yellow prisms deposited slowly, m.p. 229–230°. The fused red form recrystallized into the yellow form. The product showed no mutarotation from 4 min. to 16 hr., $[\alpha]_{5461}^{24}$ +204 ± 3° (in methanol, *c* 0.37).

Anal. Calcd. for $C_{17}H_{19}O_6N$: C, 61.26; H, 5.75. Found C, 61.21; H, 5.82.

BOSTON 14, MASS.

[CONTRIBUTION FROM THE WESTERN REGIONAL RESEARCH LABORATORY[2]]

Alkali Sensitivity of Polysaccharides: Periodate Starches, Periodate Dextran and a Polygalacturonide[1]

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Reactions in alkali of periodate dextran, periodate cornstarches of three degrees of periodation, and a polygalacturonide were studied with the chlorite method, specific for aldehyde groups. All were found to be alkali-sensitive in various ways. The periodated substances underwent reactions which, at *p*H 10.2, first increased and then decreased the aldehyde content, corresponding to hydrolysis and dialdehyde intra-action, respectively. At lower *p*H and lower dialdehyde content both reactions were slower but dialdehyde intra-action relatively more, so that hydrolysis dominated aldehyde content for a longer period. At higher *p*H, dialdehyde intra-action dominated. The polygalacturonide showed limited depolymerization, approximately five scissions per chain molecule, indicating that only one de-esterification out of eighty led to scission. These findings permit conclusions regarding the reactions occurring in alkaline reagents.

In a forthcoming article by the writers[3] it will be shown that polysaccharides such as starches, dextrans, and their periodated forms, an araban and a polygalacturonide, usually give misleading aldehyde results by alkaline methods. A typical method using iodine in a carbonate-bicarbonate buffer at *p*H 10.2 gives values from 16 to 76-fold greater than those of the *pH* 3 chlorite method. The errors are due to side-reactions in the presence of alkali.

Not all of the alkali-sensitive substances give completely erroneous results with alkaline methods: Periodate starches and periodate dextran give aldehyde values with a carbonate-iodine method employing carbonate-bicarbonate buffer at *p*H 10.2 which are only 1 to 21% higher than chlorite results. With the Willstätter-Schudel method[4] employing unbuffered sodium hydroxide at *p*H 11.9 the results are 8 to 31% higher. Although these percentages represent large absolute increases in aldehyde groups, as the initial values are high, the agreement appears fair by comparison.

Such agreement is particularly noteworthy in view of the fact that these materials, by analogy to periodate cellulose,[5-16] could be expected to be extremely sensitive to degradation by alkali by virtue of the presence of dialdehyde groups. Furthermore, Hofreiter, *et al.*[17] have shown that periodate starches of all degrees of periodation react with sodium hydroxide, and devised a method for estimating dialdehyde group content by measuring the alkali consumed.

An explanation for such agreement between iodine and chlorite results on periodated starch and dextran, aside from assuming no alkali-sensitivity, is that it is fortuitous and due to compensating reactions. Such reactions might be hydrolysis, which would create aldehyde groups, and intra-action of dialdehyde groups, which would destroy them. The dialdehyde groups would undergo mutual oxidation and reduction of a Cannizzaro-type, giving rise to a hydroxyl and a carboxyl group at the site of the former dialdehyde.

In view of this, alkaline reagents could be expected to give low results for aldehyde content because of dialdehyde intra-action, and the copper reagent (*p*H 10.2) at 100° does so, but both the carbonate-iodine (*p*H 10.2) and the hydroxide-

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(1) Presented at the American Chemical Society Meeting, Atlantic City, N. J., September 13–18, 1959.

(2) A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U.S. Dept. of Agriculture.

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iodine (pH 11.9) reagents at 25°, give high results.[3] This indicates that hydrolysis may predominate over dialdehyde intra-action under certain conditions but not under others. Such complexity again emphasizes the danger of using alkaline media for aldehyde measurement.

