Conjugate Addition of Grignard Reagents to Enones and Dienones

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Grignard reaction with 16-dehydropregnenolone acetate, **1**, results in 1,4-addition to the enone system. Introduction of even trace amounts of oxygen before the reaction has been fully quenched results in formation of 17α-hydroperoxides as well as the expected products. During saponification and Oppenauer oxidation, the intermediate hydroperoxide can decompose to form 16β-alkyl-17-keto steroids. The structure of one such byproduct, 16β-(4′-phenoxybutyl)-4-androstene-3,17-dione, **3**, was established by X-ray crystallography. Rigorous exclusion of air, even during quenching, permitted (4-phenoxybutyl)magnesium bromide and phenylmagnesium bromide to be added to **1** to form 3β-hydroxy-16α-(4′-phenoxybutyl)-5-pregnen-20-one, **7b**, and 3β-hydroxy-16α-phenyl-5-pregnen-20-one, **7c**, in good yields. Similarly, rigorous exclusion of air until quenching was complete caused copper-catalyzed Grignard addition to 17β-hydroxyandrosta-4,6-dien-3-one propionate, **9**, to become reproducible and to give greatly increased yields of 1,6-addition products. These reactions seem to reflect a tendency for enolates formed on conjugate Grignard additions to enones or to dienones to react with oxygen faster than they protonate under commonly employed reaction conditions.

Some years ago, we reported[1] that Grignard reagents prepared from 4-bromobutene, 5-bromopentene, and 3-*tert*-butoxy-1-bromopropane added in a conjugate manner to the enone system of 3β-acetoxy-5,16-pregnadien-20-one, **1**, under the conditions of Marker[2] (that is without catalysis by copper ion). At that time, we also attempted to add (4-phenoxybutyl)magnesium bromide and phenylmagnesium bromide to **1** under conditions comparable to those successfully employed in the reported cases.

An abnormal product, which we could not identify at that time, was isolated from the reaction of the (phenoxybutyl)magnesium bromide with **1**. We have reinvestigated that reaction, and we report, below, the structure of the product, the probable cause of the abnormal course of the reaction, and experimental conditions that produce the normal product of conjugate addition to this enone system. We also demonstrated that these reaction conditions give improved yields on conjugate addition of Grignard reagents to a dienone system. These findings imply that our conditions should be generally useful for all similar additions to enone and dienone systems.

In accord with our usual procedure, the Grignard reagent was prepared, and the steroid was added to the reaction mixture in ether and under nitrogen. The reaction was quenched by addition of aqueous ammonium chloride solution, and the crude product was hydrolyzed in aqueous sodium hydroxide. The crude reaction product was subjected to chromatography over a column of alumina. Phenoxybutane and other nonsteroid material eluted first. The steroidal fraction sometimes crystallized, but retrospective analysis of NMR and IR spectra indicate that both the crystalline and the noncrystalline fractions were mixtures containing, at a minimum, both the 17-keto steroid, **6**, and the expected product, **7b**. Some mixtures appear to have been rich in **6** and others in **7b**. In these experiments **7b** was always contaminated by at least 20% of **6**.[3] A portion of the more polar **6** could be obtained pure by preparative thin-layer chromatography or, in cases where the initial mixture was particularly rich in **6**, by column chromatography. Oppenauer oxidation of **6** gave **3**. The structure of **3** was established by X-ray crystallography and is in accord with its IR and proton NMR spectra and with its elemental analysis.

