Synthesis of LTalose.(1)

By Shozo FUKUNAGA and Bennosuke KUBOTA.

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Among the sixteen theoretically possible aldohexoses, l-talose has been the last one yet unknown, nor have its derivatives been described except l-talomucic acid. Although its antipode d-talose has been already formed⁽²⁾⁽³⁾ from d-galactose derivatives easily obtainable from naturally occurring d-galactose, l-talose has not yet been synthesized, because no convenient natural source or no easy synthetical method has yet been found for preparing l-galactose or its derivatives in any large quantity.

The most convenient method of obtaining l-galactose derivatives seems to us to consist first in the reduction of d-galacturonic acid into l-galactonic acid as shown below:

⁽¹⁾ l-Talonic acid and some of its derivatives were already reported, J. Chem. Soc. Japan, 57 (1936), 551. See also this Bulletin, 12 (1937), 116.

⁽²⁾ W. Bosshard, Helv. Chim. Acta, 18 (1935), 482.

⁽³⁾ P. A. Levene and T. S. Tipson, J. Biol. Chem., 93 (1931), 631; J. Komada, this Bulletin, 7 (1932), 211.

d-Galacturonic acid.

l-Galactonic acid.

While it was obtained by W. N. Haworth, E. L. Hirst and their collaborators⁽⁴⁾ from d-galactose by means of oxidation of its diacetone compound and subsequent reduction of d-galacturonic acid so formed, we have found in the course of our investigations in monoses⁽¹⁾ that it can be more easily prepared from barium salt of d-galacturonic acid directly obtained by hydrolysis of citrus pectic acid. We made attempt therefore to synthesize l-talose from l-galactonic acid prepared by this method.

Citrus pectic acid which is obtained from citrus pectin (orange pectin) by partial hydrolysis, is a polymeride of d-galacturonic acid described by K. P. Link and his collaborators. Being hydrolized with dilute sulphuric acid, it gives d-galacturonic acid which is isolated in its barium salt according to Link's method. As it is rather unstable and very difficultly purified, the crude barium salt is submitted to reduction, directly after it has been converted into sodium salt, with 2.5 percent sodium amalgam. l-Galactonic acid so formed is purified through its cadmium salt, which, treated with hydrogen sulphide, gives l-galactonic lactone in crystalline form. It forms needles melting at $109.5-111^{\circ(6)}$ with $[a]_{0}^{16}+77^{\circ}$ (c=1.76 in water, after five minutes), and seems to be anhydrous form.

There are two methods of obtaining *l*-talose from *l*-galactonic acid. As it is more difficult and roundabout to synthesize *l*-talose through triacetyl-*l*-galactal according to Levene's method⁽³⁾ in his synthesis of *d*-talose, *l*-galactonic acid is submitted to epimerization in order to turn it into *l*-talonic acid, the lactone of which is then reduced to *l*-talose in the same way as Bosshard did in his synthesis of *d*-talose,⁽²⁾ as follows:

The epimerization is carried out by refluxing *l*-galactonic acid solution directly obtained from its cadmium salt for fifty hours with pyridine in the oil bath of 135° and *l*-talonic acid partially formed is conveniently separated in its potassium salt according to Bosshard, after the greater part of unchanged *l*-galactonic acid is removed in its cadmium salt.

⁽⁴⁾ J. Chem. Soc., 1933, 1419, most recently by C. Glatthaar and T. Reichstein, Helv. Chim. Acta, 20 (1937), 1537.

⁽⁵⁾ K. P. Link and P. Nedden, J. Biol. Chem., 94 (1931), 307.

⁽⁶⁾ All the melting points given in this paper are corrected.

