Chemistry of Natural Compounds, Vol. 37, No. 2, 2001

SYNTHESIS OF 22,23-EPOXYECDYSTEROIDS FROM STIGMASTEROL

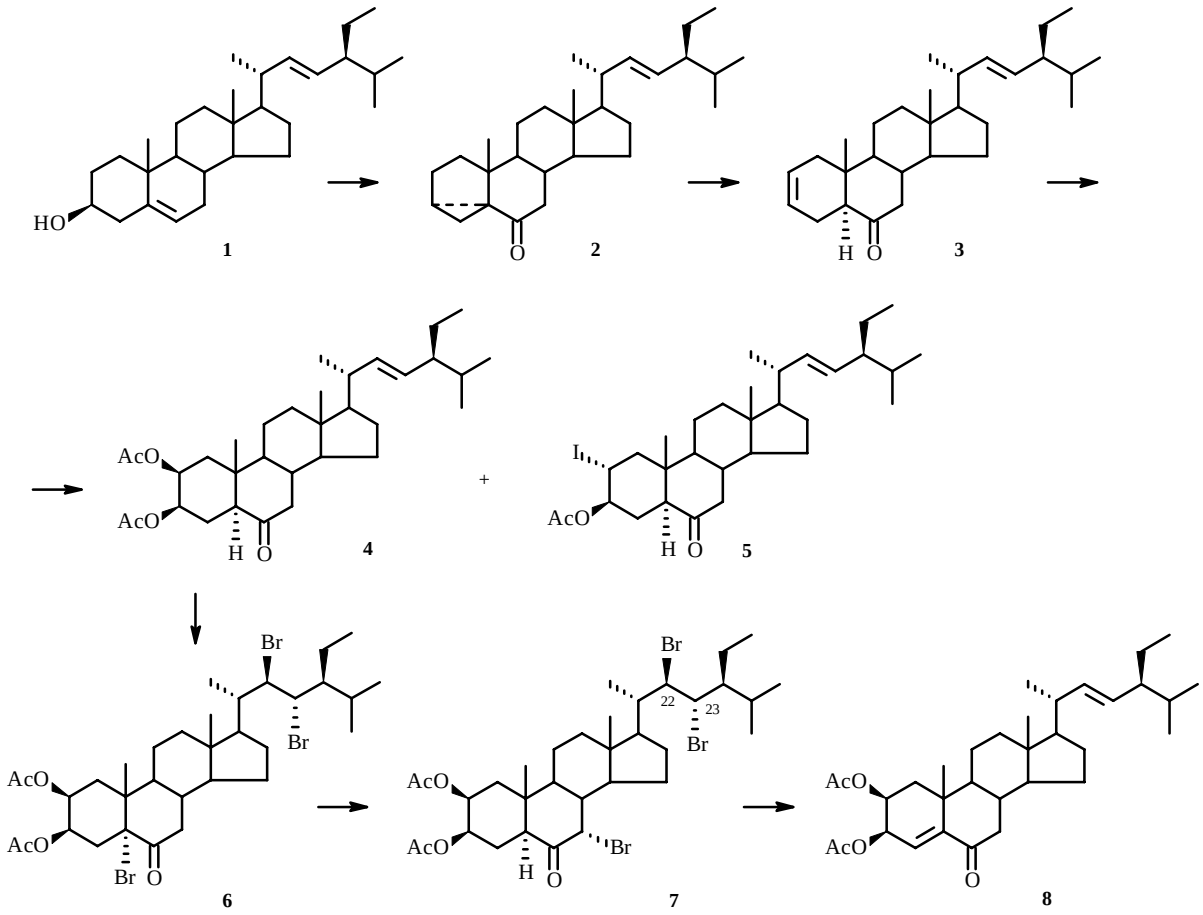
N. V. Kovganko and V. L. Survilo

UDC 547.92

A new synthetic scheme has been developed to synthesize 22,23-epoxyecdysteroids from stigmasterol.

Key words: stigmasterol, 22,23-epoxyecdysteroids.

Fungi have yielded ecdysteroids containing the functional groups typical of this type of compounds in addition to 22,23-epoxides [1]. Such compounds are polyporusterones C and E [2] and atrotosterone B and 25-hydroxyatrotosterone B [3]. The observation of these ecdysteroids in nature stimulated us to develop a chemical synthesis of them. We previously proposed a synthetic scheme [4] of ecdysteroids containing both 22,23-epoxides and 14α-hydroxy-Δ^7-6-keto groups. In continuation of this research, we developed a method for synthesizing ecdysteroids containing 22,23-epoxides in the side chain and the main functional groups in the cyclic part of the molecule.



Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, 220141, Belarus, Minsk, ul. Akad. Kuprevicha, 5/2. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 135-141, March-April, 2001. Original article submitted March 12, 2001.

0009-3130/01/3702-0154$25.00

Our plan was to first form the necessary functional grups in the tetracyclic steroidal skeleton and then to introduce the 22,23-epoxide in one of the last steps via epoxidation of a 22(23)-double bond with peracid. Thus, reaction of starting stigmasterol **1** with methanesulfonyl chloride in pyridine gives the corresponding mesylate, which was without further purification solvolyzed in aqueous acetone in the presence of KOAc to give the 3α,5-cyclo-6β-alcohol in 67.5% yield. Then, this compound was oxidized by chromic acid in acetone according to Jones. This procedure gave the 3α,5-cyclo-6-ketone **2** in 92.6% yield. The structure of **2** was confirmed by comparing its IR and ^{1}H NMR spectra with those of the authentic compound that was prepared earlier [5, 6]. In the next step, **2** was isomerized by *p*-toluenesulfonic acid in sulfolane by the literature method [7, 8]. This gave the $\Delta^{2,22}$-6-ketone **3** in 56.7% yield. The structure of **3** was confirmed by comparing its spectra with those of the authentic compound that had been synthesized previously [6, 9]. Then we succeeded in simplifying the synthesis of **3** from **1** by carrying out all reactions without purifying the intermediates. This improvement produced the required **3** in 68% overall yield calculated on the basis of starting stigmasterol.

