SYNTHESIS OF [17α, 16α]OXAZOLINO STEROIDS SPECIFICALLY LABELLED IN C-2' OF THE OXAZOLINE RING WITH ^{14}C.

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SUMMARY

The preparation of ^{14}C-labelled 11β , 21-dihydroxy-3,20-dioxy-pregna-1,4-dieno[17α, 16α-d]-2'-methyloxazoline-21-acetate and of 9α-fluoro-11β,21-dihydroxy-3,20-dioxopregna-1,4-dieno[17α,16α-d]-2'-methyloxazoline-21-acetate* is described. Specific labelling at the C-2' position was achieved reacting the corresponding 17α-amino-16α-alcohols with [1-^{14}C]-acetic anhydride.

INTRODUCTION

The [17α, 16α-d]-2'-methyloxazoline corticosteroids analogues of prednisolone acetate VIII and of 9α-fluoroprednisolone acetate VIIIa, synthesized in our laboratories [1,2] displayed high antiinflammatory activity in pharmacological[3] as well as in clinical trials [4,5,6] (Azacortid®).

For pharmacokinetics[7] and metabolic studies [8,9] the corresponding compounds ^{14}C-labelled in the C-2' position have been prepared. The key intermediate for the synthesis of the 9α-fluoro-corticosteroid VIIIa, 9α-fluoro-17α-amino-11β,16α,21-trihydroxy-3,20-dioxopregna-1,4-diene hydrochloride Va, has already been described[2].

* Note that [17α, 16α-d] does not refer to deuterium labelling

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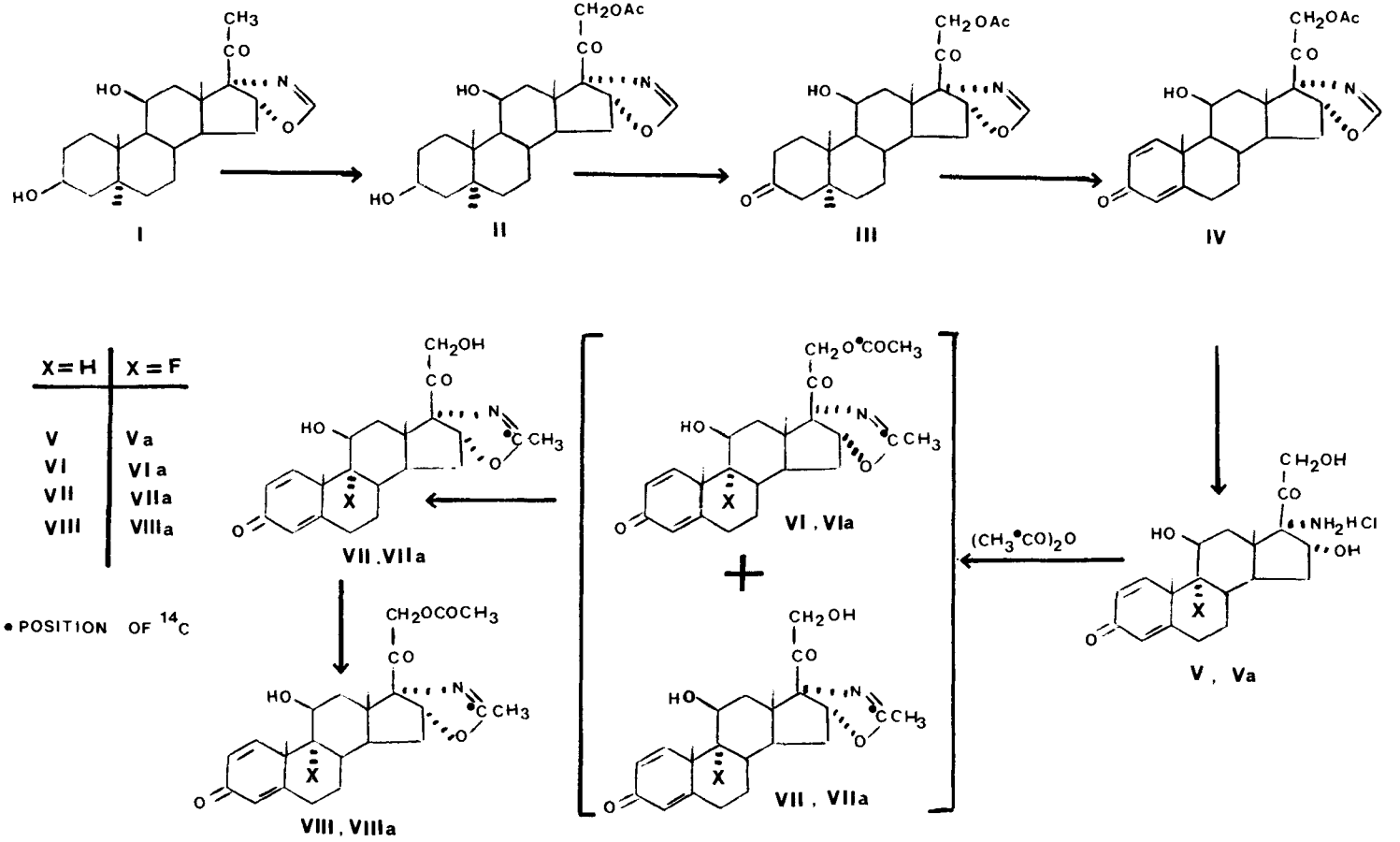

