those involved when small molecules or individual atoms are considered. Resonance might occur in spite of intermediate blankets, no immediate contact being necessary between substrate and enzyme. It might also be conceived that, depending among other things on the frequency involved, certain vibrations would be better transmitted than others by the intermediate blankets. Some types of blankets may have a stronger specific adsorption than others for the particular frequencies involved. The experiments made with blankets of evaporated gold show that such blankets are extremely efficient in preventing enzymatic as well as immunological reactions.

Thus, long range enzymatic action through a resonance phenomenon could be an explanation of the observed facts. If, as the presented data seem to indicate, long range enzymatic action occurs between films of antigen, or adsorbed molecules of antigens, and enzyme molecules, it would seem also likely that the same mechanism could apply to a substrate in solution and should be considered in a discussion of any theory of enzymatic action.[8]

Also it is important to note that in these experiments one is not justified in considering the behavior of single molecules of the substrate independently. The effect of the number and mode of deposition of monolayers of bovine albumin on its range of action is already an indication that considerable interaction takes place between the layers and presumably between the molecules within one single layer. The phenomenon of long range action could be considered as due to the cooperation of a group of molecules. Coöperation phenomena may play a role in biological processes, the degree of coöperation possibly determining the distance at which an enzyme may act. Finally, the possibility of enzymatic action through a thin cell membrane offers a new vista on physiological events.

(8) It is interesting in this connection to mention an article by Vlasow, *J. Physics, U. S. S. R.*, **9**, 25 (1945). The author shows that when considering large polyatomic systems one cannot neglect weak forces of interaction at long distances and that "these interactions reveal new dynamic properties of polyatomic systems, putting the problem of the transition from 'micro' to 'macro' anew." When collective interaction is taken into account, then follows according to the author "the presence and spontaneous origin of eigen frequencies in polyatomic systems." It is worth mentioning that in 1939 Langmuir, *Proc. Phys. Soc.*, **51**, 592 (1939), considered the possible importance of vibrations for the specificity of protein molecules.

Most of the data presented in this work were obtained with the able assistance of Miss Marjorie Hanson. I am indebted to her for her help in the preparation of this article. My thanks go also to Dr. Lyman C. Craig who read with care the manuscript and offered valuable criticism.

Summary

Multilayers of bovine albumin were submitted to the action of trypsin, and films of polysaccharide from Type III pneumococcus to that of a specific depolymerase. In both cases, following enzymatic action, the layers were altered to such a degree that they became incapable of specifically adsorbing homologous antibody. It was observed that blankets of barium stearate, of a plastic polymer (Formvar), and of polyvinyl chloride polymers deposited on the layers did not prevent enzymatic action from occurring when the enzyme solution was deposited on the blanket. The thickness of the blanket necessary to prevent any enzymatic action varied within a wide range depending on the number and mode of deposition of the underlying layers. It seemed unlikely that the enzyme molecules penetrated the blanket and the assumption was made that enzymatic action took place at a distance, the enzyme and substrate molecules being actually separated by an intervening blanket.

NEW YORK, N. Y. RECEIVED FEBRUARY 10, 1948

[CONTRIBUTION FROM EASTMAN KODAK COMPANY]

The Determination of Primary Hydroxyl Groups in Cellulose Acetate by Tosylation and Iodination

BY CARL J. MALM, LEO J. TANGHE AND BARBARA C. LAIRD

A study was undertaken of the amounts of primary and secondary hydroxyl groups in various samples of cellulose acetate to determine whether any difference could be detected depending on the history of the sample.

The method of tosylation and iodination for the determination of primary hydroxyl groups in glucose and its derivatives depends on (a) complete tosylation of primary hydroxyl groups and partial or complete tosylation of secondary hydroxyl groups, and (b) subsequent replacement of all primary tosyl groups by iodine and no reaction of secondary tosyl groups. These reactions have been applied to a commercial cellulose acetate by Purves and co-workers,[1,2] who found that slightly more than one third of the hydroxyl groups were primary. Their work indicated that the method should be suitable for comparing the amounts of primary hydroxyl in various samples.

This method, with minor modifications, was used, and the samples chosen for comparison in the

(1) F. B. Cramer and C. B. Purves, THIS JOURNAL, **61**, 3458 (1939).

(2) T. S. Gardner and C. B. Purves, *ibid.*, **64**, 1539 (1942).

TABLE I

STARTING MATERIALS

Sample		% acetyl	Hydroxyl per g. u.	Approximate % primary hydroxyl found
A	Acetylated and hydrolyzed with moderate amount of H_2SO_4	40.4	0.48	33
B	Same as A	31.6	1.27	25
C	Acetylated and hydrolyzed with $ZnCl_2$ and HCl	40.0	0.52	28
D	Acetylated with a large amount of H_2SO_4; combined sulfate split off with $MgCO_3$ and acetone	41.4	.38	60
E	From a sample similar to B, by tritylating, acetylating and detritylating	40.3	.49	33
F	Same as A	39.6	.57	33
G	Same as A	32.3	1.22	25
H	From A, deacetylated with 14% NH_4OH	None	3.00	33

present work are listed in Table I. These included cellulose acetates made by differing techniques, which possibly could affect the proportions of primary hydroxyl groups. They were tosylated with *p*-toluenesulfonyl (tosyl) chloride in the presence of pyridine for varying lengths of time (Fig. 1). The tosylated products were iodinated in acetonylacetone solution with sodium iodide, and the results obtained on the first five samples are given in Table II, from which the following points merit consideration:

1. Upon iodination of the tosylated samples, increasing amounts of iodine were introduced as the time of tosylation was extended. By determining the point at which the reaction slowed down, an approximate value for the amount of primary hydroxyl could be obtained. The estimated percentages of primary hydroxyl in the various samples are given in the last column of Table I.

