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MICROBIAL TRANSFORMATION OF CYCLOARTENOL, 24-METHYLENECYCLOARTANOL, AND LANOSTEROL, II.[1] ISOLATION AND CHARACTERIZATION OF C_{19} STEROIDS

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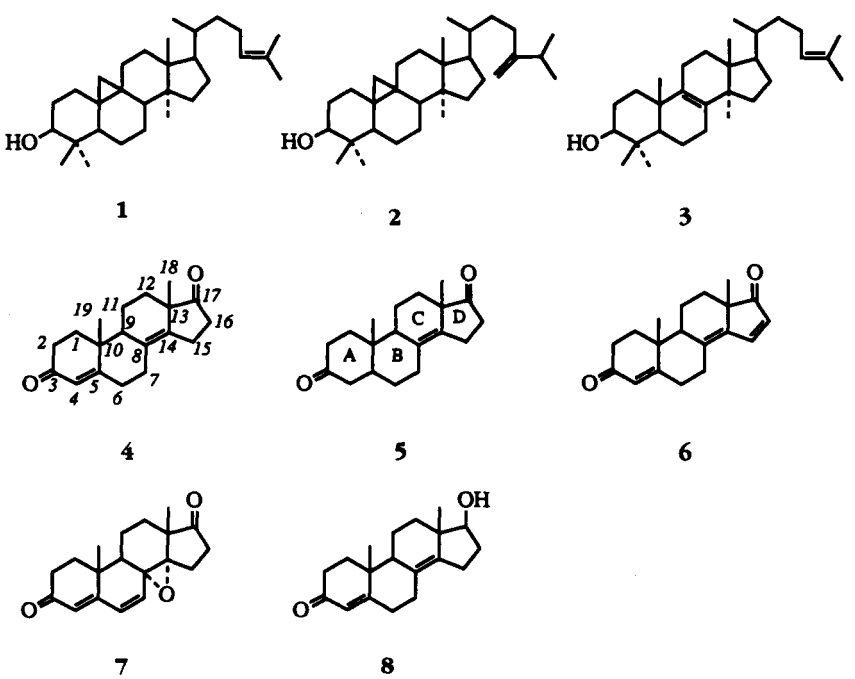
ABSTRACT.—Androsta-4,8(14)-diene-3,17-dione [**4**], 5α-androst-8(14)-ene-3,17-dione [**5**], androsta-4,8(14),15-triene-3,17-dione [**6**], 8,14-α-epoxyandrosta-4,6-diene-3,17-dione [**7**], and 17β-hydroxyandrosta-4,8(14)-dien-3-one [**8**], five new C_{19} steroids, were isolated and characterized from incubation of cycloartenol [**1**], 24-methylenecycloartanol [**2**], or lanosterol [**3**] with *Mycobacterium* sp. (NRRL B-3805).

Cycloartenol [**1**] and 24-methylenecycloartanol [**2**] are the major triterpene alcohols present in rice bran oil (2). Lanosterol [**3**] is a sterol biogenetically related to cycloartenol. In the previous communication (1), we reported the isolation (in 30–36% yield) and characterization of androsta-4,8(14)-diene-3,17-dione [**4**], as the major metabolite from the incubation of **1**, **2**, and **3**, respectively, with *Mycobacterium* sp. (NRRL B-3805). Here we present more detailed data of **4** and four additional metabolites **5–8** isolated from the same incubation mixtures.

The structure determination of compound **4** was mainly based on nOe's and a hetero long-range C-H correlation spectrum and was finally confirmed by single crystal X-ray crystallography as described previously (1). The hetero long-range COSY spectrum showed the coupling of H-18 (δ 1.11, s) to C-12 (δ 28.7, t), C-13 (δ 47.0, s), C-14 (δ 136.7, s), and C-17 (δ 220.6, s) and the coupling of H-19 (δ 1.10, s) to C-1 (δ 34.2, t), C-5 (δ 169.6, s), and C-10 (δ 39.9, d). These data designated the location of the $\Delta^{8,14}$



[1]For Part I, see Wang *et al.* (1).

and supplied very useful ^{13}C-nmr information for the structure elucidation of other metabolites. The other ^{13}C assignments of **4** were made by analyzing its COSY and hetero-COSY spectra. The nOe's of **4** designated the stereochemistry of H-9 to be α-oriented by the observation of enhancement of H-11β (δ 1.57, ddt, J = 14.0, 14.0, 10., 3.6 Hz) upon irradiation of H-18 (δ 1.11) or H-19 (δ 1.10). These observations are characteristic and were useful for the correlation of the stereochemistry of C-9 for metabolite **7** which is described later.

