Chemistry of Natural Compounds, Vol. 37, No. 5, 2001

SYNTHESIS OF 2α,3α-DIHYDROXY-$\Delta^{4,7}$-6-KETOSTEROIDS, STRUCTURAL ANALOGS OF DIAULUSTEROLS A AND B

N. V. Kovganko and S. N. Sokolov

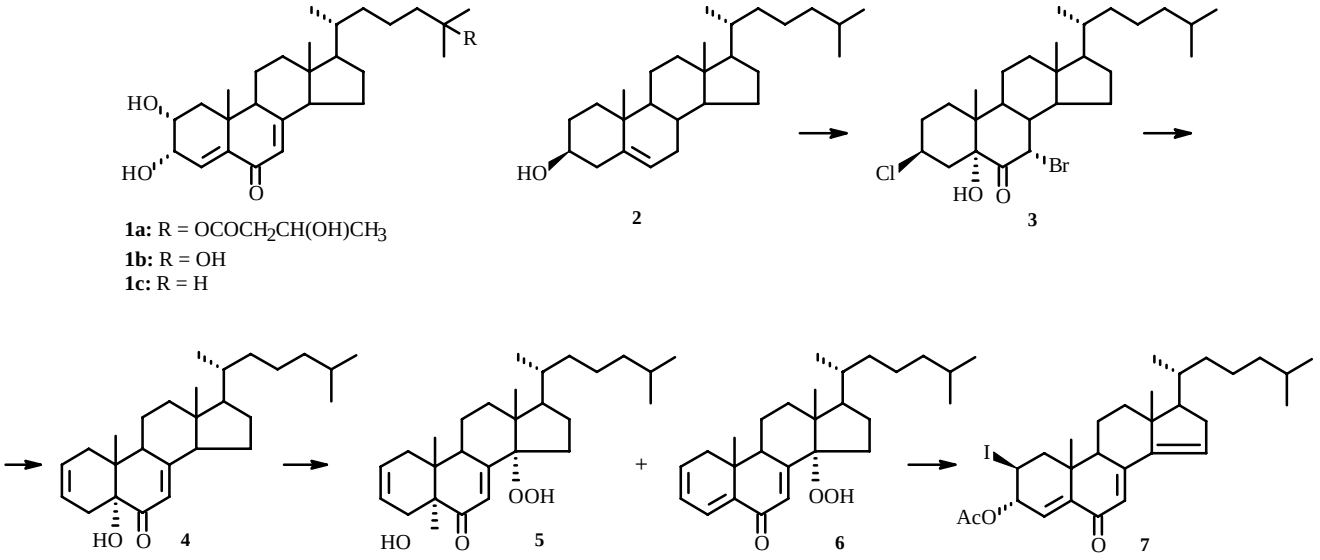
UDC 547.92

We studied various methods for synthesizing 2α,3α-dihydroxy-$\Delta^{4,7}$-6-ketosteroids, which are structural analogs of the ecdysteroids diaulusterols A and B.

Key words: 6-ketosteroids, diaulusterols A and B.

Diaulusterols A (**1a**) and B (**1b**) were isolated from skin extracts of the nudibrach mollusk *Diaulula sandiegensis* [1]. These compounds have the ecdysteroid chemical structure [2], which suggests that they may contain biologically active hormones for molting and metamorphosis of insects and crustaceans. Therefore, one reason that *D. sandiegensis* contains diaulusterols A and B is to protect it against predators, primarily crustaceans. Ingestion of these substances together with the food can cause various hormonal disturbances.

Our goal was to develop convenient methods for synthesizing ecdysteroids containing a full set of functional groups typical of diaulusterols A and B in the cyclic part. For example, **1c**, which can be synthesized from cholesterol **2**, is one compound with such a structure. We planned to use the experience gained previously during partial synthesis of ecdysteroids and related compounds. In particular, we have found [3-5] that derivatives of 2α,3α-dihydroxy-Δ^4-6-ketosteroids are prepared by *cis*-hydroxylation of 2,4-dien-6-ketosteriods by iodine and silver acetate in aqueous acetic acid by the Woodward reaction. We hypothesized that the necessary 2α,3α-dihydroxy-$\Delta^{4,7}$-6-ketosteroids could be prepared analogously from the corresponding 2,4,7-trien-6-ketosteroids. Cholesterol **2** was converted through the 3β-chloro-5α-hydroxy-7α-bromo-6-ketosteroid **3** into 5α-hydroxy-$\Delta^{2,7}$-dien-6-ketone **4**, according to the previously developed method [6]. We studied the conditions for dehydrating **4**.



1a: R = $OCOCH_2CH(OH)CH_3$
1b: R = OH
1c: R = H

Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, 220141, Minsk, ul. Akad. Kuprevicha, 5/2. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 388-393, September-October, 2001. Original article submitted October 15, 2001.

