Structure and molecular dynamics of the ternary complexes of cyclodextrins with spin-labeled indoles and hydrocarbons in the solid phase. ESR spectroscopy and quantum chemical calculations

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The ternary inclusion complexes of two spin-labeled pyrrolidine- and piperidine-containing indole derivatives (**1** and **2**, respectively) and two hydrocarbons, benzene and cyclohexane, with γ- and β-cyclodextrins (CD) (altogether eight complexes) were prepared and studied by ESR in the solid phase over a broad temperature range. For most ternary complexes, the hydrophobicity of the NO group environment is much higher than for binary complexes devoid of hydrocarbons. The rotational mobility of both spin-labeled indoles in the ternary complexes of γ-CD is much greater than in binary complexes of γ-CD, which is due to transition to the stoichiometry 2γ-CD—**1**(**2**)—C_6H_6(C_6H_{12}) from 1 : 1 stoichiometry. The guest rotational mobility in the complexes with either of the CD is higher for **2** than for **1**. The saturation transfer ESR spectra show that the rotational mobility of **1** in γ- and β-CD in the presence of C_6H_6 or C_6H_{12} has a character of fast librations in an angular cone, whose amplitude increases with temperature, whereas for radical **2**, the rotation occurs in the full solid angle. The structures and energies of all complexes were calculated for different modes of inclusion of guest molecules using the PM3 method with the standard set of parameters. The calculation results are in qualitative agreement with experimental data. The results demonstrate the possibility of substantial modification of the molecular dynamics and hydrophobicity of the environment of "functional" guest molecules by introducing a second regulatory guest molecule into the CD cavity.

Key words: γ- and β-cyclodextrins, spin-labeled indoles, inclusion complexes, ternary complexes, molecular dynamics, environment hydrophobicity, ESR spectroscopy, microwave saturation transfer ESR spectroscopy.

Studies of the inclusion complexes of cyclodextrins (CD) have aroused considerable interest in recent years. The solubility of guest molecules may increase by several orders of magnitude upon inclusion into the CD cavity; simultaneously, their physical and chemical properties considerably change.[1—10] The inclusion complexes with CD have been the subjects of numerous studies carried out by various methods (see, for example, Refs 1—7); however, important aspects of their structure and especially the dynamics of guest molecules in the CD cavities[8—12] remain obscure. The use of spin-labeled guest molecules is a promising approach to the study of the structure and molecular dynamics of the inclusion complexes. The ESR spectra of spin labels are highly sensitive to rotational dynamics over a broad range of correlation times and to the environment polarity and intermolecular contacts.[13a] Previously,[14,15] we studied the structure and molecular dynamics of the binary complexes of spin-labeled indole analogs containing piperidine and pyrrolidine radical fragments with β- and γ-CD in an aqueous solution and determined the stoichiometry, the binding constants, the polarity of the environment, and the accessibility of the reporter NO group of guest molecules for water-soluble paramagnetic ions. In the solid phase where the same complexes form a dilute solid solution in the CD, the molecular dynamics of guest molecules in the β- and γ-CD cavities have been studied over a broad temperature range.[16]

In addition to binary complexes, the ternary CD complexes containing two identical or different guest molecules can be formed.[17—22] However, there are much less data on the structures of such complexes than for binary complexes; to our knowledge, data on the dynamics of guest molecules in ternary complexes are totally missing. As regards construction of functional supramolecular systems, of particular interest is the preparation and investigation of the CD-based ternary complexes that contain guest molecules of two types, specifically, one functional

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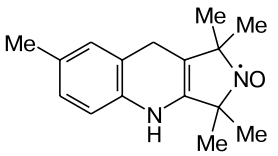
molecule and one regulatory molecule, *i.e.*, able to change the properties of the functional molecule, for example, the fluorescence yield, the catalytic activity, the redox potential, *etc*.

In this work, we attempted to achieve such regulation for spin-labeled indoles as models of functional molecules using benzene and cyclohexane as the second guest molecules. The purpose of the study was, first, to prepare ternary complexes of spin-labeled indoles with β- and γ-CD in the solid phase, second, to study by ESR the polarity of the environment and the molecular dynamics of spin-labeled guests depending on the structures of CD and guest molecules, especially, the second guest molecule, and third, to carry out quantum chemical calculations for the geometry and energy states of ternary complexes.

