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

THE PREPARATION AND PROPERTIES OF *l*-MANNONIC AND *l*-GLUCONIC LACTONES[1,2]

BY FRED W. UPSON, LILA SANDS AND CARRELL H. WHITNAH

RECEIVED OCTOBER 17, 1927 PUBLISHED FEBRUARY 4, 1928

Fischer[3] first prepared crystalline *l*-mannonic lactone from *d*-arabinose while van Ekenstein and Blanksma[4] isolated it later in the preparation of *l*-mannose.

The *l*-gluconic acid was isolated by Fischer in the form of a sirup. Nef[5] and also Hedenburg[6] prepared two crystalline modifications of *d*-mannonic lactone and also of *d*-gluconic lactone. The two modifications of *d*-mannonic lactone are fully described by Hedenberg.[6] One, which is undoubtedly the γ-lactone, possesses an initial specific rotation of $+51.8°$ in water and melts at 151°. The rotation changes only very slowly. The second lactone possesses an initial rotation of $+111.8°$ which diminishes very rapidly in water solution to a value of $+84.8°$ in less than two hours and to $+29.5°$ at the end of twenty-six hours. Hedenburg gave to this lactone provisionally a propylene oxide structure. As shown in another part of this paper this lactone should be formulated in all probability as an amylene oxide or δ-lactone.

The present paper gives an account of the properties of the corresponding lactones of *l*-mannonic and *l*-gluconic acids prepared from *l*-arabinose by the Kiliani reaction. *l*-Gluconic acid lactone has been prepared for the first time in a crystalline state and a tetramethyl mannonic acid lactone prepared and its properties determined.

Experimental Part

Addition of Hydrogen Cyanide to *l*-Arabinose.—*l*-Arabinose was treated with hydrogen cyanide according to the directions of Fischer.[3] The resulting nitriles were converted to the mixed acids by the usual process through hydrolysis with barium hydroxide. The sirup obtained after precipitation of the barium as sulfate yielded two fractions (A) of nearly pure crystalline *l*-mannonic lactone. The residual sirups were thoroughly extracted with absolute alcohol, leaving behind a thick, colloidal residue. The alcoholic extracts yielded two more fractions (B) of crystalline products which were mixtures of *l*-mannonic and *l*-gluconic lactones. The filtrates (C) resulting here were converted to the brucine salts and yielded crude brucine *l*-gluconate, from

---

[1] Presented before the Organic Division of the American Chemical Society at the Washington Meeting, April, 1924; abstract in *Science*, **60**, 49 (1924).

[2] Constructed from theses presented by Lila Sands and Carrell H. Whitnah in partial fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Nebraska in 1924 and 1925, respectively.

[3] Fischer, *Ber.*, **23**, 273 (1890).

[4] Van Ekenstein and Blanksma, *Chem. Weekblad.*, **11**, 902 (1914).

[5] Nef, *Ann.*, **403**, 309 (1914).

[6] Hedenburg, THIS JOURNAL, **37**, 345 (1915).

which crystalline *l*-gluconic lactone was first obtained. The various fractions of brucine salt were subjected to fractional crystallization either from water or alcohol. There were obtained from 100 g. of *l*-arabinose 58.8 g. of pure *l*-mannonic lactone and 8.8 g. of crystalline *l*-gluconic lactone. Fischer obtained a yield of 40 g. of *l*-mannonic lactone, but *l*-gluconic lactone was isolated only in sirupy form.

l-Mannonic Lactones

***l*-Mannonic-γ-lactone.**—The crystalline fractions (A) described in the first part of this paper, which consisted of almost pure *l*-mannonic lactone, were purified by recrystallization from absolute alcohol and then glacial acetic acid and converted to the γ-lactone according to the procedure of Nef.[6] A pure white crystalline lactone melting sharply at 150.5–151° and possessing a specific rotation of —51.8° was obtained. No significant change in rotation occurred during a period of two days.

Titration.—The lactone reacted very slowly with alkali at room temperature and required a temperature of approximately 100° to complete the titration.

Anal. 0.2064 g. required 11.85 cc. of 0.0972 *N* NaOH; calcd. for $C_6H_{10}O_6$: 11.93 cc. Calcd. for $C_6H_{10}O_6$: H, 5.61; C, 40.45. Found: H, 5.57; C, 40.69.

