

## Phycomysterols and Other Sterols from the Fungus *Phycomyces blakesleeanus*

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In the search for novel bioactive products from filamentous fungi, sterols and triterpenoids found in *Phycomyces blakesleeanus* were analyzed using semipreparative HPLC, GC–MS, and NMR techniques. Structures proposed for the three new compounds identified, phycomysterol A (**1**), phycomysterol B (**2**), and neoergosterol (**3**), were confirmed by chemical synthesis. Phycomysterols possess a new natural 19-norergostane skeleton with an aromatic B ring. Phycomysterol A showed anti-HIV activity.

Fungi have been one of the main natural sources of biologically active substances since the discovery of antibiotics. Searching for new bioactive products from filamentous fungi, chemical analysis and biological screening of metabolites from *Gibberella fujikuroi* have been the subject of our work over the past few years.<sup>1–3</sup> We recently began studying *Phycomyces blakesleeanus* (Mucoraceae), which is a fungal producer of  $\beta$ -carotene (provitamin A), riboflavine (vitamin B<sub>2</sub>), pyridoxine (vitamin B<sub>6</sub>), ergosterol (provitamin D<sub>2</sub>), biotin, and nicotinic acid.<sup>4,5</sup> Therefore, oil from *Phycomyces* could be an interesting alimentary vitamin complement, if it were proven to be free of mycotoxins. We have identified more than forty nontoxic compounds among the acidic metabolites from *P. blakesleeanus*<sup>6</sup> and are now working on neutral metabolites. In the mycelium of *P. blakesleeanus* (NRRL1555 wild strain) we have found eleven sterols previously unreported in *Phycomyces*. These include the new natural sterols (**1–3**; Chart 1) with a 19-norergostane skeleton and an aromatic B ring. Previous studies of sterols from *Phycomyces* at the University College of Wales yielded a number of new sterols (**4–13**) and certain aspects of fungal ergosterol biosynthesis were resolved.<sup>7–11</sup> However, some sterols present in the fungus were not identified.<sup>9</sup>

### Results and Discussion

Sterol and triterpene mixtures isolated from *P. blakesleeanus* were subjected to semipreparative HPLC. Fractions obtained were studied by NMR spectroscopy and aliquots were treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) to form TMS derivatives for analysis by GC–MS. The sterol ester mixture was saponified, and the nonsaponifiable fraction was analyzed as described above. Results are shown in Table 1. Proportions are only approximate because they were determined from the area of the gas chromatogram peaks, based on total ion current response data. TMS–ethers of sterols were used for GC–MS analysis, instead of free sterols, because the former have better chromatographic properties and afford highly characteristic modes of fragmentation from which structural details may be inferred.<sup>12</sup>

In the mass spectrum of **1**, as the TMS–ether, the molecular ion was consistent with the TMS derivative of a sterol with the molecular formula C<sub>27</sub>H<sub>40</sub>O, containing four double bonds. The fragment ions [M – TMSOH – C<sub>9</sub>H<sub>17</sub>]<sup>+</sup>, [M – TMSOH – C<sub>6</sub>H<sub>11</sub>]<sup>+</sup>, and [M – (C<sub>6</sub>H<sub>11</sub> + H)]<sup>+</sup> indicated

**Table 1.** Phycomysterols and Other Sterols found in *P. blakesleeanus*

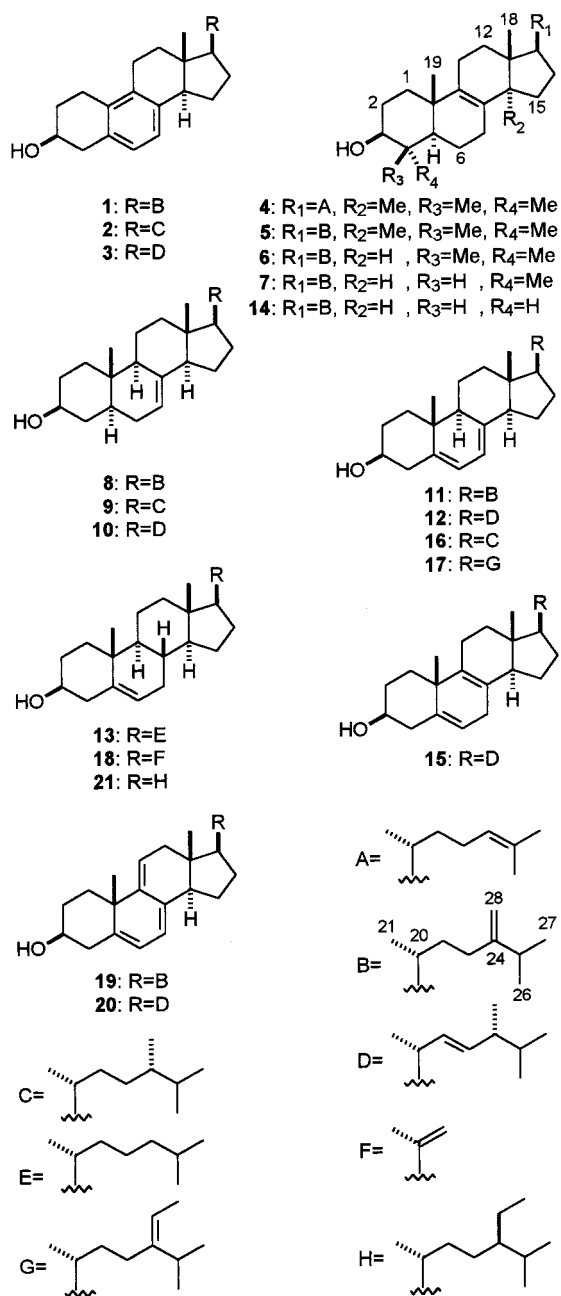
T <sub>RR</sub> <sup>a</sup>	compd	S <sup>b</sup>	SE <sup>c</sup>	ident <sup>d</sup>	ref
1.000	<b>13</b>	10	9	A, D	12
1.055	<b>20</b>	16	86	A	17
1.070	<b>15</b>	–	6	A, E	13
1.090	<b>3</b>	–	44	A, E	
1.120	<b>12</b>	trace	402	A, B, D	13
1.135	<b>19</b>	–	11	A	17
1.155	<b>10</b>	33	68	A, E	13, 20
1.175	<b>1</b>	–	13	A, E	
1.185	<b>14</b>	26	158	A	13
1.190	<b>2</b>	–	1	A, E	
1.205	<b>11</b>	17	73	A, B	8, 18
1.225	<b>16</b>	4	81	A, B	18
1.250	<b>8</b>	205	579	A, B	13, 22
1.259	<b>9</b>	–	trace	A	9
1.298	<b>4</b>	90	–	A, B	12, 19
1.325	<b>21</b>	19	86	A, D	12
1.346	<b>7</b>	96	–	A, B, C	13, 19
1.355	<b>18</b>	16	50	A	15
1.422	<b>5</b>	372	–	A, B, C	13, 19
1.425	<b>17</b>	1	trace	A	16
1.495	<b>6</b>	436	–	A, B, C	10, 21

