reasoning, McLafferty, *et al.*,[5] explained their data of *p*-phenylbenzophenone from electron-impact reactions. In their case, the transition state also receives excess vibrational energy from electron impact to overcome the steric strain. The suggestion that proximity effects can be reduced by overcoming steric strain in the transition state is also revealed in Weale's[10] study of the reaction between *o*-methyl-N,N-dimethylaniline and methyl iodide in dry methanol. He found that the "*ortho* effect" is decreased by increasing the pressure on the system. He explained this observation as due to the contribution from compression energy which overcomes the steric strain in the transition state. Certainly this study and other gas-phase studies[2] demonstrate the advantages of gas-phase studies when relating structure to chemical reactivity.

(10) K. E. Weale, *J. Phys. Soc.*, 2959 (1954).

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Carboxylate-Facilitated Acetylation of Hydroxy Acids in Aqueous Solution

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Interactions of acetic anhydride with glycolate, malate, salicylate, and similar hydroxy acid anions in dilute aqueous solutions have been shown to lead to significant formation of corresponding acetyl esters. Thus, when acetic anhydride (initial concentration 0.02 *M*) was added to 0.5 *M* solutions of sodium salicylate or sodium glycolate, 22.6% of the acetic anhydride reacted to form aspirin (acetylsalicylic acid) and 48.4% to form acetylglycolic acid, respectively. The reactions which take place competitively with the hydrolysis of the acetic anhydride are believed to be mediated by initial attack of the carboxylate function followed by an O (carboxyl) to O (hydroxy) transacetylation reaction. Salicylate ions, for example, appear to form mixed salicylic acetic acid anhydride species, which preferentially undergo rearrangement to aspirin rather than hydrolysis. These mechanisms appear to play a part in the slow hydrolysis of the corresponding acetyl esters.

In an earlier paper[1] we reported that small amounts of acetylmalate ion were obtained when acetic anhydride was added to dilute aqueous solutions of sodium malate. Further investigation of this reaction and studies on similar acetylations of other hydroxy acid anions strongly indicate that the responsible mechanism involved an O to O transacetylation process mediated by the carboxylate function. Relevant data on these systems are presented in this article.

Although unsubstituted alcohols and phenols can be acetylated by acetic anhydride in inert solvents, or in the absence of solvents, the acetylation of the hydroxy group normally competes unsuccessfully with the hydrolysis of acetic anhydride in water. For example, when acetic anhydride (0.02 *M*) was added to a solution of ethanol (0.50 *M*) in water, more than 99% of the acetic anhydride was hydrolyzed to acetic acid.

The present investigation was undertaken to examine what role a carboxylate group in the alcohol or phenol molecule played in the reaction sequence which led to the significant acetylation of these molecules by acetic anhydride in water.

Results and Discussion

Formation of Ester.—One of the simpler indications of rapid concomitant ester formation during apparent hydrolysis of acetic anhydride in, for example, aqueous sodium glycolate, is the observed reduction in the amount of acetic acid found. Thus the solution obtained 1 hr after adding acetic anhydride (0.02 *M*) to aqueous solutions of sodium glycolate (0.5 *M*) at 25° (solution 1) required a 24% smaller titer of standard sodium hydroxide solution to raise its pH to the phenolphthalein end point than did a solution obtained 1 hr after adding acetic anhydride (0.02 *M*) to water (solution 2).

When solution 1 was acidified with mineral acid and chromatographed on a silicic acid column, it was found to contain the ester, acetylglycolic acid, together with acetic and glycolic acids. The composition of solution 1 did not change appreciably between 30 min and 2 hr after mixing acetic anhydride with the sodium glycolate solution. The slow hydrolysis of acetylglycolic acid in neutral solutions has been investigated by Senter and Ward.[2]

Similar behavior was observed following addition of acetic anhydride to aqueous solutions of other hydroxy acid anions. Errors in the titration of the salicylic acid reaction mixture due to the hydrolysis of acetylsalicylic acid proved to be within the experimental error.

