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Chemical Modification of Lactose. XIII.[1] Synthesis of Lacto-N-tetraose

The protected tetrasaccharide (**6**) was synthesized in 77% yield by condensation of 1,6-anhydro-2,2′,3,4′,6′-penta-O-benzyl-β-lactose (**4**) with the oxazoline derivative of lacto-N-biose I (**5**). The protecting groups of **6** were removed by the following series of reaction to provide lacto-N-tetraose (**10**): debenzylation, acetylation, acetolysis, and de-O-acetylation. The synthetic product (**10**) was crystallized from aqueous ethanol as white needles, mp 225—228°, $[\alpha]_D^{21}$ +27° (4 min)→+21.3° (3 hr) (c=0.45, H_2O).

The homogeneity and the mobility of **10** were confirmed by the gel permeation chromatography using Bio-Gel P-4 column. The specific rotation and IR spectrum of **10** were similar to those of the natural material reported by Kuhn, Gauhe, and Baer [*Chem. Ber.*, **86**, 827 (1953)].

Keywords——human milk oligosaccharide; lactosan pentabenzylether; oxazoline; lacto-N-biose I; protected tetrassacharide; debenzylation; acetolysis; de-O-acetylation; Bio-Gel P-4 gel permeation chromatography; IR

Lacto-N-tetraose was the first aminodeoxy oligosaccharide shown to occur free in nature and that was isolated from human milk in crystalline form.[2] The methods employed to establish its structure, which is O-β-D-galactopyranosyl-(1→3)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)-D-glucopyranose, included partial hydrolysis and methylation.[3] Successive studies on the oligosaccharides in human milk have revealed that the sugar is the core structure of the more complex oligosaccharides such as lacto-N-fucopentaose I and II, lacto-N-difucohexaose I and II, LS-tetrasaccharide a and b, and disialyllacto-N-tetraose.[4]

In this communication, we wish to report a chemical synthesis of lacto-N-tetraose from lactose.

1,6-Anhydro-4′,6′-O-benzylidene-3′-O-tosyl-β-lactose (**2**), which was isolated in 15% yield by partial tosylation of 1,6-anhydro-4′,6′-O-benzylidene-β-lactose (**1**),[5] was catalytically de-

1) Part XII: H. Matsuda, H. Ishihara, and S. Tejima, *Chem. Pharm. Bull.* (Tokyo), submitted.
2) R. Kuhn, A. Gauhe, and H.H. Baer, *Chem. Ber.*, **86**, 827 (1953).
3) *a*) R. Kuhn, A. Gauhe, and H.H. Baer, *Chem. Ber.*, **87**, 289 (1954); *b*) R. Kuhn and H.H. Baer, *ibid.*, **89**, 504 (1956).
4) V. Ginsburg (ed.), "Methods in Enzymology," Vol. 28, Academic Press, New York, San Francisco, and London, 1972, p. 262; Vol. 50, 1978, p. 216.
5) T. Takamura and S. Tejima, *Chem.Pharm. Bull.* (Tokyo), **26**, 1117 (1978).

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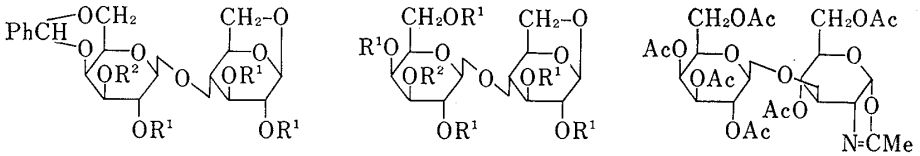
benzylidenated with palladium catalyst to yield an amorphous 1,6-anhydro-3′-O-tosyl-β-lactose (**3**) in theoretical yield. Benzylation of **3** in N,N-dimethylformamide with benzyl bromide, barium oxide, and crystalline barium hydroxide, followed by removal of the tosyl group with 2% sodium amalgam in methanol, afforded 1,6-anhydro-2,2′,3,4′,6′-penta-O-benzyl-β-lactose (**4**), $[\alpha]_D^{21}$ −25.6° (c=0.64, $CHCl_3$) as a syrup. IR ν_{max}^{Nujol} cm^{-1}: 3450 (OH). NMR $\delta_{ppm}^{CDCl_3}$: 2.44 (1H, br. s, OH), 5.46 (1H, s, H-1, β-Glc), 7.19—7.29 (25H, m aromatic protons).

The oxazoline derivative of lacto-N-biose I (**5**) was prepared according to the method of Augé and Veyrières.[6] A mixture of **4** (1 mol eq.) and **5** (1.4 mol eq.) in toluene-nitromethane (1: 1, v/v) in the presence of a trace of p-toluenesulfonic acid was stirred at 60° for 24 hr under nitrogen atmosphere. After 24 hr, further portions of **5** (1.4 mol eq.) were added, and the stirring was continued for 24 hr. The mixture was neutralized with pyridine and evaporated to dryness. The residue was chromatographed on a column of silica gel, eluting first with benzene–ether (3: 1, v/v) and secondly with $CHCl_3$–EtOH (60: 1, v/v). The protected tetrasaccharide (**6**), $[\alpha]_D^{20}$ −33° (c=0.98, $CHCl_3$), was isolated as an amorphous powder in 77% yield. IR ν_{max}^{Nujol} cm^{-1}: 3390 (NH), 1680 (amide I). NMR $\delta_{ppm}^{CDCl_3}$: 1.59, 1.94, 1.99, 2.02, 2.04, 2.09, 2.13 (21H, each s, OAc × 6, NAc), 7.24—7.33 (25H, m, aromatic protons).



