Journal of Natural Products
Vol. 58, No. 8, pp. 1222-1227, August 1995

MICROBIAL TRANSFORMATION OF LANOSTEROL DERIVATIVES WITH *MYCOBACTERIUM* SP. (NRRL B-3805)

K.C. WANG, BIH-JING YOU, JIANN-LONG YAN, and SHOEI-SHENG LEE*

School of Pharmacy, College of Medicine, National Taiwan University, Taipei 100, Taiwan, Republic of China

ABSTRACT.—Incubation of 8α,9α-epoxytetrahydrolanosterol [3] with *Mycobacterium* sp. (NRRL B-3805) gave 20(*S*)-hydroxymethyl-12α-hydroxypregn-4-en-3-one [4] and 20(*S*)-hydroxymethyl-4,4,14α-trimethylpregna-7,9(11)-dien-3β-ol [5] in 5.0% and 1.6% yields, respectively. Incubation of lanosta-7,9(11)-dien-3β-ol [6] resulted in the isolation of two C-19 steroids as the major metabolites that have been isolated previously from the incubation of lanosterol, and methyl 12α-hydroxybisnorchola-4,17(20)-dien-22-oate [9] in low yield. These results indicated that this microorganism is capable of carrying out saturation of a C=C bond, methylation of a carboxyl function, and 12α-hydroxylation in the steroid nucleus, besides earlier reported transformations.

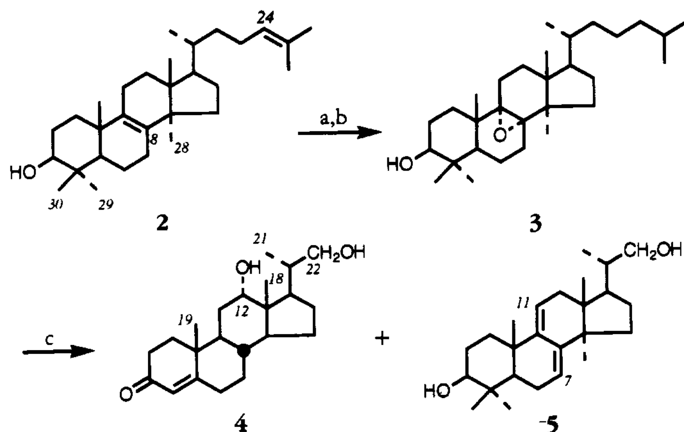
In previous reports we have disclosed that cycloartenol and 24-methylenecycloartanol are transformed by *Mycobacterium* sp. (NRRL B-3805) to give androsta-4,8(14)-diene-3,17-dione [1] as the major metabolite, in 34 and 35% yields, respectively (1,2). It was also shown that when lanosterol [2] was incubated with this microorganism **1** was isolated as the major product in 30% yield. During this transformation, a series of complicated chemical changes occurred including demethylation at the 4α,4β- and 14α- positions, formation of a $\Delta^{8(14)}$ double bond accompanied by the disappearance of the $\Delta^{8(9)}$ double bond, and cleavage of the C-17 side-chain. Among these changes, we were particularly interested in the disappearance of the $\Delta^{8(9)}$ double bond accompanied by the formation of the $\Delta^{8(14)}$ double bond in **1**. Therefore, lanosterol derivatives with substituent modification at C-8 and C-9 were prepared and subjected to transformation with this microorganism.

RESULTS AND DISCUSSION

After unsuccessful attempts in hydrogenating the $\Delta^{8(9)}$ double bond, **2** was converted smoothly with catalytic hydrogenation (H_2-Pd) and peroxidation (*m*-CPBA) to 8α,9α-epoxy-tetrahydrolanosterol [3] (3). Compound **3** showed a molecular ion at *m/z* 444 in its eims corresponding to a formula of $C_{30}H_{52}O_2$. The presence of two oxygenated quaternary carbon signals at δ 70.6 and 68.2, along with the disappearance of two olefinic carbon signals (C-8 and C-9, overlapping at δ 134.5 in dihydrolanosterol) confirmed its structure. The favored α-side attack by *m*-CPBA was confirmed by an nOe nmr study, in which a critical observation was the interaction of H-18 and H-19, which is only possible for an 8α,9α-epoxy structure in **3**.

