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SYNTHESIS OF TRITIATED DERIVATIVES OF THE DIPHENYLETHER HERBICIDES ACIFLUORFEN AND ACIFLUORFEN METHYL

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SUMMARY

Acifluorfen **1** and acifluorfen methyl **2**, two herbicides of the diphenylether family, are inhibitors of protoporphyrinogen oxidases. Two tritiated derivatives of these compounds, namely 3-[^{3}H]-5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid [^{3}H]-**1**, and methyl 3-[^{3}H]-5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate [^{3}H]-**2**, have been synthesised from 3-[^{3}H]-5-hydroxybenzoic acid, in order to probe their interactions with the target enzymes.

Key words : Diphenylether herbicide, enzyme inhibition, protoporphyrinogen oxidase, tritium labelling.

INTRODUCTION

Diphenylether herbicides, in particular acifluorfen **1** and acifluorfen methyl **2**, and some other molecules from unrelated families have been shown to inhibit protoporphyrinogen oxidases from

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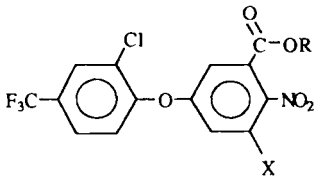
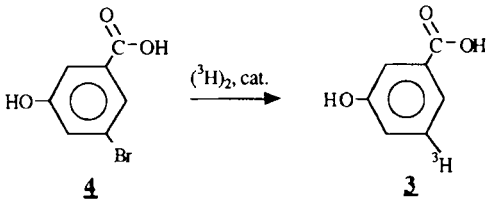
various origins (1-4). Inhibition of this enzyme in plants probably accounts for the herbicidal action of these "peroxidizing" herbicides, because their application triggers a photoinduced peroxidation of membrane lipids (5). Very low inhibition constants have been attained (IC_{50} down to 3 nM) with some compounds (4). Thus, in order to probe the interactions of these inhibitors with protoporphyrinogen oxidases, we needed radiolabelled analogues with high specific activity. We report here the synthesis of radiolabelled [^{3}H]-acifluorfen methyl [^{3}H]-**2** and [^{3}H]-acifluorfen [^{3}H]-**1**. Biological results obtained with the latter compound have been previously published (6).



1 (R = X = H) [^{3}H]-**1** (R = H ; X = ^{3}H)
2 (R = Me ; X = H) [^{3}H]-**2** (R = Me ; X = ^{3}H)

RESULTS and DISCUSSION

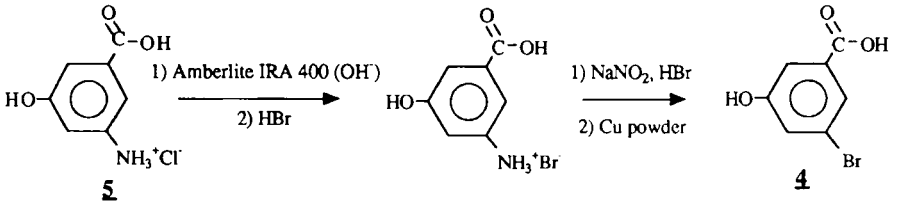
The target molecules [^{3}H]-**1** and [^{3}H]-**2** bear chloro and nitro substituents and it was not possible to introduce the tritium label at the last stage of the synthesis. Thus we started from 3-[^{3}H]-5-hydroxybenzoic acid **3** that we obtained from CEA. It was prepared by catalytic reduction of 3-bromo-5-hydroxybenzoic acid **4** by tritium gas (Scheme 1).



