The Influence of Some 1-Substituted 6-Methoxy-1,2,3,4-tetrahydro-β-carbolines on the Metabolism and Activity of 5-Hydroxytryptamine

RYSZARD J. GRYGLEWSKI, STANISLAW H. MISZTAL, JACEK A. SPLAWINSKI, AND BOGUMILA PANCZENKO

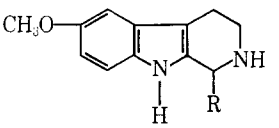
Department of Pharmacology, Medical Academy and Polish Academy of Sciences, Cracow, Grzegórzecka 16, Poland

Received January 10, 1966

1-(2-Pyridyl)-, 1-(3-pyridyl)-, and 1-(4-pyridyl)-6-methoxy-1,2,3,4-tetrahydro-β-carbolines were prepared from 5-methoxytryptamine and the corresponding pyridinealdehydes. These three compounds and six other 1-aryl- and 1-alkyl-6-methoxy-1,2,3,4-tetrahydro-β-carbolines previously obtained were tested *in vitro* and *in vivo* as antagonists of the biosynthesis and inactivation of 5-hydroxytryptamine (5-HT), and for their interaction with tryptamine receptors. The compounds did not change the activity of 5-hydroxytryptophan decarboxylase (5-HTP-D). Out of nine tested compounds only the 1-(4-pyridyl)-substituted derivative seems to inhibit the activity of monoamine oxidase (MAO). All β-carbolines tested are competitive antagonists of 5-HT, competing for a myotropic tryptamine receptor. In terms of Schild's pA_2 values the most active was the 1-(4-pyridyl)-substituted derivative. Its $pA_2 = 6.50$ compares with $pA_2 = 7.52$ of the standard compound (LSD). The least active were the 1-alkyl-substituted derivatives. 1-(4-Pyridyl)-6-methoxy-1,2,3,4-tetrahydro-β-carboline antagonized tryptamine convulsions in the rat. Unlike LSD none of β-carbolines tested contracted smooth muscles.

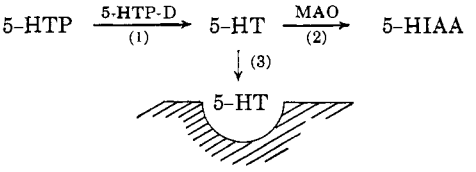
The chemical moiety, β-carboline, is common to many biologically active agents.[1] Recently evidence has been found that derivatives of 1,2,3,4-tetrahydro-β-carboline are present in the mammalian pineal gland along with 5-hydroxytryptamine (5-HT) and 5-methoxy-N_β-acetyltryptamine (melatonin).[2] As a possible physiological role of 1-methyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline, a substance isolated from pineal tissue, it was proposed that it acted as an aldosterone-releasing factor and the name "adrenoglomerulotropine" was coined.[2a] Newer experiments questioned the influence of this compound on the hormonal activity of adrenal cortex,[3] and so the name "adrenoglomerulotropine" should not be used as the synonym of this particular β-carboline. Nevertheless, 1-methyl-6-methoxy-1,2,3.4-tetrahydro-β-carboline, as well as 1-benzyl-1,2,3,4-tetrahydro-β-carboline and a number of other β-carbolines present in pineal tissue,[2b] certainly plays some physiological role, probably as moderators of 5-HT activity and metabolism.[2c,4] The similar pathway of biogenesis for 5-HT and for pineal β-carbolines makes this assumption even more probable.[2c] Moreover, the influence of plant β-carbolines on 5-HT metabolism seems to be established.[2c,4] The similarity between EEG patterns of cats treated with LSD or with 1-methyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline[5] may be considered as another proof that pineal β-carbolines are involved in the cerebral metabolism of 5-HT.

The translocation of pyridine nitrogen atom from the β to the γ position in the fused indole–pyridine system of the carboline moiety is an essential factor influencing the biological activity of carbolines.[6] This fact inspired us to synthesize three isomers of 1-pyridyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline in the hope of completing a series consisting of 1-methyl-, 1-ethyl-, 1-propyl-, 1-isopropyl-, 1-phenyl-, and 1-benzyl-6-methoxy-1,2,3,4-tetrahydro-β-carbolines which had been obtained previously.[7] All nine compounds were investigated for their influence on 5-HT metabolism and on 5-HT activity in animal tissues. The three new compounds are: 1-(2-pyridyl)-, 1-(3-pyridyl)-, and 1-(4-pyridyl)-6-methoxy-1,2,3,4-tetrahydro-β-carbolines (I, II, and III, respectively). Their synthesis was carried out according to general literature methods.[8] Figure 1 shows the infrared absorption spectrum of the isomeric carbolines.