Experimental

Commercial β-CD (Calbiochem, Germany), γ-CD hydrate, benzene, and cyclohexane (Aldrich, Germany) were used. As in previous studies, indole derivatives, *viz.*, radicals with pyrrolidine (**1**) and piperidine (**2**) rings, were used as spin-labeled guest molecules.[23]



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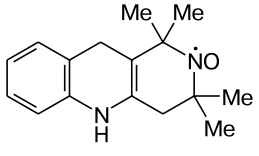

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To prepare complexes with β-CD, alcohol solutions of **1** or **2** (10^{-2} mol L^{-1}, 10 μL) were evaporated to dryness in an argon flow and then the obtained film was dissolved in benzene or cyclohexane. The label solubility in these compounds is at most 0.1—0.2 mmol L^{-1}. An aqueous solution of β-CD (23 mmol L^{-1}) was prepared with heating (50 °C). The solution of β-CD (2 mL) was mixed with heating with a 0.15 mmol L^{-1} solution of spin label (0.67 mL), which corresponded to the following component molar ratio: 1 (spin label) : 480 (β-CD) : $75 \cdot 10^3$ (C_6H_6 or C_6H_{12}). As the mixture was cooled down with vigorous stirring, the complex precipitated as a flaky white-colored solid. The mixture was stirred in a turbulent mixer for an additional 30 min and left for 24 h at ~20 °C. The settled mixture was centrifuged twice for 15 min at a rate of 8000 rpm (~6000 *g*) on a TU5.375 centrifuge (Poland). The compacted precipitate was separated from the solution and dried at 40 °C to a constant weight. In the synthesis of γ-CD complexes, the sequence of operations and the temperature conditions were the same, but 1 mL of a solution of γ-CD (50 mg mL^{-1}) and 1 mL of a 0.15 mmol L^{-1} solution of the label in C_6H_6 or C_6H_{12} were used. The complex was washed in C_6H_6 or C_6H_{12} and then dried to a constant weight. This gave eight complexes: four complexes with each of β- and γ-CD containing different pairs of guest molecules.

The ESR spectra were recorded on a Bruker ER-200D instrument in glass tubes maintained at a constant temperature to within ±0.5 °C. The temperature dependences of the ESR spectra of complexes were studied in the range of 140—340 K with reversibility check. The ESR spectra linear to the microwave field were recorded at a low microwave power (P = 32 dB, $H_1 \approx$ 0.0125 G) and a small modulation amplitude, which rule out the spectrum distortion. The ESR absorption spectra of the second harmonics of modulation in 90° phase with a modulation field under partial microwave saturation (saturation transfer ESR spectra, V_2') were recorded by a reported procedure.[24] The modulation frequency of the magnetic field in the resonator was 50 kHz, the microwave field amplitude was H_1 = 0.25 G, and the modulation amplitude was 5 G. The modulation phase was adjusted by the "zero" method.[24]

Results and Discussion

The ESR spectra linear to the microwave field for all complexes in the solid phase formally correspond to so-called "slow" rotation region (Fig. 1). The distance between the outer extrema of the spectrum ($2A_{zz}'$), which decreases upon temperature rise because of the motional averaging of the anisotropic hyperfine coupling (HFC), was used as the experimental parameter of the rotational mobility of a probe.

The polarity of the environment of spin-labeled guests in ternary complexes. The environment polarity of guest molecules was characterized using the same parameter, $2A_{zz}$, measured at a liquid nitrogen temperature where the molecular motion can be considered to be practically absent. In this limit, the $2A_{zz}'$ value is equal to twice the z-component of the HFC tensor ($2A_{zz}$). The dimensionless hydrophobicity parameter for the solid phase (h_s), by analogy with solutions,[15] is given by[16,25]

$$h_s = (2A_{zz}^{w} - 2A_{zz})/(2A_{zz}^{w} - 2A_{zz}^{HC}), \quad (1)$$

where $2A_{zz}^{w}$, $2A_{zz}^{HC}$ are the $2A_{zz}$ values in water and in a nonpolar solvent (toluene), respectively, at 77 K. Earlier,[16] a linear correlation between $2A_{zz}$ and the isotropic HFC constant (a) was established for both spin labels in solvents of different polarity (toluene, ethyl acetate, ethanol, 50% glycerol, and water). This correlation shows that the hydrophobicity parameters h in solution and h_s in the solid phase measured in the same local environment should be equal. Direct measurement of $2A_{zz}$ in frozen aqueous solutions is impossible because upon water crystallization, spin labels are displaced into a separate subphase, which produces a poorly resolved singlet ESR spectrum that arises because of strong exchange and dipole interactions. Nevertheless, we managed to determine $2A_{zz}$ values for both spin labels at 77K in aqueous environment using the above linear correlation between a_{iso} and $2A_{zz}$ and the a_{iso} values in water. The h_s values based on these $2A_{zz}$ are presented in Table 1. It can be seen that for most ternary complexes (except for γ-CD—**2**—C_6H_6 and γ-CD—**2**—C_6H_{12}), the h_s values are

