SYNTHESIS OF 1-hydroxy-3-[3-^{3}H]-(4-chlorophenyl)-2-piperidone. LABELLING OF A NEW ANTIINFLAMMATORY HYDROXAMIC ACID.

Ph. BOVY*, M. CALLAERT*, C. GILLET*, J.M. DE DECKER‡, M. WINAND‡

*Continental Pharma Research Laboratories, Louvain-la-Neuve, B-1348 Belgium.

‡Institut National des Radio-Eléments, Fleurus, B-6220 Belgium.

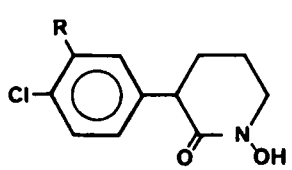
SUMMARY

Compound 1, synthezized as a potential topical antiinflammatory agent, has been labelled with tritium on the benzene ring in a position neighboring a chlorine atom. An iodinated precursor was prepared by an original six-step synthesis. Selective catalytic iodine-tritium replacement was achieved under mild reaction conditions.

Key Words : Tritium, Iodine, Antiinflammatory, Hydroxamic Acid.

INTRODUCTION

The study of the metabolic fate of 1 (CP 9337 AH), a new compound with antiinflammatory potential[1], required a tritium label. In compound 1, the phenyl group was considered as a suitable site for labelling since it combines both advantages of a selective and inexpensive tritium-halide exchange reaction and the possibility of introducing the tritium onto a metabolically stable position (see 10)[2].



1 R = H CP 9337 AH
10 R = ^{3}H

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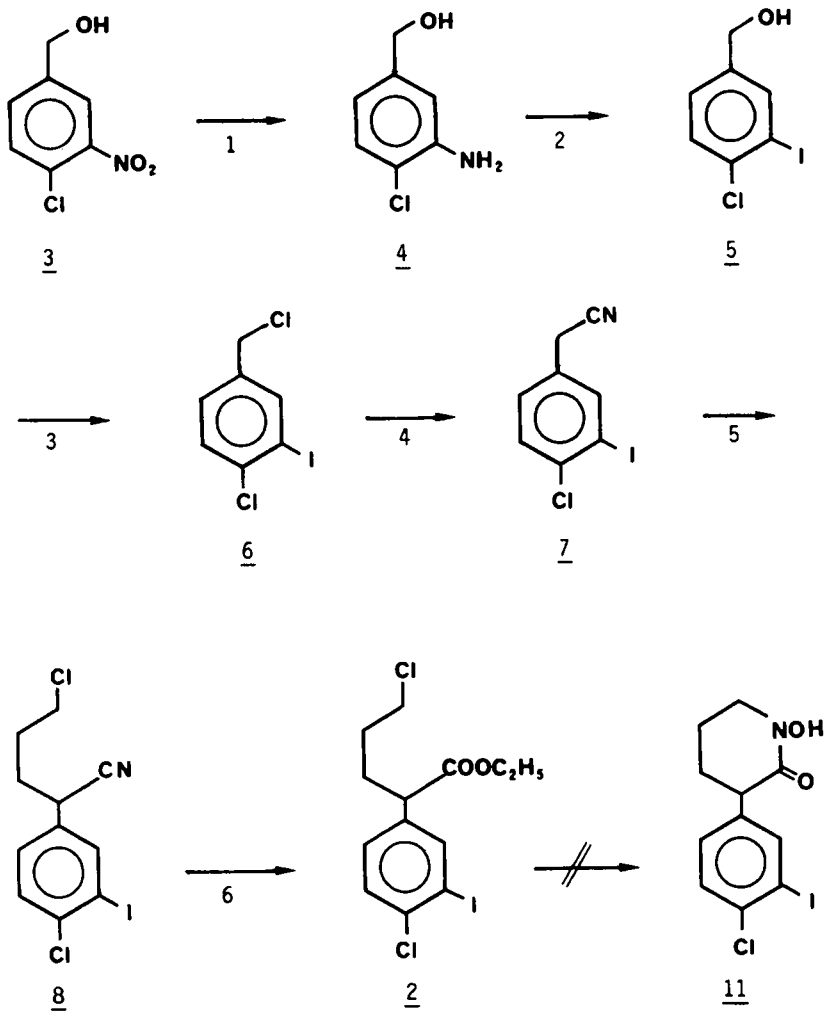
The presence of a chlorine atom on the aromatic nucleus raised the problem of selectivity during the tritiation. Since the selectivity between bromine and chlorine is not always satisfactory[3], iodine was chosen as the reactive atom for the replacement reaction. It needed to be performed in mild conditions to avoid the generation of a mixture of products in which both the halogens would be replaced by tritium.

