

NEW POLYHYDROXYSTEROLS FROM THE DICTYOCERATID  
SPONGES *HIPPOSPONGIA COMMUNIS*, *SPONGIA OFFICINALIS*,  
*IRGINIA VARIABILIS*, AND *SPONGIONELLA GRACILIS*

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**ABSTRACT.**—Six new  $3\beta,5\alpha,6\beta$ -trihydroxylated sterols **21–26** have been isolated from the sponges *Hippospongia communis*, *Spongia officinalis*, and *Ircinia variabilis*, along with the previously reported triols **12–20**. Eleven  $3\beta,6\alpha$ -dihydroxylated sterols **1–11**, isolated from the sponge *Spongionella gracilis*, are also reported. Of these sterols nine are new [**3–11**]. The structures of the new natural polyols were determined by spectral data. A partial synthesis of diol **6** confirmed the structure assignment.

In recent years much attention has been given to the polyhydroxylated sterols from marine invertebrates that have been reported from gorgonians (1–6), soft corals (1, 2–5, 7, 8), starfishes (2–5, 9), hydroids (10), bryozoans (11) and sponges (12–20).

Recently we reported (17, 18) the occurrence of new  $3\beta,6\alpha$ -dihydroxylated and  $3\beta,5\alpha,6\beta$ -trihydroxylated sterols from the sponge *Spongionella gracilis* (Vosmaer) (order Dictyoceratida, family Dysideidae) and suggested that they could derive from  $\Delta^{5,7}$ -sterols present in large amounts (21) in this organism.

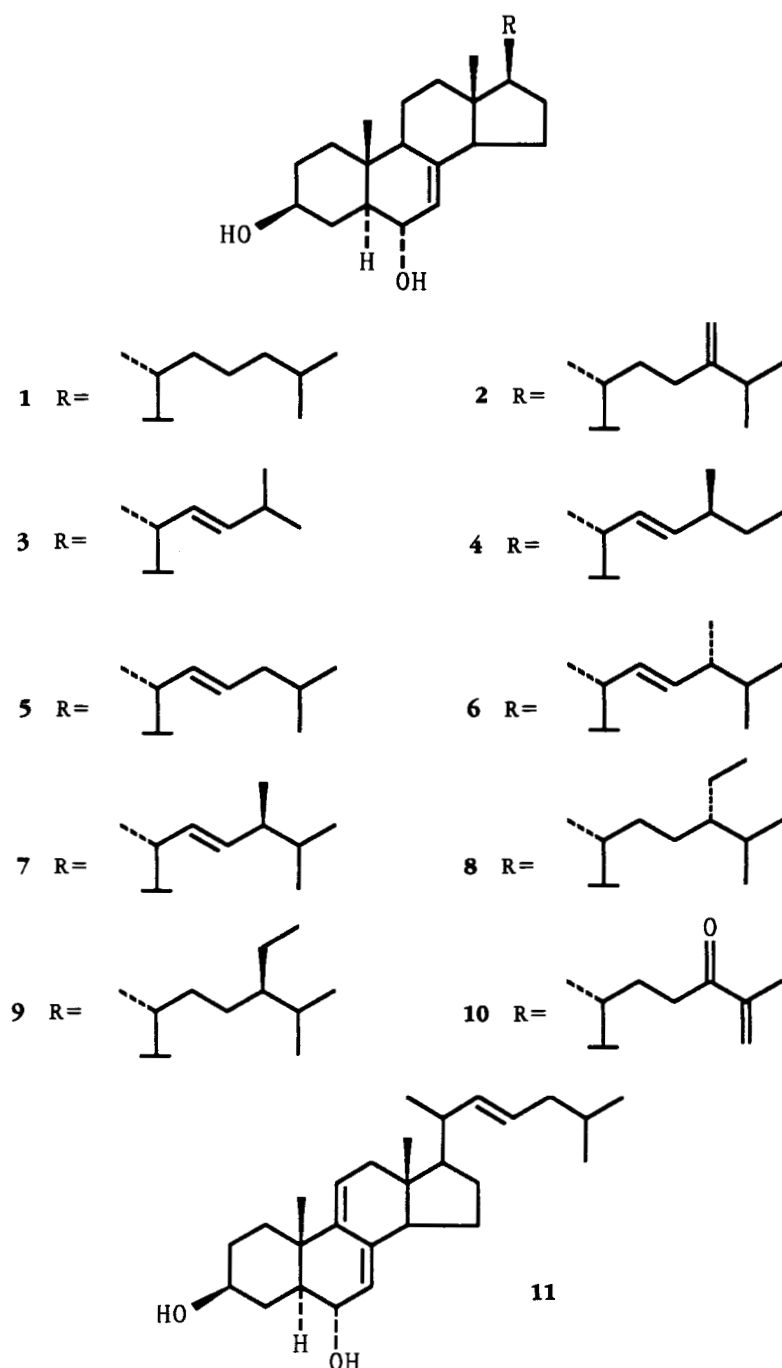
In order to substantiate this hypothesis we undertook a systematic survey of the sponges that contain  $\Delta^{5,7}$ -sterols in considerable amount. [Recently, we found that the monohydroxysterol fraction of *S. gracilis* (21) and *Ircinia variabilis* (22) consisted mainly of  $\Delta^{5,7}$ -sterols. The sponge *Hippospongia communis* also contained  $\Delta^{5,7}$ -sterols in considerable amount (unpublished results). An earlier work (23) reported that  $\Delta^{5,7}$ -sterols predominate in the sponge *S. officinalis*.] We report here that Dictyoceratid sponges *H. communis* (Lamarck) (family Spongiidae), *Spongia officinalis* L. (family Spongiidae) and *Ircinia variabilis* (Schmidt) (family Thorectidae) contain  $3\beta,5\alpha,6\beta$ -trihydroxysterols but not  $3\beta,6\alpha$ -dihydroxysterols. Further studies of the minor sterol components of the sponge *S. gracilis* yielded the new  $3\beta,6\alpha$ -dihydroxysterols **1–11**.

## RESULTS AND DISCUSSION

The total lipids extracted from the sponges *S. gracilis*, *H. Communis*, *S. officinalis*, and *I. variabilis* were chromatographed on a Si gel column to yield the polyhydroxylated sterol fractions. Separation of these polar fractions by hplc on Si gel using  $\text{CHCl}_3/\text{MeOH}$  as eluent yielded di- or trihydroxysterol mixtures that were further separated by reversed-phase hplc into pure compounds using various mixtures of  $\text{MeOH}/\text{H}_2\text{O}$  as eluent.

Sterols from *H. communis*, *S. officinalis*, and *I. variabilis* were identified as  $\Delta^7$ - $3\beta,5\alpha,6\beta$ -trihydroxysterols **12–26** on the basis of the  $\text{H}-3$ ,  $\text{H}_2-4$ ,  $\text{H}-6$ ,  $\text{H}-7$ ,  $\text{H}_3-18$ , and  $\text{H}_3-19$  chemical shift values which are typical of this nucleus; in addition, the signals of  $\text{H}-3\alpha$ ,  $\text{H}_{\text{ax}}-4$ , and  $\text{H}_3-19$  showed the typical pyridine- $d_5$ -induced deshielding due to the 1,3 diaxial interaction with the C-5 and C-6 hydroxyl groups (18, 24). These compounds are also recognized spectrally by the  $^{13}\text{C}$ -nmr signals of C-3, C-5, C-6, C-7, and C-8 at  $\delta$  67.6, 76.2, 74.3, 120.5, and 141.6, respectively (18).

