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# Synthesis of Estriol 16α-(α-D-Glucoside) by Enzymic Transglucosylation*

S. C. Pan

ABSTRACT: Formation of alkyl glucosides was demonstrated when lower alkanols and maltose were incubated together in the presence of the culture filtrate of *Aspergillus niger* (NRRL 330) or of commercial fungal amylase preparations. Of several steroidal alcohols tested, those with a 16α,17β- or a 16α,17α-diol grouping were also converted into their glucosides. The product from estriol has been isolated in crystalline form and identified as an α-glucoside of estriol. The isolation of estriol 3,17β-dimethyl ether from the acid hydrolysate of the fully methylated glucoside established the structure of the glucoside as 3,17β-dihydroxy-$\Delta^{1,3,5(10)}$-estratrien-16α-yl α-D-glucopyranoside.

Most studies of the oligosaccharide transglycosylases have dealt with the synthesis of polysaccharides or new oligosaccharides, with sugars (mono-, oligo- or polysaccharides) as the glycosyl acceptors (Hehre, 1951; Edelman, 1956; Hassid and Neufeld, 1962; Li and Shetlar, 1964; Manners *et al.*, 1968). Examples are known of compounds other than sugars acting as the glycosyl acceptors, with the resultant formation of heterosaccharides (Edelman, 1956). Syntheses of glycosides of lower alkanols (Whelan and Jones, 1953; Bealing, 1953), polyols (Whelan and Jones, 1953; Boos *et al.*, 1966), glucuronic acid (Barker *et al.*, 1959), riboflavin (Whitby, 1954; Tachibana *et al.*, 1957; Suzuki and Uchida, 1969), and aniline (Miwa *et al.*, 1960), by the use of the oligosaccharide transglycosylases have been reported. However, no steroidal alcohol has been considered capable of acting as the glycosyl acceptor in this type of reaction.

As a continuation of our earlier studies on the transglycosylase activity of the culture filtrate of *Aspergillus niger* (Pan *et al.*, 1951, 1953; Wolfrom *et al.*, 1951), we showed that the same enzyme preparation can transfer a glucosyl group from maltose not only to simple alkanols, but also to certain steroidal alcohols, *e.g.*, estriol. This paper presents a detailed account of these findings, especially on the isolation and identification of estriol 16α-(α-D-glucoside) thus synthesized.

Syntheses of steroid glucosides by conventional chemical methods have been amply documented (Miescher *et al.*, 1942; Miescher and Meystre, 1943; Huebner *et al.*, 1944; Elce *et al.*, 1967; Schneider and Bhacca, 1969). By an enzymic glycosyl transfer from uridine diphospho derivatives of monosaccharides, *e.g.*, glucose or glucuronic acid, steroid glucosides, and glucuronides have also been synthesized (Dutton, 1956; Eichenberger and Newman, 1968). The

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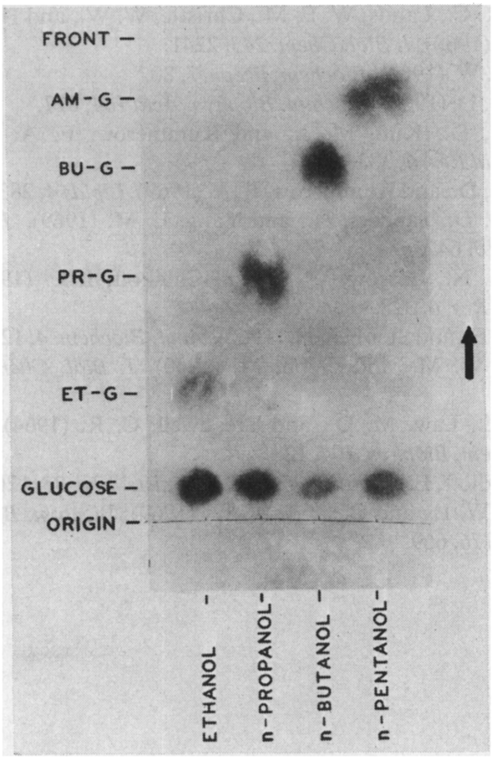

FIGURE 1: Paper chromatographic demonstration of the syntheses of alkyl glucosides.

synthesis reported in the present paper represents an entirely different approach to the problem. It involves a simple reaction and utilizes an inexpensive source of glucose. Furthermore, the α-D-glucosides in which the glucosyl group is attached to the 16α-hydroxyl group of a steroidal 16α,17-(α- or β-)diol are hitherto unreported compounds.

