ELSEVIER

Steroids 66 (2001) 33–38

Steroids

Synthesis of polyhydroxysterols (I): synthesis of 24-methylenecholest-4-en-3β,6β-diol, a cytotoxic natural hydroxylated sterol☆

JianGuo Cui[a,b,*], LongMei Zeng[a], JingYu Su[b], WeiGang Lu[a]

[a]*Department of Chemistry, Zhongshan University, Guangzhou 510275, P.R. of China*
[b]*Department of Chemistry, Guangxi Teachers College, Nanning 530001, P.R. of China*

Received 29 November 1999; received in revised form 22 May 2000; accepted 26 May 2000

Abstract

Starting from stigmasterol (**2**), 24-methylenecholest-4-en-3β,6β-diol (**1**), a cytotoxic natural dihydroxylated sterol, was synthesized via 10 steps in 20% overall yield. The introduction of a side-chain of sterol was achieved by solid-liquid phase-transfer Wittig reaction using (3-methyl-2-oxo)butyltriphenylarsonium bromide (**12**) and K_2CO_3. Construction of the steroidal nucleus was finished by the addition of 3β-acetoxycholest-5,6-en-24-one (**7**) with NBA in dioxane under ambient temperature and by the elimination of 3β, 6β-diacetoxy-5a-bromocholestane-24-one (**9**). The spectral data of the synthetic product (**1**) are completely consistent with those of the natural compound (**1**). © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Polyhydroxylated sterol; 24-Methylenecholest-4-en-3β,6β-diol; Synthesis

1. Introduction

Many naturally occurring polyhydroxylated sterols and oxysterols exhibit potent biologic activities. In our previous study on the soft coral and the sponge, some new polyhydroxylated sterols were isolated, and they showed potent cytotoxicity to cancer cells [1–3]. 24-Methylenecholest-4-en-3β,6β-diol (**1**) was obtained from the soft coral *Alcyonium patagonicum* and showed potent cytotoxicity to murine leukemia cells with an IC_{50} value of 1 μg/ml [1]. This spurred our interest in studying the synthesis of compound (**1**). This paper reports the synthesis of 24-methylenecholest-4-en-3β,6β-diol (**1**) using stigmasterol as the starting material (Scheme 1).

2. Experimental

Stigmasterol was purchased from the Merck Co. (West Point, PA, USA). All chemicals and solvents were analytical grade and solvents were purified by general methods before use. Melting points were determined on a X_4 apparatus and were uncorrected. Infrared spectra were measured with Nicolet 205 FT-IR and Nicolet FT-360 Spectrophotometers. ^{1}H NMR spectra were recorded on either a JEOL FX-90Q (90 MHz) or a Varian ^{Unity}Inova 500 NB (500 MHz) spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as the internal standard. Mass spectra were measured with a VG-ZAB-HS mass spectrometer using a 70 eV electron impact ion source.

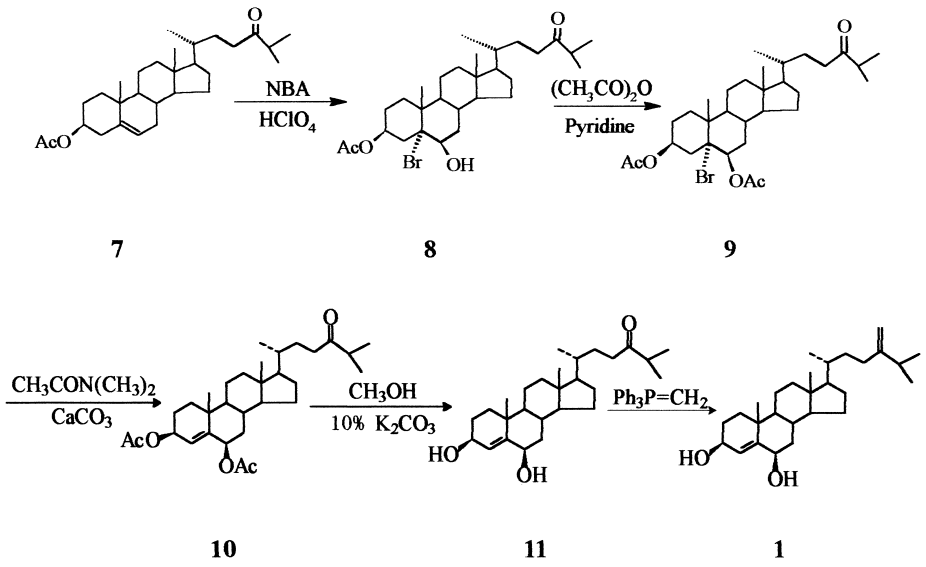
2.1. Stigmasteryl acetate (*3*)