From the incubation mixture of **3** with *Mycobacterium* sp. (NRRL B-3805), compounds **4** and **5** were isolated in relatively low yields of 5.0% and 1.6%, respectively (Scheme 1).

The hreims of **4** showed a molecular ion at *m/z* 346.2510, consistent with an elemental formula of $C_{22}H_{34}O_3$. The ^{1}H-nmr spectrum showed an olefinic proton signal at δ 5.70 (br s), typical for H-4 in a 3-one-4-ene sterol. The presence of this functionality was confirmed by an ir absorption at 1659 cm^{-1} and the uv absorption maximum at 242 nm. The ir spectrum also indicated the presence of hydroxy groups (3425 cm^{-1}). Three methyl signals in the ^{1}H-nmr spectrum of **4** including two singlets (δ 0.72, 1.13) and one doublet (δ 1.04, J=6.6 Hz), and two hydroxylated carbons (δ 72.5, d; δ 67.4, t) were observed in the ^{13}C-nmr spectrum. These data suggested the presence of a C-19 sterol



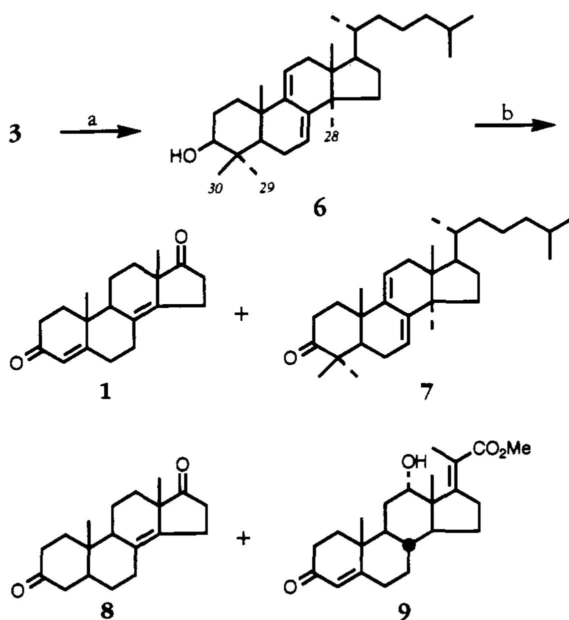
a. Pd/C, H_2, 35 psi, dioxane; b. *m*-CPBA, $CHCl_3$, 0°; c. *Mycobacterium* sp. (NRRL B-3805)

SCHEME 1. Transformation of 8α,9α-epoxytetrahydrolanosterol [**3**].

skeleton with a C-3 side-chain containing a primary alcohol. The location of the secondary alcohol at C-12 and methyl groups at C-10 and C-13 were further established by nOe observations. Irradiation of the Me-18 (δ 0.72, s) signal enhanced the Me-21 (δ 1.04, d), H-12β (δ 4.00, dd), H-11β (δ 1.63, ddd, J=3.0, 11.5, and 11.5 Hz), and the H-8 and H-20 signals (both overlapping at δ 1.53, m). The signals of H-11β and H-8 were also enhanced upon irradiation of Me-19 (δ 1.13, s) which established a trans ring junction for the B/C rings. The nOe data hence elucidated the structure of **4** to be 20(*S*)-hydroxymethyl-12α-hydroxypregn-4-en-3-one. 2D nmr spectra obtained for **4** provided further supporting evidence for the structure of this compound. In particular, the inverse heteronuclear multiple bond correlation (HMBC) spectrum linked the coupled proton and carbon signals unambiguously, in which H-4 and H-19 coupled to C-10, H-18 coupled to C-12, and H-22 coupled to C-20 and C-21, thus confirming the stereochemistry of C-10, C-12, C-13, and the isopropyl alcohol at C-17.

The hreims of **5** showed a molecular ion at *m/z* 372.3003 consistent with a molecular formula of $C_{25}H_{40}O_2$. The ir spectrum indicated hydroxyl but no ketone absorption. The uv absorption maximum at 243 nm revealed a $\Delta^{7,9(11)}$-diene chromophore (4). The ^1H-nmr spectrum showed signals ascribed to two olefinic protons (δ 5.31, m, H-7 and δ 5.46, m, H-11), a primary alcohol (CH_2OH at δ 3.65, dd, J=3.0 and 7.4 Hz and δ 3.35, dd, J=6.7 and 7.4 Hz, H-22), a secondary alcohol (C*H*OH at δ 3.23, dd, J=4.8 and 10.8 Hz, H-3), five methyl singlets, and a methyl doublet (δ 1.00, J=7.4 Hz). The chemical shifts and coupling pattern of the primary alcohol and the methyl doublet were almost identical to those of the C-17 side-chain of **4**. Analysis of these data enabled the structure of **5** to be assigned as 20(*S*)-hydroxymethyl-4,4,14-trimethylpregna-7,9(11)-dien-3β-ol. This structure was confirmed by the comparison of the ^1H- and ^{13}C-nmr data in the steroid nucleus with ganodermadiol (4).

