Chemistry of Natural Compounds, Vol. 35, No. 5, 1999

SYNTHESIS OF (24R)-3β-CHLORO-7α-BROMO-5-HYDROXY-5α-STIGMASTAN-6-ONE AND STUDY OF ITS DEHYDROHALOGENATION PRODUCTS

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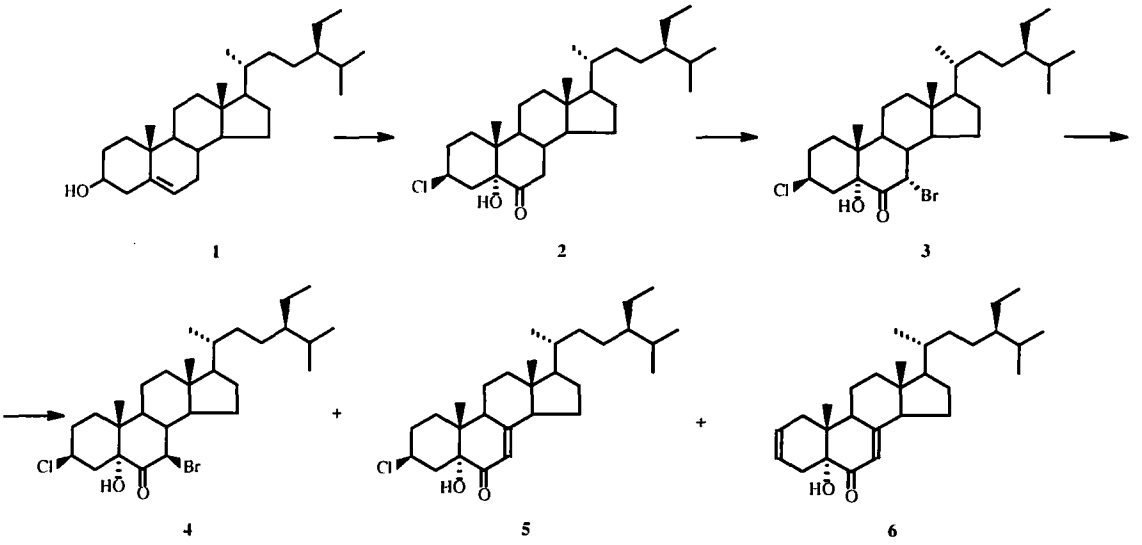
UDC. 547.92

Starting from β-cytosterol (1) and going through the intermediate 3β-chloro-5α-hydroxy-6-ketone (2), the 3β-chloro-7α-bromo-5α-hydroxy-6-ketone (3) is synthesized. Dehydrohalogenation of 3 in DMF in the presence of lithium carbonate and bromide gives the 6-ketosteroids 4-6, depending on the reaction conditions.

We have previously developed a synthetic method for brassinosteroids containing an additional 5α-hydroxy group in the structure by chemical transformation of β-cytosterol (**1**) and stigmasterol [1]. Recently brassinosteroids of this type have been found to be highly active toward phytostimulation [2]. Our proposed synthetic scheme for 5α-hydroxybrassinosteroids includes the use of 3β-chloro-5α-hydroxy-6-ketosteroids (**2**) as intermediates. It seems interesting to use compounds of this structure in the synthesis of ecdysteroids [3, 4]. A distinguishing feature of the ecdysteroid structure is the presence of a Δ^7-bond in addition to the 6-keto group. Therefore, we attempted to use the steroid **2** to prepare the 7α-bromo-6-ketone (**3**) and to effect its dehydrohalogenation to the 5α-hydroxy-2,7-dien-6-keto derivative (**6**) in order to introduce this structural feature. It should be noted that an analogous derivative of several cholestan has previously been used to synthesize ecdysteroid analogs [5].



1 **2** **3**

4 **5** **6**

Steric hindrance created by the 5α-hydroxy group prevents the 6-ketosteroid (**2**) from converting to the 7α-bromide (**3**) under conditions usually used for such reactions [3, 4]. Only prolonged bromination of **2** in acetic acid containing hydrobromic acid with heating yielded the required **3** in quantitative yield. We proved the structure of **3** using elemental analysis and IR and ^{1}H NMR spectra. In particular, the position and shape of the signal for the methine proton H-7 is very characteristic in the ^{1}H NMR spectrum of **3**. The appearance of this signal at weak field (δ 4.218 ppm) indicates that H-7 is geminal to the Br atom.

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0009-3130/99/3505-0539$22.00

The splitting constant of the H-7 signal (doublet with J = 4.0 Hz) is due to an equatorial—axial interaction with H-8 and suggests that the Br atom has the α-configuration. The presence in **3** of the tertiary 5α-hydroxy group can also be confirmed using the ^{1}H NMR spectrum. The signals of the H-3α and H-4α protons, which are spatially close to it, are shifted to weak field.

