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Short communication

Synthesis, physicochemical properties and biological studies of some substituted 2-alkoxy-4-methyl-morpholines*

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Summary — The title compounds have some structural characteristics of the piperidine-type analgesics and central sympathomimetics.

The synthesis of some substituted 2-hydroxy- and 2-alkoxy-4-methyl-morpholines is presented and studied, in terms of electronic and steric effects. Their log *P* and pK_a values were determined and are explained in terms of structural, stereochemical and electronic effects.

Acute toxicity and, for some selected cases, anti-nociceptive and central sympathomimetic activities have been evaluated in a preliminary (QSAR) study of biological properties of the synthesized compounds.

Résumé — **Synthèse, propriétés physicochimiques et études biologiques de quelques alkoxy-2 méthyl-4 morpholines substituées.** *Les composés mentionnés dans le titre ont quelques caractéristiques structurales des pipéridines analgésiques et sympathomimétiques à effets centraux.*

Nous présentons et étudions à l'aide des effets électroniques et stériques la synthèse de quelques hydroxy-2 et alkoxy-2 méthyl-4 morpholines. Nous déterminons les valeurs de log P *et de* pK_a *et nous les interprétons à l'aide des effets structuraux, stéréochimiques et électroniques.*

Leur toxicité aiguë, leurs activités analgésique et sympathomimétique centrale sont évaluées dans le cadre de l'étude préliminaire (QSAR) de leurs propriétés biologiques.

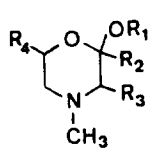
morpholines / synthesis of 2,2,4-trisubstituted morpholines / ionization constants / partition coefficients / anti-nociceptivity / central sympathomimetic activity / QSAR

Introduction

The substituted 2-alkoxy-4-methyl-morpholines, **1**, are biologically interesting since they possess most of the structural requirements of analgesics [1–4] (pethidine, **2**, [5]) and of centrally acting sympathomimetics (phendimetrazine, **3** [6–8]).

The introduction of the heterocyclic O causes major structural (intergroup relationships) and physicochemical (ionization, lipophilicity) changes, compared to the piperidine type of analgesics. Since there are few biologically active morpholines, we investigated if these changes could be offset by proper molecular manipulations.

We synthesized a number of substituted 2-alkoxy-4-methyl-morpholines in order to study further their chemistry, to determine some physicochemical properties very important for the biological activity [9–11] and to assess few biological properties, *i.e.*, LD_{50}, anti-nociceptive and central sympathomimetic activities for several selected structures, in a preliminary biological study.



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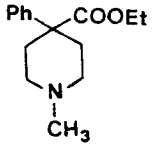

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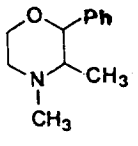

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Discussion

Chemistry

Substituted 2-hydroxy-4-methyl-morpholines

The reaction of the appropriate aminoalcohol with the

*Part of this work was presented at the 3rd Noordwijkerhout Symposium: *Innovative Approaches in Drug Research*, 3–6 September 1985: 'Pharmacochemical studies of some 2-alkoxy-2-phenyl-4,5-alkyl-morpholines. Synthesis, physicochemical properties / biological activity' P58, 118.

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corresponding bromoketone (S_N2) is accompanied by a simultaneous ring closure, which is assisted by the steric effect of the *N*-methyl, and secondarily by the −I effect of the charged N.

Attempted synthesis of 1,4- and 1,5-oxazepinium systems failed and this is attributed to the prevention of the hydroxylic chain to adopt a favorable conformation for ring closure.

The cyclic hemiketal structure was confirmed chemically (the compounds did not form 2,4-dinitrophenylhydrazones) and spectroscopically (IR, UV). There is no evidence to suggest the presence of the keto alcohol tautomer. The morpholine ring closure is not influenced by the different electronic and steric effects of the 2-substituents (Table I).

Substituted 2-alkoxy-4-methyl-morpholines

The 2-alkoxy derivatives (Table II) are formed from the corresponding 2-hydroxy-analogues (S_N1). The intermediate carbonium ion is stabilized by resonance with the phenyl group.