A study of this problem led to this report of the effect of alkali at various pH values upon the aldehyde content, measured with chlorite, of corn starch of three degrees of periodation, of periodate dextran and of a polygalacturonide. The last was of interest because its alkali-sensitivity is due to the presence of carbomethoxy groups on the C_6 atoms, aldehyde groups being absent, and because its manner of consumption of alkaline reagents indicates that a study of its alkali-sensitivity would be fruitful.

EXPERIMENTAL

Materials and methods. The periodated materials were prepared by Sloan, *et al.*[18,19] at the Northern Regional Research Laboratory, Peoria, Ill. The aldehyde contents were determined with the chlorite method[20,21] to be 1.77 aldehyde groups per AGU for the periodate dextran and 1.78, 0.78, and 0.40 aldehyde groups per AGU for the periodate starch samples, to be referred to herein as "1.8", "0.8", and "0.4", respectively.

"Polygalacturonide," a polymethyl ester of methyl polygalacturonic acid, sometimes known as "Link's compound," was synthesized[22] by R. M. McCready of this laboratory from lemon pectin. This material dissolved readily in cold water.

Solutions of the periodated materials were more difficult to prepare and were made as previously described.[20] Portions thereof (containing 3.6 mg., dry basis, of periodate dextran, as an example, and more for substances of lower aldehyde content) were then added to carbonate-bicarbonate buffers, sodium carbonate or to sodium hydroxide solutions to give the following systems: Most studies were made in the buffer of the Blom and Rosted[23] reagent (referred to in the forthcoming study as the "Carbonate-iodine" reagent) but without iodine, 0.133*M* in sodium carbonate and 0.033*M* in sodium bicarbonate giving pH 10.2. Some experiments were conducted at pH 9.3 in a buffer, 0.02*M* sodium carbonate and 0.08*M* sodium bicarbonate, some at pH 10.5 in 0.05*M* sodium carbonate, and a few at pH 11.9 in 0.04*M* sodium hydroxide (the final alkalinity of the Willstätter-Schudel reagent).

The polysaccharide-alkali mixtures, in 50-ml. red glass or foil-covered mixing cylinders or volumetric flasks, (thus excluding photochemically active light at all critical stages) were maintained at 25 ± 0.02° for the desired lengths of time. The alkali was then neutralized with sulfuric acid, the carbon dioxide when present was expelled, and the ingredients of the "3M, 50°" chlorite reagent were added. After the proper time, 16 hr., for the chlorite oxidation of aldehyde groups, a 20-ml. aliquot was freed from chlorine dioxide and iodometrically titrated for remaining chlorite as previously described.[20,21,24] During the removal of chlorine dioxide by bubbling with nitrogen gas, foaming difficulties, caused by some of the hydrolysis products, were avoided by adding a drop of capryl alcohol to test and control. The resulting aldehyde values, expressed as aldehyde groups per AGU, were plotted against time in the figures.

RESULTS AND DISCUSSION

(a) *Periodate cornstarch.* Curves 1, 2, and 3, Fig. 1, represent the aldehyde contents of the three periodate cornstarches "1.8", "0.8", and "0.4" after various time periods in the pH 10.2-buffer. All of the curves start with increases above the initial aldehyde content followed by slower but more extensive decreases. The increases are probably due to hydrolysis, either at the 1,4-bonds[11] or at the C_5-O bonds[13] and the decreases are due to dialdehyde intra-action.

