

[CONTRIBUTION FROM THE HYGIENIC LABORATORY, U. S. PUBLIC HEALTH SERVICE]

RELATIONS BETWEEN ROTATORY POWER AND STRUCTURE
IN THE SUGAR GROUP. XXIV. THE TWO CRYSTALLINE
LACTONES OF *l*-RHAMNONIC ACID^{1,2}

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Recently we undertook the preparation of *l*-rhammonic amide in order to determine its exact rotation. Chernoff³ showed that the amide is dextro-rotatory in agreement with the amide rule of rotation^{3,4} but the magnitude was not reported. Starting with pure *l*-rhamnose it was planned to prepare crystalline rhammonic lactone, which has been described by several investigators⁵ (m. p. 150–151° and $[\alpha]_D -39^\circ$), and to convert this lactone to the amide. The beautifully crystalline lactone which we obtained readily in good yield proved to be a different substance, however, as it melted much higher (172–181°) and showed an initial $[\alpha]_D -98^\circ$, which decreased fairly rapidly during the first day and then very slowly decreased to -30° at the end of eleven weeks, the temperature being 25–30°. The aqueous solution of the substance was neutral to litmus immediately after preparation at room temperature but it developed an acid reaction after about one minute. Its titration corresponded to the value calculated for rhammonic lactone. The crystals are evidently a second lactone of *l*-rhammonic acid, the first lactone being the one for which Schnelle and Tollens⁵ gave the earliest accurate description.

A thorough study of the two lactones, especially of the conditions of preparation which favor the formation of each, was undertaken. It led to a most unexpected result. The second lactone, which we at first believed to be a new substance, was unquestionably in the hands of Will and Peters forty years ago, because their accurate crystallographic measurements of the crystals of their *l*-rhammonic lactone apply closely to our crystals of the second lactone but do not agree at all with measurements, now made for the first time, of crystals of the first lactone.⁶ Will and Peters, in an article previous to the one in which they record the crystal measurements, state that their lactone melts at 148°. From this statement it seems evident at the present time that they must have had both

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² No. XXIII was published in *THIS JOURNAL*, 51, 3631 (1929).

³ Hudson, *ibid.*, 40, 813 (1918).

⁴ Hudson, *ibid.*, 39, 465 (1917).

⁵ Will and Peters, *Ber.*, 21, 1813 (1888); 22, 1704 (1889); Raýman, *ibid.*, 21, 2046 (1888); Schnelle and Tollens, *Ann.*, 271, 71 (1892); Fischer and Herborn, *Ber.*, 29, 1962 (1896).

⁶ The crystallographic measurements were made by F. E. Wright and are reported by him in the paper immediately following this.

crystalline lactones under observation and, regarding them as a single substance, measured the melting point of one sample (which happened to be the first lactone) and the crystal angles of a different sample (which happened to be the second lactone). During the progress of our investigation of these lactones, the recent article of Votoček and Beneš⁷ came to our attention and it was recognized at once that the supposedly new *l*-rhamnonic lactone which they have prepared by the reduction of *l*-rhamno-ketonic acid with sodium amalgam is the same substance that we have met with, namely, the lactone which Will and Peters measured crystallographically, the second lactone. Votoček and Beneš have shown that Kiliani⁸ recently had this lactone in hand, through the same method of preparation from *l*-rhamno-ketonic acid, though he did not recognize it as belonging in the rhamnose series but considered it to be a lactone of gulomethylonic acid. Since our study approached the subject in a different way from that undertaken by Votoček and Beneš, who have stated that they are continuing the investigation of the lactones, we decided to complete those phases of our work which concern the rotations, structures and interconversions of these lactones and the preparation of rhamnonic amide from each of them.

The first lactone ($[\alpha]_D - 39.7^\circ$) undergoes only slight change in aqueous solution in the course of several weeks at 25–30°, its specific rotation decreasing to -38.4° in six weeks. From this fact it seems reasonably certain, following the views of Nef,⁹ that the first lactone is of the 1,4-ring type. The second lactone ($[\alpha]_D - 98^\circ$) exhibits a much larger and more rapid mutarotation than does the first and is the lactone to which rhamnonic acid initially changes when liberated from solutions of its salts. These facts lead us to assign provisionally the 1,5-ring structure to the second lactone, following the views of Levene and Simms.¹⁰ The evidence for the 1,5-ring structure would be strengthened if the possibility of a 1,3-ring structure for these rapidly mutarotating lactones of the sugar group could be excluded, but in our opinion this cannot now be done.

