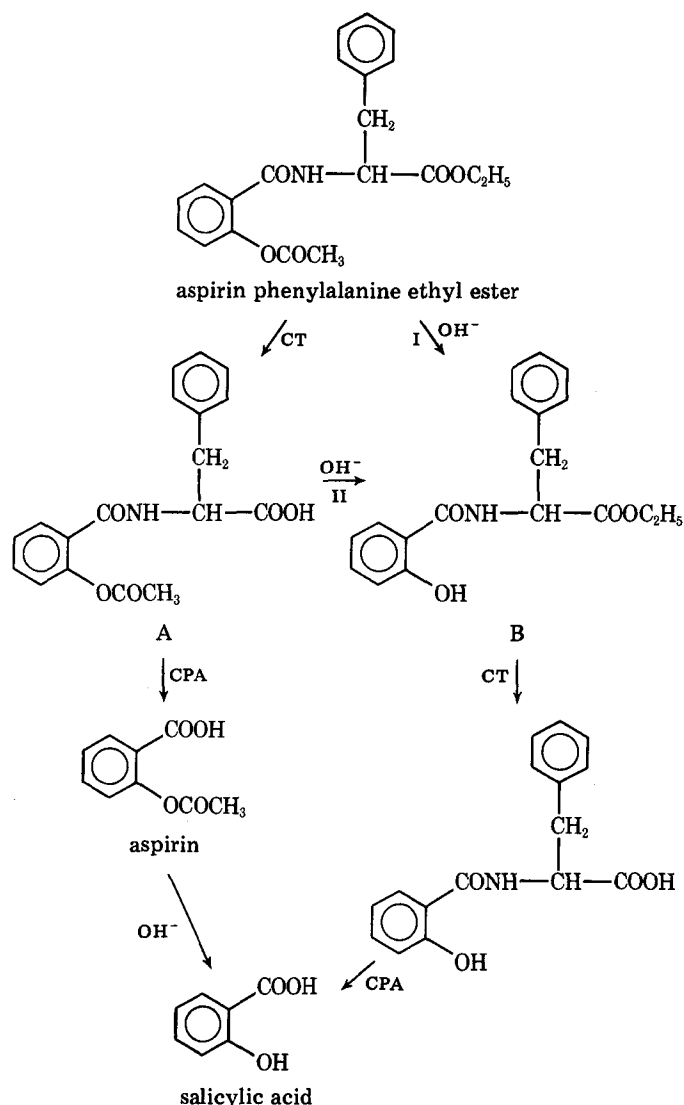
TLC plates were run in both solvents simultaneously to allow identification of all the degradation products of I in simulated intestinal fluid. All chloroform extracts were spotted on silica gel plates, 0.25 mm thick, and developed for 15 cm in both solvents. The spots were detected under a short wavelength UV detector.

Shelflife Studies of I—Compound I (5 mg) was dissolved in 1 ml of ethanol and transferred quantitatively to a 100-ml volumetric flask, and the volume was made up with 0.066 *M* phosphate buffer of pH 7.5, 6.6, or 5.6. Aliquots (5 ml) were withdrawn at timed intervals, taken in 15-ml centrifuge tubes, and extracted with 5-ml portions of chloroform. The aqueous layer was removed by aspiration. Two 0.5-ml portions of the chloroform extracts were placed in 10-ml volumetric flasks, and the solvent was evaporated with a gentle nitrogen stream. To Flask A was added 0.5 ml of ethanol and 1 ml of 5 *N* NaOH. The mixture was heated in a

¹ Monsanto Co.

² Aldrich Chemical Co.

³ Melting points were determined in capillaries and are uncorrected. NMR spectra were recorded with a Varian E-390 spectrometer with tetramethylsilane as the internal standard. All structures were confirmed by NMR and mass spectra.



