INVESTIGATION OF THE HETERODISPERSITY OF POLYSACCHARIDES, II*

DETERMINATION, BY TRITYLATION, OF THE HETERODISPERSITY OF GLUCOSIDIC BONDS IN DEXTRAN**

By

J. HOLLÓ, E. LÁSZLÓ and Á. HOSCHKE

Department of Agricultural Chemical Technology, Polytechnical University, Budapest

(Received April 5, 1968)

The methylation of the alcoholic hydroxyl groups and the oxidative cleavage with the aid of periodates represent the two methods of importance applied up to the present in the structure investigations of polysaccharides.

The data of literature referring to values obtained by the above-mentioned methods are, however, rather contradictory to each other, particularly in respect to the percentage of non 1.6-bonds, to the types of bond (1 → 3 or 1 → 4) in the branching sites and to the mean lengths of the chains.

Since methylation is in general a very cumbersome and lengthy procedure, several authors had attempted to modify the method based on oxidation with periodate, in order to obtain a method suitable for the structure investigation of dextran. BROWN et al. [1] were the first to apply the method for the determination of molecular weight. Later, a modified method has been evolved by JEANES et al. [2, 3].

Though the suggested method based on oxidation with periodate is essentially simpler and quicker than methylation, it does not afford satisfactory and reliable informations on the branching structure of dextran, due to the inaccuracy of the method (mainly due to "overoxidation").

The Jeanes method was further developed by SMITH et al. [4, 5] so that the products obtained by oxidation with periodate were reduced to polyalcohols and the latter subjected to hydrolysis. In this way, conclusions in respect to the types of the bonds of glucosidic radicals in the branching sites could be drawn from the nature of the products. However, the hazard of "overoxidation" has not been eliminated by this method, either.

Experiments carried out by the methylation and by the periodate-oxidation methods unambiguously proved, however, that in the branching sites the glucose units are linked in general by bonds of (1 → 3) type [2, 6, 7].

In our present investigations, the ether-forming reaction described by

* Part I published in Periodica Polytechnica (Chem) **12**, 69 (1968).

** 65th publication of this Department, in the series of research into polysaccharides.

HELFERICH for simple sugars [8] has been employed for the study of the branching structure of dextran.

The evolved method is based on the fact that the terminal glucose units of the branchings of the polymer molecule include free primary hydroxyl groups. These primary alcoholic hydroxyl groups can be selectively etherized by triphenyl methyl chloride (trityl chloride). The selectivity can be explained by the higher reactivity of the primary alcoholic hydroxyl group and by the particularly high place demand of the trityl group. With the aid of this method, the degree of branching and the number of non 1,6-glucosidic bonds of dextrans produced by various bacterial strains can be determined quicker and more precisely than by oxidation with periodate.

Experimental

1. Materials applied

Triphenyl methyl chloride, analytical grade (Fluka). The purity of the preparation has been checked by determining its melting point (112—113 °C).

Anhydrous formamide (analytical grade) and anhydrous pyridine (analytical grade).

Dextrans: various pulverized dextran preparations and their decomposition products.

2. Technique of tritylation

1.0 g of a sample of anhydrous dextran (previously dried for 2 days to constant weight at 105° C) was dissolved in anhydrous formamide (in the case of dextran samples of higher molecular weight, under slight heating). Separately, 1 g of trityl chloride was dissolved in 2 ml of anhydrous pyridine, and the solutions were combined in an ampoule. On sealing the ampoule, the reaction mixture was allowed to react for 2 hours in an oil-bath at 120 $\pm$ 1 °C.

After this period, the sample was cooled, and the precipitated substance (mainly unreacted trityl chloride and some trityl carbinol) separated on a G-4 filter.

To the clear filtrate, anhydrous ethanol, about 2.0 to 2.5 times its volume, was added, the precipitated trityl-dextran washed with ethanol and benzene (in order to remove trityl chloride and trityl carbinol present as contaminants). Lastly the powder-like product was washed with acetone, dried at first on a G-4 glass filter, then to constant weight at 105° C, and stored in a desiccator.

