The rates of hydration and dehydration are first order with respect to hydronium ion and to the organic compound involved.

In each case the drop in the values of the specific reaction rate constants of hydration and dehydration when hydronium ion replaces sodium ion is ascribed to the formation of an oxonium complex which is less reactive than the uncomplexed compound.

In each case the decrease in the value of the equilibrium constant with the above change is ascribed to the fact that the unsaturated compound probably is a stronger base than the corresponding hydrated compound.

Acrolein hydrates at 100° in pure water and in 0.5 *N* perchloric acid to the same extent.

The values for $\Delta H$ of hydration are: $-5.8$ kcal. in the case of acrolein, and $-6.6$ kcal. in the case of acrylic acid.

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[CONTRIBUTION FROM THE DIVISION OF INDUSTRIAL AND CELLULOSE CHEMISTRY, MCGILL UNIVERSITY]

# Studies on Reactions Relating to Carbohydrates and Polysaccharides. LXV. An Improved Technique for the Fractionation of Partially Methylated Glucosides[1]

BY IRVING LEVI, W. LINCOLN HAWKINS AND HAROLD HIBBERT

The quantitative separation of mixtures of partially methylated glucosides obtained on hydrolysis of methylated polysaccharides has been attempted by three general methods.[2,3,4] Several of the techniques described during recent years have been complicated, requiring large amounts of the methylated polysaccharide, and no procedure has been reported by which a complete quantitative separation of mixtures of tetra-, tri- and dimethylmethyl glucosides can be effected.

A description is now given of an improved procedure based on the fractionation principles and technique described by Podbielniak.[5] Several control fractionations carried out with mixtures of synthetic 2,3,4,6-tetramethyl-, 2,3,4-trimethyl- and 2,3-dimethylmethyl glucosides yielded excellent separations of the glucosides with a total recovery of 95–97%. The fractionation results on a typical mixture of these glucosides are shown in Table I. With this apparatus the 2,3,4-trimethylmethyl glucosides could be readily fractionated into the pure solid $\beta$ and the liquid $\alpha$ isomer. In no case was there any appreciable decomposition.

In a recent investigation[6] on the structure of dextran, the present procedure gave very satisfactory results; an excellent separation was obtained using only three to four grams of the mixed glucosides. Intermediate fractions were very small and there was *practically no non-volatile residue* (not more than 1% and in some cases not detectable). The application of this fractionation technique is thus of particular significance in the field of structural carbohydrate chemistry, especially where the amount of material is limited and more than one distillation undesirable.

TABLE I

FRACTIONATION OF SYNTHETIC MIXTURES OF METHYL GLUCOSIDES

| Fraction | Fraction, g. | $OCH_3$, % | "Tetra," g. | "Tri," g. | "Di," g. |
|---|---|---|---|---|---|
| 1 | 0.746 | 60.5 | 0.746 | | |
| 2 | .151 | 59.6 | .113 | 0.038 | |
| 3 | .387 | 52.5 | | .387 | |
| 4 | .665 | 52.3 | | .665 | |
| 5 | .257 | 52.4 | | .257 | |
| 6 | .300 | 50.8 | | .249 | 0.051 |
| 7 | .451 | 42.1 | | | .451 |
| Total wt. | 2.957 | | .859 | 1.596 | .502 |
| Starting wt. | | | .872 | 1.660 | .516 |

The theoretical methoxyl values for tetra-, tri- and dimethylmethyl glucosides are 62.0, 52.6 and 41.9%, respectively. The amounts of each present in the small intermediate fractions (2 and 6) were calculated on this basis.

The fractionating column used is shown in Fig. 1. It is packed with a gold-plated wire (20 gage) spiral with $^1/_8$" pitch. The vacuum-jacket, column head and delivery tube of the small condenser were wound with a heating element (nichrome ribbon) and the small condenser adapted for the passage of cold water or steam, depending on the nature of the distillate.

(1) Original manuscript received August 13, 1941.

(2) Haworth and Machemer, *J. Chem. Soc.*, 2270 (1932); Haworth and Percival, *ibid.*, 2277 (1932).

(3) Macdonald, THIS JOURNAL, **57**, 771 (1935).

(4) Hess and Neumann, *Ber.*, **70B**, 710 (1937).

(5) Podbielniak, *Ind. Eng. Chem., Anal. Ed.*, **3**, 177 (1931); **5**, 119 (1933).

(6) Levi, Hawkins and Hibbert, THIS JOURNAL, **64**, 1959 (1942).