The bulk of the entering alkoxy group influences the reaction, thus the *iso*-propoxy- or the *t*-butoxy-derivatives are not formed.

Substitution at position 2 significantly affects the formation of the 2-alkoxy-derivatives: 2-phenyl and 2-*p*-bromophenyl contribute to ketal formation, but 2-(*m*-trifluoromethyl)phenyl prevents this reaction, possibly due to electronic interactions. When there is a hydrogen at position 2 (compound **7**) no ketal is obtained. Although the 2-*t*-butyl electronically favors the carbonium ion formation, compound **9** does not yield even the 2-methoxy- or the 2-ethoxy-derivatives.

Substitution at other positions on the morpholine ring has various effects on ketal formation: 6-methyl has no effect, 3-methyl does not allow the reaction with alcohols higher than ethanol, and 5-methyl-6-phenyl-disubstitution inhibits this reaction. The above could be explained stereochemically.

Assuming that a positively charged morpholine N would facilitate the ketal formation, we tried to synthesize some 4,4-dimethyl-morpholinium derivatives by the same procedure, which yielded the 2-ethoxy-analogue, but no derivatives with higher alcohols. The 2-*n*-propoxy compound was obtained by quaternizing the corresponding tertiary morpholine. These could also be attributed to steric reasons.

The structure of the 2-alkoxy-derivatives was allocated spectroscopically (IR, UV, NMR). There was no absorption suggesting the presence of a hydroxy-group. UV band was weaker even than that of the 2-hydroxy-compounds. NMR spectra also confirmed the above structures.

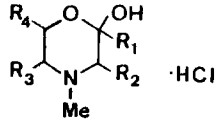
Physicochemical studies

Ionization constants

The pKa values of the morpholine derivatives (Table III) are lower than those of the piperidine analgesics, as expected [20], due to the ring oxygen.

The 2-hydroxy-morpholine derivatives are stronger bases than the 2-alkoxy-analogues, since the hydroxy group is more effectively solvated than an alkoxyl, thus lowering the C−O dipole [20, 33]. A methyl group at car-

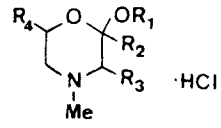
Table I. Substituted 2-hydroxy-4-methyl-morpholines, starting materials, melting points, yields and elemental analyses.



No.	R_1	R_2	R_3	R_4	Starting materials		mp(°C)	Yield (%)	Formula
					bromoketone	aminoalcohol			
1	C_6H_5	H	H	H	phenacyl bromide	2-methylamino-ethanol	163−164	72	$C_{11}H_{16}ClNO_2$[a]
2[b]	C_6H_5	H	H	H	phenacyl bromide	2-dimethylamino-ethanol	189−190	97	$C_{12}H_{18}BrNO_2$[c]
3	C_6H_5	H	H	CH_3	phenacyl bromide	3-methylamino-2-propanol[d]	132−133	50	$C_{12}H_{18}ClNO_2$
4	C_6H_4−Br(*p*)	H	H	H	*p*-bromophenacyl bromide	2-methylamino-ethanol	147−148	64	$C_{11}H_{15}BrClNO_2$
5	C_6H_5	CH_3	H	H	α-bromopropio-phenone	2-methylamino-ethanol	184−185	87	$C_{11}H_{18}ClNO_2$
6	C_6H_5	H	CH_3	C_6H_5	phenacyl bromide	ephedrine	156−157	50	$C_{18}H_{22}ClNO_2$
7	H	C_6H_5	H	H	α-bromophenyl-acetaldehyde[e]	2-methylamino-ethanol	218−219[d]	34	$C_{11}H_{16}ClNO_2$
8	C_6H_4−CF_3(*m*)	H	H	H	*m*-CF_3-α-bromo-acetophenone[f]	2-methylamino-ethanol	167−169	70	$C_{12}H_{15}ClF_3NO_2$
9	$C(CH_3)_3$	H	H	H	1-bromo-3,3-di-methyl-2-butanone[g]	2-methylamino-ethanol	156−158	50	$C_9H_{20}ClNO_2$

[a][12]. [b]Methylbromide. [c][13]. [d][14]. [e][15, 16]. [f][17]. [g][18].

