char C-190). To the colorless filtrate about 35 ml. of absolute methanol was added followed by 30 ml. of ethyl ether. The turbid solution was placed at 5° for about 24 hours; the crystals were collected by centrifugation and dried *in vacuo* over P_2O_5. The compound melted with decomposition at 146.5 to 147.5°.

Anal. Found: C, 29.44; H, 6.36; N, 7.30.

The analyst reported that the compound was hygroscopic and each portion taken for analysis was specially dried at 100°. The low nitrogen can perhaps be accounted for by this drying procedure, since the nitrogen analysis of the sample prior to shipment to the analyst was 9.00%.

ADDED IN PROOF.—Since this paper was submitted, J. L. Reissig, J. L. Strominger and L. F. Leloir, *J. Biol. Chem.*, 217, 959 (1955), have published a modification of the Morgan method which yields identical extinction coefficients for N-acetylglucosamine and N-acetylglucosamine 6-phosphate. However, we have found it necessary to heat N-acetylglucosamine 6-phosphate in the borate solution for 8 minutes, instead of 3, to obtain maximal color development. With this method, the ratio of phosphate to acetylglucosamine in the synthetic N-acetylglucosamine 6-phosphate was found to be 1.

MADISON, WISCONSIN

[CONTRIBUTION FROM THE CHEMISTRY DIVISION, CANADA DEPARTMENT OF AGRICULTURE]

The Differential Thermal Properties of Bacterial Dextrans[1]

BY HIROKAZU MORITA

RECEIVED AUGUST 25, 1955

Differential thermal analyses of some bacterial dextrans were undertaken in order to elucidate the relation between their thermographic features and molecular constitution. Specific types of dextrans exhibit characteristic thermal behavior, which also reflects changes in physical properties resulting from the method of preparation. As a result of this study it is concluded that thermal analysis, when supplemented by other data, affords valuable information relating the constitution of the dextrans with their chemical and physical properties.

This paper is the third in a series of studies pertaining to the use of differential thermal analysis and the elucidation of the factors which determine the differential thermal properties exhibited by organic substances. Previous endeavors[2,3] showed that, aside from the obvious influence of elemental composition, differential thermal reactions of certain polymers were dependent upon molecular configuration, as for example, the type of polymer linkages. The behavior of cellulose in contrast to starch, and that of amylose and amylopectin were specific instances.

In order to ascertain further the relation between molecular constitution and differential thermal property, analyses were undertaken on some bacterial dextrans representative of distinct structural types. The results constitute the main theme of this communication.

The samples described below were chosen for two reasons. First, since data relating to their chemical and physical properties were available, it was hoped some correlation might be established between these and those provided by thermal analysis. Second, there is, at present, considerable interest in the fundamental properties of the dextrans among polymer and biological chemists.

Experimental

Details of the analytical technique have been described earlier.[2] In this investigation, 100-mg. samples were used to prepare the "sandwich" packing in calcined alumina. The dextrans were provided by the Northern Utilization Research Branch and are designated by the number assigned in the NRRL Culture Collection to the bacterial strain by which the dextran was produced. Each sample was vacuum dried at 100° and 0.01 mm. pressure to constant weight. Details on the physical and chemical properties of most of the dextran samples used have been reported by Jeanes and co-workers.[4]

Results and Discussion

It has been observed[3] that the array of endotherms in the 130 to 310° region is the most prominent thermographic feature shown by glucopyranose polymers having α-1,4-linkages. Analogous patterns (Fig. 1, curves A and B) are shown by typical preparations of the water soluble, native dextran from *Leuconostoc mesenteroides* NRRL B-512. These dextrans, which were produced by the whole-culture process, contain 95% α-1,6-linked units. The salient thermographic features are the sharp endotherms at 200 and 295° followed by a diminished one at 310°.

Modifications of the B-512 thermograms, shown in Fig. 1, curves C and D, were observed for a B-1308 and an enzymatically synthesized B-512 dextran.[5] The former gave endotherms at 220, 235 and 285°, while in the latter they appeared at 215 and 280°. The only other significant way these preparations are known to differ from those of curves A and B are in intrinsic viscosity, the values corresponding to curves A, B, C and D being 1.23, 1.02, 0.463 and 1.37, respectively.[4]

Further variations of the B-512 thermograms are portrayed in Fig. 2. While the dextrans, shown by curves A and B in Fig. 2, have analogous anhydroglucose linkages, they differ markedly from those in Fig. 1 in their intrinsic viscosities, the values corresponding to curves A and B being 0.75 and 0.811. The dextrans represented by curves C and D, on the other hand, are distinct from those in Fig. 1 by virtue of their linkages, periodate analysis indicating these to be 7% 1,4-like, 3% 1,3-like and 90% 1,6-anhydroglucose units.

