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Chromatographic Separation of Sugars

VI.* A New Route to the Synthesis of Some Uronic Acids

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Crystalline methyl-α-L-*arabo*-pentodialdo-1,4-furanoside obtained by partial oxidation of methyl-β-D-galactofuranoside is now described. The dialdopentose was found to exist with an intramolecular 2,5-hemiacetal linkage.

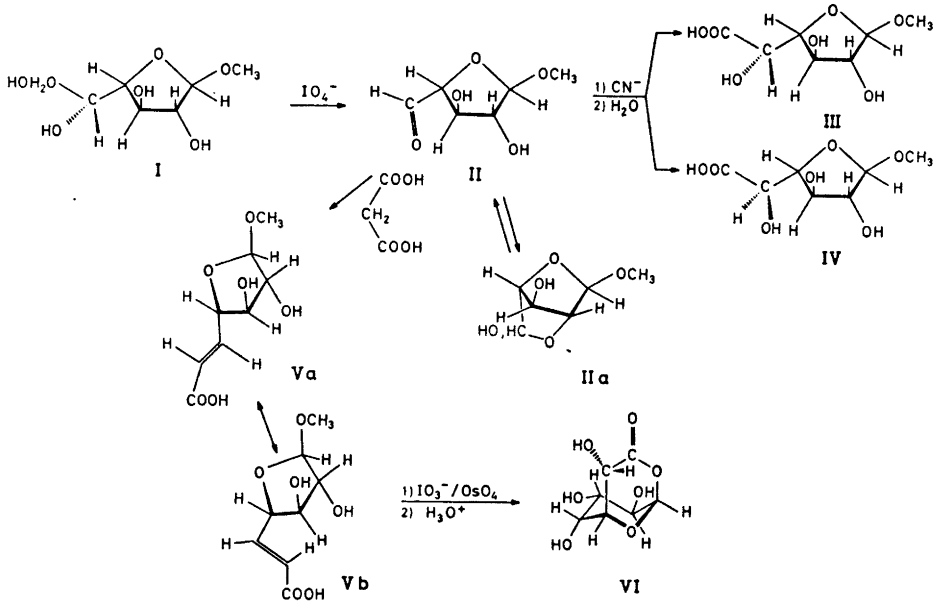
Cyanohydrin synthesis on the dialdopentoside followed by hydrolysis gave D-galacturonic and L-altruronic acids which were separated on cellulose. Knoevenagel-Doebner synthesis on the dialdehyde using malonic acid gave a *trans* unsaturated heptonic acid. Oxidation of this acid with iodic acid and osmium tetroxide as catalyst gave crystalline D-*glycero*-L-*altro*-hepturono-δ-lactone in more than 55 % yield, while the other expected epimer, L-*glycero*-D-*galacto*-hepturonic acid or its lactone has not yet been obtained crystalline.

In spite of the considerable biological importance of uronic acids only a few have so far been found in Nature. Several methods have been employed in attempts to synthesize uronic acids, *e.g.* (a) reduction of dicarboxylic acid monolactone,[2,3] (b) oxidation, using different oxidation agents [4–6] or air with rare metals as catalysts,[7] (c) epimerization of sodium uronates,[8] (d) periodate oxidation of hexono-1,4-lactones with the OH groups at C_2 and C_3 in *trans* position,[9] (e) cyanohydrin synthesis starting with a dialdehyde in which one of the aldehyde groups was protected.[10–12] By these methods only two uronic acids not found in Nature have been described, *i.e.* L-lyxuronic acid [9] and L-altruronic acid.[8] The latter seems, unfortunately, to be produced only under rather special conditions as later attempts to reproduce the method seem to have failed.[13] We also have tried the method using sodium D-galacturonate as starting material but could not detect any L-altruronic acid either by thin layer or by paper chromatography.

Some years ago one of the authors reported the partial oxidation of aldohexofuranosides having *trans* configuration at C_2-C_3 giving dialdopentoses.[14] We now report the synthesis of uronic acids using such a dialdehyde as starting material.

* Part V, Ref. 1.

Starting with methyl-β-D-galactofuranoside, I, methyl-α-L-*arabo*-pentodialdo-1,4-furanoside, II, was obtained. A cyanohydrin synthesis on compound II followed by mild hydrolysis of the cyanide group would then be expected to give methyl-β-D-galactofuranuronic acid, III, and methyl-α-L-altrofuranuronic acid, IV.



With the Knoevenagel-Doebner synthesis using malonic acid and compound II, methyl-*trans*-5,6-dideoxy-α-L-*arabo*-furano-5-heptenuronic acid was expected to be formed, which by a *cis*-hydroxylation should give two epimeric hepturonic acids.