0009-3130/01/3705-0455$25.00

TABLE 1. Chemical Shifts of C Atoms (δ, ppm) in ^{13}C NMR Spectra of **4**-**14**

Atom	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**14**
C-1	34.4	34.3	38.2	43.3	36.0	37.8	43.5	35.7	35.8	35.9
C-2	124.9	124.2	127.0		29.5	133.3		67.4	67.6	67.6
C-3	122.2	121.7	123.4	75.0	54.0	123.1	74.6	64.2	64.8	64.8
C-4	30.7	30.2	131.9	130.1	134.0	131.8	129.3	126.9	123.5	123.7
C-5	74.2	75.3	139.2	149.7	144.0	138.8	145.0	148.2	151.2	147.7
C-6	198.1	197.6	185.6	189.5	195.4	193.6	197.7	196.4	202.8	187.6
C-7	120.3	125.1	129.7	127.7	58.8	63.5	64.5	64.5	46.3	124.3
C-8	165.9	160.6	160.1	156.0	37.7	37.4	39.3	38.3	34.2	166.6
C-9	43.3	39.3	44.0	45.7	43.6	42.3	40.9	42.4	51.2	47.8
C-10	40.0	40.1	35.9	42.1	38.6		42.7	42.0	41.6	40.5
C-11	22.0	20.8	20.9	21.4	20.4	21.1	21.8	20.9	21.4	21.8
C-12	39.0	30.0	30.4	38.8	38.5	38.8	38.7	38.6	39.2	38.6
C-13	44.7	47.9	47.6	46.9	42.3	42.3	43.4	43.1	42.6	44.5
C-14	55.8	96.3	95.8	145.8	52.2	50.2	50.9	50.4	56.5	56.2
C-15	22.5	24.5	25.2	122.4	22.8	23.2	23.8	22.9	23.8	22.6
C-16	27.7	26.6	27.2	23.8	27.8	27.9	28.0	27.9	29.7	27.6
C-17	56.2	50.7	51.4	58.9	55.7	55.9	55.9	55.9	56.0	56.0
C-18	12.4	15.6	16.7	22.8	12.3	11.9	12.2	12.0	11.9	12.5
C-19	16.0	16.6	19.4	17.3	20.0	18.9	22.8	19.5	19.2	20.7
C-20	36.0	35.5	35.8	33.9	35.9	35.7	35.7	35.7	35.7	35.9
C-21	18.8	18.9	19.2	18.8	18.6	18.6	18.6	18.7	18.7	18.8
C-22	36.0	36.1	36.5	35.9	36.0	36.1	36.0	36.1	36.1	35.9
C-23	23.9	24.0	24.1	23.8	23.8	23.8	23.8	23.8	23.9	23.8
C-24	39.5	39.4	39.7	39.4	39.5	39.5	39.5	39.5	39.5	39.4
C-25	28.0	28.0	29.2	28.0	28.0	28.0	28.0	28.0	28.0	28.0
C-26	22.5	22.5	22.7	22.5	22.5	22.6	22.6	22.6	22.6	22.6
C-27	22.8	22.8	23.0	22.7	22.8	22.8	22.8	22.8	22.8	22.8
$\underline{C}H_3CO$				20.9			21.0	20.9	20.9	20.9 21.0
$CH_3\underline{C}O$				169.7			169.9	170.2	170.1	170.0 170.1
Solvent	$CDCl_3$	$CDCl_3$	C_5D_5N	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$

Several attempts to eliminate the 5α-hydroxyl in this compound using thionylchloride in pyridine did not give the expected result. A complex mixture of unstable compounds was formed, from which the desired 2,4,7-trien-6-ketosteroid was difficult to isolate. Dehydration of 5-hydroxy-5α-cholesta-2,7-dien-6-one using basic aluminum hydroxide in toluene with heating gave much better results. Depending on the reaction conditions, 5-hydroxy-14α-hydroperoxy-5α-cholesta-2,7-dien-6-one **5** and 14α-hydroperoxycholesta-2,4,7-trien-6-one **6** in yields of 20 and 30%, respectively, together with unreacted starting material were isolated.

The structures of **5** and **6** were established using spectral data. Thus, the ^{1}H NMR spectrum of **5** in deuteropyridine exhibits a 2H multiplet for vinyl protons H-2 and H-3 at δ 5.78 ppm and a doublet for vinyl proton H-7 at δ 6.31 ppm. The splitting of H-7 and the appearance at weak field (δ 3.65 ppm) of a signal for the methine proton H-9α indicate that the molecule contains an electronegative substituent in the 14α-position.

Table 1 contains ^{13}C NMR spectra for **5** that confirm its structure as a 14α-hydroperoxide. The signal for C-14 is observed at weak field (δ 96.3 ppm). Such a chemical shift for C-14, as shown by us earlier [7,8], is characteristic namely of 14α-peroxides of Δ^7-6-ketosteroids. The appearance of the signal for C-5 in **5** at δ 75.3 ppm is consistent with the presence of a 5α-hydroxyl.

