salt-like nature of the compound was shown by its solubility in water and 95% ethanol, insolubility in ether. A chloride analysis was run by adding weighed samples to water and following the usual Volhard procedure. The result corresponded to a pyridinium salt of the phenylbutadiene chlorohydrin (VIII).

Anal. Calcd. for $C_{15}H_{16}NOCl$: Cl, 13.6. Found: Cl, 13.7.

Ozonolysis of 1 g. of the salt in 50 ml. of water and decomposition of the ozonide by refluxing with 2 ml. of 30% hydrogen peroxide overnight gave an ether-soluble product and a water-soluble product. The ether extract yielded 0.2 g. of crystalline solid which was converted to the *p*-bromophenacyl derivative in the usual way.[25] This melted 112–114°, the authentic *p*-bromophenacyl ester of benzoic acid melted 119–120°, and a mixed melting point gave 116–118°. Evaporation of the water solution gave a crystalline solid which melted and decomposed at about 195°.[26] Analysis for chloride ion checked the empirical formula for pyridine–betaine hydrochloride, $[C_5H_5NCH_2COOH]Cl$ (IX).

Anal. Calcd. for $C_7H_8O_2NCl$: Cl, 20.4. Found: Cl, 21.0.

Pyridine and 1-phenyl-4-chlorobutadiene showed no evidence of reaction after standing several days.

Reduction of 1-Phenyl-1,2-epoxy-3-butene (III).—A solution of 7 g. of epoxide in absolute ether with 0.5 g. of 10% palladium on charcoal was hydrogenated in the Parr low pressure apparatus in the usual way. Distillation yielded 5.3 g. of liquid boiling 60–65° (1 mm.). The α-naphthylurethan[27] melted 101–104°, the same derivative of 1-phenyl-2-butanol (IV) melted 101–105°, and a mixed melting point gave no depression. The phenylurethans[27] melted 79–80° and 78–81°, respectively, and also showed no depression in a mixed melting point. The 1-phenyl-2-butanol was made by the Grignard reaction of ethyl bromide and phenylacetaldehyde in 70% yield, b. p. 61–66° (1 mm.).[28]

Because Abragam had obtained the isomeric 1-phenyl-1-butanol by reduction of the epoxide, this alcohol was also made. Its phenylurethan was a liquid at room temperature and its α-naphthylurethan melted 106–108°. A mixed melting point with the same derivative from the epoxide reduction was 86–94°. The 1-phenyl-1-butanol was obtained through the Grignard reaction of *n*-propyl bromide and benzaldehyde in 53% yield; b. p. 90–95° (1 mm.).[28]

Hydrolysis of 1-Phenyl-1,2-epoxy-3-butene.—Six grams of the epoxide reacted with 35 ml. of 1% hydrochloric acid at 100° for 3 hours, the solution was extracted with ether, and the ether solution distilled to give 3.2 g. boiling 107–111° (1 mm.). Crystallization from benzene gave 2.1 g. melting 40–42°. The reported melting point of the glycol 1 phenyl-1,2-dihydroxy-3-butene (V) is 43°.[29]

Phenylbutadiene Dichlorohydrin (VI).—This compound was first isolated from the higher boiling fractions of the reduction products from the monochlorohydrin as a solid melting 86–87°. One experiment with 2.0 moles of hypochlorous acid from calcium hypochlorite and 1.0 mole of phenylbutadiene run as for the monochlorohydrin gave only a small yield, less than 1%, of the dichlorohydrin, m. p. 86–87°. No attempt was made to determine the structure of this compound.

Ultraviolet Absorption Spectra.—The measurements were made on 95% ethanol solutions of the compounds in 1-cm. quartz cells with a Beckman Model DU ultraviolet spectrophotometer.

Summary

Pure monochlorohydrins from the reaction of 1-phenyl-1,3-butadiene with hypochlorous acid could not be isolated. As reported earlier, the mode of addition is 1,2 and 3,4 but *both* reactions occur under the conditions used here and very probably under all conditions. The predominant mode of addition is 3,4, as shown by the relative yields of products derived from the chlorohydrins. The ratio of 3,4 to 1,2 addition is 3–4/1. This result fulfills the prediction from an electronic mechanism and shows again the generally greater reactivity of the 3,4 double bond compared to the 1,2 in 1-phenyl-1,3-butadiene.

