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Study on Supramolecular Systems of Cyclodextrins and Magnolol, Honokiol by Thin Layer Chromatography

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The interactions between magnolol, honokiol and six cyclodextrins (CDs) have been studied by reversed-phase thin-layer chromatography. The $NH_3{\cdot}H_2O-NH_4Cl$ buffer containing various CDs (pH 9.7, 20°C) and polyamide plate are selected, respectively, as mobile and stationary phases. CDs except for α-CD and CM-β-CD can form inclusion complex with magnolol. However, the strong space resistance between honokiol and CD makes it difficult for them to form inclusion complex. The comparison of inclusion capacity of different CDs indicates that for the ionic CDs the charge interaction plays an important role in the inclusion procedure. The thermodynamic parameters of interaction imply that the inclusion process shows the enthalpy–entropy compensation effect. The $\Delta H - T\Delta S$ plot for CDs displays an excellent linear relationship, affording a very large slope ($\alpha = 1.1$) and intercept ($T\Delta S_0^0 = 16.3$), which exhibits that the enthalpic gain from the inclusion complexation is completely canceled out by the entropic loss from the conformational changes caused upon guest inclusion. The marked influence of CDs on the hydrophobicity of magnolol suggests that this interaction may modify the biological properties of magnolol.

Keywords: Cyclodextrin; Reversed-phase thin-layer chromatography; Magnolol; Honokiol supramolecular System; Enthalpy–entropy compensation effect

INTRODUCTION

Cyclodextrins (CDs) are cyclic oligosaccharides which have the ability to form inclusion complex with many organic and inorganic compounds [1].

The formation of inclusion complexes modifies the physical and chemical characteristics of guest molecules. CDs are used as novel drug carriers in the pharmaceutical field to improve drug stability, solubility and bioavailability [2–4]. The use of a parent CD is limited in pharmaceutical application just because of its low solubility. However, the chemically modified CDs have been employed successfully [5,6]. The hydrophilic CD derivatives are mainly methylated and hydroxypropylated derivatives which have stronger complexing capacity than β-CD. Now, the introduction of ionogenic groups onto the cyclodextrin ring is a new branch of research [7,8]. The chargeable CD derivatives differ in complex effect from other CD derivatives. It can sustain the release rate of drug [9] and enhance the peak concentration of drug in blood [10].

Our previous studies were aimed at the flavonoid–CD [11] and porphyrin–CD [12,13] supramolecular systems. In this paper, the inclusion complexations of magnolol, honokiol with two ionic derivatives Sulfurbutylether-β-cyclodextrin (SBE-β-CD) and Caboxymethyl-β-cyclodextrin (CM-β-CD) have been studied by means of thin-layer chromatography besides α, β, γ-CD and Hydroxypropyl-β-cyclodextrin (HP-β-CD).The inclusion constants are determined and the thermodynamic parameters of the magnolol–CD inclusion procedure are calculated. From the $\Delta H - T\Delta S$ plot, the unit slope and the large intrinsic entropic gain $T\Delta S_0^0$ jointly indicate that the enthalpic gain from the inclusion complexation is completely canceled out by the entropic loss from the conformational changes caused upon guest inclusion. As the formation of inclusion complex may influence not only the physical and chemical parameters of magnolol but also their absorption and half-life in human body, moreover, magnolol is insoluble in water [14], the

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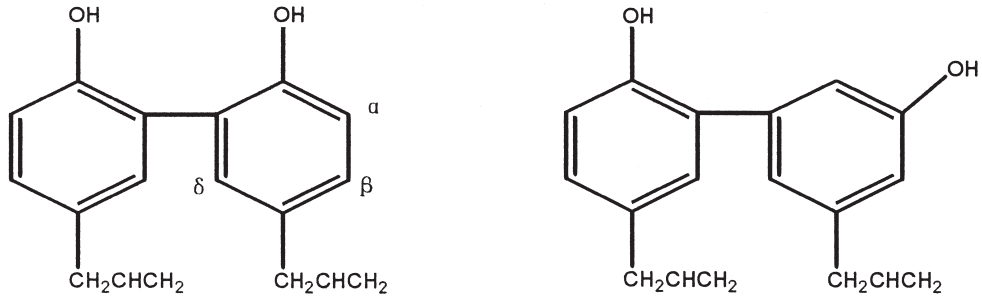


FIGURE 1 Chemical structure of magnolol and honokiol.

study of such complex inclusion may lead to the development of more effective drug with better bioavailability. The chemical structures of magnolol and honokiol are as follows in Fig. 1.