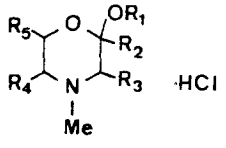
Table II. Substituted 2-alkoxy-4-methyl-morpholines, melting points, yields and elemental analyses.



No.	R_1	R_2	R_3	R_4	mp(°C)	Yield(%)	Formula
10	$CH_2CH_2CH_3$	C_6H_5	H	H	168–169	71	$C_{14}H_{22}ClNO_2$
11	$CH_2CH{=}CH_2$	C_6H_5	H	H	167–168	72	$C_{14}H_{20}ClNO_2$
12	$CH_2C{\equiv}CH$	C_6H_5	H	H	148–149	64	$C_{14}H_{18}ClNO_2$
13	CH_2CH_3	C_6H_5	CH_3	H	177–178(d)	89	$C_{14}H_{22}ClNO_2$
14	$CH_2CH_2CH_3$	C_6H_5	H	CH_3	149–150	58	$C_{15}H_{24}ClNO_2$
15	$CH_2(CH_2)_2CH_3$	C_6H_5	H	H	156–157	61	$C_{15}H_{24}ClNO_2$
16	$CH_2CH(CH_3)_2$	C_6H_5	H	H	172–173	72	$C_{15}H_{24}ClNO_2$
17	$CH_2(CH_2)_3CH_3$	C_6H_5	H	H	175–176	64	$C_{16}H_{26}ClNO_2$
18	$CH_2(CH_2)_6CH_3$	C_6H_5	H	H	143–144	54	$C_{19}H_{32}ClNO_2$
19	$CH_2(CH_2)_{14}CH_3$	C_6H_5	H	H	144–146	50	$C_{27}H_{48}ClNO_2$
20	$CH_2CH_2CH_3$	C_6H_4–Br(*p*)	H	H	186–187	70	$C_{14}H_{21}BrClNO_2$
21	$CH_2C_6H_5$	C_6H_5	H	H	165–166	62	$C_{18}H_{22}ClNO_2$
22	$CH_2CH_2C_6H_5$	C_6H_5	H	H	173–174	59	$C_{19}H_{24}ClNO_2$
23	CH_2CH_3[a]	C_6H_5	H	H	189–190	74	$C_{14}H_{22}BrNO_2$
24	$CH_2CH_2CH_3$[b]	C_6H_5	H	H	182–183	70	$C_{15}H_{24}INO_2$

[a]Methylbromide. [b]Methyliodide.

Table III. Ionization constants (pK_a), % ionization (pH 7.4), apparent and true partition coefficients (*n*-octanol / buffer) and R_f values of the substituted morpholine hydrochlorides.[a]



No.	R_1	R_2	R_3	R_4	R_5	pK_a	Ionized %	log P_{app}	log P	R_f[b]
1	H	C_6H_5	H	H	H	7.26	42.02	1.30	1.54	0.63
3	H	C_6H_5	H	H	CH_3	7.41	50.51	1.48	1.79	0.59
4	H	C_6H_4–Br(*p*)	H	H	H	7.07	31.95	1.46	1.57	0.49
5	H	C_6H_5	CH_3	H	H	7.88	75.19	1.31	1.92	0.26
6	H	C_6H_5	H	CH_3	C_6H_5	7.68	65.89	1.43	1.87	0.50
7	H	H	C_6H_5	H	H	6.02	4.00	0.97	0.99	0.81
8	H	C_6H_4–CF_3(*m*)	H	H	H	7.54	58.14	1.02	1.39	0.78
9	H	$C(CH_3)_3$	H	H	H	7.88	75.19	–	–	0.64
10	$CH_2CH_2CH_3$	C_6H_5	H	H	H	7.12	34.36	1.95	2.13	0.61
11	$CH_2CH{=}CH_2$	C_6H_5	H	H	H	7.09	32.89	1.92	2.09	0.56
12	$CH_2C{\equiv}CH$	C_6H_5	H	H	H	6.86	22.37	1.76	1.87	0.52
13	CH_2CH_3	C_6H_5	CH_3	H	H	7.79	70.92	1.74	2.27	0.61
14	$CH_2CH_2CH_3$	C_6H_5	H	H	CH_3	7.73	46.08	1.96	2.23	0.80
15	$CH_2(CH_2)_2CH_3$	C_6H_5	H	H	H	7.09	32.89	2.05	2.23	0.79
16	$CH_2CH(CH_3)_2$	C_6H_5	H	H	H	7.14	35.46	1.98	2.17	0.72
17	$CH_2(CH_2)_3CH_3$	C_6H_5	H	H	H	7.11	33.90	2.06	2.19	0.74
18	$CH_2(CH_2)_6CH_3$	C_6H_5	H	H	H	6.99	28.01	2.17	2.32	0.79
19	$CH_2(CH_2)_{14}CH_3$	C_6H_5	H	H	H	6.82	20.83	2.26	2.36	0.81
20	$CH_2CH_2CH_3$	C_6H_4–Br(*p*)	H	H	H	6.95	26.18	2.06	2.19	0.56
21	$CH_2C_6H_5$	C_6H_5	H	H	H	6.97	27.10	2.05	2.18	0.40
22	$CH_2CH_2C_6H_5$	C_6H_5	H	H	H	7.07	31.95	1.95	2.11	0.49