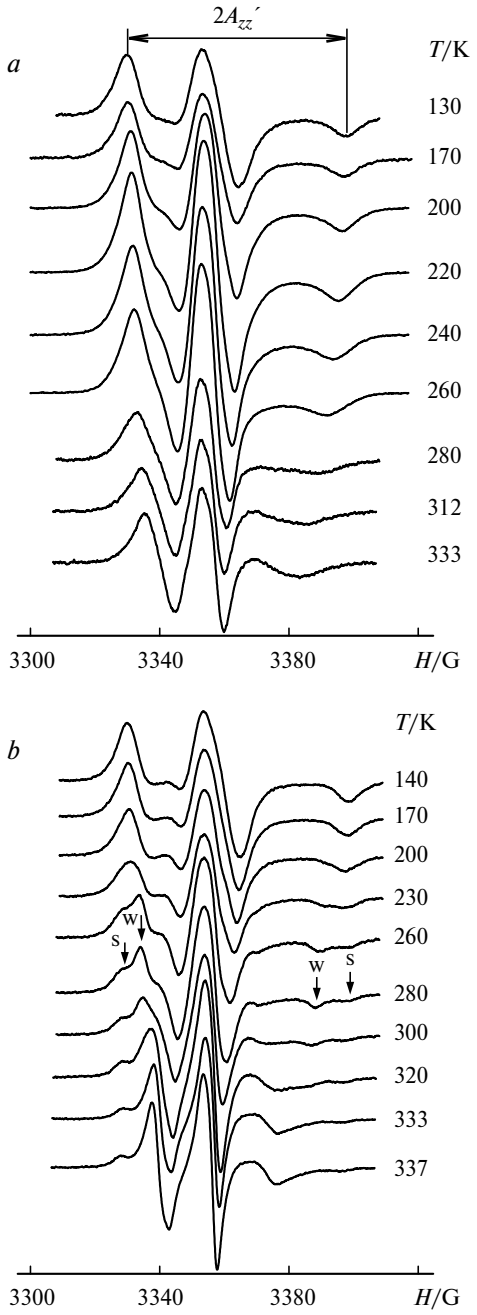

Fig. 1. ESR spectra of the β-CD—**2**—C_6H_6 (*a*) and γ-CD—**2**—C_6H_{12} (*b*) complexes in the solid phase at various temperatures. The characters "w" and "s" refer to the weakly and strongly immobilized signals, respectively.

close to unity, *i.e.*, the environment hydrophobicity of spin-labeled guests at 77 K is similar to that in a nonpolar solvent (toluene); in some complexes (β-CD—**1**—C_6H_{12} or γ-CD—**1**—C_6H_{12}), it is even somewhat greater than in this solvent. For comparison, Table 2 gives the h_s values for binary complexes of β- and γ-CD with the same spin-labeled guests **1** and **2** determined in our previous study.[16] It can be concluded that the local environment of the

Table 1. Environment hydrophobicity parameters (h_s) of spin-labeled indoles **1** and **2** in ternary complexes with β- and γ-CD and hydrocarbons in the solid phase

Spin label	Cyclo-dextrin	Hydro-carbon	$2A_{zz}$ (at 77 K)	h_s
1	β	C_6H_6	69.65	0.895
		C_6H_{12}	68.4	1.14
	γ	C_6H_6	69.6	0.895
		C_6H_{12}	69.0	1.0
2	β	C_6H_6	70.3	0.7
		C_6H_{12}	68.9	0.95
	γ	C_6H_6	72.2	0.39
		C_6H_{12}	70.6	0.6

Table 2. Environment hydrophobicity parameters (h_s) of spin-labeled indoles **1** and **2** in the binary β- and γ-CD complexes in the solid phase

Spin label	Cyclo-dextrin	$2A_{zz}$ (at 77 K)	h_s
1	β	72.2	0.41
	γ	72.32	0.38
2	β	71.8	0.45
	γ	72.64	0.315

NO group in ternary complexes is essentially more hydrophobic than in the corresponding binary complexes. This result shows that the third component (C_6H_{12} or C_6H_6) is actually incorporated into the complex and is apparently located in the vicinity of spin labels. The values $h_s \geq 1$ may be due to the fact that at 77 K (in the absence of molecular rotation), the NO group of the label occupies in the complex a fixed position in which the hydrophobicity is higher than the average environment hydrophobicity in an isotropic nonpolar solvent (toluene). In this case, one can expect that a temperature rise resulting in the onset of the spin label mobility in the CD cavity would decrease the averaged environment hydrophobicity in the cavity. As shown below, this effect is actually observed in experiments.

The data in Table 1 provide also the following conclusions. First, the environment hydrophobicity of the NO group in complexes with C_6H_{12} is distinctly higher than in the corresponding complexes with C_6H_6. Second, the h_s values for β-CD complexes are higher than for the γ-CD complexes. Third, the h_s values for all complexes with **1** are much higher than for complexes with **2**. All the above-noted differences between the h_s parameters are apparently due to differences in the structures of complexes, which can be verified by quantum chemical calculations (see below).

Molecular dynamics. The ESR spectra of two of the eight obtained complexes recorded at different tempera-

tures are presented in Fig. 1. For the β-CD—**2**—C_6H_6, β-CD—**2**—C_6H_{12} and β-CD—**1**—C_6H_6 complexes, one ESR signal is observed over the whole temperature range, whereas for complexes of both guests with γ-CD and for the β-CD—**1**—C_6H_{12} complex, two signals are recorded (shown by an arrow in Fig. 1, *b*); the signals differ substantially in the magnitude and the temperature dependence of $2A_{zz}'$. At low temperatures, the signals for both types of complexes are not completely resolved due to a large line width; however, at $T > 250$ K, the resolution is improved, so that it becomes possible to determine the $2A_{zz}'$ parameters for weakly and strongly immobilized signals, to which lower and higher $2A_{zz}'$ correspond. An increase in the spectral resolution occurs approximately in the same temperature range for radicals **1** and **2** and seems to be owing to the onset of the Me group rotation in these radicals, resulting in averaging of the dipole—dipole interaction of the unpaired electron and the Me-group protons.

