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STABILITY OF 3-GLYCOSYLOXYPROLINES IN AN ALKALINE MEDIUM. SYNTHESIS OF MODEL COMPOUNDS

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ABSTRACT

Glycosylation of the hindered secondary alcohol groups in substituted 3- and 4-hydroxyproline by reaction with 3,4,6-tri-*O*-acetyl-D-glucal and boron trifluoride etherate afforded 40–80% yields of 3- and 4-glycosyloxypyrrolidine-2-carboxylic acids. Modification of the 2,3-unsaturated glycosides of 4-hydroxyproline was achieved by hydrogenation, by addition of acetyl hypobromite, or by epoxidation with hydrogen peroxide in benzonitrile and ring-opening of the resultant 2,3-anhydro derivatives. From these model compounds was selected a 3-glycosyloxyproline that was suitable for assessment of the stability of the glycosyl linkage to base-catalyzed elimination. The model compound was *N*-acetyl-3-*O*-(4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hexopyranosyl)-*cis*-D,L-hydroxyproline ethyl ester (**12**). Homogeneity of the compounds was ascertained by high-resolution n.m.r. spectral and chromatographic methods. The glycoside (**12**) of 3-hydroxyproline is stable even in warm base at 50°. The 3- and 4-hydroxyproline glycosides are both more stable to acid hydrolysis than the analogous compounds of serine and threonine. A reliable synthesis of crystalline *cis*-D,L-3-hydroxyproline ethyl ester hydrochloride is reported that affords quantities of 250 mg or more in overall yield of 15% from inexpensive starting materials.

INTRODUCTION

Assessment of the stability of glycosyloxy amino acids toward acid, base, and enzymes is of value for structural studies on glycoproteins. Previous papers from this laboratory have considered such reactivity in model compounds[1]. The presence of glycosides of hydroxyproline in plants[2–4] and the possibility that these could exist in collagen or connective tissue prompted the synthesis of a model 4-*O*-glycosyl-hydroxyproline[5]. Specifically, this 4-*O*-glycosyl-hydroxyproline served to demonstrate the stability of the glycosidic linkage in base[5].

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Difficulties were encountered in glycosylating the sterically inaccessible 4-hydroxyl group of 4-hydroxyproline[5]. In the present study, we have sought to obtain glycosides of 3- and 4-hydroxyproline, for studies of their acid and base stability, by extending the facile glycosidation reaction of 3,4,6-tri-*O*-acetyl-D-glucal under catalysis by boron trifluoride etherate, as first reported by Ferrier and Prasad[6]. An earlier paper[1] from this laboratory showed that reasonable yields of *O*-glycosides of substituted hydroxyamino acids could be obtained by this procedure[6].

Because biosynthetic hydroxylation of proline peptides also gives small proportions of 3-hydroxyproline, in addition to the 4-hydroxy derivative[7,8], there is the possibility that glycosides of both hydroxyprolines exist. Although 4-hydroxyproline glycosylated with an arabinosyl moiety has been reported only in plants, Gallop *et al.*[8] considered that the possibility of its existence in certain mammalian collagens was presaged by this finding in the plant kingdom.

DISCUSSION OF RESULTS

This research was undertaken to discern whether or not 3-glycosyloxyprolines undergo *beta*-elimination and degradation in base. Because 3-hydroxyproline is commercially unavailable and difficult to prepare in quantity, the approach involved three phases: (*a*) to improve a route to a suitably substituted 3-hydroxyproline; (*b*) to acquire skill in producing a suitable glycosyloxy derivative of the more-accessible *trans*-4-hydroxyproline; and (*c*) to apply the best choice, from experience with 4-hydroxyproline, for producing a satisfactory glycosyl analog of 3-hydroxyproline to test its stability in base. The experimental section describes syntheses of *cis*-D,L-3-hydroxyproline, several 4-*O*-hexopyranosyl-hydroxyprolines producible in reasonable yield, and also the 3-*O*-(glycosyloxy)proline chosen as a model compound. *N*-Acetyl-3-*O*-(4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hexopyranosyl)-*cis*-D,L-hydroxyproline ethyl ester (**12**), was selected to utilize to best advantage the scarce *cis*-D,L-3-hydroxyproline.

An improvement of the 3-hydroxyproline synthesis reported by Blake and coworkers for other derivatives[9] is detailed here because a dependable source of 3-hydroxyproline is needed. Our synthesis (Chart I) uses the *N*-benzyloxycarbonyl derivative **2** during the ring closure instead of the *N*-ethoxycarbonyl analog[9] because the *N*-benzyloxycarbonyl group can be readily removed by hydrogenation. Furthermore, reduction of ethyl *N*-benzyloxycarbonyl-3-oxopyrrolidine-2-carboxylate with 5% rhodium on alumina in dilute hydrochloric acid affords a single crystalline product *cis*-D,L-3-hydroxyproline ethyl ester hydrochloride (**5**). Blake and coworkers[9] used sodium borohydride to reduce the ketone and obtained a *cis–trans* mixture. The *cis* disposition of the 3-hydroxyl and 2-ethoxycarbonyl groups in **5** was ascertained by n.m.r. spectroscopy by comparing the coupling constant ($J_{2,3} = 3.5$ Hz) with literature values[9] for *cis*-3-hydroxyproline ($J_{2,3} = 4$ Hz). The *N*-acetyl derivative of *cis*-3-hydroxyproline ethyl ester was prepared by the method of Roseman and Ludowieg[10].

