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SYNTHESIS OF CONFORMATIONALLY CONSTRAINED ANALOGUES OF BRL-32872. DETERMINATION OF STEREOCHEMISTRY AND RELATED PHARMACOLOGICAL PROPERTIES.

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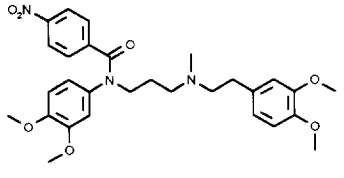
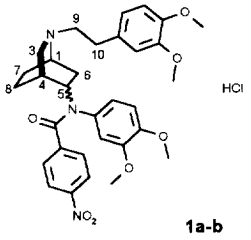
Abstract: 2-Azabicyclo[2.2.2]octane derivatives have been used as conformationally constrained structure of BRL-32872, in order to investigate the effect of molecular flexibility towards class III and class IV antiarrhythmic properties. Extensive NMR studies allowed the determination of the configuration at C5. Stereoisomerism induces variability of biological profile. © 1997 Elsevier Science Ltd.

BRL-32872 (Figure 1) is a novel antiarrhythmic agent with potassium and calcium channel blocking properties[1] and thus, has a potential use in ventricular and atrial arrhythmias therapy[2] . BRL-32872 possesses a high level of flexibility and it may be assumed that such a flexibility could be reduced in order to gain further insight into "active conformations" of the compound. To this aim, we synthesized analogues with an azabicyclo[2.2.2]octane system to introduce a rigid three-methylene link between the carboxamide function and the dimethoxy-phenethyl group of the molecule. We wish to report in this communication the synthesis of 2-azabicyclo[2.2.2]octane derivatives **1a-b**, results of the extensive NMR study leading to the determination of the stereochemistry at C5 and the pharmacological data obtained with the two epimers **1a** and **1b**.



BRL-32872



1a-b

Figure 1.

Chemistry

Preparation of the azabicyclo[2.2.2]octane nucleus has been well documented[3] as this bicyclic skeleton is found in many biologically active natural products. The synthetic pathway used to prepare **1a-b** is shown in Scheme 1. Reductive

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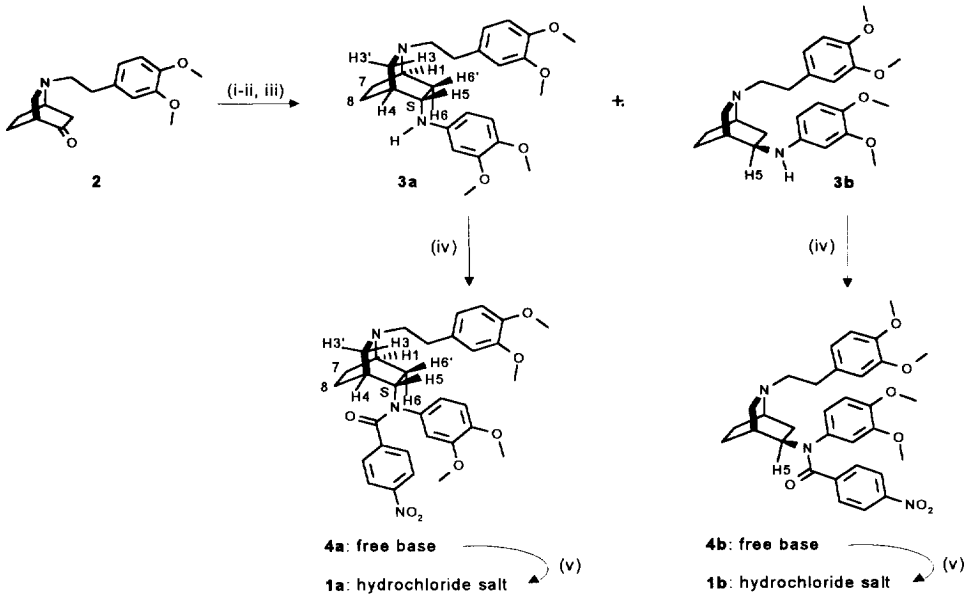
amination of the ketone **2**[3f] followed by flash chromatography led to the epimers **3a** and **3b** (ratio 2:1). Condensation of the secondary amine function of **3** with 4-nitrobenzoyl chloride afforded **4a** and **4b** which gave rise to respective hydrochloride salts **1a** and **1b**, after treatment with ethanolic hydrochloric acid.



