The reaction was carried out as described for phthaloyl-L-threonyl-L-phenylalanine methyl ester. After the acetonitrile was removed by distillation under reduced pressure, the residue was taken up in ethyl acetate and extracted with acid, bicarbonate and water. After drying and removal of the solvent the residue was crystallized from acetonitrile–water, yielding 0.980 g. (89%), m.p. 103–106°. Two recrystallizations from acetone–ether–hexane yielded an analytical sample, m.p. 109–110°, $[\alpha]^{26}D$ −16.7° (0.0770 g. in 1.5 ml. of absolute ethanol).

Anal. Calcd. for $C_{25}H_{28}N_2O_6$: C, 66.36; H, 6.24; N, 6.19. Found: C, 66.14; H, 6.25; N, 6.32.

Phthaloyl-L-threonyl-L-phenylalanine.—To a solution of 0.840 g. (0.00205 mole) of phthaloyl-L-threonyl-L-phenylalanine methyl ester in 30 ml. of acetone, there was added 10 ml. of water and 5 ml. of concentrated hydrochloric acid. The solution was refluxed for 2.5 hours and the acetone removed by distillation. Ethyl acetate was added and the product was subsequently extracted into bicarbonate solution. After acidification and re-extraction into ethyl acetate, drying and removal of the solvent under reduced pressure, the product was obtained as a crystalline mass. Recrystallization from ethanol–water yielded 0.510 g. (64%) of analytically pure material, m.p. 207–208°, $[\alpha]^{27}D$ +23.2° (0.025 g. in 1.5 ml. of absolute ethanol).

Anal. Calcd. for $C_{21}H_{20}N_2O_6$: C, 63.60; H, 5.09; N, 7.07. Found: C, 63.30; H, 5.16; N, 7.13.

Phthaloyl-L-threonyl-L-phenylalanyl-L-phenylalanine Methyl Ester.—A mixture of 0.200 g. (0.00051 mole) of phthaloyl-L-threonyl-L-phenylalanine, L-phenylalanine methyl ester[29] (0.110 g., 0.00062 mole) and N,N′-dicyclohexylcarbodiimide[15] (0.105 g., 0.00051 mole) in methylene chloride, was allowed to react as described for phthaloyl-L-threonyl-L-phenylalanine methyl ester.

The recrystallized product (ethanol–water) amounted to 0.245 g. (92%), m.p. 147.2–148°, $[\alpha]^{27}D$ −25.6° (0.0271 g. in 1.5 ml. of absolute ethanol).

Anal. Calcd. for $C_{31}H_{31}N_3O_7$: C, 66.77; H, 5.60; N, 7.54. Found: C, 66.71; H, 5.86; N, 7.48.

Phthaloyl-O-acetyl-L-seryl-L-phenylalanine Methyl Ester.—A methylene chloride solution of 0.400 g. (0.00145 mole) of phthaloyl-O-acetyl-L-serine, L-phenylalanine methyl ester[29] (0.310 g., 0.00165 mole) and N,N′-dicyclohexylcarbodiimide[15] (0.300 g., 0.0014 mole) was allowed to react as described for phthaloyl-L-threonyl-L-phenylalanine methyl ester.

Recrystallization from ethanol–water afforded a total of 0.570 g. (89%), m.p. 131–132°, $[\alpha]^{27}D$ +14.7° (0.0395 g. in 1.5 ml. of absolute ethanol).

Anal. Calcd. for $C_{23}H_{22}N_2O_7$: C, 63.01; H, 5.06; N, 6.39. Found: C, 63.21; H, 5.34; N, 6.44.

Carbobenzyloxy-L-hydroxypropyl-L-phenylalanine Methyl Ester.—A solution of carbobenzyloxy-L-hydroxyproline[10] (0.8 g., 0.0032 mole), L-phenylalanine methyl ester[29] (0.54 g., 0.0032 mole) and N,N′-dicyclohexylcarbodiimide[15] (0.625 g., 0.0033 mole) in methylene chloride was treated as described for phthaloyl-L-threonyl-L-phenylalanine methyl ester. Crystallization occurred from an ethyl acetate solution, yield 1.1 g. (86%), m.p. 108–112°. A portion of the material was recrystallized from acetone–ether–hexane, m.p. 114–115°, $[\alpha]^{27}D$ −29.2° (0.0416 g. in 1.5 ml. of absolute ethanol).

