A new method for the preparation of lactulose from lactose*

KEVIN B. HICKS† AND FREDERICK W. PARRISH‡

*Eastern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture**, Philadelphia, Pennsylvania 19118 (U.S.A.)*

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Lactulose (4-*O*-β-D-galactopyranosyl-D-fructose) is a synthetic disaccharide currently used in the medical treatment of several intestinally-related disorders[1-3]. It has been suggested that this sugar, if produced economically, could find application as a non-nutritive[4] substitute for sucrose in intermediate-moisture foods[5].

Lactulose was first prepared by a Lobry de Bruyn–Alberda van Ekenstein transformation of lactose in dilute calcium hydroxide solution[6]. This method for preparing the ketodisaccharide is tedious, as the yield is low (<20%) and the product must be isolated from unreacted starting-material, alkaline degradation-products, and calcium ion. More-recent methods for producing lactulose have involved the use of such complexing reagents as aluminate[7] and borate[8,9]. These compounds shift the pseudo-equilibrium established during base-catalyzed isomerization in favor of the ketose and prevent degradative side-reactions. The use of these reagents permits[7,8] formation of lactulose from lactose in yields as high as 70–80%. Aluminate, however, is removed from the final product with great difficulty. In addition, the use of borate, as reported by Mendicino[8], is somewhat impractical in that a borate: sugar ratio of 50:1 was necessary for optimal yield of product.

Amines have previously been utilized as basic catalysts for the conversion of lactose into lactulose. Hough *et al.*[10] used ammonia for the transformation, but the use of primary and secondary amines for this purpose leads to side reactions, including formation of glycosylamines[11] and Amadori[12] compounds. Parrish[13] eliminated this problem by using triethylamine, a tertiary amine incapable of forming adducts with reducing sugars. Yields of about 32% were reported with this reagent, but with considerable accompanying alkaline degradation.

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†Postdoctoral Fellow supported by Dairy Research Foundation, Inc.

‡Present address: Southern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, New Orleans, LA 70179, U.S.A.

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In the present work, treatment of aqueous solutions of lactose–boric acid (molar ratio 1:1) with tertiary amines gave lactulose in high yield, with minimal use of boric acid. Because degradative side-reactions were minimized and no metal ions were present, the need for extensive chromatographic purification of the product was obviated. Additionally, lactulose was prepared by a procedure that allowed for complete recycling of the reagents used.

When triethylamine was used as the basic catalyst in the presence of boric acid, the yields of lactulose were high (Table I), and alkaline degradation was almost eliminated. The rate of production of lactulose and the yield increased with the basicity of the medium (triethylamine was added to achieve the desired pH). This result is in accordance with general base-catalyzed reactions and was previously observed by Mendicino[8] during conversion of lactose into lactulose in sodium tetraborate–sodium hydroxide solutions. The absence of alkaline degradation-reactions even at pH 11 (Table I) was apparent, as the solutions were colorless, high yields of lactulose were produced, and only low levels of monosaccharides were present. Monosaccharides would arise from alkaline peeling-reactions of disaccharides[14,15].

TABLE I

EFFECT OF pH ON CONVERSION OF LACTOSE INTO LACTULOSE IN THE PRESENCE OF TRIETHYLAMINE–BORIC ACID[a]

pH[b]	*Yield of*		*Rate of production of lactulose*[e]
	Lactulose (%)[c]	*Monosaccharides (%)*[d]	
9.0	6	<1	0.03
10.0	32	5	0.16
10.5	74	3	0.37
11.0	83	5	0.41

[a]Reaction period of 96 h at 40°. [b]pH reading at room temperature. [c]Measured by quantitative l.c. [d]As galactose and tagatose, measured by quantitative l.c. [e]Expressed as mg of lactulose produced per mL of solution per h (averaged over 96 h).