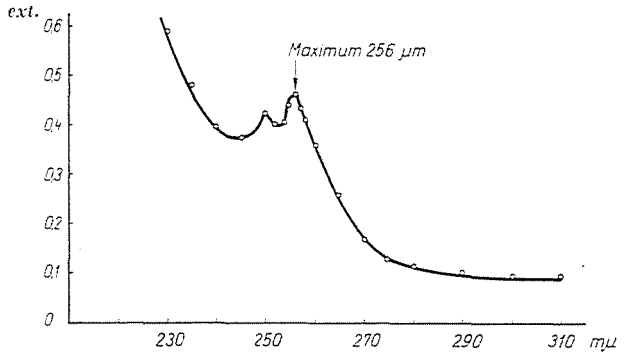

Fig. 1. Spectrum of tritylated dextran (K-22) Spectrophotometer of Spektromom 201 type. in a 1 cm quartz cell

3. *Determination of trityl content*

The trityl content of trityl-dextran was determined by spectrophotometry. The product showed an absorption peak at 256 mμ (Fig. 1).

The spectrum was established in a spectrophotometer of Spektromom 201 type, in a 1 cm quartz cell. An aqueous solution of identical concentration, prepared from a corresponding, non-tritylated dextran sample served as reference liquid.

The amount of trityl carbinol required for plotting the calibration curve has been determined by gravimetric method [9].

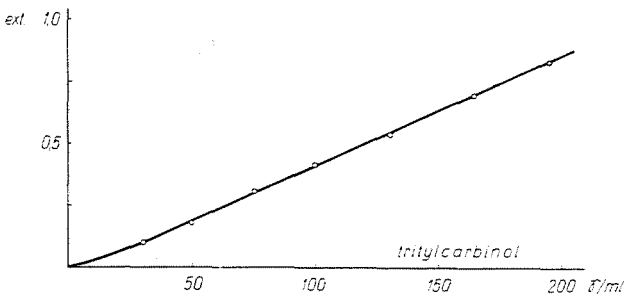

Fig. 2. Calibration curve for the spectrophotometric determination of the content of trityl carbinol (Conditions of the determination: dextran sample K-22, at 256 mμ, 1 cm quartz cell Reference liquid: non-tritylated sample of dextran K-22)

Technique of the determination

1.0 g of trityl dextran was dissolved in 20 ml of concentrated sulphuric acid under continuous stirring. Trityl carbinol (as dark grayish-black precipitate) was precipitated from the solution by adding distilled water, under

cooling and stirring. The precipitate was washed and centrifuged until it became neutral and free of sulphate. Subsequently, it was transferred into a weighing flask, dried to constant weight at 105° C and weighed.

On the basis of the data obtained in this way, the trityl carbinol content corresponding to the extinction value measured by spectrophotometry can be established and the calibration curve (Fig. 2) can be plotted.

4. Investigation of the conditions of tritylation

According to our preliminary experiments and on the basis of literature data [10, 11], the amount of trityl chloride applied is satisfactory for ether formation and that of pyridine is sufficient for binding the liberated hydrochloric acid.

The tritylation process as a function of time was studied as follows.

The reaction was carried out under completely identical conditions. Samples were withdrawn after the elapse of 0.5, 1, 2, 3 and 4 hours, the samples were processed, and their trityl contents determined (cf. Table 1).

Table 1

Investigation of tritylation plotted against time

Time of sampling hours	Trityl content mg per gram of dextran sample
0.5	33.0
1	35.5
2	35.0
3	34.5
4	27.5

It follows from the data of Table 1 that the optimum duration of reaction ranges between 1 and 2 hours. In this interval, the formation of the product appears to be constant. On applying prolonged reaction periods, the trityl content of dextran already decreases, due to side reactions.

5. Investigation of selectivity

The selectivity of the applied method has been investigated as follows. Heterogeneous dextran of high molecular weight, fermented from an identical strain was separated to homogeneous samples of different molecular weights, the aid of a column filled up with various layers of Sephadex molecular sieves

of different water retention capacities (G-10, G-25, G-75 and G-100) [12]. The gravimetric molecular weights (M_w) of the homogeneous products obtained by column chromatography were determined by the limit viscosity method [13] while the numerical molecular weights (M_n) by the iron(III) cyanide method [14]. Subsequently, the samples were subjected to tritylation. If the applied method is really selective, the degree of tritylation must increase proportionally to the decrease of molecular weights (in the case of a sample of a given weight, the number of free C_6-primary alcoholic hydroxyl groups increases).

