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New Sterol Derivatives from the Marine Sponge *Xestospongia* sp.

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Abstract

Chemical examination of a marine sponge *Xestospongia* sp. resulted in the isolation of 20 sterol derivatives (**1**-**20**), including eight new sterols namely aragusterols J-L (**1–3**), ($5\alpha,7\alpha,12\beta,22E$)-7,12,18-trihydroxystigmast-22-en-3-one (**4**), ($5\alpha,7\alpha,12\beta,24R$)- and ($5\alpha,7\alpha,12\beta,24S$)-7,12,20-trihydroxystigmastan-3-one (**5**/**6**), and ($5\alpha,7\alpha,12\beta,22E,24R$)- and ($5\alpha,7\alpha,12\beta,22E,24S$)-7,12,20-trihydroxyergost-22-en-3-one (**7**/**8**). The structures of new compounds were determined through extensive spectroscopic analyses and chemical conversion. The sterol diversity was mainly characterized by the presence of a cyclopropane unit at side chain, while compound **4** with 18-hydroxymethyl group was found in stigmasterol family for the first time. Cytotoxic test revealed the inhibitory effects of compounds **1**, **4**, and

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17 against human leukemia cell line K562 with IC_{50} values of 18.3, 24.1, and 34.3 μM, respectively.

Keywords*:* Sterols, Structure elucidation, Cytotoxic activity, *Xestospongia* sp.

Introduction.-Marine sponges have been proved to be the most diverse array of unconventional steroids derived from all organisms [1, 2]. The steroids from sponges are sometimes highly functionalized and displayed unconventional polycyclic core or unusual side chains [3], while some of them have no terrestrial counterpart. The highly functionalized steroids have attracted considerable attention due to the potential biological activities [4]. The sterols possessing a cyclopropane ring at side chain are a group of unusual natural products mainly distributed in marine sponges, such as aragusterols A-I, xestokerols A-C, 21-O-octadecanoyl-xestokerol A, 7-oxopetrosterol, 7β- and 7α-hydroxypetrosterol, and petrosterol from *Xestospongia* [5-10] and *Ianthella* sponges [11]. The potent biological activities such as aragusterols with strong inhibition against the proliferation of tumor KB cells in vitro and L1210 leukemia in vivo attracted the attention of chemists for synthesis of aragusterols A-D [12]. The sponge genus *Xestospongia* (order Haplosclerida, family Petrosiidae) widely distributed in the Pacific Ocean, Indian Ocean, and Caribbean Sea. Chemical examination of sponges revealed that the same species of a sponge collected from different location produced distinct metabolites. With the aim for the discovery of bioactive metabolites from marine invertebrates, a marine sponge *Xestospongia* sp. collected from the South China Sea was undertaken for chemical examination. The ^{1}H NMR spectrum of the AcOEt extract displayed the resonances ranging from 0 to 2 ppm typical for cyclopropane-bearing steroids. Chromatographic separation of the AcOEt extract resulted in the isolation of 20 sterol derivatives (Fig. 1).

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Results and Discussion.-The frozen sponge of *Xestospongia* sp. was extracted with EtOH to afford a crude extract, which was desalted by dissolving in MeOH. The residue was dispersed in H_2O and successively partitioned with AcOEt and BuOH. The AcOEt extract was subjected to extensive column chromatography to afford compounds **1–20**.

Aragusterol J (**1**) has a molecular formula of $C_{29}H_{46}O_4$, as established by the HR-ESI-MS (*m/z* 459.3472 $[M + H]^+$) and NMR data. The IR absorptions at 3358 and 1716 cm^{-1} suggested the presence of hydroxyl and carbonyl functionalities. The ^{1}H-NMR spectrum exhibited the resonances including four Me groups (δ(H) 0.81, s, H_3-C(18); 1.23, s, H_3-C(19); 0.95, d, J = 6.7 Hz, H_3-C(28); 1.03, d, J = 6.0 Hz, H_3-C(29)), three oxymethines (δ(H) 3.79, dt, H-C(6); 3.52, dd, J = 11.1, 4.5 Hz, H-C(12); 4.36, dd, J = 8.0, 4.4 Hz, H-C(22)), an exomethylene (δ(H) 4.96, 5.13, s), while the shielded protons at δ(H) 0.15 (*m*), 0.23 (*m*), and 0.57 (*m*) were featured by the presence of cyclopropane ring. The ^{13}C-NMR and DEPT spectra displayed a total of 29 carbon resonances, involving a ketone and two olefinic carbons for a double bond. Analyses of 1D and 2D NMR (COSY, HMQC and HMBC) data established a sterol-based gross structure, which was closely related to aragusterol E (**11**) [7], a coexisted sterol in the same fraction. The difference was attributed to a hydroxymethine C(6) (δ(C) 70.6) of **1** to replace a methylene group of **11**, as evident from the COSY correlations of H-C(6) (δ(H) 3.79, dt, J = 2.0, 4.0 Hz) with H-C(5) and H_2-C(7). The relative configuration of **1** was assigned by the NOE interactions. The NOE correlations from H-C(8) to H_3-C(18) and H_3-C(19) and between H-C(9) and H-C(5) clarified the *trans* fusion of the tetraocyclic nucleus, the same as that of **11**. Thus, the NOE relationships between H_3-C(19)/Hβ-C(4) and Hα-C(4)/H-C(6) in association with the $J_{H-5/H-6}$ value (4.0 Hz) assigned an equatorial orientation of H-C(6), indicating OH-C(6) to be β-oriented. Additional NOE correlations from H-C(12) to H-C(9), H-C(14), and H-C(17) reflected OH-C(12) to be β-oriented, while

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the side chain at C(17) was β-oriented. This was also supported by the observation of the NOE relationship between H_3-C(18) and H_2-C(21) (Fig. 2). The complete agreement of the NMR data for the side chain including the NOE interaction and *J* values led to the assignment of the same configurations for the side chain of **1** and **11**.

