Journal of Chromatography, 532 (1990) 305–319
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5440

Resolution of the enantiomers of omeprazole and some of its analogues by liquid chromatography on a trisphenylcarbamoylcellulose-based stationary phase

The effect of the enantiomers of omeprazole on gastric glands

PER ERLANDSSON*

Department of Technical Analytical Chemistry, Chemical Center, University of Lund, P.O Box 124, S-221 00 Lund (Sweden)

ROLAND ISAKSSON

Division of Organic Chemistry 3, Chemical Center, University of Lund, P O Box 124, S-221 00 Lund (Sweden)

and

PIA LORENTZON and PER LINDBERG

Department of Biology and Organic Chemistry, Hässle Gastrointestinal Research Laboratories, S-431 83 Mölndal (Sweden)

(First received March 28th, 1990, revised manuscript received June 12th, 1990)

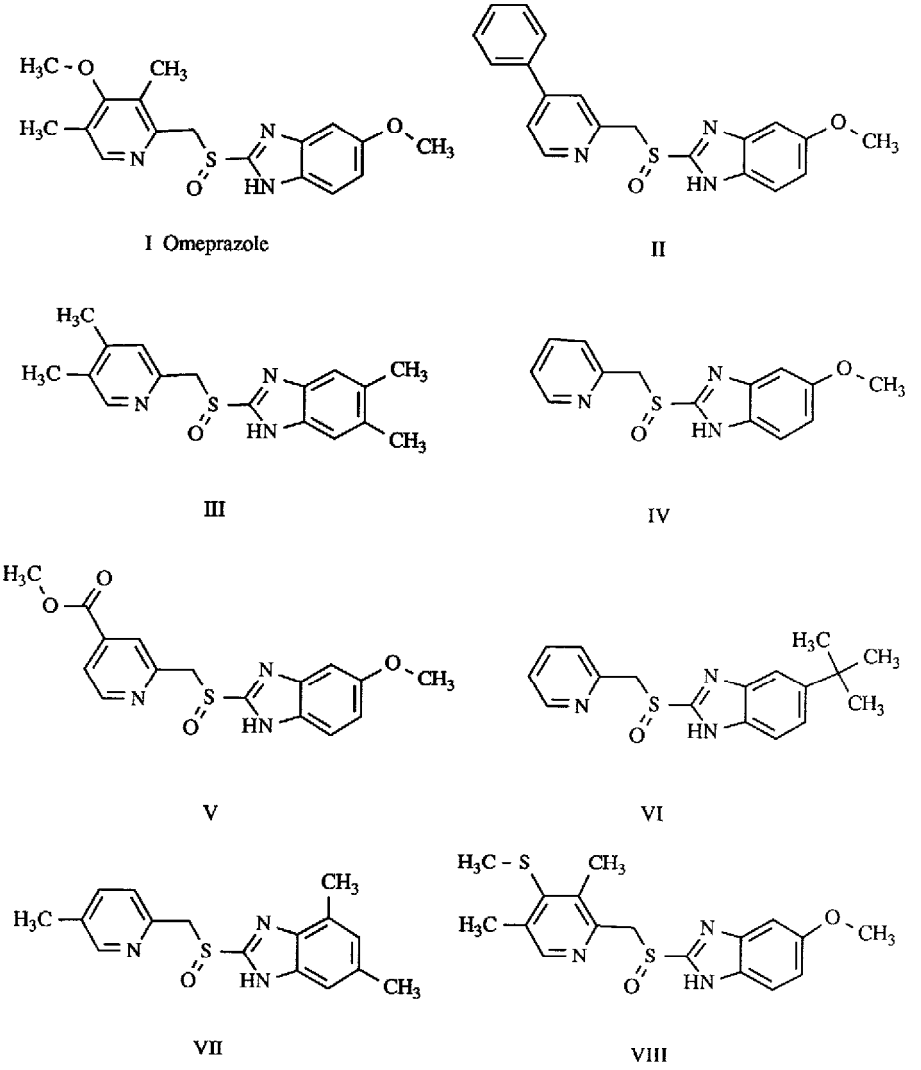
ABSTRACT

The enantiomers of omeprazole and some of its analogues have been separated on a chiral stationary phase comprising trisphenylcarbamoylcellulose coated on 3-aminopropyl silica The nature of the supporting silica has a crucial effect on the separations obtained. The racemisation half-life of omeprazole was estimated to be $1.3 \cdot 10^2$ h at 37°C. *In vitro* tests on isolated gastric glands from rabbits showed that both enantiomers of omeprazole had an inhibitory effect on acid formation.

INTRODUCTION

Omeprazole (I, Fig. 1) (trade name Losec) is the first compound of a new class of highly potent and clinically useful gastric acid secretion inhibitors [1]. This antiulcer agent differs mechanistically from the currently used histamine H_2-receptor antagonists, such as cimetidine and ranitidine, by virtue of its direct inhibition of H^+/K^+-ATPase, the gastric acid pump. The findings of mechanistic studies have shown that omeprazole is not *per se* the active inhibitor, but is itself activated *in vivo* within the acid compartments of the parietal cell to an achiral biologically active sulphenamide [2]. One should thus expect that the two

0378-4347/90/$03.50



I Omeprazole

II

III

IV

V

VI

VII

VIII

Fig. 1. Structures of analytes.

enantiomers of omeprazole have the same acid inhibitory effect at a cellular level. However, the fact that the enantiomers separate on chromatography using human (HSA) and bovine serum albumin (BSA) [3,4] may indicate a difference in the degree of plasma protein binding of the two enantiomeric forms.