The double bonds in **3** differ markedly in reactivity. Therefore, judging from the literature [10], the 2β,3β-dihydroxy group characteristic of ecdysteroids can be selectively introduced into **3** by the Woodward reaction using silver acetate and iodine in aqueous acetic acid via *cis*-hydroxylation of the sterically more accessible 2(3)-double bond. In order to introduce this functional group, we carried out this selective *cis*-hydroxylation of the 2(3)-double bond in **3** with the calculated amounts of reagents. The reaction products were not isolated but acetylated by acetic anhydride in pyridine. Chromatographic separation of the product mixture gave 2β,3β-diacetoxy-6-ketosteroid **4** and 2α-iodo-3β-acetoxy-6-ketosteroid **5** in yields of 53.3 and 7.0%, respectively. The structures of **4** and **5** were proved using spectra. The IR spectrum of **4** has absorption bands at 1755 and 1240 cm^{-1}, which are characteristic of acetoxy. The spectrum also has an absorption band for the 6-ketone at 1720 cm^{-1}. The presence in the molecule of two acetate groups was also confirmed by ^{1}H NMR, the spectrum of which contains singlets at 2.00 and 2.08 ppm in addition to signals for protons H-2 and H-3 (δ 5.30 and 4.80 ppm, respectively) geminal to the acetoxy. The splitting pattern of these signals indicates that they are both *cis* to the acetoxy. The presence of signals for H-22 and H-23 vinyl protons (δ 5.02 and 5.16 ppm) confirms that the 22(23)-double bond remains intact in the molecule.

The presence of iodine in the minor steroid **5** was determined by elemental analysis and a positive Beilstein test. The ^{1}H NMR spectrum indicates that **5** contains one acetate group (singlet at δ 2.10 ppm). The spectrum also has signals for protons geminal to I (δ 4.20 ppm) and acetoxy (δ 4.88 ppm). The splitting pattern suggests that both signals belong to axial protons. Therefore, the I and acetoxy in **5** are in equatorial positions. Taking into account the fact that *trans-A/B* fusion is present according to ^{1}H NMR, two structural variants are possible for **5**: 2α-iodo-3β-acetoxy-6-ketone and 2α-acetoxy-3β-iodo-6-ketone. The former was chosen based on double resonance results. Saturation of the proton geminal to the I greatly simplified the signal of the proton geminal to the acetoxy and changed the signal of the neighboring methylene proton (doublet of doublets with δ 2.54 ppm) to a doublet with splitting constant J = 13.5 Hz. The conclusion was drawn that the signal at 2.54 ppm corresponds to the resonance of methylene H-1β and not H-4β because the latter in this instance would have a more complicated splitting owing to coupling with H-4α and H-5α.

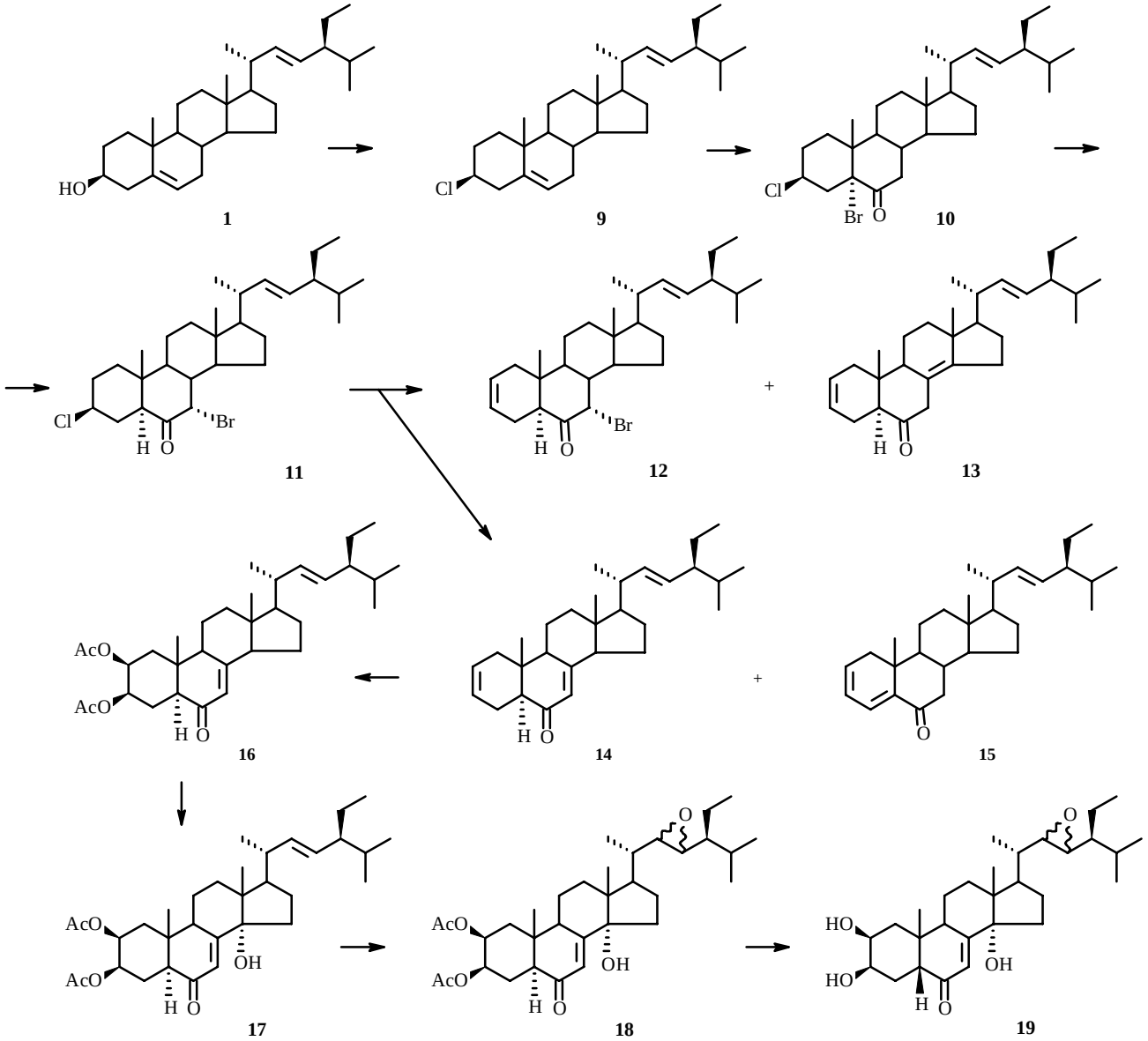
We planned to introduce the 7(8)-double bond into **4** via bromination at the position α to the 6-ketone, rearrangement of the resulting 5α-bromo-6-ketone into the more stable 7α-bromo-6-ketone, and subsequent dehydrobromination. Bromination of **4** in a mixture of acetic acid and CH_2Cl_2 gave **6** in 63% yield. It structure was unambiguously proved by ^{1}H NMR spectra, which lack signals for protons of a 22(23)-double bond. However, signals of methine protons H-22 and H-23 that are geminal to Br appear (4.40 and 4.50 ppm). The 5α-Br in this molecule shifts the signal for H-3α to weak field to 5.56 ppm in the ^{1}H NMR spectrum owing to the 1,3-diaxial coupling. Analogously, the Br on C-5 shifts the signal for methylene proton H-7α to 3.21 ppm.

