GLUCOSYLATIONS OF PREGN-5-ENE-3β,20R-DIOL

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ABSTRACT

The four possible monoglucosides of the pregn-5-ene-3β,20R-diol were synthesized along with a mixture of the four possible 3,20-diglucosides. The glucosides were characterized by HPLC and mass spectrometry.

INTRODUCTION

The discovery (1) of [^{14}C]pregn-5-ene-3β,20R-diol[a] conjugated with three molecules of glucose in eggs and ovaries of tobacco hornworms (Manduca sexta (L.)) treated as pupae with [^{14}C]cholesterol created the need for simpler glucosides of pregnenediol, both to aid in the identification of the total structure of the triglucose derivative and to evaluate in their own right for possible roles in insect sterol metabolism.

MATERIALS AND METHODS[b]

Melting points are uncorrected. Pregnenediol and pregnenolone were purchased from Sigma Chemical Co. Acetobromoglucose was prepared according to Vogel (2). Trimethylsilyl ethers of sterols were prepared with trimethylsilylimidazole in pyridine, partitioned into dichloromethane from water, and dried. Beyond thin layer chromatography (TLC) and/or gas-liquid chromatography (GLC) confirmation of purity, they were not further characterized.

(GLC) was performed on a Shimadzu model GC-9A instrument equipped with a flame ionization detector, a split-splitless injector operated at ca. 50:1 split ratio, and a 15 m DB-1 fused silica column with helium as a carrier. High-performance liquid chromatography (HPLC) was carried out on a Spectra Physics model

8700 system with a variable wavelength UV detector operated at 210 nm, fitted with a 250 x 4.6 mm Whatman Partisil 5 ODS-3 column or with a 250 x 4.6 mm Dupont Zorbax-CN column. In both cases, water containing 0.1% acetic acid and acetonitrile were the primary and secondary solvents, respectively. Mass spectra were obtained from a Finnigan model 4510 gas chromatograph-mass spectrometer. Samples were introduced either via a direct exposure (0-100 ma @ 20 ma/sec) probe or through a 30 m x 0.32-mm id DB-1 fused silica column. Electron ionization spectra were collected at 70 eV and a source block temperature of 150°C. Both ammonia and methane were used for production of chemical ionization mass spectra. Ammonia at a source temperature of 80° and pressure of 0.6 Torr yielded a reagent gas ionic distribution of 19:32:100:20, NH_4^+:$(NH_3)_2H^+$:$(NH_3)_3H^+$:$(NH_3)_4H^+$. An ionic gas distribution of 68:15:100:27, CH_5^+:H_3O^+:$C_2H_5^+$:$C_3H_5^+$ was provided by methane at 80°C and 0.3 Torr. Elevation of the source temperature to 120° shifted the distribution to 100:16:98:22. Data were analyzed via an Incos data system.

Pregnenediol-3β-O-D-glucopyranoside **1c**. A mixture of pregnenediol **1a** (0.28 g, 1 mmol), acetobromoglucose **2a** (0.82 g, 2 mmol), silver oxide (0.54 g), pulverized calcium sulfate (1 g), and benzene (12 mL) was stirred in the dark 2.5 days; then an additional portion of silver oxide was added, and stirring was continued an additional day. Chromatography on 50 g silica gel with benzene and increasing portions of ethyl acetate gave 0.26 g crystalline glucoside acetate **1b** (20-24% EtOAc) that could be recrystallized from heptane plus benzene, mp 219-221°. A solution of **1b** (139 mg) in warm methanol (4 mL) was treated with 1 N NaOH (1.25 mL). A clear solution resulted for approximately 0.5 min; then a white solid began to separate. After standing overnight at room temperature, **1c** (82 mg) was collected by filtration and washed with water, mp 283° dec (darken ca. 270°).

