Microbial Transformation of Dihydrosarsasapogenin with *Mycobacterium* sp.

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Microbial transformation of sarsasapogenin (**1**) with *Mycobacterium* sp. (NRRL B-3805) gave 25(*S*)-neospirost-4-en-3-one (**2**) as the sole product in 62% yield. Incubation of dihydrosarsasapogenin (**3**) led to the isolation of seven products in 0.5 (**4**), 6.6 (**5**), 5 (**6**), 16 (**7**), 1 (**8**), 1 (**9**), and 4.5% (**10**) yields, respectively, while 15% of **3** was recovered. Among these products, **8** and **9** were C_{22} steroids, and **10** was a C_{19} steroid. Isolation of these C_{19} and C_{22} steroids indicated that this microorganism is capable of cleaving the ether linkage between C-16 and C-22 in **3**. In addition, 12α-hydroxylation was also observed in all these three metabolites.

Previously, we disclosed that cycloartenol, 24-methylenecycloartanol, lanosterol, and lanosta-7,9(11)-dien-3β-ol were all transformed by *Mycobacterium* sp. (NRRL B-3805) to give androsta-4,8(14)-diene-3,17-dione as the major metabolite, in 34, 35, 30, and 30% yields, respectively.[1,2] During these transformations, a series of complicated chemical modifications occurred, including (a) demethylation at 4α, 4β-, and 14α- positions, (b) formation of a $\Delta^{8(14)}$ double bond accompanied by disappearance of the $\Delta^{8(9)}$ double bond in lanosterol and $\Delta^{7,9(11)}$ conjugated double bond in lanosta-7,9(11)-dien-3β-ol, (c) cleavage of the C-17 side chain, and (d) opening of the cyclopropyl ring in cycloartenol and 24-methylenecycloartanol to form the C-19 methyl group.

In 1969, Kaneko et al.[3] reported the hydroxylation of diosgenin on 7β-, 11α-, and 12β-positions, respectively, by *Cunninghamella blakesleeana* (+). In 1973, Howe et al.[4] reported the degradation of the diosgenin molecule to 9-oxode-AB-spirostane-8α-propionic acid products by *Nocardia globerula*. In both instances the spirostane structure was not degraded by these microorganisms.

In an effort to learn more about the enzymatic capabilites of *Mycobacterium* sp. (NRRL B-3805) in regard to sterol transformation, sarsasapogenin (**1**) and dihydrosarsasapogenin (**3**) were incubated with this microorganism, respectively. When sarsasapogenin (**1**) was incubated with *Mycobacterium* sp. (NRRL B-3805) for 120 h, 25(*S*)-neospirost-4-en-3-one (**2**) was isolated as the sole product in 62% yield. The EIMS of **2** showed a molecular ion at *m*/*z* $[M]^+$ 412. Comparison of spectral data (UV, IR, ^{1}H NMR, and ^{13}C NMR) with the literature value[5] confirmed its identity. This result indicated that this microorganism oxidized the 3β-hydroxyl group to a carbonyl moiety and introduced a Δ^4-double bond, but did not cleave the spirostane side chain. To facilitate the degradation at the C-17 sidechain for the possibility of conversion to useful steroid intermediates by microbial transformation, **1** was converted to dihydrosarsasapogenin (**3**) by heating with $AlCl_3$−$LiAlH_4$ in ether.[6] In **3** the spiropyran ring was opened by the action of a reducing agent, but the ether linkage between C-16 and C-22 remained unaffected. From the incubation mixture of **3** with *Mycobacterium* sp. (NRRL B-3805), **4** (0.5%), **5** (6.6%), **6** (5%), **7** (16%), **8** (1%), **9** (1%), and **10** (4.5%) were isolated, together with recovery of 15% unchanged **3**. Among these products, **4**−**7** were novel compounds.

Compound **4**, mp 135−136 °C, $[\alpha]^{21}_D$ +4.3° (*c* 1.5, $CHCl_3$), had a molecular formula $C_{27}H_{44}O_3$ as deduced from its HREIMS. Its IR absorption at 1700 cm^{-1} and a carbonyl signal (δ 213.7, s, C-3) in the ^{13}C-NMR spectrum indicated oxidation of the 3β-hydroxyl to carbonyl function. The presence of a 3525 cm^{-1} absorption in its IR spectrum and the signals for H-26's at δ 3.43 (dd, J = 5.6, 10.4 Hz) and 3.49 (dd, J = 5.1, 10.4 Hz) in the ^{1}H-NMR spectrum were indicative of the presence of intact dihydrosarsasapogenin side chain. Thus, the structure of 3-oxo-dihydrosarsasapogenin was assigned to **4**.

