Reactivity of the Thallium(I) Salts of 3-Hydroxy-1,3,5(10)-estratrien-17-one and 1,3,5(10)-Estratriene-3,17β-diol. Preparation of 17-Oxoestra-1,3,5(10)-trien-3-yl 2′-Acetamido-3′,4′,6′-tri-*O*-acetyl-2′-deoxy-β-D-glucopyranoside[1]

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Thallium(I) salts of estrone and 17β-estradiol were prepared in high yields by the reaction of estrone or 17β-estradiol with thallium(I) ethoxide. Both compounds were found to be reactive intermediates for alkylation and acylation of the steroid. The preparation and characterization of 17-oxoestra-1,3,5(10)-trien-3-yl 2′-acetamido-2′-deoxy-β-D-glucopyranoside by reaction of the thallium(I) salt of estrone with 2-trifluoroacetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl bromide is described.

Les sels de thallium(I) de l'estrone et de l'estradiol-17β ont été préparés avec de bons rendements par réaction de l'estrone ou de l'estradiol-17β avec l'éthylate de thallium(I). On a trouvé que les deux composés sont des intermédiaires réactifs pour l'alkylation et l'acylation du stéroide. On décrit la préparation et la caractérisation de l'acétamido-2′ déoxy-2′ β-D-glucopyranoside de l'oxo-17 estratriène-1,3,5(10)yle par réaction du sel de thallium(I) de l'estrone avec le bromure du trifluoroacétamido-2 tri-*O*-acétyl-3,4,6 déoxy-2 β-D-glucopyranosyle. [Traduit par le journal]

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Since the initial recognition (1) that steroid glucuronides were present in human and animal excreta, many syntheses of these compounds have been reported (2). In the past 10 years evidence has accumulated that steroid glucuronides may be important in determining the transport as well as the rate and route of excretion of hormonally active steroids (2). In addition, glucosides, galactosides, and *N*-acetylglucosaminides of the estrogens have been found in tissues of the human and other animals (3, 4). These findings have focused attention on the physiological importance of steroid glycosides and on improved methods for their preparation. These latter have almost exclusively been concerned with increasing the yields obtained in the standard Koenigs–Knorr synthesis, and Bernstein and his collaborators (5, 6) have achieved yields of up to 70% of extrogen glucuronides and *N*-acetylglucosaminides by the use of cadmium carbonate as a catalyst in this reaction.

We have been interested in the development of syntheses which might be adaptable to the preparation, for use in physiological studies, of glycosides of radioactive steroids of high specific activity. Both the Koenigs–Knorr and the modified Helferich synthesis (7) are cumbersome for this purpose, and furthermore, in our hands, the yields obtained by Bernstein's procedure (5, 6) were highly variable, being extremely dependent on the source, purity, and crystalline state of the cadmium carbonate used. We therefore explored the use of thallium(I) salts of the phenolic steroid estrogens as intermediates in glycoside synthesis, using the procedures which have been described by Taylor, McKillop, and their coworkers (8, 9) for the preparation of derivatives of several other classes of compounds.

The thallium(I) salts of estrone and the 3-monothallium(I) salt of 17β-estradiol were prepared in practically quantitative yields by the reaction of the respective steroids with thallium(I) ethoxide. In initial experiments to explore the reactivity of these salts, it was found that their reaction with acyl or alkyl halides led to the facile formation of the corresponding acyl or alkyl derivatives of the steroids. The 3-acetates and 3-benzoates of both estrone and 17β-estradiol, as well as the 3-methyl and 3-benzyl ethers of these steroids, were obtained in yields of the order of 90%. The convenience and effectiveness of the procedure might well make it preferable in some instances to the usual methods of preparing these steroid derivatives, in similar yields, by the use of the appropriate anhydride or halide in pyridine (ref. 10 p. 30 and ref. 11).