Sulphoxides, with different substituents attached to the sulphur, are chiral compounds due to their tetrahedral configuration. Pirkle and Beare [5] were the first to demonstrate that the enantiomers of the sulphoxides have non-identical nuclear magnetic resonance (NMR) spectra in the presence of a chiral fluoroalco-

hol, a non-identity which could, of course, be used for direct determinations of the optical purity of sulphoxides. A chiral stationary phase for direct separation of the enantiomers of sulphoxides was produced by Pirkle and House [6], by immobilisation of chiral 2,2,2-trifluoro-1-(9-anthryl)ethanol on 3-mercaptopropyl silica. On an analytical scale, successful resolutions of omeprazole have been obtained by Allenmark *et al.* [4] on a BSA column and by Marle *et al.* [3] by using HSA in the mobile phase. To compare biological activity and to perform racemisation experiments, a preparative-scale separation method is needed. A phase prepared at this laboratory by adsorption of albumin on silica did not enable the separation of sulphoxides [7]. The albumin phase has a relatively low preparative capacity [7] and, moreover, omeprazole is not stable at the low pH values of the mobile phase used to obtain the selectivity factors necessary for preparative-scale separation. No attempts were made to use the above-mentioned fluoroalcohol phase, as no splitting of the signals of omeprazole was obtained in an NMR experiment using the fluoroalcohol as a shift reagent.

Okamoto *et al.* [8] have prepared a great number of useful chiral stationary phases by adsorption of cellulose derivatives on macroporous silica supports. Enantiomers of chiral sulphur compounds such as sulphoxides [9], sulphinamides and sulphilimines [10] have successfully been separated on such columns. The wide range of substitution patterns possible with cellulose enables phases to be tailored in order to suit specific enantiomer separations. The relatively high loading capacities of these phases [11] also render them useful for preparative-scale separations of enantiomers.

Here we report the separation of omeprazole into its enantiomers on a preparative scale using a cellulose-based chiral phase, trisphenylcarbamoylcellulose (TPCC, Fig. 2) coated on 3-aminopropyl silica, that can be easily and inexpensively prepared using published methods [8]. The quantities of separated enantiomers obtained were used for *in vitro* pharmacological studies and for racemisation experiments.

EXPERIMENTAL

Materials

Omeprazole (I) and some of its analogues (compounds II–VIII, Fig. 1) were synthesized at the Department of Organic Chemistry of Hässle (Mölndal, Sweden). Cellulose (Avicel); 1,3,5-tri-*tert.*-butylbenzene (TTB) and phenylisocyanate were used as obtained (Merck, Darmstadt, F.R.G. and Eastman Kodak, Rochester, NY, U.S.A.). Matrex silica (500 Å pore size, 10 and 20–45 μm particle size) was obtained from Amicon-Grace (Helsingborg, Sweden), Nucleosil silica (4000 Å, 10 μm) from Macherey-Nagel (Düren, F.R.G.) and γ-aminopropyltriethoxysilane (Dynasylan AMEO) from Dynamit Nobel Chemie (Troisdorf, F.R.G.). Tetrahydrofuran (THF) (analytical grade, Riedel-de-Haën, Seelze, F.R.G.) was dried and distilled before use. Diethyl ether, methanol, pyridine and toluene were

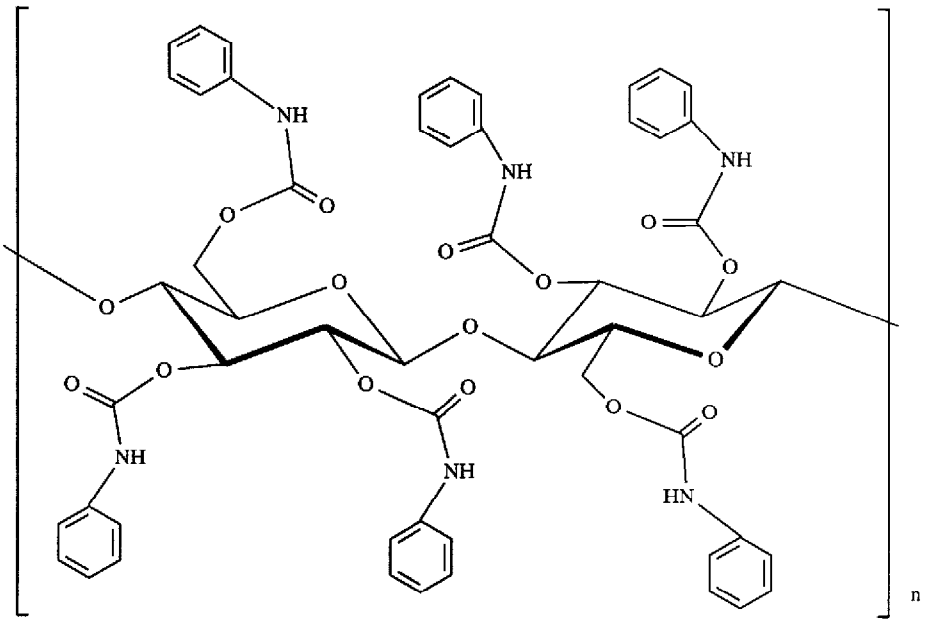


Fig. 2. Structure of trisphenylcarbamoylcellulose.

of analytical grade (Merck). Demineralised water was purified in a MilliQ filtration system (Millipore, Bedford, MA, U.S.A.) prior to use. (+)-2,2,2-Trifluoro-1-(9-anthryl)ethanol was used as obtained (Ega-Chemie, Steinheim, F.R.G.). C^2HCl_3 and $C^2H_2Cl_2$ were purchased from Dr. Glaser (Basel, Switzerland).

Use of a chiral shift reagent, (+)-2,2,2-trifluoro-1-(-9-anthryl)ethanol

A ^1H NMR-spectrum of omeprazole (5 mg) dissolved in 1 ml of C^2HCl_3 was recorded with a Varian XL-300 NMR spectrometer. To the sample was added one 1.5-mg portion of shift reagent at a time, an NMR spectrum being recorded after each addition. No splitting of the signals was obtained after four such additions (*i.e.* at more than equimolar amounts of reagent).

