4-Hydroxymethyl steroids related to aphidicolin

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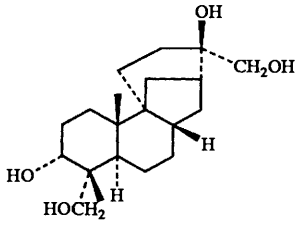
Epimeric 4α- and 4β-hydroxymethyl steroids with and without a 3-hydroxyl group have been prepared as analogues of the diterpenoid tumour inhibitor aphidicolin.

The diterpenoid fungal metabolite, aphidicolin **1**[1] is a specific inhibitor of DNA polymerase α[2] and has attracted interest because it shows a potentially useful level of antitumour and antiviral activity. A number of related metabolites have also been isolated[3] from *Cephalosporium aphidicola*, while aphidicolin has been the target of a number of total syntheses[4–6] and its biosynthesis has been studied.[7] The tetra-ol structure of aphidicolin is unusual among tumour inhibitory agents and its carbon skeleton bears a formal resemblance to that of the steroids. Bearing in mind the sensitivity of some tumours to steroid chemotherapy and the current interest in these compounds as aromatase inhibitors and in the treatment of prostate disease, we have examined routes for the introduction of the ring A functionality of aphidicolin onto the steroid nucleus. The target molecules were 4-hydroxymethyl-androstanes and 3-hydroxy-4-hydroxymethylandrostanes.



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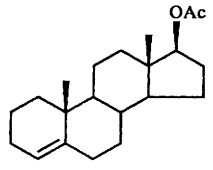

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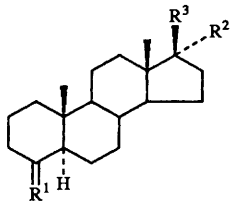
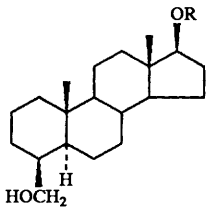
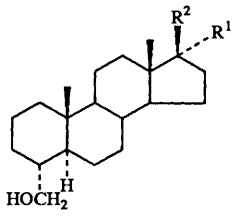


3 R^1 = O, R^2 = H, R^3 = OAc
4 R^1 = CH_2, R^2 = H, R^3 = OAc
5 R^1 = CH(OMe), R^2 = H, R^3 = OH
6 R^1 = O, R^2 = Me, R^3 = OH
7 R^1 = CH(OMe), R^2 = Me, R^3 = OH



8 R = Ac
9 R = H



10 R^1 = H; R^2 = OH
11 R^1 = Me; R^2 = OH

Results and discussion

The alkylation of saturated 5α-androstan-3-ones takes place predominantly at C-2.[8] However, 4α-methoxycarbonyl-5α-androstan-3-ones may be obtained[9] by the reductive methoxycarbonylation of androst-4-en-3-ones. Other methods which were used in the total syntheses of aphidicolin[4–6] involved trapping the equivalent of a C-4 carbanion with formaldehyde.

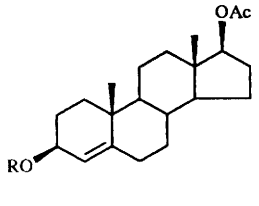
The epimeric 4-hydroxymethyl substituents were introduced onto the steroid backbone as follows. Hydroboration of 17β-acetoxyandrost-4-ene **2** and careful alkaline oxidation of the borane with hydrogen peroxide followed by oxidation to the ketone with chromium trioxide afforded a separable mixture of 17β-acetoxy-5α-androstan-4-one **3** and its 5β-epimer, in which the former predominated. Direct oxidation of the borane with chromium trioxide was not satisfactory. Methylenation of the hindered C-4 position of 17β-acetoxy-5α-androstan-4-one **3** was unsuccessful using a Wittig procedure as enolisation occurred and the isolated product contained both the 5α- and 5β-androstan-4-ones. Methylenation was best carried out using zirconocene dichloride, zinc and dibromomethane.[10] Hydroboration of the resultant 17β-acetoxy-4-methylene-5α-androstane **4** and oxidation of the borane with alkaline hydrogen peroxide gave the 4β-hydroxymethyl derivative **8** as a single isomer at C-4. Partial hydrolysis of the 17β-acetate also occurred during the reaction to give **9**, again as a single isomer.

