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IODINATION, RADIOIODINATION AND SPECTROSCOPIC IDENTIFICATION OF β-CARBOLINE DERIVATIVES.

Jose A. Sintas and Arturo A. Vitale*

PROPLAME-CONICET, Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, 1428 Buenos Aires, Argentina

SUMMARY.- The synthesis and spectral properties (MS, and NMR) of radioiodinated β-carbolines: 6- **(I)** and 8- **(II)** [^{131} I]-iodo-1-methyl-7-methoxy-β-carboline ([^{131} I]-iodo-harmine), 6- **(III)** and 8- **(IV)** [^{131} I]-iodo-1-methyl-β-carboline-7-ol ([^{131} I]-iodo-harmol), 8-[^{131} I]-iodo-1-methyl-7-methoxy-3,4-dihydro-β-carboline (8-[^{131} I]-iodo-harmaline) **(V)**, 8-[^{131} I]-iodo-3,4-dihydro-1-methyl-β-carboline-7-ol (8-[^{131} I]-iodo-harmalol) **(VI)**, 6-[^{131} I]-iodo-1-methyl-β-carboline (6-[^{131} I]-iodo-harmane) **(VII)** are described. These are compounds of biological importance and can be used for brain mapping with SPECT technology.

Keywords: [^{131} I]-Iodo-β-carbolines; preparation and characterization.

INTRODUCTION

As it is known, alteration of the monoamine oxidase (MAO) activity in the brain has been implicated in aging, Alzheimer's disease, Huntington's disease, alcoholism, suicides, and affective illness. Harmala alkaloids are known as MAO inhibitors. At least one of them is present in the pineal gland of both humans and several animals. [1-5]

The anatomical distribution of [^{3}H]-norharman binding sites was determined by quantitative autoradiography in rat brain slices,[6] suggesting that a unique class of [^{3}H]-norharman binding sites exists in the rat brain. This finding is consistent with earlier experiments which showed high-affinity binding sites for [^{3}H]-norharman in rat brain membranes. Provided the binding sites represent functional receptors, these anatomical findings may explain the biological effects of norharman.

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* Research Member of the National Research Council of Argentina (CONICET), to whom any inquiries should be addressed.

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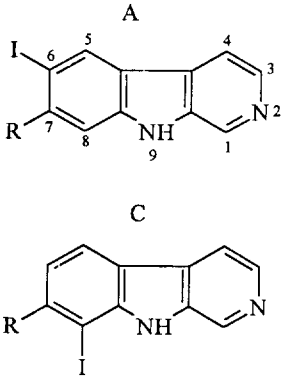
In continuation of our work on labelling compounds of biological and pharmaceutical importance,[7,8] in this paper we report the syntheis of seven new radioiodinated harmine derivatives appropriate for mapping and metabolic studies.

RESULTS AND DISCUSSION

Iodination and radioiodination. These methods have been previously developed in our laboratories. [7,8]

Two methods were used for the preparation of iodinated derivatives, the McKillop method for iodination of aromatic compounds [9] with thallium derivatives as intermediates (A), and thallium trifluoroacetate as specific oxidizing agent of molecular iodine (B). The McKillop method led to the radioiodinated products at non-carrier added levels from commercial $Na^{131}I$. Iodinated derivatives **(V-VII)** were prepared by using method (A) with a radiochemical purity over 97 % and derivatives **(I-VII)** were exchange-labelled (Method C). Derivatives **(I-IV)** were not labelled by method (A) in order to avoid the recrystallization step of the radioactive product. So this procedure proved to be useful for the preparation of seven radioiodinated derivatives of harmine that can be used for mapping and metabolic studies.

Scheme 1. Iodinated β-Carbolines.



