The present invention relates to the use of a 1,3,7-trimethylxanthine compound for the preparation of medicaments for the treatment of memory disorders and Alzheimer's disease.

More specifically, the invention relates to the use of 1,3,7-trimethyl-8-[3-(4-diethylaminocarbonylpiperazino)-propyl]xanthine and its physiologically tolerable acid addition salts for the preparation of medicaments for the treatment of memory disorders and Alzheimer's disease.

1,3,7-trimethyl-8-[3-(4-diethylaminocarbonylpiperazino)-propyl]xanthine and its physiologically tolerable acid addition salts are described in European Patent No. EP-A-0 149 578 as being usable in the treatment of migraine and asthenia at a dose of 50 mg/kg p.o., by virtue of their analgesic properties, on the one hand, and their psychotonic properties, on the other hand.

The Applicant has now found that 1,3,7-trimethyl-8-[3-(4-diethylaminocarbonylpiperazino)propyl]xanthine and its physiologically tolerable acid addition salts have valuable properties, especially properties facilitating memory acquisition and retention and anti-amnesic properties, which allow them to be used for the preparation of medicaments for the treatment of memory disorders and Alzheimer's disease, and this at specific doses that are much lower than the doses previously used for the treatment of migraine.

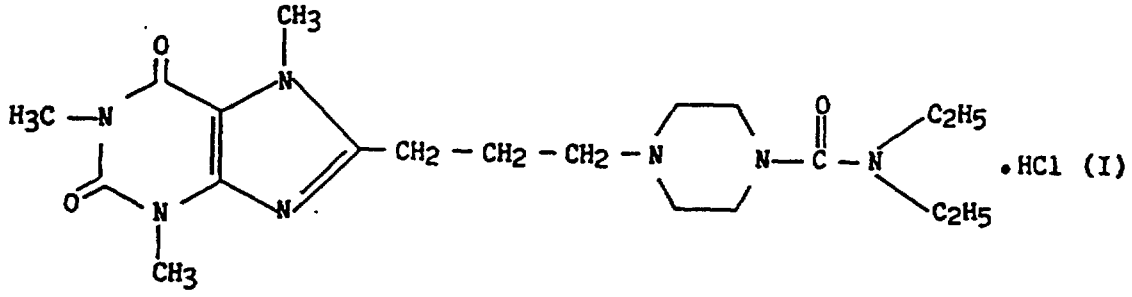
The present invention relates also to pharmaceutical compositions containing 1,3,7-trimethyl-8-[3-(4-diethylaminocarbonylpiperazino)propyl]xanthine or a physiologically tolerable salt thereof as active ingredient, in admixture or in association with a suitable pharmaceutical excipient.

The pharmaceutical compositions so obtained are advantageously in pharmaceutical forms suitable for administration orally or parenterally and especially intravenously, such as, for example, tablets, dragées, gelatin capsules, and injectable or drinkable solutions.

The dosage varies according to the age and weight of the patient, the mode of administration and the nature of the therapeutic indication and of associated treatments, and ranges from 2 to 50 mg of active ingredient per dose from 1 to 3 times per day.

The following Examples illustrate the invention and do not limit it in any way.

All the tests which follow were carried out using as active ingredient (on account especially of its good water solubility) 1,3,7-trimethyl-8-[3-(4-diethylaminocarbonylpiperazino)propyl]xanthine hydrochloride of formula I:



EXAMPLE 1:

<u>EFFECT OF PRODUCT I ON THE SPEED OF ACQUISITION AND LEARNING</u>

The procedure used, which is described by GALEY, DURKIN, SIFAKIS, KEMPF and JAFFARD in Brain Res., <u>340</u>, 171-174 (1985), makes it possible to:

1) Measure spontaneous alternation, changes in which are often predictive of the effects of a treatment on learning and spatial memory.

2) Measure the speed of acquisition of a simple spatial discrimination (right-left), which a normal animal masters in about twenty attempts.

3) Evaluate the retention of that discrimination in the memory in a test carried out 24 hours later.

A) Techniques and methods

- Animals

Fifty male mice of the BALB/c strain which had never before been exposed to experimentation and which were aged from 12 to 14 weeks were used. They were kept in individual cages in an air-conditioned animal laboratory (22°C) provided with an artificial light/dark cycle of 12 hours (0800-2000 hours).