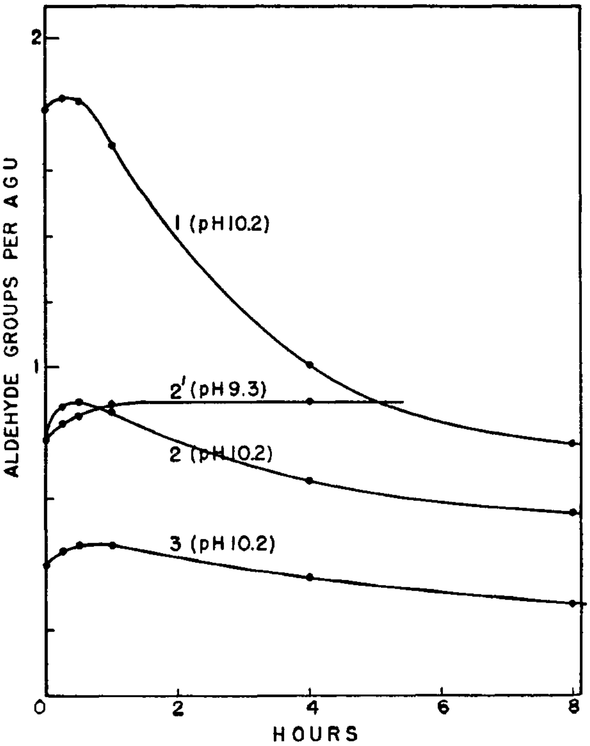

Fig. 1. Aldehyde content of periodate starches "1.8 (curve 1) "0.8" (curves 2 and 2 prime) and "0.4" (curve 3) after various periods in alkali

These reactions do not occur in acidic media and are thus types of alkali-sensitivity. They are caused by the presence of dialdehyde groups which apparently also cause alkali-sensitivity in periodate cellulose and in the simpler periodate sugars.[9,13,25] The writers find[3] that, in the absence of dialdehyde groups, the same alkaline conditions resulted in

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much smaller absolute increases in aldehyde groups of rice, wheat, and pea starch through hydrolysis, and no decreases.

Both effects determine the net aldehyde value at any time, but their simultaneous occurrence makes a quantitative evaluation of each one uncertain. As, in eight hours, more aldehyde groups are destroyed than are created, decrease by dialdehyde intra-action interferes more in the early parts of the curves than increase by hydrolysis does in the later phases. Extrapolation of curve 3 to zero time leads to a rough estimate that aldehyde content would have increased 30% because of hydrolysis. Although the steepness of the descending portions makes extrapolation, especially of curve 1, uncertain, extrapolation of curves 2 and 3 leads to approximately the same percentages. This is characteristic of first order reactions; hence, the rate of hydrolysis appears to be approximately proportional to the first power of dialdehyde content.

Considering the later parts of the curves, the 8-hour values on curves 1, 2, and 3 lead to the results that roughly 60%, 45%, and 40%, respectively, of the dialdehyde groups have disappeared, referred to the curve maxima. This indicates that this reaction is higher than first order with respect to dialdehyde content, and with decreasing dialdehyde content hydrolysis should become initially more prominent.

Thus, under these conditions, dialdehyde content should determine which effect predominates initially, and how long it lasts. Curves 1, 2, and 3 show that intra-action increased with dialdehyde content in both rate and extent, much more than did hydrolysis, and curve 1 therefore shows the smallest and briefest increase.

As was expected, the reactions decreased with *p*H. Curve 2′ shows that both hydrolysis and dialdehyde intra-action are slower at *p*H 9.3 than at *p*H 10.2, and that dialdehyde intra-action is slowed down more by decreasing *p*H than is hydrolysis. The leveling-off of the curve shows that either hydrolysis ceased and little or no dialdehyde intra-action occurred, or that the latter was operating and would have predominated if the experiment had been continued. The latter is probably the case in analogy to the results for periodate dextran in the next section. Furthermore, it is not evident why hydrolysis should have ceased if the sensitizing dialdehydes had remained undisturbed. It is known that the alkali-sensitivity of periodate cellulose is greatly lessened by oxidizing the dialdehyde with chlorite to carboxyl[6,10,12,14,15] or by reducing the dialdehyde with borohydride to hydroxyl.[15,16]

At higher alkalinities dialdehyde intra-action predominated. At the thirty-minute point, when experiments so far discussed were still showing hydrolytic increases, only extensive decreases were observable at higher *p*H. This is shown by curve 4,