In the transformation of cholesterol and phytosterols with *Mycobacterium* sp. (NRRL B-3805), 20(*S*)-hydroxymethylpregn-4-en-3-one was isolated in 1–2% yield (5,6). Inasmuch as compound **5**, isolated from the incubation of **3** in 1.6% yield, retains all the methyl groups located on the steroid nucleus, we wondered if the introduction of Δ^7 and $\Delta^{9(11)}$ double bonds in the sterol molecule might affect the enzymatic demethylation and the side-chain cleavage process in this microorganism. Therefore, the substrate lanosta-7,9(11)-dien-3β-ol [**6**] was prepared from **3** by treatment with acid. The hreims of **6** suggested a molecular formula of $C_{30}H_{50}O$. Its uv absorption maximum at 243 nm is



a. H_2SO_4, Me_2CO-H_2O, room temperature; b. *Mycobacterium* sp. (NRRL B-3805)

SCHEME 2. Transformation of lanosta-7,9(11)-dien-3β-ol [**6**].

consistent with the presence of conjugated double bonds. The ^1H-nmr spectrum displaying two olefinic protons at δ 5.44 (dd, H-11) and 5.29 (br d, H-7) for the 7,9(11)-diene moiety (4) confirmed the structure of **6**.

From the incubation mixture of **6**, four products, compounds **7** (4.2%), **1** (30%), **8** (7.1%), and **9** (0.7%) were isolated (Scheme 2). Compound **7**, with a molecular formula of $C_{30}H_{48}O$ deduced from the hreims, showed a carbonyl but no hydroxyl absorption in the ir spectrum. Its ^1H-nmr spectrum also lacked the H-3 signal. Hence, **7** is the C-3 oxidation product of **6** and its structure was confirmed by comparison of physical data (mp, uv, ir, ^1H-nmr) with those of the product of the reaction of **6** with chromium trioxide-pyridine complex.

Compounds **1** and **8** were identical to androsta-4,8(14)-diene-3,17-dione (1) and 5α-androst-8(14)-ene-3,17-dione (2), respectively, in every respect (mp, uv, ir, ^1H-nmr).

Compound **9**, with the molecular formula $C_{23}H_{32}O_4$ deduced from its hreims, showed hydroxyl and conjugated carbonyl absorptions in the ir spectrum. The uv absorption maximum at 238 nm and a ^1H-nmr olefinic proton signal at δ 5.73 (br s) were consistent with the presence of a 3-one-4-ene moiety (1). The ^1H-nmr spectrum of **9** also displayed a broad singlet at δ 4.51 (H-12β), a MeO singlet at δ 3.70 (22-OMe), and three methyl singlets at δ 2.00 (H-21), 1.17 (H-19), and 0.97 (H-18). The ^{13}C-nmr spectrum of **9** showed signals for an α,β-unsaturated ester at δ 169.4 (s, C-22), 159.9 (s, C-17), and 119.2 (s, C-20), in addition to the α,β-unsaturated ketone ($\delta_{C\text{-}3}$ 199.3, $\delta_{C\text{-}4}$ 124.0 and $\delta_{C\text{-}5}$ 170.5). The location of these structural moieties was established from the HMBC spectrum (J_{CH}=8 Hz). The critical structural information obtained was the observation of the coupling of both H-18 (δ 0.97) and H-21 (δ 2.00) to C-17 (δ 159.9) in this 2D nmr spectrum. These data confirmed the structure of **9** to be methyl 12α-hydroxybisnorchola-4,17(20)-dien-22-oate.