In view of the thermographic changes caused by pretreatment of other polysaccharides,[3] a more detailed study was made of the relation between purity and differential thermal property. In most instances, purification involving treatments to reduce ash content had negligible effects. An exception is

(1) Contribution No. 289, Chemistry Division, Science Service.

(2) H. Morita and H. M. Rice, *Anal. Chem.*, **27**, 336 (1955).

(3) H. Morita, *Anal. Chem.*, **28**, 64 (1956).

(4) (a) A. Jeanes, *et al.*, THIS JOURNAL, **76**, 5041 (1954); *J. Biol. Chem.*, **176**, 603 (1948).

(5) H. J. Koepsell and H. M. Tsuchiya, *J. Bacteriol.*, **63**, 293 (1952).

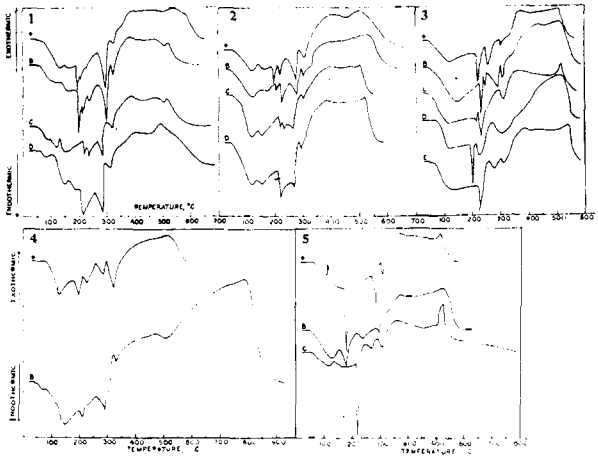

Fig. 1.—Thermograms of B-512 and related bacterial dextrans: A, B-512 dextran sample 1; B, B-512 dextran sample 2; C, B-1308 dextran sample 12; D, B-512-E dextran sample 6.

Fig. 2.—Variations in the B-512 dextran thermograms: A, B-512-E dextran sample 4; B, B-512-E dextran sample 24; C, B-1254 dextran sample 28; D, B-1254 dextran sample 29.

Fig. 3.—Thermal properties of 1,4-like linkages: A, B-1299 fraction S dextran sample 27; B, B-1299 fraction L dextran sample 11; C, B-1298 dextran sample 10; D, B-1422 dextran sample 18; E, B-1382 dextran sample 15.

Fig. 4.—Thermal properties of 1,3-like linkages: A, B-1355 fraction S dextran sample 13; B, B-1121 dextran sample 8.

Fig. 5.—Thermograms of clinical size and acetobacter dextrans: A, B-512-E dextran sample 7; B, B-512-E dextran sample 23; C, B-1225 dextran sample 9.

illustrated in Fig. 2. Curves A and B represent B-512-E[6] preparations in which B is the purified fraction containing 0.08% ash compared to the parent sample A with 0.41%. The curves reveal quantitative variations in the endotherms at 195, 215 and 300°.

Compared with the influence of impurities, changes in physical state, as manifested by solubility, noticeably altered the thermograms. A specific example is shown by the thermal curves of two B-1254 dextrans (Fig. 2, curves C and D). The samples were identical except in physical state. Sample 29 (curve D) was isolated from absolute ethanol and was difficultly soluble in water. Sample 28 was the water soluble lyophilized product. Its thermogram displays a doublet at 215 and 230° whereas sample 29 gives a sharp peak at 220°.

The results outlined in Fig. 2 lead to the conclusion that impurities do not radically alter the essential differential thermal characteristics of the dextrans. Changes in solubility may, however, produce perceptible variations in the finer details of the thermograms.

Pronounced modifications of the B-512 thermograms are caused by chain branching as depicted in Fig. 3. These dextrans were selected on the basis of their common serological behavior.[7] Like most of the highly 1,4-like linked water soluble preparations studied, the B-1382 (19%, 1,4-like linked) (curve E), the B-1422 (26% 1,4-like linked) (curve D) and the B-1298 (36% 1,4-like and 64% 1,6-linked) (curve C) dextrans all exhibit endotherms at 225 ± 5° and at 310 ± 5°.