TABLE II

CONVERSION OF LACTOSE INTO LACTULOSE IN THE PRESENCE OF BORIC ACID AND TERTIARY AMINES[a]

Amine	*pH*[b]	*Lactulose (%)*[c]	*Monosaccharides (%)*[d]
1,4-Dimethylpiperazine	9.5	21	<1
N-Methylpiperidine	10.8	81	<1
N,N,N′,N′-Tetramethylethylenediamine	9.7	33	5
Triethylamine	11.0	87	3

[a]Reaction period of 3 h at 70°, except for triethylamine, which was subjected to reaction for 4 h. [b]pH reading at room temperature. [c]Yields measured by quantitative l.c. [d]As galactose and tagatose, measured by quantitative l.c.

Several tertiary amines were found to be effective catalysts for the transformation (Table II). The yields of lactulose ranged from 21–87% when four different tertiary amines were used. In all instances in Tables I and II, lactose was the only other sugar present except for lactulose and two monosaccharides having l.c. retention-times identical to those of D-tagatose and D-galactose (3.0 and 5.5 min, respectively). Both of these monosaccharides have been identified in lactose–lactulose isomerization-mixtures[16].

It is not obvious that one tertiary amine is a more effective catalyst than any of the others. The yields and reaction rates appear to be governed largely by the pH of the reaction, which is a function of the water solubility and basicity of the amine. It is noteworthy that a quaternary amine salt, benzyltrimethylammonium methoxide, when treated with lactose at pH 10.9 exactly as described in Table II, also yielded lactulose in 83% yield.

The rate of formation of lactulose was enhanced by increasing the temperature of the reaction, with no increase in side reactions. Formation of lactulose in yields of >80% was achieved in 4 h by treatment of lactose with boric acid at 70° (Table II), as compared to 96 h at 40° (Table I).

Triethylamine and boric acid were found to be uniquely useful reagents in that they could be recovered from the mixture. Table III shows that lactulose may be prepared from lactose with reagents recycled from a previous preparation. In fact, the actual yield of lactulose was better in the second cycle than the first, although more monosaccharides were formed during the second cycle. This result was attributed to the less than quantitative (75%) recovery of boric acid (and presumably triethylamine) from the first cycle.

TABLE III

RECYCLING OF REAGENTS AFTER THE CONVERSION OF LACTOSE INTO LACTULOSE WITH TRIETHYLAMINE–BORIC ACID[a]

Cycle	*pH*[b]	*Lactulose (%)*[c]	*Lactose (%)*[c]	*Monosaccharides (%)*[d]
1	11.0	52	48	0
2	10.95	73	14	13

[a]Solutions heated for 2 h at 70°. [b]pH reading at room temperature. [c]Yields measured by quantitative l.c. [d]As galactose and tagatose, measured by quantitative l.c.

The method for production of lactulose reported here allows for facile isolation of crystalline lactulose of high purity. When calcium hydroxide is used as the isomerization reagent, lactulose is isolated only in low yields and with great difficulty.

EXPERIMENTAL

Materials and methods. — All sugars, boric acid, and tertiary (and quaternary) amines were of analytical-reagent grade. Sugars were quantitatively determined on

a μBondapak/Carbohydrate column (Waters Associates) eluted at 1 mL/min with 77:23 (w/w) acetonitrile–water, conditions that allow base-line separation of tagatose, galactose, lactulose, and lactose (Parrish, Hicks, and Doner, unpublished results).

Preparation of lactulose from lactose with triethylamine–boric acid at various pH values. — Within an efficient fume-hood, four mixtures containing 5 g of α-lactose monohydrate (14 mmol), 0.89 g of boric acid (14 mmol), and water (100 mL) were titrated in 250-mL round-bottomed flasks to pH 9.0, 10.0, 10.5, or 11.0 with 1.45, 2.40, 3.20, or 6.0 mL of triethylamine, respectively. The samples were immersed in a thermostatted waterbath at 40°, and when the samples had reached the reaction temperature (but not before) they were sealed with rubber septums. The reactions were sampled (10-mL aliquots) at timed intervals. The amine was removed by passage of the sample through a column (1 × 12 cm) of IR-120 (H^+) resin. Boric acid was removed as the methyl ester by evaporating the sample to dryness under diminished pressure, adding 40 mL of abs. methanol, re-evaporating, and repeating the whole process three times. The final residue was dissolved in water (10 mL) and acetonitrile (10 mL), and analyzed by quantitative l.c.