Scheme 1. Compounds are racemates and for clarity only one enantiomeric form is shown[4].
Methods: (i) 3,4-dimethoxyaniline, toluene, Dean Stark then (ii) $NaBH_3CN$, CH_3OH, cat. HCl, 58% overall (iii) flash chromatography on silica gel (MeOH-CH_2Cl_2, 5-95) (iv) 4-nitrobenzoyl chloride, Et_3N, CH_2Cl_2, 70-75% (v) HCl in EtOH, 68%.

Stereochemistry[5]

The determination of the stereochemistry of the structures was first attempted on the free bases **4a-b**. The difficulties in determining the configuration at C5 were twofold. Firstly, the presence of an ethylbenzene moiety as substituent of the tertiary amine increases the complexity of the ^1H NMR spectra as overlap of resonances increases. Secondly, general broadness of the spectra did not allow us to prove the stereochemistry conclusively. Only one epimer, **4a** or **4b**, afforded NMR data suitable for additional analysis (Table 1) although various experimental conditions were used[6]. Therefore, further NMR studies were performed with that stereoisomer; the results are listed in Table 2. In order to measure the spatial proximity of protons, nOe (nuclear Overhauser enhancement) experiments were used: the effects observed between H5 and H3 or H3' suggested that carbon atom 5 had the S configuration (see Scheme 1). An nOe from H5, δ 4.95 ppm, to the resonance at δ 2.40 ppm confirms the latter's assignment as H4. Despite the nOe evidence for the proximity of H5 and H3, it was not possible to distinguish between the pseudo-axial/equatorial protons H6 and H6' to confirm the

stereochemistry at C5. Therefore, an additional NMR investigation was performed on the precursor compound **3** (related to the epimer used). From the results reported in Tables 3 and 4, the following conclusions were drawn:

- The irradiation of H6 gave nOes to the geminal proton H6' and to H1. When H6' was irradiated, an additional nOe to H5 was observed. Therefore, H6' should be in the same orientation with respect to the bicyclic system as H5.

- The irradiation of H5 resulted in an enhancement of protons H4, H6' and H3. Hence, H5 is pointing in the same direction as H6', up towards proton H3[7]. The methoxy groups, which are all close in chemical shift to H5, were also irradiated (with H5) in this experiment but it is thought that they do not interfere with the analysis since they are not close in space to the protons of interest.

At this point, the NMR data have demonstrated conclusively that the epimer used for the study has the S configuration at carbon atom 5 and hence is compound **4a** (see Scheme 1).

Table 1. Assignment of ^{1}H resonances of one epimer of **4** in $CDCl_3$; chemical shifts were referenced to TMS at 0.0 ppm (accuracy for protons 1 to 10 is ± 0.05 ppm because of broad resonances).

Proton	Chemical Shift (ppm)
H5	4.95
H3 or H3'	3.35
H1, H9, H9', H10, H10', H3 or H3'	2.90
H6 or H6'	2.55
H4	2.40
H7 or H7'	2.00
H8, H8', H6 or H6'	1.60
H7 or H7'	1.40
OCH_3	3.89; 3.86; 3.82; 3.76
aromatic	4H: 8.01 and 7.39; 6H between 6.85 and 6.45

Table 2. Results of the nOe difference experiments on one epimer of **4**.

Irradiated Frequency (ppm)	Proton	nOe Effect to
4.95	H5	H3 or H3', H6 or H6', H4
3.35	H3 or H3'	H4, H5
1.40	H7 or H7', partially H8, H8', H6 or H6'	H7 or H7', resonance at 2.90 ppm
2.00	H7 or H7'	H7 or H7'
2.55	H6 or H6', partially H4	H5, H6 or H6'

Table 3. Assignment of ^1H resonances of one epimer of **3** in d_4-methanol; chemical shifts were referenced to the CHD_2OD resonance at 3.30 ppm.

Proton	Chemical Shift (ppm)
H1	3.41
H3, H3'	3.34; ≈3.30[a]
H4	2.14
H5	3.76
H6, H6'	1.52; 2.67
H7, H7', H8, H8'	1.60; 1.83; 2.03; 2.09
H9, H10	2.97; 3.30
OCH_3	3.72; 3.78; 3.79; 3.84
aromatic	6.21; 6.41; 6.77; ≈6.85; ≈6.91[b]; 6.93

[a] overlapped signals. [b]second order effects.

Table 4. Results of the nOe difference experiments on one epimer of **3**.

