3.3 Neural network method

The neural network employed in the presented experiments had the following architecture (Fig. 1): five input "neurons" characterised the drug molecule; number of carbon atoms in alkoxy-substitution in lipophilic part of molecule, position of this substitution, number of carbon atoms in alkoxy-substitution in connective chain, type of nitrogen containing substituent in hydrophilic part of molecule and LC chromatographic capacity factors. To provide good generalisation it is desirable to use the smallest number of hidden "neurons" that give satisfactory training performance. The optimal number of hidden "neurons" was two (according to the correlation index between calculated and measured data). The output was the logarithm of activity in surface anaesthesia. The program of neural network was written in Turbo-Pascal 7.0.

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Dr. J. Lehotay
Radlinského 9
81237 Bratislava
Slovakia

Institut für Pharmakognosie[1] der Leopold-Franzens-Universität, Innsbruck, und Institut für Analytische Chemie[2], Universität Wien, Austria

Fundamentals and predictions of resolution of enantiomer mixtures by crystallization in the example of phase diagrams of atenolol and atenolol hydrochloride salt

A. Burger[1], J. M. Rollinger[1] and W. Lindner[2]

Dedicated to Prof. G. Heinisch, Innsbruck, on the occasion of his 60th birthday

Enantiomers as well as racemates of atenolol and atenolol hydrochloride were investigated thermoanalytically (thermomicroscopy, DSC, TGA), by X-ray diffractometry and spectroscopy (FTIR, FTRaman). The binary phase diagrams ((*R*)-, (*S*)-) of both substances were constructed and are used in the following discussion to consider the possibilities of separating enantiomers by direct crystallization. While a solid solution according to Roozeboom type I is formed between (*R*)- and (*S*)-atenolol, the hydrochloride crystallizes as a solid solution according to Roozeboom type II, whereby an enantiomer enrichment can be achieved.

1. Introduction

Atenolol INNp, 2-[4-(2-hydroxy-3-isopropylaminopropoxy)phenyl]acetamide, represents a β_1-selective adrenoceptor antagonist (e.g. Tenormin®) and is one of the most widely used beta-blockers. Ninety percent of them are sold as racemic mixtures [1] consisting of equal moles of the (*S*)- and (*R*)-enantiomers, although there is a clinical advantage in administration of optically pure (*S*)-enantiomers relative to administration of the racemates [2]. Mainly the (*S*)-enantiomer of atenolol has hypotensive activity and activity on bradycardia [3, 4]. The administration of the inactive enantiomer will not substantially increase the desired pharmacological response but may unnecessarily increase the toxicity and adverse side effects. This has resulted in much interest in the production, isolation and purification of (*S*)-atenolol [5–7].

Asymmetric or stereoselective synthesis suffers either from low yield or insufficient enantiomeric excess rates. Separation of the enantiomers via diastereomers or diastereomeric salts is an expensive and time-consuming process that is also accompanied by technical problems. Atenolol shows a very small difference in the solubility between racemate and enantiomer [8]. Therefore, it is difficult to isolate the optically active atenolol utilizing the difference in solubility. Through, the formation of salts of atenolol with different Brønsted's acids, this difference could be increased and hence a method for isolation and purification of enantiomerically enriched atenolol could be achieved [8].

The aim of this work was to investigate the conditions underlying this behaviour, such as melting reaction and stability in the binary systems of (*R*)- and (*S*)-atenolol as well as (*R*)- and (*S*)-atenolol hydrochloride. The importance of resolution of racemates for the commercial production of enantiomerically pure chiral substances has recently been reported by Li and Grant [9]. To follow the route of crystallization prior knowledge is needed including the curve course of the enantiomers in the binary system and their thermodynamical stability in case of polymorphism [10, 11]. This information determines those parameters which can be appropriately obtained by construction of the binary phase diagram. Its curve course