I
II
III
IV
V , Va
VI , VIa
VII , VIIa
VII , VIIa
VIII , VIIIa
(CH3•CO)2O
X = H
X = F
V
Va
VI
VIa
VII
VIIa
VIII
VIIIa
• POSITION OF 14C

The synthesis of the non-fluorinated analogue VIII, was achieved in a four step synthesis starting from the 3β,11β-dihydroxy-20-oxo-5α pregnano [17α,16α-d]oxazoline[2] I (See scheme). The compound I was acetoxylated at C-21 according to the Ringold-Stork procedure. The 21-acetate II was oxidized with aluminium isopropylate (Oppenauer) to the 3-ketone III which was dibrominated and dehydrobrominated to the diene IV by known methods. Hydrolysis of the oxazoline ring with hydrochloric acid[2,10] afforded the amino alcohol V in an overall yield of 25% from I.

Compounds V and Va were then separately reacted under carefully controlled conditions with [1-^{14}C]Acetic anhydride. The mixture of VI and VII (resp. VIa and VIIa) was hydrolysed to give pure VII (resp. VII (resp. VIIa) and then treated with unlabelled acetic anhydride to give VIII (resp. VIIIa).

EXPERIMENTAL

[1-^{14}C]Acetic anhydride, 5% w/w benzene solution, was obtained from The Radiochemical Centre, Amersham with a specific activity of 104 mCi/mmol.

TLC was carried out on silica gel 60 F_{254} (Merck) plates, layer thickness 0.25 mm, (spots visualized under UV light at 254 nm) and developed in chloroform-methanol (9:1).

Melting points, IR, UV and n.m.r. spectra were in accordance with those already determined for the corresponding unlabelled compounds[1,2].

Radioactivity was measured by Liquid Scintillating Counting[11] using an Intertechnique spectrometer model SL/30.

The specific radioactivity was determined by an internal standard method[12]. The radiochemical purity was measured by radiochromatography with a Packard Scanner mod. 7201.

3β,11β,21-Trihydroxy-20-oxo-5α-pregnano-[17α,16α,-d]oxazoline-21-acetate (II)

To a stirred solution of I (31.4 g, 0.083 mole) in a 50% mixture (470ml) of tetrahydrofuran (THF) and methanol, finely ground CaO (45 g) was added. Under vigorous stirring a solution of iodine (31.4 g) in THF (155 ml) and methanol (95 ml) was added dropwise at 20°C in a hrs period. Further CaO (15 g) was added after 2 hrs. The inorganic solid was filtered off and washed with THF. The filtrate was evaporated to dryness in vacuo and the crude 21-iodocompound was heated at reflux in a mixture of acetone (640 ml), triethylamine (TEA) (540 ml) and glacial acetic acid (300 ml). After one hour most of the acetone was evaporated in vacuo and the residue was poured into ice-water (4 l). The crude 21-acetate was extracted with chloroform and purified on a silica gel column (270 g) yielding 20 g (0.046 mole, yield 55%) of pure II, m.p. 220-224°. An analytical sample melted at 230-232° (methanol).

