NOTES

Synthesis of Pregn-5-en-3β-ol-20-one-20-^{14}C

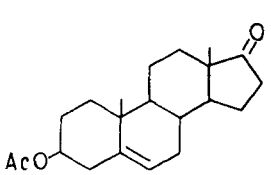
Received on 26th January 1968

In connection with our work on the biosynthesis of bufodienolides in toads ([1]) there arose the need of pregn-5-en-3β-ol-20-one-20-^{14}C (V) to be used as a precursor in one of our experiments. Because this labelled compound was not commercially available we carried out its preparation modifying in part the original method of Butenandt and Schmidt-Thomé ([2]) for the synthesis of progesterone.

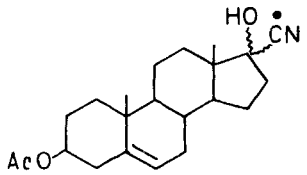
Androst-5-en-3β-ol-17-one acetate (I) reacted in a sealed tube with K^{14}CN in ethanol-acetic acid yielding the cyanohydrin II. This was the key step in the whole procedure; in the original paper a tenfold excess of KCN was used, and the reaction was conducted by boiling the solution under reflux. In our case, a similar procedure would have been prejudicial for the radiochemical yield of the reaction, so, the step was run in a Y-shape tube which was sealed before mixing the reagents. It was found that in this condition a 2 : 1 molar relation of cyanide-steroid was the best, considering both the chemical and the radiochemical yields.

Compound II was dehydrated by reaction with $POCl_3$ and pyridine in a sealed tube to the unsaturated nitrile III.

Condensation between compound III and methylmagnesium bromide led to pregna-5, 16-dien-3β-ol-20-one-20-^{14}C (IV) which on selective hydrogenation over Raney nickel produced the expected compound V with an overall yield of 15 %.



I



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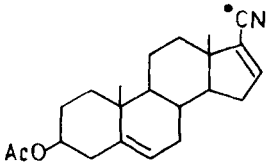
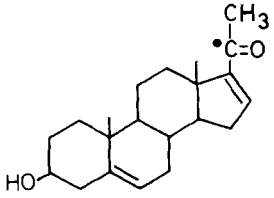
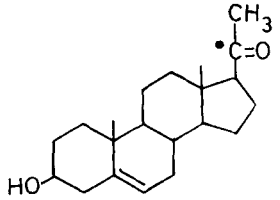


III



IV



V

EXPERIMENTAL *

17-ξ-Cyano-^{14}C-androst-5-en-3β,17-ξ-diol-3-acetate (II). In one of the branches of a Y-shape tube androst-5-en-3-β-ol-17-one acetate (330 mg), K^{14}CN (14.5 mg - 4.5 mC/mM), KCN (148 mg) and ethanol (4 ml) were placed while the other branch carried acetic acid (0.2 ml) and ethanol (1 ml). The tube was sealed, and the reagents were combined. The mixture was kept at 80° for 45 min. with occasional shaking. When cool the mixture was poured onto ice-water, and the crystalline product thus obtained was filtered off and washed with water. The substance was immediately dissolved in ethyl acetate, and the organic layer was dried over $MgSO_4$. The dry solution was boiled with decolorising charcoal and filtered, and the filtrate was evaporated. Compound II (286 mg — 80 %) had a specific activity of 0.33 mC/mM.

17-Cyano-^{14}C-androsta-5,16-dien -3β-ol acetate (III). Compound II (280 mg) pyridine (5 ml) and $POCl_3$ (0.2 ml) were heated in a sealed tube at 150° for 90 min. Once at room temperature the mixture was poured onto water-HCl (80 : 20 ml), and the solid was filtered off and washed with water. It was then dissolved in acetone, treated with charcoal and filtered. The crystalline product obtained by concentration of the solution was collected by filtration. Compound III (133 mg — 50 %) had m.p. 199-200°, its i.r. spectrum was identical to one obtained from authentic non-labelled product, sp. act. 0.30 mC/mM.

Pregna-5,16-dien-3β-ol-20-one-20-^{14}C (IV). A solution of compound III (128 mg) in benzene (0.6 ml) was added to a boiling solution of ethereal methylmagnesium bromide reagent (from 75 mg of Mg and 4 ml of CH_3Br), and the mixture was heated under reflux for 46 hs. The reaction mixture was cooled to 0°, and acetic acid (9 ml) was added dropwise followed by water (6 ml). The organic solvents were removed, and the aqueous solution was boiled under reflux for 20 min. When cool it was poured onto ice-water, and the solid thus produced was filtered off, washed with water and dried. The product was taken up in acetone, filtered from an insoluble material and the filtrate was concentrated with a stream of N_2 until crystallization. Product IV (83 mg — 70 %), m.p. 205-207°, was indistinguishable by i.r. spectroscopy from an authentic sample; sp. act. 0.28 mC/mM.

Pregn-5-en-3β-ol-20-one-20-^{14}C (V). Compound IV (70 mg) was dissolved in ethanol (10 ml) and a solution of NaOH (35 mg) in water (0.7 ml) was added. The mixture was hydrogenated for 3 hs at room temperature and atmospheric pressure over Raney nickel (W-2). The catalyst was filtered off, washing it

* Melting points were determined in a Fisher-Johns hot-plate and are uncorrected. I. r. spectra were recorded with a Perkin-Elmer Infracord spectrophotometer. Radioactivity was measured with a Packard model 3305 liquid scintillation spectrometer in the conventional scintillations solutions. Analytical T.L. C. was conducted on silica gel G with benzene-chloroform mixtures. The K^{14}CN was purchased from New England Nuclear Corp., Boston, Mass. Solvents were removed under diminished pressure below 50°.

with ethanol, and the filtrate was poured onto ice-water. The solid thus produced was filtered off, washed with water and dried. The product was recrystallized from ethanol-water to pure V (47 mg — 67 %), m.p. 178-180°, which was homogeneous by T. L. C. The i. r. spectrum was identical to the one from authentic sample; sp. act. 0.33 mC/mM.

ACKNOWLEDGEMENTS

We thank the Upjohn Company, Kalamazoo, Michigan for a generous gift of 5-androstenolone acetate and the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina for a research grant (No. 2328).

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REFERENCES

1. GROS, E. G. and DEULOFEU, V. — *Chem. Comm.*, 711 (1967); GROS, E. G. and PORTO, A. M. — *Anales Asoc. Quim. Argentina*, **55** : 177 (1967).
2. BUTENANDT, A. and SCHMIDT-THOMÉ, J. — *Ber.*, **71** : 1487 (1938); *ibid.*, **72** : 182 (1939).

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Préparation des acides L et D-α-aminoadipiques radioactifs par résolution de l'acide DL-α-aminoadipique ^{14}C-6

Reçu le 22 mars 1968.

Le dédoublement de l'acide DL-α-aminoadipique en ses isomères optiques, par hydrolyse stéréospécifique de l'acide N-chloracétyl-DL-α-aminoadipique sous l'action de l'acylase de rein de Porc, a été décrite par Greenstein et ses collaborateurs. Elle permet d'obtenir, dans un premier temps, l'acide L-α-aminoadipique, puis, après isolement de l'acide chloracétyl-D-α-aminoadipique et hydrolyse par l'acide chlorhydrique 2 N, l'acide D-α-aminoadipique.

Nous avons adapté la méthode au dédoublement de l'acide DL-α-aminoadipique ^{14}C-6, en quantités de l'ordre de quelques centigrammes.