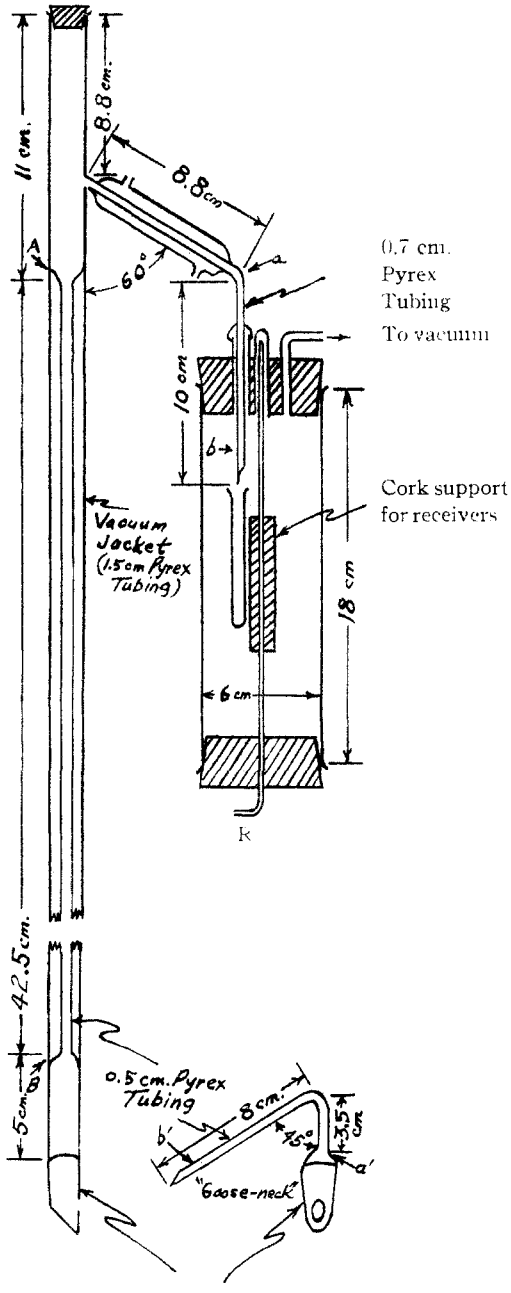


Fig. 1. No. 12/18 Pyrex ground bevelled to 45° angle and with hole in bottom as shown.

### Fractionation Technique

The glucoside mixture is placed in a pear-shaped flask (25-cc. capacity) loosely packed with glass wool to maintain smooth boiling, and fitted to the bottom of the fractionating column by means of a ground-glass joint. After evacuation of the system to a constant pressure (regulated by a "bleeder" stopcock), the bath temperature is slowly increased until the maximum height of the reflux level is attained (about one-third up the column). Heat is then applied very gradually to the column (but not to the condenser top), while the bath temperature is kept constant, until the liquid refluxes to within 2.5 cm. of the top of the column. These conditions are then maintained for several hours to establish equilibrium, the gold-plated wire becoming, meanwhile, completely wet with the liquid.

The column temperature is raised slightly (3–4°) and the tetramethylmethyl glucoside collected at the rate of 150 to 200 mg. per hour, cold water being passed through the condenser during this operation. Due to the slow rate at which the column is operated at reduced pressure, the true boiling points cannot be determined, but the thermometer installed in the column head, with its bulb opposite the condenser side arm, gives the approximate values. The approximate column temperatures are read on a thermometer placed within the column air-jacket.

After a certain number of hours (dependent on the amount of tetramethylmethyl glucoside in the mixture) of constant distillation under these conditions, a dry spot on the previously completely wet gold-plated spiral wire becomes clearly visible, usually at about one-fifth the distance from the base, and during the next one to two hours the column dries completely with the exception of several cm. at the bottom. The receiver is now changed by turning the glass rod (R), the vacuum being maintained constant. The first fraction is pure tetramethylmethyl glucoside.

The bath temperature is slowly increased and also that of the column, until the spiral wire is again wet, and equilibrium conditions maintained for four hours. The column temperature is raised a further 2–3°, thus assuring the same uniform rate of distillation. The first four or five drops of distillate suffice to remove any residual "tetra" and are retained as an intermediate fraction of "tetra" and "tri." The third fraction is pure trimethylmethyl glucoside. It was necessary at this point to insert the condenser tip (a-b, Fig. 1) into the heating system, and to pass steam instead of cold water through the condenser to prevent solidification of the $\beta$-isomer of 2,3,4-trimethylmethyl glucoside.