## Materials and Methods

All steroids and sugars are commercially available pure compounds. The Rhozyme S sample was obtained from Rohm and Haas Co. of Philadelphia, Pa., and the Clarase 900 and Clarase concentrate samples from Miles Chemical Co., Clifton, N. J. These enzymes are all fungal amylase preparations. The preparation of the culture filtrate of *Aspergillus niger* NRRL 330 was described in an earlier publication (Pan *et al.*, 1951). The maltase and emulsin samples were obtained from Nutritional Biochemicals Corp.

Descending-type chromatography on Whatman No. 1 paper was employed throughout this study. Thin-layer chromatography was carried out with precoated E. Merck AG silica gel F-254 plates (Brinkmann Instruments Inc.), 5 × 10 cm (0.25 mm thick), for routine test purposes and 20 × 20 cm plates (0.5 or 2 mm thick) for preparative scale chromatography.

Reducing sugars were detected on the papergrams with the blue tetrazolium reagent (Pan, 1962). Nonreducing (or reducing) sugars were detected by dipping the papergrams into a saturated solution of potassium metaperiodate in water and then, after air drying, into the blue tetrazolium reagent (S. C. Pan, 1967, unpublished data). Sulfuric acid charring technique (Privett and Blank, 1961) was applied to thin-layer chromatograms for detecting sugars and other organic compounds. Pauly's diazotized sulfanilic acid reagent or the phenol reagent of Folin and Ciocalteu was used to show phenolic spots and the Chromatovue (Ultra-Violet Products, Inc.) was employed to show ultraviolet-absorbing spots on thin-layer chromatogram.

All melting points were taken in capillary tubes and are uncorrected. Optical rotations were determined in chloroform, unless otherwise specified. Ultraviolet spectra were determined on a Cary 11, infrared spectra on a Perkin-Elmer 21, and nuclear magnetic resonance spectra on a Varian A-60 spectrometer in $CDCl_3$ solution (unless otherwise specified) with tetramethylsilane as the internal standard. The chemical shifts are reported as their $\tau$ values.

## Experimental Section and Discussion

*Demonstration of the Synthesis of Alkyl Glucosides.* Before attempting the synthesis of steroid glucosides, we tried to find out first whether the lower alkanols can behave as glucosyl acceptors. For this purpose, a reaction mixture containing the following ingredients per milliliter was prepared: McIlvaine's pH 5.0 buffer, 0.2 ml; maltose, 20 mg; alkanol, 0.1 ml (in the case of *n*-amyl alcohol, a saturated solution was used); culture filtrate of *A. niger* NRRL 330, 0.3 ml; and toluene, 1 drop. In experiments with Rhozyme S or Clarase samples, the enzyme concentration was 0.5 mg/ml of the mixture. The reaction mixture was incubated at 30° for 16 hr and then heated in a boiling-water bath for 5 min.

The concentrations of the alkyl glucosides formed were very low compared with those of the sugars; therefore, an aliquot of the reaction mixture was extracted three times with twice its volume of 1-butanol. Because the alkyl glucosides are much more easily extracted into 1-butanol than is glucose, such an extraction enriches the glucosides in the 1-butanol phase. The combined 1-butanol extract was evaporated to a small volume and samples equivalent to 100 μl of the reaction mixture were used for paper chromatography, with water-saturated 1-butanol as the developing solvent. Figure 1 shows a papergram in which the formation of ethyl, *n*-propyl, *n*-butyl, and *n*-amyl glucosides is fully demonstrated. A similar picture was obtained on thin-layer chromatogram (not shown), with 1-butanol–acetic acid–water (4:1:1) as the developing solvent.