Rearrangement of **6** into **7** was carried out in 71% yield using Br_2 and HBr in acetic acid. The structure of **7** was proved by the presence in the ^{1}H NMR of a doublet (δ 4.20 ppm, J = 3 Hz) for methine proton H-7β, which is geminal to Br. The shift to strong field (4.88 ppm) of the signal for methine proton H-3α is also very characteristic. This is caused by the lack of Br on C-5 in this molecule.

Dehydrobromination of **7** by lithium carbonate and bromide in the presence of phenol in DMF with boiling was suggested. It was found [11] that both dehydrobromination involving the Br atom in the position α to the ketone and debromination of the 22,23-dibromide to form the 22(23)-double bond occur under these conditions. Namely this method was used to synthesize (22S,23S)-28-homobrassinolide [6, 12] and (22E)-24-ethylcholesta-7,22-dien-3β,5α,6β-triol [13]. However, we made several unsuccessful attempts to prepare the corresponding $\Delta^{7,22}$-6-ketone from the 7α,22,23-tribromo-6-ketosteroid **7** by this method. The principal product is 4,22-dien-6-ketone **8**, isolated in >50% yield. The structure of **8** was determined

using spectra. In particular, an absorption band in the UV spectrum with a maximum at 234 nm is very characteristic of Δ^4-6-ketosteroids [14]. Such a conclusion about the structure of **8** can be made on the basis of the ^{1}H NMR spectrum, which contains a doublet of doublets for the vinyl proton H-4 with δ 5.90 ppm. The position and multiplicity of this signal correspond exactly with those of the same signal in the spectrum of the corresponding cholestane derivative [14]. The signals of H-2 and H-3 in the spectrum of **8** are unambiguously assigned using double resonance. Saturation of the signal of the proton with δ 5.36 ppm converts the signal of H-4 to a doublet. Therefore, the signal with δ 5.36 ppm belongs to H-3. The dihedral angle between H-3 and H-4 is close to 90° because the vicinal coupling constant between them is very small. The spectrum of **8** also contains signals for vinyl protons H-22 and H-23. The position and shape of the H-22 and H-23 signals in the spectra of **8** and stigmasterol coincide completely. This indicates that **8** contains a 22(23)-double bond.

It should be noted that dehydrobromination of 7α-bromo-6-ketosteroids to form Δ^4-6-ketosteroids has been observed several times [14-17]. Compounds of such structure are supposedly prepared via a 1,4-elimination reaction. Apparently the conditions necessary for debromination of the 22,23-dibromide in **7** are too forcing and enhance the occurrence of such a reaction.



The inability to transform 7α,22,23-tribromo-6-ketosteroid into **7** prompted us to find alternative methods of synthesizing 7,22-dien-6-ketosteroids from stigmasterol. We used previous experience in this area [4, 18-20]. First, **9** was prepared in ~90% yield via reaction of stigmasterol with thionyl chloride according to the literature [19]. Selective addition

at the sterically more accessible 5(6)-double bond of hypobromous acid and subsequent oxidation by chromic acid without isolating the bromohydrin converted **9** into **10** in 40-50% overall yield. The structure of **10** was easily proved because its IR and ^{1}H NMR spectra have essentially the same characteristics as those of 3β-chloro-5α-bromo-6-ketosteroids that were prepared by analogous methods [20]. The presence of the 22(23)-double bond in **10** is indicated by the presence in the ^{1}H NMR spectrum of signals for vinyl protons H-22 and H-23, the position and shape of which agree completely with the analogous signals for the same protons in the spectrum of stigmasterol.

