STEROIDS. PART III[1]

Synthesis of 16α-carbomethoxy (carboxy-c^{14}) progesterone

Data on the Mechanism of Alkaline Hydrolysis of 3β,20β-dihydroxy-16α-cyano-Δ_4-pregnene

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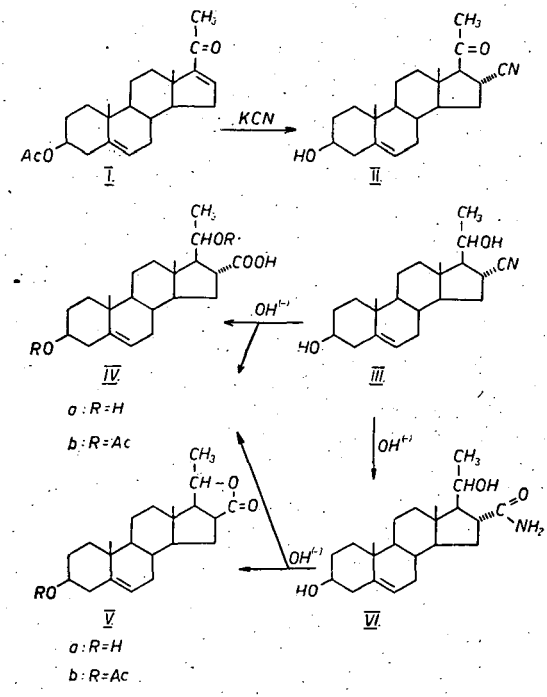
3β-hydroxy-16α-cyano-C^{14}-Δ_5-pregnene-20-one was prepared from 3β-acetoxy-$\Delta_{5,16}$-pregnadiene-20-one with $KC^{14}N$. The former was reduced with $NaBH_4$ to 3β,20β-dihydroxy-16α-cyano-C^{14}-Δ_5-pregnene and subjected to alkaline hydrolysis. By this 3β,20β-dihydroxy-16β-carboxy-C^{14}-Δ_5-pregnene-16,20-lactone was obtained together with 3β,20β-dihydroxy-16α-carboxy-C^{14}-Δ_5-pregnene. Esterification and oxidation yielded 16α-carbometoxy-(carboxy-C^{14})-progesterone The alkaline hydrolysis of 3β,20β-dihydroxy-16α-cyano-C^{14}-Δ_5-pregnene and 3β,20β-dihydroxy-Δ_5-pregnene-16α-carboxamide-(carboxy-C^{14}) were also studied, checking the ratio of the formed C_{16} isomeric acids.

In an earlier part of this series [1] we dealt in details with the mechanism of alkaline hydrolysis of 3β,20β-dihydroxy-16α-cyano-Δ_5-pregnene-20-one, obtained by treatment with KCN of 3β-acetoxy-$\Delta_{5,16}$-pregnadiene-20-one and subsequent $NaBH_4$ reduction. As it is known, the alkaline hydrolysis of 3β,20β-dihydroxy16α-cyano-Δ_5-pregnene leads to 3β,20β-dihydroxy-16α-carboxy-Δ_5-pregnene (IVa) and as a result of the epimerisation on C_{16}, 3β,20β-dihydroxy-16β-carboxy-Δ_5 pregnene-16,20-lactone (Va) was obtained. These two compounds were acetylated and the resulted diacetate (IVb) and monoacetate (Vb) were separated by chromatography.

The stereochemical changes which take place during the alkaline hydrolysis of the 16α-cyano group have been dealt with by several papers [1—8], suggesting different mechanisms. There are three possibilities of the epimerization of C_{16}:



