Chemistry of Natural Compounds, Vol. 38, No. 4, 2002

NOVEL SYNTHESIS OF 22,25-DIDEOXYECDYSONE AND ITS 5α-ISOMER

N. V. Kovganko and V. L. Survilo

UDC 547.92

*22,25-Dideoxyecdysone (**1**) and 5α-22,25-dideoxyecdysone (**1a**) were prepared from cholesterol via a new scheme using the key intermediates 3β-chloro-5α-bromo-6-ketone **3**, 3β-chloro-7α-bromo-6-ketone **4**, and 2,7-dien-6-one **7**.*

Key words: 22,25-dideoxyecdysone, 5α-22,25-dideoxyecdysone, new synthetic scheme.

The synthetic ecdysteroid 22,25-dideoxyecdysone (**1**) possesses a variety of biological activity on insects. Thus, this compound exhibits in corresponding biological tests a distinct activity as an insect molting hormone. It also significantly inhibits the growth and development of certain insects if added to food [2-4]. Despite having a structure similar to those typical natural ecdysteroids, **1** has not yet been found in any animal or plant source. It has only been identified as a metabolite of 2,22,25-trideoxyecdysone in insects and crustaceans [5]. Therefore, the constant demand for **1** can only be satisfied through chemical synthesis from cholesterol, for which several different methods have been proposed [6-8].

We previously developed a new synthetic scheme for ecdysteroids [9] in which rearrangement of 3β-chloro-5α-bromo-6-ketosteroids into the corresponding 3β-chloro-7α-bromo-6-ketosteroids is used in one of the steps. The goal of our research was to apply this scheme to the preparation of 22,25-dideoxyecdysone (**1**) and its 5α-isomer **1a**. For this, starting cholesterol was first transformed quantitatively into the 3β-chloro derivative **2** by reaction with thionylchloride according to the literature method [10]. 3β-Chloro-5α-bromo-6-ketosteroid **3** was synthesized in >70% overall yield using the method that we developed [11] for addition of hypobromous acid to the 5(6)-double bond of **2** followed by oxidation of the resulting bromohydrin by chromic acid.

Steroid **3** was transformed in 82% yield into 3β-chloro-7α-bromo-6-ketone **4** by reaction with hydrobromic acid in acetic acid. The structure of **4** was determined using spectral analysis. In particular, the PMR spectrum contains characteristic signals for methine protons H-3α (δ 3.88 ppm) and H-7β (δ 4.19 ppm), which are geminal to the Cl and Br atoms, respectively. The positions and shapes of these signals in the spectrum of **4** are the same as those of analogous signals in the spectrum of the corresponding 3β-chloro-7α-bromo-6-ketosteroid obtained earlier [9] from stigmasterol.