The metabolic pathways involved in the microbiological cleavage of the cholesterol and phytosterol side-chains have been well documented in the literature by Sih *et al.* (7–

10). In these previous studies, C-22 acid intermediates have been isolated and characterized (7). The isolation of **9**, an α,β-unsaturated C-22 acid methyl ester, demonstrated again that *Mycobacterium* sp. (NRRL B-3805) is capable of methylating the carboxylic function, as exemplified by our previous report on the microbial transformation of ceanothic acid (11). The isolation of compounds **4** and **9** revealed that hydroxylation could occur in these modified steroids (8α,9α-epoxy in **3** and $\Delta^{7,9(11)}$ diene in **6**). Without such structural modifications, hydroxylation on the steroid nucleus has never been observed with *Mycobacterium* sp. (NRRL B-3805). In addition, the present results showed that the 8α,9α-epoxy group and the $\Delta^{7,9(11)}$ diene were able to be reduced by this microorganism.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Fisher-Johns or a Buchi 510 melting point apparatus and are uncorrected. The optical rotations were recorded on a Jasco DIP-181 digital polarimeter. Ir spectra were recorded on a Jasco A-100 infrared spectrophotometer. Uv spectra were recorded on a Hitachi 150-20 uv spectrophotometer. ^{1}H- and ^{13}C-nmr spectra were obtained in $CDCl_3$ using residual solvent peaks as reference standards (δ_H 7.24 ppm, δ_C 77.0 ppm) on Bruker AM-300 or Bruker AMX-400 spectrometers. 2D Nmr (COSY-45, HETCOR, and HMBC) were recorded with Bruker's standard pulse programs. Ms spectra were recorded with a TSQ-46c GC/MS/MS/DS (eims) and JEOL JMS-HX 110 mass spectrometer (hreims) at 70 eV. Nutrient broth and other media chemicals were obtained from Difco Laboratories, Detroit, MI. Dextrose was purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. The test microorganism, *Mycobacterium* sp. (NRRL B-3805), was maintained on an agar slant (dextrose 1%, yeast extract 1%, nutrient broth 0.8%, agar 3%) at 26° for 11 days before being transferred to broth medium. Lanosterol was purchased from Tokyo Kasei Kogyo Co., Tokyo, Japan.

PREPARATION OF 8α,9α-EPOXYTETRAHYDROLANOSTEROL [**3**].—A mixture of **2** (10.0 g, 23.4 mmol), dioxane (120 ml), and 10% Pd/C (1.0 g) was hydrogenated with H_2 at 35 psi overnight at room temperature. The suspension was filtered over Celite and the filtrate was evaporated *in vacuo* to give 9.93 g (99%) of solid residue. Recrystallization of the residue with MeOH gave 24,25-dihydrolanosterol: mp 141°; ir ν max (KBr) 3306 (OH), 2952, 1467, 1372, 1029 cm^{-1}; ^{1}H- and ^{13}C-nmr data, see Emmons *et al.* (3); eims *m/z* $[M]^+$ 428 (19), 414 (25), 413 (88), 95 (44), 69 (51), 43 (100).

To a solution of *m*-CPBA (6.3 g, 18 mmol) in $CHCl_3$ (20 ml) was added dihydrolanosterol (5.28 g in 30 ml $CHCl_3$, 12 mmol) and the solution was stirred at 0° for 2 h, washed with 1 N NaOH solution (50 ml×3) and H_2O, dried over Na_2SO_4, and evaporated *in vacuo* to give a crude residue (5.26 g). Repeated crystallization of the crude product from MeOH gave **3** (3.32 g, 60%) as colorless crystals: mp 160–161°; $[\alpha]^{27}D$ +11.9° (c=0.91, $CHCl_3$); ir (KBr) ν max 3293, 2960, 1467, 1382, 1027 cm^{-1}; ^{1}H- and ^{13}C-nmr data, see Ref. (3); nOe data, Me-28 (δ 0.92) to H-3 (δ 3.20, dd, J=4.4 and 10.6 Hz) 9%, Me-29 (δ 0.76) 14%, H-5 (δ 1.61, dd, J=3.6 and 12.9 Hz) 10%, and H-6α (δ 1.38, m) 17%; Me-29 to Me-28 7% and Me-19 (δ 1.10, s) 15%; Me-19 to Me-29 11% and Me-18 (δ 0.75, s) 5%; Me-18 to Me-19 8% and H-20 (δ 1.31, m) 9%; Me-21 (δ 0.86, d, J=6.4 Hz) to H-20 16%; eims *m/z* $[M]^+$ 444 (0.5), 291 (63), 136 (100), 121 (68), 95 (34), 57 (49), 43 (81).

