TRANSFORMED STEROIDS

COMMUNICATION 28. SYNTHESIS OF Δ^4 -PREGNENE-17 α ,20-DIOL-3-ON-21-OIC ACIDS EPIMERIC AT C-20 AND THE DETERMINATION OF THEIR STEREOCHEMISTRY

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Earlier in a search for potential antagonists of aldosterone we synthesized Δ^4 -isopregnene-17 β ,20diol-3-on-21-oic acids epimeric at C-20 using rearrangement of the 17-acetate of 21,21-dibromo- Δ^4 -isopregnene-17 β -ol-3,20-dione [1]. This communication is devoted to the synthesis and stereochemistry of dihydroxy carboxylic acids having the natural (β) configuration of the side chain.

At the beginning of our investigation there were two communications in the literature [2, 3] on the synthesis of acids from 20-keto-21-aldehydes by the Cannizzaro reaction. Starting from the acetate of Reichstein's substance S (I) and using this method we synthesized both Δ^4 -pregnene-17 α ,20-diol-3-on-21-oic acids (IVa) and (IVb) epimeric at C-20.



Saponification of acetate (I) yielded compound (II), which was further oxidized with copper acetate by the known method [4] to the 20-keto-21-aldehyde (III); the latter was used in the Cannizarro reaction [2] without isolation. A mixture of acids in a yield of ~63% was obtained as a result, from which 17α -hydroxyetianic acid (V), the 20α -acid (IVa), and the 20β -acid (IVb) were isolated. Methylation of the indicated acids with diazomethane yielded their methyl esters; the methyl ester of 17α -hydroxyetianic acid (VI), the methyl ester of the 20α -acid (VIIa), and the methyl ester of the 20β -acid (VIIb). The structure of the methyl esters (VIIa) and (VIIb) was established using physical and chemical methods. IR, UV, PMR, and mass spectra confirmed the presence in them of Δ^4 -3-keto, hydroxy, and carbmethoxy groups.

The presence in them of a vicinal glycol grouping at the 17,20-positions was confirmed by oxidation of esters (VIIa) and (VIIb) with periodic acid into the known Δ^4 -androsten-3,17-dione (VIII).

N. D. Zelinskii Institute of Organic Chemistry, Academy of Sciences of the USSR. Translated from Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya, No. 8, pp. 1830-1835, August, 1970. Original article submitted February 11, 1969.

• 1971 Consultants Bureau, a division of Plenum Publishing Corporation, 227 West 17th Street, New York, N. Y. 10011. All rights reserved. This article cannot be reproduced for any purpose whatsoever without permission of the publisher. A copy of this article is available from the publisher for \$15.00. To establish the stereochemistry of the 20-center, both esters (VIIa) and (VIIb) were converted by a series of reactions not affecting the asymmetric center into the known 20,21-diacetate of Δ^4 -pregnene-17 α , 20 α ,21-triol-3-one (Xa) and the 20,21-diacetate of Δ^4 -pregnene-17 α ,20 β ,21-triol-3-one (Xb), the configurations of which were reliably established earlier [5, 6]. Reduction of ester (VIIa) with lithium aluminum hydride yielded tetrol (IXa) (mixture of C-3 epimers), which without isolation was subjected to oxidation with manganese dioxide [7] to the corresponding Δ^4 -triolone. The latter without isolation was acetylated with acetic anhydride in pyridine to give the known 20,21-diacetate of Δ^4 -pregnene-17 α ,20 α ,21-triol-3-one (Xa), which coincided with all constants described in the literature [6] and did not give a melting point depression in a mixed sample with an authentic sample.



A similar sequence of transformations with ester (VIIb) yielded the 20,21-diacetate of Δ^4 -pregnene-17 α ,20 β ,21-triol-3-one (Xb), coinciding in all constants with those described in the literature [5] and not giving a melting point depression in a mixed sample with an authentic sample.

On the basis of the obtained data the structures of Δ^4 -pregnene- 17α , 20α -diol-3-on-21-oic acid and Δ^4 -pregnene- 17α , 20β -diol-3-on-21-oic acid, respectively, should be assigned to the given pair of acids (IVa) and (IVb) epimeric at C-20.