Synthesis of triphenylcarbamoylcellulose

TPCC was prepared as described by Okamoto *et al.* [8]. A large excess of phenylisocyanate (50 ml) was added to a slurry of 5 g of cellulose (Avicel) in 50 ml of dry pyridine at ambient temperature during stirring. When the addition of the reagent was completed, the temperature of the reaction mixture was increased to 100°C and kept at that temperature for 24 h. The cooled viscous reaction mixture was poured out under vigorous stirring into 1 l of methanol. The slurry was decanted and the precipitate was carefully washed with an excess of methanol and then oven-dried at 50°C. The slightly yellowish TPCC was soluble in acetone and

THF. A sample suitable for IR spectrophotometry was prepared by grinding TPCC and potassium bromide together and compressing the mixture into a tablet. The IR spectrum showed a characteristic carbamate band at 1730 cm^{-1}; no isocyanate band (2200–2300 cm^{-1}) was detected. The reaction was monitored by recording IR spectra of the product, and after 24 h no further change was observed. A Perkin Elmer 298 infrared spectrophotometer (Perkin-Elmer, Analytical Instruments, Norwalk, CT, U.S.A.) was used to record IR spectra. The substitution of all hydroxyl groups of cellulose was checked by ^1H NMR. When no change of the NMR spectrum was observed after addition of ^2H_2O, no hydrogen–deuterium exchange had occurred and the cellulose was assumed to have been completely substituted. The NMR experiments were carried out with a 5-mg sample of TPCC dissolved in 0.5 ml of $C^2H_2CL_2$ on a Varian XL-300 NMR spectrometer.

Synthesis of 3-aminopropyl silica

Silica, 10 g, was first dried at 200°C and then dispersed in 150 ml of dry toluene. γ-Aminopropyltriethoxysilane, 10 ml, was added and the reaction mixture was refluxed for 10 h. The silica was filtered off and washed on a glass filter both with toluene, methanol and water, and with methanol and diethyl ether. The same method was adopted for preparing larger amounts of 3-aminopropyl silica for the preparative columns. The unmodified silicas used for preparation of 3-aminopropyl silicas contained 0.2% carbon and no nitrogen (as determined by elemental analysis). The 3-aminopropyl silicas contained 0.9–1.2% carbon, 0.4% nitrogen (500-Å silica) and 0.34% carbon, 0.1–0.15% nitrogen (4000-Å silica).

Immobilisation of TPCC on silica

TPCC (1 g) was dissolved in 25 ml of THF by stirring with a magnetic bar. A 2-g amount of silica or 3-aminopropyl silica was suspended in the TPCC solution and immersed for 1 min in a B220 ultrasonic bath (Branson Ultrasonics, Danbury, CT, U.S.A.). The suspension was poured into a 50-ml test tube and treated for two days on a rocker table. The TPCC was then coated on silica, either by evaporation of the solvent in a rotavapor (Büchi, Flawil, Schweiz) [12] or by precipitation by adding 50 ml of methanol to the suspension The material was carefully washed with methanol on a glass filter. The TPCC-to-silica ratio was varied from 0.15:1 to 1:1 (w/w) to study the dependence of the amount of chiral phase on retention and enantioselectivity. The TPCC silica obtained was characterized by the determination of carbon by elemental analysis. The analyses were performed at the Department of Analytical Chemistry, University of Lund (Lund, Sweden). The carbon content of the TPCC silica was taken as a measure of the amount of TPCC immobilised on the silica. Unless otherwise stated, 3-aminopropyl silica was used as support.

Preparation of columns

A 0.3-g amount of TPCC silica was suspended in 80 ml of methanol or 2-propanol and, using a descending slurry-packing technique, packed in a 100 mm × 2.1 mm I.D. stainless-steel column (Li-Chroma tubing from Skandinaviska GeneTec, Kungsbacka, Sweden) with methanol as the displacing medium at a pressure of 200 bar. Larger columns, 200 mm × 4.6 mm I.D. (Li-Chroma tubing) were packed as above, except that 3 g of TPCC silica was used in the slurry. For preparative purposes, the columns were prepared using a descending sedimentation technique [7]. TPCC silica, 16 g of 20–45 μm suspended in 200 ml of methanol, was slurry packed into a 300 mm × 10 mm I.D. Valco stainless-steel column (Scandinaviska GeneTec).

Chromatography

Mobile phases were prepared by mixing *n*-hexane (HPLC grade, Fisons, Loughborough, U.K.), 2-propanol (HPLC grade, Fisons) and diethylamine (AnalaR grade, BDH, Poole, U.K.) at different ratios. All mobile phases were degassed in an ultrasonic bath prior to use. For evaluation of the 100 mm × 2.1 mm I.D. columns, a standard chromatographic set-up was used comprising a Philips PU 4003 solvent delivery system (Pye Unicam, Cambridge, U K.), a Philips PU 4025 UV detector (equipped with a 1-μl flow cell) connected to a Philips PM 8252 recorder. The samples, 0.5 μl, were injected into the columns with a Rheodyne 7520 loop injector (Rheodyne, Cotati, CA, U.S.A.). The 200 mm × 4.6 mm I.D. 4000-Å TPCC column was used in a system comprising a Varian 5000 pump (Varian, Palo Alto, CA, U.S.A.). and a Pye Unicam LC–UV detector equipped with an 8-μl flow cell. Samples (10 μl) were injected with a Valco C6U loop-valve injector (VICI, Houston, TX, U.S.A). The 200 mm × 4.6 mm I.D. 500-Å TPCC column was used in a system comprising an LDC Constametric III pump, an LDC Spectromonitor III and a Rheodyne 7125 loop-valve injector equipped with a 20-μl loop. The chromatograms were recorded with an LKB 2210 strip chart recorded (LKB, Bromma, Sweden).

The following set-up was used for the preparative separations: a Beckman 110B pump, a Rheodyne 7125 injection valve equipped with a 5-ml loop, an LKB 2138 Uvicord-S UV detector (at 278 nm) with a 7-μl flow cell, a Perkin Elmer 241 MC polarimeter with a 1-dm, 1-ml quartz flow cell and a Rheodyne 7010 switching valve. The recycling technique has previously been described [13]. The elution orders were verified for all compounds by polarimetric detection.

