Chemistry of Natural Compounds, Vol. 37, No. 1, 2001

REACTIVITY OF HYDROXY AND KETO GROUPS ON C-6 AND C-17 OF 3α,5α-CYCLOANDROSTANES

N. V. Kovganko and Zh. N. Kashkan UDC 547.92

Convenient methods for preparing several 3α,5-cycloandrostanes have been developed in order to synthesize the phytoecdysteroid rubrosterone. Certain of their chemical transformations were studied.

Key words: androstanes, phytoecdysteroids, rubrosterone.

The phytoecdysteroid rubrosterone (**1**) is rarely encountered [1]. Reports of its isolation from plants are few [2-8]. The unavailability of rubrosterone explains why its biological properties are poorly studied, in contrast with other ecdysteroids. It is known only that **1** exhibits antidiabetic [9] and anabolic [10] activity. Chemical methods for preparing rubrosterone from more available phytoecdysteroids or from androstane steroids, for example, androstenolone (**2a**), have been proposed. However, it should be noted that existing methods have drawbacks that include multiple steps or low total yields.

We developed a convenient preparative method for synthesizing several 3α,5-cycloandrostane derivatives and studied the possibility of using them to synthesize rubrosterone. The effectiveness of using compounds of this structure to prepare rubrosterone [11] or its analogs [12] has been reported. In particular, the use of 3α,5-cyclo-6,17-dione **5** to prepare **1** has been described [11]. However, experimental details are not included. Therefore, we studied the synthesis of **5** from androstenolone **2a** in more detail. In one method, we prepared **5** via rearrangement of **2b** by KOAc in aqueous acetone with subsequent oxidation by chromic acid of the resulting 3α,5-cyclo-6β-ol **3** without isolating it from the reaction mixture. The overall yield was 61%. In another method, rearrangement of tosylate **2c** by K_2CO_3 in aqueous acetone with subsequent oxidation of intermediate **3** was used. The required diketosteroid **5** was obtained from **1a** without isolating intermediates **2c** and **3** in overall yield 74%, which is greater than the literature results [11].