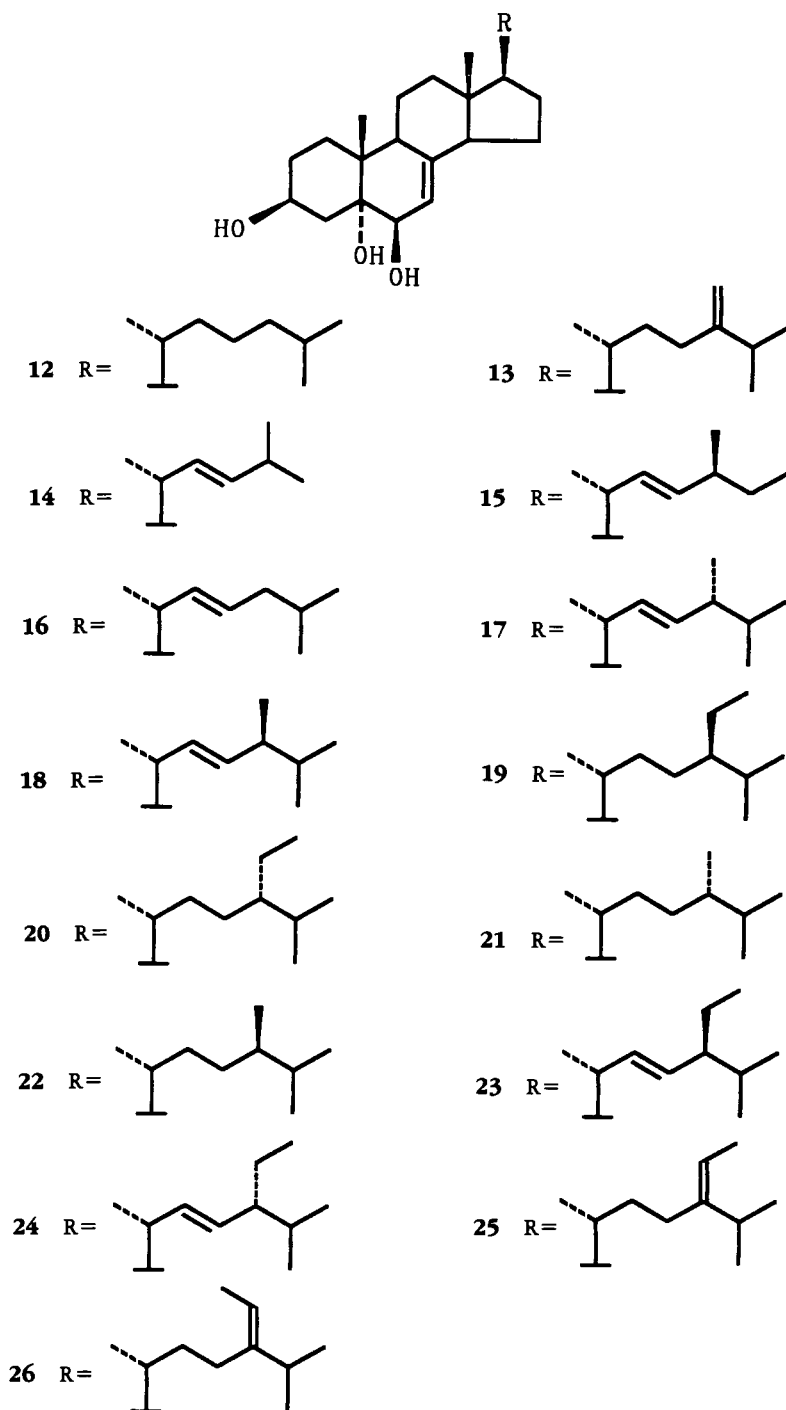
From the sponge *S. gracilis*, along with previously described  $\Delta^7$ - $3\beta,6\alpha$ -di- and  $\Delta^7$ - $3\beta,5\alpha,6\beta$ -trihydroxysterols (17, 18), were isolated the new  $\Delta^7$ - $3\beta,6\alpha$ -dihydroxy-



sterols **3–10**. The 3β,6α-dihydroxy nucleus was readily identified by the H-3, H-6, H-7, H<sub>3</sub>-18, and H<sub>3</sub>-19 <sup>1</sup>H-nmr signals (17). Furthermore, the investigation of the minor compounds of *S. gracilis* has resulted in the isolation of the novel Δ<sup>7,9(11)</sup>-3β,6α-dihydroxylated sterol **11**.

Because diols **1–10** and triols **12–26** possessed identical nuclei, it appeared that we only had to establish their side chain structure to complete their structure elucidation.

**3β,6α-DIHYDROXYLATED STEROLS.**—Sterols **1** and **2** were shown to be identical



with the 3 $\beta$ ,6 $\alpha$ -dihydroxysterols reported earlier (17), on the basis of their spectral properties.

The first new dihydroxylated sterol **3** had the molecular formula  $C_{26}H_{42}O_2$  on the basis of its high resolution mass spectrum. The electron impact mass spectrum contained the molecular ion peak at  $m/z$  386 with fragmentation ion peaks at  $m/z$  289  $[M - C_7H_{13}]^+$ , 271  $[M - H_2O - C_7H_{13}]^+$ , 253  $[M - 2H_2O - C_7H_{13}]^+$  and 211

[ $M - 2H_2O$  and ring D fission]<sup>+</sup> indicating the presence of a  $C_7H_{13}$  side chain containing one double bond. In the  $^1H$ -nmr spectrum the protons from C-20 to C-25/C-26 were readily interrelated by spin decoupling experiments. Irradiation at  $\delta$  1.99 (H-20) collapsed the doublet at  $\delta$  1.00 ( $J = 6.6$  Hz,  $H_3$ -21) to a singlet and the H-22 double doublet at  $\delta$  5.16 ( $J = 15.6$  and  $8.2$  Hz) to a doublet. The one-proton broad septet at  $\delta$  2.18 ( $J = 6.6$  Hz, H-24) was coupled with the doublet at  $\delta$  0.94 ( $J = 6.6$  Hz,  $H_3$ -25 and  $H_3$ -26) and with the H-23 double doublet at  $\delta$  5.29 ( $J = 15.6$  and  $6.6$  Hz). Consequently, the structure of this sterol was established as (22*E*)-24-nor-5 $\alpha$ -cholesta-7,22-diene-3 $\beta$ ,6 $\alpha$ -diol [**3**]. The configuration of the  $\Delta^{22}$  double bond was established as *E* from the value (15.6 Hz) of the H-22/H-23 coupling constant.

The new sterol **4** had the composition  $C_{27}H_{44}O_2$ . The mass spectrum contained the molecular ion peak at  $m/z$  400 and other fragment ions at  $m/z$  289 [ $M - C_8H_{15}$ ]<sup>+</sup>, 271, 253, and 211, indicating the presence of a  $C_8H_{15}$  side chain containing a double bond. Spin decoupling experiments established the 27-norergostane-type side chain. Irradiation at  $\delta$  1.99 (H-20) collapsed the methyl doublet at  $\delta$  1.01 ( $J = 6.6$  Hz,  $H_3$ -21) into a singlet and simplified the left portion of the multiplet centered at 5.15 (H-22 and H-23) to a doublet ( $J = 15.4$  Hz). Likewise, irradiation at  $\delta$  1.92 (H-24) collapsed the methyl doublet at  $\delta$  0.93 ( $J = 6.6$  Hz,  $H_3$ -28) into a singlet and caused the right portion of the multiplet at  $\delta$  5.15 to transform into a doublet ( $J = 15.4$  Hz). The presence in the  $^1H$ -nmr spectrum of a triplet at  $\delta$  0.836 ( $J = 7.4$  Hz,  $H_3$ -26) for a terminal ethyl group suggested a (22*E*)-27-nor-24-methyl-5 $\alpha$ -cholesta-7,22-diene-3 $\beta$ ,6 $\alpha$ -diol structure [**4**] for this sterol. The  $\Delta^{22}$  configuration was assigned as *E* on the basis of the value (15.4 Hz) of the H-22/H-23 coupling constant. The C-24 configuration was tentatively assigned as *S* because the chemical shift of its C-21 methyl doublet ( $\delta$  1.010) is almost identical with that ( $\delta$  1.008) of compound **7** whereas it differs from that of compound **6** (25.26).

Sterol **5** had molecular formula  $C_{27}H_{44}O_2$  and a  $C_8H_{15}$  side chain containing one double bond. The mass spectrum contained the molecular ion peak at  $m/z$  400 and fragment ions at  $m/z$  289 [ $M - C_8H_{15}$ ]<sup>+</sup>, 271, 253, and 211. Its  $^1H$ -nmr spectrum contained a doublet at  $\delta$  1.01 (3H,  $J = 6.6$  Hz), which is typical of a 20-Me group in a  $\Delta^{22}$ -sterol, and a doublet at  $\delta$  0.86 ( $J = 6.6$  Hz,  $H_3$ -26 and  $H_3$ -27). The  $^1H$ -nmr spectrum included two olefinic protons that appeared as a double doublet centered at  $\delta$  5.31 ( $J = 15.3$  and  $6.6$  Hz) and a multiplet centered at  $\delta$  5.20 attributable to the H-22 and H-23 protons, respectively. Irradiation at  $\delta$  2.03 (1H, m, H-20) caused the double doublet at  $\delta$  5.31 to collapse to a doublet ( $J = 15.3$  Hz) and the  $H_3$ -21 proton doublet to a singlet. Comparison of the  $^1H$ -nmr spectrum of this sterol with those of sterols having a similar side chain (27) suggested the structure (22*E*)-5 $\alpha$ -cholesta-7,22-diene-3 $\beta$ -6 $\alpha$ -diol [**5**].

