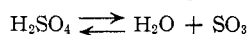
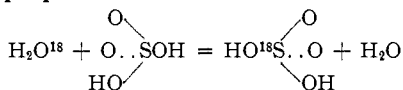


the fact that the conjugate acids of these materials should be nearly of the strength of that of the conjugate acid of sulfuric acid itself. Relatively rapid solvolysis is characteristic of these neutral esters. The rates of hydrolysis of alkyl hydrogen sulfates in strongly acidic solutions should be similar to those of dialkyl sulfates since replacement of an alkyl group by hydrogen, with its very similar electronegativity, should have no gross effect on the carbon-oxygen bond. In basic solutions much lower rates should obtain with *s*-butyl sulfate anion since it is an ester of the much weaker acid, the bisulfate ion.

**Exchange Reactions.**—The sulfate ion does not exchange in protracted periods at 100° with H<sub>2</sub>O<sup>18</sup>, the bisulfate ion exchanges slowly, sulfuric acid rapidly.<sup>19,20</sup> Most workers in this field have favored a mechanism involving



However, in the light of the present paper, reaction involving the hydrolysis of sulfuric acid may be proposed



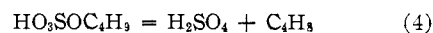
**Formation of Butylene.**—The fraction of *s*-butyl sulfate ion diverted to butylene during hydrolysis amounts to 0.08 in basic solution. In solutions of

(19) G. A. Mills, *THIS JOURNAL*, **62**, 2833 (1940).

(20) E. R. S. Winter and H. V. A. Briscoe, *J. Chem. Soc.*, 631 (1942).

sulfuric acid, it rises slowly to one-half in about 40% sulfuric acid. The rise in temperature of the refluxing solutions may be in part involved. In solutions of sulfonic acids, the relative rate of the elimination reaction rises much less than with sulfuric acid. There is some correlation between high positive configurations and low butylene yields.

These considerations are consistent with an intramolecular elimination reaction



In dilute acidic solutions, butylene yield changes but slowly with acid concentration. Thus, the elimination reaction probably has the same order as reactions (2) and (3). The relative balance among reactions (2), (3) and (4) would depend upon medium effects and upon the activity of the water molecule. The activated complex of reaction (4) involves a six-membered ring. If the elimination reaction proceeds by reaction (4), then, *under these conditions*, the reverse reaction, sulfation of butylene, must proceed by direct addition of sulfuric acid to butylene. An activated complex of this nature seems first to have been proposed by Hurd and Blunck.<sup>21</sup>

**Acknowledgment.**—The author is indebted to Dr. Ralph G. Pearson for several valuable discussions.

(21) C. D. Hurd and F. H. Blunck, *THIS JOURNAL*, **60**, 2419 (1938), for the decomposition of esters of acetic acid to acid and olefin. See also Hüchel, Tappe and Legutke, *Ann.*, **543**, 191 (1940), with regard to the decomposition of xanthates.

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## Acidic Constituents of Lactic Acid-Water Systems

BY REX MONTGOMERY<sup>2</sup>

A partition chromatographic procedure is described for estimating monomeric lactic acid, lactyllactic acid, lactyllactyllactic acid and higher polymers in solutions of lactic acid and dehydrated lactic acid. The procedure is applied in a study of the composition of lactic acid-water systems.

It is well known that the concentration of a dilute solution of lactic acid results in autoesterification of the monomer to produce lactyllactic acid, lactyllactyllactic acid and higher condensation polymers. The composition of aqueous lactic acid solutions and dehydrated lactic acid has been studied by several workers,<sup>3-5</sup> and their results have been reviewed recently by Filachione and Fisher.<sup>6</sup> In general, the method of analysis involved determination of free acidity, under conditions which minimized concomitant hydrolysis of the ester linkages, and total acidity after complete hydrolysis. Obviously

this method could not give the relative amounts of all the components, and it was assumed, therefore, that only monomeric lactic acid and lactyllactic acid were present. Bezzi, Riccoboni and Sullam<sup>3</sup> greatly extended our knowledge by a study which included determination of the dissociation constants of lactyllactic acid and the polylactic acids, and the kinetics of hydrolysis of these esters and lactide. Their results furnished more accurate data on the composition of lactic acid-water systems: they concluded that lactide was present in trace amounts only. However, the proportion of each component, with the possible exception of the monomer, could not be derived. Even in dilute solutions, polyesters were present, their average degree of polymerization being 2.0 in very dilute solutions. The degree of polymerization increased gradually to 2.6 in 95% solution and then rapidly to 6.5 in 118% total available lactic acid. It is apparent, therefore, that analyses by the general method described above decreases in accuracy as the concentration of the lactic acid increases.

