CYCLOPENTENYL ETHYLAMINES ACTIVE ON CNS(*)

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SUMMARY — *Two new cyclopentenylethylamines were prepared and were submitted to a pharmacological screening together with some others previously described and now reprepared. All compounds exhibited different degrees of depressive activity on CNS and good analgesic activity. Compound 5, bearing a phenyl group on the carbon atom to which the amino group is connected, appears rather interesting being the most active as analgesic and the least toxic. Compounds 2 and 3 are able to antagonize in a certain degree lethal doses of physostigmine and also, respectively, of pentylenetetrazole and strychnine.*

RIASSUNTO — *Sono state preparate due nuove ciclopenteniletilammine che, congiuntamente ad altre precedentemente descritte ed ora ripreparate, sono state sottoposte ad esame farmacologico. Tutte le sostanze esercitano gradi varianti di attività depressiva sul SNC ed elevata attività analgesica. Il composto 5, che contiene un fenile legato al carbonio che porta la funzione amminica, appare di un certo interesse essendo il più attivo come analgesico ed il meno tossico. I composti 2 e 3 sono capaci di antagonizzare in discreta misura dosi letali di fisostigmina, nonchè, rispettivamente, di pentilenetetrazolo e di stricnina.*

Introduction

Amino derivatives of terpenoid structures deserve from a long time a large interest for the variety of pharmacological activities that they elicit (1-3). Thus, in order to evaluate the effect of replacing the planar benzene ring with more or less puckered cycloalkyl moieties, in molecules with adrenergic and CNS stimulating activities, two of us prepared in the past (4), together with several other compounds, the β-camphylamine 1 and its homologues 2 and 3 [2-amino-1-(2,3,3-trimethylcyclopent-1-en-1-yl)-propane and 2-amino-1-(2,3,3-trimethylcyclopent-1-en-1-yl)-butane respectively].

Since from a preliminary pharmacological screening of those three amines some differences of activity depending from the nature of R were apparent, we deemed interesting to undertake a somewhat larger study of those compounds and also of the amines with R equal to n-propyl (4) and phenyl (5), where the lipophilicity and the steric hindrance on the amino function are further increased.

Scheme 1
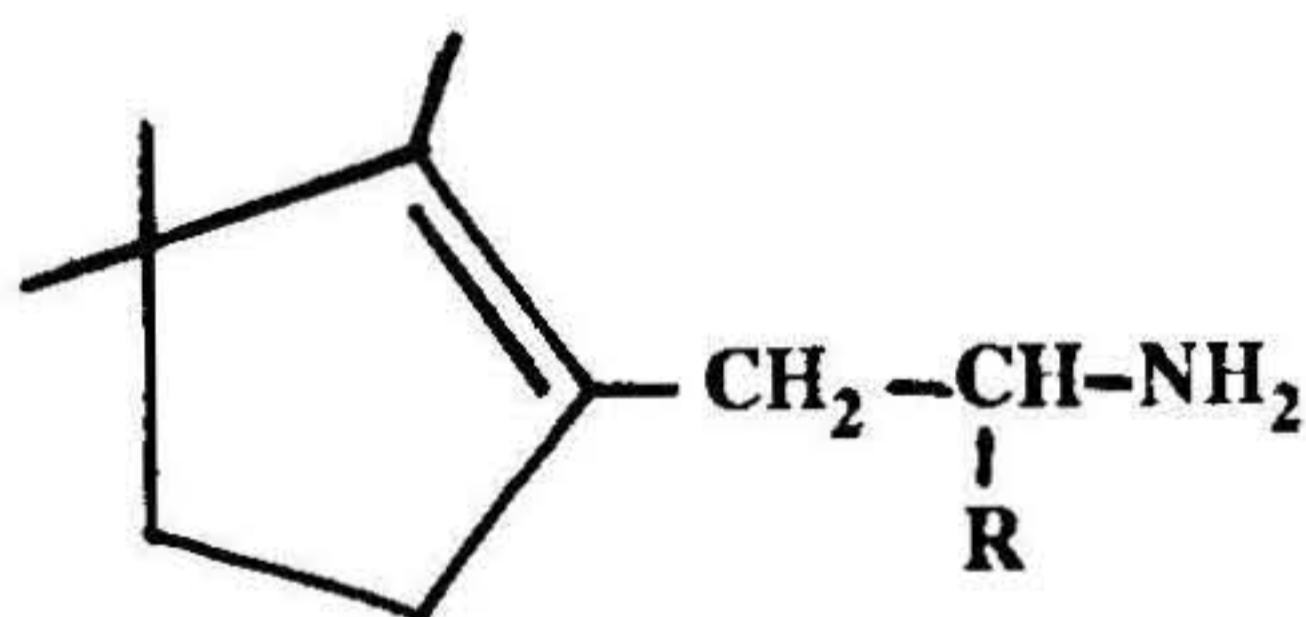
	R =	H	1
		CH_3	2
		C_2H_5	3
		$n\text{-}C_3H_7$	4
		C_6H_5	5