2. The samples showed variations in the per cent. of primary hydroxyl groups as measured by this method. Samples A and E were in qualitative agreement with the results of Gardner and Purves,[2] but sample C, made with zinc chloride, contained slightly less, and sample D, made with a large amount of sulfuric acid catalyst, contained considerably more primary hydroxyl groups.

3. Sample E, made through the trityl derivative to contain substantially all primary hydroxyl groups, actually contained no more than sample A, made by commercial methods. This is indicative of migration of acetyl groups from secondary to primary hydroxyl during the detritylation step. Note that the tosylation time curves of samples A and E were almost identical (Fig. 1).

4. In sample D the amount of tosyl reached a maximum after eight hours. Also, in the last two samples of this tosylation time series the molecular amount of iodine introduced exceeded the amount of tosyl present.

This behavior was traced to the displacement of tosyl by chlorine during tosylation, and of chlorine by iodine during iodination. Qualitative analysis[3] of the iodinated samples showed only a trace of chlorine, and the amount of iodine introduced was always less than the sum of tosyl and chlorine. Comparison of the degree of iodine substitution in Samples D-1 through D-6, Table II, where the chlorine is neglected, and in Table III, where it is taken into account, shows that the introduction of chlorine during tosylation does not

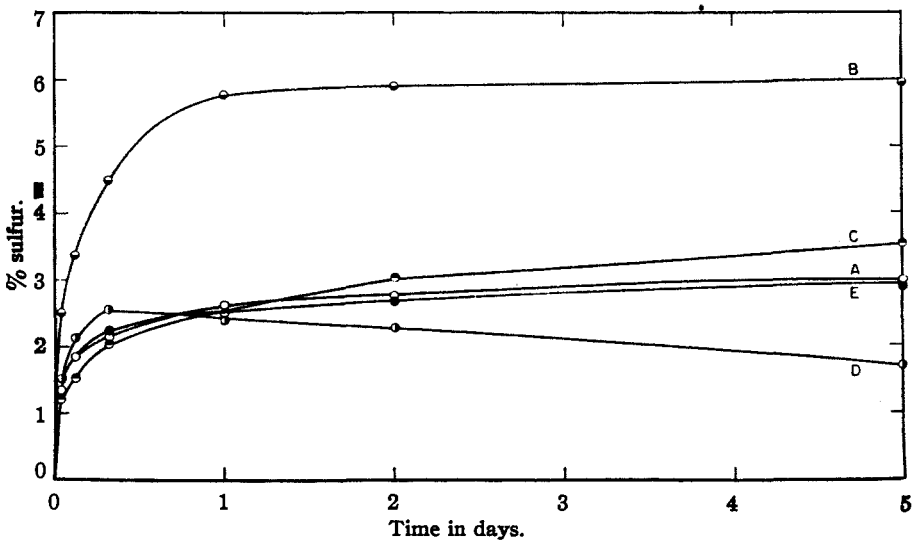

Fig. 1.—Tosylation of different samples of cellulose acetate.

(3) R. L. Shriner and R. C. Fuson, "The Systematic Identification of Organic Compounds," 2nd edition. John Wiley and Sons, Inc., New York, N. Y., 1940, p. 115.

TABLE II

TOSYLATION AND IODINATION OF VARIOUS SAMPLES OF CELLULOSE ACETATE

Sample	Tosylation: Time, hours	Tosylation: S, %	Tosylation: Tosyl per g. u.	Iodination (6 hr.): I, %	Iodination (6 hr.): Iodine per g. u.	Ratio of iodine per g. u. to original OH per g. u.
A-1	1	1.35	0.121	4.69	0.104	0.22
A-2	3	1.85	.170	6.25	.141	.29
A-3	8	2.14	.200	6.81	.156	.32
A-4	24	2.63	.252	7.08	.167	.35
A-5	48	2.77	.267	7.54	.179	.37
A-6	120	2.99	.292	8.12	.194	.40
B-1	1	2.51	.209	8.20	.167	.13
B-2	3	3.38	.295	10.00	.212	.16
B-3	8	4.49	.420	10.94	.248	.19
B-4	24	5.78	.587	14.59	.354	.27
B-5	48	5.90	.604	...	...	..
B-6	120	5.93	.608	...	...	..
C-1	1	1.20	.106	3.92	.086	.17
C-2	3	1.53	.138	4.98	.111	.21
C-3	8	2.05	.189	4.56	.127	.24
C-4	24	2.51	.237	6.63	.148	.28
C-5	48	3.04	.296	6.45	.155	.30
C-6	120	3.54	.355	7.04	.174	.33
D-1	1	1.53	.140	5.99	.136	.36
D-2	3	2.13	.202	8.63	.200	.53
D-3	8	2.55	.245	9.24	.218	.57
D-4	24	2.43	.234	9.78	.230	.61
D-5	48	2.28	.218	9.93	.231	.61
D-6	120	1.71	.159	9.68	.227	.60
E-1	1	1.29	.115	5.09	.112	.23
E-2	3	1.84	.169	6.56	.148	.30
E-3	8	2.20	.205	7.16	.164	.33
E-4	24	2.55	.243	7.52	.176	.35
E-5	48	2.73	.263	7.75	.183	.36
E-6	120	2.84	.275	8.03	.190	.38

materially affect the subsequent iodination. However, in Table III and subsequent tables, the chlorine was taken into account and assumed to be replaced by iodine.

