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SYNTHESIS OF (22E,24R)-ERGOSTA-4,7-22-TRIENE-3,6-DIONE AND ITS 14α-HYDROXY- AND 9α,14α-DIHYDROXY DERIVATIVES

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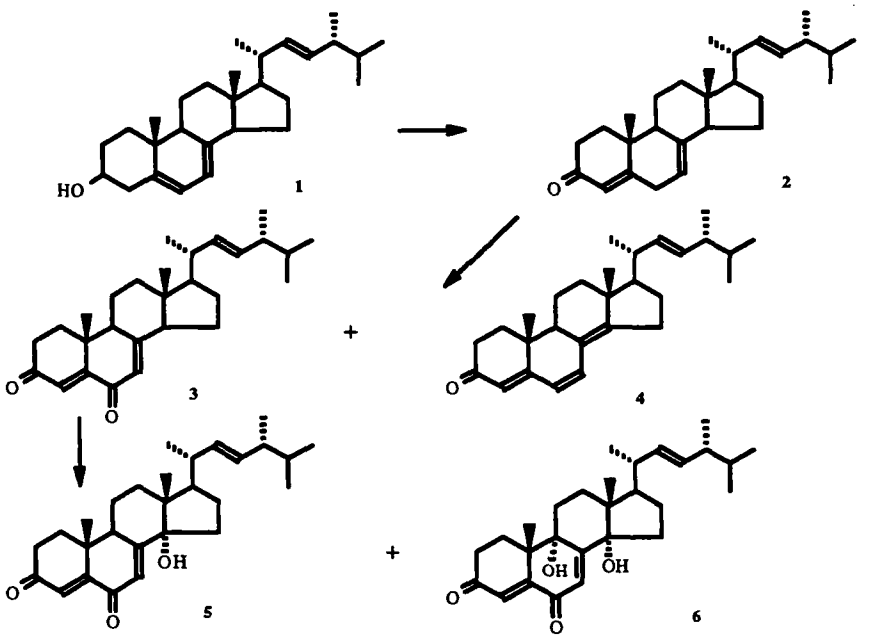
$\Delta^{4,7}$-3,6-Diketosteroids have been synthesized from ergosterol (1).

Recently, a series of ecdysteroids and substances structurally close to them — mainly to oxidized derivatives of ergosterol (**1**) — have been detected in fungi [1]. This type of compounds includes, for example, (22E,24R)-ergosta-4,7,22-triene-3,6-dione (**3**), the isolation of which from natural materials is described in [2—4]. Its hydroxy derivatives having the structure expressed by formulas (**5**) and (**6**) are also of undoubted scientific interest. In particular, it was recently shown [5] that derivatives of the cholestane series close in structure to the diketone (**5**) are involved in the biosynthesis of ecdysteroids. In its turn, compound (**6**), under the name of calvasterol B, has been isolated from *Calvatia cyathiformis* [6].



The aim of the present investigation was the development of convenient methods of synthesizing steroids (**3**), (**5**), and (**6**). It must be mentioned that several variants of the production of the diketone (**3**) have already been described in the literature [2, 7—9]. However, they are all characterized by very low yields of the desired compound. For this reason, in order to synthesize compound (**3**) we chose a scheme developed earlier in the cholestane series [10]. In accordance with this scheme, the initial ergosterol was first subjected to Oppenauer oxidation, as described in [11]. This gave a 74% yield of the $\Delta^{4,7,22}$-3-ketosteroid (**2**), the structure of which was shown with the aid of spectra. Thus, the presence in the IR spectrum of this substance of bands of the stretching vibrations of a carbonyl group at 1680 cm^{-1} and of a double bond at 1630 cm^{-1} permits it

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structure to be deduced as that of an α,β-unsaturated ketone. This was confirmed by its UV spectrum in which there was an absorption band at 241 nm. Its position is typical for Δ^4-3-ketosteroids. Characteristic for the ^{1}H NMR spectrum of compound (2) was the presence of the signal of the H-4 vinyl proton in the form of a broadened doublet at 5.81 ppm. At the same time, the signals of the other vinyl protons (H-7, H-22, and H-23, appeared in the spectrum in the form of an unresolved three-proton multiplet in the 5.08—5.26 ppm region. Moreover, in the spectrum there were two one-proton broadened doublets with their centers at δ 2.68 and 3.17 ppm having the geminal constant J = 19 Hz.