I, R = 2-pyridyl
II, R = 3-pyridyl
III, R = 4-pyridyl

The pharmacological investigation was based on the following scheme. We investigated the influence of



β-carbolines (1) on the activity of 5-hydroxytryptophan decarboxylase (5-HTP-D) in rat kidneys, (2), on the activity of monoamine oxidase in rat liver, and (3) on the 5-HT interaction with tissue receptors of isolated guinea pig ileum and rat stomach. These *in vitro* experiments were completed by the estimation of the pharmacological activity of β-carbolines in intact cats, rats, and mice.

(1) R. A. Abramovitch and I. D. Spenser, *Advan. Heterocyclic Chem.*, **2**, 79 (1964).

(2) (a) G. Farrell and W. M. McIsaac, *Arch. Biochem. Biophys.*, **94**, 543 (1961); (b) W. M. Isaac, G. Farrell, R. G. Taborsky, and A. N. Taylor, *Science*, **148**, 102 (1965); (c) W. B. Quay, *Pharmacol. Rev.*, **17**, 321 (1965).

(3) G. Farrell in "Aldosterone," E. E. Baulieu and P. Robel, Ed., Blackwell Scientific Publications, Oxford, 1964, p 243; J. Supniewski and R. Rembiesa, *Dissertationes Pharm.*, **16**, 131 (1964).

(4) N. J. Giarman and D. X. Freedman, *Pharmacol. Rev.*, **17**, 1 (1965).

(5) J. Trabka, *Dissertationes Pharm.*, **16**, 419 (1964).

(6) P. Nantka-Namirski, S. Kurzepa, J. Duszka, J. Kazimierczyk, and H. Kierylowicz, *Acta Physiol. Polon.*, **16**, 131 (1965).

(7) J. Supniewski and S. Misztal, *Dissertationes Pharm.*, **16**, 9 (1964).

(8) S. Akabori and K. Saito, *Chem. Ber.*, **63**, 2245 (1930); Z. Pelchowicz and E. D. Bergman, *J. Chem. Soc.*, 4699 (1960); Z. Pelchowicz, A. Kaluszyner, and M. Bentov, *ibid.*, 5418 (1961); M. Protiva, J. O. Jilek, E. Hachova, J. L. Novak, Z. J. Vejdelek, and E. Adlerova, *Chem. Listy*, **51**, 1915 (1957).

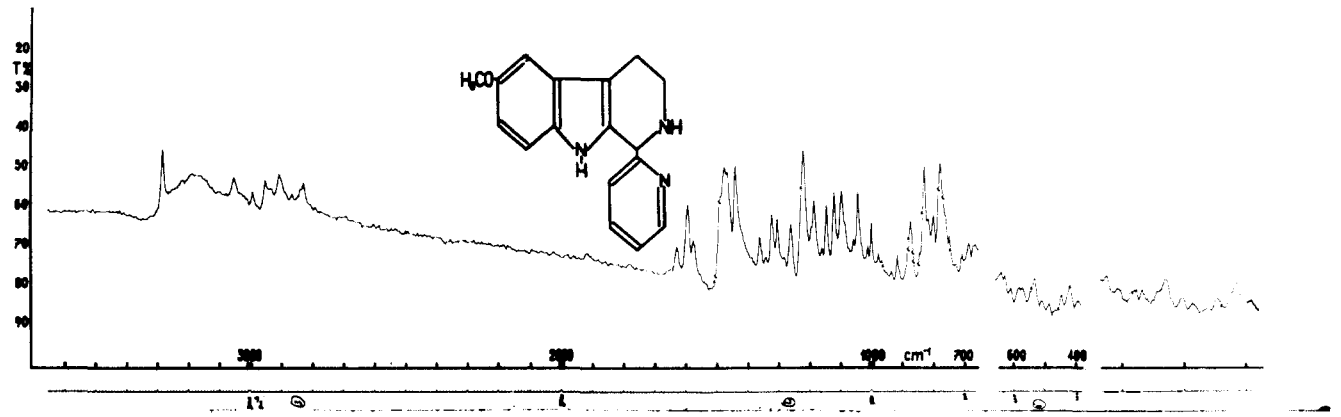


Figure 1.—Infrared absorption of the isomeric 1-(pyridyl)-6-methoxy-1,2,3,4-tetrahydro-β-carbolines.

