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Reversed retention order and other stereoselective effects in the separation of amino alcohols on Chiralcel OD

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ABSTRACT

The enantioselective resolution of a series of amino alcohols on Chiralcel OD was studied with respect to the effect of temperature, alcohol additive and water content in a mobile phase of hexane with added diethylamine. The chain length between the hydroxy and the amino groups in the solutes had a considerable influence on the stereoselectivity. For one of the amino alcohols a reversal of the retention order between the antipodes was obtained by varying the above parameters. The amino alcohols are retained by at least two chiral sites, one of which is highly dependent on hydrogen bonding. This bonding ability can be directly controlled by the water content of the mobile phase.

INTRODUCTION

The chiral stationary phase Chiralcel OD contains cellulose tris(3,5-dimethylphenylcarbamate) as a selector coated onto a macroporous silica gel [1]. It has been successfully used in liquid chromatography for the chiral separation of racemic drugs such as a series of amino alcohol derivatives with β-adrenergic blocking activity [2–5].

The chiral recognition is assumed to be due to the formation of inclusion complexes [6] and binding to the polar carbamate groups. The carbamate groups interact with the solute by hydrogen bonding with NH and C=O and dipole–dipole bonding to the C=O moiety [7–9]. The hydroxyl group of the amino alcohol seems to be of importance for the chiral recognition process. The retention and stereoselective effect of the Chiralcel OD column can be influenced by hydrogen bonding modifiers added to the mobile phase [4,5].

The chiral separations are mainly performed with mobile phases containing an alkane, such as hexane, and a lower alcohol. The presence of a small amount of an amine such as diethylamine is needed to improve the peak shape when the solutes are primary or secondary amines [1]. The alcohol additives have a low water content and it has been found that controlling the water content is essential for the optimization of the chiral resolution [10].

This paper reports the effects of the chromatographic conditions, mainly alcohol and water content in the mobile phase and temperature, on the stereoselectivity of Chiralcel OD for amino alcohols related to metoprolol. A model for the chiral recognition is suggested which is based on the assumption of two stereoselective binding sites and a specific effect of water on one of these sites.

EXPERIMENTAL

Chemicals

Metoprolol, H 170/31 and H 170/40 as tartrates, *R*- and *S*-metoprolol as sorbates and H 170/64 were obtained from Organic Chemistry, Astra Hässle AB.

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Hexane, 2,2,4-trimethylpentane, propan-2-ol and dichloromethane (HPLC grade), were from Rathburn (Walkerburn, UK), ethanol from Kemetyl (Stockholm, Sweden), propan-1-ol and butan-1-ol from E. Merck (Darmstadt, Germany), butan-2-ol, tertiary butanol and diethylamine from Fluka (Buchs, Switzerland) and diethyl ether from May & Baker (Dagenham, UK). A Milli-Q system (Millipore, Molsheim, France) was used to supply the water.

Instrumentation

The liquid chromatographic system consisted of an LKB 2150 pump (Bromma, Sweden), a Varian 9095 autosampler (Walnut Creek, CA, USA) and a Jasco 821 FP fluorescence detector (Tokyo, Japan) operated at 272 nm (excitation) and 306 nm (emission). A thermostated bath (Lauda RMS, Königshofen, Germany) controlled the water temperature in the column jacket. The chromatograms were recorded on an SP 4400 integrator (Spectra Physics, San Jose, CA, USA). A Metrohm 684 KF Coulometer (Herisau, Switzerland) was used to measure the water content of the mobile phase.

The analytical column (250 × 4.6 mm) was a Chiralcel OD column from Daicel Chemical Industries (Tokyo, Japan), which was used with flow-rates of 0.5–1.0 ml/min at 30°C, unless stated otherwise. The mobile phase contained hexane or 2,2,4-trimethylpentane with the addition of water and various alcohols in the concentrations given for each experiment. All the mobile phases contained 10 mmol/l diethylamine. The mobile phase was prepared in portions of 500 ml and was recirculated during the run. Most of the studies were performed on two different columns to confirm the validity of the observed effects.

