SYNTHESIS AND ACID-CATALYZED HYDROLYSIS OF 3-*O*-GLYCOSYL-L-SERINE AND -THREONINE[1]

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ABSTRACT

The acid stability of the glycosidic linkage of 3-*O*-β-D-xylopyranosyl-L-serine and -threonine, of import for graded acid hydrolysis of glycopeptides, was assessed through model compounds. Crystalline 3-*O*-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-L-serine and threonine were synthesized in 20% yield. Syrupy 3-*O*-(4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranosyl)-*N*-benzyloxycarbonyl-L-serine and -threonine *p*-nitrobenzyl ester afforded upon hydrogenation crystalline 3-*O*-(4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hexopyranosyl)-L-serine and -threonine in 50% yield. Extensive kinetic studies of the acid-catalyzed hydrolysis in 0.05M sulfuric acid of these four crystalline 3-*O*-glycosyl derivatives of serine and threonine and comparisons of their kinetic rate-constants and thermodynamic parameters with those of methyl α- and β-D-xylopyranosides were made. Although a protonated amino group is vicinal to the glycosidic acetal linkage in the 3-*O*-L-serine and -threonine xylosides, the rates of their hydrolysis are only slightly more than an order of magnitude lower than those of ordinary alkyl glycosides. The rate constants for the 2,3-dideoxy-α-D-*erythro*-hexopyranosides of L-serine and -threonine are similar in magnitude to those for the methyl glycosides. The $\Delta G^{\ddagger}$ values for the 2,3-dideoxy-α-D-*erythro*-hexopyranoside derivatives are nearly the same as those of the alkyl glycosides, whereas the D-xylopyranosides of L-serine and -threonine exhibit $\Delta G^{\ddagger}$ values some 2 kcal. mole^{-1} greater. All of the $\Delta S^{\ddagger}$ values are positive, but show no order interpretable in terms of structure–function relationships.

INTRODUCTION

Much structural chemistry is being conducted on the glycosyl linkage-sites in glycopeptides, and on oligosaccharide compositions and peptide sequences in the vicinity of the glycosidic attachment. Such knowledge can assist greatly in the formulation of a unified theory of glycoprotein metabolism[2,3]. As a contribution to such

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investigations, we previously reported the stability of 3-*O*-glycosyl derivatives of hydroxyamino acids in a basic medium[4,5]. In the present paper we present a detailed assessment of the stability of this same linkage in acid. Such data can be of import in structural studies for choosing conditions for the selective degradation of glycopeptides.

Investigations and reviews of acid-catalyzed hydrolysis of glycopyranosides have recently been presented by BeMiller[6,7], Capon[8], and de Bruyne[9–11]. The earlier studies by Overend and coworkers on reactions at the anomeric carbon atom of glycosides remain significant[12]. Graham and Neuberger[13] reported rate studies on the acid-catalyzed hydrolysis of 2-aminoethyl β-D-glucopyranoside. This derivative of 2-aminoethanol was contrasted in its acid-catalyzed hydrolysis with the rates of hydrolysis reported by Moggridge and Neuberger[14] for glycosides of 2-acetamido-2-deoxy-D-glucose.

The rate of acid-catalyzed hydrolysis of 1-*N*-β-L-aspartyl-(2-acetamido-2-deoxy-β-D-glucopyranosyl)amine has been discussed[2]. This *N*-glycosyl linkage is somewhat more stable in acid than an ordinary *O*-glycoside. Derevitskaya, Vafina, and Kochetkov[15] reported that cleavage of 3-*O*-serine glycosides is normal in an acid medium and results in the formation of monosaccharides and serine derivatives. The review by Marshall and Neuberger[2] misquotes Derevitskaya and coworkers[15] and implies an unusual acid-stability of 3-*O*-serine glycosides. In addition, other data are presented by Marshall and Neuberger[2] that suggest an enhanced resistance of 3-*O*-β-D-xylopyranosyl-L-serine to acid hydrolysis.

Only by having definitive kinetic and thermodynamic data can a firm physicochemical basis be established to assess chemical reactivity of the glycosidic linkage to 3-hydroxyamino acids under conditions of acid hydrolysis. An important question to be raised about these kinetics concerns the extent to which reaction occurs, under conditions of mild acid hydrolysis, between the reducing sugars liberated and the free hydroxyamino acid. The formation of glycosylamine derivatives is well known in degradations of sugars, with subsequent formation of browning polymers[16]; these side reactions could seriously interfere with accurate kinetic calculations.

EXPERIMENTAL

T.l.c. was performed by the ascending technique on Silica Gel G (E. Merck Co., Darmstadt, West Germany), 0.25-mm layers on glass plates, activated for 1 h at 110°, and on Eastman Chromatogram sheets (type 6060, silica gel with fluorescent indicator, and type 6065, cellulose with fluorescent indicator). Silica gel columns were prepared by using Silica Gel No. 7734, 0.05–0.2 mm (E. Merck Co., Darmstadt, West Germany). Solvent systems used were 2:1 petroleum ether (b.p. 30–60°)–ethyl acetate (solvent *A*), and 3:1:1; butyl alcohol–acetic acid–water (Solvent *B*). Chromatographic zones were located with 35% ammonium hydrogen sulfate spray reagent and charring; with ninhydrin (0.2% in ethanol) spray reagent; with silver nitrate–sodium hydroxide spray reagent; with iodine vapor; and with u.v. light.

