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Investigation of the Properties of Cellulose Oxidized by Nitrogen Dioxide. VI. The Effect of Alkali on the Celluronic Acids[1]

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The initial investigations[2] of the action of nitrogen dioxide on cellulose showed that the resulting celluronic acids are alkali-soluble when the carboxyl content is sufficiently great. The celluronic acids undergo further reaction in alkaline solution which complicates the application of the copper number determination or direct determination of carboxyl groups by alkaline titration.[3] The reducing values obtained by copper number determinations were exceptionally high. It appeared probable that the reducing groups were not present to such an extent in the celluronic acids, but were generated by decomposition under the severe conditions of high *p*H and temperatures used in the determinations. Attempts to prepare sodium celluronate solutions, even at *p*H values only slightly above 7, showed that whenever solution was effected, concomitant degradation had occurred. The calcium acetate and carbon dioxide evolution methods which operate at *p*H values below 7 were chosen for carboxyl determinations to avoid the degradation which appeared to accompany the direct determination by alkaline titration.[2,3]

The evidence accumulated to date indicates that free carboxyl groups in celluronic acids are substantially contained in uronic acid units. The carboxyl values by carbon dioxide evolutions are at least as high as the values from potentiometric titration or the calcium acetate method.[4] Aside from carboxyl groups which should not produce alkali-instability in celluronic acids, oxidation of hydroxyl groups could form aldehyde or ketone groups or an enediol structure by isomerization of the latter. Aldehyde groups in simple organic compounds are attacked rapidly—sometimes almost explosively—by nitrogen dioxide; hence, their presence in substantial amounts in celluronic acids is doubtful. Ketone groups in certain compounds are less readily attacked[5] by this oxidant and such groups may be present in this oxidized cellulose.

The concept of the roles of keto or ene-diol groups as a source of alkali-sensitivity in carbohydrates and oxidized celluloses is not new and has been used recently to explain the alkaline fission of cellulose oxidized by periodates.[6]

Though the presence of ketone groups has earlier been considered as the cause of alkaline breakdown of celluronic acids, this communication represents the first systematic attempt to determine such groups by direct chemical methods. Quantitative data on degradation by alkali are included.

Experimental

Materials.—Celluronic acids were prepared by treating oven-dried 500-second cotton linters (ground to 100 mesh) with carbon tetrachloride solutions of nitrogen tetroxide for various periods of time to produce the desired degree of oxidation. Products containing substantial amounts of combined nitrogen were prepared by treating the cellulose with anhydrous nitric acid in the nitrogen tetroxide–carbon tetrachloride solutions. Details of these techniques and purification of the reagents have been given previously.

Published methods were used to obtain the alginic acid and simpler reference materials.[4] The samples of periodate-oxidized cellulose were obtained by the method of Jackson and Hudson[7] as modified by Davidson.[8]

Analyses of the celluronic acid employed are shown in Table I.

TABLE I

ANALYSES OF VARIOUS CELLURONIC ACIDS AND ALGINIC ACID

Oxidation time, hours	% N dry basis[b]	% COOR (dry basis) Calcium acetate[c]	% COOR (dry basis) Uronic acid[d]	% COOR (dry basis) Potentiometric titration[e]	% carbonyl[f] by weight
1	0.29	5.14	8.26	5.06	0.57
2	.34	6.34	9.26	6.06	0.65
4	.41	10.69	13.24	10.47	1.18
8	.46	14.30	17.12	13.97	1.45
16	.27	18.73	21.78	19.16	1.70
63	.06	19.59	22.31	19.44	..[g]
1[a]	4.82	0.73	14.45	0.73	
4[a]	2.46	8.26	14.86	7.93	
8[a]	1.75	12.15	19.04	11.98	
12[a]	0.63	17.41	23.03	17.69	
Alginic acid		22.15	...	...	−0.47

[a] Oxidations in presence of anhydrous HNO_3, ref. 6. [b] deVarda method. [c] Ref. 1. [d] Ref. 3. [e] This paper. [f] Method of Ref. 9. [g] Calcium salt highly swollen, gelatinous and difficult to handle.