There is a striking relationship between the rotations of the two *l*-rhamnonic lactones and those of the two *d*-mannonic lactones which Nef⁹ prepared with much care in 1914. The *d*-mannonic lactone of $[\alpha]_D + 52^\circ$ is the one of greater stability, to which the 1,4-ring structure is commonly assigned, and the *d*-mannonic lactone of $[\alpha]_D + 111^\circ$ presumably possesses the 1,5-ring. Writing the configurations in the *d*-series and reversing therefore the signs of our measurements, which pertain to the *l*-rhamnose series, it is seen that there is a close parallelism here between the *d*-mannose

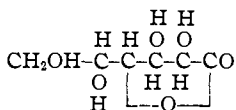
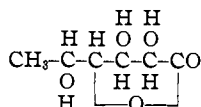
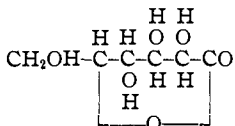
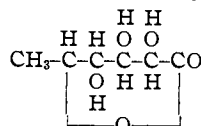
⁷ Votoček and Beneš, *Bull. soc. chim.*, [4] **43**, 1328 (1928).

⁸ Kiliani, *Ber.*, **55**, 2822 (1922).

⁹ Nef, *Ann.*, **403**, 306 (1914).

¹⁰ Levene and Simms, *J. Biol. Chem.*, **65**, 31 (1925).

and *d*-rhamnose derivatives. The change of structure of the terminal group from CH₂OH to CH₃ (this carbon atom being symmetric and with-

*d*-Mannonic (1,4)-lactone, $[\alpha]_D +52^\circ$ *d*-Rhammonic (1,4)-lactone, $[\alpha]_D +40^\circ$ *d*-Mannonic (1,5)-lactone, $[\alpha]_D +111^\circ$ *d*-Rhammonic (1,5)-lactone, $[\alpha]_D +98^\circ$

out rotation) decreases the specific rotation by about 12–13° whether the ring is 1,4 or 1,5.

In our initial preparation, which yielded the second *l*-rhammonic lactone, rhamnose was oxidized by bromine water containing barium benzoate as a buffer to prevent the occurrence of strong mineral acidity during the oxidation.¹¹ Under these conditions rhammonic acid forms its labile 1,5-lactone. If the buffer is omitted, the free hydrobromic acid which is produced during the oxidation of the sugar catalyzes the change to the stable 1,4-lactone. It has proved possible in these ways to prepare from rhamnose either crystalline lactone at will, or a mixture of them which is readily separated into its constituents by crystallization from hot acetone, in which the first lactone is much more soluble. Starting with crystals of the first or stable 1,4-lactone, the second may be produced by forming in solution a salt of rhammonic acid (*e. g.*, lead rhamnonate), decomposing this in the cold by an appropriate precipitant of the metal (*e. g.*, hydrogen sulfide) and concentrating the aqueous solution under diminished pressure to crystallization. Starting with crystals of the second or labile 1,5-lactone, dissolving them in dilute hydrochloric acid and evaporating the solution to dryness on the steam-bath yields a crystalline residue which consists almost entirely of the first lactone. Omitting the hydrochloric acid, this change takes place partially when an aqueous solution of the second lactone is evaporated on the steam-bath. These methods of preparation illustrate again the close analogy between the lactones of the rhamnose and mannose series, because the methods are indeed those which Nef⁹ devised for the interconversion of the two mannonic lactones.

The reaction of ammonia with either the first or the second *l*-rhammonic lactone dissolved in absolute alcohol yields *l*-rhammonic amide. This result confirms the view that we are dealing with two isomeric lactones of *l*-rhammonic acid. The rotation of the amide in water was found to be

¹¹ Hudson and Isbell, *THIS JOURNAL*, **51**, 2225 (1929).

$[\alpha]_D^{20} + 27.7^\circ$, which agrees with the amide rule of rotation. Its melting point is 134–134.5°.