The sample in which methanol was used as the glucosyl acceptor is not shown in Figure 1, inasmuch as methyl glucoside is not well separated from glucose (both being very close to the origin) in the solvent system used. Formation of methyl glucoside was better demonstrated on the thin-layer chromatography plate.

*Demonstration of the Synthesis of Steroid Glucosides.* After the synthesis of alkyl glucosides had been demonstrated, tests were made of different steroidal alcohols, as glucosyl acceptors. The reaction mixture was prepared as described above. The steroids were added as solutions in acetone or 50% acetone (50% water by volume), at a concentration of 1 mg/ml, to give a final concentration of 0.1 mg/ml. Clarase concentrate was used as the enzyme. After incubation overnight at 30°, the steroids and their glucosides, if any, were extracted into ethyl acetate by shaking 1 ml of the reaction mixture three times with 2-ml portions of the solvent.

TABLE I: Steroids Tested as Possible Glucosyl Acceptors.[a]

| Compounds | Characteristic Grouping | Glucoside Formation |
|---|---|---|
| Estriol ($\Delta^{1,3,5(10)}$-estratriene-3,16α,17β-triol) | 16α,17β-Diol | + |
| 17α-Epiestriol | 16α,17α-Diol | + |
| 16α-Hydroxytestosterone ($\Delta^{4}$-androstene-3-one-16α,17β-diol) | 16α,17β-Diol | + |
| 16β-Epiestriol | 16β,17β-Diol | − |
| $\Delta^{1,3,5(10)}$-Estratriene-3,16β,17α-triol | 16β,17α-Diol | − |
| Testosterone | 17β-ol | − |
| Pregnenolone | 3β-ol | − |
| Androsterone | 3α-ol | − |
| Estrone | Phenolic hydroxyl | − |
| 20α-Dihydroprogesterone | 20-α-Ol | − |
| 11α-Hydroxyprogesterone | 11α-Ol | − |
| 16α-Hydroxyprogesterone | 16α-Ol | − |
| Cortexone | 21-Ol | − |
| 11α,12α-Dihydroxyprogesterone | 11α,12α-Diol | − |
| 11α,12β-Dihydroxyprogesterone | 11α,12β-Diol | − |
| 11β,12α-Dihydroxyprogesterone | 11β,12α-Diol | − |
| 11β,12β-Dihydroxyprogesterone | 11β,12β-Diol | − |
| 5α-Pregnane-3α,11α,-17α,20α-21-pentol | 17α,20α,21-Triol | − |

[a] Some of the steroids are from the Squibb Steroid Collection.

The combined extracts were evaporated to dryness with a current of air and the residue was taken up in a small volume of a 1:1 (by volume) mixture of methanol and chloroform. Samples representing 20 μg of the steroids originally used were tested by thin-layer chromatography, the developing solvent being chloroform–methanol (10:1). Depending upon the steroid used, the spots were located by their ultraviolet-absorbing property, their phenolic nature, or the sulfuric acid charring technique. A thin-layer chromatogram that illustrates the synthesis of estriol glucoside is shown in Figure 2.

A partial list of the numerous steroids tested is given in Table I. It can be seen that only those with a 16α,17(α- or β-)diol grouping were converted into their glucosides.

*Isolation of Estriol Glucoside.* Two procedures were used for the initial extraction of estriol glucoside from the reaction mixture. Each step in either procedure was established by preliminary tests, with the results judged by thin-layer chromatography.