The structure of the methyl ester of 17α -hydroxyetianic acid (VI) was established using IR, UV, PMR, and mass spectra. Spectral data confirm the presence of Δ^4 -3-keto, hydroxy, and carbmethoxy groups in ester (VI). Comparison with an authentic sample definitely confirmed the proposed structure of ester (VI). An authentic sample of ester (VI) was obtained by the described method [8] by oxidation of (II) with periodic acid and methylation of the formed 17α -hydroxyetianic acid (V) with diazomethane.



The indicated 17α -hydroxyetianic acid (V) is evidently formed in the reaction studied by us (see first scheme) as a result of a side dehydration and decarboxylation reactions of the dihydroxy carboxylic acids (IVa) and (IVb), the primary products of molecular rearrangement of the 20,21-ketoaldehyde (III). A similar fragmentation is described in the literature for α -hydroxy carboxylic acids in the presence of oxidizing agents [9] and also of dihydroxy carboxylic acids [10, 11] upon heating. The mechanism of this reaction is as yet unclear, but it was established [9, 10] that in this case the hydroxycarbonyl compound is formed.



It is possible that in our case the hydroxy aldehyde (XI) formed initially is oxidized further by oxygen of the air under basic conditions to acid (V).



EXPERIMENTAL

All melting points were determined on a Kofler block. UV spectra were taken on an EPS-2 spectrometer; IR spectra were taken on a UR-10 spectrometer; PMR spectra were taken on a Varian HA-100D high resolution spectrometer having a working frequency of 100 MHz for 5-7% solutions of materials in $CDCl_3$ with hexamethyldisiloxane as internal standard. Al_2O_3 (act.II), prepared by the method of Akhrem and Kuznetsova [12], was used for thin-layer chromatography (TLC). Spots on plates were detected in UV light.

Saponification of the 21-Acetate of Δ^4 -Pregnene- 17α , 21-diol-3,20-dione (I). We dissolved 1 g of the acetate of Reichstein's substance S (I) having mp 234-238°C in 100 ml of CH₃OH, added a solution of 1 g of KHCO₃ in 20 ml of water, left the reaction mixture at room temperature for three days, and then heated it for 3.5 h on a water bath at 40°. The methanol was then evaporated in vacuum, and the residue was washed with water and dried in air. We obtained 0.86 g of crystals having mp 196-200°. Recrystallization from ethanol yielded Δ^4 -pregnene- 17α ,21-diol-3,20-dione (II) having mp 210-216°; R_f 0.21 in a system of moist ether. IR spectrum (ν , cm⁻¹): 1620; 1668; 1712; 3465; and 3515 (KBr).

Oxidation of Δ^4 -Pregnene-17 α , 21-diol-3,20-dione (II). To a suspension of 2.4 g of Cu(OAc)₂ in 80 ml of CH₃OH and 0.4 ml of CH₃COOH was added a solution of 2 g of Reichstein's substance S (II) in 80 ml of hot methanol. The reaction mixture was boiled with a reflux condenser for 30 min, then 30 ml of H₂O was added and the mixture was boiled an additional 20 min; the precipitate was then filtered and washed thoroughly with CH₃OH, and the filtrate was evaporated in vacuum to ~30 ml. The residue was extracted with CHCl₃. The extract was washed with water, dried with MgSO₄, and evaporated. We obtained 2 g of Δ^4 -pregnene-17 α -ol-3,20-dion-21-al (III) as an oily product.

Molecular Rearrangement of Δ^4 -Pregnene-17 α -ol-3,20-dion-21-al (III). A suspension of 2 g of (III) in 52 ml of H₂O was stirred with 11 ml of 2 N NaOH for 14 h at room temperature. Then the reaction mixture was acidified with 5% HCl to a weakly acidic reaction and extracted with ethyl acetate. The extract was thoroughly washed with a 5% solution of NaHCO₃. The bicarbonate extracts were acidified with 5% HCl to a weakly acidic reaction and extracted with ethyl acetate. The extract over MgSO₄, and evaporated. We obtained 1.29 g (yield 63% of theor.) of an oily product, a mixture of acids (IVa), (IVb), and (V), which was separated by preparative TLC.