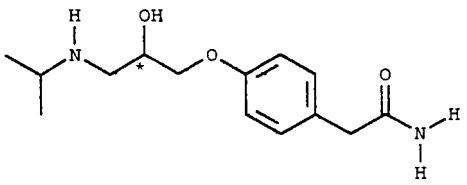

Table: Physicochemical data of atenolol hydrochloride

	Atenolol		Atenol hydrochloride	
	(*RS*)-	(*R*)-	(*RS*)-	(*R*)-
Melting point (°C), TM[a]	154	154	148–158	142–149
Melting point (°C), DSC onset	154.3	153.9	147–149	144–145
Heat of fusion (kJ mol^{-1})[b]	38.6 ± 0.9	39.7 ± 1.1	36.4 ± 2.0	38.2 ± 4.6
Entropy of fusion (J mol^{-1} K^{-1})	90.2	93.0	86.4	91.5
Specific rotation[c] (°), $[\alpha]_D^{20}$	0	+16.1	0	+14.4

[a] TM thermomicroscopy, [b] ± 95% c.i., [c] 1% in hydrochloric acid (c = 1 mol $\cdot l^{-1}$)

gives information about its belonging to one of the three basic types of racemates [12, 13]. In addition, the solubility profile can be found even without evaluation of a ternary solubility diagram, because it is strongly determined by its heats of fusion and melting points [14, 15].
In this way, proceedings to resolve an enantiomeric pair can be predicted. On the other side time-consuming solubility and crystallization experiments can be omitted or be systematically performed by making suitable salts of amines or of acids.

2. Investigations and results

2.1. Identification and characterization of the substances

2.1.1. (RS)- and (R)-Atenolol

DSC-onset temperatures of the melting peaks of racemate and enantiomer show an approximately identical m.p. (Table). Their melting equilibrium determined by thermomicroscopy is 154 °C. Residual crystals grow to rods and plates. Because X-ray diffractograms, IR- and Raman-spectra are identical, only one of them in each case is shown in Figs. 1–3.

2.1.2. (RS)- and (R)-Atenolol hydrochloride

(*RS*)- and (*R*)-atenolol hydrochloride show a broad melting range when heated by thermomicroscopy (Table). Both substances melt under decomposition (formation of bubbles) and crystallize by cooling at about 144 °C in the form of needles, small rods and rosettes. Repeated heating of the crystal film decreases the m.r. by about 4 K. The same melting behaviour results when residual crystals of the primary product remain in the melt, which are melted again after complete crystallization. By this means, the existence of a second lower-melting crystal form as a reason for the deviating melting interval can be excluded, but is due to the formation of decomposition products. Thermogravimetrical measurements confirmed their formation at temperatures higher than 120 °C. Also the presence of a solvate as reason for the weightloss during heating could be excluded by FTIR-microscopy. Contrary to (*RS*)- and (*R*)-atenolol, the DSC-curves of the hydrochlorides show no clear, but broad melting peaks. The IR- and Raman-spectra as well as X-ray diffractograms of the racemic and of the (*R*)-form of the hydrochloride differ in slight shiftings and intensities (Figs. 1–3).

2.2. Phase diagrams

Any mixture is governed by the phase rule and can therefore be characterized by evaluation of the binary phase diagram that relates the composition to the m.p. [16]. In case of enantiomers, the two participants of the phase diagram exhibit completely equivalent physical and chemical properties apart from their optical rotation. Consequently, the diagram is characterized by symmetry. Therefore, it is sufficient to make mixtures of racemate and just one of the enantiomers.
The melting behaviour of the two binary systems was examined with quantitative mixtures of (*RS*)- and (*R*)-atenolol as well as (*RS*)- and (*R*)-atenolol hydrochloride using the DSC-method. Racemization of (*R*)-atenolol and (*R*)-atenolol hydrochloride could be excluded by determi-