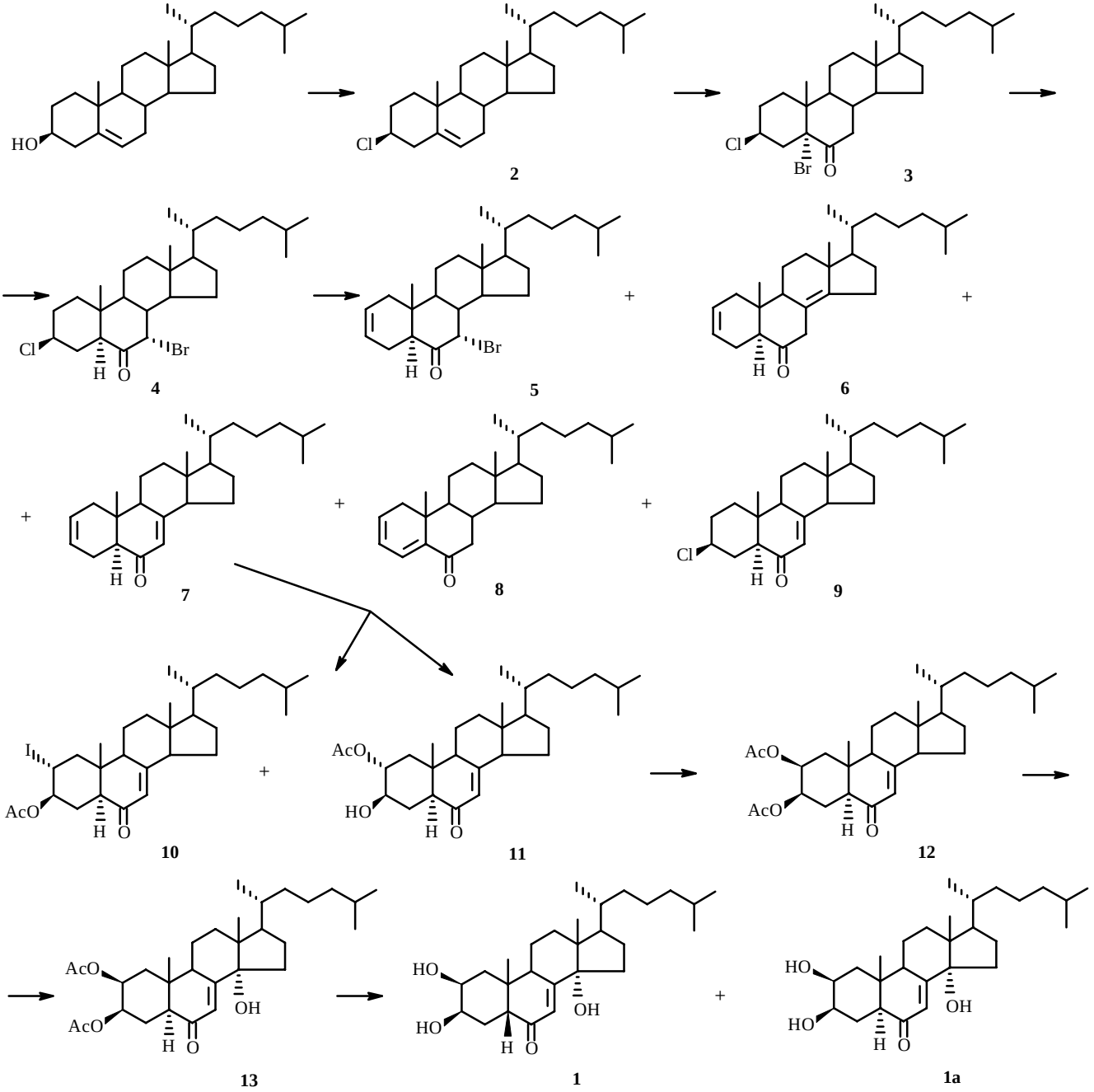
In the next step, dihaloketone **4** was dehydrohalogenated by Li_2CO_3 and LiBr in boiling DMF. Chromatographic separation of the products isolated 7α-bromo-Δ^2-6-ketone **5**, $\Delta^{2,8(14)}$-6-ketone **6**, and a mixture (3.3:1) of $\Delta^{2,7}$-6-ketone **7** and $\Delta^{2,4}$-6-ketone **8**, and 3β-chloro-Δ^7-6-ketone **9**. The structures of these follow unambiguously from the spectral data. Compounds **5** and **9** were produced in low yields (9 and 10%, respectively) by partial dehydrohalogenation of starting steroid **4**. The other steroids are formed via elimination from **4** of both halogen atoms. However, we could not separate the desired 2,7-dien-6-one **7** from 2,4-dien-6-one **8**. The latter is most probably produced by 1,4-elimination of HBr from **4**. Isomerization of 2,7-dien-6-one **7** under the reaction conditions obviously forms 2,8(14)-dien-6-one **6**.

The 2β,3β-diol group was introduced into the mixture of **7** and **8** by Woodward *cis*-hydroxylation using AgOAc and I_2 in aqueous acetic acid. The starting mixture contained mainly **7**. Therefore, the predominant reaction product is 2β-acetoxy-3β-hydroxy-Δ^7-6-ketone **11**. We isolated from the mixture of reaction products a small amount of this compound and characterized it. The structure of **11** was proved unambiguously by comparing the PMR and IR spectra with spectra of this compound that were previously published [12]. The bulk of the products from Woodward hydroxylation of **7** and **8** was then acetylated by acetic anhydride in pyridine. Purification by chromatography gave 2α-iodo-3β-acetoxy-Δ^7-6-ketone **10** and 2β,3β-diacetoxy-Δ^7-6-ketone **12** in yields 3% and 55% calculated using **7**. The structures of **10** and **12** were determined from spectral

Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, 220141, Belarus, Minsk, ul. Akad. Kuprevicha, 5/2, e-mail: kovganko@iboch.bas-net.by. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 284-287, July-August, 2002. Original article submitted July 15, 2002.