Experimental Part

Oxidation of Rhamnose with Bromine Water in the Presence of Barium Benzoate.—

Eight cc. of bromine was added to an ice cold solution of 24.4 g. of pure rhamnose monohydrate and 80 g. of barium benzoate in 1 liter of distilled water. The bromine dissolved on shaking during fifteen minutes and the solution was kept in the dark at room temperature for about forty hours. It then gave only a slight reduction of Fehling's solution. The excess bromine was removed with a stream of air, the benzoic acid was filtered off and the barium was precipitated quantitatively with 5 *N* sulfuric acid (about 82 cc.). After the addition of 10 g. of decolorizing carbon, the barium sulfate was filtered off by suction. The hydrobromic acid was neutralized with silver carbonate (54 g.), the silver bromide was filtered off and excess silver was removed as sulfide. The solution was left overnight in the refrigerator and then distilled under diminished pressure to about 250 cc. (bath temperature 75–80°). It was extracted with chloroform to remove benzoic acid and distilled under reduced pressure until crystallization began (40–50 cc.). After the solution had stood overnight in an evacuated desiccator over calcium chloride, the crystals, consisting entirely of the second rhammonic lactone, were filtered off and washed with a little acetone. The mother liquor was concentrated under reduced pressure to about 10 cc. (bath temperature 45–50°) and evaporated to dryness in a vacuum desiccator. The residue was a mixture of the two rhammonic lactones containing 0.2 g. of material insoluble in hot acetone, probably a salt. The lactones were separated by a fractional crystallization from acetone. The yield of the second lactone was 11.9 g. or 55% of the theoretical and of the first lactone was 4.8 g. or 22%. The second lactone melts at 172–181°. The melting point varies somewhat with the manner of heating and is not a sharp criterion of purity.

Anal. Calcd. for $C_6H_{10}O_6$: C, 44.43; H, 6.22. Found: C, 44.25; H, 6.11.

In aqueous solution the second lactone remains neutral to litmus for about one minute. Its titration with sodium hydroxide solution corresponds to rhammonic lactone: 0.3914 g. of substance required 24.66 cc. of 0.1 *N* sodium hydroxide, the calculated amount being 24.14 cc. It shows $[\alpha]_D^{23} - 98.4^\circ$ seven minutes after solution (0.7910 g. of substance, 25 cc. of solution in water, 2-dcm. tube; rotation, 6.23° to the left). The rotation gradually falls and after six hours at room temperature $[\alpha]_D^{24}$ is -61.0° . At the end of eleven weeks $[\alpha]_D^{28}$ is -30.1° . When a solution of the second lactone in water had stood for about seven weeks it was distilled under reduced pressure to dryness and the residue (1.8 g.) was recrystallized from acetone. This gave 1.1 g. of the first lactone and only 0.5 g. of the second lactone, showing that the more stable first lactone had formed in solution at the expense of the unstable second lactone.

The first lactone melts at 148–150° and shows $[\alpha]_D^{23} - 39.7^\circ$ eight minutes after solution (0.9856 g. of substance, 25 cc. of solution in water, 2-dcm. tube; rotation, 3.13° to the left). After seventy-five hours $[\alpha]_D^{21}$ was -38.9° and at the end of six weeks $[\alpha]_D^{23}$ was -38.4° .

The first lactone is much more soluble in acetone than the second. The solubilities were determined at 1.0 = 0.5°. Five cc. of anhydrous acetone was found to dissolve 0.0677 g. of the first lactone from undersaturation and 0.0693 g. from supersaturation, which gives 1.37 g. as the average value for 100 cc. of acetone. Ten cc. of anhydrous acetone dissolved 0.0142 g. of the second lactone from undersaturation and 0.0157 g. from supersaturation, the mean value for 100 cc. of acetone being 0.1495 g.

The two lactones do not appear to form mixed crystals from acetone. Crystals characteristic of each pure substance separated side by side during their fractional

crystallization. On one occasion these were separated mechanically as well as possible. One portion melted at 166–172° and showed $[\alpha]_D^{24} -94.7^\circ$, while the other melted at 145–147° and gave $[\alpha]_D^{24} -43.4^\circ$.

In preparing the second lactone directly from rhamnose by oxidation with bromine water in the presence of a buffering salt, it is necessary to avoid conditions of high mineral acidity acting for a prolonged time during the various separations, because the change to the first lactone is catalyzed by strong acids. For example, a preparation was carried out by the indicated directions except that the solution was kept at room temperature for about forty hours after the quantitative precipitation of the barium with sulfuric acid and before the removal of the hydrobromic acid with silver carbonate; the resulting lactone was the first or stable one, obviously because the free hydrobromic acid had catalyzed its production from the labile or second lactone during the forty hours of standing.

The oxidation of rhamnose by bromine water in the absence of a buffering salt is a slow process and the resulting lactone is principally the first form, though a small amount of the second lactone is also obtained. In one experiment the directions of Fischer and Herborn⁵ were accurately followed; from 6 g. of pure rhamnose monohydrate and 12 g. of bromine in 36 cc. of water, after standing for three days at room temperature, a yield of 57% of first lactone (m. p. 146–149°) and 6% of second lactone (m. p. 168–173°) was obtained. The lactones were separated by the use of acetone. This experiment explains how Will and Peters could have had both lactones in hand, since they oxidized rhamnose under similar conditions; it seems probable that the other earlier investigators also crystallized both lactones in the mixed state. Indeed, when the crystals of the two lactones are once recognized by the eye it seems remarkable that the separate identity of the second form has been so long overlooked.