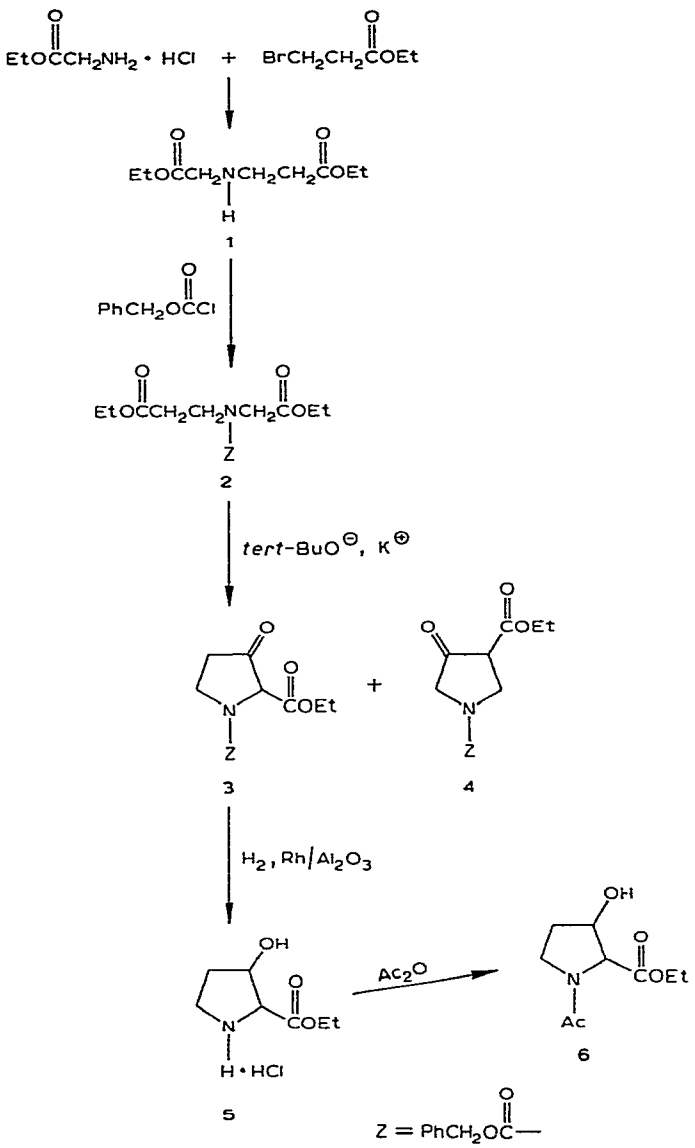

Chart 1

Criteria of homogeneity for 3- and 4-*O*-glycosylhydroxyprolines, synthesized by the method of Ferrier and Prasad[1,6] (see Chart II), were established by n.m.r. spectral, t.l.c., i.r., and optical rotary data, as given in greater detail in the earlier paper[1] for 3-*O*-(4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranosyl)-*N*-benzyloxycarbonyl-L-serine and -threonine and its hydrogenation product (the 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hexopyranoside). Extensive fractionation of the anomeric mixtures of these syrupy compounds, by column and preparative t.l.c.,

was necessary to achieve (within the limits of certitude of these methodologies) that the non-crystalline model compounds were pure. The distinct advantage of this glycosidation method over the Koenigs–Knorr reaction is that it affords 40–80% yields even with hindered secondary alcohols that are scarce. This feature is of particular utility when, as in this instance, the configuration of the glycosyl moiety is less important than assured substitution at the hindered secondary alcohol of the aglycon.