TABLE III

TOSYLATION AND IODINATION OF CELLULOSE ACETATE

Sample D; 41.4% acetyl; 2.62 acetyls per g. u.

Sample	Chlorination: % Cl	Chlorination: Chlorine per g. u.	Iodination (6 hr.): % I	Iodination (6 hr.): Iodine[a] per g. u.	Ratio of iodine per g. u. to original OH per g. u.
D-1	0.09	0.008	5.99	0.136	0.36
D-2	.09	.008	8.63	.200	.53
D-3	.37	.032	9.24	.220	.57
D-4	.80	.070	9.78	.238	.62
D-5	1.04	.090	9.93	.242	.63
D-6	1.89	.160	9.68	.237	.62

[a] Based on replacement of all the chlorine and part of the tosyl.

To obtain further evidence for the replacement of chlorine by iodine during iodination, a sample high in chlorine was prepared by treating a tosylated cellulose acetate with pyridine hydrochloride.[1] This was iodinated along with the original tosylated sample and comparable amounts of iodine were introduced to each.

The increasing amounts of iodine introduced throughout the tosylation time series were disturbing. However, when the procedure described by Gardner and Purves[2] was exactly followed using sample F, the same increase in iodine content was again observed. Small amounts of chlorine were found in the products even with short time of tosylation. The minimum amount of chlorine was introduced when the tosylation was carried out at 0°.

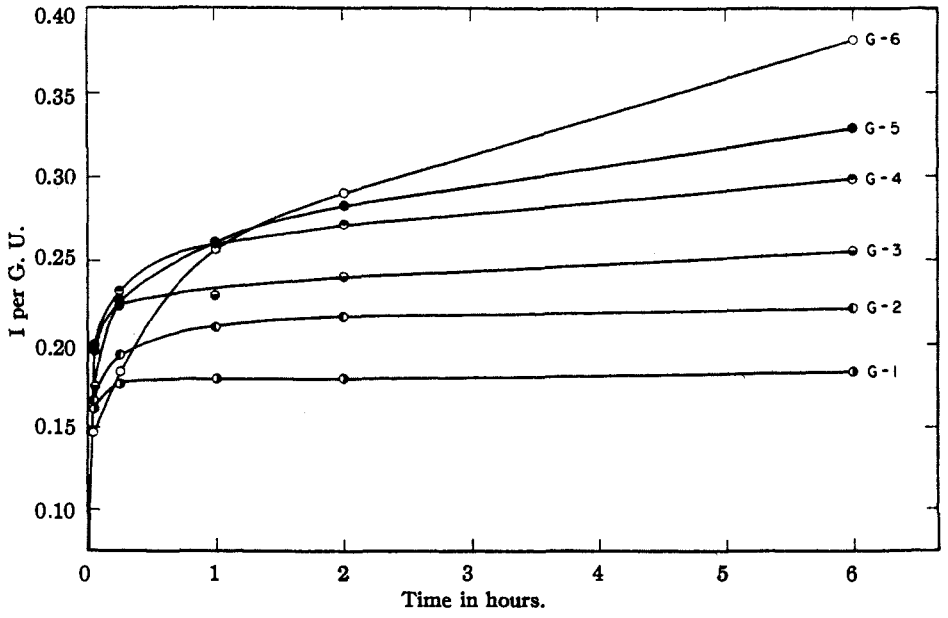

Fig. 2.—Iodination time series on tosylated cellulose acetates from Sample G, 32.3% acetyl.

TABLE IV

TOSYLATION AND IODINATION OF CELLULOSE ACETATE

Sample F; 39.6% acetyl; 2.434 acetyls per g. u.

Sample	Time, hours	Temp., °C.	Ratio TsCl: cellulose ester	S, %	Tosyl per g. u.	Cl, %	Chlorine per g. u.	Iodination (2 hr.) I, %	Iodination (2 hr.) Iodine per g. u.
F-1	1	20	13.1:1	1.66	0.149	0.12	0.009	5.7	0.127
F-2	3	20	13.1:1	2.20	.203	.16	.013	7.2	.165
F-3	8	20	13.1:1	2.54	.239	.16	.014	7.7	.179
F-4	24	20	13.1:1	3.08	.298	.23	.021	7.8	.188
F-5	48	20	13.1:1	3.43	.339	.34	.031	8.1	.199
F-6	120	20	13.1:1	4.01	.409	.79	.074	8.4	.217
F-7	48	0	13.1:1	3.14	.306	.13	.011	8.1	.193
F-8	48	20	6.6:1	3.21	.315	.43	.038	7.9	.192
F-9	48	20	3.3:1	2.61	.247	.36	.031	7.8	.184
F-10	48	20	1.7:1	1.96	.179	.20	.017	6.8	.154