(25) Shriner and Fuson, "Identification of Organic Compounds," 3rd ed., John Wiley and Sons, New York, N. Y., 1948, p. 157.

(26) The same melting characteristics are described by von Gerichter for pyridine betaine hydrochloride made from monochloroacetic acid and pyridine (*Ber.*, **15**, 1251 (1882)).

(27) Reference 25, p. 163.

(28) Lagerev, *Trudy Uzbekskogo Gosudarst. Univ.*, **6**, 71 (1936); *C. A.*, **35**, 2119 (1941).

(29) Tiffeneau and Weill, *Compt. rend.*, **200**, 1217 (1935); Deux, *ibid.*, **211**, 441 (1940).

CLEVELAND 6, OHIO RECEIVED OCTOBER 22, 1949

[CONTRIBUTION FROM EASTMAN KODAK COMPANY]

Primary Hydroxyl Groups in Hydrolyzed Cellulose Acetate[1]

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

The tritylation of cellulose has been carefully studied[2] and it has been shown that the trityl group in the "mono-trityl ether" is predominantly in the primary or number 6 position. The tritylation of a single sample of cellulose acetate has been reported.[3] About one third of the hydroxyl groups reacted.

Since the tosylation and iodination method did not give cleancut results[4] in the determination of primary hydroxyl in cellulose acetate, tritylation was used to determine whether the amount of primary hydroxyl in a sample of cellulose acetate depends on its method of preparation. The amount of trityl introduced under standardized conditions was taken as a measure of the amount of primary hydroxyl present.

Tritylation Conditions.—Trityl ethers were prepared from cellulose acetate by reaction with trityl chloride in the presence of pyridine. Since

(1) Presented before the Division of Cellulose Chemistry at the 116th Meeting of the American Chemical Society, Atlantic City, N. J., September 22, 1949.

(2) (a) W. M. Hearon, G. D. Hiatt and C. R. Fordyce, THIS JOURNAL, **65**, 2449 (1943); (b) J. Honeyman, *J. Chem. Soc.*, 168 (1947).

(3) I. Sakurada and T. Kitabatake, *J. Soc. Chem. Ind.*, Japan, Supplemental Binding, **37**, 604B (1934).

(4) C. J. Malm, L. J. Tanghe and B. C. Laird, THIS JOURNAL, **70**, 2740 (1948).

trityl chloride is known to react preferentially but not exclusively with primary hydroxyl groups, it was first necessary to show that the introduction of trityl levelled off as the reaction conditions were extended. For this purpose series were carried out in which the time, temperature, ratio of trityl chloride to cellulose acetate, and the amount and water content of pyridine were varied. The results are summarized in Tables I and II from which the following conditions were chosen for tritylation.

TABLE I

EFFECT OF REACTION CONDITIONS ON THE TRITYLATION OF CELLULOSE ACETATE[a]

Time, hours	Temp., °C.	TrCl, g.	Trityl, %	Trityl per g. u.	Primary hydroxyl, %
			Time Series		
1	70	20	11.6	0.128	10
2	70	20	18.6	.219	17
4	70	20	23.4	.294	23
8	70	20	26.0	.339	27
24	70	20	28.7	.388	31
48	70	20	29.7	.408	32
			Ratio Series		
24	70	10	24.9	.320	25
24	70	40	31.0	.434	34
			Temperature Series		
24	50	20	25.9	.337	27
24	90	20	28.5	.384	31

[a] Ten grams of cellulose acetate (31.8% acetyl, 1.26 OH groups per g. u.) in 50 ml. of anhydrous pyridine.

TABLE II

EFFECT OF DILUTION ON THE TRITYLATION OF CELLULOSE ACETATE[a]

Pyrimidine, ml.	Trityl, %	Trityl per g. u.	Primary hydroxyl, %
50	30.7	0.428	34
70	29.8	.411	33
80	29.2	.399	32
100	28.5	.384	31
150	27.0	.358	29

[a] Ten grams of cellulose acetate (31.9% acetyl, 1.24 OH groups per g. u.) and 19 g. of trityl chloride, heated to 70° for twenty-four hours.