Compound **5**, mp 143–144° from Et_2O, isolated in 16 and 7% yield from the incubation mixtures of **1** and **2**, respectively, with *Mycobacterium* sp. (NRRL B-3805), showed a molecular ion at *m/z* 286.1930, corresponding to $C_{19}H_{26}O_2$ (calcd 286.1932). The ir absorptions of **5** at 1742 and 1715 cm^{-1} indicated a five-membered and a six-membered non-conjugated carbonyl function. Its ^1H-nmr showed two methyl signals at δ 1.09 and 0.91 but lacked signals of olefinic protons. From these data, it appeared to us that **5** is the dihydro derivative of **4**. This postulation was supported by its ^{13}C-nmr and mass spectra. When comparing the spectrum of **5** to that of **4**, we noticed the absence of a trisubstituted double bond, indicating the saturation at C-4 and the presence of two extra aliphatic carbon signals at δ 44.5 (C-4, a methylene) and 46.4 (C-5, a methine). Furthermore, the two non-conjugated carbonyls, which appear at δ 210.0 (C-3) and 221.3 (C-17), together with carbon signals of the fully substituted double bond at δ 129.6 (C-8) and 135.7 (C-14), indicated the absence of Δ^4 and the presence of a β,γ-unsaturated carbonyl system in ring C and D as in compound **4**. The uv absorption maxima at 296.5 and 227.0 nm further supported the presence of a $\Delta^{8(14)}$ double bond in **5** (5,6). From above data, the structure of **5** was assigned as androst-8(14)-ene-3,17-dione, leaving only the stereochemistry at C-5 undetermined.

When compound **5** was incubated with the growing culture of *Mycobacterium* sp. for 24 h, most of the added substrate disappeared and **4** was isolated in 80% yield. On the other hand, under the same incubation conditions, most of the added **4** was recovered unchanged and **5** was not obtained. These results indicated to us that the 4,4-dimethyl groups in **1** and **2** were demethylated to give the unsubstituted C-4 as in **5**, then either by direct introduction of a Δ^4-double bond or by introduction of Δ^5 initially followed by isomerization to give the Δ^4-3-ketone as in **4**. In this situation, it is reasonable to assign the stereochemistry of H-5 to be 5α as in **1** and **2**.

Compound **6**, mp 145–147° from Et_2O, isolated in 1–2% yield from the incubation mixtures of **1**, **2**, or **3** with *Mycobacterium* sp. has $[\alpha]^{23}D + 11°$ (c = 0.10, CH_2Cl_2), showed a molecular ion at *m/z* 282.1615, corresponding to $C_{19}H_{22}O_2$ (calcd 282.1619). In comparison with the formula of **4**, **6** showed two fewer protons, which account for one more double bond equivalent. The ir spectrum of **6** showed two conjugated carbonyl absorptions at 1670 and 1700 cm^{-1}. When comparing these to those of **4** (1670 and 1742 cm^{-1} for 3- and 17-carbonyl, respectively) only the latter carbonyl absorption was shifted from 1742 cm^{-1} to 1700 cm^{-1}. This suggests that the 17-carbonyl is conjugated with two double bonds (3), i.e., it is an α,β,γ,δ-conjugated carbonyl, which was supported by uv absorption maximum at 297.6 nm, calcd 296 nm (4).