TABLE V

IODINATION OF TOSYLATED CELLULOSE ACETATE

Samples from Table IV

Sample	Tosyl per g. u.	Iodination Time, min.	Iodination I, %	Iodination Iodine per g. u.
F-2-1	0.203	15	7.1	0.163
F-2-2		30	7.1	.163
F-2-3		60	7.2 7.2	.165
F-2-4		120	7.2	.165
F-2-5		360	7.2 7.3	.166
F-3-1	.239	5	7.5	.175
F-3-2		15	7.7	.179
F-3-3		30	7.4	.173
F-3-4		60	7.8	.182
F-3-5		120	7.5	.175
F-3-6		240	7.6	.177
F-4-1	.298	15	7.6	.183
F-4-2		30	7.7	.185
F-4-3		60	7.8	.188
F-4-4		120	7.8	.188
F-4-5		240	8.3	.199
F-4-6		360	8.4	.201
F-5-1	.339	15	7.0	.173
F-5-2		30	7.5	.185
F-5-3		60	7.8	.192
F-5-4		120	8.2	.202
F-5-5		240	8.7	.214
F-5-6		420	8.7	.214
F-6-1	.409	15	6.0	.156
F-6-2		30	6.8	.178
F-6-3		60	7.5	.195
F-6-4		120	8.2	.214
F-6-5		240	8.8	.228
F-6-6		480	9.0	.233

Even including samples where variations were also made in the temperatures and the amount of tosyl chloride, the amount of iodine introduced on iodination increased with the amount of tosyl present.

Iodination time series were then carried out on some of the samples of Table IV and the results are presented in Table V. Upon iodination of a sample low in tosyl, a constant amount of iodine was introduced as the time of iodination was extended; but with increased amounts of tosyl in the sample, the amount of iodine increased considerably as the time of iodination was extended.

This behavior was even more pronounced when tosylation and iodination time series were carried out on sample G, containing 32.3% acetyl (Tables VI and VII, and Fig. 2). Increased amounts of sodium iodide in the iodination of the last sample of this series were not helpful in obtaining a sharper break in the iodination reaction.

TABLE VI

TOSYLATION OF CELLULOSE ACETATE

Sample G; 32.3% acetyl; 1.78 acetyls per g. u.; temp. 25°; ratio TsCl:cellulose ester 5.6:1

Sample	Time, hours	S, %	Tosyl per g. u.	Cl, %	Chlorine per g. u.
G-1	1	3.03	0.265	0.19	0.015
G-2	3	4.10	.379	.12	.010
G-3	8	5.04	.492	.23	.020
G-4	24	6.45	.690	.35	.034
G-5	48	6.94	.770	.59	.059
G-6	120	7.52	.878	.89	.093

The upward drift in the amount of iodine introduced as the times of tosylation and iodination were extended suggested that secondary hydroxyl groups were slowly reacting. Although the original work of Oldham and Rutherford[4] contained several examples of the non-reactivity of tosyl groups on secondary hydroxyls, a review of the more recent literature showed several instances of their reactivity. Levene and Raymond[5] treated xylose derivatives tosylated in the 3-position (*i. e.*, on secondary hydroxyl groups) with sodium iodide and found a slow iodination at 110°. Hess[6] degraded tritosyl starch to a tritosyl glucose derivative into which he was able to introduce two atoms of iodine with sodium iodide at 130°. Levene[7] replaced all three tosyl groups in tritosylglycerol by this method. Tetratosyl erythritol

(4) J. W. H. Oldham and J. K. Rutherford, THIS JOURNAL, **54**, 366 (1932).

(5) P. A. Levene and A. L. Raymond, *J. Biol. Chem.*, **102**, 317 (1933).

(6) K. Hess, O. Littman and R. Pfleger, *Ann.*, **507**, 55 (1933).

(7) P. A. Levene and C. L. Mehltretter, *Enzymologia*, **4**, II, 232 (1937).

TABLE VII

IODINATION OF TOSYLATED CELLULOSE ACETATE
(Samples from Table VI)

Sample	Tosyl per g. u.	Iodination: Ratio NaI: sample	Iodination: Time, min.	Iodination: I, %	Iodine per g. u.
G-1-1	0.265	1:1	5	7.8	0.168
G-1-2			15	8.6	.185
G-1-3			60	8.7	.188
G-1-4			120	8.7	.188
G-1-5			360	8.9	.192
G-2-1	.379	1:1	5	7.3	.166
G-2-2			15	8.5	.193
G-2-3			60	9.3	.210
G-2-4			120	9.6	.217
G-2-5			360	9.8	.221
G-3-1	.492	1:1	5	8.1	.196
G-3-2			15	9.3	.224
G-3-3			60	9.5	.228
G-3-4			120	10.0	.240
G-3-5			360	10.7	.256
G-4-1	.690	1:1	5	7.5	.200
G-4-2			15	8.7	.232
G-4-3			60	9.8	.260
G-4-4			120	10.3	.272
G-4-5			360	11.3	.298
G-5-1	.770	1:1	5	6.2	.174
G-5-2			15	8.1	.226
G-5-3			60	9.4	.261
G-5-4			120	10.2	.283
G-5-5			360	11.9	.328
G-6-1	.878	1:1	5	4.9	.147
G-6-2			15	6.4	.191
G-6-3			60	8.7	.257
G-6-4			120	9.9	.291
G-6-5			360	13.1	.381
G-6-6	.878	2:1	5	5.7	.170
G-6-7			5	7.7	.228
G-6-8			60	10.3	.303
G-6-9			120	11.4	.333
G-6-10			360	13.8	.400
G-6-11	.878	5:1	5	6.4	.191
G-6-12			15	8.0	.237
G-6-13			60	11.1	.325
G-6-14			120	13.5	.392