The per cent yield of ester, relative to moles of added acetic anhydride (relative yield, R), was calculated from the titration data on the assumption that the lower titer of sodium hydroxide against the reaction mixture (solution 1) was due to the fact that some of the acetic acid, which would result from the hydrolysis of acetic anhydride, was tied up as an acetyl group in the ester. Results in Table I show the per cent relative yield of ester obtained following the addition of acetic anhydride to aqueous solutions of hydroxy acid anions and alcohols at 25°. The importance of a carboxylate group in the molecules is evident in the fact that the un-ionized hydroxy acids and simple alcohols and phenol were not acetylated to an appreciable extent under the experimental conditions. The failure of tri-

(1) I. H. Pitman, R. B. Paulssen, and T. Higuchi, *J. Pharm. Sci.*, **57**, 239 (1968).

(2) G. Senter and T. J. Ward, *J. Chem. Soc.*, 2535 (1912).

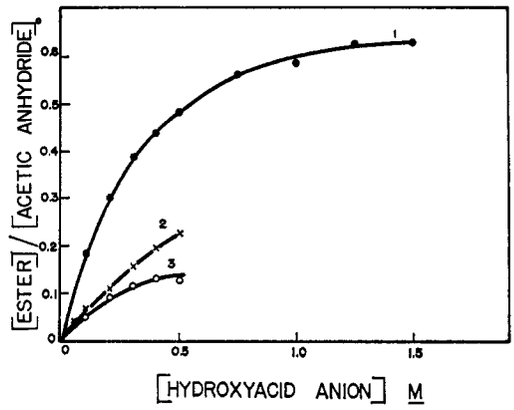


Figure 1.—Yield of (1) acetylglycolic acid, (2) aspirin, and (3) acetylmalic acid plotted against concentration of the respective hydroxy acid anions. Initial concentration of acetic anhydride = 0.02 M, T = 25°, and I = 1.50 M.

TABLE I

RELATIVE YIELDS OF ACETYL ESTERS FROM HYDROXY ACIDS AND ALCOHOLS AT 25° AND IONIC STRENGTH = 1.50 M. INITIAL CONCENTRATION OF ACETIC ANHYDRIDE 0.02 M AND OF SUBSTRATE 0.5 M

	Initial pH	[Ester]/[Ac_2O]$_T$ ×100
Acid		
Glycolic	5.50	48.4
Glycolic	1.45	<1[a]
Lactic	5.50	36.4
Lactic	2.00	<1
α-Hydroxy-*n*-butyric	7.00	45.4
β-Hydroxy-*n*-butyric	7.00	31.0
γ-Hydroxy-*n*-butyric	7.00	75.0
Salicylic	5.10	22.6
Malic	6.60	12.8
Malic	1.85	<1
Tartaric	5.70	25.6
Tartaric	1.60	<1
Citric	6.00	<1
Alcohols		
Ethanol		<1
Isopropyl alcohol[b]		<1
Xeopentyl alcohol[b]		<1
Glucose		2.0
Phenol[c]	1.60	<1

[a] Measured at 40°. [b] Concentration 0.3 M. [c] Indicator, Bromcresol purple.

ionized citric acid to be acetylated will be discussed later.

The relative yield of ester was found experimentally to be a function of the concentration of hydroxy acid anion but was independent of the initial acetic anhydride concentration between 0.01 and 0.02 M. These results indicate that the over-all reaction leading to formation of the ester and the hydrolysis of acetic anhydride are of different orders in hydroxymonocarboxylate ion, but are all first order in acetic anhydride. Plots of yield of ester relative to the initial acetic anhydride concentration against [hydroxy acid anion] are shown for several systems in Figure 1. When the data in Figure 1 were plotted as (reciprocal of yield of ester) against (reciprocal of [hydroxy acid anion]) straight lines which intersected the Y axis at values greater than 1 were obtained. Plots of this type are shown in Figure 2.