Test solutions

Standard solutions of the substances were made up in 0.01 *M* hydrochloric acid. Test solutions in the organic mobile phase were prepared from alkaline standard solutions by extraction with diethyl-ether–dichloromethane (4:1). After phase separation (freezing of the aqueous phase) and evaporation of the organic phase under nitrogen, the amines were dissolved in the mobile phase. Metoprolol was available in enantiomeric forms. Enantiomers of the other substances were obtained by separation on the Chiralcel OD column. The absolute configuration was not known. The injected solutions had a concentration between 1 and 10 μmol/l (20-μl injection).

Calculation

The parameters used in the evaluation of retention and stereoselectivity are the capacity factor, k', and the separation factor, α.

$$k' = (t_R - t_0)/t_0$$

$$\alpha = k'_S/k'_R$$

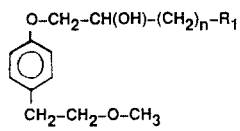
where t_R is the retention time in minutes, and t_0 is the retention time for the interstitial volume (2.5 ml); this was measured for toluene which was assumed to be unretained on the column.

RESULTS AND DISCUSSION

Alcohol modifier

The alcohol additive can affect the retention of the hydrogen bonding solutes in different ways: by improving the solvation in the mobile phase and by competing for the hydrogen bonding sites in the stationary phase. The solutes examined in this study contain amine or hydroxyl groups which can form hydrogen bonds. Their structures are summarized in Fig. 1. Metoprolol, H 170/31 and H 170/40 are closely related and differ only in the length of the carbon chain between the hydroxyl and the amino groups. H 170/64 has no amine function.

The influence of the concentration of propan-1-ol on the stereoselectivity of the column is shown in Fig. 2. Water and diethylamine, which can also



Substance	n	R_1
Metoprolol	1	NH-CH(CH$_3$)$_2$
H 170/31	2	"
H 170/40	3	"
H 170/64	4	H

Fig. 1. Structures of the solutes.

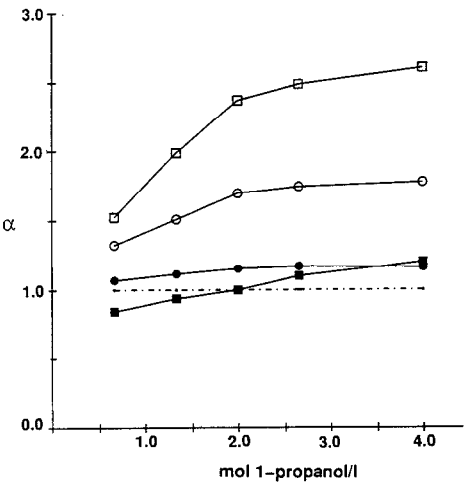


Fig. 2. Influence of propan-1-ol concentration on stereoselectivity. Mobile phase: propan-1-ol, 10 mmol/l diethylamine, 0.8 g/l water in hexane. Temperature, 30°C; flow-rate, 0.75 ml/min. □ = H 170/31; ○ = metoprolol; ● = H 170/64; ■ = H 170/40.

affect retention, were held at a constant concentration in the mobile phase. For the amino alcohols, increasing concentrations of propan-1-ol decrease the retention of all the enantiomers in a similar way. The first eluted antipode of each solute is affected to the same extent; an increase of the propan-1-ol content from 0.67 to 4.0 mol/l in the mobile phase decreased the retention by 76–78%. For metoprolol the *R*-enantiomer was eluted first and it is assumed that the same retention order was followed for the enantiomers of the other solutes.

The retention of the second elute antipode is affected to a different degree, resulting in a change in the stereoselectivity with the concentration of propan-1-ol. The influence of the chain length between the hydroxyl and the amine is remarkable; H 170/31 with a three-carbon chain shows the highest separation factors (α = 1.53–2.65) at all propan-1-ol concentrations, whereas H 170/40 with a four-

