STEROIDS

XLIV. SYNTHESIS AND D-HOMOISOMERIZATION OF 3β-ACETOXY-6α-(N-ACETYLANILINO)-17α-HYDROXYPREGN-5-EN-20-ONE

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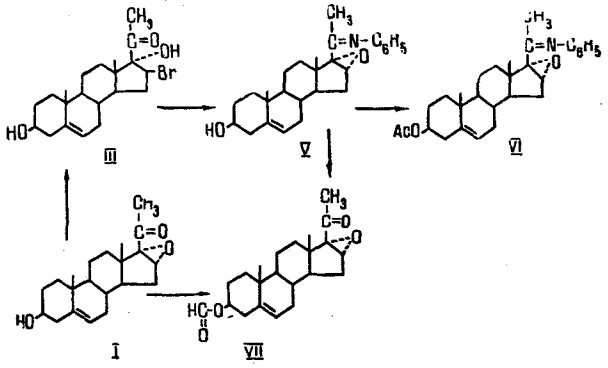
At the present time, an intensive search for biologically active compounds among the steroids by the chemical modification of the molecules of natural hormones is being carried on. In order to obtain 16-arylamino derivatives of the pregnanes, we have previously [1] studied the reaction of 16α,17α-epoxypregnanes (I) with primary aromatic amines (aniline, p-toluidine, and p-anisidine). However, it was found that the opening of the oxide ring in (I) is accompanied by an isomerization process and by the formation of the corresponding amino derivatives of D-homosteroids.

In view of this, we have studied the possibility of obtaining 16-anilino-17α-hydroxypregnenolone (II) by the reaction of 16β-bromo-3β,17α-dihydroxypregn-5-en-20-one (III) [2] and its 3,17-diacetate (IV) [3] with aniline. It was assumed that in such a reaction no D-homoisomerization would take place since the condensation was performed under relatively mild conditions.

When the reaction of substance (III) with aniline was performed a product was isolated the IR spectrum of which lacked the absorption band of the carbonyl group at C-20 and had an absorption band in the 1655 cm^{-1} region characteristic for a C = N bond.



On this basis, it could be assumed that the reaction of (III) with aniline forms not the derivative of 16-anilino-17α-hydroxypregnenolone (II), but the Schiff's base (V). The acetylation of (V) with acetic anhydride in pyridine at room temperature led to the acetate (VI). The IR spectrum of the latter had absorption bands in the 1735 cm^{-1} (ester C = O) and 1655 cm^{-1} (C = N bond) regions.

In an attempt to formylate (V) with 100% formic acid at 55-60° C, a compound was obtained the IR spectrum of which lacked the absorption band in the 1655 cm^{-1} region characteristic of a C = N bond and had absorption bands in the 1715 cm^{-1} (ester C = O) and the 1700 cm^{-1} (C = O at C – 20) regions.

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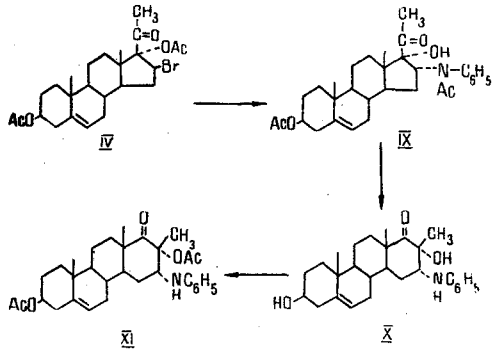
Thus, in the formylation of (V) the Schiff's base undergoes cleavage accompanied by the formylation of the hydroxy group in position 3 and the production of the formate of 16α,17α-epoxypregnenolone (VII). This compound was also obtained by the formylation of (I) with 100% formic acid.

Since attempts to obtain (II) by the reaction of (III) with aniline did not lead to the desired result, we studied the possibility of synthesizing (II) from the diacetate (IV) and aniline.

When (IV) was heated with aniline in toluene, compound (IX) was isolated. Its IR spectrum exhibited absorption bands in the 1655, 1700, 1730, and 3850 cm^{-1} regions that are characteristic for amide C = O, carbonyl C = O, ester C = O, and OH groups, respectively.



