Chemistry of Natural Compounds, Vol. 35, No. 4, 1999

SYNTHESIS OF (24R)-3β,5,14α-TRIHYDROXY-5α-STIGMAST-7-EN-6-ONE 3-BENZOATE

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UDC 547.92

An ecdysteroid analog has been synthesized from β-sitosterol.

Among various oxidized derivatives of sterols found in plants a special position is occupied by substances close in structure to the insect hormones ecdysteroids [1, 2]. The closeness of the structures of such compounds and ecdysteroids is apparently not fortuitous and makes it possible to assume that they possess ecdysteroidal or antiecdysteroidal activity in relation to insects. It is just to this type of compound that the physalones A and B, isolated from the fruit of *Physalis franchetii* [3], belong. Both these substances are derivatives of β-sitosterol (**1**) containing the Δ^7-6-keto grouping that is characteristic for ecdysteroids. The aim of the present investigation consisted in the synthesis of the Δ^7-6-ketone (**6**), structurally close to physanols A and B and having a number of the same structural fragments as in the ecdysteroids.

First, by a procedure developed previously [4], the initial β-sitosterol (**1**) was converted into the 3β,5α-dihydroxy-6-ketosteroid (**2**) as the result of *trans*-hydroxylation with hydrogen peroxide in formic acid, followed by selective oxidation of the resulting 3β,5α,6β-triol. Since the structure of physanolides A and B includes a 3β-benzoyloxy group, in the following stage of the synthesis the dihydroxyketone (**2**) was subjected to a reaction with benzoyl chloride in pyridine to form the benzoate (**3**) with a yield of 81%. The structure of compound (**3**) followed unambiguously from its spectra. In particular, in the ^{1}H NMR spectrum of the substance under discussion we observed a downfield shift to 5.30 ppm of the signal of the H-3α methine proton in comparison with its position (δ 4.00 ppm) in the spectrum of the initial dihydroxyketone (**3**) [4]. This shift was undoubtedly caused by the esterification of the 3β-hydroxy group. Another proof of the structure of compound (**3**) as a benzoate was the presence of the signals of aromatic protons in the spectrum at 7.44, 7.54, and 8.03 ppm. The IR spectrum, showing characteristic bands of a benzoyl group at 1715, 1605, and 1590 cm^{-1}, permitted a similar conclusion.

The subsequent bromination of ketone (**3**) in acetic acid gave a 92% yield of the 7α-bromoketone (**4**). In the ^{1}H NMR spectrum of this substance there was at 4.20 ppm a doublet of the H-7 proton geminal to the bromine atom, which was important for proving its structure. The coupling constant of this signal (J = 5 Hz), due to axial-equatorial interaction with the axial H-8β proton, showed the α-configuration of the bromine atom. From the ^{1}H NMR and IR spectra it was also possible to deduce the other elements of the structure of the steroid (**4**) molecule.

The dehydrobromination of the 7α-bromo-6-ketone (**4**) with lithium carbonate and bromide in dimethyl formamide gave a 22% yield of the Δ^7-6-ketone (**5**). The IR spectrum of compound (**5**) showed, in addition to the vibrational bands of the 5α-hydroxy group and of the benzoate group, bands of the stretching vibrations of the 6-keto group at 1690 cm^{-1} and of a 7(8)-double bond conjugated with it at 1630 cm^{-1}. In the UV spectrum of steroid (**5**) there was an intense absorption band of a benzoate at 230 nm. Absorption due to a Δ^7-6-keto grouping was also observed in the spectrum simultaneously, in the form of a shoulder on this band. The use of the fourth derivative of the spectrum enabled the wavelength of this peak to be determined as 259 nm. The ^{1}H NMR spectrum of the Δ^7-6-ketone (**5**) also enabled its structure to be determined unambiguously. In particular, of extreme importance for this was the presence in the spectrum, together with others, of the signal of the H-7 vinyl proton at 5.68 ppm. This signal had the form of a doublet (J = 2 Hz) as result of allyl interaction with the H-9 and H-14 methine protons.

Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus', 220141, Belarus', Minsk, ul. Akad. Kuprevich, 5/2. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 488—491, July-August, 1999. Original article submitted February 3, 1999.

