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Saccharinic Acids from D-Xylose and D-Fructose

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The saccharinic acids obtained from D-xylose and D-fructose on treatment with calcium or sodium hydroxide have been fractionated by cellulose column and gas-liquid chromatography of their lactones. The previously unknown C_5-saccharinic acids, 2-*C*-methyl-D-threonic and 2-*C*-methyl-D-erythronic acids, have been isolated and characterised. C_4-, C_5- and C_6-acids were obtained from both sugars after treatment with calcium hydroxide indicating that considerable fragmentation and recombination had occurred.

Studies on the C_6-saccharinic acids produced from D-glucose, D-mannose, and D-fructose were reported in an earlier communication.[1] The previously unknwon "*β*"-D-glucoisosaccharinic acid (3-deoxy-2-*C*-hydroxymethyl-*threo*-pentonic acid) was isolated and characterised. It has recently been demonstrated by X-ray crystallography that the "*α*"-D-glucoisosaccharinic acid has the D-*erythro*-configuration.[2] The "*β*"-D-glucoisosaccharinic acid should consequently have the D-*threo*-configuration. The L-form of the "*β*"-glucosaccharinic acid (2-*C*-methyl-arabinonic acid) was synthesised and it was demonstrated that "*β*"-D-glucosaccharinic acid was not formed on alkaline treatment of these hexoses.[1] In the present paper a more complete investigation of the different acids formed on treatment of D-xylose and D-fructose with calcium or sodium hydroxide is reported.

The acids formed on treatment of D-xylose with calcium hydroxide were converted to their lactones and separated into six fractions by cellulose column chromatography. These fractions were trimethylsilylated and analysed by gas-liquid chromatography.[1,3] The presence of 14 different lactones was demonstrated (Table 1, Fig. 1). All but one (5) could be identified by comparison of their chromatographic mobilities with those of authentic samples and by structural studies. The relative proportions of the different components were determined by gas-liquid chromatography (Table 2).

Three C_4-lactones were detected. Of these, 2-deoxy-tetrono-1,4-lactone (3) and erythronolactone (10) were formed in such low yields that they could only be detected in enriched fractions; detection was not possible in the unfractionated lactone mixture. Lactone 2 was identified as 3-deoxy-tetrono-1,4-lactone,

Table 1. Lactones from D-xylose and D-fructose and calcium hydroxide.

Fraction	Lactone No.	R_F [b]	T [c]	Reaction with $AgNO_3$ -NaOH	Reaction with $NaIO_4$ -Benzidine	Structure, 1,4-lactone
1	1	0.79	0.50	+	−	2-*C*-Methyl-threono-
	2	0.79	0.73	−	−	3-Deoxy-tetrono-
	3	0.77	1.20	+	−	2-Deoxy-tetrono-
2	4 [a]	0.71	0.80	+	+	2-*C*-Methyl-erythrono-
	5	0.71	0.73	+	−	not determined
	6	0.66	0.55	+	(+)	3-Deoxy-2-*C*-hydroxymethyl-tetrono-
3	7	0.60	1.00	+	+	2-*C*-Methyl-ribono-
	8	0.60	1.39	+	−	3-Deoxy-*erythro*-pentono-
4	9	0.51	1.81	+	−	3-Deoxy-*threo*-pentono-
	10	0.51	1.54	+	+	Erythrono-
5	11	0.41	1.24	+	+	3-Deoxy-2-*C*-hydroxymethyl-*erythro*-pentono-
	12	0.39	1.41	+	+	3-Deoxy-2-*C*-hydroxymethyl-*threo*-pentono-
	13	0.39	2.50	+	+	3-Deoxy-*ribo*-hexono-
6	14	0.30	3.00	+	+	3-Deoxy-*arabino*-hexono-

[a] Lactone 4 was detected only from D-xylose. [b] Solvent B.
[c] Relative retention times on gas-liquid chromatography. $T = 1$ for lactone 7.