Optical rotations at the sodium D line were measured with a Schmidt and Haensch polarimeter in a 2-dm tube. Optical rotations at 546 nm were measured with a Bendix 1169 and a Bendix ETL-NPL automatic polarimeter (Type 143A) in a 0.45-dm cell. Melting points were determined with Pyrex capillary tubes on a "Mel-Temp" melting point apparatus and are uncorrected. I.r. spectra were recorded on a Beckman Model IR-5A i.r. spectrometer with potassium bromide pellets. N.m.r. spectra were recorded with Varian A-60 (60 MHz) and HA-100 (100 MHz) spectrometers. The samples were measured in chloroform-*d* with tetramethylsilane as an internal reference unless otherwise indicated. For samples measured in deuterium oxide, sodium 4,4-dimethyl-4-silapentane-1-sulfonate was used as an internal reference. Samples were not degassed before use. Microanalyses were determined by Galbraith Laboratories, Inc., Knoxville, Tennessee. *N*-Benzyloxycarbonyl-L-serine and *N*-benzyloxycarbonyl-L-threonine were obtained from Pierce Chemical Co., Rockford, Illinois, and methyl glycosides from the Pfanstiehl Chemical Co., Waukegan, Illinois.

N-*Benzyloxycarbonyl*-L-*serine* p-*nitrobenzyl ester* (**1**). — Following the esterification procedure of Schwyzer[18], a mixture of α-bromo-*p*-nitrotoluene (25 g, 116 mmole), *N*-benzyloxycarbonyl-L-serine (19.8 g, 79.5 mmole), triethylamine (10 ml, 8.1 g, 80.2 mmole), and ethyl acetate (200 ml) was stirred mechanically for 6 h at 60°, after which time t.l.c. (Solvent *A*, u.v. light) indicated completion of the reaction. The mixture was filtered through a Celite pad, and the filtrate was washed successively with 2M hydrochloric acid (3 × 100 ml) and 5% sodium hydrogen carbonate (3 × 100 ml), and dried over anhydrous sodium sulfate. Crystals formed as the solution was concentrated. One recrystallization from ethyl acetate–petroleum ether (b.p. 30–60°) gave a chromatographically homogeneous compound; yield 22 g (71%), m.p. 114–115°, $[\alpha]_D^{23}$ +7.1° (*c* 1, chloroform); R_F 0.50 (solvent *A*, u.v. light); n.m.r. (60 MHz): τ 1.9 (2-proton doublet, $J_{3',2'}$, $J_{5',6'}$ 9 Hz, H-3′, H-5′), 2.58 (2-proton doublet, H-2′, H-6′), 2.72 (5-proton singlet, C_6H_5), 4.78 (2-proton singlet, of $CH_2C_6H_4NO_2$); 4.94 (2-proton singlet, $PhCH_2O$), 5.35–5.70 (1-proton multiplet, H-2), 6.05 (2-proton broad peak, H-3), 6.9 (1-proton broad singlet, removed with D_2O, OH); λ_{max}^{KBr} 3.05 (broad, N–H and O–H), 3.28 (aromatic C–H); 3.41 (aliphatic C–H), 5.72, 5.8 (C=O), 9.32 (C–O–C), 13.42, 13.68, 14.47 (Ar–), 5.98, 6.22, 6.45, 6.59, 7.45, 7.90, 8.39, 11.61, 11.81, 12.70, 12.85 μm.

Anal. Calc. for $C_{18}H_{18}N_2O_7$: C, 57.75; H, 4.81; N, 7.49. Found: C, 58.02; H, 4.97; N, 7.65.

N-*Benzyloxycarbonyl*-L-*threonine* p-*nitrobenzyl ester* (**2**). — A mixture[18] of α-bromo-*p*-nitrotoluene (20.3 g, 94 mmole), *N*-benzyloxycarbonyl-L-threonine (17 g, 67.2 mmole), triethylamine (10 ml, 8.1 g, 80.2 mmole), and ethyl acetate (250 ml) was stirred mechanically for 4 h at 60°, at which time t.l.c. (solvent *A*, u.v. light) indicated completion of the reaction. The reaction product was purified as in the preceding experiment[18] and recrystallized twice, giving a chromatographically homogeneous compound product; yield (61.5%) 16 g, m.p. 111–113°, $[\alpha]_D^{23}$ −8.5° (*c* 1, chloroform); R_F 0.64 (solvent *A*, u.v. light) n.m.r. (60 MHz): τ 1.5 (2-proton doublet, $J_{3',2'}$, $J_{5',6'}$ 9 Hz, H-3′, H-5′), 2.5 (2-proton doublet, H-2′, H-6′), 2.67 (5-proton

singlet, C_6H_5), 4.05 (1-proton doublet, $J_{3,1}$ 8 Hz, N–H), 4.73 (2-proton singlet, $CH_2C_6H_4NO_2$), 4.89 (2-proton singlet, $PhCH_2O$), 5.5–5.84 (2-proton multiplet, H-2, H-3), 6.75 (1-proton broad singlet, removed by D_2O, OH), 8.72 (3-proton doublet, $J_{4,2}$ 6 Hz, C-4 protons, methyl group at C-3); λ_{max}^{KBr} 3.0 (N–H, O–H), 5.8, 5.91 (C=O), 9.4 (C–O–C), 6.2 (Ar–), 13.19, 13.67, 14.25 (Ar–), 6.62, 7.4, 7.8, 8.18, 8.50, 9.71, 11.00, 11.60, 11.99, 12.79 μm.

Anal. Calc. for $C_{19}H_{20}N_2O_7$: C, 58.76; H, 5.15; N, 7.22. Found: C, 59.03; H, 5.24; N, 7.40.

