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Enzymatic 7β Hydroxylation of 4,4,10β-Trimethyl-*trans*-decal-3β-ol (TMD)

JAMES A. NELSON, MICHAEL R. CZARNY, AND THOMAS A. SPENCER

Department of Chemistry, Dartmouth College, Hanover, New Hampshire 03755

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4,4,10β-Trimethyl-*trans*-decal-3β-ol (TMD, **1**), an inhibitor of 2,3-oxidosqualene cyclase, is converted by S_{10} rat liver homogenate (RLH) to 4,4,10β-trimethyl-*trans*-decalin-3β,7β-diol (**2**) as its chief metabolite in 15 to 32% yield. Identification of **2** was accomplished by (a) gas–liquid chromatography/mass spectroscopy (GLC–MS) molecular-weight determination; (b) identity of GLC retention times with those of synthetic **2** and derived dione **15** on three columns; and (c) isotopic dilution and recrystallization to constant specific activity of both ^{3}H-**2** and its diacetate. Resolution of TMD was effected via formation of diastereomeric esters **18** and **19**; and both *d*-TMD and *l*-TMD were found to be metabolized in RLH to **2**, with *d*-TMD, the enantiomer having the nonsteroidal absolute configuration, being about twice as effectively hydroxylated.

We have recently reported that 4,4,10β-trimethyl-*trans*-decal-3β-ol (TMD,[1] **1**) is an effective inhibitor of the enzymatic conversion of squalene 2,3(*S*)-oxide to lanosterol in S_{10} rat-liver homogenates (RLH) (*1*) and in Chinese hamster ovary (CHO) cells (*1*, *2*). This inhibitor has proved useful both in our own laboratory, in studies which have established that squalene 2,3(*S*);22(*S*),23-dioxide and 24(*S*),25-epoxycholesterol are mammalian natural products (*3*, *4*), and in the laboratories of others (*5*). We have also preliminarily reported (*1*) that TMD is hydroxylated by S_{10} RLH at the 7β position to afford 4,4,10β-trimethyl-*trans*-decalin-3β,7β-diol (**2**) as its chief metabolite. The details of the isolation and identification of **2** are described in this paper.

In earlier studies of the oxidative demethylation at C-4 during steroid biosynthesis we have shown that the 4α (equatorial) methyl group is the site of initial enzymatic hydroxylation (*6*). We have also shown, through incubations with S_{10} RLH of various synthesized, radioactively labeled, cholestane derivatives, that there is a high degree of substrate specificity at C-4 in the enzymatic process (*7*). It seemed of interest to try to determine what effect structural changes in other, more remote, portions of the steroid structure might or might not have on the demethylation process. TMD, first prepared in 1958 (*8*), appeared to be a good model for the A and B rings of lanosterol, and was therefore selected as a substrate for incubation with S_{10} RLH.

[1] Abbreviations used: TMD, 4,4,10β-trimethyl-*trans*-decal-3β-ol; RLH, rat liver homogenate; CHO, Chinese hamster ovary; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; ORD, optical rotatory dispersion; TMS, tetramethylsilane; MS, mass spectroscopy; THF, tetrahydrofuran; PPO, 2,5-diphenyloxazole; POPOP, 1,4-bis[2-(5-phenyloxazole)]benzene.

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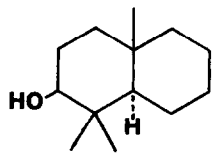
In all our earlier studies of the oxidative demethylation process (*6, 7*), we had used substrates derived from natural, optically active steroids. In the case of TMD, however, we decided for convenience initially to use synthetic racemic material, expecting, probably naively, that the nonsteroidal enantiomer would be unaffected biochemically.



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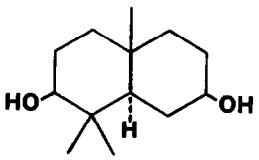

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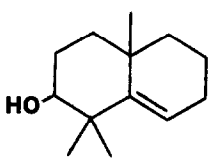

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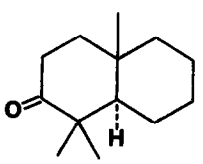

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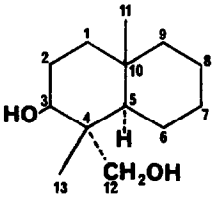
Preparation of *d,l*-TMD was performed via hydrogenation of 4,4,10β-trimethyl-Δ^5-decal-3β-ol (3), prepared by the method of Whitlock and Olson (*9*), rather than by the original route (*8*). This hydrogenation gave the desired trans ring fusion essentially stereospecifically. Radioactive labeling was introduced into *d,l*-TMD by oxidation to the corresponding ketone 4 (*8*), followed by exchange of *alpha* protons using acidic tritium oxide in THF (*10*) and reduction with $LiAlH_4$ to afford *d,l*-[^{3}H]TMD.