EXPERIMENT

Apparatus and Reagent

Magnolol and Honokiol were purchased from Beijing Drug and Biological Product Institute. β-CD (95%, YuNan Gourmet Factory) was purified by recrystallization in double distilled water. HP-β-CD, with MW = 1380 and degree of substitution (D.S.) = 0.6, was purchased from Aldrich. α-CD and γ-CD were purchased from Sigma. CM-β-CD, SBE-β-CD were synthesized employing the paper written by Jacques Reuben [15] UV_{254} lamp and pH meter were made by Leici Instrument Factory Shanghai, China.

Method

The drugs were dissolved separately in methanol at a concentration of 1 mg/ml and 1 μl of solution was plotted on the polyamide plates (the stationary phases plate). CDs were added to the $NH_3{\cdot}H_2O$–NH_4Cl buffer (pH 9.7) at $0–1\times10^{-2}$ mol/l concentrations. Developments were carried out in chambers ($6.8\times10\,cm^2$height) at room temperature, the distance of development being about 8 cm. The final position of the solutes was detected under the UV lamp.

RESULTS AND DISCUSSION

The Formation of Drug-CD Complex

The comparison of $NH_3{\cdot}H_2O$–NH_4Cl buffer with KH_2PO_4–H_3PO_4 buffer (concentration of CD = 1×10^{-2} mol/l) suggests that the R_f value of drug in the first buffer system is better than that of the second. So the $NH_3{\cdot}H_2O$–NH_4Cl buffer containing CD solution is selected as mobile phase.

Table I shows the R_f value and capacity factor (k') value (calculated according to the following relationship) of drugs.

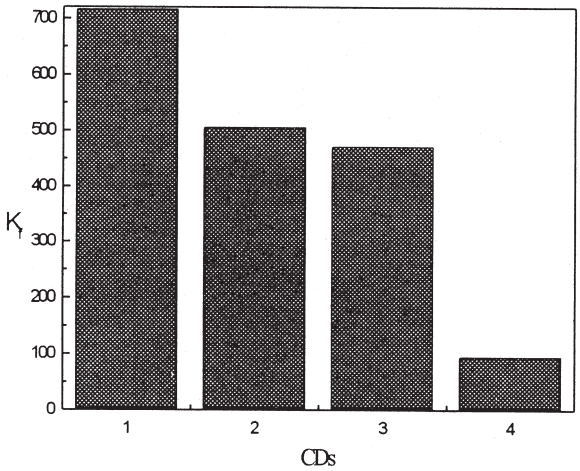
$$K' = \frac{1 - R_f}{R_f}$$

It indicates that β-CD, HP-β-CD, SBE-β-CD, γ-CD are effective agents for increasing R_f values of magnolol on polyamide plate. However, α-CD and CM-β-CD cannot increase the R_f values distinctively because α-CD have a smaller apolar cavity which cannot adjust to the drug molecular magnitude, which means that the α-CD-drug complexes cannot be formed; CM-β-CD, the ionic derivative of β-CD ($pK_a = 4–5$), and magnolol are negative ion in the condition of pH 9.7, so the charge repel interaction between CM-β-CD and drug, hampers the inclusion of CM-β-CD drug complex.

In addition, these mobile phases containing various CDs cannot increase the R_f values of honokiol remarkably. It implies that compared to magnolol, the honokiol–CD complex cannot form