Effect of the type of tertiary amine on yield. — The procedure was conducted as just described, except that the sugar–boric acid solutions were titrated with either 1,4-dimethylpiperazine (pH 9.5), *N*-methylpiperidine (pH 10.8), triethylamine (pH 11.0), or *N,N,N′,N′*-tetramethylethylenediamine (pH 9.7). After 3 h at 70° (triethylamine was allowed to react for 4 h), the solutions were sampled and treated as already described to determine the extent of aldose–ketose conversion.

Recycling of triethylamine–boric acid reagent. — A mixture of α-lactose monohydrate (5 g) and boric acid (0.89 g) in water (100 mL) was titrated in a 250-mL round-bottomed flask to pH 11 (6 mL of triethylamine was required). After heating in a water bath for 2 h at 70° (cycle 1), the sample was evaporated under diminished pressure at 35° and the distillate, containing triethylamine and water (distillate A), was collected in a cold trap (Dry Ice–acetone). The sample was then treated with anhydrous methanol to remove boric acid as already described. The distillate of methanol–methyl borate from this treatment was then mixed with an equal volume of water and evaporated under diminished pressure at 35° to yield a residue of crystalline boric acid. Distillate A was then mixed with the reclaimed boric acid, 5 g of α-lactose monohydrate was added, and the heating process was repeated (cycle 2). The lactulose preparations from each cycle were analyzed by l.c. to determine the extent of ketose formation.

Isolation of crystalline lactulose. — The triethylamine-catalyzed reaction (Table II) was treated for removal of the amine and boric acid, and the resultant, dry residue (4.8 g) was dissolved in abs. methanol (5 g). This clear solution, kept in gentle motion and incubated at 40°, yielded 6 g of wet crystals, which were isolated on a filter, washed with cold methanol, and stored overnight in a vacuum desiccator. The dried, crystalline mass (2.9 g) was slightly hygroscopic; m.p. 155–160°, and was shown by l.c. to be 90% pure lactulose. A portion of the material was recrystallized by

redissolution in water, evaporation, and treatment with methanol at 40° as already described; m.p. 168–171°; mixed m.p. with authentic lactulose (Aldrich) 168–171°.

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REFERENCES

1 J. Bircher, J. Müller, P. Guggenheim, and U. P. Haemmerli, *Lancet*, (1966) 890.
2 H. O. Conn, *Gastroenterology*, 74 (1978) 624–626.
3 U.S. Pat. 3,562,388, Feb. 9, 1971.
4 A. Dahlquist and J. D. Gryboski, *Biochim. Biophys. Acta*, 110 (1965) 635–636.
5 F. W. Parrish, F. B. Talley, K. D. Ross, J. Clark, and J. G. Phillips, *J. Food Sci.*, 44 (1979) 813–815.
6 E. M. Montgomery and C. S. Hudson, *J. Am. Chem. Soc.*, 52 (1930) 2102–2106.
7 U.S. Pat. 3,546,206, Dec. 8, 1970.
8 J. F. Mendicino, *J. Am. Chem. Soc.*, 82 (1960) 4975–4979.
9 R. Carubelli, *Carbohydr. Res.*, 2 (1966) 480–485.
10 L. Hough, J. K. N. Jones, and E. L. Richards, *J. Chem. Soc.*, (1953) 2005–2009.
11 G. P. Ellis and J. Honeyman, *Adv. Carbohydr. Chem.*, 10 (1955) 95–168.
12 J. E. Hodge, *Adv. Carbohydr. Chem.*, 10 (1955) 169–205.
13 U.S. Pat. 3,514,327, May 26, 1970.
14 I. Ziderman, J. Bel-Ayche, A. Basch, and M. Lewin, *Carbohydr. Res.*, 43 (1975) 255–263.
15 M. Lewin, I. Ziderman, N. Weiss, A. Basch, and A. Ettinger, *Carbohydr. Res.*, 62 (1978) 393–398.
16 L. A. T. Verhaar, M. J. M. van Deraalst, J. A. W. M. Beenackers, and B. F. M. Kuster, *J. Chromatogr.*, 170 (1979) 363–370.