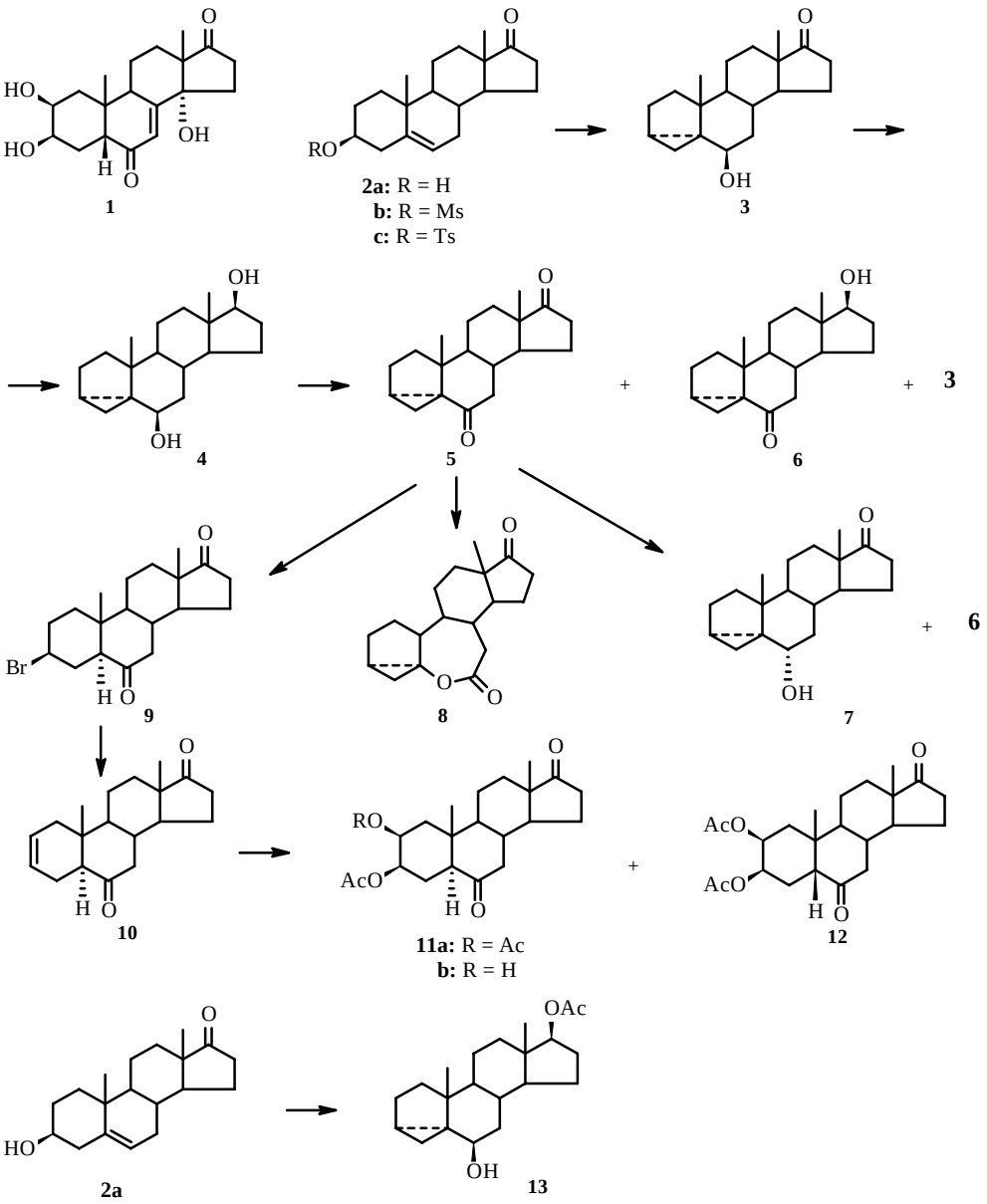
Compound **6**, a 17β-hydroxy-6-ketone, is interesting for the synthesis of **1**. Preparation of **6** via reduction of **5** by yeast has been reported [13]. We hoped to synthesize **6** by either selective oxidation of 6β,17β-diol **4** or reduction of 6,17-diketone **5**. We prepared **4** by first synthesizing the 6β-hydroxy-17-ketone (**3**) by the usual rearrangement of androstenolone **2b** mesylate in 65% yield. Subsequent reduction by $NaBH_4$ in methanol gave the required **4** in 91% yield. Further oxidation of diol **4** by chromic acid in acetone produced four compounds. The principal product was the 6,17-diketone **5** (62% yield). However, the required 17β-hydroxy-6-ketone (**6**) was isolated in a mixture with 6β-hydroxy-17-ketone (**3**) in overall yield 29%. The minor components were 6β-hydroxy-17-ketone (**3**, 5%) and starting 6β,17β-diol (**4**, 3%).

Reduction of **5** by $NaBH_4$ in methanol forms a mixture of two compounds, the required 17β-hydroxy-6-ketone (**6**) and 6α-hydroxy-17-ketone **7** with predominance of the former according to ^{1}H NMR spectra. However, the selectivity of this reaction is insufficient to use it as a synthetic method for preparing rubrosterone.

We studied oxidation of **5** by trifluoroperacetic acid. It was noted previously that this reagent reacts with stigmastane [14] and pregnane [15] 3α,5-cyclo-6-ketosteroids to form the corresponding 3α,5-cyclo-Δ^7-6-ketosteroids, which are important intermediates in the synthesis of ecdysteroids. However, the principal product in our instance was the lactone **8** (yield 32%). The structure of **8** was proved by spectral data and elemental analysis. In particular, the additional O atom is placed between C-5 and C-6 according to the observed shift to weak field in the ^{1}H NMR spectrum of protons in the 3-membered ring, as a result of which they shift into the methylene region and become indistinguishable. Apparently, **8** forms and not the Δ^7-ketone because of steric strain created by the 17-ketone. Similar examples are known in steroid chemistry [16, 17].

Institute of Bioorganic Chemistry, National Academy of Sciences, Belarus, 220141, Belarus, Minsk, ul. Akad. Kuprevicha, 5/2. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 43-47, January-February, 2001. Original article submitted January 29, 2001.