I. A, R=OCH3 II. C, R=OCH3
III. A, R=OH IV. C, R=OH
V. B, R=OCH3 VI. C, R=OH
VII.A, R=H

EXPERIMENTAL

General methods. Solvents were of the maximum purity available and their purity was checked by gas chromatography. Starting material, (all five β-carbolines) were from Aldrich, carrier-free $Na^{131}I$ from Amersham. TLC analyses were performed on aluminum baked silica gel 60 F_{254} plates (0.2 mm) obtained from Merck and were visualized using an ultraviolet light (254 and 366 nm) or I_2. Mass spectrometry was achieved on a Trio2 VG spectrometer operating at 70 eV. Melting points were recorded in a Fisher-Jones apparatus and are

uncorrected. All ^{1}H NMR spectra were recorded in a Bruker ACE 200 in DMSO-d_6 or methanol-d_4 as stated. Resonances are reported downfield from internal tetramethylsilane. Samples were counted in an automatic gamma detector (Clinigamma Pharmacia).

IODINATION

Method A. To a solution of thallium trifluoroacetate (0.33 g, 0.61 mmol) in acetonitrile, the β–carboline in 50% acetonitrile/methanol (0.68 mmol) was added slowly. The reaction mixture was heated under reflux for four hours and the solvent was evaporated under reduced pressure. The crude solid was dissolved in dichloromethane and a solution of 0.2 g (1.22 mmol) of KI was added. The reaction mixture was stirred at room temperature for 15 minutes and vacuum filtered to remove the remaining solid (TlI). A NaOH (2N) solution was added to the filtrate and the organic material extracted with CH_2Cl_2 , before being dried (anh. $MgSO_4$). Finally the solvent was removed. The crude solid was recrystallized from methanol yielding the corresponding iodinated β-carboline as a light yellow solid. Iodinated β-carbolines **(I - II)** and **(III - IV)** were purified by recrystallization from methanol.

Method B. To a solution of the β-carboline in 50% acetonitrile/methanol (0.68 mmol), the solution of thallium triflouroacetate (40 mg, 0.72 mmol), in acetonitrile was added slowly at room temperature. Some drops of trifluoroacetic acid were added in order to increase the solubility of the β-carbolines at room temperature. Then a solution of molecular iodine in methanol was added until a permanent iodine color was obtained. The reaction mixture was stirred at room temperature for 15 minutes and further vacuum filtered. The crude solid was purified in the same way as that described in method **A**.

Although both methods gave similar yields, the second method allowed the reaction to be carried out under milder conditions.

6-Iodo-harmine (I). Yield 72 %. M.S. : m/z 338 (100 %) M+, 323 (24.0 %) and 295 (26.6 %). ^{1}H-NMR (DMSO-d_6): two one-proton doublets at 8.47 ppm (J=6.2 Hz) and 8.42 ppm (J=6.2 Hz) were attributable to H-3 and H-4, respectively. It also exhibited three one-proton singlets at 8.95, 7.19 and 12.78 ppm, which were ascribed to H-5, H-8 and H-9, respectively, and a three-proton singlet at 2.98 ppm due to a 3-methyl group. Mp. 199-200 °C.

8-Iodo-harmine (II). Yield 18%. ^{1}H-NMR (DMSO-d_6): two one-proton doublets at 8.07 ppm (J=8.5 Hz) and 6.84 ppm (J=8.5 Hz) were attributable to H-5 and H-6, respectively. Mp. 188-190 °C.

6-Iodo-harmol (III). Yield 37%. M.S.: m/z 324 (60.0%) M+, 295 (18.2 %), 197 (16.4 %), 196 (16.0 %), and 169 (30.5 %). ^{1}H-NMR (MeOH- d_4): two one-proton doublets at 7.69 ppm (J=5.3 Hz) and 7.26 ppm (J=5.3 Hz) for H-3 and H-4, respectively. It also exhibited two one-proton singlets at 7.96 and 7.22 attributable to H-5 and H-8, respectively and a three-proton singlet at 2.46 ppm due to a 3-methyl group. Mp. 238-240 °C.

