Amino Acid Esters of Phenolic Drugs as Potentially Useful Prodrugs

Keyphrases □ Phenolic drugs—use of amino acid esters as prodrugs □ Amino acid esters of phenolic drugs—use as prodrugs □ Prodrugs—consideration of amino acid esters of phenolic drugs

To the Editor:

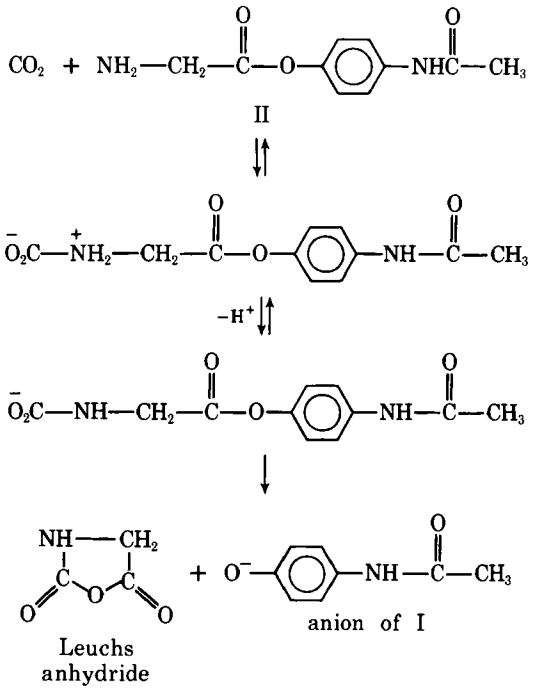
It is sometimes desirable to make temporary chemical modifications to drug molecules for the purpose of increasing their chemical stability, of changing their solubility or rate of dissolution, or of modifying their distribution within the body. If such chemically modified drugs are transformed to the parent drug before they elicit a pharmacological response, they are referred to as prodrugs (1).

It is frequently desirable to formulate phenolic drugs as prodrugs, because many of them have low water solubility as neutral molecules and they are all subject to oxidative degradations *in vitro* and conjugative metabolism *in vivo*. Amino acid esters of phenolic drugs appear to be attractive candidates for consideration as prodrugs for the following reasons:

1. Prodrugs with either higher or lower water solubility can be made following appropriate selection of the amino acid. For example, the aqueous solubilities at 25° of acetaminophen (*p*-acetamidophenol) (I), the hydrobromide salt of its glycine ester (II), and the hydrochloride salt of its β-aspartic acid ester (III) were calculated to be 0.10, 0.48, and 0.023 *M*, respectively.

2. Considerable differences in the stability against hydrolysis have been found for different amino acid esters of a particular phenol. Hence, the $t_{1/2}$ values (half-lives) for hydrolysis of II and III and for the α-aspartic acid ester of I (IV) in water at pH 7.0 and 25° were 49, 155, and 2.65 min, respectively. The $t_{1/2}$ values at pH 2.0 were 1850, 2850, and 76 min, respectively. These half-lives were observed when no general acids or bases or other catalytic species were present in the solution.

3. The hydrolyses of amino acid esters of phenols are likely to be catalyzed by general bases and nucleophilic species; even in the absence of any enzyme-facilitated hydrolysis, the hydrolyses are likely to be rapid within the body to yield the phenolic parent drug. The hydrolysis of the ester II was subject to general base and nucleophilic catalysis, and the catalytic rate constants (assuming that the rate-determining step of the reaction involves one molecule of the protonated form of the ester and one molecule of general base or nucleophilic species) were calculated to be $2.04 \times 10^{-3}\ M^{-1}\ min^{-1}$ for formate ion, $5.80 \times 10^{-3}\ M^{-1}\ min^{-1}$ for acetate ion, $120 \times 10^{-3}\ M^{-1}\ min^{-1}$ for dihydrogen phosphate ion, and $4120 \times 10^{-3}\ M^{-1}\ min^{-1}$ for tromethamine.



Scheme I

The rate laws for hydrolysis in glycine and tromethamine buffers also included a term that was second order in the amine. In addition, the rates of hydrolysis were dramatically catalyzed by dissolved carbon dioxide. Whereas the $t_{1/2}$ value for the hydrolysis of II in a $5 \times 10^{-2}\ M$ tromethamine buffer (pH 7.44) at 25° was 7.7 min, the $t_{1/2}$ value in a $2 \times 10^{-3}\ M$ solution of sodium bicarbonate in the same buffer (pH 7.44) was 4.9 min—the reaction rate constant had increased by more than 50%. Blood and other body fluids contain similar or larger concentrations of dissolved carbonic acid species (2). At pH 7.44, 6.75, and 6.00, the apparent catalytic rate constants for the total concentration of carbonic acid species (H_2CO_3, HCO_3^-, and CO_3^{-2}) were 23.07, 7.32, and 0.96 $M^{-1}\ min^{-1}$, respectively. These data can best be rationalized by postulating that carbon dioxide catalyzes the reactions *via* the sequence of reactions shown in Scheme I.