The molecular formula of aragusterol K (**2**) was determined to be $C_{31}H_{50}O_2$ on the basis of the HR-ESI-MS (*m/z* 471.3830 $[M + H]^+$) and NMR data. The NMR data of **2** (Tables 1 and 2) featured a sterol-type analogue, while analyses of 1D and 2D NMR data resulted in the gross structure of **2** to be closely related to 5α,6α-epoxy-petrosterol (**12**) [13]. The distinction was found by the presence of an acetyl group (δ(H) 2.0, s, δ(C) 21.3, 170.2) in **2**. The HMBC correlation of H-C(3) (δ(H) 4.95) with the acetyl carbonyl carbon deduced the location of acetoxy group at C(3). The similar NMR data and NOE interactions of **2** and **12** reflected the same configurations of both compounds. Conversion of **2** to **12** by deactylation with alkaline hydrolysis (Fig. 3) further supported both **2** and **12** sharing the same configurations.

The 1D and 2D NMR data in association with the HR-ESI-MS data provided the gross structure of aragusterol L (**3**) to be the same as 5α,6α-epoxy-petrosterol (**12**) [13]. The distinction was found by the NMR resonances of the epoxy group, of which the shielded C(5) (δ(C) 62.9) and deshielded C(6) (δ(C) 63.7) of **3** were observed to replace δ(C) 65.2 (C(5)) and 59.2 (C(6)) of **2** and **12**. These findings suggested that the configuration of the epoxy group in **3** differed from that of **12**. The NOE interaction from Hα-C(4) to H-C(3) and H-C(6) deduced the β-orientation of the epoxide ring [14,15]. The similar NOE interactions and NMR data regarding the tetracyclic nucleus and side chain indicated the same configurations of remaining stereogenic centers in both **3** and **12**. In addition, the structure of petrosterol (**16**) was assigned by the single-crystal X-ray diffraction using Cu Kα radiation in this work (Fig.

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4). Epoxidation of petrosterol (**16**) by 3-chloroperbenzoic acid oxidation yielded two products with a ratio of 5:1, which were identical to **3** and **12** by the comparison of their NMR, MS and specific rotation. This finding further confirmed the configurational assignment of **3**.

Compound **4** has a molecular formula of $C_{29}H_{44}O_{10}$ as determined by the HR-ESI-MS (*m/z* 461.3629 $[M + H]^+$) and NMR data. The ^{1}H- and ^{13}C-NMR spectra featured a stigmasterol nucleus, based on the resonances for an ethyl group, an ispropane unit, a methyl doublet, and two olefinic protons at δ(H) 5.52 (*dd*, *J* = 8.2, 15.4 Hz, H-22) and 5.14 (*dd*, *J* = 9.0, 15.4 Hz, H-23) for *E*-geometry of the double bond in the side chain, in association with their COSY and HMBC interactions. A ketone group resided at C(3) (δ(C) 214.4) was evident from the HMBC interactions from C(3) to H_2-C(1), H_2-C(2), H_2-C(4) and H-C(5). In addition, the chemical shifts of C(7) (δ(C) 67.6)/H-C(7) (δ(H) 3.79) and C(12) (δ(C) 82.1)/H-C(12) (δ(H) 3.45) indicated C(7) and C(12) to be hydroxylated. The absence of methyl group C(18) and the presence of hydroxymethylene (δ(C) 61.7; δ(H) 3.74, 3.97), in addition to the HMBC correlations from the hydroxymethylene protons to C(12), C(13), C(14) and C(17) clarified C(18) to be hydroxylated. The *trans* fusions of the backbone were determined by the NOE interactions as mentioned for **1**. The small *J* values of H-C(7) in association with the NOE interaction between H-C(7) and H-C(8) assigned H-C(7) to be in equatorial orientation (β-orientation), whereas the *J* values of H-C(12) (*dd*, *J* = 4,4, 11.2 Hz) reflected axial-orientation (α-orientation). The NOE interactions between H_2-C(18)/H-C(20) and H-C(12)/H-C(17) assigned α-orientation of H-C(17). The comparable NMR data of **4** and 22*E*,24*R*-stigmast-22-ene-3,7-dione [16] regarding to the side chain conducted to assign the same configuration of C(20) and C(24) of both compounds. Thus, the structure of **4** was assigned as (5α,7α,12β,22*E*)-7,12,18-trihydroxystigmast-22-en-3-one.

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Compounds **5** and **6** were obtained as a pair of inseparable analogues, whose molecular formula ($C_{29}H_{50}O_4$) was determined by the HR-ESI-MS data. The NMR data of **5** and **6** were characteristic of sitosterol-type analogues, based on the typical NMR resonances of two methyl doublets, a methyl triplet, and a methyl singlet in the side chain. Detailed analyses of 2D NMR data determined the partial structure of tetracyclic nucleus to be closely related to that of **4**, with the exception of C(18) being a methyl group instead of hydroxymethyl group. This finding was supported by the presence a ketone at C(3) (δ(C) 214.4), hydroxy groups at C(7) (δ(C) 67.7) and C(12) (δ(C) 78.7). An additional hydroxy group at C(20) (δ(C) 75.7/75.8) was defined by the HMBC correlations from H_3-C(21) (δ(H) 1.21, s) to C(17) (δ(C) 66.0), C(20), and C-22 (δ(C) 35.6/35.7). The duplicated NMR data from C(1) to C(19) (Table 2) indicated both **5** and **6** sharing the same partial structure of tetracyclic nucleus. However, the doubling NMR resonances at side chain of **5** and **6** were observed. The NOE interactions from H-C(17) to H-C(12) and H_3-C(21) were indicative of the same configuration at C(20) of **5** and **6**. In addition, the NOE correlations between H-C(12)/H_3-C(21) and H_3-C(18)/OH-C(20) in association with the absence of NOE correlation between H-C(12)/OH-C(20) indicated the side chain maintaining a dominant conformer, while OH-C(20) was spatially approximated to H_3-C(18). Thus, both **5** and **6** were supposed to be a pair of C(24) epimers. Pairwise comparison of the ^{13}C-NMR signals for side chain of **5/6** with those of sitosterol and its C(24) epimer clionasterol [17] assigned both **5** and **6** to be C(24) epimers. Thus, the structures of **5** and **6** were assigned as (5α,7α,12β,24*R*)- and (5α,7α,12β,24*S*)-7,12,20-trihydroxystigmastan-3-one.

