Nonsolvolytic Acetylation of Homatropine by Aspirin

E. SHAMI[▲], J. DUDZINSKI, L. LACHMAN, and J. TINGSTAD*

Abstract □ A nonsolvolytic (solid-state) reaction between aspirin and homatropine, yielding acetylhomatropine and free salicylic acid, is reported. A GC assay for homatropine is described. In the presence of excess aspirin, the kinetics are pseudo first order with respect to homatropine. The apparent heat of activation is estimated and a possible mechanism of the reaction is discussed.

Keyphrases □ Homatropine—kinetics and possible mechanism of nonsolvolytic (solid-state) acetylation by aspirin □ Aspirin—kinetics and possible mechanism of nonsolvolytic (solid-state) acetylation of homatropine □ Acetylhomatropine and salicylic acid—products of solid-state acetylation of homatropine by aspirin, kinetics □ Acetylation, nonsolvolytic (solid state)—reaction of aspirin and homatropine □ Solid-state (nonsolvolytic) acetylation reactions—aspirin and homatropine

Compared to the literature on aqueous solutions, there are relatively few publications on kinetics and mechanisms of reactions in the solid state. The nonsolvolytic (solid-state) acetylation of drugs containing alcoholic, aminic, or phenolic groups by aspirin has been reported (1–3). This article describes the kinetics and possible mechanism of a nonsolvolytic reaction between aspirin and homatropine, yielding acetylhomatropine and free salicylic acid.

EXPERIMENTAL

Aspirin USP (containing less than 0.05% free salicylic acid) was used. The homatropine base employed assayed 100.3% by nonaqueous titration. The two drugs were mixed in a 12:1 (aspirin–homatropine) molar ratio and were allowed to react at the desired temperatures. The 12:1 ratio was chosen because of assay convenience and because it would simplify reaction kinetics by eliminating aspirin concentration as a significant factor (4). It was felt that this experimental procedure was valid because the ratio of the two in the tablet formulation under study was even greater than 12:1.

Homatropine Assay—A gas chromatograph[1] utilizing a flame-ionization detector was used with a 1.2-m. (4-ft.) × 3-mm. i.d. glass column, using Diatoport S (80–100 mesh) coated with UC-W-98 silicone rubber (3.8%). The column temperature was 215°, and both the detector temperature and the injection port temperature were 270°. Gas flow rates were: helium, 75 ml./min.; hydrogen, 35 ml./min.; and air, 350 ml./min. Codeine was used as the internal standard.

About 1.4 g. of the aspirin–homatropine mixture was accurately weighed and dissolved in 100.0 ml. of chloroform. A 25.0-ml. aliquot was taken and extracted three times with an equal volume of distilled water adjusted to pH 11–12 with ammonia. A 20.0-ml. aliquot of the chloroform layer was pipeted into a 250-ml. distilling flask, the internal standard was added, and the solution was evaporated to dryness *in vacuo*. A standard homatropine solution was similarly treated. Ten milliliters of chloroform was pipeted into each of the sample and standard flasks and, after solution was complete, 1.8 μl. was injected into the gas chromatograph with a 1.0-μl. solvent flush. The peak height of homatropine relative to codeine was determined in both the sample and reference standard.

Free Salicylic Acid Assay—Approximately 1.4 g. of the aspirin–homatropine mixture was accurately weighed and dissolved in 100.0 ml. of methanol. An aliquot was taken and assayed by the standard ferric nitrate procedure (5).

Tropine Assay—The same procedure was used as for homatropine except that the column temperature was 115° and the detector and flash heater were both at 190°.