Carbonyl determinations by the older methods common to organic chemistry operate at high *p*H values and are not applicable to alkali-sensitive oxidized celluloses. The recent method of Meesook and Purves[9] is particularly applicable and

(1) Presented before the Cellulose Division at the Chicago Meeting of the American Chemical Society, April, 1948.

(2) Yackel and Kenyon, THIS JOURNAL, **64**, 121–127 (1942).

(3) Unruh and Kenyon, *ibid.*, **64**, 127–131 (1942).

(4) Kenyon, *et al.*, *ibid.*, **69**, 342–354 (1947).

(5) Fowler, Unruh, McGee and Kenyon, *ibid.*, **69**, 1636–1640 (1947).

(6) Ivanov and Kaversneva, *Uspekhi Khimii*, **13** (4), 281–293 (1944).

(7) Jackson and Hudson, THIS JOURNAL, **60**, 989 (1938).

(8) Davidson, *J. Textile Inst.*, **32**, T109–31 (1941).

(9) Meesook and Purves, *Paper Trade J.*, **123**, 35 (1946).

was employed, for it gives reliable results at *p*H values below 7 where the celluronic acids are stable. The data are given in Table I. Accurate carboxyl values are necessary for the calculation of carbonyl content.

Purves states that carboxyl values tend to be low when oxycelluloses react with the calcium acetate solution at *p*H values below 6.3. The effects of the *p*H of the calcium acetate solution upon the carboxyl values were examined using alginic acid and celluronic acid, as shown in Table II.

TABLE II

EFFECT OF *p*H ON CALCIUM ACETATE CARBOXYL

Polyuronide	*p*H of calcium acetate solution	Carboxyl, %
Alginic acid	6.4	21.36
Alginic acid	6.5	21.41
Alginic acid	5.3	(1) 22.04
		(2) 22.04
Celluronic acid	6.5	(1) 20.21
		(2) 20.17
Celluronic acid	5.4	(1) 20.22
		(2) 20.22

Since higher values were not obtained on these materials, when the *p*H of the calcium acetate–uronic acid reaction mixture was adjusted to between 6.3 and 7.0, this adjustment was omitted.

Dilution of the calcium acetate solution to raise the equilibrium *p*H has been recommended[10] to force the reaction with the oxidized cellulose nearer to completion. Table III shows the effect of greater and less dilution than the 50 ml. of water employed in our calcium acetate method.

TABLE III

EFFECT OF DILUTION ON THE CALCIUM ACETATE DETERMINATION OF CARBOXYL

Celluronic acid, 0.50 g.; 0.5 *N* calcium acetate, 30.0 ml.; reaction time two hours

H_2O, ml.	Aliquot titrated, ml.	0.1123 *N* NaOH used, ml.	% COOH (dry basis)	Deviation, %
500	198.70	6.23	18.92	+0.48
100	48.76	6.21	18.87	+0.21
50	30.00	6.20	18.83	0.00
25	20.63	6.12	18.58	−1.33
0	11.25	6.07	18.34	−2.60

Dilution caused a 3% change in the apparent carboxyl values over the full range studied. However, dilution of our standard analytical mixture by a factor of 10, *i. e.*, from 50 to 500 ml., caused a change of only 0.5% in carboxyl value. This deviation is probably less than the precision obtained in sampling solid products from heterogeneous oxidation systems.

Potentiometric titrations were run by the following general method with such minor changes as are noted. One (1.000) gram (moist basis) of the celluronic acid suspended in 100 ml. of 1 *N* sodium bromide, with mechanical agitation, was titrated with small increments of 0.1 *N* sodium hydroxide to the desired *p*H. Since most of the maximum *p*H values desired were over 9.5, a special lithium glass "Type E" Beckman glass electrode was used with the Laboratory Model G Beckman *p*H meter. The suspended celluronic acids usually dissolved at the high *p*H values. The solutions were allowed to stand, usually at room temperature, for the desired times, then titrated to the original *p*H with hydrochloric acid of exactly the same normality as the alkali used. The extra amounts of alkali consumed were determined from the titration curves, as indicated in Fig. 1. These amounts, hereinafter referred to as the alkali consumption, are defined as the amount, *y*, in the complete titration curve shown. All results are calculated to the dry basis.