The tritylations were carried out at 70–75° for twenty-four hours. The ratio of trityl chloride to sample was varied with the hydroxyl content of the cellulose acetate according to the formula

$$\text{Amount of trityl chloride (per 10 g. cellulose acetate)} = 10 + 12\,(\text{OH per g. u.} - 0.50)$$

For samples containing less than 0.50 hydroxyl group per glucose unit (*i. e.*, more than 40.3% acetyl) an amount of trityl chloride equal to the weight of the cellulose acetate was taken. The amount of anhydrous (less than 0.1% water) pyridine was maintained at 5 parts by weight, based on cellulose acetate, except in a few cases where the viscosity was too high.

Hydrolysis of Cellulose Acetate.—Samples were taken from several cellulose acetate hydrolysis series in which wide variations were made in the time of hydrolysis, the amount of water, and the amount of catalyst.

TABLE III

HYDROLYSIS IN ORIGINAL ESTERIFICATION BATH[a]

Sample	Catalyst[b]	Acetyl, %	Hydroxyl per g. u.	Trityl, %	Trityl per g. u.	Primary hydroxyl, % Tritylation	Tosylation iodination
A-1	0.88	43.7	0.13	5.3	0.065	50	
A-2		43.3	.17	6.6	.083	47	
A-3		42.4	.27	9.5	.12	44	
A-4		41.3	.39	12.3	.16	40	
A-5		40.7	.46	14.0	.18	40	
A-6		39.4	.59	16.6	.22	37	
A-7		37.9	.73	19.7	.26	35	
A-8		36.1	.90	21.4	.28	31	
A-9		34.3	1.06	24.3	.32	30	
A-10		32.6	1.20	25.6	.34	28	
B-1	7.0	44.0	0.09	5.2	.064	70[c]	
B-2		43.1	.20	8.6	.11	55	
B-3		42.3	.28	11.0	.14	50	41
B-4		41.8	.34	13.0	.17	50	40
B-5		41.3	.39	13.7	.18	46	37
B-6		40.6	.46	14.7	.19	42	34
B-7		40.0	.53	15.6	.20	38	31
B-8		39.5	.58	16.6	.22	37	30
B-9		39.1	.61	17.0	.22	36	30
B-10		38.7	.65	17.9	.23	36	29
B-11		38.2	.70	18.5	.24	34	28
B-12		37.8	.74	19.4	.25	34	28
B-13		37.3	.79	20.5	.27	34	28
B-14		36.7	.84	21.1	.28	33	27
B-15		33.7	1.11	23.6	.31	28	
B-16		31.6	1.28	25.5	.33	26	
B-17		30.2	1.39	27.3	.36	25	
C-1	28	42.2	0.31	12.4	.16	52	
C-2		41.2	.40	13.8	.18	45	
C-3		41.0	.42	14.5	.19	45	
C-4		40.2	.51	15.5	.20	39	
C-5		38.2	.70	18.7	.24	35	
C-6		36.1	.90	20.6	.27	30	
C-7		34.8	1.01	22.2	.29	29	
C-8		33.7	1.11	24.1	.31	28	
C-9		32.3	1.22	24.9	.32	26	
C-10		31.1	1.32	26.2	.34	26	
D		41.3	.39	18.6	.26	66[d]	

[a] Aqueous acetic acid added at the end of the esterification to provide 7–8% water in the reaction mixture. Hydrolysis at 100°F. [b] Amount of sulfuric acid catalyst, per cent., based on cellulose. [c] Poor accuracy due to low hydroxyl content of the sample. [d] Isolated after addition of magnesium carbonate and acetone as described by Malm, Tanghe and Laird, ref. 4.

In all hydrolysis series carried out directly after the acetylation of cellulose, an amount of dilute acetic acid was added to the acetylation mixture to provide 7–8% of water in the hydrolysis-bath. The amounts of sulfuric acid taken for acetylation and hydrolysis were 0.88, 7.0 and 28%, respec-

tively, based on the cellulose. The time intervals between samples varied with the amount of catalyst present. The range covered was from almost triacetate down to an acetate containing about 1.3 hydroxyl groups per glucose unit. Beyond this point the hydrolysis solution gelled when only 7–8% water was present.

