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Assignment of Absolute Configuration and Optical Purity Determination of (R)- and (S)-Econazole Nitrate by Enantioselective HPLC: Method Development and Application

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ABSTRACT A method is described for the synthesis and optical purity determination of (−)-(R)- and (+)-(S)-econazole via the optically pure intermediates, (R)- and (S)-imidazolylethanol, which are available by chromatographic resolution or by fractional crystallization of diastereomeric *O,O'*-disubstituted (R*;R*)- or (S*;S*)-tartaric acid monoesters of the parent imidazolylethanol racemate. Furthermore, this method allows the chromatographic assignment of the absolute configuration of the chiral center of the imidazolylethanol enantiomers and consequently of econazole enantiomers. In addition, a direct liquid chromatographic enantioseparation method for the determination of the optical purity of (R)- and (S)-econazole and other chiral imidazoles on a protein type CSP (OVM) is described and applied to confirm chromatographically the absolute configuration evaluations.

KEY WORDS: indirect and direct enantioseparation, chromatographic assignment of absolute configuration, econazole, miconazole, imidazolylethanol, protein type CSPs (OVM)

Racemic (±)-econazole nitrate, (±)-1-{2-[(4-chlorophenyl) methoxy]-2-(2,4-dichlorophenyl)ethyl}-1*H*-imidazole (±)-**2**, a widely used antifungal drug, is prepared from the intermediate imidazolylethanol (±)-**1**, which is the synthon of a series of azole-antimycotica[1] (see Fig. 1). The single enantiomers, however, are needed for up to date pharmacodynamic and pharmacokinetic studies. Recently, (+)- and (−)-econazole was synthesized by repetitive diastereomeric crystallization employing (2R*;3R*)- and (2S*;3S*)-tartaric acid as chiral auxiliary agents, and the assignment of the absolute configuration of (+)- and (−)-econazole has been achieved by X-ray crystal structure analysis.[2] Thus, the (S)-configuration has been assigned to the (+)-isomer and the (R)-configuration to the (−)-isomer. However, a direct liquid chromatographic enantioseparation for the optical purity determination of econazole enantiomers has not been published yet, although for the enantiomers of some structurally related compounds such as tioconazole,[3] SK&F 96365,[4] diniconazole, and uniconazole[5–8] methods can be found in the literature.

In this study, optical purity determination of (R)- and (S)-econazole was achieved by an "indirect" and a "direct" approach. First we synthesized the optically pure intermediate imidazolylethanols (+)- and (−)-**1** and thereof the (+)- and (−)-econazole enantiomers. This was accomplished by resolving the racemic intermediate **1** to the respective enantiomers applying the very general method for separating aminoalkanols via diastereomeric tartaric acid monoesters[9] to give the enantiomerically pure chiral synthons (+)- and (−)-**1**. The absolute configuration of the prepared chiral synthons was assigned by chromatographic means[10] and thus also the absolute configuration of the final products [e.g., (R)- and (S)-econazole] could be determined. Finally, an HPLC method for the direct chromatographic optical purity determination of (R)- and (S)-econazole, the intermediate imidazolylethanol, (R)- and (S)-**1**, and some other related imidazoles has been developed. These various enantioselective HPLC assays have been used to confirm the absolute configuration data which have also been validated by the data taken from the X-ray crystallography study.[2]

MATERIALS AND METHODS

Materials

Racemic (±)-econazole nitrate and (±)-miconazole nitrate were purchased from Sigma Chemicals Co. (St. Louis, MO). According to ref. 1 the synthesis of rac-**1** and of econazole enantiomers was performed employing 2,2',4'-trichloroacetophenone (97%), imidazole (99%), sodium borohydride pellets (98%), sodium hydride (80% dispersion in mineral oil), and 4-chlorobenzyl chloride (98-99%) from Aldrich-Chemie (Steinheim, Germany). (+)-(2R*;3R*)-, (−)-(2S*;3S*)-Tar-

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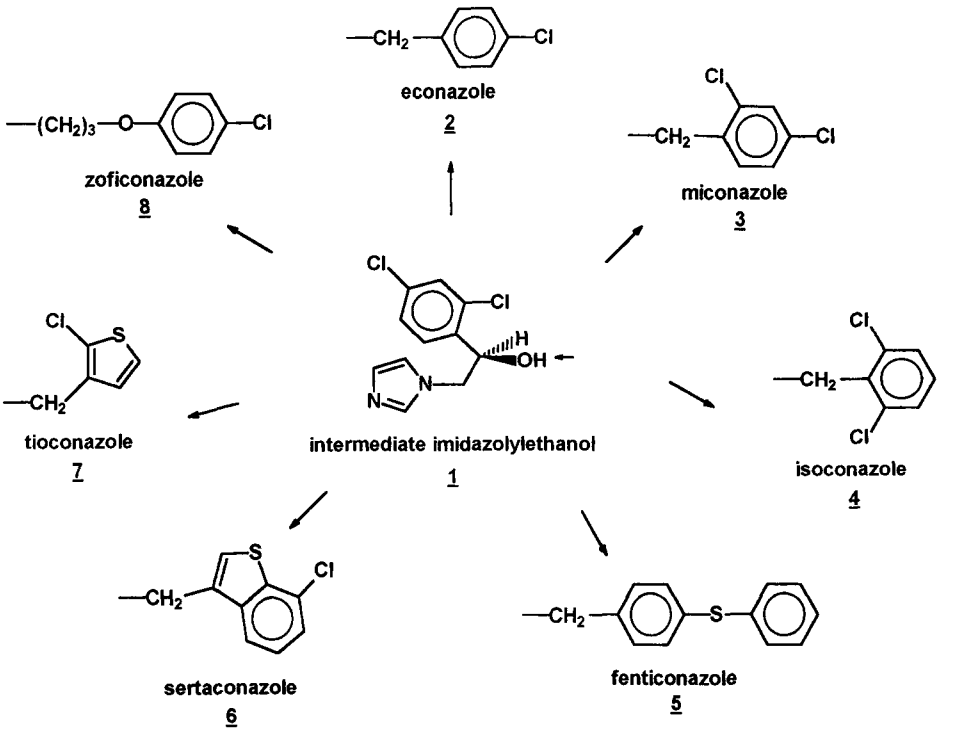

