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The structure of the ring-opened N_β-propyl-ajmaline (Neo-Gilurytmal®) at physiological pH is obviously responsible for its better absorption and bioavailability when compared with ajmaline (Gilurytmal®)

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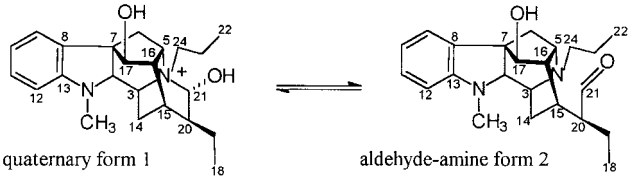
Prajmaline, the semisynthetic propyl derivative of ajmaline, shows a much better bioavailability when compared with the *Rauvolfia* alkaloid ajmaline. Early NMR and IR-studies, fluorescence spectroscopic investigations and extraction experiments combined with ion-pair chromatography proved the thesis of a tautomeric equilibrium between an aldehyde-amine and a quaternary carbinol-ammonium component. The aim of this study was to confirm this thesis by HPLC-separation and by structure-determination of both tautomeric compounds.

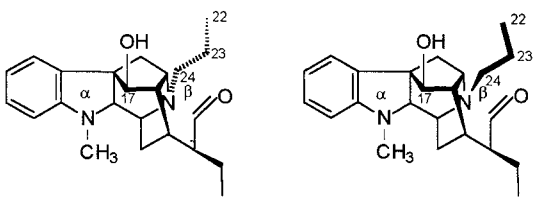
1. Introduction

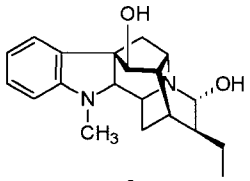
The monoterpenoid indole alkaloid ajmaline (**3**), isolated from Rauvolfiae radix, was introduced into the therapy of heart arrhythmic disorders as Gilurytmal® in 1959 by Kleinsorge [1]. Ajmaline (**3**) exhibits effects similar to the antiarrhythmic alkaloid quinidine and was therefore originally classified as a class 1 A antiarrhythmic drug. More recent results concerning its pharmacological action and particularly those concerning the recovery times of sodium channels point to the fact that ajmaline is a class 1 C antiarrhythmic drug [2]. Under conditions of oral administration the bioavailability of **3** is low and varies greatly which is clearly a therapeutic disadvantage. Therefore ajmaline was transformed into the partially synthetic N_β-propyl-ajmaline and the quaternary prajmalium-bitartrate (prajmaline = Neo-Gilurytmal®). By this procedure the water solubility of the compound could be significantly increased [3, 4]. As a salt prajmaline is highly polar but obviously is more readily absorbed than ajmaline [5]. In general text books this rather unusual phenomenon is briefly mentioned but could not be explained up to now, because of the lack of experimental work. In the present study we investigate whether the quaternary polar or a ring-opened lipophilic form of Neo-Gilurytmal® exists at physiological pH values, which might have an important influence on its absorption and bioavailability.

2. Investigations, results and discussion

Neo-Gilurytmal® tablets contain, in addition to the water soluble N_β-propyl-ajmaline-bitartrate (PBT), significant amounts of lactose, polyethylene glycol 6000 and polyvidone which makes the direct spectroscopic investigations of the structure of PBT difficult. Therefore we synthesised PBT and compared the pure PBT with a D_2O extract of the tablets by ^{1}H-NMR. The spectrum obtained clearly indicated PBT as the significant ingredient of the tablets based on the aromatic protons and the C-18 and C-22 methylgroups (data not shown). Keck has already suggested a pH-dependent tautomeric equilibrium between an aldehyde-amine (**2**) and a carbinol-ammonium (**1**) structure for PBT [3, 4]. Grundevik and Persson determined both forms by ion-pair chromatography [6].
In order to obtain a more direct evidence on the structure of PBT, Neo-Gilurytmal® tablets were powdered and extracted with ether at different pH values. Analysis of the extracts and the aqueous phases by TLC and UV absorption clearly demonstrated that at high pH the lipophilic ring-opened form (**2**) occurred and at low pH only the hydrophilic quaternary salt (**1**) existed. To provide spectroscopic evidence for the amino-aldehyde form (**2**) it was isolated from the tablets and its structure was determined by ^{1}H- and ^{13}C-NMR spectroscopy. In the proton NMR two aldehyde signals were observed at 9.38 and 9.44 ppm and were confirmed by ^{13}C data (signals at 201 and 203 ppm). In addition to these results a double set of signals in the ^{1}H spectrum for CH 17 and COH 17 supported the occurrence of a mixture of isomers at a pH value of about 8.0. Moreover, HPLC analyses on reversed phase columns supported this result. At pH 7.2 prajmaline is obviously eluted as its lipophilic, open aldehyde-amine form (**2**), because it shows even at the lower pH-value of 6.3 much longer retention times (24 min) compared to the ring closed ajmaline (11 min). In addition, at pH 7.2, two HPLC signals with similar retention times (34 min and 36 min) were observed which points to a mixture of ring-opened isomers. When both peaks were isolated and analysed by mass spectrometry the data confirmed the occur-