Chemistry

The β-camphylamine 1 was prepared as already described (4) by the lithium aluminum hydride reduction of β-campholenic amide (5). The amines 2-5 were obtained through the sequence of reactions previously set up for the preparation of 2 and 3 (Scheme 2). The α-campholenic nitrile was reacted with the suitable alkyl or phenyl magnesium halides (6) and the resulting ketones were treated with formamide and formic acid in the experimental conditions of the Leuckart reaction. Finally the formylamides 9 were hydrolized with hydrochloric acid giving place in the same time to the rearrangement of the carbon skeleton (4).

The new ketones (8 with $R = n\text{-}C_3H_7$ and C_6H_5) were characterized thorugh the elemental analysis of their semicarbazones and their I.R. and NMR spectra. The former spectra exibit bands at 2994 and 800 cm^{-1} due to the stretching and deformation vibrations of C-H on the double bond, and bands respectively,at 1706 and 1700 cm^{-1}, due to the carbonyl group. The NMR spectra show a singlet (poorly resolved triplet) at $\delta = 5.22$ arising from the olefinic proton and multiplets at $\delta = 1.80$ and 1.89 respectively, due to the cyclic CH bearing the side chain.

Scheme 2
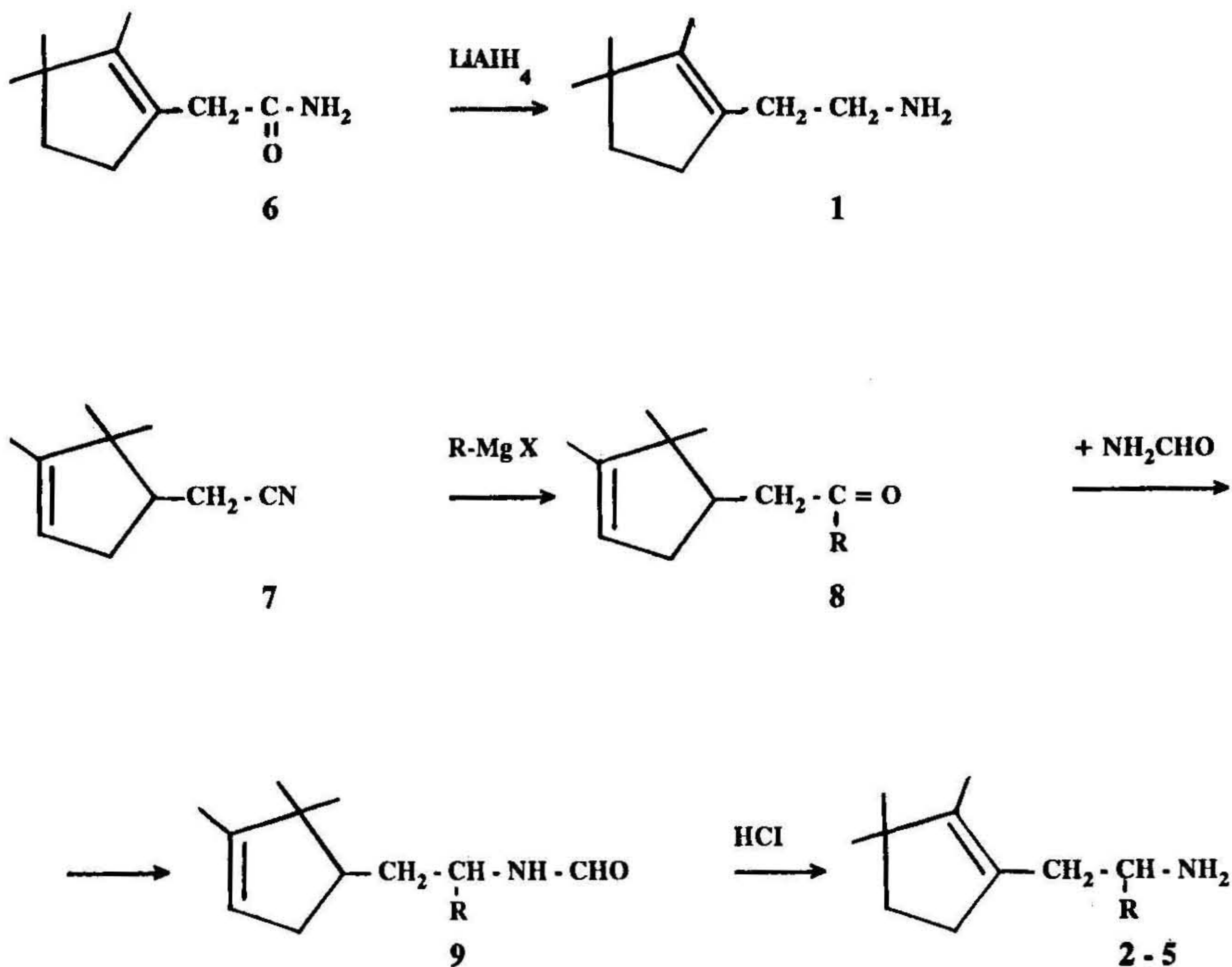
R = CH$_3$; C$_2$H$_5$; n-C$_3$H$_7$; C$_6$H$_5$

X = Br; I

The formylamines **9** were purified through chromatography on silica gel and used without further characterization; their IR spectrum (film) showed bands around 1660 cm^{-1} due to the formyl group.