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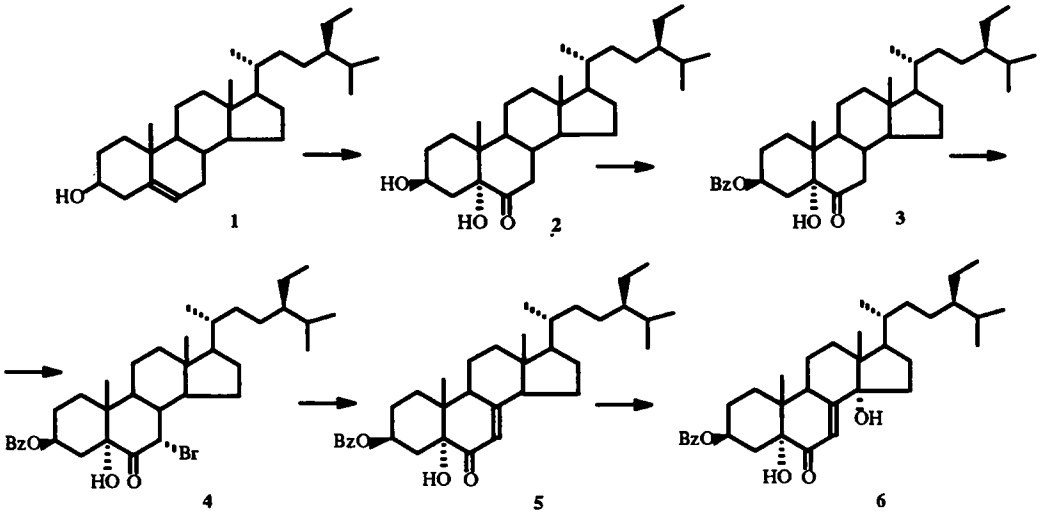

To introduce a 14α-hydroxy group into compound (**5**) we made use of allyl oxidation with selenium dioxide in dioxane [1]. As a result of this reaction we obtained the desired steroid (**6**) with a yield of 82%. The structure of compound (**6**) was confirmed by its IR and ^{1}H NMR spectra. Thus, the IR spectrum showed the presence of the main fragments of its structure in the molecule of steroid (**6**): hydroxy groups, a benzoyl group, and a Δ^7-6-keto grouping. It was possible to draw a similar conclusion from the ^{1}H NMR spectrum. Thus, for example, the signals of the aromatic protons of a benzoate group appeared in the spectrum at 7.38. 7.56, and 8.03 ppm. In addition, at 5.92 ppm there was the characteristic doublet of the H-7 vinyl proton. At the same time, the observed downfield shift in comparison with the position of the signal of this proton in the spectrum of the Δ^7-6-ketone (**5**) could serve as an indirect confirmation of the presence of the 14α-hydroxy group in compound (**6**).

It was possible to draw the same conclusion from the nature of the splitting of this signal. The fact that the signal of the H-7 proton in the spectrum had the form of a doublet showed the absence of a H-14 proton in the molecule of steroid (**6**). Furthermore, the presence of a 14α-hydroxy group in the compound concerned led to a considerable downfield shift to 2.86 ppm of the signal of the H-9α methine proton in the 1,3-diaxial position with respect to it. The use of the double-resonance method enabled this to be confirmed. Thus, irradiation of the H-7 signal caused a considerable simplification of the signal at 2.86 ppm. Analogous irradiation of the signal at 2.86 ppm led to the conversion of the H-7 signal at 5.92 ppm into a singlet. Thus, the ^{1}H NMR spectrum completely confirmed the structure of compound (**6**) as a 5α,14α-dihydroxy-Δ^7-6-ketone.

EXPERIMENTAL

Melting points were determined on a Kofler block. IR spectra were recorded on a UR-20 instrument in the 700—3600 cm^{-1} interval in KBr tablets. UV spectra were taken on a Specord M-400 spectrophotometer. ^{1}H NMR spectra 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.

(24R)-3β,5-Dihydroxy-5α-stigmastan-6-one 3-Benzoate (3). A solution of 3.0 g of (24R)-3β,5-dihydroxy-5α-stigmastan-6-one (**2**) (obtained from β-sitosterol (**1**) by the procedure of [4]) in 40 ml of pyridine was treated with 6 ml of benzoyl chloride. The reaction mixture was kept at room temperature for 21 h. The solvent was evaporated off in vacuum and the residue was carefully washed with methanol and dried. This gave 3.0 g of the benzoate (**3**). Yield 81%. mp 248—253°C.

IR spectrum (ν, cm^{-1}): 3390 (OH), 1715, 1605, 1590 (BzO and C=O). ^{1}H NMR spectrum (δ, ppm): 0.67 (3H, s, 18-Me), 0.81 (3H, d, J = 6.5 Hz, 26-Me), 0.83 (3H, d, J = 6 Hz, 27-Me), 0.84 (3H, t, J = 6 Hz, 29-Me), 0.91 (3H, d, J = 6 Hz, 21-Me), 2.73 (1H, t, J = 12.5 Hz, H-4β), 5.30 (1H, m, W/2 = 25 Hz, H-3α), 7.44 (2H, t, J = 7 Hz, arom. *meta*-H), 7.54 (1H, t, J = 7 Hz, arom. *para*-H), 8.03 (2H, d, J = 7 Hz, arom. *ortho*-H).

