tone, alkyl halide and a hydrogen halide.

Ketals react with acid anhydrides to yield mainly an ester, acid and vinyl ether.

The reaction of a ketal with *t*-butyl alcohol has been investigated and found to result in an unexpected product, methyl *t*-butyl ether. These studies are being continued.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY AND THE RESEARCH LABORATORY OF THE ATLAS POWDER COMPANY]

Crystalline Lactositol

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Glycosides of many different types of alcohols and phenols have long been known, but to our knowledge no glycoside of a sugar alcohol containing more than three carbon atoms has so far been obtained by coupling reactions. The reduction of a disaccharide offers a method for obtaining such derivatives and crystalline cellobiositol has been reported by Levene and Kuna.[1]

Various attempts to reduce lactose to the alcohol stage have been reported in the literature. Bourchadat,[2] using sodium amalgam, made the first attempt to reduce lactose. He isolated dulcitol, isopropyl alcohol and hexyl alcohol. Neuberg and Marx,[3] using calcium amalgam, reported a crystalline reduction product of lactose which they called lactobiotol. They reported that it did not melt even at 280°. No rotation was recorded.

An aqueous solution of lactose was subjected to hydrogen at seventy-four atmospheres pressure at 130° in the presence of a nickel catalyst by Ipatieff.[4] Under these conditions, dulcitol and a sirup were obtained. Karrer and Büchi[5] hydrogenated lactose with a Raney nickel catalyst under thirty atmospheres pressure and a temperature of 70°, to obtain an amorphous, hygroscopic solid exhibiting a specific rotation of +14.8° in water.[5a]

In 1920 Senderens[6] repeated the work of Ipatieff and decreased the amount of dulcitol formed. Senderens reported a crystalline monohydrate of lactositol and recorded the constants: m. p. 78°; spec. rot. +12.2° (water).

In the work herein reported, lactose was reduced with hydrogen and nickel at high pressure under acid conditions in the Research Laboratory of the Atlas Powder Company and the resultant sirup was obtained crystalline in the Chemical Laboratory of The Ohio State University. Our crystalline lactositol showed the constants: m. p. 146°; spec. rot. (23°) +14° in water. The crystalline material was anhydrous and a crystalline hydrate was not obtained. Sorbitol and galactose were identified as its hydrolytic products. A crystalline tritrityl lactositol hexaacetate was obtained. This crystalline sugar derivative is of interest because of its high molecular weight of 1323.

Our main interest in lactositol consisted in having in hand a crystalline glycoside of a sugar alcohol (4-*d*-sorbitol-β-*d*-galactopyranoside). Considerable data have been accumulated pertaining to the hydrolysis of glycosides. It is known that the nature of the aglucone portion can exert a considerable influence on the rate of glycosidic hydrolysis. It was then of interest to determine the rate of hydrolysis of a glycoside wherein the aglucone portion was a hexitol and accordingly the rate of hydrolysis of lactositol (0.05 molar) in 1 *N* hydrochloric acid was determined at three temperatures. The reactions followed the monomolecular law and from the determined velocity constants, the energy of activation for the hydrolysis was calculated. Moelwyn-Hughes[7] considers that the energy of activation is more significant for comparative purposes than the velocity constants.

The data obtained are tabulated in Table I and are therein compared with those for substances of related structure. It is seen that the velocity of hydrolysis of lactositol is of the same order of magnitude as that of the common py-

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[1] P. A. Levene and M. Kuna, *Science*, **85**, 550 (1937).

[2] G. Bourchadat, *Bull. soc. chim.*, **16**, 38 (1871).

[3] C. Neuberg and F. Marx, *Biochem. Z.*, **3**, 543 (1907).

[4] V. Ipatieff, *Ber.*, **45**, 3218 (1912).

[5] P. Karrer and J. Büchi, *Helv. Chim. Acta*, **20**, 86 (1937).

[5a] All specific rotations are herein recorded to the D-line of sodium light.

[6] J. B. Senderens, *Compt. rend.*, **170**, 47 (1920).

[7] E. A. Moelwyn-Hughes, *Trans. Faraday Soc.*, **24**, 309 (1928); *ibid.*, **25**, 81, 503 (1929).

ranosides. Lactositol is hydrolyzed more slowly at 60° and more rapidly at 70° than lactose. The two rates become equal at 69.5°. The energy of activation for the hydrolysis of lactositol is much closer to that of α-methylgalactoside than to lactose.