Dihydroxysterol **6** had the molecular formula  $C_{28}H_{46}O_2$ . The mass spectrum contained the molecular ion peak at  $m/z$  414 and significant fragment ions at  $m/z$  289 [ $M - C_9H_{17}$ ]<sup>+</sup>, 271, 253, and 211, characteristic of sterols possessing a nine-carbon side chain with one site of unsaturation. The  $^1H$ -nmr chemical shifts for the side chain protons of this sterol are consistent with those of an authentic sample of brassicasterol. Thus, the structure of the sterol was formulated as (22*E*,24*R*)-24-methyl-5 $\alpha$ -cholesta-7,22-diene-3 $\beta$ ,6 $\alpha$ -diol [**6**] and confirmed by synthesis starting from 3 $\beta$ -acetoxy-5 $\alpha$ -ergosta-7,22-dien-6-one (28). Reduction with  $LiAlH_4$  of this compound yielded the synthetic compound **6** and its 6 $\beta$ -epimer, which were separated by hplc on Si gel. Mass spectral and  $^1H$ -nmr data of one of the two C-6 epimers were identical with those of the natural product **6**.

The close similarity of the mass and  $^1H$ -nmr spectra of compound **7** with those of **6** suggested that the two compounds must be C-24 epimers. The  $H_3$ -21 doublet in the

$^1\text{H}$ -nmr spectrum of **7** appeared upfield (25,26) at  $\delta$  1.008 ( $J = 6.6$  Hz) when compared to the corresponding  $\text{H}_3$ -21 signal ( $\delta$  1.018) for the sterol **6**; hence **7** must be formulated as (22*E*,24*S*)-24-methyl-5 $\alpha$ -cholesta-7,22-diene-3 $\beta$ -6 $\alpha$ -diol [**7**].

Sterols **8** and **9** could not be separated by repeated reversed-phase hplc. They each had the molecular formula  $\text{C}_{29}\text{H}_{50}\text{O}_2$  and a  $\text{C}_{10}\text{H}_{21}$  saturated side chain. Their mass spectrum showed the molecular ion peaks at  $m/z$  430 and fragment ions at  $m/z$  289 [ $\text{M} - \text{C}_{10}\text{H}_{21}$ ] $^+$ , 271, 253, and 211. The side chain methyl signals for both isomers could clearly be seen in the  $^1\text{H}$ -nmr spectrum and were assigned by comparison with those of authentic samples of sitosterol and clionasterol. The main difference is the chemical shift for the C-29 methyl group (27). Thus the structures of **8** and **9** were formulated as (24*S*)-24-ethyl-5 $\alpha$ -cholest-7-ene-3 $\beta$ ,6 $\alpha$ -diol [**8**] and (24*R*)-24-ethyl-5 $\alpha$ -cholest-7-ene-3 $\beta$ -6 $\alpha$ -diol [**9**]. The  $^1\text{H}$ -nmr spectrum revealed the presence of the C-24 epimers in the ratio of 1:1 (29).

The mass spectrum of compound **10** contained the molecular ion at  $m/z$  414 ( $\text{C}_{27}\text{H}_{42}\text{O}_3$ ) and other fragments at  $m/z$  271 [ $\text{M} - \text{H}_2\text{O} - \text{C}_8\text{H}_{13}\text{O}$ ] $^+$ , 253, and 211 indicating the presence of a  $\text{C}_8\text{H}_{13}\text{O}$  unsaturated side chain. The ir spectrum showed a band of an  $\alpha,\beta$ -unsaturated ketone at  $1680\text{ cm}^{-1}$ . The mass spectrum contained a peak at  $m/z$  330 [ $\text{M} - 84$ ] $^+$  due to a McLafferty rearrangement of the side chain between C-22 and C-23, suggesting the presence of a carbonyl group at C-24.  $^1\text{H}$ -nmr irradiation experiments showed that the two deshielded broad signals at  $\delta$  5.95 (1H, bs,  $\text{H}_a$ -26) and 5.75 (1H, m,  $\text{H}_b$ -26) were coupled with the protons of a methyl group at  $\delta$  1.87 (3H, dd,  $J = 1.2$  and  $1.2$  Hz,  $\text{H}_3$ -27) attached to an olefinic carbon. A  $^1\text{H}$ - $^1\text{H}$  COSY-45 experiment allowed unambiguous determination of the couplings among the vicinal protons in the segment C-21/C-23. The two  $\alpha$ -to-CO protons observed at  $\delta$  2.72 (1H, ddd,  $J = 15.6, 10.2,$  and  $5.4$  Hz,  $\text{H}_a$ -23) and 2.62 (1H, ddd,  $J = 15.6, 8.8,$  and  $5.4$  Hz,  $\text{H}_b$ -23) correlated with a pair of protons centered at  $\delta$  1.77 and 1.33 ( $\text{H}_a$ -22 and  $\text{H}_b$ -22), which, in turn, showed correlations with each other and with a diffuse multiplet at  $\delta$  1.40 (H-20). The latter signal had a cross peak with a methyl signal at  $\delta$  0.94 ( $J = 6.3$  Hz,  $\text{H}_3$ -21). The protons at  $\delta$  2.72 and 2.62 were shown to be coupled with each other as well. Thus, the structure of the new sterol could be formulated as 24-keto-5 $\alpha$ -cholesta-7,25(26)-diene-3 $\beta$ ,6 $\alpha$ -diol [**10**]. The nmr data of the olefinic protons at C-26 and the C-27 methyl protons are in agreement with those reported (30) for a synthetic sterol having the same side chain.

The remaining novel sterol **11** had the molecular formula  $\text{C}_{27}\text{H}_{42}\text{O}_2$  deduced by hrms. The mass spectrum contained the molecular ion at  $m/z$  398. The presence of one degree of unsaturation in the side chain and two in the nucleus was indicated by the ions at  $m/z$  287 [ $\text{M} - \text{C}_8\text{H}_{15}$ ] $^+$ , 269 [ $\text{M} - \text{H}_2\text{O} - \text{C}_8\text{H}_{15}$ ] $^+$ , 251 [ $\text{M} - 2\text{H}_2\text{O} - \text{C}_8\text{H}_{15}$ ] $^+$ , and 209. The  $^1\text{H}$ -nmr spectrum of **11** contained the C-18 and C-19 methyl resonances at  $\delta$  0.54 and 0.95, respectively, and two olefinic signals at  $\delta$  5.54 (1H, bd, 6.6 Hz, H-11) and 5.35 (1H, bs, H-7), characteristic of two conjugated double bonds. This was confirmed by its uv spectrum that revealed maxima at 235, 242, and 250 nm typical of  $\Delta^{7,9(11)}$  sterols (31). The chemical shifts of the H-22 and H-23 olefinic protons and the doublets for the C-21, C-26, and C-27 methyl group protons were exactly the same as those of compound **5**. The slight downfield shift of the C-19 methyl signal, when compared to the corresponding signal in the sterols **1**–**10**, is in agreement with that expected for a  $\Delta^{7,9(11)}$  sterol (32). The signal of the 6-H proton suffers a slight downshift as well. Thus, the structure of this dihydroxylated sterol must be (22*E*)-5 $\alpha$ -cholesta-7,9(11),22-triene-3 $\beta$ -6 $\alpha$ -diol [**11**].

**3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -TRIHYDROXYSTEROLS.**—In the eims of 3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -trihydroxysterols **12**–**26** the molecular ion peak was absent, the highest peak being that at the  $m/z$  where

there was a loss of  $\text{H}_2\text{O}$  from the molecular ion. Compounds **12–20** were shown to be the previously described  $3\beta, 5\alpha, 6\beta$ -trihydroxysterols (11, 18).

The new trihydroxysterols **21** and **22** could not be separated by repeated reversed-phase hplc. They had molecular formula  $\text{C}_{28}\text{H}_{48}\text{O}_3$  established by hrms on the ion at  $m/z$  414  $[\text{M} - \text{H}_2\text{O}]^+$ . In their mass spectrum the ions at  $m/z$  287  $[\text{M} - \text{H}_2\text{O} - \text{C}_9\text{H}_{19}]^+$ , 269  $[\text{M} - 2\text{H}_2\text{O} - \text{C}_9\text{H}_{19}]^+$ , and 251  $[\text{M} - 3\text{H}_2\text{O} - \text{C}_9\text{H}_{19}]^+$  indicated the presence of a saturated  $\text{C}_9\text{H}_{19}$  side chain. The  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr spectra were composed of signals from the two epimers at C-24 [**21** and **22**]. The chemical shifts of the side chain carbons and methyl protons for both epimers are consistent with those of authentic samples of campesterol and 24-*epi*-campesterol, respectively. In fact, it is well established that structural changes in the ring system of sterols that are remote from the C-24 chiral center have little effect on the chemical shift of the side chain resonances, in both the  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr spectra (27, 33). A considerable difference in the chemical shift of one of the doublets of the isopropyl group was observed in the  $^1\text{H}$ -nmr spectrum for the epimeric pair **21** and **22** (27). Diagnostic differences are also noted in the chemical shift of the C-17, C-20, C-21, C-23, C-24, C-26, C-27, and C-28 signals in the  $^{13}\text{C}$ -nmr spectrum (33). These signals appeared as pairs with components in the approximate ratio of 1:1. Thus, the structures of **21** and **22** were formulated as (24*S*)-24-methyl-5 $\alpha$ -cholest-7-ene-3 $\beta, 5, 6\beta$ -triol [**21**] and (24*R*)-24-methyl-5 $\alpha$ -cholest-7-ene-3 $\beta, 5, 6\beta$ -triol [**22**].