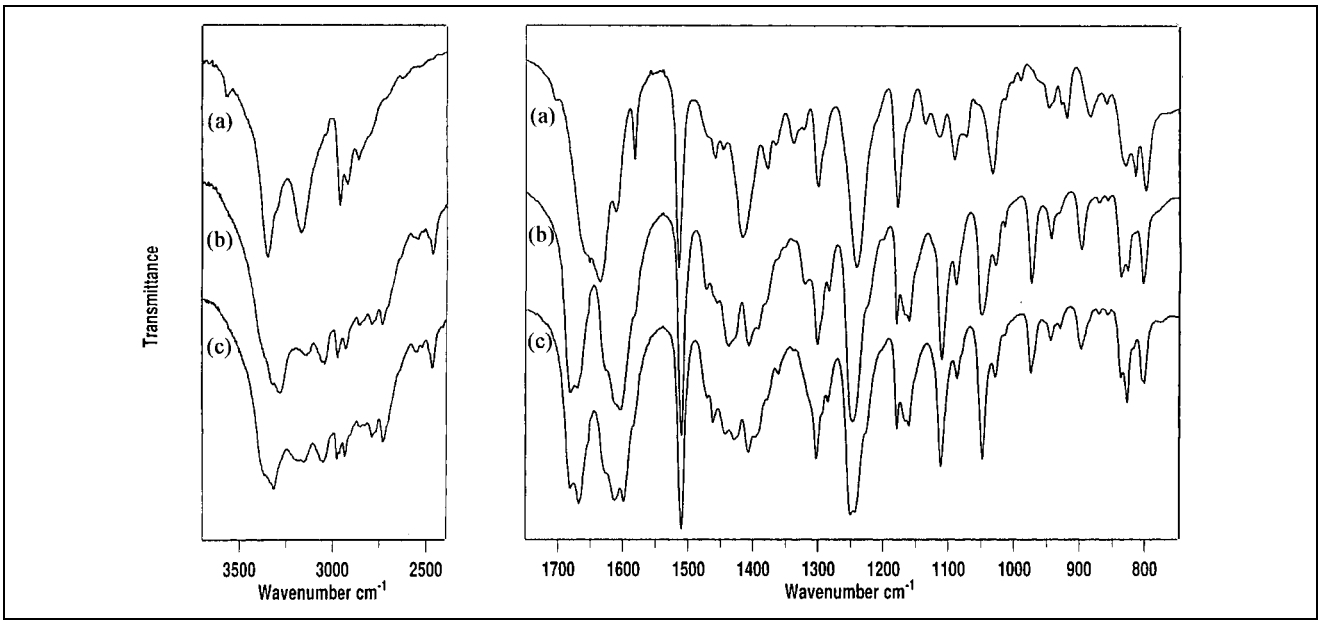

Fig. 1: FTIR spectra of (a) (*R*)-atenolol, (b) (*RS*)- and (c) (*R*)-atenolol hydrochloride

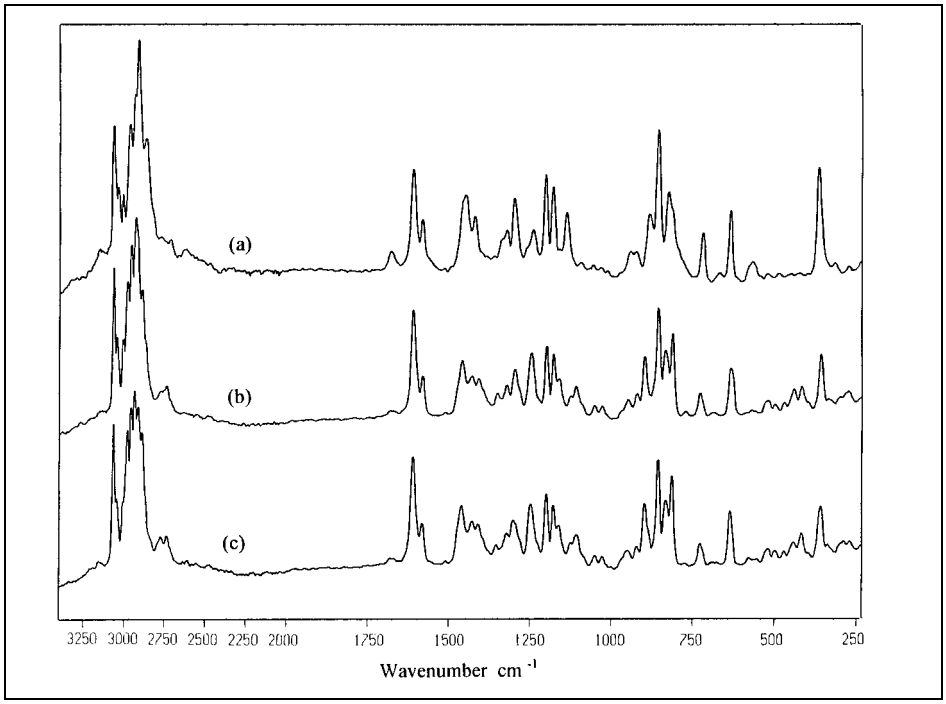

Fig. 2:
Raman spectra of (a) (*R*)-atenolol, (b) (*RS*)- and (c) (*R*)-atenolol hydrochloride

nation of their optical rotation before and after heating up to 140 °C for 30 min. Under these conditions the decomposition of (*R*)-atenolol hydrochloride was determined by TGA and lies by about 1% formation of volatile decomposition products. As the crystalline samples were used for investigation this behaviour could not significantly influence the results of the optical rotation. These nearly remained the same before and after heating, for (*R*)-atenolol and (*R*)-atenolol hydrochloride, respectively (Table).