<sup>a</sup> Relative retention time of the TMS derivatives with respect to TMS–ether of cholesterol. <sup>b</sup> Amounts ( $\mu\text{g/g}$  dry weight biomass) of nonesterified sterols and triterpenoids. <sup>c</sup> Amounts ( $\mu\text{g/g}$  dry weight biomass) of sterols found as sterol esters. <sup>d</sup> Identification: A, MS; B, <sup>1</sup>H NMR; C, <sup>13</sup>C NMR; D, comparison with an authentic sample; E, chemical synthesis.

a side chain with nine carbon atoms and a double bond. Thus, **1** must possess a nucleus with three double bonds and only eighteen carbon atoms (C<sub>18</sub>H<sub>23</sub>O). The high intensity of the [M – TMSOH]<sup>+</sup> fragment could be accounted for by the existence, in the B ring, of a conjugated system stabilizing the ion charge. Moreover, the low intensity of the [M – TMSOH – CH<sub>3</sub>]<sup>+</sup> ion, in contrast to the high intensity of the [M – 105]<sup>+</sup> ion from the TMS–ethers of 7-dehydrocholesterol and ergosterol,<sup>12</sup> suggested a 19-norsterol. On the basis of these data, we tentatively proposed structure **1**. The TMS derivate of **2** gave an MS whose molecular ion was in agreement with a sterol having molecular formula C<sub>27</sub>H<sub>42</sub>O and possessing three double bonds. The [M – TMSOH – C<sub>9</sub>H<sub>19</sub>]<sup>+</sup> ion fragment pointed to a saturated chain, and the intensities of the [M – TMSOH]<sup>+</sup> and [M – TMSOH – CH<sub>3</sub>]<sup>+</sup> ions were in agreement with an aromatic nucleus as in **1**. Therefore, structure **2** was proposed for phycomysterol B. The TMS derivate of neoergosterol (**3**) showed a mass spectrum assignable to an isomer closely related to **1**. The intensity of the [M – TMSOH – C<sub>9</sub>H<sub>17</sub>]<sup>+</sup> ion revealed the position of

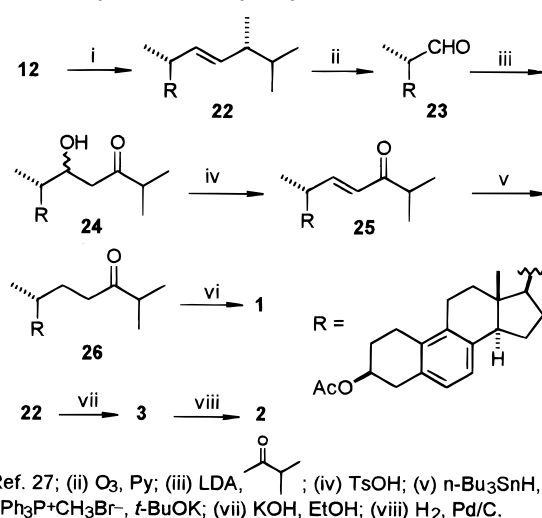
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Chart 1



the side chain double bond at  $\Delta^{22}$ , allowing the characterization of structure **3**. Mass spectra of TMS-ethers of compounds **4**, **5**, **7**, **8**, **10**, and **12–15** matched those previously described.<sup>12,13</sup> Interpretation of the MS from the TMS-ethers of compounds **6**, **9**, **11**, and **16–20** was carried out on the basis of their fragmentation patterns<sup>12,14</sup> and was facilitated by the previously reported MS of the corresponding free sterols and their acetyl derivatives.<sup>8–10,15–18</sup> Semipreparative HPLC yielded compounds **4–8**, **11**, **12**, and **16**. <sup>1</sup>H NMR spectra of **4–8** and **12**, as well as <sup>13</sup>C NMR of **6**, agreed with those previously reported.<sup>19–22</sup> In the <sup>1</sup>H NMR spectrum of **11**, two methyl singlets (H-18 and H-19), three methyl doublets (H-21, H-26, and H-27), and the signals of four olefinic protons (H-6, H-7, H-28a, and H-28b) appeared, supporting its MS-based characterization. The <sup>1</sup>H NMR spectrum of **16** showed two methyl singlets (H-18 and H-19), four methyl doublets (H-21, H-26, H-27, and H-28), and the signals of two olefinic protons (H-6 and H-7), which supported its MS-based characterization. Comparison of the <sup>13</sup>C NMR

Scheme 1. Synthesis of Phycomysterols

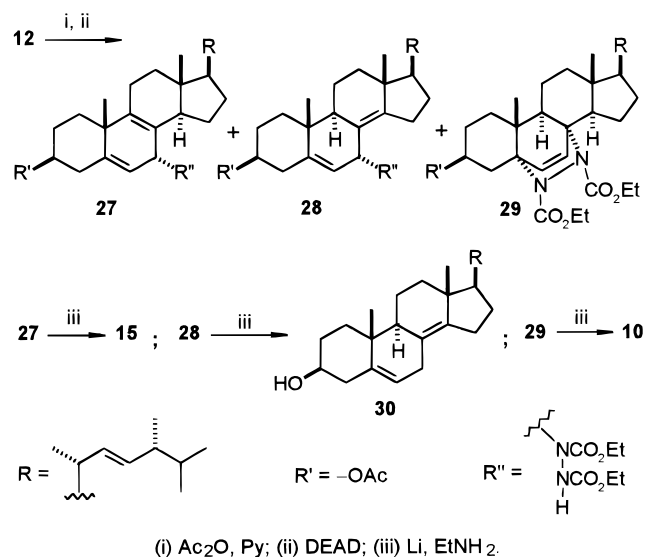


spectra for triterpenoids **5** and **7** with those of related compounds<sup>21,23</sup> confirmed their structures. The TMS-sterol at  $T_{RR'} = 1.325$  showed an MS which matched both mass spectra of TMS derivatives from sitosterol (**21**)<sup>12</sup> and clionasterol (24-*epi*-sitosterol).<sup>14</sup> The corresponding fungal sterol could not be isolated for NMR analysis, but a sample of true sitosterol was obtained from the plant *Santolina viscosa* and its 24*R* stereochemistry was confirmed by <sup>1</sup>H NMR.<sup>24</sup> Plant sitosterol was treated with BSTFA and analyzed by GC-MS, showing the same  $T_{RR'}$  as the fungal sterol **21**. The TMS-ether of clionasterol, also contained in the sterol mixture from *S. viscosa*, had a  $T_{RR'} = 1.292$  under the same GC-MS conditions.