Acetic anhydride (1.86g) was added to a solution of stigmasterol (**2**) (2.44g, 5.9 mmol) in 10 ml of pyridine, and the mixture was incubated at room temperature for 30 h. To this mixture, 30 ml of water were added to quench the excess acetic anhydride, and the mixture was subsequently extracted with ethyl acetate (20 ml × 4). The combined organic layer was washed successively with water, 1 M HCl solution, and brine, and then dried over anhydrous Na_2SO_4 and evaporated in vacuo. The residue was recrystallized

☆ The authors thank the Natural Science Foundation of China (Project: 29932030) and the Natural Science Foundation of Guangdong Province (Project: 970154) for their support.

* Corresponding author. Tel.: +86-771-5333317 (home), +86-771-3132288, ext. 8065 (office).
E-mail address: cuijg@public.nn.gx.cn (JG. Cui).

PII: S0039-128X(00)00132-X



7 **8** **9**

10 **11** **1**

Scheme 1.

from EtOAc to give **3** as colorless needle crystals (2.75 g, 96%), m.p. 148–149°C. IR (KBr)ν: 1728, 1651, 1461, 1370, 1039, 969, 800 cm^{-1}.

*2.2. 5a,6β-Dibromostigmastan-3β-yl acetate (**4**)*

Bromine (0.54 g, 3.4 mmol) was added to a solution of iodobenzene (0.66 g, 3.2 mmol) in dry n-hexane (10 ml), and the solution was cooled to −5°C.

A solution of **3** (1.35 g, 3.0 mmol) in dry n-hexane (70 ml) was also cooled to −5°C, stirred vigorously, and the reagent prepared above was added dropwise over 2.5 h under an argon atmosphere to maintain the solution a pale yellow color. The resulting solution was stirred for 2 h more, and then filtered. The solution was concentrated under vacuum until most of the bromosterol was precipitated. The mixture was heated, and the clear solution was allowed to stand overnight in the refrigerator. The resulting 5a,6β-dibromostigmastan-3β-yl acetate was filtered off and washed with MeOH. 1.78 g (97%) of **4** was obtained as colorless crystals, m.p. 128–130°C. IR (KBr) ν: 1735, 1461, 1370, 1236, 1032, 969, 561 cm^{-1}. ^{1}H NMR ($CDCl_3$, 90 MHz): δ = 0.72 (s, 3H, 18-CH_3), 0.78 and 0.85 (d, 6H, J = 4.4 Hz, 26-CH_3 and 27-CH_3), 1.01 (d, 3H, J = 6.8 Hz, 21-CH_3), 1.46 (s, 3H, 19-CH_3), 2.05 (s, 3H, 3-$OCOCH_3$), 4.84 (brd, 1H, 6α-CH), 5.04 to 5.16 (m, 2H, 22- and 23-CH), 5.32 to 5.56 (m, 1H, 3-CH).