After this *d,l*-[^{3}H]TMD was incubated in the usual manner (*6*) with standard S_{10} RLH (*11*), 50–80% of the radioactivity was recovered by extraction with ethyl acetate. Analysis of this labeled organic material by thin-layer chromatography (TLC) showed that it contained 4% recovered *d,l*-TMD and two principal product fractions, a rather broad one with R_f 0.05 (14%) and a narrow one with R_f 0.37 (32%). Efforts were focused on identifying the major product with R_f 0.37. After this material had been purified by preparative gas–liquid chromatography (GLC), mass spectral analysis indicated that it had a molecular weight of 212, indicating monohydroxylation of TMD.



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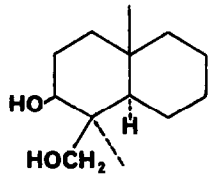

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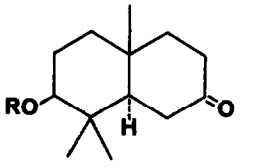
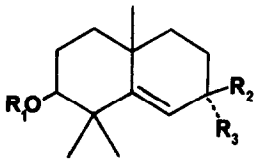


7, R = H

11, R = CH_3CO



8, R_1 = CH_3CO, R_2 = R_3 = H

9, R_1 = CH_3CO, R_2 + R_3 = O

10, R_1 = H, R_2 + R_3 = O

Our initial hope was that this metabolite was 4α-hydroxymethyl-4β,10β-dimethyl-*trans*-decal-3β-ol (5), the presumed initial intermediate in a C-4 methyl group oxidation analogous to that in the natural steroid series (*6*). However, comparison of the TLC and GLC properties of the major metabolite with those of 5 and its isomer 6, which had previously been prepared by us (*12*), showed clearly

TABLE 1

GLC RETENTION TIMES OF MAJOR METABOLITE AND VARIOUS SYNTHETIC DECALIN DIOLS[a,b]

Compound	Column[c] (temperature)		
	3% OV-17 (150°)	3% SE-30 (160°)	3% QF-1 (150°)
Metabolite	9:33	7:42	7:51
Oxidized metabolite	10:20	7:08	11:51 (165°)
2	9:33	7:44	7:50
15	10:20	7:04	11:51 (165°)
5[d]	11:30	7:25 (175°)	Decomposed
6[d]	11:55	7:35 (175°)	Decomposed

[a] GLC performed on a Varian Model 2100 instrument.

[b] Retention times are in minutes.

[c] Glass U-shaped columns, 6 ft × 4 mm; adsorbents purchased from Applied Science Laboratories, State College, Pa.

[d] Ref. (*12*).

that neither of these C-4 methyl group hydroxylation products was identical with the metabolite. The GLC data are given in Table 1.

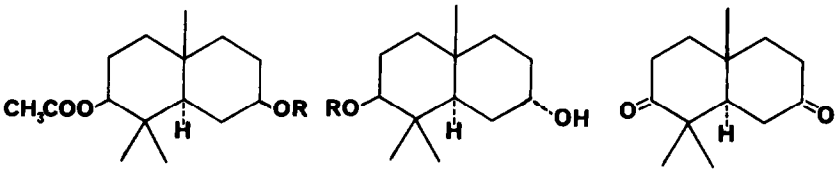
Since we had available only microgram quantities of metabolite, its identification could not be undertaken by degradation, and ultimately depended upon synthesis of possible diol products and comparison of their properties with those of the metabolite. There are 10 possible monohydroxylation sites on TMD: all carbons except C-3, C-4, and C-10 as numbered on **5**. Two of these (C-12 and C-13) had already been excluded by comparison of the metabolite with **5** and **6**.

Preliminary indication that another four of the possible sites could be eliminated from consideration was obtained by oxidizing a sample of the metabolite with Jones reagent (*13*). The product did not have strong uv absorption, which suggested that it was not a dione derived from a substance which had been hydroxylated at C-1 or C-2, because such a compound would be expected to be enolic and chromophoric. In addition, the oxidation product had chromatographic mobility which suggested that it was not the ketol derived from a C-5 hydroxylated metabolite or the keto acid derived from a C-11 alcohol. It was also found that this oxidation product was reconverted by $NaBH_4$ reduction to material which was identical by GLC to the original metabolite. This result suggested that the enzymatically introduced second hydroxyl group was equatorial (*14*).