It is interesting to note that the replacement of bromine at C-16 by aniline is accompanied by a migration of the acetyl group from the oxygen at C-17 to the nitrogen at C-16. As is well known [3], such transacetylation is possible when the substituents at C-17 and C-16 are in the cis position.

The PMR spectrum exhibited, in addition to the signals of angular methyl groups and of the methyl groups of the 3- and N-acetyl groups, signals of the protons of a methyl group at C-21 in the 1.9-2.1 ppm region which is characteristic for 20-oxopregnanes.

The mass spectrum of compound (IX) (Fig. 1a) shows the successive elimination under electron impact of the functional groups in rings A and D:

$$M^+ (m/e\ 507) \xrightarrow{-AcOH} m/e\ 447 \xrightarrow{-PhNHAc} m/e\ 312 \xrightarrow{-H_2O} \rightarrow m/e\ 294 \xrightarrow{-Ac} a\ (m/e\ 251)$$

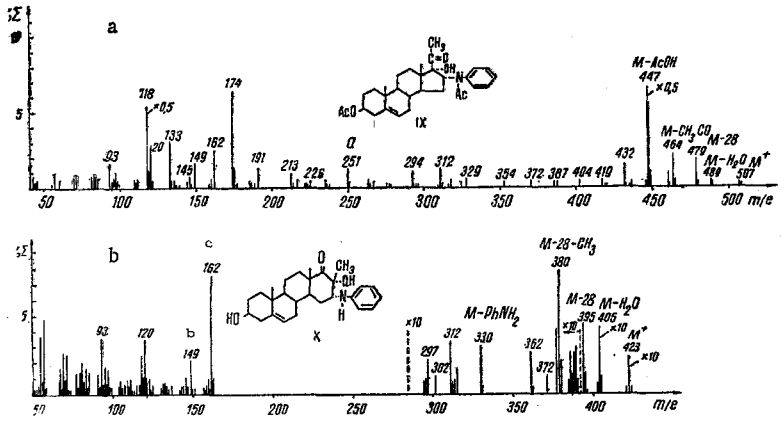
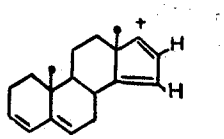


Fig. 1. Mass spectra of 3β-acetoxy-16α-(N-acetylanilino)-17α-hydroxypregn-5-en-20-one (IX) (a) and of 16α-anilino-3β,17α-dihydroxy-17β-methyl-D-homoandrosten-17a-one (X) (b).

with the formation in the final account of a tetracyclic "nuclear" fragment a (m/e 251):



a, m/e 251

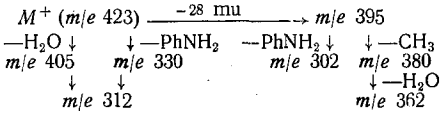
Another sequence of the ejection of functional groups can be traced, beginning with the ions $M-H_2O$ (m/e 489), M−PhNHAc (m/e 372), or $M-CH_3CO$ (m/e 464). However, in any case the final ion is the "nuclear" fragment a containing the five-membered ring D. A similar sequence of degradations has been observed [4] in the mass spectrum of 3β,16α-diacetoxy-17α-hydroxypregn-5-en-20-one, which differs from (IX) only by the nature of the substituent at C-16.

The IR, PMR, and mass spectra and the phenomenon of transacetylation show that compound (IX) is 3β-acetoxy-16α-(N-acetylanilino)-17α-hydroxypregn-5-en-20-one.

The hydrolysis of the diacetate (IX) with potassium carbonate in methanol gave as the main product compound (X), in the PMR spectrum of which there were no signals in the 1.9-2.1 ppm region corresponding to the protons in a methyl group at C-21 of 20-oxopregnanes [5]. At the same time, the spectra exhibited, in addition to the signals of the angular methyl groups, the signal of a methyl group in the 1.64-ppm region. This value of the chemical shift of the signal of a methyl group is characteristic for D-homosteroids [6]. On the basis of the PMR spectrum, it may be assumed that the hydrolysis product has the D-homosteroid structure (X).

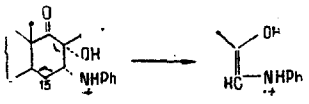
In the mass spectrum of (X) (Fig. 1b), the high-molecular-weight region has a low intensity, with the exception of the ion M-28.