(24R)-7-Bromo-3β,5-dihydroxy-5α-stigmastan-6-one 3-Benzoate (4). A solution of 0.30 g of the ketone (**3**) in 20 ml of acetic acid was treated with 6 ml of a 1.9 M solution of bromine in acetic acid. The reaction mixture was heated to

60—70°C and was kept at this temperature for 1 h. After cooling to room temperature, the mixture was diluted with 25 ml of 10% aqueous sodium sulfate solution. The reaction product was extracted with dichloroethane, and the dichloroethane solution was washed with water and was then evaporated off in vacuum. The residue was chromatographed on a column of silica gel with elution by benzene. This gave 0.317 g of the α-bromoketone (**4**). Yield 92%. mp 242—247°C (hexane—tetrahydrofuran).

IR spectrum (ν, cm^{-1}): 3420 (OH), 1720, 1610, 1595 (BzO and C=O). ^1H NMR spectrum (δ, ppm): 0.66 (3H, s, 18-Me), 2.80 (1H, t, J = 12.5 Hz, H-4β), 4.20 (1H, t, J = 5 Hz, H-7β), 5.34 (1H, m, W/2 = 25 Hz, H-3α), 7.44 (2H, t, J = 7 Hz, arom. *meta*-H), 7.54 (1H, t, J = 7 Hz, arom. *para*-H), 8.02 (2H, d, J = 7 Hz, arom. *ortho*-H).

(24R)-3β,5-Dihydroxy-5α-stigmast-7-en-6-one 3-Benzoate (5). To a solution of 0.295 g of the α-bromoketone (**4**) in 15 ml of dimethylformamide were added 0.3 g of lithium carbonate and 0.1 g of lithium bromide. The reaction mixture was boiled under reflux for 1 h. After cooling to room temperature, the deposit was filtered off, and the filtrate was diluted with water and extracted with hexane. The hexane extract was washed with water and was then evaporated off in vacuum. The residue was separated by preparative thin-layer chromatography on a silica gel plate, with elution by dichloroethane. This gave 57 mg of the enone (**5**). Yield 22%. mp 227—235°C (hexane—tetrahydrofuran).

IR spectrum (ν, cm^{-1}): 3380 (OH), 1720, 1610, 1595 (BzO), 1690 (C=O), 1630 (C=C). UV spectrum (λ_{max}^{EtOH}, nm): 230 (ε 10000), shoulder 259. ^1H NMR spectrum (δ, ppm): 0.60 (3H, s, 18-Me), 1.02 (3H, s, 19-Me), 5.34 (1H, m, W/2 = 25 Hz, H-3α), 5.68 (1H, t, J = 2 Hz, H-7β), 7.45 (2H, t, J = 7 Hz, arom. *meta*-H), 7.56 (1H, t, J = 7 Hz, arom. *para*-H), 8.03 (2H, d, J = 7 Hz, arom. *ortho*-H).

(24R)-3β,5,14β-Trihydroxy-5α-stigmast-7-en-6-one 3-Benzoate (6). A solution of 50 mg of the enone (**5**) in 3 ml of dioxane was treated with 50 mg of selenium dioxide. The reaction mixture was boiled for 30 min, after which the solvent was evaporated off in vacuum. The residue was dissolved in tetrahydrofuran, and the solution was filtered through a layer of alumina. The filtrate was evaporated off in vacuum, and the residue was purified by preparative thin-layer chromatography on a silica gel plate with elution by dichloroethane. This gave 42 mg of steroid (**6**). Yield 82%. mp 259—262°C (decomp.) (acetone—tetrahydrofuran).

IR spectrum (ν, cm^{-1}): 3429 (OH), 1720, 1610, 1595 (BzO, 1690 (C=O), 1630 (C=C). ^1H NMR spectrum (δ, ppm): 0.70 (3H, s, 18-Me), 1.04 (3H, s, 19-Me), 2.68 (1H, W/2 = 23, H-9α), 5.92 (1H, J = 2, H-7), 7.38 (2H, t, J = 7 Hz, arom. *meta*-H), 7.56 (1H, t, J = 7 Hz, arom. *para*-H), 8.03 (2H, d, J = 7 Hz, arom. *ortho*-H).

REFERENCES

1. A. A. Akhrem and N. V. Kovganko, *Ecdysteroids: Chemistry and Biological Activity* [in Russian], Nauka i Tekhnika, Minsk (1989).
2. R. Lafont and I. Wilson, *The Ecdysone Handbook, 2nd Edition*, The Chromatographic Society, Nottingham (1996).
3. N. K. Sharma, D. K. Kulshreshtha, I. S. Tandon, D. S. Bhakuni, and M. M. Dhar, *Phytochemistry*, **13**, 2239 (1974).
4. N. V. Kovganko and Zh. N. Kashkan, *Khim. Prir. Soedin.*, 771 (1990).