SYNTHESIS AND ANTIBIOTIC ACTIVITY OF SOME β-CARBOLINE ALKALOIDS.

Astréa M. Giesbrecht (*)
José R. de Sousa (**)
Leila M. G. Aguiar (**)

SUMMARY

Nine β-carboline alkaloids were synthetized and screened for antibiotic activity. Six of the compounds testes showed inhibitory activity against one or more of the microorganisms assayed.

INTRODUCTION

In a earlier paper, it was reported the isolation from **Aniba sentaladora** Ducke (Lauraceae) of 1-(p-hydroxibenzyl)-6-methoxy-β-carboline (9) the structure being confirmed by a two-step synthesis that involved 4 as an intermediate (Aguiar **et. al.**, 1980).

This paper presents the investigation of the antibiotic activity of nine β-carboline alkaloids, all of them synthetized in our laboratory.

Pharmacological interest in β-carbolines arised from the fact that the hallucinogenic properties of the beverages, known as **ayahuasca**, **caapi** or **yagé**, used by the indians in Amazonas basin are attributed partially to the presence of β-carbolines (Agurell **et al.**, 1969). Harmine, harmaline and related compounds can inhibit the enzyme monoamine oxidase and this property has been related with some of their effects in the central nervous systen (Udenfriend **et al.**, 1958).

However, the antibiotic activity of β-carbolines are poorly known since the publications are limited to the results obtained by McKenna and Towers (1981) which investigated the UV mediated cytotoxicity of β-carboline alkaloids using yeast and bacterial bioassay systems.

EXPERIMENTAL

Synthesis

1-(p-Hydroxybenzyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (3). dℓ-Tryptophan (2.04g) and p-hydroxyphenylpyruvic acid (2.0g) in a mixture of 1N H_2SO_4 (10 ml),

(*) Instituto de Ciências Biomédicas, Departamento de Farmacologia, USP, SP.
(**) Instituto de Ciências Exatas - Universidade Federal de Minas Gerais, BH - MG.

EtOH (10 ml), and H_2O (30 ml) were heated under reflux (21 hr). After evaporation of EtOH and addition of conc. NH_4OH (10 ml) and active charcoal, the mixture was boiled (30 min), cooled, diluted with NH_4OH (5 ml), filtered, washed with Et_2O, concentrated and kept at 0° (24 hr). The yellow precipitate, a mixture of stereoisomers of 3 (1.7g) melt at 198-208° dec. $\nu_{max}^{KBr}(cm^{-1})$: 3400-2500, 1720, 1610 (b), 1510, 1450, 840. ^{1}HNMR(DMSO-d_6): δ 2.70-3.32(m, CH_2), 3.35-3.88(m, CH_2), 4.90 (b, NH, H-1, H-3), 6.40-7.90(m-8H ϕ), 10.55 (br s, NH, *disappear with D_2O*).

1-(p-Hydroxybenzyl)-β-carboline(7). To a boiling solution of 3 (1.61g) in H_2O (300ml) were added 10% aq. $K_2Cr_2O_7$ (60 ml) and HOAc (10.2 ml). After reflux (30min) and cooling, 10% aq. Na_2SO_3 was added. The mixture was made alkaline with aq. NaOH and extracted with Et_2O. The Et_2O solution was dried and evaporated. The residue was crystallized from EtOH-cyclohexane to 7 (0.56g), yellow crystals, mp 220-223° dec. $\nu_{max}^{KBr}(cm^{-1})$: 3400, 3220, 1610, 1600, 1590, 1560, 1510, 1500, 1450, 820, 735. λ_{max}^{EtOH}(nm): 242, 253 sh 293, 340, 358 sh (ϵ 37260, 31510, 15070, 19730, 8220, 87770). ^{1}HNM(CD_3COCD_3): δ 4.41 (s, CH_2) 6.70 (d, J=9 Hz, H-3', H-5'), 7.20 (d, J=9 Hz, H-2', H-6'), 7.40-8.40 (m, 6H), 10.3 (br s, NH, disappear with D_2O). MS [m/z (%)] 274 (95%) M^+, 273 (100), 272(25), 256 (11), 137 (15), 136 (11), 128 (16). Found: C, 79.06; H, 5.35; N, 10.74. $C_{18}H_{14}ON_2$ requires: C, 78.01; H, 5.14; N, 10.21%.

1-(p-OAc-benzyl)-β-carboline (8), mp 169-171° (MeOH/H_2O). Found: C, 75.61; H, 5.18 N, 8.76. $C_{20}H_{16}N_2O_2$ requires: C, 75.93; H, 5.10; N, 8.85%. ν_{max}^{Kbr} (cm^{-1}): 1755, 1620, 1600 1560, 1500, 1450, 1200, 900, 820, 800, 770, 745. ^{1}NHMR ($CDCl_3$): δ 2.30 (s, CH_3), 4.35 (s, CH_2), 6.82 (d, J=9 Hz, H-3' and H-5'), 7.00-7.50 (m, H-6, H-7, H-2', H-6'), 7.85 (d J=6 Hz, H-4), 8.10 (dd, J=8 and 2 Hz, H-5), 8.40 (d, J=6 Hz, H-3), 9.15 (br s, NH, disappear with D_2O).

Determination of antibiotic activity

Compounds 1 to 9 were dissolved in dimethylsulfoxide (DMSO) and tested for antibiotic activity against seven microorganismis as listed in Table 1.