In this study we consider only the former type of complexes. A study of the nature of complexes responsible for the strongly immobilized ESR spectra is presented in the next publication.[26]

If at 77 K the motion of the spin-labeled guest is considered to be virtually frozen on the ESR time scale (*i.e.*, the rotational correlation time of the motion is $\tau \geq 10^6$ s^{-1}), it is convenient to use the value $S = 1 - A_{zz}'/A_{zz}$ as the spectral parameter characterizing the intensity of molecular rotation (more precisely, the efficiency of averaging of the A_{zz} component of the HFC tensor).[13b] The temperature dependences of this parameter for different complexes are presented in Fig. 2. It can be seen that for the γ-CD complexes with either of the hydrocarbons as a second guest, the S values are much greater than for the corresponding binary complexes.

Thus, the introduction of a second guest molecule into the γ-CD cavity results in a considerable increase rather than a decrease of the rotational mobility of guest molecules. This unusual result cannot be interpreted within the framework of the former stoichiometry (one molecule of the spin-labeled guest per γ-CD molecule), because the introduction of an additional guest (C_6H_6 or

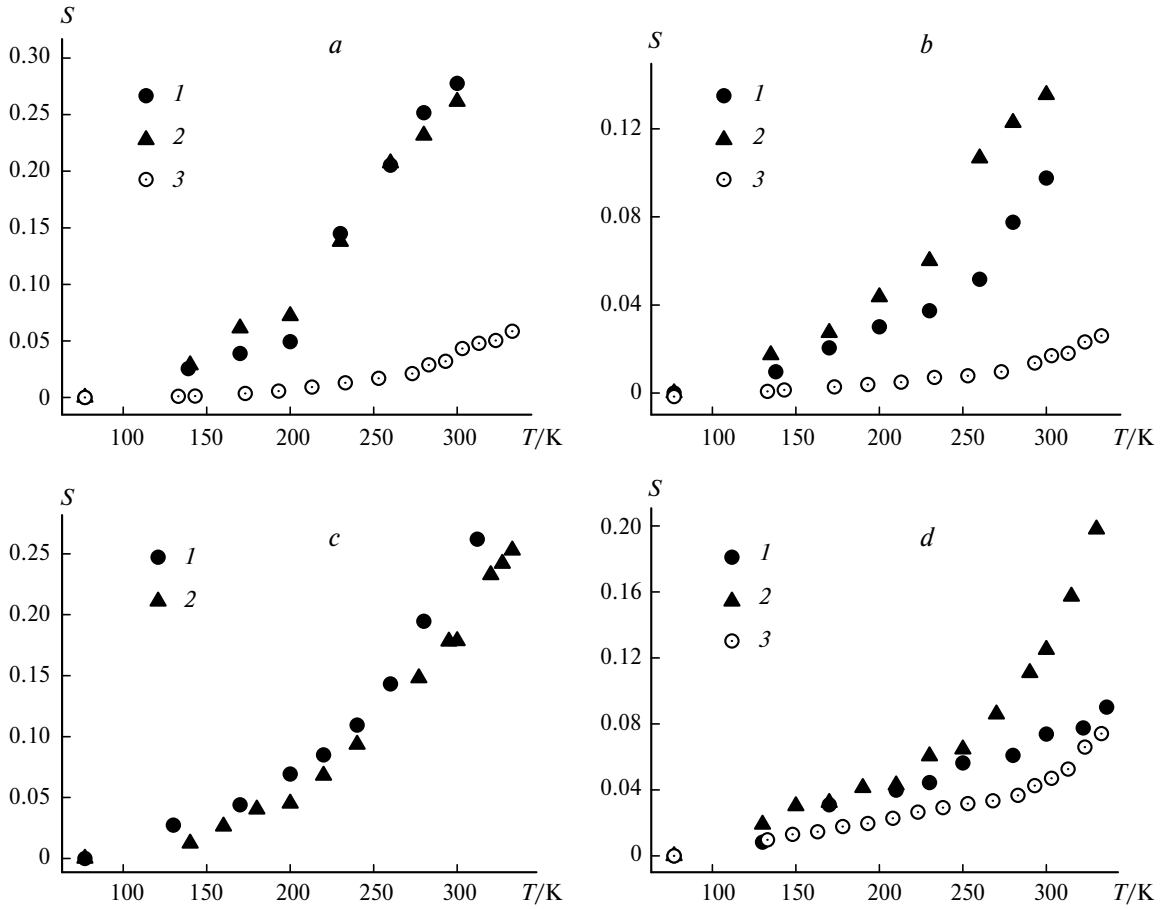

Fig. 2. Temperature dependences of the parameter $S = 1 - A_{zz}'/A_{zz}$ for 2γ-CD—**2**—C_6H_6 (*1*), 2γ-CD—**2**—C_6H_{12} (*2*), and γ-CD—**2** (*3*) (*a*), 2γ-CD—**1**—C_6H_6 (*1*), 2γ-CD—**1**—C_6H_{12} (*2*), and γ-CD—**1** (*3*) (*b*), 2β-CD—**2**—C_6H_6 (*1*) and 2β-CD—**2**—C_6H_{12} (*2*) (*c*), 2β-CD—**1**—C_6H_6 (*1*), 2β-CD—**1**—C_6H_{12} (*2*), and 2β-CD—**1** (*3*) (*d*).

C_6H_{12}) into the γ-CD cavity is expected to result in slowing down the spin label rotation. The observed increase in the mobility is due, most likely, to the formation of γ-CD dimers in which spin-label and hydrocarbon guest molecules reside. Our quantum chemical calculations (see below) have shown that 1 : 1 : 1 complexes of γ-CD are in principle possible; however, their stabilization energy is almost twice smaller than that for 2 : 1 : 1 complexes in which the spin-labeled indole and hydrocarbon are located in the γ-CD dimer cavity.