showed irregular behavior,[8] losing all of its tosyl groups with the formation of butadiene. Tosylates of certain simple secondary alcohols, such as isopropyl-*p*-toluenesulfonate showed considerable reactivity toward sodium iodide even at room temperature.[9] Hockett and co-workers[10] applied the reaction to a sorbitol derivative tosylated at positions 2 and 5 (*i. e.*, on secondary hydroxyl groups). In solvents such as acetone, acetonylacetone or acetic anhydride at 120–140°, one of the tosyl groups was replaced with iodine.

(8) R. S. Tipson and L. H. Cretcher, *J. Org. Chem.*, **8**, 96 (1943).

(9) R. S. Tipson, M. A. Clapp and L. H. Cretcher, *ibid.*, **12**, 133 (1947).

(10) R. C. Hockett, H. G. Fletcher, E. L. Sheffield and R. M. Goepp, THIS JOURNAL, **68**, 927 (1946).

Accordingly, the tosylation and iodination reactions were applied to cellulose itself where the introduction of more than one iodine per glucose unit would be clear evidence for the participation of secondary hydroxyl groups.

Previous investigators[11] have found it necessary to use an active cellulose and to avoid high temperature in the tosylation reaction. Cellulose regenerated from the acetate was chosen for this work because of its reactivity. Even then but little tosyl was introduced without suitable conditioning of the cellulose. Pretreatment with aqueous pyridine gave a starting material which reacted with tosyl chloride at room temperature to yield a soluble product containing 1.5 to 1.8 tosyl groups per glucose unit. At steam-bath temperature according to the directions of Honeyman[12] products were obtained containing large amounts of nitrogen and chlorine. At 0° less than one tosyl per glucose unit was introduced and the product failed to dissolve in the reaction mixture. Successful iodinations could be carried out, however, in acetonylacetone suspension. The results on tosylation of regenerated cellulose are given in Table VIII, and on iodination of the tosylated products in Tables IX and X.

TABLE VIII

TOSYLATION OF CELLULOSE

Sample	Ratio TsCl: Cell	Time, hours	Temp., °C.	S, %	Tosyl per g. u.	Cl, %	Chlorine per g. u.
H-1	7:1	16	R. T.	12.58	1.62	0.28	0.03
H-2	7:1	46	R. T.	13.25	1.87	0.58	.07
H-3	7:1	168	R. T.	12.95	1.80	2.58	.32
H-4	4:1	48	R. T.	12.20	1.50	0.67	.08
H-5	6:1	4	0	3.58	0.22	.11	.01
H-6	6:1	7	0	3.90	.24	.31	.02
H-7	6:1	24	0	5.31	.36	.38	.02
H-8	6:1	48	0	6.50	.48	.48	.03

Where excess tosyl had been introduced, slightly more than one iodine per glucose unit was introduced when the time of iodination was extended. Two possible explanations for the increase in iodine content beyond one iodine per glucose unit, other than replacement of secondary tosyl groups by iodine, have come to our attention[13] and have been investigated.

An increase in weight per cent. iodine could result from the loss of tosyl without entrance of iodine. Gardner and Purves[2] found that no tosyl was lost which was not replaced by iodine, and occasional analyses of our iodinated products showed the required amount of tosyl remaining. To investigate this point more fully, sulfur and iodine analyses were made on samples from two iodination time series (Table X), and the degrees of iodine and tosyl substitution were calculated.

(11) A review of earlier work is given by C. J. Malm and C. R. Fordyce in "Cellulose and its Derivatives," Emil Ott, Editor, Interscience Publishers, Inc., New York, N. Y., 1943, p. 702.

(12) J. Honeyman, *J. Chem. Soc.*, 168 (1947).

(13) E. Heuser, private communication.

TABLE IX

IODINATION OF TOSYLATED CELLULOSE

Samples from Table VIII

Sample	Ratio NaI:Sample	Time, min.	I, %	Iodine per g. u.[a]
H-1-1	1:1	15	29.2	0.87
H-1-2		30	32.4	0.95
H-1-3		60	34.8	1.01
H-1-4		120	38.2	1.10
H-1-5		360	41.2	1.18
H-1-6	2:1	15	34.9	1.02
H-1-7		30	35.3	1.03
H-1-8		60	35.4	1.03
H-1-9		120	37.2	1.08
H-1-10		360	41.1	1.18
H-2-1	1:1	60	25.8	0.86
H-2-2		120	30.4	1.00
H-2-3		360	36.1	1.17
H-3-1	1:1	360	35.1	1.21
H-4-1	1:1	5	26.1	0.74
H-4-2		15	31.7	0.89
H-4-3		30	34.8	0.98
H-4-4		60	35.5	1.00
H-4-5		120	37.3	1.03
H-5-1	1:1	5	10.5	0.16
H-5-2		15	11.7	.17
H-5-3		30	10.0	.15
H-5-4		60	11.6	.17
H-5-5		120	12.3	.18
H-8-1	1:1	5	14.0	.25
H-8-2		15	16.3	.29
H-8-3		30	18.5	.33
H-8-4		60	18.6	.33
H-8-5		120	19.0	.34

[a] Not corrected for loss of tosyl.