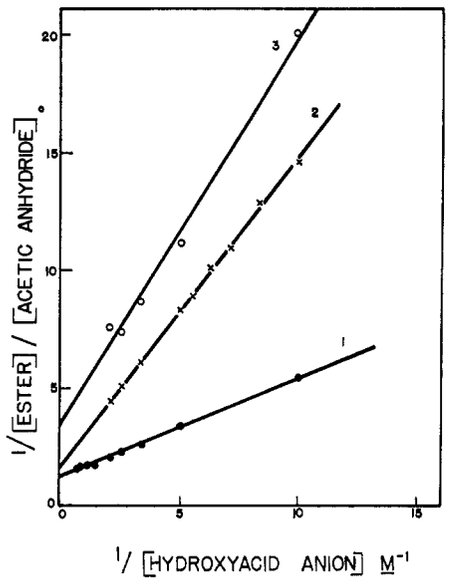


Figure 2.—Plots of 1/yield of (1) acetylglycolic acid, (2) aspirin, and (3) acetylmalic acid against 1/concentration of the respective hydroxy acid anions.

Rate of Consumption of Acetic Anhydride.—At constant pH and in the presence of a considerable excess of ionized hydroxy acids, acetic anhydride appeared to be consumed by a pseudo-first-order process. This was apparent for the glycolate and malate systems from corresponding changes in the uv spectra of the reacting solutions. The reaction with sodium salicylate could not be observed optically, but exhibited essentially the same behavior when followed titrimetrically on a pH-stat.

The second-order rate constants, k_{B^-} and $k_{B^{2-}}$, attributable to hydroxymonocarboxylate (B^-) and hydroxydicarboxylate (B^{2-}) ions, respectively, were calculated from the pseudo-first-order rate constants, k_{obsd} values, by using the equation

$$k_{obsd} = k_0 + k_{NaCl}[NaCl] + k_{B^-}[B^-] + k_{B^{2-}}[B^{2-}] \quad (A)$$

In this equation k_0 is the first-order rate constant for the hydrolysis of acetic anhydride in water and k_{NaCl} is the catalytic (negative) constant for NaCl on the observed reactions. This latter term was included because different amounts of NaCl were added to each system to maintain an ionic strength of 1.5 and NaCl is known[3a,4] to be a negative catalyst on the hydrolysis of acetic anhydride. Any catalytic effect of un-ionized hydroxycarboxylic acids was neglected because independent experiments revealed that it was very much smaller than that attributable to ionized species. The method of calculating k_{B^-} and $k_{B^{2-}}$ values is given in the Experimental Section and results are shown in Table II.

Proposed Reaction Scheme.—The proposed reaction scheme for the consumption of acetic anhydride in solutions of hydroxydicarboxylate ions is shown in Scheme I, using malate ion as an example. Similar reactions are suggested to occur in systems containing hydroxymonocarboxylate ions except that the cyclization reaction (reaction 6) would not be possible.

(3) (a) A. R. Butler and W. Gold, *J. Chem. Soc.*, 2305 (1961). (b) M. Kilpatrick, *J. Amer. Chem. Soc.*, **50**, 2891 (1928). (c) T. C. Bruice and S. Benkovic, "Bioorganic Mechanisms," Vol. 1, W. A. Benjamin, Inc., New York, N. Y. 1966.

(4) C. A. Bunton, N. A. Fuller, S. G. Perry, and I. H. Pitman, *J. Chem. Soc.*, 4478 (1962).

TABLE II

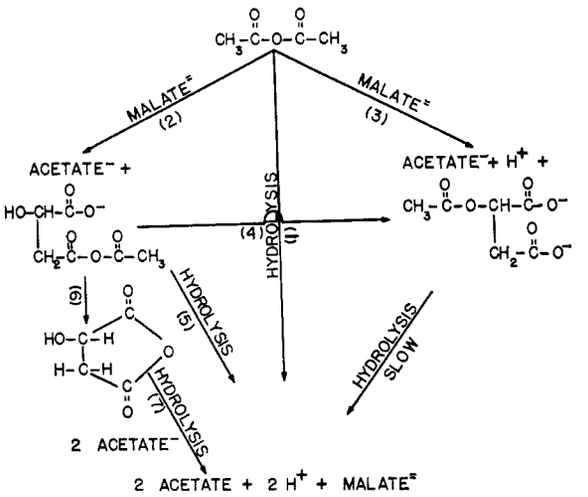
OVER-ALL EFFECT OF HYDROXY ACIDS ANIONS ON THE DISAPPEARANCE OF ACETIC ANHYDRIDE IN AQUEOUS SOLUTIONS, EXPRESSED AS SECOND-ORDER RATE CONSTANTS (I = 1.50 M, T = 25°)

Buffer acid	k_{B^-}, M^{-1} sec^{-1} × 10^3	$K_{B^{2-}}$, M^{-1} sec^{-1}
Glycolic	7.1	
Salicylic	1.8	
Malic[a]		32

[a] See ref 1.

