Note

Precipitation of neutral polysaccharides and separation of their mixtures by use of various quaternary salts

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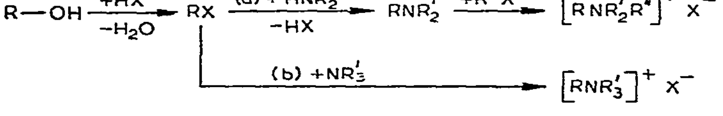
In the interactions of quaternary salts with acid polysaccharides, the significance of aliphatic radical length has been shown, and a qualitative hypothesis to explain the process of precipitation has been presented[1,2]. Hexadecyltrimethylammonium bromide (Cetavlon) and hexadecylpyridinium chloride (CPC) have been widely used for the isolation and purification of acid polysaccharides[3]. In 1957, Barker *et al.*[4] used Cetavlon (**10**) for the separation of neutral polysaccharides in borate buffer. Later Scott[5], having used hexadecyltrimethylammonium borate for the precipitation of neutral polysaccharides, showed the importance of borate complexes for the precipitation, and that pH values above 10 are essential for the dissociation of polysaccharide groups. Lindberg *et al.*[6] used Cetavlon to separate an arabinogalactan into two fractions having similar composition but different molecular weights.

An important characteristic of neutral-polysaccharide precipitation is the formation of acidic groups, due to the formation of bisdiol borate complexes[7]. The formation of polysaccharide complexes with anion groups inserted in various proportions in different positions (depending on the polysaccharide structure) opens new possibilities of fractionation by use of various concentrations.

EXPERIMENTAL

Materials and methods. — Quaternary salts were synthesized according to the scheme:



$R = C_8H_{17}$, $C_{11}H_{23}$, $C_{12}H_{25}$; $R' = CH_3$, C_2H_5 ; $R'' = C_6H_5CH_2$

$X = Cl$, Br

Methods generally used for the preparation of quaternary salts were modified so that no high-pressure nor complicated apparatus were needed for the fractionation of the intermediate products[8] (Table I).

TABLE I

QUATERNARY SALTS USED FOR THE PRECIPITATION OF POLYSACCHARIDES

Salt	*Anion radicals*			*Cation*
	R	*R'*	*R''*	
1	C_8H_{17}	$(CH_3)_2$	$CH_2C_6H_5$	Cl
2	$C_{11}H_{23}$	$(CH_3)_2$	$CH_2C_6H_5$	Cl
3	$C_{12}H_{25}$	$(CH_3)_2$	$CH_2C_6H_5$	Cl
4	$C_{12}H_{25}$	$(C_2H_5)_2$	$CH_2C_6H_5$	Cl
5	C_8H_{17}	$(CH_3)_3$		Br
6	$C_{12}H_{25}$	$(CH_3)_3$		Br
7	C_8H_{17}	$(C_2H_5)_3$		Br
8	$C_{11}H_{23}$	$(C_2H_5)_3$		Br
9	$C_{12}H_{25}$	$(C_2H_5)_3$		Br
10[a]	$C_{16}H_{33}$	$(CH_3)_3$		Br

[a] A commercial preparation of Cetavlon was used.

Rabbit-liver glycogen was prepared by the method of Stetten *et al.*[9]. Potato starch was prepared by the treatment of mashed potatoes with a 1% solution of sodium chloride. Inulin was isolated from dahlia tuber by the method of Aspinall and Hirst[10]. Eremuran was prepared as previously described[11].

Polysaccharide precipitation. — Polysaccharide (100 mg) was dissolved in distilled water (15 ml) and an equal volume of borate buffer was first added (pH 8, 10, and 12, in parallel experiments) and then solutions of quaternary salts at concentrations of 1, 2.5, and 5%. The precipitates were centrifuged, washed several times with ethanol and ether, and dried (Tables II, III, IV). The absence of quaternary salts in polysaccharide precipitates was verified by nitrogen determination.

TABLE II

GLYCOGEN PRECIPITATION WITH QUATERNARY SALTS AT pH 10[a]

Salt[b]	*Concentration (%)*		
	1	*2.5*	*5*
3	24	70	96
4	25	68	92
6	0	traces	77
9	0	0	79
10	31	74	92

[a] Yield of glycogen in %. [b] Salts **1** and **5** (Octalon) precipitate practically no glycogen; salt **2** precipitation is similar to that of **1**; salt **7** precipitation is similar to that of **4**.

TABLE III

STARCH PRECIPITATION WITH QUATERNARY SALTS[a]

Salt	*Concentration* (%)		
	1	*2.5*	*5*
1	67	69	89
3	90	94	99
4	89	94	98
5	68	71	94
6	90	91	94
9	84	86	92
10	89	91	96

[a] Yield of starch in %.

TABLE IV

EREMURAN PRECIPITATION WITH QUATERNARY SALTS AT pH 12[a]

Salt	*Concentration* (%)		
	1	*2.5*	*5*
3	38	48	80
4	36	51	80
10	44	76	81

[a] Yield of eremuran in % after the precipitate has been kept for 24 h at 10–12°.