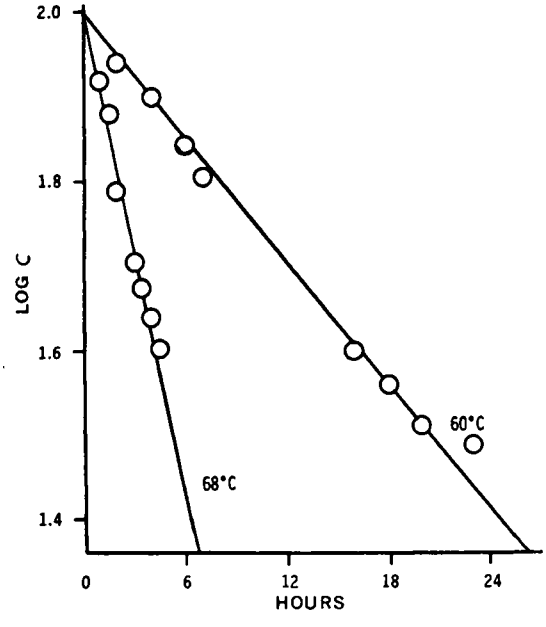

Figure 1—*Disappearance of homatropine at 60 and 68°.*

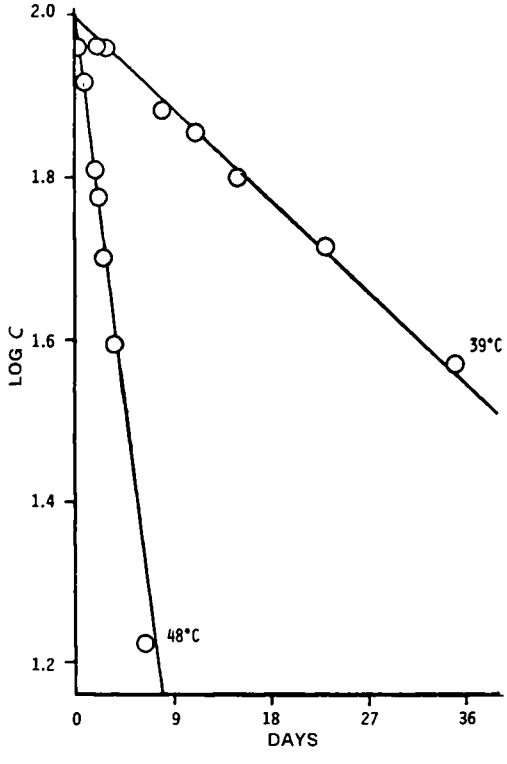

Figure 2—*Disappearance of homatropine at 39 and 48°.*

[1] F & M 402.

Table I—Reaction Rate Constants and Half-Lives for Homatropine Disappearance

Temperature	Reaction Rate Constant, hr.$^{-1}$	Half-Life, hr.
68°	0.209	3.32
60°	0.0531	13.0
48°	0.0105	65.8
39°	0.00120	577

Table II—Reaction Rate Constants and Half-Lives for Free Salicylic Acid Formation

Temperature	Reaction Rate Constant, hr.$^{-1}$	Half-Life, hr.
60°	0.0532	13.0
48°	0.0105	65.8

Kinetic Studies—Aspirin and homatropine base (12:1) were gently mixed in a glass mortar and placed in preweighed glass ampuls. Weights were accurately determined and the ampuls were sealed. Samples were stored at 39, 48, 60, and 68°, periodically removed, and assayed. Because a eutectic forms after a period of time, the entire contents of the ampul were used for each assay to ensure no loss of material. The 68° samples were maintained in a constant-temperature bath (±0.2°) while the rest were stored in hot air ovens (±1°). To ensure proper temperature control in the ovens, a recording thermal probe was placed near the samples.

Synthesis of Acetylhomatropine—Homatropine base was dissolved in a 1:1 mixture of pyridine and acetic anhydride and stored at room temperature (in the dark to prevent color formation) for 24 hr. Methanol was added to destroy the acetic anhydride; the solution was then evaporated on a flash evaporator until there was no odor of pyridine. The resulting oil was difficult to purify; consequently, the picrate salt was made. One-half gram of the oil was dissolved in 5 ml. of methanol, 5 ml. of a saturated solution of picric acid was added, and the resulting precipitate was recrystallized from boiling methanol.

Anal.—Calc. for $C_{15}H_{21}NO_4$: C, 52.75; H, 4.80; N, 10.25. Found: C, 52.79; H, 4.80; N, 10.23.

TLC System—A chamber[2] with a saturation pad was used for all chromatograms. Precoated aluminum oxide plates[3] were activated for 0.5 hr. at 100° prior to use. The developing solvent system was chloroform–methanol–diethylamine (200:10:1). For color development the plates were sprayed with Dragendorff's reagent followed by an overspray of 5% sodium nitrite.

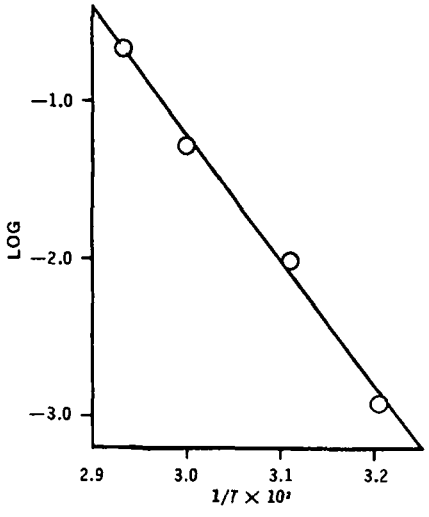

Figure 3—*Arrhenius plot for homatropine disappearance.*

[2] Gelman.
[3] Brinkmann T250μ.