*2.3. Cholest-5-en-3β-ol-22-al (**5**)*

A solution of 5α,6β-dibromostigmastan-3β-yl acetate (**4**) (0.93 g; 1.5 mmol) in dried CH_2Cl_2 (30 ml) and pyridine (0.35 ml) was cooled in a liquid nitrogen/ethyl acetate bath. A stream of ozone-rich oxygen was passed into the solution through a sintered-glass sprayer. The reaction was monitored by TLC. When the 5α,6β-dibromostigmastan-3β-yl acetate disappeared (ca. 24 min), the ozonization reaction was stopped. The reaction mixture was immediately poured into a mixture of glacial AcOH (3.8 ml) and Zn dust (2.47 g) and stirred at room temperature for 4 h. The solution was filtered, washed successively with water, 10% sodium carbonate, 5% sodium hydroxide, and brine, and then dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was dissolved in a small amount of MeOH and then combined with 15 ml of saturated $NaHSO_3$ solution. The mixture was stirred at room temperature for 6 h. Ether (15 ml) was added and the mixture was stirred for an additional 10 min. The suspension was filtered, and the solid was washed with water and dried under vacuum to give 0.60 g of the bisulfite addition product.

The bisulfite addition product was suspended in water (2 ml) and ether (40 ml), and then 10% Na_2CO_3 solution (20 ml) was added. The mixture was stirred until two clear layers formed. The organic layer was separated, and the aqueous layer was extracted with ether. The ether solution was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and dried to give 0.46 g (82%) of **5**, m.p. 107–108°C. IR (KBr) ν: 2945, 2846, 1728, 1461, 1370, 1257, 1039 cm^{-1}. ^{1}H NMR ($CDCl_3$, 90 MHz): δ = 0.73 (s, 3H, 18-CH_3), 1.03 (s, 3H, 19-CH_3), 1.13 (δ, 3H, J = 7.0 Hz, 21-CH_3), 2.03 (s, 3H, 3-$OCOCH_3$), 4.59 (m, 1H, 3a-CH), 5.38 (brd, 1H, 6-CH), 9.57(d, 1H, J = 3.2 Hz, 22-CH).

*2.4. 3β-Acetoxycholest-5, 22-diene-24-one (**6**)*

(3-Methyl-2-oxo)butyltriphenyl-arsonium bromide (**12**) (0.31 g, 0.66 mmol), K_2CO_3 (90 mg, 0.66 mmol), and 0.02 ml of H_2O were added to a solution of **5** (0.18 g, 0.48 mmol) in 4 ml of MeCN, and the mixture was

stirred at room temperature for 28 h under argon atmosphere. Water (15 ml) was added to the suspension, and the solution was extracted with ether. The ether solution was washed with water and brine and then dried over Na_2SO_4. The solvent was removed under vacuum, and the residue was purified by flash chromatography on silica gel using petroleum ether (b.p. 60–90°C)/EtOAc (19:1) as eluent to give 0.18 g (85%) of colorless needle crystals **6**, m.p. 143–144°C. IR (KBr)ν: 2938, 1735, 1693, 1623, 1243, 1039, 990 cm^{-1}. ^1H NMR ($CDCl_3$, 90Hz): δ = 0.72 (s, 3H, 18-CH_3), 1.02 (s, 3H, 19-CH_3), 1.10 (d, 9H, J = 6.8 Hz, 21-CH_3, 26-CH_3, 27-CH_3), 2.03 (s, 3H, CH_3CO), 2.84 (m, 1H, 25-CH), 4.60 (m, 1H, 3-CH), 5.38 (m, 1H, 6-CH), 6.06 (d, 1H, J = 16.1Hz, 23-CH), 6.73 (dd, 1H, J = 16.1Hz and 9.0Hz, 22-CH).

*2.5. 3β-Acetoxycholest-5-en-24-one (**7**)*

PtO_2 (16 mg) was added to a solution of **6** (0.22 g; 0.5 mmol) in 30 ml of EtOAc. Hydrogen was passed into the stirred mixture at room temperature. After 0.5 mmol H_2 was absorbed, the reaction was stopped, and the mixture was filtered. The solvent was evaporated to dryness, leaving a white solid, which was recrystallized with CH_3OH to give 216 mg (98%) of colorless needle crystals **7**, m.p. 126–127°C. IR (KBr)ν: 2938, 1728, 1707, 1644, 1468, 1370, 1243, 1032, 962 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): δ = 0.674 (s, 3H, 18-CH_3), 0.915 (d, 3H, J = 6.5 Hz, 21-CH_3), 1.016 (s, 3H, 19-CH_3) 1.091 (d, 6H, J = 7.5 Hz, 26-CH_3, 27-CH_3), 2.033 (s, 3H, CH_3CO), 2.610 (m, 1H, 25-CH), 4.607 (m, 1H, 3-CH), 5.374 (d, 1H, J = 4.5 Hz, 6-CH).

