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SYNTHESIS OF PHYSANOL A AND B ANALOGS FROM β-CYTOSTEROL

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A series of ecdysteroid analogs is synthesized from β-cytosterol.

Physanol A (**1**) and B (**2**) phytosteroids were isolated from the fruit of *Physalis franchetti* [1]. Compounds **1** and **2** have characteristic functional groups that enable them to be assigned as ecdysteroids [2, 3]. It should be mentioned that carpesterol (**3a**) [4-6] and its 14α-hydroxy derivative (**3b**) [7] that were found in *Solanum xanthocarpum* have structures similar to them. Considering that physanols A and B contain the basic C backbone of the plant sterol β-cytosterol (**4**), it seems promising that structural analogs of them can be produced from it. We previously synthesized one such compound, (24R)-3β,5,14α-trihydroxy-5α-stigmast-7-en-6-on-3-benzoate [8]. The present article presents results of further investigations in this area.

Compound (**4**) reacts in the first synthesis step with benzoyl chloride in pyridine to give the benzoate **5** in 95% yield according to the literature method [9]. The structure of **5** is unambiguously proved using spectral data. In particular, ^{1}H NMR spectra of **5** have characteristic signals for aromatic benzoate protons (δ 7.42, 7.55, and 8.04 ppm), the methine proton H-3α (4.88 ppm), and the vinylic proton H-6 (5.42 ppm).

Bromhydrin **6** is obtained in 56% yield by adding hypobromic acid to the 5-double bond of β-cytosterol benzoate. The ^{1}H NMR of **6** lacks a signal for the vinylic proton H-6. Instead, a multiplet is observed at 4.23 ppm for the methine proton H-6 geminal to the hydroxy group. The half-width of this signal (W/2 = 7 Hz) suggests an axial (i.e., β) orientation for the 6-hydroxy group. The weak-field shift to 1.38 ppm of the signal for the 19-methyl group, which occupies a 1,3-diaxial position relative to the 6β-hydroxy group, leads to the same conclusion. The weak-field shift to 5.74 ppm of the signal for H-3 compared with its position in the spectrum of the starting benzoate is interesting. Such a shift is undoubtedly due to a 1,3-diaxial arrangement of H-3α and the 5α Br atom.

Oxidation of the hydroxy group in **6** by chromic acid in acetone according to Jones gives the 5α-bromo-6-ketone **7** in quantitative yield. The ^{1}H NMR spectrum of **7** characteristically lacks a signal for H-6, which confirms that the 6-hydroxy group has been oxidized. Moreover, these spectra are consistent with the presence of the benzoate and the Br atom in **7**.

Rearrangement of **7** into the 7α-bromo-6-ketone **8** is necessary in order to introduce the 7-double bond. This reaction is usually effected by reacting with a catalytic amount of hydrobromic acid [2]. An attempt to isomerize **7** under these conditions was unsuccessful. Only starting material containing an insignificant amount of **8** was isolated according to ^{1}H NMR spectra. Heating a solution of **7** in acetic acid with a large excess of hydrobromic acid produced a much more complicated reaction. Under these conditions at least four compounds are formed. We were able to isolate **8** in a yield of only 16%. The structure of **8** was unambiguously determined from spectral data. The ^{1}H NMR spectrum of **8** contains a characteristic doublet for the methine proton H-7 geminal to the Br atom (4.22 ppm, J = 2.5 Hz). Furthermore, a signal for the methine proton H-5α (3.38 ppm) is observed. The strong-field shift to 5.00 ppm for the signal of the methine proton H-3α compared with its position in **7** is interesting. This confirms the lack in **8** of a C-5 Br atom.