The new amino compounds **4** and **5** were characterized as hydrochlorides. Their IR spectra (KBr) do not exhibit any band attributable to olefinic hydrogen. The rearrangement of the α-campholenic skeleton to the β-campholenic one was further supported by the NMR spectra where both signals at $\delta = 5.22$ and 1.89 have disappeared (see experimental part).

Chemical experimental section

Melting points were uncorrected; the mp of the amine hydrochlorides were determined in vacuum sealed capillary tubes. Elemental analyses (C,H,N) were performed at the Microanalytical Laboratory of the Istituto di Scienze Farmaceutiche of Genova University; the analytical results were within ±0.3% of the calculated values.

IR spectra were recorded with a Perkin-Elmer mod. 297 spectrophotomer, using liquid samples as film and including solid samples in KBr pellets; NMR spectra were taken on a Varian XL - 200 spectrometer, using $CDCl_3$ as solvent with TMS as internal standard. Chemical shifts are given in ppm (δ).

α-Propyl-and α-phenyl-campholenones (8; $R = nC_3H_7$ and C_6H_5)

The new ketones were obtained following the indications already described (6) for the preparation of α-methylcampholenone, starting from 0.15 moles of propylmagnesium iodide or phenylmagnesium bromide and 0.1 mole of α-campholenic nitrile. The crude ketones were distilled several times under reduced pressure (b.p. 140-145°C at 0.3 - 0.5 mmHg; air bath temp.) in a bulb to bulb apparatus, to remove as much as possible the unreacted nitrile. In both cases the yields were around 40-42%.

8 with $R = n-C_3H_7$; ¹H-NMR: 0.77(s, 3H; CH_3); 0.92(t, 3H, CH_3-CH_2); 0.99(s, 3H; CH_3); 1.52-1.70(m with superimposed s at 1.62, 5H; CH_2-CH_3 + CH_3-C = C); 1.80 (mc, 1H; CH); 2.15-2.55 (m with superimposed t centered at 2.42, 6H; $3CH_2$); 5.22 (s, 1H; CH = C).