The thallium(I) salt of estrone **1** was converted

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into various glycosides in several solvent systems. The reaction gave low yields when chlorinated solvents (chloroform, dichloromethane) were used. High yields, which were reproducible, were obtained in benzene. Only a limited number of relatively easily available sugar derivatives were investigated in the present work. The reaction of the thallium(I) salt of estrone **1** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide produced only a 16% yield of the glycoside **6**, while the analogous reaction with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide failed, as did the reaction with methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate. However, when the highly reactive 2-trifluoracetamido-3,4,6-tri-*O*-acetyl-2-deoxy-αD-glucopyranosyl bromide (**12**) was used (12), the reaction proceeded rapidly at room temperature and yielded 86% of pure 17-oxoestra-1,3,5(10)-trien-3-yl 2′-trifluoroacetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**7**). Removal of acetyl groups from this product yielded the free amine **8** in crude form which was then either submitted to selective *N*-acetylation (13) by acetic anhydride in ethanolic solution or was fully acetylated with acetic anhydride in pyridine. The glycoside **9** obtained by the latter procedure was de-*O*-acetylated by treatment overnight at 0° with dry ammonia in chloroform–methanol (14). The 17-oxoestra-1,3,5(10)-trien-3-yl 2′-acetamido-2′-deoxy-β-D-glucopyranoside (**10**) obtained was compared with that synthesized by a conventional Koenigs–Knorr synthesis from estrone and 2-acetamido-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl chloride in acetone in the presence of 1 *M* NaOH and cadmium chloride. The two compounds had identical i.r. spectra and there was no depression of the melting point on mixing of the two.

The fact that, in a 30 min reaction time at room temperature with the thallium(I) salt of estrone an 86% yield of glycoside was obtained, whereas the less reactive sodium salt of estrone gave a much lower yield of 26% under the same conditions, indicates that 2-trifluoroacetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl bromide (**12**) is a very reactive glycosylating agent for the phenolic hydroxyl group of the steroid. The trifluoracetyl group can be easily introduced or removed under mild conditions, and it has therefore often been used (12, 15, 16) in nucleoside and glycoside syntheses involving 2-deoxy-2-amino sugars. The use of the trifluoracetyl as a non-participating group to avoid the formation of an oxazoline ring has been described by Strachan *et al.* (17) and discussed by Meyer zu Reckendorf *et al.* (18).

The β-D-anomeric linkage of the steroid glycosides synthesized in this work was established by the application of Klyne's rule (19).

The formation, by way of the thallium(I) salts, of ethers, esters, and glycosides of the phenolic hydroxyl group of the estrogens proceeds rapidly at room temperature under neutral conditions. The easy removal by filtration of precipitated thallium(I) halide makes the work-up simple and convenient as compared to previously reported methods (ref. 10 p. 204 and refs. 11 and 24).

Experimental

The following procedures were used unless otherwise stated. Melting points were determined on a Thomas-Hoover Capillary melting point apparatus and i.r. spectra on a Unicam SP200 spectrometer in chloroform solution or Nujol. Nuclear magnetic resonance spectra were determined on a Varian HA-100 or a Varian T-60 spectrometer in deuterochloroform or in dimethylsulfoxide solution, with tetramethylsilane as internal standard. Specific rotations were measured on a Perkin-Elmer 141 polarimeter in chloroform or methanol. T.l.c. was carried out on plates of Silica gel H and column chromatography on Silica gel 60 (70–230 mesh, ASTM). Both adsorbents were obtained from Merck, Darmstadt.

Thallium(I) Salt of Estrone ***1***

(i) Method using Benzene–ethanol as Solvent

Thallium(I) ethoxide (20) (250 mg) was added slowly with stirring to a suspension of 267 mg of estrone in a mixture of 4 ml of benzene and 2.5 ml of ethanol. Within a few minutes estrone was dissolved and another white crystalline precipitate appeared. The reaction mixture was left overnight at 4° and was then filtered to yield 426 mg (91%) of **1**.

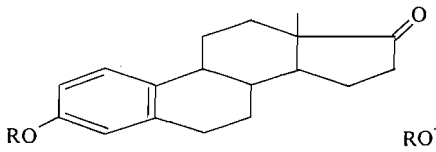
(ii) Method using Ethanol as Solvent

Thallium(I) ethoxide (270 mg) was added with stirring to a solution of 270 mg of estrone in hot ethanol. A white crystalline product was separated by filtration. A further 150 mg of thallium(I) ethoxide in ethanol was added to the mother liquor and the crystalline product was again removed by filtration. The total yield was 368 mg (78%) of the thallium(I) salt of estrone **1**, m.p. 188–195° (dec.).

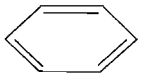
Anal. Calcd. for $C_{18}H_{21}O_2Tl.2H_2O$: C, 42.41; H, 4.15. Found: C, 42.65; H, 4.40.