0009-3130/01/3701-0047$25.00



Procedures to introduce a 2(3)-double bond and subsequently *cis*-hydroxylate it according to Woodward to form 2β,3β-diols are important in ecdysteroid synthesis [1]. The transformation of **5** into 3β-bromo-6-ketone **9** by HBr and its dehydrobromination in quinoline to form Δ^2-6,17-diketone **10** have been described [13]. We repeated these experiments by reacting HBr in acetic acid with **5** and did indeed obtain **9** in 91% yield. However, its dehydrobromination by Li_2CO_3 and LiBr in boiling DMF was unsuccessful. The desired Δ^2-steroid was isolated pure in only 23% yield. A side product from this reaction was 3α,5-cyclosteroid **5**. Direct isomerization of **5** into Δ^2-steroid **10** by *p*-toluenesulfonic acid in sulfolane with heating was a more convenient method [18]. This produced **10** in 81% yield, which is comparable with the analogous transformation of **5** by pyridinium hydrobromide in DMF [19].

Then we studied the reactivity of 2β,3β-diacetoxy-*cis*-*A*/*B*-6,17-diketosteroid **12**, which can be prepared by *cis*-hydroxylation of the Δ^2-bond in **10** by the Woodward reaction. Thus, **10** was first hydroxylated by silver acetate and iodine in aqueous acetic acid. Then the product was isomerized without further purification by K_2CO_3 in aqueous methanol. Hydrolysis of the acetoxys also occurs. Therefore, the 2,3-diols are converted to acetates by acetylation with acetic anhydride in pyridine. We succeeded in isolating a mixture of *trans*-*A*/*B*-6,17-diketosteroid **11a** and its *cis*-*A*/*B*-isomer **12** in 25% overall yield using these chemical transformations and subsequent chromatographic separation. Besides these compounds, the reaction mixture also contained small quantities of **11b**. Further acetylation of **11b** with acetic anhydride in pyridine in the presence of 4-dimethylaminopyridine for four days gave practically quantitative yield of pure **11a**. Attempts to separate a mixture of **11a** and

12 were unsuccessful. Therefore, we stopped work in this direction. The last stage included mesylation of **2a**, reduction of the 17-ketone in the resulting mesylate **2b**, acetylation of the 17β-hydroxyl, and isomerization in rings *A* and *B* by NaOAc in aqueous acetone to give 6β,17β-diol 17-monoacetate **13** in ~30% overall yield. Compound **13** was previously used [12] to synthesize structural analogs of rubrosterone. The method developed by us to synthesize it without isolating intermediates has several advantages compared with the previous method. Therefore, it is interesting for preparing androstane ecdysteroids.

We thank T. N. Netesova and Yu. G. Chernov for help with the investigation.

EXPERIMENTAL

Melting points were determined on a Kofler block. IR spectra were recorded on a UR-20 instrument at 700-3600 cm^{-1}. ^1H NMR spectra of $CDCl_3$ solutions were obtained on Bruker WM-360 and Bruker AC-200 NMR spectrometers at working frequencies 360 and 200 MHz, respectively. Chemical shifts are given relative to TMS internal standard.

Androst-5-en-3β-ol-17-one mesylate (2b) was prepared by reacting **2a** (3.1 g) and methanesulfonyl chloride (1.6 mL) in pyridine (20 mL) at room temperature for 1 d. The usual work up of the reaction mixture and column chromatography on silica gel (eluent hexane:ether and then ether) gave **2b** (3.0 g), 76%, mp 165-170°C (dec.) (acetone).

IR spectrum (KBr, ν, cm^{-1}): 1740 (C=O).

3α,5-Cyclo-5α-androstan-6β-ol-17-one (3). A solution of **2a** (1.0 g) in pyridine (10 mL) was cooled on an ice bath, treated with methanesulfonyl chloride (1.4 mL), held at room temperature for 20 h, diluted with water, and extracted with toluene. The organic layer was washed with water and evaporated under vacuum. The solid (1.1 g) was dissolved in acetone (100 mL), treated with water (10 mL) and NaOAc (0.6 g), boiled for 1 d, cooled to room temperature, diluted with water, and extracted with toluene. The toluene extract was washed with water and evaporated under vacuum. The solid was chromatographed on a silica-gel column with elution by ether:hexane (5:1). Yield of **3**, 0.65 g (65%). ^1H NMR spectrum (δ, ppm): 0.33 (1H, dd, J_1 = 8 Hz, J_2 = 5 Hz, H-4α), 0.56 (1H, t, J = 5 Hz, H-3β), 0.93 (3H, s, 18-Me), 1.10 (3H, s, 19-Me), 3.33 (1H, t, J = 2 Hz, H-6α).