The new trihydroxysterols **23** and **24** could not be separated by reversed-phase hplc. They each had the molecular formula  $\text{C}_{29}\text{H}_{48}\text{O}_3$  by hrms on the highest mass peak at  $m/z$  426  $[\text{M} - \text{H}_2\text{O}]^+$ . The ions at  $m/z$  287  $[\text{M} - \text{H}_2\text{O} - \text{C}_{10}\text{H}_{19}]^+$ , 269, and 251 established the presence of a  $\text{C}_{10}\text{H}_{19}$  side chain containing one double bond.  $^1\text{H}$ -nmr spin decoupling experiments and the  $^1\text{H}$ - $^1\text{H}$  COSY-45 spectrum delineated the connectivities among protons belonging to the segment from C-21 to C-24. Particularly, the proton at  $\delta$  5.17 (H-22) showed correlation with the protons at  $\delta$  5.04 (H-23) and 2.07 (H-20); the latter was, in turn, coupled with the methyl signal at  $\delta$  1.04 (H<sub>3</sub>-21). The signal at  $\delta$  5.04 was also coupled with a multiplet at  $\delta$  1.55 (H-24); furthermore, irradiation at  $\delta$  1.56 (H-25) collapsed two methyl doublets at  $\delta$  0.849 and 0.805, thus supporting the presence of an isopropyl group. The comparison of the methyl region of the  $^1\text{H}$ -nmr spectrum of the natural mixture with that of a 1:1 mixture of stigmasterol and poriferasterol revealed the presence of the 24*S* epimer **23** in addition to the 24*R* epimer. Two pairs of triplets at  $\delta$  0.807 and 0.818 were assigned to the C-29 methyl resonances of the 24*S* and 24*R* epimers, respectively. This was confirmed by the  $^{13}\text{C}$ -nmr spectrum which showed, as expected, that the largest chemical shift difference between the epimers is due to the carbons C-16, C-26, C-27, and C-29 (33). The relative intensities of these carbon signals established that the two epimers are present in the approximate ratio of 1:1.

The new trihydroxysterols **25** and **26** could not be separated by reversed-phase hplc. They each had the molecular formula  $\text{C}_{29}\text{H}_{48}\text{O}_3$ . The fragment ions at  $m/z$  287, 269, and 251 indicated the presence of a  $\text{C}_{10}\text{H}_{19}$  side chain containing a double bond, and the ion at  $m/z$  310  $[\text{M} - 2\text{H}_2\text{O} - \text{C}_7\text{H}_{14}]^+$  derived from a McLafferty rearrangement in a  $\Delta^{24(28)}$ -unsaturated side chain.  $^1\text{H}$ -nmr decoupling experiments confirmed the presence of ethylidene groups attached at C-24. The side chain chemical shifts for both epimers are consistent with those of authentic samples of fucosterol and 28-isofucosterol. Thus the structures of **25** and **26** were formulated as (24*Z*)-24-ethyl-5 $\alpha$ -cholesta-7,24(28)-diene-3 $\beta, 5, 6\beta$ -triol [**25**] and (24*E*)-24-ethyl-5 $\alpha$ -cholesta-7,24(28)-diene-3 $\beta, 5, 6\beta$ -triol [**26**]. The stereochemistry of the 24(28) double bond was based on the chemical shift of the H-25 protons which were characterized by a septet centered at  $\delta$  2.83 in **25** (*Z*-isomer) and at  $\delta$  2.20 in **26** (*E*-isomer) as in 28-isofucosterol and fucosterol, respec-

tively (34). The epimers **25** and **26** are produced by the sponges *H. communis* and *S. officinalis*, whereas *I. variabilis* contains only the epimer **26**.

The isolation of  $\Delta^7$ -polyhydroxylated sterols in four Dictyoceratid sponges that contain mainly  $\Delta^{5,7}$ -sterols strongly supports the hypothesis outlined earlier: that  $3\beta,6\alpha$ -dihydroxy- $\Delta^7$ -sterols (**17**) and  $3\beta,5\alpha,6\beta$ -trihydroxy- $\Delta^7$ -sterols (**18**) are formed from the corresponding  $\Delta^{5,7}$ - $3\beta$ -hydroxysterols present in the same organism. The co-occurrence in the sponge *H. communis* of  $\Delta^{5,7}$ - $3\beta$ -hydroxysterols,  $3\beta,5\alpha,6\beta$ -trihydroxylated sterols, and a mixture of 5,6-secoosterols from which we recently isolated (Z)-5,6-secocholest-7-ene- $3\beta,5\beta,6$ -triol (**20**) suggests that the sponge contains the enzyme system capable of cleaving the C-5–C-6 bond in the corresponding trihydroxylated sterols. These findings substantiate the utility of searching for new polyhydroxylated sterols whose structure could throw light on the biogenetic pathways involved in the formation of  $\Delta^7$ -polyhydroxylated sterols.

## EXPERIMENTAL

**GENERAL EXPERIMENTAL PROCEDURES.**— $^1\text{H}$ -nmr spectra were recorded on a Bruker WM-270 spectrometer operating at 270 MHz or a Bruker WM-400 at 400 MHz.  $^{13}\text{C}$ -nmr spectra were recorded on a Bruker WM-400, operating at 100.1 MHz.  $^1\text{H}$ -nmr chemical shifts were referenced to  $\text{CDCl}_3$  and  $\text{C}_5\text{D}_5\text{N}$  (7.26 and 8.71 ppm, respectively);  $^{13}\text{C}$ -nmr chemical shifts were referenced to  $\text{C}_5\text{D}_5\text{N}$  (135.5 ppm). The DEPT experiments were performed using polarization transfer pulses of  $90^\circ$  and  $135^\circ$ . Standard pulse sequences were used for COSY (35). Low resolution mass spectra were determined at 70 eV on a Kratos MS 80 mass spectrometer. High resolution mass spectra were obtained on an AEI MS 902 spectrometer. Fourier transform ir spectra were recorded with a Perkin-Elmer 1760-X FT-IR. Uv spectra were taken on a Perkin-Elmer 550S spectrophotometer. Hplc was performed on a Varian 2010 apparatus equipped with a differential refractometer.

**EXTRACTION AND ISOLATION.**—*H. communis*, *S. officinalis*, *I. variabilis*, and *S. gracilis* were collected in the Bay of Naples and supplied by the Zoological Station of Naples. Voucher specimens are on file at our laboratories. The usual procedure of extraction and isolation is as follows.

The freshly collected animal was cut into pieces and extracted at room temperature with  $\text{Me}_2\text{CO}$  and  $\text{CHCl}_3$ -MeOH (1:1) for 3 days. The combined lipid extracts were concentrated under reduced pressure to obtain an aqueous suspension which was extracted with  $\text{Et}_2\text{O}$ . The organic extract was dried over  $\text{Na}_2\text{SO}_4$  and the solvent removed to obtain an oily residue that was chromatographed on a Si gel column eluted with solvents of increasing polarity from petroleum ether through  $\text{CHCl}_3$  and increasing amounts of MeOH in  $\text{CHCl}_3$ . The fractions eluted with  $\text{CHCl}_3$  contained monohydroxylated sterols. The fractions eluted with  $\text{CHCl}_3$ -MeOH (97:3), containing mixtures of di- and/or trihydroxysterols, were further separated by hplc on a Hibar LiChrosorb Si-60 (250  $\times$  10 mm) column eluted with  $\text{CHCl}_3$ -MeOH (93:7).

**Sterols from *S. gracilis*.**—Dihydroxylated sterols (39 mg) from 13 g of extract (the air-dried sponge residue weighed 72 g) were separated by reversed-phase hplc on Hibar Superspher RP-18 (250  $\times$  4 mm) column [MeOH- $\text{H}_2\text{O}$  (88:12)] to give sterols **1** (27.4%), **2** (29.8%), **3** (4.5%), **4** (4.3%), **5** (7.4%), **6** (2.7%), **7** (2.5%), **8** and **9** (3.4%), **10** (9.8%), and **11** (3.5%).

