THE SYNTHESIS OF TWO ^{14}C-LABELLED 2-(3-ALKOXYPHENYL)-5,6-DIHYDRO-s-TRIAZOLO [5,1-a] ISOQUINOLINE COMPOUNDS, NOVEL ANTIFERTILITY AGENTS

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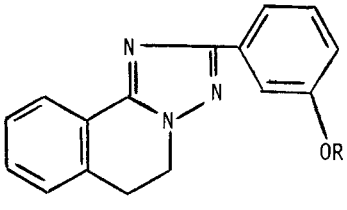
INTRODUCTION

L-10503, 2-(3-methoxyphenyl)-5,6-dihydro-s-triazolo [5,1-a] isoquinoline and DL-204 IT, 2-(3-ethoxyphenyl)-5,6-dihydro-s-triazolo [5,1-a] isoquinoline belong to a series of novel compounds developed in these laboratories[1] as non-hormonal antifertility agents [2].

They have been shown to terminate pregnancy after a single intramuscular dose in a variety of laboratory animal species [3-5], including primates[6].

Studies of pharmacokinetics, metabolism and absorption across the placenta of these compounds in animals required synthesis of ^{14}C labelled forms of both.



I, R = CH_3 L-10503

II, R = C_2H_5 DL-204 IT

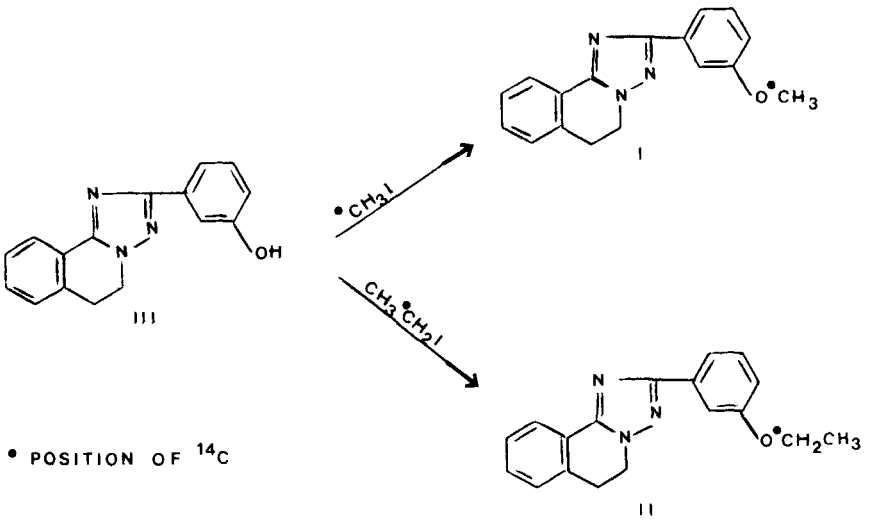
0362-4803/81/040545-09$01.00

Received December 7, 1979

DISCUSSION

The synthesis of these compounds with ^{14}C in the methyl or ethyl groups, as shown in Scheme A, was simple and inexpensive and gave I and II in fairly good yields. However, when I was administered subcutaneously to a pregnant rat, only about 30% of the radioactivity was recovered from the biological fluids. Preliminary metabolic studies showed a partial demethylation of the methoxy group, suggesting that the remaining radioactivity was exhaled as $^{14}CO_2$. The easy cleavage of the ^{14}C methyl and ethyl groups in vivo made I and II unsuitable for the planned investigations.