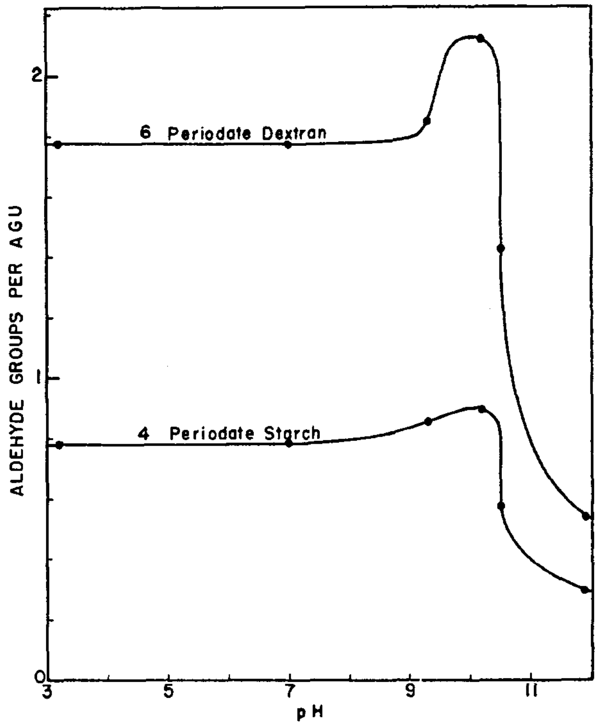

Fig. 2. Aldehyde content of the "0.8" periodate cornstarch and periodate dextran after thirty minutes at various *p*H levels

Fig. 2, in which the aldehyde content of the "0.8" periodate cornstarch sample after thirty minutes in alkali is plotted against *p*H. In the acid and neutral region the material is stable, beyond which, to approximately *p*H 10.3, there is a gradual increase in hydrolysis. At approximately *p*H 10.4 there is a point or region of rapid transition in which dialdehyde intra-action becomes very rapid and a decrease in aldehyde content is observed in thirty minutes. It is noteworthy that the curve tends to level off well above zero aldehyde content. This indicates that hydrolysis, although not observed, occurs also at the higher alkalinities and that the aldehyde endgroups so created do not inter-act.

Head[9] also observed a competitive effect of alkali upon a relatively simple periodate cellobiose derivative. For aldehyde determinations he used two iodine methods, one with sodium carbonate and one with sodium hydroxide, both at *p*H well above 10.4. Although aware of the effect of alkali upon his materials he used the methods for want of better ones. This and forthcoming[3] work of the present writers show that although quantitative results with alkaline methods should not be expected, compensatory effects for special cases may afford results which appear qualitatively reasonable.

Head did not observe an aldehyde increase in a periodate glucose derivative having no 1,4-bonds. This and the fact that he found no glyoxal indicate that the C_5-O bonds present did not hydrolyze, whereas O'Meara and Richards[13] explained their results with periodate cellulose at pH 12 on this basis. The latter, finding glycollic acid, concluded that most Cannizzaro reaction (or dialdehyde intra-action) occurred in glyoxal, a product of hydrolysis of periodate cellulose, rather than in the cellulose before hydrolysis. This is of no consequence in this study, as aldehydes, regardless of the rest of the molecule, have been found by the writers to be measurable with chlorite. However, the simple picture of glyoxal intra-acting to glycollic acid, as the principal explanation for aldehyde decrease, is not easily reconcilable with Head's finding that the rate of appearance and quantity of carboxyl was much lower than the rate of disappearance and quantity of aldehyde, unless it is assumed that the alkaline methods used were too unreliable to warrant these conclusions.