RESULTS

The original synthesis of 2-aryl-hydroxamic acid which we developed involved a suitable substituted phenyl acetic ester as the key intermediate (2 in scheme I). Originally it was planned to perform the synthesis on the iodinated precursor, leaving the iodine-tritium exchange as the last step. This scheme had to be replaced by one in which the tritium is introduced earlier in the synthetic sequence. Compound 2 was thus chosen as the crucial intermediate for introduction of tritium.

First, a classical sequence in which iodine was introduced through a Sandmeyer reaction[4] was applied to the commercially available 4-chloro-3-nitrobenzyl alcohol 3. The desired iodo-compound 5 was obtained with a yield of 30 % after a crude purification by silicagel column chromatography. This material was submitted to chlorination with thionyl chloride. Silicagel chromatography yielded a fairly pure dichloride 6 as a crystalline solid in 67 % yield. The conversion of the benzylic chloride to benzylic nitrile was achieved by heating 6 in DMF in the presence of an excess of sodium cyanide for 45 minutes. Longer reaction times or other solvents resulted in complex reaction mixtures and decomposition of the desired product. Silicagel chromatography produced a very pure crystalline material with 71 % yield. The substitution pattern on the benzene ring was carefully checked by proton NMR : simulation of an ABX system showed a superb correlation with the observed spectrum[5]. At this point, an attempt was made to alkylate 7 through an anion generated with lithium diisopropylamide (LDA). This attempt failed since the iodo substituant was found to be unstable to treatment with a strong base. Therefore another approach involving alkylation of 7 under very mild phase-transfer conditions was applied[7]. The nitrile 7 dissol-

Scheme I



1. H_2, Ra-Ni. 2. HNO_2, HCl, KI. 3. $SOCl_2$, $CHCl_3$

4. DMF, NaCN. 5. $Cl(CH_2)_3Br$, TEBA, CH_2Cl_2, NaOH 50 %.

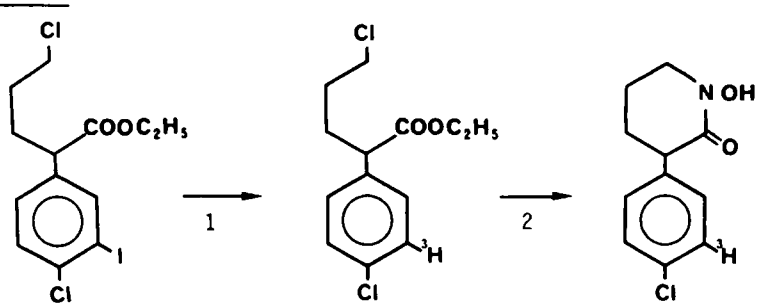
6. H_2SO_4 conc., C_2H_5OH.

ved in dichloromethane was treated with aqueous sodium hydroxide (10 N) in the presence of 3-bromochloropropane and triethylbenzylamonium chloride (TEBA). In this case, after column chromatography, the desired alkylated nitrile was isolated as a colorless oil with a yield of 44 %. This material was subjected to acid hydrolysis to give a modest yield of the expected ester 2 as an oil. This

oil was homogeneous in TLC, GLC and HPLC and showed the correct molecular ion in mass spectrometry. The proton NMR showed the distinctive pattern for the aromatic hydrogens as presented for nitrile 7. The ^{13}C NMR spectrum showed the expected thirteen signals for the different carbons; the presence of iodine was evident from the chemical shift of a meta carbon atom (98.37 ppm) which is characteristic of the heavy atom effect of iodine[8]. The subsequent step involved the cyclization to hydroxamic acid 11 in a mixture of hydroxylamine and sodium methoxide in methanol. Once again, these conditions were sufficiently basic (or nucleophilic) to induce decomposition of the iodo derivative 2. Therefore, the tritiolysis of 2, followed by cyclization was considered as the only alternative left : indeed, in this sequence, the base sensitive iodine disappears before the base catalyzed cyclization of 9 to the final compound 10 (Scheme II). The last steps of the synthesis were thus performed on microscale with very high specific radioactivity.