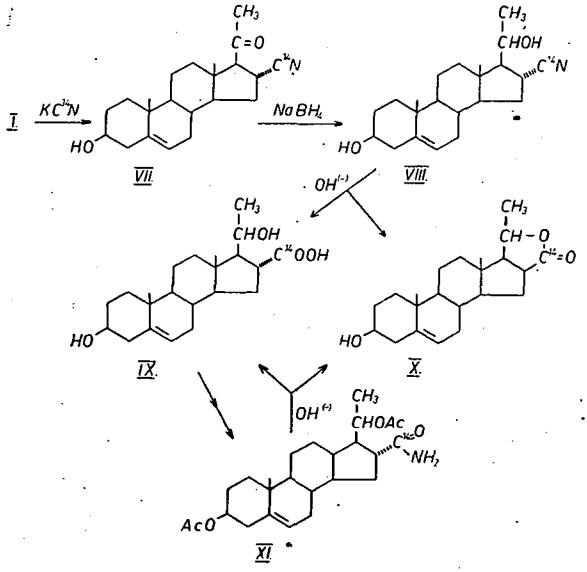
[1] Part I Acta Chim. Hung. (1965), in press.

a) Epimerization of the 16α-cyano-compound (III) may take place;

b) Isomerization of the acid amide formed during the alkaline hydrolysis of (III) nitrile;

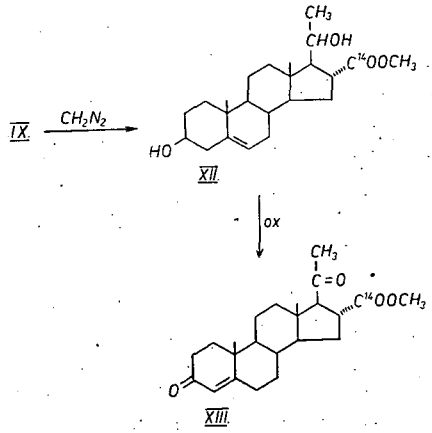
c) Interconversion of the epimeric-16-carboxylic acid salts also may take place.

There are several examples for the alkaline isomerization of these derivatives. Alkaline racemizations of cyano compounds have been published [19—24] and CRABBÉ and ROMO [13a, 13b] could separate the 16β-cyano isomer from the reaction mixture of a mild alkaline hydrolysis of 3β, 20β-dihydroxy-16α-cyano-Δ_5-pregnene (III). Earlier we succeeded [1, 26a, 26b] in isolating an acid amide (VI) from a similar alkaline reaction, the structure of which has already been proved synthetically in this laboratory. Acid amides are known to undergo racemization and epimerization, respectively [22, 23, 26] and 3β,20β-dihydroxy-16α-carboxamido-Δ_5-pregnene (IV) gives on alkaline hydrolysis 3β,20β-dihydroxy-16α-carboxy-Δ_5-pregnene (IVa) and 3β,20β-dihydroxy-16β-carboxy-Δ_5-pregnene-16,20-lactone (Va) [1]. In the course of complete alkaline hydrolysis of the 16-cyano group stereochemical changes take place in the stage containing nitrogen, that is in nitrile and acid amide states, resp., for the possible IV ⇌ V transition was excluded experimentally under the conditions applied by us [1]. The absence of the latter equilibrium was also proved by checking the hydrolysis and the proportions of the isolated end-products. The experiments show that hydrolysis was completed in 9 hours instead of 72 hours reported originally [2] while the ratios of compounds (IV) and (V) remained constant during the reaction. To get evidence that the ratio of compounds (IV) and (V) remained unchanged from the very beginning of the reaction when the concentrations are too small, the study of the hydrolysis of C^{14}-labeled nitril (III) was also carried out by activity measurements. The synthesis of labeled nitril and the hydrolysis of nitril essentially were carried out as described in [1]. 3β-acetoxy-$\Delta_{5,16}$-pregnadiene-20-one was treated with $KC^{14}N$, then the obtained labeled 16α-cyano compound (VII) was reduced with $NaBH_4$ to the labeled cyandiol. In the course of alkaline hydrolysis of the latter compound samples were taken out time-totime and run on thin-layer plates. The separated acid (IX) and lactone (X) were eluated and activities measured (Table I). 3β, 20β-diacetoxy-16α-carboxamido-C^{14}-Δ_5-pregnene (XI) was prepared from 3β,20β-dihydroxy-16α-carboxy-C^{14}-Δ_5-pregnene (IX) by acetylation and subsequent treating with thionyl chloride and ammonia. Similarly as above hydrolysis of acid amide (XI) wascarried out and the (IX:X) artio determined by measuring the activity of the products (Table II).