The ^{1}H NMR spectrum of **6** contains signals for vinyl protons H-2, H-3, and H-7 in addition to a broad doublet for the vinyl proton H-4 (δ 7.20 ppm). The structure of this compound as a 2,4,7-trien-6-ketone is confirmed by the ^{13}C NMR spectrum that contains signals for C-2, C-3, C-4, C-5, C-7, and C-8 with chemical shifts typical of vinyl C atoms. The chemical shift of

C-14 (δ 95.8 ppm) also confirms the presence of a 14α-hydroperoxyl in this steroid.

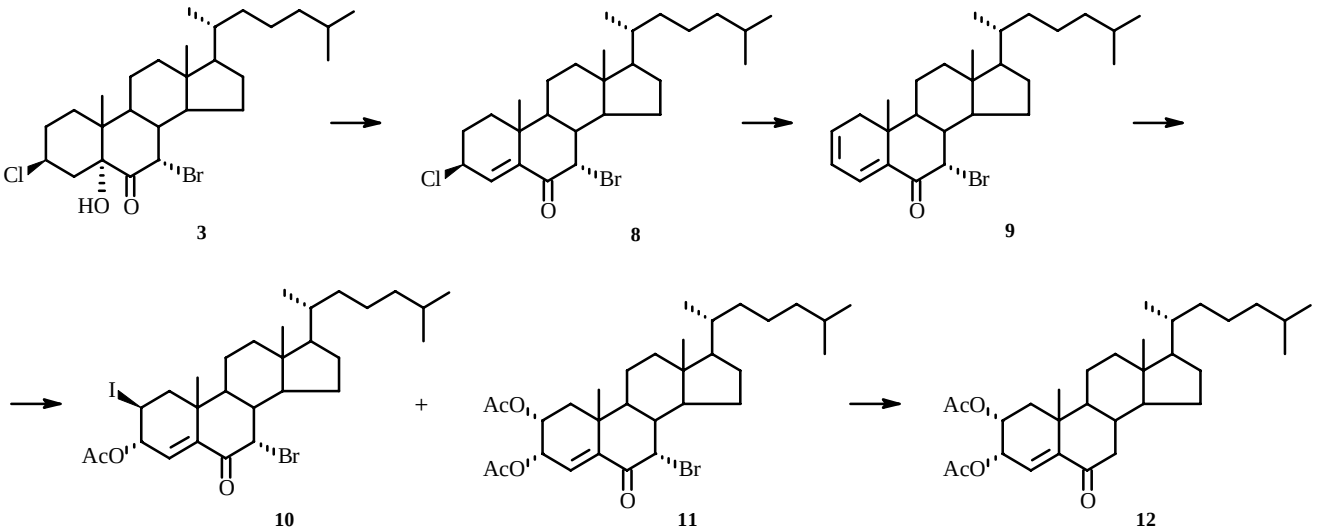
It should be noted that the formation of 14α-hydroperoxides in reactions of Δ^7-6-ketosteroids has been observed before [7, 9]. Reduction of these compounds produces rather easily 14α-hydroxy-Δ^7-6-ketosteroids, which are widely used in the synthesis of ecdysteroids [10]. However, in our instance the presence of the 14α-hydroperoxyl in **6** should surely create additional difficulties in the following synthetic step. We determined that the principal product of Woodward *cis*-hydroxylation of 2,4,7-trien-6-one **6** is 2β-iodo-3α-acetoxy-$\Delta^{4,7,14}$-6-ketosteroid **7**, which was isolated by chromatography in 36% yield. The structure was determined by analyzing spectra. Thus, the ^{1}H NMR spectrum of **7** contains signals for vinyl protons H-4 (δ 6.31 ppm), H-7 (δ 6.24 ppm), and H-15 (δ 5.99 ppm). Its structure as a 4,7,14-trien-6-one was also confirmed by the ^{13}C NMR spectrum, in which the signals for C-4, C-5, C-7, C-8, C-14, and C-15 have chemical shifts typical of vinyl C atoms. The ^{1}H NMR spectrum of this compound also contains signals for methine protons at δ 4.09 and 5.62 ppm that are geminal to the iodine and acetoxyl, respectively.

The nature of the splitting of these signals enables us to conclude that the iodine and acetoxyl in **7** have a quasi-equatorial orientation. Application of double resonance proved that these functional groups are bonded to C-2 and C-3, respectively. Thus, the doublet of doublets at δ 5.62 ppm for the proton geminal to the acetoxyl changes to a doublet with splitting constant J = 9.5 Hz upon saturation of H-4. This does not change the signal for the proton geminal to the iodine. Saturation of the signal at δ 5.62 ppm for the proton geminal to the acetoxyl changes the signal at δ 4.09 ppm into a doublet; the signal for H-4, a broad singlet. The complex multiplet at δ 2.51 ppm simplifies to a doublet of doublets upon irradiation of H-4. For this reason, this signal should be assigned to the resonance of the methine proton H-9α.

Ring A can exist in two different half-chair conformations owing to the presence of the Δ^4-bond in **7** [11]. Therefore, the stereochemistry of the substituents cannot be established only from their conformation derived from the ^{1}H NMR data. Final proof of the structure of **7** required the use of the nuclear Overhauser effect. Thus, the strengths of the signals in the difference spectrum for protons H-1, H-3, H-4, and H-9α increase upon saturation of the signal for H-2 at δ 4.09. This is possible only if H-2 has the α-orientation. Also, an Overhauser effect between H-2 and the 19-methyl should exist if H-2 has the β-orientation. However, the difference spectrum clearly shows that such an effect is lacking in **7**.