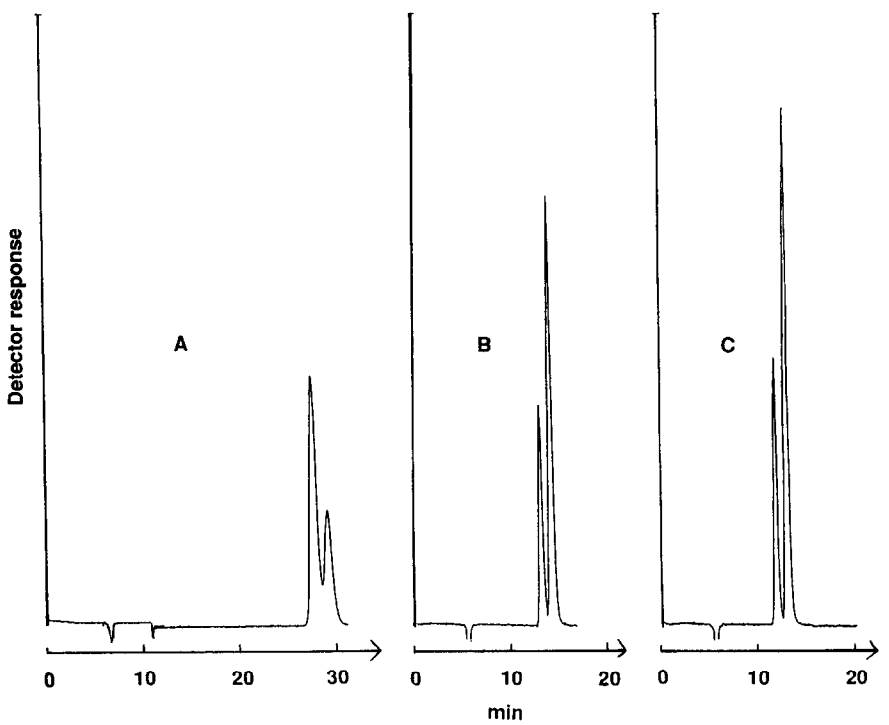


Fig. 3. Effect of propan-1-ol on the resolution of enantiomers of H 170/40. Mobile phase: 1 g/l water, 10 mmol/l diethylamine in hexane and propan-1-ol. Solutes: *R*- and *S*-enantiomers in the ratio 1:2. (A) 0.67 mol/l propan-1-ol (α = 0.94); (B) 1.33 mol/l (α = 1.13); (C) 2.0 mol/l (α = 1.17).

carbon chain shows low stereoselectivity and even a change of retention order between the enantiomers at an intermediate propan-1-ol concentration. An example is given in Fig. 3, which shows chromatograms at different propan-1-ol concentrations using samples that contained the *S*-enantiomer in an excess of 100%. The alcohol H 170/64 has a low separation factor but no reversal of the retention order with changes in alcohol content (Fig. 2).

These observations can be used to elucidate the retention processes. The strong influence of the length of the carbon chain might be due to steric effects, *e.g.*, in the binding to the cavities in the stationary phase. The reversal of the retention order indicates that at least two chiral sites take part in the binding and it seems that the binding effect of one of them, the *S*-antipode, is strongly affected when the concentration of propan-1-ol is changed. It is important to remember that the large change in propan-1-ol content will also change the distribution of water between the stationary and mobile phases and it has been observed earlier that a change in the water concentration can have a significant effect on the stereoselectivity of Chiralcel OD for amino alcohols [10]. A reversal of the elution order for the antipodes of 2-phenoxypropanoic methyl ester on changing the alcohol modifiers has also been observed on a Chiralcel OB column [8]. However, no attention was paid to the water content in this study.

The effects of different alcohol modifiers are shown in Table I. The concentrations of water and diethylamine were maintained at the same constant level as in the previous experiments. The primary and secondary alcohols show rather limited differences in their effects on the retention of the first eluted enantiomer (*R*). In general, ethanol seems to give lower separation factors than the higher alcohols. The peak shape was good for all additives except tertiary butanol.

TABLE I

INFLUENCE OF THE ADDITION OF ALCOHOL ON RETENTION OF THE AMINO ALCOHOLS

Mobile phase, 1.33 mol/l alcohol, 10 mmol/l diethylamine, 0.8 g/l water in hexane. Temperature, 30°C; flow-rate, 0.75 ml/min.