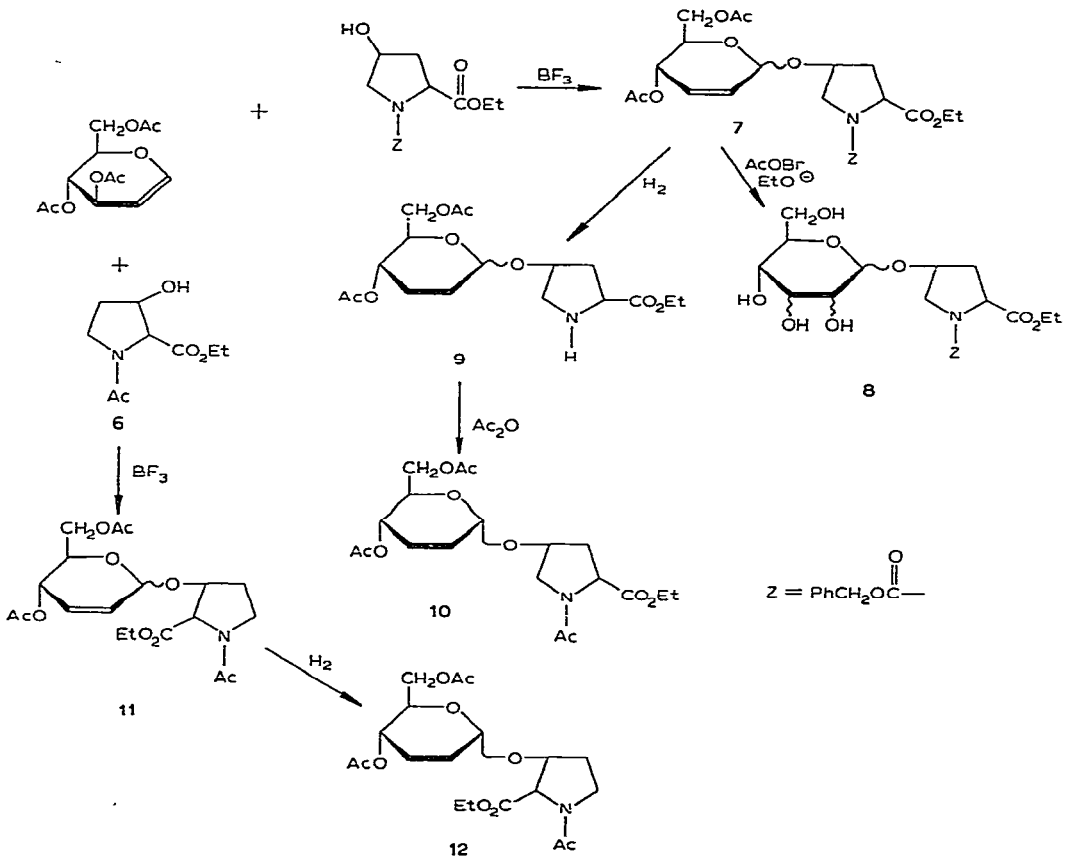

Chart 2

Addition reactions to the 2,3-double bond of the 4,6-di-*O*-acetyl-hex-2-eno-pyranosides were performed by the procedures of Albano and coworkers[11]. Although the 2,3-anhydro derivatives were not obtained by the method of Albano and co-workers[11], epoxidation did take place when hydrogen peroxide–benzonitrile was used[6]. Acetyl hypobromite gave products whose n.m.r.-spectral characteristics indicated the D-*altro* configuration[11], whereas ring opening of the 2,3-anhydro derivatives gave hexopyranosyl derivatives of undetermined configuration. One such

example of oxygen derivatization is given, namely the synthesis of compound **8**. Exact configurational assignment for **8** awaits further work.

The syntheses described provide routes, in good yield, to the isomers **10** and **12**. They were assigned the α-D-anomeric configuration by comparing their n.m.r.-spectral and optical rotatory data with those of similar α-D-linked compounds[1,6].

Unlike appropriately substituted 3-*O*-glycosyl-serine and -threonines[12,13], we have found the analogous 3-*O*-glycosyl-hydroxyproline to be stable toward base-catalyzed elimination, even in the presence of strong base. This fact is of importance for structural studies on glycopeptides that might contain glycosylated 3-hydroxyproline. Proof that the glycosidic linkage remained intact was discerned by t.l.c., polarimetry, and by n.m.r.-spectral examination of the reaction mixture. The natural 4-*O*-(arabinosyl)hydroxyproline glycopeptides were isolated after base-catalyzed hydrolysis of the peptide portion, a procedure that left the base-stable 4-*O*-(arabinosyl)hydroxyproline glycosides intact[2,3]. The present model compound demonstrates that this particular *N*-acetyl-3-*O*-hexopyranosylhydroxyproline ethyl ester does not undergo *beta*-elimination in base. If the 3-*O*-glycosyl linkage is present in the base-treated glycopeptides from the primary cell-wall material of plants, the glycosides should, therefore, withstand degradation and be isolable.

In dilute acid (pH 1.4) at 50°, the glycosides of the *N*-acetylhydroxyprolines are stable over an 8-h period, as determined by using a recording polarimeter and by chromatographic analysis of the reaction mixture. The glycosides of serine and threonine are appreciably hydrolyzed under these conditions[1], indicating that the glycosidic linkage to hydroxyproline has the greater acid-hydrolytic stability. The hydroxyproline glycosides are cleaved after 4 h in boiling 0.5M sulfuric acid. The hindrance to cleavage of this glycoside could be of hydrophobic and steric, rather than electrostatic, origin. The kinetic parameters for hydrolysis of the hydroxyproline glycosides must be determined before the relative acid stabilities can be meaningfully compared with those of other glycosides.

EXPERIMENTAL

General. — Procedures and instrumentation for characterization of compounds are as described by Egan and coworkers[1], for the chromatographic solvent systems described here. Petroleum ether refers to the fraction boiling at 30–60°. Hydrogenation was conducted with either an Aminco high-pressure shaking assembly or with a Parr shaker, series 3900. Silica gel columns were prepared with silica gel No. 7734, 0.05–0.2 mm, E. Merck Co., Darmstadt, West Germany.