A useful strategy for determining the stereochemistry at C-4 involves the use of NOE enhancements arising from irradiation of the ^{1}H NMR signal for the C-19 methyl group. The 17β-acetate **8** and 17β-alcohol **9** gave signals at δ_H 3.66 and 3.68, and δ_H 3.61 and 3.65, respectively, which were assigned to the 4-CH_2OH resonances. Irradiation of the 19-H signals (δ_H 0.76 and 0.72) produced NOE enhancements of 3.9 and 3.7% of the C-4 CH_2OH resonances. This established that hydroboration had taken place from the α-face of the molecule to generate the axial 4β-hydroxymethyl group.[11]

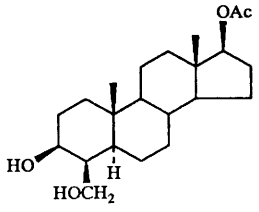
The 4α-epimer **10** was obtained by carrying out a Wittig reaction on 17β-acetoxy-5α-androstan-4-one **3** with the ylide derived from (methoxymethyl)triphenylphosphonium chloride. The 17β-acetate was cleaved during the Wittig reaction and the methoxymethylidene derivative **5** was obtained as a mixture of geometrical isomers. Hydrolysis with concentrated hydrochloric acid and reduction of the aldehyde with sodium borohydride† ($NaBH_4$) gave 4α-hydroxymethyl-5α-androstan-17β-ol **10**. As expected irradiation of the 19-H ^{1}H NMR signal at δ_H 0.83 produced no NOE enhancement of the equatorial 4-CH_2OH ^{1}H NMR signal. The sequence was repeated with 17β-hydroxy-17α-methyl-5α-androstan-4-one **6**, derived from 17α-methyltestosterone, and the Wittig reaction with (methoxy-

† Now more correctly named as sodium boranuide according to the IUPAC rules of nomenclature. Editor.

methyl)triphenylphosphonium chloride gave 4-(methoxymethylidene)-17α-methyl-5α-androstan-17β-ol **7**. Hydrolysis using a trace of concentrated hydrochloric acid in ether followed by reduction of the aldehyde with sodium borohydride gave the 4α-hydroxymethyl compound **11**. Again in accordance with the 4α-equatorial stereochemistry of the hydroxymethyl group, there was no NOE enhancement of the C-4 hydroxymethyl ^{1}H NMR signals on irradiation of the 19-H resonance.



12 R = H
13 R = $Si(Me_2)CH_2Br$



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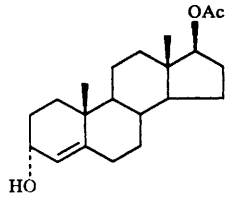

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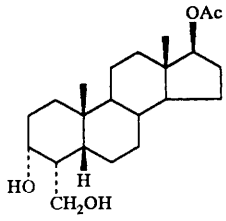

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The 3-hydroxy-4-hydroxymethyl feature of aphidicolin was introduced using the procedure developed by Stork.[12] 17β-Acetoxy-3β-hydroxyandrost-4-ene **12**, derived from testosterone acetate, was converted into its 3-(bromomethyl)dimethylsilyloxy derivative **13** with (bromomethyl)chlorodimethylsilane and immediately cyclised with tributylstannane and AIBN. The product was not purified but oxidatively cleaved with potassium fluoride and hydrogen peroxide in dimethylformamide to give 17β-acetoxy-4β-hydroxymethyl-5α-androstan-3β-ol **14**. The stereochemistry of the product was established as follows. Irradiation of the 19-H signal at δ_H 0.69 gave an NOE enhancement of 7.7% of the C-4 C*H*HOH signal at δ_H 4.02 showing that the hydroxymethyl group was β-orientated. Irradiation of the signal at δ_H 4.02 gave NOE enhancements to the other hydroxymethyl resonance (CH*H*OH; δ_H 3.58, 5.9%) and the methyl group signal (δ_H 0.69, 2.7%). Decoupling experiments established the position of the 4-H resonance (δ_H 2.12). Irradiation of the 3α-H resonance (δ_H 3.92) gave an NOE enhancement of this signal (4.5%) and of a signal at δ_H 1.3 (dt, *J* 12.9 and 2.9 Hz) assigned to the 5α-proton. The epimeric 17β-acetoxy-4α-hydroxymethyl-5β-androstan-3α-ol **16** was prepared in a similar manner from 17β-acetoxy-3α-hydroxyandrost-4-ene **15**. However in this case the method does not generate the aphidicolin stereochemistry at C-5.