The position of the signal for H-3α (4.238 ppm) is practically identical with that observed in the spectrum of the starting material (4.210 ppm). In turn the signal of the methylene proton H-4α appears as a doublet of doublets at 2.300 ppm. Double resonance was used to confirm this assignment. Irradiating this signal greatly simplifies the multiplet for H-3α. On the other hand, irradiating the signal for H-3α changes the doublet of doublets into a doublet with J = 14 Hz owing to geminal interaction with H-4β.

Dehydrohalogenation by boiling solutions of the appropriate halogen derivatives in DMF in the presence of lithium carbonate and bromide is widely used to introduce Δ^2- and Δ^7-bonds into 6-ketosteroids [3, 4]. We were unable to produce the $\Delta^{6,7}$-6-ketone from **3** under these conditions. At least 10 products are formed. The required steroid **6** could be isolated in very low yield. Therefore, we undertook a special study of this reaction under milder conditions. It was found that reducing the reaction temperature to 100°C gives mainly the product of isomerization and not elimination. As a result, the 7β-bromoketone **4** was isolated under these conditions in 65% yield in addition to unreacted **3**, with which it is isomeric.

The structure of **4** was suggested by the elemental analysis and confirmed by spectral data. The ^{1}H NMR spectrum of **4** lacks signals of vinylic protons. However, it does contain a signal for the methine proton H-3α at 4.158 ppm. The position and half-width of this signal agree well with those observed for H-3α in the spectrum of the starting steroid (**3**). This indicates that the Cl atom remains in the 3β-position in **4**. The position and shape of the signal for the methine proton H-7, which appears as a doublet at 5.103 ppm with J = 9.5 Hz in the spectrum of **4**, is also characteristic. These data agree well with the literature for 3β-acetoxy-7β-bromo-5-hydroxy-5α-cholestan-6-one [6, 7]. Such a large splitting constant for H-7α in the spectrum of **4** is due to an axial—axial vicinal interaction with the methine proton H-8β. It should be noted that the formation of 5α-hydroxy-7β-bromo-6-ketosteroids as side products during dehydrobromation of 5α-hydroxy-7α-bromo-6-ketosteroids has been previously reported [7]. The high stability of **4** compared with **3** is explained by the lack in the former of a destabilizing 1,3-diaxial interaction of the Br atom and the 5α-hydroxy group.

A third compound that was isolated from this reaction in 13% yield is the 3β-chloro-Δ^7-6-ketone (**5**). Judging from the IR and UV spectra, compound **5** contains an α,β-unsaturated keto group. The ^{1}H NMR spectrum of **5** contains a signal characteristic of the vinylic proton H-7 as a broad singlet at 5.643 ppm. Furthermore, the signal of the methine proton H-3α, which is geminal to the Cl atom, is observed at 4.244 ppm. The chemical shift and the multiplicity of the signal for H-4α (2.320 ppm) is also characteristic of the ^{1}H NMR spectrum of **5**. Irradiation of this signal significantly broadens the multiplet for H-3α. Irradiation of the signal for H-3α converts the doublet of doublets for H-4α into a doublet with J = 14.5 Hz.

Then we discovered that the reaction of **3** in DMF in the presence of only lithium carbonate increased the yield of **4** to 71%. The Δ^7-6-ketone was not formed in noticeable yields. This can be explained by the fact that the absence of lithium bromide greatly reduced the rate of dehydrobromination. As a result, isomerization dominated.

We studied the reaction of compound **3** with lithium carbonate and bromide in DMF at 130-135°C in order to increase the yield of the dehydrohalogenation products. Carrying out the reaction for 1.5 h produced the 3β-chloro-5α-hydroxy-Δ^7-6-ketone (**5**) and the 5α-hydroxy-$\Delta^{2,7}$-6-ketone (**6**) in yields of 42 and 33%, respectively. The structure of **6** was proved by analyzing spectra. A comparison of its spectra with the corresponding spectra of the previously synthesized (22E,24R)-5α-ergosta-2,7,22-trien-6-one was especially helpful [8]. According to the IR and UV spectra, **6** contains a Δ^7-6-keto group. The ^{1}H NMR spectra suggest the same conclusion. They lack a signal for a methine proton H-3α, which would be geminal to the Cl atom. However, signals for the three vinylic protons H-2, H-3, and H-7 are observed at 5.695 ppm.

