ACTA CHEMICA SCANDINAVICA 20 (1966) 2081–2085

Chromatographic Separation of Sugars

V. Separation of Some Epimeric Heptonolactones*

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It is possible to separate the epimeric heptonolactones obtained by cyanohydrin synthesis on D-glucose, D-mannose and 3-*O*-methyl-D-glucose by chromatography on cellulose. The chromatographically separated lactones have presumably been obtained in a purer state than previously described, and therefore also the 3-*O*-methyl-D-*glycero*-D-*ido*-heptono-δ-lactone was obtained crystalline.

Until about 1950 the only general method of preparing higher-carbon sugars was the Kiliani-Fischer cyanohydrin synthesis.[2] Since then some other methods, *e.g.* condensation with nitromethane [3] and the Knoevenagel-Doebner and Wittig reactions [4] have been introduced, but still the Kiliani-Fischer synthesis probably is the most convenient method.

Asymmetric induction gives rise to different ratios of the two epimeric compounds depending on the starting material. This has been investigated in some detail in order to find a relationship between the structure of the starting material and the ratios between the epimeric compounds obtained. Maltby [5] postulated that the epimer getting hydroxyl groups at carbon atoms 2 and 4 in *trans* position was likely to be the main product. This hypothesis has, however, been questioned. Thus, Isbell and coworkers [6] investigated the cyanohydrin synthesis on D-arabinose and found the ratio D-gluconic acid: D-mannonic acid varying from 72:28 to 30:70 depending on salts added. On the other hand, starting with glucose the ratio of the epimers seemed to be almost independent of the method used.[7] As the reaction conditions in some cases so easily will influence on the ratio of the epimers, Maltby's rule may seem somewhat useless. However, using hydrocyanic acid as standard reagent, Hudson [7] has found the rule to be approximately valid for a wide number of sugars.

All the methods described for fractionation of the epimers have been based on recrystallization of the compounds obtained, *i.e.* lactones,[8,9] salts,

* Part IV, Ref. 1.

either with metals [10,11] or alcaloids,[12,13] phenylhydrazides [8,9] or amides.[14,15] The best results have been obtained using a combination of two or more of these methods. All the methods are, however, time-consuming and the yields usually low, especially for the minor epimer. Except in a few cases where the two epimers could be precipitated separately using specific precipitation methods, the ratio of the epimers given in the literature is uncertain.

The separation by chromatography of the epimers obtained by the Kiliani-Fischer cyanohydrin synthesis has so far not been reported in the literature. In connection with our problems in the separation of uronic acids and their lactones [16] we were also interested in the onolactones, and the present paper reports the chromatographic separation of some epimeric heptonolactones. In the Kiliani-Fischer cyanohydrin synthesis it might be possible to separate the epimers by chromatography also at other stages, *e.g.* the cyanohydrins or the free acids. Of these, the free acids were found, like uronic acids, to be very difficult to separate by partition chromatography. The cyanohydrins, on the other hand, were separated in several systems, *e.g.* ethyl acetate-acetic acid-formic acid-water (18:3:1:4) (A), ethyl acetate-pyridine-water (10:4:3) (B), or butanol-pyridine-water (6:4:3) (C). Some tailing occurred, however, probably due to a slight hydrolysis during the development of the chromatograms. The epimeric lactones were, therefore, found to be best suited for chromatographic separation. In this case, and using γ-lactones, no hydrolysis could be detected either during the development or during the evaporation of the fractions (investigated by paper chromatography). With the δ-lactones, on the other hand, hydrolysis did occur to a certain extent during the evaporation of the fractions but no hydrolysis could be detected during the development. Therefore, also epimeric δ-lactones could be separated chromatographically.

Of the eluent systems investigated by paper chromatography the best for general purposes was found to be the system A and the system ethyl acetate-propanol-water (5:3:2) (D) (see Table 1). However, as both these eluents were relatively poor solvents, the preparative chromatography on a cellulose column of the epimers of glucoheptonolactone and of 4-*O*-methyl glucoheptono-

