Inhibitors of Steroid Biosynthesis: Preparation of 5,10-Secoestr-4-ynes

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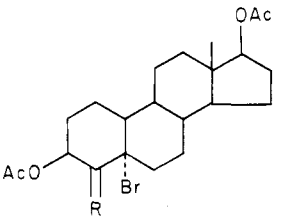
This paper describes two novel syntheses of 5,10-secoestr-4-yne-3,10,17-trione, a compound which has been shown previously to be a powerful irreversible inhibitor of both bacterial and mammalian Δ^5-3-keto steroid isomerases. One synthetic route makes available 3β-hydroxy-4-acetylenic 5,10-seco steroids and the other provides 3α-hydroxy-4-acetylenic 5,10-seco steroids. Stereospecific routes to these epimeric α,β-acetylenic alcohols were developed because of their potential utility as mechanism-based inhibitors of the corresponding 3α- and 3β-hydroxy steroid dehydrogenases.

Recently we reported that α,β-acetylenic 3-oxo-5,10-seco steroids are potent active-site-directed inhibitors of Δ^5-3-keto steroid isomerases.[1,2] We report here the preparation of these secosteroids by routes which permit the stereospecific synthesis of either 3α- or 3β-hydroxy-4-acetylenic 5,10-seco steroid precursors. The availability of these acetylenic alcohols will allow us to determine if the appropriate 3α- or 3β-hydroxy steroid dehydrogenases can enzymatically oxidize these compounds into the corresponding α,β-acetylenic 3-oxo-5,10-seco steroid. The enzyme-generated conjugated acetylenic ketone could then undergo a Michael addition reaction at the active site to inactivate the enzyme. Acetylenic alcohols[3,4] and allenic alcohols[5] have been shown previously to inactivate hydroxy steroid dehydrogenases in this manner.

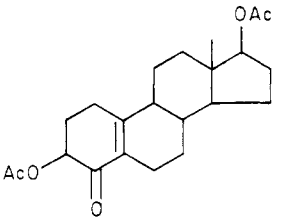
Chart I summarizes the route to the α,β-acetylenic 3-oxo-5,10-seco steroids (**7** and **8**) via precursors which maintain the β configuration for the substituent at C-3. Bromohydrin **1**[6] was converted into bromo ketone **2** in 92% yield by using Jones reagent[7] in acetone. The elimination of HBr from compound **2** to give enone **3** in 91% yield was achieved by using pyridine in refluxing toluene. Only recently has a 3-hydroxy $\Delta^{5(10)}$-4-one system been prepared in the steroid field. In that instance,[8] thermal decarboxylation of 3,17-dioxo-4β,5-epoxy-5β-androstan-19-oic acid in refluxing decalin gave an epimeric mixture of the 3-hydroxy $\Delta^{5(10)}$-4-ones in 79% yield. That the product we characterized was not a mixture of C-3 epimers was established by the different R_f values of enones **3** and **16** on TLC.

Enone **3** was converted into oxido ketone **4** in 91% yield by using *p*-nitroperbenzoic acid.[9] Attempts to carry out this reaction by using either methanolic H_2O_2–NaOH or *m*-chloroperbenzoic acid failed. The assignment of the β configuration for the epoxide is consistent with the known preference of $\Delta^{5(10)}$ steroids to undergo β-face epoxidation with peracids, the nearest analogy being the conversion of 3β,17β-diacetoxyestr-5(10)-en-6-one to the 5β,10β-epoxide by using *m*-chloroperbenzoic acid.[10] Ring opening of epoxy ketone **4** to seco steroid **5** was accomplished by the Tanabe–Eschenmoser procedure[11,12] in 63% yield by using (*p*-tolylsulfonyl)hydrazine in EtOH at 50 °C. Compound **5** was saponified to diol **6** by using aqueous methanolic

Chart I



1, R = α-H, β-OH
2, R = O



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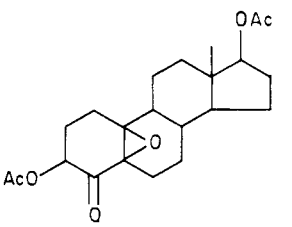