Anal. Calcd. for $C_{23}H_{26}N_2O_6$: C, 64.78; H, 6.10; N, 6.57. Found: C, 64.76; H, 6.36; N, 6.60.

CAMBRIDGE 39, MASSACHUSETTS

[CONTRIBUTION NO. 2046 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY OF THE CALIFORNIA INSTITUTE OF TECHNOLOGY]

Selective Acetylation of the Hydroxyl Groups in Gelatin

BY J. BELLO AND J. R. VINOGRAD

RECEIVED OCTOBER 15, 1955

The hydroxyl groups of gelatin have been selectively acetylated by two methods: treatment with acetic acid, acetic anhydride and perchloric acid, and treatment with acetic anhydride and trifluoroacetic acid. The latter method gives products that are less degraded and more reproducibly acetylated. Acetylation of the hydroxyl groups was shown by infrared spectra and selectivity by O-acetyl and amino nitrogen analyses and isoionic *p*H data.

As part of a study of the effect of chemical modification on the physical properties of gelatin it was decided to block the hydroxyl groups with non-ionizing substituents. Acetylation appeared to be a desirable reaction, but no methods were found in the literature for selective acylation of hydroxyl groups of proteins with carboxylic acids.

We have developed two methods for the selective acetylation of the hydroxyl groups of gelatin. One is an adaptation of the method of Sakami and Toennies[1] for the selective acetylation of the hydroxyl groups of amino acids with acetic acid, acetic anhydride and perchloric acid. The second utilizes the recently reported[2] solubility of proteins in trifluoroacetic acid and the trifluoroacetic acid catalysis of acylations of hydroxyl groups by acid anhydrides.[3]

Experimental

The gelatin used was Wilson Laboratories U-COP-CO, Special Non-Pyrogenic Gelatin, an acid extracted pigskin gelatin of isoionic point and isoelectric point of *p*H 9.2 (turbidity maximum, viscosity minimum and mixed-bed, ion-exchange resin).[4]

Acetylation with Acetic Acid, Acetic Anhydride and Perchloric Acid.—The gelatin was prepared for the reaction by freeze-drying and subsequent vacuum-drying of the porous solid for one day at room temperature. An ice-cold mixture of 150 ml. of glacial acetic acid, 8 g. of 60% perchloric acid and 35 ml. of acetic anhydride (all must be added together or the gelatin may become gummy) was added to 5 g. of gelatin in a standard Waring Blendor with stainless steel blades. The mixture was stirred for 5 minutes and then stood 20 minutes. After excess liquid was removed with a filter stick, the gelatin was washed with 50 ml. of acetic acid and five 100-ml. portions of acetone. During the last two washings the gelatin was shredded in the blendor. It was then transferred to a sintered glass funnel and washed with 200 ml. of ether. The ether was pumped off *in vacuo* and 150 ml. of water was added to the dry white powder. The remaining acidity was neutralized with a small amount of 2 *N* sodium hydroxide added dropwise. During neutralization most of the gelatin dissolved, and the remainder was dissolved by brief warming at 35°. To remove salts the solution was passed through a column 20 mm. in diameter, containing 40 ml. of Amberlite MB-3, mixed-bed, ion exchange resin. The flat portion of the *p*H *vs.* eluate volume curve indicated an isoionic point of 8.2.[4] In one run the product was freed of salts by dialysis against 2% sodium bicarbonate followed by distilled water. The salt free solutions were

(1) W. Sakami and G. Toennies, *J. Biol. Chem.*, **114**, 203 (1942).

(2) J. J. Katz, *Nature*, **174**, 509 (1954).

