The Structure–Activity Relationship of a New Anti-Arrhythmic Agent 1-[2-Acetoxy-3-(4-phenyl-1-piperazinyl)propyl]pyrrolidin-2-one

Barbara Malawska [a]*, Barbara Filipek [b], Katarzyna Stadnicka [c], and Maria Ciechanowicz-Rutkowska [d]

[a] Department of Pharmaceutical Chemistry, Collegium Medicum, Jagiellonian University, Medyczna 9, 30-688 Kraków, Poland, [b] Department of Pharmacodynamics, Collegium Medicum, Jagiellonian University, Podchorazych 1, 30-084 Kraków, Poland; [c] Faculty of Chemistry Jagiellonian University, Ingardena 3, 30-060 Kraków, Poland; [d] Regional Laboratory of Physicochemical Analysis and Structural Research, Jagiellonian University, Ingardena 3, 30-060 Kraków, Poland

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This paper reports the synthesis of the new compound 1-[2-acetoxy-3-(4-phenyl-1-piperazinyl)propyl]pyrrolidin-2-one (Ac-MG-1). Preliminary pharmacological assessment revealed that Ac-MG-1 possesses anti-arrhythmic activity and a local anesthetic effect. The crystal structure of Ac-MG-1 was determined by X-ray diffraction, and conformational analysis was performed both for Ac-MG-1 and for other derivatives of (arylpiperazinyl)propylpyrrolidin-2-one.

Die Struktur-Wirkungsbeziehungen eines neuen antiarrythmischen Verbindung 1-[2-Acetoxy-3-(4-phenyl-1-piperazinyl)propyl]pyrrolidin-2-on

Die Synthese von 1-[2-acetoxy-3-(4-phenyl-1-piperazine)propyl]pyrrolidin-2-on wurde beschrieben. Die ersten pharmakologischen Untersuchungen zeigten für Ac-MG-1 eine antiarrythmische Wirkung und auch eine lokalanästhetische Aktivität. Es wurde die Kristallstruktur von Ac-MG-1 röntgenographisch bestimmt. Die Konformationsanalyse von Ac-MG-1 und von anderen (Arylpiperazin)propylpyrrolidin-2-on-Verbindungen wurde durchgeführt.

There has been considerable interest in structural studies of pyrrolidin-2-one derivatives for the variety of their biological activity, especially anti-hypertensive and coronary vasodilatory activity. For example, a new drug cromacalim is one of the potent potassium channel openers increasingly used as vasodilators [1].

It is possible that the presence of the phenylpiperazine group plays an important role in the pharmacological activity. In fact, 1-alkyl-4-arylpiperazine and some phenylethanolamine derivatives containing the arylpiperazine moiety were reported to display anti-arrhythmic, adrenolytic, and hypotensive activity [2]. And so pipratecol, the analog of norepinephrine, is therapeutically used for its vasodilating properties [3].

Anti-hypertensive and anti-arrhythmic properties were also reported for some *N*-aminoalkyl-, *N*-hydroxyalkyl-, and *N*-acetylguanidine derivatives of pyrrolidin-2-one [4,5]. It was also found that the arylpiperazinylisopropanoloxo fragment is responsible for *beta*-adrenergic blocking and hypotensive properties [6].

In our research we were particularly interested in the effect of an arylpiperazinealkyl substituent in position 1 of the pyrrolidin-2-one on circulatory activity of the studied compounds. Among them 1-[2-hydroxy-3-(4-phenyl-1-piperazinyl)propyl]pyrrolidin-2-one (MG-1) (**2**) was found to display the most pronounced hypotensive and anti-arrhythmic properties in some models of arrhythmia [7].

To reveal the geometry of the hypothetical N-C-C-O pharmacophore the single crystal X-ray analysis of MG-1 and its *m*-chlorophenyl derivative, MG-2, was carried out [8,9]. In the crystals of MG-1 two symmetrically independent molecules of significantly different conformation were present, MG-1/I and MG-1/II, whereas the MG-2 molecule in the crystalline state adopts only one of two possible conformations which corresponds to that of MG-1/I. The conformation of Mg-1/I is antiperiplanar and that of MG-1/II is synclinal, typical for *beta*-blockers. It was interesting to find out what conformation and pharmacological properties will be shown by 1-[2-acetoxy-3-(4-phenyl-1-piperazinyl)propyl]pyrrolidin-2-one (Ac-MG-1) (**3**) in which the MG-1 hydroxyl group is replaced by the acetoxy substituent. It can be expected that such substitution should reduce the *beta*-blocking type of properties but not necessarily influence the activity of quinidine-like anti-arrhythmics.

