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STEREOSCOPIC SYNTHESIS OF 4β-DEUTERO-Δ^5-3-KETOSTEROIDS

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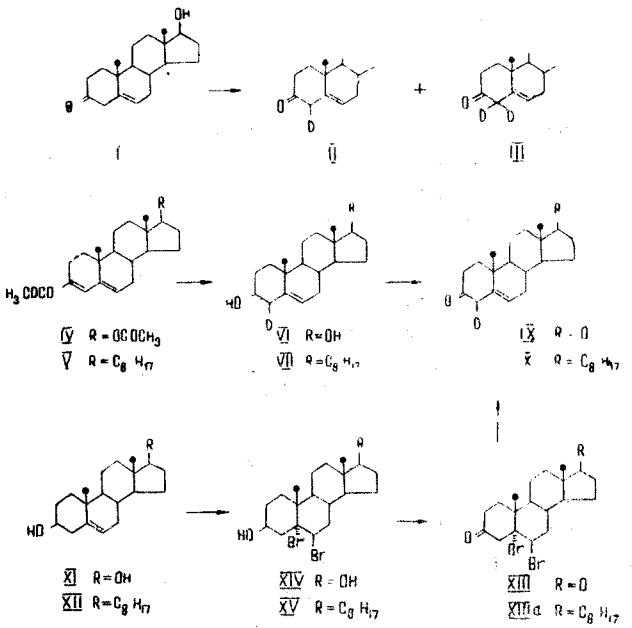
In a study of the transformation of steroids taking place under the influence of enzymes, the necessity is frequently encountered for the stereospecific introduction into the molecule of the substrate of an isotope label using deuterium or tritium. In particular, it is especially difficult to synthesize Δ^5-3-ketosteroids stereospecifically labelled in position 4. The known routes for the synthesis involve numerous stages and are fairly complex [1]. The simplest method of introducing deuterium could be the rapid exchange with the medium of the labile H-atom at C_4. Ringold et al [2] have shown previously that cholest-5-en-3-one is converted into the 4,4-dideutero-Δ^5 analog under the action of deuterium oxide in diglyme.

This induced us to study various conditions for the exchange of steroid Δ^5-3-ketones with the medium. Thus, when 17β-hydroxyandrost-5-en-3-one (I) was heated in absolute diglyme in the presence of D_2O to 80° C for 8 hr, it underwent exchange with the medium without migration of the double bond, forming a mixture of deutero analogs containing according to mass spectrometry,* 31% of the initial compound and 48% of monodeutero-(II) and 21% of dideutero--17β-hydroxyandrost-5-en-3-one (III). Since the separation of this mixture into the individual components presented certain difficulties, we studied severe conditions for the deutero exchange of steroids with the aim of obtaining clearer results. However, at 170° C(24 hr)a product was obtained which, according to the IR spectrum, contained no Δ^5-3-ketosteroids and consisted of a mixture of di- and trideutero derivatives of testosterone (mass spectrometry). Shortening the time of exchange also led to the formation of a complex mixture of deutero-Δ^5- and -Δ^4-3-ketones. Replacing the diglyme by deuteroethanol (C_2H_5OD) and by deutero-tert-butanol (tert-BuOD) did not give the desired results. When the conditions of exchange with the medium described by Ringold et al. [2] were accurately reproduced, we obtained only the Δ^4-isomer. The introduction of deuterium with the aid of the isomerization reaction of Δ^4-3-ketosteroids under the conditions given by Ringold [3] (potassium tert-butoxide in tert-BuOD) was also unsuccessful.



It is known that when 3-acetoxycholest-2-ene is reduced with lithium aluminum hydride and the complex is subsequently decomposed with deuterium oxide, 2-monodeuterocholestan-3β-ol is formed exclusively [4]. By using these conditions for the reduction of enol-acetates of Δ^4-3-ketosteroids we have synthesized Δ^5-3β-hydroxy compounds containing, according to the mass spectrometry, only one deuterium atom at C_4 (D_1 100%).

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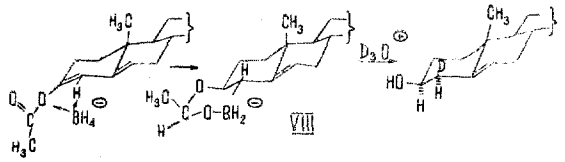
Thus, the enolacetates of testosterone (IV) and of cholest-4-en-3-one (V), on reduction with sodium borohydride in a deuterated medium (D_2O, C_2H_5OD) with decomposition of the complex with deuterium chloride, give 4-deuteroandrost-5-ene-3β, 17β-diol (VI) and 4-deuterocholesterol (VII), respectively. The inclusion of deuterium in the position adjacent to the hydroxyl group unambiguously shows the formation of an intermediate cyclic complex as a result of the bimolecular addition of the BH_4^- anion to the electrophilic C-atom of the carbonyl group and to the C_4 atom.