Compound **5**, mp 110−111 °C, $[\alpha]^{26}_D$ +8.6° (*c* 1.05, $CHCl_3$), had a molecular formula $C_{27}H_{46}O_3$ as deduced from its HREIMS. Its IR spectrum showed absorption at 3380 cm^{-1} for hydroxyl function but lacked carbonyl absorption. The ^{1}H-NMR spectrum of **5** is very similar to that of **3** except that the H-3 signal shifted from δ 4.08 (br s) in **3** to δ 3.60 (tt, J = 4.8, 11.0 Hz) in **5**, indicating that H-3 in **5** was axially oriented. These results indicated that the equatorial 3β-OH in **3** was oxidized to a carbonyl function in **4**, and then reduced to the axial 3α-OH to form dihydroepisarsasapogenin (**5**).

Compound **6**, mp 117−118 °C, $[\alpha]^{26}_D$ +56.5° (*c* 1.0, $CHCl_3$), had a molecular formula $C_{27}H_{42}O_3$ as deduced from its HREIMS. Its IR absorption at 1660 and 1605 cm^{-1}, UV absorption maximum at 241 nm, together with an olefinic proton signal at δ 5.69 (s) in the ^{1}H-NMR spectrum clearly indicated the presence of a steroidal 4-en-3-one function. Based on the spectral data (UV, IR, ^{1}H NMR, ^{13}C NMR, and EIMS), the structure of 16,22-oxido-26-hydroxycholest-4-en-3-one was assigned to **6**.

Compound **7**, colorless crystal, mp 118−119 °C, $[\alpha]^{26}_D$ +64° (*c* 1.0, $CHCl_3$), had a molecular formula $C_{25}H_{36}O_4$

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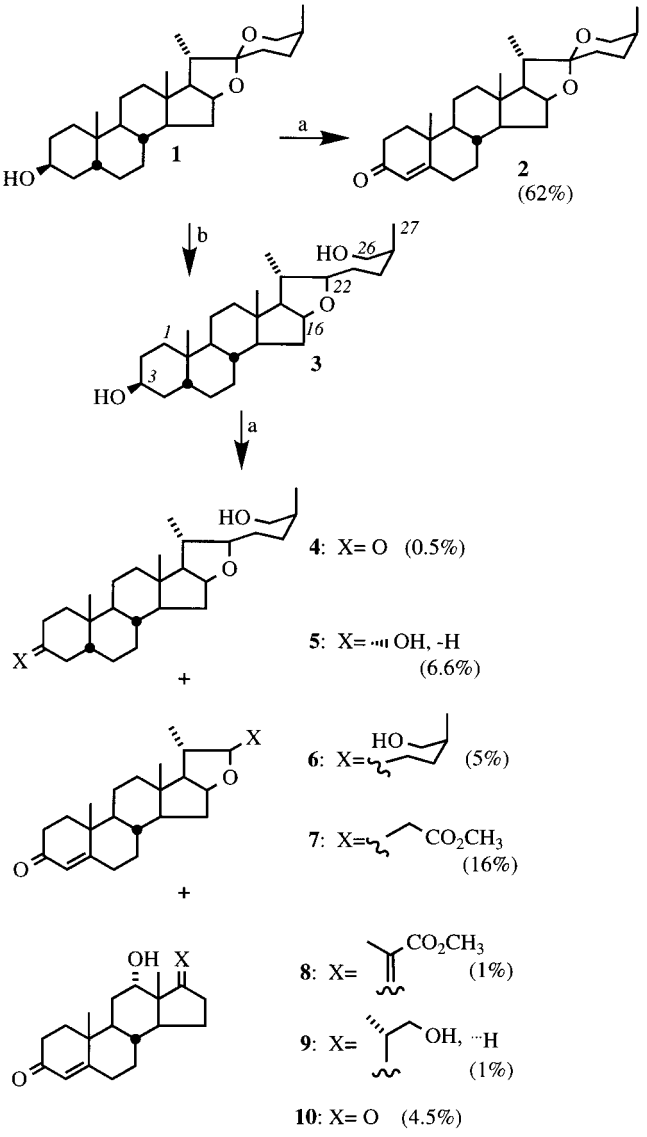