Compounds **7** and **8** were a pair of inseparable sterols with a ratio of 2:1 as detected by the NMR spectra, while the molecular formula of $C_{28}H_{46}O_4$ as determined by the HR-ESI-MS (*m/z* 447.3474 $[M + H]^+$) data. Their NMR data (Tables 1 and 2) featured the signals of

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ergosterol-type analogues. Comparison of the NMR data between **7/8** and **5/6** revealed that they possessed the same tetracyclic nucleus. The distinction was attributed to the resonances of side chain where doubling NMR resonances were observed. The position of a hydroxyl group at C(20) (δ(C) 75.1) was assigned by the HMBC interactions from H_3-C(21) (δ(H) 1.21, s) to C(17), C(20), and C(22), while the COSY and additional HMBC correlations established the side chain to be related to that of ergosterol with a 20-hydroxy group. The coupling constant $J_{H\text{-}22/H\text{-}23}$ = 15.4 Hz was in agreement with 22*E* geometry. The NOE interaction between H-C(17) and H_3-C(21) reflected the same configuration of C(20) in **7** and **8**. Additional NOE correlations between H-C(12)/H_3-C(21) and H_3-C(18)/OH-C(20) and the absence of NOE correlation between H-C(12)/OH-C(20) with the same as those observed in the NOESY spectrum of **5/6**, indicated OH-C(20) was resided in the same orientation as that of **5/6**. The doubling of side chain signals at C(22) (δ(C) 134.9/135.1), C(23) (δ(C) 132.6/132.3), C(24) (δ(C) 34.5/34.6), C(25) (δ(C) 20.2/20.3), C(25) (δ(C) 34.5/34.6), C(26) (δ(C) 20.4/20.7), and C(28) (δ(C) 17.6/17.9), was indicative of C(24) epimers of both **7** and **8**. Thus, the structures of **7** and **8** were assigned as ($5\alpha,7\alpha,12\beta$,22*E*,24*R*)- and ($5\alpha,7\alpha,12\beta$,22*E*,24*S*)-7,12,20-trihydroxyergost-22-en-3-one.

In addition, 3α-aragusterol I (**9**) [12] and petrosteryl acetate (**10**) [18], as synthetic intermediates, were isolated from nature for the first time. Chemical transformation of **14** to **9** (Fig. 6) and **16** to **10** (Fig. 7), clarified the stereogenic centers of **9** and **10**. Ten additional known analogues were identical to aragusterol E (**11**) [7], $5\alpha,6\alpha$-epoxy-petrosterol (**12**) [11], aragusterol I (**13**) [7], aragusterol B (**14**) [8], xestokerol B (**15**) [10], petrosterol (**16**) [19], aragusterol A (**17**) [9], 7-oxopetrosterol (**18**) [20], 7β-hydroxypetrosterol (**19**) [7], and 7α-hydroxypetrosterol (**20**) [9], on the basis of the comparison of their NMR, MS, and specific rotation data with those published in literature.

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The X-ray crystal data clarified petrosterol (**16**) bearing *trans*-fusion of rings A/B, B/C, and C/D, while the absolute configurations of the stereogenic centers were determined to be 3*S*, 8*S*, 9*S*, 10*R*, 13*R*, 14*S*, 17*R*, 20*R*, 24*R*, 25*R*, and 27*R*. These assignments were in agreement with known natural sterols which exclusively presented *trans*-fusion of nucleus rings. The coexistence of the cycloprepane-bearing sterols with petrosterol in the sponge conducted the biogenetic assumption of the same configurations of sterol backbone among the isolated analogues.

All steroids (**1–20**) were evaluated for their inhibitory activity against the human leukemia cell line K562 at an initial concentration of 10 μM by the MTT method. Adriamycin was used as the positive control. The bioassay results showed that nine compounds showed inhibitions more than 20% at a single dose of 10 μM (Table 3), while compounds **1**, **4**, **14**, and **17** showed moderate/weak activity with IC_{50} values of 18.3, 24.2, 34.3, and 95.6 μM, respectively.

Conclusion.-Present work provided a group of new steroids which enriched the number of sterol family derived from marine sponges. Steroids obtained from *Xestospongia* sponges exclusively possessed the analogues with a cyclopropane unit, suggesting the 26,27-cyclopropane-bearing steroids to be one of the chemotaxonomic marks of *Xestospongia* species. The weak cytotoxic activity of the isolates implied that the steroids from the sponge may play ecological role other than toxic effect.

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Experimental Part

General

TLC: *HF_{254}* silica gel (SiO_2, *Qingdao Marine Chemistry Co. Ltd.*) Column chromatography (CC): silica gel (SiO_2, 200 – 300 mesh; *Qingdao Marine Chemistry Co. Ltd.*), *Sephadex LH-20* (18 – 110 μm; Pharmacia). HPLC: semi-prep. *Prevail C_{18}* column (5 μm), *Alltech 426* pump, UV detector (*Pharmacia*). Optical rotations: *Autopol III* automatic polarimeter (*Rudolph Research Co., Ltd.*). IR Spectra: *Thermo Nicolet Nexus 470* FT-IR spectrometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Bruker Avance-400FT* NMR spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *Bruker APEX IV* 70 eV FT-MS spectrometer and on a *Thermo DFS* spectrometer using a matrix of 3-nitrobenzyl alcohol; in *m/z*. EI-MS (70 eV): *Finnigan MAT 95* mass spectrometer; in *m/z*.

Animal Material

The sponge *Xestospongia* sp. was collected at a depth of 10 m water in Yongxin Island, Hainan Province of China, in June 2012, and the fresh sample was frozen immediately. The specimen was identified by Dr. *N. J. de V.* (Department Marine Zoology, The Netherland). A voucher specimen (XSA-16) was deposited with the State Key Laboratory of Natural and Biomimetic Drugs, Peking University, P. R. China.