Semicarbazone: m.p. 119-120°C (ethanol/water); analysis for $C_{14}H_{25}NO_3$ (C,H,N).

8 with $R = C_6H_5$; ¹H-NMR: 0.88(s, 3H; CH_3); 1.05(s, 3H; CH_3; 1.62(s, 3H; CH_3-C = C); 1.89(mc, 1H; CH); 2.38(mc, 2H; CH_2-C = C); 3.00(mc, 2H; >CH-CH_2-CO); 5.23(s, 1H; CH = C); 7.48(mc, 3H arom); 7.97(mc, 2H arom).

Semicarbazone: m.p. 170-172°C (ethanol); analysis for $C_{17}H_{23}N_3O$ (C,H,N).

N-Formylamines 9 from Leuckart reaction on ketones 8

A mixture of ketones 8 (0.05 moles) with formamide (6.75 g; 0.15 moles) and 99% formic acid (2.3 g; 0.05 moles) was heated at 170°C for 48 hours under a slow stream of nitrogen. The working up was as already described (4), but the crude formylamines were chromatographed on silica gel eluting with chloroform.

The amide 9 with $R = n-C_3H_7$ was further purified by distillation under reduced pressure (b.p. about 140°C at 0.3-0.5 mmHg; air bath temp.).

The amide 9 with $R = C_6H_5$ was not distilled, it was obtained from chromatography as a low melting solid whose TLC indicated a single compound. Yields were around 60-70%.

Hydrolysis of N-formylamines:
2-amino-1-(2,3,3-trimethyl-cyclopent-1-en-1-yl)-pentane (4) and
1-amino-2-(2,3,3-trimethyl-cyclopent-1-en-1-yl)-1-phenyl-ethane (5)

The solution of 0.02 moles of formylamines 9 in 25 ml of ethanol was added with an equal volume of 6N HCl and refluxed for 10 hours. The solution was concentrated under reduced pressure until a fourth of its initial volume that was restored

with cold water. The amines **2** and **3** were isolated working up the acid solution as already described (4), while in the case of amines **4** and **5** the hydrochlorides separated spontaneously as white needles that were collected and crystallized from hot water.

4 Hydrochloride: m.p. 213-214°C; analysis for $C_{13}H_{25}N \cdot HCl$ (C,H,N).

^{1}H-NMR (hydrochloride in $CDCl_3$): 0.92 (t, 3H; CH_3-CH_2); 0.95(s, 3H, CH_3); 1.00(s, 3H; CH_3); 1.40-1.80(m with superimposed s at 1.55, 9H; $3CH_2 + CH_3$-$C=C$); 2.18(mc, 2H; CH_2-$C=C$); 2.48(mc, 2H; $C=C$-CH_2-CH-N); 3.28(m, 1H; $>CH$-N); 8.33(large s, 3H; $+NH_3$).

5 Hydrochloride: m.p. 240-245°C; analysis for $C_{16}H_{23}N \cdot HCl$ (C,H,N).

^{1}H-NMR (hydrochloride in $CDCl_3$): 0.70(s, 3H; CH_3); 0.89(s, 3H; CH_3); 1.23(s, 3H; CH_3-$C=C$); 1.47(t, 2H; CH_2-$C(CH_3)_2$); 1.92(mc, 2H; CH_2-$C=C$); 2.71(mc, 2H; $C=C$-CH_2-CH-N); 4.21(mc, 1H; $>CH$-N); 7.27(mc, 3H arom); 7.42(mc, 2H arom); 8.76(broad s, 3H; $-NH^+_3$).

Amine hydrochlorides

For the pharmacological screening the amines **1,2** and **3**, that were isolated as oily free bases, were converted in their hydrochlorides. They were dissolved in the stoichiometric volume of 1N ethanolic HCl and the solution was evaporated to dryness under reduced pressure. The residue was dissolved in the minimum volume of absolute ethanol and the salt was precipitated with dry ether.