 0009-3130/02/3804-0340$27.00

data. For example, the IR and PMR spectra of **12** were identical to those that were published earlier [12].



Next, allyl hydroxylation of **12** by SeO_2 in dioxane produced 14α-hydroxy-Δ^7-6-ketone **13** in ~90% yield. The spectra of **13** that was synthesized by us and those that appear in the literature [6, 8] were identical.

Finally, the acetoxy groups in **13** was hydrolyzed by K_2CO_3 in aqueous MeOH. This also partially epimerized C-5 to form the corresponding *cis*-A/B-isomer. This reaction produced the target 22,25-dideoxyecdysone (**1**) and its 5α-isomer **1a** in yields of 40 and 36%, respectively. The chemical shifts and shape of signals for H-2, H-3, H-19, and 19-Me in the PMR spectrum of **1** are practically identical to those of the analogous signals for the same protons in the PMR of 20-hydroxyecdysone, which we recorded under analogous conditions. This confirms the structure of 22,25-dideoxyecdysone. The presence of signals for the 19-Me at δ 1.42 ppm and other protons in the PMR are important for proving the structure of 5α-22,25-dideoxyecdysone (**1a**). This position for this signal is characteristic of ecdysteroids with *trans*-fusion of rings A and B [4].

Thus, we established that the synthetic scheme that we developed earlier [9] using stigmasterol as an example is general and applicable to the preparation of 22,25-dideoxyecdysone (**1**) and its 5α-isomer **1a**.

EXPERIMENTAL

Melting points were determined on a Kofler block. IR spectra were recorded in KBr pellets on a UR-20 instrument in the range 700-3600 cm^{-1}. UV spectra of EtOH solutions were recorded on a Specord M-400 instrument. PMR 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.

3β-Chloro-7α-bromo-5α-cholestan-6-one (4). A solution of 3β-chloro-5-bromo-5α-chloestan-6-one (**3**, 4.5 g, prepared from cholesterol by the literature method [11]) in acetic acid (270 mL) was heated to 60°C, stirred, treated with HBr (13.5 mL, 40%). The mixture was stirred at 61-63°C for 1 h 45 min, cooled to room temperature, and diluted with water. The product was extracted with benzene. The benzene extract was washed with water, $NaHCO_3$ solution (5%), and water again, and evaporated in vacuo. The solid was chromatographed over a silica-gel column with elution by petroleum ether:ethylacetate (90:1) to give **4** (3.7 g, 82%), mp 144-145°C (petroleum ether).

IR spectrum (ν, cm^{-1}): 1730 (C=O). PMR spectrum (CD_2Cl_2, δ, ppm, J/Hz): 0.70 (3H, s, 18-Me), 0.78 (3H, s, 19-Me), 0.86 (6H, d, J = 6.5, 26-Me, 27-Me), 0.92 (3H, d, J = 6, 21-Me), 3.25 (1H, dd, J_1 = 12, J_2 = 3, H-5α), 3.88 (1H, m, W/2 = 24, H-3α), 4.19 (1H, d, J = 3.5, H-7β).

Dehydrohalogenation of 3β-Chloro-7α-bromo-5α-cholestan-6-one (4). A solution of **4** (2.95 g) in DMF (25 mL) was treated with Li_2CO_3 (2.00 g) and LiBr (1.50 g). The reaction mixture was boiled for 1 h 20 min under Ar, cooled to room temperature, and filtered through a layer of silica gel. The filtrate was diluted with water and extracted with petroleum ether. The organic layer was thoroughly washed with water and evaporated in vacuo. The solid was chromatographed over a silica-gel column with elution by petroleum ether:ethylacetate (120:1). Rechromatography of a fraction over a silica-gel column with elution by the same mixture gave 7α-bromo-5α-cholest-2-en-6-one (**5**, 0.24 g, 9%), mp 82-84°C (acetone).