(3) E. J. Bourne, J. E. B. Randles, D. C. Tatlow and J. M. Tedder, *ibid.*, **168**, 942 (1951).

(4) J. W. Janus, A. W. Kenchington and A. G. Ward, *Research*, **4**, 247 (1951).

freeze-dried and stored cold. The yields were 4.0–4.1 g. (80%).

Acetylation with Acetic Anhydride in Trifluoroacetic Acid.—To a solution of 5 g. of freeze-dried, vacuum-dried gelatin in 70 ml. of trifluoroacetic acid cooled below 5° was added 6.7 ml. of acetic anhydride. The flask was stoppered and swirled to mix the contents and allowed to stand in an ice-bath for 20 minutes. The solution was poured into 1.5 l. of water cooled to 0°, the precipitate filtered, washed with three 150-ml. portions of ether and three 150-ml. portions of acetone. The gelatin was dried *in vacuo* for several hours, suspended in water and neutralized to *p*H 7 with 2 *N* sodium hydroxide. The aqueous solution was freed of salts by passage through a column of mixed-bed resin as above. The purified solution was freeze-dried and stored in the cold. The yield was 3.5 g. (70%).

Amino-acetylated Gelatin.—To a vigorously stirred solution of 68 g. of gelatin in 7 l. of water in a crock immersed in an ice-bath was added 100 ml. of acetic anhydride, dropwise during 30 minutes. A *p*H of 9.5–10.5 was maintained by dropwise addition of 2 *N* sodium hydroxide. The *p*H was measured with a glass electrode. At the end the *p*H was adjusted to 6, and the solution was dialyzed, concentrated and freeze-dried.

Analyses for Total Acetyl.—These were done by the method of Blackburn and Phillips[5] except that the gelatin hydrolysate was distilled at atmospheric pressure rather than at reduced pressure. Samples of 0.5 g. were used which contained about 0.7 mmole of acetyl. In some cases the gelatin was dissolved in water and treated with 2 g. of a strong-base, ion-exchange resin (Amberlite IRA 400) of 5.6 mmoles exchange capacity[6] to remove any free acetate that might have been present. After filtration, the gelatin content was determined by drying an aliquot at 105° overnight, and the remainder was analyzed in the usual way.

O-Acetyl Analyses.—These were done by the method of Blackburn and Phillips[5] except that the gelatins were dissolved in the 0.02 *N* sodium hydroxide whereas Blackburn and Phillips used insoluble collagen. Titrations were done electrometrically instead of with phenol red indicator.

Amino Nitrogen.—The Doherty and Ogg[7] modification of the Van Slyke method was used. These authors found an amino nitrogen content of 8.8 mg. N/g. of gelatin. We have found a value of 5.4 mg./g. in better agreement with the value of 5.7 mg./g. calculated from the amino acid content of gelatin.[8]

Viscosity.—Viscosities were measured in an Ostwald type viscometer at 40 ± 0.01°. Solutions were made up in a *p*H 5 acetate buffer containing 0.1 *M* sodium acetate and 0.15 *M* sodium chloride. Double plots of ln (η_{rel}/C) and $(\eta_{rel} - 1)/C$ against C were extrapolated to zero concentration to obtain intrinsic viscosity.

Infrared Spectra.—Infrared spectra were taken on gelatin films of 2–3 μ thickness prepared by evaporation of gelatin solutions at or slightly above room temperature. The spectra were recorded with a Perkin–Elmer Model 21 spectrophotometer.

Results and Discussion

Total acetyl contents of the products from both methods of acetylation were 6.0 ± 0.2% corresponding to 14.8 ± 0.5 acetyl groups per 10^4 g. of original gelatin. The hydroxyamino acid contents of typical gelatins have been reported as 14.2–15.6 residues per 10^4 g.[8,9] and may vary with the source of the gelatin. Acetylation of the hydroxyl groups was probably complete since the acetyl content reached a maximum which was not increased by the use of increased amounts of acetic anhydride.

