tissue by cold alkaline iodine oxidation without first isolating the lignin.

2. Iodoform has been isolated as one of the products resulting from alkaline iodine oxidation of lignins prepared from different plant materials by several different methods.

3. Carbon tetrabromide resulted from the alkaline bromine oxidation of lignin.

4. A secondary group of the type $CH_3CH(OH)$— is probably present in the lignin molecule.

AMES, IOWA RECEIVED JULY 20, 1934

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF NEBRASKA]

A Study of the Types of Lactones Formed from the Monobasic Sugar Acids under Varying Conditions

BY JOHN M. BRACKENBURY AND FRED W. UPSON

The salt of a monobasic sugar acid can in some cases be treated so as to yield either the free acid, the normal lactone (butylene oxide ring), or the abnormal lactone (amylene oxide ring).[1] This paper presents data to show that the rate, extent and type of lactone formation is dependent on the solvent, the temperature, and the concentration, as well as the particular nature or configuration of the acid under consideration. The data were obtained by means of the polariscope from solutions of the free sugar acids in water, dilute mineral acid and concentrated acetic acid. The concentration and temperature have been varied in some cases.

Hudson[2] has shown that the sign of rotation of a lactone is governed by the position of the ring and this in turn is dependent upon the position of the hydroxyl group involved in lactone formation. Therefore, if a free acid in solution undergoes lactone formation involving first a hydroxyl group to the right of the carbon chain and then lactone formation involving a hydroxyl group to the left of the carbon chain, the rotation of the solution should become first more positive and then more negative than the free acid. Since *d*-mannonic and *d*-gluconic acids were known to form two isomeric lactones, Levene and Simms[3] undertook a quantitative study of the rate and type of lactone formation of these and a number of other sugar acids. By applying Hudson's rule to the data obtained on observing the change of rotation in solution of the free acids prepared from their salts, they concluded that the δ-lactone is formed first with a much greater velocity but in smaller amount than the γ-lactone. Isbell and Frush[4] have recently published the results of an extensive study of several sugar acids and their lactones. Their paper deals primarily with methods of preparation of these compounds, using dioxane as a new solvent in this type of work. Their study includes polarimetric readings in water solution only.

The following table, graphs and discussion deal with the types and extent of lactone formation from the free sugar acids in water, in the presence of aqueous acids, and in acetic acid solutions. In some cases the influence of temperature and concentration has also been considered.

Experimental

Acetic acid solutions of *d*-mannonic, *d*-gluconic and α-*d*-glucoheptonic acids were prepared in the following manner. One gram of the anhydrous crystalline sodium salt of the sugar acid was weighed into a 25-cc. volumetric flask. After solution of the salt in 4 cc. of distilled water, sufficient glacial acetic acid was added to make a total volume of 25 cc. Polarimetric readings on the solutions were made in a two-decimeter tube.

The acetic acid solution of *d*-galactonic acid was made from sodium galactonate having two molecules of water of crystallization. The solution was made in the same manner as described above with the exception that 6 cc. of water was added instead of 4 cc. This was found necessary to prevent the galactonic acid from immediately crystallizing out of solution. Similarly, 10 cc. of water was used in making the acetic acid solution of α-*l*-rhamnohexonic acid from the crystalline sodium rhamnohexonate.

The concentrated solution (14.60%) of glucoheptonic acid was made by dissolving 4.000 g. of sodium glucoheptonate in 5 cc. of water and then adding sufficient glacial acetic acid to make a total volume of 25 cc.

The other acetic acid solutions were made from the calculated weight of the crystalline lactone or free acid dissolved in sufficient 1 *N* sodium hydroxide to effect complete neutralization. To this solution was added sufficient glacial acetic acid to make a total volume of 25 cc.

The solution in excess of mineral acid was made by dissolving the sodium salt in 0.5 *N* sulfuric acid in the cases

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[1] Brackenbury and Upson, THIS JOURNAL, **55**, 2512 (1933).

