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Studies on L-Gulonic Acid Derivatives. III.[1] Synthesis of Benzyl 2,4,5,6-Tetra-O-benzyl-L-gulonate and Its Oxidation

Michio Matsui, Masako Saito, Masashi Okada,[2] and Morizo Ishidate[2a]

Tokyo Biochemical Research Institute[2]

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Synthesis of benzyl 2,4,5,6-tetra-O-benzyl-L-gulonate (IX), a prospective starting material leading to β-keto-L-gulonic acid (II), was described. Oxidation of IX with the Pfitzner-Moffatt reagent gave a mixture of β-keto esters (X, XI) epimeric at C-2 and their enol-form, which was debenzylated by catalytic hydrogenation to afford L-xylulose (III), decarboxylation product of II, as the principal product and probably 2,3-diketo-L-gulonic acid (XII).

In the preceding paper[1] it was reported that an attempt to synthesize β-keto-L-gulonic acid (II), a metabolic intermediate in the D-glucuronic acid—L-xylulose (III) cycle of D-glucuronic acid metabolism in mammals, was unsuccessful because of the unforeseen acyl migration which took place in preparing a suitably protected starting material for the synthesis of II. To achieve this aim, therefore, another approach to the subject has been needed.

This paper deals with the synthesis of a benzyl derivative of L-gulonic acid (I) having a single hydroxyl group at C-3, and its oxidation product. Benzyl group was singled out as protecting group, because it does not have any possibility of migration and is stable to many oxidizing agents and, moreover, is easily removed in neutral condition by catalytic hydrogenation.

Treatment of 4,6-O-benzylidene-D-glucitol (IV)[3] with benzyl chloride in the presence of powdered potassium hydroxide afforded 1,2,3,5-tetra-O-benzyl-4,6-O-benzylidene-D-glucitol (V). Selective removal of the benzylidene group from V was effected by hydrolysis with ethanolic hydrochloric acid to give 1,2,3,5-tetra-O-benzyl-D-glucitol (VI). An attempt to oxidize selectively the primary hydroxyl group of VI with oxygen over platinum-on-charcoal catalyst was unsuccessful. Then it was tritylated, and the remaining secondary hydroxyl group was benzoylated. Regeneration of the primary hydroxyl group by treatment with acetic acid followed by oxidation with chromium trioxide in acetone-sulfuric acid gave 2,4,5,6-tetra-O-benzyl-3-O-benzoyl-L-gulonic acid (VII), which was treated in turn with methanolic sodium hydroxide to afford 2,4,5,6-tetra-O-benzyl-L-gulonic acid (VIII). All the foregoing compounds, V—VIII, were obtained as thin-layer chromatographically homogeneous sirup.

Treatment of VIII with benzyl alcohol and dicyclohexylcarbodiimide yielded benzyl 2,4,5,6-tetra-O-benzyl-L-gulonate (IX). It was purified by preparative thin-layer chromatography and obtained also as a sirup, which gave satisfactory result on elemental analysis and exhibited bands at 3550 and 1740 cm^{-1} in the infrared spectrum due to hydroxyl and benzyl ester group respectively. It was observed that when a methanolic solution of IX was evapo-

1) Part II: M. Matsui, M. Okada, and M. Ishidate, *Chem. Pharm. Bull.* (Tokyo), **16**, 1288 (1968).
2) Location: *Takada 3-chome, Toshima-ku, Tokyo*; *a*) Present address: *National Institute of Hygienic Sciences, Tamagawayoga-machi, Setagaya-ku, Tokyo.*
3) J.C. Sowden, *J. Am. Chem. Soc.*, **72**, 808 (1950).

rated *in vacuo*, the benzyl ester was partly converted into the methyl ester. Thus thin-layer chromatographic examination disclosed two new components with the same mobility as the methyl ester of VIII and benzyl alcohol. Furthermore, formation of the methyl ester was evident from the appearance of a singlet at 6.25 τ due to methyl ester and the disappearance of a singlet at 4.79 τ due to methylene protons of benzyl ester in the nuclear magnetic resonance spectrum. The methyl ester was actually isolated as a sirup using preparative thin-layer chromatography and was identified by direct comparison with an authentic sample prepared from VIII. In this connection it was found that addition of a few drops of formic acid to the methanolic solution of IX prevented the ester interchange.