That the acetyl actually measured was covalently bound and not free acetate which the purification procedure failed to remove was shown in several ways. Mixtures of original gelatin and the acids were neutralized and put through the dialysis and mixed-bed ion-exchange purification procedures; these showed no volatile acid. Secondly, already purified products were treated with sufficient strong-base, ion-exchange resin to combine with all the acetyl if present as acetic acid or acetate ion; analysis of the filtrates showed the same acetyl content as before, with one exception (run 2, Table I) in which a 6.4% acetyl value was reduced to 6.1% on this treatment. Control mixtures of gelatin and acid (sufficient to correspond to the acetyl of acetylated gelatin) were treated with the strong base resin and showed no volatile acid.

That the hydroxyl groups were acetylated was shown by infrared spectra.[10] Films of the products from both the perchloric and trifluoroacetic acid methods gave spectra (Fig. 1) having a new absorption band at 1730 cm.$^{-1}$ and of nearly the same intensity. This is in the region of absorption by ester carbonyl groups.[11] The band for liquid ethyl acetate is at 1738 cm.$^{-1}$, but those of other acetate esters may vary[11] by more than 10 cm.$^{-1}$. A film of non-acetylated gelatin containing glycine hydrochloride ethyl ester in an amount equivalent to the hydroxyl groups of gelatin showed a band at 1740 cm.$^{-1}$ and of nearly the same intensity as those of the acetylated gelatins. Amino-acetylated gelatin showed no new absorption bands. In addition to the band in the 1700 to 1800 cm.$^{-1}$ region, esters also have an absorption band in the 1200–1300 cm.$^{-1}$ region.[12] Gelatin also has an absorption band in this region with a maximum at 1235–1240 cm.$^{-1}$. The hydroxyl-acetylated gelatins showed increased intensity of absorption and a broadening of this absorption band. Gelatins having 0, 25, 75 and 100% of the theoretical amount of acetyl were prepared by the trifluoroacetic acid method by varying the amount of acetic anhydride used. The intensities of the 1730 and 1240 cm.$^{-1}$ bands varied almost directly with the acetyl content and can be used for a rough estimation of the latter. The film of original gelatin containing glycine hydrochloride ethyl ester also showed increased absorption and no new bands at around 1240 cm.$^{-1}$. There was no change in the 1240 cm.$^{-1}$ region of amino-acetylated gelatin.

The fact that acetylated gelatins prepared by the two methods had absorption peaks at the same wave length proves that in the trifluoroacetic acid method principally, if not entirely, acetate and not trifluoroacetate esters were formed. The ester carbonyl absorption band of ethyl trifluoroacetate lies 42 cm.$^{-1}$ higher than that of ethyl acetate.[11,13]

(5) S. Blackburn and H. Phillips, *Biochem. J.*, **38**, 171 (1949).

(6) R. Kunin and R. J. Myers, "Ion Exchange Resins," John Wiley and Sons, Inc., New York, N. Y., 1950, p. 11.

(7) D. G. Doherty and C. L. Ogg, *Ind. Eng. Chem., Anal. Ed.*, **15**, 751 (1943).

(8) H. S. Olcott in "Amino Acids and Proteins" (D. M. Greenberg, editor), C. C Thomas, Springfield, Ill., 1951, p. 105.

(9) H. C. Reitz, R. E. Ferrel, H. S. Olcott and H. Fraenkel-Conrat, THIS JOURNAL, **68**, 1024 (1946), and references cited therein.

(10) The spectra show a notch in the 1540 cm.$^{-1}$ band of O-acetylated gelatins. This has been observed in spectra of the eight preparations of O-acetylated gelatin examined. A less obvious notch has also been observed in some, but not all, films of original gelatin. The 1540 cm.$^{-1}$ band is a peptide and amide band of uncertain origin: L. J. Bellamy, "The Infrared Spectra of Complex Molecules," Methuen and Co., Ltd., London, 1954, p. 185, *et seq.* We do not know the cause of the appearance of the notch.