3-O-(2,3,4-Tri-O-acetyl-β-D-xylopyranosyl)-N-benzyloxycarbonyl-L-serine p-nitrobenzyl ester (**3**). — A modification of the Koenigs–Knorr[19] reaction was employed. A mixture of **1** (ref. 18) (10 g, 26.8 mmole), silver carbonate[20] (10 g, 36.3 mmoles, freshly prepared), Drierite (20 g, pulverized), silver perchlorate (1 g, 4.8 mmole), nitromethane (75 ml, spectroanalytical grade), and benzene (70 ml, distilled over barium oxide) was shaken mechanically in a sealed vessel for 2 h in the dark. 2,3,4-Tri-*O*-acetyl-α-D-xylopyranosyl bromide[21] (10 g, 29.5 mmole) was introduced, and after 3 days under mechanical shaking, t.l.c. (solvent *A*, u.v. light) revealed no further formation of product. More 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide (6.7 g, 19.8 mmole) and silver carbonate (10.0 g, 36.3 mmole) were added, and shaking was continued for 4 days; methanol (50 ml, abs.) was then added, and shaking was continued for one more day. The mixture was filtered under suction through a Celite pad, and the filtrate washed with 5% sodium carbonate (5 × 100 ml), dried over anhydrous sodium sulfate, and evaporated under diminished pressure. The remaining syrup was taken up in hot 95% ethanol, and the solution was allowed to cool slowly. The crystals produced were washed with cold 95% ethanol and dried under vacuum over phosphorus pentaoxide–potassium hydroxide. A chromatographically homogeneous product was obtained; yield 3.97 g (23.4%), m.p. 111–113°, $[\alpha]_D^{23.5}$ −41° (*c* 1, chloroform); R_F 0.27 (solvent *A*, u.v. light), n.m.r. (60 MHz): τ 1.3 (2-proton doublet, $J_{5',6'}$, $J_{3',2'}$ 9 Hz, H-3', H-5'), 2.2 (2-proton doublet, H-2', H-6', 2.35 (5-proton singlet, C_6H_5), 4.1 (1-proton broad peak, N–H), 4.55 (2-proton singlet, $CH_2C_6H_4NO_2$), 4.7 (2-proton singlet, $PhCH_2O$ 4.72–5.5 (5-proton complex multiplet, H-2, H-1",2",3",4"), 5.45–6.20 (4-proton multiplet, C-3 protons, H-5e", H-5a"), 7.92 (9-proton broad singlet, OAc); λ_{max}^{KBr} 3.0 (N–H), 5.67 (shoulder), 5.72, 5.82, (C=O), 7.22 (–NO_2), 9.40–9.55 (C–O–C), 6.2, 6.5, 6.58 (Ar–), 3.38 (aromatic C–H), 3.45 (aliphatic C–H), 13.12, 14.13 (monosubstituted Ar–), 11.95 (1,4-substituted Ar–), 4.3, 7.39, 7.62, 7.95, 8.08, 8.18 (shoulder), 8.95, 9.00, 10.00, 10.90–11.02, 11.38, 11.58, 12.10, 12.81, 13.32, 13.53, 13.65, 14.45 μm.

Anal. Calc. for $C_{29}H_{32}N_2O_{14}$: C, 55.06; H, 5.06; N, 4.43. Found: C, 55.11; H, 5.05; N, 4.44.

An additional crop of product crystals was obtained from the filtrate; one recrystallization from 95% ethanol gave a chromatographically homogeneous product; yield 1.68 g (9.9%). The total yield of product was 5.65 g (33%).

3-O-(2,3,4-Tri-O-acetyl-β-D-xylopyranosyl)-N-benzyloxycarbonyl-L-threonine p-nitrobenzyl ester (**4**). — Compound **2** (ref. 18) and 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl

bromide were allowed to react[19] under the same conditions as in the preparation of **3**. One recrystallization of the crystals produced on cooling of the 95% ethanolic solution gave a chromatographically homogeneous product; yield 3.07 g (17.9%); R_F 0.56 (solvent *A*, u.v. light). Two additional crops (1.82 g) were obtained from the filtrate; total yield 4.89 g (28.6%), m.p. 121–124°, $[\alpha]_D^{24}$ −39.4° (*c* 1, chloroform); n.m.r. (60 MHz): τ 1.66 (2-proton doublet, $J_{3',2'}$, $J_{5',6'}$ 9 Hz, H-3′, H-5′), 2.31 (2-proton doublet, H-2′, H-6′), 2.49 (5-proton singlet, C_6H_5), 4.0 (1-proton broad peak, N–H), 4.64 (2-proton singlet, $CH_2C_6H_4NO_2$), 4.75 (2-proton singlet, $PhCH_2O$), 4.25–5.35 (6-proton complex multiplet, H-2, H-3, H-1″,2″,3″,4″), 5.75–6.40 (2 protons, H-5e″, 5a″), 7.97 (9-proton singlet, OAc), 8.78 (3-proton doublet, $J_{4,2}$ 6 Hz, H-4, methyl group); λ_{max}^{KBr} 2.93 (N–H), 3.36 (aromatic C–H), 3.50 (aliphatic C–H), 5.66, 5.76, 5.81 (C=O), 7.21 ($-NO_2$), 9.38–9.58 (C–O–C), 13.22, 13.58, 14.35 (Ar–), 6.20, 6.51–6.61, 7.40, 7.53, 7.85, 7.96, 8.04, 8.13, 8.46, 9.82, 10.07, 10.91, 11.02, 11.58, 12.06, 15.21, 15.41, 15.62 μm.

Anal. Calc. for $C_{30}H_{34}N_2O_{14}$: C, 55.73; H, 5.26; N, 4.43. Found: C, 55.77; H, 5.24; N, 4.40.