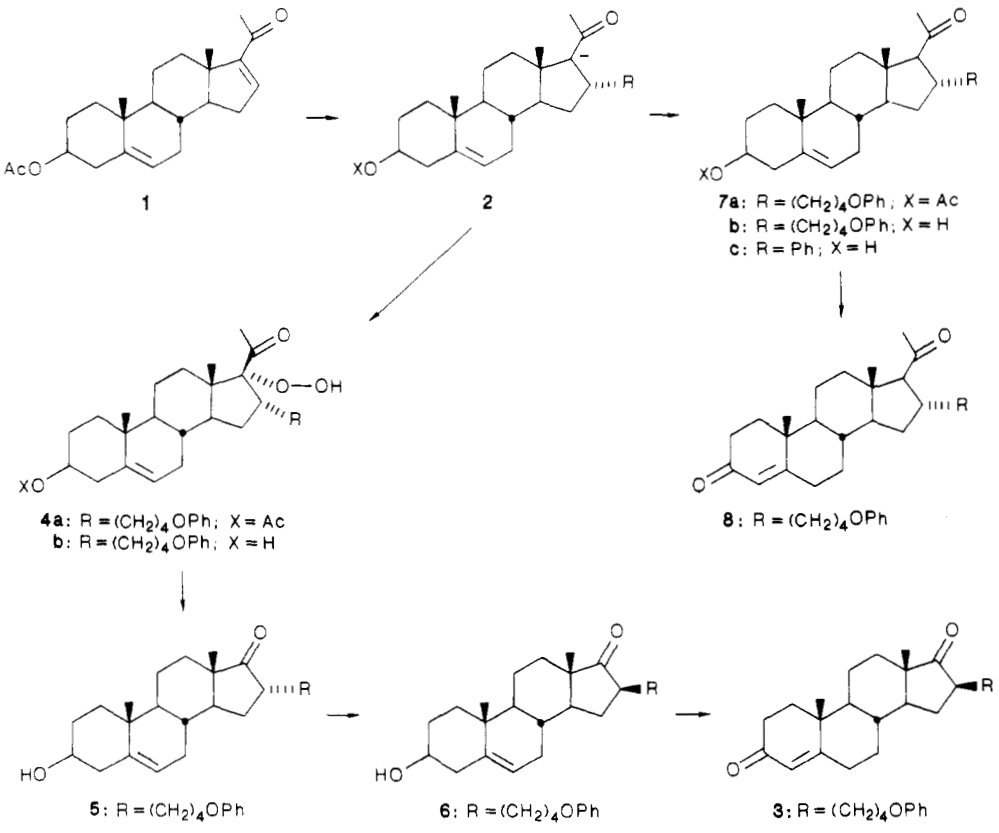
The X-ray analysis established that **3** was 16β-(4′-phenoxybutyl)-4-androstene-3,17-dione. The stereoview of the molecule, without hydrogen atoms, is given in Figure 1. The A ring has a conformation midway between a 1α-sofa and a 1α,2β-half-chair. The B and C rings have normal chair conformations. The D ring has a 14α-envelope conformation.

Carbanions such as **2** are known to react with oxygen to form hydroperoxides,[4] and the latter compounds are known to be unstable[4e,5] and to form 17-keto steroids when

(1) Solo, A. J.; Gardner, J. O. *J. Med. Chem.* **1971**, *14*, 222.
(2) Marker, R. E.; Crooks, H. M. *J. Am. Chem. Soc.* **1942**, *64*, 1280.
(3) Estimates are based on NMR spectra.
(4) (a) Bailey, E. J.; Elks, J.; Barton, D. H. R. *Proc. Chem. Soc.* **1960**, 214. (b) Bailey, E. J.; Barton, D. H. R.; Elks, J.; Templeton, J. *J. Chem. Soc.* **1962**, 1578. (c) Girsmann, H. R.; Nieuwenhuis, H. J. W.; Bickel, A. F. *Proc. Chem. Soc.* **1962**, 279. (d) Baddeley, G. V.; Carpio, H.; Edwards, J. A. *J. Org. Chem.* **1966**, *31*, 1026. (e) Gardner, J. N.; Carlon, F. E.; Gnoj, O. *J. Org. Chem.* **1968**, *33*, 3294.
(5) Siddall, J. B.; Baddeley, G. V.; Edwards, J. A. *Chem. Ind. (London)* **1966**, 25.
(6) Structure assigned on the basis of the spectroscopic properties. The NMR spectra were compared to the other spectra reported in this article, in ref 1 and 7. Corrections for changes in ring A functionality were made by using Zurcher's data.[8]
(7) Cross, A. D.; Beard, C. *J. Am. Chem. Soc.* **1964**, *86*, 5317.
(8) Bhacca, N. S.; Williams, D. H. *Applications of NMR Spectroscopy in Organic Chemistry*; Holden-Day: San Francisco, 1964; pp 19–24. (b) Zurcher, R. F. *Helv. Chim. Acta* **1963**, *46*, 2054 and loc. cit.
(9) Main, P.; Hull, S. E.; Lessinger, L.; Germain, G.; Declerq, J. P.; Woolfson, M. M. *MULTAN. A System of Computer Programs for Automatic Solution of Crystal Structures from X-ray Diffraction Data*; University of York (England) and Louvain (Belgium), 1978.
(10) Stout, G. H.; Jensen, L. H. *X-ray Structure Determination*; Macmillan: New York, 1968; p 457.