Pharmacology

On the five amines **1-5** the following investigations have been worked out: LD_{50}, explorative activity, motor coordination, antinociceptive properties (peripheral and central, with writhing and hot plate test), antagonism against physostigmine, strychnine and pentylenetetrazole toxicities.

Material and methods

For all the above mentioned tests, female Swiss albino mice were used. The animals, weighing 18-22 g, were fasting from 12 hours before tests but with free access to water and housed at constant temperature (20-22°C). The test compounds as hydrochlorides were administred *per os* (through a stomach tube) dissolved or suspended in a 1% methylcellulose solution (0.25 ml/10 g body weight), 60 minutes before tests.

Acute toxicity

For each scalar dose, groups of ten animalss were used; these were close observed during 6 hours from treatment to detect the symptomatology elicited from the test compounds. Thereafter the animals were observed for a total of 48 hours and at end of this time the mortality was detected and LD_{50} was calculated with the Sperman-Karber method (7).

Explorative activity

It was detected with the Boissier and Simon test (8) using a square board 37 cm wide with 16 equidistant holes (2.2 cm diameter), on which each animal was kept

for 5 minutes and the explored holes were counted. Compounds were administered at the dose of 50 mg/kg using 5 mice. Control animals received only methylcellulose solution. Diazepam (5 mg/kg *p.os*) was used as reference drug.

Motor coordination (Muscle relaxing activity)

This activity was evaluated with the Kinnard and Carr (9) method. using a "Rotarod" apparatus (U. Basile, Milano) turning at 16 rpm. Six hours before dosing, animals were selected; only those remaining on the turning rod for more than 120 seconds were utilized. To these animals (5 for each compound) the test substances were given at the dose of 50 mg/kg one hour before the test. Mice that remained on the turning rod for less than 2 minutes were considered incoordinate. Diazepam (5 mg/kg *p.os*) was used as reference agent.

Antinociceptive activity

a) *Writhing test*. The method of Sigmund *et al.* (10) was used with minor modifications. To groups of 8 animals the test compounds were administered *p.os* at two dose levels (25, 50 or 100 mg/kg, depending on toxicity). The control group received only the methylcellulose solution. After 1 hour to each mouse was injected *i.p.* 0.1 ml/10 g body weight of 0.25% solution of formic acid. The writhings of each animal were recorded for 20 minutes starting from the injection of the algesic agent. The antinociceptive activity was expressed as the % reduction of writhings number compared to that of the control animals.

b) *Hot plate test*. The method of Woolfe-McDonald (11) was used, employing 10 animals for each dose (25, 50, 100 mg/kg) of each compound. The animals were placed on a stainless steel plate heated at $55 \pm 0.5°C$ and mean reaction time was determined for each group of mice just before the administration of the test compounds and 1, 2 and 3 hours after the dosing. The percent reaction time variations were referred to initial values. The animals with an initial reaction time superior to 10 sec were discarded.

For both antinociceptive tests morphine was used as reference drug at the dose, respectively, of 6.25 and 12.5 mg/kg *p.os*.

Protective effects against physostigmine, strychnine and pentylenetetrazole toxicities

For all these tests, groups of 5 mice were used, each receiving a dose ranging from 12.5 to 100 mg/kg *p.os* one hour before the *i.p.* injection of the convulsant agents.

a) *Antiphysostigmine activity*. The method of Nose and Kojima (12) was used and physostigmine salicylate (corresponding to 0.75 mg/kg of base) was injected. The number of surviving animals was counted 60 minutes after the injection of the acetylcholinesterase inhibitor. Atropine sulfate (5 mg/kg of base, *p.os*) was used as reference drug.

b) *Antistrychnine activity*. The protection from a rapidly lethal dose of strychnine nitrate (2.5 mg/kg of base), was evaluated with the Morpurgo method (13). The surviving animals 5 minutes after the *i.p.* injection of strychnine were counted.

c) *Antipentylenetetrazole activity*. It was evaluated with the method of Desmedt *et al.* (14) slightly modified; the surviving animals 15 minutes after the *i.p.* injection of 100 mg/kg of pentylenetetrazole were counted.