Conversion of the Second Rhammonic Lactone to the First.—One g. of pure second lactone was dissolved in 15 cc. of distilled water to which was added 0.3 cc. of concentrated hydrochloric acid. The solution was evaporated to dryness on the water-bath and the solid crystalline residue was heated with occasional stirring until a test with litmus paper showed no volatile acidity. The substance was thrice washed with ether to remove possible traces of hydrogen chloride. The product was almost colorless; m. p., 149–151°. It showed $[\alpha]_D^{23} -40.1^\circ$. After one recrystallization from acetone it melted at 147–148° and gave $[\alpha]_D^{22.5} -39.0^\circ$, values which indicate a complete conversion of the second lactone to the first. These directions are those which Nef⁹ devised for converting the second mannonic lactone into the first and it is seen that they apply excellently to the similar transformation in the rhamnose series.

Conversion of the First Rhammonic Lactone to the Second.—A solution of 2 g. of pure first lactone in 50 cc. of distilled water in which was suspended 5 g. of pure basic lead carbonate was boiled under a reflux condenser for two hours. After filtering off excess lead carbonate the lead rhammonate was decomposed by passing hydrogen sulfide through the ice cold solution. The filtered solution was distilled immediately under reduced pressure to a thin sirup, the bath temperature at the end being 40–45°. On evaporating the sirup to dryness in a vacuum desiccator, 1.9 g. of solid resulted. After recrystallization from acetone the yield of second lactone was 1.8 g.; m. p. 168–173°. Again, these directions are the same in principle as those which Nef⁹ devised for converting the first mannonic lactone into the second; where he decomposed a calcium salt with oxalic acid we preferred to use a lead salt and hydrogen sulfide.

Preparation of *l*-Rhammonic Amide from the First and from the Second Rhammonic Lactone.—To 150 cc. of absolute ethyl alcohol, saturated with dry ammonia gas, 4.7 g. of the second lactone melting at 167–173° was added. The lactone dissolved on shaking and the rhammonic amide which crystallized from the solution was filtered off and the

filtrate concentrated under reduced pressure to a small volume. The crystals thus obtained were filtered off and the filtrate evaporated to dryness in an evacuated desiccator. The yield of crude rhammonic amide was theoretical. It was recrystallized to constant rotation from absolute alcohol, from which it crystallized as colorless plates. After drying in an evacuated desiccator over calcium chloride it melted at 126–129°. On drying to constant weight in a vacuum at 76° in the presence of phosphorus pentoxide, it lost 4.51% in weight and then melted at 134–134.5° and gave $[\alpha]_D^{20} +27.7^\circ$ (0.6043 g. of substance, 25 cc. of solution in water, 2-dcm. tube; rotation, 1.34° to the right).

Anal. Calcd. for $C_6H_{13}O_5N$: C, 40.20; H, 7.31; N, 7.82. Found: C, 40.02; H, 7.29; N (Kjeldahl), 7.89.

The repetition of this preparation, using 4 g. of the first lactone, gave a theoretical yield of the same *l*-rhammonic amide of m. p. 134–134.5° and $[\alpha]_D^{25.5} +27.6^\circ$. A mixed melting point of the amides from the two lactones showed no depression. The amide is soluble in water, nearly insoluble in cold absolute alcohol or ether and soluble in hot absolute alcohol.

Summary

The oxidation of *l*-rhamnose with bromine water containing barium benzoate as a buffer yields a second crystalline lactone of *l*-rhammonic acid, of m. p. 172–181° and $[\alpha]_D -98^\circ$ in aqueous solution. It mutarotates rapidly and therefore probably possesses the 1,5-ring. The well-known first rhammonic lactone, of m. p. 148–150° and $[\alpha]_D -40^\circ$, is of the stable type showing very slow mutarotation and it therefore probably possesses the 1,4-ring. Methods for producing either lactone at will from the oxidation of rhamnose with bromine water are shown and Nef's methods for converting each of the two lactones of mannonic acid into the other are found to be applicable to the rhammonic lactones. A comparison of the rotations of the two rhammonic lactones with the two mannonic lactones shows an interesting relation between structure and rotation. Pure *l*-rhammonic amide, of m. p. 134–134.5° and $[\alpha]_D^{20} +27.7^\circ$, is described. Its sign of rotation agrees with the amide rule.

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