The dextran fractions obtained from the B-1299 preparations have the highest proportion (48%) of 1,4-like linkages among the samples studied. Their thermograms (curves B and A) have prominent endotherms at 225 and 250°. However, the one at 290° appearing in fraction L (curve B) has diminished to an inflection point in fraction S. The differences between these fractions are more striking in their solubilities and viscosities even though the proportion of linkages is similar. Whereas water-soluble fraction S has an intrinsic viscosity of 0.469, insoluble fraction L has an estimated value of 0.873. The effect of solubility and viscosity has been noted previously in Fig. 2.

In contrast to the properties of highly 1,4-like linked dextrans, those with significant 1,3-like linkages manifest thermographic patterns exemplified by a water soluble B-1355 dextran (Fig. 4, curve A). This dextran, which has 35% 1,3-like and 56% 1,6-glucosidic linkages, shows an intense endothermic reaction at 320°.

An abnormal behavior was encountered with a B-1121 preparation (curve B). Although periodate analysis indicated that the relative proportion of 1,3-like and 1,6-linkages was not markedly different from the B-1355 samples, the thermogram displayed an extensive exothermic reaction culminating at 790°. This decided contrast is reflected in other properties. For example, the B-1355 dextran is water soluble while the B-1121 preparation requires use of 1 *N* potassium hydroxide for dissolution. The infrared spectrum of B-1121 dextran is also indicative of its unique properties.[4] Despite their dissimilarities, however, the two thermograms do show certain common features, namely, endotherms at 245 ± 5° and 285 ± 5°.

Figure 4 represents the most striking example where molecular aggregation appears to exert a dominant influence on differential thermal behavior. The surprising stability of the B-1121 sample implies that linkages which are not specifically identifiable by periodate analysis may be present. The limitations of the periodate oxidation method have been pointed out by Rankin and Jeanes.[8]

The most interesting dextrans are the clinical size preparations whose thermal characteristics are presented in Fig. 5. The preparation produced by acid hydrolysis of B-512-E dextran gave thermal reactions shown by curve A. The direct enzymatic product[9] afforded thermogram B. The curves are quite distinct but both have endotherms at 185°. This was observed with other commercial clinical dextrans.

All the dextrans described previously were produced from sucrose by *Leuconostocs* or other closely related bacteria. The B-1255 dextran produced

(6) The "B-512-E" dextran designates dextran synthesized by the dextran-sucrase enzyme system produced by B-512 strain organism.

(7) E. J. Hehre, unpublished results.

(8) J. C. Rankin and A. Jeanes, THIS JOURNAL, **76**, 4435 (1954).

(9) (a) H. M. Tsuchiya, N. N. Hellman and H. J. Koepsell, *ibid.*, **75**, 757 (1953); (b) there are reasons to suspect that the product may not be typical of this material (A. Jeanes, private communication).

from dextrin by an acetobacter strain[10] gave thermogram C. The very sharp endotherm at 220° is its most prominent feature.

The data in Figs. 1 to 5 demonstrate that the dextran thermograms show characteristic contours in the 100 to 310° region. The 200° endotherms are common to the predominantly 1,6-linked dextrans while those near the 225° region occur with enzymatic preparations. Moreover, the thermal peaks near 245° appear to be typical of the most highly 1,3-like linked dextrans. Clinical size preparations, on the other hand, are recognized by a peak at 185°. Significantly, almost all the thermograms show endotherms in the 310 ± 5° region.

Aside from the dominant influence of the type and proportion of glucosidic linkages, further variations appear to be associated with marked changes in solubility, unique viscosity properties, and to a minor extent, purity of the sample. It must be emphasized here that other unknown factors may be involved, especially in view of the structural complexities of the dextrans and more particularly in view of the undeveloped state of this thermal technique.

The differential thermal reactions responsible for the distinct endotherms appear to be similar to those encountered with starch.[3] Analogous evidence given by infrared analysis of the pyrolysis residues, as well as examination of the gaseous pyrolyzates, suggest that transglucosidation[11-13] and dehydration constitute the predominant endothermic processes. Dehydration reactions are, moreover, generally recognized as being stereospecific, particularly when they are catalytic. Consequently, the interpretation of the thermal effects due to chain branching and molecular aggregation may be based predominantly on stereochemical considerations.