0022-3263/89/1954-2317$01.50/0

Scheme I



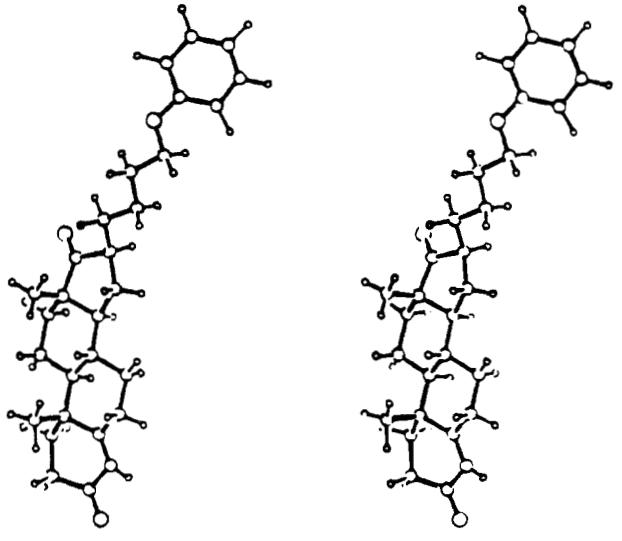


Figure 1. Stereoview of 16β-phenoxybutyl-4-androstene-3,17-dione.

treated with base.[4e,5] In spite of these precedents, we were surprised that oxygenation had occurred both because oxygen seemed to have been rigorously excluded from the reaction until it was quenched and because the reaction conditions were the same as those that we had successfully employed with other Grignard reagents.[1] The reaction was run again under similar conditions but without saponification of the crude reaction product. The principal product isolated was hydroperoxide, **4** (Scheme I).

NMR spectra of the crude reaction products, which had given the previously reported 20-keto-16α-alkyl steroids, were reexamined. All showed peaks indicative of the presence of 16β-alkyl-17-keto steroids as well as the peaks corresponding to 16α-alkyl-20-keto steroids. It may be that the type of product that is isolated depends on the relative proportions in which they are formed and on their solubilities.

We repeated the addition of (3-phenoxybutyl)magnesium bromide to **1** in an apparatus that had been flushed with argon and that was equipped with a septum. The reaction was quenched by injection of water, with care being taken to exclude air until quenching was complete. The desired product of conjugate addition, **7b**, was isolated as crystals in a yield of 63%.

In our previous attempts to add a phenyl Grignard reagent to **1**, we had obtained a mixture from which no substance having the expected properties could be isolated. In view of our demonstration of the effect of traces of oxygen on the previous reaction, we decided to investigate whether a similar problem might be affecting the addition of phenyl Grignard to **1**. Addition of phenylmagnesium bromide to **1** was carried out with rigorous exclusion of air until after the reaction had been quenched by injection of water. The desired 3β-hydroxy-16α-phenyl-5-pregnen-20-one, **7c**, was isolated in 60% yield.

In 1959, Campbell and Babcock reported[13] that methylmagnesium bromide, in the presence of cuprous chloride, undergoes 1,6-addition with $\Delta^{4,6}$-3-keto steroids, and, in the absence of an 11β-hydroxyl group, the predominant product is the 7α-methyl epimer. Their yields varied from 6–50%. Some years later, we used the procedure of Campbell and Babcock to effect conjugate addition of a series of Grignard reagents to 17β-hydroxyandrosta-4,6-dien-3-one propionate, **9**, and immediately

(11) Stewart, J. M. *The XRay System*, Technical Report TR-446; Computer Science Center: University of Maryland, College Park, MD, 1976.

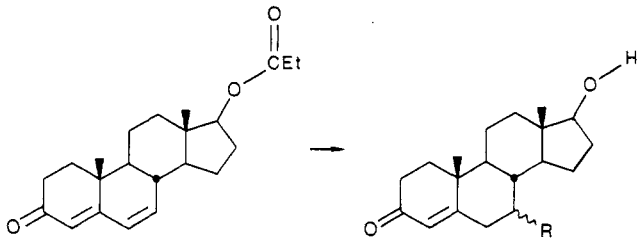
(12) *International Tables for X-Ray Crystallography*; Kynoch: Birmingham, 1974; Vol. IV, pp 71–147 (Present distributor, D. Reidel, Dordrecht).

(13) Campbell, J. A.; Babcock, J. C. *J. Am. Chem. Soc.* **1959**, *81*, 4069.

saponified the crude product.[14] Our yields were at best 25% and were erratic. Chromatography of the reaction resulted only in isolation of the desired product, dimers derived from the alkyl moiety of the alkyl halide used to make the Grignard reagent, and occassionally the hydrolysis product of the starting steroid. In all cases the material balance was poor because the steroidal byproduct remained on the chromatography column. Grunwell, Benson, Johnston, and Petrow[15] claimed that the low yields associated with the above procedure could be avoided by adding ethereal dialkylcopper lithium at 0 °C to the $\Delta^{4,6}$-3-keto steroid system. However, examination of their reported results reveals typical yields in the range of 20–50%.