*2.6. 3β-Acetoxy-5a-bromo-6β-hydroxycholestane-24-one (**8**)*

In the dark and at room temperature, four portions of *N*-bromoacetamide (NBA) (0.22g, 1.6 mmol) were added stepwise at 10-min interrals to a solution of 3β-acetoxycholest-5-en-24-one (**7**) (0.24 g, 0.54 mmol) in 2 ml of dioxane containing 70% perchloric acid (0.01 ml) and water (0.10 ml). The mixture was stirred for 30 min, and then 4 ml of freshly prepared 5% sodium sulfite were added into the reaction mixture. The mixture was extracted with methylene chloride (7 ml × 3), and the combined organic layer was successively washed with H_2O, 5% $KHCO_3$, and brine, and then dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel using petroleum ether (60–90°C)/acetone (7:1) as eluent to give 0.20 g (76%) of **8** as white solid (m.p. 145–147°C), and 25 mg of **7** were recovered. IR (KBr)ν: 3409, 2945, 1735, 1707, 1574, 1461, 1370, 1271, 1243, 1159, 1039, 969, 610 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): δ = 0.675 (s, 3H, 18-CH_3), 0.907 (d, 3H, J = 6.5 Hz, 21-CH_3), 1.091 (d, 6H, J = 7.0 Hz, 26-CH_3 and 27-CH_3), 1.318 (s, 3H, 19-CH_3), 2.039 (s, 3H, C_3-CH_3CO), 2.608 (m, 1H, 25-CH), 4.181 (brs, 1H, 6-CH), 5.471 (m, 1H, 3-CH).

*2.7. 3β, 6β-Diacetoxy-5α-bromocholestane-24-one (**9**)*

Acetic anhydride (0.90 g) was added to a solution of **8** (0.12 g, 0.22 mmol) in 1.5 ml of pyridine, and the mixture was stirred at room temperature for 40 h. 10 ml of water were then added and the mixture was extracted with ethyl acetate (8 ml × 4). The combined organic layer was washed successively with water, 1 M HCl solution, water, and brine and then dried over anhydrous Na_2SO_4. After the solvent was evaporated in vacuo, the solid residue was dissolved again using petroleum ether (b.p. 60–90°C)/EtOAc (1:1). The solution was put in the refrigerator overnight. The resulting colorless crystals were filtered and washed with petroleum ether and dried under vacuum to yield 0.11 g (85%) of **9**, m.p. 162–163°C. IR (KBr)ν: 2947, 1750, 1734, 1707, 1561, 1456, 1361, 1241, 1209, 1156, 1031, 668 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): δ = 0.680 (s, 3H, 18-CH_3), 0.906 (d, 3H, J = 6.5 Hz, 21-CH_3), 1.091 (d, 6H, J = 6.5 Hz, 26-CH_3 and 27-CH_3), 1.284 (s, 3H, 19-CH_3), 2.031 (s, 3H, C_3-CH_3CO), 2.092 (s, 3H, C_6-CH_3CO), 2.608 (m, 1H, 25-CH), 4.324 (brs, 1H, 6-CH), 5.454 (m, 1H, 3-CH).

