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Synthesis of 15α, 15β, 21, 21, 21-$^3H_5$-pregn-5-en-3β-ol-20-one

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SUMMARY

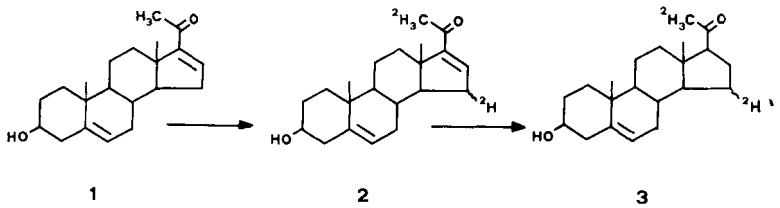
The readily accessible pregna-5,16-dien-3β-ol-20-one (1) was transformed into 3β-acetoxy-pregna-5,17-dien-20-isopropoxy-16-one (9). This compound was labelled by base-catalyzed isotopic exchange and then reconverted into labelled pregna-5,16-dien-3β-ol-20-one (13). Hydrogenation of this last one furnished the title compound (14). The labelling extent in the 15α,15β and 21 positions was determined by combined chemical and microbiological methods.

In the course of an investigation on the biosynthesis of cardenolides [1] we needed pregn-5-en-3β-ol-20-one labelled with tritium in the 15α and 15β positions. This compound had to be incorporated into cardenolides by *Digitalis lanata* plants with the aim of verifying the hypothesis according to which the introduction of the 14β-hydroxyl on the cardenolide skeleton occurs through a $\Delta^{14\,(15)}$-pregnane-type precursor. Moreover the above compound can be very useful in biosynthetic and metabolic studies in the steroid field.

Accordingly we synthesized 15α,15β,21,21,21-$^3H_5$-pregn-5-en-3β-ol-20-one (**14**).

The introduction of the label in the 15 positions is not a simple matter : attempts to prepare a C-15 labelled pregnan-20-one by acid-catalyzed [2] or base-catalyzed [3] exchange of 5α-pregn-16-en-20-one produced only the 21,21,21-d_3-labelled analog.

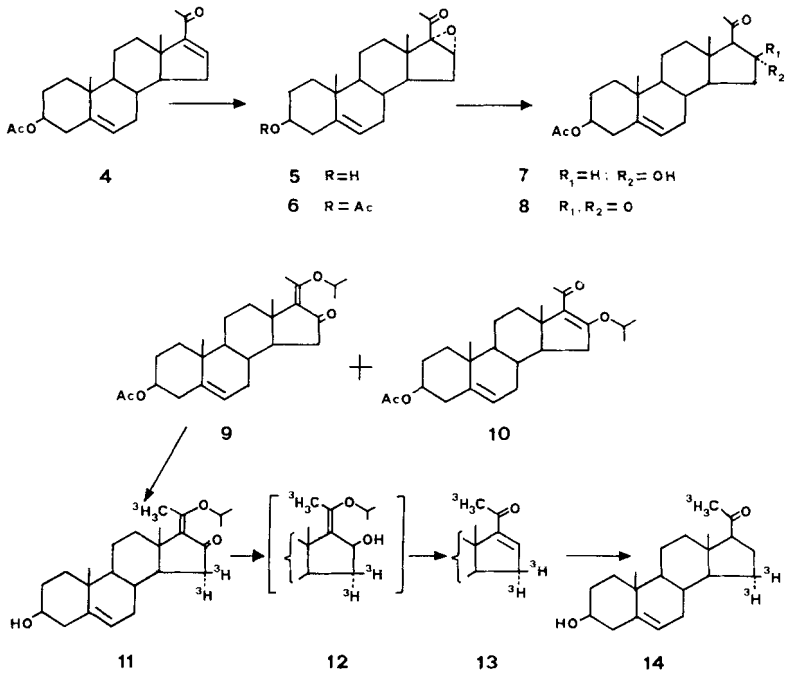
However we succeeded in introducing the label in the 15 position by exchange of pregna-5,16-dien-3β-ol-20-one (**1**) with sodium deuteroxide in deuterium oxide-dioxane under drastic conditions (when 160 mg of (**1**) were dissolved in 12 ml of dioxane and 5 ml of 0.4 N NaOD in D_2O and kept at 150° under nitrogen atmosphere in a sealed tube for 260 hr, mass-spectrometric analysis revealed a small introduction of deuterium at C-15) (Scheme 1).