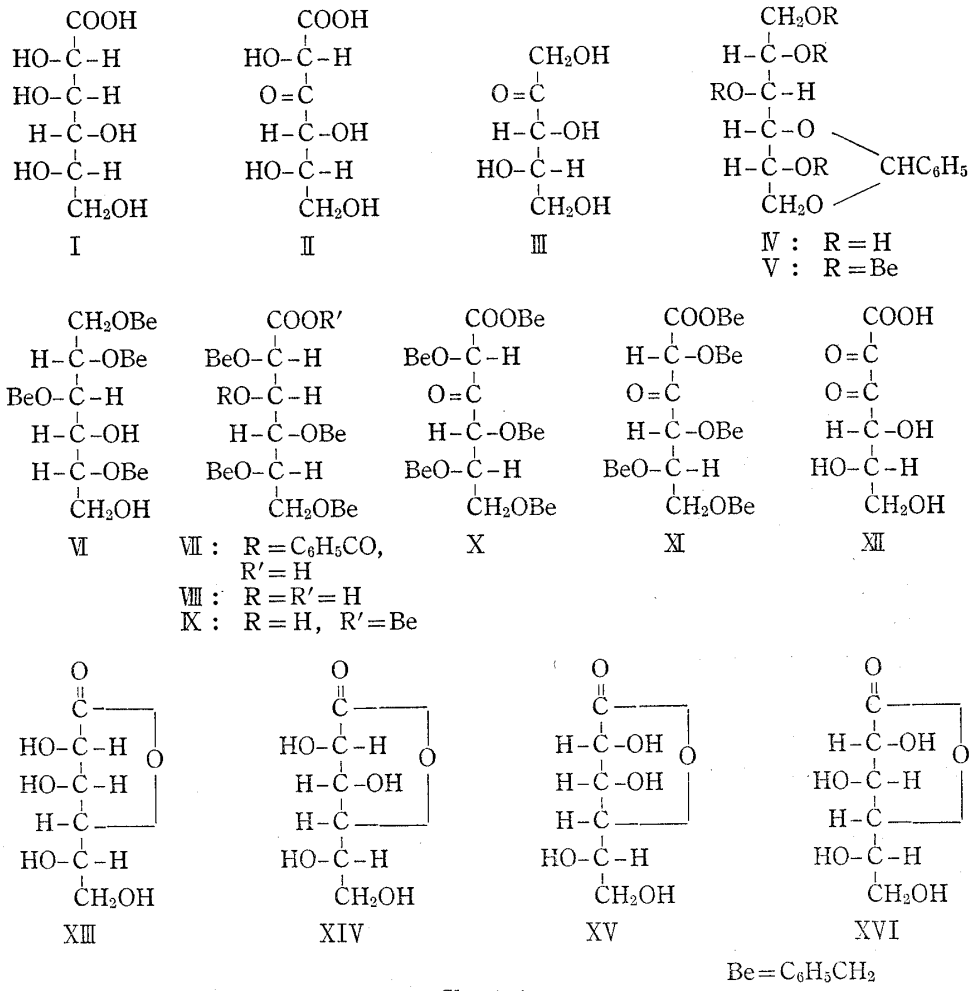

Chart 1

Catalytic hydrogenation of IX over 5% palladium-on-charcoal afforded I, giving L-gulonolactone (XIII) on treatment with methanolic hydrochloric acid which was identified by direct comparison with an authentic sample. Formation of XIII was also demonstrated by gas chromatographic analysis.[4]

Treatments of IX with several oxidizing agents were carried out to obtain benzyl 2,4,5,6-tetra-O-benzyl-L-*xylo*-3-hexuronate (X). IX was first treated with chromium trioxide in acetone[6] or chromium trioxide in acetone-sulfuric acid. Thin-layer chromatographic examination of the reaction mixture revealed the presence of two new substances in addition to IX,

4) Gas chromatographic identification of aldonolactones is reported elsewhere.[5]
5) M. Matsui, M. Okada, T. Imanari, and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **16**, 1383 (1968).
6) A. Assarsson and O. Theander, *Acta Chem. Scand.*, **18**, 727 (1964).

the minor one of which was less polar than IX and was supposed to be X, while the other one was probably degradation product derived from X. Treatment of a mixture of these oxidation products with sodium borohydride followed by debenzylation by catalytic hydrogenation and subsequent lactonization afforded galactonolactone (XIV) besides XIII, which were identified by gas chromatographic analysis,[4] thus indicating the actual formation of X in the above oxidation of IX. Since prolonged oxidation in the foregoing way did not show any apparent increase of X on thin-layer chromatogram, it was supposed that IX is rather sensitive to the oxidizing agent, resulting in the further degradation of X formed in the oxidation of IX.