This paper reports the synthesis, results of a preliminary pharmacological testing, crystal structure, and conformational analysis of Ac-MG-1. All the accessible low energy conformations of Ac-MG-1 are compared to those of MG-1/I, MG-1/II, and MG-2 in order to reveal the values of the N-C-C-O torsion angle occurring most frequently for the isolated molecules of these compounds.

Results and Discussion

Chemistry

Scheme 1 illustrates the procedure used for the synthesis of compound Ac-MG-1. The 1-(2,3-epoxypropyl)pyrrolidin-2-one (**1**) was obtained from pyrrolidin-2-one sodium salt and 1-chloro-2,3-epoxypropane in the reaction described previously [4]. The aminolysis of compound (**1**) with *N*-phenylpiperazine yielded 1-[2-hydroxy-3-(4-phenyl-1- piperazinyl)-propyl]pyrrolidin-2-one (MG-1) (**2**). Acylation of MG-1 with acetic anhydride led to 1-[2-acetoxy-3-(4-phenyl-1-piperazinyl)propyl]pyrrolidin-2-one (Ac-MG-1) which was isolated as the free base and the water-soluble dihydrochloride salt (**3**).

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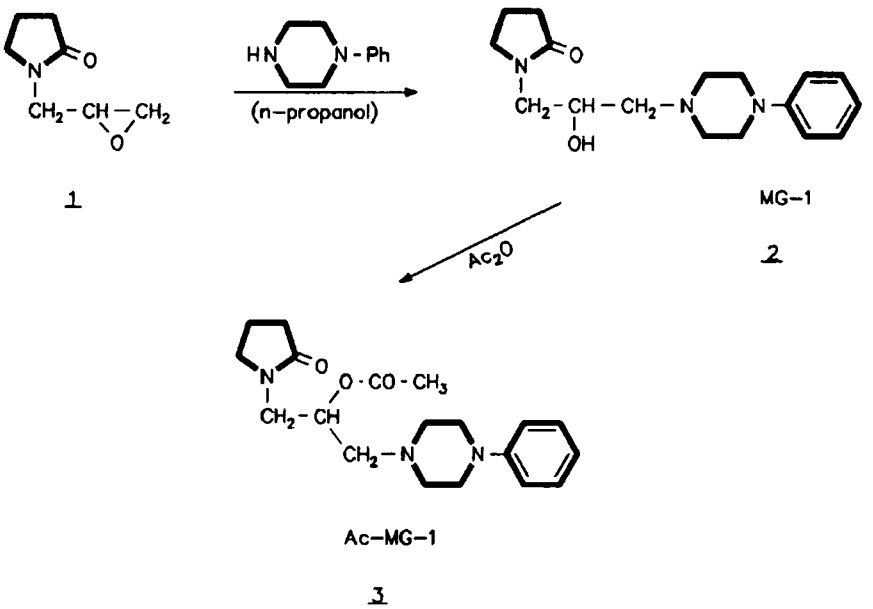

Scheme 1

Pharmacology

Acute toxicity

The LD_{50} values for the tested compounds, determined in mice or rats after intravenous and intraperitoneal administration, are presented in Table 1. All the new compounds were less toxic than propranolol and quinidine.

Table 1. Acute toxicity of the investigated compounds, according to the *Litchfield* and *Wilcoxon* approach [10]. The data are median lethal doses with 5 % confidence limits in parentheses.

Compound	Animal	Route	LD_{50} (mg kg^{-1})
MG-1	Rat	*i.v.*	160[a]
	Mouse	*i.p.*	250 (227–275)
MG-2	Mouse	*i.p.*	360[a]
Ac-MG-1	Mouse	*i.v.*	250 (232–268)
Propranolol	Rat	*i.v.*	39 (34.5–45.5)
	Mouse	*i.p.*	103 (79–135)
Quinidine	Rat	*i.v.*	55[a]
	Mouse	*i.p.*	207 (177–242)

[a] The test was carried out using the method of *Deichmann* and *Le Blanc* [11].

