Rockogenin 3-Acetate 12-Benzoate (XXIII).—Two hundred milligrams of rockogenin 3-acetate was treated at room temperature for five hours with 0.2 ml. of purified benzoyl chloride in 2 ml. of pyridine and 5 ml. of benzene. The solvents were removed *in vacuo* and the residue treated for a time with methanol and pyridine, which were removed likewise. The product was dissolved in ether, washed with dilute hydrochloric acid, then sodium bicarbonate and reisolated. It was finally purified in methanol from which it crystallized as fine needles or large prisms, m.p. 204–205°, $[\alpha]^{28}D$ −59.7°.

Anal. Calcd. for $C_{36}H_{50}O_6$: C, 74.71; H, 8.71. Found: C, 74.70; H, 8.48.

KNOXVILLE, TENNESSEE

[CONTRIBUTION FROM THE STARCH AND DEXTROSE DIVISION, NORTHERN REGIONAL RESEARCH LABORATORY[1a]]

Polysaccharide Aryl Carbamates. III. Tricarbanilates of Polyglucosans with Various Glucosidic Linkages[1b]

BY IVAN A. WOLFF, PAUL R. WATSON AND CARL E. RIST

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Tricarbanilates were prepared from water-soluble sweet corn polysaccharides no. 1 and no. 2, glycogen β-amylase limit dextrin, the polysaccharide formed by *Phytomonas tumefaciens*, laminarin, yeast polyglucosan, cellulose, lichenin and several selected types of dextrans. The various classes of polysaccharide carbanilates prepared had optical rotations in pyridine and morpholine which were dependent on the position and anomeric type of the predominant glucosidic linkages. This observation may prove useful in studies of the chemical structures of various polyglucosans.

In our previous publications[2] a relation was shown to exist between the structure of an amylaceous polysaccharide and the optical rotation of its tricarbanilate in pyridine. This paper reports the extension of this work to a number of other polyglucosans differing in their glucosidic linkages. One group of polysaccharides, comprising water-soluble sweet corn polysaccharides and glycogen β-amylase limit dextrin, had predominantly α-1,4′-linkages with some α-1,6′-branch points. A second set, having β-linkages, included 1,2′-, 1,3′-, 1,4′- and 1,6′-linked materials. Several different dextran samples (chiefly α-1,6′-linkages) were also studied. The optical rotation data obtained are of value in furnishing preliminary or confirmatory evidence of the structure of polysaccharides composed exclusively of anhydroglucose units.

Experimental

Most of the polysaccharide samples were available only in very small amounts and were used as obtained without further purification. The dextrans were isolated at this Laboratory by published procedures[3] and had very low nitrogen, phosphorus and ash contents. Those samples in Table I designated as "partially hydrolyzed" were acid degraded, alcohol-fractionated materials with a weight average molecular weight of approximately 75,000,[4] and meeting current specifications for dextran of clinical-injection type.

Each polysaccharide was dried by azeotropic distillation from pyridine dispersion, and was treated in that medium with phenyl isocyanate at 100°.[2] At the end of the reaction period the mixture was filtered through fritted glass and poured into absolute ethanol to precipitate the ester. In some cases addition of an equal volume of water to the ethanol-containing mixture was necessary to cause precipitation of the carbanilate. One treatment, for time periods varying from 5 to 24 hours, was sufficient to give trisubstitution with all of the polysaccharides except luteose, which resisted complete carbanilation after two successive reaction periods of 23 and 22 hours, each in the presence of excess reagent.

Results

In Table I are listed pertinent data on the polysaccharides used and the optical rotations of their carbanilates.

Amylaceous Polysaccharides.—Since it was found[2] previously that those predominantly 1,4′-linked polysaccharides having larger percentages of branch linkages yielded carbanilates with smaller negative optical rotations in pyridine (*e.g.*, amylose, −82.5°; amylopectin, −62°; glycogen, −31.5°) further application of this principle to available samples of this type appeared to be of interest. The water-soluble polysaccharides of sweet corn have been separated by most recent investigators into two fractions based on their solubility in 67% acetic acid.[5–7] The soluble fraction (called polysaccharide no. 2, corn glycogen or phytoglycogen) has been assigned a repeating chain length of 11–12 while the insoluble fraction (polysaccharide no. 1, glycoamylose, starch) has been found to have an average chain length of 12 by periodate end-group assay of material passed through a cotton column,[5] or of 25 by methylation study of a fraction not so treated.[6] Glycogen from animal sources is usually considered to have an average branch length of 11–13 glucose units,[8,10] and is therefore indistinguishable by end-group methods from the soluble sweet corn polysaccharides.[9,10] However, it would appear from our data (Table I and reference 2) that these sweet corn polysaccharides may have on the average a slightly lower extent of branching than animal glycogen and are intermediate in this respect between glycogen and amylopectin. The data are in agreement with those of Dvonch and

(1) (a) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted. (b) Presented before the Division of Sugar Chemistry at the 124th National Meeting of the American Chemical Society, Chicago, Ill., September, 1953.