2.2.1. (RS)- and (R)-Atenolol

Onset-temperatures (tangent method) as well as the peak temperatures were used to construct the phase diagram of atenolol. From Fig. 4 it can be deducted that the racemate and the enantiomer of atenolol can be mixed with each other in any relation without any forming of an eutecticum. The system establishes thus a continuous series of mixed crystals (solid solution). According to Bacchius-Roozeboom [12], three types of solid solutions can be distinguished, depending on the shape of melting curves. Since all mixtures of (*R*)- and (*RS*)-atenolol melt in the same temperature range as do the pure enantiomers, such a binary system can be identified according to Roozeboom type I. This behaviour could also be demonstrated by Kofler's contact method [17]. Repeated heating of the same DSC-pans (second run, peak temperatures) leads to analogous results (Fig. 4).

2.2.2. (RS)- and (R)-Atenolol hydrochloride

On account of the thermal decomposition the peak temperatures of quantitative mixtures of (*R*)- and (*RS*)-atenolol hydrochloride crystallized from ethanol are depicted in Fig. 5. All values obtained from this binary system show broad intervalls and are questionable due to its chemical

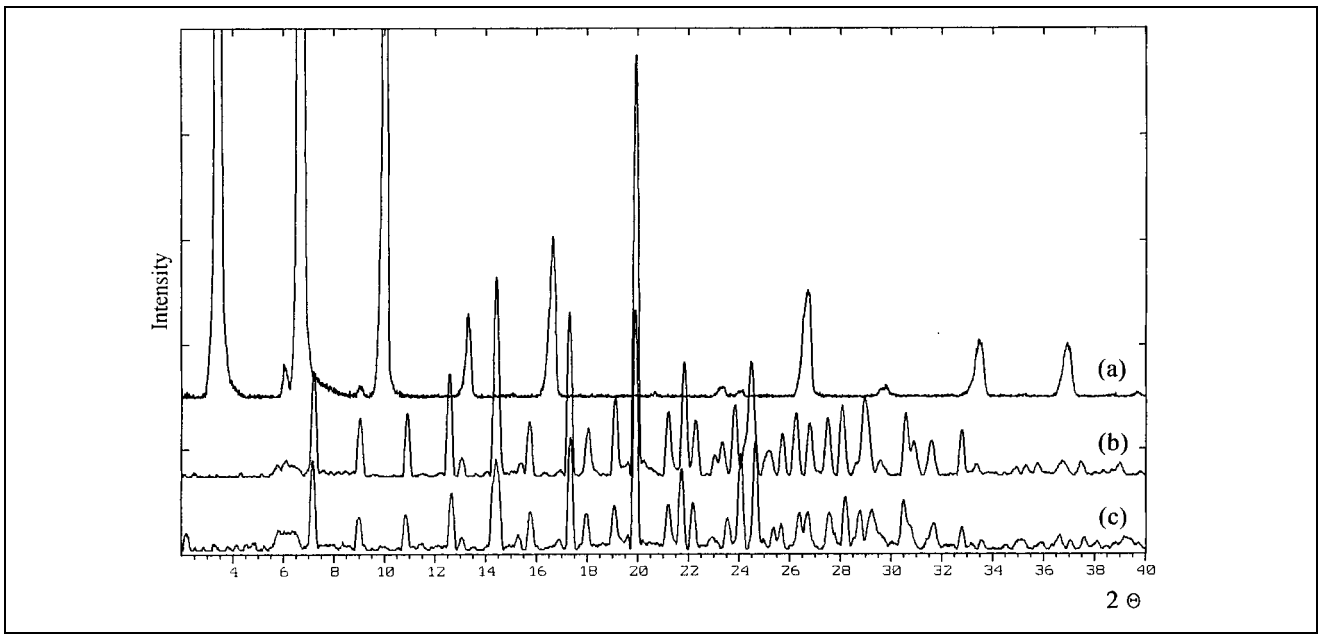

Fig. 3: X-ray diffractograms of (a) (*R*)-atenolol, (b) (*RS*)- and (c) (*R*)-atenolol hydrochloride

