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Note

Formation of β-lactose from α- and β-lactose octaacetates, and from α-lactose monohydrate

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This report shows that deacetylation of α- or β-lactose octaacetate in methanol with potassium methoxide or potassium hydroxide gives β-lactose in good yield, whereas with triethylamine or ammonia, an amorphous, anomeric mixture (α β = 55 45) is obtained α-Lactose monohydrate is converted into β-lactose with potassium methoxide or potassium hydroxide in methanol, whereas, under the same conditions, the three anhydrous forms of lactose are unaffected

In this continuation of studies[1] of the effects of methanol on lactose, we have examined the form of lactose obtained on deacetylation of α- (**1**) and β- (**2**) lactose octaacetates[2] in methanol containing potassium methoxide[3,4] Deacetylation of **2** was performed by Kunz and Hudson[5], who stated that "no difficulty was found in preparing crystalline lactose in high yield by the saponification of lactose octaacetate", however, no information was given as to the anomeric nature of the crystalline lactose or the reagent and conditions used for saponification In a prior study involving β-lactose heptaacetate, Hudson and Sayre[6] performed the saponification with alcoholic potassium hydroxide and identified the resulting lactose by its rotatory power in water, but again the anomeric nature of the lactose was not stated Later, Hudson and co-workers[7] described a synthesis of lactose involving deacetylation of **2** with barium methoxide in methanol, and crystallization from aqueous ethanol, to give α-lactose monohydrate (**3**) in 94% yield, and Cadenas and Deferrari[8] deacetylated **2** with 16% methanolic ammonia, and crystallized the product from aqueous ethanol to give **3** in 71% yield Our observation of the nature of the product of the deacetylation of **1** and **2** led us to examine the effects of potassium hydroxide and potassium methoxide in methanol on **3** and on the three forms of anhydrous lactose[9–11]

EXPERIMENTAL

Compound 3 (Sigma Chemical Co *, St. Louis, MO) was used without further purification Stable, anhydrous, α-lactose[9] (α_S) and hygroscopic, anhydrous, α-lactose[10] (α_H) were prepared by heating 3 for 3 h at 130° in air or for 16 h at 130° *in vacuo*, respectively Anhydrous α-lactose[11] (α_M) was made by boiling 3 under reflux for 2 h with ten times its weight of abs methanol, followed by drying for 48 h at 65° *in vacuo* Complexes of the anomeric forms of anhydrous lactose in the ratio $\alpha\ \beta = 4\ 1$ (ref 12) and $\alpha\ \beta = 5\ 3$ (ref 13) were also prepared from 3 β-Lactose was obtained by crystallization from a boiling, aqueous solution of 3, the crystals were washed with hot glycerol (140°) followed by boiling ethanol[14], and then dried *in vacuo* for 24 h at 65° Compounds **1** and **2**, made by the procedure of Hudson and Johnson[2], had physical constants in good agreement with those reported

The general procedure employed with the foregoing compounds was to stir the compound (15 g) with abs methanol (150 mL) containing 0 014M potassium hydroxide or potassium methoxide After a selected time (0 5–24 h) at 27° or at reflux, the solid material was removed by filtration, washed with methanol, and then dried to constant weight (16–48 h) at 65° *in vacuo*

A Dupont Model 990 thermal analyzer, operated in the differential-scanning calorimetry (d s c) mode, was used to determine melting points, and the purity of samples was established by analysis of the d s c -fusion thermograms by the procedure of Sondack[15] Intensity data and spacings of X-ray powder diffraction patterns were obtained by use of a Philips, vertical powder diffractometer and procedures similar to those described by Buma and Wiegers[16] Debye–Scherrer diffraction films of the samples mixed with aluminum powder were used in calibration, and copper radiation was used with a nickel-foil filter Intensities were estimated visually, and no corrections have been applied Optical-rotation data were obtained with 2% aqueous solutions at 589 nm in a 1-dm tube with a Perkin–Elmer Model 141 polarimeter, initial rotations were obtained by extrapolation from observations made from 2 to 12 min, and equilibrium values were read at 24 h Potassium analyses were performed by atomic absorption spectroscopy by use of a Perkin–Elmer Model 306 instrument

