# SYNTHESIS OF 3β-HYDROXY-5-CHOLENIC-24-$^{14}$C ACID.

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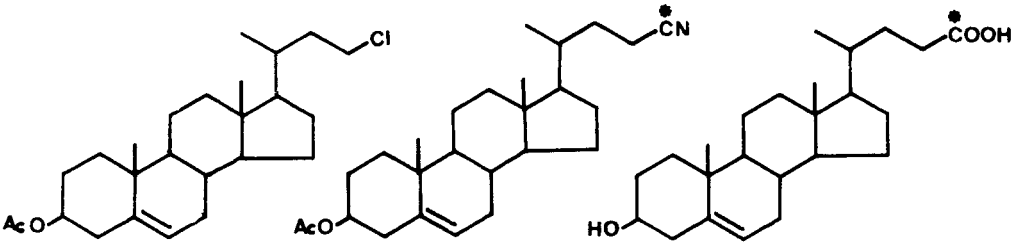
3β-Hydroxy-5-cholenic acid specifically labelled at carbon-24 is not commercially available. This labelled compound was required to continue our investigations on the biosynthesis of bufadienolides in toads (1). The preparation of the methyl ester of the title compound is described (2) in the literature but the starting material was the hyodesoxycholic acid and the synthetic transformation required several steps. On the other hand, our recent synthesis (3) of 3β-acetoxy-24-norchol-5-enyl chloride (1) provided a straightforward method for introducing a radioactive carbon-atom at C-24 of the steroid side chain. Hence, compound 1 (as an almost 1:1 mixture of 20R,20S epimers detected by spectroscopic methods) on reaction with potassium cyanide-$^{14}$C in dimethylsulphoxide (4) afforded the corresponding 3β-acetoxy-chol-5-en-24-onitrile-24-$^{14}$C (2). This was submited to alkaline hydrolysis giving the 3β-hydroxy-5-cholenic-24-$^{14}$C acid (3).




1 2 3

## EXPERIMENTAL

Melting points are uncorrected. Radioactivity was measured by the liquid scintillation technique using a Packard Tricarb model 93320

spectrometer. Potassium cyanide-$^{14}C$ was purchased from the Commissariat a L'Energie Atomique, France.

3β-Acetoxy-chol-5-en-24-onitrile-24-$^{14}C$ (2). A mixture of anhydrous $K^{14}CN$ (45 mCi/mmol, 1 mCi), inactive KCN (12.9 mg) and dry 3β-acetoxy-24-norchol-5-enyl chloride (1) (98.0 mg) was treated with freshly distilled DMSO (1 ml) and the solution was stirred at 100° for 5 hr. After cooling it was poured into water and extracted with ether. The organic extract was washed with water and dried over $MgSO_4$. The residue obtained for evaporation of the solvent was purified by preparative t.l.c. (silica gel, benzene-methylene chloride (1:1)) giving chromatographically pure compound 2 (80 mg, spec. act. $6.9 \times 10^9$ dpm/mmol).

3β-Hydroxy-5-cholenic-24-$^{14}C$ acid (3). A solution of compound 2 (78 mg) in 90% ethanol (2.2 ml) was treated with NaOH (310 mg) and heated under reflux for 48 hr. It was poured into water (150 ml), extracted with ether and the extracts were discarded. The remaining aqueous solution was acidified with diluted HCl (28 ml) and extracted with ether. The organic extract was washed with water until neutral and dried over $MgSO_4$. The residue obtained for evaporation of the solvent (69 mg) was recrystallized from ethyl acetate to m.p. 185-187° and constant specific activity of $7.3 \times 10^9$ dpm/mmol. Its IR spectrum was identical with that of an authentic sample.

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