instability. The data given in the Table should exclusively serve to comprehend the approximate course in the phase diagram of (*R*)- and (*S*)-atenolol hydrochloride. The absence of an eutecticum can obviously be seen in Fig. 5. Again, the formation of a solid solution can be observed, which shows a distinct m.p. maximum against the 50%-axis, according to Roozeboom type II, contrary to the free base of atenolol. The difference of the m.p. of racemate and enantiomer comes to about 4 K, whereas the heats of fusion are independent of the composition. From the Table it can be seen that these values do not differ significantly. The isomorphous growth [18] determined by Kofler's contact method [17] shows a continuous crystallization in the direction from racemate to the (*R*)-form and confirms this result.

3. Discussion

Most of the chiral compounds show distinctive differences in solubility between their racemates and enantiomers. In this instance, certainly the easiest method of separation of nonracemic mixtures of enantiomers is direct crystallization.

According to current knowledge, only 5–10% of all chiral organic compounds crystallize as a conglomerate and 90% as racemic compounds. The formation of solid solutions – as described for atenolol and the atenolol hydrochloride salt – is relatively rare [19]. This enantiomer behaviour is also known of camphor, camphene [20] and other derivates of camphor, such as camphorquinone [21] as well as for pantolactone [22], etc. The most favoured case occurs if a compound crystallizes as a conglomerate. Then the greatest difference in solubility between racemate and enantiomer exists and enantiomer separation by direct crystallization represents the most efficient and economical route [23].

In the case of a racemic compound or solid solution, the resolution will require derivatization procedures, the use of chiral reagents or chiral chromatography-methods which call for expensive materials and/or valuable time. Another possibility is to form a new chemical substance by derivatization, whereby again the chance of forming a conglomerate exists. By means of forming a salt, this probability increases about 2 to 3 times in comparison with covalent racemates [24]. The hydrogen-bond networks in the crystal structures of the salts of chiral primary amines and their importance in the formation of conglomerates have recently been reported by Kinbara et al. [25]. A successful application of this phenomenon was demonstrated in the formation of hydrochloride salts of bevantolol and propranolol [26]. Whereas the phase diagrams of the free bases describe a racemic compound in the racemic mixture, their hydrochloride salts form a conglomerate.

As a result, this method was applied in the case of (*R*)- and (*S*)-atenolol, because the phase diagram described here testifies to a solid solution, according to Roozeboom type I (Fig. 4). Therein the mixtures have the same solubility in all proportions and cannot be separated by crystallization in any direction. Furthermore, they can neither be distinguished with thermoanalytical methods (DSC, thermomicroscopy) nor spectroscopically; these methods are also useless for determination of the optical purity and enantiomeric excess, respectively.

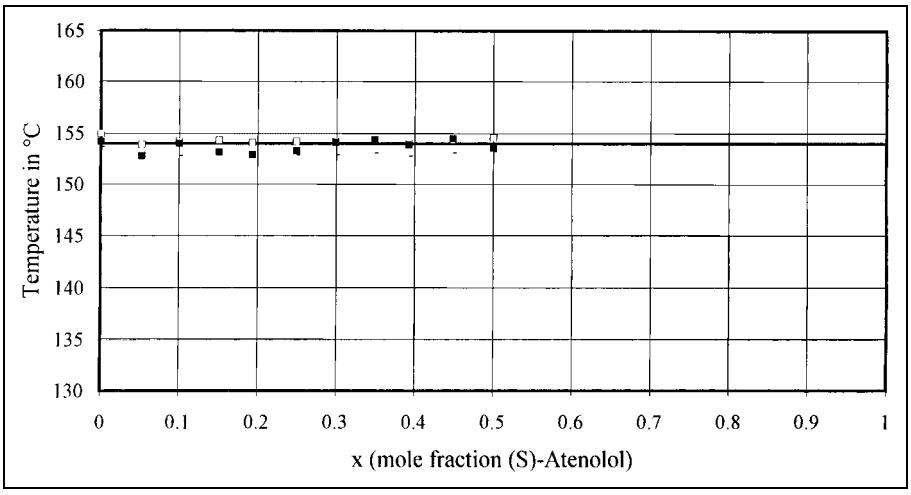

Fig. 4:
Binary (m.p.) phase diagram of (*R*)-, (*S*)-atenolol: —— ideal curve course according to Roozeboom type I using the mean value of the DSC-peak temperatures, DSC-data: – onset temperature of the 1st run, □ peak temperature of the 1st run, ■ peak temperature of the 2nd run