(11) H. M. Randall, R. G. Fowler, N. Fuson and J. R. Dangl, "Infrared Determination of Organic Structures," D. Van Nostrand Co., Inc., New York, N. Y., 1949.

(12) N. B. Colthup, *J. Optical Soc. Am.*, **40**, 397 (1950).

(13) N. Fuson, M. L. Josien, E. A. Jones and J. R. Lawson, *J. Chem. Phys.*, **20**, 1627 (1952).

A similar shift would be expected if trifluoroacetate esters had been introduced into gelatin. But no shift was found nor any new absorption band higher than 1730 cm.$^{-1}$. Also there was no indication of the 1182 cm.$^{-1}$ absorption due to the trifluoromethyl group.[13]

The 1730 cm.$^{-1}$ band of gelatin acetylated by the trifluoroacetic acid method (spectrum 4, Fig. 1) is more distinct than that of gelatin acetylated by the perchloric acid method (spectrum no. 3.) This does not imply more ester formation in the former as the ratio of the transmittance of the 1730 cm.$^{-1}$ band to that of the 1660 cm.$^{-1}$ band (amide carbonyl) is 3.2 for the former, 36 for the latter and 10 for non-acetylated gelatin. In addition, the 1240 cm.$^{-1}$ bands are of nearly identical intensities, and the analytical data (Table I) for the two methods are in agreement.

TABLE I

PROPERTIES OF ACETYLATED GELATINS

Run no.	% Acetyl	O-Acetyl, % of total acetyl	Amino N,[a] mg./g.	Intrinsic viscosity dl./g.	Isoionic point
O-Acetylated by perchloric acid method					
1[b]	5.8		5.2	0.29	
2[c]	6.4[d]	94[e]	5.4	.21	8.2[f]
3[c]	4.6	98			7.5[g]
O-Acetylated by trifluoroacetic acid method					
4[b,i]	6.1[h]			0.16	7.8[g]
5[c,j]	5.9[h]	100	5.0	.32	7.8[g]
6[c,i]	6.2[h]	97	4.9[k]	.18	8.7[f]
Amino acetylated					
7	1.8[l]	1.7	0.03	0.44	4.7[g]
Non-acetylated					
	0		5.4	.45	9.2

[a] Corrected for the weight of acetyl groups introduced, to facilitate comparison with original gelatin. [b] Freed of salts by dialysis. [c] Freed of salts by ion-exchange. [d] After resin pretreatment, 6.1%. [e] Based on total acetyl content of 6.1%. [f] By ion-exchange column method. [g] By batchwise ion-exchange method. [h] Unchanged after resin pretreatment. [i] 1.8 g. of acetic anhydride per g. of gelatin. [j] 1.4 g. of acetic anhydride per g. of gelatin. [k] Corresponds to 91% of original amino nitrogen. Titration of this material from *p*H 8.5 to *p*H 11 required 95% of alkali required by original gelatin. [l] Theory is 1.8% calculated from amino acid content of gelatin.[9]

The selectivity of the acetylation was shown by O-acetyl and Van Slyke amino nitrogen analyses and by isoionic *p*H data. In two preparations by the perchloric acid method the O-acetyl values accounted for 94 and 98% of the total acetyl. The method is based on the selective hydrolysis of ester groups by dilute sodium hydroxide.[14,15] Van Slyke amino nitrogen decreased 0 and 4% for two preparations (runs 1 and 2, Table I). The isoionic points obtained by treatment with a mixed-bed, ion-exchange resin were 7.5 to 8.2. The latter value was obtained by the column technique and the former by a batch technique. The column technique appears to give generally higher values. The isoionic point of the original gelatin was 9.2 and that of amino-acetylated gelatin was 4.7 (turbidity maximum and ion exchange). The small decrease of the isoionic point of the hydroxyl-acetylated material may be due to partial acetylation of amino groups or to some solvolysis of side-chain amide groups.