The ^1H-nmr spectrum of **6** showed the presence of two methyl signals overlapping at δ 1.20, an AX system of two olefinic protons (δ 7.92, 6.05, J_{AX} = 5.8 Hz), and the H-4 signal (δ 5.80, br s). The H-15 and H-16 in ring D constituting the AX system are the α and β protons of the conjugated carbonyl, which rationalize the chemical shifts at δ 7.92 and 6.05, respectively. The ^{13}C-nmr spectrum of **6** showed chemical shifts for C-3, C-4, and C-5, which constitute the α,β conjugated carbonyl moiety, identical to the corresponding carbon signals of **4**, whereas it showed an upfield shift of C-17 signal from δ 220.6 to 211.2. These comparisons point out that C-17 of **6** is further conju-

gated to a carbon-carbon double bond while ring A and B are apparently identical to those of **4**. The above-mentioned data and analyses led to the assignment of structure of **6** as androsta-4,8(14),15-triene-3,17-dione.

Compound **7**, mp 167–169° from Et_2O, isolated in 5% yield from the incubation of **3**, showed a molecular ion at *m/z* 298.1572, corresponding to $C_{19}H_{22}O_3$ (calcd 298.1569). The ir spectrum of **7** showed two carbonyl absorptions at 1745 and 1669 cm^{-1} similar to those of **4**. The molecular formula of **7** indicated a total of nine double bond and ring equivalents. Its ^{13}C-nmr spectrum showed four olefinic carbon signals, three doublets (δ 125.5, 133.0, and 134.7) and one singlet (δ 160.2), and two carbonyl carbon signals (δ 198.4 and 218.8). Subtraction of the four double bonds from the nine leaves five ring equivalents indicating that besides the four steroidal rings, **7** contains another ring equivalent. The presence of an additional oxygen in **7** relative to **4**, an ir absorption at 3040 cm^{-1} but lack of hydroxy absorption, and the presence of two oxygenated quaternary carbon signals at δ 64.8 and 78.9 led to the conclusion of the existence of an epoxy moiety as a partial structure of **7**. According to the property of the constituted carbons (both oxygenated quaternary) the location of the epoxy ring can be disposed at only two positions, either at C-8 and C-9, or at C-8 and C-14. The hetero long-range COSY spectrum of **7** optimized for $J = 8$ Hz showed that one of the two methyl signals (δ 1.20, s) is three-bond-coupled to C-17 (δ 218.8, s) and one of the oxygenated quaternary carbon signals (δ 78.9, s), whereas the other methyl (δ 1.32, s) is three-bond-coupled to C-5 (δ 160.2, s) and C-9 (δ 42.6, d) (Table 1). These data assigned H-18 at δ 1.20 and H-19 at δ 1.32. This 2D nmr spectrum being optimized for $J = 8$ Hz can show only the correlation spots for two-or three-bond couplings of aliphatic carbons to protons. Because the oxygenated quaternary carbon (δ 78.9) is coupled to H-18 (δ 1.20) but not to H-19 (δ 1.32), the observed result must be caused by C-14 (three-bond coupling to H-18) and not C-8, which is four-bond-coupled to both H-18 and H-19. As a result, the epoxy ring is located at C-8 and C-14.

The carbon signal of C-5 at δ 160.2 (s) in **7** designated from the hetero long-range COSY (Table 1) was upfield shifted relative to that of **4** (δ 169.6), indicating the presence of an extended double bond between C-6 and C-7. This postulation was supported by its ir absorption at 1669 cm^{-1} (3) and uv absorption maximum at 284 nm, calcd 280 nm (4), for an α,β,γ,δ-conjugated carbonyl chromophore in rings A and B. The COSY spectrum (see Experimental), which showed coupling between H-4 (δ 5.82, br s) and H-6 (δ 5.90, d. $J = 9.8$ Hz, A of the AX system), also supported the presence of this chromophore. Consequently the structure of **7** was almost determined, leaving only the stereochemistry for the epoxy ring and C-9 to be clarified.

TABLE 1. Hetero Long-Range COSY Data of **7** (CD_3OD, 300 MHz).[a]

Carbon	δ_C	δ_H
C-17	218.8 s	1.20 (H-18)
C-14	78.9 s	1.20 (H-18)
C-13	47.5 s	1.20 (H-18)
C-12	23.7 t	1.20 (H-18)
C-5	160.2 s	1.32 (H-19)
C-9	42.6 d	1.32 (H-19)
C-10	38.2 s	1.32 (H-19)
C-1	33.7 t	1.32 (H-19)

[a]Response of correlation resonance was optimized for $J_{CH} = 8$ Hz.

