Clarke (3). Indeed, some colors produced by other drugs (*e.g.*, ephedrine, amphetamine, and methamphetamine) may be identified by trained eyes, but untrained personnel should be warned of the possibility of misidentification, particularly when other extraneous material and colors are added to the drug as is the case with street drugs. As Clarke stated:

> *"The color given in any test depends on the quantity of material used and on its purity, and may be described differently by different individuals. To allow for this, the following lists must be used in as wide a sense as possible."*

Therefore, it is up to the worker to exercise his or her judgment in utilizing such color tests for the primary screening of these compounds.

Clarke's compilation of the colors produced with the Marquis reagent did not include ephedrine (3).

Cobalt Thiocyanate—Table IV in the earlier paper (2) included 14 compounds that gave a blue flaky precipitate with cobalt thiocyanate. Rorke *et al.* (1) suggested the use of stannous chloride to enhance the specificity of the test by differentiating between cocaine and procaine and between benzocaine and diphenhydramine; however, they did not mention the behavior of the other compounds listed in the table or of the many other compounds not included. They also failed to consider that many street samples contain mixtures of cocaine and procaine, which make this test worthless or, at best, very difficult to interpret.

The addition of stannous chloride to the blue flaky precipitate formed by such a mixture results in partial dissolution of the precipitate. This partial dissolution is very difficult to observe, and the mixture may be misidentified as cocaine. Rorke *et al.* (1) also failed to mention that the blue precipitate formed by methadone dissolves only partially in stannous chloride, which adds to the possibility of erroneous interpretation.

Therefore, it is our opinion that the use of stannous chloride does not add to the specificity of this test and may lead to erroneous results. For these reasons, it was not included in the scheme described in the original paper (1).

Zwikker's Test—The original paper (2) is in disagreement with Rorke *et al.* (1) and Clarke (3). We repeated the experiment using the reagents described and amobarbital, phenobarbital sodium, and secobarbital as reference standards. At the concentration of 2 mg of the barbiturates used, an instant blue-violet color developed, which was found to be very stable. At this concentration, no blue-violet color was developed with glutethimide. Instead, a yellow color developed, which changed upon standing to a gray color. However, very high concentrations of glutethimide, *i.e.*, more than 10 mg, produced a similar blue-violet color, which disappeared almost instantaneously and faded to a gray color within a few seconds. Therefore, we stand firmly by the results reported earlier (1).

In conclusion, we feel that this exchange with Rorke *et al.* (1) has clarified a number of points, which are examples of the many scientific variables between laboratories due to human differences and differences in methodology. This exchange has also demonstrated, as was pointed out previously (2), that these tests only provide preliminary information to help in the selection of the necessary confirmatory tests such as TLC and GLC. They also aid in the selection of the compound or compounds that are to be used as reference standards for the final identification by TLC or GLC.

Rorke *et al.* (1) pointed out the importance of such tests for chemists defending their results in the courts. We disagree with them in this statement. Results of spot tests should never be used as evidence in the courts.

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Asaad N. Masoud ˣ
School of Pharmacy
Creighton University
Omaha, NE 68178

Donald J. Nittskoff
Analytical Laboratories of Ohio
Cleveland, OH 44110

Received December 19, 1975.
Accepted for publication February 26, 1976.
ˣ To whom inquiries should be directed.

Intramolecular and Intermolecular Transformations of Aspirin in Nonhydroxylic Solvents

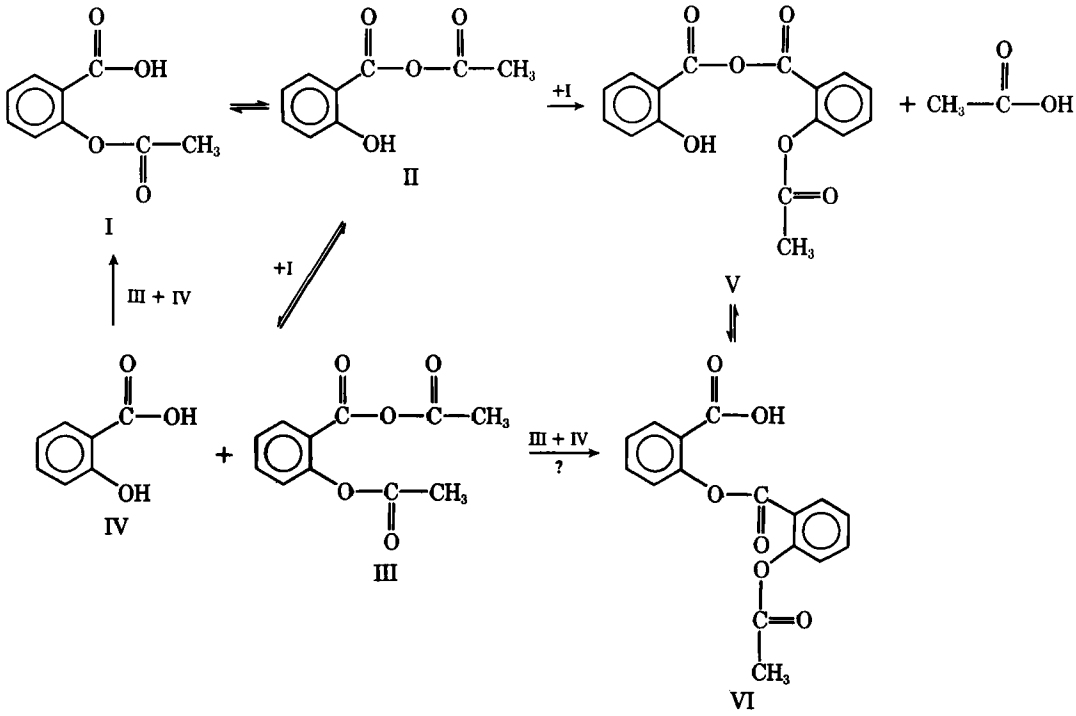
Keyphrases □ Aspirin—intra- and intermolecular transformations in nonhydroxylic solvents, reaction mechanisms □ Transformations, intra- and intermolecular—acetylsalicylic acid in nonhydroxylic solvents, reaction mechanisms