The properties of this lactone show it to be the enantiomorph of the *d*-mannonic γ-lactone obtained by Nef and by Hedenburg. The fact that neutralization proceeds very slowly at room temperature and also the slow change in rotation prove that these lactones, the one a *d*- and the other an *l*-mannonic lactone, possess in all probability the stable or butylene oxide structure.[7]

Tetramethyl-*l*-mannonic γ-Lactone.—The *l*-mannonic γ-lactone obtained as described above was methylated by means of methyl iodide and silver oxide according to the method of Purdie and Irvine.[8] The process required two treatments with methyl iodide and silver oxide using methyl alcohol as a solvent and a further treatment with methyl iodide as the solvent. This process gave a tetramethyl *l*-mannonic methyl ester. The ester group was hydrolyzed by boiling for half an hour with a slight excess of 0.5 *N* sodium hydroxide. Hydrochloric acid exactly equivalent to alkali was added and the whole distilled under reduced pressure to complete dryness at 100°. The methylated lactone was extracted from the sodium chloride with ether and the ether solution dried. Upon evaporation this solution deposited the methylated lactone in a crystalline condition. This compound was shown by analysis to be a tetramethylated lactone.

Titration.—The compound titrated like a lactone, that is, only a very small amount of alkali was taken up at room temperature and complete neutralization could be accomplished only on heating to 100°.

Anal. 0.1820 g. required 7.43 cc. of 0.103 *N* NaOH. Calcd. for $C_{10}H_{18}O_6$: 7.54 cc. Calcd. for $C_{10}H_{18}O_6$: H, 7.71; C, 51.28. Found: H, 7.8; C, 50.88.

Methoxyl determination: Calcd. for $C_{10}H_{18}O_6$: methoxyl, 52.99. Found: 53.7.

This tetramethyl *l*-mannonic lactone crystallizes in long, narrow, colorless plates which melt at 109°. It is readily soluble in ether and alcohol, less soluble in water.

The specific rotation in water solution six minutes after solution was $-65.51°$; after thirty-four hours $-64.11°$; while at the end of five days the rotation had changed to $-60.6°$, becoming constant after eighteen days with $[\alpha]_D^{20} = -47.4°$. This slow change in specific rotation, together with the fact that the titration could not be com-

---

[7] Pryde, *J. Chem. Soc.*, **123**, 1811 (1923).

[8] Purdie and Irvine, *J. Chem. Soc.*, **83**, 1026 (1903).

pleted at 25°, is evidence that the compound possesses the butylene oxide or γ-lactone structure.

Tetramethyl *d*-Mannonic γ-Lactone.—For purposes of comparison this compound was prepared by direct methylation of *d*-mannonic γ-lactone, m. p. 151°. The methylation was carried out using methyl iodide and silver oxide as already described for the *l*-mannonic lactone. The compound was obtained in the form of colorless plates; m. p. 107°.

Anal. Methoxyl determination, calcd. for $C_{10}H_{18}O_6$: methoxyl, 52.99. Found: 52.34.

Titration.—0.2120 g. required 9.1 cc. of 0.1 *N* NaOH. Calcd. 9.05 cc. The specific rotation of this compound in water solution was found to be +66.6° immediately after solution. After six hours the value was +64° and after twenty-four hours +63°. A graph in Fig. 1 shows the change of rotation with time.

The High Rotating Mannonic Lactones.—Hedenburg,[6] by the action of oxalic acid on calcium *d*-mannonate, prepared a second lactone of *d*-mannonic acid, characterized by a high initial specific rotation of +111.8° which diminished in the course of twenty-six hours to the value of +29.3°. This lactone also was neutralized much more readily with alkali. These facts indicate an unstable lactone and led Hedenberg to formulate it as a propylene oxide or β-lactone. The evidence obtained in this investigation leads to the conclusion that this lactone is in all probability an amylene oxide or δ-lactone.

The Active *l*-Mannonic Lactone.—This lactone was prepared according to Hedenburg's method and found to consist of microscopic, colorless plates which melt at 160–162°.

Anal. Calcd. for $C_6H_{10}O_6$: H, 5.62; C, 40.45. Found: H, 5.71; C, 40.68. The substance titrated more readily than did the γ-lactone; 0.1020 g. required 5.5 cc. of 0.103 *N* NaOH. Calcd. for $C_6H_{10}O_6$: 5.6 cc.

