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The synthesis of tritium-labelled tetrahydrocortexolone and tetrahydrocortexone

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SUMMARY

Cortexolone-1, 2-^3H (17α, 21-dihydroxy-4-pregnene-3,20-dione) has been used as the starting point for the preparation of tetrahydrocortexolone-1, 2-^3H and of tetrahydrocortexone-1, 2-^3H (3α, 17α, 21-trihydroxy-5β-pregnane-20-one and 3α, 21-dihydroxy-5β-pregnane-20-one). The method used involved the catalytic and $LiAlH_4$ reduction of a derivative whose side-chain was protected by bis-methylene-dioxy groups. The structural proof and radiochemical purity were determined by the radiochromatography of various derivatives and degradation products in several solvent systems.

RÉSUMÉ

Cortexolone-1, 2-^3H (17α, 21-dihydroxy-4-pregnene-3,20-dione) a été utilisée comme produit de départ pour la préparation de la tétrahydrocortexolone-1, 2-^3H et la tétrahydrocortexone-1, 2-^3H (3α, 17α, 21-trihydroxy-5β-pregnane-20-one et 3α, 21-dihydroxy-5β-pregnane-20-one).

Nous avons utilisé la réduction catalytique et par $LiAlH_4$ d'un dérivé à chaîne latérale protégée par le groupement bis-méthylène-dioxy. La preuve de structure et de la pureté radiochimique est donnée par la radiochromatographie de divers dérivés et produits de dégradation dans plusieurs systèmes de solvents.

INTRODUCTION

The steroids with a dihydroxyacetone side-chain are the main products of adrenal cortical secretion in man. Cortisol (11β, 17α, 21-trihydroxy-4-pregnene-3,20-dione) is the principal hormone secreted, but cortexolone (Reichstein's Compound "S"; 11-deoxycortisol; 11-deoxy-17-hydroxycorticosterone; 17α, 21-dihydroxy-4-pregnene-3,20-dione), although present in much smaller pro-

portions, plays an important role in certain adrenal dysfunctions. Its secretion rate and metabolic disposal are still being investigated [1, 2, 3, 4, 5]. The main metabolic disposal pathway for these hormones is via their glucurono-conjugated tetrahydro-derivatives (3-hydroxy-5α or 5β-pregnane). We suggest that the availability of these metabolites, labelled with tritium to a high specific activity might be useful in biological and clinical research.

Materials and methods

Cortexolone-1,2-^{3}H [I] can be synthetized with good yield and a high specific activity [6]. It is used as the starting point for the synthesis of tetrahydro-cortexolone-1,2-^{3}H (3α, 17α, 21-trihydroxy-5β-pregnane-20-one) [V] (Fig. 1).

