Eur. J. Med. Chem., **22** (1987) 1—4

Original paper

Synthesis and pharmacological properties of 2-azabicyclo[2.2.2]octane derivatives representing conformational restricted isopethidine analogues

Mario CARDELLINI [1], Gian Mario CINGOLANI [1], Francesco CLAUDI [1], Ugo GULINI [1]*
Franco CANTALAMESSA [2] and Fabrizio VENTURI [2]

[1] *Dipartimento di Scienze Chimiche, Universitá di Camerino, 62032 Camerino (MC), and*
[2] *Istituto di Farmacologia, Farmacognosia e Tecnica Farmaceutica, Universitá di Camerino, 62032 Camerino (MC), Italy*

(Received 12-26-1985, accepted 7-16-1986)

Summary — The synthesis and preliminary pharmacological evaluation of the epimeric 2-methyl-6-phenyl-6-carbethoxy-2-azabicyclo[2.2.2]octanes, representing conformationally restricted isopethidine analogues, are reported.

Résumé — Dérivés de l'aza-2 bicyclo[2.2.2]octane. *On décrit la synthèse et les résultats d'une étude pharmacologique préliminaire des épimères du méthyl-2-phényl-6-carbéthoxy-6-aza-2 bicyclo[2.2.2]octane, analogues de l'isopéthidine avec conformation restreinte.*

2-azabicyclo[2.2.2]octanes / isopethidine analogues / structure—activity relationship

Introduction

Phenylsubstituted piperidines were prepared and investigated in order to elucidate the role of phenyl ring conformation with respect to their biological activities. To reach this goal, suitable conformationally restricted 4-phenylpiperidine analogues [1—4] were synthesized and tested. However, only a few reports are at present available on 3-phenylpiperidines and on conformational requirements for their pharmacological effects [5, 11].

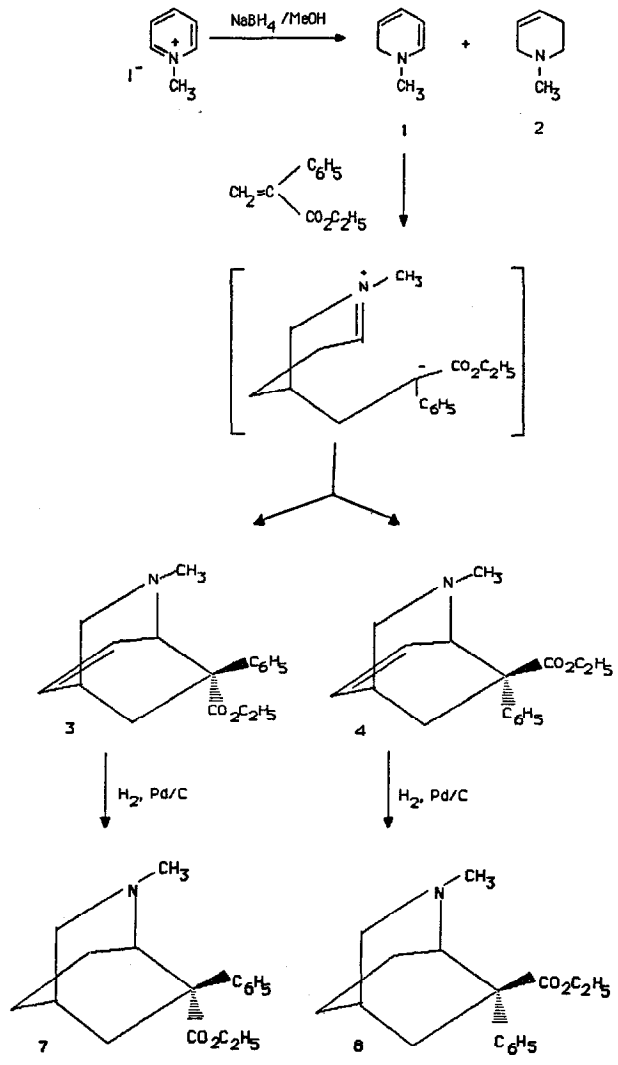
Thus, in order to explore the structure—activity relationship of 3-phenylpiperidines, our attention was drawn by the conformationally restricted isopethidine derivatives, containing the 2-azabicyclo[2.2.2]octane nucleus. Structurally this system may be regarded as a rigid analogue of the boat conformation of the piperidine in which the 2 and 5 carbon atoms are connected by a bimethylene bridge. This system is used to give conformational rigidity to a number of analgesic, muscarinic and local anesthetic agents [10, 12—14]. Using this rationale, we synthesized and tested two epimers, namely, 2-methyl-6-exo-phenyl-6-endo-carbethoxy-2-azabicyclo[2.2.2]octane **7** and 2-methyl-6-endo-phenyl-6-exo-carbethoxy-2-azabicyclo[2.2.2]octane **8**. First of all we focused our attention on the analgesic activity which is typical of phenylpiperidine derivatives even if, at least as far as epimeric pethidine analogues 2-azabicyclo[2.2.2]-heptanes and tropane esters are concerned, studies have shown that the conformational requirements are of little importance for the anti-nociceptive potency of pethidine-like compounds [2, 3]. However, since pilot experiments demonstrated that our substances as well as the reference compound isopethidine were devoid of such an effect, at least under our experimental conditions, we carried out our study by taking into consideration the anti-acetylcholine activity, that some phenylpiperidines, including isopethidine, possess.