Iodine reduction by tritium was performed with palladium on charcoal as catalyst in the presence of thiophene to ensure selectivity of iodine exchange versus chlorine exchange[9]. The crude ethyl-5-chloro-[2-([3-^{3}H]-4-chlorophenyl)] pentanoate 9 was subsequently cyclized to the hydroxamic acid 10 in the presence of hydroxylamine under basic catalysis. The hydroxamic acid is obtained in very good chemical yield and radiochemical purity as shown by thin layer chromatography (Fig. I). This crude material of high specific activity was further diluted and purified by crystallisation with cold 1 to finally isolate a product with a specific activity of 0.22 mCi/mg, or 49,6 mCi/mmol or 1,84 GBq/mmol.

Scheme II



2 9 10

1: T_2, CH_3OH, 10 % Pd/C, thiophene 2: $NH_2OH.HCl$, CH_3OH, NaOH

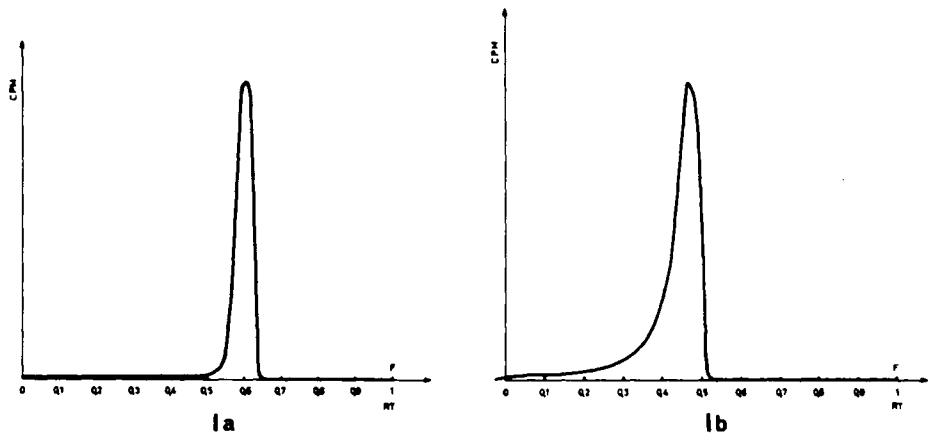


Fig. I : Radiochemical purity of 10 as assayed by TLC
(Silicagel; Ia. $CHCl_3$, MeOH, AcOH : 20/2/1 and Ib. MeOH, $CHCl_3$: 9/1).

CONCLUSION

We have described the synthesis of an original iodinated precursor for tritium-halide exchange reaction. The reaction was performed in conditions that ensure selective tritiolysis of iodine versus chlorine.
The desired tritiated chloro compound was obtained in good chemical yield and radiochemical purity.

EXPERIMENTAL PROCEDURES

Reagents were commercially available and of synthetic grade.
4-Chloro-3-nitro-benzylalcohol was obtained from Janssen Chimica.
Proton NMR spectra were recorded on a Varian T-60. Infra-red spectra were obtained as KBr pellet on a Perkin-Elmer 457 spectrometer. Mass spectra were performed on GCMS R10-10-B (Nermag) spectrometer (EI : 70 CV or CI : NH_3).
Elemental analyses were performed on a Perkin-Elmer 240 B Elemental Analyzer.
Melting points were obtained on a Mettler FP 5 and were uncorrected.

4-chloro-3-amino-benzylalcohol (hydrochloride) (4)

In a 1 l steel bomb are added successively 25.7 g ($1.4\ 10^{-1}$ moles) of 4-chloro-3-nitro-benzylalcohol, 500 ml absolute ethanol and 12 g of Raney nickel catalyst (washed with ethanol). Hydrogen is introduced to a pressure of 1000 p.s.i. and the temperature is raised to 90°C. At this point, an exothermic reaction takes place and the temperature rises further to 140°C. At the end of the exothermic reaction, the mixture is heated to 120°C and kept at this temperature for one hour. The bomb is cooled and the reaction mixture is filtered on celite. The filtrate is acidified to pH ± 2 with HCl diluted in ethylacetate and then concentrated on a rotary evaporator. When the volume is reduced to about 150 ml, the heavy precipitate is filtrered, washed with iced ethanol and dried. A second crop (± 2 g wet weight) can be collected from the mother liquor by further evaporation. The dry solids combined weighed 18.7 g ($9.6\ 10^{-2}$ moles; 70 % yield) and melted with decomposition at 180°C. Proton NMR (DMSO-d_6, ppm) : 4.5 (s, 2H); 7-7.5 (m, 3H); 8 (b.s., 4H, exchangeable).