Ethyl N-*benzyloxycarbonyl*-N-(*2-ethoxycarbonylethyl*)*glycinate* (**2**). — Ethyl *N*-(2-ethoxycarbonylethyl)glycinate (**1**) was prepared from ethyl glycinate and ethyl 3-bromopropionate according to Blake and coworkers[9]. To a solution of sodium carbonate (5.5 g) in water (70 ml) was added **1** (11 g, 0.055 mole) followed by freshly distilled benzyl chloroformate (9.3 g, 0.055 mole). The mixture was stirred for 48 h before pouring the contents into a separatory funnel and drawing off the sep-

arated lower layer. The yellow oil was dried (Na_2SO_4) before distillation; yield 5.8 g (32%), b.p. 170–178°/0.005 mm Hg; η_D^{26} 1.4930; R_F 0.64 (silica gel, 2:1, petroleum ether–ethyl acetate, iodine vapor); λ_{max}^{neat} 3.39, 5.70–5.91, 6.69, 6.83, 7.30, 8.20–8.51, 8.94, 9.69–9.60, 12.96, and 14.29–14.41 μm; n.m.r. (100 MHz, 20°, $CDCl_3$): δ 1.21 (6-proton triplet, CH_3), 2.63 (2-proton triplet, $-\overset{O}{\overset{\|}{C}}-CH_2-$), 3.61 (2-proton triplet, $-CH_2-N-$), 3.97–4.30 (6-proton multiplet, $-N-CH_2-\overset{O}{\overset{\|}{C}}-$ and ester CH_2), 5.11 (2-proton quartet, $PhCH_2O$), 7.27 (5-proton doublet, benzene ring H), and n.m.r. (100 MHz, 50°, $CDCl_3$): δ 1.21 (6-proton triplet, CH_3), 2.61 (2-proton triplet, $-\overset{O}{\overset{\|}{C}}-CH_2-$), 3.61 (2-proton triplet, $-CH_2-N-$), 3.96–4.30 (5-proton multiplet, $-N-CH_2-\overset{O}{\overset{\|}{C}}-$ and ester CH_2), 5.11 (2-proton singlet, $PhCH_2O$), and 7.28 (5-proton singlet, benzene ring H).

Anal. Calc. for $C_{17}H_{23}NO_6$: C, 60.52; H, 6.87; N, 4.15. Found: C, 60.39; H, 6.93; N, 4.22.

Ethyl N-*benzyloxycarbonyl-3-oxopyrrolidine-2-carboxylate* (3). — A three-necked flask, equipped with nitrogen inlet and pressure-equalizing dropping funnel, was oven-dried for 3 h at 125°. The flask was cooled in a nitrogen-filled drybox over phosphorus pentaoxide and Ascarite. Toluene (100 ml), dried with alumina and stored over calcium hydride, and solid potassium *tert*-butoxide (2.1 g, 18.8 mmoles, MSA Research Corporation) were mixed in the flask. The pale-yellow suspension was removed from the drybox and the nitrogen inlet and dropping funnel quickly inserted before cooling the flask in an ice bath. Compound **2** (4.5 g, 13.3 mmoles) in dry toluene (15 ml) was added over a period of 15 min to the rapidly stirred suspension. All of the solid dissolved in a few min, and the solution was stirred for 35 min in the cold under nitrogen. After this, cold glacial acetic acid (1.1 ml) was added, followed immediately by sodium dihydrogen phosphate monohydrate (7.4 g) in water (75 ml). The heterogeneous mixture was extracted with chloroform (4 × 50 ml), and the organic phase was washed with pH 7 phosphate buffer (0.1M, 2 × 20 ml), and dried (Na_2SO_4). Removal of the solvent yielded a yellow oil. The oil was dissolved in toluene (100 ml) and the mixture was cooled to 0° and then extracted with cold, pH 9.5 carbonate buffer (0.12*M*, 6 × 50 ml). The toluene solution was washed with water (20 ml), dried (Na_2SO_4), and evaporated to a yellow oil; yield 2 g (52%). T.l.c. (silica gel, 2:1 petroleum ether–ethyl acetate, iodine vapor) indicated two spots—the upper one had R_F 0.5–0.7 whereas the lower one streaked from the origin to R_F 0.2–0.3. A sample was purified by first chromatographing on a silica gel column (28 g, with 2-cm cross section) with 1:1 petroleum ether–ethyl acetate and collecting 10-ml fractions after an initial 60-ml fraction. The third and subsequent colored fractions were combined, evaporated, and the yellow liquid was rechromatographed on a silica gel column (3.5 × 9 cm) with 17:2 benzene–methanol. The first four 10-ml fractions, which contained the front-running zone, gave a yellow oil that was chro-