Experimental Section[9]

1-(2-Pyridyl)-6-methoxy-1,2,3,4-tetrahydro-β-carboline (I).—5-Methoxytryptamine (1 g), 5.5 ml of 2 *N* H_2SO_4, and 0.6 g of pyridine-2-aldehyde were dissolved in 20 ml of water. The solution was stirred and heated in 110° for 30 min, cooled, made alkaline (Na_2CO_3), and extracted three times ($CHCl_3$). The chloroform layer was washed with water and dried ($MgSO_4$). The residue from the chloroform was dissolved in a few milliliters of hot ethanol and slightly acidified with ethanolic HCl. The solution was stored overnight at −5° and yielded 1.15 g of yellow crystals, mp 228–233°. This crude hydrochloride was dissolved in water and made alkaline (Na_2CO_3), and the free base was again extracted three times with $CHCl_3$. The dry residue from the chloroform layer was dissolved in hot anhydrous benzene and filtered. From this solution crystallized 0.6 g (yield 41.7%) of yellowish product, mp 130–135°. An analytical sample was twice recrystallized from benzene; mp 138–140°.

Anal. Calcd for $C_{17}H_{17}N_3O$: C, 73.11; H, 6.13; N, 15.04. Found: C, 73.17; H, 6.05; N, 14.81.

1-(3-Pyridyl)-6-methoxy-1,2,3,4-tetrahydro-β-carboline (II). **A.**—5-Methoxytryptamine (2 g), 7 ml of 2 *N* H_2SO_4, and 1.2 g of pyridine-3-aldehyde were dissolved in 40 ml of water, and the solution was stirred and heated in 110° for 30 min. On cooling there crystallized 2 g of crude product. It was filtered, dissolved in hot water, made alkaline (Na_2CO_3), and extracted three times with chloroform. The dry residue from the $CHCl_3$ extract was dissolved in anhydrous chloroform and petroleum ether was added. The product crystallized in a yield of 1.3 g (44.3%), mp 216°. An analytical sample was twice recrystallized from ethanol; mp 219–220°.

B.—5-Methoxytryptamine (1 g), 8 ml of 2 *N* HCl, and 0.6 g of pyridine-3-aldehyde were dissolved in 20 ml of water and refluxed 1 hr. The reaction product was treated as in A. The dry residue from the chloroform extract was crystallized from ethanol; 0.6 g of colorless product, mp 210–215° (yield 44.0%).

Anal. Calcd for $C_{17}H_{17}N_3O$: C, 73.11; H, 6.13; N, 15.04. Found: C, 72.90; H, 6.23; N, 15.00.

1-(4-Pyridyl)-6-methoxy-1,2,3,4-tetrahydro-β-carboline (III). —5-Methoxytryptamine (2 g), 12 ml of 2 *N* HCl, and 1.2 g of pyridine-4-aldehyde were dissolved in 40 ml of water, refluxed 1 hr, and worked up as in the case of I to give 2.3 g of red crude hydrochloride, mp 221–244°. Finally there was obtained 0.8 g (27.3%) of a canary yellow base, mp 180–184°. An analytical sample was recrystallized many times from benzene; mp 188–192°.

Anal. Calcd for $C_{17}H_{17}N_3O$: C, 73.11; H, 6.13; N, 15.04. Found: C, 73.39; H, 5.95; N, 14.82.

Acute Toxicity.—The compounds were injected intraperitoneally to Porton mice and the acute toxicity was roughly determined. A single dose of a compound was usually injected into three mice. The behavior of animals was observed.

5-Hydroxytryptamine Biosynthesis.—Rat kidney homogenates served as the source of 5-HTP-D.[10] The incubation was carried out in Warburg apparatus. Each vessel contained about 600 mg of tissue homogenized in 2 ml of 0.2 *M* phosphate buffer (pH 8.00) and adjusted to 4 ml with distilled water, 400 μg of pyridoxal phosphate, 500 μg of iproniazid, and NaCl or a test β-carboline in a final concentration of 0.001 *M*. After 1 hr of incubation at 37° the enzymatic reaction was interrupted by bringing the pH to 5.0 with 0.1 *N* HCl. 5-HT was extracted from the incubation mixture with 20 vol of acetone.[11] The dried extracts were stored at −20° overnight and 5-HT was assayed biologically.[12] The activity of 5-HTP-D was expressed in μg of 5-HT produced by 1 g of kidney within 1 hr of incubation.