Lanosta-7,9(11)-dien-3β-ol [**6**].—Sulfuric acid (95–98%, 8 ml) was added dropwise to a solution of **3** (2.1 g, 4.72 mmol) in Me_2CO-H_2O (231 ml, 10:1) with stirring (12). After stirring at room temperature for 3 h, distilled H_2O (600 ml) was added and the precipitate was collected by suction. The air-dried **6** (2.2 g, 85%) was recrystallized from MeOH, mp 151–153°; $[\alpha]^{26}D$ +68° (c=1.0, $CHCl_3$); ir (KBr) ν max 3306 (OH), 3031, 2932, 1470, 1456, 1446, 1373 cm^{-1}; uv (MeOH) λ max (log ε) 243 (4.07) nm; ^{1}H nmr ($CDCl_3$, 400.13 MHz) δ 3.22 (1H, dd, J=4.6 and 11.2 Hz, H-3), 5.29 (1H, br d, J=6.1 Hz, H-7), 5.44 (1H, dd, J=2.7 and 5.2 Hz, H-11), 0.54 (3H, s, Me-18), 0.98 (3H, s, Me-19), 0.96 (3H, s, Me-29), 0.860 (3H, d, J=6.6 Hz, Me-21), 0.85 (3H, d, J=6.7 Hz, Me-26 or Me-27), 0.843 (3H, d, J=6.7 Hz, Me-26 or Me-27), 0.859 (3H, s, Me-28 or Me-30), 0.857 (3H, s, Me-28 or Me-30); eims *m/z* $[M]^+$ 426 (100), 411 (12), 393 (3), 313 (6), 271 (16), 253 (11), 240 (10), 225 (4), 171 (5), 157 (5), 145 (7), 119 (5), 95 (6), 57 (10), 43 (21); hreims *m/z* $[M]^+$ 426.3857 (calcd for $C_{30}H_{50}O$, 426.3861).

MICROBIAL TRANSFORMATION OF **3**.—*Mycobacterium* sp. (NRRL B-3805) was grown in Nutrient broth (1.6%)-dextrose (4%) medium (9.2 liters) contained in 23 2-liter Erlenmeyer flasks at 26–27° on a rotary shaker (250 rpm, 1-in stroke). Compound **3** (2.22 g) dissolved in DMF (104 ml) was distributed evenly among the flasks and the incubation was continued for 96 h. The culture broth was acidified with HOAc to a pH of 3.0 and was extracted with $CHCl_3$ (3.1 liters×3). The combined $CHCl_3$ layer was dried over Na_2SO_4 and evaporated to dryness *in vacuo* to give a brownish semi-solid residue (15.6 g). The residue was dissolved with Me_2CO (50 ml×3) and filtered through Celite, and evaporation of the solvent *in vacuo* gave

a yellow residue (5.1 g). This residue was chromatographed over a Si gel column (140 g) and eluted with an MeOH/$CHCl_3$ mixture. The fractions (1.6 g) obtained from $CHCl_3$ elution were purified over a Si gel column (45 g) eluted with 5 to 7% EtOAc in *n*-C_6H_{14} to obtain pure **3** (348 mg, 15.7%). The fractions (0.58 g) obtained from the 1% MeOH elution were purified over a Si gel column (30 g) eluted with *n*-C_6H_{14}/EtOAc mixtures to give **5** (30 mg, 1.6%) from the 10% EtOAc elution. The fractions (1.11 g) obtained from 5 to 7% MeOH elution were purified over a Si gel column (45 g) eluted with an MeOH/$CHCl_3$ mixture to give **4** (87 mg, 5%) from the 1% MeOH elution.