Fig. 1. The intermediate "imidazolylethanol", 1-(2,4-dichlorophenyl)-2-(1-imidazolyl)-ethanol **1** as chiral synthon of various imidazole type antimycotica (**2–8**); if optically pure it could serve as "chiral pool." (The short arrow denotes the substituent position for etherification with different aralkylhalogenids.)

taric acid (>99.5%) and (−)-(2S*;3S*)-*O,O'*-dibenzoyl tartaric acid anhydride [(S*;S*)-DBTAAN] were from Ueticon-Chemie (Ueticon, Switzerland), and (+)-(2R*;3R*)-*O,O'*-dibenzoyl tartaric acid anhydride [(R*;R*)-DBTAAN] and (+)-(2R*;3R*)-*O,O'*-diacetyl tartaric acid anhydride [(R*;R*)-DATAAN] were synthesized in our laboratories according to Zetzsche et al.[11] Dimethylformamide (DMF) was obtained from Fluka Chemie (Buchs, Switzerland), and all other chemicals and solvents for the synthesis were of p.a. quality.

Distilled water was purified by a Milli-Q-Plus filtration unit from Millipore and used for the preparation of the buffers of the mobile phases. All organic solvents used for chromatography, acetonitrile (ACN), ethanol (EtOH), methanol (MeOH), 2-propanol (iPrOH), and dichloromethane (DCM) were of HPLC grade and obtained from E. Merck (Darmstadt, Germany). Sodium dihydrogen phosphate and ammonium acetate were of p.a. quality and supplied by E. Merck and Loba Feinchemie (Fischamend, Austria), respectively.

Instrumentation

For enantioseparation of the analytes by the protein type chiral stationary phases (CSPs) the chromatographic system used consisted of an LC-Pump 410 Kontron, Jasco 875 UV detector, HP 3396A Integrator, Rheodyne 7125 injector block with 20 μl loop, and a column thermostat from W.O. electronics (Langenzersdorf, Austria). Separations were performed on a chiral AGP column (120 × 4.6 mm i.d.) from ChromTech AB (Stockholm, Sweden) and on an Ultron ES-OVM column (150 × 4.6 mm i.d.) from Shinwa Chemical Industries (Kyoto, Japan).

Preparative chromatographic resolution and analytical control of the diastereomeric monoesters, (R)- and (S)-1-(2,4-dichlorophenyl)-2-(1-imidazolyl)ethyl-(R*;R*)-*O,O'*-dibenzoyl tartaric acid monoester, were performed on a LiChrosorb Si 60 (7 μm) preparative and analytical LC column, respectively, both supplied by E. Merck (Darmstadt, Germany), with a Hitachi-Merck HPLC sytem consisting of an L-6000 Pump, L-6200 Intelligent Pump, L-4000 UV-Detector, L-4250 UV-VIS Detector, D-6000 Interface, AS-2000A Autosampler, Rheodyne injector block with 3 ml loop, column thermostat from W.O. electronics (Langenzersdorf, Austria) for the analytical column and a circulator from Haake (Berlin, Germany), for the preparative column. The chromatographic assignment of the absolute configuration of the diastereomeric monoesters was carried out on a LiChrospher 60, RP-select B, 5 μm (125 × 4 mm i.d.) reversed-phase column from E. Merck (Darmstadt, Germany) with the same HPLC system as described above.

The pH of the mobile phases (always apparent pH) was measured with an Orion-pH-meter, model 520A. Mobile phases were filtered through a Nalgene nylon membrane filter (0.2 μm) (Nalge Company, New York, NY) and degassed before use. IR spectra were recorded on a Perkin Elmer (Beaconsfield, England) 881 infrared spectrometer and NMR

TABLE 1. Chromatographic separations of diastereomeric (R*;R*)-*O,O'*-disubstituted tartaric acid monoesters of chiral aminoethanol type compounds

Entry	Analyte	C.S.[a]	Cond.	$k'_{(R;R^*;R^*)}$[b]	$k'_{(S;R^*;R^*)}$[b]	α[c]	Elution order
1	**1**[d]	NP	See Fig. 3	1.22	1.85	1.52	(R) before (S)
2	**1**[d]	RP	See Fig. 6A	3.99	3.17	1.26	(S) before (R)
3	Carvedilol[e]	RP	See Fig. 6B	2.17	3.41	1.57	(R) before (S)

[a] C.S., chromatographic system; NP, normal phase; RP, reversed phase.
[b] k' is defined as $t_r - t_0/t_0$.
[c] α is calculated as k'_2/k'_1.
[d] Derivatized with (R*;R*)-DBTAAN.
[e] Derivatized with (R*;R*)-DATAAN; for structure of carvedilol see Figure 6B.

spectra were recorded on a 360 MHz machine, Bruker (Karlsruhe, Germany) AM 360 spectrometer.

"Indirect" Approach for the Separation of the Enantiomers of the Intermediate Imidazolylethanol (±)-1

Derivatization of rac-1 according to ref. 9 Racemic 1-(2,4-dichlorophenyl)-2-(1-imidazolyl)-ethanol (±)-**1** was prepared according to the procedure described by Godefroi et al.[1] and followed by reaction with (R*;R*)-DBTAAN.[9] Thus, 10 mmol (2.6 g) of (±)-**1** and 12 mmol (4.1 g) of (R*;R*)-DBTAAN were suspended in 50 ml of dry dichloromethane (traces of water were distilled off as azeotropic mixture) and the suspension was stirred at 45°C for 4 h. The resulting brown solution was evaporated to dryness under reduced pressure.