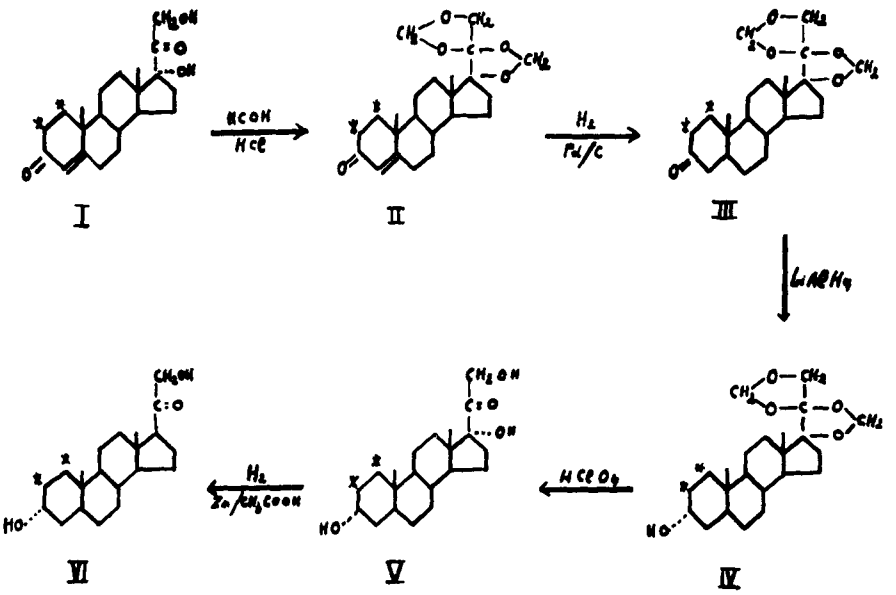

FIG. 1

The reaction sequence is the same as that used for the synthesis of tetrahydrocortisol [7]. The original compound is transformed into a bis-methylene-dioxy derivative [II] in order to preserve the side-chain [8]. Palladium catalysed hydrogenation yields the dihydro-cortexolone-bis-methylene-dioxy compound [III], which is then reduced to the tetrahydro or 3-hydroxy compound [IV] with lithium aluminium hydride. The final product is obtained after acid hydrolysis of the bis-methylene-dioxy groups.

This synthesis can easily be extended a step further : the hydrogenolysis of

the 17α-hydroxy group with zinc-acetic acid [9] giving the tetrahydrocortexone (tetrahydro-DOC; 3α, 21-dihydroxy-5β-pregnane-20-one) [VI].

Paper chromatography was used for preparation as well as for analytical purposes. Chromatography in solvent systems containing aqueous methanol as a stationary phase [10] was carried out at 32°C, using the paper pre-treatment procedure [11]. Chromatography on paper saturated with propylene glycol was carried out at room temperature [12]. The radioactivity was counted in a Tricarb Liquid Scintillation Spectrometer Mod. 3214.

The radioactivity on paper chromatograms was detected and recorded by a non-commercial scanning device, with an efficiency of about 1% on dry Whatman n° 1 filter paper [13].

The solvents used without further purification were Merck or Analar, analytical or chromatography grade. The following solvent systems were used :

B1 : petroleum ether/toluene/methanol/water : 50/50/70/30;
BA : petroleum ether/methanol/water : 100/80/20;
TP : toluene/propylene glycol;
PTP : petroleum ether/toluene (1:1)/propylene glycol.

The chemical detection on paper chromatograms was obtained by ultraviolet fluorescence quenching at 256 mμ, and by the reduction of tetrazolium blue by α-ketols [14]. The chemical determination necessary for specific activity measurements was carried out either by ultraviolet spectrophotometry at 240 mμ (Beckman DU), or by the Porter-Silber colour reaction [15] adapted in specific cases to 17-deoxy compounds [16].

EXPERIMENTAL

17, 20, 20, 21 Bis-methylene-dioxy-cortexolone-1,2-^3H [II]

500 mC (160 mg) of cortexolone-1, 2-^{3}H are stirred for 40 hrs at room temperature with 8 ml of chloroform, 2 ml of 37% formaldehyde and 2 ml of concentrated hydrochloric acid. After the addition of 50 ml of $CHCl_3$ the lower organic phase is washed with water until neutral. The solvent is evaporated in a vacuum rotatory evaporator. The residue is taken up in benzene and filtered through a chromatography column filled with 10 g of alumina. The column is washed with a further 50 ml of benzene and the combined fractions are again evaporated. The white powdery residue contains 405 mc and affords a single ultraviolet absorbing, non-reducing spot in the paper chromatography system BA. No further attempt at purification was made.

Bis-methylene-dioxy-tetrahydrocortexolone-1, 2-^3H [IV]

The crude product [II] is dissolved in 2 ml of dioxane, and 50 mg of 10% palladium on charcoal are added. After removing the air, the suspension is stirred in hydrogen at room temperature and atmospheric pressure, until the

uptake of gas has stopped. The catalyst is filtered off and washed with hot methanol. The solvents are then evaporated in vacuo. The crude product [III] is dried overnight in a dessicator, dissolved in 10 ml of anhydrous diethyl ether, and reduced with twice the theoretical amount of lithium aluminium hydride. The excess reagent is decomposed by ethyl acetate and the precipitated alumina is dissolved in HCl. The reduced product is then further extracted with ethyl acetate, and the solvent washed with water until neutral. The recovery of the crude product [IV] is 350 mC. No purification of this product has been attempted.