The lactone in aqueous solution has a high initial specific rotation, −113.6°, this diminishing to the value −102.5 after an hour and twenty minutes. At the end of three and one-half hours the value had become −78.5°. At the end of thirty-two and one-half hours the minimum value of −30.9° was reached, after which it slowly increased to the value −40.9 after twenty-eight days. The change in rotation of this *l*-mannonic lactone is thus shown to parallel the change of rotation of the active *d*-lactone as observed by Hedenberg. A curve in Fig. 1 shows more concretely the nature of the change.

Active Tetramethyl *d*-Mannonic Lactone.—Tetramethyl *d*-mannose was prepared according to the method of Haworth[9] by means of methyl sulfate and sodium hydroxide. This compound was oxidized by means of bromine as described by Pryde.[10] After removal of hydrobromic acid by means of lead carbonate and silver oxide in the usual way, the methylated oxidation product was extracted with ether and the ethereal solution subjected to fractionation finally at a pressure of 1–2 mm. The tetramethyl *d*-mannonic lactone was obtained as a colorless oil which showed no tendency to crystallize.

Anal. Calcd. for $C_{10}H_{18}O_6$: H, 7.77; C, 51.28; methoxyl, 52.99. Found: H, 7.76; C, 50.62; methoxyl, 52.83.

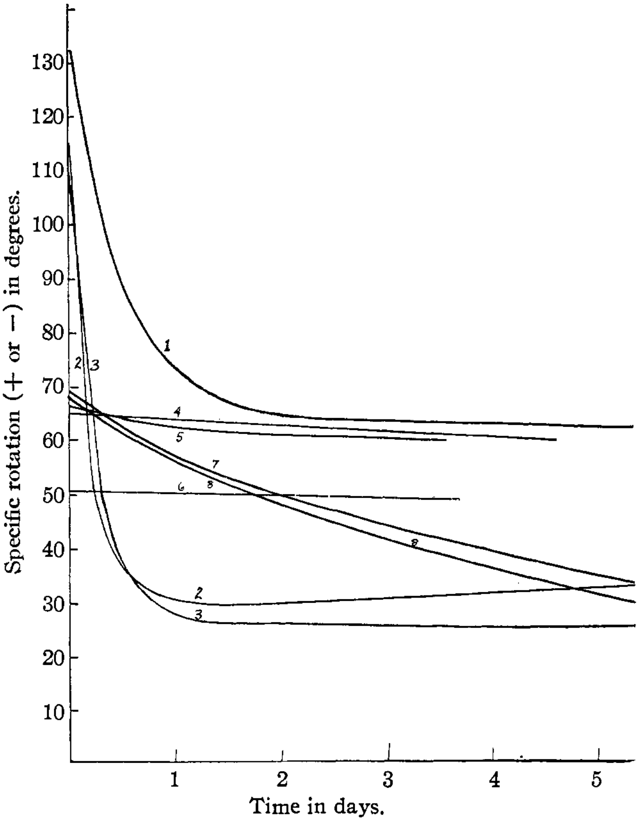
---

[9] Haworth, *J. Chem. Soc.*, **107**, 8 (1915).

[10] Ref. 7, p. 1808.

The titration proceeded rapidly in the cold until about three-fourths complete; 0.2282 g. required 9.75 cc. of 0.0972 *N* NaOH; calcd. for $C_{10}H_{18}O_6$, 9.9 cc.

The initial specific rotation five minutes after solution was +132.3°. The specific rotation diminished rapidly, changing to the value +113.6° after four and one-half hours and to −76.3° after twenty-two hours. It reached a minimum value of +59.5° after about six days and at the end of nine days the value had become +63.6°. A curve in Fig. 1 shows more concretely the change of rotation with time. An inspection of the graph brings out the fact that the curves belong to two distinct types. Those belonging to the normal lactones of *d*- and *l*-mannonic acids, as well as the methylated derivatives, are almost horizontal and show little falling off. The rotations of the two abnormal lactones and of the methoxyl derivative of the *d*- variety have a much higher initial value and the slope of the curves is very steep at first, becoming more nearly horizontal after twenty hours.