The obtained results are given in Table 2.

Table 2

Tritylation and molecular weights of products of various molecular weights

File number	M_w	M_n	Trityl content mg per 1 g of dextran	Non 1,6-bonds per cent
1	122,500	35,600	20	1.27
2	39,000	15,400	26	1.66
3	25.000	10,800	35	2.22
4	17.200	8,200	47.5	3.12
5	12,500	5,850	59.0	3.86

The data given in Table 2 indicate that the above-mentioned correlation is in accordance with the experimental values, within the errors of measurement. Thus, tritylation appears to be in fact a selective process, suitable for the determination of the degree of branching of dextrans.

Table 3

Investigation of the structure of dextrans for infusion

Designation of sample	M_w	M_n	Glucose unit branching	Non 1,6-bonds per cent
PO	65,500	19,800	72	1.39
DR	64,300	19,600	45	2.22
DV	86,200	19,700	44	2.28
CH	81,300	20,700	38	2.63
K—22	67,400	14,700	28	1.39

6. Investigation of some samples of dextran for infusion

The experiments were carried out in the way described in paragraph 2. Samples of 0.5 g of dry dextran were dissolved in 10 ml of formamide. To these solutions, trityl chloride was added (in form of a solution containing

0.5 g of trityl chloride in 1 ml of pyridine), and the mixture kept for 2 hours at 120° C. The trityl contents of the samples were established by spectrophotometry, then the number of non 1,6-bonds determined from the obtained values by calculation (Table 3).

Summary

A method based on tritylation has been evolved for the determination of the non 1.6--bonds in various dextran samples. The reaction conditions and the selectivity of ether formation were studied. The optimum conditions proved to be 2 hours at 120° C.

The results confirm that the reaction is strictly specific for primary alcoholic hydroxyl groups, and thus it is suitable for the quick and accurate determination of the branching structure of polymer molecules.

The percentages of the non 1,6-bonds of dextran samples produced by certain bacterial strains and used for purposes of infusion were established. The obtained values ranged between 1.4 and 2.6 per cent.

References

1. Brown, F., Dunston, S., Halsall, T. G., Hirst, E. L., Jones, J. K. N.: Nature **156,** 785 (1945).
2. Rankin, J. C., Jeanes, A.: J. Am. Chem. Soc. **76,** 4455 (1954).
3. Jeanes, A., Wilham, C. A., Tsuchiya, H. M., Haynes, W. C.: Arch. Biochem. Biophys. **71,** 293 (1957).
4. Adbel-Akhev, M., Hamilton, J. K., Montgomery, R., Smith, F.: J. Am. Chem. Soc. **74,** 4970 (1952).
5. Hamilton, J. K., Smith, F.: J. Am. Chem. Soc. **78,** 5910 (1956).
6. Barker, S. A., Bourne, E. J., Bruce, G. T., Neely, W. B., Stacey, M.: J. Chem. Soc. 2395 (1954).
7. Van Cleve, J. W., Schaefer, W. C., Rist, C. E.: J. Am. Chem. Soc. **78,** 4435 (1956).
8. Helferich, B., Koester, H.: Ber. **57,** 587 (1924).
9. Hearon, W. M., Hiatt, G. D., Fordyce, C. R.: J. Am. Chem. Soc. **65,** 2450 (1943).
10. Green, J. M.: in: Whistler Methods in Carbohydrate Chemistry III. 327 (1963).
11. Tanghe, L. J., Genung, L. B., Mench, J. W.: Ibid. III. 208 (1963).
12. Holló, J., László, E., Tóth, M., Ráthonyi, Z.: Periodica Polytechnica (Chem) **12,** 69 (1968).
13. Senti, F. R.: Polymer Sci. **17,** 527 (1955).
14. Park, J. T., Johnson, M. J.: J. Biol. Chem. **181,** 149 (1949).

Prof. Dr. János Holló,
Dr. Elemér László
Dr. Ágoston Hoschke
Budapest, XI.,
Gellért tér 4. Hungary