RESULTS AND DISCUSSION

Various conditions have been employed[3,4,17–19] for the deacetylation of sugar acetates with alcoholic solutions of alkali metal alkoxides We have used a solution of 14mM potassium methoxide in methanol[3,4,17] to deacetylate **1** and **2** for 24 h at 25° and have found that in each case the product, obtained in high yield (97%), was identical to β-lactose[14] by comparison of its specific optical rotation and

*Reference to brand or firm name does not constitute endorsement by the U S Department of Agriculture over others of a similar nature not mentioned

X-ray powder diffraction pattern The precipitated product from **2**, isolated after 40 min of reaction (7% yield), was mainly amorphous by d s c. and showed $[\alpha]_D^{27}$ +54 8 (initial) → +55 5° (equilibrium), the latter value being that of anhydrous lactose, and contained no acetyl groups based on i r. examination and spectrophotometric analysis[20], further treatment of this product with potassium methoxide in methanol for 24 h gave crystalline β-lactose The precipitated product from **2**, isolated after reaction for 65 min (43% yield), was mainly amorphous by d s c and showed $[\alpha]_D^{27}$ +42 1 (initial) → +55 5° (equilibrium) and was converted into crystalline β-lactose on further reaction with potassium methoxide in methanol for 24 h The precipitated product from **1**, isolated after 40 min of reaction (12% yield), was mainly amorphous by d s c and showed $[\alpha]_D^{27}$ +76 3 (initial) → +55 5° (equilibrium), namely, a ratio of α to β of 3 1 initially, and it contained no acetyl groups, again, further reaction of this product gave crystalline β-lactose The precipitated product from **1**, isolated after 90 min of reaction (50% yield), was mainly amorphous by d s c and showed $[\alpha]_D^{27}$ +50 2 (initial) → +55 5° (equilibrium) and was converted into crystalline β-lactose on further reaction with potassium methoxide in methanol for 24 h Compounds **1** or **2** with potassium methoxide in methanol at reflux[18] gave a white precipitate after a few min, and the product, isolated in 70–72% yield after 3 h, was crystalline β-lactose These results are summarized in Table I Similar results were obtained when saponification was performed with 14mM potassium hydroxide in methanol[6]

Deacetylation of **1** and **2** with 0 28M triethylamine in methanol for 17 h at 25° gave in each instance a mixture of anhydrous α- and β-lactose (87% yield) in the ratio 55 45, as determined from optical-rotation data This mixture was converted into crystalline β-lactose when boiled for 2 h under reflux with potassium methoxide in methanol

Treatment of **1** or **2** with 16% methanolic ammonia[8] gave an anhydrous

TABLE I

ANOMERIC NATURE OF THE LACTOSE OBTAINED BY DEACETYLATION OF α- AND β-LACTOSE OCTAACETATES WITH POTASSIUM METHOXIDE IN METHANOL

Starting material	*Temperature (°C)*	*Time (h)*	*Anomeric ratio (β α) of lactose product*
α-lactose octaacetate	25	0 7	26 74[a]
	25	1 5	71 29[a]
	25	24	99 1
	65	3	99 1
β-lactose octaacetate	25	0 7	63 37[a]
	25	1 1	85 15[a]
	25	24	99 1
	65	3	99 1

[a]Amorphous

lactose product, mainly amorphous, but containing approximately 20% of crystalline material having m p (216°) identical to that of α_M, the anomeric ratio was α β = 55 45 by polarimetric analysis