Extraction and Isolation

The frozen animal of (700 g, wet weight) was extracted with EtOH to give a crude extract, which was desalted by dissolving in MeOH to obtain a residue (12.5 g). The residue was dispersed in H_2O and successively partitioned with AcOEt and BuOH. The concentrated AcOEt solution (2.5 g) was subjected to a *RP-18* gel column chromatography eluting with MeOH/H_2O (1:4) to afford five fractions (*Fr. F1 – F5*). *Fr. F5* (1.1 g) was

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separated on a silica gel column with petroleum ether PE/CH_2Cl_2 (4:1) as eluent to obtain **10** (5.0 mg), **2** (4.4 mg), and **16** (286 mg). *Fr. F4* (150 mg) was subjected to a silica gel column eluting with CH_2Cl_2/MeOH (3:1, 4 ml/min) to afford **20** (16 mg), **3** (1.5 mg), **11** (2 mg), and **14** (10 mg). *Fr. F3* (90 mg) was followed by the same separation protocol as for *Fr. F1* to be separated by a silica gel column eluting with PE/AcOEt (4:1, 2 ml/min) to obtain **18** (1.5 mg), **19** (2 mg), **9** (1.8 mg), **13** (1.5 mg), **12** (8.0 mg), and **17** (3 mg). *Fr. F2* (76 mg) was separated on semi-preparative HPLC (*YMC* column) using MeOH/H_2O (4:1, 2 ml/min) as the mobile phase to yield **5/6** (4 mg, t_R 35.5 min), **15** (6 mg, t_R 35.8 min), **4** (1.5 mg, t_R 25.5 min), **1** (1.5 mg, t_R 22.4 min), and **7/8** (2 mg, t_R 27.2 min).

Aragusterol J (= **(5*S*,6*R*,8*R*,9*S*,10*R*,12*R*,13*S*,14*S*,17*R*)-6,12-Dihydroxy-17-{(3*R*,5*R*)-3-hydroxy-5-[(1*R*,2*R*)-2-methylcyclopropyl]hex-1-en-2-yl}-10,13-dimethylhexadecahydro-3*H*-cyclopenta[*a*]phenanthren-3-one**; **1**). White powder. $[\alpha]_D^{20}$ = −12.0 (c = 0.05, CH_2Cl_2). IR (KBr): 3358, 2924, 2856, 1716, 1459, 1375, 1247. ^{1}H- and ^{13}C-NMR data: see *Tables 1* and *2*, resp. HR-ESI-MS: 441.3368 ($[M - H_2O + H]^+$, $C_{29}H_{45}O_3^+$; calc. 441.3369). HR-ESI-MS: 459.3472 ($[M + H]^+$, $C_{29}H_{47}O_4^+$; calc. 459.3474). HR-ESI-MS: 481.3297 ($[M + Na]^+$, $C_{29}H_{46}NaO_4^+$; calc. 481.3294).

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Aragusterol K (= **(3*S*,4a*R*,5a*S*,6a*S*,6b*S*,9*R*,9a*R*,11a*S*,11b*R*)-9a,11b-Dimethyl-9-{(2*R*,5*R*)-5-[(1*R*,2*R*)-2-methylcyclopropyl]hexan-2-yl}hexadecahydrocyclopenta[1,2]phenanthro[8a,9-*b*]oxiren-3-yl Acetate**; **2**). White powder. $[\alpha]_D^{20} = -51.0$ ($c = 0.87$, CH_2Cl_2). IR (KBr): 2944, 2868, 1734, 1451, 1371, 1247, 1034. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 471.3830 ($[M + H]^+$, $C_{31}H_{51}O_3^+$; calc. 471.3838).

Aragusterol L (= **(3*S*,4a*S*,5a*R*,6a*S*,6b*S*,9*R*,9a*R*,11a*S*,11b*R*)-9a,11b-Dimethyl-9-{(2*R*,5*R*)-5-[(1*R*,2*R*)-2-methylcyclopropyl]hexan-2-yl}hexadecahydrocyclopenta[1,2]phenanthro[8a,9-*b*]oxiren-3-ol**; **3**). White powder. $[\alpha]_D^{20} = -4.8$ ($c = 0.46$, CH_2Cl_2). IR (KBr): 3398, 2926, 2863, 1456, 1370, 1248, 1022. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. HR-ESI-MS: 411.3624 ($[M - H_2O + H]^+$, $C_{29}H_{47}O^+$; calc. 411.3627). HR-ESI-MS: 429.3739 ($[M + H]^+$, $C_{29}H_{49}O_2^+$; calc. 429.3727).

(5α,7α,12β,22*E*)-7,12,18-Trihydroxystigmast-22-en-3-one (**4**). White powder. $[\alpha]_D^{20} = +24.7$ ($c = 0.48$, MeOH). IR (KBr): 3234, 2944, 2922, 2854, 1712, 1447, 1370, 1270. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 443.3518 ($[M - H_2O + H]^+$, $C_{29}H_{47}O_3^+$; calc. 443.3525). HR-ESI-MS: 461.3629 ($[M + H]^+$, $C_{29}H_{49}O_4^+$; calc. 461.3631).

(5α,7α,12β,24*R*)- and **(5α,7α,12β,24*S*)-7,12,20-Trihydroxystigmastan-3-one** (**5/6**). White powder. $[\alpha]_D^{20} = +7.9$ ($c = 0.28$, MeOH). IR (KBr): 3306, 2924, 2856, 1737, 1717, 1450, 1369, 1221, 1021. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 507.3687 ($[M + HCOO]^-$, $C_{30}H_{51}O_6^-$; calc. 507.3686).

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(5α,7α,12β,22*E*,24*R*)- and **(5α,7α,12β,22*E*,24*S*)-7,12,20-Trihydroxyergost-22-en-3-one** (**7/8**). White powder. $[\alpha]_D^{20}$ = +32.0 (*c* = 0.15, MeOH). IR (KBr): 3278, 2924, 2869, 1712, 1448, 1369, 1226, 1019. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 429.3355 ($[M - HO]^+$, $C_{28}H_{45}O_3^+$; calc. 429.3363). HR-ESI-MS: 447.3474 ($[M + H]^+$, $C_{28}H_{47}O_4^+$, calc. 447.3469).

3α-Aragusterol I (**9**). White powder. $[\alpha]_D^{20}$ = +46.2 (*c* = 0.39, CH_2Cl_2).