Alcohol	Metoprolol		H 170/31		H 170/40		H 170/64	
	k'_R	α	k'_1	α	k'_1	α	k'_1	α
Ethanol	1.11	1.43	1.42	1.96	1.97	1.04	1.20	1.11
Propan-1-ol	1.07	1.60	1.36	2.12	1.89	1.06	1.35	1.12
Butan-1-ol	0.94	2.15	1.16	2.37	1.77	1.10	1.35	1.14
Propan-2-ol	1.31	1.89	1.81	2.50	2.79	1.20	1.38	1.34
Butan-2-ol	1.25	2.05	1.67	2.72	2.81	1.00	1.56	1.22
tert.-Butanol	2.20	2.16	2.47	2.14	4.37	1.00	2.06	1.25

Water content

It has been emphasized that the water content of the mobile phase has a significant effect on the chiral resolution on the Chiralcel OD phase. This is seen in Fig. 4, which shows the retention of the enantiomers of H 170/40 at different water contents using a constant alcohol concentration. The *S*-enantiomer shows a decreasing value of k' with increasing water content, while k'_R seems to be almost unaffected. The effect of the water content is obvious in the range 0.1–1.0 g/l, but a higher water content does not influence the separation factors or chromatographic properties. For metoprolol, no shift of elution order is obtained as the enantiomers have too high a retention difference at low water contents. On increasing the water concentration, k'_S and the α-value decrease strongly, whereas k'_R is not influenced.

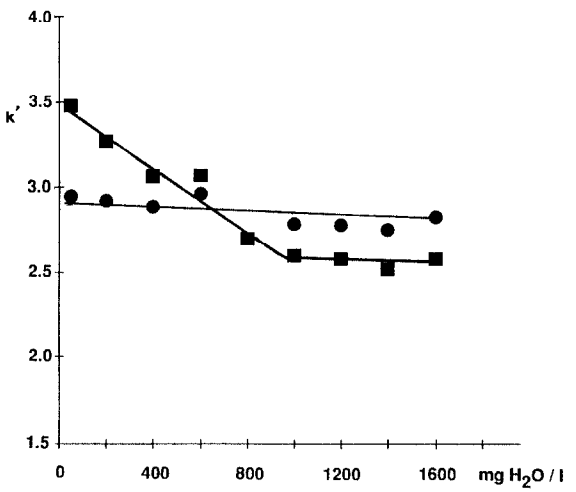


Fig. 4. Influence of water on the enantiomers of H 170/40. Mobile phase: 1.0 mol/l propan-1-ol, 10 mmol/l diethylamine and water in hexane. Temperature, 30°C; flow-rate, 0.5 ml/min. ■ = *S*-Enantiomer; ● = *R*-enantiomer.

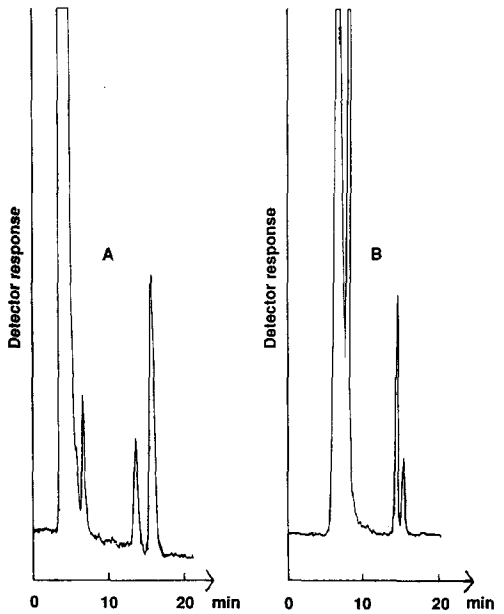


Fig. 5. Effect of temperature on the resolution of the enantiomers of H 170/40 on Chiralcel OD. Mobile phase: 1.0 mol/l propan-1-ol, 10 mmol/l diethylamine and 1 g/l water in 2,2,4-trimethylpentane. Solutes: *R*- and *S*-enantiomers in the ratio 2:1. (A) 15°C, flow-rate 1.0 ml/min; (B) 55°C, flow-rate 0.5 ml/min.

Temperature effect

A change in temperature can have a fairly strong effect on the stereoselective separation of amino alcohols on Chiralcel OD. An example is given in Fig. 5, which shows that the retention order for the enantiomers of H 170/40 is reversed when the temperature is changed from 15 to 55°C, if water is added to the mobile phase. 2,2,4-Trimethylpentane was used as the alkane component in the mobile phase to make it work at higher temperature. The injected samples contained the *R*-enantiomer in a 100% excess.