SCHEME I

REACTIONS OF ACETIC ANHYDRIDE IN MALIC ACID BUFFERS[a]



[a] All reactions are expected to be subject to general acid–base catalysis and reaction 3 is likely to also be subject to intramolecular general-base catalysis by a carboxylate group.

All the reactions in Scheme I are acyl transfer reactions and must be expected[3] to be subject to general acid, general base catalysis by the un-ionized and ionized hydroxycarboxylic acids. It is also known that salts, including inert salts such as sodium chloride,[4] effect the rate of hydrolysis of acid anhydrides. In our present treatment we have been unable to determine the effects of general acids, general bases, and salts on the individual reactions. However, we have determined the over-all effect of the concentration of hydroxycarboxylate ions on the relative yield of ester and on the rate of consumption of acetic anhydride.

The ester could be formed by either reactions 2 and 4 or by reaction 3. This latter reaction would involve attack of the hydroxy oxygen atom of the hydroxycarboxylate ion on acetic anhydride. Although such a reaction would be facilitated by the carboxylate group (general base) in the attacking molecule, we still believe that the carboxylate group would be the more nucleophilic center and that reaction 2 would predominate. There is qualitative evidence[5] to suggest that, in nonhydroxylic solvents, the carboxylate function is the more nucleophilic. Thus when salicylic acid was allowed to react with potassium hydroxide and dimethyl sulfate, only the methyl ester could be identified as a product. Therefore, it is proposed that acetic anhydride is consumed by hydrolysis, reaction 1, and by attack of the carboxylate group of the hydroxycarboxylate ion, reaction 2, to yield a mixed acetic–hydroxycarboxylic acid anhydride. To differentiate between the nucleophilic participation of hydroxycarboxylate ions, reaction 2, and the general base effect exerted by these ions on reactions 1 and 2, a specific analytical technique is required. For example, when Bunton and Fendler[6] studied the catalytic effect of fluoride ions on the hydrolysis of acetic anhydride in water at 0°, they were able to distinguish between such mechanisms by isolating acetyl fluoride which was formed by nucleophilic attack of fluoride ions on the acetic anhydride. We have made no attempts to separate the nucleophilic catalysis from the general base catalyzed component of hydrolysis because of the instability of the mixed anhydride which would be formed.

There are numerous examples of related cases of formation of mixed acid anhydrides. Reactions of acetic anhydride with ions such as propionate,[7] malate,[1] citrate,[8] and phosphate[9] have been shown to yield mixed anhydrides, and formation of similar high energy species have been postulated during carboxylate catalyzed oxidation of thioethers of sulfoxides.[10,11]

The rearrangement of the mixed anhydride to the ester reaction (reaction 4) could occur through a tetrahedral intermediate or activated complex similar to the type proposed[12] during the intramolecular nucleophilic catalyzed hydrolysis of aspirin. When discussing the likelihood of the mixed acetic salicylic anhydride being formed during the hydrolysis of aspirin, Fersht and Kirby[13] made the point that such a molecule would be very much less stable than the ester and its rate of conversion to the ester would be very rapid. Thus it seems likely that if it were formed following reaction of salicylate ion and acetic anhydride it would rapidly rearrange to yield aspirin. Analogous rearrangement of mixed anhydrides by an O (carboxy) to N (amino) acyl transfer has been utilized as a step in the synthesis of cephalosporin C.[14] In peptide synthesis this rearrangement is usually prevented by protecting the amino group in the mixed anhydride.[15a]

Kinetic Test of the Proposed Mechanism.—Hydrolysis and rearrangement of the mixed anhydrides (reactions C, D, and E) were assumed to be essentially first-order reactions with rate constants k_4, k_5, and k_6 sec^{-1}, respectively.