Scheme II—Possible hydrolytic pathways of aspirin phenylalanine in presence of chymotrypsin (CT) and carboxypeptidase A (CPA) mixture.

boiling water bath for 45 min and cooled to room temperature, and the volume was made up with distilled water. To Flask B was added 0.5 ml of ethanol, and the volume was made up with distilled water. Fluorescence of both the samples was determined at excitation/emission wavelengths of 328/400 nm.

Scheme II shows that of all possible degradation products, only I and salicylic acid phenylalanine ethyl ester (being nonionic) are extractable in chloroform. Salicylic acid phenylalanine ethyl ester has the same excitation and emission maxima as salicylic acid. Therefore, by subtracting the fluorescence of B from A, the fluorescence corresponding to salicylic acid generated by the alkali digestion of I was obtained. A standard so-

Table IV—Enzyme Kinetic Parameters for Aspirin Derivatives^a

	α -Chymotrypsin		Carboxypeptidase	
	K_m , mole/liter	K_{cat} , sec ⁻¹	K_m , mole/liter	K_{cat} , sec ⁻¹
Aspirin phenylalanine ethyl ester (I)	1×10^{-6}	2.91	1.3×10^{-4b}	8.5×10^{-2b}
Aspirin phenyllactic ethyl ester (III)	2.5×10^{-5}	3.7×10^{-3}	1×10^{-4}	25
Aspirin phenylalanine amide (II)	5.1×10^{-4}	5×10^{-3}	1.3×10^{-4}	8.5×10^{-2}

^a References 18 and 19. ^b Done at pH 8.5.

Table V—Estimated Range of *In Vivo* Reconversion Rates ($S_0 = 10^{-5} M$)

Structure	E_0 , M	pH	$t_{1/2}$	
			Maximal Rate (Zero Order), $v = k_{cat}E_0$	Minimal Rate (First Order), $v = (k_{cat}/K_m)E_0S$
X = NH R = C ₂ H ₅	10^{-4}	8.5	5.9 sec	16.8 sec
X = O R = C ₂ H ₅	10^{-4}	7.5	13.5 sec	61.9 sec
X = NH R = C ₂ H ₅	10^{-6}	8.5	10 min	28 min
X = O R = C ₂ H ₅	10^{-6}	7.5	22.5 min	103 min

lution of I carried through the described procedure served as the standard.

Solubility Determination—Compound I (~300 mg) was stirred with 100 ml of distilled water at $25.00 \pm 0.02^\circ$. Aliquots of the solution were withdrawn until no change in concentration was observed. Equilibrium was reached in 20 hr. After the aliquots were diluted 10 times, 1 ml was placed in a 10-ml volumetric flask; 1 ml of 5 N NaOH was added, and the solution was heated in a boiling water bath for 45 min. After cooling, the solution was diluted to volume with distilled water, and fluorescence measurements were taken at excitation/emission wavelengths of 328/400 nm, using a $10^{-6} M$ solution of I, taken through all the described steps, as a standard. The solubility was determined to be $4.7 \times 10^{-4} M$.

RESULTS AND DISCUSSION

Summary of Enzyme Kinetic Results—The detailed enzyme kinetic studies were reported previously (15, 16), and only the most significant results are summarized here. Of the aspirin derivatives studied, I was the best substrate for α -chymotrypsin while aspirin phenyllactic acid was the best substrate for carboxypeptidase. The kinetic parameters are given in Table IV. The values for the kinetic parameters are consistent with values for other well-known substrates of these enzymes. Table V shows the aspirin reconversion process for the ethyl esters. Given the kinetic results in Table IV, the α -chymotrypsin hydrolysis step would be rate limiting for III while the carboxypeptidase hydrolysis step would be rate limiting for I. Both steps were slow for the amide derivative.

