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SYNTHESIS OF 24-ETHYLCHOLESTA-7,22E-DIENE-3β,5α,6β-TRIOL – A NATURAL TRIHYDROXYSTEROID FROM THE BRYOZOAN Myriapora truncata

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UDC 547.92

24-Ethylcholesta-7,22E-diene-3β,5α,6β-triol – a natural trihydroxysteroid from the bryozoan Myriapora truncata – has been synthesized from stigmasterol.

Bryozoa are among the little-studied marine invertebrates. Five 3β,5α,6β-trihydroxy-7,8-dehydrosteroids having untransformed sterol side chains have been isolated from the Mediterranean bryozoan Myriapora truncata Pallas [1]. Eight trihydroxysteroids proving to be identical or close in structure to the polyhydroxysteroids of M. truncata have been isolated from the Mediterranean sponge Spongionella gracilis [2]. The 3β,5α,6β-trihydroxy-7,8-dehydrosteroids mentioned form a new and unique group of natural compounds the biological value of which, and also their distribution in the animal and, possibly, vegetable kingdoms, is yet to be elucidated.

Since these compounds are present in natural materials in only small amounts, for an all-sided study of their biological activity the development of methods for obtaining them by chemical synthesis from accessible natural sterols is necessary. With this aim, we have for the first time effected the synthesis of one of the trihydroxysteroids of M. truncata, 24-ethylcholesta-7,22E-diene-3β,5α,6β-triol (I), which was identified by Cafieri et al. [1] in

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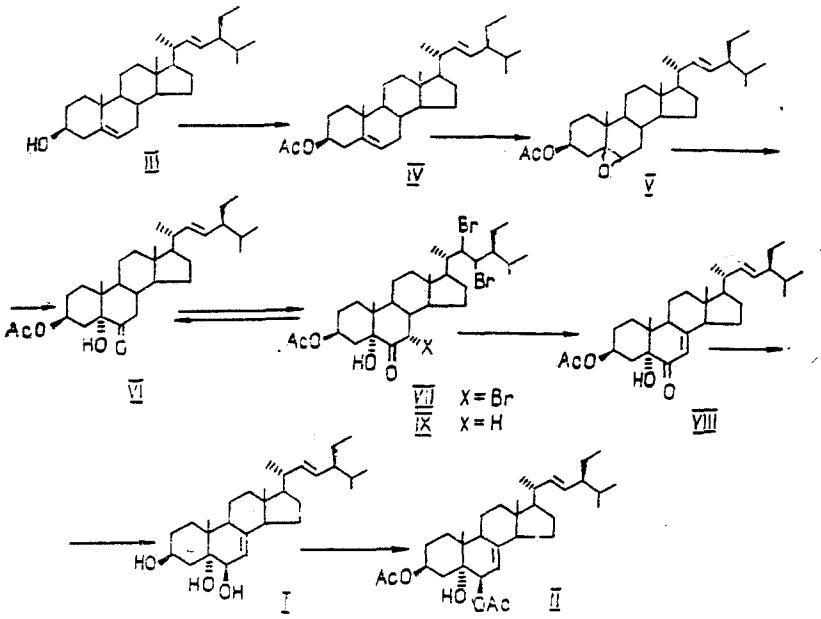
Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 664-669, September-October, 1989. Original article submitted January 2, 1989.

 0009-3130/89/2505-0564$12.50

the form of the amorphous diacetate (II). It must be mentioned that these authors [1] were unable to determine the C_{24} configuration in compound (II) accurately.

The starting compound in our synthesis was stigmasterol (III). The stigmasterol molecule contains, in addition to a suitable side chain, a 3β-hydroxy group and 5,6- and 22,23-double bonds which differ greatly in their reactivities. This makes possible the planned introduction of the appropriate functional groups characteristic for the trihydroxydiene (I).



By acetylating stigmasterol (III) with acetic anhydride in pyridine we obtained a 95% yield of the acetate (IV), the selective epoxidation of the less hindered 5,6-double bond in which with m-chloroperbenzoic acid led to the formation, with a yield of 93%, of the 5,6-monoepoxide (V) in the form of a mixture of α- and β-isomers. Proof of the fact that the epoxidation reaction in this case took place only at the 5,6-double bond was the absence from the PMR spectrum of the signal of a vinyl proton at C_6 and the presence of unchanged signals of the vinyl protons at C_{22} and C_{23} (δ 5.01 and 5.12 ppm, respectively). Also characteristic was the presence at 2.87 and 3.05 ppm of doublets due to the absorption of methine protons at C_6 geminal to the α- and β-epoxide rings, respectively.