3-O-(4,6-Di-O-acetyl-2,3-dideoxy-α-D-erythro-hex-2-enopyranosyl)-N-benzyloxycarbonyl-L-serine p-nitrobenzyl ester (**5**). — The procedure of Ferrier was utilized[22]. Boron trifluoride etherate (0.5 ml) was added to a mixture of **1** (7 g, 18.7 mmole), 3,4,6-tri-*O*-acetyl-D-glucal[23] (5.1 g, 18.7 mmole), benzene (70 ml, distilled over barium oxide, and nitromethane (50 ml). After 10 min of stirring, t.l.c. (solvent *A*, iodine vapor) indicated completion of reaction. The solution was washed successively with 5% sodium carbonate (3 × 100 ml) and cold water (3 × 100 ml), and dried over anhydrous sodium sulfate. The solvent was removed under diminished pressure. T.l.c. (solvent *A*, u.v. light) of the remaining syrup (8.0 g) showed two zones: R_F 0.37 and R_F 0.61. The zone having R_F 0.37 was identical in mobility with *N*-benzyloxycarbonyl-L-serine *p*-nitrobenzyl ester. The syrup was not crystallized, and was passed over a column of silica gel (90 g, 4-cm diameter) with 2:1 petroleum ether (b.p. 30–60°)–ethyl acetate as eluant. Fractions shown to contain the zone having R_F 0.61 by t.l.c. were combined, and the solvent was removed to give a yellow syrup; yield 5.3 g (50.2%), which could not be crystallized; n.m.r. (100 MHz): τ 1.82 (2-proton doublet, $J_{3',2'}$, $J_{5',6'}$ 9 Hz, H-3′, H-5′), 2.52 (2-proton doublet, H-2′, H-6′), 2.66 (5-proton singlet, C_6H_5), 4.02–4.22 (2-proton multiplet, H-2″, H-3″), 4.22–4.46 (1-proton multiplet, N-H), 4.70 (2-proton singlet, $CH_2C_6H_4NO_2$), 4.86 (2-proton singlet, $PhCH_2O$), 4.72 (1-proton multiplet under benzylic peak at τ 4.86, H-4″), 5.0 (1-proton broad singlet, H-1″), 5.22–5.46 (1-proton multiplet, H-2), 5.66, 5.76 (1 proton, 2 doublets, J ~3 Hz, H-5″), 5.85 (2-proton doublet, $J_{6'',5''}$ 4 Hz, H-6″), 6.08–6.32 (2-proton multiplet, H-3), 7.98–8.10 (6 protons, OAc).

Irradiation of the multiplet at τ 5.22–5.46 (H-2) simplified the multiplet at τ 6.08–6.32 (H-3). Irradiation of the doublet at τ 5.85 (H-6″) appeared to simplify the doublets at τ 5.66 and τ 5.76. Irradiation of the singlet at τ 5.0 (H-1″) simplified the multiplet at τ 4.02–4.46 (H-2″,3″).

3-O-(4,6-Di-O-acetyl-2,3-dideoxy-α-D-erythro-hex-2-enopyranosyl)-N-benzyloxy-

carbonyl-L-threonine p-*nitrobenzyl ester* (**6**). — By an adaptation of Ferrier's procedure[22], boron trifluoride etherate (0.3 ml) was added to a mixture of **2** (5 g, 12.9 mmole), 3,4,6-tri-*O*-acetyl-D-glucal (3.85 g, 14.5 mmole), and benzene (80 ml, distilled over barium oxide). The mixture was stirred for 15 min at room temperature after which time t.l.c. indicated completion of the reaction. The solution was washed with 5% sodium carbonate (3 × 50 ml) and dried over anhydrous sodium sulfate. The solvent was removed under diminished pressure to give a colorless syrup that was crystallized from benzene–ethyl ether–petroleum ether (b.p. 30–60°). Two recrystallizations from benzene–petroleum ether (b.p. 30–60°) gave a chromatographically homogeneous product; yield 3.32 g (41.3%), m.p. 95–96°, $[\alpha]_D^{25}$ +14.2° (*c* 1, chloroform); R_f 0.46 (solvent *A*, u.v. light) n.m.r. (60 MHz): τ 1.79 (2-proton doublet, $J_{3',2'}$, $J_{5',6'}$ 9 Hz, H-3', H-5'), 2.48 (2-proton doublet, H-2', H-6'), 2.68 (5-proton singlet, C_6H_5), 4.02–4.16, 4.2–4.35, 4.35–4.54 (3 protons, 3 broad peaks, H-2",3", N-H), 4.72 (2-proton singlet, $CH_2C_6H_4NO_2$), 4.86 (2-proton singlet, $PhCH_2O$), 4.65–4.83 (1-proton multiplet buried under benzylic protons, H-4"), 5.1 (1-proton, perturbed doublet, $J_{1",2"}$ ~3 Hz, H-1"), 5.4–5.7 (2-proton multiplet, H-2, H-3), 5.75–6.05 (3 protons, H-5",6"), 7.91–7.93 (6 protons, 2 peaks, OAc), 8.62 (3-proton doublet, $J_{4,3}$ 6 Hz, H-4); λ_{max}^{KBr} 2.90 (broad, N–H), 3.34 (aromatic C–H), 3.40 (aliphatic C–H), 5.63, 5.79, 5.84 (C=O), 6.18, 6.40 (shoulder), 6.54, 6.82, 7.20, 7.35, 8.32, 8.9, 9.0, 9.1, 9.7, 10.88–11.00, 11.29, 11.59, 11.70, 11.82, 12.38, 13.19, 13.34, 13.51, 14.20–14.32 μm.

Anal. Calc. for $C_{29}H_{32}N_2O_{12}$: C, 58.00; H, 5.33; N, 4.67. Found: C, 58.15; H, 5.28; N, 4.28.