**Sterols from *H. communis*.**—The trihydroxylated sterol fraction (26.3 mg) from 9 g of extract (the air-dried sponge residue weighed 189 g) was fractionated by reversed-phase hplc on Hibar Superspher RP-18 (250  $\times$  4 mm) column [MeOH- $\text{H}_2\text{O}$  (85:15)] giving sterols **12** (28.5%), **13** (9.0%), **14** (3.5%), **15** (3.6%), **16** (19.5%), **17** (2.4%), **18** (20.8%), **19** and **20** (together 6.7%), **21** and **22** (together 6.6%), **23** and **24** (together 9.2%), and **25** and **26** (traces).

**Sterols from *S. officinalis*.**—The trihydroxylated sterol fraction (137 mg) from 42.9 g of extract (dry wt of extracted sponge 569 g) was subjected to reversed-phase hplc using the same conditions employed above to give sterols **12** (33.6%), **13** (8.1%), **14** (1.9%), **15** (4.1%), **16** (8.8%), **17** (3.7%), **18** (14.7%), **19** and **20** (together 10.1%), **21** and **22** (together 2.6%), **23** and **24** (together 6.4%), and **25** and **26** (together 4.5%).

**Sterols from *I. variabilis*.**—The trihydroxylated sterol fraction (18 mg) from 4.5 g of extract (the air-dried sponge residue weighed 90.5 g) was fractionated by reversed-phase hplc using the same conditions employed above, giving sterols **12** (28.2%), **13** (8.3%), **14** (0.3%), **15** (3.8%), **16** (10.7%), **17** (7.0%), **18** (14.6%), **19** and **20** (together 12.3%), **21** and **22** (together 2.7%), **23** and **24** (together 5.5%), and **26** (6.7%).

**Compound 3.**—Mp 188–190° [petroleum ether-MeOH (8:2)]; ir (CHCl<sub>3</sub>) 3420 cm<sup>-1</sup>; <sup>1</sup>H nmr (CDCl<sub>3</sub>, 270 MHz) δ 5.29 (1H, dd, *J* = 15.6 and 6.6 Hz, H-23), 5.18 (1H, d, *J* = 1.6 Hz, H-7), 5.16 (1H, dd, *J* = 15.6 and 8.2 Hz, H-22), 3.81 (1H, bd, *J* = 7.3 Hz, H-6β), 3.60 (1H, m, H-3α), 2.18 (1H, b septet, *J* = 6.6 Hz, H-24), 1.99 (1H, m, submerged by other signals, H-20), 1.00 (3H, d, *J* = 6.6 Hz, H<sub>3</sub>-21), 0.94 (6H, d, *J* = 6.6 Hz, H<sub>3</sub>-25 and H<sub>3</sub>-26), 0.85 (3H, s, H<sub>3</sub>-19), 0.55 (3H, s, H<sub>3</sub>-18); eims *m/z* (rel. int.) [M]<sup>+</sup> 386 (78), [M - H<sub>2</sub>O]<sup>+</sup> 368 (36), [M - H<sub>2</sub>O - Me]<sup>+</sup> 353 (18), [M - 2H<sub>2</sub>O - Me]<sup>+</sup> 335 (60), [M - 2H<sub>2</sub>O and loss of C-1 to C-3]<sup>+</sup> 309 (21), [M - side chain]<sup>+</sup> 289 (16), [M - H<sub>2</sub>O - side chain]<sup>+</sup> 271 (97), [M - 2H<sub>2</sub>O - side chain]<sup>+</sup> 253 (100), 229 (52), 227 (38), [M - 2H<sub>2</sub>O and ring D fission]<sup>+</sup> 211 (60); hrms *m/z* 386.3165 (C<sub>26</sub>H<sub>42</sub>O<sub>2</sub> requires 386.3174).

**Compound 4.**—Mp 185–187° [petroleum ether-MeOH (8:2)]; ir (CHCl<sub>3</sub>) 3420 cm<sup>-1</sup>; <sup>1</sup>H nmr (CDCl<sub>3</sub>, 270 MHz) δ 5.17 (1H, d, *J* = 1.6 Hz, H-7), 5.15 (2H, m, H-22 and H-23), 3.80 (1H, bd, *J* = 7.3 Hz, H-6β), 3.59 (1H, m, H-3α), 1.99 (1H, m, submerged by other signals, H-20), 1.92 (1H, m, submerged by other signals, H-24), 1.010 (3H, d, *J* = 6.6 Hz, H<sub>3</sub>-21), 0.93 (3H, d, *J* = 6.6 Hz, H<sub>3</sub>-28), 0.845 (3H, s, H<sub>3</sub>-19), 0.836 (3H, t, *J* = 7.4 Hz, H<sub>3</sub>-26), 0.55 (3H, s, H<sub>3</sub>-18); eims *m/z* (rel. int.) [M]<sup>+</sup> 400 (100), [M - H<sub>2</sub>O]<sup>+</sup> 382 (33), [M - H<sub>2</sub>O - Me]<sup>+</sup> 367 (13), [M - 2H<sub>2</sub>O - Me]<sup>+</sup> 349 (40), [M - 2H<sub>2</sub>O and loss of C-1 to C-3]<sup>+</sup> 323 (11), [M - side chain]<sup>+</sup> 289 (15), [M - H<sub>2</sub>O - side chain]<sup>+</sup> 271 (75), [M - 2H<sub>2</sub>O - side chain]<sup>+</sup> 253 (62), 229 (28), 227 (22), [M - 2H<sub>2</sub>O and ring D fission]<sup>+</sup> 211 (30); hrms *m/z* 400.3324 (C<sub>27</sub>H<sub>44</sub>O<sub>2</sub> requires 400.3330).

**Compound 5.**—Mp 172–174° (petroleum ether-MeOH (8:2)); ir (CHCl<sub>3</sub>) 3420 cm<sup>-1</sup>; <sup>1</sup>H nmr (CDCl<sub>3</sub>, 270 MHz) δ 5.31 (1H, dd, *J* = 15.3 and 6.6 Hz, H-22), 5.20 (1H, m, overlapped to the H-7 signal, H-23), 5.18 (1H, d, *J* = 1.6 Hz, H-7), 3.80 (1H, bd, *J* = 7.3 Hz, H-6β), 3.59 (1H, m, H-3α), 2.03 (1H, m, H-20), 1.01 (3H, d, *J* = 6.6 Hz, H<sub>3</sub>-21), 0.86 (6H, d, *J* = 6.6 Hz, H<sub>3</sub>-26 and H<sub>3</sub>-27), 0.84 (3H, s, H<sub>3</sub>-19), 0.55 (3H, s, H<sub>3</sub>-18); eims *m/z* (rel. int.) [M]<sup>+</sup> 400 (100), [M - H<sub>2</sub>O]<sup>+</sup> 382 (32), [M - H<sub>2</sub>O - Me]<sup>+</sup> 367 (11), [M - 2H<sub>2</sub>O - Me]<sup>+</sup> 349 (37), [M - 2H<sub>2</sub>O and loss of C-1 to C-3]<sup>+</sup> 323 (11), [M - side chain]<sup>+</sup> 289 (14), [M - H<sub>2</sub>O - side chain]<sup>+</sup> 271 (75), [M - 2H<sub>2</sub>O - side chain]<sup>+</sup> 253 (60), 229 (29), 227 (22), [M - 2H<sub>2</sub>O and ring D fission]<sup>+</sup> 211 (29); hrms *m/z* 400.3335 (C<sub>27</sub>H<sub>44</sub>O<sub>2</sub> requires 400.3330).

**Compound 6.**—Mp 206–208° [petroleum ether-MeOH (8:2)]; ir (CHCl<sub>3</sub>) 3420 cm<sup>-1</sup>; <sup>1</sup>H nmr (CDCl<sub>3</sub>, 270 MHz) δ 5.23 (1H, dd, *J* = 14.6 and 6.6 Hz, H-23), 5.19 (1H, d, *J* = 1.6 Hz, H-7), 5.14 (1H, dd, *J* = 14.6 and 7.7 Hz, H-22), 3.80 (1H, bd, *J* = 7.3 Hz, H-6β), 3.61 (1H, m, H-3α), 1.018 (3H, d, *J* = 6.6 Hz, H<sub>3</sub>-21), 0.915 (3H, d, *J* = 6.6 Hz, H<sub>3</sub>-28), 0.85 (3H, s, H<sub>3</sub>-19), 0.838 (3H, d, *J* = 6.6 Hz, H<sub>3</sub>-26 or H<sub>3</sub>-27), 0.823 (3H, d, *J* = 6.6 Hz, H<sub>3</sub>-27 or H<sub>3</sub>-26), 0.56 (3H, s, H<sub>3</sub>-18); eims *m/z* (rel. int.) [M]<sup>+</sup> 414 (81), [M - H<sub>2</sub>O - Me]<sup>+</sup> 396 (12), [M - 2H<sub>2</sub>O - Me]<sup>+</sup> 363 (48), [M - 2H<sub>2</sub>O and loss of C-1 to C-3]<sup>+</sup> 337 (14), [M - side chain]<sup>+</sup> 289 (12), [M - H<sub>2</sub>O - side chain]<sup>+</sup> 271 (100), [M - 2H<sub>2</sub>O - side chain]<sup>+</sup> 253 (82), 229 (37), 227 (33), [M - 2H<sub>2</sub>O and ring D fission]<sup>+</sup> 211 (44); hrms *m/z* 414.3474 (C<sub>28</sub>H<sub>46</sub>O<sub>2</sub> requires 414.3486).