TABLE X

LOSS OF TOSYL ON PROLONGED IODINATION OF TOSYLATED CELLULOSE

Ratio of NaI: Sample 2:1

Sample	Time, min.	I, %	Iodine per g. u.[a]	S, %		Tosyl per g. u.	Tosyl lost per g. u.
H-2-4	5	24.1	0.78	8.4	8.1	1.06	0.10
H-2-5	15	30.0	0.97	7.2	7.1	0.91	.06
H-2-6	30	33.0	1.04	6.5	6.3	.80	.10
H-2-7	60	35.0	1.13		6.3	.81	None
H-2-8	120	37.4	1.04	4.7	4.3	.50	.40
H-2-9	360	42.2	1.19	3.9	3.7	.43	.38
H-2-10[b]	5	27.8	0.89		7.5	.95	.10
H-2-11	30	32.0	1.04		6.9	.90	None
H-2-12	60	35.8	1.15		6.2	.79	None
H-2-13	120	37.0	1.14		5.5	.67	.13
H-2-14	360	40.2	1.14		4.1	.46	.34

[a] Corrected for loss of tosyl. [b] Samples H-2-10 to H-2-14 were soaked overnight in 0.1 *N* sodium thiosulfate to remove any absorbed iodine.

When the time of iodination was one hour or less the loss of tosyl was negligible, but as the time of iodination was extended to six hours, there was a considerable loss of tosyl without entrance of iodine. Hence, the degree of iodine substitution of samples iodinated for six hours, calculated on iodine content alone (as in Table IX), is slightly high, but even when it is corrected for the amount of tosyl lost, is still slightly more than 1.00 per glucose unit. A similar correction should apply to six-hour iodinations of samples derived from cellulose acetate, since the iodine substitutions were calculated from iodine content alone, assuming no loss of tosyl without entrance of iodine.

Absorbed iodine would likewise contribute to high iodine content of the products. Extraction of iodinated samples with dilute sodium thiosulfate solution has been used[14] to remove absorbed iodine. Typical iodinated products reported herein were soaked overnight in 0.1 *N* sodium thiosulfate, and a loss of 0.6–0.8% iodine was observed. No iodine was lost, however, on refluxing the products for one hour with ethyl alcohol or with carbon tetrachloride.

In Table X, iodination time series without (Samples H-2-4 to H-2-9) and with (Samples H-2-10 to H-2-14) thiosulfate extraction gave comparable results.

The gradual increase in iodine content beyond one per glucose unit is interpreted as a slow replacement of secondary tosyl groups by iodine. This situation closely parallels that observed in a study of cellulose trityl ether[15] where slightly more than one trityl group per glucose unit could be introduced under extended reaction conditions.

Trityl derivatives were prepared from cellulose acetate sample F to determine the amount of primary hydroxyl to compare with the results of tosylation and iodination. About one-third of the hydroxyl groups could be tritylated, the exact amount of trityl introduced depending on the reaction conditions.

When applied to samples lower in acetyl, tritylation indicated slightly more primary hydroxyl than tosylation and iodination. In the preparation of sample E, about one-third of the hydroxyl groups in cellulose acetate, 31.0% acetyl, could be tritylated. In the tosylation and iodination of similar materials, samples B and G, only about 25% of the original hydroxyl groups could be readily replaced with iodine.

The results herein reported show that the tosylation and iodination method does not fulfil the conditions required for the exact determination of primary hydroxyl groups in cellulose acetate. All primary hydroxyl groups must be tosylated. However, large amounts of secondary tosyl groups are objectionable, since they interfere with the subsequent iodination, and in the iodination step the iodine content does not level off satisfactorily with the time of reaction. On samples low in tosyl where all of the primary hydroxyl groups may not have been tosylated the iodine levels off but at

(14) G. E. Murray and C. B. Purves, THIS JOURNAL, **68**, 3194 (1940).

(15) W. M. Hearon, G. D. Hiatt and C. R. Fordyce, *ibid.*, **65**, 2449 (1943).

too low a level. It was possible, however, by maintaining identical reaction conditions throughout, to determine the approximate primary hydroxyl content and to detect differences from sample to sample depending on their past histories. Especially noteworthy was the sample (D) of cellulose acetate prepared using a large amount of sulfuric acid catalyst, with subsequent removal of combined sulfate groups at the completion of the esterification. In this sample, approximately 60% of the hydroxyl groups were primary, indicating a preferential reactivity of the primary hydroxyl groups of cellulose toward the sulfuric acid catalyst during the esterification process.[16]

Experimental

Starting Materials.—Samples A, B, F and G, Table I, were made by commercial methods using sulfuric acid as the catalyst, both for the acetylation and the subsequent hydrolysis. The amount of acetyl in the products was controlled by the time of hydrolysis.