Pregnenediol 20-O-β-D-glucopyranoside **1d** was obtained by saponification of the 3-benzoate-20β-tetra-O-acetylglucoside **1e** (mp 214-219°) which could be isolated (along with unreacted **1f**) from the reaction of **1f** and **2a** under the conditions described for the preparation of **1b**. Alternatively, **1f** (100 mg) and **2a** (140 mg) were combined in dry dichloromethane (4 mL) under N_2; then tetramethylurea (150 µL) and silver trifluoromethanesulfonate (silver triflate, 92 mg) were added. The mixture was stirred in the dark 2 h, another portion (79 mg) of **2a** was added, stirring was continued an additional 1.5 h, then the mixture was filtered and the filtrate was washed with aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated to provide 0.31 g residue that was chromatographed on 15 g silica gel with benzene and increasing increments of EtOAc. The

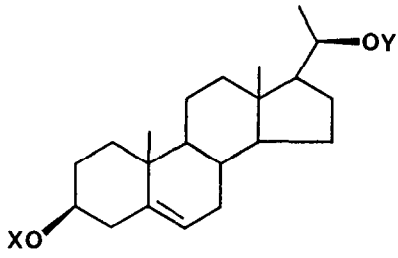
3-benzoate-20-acetate **1g** (54 mg, m.p. 195-201°, reported (3) mp 194-196°) was eluted with 5-6% EtOAc, followed by **1e** (ca. 50 mg) with 9-10% EtOAc. Saponification of **1e** gave **1d**, mp 252-257° (yellow>230°) after recrystallization from MeOH.

Reaction of **1a** and **2a** under conditions similar to those just described (overnight at room temperature in this case) gave a complex mixture (by TLC) that after column chromatography yielded only two crystalline products: pregnenediol diacetate **1h** (eluted with 4-6% EtOAc in benzene, mp 126-127° (reported (4) mp 138.5-140°), identical to a sample prepared from **1a** and Ac_2O); and the peracetylated 20β-monoglucoside **1i**, mp 218-224°, eluted with 12-15% EtOAc. Saponification of **1i** provided **1d**.

Pregnenediol-3-O-α-D-glucopyranoside **1j**. A solution of 1.2 g of the trimethylsilyl ether **1k** of the 20-monobenzoate **1l** (4) and 0.99 g of 1-fluoro-2,3,4,6-tetra-O-benzyl-1-deoxyglucose **2b** (8) in dry dichloromethane (8 mL) was treated with 50 µL of BF_3-Et_2O, stirred overnight at room temperature, and then partitioned between aqueous $NaHCO_3$ and dichloromethane. Concentration of the dried organic phase gave 1.93 g of a glass that contained (by HPLC) a 5:4 mixture of **1n** and **1o**. Column chromatography on silica gel gave two main fractions: 1.5 g of **1n** + **1o** eluted with 4% EtOAc in benzene, and 0.4 g of **1l** eluted with 8% EtOAc. The **1n** + **1o** mixture was rechromatographed on 100 g silica gel with slowly increasing increments of ether in benzene: a fraction (0.08 g) of 99% **1n** was eluted with 2% ether; subsequent fractions contained increasing percentages of **1o** such that 0.12 g of later-eluting (3% ether) fractions consisted of >92% **1o**. A portion of early-eluting material (enriched in **1n**; 0.67 g) was refluxed overnight in a solution of 6 mL THF and 2.5 mL of 1 N KOH in MeOH. The mixture was diluted with water and partitioned into dichloromethane to provide 0.61 g crude **1p** + **1q**; chromatography on silica gel provided 0.26 g essentially pure **1p** as a glass (6% ether in benzene) followed by mixtures of **1p** and **1q**. A solution of ca. 0.25 g **1p** in THF (4 mL) + 2-propanol (1 mL) was added to 10 mL condensed NH_3; then small pieces of Li were added over 1.5 h. The NH_3 was allowed to evaporate, aqueous NH_4Cl was added, and the mixture was evaporated to dryness. Water was added and crude **1j** was collected by filtration; addition of MeCN to a solution of **1j** in warm MeOH, followed by chilling, gave 104 mg of a white solid that was then recrystallized from H_2O + 2-propanol, mp 208-213° (shrink >200°).