SCHEME 1.

The above method is experimentally simple, but it does not allow to obtain sufficient amounts of doubly-labelled product.

In order to increase the isotopic incorporation we effected the exchange reaction on a compound such as (**9**), where a ketonic function was adjacent to the 15-methylenic group and the 20-keto group was protected as enol-isopropylether (Scheme 2).



SCHEME 2.

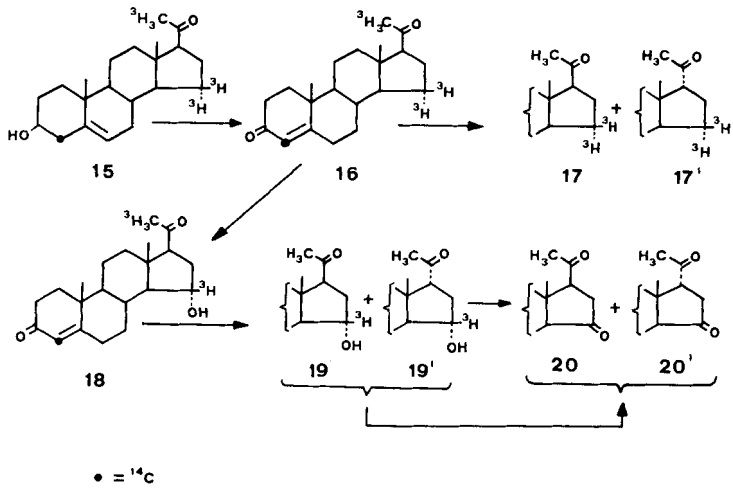
This product was obtained as follows : 3β-acetoxy-pregna-5,16-dien-20-one (**4**) was transformed into the corresponding 16α,17α-epoxyderivative (**5**) [4], which, after acetylation with acetic anhydride, was reduced to 3β-acetoxy-pregn-5-en-16α-ol-20-one (**7**) with chromous acetate [5]; Jones'oxidation of (**7**) furnished the dione (**8**). This last compound was converted into a mixture of the enol-isopropylethers (**9**) and (**10**), which were separated by careful chroma-

tography on Al_2O_3; the structures (**9**) and (**10**) were assigned on the basis of their UV, IR and NMR spectra.

The introduction of the label into the 15 and 21 positions of the enol-ether (**9**) was effected by exchange with sodium hydroxide in isopropyl alcohol-tritiated water *.

The labelled enol-ether (**11**) was reduced with $NaBH_4$ and the resulting crude material was dehydrated with H_2SO_4 in tetrahydrofuran to yield labelled pregna-5,16-dien-3β-ol-20-one (**13**). Homogeneous catalytic hydrogenation (Wilkinson catalyst) [6] afforded the labelled pregn-5-en-3β-ol-20-one (**14**) which, after purification and crystallization, showed a molar activity of 110 mC/mM.

For our biosynthetic work the relative amount of the label in the 15α and 15β positions had to be known. To do that, 4.5 mC of 15α,15β,21,21,21-$^3H_5$-pregn-5-en-3β-ol-20-one (**14**) were mixed with 0.1 mC of 4-^{14}C-pregn-5-en-3β-ol-20-one; a portion of the above mixture (4 μC of ^{14}C) was diluted with 2 g of carrier pregn-5-en-3β-ol-20-one : a fraction of the diluted labelled pregn-5-en-3β-ol-20-one (**15**) was converted, by Oppenauer oxidation, into progesterone (**16**), part of which was back-exchanged with sodium hydroxide in methanol-water, until a constant $^3H/^{14}C$ ratio was reached (Scheme 3).



SCHEME 3.