Recently, a number of procedures have been worked out for oxidation of secondary hydroxyl groups in suitably protected derivatives of monosaccharides leading to the corresponding keto-sugars.[7] Oxidation with ruthenium tetroxide[8] was not applicable to IX, because the ether bond of IX was attacked by the reagent. Among several oxidation procedures using dimethyl sulfoxide,[9] the Pfitzner-Moffatt reagent[10] was found to be very effective. Thus IX was treated with dimethyl sulfoxide-dicyclohexylcarbodiimide-pyridinium trifluoroacetate at room temperature. Thin-layer chromatographic examination indicated almost complete disappearance of IX within an hour. The oxidation product was treated with sodium borohydride and the reduction product was debenzylated by catalytic hydrogenation. Subsequent lactonization followed by gas chromatographic examination[4] revealed the presence of talonolactone (XV) and idonolactone (XVI) in addition to XIII and XIV. Based on this finding it was evident that partial epimerization of the benzyl ether group at C-2 took place in the oxidation, resulting in the formation of X and its C-2 epimer, benzyl 2,4,5,6-tetra-O-benzyl-L-*lyxo*-3-hexuronate (XI). An example of the similar epimerization had been recorded.[11]

The epimerization seems to proceed through the enol-form. Its presence was evident from the observation that the oxidation product of IX gave an intense red color on treatment with ferric chloride. Furthermore, the purified oxidation product by preparative thin-layer chromatography exhibited a very weak or an almost negligible band due to hydroxyl group at about 3500 cm^{-1} besides strong bands at 1750, 1730, and 1655 cm^{-1} in the infrared spectrum, which were ascribable to ester, ketone, and enolic double bond conjugated with carbonyl group respectively. Repeated thin-layer chromatographic purification of the oxidation product resulted in an increase of the enol-form content, which was indicated by the more intense absorption band at 1655 cm^{-1} in the infrared spectrum and also by the finding described below.

Then, next step was to examine the oxidation product of IX after debenzylation. There were three possibilities: 1) β-keto acids, 2) their enol-form (enediol) or its further oxidation product, probably 2,3-diketo-L-gulonic acid (XII), 3) L-xylulose (III), the decarboxylation product of the β-keto acids.

Thus the oxidation product was debenzylated by hydrogenation over palladium-on-charcoal, and the product was extracted with cold water and the aqueous layer was assayed immediately according to the following ways: III was determined by the cysteine-carbazole method;[12] the β-keto acids were estimated as III after decarboxylation by heating at 100° for one minute according to the procedure reported previously;[13] the enol-form of the β-keto

7) J.S. Brimacombe, *Chemistry in Britain*, **2**, 99 (1966); D. Horton and J.S. Jewell, *Carbohydrate Res.*, **2**, 251 (1966).
8) L.M. Berkwitz and P.N. Rylander, *J. Am. Chem. Soc.*, **80**, 6682 (1958); P.J. Beynon, P.M. Collins, and W.G. Overend, *Proc. Chem. Soc.*, **1964**, 342.
9) W.W. Epstein and F.W. Sweat, *Chem. Rev.*, **67**, 247 (1967).
10) K.E. Pfitzner and J.G. Moffatt, *J. Am. Chem. Soc.*, **87**, 5670 (1965).
11) A.F. Krasso, Ek. Weiss, and T. Reichstein, *Helv. Chim. Acta*, **46**, 2538 (1963).
12) C. Ashwell and J. Hickman, *J. Biol. Chem.*, **226**, 65 (1957).
13) J.D. Smiley and G. Ashwell, *J. Biol. Chem.*, **236**, 357 (1961); J. Winkelman and G. Ashwell, *Biochim. Biophys. Acta*, **52**, 170 (1961).

acids was determined as L-ascorbic acid by the 2,4-dinitrophenylhydrazine method.[14] Results of these assays indicated that almost 90% of the β-keto acids formed by debenzylation were decarboxylated to give III, which was characterized further by the fact that reduction with sodium borohydride followed by gas chromatographic analysis[15] showed the presence of xylitol and arabinitol in about equal amount. It was found by the L-ascorbic acid assay that samples derived from the oxidation product of IX rich in the enol-form which was purified by preparative thin-layer chromatography had the higher XII content than those derived from the crude oxidation product of IX. It seems reasonable that the enediol grouping resulted from the debenzylation was further oxidized by the charcoal of palladium-on-charcoal catalyst to afford XII, because the above samples derived from the purified oxidation product did not decolorize 2,6-dichlorophenolindophenol.