The use of the double-resonance procedure enabled these signals to be assigned to the resonance absorption of the allyl protons H-6β and H-6α, respectively. Thus, irradiation of the signal at 2.68 ppm led, in the first place, to the conversion of the signal at 3.17 ppm into a broadened singlet and, in the second place, to a very slight change in the form of the H-4 signal. In its turn, irradiation of the signal with δ 3.17 ppm caused the transformation of the signal with δ 2.68 ppm into a broadened singlet. Under these conditions, the H-4 signal was likewise observed in the form of a broadened singlet. From this it may be concluded that the splitting of the H-4 signal (J = 1.5 Hz) in the spectrum was due to an allyl interaction with just this last proton A consideration of molecular models permits the conclusion that the signal with δ 3.17 ppm was due to the resonance absorption of the H-6α proton, since, on the other hand, the dihedral angle between H-4 and H-6β amounts to approximately 90° and, thus, the constant of allyl coupling between them must very small.

In the following stage of the synthesis we oxidized steroid (2) with chromium trioxide in a mixture of methylene chloride and pyridine. We investigated several variants of this reaction, two of which are given in the Experimental part. It was established that the best conditions were those under which the reaction mixture still contains a considerable amount of the initial substance, which can be isolated from the mixture and be re-used subsequently. We established that the main reaction products isolated in addition to the initial steroid (2) were the desired $\Delta^{4,7,22}$-3,6-diketone (3) and the $\Delta^{4,6,8(14),22}$-3-ketone (4).

The structures of compounds (3) and (4) were confirmed by their spectra. Thus, in the IR spectrum of steroid (3) there were bands of the stretching vibrations of carbonyl groups at 1685 and 1660 cm^{-1} and of double bonds conjugated with them at 1625 and 1605 cm^{-1}. An absorption band at 277 nm observed in the UV spectrum of this substance is very characteristic for $\Delta^{4,7}$-3,6-diketosteroids [2, 3, 9, 10]. The ^{1}H NMR spectrum of the trienedione (3) also gave confirmation of the presence of the main structural elements. In particular, it contained signals of the vinyl protons H-4 (δ 6.48 ppm) and H-7 (δ 5.99 ppm). The latter appeared in the form of a triplet with the splitting constant J = 2 Hz, due to allyl interaction with the methine protons H-9 and H-14. It must be mentioned that the IR, UV, and ^{1}H NMR spectra of the steroid (3) that we had synthesized corresponded well to those described in the literature [2, 3, 7].

Analogously, it was possible to determine the structure of compound (4) by spectral analysis. In particular, the presence in the IR spectrum of this substance of a band of stretching vibrations of a carbonyl group at 1665 cm^{-1} and of double bonds conjugated with it at 1635 and 1590 cm^{-1} permitted the assumption of the presence in it of a $\Delta^{4,6,8(14)}$-3-keto grouping. The UV spectrum, in which there were bands at 239, 281, and 350 nm, led to the same conclusion. Judging from the literature [13—18], it is just the presence of such bands that is characteristic for the UV absorption of compound (4). In the ^{1}H NMR spectrum of the tetraenone (4) there are the characteristic signals of the vinyl protons H-4 (δ 5.74 ppm), H-6 (δ 6.61 ppm), H-7 (δ 6.03 ppm) and H-22 and H-23 (δ 5.18—5.30 ppm). The positions and forms of the signals agree well with the analogous parameters of the spectrum of compound (4) described in the literature [13, 14, 16, 19].

It must be mentioned that the formation of a $\Delta^{4,6,8(14)}$ -3-ketone as a by-product of the oxidation of a $\Delta^{4,7}$-3-ketone has also been observed in the cholestane series [10]. Attention is attracted by the fact that the tetraenone (4), like the diketone (3), is also a natural substance. The isolation of compound (4) has been described in, for example, [13—22].

In the completing stage of the synthesis, the trienedione (3) was oxidized with selenium dioxide in dioxane. This led to the formation of the 9α-hydroxy-$\Delta^{4,7,22}$-3,6-diketone (5) and 9α,14α-dihydroxy-$\Delta^{4,7,22}$-3,6-diketone (6), isolated from the reaction mixture with yields of 24 and 23%, respectively. It was possible to demonstrate the structures of compounds (5) and (6) unambiguously with the aid of their spectra. Thus, in the ^{1}H NMR spectrum of steroid (5) we observed a downfield shift of the signals of the vinyl protons H-4 and H-7 in comparison with their positions in the spectrum of the initial substance caused by the presence of the 14α-hydroxy group. At the same time, the signal of the H-7 proton had the form not of a triplet but of a doublet. It was shown by the double-resonance method that the splitting of this signal was due to an allyl interaction with the H-9 methine proton, which appeared in the spectrum at 2.95 ppm in the form of a doublet of doublets. When the H-9α signal was irradiated the H-7 signal was converted into a singlet. At the same time, irradiation of H-7 led to the H-9α signal being observed in the spectrum in the form of a doublet of doublets. We may note that such a downfield shift of the H-9α signal is typical for ecdysteroids [1, 23] and is due to the presence of the 14α-hydroxy group, assuming the 1,3-diaxial orientation in

relation to it.