Our observations on the effect of traces of oxygen on the conjugate addition of Grignard reagents to 1 led us to investigate the possibility that the low, erratic yields that we had encountered on conjugate addition of Grignard reagents to the steroidal $\Delta^{4,6}$-3-ketone system also might be the consequence of the intermediate enolates reacting with oxygen faster than they protonate during quenching of the reactions. Formation of such hydroperoxides and/or their decomposition products would be accord with the fact that all byproducts of the reactions are more polar than the desired products and remain bound to alumina chromatography columns during isolation of the desired products.

Excess Grignard reagent (ethyl, *n*-propyl, and *n*-butyl, respectively) was allowed to react with a catalytic amount of cuprous chloride in ether–THF at 0 °C for 5 min. Then 17-hydroxyandrosta-4,6-dien-3-one propionate, **9**, in tetrahydrofuran was added and stirred at 0 °C for 30 min. The reactions were quenched by injection of water or aqueous hydrochloric acid with rigorous exclusion of air. The crude products were saponified and then chromatographed over silica gel to give mixture of the 7α- and 7β-adducts, **10a–c**, in yields of 68–78%.



The isolation of products of 1,6-addition of the Grignard reagents to the steroidal 3-keto-$\Delta^{4,6}$-diene system in yields that were at least three times greater than had been observed previously again illustrates that the carbanions formed by conjugate addition of Grignard reagents to enones or to dienones faster with oxygen than they react with water. To obtain good yields in such reactions, it is necessary to quench the reaction while rigorously excluding oxygen.

Experimental Section

Melting points were determined in open capillary tubes on a Meltemp apparatus and are uncorrected. IR spectra were determined on a Beckman IR-8 spectrophotometer or a Nicolet 1180 Fourier-transform infrared spectrometer. NMR spectra were determined on a Varian A-60, T-60, or EM-390 spectrometer. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, or by Galbraith Microanalytical Lab., Knoxville, TN.

16β-(4′-Phenoxybutyl)-4-androstene-3,17-dione, 3. A mixture of 2 g of twice recrystallized phenoxybutyl bromide, 250 mg of magnesium, 60 mL of anhydrous ether, and a crystal of iodine was heated to reflux, under nitrogen, for 45 min. The resulting Grignard reagent was allowed to cool to room temperature. Then 1.15 g of 16-dehydropregnenolone acetate, 1, in 60 mL of anhydrous ether was added dropwise. A precipitate formed immediately. The mixture was stirred under nitrogen at room temperature for 30 min, and then a saturated aqueous solution of ammonium chloride was added. The reaction product was partitioned between ether and water. The ether layer was washed with water, dried over magnesium sulfate, and concentrated to an oil, which was dissolved in 70 mL of methanol. A solution of 2 g of sodium hydroxide in 10 mL of methanol was added, and the mixture was heated to reflux for 30 min. The methanol was distilled, and the residue was partitioned between ether and water. The organic layer was washed with water and dried. The solvent was distilled, and the residue was chromatographed over a column of 100 g of alumina. The first fraction eluted contained phenoxybutane. This usually was followed by a small quantity of 6-phenoxy-2-hexanone. The most polar fraction contained a mixture of adducts of the steroid and the Grignard reagent. Some component(s) of the mixture retained the acetyl side chain, other components showed loss of this side chain and presence of a new carbonyl peak at 1735 cm^{-1}. In some cases, crystals were isolated, but NMR and TLC showed that they were composed of mixtures of the above components. On one occassion, an aliquot was subjected twice to thick-layer chromatography over alumina. The slowest moving component seemed[6] to be 6: IR ($CHCl_3$) 1724 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (s, 3 H), 1.04 (s, 3 H), no peak in C=O CH_3 range.

A mixture of 100 mL of toluene, 10 mL of cyclohexanone and 1.2 g of the above mixed steroids was heated to reflux under a Dean–Stark head for 30 min. After 1.4 g of aluminum isopropoxide had been added, heating was resumed for 2 h. The solvent then was distilled, and the residue was partitioned between ether and aqueous hydrochloric acid. The ether layer was washed with water, dried, and concentrated under vacuum. After most of the cyclohexanone had distilled, the residue was chromatographed over alumina. The steroidal fraction sometimes partially crystallized from hexane/ethyl ether, but both the crystals and the liquors were shown by TLC to contain two difficulty separable components. The more polar of the components could be isolated either by column or by thin-layer chromatography over alumina. It crystallized from hexane to give 3: mp 115.5–116 °C; ^1H NMR δ 0.89 (s, 3 H), 1.23 (s, 3 H), 3.98 (t, J = 6 Hz, 2 H), 5.75 (s, 1 H), 6.72–7.45 (m, 5 H); IR (CCl_4) 1735, 1670 cm^{-1}. Anal. Calcd for $C_{29}H_{38}O_3$: C, 80.14; H, 8.81. Found: C, 80.53; H, 9.20.