Petrosteryl Acetate (**10**). White powder. $[\alpha]_D^{20}$ = −37.5 (*c* = 0.22, CH_2Cl_2).

Chemical Transformation of **2** *to* **12**. To a stirred solution of **2** (1 mg) in 1 ml MeOH, 0.5 mg NaOH was added. The mixture was stirred at r.t. for 0.5 h and then evaporated. The residue was subjected to *Sephadex LH-20* using EtOH as eluent to afford a product, which was identified to **12** by the comparison of the ^{1}H-NMR data, R_f, and $[\alpha]_D^{25}$ values with those reported in literature.

Acetylation of **16** *to* **10**. Acetic anhydride (200 μl) was added to a stirred solution of compound **16** (1 mg) in freshly distilled pyridine (0.5 ml). The reaction was stirred at r.t. for 12 h and quenched by adding 0.1 ml of H_2O. After removal of solvent under vacuum, the residue was purified on a flash silica gel column eluting with CH_2Cl_2 to afford **10**.

Epoxidation of **16**

To a stirred solution of **16** (10 mg, 0.02 mmol) in CH_2Cl_2 (1 ml) at 0°, 3-chloroperbenzoic acid (10 mg) was added. The mixture was stirred at r.t. for 2 h and then evaporated. The mixture was subject to a silica gel CC (CH_2Cl_2/MeOH, 100:1, 2 ml/min) to afford **3** (1 mg) and **12** (5 mg).

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Chemical Transformation of **14**

To a stirred solution of **12** (3 mg) in MeOH (0.5 ml), $NaBH_4$ (2 mg) was added and was stirred for 15 min at r.t. The mixture was stirred sequentially at r.t. for 20 min and then purified on *Sephadex LH-20* using EtOH as eluent to afford two products (8:1). The mixture was further purified using a slica gel CC (CH_2Cl_2/MeOH, 80:1) to obtain **9** (1.5 mg) and **13** (0.4 mg).

Cytotoxicity Assays

The cytotoxicity against human leukemia cell line K562 cell lines was evaluated using the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) method [21]. Adriamycin was used as a positive control.

X-Ray Crystal Data of **16**

Colorless crystals of **16** were obtained from PE/AcOEt (1:1) using the vapor diffusion method. A colourless crystal (0.65 × 0.65 × 0.08) was used for X-ray diffraction on an *Agilent Gemini E* single crystal X-ray diffractometer with graphite monochromated CuK_α radiation at 103.5 K. Crystal Data: $C_{29}H_{48}O$, M = 412.67, monoclinic, a = 11.6546(3) Å, b = 5.99382(15) Å, c = 18.3691(6) Å, β = 96.828(3)°, U = 1274.08(6) Å^3, T = 103.5, space group $P2_1$ (no. 4), Z = 2, μ (CuK_α) = 0.462, 8054 reflections measured, 4782 unique (R_{int} = 0.0270) which were used in all calculations. The final $wR(F_2)$ was 0.1157 (all data). Flack parameters = −0.2 (3). The Crystallographic data for **16** have been deposited in Cambridge Crystallographic Data Center [deposition number: CCDC 1417552]. Copy of the data can be obtained free of charge from the CCDC *via* www.ccdc.cam.ac.uk.

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Table 1. [1]H-NMR Data (400 MHz, in $CDCl_3$) of **1 – 8**. δ in ppm, *J* in Hz.

Position	**1**	**2**	**3**	**4**	**5/6**	**7/8**
1	1.93 – 1.87 (*m*),	1.69 – 1.65 (*m*),	1.97 – 1.95 (*m*),	2.06 – 2.02 (*m*),	2.04 – 2.01 (*m*),	2.04 – 2.00 (*m*),
	1.34 – 1.30 (*m*)	1.42 – 1.39 (*m*)	1.26 – 1.23 (*m*)	1.54 – 1.50 (*m*)	1.44 – 1.40 (*m*)	1.41 – 2.38 (*m*)
2	2.41 – 2.37 (*m*),	1.69 – 1.63 (*m*),	1.81 – 1.78 (*m*),	2.61 (*dt*, *J* = 12.0,4.0),	2.50 (*dt*, *J* = 12.0,4.0),	2.49 (*dt*, *J* = 12.0,4.0),
	2.34 – 2.30 (*m*)	1.42 – 1.38 (*m*)	1.45 – 1.42 (*m*)	2.26 – 2.22 (*m*)	2.25 – 2.20 (*m*)	2.25 – 2.21 (*m*)
3		4.95 – 4.90 (*m*)	3.75 – 3.70 (*m*)			
4	2.80 (*t*, *J* = 12),	2.16 (*t*, *J* = 12.0),	2.19 (*t*, *J* = 12.0),	2.36 (*t*, *J* = 12.0),	2.36 (*t*, *J* = 12.0),	2.35 (*t*, *J* = 12.0),
	2.12 (*dd*, *J* = 12.0, 2.0)	1.32 (*dd*, *J* = 12.0, 4.0)	1.43 (*dd*, *J* = 12.0, 4.0)	1.95 (*dd*, *J* = 12.0, 4.0)	1.95 (*dd*, *J* = 12.0, 2.0)	1.95 (*dd*, *J* = 12.0, 2.0)
5	1.57 – 1.50 (*m*)			2.02 – 1.98 (*m*),	2.04 – 2.00 (*m*)	2.04 – 2.00 (*m*)
6	3.79 (*td*, *J* = 2.0, 4.0)	2.89 (*d*, *J* = 4.4)	3.06 (*d*, *J* = 2.3)	1.57 – 1.53 (*m*),	1.59 – 1.55 (*m*),	1.57 – 1.54 (*m*),
				1.46 (*m*)	1.48 – 1.45 (*m*)	1.47 – 1.44 (*m*)
7	1.87 – 1.82 (*m*),	1.91 – 1.87 (*m*),	2.08 – 2.05 (*m*),	3.79 (*td*, *J* = 3.0, 4.0)	3.82 – 3.80 (*m*)	3.81 – 3.79 (*m*)
	1.18 – 1.12 (*m*)	1.49 – 1.45 (*m*)	1.40 – 1.35 (*m*)			
8	1.81 – 1.75 (*m*)	1.35 – 1.31 (*m*)	1.48 – 1.44 (*m*)	1.40 – 1.37 (*m*)	1.42 – 1.39 (*m*)	1. 42 – 1.38 (*m*)
9	0.92 – 0.85 (*m*)	1.30 – 1.25 (*m*)	0.61 – 0.58 (*m*)	1.45 – 1.41 (*m*)	1.43 – 1.40 (*m*)	1.42 – 1.38 (*m*)
11	1.72 – 1.68 (*m*),	1.37 – 1.32 (*m*),	1.38 – 1.34 (*m*),	1.85 – 1.82 (*m*),	1.72 – 1.68 (*m*),	1.73 – 1.69 (*m*),
	1.46 – 1.40 (*m*)	1.27 – 1.23 (*m*)	1.26 – 1.24 (*m*)	1.70 – 1.66 (*m*)	1.36 – 1.33 (*m*)	1.33 – 1.30 (*m*)
12	3.52 (*dd*, *J* = 11.1, 4.5)	1.95 – 1.92 (*m*),	1.97 – 1.94 (*m*),	3.45 (*dd*, *J* = 11.2, 4.4)	3.33 (*dd*, *J* = 11.2, 4.4)	3.31 (*dd*, *J* = 11.2, 4.4)
		1.12 – 1.08 (*m*)	1.06 – 1.03 (*m*)			
14	1.21 – 1.17 (*m*)	0.98 – 0.95 (*m*)	0.87 – 0.83 (*m*)	1.40 – 1.36 (*m*)	1.43 – 1.40 (*m*)	1.42 – 1.39 (*m*)
15	1.72 – 1.67 (*m*),	1.56 – 1.52 (*m*),	1.58 – 1.54 (*m*),	1.78 – 1.75 (*m*),	1.86 – 1.82 (*m*),	1.85 – 1.81 (*m*),