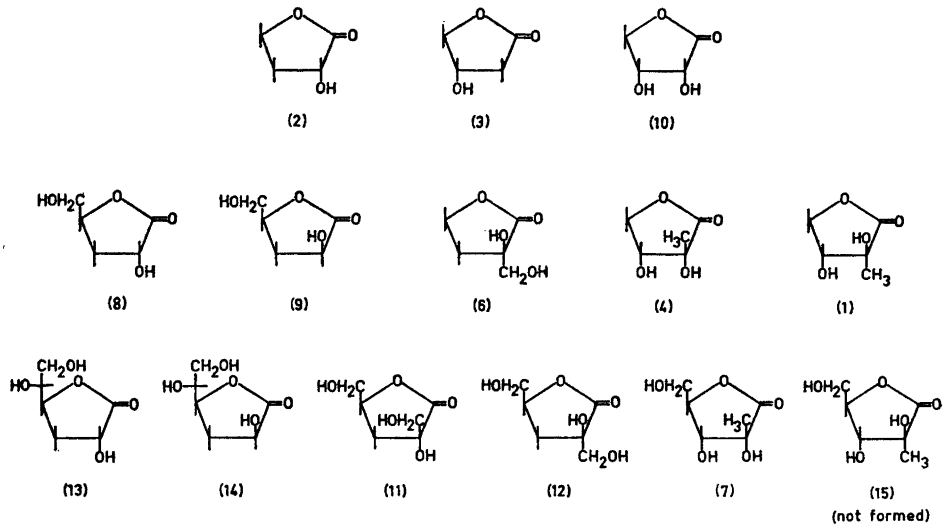

Fig. 1. 1,4-Lactones obtained from D-xylose and calcium hydroxide. (Only the D-forms are given although some of the lactones obtained were complete or partial racemates. Numbers of lactones correspond to those in Table 1.

Table 2. Lactones formed on alkaline treatment of D-xylose, L-arabinose, and D-fructose.

Sugar	Reagent	Temp. (°C)	Time (h)	% Lactone 1	2+5	4	6	7	8+12	9	11	13	14
D-Xylose	0.02 M $Ca(OH)_2$	25	650	5	5	5	4	10	26	31	+	7	8
	M NaOH	25	650	—	7	—	2	+	31	60	+	+	+
	8 M NaOH	100	8	—	21	—	1	—	34	44	—	—	—
L-Arabinose	8 M NaOH	100	8	—	16	—	1	—	36	48	—	—	—
D-Fructose	0.02 M $Ca(OH)_2$	25	1700	2	3	—	1	[a]	25	15	21	15	18
	8 M NaOH	100	9.5	—	10	—	—	—	+	7	5	27	51

[a] In this experiment lactone 7 predominated and the figures refer to the relative percentages of the other lactones present.

but in the quantitative analysis by gas-liquid chromatography it was not separated from the unidentified lactone 5. Lactones 3 and 10 are cleavage products of the intermediates in the formation of glucoisosaccharinic [4] and glucosaccharinic acids,[5] respectively. Lactone 2 was obtained previously by Nef [6] on treatment of D-arabinose with hot alkali. 2-Deoxy-D-*erythro*-pentono-1,4-lactone (R_F 0.64, T 1.55), which was formed in a low yield from the dicar-

bonyl intermediate leading to the glucometasaccharinic acids,[7] was not observed.

Two of the lactones (8 and 9) were chromatographically indistinguishable from 3-deoxy-*erythro*- and 3-deoxy-*threo*-pentono-1,4-lactone, which are obtained on treatment of 3-*O*-methyl-D-xylose with calcium hydroxide.[8] They were further characterised as brucine salts, benzoate of lactone 9 and by their physical properties. These lactones were interconvertible on treatment with 5 % sodium hydroxide at 175° for 2 h, followed by acidification. By reduction of lactone 8 with sodium amalgam, a product indistinguishable from 3-deoxyribose was obtained. Periodate oxidation studies of the free acids gave results consistent with the assigned structures. As expected, these two acids were formed in the highest yields (Table 2).

Lactone 6 was chromatographically indistinguishable from an authentic sample of D,L-3-deoxy-2-*C*-(hydroxymethyl)-tetrono-1,4-lactone. This lactone has been obtained by alkaline degradation of β-(1→4)-linked oligosaccharides composed of D-xylose residues.[9,10]

Lactone 1 was isolated as its amorphous benzoate by preparative thin-layer chromatography. The benzoate, $[\alpha]_D$ −57°, showed a strong IR-absorption at 1795 cm^{-1}. The free lactone, $[\alpha]_D$ −11°, absorbed at 1770 cm^{-1}. The IR-absorption thus indicated a 1,4-lactone structure. NMR of the benzoate (Fig. 2) shows the presence of 10 aromatic protons, a three-proton singlet at τ 8.27 and three quartets around τ 3.87, 5.00 and 5.83 corresponding to one proton each. This indicates the presence of two benzoate groups, a non-coupled methyl group and three protons giving an AMX-type spectrum. From the τ-values, the carbon atoms to which the protons are linked are substituted by oxygen atoms. The carbon atom to which the proton with the lowest τ-values is linked probably carries a benzoate ester group. Periodate oxidation of the free acid yielded one mole of formaldehyde and one mole of pyruvic