[2] Hudson, *ibid.*, **32**, 338 (1910).

[3] Levene and Simms, *J. Biol. Chem.*, **65**, 31 (1925).

[4] Isbell and Frush, *Bur. Standards J. of Res.*, **11**, 649 (1933).

of mannonic, gluconic, glucoheptonic, rhamnohexonic and galactonic acids. In all the other cases the 0.5 *N* sulfuric acid was added to the concentrated solution of the sodium salt made from the free acid or lactone as described above. In both cases a sufficient amount of the acid was added to make a total volume of 25 cc. and the calculated amount of material was used to make a 4% solution of the sodium salt.

The solution of free rhamnohexonic acid in water was made by dissolving 1 g. of the recrystallized acid in distilled water at 25°.

The solution of free *l*-rhamnonic acid in water was made by warming 0.6417 g. of rhamnonic γ-lactone in a 20-cc. volumetric flask with 5.1 cc. of 1 *N* sodium hydroxide and allowing the solution to stand for twenty hours. The flask was immersed in water at 25° and 10.2 cc. of 0.5 *N* hydrochloric acid added and the sufficient distilled water to make a total volume of 20 cc.

The data in Table I were obtained by dissolving 1 g. of *d*-mannonic γ-lactone in 8 cc. of water and quickly adding glacial acetic acid to make a total volume of 25 cc. It was possible to keep the lactone in solution when this quantity of water was used provided the solution was not stirred.

No mechanism for temperature control was used for the determinations made at 25° since room temperature remained close to this point. For the determination made at 0°, the tube was placed with a thermometer in a tray in the refrigerator. The temperature was noted to remain near 0°. Polarimetric readings were made in 2-dm. tubes with the aid of a high grade triple-field polariscope. These readings were reproducible with a maximum variation of $\alpha \pm 0.03°$.

In Table I are given the data obtained with the various solutions of mannonic acid. In the case of the other acids and lactones studied the data were obtained in just as complete form but are here omitted and expressed only in the curves of Figs. 1, 2 and 3. Thus the number of polarimetric readings varied from twenty-five in the case of *l*-rhamnonic acid to thirty-six in the case of *l*-arabonic acid. From four to six readings were taken during the first hour when mutarotation is most rapid. About ten additional readings were taken during the first twenty-four hours, after which they were taken at daily intervals for eight to ten days. Final readings were taken at the end of nine or ten days in some instances and in others after eighteen to twenty-four days.

TABLE I

d-MANNONIC ACID

Time	$[M]^{25}_D/100$ in acetic acid soln.	$[M]^{25}_D/100$ in water solution[a]	$[M]^{25}_D/100$ in 0.5 *N* H_2SO_4	$[M]^{0}_D/100$ in acetic acid soln.
0 min.		−0.6		
5 min.	+4.1	+2.4	+25.6	+3.9
15 min.	12.0	9.6	40.3	
30 min.	25.0	17.0	60.3	
45 min.	37.3	24.0	62.7	
1 hr.	46.5	28.0	65.5	
1.5 hr.	63.5	38.0	66.3	
2 hr.	78.2	44.0	67.8	
3 hr.	93.2		69.8	
4 hr.	102.7	55.0	71.8	
5 hr.	108.3	58.0	73.2	
6 hr.	111.1[b]		75.0	
7 hr.	113.4		76.2	
8 hr.	113.7		77.1	
24 hr.	111.0	62.6	85.1	54.0
30 hr.	106.5	64.0	85.2	
2 days	101.0	66.0	85.2	75.0
4 days	92.0	73.4		94.0
6 days	84.8	77.2		105.0
7 days		79.0		103.0
13 days				102.0[d]
Final	84.8[c]	84.0	85.2	...

[a] Levene and Simms, *J. Biol. Chem.*, **65**, 31 (1925).