3α,5-Cyclo-5α-androstan-6β,17β-diol (4). A solution of **3** (0.60 g) in methanol (14 mL) was treated with $NaBH_4$ (0.07 g), stirred and cooled on an ice bath for 1 h, and treated with acetic acid (3 mL). The solvent was removed under vacuum. The solid was chromatographed on a silica-gel column with elution by THF:hexane (1:1). Yield of **4**, 0.55 g (91%). ^1H NMR spectrum (δ, ppm): 0.30 (1H, dd, J_1 = 8.5 Hz, J_2 = 5 Hz, H-4α), 0.53 (1H, t, J = 5 Hz, H-3β), 0.80 (3H, s, 18-Me), 1.06 (3H, s, 19-Me), 3.26 (1H, t, J = 2 Hz, H-6α), 3.64 (1H, m, W/2 = 20 Hz, H-17α).

Oxidation of 3α,5-Cyclo-5α-androstan-6β,17β-diol (4). A solution of **4** (0.492 g) in acetone (10 mL) was cooled to 0°C, treated with chromic acid (0.636 mL, 8 N), stirred and cooled for 20 min, and treated with isopropanol (1 mL). After 10 min the mixture was diluted with water and extracted with toluene. The toluene extract was washed with water and evaporated under vacuum. The solid was chromatographed on a silica-gel column with elution by THF:petroleum ether (1:10). Yield of **5** in fraction 1, 0.300 g (62%), mp 187-190°C; of **3** in fraction 2, 0.023 g (5%); of **3** and **6** in fraction 3, 0.143 g (29%); of **4** in fraction 4, 0.013 g (3%).

3β,5-Cyclo-5α-androstan-6,17-dione (5). A. A solution of **2b** mesylate (2.8 g) and KOAc (2.0 g) in acetone (500 mL) and water (50 mL) was refluxed for 15 h, cooled to room temperature, stirred, and treated with chromic acid (10 mL, 8 N). After 10 min the excess of oxidant was decomposed by stirring, adding ethanol (10 mL), and diluting with water (500 mL). The product was extracted with $CHCl_3$. The organic layer was evaporated under vacuum. The solid was chromatographed on a silica-gel column with elution by hexane:ether (1:1). Yield of **5**, 1.7 g (61%), mp 187-196°C (petroleum ether), lit. [13] mp 182-183°C.

IR spectrum (KBr, ν, cm^{-1}): 1740 (C_{17}=O), 1690 (C_6=O). ^1H NMR spectrum (δ, ppm): 0.77 (1H, t, J = 5 Hz, H-3β), 0.94 (3H, s, 18-Me), 1.05 (3H, s, 19-Me).

B. A solution of **2a** (5.0 g) and *p*-toluenesulfonyl chloride (5.0 g) in pyridine (50 mL) was held at room temperature for 1 d and diluted with water. The precipitate of androst-5-en-3β-ol-17-one tosylate (**2c**) was filtered off, washed on the filter with water, and dissolved without further purification in acetone (1000 mL). The solution was treated with water (50 mL) and K_2CO_3 (3.0 g), refluxed for 1 d, held at room temperature for 2.5 d, stirred, treated at room temperature with chromic acid (30 mL, 8 N), treated after 2 h with isopropanol (10 mL), and diluted with water. The product was extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated under vacuum. The solid was chromatographed on a silica-gel column with elution by ether to

give **5** (3.7 g, 74%).