Of the possible hydroxylated products at the four remaining sites, C-6, C-7, C-8, or C-9, we chose the C-7 equatorially oxygenated **2** as the initial target primarily because it appeared to be the easiest to prepare. Synthesis of *d,l*-**2** was readily accomplished by $LiAlH_4$ reduction of the known (*15*) hydroxyketone **7**, which was prepared by the sequence **3**→**8**→**9**→**10**→**7**, basically following the pathway of Mukherjee and Dutta (*16*) to **9**. Confirmation that the hydride reduction of **7** afforded the expected (*14*) 7β equatorial hydroxyl group as shown in **2** was obtained as follows.

The C-7 α H of compound **2** would be readily distinguishable from the C-7 β H

of the C-7 epimer of **2** on the basis of [^{1}H]nmr chemical shift and splitting pattern (*17*). To observe these distinctions clearly, however, it was necessary to cause the nmr signal of the C-3 α H of **2** to shift out of the same region of the spectrum. This was accomplished by acetylation of hydroxyketone **7** to afford **11**, which had previously been prepared by another route (*15*). In **11** the C-3 α H appears at δ 4.55 ppm. Reduction of **11** with $NaBH_4$ gave 91% of **12**, which had a complex broad multiplet at 3.5–4.0 ppm. On the other hand, reduction of **11** with the hindered $K(s\text{-}Bu)_3BH$ (*18*) gave 63% of **13**, which had a broad singlet at 4.1, characteristic in both chemical shift and splitting of an equatorial 7β H. As expected, treatment of **12** with $LiAlH_4$ gave **2**, and treatment of **13** with $LiAlH_4$ gave the previously unknown 3β,7α-diol **14**.



12, R = H
16, R = $OCCH_3$

13, R = CH_3CO
14, R = H

15

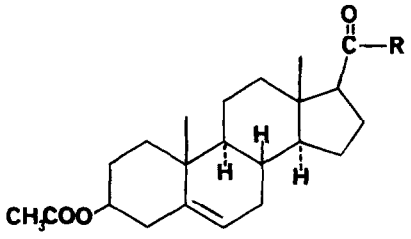
The retention times of **2** on three different GLC columns were essentially identical with those of the major metabolite, as shown in Table 1. Furthermore, the retention times of dione **15** (*16*) prepared by Jones oxidation (*13*) of **7**, also corresponded closely to those of the product obtained by analogous oxidation of the metabolite. Little doubt remained that the major metabolite from TMD was **2** and that we had been fortunate indeed in our initial choice of a synthetic target.

Final confirmation of the identity of the metabolite was achieved through isotopic dilution experiments. A metabolite product fraction obtained by preparative TLC was diluted with a large excess of unlabeled **2** and the mixture was recrystallized to constant specific activity. The amount of radioactivity incorporated in the recrystallized **2** indicated that at least 15% of the tritium in the incubated *d,l*-TMD had been isolated as **2**. Finally, the recrystallized **2** was isotopically diluted further and then converted to its diacetate **16**, which was also recrystallized to constant specific activity.

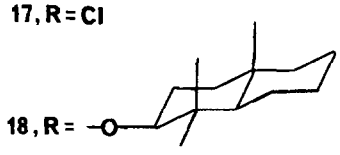
The fact that almost all of the *d,l*-TMD incubated had been metabolized and the relatively high yield of **2** raised the question whether *both* enantiomers of TMD were being enzymatically hydroxylated at the 7β position. To determine if this was the case, resolution of *d,l*-TMD was undertaken.

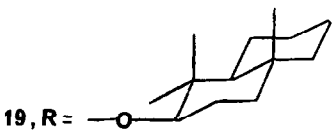
3β-Acetoxyetienic acid chloride **17** (*19*) was used to convert *d,l*-TMD into diastereomeric esters **18** and **19**. Separation of these diastereomers was laboriously accomplished by dry-column chromatography (*20*). Once pure **18** and **19** had been obtained they were converted by $LiAlH_4$ reduction to the pure enantiomers *d*-TMD and *l*-TMD. These enantiomers, and the derived ketones, *d*-**4** and *l*-**4**, displayed the appropriate and opposite optical rotatory dispersion (ORD) curves. The ORD data for *d*-TMD and *d*-**4** were consistent with those previously reported

by Djerassi and Marshall (*21*) for samples of these materials derived from a natural product.



17, R = Cl





Incubations of *d*-[^{3}H]TMD and *l*-[^{3}H]TMD with S_{10} RLH were conducted in the same manner as incubation of *d,l*-TMD. Analysis of the product mixtures by TLC showed that *both* *d*-TMD and *l*-TMD did indeed afford metabolite **2**, but in different amounts. The "unnatural" enantiomer, *d*-TMD, yielded in two runs an average of 43% of material with the R_f value of **2**, and *l*-TMD afforded an average of 24% of material with the R_f value of **2**, presumably enantiomeric to that obtained from *d*-TMD.