Resolutions (R_s), separation factors (α) and capacity factors (k') were calculated as previously described [14]. Plate heights (H) were calculated using peak widths at the baseline [15]. The capacity factors for compounds II and III were estimated by fitting the experimental chromatograms to skewed overlapping Gaussian curves.

Determination of the optical purity of omeprazole

The enantiomeric purity of the collected fractions of omeprazole was checked as described by Allenmark *et al.* [4], using the commercial chiral albumin phase Resolvosil® BSA-7 from Macherey-Nagel (Düren, F.R.G.). The enantiomers of omeprazole were isolated by evaporation of the mobile phase in a rotavapor. Approximately 1 μg of the solute in 40 μl of mobile phase (phosphate buffer, pH 6.6, ionic strength 0.05 and 2% 1-propanol as modifier) was injected on the column. The eluent was monitored with a UV detector at wavelengths both of 229 and 280 nm, at a flow-rate of 1 ml/min. The UV signal was fed to an electronic integrator to calculate the enantiomeric purity.

Racemisation of omeprazole

As mentioned above, omeprazole (I) is a chiral compound due to the pyramidal structure of the sulphoxide group. The racemisation of sulphoxides takes place by means of pyramidal inversion without breaking the bonds [16]. The omeprazole enantiomer that eluted first from the column was isolated from the mobile phase by evaporation of the solvents. The isolated enantiomer was then dissolved in a few drops of 2-propanol, and the solution was diluted with 0.05 *M* sodium phosphate buffer, pH 7.0, to a suitable concentration (the exact concentration was not measured). Racemisation experiments were performed at different temperatures and monitored at a wavelength of 265 nm using a CD instrument (Jasco J-500A spectropolarimeter, Tokyo, Japan) equipped with a thermostatic 1-mm quartz cell. A Haake B thermostat (Haake, Karlsruhe, F.R.G.), with a Haake N3 digital regulator, was used for keeping the temperature constant in the CD cell. The temperatures were also checked with a calibrated thermometer during the experiments. The decline in optical activity at 265 nm at different temperatures was taken up on the recorder of the CD instrument. The racemisation experiments were performed at 25, 37, 50 and 75°C, and the racemisation half-lives at these temperatures were estimated from CD data. By using the half-life estimated at 75°C, the racemisation barrier, ΔG^*, was obtained from Eyring's equations [17]:

$$k = k_b\, T/h \exp(-\Delta G^*/\mathrm{RT}) \quad (1)$$

$$t_{1/2} = \ln 2/k = 3.3265 \cdot 10^{-11}\, T^{-1} \exp(\Delta G^*/\mathrm{RT}) \quad (2)$$

where k is the rate constant, k_b the Boltzman constant and h the Planck's constant.

Determination of effect on acid formation in isolated gastric glands

The biological effect of omeprazole and its enantiomers was evaluated *in vitro* using isolated rabbit gastric glands [18]. Acid formation in the glands was assessed by using the weak base [^{14}C]aminopyrine (AP) as previously described [19]. Briefly, the basis of this method is that the base is freely permeable across the cell membranes, but becomes impermeable when protonated. The pK_a of AP (5)

allows it to selectively accumulate in the acidic compartments of the parietal cell. The secretory state of the glands, *i.e.* the extent of AP accumulation, can thus be determined by measuring the ratio of AP in intraglandular water to AP in medium.

RESULTS AND DISCUSSION

Preparation of stationary phases

A comparison was made between unmodified silica and 3-aminopropyl silica as support to TPCC. Chromatography of omeprazole on TPCC 3-aminopropyl silica showed two partly overlapping bands, but on unmodified silica no separation could be observed by UV detection (see Fig. 3). One explanation for the superiority of the 3-aminopropyl silica support in this case could be that the amino groups assist in the chiral recognition. Another explanation could be that the amino groups govern the arrangement of TPCC on the surface to form a

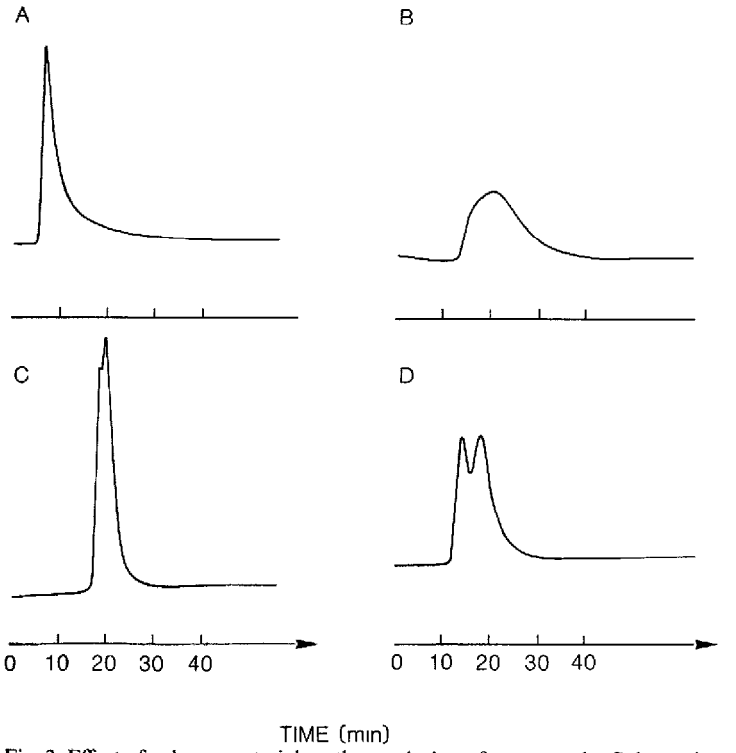


Fig 3. Effect of column material on the resolution of omeprazole Column dimensions, 100 mm × 2.1 mm I.D., particle size, 10 μm. Column A, 500-Å unmodified silica as the support, 1 5–2% carbon, column B, 500-Å unmodified silica as the support, 8.4% carbon; column C, 500-Å 3-aminopropyl silica as the support, 4.8% carbon; column D, 500-Å 3-aminopropyl silica as the support, 12% carbon. Chromatographic conditions: mobile phase, *n*-hexane–2-propanol–diethylamine (80 20:0 1, v/v), injection volume, 0.5 μl of mobile phase containing 0.5 μg; UV detection, 300 nm.

more homogeneous or well ordered phase and, as a consequence, an improved stereoselectivity. A similar phenomenon has been observed when omeprazole was separated with different types of columns based on BSA. When BSA was adsorbed on unmodified silica, no separation of the enantiomers was observed [7]. Comparison of BSA cross-linking with glutaraldehyde on unmodified silica and on 3-aminopropyl silica showed the latter to give a much better separation of omeprazole [20].