matographed on a preparative silica gel plate (20 × 20 cm) with 17:2 benzene–methanol, to give 4 zones (u.v. and visible light) that were eluted with abs. ethanol. The fastest-moving zone yielded a yellow oil, whereas the other zones gave only traces of material. The oil was chromatographed twice again on a silica gel column (1.5 × 5 cm) with chloroform as eluant. The 1-ml fractions collected were combined on the basis of t.l.c. to give a pale-yellow oil (**3**). Another sample, which had been chromatographed only once on a silica gel column (preceding front-running zone) and then distilled, had the same physical properties, and this method is recommended for preparative purposes. Samples purified by either method always showed a trace amount of the slower-moving spot on t.l.c. The physical properties of **3** were: b.p. 168–178° at 0.05 torr, η_D^{25} 1.5184; R_F 0.48 (silica gel, 2:1 petroleum ether–ethyl acetate, iodine vapor), R_F 0.93 (silica gel, chloroform, iodine vapor); no optical rotation (*c* 1 in ethanol); λ_{max}^{neat} 3.37, 5.65, 5.75 (–CO_2Et), 5.86 (C=O), 6.67, 6.81, 6.90, 7.00–7.10, 7.30, 7.40, 8.09, 9.60, 9.71–9.90, 12.98, 13.40, and 14.30 μm; n.m.r. (100 MHz, $CDCl_3$): δ 1.00–1.50 (3-proton multiplet, CH_3), 2.64 (2-proton triplet, H-4), 3.50–4.40 (4-proton multiplet, H-5 and ester CH_2), 4.54 (1-proton singlet, exchanges with D_2O, H-2), 5.12 (2-proton quartet, $PhCH_2O$), and 7.30 (5-proton singlet, benzene ring H).

Acceptable elementary analyses (for carbon) of the high-boiling oil (**3**) were not obtained.

Anal. Calc. for $C_{15}H_{17}NO_5$: N, 4.81. Found: N, 4.56.

The carbonate buffer solution was acidified to pH 3 with phosphoric acid and extracted with chloroform (3 × 100 ml). The dried (Na_2SO_4) extract afforded a yellow oil that crystallized after one day. The compound, tentatively identified as ethyl *N*-benzyloxycarbonyl-4-oxopyrrolidine-3-carboxylate (**4**), was recrystallized from cyclohexane by the addition of ether and petroleum ether (30–60°) to yield white crystals; m.p. 56–59°; R_F 0.0–0.3 (silica gel, 2:1 petroleum ether–ethyl acetate, iodine vapor); λ_{max}^{KBr} 2.80–2.94, 5.65, 5.85, 5.96, 7.00, 7.32, 7.42, 7.60, 8.70, 9.30–9.60, 13.00, 14.22, and 14.40 μm; n.m.r. ($CDCl_3$): δ 1.26 (3-proton triplet, CH_3), 3.82–4.45 (7-proton multiplet, H-2, H-3, H-5, and ester CH_2), and 5.16 (2-proton singlet, $PhCH_2O$, 7.31 (5-proton singlet, benzene ring H).

Condensation ring-closure of **2** was attempted by using sodium hydride and *n*-butyllithium as the base in place of potassium tert-butoxide; in both cases the yield consisted mostly of the 4-oxopyrrolidine product (**4**). A low yield of **3** was obtained when the reaction was conducted at room temperature and potassium tert-butoxide solution added to the solution of **2** (reverse addition).

cis-D,L-*3-Hydroxyproline ethyl ester hydrochloride* (**5**). — Removal of the *N*-benzyloxycarbonyl group and reduction of the carbonyl group were effected in one step by dissolving **3** (1.1 g, 3.78 mmoles) in abs. ethanol (18 ml) and adding water (1 ml), 2M HCl (1 ml), and 5% rhodium on alumina (1.5 g) before hydrogenating for 1 h at 100 lb.in^{-2}. The vessel was washed with ethanol, and the contents, after treatment with charcoal, gave a light-yellow solution. The solution was concentrated to about 10 ml, and ether (200 ml) was added to cloudiness followed by 30 drops of

2M HCl to complete precipitation of **5**. The white crystals were recrystallized twice by dissolving them in a minimal amount of ethanol and precipitating the product with ether; yield 0.25 g (30%), m.p. 166–169° with initial browning; R_F 0.50 (silica gel, 1:2:1, 1-butanol–1-propanol–0.1M HCl, ninhydrin spray); λ_{max}^{KBr} 2.84–3.04, 3.35, 3.50, 3.67, 5.75 (C=O), 6.30, 6.80, 7.10, 7.20, 7.79, 8.06, 8.52, 9.48, 11.08, 11.32, and 11.65 μm; n.m.r. (100 MHz, Me_2SO-d_6 with benzene added for downfield lock): δ 1.00–1.30 (3-proton triplet, CH_3), 1.90–2.15 (2-proton multiplet, H-4), 3.10–3.65 (3-proton multiplet, H-2 and H-5), 4.00–4.25 (2-proton quartet, ester CH_2), and 4.27–4.45 (1-proton doublet, H-3, $J_{2,3}$ 3.5 Hz). A sample submitted for analysis was first recrystallized as before and then dried for 6 h (NaOH and P_2O_5) in an Abderhalden drying-pistol over refluxing acetone.

Anal. Calc. for $C_7H_{13}NO_3 \cdot HCl$: C, 42.97; H, 7.16; N, 7.16; Cl, 18.18. Found: C, 42.85; H, 7.03; N, 6.93; Cl, 17.96.