We transferred 200 mg of the acid mixture to a plate $(20 \times 25 \text{ cm})$ containing Al_2O_3 (act. II), layer thickness 2 mm. Separation was carried out in a system of n-PrOH – NH₄OH (67:33). Elution of the ammonium salts was carried with distilled water, which was then acidified with 5% HCl to a weakly acidic reaction and extracted with ethyl acetate. The extract was washed with water, dried over MgSO₄, and evaporated. The upper band yielded 18 mg of acid (V) having mp 240-242° (dec.); R_f 0.60 (in the system indicated above); a mixed sample with an authentic sample of 17α -hydroxyetianic acid (V) did not give a melting point depression.

The middle band yielded 43 mg of Δ^4 -pregnene- 17α , 20α -diol-3-on-21-oic acid (IVa) having mp 229-231° (acetone -hexane); Rf 0.28 (in the system indicated above). IR spectrum (ν , cm⁻¹): 1620; 1638; 1702; and 3430 (KBr). UV spectrum: λ_{max} 243 nm, ε 15,000 (ethanol).

The bottom band yielded 93 mg of Δ^4 -pregnene- 17α , 20β -diol-3-on-21-oic acid (IVb) having mp 219-221° (acetone - hexane); Rf 0.20 (in the system indicated above). IR spectrum (ν , cm⁻¹): 1612; 1638; 1702; and 3435 (KBr). UV spectrum: λ_{max} 243 nm, ε 15,800 (ethanol).

<u>Methyl Ester of 17α -Hydroxyetianic Acid (VI)</u>. We methylated 50 mg of 17α -hydroxyetianic acid (V) with diazomethane. We obtained 50 mg of the methyl ester (VI) having mp 214-215.5° (ether – petroleum ether); $[\alpha]_D^{22}$ +56.5° (C 0.527, acetone); R_f 0.74 (in a system of moist ether); mass number 346. A mixed sample with an authentic sample of the methyl ester of 17α -hydroxyetianic acid (VI) did not give a melting point depression.

Methyl Ester of Δ^4 -Pregnene-17α, 20β-diol-3-on-21-oic Acid (VIIb). Methylation with diazomethane of 200 mg of acid (IVb) yielded 200 mg of ester (VIIb) having mp 174-175.5° (acetone – hexane); $[\alpha]_D^{26} + 41.2°$ (C 0.992, acetone). IR spectrum (ν , cm⁻¹): 1618; 1678; 1720; 1738; and 3425 (KBr). UV spectrum: λ_{max} 243 nm, ε 16,500 (ethanol). PMR spectrum (δ , ppm): 0.839 (CH₃-18); 1.119 (CH₃-19); 5.661 (H at a double bond); 4.283 (H at C-20). Found: C 69.86; H 8.51%. C₂₂H₃₂O₅. Calculated: C 70.18; H 8.57%. Mass number 376. A mixed sample with the 20α-ester (VIIa) melted at 169-180°.

Oxidation of Esters (VIIa) and (VIIb) with Periodic Acid. In two small flasks, each containing 10 ml of methanol, were dissolved 100-mg samples of esters (VIIa) and (VIIb), respectively. To each of the obtained solutions were added 2 ml of H_2O and 6 ml of 4% HIO_4 in 0.2 N H_2SO_4 , and the solutions were left at room temperature overnight. Then the solutions were diluted with 100 ml of water and extracted with CH_2Cl_2 . The extracts were washed with a dilute solution of NaHCO₃ and water, dried with MgSO₄, and evaporated. The experiment with ester (VIIa) yielded 91 mg and the experiment with ester (VIIb) yielded 92 mg of Δ^4 -androstene-3,17-dione (VIII) having mp 169-171° (acetone – hexane). A mixed sample with an authentic sample of Δ^4 -androstene-3,17-dione did not give a melting point depression.