These measurements show that in the alkaline hydrolysis both of (VIII) and (XI) the IX:X ratio *i. e.* the originally examined IVa:Va ratio does not change during the hydrolysis, thus we suppose that in the alkaline hydrolysis of 3β, 20β-dihydroxy-16α-cyano-Δ_5-pregnene isomerization of the C_{16}-function takes place either in nitril or in acid amide phase, perhaps in both. The secondary stereochemical transformation of the carboxyl group in 16α or 16β position can be excluded.

We prepared 3β,20β-dihydroxy-16α-carboxymetoxy-carboxy-C^{14}-(-Δ_5-pregnene (XII)). Oxidation of this ester (XII) leads to 16α-carbomethoxy-(carboxy-C^{14})-progesterone (XIII). Biological studies of this compound are planned.



Experimental[2]

3β-hydroxy-16α-cyano-C^{14}-Δ_5-pregnene-20-one (VII)

500 mg of 3β-acetoxy-$\Delta_{5,16}$-pregnadiene-20-one (I) was dissolved in 10 ml of methanol, 1 ml of ethyl acetate and 1 ml of water and then 500 mg of KCN containing $KC^{14}N$ were added. The solution was kept boiling in a vapour-bath for two and half hours. After cooling it was poured in 50 ml of water, filtered and washed with 3×10 ml water and dried. 370 mg of 3β-hydroxy-16α-cyano-C^{14}-Δ_5-pregnene-20-one (VII) was obtained. Mp.: 231—235°; $(\alpha)_D^{20}$: +16°; (c: 0,5, chloroform). Analysis: Calcd.: $C_{22}H_{31}NO_2$ (341,47) C 77,38 H 9,15 Found: C 77,35 H 9,09%. Spec. activity: 9595 cpm/mg.

3β,20β-dihydroxy-16α-cyano-C^{14}-Δ_5-pregnene (VIII)

340 mg of 3β-*hydroxy*-16α-cyano-C^{14}-Δ_5-pregnene-20-one (VII) was dissolvedin 6 ml of boiling methanol and 20 mg of $NaBH_4$ dissolved in water was added. The solution was boiled for half an hour and the solvent decanted from the separated syrup. After cooling 0,1 ml of glacial acetic acid was added and eva-

[2] Measurements of activity were carried out with 1000 decadic scaler (Orion EMG 1872) by a (Gamma) scintillation crystal.

porated under reduced pressure. The crystalline mixture obtained was transferred on to a filter with 5 ml of water, washed and dried. It was recrystallized from methanol. 278 mg of 3β,20β-dihydroxy-16α-cyano-C^{14}-Δ_5-pregnene (VIII) was obtained. Mp.: 231—233°; $(\alpha)^{D}_{20}$: −74°; (c: 0,5, dioxane). Anal.: Calcd. $C_{22}H_{33}NO_2$ (343,49) C 76,92 H 9,68 Found: C 76,82 H 9,59%. Spec. activity 8731 cpm/mg.

Alkaline hydrolysis of 3β,20β-dihydroxy-16α-cyano-C^{14}-Δ_5-pregnene (VIII).

200 mg of 3β,20β-dihydroxy-16α-cyano-C^{14}-Δ_5-pregnene (VIII) was dissolved in 5 ml of ethanol and 1 g of KOH dissolved in 2,5 ml of water was added. The solution was boiled and sampleswere taken time-by-time. The samples were treated with diluted HCl (3 volumes) then extracted with double volume ethyl acetate and the extract washed with water, dried (Na_2SO_4 sicc.) and evaporated. The residue was divided into two thin-layer plates, chromatographed (non-bonded alumina, neutral, III.: layer thickness: 0,5 mm, solvent system: benzene: ethyl acetate 9:1). Parts of the adsorbent were separated in the area corresponding to Rf:0,00 and Rf: 0,15, respectively, extracted with hot ethyl acetate and evaporated. The activities of the residues are tabulated in Table I.

