Laboratorio de Síntesis Orgánica. Facultad de Farmacia[1], Laboratorio de Ciencias Biológicas[2], Facultad de Farmacia, Universidad Central de Venezuela, Caracas, Venezuela. Laboratorio de Síntesis Orgánica y Diseño Molecular, Departamento de Química[3], Facultad de Ciencias, Universidad del Zulia, Maracaibo, Venezuela

Synthesis of 1-amino-6,7,8,8a-tetrahydroacenaphthene and its effect on the inhibition of the MAO-enzyme at the brain cortex and liver level

J. Charris[1], J. Pérez[1], Z. Duerto de Pérez[1], R. Companogne, C. Ayala[2], A. Israel Stern[2], B. Migliore de Angel[2], E. Michelena de Báez[3], J. Caldera[3], D. Avila[3] and J. Angel[3]

(±)-1-Amino-6,7,8,8a-tetrahydroacenaphthene was synthesized and evaluated as a novel drug acting on the dopaminergic system. It was shown that the new compound displays activity as MAO inhibitor.

1. Introduction

Dopamine **1** (DA) has been related to several pathological conditions such as Parkinson's disease, schizophrenia, and Huntington's disease [1]. Several evidences suggest a role of DA in the neuroendocrine function and in the regulation of fluid and electrolyte metabolisms [2]. In the past years, a number of approaches have been made in the development of drugs that act like dopaminergic agonist. There are two subtypes of the dopaminergic receptor, D-1 and D-2, and it has been proposed that most of the desirable dopaminergic effects arise from the activation of D-2 receptors in the striatum, which contribute to a therapeutic response. Although DA agonists have been used in the treatment of Parkinson's disease, another approach in the development of anti-Parkinson drugs is the use of MAO-inhibitors [3]. It is well known that the phenylethylamine moiety is a selective substrate of the MAO-B enzyme [4].

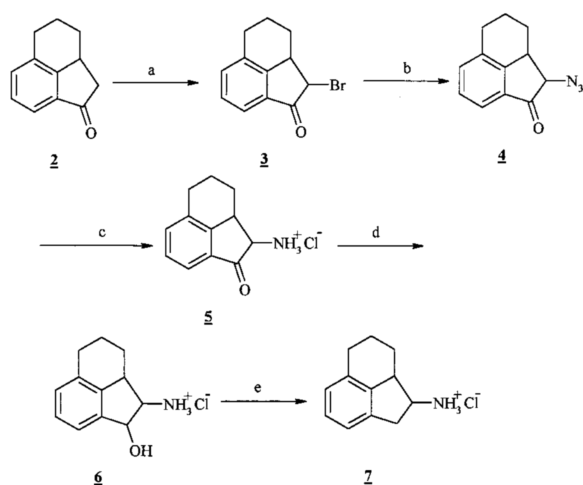
The (±)-1-amine-6,7,8,8a-tetrahydroacenaphthene (**7**) was synthesized and biologically evaluated, resulting in a novel compound that acts on the dopaminergic system [5, 6]. As a consequence, in the present work we report another synthetic route and a biological test to understand the mechanism of action of the dopaminergic central system.

2. Investigations, results and discussion

The synthesis of **7** is outlined in the Scheme. Ketone **2** reacted with pyridinium hydrobromide perbromide according to Nedelec et al. [7], affording compound **3** as a mixture of diastereomers. The following treatment with sodium azide yields compound **3** as a mixture of diastereomers. Catalytic hydrogenation of the mixture in HCl/EtOH, using 10% Pd/C, afforded **4**. Subsequent reduction with sodium borohydride yielded mixture **5**. This diasteroisomeric mixture upon catalytic reduction in acetic acid-perchloric acid (60%) using Pd/C at 10% as a catalyst produced **7**, as a mixture of diastereomers.

Our results about the inhibition effect of compound **7** on the enzymatic activity of MAO at the brain cortex and liver levels are shown in the Table.