Anal.Calcd. for $C_{24}H_{35}NO_6$: C 66.49; H 8.14; N 3.23. Found: C 66.21; H 8.28; N 3.27.

11β,21-Dihydroxy-3,20-dioxo-5-pregnano-[17α,16α-d]-oxazoline-21-acetate(III).

From a mixture of II (24 g, 0.055 mole) in toluene (920 ml) and cyclohexanone (180 ml), 100 ml were distilled off and aluminium isopropylate (12 g) in toluene (240 ml) was added. The reaction mixture was heated at reflux temperature for 3 hrs. The solvent was distilled in vacuo and the residue was submitted to steam distillation and collected by filtration.

The dry solid was extracted with hot chloroform yielding crude 3-ketone III which was crystallized from methanol (13.5 g, 0.031 mole, yield 57%). M.p. 233-236°. An analytical sample melted at 238-240° (methanol). Anal. Calcd. for $C_{24}H_{33}NO_6$: C 66.3; H 7.71; N 3.25. Found: C 66.89; H 7.68; N 3.22.

11β, 21-Dihydroxy-3,20-dioxopregna-1,4-dieno[17α,16α-d]oxazoline 21-acetate (IV).

To a solution of III (10.36 g, 0.024 mole) in a mixture of dioxane (200 ml), glacial acetic acid (40 ml) and hydrobromic acid (4 ml of a 24% solution in acetic acid), bromine (3.84 g) in acetic acid (38,4 ml) was added dropwise at 10° C. After 30 min. the reaction mixture was poured into ice-water (1 l) containing potassium acetate (20 g).

The crude dibromo derivative was collected, dried at 50° C and dissolved in dimethylformamide (DMF) (200 ml). Lithium bromide (4.36 g), and lithium carbonate (8.74 g) were added.

The mixture was heated at 130° C for 6 hrs. The cooled reaction mixture was poured into ice-water (1.5 l), the suspension was neutralized with hydrochloric acid and extracted with ethyl acetate (3x1 l). The solvent was washed with saturated sodium chloride solution (300 ml); dried and evaporated to dryness. Crude IV was crystallized first from acetone and then from a methylene chloride-methanol mixture (1:4) yielding IV (6.1 g, 0.014 mole, 59%). M.p. 270-276°. Anal. Calcd. for $C_{24}H_{29}NO_6$ C 67.43, H 6.84, N 3.28. Found: C 67.60; H 7.05; N 3.39.