In the case of complexes of **2** with β-CD, it is difficult to accurately determine the S values for binary complexes because of substantial broadening of the $m = \pm 1$ components; therefore, this $S(T)$ dependence is not shown in Fig. 2. However, comparison of the ESR spectra of ternary and binary β-CD complexes at equal temperatures shows that the spectral line shapes are substantially different (Fig. 3). Despite the greater linewidth of the $m = -1$ components, it can be seen that the distances between the outer extrema are greater for the binary complexes than for the ternary ones. The shifts of the $m = \pm 1$ components with respect to the central line in the binary complexes are approximately equal; in addition, the widths of these components are much greater than those for ternary complexes. These features are distinctive of the jump rotation mechanism.[13a,16] In the spectra of ternary complexes (see Fig. 3), the linewidths of the $m = \pm 1$ components are much smaller and the shifts with respect to the central line are much greater for $m = -1$ than for $m = +1$, which is indicative of the diffusion rotation or fast librations of guest molecules.

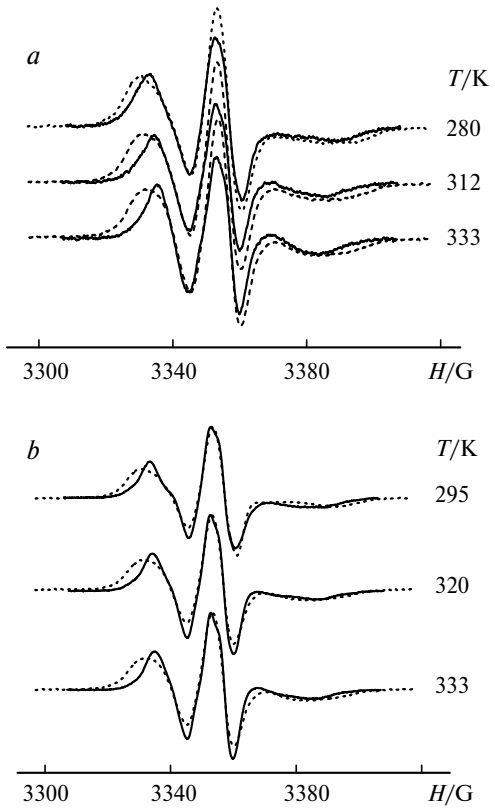

Fig. 3. Comparison of the ESR line shapes for ternary and binary β-CD complexes: 2β-CD—**2**—C_6H_6 (solid line) and 2β-CD—**2** (dashed line) (*a*), 2β-CD—**2**—C_6H_{12} (solid line) and 2β-CD—**2** (dashed line) (*b*).

One can distinguish between libration and overall diffusion motions of **1** and **2** in ternary complexes using saturation transfer ESR spectra (ST ESR) (see below).

It can be seen from Fig. 2 that the S values for **2** are greater than for **1** and that, at the same temperature, they are greater for γ-CD than for β-CD. The effect of the second guest (the hydrocarbon molecule) depends on the nature of other components: it is insignificant for **2**, although for both CD its mobility is somewhat higher in complexes with C_6H_6 than C_6H_{12}. Conversely, for **1**, the effect of a second guest is much more pronounced and, moreover, unlike **2**, a higher mobility is found for C_6H_{12} rather than C_6H_6 complexes for both CD.

The effect of CD and spin-labeled guest structures on the guest rotational mobility can be explained, at a qualitative level, as follows. The piperidine ring in molecule **2** undergoes intramolecular conformational transitions, which contribute to the rotational mobility of the NO group, whereas **1** is planar and relatively rigid; in addition, the Me group present in the indole part of **1** may give rise to additional immobilization of this label caused by steric hindrance in the γ- and β-CD dimers. The high mobility in the complexes of both labels in γ-CD dimers is naturally attributable to the greater dimensions of the γ-CD dimer cavity compared to the β-CD dimer cavity.