Scheme



a. Py-HBr perbromide/$CHCl_3$
b. NaN_3, H_2O/DMF
c. H_2/Pd-C 10%/EtOH-HCl
d. $NaBH_4$/MeOH
e. H_2/Pd-C 10% AcOH-$HClO_4$

Table: Effect of the diastereomeric mixture of compound 7 on the inhibition of MAO

Inhibition of MAO

Tissue	Control	10^{-3} M	10^{-4} M	10^{-5} M
Brain cortex	82.4 ± 3.44 N = 6	1.5 ± 0.036** N = 6	18.5 ± 2.35** N = 6	56.43 ± 6.32** N = 6
Liver	104.48 ± 8.89 N = 5	0.48 ± 0.08** N = 5	33.62 ± 4.35* N = 4	78.80 ± 14.29 n.s. N = 4

Percentage of inhibition

Tissue	Control	10^{-3} M	10^{-4} M	10^{-5} M
Brain cortex	–	98.17	77.54	31.51
Liver	–	99.54	67.82	24.57

The numbers represent the X ± Es of n-determinations for duplicate. They are expressed in ng of 4-OHQ/mg Protein × min.
n.s. = not significant according to control
** $P < 0.05$ on T-Switched test
* $P < 0.05$ on T-non Switched test

Compounds **7** inhibited the enzymatic activity of MAO at concentrations of 10^{-3}, 10^{-4} and 10^{-5} M, either of brain cortex or liver, with a 90% inhibition at the concentration of 10^{-3} M and with 30% at the concentration of 10^{-5} M in these tissues, with $p < 0.05$ of significance. These results suggest that the diuretic and natriuretic response practiced with compounds **7** under ICV administration [5], could be the results of the endogenous dopamine increase, due to its metabolism inhibition, and they support the presynaptic action hypothesis of **7**. With this outcome, we can have a closest approach, although we cannot conclude about the possible mechanism of action. The fact that the diuretic and natriuretic responses are inhibited after dopaminergic denervation suggests that **7** might be acting at the presynaptic level, increasing the release, altering the storage, the metabolism and the synthesis of endogenous dopamine [5]. This action might possibly occur either by inhibiting the MAO enzyme or allowing the dopamine to be released from the storage sites in the presynaptic neuron. We can deduce that under these experimental conditions the metabolic activation is not happening and as a consequence the *m*-hydroxyphenylethylamine moiety has not been formed, which would be responsible for the interaction on the postsynaptic receptor. Our results obtained after the denervation also support this suggestion. However, we do not discard the possibility that compounds **7** are metabolically activated [6], because when the selective presynaptic dopaminergic denervation happens, the dead of the nervous terminal is produced, even of the enzymatic system present. This is the reason why the enzymatic activation on compounds **7** did not occur. As a consequence, the final result after denervation is the inhibition of the diuretic and natriuretic response. Although, compounds **7** belong to the acenaphthene nuclei, which are considered as a rigid amino indan non hydroxylated, they keep in part the structural disposition of the dopaminergic pharmacophore. As we previously mentioned and according to other reports [6, 9–13], a possible metabolic activation will result in a *m*-hydroxyphenylethylamine fragment in compounds **7**, and as a consequent it could have activity as a dopaminergic postsynaptic agonist.
In the future it will be investigated which stereoisomer of compound **7** is responsible for the biological activity.

3. Experimental

3.1. Synthesis

IR were recorded on a Shimadzu 435 spectrometer (using pure fluid compounds as film and the solid ones as KBr tablets). ^{1}H NMR spectra were measured at 270 MHz on a JEOL JNM Eclipse spectrometer using tetramethylsilane as internal standard (chemical shift in ppm) and in a Varian EM-360L at 60 MHz. MS were performed on a Dupont 21-49b mass spectrometer operated at 70 eV (the main peaks of the fragments are expressed in m/z units). M.p.'s were determined using a Thomas Hoover apparatus and are not corrected.