Other hydrolysis series were initiated with a commercial cellulose acetate containing 40.4% acetyl. In this case the amount of water in the hydrolysis-bath was varied from 2.5 to 40%. At high water content samples could be obtained over a wider range of hydrolysis.

TABLE IV

FURTHER HYDROLYSIS OF COMMERCIAL CELLULOSE ACETATE

Different amounts of water in hydrolysis bath

Sample	Catalyst[a]	Water,[b] %	Acetyl, %	Hydroxyl per g. u.	Trityl, %	Trityl per g. u.	Primary hydroxyl, % Tritylation	Primary hydroxyl, % Tosylation iodination
Original			40.4	0.50	14.7	0.19	39	
E-1	7	2.5	38.5	.68	14.6	.18	27	
E-2			36.8	.84	16.8	.21	25	
E-3			35.8	.95	17.5	.23	24	
F-1	7	6	38.3	.69	17.7	.23	33	
F-2			36.5	.86	20.8	.27	31	
F-3			35.2	.98	21.8	.28	29	
F-4			34.8	1.01	23.3	.31	30	
F-5			33.2	1.15	25.3	.33	29	
F-6			31.7	1.27	26.6	.35	28	
G-1	7	10	37.8	0.74	20.3	.27	36	
G-2			35.7	0.94	24.5	.33	35	
G-3			33.8	1.09	27.2	.37	35	
H-1	7	20	37.3	0.79	23.0	.31	40	
H-2			35.0	0.99	28.1	.40	40	
H-3			33.6	1.11	30.8	.44	40	
I-1	7	30	38.7	0.66	20.8	.28	43	
I-2			38.4	.69	22.6	.31	45	
I-3			37.2	.80	25.8	.37	46	
I-4			36.1	.90	27.8	.40	44	
I-5			33.1	1.16	33.9	.50	43	
I-6			32.0	1.25	36.7	.56	45	
I-7			28.3	1.53	41.1	.64	42	
J	22	26	33.1	1.16	32.9	.48	42	38
K	11	40	32.2	1.23	34.9	.52	42	36
L	11	10	31.9	1.25	29.2	.40	32	27
M	22	30	29.1	1.47	40.1	.62	42	38
N	22	34	26.6	1.64	43.2	.68	41	39
O	22	37	24.2	1.81	46.9	.77	42	39
P	22	40	20.3	2.05	49.9	.82	41	39
Q	22	40	15.9	2.29	54.9	.95	42	39

[a] Amount of sulfuric acid catalyst, per cent., based on cellulose content. [b] In the hydrolysis bath. The starting cellulose acetate was not soluble at 100° F. in acetic acid–water mixtures containing more than 30% water. Where more than that amount of water is indicated, part of the water was added during the course of the hydrolysis.

Results

With increasing hydrolysis in the presence of 7–8% water the amount of primary hydroxyl per glucose unit steadily increased, and the ratio of primary to total hydroxyl decreased. The results are given in Table III and Fig. 1. In the range where there were more than 0.50 hydroxyl group per glucose unit, the percentage of primary hydroxyl was independent of the esterification catalyst. With less than 0.50 hydroxyl per glucose unit, a smaller percentage of primary hydroxyl was found with the lowest amount of catalyst. This indicates that the sulfuric acid which has been shown to be combined to the cellu-

TABLE V

VARIATION IN PERCENTAGE OF PRIMARY HYDROXYL IN CELLULOSE DIACETATE WITH THE WATER CONTENT DURING HYDROLYSIS

Water in hydrolysis bath, %	Primary hydroxyl at the diester stage of hydrolysis, %
2.5	24
6	29
10	34
20	40
30	44