Rearrangement of **10** into the thermodynamically more stable **11** should be carried out using HBr in acetic acid. It has been shown earlier [4, 18] that this reaction occurs without involving the 22(23)-double bond. In fact, it has been found that **11** is formed in 86% yield from **10** under these conditions. The structure was confirmed by the presence in the ^{1}H NMR of **11** of signals for methine protons H-3α (δ 3.86 ppm) and H-7β (δ 4.20 ppm) and vinyl protons H-22 (δ 5.02 ppm) and H-23 (δ 5.16 ppm).

Dehydrohalogenation of **11** using lithium carbonate and bromide in DMF is rather complicated. The reaction produces 7α-bromo-$\Delta^{2,22}$-6-ketone **12**, $\Delta^{2,8(14),22}$-6-ketone **13**, $\Delta^{2,7,22}$-6-ketone **14**, and $\Delta^{2,4,22}$-6-ketone **15** in yields of 11, 28, 32, and 10%, respectively. The last two compounds were obtained as a mixture that could not be separated despite repeated attempts. The structures of **12-15** were established by analyzing spectra. Thus, the presence in the ^{1}H NMR spectrum of **12** of the signal for methine proton H-7β at 4.10 ppm indicates that the Br is on C-7. Signals for vinyl protons H-2, H-3, H-22, and H-23 indicate that the structure is a $\Delta^{2,22}$-steroid. Such signals are also characteristic for the ^{1}H NMR spectrum of **13**. This confirms that **13** contains 2(3)- and 22(23)-double bonds. This compound is a saturated 6-ketone according to a band at 1715 cm^{-1} in the IR spectrum. The position of the third double bond was established using ^{13}C NMR spectra, in which the signals for C-8 and C-14 with chemical shifts 122.5 and 145.7 ppm, respectively, were determined. The presence of these signals at weak field indicates that both these atoms are bonded to the double bond. It should be noted that the chemical shifts of C-8 and C-14 in the ^{13}C NMR spectrum of **13** agree well with the literature data for the signals of analogous atoms in the spectrum of a $\Delta^{8(14)}$-6-ketoecdysteroid [21].

The presence in the UV spectrum of bands at 245 and 315 nm indicates that **14** and **15** are a Δ^7-6-ketone and $\Delta^{2,4}$-6-ketone, respectively, with the former predominating. This same conclusion results from the presence in the ^{1}H NMR spectrum of signals for the H atoms of the main compound. These are mainly multiplets of vinyl protons H-2 and H-3 (δ 5.58 and 5.72 ppm) and a triplet of H-7 (δ 5.76 ppm). The structure of the minor compound **15** was proved by the presence in the spectrum of signals for vinyl protons H-2 and H-3 (δ 6.08 ppm) and H-4 (δ 6.84 ppm). These signals are characteristic of the ^{1}H NMR spectrum of 2,4,22-trien-6-one **15**, which was synthesized earlier [11].

Hydroxylation of 2,4-dien-6-one **15** according to Woodward with subsequent acetylation is known [22] to form not the diacetate 2β,3β-diol but the diacetate 2α,3α-diol, whose properties differ considerably from those of **15**. Therefore, we decided to carry out this reaction without separating **14** and **15**. Acetylation of the product mixture with acetic anhydride in pyridine and subsequent chromatographic purification gave the desired 2β,3β-diacetoxy-$\Delta^{7,22}$-6-ketone **16** in >40% yield. The structure was determined from spectra. Thus, the presence in the UV spectrum of a strong absorption at 245 nm indicates that **16** is a Δ^7-6-ketosteroid. This is confirmed by the ^{1}H NMR spectrum, which contains a triplet characteristic of vinyl proton H-7 with δ 5.76 ppm. This signal is split by allylic coupling of H-7 to methine protons H-9 and H-14. The presence in the ^{1}H NMR spectrum of signals for methyl protons with δ 2.03 and 2.09 ppm convincingly indicates that **16** contains two acetoxy groups. The position and shape of the H-2 and H-3 signals in the spectra of **4** and **16** are practically the same. Therefore, the structure of the latter is the diacetate of the 2β,3β-diol.

The 14α-hydroxy was introduced in the next step via allylic hydroxylation of **16** with selenium dioxide in dioxane. The structure of **17** that was prepared this way in 64% yield was unambiguously determined using spectra. In particular, the 14α-hydroxy in **17** shifts the signal for methine proton H-9α, which is 1,3-diaxial to it, to weak field (2.73 ppm) in the ^{1}H NMR spectrum. The signal for vinyl proton H-7 in the spectrum of **17** is observed as a doublet, which is explained by the lack of a proton on C-14. The shift to weak field (5.94 ppm) of the signal for H-7 in the ^{1}H NMR spectrum of **17** that is caused by the 14α-hydroxy is interesting when compared with its position in the spectrum of starting **16**.

Epoxidation of the 22(23)-double bond in **17** with *m*-chloroperbenzoic acid produced the 22,23-epoxide **18** as a mixture of the (22R,23R)- and (22S,23S)-isomers with the former predominant. The presence of the 22,23-epoxy in **18** causes signals of protons H-22 and H-23 that are geminal to it to appear at 2.50-3.00 ppm in the ^{1}H NMR spectrum.

In the final step, the acetates in **18** are hydrolyzed by K_2CO_3 in aqueous methanol. This also isomerizes C-5 to form the more stable *cis-A/B*-steroid **19**, which we isolated in 38% yield. The UV spectra and ^{1}H NMR of **19** unambiguously confirm

that it contains the principal structural groups. It should be noted that the spectral parameters obtained by us for **19** agree with the analogous characteristics for the 22,23-epoxyecdysteroid polyporusterone E [2]. This also confirms the structure of the compound synthesized by us.