Prophylactic anti-arrhythmic activity

Ac-MG-1 was examined using two models: adrenaline- and barium chloride-induced arrhythmia. Ac-MG-1 administered *i.v.* (10–40 mg kg^{-1}) 15 min before arrhythmogen markedly protected the animals against arrhythmia produced by adrenaline. ED_{50} (a dose producing a 50 % inhibition of arrhythmia) values of Ac-MG-1 were higher than that of MG-1, propranolol, or quinidine. The therapeutic index of Ac-MG-1 was similar to that of MG-1 and about two times lower than that of propranolol, but about three times higher than that of quinidine (Table 2). In contrast to MG-1, Ac-MG-1 applied *i.v.* (20–40 mg kg^{-1}) was ineffective at preventing cardiac arrhythmias caused by barium chloride.

Table 2. Prophylactic anti-arrhythmic effects of Ac-MG-1, MG-1, MG-2, propranolol, and quinidine on adrenaline-induced arrhythmia [12] in rats. Every value was obtained from 3 experimental groups consisting of 8-10 animals each.

Compound	Route	ED50 (mg/kg)	IT = LD_{50}/ED_{50}
Ac-MG-1	*i.v.*	13.7 (9.1–20.5)	18.2
MG-1	*i.v.*	7.6 (6.9–8.4)	21
MG-2	*i.p.*	29.0 (21.3–9.4)	12.4[a]
Propranolol	*i.v.*	1.05 (0.64–1.73)	37
Quinidine	*i.v.*	8.7 (8.0–9.4)	6

[a] according to *Malawska et al.* [7]

Local anesthetic activity

Ac-MG-1, administered into the conjunctival sac (corneal anesthesia) was not effective in local anesthetic activity. The data reported in Table 3 indicate that Ac-MG-1 had stronger local anesthetic properties when applied intradermally to the conscious guinea pig; it was considerably less effective than propranolol and quinidine in this test.

Table 3. Inhibitory concentration (IC_{50}) of MG-1, Ac-MG-1, lidocaine, propranolol, and quinidine in the corneal responses (surface anesthesia) and the dorsal skin responses (infiltration anesthesia) [13] in guinea pigs.

Compound	Surface anesthesia IC_{50} (% conc.)	Infiltration anesthesia IC_{50} (% conc.)
MG-1	> 2	0.98 (0.65–1.47)
Ac-MG-1	–	0.38 (0.16–0.91)
Lidocaine	0.86 (0.68–1.1)	0.36 (0.33–0.39)
Propranolol	0.56 (0.50–0.63)[a]	0.14 (0.12–1.15)[a]
Quinidine	0.39 (0.27–0.55)	0.05 (0.0037–0.067)

[a] according to *Kato et al.* [14].

Crystal structure determination

Crystal data for Ac-MG-1: $C_{19}H_{27}N_3O_3$, M.W. = 345.44, triclinic, $P\bar{1}$ (C_i^1), a = 11.315(2), b = 12.122(2), c = 8.250(2) Å, α = 79.92(2), β = 111.21(2), γ = 117.43(2)°, V = 936.3(4) $Å^3$, Z = 2, D = 1.225 Mgm^{-3}, λ(CuKα) = 1.54056 Å, μ = 0.64 mm^{-1}, F(000) = 372, T = 296 K. The structure was refined to R = 0.0569 and wR = 0.0539 for 3202 unique observed reflections ($|Fo| > 3\sigma(Fo)$).

Table 4 gives the final atomic coordinates of non-hydrogen atoms and their equivalent isotropic thermal parameters. The molecular structure of Ac-MG-1 is shown in Fig. 1 [28].

Table 4. Fractional atomic coordinates and equivalent isotropic vibration parameters U_{eq} ($Å^2$) for non-H atoms with estimated standard deviations in parentheses. $U_{eq} = (1/3)\Sigma_i\Sigma_j a_i a_j {}^*\mathbf{a}_i \cdot \mathbf{a}_j$.