Another portion of the progesterone (**16**) was microbially transformed with *Fusarium lini* (Scheme 3) into 15β,21,21,21-$^3H_4$-4-^{14}C-15α-hydroxy-progesterone (**18**) [7]. This compound was equilibrated with 1 N NaOH in

* During preliminary experiments, use of methanol instead of isopropyl alcohol resulted in complete conversion of the enol-ether (**9**) into the dione (**8**); the quantity of water in the isopropyl alcohol-water mixture reaches a critical values at 10%, beyond which total transformation of the enol-ether into the corresponding dione occurs.

methanol-water to the mixture of **19** and **19′**, which was crystallized to constant specific activity.

The mixture of **19** and **19′** was oxidized to the mixture of the corresponding 15-ketones (**20** + **20′**), which was crystallized and counted.

The $^{3}H/^{14}C$ ratios of the above mentioned products are reported in Table 1.

TABLE 1.

Products	$^{3}H/^{14}C$ ratios
15α,15β,21,21,21-$^{3}H_5$-4-^{14}C-pregn-5-en-3β-ol-20-one (**15**)	45.0
15α,15β-$^{3}H_2$-4-^{14}C-progesterone (**17**) + 15α-15β-$^{3}H_2$-4-^{14}C-17-isoprogesterone (**17′**)	9.49
15β-^{3}H-4-^{14}C-15α-hydroxyprogesterone (**19**) + 15β-^{3}H-4-^{14}C-15α-hydroxy-17-isoprogesterone (**19′**)	7.34
4-^{14}C-pregn-4-en-3,15,20-trione (**20**) + 4-^{14}C-17-iso-pregn-4-en-3,15,20-trione (**20′**)	0

The values of Table 1 indicate that the tritium present on the labelled pregnenolone (**15**) was located as follows :

$$\text{position 21} : \frac{45 - 9.49}{45} \times 100 = 78.9\%$$

$$\text{position 15}\beta : \frac{7.34}{45} \times 100 = 16.3\%$$

$$\text{position 15}\alpha : \frac{9.49 - 7.34}{45} \times 100 = 4.8\%.$$

The better incorporation in the 15β position, with respect to the 15α one, is in agreement with the easier protonation of the enolate of (**9**) from the β side, as revealed by model examination.

The method used to determine the label extent in the 15α and 15β positions is based on the assumption that, in the microbial hydroxylation of progesterone (**16**) to 15α-hydroxyprogesterone (**18**), the hydroxyl group introduced assumes, as usually happens, the stereochemistry of the hydrogen removed.

In order to control that this occurs also in our experiment we transformed 15α-hydroxyprogesterone, obtained by microbial hydroxylation of progesterone with *Fusarium lini*, into 15β-^{3}H-progesterone according to Ramm and Caspi [8]. The assignment of the 15β configuration to the tritium derives from the known inversion which occurs in the $LiAlH_4$ hydrogenolysis of tosyl esters [9]. The so

obtained 15β-^{3}H-progesterone (17.08 μC) was mixed with 4-^{14}C-progesterone (4 μC; $^3H/^{14}C$ ratio = 4.27), diluted with non radioactive progesterone and again hydroxylated with *Fusarium lini* to give 15β-^{3}H-4-^{14}C-15α-hydroxy-progesterone, which had the same $^3H/^{14}C$ ratio (4.24) of the starting progesterone : this result demonstrates that the hydroxylation occurs with retention of configuration.

EXPERIMENTAL.

Melting points are uncorrected. All the compounds gave satisfactory elemental analysis. The rotations are taken in chloroform.

16α,17α-epoxy-pregn-5-en-3β-ol-20-one (5).

To a solution of 3 g of 3β-acetoxy-pregna-5,16-dien-20-one (**4**) in 200 ml of methanol were added at 10° C, 6 ml of 4N NaOH and, with stirring, 12 ml of 30 % H_2O_2 [4]. The mixture was allowed to stand at 0° C for 4 days and then poured into water : the precipitate was filtered and washed with additional water to yield 2.7 g of (**5**) which, after crystallization from methanol, had m.p. 187-90°; IR (nujol) : 1730, 1695 cm^{-1}; NMR (C_5D_5N) : 5.38 δ (m, 1 H), 3.70 (m, 1 H), 3.69 (s, 1 H), 1.99 (s, 3 H), 1.05 (s, 3 H), 1.02 (s, 3 H).