*2.8. 3β, 6β-Diacetoxycholest-4-en-24-one (**10**)*

The solution of bromo-ketone (**9**) (80 mg) in 1.5 ml of dimethylacetamide (DMA) was added to a stirred suspension of calcium carbonate (140 mg) in refluxing DMA (3 ml). Refluxing and stirring were continued for 30 min. The cooled solution was filtered, and the calcium carbonate was washed with ether. To the filtrate, 8 ml of water were added, and the resulting solution was extracted with ether (10 ml × 3). The combined organic layer was washed with water and brine, and then dried over anhydrous Na_2SO_4. After the solvent was evaporated in vacuo, the residue was chromatographed on silica gel using petroleum ether (60–90°C)/acetone (7:1) as eluent to give 65 mg (95%) of **10** as colorless needle crystals, m.p. 143–145°C. IR (KBr)ν: 2938, 1735, 1700, 1468, 1370, 1243, 1166, 1096, 1032, 969, 934, 892 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): δ = 0.719 (s, 3H, 18-CH_3), 0.912 (d, 3H, J = 6.5 Hz, 21-CH_3), 1.092 (d, 6H, J = 6.5 Hz, 26-CH_3 and 27-CH_3), 1.163 (s, 3H, 19-CH_3), 2.030 (s, 3H, C_3-CH_3CO), 2.061 (s, 3H, C_6-CH_3CO), 2.609 (m, 1H, 25-CH), 5.239 (ddd, 1H, J = 10.0, 7.0 and 2.0 Hz, 3-CH), 5.284 (dd, 1H, J = 3.0 and 1.5 Hz, 6-CH), 5.606 (s, 1H, 4-CH).

*2.9. Cholest-4-en-3β,6β-diol -24-one (**11**)*

A total of 1.6 ml of 10% potassium carbonate was added to a solution of 3β, 6β-diacetoxycholest- 4-en-24-one (**10**) (55 mg, 0.11 mg) in 6 ml of MeOH, and the

mixture was refluxed for 2.5 h. The MeOH was evaporated under vacuo, and the residue was dissolved in 20 ml of CH_2Cl_2. The methylene chloride layer was washed with water and brine, dried over anhydrous sodium sulfate, and evaporated in vacuo to give a white solid. The crude product was recrystallized with acetone/CH_3OH (1:1) to give 35 mg (77%) of colorless needle crystals **11**, m.p. 211–212°C. IR (KBr)ν: 3291, 2931, 1716, 1635, 1466, 1382, 1093, 1036, 1019, cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): δ = 0.707 (s, 3H, 18-CH_3), 0.907 (d, 3H, J = 7.0 Hz, 21-CH_3), 1.090 (d, 6H, J = 6.5 Hz, 26-CH_3 and 27-CH_3), 1.255 (s, 3H, 19-CH_3), 2.33–2.50 (m, 2H, 23-CH_2), 2.607 (m, 1H, 25-CH), 4.180 (ddd, 1H, J = 9.0, 6.5 and 1.0 Hz, 3-CH), 4.227 (dd, 1H, J = 3.0 and 2.5 Hz, 6-CH), 5.543 (s, 1H, 4-CH).

*2.10. 24-Methylenecholest-4-en-3β,6β-diol (**1**)*

Of 80% NaH-paraffin (2.33 mmol of sodium hydride), 56 mg was placed in a two-neck flask. After the paraffin was washed away with petroleum ether (30–60°C), 4 ml of anhydrous dimethyl sulfoxide were added to the dried NaH under an argon atmosphere. The mixture was stirred at 80°C for 40 min, and a dark green solution was produced. After cooling to room temperature, a solution of 350 mg (0.88 mmol) of methyltriphenylphosphonium iodide (**13**) in 2 ml of anhydrous dimethyl sulfoxide was added, and the solution turned yellowish in color immediately. After 10 min, a solution of 28 mg (0.067 mmol) of cholest-4-en-3β,6β-diol -24-one (**11**) in 2 ml of anhydrous dimethyl sulfoxide was added, and the resulting mixture was stirred at 80°C for 4 h. The reaction mixture was cooled, diluted with 15 ml of cold water, and extracted with ether. The combined organic extracts were washed with water and then brine. After drying over anhydrous sodium sulfate, the solvent was removed under reduced pressure, and the resulting crude product was purified by flash chromatography on silica gel using petroleum ether (b.p. 60–90°C)/acetone (3:1) as eluent to give 18 mg (65%) of colorless needle crystals **1**, m.p. 224–225°C. IR (KBr)ν: 3301, 1639, 1463, 1382, 1274, 1158, 1089, 1029, 879, 836 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ = 0.715 (s, 3H, 18-CH_3), 0.941 (d, 3H, J = 6.5 Hz, 21-CH_3), 1.017 (d, 3H, J = 2.5 Hz, 26-CH_3 or 27-CH_3), 1.031 (d, 3H, J = 2.5 Hz, 26-CH_3 or 27-CH_3), 1.259 (s, 3H, 19-CH_3), 2.227 (m, 1H, 25-CH), 4.181 (ddd, 1H, J = 9.0, 6.5 and 1.0 Hz, 3α-CH), 4.231 (brt, 1H, J = 2.5 Hz, 6α-CH), 5.545 (brs, 1H, 4-CH).