l-Talonic acid crystallizes in needles with half a molecule of water like its antipode, melting at 124.5-125°, but when recrystallized from absolute ethyl alcohol, the anhydrous acid is obtained, white powder melting at 137–138° with $[a]_{\rm D}^{\rm HS}$ – 18.2° (c=3.53 in water, after five minutes). *l*-Talonic lactone can be crystallized in prisms melting at 133-134.5° with $[a]_{0}^{pp} + 34.4^{\circ}$ (c = 1.17 in water, after five minutes). Following some derivatives of l-talonic acid are obtained. Amide, white powder melting at 119-120.5° with $[a]_{D}^{18} + 13.3^{\circ}$ (c = 1.59 in water). Phenylhydrazide, prisms melting at 161–162° with $\lceil a \rceil_{D}^{B^{\circ}} + 26.0^{\circ}$ (c = 2.21 in water). According to O. F. Hedenburg and L. H. Cretscher, (7) the brucine salt of d-talonic acid is not a suitable derivative for the identification of the acid though many workers have written about it, because of its hygroscopic property and some uncertainty about its purity. On the contrary, the brucine salt of l-talonic acid is obtained in beautiful crystals from dilute ethyl alcohol which is by no means hygroscopic. It forms plates melting at 139-140° with $[\alpha]_{\rm D}^{\rm Ho}$ - 23.7° (c = 3.25 in water), having the composition $C_6H_{12}O_7 \cdot C_{23}H_{26}O_4N_2 \cdot 2H_2O$.

The reduction of l-talonic lactone is carried out according to M. Steiger's advice described in preparation of d-ribose. (8) l-Talose is separated in its o-nitrophenylhydrazone. It forms orange leaflets melting at 148–149° with $[a]_D^{40} - 88^\circ$ (c = 0.171 in methyl alcohol).

⁽⁷⁾ J. Am. Chem. Soc., 49 (1927), 478.

⁽⁸⁾ Helv. Chim. Acta, 19 (1936), 193.

l-Talose is obtained in a colourless syrup with sweet taste from the o-nitrophenylhydrazone by decomposition with benzaldehyde. We have not yet succeeded in crystallizing it within a month notwithstanding our utmost efforts, while W. Bosshard described also that d-talose obtained by the similar method did not crystallize of itself without seeding. (9)

The equilibrium rotation of l-talose is calculated therefore from the reading of the l-talose solution directly obtained by the decomposition of known weight of its o-nitrophenylhydrazone, on the assumption that the conversion is quantitative. The results are $[a]_{D}^{H^o} - 18.6^{\circ}$ (c = 0.809 in water, an hour after decomposition) and $[a]_{D}^{H^o} - 19.8^{\circ}$ (after ten days), quite in agreement with the equilibrium rotation of Bosshard's d-talose, $[a]_{D}^{H^o} + 20.6^{\circ}$ or Levene's, $[a]_{D}^{H^o} + 19.7^{\circ}$.

It seems clear that l-talose, l-talonic acid, l-talonic lactone and their derivatives are now formed. The comparison of their properties with those of d-isomers are shown in Table 1 and 2.

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		Talose	o-Nitrophenylhydrazone			
Authors' l-form	M.p. [α]D	_ -19.8°(14°)	148-149° -88°(14°) (in methyl alcohol)			
Bosshard's d-form(2)	M.p. [α]D	128−130° +ca. 30°→+20.6°(20°)	148.5-149° · · · · · · · · · · · · · · · · · · ·			

Table 1. Talose and its o-nitrophenylhydrazone.

Table 2.	Talania	ooid	and	ita	derivatives.
Table 2.	Laionic	aciu	anu	ıts	derivatives.

		Acid anhydrous	Lactone	Phenyl- hydrazide	Amide	Brucine salt
Authors' l-form	M.p.	137—138°	133—134.5°	161-162°	119-120 5°	139 - 140°
	[α]D	—18.2°(15°)	+34.4°(17°)	+26.6°(13°)	+13.3°(18°)	- 23.7°(14°)
d-Form(10)	M.p.	138°	132—134°	159°	121°	132° (11)
	[α]D	+19.0°(25°)	-34.7°(25°)	-25.4°(25°)	-13.1°(25°)	- 26.15°(20°)

⁽⁹⁾ The seeding crystal he used was obtained by the other method by Levene, see foot-note (2).
(10) From Tollens Elsner, "Kurzes Handbuch der Kohlenhydrate," 4th Ed., 364, Leipzig

<sup>(1935).

(11)</sup> The highest value recorded, but its purity was doubtful, P. A. Levene and G. M. Meyer J. Biol. Chem., 26 (1916), 362: 31 (1917), 624.

Experimental.