TABLE VI

FURTHER HYDROLYSIS OF COMMERCIAL CELLULOSE ACETATE

10% Water in hydrolysis bath, temperature and catalyst varied

Sample	Catalyst	Temp., °F.	Acetyl, %	Hydroxyl per g. u.	Trityl, %	Trityl per g. u.	Primary hydroxyl, %
G-4	H_2SO_4	77	38.6	0.68	19.9	0.27	40
G-5			36.7	0.85	24.2	.33	39
G-6			34.8	1.02	26.2	.37	36
G-1	H_2SO_4	100	37.8	0.74	20.3	.27	36
G-2			35.7	0.94	24.5	.33	35
G-3			33.8	1.09	27.2	.37	35
G-7[a]	H_2SO_4	100	37.7	0.75	20.8	.28	37
G-8			35.9	0.92	24.9	.34	37
G-9			33.8	1.10	27.9	.38	35
G-10	H_2SO_4	120	39.5	0.58	17.6	.23	40
G-11			38.4	.69	20.1	.27	39
G-12			35.3	.97	25.7	.35	37
G-13	H_2SO_4	140	37.9	.73	20.6	.28	38
G-14			36.5	.86	22.9	.31	36
G-15			35.5	.96	25.4	.34	36
G-16	H_2SO_4	160	37.3	.78	21.8	.29	37
G-17			35.5	.95	25.5	.35	37
G-18			33.4	1.13	28.4	.39	35
G-19	None	160	38.1	0.71	20.4	.27	38
G-20			36.3	0.88	24.2	.33	37
G-21			34.8	1.01	26.8	.37	37
G-22	HCl	100	38.9	0.64	18.0	.24	37
G-23			37.4	.78	20.3	.27	35
G-24			36.7	.84	22.0	.29	35
G-25	$HClO_4$	100	39.0	.63	18.9	.25	40
G-26			37.5	.77	21.5	.29	38
G-27			35.9	.92	24.3	.33	36

[a] The solvent:solid ratio was increased from 7:1 to 14:1 for samples G-7 to G-9. The concentration of sulfuric acid was held constant.

lose in the acetylation process[5] is attached predominantly to the primary hydroxyl group.

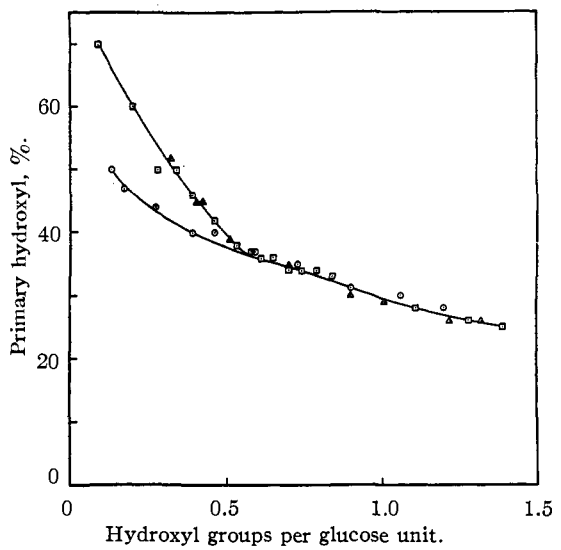

Fig. 1.—Hydrolysis of cellulose acetate after addition of 8% water to original esterification bath: ⊙, 0.88% H_2SO_4 catalyst; ⊡, 7%; ⧋, 28%.

The results obtained on hydrolyzing a commercial cellulose acetate with varying water content in the hydrolysis bath are given in Table IV and Fig. 2. The percentage of primary hydroxyl increased regularly with the amount of water present. Interpolated values (from Fig. 2) for the percentage of primary hydroxyl at the di-ester stage of hydrolysis with variations in water content in the hydrolysis bath are given in Table V and Fig. 3.

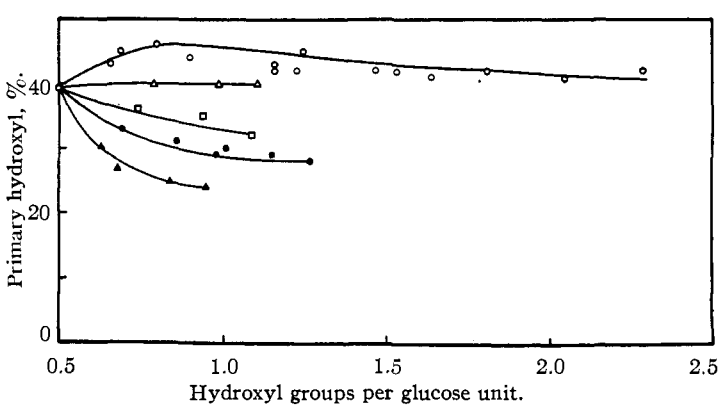

Fig. 2.—Hydrolysis of commercial cellulose acetate: O, 30–40% H_2O; △, 20% H_2O; □, 10% H_2O; ●, 6% H_2O; ▲, 2.5% H_2O.