It should be noted that *trans*-iodoacetoxy derivatives like **7** are known as intermediates in the synthesis of *cis*-diols via the Woodward reaction. Apparently **7** can be converted into the necessary 2α,3α-diol. However, this is very difficult owing to its instability.

We used 5α-hydroxy-6-ketosteroid **3** in further attempts to synthesize 2α,3α-dihydroxy-$\Delta^{4,7}$-6-ketosteroids. Dehydration of **3** by thionylchloride in pyridine produced Δ^4-6-ketosteroid **8** in ~60% yield. The structure of **8** was proved based on spectral data. In particular, the ^{1}H NMR spectrum of this compound contains signals for the methine protons H-3α (δ 4.62 ppm) and H-7β (δ 4.35 ppm) geminal to the halogens and a signal for the vinyl proton H-4 (δ 6.27 ppm). The ^{13}C NMR spectrum, which has signals for C-4 and C-5 with chemical shifts of δ 134.0 and 144.0 ppm, respectively, is consistent with the presence of a 4-double bond in this compound.



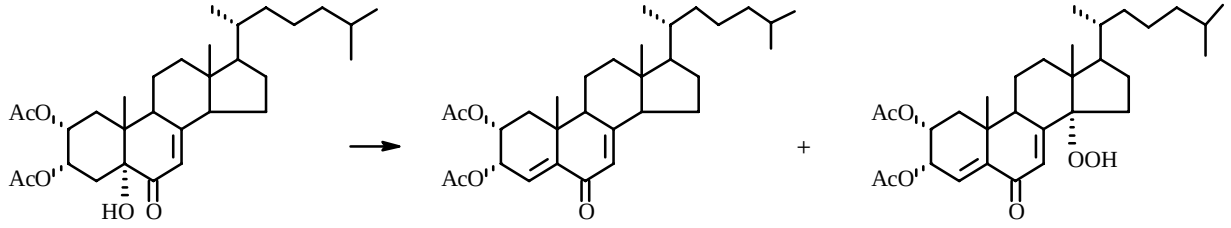
In the next step, 7α-bromo-$\Delta^{2,4}$-6-ketosteroid **9** was isolated in >20% yield upon elimination of the more available and reactive allyl Cl by reaction with Li_2CO_3 and LiBr in DMF with heating. Better results were obtained upon dehydrochlorination of **8** by heating in 2,6-lutidine. This gave the 2,4-dien-9-one **9** in >40% yield. The ^{1}H NMR spectrum of **9** has a doublet at δ 4.21 ppm for the methine proton H-7β geminal to the Br. The presence of a 2H multiplet for H-2 and H-3 (δ 6.11 ppm) and a 1H multiplet for H-4 (δ 6.89 ppm) proves that **9** contains the 2,4-dien-6-one group. The significant shift to weak field of the latter signal compared with its position in the spectrum of the starting Δ^4-6-ketone **8** is interesting. Such a shift is surely caused by the presence of the highly conjugated 2,4-dien-6-one group in **9**. The analogous conclusion can be drawn from the ^{13}C NMR spectrum, which typically has signals for C-2 to C-5 at weak field near δ 120-140 ppm.

It was found that the principal products of Woodword *cis*-hydroxylation of **9** and subsequent acetylation are 2β-iodo-3α-acetoxy-Δ^4-6-ketosteroid **10** and 2α,3α-diacetoxy-Δ^4-6-ketosteroid **11**, which were isolated in yields of 18 and 42%, respectively. The ^{1}H NMR spectra of both compounds contain signals for methine proton H-7β at δ 4.14 and 4.21 ppm, respectively. This indicates that the Br is retained in them. The analogous conclusion follows from the ^{13}C NMR spectra of **10** and **11** in which the signals for C-7 appear at δ 64.5 ppm. The structure of **10** as a 2β-iodo-3α-acetoxy-Δ^4-6-ketone was determined by comparing its ^{1}H NMR spectrum with that of **7**. Good correspondence of the chemical shifts and multiplicity of the signals for methine protons H-2α and H-3β, which are geminal to the iodine and acetoxyl, respectively, is observed.

The ^{1}H and ^{13}C NMR spectra indicate that **11** contains a Δ^4-bond and two acetoxyls on C-2 and C-3. The multiplicity in the ^{1}H NMR spectrum of the protons geminal to them enable us to conclude that one acetoxyl is quasi-axial whereas the other is quasi-equatorial. Of the two possible *cis*-orientations of the acetoxyls (i.e., 2α,3α or 2β,3β) in **11**, the former was chosen based on the ^{1}H NMR data. The appearance of the signal for H-4 as a doublet at δ 6.17 ppm with J = 5.5 Hz is characteristic for 2α,3α-dihydroxy-Δ^4-6-ketosteroids [3-5]. Also, this signal in proton spectra of 2β,3β-diacetoxy-Δ^4-6-ketosteroids is usually observed at δ 5.88 ppm as a doublet of doublets with J_1 = 3.0 and J_2 = 1.1 Hz [4].