Separation of the above two-component mixture by thick-layer chromatography over alumina with repeated (5×) development by 50% ethyl ether/hexane gave in addition to 3, a slightly less polar fraction as an oil, which had spectroscopic properties consistent with those expected[6] for 8: IR (CCl_4) 1703, 1673 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.68 (s, 3 H), 1.18 (s, 3 H), 2.13 (s, 3 H), 3.94 (t, J = 6 Hz, 2 H), 5.75 (s, 1 H), 6.7–7.5 (m, 5 H).

3β-Acetoxy-17α-hydroperoxy-16α-(4′-phenoxybutyl)-5-pregnen-20-one, 4a. To a flame-dried three-neck 250-mL Morton flask fitted with a reflux condenser were added 1.0 g of 4-phenoxybutyl bromide and 40 mL of anhydrous ether followed by addition of 0.125 g of magnesium and a crystal of iodine. The mixture was refluxed under argon for 4 h with stirring. After the mixture had cooled to room temperature, a rubber septum was fitted onto one neck, and the reflux condenser was replaced with an addition funnel containing 0.55 g of 16-dehydropregnenolone acetate, 1, dissolved in 30 mL anhydrous ether. The latter solution was added, dropwise, to the reaction mixture, which then was stirred overnight at room temperature under argon. A syringe was loaded with saturated aqueous ammonium chloride solution. Some air bubbled through the syringe during the loading. The aqueous solution was injected into the reaction flask to quench the reaction. The reaction mixture was partitioned between ether and water. The organic phase was washed twice with water, dried over $MgSO_4$, and concentrated to give 1.00 g of a white solid. The

(14) Solo, A. J.; Caroli, C.; Darby, M. V.; McKay, T.; Slaunwhite, W. D.; Hebborn, P. *Steroids* **1982**, *40*, 603.
(15) Grunwell, J. F.; Benson, H. D.; Johnston, J. O.; Petrow, V. *Steroids* **1976**, *27*, 759.

solid was recrystallized from ethyl acetate to give **4a** in a yield of 0.29 g (37%), mp 156–157 °C. Two additional recrystallizations from ethyl acetate raised the melting point to 160.5–161 °C: IR ($CHCl_3$) 1725, 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.69 (s, 3 H), 0.99 (s, 3 H), 2.01 (s, 3 H), 2.25 (s, 3 H), 3.96 (t, J = 6 Hz, 2 H), 5.35 (m, 1 H), 6.7–7.5 (m, 5 H), 8.41 (s, 1 H). Anal. Calcd for $C_{33}H_{46}O_6$: C, 73.57; H, 8.61. Found: C, 73.45; H, 8.70.

The mother liquors were chromatographed over silica gel and were eluted with a gradient, which varied from benzene to 7% ethyl acetate in benzene. The first fraction eluted gave 60 mg (7.7%) of solid, mp 130–131 °C, which was assigned structure **7a** on the basis of its spectroscopic properties:[6] IR ($CHCl_3$) 1725, 1700 cm^{-1}; ^1H NMR δ 0.65 (s, 3 H), 1.01 (s, 3 H), 2.02 (s, 3 H), 2.12 (s, 3 H), 3.92 (t, J = 6 Hz, 2 H), 5.37 (s, 1 H), 6.75–7.4 (m, 5 H). Anal. Calcd for $C_{33}H_{46}O_4$: C, 78.22; H, 9.15. Found: C, 77.68, 77.61; H, 9.21, 9.23.

The next fraction eluted consisted of an additional 117.5 mg (15%) of the hydroperoxide **4a**. Further elution resulted in recovery of an additional 183 mg of unidentified material.

3β-Hydroxy-16α-(4′-phenoxybutyl)-5-pregnen-20-one, 7b. A 100-mL three-neck round-bottom flask equipped with a condenser, an addition funnel, a septum, and a magnetic stirrer was flame-dried and cooled under argon. Dry ether, 5 mL, and 202 mg (8.4 mM) of magnesium turnings (freshly cut) were placed in the reaction flask. To this was added dropwise 1.76 g (7.7 mM) of 4-phenoxybutyl bromide in 7 mL of dry ether over a 5-min period. This mixture was refluxed to initiate Grignard formation. Reflux was continued until most of the magnesium turning went into solution (4–5 h). To this Grignard reagent was added dropwise 500 mg (1.4 mM) of 16-dehydropregnenolone acetate in 20 mL of dry ether over 5 min, and the resulting mixture was refluxed overnight. This mixture was cooled and carefully quenched with water introduced by a syringe and needle. It was acidified with 10% H_2SO_4 and was extracted with ether. The organic layers were combined, washed with water and saturated NaCl solution, and then dried with $MgSO_4$. Solvents were evaporated under vacuum to yield a solid, which was chromatographed (silica gel, 5% ethyl–acetate in benzene) to give 0.410 g (63%) of **7b**, which spontaneously crystallized: mp 132.5–133.5 °C; IR (KBr) 3500–3232, 1703, 1682 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.66 (s, 3 H), 1.01 (s, 3 H), 2.12 (s, 3 H), 3.55 (broad m, 1 H), 3.91 (t, J = 6.3 Hz, 2 H), 5.34 (m, 1 H), 6.90 (m, 3 H), 7.24 (m, 2 H). Anal. Calcd for $C_{31}H_{44}O_3$: C, 80.17; H, 9.48. Found: C, 80.03; H, 9.55.