Preparation of Barium d-Galacturonate. Citrus pectic acid was hydrolized with dilute sulphuric acid according to K. P. Link and P. Nedden, but their method was a little modified. Citrus pectic acid (80 g.) was gradually added with stirring to boiling 2.5 percent sulphuric acid (4 litres) in a 5 litre flask equipped with a reflux condenser and a stirrer. The solution was refluxed in the oil bath of 110° for fifteen hours, being well stirred to prevent from charring and bumping. After the heating was finished, it was added with some kieselguhr, decanted and filtered from gelatinous residue. The filtration was very difficult, the solution was therefore filtered with a large filter paper over kieselguhr. The filtrate was then neutralized by heating to 70-80° with barium carbonate which was gradually added to the solution with stirring. Thus, we used barium carbonate in place of barium hydroxide solution for the neutralization of the solution, as this method had the advantages that neutralization of sulphuric acid and conversion of d-galacturonic acid formed into its barium salt were carried out at the same time and increase of volume of the solution was avoided. When the solution became not acid to congo red, it was decolourized with active carbon and filtered from barium sulphate after cooled. The filtrate and washings with hot water were united and concentrated in the bath below 50° in vacuum. During the concentration, brown gummy substance was precipitated which was filtered and the filtrate was decolourized twice with active carbon, further concentrated to about 100 c.c. in the bath below 45° in vacuum. After it was warmed with active carbon if necessary, the light brown solution was then poured into four volumes of 95 percent ethyl alcohol. Barium d-galacturonate was precipitated in almost white powder, which was filtered after standing overnight and dehydrated by washing successively with hot 95 percent and absolute ethyl alcohol. As the precipitate was very hygroscopic, the filtration and washing were carried out continuously lest it should be filtered off. But, when it was completely dehydrated, it became not so hygroscopic and was quickly dried by washing with dry ether. Faint yellow powder. Yield about 30 g. This was impure, but used directly for the reduction.

Reduction to l-Galactonic Acid and Separation in its Cadmium Salt. barium d-galacturonate (25 g.) was dissolved in small volume of warm water, filtered from flocculent residue after it was warmed with active carbon for a while and excess of sodium sulphate was added to convert it into sodium salt. The filtrate and washings with hot water were united and the solution (about 500 c.c.) was reduced with 2.5 percent sodium amalgam at room temperature as follows: It was stirred vigorously in a thick-walled flask and 50 g. of the amalgam were added, while the solution was kept all the time to be slightly alkaline being not red to phenolphthalein by neutralizing with 20 percent sulphuric acid. The reduction was continued by adding 50 g. of fresh sodium amalgam each time after the amalgam was almost consumed, until the solution showed no reducing power against Fehling's solution. It took about forty hours and about 800 g. of the amalgam were consumed. The solution was then separated from mercury and filtered over active carbon. The filtrate was neutralized with dilute sulphuric acid to be acid to litmus but not acid to congo red and concentrated in vacuum until sodium sulphate separated out. After it was acidified with 20 percent sulphuric acid to become distinctly blue to congo red, it was poured into two litres of hot 95 percent ethyl alcohol. The alcoholic solution was filtered after standing overnight and the filtrate was concentrated in vacuum to expel alcohol after it was

diluted with water and finally concentrated to about 50 c.c. The excess of sulphuric acid was then removed quantitatively by warming the solution on the water bath with freshly precipitated wet barium carbonate. It was filtered over active carbon, the resulting l-galactonic acid solution was further concentrated in vacuum and heated on the boiling water bath for an hour with the excess of cadmium carbonate. After the evolution of carbon dioxide was ceased, some cadmium hydroxide was added to complete the neutralization. Carbon dioxide was then passed in the hot solution in order to remove the excess of cadmium hydroxide. The hot filtrate and washings with hot water separated cadmium l-galactonate in the cold, which was filtered after left for two days in cold place and washed with cold water. Some quantity of the salt was recovered from the mother liquor when concentrated and left for a long time. Recrystallized from large volume of boiling water. Yield about 9 g. White powder hardly soluble in cold water, having one molecule of water of crystallization. (Found: residue, 39.47. Calc. for (C₀H₁₁O₇)₂Cd·H₂O: CdSO₄, 40.05 %.) When crystallized from dilute solution by gradual evaporation in the air at room temperature, needle crystals aggregating in cubes were obtained, which seemed to be tetrahydrate. (Found: residue, 35.66. Calc. for (C₀H₁₁O₇)₂Cd·4H₂O: CdSO₄, 36.27 %.)