5-Hydroxytryptamine Inactivation.—Rat liver homogenates served as the source of MAO. The activity of this enzyme was determined manometrically as described previously.[13] However, tryptamine hydrochloride instead of tyramine hydrochloride was used as the substrate.

Antiserotonin Activity *in Vitro.*—The investigation was carried out on isolated guinea pig ileum and isolated rat stomach strips[14] bathed in air-bubbled Tyrode solution at 38°. As an agonist of 5-hydroxytryptamine, creatinine sulfate in a concentration of 0.1–10 ng/ml was used. LSD (10–100 ng/ml) and morphine hydrochloride (0.5–2 μg/ml) were used as the standard myotropic and neurotropic tryptamine receptor antagonists, respectively.[14] The time of contact with antagonist was 2 min. The antiserotonin activity of β-carbolines was determined using Ariëns' plots[15] and the method of Schild's pA_x values.[16] pA_x values were determined graphically.[17]

Antiserotonin Activity *in Vivo.*—Cats and Wistar rats were anesthetized with chloralose and amobarbital. Blood pressure was recorded from the carotid artery with a Condon manometer. In cats the contractions of the nictitating membrane were registered. All drugs were injected into the femoral vein. Blood pressure response to injection of 5-HT in doses 20–50 μg/kg was considered as the standard agonist effect. Antagonists, *i.e.*, β-carbolines, were injected in doses of 1–80 mg/kg 2 min previous to the application of the agonist. The ability of the β-carbolines to antagonize the tryptamine receptor in cerebral tissue was followed by the procedure of Tedeschi and co-workers.[18]

Results

Acute Toxicity and Effect on Behavior.—The LD_{50} values were studied between 200 and 500 mg/kg for all compounds. Acute toxicity for the 1-methyl-sub-

(9) Melting points were determined with Kofler block and are not corrected.

(10) J. H. Gaddum and N. J. Giarman, *Brit. J. Pharmacol.*, **11**, 88 (1956); S. A. P. Price and G. B. West, *J. Pharm. Pharmacol.*, **12**, 617 (1960).

(11) A. H. Appin, T. B. B. Crawford, and J. H. Gaddum, *J. Physiol. (London)*, **126**, 596 (1954).

(12) J. R. Vane, *Brit. J. Pharmacol.*, **12**, 344 (1957).

(13) R. Gryglewski and K. Kudzieława, *Dissertationes Pharm.*, **13**, 111 (1961).

(14) J. H. Gaddum and Z. P. Picarelli, *Brit. J. Pharmacol.*, **12**, 323 (1957).

(15) E. J. Ariëns, A. M. Simonis, and J. M. von Rossum in Molecular Pharmacology, E. J. Ariëns, Ed., Academic Press Inc., New York, N. Y., 1964, p 383.

(16) H. O. Schild, *Brit. J. Pharmacol.*, **2**, 189 (1947); **4**, 277 (1949).

(17) R. Gryglewski, S. Wolfarth, and M. Zielińska, *Dissertationes Pharm.*, **17**, 467 (1955).

(18) D. H. Tedeschi, R. E. Tedeschi, and E. J. Fellows, *J. Pharmacol. Exptl. Therap.*, **126**, 223 (1956).

