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Note

Reaction of lactose with urea

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Simple chemical modification of lactose may lead to the further utilization of this surplus carbohydrate. Although the acid-catalyzed reaction of lactose with urea in aqueous solution was first reported[1] in 1903, the reaction products have not been completely characterized. We have reinvestigated this reaction and have employed chromatography for analysis and separation of the products. The reaction was performed at an initial pH of 2 for 5 days at 50° (ref. 1) or for 4 h at reflux temperature. Little difference in the products was observed with variation of temperature, and the higher temperature obviated the need for a long reaction-time. Maximal yields (40%) of lactosylurea were obtained, together with unreacted lactose (50%), with an initial pH of 2.0 (final pH 3.0). At this low pH, lactose was partially hydrolyzed to D-glucose and D-galactose, which reacted with urea to yield minor, urea-containing compounds. The composition of the products was different at various pH values. As estimated by paper chromatography, the yield of lactosylurea decreased as the pH was raised from 3.0 to 7.0; no lactosylurea was produced at pH 7.0. Lactulose was the principal secondary product formed above pH 4, with the yield increasing to 15% at pH 7.0.

Segal *et al.*[2], using i.r. spectroscopy, postulated that D-glucosylurea exists in an acyclic form; however, Jones and Ross[3] presented chemical evidence, as well as i.r. and u.v. spectra, which showed that the D-glucosyl residue exists in the pyranosyl form. ^{13}C-N.m.r. spectra of lactosylurea demonstrated that neither the glucose nor the galactose rings had undergone opening or rearrangement. The assigned chemical shifts observed for lactosylurea were compared with those of β-lactose and β-D-glucosylurea (Table I). With the exception of the C-1 and C-2 resonances of the urea derivatives, which appear at somewhat higher fields (presumably due to the lower electronegativity of nitrogen relative to oxygen), all other shift-positions for lactosylurea show good agreement with those of β-lactose. Furthermore, the observation of no low-field, C=N carbon resonance for the urea derivatives definitively rules out the possibility of the open-chain form postulated earlier by Segal *et al.*[2] and corroborates the findings of Jones and Ross[3]. A gated-decoupling experiment, with suppressed NOE (nuclear Overhauser enhancement), confirmed the 1:1 ratio of carbonyl carbon

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to each ring carbon atom. ^{1}H-N.m.r. spectra in D_2O displayed the characteristic lactosyl anomeric H-1 resonance at δ 4.82 (1, d, $J = 9.0$ Hz). The large 9.0-Hz coupling confirmed the assignment of the β configuration at C-1. The β-glycosidic linkage was confirmed by the H-4′ resonance at δ 4.45 (1, d, $J = 8.4$ Hz).

TABLE I

CHEMICAL SHIFTS FROM ^{13}C-N.M.R. SPECTRA

Compound	Chemical shifts of carbon atoms, δ (p.p.m.)[a]												
	1′	*2′*	*3*	*4′*	*5′*	*6′*	*1*	*2*	*3*	*4*	*5*	*6*	*C=O*
β-Lactose[b]	104.0	72.2	73.9	69.8	76.3	62.1	97.0	75.2	75.9	79.9	75.6	61.4	—
β-Lactosylurea	103.7	71.8[c]	73.4	69.4	76.1	61.9	81.7	72.6[c]	76.7	78.9	76.0	60.9	161.5
β-D-Glucosylurea							81.9	72.9	77.7	70.3	77.9	61.9	161.5

[a]Relative to a reference of 1,4-dioxane (1%) taken as δ 67.4 from external tetramethylsilane. [b]See ref. 4. [c]These assignments may be reversed.

The occurrence of lactulose at pH values above pH 4 is consistent with the observation of Hynd[5], who noted that D-glucose is converted into D-fructose (approximately 20%) upon incubation in aqueous urea for 20 days at 50°. In addition, ketohexoses do not appear to react with urea, even in the presence of acid[1,6]; thus, in experiments at initial pH values of 4.0 to 7.0, some lactose was converted into lactulose (approximately 10–15% at pH 7.0).

EXPERIMENTAL

General methods. — Paper chromatograms (Whatman No. 1 paper) were developed by descending irrigation. Silica Gel G was used for t.l.c. plates. Solvents employed for p.c. and t.l.c. were: *A*, 7:1:2 1-propanol–ethyl acetate–water; and *B*, 6:4:3 1-butanol–pyridine–water. The spots on paper were detected with (*a*) *p*-anisidine[7], (*b*) urea–sulfuric acid[8], and (*c*) *p*-(dimethylamino)benzaldehyde[9]; t.l.c. plates were sprayed with sulfuric acid.

Enzymic hydrolysis with lactase (Enzyme Development Corporation*, New York) was performed on paper chromatograms by applying a drop of lactase solution to a spot, waiting for 15 min, and then developing the chromatogram.

Mixtures of lactose (10 g) and urea (10 g) in water (100 ml) were adjusted to the desired pH with phosphoric acid and boiled for 4 h under reflux. Similar results were obtained with sulfuric acid. The reactions were also conducted for 5 days at 50° (ref. 1).

Both ^{13}C- and ^{1}H-n.m.r. spectra were performed with a JEOL FX 60-Q pulsed Fourier-transform spectrometer, operating at 15.04 and 59.80 MHz, respectively. All

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^{13}C-n.m.r. spectra were obtained during complete proton-noise decoupling at 35° in 79% H_2O, 20% D_2O containing 1% of 1,4-dioxane as internal reference. Quantitative area-relationships among carbon resonances were obtained from NOE-suppressed spectra, with 30-sec repetition rates and 90° pulses. ^{1}H-Spectra were recorded in D_2O. All shifts are reported relative to sodium 4,4-dimethyl-4-silapentane-1-sulfonate as internal reference. The H-1 resonance was obscured by the DHO resonance at 30°; however, at 50°, the DHO absorption was shifted upfield sufficiently that the anomeric proton resonance could be observed.

Identification of 1-(4-O-β-D-galactopyranosyl-β-D-glucopyranosyl)urea (lactosylurea). — The product formed at pH 3 after 4 h at reflux temperature was isolated by chromatography on cellulose columns (1 × 30 cm) eluted with solvent *A*. Thin-layer and paper chromatography showed one component. Enzymic hydrolysis by lactase gave galactose and glucosylurea. Acid hydrolysis gave galactose, glucose, and urea. The product had m.p. 230–240° (dec.) (lit.[1] m.p. 230–240°), $[\alpha]_D^{25}$ +1.5° (water). The chemical composition found is consistent with that of lactosylurea.

Anal. Calc. for $C_{13}H_{24}N_2O_{11}$: C, 40.62; H, 6.30; N, 7.29; O, 45.80. Found: C, 40.62; H, 6.32; N, 6.90; O, 46.62.

Identification of lactulose. — The byproduct (lactulose) contained some traces of lactose, but the major component (m.p. 156–157°, lit.[1] m.p. 168°) displayed identical mobility and color reactions with a known specimen of lactulose on paper chromatograms; acid and enzymic hydrolysis gave galactose and fructose. The chemical composition found was consistent with that of lactulose.

Anal. Calc. for $C_{12}H_{22}O_{11}\cdot 1.5H_2O$: C, 39.02; H, 6.83; O, 54.15. Found: C, 38.96; H, 6.83; O, 54.15.

The lower m.p. may arise from impurities or water of hydration.

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