Chemistry

The synthesis of the compounds **7** and **8** was carried out as outlined in Scheme 1. Reduction of *N*-methylpyridinium iodide with sodium borohydride in a two-phase system (*n*-hexane and aqueous methanol containing sodium hydroxide) provided a mixture of *N*-methyl-1,2-dihydropyridine **1** and *N*-methyl-1,2,5,6-tetrahydropyridine **2**.

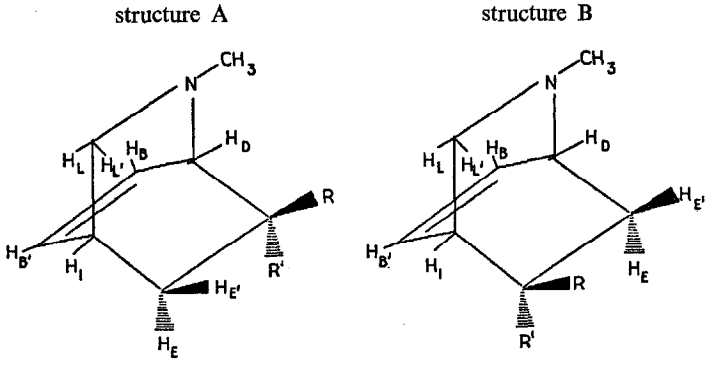
Treatment of this mixture with ethyl atropate provided the two possible diastereoisomers of 2-methyl-6-phenyl-6-carbethoxy-2-azabicyclo[2.2.2]oct-7-ene (**3** and **4**) (structure A) presumably by a polar non-concerted two-step cycloaddition mechanism [15]. On the basis of the NMR spectra, the formation of compounds **5** and **6** (structure B), arising

* *To whom correspondence should be addressed.*



Scheme 1.

from a possible Diels—Alder reaction was excluded. Indeed, NMR analysis (Table I) of compounds **3** and **4** revealed a double doublet at δ 4.24 and δ 4.23 ppm respectively due to the coupling between H_D and the olefinic protons and a multiplet at δ 2.61 and δ 2.56 ppm respectively due



structure A structure B

3: R = Ph; R′ = $CO_2C_2H_5$ **5**: R = Ph; R′ = $CO_2C_2H_5$
4: R = $CO_2C_2H_5$; R′ = Ph **6**: R = $CO_2C_2H_5$; R′ = Ph