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(10) M. Abdel-Akher and F. Smith, THIS JOURNAL, **73**, 994 (1951).

TABLE I

PROPERTIES OF THE POLYSACCHARIDES AND THEIR CARBANILATES

Polysaccharide	Type of linkage	Carbanilate properties[a] $[\alpha]^{25}D$ (c 1) in Pyridine	Morpholine
Sweet corn polysaccharide no. 1	α-1,4′ and α-1,6′	−58°	−7°
Sweet corn polysaccharide no. 2	α-1,4′ and α-1,6′	−54	−9
β-Amylase limit dextrin from glycogen	α-1,4′ and α-1,6′	−7	+22
Polysaccharide from *Phytomonas tumefaciens*	β-1,2′	+50	−11
Laminarin	β-1,3′	−63	−63
Yeast polyglucan	β-1,3′	−75[b]	−41[b]
Cellulose[c]	β-1,4′	−152	−85[f]
Lichenin	73% β-1,4′	−133	−83
Luteose	27% β-1,3′, β-1,6′	...	−56[g]
Dextran from *L. mesenteroides*, NRRL B-512	95% α-1,6′[d]	...	+343
Partially hydrolyzed B-512 dextran	96% α-1,6′	...	+348
Autolyzed B-512 dextran	96% α-1,6′[d]	...	+349
Dextran B-512 prepared in presence of $CaCO_3$ buffer	94% α-1,6′[d]	...	+349
Dextran from *S. dextranicum* NRRL B-1254	89% α-1,6′	...	+335[e]
Partially hydrolyzed B-1254 dextran	91% α-1,6′	...	+335
Partially hydrolyzed dextran from *L. mesenteroides*, NRRL B-742	70% α-1,6′	+275	+245

[a] All of the carbanilates were found to contain between 7.90 and 8.20% nitrogen, with the exception of the luteose carbanilate which had 7.25%. The calculated nitrogen content for a polyglucosan tricarbanilate is 8.09%. [b] Determined on the soluble portion; c 0.7 in pyridine and c 0.15 in morpholine. [c] U.S.P. absorbent cotton ground to 60 mesh in a laboratory Wiley mill. [d] A. Jeanes and C. A. Wilham, THIS JOURNAL, **72**, 2655 (1950). [e] Value ± about 8°; solution very turbid. [f] c 0.6. [g] c 0.25.

Whistler in assigning a generally similar structure to the two sweet corn polysaccharide fractions and in indicating that polysaccharide no. 1 as originally precipitated contains more linear material than no. 2. The β-amylase limit dextrin of glycogen, resulting after 37% of the glycogen was converted to maltose by wheat β-amylase, was the most branched material examined. Its tricarbanilate had a very low negative rotation in pyridine and the shift to a positive rotation in morpholine which might be expected, on the basis of our previous results, from a material with an average branch length of only 5–5.5.[8,11]

β-Linked Polyglucans.—The detailed results on some of the rarer polysaccharides should be interpreted with caution since the isolation of only small quantities has not permitted perfection of purification procedures to free them from ash and other trace constituents. Moreover, their structures and molecular weights are currently less well known than those of starch and cellulose. Still, accepting such limitation, we find characteristic rotational differences between the various polysaccharides and can interpret our data as follows.

The β-1,2′-linkage for the *Phytomonas tumefaicens* polysaccharide[12,13] recently has been established by methylation analysis.[14] Our optical rotation of +50° on its carbanilate in pyridine is so different from the rotations of the polysaccharide carbanilates having other linkages that it appears to be in a class by itself. The difference in rotation between laminarin and yeast polysaccharide carbanilates, both having predominantly the β-1,3′-structure,[15] may be due to higher molecular weight of the yeast polysaccharide, to branching, or to other fine points in the structure which have not yet been elucidated. The yeast polysaccharide carbanilate was incompletely soluble and the rotation therefore represents that of the major fraction (approximately 90%) of the original material. Lichenin, with its combination of linkage types,[16] had the expected intermediate rotation between cellulose and the β-1,3′-linked polysaccharides. The specific rotation of lichenin carbanilate in pyridine calculated from its postulated structure (assuming rotational effects to be additive and using cellulose as characteristic of the β-1,4′-linkage and the average for laminarin and yeast polyglucosan for the β-1,3′) was −130°, in close agreement with the value found. Its rotation in morpholine was closer to that of the cellulose tricarbanilate. The failure to obtain complete esterification of luteose parallels the difficulties obtained in its methylation[17] which prompted Anderson to postulate the presence of cross-linkages or aggregating bonds between the chains. Since the degree of substitution here attained was only 2.2, the properties of this ester are not of interest for comparative purposes. Comparison of α- and β-linked polysaccharides is possible in the case of amylose[2] and cellulose, both having only 1,4′-linkages. The latter has a considerably higher negative rotation in both pyridine and morpholine than does amylose.