1. Tetramethyl *d*-mannonic δ-lactone; 2. *d*-mannonic δ-lactone; 3. *l*-mannonic δ-lactone; 4. tetramethyl *d*-mannonic γ-lactone; 5. tetramethyl *l*-mannonic γ-lactone; 6. *d*-mannonic and *l*-mannonic γ-lactones; 7. *l*-gluconic γ-lactone; 8. *d*-gluconic γ-lactone.

Fig. 1.—Curves showing change of rotation of various lactones with time.

Structure of the Active Mannonic Lactones.—The common lactones of the sugar acids are formulated as butylene oxides of γ-lactones because of their comparative stability towards alkalies and because of the slow change in rotation which they undergo in water solution. Evidence of this view is presented by Hedenberg[6] and by Pryde,[7] and the theory is further supported by Hudson's rule.[11]

According to the rule of Hudson the position of the lactone bridge in the sugar acid lactones determines the sign of rotation. Pryde has shown in the case of galactonic lactone that the two tetramethyl derivatives, the one obtained by the oxidation of methylated galactose and the other by direct methylation of the lactone, differ in sign of rotation. According to Hudson's rule the 1-5 lactone should rotate to the right and the 1-4 to the left. In the case of the lactones of mannonic acid both the 1-4 and the 1-5 should be dextro-rotatory. It seems certain, therefore, that the active *d*-mannonic lactone of Nef and Hedenburg as well as the active *l*-mannonic lactone here reported together with the tetramethyl lactone obtained by oxidation of tetramethyl mannose, all possess the amylene oxide or 1-5 lactone structure. This leads logically to the conclusion that crystalline mannose possesses the amylene oxide or 1-5 lactone structure. Levene and Meyer[12] have reached the same conclusion based on a study of these same methylated lactones. The constants of their tetramethyl 1,4-lactone agree closely with those determined by us. The second compound of Levene and Meyer, obtained on oxidation of tetramethyl mannose, shows a lower specific rotation, +105°, than the value found in this Laboratory, +132°. It is possible that their product contained some free acid, which accounts for the lower rotation. They give no data showing the change of rotation with time.

Lewis and Greene[13] have recently prepared tetramethyl mannose in a crystalline form. From this they have obtained by oxidation with bromine a tetramethyl lactone in the form of a sirup which shows an initial specific rotation of +136.4° and a final value of +62.4°. These results correspond closely with those found in this Laboratory and their lactone is undoubtedly identical with the one reported above.

It should be noted that Nef[14] in 1914 first suggested that other lactone bridges than those of the γ or butylene oxide type are possible among the members of the sugar group and that Nef and also Hedenburg[6] were the first to obtain representatives of these abnormal lactone types in their studies of mannonic and gluconic lactones. The methylation studies on the mannonic lactones both in this Laboratory and elsewhere connect

---

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

[12] Levene and Meyer, *J. Biol. Chem.*, **60**, 167 (1924).

[13] Lewis and Greene, *Science*, **64**, 206 (1926).

[14] Ref. 5, p. 317.

definitely the one lactone type of the acid with that of its parent sugar. As pointed out by Charlton, Haworth and Peat,[15] the normal lactone type of the sugar apparently belongs to the γ or amylene oxide form, while the stable normal lactones of the monobasic sugar acids belong to the γ or butylene oxide type. In only one other case, that of glucose, has the unmethylated acid lactone been obtained which corresponds to the normal lactone type of the sugar.[16]

l-Gluconic Lactone

l-Gluconic lactone, as already indicated in the first part of this paper, has been isolated for the first time in a crystalline condition. The mother liquors (C) obtained as already described, from which no more crystalline mannonic lactone could be obtained were converted to brucine salt in the usual way. On crystallization of the brucine salts from absolute alcohol, several fractions melting at temperatures ranging from 150–180° were obtained. As the larger portion of the mannonic lactone had already been removed by crystallization, the crystalline brucine salts obtained here contained very little brucine *l*-mannonate. The whole of the crystalline brucine salt was converted to the free lactone in the usual way through treatment with barium hydroxide and subsequent removal of the barium through precipitation as sulfate. The gluconic lactone was obtained first as a thick gum which showed evidence of crystallization after standing for some days under absolute alcohol. On long standing there was obtained a considerable quantity of crystalline lactone, which could be removed by filtration with vacuum. After the lactone was once obtained it was readily recrystallized from absolute alcohol or glacial acetic acid. The latter solvent was found to give a pure product. The lactone was obtained in the form of colorless plates melting at 134–135°. The initial specific rotation in 3.79% solution was −68.7°. The rotation diminished to the value of −62.5° after twenty-four hours, to the value −58.2° after two days and continued to diminish for some days. The minimum value −13.7° was reached after a period of fifteen days, after which there was a slow increase in the value. This change in rotation closely paralleled that for the *d*-gluconic lactone as recorded by Hedenburg,[6] who found an initial value of −67.2°.