Pregnenediol-20-O-α-D-glucopyranoside **1r**. The TMS-ether **1s** (0.90 g) of the 3-monobenzoate **1f** was combined in dichloromethane with 1.05 g **2b** as described for the preparation of **1j** except that in this case trimethylsilyl

Scheme 1



1a X = Y = H

1b X = tetra-O-acetyl-β-D-glucopyranoside, Y = H

1c X = β-D-glucopyranoside, Y = H

1d X = H, Y = β-D-glucopyranoside

1e X = C_6H_5CO, Y = tetra-O-acetyl-β-D-glucopyranoside

1f X = C_6H_5CO, Y = H

1g X = C_6H_5CO, Y = CH_3CO

1h X = Y = CH_3CO

1i X = CH_3CO, Y = tetra-O-acetyl-β-D-glucopyranoside

1j X = α-D-glucopyranoside, Y = H

1k X = $(CH_3)_3Si$, Y = C_6H_5CO

1l X = H, Y = C_6H_5CO

1n X = tetra-O-benzyl-α-D-glucopyranoside, Y = C_6H_5CO

1o X = tetra-O-benzyl-β-D-glucopyranoside, Y = C_6H_5CO

1p X = tetra-O-benzyl-α-D-glucopyranoside, Y = H

1q X = tetra-O-benzyl-β-D-glucopyranoside, Y = H

1r X = H, Y = α-D-glucopyranoside

Scheme 1, continued

1s X = C_6H_5CO, Y = $(CH_3)_3Si$

1t X = C_6H_5CO, Y = tetra-O-benzyl-α-D-glucopyranoside

1u X = C_6H_5CO, Y = tetra-O-benzyl-β-D-glucopyranoside

1v X = Y = β-glucopyranoside

1w X = β-D-glucopyranoside, Y = α-D-glucopyranoside

1x X = α-D-glucopyranoside, Y = β-D-glucopyranoside

1y X = Y = α-D-glucopyranoside

1z X = Y = $(CH_3)_3Si$

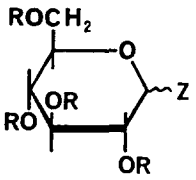
1aa X = Y = tetra-O-benzyl-β-D-glucopyranoside

1bb X = tetra-O-benzyl-β-D-glucopyranoside, Y = tetra-O-benzyl-α-D-glucopyranoside

1cc X = tetra-O-benzyl-α-D-glucopyranoside, Y = tetra-O-benzyl-β-D-glucopyranoside

1dd X = Y = tetra-O-benzyl-α-D-glucopyranoside

1ee X = Y = tetra-O-acetyl-β-D-glucopyranoside



2a. R = CH_3CO; Z = α-Br

2b. R = $C_6H_5CH_2$; Z = α + β-F

trifluoromethanesulfonate (75 µL) was employed as catalyst. From chromatography, first on alumina and then on silica gel, it was possible to obtain pure samples of both the 20α-anomer **1t** and the 20β-anomer **1u**. Lithium/ammonia reduction of **1t** as described for **1p** and saponification of the benzoate gave **1r**, mp 264° dec (darken>250°) from MeOH. Similar Li/NH_3 debenzylation of **1u** followed by saponification gave the 20β-monoglucoside **1d**, identical to that prepared from acetobromoglucose **2a**, further confirming these stereochemical assignments.

3,20-Diglucopyranosides **1v,1w,1x,1y**. A solution of di-TMS ether **1z** (1.09 g) and **2b** (2.85 g) in dichloromethane (10 mL) was treated at room temperature with 100 µL BF_3-Et_2O. After stirring 1.5 h, the usual workup gave 3.18 g of a viscous oil. Chromatography on silica gel (1-2% Et_2O in benzene) separated **1aa-1dd** from extraneous materials, but separation of individual isomers was not achieved. Accordingly, 1.65 g of the mixture was debenzylated with Li/NH_3 as described earlier. The diglucosides **1v-1y** were quite water-soluble and difficult to separate from inorganic by-products; therefore, a portion of the material in water was added to a C18 extraction cartridge that was rinsed with water, then eluted with methanol. The methanol eluate was chromatographed on a small Florisil column with increasing amounts of 95% ethanol in chloroform; most of **1v-1y** eluted in the 40-50% ethanol fractions. HPLC analysis showed comparable amounts of each of the isomers, and small samples of each, particularly **1v**, were obtained by preparative HPLC for mass spectral analysis.