N-*Acetyl*-cis-D,L-*3-hydroxyproline ethyl ester* (**6**). — Following the procedure of Roseman and Ludowieg[10], a water solution (10 ml) of **5** (0.2 g, 1 mmole) was stirred with ethanol (5 ml) and Dowex-1 (6.5 ml, carbonate form) in an ice bath before adding acetic anhydride (10 drops). T.l.c. showed no ninhydrin-active material after 10 min (solvent as for **5**). The suspension was filtered, and the resin was washed successively with ethanol (3 ml) and water (3 ml). The filtrate was evaporated to give a yellow oil, which was chromatographed on a silica gel column (1 × 7 cm) with 1:7 chloroform–acetone. The 1-ml fractions were combined on the basis of t.l.c. Removal of the solvent gave a clear oil that crystallized; yield 0.18 g (87%). m.p. 60–62°; R_F 0.72 (silica gel, 15:3 chloroform–acetone, iodine vapor); λ_{max}^{neat} 2.90–3.05, 3.38, 5.74, 6.03, 6.09, 6.81, 7.24, 8.19–8.50, 8.55, 8.92, and 10.00 μm; n.m.r. ($CDCl_3$): δ 1.28 (3-proton doublet of triplets, CH_3, racemic modification at $-CO_2Et$), 1.92–2.31 (2-proton multiplet, H-4), 2.08 (3-proton singlet, Ac), 3.48–3.91 (2-proton multiplet, H-5), and 4.04–4.82 (5-proton multiplet, H-2, H-3, –OH, and ester CH_2). Although compound **6** was chromatographically homogeneous and its n.m.r. spectrum showed the anticipated proton integrals, acceptable elementary analyses were not obtained for this compound.

N-*Benzyloxycarbonyl-4*-O-(*4,6-di*-O-*acetyl-2,3-dideoxy*-D-erythro-*hex-2-enopyranosyl*-trans-D,L-*hydroxypriline ethyl ester* (**7**). — Boron trifluoride etherate (3 drops) was added to a solution of *N*-benzyloxycarbonyl-4-*trans*-D,L-hydroxyproline ethyl ester (16 g, 54.6 mmoles, synthesized by the usual methods) and tri-*O*-acetyl-D-glucal (14.8 g, 54.5 mmoles) in dry benzene (100 ml)[1,6]. When additional boron trifluoride etherate (25 drops) was added, the solution became purple in 1 h. Solid anhydrous sodium carbonate was added until the purple color disappeared, and the solids were filtered and washed with benzene (20 ml). The benzene was evaporated to give a chromatographically homogeneous, viscous yellow oil; yield 17.2 g (64%); R_F 0.72 (silica gel, 2:1 petroleum ether–ethyl acetate, iodine vapor); λ_{max}^{neat} 3.30, 3.35, 3.40, 5.68, 5.76, 5.83, 6.64, 6.88, 7.00, 7.05, 7.28, 8.10, 9.49, 9.70, 10.98, 13.00, 13.53, 14.31, and 14.60 μm; n.m.r. ($CDCl_3$): δ 0.90–1.50 (3-proton multiplet, ester CH_3), 2.09 (6-proton singlet, acetate CH_3), 2.09–2.53 (2-proton multiplet, H-3), 3.70

(2-proton doublet, H-5), 3.89–4.78 (7-proton multiplet, H-2, H-4, H-5′, H-6′, and ester CH_2), 5.02–5.50 (4-proton multiplet, $PhCH_2O$, H-1′ and H-4′), 5.71–6.00 (2-proton multiplet, H-2 and H-3′), and 7.31 (5-proton singlet, benzene ringH′).

Proton-n.m.r. integrals were in agreement with the elementary compositions of compound **7**. Further characterization of the product was not effected at this stage and **7** was used as such for the synthesis of **8**.

N-*Benzyloxycarbonyl-4*-O-(D-erythro-*hexopyranosyl*)-trans-D,L-*hydroxyproline ethyl ester* (**8**). — Acetyl hypobromite addition to **7** was performed according to the procedure of Albano and coworkers[11]. An ethanolic solution (15 ml) of the acetyl hypobromite addition-product of **7** (1 g, 1.5 mmoles) was treated with sodium ethoxide solution (0.09 g of sodium in 15 ml of ethanol). The solution was stirred for 40 min at 60° and then overnight at room temperature prior to neutralization with Dowex-50 X8 (H^+ form). After filtering the mixture and treating the filtrate with charcoal, evaporation of the solvent gave a yellowish-white liquid that was chromatographed on a silica gel column (1.5 × 8 cm) with 17:2 benzene–methanol as eluant. The column effluent contraining the second yellowish band to be eluted was evaporated to an oil that was rechromatographed on a silica gel column (1.5 × 7 cm) with 1:7 chloroform–acetone. Removal of the solvent yielded a chromatographically homogeneous syrup that was the product of epoxide-ring opening; yield 0.3 g (44%); R_F 0.36 (silica gel, 17:1 benzene–methanol, iodine vapor); n.m.r. (100 MHz, $CDCl_3$) δ 1.00–1.40 (3-proton overlapping triplets, ester CH_3), 2.00–2.60 (2-proton multiplet, H-3), 3.20–4.70 (16-proton multiplet, H-2, H-4, H-5, H-2′, H-3′, H-4′, H-5′, H-6′, –OH′ and ester CH_2), 5.10 (3-proton quartet, H-1′ and $PhCH_2O$), and 7.30 (5-proton doublet, benzene ring H).