VIII

If we take account of steric hindrance and thermodynamic considerations, the most favorable direction of attack of the molecule of the enol-acetate by the BH_4^- ion is from the α-side. Under these conditions the formation of an equatorial-equatorial cyclic transition complex (VIII), already possessing considerable steric hindrance on the α-side, is completely probable. Consequently it is reasonable to assume that in the second stage of the reaction a stereospecific attack by solvated deuterium from the β-side takes place. As the result of an ordinary Walden inversion at C_4 the β-deuteroanalog (VI) or (VII), respectively, must be formed. Indeed, the oxidation of the 4β-deutero compound VI by Jones' method leads to 4β-deuteroandrost-5-ene-3, 17-dione (IX), with the complete retention of the deuterium at C_4.

In the second method of synthesis, we used Fieser's well-known scheme [5] which he proposed for the synthesis of cholest-5-en-3-one. The initial compounds were androst-5-ene-3β, 17β-diol (XI) and cholesterol (XII), from which, by bromination and subsequent oxidation with sodium dichromate in acetic acid, the dibromoketones XIII and XIIIa were obtained. Debromination of the latter with zinc dust in deuteroacetic acid (CH_3COOD) gave the 4-deuteroketones IXX and X containing (according to mass spectrometry) only one atom of deuterium.

This fact also shows the stereospecificity of the inclusion of deuterium. The deuteroketones IX and X would contain a mixture of the 4α- and 4β-epimers only if the rates of exchange with the medium or the 4α- and 4β-H atoms were were comparable. But then the 4, 4-dideuteroketones should be formed, which is quite possible with an excess of CH_3COOD and a sufficiently long reaction time. Since the axial H-atom present in the α-position to the keto group takes part in exchange with the medium considerably more readily [6], we ascribed to the 4-deuteroketones IX and X the configuration of 4β-deuterosteroids. Physicochemical methods were used to confirm their structures.

The IR spectrum of 4β-deuteroandrost-5-ene-3β, 17β-diol (VI) has a sharp absorption band of a C—D bond at 2175 cm^{-1} (paraffin oil) and 2145 cm^{-2} (chloroform). In the IR spectrum of compound IX this absorption band is practically absent, although mass spectrometry unambiguously showed the complete retention of the deuterium in the molecule of the steroid. Similar results were obtained for 4-deuterocholesterol (VII) and for 4-deuterocholestenone (X). Consequently, in contradiction to Ringold's statement [2], we consider that IR spectrophotometry cannot be used to analyze the content and to determine the orientation of deuterium in such systems. We used magnetic resonance to determine the orientation of the deuterium.

It is known [7, 8] that the introduction of deuterium into the axial position causes a considerable change in the signal of the neighboring proton, while with the equatorial substitution of deuterium this effect is very slight. The NMR spectra of cholesterol acetate and the 4-deutero compound corresponding to it, obtained by the reduction of the enol-acetate of cholestenone with sodium borohydride in a deuterated medium, were recorded. It was found that the half-width of the signal of the C_3 proton in the nondeuteriated substance was 31 Hz while in the deutero compound it was 16 Hz, which shows the disappearance of the strong axial-axial interaction of the protons, and consequently, the axial 4β position of the deuterium.

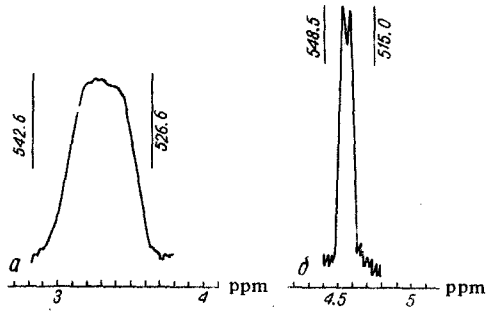
In considering the NMR spectra (see figure) of cholest-5-en-3-one and its 4-deutero analog (X) obtained by debrominating the dibromoketone XIIIa in a deuterated medium, it was found that in the deuteriated compound the signal of the vinyl proton of C_6 gives a sharp doublet, in contrast to the corresponding signal in the NMR spectrum of the non-deuteriated ketone, which shows the disappearance of the strong spin-spin coupling and, consequently, the axial 4β position of the deuterium.

Thus, both methods of introducing deuterium into the C_4 position of the steroid nucleus lead stereospecifically to 4β-deuterio-Δ^5-3-ketosteroids

Experimental

Enol diacetate of testosterone (IV). A mixture of 800 mg of testosterone, 16 mg of acetic anhydride, and 24 ml of acetyl chloride was kept at a gentle boil in an atmosphere of argon for 4.5 hr and was then evaporated in vacuum. This gave 820 mg of the enol diacetate IV, with mp 156–158° C (from ethanol).