- Apparatus

This is a T-shaped labyrinth made of grey plexiglass. The central corridor and the two arrival arms are 35 cm long, 10 cm wide and 25 cm high. The departure compartment (10 x 12 cm) is separated from the central corridor by means of a raisable gate. The entry to each arm is likewise provided with a gate.

- Procedure

Habituation: The actual experiment is preceded by two 10 minute habituation sessions (free exploration;

one session per day).

Spontaneous alternations and acquisition of the spatial discrimination: These two tests take place on the following day, without interruption. During the first 6 attempts, food is provided at the ends of the two arms. At each attempt, the animal is placed in the departure compartment for 30 seconds (interval between attempts). The gate allowing access to the central corridor is opened and the animal is able to choose between the two arms, where it remains enclosed for 30 seconds. It is found that a normal animal alternates its successive choices (approximately 65% alternation). Starting from the 7th attempt, food is provided in only one of the arms, always the same one. The test is continued until the animal succeeds in 5 successive attempts without making a mistake (that is to say, the criterion is that it goes into the filled arm 5 times).

Retention of the spatial discrimination: This test is carried out under the same conditions as the acquisition test, after a period of 24 hours. The test is continued until the animal again reaches the criterion of 5/5.

- Groups

The 50 animals were divided into 5 groups of 10 subjects:

- control-control (no treatment)
- solvent-solvent (0.1 ml/10 g of 7% NaCl under the same conditions)
- drug-drug (injection of product I 30 minutes before each test : 0.0625 - 0.25 - 1 $mg.kg^{-1}$ intraperitoneally)

B) Results

1) Spontaneous alternations

The results of the 5 groups are summarised in Figure A. The minimum dose (D1 : 0.0625 $mg.kg^{-1}$) improves the percentage of alternations (from 59.0 ± 3.1 % to 72.0 ± 5.3 %).

2) Acquisition of the spatial discrimination

The results are summarised in Figure B. It is found that administering product I at a dose of 0.0625 $mg.kg^{-1}$ (D1) reduces the number of attempts at the criterion in a highly significant manner. Product I improves the speed of acquisition in a dose-dependent manner, dose D1 giving a greater speed of acquisition than dose D2 and dose D3.

3) Retention of the spatial discrimination

The results are summarised in Figure C. At a dose of 0.0625 $mg.kg^{-1}$, the product reduces the number of attempts at the criterion very significantly, which is not the case with the higher doses. Here, too, there is a dose-dependent facilitating effect, dose D1 causing the criterion to be reached significantly more quickly than doses D2 and D3.

4) Conclusion

The i.p. administration of product I 30 minutes before each test improves spontaneous alternation, acquisition of a spatial discrimination and retention of that spatial discrimination 24 hours later (tests carried out in the T-shaped labyrinth). These facilitations are observed at the lowest dose used in the procedure (0.0625 $mg.kg^{-1}$)

and not at the two higher doses (0.025 and 1 $mg.kg^{-1}$).

5) Graphic expression of the results

Figure A : Spontaneous alternations
(mean ± SD)

C : control
S : solvent
D1 : 0.0625 $mg.kg^{-1}$
D2 : 0.25 $mg.kg^{-1}$
D3 : 1.0 $mg.kg^{-1}$

* significantly different
from C + S ; $p < 0.05$

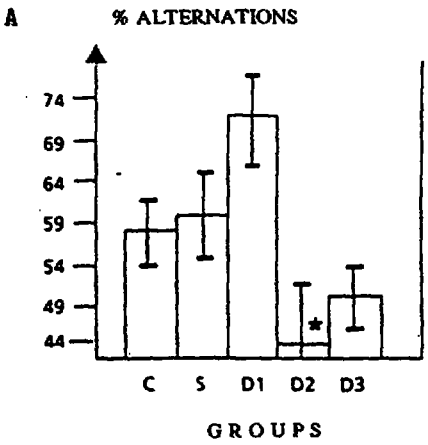


Figure B : Acquisition of the discrimination

(attempts at criterion : mean ± SD)