[b] At this time a part of this solution yielded the δ-lactone when a crystal of the δ-lactone was stirred into the solution.

[c] No δ-lactone could be obtained from this solution. On evaporation crystals of the γ-lactone formed.

[d] At this time the solution containing 1.0 g. of the sodium salt yielded 0.135 g. of the δ-lactone on stirring with a crystal of the δ-lactone and allowing the mixture to stand in the refrigerator overnight.

Discussion

The experimental data for mannonic acid are presented in Table I and expressed graphically in Fig. 1 together with that for gluconic acid.

Table I and Fig. 1, curves I–III, show that a higher positive maximum rotation is reached in an acetic acid solution of mannonic acid than in water solution. Since the δ-lactone has a higher positive value than the γ-lactone or the free acid, the data indicate that mannonic acid is converted more slowly but to a greater extent into the δ-lactone in acetic acid solution than in water solution. The decrease in molecular rotation after ten hours may be explained by the simultaneous formation of the γ-lactone, which has a lower positive value than the δ-lactone. Isolation of the γ-lactone from the final equilibrium mixture shows that a gradual conversion of the δ-lactone into the γ-lactone must be taking place.

That this conversion takes place also when the pure δ-lactone is dissolved in an acetic acid was established by independent experiment, since the rotation of the δ-lactone is lower than that of the γ. There is a change of molecular rotation in acetic acid solution from an original value of +189.2° to +102° in twenty hours and to a final value of +75° after fifteen days. The values obtained for the pure δ-lactone also show that the molecular rotation of the pure δ-lactone in acetic acid solution is comparable with the molecular rotation in water solution at the same temperature.

A comparison of curves I–III of Fig. 2, with curves I–III of Fig. 1, shows the striking similarity between the behavior of solutions of *d*-mannonic and *l*-rhamnonic acids. Since rhamnonic acid has the enantiomorphic configuration of mannonic acid, except for a hydrogen in place of the hydroxyl on the terminal carbon atom, the tendency toward lactone formation should be similar for the two acids.

Curves IV, V and VI of Fig. 1 show that gluconic acid has a higher final rotation in acetic acid solution than in water solution. Since the lactones both have higher positive rotations than the free acid, the data indicate that gluconic acid is converted into its lactones to a greater extent in acetic acid solution than in water solution. Isolation of the δ-lactone from an acetic acid solution of the free acid indicates that in this solution the final equilibrium mixture contains the δ-lactone as possibly the chief constituent.

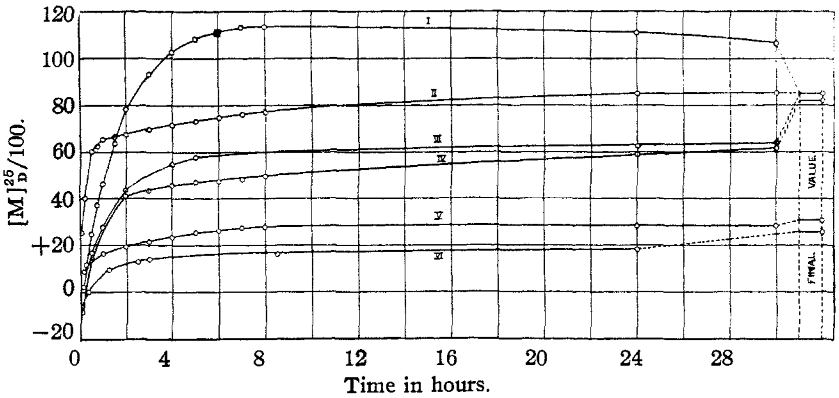

Fig. 1.—*d*-Mannonic acid: I, in acetic acid at 25°; II, in 0.5 *N* H_2SO_4 at 25°; III, in water at 25° [Levene and Simms, *J. Biol. Chem.*, **63**, 31 (1925)]. *d*-Gluconic acid: IV, in acetic acid at 25°; V, in 0.5 *N* H_2SO_4 at 25°; VI, in water at 25° [Rehorst, *Ber.*, **61**, 163 (1928)]. (■, At this point the mannonic δ-lactone crystallized from a portion of the solution on inoculation with a trace of the crystalline substance.)