8-Iodo-harmol (IV). Yield 55%. ^{1}H-NMR (MeOH- d_4): two one-proton doublets at 7.34 ppm (J=8.5 Hz) and 6.35 ppm (J=8.5 Hz) attributable to H-5 and H-6, respectively. Mp. 231-233°C.

8-Iodo-harmaline (V). Yield 95%. M.S.: m/z 340 (100%) M+, 339 (68.3 %), 325 (16.9 %), 324 (22.3 %), and 185 (90.5 %).^{1}H-NMR (MeOH- d_4): two one-proton triplets at 3.90 ppm (J=7.5 Hz) and 3.20 ppm (J=7.5 Hz) for H-3 and H-4, respectively. It also exhibited two one-proton doublets at 7.62 ppm (J=8.7 Hz) and 6.95 ppm (J=8.7 Hz) attributable to H-5 and H-6, respectively, and a three-proton singlet at 2.75 ppm due to the 3-methyl group. Mp. 98-100°C.

8-Iodo-harmalol (VI). Yield 96 %. M.S.: m/z 326 (21.5 %) M+, 325 (21.1 %), 309 (15.0 %), 298 (13.5 %), 182 (35.0 %) and 170 (11.3 %). ^{1}H-NMR (DMSO-d_6): two one-proton triplets at 3.81 ppm (J=7.1 Hz) and 3.11 ppm (J=7.1 Hz) for H-3 and H-4, respectively. It also exhibited two one-proton doublets at 7.58 ppm (J=8.5 Hz) and 6.94 ppm (J=8.5 Hz) attributable to H-5 and H-6, respectively, one-proton singlet at 11.46 ppm corresponding to H-9, and a three-proton singlet at 2.75 ppm due to the 3-methyl group. Mp. 167-168 °C.

8-Iodo-harmane (VII). Yield 88%. M.S.: m/z 308 (6.9 %) M+, 182 (100 %) and 181 (40.5 %). ^{1}H-NMR (DMSO-d_6): two one-proton doublets at 8.42 ppm (J=6.5 Hz) and 8.38 ppm (J=6.5 Hz) for H-3 and H-4, respectively. It also exhibited one-proton doublet at 8.78 ppm (J=1.9 Hz) and one one-proton double doublet at 7.90 (J=8.5/1.9 Hz) attributable to H-5 and H-7, respectively, one one-proton doublet at 7.55 ppm (J=8.5 Hz) corresponding to H-8, one-proton singlet at 12.38 ppm corresponding to H-9, and a three-proton singlet at 2.90 ppm due to the 3-methyl group. Mp. 161-162 °C.

RADIOIODINATION

Although both methods provided similar yields, for obtaining [131-I]-radioiodinated β-carbolines from commercial [131-I]-NaI, we selected method A. The general procedure for radioiodination by McKillop's method (A) was similar to the synthesis of the corresponding nonradioactive derivatives (Table 1). As an alternative procedure, direct isotopic exchange from the iodinated precursor catalyzed by Cu (I) was performed as follows. The iodinated

amine was dissolved in 2 ml of 50% PBS (phosphate buffer saline) pH 6.5/ethanol 0.100 ml followed by addition of a 0.1 M solution of Cu_2Cl_2 ; and 3 mCi of carrier-free [131-I]-NaI. The reaction mixture was heated under reflux for 10 minutes and examined by TLC. The radiochemical purity was always over 96% (Table 1).

Table 1. Rf (Silicagel/Ethylacetate)* and radiochemical purity (RCP) of radioiodinated β-carbolines.

[131–I]–β-carboline	RCP %	Rf	Rf (Substrate)
6-Iodo-harmine	96	0.22	0.00
8-Iodo-harmine	96	0.44	0.00
6-Iodo-harmol	97	0.35	0.08
8-Iodo-harmol	96	0.50	0.08
8-Iodo-harmaline	97	0.20	0.00
8-Iodo-harmalol	96	0.14	0.00
8-Iodo-harmane	97	0.19	0.50

***Rf ([131-I]-Iodine: 0.08**

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