Resolution of the resulting diastereomeric monoesters, (R)- and (S)-1-(2,4-dichlorophenyl)-2-(1-imidazolyl)ethyl-(R*;R*)-*O,O'*-dibenzoyl tartaric acid (structure depicted in Fig. 2) The mixture of diastereomeric monoesters has been resolved into the single diastereomers by chromatographic means on a preparative silicagel column (LiChrosorb Si 60—7 μm) using a mixture of dichloromethane, methanol, acetonitrile, and glacial acetic acid (70/23/5/2) (v/v) as eluent. The two fractions containing the diastereomeric monoesters were collected separately and the eluent was removed by vacuum. The analytical control of the two fractions confirmed a diastereomeric excess (de) of 99.2% for the first eluted and 83.8% for the second eluted monoester, respectively, and no further chemical impurities were detected (see Table 1, entry 1 and Fig. 3). However, the purity of the latter compound can be increased by cutting the fractions more precisely, but at the expenses of yield. Crystallization of the first eluted fraction with a mixture of acetonitrile/diethylether gave slightly yellow crystals, while the second eluted fraction remained as a yellow viscous oil and could not at all be crystallized. The absolute configuration of the first eluting compound was determined as (R;R*;R*) according to the rule established by W. Lindner and co-workers.[9,10] The rationale for this rule will be explained later (see Results and Discussion).

Resolution of the mixture of the diastereomeric monoesters by fractional crystallization was feasible but relying on seedling crystals of the optically pure (R;R*;R*)-diastereomer, which have been obtained by the above mentioned preparative chromatographic resolution method. Dissolving the crude reaction product in acetonitrile/diethylether/dichloromethane and adding a spatula of the crystals of the (R;R*;R*)-diastereomer affected crystallization. Crystallization of the (S;R*;R*)-diastereomer could not be achieved. In order to obtain the optically pure crystalline (S)-isomer, (S*;S*)-DBTAAN had to be used as chiral derivatizing agent applying the similar procedure as described above.

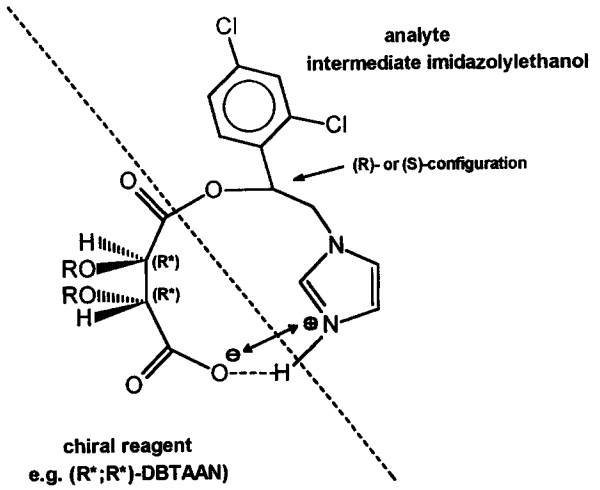

Fig. 2. Postulated twelve membered ring structure of (R)- and (S)-1-(2,4-dichlorophenyl)-2-(1-imidazolyl)ethyl-(R*;R*)-*O,O'*-disubst. tartaric acid monoesters (pair of diastereomers) formed by intramolecular ion-pairing (R could be: benzoyl-, acetyl-, *p*-toluoyl-, benzyl-, etc.).

Physical properties of (R)-1-(2,4-dichlorophenyl)-2-(1-imidazolyl)ethyl-(R*;R*)-*O,O'*-dibenzoyl tartaric acid monoester (first eluting compound from the silica gel column) Yield, 90%. mp 158–160°C. $[\alpha]^{20}_{Na589}$, − 88; $[\alpha]^{20}_{Hg546}$, − 109; (c = 0.884, MeOH/glacial acetic acid = 10/1). IR (KBr), 3431 (m,b), 2900–3180 (multip.,w), 1775 (s), 1727 (s), 1645 (m), 1601 (m) cm^{-1}. ^{1}H-NMR (dDMSO), 8.04 (2H, d), 7.82 (2H, d), 7.75 (2H, m), 7.58 (5H, m), 7.16 (3H, m), 6.97 (1H, s), 6.71 (1H, s), 6.18 (1H, m), 6.14 (1H, d), 5.92 (1H, d), 4.44 (2H, m).

Synthesis of (R)- and (S)-econazole 2 via (R)- and (S)-imidazolylethanol 1 Hydrolysis of the (R*;R*)- and (S*;S*)-*O,O'*-dibenzoyl tartaric acid monoesters of **1**: 2 mmol of the monoester was dissolved in 50 ml methanolic potassium hydroxide (8 mmol) and stirred at 40°C for 3 h. The precipi-

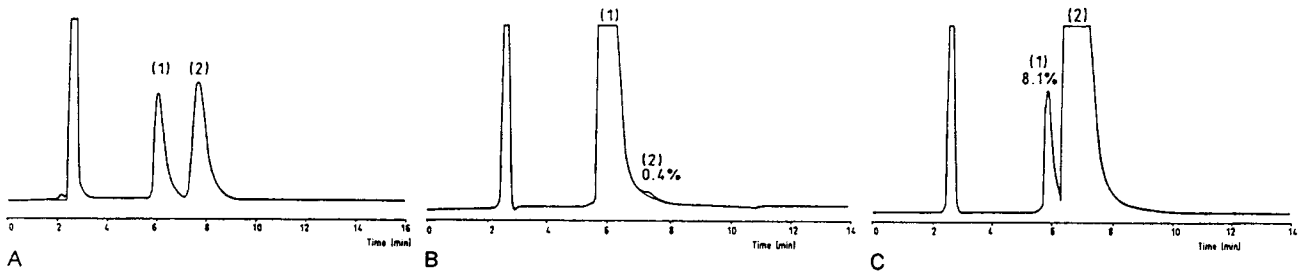

Fig. 3. Resolution of (R)- and (S)-1-(2,4-dichlorophenyl)-2-(1-imidazolyl)ethyl-(R*;R*)-*O,O'*-dibenzoyl tartaric acid monoester. **(A)** Mixture of diastereomers, **(B)** analysis of the chromatographically resolved (R;R*;R*)-fraction, **(C)** analysis of the (S;R*;R*)-fraction. Peak (1), (R;R*;R*)-diastereomer; peak (2), (S;R*;R*)-diastereomer. Chromatographic conditions: stat. phase: LiChrosorb Si 60—7 μm; mob. phase, 70% dichloromethane, 23% methanol, 5% acetonitrile, 2% glacial acetic acid (v/v); flow rate, 1.00 ml/min; temperature, 30°C; detection, UV 230 nm.

tate (potassium tartrate) was filtered off and washed, the combined filtrate evaporated to dryness, the residue suspended in water and extracted with ether. Addition of a slight excess of concentrated HCl to the organic phase led to a precipitate of the enantiomerically pure (enriched) imidazolylethanol [(R)- or (S)-**1**] as HCl salt. IR and NMR spectra were in agreement with those of the racemic compound.