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

(2) University of Minnesota, Department of Agricultural Biochemistry, St. Paul 1, Minnesota.

(3) S. Bezzi, L. Riccoboni and C. Sullam, *Mem. accad. Italia, Classe sci., fis., mat. nat.*, **8**, 127 (1937).

(4) R. Eder and F. Kutter, *Helv. Chim. Acta*, **9**, 355 (1926).

(5) (a) R. Dietzel and R. Krug, *Ber.*, **58B**, 1307 (1925); (b) G. I. Thurmond and G. Edgar, *Ind. Eng. Chem.*, **16**, 823 (1924); (c) P. D. Watson, *ibid.*, **32**, 399 (1940).

(6) E. M. Filachione and C. H. Fisher, *ibid.*, **36**, 223 (1944).

To analyze a polymeric series such as that found in lactic acid systems, it is necessary to employ a fractionation procedure. Application of the partition chromatographic technique, described by Marvel and Rands,<sup>7</sup> to aqueous lactic acid solutions and dehydrated lactic acid is the subject of this paper. In this way, it was possible to determine monomeric lactic acid; the dimer, lactylactic acid; the trimer, lactyllactylactic acid; and the higher esters, hereafter referred to as "*n*-mer" and taken as a single component.

The fractionation procedure used was essentially that described by Marvel and Rands.<sup>7</sup> The acidic components were partitioned between an immobile aqueous phase adsorbed on silicic acid and a developing phase which consisted of mixtures of chloroform and *n*-butanol. For complete separation of the four components it was necessary to take aliquots which contained no more than 4.5 mg. of total available lactic acid per gram of silicic acid. Overloading of the column produced a fraction of *n*-mer and trimer which flowed with the solvent front of the first developing solution.

Development of the chromatogram was followed by titrating the free acidity in 10-ml. unit fractions of eluate. A graph of milliequivalents of free acid versus volume of effluent indicates which unit fractions constitute each component. Figure 1 shows results of a typical chromatograph for 117.4% total available lactic acid. It will be noted that the acid peaks have trailing edges, particularly the *n*-mer component, which is a mixture of the higher molecular weight esters. The ester in the trailing edge should be principally the tetramer. On this assumption, extrapolation of the *n*-mer peak indicates that the error in the proportion of trimer due to tailing is 2-3%. The tailing effect may be reduced in normal circumstances by using dilute sulfuric acid as the stationary aqueous phase.<sup>7</sup> In the lactic acid systems, however, such high hydrogen ion concentrations would displace the ester equilibrium. It was thought that the equilibrium

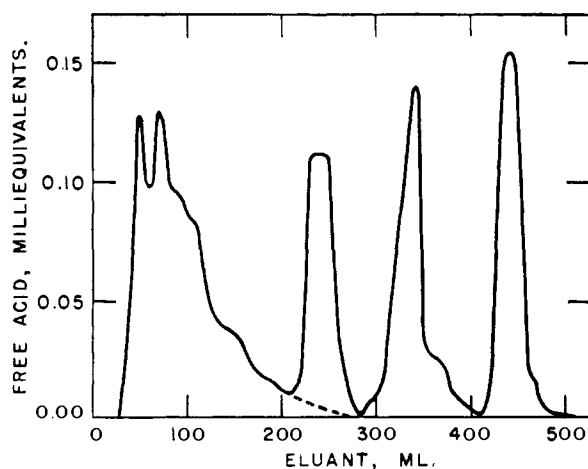


Fig. 1.—Separation of acid components of 117.4% lactic acid showing peak effluent volumes of polymers (50 and 70 ml.); trimer (230 ml.); dimer (340 ml.); monomer (440 ml.).

(7) C. S. Marvel and R. D. Rands, Jr., *THIS JOURNAL*, **72**, 2642 (1950).

on the column may be disturbed either by hydrolysis of the ester linkages in the stationary aqueous phase or esterification and alcohol interchange by the *n*-butanol in the developing solutions. Lactide was recovered from the column in 99.1% yield, however, without any indication of a lactyllactic acid peak. Also, replacing the *n*-butanol by the tertiary isomer gave the same results. Therefore, the equilibrated system must undergo negligible change during the fraction procedure.

On the average, 94% of the total available lactic acid was accounted for in the analysis. The losses may have been due to (1) transference, (2) steam distillation during removal of chloroform and ethanol prior to saponification, (3) cumulative titration errors; but the losses occurred to approximately an equal extent in each component. Since the proportion of each component was calculated from the total acid eluted from the column and not the weight of acid sample put on the column, the errors in composition must be much less than that indicated by a 6% loss of acid.