	X/A	Y/B	Z/C	U_{eq}
N(1)	0.3871(2)	0.8117(2)	0.4276(2)	0.0349(4)
C(2)	0.3430(2)	0.8369(2)	0.5419(3)	0.0367(5)
O(2)	0.3641(2)	0.8035(2)	0.6938(2)	0.0543(5)
C(3)	0.2618(3)	0.9125(2)	0.4475(3)	0.0450(5)
C(4)	0.2569(3)	0.9212(3)	0.2628(3)	0.0740(5)
C(5)	0.3492(3)	0.8648(2)	0.2546(3)	0.0449(6)
C(6)	0.4651(2)	0.7370(2)	0.4733(3)	0.0379(5)
C(7)	0.3784(2)	0.6063(2)	0.3990(3)	0.0348(5)
O(7)	0.3543(2)	0.6144(1)	0.2127(2)	0.0372(4)
C(8)	0.2361(3)	0.5407(2)	0.4253(3)	0.0424(6)
N(9)	0.1540(2)	0.4180(2)	0.3452(2)	0.0384(5)
C(10)	0.0034(3)	0.3783(2)	0.2959(3)	0.0506(6)
C(11)	–0.0751(3)	0.2588(2)	0.1953(3)	0.0470(5)
C(12)	0.1885(3)	0.3224(2)	0.4563(3)	0.0489(G)
C(13)	0.1140(3)	0.2011(2)	0.3587(3)	0.0457(5)
N(14)	–0.0383(2)	0.1608(2)	0.2960(2)	0.0387(5)
C(15)	–0.1220(2)	0.0420(2)	0.2189(3)	0.0374(5)
C(16)	–0.2688(3)	–0.0053(2)	0.1625(3)	0.0419(5)
C(17)	–0.3535(3)	–0.1207(2)	0.0846(3)	0.0504(6)
C(18)	–0.2949(3)	–0.1929(2)	0.0615(3)	0.0569(5)
C(19)	–0.1514(3)	–0.1479(2)	0.1190(3)	0.0579(5)
C(20)	–0.0644(3)	–0.0320(2)	0.1964(3)	0.0488(5)
C(21)	0.3765(3)	0.5354(2)	0.1431(3)	0.0402(5)
O(21)	0.4317(2)	0.4707(2)	0.2283(2)	0.0622(5)
C(22)	0.3222(3)	0.5392(2)	–0.0491(2)	0.0547(5)

The molecule has an asymmetric C(7) atom and so both configurations, *R* and *S*, are present in the centrosymmetric structure (racemate). The conformation of the molecule in the crystalline state is close to that of MG-1/II and it is stabilized by a weak intramolecular H-bond C(6)...O(2) of 2.852(4) Å, [C(6)-H(061) = 1.053(5) Å, H(061)...O(2) = 2.410(9) Å and C(6)-H(061)...O2) = 103.8(4)°]. The hydrogen bond is not linear since it follows the requirements imposed by the geometry of the five-membered quasi-ring [O(2)-C(2)-N(1)-C(6)-H(061)], unique for this conformation. The important feature of this fragment seems to be the electron withdrawing ability of O(2) leading to the typical shortening of the N(1)-C(2) bond length to 1.347(4) Å due to the involvement of the electron lone-pair of N(1). The displacement of the electron density is stabilized by the presence of the hydrogen bond discussed above. This type of hydrogen bonding is encountered in other structures [15,16]. All bond lengths have values in a good agreement with the statistical average values listed for organic compounds in [17]. A typical for *beta*-adrenolytics, synclinal conformation of the O(7)-C(7)-C(8)-N(9) chain with torsion angle of 57.3(3)° is observed. The other torsion angles important for the description of molecular conformation are N(1)-C(6)-C(7)-C(8) and C(6)-C(7)-C(8)-N(9) of –47.1(3)° and 177.4(2)°, respectively. The piperazine ring adopts the chair conformation with a pseudo-mirror plane through the N atoms (ring puckering parameters, defined according to *Cremer* and *Pople* [18], are $q_2 = 0.057(3)$ and $q_3 = 0.574(3)$ Å, $\phi_2 = -2(3)°$, QT = 0.576(3) Å and $\theta_2 = 5.7(3)°$ while for the ideal chair conformation $q_2 = 0°$, $q_3 = \pm QT$ and θ = 0 or 180°; the asymmetry parameter of the pseudo-mirror plane through N(9) and N(14) is 0.002(2)°). The conformation of the pyrrolidine five-membered ring is intermediate between an envelope and twist form (ring puckering parameters are q_2 = 0.072(3) Å and ϕ_2 = 117(2)° whereas for pure envelop conformation ϕ_2 may adopt 0, 36, 72, 108°, *etc.* and for pure twist form ϕ_2 = 18, 54, 90, 126° *etc.*; a pseudo-diad axis through C(2) and a pseudo-mirror plane through C(4) have the asymmetry parameters 0.006(1) and 0.009(1)°, respectively). The pyrrolidine nitrogen atom N(1) has sp^2 hybridization (the sum of the appropriate bond angles at N(1) is 360° within the limit of error) whereas piperazine nitrogen atoms, N(9) and N(14) have sp^3 hybridization with the sum of their bond angles being 333.8 and 342.1°, respectively. The phenyl ring is planar, with atoms deviating from the best least-squares plane by between 0.006(2) and –0.005(2) Å. It forms the angle of 30.2(1)° with the mean piperazine plane and 101.6(1)° with that of pyrrolidine ring.