In its turn, in the ^{1}H NMR spectrum of the 9α,14α-dihydroxy derivative (**6**) a greater downfield shift of the signals of the vinyl protons H-4 and H-7 as compared with their positions in the spectra of steroids (**3**) and (**5**) was observed. A convincing proof of the presence in the molecule of compound (**6**) of an additional 9α-hydroxy group was the absence from the spectrum of the signal of the H-9α proton and the conversion of the H-7 signal into a singlet. Attention is attracted by the considerable hypsochromic shift, to 267 nm, of the absorption band in the UV spectrum of compound (**6**) in comparison with its position in the spectra of the $\Delta^{4,7}$-3,6-diketones (**3**) and (**5**). Such a shift is probably also due to the presence of a 9α-hydroxy group in the structure of steroid (**6**).

EXPERIMENTAL

Melting points were determined on a Kofler block. IR spectra were recorded on a UR-20 instrument in the range of 700—3600 cm^{-1} in KBr tablets. UV spectra in ethanol were taken on a Specord M-400 instrument. The ^{1}H NMR spectra of solutions in deuterochloroform were obtained on a Bruker AC-200 NMR spectrometer with a working frequency of 200 MHz. Chemical shifts are given relative to TMS as internal standard.

(22E,24R)-Ergosta-4,7,22-trien-3-one (2). A mixture of 0.5 g of ergosterol (**1**), 1.83 ml of cyclohexanone, and 0.5 g of 4 Å molecular sieves in 30 ml of toluene was boiled under reflux for 20 min. To this mixture was added 1.31 g of aluminum isopropoxide and 5 ml of toluene, and boiling was continued for another 25 min. After the reaction mixture had cooled to room temperature, 1 ml of water and 0.5 ml of 2 M caustic soda solution was added to it, and it was filtered through a layer of alumina which was then washed with 50 ml of hot toluene and 30 ml of dichloroethane. The combined filtrate was evaporated under reduced pressure. The residue was chromatographed on a column of silica gel, with elution by petroleum ether—ethyl acetate (10:1).

This gave 0.37 g of the trienone (**2**). Yield 74%. mp 129—131°C (hexane—ethyl acetate); lit. [11]: mp 129—132°C. IR spectrum (cm^{-1}): 1680 (C=O, 1630 (C=C). UV spectrum: λ_{max} 241 nm (ε12800). ^{1}H NMR spectrum (δ, ppm): 0.61 (18-Me, s), 0.84 (26-Me, d, J=6.5 Hz), 0.86 (27-Me, d, J=6.5 Hz), 0.93 (28-Me, d, J=6.5 Hz), 1.04 (21-Me, d, J=6.5 Hz), 1.19 (19-Me, s), 2.68 (H-6β, br, d, J=19 Hz), 3.17 (H-6α, br, d, J=19 Hz), 5.08-5.26 (H-7, H-22, H-23, m), 5.81 (H-4, br, d, J=1.5 Hz).

Oxidation of (22E,24R)-Ergosta-4,7,22-trien-3-one (2). A. With stirring, 5.75 ml of pyridine was added to a suspension of 0.89 g of chromium trioxide in 30 ml of methylene chloride at 15°C. After 50 min, a solution of 0.44 g of the trienone (**2**) in 10 ml of methylene chloride was added to this mixture at 0°C. The reaction mixture was stirred at 0°C for 45 min and was filtered through a layer of alumina, which was then washed with dichloroethane. The combined filtrate was evaporated under reduced pressure, and the residue was chromatographed on a column of silica gel with elution by hexane—ethyl acetate (from 20:1 to 8:1). Three fractions were obtained.

Fraction 1 — 0.16 g (yield 36%) of the initial trienone (**2**).