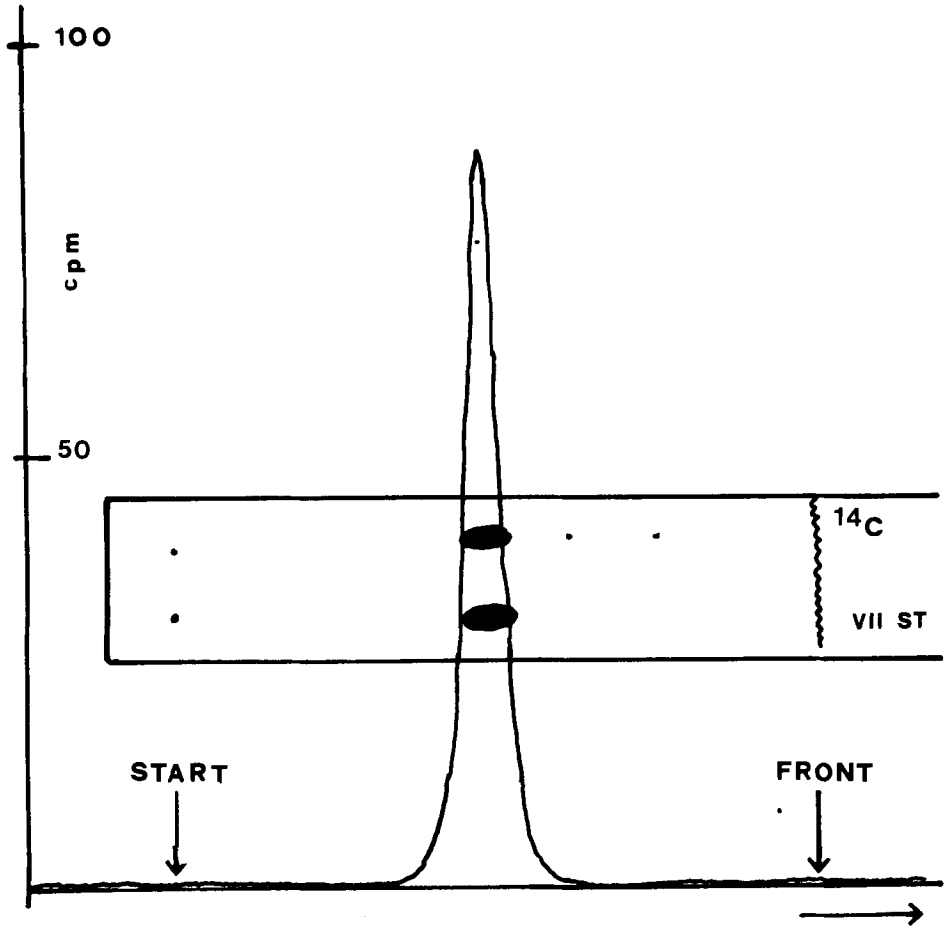
17α-Amino-11β, 16α, 21-trihydroxy-3,20-dioxopregna-1,4-diene hydrochloride (V).

A mixture of the 1,4-diene IV (4.0 g, 0.0093 mole) in acetone (80 ml) and 10% hydrochloric acid (80 ml) was stirred at room temperature for 16 hrs. The solvent was distilled *in vacuo* and the residue was repeatedly taken up in ethanol and evaporated to dryness. Compound V was obtained by crystallization from methanol yielding 3.5 g (0.0085 mole, 91.0%). M.p. 310°.

Anal. Calcd. for $C_{21}H_{29}$.HCl: C 61 23; H 7.34; N 3.40; Found: C 60.95; H 7.26; N 3.52.

11β,21-Dihydroxy-3,20-dioxopregna-1,4-dieno/17α,16α-d/ /2'-^{14}C/oxazoline--methyl 21-acetate (VIII).

To a stirred solution of V (382 mg, 0.80 mmole) in anhydrous piridine (3 ml) was added /1-^{14}C/ acetic anhydride (123.2 mg of unlabelled compound plus 9.8 mg, 10 mCi, of the ^{14}C-labelled compound) dissolved in anhydrous pyridine (3 ml). Stirring was continued at 95° C for 30 min. and the solution poured into ice-water (30 ml) containing 5.4 ml of concentrated hydrochloric acid and extracted with chloroform (5x15 ml).



The organic layer was washed with saturated solution of NaCl (3x15 ml), dried and evaporated to dryness. Crude labelled compounds VI + VIII were obtained. (350 mg, 4.86 mCi, radiochemical yield 48.6 %). TLC and radiochromatogram scan see Fig. 1.

To a stirred solution of crude labelled VI + VII (350 mg) in 2 ml of methylene chloride and 3 ml of methanol, 0.5 ml of 1N NaOH was added dropwise at 0° C. After 15 min. the mixture was neutralised with few drops of diluted acetic acid, poured into ice-awter and extracted with chloroform (5x15 ml). The organic layer was washed with saturated sodium chloride solution (2x10), dried and evaporated to dryness. Crude labelled VII was obtained (274 mg, 2.89 mCi, radiochemical yield 28.9%).
TLC and radiochromatogram see Fig. 2