RESULTS AND DISCUSSION

The β-anomers of the monoglucosides were addressed first because it is well known that reaction of acetobromoglucose (**2a**) and alcohols with silver catalysts gives nearly exclusively β-substitution (5). It is also well known (6) that of the two hydroxyls of **1a** (Scheme 1), the 3-OH is by far the more reactive, and indeed, mono(peracetyl)glucosylation at the 3-OH was achieved with silver oxide and calcium sulfate without protection of the 20-OH, giving **1b** from which the 3-mono-β-glucoside **1c** was obtained by saponification. The

mono(peracetylated)-β-glucoside of pregnenolone could similarly be prepared; saponification and $NaBH_4$ reduction also provided **1c**, contaminated in this case with a small amount of the 20-S-epimer.

The 20-mono-β-glucoside **1d** was prepared via **1e** from the 3-monobenzoate **1f** (3) and **2a**, either under conditions similar to those used to prepare **1b**, or using the procedure of Hanessian and Banoub (7) that employs silver trifluoromethanesulfonate (triflate) and tetramethylurea. The latter conditions promoted a much more rapid reaction, but transacetylation competed with glucosylation, and two major products were separated by chromatography on silica gel: the 3-benzoate, 20-acetate **1g** (3) and the desired 3-benzoate of the peracetylated 20β-glucoside **1e**. Saponification of **1e** gave the 20-mono-β-glucoside **1d**.

Treatment of diol **1a** with excess **2a** and silver triflate/tetramethylurea was not a useful route to the peracetylated diglucoside **1ee**; a rather complex mixture resulted that, after column chromatography, yielded the 3,20-diacetate **1h** (4) as a major product and a smaller amount of the peracetylated 20-mono-β-glucoside **1i**. Saponification of **1i** also provided **1d**.

To obtain the α-glucosides, we employed 1-fluoro-2,3,4,6-tetra-O-benzyl-1-deoxyglucose **2b** (8) (obtained and used as a

presumed mixture of anomers). Hashimoto et al (9) described reactions of **2b** with TMS ethers of various alcohols catalyzed by SiF_4 or TMS trifluoromethanesulfonate to give mixtures of α- and β-(benzylated)-glucosides, and reported that the α/β ratio could be easily manipulated by simply changing the solvent: ether gave predominantly α-glucoside derivatives, whereas acetonitrile gave largely β-glucoside derivatives. Kunz and Sager (10) found that similar reactions in dichloromethane with boron trifluoride etherate gave predominantly α-anomers in some instances (evidently the orientation of the fluorine in **2b** does not affect the anomeric disposition of the product). In our experiences with **2b** and TMS ethers related to **1a**, the couplings proceeded fairly smoothly to give mixtures of anomers whose ratios were in fact influenced by solvent, but to a less useful extent than indicated by Hashimoto et al (9). Accordingly, most reactions were carried out in methylene chloride with BF_3-Et_2O for convenience, and column chromatography was used to separate anomeric products. The 20-monobenzoate **1l** (4) was converted to its TMS ether **1k**, which in turn was reacted with **2b** plus BF_3-Et_2O to give a mixture of **1n** (α) and **1o** (β) in an approximate ratio of 4:5 (by HPLC). Separation by chromatography on silica gel (**1n** eluted before **1o** both from silica gel and from reverse-phase HPLC columns) followed by saponification and

Li/NH_3 debenzylation of **1n** gave the 3-mono-α-glucoside **1j** (the benzoates were incompletely cleaved by the Li/NH_3 conditions, and a separate saponification was necessary).