An increase in the amount of immobilised TPCC has virtually no effect on the retention of omeprazole, whereas the stereoselectivity is increased (Fig. 3C and D). We did not succeed in making well functioning TPCC silica with a carbon content higher than 14%. The coating process probably constitutes a combination of adsorption and precipitation of TPCC on silica. No significant difference was observed between the immobilisation procedures (*i.e.* by evaporation or by precipitation with methanol). No attempts to immobilise TPCC covalently or by cross-linking on silica were made, as Okamoto *et al.* [21] have reported that these phases generally manifest poorer resolution, the proposed explanation of which is that too strong a fixation prevents the cellulose derivative from forming an ordered structure on the surface on the silica gel. Cellulose derivatives can form liquid crystal phases, a characteristic essential for the derivatives to exhibit a high degree of chiral recognition [22,23].

The effect of polarity of the mobile phase was studied by using different proportions between *n*-hexane and 2-propanol, as shown in Table I. Diethylamine was added to the mobile phase to enhance omeprazole stability. The mobile phase comprising *n*-hexane–2-propanol–diethylamine (80:20:0.1, v/v) was chosen for further studies to obtain a reaonable resolution and out-put per unit of time. The resolution of omeprazole decreases with an increase of flow-rate, as illustrated in Fig. 4.

A comparison was also made of 3-aminopropyl silica with a 500-Å *versus* a 4000-Å pore size as support materials. The 4000-Å material showed somewhat better resolution (see Fig. 5). Although the molecular size distribution of TPCC

TABLE I

EFFECT OF MOBILE PHASE COMPOSITION

A 0.5-μg amount of omeprazole in 0.5 μl of mobile phase was injected on a 100 mm × 2.1 mm I.D column packed with 10-μm TPCC 3-aminopropyl silica, 500 Å pore size, containing 12% carbon. Mobile phase, *n*-hexane–2-propanol–diethylamine (80.20:0.1, v/v); flow-rate, 0.2 ml/min, UV detection, 300 nm

n-Hexane–2-propanol–diethylamine	k'_1	k'_2	α	R_s
60:40:0 1	4.4	5.3	1.2	<0.4
70:30:0.1	6.4	7.9	1 2	<0.4
80·20:0 1	9.7	13.1	I 3	0 69
90:10:0.1	36	45	I 2	0 65

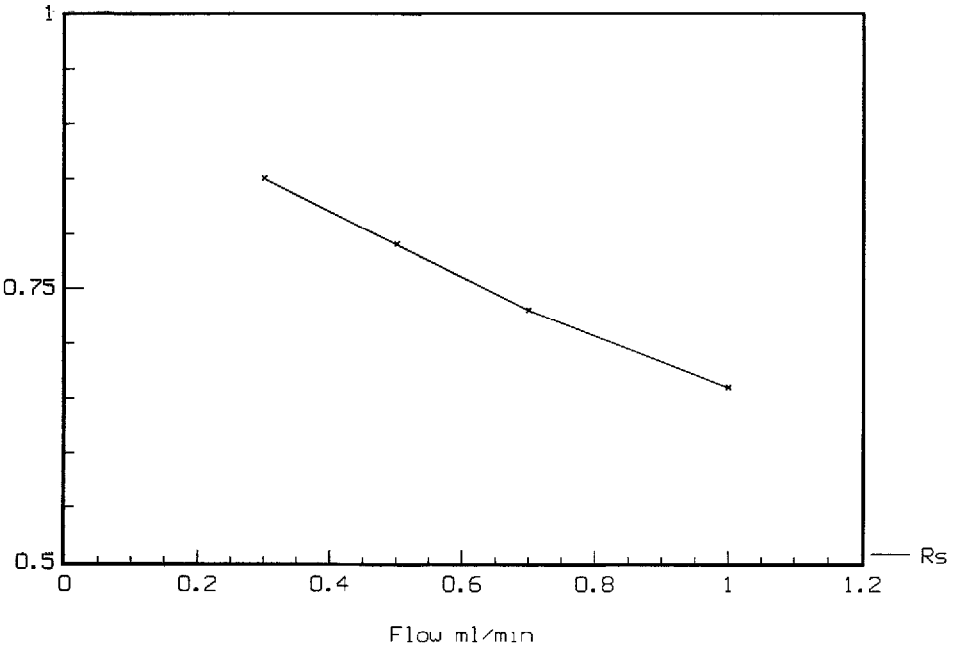


Fig. 4. Resolution (R_s) of omeprazole as a function of flow-rate. Chromatographic conditions: mobile phase, *n*-hexane–2-propanol–diethylamine (80:20:0.1, v/v), injection volume, 10 μl of mobile phase containing 10 μg; column, 200 mm × 4.6 mm I.D., 10 μm particle size, 4000-Å 3-aminopropyl silica as the support, 5.1% carbon, UV detection, 300 nm.