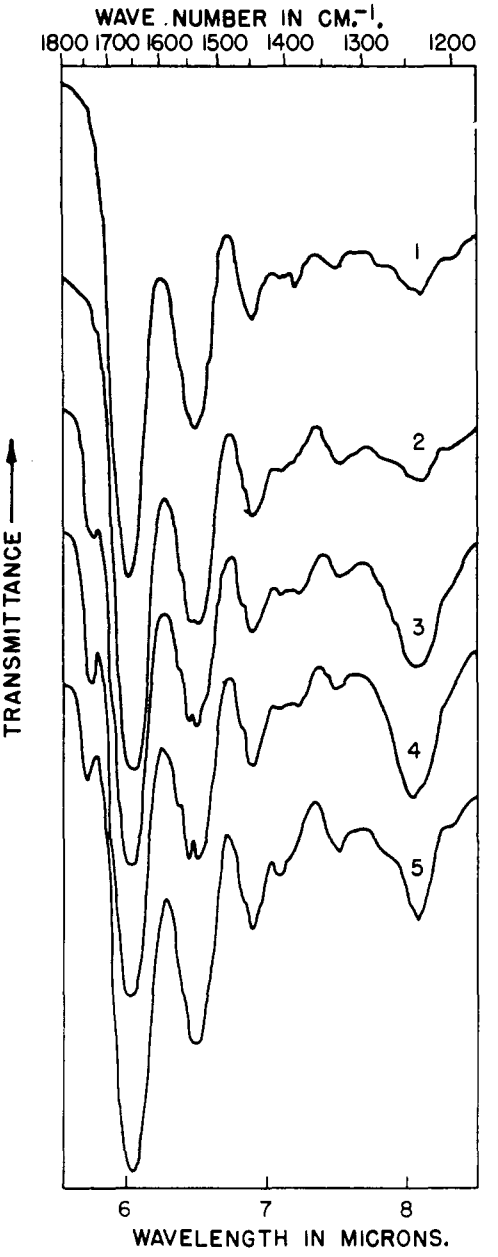

Fig. 1.—Infrared spectra of gelatin and acetylated gelatins: 1, original gelatin; 2, amino-acetylated gelatin; 3, O-acetylated by perchloric acid method; 4, O-acetylated by trifluoroacetic acid method; 5, original gelatin containing glycine hydrochloride ethyl ester. All curves are drawn to the same scale.

In two preparations acetylated by treatment with trifluoroacetic acid and acetic anhydride O-acetyl values accounted for 100 and 97% of the total acetyl (runs 5 and 6, Table I). The Van Slyke amino nitrogen of the two preparations decreased by 7 and 9%. The isoionic points were 7.5 to 8.7. The somewhat greater reduction of amino nitrogen in the trifluoroacetic acid method

(14) M. L. Wolfrom, M. Konigsberg and S. Soltzberg, THIS JOURNAL, **58**, 490 (1936).

(15) R. L. M. Synge, *Biochem. J.*, **33**, 1913 (1939).

may indicate less selectivity than in the perchloric acid method, but the O-acetyl analysis and isoionic points indicate at least equal selectivity.

The O-acetyl and Van Slyke nitrogen analyses permit the conclusion that, within experimental error of about 5%, acetylation by both methods has taken place selectively at the hydroxyl groups. Although no analyses for guanidino groups were made, it is assumed that they were not attacked, for theoretical reasons which apply as well to the amino groups, discussed below, and because of the correspondence between the total acetyl and the O-acetyl values.

Partial degradation appeared to take place in both acetylation procedures as indicated by the reduction of intrinsic viscosity (Table I). Attempts to reduce degradation by reducing the reaction time by 50% gave, in both procedures, products that were about 50–60% acetylated.