*2.11. (3-Methyl-2-oxo)butyltriphenylarsonium bromide (**12**)*

A solution of triphenylarsine (8.80 g, 29 mmol) and 1-bromo-3-methyl-2-butanone (4.50 g, 27 mmol) in 3 ml of benzene was stirred at about 65°C under an argon atmosphere for 10 h. The white solid formed was filtered and washed with benzene. The crude product was recrystallized from $CHCl_3$ /EtOAc (2:1) to give 7.61 g (60%) of colorless crystals **12**, m.p. 182 (decomposition). IR (KBr) ν: 2797, 1693, 1440, 1032, 744, 688, 477 cm^{-1}. ^1H NMR ($CDCl_3$, 90 MHz): δ = 1.16 (d, 6H, J = 7.1 Hz, $C(CH_3)_2$), 2.96 to 3.44 (m, 1H, CH), 5.97 (s, 2H, CH_2), 7.33 to 7.96 (m, 15H, Ph-H).

*2.12. Methyltriphenylphosphonium iodide (**13**)*

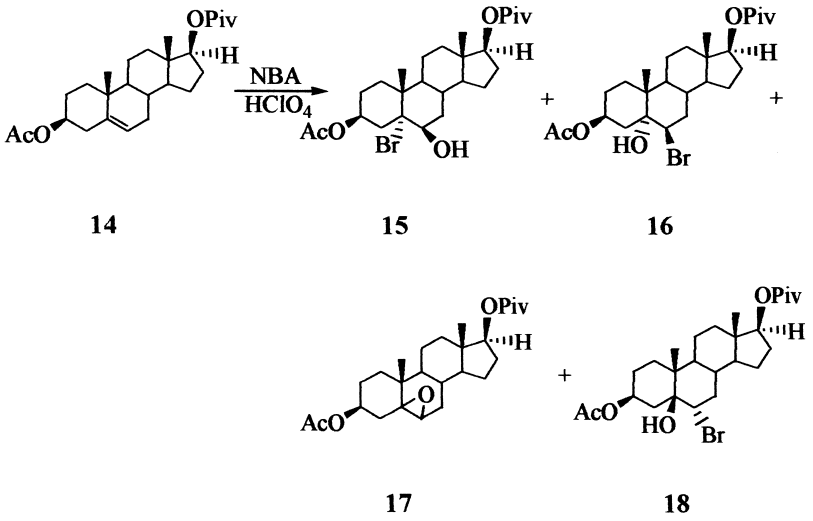
Triphenylphosphine (5.24 g, 20 mmol) was dissolved in 20 ml dry benzene. Iodomethyl (2.67 g, 21 mmol) was added, and the mixture was stirred at room temperature for 16 h. The resulting white solid was isolated with suction and washed with benzene. The crude product was recrystallized from $CHCl_3$/EtOAc (1:1) to afford 7.53 g (97%) of colorless crystals **13**, m.p. 193–194°C. IR (KBr) ν: 1586, 1484, 1439, 1116, 915, 749 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): δ = 3.211 (d, 3H, J = 13.0 Hz, CH_3), 7.699 to 7.840 (m, 15H, arom-H).