EXPERIMENTAL

Melting points were determined on a Kofler block. IR spectra were recorded on a UR-20 instrument in the range 700-3600 cm^{-1} in KBr pellets. UV spectra of ethanol solutions were recorded on a Specord M-400 instrument. ^1H and ^{13}C NMR spectra were obtained on a Bruker AC-200 NMR spectrometer at working frequencies of 200 and 50.32 MHz, respectively. Chemical shifts are reported relative to TMS internal standard.

Woodward Hydroxylation of (22E,24S)-5α-Stigmasta-2,22-dien-6-one (3). A solution of **3** (3.07 g) in acetic acid (50 mL) was treated with silver acetate (2.90 g), iodine (2.10 g), and water (5 mL). The reaction mixture was stirred at 50-60°C for 2 h, cooled to room temperature, filtered through a layer of aluminum oxide, and evaporated under vacuum. The solid was dried at 60°C under vacuum for 2 h, dissolved in pyridine (12 mL), treated with acetic anhydride (3 mL), left for 15 h, diluted with water, and extracted with dichloroethane. The organic layer was washed with water and evaporated under vacuum. The solid was chromatographed on a silica-gel column with elution by hexane—THF (10:1). Yield of **5**, 0.30 g (7.0%), mp 147-150°C (hexane—acetone). IR spectrum (ν, cm^{-1}): 1750, 1240 (AcO), 1720 (C=O).

^1H NMR ($CDCl_3$, δ, ppm, J/Hz): 0.68 (3H, s, 18-Me), 0.80 (3H, s, 19-Me), 1.04 (3H, d, J = 6.5, 21-Me), 2.10 (3H, s, AcO), 2.54 (1H, dd, J_1 = 13.5, J_2 = 4.5, H-1β), 4.20 (1H, td, J_1 = 12, J_2 = 4.5, H-2β), 4.88 (1H, td, J_1 = 11.5, J_2 = 5, H-3α), 5.02 (1H, dd, J_1 = 15, J_2 = 7.5, H-22), 5.16 (1H, dd, J_1 = 15, J_2 = 7.5, H-23). Found, %: C 64.89, H 8.78, I 20.82; calc. for $C_{31}H_{49}O_3I$, %: C 62.41, H 8.28, I 21.27.

Then, **4** was isolated. Yield 2.18 g (53.3%), mp 180-184°C (hexane—acetone). IR spectrum (ν, cm^{-1}): 1755, 1240 (AcO), 1720 (C=O). ^1H NMR ($CDCl_3$, δ, ppm, J/Hz): 0.68 (3H, s, 18-Me), 0.95 (3H, s, 19-Me), 1.02 (3H, d, J = 6.5, 21-Me), 2.00 (3H, s, AcO), 2.08 (3H, s, AcO), 4.80 (1H, dt, J_1 = 11.5, J_2 = 4.5, H-3α), 5.02 (1H, dd, J_1 = 15, J_2 = 7.5, H-22), 5.16 (1H, dd, J_1 = 15, J_2 = 7.5, H-23), 5.30 (1H, br. d, J = 2.5, H-2α).

(22R,23S,24S)-2β,3β-Diacetoxy-5,22,23-tribromo-5α-stigmastan-6-one (6). Diacetoxyketone **4** (1.00 g) was dissolved in dichloroethane (2.5 mL) and treated with acetic acid (10 mL) and bromine in acetic acid (2.5 mL, 2 M). The reaction mixture was held for 10 min at 40-50°C, left at room temperature for 24 h, stirred, treated with sodium sulfite solution (2 mL, 1.6 M), diluted with water after 10 min, and extracted with dichloroethane. The organic layer was washed with water and evaporated under vacuum. The solid was chromatographed on a silica-gel column with elution by hexane—THF (10:1). Yield 0.89 g (63%) of **6**, mp 200-205°C (dec.) (hexane—THF). IR spectrum (ν, cm^{-1}): 1750, 1250 (AcO), 1720 (C=O). ^1H NMR ($CDCl_3$, δ, ppm, J/Hz): 0.73 (3H, s, 18-Me), 0.98 (3H, s, 19-Me), 1.01 (3H, d, J = 6.5, 21-Me), 2.00 (3H, s, AcO), 2.08 (3H, s, AcO), 3.21 (1H, dd, J_1 = 15, J_2 = 12, H-7α), 4.40 (1H, dd, J_1 = 11.5, J_2 = 1.5, H-22/23), 4.50 (1H, d, J = 11.5, H-22/23), 5.39 (1H, d, J = 3, H-2α), 5.56 (1H, dt, J_1 = 11.5, J_2 = 5, H-3α).

(22R,23S,24S)-2β,3β-Diacetoxy-7α,22,23-tribromo-5α-stigmastan-6-one (7). Tribromide **6** (0.70 g) was dissolved in acetic acid (25 mL) and treated with HBr (0.1 mL, 40%) and bromine in acetic acid (0.2 mL, 2 M). The reaction mixture was held at 45-60°C for 2 h, cooled to room temperature, stirred and treated with sodium sulfite (0.25 mL, 1.6 M), diluted with water after 10 min, and extracted with dichloroethane. The organic layer was washed with water and evaporated under vacuum. The solid was chromatographed on a silica-gel column with elution by hexane—THF (10:1). Yield of **7** 0.5 g (71%), mp 196-200°C (dec.) (hexane—$CHCl_3$). IR spectrum (ν, cm^{-1}): 1745, 1250 (AcO), 1720 (C=O).