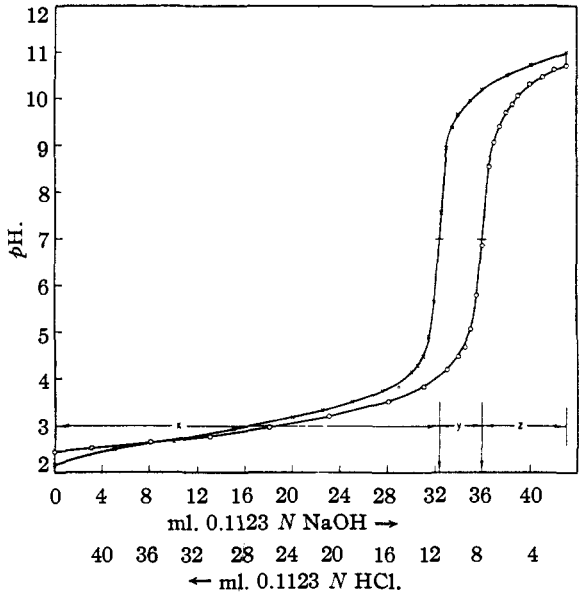


Fig. 1.—Potentiometric titration of a 1-g. sample of a sixteen-hour oxidized cellulose showing acidity developed in two hours at *p*H 11: X, titration with NaOH; O, back titration with HCl; ×, ml. alkali to reach equivalence in initial titration (zero time); Z, ml. acid to reach equivalence in final titration (two hours time); Y, ml. extra alkali consumed at *p*H 11 in two hours.

Air was not excluded during the action of alkali on the celluronic acids, but this was not a factor in the decomposition, as shown by the data of Table IV.

Kinetics data for determining the mechanism by which celluronic acids are acted upon by hydroxyl ion were obtained by a modified procedure. The celluronic acid was titrated with 0.1 *N* sodium hydroxide to a *p*H value representing the maximum desired in the experiment. These data were plotted and the resulting "master" titration curve was used for reading off the volumes of standard alkali necessary to produce definite *p*H values with a standard weight of sample. Samples of the same celluronic acid were suspended in 1 *N* so-

(10) Davidson and Nevell, *Shirley Inst. Mem.*, **21**, 85–99 (1947).

TABLE IV

ACIDITY DEVELOPED IN CELLURONIC ACIDS AT *p*H 11 IN TWO HOURS UNDER DIFFERENT ATMOSPHERES

Basis: 1.0000 g. anhydrous celluronic acid

Oxidation time, hours	*y* in ml. 0.1123 *N* NaOH	*y* in mole NaOH × 10^4	Atmosphere
4	2.42	2.72	Nitrogen
16	3.84	4.31	Nitrogen
63	8.25	9.25	Nitrogen
4	2.50	2.82	Air
16	4.35	4.87	Air
63	8.24	9.24	Air

dium bromide solution, and the amounts of alkali added very rapidly to produce solutions (or suspensions) at the *p*H desired. This procedure minimizes the decomposition encountered while bringing the solutions to the proper high *p*H of the experiment. After two hours at room temperature, the samples were titrated rapidly with 0.1 *N* hydrochloric acid to a *p*H of 7.0. Calculation of extra alkali consumption was done as explained in Fig. 1. For a more meaningful comparison between samples of different degrees of oxidation, the ratio, *R*, between moles of extra alkali consumed and initial moles of alkali needed for equivalence is used in this paper.