There is at least one precedent for such an "enantiomeric" enzymatic hydroxylation. Johnson *et al.* (*22*) have reported that both enantiomers of 1-benzoyl-*trans*-decahydroquinoline gave the corresponding enantiomers of 1-benzoyl-*trans*-decahydroquinolin-6-ol upon treatment with *S. sulfurescens*, in one case as the major product and in the other as a minor product.

No effort has been made to identify the metabolite or metabolites which comprise the more polar, R_f 0.14, product fraction. It is also probable, in view of the loss of radioactivity in the isotopic dilution experiment, that there is at least one other product in the narrow TLC band containing metabolite **2**. Of greater importance than attempts to identify the minor products from TMD, however, will be efforts to determine the relationship, if any, between the metabolism of TMD and its effect on steroid biosynthesis. In this connection, it is worth noting that TMD is not converted to **2** by CHO cells (*23*), in which its inhibitory properties have been most extensively studied (*2*).

EXPERIMENTAL

General

Melting points were determined in open capillaries in a Thomas–Hoover apparatus and are uncorrected. Elemental analyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, Michigan. Infrared (ir) spectra were recorded on a Perkin–Elmer Model 137 or 337 spectrometer. Unless otherwise specified, ir spectra of liquids were taken as thin films between NaCl plates and solids as KBr pellets. Ultraviolet (uv) spectra were recorded on a Unicam SP 800B spectrometer. Nuclear magnetic resonance (nmr) spectra were recorded on a

Perkin–Elmer R-24 instrument. Unless otherwise noted, all spectra were recorded using $CDCl_3$ as solvent. Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet, and coupling constants are given in hertz. Tetramethylsilane (TMS) was used as an internal standard. Mass spectra were determined at the MIT Mass Spectra Facility, sponsored by the U.S. Public Health Service Division of Research Resources through Grant RR 00317. Optical rotatory dispersion (ORD) curves were determined on a Cary Model 60 C D spectropolarimeter. Optical rotations were determined on a Zeiss–Ikon 3015M polarimeter.

Preparative thin-layer chromatography (TLC) was performed on 20 × 20-cm plates coated with 1.45-mm-thick layers of silica gel $PF_{(254+366)}$ (Brinkman Instruments, Westbury, N.Y.) which had been mixed with 0.002% Rhodamine 6G dye (Eastman Kodak Co., Rochester, N.Y.). Ultraviolet light was used to visualize TLC plates. Qualitative plates (0.25-mm-thick layers of silica gel $PF_{(254+366)}$) were sprayed with a 5% methanol:water (70:30) solution of phosphomolybdic acid (Eastman Kodak) and heated briefly at 110°. Details of gas–liquid chromatographic (GLC) analyses are given with Table 1.

Brine refers to saturated aqueous sodium chloride solution. Bicarbonate refers to saturated aqueous $NaHCO_3$ solution. A "normal workup" is defined as follows. The material was dissolved in ether which was washed twice with water. The aqueous layer was extracted again with ether. The combined ethereal layers were washed with brine, dried over $MgSO_4$, and evaporated. All compounds are racemic (*d,l*) mixtures unless otherwise specified. Rats (Charles Breeding Laboratories, Wilmington, Mass.) were male, Sprague–Dawley strain, CD, weighing 110–130 g.

4,4,10β-Trimethyl-Δ⁵-decal-3β-ol (**3**). 10-Methyl-$\Delta^{1,9}$-octal-2-one, prepared by the method of Ross and Levine (*24*), was converted to **3** by the method of Whitlock and Olson (*9*), except that NaO*t*Bu was used instead of KO*t*Bu in the procedure of Ringold and Rosenkranz (*25*), for the alkylation of 10-methyl-$\Delta^{1,9}$-octal-2-one.

4,4,10β-Trimethyl-trans-decal-3β-ol (**1**). A solution of 7.76 g (0.040 mol) of **3** and 3 ml of triethylamine in 500 ml of hexane was hydrogenated over 10% palladium-on-carbon at atmospheric pressure. After hydrogen uptake ceased, the catalyst was removed by filtration and the filtrate was evaporated to afford 7.8 g (100%) of **1** as a semisolid. Sublimation at 80°/15 Torr afforded 7.29 g (92%) of **1**, mp 61–62° (lit. (*8*) mp 60–65°); ir 3400 cm^{-1}; nmr δ 0.80 (s, 3), 0.95 (s, 3), 1.00 (s, 3), and 3.25 ppm (*d* of *d*, 1, J = 9, and 5 Hz).