Van 't Hoff plots for the same substances in the temperature range 8–55°C are shown in Fig. 6. These plots show the highly significant effect of water on the stereoselectivity; a reversal of the retention order is only obtained in the presence of a higher water content. Under identical temperature conditions and across the shared temperature range, the overall reactions, as indicated by the capacity factor, are always less for the *R*-enantiomer than for the *S*-enantiomer.

The Gibbs free energy for the solute–stationary phase interaction, ΔG^0, is related to the natural logarithm of the capacity factor by

$$\ln k' = -\Delta G^0/RT + \Phi \quad (1)$$

where R is the gas constant, T is the absolute temperature and Φ is the natural logarithm of the phase ratio in the column. The equation can be written with the appropriate enthalpy, ΔH^0, and entropy, ΔS^0, as

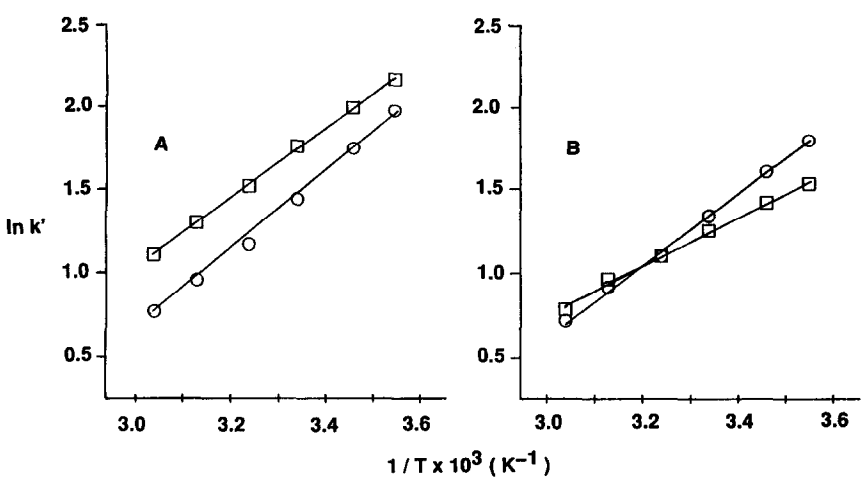


Fig. 6. Van 't Hoff plots for the enantiomers of H 170/40. Temperature range, 8–55°C. Mobile phase, 0.93 mol/l propan-1-ol, 10 mmol/l diethylamine in 2,2,4-trimethylpentane. Flow-rate, 0.5 ml/min. (A) No water added; (B) 1 g/l water added to the system. (□) *S*-Enantiomer; (○) *R* -enantiomer.

$$\ln k' = -\Delta H^0/RT + \Delta S^0/R + \Phi \quad (2)$$

The enthalpy can be estimated from the slope of the Van 't Hoff plot. The values presented in Table II show that the water added to the mobile phase has a different influence on the enantiomers metoprolol, H 170/31 and H 170/40. For the *R*-antipode the decrease of the slope is fairly low and the change in $-\Delta H^0$ does not exceed 2.1 kJ/mol. The effect on the *S*-enantiomer is considerably larger and the decrease of $-\Delta H^0$ is in the range 5–8 kJ/mol. For the alcohol H 170/64, the water has about the same effect on both enantiomers.

Contributions of the enthalpy parameter to the enantioselectivity can only be inferred from the differences between values for the individual enantiomers. However, the results of the Van 't Hoff plots support the view that the amino alcohols rather than the alcohol are retained by two chiral sites. The effect of one of these sites seems to be due to hydrogen bonding as its binding ability is highly affected by water. This site has a significantly different influence on the *R*- and *S*-enantiomers.

Retention processes

The chiral selective stationary phase Chiralcel can be assumed to consist of different sites, as discussed above and suggested by Gaffney *et al.* [8]. Our studies on Chiralcel OD show changes in the separation factor, α, by varying the alcohol concentration, temperature or water content. These changes in selectivity also lead to changes in the retention order between the enantiomers of an amino alcohol when the separation factor is fairly low, as for H 170/40.