[a]pK_a s (25°C) of pethidine and phendimetrazine were found [23], using the appropriate Taft equation to be 8.23 and 7.63, respectively. Log *P*'s for these compounds were found [24], using Rekker's *f* system to be 2.67 and 1.62, respectively.
[b]Ammonia-saturated chloroform.

bon atoms adjacent to the nitrogen electronically enhances basicity; substitution at more remote positions has little effect, with the exception of the *t*-butyl group (compound **9**), which significantly increases pK_a (ΔpK_a 0.62), compared to the 2-phenyl substituent. A 3-phenyl group lowers pK_a, due to electronic and steric effects of the aromatic group.

Partition coefficients

Lipophilicity of the morpholine derivatives, as expected, increases as the 2-hydroxyl is replaced by a 2-alkoxyl and as the alkoxyl becomes larger. In general, branching lowers and morpholine ring substitution increases lipophilicity. Addition of a methyl group to an already long methylene chain produces a less expressed increase of log *P* than that caused by the introduction of the first methyl in a polar group, as reported previously [34] (Table III).

Biological testing

Acute toxicity was determined for all 2-alkoxy derivatives (Table IV), and for one representative 2-hydroxy-compound, which was found to be less toxic, probably due to its lower lipid solubility. All tested compounds demonstrated, depending upon administered doses, a series of symptoms with only small variations: stereotyped behavior, hyperkinesia, piloerection, convulsions, coma.

The two central actions (anti-nociceptive, sympathomimetic) were confirmed by specific tests. An action peak was observed 20–30 min post-injection.

The compounds tested for anti-nociceptivity showed a statistically significant activity, compared to controls. Approximately the same response was received after pethidine administration, at a dose 1/2–1/3 of those administered. Naloxone antagonized the activity of compound **17** (Table V).

Experimental animals demonstrated hyperkinesia and stereotyped behavior at statistically significant levels (Table V).

Preliminary QSAR study

The partition coefficient is of major importance for activity [35, 36]. In a preliminary QSAR study, we found that anti-nociceptive activity, expressed as $\log 1/C$, correlates very well with $\log P$: $\log 1/C = 0.091(\pm 0.042)(\log P)^2 - 0.513(\pm 0.203) \log P - 0.902$; $n=5$, $R=0.984$, $s=0.021$, $F=29.89$.

The pK_a values could not be used as a parameter in a QSAR analysis because the morpholine derivatives possess similar ionization constants. This has been reported to be the case for most piperidine and piperazine morphinomimetics [37].

In conclusion, it appears that the changes made, piperidines to morpholines, with further modifications in substitution, have led to compounds with anti-nociceptive activity. It remains, therefore, to optimize potential useful anti-nociceptivity through systematic QSAR studies.