Results of analyzing a series of lactic acid solutions and dehydrated lactic acid (Fig. 2) show that even in dilute solutions both dimer and trimer were present; the latter, however, was in low concentration. As the concentration of the solution increased, the proportions of dimer and trimer increased at the expense of the monomer. At about 100% total available lactic acid, the dimer reached

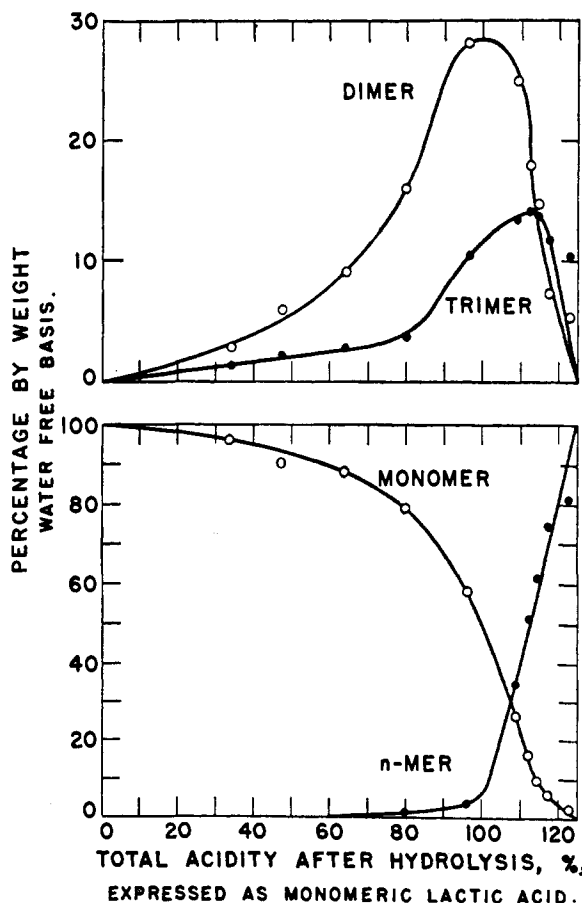


Fig. 2.—Percentage of monomer, dimer, trimer and *n*-mer in lactic acid-water systems.

a maximum of 28% of the total. The trimer reached a maximum of 14% at 113% total available lactic acid. At the concentration of lactic acid in which the amount of dimer was maximum, the proportion of the *n*-mer component began to increase rapidly; and at the hypothetical 125% total available lactic acid, the *n*-mer would be the sole component. This condition is approached in the concentrated lactic acids studied. The highest concentration of available lactic acid investigated, 123%, contained less *n*-mer and more trimer, dimer, and monomer than would be predicted from Fig. 2. This condition would suggest an unequilibrated system, which was somewhat surprising, since this sample was heated at 125° for 73 hours.

Esters of molecular weight higher than lactyl-lactylactic acid cannot be separated by this procedure. This is not surprising when one considers the similarity in solubilities of homologous polymers of comparable molecular weight. Bezzi<sup>3</sup> fractionated the polymers by adding water or petroleum ether to an acetone solution of polylactic acid. Watson<sup>5c</sup> added petroleum or terpene hydrocarbons to acetone or benzene solutions. Because of these results, fractionation by partition chromatography of the *n*-mers was attempted; mixtures of petroleum ether (b.p. 63–70°) and chloroform were used for development. In this way, it was possible to isolate a series of fractions of esters which gradually decreased in degree of polymerization, but in no case was the isolation of a single component possible.

### Experimental

**Preparation of Lactic Acid Solutions.**—All solutions were prepared from 79.6% edible grade lactic acid. The solutions of lower concentration were equilibrated by heating the diluted solution under reflux for 24 hours. Lactic acid solutions of 95 to 118% total available acidity were prepared by heating the 79.6% solution at 80–115° at 0.1–12 mm. pressure until the required degree of dehydration had been reached. The highest concentration of lactic acid prepared (122.9%) was the result of distilling the 79.6% solution in a tensimeter still<sup>8</sup> at 0.02–0.20 mm. pressure. The distillation was continued until the temperature of the still residue reached 175°. During the latter stages of the distillation, lactide crystallized on the cooler parts of the still. The non-volatile residue, a dark brown glass-like material at room temperature, was equilibrated at 125° for 73 hours.

The total lactic acid content in each solution prepared above was determined in the usual manner by saponification with 0.1 *N* sodium hydroxide at 100° for 30 minutes.