Determination of the Configuration of Ester (VIIa). To a suspension of 200 mg of LiAlH₄ in 10 ml of tetrahydrofuran (THF) was added a solution of 150 mg of ester (VIIa) in 10 ml of THF. The reaction mixture was boiled for 30 min, then cooled, and the excess LiAlH₄ was decomposed with ethyl acetate; 5 g of the solid Na₂SO₄ and 5 ml of a saturated solution of Na₂SO₄ were added. The reaction mass was filtered, the precipitate was thoroughly washed with THF and CH₃OH, the filtrate was evaporated, and the residue was dissolved in ethyl acetate, dried over MgSO₄, and evaporated. We obtained 128 mg of an oily reduction product, which was dissolved in 10 ml of THF; 0.6 g of activated MnO₂ was added and the reaction mixture was stirred for 24 h at room temperature. Next the MnO₂ was filtered and the precipitate was washed thoroughly with methanol. The filtrate was evaporated to give 95 mg of an oily product which was acetylated with 0.5 ml of (CH₃CO)₂O in 1 ml of pyridine, then chromatographed on a plate (13 × 18 cm) of Al₂O₃, layer thickness 1 mm, in a system of moist ether with three passes. Elution was carried out with chloroform. We obtained 38 mg of the 20,21-diacetate of Δ^4 -pregnene-17 α ,20 α ,21-triol-3-one (Xa) having mp 243-245° (acetone – hexane). A mixed sample with an authentic sample of the 20 α ,21-diacetate did not give a melting point depression.

Determination of Configuration of Ester (VIIb). To a suspension of 200 mg of LiAlH₄ in 10 ml of THF was added a solution of 160 mg of ester (VIIb) in 10 ml of THF. The reaction mixture was boiled for 30 min and then treated as described above for (VIIa). We obtained 123 mg of an oily product, which was oxidized with MnO₂ under the conditions described above for isomer (VIIa). We obtained 94 mg of an oily product which was acetylated with 0.5 ml of (CH₃CO)₂O in 1 ml of pyridine and then chromatographed on a plate (13 × 18 cm) of Al₂O₃, layer thickness 1 mm, in a system of moist ether with three passes. Elution was carried out with CHCl₃. We obtained 30 mg of the 20β ,21-diacetate of Δ^4 -pregnene- 17α ,20 β ,21-triol-3-one (Xb) having mp 191-192° (ether – hexane). A mixed sample with an authentic sample of the 20β ,21-diacetate did not give a melting point depression.

<u>17 α -Hydroxyetianic Acid (V)</u>. To a solution of 500 mg of Reichstein's substance S (II) in 15 ml of CH₃OH was added a solution of 350 mg of HIO₄ in 2.5 ml of H₂O and the mixture was left at room temperature for 18 h. Then the CH₃OH was evaporated in vacuum and the residue was dissolved in CHCl₃. The chloroform solution was extracted with a solution of NaHCO₃. The bicarbonate extracts were acidified with 5% HCl and extracted with ethyl acetate. The extract was washed with water, dried with MgSO₄, and evaporated. We obtained 300 mg of crystals of 17 α -hydroxyetianic acid (V) having mp 210-225° (dec.). The material was crystallized from acetone – hexane, then from methanol, mp 236-238° (dec.); $[\alpha]_D^{23} + 66.3°$ (C 0.927, acetone). Found: C 68.60; H 8.80%. C₂₀H₂₈O₄·H₂O. Calculated; C 68.57; H 8.57%.

<u>Methyl Ester of 17α -Hydroxyetianic Acid (VI)</u>. We dissolved 140 mg of acid (V) in 5 ml of CH₃OH and added an ether solution of diazomethane. The reaction mixture was left overnight, then evaporated in vacuum to dryness, and the methyl 17α -hydroxyetianate (VI) residue was crystallized from acetone – hexane, mp 214-215.5° $[\alpha]_D^{22}$ + 56.5° (C 0.527 acetone). IR spectrum (ν , cm⁻¹): 1620; 1675; 1720; and 3522 (KBr). PMR spectrum (δ , ppm): 0.63 (CH₃-18); 1.12 (CH₃-19); 5.68 (H at a double bond); 3.69 (COOCH₃).

CONCLUSIONS

1. Both Δ^4 -pregnene-17 α ,20-diol-3-on-21-oic acids, epimeric at C-20, and also 17 α -hydroxyetianic acid are formed upon the Cannizzaro rearrangement of Δ^4 -pregnen-17 α -ol-3,20-dion-21-al.

2. The configurations of the 20α - and 20β -dihydroxy carboxylic acids and their esters were established by their conversion to the known 20α , 21- and 20β , 21-diacetates of Δ^4 -pregnene- 17α , 20, 21-triol-3-ones by a series of reactions not affecting the asymmetric 20-center and was confirmed using IR, UV, PMR, and mass spectra.

3. The structure and configuration of 17α -hydroxyetianic acid and its methyl ester were established using IR, UV, PMR, and mass spectra, and also by countersynthesis.

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