The Δ^7-6-ketone **9** is isolated in 62% yield by dehydrobromination of **8** using lithium carbonate and bromide in boiling DMF. Its structure was determined from spectral data. The IR spectrum of **9** exhibits a band for stretching vibrations of the keto group at 1680 cm^{-1}. The ^{1}H NMR spectra of **9** contain a characteristic triplet for the vinylic proton H-7 (5.76 ppm, J = 2 Hz). Comparison of the ^{1}H NMR spectra of the Δ^7-6-ketone **9** and the isomeric Δ^4-6-ketone **10**, prepared in 87% yield by

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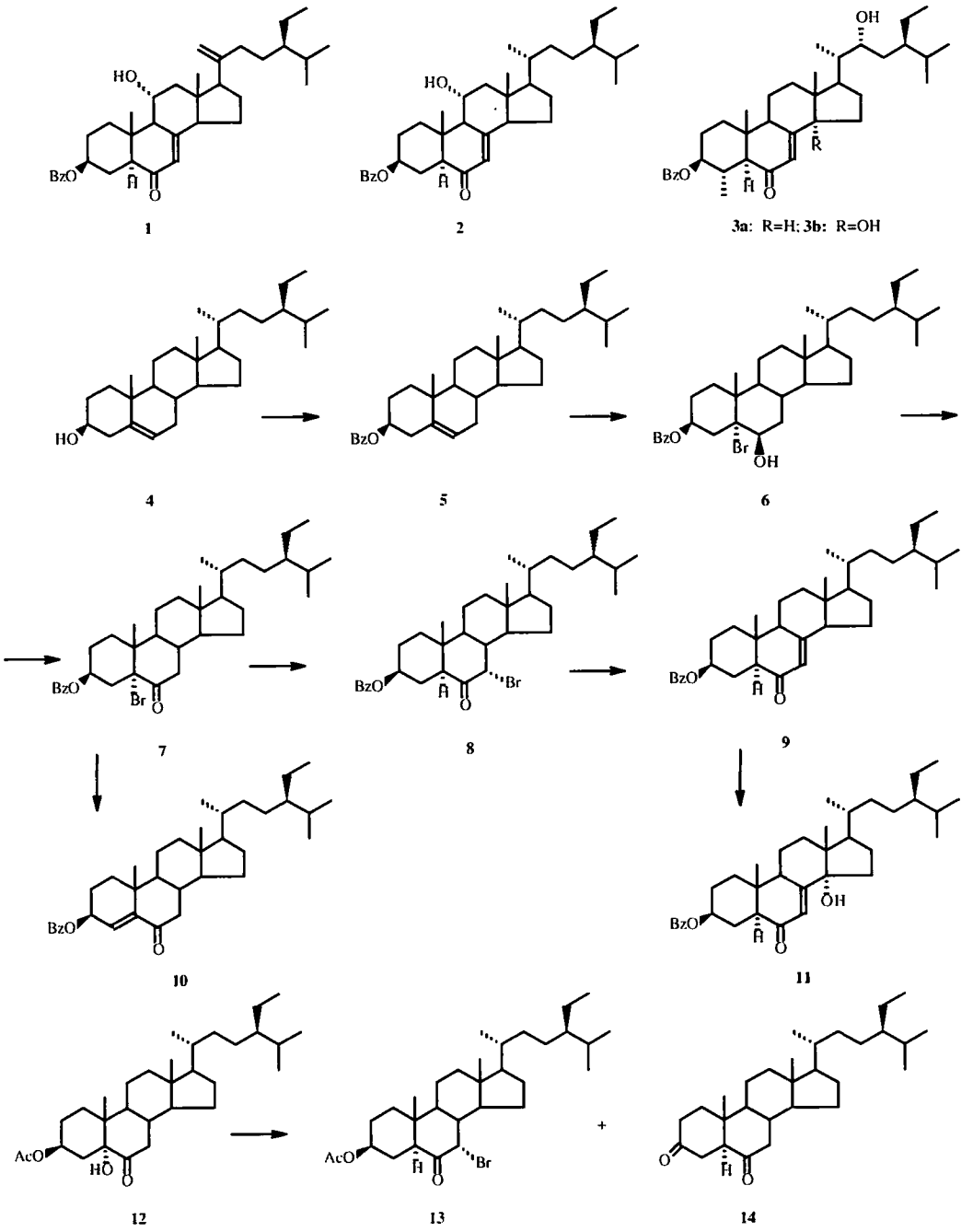