Pregnenediol-20α-glucopyranoside **1r** was similarly prepared from the 3-monobenzoate **1f** via its TMS ether **1s** except that in this case trimethylsilyl trifluoromethanesulfonate was used as the catalyst. In this case too, the benzylated 3-mono-α-glucoside **1t** eluted earlier than the β-anomer **1u** from both silica and reverse-phase columns. Saponification and debenzylation with Li/NH_3 of **1t** gave the 20-mono-α-glucoside **1r.** The bis-TMS ether **1z** was also reacted with **2b** and BF_3-Et_2O to give a mixture of the four isomeric perbenzylated diglucosides in the approximate ratio of (in order of elution from reverse-phase-HPLC) 33:26:24:17. The mixture was not separated but rather was debenzylated with Li/NH_3 to give a mixture of **1v, 1w, 1x, 1y.**

HPLC The isomeric monoglucosides of pregnenediol could be separated on either C18 or CN (reverse-phase) columns; frequently employed conditions consisted of 30% acetonitrile (containing 0.1% acetic acid) at 1.5 mL/min and a 250 x 4.6 mm C18 column. Figure 1 illustrates a chromatogram of a mixture of the four isomeric monoglucosides; it can be seen that here (in contrast to the benzylated derivatives) β-anomers eluted earlier than α-anomers (3β>3α, 20β>20α) and also that the 3-glucosides eluted

earlier than the 20-glucosides (3α>20α, 3β>20β). Figure 2 illustrates a chromatogram of the mixture of 3,20-diglucosides **1v**, **1w**, **1x** and **1y** from a CN column with 20% acetonitrile (very similar results were obtained with the C18 column); from the elution orders discussed above, it is evident that the first-eluting diglucoside must be the 3β,20β isomer **1v**, and the last the 3α,20α isomer **1y** (we have not attempted to assign the other two isomers **1w** and **1x**).

Mass Spectra of the four monoglucosides **1c**, **1d**, **1j**, and **1r**, along with that of diglucoside **1v**, were evaluated under several conditions to determine whether they would be of diagnostic value in the identification of our isolated triglucoside and other polysaccharides. Ammonia chemical ionization (NH_3 CI) at 0.6 Torr and a source temperature of 80° of all four monoglucosides yielded ammonium adduct ions at m/z 498 with little or no fragmentation. The β,β-diglucoside **1v** provided an ammonium adduct ion at m/z 660 (100%, $M + NH_4^+$) along with a cluster of moderately abundant ions at: m/z 498 (28%), m/z 480 (42%, $M + NH_4^+$-glucose), m/z 463 (18%, loss of water from a protonated monoglucoside neutral fragment). Also present were ions indicative of the pregnenediol aglycone (Agly) moiety: m/z 318 (10%, Agly), m/z 301 (14%, Agly + H^+-H_2O), m/z 283 (30%, Agly + H^+-$2H_2O$), as well as an ion at m/z 180 (45%) from the glucose groups.

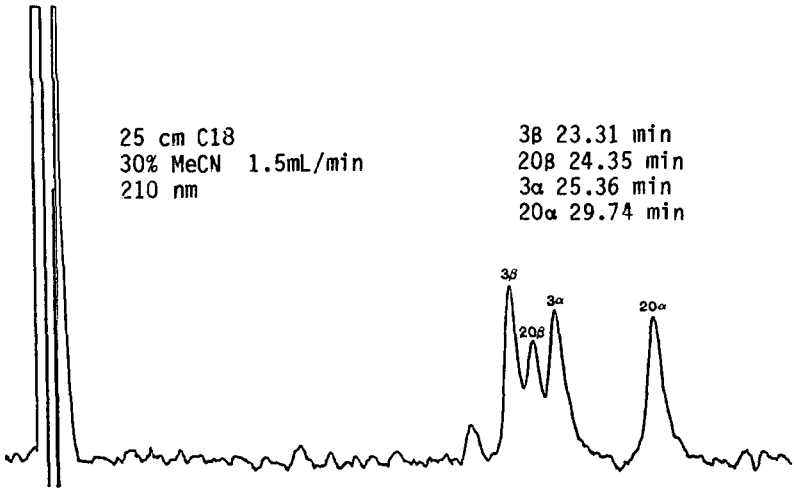


Figure 1. HPLC of pregnenediol monoglucosides.