On the basis of these results, it may be concluded as follows: 1) Oxidation of IX with the Pfitzner-Moffatt reagent gave a mixture of X, XI and their enol form; 2) Hydrogenolytic debenzylation of these oxidation products resulted mainly in the formation of III and XII; 3) Therefore, the synthesis of II seems to be quite difficult because of its marked susceptibility to decarboxylation as well as enolization.

Experimental[16]

1,2,3,5-Tetra-O-benzyl-4,6-O-benzylidene-D-glucitol (V)——A suspension of 4,6-O-benzylidene-D-glucitol[3] (IV) (5 g), finely powdered KOH (10 g) and freshly distilled benzyl chloride (20 ml) was stirred vigorously at 100—105° for 4 hr. The reaction mixture was cooled, poured into water and extracted with benzene. The extract was washed with water, dried over Na_2SO_4, and concentrated *in vacuo* at 20 mmHg and then at 0.3 mmHg to give a sirup (11.4 g). The sirup was chromatographed on a column of aluminum oxide (550 g) using benzene as solvent. The appropriate fractions containing the main component were combined and concentrated *in vacuo* to give a thin-layer chromatographically homogeneous colorless sirup (8.4 g). TLC with solvent $CHCl_3$–MeOH (40:1), *Rf*: 0.86.

1,2,3,5-Tetra-O-benzyl-D-glucitol (VI)——A solution of V (5.7 g) in 80% EtOH–36% HCl (100:1) (100 ml) was refluxed for 2 hr. The reaction mixture was neutralized with 1N NaOH and concentrated *in vacuo* to afford a sirup. It was tipped into water and extracted with benzene. The extract was washed with water, dried over Na_2SO_4, and concentrated *in vacuo* to yield a sirup (4.6 g). The sirup was chromatographed on a column of aluminum oxide (200 g) using $CHCl_3$ as solvent. The appropriate fractions containing the main component were combined and concentrated *in vacuo* to afford a thin-layer chromatographically homogeneous colorless sirup (3.9 g). IR ν_{max} cm^{-1}: 3550 (OH). TLC with solvent $CHCl_3$–MeOH (40:1), *Rf*: 0.20.

2,4,5,6-Tetra-O-benzyl-3-O-benzoyl-L-gulonic Acid (VII)——To a solution of VI (8.0 g) in pyridine (45 ml) was added trityl chloride (7.0 g) and the reaction mixture was kept at room temperature for 3 days. Examination by TLC showed almost complete disappearance of VI at the end of this period. Then, the reaction mixture was further treated with benzoyl chloride (3.0 ml) at room temperature for 15 hr. The reaction mixture was poured into water and extracted with benzene. The extract was washed with water, 5% Na_2CO_3, and water, then dried over Na_2SO_4, and concentrated *in vacuo* to give a sirup. A solution of the sirup in 80% AcOH (150 ml) was heated at 100° for 1 hr and kept at room temperature overnight. Triphenyl carbinol separated was filtered off, and the filtrate was concentrated *in vacuo* to give a sirup. To a solution of the sirup in acetone (150 ml) was added chromium trioxide (6.0 g) in 7N H_2SO_4 (15 ml) over a period of 10 min.

14) A. Polk, T.L. Flanagan, and E.J.V. Loon, *Clin. Chem.*, 6, 558 (1960).

15) Gas chromatographic identification of these pentitols is reported elsewhere.[5]

16) L-Xylulose (III) and the β-keto acids were assayed by the procedures reported previously.[12,13] 2,3-Diketo-L-gulonic acid (XII) was determined by the 2,4-dinitrophenylhydrazine method.[14] Thin-layer chromatography (TLC) was carried out on 5×20 cm glass plates coated with a 0.25 mm layer of silica gel G (Merck) with detection by ammonium metavanadate-sulfuric acid.[17] Preparative TLC was done on 20×20 cm glass plates coated with a 0.5 mm layer of silica gel G, and column chromatography on aluminum oxide (Merck) or silicic acid (Mallinckrodt, 100 mesh). Gas chromatography (GC) of the trimethylsilyl derivatives of aldonolactones and of the trifluoroacetyl derivatives of pentitols was carried out by the procedure reported elsewhere.[5] Infrared (IR) spectra were recorded for carbon tetrachloride solutions on Hitachi EPI-S2 spectrophotometer. Nuclear magnetic resonance spectra were obtained using solutions in deuteriochloroform with tetramethylsilane as internal standard and Japan Electron Optics Laboratory 3H-60 spectometer.