Four structures for **7** were possible, considering the stereochemistry of C-9 and epoxy at C-8 and C-14. From comparison of the biotransformation products of **1**, **2**, and **3**, compound **7** possessed the same stereochemistry at C-9 as **4**, i.e., H-9 was α-oriented (1). From a stereochemical viewpoint the epoxy at C-8 and C-14 would favor the α-oriented form. These two arguments were confirmed by nOe studies (Figure 1). From Dreiding model studies, if H-9 is β-oriented and the epoxy is either α- or β-oriented, the two methyls (H-18 and H-19) will be far apart. If H-9 is α-oriented and the epoxy is β-oriented, the two methyls will be also far apart. The above three structures will not give rise to an nOe between the two methyl protons. Only if both H-9 and the epoxy are α-oriented, both methyls can be spatially close enough to cause an nOe to each other. In addition, the structure of these arrangements showed spatial closeness of H-11β to both methyl protons. Thus, the enhanced observation of H-18 upon irradiation of H-19 and the enhancement of H-11β (δ 1.57, dddd, $J = 9.7, 9.7, 9.7, 4.8$ Hz) upon irradiation of H-18 or H-19 as shown in Figure 1 supported the configuration of

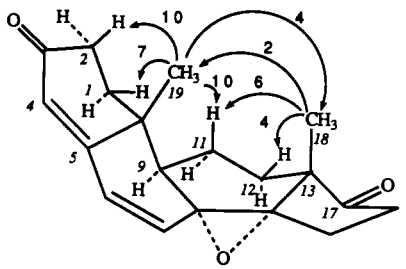


FIGURE 1. The nOe's (%) of **7** (CD_3OD, 300 MHz).

H-9α and 8α-14α-epoxy assignments. These establish **7** as 8,14-α-epoxyandrosta-4,6-diene-3,17-dione.

Compound **8**, mp 133–134° from Et_2O, isolated in up to 5% yield from the incubation of **1**, **2**, and **3**, has $[\alpha]^{24}D + 149°$ ($c = 0.57$, CH_2Cl_2) and showed a molecular ion at *m/z* 286.1936, corresponding to a formula of $C_{19}H_{26}O_2$ (calcd 286.1932). The ir absorptions at 3470 and 1680 cm^{-1} indicated the presence of a conjugated carbonyl and a hydroxy function but absence of a five-membered ketone group. Its ^1H nmr showed two methyl signals at δ 1.07 and 0.93, an olefinic proton signal at δ 5.74, and an aliphatic proton at δ 3.57 (dd, $J = 10.6$ and 7.4 Hz, H-17α). These data suggested that the structure of **8** was most probably the 17β-hydroxy derivative of **4**. Its ^{13}C nmr, which showed the presence of an extra methine signal (δ 82.1) but absence of a 17-ketone signal, also supported this proposal. Previously, we reported that in the incubation of cholesterol with this microorganism, the addition of 4% dextrose in nutrient broth medium resulted in the isolation of a 17β-hydroxy steroid, testosterone, in 10 to 11% yield (7). Therefore, it is possible to obtain **8** from this incubation mixture. When **4** was reduced with $NaBH_4$ (0°, MeOH) (8), a sole product identical to **8** (mmp and nmr) was obtained. This result unequivocally establishes the structure of compound **8** as 17β-hydroxyandrosta-4,8(14)-dien-3-one.

This report describes for the first time the isolation of compounds **5**–**8**. Although **7** has not been isolated from the incubation mixtures of either **1** or **2**, it might be a relatively minor metabolite, and we will look for its presence during future workup of larger scale incubation mixtures.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were measured on a Fisher-Johns melt-

ing point apparatus and not corrected. Optical rotations were measured on a JASCO DIP-181 digital polarimeter. Ir spectra were recorded on a Perkin-Elmer 1760-X Infrared FT spectrometer. The uv spectra were recorded on a Hitachi 150-20 spectrophotometer. Eims were recorded on a Finnigan Mat 4500 series GC/MS and JEOL JMS-HX 110 mass spectrometer. The ^{1}H-nmr and ^{13}C-nmr spectra were recorded on Bruker AC-80 and AM-300 spectrometers. They were measured in appropriate solvents using each solvent peak as internal standard. The 2D nmr spectra were recorded by using Bruker's standard pulse programs. In the hetero long-range COSY experiments, a 1-sec delay was allowed between each scan, and the coupling constant was optimized for $J = 8$ Hz. The COSY-45 correlation map consisted of 512 × 1K data points per spectrum, each composed of 32 transients. The hetero-COSY correlation maps consisted of 512 × 1K data points per spectrum, each composed of 256 transients.