Table I

Rates of Hydrolysis and Energies of Activation of Lactositol and Related Glycosides

Substance	$k \times 10^3$ 60°	70°	Energy of activation, cal.
Lactositol	0.873[a]	4.00[a]	33,600
Lactose[b]	1.19	3.89	26,900
α-Methyl-*d*-galactoside[c]	0.430	2.01	35,000
Maltose[b]	.978	3.83	31,000
α-Methyl-*d*-glucoside[b]	.079	0.425	38,200
β-Methyl-*d*-glucoside[b]	.205	0.905	33,700

[a] Monomolecular *k*, minutes and decimal logarithms. [b] Data of Moelwyn-Hughes.[7] [c] Data of Morell and Link.[11]

Experimental

Lactositol.—Fifty grams of supported nickel catalyst was reduced for one hour in a stream (100 cc. per minute) of hydrogen at 450° and allowed to cool for twenty minutes in a stream of hydrogen. A lactose monohydrate solution having 400 g. of lactose monohydrate, 600 g. of water and a *p*H of 3.4 was prepared. This solution was put into a 3-liter steel shaking autoclave (American Instrument Company) and the reduced catalyst introduced into the solution. The *p*H of the mixture then became 6.3.

The autoclave was closed and hydrogen admitted to a pressure of 1500 lb. per sq. in. (102 atm.). The initial temperature of the solution was 30°. Shaking and heating were started. The maximum pressure of 2025 lb. per sq. in. (138 atm.) was reached after one hour, at which time the temperature was 143°. At the end of sixty-five minutes the temperature was 150° and a temperature of 146–150° was maintained for a further three and one-half hours. The pressure at this time was 1875 lb. per sq. in. (128 atm.) and represented a total pressure drop of 150 lb. per sq. in. (10 atm.). The autoclave was cooled, the pressure released and the autoclave was opened. The *p*H of the unfiltered, reduced solution was 5.0. The reduced solution was filtered and the colorless filtrate was concentrated under reduced pressure to an 80% sirup. Sugar analysis of the filtrate showed that 99.7% of the lactose had been reduced.[8]

The concentrate received at Columbus was diluted to a 25% total solid content, and, after the addition of a small amount of lead acetate, the solution was heated short of boiling and treated with hydrogen sulfide until precipitation was complete. Decolorizing charcoal was then added and the mixture was boiled for an hour and filtered. The filtrate was allowed to stand for several days and again filtered from separated colloidal sulfide, whereupon it was concentrated under reduced pressure to a thick sirup.

The lactositol sirup was extracted repeatedly with boiling absolute ethanol and a very hygroscopic, amorphous solid resulted. After standing in absolute ethanol for about a month, the material crystallized; yield approximately 80%. It was recrystallized by dissolving in a small amount of water and adding about an equal volume of ethanol. On standing overnight at ice-box temperature, the substance separated in the form of small, hard, tetrahedral-like crystals; m. p. 146°; spec. rot. (23°) + 14° (*c*, 4; H_2O). The crystalline material reduced Fehling's solution only after acid hydrolysis. It was soluble in water and pyridine but was practically insoluble in methanol and ethanol.

Lactositol crystallized in the anhydrous form and a hydrate was not obtained. A sample of lactositol showed a negligible loss in weight on heating under reduced pressure for fifty-four hours over phosphorus pentoxide at the temperature of boiling xylene (140°). Five aqueous solutions containing various concentrations of lactositol were allowed to crystallize spontaneously at different times. The resulting products were allowed to dry under atmospheric conditions at room temperature. In all cases, the crystals melted within the range 144–146°.

Anal. Calcd. for $C_{12}H_{24}O_{11}$: C, 41.84; H, 7.03. Found: C, 41.55; H, 7.12.

Tritrityl-lactositol Hexaacetate.—Two grams (1 mol) of lactositol, 4.9 g. (3 mols) of triphenylmethyl chloride and 30 cc. of dry pyridine were heated at 90° for sixteen hours. The solution was then cooled to 0°, 15 cc. of acetic anhydride was added slowly and the solution kept overnight at room temperature. At the end of this period, the solution was poured into ice and water. The sirupy product that separated hardened upon standing and was washed with dilute acetic acid and then with water.

The dried, crude product was obtained crystalline from xylene; yield 3.1 g.; m. p. 248–250°; spec. rot. (27°) −40.7° (*c*, 2; $CHCl_3$). Further recrystallization from xylene altered these constants but slightly; m. p. 249–251°; spec. rot. (27°) −40.1° (*c*, 1; $CHCl_3$). The substance was quite soluble in chloroform, pyridine and hot xylene; it was only slightly soluble in hot alcohol and was practically insoluble in petroleum ether. The compound did not reduce Fehling's solution until after acid hydrolysis. An amount of 20 mg. of the substance was dissolved in hot 80% acetic acid and the solution heated for five minutes. The trityl carbinol that separated on cooling was removed by filtration and the filtrate was treated with hydrochloric acid and boiled. The neutralized solution then reduced Fehling's solution.

Anal. Calcd. for $C_{12}H_{15}O_{11}(C(C_6H_5)_3)_3(COCH_3)_6$: $C(C_6H_5)_3$, 55.17; CH_3CO, 4.54 cc. 0.1 *N* NaOH per 100 mg. Found: $C(C_6H_5)_3$, 54.20; CH_3CO, 4.52 cc.