The optical purity of the resulting imidazolylethanol enantiomers, (R)- and (S)-**1**, was determined by derivatization with (R*;R*)-DBTAAN and reanalysis on an analytical scale silica gel column as mentioned above. No racemization was diagnosed in the course of hydrolysis, provided the temperature was controlled not to exceed 40°C. Physical properties of (R)-**1** and (S)-**1** (base) prepared by the above outlined procedure employing the (S*;S*)-reagent: (R)-**1** (ee%: 90.2): mp: 102–106 (base): $[\alpha]^{20}_{Na589}$, − 85; $[\alpha]^{20}_{Hg546}$, − 100 (c = 1.016, MeOH). (S)-**1** (ee%: 98.8): mp: 112–118 (base): $[\alpha]^{20}_{Na589}$, + 88; $[\alpha]^{20}_{Hg546}$, + 110 (c = 1.056, MeOH). By etherification of (−)-(R)- and (+)-(S)-**1** with 4-chlorobenzyl chloride according to the method described by Godefroi et al.[1] (see Fig. 1) (−)-(R)- and (+)-(S)-econazole nitrate **2** with the same degree of optical purity as determined for the corresponding tartaric acid monoesters was obtained.

Alternatively, (−)-(R)- and (+)-(S)-econazole was prepared by resolution via fractional crystallization of the respective (R*;R*)- and (S*;S*)-tartaric acid salts.[2] These reference compounds were also used for the determination of the elution order on the protein type chiral stationary phases and to confirm the results of the chromatographic assignment of the absolute configuration of the chiral synthons (R)- and (S)-**1** and their following products (R)- and (S)-**2**. Physical properties of econazole enantiomers: (R)-**2** nitrate (ee%: 97): mp, 178–184 (decomp.): $[\alpha]^{20}_{Na589}$, − 78; $[\alpha]^{20}_{Hg546}$, − 93 (c = 1.056, MeOH). (S)-**2** nitrate (ee%: 97): mp, 166–172 (decomp.): $[\alpha]^{20}_{Na589}$, + 79; $[\alpha]^{20}_{Hg546}$, + 93 (c = 1.056, MeOH).

RESULTS AND DISCUSSION

"Indirect" Approach

The general technique for resolving "aminoalkanol" type enantiomers involving tartaric acid monoester formation[9] provides a good means to prepare optically pure (+)- and (−)-**1**, to determine the absolute configuration chromatographically[10] and to judge the optical purity of thus prepared chiral synthons (R)- and (S)-**1** (Fig. 3A–C). Based on these chiral intermediates a large number of optically pure azole-antimycotica could be synthesized in a straight forward manner (Fig. 1). The remarkable good separation of the diastereomers is most probably due to a 12-membered ring structure formed by intramolecular ion-pairing of the free carboxyl function and the amino group as it was postulated by W. Lindner et al.[9] in the course of the indirect resolution of β-blockers; later on this hypothesis was confirmed by X-ray crystallographic examinations and NMR data.[12] This general concept could also be adapted for the formulation of the conformation of (R)- and (S)-1-(2,4-dichlorophenyl)-2-(1-imidazolyl)-ethyl-(R*;R*)-*O,O'*-dibenzoyl tartaric acid monoester (see Fig. 2) although crystal structure data of the compounds have not been established yet. Since the aromatic imidazole of the imidazolylethyl tartaric acid monosters is less basic than the secondary aliphatic amine of the "aminoethanols" derived from β-blockers, the intramolecular ion-pairing and thus the ring structure should be somewhat less distinct and consequently a lower chromatographic (dia)stereoselectivity was observed. However, the conformational rigidity of the diastereomers owing to their ring structure results in remarkable differences in the overall lipophilicity and good resolution by crystallization and chromatography (reversed phase and normal phase) is still possible. Working in the reversed phase mode, the pH of the eluent has a great influence on the (dia)stereoselectivity, and it becomes quite clear that the maximum of α-values will be reached within a pH range close to the isoelectric point, which is around pH 5 (see Fig. 4). For more detailed information concerning pH dependence, influence of substituents on the two oxygens of (R*;R*)- and (S*;S*)-tartaric acid (R: e.g., benzoyl, acetyl, etc.), of substituents on the amino function, of chain length (*n*) and of substituents on the chiral center of the aminoalkanol (R_1) (for general structure and substituents see Fig. 5) (see Lindner et al.[9,10]).

Conformational studies of tartaric acid monoesters of aminoalkanols[12] proved that intramolecular ring formation occurs leading to a conformationally rigid cyclic skeleton; this seems to be true for all tartaric acid monoesters of aminoalkanols fitting to the structural model presented in Figure 5. Hence, lipophilicity strongly correlates with the configuration of the