LGalactonic Acid and its Lactone. Cadmium l-galactonate was dissolved in about twenty times of boiling distilled water and quickly cooled. When it began to be turbid, it was decomposed with hydrogen sulphide, excess of which was avoided by testing with lead acetate in order to facilitate the filtration from cadmium sulphide. The filtrate was concentrated in vacuum and filtered over active carbon. Thus we obtained l-galactonic acid solution which separated needle crystals of the acid in the cold. It was used directly for the epimerization in the preparation of l-talonic acid.

The crystalline l-galactonic lactone was obtained as follows: The solution was concentrated in vacuum to a thick syrup and it was heated on the water bath for an hour in vacuum in order to complete the lactonization. The resulting lactone syrup was dissolved in absolute ethyl alcohol, filtered from flocculent precipitate and concentrated to a thick syrup, which began to crystallize in a few days in a vacuum desiccator over sulphuric acid. It was recrystallized twice from absolute alcohol, finally from dry ethyl acetate, and dried over P_2O_5 at 60° in vacuum. Small needles melting at $109.5-111^\circ$, with $[\alpha]_{10}^{18} + 77^\circ$ (35.3 mg. in 2 c.c. aqueous solution, l=2 dm., $\alpha=+2.72^\circ$).

Potassium l-Talonate, Epimerization of l-Galactonic Acid. The l-galactonic acid solution obtained from 30 g. of cadmium l-galactonate as described above, was concentrated to about 120 c.c., added with 9 c.c. pyridine and refluxed in the oil bath of 135° for fifty hours. The reflux condenser was then removed and the brown solution was heated for about twenty minutes with supply of water. When the greater part of pyridine was expelled, 10 g. of cadmium carbonate were gradually added to the solution and it was boiled gently with supply of water for half an hour more and some cadmium hydroxide freshly precipitated was added to neutralize the solution completely. The heating was then ceased and carbon dioxide was passed in the hot brown muddy solution to remove the excess of cadmium hydroxide. It was then left to stand two days in cold place and filtered from cadmium l-galactonate and carbonate. After the brown filtrate and washings with cold distilled water were decolourized with active carbon, the solution was concentrated to a thin syrup in vacuum. This syrup was left in ice-chest for two days and filtered from separated cadmium l-galactonate. Thus, the greater part of unchanged l-galactonic acid was removed, about 9 g.

of its cadmium salt being recovered. The filtrate and washings with small volume of cold distilled water were united and decomposed with hydrogen sulphide. The filtrate from cadmium sulphide was concentrated in vacuum, decolourized with active carbon and treated after W. Bosshard's method for separating talonic acid in its potassium salt as follows: The solution was divided in two portions, the nine tenths of its volume and the remainder. The former was exactly neutralized with carbonate-free potassium hydroxide solution and mixed with the latter. Thus, 90 percent of the acid was converted into its potassium salt. The solution was then concentrated in vacuum, decolourized and finally concentrated to a thin syrup. To this syrup methyl alcohol was added so far as constant turbidity did not occur and it was left in ice-chest. Potassium *l*-talonate crystallized out and was filtered after two days, washed with 70, 80 and 90 percent methyl alcohol respectively. It was dried in the air. Yield 4.7 g., some was recovered from the mother liquor when treated as above. Prisms melting at $166-168^{\circ}$ with $[\alpha]_{10}^{10}-2^{\circ}$, sufficiently pure for the next experiment.

Potassium l-talonate was dissolved in LTalonic Acid from its Potassium Salt. distilled water and a volume of standard sulphuric acid solution slightly less than the calculated was added. The solution was concentrated in the bath below 50° in vacuum until sodium sulphate began to separate and precipitated by pouring into large volume of hot absolute ethyl alcohol. The warm solution was filtered from the precipitated salt, which was redissolved in small volume of warm water and precipitated again with absolute alcohol. The united warm alcoholic extracts separated needle crystals of l-talonic acid in the cold. When the filtrate was concentrated to a thin syrup at low temperature and added with several volumes of absolute alcohol, l-talonic acid crystallized out again. Recrystallized from dilute ethyl alcohol. Needles melting at 124.5-125°. But, when recrystallized from absolute ethyl alcohol, white powder melting at 137-138° with $[a]_{\mathbf{D}}^{\mathbf{D}}$ -18.2° (35.3 mg. in 1 c.c. aqueous solution, l=1 dm., $\alpha = -0.64^{\circ}$ after five minutes) was obtained. The former was hydrate and the latter anhydrous form. (Hydrate, found: C, 35.48; H, 6.63. Calc. for $C_0H_{12}O_7$: $\frac{1}{2}H_2O$: C, 35.28; H, 6.42%. Anhydrous acid, found: C, 36.87; H, 6.23. Calc. for $C_6H_{12}O_7$: C, 36.71; H, 6.17%.)