3β-Hydroxy-16α-phenylpregn-5-en-20-one, 7c. A three-necked 100-mL round-bottom flask equipped with a condenser, an addition funnel, a septum, and a magnetic stirrer was flame-dried and cooled under argon. Dry ether, 8 mL, and 200 mg (8.4 mM) of freshly cut magnesium turnings were added to the flask. A solution of 0.8 mL (7.4 mM) of phenyl bromide in 6 mL of dry ether was added to the flask over 5 min. This mixture was refluxed to initiate Grignard formation and was refluxed for 1 h. Then, 400 mg (1.12 mM) of 16-dehydropregnenolone in 20 mL of dry ether was added over 5 min. The mixture was refluxed overnight. The mixture was cooled and quenched carefully with distilled water by using a syringe and needle. It was acidified with 10% H_2SO_4 and was stirred until a clear solution resulted. Two layers were separated. The aqueous layer was extracted with ether. All organic layers were combined, washed with water and brine solution, and dried with $MgSO_4$. The solvent was evaporated to leave a yellow oil, which was chromatographed (silica gel, 25% ethyl acetate in benzene) to give 270 mg (60%) of **7c** as an oil, which crystallized from ether as colorless plates: sintered 79 °C; IR (KBr) 3591–3197, 1703 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75 (s, 3 H), 1.01 (s, 3 H), 1.98 (s, 3 H), 3.31–3.91 (br, 2 H), 5.33 (m, 1 H), 7.12 (m, 5 H). Anal. Calcd for $C_{27}H_{35}O_2$: C, 82.86; H, 8.95. Found: C, 82.69; H, 9.06.

17β-Hydroxy-7-ethyl-4-androsten-3-one, 10a. To a 100-mL three-neck round-bottom flask that had been flame-dried and cooled under argon was added a solution of 2.1 mL of 3.0 M ethylmagnesium bromide in ether and 15 mL of dry THF. The mixture was cooled to 0 °C in an ice bath. Then 80 mg of cuprous chloride was added all at once. The resulting blue solution was stirred for 5 min. Then a solution of 0.500 g (1.46 mM) of 17-hydroxy-4,6-androstadien-3-one propionate, **9**, in 13 mL of dry THF was added with stirring. After the addition, stirring was continued for 0.5 h at 0 °C. The reaction was quenched by injection through a septum of 5 mL of distilled water. The reaction mixture was acidified with 30% aqueous HCl and was extracted with ether. The organic layers were combined, washed with water, and then with brine, and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to yield a solid, which was taken up in 30 mL of methanol, 3 mL of water, and 0.3 g of KOH. This mixture was refluxed under argon for 45 min, concentrated, partitioned between water and chloroform, and washed with saturated sodium chloride solution. The chloroform solution was dried over $MgSO_4$ and concentrated to give a solid. The latter was chromatographed over silica gel (5% to 25% ethyl acetate in benzene) to give three fractions. The first, which proved to be 17β-hydroxy-7α-ethyl-4-androsten-3-one, was isolated in a yield of 230 mg (50%) as a white solid, which was recrystallized from ether: mp 220–222 °C (lit.[14] mp 219–221 °C).

The second fraction was proved by TLC to be a mixture of α- and β-isomers and was isolated in a yield of 55 mg (12%).

The third fraction, which consisted of 17β-hydroxy-7β-ethyl-4-androstene-3-one was isolated in a yield of 60 mg (13%) as a white solid, which was recrystallized from ether: mp 175–176 °C (lit.[15] mp 170–172 °C).