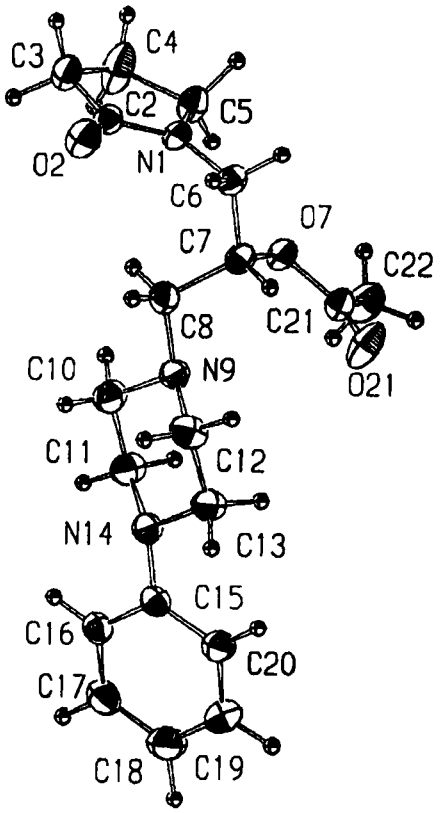

Fig. 1. Conformation of the Ac-MG-1 molecule in the crystalline state shown in *R* configuration. Atom numbering is given. Thermal-vibration ellipsoids are scaled to enclose 40 % probability.

The intermolecular interactions are purely weak dispersive. The shortest intermolecular distances found in the structure, H(07)...H(07) [–X+1,Y+1,–Z+1] = 2.393(12) Å and H(052)...H(112) [–X,–Y+1,–Z] = 2.462(6) Å, correspond, in the limit of error, to the sum of the appropriate *van der Waals* radii.

Conformational analysis

Conformational analysis was carried out in order to find how VdW energy of the studied molecules MG-1/I, MG-1/II, Ac-MG-1, and MG-2 changes with the variation of torsional angle τ = O(7)-C(7)-C(8)-N(9). It is apparent that for the two symmetrically independent molecules of MG-1 taken out of the crystal force field the τ angle has basically the same values for the low energy conformations, and that the synclinal conformations ($\tau = 60°$ and $-60°$) are nearly equally probable as the antiperiplanar conformation ($\tau = 180°$). Similar is true for Ac-MG-1 and MG-2.

It is interesting to note that there are no other conformations accessible for an isolated molecule than synclinal and antiperiplanar and that, regardless of the derivative and the starting conformation, the optimization of energy leads to only three possible conformations: two synclinal and one antiperiplanar.