4,4,10β-Trimethyl-3β-hydroxy-trans-decal-7-one (**7**). Acetylation of **3** to give **8** and oxidation of **8** to give **9** were conducted according to the procedures of Mukherjee and Dutta (*16*). The conversion of **8** to **9** afforded 84% of **9** as an oil, bp 153–155°/0.15 Torr (lit. (*16*) bp 150°/0.1 Torr). This material eventually solidified, and recrystallization from ether–pentane gave 59% of **9**, mp 61–64°; ir 1740 and 1685 cm^{-1}; nmr δ 1.11 (s, 3), 1.22 (s, 3), 1.40 (s, 3), 2.10 (s, 3), 4.60 (*t*, 1, J = 7 Hz), and 5.97 ppm (s, 1). Hydrolysis of **9** by treatment with 1.5 eq of KOH in CH_3OH for 12 hr at room temperature afforded 97% of **10**, mp 109–111°. Recrystallization from ether gave 90% of **10**, mp 119–121° (lit. (*16*) bp 165–170°/4 Torr; lit. (*15*) mp

48–49°; lit. (*26*) mp 108–116°); ir 3500 and 1680 cm^{-1}; nmr 1.10 (s, 3), 1.22 (s, 3), 1.31 (s, 3), 3.47 (*d* of *d*, 1, J = 7, and 5 Hz) and 5.87 ppm (s, 1).

Reduction of **10** to **7** was effected, as it was by Levisalles and Rudler (*15*), with lithium in ammonia except that only ether was used as solvent, and at the end of the reaction the ammonia was evaporated and aqueous HCl was added. Normal workup afforded a crude product which was recrystallized from ether to give 84% of prisms of **7**, mp 121–123°. Sublimation of the crude product gave needles of **7**, mp 86–87° (lit. (*15*) mp 85–86°); ir 3400 and 1710 cm^{-1}; nmr δ 0.80 (s, 3), 0.95 (s, 3), 1.12 (s, 3), and 3.27 (*d* of *d*, 1, J = 5, and 6 Hz).

4,4,10β-Trimethyl-trans-decalin-3β,7β-diol (**2**). A mixture of 1.1 g (5.2 mmol) of **7** and 0.15 g (0.4 mmol) of $LiAlH_4$ in 75 ml of ether was stirred for 1.5 hr at room temperature. Excess $LiAlH_4$ was destroyed by addition of ethyl acetate, followed by 30 ml of 1 *M* hydrochloric acid. Normal workup afforded 1.1 g of crude solid **2**. Recrystallization from ether afforded 0.82 g (74%) of **2**, mp 147–149°; ir 3300 cm^{-1}; nmr δ 0.80 (s, 3), 0.98 (s, 3), 1.00 (s, 3), and 3.10–3.85 ppm (bm, 2). *Anal.* Calcd for $C_{13}H_{24}O_2$: C, 73.54, H, 11.39. Found: C, 73.50; H, 11.35.

4,4,10β-Trimethyl-3β-acetoxy-trans-decal-7-one (**11**). A solution of 127 mg (0.61 mmol) of **7** and 100 mg (0.98 mmol) of acetic anhydride in 20 ml of pyridine was refluxed for 20 hr. The solution was cooled and the pyridine was removed by repeated azeotropic distillation *in vacuo* with benzene. Normal workup afforded 143 mg of yellow oil which solidified on standing. This material was sublimed at 115°/20 Torr to afford 120 mg (78%) of pure **11**, mp 110–111° (lit. (*16*) mp 113–114°); ir 1740–1700 cm^{-1}; nmr δ 0.85 (s, 3), 0.94 (s, 3), 1.17 (s, 3), 2.06 (s, 3), and 4.55 ppm (*d* of *d*, 1, J = 5, and 6 Hz).

4,4,10β-Trimethyl-3β-acetoxy-trans-decal-7β-ol (**12**). A mixture of 106 mg (0.42 mmol) of **11**, 10 mg (0.3 mmol) of $NaBH_4$, and 10 ml of methanol was stirred at room temperature for 90 min. The mixture was concentrated *in vacuo* and the residue was partitioned between 30 ml of ether and 20 ml of 5% HCl solution. Normal workup afforded 120 mg of colorless oil. Preparative TLC (1:1 hexane:ether, twice) afforded 9 mg of **11** and 100 mg (91%) of colorless oily **12**: ir 3450 and 1735 cm^{-1}, nmr δ 0.85 (s, 6), 1.00 (s, 3), 2.10 (s, 3), 3.2–4.0 (bm, 1), and 4.50 ppm (*d* of *d*, 1, J = 5, and 6 Hz). MS *m/e* M^+ 254.1879 (Calcd for $C_{15}H_{26}O_3$: 254.1882).