Separation of the polysaccharide mixture. — Some experiments with Cetavlon (**10**) and benzyldimethyldodecylammonium chloride (**3**) were carried out according to a simplification of the procedure of Barker[4], because the quaternary salts synthesized were more soluble than Cetavlon.

RESULTS AND DISCUSSION

Polysaccharide precipitation. — As shown in Tables II, III, and IV, the precipitation of starch is more complete than that of glycogen, and the latter is more complete than that of eremuran. The yield of precipitate is proportional to the length of the radical, as previously shown for acid polysaccharides. The decrease of a long-chain radical from 16 to 12 carbon atoms seems to be compensated by the substitution of one methyl by a benzyl group (**3**).

It is noteworthy that octyltrimethylammonium bromide (**5**, Octalon) precipitates starch easily, but does not precipitate glycogen and eremuran.

In most cases of glycogen and starch precipitation, optimum conditions were reached at pH 10 and at pH 12, respectively, and at higher pH for eremuran.

Separation of polysaccharide mixtures. — The separation of a mixture of glycogen and inulin was performed with three different procedures (Table V): (a) Separation with Cetavlon (**10**) by the method of Barker[4], (b) separation with benzyldimethyldodecylammonium chloride (**3**) under the conditions described by Barker[4], and (c) separation with **3** (more soluble than **10**) by a new modification: Instead of being washed with ethanol and acetic acid, the glycogen precipitate was washed with ethanol and ether; the dialysis of the inulin solution was omitted as well as the cation-exchange resin treatment and the concentration *in vacuo*. The inulin precipitate formed after the addition of ethanol was washed with ethanol and ether. Paper chromatography of the hydrolyzates of the compounds isolated from the polysaccharide mixture showed in the first case glucose only, and in the second case, fructose only.

TABLE V

THE SEPARATION OF MIXTURES OF GLYCOGEN AND INULIN WITH **10** (CETAVLON) AND **3**[a]

Polysaccharide	**10**		**3**	
	This work	*Lit. (Ref. 4)*	*Method (b)*	*Method (c)*
Glycogen	74	68	84	91
Inulin	57	57	54	92

[a]The concentration of reagents was 5% and the pH was 10. The yields are reported in %.

The separation of the mixtures of starch–eremuran and starch–glycogen (Table VI) was performed with Octalon (**5**). The second polysaccharide remaining in the solution was precipitated with ethanol. Paper chromatography of the hydrolyzates of the polysaccharides isolated showed only glucose in the preparation of starch, and glucose and mannose in the eremuran preparation. The spectra of the iodine–polysaccharide complexes before and after the isolation from the starch–glycogen mixture are practically identical (Fig. 1).

TABLE VI

SEPARATION OF MIXTURES OF STARCH–EREMURAN AND STARCH–GLYCOGEN WITH **5**

Polysaccharides	*Yield* (%)
Starch	92
Eremuran	94
Starch	96
Glycogen	93

Because of the lack of experimental data, we can suggest only limited explanations for the dependence of polysaccharide precipitation on their structure. In the case of homopolysaccharides (α-D-glucans: starch and glycogen), the ease of forma-

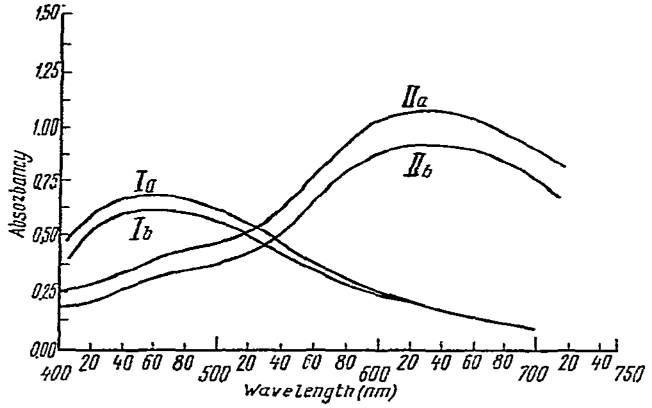


Fig. 1. Absorbtion curves of glycogen (I) and starch–iodine (II) complexes; (*a*) original preparations, (*b*) after isolation from the mixture.

tion of starch precipitate indicates that the degree of branching influences the reaction. When bisdiol borate complexes are formed, the boron atom seems to link two pairs of oxygen atoms with the two vicinal hydroxyl groups in two vicinal polysaccharide chains[7]. It is possible that, in the case of starches containing only the linear molecules of amylose and the slightly-branched amylopectin, the conditions of borate complex formation are more favorable.

If the anion groups formed are situated close to each other, cations of the quaternary salts having comparatively short aliphatic chains (such as 5, Octalon) can overlap and cause precipitation, as suggested by Scott[1] in his theory of acid polysaccharide precipitation.

In highly branched glycogen, the conditions for the formation of borate complexes are not so favorable, which is confirmed by our experiments showing that amylose is precipitated much more easily than amylopectin.

The explanation for the poor precipitation of eremuran with quaternary salts may be its low molecular weight (D.P. 200), the presence of β-D-glucosidic linkages, or the presence at C-2 of a configuration different from that of glucose.

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