dehydrobromination of 5α-bromo-6-ketone **7**, is interesting. The ^{1}H NMR spectrum of the enone **10** contains a singlet for the vinylic proton H-4 at 6.02 ppm. Furthermore, the significant weak-field shift to 5.61 ppm of the signal for the methine proton H-3α compared with its position in the spectrum of **9** (5.00 ppm) is noteworthy. Such a shift in the spectrum of **10** is undoubtedly due to its allylic position.

In the final synthesis step, the 14α-hydroxy derivative **11** is produced in 37% yield by allylic hydroxylation of **9** by selenium dioxide in dioxane. Introducing the additional 14α-hydroxy group into **9** to form **11** produces characteristic changes in the ^{1}H NMR spectrum. First, the presence of the 14α-hydroxy group causes a weak-field shift to 2.70 ppm of the signal for the methine proton H-9α, which is situated in a 1,3-diaxial position relative to it. Second, the signal for H-7 appears as a doublet (J = 2.5 Hz) owing to the lack of an H-14α proton. The splitting is due to allylic interaction with H-9α. Furthermore,

the slight shift to weak field of the signal for H-7 to 5.94 ppm compared with its position in **9** should be noted.

The results from the reaction of 5α-bromo-6-ketone **7** with hydrobromic acid prompted us to study the analogous reaction of 5α-hydroxy-6-ketone **12**. It is known [10] that such a reaction of cholestanes would enable the corresponding 7α-bromo-6-ketone to be obtained in high yields. We found that heating **12** with hydrobromic acid in acetic acid also gives the 7α-bromo-6-ketone **13** in 38% yield. A second compound isolated from this reaction in 40% yield is the 3,6-diketone **14**. The structures of **13** and **14** were confirmed using spectral data. Furthermore, the structure of **14** was proved by comparing its IR and ^{1}H NMR spectra with those in the literature [11].

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} in KBr pellets. ^{1}H NMR spectra of $CDCl_3$ solutions were obtained on a Bruker AC-200 NMR-spectrometer at 200 MHz working frequency. Chemical shifts are given relative to an internal standard of TMS.

(24R)-5-Bromo-5α-stigmast-3β,6β-diol-3-benzoate (6). A solution of β-cytosterol (**5**, 6.0 g, prepared from β-cytosterol **4** by the literature method [4]) in dioxane (300 ml) was treated with constant stirring at room temperature successively with water (20 ml), perchloric acid (5 ml, 70%), and N-bromoacetamide (4.0 g). The reaction mixture was stirred at room temperature for 1 h. Then water (300 ml) was added. The mixture was treated with sodium sulfite (3.0 g) and extracted with benzene. The benzene extract was washed with water and evaporated under vacuum. Yield of **6**, 4.0 g, 56%, mp 252-254°C (hexane).

IR spectrum (ν, cm^{-1}): 3500 (OH), 1725, 1700, 1610, 1595 (benzoate). ^{1}H NMR spectrum (δ, ppm): 0.68 (18-Me, s), 0.81 (26-Me, d, J = 6.5 Hz), 1.38 (19-Me, s), 4.23 (H-6α, m, W/2 = 7 Hz), 5.74 (H-3α, m, W/2 = 21 Hz), 7.43 (2H, t, J = 7 Hz, arom. *m*-H), 7.55 (1H, t, J = 7 Hz, arom. *p*-H), 8.04 (2H, d, J = 7 Hz, arom. *o*-H).