Since, according to Hudson's "Lactone Rule,"[5] the δ-lactone of glucoheptonic acid should have a positive rotation, while the γ-lactone has been shown to have a negative rotation, optical data on this acid may be interpreted readily. The data expressed in curves IV–VIII of Fig. 2 were obtained by varying the conditions under which lactone formation of the free acid took place.

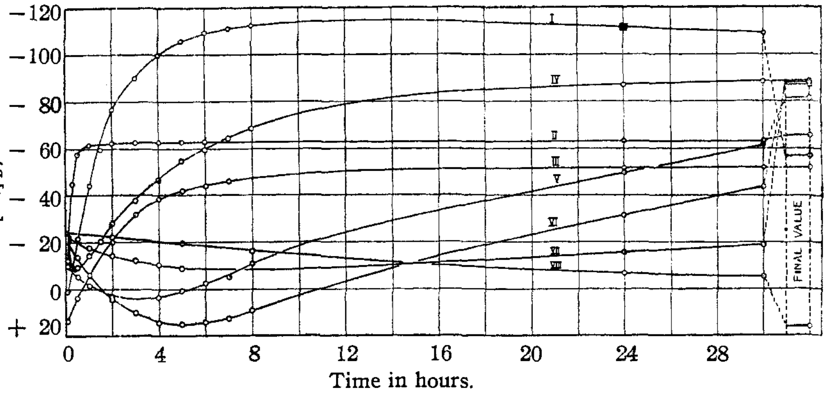

Fig. 2.—*l*-Rhamnonic acid: I, in acetic acid at 25°; II, in 0.5 *N* H_2SO_4 at 25°; III, in water at 25°. α-*d*-Glucoheptonic acid: IV, in 0.5 *N* H_2SO_4 at 25°; V, in 14.6% soln. in acetic acid at 25°; VI, in 3.65% soln. in acetic acid at 25°; VII, in water at 25° [Levene and Simms, *J. Biol. Chem.*, **65**, 31 (1925)]; VIII, in acetic acid at 0°. (■, At this point rhamnonic δ-lactone crystallized from a portion of the solution on inoculation with a trace of the pure substance.)

The data show: (1) a gradual mutarotation to a higher positive maximum value in acetic acid solution than in water solution, followed by a change to a high negative value. This indicates that the formation of the δ-lactone takes place more slowly but to a greater extent in acetic acid solution than in water or dilute mineral acid, since the δ-lactone has a positive value while the γ-lactone and free acid have negative values for their rotations; (2) a more gradual mutarotation in the positive direction in acetic acid at 0° such that even after eight days no γ-lactone has formed. This indicates that the reaction is much slower at 0° than at 25° and eventually reaches an equilibrium between the δ-lactone and the free acid; (3) that in a 14.60% solution of the free glucoheptonic acid in acetic acid solution, a positive maximum value for the rotation is reached. This is not quite as large in a positive direction as the maximum obtained from a 3.65% solution. This indicates that in a 14.60% solution of the free acid in acetic acid the formation of the δ-lactone is less than in a 3.65% solution.

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[5] Hudson, THIS JOURNAL, **32**, 338 (1910).

Since the data for α-*l*-rhamnohexonic acid, expressed in curves I and II of Fig. 3, show that the rotation of the acid becomes first more negative and then more positive, and since the γ-lactone has been shown to have a positive rotation,[6] the data indicate that the δ-lactone has a negative rotation. This is in accord with Hudson's "Lactone Rule." The data show a greater negative value for the acetic acid solution than for the water solution. This indicates that the δ-lactone is formed to a greater extent in the acetic acid solution than it is in the water solution.