Conclusion

Preliminary pharmacological assessment revealed that Ac-MG-1, similarly to MG-1, displays strong anti-arrhythmic action in adrenaline-induced arrhythmia. In contrast to MG-1, Ac-MG-1 did not prevent cardiac arrhythmia caused by the administration of barium chloride. Ac-MG-1 has a local anesthetic effect stronger than that of MG-1 in infiltration anesthesia. Our earlier hypothesis, that MG-1 possesses *beta*-adrenergic antagonist properties [7] typical for class II agents (*beta*-adrenergic blockers) and depending on the presence of the hydroxyl group was not confirmed. Ac-MG-1, in which the hydroxyl group of MG-1 is replaced by the acetoxy substituent, remains active. This result shows that the presence of the hydroxyl group is not necessary for the anti-arrhythmic activity. On the other hand, the analysis of the hypotensive properties of MG-1 in rats indicated that the blockage of the vascular alpha-adrenoreceptors is probably responsible for this action and, although MG-1 has resulting effect similar to cromacalim they display different mechanism of activity [19]. From the point of view of the classification of *Vaughan Williams* [20], MG-1 could be considered as class I anti-arrhythmic drug. It seems that Ac-MG-1 possesses the same pharmacological profile.

In the crystal structure the Ac-MG-1 molecule exhibits a synclinal conformation for O(7)-C(7)-C(8)-N(9) chain, similar to that of molecule II of MG-1. However, for an isolated molecule in both cases the antiperiplanar conformation is more populated. The antiperiplanar conformation was observed in crystal structure for molecule I of MG-1 and for MG-2. In contrast, the molecule of cromacalim in its crystal structure [21] adopts a synclinal conformation (O(2)-C(2)-C(3)-N(1) = 62.9°) which cannot be switched out towards the antiperiplanar one.

The conformational analysis of the isolated molecules for all compounds (MG-1/I, MG-1/II, Ac-MG-1, and MG-1) shows that two synclinal and one antiperiplanar conformations are accessible and they are the only possible ones. The fact that the antiperiplanar conformation for Ac-MG-1 is more populated supports the pharmacological conclusion regarding its quinidine-like anti-arrhythmic activity (class I).

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Experimental Part

Chemistry

Satisfactory elemental analysis ±0.4 % of calculated values were obtained for the new compounds. Melting points were determined with a Boetius melting-point apparatus (VEB Analytic Dresden) and were uncorrected. The purity of synthesized compounds was checked by thin-layer chromatography on silica gel plates (5 × 10 cm, 0.25 mm) Kieselgel GF_{254} (Merck). ^1H NMR spectra were recorded on Bruker spectrometer at 300 MHz using TMS as internal standard, [D_6]DMSO was used as a solvent. The IR spectrum was taken with a Specord 80 IR (VEB Carl Zeiss Jena) using KBr disk (1:300 mg KBr). The mass spectrum was obtained on a GCMS 2091 LKB mass spectrometer operating at an ionizing energy of 70 eV.

1-[2-Acetoxy-3-(4-phenyl-1-piperazinyl)propyl]-pyrrolidin-2-one

2 ml (0.02 mol) of acetic anhydride and 1 ml (0.01 mol) of pyridine were added to 0.6 g (0.002 mol) of 1-[2-hydroxy-3(4-phenyl-1-piperazinyl)propyl]pyrrolidin-2-one. The mixture was heated for 1.5 h at 50 °C and then evaporated under vacuum. The residue was dissolved in 20 ml of ether, and the organic layer was washed with sodium bicarbonate and water and dried (with sodium sulfate). Removal of the solvent left the crude product which was recrystallized from 60:80 petroleum ether and ethyl acetate to give 0.5 g (75 %) of colourless crystals: m.p. 84–86 °C. Formula $C_{19}H_{27}N_3O_3$, M.w. = 345.43.