IR spectrum (ν, cm^{-1}): 1725 (C=O). PMR spectrum (CD_2Cl_2, δ, ppm, J/Hz): 0.70 (3H, s, 18-Me), 0.73 (3H, s, 19-Me), 0.87 (6H, d, J = 6.5, 26-Me, 27-Me), 0.94 (3H, d, J = 6, 21-Me), 3.17 (1H, dd, J_1 = 11, H-5α), 4.10 (1H, d, J = 3 Hz, H-7β), 5.57 (1H, m, W/2 = 9, H-2/3), 5.70 (1H, m, W/2 = 9, H-2/3).

Then, we isolated 5α-cholest-2,8(14)-dien-6-one (**6**, 0.39 g, 17%), mp 74-78°C (acetone), lit. [13] mp 78-80°C. IR spectrum (ν, cm^{-1}): 1720 (C=O). PMR spectrum (CD_2Cl_2, δ, ppm, J/Hz): 0.58 (3H, s, 18-Me), 0.84 (3H, s, 19-Me), 0.86 (6H, d, J = 6.5, 26-Me, 27-Me), 0.96 (3H, d, J = 6, 21-Me), 5.66 (2H, m, W/2 = 15, H-2, H-3). ^{13}C NMR spectrum (CD_2Cl_2, δ, ppm): 122.9 (C-8), 125.0 (C-2), 125.2 (C-3), 145.8 (C-14), 210.3 (C-6).

Elution by petroleum ether:ethylacetate (90:1) isolated a mixture of 5α-cholest-2,7-dien-6-one (**7**) and cholest-2,4-dien-6-one (**8**) (0.84 g, 37%, **7**:**8** = 3.3:1). UV spectrum (λ_{max}, nm): 245, 315. PMR spectrum (CD_2Cl_2, δ, ppm, J/Hz): for **7**: 0.64 (s, 18-Me), 0.82 (s, 19-Me), 0.86 (6H, d, J = 6.5, 26-Me, 27-Me), 1.00 (d, J = 6, 21-Me), 5.52-5.66 (m, W/2 = 9, H-2/3), 5.66-5.72 (m, W/2 = 9, H-2/3), 5.68 (br.t, J = 2, H-7); for **8**: 0.72 (s, 18-Me), 0.86 (6H, d, J = 6.5, 26-Me, 27-Me), 0.96 (s, 19-Me), 6.08 (m, W/2 = 8, H-2, H-3), 6.77 (m, W/2 = 9, H-4).

Then, elution by petroleum ether:ethylacetate (60:1) isolated 3β-chloro-5α-cholest-7-en-6-one (**9**, 0.25 g, 10%). UV spectrum (λ_{max}, nm): 245. IR spectrum (ν, cm^{-1}): 1680 (C=O), 1630 (C=C). PMR spectrum (CD_2Cl_2, δ, ppm, J/Hz): 0.60 (3H, s, 18-Me), 0.85 (6H, d, J = 6.5, 26-Me, 27-Me), 0.86 (3H, d, 19-Me), 0.94 (3H, d, J = 6, 21-Me), 2.77 (1H, dd, J_1 = 12, J_2 = 3.5, H-5α), 3.88 (1H, m, W/2 = 24, H-3α), 5.67 (1H, br.t, J = 2, H-7).

Woodward Hydroxylation of 5α-Cholest-2,7-dien-6-one (7) and Cholest-2,4-dien-6-one (8). A solution of **7** and **8** (0.80 g, 3.3:1) in acetic acid (20 mL) was heated to 60°C, treated successively with water (1 mL), I_2 (0.66 g), and AgOAc (1.00 g), stirred at 61-63°C under Ar for 2 h, cooled to room temperature, filtered through a layer of silica gel, diluted with water, and extracted with CH_2Cl_2. The organic layer was thoroughly washed with water and evaporated in vacuo to give a mixture of products (0.86 g). Recrystallization of the mixture (0.050 g) from hexane:ether gave pure 2β-acetoxy-3β-hydroxy-5α-cholest-7-en-6-one (**11**, 0.035 g). UV spectrum (λ_{max}, nm): 245. PMR spectrum (CD_2Cl_2, δ, ppm, J/Hz): 0.60 (3H, s, 18-Me), 0.87 (6H, d, J = 6.5, 26-Me, 27-Me), 0.95 (3H, s, 19-Me), 2.07 (3H, s, AcO), 2.32 (1H, dd, J_1 = 12, J_2 = 3.5, H-5α), 3.70 (1H, m, W/2 = 24, H-3α), 5.10 (1H, br.d, J = 2.5, H-2α), 5.70 (1H, br.t, J = 2, H-7).