** significantly different
from C and S ; $p < 0.001$

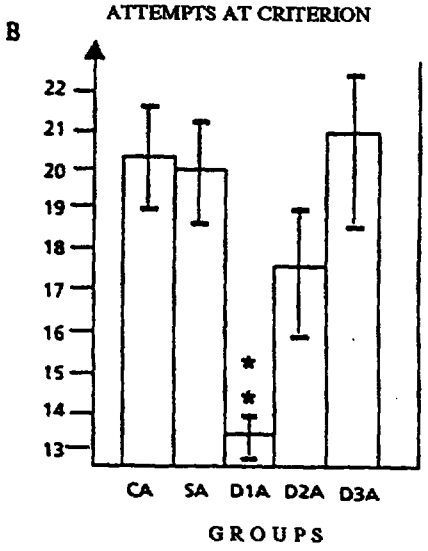
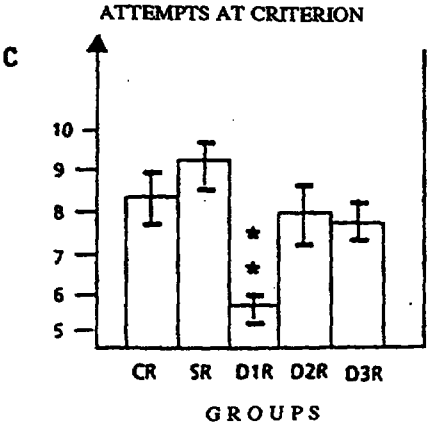


Figure C : Retention of the discrimination
(cf. Fig. B)

** significantly different
from C and S ; $p < 0.001$



EXAMPLE 2:

EFFECT OF PRODUCT I ON RETENTION OF THE MEMORY PHENOMENON

1) STUDY OF PASSIVE AVOIDANCE CONDITIONING IN RATS

A) Methodology:

In this test, the rats are able to explore freely a large white compartment and a small black compartment for 5 minutes. The time spent in each zone is noted, as is the time taken by the rats released into the white compartment to enter the black compartment for the first time. Each rat is then trapped in the black compartment and receives an electric shock to the paws which is impossible to avoid. One minute later, the rat undergoes another test for 5 minutes, in which it is free to go into the white or the black compartment. The length of time the rat takes to enter the black compartment and the length of time it remains in the latter are noted. The fixation of this passive avoidance response in the memory is then tested in all the rats after 48 hours.

The following doses of product I (expressed as base) were tested: 0.0625, 0.25, 1 and 4 mg/kg. The control rats received distilled water; all the injections were administered intraperitoneally 30 minutes before the acquisition sessions and the test of memory fixation after 48 hours. Ten rats were tested for each dose.

B) Results:

If the time taken to enter the black compartment is considered, it can be seen that the control animals exhibited no fixation in the test carried out 48 hours later. However, the groups of animals taken as a whole

exhibited memory fixation.

If the data for all the groups are analysed, the animals spend significantly less time on the black side after 48 hours, as compared with the situation before the shock, but the product x test interaction is of marginal significance. When only the data of the controls and of the 0.0625 mg/kg group are analysed, the product x test interaction reaches statistical significance.

2) WORKING AND REFERENCE MEMORY

A) Methodology:

In this test, the rats were not deprived of food, since they were rewarded by a sugary titbit. Before the start of training, the rats were given such titbits in their cages for five days in order to accustom them to the titbits.

In each test, the sugary titbits were placed opposite 3 holes out of a possible 9 holes; the task for the rat was to visit only the holes with titbits, to take and eat the latter, and not to return to the hole once the titbit had been taken from there. These are the same holes which had received the bait each day, so that visits to the holes without bait indicate errors of reference memory. Further visits to a hole where the titbit has already been removed and consumed indicate an error of working memory in this study. The performances are analysed by the time taken to complete the task (remove and consume the three titbits) and by the number and nature of the errors made. One test was carried out per day. When the animals had met the criteria (carrying out the task in less than 60 seconds, taking and eating the three sugary titbits and not making more than one

mistake), they were left quietly in their cages before undergoing a further test 7 days later. Memory fixation was tested three weeks later.

Male Lister rats were used; ten of them were assigned to each of the following groups: control (water); product I (0.0625, 0.25, 1 and 4 mg/kg; all doses are expressed as base). The injections were administered intraperitoneally each day, 30 minutes before the test.

B) Results:

All the animals learned to carry out the task. On the whole, product I did not significantly alter the time required to complete the task.

On the whole, the rats took significantly longer to complete the task when they were tested again after an interval of 7 days.

Product I had no overall effect on the number of mistakes made.

The groups exhibited differences in the total number of mistakes made on day 7. In this case, the 0.0625 mg/kg group made significantly fewer total mistakes than the control group. If the different types of mistakes made in this memory fixation test are analysed, it is found that the 0.0625 mg/kg group made fewer mistakes of each type, and that the difference is close to statistical significance for errors of reference memory.