When the antipodes of a solute are retained by two different kinds of chiral sites, their effects can be illustrated by the partial capacity factors, k'_{S1} and k'_{R1} for one of the sites and k'_{S2} and k'_{R2} for the other. This gives the following expression for the separation factor:

$$\alpha = (k'_{S1} + k'_{S2})/(k'_{R1} + k'_{R2}) \quad (3)$$

The effects of adding water and alcohol to the mobile phase can be characterized by a simplified retention model based on the following assumptions: (1) the water content in the mobile phase has an influence on the binding effect of only one of the chiral sites; (2) the alcohol has a direct influence on the retention of the enantiomers only by solvation in the mobile phase; and (3) the alcohol binds or solvates water in the mobile phase, thereby influencing the accessible (free) concentration of water. The solvation of water is temperature-dependent.

If the effect of water is due to Langmuir adsorption, the retention of the *S*-enantiomer can be expressed by

$$k'_S = qK_0K_S/[1 + K_{H_2O}(H_2O)_m] \quad (4)$$

where q is the phase volume ratio, K_0 the total binding capacity of the site, K_S the binding constant for the solute, K_{H_2O} the binding constant for water and $(H_2O)_m$ the accessible water concentration in the mobile phase.

k' of the *S*-antipode decreases with increased water content (Fig. 4). The effect levels off at about 1000 mg/l of water, which supports the assumption of adsorption according to a Langmuir isotherm. For the *R*-antipode there is no significant effect of water, which indicates that the *R*-antipode is not retained by the site affected by water. Assuming that only k'_{S2} in eqn. 3 changes with the water content, whereas k'_{S1} and k'_{R1} are unchanged and k'_{R2} is negligible, a combination of eqns. 3 and 4 gives

$$\alpha = A + B/[1 + K_{H_2O}(H_2O)_m] \quad (5)$$

where $A = k'_{S1}/k'_{R1}$ and $B = qK_0K_S/k'_{R1}$ are constant.

Eqn. 5 shows the prerequisites for the reversal of the retention order between the enantiomers. A must be less than 1 whereas $(A + B)$ must be greater than 1. α is controlled by A at high water content and

TABLE II

ENTHALPY ($-\Delta H^0$ kJ/mol) ESTIMATED FROM THE SLOPE OF VAN 'T HOFF PLOTS

Mobile phase, 0.93 mol/l propan-1-ol, 10 mmol/l diethylamine in 2,2,4-trimethylpentane. Temperature range, 8–55°C. (A) No water added; (B) 1 g/l water added to the system.

Amino alcohol	*R*-Enantiomer		*S*-Enantiomer	
	A	B	A	B
Metoprolol	8.6	8.6	15.4	10.1
H 170/31	13.8	12.6	26.4	18.4
H 170/40	19.7	17.6	17.5	12.1
H 170/64	13.6	12.4	14.3	13.0

($A + B$) at low water contents. It must be emphasized that eqn. 5 is based on several simplified assumptions. The non-linear relationship between α and $(H_2O)_m$ makes a control of its quantitative validity difficult.

The alcohol in the mobile phase contributes to the transport of the solutes through the column. The elution strength is different for different alcohols, as can be anticipated (Table I), straight-chain alcohols being stronger eluents. An increased alcohol content gives a decreased retention of the solutes, as expected (Fig. 3). However, in a non-polar organic mobile phase, as in the systems studied here, the alcohol is responsible for solvation of the water in the mobile phase. The degree of complexation is dependent on the concentration and the nature of the alcohol. The extent of accessible of free water, $(H_2O)_m$, is a function of this and decreases with increased alcohol content. The effect of the alcohol concentration on the chiral selectivity is thus mediated through water. The influence of the structure of the alcohol on the enantioselectivity is more complex, but is probably related to the accessible water content.

The effect of temperature on the selectivity may be due to the influence on the chiral sites of the stationary phase. However, this effect might be mediated through the free water content by affecting the degree of solvation of water by the alcohol in the mobile organic phase.

CONCLUSIONS

The stereoselectivity of Chiralcel OD for amino alcohols related to metoprolol can be controlled by alcohol additives and the water content of the mobile phase in addition to temperature. For solutes with low separation factors it is even possible to achieve a reversal of the retention order. It has been shown that the amino alcohols are retained by at least two chiral sites. The binding ability of one of these is highly dependent on hydrogen bonding and its effect can be directly controlled by the water content of the mobile phase.

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