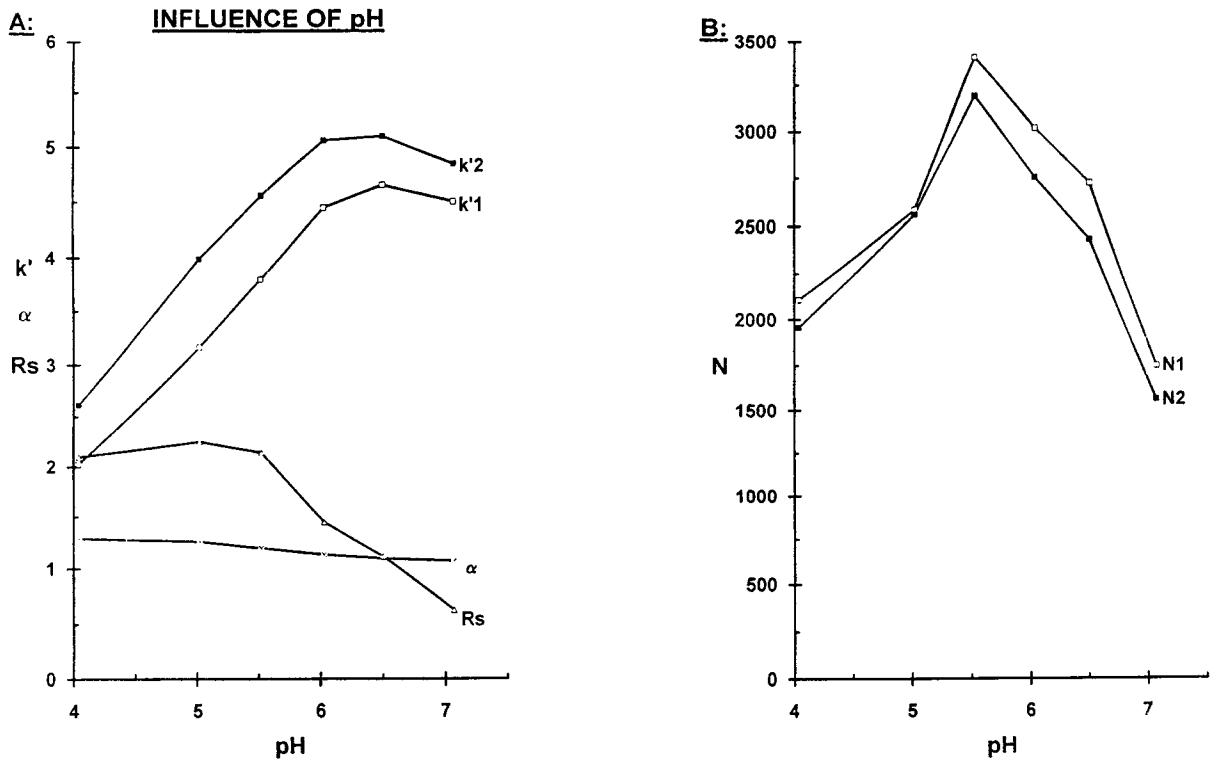

Fig. 4. Influence of pH (apparent pH) on the chromatographic parameters (k', α, R_s, and N) of the resolution of rac-**1** using (R*;R*)-DBTAAN as chiral derivatizing agent. Chromatographic conditions: stat. phase, RP-select B; mob. phase, 60% MeOH/40% 0.1 *M* ammonium acetate; T, 30°C; flow rate, 1.00 ml/min; detection, UV 230 nm. For calculation of k' and α see Table 1. R_s is calculated by the formula: $R_s = 1.18\,(t_{r2} - t_{r1})/(w_{1/2\,1} + w_{1/2\,2})$; $w_{1/2}$ = peak width at half peak height. The plate numbers (N) are calculated by the formula: $N = 5.54\,(t_R/w_{1/2})^2$.

monoester molecules, allowing the chromatographic assignment of the absolute configuration of the chiral center of aminoalkanols by the elution order of its tartaric acid monoesters on a reversed phase system. The conformation of the cyclic skeleton of tartaric acid monoesters with structure and configurations depicted in Figure 5A is the same. They are less lipophilic than their corresponding diastereomers presented in Figure 5B and therefore elute first from a RP column. On the other hand, tartaric acid monoesters with structure and configurations illustrated in Figure 5B have also the same conformation regarding the cyclic skeleton, but different conformations to their corresponding diastereomers in Figure 5A. They are more lipophilic and therefore elute second. Accordingly, considering strictly the Cahn–Ingold–Prelog priority rules the elution order of (R*;R*)-tartaric acid monoesters of aminoalkanols on a reversed phase column is A-1 before B-1 (Fig. 5) and, consequently, (S)-amino alkanol elutes before (R)-amino alkanol, if substituent *b* has higher priority than substituent *c*, e.g., if applying the concept to imidazolylethanol **1** (see Table 1, entry 2 and Fig. 6A), and (R)-aminoalkanol elutes before (S)-aminoalkanol, if substituent *c* has higher priority than substituent *b*, e.g., if applying the concept to β-blockers like carvedilol (see Table 1, entry 3 and Fig. 6B).

It must be borne in mind that this reversal of elution order is not indicative to a change of the overall conformation of the total molecule, but only to a change in the priority of the substituents.

The elution order is reversed, if using (S*;S*)-tartaric acid anhydride as chiral derivatizing agent (see Fig. 5, A-2 before B-2), because (R;S*;S*) is enantiomeric to (S;R*;R*) and S;S*;S*) is enantiomeric to (R;R*;R*) (compare Fig. 5, A-1 with A-2 and B-1 with B-2). The conformation of the corresponding enantiomers is equal, and therefore they are coeluting. On the other hand the conformation of the corresponding diastereomers is different, and therefore separation becomes possible.

The present example deals with the chromatographic separation of diastereomers with different conformation, lipophilicity and polarity—consequently the elution order will also depend on the type of chromatographic system: in normal phase mode the elution order will be reversed compared to the reversed phase mode (compare Table 1, entry 1 and 2). All these relationships should be illustrated by Figure 5.