To the Editor:

Much evidence has been presented (1–3) to suggest that the hydrolysis of ionized acetylsalicylic acid (aspirin) involves intramolecular general-base catalysis by the carboxylate anion and not, as previously supposed (4–8), an intramolecular nucleophilic catalysis with a kinetically significant intermediary formation of the mixed anhydride of salicylic and acetic acids. There seems, however, to be an equilibrium between acetylsalicylate and the anion of the mixed salicylic acetic anhydride (3, 9). But since the anhydride reverts to the starting ester much faster than it is hydrolyzed, the nucleophilic pathway is not a feasible hydrolytic route.

The hydrolysis of the ester group in acetylsalicylic acid is also catalyzed by the unionized carboxyl group (2, 10). In this case, nucleophilic catalysis may be involved because of a more favorable equilibrium constant for the formation of the protonated form rather than the ionized one of the mixed anhydride intermediate (10).

We have observed, and now wish to report, novel and unusual reactions of acetylsalicylic acid occurring in solutions of the acid in nonhydroxylic solvents.



Scheme I

Acetylsalicylic acid (I, Scheme I) dissolved in various nonhydroxylic solvents (*e.g.*, benzene, ether, chloroform, ethyl acetate, acetone, and acetonitrile) was found to convert into several products. In alcohols such as methanol and propanol, I remained unchanged. This preliminary report presents only results obtained in benzene solutions.

On heating a solution of I in benzene (2–20 mg/ml) at 60°, an equilibrium between I and the mixed acetylsalicylic acetic anhydride (III) and salicylic acid (IV) was established after a few hours at concentrations corresponding to 74, 13, and 13 mole %, respectively, of the initial I concentration. At constant temperature, the rates of the reversible formation of both III and IV followed first-order kinetics for periods corresponding to two to three half-lives. Besides being formed in equimolar amounts, III and IV were produced at identical rates. The mixed anhydride III was determined quantitatively by an anhydride assay (11) after prior removal of the acid products (I and IV) by extraction with an aqueous phosphate buffer solution of pH 8 (11). Salicylic acid (IV) was determined either by direct spectrophotometric measurement at 320 nm (where I and IV do not interfere) after appropriate dilution of the reaction solution with benzene or by spectrophotometric analysis of an aqueous buffer (pH 7.4)-extracted reaction solution [this procedure, which is similar to that described by Gore *et al.* (12), was also used to determine undegraded I].

The identification of III was accomplished by IR (chloroform) and NMR (deuterochloroform) spectroscopy as well as by a quantitative UV determination of the amount of IV released by alkaline hydrolysis. In addition, by using a modification of the anhydride assay (11), which allows a selective determination of both an anhydride moiety and a reactive ester group like that in I (to be reported later), the compound was shown to contain equimolar amounts of anhydride and ester groups. On reaction with glycine in aqueous solution at pH 9.5, the anhydride produced *N*-salicyloylglycine in a yield of 25%.

We previously (13) thought, as have others (14), that the anhydride formed in benzene solutions of I was acetylsalicylic anhydride, but this is not correct. On prolonged standing of the solutions, however, a disproportionation of III to the symmetrical acetylsalicylic anhydride (and acetic anhydride) was observed to take place.

On further standing of the benzene solutions of I, a product shown to be acetylsalicylsalicylic acid (VI) was formed in considerable amounts. An approximate first-order half-life of 23 hr was observed at 60°. The product reached a maximal concentration corresponding to a net conversion of 58% of I. The identity of VI was established by comparison with authentic acetylsalicylsalicylic acid (15) by TLC using several developing systems, by GLC (16), and by alkaline hydrolysis to give first salicylsalicylic acid (a reaction used for spectrophotometric quantitation of VI) and then IV (15). Although more stable than I, VI, once formed, readily transformed as observed by means of TLC.