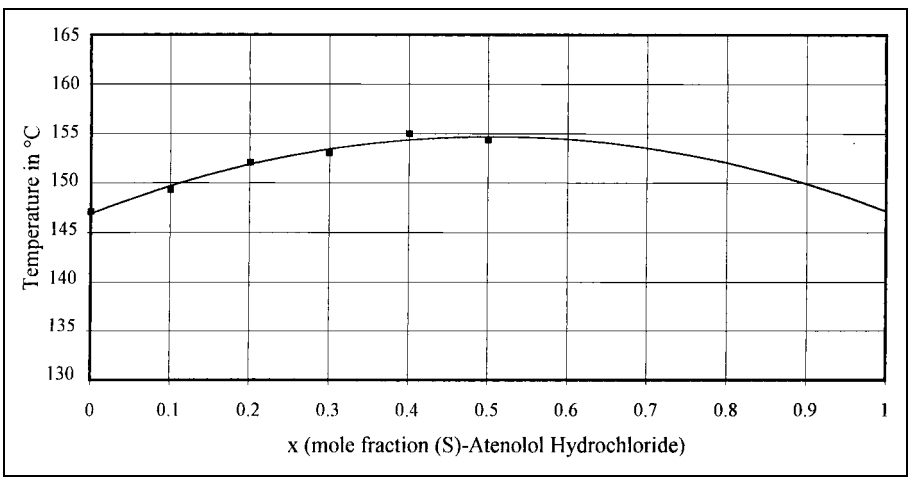

Fig. 5:
Binary (m.p.) phase diagram of (*R*)-, (*S*)-atenolol hydrochloride: —— ideal curve course according to Roozeboom type II. The curve was fitted with the method of least squares using the DSC-peak temperatures, DSC-data: ■ peak temperature of the mixtures of (*R*)- and (*RS*)-atenolol hydrochloride obtained from ethanol

By means of forming a salt of atenolol with hydrochloric acid no conglomerate could be achieved. The phase diagram of atenolol hydrochloride (Fig. 5) shows the forming of a solid solution at all concentrations with a m.p. maximum, according to Roozeboom type II. For example, (*R*)- and (*S*)-kawain [27]), atropine ((*R*)- and (*S*)-hyoscyamine) [28] and also pindolol [26] belong to this type. In these binary systems the intermolecular interaction of molecules is more or less raised against the 50%-axis. This instance expresses itself in the difference of the m.p. of enantiomer and racemate (see ideal curve course in Fig. 5) as well as in the slight but significant differences of FTIR-, Raman spectra and X-ray diffractograms (Figs. 1–3). The continuous alteration could be pursued in mixtures of (*R*)- and (*RS*)-atenolol hydrochloride, according to 0, 10, 20, 30, 40 and 50% (*S*)-atenolol hydrochloride, by FTIR and Raman (data not shown). In Fig. 1 (spectra (b) and (c)) this fact can be observed in the variance of the position of the hydrogen valence bands by a wave number of about 3300 cm^{-1} as well as in changes of intensities of the carbonyl valence bands of the primary amide (1684 cm^{-1} and 1668 cm^{-1}) and of the bands of the two adjacent aromatic C–H (837 cm^{-1} and 826 cm^{-1}). The differences in solubility are also due to this behaviour, which permits an enantiomer enrichment [8], although this binary system does not represent a suitable solution for separation an enantiomer, merely a possibility for its enrichment.

An aspired aim is the production of salts of atenolol, which form a conglomerate. This character facilitates an easy way of resolution. Characterizing the crystalline nature of the racemate type is achieved most efficiently through evaluation of the binary phase diagram. Its knowledge leads to the selection of promising salts, which consequently may lead to resolution methods that are reasonable and economical.