The insignificant effect of hydrocarbons on the mobility of **2** in ternary complexes is, apparently, due to the above-mentioned intramolecular transitions in the piperidine ring of **2**. Unlike **2**, the mobility of the rigid **1** in the CD cavity is largely determined by packing and dynamics of the second guest; therefore, in the presence of C_6H_{12}, which undergoes intramolecular chair-to-boat transitions, the mobility of **1** is higher than in the presence of C_6H_6.

As noted in the previous study,[16] the quantitative parameters of the molecular mobility cannot be determined directly from the variation of A_{zz}' (or S) value, because the same S values can be due to either an increase in the angular amplitude of fast librations of the z axis of the guest molecule or an increase in the slow rotation frequency of the molecule in the full solid angle 2π. In the previous study[16] dealing with binary complexes of **1** and **2** with β- and γ-CD, the choice between these two limiting models of molecular rotation of spin-labeled indoles was made using ST ESR spectroscopy. This approach[16,27,28] is based on the fact that the ST ESR spectra, namely, those for the second harmonics absorption detected in the 90° phase with modulating field (V_2'), differ substantially in

their line shapes for the two above motion models, *viz.*, fast librations of the limited angular amplitude and slow reorientations in the full solid angle (or in the plane) even when the linear ESR spectra have the same S values. It was found previously[16] that the rotational motions of radicals **1** and **2** in the binary γ-CD complexes are essentially fast librations of a relatively small amplitude, while the reorientation of these radicals in β-CD dimers is better described by the jump rotation model in the full solid angle with frequencies of ~10^7 s^{-1} at room temperature. We used the same approach to choose between these limiting models of rotation of guest molecules in CD ternary complexes. The experimental V_2' spectra for ternary complexes of **1** (2γ-CD—**1**—C_6H_6 and 2β-CD—**1**—C_6H_{12}) and the best simulated spectra are presented in Fig. 4. Due to mathematical difficulties, the V_2' spectra were simulated as previously,[16] in terms of the jump rotation model in the adiabatic approximation or the resonance field using axially symmetric magnetic tensors A, g.[27,28] Therefore, the L''/L intensity ratio (see Fig. 4, *a*), which is only weakly sensitive to these approximations, was used as the diagnostic parameter characterizing the rotation model.[16,27,28] The calculations showed that in the fast libration model, this ratio is greater than zero, which is actually observed in experiments for the ternary complexes containing **1**. This provides the qualitative conclusion that the character of motion of **1** in all complexes is a libration of a limited amplitude. It can be seen from Fig. 4, *a* that agreement between the experimental and simulated spectra is insufficiently good in the central part, which is apparently due to approximations made in the calculation, in particular, to the axial symmetry of the g-tensor and small modulation amplitude. A similar character of motion of **1** in other complexes follows from simulation of their V_2' spectra. Within the framework of this model, the average angular amplitude of the librations of z axis (Δθ) is determined from the ratio (see, for example, Refs 13c, 16, and 29)

$$\langle \sin^2\Delta\theta \rangle = (A_{zz} - A_{zz}')/(A_{zz} - A_{\perp}),$$

where $A_{\perp}$ is the perpendicular component of the HFC tensor in the absence of molecular motion (at 77 K). The temperature dependences of the Δθ values calculated from

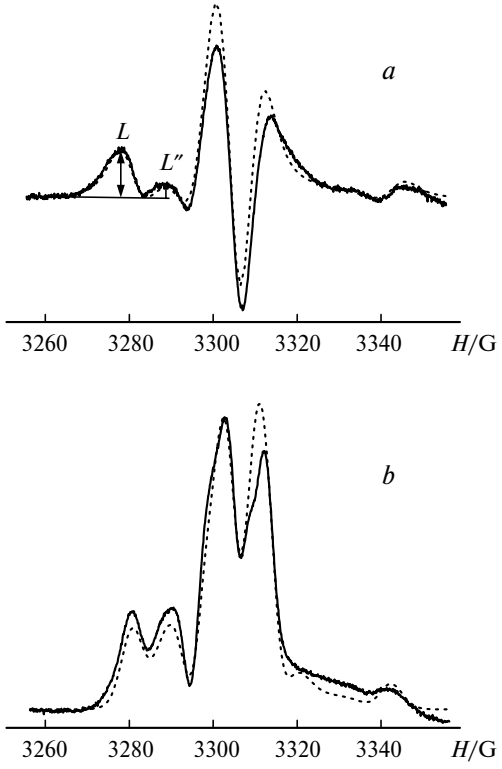

Fig. 4. Experimental ESR spectra of the second harmonics absorption detected in the 90° phase with modulating field (V_2') at 295 K (solid lines) and simulated V_2' spectra (dashed lines) for the 2γ-CD—**1**—C_6H_6 (*a*) and 2β-CD—**1**—C_6H_{12} (*b*) ternary complexes; L and L'' are the intensities at field positions corresponding to the low and high rates of variation of the resonance magnetic field with the angle between the spin label z axis and magnetic field direction.