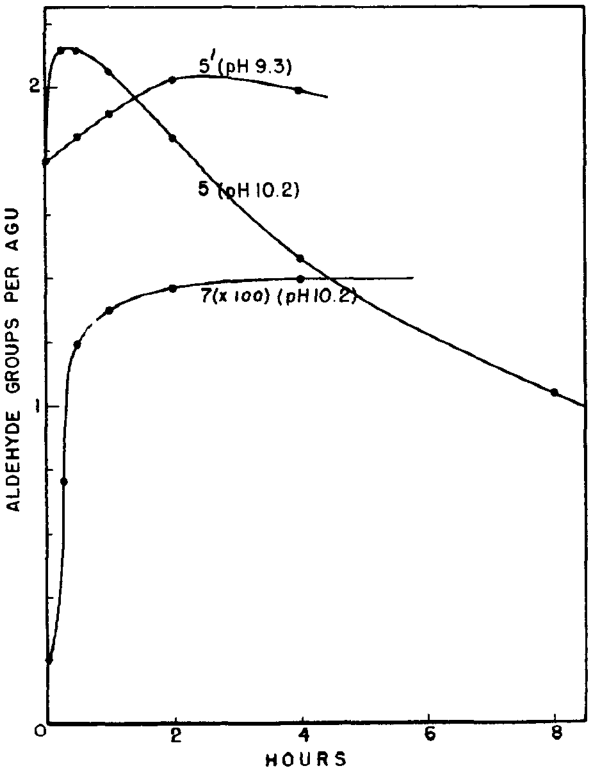

Fig. 3. Aldehyde content of periodate dextran (curves 5 and 5 prime) and polygalacturonide (curve 7) after various periods in alkali. The ordinates on curve 7 have been increased 100-fold to allow inclusion in the figure

(b) *Periodate dextran.* Curve 5, Fig. 3 shows the aldehyde content of periodate dextran after various periods in the pH 10.2-buffer. The qualitative resemblance to curve 1 for periodate cornstarch of the same initial dialdehyde content is striking, although 95% of the glucose monomers in this dextran were 1,6 linked, whereas in starch the monomers are mainly 1,4 linked.

The much greater initial rise shows that hydrolysis at pH 10.2 is much faster for the 1,6-bonds than for the 1,4-bonds of starch. This further indicates that the C_6—O bonds, which are common to both polymers, probably do not dominate the hydrolytic process in either substance.

After the initial rise, dialdehyde intra-action predominates. The rate of intra-action is approximately one-half of that in starch, possibly because the dialdehyde groups in starch, on carbon atoms C_2 and C_3, are closer together, whereas the aldehyde groups in dextran are on the C_2 and C_4 carbon atoms, C_3 having been eliminated as formic acid during periodation. This, and the fact that the slower removal of dialdehydes from periodate dextran should result in a longer retention of sensitivity to hydrolysis, would qualitatively if not quantitatively explain the greater hydrolysis.

Curve 5' shows that at pH 9.3 both hydrolysis and dialdehyde intra-action are much slower than at pH 10.2. The pH effect appears to be greater than for periodate starch. This is also shown by curve 6, Fig. 2, which by comparison with curve 4 shows that periodate dextran is more alkali-sensitive than periodate cornstarch in regard to hydrolysis and dialdehyde intra-action.

(c) *Polygalacturonide.* This substance owes the alkali-sensitivity of its 1,4-bonds to the presence of methoxy groups on the C_6 carboxyl groups, according to Vollmert,[26] who from osmotic measurements of the nitrate, concluded that depolymerization in alkali ceases when concurrent de-esterification becomes complete, leaving the resulting pectic acid stable.

Curve 7, Fig. 3, shows the aldehyde content of this substance before and after various periods in the pH 10.2-buffer at 25°. Aldehyde content is seen to increase rapidly until at about thirty minutes, it becomes practically stable. This is qualitatively in accord with Vollmert's osmotic results. The final aldehyde content corresponds to DP 80, or about five scissions per chain molecule. As originally every carboxyl group was esterified and practically every ester group may be assumed to have been removed by the alkali, it is evident that only about one de-esterification in eighty resulted in a scission.