Hour	Activity (cpm)		IX:X ratio
	16α-isomer (IX)	16β-isomer (X)	
1/2	2420	1792	1,35
1	4552	3422	1,33
2	6618	4938	1,34
3	7602	5759	1,32
7	9439	6940	1,36
10	10020	7367	1,36
12	9870	7421	1,33
15	9912	7342	1,35
30	9895	7222	1,37
50	9770	7291	1,34

3β,20β-dihydroxy-16β-carboxy-C^{14}-Δ_5-pregnene-3,20-lactone (I)

400 mg of 3β,20β-dihydroxy-16α-cyano-C^{14}-Δ_5-pregnene (VIII) was dissolved in 10 ml of ethanol, 2 g KOH dissolved in 5 ml of water was added. The solution was boiled for 12 hours, after cooling the solution was treated with diluted hydrochloric acid (1:1). The separated amorphous solid was filtered, washed with water, dried and chromatographed on Al_2O_3 (neutr. III) with ethyl acetate. The residue, obtained after removal of the solvent was recrystallized from ethanol. 82 mg of 3β, 20β-dihydroxy-16β-carboxy-C^{14}-Δ_5-pregnene-3,20-lactone (X) was obtained. Mp.: 241—242°; $(\alpha)^{20}_{D}$: −31° (c: 0,5, dioxane). Anal.: Calcd. $C_{22}H_{32}O_3$ (344,47) C76,70 H 9,34 Found: C 76,58 H 9,20%. Spec. activity: 7502 cpm/mg.

3β,20β-dihydroxy-16α-carboxy-C^{14}-Δ_5-pregnene (IX)

Al_2O_3 remaining from the chromatographic experiment described in the former case was extracted several times with hot ethyl acetate. The residue remained after

evaporation of the extracts was recrystallized from methanol. 106 mg of 3β,20β-dihydroxy-16α-carboxy-C^{14}-Δ_5-pregnene (IX) was obtained. Mp.: 287—291°; $(\alpha)_D^{20}$: −74°; (c: 0,4, dioxane). Anal.: Calcd.: $C_{22}H_{34}O_4$ (362,49) C 72,89 H 9,45 Found: C 72,79 H 9,41% Spec. activity: 7802 cpm/mg.

3β,20β-diacetoxy-16α-carboxamido-C^{14}-Δ_5-pregnene (XI)

According to CRABBÉ and his coworkers [8], 3β, 20β-dihydroxy-16α-carboxy-C^{14}-Δ_5-pregnene (IX) after acetylation in the usual way with acetic anhydride was transformed in benzene into acid chloride with thionylchloride, then into acid amide with ammonia. After recrystallization 3β,20β-diacetoxy-16-carboxamido-C^{14}-Δ_5-pregnene (XI) was obtained. Mp.: 202—204°; $(\alpha)_D^{20}$: −56°; (c: 0,5 chloroform). Anal.: Calcd. $C_{22}H_{39}O_5N$ (445,57) C 70,08 H 8,82 Found: C 70,02 H 8,69%. Spec. activity: 6519 cpm/mg.

Alkaline hydrolysis of 3β,20β-diacetoxy-16α-carboxamido-C^{14}-Δ_5-pregnene (XI).

100 mg of 3β,20β-diacetoxy-16α-carboxamido-C^{14}-Δ_5-pregnene (XI) was hydrolized in a completely similar way as described in the alkaline hydrolysis of 3β, 20β-dihydroxy-16α-cyano-C^{14}-Δ_5-pregnene (VIII). Values of activity obtained from evaluation of samples taken time by time are tabulated in Table II.

