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Chromatographic Separation of Anomeric Glycosides

II. New Crystalline Methylfuranosides of Galactose, Arabinose and Xylose

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In Part I of this series Augestad, Berner and Weigner [1] gave a preliminary report on the chromatographic separation of isomeric glycosides on columns of powdered cellulose. It was found that by using a suitable mixture of solvents not only ring-isomeric but even anomeric glycosides could be separated in a single operation. Previously Hough, Jones, and Wadman [2] had reported the separation of α- and β-methyl-L-rhamnopyranosides by partition chromatography on a column of "hydrocellulose". As the methylpyranosides of the common sugars are known in the pure state, the investigation was concentrated on the isolation of such methylfuranosides which have not as yet been obtained in crystalline form. In Part I the results obtained with D-fructose and D-galactose were briefly described. The β-methylfructofuranoside which was isolated as a thick syrup having a specific rotation in water of —46.9° has still not crystallized. As analysis showed the purity to be only 97 % further purification is now being attempted. In this report details are given of the isolation of the crystalline methylfuranosides of D-galactose, D- and L-arabinose, and D-xylose, of which the following are new crystalline substances:

α-Methyl-D-galactofuranoside,	m. p.	91—92°,	$[\alpha]_D^{20}$ =	+104° (in water)
α-Methyl-D-xylofuranoside	»	84°	»	+182° (» »)
β-Methyl-L-arabofuranoside	»	58°	»	+118° (» »)
β-Methyl-D-arabofuranoside	»	56—57°	»	—119° (» »)

The general procedure, details of which will be found in the experimental part, can briefly be summarized as follows. The process of glycosidation was first studied in order to find the optimal conditions for the formation of furanosides. Generally it was found that boiling a methanolic solution containing very little hydrogen chloride gave the best results. The process was followed by measuring the rotation and the amount of free sugar, and by taking paper chromatograms using the known methylpyranosides of each sugar as reference substances. The chromatographic examination at the same time enabled the solvent or solvent-mixture best suited for the subsequent

separation of the glycosides on the cellulose column to be found. Having removed the hydrogen chloride by means of silver carbonate and the methanol by evaporation a syrup was obtained, a concentrated solution of which was put directly on top of a column of powdered cellulose (Whatman, Standard Grade). The eluant was taken up in an automatic fraction-collector as described by Hough, Jones and Wadman [3]. Polarimetric readings permitted a curve to be drawn showing the main distribution of the isomeric glycosides in the fractions. In order to be able to collect those fractions which contained one glycoside only chromatographic examinations were taken on the border between subsequently eluted glycosides. From the fractions containing practically one single glycoside a syrup was obtained which in several cases crystallized spontaneously on standing for a shorter or longer time, eventually at low temperature. In repeated experiments when crystals were available for seeding crystallization took place rapidly. The glycosides were recrystallized from dry ethyl acetate.

EXPERIMENTAL

Methyl-D-galactosides

Haworth *et al.*[4,5] first prepared syrupy mixtures rich in methyl-galactofuranosides by glycosidation in methanol containing hydrochloric or sulphuric acid. Whether the reaction was carried out at room temperature or by boiling, the syrup obtained had a specific rotation of about −50°. The syrup was methylated to tetramethylmethylgalactoside the ring structure of which was proved to be that of a furanoside.

Green and Pacsu [6] prepared crystalline β-methyl-D-galactofuranoside, m. p. 63−65°, $[\alpha]_D = -108°$, by treatment of galactose-ethylmercaptal with methanol in the presence of mercuric chloride and oxide.

In our experiments a syrup rich in galactofuranosides was prepared by boling, for 6 hours, a methanolic solution containing 2 % D-galactose (C. P. quality, Pfanstiehl) and 0.013 % HCl (0.0037 *N*) when a maximum of levorotation (−44°) was reached. The solution then showed no reducing power. The hydrogen chloride was neutralized with silver carbonate. A little discoloration by silver salt was removed by heating with charcoal. On evaporating the filtered solution a completely colourless syrup was obtained. Chromatographic examinations on paper showed that a mixture of ethyl acetate, propanol-1, and water (5 : 3 : 2) gave a good separation. α-Methylgalactopyranoside prepared according to Michel and Litman [7] and β-pyranoside prepared according to Fischer [8] were used as reference substances. The paper chromatograms revealed that the syrup contained mainly furanosides, a small amount of α-pyranoside and very little β-pyranoside.