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	1.41 – 1.38 (*m*)	0.98 – 0.94 (*m*)	1.07 – 1.03 (*m*)	1.10 – 1.08 (*m*)	1.20 – 1.17 (*m*)	1.25 – 1.22 (*m*)
16	1.91 – 1.85 (*m*), 1.65 – 1.60 (*m*)	1.83 – 1.80 (*m*), 1.22 – 1.18 (*m*)	1.83 – 1.80 (*m*), 1.26 – 1.22 (*m*)	1.63 – 1.60 (*m*), 1.47 – 1.43 (*m*)	1.78 – 1.74 (*m*), 1.55 – 1.51 (*m*)	1.78 – 1.75 (*m*), 1.62 – 1.59 (*m*)
17	2.37 – 2.30 (*m*)	1.07 – 1.04 (*m*)	1.07 – 1.03 (*m*)	1.62 – 1.58 (*m*)	1.69 – 1.65 (*m*)	1.71 – 1.67 (*m*)
18	0.81 (*s*)	0.61 (*s*)	0.64 (*s*)	3.97 (*d*, J = 11.3), 3.74 (*d*, J = 11.3)	0.85 (*s*)	0.73 (*s*)
19	1.23 (*s*)	1.07 (*s*)	1.00 (*s*)	1.09 (*s*)	1.07 (*s*)	1.05 (*s*)
20		1.32 – 1.29 (*m*)	1.34 – 1.30 (*m*)	2.89 – 2.84 (*m*)		
21	4.96 (*s*), 5.13 (*s*)	0 .89 (*s*)	0.90 (*d*, J = 6.5)	1.19 (*d*, J = 6.8)	1.13 (*s*)	1.21 (*s*)
22	4.36 (*dd*, J = 8.0, 4.4)	1.46 – 1.42 (*m*), 0.98 – 0.95 (*m*)	1.46 – 1.42 (*m*), 1.00 – 0.95 (*m*)	5.52 (*dd*, J = 15.4, 8.2)	1.61 – 1.57 (*m*), 1.46 – 1.42 (*m*)	5.70 (*d*, J = 15.8)
23	1.73 – 1.65 (*m*), 1.42 – 1.38 (*m*)	1.33 – 1.30 (*m*), 1.23 – 1.20 (*m*)	1.29 – 1.25 (*m*), 1.26 – 1.23 (*m*)	5.14 (*dd*, J = 15.4, 9.0)	1.57 – 1.53 (*m*)	5.56 (*dd*, J = 15.8, 7.7)
24	0.83 – 0.78 (*m*)	0.59 – 0.54 (*m*)	0.60 – 0.56 (*m*)	1.62 – 1.58 (*m*)	0.98 – 0.95 (*m*)	2.0 – 1.97 (*m*)
25	0.23 – 0.21 (*m*)	0.13 – 0.10 (*m*)	0.13 – 0.10 (*m*)	1.57 – 1.52 (*m*)	1.70 – 1.65 (*m*)	1.58 – 1.54 (*m*)
26	0.15 – 0.13 (*m*), 0.23 – 0.20 (*m*)	0.09 – 0.05 (*m*), 0.15 – 0.12 (*m*)	0.08 – 0.04 (*m*), 0.14 – 0.11 (*m*)	0.83 (*d*, J = 6.6)	0.88 (*d*, J = 6.6)	0.89 (*d*, J = 6.8)
27	0.57 – 0.52 (*m*)	0.44 – 0.41 (*m*)	0.45 – 0.42 (*m*)	0.89 (*d*, J = 6.6)	0.88 (*d*, J = 6.6)	0.89 (*d*, J = 6.8)
28	0.95 (*d*, J = 6.7)	0.88 (*d*, J = 6.7)	0.88 (*d*, J = 6.5)	1.46 – 1.43 (*m*), 1.25 – 1.21 (*m*)	1.38 – 1.35 (*m*), 1.25 – 1.21 (*m*)	0.97 (*d*, J = 6.8)
29	1.03 (*d*, J = 6.0)	0.99 (*d*, J = 5.9)	1.01 (*d*, J = 6.0)	0.86 (*t*, J = 7.4)	0.89 (*t*, J = 7.0)	
AcO		2.00 (*s*)				

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Table 2. ^{13}C-NMR Data (100 MHz, in $CDCl_3$) of **1 – 8**. δ in ppm.