Sample C, Table I, was made by acetylation of cotton linters using acetic anhydride and zinc chloride catalyst. The amount of zinc chloride used was equal to the weight of the cellulose. In addition, 20 ml. of concentrated hydrochloric acid was added per pound of cellulose to aid the esterification. At the completion of the esterification of the cellulose, water was added to hydrolyze the product to the desired acetyl content.

Sample D, Table I, was made by acetylation of cotton linters using acetic anhydride and a comparatively large amount of sulfuric acid (28% of the weight of the cellulose). With this large amount of catalyst, good cooling was necessary to prevent excessive rise in temperature. The esterification was complete after a reaction time of one-half hour, as indicated by absence of fiber and grain. At this point an amount of magnesium carbonate was added, equivalent to three-fourths of the sulfuric acid catalyst. After stirring fifteen minutes at 100° F. the reaction mixture was diluted with half its volume of acetone, and the mixing was continued for one hour at the same temperature. The magnesium carbonate and the acetone aided the removal of combined sulfate without hydrolysis of acetyl. The product was then precipitated and washed in water.

Sample E, Table I, was prepared in three steps, by tritylation, acetylation, and detritylation of cellulose acetate according to the details immediately following:

1. **Tritylation.**—Twenty grams of cellulose acetate (31.0% acetyl; 1.68 acetyl groups per glucose unit) was dissolved in 100 ml. of anhydrous pyridine and 30 g. of triphenylchloromethane was added. The reaction mixture was heated at 70° for sixteen hours, at which time it was diluted with acetone, precipitated and washed in alcohol. The yield was 25.8 g. of a white, fluffy product containing 22.1% acetyl.[17] When several similar products were combined and tritylated again with fresh reagents, there was a slight increase in the amount of trityl introduced.

Anal. Calcd. for tritylation of one-third of the available hydroxyl groups (1.68 acetyl and 0.44 trityl per glucose unit): acetyl, 21.2; trityl, 31.6. Found: acetyl, 21.4; trityl, 31.4, 32.0.[18]

2. **Acetylation.**—Forty grams of the above product was dissolved in 200 ml. of anhydrous pyridine, and 40 ml. of acetic anhydride was added. After seventy-two hours at 50° the reaction mixture was diluted with 200 ml. of methanol. Considerable heat was evolved, indicating an excess of acetic anhydride. The product was precipitated and washed in alcohol, yielding 43.1 g. of a white, fluffy product.

Anal. Calcd. for complete acetylation of remaining hydroxyl groups (2.56 acetyl and 0.44 trityl per glucose unit): acetyl, 29.2; trityl 28.5. Found: acetyl, 29.4, 29.5; trityl, 27.8, 28.2.

3. **Detritylation.**—Ten grams of the above product (2.56 acetyl and 0.44 trityl per glucose unit) was dissolved in 70 ml. of acetic acid. A mixture of 14 ml. of acetic acid and 7 ml. of concentrated hydrochloric acid was added with stirring. Upon standing two hours at room temperature, crystals of triphenylcarbinol had separated out. The reaction mixture was diluted with 25 ml. of acetone and the product was precipitated and washed in methanol. The dried product was redissolved in acetone and precipitated in methanol to ensure complete removal of triphenylcarbinol. The yield was 6.3 g. of a white, fluffy product.

Anal. Calcd. for 2.56 acetyl per glucose unit: acetyl, 40.8. Found: acetyl, 40.2, 40.3; trityl, absent.

The above material represents sample E, Table I. It displayed the same solubilities in organic solvents as commercial cellulose acetate of comparable acetyl content, such as sample A, Table I.

Sample H, Table I, was prepared by the deacetylation of sample A by treatment with 14% ammonium hydroxide for two days at room temperature. The product remained in suspension throughout the process and retained the flaky appearance of the cellulose ester. The viscosity at 25° of the regenerated cellulose was 14 centipoises when dissolved in 2.5% concentration in cuprammonium solution.

Tosylation of Cellulose Acetate.—All tosylations were carried out at room temperature or lower with the cellulose acetate dissolved in anhydrous pyridine.

For the samples of Table II, the cellulose acetate was dissolved in 10 parts of pyridine and 2 g. of tosyl chloride per gram of cellulose acetate was added. After stirring for a few minutes to dissolve the latter, the bottles were placed in the 25 ± 0.1° bath. At the times indicated samples were diluted with acetone and precipitated and washed in alcohol. The tosylated products[18] acquired a slight color after a long time of reaction, but were all obtained in a fluffy, fibrous texture.

When small amounts of chlorine were found[19] in the tosylated materials measures were taken to eliminate or minimize this side-reaction. The tosyl chloride was dissolved in pyridine and the solution was cooled before it was added to the solution of the cellulose ester. Various samples of tosyl chloride were compared, and one sample was recrystallized from cyclohexane before use. These measures were without avail, although lowering of the reaction temperature to 0° did decrease the amount of chlorine entering.

In repeating the experiment of Gardner and Purves[2] a sample of cellulose acetate of comparable acetyl content was selected and all details were modified to conform to their experimental conditions. The results are given in samples F-1 through F-6, Table IV, and in Fig. 2. In the remaining samples of Table IV, variations were made in the temperature and in the amount of tosyl chloride.

In an attempt to eliminate the introduction of chlorine, some experiments were made with *p*-toluenesulfonic anhydride as the tosylating agent. This material was prepared from the acid and thionyl chloride, and melted

(16) C. J. Malm, L. J. Tanghe and B. C. Laird, *Ind. Eng. Chem.*, **38**, 77 (1946).