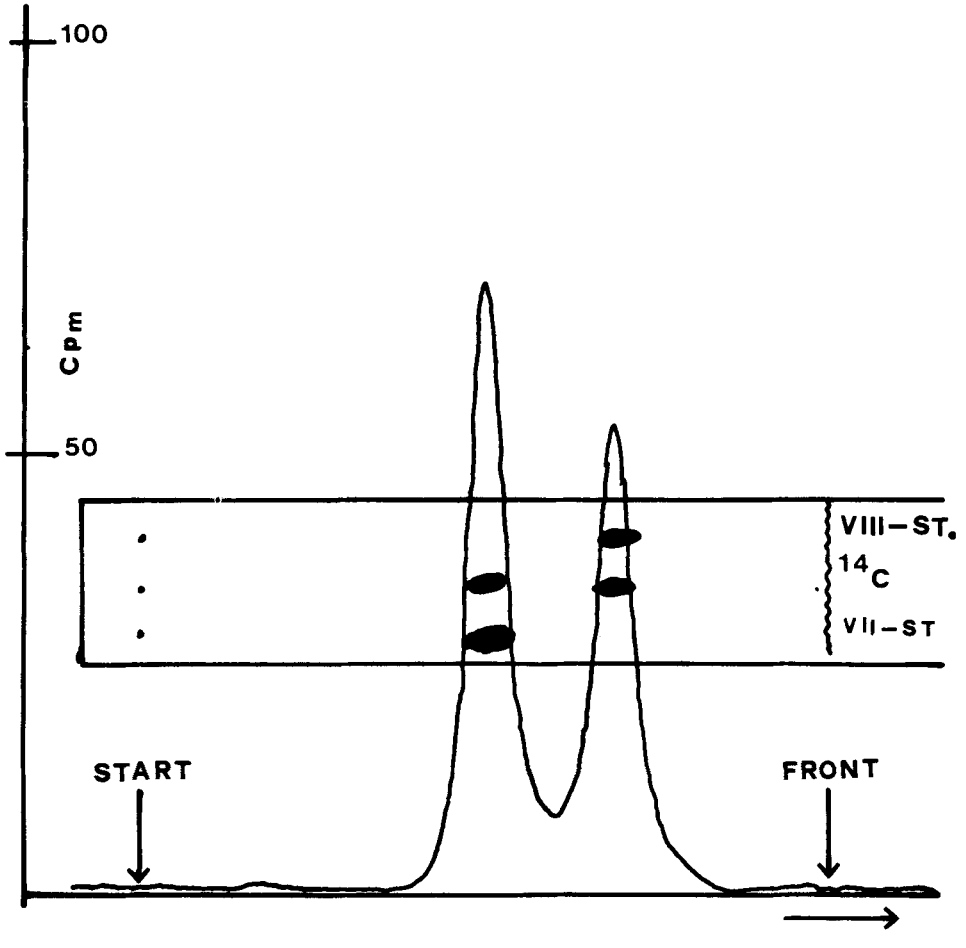


Fig. 2 - TLC and radio-TLC-chromatogram of crude labelled compound VII

To a stirred solution of crude labelled VII (274 mg, 2.89 mCi) in 2 ml of anydrous pyridine was added 0.2 ml of acetic anhydride. Stirring at room temperature was continued for 6 hrs, the solution was poured into ice-water containing 2 ml of concentrated hydrochloric acid and extracted with chloroform (5x15 ml). The organic phase was washed with saturated sodium chloride solution (2x10 ml), dried and evaporated to dryness.

Crystallization from ethyl acetate yielded labelled VIII (198 mg, 0.448 mmole, chemical yield 37.4% based on acetic anhydride) with a specific activity of 4.48 mCi/mmol (2.01 mCi, radiochemical yield 20.1 %). TLC and radiochromatogram see Fig. 3.