Position	**1**	**2**	**3**	**4**	**5/6**	**7/8**
1	39.7	32.1	37.2	39.5	39.6	39.5
2	38.1	27.2	31.1	38.9	38.9	38.9
3	212.2	71.4	69.4	214.4	214.4	214.4
4	41.9	36.1	42.2	45.0	45.0	45.0
5	48.8	65.2	62.9	40.6	40.6	40.6
6	70.6	59.2	63.7	37.9	38.0	37.9
7	39.4	28.8	32.6	67.6	67.7	67.7
8	29.9	29.9	29.8	39.1	39.3	39.3
9	52.9	42.5	51.4	45.8	45.5	45.4
10	35.7	35.0	34.9	36.8	36.8	36.8
11	30.0	20.6	22.0	33.4	29.8	29.9
12	78.5	39.4	39.9	82.1	78.7	78.6
13	49.3	42.3	42.3	50.2	48.9	49.5
14	54.6	56.8	56.3	50.0	50.1	49.9
15	24.2	24.1	24.2	24.4	23.8	23.9/23.7
16	32.0	28.1	28.2	26.9	26.0	25.5
17	47.9	55.9	56.2	58.4	66.0/66.1	64.3
18	8.3	11.9	11.8	61.7	10.0	9.98/10.0

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19	14.8	15.9	17.0	10.6	10.6	10.6
20	151.1	35.9	35.9	39.2	75.7/75.8	75.1
21	113.8	18.6	18.7	23.7	27.9/27.9	31.2
22	76.9	33.5	33.4	138.6	35.6/35.7	134.9/135.1
23	45.0	34.0	33.9	131.4	25.1/25.2	132.6/132.3
24	34.9	38.7	38.7	53.0	47.7/47.8	44.1/44.8
25	27.3	27.4	27.4	33.1	30.6/30.7	34.5/34.6
26	11.7	12.8	11.6	19.4	19.79/19.83	20.2/20.3
27	12.9	11.6	12.8	21.5	19.8/20.0	20.4/20.7
28	20.1	19.8	19.8	26.7	24.3	17.6/17.9
29	19.1	19.1	19.1	12.8	12.5/12.7	
3-AcO		21.3				
		170.2				

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Table 3. Inhibitory effects of compounds against human tumor cells K562.

Compound	Inhibition ([%], 10 μM)	IC_{50} [μM]
1	50.28	34.31
4	65.24	18.32
5/6	25.73	> 50
7/8	41.32	> 50
9	46.43	> 50
11	33.47	> 50
13	38.11	> 50
14	48.73	> 50
17	53.21	24.19

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Captions:

Fig. 1. Structures of sterols isolated from *Xestospongia* sp.

Fig. 2. Key NOE correlations of **1 – 4**.

Fig. 3. Alkaline conversion of **2** to **12**.

Fig. 4. X-ray structure of **16**.

Fig. 5. Epoxidation of **16** to **3** and **12**.

Fig. 6. Chemical conversion of **14** to **9** and **13**.

Fig. 7. Acetylation **16** to **10**.

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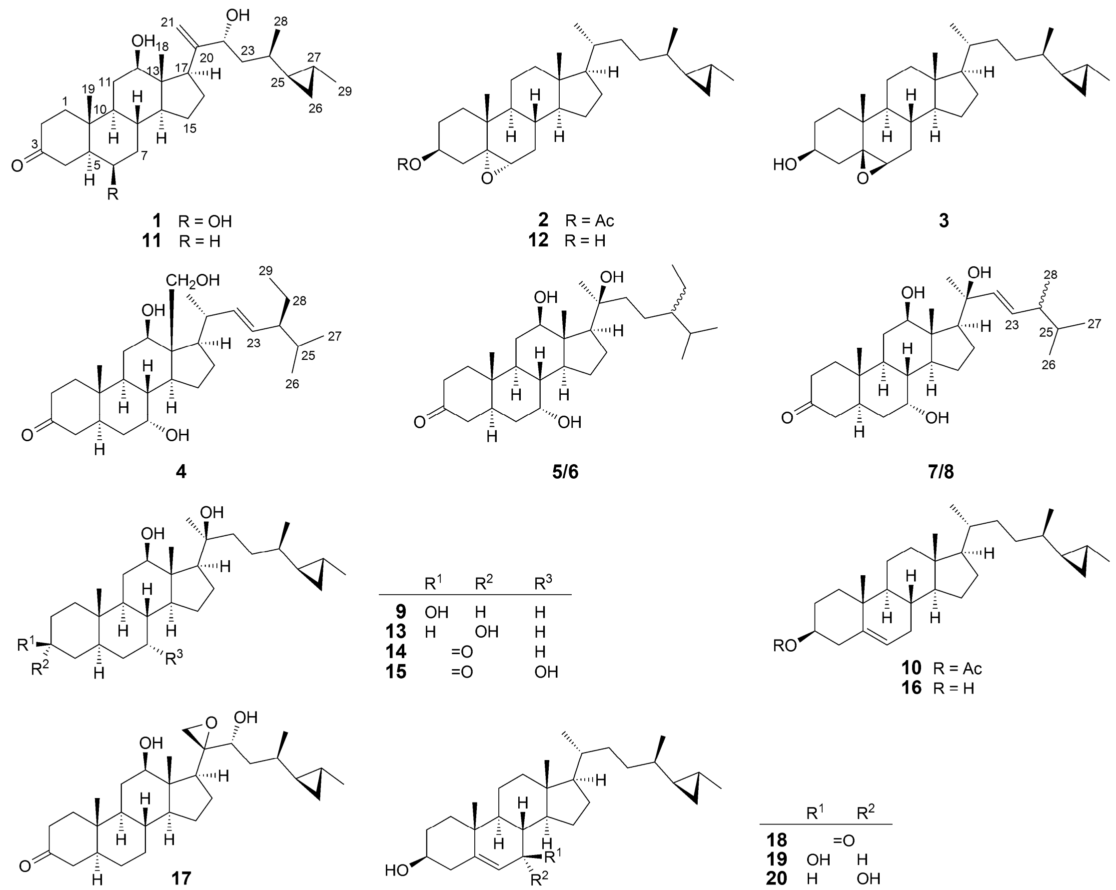