TABLE I

THE INFLUENCE OF 1-SUBSTITUTED 6-METHOXY-1,2,3,4-TETRAHYDRO-β-CARBOLINES ON MAO ACTIVITY IN RAT LIVER HOMOGENATES

Radical	Concn, M	% inhib (−) or activation (+) of MAO ± SE[a]	n[b]	t	P
1-Methyl	0.001	−11.78 ± 2.25	17	2.153	0.02–0.05
	0.01	−26.63 ± 1.94	11	5.230	<0.001
1-Ethyl	0.001	+2.68 ± 1.64	5	0.591	
	0.01	−19.83 ± 0.72	6	6.177	0.001–0.01
1-Propyl	0.001	+2.63 ± 2.19	5	0.154	
	0.01	−36.52 ± 1.43	5	16.907	<0.001
1-Isopropyl	0.001	+8.24 ± 2.89	6	1.405	
	0.01	−27.18 ± 2.89	6	7.171	<0.001
1-Phenyl	0.001	−6.45 ± 2.89	6	1.282	
	0.01		Insoluble		
1-Benzyl	0.001	−16.83 ± 3.95	16	3.181	0.001–0.01
	0.01		Insoluble		
1-(2-Pyridyl)	0.001	−13.06 ± 2.65	11	2.622	0.02–0.05
	0.01	−40.46 ± 3.88	6	10.039	<0.001
1-(3-Pyridyl)	0.001	−1.30 ± 0.47	6	0.121	
	0.01	−35.67 ± 2.34	6	9.386	<0.001
1-(4-Pyridyl)	0.001	−16.99 ± 1.09	12	4.910	<0.001
	0.01	−69.65 ± 1.41	6	43.505	<0.001
Iproniazid	0.0001	−19.06 ± 2.95	6	6.100	0.001–0.01
	0.001	−82.64 ± 1.41	6	—	<0.001

[a] The average MAO activity in $n = 18$ control samples is expressed by the value 119.2 ± 4.7 μl of O_2/1 g of wet tissue per 1 hr. [b] Number of estimations.

stituted derivative was 350 mg/kg.[19] The difference in toxicity between the particular compounds depends on the chemical nature of the radical at position 1. The toxicity rose successively for 1-alkyl, 1-pyridyl, and 1-aryl derivatives.

With subtoxic doses, a striking difference in the behavior between the animals treated with 1-alkyl and 1-aryl derivatives on the one hand and 1-pyridyl derivatives on the other hand was observed. The first group of β-carbolines produced an excitation followed by atetotic movements and clonic seizures, while the second group was responsible for generalized depression and sleepiness in a nonnarcotic state. Both types of central activity developed 10–20 min after β-carboline injection.

The Effect on 5-HT Biosynthesis.—Under our conditions the activity of 5-HTP-D can be expressed as 26.0 ± 2.2 μg of 5-HT produced by 1 g of rat kidney within 1 hr for $n = 9$ estimations. Except for 1-ethyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline, which slightly stimulated the activity of 5-HTP-D, none of the test compounds affected significantly 5-HT biogenesis. The average activity of 5-HTP-D in the presence of eight β-carbolines studied at 0.001 M varied around 104.5 ± 5.4% of 5-HTP-D activity in control homogenates.

The Effect on 5-HT Inactivation.—The results are presented in Table I. In control homogenates the activity of MAO was expressed by the value of 119.2 ± 4.7 μl of O_2 consumed by 1 g of rat liver within 1 hr for $n = 18$ determinations. All β-carbolines were tested at 0.001 and 0.01 M concentrations, while the standard MAO inhibitor, iproniazid, was tested at 0.0001 and 0.001 M concentrations. In lower concentrations only the 1-(4-pyridyl) and 1-benzyl derivatives and iproniazid inhibited the activity of MAO. In higher concentration all soluble compounds inhibited the enzyme. The concentrations employed in this study were rather high, and so β-carbolines are not to be considered as true MAO inhibitors. Only in the case of the 1-(4-pyridyl) and 1-benzyl derivatives was this effect reproducible *in vivo*. Generally speaking the MAO-inhibiting effect of β-carbolines, if any, was obscured *in vivo* by their antiserotonin activity.

The Effect on 5-HT Receptor *in Vitro*.—There existed a competitive, reversible antagonism for the myotropic tryptamine receptor between 5-HT and the β-carbolines studied, which could be proved using Ariëns' plots.[15] As the pA_2–pA_{10} difference was not significantly smaller than log 9, it could be expected that the antagonism was really competitive and monomolecular.[20] There was no need to use the pK_i value (the inverse logarithm of the apparent dissociation constant of the complex antagonist–receptors), which should be used in the case of a multimolecular type of antagonism. The β-carbolines did not act on neurotropic tryptamine receptors in guinea pig ileum, since the presence of morphine hydrochloride in Tyrode solution did not change the nature of competition.[14]

The pA_2 values were the real measure for antiserotonin activity of β-carbolines on myotropic receptors in rat stomach strips. LSD was used as the standard myotropic antagonist. The results are presented in Figure 2. The most potent 5-HT antagonists proved to be the 1-(4-pyridyl) and 1-benzyl derivatives, the pA_2 values of which were 6.50 and 6.12, respectively.

