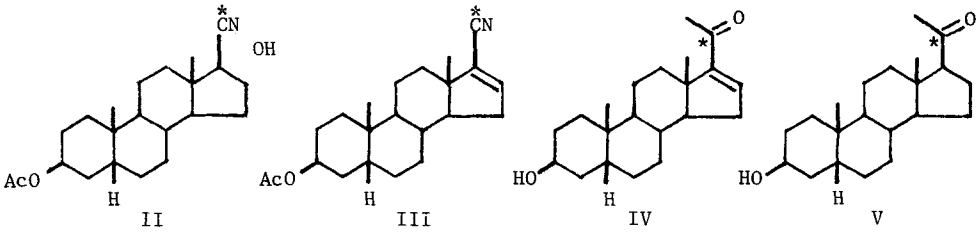
NOTES

SYNTHESIS OF |20-^{14}C|3β-HYDROXY-5β-PREGNAN-20-ONE

H. M. Garraffo and E. G. Gros
Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pab. 2, Ciudad Universitaria, 1428 Buenos Aires, Argentina

The details of the biosynthetic pathway leading to the animal bufadienolides are less known than those conducting to plant bufadienolides[1]. In a study of the biosynthesis of toad bufadienolides, 3β-hydroxy-5β-pregnan-20-one labelled at the side chain was required. The synthesis of this compound labelled at C-20 was performed following a procedure previously used by us for a closely related steroid[2]. Condensation of 3β-acetoxy-5β-androstan-17-one (I) with potassium |^{14}C|cyanide produced the cyanohydrin (II)[3]. Dehydration of II afforded the α,β-unsaturated nitrile (III)[3] that was treated with methylmagnesiumiodide producing the 3β-hydroxy-5β-pregn-16-en-20-one (IV)[4]. Hydrogenation of IV gave the title product (V) with identical properties than those from an authentic sample[5], and specific activity of 0.65 mCi/mmol.



EXPERIMENTAL

Melting points are uncorrected. IR spectra were obtained in Nujol dispersions. Mass spectra at 70 eV. Radioactivity was measured by liquid scintillation.

|20-^{14}C|17ξ-Hydroxy-17ξ-cyano-5β-androstan-3β-yl acetate (II). Compound I (200 mg) was suspended in EtOH (3 ml) and a mixture of potassium cyanide (99 mg) and potassium|^{14}C|cyanide (1.06 mg; 1 mCi) was added. The reaction mixture was kept in a flask closed by means of a rubber septum at 10°C. While it was being stirred, acetic acid (1 drop) was injected through the septum; after 15 min acetic acid (0.7 ml) was added dropwise at a rate of 6 drops during 1 hr and the rest during a second hour. The mixture was maintained at room temperature for 2 hr, poured into water-HCl (50:5 ml), and the solid was filtered off.

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It was dissolved in EtOAc (50 ml), washed with water (3 x 30 ml) and dried over $MgSO_4$. Evaporation of the solvent gave II (198 mg) with physical properties identical to those from the unlabelled product; sp. act.: 0.65 mCi/mmol.

|20-^{14}C|17-cyano-5β-androst-16-en-3β-yl acetate (III). Compound II (190 mg) was dissolved in pyridine (4 ml), treated with phosphorous oxychloride (0.2 ml) and the mixture was heated in a sealed tube for 30 min at 150°C. It was poured into water-HCl (52:8 ml) and extracted with methylene chloride (2 x 40 ml). The extract was washed with water (3 x 30 ml) and dried over $MgSO_4$. Evaporation of the solvent afforded a residue (187 mg) that was purified through a silica gel column giving III (164 mg) with physical properties (IR. MS) identical to those from an authentic sample; sp. act.: 0.66 mCi/mmol.

|20-^{14}C|3β-Hydroxy-5β-pregn-16-en-20-one (IV). To a solution of methylmagnesiumiodide prepared from Mg (1.5 g), iodine (0.1 mg) and methyl iodide (4.4 ml) in ether (25 ml), a solution of III (158 mg) in benzene (3 ml) was added. The mixture was refluxed under nitrogen for 48 hr. It was cooled to 0°C and treated dropwise with acetic acid (12 ml) followed by water (18 ml). The organic solvent was removed, and the aqueous solution was boiled under reflux for 20 min. It was poured into ice-water and the solid thus produced was extracted with methylene chloride (3 x 30 ml). The extract was washed with water (3 x 30 ml) and dried over $MgSO_4$. Evaporation of the solvent gave a residue that was chromatographed through a silica gel column eluting it with benzene and benzene-EtOAc (95:5 and 90:10). Pure IV (104 mg) of m.p. 170-173°C resulted indistinguishable by IR and MS from an authentic sample; sp. act.: 0.66 mCi/mmol.

|20-^{14}C|3β-Hydroxy-5β-pregnan-20-one (V). A solution of IV (93.5 mg) in EtOAc (20 ml) was hydrogenated for 12 hr at room temperature and atmospheric pressure over 10% Pd/C. The catalyst was filtered off and washed with EtOAc. Evaporation of the solvent afforded V (93 mg) that was recrystallized from EtOH-water to m.p. 141-144°C, homogeneous by TLC and physical properties (IR, MS) identical to those from an authentic sample; sp. act.: 0.65 mCi/mmol.

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