4-chloro-3-iodo-benzylalcohol (5)

The hydrochloride (19 g, 10^{-1} mole) prepared as above is dissolved in 25.6 ml of concentrated HCl and 25.6 ml H_2O in a 250 ml erlenmeyer flask. The solution is cooled on crushed ice (T° : 0-5°C). A solution of 7 g $NaNO_2$ (0.11 mole) in 35 ml iced water is then added in small portions (2.3 ml). The addition is performed slowly so that the reaction mixture temperature does not rise above 10°C. The reaction is allowed to stir at 0°C for an additional 15 minutes. A solution of potassium iodide (16.6 g, 10^{-1} mole) in 20 ml H_2O was added to the solution of diazonium chloride; addition is completed in about 5 minutes. After stirring for an additional half hour, the reaction mixture is extracted twice with 50 ml chloroform. The organic phase is washed with 10 % NaOH, satured with $Na_2S_2O_5$ and water, separated, and dried on $MgSO_4$. After evaporation of the solvents, the residual red oil (18 g) is chromatographed (500 g Silicagel, 43-40 mesh, eluant hexane/ethylacetate; 4/1). The collected iodide (8.5 g) is pure enough for the next step (85 % homogeneous as shown by HPLC), NMR ($CDCl_3$, ppm) : 4.7 (s, 2H); 4.8 (b.s., 1H); 7.1-8 (m, 3H). TLC (SiO_2, hexane/ethylacetate; 4/1, r.f. 0.2).

3-iodo-4-chloro-benzylchloride (6)

The crude benzylalcohol (7.5 g, $2.8\ 10^{-2}$ moles) is dissolved in 200 ml dry chloroform. The resulting solution is cooled to 0°C, 16.6 g ($1.4\ 10^{-1}$ moles, 5 equivalents) of $SOCl_2$ are added and the reaction mixture is refluxed for two hours. After cooling, the solution is concentrated under vacuum and the resul-

ting brown oil (7.2 g) is chromatographed (250 g Silicagel 60, 60-230 mesh; hexane as eluant) to give 5.2 g (yield : 67 % of a crystalline white product, mp. 36.3-36.9°C. Proton NMR ($CDCl_3$, ppm) : 4.8 (s, 2H); 7.1-8 (m, 3H). The product was examined by GCMS on a capillary column (25 m, fused silica cap. CP SIL 5 CB, electron impact ionization). The main product (96.5 %) is the expected chloride: m/e (relative intensity) 286 (M^+ , 35 %); 251 (100); 252 (10), 253 (30), 254 (5), 159 (10), 161 (7). Two other products were detected : 3-iodo-benzylchloride (1.5 %) m/e : 252 (M^{+1}, 50) and 4-chloro-benzylchloride (2 %).

(4-chloro-3-iodo-phenyl) acetonitrile (7)

An aliquot of 12 ml dry dimethylformamide, 5 g 3-iodo-4-chloro-benzylchloride ($1.6\ 10^{-2}$ moles) and 2.5 g sodium cyanide are mixed in a 25 ml flask. The reaction flask is heated at 60°C for 45 minutes. The solvent is distilled off under reduced pressure and the residue is partitioned between water and diethylether phases. The decanted organic phase is dried on $MgSO_4$ and the solvent evaporated The residual oil is chromatographed (150 g Silicagel 60, 40-43 mesh; eluant : benzene) to yield 3.15 g (71 %) of pure nitrile as a pale yellow solid (mp 92.6-93). Proton-NMR ($CDCl_3$, ppm) : 3.70 (s, 2H); 7.1-8 (m, 3H). IR (film, cm^{-1}) : 2260 (CN, w), 1400, 1460 (m), low-resolution mass-spectrum m/e (relative intensities) : 280 (5), 279 (35), 278 (15), 277 (M^+, 100), 242 (20), 152 (35), 150 (100).

Anal. calcd. for C_8H_5ClIN (found) : C 34.62 (34.70), H 1.82 (1.84), N 5.05 (5.00).