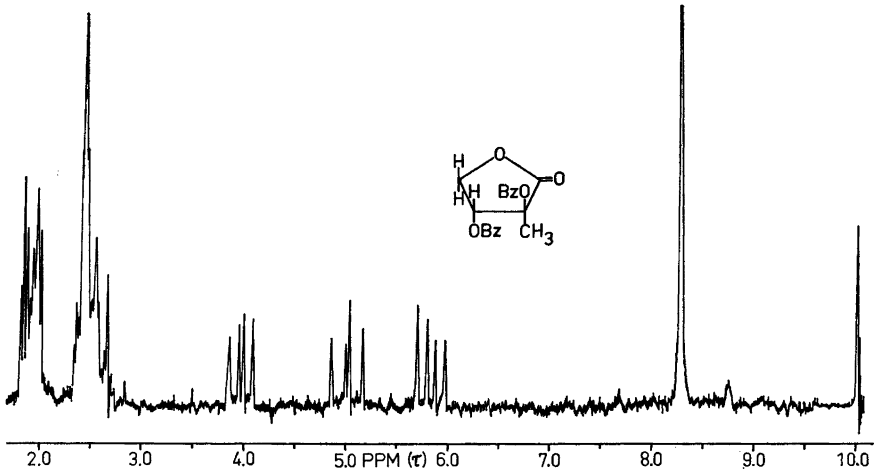

Fig. 2. NMR spectrum of dibenzoate of lactone 1.

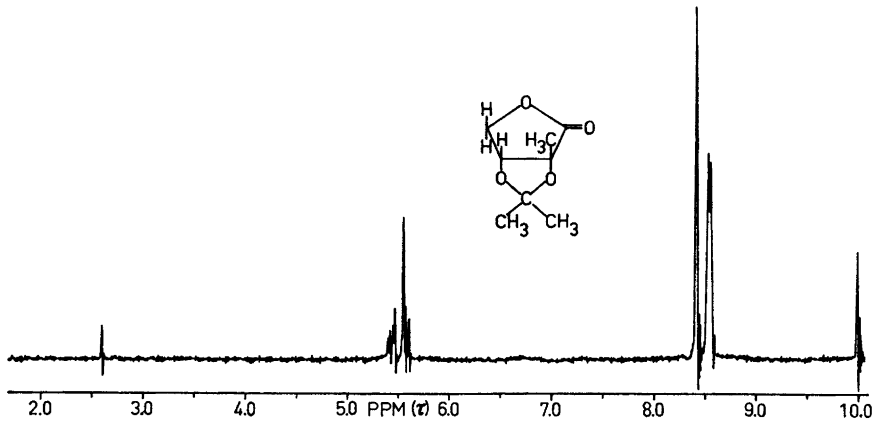

Fig. 3. NMR spectrum of isopropylidene of lactone 4.

acid. These facts show that lactone 1 is a 2-*C*-methyl-tetronolactone. The lactone could not be detected with periodate-benzidine reagent and its paper chromatographic mobility was not enhanced on addition of phenylboronic acid to the chromatographic solvent.[11] These results show that the hydroxyls at C-2 and C-3 are *trans*. As the lactone is optically active and obtained from D-xylose, it seems reasonable to assume that it belongs to the D-series and therefore is 2-*C*-methyl-D-threono-1,4-lactone.

Purification of lactone 4 as its benzoate was not successful as decomposition occurred during thin-layer chromatography; however, the lactone formed an isopropylidene derivative and was purified *via* that substance. The isopropylidene derivative had $[\alpha]_D$ −40°, and a strong IR-absorption at 1790 cm^{-1}. The corresponding values of the free lactone were −18° and 1765 cm^{-1}. The NMR spectrum of the isopropylidene derivative (Fig. 3) showed a six-proton doublet at τ 8.54, a three-proton singlet at τ 8.44 and a three proton multiplet around τ 5.5. This is in agreement with the structure of the 2,3-di-*O*-isopropylidene derivative of 2-*C*-methyl-erythrono-1,4-lactone. This *cis*-relationship between the hydroxyls at C-2 and C-3 is obvious because the lactone gives an isopropylidene derivative. In agreement with this the lactone gives a positive reaction with periodate-benzidine reagent and its paper chromatographic mobility is enhanced on addition of phenylboronic acid to the chromatographic solvent. Periodate oxidation of the acid yielded one mole of formaldehyde and one mole of pyruvic acid, as expected from the assigned structure. By the same arguments as above for the corresponding threono-lactone, it should belong to the D-series.