Table I. Chemical shifts of 2-azabicyclo[2.2.2]oct-7-enes. *

Compound	H_D	NCH_3	H_L	$H_{L'}$	H_I	H_E	$H_{E'}$	H_B-$H_{B'}$
3	4.24 dd	2.25 s	1.73 dt	3.03 dd	2.61 m	2.82 dq	1.78 dd	6.40 m
4	4.23 dd	2.30 s	1.70 dt	3.18 dd	2.56 m	1.50 dt	3.06 dd	6.35 m

* Chemical shifts are given in δ. The centers of multiplet were taken as values for chemical shifts. $CDCl_3$ used as the solvent.

to coupling between H_I and H_L, $H_{L'}$, H_E, $H_{E'}$ and the olefinic protons. While decoupling of the H_D doublet led to the collapse of the olefinic protons only, decoupling the H_I multiplet collapsed the H_L, $H_{L'}$, H_E, $H_{E'}$ and the olefinic protons. This behavior is in agreement with structure A but not with structure B.

Further support for structure A is the $J_{H_L,H_E} \simeq 2.5$ Hz which is typical for long range proton—proton coupling through a ' W ' arrangement [16, 17].

It is therefore possible to distinguish $H_{E'}$ from H_E since H_E only couples with H_L. Stereochemical assignment of the diastereoisomers **3** and **4** was based upon the chemical shift of the H_E and $H_{E'}$ (geminal protons) (see Table I).

The NMR analysis of other related systems [18, 19] revealed that the carbonyl group deshields the *cis* vicinal proton more, whereas the benzene ring deshields the *trans* vicinal proton more. As a consequence, the configurations of 2-methyl-6-exo-phenyl-6-endo-carbethoxy-2-azabicyclo[2.2.2]oct-7-ene and 2-methyl-6-endo-phenyl-6-exo-carbethoxy-2-azabicyclo[2.2.2]oct-7-ene were assigned to **3** and **4** respectively.

In addition, the shift of the carbonyl group in the IR-spectra of the ester **3** (endo carbonyl) to a lower wavelength compared to the shift of compound **4** (exo carbonyl) (Table II) is in agreement with those reported in a related system by Ibuka and Grunewald [20, 21].

Table II. Characteristics of 2-azabicyclo[2.2.2]oct-7-enes and 2-azabicyclo[2.2.2]octanes.

Compound	bp/mm or mp (°C)	IR (cm^{-1})	mp HCl (°C)
3	90—91 [a]	1715 [b]	121—124 [d]
4	63—64 [a]	1740 [b]	120—122 [d]
7	80—81 [a]	1730 [b]	205—208 [e]
8	118/0.2	1738 [c]	206—207 [e]

[a] Recrystallized from ethanol.
[b] Nujol.
[c] Liquid film.
[d] Recrystallized from ethyl acetate—isopropanol.
[e] Recrystallized from isopropanol.

Compounds **7** and **8** were obtained using two methods: (1) reduction of the isolated compounds **3** and **4**, respectively (Method A); (2) reduction of the mixture of **3** and **4** and subsequent chromatographic separation of the epimers (Method B).

The latter method allowed us to work more quickly, and eliminated the troublesome separation of **3** and **4** before reduction. Compounds **3**, **4**, **7** and **8** were converted into their hydrochlorides.

Results

In vitro *experiments*

Both compounds **7** and **8** elicited clear-cut inhibition of the guinea pig ileal contraction induced by acetylcholine. The effect was short-lived and was easily abolished by washing the gut with Krebs solution. Both compounds were about 4 times more effective than the comparison substance, isopethidine. The results of these experiments are summarized in Table III.

Table III. Inhibitory concentrations on acetylcholine stimulated guinea pig ileum. *

Compound	ID_{50} (μg/ml)
Isopethidine	7.34 ± 0.84
7	2.38 ± 0.14
8	1.89 ± 0.25

* All values, referred to the free bases, are the means ± SE.