4 **3**

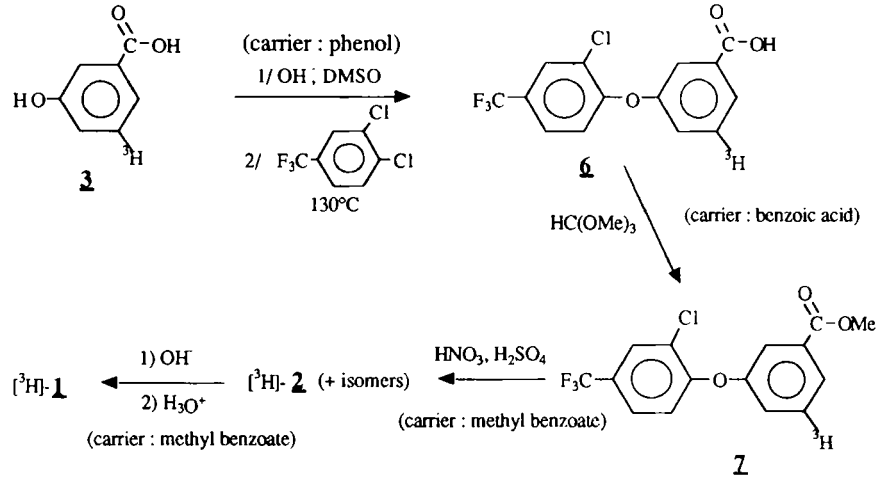
Scheme 1

We could not reproduce the published synthesis of the precursor 3-bromo-5-hydroxybenzoic acid **4** (7), thus this compound was prepared by a different route, from 3-amino-5-hydroxybenzoic acid hydrochloride **5**, which was transformed to its hydrobromide and then submitted to the Gatterman reaction (Scheme 2).



Scheme 2

[^3H]-acifluorfen methyl [^3H]-**2** and [^3H]-acifluorfen [^3H]-**1**, were synthesised from the tritiated 5-hydroxybenzoic acid **3** according to Scheme 3 :



Scheme 3

This Scheme was deduced from the general method of synthesis of diphenylether herbicides (8) It was previously checked by preparing the cold products (**6**, **7**, **1**, **2**), which served as references for chromatographic separations. At each step of the synthesis we used a carrier bearing the same functional group as that to be transformed in the corresponding radiolabelled compound. After each reaction, the product resulting from the carrier was first separated from the radioactive materials by using preparative reversed phase chromatography. The radiolabelled compounds were finally purified by chromatography on an analytical column.

The acid **6** was prepared by coupling crude 3-[^3H]-5-hydroxybenzoic **3** (40 mCi, 75% radiochemical purity) in its dibasic form, with 1,2-dichloro-4-(trifluoromethyl)benzene in dimethyl sulfoxide at 130°C. Sodium phenoxide was used as the carrier. After two chromatographic separations, a radiochemical yield of 37% was obtained for the 3-[^3H]-5-[2-chloro-4-

(trifluoromethyl)phenoxy]benzoic acid **6**. Its radiochemical purity was over 95% (HPLC).

The ester **7** was obtained by refluxing the acid **6** in methyl orthoformate in the presence of benzoic acid as the carrier. From 14.9 mCi of **6**, we obtained 10.2 mCi of ester **7** with a radiochemical purity greater than 95% (HPLC).

Nitration of the ester **7** was effected with fuming nitric acid in the presence of sulfuric acid, in dichloromethane solution. The carrier was methyl benzoate. After removal of the methyl nitrobenzoate (resulting from the carrier) by preparative chromatography, HPLC analysis of the radioactive fractions revealed the presence of a group of peaks corresponding to [^{3}H]-**2** and to its nitro isomers. The major peak which was collected in the final chromatography was attributed to [^{3}H] -acifluorfen methyl [^{3}H]-**2**. From 8 mCi of **7**, 2.5 mCi of the expected product [^{3}H]-**2** were obtained, with a radiochemical purity over 97%.

[^{3}H]-acifluorfen [^{3}H]-**1** was obtained by boiling its ester [^{3}H]-**2** in a sodium hydroxide solution in the presence of methyl benzoate as the carrier. A yield of 1.8 mCi was obtained from 2.5 mCi of **2**. The radiochemical purity of the product was over 99%. The specific activity (which was the same as that of the precursor **3** if no tritium-hydrogen exchange occured) determined by UV spectrometry and radioactivity counting was found to be 9.5 Ci.mmol^{-1}.