Acetylation with trifluoroacetic acid and acetic anhydride is the preferred procedure as it occurs in a homogeneous reaction mixture. The heterogeneous perchloric acid method gave, in some cases, products of low acetyl content, *e.g.*, run 3, Table I, probably owing to inability of the reagents to penetrate the gelatin thoroughly in the short reaction time employed. In addition, while the use of perchloric acid in acetic acid at 0° appears to be a safe procedure, explosion hazards may be involved if it is used in some other acids. The presence of acetic acid is necessary to swell the gelatin and carry the reagents into it. Since the function of the perchloric acid is to inactivate the amino groups through salt formation and to catalyze the reaction of the hydroxyl groups with the anhydride,[1] it may be possible to replace it with other strong acids known to catalyze esterifications.

Since trifluoroacetic acid is also a very strong acid, it will prevent acetylation of amino and guanidino groups by salt formation and catalyze the acetylation of the hydroxyl groups, possibly through the formation of a mixed trifluoroacetic–acetic anhydride.[3]

Since the trifluoroacetic acid method was superior, it was investigated in greater detail. It was found that trifluoroacetic acid alone does not cause a decrease of viscosity. The effect of increasing amounts of acetic anhydride on the viscosity and extent of acetylation was investigated, and the results are shown in Table I. Runs 4 and 6, in which a larger amount of acetic anhydride was used than in run 5, have lower viscosities than run 5. The lower viscosities of the acetylated gelatins probably represent lower molecular weights due to chain degradation. Whether the degradation is due to acetic anhydride itself, to the mixed anhydride or to acetyl carbonium formed by ionization of the mixed anhydride[3] is not clear. When gelatin swelled in acetic acid was treated with a quantity of mixed acetic–trifluoroacetic anhydride (prepared from trifluoroacetic anhydride and excess acetic acid) equivalent to that of run 5, Table I, there was only a 10% decrease of intrinsic viscosity, in spite of tripled reaction time and a 20° higher temperature. (The product was partially and non-selectively acetylated.) It seems therefore that the mixed anhydride alone does not cause degradation although it might do so in such a highly acidic medium as trifluoroacetic acid.

Acknowledgment.—This work was supported by the Office of the Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-298. We wish to thank Miss Helene Riese for technical assistance.

PASADENA, CALIFORNIA

[CONTRIBUTION FROM THE DEPARTMENTS OF PHYSIOLOGICAL CHEMISTRY AND OF MICROBIOLOGY, WAYNE UNIVERSITY COLLEGE OF MEDICINE]

The Metabolism of Squalene by Cell-free Yeast Extracts[1,2]

BY LAURENCE M. CORWIN,[3] L. J. SCHROEDER AND WILLARD G. MCCULLOUGH

RECEIVED AUGUST 31, 1955

A cell-free yeast extract capable of synthesizing sterols from acetate was developed. On addition of the cell-free extract to a medium in which 1-C^{14}-acetate was present, squalene was isolated and it was found to have a lower specific activity than the sterols, a result contrary to that obtained with whole yeast cells. C^{14}-Labeled squalene obtained from whole yeast cells, when incubated with the cell-free yeast extract, was mainly oxidized to the fatty acids with a lesser conversion to sterols. The reduction of 1-C^{14}-acetate conversion to the sterols by addition of non-radioactive squalene was not considered significant in the light of other data. It was concluded that in yeast, squalene is not an obligatory intermediate in the synthesis of sterols.

Introduction

The question of the role of squalene as a precursor of the sterols has been the subject of much recent experimentation. Langdon and Bloch[4] were able to isolate radioactive squalene from rats fed 1-C^{14}-acetate and carrier squalene. Upon feeding of this radioactive squalene to rats, radioactive cholesterol was isolated from the rat liver and little activity was demonstrated in the fatty acid fraction. They were also able to show that feeding of natural squalene to rats, for several days previous to giving radioac-

(1) Abstracted from a dissertation submitted by Laurence M. Corwin to Wayne University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1956.

(2) A preliminary report of this work was presented at the 128th National American Chemical Society Meeting at Minneapolis, September, 1955.

(3) United States Public Health Pre-doctoral Fellow; present address, Division of Biology, Cal. Inst. of Tech., Pasadena, Cal.

(4) R. G. Langdon and K. Bloch, *J. Biol. Chem.*, **200**, 129, 135 (1953).