"Direct" Chromatographic Enantioseparation of Compounds 1, 2, and 3

Due to the basic character of the analytes we selected as first choice the two protein type CSPs, chiral AGP and OVM. Both CSPs have acidic properties and are operated at reversed phase conditions. Hydrophobic and ion-exchange type

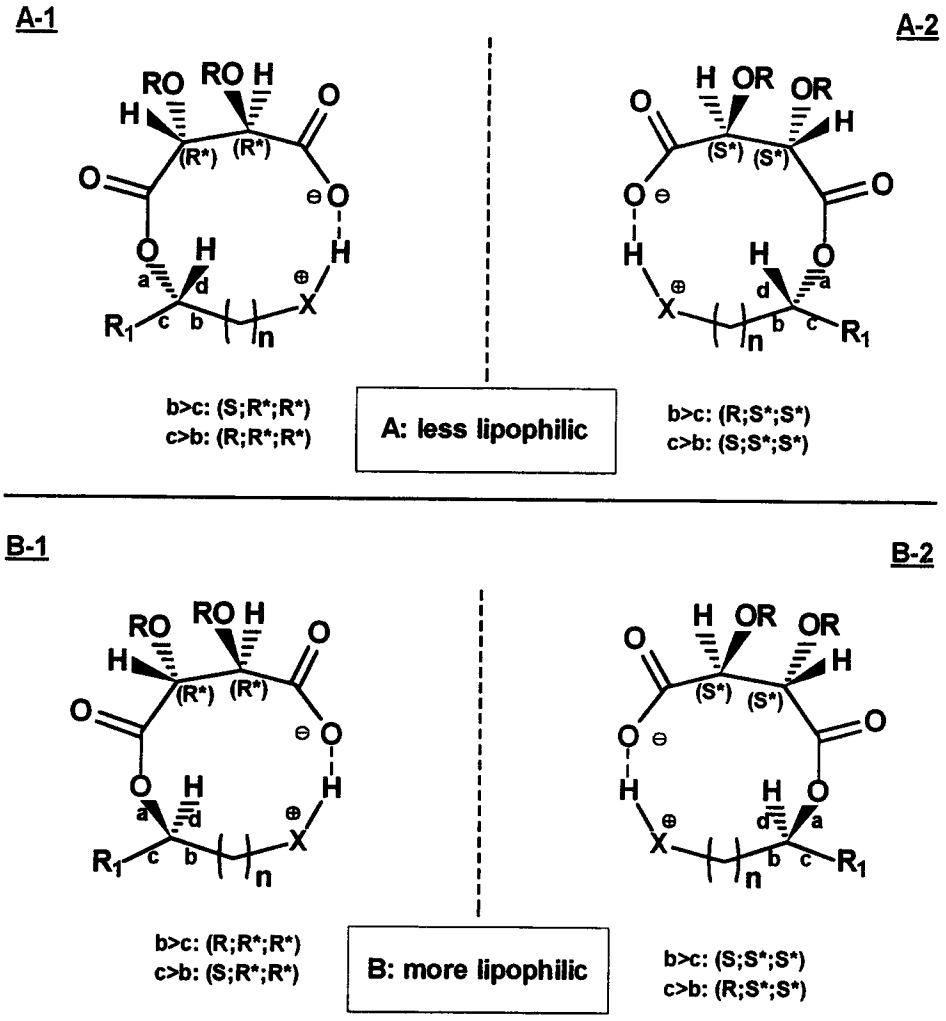

Fig. 5. General concept for the chromatographic assignment of the absolute configuration of chiral aminoalkanols by the elution order of diastereomeric (R*;R*)- or (S*;S*)-*O,O'*-disubstituted tartaric acid monoesters on a reversed-phase system: Considering strictly the Cahn–Ingold–Prelog priority rules the elution order of (R*;R*)-tartaric acid monoesters is A-1 before B-1 and, consequently, if b > c, (S)-aminoalkanol before (R)-isomer, e.g., if applying the concept to the intermediate **1** (X, 1-imidazolyl-; *n*, 1; R_1, 2,4-dichlorophenyl-) (see Table 1, entry 2 and Fig. 6A) and if c > b, (R)-aminoalkanol before (S)-isomer, e.g., if applying the concept to β-blockers like carvedilol [X, 2-(2-methoxyphenoxy)ethylamino-; *n*, 1; R_1, 1-carbazolyl-oxymethyl-) (see Table 1, entry 3 and Fig. 6, B). On the other hand the elution order is inversed, if handling with (S*;S*)-tartaric acid anhydride as derivatizing agent (A-2 before B-2) or if working in the normal phase mode. (R, acetyl-, benzoyl-, *p*-toluoyl-, benzyl-, etc.; R_1, aryl-, aryloxymethyl-; *n*, 1–3; X, basic aliphatic or aromatic amines).

interaction of the basic solutes with the CSP are the predominant principles for retention and enantioselectivity.

Optimization of the chromatographic conditions Preliminary studies to resolve the enantiomers of econazole **2** were performed with chiral AGP. However, because of small α-values (in the range of 1.0 to 1.2), low resolution (R_s below 1.0), a relatively marked peak tailing associated with pH of the mobile phase, the chiral AGP column was of limited usefulness for the present task. Therefore, we continued our studies with OVM as CSP. The results of the separations with OVM are presented in Table 2 and 3. As poined out in Table 2 acetonitrile exhibits the best chromatographic response (high enantioselectivity and elution strength) as well as the best chromatographic behaviour (less tailing). The influence of the pH on the chromatographic parameters for econazole is displayed in Figure 7; a similar relationship has been found for the other analytes studied (**1**, **2**, and **3**). Variation of the pH from acidic to basic range revealed the normal retention characteristics for basic compounds on the OVM column. The higher retention in the basic range caused also higher enantioselectivity and higher resolution. Retention, enantioselectivity, resolution and column efficiency were also remarkedly influenced by temperature. Usually, by lowering the temperature the enantioselectivity increases, while the column efficiency decreases, often to a greater extent, revealing the optimum resolution at higher temperatures. However, for the separation of the solutes **1**, **2**, and **3** on OVM both enantioselectivity (α) and resolution (Rs) increase with decreasing temperature (see Fig. 7), whereby the optimum of enantioselectivity and resolution can be expected most probably below 10°C; however, for practical reasons we stopped at this point.