3.1.1. *2-Bromo-2a,3,4,5-tetrahydro-1-acenaphthene* (**3**)

Pyridine hydrobromide perbromide (2.77 g, 8.6 mmol) was added to a solution of ketone **2** (1.12 g, 6.5 mmol) [6], in $CHCl_3$ (50 ml). The mixture was heated for to 50 °C 15 min, then poured into H_2O, extracted with benzene, washed with H_2O, dried ($MgSO_4$), and evaporated. The residual oil obtained was purified by CC with silica gel 60 (0.04–0.06 mm) and eluted with benzene: yield 1.40 g (85%) of a brown oil (rf = 0.4285). IR cm^{-1} (film) 3059 (Ar); 1692 (CO). ^{1}H NMR (CCl_4/TMS) δ: 0.60–2.40 (m, 4 H, $C\underline{H}_2C\underline{H}_2$); 2.45–3.60 (m, 3 H, $ArC\underline{H}_2$, ArH); 4.0–4.6 (m, 1 H, $COC\underline{H}Br$); 6.8–8.0 (m, 3 H, ArH).

3.1.2. *2-Azido-2a,3,4,5-tetrahydro-1-acenaphthenone* (**4**)

Sodium azide (0.51 g, 7.8 mmole) in H_2O (6 ml) was added to a solution of **3** (0.7 g, 2.7 mmol) in DMF (20 ml) and acetic acid (1 ml), cooled to 10 °C. The temperature rose to 20 °C, and the reaction was stopped after 20 h. After adding H_2O, the solution was extracted with $CHCl_3$, washed with H_2O, dried ($MgSO_4$) and evaporated to afford a red oil (0.47 g, 80%). This compound was obtained as a crude material due to its tendency to decompose quickly in the presence of air. IR cm^{-1} (film) 2099 (N_3); 1675 (CO). ^{1}H NMR (CCl_4/TMS) δ: 0.6–2.6 (m, 4 H, $C\underline{H}_2C\underline{H}_2$); 2.61–4.30 (m, 4 H, $ArC\underline{H}_2$, $ArC\underline{H}$, $COC\underline{H}N_3$); 6.80–8.10 (m, 3 H, $Ar\underline{H}$).

3.1.3. *2-Amino-2a,3,4,5-tetrahydro-1-acenaphthenone* (**5**)

A mixture of 10% Pd/C (0.047 g), ketoneazide **4** (0.47 g, 2.2 mmol) of C_2H_5OH (98 ml), and conc. HCl (2 ml) was hydrogenated at room temperature for 5 h. After, separation of the catalyst, the solvent was evaporated and the aminoketone **5** was obtained as its hydrochloride. It was purified over active charcoal to afford a highly hygroscopic yellow solid (0.29 g, 60%). IR cm^{-1} (film) 3010, 2910, 1656 ($NH_3^+Cl^-$); 1700 (CO). ^{1}H NMR (DMSO-d_6/TMS) δ 0.40–2.10 (m, 4 H, $C\underline{H}_2C\underline{H}_2$); 2.2–3.60 (m, 4 H, $ArC\underline{H}_2$, $ArC\underline{H}$, $COC\underline{H}NH_3{}^+Cl^-$); 4.20–5.6 (bd, $NH_3^+Cl^-$); 6.6–8.2 (m, 3 H, $Ar\underline{H}$).

3.1.4. *1-Amino-2-hydroxy-6,7,8,8a-tetrahydroacenaphthene* (**6**)