5-chloro-[2-(3-iodo-4-chlorophenyl)]-pentanenitrile (8)

In a 100 ml flask under nitrogen, one add successively 1.9 g ($6.8\ 10^{-3}$ moles) 3-iodo-4-chlorophenylacetonitrile, 20 ml dichloromethane, 1.3 g 1-chloro-3-bromopropane ($8.2\ 10^{-3}$ moles) and 50 mg triethylbenzylammonium chloride. Stirring is trarted and 1.09 g 50 % NaOH ($2.7\ 10^{-2}$ moles) are added. The reaction mixture is stirred for 16 hours at room temperature. The methylene chloride is then decanted, washed with 20 % ammonium chloride, with water and dried on $MgSO_4$. After removal of the solvent, the residual oil (2.7 g) is chromatographed (100 g Silicagel 60, 40-60 mesh, benzene eluant) to give 1.07 mg, (44 % yield) of a colorless oil homogeneous (100 %) by GLC. NMR ($CDCl_3$, ppm) : 2 (m, 4H), 3.5 (m, 2H), 3.9 (m, 1H), 7.8 (m, 3H).

Ethyl-5-chloro-[2-(3-iodo-4-chlorophenyl)]-pentanoate (2)

In a 10 ml flask under nitrogen, 1.07 g (3.02 moles) of the nitrile 8 is dissolved in 2 ml 95 % ethanol and 2 ml concentrated H_2SO_4. The mixture is stirred

for 4 1/2 hours in a bath at 110°C. After cooling, 20 ml water are added and the mixture is extracted with methylene chloride and washed with 5 % $NaHCO_3$. After drying on $MgSO_4$ and removal of the solvant, the residual brown oil (1.2 g) is chromatographed (50 g Silicagel 60, 40-60 mesh, benzene/hexane, 1/1 as eluant) to give 520 mg (45 % yield) of a colorless oil which is homogeneous (> 95 % by HPLC and GLC). NMR ($CDCl_3$, ppm) : 1.12 (t, 3H, J = 7.5 Hz), 1.7-2.3 (m, 4H), 3.5 (m, 3H), 4.2 (9.2H, J = 7.5 Hz), 7.1-8 (m, 3H). Low resolution mass spectroscopy m/e (relative intensities, %) : 400 ($M^{+\cdot}$, 45), 327 (50), 251 (100).

Ethyl-5-chloro-[2-([3-^{3}H]-4-chlorophenyl]-pentanoate (9)

The iodo ester 2 (0.02 g, 0.05 mmoles) is dissolved in a mixture of dioxane (0.5 ml) and TEA (0.1 ml) containing thiophene (20 µl of 4 % solution in THF). The mixture is tritiated (30 Ci of T_2) for 20 hours at room temperature in the presence of 10 % palladium on charcoal (20 mg). The reaction mixture is then lyophilized twice from methanol. The catalyst is removed by filtration and the residue (1.9 Ci) is finally dissolved in 10 ml methanol. The purity of the product was checked by TLC (SiO_2, eluant : $CHCl_3$) : a single spot corresponding to the cold chloro compound amounts to 90 % of the radioactivity.

1-Hydroxy-3[3-^{3}H]-(4-chlorophenyl) piperidine-2-one (10)

A solution of hydroxylamine is prepared by mixing NH_4OH-HCl (10 mg) with 1.1 ml of a 0.48 M solution of sodium methanolate in methanol. The mixture is stirred at room temperature for one hour and the excess NaCl is allowed to settle. An aliquot (0.6 ml) of this freshly prepared solution is added to an ampulla containing the methanolic solution of 9. After sealing under vacuo, the reaction mixture is stirred for 3 hours at room temperature and then at 60°C for 20 hours. The resulting solution was dried by lyophilization, the residue dissolved in 10 ml HCl 0.1 N, and the solution extracted with ethylacetate. After drying on magnesium sulfate, the solution (1.1 Ci) is checked by TLC. Elution with cyclohexane:ethylacetate (4:1) on silicagel indicated complete disparition of the ester 9. Elution with CH_2Cl_2 : MeOH:HOAc (20:1:0.5) on silicagel indicated that the expected product contains 70 % of the radioactivity. An aliquot of this solution (0.55 Ci) is lyophilized and recrystallized with one gram of cold product in 2.5 ml ethylacetate (overnight at -18°C). The crystals were filtered, washed and analyzed. Total radioactivity amounted to 120 mCi in 550 mg of material (specific activity : 0.22 mCi/mg or 49.6 mCi/mmol or 1.84 GBq/mmol; yield : 21,8 %).

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