TABLE I The effect of [CD] on the R_f and k' value of magnolol

CDs		Parameters [CD] = 0,2,4,5,8,10 × 10 − 3 mol/l						r
β-CD	R_f	0.103	0.162	0.220	0.264	0.310	0.342	0.988
	K'	8.708	5.173	3.545	2.788	2.225	1.923	
HP-β-CD	R_f	0.076	0.184	0.280	0.300	0.343	0.377	0.990
	K'	12.15	4.435	2.571	2.333	1.915	1.653	
SBE-β-CD	R_f	0.081	0.151	0.140	0.171	0.196	0.294	0.977
	K'	11.35	5.622	6.142	4.847	4.102	2.401	
γ-CD	R_f	0.066	0.093	0.107	0.100	0.106	0.123	0.973
	K'	14.15	9.753	8.345	9.000	8.434	7.130	
α-CD			$R_{f\ 0.081}$ ([CD] = 0 mol/l)			$R_{f\ 0.088}$ ([CD] = 0.01 mol/l		
CM-β-CD			$R_{f\ 0.076}$ ([CD] = 0 mol/l)			$R_{f\ 0.073}$ ([CD] = 0.01 mol/l		



1 HP-*B*-CD, 2 *B*-CD, 3 SBE-*B*-CD, 4 *r*-CD

FIGURE 2 Show of the inclusion constants of drug-CD complexes.

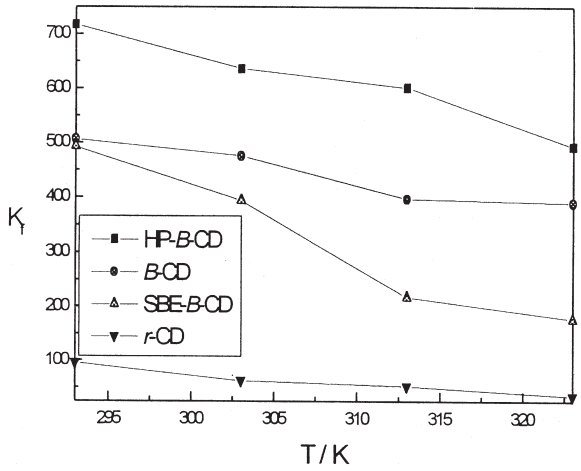


FIGURE 3 The effect of temperature on inclusion constants.

just because of the structure differences between magnolol and honokiol. At the same time, it also demonstrates that the binding site is at the different part of the chemical structure of magnolol and honokiol.

The Determination of Inclusion Constant

Assume that magnolol can form 1:1 complex with these CDs. The R_f value increases sharply with the increasing concentrations of CDs, and the inclusion constants of drug with the several CDs are determined by the following equation [16].

$$\frac{R_f}{1-R_f} = \frac{1}{\Phi K[A]} + \frac{K_f}{\Phi K[A]}[\mathrm{CD}]$$

where Φ is the phase ratio (the ratio of mobile-phase volume and stationary-phase volume) and R_f is the retardation factor of a solute in thin-layer chromatography, A is a stationary phase adsorption, [CD] is the concentration of cyclodextrin, equilibrium constant between drugs and stationary phase is K and K_f is the inclusion constant. This equation shows linear behavior.

Plots of $R_f/1-R_f$ vs. [CD] give curves in which the slope corresponds to $K_f/\Phi K[A]$ and the intercept is $1/\Phi K[A]$. From the ratio of slope over intercept we can calculate K_f. The good linearity of the plots in Table I supports the existence of 1:1 inclusion complex.

TABLE II The thermodynamic parameters of different cyclodextrins

	ΔG (kJ/mol)	$-\Delta H$ (kJ/mol)	ΔS (kJ/mol)
HP-β-CD	−16.02	9.8	0.02145
β-CD	−15.16	8.1	0.02425
SBE-β-CD	−15.09	30.5	−0.0519
γ-CD	−11.10	27.6	−0.05615

Comparison of Inclusion Capacity of Different CDs

The inclusion constants of different CD-drug complexes in Fig. 2 manifest that the consequence of inclusion capacity of different CDs is HP − β − CD > β − CD > SBE − β − CD > γ − CD. It indicates that the hydrophilic derivative of β-CD (HP-β-CD) has remarkably strong inclusion capacity, while γ-CD which has a bigger cavity that cannot be matched with the size of magnolol shows lower inclusion capacity than that of β-CD. However, the charges repel interaction between SBE-β-CD and magnolol decreases the inclusion capacity of SBE-β-CD, which implies that for the ionic cyclodextrins the charge interaction plays an important role in the inclusion procedure.