3β-acetoxy-pregn-5-en-16α-ol-20-one (7).

The epoxy derivative (**5**) was acetylated with 6 ml of acetic anhydride in 30 ml of pyridine. After 20 hr at r.t. traditional work-up yielded 2.5 g of crude 3β-acetoxy-16α,17α-epoxy-pregn-5-en-20-one (**6**), which was dissolved into 54 ml of acetic acid. To this solution a solution of $Cr(OCOCH_3)_2$ (prepared from 15 g of $CrCl_3 . 6H_2O$) [5] was added under nitrogen. After stirring under nitrogen for 16 hr at r.t. the mixture was poured into water, the precipitate collected dissolved into ether and the solution washed with water. The ethereal layer, dried on Na_2SO_4 and evaporated, yielded 2.25 g of crude product. This was chromatographed on 135 g of silica gel-celite (1 : 1) : elution with ether-ethanol (8 : 2) afforded 1.65 g of 3β-acetoxy-pregn-5-en-16α-ol-20-one(**7**), which, after two crystallizations from acetone, had m.p. 163-4°; IR (nujol) : 3380, 1725, 1695 cm^{-1}.

3β-acetoxy-pregn-5-en-16,20-dione (8).

A solution of 1.60 g of (**7**) in 200 ml of acetone distilled over CrO_3 was treated with 1.5 ml of Jones' reagent and stirred at 5° C for 10 min. After addition of few drops of isopropyl alcohol the solution was poured into water and extracted three times with ether. The combined ethereal layers were washed with water, dried on Na_2SO_4 and evaporated *in vacuo* to yield 1.4 g of crude product, which after crystallization from acetone had m.p.

151-3°; $[\alpha]_D^{20°} = -124°$ (c = 1.27); IR (nujol) : 1 740, 1 730, 1 710, 1 690, 1 650, 1 610 cm^{-1}; $\lambda_{max}^{CH_3OH}$: 287 ($\varepsilon = 3\,350$) and 310 ($\varepsilon = 17\,130$) mμ; the NMR ($CDCl_3$) showed that the product was a mixture of the ketonic and enolic tautomers : 5.30 δ (m, 1 H), 4.60 (m, 1 H), 2.28 (s, 1.5 H, $C_{21}H_3$—CO—), 2.02 (s, 4.5 H, $C_{21}H_3$—C(OH)=C + CH_3COO), 1.08 (s, 4.5 H, $C_{19}H_3$ + $C_{18}H_3$) 0.98 (s, 1.5 H, $C_{18}H_3$).

3β-acetoxy-pregna-5,17-dien-20-isopropoxy-16-one (9) and *3β-acetoxy-pregna-5,16-dien-16-isopropoxy-20-one (10).*

1.3 g of the diketone (**8**), 2.6 g of anhydrous K_2CO_3 and 2.6 ml of isopropyl jodide in 50 ml of anhdyrous acetone were refluxed for 16 hr. The mixture was concentrated *in vacuo*, water and benzene were added and the aqueous layer was extracted with benzene. The benzene extracts yielded, after drying on Na_2SO_4 and evaporation, 1.24 g of residue.

The crude residue was chromatographed on 124 g of Al_2O_3 Woelm III. Petroleum-ether-benzene (55 : 45, 11 × 100 ml) eluted 500 mg of the 16-enol ether (**10**), petroleum ether-benzene (50 : 50, 4 × 100 ml) eluted 300 mg of mixture of (**9**) and (**10**), whereas petroleum ether-benzene (45 : 55, 15 × 100 ml and 30 : 70, 2 × 500 ml) eluted 320 mg of the 20-enol ether (**9**).