The 7-double bond was introduced in the next step by reacting **11** with Li_2CO_3 and LiBr in DMF with heating. Unfortunately, the main product turned out to be Δ^4-6-ketone **12**, which is formed by reduction and not elimination. The structure of **12** was easily determined. Its spectral properties are identical to a previously obtained compound [4], the structure of which was positively established by an x-ray structural analysis [12].

It should be noted that the products of reduction upon dehydrobromination of 7α-bromo-6-ketosteroids were also formed previously [13]. Apparently this process becomes dominant because of the presence of the additional Δ^4-bond in **11**.



13 **14** **15**

We also studied dehydration of 2α,3α-diacetoxy-5α-hydroxy-Δ^7-6-ketosteroid **13**, which has been isolated previously [6], in the final stage of the investigation in order to synthesize 2α,3α-dihydroxy-$\Delta^{4,7}$-6-ketosteroids. Reaction of **13** with thionylchloride in pyridine produces **14** in >70% yield. A minor product of this reaction (8% yield) is 14α-peroxide **15**. The spectra of **14** agree with those in the literature [1] for analogous derivatives of 2α,3α-dihydroxy-$\Delta^{4,7}$-6-ketosteroids. This proves its structure. The main properties of the ^{1}H NMR spectrum of **15** agree fully with those of the 14α-peroxide of the Δ^7-6-ketosteroid described previously by us [7].

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}. UV spectra of ethanol solutions were taken on a Specord M-400 instrument. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AC-200 NMR spectrometer at working frequencies 200 and 50.32 MHz, respectively. Chemical shifts are given relative to TMS internal standard.

Dehydration of 5-Hydroxy-5α-cholesta-2,7-dien-6-one (4). A. A solution of **4** (0.30 g, obtained from cholesterol

2 by the literature method [6]) in toluene (30 mL) was treated with basic aluminum oxide (0.60 g). The reaction mixture was stirred and boiled for 1.5 h. The aluminum oxide was filtered off. The filtrate was evaporated in vacuum. The solid was treated with cyclohexane (15 mL). The precipitate was filtered off. Yield of **6** 0.015 g (5%), mp 175-176°C (hexane), 181-182°C (acetone).

IR spectrum (KBr, ν, cm^{-1}): 3440, 3220 (OH), 1660 (C=O), 1635, 1615, 1560 (C=C), 865 (O–O). UV spectrum (λ_{max}, nm): 271 (ε 9700), 335 (ε 9600).

^{1}H NMR spectrum (C_5D_5N, δ, ppm, J/Hz): 0.80 (3H, s, 18-Me), 0.88 (6H, d, J = 6.5, 26-Me, 27-Me), 0.97 (3H, d, J = 6, 21-Me), 0.99 (3H, s, 19-Me), 2.77 (1H, dt, J_1 = 2, J_2 = 10, H-9α), 5.97 (1H, m, J_1 = 9.5, J_2 = 5.5, J_3 = 3, J_4 = 1, H-2), 6.10 (1H, ddd, J_1 = 9.5, J_2 = 5.5, J_3 = 3, H-3), 6.55 (1H, d, J = 2, H-7), 7.20 (1H, br.d, J_1 = 5.5, J_2 = 1, H-4).

Then, the filtrate was evaporated in vacuum. The solid was chromatographed over a silica-gel column with elution by a cyclohexane:ethylacetate mixture of increasing polarity (from 10:1 to 6:1).

Fraction 1: 0.080 g (27%) of amorphous starting **4**.

IR spectrum (film, ν, cm^{-1}): 1680 (C=O), 1630 (C=C). UV spectrum (λ_{max}, nm): 249 (ε 10,000).

^{1}H NMR spectrum (C_5D_5N, δ, ppm, J/Hz): 0.59 (3H, s, 18-Me), 0.90 (6H, d, J = 6.5, 26-Me, 27-Me), 0.99 (3H, d, J = 6, 21-Me), 1.00 (3H, s, 19-Me), 3.00 (1H, br.dt, J_1 = 2.5, J_2 = 10, H-9α), 5.80 (2H, m, W/2 = 5.5, H-2, H-3), 5.97 (1H, t, J = 2.5, H-7), 7.37 (1H, s, 5α-OH).

Fraction 2: 0.045 g (14%) of amorphous **5**.

IR spectrum (film, ν, cm^{-1}): 1685 (C=O), 1665, 1640 (C=C). UV spectrum (λ_{max}, nm): 245 (ε 5800).

^{1}H NMR spectrum (C_5D_5N, δ, ppm, J/Hz): 0.80 (3H, s, 18-Me), 0.87 (6H, d, J = 6.5, 26-Me, 27-Me), 0.96 (3H, d, J = 6, 21-Me), 1.03 (3H, s, 19-Me), 3.65 (1H, dt, J_1 = 3, J_2 = 9.5, H-9α), 5.78 (2H, m, W/2 = 5.5, H-2, H-3), 6.31 (1H, d, J = 3, H-7).