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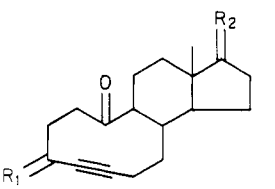
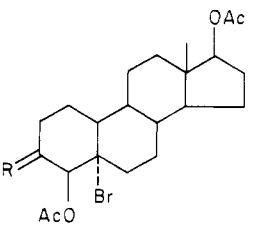
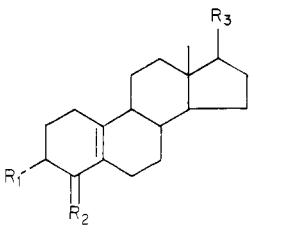


5, R_1 = R_2 = α-H, β-OAc
6, R_1 = R_2 = α-H, β-OH
7, R_1 = O; R_2 = α-H, β-OH
8, R_1 = R_2 = O

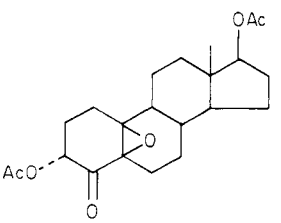
Chart II



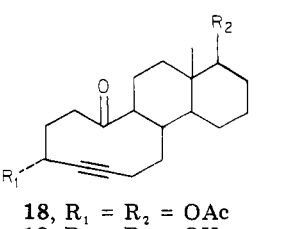
9, R = O
10, R = β-H, α-OH



11, R_1 = OH; R_2 = α-H, β-OAc; R_3 = OAc
12a,b, R_1 = OTHP; R_2 = α-H, β-OAc; R_3 = OAc
13a,b, R_1 = OTHP; R_2 = α-H, β-OH; R_3 = OH
14a,b, R_1 = OTHP; R_2 = O; R_3 = OH
15, R_1 = OH; R_2 = O; R_3 = OH
16, R_1 = OAc; R_2 = O; R_3 = OAc



17



18, R_1 = R_2 = OAc
19, R_1 = R_2 = OH

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K_2CO_3 in 80% yield. Conjugated acetylenic ketones **7** or **8** were prepared by activated MnO_2[13] or Jones[7] oxidation of acetylenic alcohol **6** in 53% yields by each method.

The alternative and lower yield (<5% vs. ~20%) route to conjugated acetylenic ketone **8** via precursors which maintain the α configuration for the substituent at C-3 is presented in Chart II. Reduction of the C-3 carbonyl group in compound **9**[6] with $NaBH_4$ in EtOH at 0 °C gave compound **10** (96% yield). The α configuration for the hydroxyl group was established by comparison of the ^1H NMR spectra of compound **10** and authentic 5-bromo-4β,17β-diacetoxy-5α-estran-3β-ol.[6] In this latter compound the protons of the C-4 acetoxy group occur as a singlet at δ 2.15 whereas in compound **10** the corresponding resonance is shifted upfield to δ 2.10 due to the absence of the deshielding effect of the 3β-hydroxyl group. The difference in the configuration of the C-3 hydroxyl group is further indicated by the chemical shift of the C-3 protons in these compounds. In 5-bromo-4β,17β-diacetoxy-5α-estran-3β-ol this resonance is obscured by the superimposition of the C-17 proton resonance and appears as a complex multiplet centered at δ 4.46, while in compound **10** this resonance is found as an unobscured multiplet centered at δ 3.78. This upfield shift presumably results from the loss of the deshielding effect of the 1,3-diaxial interaction between the C-3 proton and the C-5 bromo group when the configuration of the C-3 hydroxyl group is changed from 3β to 3α.

Elimination of HBr from comound **10** by using pyridine in refluxing toluene gave compound **11** in 63% yield. The hydroxyl group of compound **11** was then protected as a tetrahydropyranyl (THP) ether while the C-4 acetoxy group was saponified and subsequently oxidized to generate the $\Delta^{5(10)}$-4-one system. The unresolved diastereomeric THP ethers **12a** and **12b** were prepared in 90% yield by using dihydropyran and pyridinium *p*-toluenesulfonate (PPTS) in CH_2Cl_2.[14] Saponification of unresolved compounds **12a** and **12b** by using aqueous methanolic K_2CO_3 gave quantitatively THP ethers **13a** and **13b** which were readily separated by either TLC or column chromatography as reported in the experimental section.[15] Pure samples of the precursor compounds **12a** and **12b** were prepared and characterized by esterification of resolved compounds **13a** and **13b**.