a. *Mycobacterium* sp. (NRRL B-3805); b. $AlCl_3$, $LiAlH_4$, ether, reflux

Figure 1. Transformation of sarsasapogenin (**1**) and dihydrosarsasapogenin (**3**) with *Mycobacterium* sp. (NRRL B-3805).

as deduced from the HREIMS. It possessed all the spectral characteristics of a steroidal 4-en-3-one function as mentioned above for compound **6**. In addition, carbonyl absorption at 1739 cm^{-1} in the IR spectrum and a methoxy singlet at δ 3.68 in the ^{1}H-NMR spectrum was indicative of the presence of a methyl ester ($-COOCH_3$) in the molecule.

It is well documented by Sih et al.[7] that, during the early stage of cholesterol side-chain degradation by microorganisms, carbon−carbon bond fission between C-24 and C-25 led to the formation of one mole of propionic acid and formation of the cholic acid side chain. Also, in our previous report[2] we indicated that this microorganism is capable of methylating the carboxylic acid intermediates formed during the sterol side-chain degradation process. Therefore, based on the spectral data obtained, the structure of methyl 3-oxo-16,22-oxidocholan-4-en-24-oate was assigned to **7**.

Using the 5β-spirostane smilagenin and epismilagenin, 3β-acetoxy-26-hydroxy-5α-furostane,[8] 5β-pregnane-3,20-dione, and progesterone[9] as model compounds, the ^{13}C-NMR data of **3**−**7** were completely assigned (Table 1).

Table 1. ^{13}C-NMR Data of **3**−**7** (δ in ppm, mult.[a]) ($CDCl_3$)

position	3	4	5	6	7
1	29.9 t	37.1 t	35.4 t	35.7 t	35.7 t
2	27.8 t	36.9 t	30.5 t	33.9 t	33.9 t
3	67.1 d	213.3 s	71.7 d	199.5 s	199.5 s
4	33.5 t	42.3 t	36.4 t	123.8 d	123.9 d
5	36.5 d	44.2 d	42.0 d	171.2 s	171.1 s
6	26.5 t	25.9 t	27.1 t	32.8 t	32.7 t
7	26.5 t	26.5 t	26.6 t	32.0 t	32.0 t
8	35.4 d	35.3 d	35.6 d	35.3 d	35.3 d
9	39.86[b] d	40.8 d	40.5 d	53.8 d	53.7 d
10	35.2 s	35.0 s	34.7 s	38.6 s	38.6 s
11	20.7 t	20.8 t	20.4 t	20.6 t	20.6 t
12	39.91[b] t	39.7 t	39.8 t	39.2 t	39.1 t
13	41.1 s	41.1 s	41.1 s	40.8 s	40.7 s
14	56.9 d	56.7 d	56.8 d	56.1 d	56.0 d
15	32.2 t	32.1 t	32.2 t	32.1 t	32.0 t
16	83.3 d	83.1 d	83.3 d	83.0 d	83.4 d
17	65.4 d	65.3 d	65.3 d	65.1 d	64.7 d
18	16.6 q	16.5 q	16.6 q	16.4 q	16.4 q
19	23.9 q	22.6 q	23.4 q	17.4 q	17.4 q
20	37.9 d	37.9 d	37.9 d	37.9 d	37.7 d
21	19.0 q	19.0 q	19.0 q	18.9 q	18.5 q
22	90.5 d	90.5 d	90.5 d	90.5 d	85.5 d
23	30.7 t	30.7 t	30.7 t	30.7 t	38.4 t
24	30.0 t	30.0 t	30.0 t	30.0 t	171.9 s
25	36.0 d	36.0 d	36.0 d	35.9 t	
26	67.9 t	67.9 t	67.9 t	67.9 t	
27	16.7 q	16.7 q	16.8 q	16.7 q	
24-OMe					51.8 q

[a] Multiplicities were obtained from DEPT experiments. [b] Interchangeable assignment in **3**.