Experimental

Light petroleum refers to the fraction bp 60–80 °C; silica for chromatography was Merck Kieselgel 60 type 9380. IR spectra were determined as Nujol mulls; ^{1}H NMR spectra were determined at 360 or 500 MHz in deuteriochloroform on Bruker WM 360 or AMX 500 spectrometers. δ Values quoted are relative to tetramethylsilane and *J* values are given in Hz. Extracts were dried over anhydrous sodium sulfate.

Preparation of 17β-acetoxy-5α-androstan-4-one 3

A solution of 17β-acetoxyandrost-4-ene **2** (2 g)[13] in dry tetrahydrofuran (50 cm^3) was treated with borane in tetrahydrofuran (1 mol dm^{-3}; 40 cm^3) at 0 °C for 2 h (TLC control). Water *ca.* 10 cm^3 was added to the mixture followed by hydrogen peroxide (30%; 20 cm^3) and aqueous sodium hydroxide (10%; 20 cm^3); after this it was kept at 0 °C for 1 h. Aqueous sodium sulfite (10%; 30 cm^3) and hydrochloric acid (10%; 20 cm^3) were then added to the mixture which was then stirred for 30 min. The product was extracted with ethyl acetate and the extract was washed with water, aqueous sodium hydrogen carbonate and brine, dried and evaporated under reduced pressure. The residue was dissolved in acetone (100 cm^3) and the chromium trioxide reagent[14] (10 cm^3) was added to the solution until the orange colour persisted. After 30 min, methanol (5 cm^3) was added to the mixture which was then evaporated the residue was dissolved in ethyl acetate, and the solution washed with aqueous sodium hydrogen carbonate and water, dried and evaporated. The residue was chromatographed on silica (5% ethyl acetate–light petroleum) to give 17β-acetoxy-5β-androstan-4-one **3** (500 mg), mp 119–121 °C (Found: C, 75.8; H, 9.8. $C_{21}H_{32}O_3$ requires C, 75.9; H, 9.7%); ν_{max}/cm^{-1} 1734 and 1703; δ_H 0.77 (3 H, s, 18-H), 1.12 (3 H, s, 19-H), 2.03 (3 H, s, OAc) and 4.56 (1 H, t, *J* 8.1, 17-H). Further elution gave mixed fractions followed by 17β-acetoxy-5α-androstan-4-one (650 mg), mp 145 °C (lit.,[15] 135–137 °C), ν_{max}/cm^{-1} 1740 and 1708; δ_H 0.75 (3 H, s, 18-H), 0.78 (3 H, s, 19-H), 2.04 (3 H, s, OAc) and 4.60 (1 H, t, *J* 8, 17-H).

Methylenation of 17β-acetoxy-5α-androstan-4-one 3

A dry flask was flushed with nitrogen and sequentially charged with activated zinc dust (0.65 g), zirconocene dichloride (2.9 g), tetrahydrofuran (25 cm^3), 17β-acetoxy-5α-androstan-4-one **3** (300 mg) and dibromomethane (0.7 cm^3). The solution was stirred at room temperature for 3 h, quenched with water (20 cm^3) and the products recovered with ethyl acetate. The extract was washed with water, aqueous sodium hydrogen carbonate and brine, dried and evaporated. The residue was chromatographed on silica (5% ethyl acetate–light petroleum) to give 17β-acetoxy-4-methylene-5α-androstane **4** (90 mg) which crystallised from light petroleum as plates, mp 121–122 °C (Found: C, 77.6; H, 10.2. $C_{22}H_{34}O_2 \cdot 0.5H_2O$ requires C, 77.8; H, 10.4%); ν_{max}/cm^{-1} 1741 and 1644; δ_H 0.70 (3 H, s, 18-H), 0.79 (3 H, s, 19-H), 2.05 (3 H, s, OAc), 4.45 (1 H, br s, 4=CH*H*), 4.50 (1 H, t, *J* 8.5, 17-H) and 4.73 (1 H, br s, 4=CH*H*).