3-O-(2,3,4-Tri-O-acetyl-β-D-xylopyranosyl)-L-serine (**7**). — A mixture of **3** (3.9 g, 6.17 mmole), *p*-dioxane (20 ml), water (5 ml), hydrochloric acid (12M, 4 drops) and 10% palladium on carbon (0.25 g) was hydrogenated at atmospheric pressure under a stream of hydrogen for 10 h[24]. The mixture was filtered through a fritted glass funnel, and the filtrate extracted with chloroform (10 ml). Crystals formed upon concentration of the aqueous layer. These were collected, washed with acetone, and dried under vacuum over phosphorus pentaoxide–potassium hydroxide to give a chromatographically homogeneous product; yield 327 mg (23.1%), m.p. 180–190° (decomp), $[\alpha]_{546}^{28}$ −84.3° (*c* 0.1, water); R_F 0.19 (solvent *B*, ninhydrin); n.m.r. (100 MHz, D_2O): τ 4.70–5.3 (4-proton multiplet, H-1', 2', 3', 4'), 5.6–6.5 (5-proton multiplet, H-2, H-3', H-5e', 5a'), 7.82 (9 protons, 2 peaks, OAc); λ_{max}^{KBr} 2.93, 3.08, 3.48–4.00 (broad), 5.71, 5.75 (C=O), 6.30, 6.38, 6.42, 7.28, 7.46, 7.70, 7.93, 8.12, 8.22, 8.42, 9.02, 9.27, 10.07, 11.01, 11.45 μm.

Anal. Calc. for $C_{14}H_{21}NO_{10}$: C, 46.28; H, 5.79; N, 3.86. Found: C, 46.25; H, 5.86; N, 3.72.

3-O-(2,3,4-Tri-O-acetyl-β-D-xylopyranosyl)-L-threonine (**8**). — Compound **8** was prepared from compound **4** under conditions[24] similar to those used for preparation of **7**. Chromatographically homogeneous crystals were obtained; yield 700 mg (47.4%), m.p. 165–180° (decomp), $[\alpha]_{546}^{28}$ −80.7° (*c* 0.1, water); R_F 0.27 (solvent *B*, ninhydrin); n.m.r. (100 MHz, D_2O): τ 4.60–6.00 (6-proton multiplet, H-1', 2', 3', 4', H-2, H-3), 6.20–6.60 (2-proton multiplet, H-5e', 5a'), 7.86 (9 protons, 3 peaks, OAc),

8.64 (3-proton doublet, $J_{4,3}$ 6 Hz, H-4, methyl group); λ_{max}^{KBr} 2.90–3.00 (broad), 3.40, 5.75 (C=O), 6.05–6.12, 6.41, 6.90–6.94,7.33, 7.96, 8.08–8.18, 8.91, 9.20, 9.51–9.67, 9.98, 11.00, 11.40 μm.

Anal. Calc. for $C_{15}H_{23}NO_{10}$: C, 47.75; H, 6.10; N, 3.71. Found: C, 47.57; H, 6.22; N, 3.81.

*3-O-(4,6-Di-O-acetyl-2,3-dideoxy-α-*D-erythro-*hexopyranosyl)-*L-*serine* (**9**). — Hydrogenation of compound **5** under the conditions just described produced a chromatographically homogeneous product, yield 896 mg (55%), m.p. 155–190° (decomp), $[\alpha]_{546}^{28}$ +75.5° (*c* 0.1, water); R_F 0.20 (solvent *B*, ninhydrin); n.m.r. (100 MHz, D_2O) τ 4.02, 4.58, 4.68, 4.82 (5 protons, 4 singlets or 2 singlets and 1 doublet, carbohydrate-ring protons), 5.62–6.20 (6-proton multiplet, H-2, H-3, carbohydrate-ring protons), 5.26 (possible 1-proton pattern under D_2O peak), 7.85 (6-proton singlet, OAc); λ_{max}^{KBr} 2.95, 3.12, 3.38–3.43, 5.78 (C=O), 5.81–5.91 (shoulder, C=O), 6.2, 6.40, 6.58, 7.08, 7.28, 7.40, 7.15, 7.95, 8.2, 9.08, 9.5, 10.35, 11.15, 12.40 μm.

Anal. Calc. for $C_{13}H_{21}NO_8$: C, 48.90; H, 6.58; N, 4.39. Found: C, 48.79; H, 6.46; N, 4.25.

*3-O-(4,6-Di-O-acetyl-2,3-dideoxy-α-*D-erythro-*hexopyranosyl)-*L-threonine* (**10**). — A mixture of **6** (2.7 g, 4.5 mmoles), *p*-dioxane (36 ml), water (9 ml), hydrochloric acid (12M, 3 drops), and 10% palladium on carbon (0.3 g) was hydrogenated for 48 h at atmospheric pressure under a hydrogen stream[24]. The mixture was filtered through a fritted glass funnel, and the filtrate was extracted with chloroform (40 ml). Crystals formed upon concentration of the aqueous layer. These were collected, washed with propyl alcohol, and dried under vacuum over phosphorus pentaoxide–potassium hydroxide to give a chromatographically homogeneous product; yield 792 mg (55%), m.p. 165–185° (decomp), $[\alpha]_{546}^{28}$ +62.6° (*c* 0.1, water); R_F 0.30 (solvent *B*, ninhydrin); n.m.r. (100 MHz, D_2O): τ 4.04, 4.61, 4.69, 4.78 (5 protons, 4 singlets or 2 singlets and 1 doublet, carbohydrate-ring protons), 5.42–6.00 (4-protons, H-3 and carbohydrate-ring protons), 6.26 (1-proton doublet, $J_{1,2}$ 4 Hz, H-2), 5.28 (possible 1-proton pattern under D_2O peak), 7.84 (6-proton singlet, OAc), 8.55 (3-proton doublet, *J* 6 Hz, H-4, methyl group); λ_{max}^{KBr} 2.92, 3.29, 3.45, 5.75, 5.82 (C=O), 6.15, 6.56, 6.62, 7.30, 8.10, 8.40, 8.92, 9.58, 9.76, 10.20, 11.20 μm.

Anal. Calc. for $C_{14}H_{23}NO_8$: C, 50.45; H, 6.91; N, 4.20. Found: C, 50.26; H, 7.02; N, 4.29.