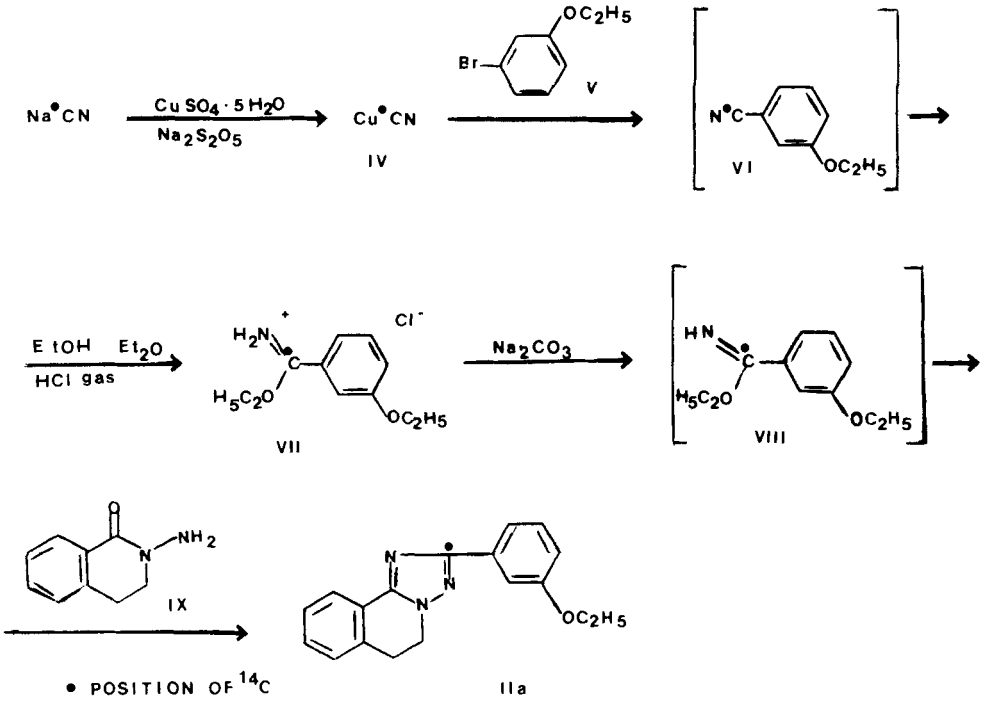
Scheme A



Incorporation of ^{14}C into the triazole ring appeared to be better for retention of the label during subsequent biological studies.

Using Na^{14}CN as radioactive starting material, DL-204 IT labelled in position 2 was synthetized by the route outlined in Scheme B. The conversion of sodium cyanide to cuprous cyanide by the method of Berber[7], was quantitative also on a small scale. By nucleophylic exchange of 3-ethoxybromobenzene V [8] in N-methylpyrrolidone [9], 3-ethoxybenzonitrile VI was obtained, and it was converted without purification to the corresponding imino ester hydrochloride VII by addition of dry hydrogen choride in ethanol-ether at 0°C.
The salt was converted to the free imino ester VIII by treatment with sodium carbonate. 2-Amino-3,4-dihydroisoquinoline-1(2H)one IX[10] was condensed under basic catalysis with VIII. Purification on the crude product by column chromatography on silicagel gave labelled IIa in a 31% radiochemical yield, with radiochemical purity greater than 98%.

SCHEME B



EXPERIMENTAL

^{14}C-Methyl Iodide (55 mCi/mmol), (1-^{14}C)-Ethyl Iodide, (24 mCi/mmol), and Sodium ^{14}C-Cyanide (60 mCi/mmol) were purchased from the Radiochemical Center, Amersham, England.

The radioactivity of the samples was measured by liquid scintillation counting [11,12] in the Intertechnique mod. SL/30 spectrometer.

The specific activities were determined by an internal standard method[11].

The radiochemical purity was determined by radiochromatography[13] using a Packard mod. 7201 Scanner. TLC was carried out on silicagel 60 F_{254} (Merck) plates and the spots were visualized under UV light at 254 nm.

IR, UV, n.m.r. spectra and melting points were in accordance with those already determined for unlabelled standards[1].

5,6-Dihydro-2/3-(^{14}C-methoxy)phenyl/-s-triazolo-[5,1-a]-isoquinoline I, M.W. 277.33

A stirred mixture of compound III (158 mg, 0.6 mmole) and K_2CO_3 (83 mg, 0.6 mmole) in DMF (5 ml) was treated with methyl iodide (73.55 mg, 5mCi or 0.055 mmole of the labelled plus 0.445 mmole of unlabelled).

The reaction mixture was stirred at 60°C for 7 hours and then the solvent was removed in vacuo. The residue was taken up in water (20 ml) and extracted with dichloromethane (5x20 ml). The extracts were washed with water (2 ml), 1N NaOH (2x5 ml) and water again(to pH 7.0), dried over sodium sulfate, filtered and concentrated to a small volume.

The crude I was purified by preparative TLC (Merck 5717, developed with chloroform-ethyl acetate 9:1 and eluted with dichloromethane) to give the labelled I (108.5 mg, 0.39 mmole , yield 76.5%) with a specific activity of 34.7 μCi/mg (9.64 mCi/mmol , radiochemicalyield 75.1%).

For the TLC and radiochromatogram: see fig. 1.

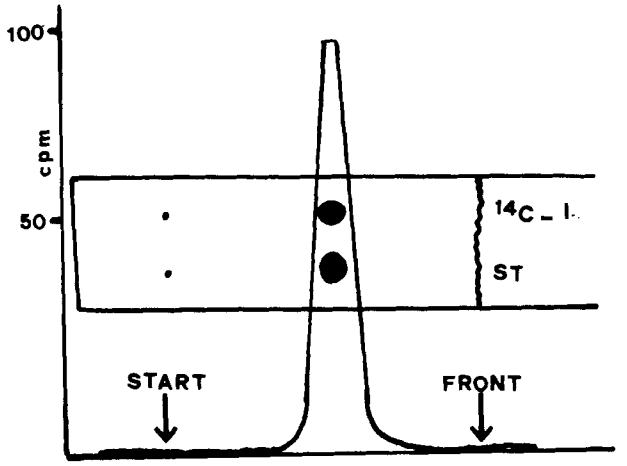


Fig. 1 TLC and radio-TLC-chromatogram of labelled ^{14}C-I developed in the solvent system chloroform-ethyl acetate 9:1