All the investigated chiral imidazoles (**1**, **2**, and **3**, see Fig. 1) could be resolved on the OVM column: the solutes seem to share structural features for interaction with the chiral selector, and most probably its basic imidazole drives the interaction with negatively charged groups of the selector protein,

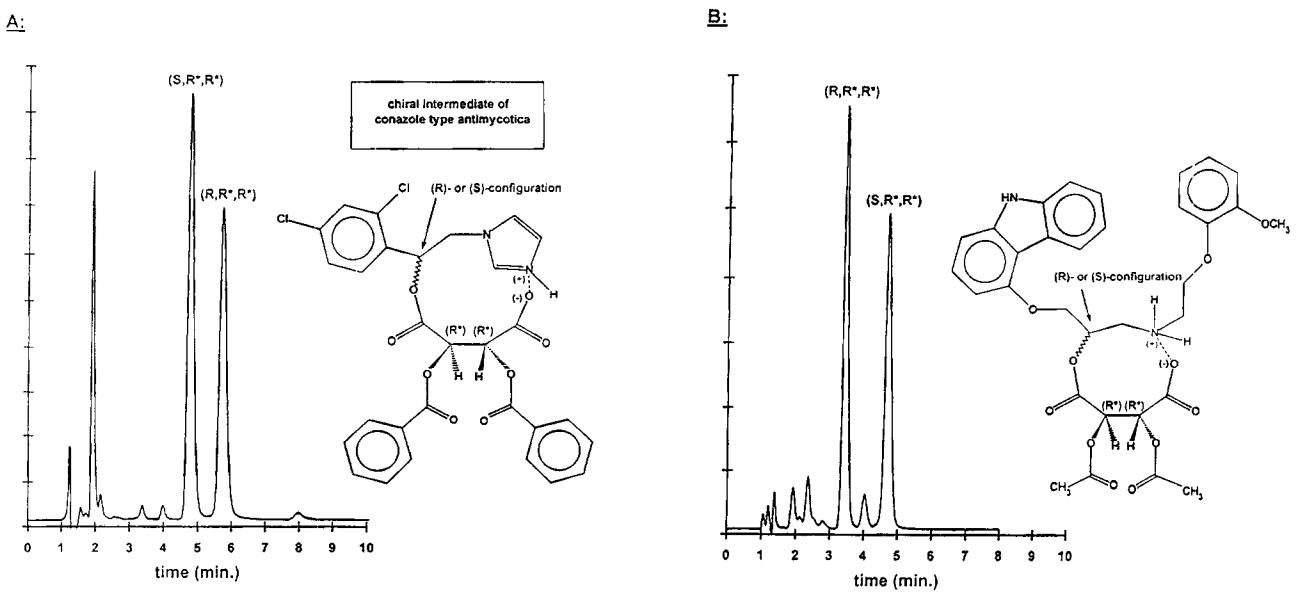

Fig. 6. Indirect resolution of aminoalkanols. **(A)** rac-**1** derivatized with (R*;R*)-DBTAAN. **(B)** rac-Carvedilol derivatized with (R*;R*)-DATAAN. Chromoatographic conditions: stat. phase, RP-select B; T, 30°C; flow rate, 1 ml/min; detection, UV 230 nm; mob. phase, MeOH/0.1 *M* ammonium acetate. **(A)** 60/40 (pH_a 5.0) **(B)** 55/45 (pH_a 5.3).

TABLE 2. HPLC separation of enantiomers of econazole and micronazole on OVM column using various types of uncharged organic modifier[a]

Compound	o.m.[a]	pH_a[b]	k_1'[c]	k_2'[c]	α[d]	R_s[e]
Econazole	ACN	4.20	1.13	1.33	1.18	—
	iPrOH	4.20	4.21	4.21	1.00	—
	EtOH	4.20	7.02	8.30	1.18	1.18
	MeOH	4.20	n.e.[f]	n.e.[f]	—	—
Miconazole	ACN	4.20	2.06	2.86	1.39	1.43
	iPrOH	4.20	7.55	12.47	1.65	2.30
	EtOH	4.20	12.29	20.10	1.63	3.37
	MeOH	4.20	n.e.[f]	n.e.[f]	—	—

[a] Eluent, 80% 0.01 *M* phosphate buffer/20% organic modifier (o.m.); T, 25°C; flow rate, 1.00 ml/min; det., UV 230 nm.
[b] pH_a, apparent pH.
[c] k' is defined as $t_r - t_0/t_0$.
[d] α is calculated by k_2'/k_1'.
[e] R_s is calculated by the formula: $1.18\ (t_{r2} - t_{r1})/(w_{1/2\,1} + w_{1/2\,2})$; $w_{1/2}$ = peak width at half peak height.
[f] n. e., not eluted ($k' > 30$).

TABLE 3. Enantioseparation of some chiral imidazoles on OVM type CSP[a]

Compound	Acetonitrile (%)	pH_a	k_1'	k_2'	α	R_s
1	15	7.0	3.07	4.04	1.31	0.79
2	30	6.5	5.37	8.28	1.54	1.69
3	20	4.2	3.04	4.46	1.47	1.82

[a] Eluent: 0.01 *M* phosphate buffer/acetonitrile; T, 10°C; flow rate, 1.00 ml/min; det., UV 230 nm.

but the lipophilic chlorophenyl and dichlorophenyl groups must also interact with the hydrophobic and/or "aromatic" sites of the protein.

Application Using the empirically optimized chromatographic conditions (low temperature, high pH) (see Table 3) trace optical purity analysis of econazole **2** could easily be performed. An enantiomeric excess (ee) of 98.8% was determined for both the (S)- and (R)-enantiomer of econazole synthesized by fractional crystallization of the respective tartaric acid salts (see Fig. 8). (R)- and (S)-Econazole synthesized via the optically enriched intermediate **1** reached ee values of 99.2 and 83.8%, respectively.