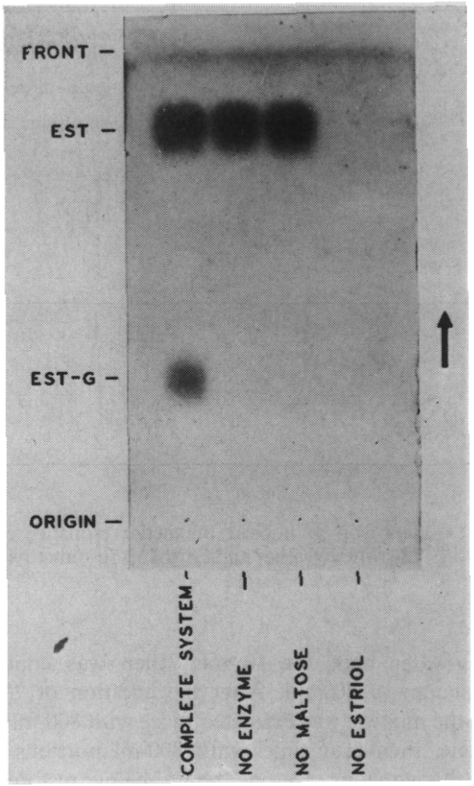

FIGURE 2: Thin-layer chromatographic demonstration of the synthesis of estriol glucoside. The spots were made visible with the phenol reagent of Folin and Ciocalteu.

METHOD 1. Ten liters of the reaction mixture, to which 1 g of estriol had been added as a solution in 1 l. of 50% acetone, was prepared and incubated overnight. The mixture was mechanically agitated with 40 g of active charcoal (Darco G-60) for 1 hr. The charcoal was filtered off on a Celite pad, washed with water, and then shaken on a reciprocating shaker four times with 400-ml portions of 50% acetone and filtered. The aqueous acetone, which contained all the estriol and estriol glucoside, was shaken with an equal volume of benzene. The aqueous acetone phase was restored to its original volume with 90% acetone (10% water) and shaken again with an equal volume of benzene. More than 90% of the estriol was extracted into benzene, but practically all of the estriol glucoside remained in the aqueous acetone phase, which was then evaporated to dryness. The residue was taken up in a small volume of methanol and chromatographed on six 20 × 20 cm, 2 mm thick silica gel plates. The upper phase of a mixture of ethyl acetate–1-butanol–water (4:1:1) was used as the developing solvent. The estriol glucoside band, located by virtue of its weak ultraviolet-absorbing property, was eluted with 50% acetone. The eluate was partitioned four times with twice its volume of ethyl acetate, the aqueous phase being restored each time to its original volume with 90% acetone. The combined ethyl acetate extracts were clarified with anhydrous sodium sulfate. Evaporation to dryness under vacuum left a crystalline residue, which was recrystallized several times from methanol–ethyl acetate to give 102 mg of pure estriol 16α-(α-D-glucopyranoside), mp 255–257°.

METHOD 2. The reaction mixture (4000 ml) was heated in

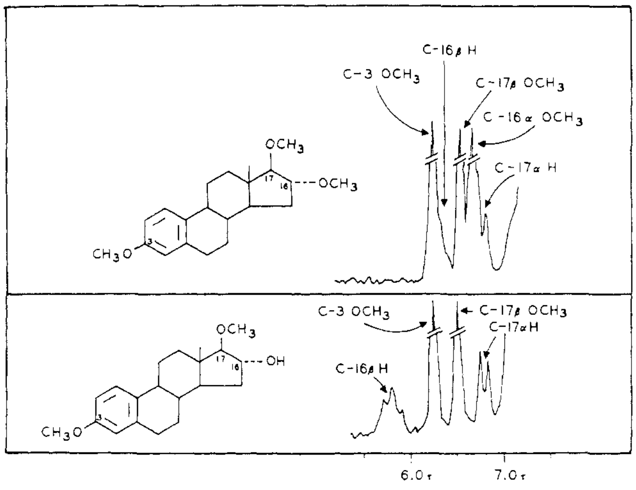

FIGURE 3: Comparison of nuclear magnetic resonance spectra of estriol 3,16α,17β-trimethyl ether and estriol 3,17β-dimethyl ether.

a boiling-water bath for 10 min, then was concentrated under vacuum to 200 ml. After the addition of 200 ml of acetone, the mixture was extracted twice with 800-ml portions of benzene, then four times with 800-ml portions of ethyl acetate, the aqueous acetone phase being restored to its original volume after each extraction, as described above. Most of the estriol was carried in the benzene extract, while the glucoside was in the ethyl acetate, well separated from water-soluble impurities. The ethyl acetate extract was then chromatographed and processed (as described in method 1) to yield 56 mg of pure estriol 16α-(α-D-glucoside), mp 256–257°.