17) M. Ishidate, M. Matsui, and M. Okada, *Anal. Biochem.*, 11, 176 (1965).

The reaction mixture was kept at room temperature for 1 hr and then poured into water and extracted with benzene. The extract was washed with water, dried over Na_2SO_4, and concentrated *in vacuo* to yield a sirup (11.0 g). The sirup was chromatographed on a column of silicic acid (350 g) using $CHCl_3$ as solvent. The appropriate fractions containing the main acidic component were combined and concentrated under reduced pressure to afford a thin-layer chromatographically homogeneous sirup (4.7 g). TLC with solvent benzene–acetone–formic acid (40:4:1), *Rf*: 0.35.

2,4,5,6-Tetra-O-benzyl-L-gulonic Acid (VIII)——A solution of VII (4.0 g) in MeOH (32 ml) was treated with 2N NaOH (30 ml) at 90—95° for 30 min. The reaction mixture was concentrated *in vacuo* to give a sirup. The sirup was extracted with ether,[18] and the extract was re-extracted with water. The aqueous layer was acidified with 2N HCl and then extracted with benzene. The benzene extract was washed with water, dried over Na_2SO_4, and concentrated *in vacuo* to afford a colorless sirup (2.7 g). TLC with solvent benzene–acetone–formic acid (40:4:1), *Rf*: 0.23.

Benzyl 2,4,5,6-Tetra-O-benzyl-L-gulonate (IX)——To a mixture solution of VIII (2.0 g) in pyridine (4.0 ml) and benzyl alcohol (20 ml) was added dicyclohexylcarbodiimide (750 mg). The reaction mixture was kept at room temperature for 15 hr. Precipitated dicyclohexylurea was filtered off and washed with benzene, and the combined filtrate was concentrated at 20 mmHg and then at 0.3 mmHg to give a sirup (2.8 g). The sirup was purified by preparative TLC with fourteen plates using benzene–acetone (20:1) as solvent, and the appropriate portions containing the main component were combined and extracted with MeOH. To the methanolic extract was added a few drops of formic acid, and the solvent was evaporated *in vacuo* to afford a thin-layer chromatographically homogeneous sirup (1.2 g). *Anal.* Calcd. for $C_{41}H_{42}O_7$: C, 76.14; H, 6.55. Found: C, 76.50; H, 6.66. $[\alpha]_D^{20}$ −22° (*c*=1.07, $CHCl_3$). IR ν_{max} cm^{-1}: 3550 (OH), 1740 (CO). TLC with solvent benzene–acetone–formic acid (40:4:1), *Rf*: 0.54.

A methanolic solution of IX was concentrated *in vacuo* without prior addition of formic acid to dryness. Thin-layer chromatographic examination of the residue using benzene–acetone–formic acid (40:4:1) as solvent showed three spots (*Rf*: 0.32, 0.42, 0.54) which corresponded to benzyl alcohol, methyl ester of VIII, and IX respectively. The residue was further separated by preparative TLC and the portions corresponding to the component of *Rf* 0.42 were combined and extracted with MeOH. Evaporation of the solvent gave a sirup, whose IR spectrum was identical with that of the methyl ester of VIII prepared from VIII by methylation with diazomethane in the usual way.

Catalytic Hydrogenation of IX——A solution of IX (162 mg) in AcOEt (7.5 ml) was hydrogenated over 5% Pd-C (500 mg). Hydrogen uptake ceased within 1.5 hr, and the catalyst was removed by filtration and washed with water. The organic layer was extracted with water, and the combined aqueous layer was concentrated *in vacuo* to give a sirup (52 mg). A solution of the sirup in MeOH (5.0 ml) and 36% HCl (0.05 ml) was warmed at 50° for 30 min, and subsequent concentration gave a sirup. Addition of MeOH (10 ml) and evaporation of the solvent were repeated three times to remove remaining HCl. Then, the sirup was dissolved in a little MeOH and kept in a refrigerator to afford crystals (29 mg) melting at 183—185°, which were recrystallized from MeOH to yield needles of XIII (22 mg), mp 184—186°. The melting point of the mixture with an authentic sample showed no depression, and the IR spectra of the two samples were identical in all respects. Examination of the crystals and the residual sirup by GC also revealed the presence of XIII alone.