INCUBATION OF LANOSTEROL [3] AND SEPARATION OF **4**, **5**, **6**, **7**, AND **8**.—To the incubation medium of *Nycobacterium* sp. growing in a medium consisting of nutrient broth and dextrose (16 g/liter and 40 g/liter, respectively), lanosterol (1.0 g dissolved in 45 ml of DMF to make a final concentration of 250 μg/ml) was added. The resultant mixture was incubated on a rotary shaker (250 rpm, 1-in. stroke) in ten 2-liter Erlenmeyer flasks containing 400 ml medium at 26–28° for 5 days. The incubation medium (4 liters) was then acidified with HOAc and extracted with $CHCl_3$ (1.3 liters × 3). The $CHCl_3$ layer, after centrifugation (4000 rpm, 10 min, 10°) to remove the solid material, was washed with H_2O (1 liter × 2), dried over Na_2SO_4, and evaporated to give a viscous residue. To the residue was then added Me_2CO (50 ml) to eliminate the residual protein. The filtrate was evaporated under reduced pressure to give 0.90 g of residue, showing six spots in a Si gel tlc plate with R_f value of 0.61 for **5** (71 mg), 0.51 for **4** (198 mg), 0.43 for **6** (10 mg) and **7** (32 mg), 0.40 (trace), 0.30 for **8** (trace) and 0.22 (trace), developing with 5% Me_2CO in $CHCl_3$. They were separated by a Si gel column (45.0 g, 230–400 mesh) eluted with $CHCl_3$ and increasing amounts of Me_2CO (from 0% to 2%). The fraction s containing **6** and **7** with the same R_f value were further separated as follows. Compound **7** (32 mg) was obtained directly via recrystallization of the mixture from Et_2O. The mother liquid after purification via a Si gel column (6.0 g) eluted with 0.5% Me_2CO in $CHCl_3$ afforded 10 mg of **6**.

Compound **8** was mainly obtained from the incubation mixture of cycloartenol and 24-methylenecycloartenol in a similar condition. The yield of **8** of this incubation is about 4%.

ANDROSTA-4,8(14)-DIENE-3,17-DIONE [**4**].—Mp 141–142° from Et_2O; $[\alpha]^{20}D$ +316° ($c = 0.25$, EtOH); ir ν max (KBr) cm^{-1} 2961, 1742 (s), 1669 (s), 1610, 1447, 1370; uv λ max MeOH (log ε) 233.0 (4.07); eims *m/z* (rel. int.) $[M + 1]^+$ 285 (68), $[M]^+$ 284 (100), 227 (24), 212 (81), 209 (61), 199 (22); hrms *m/z* $[M]^+$ 284.2765 (calcd for $C_{19}H_{24}O_2$, 284.1776); ^{1}H nmr ($CDCl_3$) δ 1.80 (m, H-1α), 1.95 (m, H-1β), 2.35 (m, H-2α and H-2β), 5.75 (br s, H-4), 2.39 (m, H-6α and H-6β), 1.91 (m, H-7α), 2.71 (m, H-7β), 2.09 (m, H-9), 1.73 (m, H-11α), 1.57 (m, H-11β), 1.21 (m, H-12α), 1.78 (m, H-12β), 2.18 (m, H-15α), 2.58 (m, H-15β), 2.78 (m, H-16α), 2.55 (m, H-16β), 1.11 (s, H-18), 1.10 (s, H-19); ^{13}C nmr ($CDCl_3$) δ 34.2 (t, C-1), 33.8 (t, C-2), 198.7 (s, C-3), 123.4 (d, C-4), 169.6 (s, C-5), 32.3 (t, C-6), 28.8 (t, C-7), 127.8 (s, C-8), 47.6 (d, C-9), 39.9 (s, C-10), 18.4 (t, C-11), 28.7 (t, C-12), 47.0 (s, C-13), 136.7 (s, C-14), 35.8 (t, C-15), 22.9 (t, C-16), 220.6 (s, C-17), 21.8 (q, C-18), 18.2 (q, C-19).