Hydroboration of 17β-acetoxy-4-methylene-5α-androstane 4

The alkene **4** (200 mg) in dry tetrahydrofuran (5 cm^3) was treated with borane in tetrahydrofuran (1 mol dm^{-3}; 5 cm^3) at 0 °C. After 4 h (TLC control), water, was added to the mixture followed by hydrogen peroxide (30%; 3 cm^3) and aqueous sodium hydroxide (10%; 3 cm^3) the mixture was stirred overnight after which aqueous sodium sulfite (10%; 10 cm^3) and dil. hydrochloric acid (5 cm^3), were added to it; the mixture was then left for 30 min. The product was recovered with ethyl acetate, and the extract was washed with aqueous sodium hydrogen carbonate, water and brine, dried and evaporated. The residue was chromatographed on silica (10% ethyl acetate–light petroleum) to give 17β-acetoxy-4β-hydroxymethyl-5α-androstane **8** (30 mg) mp 180–182 °C (Found: C, 73.8; H, 10.2. $C_{22}H_{36}O_3 \cdot 0.5H_2O$ requires C, 73.9; H, 10.4%); ν_{max}/cm^{-1} 3456 and 1743; δ_H 0.72 (3 H, s, 18-H), 0.76 (3 H, s, 19-H), 3.66 (1 H, t, *J* 10.5, 4-C*H*HOH), 3.68 (1 H, dd, *J* 6.8 and 10.5, 4-CH*H*OH) and 4.58 (1 H, t, *J* 8, 17-H). Further elution (30% ethyl acetate–light petroleum) gave 4β-hydroxymethyl-5α-androstan-17β-ol **9** (90 mg) which crystallised from acetone–light petroleum as needles, mp 214–216 °C (Found: C, 74.1; H, 10.5. $C_{20}H_{34}O_2 \cdot H_2O$ requires C, 74.0; H, 10.6%); ν_{max}/cm^{-1} 3328

and 3280; δ_H 0.71 (3 H, s, 18-H), 0.72 (3 H, s, 19-H), 3.58 (1 H, t, *J* 8.5, 17-H), 3.61 (1 H, t, *J* 10.5) and 3.65 (1 H, dd, *J* 6.5 and 10.5) (4-CH_2OH).

Preparation of 4-methoxymethylidene-5α-androstan-17β-ol 5

Methoxymethyl(triphenyl)phosphonium chloride (0.9 g) in dry tetrahydrofuran (50 cm^3) under nitrogen was treated with phenyllithium in cyclohexane–ether (7:3) (1.8 mol dm^{-3}; 3 cm^3) for 1 h. 17β-Acetoxy-5α-androstan-4-one **3** (300 mg) in dry tetrahydrofuran (30 cm^3) was added dropwise over 30 min to this mixture which was then stirred overnight. After this the mixture was poured into water and the steroids were recovered with dichloromethane. The extracts were dried and evaporated and the residue was chromatographed on silica. Elution with 5% ethyl acetate–light petroleum to give 4-methoxymethylidene-5α-androstan-17β-ol **5** (170 mg) which crystallised from ethyl acetate–light petroleum as needles, mp 148–151 °C (Found: C, 78.7; H, 10.6. $C_{21}H_{34}O_2$ requires C, 79.2; H, 10.8%); ν_{max}/cm^{-1} 3370 and 1700 (C=C); δ_H 0.71 (3 H, s, 18-H), 0.73 and 0.84 (together 3 H, s, 19-H), 3.42 and 3.84 (together 3 H, s, OMe), 3.64 (1 H, m, 17-H), 5.50 and 5.81 (together 1 H, s, 4=CH).