Compounds **8** and **9** were identical in every respect (mp, UV, IR, ^{1}H NMR)[2] to methyl 12α-hydroxybisnorchola-4,17(20)-dien-22-oate and 20(*S*)-hydroxymethyl-12α-hydroxypregn-4-en-3-one, respectively. The isolation of these two metabolites indicated that the ether linkage between C-16 and C-22 in **3** was cleaved by this microorganism, although in low yields.

Compound **10**, mp 177−178 °C, $[\alpha]^{26}_D$ +206° (*c* 0.53, $CHCl_3$), had a molecular formula $C_{19}H_{26}O_3$ as deduced from its HREIMS. It also contained a steroidal 4-en-3-one function as exemplified by the IR absorption at 1680 and 1620 cm^{-1}, UV absorption maximum at 240 nm, and a proton signal at δ 5.74 (s, 1H). Its ^{1}H-NMR spectrum also revealed a broad singlet at δ 4.15 for a carbinoyl proton and two methyl singlets at δ 1.17 and 0.89, assignable to H-19 and H-18, respectively. An additional IR absorption at 1730 cm^{-1} suggested a five-membered ketone at C-17, supported by a signal at δ 219.8 (s) in the ^{13}C-NMR spectrum. From these data we concluded that the C-17 side-chain has been cleaved and that **10** contained an additional secondary hydroxyl and a carbonyl group at C-17. This hydroxy function was located at the 12α-position by analysis of the coupling pattern of the carbinoyl proton (br s) in the ^{1}H-NMR spectrum and by the three-bond coupling of C-12 (δ 69.1, d) to H-18 (δ 0.89) in the HMBC spectrum. Based on these results, 12α-hydroxyandrost-4-en-3,17-dione was assigned to **10**, which was previously isolated by Bilton et al. as one of the minor products during the degradation of deoxycholic acid by *Pseudomonas* sp. (NCIB 10590).[10] Based on analysis of the HMQC and

HMBC spectra, the ^{1}H- and ^{13}C-NMR data of **10** were also unambiguously assigned (see Experimental Section).

In this present report, we showed that *Mycobacterium*. sp. (NRRL B-3805) is unable to cleave the spirostane side chain in sarsasapogenin as those reported microorganisms. However, it is capable of cleaving the 16, 22-oxido linkage in dihydrosarsasapogenin to some extent and resulted in the isolation of **8**, **9**, and **10** in 1, 1, and 4.5% yields, respectively.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher−Johns or a Büchi 510 melting point apparatus and were uncorrected. The optical rotations were recorded on a JASCO DIP-181 digital polarimeter. IR spectra were recorded on a JASCO-100 IR spectrometer. UV spectra were recorded on a Hitachi 150-20 UV spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were obtained in $CDCl_3$ using the residual solvent peak as reference standard on a Bruker AMX-400 spectrometer. MS spectra were recorded with a TSQ-46c GC/MS/MS/DS (EIMS) and a JEOL JMS−HX 100 mass spectrometer (HREIMS).

Biological Material. 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 °C for 11 days before being transferred to broth medium (nutrient broth 1.6%, dextrose 4%) for microbial transformation study. The biotransformations were carried out in the 2-L Erlenmeyer flasks containing 400 mL of the broth at 26−28 °C on a rotary shaker (250 rpm, l-in stroke). The substrate dissolved in *N*,*N*-dimethylformamide (DMF) was added to the growing microorganism and incubated.

Microbial Transformation of 1. The substrate **1** (1.025 g, Aldrich #34, 814−7), dissolved in DMF (30 mL), was distributed evenly among 10 2-L Erlenmeyer flasks and the incubation continued for 120 h. At the end of the incubation period, the culture broth was acidified with HOAc to a pH of 3.0 and extracted with $CHCl_3$ (1.4 L × 3). The combined $CHCl_3$ layer was washed with distilled H_2O and dried over Na_2SO_4, then evaporated to dryness *in vacuo* to give a brownish semisolid residue (3.5 g). The residue was dissolved with Me_2CO (50 mL × 3) and filtered through Celite. Evaporation of the filtrate *in vacuo* gave a yellowish residue (2.15 g) that was chromatographed over a Si gel column (70 g) and eluted with $CHCl_3$, then with Me_2CO−$CHCl_3$ mixtures. The 10% Me_2CO elution (785 mg) contained mainly the major product **2**. Purification of this residue over a Si gel column (22 g) eluted with 0−10% Me_2CO in $CHCl_3$ gave pure **2**[5] (625 mg, 62%): mp 180−182 °C, $[\alpha]^{25}_D$ −23° (*c* 1.0, $CHCl_3$).