5α-ANDROST-8(14)-ENE-3,17-DIONE [**5**].—Mp 143–144° from Et_2O; $[\alpha]^{23}D$ +248° ($c = 0.26$, CH_2Cl_2); ir ν max (KBr) cm^{-1} 2960, 1742, 1615, 1460, 1250; uv λ max (MeOH, log ε) 296.4 nm (2.35), 226.8 nm (3.40); eims *m/z* (rel. int.) $[M]^+$ 286 (100), $[M - CO]^+$ 258 (40), 243 (19), 229 (52), 171 (52); hrms *m/z* 286.1930 (calcd for $C_{19}H_{26}O_2$, 286.1932); ^{1}H nmr ($CDCl_3$) δ 1.09 (H-18, s), 0.91 (H-19, s); ^{13}C nmr ($CDCl_3$) δ 38.0 (t, C-1), 37.9 (t, C-2), 211.0 (s, C-3), 44.5 (t, C-4), 46.4 (d, C-5), 29.1 (t, C-6), 28.8 (t, C-7), 129.6 (s, C-8), 49.4 (d, C-9), 37.3 (s, C-10), 19.1 (t, C-11), 28.7 (t, C-12), 47.3 (s, C-13), 135.7 (s, C-14), 36.1 (t, C-15), 23.1 (t, C-16), 221.3 (s, C-17), 22.0 (q, C-18), 11.9 (q, C-19). The ^{13}C-nmr assignment of **5** is made by correlation with that of **4** (1).

ANDROSTA-4,8(14),15-TRIENE-3,17-DIONE [**6**].—Mp 145–147° from Et_2O; $[\alpha]^{23}D$ +11° ($c = 0.10$, CH_2Cl_2), ir ν max (KBr) cm^{-1} 3080, 2970, 2940, 1700 (s), 1670 (s), 1618, 1540, 1435, 1372, 1230, 1190, 1140, 1078, 918, 868, 845, 715; uv λ max MeOH (log ε) 297.6 nmr (4.36), 228.8 (4.24); eims *m/z* (rel. int.) $[M]^+$ 282 (59), 249 (15), 159 (10), 148 (10), 147 (100), 136 (24); hrms *m/z* $[M]^+$ 282.1615 (calcd for $C_{19}H_{22}O_2$, 282.1619); ^{1}H nmr ($CDCl_3$) δ 7.92 (H-15, d, $J = 5.8$ Hz), 6.05 (H-16, d, $J = 5.8$ Hz), 5.80 (H-4, br s), 1.20 (6H, H-18 and H-19, s); ^{13}C nmr ($CDCl_3$) δ 34.7 (t, C-1), 34.0 (t, C-2), 198.8 (s, C-3), 124.3 (d, C-4), 168.1 (s, C-5), 32.7 (t, C-6), 29.4 (t, C-7), 140.8 (s, C-8), 49.4 (d, C-9), 41.0 (s, C-10), 19.1 (t, C-11), 27.4 (t, C-12), 45.6 (s, C-13), 133.3 (s, C-14), 152.1 (d, C-15), 129.3 (d, C-16), 211.2 (s, C-17), 23.1 (q, C-18), 18.5 (q, C-19). The ^{13}C-nmr assignment of **6** is made by correlation with that of **4** (1).