TLC: R_F = 0.26 (chloroform–methanol–acetic acid, 60:10:5), R_F = 0.72 (methanol–ammonium hydroxide 25 %; 10 ml : 3 drops).– MS m/z : 345 (0.13), 303 (6.61) M^+ – CO-CH_3, 285 (5.75), 205 (4.55), 184 (2.93), 175 (100) – CH_2-Piperazine-Ph, 160 (9.4), 132 (25.44), 120 (4.74), 104 (9.79), 98 (7.06), 77 (6.19), 70 (61.56), 56 (7.38), 42 (8.46), 38 (20.99), 36 (69.26), 28 (5.88).– ^1H NMR ([D_6]DMSO) δ (ppm) = 1.94 [2H, m, CH_2(C4)]; 2.24 [2H, m, CH_2(C3)]; 2.51 [3H, s, CH_3(C22); 3.07–3.22 [8H, m, piperazine protons (C10, C11, C12, C13); 3.45– 3.47–3.49 [2H, t, CH_2(C5)] J = 6.9; 3.50, 3.57, 3.68 [2H, m, CH_2(C8)]; 3.79–3.82 [2H, d, CH_2(C6)] J = 9.2; 4.31–4.33 [1H, d, CH(C7)]; 6.85–6.89, 7.00–7.03, 7.24–7.30 [5H, aromatic protons, (C16, C17, C18, C19, C20)].

The free base (Ac-MG-1) thus obtained was converted into the hydrochloride salt by treatment with 1 equiv. of concentrated HCl in ethanol. It was recrystallized from ethanol to give colourless crystals, m.p. = 224–226 °C. Formula $C_{19}H_{29}N_3O_3Cl_2$, M.w. = 418.36.– IR $\tilde{\nu}$ (cm^{-1}) 1680 C=O in pyrrolidinone ring; 1648 C=O in acetoxy group.

Pharmacology

Materials and methods

Compounds: Adrenaline (adrenalinum hydrochloricum, Polfa), amobarbital (amytal, Lilly), barium chloride (POCH Gliwice), lidocaine (lignocainum hydrochloricum, Polfa), propranolol (propranololum hydrochloricum, Polfa), quinidine sulfate (Polfa). The drugs were dissolved or diluted with 0.9 % saline.

Animals: The experiments were carried out on male albino Swiss mice (18–25 g), male Wistar rats (180–250 g), and male and female guinea-pigs (300–450 g). Animals were housed in wire mesh cages in a room at 20 ± 2 °C with natural light-dark cycles. The animals had free access to standard pellet diet and water and used after a minimum of 3 days acclimatization to the housing conditions. Control and experimental group consisted of 8–10 animals each.

Doses and routes of administration: Depending on the experimental method, compounds were given intravenously (*i.v.*), in doses corresponding to 0.05 and 0.2 of LD_{50} and lower, in a volume of 10 ml kg^{-1} (mice) or 1 ml kg^{-1} (rats).

Reference compounds: Propranolol, quinidine and lidocaine were used as a reference compounds.

Statistics: LD_{50} and ED_{50} values and their confidence limits were calculated according to the method of *Litchfield* and *Wilcoxon* [10].

Acute toxicity was calculated according to the method of *Litchfield* and *Wilcoxon* [10]. The compound dissolved in 0.9 % saline solution was administered *i.v.* and *i.p.* to mice or rats and the animals were observed for 6 h. The number of dead animals were counted 24 h after administration.

Prophylactic anti-arrhythmic activity:

a) Adrenaline-induced arrhythmia (according to *Szekeres* [12]). The arrhythmia was evoked in rats anesthetized with amobarbital (75 mg kg^{-1}, *i.p.*) by *i.v.* injection of adrenaline (20 µg kg^{-1}) Ac-MG-1 was administered *i.v.* 15 min and 1 h before adrenaline. The criterion of anti-arrhythmic activity was the lack of premature beats and inhibition of cardiac arrhythmia in comparison with the control group.

b) Barium chloride-induced arrhythmia (according to *Szekeres* [12]). Barium chloride solution was injected into the caudal vein of rat (32 mg kg^{-1}, in a volume of 1 ml kg^{-1}). Ac-MG-1 was given *i.v.* 15 min before the arrhythmogen. The criterion of anti-arrhythmic activity was gradual disappearance of the arrhythmia and restoration of the sinus rhythm.