For tests **b)** and **c)** diazepam (10 mg/kg; *p.os*) was used as reference substance.

Results and discussion

The results of the pharmacological screening on cyclopentenylethylamines **1-5** are illustrated in Tables I-V. It is worthy to note that all compounds were rather toxic (oral LD_{50} between 83 and 332 mg/kg) and were generally endowed with CNS depressant activity, although these properties do not seem to be correlated to each other. Infact, in compounds **2, 3** and **4** by increasing the aliphatic chain length the toxicity was lowered while the CNS depressant activity was progressively more intense and long lasting, as it was observed during the DL_{50} determination (decreased spontaneous activity, hypotonicity, ataxia, ptosis) and in the explorative activity, motor coordination and central analgesic tests (Tables II and IV).

The compound **1** exibited a depressive symptomatolgy comparable with that of the homologue **2**, but it was much less toxic probably on account of the easier oxidative metabolism of the unhindered aminogroup. On the other hand the compound **5** containing a benzene ring was the least toxic and the least active as CNS depressant, so far the motor activity and coordination were considered, while it was very active as analgesic.

Concerning the antinociceptive activity it is worth noting that all compounds exhibited some degree of analgesia in both used tests; the activity against a chemical stimulus (peripheral analgesia) was stronger in compounds **2** and **3**, while that against a thermic stimulus (central analgesia) was higher in compounds **4** and **5**. Particulary the compound **5** deserves further investigations for its high and long lasting analgesic activity joined with a moderate toxicity. Actually, this amine shows some structural analogies with 1-amino-1,2-diphenylethane (15,16) and with lefetamine (17,18), a well known central analgesic, where a benzene ring is replaced by a cyclopentene nucleus.

Finally we mention some degree of protection against lethal doses of physostigmine, strychnine and pentylenetetrazole although the protective activities were not always dose dependent (Table V).

Thus the compound **2** at 25 mg/kg antagonized physostigmine (4 survivors/5) and pentylenetetrazole (3/5), while compound **3** at 50 mg/kg partially antagonized physostigmine (3/5) and strychnine (2/5).

Without excluding the possibility that the antagonism to physostigmine is the result of non specific mechanism, as established in several cases by Niemegeer *et al.* (19) it seems worthwhile to investigate if such activity of the amines **1-5** is expression of a central anticholinergic activity that could be very interesting as prerequisite for antiparkinson activity.

Concluding, the results of these preliminary assays support our interest on these cyclopentenylethylamines, whose pharmacogenic potentialities will be further investigated.

TABLE I

Oral acute toxicity of amines 1-5

Compounds	R	LD$_{50}$ (95% confidence limits) mg/kg, p. os		
1	H	260.0 (	268.5 - 251.6	)
2	CH_3	83.2 (	95.2 - 72.6	)
3	C_2H_5	172.8 (	194.6 - 153.4	)
4	$n-C_3H_7$	304.8 (	355.3 - 261.4	)
5	C_6H_5	332.3 (	384.4 - 287.2	)

TABLE II

CNS activities of amines 1-5

Compounds (50 mg/kg,p.os)	% of LD$_{50}$	Explorative activity		Muscle relaxing activity
		Mean number of explored holes ± s.e.	Variation %	Incoordinate animals/ treated animals
Controls		43.4 ± 1.25		0 / 5
Diazepam(a)		7.6 ± 1.77	-82	5 / 5
1	19,2	22.4 ± 2.54	-48	2 / 5
2	60,1	18.4 ± 2.72	-57	2 / 5
3	28,9	6.0 ± 1.22	-86	3 / 5
4	16,4	3.75 ± 0.62	-91	5 / 5
5	15,0	31.8 ± 3.21	-26	0 / 5

(a) 5 mg/kg,p.os

TABLE III

Antinociceptive activity of amines 1-5 (writhing test)