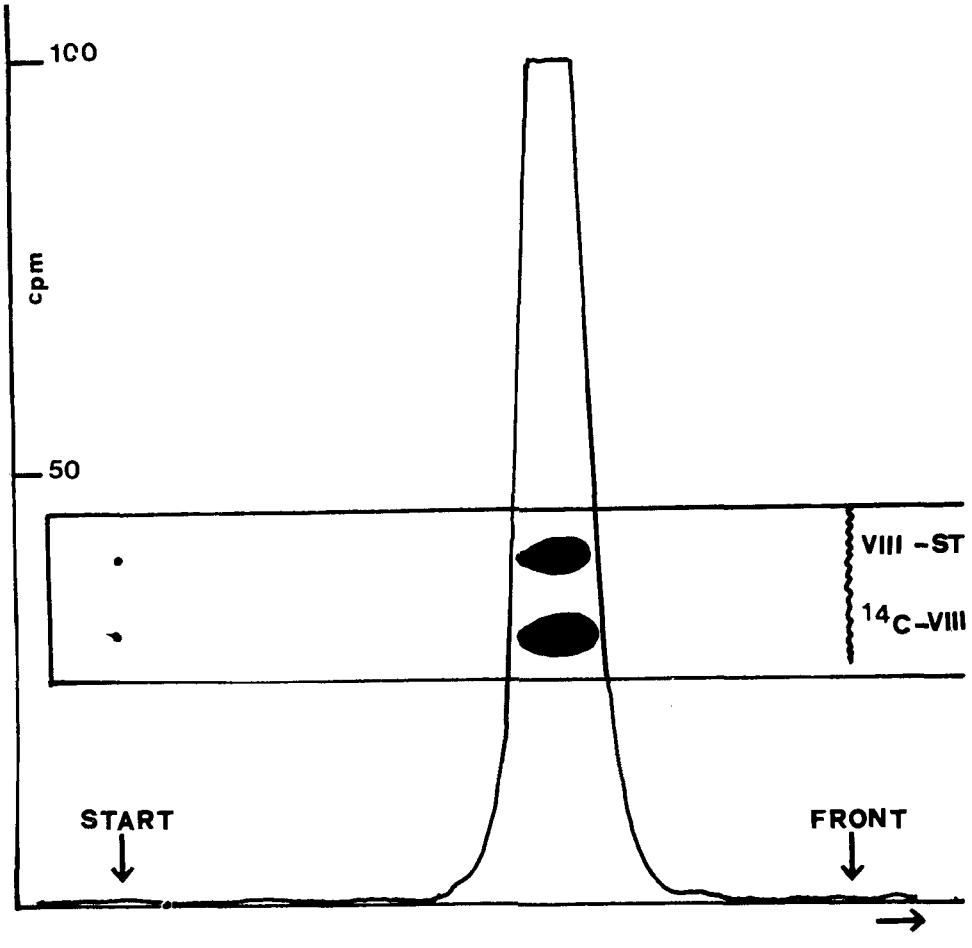


Fig. 3 - TLC and Radio-TLC chromatogram of labelled compound VIII.

9α-Fluoro-11β,21-dihydroxy-3,20-dioxopregna-1,4-dieno[17α,16α-d]-[2'-^{14}C]-oxazoline methyl 21-acetate (VIIIa).

The labelled compound VIIIa was obtained as described above for VIII. From compound Va (172 mg, 0.4 mmole) and [1-^{14}C]acetic anhydride (66 mg, 0.6 mmole, 3 mCi) 83.2 mg of labelled VIIIa were obtained. (0.18 mmole, chemical yield 30.17%) with a specific activity of 2.45 mCi/mmol (0.441 mCi, radiochemical yield 14.7%). TLC and radiochromatogram see Fig. 4.