3-Monothallium(I) Salt of 17β-Estradiol ***1′***

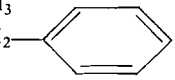
Thallium(I) ethoxide 925 mg was added to a suspension of 1 g of 17β-estradiol in a mixture of 15 ml of benzene and 10 ml of ethanol. The mixture was stirred at room temperature overnight and was then evaporated under vacuum to one quarter of its original volume. On the



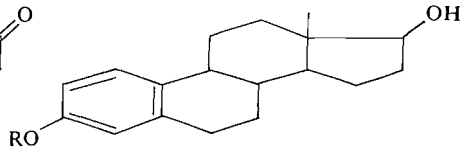
1 R = Tl
2 R = $COCH_3$



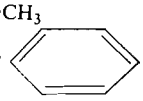
3 R = CO



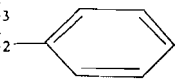
4 R = CH_3
5 R = CH_2



1' R = Tl
2' R = $COCH_3$

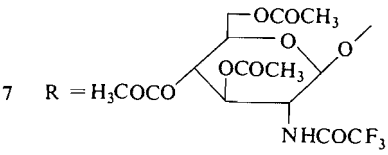


3' R = CO

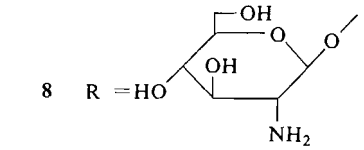


4' R = CH_3
5' R = CH_2

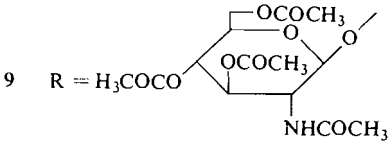
6 R = H_3COCO



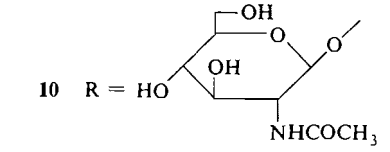
7 R = H_3COCO



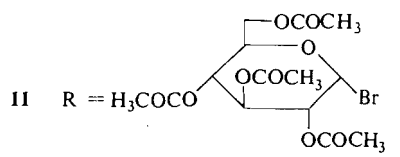
8 R = HO



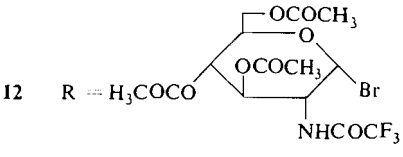
9 R = H_3COCO



10 R = HO



11 R = H_3COCO



12 R = H_3COCO

addition of 10 ml of ether a white crystalline solid was separated by filtration. A yield of 1.734 g (98%) of **1′** was obtained: m.p. (dec.) 180–200°; $[\alpha]_D^{25}$ +27.4° (*c*, 1.66 in pyridine). Analysis: drying under elevated temperature to remove solvent resulted in decomposition, precluding valid elemental analysis.

3-Acetoxyestra-1,3,5(10)-trien-17-one (2) and 3-Benzoyloxyestra-1,3,5(10)-trien-17-one (3). 3-Acetoxy-17β-hydroxyestra-1,3,5(10)-triene (2′) and 3-Benzoyloxy-17β-hydroxyestra-1,3,5(10)-triene (3′)

The thallium(I) salt of the steroid was suspended in benzene and a slight excess of acetylchloride or of benzoyl chloride in benzene solution was added slowly with stirring. The reaction vessel was kept in cold water during the addition. The inorganic precipitate was filtered off, washed with benzene, and the washings were added to the filtrate which was washed with a 10% (w/v) aqueous solution of sodium carbonate and then with 5% hydrochloric acid and with water. The organic layer was dried over sodium sulfate and concentrated. The following esters were obtained in crystalline form (>90%) by this procedure: **2**, m.p. 124° (lit. (21) 125°); **3**, m.p. 216° (lit. (21) m.p. 218°); **2′**, m.p. 134–136° (lit. (22) 136–138°); **3′**, m.p. 193° (methanol–water) (lit. (23) 192–193°) (methanol–water). The i.r. spectra of chloroform solutions were in accord with these structures.