Although pure samples of the chiral THP ethers **14a** and **14b** were prepared by activated MnO_2 oxidation of resolved compounds **13a** and **13b**, these compounds were typically prepared as a mixture (64% yield) by MnO_2 oxidation of unseparated compounds **13a** and **13b**. The THP ether group was then removed by using PPTS in EtOH at 55 °C,[14] and the resulting compound **15** (72%) was esterified to give enone **16** (72%). The *p*-nitroperbenzoic acid oxidation of enone **16** to epoxy ketone **17** was quantitative, and the fragmentation of this compound to give seco steroid **18** was accomplished in 40% yield. Hydrolysis of the acetates gave the desired diol **19** (67%). Jones oxidation of diol **19** gave seco steroid **8**, and this fact when considered together with the spectroscopic data presented in the experimental section unambiguously established the epimeric relationship at C-3 between compounds **6** and **19**.

Experimental Section

General Methods. Melting points were determined on a Kofler micro hot stage and are uncorrected. Proton magnetic resonance spectra were recorded in $CDCl_3$ with tetramethylsilane internal standard on a Varian Associates Model T-60 spectrometer. Infrared spectra were recorded in KBr. Unless indicated otherwise, ultraviolet spectra were recorded in methanol. HPLC columns were purchased from Waters Associates. Dry-column grade silica gel was purchased from Universal Scientific Inc. Silica gel GF thin-layer plates (250-μm thickness) were purchased from Analtech Inc., and unless indicated otherwise the R_f values reported are for plates run in 9:1 $CHCl_3$/EtOAc. The *p*-nitroperbenzoic acid was purchased from Aldrich Chemical Co. Elemental analyses were performed by Micro-analysis, Inc., Wilmington, DE.

5-Bromo-3β,17β-diacetoxy-5α-estran-4-one (2). A stirred solution of bromohydrin **1**[6] (300 mg, 0.66 mmol) in acetone (25 mL) was oxidized at room temperature for 1.5 h with Jones reagent. Excess oxidant was destroyed with MeOH. Following the addition of water (100 mL) and during removal of the acetone on a rotary evaporator, the product precipitated and was recovered by filtration (276 mg, 92%). Recrystallization from acetone gave compound **2** as white crystals: mp 149–151 °C; IR 1760 (s, C=O), 1730 (s, ester C=O), 1240 cm^{-1} (s, CO); ^1H NMR δ 0.80 (s, 3, CH_3), 2.07 (s, 3, $OCOCH_3$ C-17), 2.18 (s, 3, $OCOCH_3$ C-3), 4.70 (m, 1, CHOAc C-17), 6.30 (dd, 1, J = 8, 12 Hz, CHOAc C-3); R_f = 0.75. Anal. Calcd for $C_{22}H_{31}O_5Br$: C, 58.02; H, 6.86; Br, 17.25. Found: C, 58.16; H, 6.91; Br, 17.23.

3β,17β-Diacetoxy-5(10)-estren-4-one (3). Bromo ketone **2** (2.7 g, 5.93 mmol) was dissolved in toluene (21.5 mL), pyridine (5.5 mL) was added, and the solution was refluxed for 3 h. The solution was cooled and ether (20 mL) was added, and after the mixture was washed with 5% HCl and 5% $NaHCO_3$, the solvent was dried and removed. Enone **3** was recrystallized from hexane/ether to give white crystals: 2.0 g (91%); mp 160–162 °C; IR 1735 (s, ester C=O), 1690 (s, C=O), 1620 (m, C=C), 1235 cm^{-1} (s, CO); ^1H NMR δ 0.83 (s, 3, CH_3), 2.07 (s, 3, $OCOCH_3$ C-17), 2.18 (s, 3, $OCOCH_3$ C-3), 4.72 (m, 1, CHOAc C-17), 5.30 (m, 1, CHOAc C-3); UV λ_{max} 250 nm (ϵ 12 100); R_f 0.56. Anal. Calcd for $C_{22}H_{30}O_5$: C, 70.56; H, 8.08. Found: C, 70.54; H, 7.99.