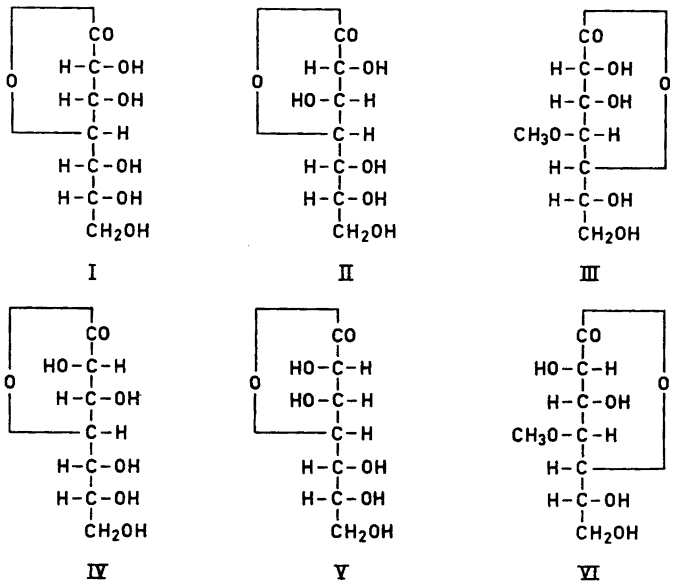
Table 1. R_G-values of some heptonolactones in different eluent systems.

Lactones	system A	system D	system E
D-*Glycero*-D-*gulo*-heptono-γ-lactone	0.76	1.27	1.42
D-*Glycero*-D-*ido*-heptono-γ-lactone	1.24	1.57	1.79
D-*Glycero*-D-*galacto*-heptono-γ-lactone	0.84	1.08	1.50
D-*Glycero*-D-*talo*-heptono-γ-lactone	1.20	1.31	1.51
4-*O*-Methyl-D-*glycero*-D-*gulo*-heptono-δ-lactone	1.72	1.97	2.05
4-*O*-Methyl-D-*glycero*-D-*ido*-heptono-δ-lactone	1.96	2.12	2.30

lactones was carried out using methyl ethyl ketone-ethanol-water (5:2:1) (E) as eluent. Unfortunately, this system did not separate the epimeric manno-heptonolactones (see Table 1) and here the system A was used.

According to Isbell *et al.*[6] one of the critical steps in the Kiliani-Fischer cyanohydrin synthesis is the hydrolysis of the cyanohydrins as a partial reversal of the condensation may occur. However, using the conditions described by Isbell *et al.*[6] for the hydrolysis no appreciable reversal could be detected chromatographically. On the other hand, during the lactonization step some side reactions seemed to have taken place.

Chromatography of glucoheptonolactone gave 56 % D-*glycero*-D-*gulo*-heptono-γ-lactone (I) m.p. 151°C $[\alpha]_D^{20}$ + 54.1° (*c* 0.2, water) ν_{max} 1756 cm^{-1} (see Barker [17]) and 27 % D-*glycero*-D-*ido*-heptono-γ-lactone (IV) m.p. 152°C, $[\alpha]_D^{20}$ −74.9° (*c* 0.2, water), ν_{max} 1759 cm^{-1}. Starting with mannose the result was 82 % D-*glycero*-D-*galacto*-heptono-γ-lactone (II), m.p. 150°C, $[\alpha]_D^{20}$ + 61.5° (*c* 0.2, water), ν_{max} 1770 cm^{-1} and 4.3 % D-*glycero*-D-*talo*-heptono-γ-lactone (V), m.p. 152°C, $[\alpha]_D^{20}$ + 35.3° (*c* 0.2, water), ν_{max} 1770 cm^{-1}. There was also isolated a brown syrup, which on the chromatogram moved faster than both the epimeric lactones. This syrup has not yet been analyzed in detail but some tests seem to indicate that it might be an anhydro uronolactone.



The lactones obtained starting with 3-*O*-methyl glucose are δ-lactones and therefore more difficult to prepare. The syrup used for chromatography contained some free acid and consequently the yield of the separated lactones was low: 13 % of 4-*O*-methyl-D-*glycero*-D-*ido*-heptono-δ-lactone (VI), m.p. 147—149°C, $[\alpha]_D^{20}$ + 37.4° (*c* 0.2, water), ν_{max} 1730 cm^{-1} (δ-lactone [17]) was obtained together with 43 % of 4-*O*-methyl-D-*glycero*-D-*gulo*-heptono-δ-lactone

(III), m.p. 198°C, $[\alpha]_D^{20}$ + 40.4° (*c* 0.2, water), ν_{max} 1725 cm^{-1}, and 16 % of a brown syrup moving faster than the two epimeric lactones.

Assuming the side reactions to occur proportionally to the amounts of the epimers, and, in the case of the 4-*O*-methyl compounds, that one has the same ratio of the epimeric heptonic acids as of the corresponding epimeric lactones, the percentage of the fractions given above represents the true ratio of the epimers. We therefore conclude that with the procedure used (sodium cyanide as reagent), Maltby's rule is valid for the cyanohydrin synthesis starting with glucose as well as mannose, since the ratio of the gluco-heptonolactone epimers in the relationship given above is 2.1:1 in favour of the *gulo*-configuration and for the mannoheptonolactones 19:1 in favour of the *galacto*-configuration.