1: R^1=R^2=H
2: R^1=H, R^2=Ts
3: R^1=H, R^2=Ts
4: R^1=Bn, R^2=H
5

Ac=acetyl, Bn=benzyl, Me=methyl, Ts=tosyl, Ph=phenyl

Chart 1

Catalytic debenzylation of **6** in methanol with palladium catalyst and successive acetylation of the debenzylated product with acetic anhydride and pyridine provided the acetylated tetrasaccharide (**7**), $[\alpha]_D^{21}$ +2.1° (c=1.2, $CHCl_3$), in 97.5% yield as an amorphous powder. IR ν_{max}^{Nujol} cm^{-1}: 3360 (NH), 1675 (amide I), 1540 (amide II). NMR $\delta_{ppm}^{CDCl_3}$: 1.98, 2.04, 2.06, 2.11, 2.12, 2.14 (36H, each s, OAc × 11, NAc), 6.28 (1H, d, $J_{NH,2''}$=7 Hz, NH).

Acetolysis of **7** was performed with an acetolysis mixture, Ac_2O–AcOH–96% H_2SO_4 (70: 30: 1, v/v), at 8° for 2 hr. The product was chromatographed on a column of silica gel, eluting with benzene–ether–MeOH (7: 7: 1, v/v). The β-acetate (**8**) was eluted first and isolated as an amorphous powder, $[\alpha]_D^{19}$ +22.9° (c=0.53, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3380 (NH), 1670 (amide I), 1540 (amide II). NMR $\delta_{ppm}^{CDCl_3}$: 1.97, 1.99, 2.04, 2.06, 2.11 (42H, each s, OAc × 13, NAc), 5.71 (1H, d, $J_{1,2}$=8 Hz, H-1, β-Glc), 6.06 (1H, d, $J_{NH,2''}$=7 Hz, NH).

After the β-acetate (**8**) emerged, the α-acetate (**9**) was eluted with the same solvent and isolated as an amorphous powder, $[\alpha]_D^{20}$ +49.3° (c=0.5, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3400 (NH), 1670 (amide I), 1540 (amide II). NMR $\delta_{ppm}^{CDCl_3}$: 1.95, 1.99, 2.05, 2.13, 2.17 (42H, each s, OAc × 13, NAc), 5.81 (1H, d, $J_{NH,2''}$=8 Hz, NH), 6.26 (1H, d, $J_{1,2}$=3.5 Hz, H-1, α-Glc). The total yield of the acetates (**8**+**9**) was 88.2% yield.

A mixture of **8** and **9** in MeOH–H_2O–triethylamine (2: 3: 1, v/v) was left to stand at room temperature for 48 hr to de-O-acetylation. After removal of the solvent, treatment of the residue with aqueous ethanol induced crystallization of lacto-N-tetraose (**10**), mp 225—228°, $[\alpha]_D^{21}$ +27° (4 min)→+21.3° (3 hr) (c=0.45, H_2O), as white needles in 81.5% yield. IR ν_{max}^{KBr} cm^{-1}: 3250 (br. OH, NH), 1635 (amide I), 1588 (amide II), 1260 (amide III) [lit. mp

6) C. Augé and A. Veyrières, *Carbohyd. Res.*, **46**, 293 (1976).

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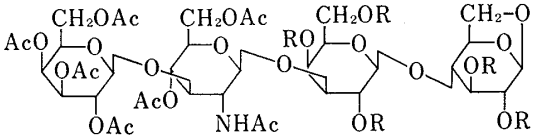
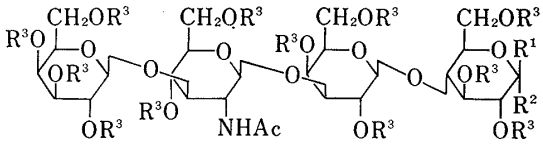
204—205°,[7] 205±10° (dec.),[3b] $[\alpha]_D^{24}$ +25.2° (final value) (c=1.5, H_2O),[3b] $[\alpha]_D^{23}$ +38° (0 min) →+25.5° (final value) (H_2O)[2]].

The homogeneity and mobility of **10** were confirmed by the gel permeation chromatography using Bio-Gel P-4 column (2×175 cm, under 400 mesh) at 53°.[8] Furthermore, the specific rotation and IR spectrum of **10** were similar to those of the natural material reported by Kuhn, Gauhe, and Baer [*Chem. Ber.*, **86**, 827 (1953)].



6: R=Bn
7: R=Ac



8: R^1=OAc, R^2=H, R^3=Ac
9: R^1=H, R^2=OAc, R^3=Ac
10: R^1=R^3=H, R^2=OH

Chart 2

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7) F.H. Malpress and F.E. Hytten, *Biochem. J.*, **68**, 708 (1958).
8) K. Yamashita, Y. Tachibana, and A. Kobata, *Arch. Biochem. Biophys.*, **182**, 546 (1977).

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