20(S)-Hydroxymethyl-12α-hydroxypregn-4-en-3-one [**4**].—Amorphous solid, $[\alpha]^{27}D$ +12.4° (c=1.17, $CHCl_3$); ir (KBr) ν max 3425 (OH), 2942, 1659 (C=O, conjugated ketone), 1451, 1380, 1046 cm^{-1}; uv (MeOH) λ max (log ε) 242 (3.99) nm; ^1H nmr ($CDCl_3$, 300 MHz) δ 5.70 (1H, br s, H-4), 1.63 (1H, ddd, J=3.0, 11.5, and 11.5 Hz, H-11β), 4.00 (1H, dd, J=2.9 and 5.7 Hz, H-12β), 0.72 (3H, s, Me-18), 1.13 (3H, s, Me-19), 1.04 (3H, d, J=6.6 Hz, Me-21), 3.43 (1H, dd, J=6.0 and 10.5 Hz), 3.57 (1H, dd, J=4.0 and 10.5 Hz) for H_2-22; ^{13}C nmr ($CDCl_3$, 75.4 MHz) δ 35.4 (t, C-1), 33.8 (t, C-2), 199.6 (s, C-3), 123.8 (d, C-4), 171.3 (s, C-5), 32.8 (t, C-6), 31.8 (t, C-7), 35.8 (d, C-8), 47.2 (d, C-9), 38.1 (s, C-10), 28.5 (t, C-11), 72.5 (d, C-12), 46.4 (s, C-13), 47.2 (d, C-14), 23.7 (t, C-15), 26.8 (t, C-16), 43.2 (d, C-17), 12.8 (q, C-18), 17.2 (q, C-19), 38.3 (d, C-20), 15.5 (q, C-21), 67.4 (t, C-22); major HMBC data (J=8 Hz) H-4 (δ 5.70) to C-6 (δ 32.8) and C-10 (δ 38.1); H-18 (δ 0.72) to C-12 (72.5), C-13 (δ 46.4), C-14 (δ 47.2), and C-17 (δ 43.2); H-19 (δ 1.13) to C-1 (δ 35.4), C-5 (δ 171.3), C-9 (δ 47.2), and C-10 (δ 38.1); H-21 (δ 1.04) to C-17 (δ 43.2), C-20 (δ 38.3) and C-22 (δ 67.4); H-22 (δ 3.43 and 3.57) to C-17 (δ 43.2), C-20 (δ 38.3) and C-21 (δ 15.5); eims *m/z* $[M]^+$ 346 (7), 286 (33), 269 (40), 124 (99), 107 (100); hreims *m/z* $[M]^+$ 346.2510 (calcd for $C_{22}H_{34}O_3$, 346.2508).

20(S)-Hydroxymethyl-4,4,14α-trimethylpregna-7,9(11)-dien-3β-ol [**5**].—Mp 208–210°; $[\alpha]^{27}D$ +16.9° (c=0.74, $CHCl_3$); ir (KBr) ν max 3436 (OH), 2927, 1731, 1374, 1032 cm^{-1}; uv (MeOH) λ max (log ε) 243 (4.15), 252 (3.98, sh) nm; ^1H nmr ($CDCl_3$, 300 MHz) δ 3.23 (1H, dd, J=4.8 and 10.8 Hz, H-3), 5.31 (1H, m, H-7), 5.46 (1H, m, H-11), 3.35 (1H, dd, J=6.7 and 7.4 Hz), 3.65 (1H, dd, J=3.0 and 7.4 Hz) for H_2-22, 0.57 (3H, s, Me-18), 0.99 (3H, s, Me-19), 0.96 (3H, s, Me-4α), 0.86 (3H, s, Me-4β), 0.86 (3H, s, Me-14α), 1.00 (3H, d, J=7.4 Hz, Me-21); ^{13}C nmr ($CDCl_3$, 75.4 MHz) δ 35.8 (t, C-1), 27.9 (t, C-2), 79.0 (d, C-3), 38.7 (s, C-4), 49.2 (d, C-5), 23.1 (t, C-6), 120.5 (d, C-7), 142.6 (s, C-8), 146.1 (s, C-9), 37.5 (s, C-10), 116.2 (d, C-11), 37.8 (t, C-12), 44.0 (s, C-13), 50.1 (s, C-14), 31.7 (t, C-15), 27.4 (t, C-16), 47.3 (d, C-17), 15.8 (q, C-18), 22.7 (q, C-19), 39.3 (d, C-20), 16.6 (q, C-21), 68.2 (t, C-22), 28.2 (q, 4α-Me), 15.8 (q, 4β-Me), 25.5 (q, 14-Me); eims *m/z* $[M]^+$ 372 (100), 373 (48), 271 (62), 253 (48), 111 (53); hreims *m/z* $[M]^+$ 372.3003 (calcd for $C_{25}H_{40}O_2$, 372.3028).