*Characterization of Estriol 16α-(α-D-Glucoside).* The physical and chemical constants of the pure compound are: mp 255–257°; $[\alpha]_D$ +143° (*c* 0.40, MeOH); $\lambda_{max}^{alcohol}$ 281 mμ (ε 2190), 286 mμ (ε 2000); $\lambda_{max}^{alcohol}$ (0.4N KOH) 297 mμ (ε 3170); $\nu_{max}^{KBr}$ 3485, 3360, 1604, 1575, 1490 $cm^{-1}$; solubility in water at 23°, 132 mg/l. *Anal.* Calcd: C, 64.24; H, 7.70. Found: C, 64.00; H, 7.57. The nuclear magnetic resonance spectra (in pyridine) showed the following: Three protons as multiplet at 2.6–3.05 (C-1 H, C-2 H, and C-4 H), one proton in doublet at 4.06 (*J* = 3.5 Hz, C-1′ H), approximately eight protons merging at 5.3–5.8 (C-16β H, C-17α H, C-2′ H, C-3′ H, C-4′ H, C-5′ H, and C-6′ H), three protons in singlet at 9.06 (C-18 H).

The ultraviolet spectrum, together with the bathochromic shift, confirms that the compound is of phenolic nature. To determine whether it was a glucoside of estriol, the compound was hydrolyzed by heating 1 mg dissolved in 0.2 ml of methanol with 0.8 ml of 1.25 N HCl in a boiling-water bath for 1 hr. The presence of estriol in the hydrolysate was demonstrated in a thin-layer chromatogram of an ethyl acetate extract. Formation of glucose was demonstrated by chromatographing the aqueous phase, which had been evaporated to dryness under vacuum, on paper with 1-butanol–pyridine–water (6:3:2) as the developing solvent (Jeanes *et al.*, 1951) and also on thin-layer chromatogram, with 1-butanol–acetic acid–water (4:1:1) as the solvent system. A spot moving with $R_F$ 0.2 on the paper gram, as detected by tetrazolium reagent, or with $R_F$ 0.22 on thin-layer chromatogram, as detected by the sulfuric acid charring technique, was shown to be present in the hydrolysate. Pure glucose, used as reference spot, moved with the same $R_F$. No spots corresponding to estriol or glucose were detected in a control experiment, with the acid and heating omitted. This result, together with the elemental analysis, showed conclusively that the compound isolated was a monoglucoside of estriol, with the glucosyl group attached at either the 16α or 17β position of the steroid moiety.

*Configuration of the Glucosidic Bond.* To determine the configuration of the glucosidic linkage, the glucoside was treated with maltase (α-glucosidase) and emulsin (β-glucosidase) as follows: To a test tube containing 1.0 mg of the glucoside dissolved in 0.1 ml of methanol was added 0.9 ml of an aqueous solution containing 0.5 mg of the enzyme and 0.25 ml of McIlvaine's pH 5.0 buffer. The mixture was incubated at 30° for 4 hr and then extracted twice with 2-ml portions of ethyl acetate. An aliquot of the ethyl acetate extract representing 20 μg of the glucoside was tested on thin-layer chromatogram. Spraying with Pauly's reagent revealed that estriol had been produced in the sample containing maltase, but not in that with emulsin. Control tests in which methyl α-glucoside and methyl β-glucoside were used in place of estriol glucoside showed that methyl α-glucoside was hydrolyzed by the maltase sample but not by the emulsin. The reverse was true for methyl β-glucoside.