Microbial Transformation of 3. Dihydrosarsasapogenin (**3**) (2.4 g), obtained by reacting **1** with $AlCl_3$−$LiAlH_4$ in ether under reflux,[6] was dissolved in DMF (48 mL). The solution was distributed evenly among 24 2-L Erlenmeyer flasks and incubated for 80 h. At the end of incubation period, the broth was acidified with HOAc and extracted with $CHCl_3$ (3.3 L × 3). Workup of the combined $CHCl_3$ extract gave a yellow residue (4.7 g) that was chromatographed over a Si gel column (130 g) eluted with a MeOH−$CHCl_3$ mixture. In the fractions eluted with 1−6% MeOH, 1.6 g of residue A was obtained after evaporation of the solvent. And in the fractions eluted with 8−16% MeOH, 1.5 g of residue B was obtained. The residue A was rechromatographed over another Si gel column (130 g) and eluted with an EtOAc−C_6H_{14} mixture. In the fractions eluted with 10% EtOAc, 0.5 g of residue containing mainly **7** was obtained after evaporation of the solvent. This residue, after repeated crystallization with Me_2CO, gave an analytical sample of **7** (368 mg, 16%). In the fractions eluted with 17% EtOAc, **6** was obtained, which gave an analytical sample of **6** after recrystallization from an Me_2CO−petroleum ether mixture (119 mg, 5%). In the fractions eluted with 20% EtOAc, **4** was obtained; recrystallization from Me_2CO−petroleum ether gave an analytical sample of **4** (12 mg, 0.5%). In the fractions eluted with 25−30% of EtOAc, **8** (23 mg, 1%) was obtained after purification of the residue over a small Si gel column (10 g) and recrystallization. Residue B was rechromatographed over another Si gel column (130 g) and eluted with Me_2CO−$CHCl_3$ mixtures. In the fractions eluted with 5% Me_2CO, 450 mg of residue containing mainly substrate **3** was obtained, which gave pure **3** (361 mg, 15%) after recrystallization from ether. In the fractions eluted with 8% Me_2CO, **5** (159 mg, 6.6%) was obtained. In the fractions eluted with 10% Me_2CO, 120 mg of residue containing mainly **10** was obtained. Rechromatography of this residue over a small Si gel column (20 g) and elution with Me_2CO−$CHCl_3$ (1:9) gave crystalline **10** (78 mg, 4.5%). In the fractions eluted with 20−25% Me_2CO in $CHCl_3$, **9** (21 mg, 1%) was obtained after purification over a Si gel column (10 g) and recrystallization from Me_2CO−petroleum ether.

3-Oxo-dihydrosarsasapogenin (4): mp 135−136 °C; $[\alpha]^{21}_D$ +4.3° (*c* 1.51, $CHCl_3$); IR (KBr) ν_{max} 3525, 2925, 1700, 1445, 1375, 1340, 1320, 1265, 1240, 1220, 1150, 1130, 1095, 1045 cm^{-1}; ^{1}H NMR δ 4.29 (1H, dt, *J* = 5.2, 7.8 Hz, H-16), 3.49 (1H, dd, *J* = 5.1, 10.4 Hz, H-26a), 3.43 (1H, dd, *J* = 5.6, 10.4 Hz, H-26b), 3.30 (1H, dt, *J* = 3.5, 8.1 Hz, H-22), 2.65 (1H, dd, *J* = 13.8, 14.7 Hz, H-4α), 2.29 (1H, dt, *J* = 5.3, 14.6 Hz, H-2α), 2.14 (1H, ddd, *J* = 3.9, 5.2, 14.0 Hz, H-2*β*), 1.01 (3H, s, H-19), 0.98 (3H, d, *J* = 6.7 Hz, H-21), 0.91 (3H, d, *J* = 6.6 Hz, H-27), 0.79 (3H, s, H-18); ^{13}C-NMR data, see Table 1; EIMS *m*/*z* [M + 1]$^+$ 417 (45), [M]$^+$ 416 (17), 398 (47), 383 (42), 356 (60), 342 (60), 329 (25), 271 (100), 253 (10), 161 (16), 144 (40); HREIMS *m*/*z* [M$^+$] 416.3285 (calcd for $C_{27}H_{44}O_3$, 416.3290).