3β,17β-Diacetoxy-5β,10β-epoxyestran-4-one (4). Enone **3** (987 mg, 2.53 mmol) and *p*-nitroperbenzoic acid (2.0 g, 2.53 mmol) were dissolved in benzene (100 mL) and stirred at ambient temperature for 24 h. Water was added, and the organic phase was washed successively with 2 M $NaHSO_3$, 5% $NaHCO_3$, and H_2O. The organic solvent was dried and removed, and the residue was crystallized from hexane/ether to give epoxy ketone **4** as white crystals: 941 mg (91%); mp 185–187 °C; IR 1760 (s, C=O), 1730 (s, ester C=O), 1229 cm^{-1} (s, CO); ^1H NMR δ 0.82 (s, 3, CH_3), 2.07 (s, 3, $OCOCH_3$ C-17), 2.17 (s, 3, $OCOCH_3$ C-3), 4.42–5.23 (overlapping m, 2, CHOAc C-3 and C-17); R_f 0.58. Anal. Calcd for $C_{22}H_{30}O_6$: C, 67.67; H, 7.74. Found: C, 67.40; H, 7.78.

3β,17β-Diacetoxy-5,10-secoestr-4-yn-10-one (5). Epoxy ketone **4** (600 mg, 1.54 mmol) and (*p*-tolylsulfonyl)hydrazine (324 mg, 1.74 mmol) were dissolved in absolute ethanol and stirred at 50 °C for 34.5 h. The seco steroid crystallized (361 mg, 63%) when the reaction solution was cooled to −20 °C. The analytical sample was recrystallized from methanol: mp 208–209.5 °C; IR 2245 (w, C≡C), 1735 (s, ester C=O), 1705 (s, C=O), 1235 cm^{-1} (s, CO); ^1H NMR δ 0.83 (s, 3, CH_3), 2.07 (s, 6, $OCOCH_3$ C-3 and C-17), 4.72 (m, 1, CHOAc C-17), 5.32 (m, 1, CHOAc C-3); R_f 0.61. Anal. Calcd for $C_{22}H_{30}O_5$: C, 70.56; H, 8.08. Found: C, 70.50; H, 8.20.

3β,17β-Dihydroxy-5,10-secoestr-4-yn-10-one (6). Seco steroid **5** (315 mg, 0.84 mmol) was stirred for 4 h at ambient temperature in 0.18 M aqueous methanolic K_2CO_3 (25 mL, 70% MeOH). After the addition of H_2O (25 mL) and the removal of MeOH under vacuum, diol **6** was isolated by $CHCl_3$ extraction as an oily foam which was further purified by HPLC (3.9 mm × 30 cm μ-Bon-

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(15) A difference in the extent of intramolecular hydrogen bonding between the C-4 hydroxyl group and the oxygen of the tetrahydropyran ring in compounds **13a** and **13b** may explain the large difference in chromatographic mobility observed for these diastereomers. Models show that such bonding is readily achieved only when the chiral carbon of the tetrahydropyran ring has the *S* configuration.

dapak C_{18} column eluted with 60% MeOH/40% H_2O). The recovered seco steroid **6** (195 mg, 80%) was recrystallized from acetone: mp 188–189 °C; IR 3495 (s, free OH), 3370 (s, br, bonded OH), 2190 (w, C≡C), 1705 cm^{-1} (s, C=O); ^1H NMR δ 0.82 (s, 3, CH_3), 3.77 (m, 1, CHOH C-17), 4.39 (m, 1, CHOH C-3); R_f 0.20 (1:1 benzene/EtOAc). Anal. Calcd for $C_{18}H_{26}O_3$: C, 74.45; H, 9.02. Found: C, 74.68; H, 8.99.

17β-Hydroxy-5,10-secoestr-4-yne-3,10-dione (7). Diol **6** (194 mg, 0.67 mmol) was stirred with activated MnO_2 (1.0 g, 11.5 mmol) in CH_2Cl_2 (10 mL) overnight (ca. 19 h) at ambient temperature. Filtration and solvent removal gave the crude product (161 mg) which was further purified by HPLC (3.9 mm × 30 cm μ-Porasil column eluted with 20% acetone/80% hexane). Recovered seco steroid **7** (101 mg, 53%) was recrystallized from ether: mp 150.5–153 °C; IR 3490 (s, br, bonded OH), 2190 (s, C≡C), 1700 (s, C=O), 1655 cm^{-1} (s, C=O); ^1H NMR δ 0.85 (s, 3, CH_3), 3.73 (m, 1, CHOH); UV (CH_3CN) λ_{max} 218 nm (ϵ 6400) 211 (7300); R_f 0.16. Anal. Calcd for $C_{18}H_{24}O_3$: C, 74.97; H, 8.39. Found: C, 74.82; H, 8.54.