(24R)-5-Bromo-3β-hydroxy-5α-stigmastan-6-one benzoate (7). A solution of **6** (3.6 g) in acetone (300 ml) was treated with stirring on a magnetic stirrer at room temperature with chromic acid (4 ml, 8 N). The reaction mixture was stirred at room temperature for 20 min. Then isopropanol (10 ml) was added to remove the excess of oxidant. The mixture was diluted with water and extracted with benzene. The extract was washed with water and evaporated in vacuum. Yield: amorphous **7**, 3.75 g (quantitative).

IR spectrum (ν, cm^{-1}): 1720, 1610, 1595 (C=O and benzoate). ^{1}H NMR spectrum (δ, ppm): 0.74 (18-Me, s), 0.82 (26-Me, J = 6.5 Hz), 0.84 (27-Me, d, J = 6 Hz), 0.92 (21-Me, d, J = 6 Hz), 1.03 (29-Me, t, J = 6 Hz), 1.06 (19-Me, s), 3.20 (H-4β, dd, J_1 = 14 Hz, J_2 = 12 Hz), 5.58 (H-3α, m, W/2 = 14 Hz), 7.44 (2H, t, J = 7 Hz, arom. *m*-H), 7.56 (1H, t, J = 7 Hz, arom. *p*-H), 8.04 (2H, d, J = 7 Hz, arom. *o*-H).

Reaction of α-Bromoketone 7 with Hydrobromic Acid. A solution of **7** (2.0 g) in acetic acid (25 ml) was treated with hydrobromic acid (2 ml, 40%). The reaction mixture was heated at 80°C for 1 h. The mixture was cooled to room temperature, diluted with water, and extracted with benzene. The extract was successively washed with saturated sodium bicarbonate and water. The solvent was removed under vacuum. The solid was chromatographed on a silica-gel column with elution first by mixtures of hexane and benzene with increasing polarity (10:1, 5:1, 1:1) and then by benzene. Yield of **8**, 0.312 g, 16%, mp 140-142°C (hexane).

IR spectrum (ν, cm^{-1}): 1720, 1610, 1595 (C=O and benzoate). ^{1}H NMR spectrum (δ, ppm): 0.70 (18-Me, s), 0.81 (26/27-Me, d, J = 6 Hz), 0.83 (19-Me, s), 0.85 (29-Me, t, J = 5 Hz), 0.93 (21-Me, d, J = 7 Hz), 3.38 (H-5α, dd, J_1 = 13 Hz, J_2 = 3 Hz), 4.22 (H-7α, d, J = 2.5 Hz), 5.00 (H-3α, m, W/2 = 24 Hz), 7.44 (2H, t, J = 7 Hz, arom. *m*-H), 7.56 (1H, t, J = 7 Hz, arom. *p*-H), 8.04 (2H, d, J = 7 Hz, arom. *o*-H).

(2R)-3β-Hydroxy-5α-stigmast-7-en-6-one benzoate (9). A solution of **8** (0.166 g) in DMF (10 ml) was treated with lithium carbonate (0.2 g) and lithium bromide (0.070 g). The mixture was refluxed for 1 h 20 min and then cooled to room temperature. The solid was filtered off through a layer of silica gel. The filtrate was diluted with water and extractede with hexane. The hexane extract was washed with water and evaporated under vacuum. The solid was separated by preparative TLC on a silica-gel plate. The eluent was a mixture of hexane and THF (10:1). Yield of **9**: 0.089 g, 62%, mp 152-162°C (hexane).

IR spectrum (ν, cm^{-1}): 1720, 1680 (benzoate and C=O). ^{1}H NMR spectrum (δ, ppm): 0.63 (18-Me, s), 0.83 (26-Me, d, J = 5.5 Hz), 0.84 (27-Me, d, J = 5.5 Hz), 0.85 (29-Me, t, J = 5.5 Hz), 0.88 (21-Me, d, J = 5 Hz), 0.94 (19-Me, s), 5.00 (H-3α, m, W/2 = 24 Hz), 5.76 (H-7, t, J = 2 Hz), 7.44 (2H, t, J = 7 Hz, arom. *m*-H), 7.57 (1H, t, J = 7 Hz, arom. *p*-H), 8.05 (2H, d,

J = 7 Hz, arom. *o*-H).