The main degradations of (X) are connected with the expulsion of the particles H_2O, $C_6H_5NH_2$, or a CH_3 group from the molecular ion and from the M-28 ion.

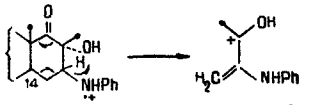


The fragments in the low-molecular-weight region (b, m/e 149, and c, m/e 162) are formed as the result of α cleavage ($C_{17}-C_{17a}$) and $C_{15}-C_{16}$ and $C_{14}-C_{15}$ cleavage, respectively, as shown below.

Both ions contain a C-17 atom with methyl and hydroxy groups and therefore the oxo group is in the C-17a position. Thus, in the hydrolysis of (IX), 16-anilino-3β,17-dihydroxy-17-methyl-D-homoandrost-5-en-17a-one (X) is formed.



b m/e 149



c, m/e 162

The acetylation of (X) with acetic anhydride in pyridine at room temperature led to the formation of a diacetate, the IR spectrum of which shows that only the hydroxy groups are acetylated.

As can be seen from the paper by Fukushima et al. [7], acetylation of a hydroxy group at C-17 under mild conditions takes place where the hydroxy group is in the equatorial (α) position. Consequently, it may be considered that in compound (X) the tertiary hydroxy group at C-17 has the equatorial (α) position and the methyl group the axial (β) position.

It is known [8] that the D-homoisomerization of 3β,16α,17α-trihydroxypregn-5-en-20-one takes place without a change in the configuration of the hydroxy group at C-16. It may be considered that the hydrolysis of (IX) forms the D-homo compound (X) containing a 16α-anilino group.

On the basis of what has been said above, we have constructed molecular models of (X) and (XI). It can be seen from a consideration of these models that the diacetate (XI) can be formed from (X) only if the anilino group at C-16 is in the axial (α) position and the hydroxy group at C-17 in the equatorial (α) position. Otherwise, as its model shows in the case of the 16β-anilino-17β-hydroxy derivative, acetylation should take place at the nitrogen atom and not at the 17β-hydroxy group, since a hydroxy group in the 17β position is strongly screened.

The facts presented confirm that the hydrolysis of the diacetate (IX) with potassium carbonate in aqueous methanol leads to the formation of 16α-anilino-3β,17α-dihydroxy-17β-methyl-D-homoandrost-5-en-17a-one (X), while together with (X) two as yet unidentified substances have been found.

EXPERIMENTAL

The mass spectra were obtained on an industrial MKh-1306 instrument fitted with a system for the direct introduction of the sample into the ion source at a temperature of 170° C with an ionizing voltage of 30 V; the PMR spectra were taken on a JNM-4H100 instrument in deuteropyridine, and the IR spectra on a UR-10 instrument in paraffin oil. The purities of the substances isolated were checked by thin-layer chromatography. The analyses of all the compounds corresponded to the calculated figures.

The 20-Anil of 16α,17α-Epoxypregnenolone (V). A solution of 4 g of the bromohydrin (III) and 3 ml of aniline in 20 ml of dry toluene was boiled with stirring in a current of nitrogen under reflux with a calcium chloride tube for 5 h. The reaction mixture was cooled to 20° C, the aniline hydrobromide was separated off, and the precipitate was washed with toluene. The toluene and the excess of aniline were driven off from the combined filtrate in vacuum. The residue was crystallized from absolute ethanol with the addition of carbon. This gave 3.1 g (78.6%) of (V), $C_{27}H_{35}NO_2$, mp 175-176.5° C, $[\alpha]_D^{20}$ + 30° (here and below, in 1% chloroform), ν 3400 cm^{-1} (OH), 1655 cm^{-1} (C = N), and 1600 cm^{-1} (C = C).

3-Acetate of the 20-Anil of 16α,17α-Epoxypregnenolone (IV). A solution of 1.5 g of (V) in a mixture of 15 ml of acetic anhydride and 30 ml of pyridine was left at room temperature for 24 h and was then diluted with water, and the precipitate was filtered off, washed with water, and dried. This gave 1.4 g (84.8%) of (VI), $C_{29}H_{37}NO_3$, mp 216.5-218° C (from absolute ethanol) $[\alpha]_D^{20}$ + 32°, ν 1730 cm^{-1} (ester CO), 1655 cm^{-1} (C = N), and 1600 cm^{-1} (C = C).