Results and Discussion

The oxidation of the number six carbon atom in the anhydro-glucose unit of cellulose by nitrogen dioxide is essentially complete at the end of twenty-four hours reaction time under the experimental conditions used in preparing these particular samples. However, it is obvious that a secondary oxidation continues. While the calcium acetate, uronic acid, and titrated carboxyl values of Table I have about reached their maxima at the end of twenty-four hours reaction time, the data of Fig. 2 show that the extra moles of alkal. consumed continued to increase beyond this time of oxidation. A sample which has been oxidized for sixty-three hours consumed roughly twice as much extra alkali as that consumed by a sample having a sixteen-hour oxidation.

The celluronic acids contain small amounts of nitrogen presumably combined as nitrate groups. It is well known[11] that cellulose nitrates decompose intramolecularly in alkali with reduction of nitrate nitrogen to nitrite and oxidation of the cellulose molecule to simple organic acids. The data of Table V show that this is not the mechanism of alkali consumption by celluronic acids, since the consumption increases (column 4) and the nitrogen content decreases (column 2) with increase in time of oxidation.

TABLE V

ACIDITY DEVELOPED IN CELLURONIC ACIDS OF VARIOUS NITROGEN CONTENTS AT *p*H 11 IN TWO HOURS

Basis: 1.0000 g. anhydrous celluronic acid

Oxidation time, hours	Nitrogen, %	*y* in ml. 0.1123 *N* NaOH	*y* in moles NaOH × 10^4
1	4.82	1.53	1.72
4	2.46	2.74	3.08
8	1.73	4.22	4.74
12	0.63	5.19	5.83
1	.29	1.65	1.86
4	.41	3.06	3.45
8	.46	3.99	4.48
16	.27	5.27	5.86

The alkali consumption is not due to oxidation of the alkaline solutions by atmospheric oxygen (Table IV). Dissolved oxygen is present but its role is believed to be negligible in this instance.

Typical curves of the generation of acidity as measured by alkali consumption are shown in Fig. 3, using the celluronic acid resulting from sixteen hours oxidation with nitrogen dioxide. Curve A shows that consumption during initial alkaline decomposition at *p*H 11 is nearly linear with respect to time. Extended reaction at *p*H 12 indicates that the consumption may approach completion at very long reaction times.

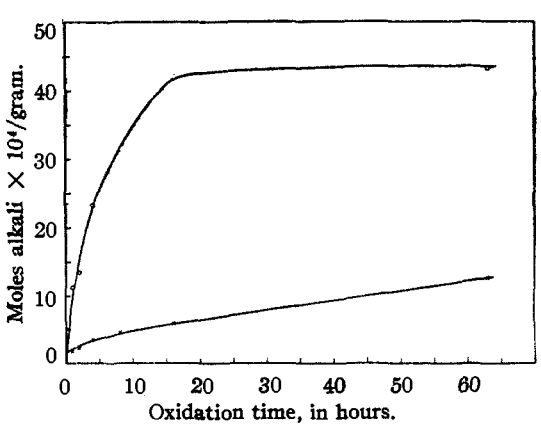


Fig. 2.—Relation between oxidation time and consumption of alkali in two hours at *p*H 11: O, initial equivalence; ×, extra moles alkali.

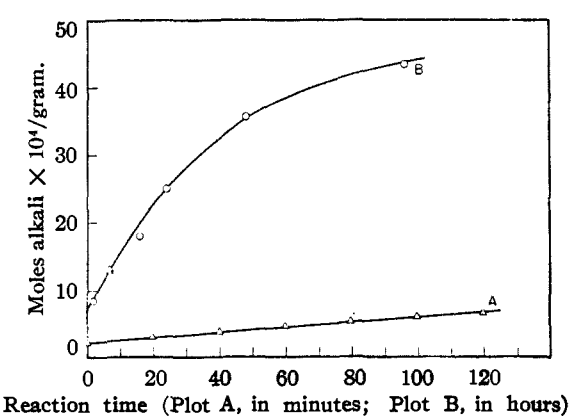


Fig. 3.—Rate of alkali consumption at high *p*H: plot A at *p*H 11; plot B at *p*H 12.

(11) Kenyon and Gray, THIS JOURNAL, 58, 1422 (1936).