LTalonic Lactone from LTalonic Acid. The alcoholic solution of *l*-talonic acid obtained in the above experiment was directly used. It was concentrated to a thick syrup in vacuum, which was heated on the boiling water bath for an hour in vacuum to complete the lactonization. The syrup was then dissolved in absolute ethyl alcohol, filtered from flocculent precipitate and concentrated to a thin syrup in vacuum, which was easily crystallized by seeding or by concentrating in a vacuum desiccator. Recrystallized from absolute ethyl alcohol. Prisms melting at $133-134.5^{\circ}$ with $[\alpha]_D^{170} + 34.4^{\circ}$ (23.4 mg. in 2 c.c. aqueous solution, l = 2 dm., $\alpha = +1.61^{\circ}$, after five minutes) changing to $+29.3^{\circ}$ after four days, showing that it was γ-lactone.

Derivatives of LTalonic Acid. Amide. It was precipitated when dry ammonia was passed in the lactone solution in absolute alcohol. Recrystallized twice from absolute alcohol. White powder melting at 119-120.5° with $[\alpha]_D^{18} + 13.3^\circ$ (15.9 mg. in 1 c.c. aqueous solution, l = 1 dm., $\alpha = +0.21^\circ$). (Found: N, 6.80. Calc. for $C_0H_{12}O_7N$: N, 6.63%.)

Phenylhydrazide. The equal weights of l-talonic acid and phenylhydrazine in a small volume of water were heated on the water bath for an hour with one drop of

dilute acetic acid. The phenylhydrazide separated when absolute alcohol was added to the solution. Recrystallized from absolute alcohol. Prisms melting at $161-162^{\circ}$ with $[\alpha]_{18}^{18} + 26.0^{\circ}$ (44.2 mg. in 2 c.c. aqueous solution, l = 2 dm., $\alpha = +1.16^{\circ}$). (Found: N, 9.35. Calc. for $C_{12}H_{18}O_7N_2$: N, 9.27%.)

Brucine salt. The equivalent weights of the lactone and brucine in small volume of water were heated for a while and several volumes of absolute alcohol were added to the solution. The brucine salt was crystallized out when it was left in cold place. Recrystallized by dissolving it in small volume of cold water and adding absolute alcohol. Plates melting at $139-140^{\circ}$ with $[\alpha]_D^{1d^{\circ}}-23.7^{\circ}$ (65.0 mg. in 2 c.c. aqueous solution, l=2 dm., $\alpha=-1.54^{\circ}$). It was dihydrate, but not completely dehydrated by heating at 110° over P_2O_5 in vacuum (weight loss, 4.63%). When heated over its melting point in ordinary pressure, it solidified again after foaming, being completely dehydrated to the anhydrous form which melted towards 180° with decomposition. (Found: N, 4.61; H_2O , 5.79. Calc. for $C_0H_{12}O_7\cdot C_{23}H_{20}O_4N_2\cdot 2H_2O$: N, 4.47; H_2O , 5.75%.)