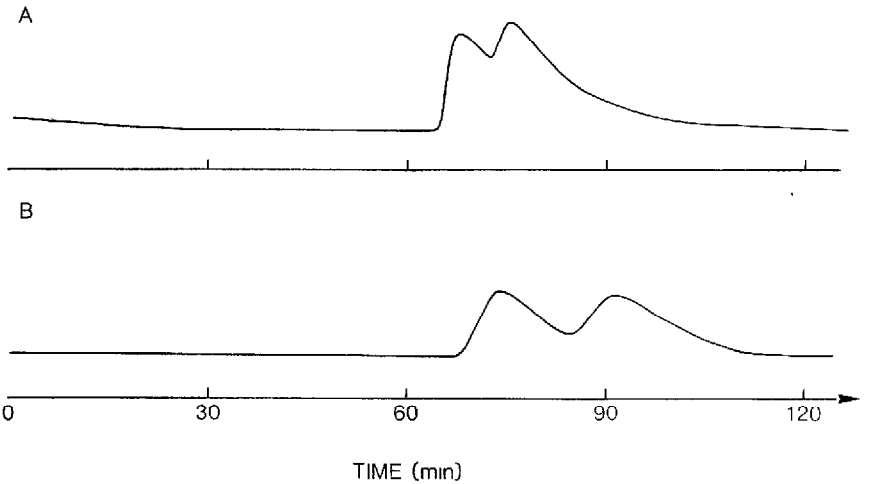


Fig. 5. Comparison of different pore sizes of the silica. Column dimensions, 200 mm × 4.6 mm I.D; particle size, 10 μm Column A, pore size 500 Å, 4.5% carbon, column B, pore size 4000 Å, 5.1% carbon. Chromatographic conditions: 20 μg in 20 μl (column A) or 10 μg in 10 μl (column B) of mobile phase were injected; mobile phase, *n*-hexane–2-propanol–diethylamine (80 20:0 1, v/v), flow-rate, 0.5 ml/min, UV detection, 300 nm (column A), 254 nm (column B)

was not investigated, its molecular size is probably considerable, as indicated by the viscous solutions obtained by dissolving TPCC in THF. Possibly, the pores of the 500-Å silica clog more easily than those of the 4000-Å material, and the 500-Å pores may restrict the mobility of TPCC owing to its molecular size, thus preventing it from forming an ordered structure in the pores, as mentioned above. Ichida and Shibata [24] have also reported that cellulose derivatives with identical structures behave differently depending on physical factors such as the molecular weight of the polymer, the solvent used in the coating, the history of previous contact of the polymer with liquids and the nature of the support.

The columns were generally used for periods varying from a week to a month and their properties did not change during that time. After six months of storage, however, the eluted bands obtained were very broad, suggesting that the stationary phase had degraded or that the TPCC had been lost from the silica gel.

Comparative study between different omeprazole analogues

Omeprazole (I) and its analogues (compounds II–VIII) were chromatographed on an analytical scale on a 200 mm × 4.6 mm I.D. TPCC column with a 4000-Å pore size; the results are summarised in Table II. The column showed a relatively low efficiency, and the resolution (R_s) varied from 0.39 to 1.13. The resolution of compound VII (R_s = 1.13) is illustrated in Fig. 6. The most notable result is the large differences in retention, the capacity factors k'_1 varying between 2.5 and 19.2 and k'_2 between 3.0 and 23.7. Interestingly, the retention seems to vary with the pK_a for the abstraction of a proton from the nitrogen atom in the benzimidazole

TABLE II

CHROMATOGRAPHIC DATA

A 10-μg amount of each compound in 10 μl of mobile phase was injected on a 200 mm × 4.6 mm I.D column, packed with 10-μm TFK silica, (5 1% carbon, 4000 Å pore size) Mobile phase, *n*-hexane–2-propanol–diethylamine (80:20:0.1, v/v); flow-rate, 0 5 ml/min; UV detection, 254 nm. The elution orders were determined by injecting 2 mg of the racemic analytes on the preparative columns using the same mobile phase. In all cases the (−)-enantiomer eluted first. TTB was used as the t_0 marker The pK_a values presented for the abstraction of a proton from the nitrogen in the benzimidazole have been calculated according to ref. 25.

Compound	k'_1	k'_2	α	R_s	pK_a
I	8.3	10.0	1.2	0.67	8.72
II	12.0	13.7	1 1	0.48	8.57
III	4.7	5.4	1 1	0 39	9.08
IV	10.5	12.2	1.2	0 48	8.55
V	19.2	23.7	1.2	0.91	8.31
VI	2 5	3.0	1 2	0 57	8.81
VII	2 6	3.6	1 4	1 13	8 97
VIII	8.5	11.1	1.3	0 84	8.65

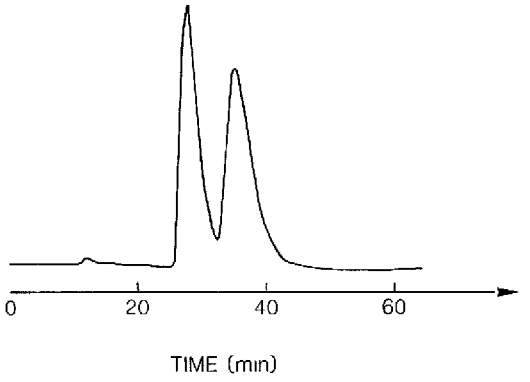


Fig. 6. Resolution of compound VII on a 200 mm × 4 6 mm I.D 4000-Å TPCC column Conditions are given in Table II Plate height H_1 0 51 mm, plate height H_2 = 0 62 mm

(see Fig. 7). Thus, the compounds containing alkyl substituents in the benzimidazole ring, having the highest pK_a values, show low retention while the other compounds containing a 5-methoxy substituent, having lower pK_a values, show higher retention. Accordingly, the lowest pK_a values in this series, that of compound V, correspond to the highest capacity factors. The diethylamine in the