In vivo *experiments*

Acute single dose toxicity. The intraperitoneal injection of compounds **7** and **8** was immediately followed by excitation, increased motor activity, tremors and gasping. Deaths always occurred in the first 10 min, with symptoms of asphyxia. When the animals survived the treatment, all the above symptoms completely disappeared in about 30 min. LD_{50} and confidence limits (95%) of compounds **7** and **8**, as well as those of isopethidine, are reported in Table IV.

Table IV. Acute toxicity. *

Compound	LD_{50} (mg/kg)	Confidence limits (95%)
Isopethidine	156.60	166.40—147.30
7	130.80	147.14—116.40
8	143.44	165.60—127.16

* All values are referred to the free bases.

Conclusions

The results of our experiments demonstrated that the compounds **7** and **8** share practically the same pharmacological activities and that their potencies relative to the comparison substance, isopethidine, are almost equal.

In the same dose range, both compounds **7** and **8** elicit *in vitro* evident inhibitory effects on guinea pig ileal contractions induced by acetylcholine. Both compounds (which, as stated above, in a pilot experiment appear to be devoid of any anti-nociceptive effect) produce, again in the same dose range, similar toxicological symptoms. Thus, our results suggest that it is not possible to differentiate between the conformations of the two epimers on the basis of their pharmacological and toxicological effects.

Experimental protocols

Chemical synthesis

Boiling points are uncorrected. Melting points were taken on a Büchi SMP-20 apparatus and are uncorrected. NMR spectra were recorded on a Varian EM-390, 90 MHz instrument, using TMS as the internal standard. IR-spectra were obtained with a Perkin—Elmer 297 spectrophotometer. Mass spectra were determined on a low resolution Hewlett—Packard 5980 A mass spectrometer. The elemental analyses were performed by the Microanalytical Laboratory of the Department of Chemical Sciences of the University of Camerino and were within ± 0.4% of the calculated values. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.063—0.200 mm, Merck). R_f values were determined with silica gel TLC plates (Kieselgel 60, F_{254}, layer thickness 0.25 mm, Merck).

N-Methyl-1,2-dihydropyridine ***1*** and *N-methyl-1,2,5,6-tetrahydropyridine* ***2***

A solution of 50 g (0.226 mol) of *N*-methylpyridinium iodide in 14 ml of water was added rapidly, under nitrogen at 15°C, to a stirred mixture of 130 ml of 11.24 N NaOH, 130 ml of methanol, 300 ml of *n*-hexane and 5.6 g (0.148 mol) of $NaBH_4$. The mixture was stirred for 6 min, the *n*-hexane layer was separated and the aqueous phase was extracted with two 100 ml portions of *n*-hexane. The combined *n*-hexane extracts were dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to yield a mixture of **1** and **2** (6.48 g) which was not resolved, but their percent ratio was established by NMR analysis immediately after evaporation of the solvent with the following results: *N*-methyl-1,2-dihydropyridine **1** 75%; *N*-methyl-1,2,5,6-tetrahydropyridine **2** 25%. NMR were virtually identical with those reported in the literature [25, 26].

2-Methyl-6-exo-phenyl-6-endo-carbethoxy-2-azabicyclo[2.2.2] oct-7-ene ***3*** and *2-methyl-6-endo-phenyl-6-exo-carbethoxy-2-azabicyclo[2.2.2]oct-7-ene* ***4***

A solution of 29 g (0.164 mol) of ethyl atropate in 40 ml of *n*-hexane was added dropwise under nitrogen atmosphere at room temperature to 6.4 g of a stirring mixture of 4.8 g (50 mmol) of *N*-methyl-1,2-dihydropyridine **1** and 1.6 g (16 mmol), of *N*-methyl-1,2,5,6-tetrahydropyridine **2** (according to ^1H NMR analysis) in 500 ml of *n*-hexane. Stirring at 20°C was continued for 30 h, the reaction mixture was filtered, the filtrate was evaporated *in vacuo* and the residue acidified (4 N HCl) and washed with diethyl ether. The acidic layer was made basic (30% NH_4OH) and extracted several times with diethyl ether. The combined extracts were washed (brine), dried over anhydrous sodium sulfate, filtered (silica gel) and concentrated under reduced pressure to give 8 g (58.4%) of a crude mixture of the isomers **3** and **4**, which after several fractional crystallizations from ethanol gave the pure isomers **3** (2.34 g, 7.2%) and **4** (0.8 g, 5.8%) with identical molecular weights (m/e = 271 from mass spectrum) but different melting points, NMR (Table I) and IR-spectra (Table II).