When the 5,6-epoxide (V) was subjected to Jones oxidation we obtained the corresponding 5α-hydroxy-6-ketone (VI) in 34% yield. We planned to introduce a 7,8-double bond into the 6-ketosteroid (VI) by bromination in the α-position to the keto group with the formation of the corresponding 7α-bromo-6-ketone, followed by dehydrobromination. This sequence of reactions as a method of introducing a Δ^7-6-keto grouping finds wide use in the synthesis of ecdysteroids and their analogues [3]. However, the presence in compound (VI) of an additional 22,23-double bond capable of adding a molecule of bromine with the formation of a 22,23-dibromide is a complicating factor in this case.

As we had established previously [4], the selective debromination of 22,23-dibromostigmastanes under the conditions for the dehydrobromination of α-bromoketones under the action of lithium carbonate in dimethylformamide is possible if phenol is added to the reaction mixture to bind the bromine liberated. This expedient was utilized in our synthesis. When the Δ^{22}-6-ketosteroid (VI) was brominated in a mixture of acetic acid and chloroform for 12 h, the 7α,22,23-tribromo-6-ketosteroid (VII) was formed in quantitative yield. Its structure followed unambiguously from its PMR spectrum which lacked the absorption of the protons of a 22,23-double bond and exhibited the signals of methine protons geminal to bromine atoms at C_7 (δ 4.19 ppm) and C_{22} and C_{23} (δ 4.35 and 4.49 ppm). As a result of the interaction of the tribromide (VII) with lithium carbonate and phenol in boiling dimethylformamide, the 7,22-dien-6-one (VIII) was formed, and this was isolated after the chromatographic purification of the mixture of reaction products with a yield of 26%. The presence of a conjugated Δ^7-6-keto grouping in steroid (VIII) followed from the presence of absorption at 251 nm in the UV spectrum and also from the shift in the band of the stretching vibrations of the 6-keto group

to 1680 cm^{-1} and the appearance of a band of the stretching vibrations of a C=C bond conjugated with it at 1625 cm^{-1} in the IR spectrum. Also characteristic was the presence in the PMR spectrum of the signal of a vinyl proton at C_7 (δ 5.64 ppm). The fact that compound (VIII) contained a regenerated 22,23-double bond followed from the presence in the PMR spectrum of the signals of vinyl protons at C_{22} and C_{23}. The C_{22}–H signal in the PMR spectrum partially overlapped with the broad signal of the axial proton at C_3. However, from the nature of the splitting of the signal of the proton at C_{23} (δ 5.15 ppm), having the form of a doublet of doublets with the constants J_1 = 14.4 Hz and J_2 = 8.4 Hz, due to vicinal interaction with C_{22}–H and C_{24}–H it was possible to conclude that the 22,23-double bond formed had the (E)-geometry, as in the initial stigmasterol.

For a more convincing determination of the configuration of the side chain in compound (VIII) it would be necessary to obtain not only spectroscopic but also purely chemical proofs of the stereochemical occurrence of the debromination of the 22,23-dibromide grouping. With this aim, as the result of the bromination of compound (VI) for a short time we obtained the 22,23-dibromide (IX). Its dehydrobromination under the conditions analogous to those described above for the 7,22,23-tribromide (VII) led with a yield of 91% to the Δ^{22}-6-ketosteroid (VI). The steroid (VI) obtained in this way was identical in all respects with the substance formed on the oxidation of the epoxide (V). This unambiguously proved the identity of the side chains of the $\Delta^{7,22}$-6-ketosteroid (VIII) and stigmasterol (III).

The reaction of the 3-acetoxy-6-ketosteroid (VIII) with lithium tetrahydroaluminate in ether led to the reduction of the 6-keto group with the formation mainly of a 6β-hydroxy group and the removal of the protective grouping from the 3β-hydroxy group. As a result of the chromatographic purification of the reaction mixture we isolated with a yield of 46% a triol to which, on the basis of an analysis of its IR, PMR, and mass spectra, it was necessary to assign the structure of 24-ethylcholesta-7,22E-diene-3β,5α,6β-triol (I). The acetylation of the triol (I) formed the 3,6-diacetate (II). The PMR spectrum of the compound (II) that we had obtained coincided practically completely with that given in [1] for the diacetate of the natural steroid. It may therefore be concluded that the (24S)-configuration is the most probable one for the natural steroid (I) from M. truncata but this can be decided definitively by a direct comparison of the natural and synthetic samples.