The Effect of Temperature on Inclusion Capacity

At different temperature (from 293 to 323 K), the inclusion constants of magnolol–CD complexes are obtained. Figure 3 shows the effect of temperature on the value of inclusion constants. The curves descend sharply. That means the high temperature is not suitable for inclusion procedure. At high temperature, the drug molecule moves violently, which lead to the drug entering and coming out the cavity of CD more frequently. Accordingly, the inclusion capacity of CD is subdued.

Enthalpy–entropy Compensation Effect

From the inclusion constant, the ΔG of the inclusion process can be calculated easily. According to following formula: $\Delta G/T = \Delta H/T - \Delta S$, plots of $\Delta G/T$ vs. $1/T$ give a curve in which the slope corresponds to ΔH and the intercept is $-\Delta S$. Table II shows the thermodynamic parameters of different CDs. As can be seen from,Table II the inclusion

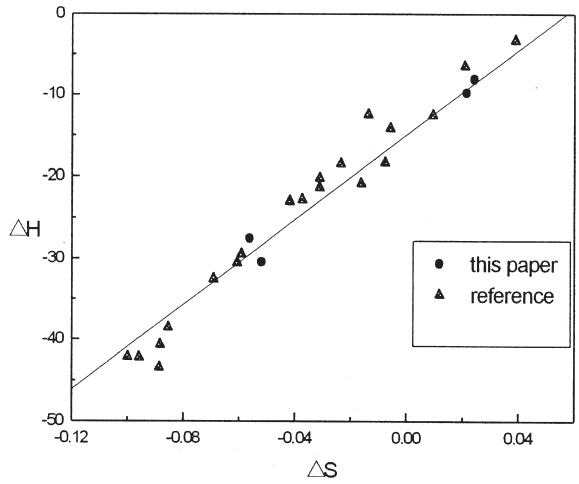


FIGURE 4 Enthalpy–entropy compensation plot.

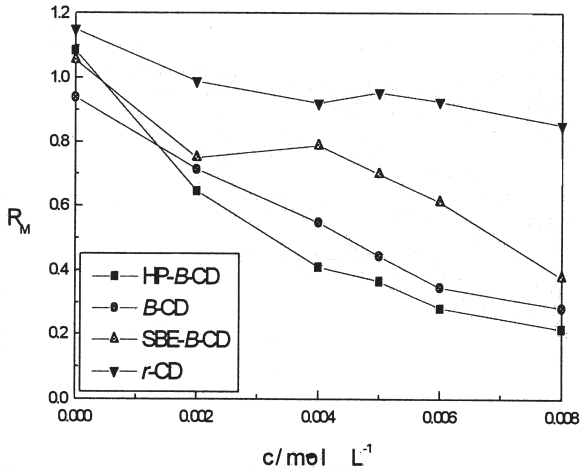


FIGURE 5 The change of R_M value with the increase of [CD].

complexation of magnolol with CDs is exclusively exothermic and mostly enthalpy driven with varying positive or negative entropic contribution.

According to the equation [17] $\Delta(\Delta G)=\Delta(\Delta H) - T\Delta(\Delta S)$, $T\Delta S^0=\alpha\Delta H+T\Delta S^0{}_0$ with all the thermodynamic data obtained in this paper and reported elsewhere [18], plot of ΔH vs. ΔS gives a good linear relationship and the slope of the curve is the compensation temperature, here, $T = 290\,\mathrm{K}$. Fig. 4 indicates that the resulting change in ΔS is proportional to the accompanying change in ΔH, and the inclusion process shows the enthalpy–entropy compensation effect [19]. In accordance with the literature [18], the $T\Delta S^0$ are plotted against the ΔH to offer a good straight line with a correlation coefficient of $r = 0.989$. The slope very close to unity $\alpha = 1.1$ indicates that despite the apparently rigid skeleton of CD, the inclusion complexation causes substantial conformational changes involving the reorganization of the original hydrogen bond network, while the intermediate intercept ($T\Delta S^0{}_0 = 16.3\,\mathrm{kJ\,mol^{-1}}$) means fairly extensive dehydration occurring upon inclusion. The unit slope and the large intrinsic entropic gain $T\Delta S_0^0$ jointly indicates that the enthalpic gain from the inclusion complexation is completely canceled out by the entropic loss from the conformational changes caused upon guest inclusion [16].