^1H NMR ($CDCl_3$, δ, ppm, J/Hz): 0.76 (3H, s, 18-Me), 0.98 (3H, s, 19-Me), 1.02 (3H, d, J = 6.5, 21-Me), 2.00 (3H, s, AcO), 2.08 (3H, s, AcO), 3.40 (1H, dd, J_1 = 12.5, J_2 = 2, H-5α), 4.20 (1H, d, J = 3, H-7β), 4.23-4.49 (2H, m, H-22 and H-23), 4.88 (1H, dt, J_1 = 11.5, J_2 = 4.5, H-3α), 5.30 (1H, br. d, J = 2.5, H-2α).

(22E,24S)-2β,3β-Diacetoxystigmasta-4,22-dien-6-one (8). Tribromide **7** (0.63 g) was dissolved in DMF (10 mL) and treated with Li_2CO_3 (0.30 g), LiBr (0.10 g), and phenol (0.10 g). The reaction mixture was boiled for 30 min, cooled to room temperature, filtered, diluted with water, and extracted with dichloroethane. The organic layer was washed with saturated aqueous $NaHCO_3$ and filtered through a layer of silica gel. The solvent was removed in a rotary evaporator. The solid was chromatographed on a silica-gel column with elution by hexane—THF (15:1). Yield of **8** 0.24 g (56%), mp 196-200°C (hexane—$CHCl_3$). UV spectrum (λ_{max}, nm): 234. ^1H NMR ($CDCl_3$, δ, ppm, J/Hz): 0.72 (3H, s, 18-Me), 0.81 (3H, s, 19-Me),

1.02 (3H, d, J = 6.5, 21-Me), 2.06 (3H, s, AcO), 2.08 (3H, s, AcO), 5.02 (1H, dd, J_1 = 15, J_2 = 7.5, H-22), 5.16 (1H, dd, J_1 = 15, J_2 = 7.5, H-23), 5.36 (1H, m, W/2 = 14, H-3α), 5.46 (1H, br. t, J = 3, H-2α), 5.90 (1H, dd, J_1 = 2.5, J_2 = 1, H-4).

(22E,24S)-3β-Chloro-5-bromo-5α-stigmast-22-en-6-one (10). A solution of **9** (2.31 g, obtained by reaction of **1** and thionyl chloride by the literature method [19]) in dioxane (120 mL) was treated with water (3.2 mL) and perchloric acid (1.2 mL, 32%), stirred, treated over 40 min with portions of N-bromoacetamide (1.00 g), after 30 min with chromic acid (4 mL, 8 M), and stirred for another 55 min. The excess of oxidant was neutralized with isopropanol (10 mL). The solution was filtered through a layer of aluminum oxide. Most of the solvent was evaporated in a rotary evaporator. The solid was diluted with water and extracted with toluene. The organic layer was washed with water and evaporated under vacuum. The solid was chromatographed on a silica-gel column with elution by petroleum ether. Yield of **9**, 0.47 g (20%).

Then, elution by petroleum ether—ethylacetate (80:1) gave **10**, 1.12 g (40%), mp 142-145°C (acetone). IR spectrum (ν, cm^{-1}): 1720 (C=O).

^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 0.68 (3H, s, 18-Me), 0.99 (3H, s, 19-Me), 1.03 (3H, d, J = 6.5, 21-Me), 4.46 (1H, m, W/2 = 25, H-3α), 5.02 (1H, dd, J_1 = 15, J_2 = 7.5, H-22), 5.16 (1H, dd, J_1 = 15, J_2 = 7.5, H-23).

(22E,24S)-3β-Chloro-7α-bromo-5α-stigmast-22-en-6-one (11). A solution of **10** (0.51 g) in acetic acid (35 mL) was heated to 60°C, stirred, treated with HBr (1.4 mL, 40%), stirred at 56-58°C for 1.5 h, cooled to room temperature, diluted with water, and extracted with toluene. The organic layer was washed with water and evaporated under vacuum. The solid was chromatographed on a silica-gel column with elution by cyclohexane—THF (40:1). Yield of **11**, 0.44 g (86%), mp 167-169°C (acetone). IR spectrum (ν, cm^{-1}): 1725 (C=O).

^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 0.71 (3H, s, 18-Me), 0.80 (3H, s, 19-Me), 1.02 (3H, d, J = 6.5, 21-Me), 3.25 (1H, dd, J_1 = 12.5, J_2 = 3, H-5α), 3.86 (1H, m, W/2 = 25, H-3α), 4.20 (1H, d, J = 3.5, H-7β), 5.02 (1H, dd, J_1 = 15, J_2 = 7.5, H-22), 5.16 (1H, dd, J_1 = 15, J_2 = 7.5, H-23).

Dehydrohalogenation of 11. A solution of **11** (0.69 g) in DMF (10 mL) was treated with Li_2CO_3 (0.70 g) and LiBr (0.35 g), boiled for 1.5 h under Ar, cooled to room temperature, and filtered through a layer of aluminum oxide. The filtrate was diluted with water and extracted with petroleum ether. The organic layer was thoroughly washed with water and evaporated under vacuum. The solid was chromatographed on a silica-gel column with elution by petroleum ether—ethylacetate (60:1). Yield of **12**, 0.068 g (11%), mp 148-149°C (acetone). IR spectrum (ν, cm^{-1}): 1720 (C=O).

^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 0.74 (3H, s, 18-Me), 0.76 (3H, s, 19-Me), 1.02 (3H, d, J = 6.5, 21-Me), 3.25 (1H, dd, J_1 = 11, J_2 = 5, H-5α), 4.10 (1H, d, J = 3.5, H-7β), 5.02 (1H, dd, J_1 = 15, J_2 = 7.5, H-22), 5.16 (1H, dd, J_1 = 15, J_2 = 7.5, H-23), 5.56 (1H, m, W/2 = 9, H-2/3), 5.70 (1H, m, W/2 = 9, H-2/3).