4,4,10β-Trimethyl-3β-acetoxy-trans-decal-7β-ol (**13**). To a solution of 175 mg (0.69 mmol) of **11** in 20 ml of anhydrous THF, cooled to 0° and stirred under nitrogen, was added 1.5 ml (0.75 mmol) of 0.5 *M* K-Selectride (Aldrich). The mixture was allowed to warm to room temperature and was stirred for an additional 2 hr. The resulting yellow solution was partitioned between 100 ml of ether and 50 ml of water. Normal workup afforded of 203 mg of yellow oil. Preparative TLC (1:1 hexane:ether, twice) afforded 30 mg of **11**, 111 mg (63%) of **13**, and 9 mg of polar material. Compound **13** was a colorless oil: ir 3500 and 1730 cm^{-1}; nmr δ 0.81 (s, 3), 0.91 (s, 6), 2.01 (s, 3), 4.11 (bs, 1), and 4.45 ppm (*t*, 1, J = 6 Hz). MS *m/e* M^+ 254.1884 (Calcd for $C_{15}H_{26}O_3$: 254.1882).

4,4,10β-Trimethyl-trans-decalin-3β-7α-diol (**14**). A mixture of 100 mg (0.39 mmol) of **13** and 30 mg (0.81 mmol) of $LiAlH_4$ in 30 ml of ether was stirred for 2 hr at room temperature. The excess $LiAlH_4$ was decomposed with 5% sulfuric acid

and the mixture was partitioned between 20 ml of water and 20 ml of ether. Normal workup afforded 86 mg of a white solid which was homogeneous by TLC (2:1 ether:hexane). This material was sublimed at 150°/20 Torr to afford 75 mg (90%) of **14**, mp 164–166°; ir 3350 cm^{-1}, nmr δ 0.65 (s, 3), 0.81 (s, 3), 0.87 (s, 3), 3.17 (*d* of *d*, 1, $J = 6$ and 5 Hz), and 4.05 ppm (bs, 1). *Anal.* Calcd for $C_{13}H_{24}O_2$: C, 73.54; H, 11.39. Found: C, 73.60; H, 11.47.

Conversion of **12** *to* **2**. Exactly as in the conversion of **13** to **14**, $LiAlH_4$ reduction of **12** afforded 69% of purified **2**.

4,4,10β-Trimethyl-3β,7β-diacetoxy-trans-decalin (**16**). Treatment of **2** with acetic anhydride, acetyl chloride, and pyridine according to the procedure of Mukherjee and Dutta (*16*) afforded 89% of **16**, mp 84–85°, after recrystallization from hexane; ir 1740 cm^{-1}; nmr δ 0.85 (s, 6), 1.00 (s, 3), 2.05 (s, 6) and 4.4–4.8 ppm (bm, 2). *Anal.* Calcd for $C_{17}H_{28}O_4$: C, 68.89; H, 9.52. Found: C, 69.20; H, 9.59.

4,4,10β-Trimethyl-trans-decalin-3,7-dione (**15**). To a solution of 45 mg (0.21 mmol) of **7** in 20 ml of acetone was added 0.3 ml of Jones reagent (*13*). The mixture was stirred for 20 min and then concentrated. Normal workup afforded 46 mg of yellow solid which was sublimed at 110°/20 Torr to afford 33 mg of **15**, mp 120–121° (lit. (*16*) mp 121–122°); ir 1715 cm^{-1}; nmr δ 1.03 (s, 6) and 1.33 ppm (s, 3).

Diastereomeric Esters **18** *and* **19**. 3β-Acetoxyetienic acid was prepared from pregnenolone (Steraloids, Wilton, N. H.) by the following sequence: acetylation to pregnenolone acetate in 94% yield, mp 146–147° (lit. (*27*) mp 147°); hypobromite oxidation (*28*) in 98% yield to 3β-hydroxyetienic acid, mp 263–265° (lit. (*29*) mp 273–274°); and acetylation (*28*) in 62% yield to 3β-acetoxyetienic acid, mp 236–238° (lit. (*28*) mp 235–238°); ir 3300–3100, 1735, and 1710 cm^{-1}; nmr δ 0.75 (s, 3), 1.03 (s, 3), 2.01 (s, 3), 4.3–4.8 (bm, 1), 5.40 (bs, 1) and 10.1 ppm (bs, 1).

A mixture of 5.00 g (0.014 mol) of 3β-acetoxyetienic acid, 250 ml of CH_2Cl_2 and 25 ml (0.350 mol) of freshly distilled $SOCl_2$ was allowed to stir for 12 hr at room temperature. The solution was evaporated and a vacuum pump was used to remove the last traces of $SOCl_2$. A solution of 2.50 g (0.013 mol) of TMD (**1**) in 25 ml of CH_2Cl_2 was added to the resulting solid acid chloride **17** with rapid stirring followed by an additional 250 ml of CH_2Cl_2. The resulting suspension was stirred for 12 hr; after 3 hr all the solid material had dissolved and the solution took on a light red color. The solution was poured into 200 ml of water and the aqueous layer was extracted with an additional 150 ml of CH_2Cl_2. The combined organic layers were washed with 2 × 100 ml of brine, dried over $MgSO_4$ and evaporated to give 7.54 g of yellow solid, which was chromatographed on 200 g of silica gel. Elution with 10% ether–hexane afforded 3.47 g (51%) of a mixture of **18** and **19** as a white solid. Elution with 15% ether–hexane gave 1.11 g of TMD.

