Reaction of Aspirin with Amines. Potential Mechanism for Aspirin Allergy

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A possible explanation for the lack of hypersensitivity to salicylic acid by patients known to be allergic to aspirin is offered. It is shown that while aspirin reacts with amino acids to produce salicylamide derivatives, salicylic acid does not. This may be the mechanism by which aspirin combines with protein to form antigen.

IT IS NOT yet clear whether hypersensitivity to aspirin is immunologic in origin or is a direct effect of aspirin on tissues which is not mediated by an antigen–antibody reaction. On the hypothesis that an allergic reaction is involved, several studies have been made of the immunogenicity of synthetic aspirin–protein conjugates in animals (1–4). These conjugates were prepared by treatment of protein with acetylsalicyl chloride or azide. In each case it was shown that antibodies to the aspiryl or salicyl hapten were produced, but attempts to demonstrate the presence of these antibodies in humans known to be aspirin hypersensitive were not successful.

One remarkable characteristic of aspirin hypersensitivity is the fact that individuals who react to aspirin rarely do so with salicylate (5). If it is assumed that aspirin hypersensitivity is mediated by an antigen–antibody reaction, then the antigen must be formed *in vivo* by coupling of aspirin to protein. One explanation of the lack of reactivity to salicylate may be that salicylate is unable to react with protein to form the antigenic conjugate as does aspirin.

The anhydride nature of aspirin has been noted by Davidson and Auerbach (6) who found that aspirin reacted in pyridine at 100° with *p*-toluidine to produce a small yield (0.13%) of *N*-salicyloyl-*p*-toluidide. Salicylic acid itself could not react in the same way. Surprisingly little attention has been paid to the reactions of aspirin with nucleophiles in aqueous media. Troup and Mitchner (7) have found acetylated phenylephrine resulting from reaction of aspirin with the latter in tablet formulations and Jacobs *et al.* (8) recently reported a similar reaction between codeine and aspirin. There has been no demonstration of any salicyloyl derivative being formed in these reactions.

The present work reports the results of experiments in model systems where a deliberate search was made for salicyloyl derivatives in the reaction of aspirin with amino acids.

EXPERIMENTAL

Preparation of N-Salicyloyl Derivatives of Amino Acids.—To a solution of 0.03 mole of the amino acid in 25 ml. of water was added 3.2 Gm. (0.08 mole) sodium hydroxide and 4.4 Gm. (0.02 mole) phenyl salicylate and the mixture was refluxed 2–4 hr. (until the phenyl salicylate had dissolved). The mixture was cooled, acidified, and the precipitate collected and recrystallized from an appropriate solvent. Details are listed in Table I.

Preparation of N-Acetyl Derivatives of Amino Acids.—In 4 ml. of glacial acetic acid was suspended 1.5–2.0 Gm. of the amino acid and 2.0 ml. acetic anhydride was added. The mixture was heated gently until all the amino acid had dissolved and on cooling the *N*-acetyl derivatives crystallized. Details of each compound are listed in Table I.

For both the salicyloyl and acetyl derivatives, infrared spectra showed those bands expected.

Products of Reaction of Aspirin with Amino Acids.—*ε-Aminocaproic Acid.*—Six and one-half grams of ε-aminocaproic acid was mixed with one equivalent of sodium hydroxide and 1.8 Gm. aspirin was added. The reaction was allowed to proceed 15 min. at 40° and then acidified to pH 2, the precipitate filtered off, and the filtrate evaporated to dryness. The residue was extracted with benzene and the benzene removed under reduced pressure. The resulting residue was dissolved in chloroform and subjected to thin-layer chromatography.

Glycine.—The reaction was carried out exactly as above except that methanol was substituted for benzene and acetone for chloroform.

Thin-Layer Chromatography.—Separation of the products of reaction of aspirin with glycine and ε-aminocaproic acid was accomplished by thin-layer chromatography on Silica Gel G using either benzene–acetic acid–water, 2:2:1 (I) or 4:8:3 (II), as the developing solvent. The spots were detected by exposing the plates to iodine vapor, and the R_f values are given in Table II. As a control, the experiment with glycine was repeated substituting salicylic acid for aspirin.