Two experiments were made, hydrolyzing cellulose acetate (40.4% acetyl, 0.50 hydroxyl per g. u.) in aqueous dioxane so that the possibility of any reacetylation during the hydrolysis step would be ruled out. However, the results were the same as in aqueous acetic acid. With 6% water in the hydrolysis bath and hydrolyzing to 36.9% acetyl (0.82 hydroxyl per g. u.), 33% of the hydroxyl was found to be primary. With 25% water in the hydrolysis bath and hydrolyzing to 38.7% acetyl (0.65 hydroxyl per g. u.), 42% of the hydroxyl was primary.

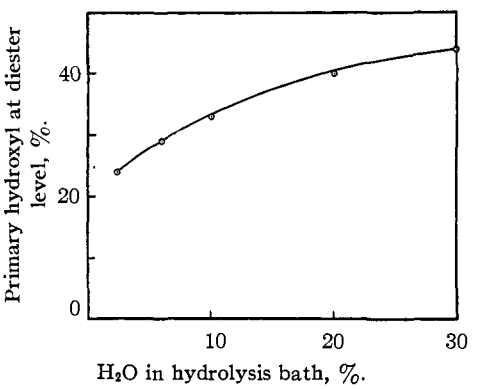

Fig. 3.—Effect of water content on per cent. primary hydroxyl.

Hydrolysis series were carried out with sulfuric acid catalyst in acetic acid:water (90:10) over a temperature range from 77 to 160°F. (Table VI, Samples G-1 to G-18). The times of hydrolysis were varied to give comparable acetyl ranges at the different temperatures. Variations in temperature in this range were without effect on the primary hydroxyl content.

Doubling the solvent:solid ratio in the hydrolysis bath had no effect on primary hydroxyl content. This shows that the primary hydroxyl content depends on the ratio of water to acetic acid and not on the ratio of water to cellulose acetate.

Hydrolysis series were carried out at 100°F. with hydrochloric and with perchloric acids, and at 160°F. without catalyst (Table VI, Samples G-19 to G-27.) In all cases the primary hydroxyl content was the same as with the use of sulfuric acid catalyst.

Comparison with the Tosylation and Iodination Method.—It has been recently shown[4] that the tosylation and iodination method gives variable results depending on the conditions chosen in each of the two steps. By standardizing on reaction conditions the method was found useful for comparing the primary hydroxyl content of different samples.

The primary hydroxyl contents of several samples determined by this method are included in Tables III and IV and are consistently lower than the tritylation results. The reason for this discrepancy probably lies in the fact that both of these methods are empirical in their choice of conditions, and neither is entirely specific for primary hydroxyl.

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

The tritylation method is preferred over the tosylation and iodination method since it is a single step reaction and fewer variables are involved.

Experimental

Materials

Hydrolyzed Cellulose Acetate.—Cotton linters were acetylated as previously described[5] with acetic anhydride and an amount of sulfuric acid equal to 7% of the weight of the cellulose. At the completion of the esterification sufficient acetic acid:water (2:1) was added to provide 7–8% water in the system. The hydrolysis solution was maintained at 100° F. and samples were removed from time to time. The cellulose acetate was precipitated and washed in distilled water.

Some changes were made in the acetylation conditions when variations were made in the amount of catalyst used. The liquid–solid ratio in the esterification bath was maintained at 9.2 to 1, and the amount of water in the hydrolysis bath at 7–8%.

Other hydrolysis series were initiated with commercial cellulose acetate to obtain higher water content during hydrolysis. As an example, 400 g. of cellulose acetate, 40.4% acetyl, was dissolved in 2400 ml. of 70% acetic acid at 100°F. A solution of 9.6 ml. of concentrated sulfuric acid (7% by weight, based on the cellulose content of the cellulose acetate) in 400 ml. of 70% acetic acid was added rapidly with stirring and the solution maintained at 100° F. during hydrolysis. Samples containing less than 1.5 acetyl groups per glucose unit were very water susceptible and were precipitated and washed in methanol.