Three celluronic acids of diverse oxidation history (*i. e.*, one-, four- and sixteen-hour oxidation, Table I) were each treated with alkali for two hours at several *p*H values greater than 7. The log of alkali consumption expressed as R was a linear function of *p*OH of the solution (Fig. 4). The equation for these curves is

$$\log R = -B[p\mathrm{OH}] + \log A \qquad (1)$$

where $-B$ is the slope of each curve and $\log A$ is the $\log R$ intercept. Equation (1) converts to

$$R = A[\mathrm{OH}^-]^B \qquad (2)$$

The constants A and B for these three celluronic acids are given in Table VI.

TABLE VI

CONSTANTS RELATING HYDROXYL-ION DEPENDENCY OF ACIDITY GENERATED BY CELLURONIC ACIDS AT *p*H 7

Oxidation time, hours	A	B
1	3.72	0.50
4	1.21	.34
16	1.31	.33

Thus, the catalyst in the generation of extra acidity at high *p*H appears to be hydroxyl ion. When a *p*H of 7 is exceeded, the celluronic acids begin to decompose even before dissolving.

The evidence thus accumulated indicates that the extensive oxidation of the primary hydroxyls of cellulose by nitrogen dioxide to form carboxyl groups is accompanied by some oxidation at another position forming an alkali-labile linkage. Systematic consideration will show that a variety of structures are theoretically capable of formation by oxidizing one or more positions in the anhydroglucose unit.[12] Oxidation of the glucoside links should be considered, as nitrogen dioxide is a powerful oxidant for simple ethers. All the possible resulting structures could not be examined, but data on a few typical ones are shown in Table VII.

TABLE VII

ACIDITY DEVELOPED IN REFERENCE SUBSTANCES AT ELEVATED *p*H (DRY BASIS)

Substance	*p*H max.	Time at *p*H max., hours	% COOH—Theory	% COOH—Potentiom. titra.	Extra moles alk. consumed per gram
Potassium acid saccharate	10.93	2	18.14	18.20	3.5×10^{-5}
D-Galacturonic acid monohydrate	10.92	2	21.22	20.41	6.6×10^{-5}
L-Ascorbic acid	11.12	2	25.56	25.42	1.07×10^{-3}
L-Ascorbic acid	11.04	48	25.56	25.42	7.80×10^{-3}
L-Ascorbic acid	11.20	96	25.56	25.42	8.15×10^{-3}
Tartaric acid	10.92	2	60.00	58.91	1.1×10^{-5}
D-Gluconic acid	10.98	2	22.96	24.76	6.0×10^{-5}
D-Glucono-γ-lactone	11.00	2	25.30	24.22	6.2×10^{-5}
Periodic acid-oxidized cellulose	12.00	64			5.79×10^{-3}

(12) Unruh and Kenyon, *Textile Research J.*, **16**, 1–12 (1946).

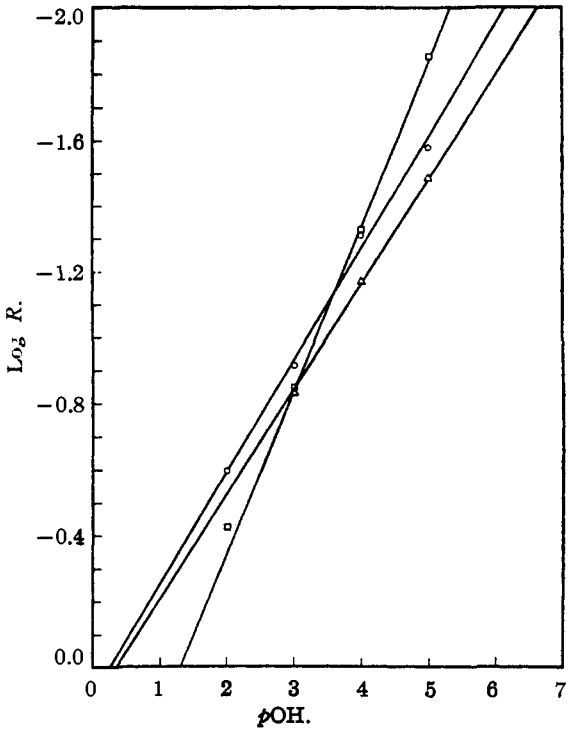


Fig. 4.—Relation between log of ratio of extra and initial moles alkali needed for equivalence and *p*OH: △, sixteen-hour oxidation; ○, four-hour oxidation; □, one-hour oxidation.