Thus, in the presence of fully ionized hydroxymonocarboxylic acids, the yield of ester relative to the moles of added acetic anhydride, R, would be

$$R = \frac{k_{B^-}'k_4[B]_T}{(k_4 + k_5)(k_0 + 1.50k_{NaCl}) + (k_4 + k_5)(k_{B^-} - k_{NaCl})[B]_T} \quad (B)$$

or

$$1/R = \frac{(k_4 + k_5)(k_0 + 1.50k_{NaCl})}{k_{B^-}'k_4[B]_T} + \frac{(k_4 + k_5)(k_{B^-} - k_{NaCl})}{k_{B^-}'k_4} \quad (C)$$

(5) R. Nodzu, M. Hamada, M. Hosino, and T. Kinosita, *J. Chem. Soc. Jap.*, **60**, 1189 (1939); *Chem. Abstr.*, **36**, 6513 (1942).

(6) C. A. Bunton and J. H. Fendler, *J. Org. Chem.*, **32**, 1547 (1967).

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(9) T. Higuchi, G. L. Flynn, and A. C. Shah, *J. Amer. Chem. Soc.*, **89**, 616 (1967).

(10) T. Higuchi and K.-H. Gensch, *ibid.*, **88**, 3874 (1966).

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(13) A. R. Fersht and A. F. Kirby, *ibid.*, **89**, 4857 (1967).

(14) R. B. Woodward, K. Heusler, J. Gosteli, P. Naegeli, W. Oppolzer, R. Ramage, S. Ranganathan, and H. Vorbruggen, *ibid.*, **88**, 852 (1966).

(15) (a) N. F. Albertson, *Org. Reactions*, **12**, N. Y., 1962. (b) T. Higuchi and A. Drubulis, *J. Pharm. Sci.*, **50**, 905 (1961).

where k_{B^-}' is the second-order rate constant for nucleophilic attack of hydroxymonocarboxylate ion on acetic anhydride as distinct from the over-all catalytic effect of the ions on the consumption of acetic anhydride. A plot of $1/R$ against $1/[B]_T$ should give a straight line with a value of slope/intercept on the Y axis.

$$\frac{\text{slope}}{\text{intercept}} = \frac{k_0 + 1.50k_{NaCl}}{k_{B^-} - k_{NaCl}} \quad (D)$$

Equation D was derived on the basis that as $[B^-]$ varied, appropriate amounts of sodium chloride were added or deleted to maintain the ionic strength at 1.50 M. The salt effect has to be taken into account in our results, but if it could be ignored, the slope/intercept ratio would be approximately equal to the ratio of the specific rate of spontaneous hydrolysis of acetic anhydride to the over-all second-order reaction rate constant of the attacking hydroxymonocarboxylate ion.

For reactions in the presence of hydroxydicarboxylate ions a similar straight line relationship would be expected and the slope/intercept would be

$$\frac{\text{slope}}{\text{intercept}} = \frac{k_0 + 1.50k_{NaCl}}{k_{B^{2-}} - 3k_{NaCl}} \quad (E)$$

It is possible to check the approximate validity of eq D for the glycolate system for which we have the most data. The ratio of slope to intercept for this system as plotted in Figure 2 corresponds to 0.3 M. Independently determined value of $(k_0 + 1.50k_{NaCl})$ is 1.90×10^{-3} and k_{B^-} from Table II is 7.1×10^{-3} M^{-1} sec^{-1}. The measured value of $-k_{NaCl}$ for acetic anhydride at 25° in water was 6×10^{-4} M^{-1} sec^{-1}. Thus the directly determined value of $(k_0 + 1.50k_{NaCl})/(k_{B^-} - k_{NaCl})$ is 0.2 M.

The comparison for the salicylate system is much less satisfactory, being 0.7 M *vs.* 1.6 M, respectively. This may be due, at least in part, to the known[15b] role of salicylate anion in greatly modifying the thermodynamic activities of other organic solutes in solution. The malate system shows somewhat greater discrepancy. The very large corrections associated with ionic strength adjustments in these systems make more than qualitative agreements for this crude test rather difficult.

Effect of Structure of the Hydroxy Acid on the Degree of Acetylation.—The proposed reaction scheme contains a number of competitive reactions and the relative amount of acetic anhydride which reacts by a given route will be largely influenced by the structures of the hydroxy acid anions and the mixed anhydrides which would be formed.