That the estriol glucoside synthesized is truly an α-glucoside is also shown by its nuclear magnetic resonance spectrum. As described previously, while the resonance peaks for the protons on hydroxyl-bearing carbon atoms are not distinguishable from each other, that for the proton on the anomeric carbon atom is readily recognized at 4.06. It appears as a doublet (*J* = 3.5 Hz). It is known that a spin–spin coupling constant of approximately 3 Hz indicates the coupling of an equatorial proton with an axial or equatorial proton (Lemieux *et al.*, 1958). Based on the established conformation of glucopyranose, such a coupling constant would mean that the glucosidic bond is of α configuration, as is evident from formula III of Figure 4.

*Estriol 3,16α,17β-Trimethyl Ether.* To determine whether the glucosido group is attached at the 16α or 17β position, the conventional methylation and hydrolysis technique was followed. For this purpose, a sample of estriol 3,16α,17β-trimethyl ether was first prepared by a modification of the method of Kuhn and Baer (1958). The reaction mixture which contained 50 mg of estriol, 0.5 ml of dimethyl formamide, 200 mg of powdered barium oxide, and 0.25 ml of methyl iodide was refluxed at 140–150° under stirring for 2 hr. After cooling, the mixture was taken up in 5–10 ml of water and extracted three times with 10-ml portions of ethyl acetate. Thin-layer chromatograms, developed with chloroform–methanol (100:1), showed that a fair yield of the trimethyl ether ($R_F$ 0.65) was present and that at least as much of the three dimethyl ethers ($R_F$ 0.25) was also formed. The ethyl acetate extract was evaporated to dryness and the methylation procedure was repeated. The ethyl acetate extract after the second methylation was chromatographed on four 20 × 20 cm, 0.5 mm thick, silica gel plates with chloroform–methanol (100:1) as the developing solvent. The trimethyl ether band, located by virtue of its ultraviolet-absorbing property, was eluted with a mixture of methanol and chloroform (1:1). The eluate was partitioned