(24R)-3β-Hydroxystigmast-4-en-6-one benzoate (10). A solution of **7** (3.3 g) in DMF (100 ml) was treated with lithium carbonate (3.0 g) and lithium bromide (1.0 g). The mixture was refluxed for 1 h and then cooled to room temperature. The solid was filtered off. The filtrate was diluted with water (100 ml) and extracted with hexane. The hexane extract was washed with water and evaporated under vacuum. Yield of **10**: 2.5 g, 87%, mp 225-228°C (hexane).

IR spectrum (ν, cm^{-1}): 1720, 1700, 1610, 1595 (C=O and benzoate), 1640 (C=C). ^{1}H NMR spectrum (δ, ppm): 0.70 (18-Me, s), 0.93 (21-Me, d, J = 6 Hz), 1.07 (19-Me, s), 5.61 (H-3α, m, W/2 = 18 Hz), 6.02 (H-4, s), 7.44 (2H, t, J = 7 Hz, arom. *m*-H), 7.58 (1H, t, J = 7 Hz, arom. *p*-H), 8.04 (2H, d, J = 7 Hz, arom. *o*-H).

(24R)-3β,14α-Dihydroxy-5α-stigmast-7-en-6-one benzoate (11). A solution of **9** (0.061 g) in dioxane (5 ml) was treated with selenium dioxide (0.062 g). The mixture was refluxed at 60-80°C for 30 min. Then the solvent was removed under vacuum. The solid was purified by preparative TLC on a silica-gel plate using first a mixture of hexane and dichloroethane (1:1) and then dichloroethane as eluent. Yield of **11**: 0.023 g, 37%, mp 175-182°C (EtOH).

IR spectrum (ν, cm^{-1}): 3450 (OH), 1715, 1605, 1590 (benzoate), 1660 (C=O), 1630 (C=C). ^{1}H NMR spectrum (δ, ppm): 0.70 (18-Me, s), 0.94 (19-Me, s), 2.70 (H-9α, m, W/2 = 25 Hz), 5.00 (H-3α, m, W/2 = 28 Hz), 5.94 (H-7, d, J = 2.5 Hz), 7.46 (2H, t, J = 7 Hz, arom. *m*-H), 7.58 (1H, t, J = 7 Hz, arom. *p*-H), 8.04 (2H, d, J = 7 Hz, arom. *o*-H).

Reaction of (24R)-3β,5-Dihydroxy-5α-stigmastan-6-on-3-acetate (12) and Hydrobromic Acid. A solution of **12** (0.428 g, prepared from β-cytosterol **4** by the literature method [11, 12]) in acetic acid (10 ml) was treated with hydrobromic acid (0.5 ml, 40%). The mixture was heated to 60-80°C and held at that temperature for 1 h. The mixture was cooled to room temperature, diluted with water, and extracted with benzene. The benzene extract was washed first with saturated sodium bicarbonate and then with water and evaporated under vacuum. The solid was chromatographed on a silica-gel column with mixtures of hexane and benzene of increasing polarity (5:1, 1:1) as eluent. Two fractions were obtained.

Fraction 1. Yield of **13**: 0.185 g, 38%.

^{1}H NMR spectrum (δ, ppm): 0.68 (18-Me, s), 0.77 (19-Me, s), 0.82 (26-Me, d, J = 6 Hz), 0.83 (27-Me, d, J = 6 Hz), 0.85 (29-Me, t, J = 6 Hz), 0.92 (21-Me, d, J = 6 Hz), 2.50 (acetate, s), 4.20 (H-7β, d, J = 3 Hz), 4.74 (H-3α, m, W/2 = 24 Hz).

Fraction 2. Yield of **14**: 0.149 g, 40%, mp 205-206.5°C. The sample has IR and ^{1}H NMR spectra identical to those in the literature [11].

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