Synthetic compounds **2** and **3** had previously been obtained for the preparation of oestrone,<sup>25</sup> but no previous reports about structure **1** have been found. To confirm the structure proposed for phycomysterol A (**1**) and to study its biological activity, chemical synthesis of **1**, starting from commercially available ergosterol (**12**), was carried out (Scheme 1). The acetate **22** was prepared following the modified photochemical procedure of Mosettig and Scheer.<sup>26,27</sup> Aldehyde **23** was obtained from **22**, without isomerization at C-20, by ozonolysis in the presence of pyridine. The condensation of **23** with the kinetic enolate of 3-methyl-2-butanone yielded a mixture (1:2.7) of the 22*R* and 22*S* stereoisomers of the aldol **24**. Dehydration of the epimeric mixture led to the conjugated enone **25** and selective reduction of its conjugated double bond furnished the ketone **26**. Finally, the Wittig olefination of **26** afforded **1**. Moreover, saponification of **22** generated **3**, and selective hydrogenation of **3** led to **2**. Synthetic **1**, **2** and **3** were treated with BSTFA and were analyzed by GC-MS. Mass spectra and relative retention times of the synthetic TMS-ethers were identical to those of the TMS derivatives of the corresponding fungal sterols.

Recently, substantial levels of 19-norcholesta-5,7,9-trien-3 $\beta$ -ol have been found, by means of GC-MS techniques, in blood and feces from patients with the Smith-Lemli-Opitz syndrome.<sup>28</sup> However, it has been reported that a part of the 19-norcholesta-5,7,9-trien-3 $\beta$ -ol measured derives from cholesta-5,8-dien-3 $\beta$ -ol by an aromatization process which takes place during the GC-MS analysis. The artifact proportion varied directly with the amount of material injected in the GC-MS apparatus, and inversely with the injector head pressure.<sup>29</sup> Equally, neoergosterol (**3**) found in *P. blakesleeanus* might be an artifact from lichesterol (**15**). Thereby, an authentic sample of lich-

## Scheme 2. Synthesis of Sterols 10, 15, and 30



sterol was synthesized in order to study the possible transformation of **15** into **3** under our GC–MS conditions. Reaction between ergosteryl acetate and diethyl azodicarboxylate (DEAD) gave a mixture of **27**,<sup>30</sup> **28**,<sup>30</sup> and the unexpected Diels–Alder type adduct **29** (Scheme 2). Treatment of **27**, **28**, and **29** with lithium in ethylamine afforded **15**,<sup>30</sup> **30**, and **10**, respectively. In the <sup>1</sup>H NMR spectra of **10** and **30**, chemical shifts of methyl singlets (H-18 and H-19) and olefinic hydrogens indicated localization of the double bonds.<sup>31</sup> <sup>13</sup>C NMR spectra of **10** and **30**, confirmed their structures. Derivatization of synthetic **10** and subsequent analysis by GC–MS, confirmed the structure proposed for the corresponding fungal sterol.

Synthetic **15** was treated with BSTFA and analyzed by GC–MS, under the same conditions used for the fungal sterol analysis. In the chromatogram, a major and a minor peak appeared. The major peak showed MS and  $T_{\text{RR}}$  identical to those for fungal lichesterol, confirming the proposed structure **15**. The minor peak had  $T_{\text{RR}}$  and MS which match those of **3**. The area ratio between the two peaks of the chromatogram was  $15/3 = 6:1$ , whereas relative proportions between the sterols measured in *P. blakesleeanus* was  $15/3 = 1:7$ . These facts point to a true metabolic origin for the major part of neoergosterol (**3**) found in *Phycomyces*.

Phycomysterols A (**1**) and B (**2**), as well as neoergosterol (**3**), co-occur with sterols **11**, **12**, and **16** in *P. blakesleeanus*. From a structural point of view, biogenesis of **1**–**3**, from **11**, **16**, and **12**, respectively, seems plausible.

Finally, the biological activity of phycomysterol A (**1**) was tested against the human immunodeficiency virus (HIV)<sup>32</sup> and on several human and mouse tumor cell lines.<sup>33</sup> Sterol **1** displayed medium anti-HIV activity at a concentration nontoxic for the infected human cells. Several HIV-inhibitory sulfated sterols from marine invertebrates have been reported previously<sup>34</sup> but anti-HIV activity of a nonsulfated aromatic sterol is novel.

## Experimental Section

**General Experimental Procedures.** Optical rotations were determined on a Perkin-Elmer 141 polarimeter. IR spectra were obtained, in liquid film between NaCl plates, on a 983 G Perkin-Elmer apparatus. HRMS were measured on an Autospec-Q VG-Analytical (FISONS) mass spectrometer, and LRMS were determined on a 5988A Hewlett-Packard instrument. NMR spectra were recorded on Bruker AM 300

and Bruker ARX 400 spectrometers. Chemical shifts are reported in parts per million ( $\delta$ ) relative to TMS and coupling constants ( $J$ ) are in Hertz. <sup>13</sup>C NMR assignments are tentative unless otherwise stated. Carbon substitution degrees were established by DEPT multipulse sequence. Thin-layer chromatography (TLC) was performed on precoated 0.25-mm thick Merck plates of Si gel 60 F<sub>254</sub>, using a 7% phosphomolybdic acid solution (EtOH) to visualize the spots. Semipreparative TLC was performed on precoated 0.5-mm thick Scharlau plates of Si gel F<sub>254</sub>. Gravity column chromatography was carried out on Brockmann type Al<sub>2</sub>O<sub>3</sub> and flash chromatography was performed as described previously.<sup>35</sup> All solvents were purified and dried following standard procedures.<sup>36</sup> Cholesterol was purchased from BDH Chemicals Ltd and ergosterol from Aldrich. Other reagents were purchased from Aldrich, Merck, or Fluka and were used as received.

**Organism.** An aqueous spore suspension ( $1.2 \times 10^7$  spores per mL) of *P. blakesleeanus* (NRRL1555 wild strain) was obtained from Prof. E. Cerdá-Olmedo, Departamento de Genética, Universidad de Sevilla, Sevilla, Spain.

**Culture and Extraction Procedures.** Minimal medium<sup>37</sup> (4 L) was autoclaved at 120 °C for 15 min (glucose was autoclaved separately) and distributed in forty 500 mL Erlenmeyer flasks. Fungal spores were activated by heat shocking the spore suspension (48 °C for 10 min) immediately before inoculation. The liquid medium was inoculated ( $7 \times 10^5$  spores per L) with the activated spore suspension. The flasks were incubated at 23 °C, under the light of four 40 W fluorescent lamps, at 200 rpm for 5 days. The fungal mycelium, harvested by vacuum filtration and then lyophilized, weighed 26.56 g. The lyophilized mycelium was ground and extracted with *t*-BuOMe. The extract was divided into neutral (5.66 g) and acidic (2.25 g) parts. The neutral part was chromatographed over an Al<sub>2</sub>O<sub>3</sub> column, using hexane/*t*-BuOMe mixtures of increasing polarity. Sterol esters (139 mg) were eluted with hexane/*t*-BuOMe (8:2), triterpenoids (33 mg) were eluted with hexane/*t*-BuOMe (2:8), and free sterols (24 mg) were eluted with *t*-BuOMe. The sterol esters were saponified and the nonsaponifiable fraction (57 mg) was rechromatographed in eight subfractions using reverse phase HPLC (Spherisorb ODS-2 column, 250  $\times$  4.6 mm i.d.) and MeOH as eluant (4 mL per min). These subfractions were derivatized and analyzed by GC–MS together with the free sterol fraction.