Reduction of l-Talonic Lactone into l-Talose. l-Talonic lactone (1.6 g.) was dissolved in distilled water (16 c.c.) and cooled to -2° in the freezing mixture. Sodium amalgam (38 g.) prepared from pure mercury in a porcelain mortar was added at a time with vigorous mechanical stirring, while the solution was kept constantly grey to congo red by neutralizing with 20 percent sulphuric acid drop by drop. In order to see the acidity all the time, we used fibres of congo red paper which were well washed and added in the flask at a white background at the beginning. The stirring was continued until the solution showed the reducing power corresponding to about 5 percent glucose solution against Fehling's solution, while the temperature was gradually raised to $+6^{\circ}$. The reduction was then ceased, it took about 25 minutes and the amalgam was almost consumed. It was then separated from mercury, filtered over active carbon and neutralized with dilute sulphuric acid to become acid to litmus, but not acid to congo red. It was concentrated until sodium sulphate separated out, acidified with 20 percent sulphuric acid to become distinctly blue to congo red and gradually added with about eight volumes of absolute ethyl alcohol with stirring. It was filtered from sodium sulphate, which was digested with small volume of water on the water bath and precipitated again with absolute alcohol. The united alcoholic solution was concentrated over freshly precipitated barium carbonate in vacuum after diluted with water. It was then heated on the water bath for an hour with the excess of fresh barium carbonate. After the solution became neutral to litmus, it was filtered over active carbon and concentrated to a thin syrup, to which ten volumes of methyl alcohol were gradually added, digested in a mortar and filtered from precipitated barium l-talonate. The precipitate was dissolved in small volume of water and treated again as above with methyl alcohol. Thus, unchanged lactone was recovered as barium salt (about 0.35 g.). The united methyl alcoholic solution was concentrated to a thin syrup in vacuum. After it was precipitated again with methyl alcohol, the filtrate was concentrated to a syrup in vacuum. This syrup was then dissolved in absolute ethyl alcohol, filtered after standing for a while and concentrated again to a thick syrup. Thus, crude l-talose syrup (about 1.3 g.) was obtained.

l-Talose-o-nitrophenylhydrazone. The l-talose syrup (1.3 g.) was converted into its o-nitrophenylhydrazone by heating it with o-nitrophenylhydrazine in methyl alcohol exactly after Bosshard's method for the d-isomer. Yield 1.4 g., corresponding to 46

percent of the theory when calculated from l-talonic lactone taken in the reduction. Recrystallized from absolute ethyl alcohol. Orange leaflets, melting at 148–149° with $[\alpha]_D^{H^0} - 88^\circ$ (17.1 mg. in 10 c.c. methyl alcohol solution, l = 2 dm., $\alpha = -0.30^\circ$). (Found: N, 13.47. Calc. for $C_{12}H_{17}O_7N_3$: N, 13.33%.)

LTalose from its o-Nitrophenylhydrazone. One gram of the o-nitrophenylhydrazone was heated on the water bath for an hour with 0.65 g. of benzaldehyde and 0.15 g. of benzoic acid in 40 c.c. of distilled water. It was then filtered from separated red voluminous precipitate of benzaldehyde-o-nitrophenylhydrazone and the filtrate was extracted several times with ether. When the aqueous solution was concentrated in vacuum, l-talose was obtained in a colourless syrup with sweet taste. Though we have left the syrup in a vacuum desiccator for a month and endeavoured to crystallize it, it has not yet been crystallized.

The Equilibrium Rotation of *l*-Talose. *l*-Talose-*o*-nitrophenylhydrazone (0.1532 g.) was decomposed with benzaldehyde in 6 c.c. of distilled water and treated as above. This *l*-talose solution was exactly diluted to 10 c.c. With this solution the rotation was directly measured and the specific rotation was calculated assuming that *l*-talose was formed quantitatively. The results were $[\alpha]_D^{46} - 18.6^\circ$ (c = 0.809 in water, l = 1 dm., $\alpha = -0.15^\circ$, an hour after decomposition) and $[\alpha]_D^{46} - 19.8^\circ$ ($\alpha = -0.16^\circ$, after ten days).

Summary.

- (1) l-Talose is formed by the reduction of l-talonic lactone obtained from l-galactonic acid by means of epimerisation. It is a colourless syrup with sweet taste having a specific rotation of -19.8° at 14° .
- (2) *l*-Talonic acid (m.p. 137–138°, $[\alpha]_D^{15^\circ} 18.2^\circ$) and *l*-talonic lactone (m.p. 133–134.5°, $[\alpha]_D^{17^\circ} + 34.4^\circ$) are obtained.
- (3) Brucine salt of *l*-talonic acid (m.p. 139–140°, $[a]_D^{\text{M}^\circ} 23.7^\circ$) is suited for the identification of the acid. The amide (m.p. 119–120.5°, $[a]_D^{\text{M}^\circ} + 13.3^\circ$) and the phenylhydrazide (m.p. 161–162°, $[a]_D^{\text{M}^\circ} + 26.0^\circ$) are also obtained.
- (4) l-Galactonic acid is conveniently prepared from citrus pectic acid.

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Chemical Institute, Faculty of Science, Imperial University of Tokyo.