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CATALYTIC HYDROGENATION OF DEHYDROEPIANDROSTERONE

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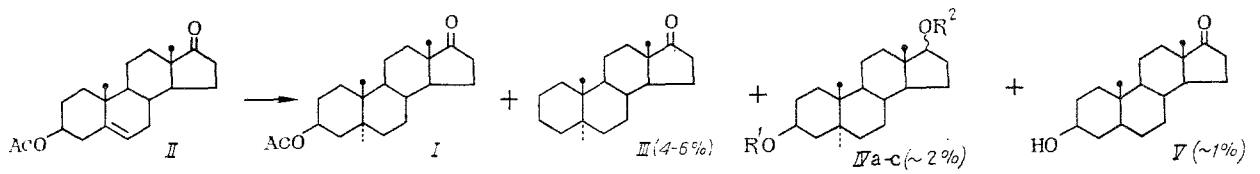
UDC 615.357.453.012.1.002.62

3β-Acetoxy-5α-androstan-17-one (I) (epiandrosterone acetate) may be used as starting material for the synthesis of various therapeutic preparations of the 5α-androstane series such as pancuronium bromide [1], testololactone [2], and 2α-methyldihydrotestosterone [3].

We have developed a practically convenient method of obtaining I from the industrially available dehydroepiandrosterone acetate (II) by the catalytic hydrogenation of the double bond in the 5,6 position in the presence of 5% Pd/C with an 80% yield of recrystallized product. Hydrogenation was carried out in an autoclave at a pressure of 50 atm and a temperature of 50° in a medium of ethyl, methyl, and technical isopropyl alcohols. The previously described methods for the catalytic hydrogenation of I specify the use of a large quantity of catalyst with a higher palladium content (2 g $PdCl_2$ on 8 g carbon [2], 10% Pd/C [4]). Technical I obtained after hydrogenation contained 7-9% total contaminants of the 5α-androstane series (determined by quantitative thin-layer chromatography).

Under the proposed conditions of hydrogenation of II practically no compounds containing a double bond in the 5,6 position remained. This was checked by the absence of signals in the 5.3 ppm region, characteristic of vinylic protons at C-6, and the semiquantitative color reaction with concentrated sulfuric acid. This fact is very important since it is difficult to detect and determine chromatographically compounds of the 5α series in the presence of their unsaturated analogs.



a) R^1=Ac, R^2=H 17(configuration unknown)
b) R^1=R^2=Ac 17β
c) R^1=R^2=H 17β

The main contaminant in the process of hydrogenating (II) was 5α-androstan-17-one (III), the product of hydrogenolysis of the 3β-acetoxy group. Its structure was confirmed by data of elemental analysis, by the presence in the IR spectrum of only one band at 1750 cm^{-1}, the absence of a band at 1250 cm^{-1}, and the fact that there were no signals in the PMR spectrum connected with the acetoxy group in position 3. The content of III in the reaction mass depended on the temperature of hydrogenation; the amount of II rose with increasing tempera-

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TABLE 1. PMR Spectra of Compounds (I, III, IVa-c) (δ, ppm)

Compound	CH_3		OAc	H_{C_3}		$H_{C_{17}}$		OH
	C_{19}	C_{18}		OH	OAc	OH	OAc	
I	—	0,81	1,97	—	4,62	—	—	—
III	0,75	0,8	—	—	—	—	—	
IVa	0,66	0,78	1,95	—	4,62	3,58	—	
IVb	0,72	0,80	2,0 (doubling)	—	4,57	—	4,57	—
IVc	0,7	0,83	—	3,79	—	3,79	—	5,8

ture. Recrystallization of technical I from hexane, pentane, or petroleum ether permitted a reduction in the content of III in I to 1-2%.

When hydrogenating II a sterically nonspecific reduction of the carbonyl group in position 17 also occurred with the formation of a mixture of isomeric 3β-acetoxy-5α-androstan-17-ols (IVa) (amount 2%) with one predominating. The structure of IVa was confirmed by data of elemental analysis, IR, and PMR spectra (see Table 1). The chromatographic heterogeneity of IVa (Rf 0.66, 0.71) and its low melting point indicated the presence of a mixture of two stereoisomers. However, there was no doubling of signals in the PMR spectrum from which the content of each isomer might have been determined.

On acetylation of IVa with acetic anhydride in pyridine a mixture of 3β,17ε-diacetoxy-5α-androstanes (IVb) with melting point 122-124° (R_f 0.83) was also obtained after recrystallization which made it possible to confirm the predominance in the mixture of the 17β isomer the melting point of which is 127-128° [5]. The PMR spectrum of IVb also had no doubling of signals. Only after hydrolyzing IVb with 5% potassium hydroxide solution in methanol and subsequently crystallizing it was an individual isomer successfully isolated. This was 5α-androstan-3β,17β-diol (IVc) with melting point identical with the melting point of the 17β isomer in [5], and its acetylation led to 3β,17β-diacetoxy-5α-androstane mp 126-127°. Thus the 17β-equatorial isomer is the predominant isomer on catalytic hydrogenation of the carbonyl group in the presence of 5% Pd/C.