(19) A. J. Poleshaieva, *Farmakol. Toksykol.*, **27**, 165 (1964).

(20) M. Rocha e Silva and J. G. Leme in "Recent Advances in the Pharmacology of Toxins," H. W. Raudonat, Ed., Pergamon Press Ltd., London, 1964, p 33.

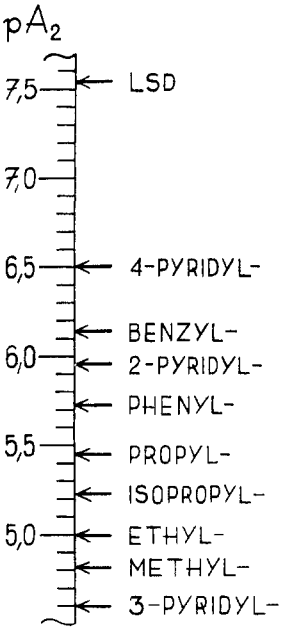


Figure 2.—The antiserotonin activity of LSD and of nine 1-substituted 6-methoxy-1,2,3,4-tetrahydro-β-carbolines expressed as pA_2 values.[16] The compounds were tested on the myotropic tryptamine receptor of rat stomach strips.[12] Each determination was repeated at least three times. The mean deviation of these readings was smaller than 0.15 pA_2 unit.

Since the pA_2 value for LSD is 7.52, the most active β-carboline was still only one-tenth as active as the standard antagonist. The least active were 1-alkyl derivatives (see Figure 2).

The Effect on 5-HT Receptor *in Vivo*.—Most of investigated β-carbolines reduced markedly the 5-HT effect on blood pressure in the cat and rat, when the compounds were applied in doses 20–30 mg/kg. More active were only the 1-(4-pyridyl) and 1-benzyl derivatives, which when applied at 5–10 mg/kg not only abolished the effect of 5-HT on blood pressure, but also the 5-HT effect on the nictitating membrane in the cat. The antiserotonin effect of the β-carbolines on rat blood pressure lasted much shorter than the antinictitating membrane effect.

The 1-(4-pyridyl) derivative applied intraperitoneally antagonized tryptamine convulsions, ED_{50} = 23.5 (13.1–42.3) mg/kg. Other tryptamine-induced side effects were not antagonized.[18]

Discussion

The most prominent pharmacological activity of the compounds was on the myotropic tryptamine receptor. All β-carbolines possessed an affinity to the 5-HT receptor but no intrinsic activity when compared with 5-HT. The antiserotonin effect is therefore based on the principles of competitive, monomolecular, and reversible antagonism with 5-HT. The activity of test compounds was confined to values between 4.60 and 6.50 pA_2. Interestingly the most active was the 1-(4-pyridyl) and the least active the 1-(3-pyridyl) derivative (Figure 1). Thus the change of the position of the nitrogen in the pyridine ring attached to the β-carboline moiety can intensify the antiserotonin activity of the compound nearly 100 times. Generally 1-alkyl-substituted derivatives were less active than 1-aryl and 1-pyridyl compounds. In the series of 1-alkyl derivatives longer carbon chain produced stronger antiserotonin properties. The least active was 1-methyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline previously called "adrenoglomerulotropine." This scale of antiserotonin activity *in vitro* was checked *in vivo* and good agreement was found. All β-carbolines tested were less active than the standard antiserotonin compound. However, LSD in concentrations above 0.1 μg/ml caused long-lasting contractions of guinea pig ileum, rat stomach, and uterus, while β-carbolines applied even in the high concentration of 100 μg/ml did not contract smooth muscles. Since this is a very convenient property for an antiserotonin drug, the investigation in the group of β-carbolines could be promising from a practical point of view.

We were not able to find any effect on 5-HT biosynthesis. The slight inhibitory effect of β-carbolines on MAO activity is more pronounced only in the case of the 1-(4-pyridyl) derivative. This enzymatic effect can hardly be demonstrated *in vivo* as the antiserotonin effect of β-carbolines is much stronger.

It should be noted that substitution of 1-alkyl or 1-aryl radicals for one of the 1-pyridyl isomers produced compounds with different central activity. This phenomenon needs further study.