The mixture of **18** and **19** was separated by dry column chromatography (*20*). In a typical run 2.937 g of mixture was chromatographed on acid-washed activity III alumina (5.0 × 60.0 cm) with 2:1 hexane:ether to give 0.783 g of **18**, then 0.880 g of a mixture of **18** and **19**, and finally 0.657 g of **19**.

Diastereomer **18** was recrystallized from ether–hexane thrice to afford pure **18**, homogeneous by tlc on silica gel and alumina, mp 194–196°; ir 1730 cm^{-1}; nmr δ 0.65 (s, 3), 0.76 (s, 6), 0.94 (s, 3), 1.01 (s, 3), 2.00 (s, 3), 4.35–4.7 (bm, 2) and 5.40

ppm (bs, 1); $[\alpha]_D^{31}$ − 11.5° ($CHCl_3$). *Anal.* Calcd for $C_{35}H_{54}O_4$: C, 78.02; H, 10.10. Found: C, 77.96; H, 9.90.

Diastereomer **19** was recrystallized from ether–hexane thrice to afford pure **19**, homogeneous by TLC on silica gel and alumina, mp 204–206°; ir 1735 cm^{-1}; nmr δ 0.74 (s, 3), 0.85 (s, 3), 0.87 (s, 3), 0.93 (s, 3), 1.02 (s, 3), 2.00 (s, 3), 4.5 (bt, 2), and 5.40 ppm (bs, 1); $[\alpha]_D^{31}$ −13.5° ($CHCl_3$). *Anal.* Calcd for $C_{35}H_{54}O_4$: C, 78.02; H, 10.10. Found: C, 78.08; H, 10.02.

l-TMD. A mixture of 0.300 g (0.56 mmol) of **19**, 25 ml of THF, and 0.20 g (5.40 mmol) of $LiAlH_4$ was stirred for 5 hr at room temperature. Normal workup afforded 0.254 g of white solid which was purified by preparative TLC (3 : 1 hexane : ether, twice) to give 0.106 g (97%) of crystalline *l*-TMD and 133 mg of what is assumed to be 3β-hydroxy-17β-hydroxymethylandrost-$\Delta^{5,6}$-ene, which was not characterized. Sublimation of the *l*-TMD afforded 78 mg (90%) of pure *l*-TMD, mp 87–88°; $[\alpha]_D^{26}$ −16.9° ($CHCl_3$); ir 3500 cm^{-1}; nmr δ 0.72 (s, 3), 0.91 (s, 3), 0.96 (s, 3), and 3.20 ppm (bt, 1); MS *m/e* M^+ 196.1826 (Calcd for $C_{13}H_{24}O$: 196.1827). ORD (CH_3OH) negative plain dispersion curve rising to about 115° at 250 nm; $[\alpha]_{589}$ −11.7°.

d-TMD. A mixture of 1.400 g (2.60 mmol) of **18**, 50 ml of THF, and 0.300 g (8.10 mmol) of $LiAlH_4$ was stirred for 5 hr at room temperature. Normal workup afforded 1.330 g of white solid which was purified by preparative TLC (3 : 1 hexane : ether, twice) to give 0.502 g (98%) of crystalline *d*-TMD and 300 mg of the presumed 3β-hydroxy-17β-hydroxymethylandrost-$\Delta^{5,6}$-ene. Sublimation of the *d*-TMD afforded 446 mg (88%) of pure *d*-TMD, mp 87–88° (lit. (*21*) mp 87–89°); $[\alpha]_D^{26}$ +15.9° ($CHCl_3$); ir and nmr; same as *l*-TMD; MS *m/e* M^+ 196.1827); ORD (CH_3OH); positive plain dispersion curve rising to about 111° at 250 nm; $[\alpha]_{589}$ +12.2°.

l-4,4,10β-Trimethyl-trans-decal-3-one (*l*-**4**). A solution of 98 mg (0.05 mmol) of *l*-TMD in 5 ml of acetone was treated with 5 drops of Jones reagent (*13*) and stirred for 30 min at room temperature. The solution was concentrated and the residue was subjected to normal workup to afford 85 mg of colorless oil. Preparative TLC (3 : 1 hexane : ether) afforded 80 mg (83%) of oily *l*-**4**: ir 1710 cm^{-1}; nmr δ 1.00 (s, 3), 1.06 (s, 3), 1.14 (s, 3); MS *m/e* M^+ 194.1669 (Calcd for $C_{13}H_{22}O$: 194.1671); ORD (CH_3OH) $[\alpha]_{600}$ −36°, $[\alpha]_{589}$ −40°, $[\alpha]_{320}$ −370°.