The syrup (3.5 g), diluted with a little of the eluant, was put at the top of a column (56 × 3.5 cm) of powdered cellulose and covered with a layer of cellulose. The mixture mentioned above served as mobile phase. The eluate left the column at a rate of 80 ml per hour and was collected automatically in fractions of 10 ml. After 6 hours the eluate aquired a negative rotation. The readings of the subsequent fractions were plotted against the number of the fractions as shown in Fig. 1.

Chromatographic examinations indicated where to divide the series of fractions in order to obtain pure substances. Fractions 1−12 gave 1.85 g (53.5 % of original syrup) syrup of β-furanoside, fractions 19−29 0.50 g (14.2 %) syrup of α-furanoside and fractions 34−60 0.37 g (10.8 %) syrup of α-pyranoside. The fractions containing the α-pyranoside were not worked up.

Crystalline α-methyl-D-galactofuranoside: The syrup from fractions 19−29 did not crystallize when left for a week in a vacuum desiccator. It was therefore dissolved in warm ethyl acetate and the solution placed in a refrigerator. Over night crystals (0.4 g) had developed which were recrystallized from 25 ml dry and alcohol-free ethyl acetate, m.p. 91−92°, $[\alpha]_D^{20} = +104°$ ($c = 1$ in water) and $[\alpha]_D^{20} = +101°$ ($c = 2$ in methanol). A final chromatographic examination gave only one spot. By hydrolysis at room tem-

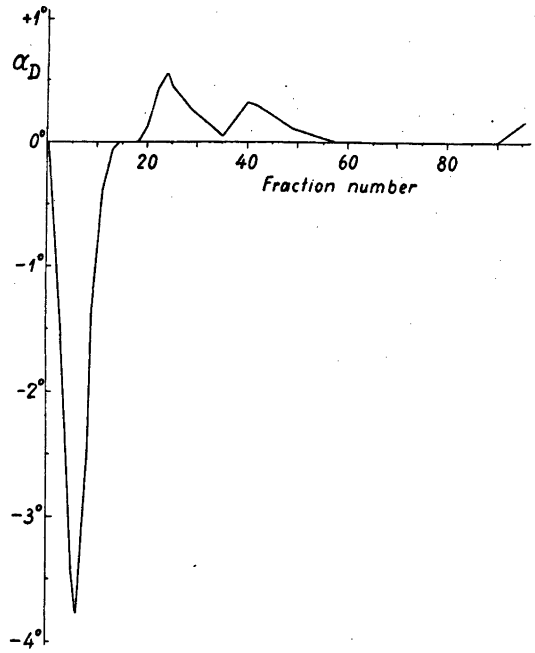

Fig. 1. Chromatographic separation of methylgalactosides: optical rotation of the fractions.

perature of a 0.5 % solution in 1.1 *N* HCl the time of half life was found to be $6^3/_4$ hours. The determination of free galactose was made by Shaffer-Hartmann's method, using the table given by Schoorl [9].

*Crystalline β-methyl-*D*-galactofuranoside:* The syrup from fractions 1—12 crystallized spontaneously when kept at −5° for a fortnight. After recrystallization from ethyl acetate the compound had, m.p. 69°, $[\alpha]_D^{20} = -112°$ ($c = 3$ in water), $[\alpha]_D^{20} = -140°$ ($c = 2.5$ in methanol).

The syrup from fractions 61—81 crystallized spontaneously overnight, and the β-methyl-D-galactopyranoside thus obtained after recrystallization had m. p. 178° and practically no optical rotation.

Methyl-D-xylosides

Haworth and Westgarth [10] by treatment of xylose at room temperature with methanol containing 1 % HCl obtained a liquid mixture of methylxylofuranosides having a specific rotation of +62°. We prepared the raw-material for the chromatographic separation by boiling, for $5^3/_4$ hours, a methanolic solution containing 2 % D-xylose (C. P. quality, Pfanstiehl) and 0.012 % HCl (0.0036 *N*). By this treatment 99 % of the sugar had reacted and the syrup isolated as described for galactose had $[\alpha]_D = +32°$.

Paper chromatograms of the syrup showed that the use of methyl ethyl ketone saturated with water as mobile phase led to a good separation of the xylosides. This solvent was therefore also used for the separation on the column. All four methyl xylosides were found to be present, the β-pyranoside, however, in minor quantity. The α- and β-methylpyranosides used for comparison were prepared according to Hudson [11] and Fischer [8] respectively. Starting with 2.3 g syrup the separation on a column of cellulose was carried out as described above. The curve for the rotation of the fractions showed that also in

this case the β-furanoside was eluted first; then followed the α-furanoside and after this the α-pyranoside. The elution was not continued until the small amount of β-pyranoside present appeared.