8,14-α-EPOXYANDROSTA-4,6-DIENE-3,17-DIONE [**7**].—Mp 167–169° from Et_2O; $[\alpha]^{25}D$ +405° ($c = 0.52$, CH_2Cl_2), ir ν max (KBr) cm^{-1} 3040, 2970, 2950, 1745 (s), 1669 (s), 1655 (s, sh), 1625, 1590,

1453, 1420, 1378, 1275, 1230, 1198, 1058, 1020, 890, 880; uv λ max MeOH (log ε) 283.6 (4.36); eims *m/z* (rel. int.) $[M]^+$ 298 (74), 283 (33), 255 (45), 237 (74), 209 (49), 195 (45), 181 (82), 166 (88), 165 (82); hrms *m/z* $[M]^+$ 298.1572 (calcd for $C_{19}H_{22}O_3$, 298.1569); ^1H nmr (CD_3OD) δ 2.08 (H-1β, m), 2.54 (H-2β, ddd, J = 17.7, 14.5, 5.1 Hz), 5.82 (H-4, br s), 1.57 (H-11β, dq, J = 4.8, 9.7, 9.7, 9.7 Hz), 1.45 (H-12β, m), 1.20 (H-18, s), 1.32 (H-19, s). The above assignments were made from nOe difference studies. Other ^1H-nmr assignments were made by a COSY-45 experiment: 1.84 (H-1α), 2.49 (H-2α), 5.90 (H-6, d, J = 9.8 Hz), 6.53 (H-7, d, J = 9.8 Hz), 1.64 (H-9), 2.12 (H-11α), 1.69 (H-12α), 2.43 (H-15α), 2.64 (H-15β), 2.58 (H-16β), 2.00 (H-16α). ^{13}C nmr ($CDCl_3$) δ 33.7 (t, C-1), 33.5 (t, C-2), 198.4 (s, C-3), 125.5 (d, C-4), 160.2 (s, C-5), 133.0 (d, C-6), 134.7 (d, C-7), 64.8 (s, C-8), 42.6 (d, C-9), 38.2 (s, C-10), 18.1 (t, C-11), 23.7 (t, C-12), 47.5 (s, C-13), 78.9 (s, C-14), 34.4 (t, C-15), 24.6 (t, C-16), 218.8 (s, C-17), 19.1 (q, C-18), 17.9 (q, C-19). The ^{13}C-nmr assignment of **7** is made by a hetero long-range COSY experiment and correlation with that of **4** (1).

17β-HYDROXYANDROSTA-4,8(14)-DIEN-3-ONE [**8**].—Mp 133–134° from Et_2O; $[\alpha]^{24}D$ +149° (c = 0.57, CH_2Cl_2); ir ν max (KBr) cm^{-1} 3470, 2970, 1680, 1663, 1615, 1435, 1375, 1225; uv λ max (MeOH, log ε) 234 nm (4.36); eims *m/z* (rel. int.) $[M]^+$ 286 (100), $[M-H_2O]^+$ 268 (18); hrms *m/z* 286.1936 (calcd for $C_{19}H_{26}O_2$, 286.1932); ^1H nmr ($CDCl_3$) δ 5.74 (H-4, br s), 3.57 (H-17, dd, J = 10.7, 7.4 Hz), 1.07 (H-19, s), 0.93 (H-18, s); ^{13}C nmr ($CDCl_3$) δ 34.6 (t, C-1), 34.1 (t, C-2), 199.3 (s, C-3), 123.5 (d, C-4), 170.9 (s, C-5), 32.5 (t, C-6), 29.5 (t, C-7), 126.9 (s, C-8), 47.8 (d, C-9), 41.0 (s, C-10), 19.5 (t, C-11), 29.2 (t, C-12), 42.9 (s, C-13), 139.6 (s, C-14), 34.6 (t, C-15), 23.6 (t, C-16), 82.1 (d, C-17), 17.0 (q, C-18), 18.5 (q, C-19). The ^{13}C-nmr assignment of **8** is made by correlation with that of **4** (1).

PREPARATION OF **8** FROM **4**.—Compound **4** (9.5 mg) dissolved in 1 ml of MeOH in a 10 ml round bottom flask to which was added $NaBH_4$ (12.7 mg) stepwise in an ice bath. After stirring 10 min, the mixture was partitioned between 6% NH_4OH (40 ml) and $CHCl_3$ (40 ml × 2). The $CHCl_3$ extract was dried over $MgSO_4$ and evaporated to give a viscous residue. The product was then purified via a Si gel column (5 g) eluted with 1.5% to 2% Me_2CO in $CHCl_3$. The product (8.2 mg, 86% yield) shows identical mp, R_f, and nmr data to those of **8**.

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