The 16-enol ether (**10**), after crystallization from methanol, had m.p. 178-81°; $[\alpha]_D^{20°} = -48°$ (c = 1.95); IR (nujol) : 1 730, 1 635, 1 590 cm^{-1}; $\lambda_{max}^{CH_3OH}$: 278 mμ ($\varepsilon = 13{,}380$); NMR ($CDCl_3$) : 5.4 δ (m, 1 H), 4.6 (m, 1 H), 4.44 (m, 1 H), 2.3 (s, 3 H), 2.05 (s, 3 H), 1.34 (d, 3 H, J = 6 Hz), 1.32 (d, 3 H, J = 6 Hz), 1.08 (s, 3 H), 0.98 (s, 3 H).

The 20-enol ether (**9**), crystallized from petroleum ether, had m.p. 137-9°, $[\alpha]_D^{20°} = -146°$ (c = 1), IR (nujol) : 1 730, 1 690, 1 600 cm^{-1}; $\lambda_{max}^{CH_3OH}$ 284 mμ ($\varepsilon = 14\,450$); NMR ($CDCl_3$) : 5.4 δ (m, 1 H), 4.6 (m, 1 H), 4.44 (m, 1 H), 2.3 (s, 3 H), 2.05 (s, 3 H), 1.3 (d, 3 H, J = 6 Hz), 1.28 (d, 3 H, J = 6 Hz), 1.08 (s, 3 H), 0.98 (s, 3 H).

15α,15β,21,21,21-$^3H_5$-pregna-5,17-dien-20-isopropoxy-3β-ol-16-one (11).

80 mg of the 20-enol ether (**9**) were dissolved into a mixture of isopropyl alcohol (7 ml) and tritiated water (0.5 ml; 20 C/ml) in which 23 mg of Na had been previously dissolved. After refluxing for 3 hr under nitrogen atmosphere the tritiated solvent was recovered by evaporation at r.t. and collection into a cold trap. The solid residue was dissolved into chloroform, the solution washed with water, dried on Na_2SO_4 and evaporated to dryness *in vacuo* to yield 66 mg of the tritiated enol ether (**11**).

15α,15β,21,21,21-$^3H_5$-pregna-5,16-dien-3β-ol-20-one (13).

66 mg of (**11**) in 5.5 ml of 95% ethanol were treated with 73 mg of $NaBH_4$ and the mixture stirred at r.t. overnight. Dilution with water followed by extraction with chloroform gave 70 mg of crude product which was dissolved in 7.7 ml of anhydrous tetrahydrofuran; after addition of 0.66 ml of 10% H_2SO_4 and stirring for 4 hr, the mixture was diluted with water, concentrated *in vacuo* and extracted with chloroform; evaporation of the solvent afforded 56 mg of an oily material which was chromatographed on 6 g of silica gel-celite (1 : 1). Elution with benzene ethyl acetate (95 : 5, 7 × 25 ml) gave 27 mg of (**13**).

15α,15β,21,21,21-$^3H_5$-pregn-5-en-3β-ol-20-one (14).

29 mg of tristriphenylphosphinerhodium chloride [6] were added to 27 mg of (**13**) into 5 ml of benzene and the solution hydrogenated for 8 days. The solution was filtered on a column of florisil; elution with hexane-ether (7 : 3) yielded 25 mg of pure (**14**), which was crystallyzed from petroleum ether to constant specific activity. The molar activity was of 110 mC/mM.

Determination of the labelling extent in the 15α, 15β and 21 positions of of 15α,15β,21,21,21-$^3H_5$-pregn-5-en-3β-ol-20-one (14).

4.5 mC of 15α,15β,21,21,21-$^3H_5$-pregn-5-en-3β-ol-20-one (**14**) were mixed with 0.1 mC of 4-^{14}C-pregn-5-en-3β-ol-20-one : a portion (4 μC of ^{14}C) of the doubly labelled compound (**15**) was diluted with 2 g of non radioactive pregn-5-en-3β-ol-20-one and crystallized from petroleum ether.

15α,15β,21,21,21-$^3H_5$-4-^{14}C-pregn-4-en-3,20-dione (16).

