pared to amylose. The hydrolysis of amylopectin and amylose is compared in Fig. 1, which graphically shows that the hydrolysis rate for the amylopectin decreased abruptly to a definitely lower value as the level of 60% conversion was approached, a striking analogy to the well known behavior of β-amylase on this substrate. These results may be taken as further evidence that the amylo-glucosidase does not operate at random but, rather, by a terminalwise attack on the non-reducing end of starch molecules, and that the rate of hydrolysis for anomalous linkages, at points of branching, is very much less than the rate for normal glucoside linkages.

It appears quite possible in light of these experiments, that the limit-dextrinase activity noted in early work for some relatively crude enzyme preparations may have been due in part at least to the combined action of an amylo-glucosidase and an α-amylase.

The very large and no doubt slightly branched amyloses, such as tapioca A-fraction, gave hydrolysis rates intermediate between that of corn amylose and amylopectin. Using *equimolar* concentrations, the rate of hydrolysis for these larger amylose molecules, as mg. of glucose produced per unit time, was initially greater than the rate for corn amylose, but decreased to values approaching that of the corn samples as hydrolysis progressed. These experiments will be detailed in a future communication.[22]

It is possible that the final amylo-glucosidase preparation, which was used to obtain the data in Tables VI and VII and for Fig. 1, still contained a trace of α-amylase activity. Even a trace of α-amylase would modify the results and may account for the slight drop in peak light absorption values during the corn A-fraction hydrolysis as well as a part, at least, of the secondary phase of the amylopectin hydrolysis. However, it is believed that the data given in Tables VI and VII, when considered together with the data in preceding tables, showing the manner in which the kinetics of the hydrolysis tend to change as the enzyme preparation was progressively freed of α-amylase activity and the residual activity became more exclusively that of the amylo-glucosidase factor, clearly indicate the general pattern of the action of this latter enzyme and distinguish it from other carbohydrases.

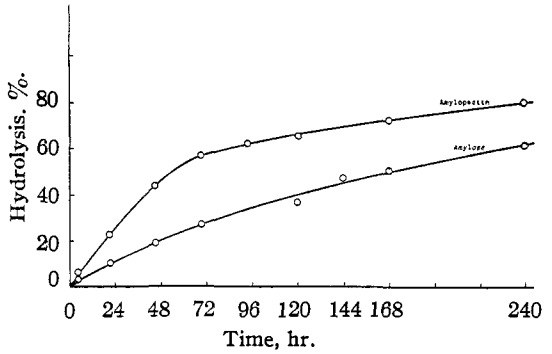

Fig. 1.—Hydrolyses of amylose and amylopectin at equal weight concentration by amylo-glucosidase.

(22) R. W. Kerr and F. C. Cleveland, manuscript in preparation.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY AND NUTRITION, TEXAS AGRICULTURAL EXPERIMENT STATION TEXAS AGRICULTURAL AND MECHANICAL COLLEGE SYSTEM]

The Synthesis of C^{14}-Labeled Glycerol[1,2]

BY HERMANN SCHLENK AND BERNICE WALLACE DEHAAS

Glycerol-1,3-C_2^{14} has been prepared in over-all yield of 19%, by condensing nitromethane with paraformaldehyde-C^{14}, followed by reduction and diazotization.

Glycerol, labeled with carbon-14, was needed for projected studies in this Laboratory on the metabolism of glycerides. Although there are several synthetic routes[3] which might have been employed, the scheme shown in Fig. 1 was chosen because of the availability of both nitromethane-C^{14} [4] and formaldehyde-C^{14}.[5]