Reduction of 3α,5-Cyclo-5α-androstan-6,17-dione (5). A cooled (0°C) solution of **5** (0.10 g) in methanol (7 mL) was treated with $NaBH_4$ (0.013 g), stirred at 0°C for 0.5 h, and treated with acetic acid (0.5 mL). The solvent was removed under vacuum. The solid was dissolved in THF (3 mL). The solution was filtered through a layer of aluminum oxide. The filtrate was evaporated to dryness under vacuum. Yield of **6** and **7** (4:3 ratio according to ^{1}H NMR), 0.118 g. IR spectrum (film, ν, cm^{-1}): 1750 (C_{17}=O), 1695 (C_6=O). ^{1}H NMR spectrum (δ, ppm): **6**: 0.76 (3H, s, 18-Me), 0.94 (3H, s, 19-Me), 3.68 (1H, m, W/2 = 27 Hz, H-17α); **7**: 0.81 (3H, s, 18-Me), 1.07 (3H, s, 19-Me), 3.92 (1H, m, W/2 = 19 Hz, H-6β).

3α,5-Cyclo-B-homo-6-oxa-5α-androstan-6α,17-dione (8). A solution of **5** (0.30 g) in CH_2Cl_2 (10 mL) was treated with a solution of trifluoroacetic acid (prepared by dissolving 1.0 mL trifluoroacetic anhydride in 15 mL CH_2Cl_2 with subsequent dilution with 0.3 mL of 30% H_2O_2). The mixture was stirred at room temperature for 0.5 h and filtered through a layer of aluminum oxide. The filtrate was evaporated under vacuum at room temperature. The solid was chromatographed on a silica-gel column with elution by ether:hexane (1:1). Yield of **8**, 0.10 g (32%), mp 196-199°C (hexane—acetone). Found (%): C 75.20, H 8.74. Calc. for $C_{19}H_{26}O_3$ (%): C 75.43, H 8.66. IR spectrum (KBr, ν, cm^{-1}): 1740 (C=O). ^{1}H NMR spectrum (δ, ppm): 0.92 (3H, s, 18-Me), 1.08 (3H, s, 19-Me).

3β-Bromo-5α-androstan-6,17-dione (9). A solution of **5** (1.0 g) in glacial acetic acid (10 mL) was treated dropwise with HBr (1.5 mL), stirred at 13°C for 15 min, and diluted with water (15 mL). The product was extracted with CH_2Cl_2. The organic layer was washed successively with water, saturated $NaHCO_3$ solution, and water, was filtered through a layer of aluminum oxide, and was evaporated under vacuum to dryness. Yield of **9**, 1.17 g (91%), mp 187-189°C (hexane—acetone), lit. [13] mp 184°C.

IR spectrum (KBr, ν, cm^{-1}): 1750 (C_{17}=O), 1725 (C_6=O). ^{1}H NMR (δ, ppm): 0.84 (3H, s, 19-Me), 0.88 (3H, s, 18-Me), 3.96 (1H, m, W/2 = 26 Hz, H-3α).

5α-Androst-2-en-6,17-dione (10). A. A solution of **9** (0.90 g) in DMF (3 mL) was treated with Li_2CO_3 (0.90 g) and LiBr (0.30 g), boiled for 0.5 h, cooled to room temperature, diluted with water, and extracted with benzene. The benzene extract was washed with water and evaporated under vacuum. The solid was chromatographed on a silica-gel column with elution by benzene:hexane (3:1).

Yield of **10** in fraction 1: 0.16 g (23%). IR spectrum (KBr, ν, cm^{-1}): 1750 (C_{17}=O), 1720 (C_6=O), 1670 (C=C). ^{1}H NMR spectrum (δ, ppm): 0.74 (3H, s, 19-Me), 0.89 (3H, s, 18-Me), 5.60 and 5.69 (1H, m, W/2 = 11 Hz, H-2 and H-3); of **10** and **5** in fraction 2, 0.35 g (50%); of **5** in fraction 3, 0.21 g (30%).

B. A solution of **5** (78.8 mg) in sulfolane (4 mL) was treated with *p*-toluenesulfonic acid (6 mg), heated at ~170°C for 3 h, cooled to room temperature, diluted with water (10 mL), and extracted with hexane. The extract was washed with water, filtered through a layer of aluminum oxide, and evaporated under vacuum to dryness. Yield of **10**, 63 mg (81%).