**Preparation of TMS Derivatives and Analysis by GC–MS:** Anhydrous THF (10  $\mu$ L) and 10  $\mu$ L of BSTFA were added per mg of sample, and the mixture was heated at 110 °C in a sealed vial for 20 min. The solvent and reagents were evaporated, and the residue was redissolved in THF (0.1 mL per mg). One  $\mu$ L of sample was injected into a 25 m  $\times$  0.32 mm i.d. HP-1 methylsilicone capillary column (He at 0.6 mL·min<sup>-1</sup>; temperature programmed from 80 to 260 °C at 40 °C·min<sup>-1</sup>, from 260 to 280 °C at 3 °C·min<sup>-1</sup>, and 30 min hold at 280 °C; injector temperature 260 °C; 70 eV; ion source at 150 °C).

**EMS Data of TMS–Ethers from Compounds 6, 9, 11, and 16–20:** **6:**  $m/z$  498 [M]<sup>+</sup> (100), 483 (28), 408 (77), 393 (97), 371 (18), 309 (17), 283 (37), 281 (38), 255 (73), 241 (93). **9:**  $m/z$  472 [M]<sup>+</sup> (94), 457 (15), 382 (8), 367 (18), 255 (100), 229 (27), 213 (36). **11:**  $m/z$  468 [M]<sup>+</sup> (30), 453 (2), 378 (19), 363 (100), 339 (4), 337 (72), 294 (9), 253 (14), 251 (12), 227 (5), 226 (11), 225 (11), 211 (38), 131 (62), 129 (48). **16:**  $m/z$  470 [M]<sup>+</sup> (21), 455 (2), 380 (20), 365 (100), 341 (6), 339 (80), 255 (14), 253 (2), 229 (6), 228 (8), 227 (6), 213 (31), 131 (55), 129 (37). **17:**  $m/z$  482 [M]<sup>+</sup> (19), 392 (15), 377 (95), 351 (46), 294 (41), 253 (21), 226 (23), 225 (21), 211 (53), 131 (100), 129 (80). **18:**  $m/z$  386 [M]<sup>+</sup> (25), 296 (24), 281 (27), 257 (12), 227 (7), 129 (100). **19:**  $m/z$  466 [M]<sup>+</sup> (30), 451 (12), 376 (98), 361 (35), 292 (8), 251 (65), 249 (69), 225 (16), 224 (24), 223 (27), 209 (100). **20:**  $m/z$  466 [M]<sup>+</sup> (8), 451 (2), 376 (29), 361 (6), 251 (100), 249 (17), 225 (3), 224 (4), 223 (6), 209 (20).

**<sup>1</sup>H NMR Data of 11:** (CDCl<sub>3</sub>, 300 MHz)  $\delta$  5.57 (1H, m, H-6), 5.38 (1H, m, H-7), 4.71 (1H, br s, H-28a), 4.65 (1H, br d,  $J = 1.2$  Hz, H-28b), 3.63 (1H, m, H-3), 1.02 (3H, d,  $J = 6.8$  Hz,

**Table 2.**  $^{13}\text{C}$  NMR Data ( $\delta$ ,  $\text{CDCl}_3$ ) for Compounds **1–3**, **5**, **7**, **10**, **15**, and **23–26**

compd	<b>1</b>	<b>2</b>	<b>3</b>	<b>5</b>	<b>7</b>	<b>10</b>	<b>15</b>	<b>23</b> <sup>a</sup>	<b>24a</b>	<b>24b</b>	<b>25</b>	<b>26</b>
C												
<b>1</b>	24.3	24.3	24.3	35.6	35.2	37.3	35.8	24.1	24.3	24.1	24.2	24.2
<b>2</b>	31.7	31.8	31.8	27.8	31.3	31.6	32.1	27.9	28.2	27.9	28.1	28.1
<b>3</b>	67.2	67.2	67.2	78.9	76.6	71.2	71.6	69.7	69.8	69.8	69.8	69.9
<b>4</b>	39.0	39.1	39.1	38.8	39.3	38.1	42.4	35.0	35.1	35.0	35.1	35.1
<b>5</b>	131.2	131.2	131.3	50.4	47.0	40.4	139.0	130.9	131.0	130.7	130.9	130.8
<b>6</b>	126.9	126.9	126.9	18.2	21.0	29.8	119.6	126.7	126.8	126.6	126.7	126.7
<b>7</b>	124.0	124.0	124.0	28.2	27.6	117.6	29.2	123.8	123.9	123.8	123.9	124.0
<b>8</b>	133.1	133.2	133.2	134.4	128.2	139.7	126.6	133.1	133.1	132.9	133.1	133.0
<b>9</b>	134.2	134.3	134.2	134.4	135.1	49.6	132.1	133.7	134.0	133.9	133.8	133.1
<b>10</b>	138.0	138.1	138.1	37.0	32.0	34.3	37.5	137.1	137.6	137.8	137.5	137.9
<b>11</b>	24.7	24.7	24.8	21.0	22.8	21.6	22.4	24.9	25.1	25.0	25.0	25.1
<b>12</b>	37.0	37.1	37.0	26.5	37.1	39.6	36.9	36.6	37.0	36.9	36.9	37.0
<b>13</b>	41.9	41.9	41.8	44.5	42.2	43.4	42.0	42.4	42.4	41.6	42.2	42.0
<b>14</b>	51.8	51.8	51.9	49.8	51.9	55.2	52.0	50.9	51.5	51.4	51.7	51.8
<b>15</b>	25.2	25.2	24.6	31.0	23.8	23.0	23.1	24.5	24.2	24.1	24.2	24.2
<b>16</b>	28.9	28.9	29.3	30.8	28.9	28.2	29.1	27.7	28.1	27.9	28.1	28.1
<b>17</b>	55.1	55.2	55.7	50.4	54.8	56.1	54.7	50.0	52.3	51.5	54.1	55.1
<b>18</b>	11.1	11.2	11.4	15.7	11.3	12.18	11.6	11.4	11.2	11.0	11.4	11.2
<b>19</b>				18.7	18.8	13.12	23.0					
<b>20</b>	36.2	36.7	40.6	36.5	36.4	40.6	40.6	49.8	41.8	41.5	40.3	35.8
<b>21</b>	18.9	15.6	21.1	19.1	19.0	21.1	21.0	13.5	12.9	12.5	19.5	18.7
<b>22</b>	34.7	33.8	135.7	35.0	34.7	135.8	135.8	204.7	69.1	68.8	152.1	29.9
<b>23</b>	31.0	30.7	132.2	31.2	31.2	132.0	132.0		40.1	45.2	126.4	37.3
<b>24</b>	156.8	39.2	43.0	156.9	157.0	42.9	42.9		216.9	216.2	204.4	215.4
<b>25</b>	33.9	31.6	33.2	33.8	33.9	33.2	33.2		41.8	41.1	38.4	41.0
<b>26</b>	21.9	19.1	19.8	21.9	22.0	19.7	19.8		18.2	18.0	18.5	18.4
<b>27</b>	22.0	20.7	20.1	22.0	22.1	20.0	20.1		18.2	18.0	18.6	18.5
<b>28</b>	106.1	17.7	17.7	105.9	106.0	17.7	17.7					
<b>29</b>				28.0	15.1							
<b>30</b>				15.4								
<b>31</b>				24.3								
H <sub>3</sub> CCO <sub>2</sub>								21.4	21.5	21.4	21.5	21.5
H <sub>3</sub> C CO <sub>2</sub>								170.8	170.9	170.7	170.4	170.9

<sup>a</sup> Assignments were made on the basis of 2D NMR experiments: direct (HETCOR) and long-range (COLOC) heteronuclear  $^1\text{H}/^{13}\text{C}$  correlations.