MICROBIAL TRANSFORMATION OF **6**.—Compound **6** (2.1 g) dissolved in DMF (84 ml) was distributed evenly in Nutrient broth-dextrose medium (8.4 liters) in 21 2-liter Erlenmeyer flasks with viable *Mycobacterium* sp. (NRRL B-3805), and was incubated as mentioned above for 120 h. Acidification and extraction of the incubation mixture with $CHCl_3$ (2.8 liters×3) and workup of the combined $CHCl_3$ extract gave a brownish semi-solid residue (4.49 g). This residue dissolved in $CHCl_3$ was chromatographed over a Si gel column (135 g) eluted successively with C_6H_6, $CHCl_3$, and then an Me_2CO/$CHCl_3$ mixture. The C_6H_6 elution (0.25 g) yielded crystalline **7** (88.5 mg, 4.2%) after purification over a Si gel column (15 g) eluted with *n*-C_6H_{14}-C_6H_6 (1:2). The C_6H_6-$CHCl_3$ (1:1) elution (0.22 g) was purified over a Si gel column (18 g) eluted with C_6H_6 and then with C_6H_6-$CHCl_3$ (1:1) to give the starting material (**6**, 87.6 mg, 4.2%) and **8** (99.4 mg, 7.1%), respectively. The $CHCl_3$ elution (0.48 g) was purified over a Si gel column (24 g) eluted with C_6H_6/$CHCl_3$ to give **1** (417 mg, 30%). The Me_2CO-$CHCl_3$ (1:9) elution (96 mg) was purified over a Si gel column (10 g) eluted with MeOH-$CHCl_3$ (1:9) to give **9** (13 mg, 0.7%).

Lanosta-7,9(11)-dien-3-one [**7**].—Mp 126–128°; $[\alpha]^{27}D$ +27° (c=1.48, $CHCl_3$); ir (KBr) ν max 2957, 2927, 2854, 1734, 1713, 1466, 1379, 1287 cm^{-1}; uv (MeOH) λ max (log ε) 242 (4.06) nm; ^1H nmr ($CDCl_3$, 400.13 MHz) δ 2.75 (1H, ddd, J=5.7, 14.5, and 14.5 Hz, H-2β), 5.37 (1H, br d, J=5.5 Hz, H-7), 5.49 (1H, br d, J=6.4 Hz, H-11), 0.57 (3H, s, Me-18), 1.07 (3H, s, Me-19), 0.87 (3H, d, J=6.7 Hz, Me-21), 0.850 (3H, d, J=6.4 Hz, Me-26 or Me-27), 0.854 (3H, d, J=6.4 Hz, Me-26 or Me-27), 0.86 (3H, s, Me-28), 1.11 (3H, s, Me-29), 1.18 (3H, s, Me-30); eims *m/z* $[M]^+$ 424 (18), 409 (4), 311 (6), 269 (21), 256 (17), 244 (8), 229 (5), 213 (4), 185 (7), 171 (10), 157 (15), 145 (14), 133 (18), 123 (14), 119 (14), 105 (12), 95 (14), 83 (16), 69 (30), 55 (43), 43 (100); hreims *m/z* $[M]^+$ 424.3704 (calcd for $C_{30}H_{48}O$, 424.3705).

CHROMIUM TRIOXIDE-PYRIDINE OXIDATION OF **6**.—The mixture of **6** (41 mg, 0.096 mmol) and CrO_3/pyridine complex (29 mg, 0.29 mmol) in pyridine (5.5 ml) (13) was stirred overnight at room temperature and then heated under reflux for 3 h. After cooling, the mixture was poured into cold dilute H_2SO_4 (10 ml) and the product was extracted with $CHCl_3$ (5 ml×3). Evaporation of the $CHCl_3$ extract gave crude **7** that was purified over a Si gel column eluted with C_6H_6 to give **7** (35.8 mg, 88%) with identical physical data to the isolated metabolite.