Carrying out this reaction of **3** at 135°C for 6 h suppressed the formation of **5**. The 5α-hydroxy-$\Delta^{2,7}$-6-ketone **6** was isolated in 50% yield. We demonstrated through separate experiments that both the 3β-chloro-7β-bromo-6-ketone **4** and the 3β-chloro-Δ^7-6-ketone **5** undergo dehydrohalogenation at 135°C for 6 h. In both instances the main product is the 2,7-diene-6-ketone **6**, isolated in yields of 47 and 38%, respectively.

Thus, our investigations demonstrate that products of both isomerization and dehydrohalogenation can be produced depending on the reaction temperature. This significantly expands the potential of this approach to synthesizing various steroids.

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 and CCl_4 solutions. UV spectra of ethanol solutions were recorded on a Specord M-400 instrument. ^1H 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)-3β-Chloro-7α-bromo-5-hydroxy-5α-stigmastan-6-one (3). A solution of **2** (5.27 g, prepared from **1** by the literature method [1]) and hydrobromic acid (1.2 ml, 40%) in acetic acid (180 ml) at 45°C was treated with stirring over 5 h with several portions of solution Br_2 in acetic acid (6.8 ml, 2 M). Then the reaction mixture was cooled to room temperature and held there for 19 h. The precipitate was filtered off. The filtrate was diluted with water (100 ml). The precipitate was separated and combined with that obtained previously. Yield of **3**: 6.02 g (quantitative), mp 162-164°C (ethanol). IR spectrum (cm^{-1}): 1720 (C=O). ^1H NMR spectrum (δ, ppm): 0.688 (18-Me, s), 0.850 (19-Me, s), 0.930 (21-Me, d, J = 6 Hz), 2.300 (H-4α, dd, J_1 = 4 Hz, J_2 = 14 Hz), 4.218 (H-7β, d, J = 4.0 Hz), 4.238 (H-3α, m, W/2 = 30 Hz). Found, %: C 64.09, H 8.65, Br + Cl 21.43. Calc. for $C_{29}H_{48}BrClO_2$, %: C 64.02, H 8.89, Br + Cl 21.20.

Dehydrohalogenation of 3. A. A solution of **2** (1.0 g) in DMF (40 ml) was treated with lithium carbonate (1.36 g) and lithium bromide (0.32 g). The mixture was heated to 100°C and stirred at that temperature for 40 min. Then the precipitate was filtered off. The filtrate was diluted with water (50 ml) and extracted with ethylacetate (3×50 ml). The ethylacetate extract was washed with water (3×50 ml) and dried over anhydrous $MgSO_4$. The desiccant was filtered off. The filtrate was evaporated under vacuum. The solid was chromatographed on a silica-gel column using mixtures of hexane and ethylacetate of increasing polarity (from 35:1 to 20:1) as eluent. Three fractions were obtained:

Fraction 1. Yield of **3**: 0.12 g, 12%.

Fraction 2. Yield of **4**: 0.65 g, 65%, mp 162-163°C (dec.) (hexane—ethylacetate). IR spectrum (cm^{-1}): 1730 (C=O). ^1H NMR spectrum (δ, ppm): 0.680 (18-Me, s), 0.810 (19-Me, s), 0.915 (21-Me, d, J = 6 Hz), 4.158 (H-3α, m, W/2 = 28 Hz), 5.103 (H-7α, d, J = 9.5 Hz). Found, %: C 64.07, H 9.13, Br + Cl 20.80. Calc. for $C_{29}H_{48}BrClO_2$, %: C 64.02, H 8.89, Br + Cl 21.20.

Fraction 3. Yield of **5**: 0.11 g, 13%, mp 173-175°C (hexane). IR spectrum (cm^{-1}): 1650 (C=O), 1620 (C=C). UV spectrum (λ_{max}, nm): 250 (ε 12,600). ^1H NMR spectrum (δ, ppm): 0.604 (18-Me, s), 0.970 (19-Me, s), 2.320 (H-4α, dd, J_1 = 4 Hz, J_2 = 14.5 Hz), 4.244 (H-3α, m, W/2 = 29 Hz), 5.643 (H-7, br. s).

B. A solution of **3** (1.0 g) in DMF (40 ml) was treated with lithium carbonate (1.36 g). The mixture was stirred at 100°C for 2 h. Then the precipitate was filtered off. The filtrate was diluted with water (50 ml) and extracted with ethylacetate (3×50 ml). The ethylacetate extract was washed with water (3×30 ml) and dried over anhydrous $MgSO_4$. The desiccant was filtered off. The filtrate was evaporated under vacuum. The solid was chromatographed on a silica-gel column using mixtures of hexane and ethylacetate with increasing polarity (from 30:1 to 20:1) as eluent. Two main fractions were obtained.

Fraction 1. Yield of **3**: 0.08 g, 8%.

Fraction 2. Yield of **4**: 0.71 g, 71%.