Thus the first competitive process would be between hydrolysis of acetic anhydride and nucleophilic attack of a hydroxy acid anion on acetic anhydride to yield a mixed anhydride. To a first approximation, the more nucleophilic the hydroxy acid anion, the more acetic anhydride would be expected to be consumed *via* formation of a mixed anhydride. Comparison of rate constants in Table II shows that the rate of mixed anhydride formation in these systems would be in the order malate > glycolate > salicylate. The ultimate yield of ester will not be determined by this reaction alone and will also depend on the result of competition between intramolecular acetylation, hydrolysis, and other reactions of the mixed anhydride. Thus malate ions are probably stronger nucleophiles than glycolate ions and would be expected to react with acetic anhydride to form more mixed anhydride. However, the resulting anhydride readily cyclizes to give malic anhydride[1] and the yield of acetyl malate is therefore considerably smaller than that of acetyl glycolate. The mixed anhydride precursor of this latter species could not cyclize in a similar way to acetic–malic anhydride. Similarly, the failure of citrate ions to yield any acetyl citrate is believed to be due to the fact that, at this pH value, the mixed citric acetic anhydride would preferentially cyclize to yield highly reactive citric anhydride[8] rather than rearrange to the ester.

The position of the hydroxy group relative to the acetyl group in the mixed hydroxy acid–acetic acid anhydride also affects the competition between intramolecular acetylation and hydrolysis. Thus, β- and γ-hydroxy-*n*-butyric acids have similar pk_a values and the nucleophilicities of their ions are expected to be very close. The higher yield of γ-acetyl butyrate ions relative to β-acetyl butyrate ions suggests that intramolecular acetylation of the mixed anhydride precursor is more favorable compared to hydrolysis in the former case than in the latter.

The number of hydroxy groups also appears to be important and thus the per cent yield of ester obtained from reactions of tartarate ion (2,3-dihydroxy succinate) is approximately double that obtained from reactions on malate ion (2-hydroxy succinate), although the former is expected to be the weaker nucleophile.

The Y intercept for the salicylate system (Figure 2) is 1.75, corresponding to 57% consumption of the acetic anhydride in formation of aspirin in an infinite concentration of sodium salicylate, assuming acetic anhydride only formed the mixed acetic–salicylic acid anhydride. Thus the limiting value indicates that intramolecular acetylation of the mixed anhydride was preferred to direct hydrolysis of this species. Any positive catalytic effect by sodium salicylate on the hydrolysis of acetic anhydride, reaction 1, would increase the value of $k_4/(k_4 + k_5)$ which reflects the preferred rearrangement of the mixed anhydride to the hydrolysis of the same species.

Experimental Section

Equipment.—Spectrophotometric studies were performed using commonly employed technique on Cary Models 11 and 14. pH-Stat experiments were done on a Radiometer TTT1 automatic titrator utilizing a SBR titrigraph and an ABU1 autoburet with a TTA3 titration assembly. All thermostated water baths were regulated within ±0.1°. In tlc, Eastman chromagram sheet 6060, silica gel, was employed.

Reagents.—All reagents used were of analytical grade unless otherwise stated. α- and β-hydroxy-*n*-butyric acids were obtained from K & K Laboratories, Plainview, N. Y., and employed without further purifications. The sodium salt of γ-hydroxybutyric acid was made from γ-butyrolactone. Acetic anhydride was distilled over magnesium turnings and the fraction boiling between 137.8 and 138.2° was collected and sealed in ampoules. Commercial grade *p*-dioxane was purified by the method described in Vogel.[16] All water used was redistilled from acid permanganate using an all-glass still. Acetylglycolic acid was synthesized[17] by refluxing glycolic acid with acetyl chloride, mp 65.5–66.5° (lit.[18] mp 67–68°).

Acetyllactic Acid.—Freshly distilled lactic acid was refluxed with acetyl chloride and the acetyllactic acid formed was purified

(16) A. I. Vogel, "Practical Organic Chemistry," 3rd ed, John Wiley & Sons, Inc., New York, N. Y., 1962.

(17) A. Anschutz and W. Bertram, *Chem. Ber.*, **36**, 467 (1903).