To a suspension of the hydrochloride **5** (0.4 g, 1.7 mmol) a solution of sodium borohydride (0.129 g, 3.4 mmol) in CH_3OH (10 ml) was added slowly at 5 °C. After 4 h, the mixture was acidified with 2 N HCl and then evaporated under vacuum; H_2O was first added solution to the residual product and then aqueous 2 N NaOH to basily the mixture. The organic phase was extracted with $CHCl_3$, washed with H_2O dried ($MgSO_4$) and evaporated. The resulting semisolid was treated with ether/ethereal-HCl solution and recrystallized from isopropanol-ether; yield 0.24 g (60%) of diastereomeric mixture of **6** as a white solid: m.p. 241–242 °C; IR cm^{-1} (KBr) 3221 (OH); 2925, 1616 ($NH_3^+Cl^-$); ^{1}H NMR (DMSO-d_6/TMS) δ 1.15 (m, 2 H, CH_2); 2.03 (m, 2 H, CH_2); 2.42 (m, 2 H, CH_2); 1.4 (m, 1 H, CH); 3.48 (m, 1 H, CH); 4.8 (d, 1 H, NH, J = 5.19 Hz); 5.23 (m, 1 H, CHOH); 5.9 (bd, 1 H, OH); 6.05 (d, 1 H, NH, J = 4.20 Hz); 7.09 (m, 3 H, Ar). MS (m/z) 189 (M-HCl, $C_{12}H_{15}NO$, 6.5%); 188 (M-1, $C_{12}H_{14}NO$, 1%); 172 ($C_{12}H_{12}O$, 8%); 160 ($C_{11}H_{14}N$, 100%); 115 (C_9H_7, 25%); 91 (C_7H_7, 19%); 77 (C_6H_5, 10%).

3.1.5. *1-Amino-6,7,8,8a-tetrahydroacenaphthene hydrochloride* (**7**)

A mixture of **6** (0.3 g, 1.4 mmol), acetic acid (95 ml) and, 60% $HClO_4$ (5 ml) was hydrogenated over 10% Pd/C (0.12 g) at room temperature, and at initial pressure of 50 psi for 6 h. After absorption of the calculated amount of hydrogen, the reaction mixture was stirred for 5 min with 3 g of potassium acetate and then filtered. The residue was diluted with H_2O, made basic (pH 10) with 2 N NaOH solution, and extracted with CH_2Cl_2. The organic extracts were combined, dried over $MgSO_4$ and concentrated under reduced pressure, affording a purple oil, which after treatment with 10 ml of diethylether and treated with ethereal-HCl, yielded the hydrochloride as an amorphous solid. The crude product was recrystallized from isopropanol-ether affording a diastereomeric mixture of **7** as a white solid (0.195 g, 70%; m.p.: 190–191 °C). IR cm^{-1} (KBr) 2925, 2762, 2592, 1617 (NH_3^+); ^{1}H NMR (DMSO-d_6/TMS) δ: 1.18 (m, 2 H, $C\underline{H}_2$); 1.71 (m, 2 H, CH_2); 2.61 (m, 2 H, CH_2); 2.75 (m, 2 H, CH_2); 3.12 (m, 1 H, CH); 3.35 (m, 1 H, CHN^+H_3); 7.06 (m, 3 H, Ar); 8.63 (bd, 3 H, $^+NH_2ClH$). MS (m/z) 173 (M-HCl, $C_{12}H_{15}N$, 100%); 174 (M + 1, $C_{12}H_{16}N$, 8%); 172 (M-1, $C_{12}H_{14}N$, 27%); 156 ($C_{12}H_{12}$, 80%); 142 ($C_{11}H_{10}$, 62%); 130 ($C_{10}H_{10}$, 72%); 115 (C_9H_7, 46%); 91 (C_7H_7, 25%); 77 (C_6H_5, 16%); 63 (C_5H_3, 28%); 51 (C_4H_3, 24%); 44 (C_2H_6N, 63%).