4β-Deuteroandrost-5-ene-3β, 17β-diol (VI). To a solution of 820 mg of the enol diacetate (IV) in 84 ml of absolute ether were added 15 ml of C_2H_5OD and 6.3 ml of D_2O. The mixture was cooled to 0° C and a solution of 1.5 ml of $NaBH_4$ in 21 ml of deuteroethanol (C_2H_5OD) and 15 ml of D_2O was added. After 2 hours' stirring at 18° C and 20 hours' cooling, 6.5 ml of DCl was added (to give pH 6.5). The reaction mixture was boiled for 1 hr, after which acidification gave a white precipitate. Exhaustive extraction with ether yielded 620 mg of product which was saponified by being boiled with 0.3 g of KOH in 10 ml of methanol for 4 hr. The reaction mixture was poured into water and 250 mg of the 4β-deuterodiol VI was filtered off with mp 185–187° C (from ethanol), D_1 = 100% (mass spectrometry), IR spectrum (paraffin oil; ν, cm^{-1}): 3480 (OH group), 3390 (OH group), 2175 (C—D bond); IR spectrum in chloroform (c 0.3; l = 0.5 cm); ν 2145 cm^{-1} (C—D bond).



NMR spectra of cholest-5-en-3-one (a) and of 4β-deuterocholest-5-en-3-one (b).

5α, 6β-Dibromoandrostane-3β, 17β-diol (XIV). A solution of 26 mg of anhydrous sodium acetate in 3.6 ml of glacial acetic acid and 0.12 ml of bromine were added to a solution of 750 mg of androst-5-ene-3β, 17β-diol (XI) in a mixture of 10 ml of absolute ether and 15 ml of absolute tetrahydrofuran. After 30 min, the reaction mixture was evaporated to half bulk, ether was added, and 850 mg of the dibromide XIV with mp 129–131° C (decomp.) was filtered off. IR spectrum: ν, cm^{-1}: 3280, 563. The dibromide XIV was used for oxidation without further purification.

5α, 6β-Dibromoandrostane-3, 17-dione (XIII). A solution of 0.3 g of sodium dichromate in 10 ml of acetic acid was added to a suspension of 200 mg of the dibromide XIV in 3 ml of acetic acid. The mixture was kept at 60° for 40 min, cooled to 20° C, and diluted with water. The precipitate that deposited was filtered off and washed with cold dilute ethanol (1:1). This gave 150 mg of the dibromoketone XIII. It proved to be a very unstable compound and it was therefore rapidly subjected to debromination.

4β-Deuteroandrost-5-ene-3, 17-dione (IX). A) Four drops of freshly-prepared Jones reagent was added to a solution of 75 mg of the 4β-deuterodiol VI in 15 ml of acetone at 0° C. The mixture was kept at the same temperature for 10 min and was then diluted with ether. After the usual working up, 52 mg of 4β-deuteroandrost-5-ene-3,17-dione (IX) was obtained with mp 159–160° C, D_1 = 100% (mass spectrometry), IR spectrum (paraffin oil); ν, cm^{-1}: 1720 (3-CO), 1750 (17-CO).

B) A solution of 150 mg of the dibromoketone XIII in 6 ml of absolute ether was treated with 0.11 ml of CH_3COOD and 0.11 ml of D_2O (a mixture of 0.09 ml of acetic anhydride and 0.12 ml of D_2O was boiled for 25–30 min), and then 0.1 g of activated zinc dust was added and the mixture was boiled for 1 hr. After cooling, a drop of pyridine was added to the reaction mixture, and the precipitate was filtered off and carefully washed with ether. The combined filtrates were treated in the usual way, giving 56 mg of 4β-deuteroandrost-5-ene-3, 17-dione (IX) with mp 158.5–159° C, D_0 = 50%, D_1 = 50% (mass spectrometry), IR spectrum (paraffin oil); ν, cm^{-1}: 1720, 1750.

4β-Deuterocholesterol (VII). The enol acetate of cholest-4-en-3-one (V) was obtained [yield 81%, mp 82–83° C (from ethanol)] in the same way as the enoldiacetate of testosterone.

The enol acetate V (0.2 g) was reduced with $NaBH_4$ (0.35 g) as described for 4β-deuteroandrost-5-ene-3β, 17β-diol (VI), giving 180 mg of 4β-deuterocholesterol (VII), with mp 128–130° C (from ethanol), IR spectrum, cm^{-1}: 3400, 2140.

When 135 mg of 4β-deuterocholesterol (VII) and 0.55 ml of acetic anhydride were boiled for 1 hr, subsequent working up yielded 97 mg of 4β-deuterocholesterol acetate with mp 113–115° C (from acetone).

4β-Deuterocholest-5-en-3-one (X). Cholesterol dibromide (XV) (3.8 g) obtained by Fieser's method [5] was oxidized with sodium dichromate in a similar manner to the dibromoketone XIII. This gave 3 g of a labile dibromide (XIIIa) which was rapidly debrominated by the action of zinc dust in deuteroacetic acid (CH_3COOD) [conditions identical with those for preparing the deuterodiketone IX]. From 3 g of the dibromide XIIIa was obtained 1 g of 4β-deuterocholest-5-en-3-one (X) with mp 125–126° C (from ethanol).

Conclusions

Simple methods for the stereospecific synthesis of 4β-deutero-Δ^5-3-ketosteroids have been developed involving the reduction of 3-acetoxy-$\Delta^{3,5}$-steroids with lithium aluminum hydride and subsequent decomposition with deuterium oxide and Jones oxidation and also the boiling of 5α, 6β-dibromo-3-ketosteroids with zinc dust in deuteroacetic acid.

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