B. A solution of **4** (0.26 g) in toluene (30 mL) was treated with diphenylamine (0.1 g) and basic aluminum oxide (0.6 g). Air was bubbled through the reaction mixture for 1 h with constant boiling and stirring. The aluminum oxide was filtered off. The filtrate was evaporated in vacuum. The solid was dissolved in hexane. Crystals that formed after 96 h were filtered off. Yield of **6** 0.072 g (27%).

Woodward *cis*-Hydroxylation of 6. A solution of **6** (0.15 g) in a heated (40°C) mixture of acetic acid (30 mL) and THF (15 mL) was stirred and treated successively with water (0.2 mL), silver acetate (0.18 g), and iodine (0.16 g). The precipitate that formed after 50 min at 40°C was filtered off. The filtrate was evaporated in vacuum to one fourth the volume. The solid was dissolved in $CHCl_3$ (30 mL), washed successively with water (10 mL), saturated $NaHCO_3$ solution (10 mL), and water (15 mL), and dried over anhydrous $MgSO_4$. The desiccant was removed. The solvent was evaporated in vacuum. The solid was chromatographed over a silica-gel column with elution by hexane:ethylacetate mixtures of increasing polarity (from 50:1 to 40:1). Yield of **7** 0.073 g (36%), mp 123°C (dec.) (hexane).

IR spectrum (KBr, ν, cm^{-1}): 1760, 1225 (AcO), 1670 (C=O), 1640, 1620, 1590 (C=C). UV spectrum (λ_{max}, nm): 260 (ε 6600), 313 (ε 15,700).

^{1}H NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.88 (6H, d, J = 6.5, 26-Me, 27-Me), 0.92 (3H, s, 18-Me), 0.95 (3H, d, J = 6, 21-Me), 1.12 (3H, s, 19-Me), 2.13 (3H, s, AcO), 2.51 (1H, m, W/2 = 22, H-9α), 2.75 (1H, dd, J_1 = 15, J_2 = 3.5, H-1β), 4.09 (1H, ddd, J_1 = 14, J_2 = 9.5, J_3 = 3.5, H-2α), 5.62 (1H, dd, J_1 = 9.5, J_2 = 1.5, H-3β), 5.99 (1H, m, W/2 = 7, H-15), 6.13 (1H, d, J = 1.5, H-4), 6.24 (1H, d, J = 2.5, H-7).

3β-Chloro-7α-bromocholest-4-en-6-one (8). A solution of **3** (1.90 g) in pyridine (20 mL) was treated with thionylchloride (0.66 mL). The reaction mixture was diluted after 30 min with water (150 mL) and extracted with $CHCl_3$ (3×40 mL). The $CHCl_3$ extract was successively washed with water (50 mL), HCl (150 mL, 2 N), and water (50 mL), and dried over $MgSO_4$. The desiccant was removed. The solvent was evaporated in vacuum. The solid was chromatographed over a silica-gel column with elution by hexane:dichloroethane mixtures of increasing polarity (from 50:1 to 10:1). Yield of **8** 1.07 g (58%), mp 108-111°C (hexane).

IR spectrum (film, ν, cm^{-1}): 1710 (C=O), 1640 (C=C). UV spectrum (λ_{max}, nm): 252 (ε 4000).

^{1}H NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.72 (3H, s, 18-Me), 0.87 (6H, d, J = 6.5, 26-Me, 27-Me), 0.92 (3H, d, J = 6, 21-Me), 1.04 (3H, s, 19-Me), 4.35 (1H, d, J = 2.5, H-7β), 4.62 (1H, ddd, J_1 = 10, J_2 = 6, J_3 = 2.5, H-3α), 6.27 (1H, m, W/2 = 5, H-4).

7α-Bromocholesta-2,4-dien-6-one (9). A. A solution of **8** (0.2 g) in DMF (20 mL) was treated with Li_2CO_3 (0.3 g) and LiBr (0.077 g). The reaction mixture was heated to 135°C and stirred for 1 h. The precipitate was filtered off after cooling

the mixture to room temperature. The filtrate was diluted with water (50 mL) and extracted with benzene (2×40 mL). The benzene extract was washed with water (2×20 mL) and dried over $MgSO_4$. The desiccant was removed. The solvent was evaporated in vacuum. The solid was chromatographed over a silica-gel column with elution by cyclohexane:dichloroethane (10:1). Yield of **9** 0.041 g (22%).

IR spectrum (film, ν, cm^{-1}): 1685 (C=O), 1635, 1560 (C=C). UV spectrum (λ_{max}, nm): 236 (ε 8500), 320 (ε 5600).

^1H NMR spectrum ($CDCl_3$, δ, ppm): 0.71 (3H, s, 18-Me), 0.87 (6H, d, J = 6.5, 26-Me, 27-Me), 0.93 (3H, d, J = 6, 21-Me), 0.99 (3H, s, 19-Me), 4.21 (1H, d, J = 3, H-7β), 6.11 (2H, m, W/2 = 7, H-2, H-3), 6.89 (1H, m, W/2 = 8, H-4).