Then, **13** was isolated. Yield 0.151 g (28%), mp 129-131°C (acetone). IR spectrum (ν, cm^{-1}): 1715 (C=O).

^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 0.60 (3H, s, 18-Me), 0.88 (3H, s, 19-Me), 1.06 (3H, d, J = 6.5, 21-Me), 5.05 (1H, dd, J_1 = 15, J_2 = 7.5, H-22), 5.22 (1H, dd, J_1 = 15, J_2 = 7.5, H-23), 5.66 (2H, m, W/2 = 19, H-2 and H-3).

^{13}C NMR ($CDCl_3$, δ, ppm): 122.5 (C-8), 124.5 (C-2), 124.9 (C-3), 129.9 (C-23), 137.8 (C-22), 145.7 (C-14), 210.5 (C-6).

Then, elution with petroleum ether—ethylacetate (50:1) gave a mixture of (22E,24S)-5α-stigmasta-2,7,22-trien-6-one (**14**) and (22E,24S)-stigmasta-2,4,22-trien-6-one (**15**) in total yield 42% (**14**:**15** = 3.3:1). UV spectrum (λ_{max}, nm): 245, 315.

^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): for **14**: 0.63 (s, 18-Me), 0.86 (s, 19-Me), 1.03 (d, J = 6.5, 21-Me), 4.94-5.28 (m, H-22 and H-23), 5.58 (m, W/2 = 10, H-2/3), 5.72 (m, W/2 = 10, H-2/3), 5.74 (br. t, J = 2.5, H-7), for **15**: 0.73 (s, 18-Me), 1.02 (s, 19-Me), 6.08 (m, H-2 and H-3), 6.84 (m, H-4).

(22E,24S)-2β,3β-Diacetoxy-5α-stigmasta-7,22-dien-6-one (16). A mixture of **14** and **15** (3.3:1, 0.215 g) was dissolved in acetic acid (10 mL), heated to 60°C, and treated with water (0.2 mL), iodine (0.150 g), and silver acetate (0.220 g). The reaction mixture was stirred at 60-65°C under N_2 for 2 h, cooled to room temperature, filtered through a layer of aluminum oxide, diluted with water, and extracted with $CHCl_3$. The organic layer was thoroughly washed with water and evporated under vacuum. The solid was dissolved in pyridine (4 mL), treated with acetic anhydride (2 mL), left for 18 h, diluted with water, and extracted with $CHCl_3$. The organic layer was washed with water and evaporated under vacuum. The solid was chromatographed on a silica-gel column with elution by petroleum ether—ethylacetate of increasing polarity (50:1, 10:1, 7:1, 6:1, 5:1). Yield of **16**, 0.091 g (43% based on pure **14**), mp 182-184°C (petroleum ether). UV spectrum (λ_{max}, nm): 245 (ε 15,100).

^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 0.63 (3H, s, 18-Me), 1.02 (3H, s, 19-Me), 1.05 (3H, d, J = 6.5, 21-Me), 2.03 (3H, s, AcO), 2.09 (3H, s, AcO), 4.84 (1H, dt, J_1 = 12, J_2 = 4, H-3α), 5.04 (1H, dd, J_1 = 15, J_2 = 7.5, H-22), 5.18 (1H, dd, J_1 = 15,

J_2 = 7.5, H-23), 5.30 (1H, br. d, J = 2.5, H-2α), 5.76 (1H, br. t, J = 2.5, H-7).

(22E,24S)-2β,3β-Diacetoxy-14α-hydroxy-5α-stigmasta-7,22-dien-6-one (17). A heated (50°C) solution of **16** (0.089 g) in dioxane (4 mL) was treated with a boiling solution of SeO_2 (0.085 g) in dioxane (6 mL). The mixture was held at 81-85°C for 30 min, cooled to room temperature, and filtered through a layer of silica gel. The filtrate was evaporated under vacuum. The solid was chromatographed on a silica-gel column with elution by petroleum ether—ethylacetate (4:1). Yield of **17**, 0.058 g (64%), mp 211-213°C (petroleum ether—acetone). UV spectrum (λ_{max}, nm): 242 (ε 10,800).

^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 0.68 (3H, s, 18-Me), 1.00 (3H, s, 19-Me), 1.04 (3H, d, J = 6.5, 21-Me), 2.02 (3H, s, AcO), 2.06 (3H, s, AcO), 2.42 (1H, dd, J_1 = 12, J_2 = 3.5, H-5α), 2.73 (1H, m, W/2 = 22, H-9α), 4.84 (1H, m, W/2 = 22, H-3α), 5.06 (1H, dd, J_1 = 15, J_2 = 7.5, H-22), 5.20 (1H, dd, J_1 = 15, J_2 = 7.5, H-23), 5.30 (1H, br. d, J = 2.5, H-2α), 5.94 (1H, d, J = 2.5, H-7).

(22RS,23RS,24S)-2β,3β-Diacetoxy-14α-hydroxy-22,23-epoxy-5α-stigmast-7-en-6-one (18). Dienone **17** (0.055 g) was dissolved in $CHCl_3$ (5.5 mL), stirred, treated with $NaHCO_3$ (0.050 g) and *m*-chloroperbenzoic acid (0.042 g, 85%), stirred for 19 h at room temperature, and evaporated under vacuum. Preparative TLC of the solid on a silica-gel plate with elution by petroleum ether—ethylacetate (4:1 and 3:1) and subsequent treatment with aqueous $NaHCO_3$ of a $CHCl_3$ solution of the resulting mixture of **18** and *m*-chloroperbenzoic acid gave **18**, 0.054 g (96%), mp 176-182°C (petroleum ether—ethylacetate). UV spectrum (λ_{max}, nm): 241 (ε, 17,900).

