SYNTHESIS OF 5α-PREGNANE-3β,20α-DIOL AND OF 5α-PREGNANE-3α,20α-DIOL LABELLED SPECIFICALLY AT THE 20β-POSITION

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SUMMARY

A convenient synthesis of 5α-[20β-^{2}H]pregnane-3α,20α-diol and 5α-[20β-^{2}H]pregnane-3β,20α-diol from 3β-hydroxy-5α-pregn-16-en-20-one is described. The reaction products are characterized by combined gas chromatography-mass spectrometry.

INTRODUCTION

Pregnane-3,20α-diols are encountered in various biological fluids where they arise from the reductive metabolism of progesterone. In connection with studies on the biological role of such compounds, pregnanediols containing a 20α-hydroxyl-[20β-^{2}H]function in high isotopic and epimeric purity were required.

Reagents commonly used for the reduction of 20-ketosteroids to the corresponding alcohols e.g. complex metal hydrides, sodium and alcohol, and hydrogenation over nickel catalyst, generally afford a mixture of 20α and 20β epimers (3,4). Although sodium borohydride reductions of 20-ketones give mainly the more hindered

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20β-alcohol, the ratio of 20α to 20β epimers obtained from reductions with lithium aluminium hydride has been shown to be influenced by the nature of neighbouring functional groups. Thus, in the presence of a Δ^{16} double-bond, reduction of the 20-ketone yields mainly the corresponding 20α-alcohol (5), which can be hydrogenated to the corresponding 20α-hydroxy-17β-pregnane. The purpose of this investigation was to determine whether this sequence of reactions could be applied to the preparation of 20α-hydroxy-[20β-^{2}H]pregnanes in high isotopic purity.

MATERIALS AND METHODS

Reference samples of 5α-pregnane-3β,20α-diol and 5α-pregnane-3α,20α-diol were purchased from Ikapharm (Ramat-Gan, Israel). Diethylazodicarboxylate was obtained from Aldrich-Europe (Beerse, Belgium), and lithium aluminium deuteride (≥ 90% $^2H_4$) from Fluka AG (Buchs, Switzerland). Lipidex^R lipophilic dextran gels with properties corresponding to those of the gels described by Ellingboe et al. (6) were obtained from Packard Instrument Co., Inc. (Downers Grove, Ill., USA). Tetrahydrofuran was distilled from lithium aluminium hydride immediately before use.

Thin-layer chromatography (TLC) was carried out on glass plates (5 x 20 cm), precoated with 0.25 mm layers of silica gel (Merck AG, Darmstadt, Germany), using chloroform as mobile phase.

Gas-liquid chromatography (GLC) was carried out with a Pye Series 104 gas chromatograph, equipped with flame ionization detectors. Columns (3 m x 3 mm i.d.), packed with 1.5% SE-30 on Chromosorb W HP were used with nitrogen as carrier gas. Column temperature was 240°. Steroids were analysed as trimethylsilyl (TMS) ethers (7), and where appropriate, as O-methyloxime trimethylsilyl (MO-TMS) derivatives (8). Retention times were measured relative

to that of 5α-cholestane.

Gas chromatography-mass spectrometry (GC-MS) was performed on an LKB 9000 instrument, also equipped with a 1.5% SE-30 column. Analyses were carried out with an oven temperature of 210°, while the molecular separator and ion source were held at 250° and 290°, respectively. The energy of the bombarding electrons was 22.5 eV and the ionizing current was 60 μamp. Multiple spectra were recorded on magnetic tape and average isotope content was determined by comparison with the unlabelled compounds using an IBM 1800 computer (9).

EXPERIMENTAL

5α-[20ξ-^{2}H]Pregn-16-ene-3β,20ξ-diol (II) (5)

3β-Hydroxy-5α-pregn-16-en-20-one (I; 100 mg) in dry tetrahydrofuran (5 ml) was added dropwise to a stirred suspension of lithium aluminium deuteride (364 mg) in dry tetrahydrofuran (5 ml). Addition was complete in 20 min. The resulting mixture was stirred at room temperature, under exclusion of moisture, for 40 min, and then heated under reflux for 3 h. The product was cooled to 0° and treated with acetone (5 ml), followed by saturated ammonium chloride solution (10 ml). After centrifugation, the supernatant was decanted, and the residue treated with water (15 ml). The aqueous phase was extracted with methylene chloride (3 x 10 ml), and the combined organic phases were washed with water (10 ml), dried and evaporated *in vacuo* to give the product as a colourless oil. TLC and GLC analysis indicated complete reduction of the starting material. The mixture of epimers obtained was not separated, but used directly for the next step.