Hydrolytic studies on glycosides **7–10**. — The extent of hydrolysis was determined by use of an electronic recording polarimeter that was calibrated by means of a variable potentiometer. A water-jacketed polarimeter cell and cell holder was employed for the solutions, and temperatures were maintained within ±0.05° by means of a Tamson Thermostatic Bath (Neslab Instruments, Inc., Portsmouth, N.H.). Hydrolyses were performed at 50, 60, and 70° at a sample concentration of 3mM. The polarimeter was set at zero with 0.05M sulfuric acid at the operating temperature. Samples (weighed in 10 ml volumetric flasks) were diluted to 10 ml with 0.05M sulfuric acid and injected into the cell; this step generally required about 4 min. Alternatively, the hydrolytic mixture was continuously pumped through the thermo-

stated polarimeter-cell from an external reservoir heated by a constant-temperature bath. Either method yielded identical results. In terms of convenience, the latter method of external heating was preferred to permit the greatest stability of the polarimeter readings.

The cell was held secure by a plane-parallel, machined aluminum sleeve fitted with two retainer screws. The sleeve was also fitted with two arms so machined that the sample cell could be inserted and withdrawn without changing its azimuth position. This was done to minimize the effects of birefringence in the cell windows.

Because the electronic polarimeter was so sensitive, the concentration of substrate low, and the cell path-lengths short, the angular rotations measured (± 0.5 degree relative to a fixed angle obtained during zeroing procedures) were generally very small, and the effects of birefringence became a factor of considerable significance. Birefringence could not be avoided entirely, but its effects could be balanced out by the continuous-flow method.

Rotations *versus* time were recorded and, in general, no points before 10 min of reaction were plotted, to permit temperature equilibration and the removal of air bubbles. By use of a computer, the least-squares regression lines were determined for a regression equation[17]. Calculations to give rate constants and thermodynamic data were conducted as described by Overend and co-workers[12,25]. These data are recorded in Table I.

TABLE I

RATE CONSTANTS AND HALF LIVES FOR HYDROLYSES OF 3-GLYCOSYLOXY AMINO ACIDS

Compound no.	*Name*	*Kinetic data*[a]			
		k(10^4) (sec^{-1})			$t_{\frac{1}{2}}$ (70°) ($sec\ 10^4$)
		50°	*60°*	*70°*	
7	3-*O*-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-L-serine	0.042	0.102	0.407	1.70
8	3-*O*-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-L-threonine	0.014	0.065	0.296	2.30
9	3-*O*-(4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hexopyranosyl)-L-serine	0.385	1.74	7.88	0.088
10	3-*O*-(4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hexopyranosyl)-L-threonine	0.58	2.09	8.21	0.085

[a]No acidity function used in these calculations.

Gas-chromatographic determination of furfural derivatives in hydrolyzates. — The reaction mixtures from the acidic hydrolyses of **7–10** were neutralized with M sodium hydroxide and extracted with chloroform. The chloroform extract was concentrated under a stream of dry nitrogen. The samples were injected into a Varian Aerograph Model 600-C gas chromatograph equipped with hydrogen flame-ionization detector. The column was of 3.5-mm stainless-steel tubing packed with 3% diethylene

glycol succinate on Poropak Q. Standards were run with commercial samples of furfural or 5-(hydroxymethyl)-2-furaldehyde. No detectable quantity of either aldehyde was found in the hydrolyzates under the conditions of hydrolysis and determination.

DISCUSSION AND RESULTS

The scope of the present hydrolytic study does not allow strict delineation between steric and electronic effects in the cleavage of 3-glycosyloxy amino acids. However, by using simple kinetic rate-constants and Arrhenius kinetic parameters, a relationship in acid stability was sought concerning (*i*) glycosides of primary and secondary alcohols (compounds **7** and **8**), (*ii*) the relative Coulombic effect of a positively charged ammonium group vicinal to the acetal oxygen atom of the aglycon, and (*iii*) the effect of the electron-withdrawing acetoxyl substituents at positions 2 and 3 of the pyranosyl ring (compounds **9** and **10**). The 2,3-dideoxyglycoside would partially reflect the effect of shielding of the acetal oxygen atom by the ammonium group because of the absence of 2- and 3-acetoxy substituents. To achieve these ends, compounds **7**–**10** were synthesized. Observations concerning the proofs of structure for **7**–**10** and the rationale of the approach to their synthesis are made first.

During earlier studies on similar glycosides of hydroxyamino acids[4,5], difficulties were encountered in purification and crystallization when diastereoisomeric 3-D-glycosyloxy-D,L-amino acids were formed. Therefore, the use of pure L-amino acids and strict limitation of racemization are imperative. To derivatize L-serine as the *N*-benzyloxycarbonyl benzyl ester, low yields result in loss of the costly L-serine, and this prompted use of the *p*-nitrobenzyl ester to afford much higher yields of crystalline product. Benzyl esters are easily cleaved by hydrogenation, thus avoiding racemization or elimination reactions. The procedure for *N*-benzyloxycarbonyl-L-serine *p*-nitrobenzyl ester (**1**) described by Theodoropoulos and Tsangaris[26] uses the silver salt of the amino acid, which is treated with *p*-nitrobenzyl *p*-toluenesulfonate. In our hands, this procedure led to impure product in very low yield. The esterification method of Schwyzer and Sieber[18], for *p*-nitrobenzyl esters of *N*-substituted amino acids, uses α-bromo-*p*-nitrotoluene in the presence of an equimolar concentration of triethylamine; it has not previously been reported for *N*-benzyloxycarbonyl-L-serine and -threonine. Physical constants, data in support of optical purity, and structural assignment, for products obtained by this route, are given in the experimental section.