Compounds	Dose (mg/kg) p. os	% LD$_{50}$	Number of writhings at the indicated time after formic acid injection ± s.e. (% variation compared to controls)			
			5'	10'	15'	20'
Controls			5.25±0.45	13.25±1.12	18.35±1.60	23.67±1.89
Morphine	6.25		0.87±0.29	2.50±0.80	3.50±0.73	4.75±0.99(-80)
1	50	19,2	1.57±0.57	5.00±1.29	7.57±1.87	9.17±2.34(-61)
1	100	38,5	1.12±0.66	4.25±1.70	7.00±2.28	7.75±2.44(-67)
2	25	30,0	1.25±0.49	4.63±1.08	7.25±1.39	8.75±1.85(-63)
2	50	60,1	0.14±0.14	1.28±0.74	3.28±1.37	4.14±1.79(-82)
3	50	28,9	1.25±0.97	2.75±1.37	4.75±2.08	6.00±2.63(-75)
3	100	57,9	0.62±0.26	1.25±0.45	1.50±0.49	1.62±0.56(-93)
4	50	16,4	0.37±0.18	3.37±1.06	7.12±1.44	9.75±1.73(-59)
5	50	15,0	0.25±0.16	3.12±0.91	7.25±1.78	10.00±2.39(-58)
5	100	30,1	0.12±0.12	1.25±0.45	3.00±0.62	4.37±0.88(-82)

TABLE IV

Antinociceptive activity of amines 1-5 (Hot plate test)

Compounds	Dose (mg/kg) p. os (a)	Reaction time (in sec.) ± s.e. after the indicated hours from treatment (% variation compared to the initial reaction time)			
		0'	1 h	2 h	3h
Controls		8.75±0.64	8.98±0.43 (+ 3)	10.49±0.66 (+20)	11.32±0.44 (+ 29)
Morphine	12.5	8.21±0.50	13.97±1.39 (+70)	14.08±1.04 (+71)	16.62±0.89 (+102)
1	50	8.57±0.48	12.63±0.96 (+47)	13.08±1.14 (+53)	11.75±1.48 (+ 37)
1	100	7.42±0.46	8.23±0.94 (+14)	11.75±1.38 (+58)	12.68±0.76 (+ 71)
2	25	8.57±0.48	8.38±0.62 (− 2)	11.70±0.77 (+36)	11.06±0.75 (+ 29)
2	50	8.31±0.32	12.33±1.28 (+48)	14.93±1.34 (+80)	13.15±1.55 (+ 58)
3	50	8.64±0.43	12.92±0.73 (+49)	14.32±1.29 (+66)	11.80±0.85 (+ 36)
3	100	8.07±0.42	15.37±1.19 (+90)	16.03±1.20 (+99)	14.86±1.21 (+ 84)
4	50	8.75±0.22	15.87±3.75 (+81)	15.59±0.88 (+78)	16.00±1.19 (+ 83)
5	50	7.65±0.53	14.95±1.19 (+95)	14.15±1.16 (+85)	16.77±0.90 (+119)
5	100	7.98±0.45	15.46±1.27 (+94)	15.48±1.40 (+94)	14.02±1.15 (+ 76)

(a) For % of DL_{50} see Table III

TABLE V

Protective effect of the pretreatment with amines 1-5 against drug toxicity

Compounds	Dose (mg/kg) p. os (a)	Surviving animals / treated animals after i.p. administration of		
		physostigmine (0.75 mg / kg)	strychnine (2.5 mg / kg)	pentylenetetrazole (100 mg / kg)
Controls		0 / 5	0 / 5	0 / 5
Atropine	5	5 / 5	n.d.	n .d.
Diazepam	10	n.d.	3 / 5	5 / 5
1	50	1 / 5	2 / 5	0 / 5
1	100	2 / 5	1 / 5	1 / 5
2	12.5	2 / 5	n.d.	n.d.
2	25	4 / 5	0 / 5	3 / 5
3	50	3 / 5	2 / 5	0 / 5
3	100	2 / 5	2 / 5	0 / 5
4	50	3 / 5	1 / 5	1 / 5
4	100	1 / 5	0 / 5	0 / 5
5	75	0 / 5	0 / 5	1 / 5
5	100	0 / 5	2 / 5	0 / 5

(a) For % of LD_{50} see Table III

n.d.= not determined

Acknowledgment

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