Irradiated Frequency (ppm)	Proton	nOe Effect to
1.52	H6	H6', H1
2.67	H6'	H6, H1, H5
3.76	H5 and OCH_3	H4, H6', H3

Pharmacological Data

Pharmacological effects of **1a** and **1b** were assessed using standard microelectrode (Figures 2A and 2B) and patch-clamp techniques (Figures 2C and 2D) on cardiac tissue isolated from guinea-pig hearts. Figures 2A and 2B show the results obtained on action potential duration measured at 30% and at 90% of repolarization. The former parameter is particularly sensitive to inhibition of the calcium current and its decrease may be considered as an index of class IV antiarrhythmic activity, whereas the latter parameter increases as repolarizing potassium current is inhibited and is considered as an index of class III antiarrhythmic activity of tested compounds. The main effect of **1b** was to prolong action potential duration though this prolongation declined at higher concentrations, resulting in a bell-shaped dose-response curve (Figure 2B) similar to BRL-32872 (see Reference 1). Both the increase in action potential duration for low concentrations and the latter decline were less pronounced with **1a** (Figure 2A) which may thus be considered as a less potent class III and class IV antiarrhythmic agent. These results are confirmed with the patch-clamp experiments which show that the effective concentrations of **1b** on the potassium and the calcium currents (0.10 and 11.78 μM respectively; Figure 2D) are lower than those of **1a** (0.24 and 41.11 μM respectively; Figure 2C). In summary, the profile of BRL-32872 is maintained for both compounds but **1b** is closer to the lead than **1a**; this result could be explained by the difference in conformational

spaces generated by the two compounds; for instance, because of the pseudo-axial position of its amide **1a** cannot access the conformation of **1b** shown in Figure 3 where the intramolecular hydrogen bond strengthens the core of the structure. Superimposition of BRL-32872 on **1b** gave rise to a close low energy conformation after minimization (Figure 3), which might be considered as an "active conformation" of the lead.

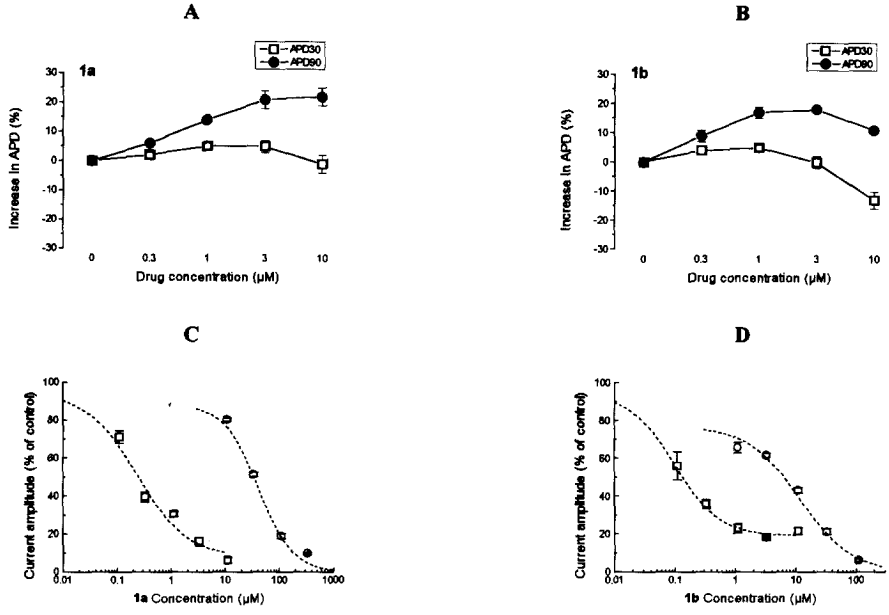


Figure 2: **A** and **B** : Action potential duration was recorded on guinea-pig papillary muscles at 30% and 90% of repolarization (APD30 and APD90, respectively) using standard microelectrode technique. Effect of each concentration was measured after steady-state was reached (30 min exposition). **C** and **D** : L-type calcium (circles) and delayed rectifier potassium (squares) currents were isolated and recorded according to standard procedures (see Reference 1). Calcium and potassium currents were inhibited by **1b** (right panel) with EC_{50s} of 11.78 and 0.10 μM respectively (Hill coefficients : 1.01 and 1.18, respectively) and by **1a** (left panel) with EC_{50s} of 41.11 and 0.24 μM respectively (Hill coefficients : 1.37 and 0.93, respectively)

