TABLE II

BIOSYNTHESIS OF β-HYDROXYISOVALERIC ACID FROM 2-C^{14}-NaOAc AND 3′-C^{14}-HMG BY RAT LIVER

Each flask contained 5 ml. of enzyme. The additions were 1 mg. each of AMP, DPN and substrates. Following incubation for 3 hours at 37°, 30 mg. of HIV carrier was added to each flask. Gas phase was O_2. In each system aliquots of the same enzyme system were used.

System	Experiment	Radioactivity recovered, c.p.m./mg. C in HIV Precursors: 2-C^{14}-NaOAc (1.5×10^5 c.p.m./MgC)	3′-C^{14}-HMG (1.0×10^4 c.p.m./MgC)
Cell-free homogenate	1	1110	1830
	2	770	710
Particle-free extract	3	640	710
	4	890	700

HIV obtained biosynthetically from HMG this can be accounted for by the formation of some radioactive CO_2. The carboxylation reaction[12] would thus account for the possible formation of carboxyl labeled AcAcOH.

TABLE III

DISTRIBUTION OF C^{14} IN BIOSYNTHETIC HIV[a] OBTAINED FROM 2-C^{14}-NaOAc AND 3′-C^{14}-HMG

β-Hydroxyisovaleric acid	Radioactivity recovered as $BaCO_3$, c.p.m./mg. C: HIV from 3′-C^{14}-HMG precursor	HIV from 2-C^{14}-HOAc precursor
COOH	50- 60 (3%)	110–130 (5%)
CH_2	190–230 (14%)	690–760 (30%)
HCOH	100–120 (7%)	80–100 (4%)
CH_3 CH_3	560–600 (75%)	780–840 (61%)
Total oxidation found	300–320	510–530
Total oxidation calcd.	240	590

[a] Pooled materials obtained from cell-free homogenates and particle-free systems.

Experimental

β-Hydroxyisovaleric acid[13–15] was prepared by a modification of Saizew's[13] method; dimethylallylcarbinol was ozonized in ethyl acetate. The index of refraction of HIV obtained by each synthetic method as well as the biosynthetic HIV was identical, $n^{20}D$ 1.435.

The preparation of tissue slices, cell-free homogenates and aqueous extracts of rat liver has been described earlier.[9–11] The only modification involved the preparation of the aqueous extract of the mitochondrial fraction. This fraction was treated with 3 volumes of H_2O and rehomogenized for 30 seconds instead of the previous method which involved intermittent shaking for 45 minutes. The buffers utilized, co-factors and methods of separation have all been described.[4–8] The purity of the isolated HIV was demonstrated by the constant activity of the original preparation as compared to the activity shown by the Ag and Cu salts[13] as well as the eluate obtained after paper chromatography.[12] (None of these preparations absorbed bromine; therefore, contamination of HIV with SA was considered minimal.) Incubation of inactive enzyme preparations with C^{14}-labeled substrates yielded no radioactive HIV.

The degradation of HIV was accomplished by the utilization of several standard methods. The Schmidt reaction yielded the carboxyl carbon as CO_2. Dehydration of HIV to SA was accomplished with H_2SO_4[15]; the product was then sublimed and subsequently ozonized in ethyl acetate, yielding acetone and oxalic acid. The acetone fragments obtained from this ozonization was isolated with Denige's reagent. The resulting Hg–acetone complex was dissolved in acid and the acetone distilled; an aliquot of the distillate was treated with NaOI; the resulting iodoform was isolated, recrystallized and oxidized to CO_2. The remaining NaOAc fragment (derived from the acetone) was isolated and an aliquot of this material was oxidized to CO_2; another aliquot of this acetate was converted into the Ba salt and pyrolyzed to yield the carboxyl carbon of acetate as $BaCO_3$. This carbon was derived from the carbonyl group of the acetone. The oxalic acid was converted to its Ag salt and directly oxidized to CO_2. (The only carbon not directly isolated was carbon 2. This value was obtained from the difference in specific activities between the carboxyl carbon of HIV and the oxalic acid carbons.)

Cholesterol was isolated as the digitonide and plated as such. Most of the other materials were oxidized to CO_2 and radioassayed as $BaCO_3$ on a flow counter and corrected to infinite thinness. Variations in the activity of different derivatives of the same sample were within statistical counting error.