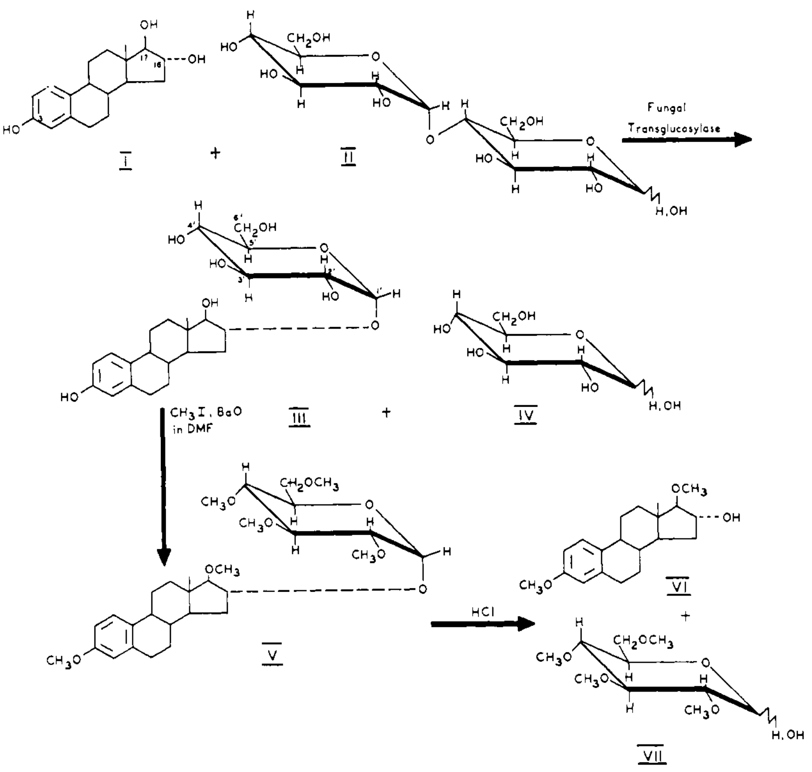

FIGURE 4: Reaction sequence for the synthesis and identification of estriol 16α-(α-D-glucoside). Estriol ($\Delta^{1,3,5(10)}$-estratriene-3,16α,17β-triol) (I), maltose [4-*O*-(α-D-glucopyranosyl)-D-glucopyranose] (II), estriol 16α-(α-D-glucoside) (3,17β-dihydroxy-$\Delta^{1,3,5(10)}$-estratriene-16α-yl α-D-glucopyranoside) (III), D-glucopyranose (IV), estriol 16α-(α-D-glucoside) 3,17β,2′,3′,4′,6′-hexamethyl ether (V), estriol 3,17β-dimethyl ether (3,17β-dimethoxy-$\Delta^{(1,3,510)}$-estratriene-16α-ol) (VI), and 2,3,4,6-tetra-*O*-methyl-D-glucopyranose (VII).

between chloroform and 50% methanol (50% water). The chloroform phase was dried over anhydrous $Na_2SO_4$. Evaporation to dryness under vacuum left a crystalline residue which was recrystallized several times from acetone–*n*-hexane to yield 30 mg of pure estriol 3,16α,17β-trimethyl ether, mp 94–95°; $[\alpha]_D$ +7.5° (*c* 0.4); $\lambda_{max}^{MeOH}$ 278 mμ (ε 2030), 287 mμ (ε1415); $\nu_{max}^{KBr}$ 1607, 1572, 1491 $cm^{-1}$; nuclear magnetic resonance spectrum[1]: one proton in doublet at 2.80 ($J$ = 8.5 Hz, C-1 H), two protons in multiplet at 3.3 (C-2 H, C-4 H), three protons in singlet at 6.21 (C-3 $OCH_3$), one proton, hidden partly under the peak for the C-3 $OCH_3$ at approximately 6.3 (C-16β H), three protons in singlet at 6.52 (C-17β $OCH_3$), three protons in singlet at 6.65 (C-16α $OCH_3$), one proton in doublet at 6.75 ($J$ = 5.3 Hz, C-17α H), and three protons in singlet at 9.20 (C-18 H). *Anal.* Calcd for $C_{21}H_{30}O_3$: C, 76.30; H, 9.16. Found: C, 76.15; H, 9.22.

*Estriol 16α-(α-D-Glucoside) Hexamethyl Ether.* The same methylation procedure was applied to 50 mg of estriol 16α-(α-D-glucoside) to yield 30 mg of glassy material. Attempts at crystallizing the compound have not been successful. Its ultraviolet absorption spectrum exhibited at 278 and 284 mμ the characteristic peaks for an estrogen 3-methyl ether. Its nuclear magnetic resonance spectrum showed six distinctive $OCH_3$ peaks at 6.22, 6.36, 6.45, 6.51, 6.54, and 6.60, besides the three aromatic protons at 2.76 (one proton) and 3.3 (two protons) and an 18-$CH_3$ peak at 9.22. A characteristic C-1′ proton was also observed at 4.84 as a doublet ($J$ = 3.0 Hz). This material was used for acid hydrolysis, as described below.