3) CONCLUSION

The tasks selected for this study measure different types of learning. It is clear that product I has no effect on

habituation in the long term, even when a long interval of 48 hours is used between the tests.

In the learning task with reward, product I had a tendency (in particular at the lowest dose) to hinder acquisition. However, in the test of memory fixation after one week, the lowest dose (0.0625 mg/kg) significantly reduced the number of mistakes made (in particular errors of reference memory), which indicates an improvement in the long term memory fixation of this task. The same dose improved the long term (48 hours) fixation of the passive avoidance response. It may therefore be concluded that product I improves long term memory fixation of rewarded or punished tasks.

EXAMPLE 3:

ANTI-AMNESIC ACTION OF PRODUCT I

1) EFFECTS OF PRODUCT I ON SCOPOLAMINE-INDUCED AMNESIA IN THE PASSIVE AVOIDANCE TEST IN MICE:

A) Methodology:

The test was carried out on male mice of the N.M.R.I. strain having an average weight per group of from 24 to 27 g. The compounds used were:
- product I, in solution in distilled water;
- piracetam (reference product) in solution in distilled water;
- scopolamine hydrobromide in solution in a 0.9% NaCl solution.

All the tests were carried out at ambient temperature (20-21°C) in artificial light.

The test, according to the technique of LENEGRE A. et al., Pharmacol. Biochem. Behar., 29, 625-629 (1988), was carried out as follows:

A mouse is introduced into the illuminated compartment (10 x 10 x 29 cm) of a box with two compartments. When it has passed with all four paws through the passage giving access to the dark compartment of larger size (19.5 x 16.5 x 29 cm), it receives a slight electric shock (0.35 mA) until it returns to the illuminated compartment, from which it is immediately removed (S1). When the mouse is introduced into the system again 24 hours later (S2), it avoids entering the dark compartment. The time it takes to enter the latter is measured, up to 180 seconds.

An i.p. injection of scopolamine (1 mg/kg) 30 minutes before S1 reduces the memory, which is measured by the time the mouse takes to enter the dark compartment in session S2.

This amnesia is reduced by nootropic agents such as piracetam, cf. SCHINDLER U. et al., Drug Dev. Res., 4, 567-576, (1984). 18 animals are studied in each group. The experiment is carried out blind.

Product I was studied at the following doses: 0.25, 1.4 and 16 mg/kg (first experiment) and 0.0625, 0.125, 0.25 and 0.5 mg/kg (second experiment), administered p.o. 60 minutes before S1.

Piracetam (2048 mg/kg p.o.), administered under the same experimental conditions, was used as reference compound in the two experiments.

B) Results:

Under the experimental conditions, product I at doses ranging from 0.0625 to 0.5 mg/kg significantly antagonised scopolamine-induced amnesia. At higher doses (1.4 and 16 mg/kg), no significant effect was observed. According to these results, it may therefore be concluded that product I has an anti-amnesic activity over a wide range of doses.

2) EFFECTS OF PRODUCT I ON DIAZEPAM- AND ELECTRIC SHOCK-INDUCED AMNESIA IN THE PASSIVE AVOIDANCE TEST IN MICE:

A) Methodology:

The working conditions and the test used are identical to those described above [see 1)-A], but here amnesia is induced not by scopolamine but:

- either by diazepam used in dispersion in a 5% suspension of gum arabic,

  in which case:

  An i.p. injection of diazepam (1 mg/kg) 30 minutes before S1 reduces the memory, which is measured by the time the mouse takes to enter the dark compartment in session S2. This amnesia is reduced by nootropic agents such as piracetam, cf. SCHINDLER U., Drug Dev. Res., 4, 567-576, (1984).

  18 animals are studied in each group. The experiment is carried out blind.

  Product I was studied at the following doses: 0.0312, 0.0625, 0.125 and 0.25 mg/kg, administered p.o.

60 minutes before S1.

Piracetam (2048 mg/kg p.o.), administered under the same experimental conditions, was used as reference compound.

- or by electric shock,

in which case:

An electric shock (square-wave current, 0.4 s, 50 mA, 50 Hz, by means of temporal electrodes connected to a Ugo Basile shock generator) administered immediately after S1 reduces the memory, which is measured by the time the mouse takes to enter the dark compartment in session S2. This amnesia is reduced by nootropic agents such as piracetam, cf. BANFI et al., J. Pharmacol. Meth., 8, 255-263, (1982).

