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Synthesis of ^{14}C-(Carbonyl)-N-(4-iodo-2,6-diethylphenylcarbamoylmethyl)-iminodiacetic acid

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SUMMARY

The synthesis of the N-(4-iodo-2,6-diethylphenylcarbamoylmethyl)iminodiacetic acid labelled with ^{14}C at the carbonyl carbon atom which was required for metabolism studies is described. The complex formation between the title product and Tc-99m is also presented.

In 1975, Harvey *et al.* (1) reported the preparation of 2,6-dimethylphenylcarbamoylmethyl-iminodiacetic acid and its properties as ^{99m}Tc complex formation agent for the study of the hepatobiliar trak. Since then, several authors in an attemp to improve the biological properties of the above mentioned product, have prepared a considerable number of similar compounds. In particular, Subramanian *et al.* (2) prepared an iodinated derivative where the iodine is in the aromatic ring, namely 3-iodo-2,4,6-trimethylphenyl carbamoylmethyl-iminodiacetic acid (Iodofenin) while Nunn *et al.* (3) following this approach introduced bromine in the same 3-position of the aromatic ring (Mebrofenin). Iodofenin has better biological properties than Bromofenin while both halogenated products, being more lipophylic than their non-halogenated counterparts, have better transport properties and higher hepatic absorption than similar compounds lacking halogen in their respective structures.

In order to test the metabolism of those halogenated products and of its complex with ^{99m}Tc, we prepared a ^{14}C-labelled agent, namely ^{14}C-*carbonyl* N-(4-iodo-2,6-diethylphenylcarbamoylmethyl)iminodiacetic acid (**3**) and its ^{99m}Tc-complex.

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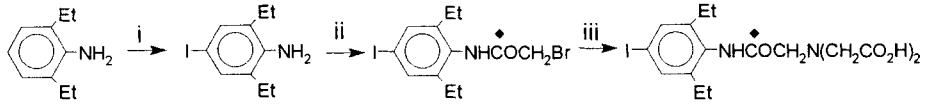
RESULTS AND DISCUSSION

The title compound, needed for metabolism studies, was prepared by reaction of 4-iodo-2,6-diethylaniline (**1**) (see Scheme) with [1-^{14}C] α-bromoacetic acid obtained by bromination of [^{14}C] acetic anhydride (4). The resulting derivative **2** was condensed with iminodiacetic acid following a previously described procedure (5). Compound **3** was obtained in 41.4 % yield with more than 95% of radiochemical purity.

The formation of the complex with ^{99m}Tc was performed as previously described (5) and was purify through Sep-Pack C-18 (Waters) cartridges as described in Experimental. The complex, stable for not less than 3 h, was obtained with a radiochemical purity higher than 95 % (6).



1 **2** **3**

i= I_2
ii= Br_2/[^{14}C]Ac_2O/Et_3N
iii= $NH(CH_2CO_2H)_2$/EtOH/H_2O/NaOH
• = ^{14}C

Fig. 1. Synthesis of ^{14}C-(carbonyl)-N-(4-iodo-2,6-diethylphenylcarbamoylmethyl)-iminodiacetic acid.

EXPERIMENTAL

Melting points are uncorrected. ^{1}H-NMR spectra were obtained in $CDCl_3$ solutions using TMS as internal standard and were registered on a Varian XL-100-15 spectrometer operating in the FT mode. Infrared spectra were recorded for KBr pellets on a Jasco A-100 spectrophotometer. Gas chromatographic analyses were performed with a Hewlett-Packard 5890 GC having a FID detector and using a methyl silicone capillary column (50 m x 0.31 mm). HPLC analyses were conducted with a Hewlett-Packard 1090L liquid chromatograph having a DA detector. ^{14}C-Radioactivity was measured on a Packard TriCarb 1500 liquid scintillation counter. ^{99m}Tc-Radioactivity was measured on a monochannel γ-spectrometer. Microanalysis were performed at UMYMFOR (CONICET-FCEN).