**Compound 7.**—Mp 196–198° [petroleum ether-MeOH (8:2)]; ir (CHCl<sub>3</sub>) 3420 cm<sup>-1</sup>; <sup>1</sup>H nmr (CDCl<sub>3</sub>, 270 MHz) δ 5.18 (1H, d, *J* = 1.6 Hz, H-7), 5.17 (2H, m, H-22 and H-23), 3.81 (1H, bd, *J* = 7.3 Hz, H-6β), 3.60 (1H, m, H-3α), 1.008 (3H, d, *J* = 6.6 Hz, H<sub>3</sub>-21), 0.912 (3H, d, *J* = 6.6 Hz, H<sub>3</sub>-28), 0.85 (3H, s, H<sub>3</sub>-19), 0.842 (3H, d, *J* = 6.6 Hz, H<sub>3</sub>-26 or H<sub>3</sub>-27), 0.823 (3H, d, *J* = 6.6 Hz, H<sub>3</sub>-27 or H<sub>3</sub>-26), 0.56 (3H, s, H<sub>3</sub>-18); eims *m/z* (rel. int.) [M]<sup>+</sup> 414 (40), [M - H<sub>2</sub>O]<sup>+</sup> 396 (43), [M - H<sub>2</sub>O - Me]<sup>+</sup> 381 (11), [M - 2H<sub>2</sub>O - Me]<sup>+</sup> 363 (83), [M - 2H<sub>2</sub>O and loss of C-1 to C-3]<sup>+</sup> 337 (25), [M - side chain]<sup>+</sup> 289 (14), [M - H<sub>2</sub>O - side chain]<sup>+</sup> 271 (92), [M - 2H<sub>2</sub>O - side chain]<sup>+</sup> 253 (100), 229 (35), 227 (40), [M - 2H<sub>2</sub>O and ring D fission]<sup>+</sup> 211 (60); hrms *m/z* 414.3470 (C<sub>28</sub>H<sub>46</sub>O<sub>2</sub> requires 414.3486).

**Compounds 8 and 9.**—Ir (CHCl<sub>3</sub>) 3420 cm<sup>-1</sup>; <sup>1</sup>H nmr (CDCl<sub>3</sub>, 270 MHz) δ 5.18 (1H, d, *J* = 1.6 Hz, H-7), 3.81 (1H, bd, *J* = 7.3 Hz, H-6β), 3.60 (1H, m, H-3α), 0.93 (3H, d, *J* = 6.2 Hz, H<sub>3</sub>-21), 0.852 (3H, t, *J* = 7.3 Hz, H<sub>3</sub>-29 of **8**), 0.85 (3H, s, H<sub>3</sub>-19), 0.845 (3H, t, *J* = 7.3 Hz, H<sub>3</sub>-29 of **9**), 0.835 (3H, d, *J* = 6.2 Hz, H<sub>3</sub>-26 or H<sub>3</sub>-27), 0.813 (3H, d, *J* = 6.2 Hz, H<sub>3</sub>-27 or H<sub>3</sub>-26), 0.54 (3H, s, H<sub>3</sub>-18); eims *m/z* (rel. int.) [M]<sup>+</sup> 430 (36), [M - H<sub>2</sub>O]<sup>+</sup> 412 (39), [M - H<sub>2</sub>O - Me]<sup>+</sup> 397 (30), [M - 2H<sub>2</sub>O - Me]<sup>+</sup> 379 (100), [M - 2H<sub>2</sub>O and loss of C-1 to C-3]<sup>+</sup> 353 (35), [M - side chain]<sup>+</sup> 289 (9), [M - H<sub>2</sub>O - side chain]<sup>+</sup> 271 (98), [M - 2H<sub>2</sub>O - side chain]<sup>+</sup> 253 (62), 229 (43), 227 (46), [M - 2H<sub>2</sub>O and ring D fission]<sup>+</sup> 211 (62); hrms *m/z* 430.3788 (C<sub>29</sub>H<sub>50</sub>O<sub>2</sub> requires 430.3798).

**Compound 10.**—Ir (CHCl<sub>3</sub>) 3420 and 1720 cm<sup>-1</sup>; <sup>1</sup>H nmr (CDCl<sub>3</sub>, 400 MHz) δ 5.95 (1H, bs, H<sub>a</sub>-26), 5.75 (1H, m, H<sub>b</sub>-26), 5.19 (1H, bd, *J* = 1.6 Hz, H-7), 3.81 (1H, bd, *J* = 7.3 Hz, H-6β), 3.59 (1H, m, H-3α), 2.72 (1H, ddd, *J* = 15.6, 10.2 and 5.4 Hz, H<sub>a</sub>-23), 2.62 (1H, ddd, *J* = 15.6, 8.8, and 5.4 Hz, H<sub>b</sub>-23), 1.87 (3H, dd, *J* = 1.2 and 1.2 Hz, H<sub>3</sub>-27), 0.94 (3H, d, *J* = 6.3 Hz, H<sub>3</sub>-21), 0.84 (3H, s, H<sub>3</sub>-19), 0.55 (3H, s, H<sub>3</sub>-18); eims *m/z* (rel. int.) [M]<sup>+</sup> 414 (19), [M - Me]<sup>+</sup> 396 (34), [M - H<sub>2</sub>O - Me]<sup>+</sup> 381 (30), [M - 2H<sub>2</sub>O - Me]<sup>+</sup> 363 (67), [M - 2H<sub>2</sub>O and loss of C-1 to C-3]<sup>+</sup> 337 (25), [M - side chain - 2H]<sup>+</sup> 287 (38), [M - H<sub>2</sub>O - side chain]<sup>+</sup> 271 (100), [M - 2H<sub>2</sub>O - side chain]<sup>+</sup>



253 (74), 229 (68), 227 (57),  $[M - 2H_2O \text{ and ring D fission}]^+ 211$  (87); hrms  $m/z$  414.3114 ( $C_{27}H_{42}O_3$  requires 414.3123).

**Compound 11.**—Mp 184–186° [petroleum ether-MeOH (8:2)]; ir (CHCl<sub>3</sub>) 3460 cm<sup>-1</sup>; <sup>1</sup>H nmr (CDCl<sub>3</sub>; 270 MHz)  $\delta$  5.54 (1H, bd,  $J = 6.6$  Hz, H-11), 5.35 (1H, bs, H-7), 5.33 (1H, dd, overlapped to the 7-H signal,  $J = 15.0$  and  $6.3$  Hz, H-22), 5.21 (1H, m, H-23), 3.90 (1H, bd,  $J = 7.3$  Hz, H-6 $\beta$ ), 3.59 (1H, m, H-3 $\alpha$ ), 1.01 (3H, d,  $J = 6.3$  Hz, H<sub>3</sub>-21), 0.95 (3H, s, H<sub>3</sub>-19), 0.87 (6H, d,  $J = 6.6$  Hz, H<sub>3</sub>-26 and H<sub>3</sub>-27), 0.54 (3H, s, H<sub>3</sub>-18); eims  $m/z$  (rel. int.)  $[M]^+ 398$  (28),  $[M - H_2O]^+ 380$  (10),  $[M - H_2O - Me]^+ 365$  (100),  $[M - 2H_2O - Me]^+ 347$  (9),  $[M - H_2O - \text{side chain}]^+ 269$  (28),  $[M - 2H_2O - \text{side chain}]^+ 251$  (46), 227 (26), 225 (17),  $[M - 2H_2O \text{ and ring D fission}]^+ 209$  (26); hrms  $m/z$  398.3168 ( $C_{27}H_{42}O_2$  requires 398.3174).