In the process of catalytic hydrogenation of II we also observed the hydrolysis of the 3-acetyl group with the formation of 3β-hydroxy-5α-androstan-17-one (V) to 1%. Hydrolysis probably proceeded under the action of the small amount of acetic acid formed as a result of the hydrogenolysis of I (the content of acid in alcohol after hydrogenation was 0.02%).

Thus on catalytic hydrogenation of I in the presence of 5% Pd/C catalyst the following side reactions occurred in addition to the main process of reduction of the isolated double bond in the 5,6 positions: a hydrogenolysis reaction of the 3-acetoxy group, a sterically nonspecific reduction of the 17-keto group to a hydroxyl with a preponderance of the quasi-equatorial isomer, and hydrolysis of the acetyl group.

EXPERIMENTAL

IR spectra were taken on a Perkin-Elmer instrument in Nujol. PMR spectra were taken on a JNM-4H-100 instrument with tetramethylsilane in deuterochloroform unless specified otherwise. Chromatography was carried out on Silufol thin layers in the system cyclohexane–acetone (2:1) with visualization with 1% vanillin solution in 10% perchloric acid at 100°. Preparative chromatography was carried out on type L40/100 silica gel (Chemapol).

The authors are grateful to H. M. Ivanova for determining the content of contaminants by thin-layer chromatography (TLC) and to T. Ya. Filipenko for taking the PMR spectra.

Hydrogenation of 3β-Acetoxyandrost-5-en-17-one (II). A solution of II (70 g: 0.212 mole) in ethyl alcohol (140 ml) was hydrogenated in an autoclave with 5% Pd/C (14 g) at 50 atm and 50° for 4 h. Absorption of hydrogen had finished after 2-3 h. The cooled solution was filtered from catalyst, evaporated to dryness in vacuum, and the residue was recrystallized from hexane with 1:3 ratio of carbon. Compound I (53.7 g: 76% was obtained having mp 111-113°, $[\alpha]_D^{20}$ = +65° (concn. 1.0, chloroform). Literature data [4]: 116-117°, 104-105°, $[\alpha]_D^{20}$ = +65° (concn. 1.0, chloroform). Further I (2.95 g) was isolated from the mother liquor giving a total yield of 80.5%, R_f = 0.76. The total content of contaminants III-V

was 2-3% (determined by TLC), and of II traces (test for unsaturated compounds with concentrated sulfuric acid).

The residue after evaporation of the hexane mother liquor was chromatographed on a column of silica gel. By taking many fractions the following were isolated; compound III (0.3 g) mp 115-117° (from hexane). Literature data [6]: 121-122°, R_f = 0.84; compound IVa (0.25 g) mp 96-100° (from 80% methanol), R_f = 0.66, 0.71, IR spectrum: 3400, 1725 cm^{-1}. Found, %: C 74.81; H 10.47. $C_{21}H_{34}O_3$. Calculated, %: C 75.40; H 10.24. Further, compound V (0.1 g) was obtained of mp 172-174° (from methanol), R_f = 0.61. A mixing test with an authentic specimen revealed no depression.

3β-17β-Diacetoxy-5α-androstane (IVb). a. Compound IVa (0.2 g) was acetylated with acetic anhydride (0.2 ml) in pyridine (1 ml) at room temperature for 20 h. The mixture was poured into water, the solid filtered off, and recrystallized from hexane. Compound IVb (0.16 g) was obtained of mp 122-124°. IR spectrum: 1730 cm^{-1}. R_f = 0.83. Found, %: C 73.23; H 9.34. $C_{23}H_{36}O_4$. Calculated, %: C 73.36; H 9.63.

b. Compound IVc (0.05 g) was acetylated as described above. After crystallization from hexane compound IVb (0.02 g) was obtained and had mp 127-128°.

3β-17β-Dihydroxy-5α-androstane (IVc). Compound IVb (0.13 g) was boiled in 5% potassium hydroxide in methanol solution (3 ml), the solution was neutralized with acetic acid, poured into water, the solid filtered off, and recrystallized from aqueous methanol. Compound IVc (0.08 g) was obtained and had mp 162-163°. Literature data [5]: mp 163-164°, R_f = 0.58. IR spectrum: 3300, 3380, and 3480 cm^{-1}.

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COMPARATIVE STUDY OF THE BIOSYNTHESIS OF OXYTETRACYCLINE AND LINCOMYCIN IN FLASKS AND FERMENTERS

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UDC 615.332(OXYTETRACYCLINUM + LINCOMYCINUM,012.6)

It is known that when the biosynthesis of antibiotics is performed in apparatuses of different capacities and designs considerable differences are observed in the dynamics of the process and in the level of antibiotic formation. This appears particularly clearly on passing from flasks to fermenters [1-3]. The problem is of fundamental importance in connection with the limited possibilities of experimentation in fermenters and the presence, as a rule, of the stage of studying the process in the flasks on a shaking machine. Because flasks and fermenters are difficult to compare with respect to conditions for ensuring the biosynthetic process, especially the supply of oxygen, concrete processes must be studied in this apparatus.

All-Union Scientific-Research Institute of Antibiotics, Moscow. Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 10, No. 12, pp. 91-95, December, 1976. Original article submitted June 1, 1976.