Though the number of possible compounds or structures resulting from oxidation of the glucose unit is large, these compounds must contain, in addition to unreacted hydroxyls, one or more of the three following groups, *i. e.*, carboxyl (ester or lactone), ketone (ene-diol) or aldehyde (enol). Carboxylic acids typified by saccharic, gluconic and tartaric did not generate significant acidity in alkali. The slow hydrolysis of lactones is not the source of the acidity, for glucono-γ-lactone opened completely and was titrated as the free acid as the *p*H was raised. It is doubtful whether generation of acidity is due to a slow hydrolysis of ester groups. The saponification of esters is the the classical illustration of a bimolecular reaction. Mathematical analysis of the acidity generated at constant time as a function of alkali concentration (Fig. 4) did not fit the second-order kinetics.[13] Galacturonic acid generated only little acidity even though an aldehyde group is present. Periodate cellulose known to contain a high percentage of aldehyde groups, generated much acidity but as previously stated, aldehyde groups are so vigorously oxidized by nitrogen dioxide that their presence in celluronic acids must be considered with reserve.

Ketone groups appear the most probable source of alkali-lability. Measurement of their presence by the methyl hydroxylamine method

(13) We wish to thank Dr. L. K. J. Tong, of these Laboratories, for this analysis.

of Purves indicated small but significant amounts as shown in Table I. A celluronic acid from a sixteen-hour oxidation appeared to possess 1.7% of carbonyl group by weight, or about one carbonyl for each ten of the original glucose units. Analyses of the data indicate a possible mechanism by which extra alkali is consumed. Extrapolation of Fig. 3, Plot B, to infinite time indicates that a total of about 50×10^{-4} mole of alkali would finally be neutralized. The same sample contains approximately 6×10^{-4} mole of carbonyl per gram (Table I). If this is in the form of a keto-glucuronic acid, it may be calculated that there are 37.3×10^{-4} mole of carbonyl per gram. Therefore, one mole of keto-glucuronic acid unit consumes 1.34 moles of alkali. *l*-Ascorbic acid (Table VII) will ultimately consume approximately 80×10^{-4} mole of alkali per gram, or about 1.41 moles per mole.

Figure 5 shows that the relation between the carbonyl content and the extra alkali consumption in two hours of the various celluronic acid is linear, the slope being 1.02. The small positive intercept of the experimental curve (plotted by a least-squares analysis of the data) lies within the experimental error of the measurements.

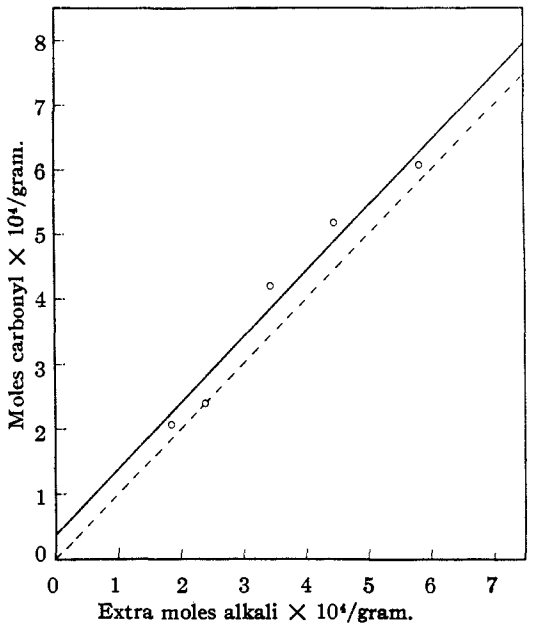
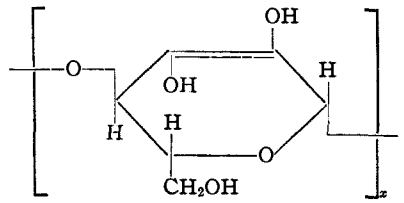


Fig. 5.—Relation between moles of carbonyl and extra moles of alkali consumed in two hours by various celluronic acids: solid line is experimental, broken line is exact equimolar relation.