*Acid Hydrolysis of Estriol 16α-(α-D-Glucoside) Hexamethyl Ether and Isolation of Estriol 3,17β-Dimethyl Ether.* Estriol 16α-(α-D-glucoside) hexamethyl ether obtained above was dissolved in 20 ml of 95% ethanol and 6 ml of concentrated HCl was added. After the mixture had been heated in a boiling-water bath under reflux for 1 hr, thin-layer chromatography showed hardly any of the glucoside methyl ether left. The mixture was diluted with 50 ml of water and extracted twice with 50-ml portions of ethyl acetate. The ethyl acetate extract, after evaporation to dryness, was chromatographed on four 20 × 20 cm, 0.5 mm thick silica gel plates, with chloroform–methanol (100:1) as the developing solvent. The ultraviolet-absorbing band, whose $R_F$ (0.25) is slightly slower than that of estriol 16α-(α-D-glucoside) hexamethyl ether, was eluted with a mixture of chloroform and methanol (1:1). The eluate was partitioned between equal volumes of chloroform and 50% methanol and the chloroform phase was dried over anhydrous $Na_2SO_4$. Evaporation to dryness under vacuum yielded a crystalline residue which was recrystallized several times from acetone–*n*-hexane to yield 15 mg of pure estriol 3,17β-dimethyl ether, mp 165–166°; $[\alpha]_D$ +35° (*c* 0.4); $\lambda_{max}^{MeOH}$ 278 mμ (ε 2230), 287 mμ (ε 2110); $\nu_{max}^{KBr}$ 3445, 1608, 1571, 1496 $cm^{-1}$, nuclear magnetic resonance spectrum: one proton in doublet at 2.75 ($J$ = 9 Hz, C-1 H), two protons in multiplet at 3.18 and 3.36 (C-2 H, C-4 H), one proton in multiplet at 5.78 (C-16β H); three protons in singlet at 6.22 (C-3 $OCH_3$), three protons in singlet at 6.47 (C-17β $OCH_3$), one proton in doublet at 6.77 ($J$ = 5.5 Hz, C-17α H), and three protons in singlet at 9.20 (C-18 H). *Anal.* Calcd for $C_{20}H_{28}O_3$: C, 76.00; H, 8.87. Found: C, 75.92; H, 8.70.

The partial nuclear magnetic resonance spectra of estriol 3,16α,17β-trimethyl ether and of estriol 3,17β-dimethyl

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[1] For the assignment of the nuclear magnetic resonance peaks to the protons at 16β and 17α positions and to the methoxyls at 16α and 17β positions, see discussion concerning Figure 3.

ether are compared in Figure 3. In the spectrum for the dimethyl ether (lower spectrum), the resonance peak at 6.77 is unequivocally assigned to the 17$\alpha$ proton because of its doublet nature and that at 5.78 to the 16$\beta$ proton because of its multiplet nature. In the spectrum for the trimethyl ether (upper spectrum), the 6.77 peak (17$\alpha$ proton) remains the same as in the dimethyl ether, while that at 5.78 (16$\beta$ proton) has now become one at 6.33 (partly hidden under the C-3 $OCH_3$ peak at 6.21). These data provided unequivocal evidence that the methoxyl group in the dimethyl ether is at 17$\beta$ position and the glucosido group must be at 16$\alpha$ position in the estriol glucoside. On the same basis, the resonance peak at 6.52 in the trimethyl ether or at 6.47 in the dimethyl ether is assigned to the C-17$\beta$ $OCH_3$ protons and that at 6.65 in the trimethyl ether is assigned to the C-16$\alpha$ $OCH_3$ protons.

To demonstrate that 2,3,4,6-tetra-*O*-methylglucose was also formed in the hydrolysate, a spot of the ethyl acetate extract (see above) was tested on the thin-layer chromatogram with chloroform–methanol (20:1) as the developing solvent. A spot of authentic 2,3,4,6-tetra-*O*-methylglucopyranose[2] was chromatographed on the same plate. By means of the sulfuric acid charring technique, a spot of the tetramethylglucose was detected on the thin-layer chromatogram with mobility identical with that of the authentic sample. When a spot of the ethyl acetate extract of the hydrolysate was chromatographed on paper in a Zaffaroni-type system (Zaffaroni *et al.*, 1950; Neher, 1959) of benzene *vs.* ethylene glycol, the tetramethylglucose spot was also shown to be present, with an $R_F$ 0.28, identical with that of the authentic sample. The spot was made visible by either aniline phthalate or the tetrazolium reagent.

## Conclusion

The experimental results discussed above establish fully that the reaction sequence carried out in this study is that shown in Figure 4. We have demonstrated that steroidal 16$\alpha$,17($\alpha$- or $\beta$-)diols can behave as glucosyl acceptors from maltose in a fungal enzyme-catalyzed transglucosylation reaction, resulting in the formation of steroidal $\alpha$-D-glucosides with the glucosido group attached at the 16$\alpha$ position of the steroid moiety.

The same enzyme also catalyzes the syntheses of alkyl glucosides from lower alkanols and maltose.

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