Relation of results to those of alkaline methods. These results help to shed light upon the reactions occurring in the "Copper" (pH 10.2), "Carbonate-iodine" (pH 10.2), and "Hydroxide-iodine" (pH 11.9) reagents used upon the same substances.[3] In such methods the active agent disappearing is assumed to indicate aldehyde content. With the iodine method no decreases are observed, only large increases with time in iodine consumed by the "1.8" periodate cornstarch. Apparently, oxidation of dialdehyde groups by iodine is rapid enough to prevent the dialdehyde intra-action, which would have reduced aldehyde content, but not rapid enough to prevent hydrolysis by alkali,

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[26] B. Vollmert, *Makromol. Chemie,* 5, 110 (1950).

followed by reaction of iodine with the aldehyde endgroups created by hydrolysis. Reaction of iodine with dialdehydes must be very rapid at *p*H 11.9, for, in the absence of iodine, extensive aldehyde decrease at this *p*H is shown by curve 4 to occur.

In the "0.8" periodate cornstarch only slight overconsumption of iodine is observed and the effect of *p*H was much less. This reflects the lower alkali-sensitivity at lower dialdehyde content, at which all reactions including hydrolysis are slower, although the oxidation by iodine of dialdehyde groups still predominates over their intra-action.

The consumption of iodine by periodate dextran is approximately 15% less than for the "1.8" periodate cornstarch, although hydrolysis and therefore aldehyde end-group content, which should cause greater consumption of iodine, are greater in periodate dextran. This indicates either that iodine is consumed by nonaldehydic structures, or that the removal of the hydrolysis-engendering dialdehydes by iodine is more rapid in periodate dextran. Actually, the consumption of iodine in all cases may be due to reaction(s) with nonaldehydic structures—*i.e.* due entirely to side-reactions—but the correlation with aldehyde content, however rough, makes this appear improbable.

On the other hand, the copper reagent responds to only about 10% of the dialdehyde groups known to be present.[3] This may be due to the disappearance of most of the dialdehyde groups by intra-action in the hot alkaline medium before extensive reduction of copper could take place. The slow final consumption of copper would then be due to hydrolysis of structures which are no longer very alkali-sensitive after the dialdehyde groups had disappeared. It may be mentioned that dicarboxyl groups in cellulose have been found to be removable with hot water,[27] resulting in cellulose structures which reduce copper.

With regard to the polygalacturonide, these results show that both the iodine and the copper reagents give misleading values for different reasons. Apparently the alkali of the iodine reagent, at 25°, created aldehyde endgroups by hydrolysis, with which the iodine then reacts and continues to react slowly with nonaldehydic structures. The hot copper reagent, on the other hand, causes much more rapid and extensive depolymerization, ceasing abruptly at approximately DP 15, after which the reduction of copper becomes very slow.

The results of Hofreiter, *et al.* are compatible with the mechanisms that have been considered herein, although no intermediate or final aldehyde measurements were made. Rapid consumption of sodium hydroxide occurs within the first minute, during which, in view of the relatively drastic conditions, 70° and 0.25 *N*, rapid dialdehyde intra-action would be the expected main primary reaction. This would be followed by the very rapid secondary reaction of sodium hydroxide with the newly created carboxyl groups, which alone would demand sodium hydroxide: dialdehyde ratio of 1:1 unless unsuspected complications occur. As their results do indicate a nearly 1:1 ratio, no other major consumption of sodium hydroxide is indicated. Slow, concurrent hydrolysis, apparently also consuming sodium hydroxide, probably occurs, which continues after the main consumption of sodium hydroxide, and presumably causes the somewhat indefinite final tapering-off.

Finally the results show that reactions caused by alkali can be so complex that any agreement between alkaline reagent consumed and original aldehyde content is a coincidence which may well depend upon compensating side-reactions.

ALBANY, CALIF.

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(27) G. F. Davidson and H. A. Standing, *J. Textile Inst.*, 42, T141 (1951).