rence of a pair of isomers but did not allow any conclusion about the nature of the stereoisomers. As discussed in the literature [7] the quaternary ring-closed clinically available prajmaline (**1**) exists as a 55% to 45% mixture of diastereomers due to the N_β chirality. Therefore, it seems obvious that after ring opening at higher pH this stereocentre still exists, because of the fixation of N_β in two ring systems which could lead to a restricted inversion at N_β.

Indirect evidence for this assumption is provided by the HETCOR NMR spectrum of the reduced prajmaline (N_β-propyl-dihydro-chanoajmaline). In this spectrum both CH 24 protons show different ppm values indicating a diastereotopic methylene group (data not shown). For that reason the stereocentre is again expected to be at N_β, because of the restricted inversion.

In conclusion, at physiological pH values of about pH 8–9 in the small intestine [8] prajmaline exists in the ring-opened more lipophilic form **2** and not in the highly polar ring-closed form **1**. This result is most probably one important reason to explain the better absorption and bioavailability of Neo-Gilurymal® when compared to ajmaline.

3. Experimental

3.1 Materials and reagents

Acetonitrile, 1-propyliodide and the TLC plates Si 60 $_{F254}$ were obtained from Merck (Darmstadt, Germany). Ajmaline was obtained from Roth (Karlsruhe, Germany) and the deuterated NMR solvents from Deutero (Kastellaun, Germany)

3.2 Synthesis of N_β-propyl-ajmaline-bitartrate

The synthesis of PBT was carried out as described by Keck [3, 4] but 1-propyliodide was used instead of 1-propylbromide for quaternisation.

3.2.1. Synthesis of N_β-propyl-ajmaline-iodide

1-Propyliodide (2 ml) was added to 2 g ajmaline (6.1 mmol) in acetonitrile (20 ml) and stirred under reflux for 7 h yielding white crystals. The product was washed with chloroform and ether and finally recrystallized from methanol/ether to give 2.4 g (4.8 mmol) of N_β-propyl-ajmaline-iodide.

3.2.2. Synthesis of N_β-propyl-ajmaline-bitartrate

N_β-propyl-ajmaline-iodide (2.4 g, 4.8 mmol) was suspended in an aqueous solution of sodium-bicarbonate and stirred with 500 ml ethyl acetate for 6 h. The organic layer was separated and evaporated to dryness. The yellow oily residue was dissolved in acetone and treated at 0 °C with 0.7 g (4.9 mmol) L-(+)-tartaric acid in acetone. The crude product was washed with ether and recrystallized from ethanol/ether to give 1.5 g (2.8 mmol) of N_β-propyl-ajmaline-bitartrate.

3.3. HPLC

The HPLC experiments were performed using a Merck-Hitachi System (D2500 Integrator, AS 2000 Autosampler, UV/VIS Detector L 4250 and a L 6200 Gradient Pump) and a LiChrospher® column with a suitable precolumn. The flow rate was 0.5 ml/min and isocratic elution was carried out using a mixture of acetonitrile and phosphate buffer (25 mM, pH 7.2) in a ratio 1 : 1. UV-detection was at 254 nm.

3.4. Spectroscopic methods

3.4.1. Mass spectrometry

Mass spectra were measured on a Finnigan MAT 44 S at 70 eV.

3.4.2. NMR spectroscopy

NMR analyses were performed on Bruker instruments (AM 400, ARX 400 or AC 300) at 400 or 300 MHz for ^{1}H spectra and 100.6 or 75 MHz for ^{13}C spectra. Solvents used were D_2O or DMSO-d_6.