EXPERIMENTAL

Melting points were determined on a Kofler block. IR spectra (of tablets in KBr) were obtained on a UR-20 instrument. PMR spectra were recorded on a Bruker WM-360 NMR spectrometer with a working frequency of 360 MHz. Chemical shifts are given relative to TMS as internal standard. The mass-spectrometric results were obtained on a Varian MAT-311 instrument at an energy of the ionizing electrons of 70 eV. UV spectra were recorded on a Specord UV-Vis instrument.

(24S)-3β-Acetoxy-24-ethyl-5,6-epoxycholest-22E-ene (V). A solution of 4.96 g of stigmasterol acetate (IV) [obtained with a yield of 95% on the acetylation of stigmasterol (III), mp 140-144°C (hexane); according to the literature [5]: mp 141°C] in 115 ml of chloroform was treated with 3.11 g of m-chloroperbenzoic acid (with a purity of 85%). The reaction mixture was kept at room temperature for 12 h and was then filtered through a layer of alumina, and the filtrate was evaporated in vacuum. The residue was chromatographed on a column of silica gel with elution by hexane–ether (5:1). This gave 4.75 g (93%) of the monoepoxide (V), mp 131-136°C (hexane). IR spectrum (cm^{-1}): 1730 (AcO). PMR spectrum ($CDCl_3$, δ, ppm): 0.62 s, 0.65 s (18-Me), 0.79 d (J = 6 Hz, 3H, 26-Me), 0.80 t (J = 7.2 Hz, 3H, 29-Me), 1.00 d (J = 6.6 Hz, 3H, 21-Me), 1.01 s, 1.07 s (19-Me), 0.84 d (J = 6 Hz, 3H, 27-Me), 2.00 s, 2.01 s (AcO), 2.87 d (J = 4.8 Hz, C_6–H_β), 3.05 br.d (J = 1.2 Hz, C_6–H_α), 5.01 br.dd (J_1 = 14.4 Hz, J_2 = 8.4 Hz, 1H, C_{22}–H), 5.12 dd (J_1 = 14.4 Hz, J_2 = Hz [sic], 1H, C_{23}–H), 4.69-4.80 m, 4.93 m(C_3–H_α). Mass spectrum (m/z): 470 (M^+).

(24S)-3β-Acetoxy-24-ethyl-5α-hydroxy-5α-cholestan-22E-en-6-one (VI). With stirring at room temperature, 22.5 ml of an 8N solution of chromic acid was added to a solution of 4.25 g of the epoxide (V) in 300 ml of acetone. The mixture was stirred for 30 min, and then 10 ml of methanol was added to eliminate the excess of oxidant and stirring was continued for 40 min. The reaction mixture was diluted with water and extracted with chloroform. The chloroform extract was evaporated in vacuum and the residue was chromatographed on a column of silica gel with elution by hexane–chloroform (5:1). This gave 1.48 g (34%) of the hydroxyketone (VI), mp 248-249°C (hexane). Found, %: C 76.76; H 10.38. $C_{31}H_{50}O_4$. Calculated, %:

C 76.49; H 10.35. IR spectrum (cm^{-1}): 3420 (OH), 1730 (AcO), 1710 (C=O). PMR spectrum ($CDCl_3$, δ, ppm): 0.65 s (3H, 18-Me), 0.79 d (J = 7.2 Hz, 3H, 26-Me), 0.80 t (J = 7.2 Hz, 3H, 29-Me), 0.81 s (3H, 19-Me), 0.83 d (J = 6 Hz, 3H, 27-Me), 1.01 d (J = 6 Hz, 3H, 21-Me), 2.01 s (3H, AcO), 4.96-5.08 m (2H, C_3–H_α and C_{22}–H), 5.12 dd (J_1 = 8.4 Hz, J_2 = 72. Hz, 1H, C_{23}–H). Mass spectrum (m/z): 486 (M^+).