Trityl Chloride.—This product was prepared by the Friedel and Crafts reaction.[6] Additional purification was effected by recrystallization from cyclohexane; m. p., 111° from the cooling curve of the melt.

Tritylation.—Ten-gram samples of cellulose acetate, dried at 105°, were dissolved in 50 ml. of anhydrous pyridine (water content not over 0.1% by the Karl Fischer method) and an amount of trityl chloride added according to the hydroxyl content of the cellulose acetate. The mixture was heated to 70–75° and the trityl chloride brought into solution by occasional stirring or inverting of the container. After maintaining at that temperature for twenty-four hours in an electric oven, the solutions were diluted with acetone, precipitated and washed in methanol. Reprecipitation of representative samples from acetone into methanol showed trityl content unchanged, hence this step was not included in routine procedures.

Trityl analyses were carried out according to the method of Hearon.[2a]

(6) "Organic Syntheses," **23**, 102 (1943).

Summary

1. The primary hydroxyl contents of hydrolyzed cellulose acetates of different acetyl contents have been determined by reaction with trityl chloride.

2. The percentage of primary hydroxyl is influenced by the amount of water in the hydrolysis bath. An increase in water concentration gives an increase in percentage of primary hydroxyl.

3. The percentage of primary hydroxyl is not affected by the temperature, or by the amount and nature of the catalyst during the hydrolysis.

4. The amount of sulfuric acid catalyst in the *esterification* influences the percentage of primary hydroxyl in the early part of the hydrolysis.

ROCHESTER 4, N. Y. RECEIVED OCTOBER 3, 1949

[CONTRIBUTION FROM THE HENRY PHIPPS INSTITUTE, UNIVERSITY OF PENNSYLVANIA]

Comparison of the Filtrate Fractions and of Certain Bacillary Extracts of the H 37, H 37 RV and H 37 RA Strains of Tubercle Bacilli[1]

BY FLORENCE B. SEIBERT, CRETYL CRUMB AND MABEL V. SEIBERT

The electrophoretic technique furnishes a simple means of determining the proportions of different components in any tuberculin. This is conspicuously shown in the case of samples of culture filtrates from tubercle bacilli grown on synthetic medium, filtered free of bacilli through a Seitz filter, and concentrated by ultrafiltration in the cold. Fractions with similar mobilities have already been isolated and studied separately and their chemical properties ascertained.[2] For example, the accompanying picture (Fig. 1) shows these components, which have been studied under identical electrophoretic conditions of *p*H 7.6, μ 0.1, in phosphate buffer, superimposed over each other so that the relative distances traveled from the start are indicative of their relative mobilities. They have been characterized further in order of slowest to fastest components, as polysaccharides I and II, A protein, B and C proteins with approximately the same mobility, a number of nucleo-proteins, and finally free nucleic acid itself.

(1) Presented before the Division of Biological Chemistry of the American Chemical Society, Atlantic City, September 1949.

(2) F. B. Seibert, *Am. Rev. Tuberc.*, **59**, 86 (1949).

Experimental

Comparison of Components in Different Batches of Filtrates from the Same Strain or from Different Strains.—It is possible to see the relative proportions of the components in the filtrates from the same strain made under identical conditions at different times, as well as in the filtrates made from different strains. It was interesting to find a very close similarity in the filtrates made from the same strain at different times but not in the filtrates from certain other strains; see Fig. 2. For example, it is clear, even without any isolation studies or mathematical evaluation of the curves, that the DT strain yields far more protein in proportion to polysaccharide than does the H 37 strain, which yields a relatively high percentage of polysaccharide. Furthermore these results were reproducible.

Comparison of Components in Filtrates of H 37, H 37 Rv and H 37 Ra.—A study similar to the one above was made of the H 37 strain, as well as of the virulent (H 37 Rv) and avirulent (H 37 Ra) dissociates of this strain, isolated by W. Steenken at the Laboratory of the Trudeau Sanatorium. We express our thanks to him for furnishing us with these dissociants.

About 100-liter bottles each of Long synthetic medium were planted with the three strains of tubercle bacilli and incubated eight weeks. Then the bacilli were filtered off