Experimental protocols

Chemistry

Melting points (uncorrected) were taken with a Carl Zeiss melting point microscope. Thin-layer chromatography (TLC) was performed on silica gel (60 F_{254}, Merck). IR, UV and ^1H NMR (in CF_3COOH or DMSO-d_6, tetramethylsilane as the internal standard) spectra were recorded with Perkin-Elmer 597 and 554 double beam spectrophotometers and a Varian EM-60 spectrophotometer at 60 MHz, respectively. Elemental analyses (C, H, N) were obtained on a Perkin-Elmer 240 analyzer and are within ±0.4% of the calculated values. For the pK_a determination, a digital Radiometer PHM63 pH meter and a combined Radiometer GK2401C glass electrode were used. In biological experiments, male Wistar rats (160–220 g) were used. A hot plate Ridi 85 (Greece) type and a 25×30×25 cm electrical activity cage, model Ugo Basile Biological Research Apparatus, were used. Starting materials were purchased from Aldrich Chemical Co., USA.

Substituted 2-hydroxy-4-methyl-morpholine hydrochlorides

2-Methylamino-(1,2-substituted)-ethanol (0.22 mol) reacted at room temperature with the appropriate bromoketone (0.10 mol) in dry ether. The mixture was left for 16–24 h, the precipitated aminoalcohol hydrobromide was removed by filtration, the ethereal solution was washed with saturated sodium chloride solution, dried (K_2CO_3), the ether was evaporated and the residue was converted into hydrochloride (using ethereal hydrogen chloride) and recrystallized (acetone or *iso*-propanol and ether) to give the corresponding 2-hydroxy-morpholine hydrochloride.

The synthesized compounds, yields, melting points and elemental analyses are given in Table I. Starting materials were purchased unless otherwise stated (Table I).

Upon addition of a methanolic solution of 2,4-dinitrophenylhydrazine and H_2SO_4 to a methanolic solution of these compounds, no solid precipitated.

The IR spectra (nujol mull) showed a strong band at 3400 cm^{-1}, no band at 1680–1710 cm^{-1}. UV spectra ($0.15-0.70\times10^{-3}$ M in *iso*-propanol) showed λ_{max} 210–220 nm; $\epsilon_{\lambda_{max}}$ 6200 (compounds with one phenyl) or 12 300 (compounds with two phenyls), ϵ_{240} 740–1100.

By the above procedure, using equimolar quantities of phenacyl bromide and 2-dimethylaminoethanol, 2-hydroxy-2-phenyl-4,4-dimethyl morpholinium bromide was precipitated, filtered and recrystallized [13] (**2**). Reaction of 2-dimethylaminoethanol (8.8 g, 0.1 mol) with 3-chloropropiophenone (17 g, 0.1 mol) gave 3-phenyl-3-oxopropyl 2-hydroxyethyl dimethylammonium chloride, yield 54%, mp: 123–124°C, R_f 0.53 (CH_3COOH). Anal. calcd. for $C_{13}H_{20}ClNO_2$: C: 60.58; H: 7.77; N: 5.44%; found: C: 60.69; H: 8.01; N: 5.46%. Reaction of dimethylamino-1-propanol (6.4 g, 0.06 mol) with phenacyl bromide (12 g, 0.06 mol) gave phenacyl 2-hydroxypropyl dimethylammonium bromide, yield 50% (8 g); mp: 115–116°C. R_f 0.32 (CH_3OH:CH_3COOH 10:1). Anal. calcd. for $C_{13}H_{20}BrNO_2$: C: 51.66; H: 6.62; N: 4.63%; found: C: 51.81; H: 6.78; N: 4.45%.

The latter two open chain compounds showed a band at 1680 cm^{-1} (IR, nujol mull), ϵ_{240} 13 000 (UV, 1.7×10^{-3} M in *iso*-propanol).

Substituted 2-alkoxy-4-methyl-morpholine hydrochlorides

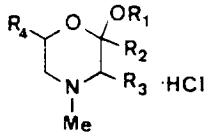
The appropriate 2-hydroxy-morpholine hydrochloride was heated with traces of acid in the corresponding alcohol or in *iso*-propanol with an excess (1:3–1:6) of the alcohol (in the case of high boiling or solid alcohols) for 10–24 h. Using unsaturated alcohols (**11, 12**) the reaction was carried out in a nitrogen atmosphere. The solvent was evaporated *in vacuo* to yield the 2-alkoxy-substituted morpholine hydrochlorides.