Although the reactions of I to give III and IV followed first-order kinetics, the observed rate constants associated with the production of either III or IV depended on the initial concentration of I (Fig. 1). However, this apparent paradoxical feature becomes understandable when it is borne in mind that the total change of the concentration of I is relatively small (26% of I is transformed). The pronounced saturation effect of the plot in Fig. 1 is indicative of the

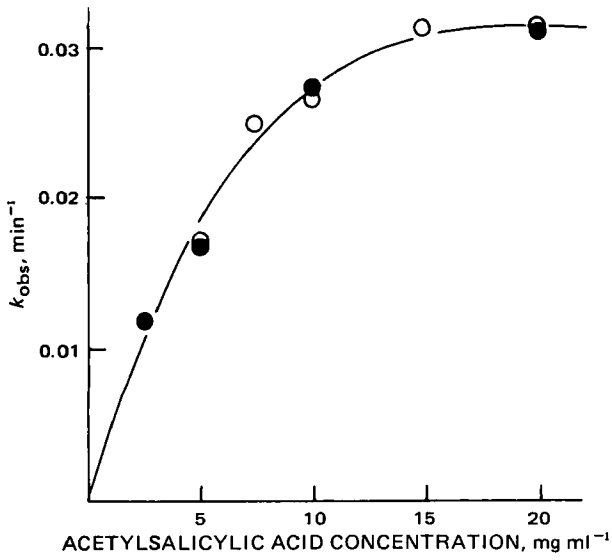


Figure 1—*Plot of observed first-order rate constants for formation of salicylic acid (●) and mixed acetylsalicylic acetic anhydride (○) at 60° in benzene solutions of acetylsalicylic acid as a function of the initial concentration of acetylsalicylic acid.*

presence of a metastable intermediate in the reactions and of a change in the rate-determining step with increasing concentrations of I; *i.e.*, the formation of the intermediate becomes rate limiting at the higher concentrations of I.

We interpret these results to mean that I, through an intramolecular addition of the carboxyl group to the ester carbonyl moiety, is converted into the mixed salicylic acetic anhydride (II), which then either returns to I or reacts at the anhydride moiety with the carboxyl group of a second molecule of I to produce III and IV (with attack on the acetyl carbonyl moiety of the anhydride) or to produce V (with attack on the salicyloyl carbonyl moiety) which, in turn, rearranges into VI (like II to I, Scheme I). Admittedly, other pathways can be imagined to lead to the formation of III, IV, and VI (*e.g.*, a reaction between two molecules of II), but it seems inevitable to postulate an intermediate formation of a mixed salicylic acetic anhydride (II). The *para*-substituted analog of I, *p*-acetoxybenzoic acid, completely fails to undergo any degradation in the present conditions.

A catalysis of all reactions shown in Scheme I was observed by triethylamine and also by benzene-insoluble materials such as magnesium hydroxide and magnesium carbonate. This finding apparently suggests that the ionized acetylsalicylate undergoes reactions *via* the anhydride II in nonhydroxylic solvents. A similar suggestion was advanced previously (17, 18).

The observations described may possibly contribute to an understanding of the mechanism of catalysis of reactions of I and related compounds in aqueous as well as in nonaqueous solutions [*cf.*, the recent paper by Kömives *et al.* (19) on aminolysis of I in acetonitrile]. Since preliminary experiments indicated that similar transformations of I can occur in the solid state at elevated temperatures, the results may become of relevance for the assessment of the stability of I formulations. This assessment has so far mostly been based on the conviction that IV and acetic acid are the only products of degradation (20).

As far as allergy to I is concerned, the formation of even small amounts of the immunogenic acetylsalicylsalicylic acid and acetylsalicylic anhydride should be avoided (21, 22). On the basis of the ready ability of the mixed acetylsalicylic acetic anhydride to salicyloylate amino groups as described here, this compound may most likely be as immunogenic as acetylsalicylsalicylic acid and acetylsalicylic anhydride.

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Hans Bundgaard ˣ
Claus Larsen
Department of Pharmaceutics
Royal Danish School of Pharmacy
2 Universitetsparken
DK-2100 Copenhagen
Denmark

Received December 22, 1975.
Accepted for publication February 9, 1976.
ˣ To whom inquiries should be directed.

Two New Diterpenes from *Stemodia maritima* L.

Keyphrases □ Diterpenes—isolated from aboveground portion of *Stemodia maritima*, PMR, IR, mass, and circular dichroism spectral identification □ *Stemodia maritima*—aboveground portion extracted, diterpenes isolated and identified □ Maritimol—isolated from *Stemodia maritima*, PMR, IR, mass, and circular dichroism spectral identification □ Stemodinol—isolated from *Stemodia maritima*, PMR, IR, mass, and circular dichroism spectral identification

To the Editor:

An investigation of the aboveground portion of