Experimental

Sodium-2-nitro-1,3-propanediol (I).—According to the procedure of Schmidt and Wilkendorf,[6] 13.47 g. of paraformaldehyde-C^{14} (0.449 mole)[7] was allowed to react with 8.54 g. of nitromethane (0.14 mole) in 110 ml. of absolute methanol by the addition of 8–10 drops of 50% KOH. When Eastman Kodak Co. paraformaldehyde was used, the reaction began immediately upon heating on a steam-cone, and with mechanical stirring, the solution became clear within ten minutes. When the reaction was performed with the isotopic paraformaldehyde, there were still some unreacted particles after 45 minutes heating. After decanting from the insoluble particles the mixture was cooled to −5°. A solution containing 4.02 g. of sodium dissolved in 65 ml. of absolute methanol was added during a period of 20 minutes with mechanical stirring. After standing for six hours at −5°, the precipitate was filtered off. The yield of the sodium salt of 2-nitro-1,3-propanediol, containing 2 molecules of methanol of crystallization, was 27 g. or 58%. The filtrate was neutralized with 7.8 *N* HCl in methanol, mixed with the unreacted paraformaldehyde and the whole procedure was repeated by adding 3.05 g. (0.05 mole) of nitromethane. The yield in this recycling was 8 g. A second recycling yielded 1 g. The total yield was 36 g. or 77% based on the paraformaldehyde.

2-Amino-1,3-propanediol Hydrochloride (II).—Hydrogenation of I was carried out in two batches of about 18 g. each, using an apparatus similar to the Parr apparatus. Each batch was dissolved as completely as possible in 225 ml. of absolute methanol, and 2.1 equivalents of glacial acetic acid was added, followed by 4 g. of Raney nickel. The pressure was kept between 37 and 20 lb./sq. in. Hy-

(1) This investigation was supported in part by a grant from the United States Atomic Energy Commission.

(2) Presented at the 118th Meeting of the American Chemical Society, Chicago, Illinois, September, 1950.

(3) Since the writing of this manuscript the preparation of glycerol-1-C^{14} was reported with a yield in radioactivity of 12.3%: A. P. Doerschuk, THIS JOURNAL, **73**, 821 (1951).

(4) L. G. Sowden, *J. Biol. Chem.*, **180**, 56 (1949).

(5) A. R. Jones and W. J. Skraba, *Science*, **110**, 332 (1949).

(6) E. Schmidt and R. Wilkendorf, *Ber.*, **52**, 395 (1919).

(7) Isotopes Division, U. S. Atomic Energy Commission, Oak Ridge, Tenn. The material was lumpy and sticky, whereas Eastman paraformaldehyde is powdery. Its activity was specified as approximately 5 microcuries per millimole.

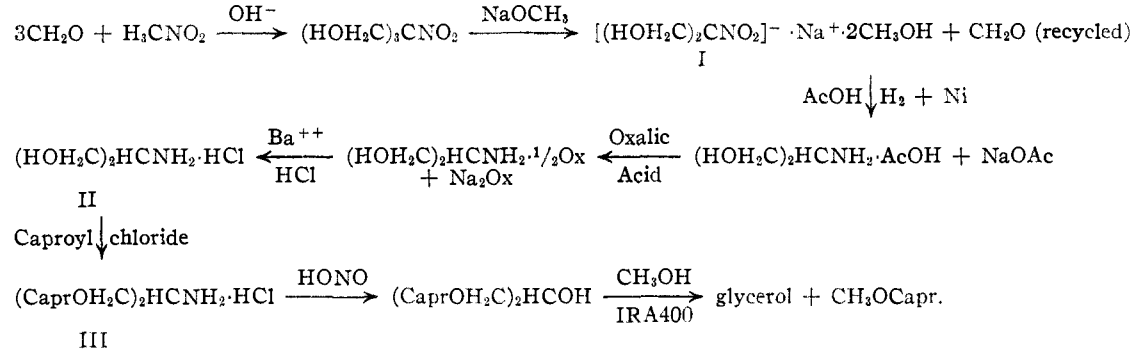

Fig. 1.

drogenation at room temperature under these conditions required about six hours. The amine and sodium salt were precipitated as oxalates from the filtered solution by addition of a 10% molar excess of anhydrous oxalic acid dissolved in a minimum of absolute ether. The mixture of oxalates was filtered after standing overnight at −20°. The total product of both batches was 34 g. This was dissolved in 315 ml. of water and sufficient $BaCl_2$ solution was added to react with approximately 50% of the oxalate. $Ba(OH)_2$ solution was then added until the *p*H was above 11.5. The barium oxalate was removed by filtration and the excess of barium ions was removed in the form of $BaCO_3$. The solution was then acidified with hydrochloric acid to a *p*H of 1.9. The water was removed in a freeze drying apparatus and the residue, a slightly yellow mixture of 2-amino-1,3-propanediol hydrochloride and sodium chloride, was dried over KOH and P_2O_5. This was extracted with a total of 120 ml. of hot absolute ethanol and filtered to separate the soluble amino-salt from sodium chloride. This separation should be performed in as dry an atmosphere as practicable. The salt was crystallized at 5°, filtered, and a second crop was obtained by adding 55 ml. of absolute ether in small amounts to avoid separation of oil. The total yield of II was 13.17 g. or 59% from I. The crude radioactive product described had a melting point of 93–97° after drying and was pure enough for the subsequent reaction.