The synthesis of glycosides **7** and **8** followed procedures and used structural assignments essentially the same as those reported earlier[4,5] by this laboratory and also by Kum and Roseman[24]. The route to the 2,3-dideoxy glycosides (**9** and **10**) followed the procedure of Ferrier and Prasad[22], by boron trifluoride etherate-catalyzed allylic displacement of the 3-acetoxy group of 3,4,6-tri-*O*-acetyl-D-glucal by an alcohol; optimum yields of **5** and **6** were only achieved by trial and error. It is noteworthy that the reactions proceeded well for the synthesis of **5** and **6**, even in the presence of an amide nitrogen atom in the aglycon. The amide group in these sensi-

tive amino acids might have formed irreversible complexes with the boron trifluoride or have undergone degradation. Hydrogenation to produce the 2,3-dideoxyglycosides having unprotected amino acids proceeded smoothly. Assignment of the α-D configuration to compounds **5** and **6** was made by comparison of the n.m.r. data with those recorded by Ferrier and Prasad[22] for several 2,3-unsaturated glycosides synthesized in the same manner. Comparison of the n.m.r. data for **9** and **10** with configurational assignments recently made by Horton and Thomson[27] for methyl 2,3,6-trideoxy-α-D-*erythro*-hexopyranoside derivatives, by use of a lanthanide shift-reagent, reinforced these conclusions for **9** and **10**. Although high positive values of optical rotation were observed for both **9** and **10**, such values are less significant than n.m.r. data in assigning configurations to these compounds[22].

Difficulty was encountered in obtaining crystalline glycosides after *O*-deacetylation of **7–10**, and the deacetylated products tended to be impure. The crystalline, acetylated **7–10** were quite soluble in aqueous solutions. As they were pure and synthetically reproducible before *O*-deacetylation, the hydrolytic studies were conducted on the acetates. About 5 g of each substrate (**7–10**) was required for all aspects of the study.

TABLE II

COMPARISON OF RATE CONSTANTS WITH ACIDITY-FUNCTION CORRECTIONS

Compound no.	*Name*	*70°* k (sec^{-1})	*60°* k (sec^{-1})	*60°* k_2 (sec^{-1})[a]
7		4.07×10^{-5}	1.02×10^{-5}	7.56×10^{-6}
8		2.96×10^{-5}	6.50×10^{-6}	4.81×10^{-6}
9		7.88×10^{-4}	1.74×10^{-4}	1.29×10^{-4}
10		8.21×10^{-4}	2.09×10^{-4}	1.55×10^{-4}
11	methyl α-D-glucopyranoside[b]	3.54×10^{-4}	8.99×10^{-5}	6.66×10^{-5}
12	methyl α-D-xylopyranoside[b]	4.11×10^{-4}	9.20×10^{-5}	6.81×10^{-5}
13	methyl β-D-xylopyranoside[b]	6.81×10^{-4}	1.56×10^{-4}	1.16×10^{-4}

[a] $h_0 = 1.35$ (see refs 25, 28); compare Overend and coworkers, ref. 12. These values were used to calculate the Arrhenius parameters in Table III. Rate constants for hydrolyses of **11–13** are comparable with those reported in ref. 12. [b] Half lives, 70°, 10^4 sec: Compound **11**, 0.19; **12**, 0.16; **13**, 0.10.

TABLE III

ARRHENIUS PARAMETERS CALCULATED FOR COMPOUNDS **7–13**

Compound	$E_a\left(\frac{kcal}{mole}\right)$	$\Delta H^{\ddagger}\left(\frac{kcal}{mole}\right)$	$\Delta G^{\ddagger}\left(\frac{kcal}{mole}\right)^{a}$	$\Delta S^{\ddagger}\left(\frac{cal}{deg\text{-}mole}\right)^{a,b}$
7	31.4	30.8	27.4	+10.36
8	34.4	33.8	27.8	+18.36
9	34.3	33.7	25.5	+24.52
10	31.1	30.4	25.4	+15.14
11	31.1	30.5	25.9	+13.67
12	34.0	33.3	25.9	+22.31
13	33.4	32.8	25.5	+21.88

[a] Calculated from k_2 in Table II. [b] Calculated for $\Delta S^{\ddagger}$ at 60°.

The kinetic data from hydrolyses of **7–10** are given in Tables I, II, and III. In order to avoid reactions of the Maillard type[16], the substrates were kept at low concentration (3 mM) in dilute acid solution (0.05M sulfuric acid, pH 1.38). Only the sugar and amino acid were found to be present in the hydrolyzates, with no formation of 5-(hydroxymethyl)-2-furaldehyde, furfural, or browning polymers. All of the kinetic runs were conducted in triplicate or quadruplicate. The composite data for each compound were then treated by a computer program with analysis by a regression equation[17]. Standard deviations were determined by this program and weighting values assigned for computer reruns, which resulted in the final values used for calculation of the kinetic rate-constants given in Tables I, II, and III.

Graham and Neuberger[13] reported the following kinetic parameters for 2-aminoethyl β-D-glucopyranoside upon hydrolysis in 2M hydrochloric acid: $k_{70°}$, 2.2×10^{-5} sec^{-1}; $k_{80°}$, 8.2×10^{-5} sec^{-1}; $k_{90°}$, 2.7×10^{-4} sec^{-1}; $E_a = +31.4$ kcal.mole^{-1}, and $\Delta S^{\ddagger}_{90°} = +8.7$ cal.mole^{-1} deg^{-1}. By our calculations from Graham and Neuberger's data[13], $\Delta H^{\ddagger} = 30.7$ kcal. mole^{-1} and $\Delta G^{\ddagger} = 27.5$ kcal.mole^{-1} for 2-aminoethyl β-D-glucopyranoside.