(24S)-3β-Acetoxy-7α,22,23-tribromo-24-ethyl-5α-hydroxy-5α-cholestan-6-one (VII). A solution of 1.0 g of the enone (VI) in 30 ml of acetic acid and 30 ml of chloroform was treated with 15 ml of a 1.9 M solution of bromine in acetic acid. The reaction mixture was kept at room temperature for 12 h and was then treated with a solution of 4.0 g of sodium sulfate in 20 ml of water and was diluted with water. After extraction with chloroform, the chloroform extract was washed with water and was then evaporated in vacuum. The residue was chromatographed on a column of silica gel with elution by hexane–chloroform (1:1). This gave 1.5 g (quantitative yield) of the tribromide (VII), amorphous. IR spectrum (cm^{-1}): 3440 (OH), 1730 (AcO), 1710 (C=O). PMR spectrum ($CDCl_3$, δ, ppm): 0.77 s (3H, 18-Me), 0.85 t (J = 2.4 Hz, 3H, 29-Me), 1.01 d (J = 7.2 Hz, 3H, 21-Me), 0.94 s (3H, 19-Me), 0.98 d (J = 7.2 Hz, 6H, 26/27-Me), 2.03 s (3H, AcO), 4.19 d (J = 4.8 Hz, 1H, C_1–H_β), 4.35 dd (J_1 = 12 Hz, J_2 = 2.4 Hz, 1H, C_{22}– or C_{23}–H), 4.49 dd (J_1 = 12 Hz, J_2 = 1.8 Hz, 1H, C_{22}– or C_{23}–H), 5.03 m (W/2 = 24 Hz, 1H, C_3–H_α).

(24S)-3β-Acetoxy-24-ethyl-5α-hydroxy-5α-cholesta-7,22E-dien-6-one (VIII). A mixture of 1.5 g of the tribromide (VII), 1.0 g of lithium carbonate, 0.3 g of phenol, and 50 ml of dimethylformamide was boiled for 30 min and was then cooled to room temperature and the precipitate was filtered off. The filtrate was diluted with water and extracted with hexane. The hexane extract was evaporated in vacuum and the residue was chromatographed on a column of silica gel with elution by hexane–chloroform (3:1). This gave 0.26 g (26%) of the dienone (VIII), mp 238-240°C (hexane–chloroform). IR spectrum (cm^{-1}): 3420 (OH), 1735 (AcO), 1680 (C=O), 1625 (C=C). UV spectrum: ε_{251} 16,000 (MeOH). PMR spectrum ($CDCl_3$, δ, ppm): 0.61 s (3H, 18-Me), 0.80 d (J = 6 Hz, 3H, 26-Me), 0.81 t (J = 7.2 Hz, 3H, 29-Me), 0.85 d (J = 6 Hz, 3H, 27-Me), 0.96 s (3H, 19-Me), 1.04 d (J = 6 Hz, 3H, 21-Me), 2.02 s (3H, AcO), 5.00-5.11 m (2H, C_3–H_χ and C_{22}–H), 5.15 dd (J_1 = 14.4 Hz, J_2 = 8.4 Hz, 1H, C_{23}–H), 5.64 s (1H, C_7–H). Mass spectrum (m/z): 424 (M^+ – AcOH).

(24S)-3β-Acetoxy-22,23-dibromo-24-ethyl-5α-hydroxy-5α-cholestan-6-one (IX). With stirring at 50°C, 5 ml of a 1.9 M solution of bromine in acetic acid was added to solution of 1.3 g of the enone (VI) in 145 ml of acetic acid and 46 ml of chloroform. The mixture was stirred at 50°C for 5 min and then, after cooling to room temperature, it was treated with a solution of 2.0 g of sodium sulfite in 10 ml of water and was diluted with water. After extraction with chloroform, the chloroform extract was washed with water and was then evaporated in vacuum. The residue was chromatographed on a column of silica gel with elution by hexane–chloroform (1:1). This gave 1.5 g (86%) of the 22,23-dibromide (IX), mp 216°C (decomp.). IR spectrum (cm^{-1}): 3420 (OH), 1735 (AcO), 1710 (C=O). PMR spectrum ($CDCl_3$, δ, ppm): 0.72 s (3H, 18-Me), 0.81 t (J = 2.4 Hz, 3H, 29-Me), 0.93 s (3H, 19-Me), 0.96 d (J = 3.6 Hz, 3H, 26-Me), 0.97 d (J = 4.8 Hz, 3H, 27-Me), 1.00 d (J = 7.2 Hz, 1H, 21-Me), 2.01 s (3H, AcO), 4.40 dd (J_1 = 11.4 Hz, J_2 = 2.4 Hz, 1H, C_{22}– or C_{23}–H), 4.49 dd (J_1 = 12 Hz, J_2 = 1.2 Hz, 1H, C_{22}– or C_{23}–H). 5.01 m (W/2 = 24 Hz, 1H, C_3–H_α). Mass spectrum (m/z): 648, 646, 644 (M^+).