This equimolar relationship would be greatly exceeded at longer reaction times in the alkali and at high *p*H values. Figure 3, Plot B, shows that several times as much alkali is consumed at about 100 hours as that consumed at two hours.

Evans and his collaborators[14] have explained the steps by which both simple sugars and glycosides are acted upon by alkali. Ivanov and Kaversneva[6] have applied these findings to the reaction of various oxidized celluloses with aqueous alkaline solutions so as to arrive at satisfactory mechanisms for explaining the changes in physical properties resulting from treatment of cellulose with several different oxidizing agents. They ascribed the increase in fluidity of oxidized celluloses after treatment with alkali to the shift of the keto form of different oxidized carbon atoms to the enols which render the glycosidic link unstable. Applying this concept to celluronic acids, the presence of a ketone group would constitute a weak point[15] in the chain as indicated by the enol structure shown.



Evans has shown that such enediols in simpler carbohydrates are ruptured by alkali, the generated aldehyde groups enolize, then the adjacent glucoside groups become alkali-labile. In an analogous manner, the units of celluronic acids containing ketone groups are readily split by alkali and the groups so split as well as the lower polyuronic acid fragments may undergo fission into reducing and acidic fragments. In other words, once the alkaline scission is initiated it continues, thus producing degradation far more extensive than indicated by the initial ketone content. Such fission along the chain would account for the extreme decrease in viscosity often observed even in lightly oxidized celluloses.[16] Davidson,[17] using oxidized celluloses prepared by a number of methods other than those involving the use of nitrogen dioxide, has demonstrated that the decrease in viscosities of nitrated oxidized celluloses in cuprammonium is not necessarily due to oxidative fission of the cellulose, but may be ascribed to the scission of alkali-sensitive links in the oxidized celluloses. The alkali-sensitivity of the periodic acid-oxidized celluloses appears to be related to the instability of the glyoxal and/or erythrose units (aldehyde groups).

Pacsu[18] has postulated that periodate-oxidized celluloses are reacted upn by alkaline solutions in the manner of an inner Cannizzaro reaction, supporting the theory in a qualitative way by showing that alkali reacted with such an oxidized cellulose to generate acidity. Since Davidson[8] has

(14) Evans and Benoy, This Journal, **52**, 294 (1930); Evans and Hockett, *ibid.*, **53**, 4384 (1931); Gehman, Kreider and Evans, *ibid.*, **58**, 2388 (1936).

(15) Staudinger [*Ber.*, **72**, 1709 (1939)] has postulated such "defective" celluloses with carboxyl instead of carbonyl units.

(16) We wish to thank Dr. D. D. Reynolds, of these Laboratories, for criticism helpful in arriving at these conclusions.

(17) Davidson, *J. Textile Inst.*, **32**, 132–148 (1941); **32**, T109–131, (1931); **29**, T195–218 (1938).

(18) Pacsu, *Textile Research J.*, No. 10, **15**, 354 (1945).

demonstrated that a variety of breakdown products (such as formaldehyde and carbon dioxide) are produced during this type of cellulose oxidation, indicating that the oxidizing agent is not confined in its action to oxidation at the number two and three carbon atoms of the anhydro-glucose unit, this hypothesis may be viewed with some reserve. Head[19] recently has shown that in both mono- and polysaccharides, dialdehydes are converted to carboxyl slowly in the presence of dilute alkali.

The criticisms of Davidson and Purves on the calcium acetate method for carboxyl determination do not appear valid when the method is used for celluronic acids.