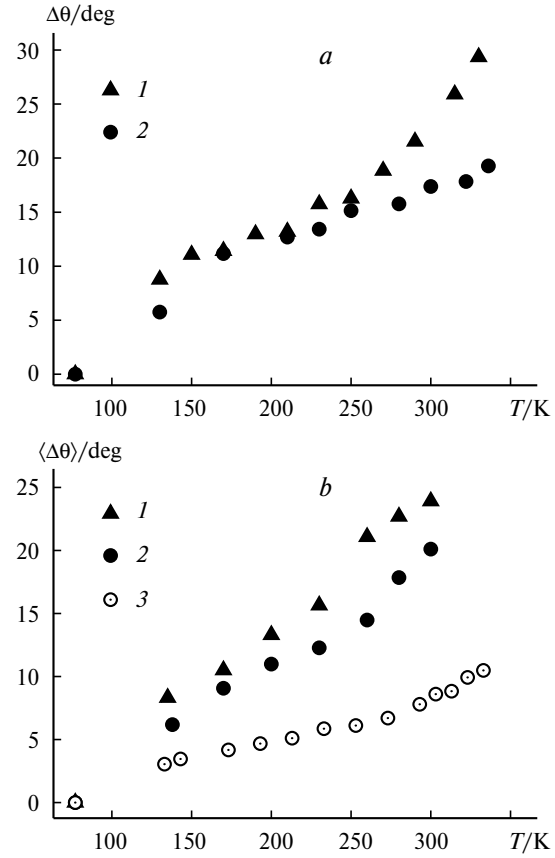

Fig. 5. Temperature dependences of the average libration amplitudes of the HFC axial anisotropy axis (z axis) for the complexes of **1** with β- (*a*) and γ-CD (*b*): 2CD—**1**—C_6H_{12} (*1*), 2CD—**1**—C_6H_6 (*2*), and CD—**1** (*3*).

this ratio for all ternary complexes of **1** are presented in Fig. 5.

Like the S values, the libration amplitudes of **1** in the ternary complexes with γ-CD are much greater than in the corresponding binary complexes (see Ref. 16). Besides, as expected, the effect of the CD and second guest structures on the libration amplitudes of **1** is qualitatively the same as the effect of these components on the S parameters considered above: for equal temperature, the libration amplitudes of **1** are greater in γ-CD complexes than in β-CD complexes and greater in complexes with C_6H_{12} than with C_6H_6.

The ST ESR line shapes of ternary complexes with **2** differ considerably from those of complexes with **1**. Examination of the V_2' spectra of β-CD—**2**—C_6H_6 and γ-CD—**2**—C_6H_6 (Fig. 6) shows that the L''/L parameter is negative, which is indicative of motion of the spin-labeled guest over the full solid angle.[27,28] The agreement between the simulated and experimental V_2' spectra is not completely satisfactory, in particular, due to the presence of the second, strongly immobilized signal; nevertheless, the negative L''/L value is not related to its presence.

As noted above, the line shapes of the linear first harmonics ESR spectra (V_1) for the complexes with **2** is in better agreement with the Brownian (*i.e.*, continuous) rather than jump diffusion mechanism. However, detailed simulation of the V_1 ESR spectra for the complexes with **2** is hampered by the presence of the second signal corresponding to the to the strongly immobilized molecules of **2**. Therefore, we determined the rotational diffusion coefficients for for the complexes with weakly immobilized **2** using the S parameter. To this end, we calculated the calibration dependence of S on the rotational diffusion coefficient (D_R) in terms of the isotropic Brownian diffusion model using a reported program[30] and based on A_{zz} tensor components determined at 77 K and the individual line widths determined from the ESR spectra of the complexes. This dependence is satisfactorily approximated by a straight line in double logarithmic coordinates (Fig. 7) and can be fitted to relation:

$$\log S = -7.68(\pm 0.14) + 0.93(\pm 0.02)\log D_R.$$

The above calibration was used to determine the D_R values for all four complexes of **2** as functions of temperature (Fig. 8). For complexes with γ-CD, these dependences were plotted for high-temperature region (>240 K) where the w- and s-signals are resolved and the $2A_{zz}'$ values can be measured for the weakly immobilized w-signal. For the complexes of **2** with β-CD whose ESR spectra exhibit only one signal, the temperature dependences of D_R were plotted over the whole temperature range studied(140—338 K). As can be seen in Fig. 8, *b*, the D_R dependences for the γ-CD complexes with C_6H_6 or C_6H_{12} as the second guest produce satisfactory straight lines in the semilogarithmic Arrhenius coordinates. A similar Arrhenius dependence holds for the 2β-CD—**2**—C_6H_{12} complex (see Fig. 8, *a*). For the 2β-CD—**2**—C_6H_6 complex, this dependence is slightly nonlinear, in particular, at elevated temperatures it resembles the dependence for 2β-CD—**2**—C_6H_{12}, but deviates from it at lower tem-