Fraction 2 — 0.04 g (9%) of (22E,24R)-ergosta-4,6,8(14),22-tetraen-3-one (**4**). mp 114—115°C (methanol); lit.: mp 113-114°C [12, 13], 113.5-114.5°C [16], 113.0-113.5°C [17], 112-113°C [8], 114-115°C [24]. IR spectrum (cm^{-1}): 1665 (C=O), 1655, 1635, 1590 (C=C). UV spectrum (nm): λ_{max} 239 (ε 7000), 281 (ε 9700), 350 (ε 34800). ^{1}H NMR spectrum (δ, ppm): 0.83 (26-Me, d, J=6.5 Hz), 0.85 (27-Me, d, J=6.5 Hz), 0.92 (18-Me, s), 0.95 (19-Me, s), 0.98 (28-Me, d, J=6.5 Hz), 1.06 (21-Me, d, J=6.5 Hz), 5.18-5.30 (H-22, H-23, m), 5.74 (H-4, s), 6.03 (H-7, d, J=9.5 Hz), 6.61 (H-6, d, J=9.5 Hz).

Fraction 3 — 0.09 g (20%) of (22E,24R)-ergosta-4,7,22-triene-3,6-dione (**3**). mp 144—155°C (decomp.) (hexane—ethyl acetate); lit.: mp 140-152°C [2], 156-159°C [3], 145-146°C [7], 144-146°C [8]. IR spectrum (cm^{-1}): 1685 (C=O), 1660, 1625, 1605 (C=C). UV spectrum: λ_{max} 227 nm (ε 8500). ^{1}H NMR spectrum (δ, ppm): 0.70 (18-Me, s), 0.84 (26-Me, d, J=6.5 Hz), 0.86 (27-Me, J=6.5 Hz), 0.93 (28-Me, d, J=6.5 Hz), 1.06 (21-Me, d, J=6.5 Hz), 1.32 (19-Me, s), 5.08-5.28 (H-22, H-23, m), 5.99 (H-7, t, J=2 Hz), 6.48 (H-4, s).

B. With stirring, 5.91 ml of pyridine and, after 15 min, a solution of 0.45 g of the trienone (**2**) in 10 ml of methylene chloride were added to a suspension of 0.91 g of chromium trioxide in 30 ml of methylene chloride at 0°C. The reaction mixture was stirred at 0°C for 2 h and was then filtered through a layer of alumina, which was washed with dichloroethane. The combined filtrate was evaporated under reduced pressure, and the residue was chromatographed on a column of silica gel with elution by hexane—ethyl acetate (from 20:1 to 8:1). Three fractions were obtained: fraction 1 — 1.32 g (yield 71%) of the initial compound (**2**); fraction 2— 0.03 g (7%) of the tetraenone (**3**); and fraction 3 — 0.08 g (17%) of the trienedione (**4**).

Oxidation of the Trienedione (3) with Selenium Dioxide. With stirring, a solution of 0.040 g of steroid (**3**) in 5 ml of dioxane was added to a solution of 0.050 g of selenium dioxide in 20 ml of dioxane at 70°C. After 20 min, the reaction mixture was evaporated under reduced pressure. The residue was separated by preparative thin-layer chromatography on a silica gel plate with elution by benzene—ethyl acetate mixtures of increasing polarity (from 30:1 to 18:1). Two fractions were obtained.

Fraction 1 — 0.010 g of (22E,24R)-14α-hydroxyergosta-4,7,22-triene-3,6-dione (**4**). Yield 24%. mp 158—165°C (hexane). UV spectrum: λ_{max} 276 nm (ε 15800). ^{1}H NMR spectrum: (δ, ppm): 0.77 (18-Me, s), 0.84 (26-Me, d, J=6.5 Hz), 0.86 (27-Me, d, J=6.5 Hz), 0.94 (28-Me, d, J=7 Hz), 1.06 (21-Me, d, J=7 Hz), 1.32 (19-Me, s), 2.95 (H-9a, ddd, J_1=10.8 Hz, J_2=7.2 Hz, J_3=1.8 Hz), 5.12-5.38 (H-22, H-23, m), 6.16 (H-7, d, J=1.8 Hz), 6.46 (H-4, s).

Fraction 2 — 0.010 g of (22E,24R)-9α,14α-dihydroxyergosta-4,7,22-triene-3,6-dione (**6**). Yield 23%. mp 176—185°C (hexane). UV spectrum: λ_{max} 267 nm (ε 11100). ^{1}H NMR spectrum: (δ, ppm): 0.78 (18-Me, s), 0.85 (26-Me, d, J=6.5 Hz), 0.87 (27-Me, d, J=6.5 Hz), 0.94 (28-Me, d, J=6.5 Hz), 1.07 (21-Me, d, J=6.5 Hz), 1.39 (19-Me, s), 5.12-5.34 (H-22, H-23, m), 6.20 (H-7, s), 6.61 (H-4, s).

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