Hydrolysis and reduction of 4-methoxymethylidene-5α-androstan-17β-ol 5

The above steroid (100 mg) in dry diethyl ether (20 cm^3) was treated with conc. hydrochloric acid (6 drops). The solution was treated with aqueous sodium hydrogen carbonate and brine, dried and evaporated. The residue was dissolved in methanol (10 cm^3) and the solution was treated with sodium borohydride (50 mg) for 1 h followed by acetic acid (3 drops). It was then diluted with ethyl acetate, washed with aqueous sodium hydrogen carbonate and water, dried and evaporated. The residue was chromatographed on silica with (30% ethyl acetate–light petroleum) to give 4α-hydroxymethyl-5α-androstan-17β-ol **10** (70 mg) which crystallised from methanol as needles, mp 207–209 °C (Found: C, 78.0; H, 11.4. $C_{20}H_{34}O_2$ requires C, 78.4; H, 11.2%); ν_{max}/cm^{-1} 3294; $\delta_H(C_5D_5N)$ 0.73 (3 H, s, 18-H), 0.83 (3 H, s, 19-H), 3.68 (1 H, dd, *J* 6.0 and 11.1, 4-C*H*HOH), 3.80 (1 H, t, *J* 8.7, 17-H) and 3.81 (1 H, dd, *J* 5.5 and 11.1, 4-CH*H*OH). Using the same procedure as above 17β-hydroxy-17α-methyl-5α-androstan-4-one **6** (300 mg) gave 4α-hydroxymethyl-17α-methyl-5α-androstan-17β-ol **11** (70 mg) which crystallised from methanol as needles, mp 194–196 °C (Found: C, 78.5; H, 11.2. $C_{21}H_{36}O_2$ requires C, 78.7; H, 11.3%); ν_{max}/cm^{-1} 3294; δ_H 0.77 (3 H, s, 19-H), 1.07 (3 H, s, 18-H), 1.41 (3 H, s, 20-H), 3.94 (1 H, dd, *J* 4.5 and 11.1, 4-C*H*HOH) and 3.96 (1 H, dd, *J* 6.0 and 11.1, 4-CH*H*OH).

Preparation of 17β-acetoxy-4β-hydroxymethyl-5α-androstan-3β-ol 14

17β-Acetoxy-3β-hydroxyandrost-4-ene **12** (1 g)[16] in dry benzene (35 cm^3) was treated with triethylamine (3.5 cm^3) and bromomethyl(chlorodimethyl)silane (1 g) for 1 h (TLC control). The mixture was diluted with benzene and washed with aqueous sodium hydrogen carbonate and water, dried and concentrated. The mixture was heated under nitrogen to reflux, after which it was treated with freshly crystallised azoisobutyronitrile (AIBN) (0.1 g) and tributylstannane (Bu_3SnH) (1.2 cm^3) in benzene (8 cm^3) dropwise over 2 h. The mixture was then heated under reflux for a further 2 h (TLC control). The benzene was removed under reduced pressure and the residual gum was taken up in dimethylformamide (5 cm^3) and treated with hydrogen peroxide (30%; 1.2 cm^3) and potassium fluoride (0.7 g). The mixture was heated for 8 h at 60 °C (TLC control), after which it was poured into water and the product recovered with ethyl acetate. The extract was washed with aqueous sodium hydrogen carbonate and water, dried and evaporated. The residue was chromatographed on silica (30% ethyl acetate–light petroleum) to give 17β-acetoxy-4β-hydroxymethyl-5α-androstan-3β-ol **14** (450 mg) which crystallised from ethyl acetate–light petroleum as needles, mp 219–221 °C (Found: C, 72.1; H, 9.75. $C_{22}H_{36}O_4$ requires C, 72.5; H, 9.95%); ν_{max}/cm^{-1} 3583, 3372 and 1739; δ_H 0.69 (3 H, s, 19-H), 0.77 (3 H, s, 18-H), 2.03 (3 H, s, OAc), 3.58 (1 H, d, *J* 10.3, 4-C*H*HOH), 3.92 (1 H, td, *J* 5.0 and 10.4, 3-H), 4.02 (1 H, t, *J* 10.3, 4-CH*H*OH) and 4.57 (1 H, t, *J* 8.9, 17-H). Under similar conditions, 17β-acetoxy-3α-hydroxyandrost-4-ene **15**[17] (1 g) gave 17β-acetoxy-4α-hydroxymethyl-5β-androstan-3α-ol **16** (685 mg) which crystallised from acetone–light petroleum as fine needles, mp 198–200 °C (Found: C, 72.35; H, 9.95. $C_{22}H_{36}O_4$ requires C, 72.5; H, 9.95%); ν_{max}/cm^{-1} 3583, 3212 and 1735; δ_H 0.76 (3 H, s, 18-H), 0.91 (3 H, s, 19-H), 2.04 (3 H, s, OAc), 3.69 (1 H, dd, *J* 4.0 and 10.5, 4-C*H*HOH), 3.90 (2 H, m, 3-H and 4-CH*H*OH) and 4.58 (1 H, t, *J* 8.5, 17-H).

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