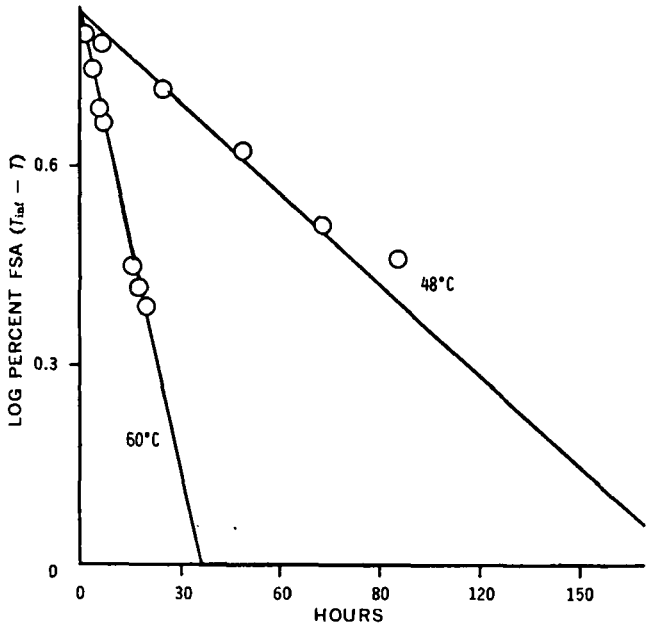

Figure 4—*Appearance of free salicylic acid (FSA) at 48 and 60°. See text for explanation.*

RESULTS

TLC and GLC analyses indicated that the partially reacted mixtures contained only homatropine, aspirin, salicylic acid, and acetylhomatropine, with trace amounts of acetic acid. The completely reacted mixtures (at time infinity) contained only aspirin, salicylic acid, and acetylhomatropine, with trace amounts of acetic acid. No tropine was found in any of the reaction mixtures; consequently, hydrolysis of homatropine can be ignored (6, 7).

Disappearance of Homatropine—Based on the kinetic plots shown in Figs. 1 and 2, the reaction appears to be pseudo first order. An Arrhenius plot (Fig. 3) yields an activation energy of 36.4 kcal./mole. The reaction rate constants and half-lives at the various temperatures are given in Table I.

Appearance of Free Salicylic Acid—The rates of appearance of free salicylic acid, plotted as percentage free salicylic acid at time infinity minus percentage at time t for 48 and 60°, are shown in Fig. 4. The experimentally determined values of the free salicylic acid infinity term (6 days, 60° = 6.93% and 8 weeks, 48° = 6.50%) are in good agreement with the theoretical value of 6.39%. Based