From fractions 1—15 the β-methyl-D-xylofuranoside (1.03 g or 45 % of original syrup) was obtained as a clear syrup having $[\alpha]_D = -82°$ in water. A paper chromatogram showed that one sugar derivative only was present, but the syrup has as yet not crystallized.*

Crystalline α-methyl-D-xylofuranoside: From fractions 21—34 a syrup (0.51 g or 22 %) was isolated which crystallized on standing overnight in a vacuum desiccator. Recrystallized from ethyl acetate it had, m. p. 84°, $[\alpha]_D^{20} = +182°$ ($c = 4$ in water). A chromatographic control gave a single spot only. The α-methylfuranoside was very easily hydrolyzable. For a 0.5 % solution in 1.06 *N* HCl the time of half life was 45 min. at room temperature.

The fractions 35—60 gave 0.39 g (17 %) syrup which crystallized in the course of a week. Recrystallized from ethyl acetate it had, m. p. 80—82°, $[\alpha]_D^{20} = +137°$ ($c = 1.2$ in water). A chromatogram showed that it was an impure α-methylpyranoside.

Methyl-L-arabinosides

Baker and Haworth [12] prepared a syrup rich in methyl-L-arabofuranosides by letting the sugar react for 17 hours at room temperature with methanol containing 2 % HCl. The syrup after having been distilled at 173—175° (0.15 mm) had $[\alpha]_D = -71.3°$ (in methanol). The rotation decreased in six months to −51.9°. This may have been due to a contamination by a small quantity of hydrogen chloride. We have found that in order to obtain stable syrups considerable attention must be paid to the removal of the hydrogen chloride.

Crystalline α-methyl-L-arabofuranoside, $[\alpha]_D = -125°$ (in water) was prepared by Green and Pacsu [6] from L-arabinoseethylmercaptal. The melting point was not determined owing to the strongly hygroscopic nature of the substance.

The α- and β-methylpyranosides of L-arabinose necessary as reference substances in our experiments were prepared according to Hudson [11].

A solution of 7 g L-arabinose (E. Merck) in 250 ml methanol containing 0.036 % HCl (0.01 *N*) was boiled for 2 h 50 min. when maximum of negative rotation ($[\alpha]_D = -48°$) was reached. The syrup was isolated as described above. Again methyl ethyl ketone saturated with water was found to be an effective mobile phase, the glycosides being eluted from the column in the order: α-furanoside, β-furanoside, β-pyranoside, and α-pyranoside. Starting with 3.3 g syrup and eluting at a rate of 200 ml per hour, the first rotation was observed after 4 hours. Fractions 1—20 gave 1.84 g (55.8 %) of levorotatory α-furanoside and fractions 37—84 0.71 g (21.5 %) of dextrorotatory β-furanoside, both as colourless syrups. Fraction 90 on chromatographic examination, besides the spot of the β-furanoside, showed a weak spot which did not coincide with those of the pyranosides and therefore obviously was due to an impurity present in the L-arabinose used. Fractions 85—110 on evaporation yielded a small quantity of crystalline β-methyl-L-arabopyranoside having m. p. 169°.

Crystalline β-methyl-L-arabofuranoside: The syrup from fractions 37—84 was dissolved in 100 parts of warm ethyl acetate and the solution placed in a refrigerator. As no crystals had appeared in four weeks one half of the solvent was distilled off. After standing a

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* *Added in proof:* The syrupy β-furanoside was later separated on a column in two nearly equal fractions having the specific rotations −85° and −87.2° respectively. The latter fraction was dissolved in 50 ml dry ethyl acetate of which 35 ml were distilled off. Placing the concentrated solution at −20° for six weeks and scratching with a glass rod the furanoside crystallized. Also the first fraction then crystallized on inoculation. After recrystallization from ethyl acetate and from ether the *β-methyl-D-xylofuranoside* had m.p. 45°, $[\alpha]_D^{21} = -89.5°$ ($c = 4.5$ in water). On hydrolyzing at room temperature with 1.09 *N* HCl the time of half life was 80 min.

further week in the refrigerator needles had been formed. Another sample of syrupy β-furanoside which had been kept for some time in a vacuum desiccator over phosphorus pentoxide crystallized overnight when inoculated with the needles. The crystals were extremely hygroscopic and necessary precautions had to be taken in handling them. After recrystallization from ethyl acetate they had, m.p. 58°, $[\alpha]_D^{20} = +118°$ ($c = 2.2$ in water). The hydrolysis of the β-furanoside in 1 *N* HCl proceeded at room temperature with a time of half life of 4 ¾ hours.