[^{3}H]-acifluorfen [^{3}H]-**1** was stable in methanol solution (1 mCi.mL^{-1}). A decrease of less than 1% of the radiochemical purity was measured over a 6 months period.

EXPERIMENTAL

Radioactivity was determined with a Beckman Model 1800 liquid scintillation counter, using the Beckman Ready Value® cocktail as scintillation medium. UV spectra were recorded on a Perkin Elmer Model Lambda 2 spectrometer using 1 cm path length quartz cells. NMR spectra were recorded on a Varian EM 360 spectrometer using tetramethylsilane as an internal standard. HPLC purifications were performed on a Waters apparatus including two pumps (M6000A and M45 models), a solvent programmer M660, a Rheodyne injector equipped with a 2 ml sample loop and an UV detector M440 (254 nm). The recordings of the chromatograms were taken on a Shimadzu CR-3A integrator. The following solvents were used for the purification of labelled compounds: solvent A was a mixture of methanol-water-acetic acid (40:60:1); solvent B was a mixture of water-methanol (40:60). Three

Merck columns were used for the purifications: column A (310x25 mm), and column B (240x10) were preparative glass columns loaded with Lichroprep RP 18 (40-63 μm) and column C (125x4) was a steel analytical column loaded with Lichrospher 100 RP 18 (5 μm). The flow rate of the eluents was 1 mL.min^{-1}. Unlabelled substances which served as references were prepared following the Patent Literature (8) and unpublished information from Rohm and Haas Company. 3-[^{3}H]-5-hydroxybenzoic acid **3** was provided by the Service des Molécules Marquées du CEA (France). It was prepared (77% yield) by catalytic reduction (10% Pd on carbon; KOH 10^{-2} M ethanolic solution) with tritium gas (20 Ci) of 3-bromo-5-hydroxybenzoic acid **4** (5 mg). The radiochemical purity of compound **3** was determined by TLC on cellulose plates using a mixture of 20% KCl in water-acetic acid (100:1) as the eluent, and scanning the radioactivity on the plate. It was found to be 75%.

3-bromo-5-hydroxybenzoic acid **4**.

1.9 g (10 mmol) of 3-amino-5-hydroxybenzoic acid hydrochloride **5** (Fluka) was dissolved in 100 mL of a solution of 0.8 g (20 mmol) of sodium hydroxide. 10 g of Amberlite IRA400 ion exchange resin (OH^- form) were added, and the suspension stirred for 3 h. The resin was filtered off, washed with distilled water and poured into 10 mL of a 6% solution of hydrobromic acid. The resin was filtered off and the filtrate evaporated under reduced pressure, leaving a solid residue (1.61 g of 3-amino-5-hydroxybenzoic acid hydrobromide). This residue was dissolved in 20 mL of a 47% HBr solution in water, and the solution cooled in an ice-water bath. A solution of 1 g of sodium nitrite (14.5 mmol) in 10 mL of water was added in small portions, the temperature being kept between 0-5°C. The solution was then stirred for 0.5 h at room temperature, 0.5 g of copper powder was added, and the suspension kept at room temperature for 2.5 h, then heated at 50°C for 1 h. 200 mL of water were added and the mixture extracted with diethyl ether (2x100 mL). The extracts were evaporated at atmospheric pressure and the solid residue dissolved in a solution of sodium hydroxide (0.8 g) in water (50 mL). After extraction with diethyl ether (50 mL), the aqueous solution was acidified with a 10% HCl solution and extracted again with two portions of 50 mL of diethyl ether. The extracts were dried over sodium sulfate. Evaporation of the solvent left a solid which was chromatographed through a silica gel column using a mixture of methanol- dichloromethane-acetic acid (10:90:1) as eluent. The fractions of the eluate were analyzed by TLC on silica gel using the above eluent. The eluate fractions which contained the two fastest migrating products, which were