CONCLUSION

Protein type CSPs seem to exhibit good enantioselectivity for chiral imidazoles, with the OVM phase being somewhat more selective than AGP. The chromatographic assignment of the absolute configuration of the chiral intermediate (R)- and (S)-imidazolylethanol **1** and thus of (R)- and (S)-econazole **2** matches with the assignment performed by X-ray crystal structure analysis for (R)-econazole.[2]

Preliminary results indicate that optically pure econazole and the intermediate imidazolylethanol seem to be temperature and pH labile with respect to racemization of the stereogenic center. However, so far no differences in antifungal activity (inhibition of the cytochrome P-450-dependent monooxygenase, which removes the C-14α methyl group in fungal sterol biosynthesis) have been found for the optical antipodes of econazole and structurally related compounds (miconazole, tioconazole sertaconazole, isoconazole, fenticonazole, zoficonazole). But for some related imidazoles which are less labile concerning the chiral center (diniconazole, uniconazole, M 14360, valconazole, etaconazole, propiconazole, etc.) the (R)-isomer is the major determinant of fungitoxicity.[13–17] This raises the question whether or not the identical antifungal activity of the two enantiomers of econazole[18] is real or has to

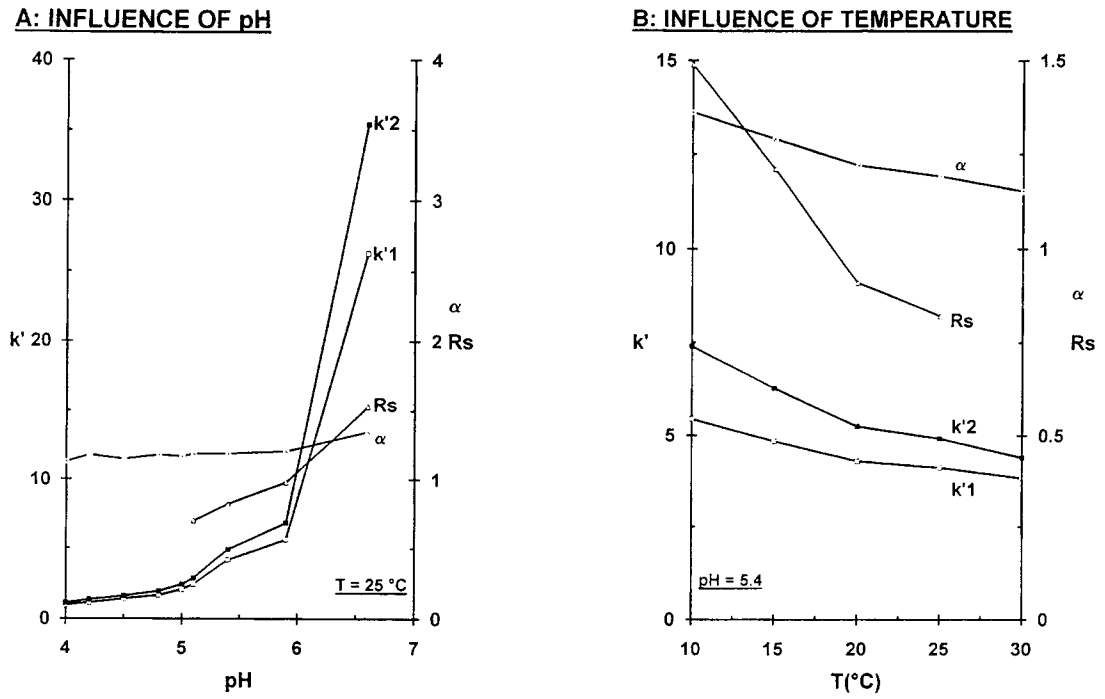

Fig. 7. Chromatographic parameters of enantioseparation of econazole as function of pH **(A)** and temperature **(B)**. Chromatographic conditions: stat. phase, OVM; mob. phase, 80% 0.01 *M* phosphate buffer/20% acetonitrile; flow rate, 1.00 ml/min; detection, UV 230 nm. (The missing R_s values are not calculated due to too low resolution.)

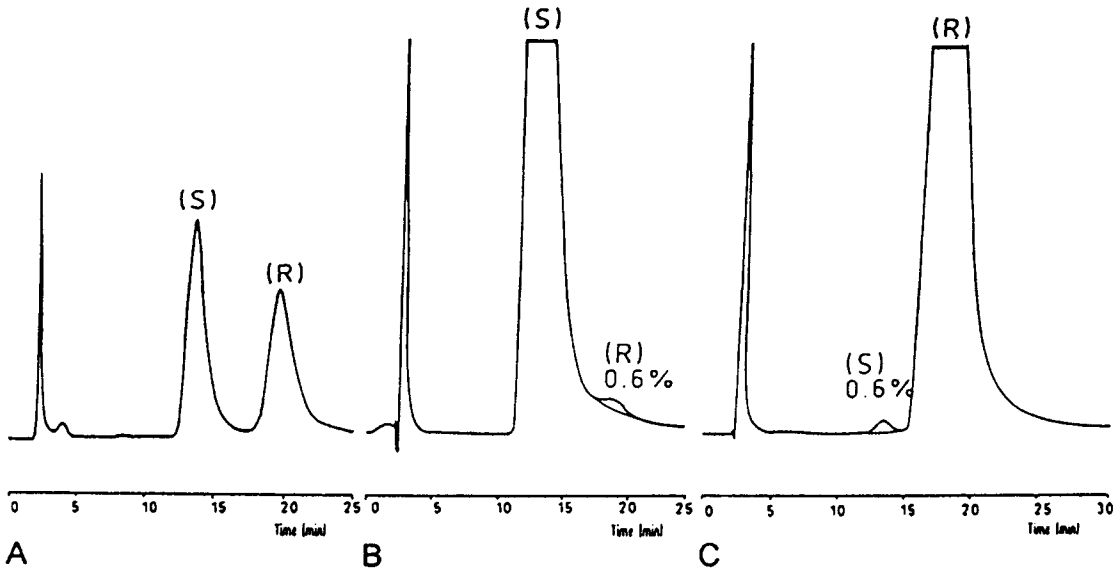

Fig. 8. Resolution of racemic econazole **(A)** and chiral analysis of (S)- and (R)-econazole synthesized by fractional crystallization of the (S*;S*)- and (R*;R*)-tartaric acid salts, respectively **(B** and **C)**. Chromatographic conditions: stat. phase, OVM; mob. phase, 70% 0.01 *M* phosphate buffer/30% acetonitrile, pH: 6.5; temperature, 10°C; flow rate, 1.00 ml/min; detection, UV 230 nm.

be attributed to in vivo racemization phenomena not investigated carefully enough. Our findings would support the latter, but more studies have to be undertaken to clarify this speculation.

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