5,6-Dihydro-2 [3-(2-^{14}C)ethoxyphenyl]-s-triazolo-[5,1-a]isoquinoline

II, M.W. 291.355

The labelled compound II was prepared as described above for I. Starting from III (158 mg, 0.6 mmole) and (1-^{14}C) ethyl iodide (84.44 mg, 5 mCi or 0.20 mmole labelled plus 0.34 mmole of unlabelled), 64.62 mg of labelled II were obtained, (0.22 mmole,yield 41.0%) with a specific activity of 31,67 μCi/mg (9.23 mCi/mmol , 2.05 mCi, radiochemical yield 41.1%). For the TLC and radiochromatogram see Fig. 2.

Cuprous ^{14}C-cyanide - IV - M.W. 89.59

Copper sulphate (1250 mg, 5 mmole) was dissolved in water (4 ml) containing 0.1% H_2SO_4 (0.02 ml) and stirred at 60°C. A solution of sodium metabisulphite (350 mg, 1.84 mmole) in water (0.8 ml) was added dropwise over 3 min immediately followed by a solution of sodium ^{14}C-cyanide (8.16 mg, 0.167 mmole, 10 mCi diluted with unlabelled sodium cyanide, 237 mg, 4.833 mmole) in water (1 ml).

The reaction mixture was stirred for 10 min at 60°. The labelled cuprous cyanide was filtered off, washed with hot water, then absolute ethanol and then dried in vacuo at 70°C to give 436 mg IV (4.85 mmole, yield 97.0%) with a specific activity of 22.2 μCi/mg (1.997 mCi/mmol , 9.68 mCi, radiochemical yield 96.8%).

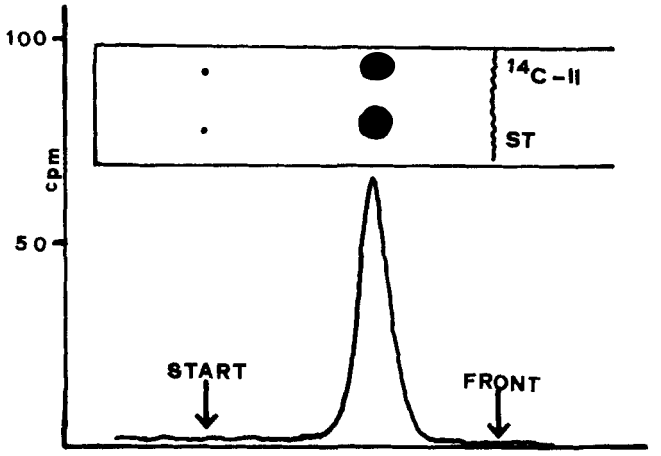


Fig. 2. TLC and Radio-TLC-chromatogram of labelled ^{14}C-II developed in the solvent system benzene-ethyl acetate 7:3

3-Ethoxybenzimidic acid ethyl ester, hydrochloride VII, M.W. 229,71

A mixture of 3-ethoxybromobenzene, V, (1005 mg, 5 mmole) in N-methyl-pyrrolidone (5 ml) and Cu^{14}CN (450 mg, 5 mmole, 10 mCi) was put in an oil bath preheated to 80°C. The temperature was then raised to 210°C within 30 min. After 1 hour the reaction mixture was cooled to 50°C and 6 ml of an aqueous solution containing 2 gm of $FeCl_3$ and 3 ml of 37% HCl was added. The mixture was stirred at 65°C for 20 min and after the addition of 20 ml of water was extracted with ethyl ether (5x25 ml).

The extracts were dried over Na_2SO_4, filtered and evaporated to dryness. The residue was dissolved in anhydrous ethyl ether (20 ml) and anhydrous ethyl alcohol (1 ml), cooled at 10°C, and dry hydrogen chloride was bubbled in for 8 hours. After crystallization at 4°C for 3 days,the labelled compound VII was filtered out dried in vacuo to give 710 mg (3.09 mmole, yield 61,8%) of ^{14}C-VII with a specific activity of 8.74 μCi/mg (6.2 mCi, radiochemical yield 62%). For the TLC and radiochromatogram: see fig. 3.