Hour	Activity (cpm)		IX:X ratio
	16α-isomer (IX)	16β-isomer (X)	
1/2	763	592	1,29
1	1521	1170	1,30
2	2481	1924	1,29
3	3389	2648	1,28
7	5307	4021	1,32
10	5950	4542	1,31
15	5968	4627	1,29
30	5530	4318	1,29
50	5484	4219	1,30

3β,20β-dihydroxy-16α-carbomethoxy-(carboxy-C^{14})-Δ_5-pregnene (XII)

100 g of 3β,20β-dihydroxy-16α-carboxy-C^{14}-Δ_5-pregnene (IX) was dissolved in 40 ml of methanol and treated in the usual way with ethereal solution of diazomethane. After standing for an hour the solution was evaporated and the residue recrystallized from a mixture of methanol-ether. 81 mg of 3β,20β-dihydroxy-16α-carbomethoxy-(carboxy-C^{14}-)-Δ_5-pregnene (XII) was obtained. Mp.: 177—179°; $(\alpha)_D^{20}$: −74°; (c: 0,2 chloroform). Anal.: Calcd: $C_{23}H_{36}O_4$ (376,55) C 73,36 H 9,64 Found: C 73,32 H 9,52%. Spec. activity: 6188 cpm/mg.

16α-carbomethoxy-(carboxy-C^{14})-progesterone (XIII)

500 mg of 3β,20β-dihydroxy-16α-carbomethoxy-(carboxy-C^{14})-Δ_5-pregnene (XII) was dissolved in 30 ml of toluene, 10 ml of freshly distilled cyclohexanone

and 200 mg aluminumisopropoxide dissolved in 10 ml of anhydrous toluene were added. The reaction mixture was boiled for two hours, filtered, washed with 2×10 ml of 5% hydrochloric acid and then with 2×20 ml of water. The solution was then steam distilled, the residue was extracted with 3×20 ml of chloroform, the extract filtered, dried (Na_2SO_4 sicc.) and distilled. The amorphous residue was dissolved in 7 ml of glacial acetic acid and while constantly shaking and cooling, 0,5 g chromic acid anhydride dissolved in 5 ml of water and 20 ml glacial acetic acid were added in 20 min. The solution was shaken for 20 min., during this time the temperature of the bath was raised to room temperature. The reaction mixture was poured into 40 ml ice-water and extracted with 5×30 ml ether. The extract was washed with 5% sodium carbonate solution and water, dried and distilled. The residue was recrystallized from acetone-n-hexane mixture. 22 mg of 16α-carbomethoxy-(carboxy-C^{14})-progesterone (XIII) was obtained. Mp.: 143—144°; $(\alpha)_D^{20}$: +133°; (c: 0,5 $CHCl_3$). Anal: Calcd. $C_{23}H_{32}O_4$ (372,48) C 74,16 H 8,66 Found: C 74,00 H 8,41%. Spec. activity: 6002 cpm/mg.

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СТЕРОИДЫ. III

Синтез 16α-карбометокси (карбокси-C¹⁴)-прогестерона

Данные о механизме алкалического гидролиза 3*β*, 20*β*-дигидрокси-16α-циан-Δ_5-прегнена

М. Гальмоши и Й. Сабо

Авторами было синтезировано 3*β*-гидроски-16α-циан-C¹⁴-Δ_5-прегнен-20-он из 3*β*-ацетокси-$\Delta_{5,16}$-прегнадиен-20-он с KC¹⁴N. После редукции с $NaBH_4$ получили 3*β*,20*β*-дигидрокси-16α-циан-C¹⁴-Δ_5-прегнен, и алкалический гидролиз последнего был изучен. В результате алкалического гидролиза 3*β*,20*β*-дигидрокси-16*β*-карбокси-C¹⁴-Δ_5-прегнен-16,20-лактон и 3*β*,20*β*-дигидрокси-16α-карбокси-C¹⁴-Δ_5-прегнен были получены. Эстерированием и окислением последнего 16α-карбометокси-(карбокси-C¹⁴)-прогестерон получили. Изучался алкалический гидролиз 3*β*, 20*β*-дигидрокси-16α-циан-C¹⁴-Δ_5-прегнена, и также изучали соотношение полученных C_{16} изомерных окисей во времени и также соотношение C_{16} изомерных окисей полученных в алкалическом гидролизе 3*β*, 20*β*-диацетокси-16α-карбоксамидо-C¹⁴-Δ_5-прегнена полученного из 3*β*, 20*β*-дигидрокси-16α-карбокси-C¹⁴-Δ_5-прегнена путем измерения активности.