Lactones 1 and 4 are thus the 2-*C*-methyl-tetrono-1,4-lactones, which are analogous to the glucosaccharino-1,4-lactones (7 and 15). Of the latter only the *ribo*-form was formed on alkaline treatment of fructose and it is remarkable that both the *erythro*- and *threo*-forms are formed in the reaction between D-xylose and calcium hydroxide. The C_6-*ribo*-lactone (7) has a higher mobility

than the C_6-*arabino*-lactone (15) on paper and gas-liquid chromatography with the systems used in this study. Therefore one would expect that the C_5-*erythro*-lactone (4) would also have a higher mobility than the C_5-*threo*-lactone (1), but the reverse was observed.

The benzoate of lactone 5 was isolated by preparative thin-layer chromatography. It was optically inactive, but due to the low yield of the substance it was not further investigated.

When D-xylose was treated with sodium hydroxide, either at 25° or 100°, the products formed were not as varied (Table 2). Only traces of C_6-acids were formed at 25° and none at 100°, in addition the 2-*C*-methyl-tetronic acids were not formed. The same picture was obtained when L-arabinose was treated with sodium hydroxide at 100°.

D-Fructose was treated with calcium hydroxide and the lactone mixture worked up using the same methods as in the analogous experiment with D-xylose. All the lactones observed in the experiment with D-xylose were formed except lactone 4; however, the relative proportions were different. The C_6-acids, especially 2-*C*-methyl-ribono lactone (7) predominated (Table 2). Fewer products were observed when D-fructose was treated with sodium hydroxide. No 2-*C*-methyl-ribono lactone (7) and only small amounts of the two C_5-lactones, 8 and 9, were formed.

It is known that part of the saccharinic acids formed on alkaline treatment of a sugar derive from modified sugars, formed by extensive isomerisation or by fragmentation and recombination of the starting material. Different possibilities have been discussed by Sowden.[12] As C_6-acids were formed from D-xylose on treatment with calcium hydroxide (Table 2), it is obvious that fragmentation and recombination must be considerable under these conditions. The formation of C_6-saccharinic acids from pentoses was also indicated by Green.[13] The formation of xylose and arabinose during the early stages of the reaction of D-fructose with calcium hydroxide was demonstrated by paper chromatography, thus indicating similar reactions.

In order to study this problem further 3-deoxy-*threo*-pentono-1,4-lactone (9) was isolated from four different reaction mixtures. The crystalline benzoate derivative was prepared and its optical rotation determined (Table 3). The different samples of the lactone and its derivatives were indistinguishable by paper, thin-layer, and gas-liquid chromatography, and identical IR and

Table 3. Benzoates of lactone 9 from different origins.

Reaction conditions [a]			Benzoate	
Sugar	Base	Time (h)	m.p. (°)	$[\alpha]_D^{22}$
D-Fructose	$Ca(OH)_2$	1700	135.5–137.5	+ 6°
D-Xylose	$Ca(OH)_2$	300	128–133	+ 15°
D-Xylose	NaOH	300	131.5–133.5	+ 43°
3-*O*-methyl-D-xylose	$Ca(OH)_2$	50	137–138	+ 52°

[a] Temp. 25°C.

NMR spectra were obtained. The substance prepared from 3-*O*-methyl-D-xylose and calcium hydroxide had the highest optical purity, followed by the product obtained from D-xylose and sodium hydroxide. The products obtained from D-xylose or D-fructose by treatment with calcium hydroxide were extensively racemised. Except when one reaction route is facilitated by a substituent, *i.e.* with 3-*O*-methyl-D-xylose, the reactions in calcium hydroxide are more complicated than the reactions in sodium hydroxide. The complete analysis of the reaction mixtures, as discussed above, shows that fragmentation and recombination is more important with calcium hydroxide than with sodium hydroxide. A contributing factor could be that fragments, *e.g.* trioses, are preferentially rearranged into acids in sodium hydroxide (which was also used in a higher concentration than could be obtained with calcium hydroxide) and thus are withdrawn before they can undergo recombination (aldol condensation). The fact that C_6-acids could be detected in products from the reaction of D-xylose with 1 M sodium hydroxide at 25°, but were not formed by treatment in 8 M sodium hydroxide at 100°, lends some support to this assumption.