3. Results and discussion

In the synthesis of steroids, the commercially available stigmasterol is often used as a starting material because the double bond in the side chain of stigmasterol can be easily oxidized to an aldehyde by ozonization. The advantages of this process are that various types of side chains can be constructed via this aldehyde, and the configuration of C-17 and C-20 of the natural steroids will be maintained [4–5]. Therefore, stigmasterol was chosen as a starting material for the synthesis of 24-methylenecholest-4,5-ene-3β,6β-diol (**1**).

The planned synthetic route from stigmasterol (**2**) to the target compound 24-methylenecholest-4-en-3β,6β-diol (**1**) is shown in Scheme 1. The 3β-hydroxy group of **2** was protected by acetylation, then bromination of 3β-acetoxystigmasterol (**3**) was performed by using iodobenzene dibromide to give the 5,6-dibromide (**4**), which is pure enough for ozonolysis [6]. When the ozonide was decomposed with zinc and acetic acid at room temperature, debromination of **4** was simultaneously carried out to give compound (**5**). For the conversion of aldehyde **5** to ketone **6**, a solid-liquid phase-transfer Wittig reaction was used [7]. The reaction was performed at room temperature using (3-methyl-2-oxo)butyltriphenylarsonium bromide (**12**) as reagent and K_2CO_3 as base in CH_3CN. The addition of a trace of H_2O or $HCONH_2$ to the reaction mixture greatly accelerates this reaction. The coupling constant ($^3J_{H\text{-}H}$ = 15.5Hz) at the 22, 23-double bond revealed that this reaction was stereoselective and produced only the trans-isomer. Catalytic hydrogenation of **6** gave nearly quantitative amounts of compound (**7**).

The 3β-acetoxycholest-5-en-24-one (**7**) was converted to bromohydrin (**8**) with NBA containing a catalytic amount of 70% $HClO_4$ in dioxane/water under dark conditions. Rodewald et al. studied the reaction of compound (**14**) with NBA and showed that in addition to the major product bromohydrin (**15**), the reaction mixture contained the minor, more

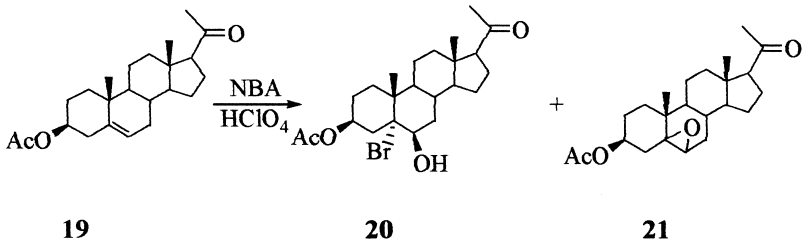


Scheme 2.

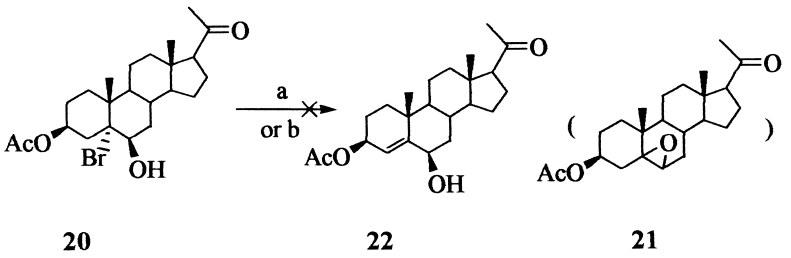
mobile products (**16**), (**17**), and (**18**) (Scheme 2). The mechanism of this reaction was also discussed [8].

The model compound (**19**) was used to study this reaction, and 61% of bromohydrin (**20**) was obtained when the ratio of **19** (0.50 mmol): 70% $HClO_4$ (0.01 ml): H_2O (0.05 ml) was used (Scheme 3). This showed that the amount of $HClO_4$ had a key effect on the reaction. If the acidity was either too strong or too weak, it was unfavorable for the formation of **20**. A better reaction condition was obtained through the model reaction. Using the same reaction condition as for compound (**19**), bromohydrin (**8**) was obtained in 76% yield from **7**.