5α-[20β-^{2}H]Pregnane-3β,20α-diol (III)

The mixture of pregnenediols (II; 35 mg) was dissolved in ethanol (5 ml) and hydrogenated over 10% Pd/C (11 mg) during 1 h

at room temperature. The product was filtered and evaporated to give an oil (yield = 29 mg, 83%).

The mixture of pregnanediols was applied to an 8 g Lipidex^{R}-1000 column, prepared in methanol/water/chloroform, 60:40:10 (by vol.) (6). The column was eluted with the same solvent and 2 ml fractions were collected. Pure 5α-[20β-^{2}H]pregnane-3β,20α-diol (III; 6 mg) was obtained in fractions 83-91, after which elution of the 20β-epimer (IV) commenced.

Compound III so obtained had the same TLC and GLC properties as those of authentic (unlabelled) 5α-pregnane-3β,20α-diol.

3α-Hydroxy-5α-pregn-16-en-20-one (V) (10)

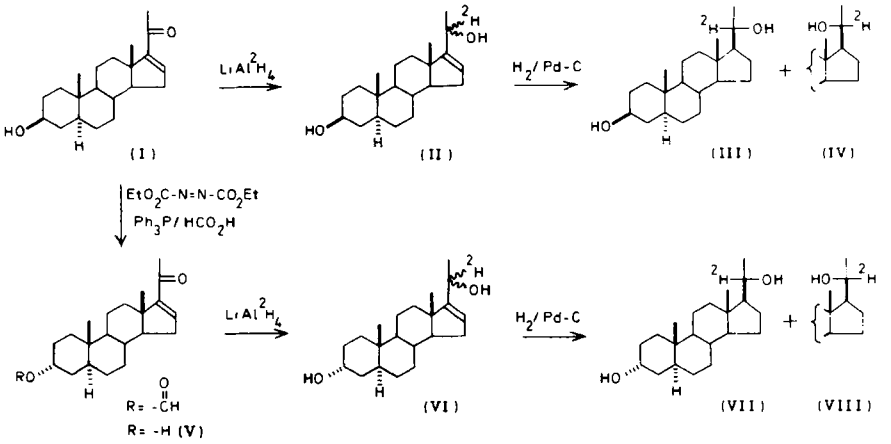
To a stirred solution of 3β-hydroxy-5α-pregn-16-en-20-one (I; 40 mg), triphenylphosphine (135 mg) and two drops (28 mg) formic acid in dry tetrahydrofuran (3 ml) was added a second solution containing diethylazodicarboxylate (102 mg) in dry tetrahydrofuran (2 ml). The addition, which took place over a period of 1 h, was carried out under anhydrous conditions. Stirring was continued at room temperature overnight (18 h), after which the reaction mixture was evaporated. The residue was taken up in ether, washed successively with 2 x 10 ml portions of 2 N aq. KOH, 2 N HCl and water, dried and evaporated. The product was treated with a mixture of hexane and 10% aq. methanol (1:1, v/v), and the hexane phase evaporated to afford 3α-hydroxy-5α-pregn-16-en-20-one formate as an oil.

Saponification of this ester was carried out using KOH (170 mg) in 15% aq. t-butanol (3.8 ml), during 30 min. at 55°. The hydrolysis product was purified by chromatography on Lipidex^{R}-5000 (12 g column, solvent system: hexane/chloroform, 8:2, v/v). Fractions of 5 ml were collected, and 3α-hydroxy-5α-pregn-16-en-20-one (V; 9 mg) obtained in fractions 10 and 11.

5α-[20β-^{2}H]Pregnane-3α,20α-diol (VII)

This compound was obtained in the same manner as III, as shown in the Scheme.

SCHEME



RESULTS AND DISCUSSION

The reaction sequence is shown in the Scheme, while gas chromatographic and mass spectrometric data are summarized in Table 1.