The activity was assayed by dipping antibiotic assay (AA) discs into the test sample draining and then transferring the discs to the surface of an agar plate(Trypticase-soy agar) previously seeded with the test organism.

For quantitative bioassays, disc containing respectively 500 µg, 100 µg, 50 µg, 25 µg and 10 µg of the samples were used. A solvent blank AA disc was included in all bioassays and discs containing 100 µg of streptomycin sulfate and 100 µg of amphotericin B were included in plates inoculated respectively with bacteria or yeast. The plates were incubated at 37°C and examined after 24 and 48 hours for growth.

RESULTS AND DISCUSSION

Synthesis of the compounds

Compounds 1 and 2 (known as synthetic products) were synthetized by condensation *of dℓ-tryptophan with acetaldehyde and phenyl-acetaldehyde.* Oxidation/decarboxilation of these two compounds with $K_2Cr_2O_7/CH_3COOH/H_2O$ led to compounds 5 (known as natural and synthetic product) and 6 (known as synthetic product) (Snyder **et al.**, 1948). Compound 3 was synthetized using the same procedure as for 9 that is, by condensation of dℓ-tryptophan with p-hydroxyphenylpyruvic acid (Aguiar **et al.**, 1980). Oxidation/decarboxilation with $K_2Cr_2=_7/CH_3COOH/H_2O$ led to compound 7. Compound 8 was obtained by acetilation of 7. Compounds 3, 7 and 8 are new products.

Bioassays

From the nine alkaloids tested, only compounds 1, 8 and 9 were totally inactive. Compound 5 (harman) exhibited growth inhibitory activity against all the microorganisms assayed. Compunds 3 and 4 showed activity only in concentrations above 500 µg per disc.

A summary of the results are presented in Table 1, where the minimum inhibitory concentrations are expressed in µg per disc.

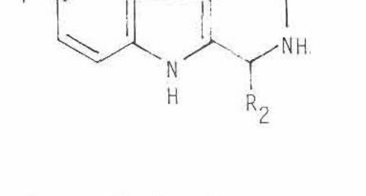
It is difficult to correlate structure and activity. It seems that activity is reduced when the piridine ring looses the aromaticity. An exception is compound 2 which still mantains the activity.

ACKNOWLEDGEMENTS

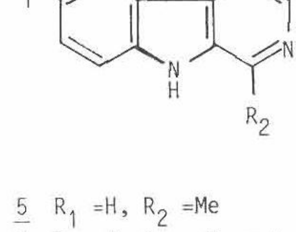
This work was partially supported through financial aid by FINEP and through fellowships by CNPq and CAPES.

RESUMO

Nove alcalóides β-carbolínicos foram sintetizados e avaliados quanto à sua ação antibiótica. Seis dos compostos inibiram o crescimento de um ou mais dos microorganismos ensaiados.



1 R_1 =H, R_2 =Me
2 R_1 =H, R_2 =Benzyl
3 R_1 =H, R_2 =p-OH-Benzyl
4 R_1 =OMe, R_2 =p-OH-Benzyl



5 R_1 =H, R_2 =Me
6 R_1 =H, R_2 = Benzyl
7 R_1 =H, R_2 =p-OH-Benzyl
8 R_1 =H, R_2 =p-OAc-Benzyl
9 R_1 =OMe, R_2=p - OH-Benzyl

Table 1 - Antibiotic activity of β-carbolines (minimum inhibitory concentrations in μg per disc)

Organism	Compound								
	1	2	3	4	5	6	7	8	9
C. albicans	-	250	+	+	50	250	250	-	-
M. smegmatis	-	25	-	+	100	50	100	-	-
E. coli	-	-	-	-	50	-	-	-	-
S. epidermidis	-	+	-	-	10	-	-	-	-
S. aureus	-	-	-	-	25	-	-	-	-
K. pneumoniae	-	-	-	-	25	-	-	-	-
S. paratyphosa	-	-	-	-	50	-	-	-	-

- no inhibition

+ inhibition only concentrations above 500 μg per disc.

References

Aguiar, L. M. G.; Braz, F. R.; Gottlieb, O. R.; Maia, J. G. S.; Pinho, S. L. V.; Sousa, J. R. - 1980. Cecilin, a 1-benzyl-β-carboline from Aniba santaladora. **Phytochemistry** 19: 1859-1860.

Agurell, S.; Holmsted, B.; Lindgren, J. E.; Schultes, R. E. - 1969. Alkaloids in certain species of **Virola** and other South American plants of ethnopharmacological interest. **Acta Chem. Scand.**, 23: 903.

McKenna, D. J. & Towers, G. H. N. - 1981. Ultra-violet mediated citotoxic activity of β-carboline alkaloids. **Phytochemistry**, 20: 1001-1004.

Snyder, H. R.; Hansch, C.´H.; Katz, L.; Parmenter, S. M.; Spaeth, E. C. -1984. Synthesis of derivatives of β-carboline. II. Synthesis from dℓ-tryptophan and aldehydes. **J. Amer. Chem. Soc.**, 70: 219-221.

Udenfriend, S.; Witkop, B.; Redfield, B. G.; Weissbach, H. - 1958. Studies with reversible inhibitors of monoamine oxidase. **Biochem. Pharmacol.**, 1: 160-165.