3.2. Pharmacology

Sprague-Dawley male rats of 250 g were used coming from the Venezuelan Central University Pharmacy Faculty. The animals were sacrified by decapitation and brain, liver and kidneys were separated. The sample was placed in a Trizma base buffer (Tris[hydroxymethyl]aminomethane, pH 10.5–12.0) solution and kept in ice. The method of Drujan and Diaz Borges was applied for the Monoamine Oxidase (MAO) determination [8]. It includes the measurement of the fluorescence emitted by 4-hydroxyquinoline (4-OHQ), which is the MAO and Kinuramine reaction product.
The tissues (brain, and liver) (0.5 g) were homogenized preparing a 15% p/v solution of Trizma buffer (Sigma) using a Wheaton homogenizer with a Teflon piston activated by a motor at 2500 rpm (Cole Palmer, 62026). Equal parts of each assay (0.4 ml) were taken for duplicated, mixed up with of Kinuramine (0.1 ml, 0.2 mg/ml) or with distilled H_2O (target tissue) (0.1 ml) and Trizma buffer solution was added until 0.6 ml, which was the total volume of incubation environment. The samples were incubated at 28 °C for 30 min in a thermal incubator under continuous stirring (Dubnoff). The reaction was stopped and the tubes were placed on ice, and absolute C_2H_5OH (Fisher) was added (1.8 ml). The samples were centrifuged at 4500 rmp for 45 min in a refrigerated centrifuge (Sorvell RC-5) with a rotor (Sorvall, SS-34). Next, 1 ml of the upper layer was mixed with 3 ml of NaOH 0.1 N (Merck), and after 3 min the sample was analyzed in order to read the fluorescence in a spectrometer (A Minco-Bow-

man) at 310 nm of wavelength for excitation and 390 nm wavelength for emission. The activity was evaluated in terms of the 4-OHQ formed. A calibration curve was prepared using five points, the concentrations on which were between 0.01 μg/μl and 100 μg/μl of 4 OHQ (Koch-Light). The previous procedure was followed, including in the incubation environment compound **7**, in the final concentrations of 10^{-3}, 10^{-4} and 10^{-5} M.

Acknowledgements: We are indebted to the CONICIT Venezuela, Grants No. S1 97000394 and LAB 97000665.

References

1 Koe, K.; Vinick, F.; in: Bailey, D. M. (ed.): Annual Reports in Medicinal Chemistry, vol. 19, p. 46, Academic Press 1984
2 Hornykiewicz, O.: Pharmacol. Rev. **18**, 925 (1996)
3 Youdin, M. B. H.; Finberg, J. P. M.; Wajsbort, M. D.; in: Ellis, G. P.; West, G. B.: Progressin Medicila Chemistry, vol. 21, p. 138, Elsevier Science Publishers, B. V. 1984
4 Yang, H. Y.; Neff, H. N.: J. Pharmac. Exper. Ther. **187**, 365 (1973)
5 Orfila, L.; Angel, J. E.; Torres, M.; Barbella, Y.; Israel, A.: J. Pharm. Pharmacol. **46**, 397 (1994)
6 Pérez, J.; Pérez, Z.; Angel, J.; Charris, J.; Torres, M.; Israel, A.: Boll. Chim. Farm. **134**, 329 (1995)
7 Nedelec, L.; Pierdet, A.; Fauveau, P.; Euvrad, C.; Proulex-Ferland, L.; Dumont, C.; Labrie, F.; Boisser, J. R.: J. Med. Chem. **26**, 522 (1983)
8 Drujan, B. O.; Diaz Borges, J. M.: Biochem. Anal. **243**, 662 (1968)
9 Foller, R. W.; Perry, K. W.; Baker, J. C.; Parli, C. J.; Lee, N.; Dag, W. A.; Molloy, B.: Biochem. Pharmacol. **23**, 3267 (1967)
10 Foller, R. W.; Hines, C. W.: J. Pharm. Sci. **56**, 302 (1967)
11 Foller, R. W.; Baker, J. C.; Molloy, B. B.: J. Pharm. Sci. **66**, 271 (1977)
12 Cannon, J. G.; Long, J. P.; Bhatnagar, R.: J. Med. Chem. **24**, 1113 (1981)
13 Irino, O.; Tateshi, M.; Miura, C.; Fukawa. K.: Chem. Pharm. Bull. **20**, 734 (1980)

Received January 11, 1999
Accepted April 6, 1999

Dr. Jorge Angel
Laboratorio de Síntesis Orgánica y Diseño Molecular
Departamento de Química
Facultad de Ciencias, Universidad del Zulia, Ap. 526
Maracaibo, Venezuela
jangel@solidos.ciens.luz.ve