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RADIOISOTOPE UNIT, VETERANS ADMINISTRATION HOSPITAL
PHILADELPHIA, PENNSYLVANIA, AND
DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY
SCHOOL OF MEDICINE
UNIVERSITY OF PENNSYLVANIA

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Crystallized N,N′-Diacetylchitobiose[1]

BY F. ZILLIKEN, G. A. BRAUN, C. S. ROSE AND P. GYÖRGY

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Among the partial breakdown products of chitin, N,N′-diacetylchitobiose was described for the first time in 1931.[2] It was obtained as an amorphous product having a melting point above 185° from decalcified chitin by acetolysis[2] followed by saponification of the crystalline hexa-*O*-acetyl-di-N-acetylchitobiose with KOH. The known structure of chitin[3,4] leads one to expect an N,N′-diacetylchitobiose to be a 2-acetamido-2-deoxy-4-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-glucose.

Upon saponification of hexa-*O*-acetyl-di-N-acetylchitobiose, m.p. 290–291° and $[\alpha]^{26}D$ 55.3°, with dry methanolic ammonia, an amorphous product was obtained. It was demonstrated by means of paper chromatography to consist of five different components.

After chlorination of the paper, followed by spraying with an alcoholic solution of benzidine dihydrochloride[5] there were in addition to N,N′-diacetylchitobiose (IV, R_{lac} = 1.49) one faster (V, R_{lac} = 2.38) and 3 slower moving spots (I, R_{lac} = 0.51; II, R_{lac} = 0.66; III, R_{lac} = 0.96) visible, indicating that all five components possess the –NH–CO– group. The fastest moving spot V was identified as acetamide. Upon spraying with aniline oxalate I, II, III and IV gave a positive test, all four spots were negative to ninhydrin.

Chromatography of this amorphous mixture on a charcoal/Celite column[6] using aqueous ethanol of

(1) Supported by a grant from the Wyeth Laboratories, Division of the American Home Products Corporation.
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increasing ethanol concentration as eluent, resulted in the isolation of a chromatographically homogeneous N,N′-diacetylchitobiose which crystallized from aqueous methanol in long, slender needles.

Crystallized N,N′-diacetylchitobiose melts at 245–247° with decomposition. The crystals exhibit mutarotation in aqueous solution: $[\alpha]^{25}D$ extrapolated to zero time was +39.5° (*c* 1.0, H_2O). The equilibrium rotation, reached after 60 minutes was $[\alpha]^{25}D$ +18.5° (*c* 1; H_2O). Therefore the crystalline N,N′-diacetylchitobiose is presumably the α-isomer. The crystals take up water readily thereby losing their crystalline structure. They are only slightly soluble in dry methanol. One mole of NaOI was consumed per mole of disaccharide.[7] The Morgan–Elson reaction[8] was negative with and without prior treatment with Na_2CO_3. This is consistent with a recent observation by Kuhn, *et al.*,[9] for 4-substituted N-acetyl-D-glucosamines. One mg. of the crystallized disaccharide produced a color equivalent to only 30 γ of free N-acetyl-D-glucosamine. This is due to a slight formation of N-acetyl-D-glucosamine by the action of Na_2CO_3.

Crystallized N-N′-diacetylchitobiose is hydrolyzed into two moles of N-acetyl-D-glucosamine by a crude enzyme preparation from *Lactobacillus bifidus* var. *Penn*,[10] which is known to split alkyl-N-acetyl-β-D-glucosaminides with liberation of N-acetyl-D-glucosamine.[11] The same enzyme does not attack the corresponding alkyl-N-acetyl-α-D-glucosaminides.[11] The growth promoting activity for *Lactobacillus bifidus* var. *Penn* when autoclaved with the medium was 800 γ per unit. When added under sterile conditions one growth unit was contained in 2.2 mg.

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Experimental

Hexa-*O*-acetyl-di-N-acetylchitobiose.—Hexa-*O*-acetyl-di-N-acetylchitobiose was obtained from decalcified lobster chitin according to Bergmann, *et al.*,[2] in long colorless needles. Upon three recrystallizations the melting point and optical rotation remained unchanged; m.p. 290–291° dec., $[\alpha]^{30}D$ +55.3° (*c* = 1, CH_3COOH).