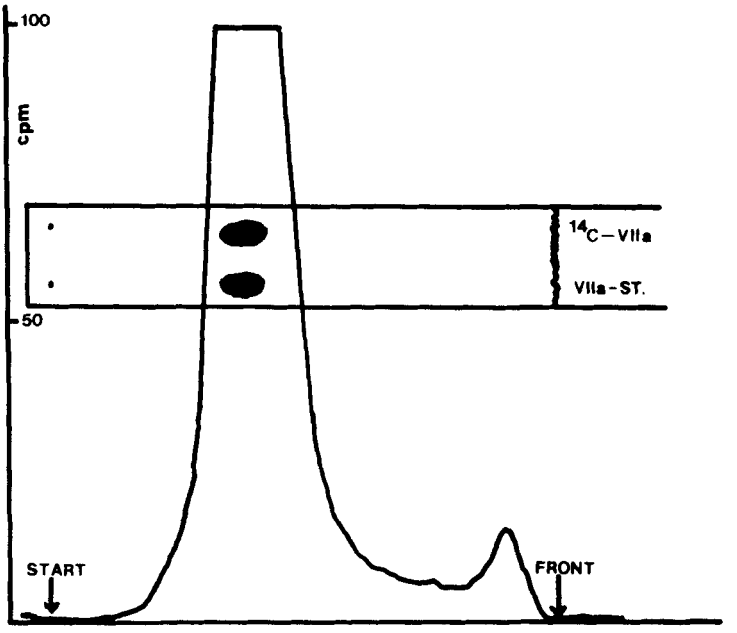


Fig. 3 TLC and Radio-TLC-chromatogram of ^{14}C-VII developed in the solvent system benzene-ethyl acetate 8:2

5,6-Dihydro-(2-^{14}C) [3-ethoxyphenyl]-s-triazolo-[5,1-a]isoquinoline. IIa M.W. 291.335

A mixture of labelled VII (710 mg, 3.09 mmole, 6.2 mCi), ethyl ether (15 ml) and a 15% aqueous solution of Na_2CO_3 (2 ml) was stirred for 2 min. The organic layer was separated off and the aqueous phase (pH 9.0) extracted with ethyl ether (2x10 ml).

The extracts were dried over Na_2SO_4, filtered and evaporated to dryness. Compound IX (490 mg, 3.03 mmole) was added to the oily residue VIII and the mixture was stirred at 90°C for 5 hours and then at 130°C for 16 hours. The mixture was cooled and, after addition of absolute ethanol (5 ml) and 55% NaH (27 mg, 0.60 mmole) was refluxed for 5 hours. The solvent was evaporated off in vacuo and the residue, dissolved in ethyl ether (20 ml), was washed with brine (3x5 ml), a 2% solution of HCl (1x3 ml) then with a saturated solution of $NaHCO_3$ (1x3 ml) and dried (Na_2SO_4). Removal of the solvent in vacuo gave a residue which was chromatographed on a silicagel column eluted with benzene-ethyl acetate 99:1. Fractions containing labelled IIa were pooled and evaporated to dryness to give labelled (2-^{14}C)- IIa (465.3 mg, 1.6 mmole, yield 31.9%) with a specific activity of 6.57 μCi/mg (1.92 mCi/mmol , 3.06 mCi, radiochemical yield 30.6%). For the TLC and radiochromatogram see Fig. 4.

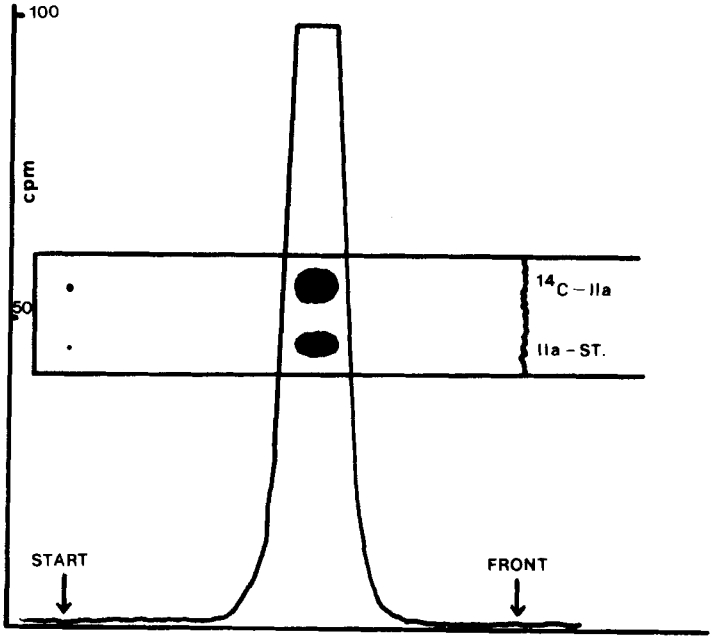


Fig. 4 TLC and Radio-TLC-chromatogram of (2-^{14}C)-IIa developed in the solvent system: chloroform-ethyl acetate 9:1

R E F E R E N C E S

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