poorly separated, were pooled and the solvent evaporated to dryness. The residue was chromatographed through column A using solvent A as eluent. The first eluted product was the expected 3-bromo-5-hydroxybenzoic acid **4** (0.30 g, 14% yield); mp 255 °C [Litt. (6) 233.5 °C]. Molecular weight determination by acidimetric titration gave 216.8 (theoretical 217); ^{1}H NMR (DMSO-d_6) δ 7.23 (m, 1H, phenyl H), 7.40 (m, 1H, phenyl H), 7.57 (m, 1H, phenyl H).

3-[^{3}H]-5-[2-chloro-4-(trifluoromethyl)phenoxy]benzoic acid **6**.

A solution containing 40 mCi of crude 3-[^{3}H]-5-hydroxybenzoic acid **3** (75% of radiochemical purity) in 19 mL of ethanol was mixed with 31.1 mg of a 46.2% aqueous solution of sodium hydroxide (0.36 mmol). The solvents were evaporated under vacuum to dryness. To the residue were added 136.8 mg of phenol (1.475 mmol), 126.3 mg of the 46.2% aqueous solution of sodium hydroxide (1.459 mmol) and 10 mL of methyl sulfoxide (DMSO). 3 mL of a water-DMSO mixture were distilled off under reduced pressure through a short Claisen column. 1,2-Dichloro-4-(trifluoromethyl)benzene (385.5 mg, 1.793 mmol) was added and the solution heated for 4 h at 130 °C. After cooling to room temperature, DMSO was distilled off as above, and 3 mL of water added to the residue. The aqueous solution was extracted with 2x3 mL of diethyl ether, then acidified to pH 3 with HCl 1M. The aqueous layer was concentrated in a rotatory evaporator to about 1 mL. 3 mL of solvent A were added and the resulting solution chromatographed through column B, with a linear elution gradient (solvent A vs methanol 30% to 100% over 1.5 h). Radioactivity eluted under two main peaks. The fractions corresponding to the second peak were pooled and the solvents evaporated under reduced pressure. The residue was dissolved in 2 mL of solvent A and the resulting solution chromatographed through column C, with a linear elution gradient (solvent A vs methanol 30% to 100% over 20 min). The peak with retention time of 14.9 min corresponding to 3-[^{3}H]-5-[2-chloro-4-(trifluoromethyl) phenoxy]benzoic acid **6** was collected and contained 14.9 mCi. The product assayed by HPLC was found to have a radiochemical purity of 95%.

Methyl 3-[^{3}H]-5-[2-chloro-4-(trifluoromethyl)phenoxy]benzoate **7**.

The above eluate solution of 3-[^{3}H]-5-[2-chloro-4-(trifluoromethyl)phenoxy]benzoic acid **6** (14.9 mCi) was evaporated to dryness. To the residue were added 8 mL of methyl orthoformate and

50 mg of benzoic acid. The solution was heated to reflux for 9 days. The solvent was evaporated to dryness, and the residue was taken up in 2 mL of solvent A. The solution was injected in column B which was eluted using a linear gradient (solvent A vs methanol 30% to 100% over 1.5 h). The fractions containing the major peak of radioactivity were pooled and the solvents evaporated. The residue was dissolved in 2 ml of solvent B and the solution chromatographed through column C with a linear elution gradient (solvent B vs methanol 30% to 100% over 15 min). The peak with retention time of 16.7 min corresponding to methyl 3-[^{3}H]-5-[2-chloro-4-(trifluoromethyl) phenoxy]benzoate **7** was collected and contained 10.2 mCi. The compound **7** was assayed by HPLC and its radiochemical purity was foud to be over 95%.

Methyl 3-[^{3}H]-5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate ([^{3}H]-acifluorfen methyl) [^{3}H]-**2**.