The very approximate estimates of the *in vitro* reconversion half-lives in Table V are based on the kinetic parameters for the appropriate rate-limiting step. Whether such estimates reflect the *in vivo* situation is not yet known. However, the results indicate that regeneration of aspirin *in vivo* is at least possible. Compound I was selected for further studies. This choice was based in part on the rapid first step of the reconversion. Since all derivatives had relatively low aqueous solubilities by design, the rapid hydrolysis of the ester, to produce an acid, would increase the dissolution rate of the ethyl ester and perhaps give better *in vivo* performance.

Hydrolytic Pathway of I—Scheme II shows the possible hydrolytic pathways for I. Figure 1 shows the TLC results on a solution containing the prodrug and α -chymotrypsin and carboxypeptidase. The results

Table VI—Kinetic Rate Constants and Shelflife of I in Aqueous Solutions

pH	$k_{obs} \times 10^3$, hr ⁻¹	t_{90} , hr
7.5	9.84	10.8
6.6	2.77	38.3
5.6	1.92	55.2

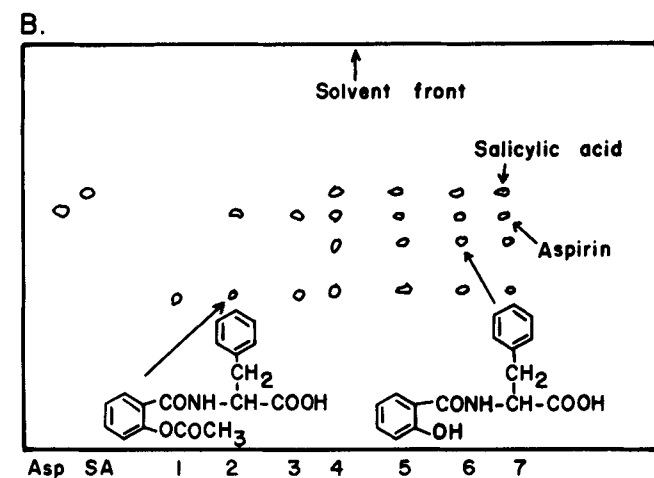
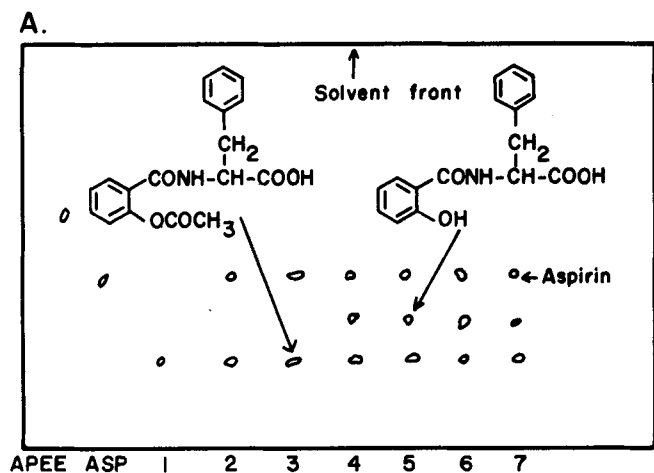


Figure 1.—Thin-layer chromatogram of the chloroform extract of a mixture of I, α -chymotrypsin (50 mg/100 ml), and carboxypeptidase A (25 mg/100 ml) in pH 7.5 phosphate buffer. The support was silica gel with fluorescent indicator UV₂₅₄. The solvents were cyclohexane-benzene-acetic acid (4:5:1) (A) and methanol-acetic acid-ether-benzene (1:18:60:20) (B). Key: spot 1, 1 min; spot 2, 2 min; spot 3, 5 min; spot 4, 10 min; spot 5, 20 min; spot 6, 45 min; and spot 7, 2 hr. APEE = aspirin phenylalanine ethyl ester; ASP = aspirin; and SA = salicylic acid.

indicate that the ethyl ester group is lost immediately and that aspirin phenylalanine appears. With time, aspirin appears, followed by salicylic acid and the phenylalanine derivative of salicylic acid. While more detailed kinetic studies are needed, these results demonstrate that aspirin is regenerated in solution in the presence of the enzymes.