This reaction sequence is similar to one postulated by Hay and Main (3) to account for the effects of carbon dioxide on the hydrolysis of α-amino acid esters of *p*-nitrophenols. The only difference is that whereas Hay and Main concluded that the formation of the carbamate was the sole rate-determining step for hydrolysis of *p*-nitrophenol esters, the reactions of II can best be rationalized by concluding that both this step and the step that results in the formation of the Leuchs anhydride are rate determining.

The rate of hydrolysis of II was significantly increased over what was found in water at 25° when the solution temperature was increased to 37° and rabbit blood plasma was added to the solution. In a 3×10^{-2} *M* solution of tromethamine (pH 7.40) to which 1 and 3% blood plasma had been added, the $t_{1/2}$ values were

14 and 4.9 min, respectively, at 37°. Catalysis in these solutions is probably the result of a combination of general base, nucleophilic, carbon dioxide, and even some enzyme catalysis. Therefore, it is expected that many amino acid esters of phenolic drugs would be rapidly tranformed to the drug under *in vivo* conditions.

4. Many amino acids are normal dietary constituents or are substances with little toxicity. Thus, the compound formed along with the drug when the prodrug is hydrolyzed is unlikely to cause toxic reactions.

Detailed results of a kinetic study of the reactions of II, III, and IV in aqueous solutions together with some mechanistic considerations will be published subsequently.

(1) A. Albert, "Selective Toxicity," Wiley, New York, N.Y., 1965.

(2) "Biology Data Book," P. L. Altman and D. S. Dittmer, Eds., Federation of American Societies for Experimental Biology, Washington, D.C., 1964

(3) R. W. Hay and L. Main, *Aust. J. Chem.*, **21**, 155(1968).

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Phenol Formation from Alkylparabens by Bacteria

Keyphrases ◻ Phenol—formation from alkylparabens by bacteria ◻ Parabens, alkyl—role in formation of phenol by bacteria ◻ Preservatives—phenol formation from alkylparabens by bacteria

To the Editor:

Alkylparabens are widely used as preservatives for various pharmaceutical preparations such as injections, solutions, emulsions, and suspensions. They are also used in cosmetic preparations containing various fats and oils susceptible to microbial attack. Alkylparabens are also involved in gelatin preparation from animal sources to prevent proliferation of contaminated bacteria.

When a paste of gelatin containing alkylparabens was incubated at room temperature, we found that phenol formation was followed by the liquefaction of gelatin.

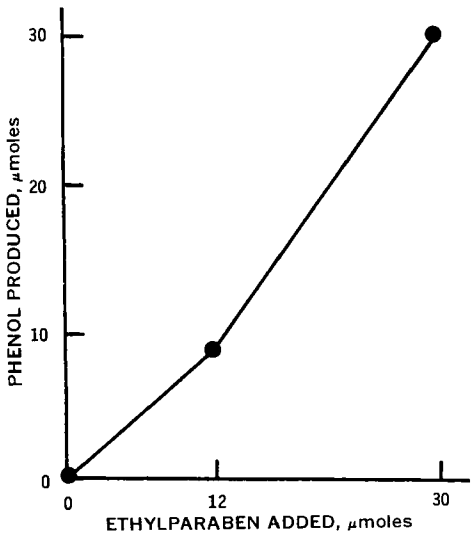

Figure 1—*Effects of various levels of ethylparaben on phenol formation, using resting cells as the enzyme source. Reaction mixtures contained ethylparaben and 7.6 × 10^{10} cells in a final volume of 12 ml. The mixed cell suspension containing strains 3, 4, and 5 was added. Reaction mixtures were incubated at 37° without shaking for about 110 hr. Viable cell counts in the reaction mixtures after incubation at the lowest and highest levels were estimated as 2.9 × 10^9 and 1.7 × 10^9, respectively. Each experiment was carried out with two flasks.*

With the advancement of the Good Manufacturing Practices, microbial contamination, even in nonsterile drugs in either final products or raw materials, has become of concern for the quality control of pharmaceutical manufacturing processes. This communication is concerned with the finding that alkylparabens are decomposed to phenol by the cooperative action of two different bacteria.

Three microorganisms were isolated from the pharmaceutical manufacturing processes using gelatin; they were identified as *Klebsiella aerogenes* (strain 3), *Pseudomonas aeruginosa* (strain 4), and *Pseudomonas aeruginosa* (strain 5). They are able to grow in a gelatin paste containing alkylparabens.

Each microorganism was grown on soybean casein digest agar slant medium (1) for 18 hr at 37° without shaking. The grown cells were harvested aseptically by centrifugation, washed once with sterile water, and suspended in sterile water. The cell suspensions thus prepared were kept in a refrigerator until use and were used for the following experiments.

Counting of viable cells was carried out by means of the plate method, using soybean casein digest agar as the test medium as described in USP XVIII (1). Various alkylparabens[1] and *p*-hydroxybenzoic acid[2] were obtained commercially. Other reagents used were of the best quality commercially available. TLC[3] was carried out according to the methods described by Sadamatsu *et al.* (2). Phenol was determined by literature methods (3, 4).

[1] Wako Pure Chemicals Co.
[2] Tokyo Kasei Co.
[3] DC Fertigplatten Kiesel gel G 60 F 254 from Merck was used.