To 8 mCi of an ice-water cooled solution of methyl 3-[^{3}H]-5-[2-chloro-4-(trifluoromethyl) phenoxy]benzoate **7** in 3 mL of dichloromethane, were added 113 mg (0.83 mmol) of methyl benzoate, 300 µL (3.2 mmol) of acetic anhydride, 15 µL of concentrated sulfuric acid (0.27 mmol) and fuming white nitric acid (65 µL, 1.39 mmol). The mixture was stirred at 0 °C for 1 h. Water (3 mL) was added and the mixture was shaken. The water layer was pipeted off and the organic phase washed again with 2x3 mL of water. The solvent was evaporated and 2 mL of solvent B were added to the residue. The resulting suspension was filtered through a Millex® HV (0.45 µm) filter. The filtrate was injected in column A. Elution was carried out using a concave gradient (solvent B vs methanol 60% to 100% over 1 h). The collected fractions containing the peak of radioactivity were pooled and the solvent evaporated. The residue was taken up in 2 mL of solvent A and the solution chromatographed through column C using a concave elution gradient (solvent A vs methanol 60% to 100% over 15 min).The peak with a retention time of 11.6 min corresponding to [^{3}H]-acifluorfen methyl [^{3}H]-**2** was collected and it contained 2.5 mCi. The obtained product [^{3}H]-**2** was assayed by HPLC and its radiochemical purity was found to be over 97%.

3-[^{3}H]-5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid ([^{3}H]-acifluorfen) [^{3}H]-**1**.

To 2.5 mCi of 3-[^{3}H]-acifluorfen methyl [^{3}H]- **2** in 1 mL of solvent A were added 1.5 mL of a

1M solution of sodium hydroxide and 96 mg (0.706 mmol) of methyl benzoate. The solution was heated at reflux for 45 min. After cooling to room temperature, the solution was acidified by 1 ml of a 2 M solution of HCl, and passed through a C18 Seppak® cartridge. After being washed by 2 mL of water, the cartridge was eluted by 2 mL of methanol. The solvent of the eluate was evaporated off and the residue dissolved in 2 mL of solvent A. The resulting solution was chromatographed through column B with a linear elution gradient (solvent A vs methanol 30% to 100% over 1 h). The collected fractions corresponding to the peak of radioactivity were pooled and the solvent evaporated. The residue was dissolved in 2 mL of solvent A, and the resulting solution chromatographed through column C with a linear elution gradient (solvent vs methanol 30% to 100% over 15 min). The peak at 8.5 min which corresponded to [^{3}H]-acifluorfen [^{3}H]-**1** was collected. It contained 1.8 mCi of pure [3H]-**1**. The radiochemical purity of the product was over 99%. Specific activity was determined by measuring UV absorbance of the solution at 285 nm (ε_{max} = 6.23x10^3 cm^{-1}.mol^{-1}.L for acifluorfen) and counting the radioactivity of an aliquot, and was found to be 9.5 Ci.mmol^{-1}.

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REFERENCES

1. Matringe M., Camadro J.M., Labbe P. and Scalla R. - FEBS Lett. 245: 35 (1989)
2. Matringe M., Camadro J.M., Labbe P. and Scalla R. - Biochem. J. 260: 231 (1989)
3. Witkowski D.A. and Halling B.P. - Plant Physiol. 90: 1239 (1989)
4. Camadro J.M., Matringe M., Scalla R. and Labbe P. - Biochem. J. 277: 17 (1991)
5. Scalla R., Matringe M., Camadro J.M. and Labbe P. - Z. Naturforsch. 45c: 503 (1990)
6. Varsano R., Matringe M., Magnin N., Mornet R. and Scalla R. - FEBS Lett. 272: 106 (1990)
7. Inubushi Y. and Amano T. - J. Pharm. Soc. Japan 72: 1459 (1952)
8. Swithenbank C. and Yih R.Y. - US Pat. 3,798,276 (1974)
   Johnson, W.O. - US Pat. 4,031,131 (1977)