RESULTS AND DISCUSSION

The thin-layer chromatograms of the extracts from workup of the reaction mixtures are shown in Fig. 1. It is quite clear from these plates that the *N*-salicyloyl derivatives of both glycine and ε-aminocaproic acid are formed in the reactions of aspirin with the respective amino acids. This is the first demonstration of a salicylamide derivative resulting from reaction of aspirin with an amine. The amount formed is quite small. From the size of the spots on the thin-layer plates, it is estimated that only about 0.01–0.1% of the aspirin reacted by this route. Nevertheless, these results show the feasibility of reaction of aspirin with amino groups on proteins to produce conjugates which may be antigenic. When salicylic acid was substituted for aspirin, no salicyluric acid could be detected in the reaction mixture. The over-all reaction taking place may be depicted as in Scheme I. Direct nucleophilic attack by amine will result in aminolysis of the ester producing salicylic acid and an acet-

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TABLE I.—DETAILS FOR EACH COMPOUND

Compd.	Solvent for Recrystallization	M.p. Found	M.p. Lit.
N-Salicyloyl glycine	$CHCl_3$–ethyl acetate, 3:1	162–164	170.2 (164)[a]
N-Salicyloyl-ε-amino-caproic acid	$CHCl_3$–CCl_4	105–108	...
N-Acetylglycine	...	205–207	206–208[b]
N-Acetyl-ε-aminocaproic acid	$CHCl_3$	97–99	...

[a] Heilbron, I., "Dictionary of Organic Compounds," vol. 2, Oxford University Press, New York, N. Y., 1953, p. 775. [b] *Ibid.* vol. 1, p. 206.

TABLE II.—R_f VALUES FOR COMPOUNDS

Compd.	R_f Value in Solvent System I	R_f Value in Solvent System II
Salicylic acid	0.47	0.60
N-Salicyloyl glycine	...	0.16
N-Salicyloyl-ε-aminocaproic acid	0.23	...
N-Acetylglycine	...	0
N-Acetyl-ε-aminocaproic acid	0	...

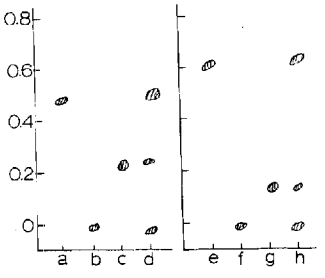
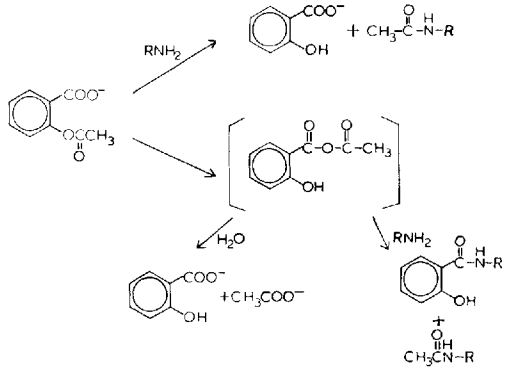


Fig. 1.—Diagram of TLC plates utilized in identification of reaction products. Key: a, salicylic acid; b, *N*-acetyl-ε-aminocaproic acid; c, *N*-salicyloyl-ε-aminocaproic acid; d, extract of products of reaction of aspirin with ε-aminocaproic acid; e, salicylic acid; f, *N*-acetylglycine; g, *N*-salicyloyl glycine; h, extract of products of reaction of aspirin with glycine.

amide derivative. Competing with this reaction is the intramolecular hydrolysis of aspirin which proceeds through an anhydride of acetic and salicylic acids (9). It seems likely that in the presence of amine both acetyl and salicyloyl derivatives might be formed depending on the relative electrophilicity of the respective carbonyl carbons. In the presence of H_2O^{18} it was found that aspirin hydrolysis produced salicylic acid containing 6% of the O^{18} which had taken part (10). Of course the amine competes with water as the nucleophile, and as a result only a very small proportion of the



Reaction of Aspirin with Amines
Scheme I

original aspirin would become salicylamide derivative.

If salicyloyl–protein is indeed proved to be the antigenic determinant in hypersensitivity to aspirin in humans, then it can be intimated that such compounds as salicyl–salicylic acid and aspirin anhydride would be potentially allergenic drugs. The product of reaction of the former, as can be seen by analogy with Scheme I, would be a salicyloyl amide by both pathways. Aspirin anhydride is quite labile (11) and probably subject to aminolysis to produce acetyl–salicyloyl amide derivatives.

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