According to Maltby the substituents at carbon atom 3 in the starting material are the determining factor for the ratio between the epimeric compounds obtained by the cyanohydrin synthesis. Starting with 3-*O*-methyl glucose, having the more bulky methoxyl group in 3 position, one would expect even more of the favoured epimer, which also seems to be the case (ratio 3.4:1).

The physical data obtained are in fair agreement with those reported earlier but our data indicate that somewhat purer compounds have been obtained by the chromatographic separation. By this method we also succeeded in crystallizing the compound VI.

In spite of the general nature of the Kiliani-Fischer cyanohydrin synthesis certain higher-carbon sugars have been practically inaccessible by this method due to difficulties in isolating the minor components. By chromatography of the epimers, also these minor components can easily be isolated and accordingly the cyanohydrin synthesis is given an even wider scope.

EXPERIMENTAL

Paper chromatography was carried out using Whatman No. 1 paper and one of the eluents A, B, C, D, or E. As spray reagent served either silver nitrate as described by Trevelyan *et al.*[18] or the hydroxylamine ferric chloride reagent as described by Abdel-Akher and Smith.[19]

The preparative separation was effected using Whatman cellulose powder, standard grade, in a column (40 × 3 cm) where the effluent left at a rate of *ca.* 50 ml/h and was collected automatically in fractions of 10 ml.

The reaction of the sodium cyanide with the monosaccharide was followed by estimation of reducing sugar by the Somogyi method.[20] With all the three sugars the reaction was complete (100 %) within 40 h but the reaction was continued for further 8 h before hydrolysis of the cyanide. Specific rotation was measured using a Perkin-Elmer Model 141 polarimeter.

The infrared spectra were determined in Nujol and potassium bromide using a Beckman-IR5A spectrophotometer.

D-*Glucoheptono-γ-lactone*. The method of Pratt and Richtmeyer[21] was used for the synthesis of the lactone. D-Glucose (10 g) was dissolved in water (70 ml) and sodium cyanide (5 g dissolved in 30 ml) was added at 0°C and the solution kept at 0°C for 48 h. The hydrolysis was achieved by heating the solution on a boiling water bath for 6 h keeping the volume constant by addition of water. Sodium ions were removed with Amberlite IR-120 ion exchange resin and the solution was concentrated to a small volume *in vacuo*. The lactonization was achieved by heating the syrup to 100°C for 27 h with mechanical stirring. Yield 10.5 g. The chromatography was carried out by applying the

syrup (1.5 g) to the column and using solvent E as eluent. Analysis of the fraction in the polarimeter and by paper chromatography showed two distinct fractions, which on evaporation gave, respectively, 468 mg of the crystalline compound IV and 950 mg of compound I, also crystalline. Prior to determination of the physical data, the lactones were recrystallised; compound IV from methyl cellosolve and compound I from alcohol, and then dried in a vacuum desiccator over calcium chloride.

D-*Mannoheptono-γ-lactone*. The synthesis was carried out as described for the preparation of glucoheptonolactone. Starting with 10 g of D-mannose the yield was 11.2 g of syrupy mannoheptonolactone. The chromatography was carried out on 517 mg of the syrup with solvent A as eluent.

Three different fractions were obtained; the second giving 434 mg of crystalline compound II and the third gave 17.6 mg of compound V as a syrup which crystallized from alcohol. The first fraction gave 62.6 mg of a dark brown syrup. We have not yet been able to crystallize or decolourize this syrup.

4-O-Methyl-D-*glucoheptone-δ-lactone*. 3-*O*-Methyl-D-glucose was obtained from D-glucose in the usual way as described by Glen *et al.*[22] by methylation of 1,2:5,6-di-*O*-isopropylidene-D-glucofuranoside. After hydrolysis of the isopropylidene groups the 3-*O*-methyl-D-glucose was purified by chromatography on a cellulose column using butanol-ethanol-water (5:1:4) as an eluent.

3-*O*-Methyl-D-glucose (5 g) dissolved in water (35 ml) was treated with sodium cyanide (5 g in 15 ml of water) at 0°C for 65 h and the heptonolactone isolated as described for D-glucose. Yield 5.5 g. The chromatography was carried out in the usual way. Starting with 1.5 g and using solvent E as an eluent there was obtained three distinct fractions; the first one giving a brown syrup (168 mg), the second 192 mg of crystalline compound VI, and the third 649 mg of crystalline compound III. Compound VI was recrystallized from abs. ethanol and compound III from ethanol (96 %). The free acids present in the syrup moved extremely slowly in the column and separation or isolation were not attempted.

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Received March 30, 1966.