The Enhancement of CD on the Solubility of Magnolol

The R_M value characterizing the molecular hydrophobicity in reversed-phase thin-layer chromatography is calculated for magnolol in each eluent [12].

$$R_M = \log(1/R_f - 1)$$

The effect of the concentration of CDs on the R_M value can be seen in Fig. 5. It suggests that the hydrophobicity of magnolol decrease with the increasing concentration of CD, which proves that CDs can enhance the solubility of magnolol.

Conformation Analysis by NMR Spectroscopy

Additional evidence for the formation of magnolol–CD complex can be obtained from changes of the chemical shifts of ^{1}HNMR spectra. The complex was prepared by adding the methanol solution of magnolol to the water solution of HP-β-CD. Keep both the magnolol and HP-β-CD at the same concentration (1×10^{-3} mol/l) in this mixed solution and stir it, then place the mixture in refrigerator for more than 24 h, and the solidified complex is formed when all solvents are removed. In this experiment CD_3COCD_3 is selected as the solute of magnolol and D_2O is selected for the complex, and all data are obtained under the condition of 300 MHZ. The ^{1}HNMR spectrum of HP-β-CD, magnolol and their complex are shown in Fig. 6. As shown in Fig. 6, the chemical shift of the interior protons H-3, H-5, move upfield by 0.09 and 0.06 ppm, which is perhaps due to the direct interaction of these protons with magnolol molecule. By contrast, the chemical shifts of outer protons H-2 and H-4 are relatively unchanged, indicating the interaction occurs inside the cavity instead of exterior of the torus. Furthermore, the downfield (0.04 ppm) chemical shift of δ protons of phenyl, the upfield (0.06, 0.12 ppm) chemical shift of α and β phenyl protons and the upfield (0.22 ppm) of methylene protons (near the benzene ring), indicate the phenyl groups of magnolol enter the cavity of HP-β-CD.

Discussion

To compare TLC method with other methods that are often used to study the supramolecular system, such