EXPERIMENTAL

Concentrations were done under reduced pressure at a bath temperature not exceeding 45°. Melting points are corrected.

Paper chromatography. Paper: Whatman No. 1. Solvents: A. Ethyl acetate-pyridine-water, 8:2:1, B. Butanone, saturated with water, C. Butanol-ethanol-water, 4:1:5, upper phase, D. Same as C, but containing 5 % phenylboronic acid.[11]

Paper electrophoresis. Paper: Whatman No. 1. Buffer: 0.1 M sodium borate pH 10.

Thin-layer chromatography. Adsorbent: "Kieselgel HF_{254} nach Stahl". Solvent: Ethyl acetate-light petroleum (60—70°), 1:2. Benzoates were detected with UV-light.

Spraying reagents. Conventional spraying agents for acids, lactones and sugars were used. Periodate-benzidine reagent [14] was used for detection of glycols.

Gas-liquid chromatography of trimethylsilyl ethers, prepared according to Sweeley and coworkers,[3] was carried out on a butanediol-succinate column at 160°, using a Perkin Elmer 820 instrument.

NMR spectra were recorded at 60 Mc/sec on a Varian A 60 spectrometer. The spectra were determined in deuterochloroform, using tetramethylsilane as an internal reference.

Periodate oxidation of some saccharinic acids and analysis of the products formed were performed by methods recently developed by Barker and coworkers [15] and will be reported separately.

Alkaline treatment of sugars. The purity of the sugars used in this study was determined by paper and gas-liquid chromatography and no traces of contaminating sugars were detected.

D-Fructose (200 g) was treated with calcium hydroxide (100 g) in water (2 l) for 1700 h at 25°. The product was worked up as described by Whistler and BeMiller [16] and fractionated.

D-Xylose (24 g) was treated either with calcium hydroxide (74 g) in 1.9 l water or M sodium hydroxide (0.8 l) for about 300 h at 25°. The solutions were passed through columns of Dowex 50 (H^+). In the experiment with calcium hydroxide this treatment was preceeded by filtration, neutralisation with carbon dioxide and filtration.

When the lactone mixture was to be investigated by gas-liquid chromatography only, a smaller amount of sugar (about 0.5 g) was treated with calcium hydroxide or M sodium hydroxide as described above or with 8 M sodium hydroxide (18 ml) at 100° in a cylinder of stainless steel (Table 2). Cations were removed as described above and the concentrated solution was given a brief treatment with Dowex 3 (free base) in order to remove free acids, and concentrated.

In a separate experiment a mixture of D-fructose (10 g) and calcium hydroxide (2 g) in oxygen free water (100 ml) was kept at 25° and agitated occasionally. After 1, 2, and

6 days, 10 ml samples were withdrawn, deionised and concentrated. Sugars with paper chromatographic mobilities and colour reactions corresponding to xylose and arabinose were detected in all samples but seemed to be present in highest concentration after 6 days. The substances corresponding to xylose and arabinose were isolated by preparative paper chromatography in solvent A and their identity established by paper chromatography in different solvents and paper electrophoresis.

Fractionation of lactone mixtures. After the different alkaline treatments no reducing sugars remained in the products. The product formed by alkaline treatment, corresponding to about 5 g of the original sugar, was dissolved in water, given a brief treatment with Dowex 3 (free base) to remove acids, and concentrated. The concentrate was placed on top of a cellulose column (6 × 70 cm), and eluted with solvent B. Fractions were collected and investigated by paper chromatography and gas-liquid chromatography.

Quantitative analysis of lactone mixtures. The lactone mixtures were trimethylsilylated, pyridine was removed by distillation and the residue dissolved in cyclohexane before injection. The removal of pyridine was essential in order to detect the lactones with lower retention times. The concentration of the different components was assumed to be proportional to the corresponding peak areas. Two pairs of lactones, 2—5 and 8—12, were not separated. In a previous investigation it was shown that lactones 11 and 12 are formed in comparable amounts.[1] It may therefore be assumed that the yield of lactone 12 is similar to that of lactone 11 in the present investigation.

Characterisation of products. Lactones 2, 3, 6, 7, 10, 11, 12, 13, and 14 were identified by comparison of their mobilities during paper and gas-liquid chromatography and their colour reactions with those of authentic substances. The optical purity of these lactones is consequently not known.