17β-Hydroxy-7-propyl-4-androsten-3-one, 10b. A 100-mL three-flask equipped with a condenser was flame-dried and cooled under argon. Activated magnesium turnings (400 mg, 16.7 mM), a crystal of iodine, 12 mL dry ether (transferred through a needle), and freshly distilled n-propyl iodide (2.0 mL, 20.5 mM) were placed in the reaction flask. The mixture was refluxed until all the magnesium dissolved. After the mixture was cooled to 0 °C, 8 mL of dry THF was added followed by 100 mg of cuprous chloride. A dark blue solution resulted. Then, a solution of 0.500 g (1.46 mM) of 17β-hydroxy-4,6-androstadien-3-one propionate, **9**, in 10 mL of THF was added through a syringe into the reaction mixture. After the addition, stirring at 0 °C was continued for 1 h. The mixture was quenched by injecting 30% aqueous HCl. The mixture was extracted with ether. Ether layers were combined, washed with water and brine, and dried over $MgSO_4$. The solvents were evaporated under reduced pressure to give a semisolid, which was taken up in 30 mL of dry methanol, 0.35 g KOH, and 3 mL of water. The resulting solution was refluxed under argon for 1 h and then concentrated. The residue was partitioned between chloroform and water, dried over $MgSO_4$, and concentrated under vacuum to give 0.57 g of foam. The latter was chromatographed over silica gel. Benzene–ethyl acetate eluted 75 mg (15.5%) of the α-isomer as a white solid, which was recrystallized from ether: mp 202–204 °C (lit.[14] mp 202.5–204 °C). Further elution with 50% ethyl acetate in benzene provided 300 mg (62%) of a mixture of α- and β-isomers, which was not separated by either selective crystallization or column chromatography.

17β-Hydroxy-7-butyl-4-androsten-3-one, 10c. A 100-mL three-neck flask equipped with a condenser was flame-dried and cooled under argon. Activated magnesium turnings (400 mg, 16.7 mM), a crystal of iodine, 15 mL dry ether, and freshly distilled n-butyl bromide (1.8 mL, 16.7 mM) was placed in the reaction flask. The Grignard reagent was prepared by refluxing the mixture until all the magnesium went into solution. The mixture was diluted with 6 mL of dry THF and cooled in an ice bath. Then 100 mg of cuprous chloride followed by 0.500 g (1.46 mM) of 17β-hydroxy-4,6-androstadien-3-one propionate, **9**, in 12 mL dry THF was added with stirring. The mixture was stirred at 0 °C for 2 h and then quenched carefully with 30% aqueous HCl through a syringe and needle. A colorless solution resulted, which was partitioned between water and ether. Ether layers were combined, washed with water and brine, and then dried over $MgSO_4$. The solvents were evaporated under vacuum to give an oil, which was dissolved in 30 mL of methanol, 3 mL of water, and 0.3 g of KOH. The reaction mixture was refluxed for 1 h under argon and then concentrated. The residue was partitioned between water and chloroform, dried, and concentrated under vacuum to give an oil. The latter was chromatographed over silica gel. Benzene–ethyl acetate eluted the α-isomer in a yield of 0.100 mg (20%) as a solid: mp 179–180.5 °C (lit.[14] mp 182–184 °C). A mixture of α- and β-isomers was collected in a yield of 0.15 g (30%). Further elution resulted in the isolation of β-isomer in a yield of 0.09 g (18%) as a white solid, which was recrystallized from ether: mp 149–151 °C (lit.[15] mp 156–157 °C).

X-ray Crystal and Molecular Structure Determination of 16β-(4'-Phenoxybutyl)-4-androstene-3,17-dione, 3. A small (0.12 × 0.16 × 0.60 mm) single crystal of **3** grown from acetone solution was determined to be in the monoclinic space group $P2_1$. The unit cell parameters determined from least-squares analysis of the 20 values for 25 reflections are a = 19.803 (4), b = 7.139 (F), and c = 8.849 (2) Å; β = 102.61 (2)°; unit cell volume V = 1221 (2) Å^3. Integrated intensities for 2706 independent reflections having $\theta < 75°$ ($-l \le 24$, $0 \le k \le 8$, $-11 \le l \le 11$) were measured by 20 scans on an Enraf-Nonius CAD-4 diffractometer using Cu Kα radiation. Reflections 151, 125, 1011, and 622 maintained intensity within 8% during data collection. Lp and polarization corrections applied. The structure was solved with the MULTAN program.[9] All hydrogen atoms were found from ΔF maps. The positional parameters of all atoms and anisotropic displacement parameters for non-hydrogen atoms (402 variables) were refined by full-matrix least-squaring with the 2122 reflections for which $F_o^2 > 1.75\sigma_F$. The isotropic displacement parameters for hydrogen atoms were not refined and fixed equal to the values of equivalent displacement parameters for correspondent non-hydrogen atoms. The final R, R_w, and S factors were 7.6%, 7.8%, and 2.36, respectively. The weights used were the quantities $1/\sigma_F^2$,[10] using 0.03 rather than 0.01 as the instability correction. Final difference maps showed the strongest peak of ±0.55 e Å^{-3}. The ratio of maximum least-squares shift to error in final refinement cycle $(\Delta/\sigma)_{max}$ was 0.8. The relatively high reliability index is due to thermal liberation of the extended chain, the absence of strong packing forces such as hydrogen bonding, and the small crystal size. Computations were performed with the XRAY76.[11] Atomic scattering factors were taken from the *International Tables for X-ray Crystallography*.[12]