3-Methoxyestra-1,3,5(10)-trien-17-one (4) and 3-Benzyloxyestra-1,3,5(10)-trien-17-one (5). 3-Methoxy-17β-hydroxyestra-1,3,5(10)-triene (4′) and 3-Benzyloxy-17β-hydroxyestra-1,3,5(10)-triene (5′)

The 3-monothallium(I) salt of steroid (100 mg) was dissolved in 2 ml of dimethylsulfoxide and a slight excess of methyl iodide or benzyl bromide was added. After 10 min 5 ml of chloroform and a small amount of Celite were added. The inorganic precipitate was filtered off and washed with chloroform. The filtrate and washings were combined and concentrated by evaporation, aqueous methanol was added, and the crystalline precipitate was separated by filtration and recrystallized from methanol–water. The following ethers were obtained: **4** (92%), m.p. 165° (lit. (24) m.p. 161–162°); **5** (80%), m.p. 134° (lit. (21) m.p. 136°); **4′** (94%), m.p. 96–98° (fuse), 118–120° (melting) (lit. (23) m.p. 97–98°); **5′** (94%), m.p. 83° (lit. (23) m.p. 82–84°). Infrared spectra in chloroform were in accord with these structures.

Etherifications in Dimethylformamide

The thallium(I) salt of estrone (100–110 mg) was dissolved in dimethylformamide and a slight excess of methyl iodide or of benzyl bromide was added. The reaction mixture was heated with stirring at 70° for 3 h in the methylation reaction and at 55° for 4 h in the benzylation reaction. The inorganic precipitate was removed by filtration through a pad of Celite and was washed with chloroform. The washings were added to the filtrate, which was concentrated by evaporation. Crystalline products **4** and **5** were obtained in the yields over 90%.

17-Oxoestra-1,3,5(10)-trien-3-yl 2′,3′,4′,6′-Tetra-O-acetyl-β-D-glucopyranoside (6)

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**11**) (411 mg) was added in two portions to a suspension of 470 mg of the thallium(I) salt of estrone **1** in 10 ml of benzene. The reaction mixture was stirred at room temperature for 3 days. The inorganic precipitate was removed by filtration. After evaporation and separation of a small amount of unreacted steroid, 97 mg of glycoside **6** was obtained (16%), m.p. 139–141° (lit. (5, 7) m.p. 140–142°).

17-Oxoestra-1,3,5(10)-trien-3-yl 2-Trifluoroacetamido-3′,4′,6′-tri-O-acetyl-2-deoxy-β-D-glucopyranoside (7)

(i) From the Thallium(I) Salt of Estrone

The thallium(I) salt of estrone (650 mg) was added in small portions to a solution of 634 mg of 2-trifluoroacetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl bromide (**12**) in 10 ml of dry benzene. The reaction mixture was stirred at room temperature for 30 min. The inorganic product was removed by filtration through a Celite pad and washed with benzene. The filtrate and washings were combined and washed with a small amount of water, dried over sodium sulfate, and evaporated to dryness. On crystallization from dichloromethane–ether–hexane 865 mg of crude material yielded 765 mg (86%) of **7**: m.p. 203–205°; $[\alpha]_D^{27}$ +51° (*c*, 0.5 in chloroform). These constants remained unchanged on further crystallization. Infrared (chloroform) ν_{max} 1720–1745, 1545, 1510, 1200–1250, and 1180 cm^{-1}; n.m.r. (Varian HA-100, $CDCl_3$) 7.22 (H-1 and N—H; 2 protons, spacing 20 Hz), 6.75 (H-2, -4; 2 protons, m, 12 Hz), broad signal from 5.4 to 5.02 (H-1′, -3′, -4′, 3 protons), 4.2 (Hs-6′ and H-2′; 3 protons; m, spacing 16 Hz), 2.06, 2.0 and 1.99 (3 acetates, 9 protons, 3 singlets) and 0.84 δ (C-18 methyl group; 3 protons, singlet).

Anal. Calcd. for $C_{32}H_{38}F_3NO_{10}$: C, 58.80; H, 5.86; N, 2.14. Found: C, 58.57; H, 6.03; N, 2.08.

(ii) From the Sodium Salt of Estrone

To a solution of 296 mg of the sodium salt of estrone (prepared from sodium methoxide and estrone in methanol) in benzene 471 mg of bromide **12** was added in three portions. The reaction mixture was stirred at room temperature for 3 days and the inorganic material was then removed by filtration. The filtrate and washings were washed with a small amount of water, dried over sodium sulfate, and evaporated to dryness. During the evaporation 88 mg of unreacted steroid was separated. The residue was chromatographed on a column containing 10 times its weight of silica gel. Benzene eluates gave 172.6 mg (26%) of crystalline **7** (m.p. 200–203°). Further eluates contained a mixture of unreacted steroid and of a partly decomposed sugar component and were not further investigated.