**General Analytical Procedure.**—The procedure used for preparing the chromatographic column was similar to that of Marvel and Rands.<sup>7</sup> To 20 g. of silicic acid in a mortar was added 11 ml. of distilled water, dropwise with constant grinding until thoroughly mixed. A fine slurry, prepared by stirring in 80 ml. of chloroform (saturated with water), was poured into a chromatographic tube of 2.1-cm. inside diameter and 48 cm. long. The tube was tapped to facilitate the escape of air bubbles. An air pressure of 20–30 cm. was applied until 30–40 ml. of chloroform was collected, at which point the sides of the tube were cleaned and rinsed with 20 ml. of wet chloroform. The final settling of the column was carried out at a pressure of 150–175 cm.

A sample of lactic acid solution containing 70–90 mg. of total available acid was dissolved in 1 ml. of wet chloroform. If the concentration of the solution was below 97%, 0.4 ml. of *n*-butanol was added to the sample, followed by 0.6 ml. of dry chloroform, thus preventing the formation of two layers because of the water present. The solution was carefully added to the top of the column and forced into the

column under a little pressure. The sample container was rinsed with two 1-ml. aliquots of wet chloroform, each being forced into the column as before.

The chromatogram was developed with the following sequence of solvent mixtures, each of which had been saturated with water: 1, 200 ml. chloroform; 2, 100 ml. 5% *n*-butanol–95% chloroform v./v.; 3, 100 ml. 10% *n*-butanol–90% chloroform v./v.; 4, 150 ml. 25% *n*-butanol–75% chloroform v./v.

For the complete separation of *n*-mer in 123% lactic acid, it was necessary to precede this development by that described below for the fractionation of higher polymers. The free acidity of each 10-ml. unit fraction of eluate was determined by titration with 0.02 *N* aqueous sodium hydroxide solution to phenol red end-point. A small predetermined blank was applied to each titration to correct for acidic materials in the solvents and from the column. Ethanol was added before and during titration to maintain a homogeneous system. Figure 1 shows a typical plot of milliequivalents of free acid per unit fraction versus milliliter of effluent.

The unit fractions corresponding to each component in the sample were combined and boiled to remove the chloroform and ethanol, water being added periodically to the solution. The resulting aqueous solution was boiled long enough to ensure that the chloroform was completely removed, by which time the lactic acid esters were partially hydrolyzed. The hydrolysis was completed by saponification with 0.02 *N* sodium hydroxide. The total available lactic acid in each component was given by the total free acidities of the corresponding unit fractions together with the acid produced by hydrolysis of the ester linkages. A small amount of ester was usually present in the monomer fraction, and it was assumed that the dimer had not been completely separated. This ester titration was therefore added to that of the dimer.

The amount of each component, calculated as monomeric lactic acid, was expressed as percentage of the total acid eluted from the column. Variations of the four components with the concentration of the lactic acid solutions are shown in Fig. 2.

The degree of polymerization of the components in the solution was calculated from the free acidity and total acidity after hydrolysis. The average value for the series of analyses was 2.0 for the dimer and 2.9 for the trimer. The degree of polymerization of the *n*-mer increased with the concentration of lactic acid; it was 9.1 for 122.9% lactic acid.

**Fractionation of the Higher Polymers in 117.4% Lactic Acid.**—The chromatographic column was prepared as before, except that wet petroleum ether (b.p. 63–70°) was used to slurry the silicic acid–water mixture. The lactic acid (0.135 g.) was placed on the column in the usual way, and the developing liquid was increased in polarity as follows: 1, 100 ml. 40% chloroform–60% petroleum ether (v./v.); 2, 200 ml. 50% chloroform–50% petroleum ether (v./v.); 3, 100 ml. 60% chloroform–40% petroleum ether (v./v.); 4, 100 ml. 70% chloroform–30% petroleum ether (v./v.); 5, 100 ml. 80% chloroform–20% petroleum ether (v./v.); 6, 100 ml. 90% chloroform–10% petroleum ether (v./v.). The fractionation data are summarized in Table I.

Unit 10-ml. fractions	Average degree of polymerization	Total available acid, %
6–11	13.2	6.3
12–14	11.6	8.6
15–23	9.2	18.2
24–30	>7.6 <sup>a</sup>	>10.1 <sup>a</sup>
31–37	5.9	9.4
38–55	4.8	5.8
56–61	2.5	6.3
62–71	2.6	6.4

<sup>a</sup> Some of this fraction was lost during hydrolysis.

**Acknowledgment.**—The author is indebted to E. M. Filachione for helpful discussions and to R. A. Ronca for technical assistance.

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(8) W. P. Ratchford and C. E. Rehberg, *Anal. Chem.*, **21**, 1417 (1949).