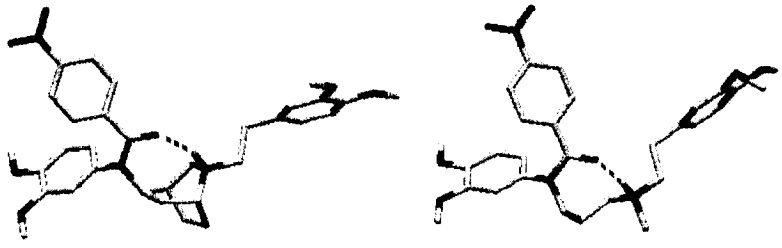


Figure 3. Low energy conformations of **1b**[a] (left) and BRL-32872 (right) showing intramolecular hydrogen bonds displayed in dotted line.

Conclusion

Stereoisomers of 2-azabicyclo[2.2.2]octanes have been synthesized and used as constrained analogues of BRL-32872 to investigate some structural requirements for the inhibition of the potassium and calcium currents. Our results have clearly shown that stereoisomerism is involved, to some extent, in modulating potency on both currents. We have been able to identify the respective stereochemistry of each epimers and this gave rise to the design of a relevant conformational feature for the lead compound.

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References and Notes

1. Bril, A.; Faivre J.-F.; Forest M. C.; Cheval B.; Gout B.; Linée P.; Ruffolo R. R. Jr; Poyser R. H. *J. Pharm. and Exp. Ther.* **1995**, *273*, 1264-1272.

2. Gout, B.; Nichols A. J.; Feuerstein G. Z.; Bril A. *J. Cardiovasc. Pharmacol.* **1995**, *26(6)*, 636-644.

3. a) Bakunov S. A.; Denisov A. Y.; Tkachev A. V. *Tetrahedron* **1995**, *51*, 8565-8572. b) Asaoka M.; Ohkura N.; Yokota M.; Sonoda S.; Takei H. *Heterocycles*, **1994**, *38*, 2455-2462. c) Szantay C.; Bolcskei H.; Gacs-Baitz E. *Tetrahedron* **1990**, *46*, 1711-1732. d) Mehmandoust M.; Marazano C.; Singh R.; Gillet B.; Césario M.; Fourrey J.-L.; Das B. C. *Tetrahedron Lett.* **1988**, *29*, 4423-4426. e) Trost B. M.; Romero A. G. *J. Org. Chem.* **1986**, *51*, 2332-2342. f) Law, S.-J.; Lewis D. H.; Borne R. F. *J. Heterocyclic Chem.* **1978**, *15*, 273-280.

4. Analytical data. **2**: oil. Anal. Calc. for $C_{17}H_{23}NO_3$ (289.4): C 70.56, H 8.01, N 4.84; Found: C 70.38, H 8.01, 4.89. **3a-b**: amorphous solid. Anal. Calc. for $C_{25}H_{34}N_2O_4$ (426.6): C 70.40, H 8.03, N 6.57; Found: C 70.19, H 8.23, N 6.21. **1a**: amorphous solid. Anal. Calc. for $C_{32}H_{37}N_3O_7$.HCl (612.1): C 62.80, H 6.26, N 6.86, Cl 5.79; Found: C 62.51, H 6.38, N 6.73, Cl 5.69. **1b**: amorphous solid. Anal. Calc. for $C_{32}H_{37}N_3O_7$.HCl.0.5H_2O (621.1): C 61.88, H 6.33, N 6.77, Cl 5.71; Found: C 61.89, H 6.33, N 6.81, Cl 5.54.

5. Identification of stereochemistry in a simpler series has been reported: Blaney F. E.; Hadley M. S.; King F. D.; Watts E. A. *Eur. Patent 115 933*, **1984**.

6. NMR was performed at different field strengths (JEOL GX270, Bruker AMX400 and AMX500) operating at different temperatures (up to 370 K) in various solvents (DMSO, $CDCl_3$ in addition to d_4-MeOH).

7. The result from the nOe difference study was confirmed by the coupling pattern of the protons H6 and H6'. The coupling pattern of the resonance of H6 δ 1.52 ppm results from a large geminal coupling and a small vicinal coupling whereas the resonance of H6' δ 2.67 ppm exhibits two large coupling constants.

8. The presence of intramolecular hydrogen bond decreases the resulting conformational energy of **1b** up to 9.5 Kcal.mol^{-1} compare to the closest conformation without hydrogen bond. Calculations were performed using MM2 force-field from within MacroModel 5.0 software (Columbia University New York, NY 10027).

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