4. Experimental

4.1. Materials and solvents

(*RS*)-Atenolol was purchased from Schweizerhall (Basel, Switzerland). The respective enantiomers were separated via diastereomeric monoesters of tartaric acid as described in [29]. The racemic and enantiomeric atenolol hydrochloride salts were gained by respectively solving in methanol with hydrochloric acid 1% (excessive hydrochlorid acid about 2 $mol \cdot l^{-1}$). Methanol was evaporated and the residue recrystallized from ethyl acetate. All solvents used were of analytical grade.

4.2. Instruments and methods

4.2.1. Thermoanalysis

Kofler-hot-stage microscope: Thermovar® (Reichert, Vienna, Austria). Kofler-hot-bench: (Reichert, Vienna, Austria) for the preparation of Kofler's contact preparates.

Differential scanning calorimetry: DSC-7 (Perkin-Elmer, Norwalk, Ct., USA); Aluminium-sample pans (25 µl), sample mass each about 2 ± 0.0005 mg (ultramicroscales UM 3, Mettler, CH-Greifensee, Switzerland). Nitrogen 4.0 was used as purge gas (20 $ml \cdot min^{-1}$). Registration of the DSC-signals by means of EDP (7 series/UNIX DSC-7 Lab system, Perkin-Elmer). The general heating rate was 5 $K \cdot min^{-1}$. The calibration of the temperature axis was carried out with caffeine (m.p. 236.2 °C, tight closed sample capsule) and with benzophenone (m.p. 48.0 °C, perforated sample capsule), the enthalpy calibration of the DSC-Signal with indium 99.999% (Perkin-Elmer, Norwalk, Ct., USA).

Thermogravimetry: TGA-7 thermogravimetric system (Perkin-Elmer, Norwalk, Ct., USA), sample mass about 2 ± 0.0005 mg, aluminium-sample pan in platinum pan, nitrogen 4.0 was used as purge gas (20 $ml \cdot min^{-1}$), heating rate 10 $K \cdot min^{-1}$. Mass calibration with 100-mg-calibration weight (Perkin-Elmer), temperature calibration with benzophenone (m.p. 48.0 °C) and caffeine (m.p. 236.2 °C).

4.2.2. Spectroscopy

FTIR-Spectroscopy: Bruker IFS 25 FTIR spectrometer (Bruker Analytische Messtechnik GmbH, Karlsruhe, Germany) connected with a Bruker FTIR-microscope (15 × Cassegrain-objective and visible polarization). Samples were scanned as potassium bromide disks at an instrument resolution of 2 cm^{-1} (50 interferograms) or rolled on a zinc selenide window (13 × 1 mm) at an instrument resolution of 4 cm^{-1} (100 interferograms). Spectral range 4000 to 400 cm^{-1}.

Raman-Spectroscopy: Bruker IFS 88 (Bruker Analytische Meßtechnik GmbH, Karlsruhe, Germany), Raman modul FRA 106 (300 mW Nd: YAG Laser at 1064 nm). Spectra were recorded over the range 4000 to 100 cm^{-1}. The powder samples were packed into small aluminium cups. Spectral resolution 4 cm^{-1} (100 interferograms).

4.2.3. Powder X-ray diffractometry

X-ray diffractometer: Siemens D-5000, Diffrac/AT with θ/θ-goniometer (Siemens AG, Karlsruhe, Germany), using monochromatic CuK_α-radiation (at a acceleration rate of 40 kV, tube current 40 mA), rotation of the sample during the measurement, szintillation counter, angle range 2° to 40° 2θ at a rate of 0.005° 2θ s^{-1}. Atenolol samples were pressed into the cavity of a sample holder and smoothed with a glass side. Atenolol hydrochloride samples have been recorded by means of a one crystal sample carrier (Siemens AG, Karlsruhe, Germany) on account of little substance, therefore less quality (Fig. 3 (b) and (c)).

4.2.4. Polarimetry

Polarimeter: Perkin Elmer Polarimeter 341 (Überlingen, Germany) using the microcell (pathlength 100 mm, volume 1 ml). Measurements were carried out by 20 °C, wavelength 589 nm. Substances were solved 1% in hydrochloric acid (c = 1 mol/l).

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Prof. Dr. Artur Burger
Institut für Pharmakognosie
Innrain 52
A-6020 Innsbruck
Austria
Artur.Burger@uibk.ac.at