C. A solution of steroid **3** (0.5 g) in DMF (20 ml) was treated with lithium carbonate (0.68 g) and lithium bromide (0.16 g). The mixture was stirred at 130°C for 1 h 30 min and then cooled to room temperature. The precipitate was filtered off. The filtrate was diluted with water (40 ml) and extracted with ethylacetate (3×30 ml). The ethylacetate extract was washed with water (3×20 ml) and dried over anhydrous $MgSO_4$. The desiccant was filtered off. The filtrate was evaporated under vacuum. The solid was chromatographed on a silica-gel column using mixtures of hexane and ethylacetate with increasing polarity (from 30:1 to 8:1) as eluent. Two fractions were obtained.

Fraction 1. Yield of **5**: 0.18 g, 42%.

Fraction 2. Yield of **6**: 0.13 g, 33%, mp 156-159°C (hexane). IR spectrum (cm^{-1}): 1690 (C=O), 1640 (C=C). UV spectrum (λ_{max}, nm): 250 (ε 10,600). ^1H NMR spectrum (δ, ppm): 0.603 (18-Me, s), 0.875 (19-Me, s), 5.695 (m, H-2, H-3, H-7).

D. A solution of steroid **3** (0.56 g) in DMF (30 ml) was treated with lithium carbonate (0.76 g) and lithium bromide (0.18 g). The mixture was heated to 135°C and stirred at that temperature for 6 h. The mixture was cooled to room temperature. The precipitate was filtered off. The filtrate was diluted with water (50 ml) and extracted with ethylacetate (3×35 ml). The ethylacetate extract was washed with water (3×25 ml) and dried over anhydrous $MgSO_4$. The desiccant was filtered off. The filtrate was evaporated under vacuum. The solid was chromatographed on a silica-gel column using mixtures

of hexane and ethylacetate with increasing polarity (from 30:1 to 8:1) as eluent. Yield of **6**: 0.22 g, 50%.

Dehydrohalogenation of 3β-Chloro-7β-bromo-6-ketone (4). A solution of steroid **4** (0.30 g) in DMF (25 ml) was treated with lithium carbonate (0.40 g) and lithium bromide (0.09 g). The mixture was heated to 135°C and stirred at that temperature for 6 h. Then the mixture was cooled to room temperature. The precipitate was filtered off. The filtrate was diluted with water (40 ml) and extracted with ethylacetate (3×30 ml). The ethylacetate extract was washed with water (3×20 ml) and dried over anhydrous $MgSO_4$. The desiccant was filtered off. The filtrate was evaporated under vacuum. The solid was chromatographed on a silica-gel column using mixtures of hexane and ethylacetate with increasing polarity (from 20:1 to 10:1) as eluent. Yield of **6**: 0.11 g, 47%.

Dehydrohalogenation of 3β-Chloro-Δ^7-6-ketone (5). A solution of steroid **5** (0.23 g) in DMF (20 ml) was treated with lithium carbonate (0.36 g) and lithium bromide (0.08 g). The mixture was heated to 135°C and stirred at that temperature for 6 h. Then the mixture was cooled to room temperature. The precipitate was filtered off. The filtrate was diluted with water (40 ml) and extracted with ethylacetate (3×30 ml). The ethylacetate extract was washed with water (3×20 ml) and dried over anhydrous $MgSO_4$. The desiccant was filtered off. The filtrate was evaporated under vacuum. The solid was chromatographed on a silica-gel column using mixtures of hexane and ethylacetate of increasing polarity (from 25:1 to 10:1) as eluent. Yield of **6**: 0.08 g, 38%.

REFERENCES

1. N. V. Kovganko and S. K. Ananich, *Zh. Org. Khim.*, 103 (1991).
2. C. Brosa, L. Soca, E. Terricabras, J. C. Ferrer, and A. Alsina, *Tetrahedron*, **54**, 12337 (1998).
3. . A. A. Akhrem and N. V. Kovganko, *Ecdysteroids: Chemistry and Biological Activity* [in Russian], Nauka i Tekhnika, Minsk (1989).
4. N. V. Kovganko, *Khim. Prir. Soedin.*, 655 (1997).
5. H. Lettre, J. Greiner, K. Rutz, L. Hoffman, A. Egle, and W. Bieger, *Liebigs Ann. Chem.*, **758**, 89 (1972).
6. R. Hanna, E. Maalouf, and B. Muckensturm, *Tetrahedron*, **29**, 2297 (1973).
7. W. J. Rodewald, W. J. Szczepek, and J. Gumulka, *Pol. J. Chem.*, **53**, 139 (1979).
8. N. V. Kovganko and S. N. Sokolov, *Khim. Prir. Soedin.*, 354 (1998).