Dihydroepisarsasapogenin (5): mp 110−111 °C; $[\alpha]^{26}_D$ +8.6° (*c* 1.05, $CHCl_3$); IR (KBr) ν_{max} 3380, 2925, 1470, 1445, 1375, 1340, 1250, 1165, 1125, 1095, 1050, 1010 cm^{-1}; ^{1}H NMR δ 4.27 (1H, dt, *J* = 5.1, 7.7 Hz, H-16), 3.60 (1H, tt, *J* = 4.8, 11.0 Hz, H-3), 3.47 (1H, dd, *J* = 4.5, 10.6 Hz, H-26a), 3.42 (1H, dd, *J* = 6.0, 10.6 Hz, H-26b), 3.29 (1H, dt, *J* = 3.4, 8.1 Hz, H-22), 0.96 (3H, d, *J* = 6.7 Hz, H-21), 0.904 (3H, s, H-19), 0.90 (3H, d, *J* = 6.4 Hz, H-27), 0.74 (3H, s, H-18); ^{13}C-NMR data, see Table 1; EIMS *m*/*z* [M]$^+$ 418 (6), 344 (4), 331 (42), 313 (6), 285 (10), 273 (100), 255 (32), 161 (10), 144 (80), 133 (10), 122 (16), 107 (23), 93(21), 81 (28), 67 (18), 55 (24); HREIMS *m*/*z* [M]$^+$ 418.3447 (calcd for $C_{27}H_{46}O_3$, 418.3447).

16,22-Oxido-26-hydroxycholest-4-en-3-one (6): mp 117−118 °C; $[\alpha]^{26}_D$ +57° (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 241 (4.08) nm; IR (KBr) ν_{max} 3480, 2955, 1660, 1605, 1450, 1380, 1325, 1275, 1230, 1190, 1180, 1125, 1105, 1045 cm^{-1}; ^1H NMR δ 5.69 (1H, s, H-4), 4.27 (1H, dt, J = 5.2, 7.7 Hz, H-16), 3.48 (1H, dd, J = 5.4, 10.6 Hz, H-26a) and 3.42 (1H, dd, J = 6.0, 10.6 Hz, H-26b), 3.29 (1H, dt, J = 3.5, 8.1 Hz, H-22), 1.16 (3H, s, H-19), 0.97 (3H, d, J = 6.7 Hz, H-21), 0.90 (3H, d, J = 6.5 Hz, H-27), 0.81 (3H, s, H-18); ^{13}C-NMR data, see Table 1; EIMS *m/z* $[M]^+$ 414 (30), 396 (12), 368 (14), 355 (18), 340 (22), 327 (23), 269 (71), 175 (11), 159 (9), 144 (44), 55 (100); HREIMS *m/z* $[M]^+$ 414.3113 (calcd for $C_{27}H_{42}O_3$, 414.3134).

Methyl-3-oxo-16,22-oxidocholan-4-en-24-oate (7): mp 118−119 °C; $[\alpha]^{26}_D$ +64° (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 241 (4.08) nm; IR (KBr) ν_{max} 2950, 1735, 1670, 1610, 1540, 1445, 1340, 1195, 1175 cm^{-1}; ^1H NMR δ 5.70 (1H, s, H-4), 4.34 (1H, dt, J = 5.2, 7.8 Hz, H-16), 3.76 (1H, dt, J = 4.8, 8.1 Hz, H-22), 3.68 (3H, s, 24-OMe), 2.58 (1H, dd, J = 7.2, 15.0 Hz, H-23a), 2.53 (1H, dd, J = 4.7, 15.0 Hz, H-23b), 1.17 (3H, s, H-19), 1.00 (3H, d, J = 6.8 Hz, H-21), 0.82 (3H, s, H-18); ^{13}C-NMR data, see Table 1; EIMS *m/z* $[M]^+$ 400 (38), 382 (33), 368 (6), 350 (11), 340 (23), 327 (43), 284 (18), 271 (10), 130 (100); HREIMS *m/z* $[M]^+$ 400.2484 (calcd for $C_{21}H_{36}O_4$, 400.2614); *anal.* C 74.95%, H 9.06%; calcd for $C_{21}H_{36}O_4$, found C 74.73%, H 9.03%.