Tetrahydrocortexolone-1,2-^3H [V]

The crude product [IV] is dissolved in dioxane and an equal volume of 1 N $HClO_4$ is added. This solution is kept for 30 minutes at 100 °C in a sealed, evacuated phial. The seal is then broken, the acid neutralised by the addition of sodium carbonate solution and the steroids are extracted with ethyl acetate. The analytical paper radiochromatography in the solvent system B1 shows the presence of small amounts of 3α-hydroxy-5α-pregnane isomer, some unreduced 3-ketone (5α and 5β) isomers and a considerable amount of less polar material (Fig. 2).

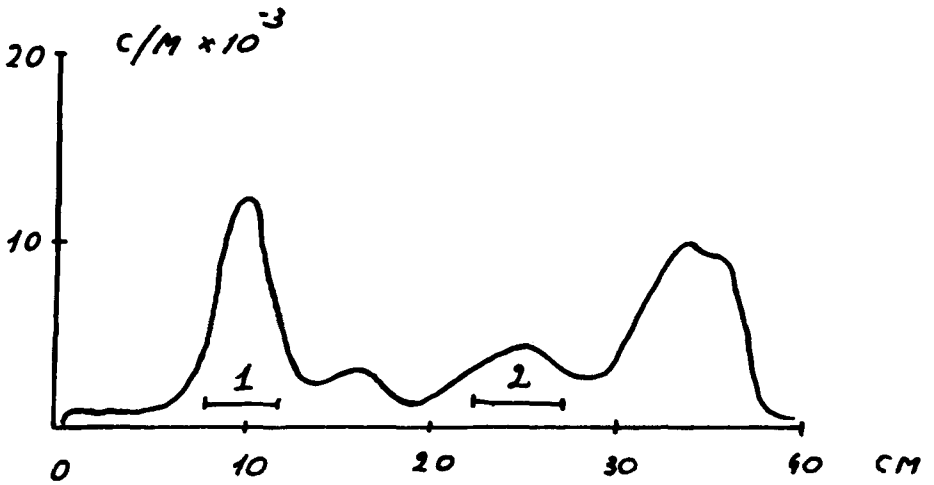

FIG. 2
Solvent system B1; 32 °C
Carriers : 1 = tetrahydrocortexolone
2 = dihydrocortexolone
Detection : tetrazolium blue

After purification by paper chromatography, first in the solvent system TP and then in the previously mentioned B1 system, the pure tetrahydrocortexolone

[V] is obtained with a 26% yield in relation to [IV] i.e. with an 18% overall yield. The specific activity was 2.8 mc/mg i.e. 980 mC/mM.

Radiochemical purity and structural proof — 10 μC of the product were chromatographed on the solvent systems B1 and TP. In both instances a single symmetrical radioactive peak was recorded, coincident with the position of 100 μg of the authentic non-radioactive 3α, 17α, 21-trihydroxy-5β-pregnane-20-one (Fig. 3). Acetylation with acetic anhydride/pyridine for 18 hrs at room

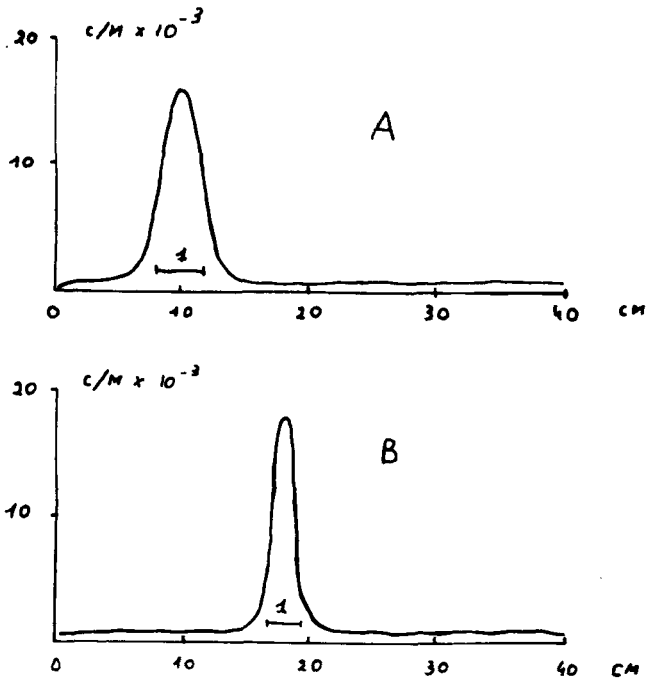

FIG. 3

A : solvent system B1; 32 °C
B : solvent system TP; 16 hrs
Carrier 1 = tetrahydrocortexolone
Detection : tetrazolium blue

temperature yielded a single symmetrical peak in the solvent system BA, coincident with the position of the carrier tetrahydrocortexolone-3, 21-diacetate (Fig. 4). Sodium bismuthate oxidation and chromic anhydride oxidation yielded single radioactive peaks coincident with the position of, respectivily 3α-hydroxy-