Debromination of the 22,23-Dibromide (IX). A mixture of 1.5 g of the 22,23-dibromide (IX), 1.0 g of lithium carbonate, 0.3 g of phenol, and 50 ml of dimethylformamide was boiled for 1 h and then, after being cooled to room temperature, it was filtered. The filtrate was diluted with water and extracted with chloroform. The chloroform extract was washed with water, and was evaporated in vacuum. The residue was chromatographed on a column of silica gel with elution by hexane–chloroform (3:1). This gave 1.2 g (91%) of the enol (VI), mp 248-250°C (hexane–chloroform). The same had IR and PMR spectra identical with the spectrum of the authentic substance and showed no depression of the melting point in admixture with it.

(24S)-24-Ethylcholesta-7,22E-diene-3β,5α,6β-triol (I). With stirring, 0.36 g of lithium tetrahydroaluminate was added in portions to a solution of 0.25 g of the ketone (VIII) in 70 ml of anhydrous ether. The reaction mixture was stirred at room temperature for 40 min and then the excess of reagent was neutralized by the addition of ethyl acetate and the whole was evaporated in vacuum. The residue was chromatographed on a column of silica gel with elution by chloroform–methanol (30:1). This gave 0.105 g (46%) of the triol (I), mp 227-232°C (hex-

ane–chloroform). IR spectrum (cm^{-1}): 3435 (OH), 1660 (C=C). PMR spectrum ($CDCl_3$, δ, ppm): 0.60 s (3H, 18-Me), 0.80 d (J = 6 Hz, 3H, 26-Me), 0.81 t (J = 7.2 Hz, 3H, 29-Me), 0.85 d (J = 6 Hz, 3H, 27-Me), 1.03 d (J = 6 Hz, 3H, 21-Me), 1.08 s (3H, 19-Me), 3.62 br.s (1H, C_6–H_α), 4.07 m (W/2 = 24 Hz, 1H, C_3–H_α), 5.03 dd (J_1 = 14.4 Hz, J_2 = 8.4 Hz, 1H, C_{22}–H), 5.14 dd (J_1 = 14.4 Hz, J_2 = 9.0 Hz, 1H, C_{23}–H), 5.35 dd (J_1 = 4.8 Hz, J_2 = 4.5 Hz, 1H, C_7–H); (C_5D_5N, δ, ppm) 0.70 s (3H, 18-Me), 0.85 d (J = 6 Hz, 3H, 26-Me), 0.87 t (J = 6 Hz, 3H, 29-Me), 0.92 d (J = 6 Hz, 3H, 27-Me), 1.08 d (J = 6 Hz, 3H, 21-Me), 1.55 s (3H, 19-Me), 4.34 br.s (1H, C_6–H_α), 4.87 m (W/2 = 24 Hz, 1H, C_3–H_α), 5.05-5.23 m (2H, C_{22}– and C_{23}–H), 5.75 br.s (1H, C_7–H). Mass spectrum (m/z): 444 (M^+).

3,6-Diacetate of (24S)-24-ethylcholesta-7,22E-diene-3β,5α,6β-triol (II). A solution of 0.072 g of the triol (I) in 3 ml of pyridine and 0.5 ml of acetic anhydride was kept at room temperature for 12.5 h and was then evaporated in vacuum. The residue was chromatographed on a column of silica gel with elution by hexane–ether (3:1). This gave 0.071 g (84%) of the diacetate (II), amorphous. IR spectrum (cm^{-1}): 3450 (OH), 1735 (AcO), 1660 (C=C). PMR spectrum ($CDCl_3$, δ, ppm): 0.59 s (3H, 18-Me), 0.80 d (J = 6 Hz, 3H, 26-Me), 0.8 t (J = 7.2 Hz, 3H, 29-Me), 0.85 d (J = 6 Hz, 3H, 27-Me), 1.03 d (J = 6 Hz, 3H, 21-Me), 1.07 s (3H, 19-Me), 2.04 s (3H, AcO), 2.07 s (3H, AcO), 4.80 br.d (J = 4.8 Hz, 1H, C_β–H_α), 5.06 dd (J_1 = 15 Hz, J_2 = 7 Hz, 1H, C_{22}–H), 5.10-5.15 m (2H, C_3–H_α and C_{23}–H), 5.27 br.d (J = 5 Hz, 1H, C_7–H). Mass spectrum (m/z): 486 ($M^+ - CH_2CO$), 468 (M^+ – AcOH).

SUMMARY

24-Ethylcholesta-7,22E-diene-3β,5α,6β-triol – a natural steroid from the bryozoan Myriapora truncata – has been synthesized from stigmasterol.

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