NMR-Data of the carbinol-ammonium-ion (**1**): ^{1}H-NMR (D_2O, 300 MHz): δ [ppm] 7.62, (d, 1 H, $J_{H\text{-}9/H\text{-}10} = 7.2$, H-9); 7.41.(dd, 1 H, $J_{H\text{-}11/H\text{-}12} = 7.9$, $J_{H\text{-}11/H\text{-}10} = 7.5$, H-11); 7.06 (dd, 1 H, $J_{H\text{-}10/H\text{-}9} = 7.2$, $J_{H\text{-}10/H\text{-}11} = 7.5$, H-10); 6.97 (d, 1 H, $J_{H\text{-}12/H\text{-}11} = 7.9$, H-12); 4.80 (s, 1 H, H-21); 4.60 (s, 1 H, H-17); 4.08 (d, 1 H, $J_{H\text{-}3/H\text{-}14B} = 10$, H-3); 4.01 (td, 1 H, $J_{H\text{-}24B/H\text{-}23} = 9.1$, $J_{H\text{-}24B/H\text{-}24A} = 4.2$, H-24B), 3.86 (t, 1 H, H-5); 3.58 (td, 1 H, $J_{H\text{-}24A/H\text{-}23} = 9.1$, $J_{H\text{-}24A/H\text{-}24B} = 4.2$, H-24A); 3.02 (s, 1 H, H-2); 2.88 (s, 3 H, N_α-CH_3); 2.81 (m, 2 H, H-15, H16); 2.38 (m, 3 H, H-6A, H-6B, H-14); 2.10 (m, 2 H, H-20, H-14); 1.85 (m, 4 H, H-23, H-19); 1.12 (t, 3 H, $J_{H\text{-}18/H\text{-}19} = 7.3$; H-18); 1.02 (3H, t, $J_{H\text{-}22/H\text{-}23} = 7.3$, H-22)

^{13}C-NMR (D_2O, 75 MHz): δ [ppm] 156.0 (C-13); 133.9 (C-8); 131.7 (C-11); 126.4 (C-9); 123.6 (C-10); 113.5 (C-12); 93.2 (C-21); 79.9 (C-2); 78.8 (C-17); 61.8 (C-5); 57.8 (C-7); 57.2 (C-24); 57.0 (C-3); 51.8 (C-20); 49.3 (C-16); 36.8 (N_α-CH_3); 33.4 (C-6); 32.9 (C-14); 29.4 (C-15); 27.5 (C-19); 17.34 (C-23); 13.9 (C-18); 13.0 (C-22).

NMR-data of the aldehyde-amine (**2**) (mixture of isomers): ^{1}H-NMR (DMSO-d_6, MHz 300): δ [ppm] 9.44 (d, 1 H, $J_{H\text{-}21/H\text{-}20} = 4.4$, H-21); 9.38 (d, 1 H, $J_{H\text{-}21/H\text{-}20} = 4.4$, H-21), 7.38 (d, 1 H, $J_{H\text{-}9/H\text{-}10} = 7.2$, H-9); 7.41 (dd, 1 H, $J_{H\text{-}11/H\text{-}12} = 7.8$, $J_{H\text{-}11/H\text{-}10} = 7.6$, H-11); 7.02 (q, 2 H, H-10, H-12), 5.20 (d, 1 H, $J_{OH\text{-}17/H\text{-}17} = 4.3$, OH-17), 5.16 (d, 1 H, $J_{OH\text{-}17/H\text{-}17} = 4.3$, OH-17); 3.88 (d, 1 H, $J_{H\text{-}17/OH\text{-}17} = 4.3$, H-17); 3.24 (d, 1 H, $J_{H\text{-}3/H\text{-}14B} = 8.6$, H-3); 3.04 (m, 1 H, H-5), 2.72 (m, 1 H, H-24B); 2.62 (s, 3 H, N_α-CH_3); 2.39 (m, 4 H, H-24A, H-16, H-14B, H-2); 2.07 (m, 1 H, H-15); 1.87 (d, 1 H, $J_{H\text{-}6B/H\text{-}6A} = 11.5$, H-6B); 1.66 (m, 7 H, H-23, H-20, H-19, H-14A, H-6A); 0.83 (m, 6 H, H-22, H-18).

^{13}C-NMR (DMSO-d_6, 75 MHz): δ [ppm] 204 (C-21); 154.8 (C-13); 134.5 (C-8); 126.9 (C-11); 123.5 (C-9); 118.9 (C-10); 109.1 (C-12); 80.8 (C-17); 76.9 (C-2); 59.2 (C-5); 54.3 (C-24); 54.0 (C-7); 50.8 (C-16); 47.8 (C-3); 47.5 (C-20); 34.5 (N_α-CH_3); 33.6 (C-15); 29.9 (C-14); 28.6 (C-6); 20.9 (C-19); 20.8 (C23); 12.3 (C-18); 11.8 (C-22)

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