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Supplementary Material Available: Tables of positional and equivalent isotropic thermal parameters, anisotropic thermal parameters for non-hydrogen atoms, figures showing selected bond angles and torsion angles in **3**, and crystal and molecular packing diagram for **3** (5 pages). Ordering information is given on any current masthead page.

General Method for the Preparation of N^3- and O^4-Substituted Uridine Derivatives by Phase-Transfer Reactions

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A general method for regioselective introduction of a variety of protecting groups into the uracil residue of 1 has been developed by use of the phase-transfer reaction. Under such conditions, acyl groups such as benzoyl, *p*-toluyl, *p*-anisoyl, (2,2,2-trichloro-1,1-dimethylethoxy)carbonyl, and (allyloxy)carbonyl were introduced to both the N^3- and O^4-positions of 1. The O^4-acylated species (**3a–f**) initially formed could be readily converted upon warming at 60–70 °C to the N^3-acylated products (**2a–f**), which were obtained ultimately in high yields. N^3-Alkylation occurred under similar conditions when alkylating agents such as methyl iodide, benzyl bromide, and allyl bromide were employed in place of acylating agents. Reactions of 1 with 2-nitrobenzenesulfenyl chloride and triphenylmethanesulfenyl chloride gave exclusively N^3-sulfenylated products (**11** and **12**) in quantitative yields, while sulfonylation of 1 with 2,4,6-triisopropylbenzenesulfonyl chloride led to the O^4-substituted product (**13**), which reacted with ammonia to give a cytidine derivative (**14**).

Introduction

In the current strategy for oligoribonucleotide synthesis, a number of research groups have suggested that the imide moiety of uridine should be protected during chain elongation to avoid side reactions.[1] It is apparently desirable that such a protecting group can be introduced conveniently and, if possible, selectively without modification of other functional groups such as ribose hydroxyl groups. In this respect, Ogilvie[2] reported a suggestive study of tetrabutylammonium fluoride catalyzed N^3-alkylations of uridine and thymidine derivatives involving the 2-cyanoethylation[2d] of thymidine and 5'-*O*-monomethoxytrityl-thymidine with acrylonitrile without O-alkylation at the sugar moieties. On the other hand, Claesen[3] has recently reported the regioselective introduction of the 2-((4-nitrophenyl)sulfonyl)ethyl group into the O^4-position of uridine by treatment with 4-nitrophenyl vinyl sulfone in the presence of tetrabutylammonium hydroxide as the base. Later, Engels[4] showed from his 2D NMR study that

(1) (a) Reese, C. B.; Ubasawa, A. *Tetrahedron Lett.* **1980**, *21*, 2265. (b) Reese, C. B.; Ubasawa, A. *Nucleic Acids Symp. Ser.* **1980**, *7*, 5. (c) Hartog, J. A. J.; Wille, G.; Scheublin, R. A.; van Boom, J. H. *Biochemistry* **1982**, *21*, 1009. (d) Rayner, B.; Reese, C. B.; Ubasawa, A. *J. Chem. Soc., Chem. Commun.* **1980**, 972. (e) Ohtsuka, E.; Wakabayashi, T.; Ikehara, M. *Chem. Pharm. Bull.* **1981**, *29*, 759. (f) Divaker, K. J.; Reese, C. B. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1171. (g) Reese, C. B.; Richards, K. H. *Tetrahedron Lett.* **1985**, *26*, 2245.

(2) (a) Ogilvie, K. K.; Beaucage, S. L.; Gillen, M. F. *Tetrahedron Lett.* **1978**, 1663; (b) **1978**, 3203; (c) Ogilvie, K. K.; Beaucage, S. L.; Gillen, M. F.; Entwistle, D. W. *Nucleic Acids Res.* **1979**, *6*, 2261. (d) Ogilvie, K. K.; Beaucage, S. L. *Ibid.* **1979**, *7*, 805.

(3) Claesen, C. A. A.; Pistorius, A. M. A.; Tesser, G. I. *Tetrahedron Lett.* **1985**, *26*, 3859.

(4) Mag, M.; Engels, J. W. *Nucleic Acids Res.* **1988**, *16*, 3525.

0022-3263/89/1954-2321$01.50/0