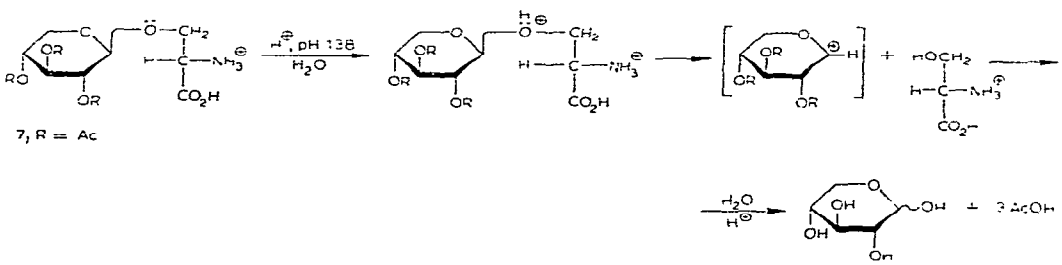

Chart 1. Mechanism of hydrolysis for 3-glycosyloxy amino acids

At pH 1.38, the amino acid portion of **7–10** is completely protonated (see Chart 1). Upon protonation of the glycosidic oxygen atom, an electrostatic repulsion should result from the double positive charge on vicinal atoms, which then should lead to the departure of the alcohol leaving-group and formation of a D-xylosyl carbonium ion. This process could conceivably lead to rate acceleration for the hydrolysis[2], but the kinetic rate-constants observed for the hydrolysis of **7** and **8** (Tables I and II) show that the hydrolytic rate is ten to fifteen times lower than that for the simpler methyl glycosides. Significantly, the 2,3-dideoxyglycosides (**9** and **10**) are hydrolyzed one to three times faster than the methyl glycosides. It is well known that 2-deoxyglycosides exhibit rates of hydrolysis several hundred times those of the 2-hydroxy-glycosides[12]. Compounds **9** and **10** evidently have lower hydrolytic rates than other alkyl 2-deoxy glycosides. In comparing rate data for compounds **7–10** in Tables I and II, no specific difference is discernible resulting from the serine aglycon (primary alcohol) and the threonine analog (secondary alcohol and an extra methyl group).

Many workers in the field of carbohydrate chemistry[6–12] have used various

values for the acidity function, H_0. In Table II, the kinetic rate-constant is corrected to $k_2 = k_\psi / h_0$, or rate $= k_2$(glycoside)h_0, and the calculations are reported for $h_0 = 1.35$, the magnitude of correction for acidity functions used by Bunnett[28] for dilute acid solutions. The values after this correction (k_2 in Table II) do not vary considerably from the uncorrected forms. Since the protonated amino acid is itself an acid, this acidity function could be an important consideration. However, it is also possible that, at the low concentrations of acid used in the present work, the acidity function has little significance.

In Table III, Arrhenius activation-parameters are given for the reactions of **7–13**. By using k_2 as corrected for the acidity function, the E_a, $\Delta H^\ddagger$, $\Delta G^\ddagger$, and $\Delta S^\ddagger$ values are given. A positive entropy of activation in the hydrolysis of glycosides has been interpreted[6–12] as being indicative of a unimolecular mechanism (A-1). In the present work, a strongly electron-withdrawing ammonium group vicinal to the glycosidic oxygen atom could induce a bimolecular (A-2) mechanism. Although the A-2 mechanism in the hydrolysis of furanosides has a negative entropy of activation[12], the positive $\Delta S^\ddagger$ value reported here can only be interpreted in terms of glycoside hydrolyses that follow the A-1 mechanism[8].

BeMiller and Doyle[7] have suggested that, as E_a, $\Delta H^\ddagger$, and $\Delta S^\ddagger$ are not sufficient to explain the differences in rates of hydrolysis for primary and secondary α-D and β-D-glycopyranosides, the values of $\Delta G^\ddagger$ may be best suited to explain differences between anomeric configuration and aglycon groups. By using the $\Delta G^\ddagger$ values for **7–13** (Table III) as being indicative of the relationship between hydrolytic rate and equilibrium constant for the reaction, it is seen that compounds **7** and **8** have much higher free-energies of activation (by $\sim$2 kcal mole^{-1}) than do **11–13**. That **9** and **10** have $\Delta G^\ddagger$ values nearly equivalent to those of **11–13** is indicative that the rates of hydrolysis for both **9** and **10** were lower (Table II) than normal for 2-deoxyglycosides. Also, the transition states for **9** and **10** must be achieved with as much energy of activation as for those of the simple alkyl glycosides. This could be explained on the basis of influence on protonation of the glycosidic oxygen atom by the positive charge on the aglycon.

Entlicher and BeMiller[29] have recently reported that deacylation at C-2, C-3, and C-6 of acylated D-glucopyranosyl groups results in a significant change in optical rotation; a marked initial decrease in positive rotation was observed as a result of this deacylation. These investigators suggested that deacylation of compounds containing α-D-glucopyranose residues results in a change in optical rotation, whereas the effect of deacylation on the optical rotation of β-D-glucopyranose derivatives was questionable. However, it was shown that the degree of stability of these compounds to hydrolysis in an acidic, aqueous ethanol medium is directly related to the degree of substitution. Even though the effects of acylation of compounds **7**, **8**, **9**, and **10** did not result in non-first order or adverse optical rotational changes during hydrolysis, the fact that these compounds contained acyl substituents certainly must be considered in assessing the lower reaction rates as compared with those of the simple unacylated, alkyl glycosides, **11**, **12**, and **13**. A point of difference between these hydrolytic studies

and those of Entlicher and BeMiller[29] is that the present hydrolyses were conducted a fully aqueous solution containing no organic solvent. This was possible because the acylated derivatives **7–10** possessed an electrostatic charge.

In conclusion, therefore, evidence is presented in Tables I–III that the 3-glycosyloxy amino acids **7–10** are somewhat more stable toward acid hydrolysis than are simpler glycosides. Whether this stabilization is due to shielding of the glycosidic oxygen to protonation, solvation effects about the aglycon, or simply an alteration in mechanism that affects the molecularity of reaction, cannot be determined from the present data alone. Experimentation with a wider variety of acid concentrations and substitution patterns on the aglycon could shed some light on these effects.

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