**Compounds 21 and 22.**—Ir (CHCl<sub>3</sub>) 3460 cm<sup>-1</sup>; <sup>1</sup>H nmr (CDCl<sub>3</sub>, 270 MHz)  $\delta$  5.35 (bd,  $J = 4.9$  Hz, H-7), 4.07 (m, H-3 $\alpha$ ), 3.63 (bs, H-6 $\alpha$ ), 2.14 (dd,  $J = 12.8$  and  $12.8$  Hz, H<sub>xx</sub>-4), 1.08 (s, H<sub>3</sub>-19), 0.931 (d,  $J = 6.6$  Hz, H<sub>3</sub>-21 of **21**), 0.925 (d,  $J = 6.6$  Hz, H<sub>3</sub>-21 of **22**), 0.857 (d,  $J = 6.6$  Hz, H<sub>3</sub>-26 or H<sub>3</sub>-27 of **21**), 0.853 (d,  $J = 6.6$  Hz, H<sub>3</sub>-26 or H<sub>3</sub>-27 of **22**), 0.805 (d,  $J = 6.6$  Hz, H<sub>3</sub>-27 or H<sub>3</sub>-26 of **22**), 0.784 (d,  $J = 6.6$  Hz, H<sub>3</sub>-27 or H<sub>3</sub>-26 of **21**), 0.778 (d,  $J = 6.6$  Hz, H<sub>3</sub>-28), 0.59 (s, H<sub>3</sub>-18); <sup>1</sup>H nmr (pyridine-*d*<sub>5</sub>, 400 MHz)  $\delta$  5.74 (bd,  $J = 5.5$  Hz, H-7), 4.83 (m, H-3 $\alpha$ ), 4.31 (bd,  $J = 5.5$  Hz, H-6 $\alpha$ ), 3.02 (dd,  $J = 12.2$  and  $12.2$  Hz, H<sub>xx</sub>-4), 1.53 (s, H<sub>3</sub>-19), 1.06 (d,  $J = 6.6$  Hz, H<sub>3</sub>-21), 0.67 (s, H<sub>3</sub>-18); <sup>13</sup>C nmr (pyridine-*d*<sub>5</sub>, 100.1 MHz)  $\delta$  141.6 (C-8), 120.5 (C-7), 76.2 (C-5), 74.3 (C-6), 67.6 (C-3), 56.5 (C-17 of **22**), 56.4 (C-17 of **21**), 55.2 (C-14), 43.8 (C-9 and C-13), 42.0 (C-4), 40.0 (C-12), 39.4 (C-24 of **21**), 39.1 (C-24 of **22**), 38.1 (C-10), 37.0 (C-20 of **21**), 36.7 (C-20 of **22**), 34.9 (C-22), 33.8 (C-2), 32.6 (C-1 and C-25 of **22**), 31.7 (C-25 of **21**), 31.2 (C-23 of **21**), 30.8 (C-23 of **22**), 28.2 (C-16), 23.5 (C-15), 22.4 (C-11), 20.7 and 17.7 (C-26 and C-27 of **21**), 20.4 (C-27 of **22**) 19.3 (C-21 of **21**), 19.1 (C-21 of **22**), 18.8 (C-19), 18.4 (C-26 of **22**), 15.7 (C-28 of **21**), 15.6 (C-28 of **22**) and 12.2 (C-18); multiplicities were assigned on the basis of DEPT experiments; eims,  $m/z$  (rel. int.)  $[M - H_2O]^+ 414$  (98),  $[M - H_2O - Me]^+ 399$  (80),  $[M - 2H_2O]^+ 396$  (92),  $[M - H_2O - Me]^+ 381$  (92),  $[M - H_2O - \text{side chain}]^+ 287$  (37),  $[M - 2H_2O - \text{side chain}]^+ 269$  (78),  $[M - 3H_2O - \text{side chain}]^+ 251$  (100); hrms  $m/z$  414.3495 ( $C_{28}H_{46}O_2$  requires 414.3486).

**Compounds 23 and 24.**—Ir (CHCl<sub>3</sub>) 3460 cm<sup>-1</sup>; <sup>1</sup>H nmr (CDCl<sub>3</sub>, 270 MHz)  $\delta$  5.35 (bd,  $J = 4.9$  Hz, H-7), 5.17 (dd,  $J = 14.8$  and  $7.8$  Hz, H-22), 5.04 (dd,  $J = 14.8$  and  $7.8$  Hz, H-23), 4.08 (m, H-3 $\alpha$ ), 3.63 (bs, H-6 $\alpha$ ), 2.14 (dd,  $J = 12.8$  and  $12.8$  Hz, H<sub>xx</sub>-4), 2.07 (m, submerged by other signals, H-20), 1.08 (s, H<sub>3</sub>-19), 1.04 (d,  $J = 6.6$  Hz, H<sub>3</sub>-21), 0.849 (d,  $J = 6.6$  Hz, H<sub>3</sub>-26), 0.818 (t,  $J = 7.0$  Hz, H<sub>3</sub>-29 of **24**), 0.807 (t,  $J = 7.0$  Hz, H<sub>3</sub>-29 of **23**), 0.805 (d,  $J = 6.6$  Hz, H<sub>3</sub>-27), 0.60 (s, H<sub>3</sub>-18); <sup>1</sup>H nmr (pyridine-*d*<sub>5</sub>, 400 MHz)  $\delta$  5.74 (bd,  $J = 5.5$  Hz, H-7), 4.83 (m, H-3 $\alpha$ ), 4.31 (bd,  $J = 5.5$  Hz, H-6 $\alpha$ ), 3.02 (dd,  $J = 12.2$  and  $12.2$  Hz, H<sub>xx</sub>-4), 1.53 (s, H<sub>3</sub>-19), 1.06 (d,  $J = 6.6$  Hz, H<sub>3</sub>-21), 0.67 (s, H<sub>3</sub>-18); <sup>13</sup>C nmr (pyridine-*d*<sub>5</sub>, 100.1 MHz)  $\delta$  141.6 (C-8), 138.6 (C-22), 129.7 (C-23), 120.5 (C-7), 76.2 (C-5), 74.3 (C-6), 67.6 (C-3), 56.1 (C-17), 55.3 (C-14), 51.5 (C-24), 43.8 (C-13 and C-9), 42.0 (C-4), 41.1 (C-20), 39.9 (C-12), 38.1 (C-10), 33.8 (C-2), 32.6 (C-1), 31.2 (C-25), 28.84 (C-16 of **23**), 28.77 (C-16 of **24**), 25.7 (C-28), 23.6 (C-15), 22.4 (C-11), 21.7 (C-21), 21.29 (C-26 of **23**), 21.14 (C-27 of **24**), 19.26 (C-27 of **23**), 19.17 (C-26 of **24**), 18.8 (C-19), 12.66 (C-29 of **24**), 12.52 (C-18), and 12.49 (C-29 of **23**); eims  $m/z$  (rel. int.)  $[M - H_2O]^+ 426$  (71),  $[M - H_2O - Me]^+ 411$  (24),  $[M - 2H_2O]^+ 408$  (71),  $[M - 2H_2O - Me]^+ 393$  (90),  $[M - H_2O - \text{side chain}]^+ 287$  (24),  $[M - 2H_2O - \text{side chain}]^+ 269$  (90),  $[M - 3H_2O - \text{side chain}]^+ 251$  (100); hrms  $m/z$  426.3498 ( $C_{29}H_{46}O_2$  requires 426.3486).

**Compounds 25 and 26.**—Ir (CHCl<sub>3</sub>) 3460 cm<sup>-1</sup>; <sup>1</sup>H nmr (CDCl<sub>3</sub>, 400 MHz)  $\delta$  5.35 (d,  $J = 4.9$  Hz, H-7), 5.19 (q,  $J = 6.8$  Hz, H-28 of **26**), 5.11 (q,  $J = 6.8$  Hz, H-28 of **25**), 4.08 (m, H-3 $\alpha$ ), 3.63 (bs, H-6 $\alpha$ ), 2.83 (septet,  $J = 6.8$  Hz, H-25 of **25**), 2.20 (septet,  $J = 6.8$  Hz, H-25 of **26**), 2.14 (dd,  $J = 12.8$  and  $12.8$  Hz, H<sub>xx</sub>-4), 1.59 (d,  $J = 6.8$  Hz, H<sub>3</sub>-29 of **25**), 1.57 (d,  $J = 6.8$  Hz, H<sub>3</sub>-29 of **26**), 1.08 (s, H<sub>3</sub>-19), 1.00 (d,  $J = 6.6$  Hz, H<sub>3</sub>-21 of **26**), 0.984 (d,  $J = 6.8$  Hz, H<sub>3</sub>-26 and H<sub>3</sub>-27 of **25**), 0.980 (d,  $J = 6.8$  Hz, H<sub>3</sub>-26 and H<sub>3</sub>-27 of **26**), 0.960 (d,  $J = 6.6$  Hz, H<sub>3</sub>-21 of **25**), 0.601 (s, H<sub>3</sub>-18 of **26**), 0.598 (s, H<sub>3</sub>-18 of **25**). **Compound 26:** <sup>1</sup>H nmr (pyridine-*d*<sub>5</sub>, 400 MHz)  $\delta$  5.73 (1H, bd,  $J = 5.5$  Hz, H-7), 5.25 (1H, q,  $J = 6.7$  Hz, H-28), 4.83 (1H, m, H-3 $\alpha$ ), 4.33 (1H, bd,  $J = 5.5$  Hz, H-6 $\alpha$ ), 3.03 (1H, dd,  $J = 12.2$  and  $12.2$  Hz, H<sub>xx</sub>-4), 1.62 (3H, d,  $J = 6.7$  Hz, H<sub>3</sub>-29), 1.53 (3H, s, H<sub>3</sub>-19), 1.030 (6H, d,  $J = 6.7$  Hz, H<sub>3</sub>-26 and H<sub>3</sub>-27), 1.016 (3H, d,  $J = 6.7$  Hz, H<sub>3</sub>-21), 0.64 (3H, s, H<sub>3</sub>-18); eims  $m/z$  (rel. int.)  $[M - H_2O]^+ 426$  (53),  $[M - H_2O - Me]^+ 411$  (33),  $[M - 2H_2O]^+ 408$  (53),  $[M - 2H_2O - Me]^+ 393$  (83),  $[M - 2H_2O - C_7H_{14}]^+ 310$  (33),  $[M - 2H_2O - C_7H_{14} - Me]^+ 295$  (27),  $[M - H_2O - \text{side chain}]^+ 287$  (17),  $[M - 2H_2O - \text{side chain}]^+ 269$  (43),  $[M - 2H_2O - \text{side chain}]^+ 251$  (100); hrms  $m/z$  426.3482 ( $C_{29}H_{46}O_2$  requires 426.3486).