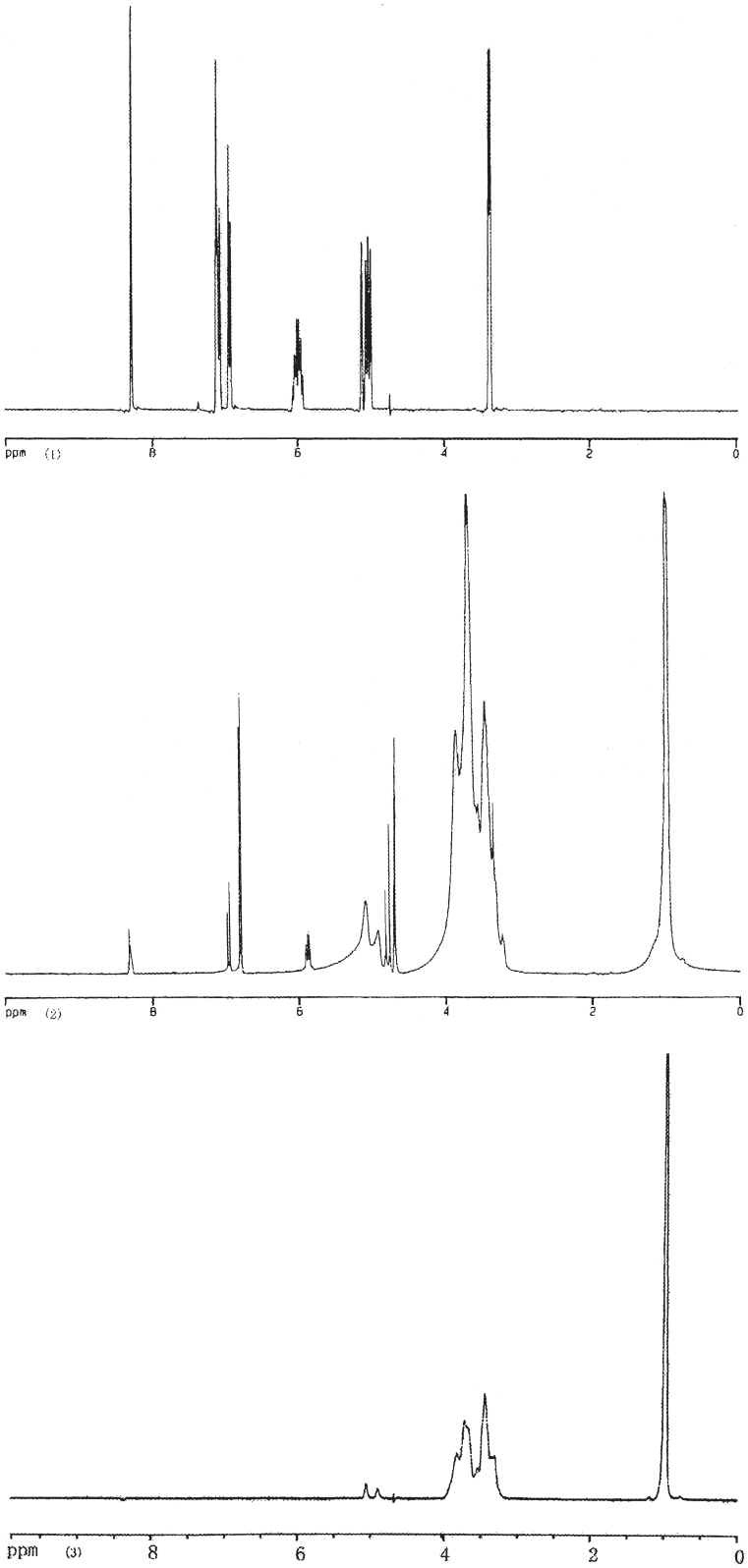


FIGURE 6 ^{1}HNMR spectra of magnolol (in CD_3COCD_3), HP-β-CD and the complex (in D_2O) (1) 1×10^{-3} M magnolol (2) complex (3) 1×10^{-3} M HP-β-CD.

as UV spectrophotometry, spectrofluorimetry, polarography, HPLC, NMR, free solution capillary electrophoresis (FSCE) and so on. Although we cannot perform all these methods mentioned here, the essential difference shed light on advantages and disadvantages, respectively.

The UV spectrophotometry is limited to UV absorbing analytes but appears very easy to perform, moreover, there are few constraints over the choice of the medium except those required for analyte solubility [20].

For spectrofluorimetry, generally, the variations of fluorescence intensity between free and complexed guest are large. So the determination of K_f is precise and easy. The method is limited to fluorescent analytes but requires only a low concentration of guest [21].

All these two spectroscopy methods have the same drawback; the slight shifts in wavelengths (adsorption and fluorescence emission) observed for the various concentrations of CDs influence the spectra.

For polarography, the procedure is easy to operate and the change of peak current (*i*p) is remarkable. But just on the condition that the variation of *i*p must be controlled by diffusion [22], the inclusion constant can be calculated by "electric current method" [12]. As a result, the concentration of guest is higher than other methods.

HPLC is less easy to perform and unsuited to ionic analytes (constraint of pH). The necessity of preparing different mobile phases is tedious and column equilibration subsequent to each change in CD concentration is time-consuming [23].

^{1}HNMR and ^{13}CNMR are good methods for the study of the inclusion mechanism and the determination of the binding site. According to the change of the chemical shifts (guest) in the presence of different concentrations of CD, inclusion constant can be calculated. The apparent disadvantage is too expensive to afford [24,25].

For FSCE, the buffer is easy to prepare and the time to equilibrate is short. Moreover, only small quantities of analyte and reagents are needed. However, the method is limited to ionizable analytes [26].

As for TLC, the value of K_f lacks precision because the performance is easily influenced by other conditions such as temperature and vibration etc. However, the time spend on development is short and the reagents needed is less.

CONCLUSIONS

Because of the enhancement of CD on the solubility of magnolol, it is probable that the complex inclusion of drug with CDs modifies the various biological parameters of magnolol in living organisms, at least, the bioavailability. And for the ionic CDs the charge interaction played an important role in the inclusion procedure. The thermodynamic parameters of interaction, implies that the inclusion process shows the enthalpy–entropy compensation effect. The thin-layer chromatography is proved to be available, easy to perform, less time consuming and less reagent consuming for the study on the inclusion interaction.

Acknowledgements

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