(17) Acetyl analyses were carried out by the Eberstadt method as described by L. B. Genung and R. Mallatt, *Ind. Eng. Chem., Anal. Ed.*, **13**, 369 (1941).

(18) Sulfur analyses were carried out by the Parr bomb or by the method described by C. J. Malm and L. J. Tanghe, *Ind. Eng. Chem., Anal. Ed.*, **14**, 940 (1942).

(19) The chlorine determinations were carried out by a saponification procedure developed in this Laboratory by Dr. J. W. Mench. This has been found adaptable to small amounts of chlorine in cellulose esters and has given results in agreement with a standard combustion method.

at 128°, in good agreement with the literature.[20] A tosylation reaction was carried out in pyridine solution using four parts of the anhydride to one part of cellulose acetate (Sample A, Table I). When only 0.30% sulfur was found in the product after a reaction time of twenty-four hours on the steam-bath, this method was abandoned.

Tosylation of Regenerated Cellulose.—When dried regenerated cellulose was treated with tosyl chloride in pyridine suspension, only a small amount of sulfur was introduced after several days of reaction at room temperature.

For activation, 30 g. of regenerated cellulose (Sample H, Table I) was tumbled overnight with 450 g. of pyridine and 150 g. of water. The water was then displaced by four changes of anhydrous pyridine, the cellulose being pressed out on a Buchner funnel with a sheet of rubber after each treatment.

To obtain samples H-1, H-2 and H-3, Table VIII, the cellulose, wet with pyridine, was divided into three parts of 48 g. each. To each was added 100 ml. of pyridine and 70 g. of tosyl chloride, and the mixtures were tumbled at room temperature for the times indicated. Sample H-1 gave a reaction dope with considerable grain. As the reaction time was extended the grain diminished, and was absent from sample H-3. In each case the reaction mixture was diluted with an equal part of pyridine and the product precipitated and washed in alcohol. The products were all light tan in color and fluffy in texture.

In order to obtain samples with lower amounts of tosyl and to minimize the introduction of chlorine, a reaction time series was carried out at 0°. The regenerated cellulose was activated in the same way, and the mixture was stirred continuously throughout the reaction (Samples H-5 through H-8, Table VIII). Less than one tosyl group per glucose unit was introduced under these conditions, and the cellulose failed to dissolve. The products were washed in alcohol and were unchanged in appearance from the starting material.

Iodination of Tosylated Cellulose Derivatives.—After a few trials using acetone at 100° in sealed tubes as the solvent for the iodination, this solvent was abandoned in favor of acetonylacetone.[2]

Where iodination time series were not taken, 1 g. of the tosylated sample and 1 g. of sodium iodide were dissolved in 30 ml. of acetonylacetone and heated in the electric oven at 120 ± 2°. In Table II the reaction time was six hours, and in Table IV, two hours, with occasional stirring. Crystallization of sodium *p*-toluenesulfonate took place during the course of the reaction. The products were isolated by precipitation and washing in distilled water.

A yellow color was always indicative of a large amount of iodine in the product. Most of these iodinated products were fluffy in texture and isolated in good yield. However, iodinated derivatives of Samples B-5 and B-6, Table II were too powdery to be isolated after iodination for six hours.

The iodinated derivatives of tosyl cellulose (Table IX) were isolated in poor yield when the time of iodination was extended to six hours. With shorter times of iodination, good yields were obtained on these products.

For the iodination time series 2.5 g. of tosylated derivative was dissolved in 75 ml. of acetonylacetone and heated in an oil-bath to 120 ± 1.0° in a three-necked flask fitted with thermometer and stirrer. After the solution had come to temperature 2.5 g. of sodium iodide was added. Approximately 15-ml. portions were pipetted out at the indicated intervals, and the products were precipitated and washed in distilled water.

All the tosylated derivatives except the cellulose *p*-toluenesulfonates containing less than one tosyl per glucose unit were soluble in hot acetonylacetone. Satisfactory iodinations were achieved in insoluble derivatives by carrying out the reaction in suspension.

Chlorination of Tosylated Cellulose Acetate.—Sample F-9, Table IV, was treated on the steam-bath with an equal weight of pyridine hydrochloride in pyridine solution. A large portion of the tosyl was replaced by chlorine, yielding a product containing 2.7% chlorine. Upon iodination, a product was obtained with 7.1% iodine, the chlorine having undergone the same displacement reaction as the tosyl.

Summary

The method of tosylation and iodination did not give exact results in the determination of primary hydroxyl in cellulose and cellulose acetate; since as the reaction conditions in both the tosylation and iodination steps were extended, increasing amounts of primary hydroxyl were indicated.

In view of these difficulties the reaction conditions must be standardized in comparing the amounts of primary hydroxyl in different samples of cellulose acetate.

Different proportions of primary hydroxyl were found in samples of cellulose acetate depending on their methods of preparation.

The introduction of slightly more than one iodine per glucose unit into tosylated cellulose indicated a slow participation of secondary hydroxyl in the tosylation–iodination reaction.

ROCHESTER, N. Y. RECEIVED MARCH 17, 1948

(20) A. L. Bernouilli and H. Stauffer, *Helv. Chim. Acta*, **23**, 627 (1940).