**SYNTHESIS OF 6 AND ITS 6 $\beta$ -EPIMER.**—3 $\beta$ -Acetoxy-5 $\alpha$ -hydroxyergosta-7,22-dien-6-one (**28**) treated with Zn dust in HOAc for 1 h yielded 3 $\beta$ -acetoxy-5 $\alpha$ -ergosta-7,22-dien-6-one and 3 $\beta$ -acetoxy-5 $\beta$ -ergosta-7,22-dien-6-one, which were separated by hplc on a Hibar LiChrosorb Si-60 (250  $\times$  4 mm)

using *n*-hexane-EtOAc (95:5) as eluent. All spectral data were in agreement with the literature values (28,36). Reduction of the 5 $\alpha$ -epimer with LiAlH<sub>4</sub> at room temperature for 15 min afforded a 3:1 mixture of the 6 $\alpha$ - and 6 $\beta$ -epimers of ergosta-7,22-diene-3 $\beta$ ,6-diol, which were separated by hplc on a Hibar Li-Chrosorb Si-60 (250  $\times$  4 mm) column using CHCl<sub>3</sub>-MeOH (95:5) as eluent. The 6 $\alpha$ -epimer was identical in all respect to the natural product **6**. The 6 $\beta$ -epimer showed the following spectral features: Mp 119–120° [petroleum ether-MeOH (8:2)]; [ $\alpha$ ]<sub>D</sub> –120 (*c* = 0.1, CHCl<sub>3</sub>); <sup>1</sup>H nmr (CDCl<sub>3</sub>)  $\delta$  5.44 (1H, bd, *J* = 5.1 Hz, H-7), 5.23 (1H, dd, *J* = 15.3 and 7.3 Hz, H-23), 5.15 (1H, dd, *J* = 15.3 and 7.3 Hz, H-22), 3.91 (1H, bs, H-6 $\alpha$ ), 3.67 (1H, m, H-3 $\alpha$ ), 1.02 (3H, d, *J* = 6.7 Hz, H<sub>3</sub>-21), 0.98 (3H, s, H<sub>3</sub>-19), 0.91 (3H, d, *J* = 6.7 Hz, H<sub>3</sub>-28), 0.83 (3H, d, *J* = 6.7 Hz, H<sub>3</sub>-26 or H<sub>3</sub>-27), 0.82 (3H, d, *J* = 6.7 Hz, H<sub>3</sub>-27 or H<sub>3</sub>-26), 0.59 (3H, s, H<sub>3</sub>-18).

#### ACKNOWLEDGMENTS

We thank Stazione Zoologica di Napoli for supplying the sponges. This work was supported by Ministero della Pubblica Istruzione, Italy. Mass spectral data were provided by Servizio di Spettrometria di Massa del CNR e dell'Università di Napoli. The assistance of the staff is gratefully acknowledged.

#### LITERATURE CITED

1. F.J. Schmitz, in: "Marine Natural Products: Chemical and Biological Perspectives." Ed. by P. Scheuer, Academic Press, New York, 1978, Vol. I, p. 241.
2. D.J. Faulkner, *Nat. Prod. Rep.*, **1**, 551 (1984).
3. D.J. Faulkner, *Nat. Prod. Rep.*, **3**, 1 (1986).
4. D.J. Faulkner, *Nat. Prod. Rep.*, **4**, 539 (1987).
5. D.J. Faulkner, *Nat. Prod. Rep.*, **5**, 613 (1988).
6. C.B. Rao, K.V. Ramana, D.V. Rao, E. Fahy, and D.J. Faulkner, *J. Nat. Prod.*, **51**, 954 (1988).
7. M.B. Ksebati and F.J. Schmitz, *Steroids*, **43**, 639 (1984).
8. B.M. Jagodzinska, J.S. Trimmer, W. Fenical, and C. Djerassi, *J. Org. Chem.*, **50**, 2988 (1985).
9. R. Riccio, O. Squillace Greco, L. Minale, D. Laurent, and D. Duhet, *J. Chem. Soc., Perkin Trans. 1*, 665 (1986).
10. E. Fattorusso, V. Lanzotti, S. Magno, and E. Novellino, *J. Org. Chem.*, **50**, 2868 (1985).
11. F. Cafieri, E. Fattorusso, M. Gavagnin, and C. Santacroce, *J. Nat. Prod.*, **48**, 944 (1985).
12. R. Manconi, V. Piccialli, R. Pronzato, and D. Sica, *Comp. Biochem. Physiol.*, **91B**, 237 (1988).
13. N. Fusetani, S. Matsunaga, and S. Konosu, *Tetrahedron Lett.*, **22**, 1985 (1981).
14. S.P. Gunasekera and F.J. Schmitz, *J. Org. Chem.*, **48**, 885 (1983).
15. T.N. Makarieva, L.K. Shubina, A.I. Kalinovskiy, V.A. Stonik, and G.B. Elyakov, *Steroids*, **42**, 267 (1983).
16. R.J. Capon and D.J. Faulkner, *J. Org. Chem.*, **50**, 4771 (1985).
17. V. Piccialli and D. Sica, *J. Nat. Prod.*, **49**, 779 (1986).
18. V. Piccialli and D. Sica, *J. Nat. Prod.*, **50**, 915 (1987).
19. R.R. West and J.H. Cardellina II, *J. Org. Chem.*, **53**, 2782 (1988).
20. A. Madaio, V. Piccialli, and D. Sica, *Tetrahedron Lett.*, **29**, 5999 (1988).
21. D. Sica and V. Piccialli, *Comp. Biochem. Physiol.*, **81B**, 115 (1985).
22. A. Dini, D. Sica, and L. Boniforti, *Comp. Biochem. Physiol.*, **78B**, 741 (1984).
23. M. De Rosa, L. Minale, and G. Sodano, *Comp. Biochem. Physiol.*, **46B**, 823 (1973).
24. Y. Fujimoto, T. Yamada, and N. Ikekawa, *Chem. Pharm. Bull.*, **33**, 3129 (1985).
25. Y. Hirano and C. Djerassi, *J. Org. Chem.*, **47**, 2420 (1982).
26. T. Itoh, D. Sica, and C. Djerassi, *J. Chem. Soc., Perkin Trans. 1*, 147 (1983).
27. I. Rubinstein, L.J. Goad, A.D.H. Clague, and L.J. Mulheirn, *Phytochemistry*, **15**, 195 (1976).
28. D.H.R. Barton and C.H. Robinson, *J. Chem. Soc.*, 3045 (1954).
29. P.L. Chiu and G.W. Patterson, *Lipids*, **16**, 203 (1981).
30. T. Gebreyesus, I. Stoilov, F.T. Luo, and C. Djerassi, *Steroids*, **45**, 447 (1985).
31. J. Zielinski, T. Milkova, S. Popov, N. Marekov, and C. Djerassi, *Steroids*, **39**, 675 (1982).
32. W. Arnold, W. Meister, and G. Englert, *Helv. Chim. Acta*, **57**, 1559 (1974).
33. J.L.C. Wright, A.G. McInnes, S. Shimizu, D.G. Smith, J.A. Walter, D. Idler, and W. Khalil, *Can. J. Chem.*, **56**, 1898 (1978).
34. D.J. Frost and J.P. Ward, *Tetrahedron Lett.*, 3779 (1968).
35. A. Bax, R. Freeman, and G.A. Morris, *J. Magn. Reson.*, **42**, 164 (1981).
36. H. Scherrer, *Helv. Chim. Acta*, **52**, 2428 (1969).