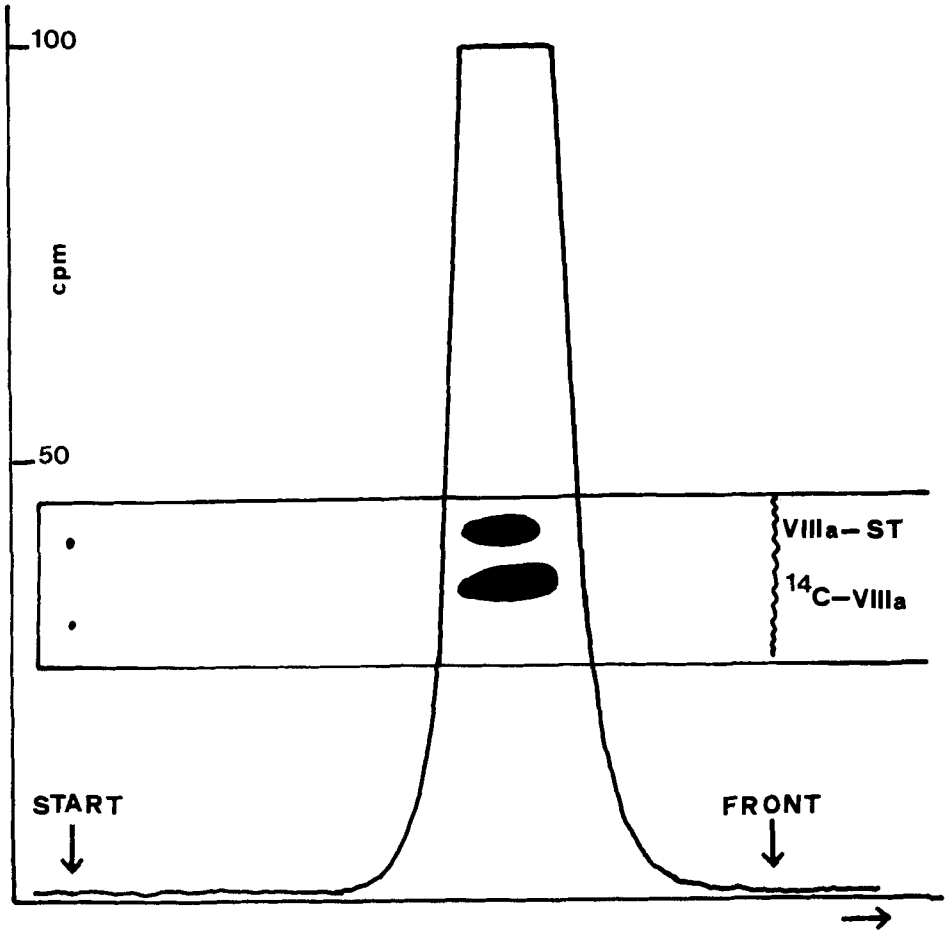


Fig. 4 - TLC and Radio-TLC-chromatogram of labelled compound VIIIa.

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REFERENCES

1) G. Nathansohn, G. Winters and E. Testa - J. Med. Chem. 10:799 (1967).

2) G. Nathansohn, G. Winters and V. Aresi - Steroids 13: 383 (1969).

3) G. Nathansohn, C.R. Pasqualucci, P. Radaelli, P. Schiatti, D. Selva and G. Winters - Steroids 13: 365 (1969).

4) F.B. Nicolis, G. Buniva and L. Bonollo - Rass. Med. Sper. 15: 249 (1968).

5) M. Marchetti, L. Bonollo and F.B. Nicolis - Boll. Chim. Farm. 111: 24 (1972).

6) L. Bonollo, G. Acocella, A. Manzini, M. Marchetti and F.B. Nicolis - Bull. Chim. Pharm. 111: 35 (1972).

7) J.D. Lewis, B.D. Cameron, D.R. Hawkins, L.F. Chasseaud and E.R. Franklin- Arzneim.-Forsch. (Drug. Res.) 25: 1646 (1975).

8) A. Assandri, G. Buniva, V. Pagani - Drug Metab. and Disposition (In press).

9) E. Martinelli, A. Assandri, P. Ferrari, G. Tuan, A. Perazzi and A. Ripamonti - Drug Metab. and Disposition (In press).

10) G. Winters, M. Cannas and G. Nathansohn - Ann. Chimica 62: 803 (1972).

11) R.D. Stubbs - Radioisotope Laboratory Techniques (Third Edition) - Butterworths, London (1973).

12) V.F. Raaen, G.A. Rapp and H.P. Raaen - Carbon 14 page 209, Mc Graw-Hill, Book Company, New York.