(18) M. S. Kharasch, H. N. Friedlander, and W. H. Urry, *J. Org. Chem.*, **16**, 533 (1951).

by fractionated distillation.[19] α- and β-acetoxy-*n*-butyric acids were synthesized by refluxing the parent acid with excess acetyl chloride. The liquid products were isolated by vacuum distillation. Nmr and ir spectra were consistant with the assumed structure. Salicylic acid was recrystallized from hot water, mp 158–159°. Titration with sodium hydroxide showed 99.69% salicylic acid. Acetylsalicylic acid was recrystallized from benzene and the crystals were washed with cyclohexane, mp 133–134.5°. Titration with sodium hydroxide showed 100.21% acetylsalicylic acid.

Isolation and Identification of Reaction Products.—In order to isolate reaction products, the amount of reactants was scaled up, but the [hydroxy acid] was kept in large excess over [acetic anhydride] by repeatedly adding small aliquots of acetic anhydride to the reaction mixture. The pH of the solution was kept constant by addition of sodium hydroxide.

Glycolic Acid.—The final reaction mixture was evaporated to dryness, and the crystalline residue was dissolved in 3 *M* sulfuric acid. Inorganic salt was precipitated by adding methanol. The filtrate was again evaporated to dryness and after acidification, extracted with benzene. Upon concentration and cooling, the mixture solidified and acetylglycolic acid was obtained after recrystallization from benzene, mp 66.5–67°. No depression in melting point was observed when isolated material was mixed with synthesized material. Paper chromatography (*n*-butyl alcohol form sprayed with bromcresol green) and tlc (ethyl ether–formic acid–water, 18:5:9 v/v, sprayed with bromcresol green) of isolated material showed spots corresponding to synthesized acetoxyglycolic acid.

α- and β-Hydroxy-*n*-butyric Acids.—The reaction mixture was acidified with sulfuric acid and extracted several times with ethyl ether. The residues after evaporation of the ether were tested for acetoxy derivatives by tlc. In three different solvents (*n*-butyl alcohol–formic acid–water, 10:2:5 v/v, acetone, and ethyl acetate), R_f values were obtained identical with those of the synthesized acetyl esters. The acetoxy derivatives always moved faster than the parent acid on the tlc sheets.

Acetylsalicylic Acid.—An 8.0-ml sample of 1.2 *M* acetic anhydride in dioxane was added to 50.0 ml of 0.5 *M* salicylate buffer of pH 5.1. This addition was repeated four times with 20-min intervals. The reaction mixture was shaken with chloroform after acidification with sulfuric acid and the chloroform was evaporated. The residue was suspended in water and 6.0 ml of 3 *N* sulfuric acid was added. Precipitated salicylic acid was filtered off and the filtrate was shaken with chloroform until no more salicylic acid could be detected with ferric chloride. The collected chloroform was pooled and shaken with 1% ferric chloride until the water layer was no longer purple. The chloroform solution, this way freed for salicylic acid, was dried and evaporated to dryness. The residue was recrystallized from isopropyl alcohol and melted at 135–137° (lit.[13] mp 135°). No depression in the melting point was observed when the isolated crystals were mixed with authentic acetylsalicylic acid. Uv spectrum of the isolated material matched that of known acetylsalicylic acid. λ_{CHCl_3} 278 mμ (log ε = 3.125). (A value of log ε = 3.120 was found for authentic material.)

Acetylation Followed by Titration.—A 0.1-ml sample of 0.6 *M* acetic anhydride in dioxane was allowed to react with 3.0 ml of buffer solution in a closed vial for 1 hr. The reaction mixture was then diluted with 2.0 ml of water and titrated with standard solution of sodium hydroxide to phenolphthalein (or phenol red) end point, volume of required titrant = *a* ml. A 3.0-ml sample of buffer solution and 2.0 ml of water was titrated to the same end point, volume of titrant = *b* ml. An equivalent amount of acetic anhydride was titrated after hydrolysis in 5.0 ml of water, volume of titrant = *c* ml. Error in the titration was estimated to be less than ±2%.