Titration.—0.2017 g. of lactone required 11.24 cc. of 0.0972 *N* NaOH; calcd., 11.48 cc.

A number of derivatives of *l*-gluconic lactone were prepared. Two g. of lactone was converted to the brucine salt. After recrystallization from 90% alcohol and drying *in vacuo* the salt melted sharply at 181–182°; $[\alpha]_D^{20} = -25.43$ in 4.01% solution.

The brucine salt of *l*-mannonic acid was also prepared for purposes of comparison. Its melting point was found to be 161–162° and $[\alpha]_D^{20} = -15.78°$ in 3.98% solution.

The phenylhydrazide of *l*-gluconic acid was prepared in the usual way. On recrystallization from water it melted sharply at 200° and $[\alpha]_D^{20} = -11.7°$ in water solution of 2.97%. The melting point of the hydrazide of *d*-gluconic acid is 200° and the specific rotation is +12°. The results recorded above indicate very definitely that the compound is the phenylhydrazide of *l*-gluconic acid. The properties of the lactone, together with those of its brucine salt and phenylhydrazide, indicate clearly that the above-described compound is the crystalline lactone of *l*-gluconic acid.

Summary

1. The lactones of *l*-mannonic and *l*-gluconic acids were prepared from *l*-arabinose in a somewhat better yield than had been obtained previously.

---

[15] Charlton, Haworth and Peat, *J. Chem. Soc.*, **129**, 97 (1926).

[16] Ref. 5, p. 323.

2. Two lactones of *l*-mannonic acid were prepared: the common lactone having a butylene oxide ring and the less stable lactone having an amylene oxide ring.
3. Evidence for the structure of the two lactones was substantiated by the preparation of two distinct tetramethyl mannonic lactones.
4. *l*-Gluconic lactone was prepared for the first time in crystalline form and its constants were determined.

LINCOLN, NEBRASKA

[CONTRIBUTION FROM THE LABORATORY OF PHYSICAL CHEMISTRY OF THE UNIVERSITY OF UPSALA]

THE MOLECULAR WEIGHTS OF PHYCOERYTHRIN AND OF PHYCOCYAN

BY THE SVEDBERG AND N. B. LEWIS[1]

RECEIVED OCTOBER 27, 1927 PUBLISHED FEBRUARY 4, 1928

Phycoerythrin and phycocyan are closely related proteins of the globulin type which occur together in certain algae. They are both fluorescent and have characteristic colors; the color and fluorescence are quite strong even in 0.01% solution. On account of this strong light absorption both in the visible and the ultraviolet and of the relative ease of preparing them in a pure crystalline state they appeared to be particularly suitable for molecular weight determinations by means of the ultracentrifuge.

There is a series of references in the literature to these substances dating back to that of Kützing;[2] but they appear to have first been prepared in a pure state by Kylin.[3] In this paper he gives an account of their preparation from *Ceramium rubrum*, of their chemical properties, absorption spectra and a quantitative analysis of phycoerythrin. It also includes a complete list of the earlier references.

Kitasato[4] used another alga, *Porphyra tenera* (the sun-dried material being an article of commerce in Japan) as raw material, and the substances were prepared by a method analogous to that of Kylin. His observations in general, and his analysis of phycoerythrin, agree with those of Kylin. Further, he was able to make an analysis of phycocyan, which occurs in much smaller quantities (*Ceramium rubrum*, according to Kylin, about $^1/_{10}$ of that of phycoerythrin); his figures for this are very close to those for phycoerythrin.

Experimental

Preparation of Material.—*Ceramium rubrum* was extracted with water as described by Kylin. The extract, consisting of a solution of the mixed substances as well as

---

[1] Fellow of the International Education Board.

[2] Kützing, *Phycologia Generalis*, Leipzig, **1843**.

[3] Kylin, *Z. physiol. Chem.*, **69**, 169 (1910).

[4] Kitasato, *Acta Phytochimica*, **2**, 75 (1925).