2-Methyl-6-exo-phenyl-6-endo-carbethoxy-2-azabicyclo[2.2.2] octane ***7*** *(Method A)*

A solution of 1.74 g (6.4 mmol) of **3** in 220 ml of ethanol was hydrogenated over 0.33 g of 5% palladium-on-charcoal for 5 h at room temperature and 3.4 atm. The reaction mixture was filtered and the filtrate evaporated *in vacuo* to give 1.65 g (94%) of **7** (Table II).

^1H NMR in $CDCl_3$: δ 7.45—7.0 (m, 5H, aromatic), 3.95 (q, 2H, $—OCH_2CH_3$), 2.36 (s, 3H, $—NCH_3$), 1.04 (t, 3H, $—OCH_2CH_3$) ppm. MS: m/e = 273 (M^+).

2-Methyl-6-endo-phenyl-6-exo-carbethoxy-2-azabicyclo[2.2.2] octane 8 (Method A)

A solution of 3.12 g (11.5 mmol) of **4** in 300 ml of ethanol was hydrogenated over 0.6 g of 5% palladium-on-charcoal for 5 h at room temperature and 3.4 atm. The catalyst was filtered off and the solvent removed *in vacuo*. Distillation of the residue afforded 2.8 g (90%) of pure **8** as a colorless oil (Table II).

^{1}H NMR in $CDCl_3$: δ 7.5—7.0 (m, 5H, aromatic), 4.0 (q, 2H, —OCH_2 CH_3), 3.51 (t, 1H, H_D), 2.38 (s, 3H, —NCH_3), 1.05 (t, 3H, —OCH_2-CH_3) ppm. MS: m/e = 273 (M^+).

2-Methyl-6-exo-phenyl-6-endo-carbethoxy-2-azabicyclo[2.2.2] octane 7 and 2-methyl-6-endo-phenyl-6-exo-carbethoxy-2-azabicyclo[2.2.2]octane 8 (Method B)

A solution of 1.45 g (5.35 mmol) of the isomers **3** and **4** in 150 ml of ethanol was hydrogenated over 0.3 g of 5% palladium-on-charcoal for 5 h at room temperature and 3.4 atm. The catalyst was filtered off, the solvent removed *in vacuo* and the residue was chromatographed on silica gel. Elution with a mixture of ammonium hydroxide/ethanol/methylene chloride/diethyl ether/petroleum ether (1:10:40:40:100) yielded 0.89 g (59.3%, R_f = 0.76) of **7** and 0.29 g (20%, R_f = 0.65) of **8**.

Hydrochlorides of 2-azabicyclo[2.2.2]oct-7-enes 3, 4 and 2-azabicyclo-[2.2.2]octanes 7, 8

Dry hydrochloric acid was bubbled into a solution of the base in anhydrous ethyl ether. The white precipitate was filtered and recrystallized (Table II).

Biological methods

The single-dose toxicity and the *in vitro* anti-acetylcholine activity of compounds **7** and **8** (hydrochlorides) were studied.

The single-dose toxicity was determined in male albino mice of the Swiss strain (Charles River, Como, Italy), while the anti-acetylcholine effect was studied on the isolated, acetylcholine-stimulated, guinea pig ileum.

The drugs were always dissolved in distilled water, and for *in vivo* experiments, they were administered intraperitoneally to 24 h fasted mice, in a volume of 1 ml/100 g of bw. Isopethidine hydrochloride [22] was employed as a comparison substance.