Reduction of 3β-hydroxy-5α-pregn-16-en-20-one (I) with lithium aluminium deuteride afforded a mixture of the corresponding pregnenediols (II), epimeric at C-20. The TMS ethers of these isomers did not separate on GLC, but were eluted from the column as a single, unresolved peak. Their mass spectrum is illustrated in Fig. 1. Incorporation of a single deuterium atom at C-20 is indicated by the ion at m/e 118, which corresponds to the fragment at m/e 117 in the mass spectra of unlabelled 20-trimethylsilyloxy pregnanes

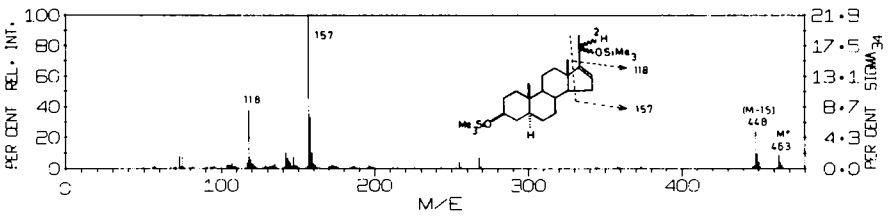
(11). The base peak at m/e 157 arises from cleavage through ring D, as shown.

Table 1. Gas Chromatographic and Mass Spectrometric Data

Compound		t_R*	M/e values and relative abundances of characteristic ions							^2H excess
			$M^{+\cdot}$	Fragment ions						atoms%
I	TMS	0.75	388 (68)	373 (54)	345 (50)	298 (7)	283 (53)	255 (100)		-
	MO-TMS	0.94	417 (53)	402 (76)	386 (100)	312 (37)	296 (26)	255 (29)		-
II	TMS	0.88	463 (9)	448 (24)	358 (1)	268 (8)	255 (4)	157 (100)	118 (38)	not determ.
III	TMS	1.11	465 (0)	450 (0.5)	360 (0.3)	347 (0.6)	285 (0.3)	270 (1.4)	118 (100)	99.4
IV	TMS	1.05	465 (0)	450 (0.5)	360 (0.1)	347 (0.5)	285 (0.4)	270 (1.1)	118 (100)	96.8
V	TMS	0.59	388 (43)	373 (12)	345 (24)	298 (19)	283 (48)	255 (100)		-
	MO-TMS	0.76	417 (52)	402 (60)	386 (100)	312 (75)	296 (51)	255 (42)		-
VI	TMS	0.70	463 (9)	448 (22)	358 (3)	268 (8)	255 (6)	157 (100)	118 (35)	not determ.
VII	TMS	0.91	465 (0)	450 (0.1)	360 (0.4)	346 (1)	285 (0.6)	270 (3)	118 (100)	96.4
VIII	TMS	0.88	465 (0)	450 (0.1)	360 (0.2)	346 (1)	285 (0.5)	270 (2)	118 (100)	97.6

* t_R of 5α-cholestane = 1.00

Fig. 1. Mass spectrum of 5α-[20ξ-^{2}H]pregn-16-ene-3β,20ξ-diol trimethylsilyl ether.



Catalytic hydrogenation of the mixture of pregnenediols gave the corresponding labelled pregnanediols, where the ratio of 20α- to 20β-hydroxy epimer was about 4:1 as judged by GLC. Mass spectrometry again confirmed that deuterium had been incorporated in the desired manner (base peak at m/e 118), while the deuterium content (> 95% in each isomer) showed that negligible scrambling of label had taken place during hydrogenation of the allylic double bond.

Finally, separation of the pregnanediol isomers was achieved by reversed-phase chromatography on Lipidex^R-1000 (6), when 5α-[20β-^{2}H]pregnane-3β,20α-diol (III) was eluted before its 20β-hydroxy-[20α-^{2}H]epimer (IV).

3α-Hydroxy-5α-pregn-16-en-20-one (V), obtained from compound (I) by the method of Bose *et al.* (10), was subjected to the same sequence of reactions as described above. Chromatography of the product yielded 5α-[20β-^{2}H]pregnane-3α,20α-diol (VII) in the expected manner.

Thus, lithium aluminium deuteride reduction of Δ^{16}-20-ketones, followed by hydrogenation over Pd/C and reversed-phase chromatography on Lipidex^R-1000 affords a practical method for the preparation

of 20α-hydroxy-[20β-^{2}H]pregnanes in high isotopic and epimeric purity.

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