5β-androstane-17-one and 5β-androstane-3, 17-dione; the solvent systems used being BA and PTP (Fig. 5 and 6).

Tetrahydrocortexone-1,2-^3H [VI]

6 mc of [V] were dissolved in 2 ml of 50% aqueous acetic acid. 1g of zinc powder was added and the suspension was kept boiling in an oil bath for 90 minutes under reflux. After cooling, dilution with water, extraction with ethyl acetate and chromatography in solvent system B1, the chromatographically

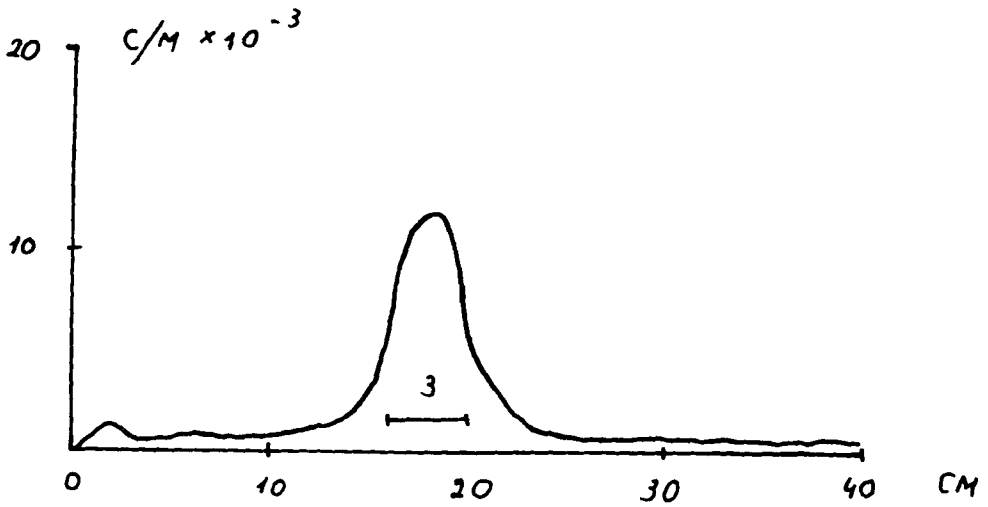

FIG. 4
Solvent system BA; 30°C
Carrier 3 = tetrahydrocortexolone-3,21-diacetate
Detection : tetrazolium blue

pure tetrahydrocortexone was isolated with a 50% yield in relation to [V]. The molar specific activity remained unchanged. The specific activity per milligram, however, was slightly higher (2.9 mC/mg) due to the lower molecular weight of the product.

Radiochemical purity and structural proof — The chromatography of the product and of its 3,21-diacetate was carried out in 2 different solvent systems, and the single radioactive peak coincided with the authentic non-radioactive carrier and with its diacetate in each case. After chromic acid oxidation the radioactivity moved with the carrier 5β-androstane-3,17-dione.

4. Discussion

Radiosynthesis is quite often a straight-forward application of classical organic chemistry, adapted to particular requirements of radioisotopic work. These adaptations, however, can be rather significant. This is particularly true as far as the methodology of the structural proof is concerned. Obviously, the melting point, the infrared and ultraviolet spectra and several other methods of