In the previous communication [14] the dialdofuranoside II was not isolated in a pure state. That has now been done prior to further synthesis, and we now report the molecular structure of the compound II. A somewhat similar dialdehyde, 5-aldo-1,2-*O*-isopropylidene-D-*xylo*-pentofuranose has been prepared by Brocca and Dansi [15] and later by Schaffer and Isbell [16] who by molecular weight determination found the compound to exist as a dimer. From this finding and the absence of indications of a free aldehyde group on the infrared spectrum they concluded that the dimer existed with two hemiacetal linkages. Molecular weight determination of the compound II by the Beckmann method (M.W. 172) and the molecular ion ($[M+1]^+$ 163) from mass spectroscopy showed that our dialdehyde was a monomer. The infrared spectrum in potassium bromide gave only a very small peak at 1730 cm^{-1} indicating no or very little free aldehyde group. Proton magnetic resonance in deuterium oxide gave a singlet at $\delta = 3.40$ equal to three protons ($-OCH_3$), a doublet (one proton) at $\delta = 5.06$ ($J \sim 2$ cps), a doublet (one proton) at $\delta = 4.98$ ($J \sim 1$ cps), and a complex multiplet at $\delta = 3.8 - 4.3$ containing three protons,

presumably the hydrogens at C_2, C_3, and C_4. In DMSO a small peak at $\delta = 9.55$ was obtained indicating a small amount of free aldehyde. In this solvent the spectrum was very complex and no attempt at interpretation was made.

From these data we conclude that the compound II preferentially occurs as a hemiacetal but with an intramolecular hemiacetal linkage IIa. The hydrogens at C_1 and C_5 would then account for the two doublets at 5.06 and 4.98 ppm.

The application of the cyanohydrin synthesis to a dialdehyde, 5-aldo-1,2-*O*-isopropylidene-D-*xylo*-pentofuranose, is described by Sowden [10] who isolated D-glucuronic acid in 20 % yield. The method has later been improved by Wolfrom *et al.*[11] who by adsorption chromatography on column isolated 1,2-*O*-isopropylidene-D-glucofuranurono-γ-lactone and 1,2-*O*-isopropylidene-L-idofuranurono-γ-lactone.

By application of the cyanohydrin method to compound II, methyl-β-D-galactofuranuronic acid and methyl-α-L-altrofuranuronic acid were formed. Separation of the two epimers by paper chromatography as described previously [1] was attempted in spite of the fact that lactones of galacturonic and altruronic acid sterically are unfavourable. After a lactonization process as described previously [1] the chromatogram showed two fast moving spots which both gave reaction with hydroxamic acid indicating that methyl-hexofuranurono-lactones had been formed. However, the heavy tailing on the chromatogram showed that hydrolysis presumably had taken place during the development, and therefore the method was found unsuitable for preparative separation of the two isomers. In this case the best way for separation of the epimers was found to be paper chromatography on the free uronic acids after hydrolysis of the glycosides using solvent D (see experimental). Three distinct spots were obtained with R_{GalA} 1.00 (a), 1.29 (b), and 1.75 (c), respectively, in proportion 1:1.1:2. Physical data of the substances a, b, and c and some of their oxidation and reduction products are given in Table 1.

Table 1. Physical data of uronic acids and their oxidation and reduction products.

Substance	m.p. °C	$[\alpha]_D^{20}$	After oxidation $[\alpha]_D^{20}$	After reduction $[\alpha]_D^{20}$	m.p.	Equiv. weight
a	152 – 156 dec.	+51.3°	0.0°	–	–	–
b	–	−7.4°	+7.7°	+6.2°	121 – 126	160
c	124 – 127	+196→+2.7°	−23.0°	−5.6°	–	184

From the melting point, specific rotation, the chromatographic mobility, and the fact that fraction a gave an inactive dicarboxylic acid on oxidation, it was concluded that fraction a was identical with galacturonic acid. (Lit. m.p. 159 – 160° dec. $[\alpha]_D^{20} + 53.6°$.)