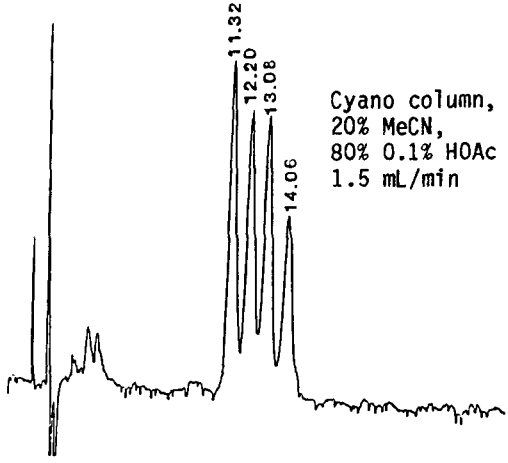


Figure 2. HPLC of pregnenediol of 3,20-diglucosides.

Methane Chemical Ionization (CH_4 CI) The CH_4 CI spectra of the 20α- and 20β-monoglucosides were very similar, each displaying prominent ions at m/z 463 (25-55%, $M+H^+-H_2O$), 301 (100%, $M+H^+$-glucose), and 283 (35-65%, $M+H^+$-glucose-H_2O). In each case, reduction of the source temperature from 120° to 80° resulted in an increase (nearly doubling) of the m/z 463 peak with a concomitant decrease in the m/z 283 peak. In contrast, the m/z 463 ion was absent or barely detectable in the CH_4 CI spectra of both the 3α- and 3β-isomers; again the m/z 301 was the most intense ion, although in the spectrum of the 3α-anomer **1j**, the m/z 283 ion was almost as intense. The 3β,20β-diglucoside **1v** gave the same three major ions (m/z 463, 301, 283), although m/z 463 was predominant, with m/z 643 ($M+H^+$) being barely detectable; weaker ions (≤10%) were also visible at m/z 481, 353, and 325.

Participation of the Δ^5-double bond, either in ionization of the 3-substituent or in stabilization of the resulting ion, is almost certainly the reason that $M+H^+$-glu (m/z 301) constitutes the highest mass ions observed in the CH_4 CI mass spectra of the 3-glucosides, whereas ions resulting from loss of water (m/z 463, glucose retained) were observed for the 20-glucosides.

The electron impact spectra of the 3α- and 3β-monoglucosides were very similar to each other and remarkably simple, exhibiting ions at m/z 300 (100%, M^+-glucose), m/z 301 (ca. 30%), and 283

(20-25%, M^+-glucose - OH), with virtually no ions of higher mass and no lower mass (>m/z 50) ions of relative intensities over 20%. The EI spectra of the 20α- and 20β-monoglucosides were also rather similar to each other, but were noticeably different from those of the 3-monoglucosides. In each of the 20-glucoside spectra, m/z 283 had a relative intensity of 100%, and each had m/z 300 of 68-80%; but in each case (in contrast to the spectra of the 3-glucosides) m/z 301 was more abundant than m/z 300. Again there were no higher mass ions of significance, but in each of these cases a series of lower mass ions was evident, with m/z 163, 151, 145, and 133 being particularly noteworthy.

In summary, stereochemistry of the anomeric linkage had minimal effect on the mass spectra of the glucosides, whereas position (3 vs 20) had a profound effect. Presumably participation of the Δ^5-double bond in ionization (and ion stabilization) at the 3-position is a principal factor. Thus, mass spectra can be useful to distinguish between possible sites of substitution, but perhaps less so in determining anomeric configurations.

ACKNOWLEDGMENTS

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NOTES

[a]All pregnenediol derivatives described in this paper have the Δ^5-3β,20R (i.e. 3β,20β)-configuration. To avoid possible

confusion, this stereochemistry is to be understood, and all subsequent use of the α- and β-descriptors will be reserved for discussion of stereochemistry at the anomeric carbons of glucose units.

[b]Mention of a proprietary product does not necessarily constitute an endorsement by the USDA.

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