B. A solution of **8** (0.77 g) in 2,6-lutidine (10 mL) was boiled for 1 h 45 min, cooled to room temperature, and diluted with water (50 mL). The reaction product was extracted with benzene (3×30 mL). The benzene extract was washed successively with HCl (50 mL, 2 N) and water (50 mL) and dried over $MgSO_4$. The desiccant was removed. The solvent was evaporated in vacuum. The solid was charomatographed over a silica-gel column with elution by hexane:dichloroethane (9:1). Yield of **9** 0.30 g (42%).

Woodward *cis*-Hydroxylation of 7α-Bromocholesta-2,4-dien-6-one (9). A heated (40°C) solution of **9** (0.36 g) in acetic acid (60 mL), THF (20 mL), and water (1 mL) was stirred and treated with silver acetate (0.39 g) and then iodine (0.40 g). The reaction mixture was stirred for 2 h 40 min at 40°C. The precipitate was filtered off. The filtrate was evaporated in vacuum. The solid was dissolved in $CHCl_3$ (50 mL). The $CHCl_3$ solution was washed successively with water (20 mL), saturated $NaHCO_3$ solution (2×20 mL), sodium thiosulfate solution (5%, 10 mL), and water (20 mL) and dried over $MgSO_4$. The desiccant was removed. The solvent was removed in vacuum. The solid was dissolved in a mixture of pyridine (2 mL) and acetic anhydride (2 mL). The reaction mixture was kept at room temperature for 19 h. The solvent was removed by azeotropic distillation with toluene. The solid was chromatographed over a silica-gel column with elution by cyclohexane:ethylacetate mixtures of increasing polarity (from 40:1 to 10:1).

Fraction 1: Yield of **10** 0.09 g (18%), mp 140-142°C (hexane).

IR spectrum (film, ν, cm^{-1}): 1755, 1235 (AcO), 1720 (C=O), 1650 (C=C). UV spectrum (λ_{max}, nm): 238 (ε 6100).

^1H NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.70 (3H, s, 18-Me), 0.87 (6H, d, J = 6.5, 26-Me, 27-Me), 0.92 (3H, d, J = 6, 21-Me), 1.05 (3H, s, 19-Me), 2.15 (3H, s, AcO), 2.78 (1H, dd, J_1 = 14.5, J_2 = 3.5, H-1α), 4.14 (1H, d, J = 3, H-7β), 4.14 (1H, ddd, J_1 = 13, J_2 = 9, J_3 = 3.5, H-2α), 5.54 (1H, dd, J_1 = 9, J_2 = 1.5, H-3β), 5.89 (1H, d, J = 1.5, H-4).

Fraction 2: Yield of amorphous **11** 0.19 g (42%).

IR spectrum (film, ν, cm^{-1}): 1755, 1250, 1235 (AcO), 1715 (C=O), 1645 (C=C). UV spectrum (λ_{max}, nm): 238 (ε 6000).

^1H NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.71 (3H, s, 18-Me), 0.87 (6H, d, J = 6.5, 26-Me, 27-Me), 0.93 (3H, d, J = 6, 21-Me), 1.09 (3H, s, 19-Me), 2.04 (3H, s, 2α-AcO), 2.12 (3H, s, 3α-AcO), 4.21 (1H, d, J = 3.5, H-7β), 5.11 (1H, dt, J_1 = 12, J_2 = 4, H-2β), 5.55 (1H, t, J_1 = 5.5, J_2 = 4, H-3β), 6.17 (1H, d, J = 5.5, H-4).

2α,3α-Diacetoxycholest-4-en-6-one (12). A solution of **11** (0.20 g) in DMF (15 mL) was treated with Li_2CO_3 (0.25 g) and LiBr (0.066 g). The reaction mixture was heated to 125-130°C and stirred for 6 h. The solid was filtered off after cooling to room temperature. The filtrate was poured into water (50 mL) and extracted with $CHCl_3$ (2×20 mL). The $CHCl_3$ extract was washed with water (6×30 mL) and evaporated in vacuum. The solid was chromatographed over a silica-gel column with elution by hexane:ethylacetate mixtures of increasing polarity (from 30:1 to 15:1). Yield of **12** 0.073 g (42%), mp 149-151°C (hexane), lit. [3-4] mp 153-154°C (acetone).

IR spectrum (film, ν, cm^{-1}): 1755, 1250, 1230 (AcO), 1700 (C=O), 1640 (C=C). UV spectrum (λ_{max}, nm): 230 (ε 8000), 317 (ε 830).

^1H NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.71 (3H, s, 18-Me), 0.87 (6H, d, J = 6.5, 26-Me, 27-Me), 0.92 (3H, d, J = 6, 21-Me), 1.09 (3H, s, 19-Me), 2.03 (3H, s, 2α-AcO), 2.10 (3H, s, 3α-AcO), 2.58 (1H, dd, J_1 = 15, J_2 = 3.5, H-7β), 5.12 (1H, m, W/2 = 23, H-2β), 5.55 (1H, m, W/2 = 12, H-3β), 6.10 (1H, d, J = 5.5, H-4). IR spectra and ^1H NMR spectra of this compound agree with those of that synthesized earlier [3, 4].