H-26), 1.01 (3H, d,  $J = 6.8$  Hz, H-27), 0.97 (3H, d,  $J = 6.5$  Hz, H-21), 0.94 (3H, s, H-19), 0.62 (3H, s, H-18).

**$^1\text{H}$  NMR Data of 16:** ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  5.56 (1H, dd,  $J = 5.9, 2.7$  Hz, H-6), 5.39 (1H, m, H-7), 3.63 (1H, m, H-3), 0.93 (3H, s, H-19), 0.93 (3H, d,  $J = 6.4$  Hz, H-21), 0.85 (3H, d,  $J = 6.8$  Hz, H-26), 0.78 (3H, d,  $J = 6.8$  Hz, H-27), 0.77 (3H, d,  $J = 6.8$  Hz, H-28), 0.61 (3H, s, H-18).

**19-Norergosta-5,7,9,22-tetraen-3 $\beta$ -yl Acetate (22).** This compound was obtained from ergosterol (**12**) by the photochemical procedure previously reported.<sup>27</sup>

**20-Formyl-19-norpregna-5,7,9-trien-3 $\beta$ -yl Acetate (23).** A current of freshly generated ozone was passed through a solution of **22** (800 mg) in anhydrous  $\text{CH}_2\text{Cl}_2$  (65 mL) and pyridine (0.32 mL) at  $-50$  °C for 6 h. An 8 mL amount of  $\text{Me}_2\text{S}$  was then added, and the stirred mixture was allowed to return to room temperature. The reaction mixture was washed with saturated solutions of  $\text{KHSO}_4$  and  $\text{NaHCO}_3$  and dried over anhydrous  $\text{Na}_2\text{SO}_4$ . The solvent was removed, and the crude residue was flash chromatographed (hexane, *t*-BuOMe 8:2) giving 525 mg of **23**: oil; IR (film)  $\nu_{\text{max}}$  2707, 1729, 1718  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  9.63 (1H, d,  $J = 3.1$  Hz, H-22), 6.89 (1H, d,  $J = 7.8$  Hz, H-6), 6.85 (1H, d,  $J = 7.8$  Hz, H-7), 5.14 (1H, m, H-3), 3.09 (1H, dd,  $J = 16.3, 4.8$  Hz, H-4 $\alpha$ ), 2.86 (1H, dd,  $J = 16.3, 7.7$  Hz, H-4 $\beta$ ), 2.45 (1H, ddq,  $J = 10.0, 6.8, 3.1$  Hz, H-20), 2.06 (3H, s,  $\text{CH}_3\text{CO}$ ), 1.21 (3H, d,  $J = 6.8$  Hz, H-21), 0.65 (3H, s, H-18);  $^{13}\text{C}$  NMR (Table 2); EIMS  $m/z$  326 [ $\text{M} - \text{CO}$ ]<sup>+</sup> (10), 324 (12), 310 (100), 294 (41), 237 (10), 236 (8), 212 (20), 210 (12), 195 (18), 183 (15), 141 (12); HRFABMS  $m/z$  377.2094 (calcd for  $\text{C}_{23}\text{H}_{30}\text{O}_3\text{Na}$ , 377.2093).

**22-Hydroxy-19-nor-24-oxoergosta-5,7,9-trien-3 $\beta$ -yl Acetate (24 a,b).** Butyllithium (2.4 mL of a 2.5 M solution in hexane) was added to a solution of diisopropylamine (0.84 mL) in anhydrous THF (6.6 mL) at  $-78$  °C under Ar. After 20 min a solution of 3-methyl-2-butanone (0.64 mL) was added and the mixture stirred for 15 min. The resulting enolate was

transferred by means of a cannula into a previously cooled solution ( $-78$  °C) of **23** (500 mg) in THF (4 mL). The reaction mixture was left at 0 °C for 1 h and was poured into a saturated solution of  $\text{NH}_4\text{Cl}$  (20 mL). The solution was extracted with AcOEt (40 mL  $\times$  4), and the organic layers were washed with 1 M HCl, saturated  $\text{NaHCO}_3$ , and brine and then dried over anhydrous  $\text{Na}_2\text{SO}_4$ . When the solvent was eliminated, a crude residue (799 mg) was obtained, which was flash chromatographed (hexane, *t*-BuOMe 7:3) yielding 162 mg of 22*R*-hydroxy-19-nor-24-oxoergosta-5,7,9-trien-3 $\beta$ -yl acetate (**24a**): oil; IR (film)  $\nu_{\text{max}}$  3500, 1731, 1713  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  6.88 (1H, d,  $J = 7.9$  Hz, H-6), 6.83 (1H, d,  $J = 7.9$  Hz, H-7), 5.13 (1H, dddd,  $J = 9.2, 7.7, 4.8, 3.0$  Hz, H-3), 4.16 (1H, ddd,  $J = 10.1, 3.3, 2.0$  Hz, H-22), 3.08 (1H, dd,  $J = 16.3, 4.8$  Hz, H-4 $\alpha$ ), 2.85 (1H, dd,  $J = 16.3, 4.8$  Hz, H-4 $\beta$ ), 2.58 (1H, dd,  $J = 17.4, 2.0$  Hz, H-23 $\alpha$ ), 2.46 (1H, dd,  $J = 17.4, 10.1$  Hz, H-23 $\beta$ ), 2.05 (3H, s,  $\text{CH}_3\text{CO}$ ), 1.13 (6H, d,  $J = 6.9$  Hz, H-26, H-27), 1.03 (3H, d,  $J = 6.7$  Hz, H-21), 0.62 (3H, s, H-18);  $^{13}\text{C}$  NMR (Table 2); EIMS  $m/z$  380 [ $\text{M} - \text{AcOH}$ ]<sup>+</sup> (100), 362 (18), 294 (46), 237 (8), 236 (8), 210 (18), 195 (25), 183 (16), 141 (11), 115 (6), 71 (10). From the flash chromatography 428 mg of 22*S*-hydroxy-19-nor-24-oxoergosta-5,7,9-trien-3 $\beta$ -yl acetate (**24b**) were also obtained: oil; IR (film)  $\nu_{\text{max}}$  3491, 1731, 1712  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  6.88 (1H, d,  $J = 7.9$  Hz, H-6), 6.83 (1H, d,  $J = 7.9$  Hz, H-7), 4.53 (1H, dddd,  $J = 9.2, 7.7, 4.8, 3.0$  Hz, H-3), 4.22 (1H, br d,  $J = 9.5$  Hz, H-22), 3.08 (1H, dd,  $J = 16.3, 4.8$  Hz, H-4 $\alpha$ ), 2.85 (1H, dd,  $J = 16.3, 7.7$  Hz, H-4 $\beta$ ), 2.74 (1H, dd,  $J = 9.5, 7.5$  Hz, H-23 $\alpha$ ), 2.66 (5H, m, H-1, H-11, H-14), 2.49 (1H, dd,  $J = 17.5, 2.3$  Hz, H-23 $\beta$ ), 2.05 (3H, s,  $\text{CH}_3\text{CO}$ ), 1.12 (6H, d,  $J = 6.9$  Hz, H-26, H-27), 1.03 (3H, d,  $J = 6.7$  Hz, H-21), 0.59 (3H, s, H-18);  $^{13}\text{C}$  NMR (Table 2); EIMS  $m/z$  380 [ $\text{M} - \text{AcOH}$ ]<sup>+</sup> (100), 362 (13), 294 (41), 237 (8), 236 (9), 235 (8), 210 (18), 195 (25), 183 (18), 141 (11), 115 (7), 71 (12).