17-Oxoestra-1-3-5(10)-trien-3-yl 2′-Amino-2′-deoxy-β-D-glucopyranoside (8)

A solution of 200 mg of glycoside **7** in 15 ml of chloroform and 30 ml of methanol was saturated with dry ammonia at 0° and allowed to stand at room temperature for 7 days. The solution was evaporated under reduced pressure to an oily residue. Water was added and the product was isolated by extraction into chloroform – isopropyl alcohol (3 : 2). Evaporation gave 100 mg (76%) of amorphous material **8**: $[\alpha]_D^{24}$ +59° (*c*, 0.2 in methanol); i.r. ν_{max} 3500–2800, 1720, and 1497 cm^{-1}.

17-Oxoestra-1,3,5(10)-trien-3-yl 2′-Acetamido-3′,4′,6′-tri-O-acetyl-2′-deoxy-β-D-glucopyranoside (9)

Compound **7** (100 mg) was converted into 17-oxoestra-1,3,5(10)-trien-3-yl 2′-amino-3′4′6′-trihydroxy-2′-deoxy-β-D-glucopyranoside (**8**) by means of ammonia as described above, and the crude product was dissolved in pyridine and acetylated by the addition of 3 ml of acetic anhydride. The reaction mixture was allowed to stand overnight at room temperature. After work-up 88 mg of *O,N*-acetate **9** was obtained (96%). The product was chromatographed on a column containing 50 times its weight of silica gel. Chloroform eluates contained 80 mg (87%) of **9** as a white amorphous solid: $[\alpha]_D^{24}$ +70° (*c*, 0.129 in chloroform) $[M]_D$ +419°, $[M]_D$ calcd. +351° for β-D-glycosidic linkage ($[M]_D$ of methyl 3,4,6-tri-*O*-acetyl-*N*-acetyl-glucosaminide −75.8°, $[M]_D$ of estrone +427°); i.r. ν_{max} 1720–1760, 1545–1510, 1200–1250, 1180 cm^{-1}; n.m.r. (Varian HA-100) 7.1 (1 proton; d; $J_{1,2}$ = 8 Hz), 6.8 (H-2, -4; 2 protons, m, spacing 10 Hz), 5.7 (N—H; 1 proton; d, J = 9 Hz), broad signal from 5.53 to 5.02 (H-1′, -3′, -4′; 3 protons), 4.2 (H-2′, Hs-6′; 3 protons; m, spacing 13 Hz), 3.89 (H-5′, 1 proton; m over 14 Hz), 2.86 (H-6; 2 protons, m, spacing 12 Hz), 2.06, 2.03, 2.01 (3 acetates, 9 protons, 3 singlets), 1.93 (*N*-acetate; 3 protons; singlet), and 0.89 δ (C-18 methyl; 3 protons; singlet).

17-Oxoestra-1,3,5(10)-trien-3-yl 2′-Acetamido-2′-deoxy-β-D-glucopyranoside (10)

(i) By Selective Acetylation of the Free Amine 8

A solution of 90 mg of **8** in 2 ml of absolute ethanol was treated with 0.02 ml of acetic anhydride. After 2 h at room temperature the solvents were removed under vacuum, the residue was dissolved in a 5% (v/v) solution of ethanol in benzene, and filtered through twice its weight of silica gel. The crude product was crystallized from aqueous methanol to yield 45 mg (45%) of **10**: m.p. 225° (softening), 228–230°; $[\alpha]_D^{25}$ +130° (*c*, 0.1 in methanol); i.r. (Nujol) ν_{max} 3500–2800, 1728, 1680, 1510 cm^{-1}.

Anal. Calcd. for $C_{26}H_{35}NO_7$ $1\frac{1}{2}$ CH_3OH: C, 63.32; H, 7.92; N, 2.68. Found: C, 63.05; H, 7.63; N, 2.82.

(ii) By Selective De-O-acetylation of the Fully Acetylated Glycoside 9

A solution of 60 mg of 17-oxoestra-1,3,5(10)-trien-3-yl 2′-acetamidoacetyl-2′-deoxy-β-D-glucopyranoside (**9**) in 3 ml of chloroform and 10 ml of methanol was saturated with dry ammonia at 0° and allowed to stand at 0° overnight. The product was extracted into chloroform – isopropyl alcohol (3:2). After the addition of water to the reaction mixture 43 mg of crude material was obtained. Crystallization from ethanol–water yielded 40 mg (85%) of **10**.

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