In vitro *experiments*

Terminal pieces of guinea pig ileum (350—450 g) were suspended in 15 ml of aerated Krebs solution, kept at 32°C and washed by overflow. The Krebs solution was modified according to G. Bertaccini and P. Zamboni [23]. The movements of the gut were recorded with a frontal-writing lever with a two-fold magnification. The dose which reduced the ileal response by 50% to a fixed concentration (5 ng/ml) of acetylcholine was determined for each substance.

In vivo *experiments*

Acute single dose toxicity. For each substance, 40 mice, divided into groups of 10 animals each, were used. The median lethal dose (LD_{50}) was determined by evaluating the effect of single intraperitoneal doses, 14 days after the administration of the drugs. The LD_{50} was estimated according to the method of Weil [24].

Acknowledgements

Supported by the National Research Council (CNR) Rome.

References

1 Smissman E. E. & Steinman M. (1966) *J. Med. Chem.* 9, 455
2 Portoghese P. S., Mikhail A. A. & Kupferberg H. J. (1968) *J. Med. Chem.* 11, 219
3 Daum S. J., Martini C. M., Kullnig R. K. & Clarke R. L. (1975) *J. Med. Chem.* 18, 496
4 Clark C. R., Holbrook J. M. & Borne R. F. (1976) *J. Med. Chem.* 19, 852
5 Macdonald A. D., Woolfe G., Bergel F., Morrison A. L. & Rinderknecht H. (1946) *Brit. J. Pharmacol.* 1, 4
6 Kugita H. & Oine T. (1963) *Chem. Pharm. Bull.* 11, 253
7 Kugita H., Inoue H., Oine T., Hayashi G. & Nurimoto S. (1964) *J. Med. Chem.* 7, 298
8 Kugita H., Oine T., Inoue H. & Hayashi G. (1965) *J. Med. Chem.* 8, 313
9 Iorio M. A. & Casy A. F. (1975) *J. Pharm. Pharmac.* 27, 140
10 Takeda M., Kawamori M., Inoue H., Noguchi K. & Nurimoto S. (1977) *Chem. Pharm. Bull.* 25, 775
11 Iorio M. A. & Casy A. F. (1978) *J. Med. Chem.* 21, 812
12 Nelson W. L. & Wilson R. S. (1970) *J. Pharm. Sci.* 59, 98
13 Borne R. F., Clark C. R. & Holbrook J. M. (1973) *J. Med. Chem.* 16, 853
14 Borne R. F., Clark C. R. & Waters I. W. (1974) *J. Pharm. Sci.* 63, 1559
15 Schenker K. & Druey J. (1962) *Helv. Chem. Acta* 45, 1344
16 Liberatore F., Casini A., Carelli V., Arnone A. & Mondelli R. (1971) *Tetrahedron Lett.* 26, 2381
17 Beckett H. A. & Reid R. E. (1972) *Tetrahedron* 28, 5555
18 Fraser R. R. (1962) *Can. J. Chem.* 40, 78
19 Erman W. F. & Flautt T. J. (1962) *J. Org. Chem.* 27, 1526
20 Ibuka T., Mori Y., Aoyama T. & Inubushi Y. (1978) *Chem. Pharm. Bull.* 26, 456
21 Grunewald G. L., Kuttab S. H., Pleiss M. A., Mangold J. B. & Soine P. (1980) *J. Med. Chem.* 23, 754
22 Jacoby R. L., Nieforth K. A. & Willette R. E. (1974) *J. Med. Chem.* 17, 453
23 Bertaccini G. & Zamboni P. (1961) *Arch. Int. Pharmacodyn. Ther.* 133, 138
24 Weil C. S. (1952) *Biometrics* 8, 249
25 Fowler F. W. (1972) *J. Org. Chem.* 37, 1321.
26 Ksandr Z., Samek Z., Spirko V. & Ferles M. (1966) *Collect. Czech. Chem. Commun.* 31, 3003