Heating compound 2 in acidified *n*-propanol gave the starting 2-hydroxy-compound. The 2-*n*-propoxy-morpholinium derivative (**24**) was obtained from compound **10** by liberation of the base and quaternization with CH_3I. Heating compounds **6, 7, 8** and **9** in either acidified methanol or ethanol gave the starting materials. Heating compound **2** in alcohols higher than ethanol gave the starting material. None of the 2-hydroxy-derivatives reacted with either *iso*-propanol or *t*-butanol, and the starting materials were obtained.

The synthesized substituted 2-alkoxy-4-methyl-morpholine hydrochlorides, yields, melting points and elemental analyses are given in Table II.

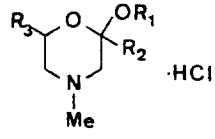
The synthesized compounds showed no band at 3400 cm^{-1} (IR in

Table IV. LD_{50} values of the substituted morpholines.



No.	R_1	R_2	R_3	R_4	LD_{50} / 100 g b.w.	
					mg	μmol
1	H	C_6H_5	H	H	50.0	217.9
10	$CH_2CH_2CH_3$	C_6H_5	H	H	18.2	67.0
11	$CH_2CH=CH_2$	C_6H_5	H	H	22.0	81.6
12	$CH_2C\equiv CH$	C_6H_5	H	H	14.0	89.7
13	CH_2CH_3	C_6H_5	H	H	19.0	70.0
14	$CH_2CH_2CH_3$	C_6H_5	CH_3	CH_3	15.5	54.3
15	$CH_2(CH_2)_2CH_3$	C_6H_5	H	H	19.0	66.5
16	$CH_2CH(CH_3)_2$	C_6H_5	H	H	22.0	77.1
17	$CH_2(CH_2)_3CH_3$	C_6H_5	H	H	16.0	53.4
18	$CH_2(CH_2)_6CH_3$	C_6H_5	H	H	20.0	58.6
19	$CH_2(CH_2)_{14}CH_3$	C_6H_5	H	H	81.7	180.2
20	$CH_2CH_2CH_3$	C_6H_4–Br(*p*)	H	H	14.8	42.2
21	$CH_2C_6H_5$	C_6H_5	H	H	15.0	46.9
22	$CH_2CH_2C_6H_5$	C_6H_5	H	H	14.0	42.0

Table V. Anti-noceptive, locomotor activity and stereotyped behavior of some substituted morpholines in rats.



No.	R_1	R_2	R_3	Anti-nociceptive activity			Locomotor activity			Stereotyped behavior[a]
				dose / 100 g		degree of	dose / 100 g b.w.		no. of	
				mg	μmol	analgesia[a]	mg	μmol	movements[a]	
Controls					(water)	7.52			51.50	0.27
10	$CH_2CH_2CH_3$	C_6H_5	H	7.5	27.6	38.70*	8.0	29.0	363.14*	11.29*
14	$CH_2CH_2CH_3$	C_6H_5	CH_3	–	–	–	3.0	11.0	164.00*	8.75*
15	$CH_2(CH_2)_2CH_3$	C_6H_5	H	6.5	21.0	37.12*	–	–	–	–
17	$CH_2(CH_2)_3CH_3$	C_6H_5	H	5.5	18.4	40.80*	6.0	20.0	402.30*	9.00*
20	$CH_2CH_2CH_3$	$C_6H_4Br(p)$	H	6.5	18.5	25.33*	–	–	–	–
21	$CH_2C_6H_5$	C_6H_5	H	–	–	–	3.0	9.4	169.70*	0.00*
22	$CH_2CH_2C_6H_5$	C_6H_5	H	6.0	18.0	37.22*	–	–	–	–
Pethidine (as hydrobromide)				2.2(base)	9.0	39.00*	–	–	–	–
Naloxone (as hydrochloride)				1.0(base)	3.0	10.25[b]	–	–	–	–
Naloxone + **17** (as above)						11.33**	–	–	–	–