Local anesthetic activity (according to Bülbring and Wajda [13]):

a) Corneal anesthesia. 0.05 ml volumes of tested solutions were applied into the conjuctival sacs of male and female guinea-pigs, and the presence or absence of corneal anesthesia was checked every 5 min by means of a test hair poked six times into the cornea. The trial was performed every 5 min for 30 min.

b) Infiltration anesthesia. The backs of female guinea pigs were depilated on the day before the experiment. On the day of the study the naked surface was divided into two equal fields with Indian ink and 0.1 ml of the test solutions were injected intracutaneously into the center of each field. The skin responses to pain prick were tested 5 min after the injection. Three pricks were applied to each injected area at 5 s intervals. The trial was performed every 5 min for 30 min. In both methods, the criterion used to calculate the IC was percentage of negative responses for each concentration [14].

X-Ray crystal structure analysis

Crystals of Ac-MG-1 suitable for X-ray analysis were obtained from solution in a mixture of *n*-hexane and ethyl acetate. A colorless crystal of dimensions 0.55 × 0.55 × 0.30 mm was used for X-ray measurements. Data were collected with a KM4 (kappa geometry) diffractometer (KUMA diffraction) at room temperature (296 K) using graphite-monochromated CuKα radiation. Cell parameters were determined with the diffractometer using 25 reflections in the range $8 < \theta < 68°$. Although, it is possible to transform the unit cell to a pseudo-monoclinic one, the *Laue* class was checked not to be higher than $\bar{1}$. Later, the statistics of $< | |E|^2 - 1 | >$ confirmed the centrosymmetric space group, *i.e.*, $P\bar{1}$. Intensity measurements were carried out in the range $2 \le \theta \le 72°$ (the range of indices $0 \le h \le 11$, $-11 \le k \le 14$, $-10 \le l \le 10$) with scan mode $\omega/2\theta$. Standards ($4\ \bar{5}\ \bar{3}$, $1\ 3\ \bar{4}$ and 640) were checked every 50 reflections and no changes in their intensity greater than 2 % were found throughout the data collection. *Lorentz* and polarization corrections were applied. No corrections were made for absorption or secondary extinction. The structure was solved using the program SHELXS-86 [22] and positions of all the non-H atoms were found from E-map followed by one cycle of peak-list optimization. From 3781 reflections measured, 3202 unique observed reflections ($|F| \ge 3\sigma(F)$) were used in the refinement performed with SHELX-76 program [23]. After the refinement of non-H atom coordinates with anisotropic vibration parameters all H atoms were located from difference Fourier map and included into refinement with isotropic temperature factors. The full-matrix refinement (on $|F|$) converged at R = 0.057 and wR = 0.054, w = 1.4114 $[\sigma^2(F)]^{-1}$ for 334 refined parameters; max. and min. peak on final difference Fourier map were 0.18 and −0.11 $e\text{Å}^{-3}$, respectively. Scattering factors were taken from International Tables for X-ray Crystallography, Vol. IV (1974). Geometric calculations were carried out using PARST program [24] and drawings were done with ORTEPII [25] using an IBM-type PC 486 computer.

Conformational analysis

Conformational analysis of two symmetrically independent molecules of MG-1 (*i.e.* MG-1/I and MG-1/II), Ac-MG-1 and MG-2 was carried out with CHEMX [26]. First the crystallographic structures with *Gasteiger's* charges [27] assigned to atoms were optimized using CHEMX's molecular mechanics force field with the restraints applied to the heterocyclic rings preserving their X-ray coordinates. The geometries thus obtained were used as starting points for the generation of 18 × 18 × 6 (1944) different conformations by stepwise rotation of 20° around both N9-C8 and C8-C7 bonds and 30° around C6-C7 bond. Next, the energy was calculated for all the conformations taking into account both the molecular mechanics and Van der Waals interactions. Then the geometries of conformations with a relative VdW energy within 10 kcal mol^{-1} (248.9 kJ mol^{-1}) above the global minimum were optimized using molecular mechanics and analyzed. The information, most relevant to the subject of our investigation, is obtained by the inspection of the scatter plots of VdW energy *vs* torsional angle τ = O7-C7-C8-N9.

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28 Further information can be obtained from the Fachinformationszentrum Energie, Physik, Mathematik, D-76344 Eggenstein-Leopoldshafen 2, citing the deposit no., the authors and the journal.

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