Woodward *cis*-Hydroxylation of 10. A stirred solution of **10** (0.512 g) in acetic acid (20 mL) and water (1 mL) at 43°C was treated successively with silver acetate (0.9 g) and iodine (0.5 g). The temperature of the reaction mixture was adjusted to 55-60°C. Stirring was continued for 2 h. The reaction mixture was cooled to room temperature and filtered through a layer of aluminum oxide. The filtrate was diluted with water (20 mL). The product was extracted with dichloroethane. The organic layer was washed with water. The solvent was removed under vacuum. The solid was treated with methanol (10 mL), water (0.5 mL), and K_2CO_3 (0.2 g). The mixture was refluxed for 1 h, cooled to room temperature, and filtered through a layer of aluminum oxide. The filtrate was diluted with water (25 mL) and extracted with dichloroethane. The extract was washed with water. The solvent was removed completely under vacuum. The solid was dissolved in pyridine (3 mL), added to acetic acid solution (2 mL), held at room temperature for 20 h, diluted with water, and extracted with dichloroethane. The extract was washed with water. The solvent was removed under vacuum. The solid was separated on a silica-gel column with elution by dichloroethane:THF (1:1).

Yield of **11a** and **12** in fraction 1, 183 mg (25%) (according to ^{1}H NMR). ^{1}H NMR spectrum (δ, ppm): **11a**: 0.88 (3H, s, 18-Me), 0.98 (3H, s, 19-Me), 2.02 (3H, s, AcO), 2.09 (3H, s, AcO), 4.82 (1H, m, W/2 = 22 Hz, H-3α), 5.32 (1H, m, W/2 = 10 Hz, H-2α); **12**: 0.88 (3H, s, 18-Me), 1.06 (3H, s, 19-Me), 2.02 (3H, s, AcO), 2.13 (3H, s, AcO), 4.95 (1H, m, W/2 = 22 Hz, H-2α), 5.38 (1H, m, W/2 = 10 Hz, H-3α); of **11b** in fraction 2, 52 mg (8%), mp 224-227°C (methanol). IR spectrum (KBr, ν, cm^{-1}): 1740 (AcO, C_{17}=O), 1715 (C_6=O). ^{1}H NMR spectrum (δ, ppm): 0.89 (3H, s, 18-Me), 1.05 (3H, s, 19-Me), 2.11 (3H, s, AcO), 3.51 (1H, d, J = 5 Hz, H-4β), 4.12 (1H, m, W/2 = 9 Hz, H-2α), 4.77 (1H, m, W/2 = 22 Hz, H-3α).

Acetylation of 11b. A solution of **11b** (43 mg) in pyridine (3 mL) was treated with acetic anhydride (1 mL) and 4-dimethylaminopyridine (5 mg), held for 4 d at room temperature, diluted with water, and extracted with dichloroethane. The

extract was washed with water. The solvent was removed under vacuum. The solid was dissolved in dichloroethane 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 THF:dichloroethane (1:1). Yield of **11a**, 46 mg (96%), mp 260°C (methanol). IR spectrum (KBr, ν, cm^{-1}): 1745 (AcO, C_{17}=O), 1710 (C_6=O). ^{1}H NMR spectrum (δ, ppm): 0.88 (3H, s, 18-Me), 0.98 (3H, 19-Me), 2.02 (3H, s, AcO), 2.09 (3H, s, AcO), 4.80 (1H, m, W/2 = 22 Hz, H-3α), 5.30 (1H, m, W/2 = 8 Hz, H-2α).