d-4,4,10β-*Trimethyl-trans-decal-3-one* (*d*-**4**). In exactly the same manner *d*-TMD was converted in 77% yield to *d*-**4**; ir and nmr same as *l*-**4**; MS *m/e* M^+ 194.1668; ORD (CH_3OH) $[\alpha]_{600}$ +44°, $[\alpha]_{589}$ +45°, $[\alpha]_{320}$ +300°.

[^{3}H]TMD (d,l, or d,l). For introduction of radioactive labeling, TMD was oxidized to **4** as described above. Tritium was introduced at C-2 by treatment with acidic tritium oxide in THF (*10*). Reduction of [^{3}H]-**4** with $LiAlH_4$, as in the conversion of **7** to **2**, afforded [^{3}H]TMD, which was purified by preparative TLC (1 : 2 ether–hexene, twice); GLC analysis, 3% OV-225, 6 ft × 4 mm, 110° and 3% OV-17, 5.5 ft × 4 mm, 135°, indicated 97% pure; specific activity typically 77,500 dpm/μg.

Incubation of [^{3}H]TMD. S_{10} Rat-liver homogenates (RLH) were prepared by the procedure of Popjak (*11*). A 150-μl aliquot (containing 34 μg of [^{3}H]TMD) of a solution of 1.2 mg of [^{3}H]TMD and 15 mg of polyoxyethylene sorbitan monooleate

(Tween-80; Sigma Chemical Co., St. Louis, Mo.) in 5.0 ml of distilled water was added to 5 ml of S_{10} RLH and the mixture was incubated at 37° for 3 hr, cooled to 0°, and extracted with 4 × 30 ml of ethyl acetate. The combined organic layers were evaporated to afford a residue containing 50–80% of the initial radioactivity. Control runs with boiled RLH gave 80–90% recovery of unchanged TMD.

Product analysis. The products were analyzed by TLC using ether. About 0.5% of the extract residue was used. When possible, 10–20 μg of nonradioactive reference compounds were spotted beside the product mixture. After the plate was developed, the components were located using visible and uv light, iodine, or reference compound, and the plate was cut into 0.25- to 1.0-cm sections and each section was scraped into a scintillation vial. To each vial was added 10 ml of a toluene fluor (PPO + POPOP) containing 2% ethanol. Usually 85–100% of the total extracted radioactivity was accounted for in the vials.

In a typical incubation of *d,l*-TMD with S_{10} RLH, the extracted radioactivity was partitioned into 7 principal fractions, the one with lowest mobility containing 22% of the ^3H, 3 fractions with the R_f of **2** containing 54% of the ^3H, and 3 fractions of the R_f of TMD containing 6.8% of the ^3H, with minima in other fractions between these peaks. The yield of a metabolite was then calculated as the percent radioactivity extracted × percent in the TLC band; e.g., for **2**, 60% × 54% = 32% yield.

The band containing product [^3H]-**2** was obtained on a larger scale by preparative TLC, and it was then purified by preparative GLC on a 3% OV-17, 5½ ft × 4-mm column at 170°. GLC–MS analysis of this material give m/e M^+ = 212.

Identification of the chief metabolite as **2** was accomplished by GLC comparison of the metabolite with various synthesized diols. The data are given in Table 1. Confirmation was obtained by addition to 0.6 mg of crude metabolite from *d,l*-[^3H]TMD, which had been isolated by preparative TLC and which had 1.7×10^6 dpm, of 200 mg of unlabeled *d,l*-**2**, and this mixture was recrystallized from ether. These recrystallizations afforded **2** with mp 147–149° and, successively, 3840, 3490, 3450, and 3450 dpm/mg. The residue from the mother liquor from the fourth recrystallization had 3480 dpm/mg.

As final confirmation, 39 mg of *d,l*-**2** from the fourth recrystallization above was diluted with 161.4 mg of unlabeled *d,l*-**2** and this mixture was converted as previously described to 270.5 mg (97%) of diacetate **16**, which was purified by preparative TLC (1:1 ether–hexane) and recrystallized from hexane to give 248.5 mg (89%) of **16**, mp 84–85°, with specific activity 485 dpm/mg. Recrystallization afforded **16**, mp 84–85°, with 485 dpm/mg. The calculated specific activity, based on the amount of *d,l*-[^3H]-**2** used for acetylation, was 480 dpm/mg.

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