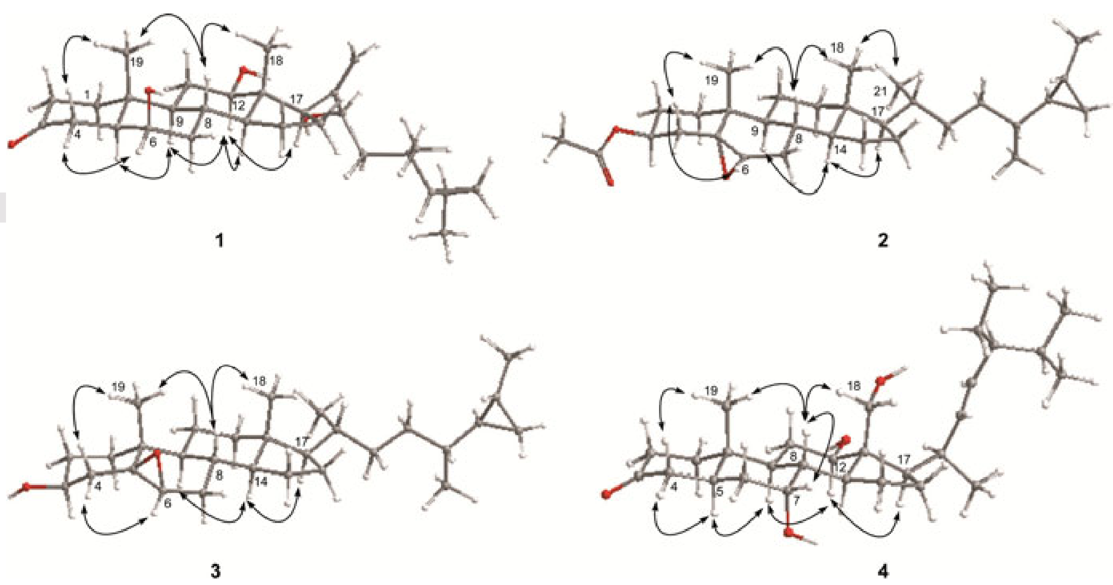

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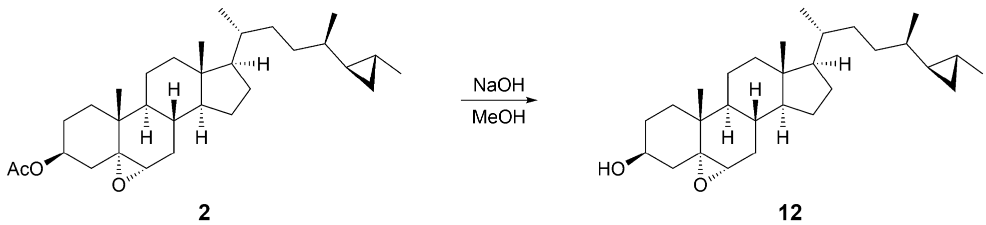

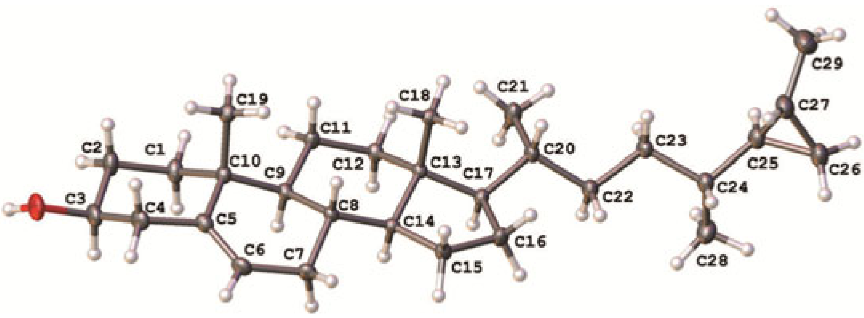

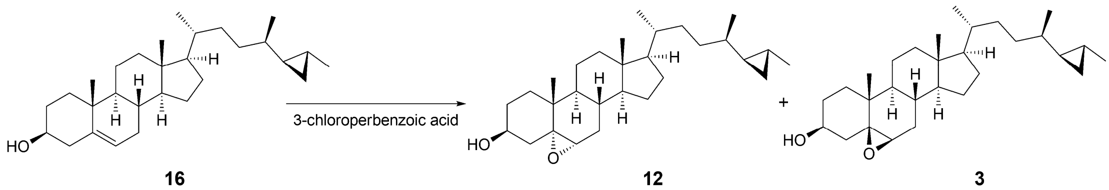

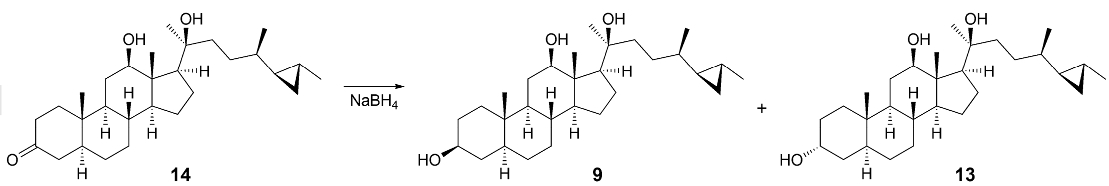

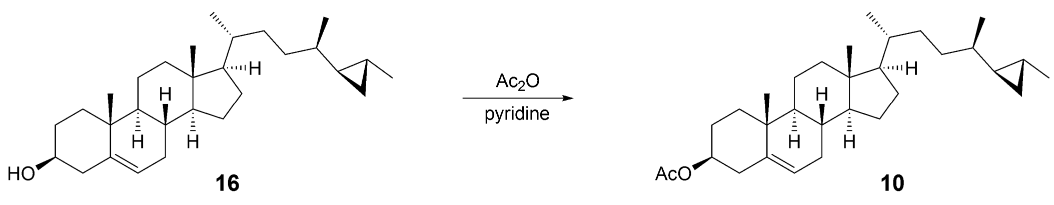

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