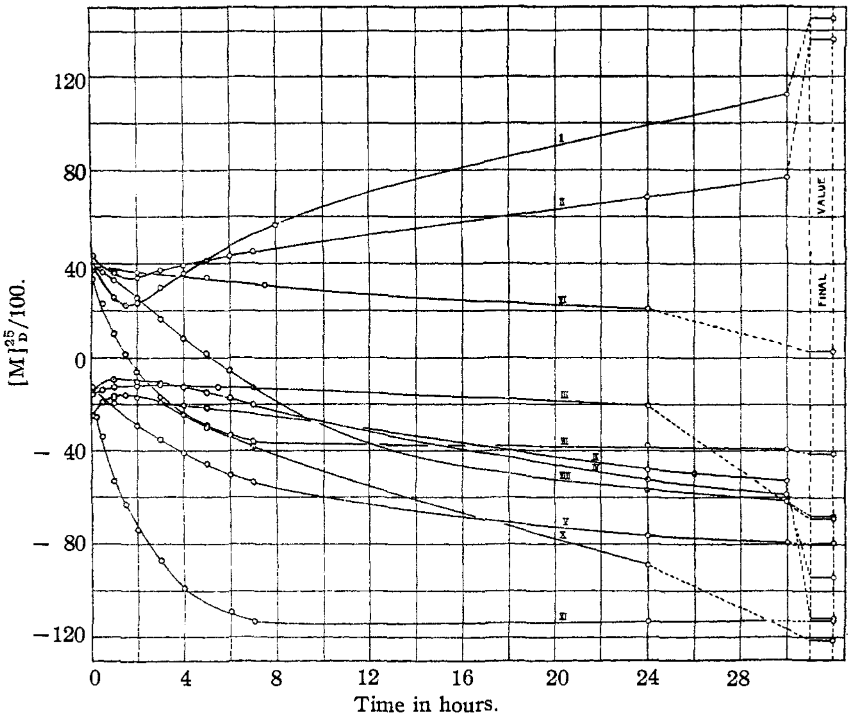

Fig. 3.—α-*l*-Rhamnohexonic acid: I, in acetic acid at 25°; II, in water at 25°. *l*-Arabonic acid: III, in water at 25° [Rehorst, *Ber.*, **63**, 2279 (1930)]; IV, in acetic acid at 25°; V, in 0.5 *N* H_2SO_4 at 25°. *d*-Talonic acid: VI, in water at 25° [Cretcher and Renfrew, THIS JOURNAL, **54**, 1590 (1932)]; VII, in 0.5 *N* H_2SO_4 at 25°; VIII, in acetic acid at 25°. *d*-Galactonic acid: IX, in water at 25° [Levene and Simms, *J. Biol. Chem.*, **65**, 31 (1925)]; X, in acetic acid at 25°; XI, in 0.5 *N* H_2SO_4 at 25°.

The data expressed in curves III–V of Fig. 3, for arabonic acid, in curves VI–VIII for talonic acid, and in curves IX–XI for galactonic acid, indicate that δ-lactone formation is not much greater in acetic acid solution than in water solution.

Summary

1. The lactone formation of the free monobasic sugar acids in acetic acid solution has been observed polarimetrically.

2. A comparison of the data obtained in acetic acid solution with those recorded in the literature has been made and the conclusions drawn that the solvent may influence the rate, type and extent of lactone formation. The effect of temperature and concentrations has also been considered.

3. Data have been derived for the lactone formation of *l*-rhamnonic acid and α-*l*-rhamnohexonic acid in water solution.

4. The existence of a δ-lactone of α-*l*-rhamnohexonic acid having the sign of rotation in accord with Hudson's "Lactone Rule" has been shown.

LINCOLN, NEBRASKA RECEIVED JULY 26, 1934

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[6] Fischer and Piloty, *Ber.*, **23**, 3102 (1890).