[a]For estimation see text.
[b]The apparent anti-nociceptive activity of naloxone (possibly a mere placebo effect) is statistically not significant compared to controls.
*$p<0.001$ (compared to controls).
$p<0.001$ (compared to **17).

nujol), λ_{max} 210–220 nm, $\epsilon_{\lambda_{max}}$ 7500 or 13 000 (compounds with 1 or 2 phenyls, respectively), ϵ_{240} *ca.* 170 (UV, solutions of $0.15-0.70\times10^{-3}$ M in *iso*-propanol). ^{1}H NMR spectra showed multiplets for protons of the methylenic group at δ 2.8–3.8, protons of the allylic and propargylic groups are found at δ 4.8–5.5 or 2.5, respectively. Aromatic protons appeared at δ 7.5–7.7 as a singlet or doubled (mono-an diaryl derivatives, respectively). The aliphatic methyl protons are found as triplet at δ 0.8–0.9, and the proton of the protonated N is sometimes found at δ 10.3.

Physicochemical studies

Determination of ionization constants

Ionization constants of the conjugated acids of the synthesized morpholine bases were determined by potentiometric titration [19], with 0.1 N KOH in 50% aqueous ethanol, neutral and CO_2-free, at 37±0.05°C, requiring an increase of 0.54 for evaluating pK_a in aqueous solution [19, 20]. Determination of pK_a values for a few randomly selected compounds in 4 ethanol concentrations and extrapolation to aqueous solution gave an increase of 0.53±0.05. The accuracy of the apparatus and technique was checked by measuring the pK_a of benzoic acid in water at 25°C (found 4.09, reported [21] 4.05) and of 2-ethoxy-2-phenyl-4-phenethyl-morpholine in 50% aqueous ethanol at 25°C (found 6.61, reported [20] 6.67).

Determination of partition coefficients

Apparent partition coefficients were determined between *n*-octanol (analytical grade) and buffer solution (pH 7.4) mutually saturated, after vigorous agitation (10 min), centrifugation and determination of the concentration spectroscopically (220 nm, 4–6 individual measurements, standard deviation of P less than 10%). True partition coefficients were calculated using the expression $P = P_{app}/(1-\alpha)$, where α is percent ionization [22].

The pK_a, α, log P and R_f values of the synthesized compounds are given in Table III.

Biological studies

The test compounds were administered intraperitoneally as aqueous solutions. Controls received water. Each group was composed of 6–15 rats.

Acute toxicity tests

Rats were divided into 6 groups to which 6 different doses of the test compounds at the lethal range were injected. Mortality was recorded 24 h post-injection. LD_{50} s (Table IV) were estimated by probit analysis [25]. (Confidence limits are within 4 mg).

Pharmacological tests

The test compounds were administered at a 3 dose regime (15–30 μmol / 100 g for anti-nociceptivity, 5–30 μmol / 100 g for the central sympathomimetic action) and the smallest dose giving the optimal response was selected.

Anti-nociceptive activity for selected morpholine derivatives was determined by the hot plate test at 60°C [26, 27]. Hind limb movement was used as the end point. Reaction time was recorded at 5, 10, 20, 30, 40 and 50 min post-injection. Anti-nociceptivity was evaluated using an arbitrary scale of 1–10 [28]. Degrees of analgesia were given as follows: reaction time up to 4 s (controls), 1 degree; 4.1–5 s, 3 degrees; 5.1–6 s, 6 degrees; 6.1–10 s, 10 degrees. The mean of the sum of the degrees for 50 min given to each group was recorded.

Pethidine (hydrobromide) was used as a reference compound at a dose giving about the same response.

Naloxone (1 mg / 100 g, subcutaneously) was given 20 min before the administration of compound **17** (Table V) [29, 30].

Locomotor activity for some morpholine derivatives was measured using an electrical activity cage, in which 1 rat at a time was placed. The number of movements was recorded for 45 min in 5 min intervals, after a 15 min adaptation period in the cage [31].

Stereotyped behavior was estimated in 5 min intervals for 45 min, using an arbitrary scale of 1–5 [32].

Anti-nociceptivity and antagonism by naloxone, locomotor activity and stereotyped behavior are shown in Table V.

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