5,10-Secoestr-4-yne-3,10,17-trione (8). A stirred solution of seco steroid **6** (250 mg, 0.86 mmol) in acetone (20 mL) was oxidized at ambient temperature with Jones reagent. Excess oxidant was destroyed with MeOH, water (100 mL) was added, the acetone was removed under vacuum, and the water was extracted with EtOAc. The EtOAc was dried and removed to give crude product (183 mg) which was further purified by HPLC (3.9 mm × 30 cm μ-Porasil column eluted with 15% acetone/85% hexane). Recovered seco steroid **8** (131 mg, 53%) was recrystallized from ether/hexane: mp 146–148 °C; IR 2205 (s, C≡C), 1735 (s, C=O), 1705 (s, C=O), 1675 cm^{-1} (s, C=O); ^1H NMR δ 0.97 (s, 3, CH_3); UV (CH_3CN) λ_{max} 218 nm (ϵ 7500), 211 (8700); R_f 0.33. Anal. Calcd for $C_{18}H_{22}O_3$: C, 75.50; H, 7.74. Found: C, 75.44; H, 7.76.

5-Bromo-4β,17β-diacetoxy-5α-estran-3α-ol (10). Steroid **9**[6] (3.0 g, 6.59 mmol) was dissolved in dioxane (125 mL) and absolute EtOH (125 mL) and cooled to 0 °C. $NaBH_4$ (300 mg, 7.93 mmol) was added, and the reaction mixture was further stirred for 20 min at 0 °C. Excess $NaBH_4$ was consumed by the addition of acetone (10 mL), and the reaction mixture was acidified with 5% HCl (20 mL). Water was added (100 mL), and organic solvent removal on a rotary evaporator was accompanied by precipitation of the product (2.9 g, 96%). Steroid **10** was recrystallized from hexane/ether: mp 164–166 °C; IR 3630 (s, br, OH), 1730 (s, ester C=O), 1230 cm^{-1} (s, CO); ^1H NMR δ 0.80 (s, 3, CH_3), 2.02 (s, 3, $OCOCH_3$ C-17), 2.10 (s, 3, $OCOCH_3$ C-4), 3.78 (m, 1, CHOH), 4.62 (m, 1, CHOAc C-17), 5.33 (d, 1, J = 2 Hz, CHOAc C-4); R_f 0.53. Anal. Calcd for $C_{22}H_{33}O_5Br$: C, 57.77; H, 7.27; Br, 17.47. Found: C, 57.67; H, 7.17; Br, 17.62.

4β,17β-Diacetoxy-5(10)-estren-3α-ol (11). Steroid **10** (2.9 g, 6.3 mmol) was dissolved in toluene (70 mL). Pyridine (6 mL) was added, and the solution was refluxed for 4 h. After cooling, ether (50 mL) was added and the reaction mixture was washed successively with 5% HCl and 5% $NaHCO_3$. After solvent removal the crude product was purified by silica gel column chromatography (3.2 cm × 59 cm column eluted with 9:1 $CHCl_3$/EtOAc). Recovered steroid **11** (1.5 g, 63%) was recrystallized from hexane: mp 166–167 °C; IR 3600 (s, OH), 1735 (s, ester C=O), 1710 (s, ester C=O), 1225 cm^{-1} (s, CO); ^1H NMR δ 0.80 (s, 3, CH_3), 2.03 (s, 3, $OCOCH_3$ C-17), 2.12 (s, 3, $OCOCH_3$ C-4), 3.72 (m, 1, CHOH), 4.63 (m, 1, CHOAc C-17), 5.23 (d, 1, J = 7 Hz, CHOAc C-4); R_f 0.22. Anal. Calcd for $C_{22}H_{32}O_5$: C, 70.19; H, 8.57. Found: C, 70.10; H, 8.59.