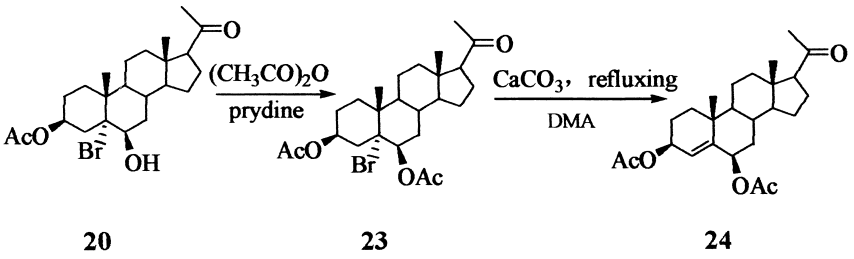
The elimination of HBr from **20** to **22** using a method from the literature [9–10] yield compound (**21**) as the major product, but not the desired compound (**22**) (Scheme 4). We suppose that in compound (**20**), the C_6-OH trans to C_5-Br might accelerate the formation of 5,6-β-epoxide (**21**). Therefore, the C_6-OH of **20** was protected by acetylation to give **23**. In compound (**23**), HBr can be eliminated by boiling in dimethylacetamide (DMA) containing calcium carbonate to give **24** in 87% overall yield (Scheme 5). Referring to the reaction from **20** to **24**, compound **10** was prepared from the bromohydrin **8** in 81% yield. The preparation of **1** directly from compound **10** was unsuccessful. Therefore, **10** was hydrolyzed to compound **11**, methylenation of **11** was completed by Wittig reaction with methylenetriphenylphosphonium iodide **13**, and the target compound **1** was obtained. The IR and ^1H NMR spectra data of **1** are completely consistent with those of the natural compound **1**. This synthesis unambiguously confirmed the struc-



Scheme 3.



Scheme 4. a. Heated at 105°C for 30 min in glacial acetic acid containing the catalytic amount of HCl. b. Heated under reflux for 9 h in dry pyridine.



20 23 24

Scheme 5.

ture of the natural product **1** as 24-methylenecholest-4-en-3β,6β-diol.

References

[1] Zeng Long–Mei, Li Xiao–Qing, Su Jing–Yu, Fu Xiong, Schmitz FJ. A new cytotoxic dihydroxy sterol from the soft coral alcyonium patagonicum. J Nat Prod 1995;58(2):296.

[2] Zhong Yl, Su JY, Zeng LM, Shen W, Wang Q-W. Structure of a new sterol from the south China sponge *Dysidea fragilis*. Chinese J Chem 1993;11(6):560.

[3] Wang GS, Li FY, Zeng LM, Ma LB, Tu GZ. Structure determination of nephalsterol A and B with 19-hydroxy group from soft corals Nephthea. Chem J Chinese Uni 1992;13:623.

[4] Jung ME, Johnson TW. Synthesis of 7-deoxyxestobergestrol A. A novel pentacyclic steroid of the xestobergestrol clase. J Am Chem Soc 1997;119(50):12412.

[5] David MP, Jerzy W. Various approaches to the construction of aliphatic side chains of steroids and related compounds. Chem Rev 1978;78:199.

[6] Fryberg M. Oehlschlager AC. Unrau AM. The synthesis of possible polyene intermediates in phytosterol biosynthesis. Tetrahedron 1971; 27:1261.

[7] Yao–Zeng H, Li–lan Shi, Sao–Wei Li. An efficient and stereoselective synthesis of (E)-a-enones via synthesis of brassinosteroid and prostaglandin. Synthesis 1988;(12):975.

[8] Rodewald WJ. Jaszczynski JR. Sicinski, RR. 19-functionalized steroids. Part I. An improved synthesis of androst-5-ene-3β, 17β, 19-triol derivatives. Polish J Chem 1978;52:501.

[9] Chorvat RJ. Pappo R. Synthesis of 2-azaestratrienes. J Org Chem 1976;41:2864.

[10] Heilbron IM. Jones ERH. Spring FS. Studies in the sterol group. Part XXXII. The bromination of 6-ketocholestanyl acetate. J Chem Soc 1937:801.