Hydrolysis of Lactositol (Qualitative).—A solution of 2 g. of crystalline lactositol in 40 cc. of 5% hydrochloric acid was heated at 90° for two hours. After solvent removal under reduced pressure, the resultant sirup was treated with benzaldehyde and concentrated hydrochloric acid according to the procedure of Karrer and Büchi.[6]

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(8) The reduction of the lactose was carried out in the Research Laboratory of the Atlas Powder Company by Dr. Robert S. Rose, Jr., under the direction of Mr. K. R. Brown. The concentrate was then forwarded to the Chemical Laboratory of The Ohio State University.

The purified reaction product was characterized by melting point (191–192°) and mixed melting point as tribenzylidene-sorbitol, for which Karrer and Büchi record the melting point of 190–191°.

Anal. Calcd. for $C_6H_8O_6(C_6H_5CH)_3$: C_6H_5CH, 60.5. Found: C_6H_5CH, 58.2.

Another portion of lactositol (1.5 g.) was hydrolyzed under the above-described conditions and the resultant sirup was dissolved in 2.5 cc. of concentrated hydrochloric acid (*d*, 1.19) and shaken with 1 cc. of ethyl mercaptan. A crystalline product began to separate after fifteen minutes and crystallization was completed in an ice–salt bath. The filtered product was washed with cold water and recrystallized twice from ethanol and twice from water. The product was identified by melting point (141–142°) and mixed melting point as galactose diethyl mercaptal, for which Fischer[9] records the melting point of 140–142°.

Hydrolysis of Lactositol (Quantitative).—The hydrolysis of lactositol to *d*-sorbitol and *d*-galactose was followed at three temperatures by determining the amount of galactose present by hypoiodite oxidation. The data are tabulated in Table II. The lactositol solutions were 0.05 molar at the indicated temperatures. The titer samples were exactly neutralized with sodium hydroxide and their aldose content then determined by the method of Cajori.[10]

The form of the equation for determining the monomolecular velocity constants is that of Morell and Link.[11] The energy of activation (Table I) was calculated from the Arrhenius equation[12]

$$\log_{10}\frac{k_2}{k_1} = \frac{E(T_2 - T_1)}{2.303RT_1T_2}$$

wherein R = molar gas constant, E = energy of activation, k_1 = velocity constant at temperature T_1.

By plotting $10^3/T$ against $\log_{10}(k \times 10^4)$, the three points fall along a straight line, the slope of which is $-E/R$.

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TABLE II

VELOCITY OF HYDROLYSIS OF LACTOSITOL (0.05 MOLAR) IN 1 N HYDROCHLORIC ACID

Time, min.	Temp., °C.	Titer,[a] cc. thiosulfate (N, 0.0984)	$k \times 10^3$[b] (min. and dec. log.)	
0	50	25.86		
454		25.05	0.183	
790		24.40	.196	
1578		23.25	.188	
1822		22.88	.190	
2220		22.33	.192	
∞		15.68 (calcd.)	Av.	0.190
0	60	26.15		
244		24.21	0.865	
453		22.81	0.878	
998		20.25	0.870	
1610		18.46	0.877	
2620		17.01	0.874	
∞		15.99 (calcd.)	Av.	0.873
0	70	26.15		
124		22.02	4.07	
219		20.14	4.09	
276		19.50	3.85	
362		18.40	3.98	
∞		15.99 (calcd.)	Av.	4.00

[a] Per 10 cc. soln. measured at the indicated temperature

[b] $$k = \frac{2.303}{t}\log_{10}\frac{\text{cc. } Na_2S_2O_3 \text{ at } t_0 - \text{cc. } Na_2S_2O_3 \text{ at } t_\infty}{\text{cc. } Na_2S_2O_3 \text{ at } t - \text{cc. } Na_2S_2O_3 \text{ at } t_\infty}$$

$$k_{454} = \frac{2.303}{454}\log_{10}\frac{25.86 - 15.68}{25.05 - 15.68} = 0.000183$$

$$\log_{10}\frac{4.02}{0.190} - \frac{E\,(343 - 323)}{2.303 \times 1.987 \times 343 \times 323};\ E = 33{,}600 \text{ calories}$$

Summary

1. Anhydrous lactositol has been prepared in crystalline form and further characterized as its crystalline tritrityl ether hexaacetate.

2. Sorbitol and galactose have been identified as the hydrolytic products of crystalline lactositol.

3. The velocity of hydrolysis of lactositol has been measured at three temperatures and the energy of activation of the hydrolysis calculated.

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[9] E. Fischer, *Ber.*, **27**, 673 (1894).

[10] F. A. Cajori, *J. Biol. Chem.*, **54**, 617 (1922).

[11] S. Morell and K. P. Link, *ibid.*, **104**, 186 (1934).

[12] S. Arrhenius, *Z. physik. Chem.*, **4**, 226 (1889).