The remainder was dissolved in pyridine (4 mL), treated with acetic anhydride (2 mL), and left for 12 h at 0°C. The solution was diluted with water and extracted with benzene. The organic layer was washed with water and evaporated in vacuo. The solid was chromatographed over a silica-gel column with elution by petroleum ether:ethylacetate of increasing polarity (from 20:1 to 5:1) to isolated 2α-iodo-3β-acetoxy-5α-cholest-7-en-6-one (**10**, 0.030 g, 3% calculated for pure **7**), mp 148-150°C (petroleum ether:ethylacetate). UV spectrum (λ_{max}, nm): 245. PMR spectrum (CD_2Cl_2, δ, ppm, J/Hz): 0.60 (3H, s, 18-Me),

0.87 (6H, d, J = 6.5, 26-Me, 27-Me), 0.89 (3H, s, 19-Me), 0.96 (3H, d, J = 6, 21-Me), 2.05 (3H, s, AcO), 2.47 (1H, dd, J_1 = 12, J_2 = 3.5, H-5α), 2.60 (1H, dd, J_1 = 13, J_2 = 4.5, H-1β), 4.22 (1H, ddd, J_1 = 14, J_2 = 12, J_3 = 4, H-2β), 4.92 (1H, td, J_1 = 11.5, J_2 = 5, H-3α), 5.68 (1H, br.t, J = 2, H-7).

Elution by petroleum ether:ethylacetate (3:1) isolated the target 2β,3β-diacetoxy-5α-cholest-7-en-6-one (**12**, 0.440 g, 55% calculated for pure **7**), mp 180-184°C, lit. [12] mp 213-216°C. UV spectrum (λ_{max}, nm): 245. IR spectrum (ν, cm^{-1}): 1750, 1250 (AcO), 1680 (C=O), 1630 (C=C). PMR spectrum (CD_2Cl_2, δ, ppm, J/Hz): 0.60 (3H, s, 18-Me), 0.88 (6H, d, J = 6.5, 26-Me, 27-Me), 0.94 (3H, d, J = 6, 21-Me), 0.99 (3H, s, 19-Me), 2.00 (3H, s, AcO), 2.05 (3H, s, AcO), 2.40 (1H, dd, J_1 = 12, J_2 = 3.5, H-5α), 4.81 (1H, ddd, J_1 = 12, J_2 = 5, J_3 = 3.5, H-3α), 5.27 (1H, br.d, J = 2.5, H-2α), 5.70 (1H, br.t, J = 2, H-7).

2β,3β-Diacetoxy-14α-hydroxy-5α-cholest-7-en-6-one (13). A solution of **11** (0.39 g) in dioxane (5 mL) was treated with a boiling solution of SeO_2 (0.30 g) in dioxane (8 mL). The mixture was held at 81-85°C for 35 min, cooled to room temperature, and filtered through a layer of silica gel. The filtrate was evaporated in vacuo. The solid was chromatographed over a silica-gel column with elution by petroleum ether:ethylacetate of increasing polarity (from 5:1 to 3:1) to give **13** (0.36 g, 89%), mp 221-223°C, lit. [8] mp 230-232°C. UV spectrum (λ_{max}, nm): 242. PMR spectrum (CD_2Cl_2, δ, ppm, J/Hz): 0.70 (3H, s, 18-Me), 0.88 (6H, d, J = 6.5, 26-Me, 27-Me), 0.92 (3H, d, J = 6, 21-Me), 1.00 (3H, s, 19-Me), 1.98 (3H, s, AcO), 2.04 (3H, s, AcO), 2.42 (1H, dd, J_1 = 12, J_2 = 3.5, H-5α), 2.72 (1H, m, W/2 = 22, H-9α), 4.81 (1H, ddd, J_1 = 12, J_2 = 5, J_3 = 3.5, H-3α), 5.26 (1H, br.d, J = 2.5, H-2α), 5.89 (1H, d, J = 2.5, H-7).