Dehydration of 13. A solution of **13** (0.12 g, prepared by the literature method [6]) in pyridine (3 mL) cooled to -19°C was treated with thionylchloride (0.12 mL). After 15 min the reaction mixture was poured into water (30 mL) and extracted with $CHCl_3$ (5×10 mL). The $CHCl_3$ extract was washed successively with HCl (20 mL, 2 N) and water (2×20 mL) and dried over $MgSO_4$. The desiccant was removed. The solvent was evaporated in vacuum. The solid was chromatographed over a silica-gel column with elution by hexane:ethylacetate mixtures of increasing polarity (from 15:1 to 5:1).

Fraction 1: Yield of **14** 0.082 g (71%), mp 139-141°C (dec.) (hexane).

IR spectrum (KBr, cm^{-1}): 1755, 1250, 1240 (AcO), 1675 (C=O), 1640 (C=C). UV spectrum (λ_{max}, nm): 267 (ε 16,300).

^{1}H NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.66 (3H, s, 18-Me), 0.87 (6H, d, J = 6.5, 26-Me, 27-Me), 0.96 (3H, d, J = 6, 21-Me), 1.24 (3H, s, 19-Me), 2.04 (3H, s, 2α-AcO), 2.09 (3H, s, 3α-AcO), 2.43 (1H, br.t, J_1 = 9.5, J_2 = 2, H-9α), 5.11 (1H, dt, J_1 = 12, J_2 = 4, H-2β), 5.61 (1H, t, J_1 = 5.5, J_2 = 4, H-3β), 5.89 (1H, t, J = 2, H-7), 6.43 (1H, d, J = 5.5, H-4).

Fraction 2: Yield of amorphous **15** 0.0094 g (8%).

IR spectrum (film, ν, cm^{-1}): 1750, 1245 (AcO), 1680 (C=O), 1635 (C=C). UV spectrum (λ_{max}, nm): 254 (ε 6100).

^{1}H NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.87 (9H, d, J = 6.5, 18-Me, 26-Me, 27-Me), 0.93 (3H, d, J = 6, 21-Me), 1.26 (3H, s, 19-Me), 2.03 (3H, s, 2α-AcO), 2.08 (3H, s, 3α-AcO), 5.11 (1H, dt, J_1 = 12, J_2 = 4, J_3 = 2, H-2β), 5.62 (1H, t, J_1 = 5.5, H-3β), 6.08 (1H, d, J = 2.5, H-7), 6.46 (1H, d, J = 5.5, H-4).

ACKNOWLEDGMENT

The work was supported by the Belorussian Republic Foundation for Basic Research (Grant 00-X025).

REFERENCES

1. D. E. Williams, S. W. Ayer, and R. J. Andersen, *Can. J. Chem.*, **64**, 1527 (1986).
2. R. Lafont and I. Wilson, *The Ecdysone Handbook*, 2nd Ed., The Chromatography Society, Nottingham (1996), p. 525.
3. A. A. Akhrem, F. A. Lakhvich, V. A. Khripach, and N. V. Kovganko, *Dokl. Akad. Nauk SSSR*, **257**, 1133 (1981).
4. A. A. Akhrem, F. A. Lakhvich, V. A. Khripach, and N. V. Kovganko, *Zh. Org. Khim.*, **19**, 1249 (1983).
5. A. A. Akhrem, F. A. Lakhvich, V. A. Khripach, N. V. Kovganko, and V. N. Zhabinskii, *Zh. Org. Khim.*, **25**, 1661 (1989).
6. N. V. Kovganko and S. N. Sokolov, *Khim. Prir. Soedin.*, 470 (2000).
7. N. V. Kovganko and S. H. Sokolov, *Khim. Prir. Soedin.*, 244 (2000).
8. N. V. Kovganko and S. H. Sokolov, *Khim. Prir. Soedin.*, 142 (2001).
9. L. Canonica, B. Danieli, G. Lesma, G. Palmisano, and A. Mugnoli, *Helv. Chim. Acta*, **70**, 701 (1987).
10. A. A. Akhrem and N. V. Kovganko, *Ecdysteroids: Chemistry and Biological Activity* [in Russian], Nauka i Tekhnika, Minsk (1987).
11. V. Cerny, M. Budesinsky, and F. Sorm, *Collect. Czech. Chem. Commun.*, **38**, 565 (1973).
12. A. A. Akhrem, N. M. Galitskii, G. A. Yasnitskii, V. A. Gritsuk, F. A. Lakhvich, V. A. Khripach, and N. V. Kovganko, *Zh. Org. Khim.*, **26**, 771 (1990).
13. W. Rodewald, W. J. Szczepek, and J. Gumulka, *Pol. J. Chem.*, **53**, 139 (1979).