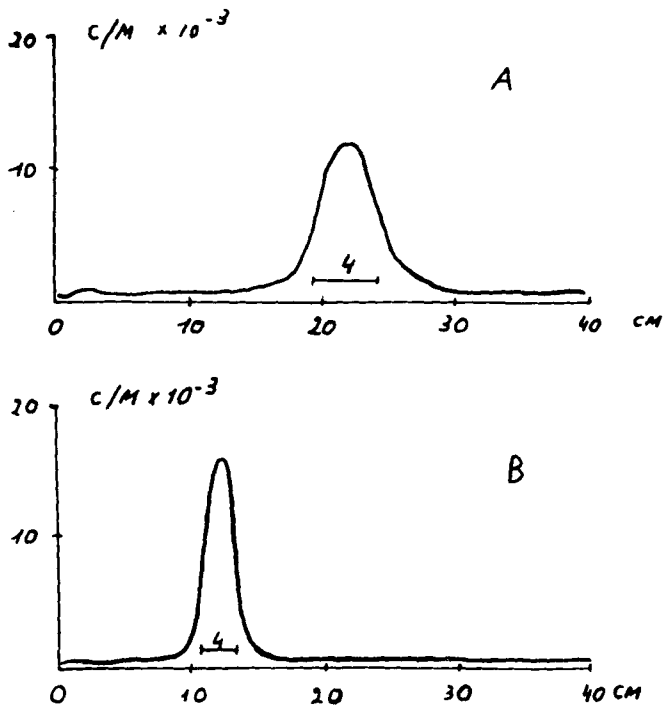

Fig. 5

A : solvent system BA; 30 °C
B : solvent system PTP; 14 hrs
Carrier 4 = 3α-hydroxy-5β-androstane-17-one
Detection : alcaline *m*-dinitrobenzene

identification are not easily applied to substances containing high amounts of a radioisotope per unit weight and not available in large quantities. Since identification is in any case an accumulation of experimental data making any other structure highly improbable, we feel that the method of carrying out repeated chromatography of both the compound and several of its derivatives and degradation products in different solvent systems, may afford as high a degree of certitude as any other method.

Moreover, this method, whilst using very small amounts of material, produces valuable data on the radiochemical purity and the stability of the radioisotope. There seems to be very little loss of the radioisotope during the entire sequence of reactions, although it is well known that the hydrogen on the carbon 2 may exchange in a saturated 3-ketone structure. Therefore the final specific activity will be solely a function of the isotope content of the original cortexolone, up to the theoretical limit of 60 C/mM.

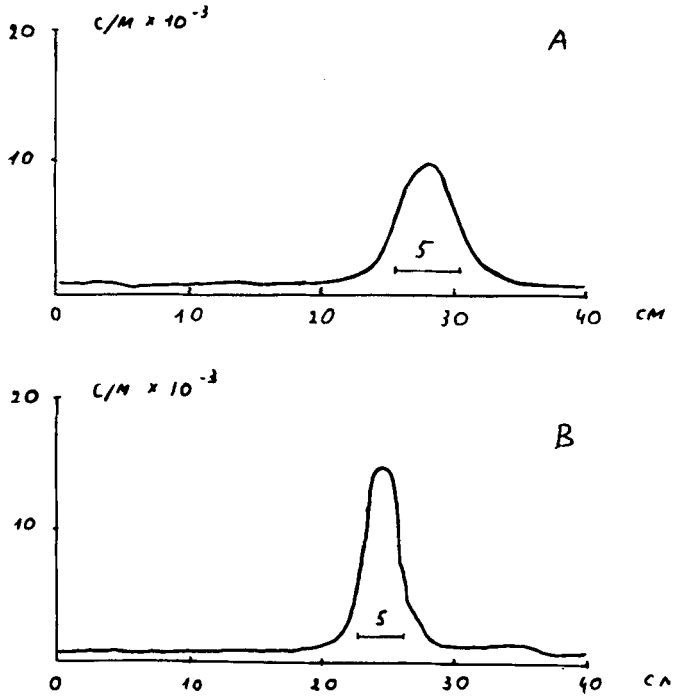

FIG. 6

A : solvent system BA; 30 °C
B : solvent system PTP; 12 hrs
Carrier 5 = 5β-androstane-3,17-dione
Detection : alcaline *m*-dinitrobenzene

The hydrolysis of bis-methylene-dioxy derivatives is usually performed in 60% formic acid at 100 °C. Under these conditions, however, the 3-hydroxy group is easily formulated. We therefore found it preferable to use dilute perchloric acid, which does not increase the yield significantly, but gives a much cleaner chromatogram and avoids the additional step of alcaline hydrolysis of the formyl esters.

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