5(10)-Estrene-3α,4β,17β-triol 4,17-Diacetate 3-(Tetrahydro-2*H*-pyran-2-yl) Ether (12a,b). Steroid **11** (1.5 g, 3.98 mmol) was dissolved in CH_2Cl_2 (100 mL). Dihydropyran (3 mL) and PPTS (75 mg) were added, and the reaction mixture was stirred overnight at ambient temperature. Ether (100 mL) was added, and the reaction mixture was washed with H_2O (100 mL) and dried. The organic solvent was removed, and the product was recrystallized from methanol to give white crystals (1.65 g, 90%). The epimeric compounds **12a** and **12b** could not be directly separated. However, esterification (pyridine/acetic anhydride) of the easily separated compounds **13a** and **13b** gave pure THP ethers **12a** and **12b**, respectively.

Steroid **12a** was recrystallized from hexane/ether: mp 148–149.5 °C; IR 1735 (s, ester C=O), 1225 cm^{-1} (s, CO); ^1H NMR δ 0.83 (s, 3, CH_3), 2.07 (s, 3, $OCOCH_3$ C-17), 2.10 (s, 3, $OCOCH_3$ C-4), 3.30–4.23 (overlapping m, 3, CHOTHP, CH_2O), 4.47–4.87 (overlapping m, 2, CHOAc C-17, OCHO), 5.48 (d, 1, J = 7 Hz, CHOAc C-4); R_f 0.63. Anal. Calcd for $C_{27}H_{40}O_6$: C, 70.41; H, 8.75. Found: C, 70.67; H, 8.54.

Steroid **12b** was recrystallized from hexane/ether: mp 160.5–163.5 °C; IR 1735 (s, ester C=O), 1230 cm^{-1} (s, CO); ^1H NMR δ 0.83 (s, 3, CH_3), 2.08 (s, 3, $OCOCH_3$ C-17), 2.13 (s, 3, $OCOCH_3$ C-4), 3.25–4.35 (overlapping m, CHOTHP, CH_2O), 4.47–5.03 (overlapping m, 2, CHOAc C-17, OCHO), 5.60 (d, 1, J = 7 Hz, CHOAc C-4); R_f 0.63. Anal. Calcd for $C_{27}H_{40}O_6$: C, 70.41; H, 8.75. Found: C, 70.55; H, 8.72.

5(10)-Estrene-3α,4β,17β-triol 3-(Tetrahydro-2*H*-pyran-2-yl) Ether (13a,b). Unresolved compounds **12a** and **12b** (1.60 g, 3.47 mmol) were stirred for 4 h at ambient temperature in 0.18 M aqueous methanolic K_2CO_3 (100 mL, 70% MeOH). After the addition of H_2O (50 mL) and the removal of MeOH under vacuum, compounds **13a** and **13b** were isolated by EtOAc extraction as a white foam (1.29 g, 99%). Pure samples of each epimer were obtained by column chromatography on silica gel with 9:1 $CHCl_3$/EtOAc as the eluent.

Steroid **13a** was recrystallized from hexane/acetone: mp 170–173 °C; IR 3480 cm^{-1} (s, br, OH); ^1H NMR δ 0.75 (s, 3, CH_3), 3.27–4.23 (overlapping m, 5, CHOH C-4 and C-17, CHOTHP, CH_2O), 4.87 (m, 1, OCHO); R_f 0.14. Anal. Calcd for $C_{23}H_{36}O_4$: C, 73.37; H, 9.64. Found: C, 73.32; H, 9.58.

Steroid **13b** was recrystallized from hexane/acetone: mp 165–167 °C; IR 3510 (s, free OH), 3390 cm^{-1} (s, br, bonded OH); ^1H NMR δ 0.73 (s, 3, CH_3), 3.17–4.33 (overlapping m, 4, CHOH C-17, CHOTHP, CH_2O), 4.33–4.77 (overlapping m, 2, CHOH C-4, OCHO); R_f = 0.25. Anal. Calcd for ($C_{23}H_{36}O_4$): C, 73.37; H, 9.64. Found: C, 73.43; H, 9.54.