The salt is extremely hygroscopic and may be recrystallized from absolute ethanol. The melting point of a "cold" preparation dried *in vacuo* over P_2O_5 at 70° was 101–103° (uncor., evacuated melting point tube).

Anal. Calcd. for $C_3H_{10}O_2HCl$: N, 10.98; Cl, 27.79. Found: N, 10.70; Cl, 27.75.

2-Amino-1,3-dicaproxypropane Hydrochloride (III).—The dried compound II was allowed to react with 1.95 mole equivalents of caproyl chloride at 90°. As hydrogen chloride evolution ceased, the mixture became solid. A small amount of unreacted caproyl chloride was removed over KOH in a vacuum desiccator. The ester was recrystallized from absolute dioxane after separation from traces of unreacted II which is insoluble in this solvent. The mother liquor from the first crop of III was concentrated and crystallization was completed by adding absolute ether and by cooling. This was repeated twice, to give a total yield of 27.4 g. of III or 81.8% based on II. The melting points of the crude fractions were between 113 and 118° (uncor.). A corresponding product of a "cold" run was recrystallized to a constant m.p. of 125–127°.

Anal. Calcd. for $C_{15}H_{30}O_4NCl$: N, 4.33; Cl, 10.95. Found: N, 4.25; Cl, 10.75.

Diazotization.—The solution of III (0.0847 mole) in 138 ml. of 50% acetic acid was cooled in an ice-bath, and 11.66 g. (0.169 mole) of $NaNO_2$ in concentrated aqueous solution was added slowly. By holding the temperature below 8°, only traces of nitrous oxides were evolved. The reaction was nearly complete within ten minutes. The resulting yellow oily upper layer was then separated. An additional 5.83 g. of $NaNO_2$ was added to the aqueous layer. After standing for 1.5 hours the mixture was extracted with ether. Ether was also added to the oil and the small amounts of nitrous oxides present were destroyed by washing with a 10% solution of sulfamic acid. The ether extract of the diazotization mixture contained large amounts of nitrous oxides which were removed in the same manner. The combined ether solutions were dried over sodium sulfate and the ether and most of the caproic acid were evaporated under vacuum. The weight of the crude dicaproin was 24.89 g.

Interesterification.—The crude dicaproin was dissolved in 300 ml. of methanol and 60 g. of activated Amberlite IR-A-400 was added, changing the *p*H from 3.5 to 5.9. The odor of methyl caproate was immediately noticeable. After shaking for 22 hours, the resin was filtered and extracted with 100 ml. of methanol. Colloidal particles of the Amberlite were absorbed from the combined methanol solutions on a charcoal–Hyflo mixture. The solvent and most of the methyl caproate were removed under vacuum at room temperature. Caproic acid, methyl caproate and water were removed in a vacuum desiccator over KOH and P_2O_5, yielding 4.82 g. of crude glycerol.

Glycerol.—The yellow viscous crude product was distilled at 3 mm. at 140–155° bath temperature. The amount of distilled material was 3.95 g. or 50.6% based on III, or 19.1% based on formaldehyde. The product was slightly yellow, was free of chlorine and nitrogen and showed no reduction with Fehling solution.

In a "cold" run the acid value of similarly prepared glycerol was negligible and the saponification value was 11.0. This value is equivalent to the presence of 3.8% monocaproin. For further characterization of the synthetic glycerol, "cold" preparations were treated with palmitic acid to form tripalmitin, which corresponded in yield and properties to the compound prepared from authentic glycerol.

Radioactive Assay.—Radioactive paraformaldehyde used for the synthesis was converted to the bis-methone derivative, which had a specific activity of 3.50×10^4 counts/minute/milligram. The same compound formed from glycerol after oxidation with periodic acid had a specific activity of 3.44×10^4 counts/minute/milligram.

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