While this paper was being prepared, an investigation appeared of the structure of celluronic acids by absorption spectra.[20] The data indicate large contents of carboxyl groups with possibly a small carbonyl group content. These results agree with our chemical findings.

(19) Head, *Shirley Inst. Mem.*, **21**, 11 (1947).

(20) Rowen, Hunt and Plyler, *J. Research Natl. Bur. Standards*, **39**, 133–140 (1947).

Summary

1. Potentiometric investigations show that celluronic acids degrade in alkaline solutions to generate acidity.

2. Small amounts of carbonyl groups appear to be present in the celluronic acids.

3. The acidity generated in alkaline solution at a constant reaction time appears directly related to the carbonyl group content and is an exponential function of the alkali concentration.

4. The ketone groups are believed to enolize in alkali, the enediols split as in simple carbohydrates, the adjacent glucoside links hydrolyze and an extensive alkaline degradation is thus initiated which continues along the chain producing reducing and acidic substances.

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[CONTRIBUTION FROM THE COATES CHEMICAL LABORATORY OF LOUISIANA STATE UNIVERSITY]

The Migration of Acetyl and Benzoyl Groups in o-Aminophenol

BY ARTHUR L. LEROSEN AND EDGAR D. SMITH

A considerable amount of experimental data has been collected on the general subject of acyl migrations in *o*-aminophenols since interest was first drawn to this problem by Stieglitz[1] in 1898. The great majority of this work has been published by Raiford and co-workers[2–4] and has indicated that when two different acyl groups, derived from carboxylic acids, are introduced into an *o*-aminophenol generally the same acyl derivative is obtained regardless of the order of introduction. On hydrolysis the heavier acyl group has usually been found on nitrogen.

No satisfactory general explanation has been given for all the phenomena observed in the acylation or hydrolysis of these compounds. The best discussion to date, in the opinion of the authors, was that of Bell,[5] and this work has generally been neglected by other workers in this field.

The present work was undertaken because it seemed probable that it would be possible to give an adequate explanation of these acyl migrations in terms of a combination of the theory of resonance and inductive effects. Accordingly a general theory was derived for these reactions and was found to agree in many respects with the data found in the literature. Nevertheless there were discrepancies and these were of such a nature that a reëxamination of the reported data was advisable, especially since at present new and powerful aids to this study are available in the form of absorbtion spectroscopy for the determination of structure, and chromatographic techniques for the quantitative analysis of mixtures.

It is premature to present the details of our theories of acyl migrations here, but three resulting conclusions are important: first, no migration should be complete, but instead there should be a reversible equilibrium; second, in the acetyl–benzoyl migration the N-acetyl isomer should predominate; and third, if a "migration" occurs in acylation, the opposite migration should be observed during hydrolysis. There are no conclusive data on this first point in the literature, Raiford found only one product in the acetyl–benzoyl mixed acyl derivative, while Bell reported the formation of different isomers, depending on the acylation sequence. Bell based his conclusions on mixed melting point data. Both of these men reported that hydrolysis yielded only *o*-benzoylaminophenol.

The experiments here were concerned with the determination of the nature of the acylation product when acetyl and benzoyl groups were introduced into *o*-aminophenol in different sequence. The method was to prepare the crude derivatives by acylation with the corresponding anhydrides in pyridine solution, and to separate the product into its constituents chromatographically. The determination of structure was accomplished by comparison with spectroscopic curves determined for all of the possible mono- and diacyl deriva-

(1) Julius Stieglitz, *Am. Chem. J.*, **21**, 111 (1898).

(2) L. C. Raiford, THIS JOURNAL, **41**, 2068 (1919).

(3) L. C. Raiford and J. R. Couture, *ibid.*, **46**, 2305 (1924); **44**, 1792 (1922).

(4) L. C. Raiford and H. P. Lankelma, *ibid.*, **47**, 1111 (1925).

(5) Frank Bell, *J. Chem. Soc.*, 2966 (1931).