17β-Hydroxy-3α-[(tetrahydro-2*H*-pyran-2-yl)oxy]-5(10)-estren-4-one (14a,b). Unresolved steroids **13a** and **13b** (1.29 g, 3.42 mmol) were dissolved in CH_2Cl_2 (150 mL), activated MnO_2 (7.8 g, 89.7 mmol) was added, and the reaction mixture was stirred at ambient temperature for 2 days. Filtration and solvent evaporation left a light yellow oil (1.29 g) which was further purified by column chromatography (1.6 cm × 49 cm column eluted with 9:1 $CHCl_3$/EtOAc) to give a white solid (0.82 g, 64%). Pure samples of epimeric compounds **14a** and **14b** were obtained by using this procedure and pure steroids **13a** and **13b**, respectively.

Steroid **14a** was recrystallized from hexane/acetone: mp 175–178 °C; IR 3629 (s, OH), 1668 (s, C=O), 1619 cm^{-1} (m, C=C); ^1H NMR δ 0.77 (s, 3, CH_3), 3.20–4.40 (overlapping m, 4, CHOH C-17, CHOTHP C-3, CH_2O), 4.82 (m, 1, OCHO); UV λ_{max} 249 nm (ϵ 12400); R_f 0.14. Anal. Calcd for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.77; H, 9.00.

Steroid **14b** was recrystallized from hexane/acetone: mp 180–183 °C; IR 3552 (s, OH), 1657 (s, C=O), 1619 cm^{-1} (m, C=C); ^1H NMR δ 0.78 (s, 3, CH_3), 3.25–4.43 (overlapping m, 4, CHOH C-17, CHOTHP C-3, CH_2O), 4.95 (m, 1, OCHO); UV λ_{max} 249 nm (ϵ 13000); R_f 0.15. Anal. Calcd for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.81; H, 9.08.

3α,17β-Dihydroxy-5(10)-estren-4-one (15). An unseparated mixture of compounds **14a** and **14b** (760 mg, 2.02 mmol) was stirred with PPTS (700 mg, 2.79 mmol) in EtOH (15 mL) for 3 h at 55 °C. The EtOH was removed, and ether and water were added to the residue. The ether layer was dried, and the crude product was isolated and purified by column chromatography on silica gel (1.6 cm × 49 cm column eluted with 9:1 $CHCl_3$/EtOAc). The recovered pure steroid **15** (422 mg, 72%) was recrystallized from CH_2Cl_2/ether: mp 219–221 °C; IR 3485 (s, OH), 1640 (s, C=O), 1610 cm^{-1} (s, C=C); ^1H NMR δ 0.80 (s, 3, CH_3), 3.52–4.35 (overlapping m, 3, CHOH C-3 and CHOH C-17); UV λ_{max} 249 nm (ϵ 12900); R_f 0.32 (1:1 benzene/EtOAc). Anal. Calcd for $C_{18}H_{26}O_3$: C, 74.45; H, 9.02. Found: C, 74.44; H, 9.28.

3α,17β-Diacetoxy-5(10)-estren-4-one (16). Enone **15** (155 mg, 0.53 mmol) was stirred in pyridine (2 mL) and acetic anhydride (2 mL) at room temperature for ca 16 h. Water was added and the oily steroid extracted into $CHCl_3$. After the mixture was washed with 5% HCl and 5% $NaHCO_3$, the solvent was dried and removed, and the crude product (183 mg) was purified by column chromatography on silica gel (1.2 cm × 30 cm column eluted with CH_2Cl_2). The recovered enone **16** (143 mg, 72%) was recrystallized from hexane/ether and had: mp 142–144.5 °C; IR

1745 (s, ester C=O), 1730 (s, ester C=O, 1670 (s, C=O), 1615 (m, C=C), 1240 cm^{-1} (s, CO); ^1H NMR δ 0.83 (s, 3, CH_3), 2.07 (s, 3, $OCOCH_3$ C-17), 2.20 (s, 3, $OCOCH_3$ C-3), 4.73 (m, 1, CHOAc C-17), 5.35 (dd,1, J = 7, 13 Hz, CHOAc, C-3); UV λ_{max} 249 nm (ϵ 13 800); R_f 0.61. Anal. Calcd for $C_{22}H_{30}O_5$: C, 70.56; H, 8.08. Found: C, 70.50; H, 8.20.