^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 0.67, 0.68 (3H, s, 18-Me), 1.00 (3H, s, 19-Me), 1.02, 1.06 (3H, d, J = 6.5, 21-Me), 2.42 (1H, dd, J_1 = 12, J_2 = 3.5, H-5α), 2.58 (m, W/2 = 11) and 2.81 (dd, J_1 = 7, J_2 = 2) (total 2H, H-22 and H-23), 2.73 (1H, m, W/2 = 23, H-9α), 4.84 (1H, m, W/2 = 22, H-3α), 5.30 (1H, br. d, J = 2.5, H-2α), 5.96 (1H, d, J = 2.5, H-7).

(22RS,23RS,24S)-2β,3β,14α-Trihydroxy-22,23-epoxy-5β-stigmast-7-en-6-one (19). A solution of **18** (0.049 g) in CH_3OH (6 mL) was treated with water (0.3 mL) and K_2CO_3 (0.050 g), refluxed for 27 min, cooled to room temperature, and evaporated under vacuum. The solid was chromatographed on a silica-gel plate with elution by ethylacetate—$CHCl_3$ (1.2:1). Yield of **19**, 0.015 g (38%), mp 189-192°C (ethylacetate). UV spectrum (λ_{max}, nm): 243 (ε 11,300).

^{1}H NMR (C_5D_5N, δ, ppm, J/Hz): 0.70 (3H, s, 18-Me), 1.06 (3H, s, 19-Me), 1.18 (3H, d, J = 6.5, 21-Me), 2.56 (m) and 2.86 (br. d, J = 7) (total 2H, H-22 and H-23), 3.02 (1H, dd, J_1 = 12, J_2 = 3, H-5β), 3.58 (1H, m, W/2 = 24, H-9α), 4.14 (1H, m, W/2 = 17, H-2α), 4.23 (1H, m, W/2 = 12, H-3α), 6.23 (1H, d, J = 2.5, H-7).

REFERENCES

1. N. V. Kovganko, *Khim. Prir. Soedin.*, 691 (1999).
2. T. Ohsawa, M. Yukawa, C. Takao, M. Marayama, and H. Bando, *Chem. Pharm. Bull.*, **40**, 143 (1992).
3. K. Vokac, M. Budesinsky, J. Harmatha, and J. Pis, *Tetrahedron*, **54**, 1657 (1998).
4. N. V. Kovganko and V. L. Survilo, *Khim. Prir. Soedin.*, 309 (2000).
5. Disclosure No. 1162816 (USSR), *Byull. Izobret.*, No. 23 (1985); *Chem. Abstr.*, **105**, 97813q (1986).
6. A. A. Akhrem, F. A. Lakhvich, V. A. Khripach, N. V. Kovganko, and V. N. Zhabinskii, *Zh. Org. Khim.*, **23**, 762 (1987).
7. R. B. Mitra, B. G. Hazra, and V. M. Kapoor, *Indian J. Chem., Sect. B*, **23**, 106 (1984).
8. R. B. Mitra and V. M. Kapoor, *Synth. Commun.*, **15**, 1087 (1985).
9. A. A. Akhrem, F. A. Lakhvich, V. A. Khripach, V. N. Zhabinskii, and N. V. Kovganko, *Dokl. Akad. Nauk SSSR*, **275**, No. 5, 1089 (1984).
10. C. Brosa, S. Nusimovich, and R. Peracaula, *Steroids*, **59**, No. 8, 463 (1994).
11. A. A. Akhrem, F. A. Lakhvich, V. A. Khripach, V. N. Zhabinskii, and N. V. Kovganko, *Zh. org. Khim.*, **20**, No. 10, 2140 (1984).
12. A. A. Akhrem, F. A. Lakhvich, V. A. Khripach, N. V. Kovganko, and V. N. Zhabinskii, *Dokl. Akad. Nauk SSSR*, **283**, No. 1, 130 (1985).
13. N. V. Kovganko and S. K. Ananich, *Khim. Prir. Soedin.*, 664 (1989).
14. A. A. Akhrem, F. A. Lakhvich, V. A. Khripach, and N. V. Kovganko, *Zh. Org. Khim.*, **19**, No. 6, 1249 (1983).
15. A. Furlenmeir, A. Furst, A. Langemann, G. Waldvogel, U. Kerb, P. Hocks, and R. Wiechert, *Helv. Chim. Acta*, **49**, No. 5, 1591 (1966).

16. W. J. Rodewald, J. Szczepek, and J. Gumulka, *Pol. J. Chem.*, **53**, No. 1, 139 (1979).
17. Y.-M. Lee and K. Nakanishi, *Tetrahedron Lett.*, **21**, No. 45, 4323 (1980).
18. N. V. Kovganko and V. L. Survilo, *Khim. Prir. Soedin.*, 402 (2000).
19. N. V. Kovganko and S. K. Ananich, *Zh. Org. Khim.*, **27**, No. 1, 103 (1991).
20. A. A. Akhrem, F. A. Lakhvich, V. A. Khripach, and N. V. Kovganko, *Vestsi Akad. Navuk BSSR, Ser. Khim. Navuk*, No. 2, 65 (1983).
21. L. Canonica, B. Danieli, G. Lesma, G. Palmisano, and A. Mugnoli, *Helv. Chim. Acta*, **70**, No. 3, 701 (1987).
22. A. A. Akhrem, F. A. Lakhvich, V. A. Khripach, N. V. Kovganko, and V. N. Zhabinskii, *Zh. Org. Khim.*, **25**, No. 8, 1661 (1989).