18 animals are studied in each group. The experiment is carried out blind.

Product I was studied at the following doses: 0.0312, 0.0625, 0.125 and 0.25 mg/kg, administered p.o. 60 minutes before S1.

Piracetam (2048 mg/kg p.o.), administered under the same experimental conditions, was used as reference compound.

B) Results:

Under the above working conditions, product I:

- antagonised the amnesia-producing effects of diazepam in a dose-dependent manner, with an antagonism of 97%

for the highest dose tested (0.25 mg/kg);

- did not antagonise the amnesia-producing effects of the electric shock, whatever the dose.

Piracetam (2048 mg/kg p.o.), under the same experimental conditions, antagonised the amnesia-producing effects of diazepam by 89% and those of the electric shock by 83%.

3) EFFECTS OF SINGLE AND REPEATED ADMINISTRATION OF PRODUCT I ON SCOPOLAMINE-INDUCED AMNESIA IN THE PASSIVE AVOIDANCE TEST IN MICE:

A) Methodology:

This test, which shows the effects of product I in acute treatment, on the one hand, and in chronic treatment, on the other hand, was carried out on male mice of the N.M.R.I. strain having an average weight per group of from 25 to 26 g.

The working conditions and the test used are identical to those described above (see 1)-A).

The doses of product I studied were:

- Single administration: 0.0312 mg/kg, administered 60 minutes before the learning test (S1) on day A. From D1 to D3 the animals received distilled water twice a day (at 0900 and 1600 hours).

- Repeated administration: 0.0312 and 0.25 mg/kg, administered twice a day (at 0900 and 1600 hours) for three

days and 60 minutes before S1 on day 4.

Piracetam (2048 mg/kg), administered only once under the same experimental conditions, was used as reference compound.

B) Results:

Under the above experimental conditions, product I, administered only once at the two doses tested, clearly antagonised scopolamine-induced amnesia (antagonism of 87% at a dose of 0.0312 mg/kg and 91% at 0.25 mg/kg).

Repeated administration of the same doses over a period of four days resulted in a more marked antagonism of scopolamine-induced amnesia, under the same experimental conditions (93% and 106%, respectively). The difference between single administration and repeated administration was significant at a dose of 0.25 mg/kg.

According to these results, it may be concluded that the repeated administration of product I:

- does not induce exhaustion of the anti-amnesic effects observed on single administration,

- potentiates its anti-amnesic effects.

However, since the antagonism of amnesia after repeated administration at the lowest dose was not maximum and remained lower than that obtained with the reference compound (piracetam), this potentiation is not very marked.

4) STUDY OF THE SPECIFICITY OF THE ANTI-AMNESIC EFFECT OF PRODUCT I:

In addition to the tests described above, product I was studied after oral administration to male mice of the N.M.R.I. strain in order to evaluate any possible interaction with the effects of scopolamine (1 mg/kg i.p.) on spontaneous locomotor activity and with the effects of diazepam (1 mg/kg i.p.) in the four-plate test (anxiolytic activity).

The dose used was 0.25 mg/kg, administered 60 minutes before the tests (i.e. 30 minutes before the i.p. injection of scopolamine or diazepam). This is the highest dose of product I that antagonised scopolamine- or diazepam-induced amnesia in the passive avoidance test (1, 2).

The results obtained show:

- that there is no antagonism of the hypermotility induced by scopolamine in activity/metre;

- that there is no antagonism of the unblocking, induced by diazepam, of the behaviour punished in the four-plate test.

These results confirm that the anti-amnesic effects of product I are not linked to the specific nature of these two amnesia-inducing agents, and make it possible to conclude that the anti-amnesic action of product I at low doses is specific.

* C L M S *

CLAIMS

1. Use of 1,3,7-trimethyl-8-[3-(4-diethylaminocarbonyl-piperazino)propyl]xanthine and its physiologically tolerable acid addition salts for the preparation of medicaments for the treatment of memory disorders and Alzheimer's disease.

2. Use according to claim 1 of 1,3,7-trimethyl-8-[3-(4-diethylaminocarbonylpiperazino)propyl]xanthine hydrochloride for the preparation of medicaments for the treatment of memory disorders and Alzheimer's disease.