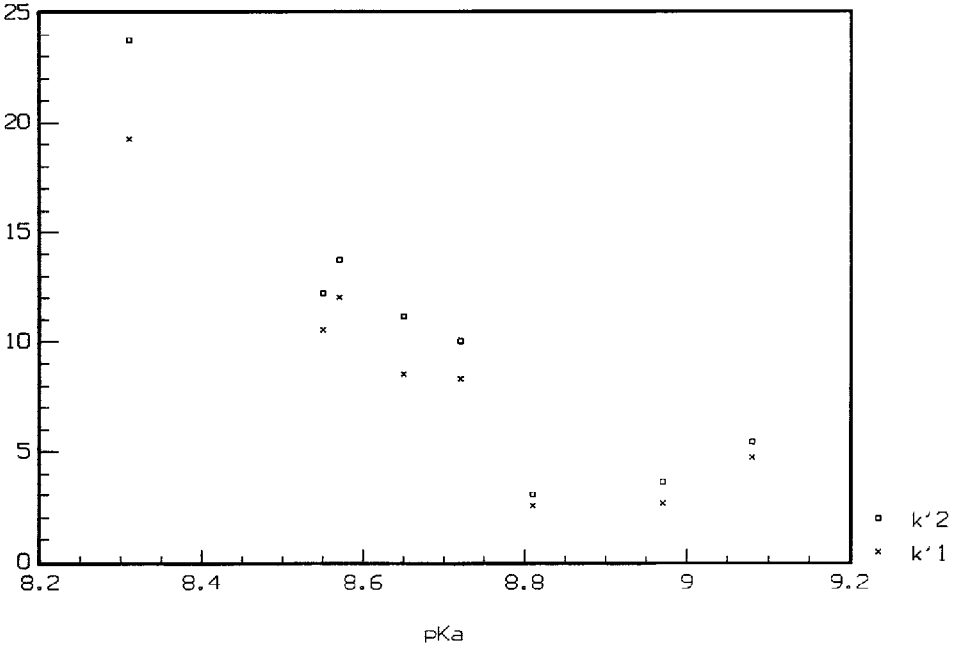


Fig 7 Retention of compounds I–VIII as a function of pK_a for the abstraction of a proton from the nitrogen atom in the benzimidazole.

eluent will partially deprotonate the benzimidazole. The higher the pK_a, the higher the fraction of the protonated form, possibly giving higher tendency for hydrogen bonding to the 2-propanol in the mobile phase. This hypothesis is supported by the decreased retention when increasing the amount of 2-propanol in the mobile phase. The selectivity factors (α) were more or less constant. The stereochemistry at the asymmetric sulphur of the analogues is probably the same which would explain the similarity in enantioselectivity.

Preparative resolution of omeprazole

The TPCC silica used for the preparative columns contained 6 1% carbon, and a separation is illustrated in Fig. 8. The collected (+)- and (−)-fractions were evaporated to dryness in a rotavapor. After six injections approximately 3 mg of (+)-omeprazole with an enantiomeric purity of 82% and approximately 4 mg of (−)-omeprazole with an enantiomeric purity of 95.6% were obtained. A limiting factor was the low solubility of omeprazole in the mobile phase.

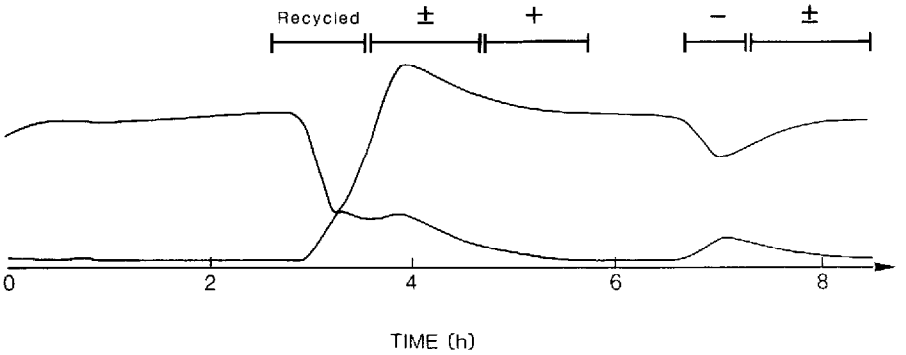


Fig. 8. Preparative resolution of omeprazole using the recycling technique Chromatographic conditions: two 300 mm × 10 mm I.D. columns connected in series; mobile phase, *n*-hexane–2-propanol–diethylamine (80:20:0.1, v/v); flow-rate 1.5 ml/min; 5 mg omeprazole in 5 ml of mobile phase were injected. (Upper trace) polarimetric detection at 365 nm, (lower trace) UV detection at 278 nm, (−), (+) and (±) denote collection of the respective fractions. The recycled fraction is eluted at approximately 7 h.

Racemisation of omeprazole

The racemisation half-lives at the different temperatures were estimated from the CD data. By using the half-life at 75°C ($3.8 \cdot 10^3$ s), the racemisation barrier (ΔG^*) was calculated using Eyring's equations (eqns. 1 and 2): 26 kcal/mol. The racemisation half-life at 37°C has thus been estimated, using eqn. 2, to be $1.3 \cdot 10^2$ h (assuming the activation entropy to be negligible within the temperature interval 37–75°C).

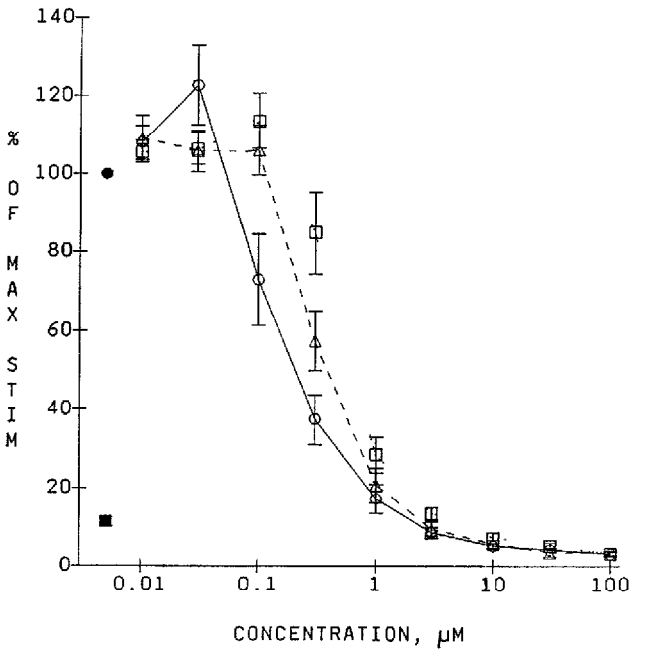


Fig 9. Effect on aminopyrine accumulation in isolated gastric glands (n = 5) (○) (±)-Omeprazole (H 168/68), IC_{50} = 0.248 ± 0.068 μM, (□) (+)-omeprazole (H 199/19), IC_{50} = 0.781 ± 0 151 μM; (△) (−)-omeprazole (H 199/18), IC_{50} = 0.470 ± 0.090 μM; (■) basal, (●) 0.1 mM histamine