After complete distillation of the trimethylmethyl glucoside, the column again becomes "dry," only the lowest 5 cm. being wet with dimethylmethyl glucoside. The column is now allowed to cool to room temperature and the vacuum released.

The pear-shaped distilling cup is removed and the column and condenser washed by distilling through them a small amount of chloroform. The chloroform distillate and washings from the column are combined and taken to dryness, this residue constituting the fraction intermediate between tri- and dimethylmethyl glucosides.

The dimethylmethyl glucoside should not be removed through the column because of its high boiling point but is distilled out of the flask through a short "goose-neck" (Fig. 1). This has a glass joint ground to fit the distilling flask and is wound with nichrome ribbon. This heating element is inserted in series with the heating unit of the column, thus permitting temperature control. The temperature of the "goose-neck" is kept at 100° during the distillation and the pressure reduced to 0.010 mm.

The authors are indebted to Dr. L. M. Cooke for designing the column described.

## Summary

An improved apparatus is described and pro-

cedure outlined for the separation of small quantities of partially methylated glucosides whereby an almost quantitative recovery (95–97%) can be effected.

The glucosides can be separated in a high degree of purity and with accompaniment of only very small intermediate fractions.

The amount of non-volatile residue formed was never more than one per cent.

MONTREAL, CANADA RECEIVED MAY 12, 1942

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[CONTRIBUTION FROM THE DIVISION OF INDUSTRIAL AND CELLULOSE CHEMISTRY, MCGILL UNIVERSITY]

# Studies on Reactions Relating to Carbohydrates and Polysaccharides. LXVI. Structure of the Dextran Synthesized by the Action of *Leuconostoc Mesenteroides* on Sucrose[1]

BY IRVING LEVI, W. LINCOLN HAWKINS AND HAROLD HIBBERT

In a previous communication[2] an investigation of the structure of dextran synthesized by the action of *L. mesenteroides* on sucrose was described. Hydrolysis of the trimethyl dextran by the action of methanolic hydrogen chloride yielded dimethyl, trimethyl and tetramethyl methyl glucosides in the approximate ratio of 1:3:1. The products of hydrolysis were identified as 2,3-dimethyl methyl glucoside, 2,3,4-trimethyl methyl glucoside and 2,3,4,6-tetramethyl methyl glucoside. Based on these results a branched chain structure for the dextran was proposed.

These results were subsequently criticized by Brauns[3] on the following grounds: (a) the dextran was incompletely methylated; (b) the ratio of tetra- to tri- to dimethyl methyl glucosides of 1:3:1 was not conclusive because of the inefficient fractional distillation employed; and (c) the large percentage (18.4%) of material lost during fractionation. A re-investigation of this dextran was therefore made in order definitely to establish its structure.

## Discussion of Results

Most complex polysaccharides such as mannan,[4,5] glycogen,[6,7,8] and araban[9] contain intricately branched chains, every branching position of which yields a dimethyl methyl glycoside in the case of hexosans and a monomethyl methyl glycoside in the case of pentosans. In methylation studies of such polysaccharides a final methoxyl value of one or two per cent. lower than the theoretical can render the results worthless with respect to the extent of branching and so preclude an accurate structural assignment. The great importance of complete methylation of polysaccharide products prior to structural determination by hydrolysis cannot be over-stressed and has been all too frequently neglected[10,11] with the result that conclusions drawn have only a restricted value.

Throughout this investigation every precaution was taken to obtain yields of material as nearly quantitative as possible in order that the results obtained could be based on a very high percentage of the starting material and therefore be of true significance. With dextran and its derivatives this involved reducing experimental manipulations and transfers to a minimum since these compounds are very difficult to handle because of their physical properties.

Dimethyl sulfate and alkali yielded a partially methylated dextran (40–41% $OCH_3$) which was further methylated to the theoretical value of 45.6% $OCH_3$ (calcd. for $C_6H_7O_2(OCH_3)_3$) in 71.4% over-all yield by a modified Muskat technique.[12] Hydrolysis of the completely methylated dextran was carried out with methanol–hydrogen chloride, in sealed glass bombs, heated to 140–142° for sixty to sixty-five hours in a tilting electric oven. The resulting glucosidic mixture, obtained in 95% yield, was fractionated by the new technique described in the preceding communication[13] using the modified Podbielniak column,[14] and an excellent separation of the glucosides effected with an over-all recovery of 97%. Of the 5% lost during

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(14) Podbielniak, *Ind. Eng. Chem., Anal. Ed.*, **3**, 177 (1931); *ibid.*, **5**, 119 (1933).