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Dextran Carbanilates.—The rotation of dextran carbanilates was found to be quite insensitive to variations in the cultural conditions under which the dextran was formed[3] or to partial hydrolytic breakdown. However, the presence of increased amounts of non-1,6′-linkages was accompanied by a decrease in the high positive rotation in morpholine found to be characteristic of dextran tricarbanilates. The chemical structures of the NRRL B-742 and NRRL B-1254 dextrans are not yet well enough defined to enable a statement as to whether or not the non-1,6′-linkages occur as points of branching.

Discussion

In the starch series of polysaccharides, the rotational differences between the polysaccharides themselves or their aliphatic triester derivatives[18] is small. This difference was greatly accentuated by conversion of the polysaccharides to the tricarbanilates.[2] A similar advantage is apparent for the polysaccharides discussed here. For example, in water the specific rotations of the dissimilar polysaccharides laminarin ($-14°^{15}$) and of the *Phytomonas tumefaciens* polysaccharide ($-10°^{14}$) differ only slightly while the specific rotations of their tricarbanilates in pyridine differ by more than 100° (Table I). The differences between the rotations of dextrans and amylaceous polysaccharides are likewise enhanced by use of the tricarbanilate rotations in morpholine.

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[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF STANFORD UNIVERSITY]

The Phosphorylation of Chloromycetin[1]

BY HARRY S. MOSHER, JOAN REINHART AND H. C. PROSSER

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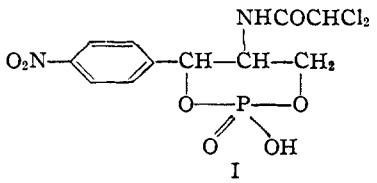
In an attempt to obtain a water-soluble derivative of Chloromycetin, its phosphorylation was studied. With phosphorus oxychloride and tetraphosphoric acid, a cyclic phosphoric acid ester I was formed. This compound was water soluble, very stable to acid hydrolysis and showed no antibiotic activity. Use of crystalline orthophosphoric acid or non-anhydrous phosphorylating conditions was unsuccessful, and often resulted in cleavage of the amide linkage.

The antibiotic Chloromycetin has a low water solubility, which increases its difficulty of administration by the parenteral route. Since many physiologically active compounds exist *in vivo* as the phosphoric acid esters, and since enzymes are present *in vivo* for hydrolyzing such esters, it was desirable to prepare a soluble, phosphoric acid ester of Chloromycetin. Such an ester has been obtained by use of phosphorus oxychloride[2] in pyridine at 0°. Our evidence indicates that this crystalline product has the cyclic structure I.



I

The ester gives an unequivocal monobasic titration curve indicating a single replaceable hydrogen atom. Analytical data as well as neutral equivalent, support the cyclic structure. The ultraviolet absorption spectra for the phosphorylated product is identical to that of Chloromycetin except for a 5 mμ displacement toward the shorter wave lengths. The infrared absorption spectra for the phosphorylated product shows the general absorption around 10 μ, reported to be characteristic of phosphoric acid esters.[3]

Similar types of cyclic phosphate esters have recently been reported, such as the riboflavin cyclic 4′,5′-phosphate,[4] catechol phosphate[5] and the 2′,3′-cyclic phosphates of adenosine, cytidine and uridine.[6] A cyclic phosphate has been postulated as an intermediate in the acid rearrangement of glycerol 2-phosphate to glycerol 1-phosphate.[7]

Since these are all α,β-cyclic phosphates (five-membered ring), it is surprising, in view of the favorable configuration of α,γ-carbon atoms,[8] that the only other isolation of an α,γ-cyclic phosphate (six-membered ring) so far reported, is that of Baddiley and Thain[9] who have prepared a cyclic D-pantothenic acid 2′,4′-phosphate.

An interesting stability correlation exists between the cyclic pantothenic phosphate and cyclic Chloromycetin phosphate. With both compounds the phosphate group was hydrolyzed very slowly in acid media. The hydrolysis curve of Chloro-

(1) Chloromycetin is the registered trademark which Parke, Davis and Company has adopted for the antibiotic drug, chloramphenicol. See M. C. Rebstock, *et al.*, THIS JOURNAL, **71**, 2460 (1949).

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