Anal. Calcd. for $C_{28}H_{40}O_{17}N_2$ (676.3): C, 49.68; H, 5.96; N, 4.14. Found: C, 49.68; H, 6.03; N, 4.16.

N,N′-Diacetylchitobiose.—2.3 g. of hexa-*O*-acetyl-di-N-acetylchitobiose was dissolved in 50 ml. of dry methanol, cooled to 0° and 50 ml. of methanol saturated with ammonia at 0° was added immediately. The solution was kept for 24 hours at room temperature and then evaporated to dryness below 50° *in vacuo*. Dry methanol was added and the material again evaporated to dryness. The amorphous residue was reprecipitated from dry methanol.

1.3 g. of the amorphous product was dissolved in 20 ml. of water and chromatogrammed on a charcoal/Celite column, prepared from 80 g. of Norite A and 80 g. of Celite.[12] The flow rate of the column was 1.5–2 ml. per minute. After washing the column with 2 l. of water, 2.5 l. of 2.5% and 3 l. of 5% ethanol, a chromatographically homogeneous N,N′-diacetylchitobiose (R_{lac} = 1.49) was eluted in the first three 500-ml. fractions of 7.5% ethanol. The three 7.5% ethanolic fractions were combined and evaporated to dryness *in vacuo*. The residue was dissolved in 30 ml. of dry methanol, filtered and again evaporated. During this operation very tiny needles were visible. For a final recrystallization the needles were treated with 10 ml. of hot dry methanol, then water added dropwise until they dissolved. The solution was kept for crystallization at room temperature, 230 mg. of slender needles were obtained having a melting point at 245–247° with decomposition. (The crystals become brown at 237–239°); $[\alpha]^{25}D$ (extrapolated to zero time) +39.5 (*c* 1, H_2O). The equilibrium rotation reached after 60 minutes was $[\alpha]^{25}D$ +18.5° (*c* 1, H_2O).

Anal. Calcd. for $C_{16}H_{28}O_{11}N_2$ (424.2): C, 45.26; H, 6.65; N, 6.60. Found: C, 44.93; H, 6.72; N, 6.44.

Paper chromatography was carried out on Whatman filter paper No. 1. The solvent used was the upper layer of an ethyl acetate:pyridine:H_2O = 2:1:2 mixture.

(12) Johns-Manville No. 535.

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY
AND PEDIATRICS
SCHOOL OF MEDICINE
UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA, PA.

[CONTRIBUTION FROM THE CHEMISTRY DIVISION, ARGONNE NATIONAL LABORATORY]

Hydrogen Isotope Effects in the Alkaline Cleavage of Triorganosilanes[1]

BY LOUIS KAPLAN AND K. E. WILZBACH

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The effects of isotopic substitution on the rates of hydrolysis of triphenyl- and tripropylsilane in piperidine–water and ethanol-water solvents have been studied in order to acquire information concerning the reaction mechanisms. Substitution of deuterium or tritium for protium in either the silane or the solvent in every case reduced the reaction rate. The results indicate that the rate-determining step in the reaction involves the rupture of the silicon–hydrogen bond and that in the transition state the hydrogen atom from the silane is strongly bonded to that from the solvent.

The alkaline cleavage of the silicon–hydrogen bond in trialkyl- or triarylsilanes has been the subject of several recent investigations. The reactions can be represented stoichiometrically by the equation

$$R_3SiH + OH^- + HS \longrightarrow R_3SiOH + H_2 + S^-$$

where HS represents a molecule of the solvent. Price[2] found that the evolution of hydrogen from trialkylsilanes dissolved in alcoholic potassium hydroxide was essentially quantitative, and that the reaction was first order with respect to silane, hydroxide and, probably, solvent. Gilman and Dunn[3] showed that the closely related hydrolysis of triarylsilanes in piperidine–water was a pseudo first-order reaction which had a positive value of ρ in the Hammett equation. These results, together with others,[4] indicate that the reactions of silanes with

(1) Presented at the Los Angeles Meeting of the American Chemical Society, March 15–19, 1953.
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