According to Fischer and Schmidt [10] the specific rotation of L-altruronic acid is +5.3° but no other data for the acid are given. Oxidation of L-altruronic acid with bromine water should give L-talaric acid $[\alpha]_D^{20} - 28.6°$ (D-talaric acid, $[\alpha]_D^{20} + 28.6°$)[17] while reduction would lead to L-talonic acid, $[\alpha]_D^{20} - 19°$, or

the corresponding lactone, $[\alpha]_D^{20} + 35°$, m.p. 134°. By comparison of fractions b and c with these data and M. W. determination it seems most reasonable to conclude that fraction c is L-altruronic acid. As the amount of sugars available did not permit further attempts of purification, small amounts of impurities may be present and account for the small disagreement between the oxidation product of fraction c and the data given in the literature for D-talaric acid. The discrepancy in specific rotation between L-talonic acid and the reduction product of fraction c may be explained by assuming the reduction product to be a mixture of the free acid and the corresponding lactone. This is also indicated by infrared as the reduction product of c gave a small absorption at ν_{max} 1770 cm^{-1} and a larger absorption at ν_{max} 1730 cm^{-1} in potassium bromide. Also the chromatographic mobilities of the fractions seem to indicate that fraction c is L-altruronic acid as one might expect higher mobility for L-altruronic acid compared with galacturonic acid than 1:1.29, which is the value for fraction b. By comparison between galacturonic and mannuronic acid (R_{GalA} 1.48) a value of 1.75 for altruronic acid seems reasonable. The fraction b is still not identified, but further work is in progress.

The Knoevenagel synthesis was carried out as described by Kochetkov *et al.*[18] Chromatography of the reaction product using solvent C as eluent gave a fraction which was shown to be unsaturated, and by PMR to have a *trans* configuration; one allylic proton giving a quartet at $\delta = 7.10$ ($J = 17$ cps), equal to C_5 and one giving a doublet at $\delta = 6.16$ ($J = 17$ cps) equal to C_6. The fraction was found to contain a small amount of the compound II but no attempts were made to remove this impurity prior to further synthesis. Hydroxylation of the alkene was carried out as a *cis*-hydroxylation using iodic acid with osmium tetroxide as a catalyst as described by Dmitriev and Kochetkov.[19] As the reaction is known to be almost quantitative we found that a better result could be obtained by using only slight excess of iodic acid compared with the theoretical molar ratio alkene:iodic acid (5:2) instead of molar ratio (1:1) as stated by Kochetkov *et al.*[19] By our method the excess of iodic acid was much easier to remove. After hydrolysis of the methyl group, part of the syrup (55 %) crystallized from ethyl acetate and the crystalline product gave positive reaction for a lactone, m.p. 138 – 139.5°C, $[\alpha]_D^{20} + 9.2°$ (*c* 0.5 water) ν_{max} 1730 cm^{-1} and M.W. 210 (by titration). Oxidation with bromine water gave a product which in alkaline solution was optically inactive. From the result of the oxidation and the infrared spectrum indicating a δ-lactone, it seems reasonable to conclude that the crystalline compound is D-*glycero*-L-*altro*-heptopyranurono-7,1-lactone (VI).

The residue after the crystallization was also oxidized giving a solution which was optically active. This may indicate the presence of the other isomer (L-*glycero*-D-*galacto*-hepturonic acid) in the syrup. Paper chromatography of the residue showed the presence of some pentodialdose, some of compound VI beside a third main component. In none of the eluents used was this component well enough separated from compound VI for a preparative separation.

The yield indicates that compound VI is the isomer which preferentially has been formed during the *cis*-hydroxylation. Because of steric hindrance the oxidation reaction must take place from the opposite side of the double bond from the ring. Getting most of the compound VI should therefore indicate

that the conformer Va is preferred to the conformer Vb. Also by looking at the two conformers, it seems reasonable to assume that Va with C_3 and C_6 in *anti* position will be a more preferred conformer than the Vb with C_3 and C_6 in *syn* position. The configuration at C_3 may also play a role in the stability of the two conformers and with an opposite configuration at C_3 one might therefore get another ratio between the D-*glycero* and the L-*glycero* isomers. This will be further investigated.

EXPERIMENTAL

Chromatography was carried out using Whatman paper No. 1 or 3 MM or cellulose powder standard grade and one of the following eluent systems (v/v): A, ethyl acetate : propanol : water 5 : 3 : 2. B, methyl ethyl ketone, saturated with water. C, methyl ethyl ketone : ethanol : water 5 : 2 : 1. D, ethyl acetate : pyridine : acetic acid : water 5 : 5 : 1 : 3. As spray reagent served either silver nitrate as described by Trevelyan *et al.*[20] or the hydroxylamine-ferric chloride reagent as described by Abdel-Akher and Smith.[21]

The equivalent weights were determined by potentiometric titration under nitrogen to pH 8.0 using 0.01 N potassium hydroxide, and specific rotation was measured using a Perkin-Elmer Model 141 polarimeter. The infrared spectra were determined in potassium bromide using a Perkin-Elmer IR 457 spectrophotometer, PMR spectra were determined using a Varian A60 and the mass spectra were obtained from an AEI MS 902 mass spectrometer.