Known Compounds with Additional Data. Dihydrosarsasapogenin (3): IR (KBr) ν_{max} 3350, 2925, 1450, 1375, 1340, 1320, 1240, 1170, 1095, 1045, 1010 cm^{-1}; ^1H NMR δ 4.26 (1H, dt, J = 5.2, 7.8 Hz, H-16), 4.08 (1H, br s, H-3), 3.48 (1H, dd, J = 5.5, 10.6 Hz, H-26a), 3.42 (dd, J = 6.0, 10.6 Hz, H-26b), 3.29 (1H, dt, J = 3.5, 8.2 Hz, H-22), 1.56 (1H, J = 5.1, 8.0 Hz, H-17), 0.97 (3H, d, J = 6.8 Hz, H-21), 0.95 (3H, s, H-19), 0.91 (3H, d, J = 6.6 Hz, H-27), 0.75 (3H, s, H-18); ^{13}C-NMR data, see Table 1; EIMS *m/z* $[M]^+$ 418 (32), 400 (26), 385 (10), 368 (24), 357 (35), 344 (60), 331 (26), 273 (55), 255 (12), 161 (14), 144 (75), 133 (15), 122 (26), 107 (43), 93 (43), 81 (55), 67 (55), 55 (100); HREIMS *m/z* $[M]^+$ 418.3416 (calcd for $C_{27}H_{46}O_3$, 418.3447).

12α-Hydroxyandrost-4-en-3,17-dione (10): mp 177−178 °C; $[\alpha]^{26}_D$ +206° (*c* 1.05, $CHCl_3$); IR (KBr) ν_{max} 3450, 2925, 1730, 1680, 1620, 1455, 1375, 1265, 1230, 1040 cm^{-1}; ^1H NMR δ 5.74 (1H, s, H-4), 4.13 (1H, br s, H-12), 1.17 (3H, s, H-19), 0.89 (3H, s, H-18); ^{13}C NMR δ 219.8 (s, C-17), 199.2 (s, C-3), 170.0 (s, C-5), 124.3 (d, C-4), 69.1 (d, C-12), 53.0 (s, C-13), 47.9 (d, C-9), 42.8 (d, C-14), 38.2 (s, C-10), 36.1 (t, C-16), 35.5 (t, C-1), 35.1 (d, C-8), 33.8 (t, C-2), 32.5 (t, C-6), 30.5 (t, C-7), 27.3 (t, C-11), 20.9 (t, C-15), 17.2 (q, C-19), 13.8 (q, C-18); HMQC data, H-1 (δ 1.98 and 1.73) to C-1, H-2 (δ 2.35 and 2.40) to C-2, H-4 (δ 5.74) to C-4, H-6 (δ 2.35 and 2.40) to C-6, H-7 (δ 1.15 and 1.19) to C-7, H-8 (δ 1.73) to C-8, H-9 (δ 1.42) to C-9, H-11 (δ 1.59 and 1.74) to C-11, H-12 (δ 4.13) to C-12, H-14 (δ 2.02) to C-14, H-15 (δ 1.58 and 1.99) to C-15, H-16 (δ 2.09 and 2.48) to C-16, H-18 (δ 0.89) to C-18, H-19 (δ 1.17) to C-19; HMBC data, H-2 to C-1 and C-3; H-4 to C-2, C-6, and C-10; H-6 to C-5, C-7, and C-8; H-12 to C-9 and C-14; C-18 to C-12, C-13, C-14, and C-17; C-19 to C-1, C-5, C-9, and C-10; EIMS *m/z* $[M]^+$ 302 (33), 284 (35), 269 (9), 256 (13), 242 (64), 227 (31), 213 (11), 199 (10), 184 (19), 177 (36), 161 (25), 147 (21), 133 (23), 124 (100); HREIMS *m/z* 302.1874 (calcd for $C_{19}H_{26}O_3$, 302.1882).

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