Lactone 1. Cellulose column chromatography of the lactones formed by treatment of D-xylose with calcium hydroxide yielded the fraction 1 (Table 1), enriched in lactone 1. A portion (118 mg) of this fraction was benzoylated and the benzoate separated by thin-layer chromatography. A pure component (60 mg), which was extracted from the main zone with ethanol but did not crystallise, was obtained. It showed $[\alpha]_D^{22}$ −57° (*c* 1.9, acetone). The IR-spectrum showed a strong absorption at 1795 cm^{-1} and no absorption in the hydroxyl region.

The benzoate (62 mg) was suspended in 75 % aqueous ethanol (5 ml) and 0.2 M sodium ethoxide in ethanol (10 ml) was added. The mixture was kept overnight at room temperature, diluted with water (15 ml) and passed through a column of Dowex 50 (H^+). The concentrated solution was extracted with ether, concentrated to a syrup and kept at 55° for 3 h to complete lactonisation. The product was further purified by paper chromatography (solvent B) to yield the amorphous lactone (8 mg). It showed $[\alpha]_D^{22}$ −11° (*c* 0.5, water).

Lactone 4. The fraction 2 (Table 1), enriched in lactone 4 was obtained by treatment of D-xylose with calcium hydroxide. A portion (190 mg) of this fraction was dissolved in acetone (10 ml), and conc. sulphuric acid (0.1 ml) and anhydrous cupric sulphate (400 mg) were added to the solution. The mixture was shaken at room temperature for 46 h, neutralised with aqueous ammonia, water (5 ml) was added and the acetone removed by distillation. The aqueous solution was extracted with chloroform, the chloroform extract was dried, concentrated and distilled at 12 mm Hg and a bath temperature at 100—105°. The distillate, which showed two spots on a thin-layer chromatogram, was purified by chromatography on a silicic acid column (1.5 × 18 cm), using ethylacetate-light petroleum (1:2) as irrigant. The main component (34 mg) was a colourless syrup having $[\alpha]_D^{22}$ −40°. The isopropylidene derivative on mass spectrometry gave the expected peak at 157 (M−15). (Found: 157.053. $C_8H_{12}O_4$ requires: 157.050 (for C = 12.000)). The IR-spectrum (CCl_4) showed a strong absorption at 1790 cm^{-1} and no absorption in the hydroxyl region.

The isopropylidene derivative (33 mg) was hydrolysed with 0.5 M sulphuric acid (4 ml) at 100° for 1 h. After neutralisation with barium carbonate and filtration through a column of Dowex 50 (H^+), the free lactone was further purified by paper chromatography as described above for lactone 1. It had $[\alpha]_D^{22}$ −18° (*c* 0.2, water).

Lactone 8. A solution of lactone 8 (100 mg), obtained by treatment of D-xylose with M sodium hydroxide, and brucine (350 mg) in 50 % aqueous acetone (10 ml) was refluxed for 2 h. The acetone was distilled off and the remaining aqueous solution treated with charcoal. The brucine salt was precipitated from the aqueous solution by addition of

acetone and the product (180 mg) was recrystallised from ethanol to yield the pure salt (120 mg). The m.p. 195—198° and the optical rotation $[\alpha]_D^{22}$ −21.5° (*c* 1.0, water) are in good agreement with previously recorded values for the brucine salt of 3-deoxy-D-*erythro*-pentonic acid.[8,17]

Part of the lactone was reduced with sodium amalgam as previously described.[1] A sugar which was chromatographically and electrophoretically indistinguishable from 3-deoxyribose was produced.

Lactone 9. Lactone 9, prepared from D-xylose and M sodium hydroxide, was converted to the brucine salt of the acid as described above. On heating the salt to about 148°, a crystalline modification, which melted at 166—171°, occurred. The optical rotation, $[\alpha]_D^{22}$ −29° (*c* 1.0, water), is in reasonably good agreement with previously reported values; however, Nef [6] reported a m.p. of 145—150° and Corbett and coworkers [8] a value of 133—135°.

The crystalline benzoate of lactone 9 was prepared. Different samples, prepared from 3-*O*-methyl-D-xylose (Found: C 66.7; H 4.82; O 28.5. Calculated: C 67.1; H 4.71; O 28.2), D-xylose, and D-fructose treated with calcium hydroxide and from D-xylose treated with sodium hydroxide had similar melting points, but different optical rotations (Table 3).

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