Hydrolysis of 2β,3β-Diacetoxy-14α-hydroxy-5α-cholest-7-en-6-one (13). A solution of **13** (0.30 g) in MeOH (25 mL) at 50°C was treated with K_2CO_3 (0.30 g) in aqueous MeOH (4 mL, 1:1). The reaction mixture was held at 45-50°C for 25 min, cooled to room temperature, and diluted with water. The precipitate was filtered off. The mother liquor was extracted with ethylacetate. The organic layer was washed with water and evaporated in vacuo. The solid was combined with the previously obtained product and chromatographed over a silica-gel column with elution by $CHCl_3$:CH_3OH of increasing polarity (from 20:1 to 15:1) to give 5α-22,25-dideoxyecdysone (**1a**, 0.092 g, 36%), mp 231-233°C ($CHCl_3$), lit. [8] mp 245-249°C (CH_3OH). UV spectrum (λ_{max}, nm): 242. PMR spectrum (C_5D_5N, δ, ppm, J/Hz): 0.72 (3H, s, 18-Me), 0.88 (6H, d, J = 6.5, 26-Me, 27-Me), 1.04 (3H, d, J = 6.5, 21-Me), 1.42 (3H, s, 19-Me), 3.03 (1H, m, W/2 = 24, H-9α), 3.90 (1H, m, W/2 = 17, H-3α), 4.40 (1H, m, W/2 = 11, H-2α), 6.24 (1H, d, J = 2.5, H-7).

Continued elution by $CHCl_3$:CH_3OH (12:1, 10:1) isolated 22,25-dideoxyecdysone (**1**, 0.101 g, 40%), mp 206-208°C (ethylacetate), lit. [8] mp 208-210°C. UV spectrum (λ_{max}, nm): 243. PMR spectrum (C_5D_5N, δ, ppm, J/Hz): 0.75 (3H, s, 18-Me), 0.88 (6H, d, J = 6.5, 26-Me, 27-Me), 1.02 (3H, d, J = 6.5, 21-Me), 1.06 (3H, s, 19-Me), 3.04 (1H, br.d, J = 13, H-5β), 3.59 (1H, m, W/2 = 24, H-9α), 4.20 (1H, br.d, J = 12, H-3α), 4.28 (1H, m, W/2 = 11, H-2α), 6.25 (1H, d, J = 2.5, H-7).

REFERENCES

1. R. Bergamasco and D. H. S. Horn, in: *Progress in Ecdysone Research*, J. A. Hoffmann, ed., Elsevier/North Holland Biomed. Press, Amsterdam (1980), p. 299.
2. W. E. Robbins, J. N. Kaplanis, M. J. Thompson, T. J. Shortino, and S. C. Joyner, *Steroids*, **16**, 105 (1970).
3. W. F. Walker and M. J. Thompson, *J. Econ. Entomol.*, **66**, 64 (1973).
4. A. A. Akhrem and N. V. Kovganko, *Ecdysteroids: Chemistry and Biological Activity* [in Russian], Nauka i Tekhnika, Minsk (1989).
5. R. Lafont and I. Wilson, *The Ecdysone Handbook, 2nd Ed.*, The Chromatographic Society, Nottingham (1996).
6. A. Furlenmeier, A. Fuerst, A. Langemann, G. Waldvogel, U. Kerb, P. Hocks, and R. Wiechert, *Helv. Chim. Acta*, **49**, 1591 (1966).
7. H. Mori, K. Shibata, K. Tsuneda, and M. Sawai, *Chem. Pharm. Bull.*, **16**, 1593 (1968).
8. M. J. Thompson, W. E. Robbins, J. N. Kaplanis, C. F. Cohen, and S. M. Lancaster, *Steroids*, **16**, 85 (1970).
9. N. V. Kovganko and V. L. Survilo, *Khim. Prir. Soedin.*, 135 (2001).
10. K. T. Alston, P. M. Bebington, S. E. Green, E. D. Morgan, and C. F. Poole, *Steroids*, **27**, 609 (1976).
11. N. V. Kovganko and V. L. Survilo, *Khim. Prir. Soedin.*, 216 (2002).
12. A. A. Akhrem, F. A. Lakhvich, V. A. Khripach, and N. V. Kovganko, *Zh. Org. Khim.*, **19**, 1249 (1983).
13. B. Rezabova, J. Hora, V. Landa, V. Cerny, and F. Sorm, *Steroids*, **11**, 475 (1968).