**19-Nor-24-oxoergosta-5,7,9,22-tetraen-3 $\beta$ -yl Acetate (25).** A solution of **24** (550 mg of epimeric mixture) in toluene/ $\text{CHCl}_3$

(3:1) (12 mL) was treated with *p*-toluenesulfonic acid (18 mg) and anhydrous  $\text{MgSO}_4$  (370 mg), and the solution was stirred at 70 °C for 1 h. The reaction mixture was then filtered, diluted with ether (50 mL), and washed with a saturated solution of  $\text{NaHCO}_3$  (25 mL  $\times$  3) and then brine. The organic layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and filtered, and the solvent was removed to give a residue which was flash chromatographed (hexane, *t*-BuOMe 85:15) obtaining 375 mg of **25**: oil; IR (film)  $\nu_{\text{max}}$  1735, 1696, 1672  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  6.88 (1H, d,  $J = 7.9$  Hz, H-6), 6.83 (1H, d,  $J = 7.9$  Hz, H-7), 6.77 (1H, dd,  $J = 15.8$ , 9.0 Hz, H-22), 6.11 (1H, d,  $J = 15.8$  Hz, H-23), 5.14 (1H, dddd,  $J = 9.7$ , 7.1, 4.8, 3.2 Hz, H-3), 3.08 (1H, dd,  $J = 16.3$ , 4.8 Hz, H-4 $\alpha$ ), 2.86 (1H, dd,  $J = 16.3$ , 7.1 Hz, H-4 $\beta$ ), 2.84 (1H, heptuplet,  $J = 6.9$  Hz, H-25), 2.05 (3H, s,  $\text{CH}_3\text{CO}$ ), 1.18 (3H, d,  $J = 6.6$  Hz, H-21), 1.12 (6H, d,  $J = 6.9$  Hz, H-26, H-27), 0.63 (3H, s, H-18);  $^{13}\text{C}$  NMR (Table 2); EIMS  $m/z$  362 [ $\text{M} - \text{AcOH}$ ] $^+$  (100), 237 (2), 221 (9), 210 (11), 195 (20), 181 (13), 141 (14).

**19-Nor-24-oxoergosta-5,7,9-trien-3 $\beta$ -yl Acetate (26).** A mixture of **25** (300 mg),  $\text{Bu}_3\text{SnH}$  (240 mg, 97% pure), and azaisobutyronitrile (AIBN, 2 mg) in 1 mL of toluene was stirred under Ar for 6 h at 80 °C. The reaction mixture was cooled, the solvent removed, and the residue purified by flash chromatography (hexane, *t*-BuOMe 85:15) yielding **26** (210 mg). Oil;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  6.88 (1H, d,  $J = 7.9$  Hz, H-6), 6.84 (1H, d,  $J = 7.9$  Hz, H-7), 5.13 (1H, dddd,  $J = 9.0$ , 7.8, 4.9, 3.2 Hz, H-3), 3.08 (1H, dd,  $J = 16.3$ , 4.9 Hz, H-4 $\alpha$ ), 2.85 (1H, dd,  $J = 16.3$ , 7.8 Hz, H-4 $\beta$ ), 2.05 (3H, s,  $\text{CH}_3\text{CO}$ ), 1.01 (6H, d,  $J = 6.9$  Hz, H-26, H-27), 0.99 (3H, d,  $J = 6.4$  Hz, H-21), 0.58 (3H, s, H-18);  $^{13}\text{C}$  NMR (Table 2); EIMS  $m/z$  364 [ $\text{M} - \text{AcOH}$ ] $^+$  (100), 278 (7), 237 (7), 236 (4), 235 (9), 210 (19), 195 (24), 183 (14), 141 (7), 71 (3); HRFABMS  $m/z$  447.2878 (calcd for  $\text{C}_{28}\text{H}_{40}\text{O}_3\text{Na}$ , 447.2875).

**19-Norergosta-5,7,9,24(28)-tetraen-3 $\beta$ -ol (1).** *t*-BuOK (0.5 mL of a solution 1 M in *t*-BuOH) was added to a suspension of methyltriphenylphosphonium bromide (175 mg) in anhydrous toluene (1.25 mL) and heated at 70 °C for 40 min. A solution of **26** (50 mg) in toluene (1 mL) was then added, and the mixture stirred at 70 °C for 3 h. The reaction mixture was allowed to cool and was flash chromatographed with hexane: *t*-BuOMe (60:40), yielding 20 mg of **1**: oil;  $[\alpha]_{\text{D}}^{25} + 6.5$  (*c* 0.4,  $\text{CHCl}_3$ ); IR (film)  $\nu_{\text{max}}$  3339, 3081, 2958, 2870, 1639, 1462, 1233, 1051, 887, 810  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  6.90 (1H, d,  $J = 7.8$  Hz, H-6), 6.85 (1H, d,  $J = 7.8$  Hz, H-7), 4.74 (1H, br s, H-28a), 4.68 (1H, d,  $J = 1.4$  Hz, H-28b), 4.11 (1H, dddd,  $J = 10.3$ , 8.0, 4.2, 3.1 Hz, H-3), 3.06 (1H, dd,  $J = 15.5$ , 4.2 Hz, H-4 $\alpha$ ), 2.76 (1H, dd,  $J = 15.5$ , 8.0 Hz, H-4 $\beta$ ), 1.05 (3H, d,  $J = 6.8$  Hz, H-26), 1.04 (3H, d,  $J = 6.8$  Hz, H-27), 1.03 (3H, d,  $J = 6.0$  Hz, H-21), 0.59 (3H, s, H-18);  $^{13}\text{C}$  NMR (Table 2); EIMS, as TMS-ether,  $m/z$  452 [ $\text{M}$ ] $^+$  (17), 437 (10), 362 (100), 347 (11), 278 (59), 237 (45), 235 (59), 210 (81), 195 (99); HREIMS  $m/z$  380.3072 (calcd for  $\text{C}_{27}\text{H}_{40}\text{O}$ , 380.3079). Cholesterol (0.5 mg) was added to 2 mg of **1**. The mixture was derivatized with BSTFA and analyzed by GC-MS.  $T_{\text{RR}}$  (1.175) and the mass spectrum of TMS derivative of **1** coincided with those obtained from phycosterol A.