The formation of β-lactose on deacetylation of **1** or **2** (refs 5–8) has not been reported previously, because of final isolation involving crystallization from aqueous ethanol to give **3** In view of the subsequent conversion into β-lactose of the intermediate, amorphous products from the deacetylation of **1** or **2**, we examined the effects of the foregoing deacetylation reagents and conditions on the various forms of lactose

When **3** was stirred at 25° or boiled under reflux with 14mM potassium methoxide in methanol, conversion into β-lactose was complete in 1–2 h Boiled for 2 h under reflux with concentrations of potassium methoxide in methanol of 1 4, 2 8, 7 0, and 14mM gave conversions into β-lactose, as judged by polarimetric analysis, of 9, 12, 52, and 98%, respectively The remainder of **3** was converted into anhydrous (based on Karl Fischer moisture and polarimetric analyses) α-lactose, with thermal properties by d s c close to those[11] of α_M Complete conversion into β-lactose also occurred when **3** was treated with 14mM potassium or sodium methoxides in ethanol or 1-propanol instead of methanol[21] Treatment of **3** with 14mM potassium hydroxide in methanol at 27° or at the b p under reflux also gave β-lactose, but on boiling under reflux with 14mM potassium hydroxide in oxolane, **3** was recovered unchanged Treatment of compound **3** with 0 2M potassium hydroxide in 95% aqueous methanol for 16 h at 27° gave a mixture of unchanged **3** and anhydrous lactose (α_M), as judged by d s c analysis, whereas on boiling under reflux for 2 h with 14mM potassium hydroxide in 95% aqueous methanol, **3** was converted into β-lactose The use of 0 07M triethylamine in methanol gave a mixture of unchanged **3** (37%) and anhydrous α-lactose (α_M) (63%), but no β-lactose, as based on Karl Fischer moisture, polarimetric, and d s c analyses

None of the three anhydrous forms of α-lactose[9–11] were converted into β-lactose by treatment with 14mM potassium methoxide in methanol or 95–99% aqueous methanol, or with up to 0 2M potassium hydroxide in methanol at 27° or at reflux

Complexes of the anomeric forms of anhydrous lactose in the ratios α β = 4 1 (ref 12) and α β = 5 3 (ref 13) gave β-lactose on boiling for 3 h under reflux with 14mM potassium hydroxide or potassium methoxide in methanol

Treatment of **3** with 16% methanolic ammonia[8] gave a mainly amorphous mixture of the anomers of lactose in the ratio α β = 55 45, with about 20% of the material similar to α_M, and similar products were obtained from the anhydrous forms of lactose (α_S and α_H) Thus, these products are closely similar to those from **1** and **2**

In no instance, under the conditions we have used, have we found complexes of lactose with potassium methoxide[4 17] or potassium acetate[22], the highest level of potassium contamination determined by atomic absorption spectroscopy being 0 011 g-atom of potassium/mol of lactose

The diffraction spacings and relative intensities of β-lactose prepared by treatment of **3** with methanolic potassium methoxide agreed with those reported[16] for

β-lactose prepared by crystallization from boiling water; similarly, we have established that the anhydrous forms of α-lactose produced by heating in air (α_S)[9] or by heating in methanol (α_M)[11] or ethanol give identical diffraction spacing and relative intensities, the data agreeing with those reported[16] for α_S

β-Lactose produced by treatment of 3 with potassium methoxide had m.p 232° and $[\alpha]_D^{27}$ +33.9 (initial) → +55.7° (equilibrium), in good agreement with reported values[14] of +33.5 → +55.5°; by determination from d.s.c. data[15], the purity was 98%, the impurities being α-lactose and methanol. The crystallite diameter in the product from treatment with potassium methoxide was 2×10^{-5} to 10^{-3} cm, whereas β-lactose from aqueous solution[14] ranged in size from $4–8 \times 10^{-3}$ cm, both products being very readily soluble in water. Thus, treatment of 3 with potassium methoxide provides a rapid and convenient procedure for the preparation of β-lactose in excellent yield

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