Crystalline α-methyl-L-arabofuranoside: The syrup from fractions 1—20 was kept in a desiccator for one month without crystallizing. On boiling with dry ether some of it dissolved. The remainder was dissolved in warm ethyl acetate but no crystals were formed on keeping the solution in a refrigerator for a fortnight. One half of the solvent was therefore distilled off whereupon some syrup separated which crystallized rapidly on standing in the refrigerator. The syrup recovered from the ethereal solution also crystallized on seeding. Owing to the strongly hygroscopic nature of the substance the same precautions had to be taken as in handling the β-furanoside. After recrystallization from ethyl acetate it had, m. p. 52°, $[\alpha]_D^{20} = -128°$ ($c = 4.7$ in water).

Methyl-D-arabinosides

Crystalline α-methyl-D-arabofuranoside was prepared by Montgomery and Hudson [18]. They treated the sugar with methanolic hydrogen chloride (0.2 *N*) at room temperature and stopped the reaction after 17 hours when a maximum of rotation of +45° had been observed. The crystalline α-furanoside which was described as quite hygroscopic had m. p. 65—67° (closed tube) and $[\alpha]_D = +123°$ ($c = 1.2$ in water).

In the present case a syrup rich in furanosides was prepared by boiling a solution of 6.7 g D-arabinose (C. P. quality, Pfanstiehl) in 250 ml methanol containing 0.036 % HCl (0.01 *N*) until a maximum specific rotation of +43.7° had been reached in 1 h 40 min. Analysis showed that 99.2 % of the sugar had reacted. The isolation of the syrup was carried out as described above.

The methylpyranosides used as reference substances were prepared as described by Hudson [11] for the corresponding derivatives of L-arabinose.

In the column chromatography of 3.5 g syrup methyl ethyl ketone saturated with water was used as mobile phase. The glycosides were eluted in the same sequence as in the case of L-arabinosides, the only difference being the reversed sign of rotation. Fractions 2—28 gave 1.78 g (51 %) of dextrorotatory α-furanoside and fractions 51—94 0.67 g (19.2 %) of levorotatory β-furanoside, both as syrups. From fraction 124 a small amount of crystalline β-methyl-D-arabopyranoside was obtained having m. p. 170° and a specific rotation in water of about −240°.

Crystalline β-methyl-D-arabofuranoside: 0.4 g of the syrup from fractions 51—94 was boiled with 30 ml dry ether. The undissolved part was dissolved in 30 ml ethyl acetate 25 ml of which were afterwards distilled off. The remaining mixture of syrup and solution crystallized on standing over night in a refrigerator. The hygroscopic crystals were rapidly collected and dried over phosphorus pentoxide. M. p. 56—57°, $[\alpha]_D^{20} = -119°$ ($c = 2.6$ in water). By hydrolysis at room temperature of a 0.65 % solution of the β-furanoside in 1 *N* HCl the time of half life was found to be 3 hours.

Crystalline α-methyl-D-arabofuranoside: The syrup from fractions 2—28 crystallized spontaneously when left for a couple of months in a desiccator over phosphorus pentoxide. Recrystallized from ethyl acetate, they formed needles, m. p. 52°, $[\alpha]_D^{20} = +128°$ ($c = 4$ in water); the same value was found for $c = 1.2$ in water. On recrystallization from dry ether the same kind of needles having m. p. 52° were obtained. As mentioned above Montgomery and Hudson found the m. p. 65—67°. They described their preparation as well formed transparent prisms, and it therefore seems probable that we have obtained a dimorphic form. It should be remarked that in the case of the enanthiomorphic form, the α-methyl-L-arabofuranoside, we also obtained needles with the same melting point (52°).

SUMMARY

Chromatography on a column of powdered cellulose using an appropriate solvent or solvent-mixture as mobile phase was found to give a good separation of isomeric glycosides. By this method the following new crystalline methyl-furanosides have been prepared: α-methyl-D-galactofuranoside, α-methyl-D-xylofuranoside, β-methyl-L-arabofuranoside, and β-methyl-D-arabofuranoside.

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