**19-Norergosta-5,7,9,22-tetraen-3 $\beta$ -ol (3).** The acetate **22** (400 mg) was dissolved in 25 mL of 1 N KOH/MeOH. The solution was refluxed for 30 min, and then the EtOH was removed and 50 mL of water was added. The mixture was extracted with ether (40 mL  $\times$  3), and the organic layers washed together with brine and dried over anhydrous  $\text{Na}_2\text{SO}_4$ . When the solvent was eliminated, 341 mg of **3** was obtained. Colorless needles; mp 150–152 °C;  $[\alpha]_{\text{D}}^{25} - 7^\circ$  (*c* 0.5,  $\text{CHCl}_3$ ); IR (film)  $\nu_{\text{max}}$  3338, 970  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  6.90 (1H, d,  $J = 6.8$  Hz, H-6), 6.84 (1H, d,  $J = 6.8$  Hz, H-7), 5.24 (2H, m, H-22, H-23), 4.11 (1H, dddd,  $J = 9.6$ , 8.1, 4.9, 3.2, H-3), 3.06 (1H, dd,  $J = 16.0$ , 4.9 Hz, H-4 $\alpha$ ), 2.76 (1H, dd,  $J = 16.0$ , 8.1 Hz, H-4 $\beta$ ), 1.10 (3H, d,  $J = 6.6$  Hz, H-21), 0.94 (3H, d,  $J = 6.8$  Hz, H-28), 0.86 (3H, d,  $J = 6.8$  Hz, H-26), 0.84 (3H, d,  $J = 6.8$  Hz, H-27), 0.61 (3H, s, H-18);  $^{13}\text{C}$  NMR (Table 2); EIMS, as TMS-ether,  $m/z$  452 [ $\text{M}$ ] $^+$  (18), 437 (7), 362 (100), 237 (64), 235 (20), 210 (34), 195 (49); HREIMS  $m/z$  380.3076 (calcd for  $\text{C}_{27}\text{H}_{40}\text{O}$ , 380.3079). Cholesterol (0.5 mg) was added to 2 mg of **3**. The mixture was derivatized with

BSTFA and analyzed by GC-MS.  $T_{\text{RR}}$  (1.090) and the mass spectrum of the TMS derivative of **3** coincided with those obtained from fungal neoergosterol.

**19-Norergosta-5,7,9-trien-3 $\beta$ -ol (2).** The catalyst (10% Pd/C, 40 mg) was added to a solution of **3** (167 mg) in EtOH (35 mL). The mixture was stirred for 2 days under  $\text{H}_2$  at 1 atm. The suspension was filtered, and the solvent was removed, obtaining 135 mg of **2**:  $[\alpha]_{\text{D}}^{25} - 2^\circ$  (*c* 1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  6.90 (1H, d,  $J = 8.0$  Hz, H-6), 6.85 (1H, d,  $J = 8.0$  Hz, H-7), 4.11 (1H, dddd,  $J = 9.5$ , 8.0, 4.5, 3.3 Hz, H-3), 3.06 (1H, dd,  $J = 16.0$ , 4.5 Hz, H-4 $\alpha$ ), 2.76 (1H, dd,  $J = 16.0$ , 8.0 Hz, H-4 $\beta$ ), 1.00 (3H, d,  $J = 6.3$  Hz, H-21), 0.87 (3H, d,  $J = 6.8$  Hz, H-28), 0.799 (3H, d,  $J = 6.8$  Hz, H-26), 0.797 (3H, d,  $J = 6.8$  Hz, H-27), 0.56 (3H, s, H-18);  $^{13}\text{C}$  NMR (Table 2); EIMS, as TMS-ether,  $m/z$  454 [ $\text{M}$ ] $^+$  (7), 439 (5), 364 (100), 349 (3), 237 (35), 210 (33), 195 (49); HREIMS  $m/z$  382.3247 (calcd for  $\text{C}_{27}\text{H}_{42}\text{O}$ , 382.3236). Cholesterol (0.5 mg) was added to 2 mg of **2**. The mixture was derivatized with BSTFA and analyzed by GC-MS.  $T_{\text{RR}}$  (1.190) and the mass spectrum of TMS derivative of **2** coincided with those obtained from phycosterol B.

**Reaction of Ergosteryl Acetate with Diethyl Azodicarboxylate:** Acetic anhydride (5 mL) was added to a solution of ergosterol (**12**, 500 mg) in 5 mL of pyridine. The reaction mixture was stirred at room-temperature overnight. Then it was poured onto crushed ice and extracted with ether. The organic layer was washed with saturated solutions of  $\text{KHSO}_4$  and  $\text{NaHCO}_3$  and brine and dried over anhydrous  $\text{Na}_2\text{SO}_4$ , yielding 360 mg of ergosteryl acetate. Diethyl azodicarboxylate (0.15 mL) was added to a solution of ergosteryl acetate (360 mg) in sodium-dried benzene (5 mL), and the solution was refluxed under argon for 8 h. Removal of the solvent gave a mixture of compounds **27** (93 mg), **28** (44 mg), and **29** (72 mg), which were isolated using semipreparative TLC (hexane, *t*-BuOMe 7:3). Spectroscopic data for compounds **27** and **28** were in agreement with those previously reported.<sup>30</sup> Compound **29**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  6.50 (1H, d,  $J = 8.3$  Hz, H-7 or H-6), 6.11 (1H, d,  $J = 8.3$  Hz, H-6 or H-7), 5.20 (1H, m, H-3), 5.20 (1H, dd,  $J = 15.3$ , 8.0 Hz, H-23), 5.12 (1H, dd,  $J = 15.3$ , 8.0 Hz, H-22), 2.01 (3H, s,  $\text{CH}_3\text{CO}$ ), 1.22 (3H, t,  $J = 7.1$  Hz,  $\text{CO}_2\text{CH}_2\text{CH}_3$ ), 1.13 (3H, t,  $J = 7.1$  Hz,  $\text{CO}_2\text{CH}_2\text{CH}_3$ ), 0.97 (3H, d,  $J = 6.6$  Hz, H-21), 0.92 (3H, s, H-19), 0.89 (3H, d,  $J = 6.8$  Hz, H-28), 0.82 (3H, d,  $J = 6.6$  Hz, H-27), 0.801 (3H, d,  $J = 6.5$  Hz, H-26), 0.799 (3H, s, H-18);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  170.3 (s,  $\text{CH}_3\text{CO}$ ), 159.9 (s,  $\text{CO}_2\text{Et}$ ), 155.9 (s,  $\text{CO}_2\text{Et}$ ), 140.8 (d, C-7 or C-6), 135.5 (d, C-22), 132.1 (d, C-23), 128.8 (d, C-6 or C-7), 70.3 (d, C-3), 66.3 (s, C-8 or C-5), 65.1 (s, C-5 or C-8), 62.0 (t,  $\text{CO}_2\text{CH}_2\text{CH}_3$ ), 61.2 (t,  $\text{CO}_2\text{CH}_2\text{CH}_3$ ), 57.0 (d, C-17), 50.8 (d, C-14), 50.0 (d, C-9), 43.9 (s, C-13), 42.8 (d, C-24), 40.5 (s, C-10), 39.9 (d, C-20), 39.5 (t, C-12), 34.8 (t, C-1), 33.2 (d, C-25), 32.0 (t, C-2), 28.4 (t, C-16), 26.8 (t, C-4), 24.8 (t, C-15), 21.5 (q,  $\text{CH}_3\text{CO}$ ), 21.2 (t, C-11), 20.8 (q, C-21), 20.0 (q, C-27), 19.8 (q, C-26), 19.0 (q, C-19), 17.6 (q, C-28), 14.7 (q,  $\text{CO}_2\text{CH}_2\text{CH}_3$ ), 14.4 (q,  $\text{CO}_2\text{CH}_2\text{CH}_3$ ), 13.4 (q, C-18);  $^1\text{H}$  and  $^{13}\text{C}$  NMR data were assigned on the basis of 2D NMR experiments:  $^1\text{H}/^1\text{H}$  homonuclear correlation (COSY), as well as direct (HETCOR) and long-range (COLOC) heteronuclear  $^1\text{H}/^{13}\text{C}$  correlations; HRFABMS  $m/z$  635.4023 (calcd for  $\text{C}_{36}\text{H}_{56}\text{N}_2\text{O}_6\text{Na}$ , 635.4036).