*Methyl-β-*D*-galactofuranoside.* The method as described by Augestad and Berner [22] was used for the preparation of the furanoside. The preparative separation of the anomers was effected on a column (40 × 3 cm) cellulose powder using solvent A or B as eluent. The effluent left the column at a rate of *ca.* 80 ml/h and was collected automatically in fractions of 13 ml. The furanoside was recrystallized three times from ethyl acetate.

*Methyl-α-*L*-arabo-pentodialdo-1,4-furanoside.* The periodate oxidation of methyl-β-D-galactofuranoside was carried out as described previously;[14] the reaction was stopped after 40 min when 0.97 mol periodate had been consumed per mol furanoside. After all the inorganic material had been removed a syrup was obtained which crystallized from ethanol. The dialdehyde decomposed before melting. $[\alpha]_D^{20} = -93.5°$ (*c* 0.1, water). M.W. 163. (Found: C 44.2; H 6.20. Calc. for $C_6H_{10}O_5$: C 44.5; H 6.18.)

D-*Galacturonic* and L-*altruronic acid.* The method of Pratt and Richtmeyer [23] for the preparation of heptonic acids from a hexose was applied on the pentodialdofuranoside, and the reaction was followed by estimation of the reducing sugar by the Somogyi method.[24] Methyl pentodialdofuranoside (2.0 g) was dissolved in water (10 ml) and sodium cyanide (1.2 g dissolved in 10 ml) was added at 0°C and the solution kept at 0°C for 60 h. The hydrolysis was achieved by heating the solution (60 – 80°C) for 8 h keeping the volume constant by addition of water. Sodium ions were removed with Amberlite IR-120 ion exchange resin and the solution was evaporated to dryness *in vacuo*. Hydrolysis of the methoxyl group was achieved by heating the uronic acid mixture (130 mg) in sulphuric acid (5 ml; 2 N) at 60°C for 2 h. The solution was neutralized with barium carbonate, filtered and evaporated to a small volume *in vacuo*. The solution was then centrifuged, treated with activated carbon and the free uronic acids were obtained from the barium salts by treatment with Amberlite IR-120 ion exchange resin (100 % excess of resin). After evaporation *in vacuo* and drying, the yield was 88 mg. Preparative separation of the epimeric uronic acids was carried out on a pre-washed Whatman 3MM paper using solvent D as eluent. From 52 mg applied on one paper it was obtained 10.9 mg of fraction a, 12 mg of fraction b, and 22 mg of fraction c. The fractions a, b, and c (8.5 mg) were oxidized to the corresponding aldaric acids by bromine water (0.5 ml water saturated with bromine) by keeping the solution at room temperature for 6 h. Hydrogen bromide and excess of bromine was removed by successive addition and evaporation of water (3 × 2 ml). Reduction of fraction b and c was carried out with sodium borohydride using the method as described by Perry and Hulyalkar.[25]

trans-*5,6-Dideoxy-methyl-α-*L*-arabo-5-heptenuronic acid.* The method as described by Kochetkov and Dmitriev [18] for the preparation of unsaturated onic acids was used. The

methyl pentodialdoside (2.5 g) was dissolved in pyridine (4 ml), piperidine (3 drops) and malonic acid (1.85 g) were added and the mixture heated at 100°C for 90 min. After evaporation of pyridine, the mixture was separated on a cellulose column (40 × 3 cm) in the standard way using solvent C as eluent. The fastest moving component, which was isolated as a syrup (65 % of the applied mixture), was the only fraction which by PMR indicated an unsaturated compound with a *trans* coupling constant. No further attempts were made for the purification of this fraction.

D-glycero-L-altro-*Hepturonic acid*. The heptenuronic acid (200 mg) was dissolved in a water-propanol (1:3) mixture (4 ml), one crystal of osmium tetroxide and iodic acid (65 mg) were added. After 2 days at room temperature the solution was evaporated to dryness and the excess of iodine removed by four times successive addition and evaporation of water (5 ml). The resulting syrup showed only a very small amount of the starting material by chromatography using either solvent A or D as eluent. Without further purification the mixture was hydrolyzed by heating a solution (380 mg) in sulphuric acid (15 ml; 2 N) at 60°C for 2 h. Sulphuric acid and excess of iodic acid was removed by precipitation with barium ions and the free hepturonic acid isolated from its barium salt as described for the hexuronic acids. A syrup (228 mg) was obtained which partly crystallized. Recrystallization two times from ethyl acetate gave m.p. 138–139.5°. (Found: C 40.2; H 4.77. Calc. for $C_7H_{10}O_7$: C 40.7; H 4.85.)

Oxidation to the corresponding dicarboxylic acid was achieved using bromine as described for the oxidation of D-galacturonic acid.

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