3-Formate of 16α,17α-Epoxypregnenolone (VII). A solution of 2 g of (V) in 30 ml of 100% formic acid was stirred in a current of nitrogen at 55-60° C in a flask fitted with a reflux condenser and a calcium chloride tube for 2.5 h. The excess of formic acid was distilled off in vacuum, and the residue was cooled and mixed with water. The precipitate was filtered off, washed with water, and dried. This gave 1.58 g (90%) of (VII), $C_{22}H_{30}O_4$, mp 166-167° C (from absolute ethanol), $[\alpha]_D^{20}$ − 19°, ν 1715 cm^{-1} (ester CO) and 1700 cm^{-1} (carbonyl CO).

3-Acetoxy-16α-(N-acetylanilino)-17α-hydroxypregn-5-en-20-one (IX). A solution of 7.5 g of the diacetate (IV) and 5 ml of aniline in 100 ml of dry toluene was boiled with stirring in a current of nitrogen in a flask fitted with a reflux condenser and calcium chloride tube for 6 h. Then it was cooled to 20° C, the aniline hydrobromide was filtered off, and the residue was washed with toluene. The toluene and the excess of aniline were distilled off from the combined filtrate in vacuum. The residue was crystallized from absolute ethanol with the addition of carbon. This gave 5 g (76.8%) of (IX), $C_{31}H_{41}NO_5$, mp 218-219.5° C, $[\alpha]_D^{20}$ − 90°, ν 3580 cm^{-1} (OH), 1730 cm^{-1} (ester CO), 1700 cm^{-1} (carbonyl CO), 1655 cm^{-1} (amide), and 1600 cm^{-1} (C = C).

16α-Anilino-3β,17α-dihydroxy-17β-methyl-D-homoandrost-5-en-17a-one (X). A solution of 3 g of potassium carbonate in 20 ml of water was added to a suspension of 1.8 g of (IX) in 100 ml of methanol, and the mixture was boiled with stirring under reflux for 2 h. The methanol was evaporated off in vacuum, and the precipitate was filtered off, washed with water, and dried. This gave 0.95 g (63.3%) of (X), $C_{27}H_{37}NO_3$, mp 245-246° C (from methanol), $[\alpha]_D^{20}$ − 61°, ν (cm^{-1}) 3540, 3510 (OH), 3380 (NH), 1705 (C = C) and 1600 (C = C).

3β,17α-Diacetoxy-16α-anilino-17β-methyl-D-homoandrost-5-en-17a-one (XI). A solution of 1.5 g of (X) and 15 ml of acetic anhydride in 30 ml of pyridine was left at room temperature for 24 h, and it was then diluted with water and the precipitate was filtered off, washed with water, and dried. This gave 1.45 g (81%) of (XI), $C_{31}H_{41}NO_5$, mp 260-262° C (from absolute ethanol), $[\alpha]_D^{20} - 68°$, ν (cm^{-1}) 3410 (NH), 1735 (ester CO), 1705 (ketone CO), and 1605 (C = O).

SUMMARY

1. The reactions of 16β-bromo-3β,17α-dihydroxypregn-5-en-20-dione (III) and its 3,17-diacetate (IV) with aniline have been studied. The reaction of (III) with aniline leads to the formation of the 20-anil of 16α,17α-epoxypregnenolone (V) and the reaction of (IV) with aniline to the formation of 3-acetoxy-16α-(N-acetylanilino)-17α-hydroxypregn-5-en-20-one (IX).

2. The hydrolysis of the diacetate (IX) with potassium carbonate in aqueous methanol has been studied. The hydrolysis is accompanied by D-homoisomerization with the formation of 16α-anilino-3β,17α-dihydroxy-17β-methyl-D-homoandrost-5-en-17a-one.

3. The structures and configurations of the compounds obtained (V, VI, VII, IX, X, and XI) have been shown by a series of chemical transformations and by physicochemical methods.

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