3*α*,5-Cyclo-5*α*-androstan-6*β*,17*β*-diol 17-Acetate (13). A solution of **2a** (7.3 g) and methanesulfonyl chloride (10.5 mL) in pyridine (40 mL) was held at room temperature for 20 h, diluted with water, and extracted with toluene. The toluene extract was washed with water and evaporated under vacuum. A solution of **2b** mesylate in methanol (100 mL) was cooled on an ice bath, treated with $NaBH_4$ (0.7 g), stirred at 0°C for 40 min, treated with acetic acid (30 mL), and stirred for 10 min. The solvent was completely evaporated under vacuum. The solid (7.9 g) was dissolved in pyridine (40 mL), treated with acetic anhydride (14 mL), held at room temperature for 18 h, diluted with water, and extracted with toluene. The organic layer was washed with water and evaporated under vacuum. The solid (7.85 g) was dissolved in acetone (110 mL) and water (25 mL), treated with NaOAc (4.5 g), and refluxed for 9 h. Part of the acetone was removed at reduced pressure. The remainder was diluted with water and extracted with toluene. The organic extract was washed with water and evaporated under vacuum. The solid was chromatographed on a silica-gel column with elution by THF:petroleum ether (1:1). Yield of **13**, 2.4 g (29%), mp 124-128°C, lit. [12] mp 130-132°C. IR spectrum (KBr, ν, cm^{-1}): 1745 (AcO). ^{1}H NMR (δ, ppm): 0.30 (1H, dd, J_1 = 9 Hz, J_2 = 6 Hz, H-4α), 0.53 (1H, t, J = 5 Hz, H-3β), 0.85 (3H, s, 18-Me), 1.09 (3H, s, 19-Me), 2.05 (3H, s, AcO), 3.29 (1H, m, W/2 = 8 Hz, H-6α), 4.63 (1H, m, W/2 = 20 Hz, H-17α).

REFERENCES

1. A. A. Akhrem and N. V. Kovganko, *Ecdysteroids: Chemistry and Biological Activity* [in Russian], Nauka i Tekhnika, Minsk (1989).
2. T. Takemoto, Y. Hikino, H. Hikino, S. Ogawa, and N. Nishimoto, *Tetrahedron Lett.*, 3053 (1968).
3. T. Takemoto, Y. Hikino, H. Hikino, S. Ogawa, and N. Nishimoto, *Tetrahedron*, **25**, 1241 (1969).
4. Jpn. Pat. No. 7127472 (1971); *Chem. Abstr.*, **76**, 23177w (1972).
5. M. T. Chua, A. C. Santos, C. Abela, and U. Wagner, *Philipp. J. Sci.*, **111**, 1 (1982).
6. Y. Shiobara, S. S. Inoue, K. Kato, Y. Nishiguchi, Y. Oishi, N. Nishimoto, F. de Oliveira, G. Akisue, M. K. Akisue, and G. Hashimoto, *Phytochemistry*, **32**, 1527 (1993).
7. C. Kusamba, M. Nicoletti, E. Federici, G. Multari, G. Galeffi, and G. Palazzino, *Fitoterapia*, **66**, 175 (1995).
8. J.-P. Girault, M. Bathori, E. Varga, K. Szendrei, and R. Lafont, *J. Nat. Prod.*, **53**, 279 (1990).
9. Jpn. Pat. No. 7342872 (1973); *Chem. Abstr.*, **81**, 49923q (1974).
10. K. Slama and R. Lafont, *Eur. J. Entomol.*, **92**, 355 (1995).
11. W. Van Bever, F. Kohen, V. V. Ranade, and R. E. Counsell, *J. Chem. Soc., Chem. Commun.*, 758 (1970).
12. L. Labler, K. Slama, and F. Sorm, *Collect. Czech. Chem. Commun.*, **33**, 2226 (1968).
13. A. Butenandt and L. A. Suranyi, *Ber.*, **75B**, 591 (1942); *Chem. Abstr.*, **37**, 3444[4] (1943).
14. A. A. Akhrem, F. A. Lakhvich, V. A. Khripach, V. N. Zhabinskii, and N. V. Kovganko, *Dokl. Akad. Nauk SSSR*, **275**, 626 (1984).
15. A. A. Akhrem, O. P. Kananovich, and N. V. Kovganko, *Zh. Org. Khim.*, **26**, 1050 (1990).
16. E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, *Conformational Analysis*, Interscience (Div. of Wiley), New York (1965).
17. M. Paluchowska and J. Mokrosz, *Wiad. Chem.*, **34**, 789 (1980).
18. D. H. R. Barton, P. G. Feakins, J. P. Poyser, and P. G. Sammes, *J. Chem. Soc.*, 1584 (1970).
19. M. Anastasia, P. Allevi, P. Ciuffreda, and A. Oleotti, *Steroids*, **45**, 561 (1985).