Shelflife Studies of I in Aqueous Solutions—Logarithms of concentrations of I remaining in the aqueous solutions at various pH values were plotted against time. Reactions were followed up to 10% degradation.

From the slopes of these plots, the pseudo-first-order rate constant, k_{obs} , was determined. The pseudo-first-order rate constants at several pH values and the corresponding shelflives are given in Table VI.

In case of suspension, the degradation rate is given by:

$$\frac{dc'}{dt} = k_1 s \quad (\text{Eq. 1})$$

where c' is the concentration of the drug in solution (mass/volume), k_1 is the first-order rate constant, and s is the solubility of the drug expressed in the same unit as c' . For v milliliters of suspension:

$$v \frac{dc'}{dt} = \frac{dc}{dt} (\text{mass/time}) = -k_1 v s = -k_0 \quad (\text{Eq. 2})$$

where k_0 is the zero-order rate constant for the drug in suspension.

The solubility of I was $4.7 \times 10^{-4} M$ (i.e., 0.167 mg/ml) compared to that of $1.85 \times 10^{-2} M$ (i.e., 3.33 mg/ml) of aspirin. The calculated shelflife of a 600 mg/5 ml of suspension of I (i.e., an equivalent of 300 mg/5 ml of aspirin) was found to be 4.27 years at pH 5.6 compared to 38 days for 300 mg/5 ml of aspirin suspension at its optimum pH (2.25) for stability.

REFERENCES

- (1) G. L. Amidon, R. S. Pearlman, and G. D. Leesman, in "Design of Biopharmaceutical Properties through Prodrugs and Analogs," E. B. Roche, Ed., American Pharmaceutical Association, Washington D.C., 1977.
- (2) C. T. Baugess, J. H. Fincher, F. Sadik, and C. W. Hartman, *J. Pharm. Sci.*, **64**, 1489 (1975).
- (3) L. Cunningham, *Comp. Biochem.*, **16**, 85 (1965).
- (4) M. Rovey, M. Poilroux, A. Yoshida, and P. Desnuelle, *Biochim. Biophys. Acta*, **23**, 608 (1957).
- (5) K. S. V. Sampath Kumar, J. B. Cleag, and K. A. Walsh, *Biochemistry*, **3**, 1728 (1964).
- (6) J. H. Rupley and H. Neurath, *J. Biol. Chem.*, **235**, 609 (1960).
- (7) H. Neurath, in "The Enzymes," 2nd ed., vol. IV, Academic, New York, N.Y., 1950, p. 11.
- (8) H. Neurath and G. W. Schwert, *Chem. Rev.*, **46**, 69 (1950).
- (9) L. J. Edwards, *Trans. Faraday Soc.*, **46**, 723 (1950).
- (10) *Ibid.*, 696 (1952).
- (11) E. R. Garrett, *J. Am. Chem. Soc.*, **79**, 3401 (1957).
- (12) K. A. Connors, G. L. Amidon, and L. Kennon, "Chemical Stability of Pharmaceuticals," Wiley, New York, N.Y., 1979, p. 151.
- (13) T. S. Pierre and W. P. Jencks, *J. Am. Chem. Soc.*, **90**, 3817 (1968).
- (14) G. L. Amidon, G. D. Leesman, and R. L. Elliott, *J. Pharm. Sci.*, **69**, 1363 (1980).
- (15) J. P. Leonards and G. Levy, *ibid.*, **59**, 1511 (1970).
- (16) K. W. Anderson, *Arch. Int. Pharmacodyn. Ther.*, **152**, 379 (1964).
- (17) C. Davison, D. H. Hertig, and R. DeVine, *Clin. Pharmacol. Ther.*, **7**, 329 (1966).
- (18) P. K. Banerjee and G. L. Amidon, *J. Pharm. Sci.*, **70**, 1304 (1981).
- (19) *Ibid.*, **70**, 1307 (1981).

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