**Synthesis of Ergosta-5,8,22-trien-3 $\beta$ -ol (15).** This sterol was obtained from compound **27** by the procedure previously described.<sup>30</sup>  $^1\text{H}$  NMR data, mp, and  $[\alpha]_{\text{D}}^{25}$  were in agreement with those previously reported;<sup>30</sup>  $^{13}\text{C}$  NMR (Table 2); HREIMS  $m/z$  396.3392 (calcd for  $\text{C}_{28}\text{H}_{44}\text{O}$ , 396.3392). Cholesterol (0.25 mg) was added to 1 mg of **15**. The mixture was derivatized with BSTFA and analyzed by GC-MS. The chromatogram showed two peaks: the major peak had  $T_{\text{RR}}$  (1.070) and MS identical to those for TMS ether of fungal lichesterol, whereas  $T_{\text{RR}}$  (1.090) and MS of the minor peak (17% abundance relative to the major peak) coincided with those obtained from TMS-ether of neoergosterol.

**Synthesis of Ergosta-5,8(14),22-trien-3 $\beta$ -ol (30).** The intermediate **28** (44 mg) was dissolved in ethylamine (2 mL), treated with lithium (35 mg), and stirred at -20 °C for 1 h. A 4 mL amount of water was then added, and the suspension

was extracted with t-BuOMe. The organic layer was washed with brine and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. After removal of the solvent a crude (27 mg) was obtained, which was flash chromatographed (hexane/t-BuOMe 1:1) yielding 13 mg of the sterol **30**: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 5.31 (1H, m, H-6), 5.22 (2H, m, H-22, H-23), 3.60 (1H, m, H-3), 1.55 (3H, s, H-19), 1.04 (3H, d, *J* = 6.7, H-21), 0.93 (3H, d, *J* = 6.8 Hz, H-28), 0.89 (3H, s, H-18), 0.84 (3H, d, *J* = 6.7 Hz, H-26), 0.83 (3H, d, *J* = 6.7 Hz, H-27); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 143.4 (s, C-14), 141.0 (s, C-5) 135.8 (d, C-22), 132.1 (d, C-23), 123.6 (s, C-8), 120.7 (d, C-6), 71.3 (d, C-3), 57.5 (d, C-17), 46.8 (d, C-9), 43.0 (d, C-24), 42.7 (s, C-13), 42.0 (t, C-4), 39.4 (d, C-20), 37.7 (t, C-12), 37.6 (s, C-10), 36.4 (t, C-1), 33.2 (d, C-25), 31.9 (t, C-2), 29.4 (t, C-7), 28.0 (t, C-16), 26.1 (t, C-15), 21.4 (q, C-21), 20.1 (q, C-27), 19.8 (q, C-26), 19.5 (t, C-11), 19.2 (q, C-19), 18.8 (q, C-18), 17.7 (q, C-28); HREIMS *m/z* 396.3399 (calcd for C<sub>28</sub>H<sub>44</sub>O, 396.3392).

**Synthesis of Ergosta-7,22-dien-3β-ol (10).** The intermediate **29** (72 mg) was dissolved in ethylamine (3 mL), treated with lithium (30 mg) and stirred at -20 °C for 1 h. Then 6 mL of water was added and the suspension was extracted with t-BuOMe. The organic layer was washed with brine and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. After removal of the solvent a crude (46 mg) was obtained, which was flash chromatographed (hexane, t-BuOMe 1/1) giving 27 mg of the sterol **10**: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 5.19 (2H, m, H-22, H-23), 5.15 (1H, m, H-7), 3.60 (1H, m, H-3), 1.02 (3H, d, *J* = 6.6, H-21), 0.91 (3H, d, *J* = 6.8 Hz, H-28), 0.84 (3H, d, *J* = 6.7 Hz, H-26), 0.82 (3H, d, *J* = 6.7 Hz, H-27), 0.80 (3H, s, H-19), 0.55 (3H, s, H-18); <sup>13</sup>C NMR (Table 2); HREIMS *m/z* 398.3549 (calcd for C<sub>28</sub>H<sub>46</sub>O, 398.3549). Cholesterol (0.5 mg) was added to 2 mg of **10**. The mixture was derivatized with BSTFA and analyzed by GC-MS. *T*<sub>RR</sub> (1.155) and the mass spectrum of **10** TMS-ether coincided with those obtained from TMS-ether of fungal ergosta-7,22-dien-3β-ol.

**Biological Activity Tests.** Anti-HIV activity of phycomysterol **1** was tested following a procedure previously described.<sup>32</sup> Compound **1** displayed 64% antiviral inhibition at a concentration of 0.64 μg per well (200 μL). In vitro antitumoral activity was assayed<sup>33</sup> on P338 and SCHABEL mouse lymphomas, as well as A549 (lung carcinoma), HT29 (colon carcinoma), and MEL28 (melanoma) human cell lines. Phycomysterol A showed IC<sub>50</sub> = 5.0 μg/mL against both mouse lymphomas, and IC<sub>50</sub> = 10 μg/mL against the three human cell lines.

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