Anal. Calc. for $C_{21}H_{29}NO_{10}\cdot 0.5H_2O$: C, 54.40; H, 6.47; N, 3.02. Found: C, 54.48; H, 6.17; N, 2.90.

Drying under vacuum in a drying pistol (P_2O_5) to constant weight gave a loss in weight in agreement with 0.5 mole of water of hydration per mole of **8**. The configuration of **8** was not assigned.

4-O-(*4,6-Di*-O-*acetyl-2,3-dideoxy*-D-erythro-*hexopyranosyl*)-trans-D,L-*hydroxyproline ethyl ester* (**9**). — Compound **7** (1 g, 1.98 mmoles), dissolved in ethanol (18 ml) and water (2 ml), was hydrogenated over 5% rhodium on alumina catalyst (1.5 g) for 1.5 h at 190 $lb.in^{-2}$ (cleavage of the glycosidic acetal linkage was detected at 480 $lb.in^{-2}$). After filtering off the catalyst and evaporating the filtrate, a viscous yellow syrup resulted, which was chromatographed on a silica gel column (1 × 10 cm) with 1:2:1, 1-butanol–1-propanol–water. After combining all but the first 1-ml fraction, the solvent was removed, and the oil was rechromatographed on a silica gel column (1 × 15 cm) with 17:2 benzene–methanol as eluant with the collection of fractions as follows (in order of elution): 1, 1 ml; 2, 2 ml; 3, 5 ml; 4, 2 ml; and 5, 20 ml. Fractions 3–5 were combined and evaporated before chromatographing a final time. A silica gel column (1 × 20 cm) was used, and the elution with 3:2, chloroform–acetone was monitored by t.l.c. Removal of solvent gave a chromatographically

homogeneous, yellow oil; yield 0.17 g (23%); R_F 0.49 (silica gel, 3:2 chloroform–acetone, ninhydrin spray); n.m.r. ($CDCl_3$): δ 1.26 (3-proton triplet, ester CH_3), 1.60–2.55 (12-proton multiplet, H-3, H-2′, H-3′, and acetate CH_3), 3.00–3.25 (2-proton triplet, H-5), and 3.70–5.00 (9-proton multiplet, H-2, H-4, H-1′, H-4′, H-5′, H-6′, and ester CH_2). This syrupy compound was used without further characterization in the synthesis of **10**.

N-*Acetyl-4-O-(4,6-di-O-acetyl-2,3-dideoxy-α-*D-erythro-*hexopyranosyl)*-trans-D,L-*hydroxyproline ethyl ester* (**10**). — A solution of **9** (0.17 g, 0.46 mmoles) was *N*-acetylated[10] as before for **6**. The yellow oil was first chromatographed on a silica gel column (1.5 × 19 cm) with chloroform as eluant, collecting fractions until t.l.c. (silica gel, 15:3 chloroform–acetone, iodine vapor) showed only a faint spot below the solvent front. Ethanol was then used to remove a yellow band at the top of the column; evaporation of the ethanol yielded a pale-yellow oil that was chromatographed on a preparative silica gel plate (20 × 20 cm) with 15:3, chloroform–acetone. Separation of the plate into four zones (u.v. and visible light) and elution of each zone with acetone led to a pale-yellow oil from zone 3; yield 52 mg (28%), $[\alpha]_D^{26}$ +36.7° (*c* 0.62 in ethanol); R_F 0.68 (silica gel, 15:3 chloroform–acetone, iodine vapor); λ_{max}^{neat} 3.41, 5.75, 5.78, 6.02, 6.09, 6.82, 6.90, 7.08, 7.29, 8.00, 8.38, 9.12, 11.51, and 12.51 μm; n.m.r. $CDCl_3$): δ 1.28 (3-proton doublet-of-triplets, ester CH_3), 1.78–2.63 (15-proton multiplet, H-3, H-2′, H-3′, and acetate CH_3), and 3.41–4.90 (11-proton multiplet, H-2, H-4, H-5, H-1′, H-4′, H-5′, H-6′, and ester CH_2). The n.m.r. spectrum of **10** displayed proton integrals consistent with its assigned structure.

Anal. Calc. for $C_{19}H_{29}NO_9$: C, 54.93; H, 7.04; N, 3.37. Found: C, 54.65; H, 6.84; N, 3.18.

The anomeric configuration of **10** was adequately established by analogy with previous work[1] by using n.m.r. and optical rotatory data.