4-Iodo-2,6-diethyl-aniline (**1**). 4-Iodo-2,6-diethyl-aniline (**1**) was prepared by shaking overnight at 0°C, 2,6-diethylaniline (Aldrich)(3 g, 20 mmol) and iodine (12.7 g, 50 mmol) in the presence of cationic resin (Dowex 50-X12) (40

meq) suspended in water (40 ml). The filtered and washed (water and CH_2Cl_2) resin was treated with 0.1 M NaOH (30 ml) and compound **1** was extracted with CH_2Cl_2 yielding 1.9 g, 7 mmol (35%).

^{14}C-(Carbonyl)-N-(4-Iodo-2,6-diethylphenyl)-2-bromo-acetamide (**2**). [^{14}C]Acetic anhydride (CEA-CMM-39A) (6.3 mCi/mmol, 1 mCi) diluted with acetic anhydride (2.04 g, 20 mmol) was treated with dry Br_2 (2 ml) and the mixture heated at 100°C for 1 h. After removing the excess of Br_2 by a stream of dry N_2, and addition of CH_2Cl_2 (3 ml) and triethylamine (1 g, 10 mmol), the mixture was cooled to -30°C. Compound **1** (1.5 g, 5.5 mmol) was added, and after stirring for 1 h the solvent was evaporated and the precipitate was filtered, washed with water (50 ml), with EtOH-water (1:1, 30 ml) and dried. Compound **2** (1.6 g, 4 mmol) (73% yield) had mp 208-210°C; IR: 3250 (NH), 1660 (C=O), 980 (CN) cm^{-1}.

^{14}C(Carbonyl)-N-(4-iodo-2,6-diethylphenylcarbamoylmethyl)-iminodiacetic acid (**3**). Compound **2** (1.6 g, 4 mmol) and iminodiacetic acid (1.1 g, 8 mmol) were dissolved in EtOH-water (20:15 ml) containing NaOH (0.64 g, 16 mmol). To this solution, K_2CO_3 (0.275 g) in water (3.2 ml) was added dropwise keeping a pH=11. After stirring 24 h at room temp, the ethanol was removed, water (40 ml) was added and the soln was made acid to pH 2.5 by addition of HCl. The precipitate was filtered, washed with diluted HCl and with cold EtOH-water (1:1, 30 ml), and dried. HPLC analysis (RP-18 ODS-Hypersil 5 μm, 100 x 6 mm column) eluting with THF-MeOH (1:1) showed a single signal with tr: 0.85-0.90 min. Compound **3** (1.3 g; 2.9 mmol)(73% yield) had mp 213-218°C and specific radiactivity of 28 μCi/mmol.
IR: 3450 (OH), 3250 (NH), 1660 (C=O), 980 (CN) cm^{-1}.
^{1}H-NMR (DMSO-d_6): δ 1.05 (6H, t, J=7 Hz, $C\underline{H}_3CH_2Ph$), 2.45 (4H, q, J=7 Hz, $CH_3C\underline{H}_2Ph$), 3.49 (2H, s, $COCH_2N$), 3.56 (4H, s, NCH_2COOH), 7.45 (2H, s, aromatic protons), 9.48 (2H, s, COOH). Analysis: calc. for $C_{16}H_{21}N_2O_5I$, C: 42.87; H: 4.72; N: 6.25; I: 28.31. Found, C: 43.16; H: 4.80; N: 6.24; I: 28.19 %

Preparation of ^{99m}Tc-complex. Compound **3** (27 mg, 0.060 mmol) was dissolved in 0.25N NaOH (1.5 ml); to the stirred solution, $SnCl_2$ (10 mg Sn^{++}/ml) in 1N HCl (50 μl) was added bringing a final pH of 5.5-6.0, followed by a soln of $Na^{99m}TcO4$ (20 mCi) in water (1 ml). After 15 min the radiochemical purity of the complex was controlled as described elsewhere (5,6) giving values higher than 95%. Further purification was attained using Sep-Pak C18 cartridges. For this purpose, the reaction mixture was inoculated in a water-washed cartridge which was then washed with saline solution (2 x 5 ml) to eliminate excess of salts ($SnCl_2$ and $Na^{99m}TcO_4$); elution with 40% EtOH (2 x 4 ml) removed compound

3 in excess, the complex was then eluted with 90% EtOH (2 x 1 ml); to this fraction, 0.1M phosphate buffer (pH=5.8) was added to stabilize the pure complex.

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