Chromium Trioxide Oxidation of IX——A solution of IX (36 mg) in acetone (1.0 ml) was treated with chromium trioxide (30 mg) in water (0.2 ml) and acetone (1.0 ml). The reaction mixture was kept at room temperature for 2.5 hr, then tipped into water, and extracted with benzene. The extract was concentrated *in vacuo* at 30° to give a sirup. It was reduced with $NaBH_4$ as described below, and subsequent hydrogenolytic debenzylation and lactonization followed by GC as described above indicated the presence of XIV (minor peak) besides XIII.

Dimethyl Sulfoxide Oxidation of IX——To a solution of IX (260 mg) in benzene (0.65 ml) were added dicyclohexylcarbodiimide (248 mg), dimethyl sulfoxide (0.64 ml), pyridine (0.032 ml) and trifluoroacetic acid (0.016 ml), and the mixture was kept at room temperature for 1 hr. Examination by TLC using benzene–acetone (20:1) as solvent indicated almost complete disappearance of IX at the end of this period (*Rf*: IX, 0.41; oxidation product, 0.63). To the reaction mixture were added benzene (10 ml) and oxalic acid (109 mg) in MeOH (1.0 ml) and the resultant mixture was kept at room temperature for 30 min and then it was poured into water (6.0 ml) and filtered. Benzene layer was washed with water, cold 5% $NaHCO_3$, and water, then dried over Na_2SO_4, and concentrated *in vacuo* at 20° to give a sirup. This sirup was purified by preparative TLC with three plates using cyclohexane–acetone (5:1) as solvent. The appropriate portions containing the main component were combined and extracted with ether. The extract was concentrated *in vacuo* to afford a sirup (140 mg). IR ν_{max} cm^{-1}: 1750, 1730 (CO), 1655 (C=C–C=O). Both crude and purified sirups gave an intense red color on treatment with ferric chloride.

A solution of the purified sirup (120 mg) in MeOH (2.0 ml) was cooled in an ice-water and treated with $NaBH_4$ (20 mg) in MeOH (1.0 ml). During the reduction MeOH containg 1% AcOH was added to keep pH

18) The sodium salt of VIII was extractable with ether.

of the medium below 7.5. The resultant solution was further treated with another $NaBH_4$ and kept in an ice–water for 1 hr. Examination by TLC showed complete disappearance of the starting material (*Rf*:0.63) and appearance of a component with the same mobility (*Rf*:0.41) as IX. The reaction mixture was tipped into water and extracted with benzene. The extract was washed with water, dried over Na_2SO_4, and concentrated *in vacuo* at 30° to give a sirup (115 mg). Hydrogenation of the sirup in AcOE (0.8 ml) over 5% Pd–C (500 mg) followed by lactonization with methanolic hydrochloric acid by the procedure used in the foregoing experiment afforded a sirup (35 mg), which stained red on treatment with ferric hydroximate. Examination by GC indicated the presence of four aldonolactones, XIII, XIV, XV and XVI.

Debenzylation of the Oxidation Product of IX and Quantitative Determination of the Products after Debenzylation——a) A solution of the crude oxidation product of IX (81 mg) in AcOEt (3.0 ml) was hydrogenated over 5% Pd–C (250 mg). After 30 min the same amount of catalyst was added to the reaction mixture and hydrogenation was continued further for 2 hr, and then the reaction mixture was extracted with cold water. The resulting aqueous layer (30 ml) was assayed immediately for III, XII and the β–keto acids: III, 3.5 mg (23 μmole); XII, 0.6 mg (3 μmole); β–keto acids, 0.3 mg (2 μmole).

On the other hand, the aqueous layer (20 ml) was treated with $NaBH_4$ (10 mg) for 1 hr in an ice–water. Then, the reaction mixture was treated by the procedure reported in the previous paper[1] to give a sirup. Examination of the sirup by GC revealed the presence of two pentitols, xylitol and arabinitol, in about equal amount, which were derived from III, and the presence of four aldonolactones, XIII, XIV, XV and XV, which were derived from the β–keto acids and XII.

b) A solution of the purified oxidation product (47 mg) by preparative thin–layer chromatography in AcOEt (3.0 ml) was hydrogenated over 5% Pd–C (250 mg) as described above. The resulting aqueous solution was immediately subjected to assays: III, 1.8 mg (12 μmoles); XII, 1.6 mg (8 μmole); β–keto acids, 0.2 mg (1 μmole).

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