Determination of the effect on acid formation in isolated glands

The *in vitro* tests showed that omeprazole and its two optical isomers potently inhibited the histamine-stimulated acid formation in the gastric glands (Fig. 9). The concentrations needed for half maximal inhibition (IC_{50}) were found to be 0.248 ± 0.068, 0.781 ± 0.151 and 0.470 ± 0.090 μM for omeprazole (H 168/68), the (+)-enantiomer (H 199/19) and the (−)-enantiomer (H 199/18), respectively (values are mean ± S.E.M., n = 5).

Thus, in similarity with the racemate both enantiomers were found to inhibit acid secretion in this model. To statistically compare the effects of the three drugs a Newman−Keuls multiple range test of the IC_{50} values obtained from the five different experiments was performed. This test showed a difference between omeprazole and the (+)-enantiomer (significance level, p = 0.05). However, the IC_{50} values of the (+)-enantiomer did not differ from those of the (−)-enantiomer at this significance level, and omeprazole was accepted as equal in potentency to the (−)-enantiomer. In conclusion, the inhibitory effect on acid formation in the isolated glands of the racemate (H 168/68) should be ascribed to the inhibitory action of both of its enantiomers.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Swedish Board for Technical Development and the Swedish Natural Science Research Council. Dr Sam Larsson (Hässle Gastrointestinal Research Laboratories) is greatfully acknowledged for the determination of the optical purity of the omeprazole enantiomers and M. Rachel Suissa (Department of Organic Chemistry, The Hebrew University of Jerusalem) for performing the NMR experiments.

REFERENCES

1 P. Lindberg, A. Brändström, B. Wallmark, H. Mattsson, L Rikner and K.-J Hoffman, *Med Res. Rev.*, 10 (1990) 1–54
2 P. Lindberg, P. Nordberg, T Almınger, A. Brändström and B Wallmark, *J Med Chem* , 29 (1986) 1327–1329
3 I. Marle, C. Pettersson and T. Arvidsson, *J. Chromatogr.*, 456 (1988) 323–336
4 S. Allenmark, B. Bomgren, H. Borén and P -O Lagerström, *Anal Btochem* , 136 (1984) 293–297
5 W. H. Pırkle and S. D. Beare, *J Am. Chem Soc* , 90 (1968) 6250–6251.
6 W. H. Pirkle and D W. House, *J. Org Chem.*, 44 (1979) 1957–1960
7 P. Erlandsson, L. Hansson and R. Isaksson, *J. Chromatogr.*, 370 (1986) 475–483.
8 Y. Okamoto, M. Kawashima and K. Hatada, *J. Am Chem. Soc.*, 106 (1984) 5357–5359
9 A. Ichıda, T. Shıbata, I Okamoto, Y Yuki, H. Namikoshi and Y. Toga, *Chromatographıa*, 19 (1984) 280–284.
10 I. W. Wainer, M. C. Alembik and C. R. Johnson, *J. Chromatogr.*, 361 (1986) 374–378
11 Y. Okamoto, M Kawashima, R Aburatani, K. Hatada, T. Nıshiyama and M Masuda, *Chem Lett.*, (1986) 1237–1240.
12 Y. Okamoto, M. Kawashima and K. Hatada, *J. Chromatogr.*, 363 (1986) 173–186.
13 R. Isaksson and R. Rochester, *J. Org. Chem.*, 50 (1985) 2519–2521.
14 L. R. Snyder and J. J Kırkland, *Introduction to Modern Lıquid Chromatography*, Wiley, New York, 2nd ed., 1979, Ch 2, pp. 22–37.
15 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Lıquid Chromatography*, Wiley, New York, 2nd ed , 1979, Ch 5, p. 222
16 M. Mıkolajczyk and J Drabowicz, ın N L Allınger, E. L Eliel and S H Wılen (Edıtors), *Topıcs ın Stereochemıstry*, Vol 13, Wiley, New York, 1982, pp 406–407
17 S. Glasstone, K. Laıdler and H. Eyrıng, *The Theory of Rate Processes*, McGraw-Hıll, New York, 1941.
18 T. Berglindh and K J. Öbrink, *Acta Physiol. Scand* , 96 (1976) 150–159
19 T. Berglindh, H. F. Helander and K J. Öbrink, *Acta Physiol Scand* , 97 (1976) 401–414.
20 S. Andersson, S. Allenmark, P Erlandsson and S Nilsson, *J Chromatogr* , 498 (1990) 81–91
21 Y. Okamoto, R. Aburatani, S Miura and K. Hatada, *J. Lıq. Chromatogr.*, 10 (1987) 1613–1628.
22 D. G. Gray, *J Appl Polym Sci., Appl. Polym. Symp* , 37 (1983) 179–192
23 Y. Okamoto, Z-K. Cao, R. Aburatani and K Hatada, *Bull. Chem Soc. Jpn* , 60 (1987) 3999–4003
24 A. Ichida and T. Shibata, in M. Zıef and L. J Crane (Editors), *Chromatographıc Chiral Separatıons*, Marcel Dekker, New York, 1988, Ch. 9
25 A. Brändström, N.-Å. Bergman, I Grundevık, S. Johansson, L Tekenbegs-Hjelte and K Ohlson, *Acta. Chem. Scand.*, 43 (1989) 569–576