Methyl 12α-hydroxybisnorchola-4,17(20)-dien-22-oate [**9**].—Mp 185–186°; $[\alpha]^{25}D$ +125° (c=0.20, $CHCl_3$); ir (KBr) ν max 3692, 3000, 2934, 2856, 1700, 1658, 1618, 1433, 1350, 1272 cm^{-1}; uv (MeOH) λ max (log ε) 238 (4.39) nm; ^1H nmr ($CDCl_3$, 400.13 MHz) δ 5.73 (1H, br s, H-4), 4.51 (1H, br s, H-12), 3.70 (3H, s, OMe-22), 2.00 (3H, s, Me-21), 1.17 (3H, s, Me-19), 0.97 (3H, s, Me-18); ^{13}C nmr ($CDCl_3$, 100.61 MHz) δ 35.4 (t, C-1), 33.8 (t, C-2), 199.3 (s, C-3), 124.0 (d, C-4), 170.5 (s, C-5), 32.7 (t, C-6), 31.5 (t, C-7), 34.8 (d, C-8), 47.3 (d, C-9), 38.0 (s, C-10), 27.7 (t, C-11), 71.7 (d, C-12), 52.3 (s, C-13), 46.9 (d, C-14), 24.0 (t, C-15), 33.3 (t, C-16), 159.9 (s, C-17), 15.9 (q, C-18), 17.1 (q, C-19), 119.2 (s, C-20), 13.8 (q, C-21), 169.4 (s, C-22), 51.3 (q, OMe-22); major HMBC data (J=8 Hz), H-4 (δ 5.73) to C-6 (δ 32.7) and C-10 (δ 38.0); H-18 (δ 0.97) to C-12 (71.7), C-13 (δ 52.3), C-14 (δ 46.9), and C-17 (δ 159.9); H-19 (δ 1.17) to C-1 (35.4), C-5 (δ 170.5) C-9 (δ 47.3), and C-10 (δ 38.0); H-21 (δ 2.00) to C-17 (δ 159.9), C-20 (δ 119.2), and C-22 (δ 169.4); OMe-22 (δ 3.70) to C-22 (δ 169.4); eims *m/z* $[M]^+$ 372 (4), 373 (8), 354 (51), 339 (30), 322 (26), 307 (11), 279 (7), 251 (7), 225 (10), 196 (16), 185 (9), 178 (11), 171 (16), 161 (15), 157 (11), 155 (11), 147 (19), 133 (29), 119 (32), 105 (56), 91 (100), 79 (70), 55 (67), 43 (92); hreims *m/z* $[M]^+$ 372.2287 (calcd for $C_{23}H_{32}O_4$, 372.2300).

ACKNOWLEDGMENTS

This research was supported by the National Science Council of the Republic of China, under Grant NSC 83-0412-B002-083 to K.C.W.

LITERATURE CITED

1. K.C. Wang, L.-H. Young, Y. Wang, and S.-S. Lee, *Tetrahedron Lett.*, **31**, 1283 (1990).
2. S.-S. Lee, L.-H. Young, and K.C. Wang, *J. Nat. Prod.*, **54**, 178 (1991).
3. G.T. Emmons, W.K. Wilson, and G.J. Schroepfer, Jr., *Magn. Reson. Chem.*, **27**, 1012 (1989).
4. M. Arisawa, A. Fujita, M. Saga, H. Fukumura, T. Hayashi, M. Shimizu, and N. Morita, *J. Nat. Prod.*, **49**, 621 (1986).
5. W.J. Marsheck, S. Krachy, and R.D. Muir, *Appl. Microbiol.*, **23**, 72 (1972).
6. K.C. Wang, C.-S. Gau, and R.R.-L. Chen, *J. Taiwan Pharm. Assoc.*, **34**, 129 (1982).
7. C.J. Sih, K.C. Wang, and H.H. Tai, *J. Am. Chem. Soc.*, **89**, 1956 (1967).
8. C.J. Sih, H.H. Tai, and Y.Y. Tsong, *J. Am. Chem. Soc.*, **89**, 1957 (1967).
9. Y. Fujimoto, C.S. Chen, Z. Szeleczky, D. Di'Tullio, and C.J. Sih, *J. Am. Chem. Soc.*, **104**, 4718 (1982).
10. Y. Fujimoto, C.S. Chen, A.G. Gopalan, and C.J. Sih, *J. Am. Chem. Soc.*, **104**, 4720 (1982).
11. S.-S. Lee, B.-J. You, and K.C. Wang, *J. Chin. Chem. Soc.*, **40**, 213 (1993).
12. A.T Rowland and H.R. Nace, *J. Am. Chem. Soc.*, **82**, 2833 (1960).
13. S. Sarel and M.S. Newman, *J. Am. Chem. Soc.*, **78**, 5416 (1956).

Received 14 March 1995