3α,17β-Diacetoxy-5β,10β-epoxyestran-4-one (17). Enone **16** (425 mg, 1.13 mmol) was converted into epoxy ketone **17** in 99% yield using the procedure described earlier for the preparation of epoxy ketone **4**. Compound **17** was recrystallized from MeOH and had: mp 213-215 °C; IR 1740 (s, ester C=O), 1720 (s, C=O), 1230 cm^{-1} (s, CO; ^1H NMR δ 0.83 (s, 3, CH_3), 2.07 (s, 3, $OCOCH_3$ C-17), 2.15 (s, 3, $OCOCH_3$ C-3), 4.67 (m, 1, CHOAc C-17), 5.73 (dd, 1, J = 6, 10 Hz, CHOAc C-3); R_f 0.65. Anal. Calcd for $C_{22}H_{30}O_6$: C, 67.67; H, 7.74. Found: C, 67.80; H, 7.91.

3α,17β-Diacetoxy-5,10-secoestr-4-yn-10-one (18). Epoxy ketone **17** (446 mg, 1.14 mmol) was converted into seco steroid **18** using the method described earlier for the preparation of compound **5**. Seco steroid **18** was isolated from the reaction by ether extraction and purified by column chromatography on silica gel (2.0 cm × 44 cm column eluted with CH_2Cl_2). Recovered seco steroid **18** (171 mg, 40%) was recrystallized from $MeOH/H_2O$: mp 151-153 °C; IR 1740 (s, ester C=O), 1700 (m, C=O), 1236 cm^{-1} (s, CO); ^1H NMR δ 0.83 (s, 3, CH_3), 2.03 (s, 3, $OCOCH_3$ C-17), 2.08 (s, 3, $OCOCH_3$ C-3), 4.68 (m, 1, CHOAc C-17), 5.28 (m, 1, CHOAc C-3); R_f 0.61. Anal. Calcd for $C_{22}H_{30}O_5$: C, 70.56; H, 8.08. Found: C, 70.37; H, 8.01.

3α,17β-Dihydroxy-5,10-secoestr-4-yn-10-one (19). Seco steroid **18** (170 mg, 0.45 mmol) was converted into diol **19** using the method described earlier for the preparation of compound **6**. The product was isolated by EtOAc extraction and recovered as a solid (87 mg, 67%) which was recrystallized from hexane/acetone: mp 186-188 °C; IR 3425 (s, br, OH), 1705 cm^{-1} (s, C=O); ^1H NMR δ 0.78 (s, 3, CH_3), 3.68 (m, 1, CHOH C-17), 4.37 (m, 1, CHOH C-3); R_f 0.16 (1:1 benzene/EtOAc). Anal. Calcd for $C_{18}H_{26}O_3$: C, 74.45; H, 9.02. Found: C, 74.59; H, 9.41.

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Solid-State Carbon-13 Nuclear Magnetic Resonance Study of Ribonucleosides and Ribonucleic Acid

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Natural-abundance ^{13}C NMR spectra of cytidine, uridine, adenosine, guanosine, and Torula yeast ribonucleic acid in the solid state could be easily measured by using the combined techniques of high-power proton decoupling, cross-polarization, and magic angle spinning. A proton flip-back pulse sequence was employed to overcome the sensitivity problem caused by the long proton spin-lattice relaxation and yet retain the resolution. The solid-state ^{13}C spectra of uridine and guanosine reveal two distinct conformations of the ribose which could not be detected in the solution state.

Nuclear magnetic resonance spectroscopy has been routinely used in the configurational and conformational analysis of nucleosides, nucleotides, and nucleic acids in solutions.[1,2] However, there exist few solid-state carbon-13,[3,4] phosphorus-31,[5,6] and nitrogen-15[7] NMR studies that have been performed by using the combined techniques of high-power proton decoupling, cross-polarization (CP),[8] and magic angle spinning (MAS) to enhance resolution and sensitivity. The applicability of solid-state NMR is also hampered in part by the lack of a direct spectral correlation between solid and liquid samples. The additional spectral complications involving solid-state spectra may result from the subtle intramolecular and intermolecular interactions inherent in microcrystalline powders and the second-order splittings cause by quadrapolar nuclei. In the present study, we report the solid-state ^{13}C NMR spectra analysis of ribonucleosides and Torula yeast RNA and briefly discuss the approaches employed to resolve the

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