1 g of (**15**) was dissolved into a mixture of 52 ml of anhydrous toluene, 17 ml of cyclohexanone and 380 mg of aluminum isopropoxide; from this solution few ml of solvent were slowly distilled during 2 hr. The reaction mixture was diluted with benzene, washed with 10% HCl and finally with water. The organic layers, after drying and evaporation to dryness, yielded a residue which was chromatographed on 100 g of Al_2O_3 II. Benzene-ethyl acetate (95 : 5, 8 × 100 ml) eluted 800 mg of 15α, 15β,21,21,21-$^3H_5$-4-^{14}C-pregn-4-en-3,20-dione (**16**), which, after crystallization from acetone-petroleum ether exhibited a specific activity of 1.3×10^3 dpm of ^{14}C/μM; ^{3}H/^{14}C ratio = 14.65.

Equilibration of 15α,15β,21,21,21-$^3H_5$-4-^{14}C-pregn-4-en-3,20-dione (16).

The equilibration was repeated three times until the ^{3}H/^{14}C ratio was constant.

To a solution of 115 mg of Na in 5 ml of 90% aqueous methanol, 100 mg of (**16**) were added and the solution was refluxed under nitrogen atmosphere for 4 hr, poured into water, concentrated *in vacuo* and extracted with chloroform. Evaporation of the solvent under reduced pressure yielded 90 mg of a mixture of 15α,15β-$^{3}H_2$-4-^{14}C-pregn-4-en-3,20-dione (**17**) and 15α,15β-$^{3}H_2$-4-^{14}C-17-iso-pregn-4-en-3,20-dione (**17'**) which was repeatedly crystallized from acetone-petroleum ether.

The following $^{3}H/^{14}C$ ratios were obtained :

1st equilibration : $^{3}H/^{14}C$ ratio = 9.69

2nd equilibration : $^{3}H/^{14}C$ ratio = 9.58

3rd equilibration : $^{3}H/^{14}C$ ratio = 9.49; specific activity = 1.29×10^3 dpm of $^{14}C/\mu M$.

15β,21,21,21-$^{3}H_4$-4-^{14}C-pregn-4-en-15α-ol-3,20-dione (18).

1.5 lt of Czapek-Dox medium were inoculated with *Fusarium lini* (Bolley). After shaking for 72 hr at 27° C, 0.5 g of progesterone (**16**), dissolved in 15 ml of acetone, were added; the culture was incubated for 48 hr and harvested by filtration; the filtrate was extracted with dichloromethane (5 × 5 lt). The combined extracts were dried, the solvent was evaporated and the 426 mg of residue chromatographed on 30 g of Al_2O_3 II. Elution with benzene-ethyl acetate (5 : 5) yielded 200 mg of 15β-21.21,21-$^{3}H_4$-4-^{14}C-pregn-4-en-15α-ol-3,20-dione (**18**) which, after crystallization from ethyl acetate had m.p. 220-5° and a $^{3}H/^{14}C$ of 10.8.

Equilibration of 15β,21,21,21-$^{3}H_4$-4-^{14}C-pregn-4-en-15α-ol-3,20-dione (18).

60 mg of (**18**) were equilibrated three times as previously described for (**16**). The following $^{3}H/^{14}C$ ratios were obtained :

1st equilibration : $^{3}H/^{14}C$ ratio = 7.60

2nd equilibration : $^{3}H/^{14}C$ ratio = 7.40

3rd equilibration : $^{3}H/^{14}C$ ratio = 7.34; specific activity = 1.28×10^3 dpm of $^{14}C/\mu M$.

4-^{14}C-pregn-4-en-3,15,20-trione (20) and *4-^{14}C-17-iso-pregn-4-en-3,15,20-trione (20').*

A solution of 60 mg of the material obtained in the previous equilibration in 20 ml of acetone was treated with Jones' reagent at 10° C for 10 min. After addition of few drops of methanol, the solution was poured in water, concentrated *in vacuo* and extracted with chloroform. Evaporation of the solvent yielded a crude residue (58 mg) which was chromatographed on 6 g of Al_2O_3 II. Benzene-ethyl acetate (9 : 1) eluted 45 mg of a mixture of (**20**) and (**20'**) which, after crystallization from acetone-hexane had a specific activity of 1.29×10^3 dpm of $^{14}C/\mu M$ ($^{3}H/^{14}C$ ratio = 0).

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