The present investigation has shown that there may be some relationships between the thermal property of the bacterial dextrans and their molecular constitution which cannot be deduced readily by other analytical techniques. The results also indicate that differential thermal analysis may become an invaluable aid in elucidating the constitution of other polysaccharides and biologically important polymers.

Acknowledgment.—The author thanks Dr. Allene Jeanes of the Northern Utilization Research Branch, United States Department of Agriculture, for her helpful advice and material assistance during this investigation.

(10) E. J. Hehre, *J. Biol. Chem.*, **192**, 161 (1951).

(11) B. Brimhall, *Ind. Eng. Chem.*, **36**, 72 (1944).

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(13) I. A. Wolff, *et al.*, *Ind. Eng. Chem.*, **45**, 755 (1953).

OTTAWA, CANADA

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

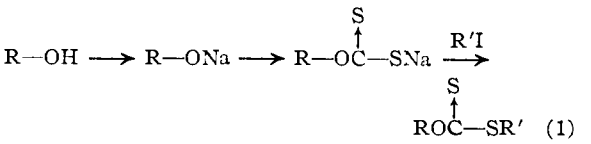
Dithiocarbonate Esters of Arabinose[1]

BY M. L. WOLFROM AND A. B. FOSTER[2]

RECEIVED SEPTEMBER 22, 1955

Certain 2-*O*-(*S*-alkyl dithiocarbonate) esters ("xanthates") of methyl 3,4-*O*-isopropylidene-β-D- and L-arabinopyranoside (I, Ia, II) and of methyl 4,6-*O*-benzylidene-3-*O*-methyl-α-D-altropyranoside (IV) have been prepared and their behavior on pyrolysis studied. The derivatives did not undergo a Chugaev type elimination reaction, but the 2-*O*-(*S*-methyl dithiocarbonate) of III rearranged to the isomeric 2-*S*-(*S*-methyl dithiocarbonate) (V) on distillation; V was reductively desulfurated and debenzylidenated to 2-deoxy-D-*erythro*-aldopentose ("2-deoxy-D-ribose") in low yield. The relation of the rearrangement (of which other examples are known) to the Chugaev reaction is discussed.

The production of olefins by the controlled thermal decomposition of the *O*-(*S*-alkyl dithiocarbonate) or "xanthate" esters of certain alcohols (Chugaev reaction)[3] is now well known. Numerous examples of the Chugaev reaction have been described in the literature, but the thermal decomposition of 2-*O*-(*S*-alkyl dithiocarbonate) esters of simple carbohydrates has been little studied. In fact, few such esters are known. Freudenberg and Wolf[4] described 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose 6-*O*-(*S*-methyl dithiocarbonate), 2,3:5,6-di-*O*-isopropylidene-D-mannofuranose 1-*O*-(*S*-methyl dithiocarbonate) and 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose 3-*O*-(*S*-methyl dithiocarbonate), of which the latter two derivatives were crystalline. These esters were prepared by the general route (1) as was methyl α-D-glucopyranoside 2-*O*-(*S*-methyl dithiocarbonate).[5] *O*-Acetyl-1-thioaldose 1-*S*-(*O*-alkyl dithiocarbonate) esters have been prepared by the action of potassium *O*-ethyl dithiocarbonate on *O*-acetylglycosyl halides.[6]



(1)

Pyrolysis of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose 3-*O*-(*S*-methyl dithiocarbonate)[4] under the conditions originally employed by Chugaev[3] did not give the desired 3,4-unsaturated compound.

The purpose of this investigation was the preparation of other dithiocarbonate ("xanthate") esters of simple carbohydrates, which might reasonably be expected to undergo a Chugaev type reaction (5) and a study of their behavior on pyrolysis.

(1) A preliminary report of this work appears in *Abstracts Papers Am. Chem. Soc.*, **126**, 23D (1954).

(2) Rockefeller Foundation Fellow, 1953-1954.

(3) L. Chugaev, *Ber.*, **32**, 3332 (1899).

(4) K. Freudenberg and A. Wolf, *ibid.*, **60**, 232 (1927).

(5) T. Lieser and E. Leckzyck, *Ann.*, **519**, 279 (1935); M. L. Wolfrom and M. A. El-Taraboulsi, THIS JOURNAL, **75**, 5350 (1953).

(6) W. Schneider, R. Gille and K. Eisfeld, *Ber.*, **61**, 1244 (1928).