N-*Acetyl-3-*O-*(4,6-di-*O-*acetyl-2,3-dideoxy-*D-erythro-*hex-2-enopyranosyl)*-cis-D,L-*hydroxyproline ethyl ester* (**11**). — 3,4,6-Tri-*O*-acetyl-D-glucal (0.157 g, 0.58 mmoles) and **6** (0.116 g, 0.58 mmoles) were stirred together in dry benzene (25 ml), and boron trifluoride etherate (20 drops) was added[1,6]. A drying tube was then inserted. The mixture became purple after 20 min and an oily precipitate adhered to the walls of the flask. No. 3,4,6-tri-*O*-acetyl-D-glucal was present after this time (t.l.c., silica gel, 2:1 petroleum ether–ethyl acetate, iodine vapor). Solidanhydrous sodium carbonate was added to discharge the purple color and decompose any excess boron trifluoride etherate. The solids were filtered off and washed with benzene (20 ml); evaporation of the filtrate produced a yellow oil that was chromatographed on a column (1 × 10 cm) of silica gel with 15:1 chloroform–acetone, as eluant with the collection of 5-ml fractions. Fractions 3–10 were combined, and the solvent was removed to give a chromatographically homogeneous yellow oil; yield 0.1 g (42%); R_F 0.51 (silica gel, 2:1 petroleum ether–ethyl acetate, iodine vapor); λ_{max}^{neat} 3.38–3.43, 5.72, 5.79, 5.92, 6.02, 6.08, 7.07, 7.30, 8.28, and 10.98 μm; n.m.r. ($CDCl_3$): δ 1.27 (3-proton triplet, ester CH_3), 1.93–2.19 (11-proton multiplet, H-4 and acetate CH_3), 3.54–3.78 (2-proton

multiplet, H-5), 4.03–4.50 (5-proton multiplet, H-5′, H-6′, and ester CH_2), 4.55–4.81 (2-proton multiplet, H-2 and H-3), 5.10–5.33 (1-proton multiplet, H-4′), 5.37–5.50 (1-proton multiplet, H-1′), and 5.80–6.00 (2-proton multiplet, H-2′ and H-3′).

The oily precipitate was removed from the flask with chloroform. After treating with charcoal it was found to be unreacted **6**. Although n.m.r. spectroscopy displayed acceptable proton integrals for compound **11**, no further characterization of the product was effected and it was used as such in the synthesis of **12**.

N-*Acetyl-3*-O-(*4,6-di*-O-*acetyl-2,3-dideoxy*-α-D-erythro-*hexopyranosyl*)-cis-D,L-*hydroxyproline ethyl ester* (**12**). — Compound **12** was synthesized by hydrogenating **11** (0.1 g, 0.24 mmoles) in abs. ethanol (10 ml) at 110 lb.in^{-2} for 50 min with 5% rhodium on alumina (1 g) catalyst. The vessel was washed out with ethanol, the mixture was filtered, and the filtrate was condensed to a white oil. The oil was purified on a preparative silica gel plate (10 × 20 cm) with 15:3 chloroform–acetone as eluant.The layer was separated into four zones, which were located by u.v. and visible light, and eluted with acetone. The third zone with respect to the fastest-migrating zone co tained the desired product, which was isolated as a chromatographically homogeneous, hygroscopic syrup; yield 14 mg (14%); $[\alpha]_D^{26}$ +41.7° (*c* 0.24 in ethanol); R_F 0.60 (silica gel, 15:3 chloroform–acetone, iodine vapor); λ_{max}^{neat} 3.41, 5.72, 5.80, 6.09, 6.81, 6.99, 7.30, 7.62, 10.66, and 10.77 μm; n.m.r. (100-MHz, $CDCl_3$): δ 1.31 (3-proton multiplet, ester CH_3), 1.67–1.91 (4-proton multiplet, H-2′ and H-3′), 1.94–2.20 (11-proton multiplet, H-4 and acetate CH_3), 3.55–3.90 (2-proton multiplet, H-5), 4.00–4.40 (6-proton multiplet, H-4′, H-5′, H-6′, and ester CH_2), 4.50–4.75 (2-proton multiplet, H-2 and H-3), and 5.00–5.10 (1-proton multiplet, H-1′).

The anomeric configuration of **12** was assigned by analogy with previous work[1], by using n.m.r. and optical rotatory data.

Anal. Calc. for $C_{19}H_{29}NO_9 \cdot 0{,}5H_2O$: C, 52.82; H, 6.99; N, 3.24. Found: C, 53.00; H, 6.47; N, 3.34.

Drying under vacuum in a drying pistol (P_2O_5) to constant weight gave a loss in weight in agreement with 0,5 mole water of hydration per mole of **12**.

*Assessment of stabilities of 3- and 4-*O*-glycosylprolines toward base and acid.* — Following the procedures outlined in ref. 13, treatment of the 3- and 4-*O*-(glycosyl) hydroxyprolines (**7–12**) with base (0.05M sodium hydroxide in 10% ethanol), or more-vigorous reaction conditions, even at 50°, gave no indication of the base-catalyzed degradation or *beta*-elimination previously described for appropriately substituted 3-*O*-(glycosyl)-serine and -threonine[13]. Reaction mixtures were monitored by t.l.c., polarimetry, and by n.m.r. analyses of recovered starting material.

Unlike the analogous serine and threonine derivatives[1], compounds **6–12** were stable in 10 mM sulfuric acid for 8 h at 50°, within the limits of detection by t.l.c. and polarimetry. At ~100° in the same acid medium, these glycosides were cleaved at rates analogous to those for other glycosides[1].

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