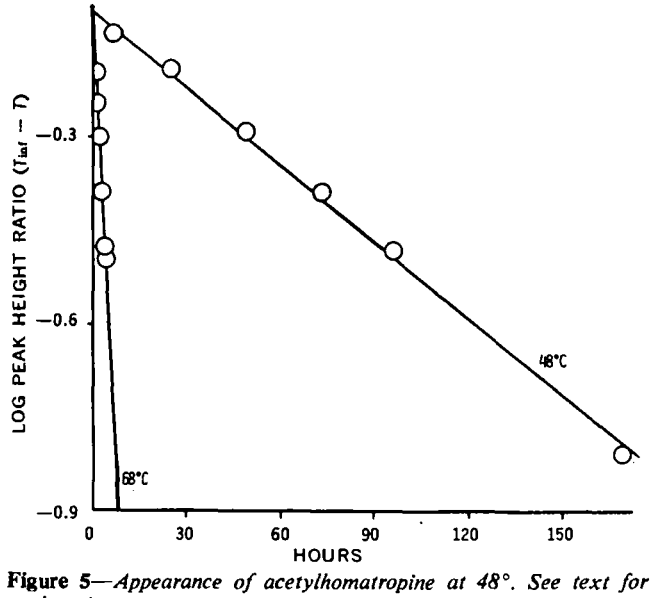

Figure 5—*Appearance of acetylhomatropine at 48°. See text for explanation.*

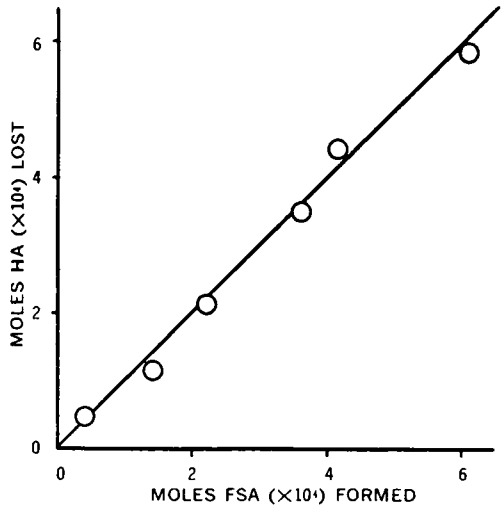

Figure 6—*Correlation of disappearance of homatropine (HA) with appearance of free salicylic acid (FSA) at 48°.*

upon the appearance of acetylhomatropine and the disappearance of homatropine, the reaction seemed to be complete at 48° in at least 5 weeks. The fact that 3 additional weeks at this temperature did not produce significant amounts of excess free salicylic acid indicates that degradation of aspirin to salicylic acid apart from its reaction with homatropine was not a significant factor in these studies. This is further confirmed by the fact that the reaction rate constants and half-lives for the disappearance of homatropine (Table I) are in good agreement with those found for the appearance of salicylic acid (Table II).

Appearance of Acetylhomatropine—The rate of acetylhomatropine formation, plotted as the peak height ratio (acetylhomatropine–codeine after normalizing all sample weights to 1 g.) at time infinity minus that at time t for 48 and 68°, is shown in Fig. 5. The infinity value was taken as the average normalized peak height ratio at 5 and 8 weeks at 48°, and the two values agreed within 3%. These figures were confirmed by the fact that at 5 weeks, at 48°, no homatropine could be detected in the reaction mixture. The reaction rate constants and half-lives for acetylhomatropine appearance are given in Table III; they are in good agreement with those of Tables I and II.

Correlation of Homatropine Loss and Free Salicylic Acid Formation—The tabular data indicate a 1:1 molar relationship in the reaction. This is further emphasized in Fig. 6 where, at 48°, the moles of free salicylic acid formed is plotted against moles of homatropine lost; the correlation coefficient is 0.99. Similar plots of 39 and 60° data yield coefficients of 1.06 and 0.96, respectively. The coefficient for 68° is 0.87, indicating some possible variations in the reaction not observed at lower temperatures.

Confirmation of Acetylhomatropine Synthesis—NMR analysis of the synthesized homatropine picrate showed the disappearance of the alcohol proton and the appearance of three methyl protons on the acetate group. TLC results for the "known" acetylhomatropine base (the oil resulting from the reaction mixture) and unknown samples (the heat-treated mixtures of aspirin and homatropine base) were identical (no tropine present in the unknown). The following R_f values were obtained: acetylhomatropine, 0.64; homatropine, 0.42; and tropine, 0.20.

Table III—Reaction Rate Constants and Half-Lives for Acetylhomatropine Formation

Temperature	Reaction Rate Constant, $hr.^{-1}$	Half-Life, hr.
68°	0.202	3.43
48°	0.00946	73.3

DISCUSSION

There are two probable mechanisms for the reaction. In the first version, there is liquefaction followed by subsequent reaction. The low water content (less than 0.05%) and the absence of excess free salicylic acid in the reaction mixture eliminate water as a factor, either in liquefaction or in the reaction kinetics. The eutectic contains no solid homatropine (as shown by differential scanning calorimetry); aspirin is present both in the liquid phase and as a crystalline solid. The "saturated" solution with respect to aspirin (with excess solid phase present) accounts for the pseudo-first-order character of the reaction.

The second version of the proposed mechanism is identical to the first except that after liquefaction the reaction (probably intramolecular transesterification) is preceded by salt formation. A similar "solid-state" salt formation between aspirin and antipyrine has been reported (8). The second version, involving salt formation, seems more likely for the following reasons:

1. If a 1:1 molar mixture of aspirin and homatropine is dissolved in a chloroform–methanol mixture and evaporated to dryness, the resulting oil is soluble in water.
2. When a 1:1 molar mixture of the two is gently heated, an oily liquid results which does not solidify upon cooling. IR scans of this oil and of a solid 1:1 mixture indicate salt formation.
3. The relatively high heat of activation (36.4 kcal./mole) points toward a possible contribution from heat of ionization and/or fusion.

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