$$\frac{[\text{ester}]}{[Ac_2O]_{\text{added}}} = 2\,\frac{c-(a-b)}{c}$$

Spectrophotometric Determination of Aspirin in the Presence of Salicylic Acid.—Aspirin, ASA (20 mg), dissolved in 1 ml of dioxane was added to 25.0 ml of salicylate buffer of pH 5.40. Sulfuric acid (6 *N*, 2 ml) was added and the precipitated salicylic acid, SA, was filtered off. A 20.0-ml sample of the filtrate was then extracted with chloroform to 100.0 ml of solution. After a proper dilution, the absorbance was read at 277.5 and 308 mμ. The concentration of aspirin, c_{ASA}, was calculated from

$$c_{ASA} = \frac{A_{277.5}\,\epsilon_{SA}^{308} - A_{308}\,\epsilon_{SA}^{277.5}}{\epsilon_{ASA}^{277.5}\,\epsilon_{SA}^{308} - \epsilon_{SA}^{308}\,\epsilon_{SA}^{277.5}}$$

TABLE III
MOLAR ABSORPTIVITIES, ε VALUES, OF SALICYLIC AND ACETYLSALICYLIC ACIDS[a]

	Wavelength, mμ	
	277.5	308
Acid	ε	ε
Salicylic acid	725	4125[b]
Acetylsalicylic acid	1325[b]	15

[a] Measured in chloroform. [b] Maxima in the absorbance.

Comparison of the Spectrophotometric and the Titrimetric Determination of Aspirin in the Reaction Mixture.—A 1.0 ml sample of 1.2 *M* acetic anhydride was added to 25.0 ml of salicylate buffer of pH 5.40. After 1 hr the reaction mixture was worked up as described above. The expected amount of aspirin was calculated on the basis of a simultaneous titration of acetic acid in an aliquot of the reaction mixture. The analysis showed that 85% of the calculated aspirin was found by the spectrophotometric determination compared to 87% recovery of aspirin added to the salicylate buffer.

Calculation of Rate Constants.—The pseudo-first-order rate constants for the reactions of acetic anhydride in aqueous hydroxymonocarboxylate ions could be accounted for by eq A. Because the ionic strength of each system was 1.5, [NaCl] could be related to [B^-] by the identity

$$[\text{NaCl}] = 1.5 - [B^-]$$

Also, the total buffer concentration $[B]_T$ was related to concentrations of un-ionized and ionized buffer species by the identity

$$[B]_T = [BH] + [B^-]$$

Using these two identities, eq A could be rearranged to

$$k_{obsd} = k_0 + 1.5k_{NaCl} + (k_{B^-} - k_{NaCl})\,\frac{[B]_T K_a}{K_a + [H^+]}$$

Plots of $[B]_T K_a/(K_a + [H^+])$ against k_{obsd} for systems in which either $[B]_T$ or $[H^+]$ was varied and the other term kept constant gave straight lines from whose slope and intercept on the *Y* axis values of $(k_{B^-} - k_{NaCl})$ and $(k_0 + 1.5k_{NaCl})$, respectively, were calculated. By using an independently measured value of the rate constant for hydrolysis of acetic anhydride in pure water at 25°, $k_0 = 2.80 \times 10^{-3}$ sec^{-1}, values of k_{B^-} could then be calculated. This method is only valid if the hydrolysis of acetic anhydride is not subject to specific acid or base catalysis in the pH region being investigated. Outside of this pH region, only $[B]_T$ can be varied, and the effective rate constant for acetic anhydride hydrolysis at the particular pH value used as k_0.

Determination of Ionization Constants.—The ionization constant of salicylic acid was determined spectrophotometrically[20] in aqueous solutions of sodium chloride. At 25° and $I = 1.50$ *M*, $pK_a = 2.55 \pm 0.06$. The ionization constant of glycolic acid was determined potentiometrically under the same conditions, $pK_a = 3.44 \pm 0.04$.

Registry No.—Glycolic acid, 79-144; lactic acid, 50-21-5; α-hydroxy-*n*-butyric acid, 565-70-8; β-hydroxy-*n*-butyric acid, 300-85-6; γ-hydroxy-*n*-butyric acid, 591-81-1; salicylic acid, 69-72-7; malic acid, 97-67-6; tartaric acid, 526-83-0; citric acid, 77-92-9; ethanol, 64-17-5; isopropyl alcohol, 67-63-0; neopentyl alcohol, 75-84-3; glucose, 50-99-7; phenol, 108-95-2.

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