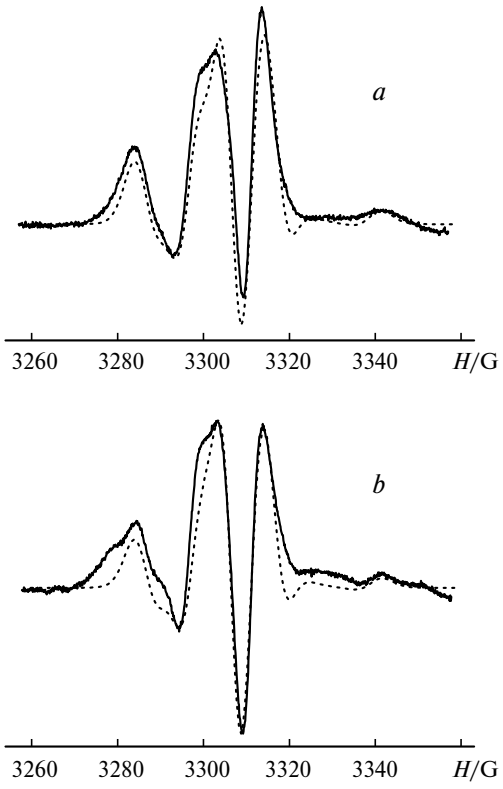

Fig. 6. Experimental (295 K) and simulated ESR spectra of the second harmonics absorption in the 90° phase with modulating field (V_2') for the 2β-CD—**2**—C_6H_6 (*a*) and 2γ-CD—**2**—C_6H_6 (*b*) complexes.

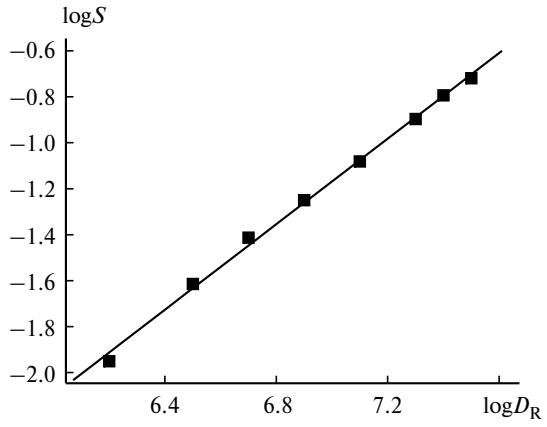

Fig. 7. Calibration dependence of the parameter $S = 1 - A_{zz}'/A_{zz}$ on the rotational diffusion coefficient (D_R). Calculations were carried out in the isotropic rotational diffusion model using reported software.[30]

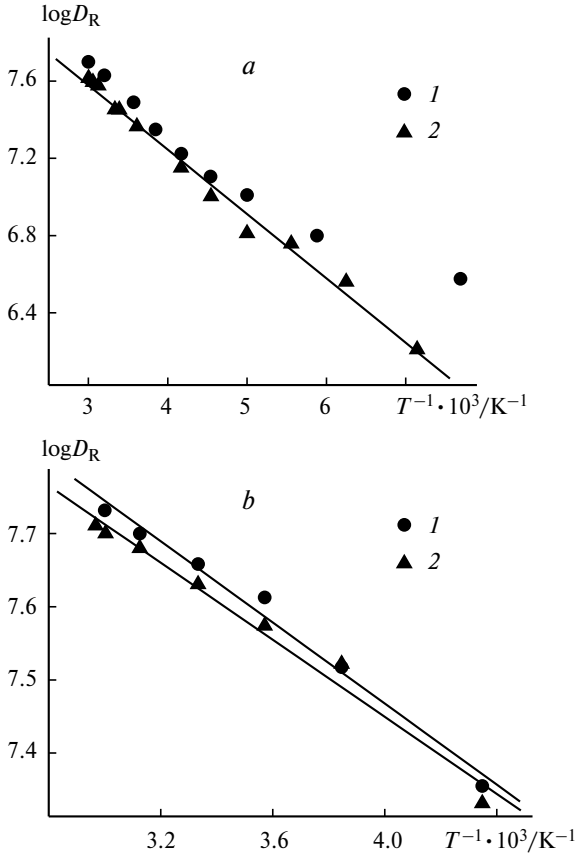

Fig. 8. Temperature dependences of the rotational diffusion coefficients (D_R) of spin-labeled indole **2** in solid-phase complexes with β- (*a*) and γ-CD (*b*): (*a*) 2β-CD—**2**—C_6H_6 (*1*), 2β-CD—**2**—C_6H_{12} (*2*); (*b*) 2γ-CD—**2**—C_6H_6 (*1*) and 2γ-CD—**2**—C_6H_{12} (*2*).

peratures. The activation energies and the pre-exponential factors (log*A*) of D_R for the rotation of **2** in β- and γ-CD complexes with both hydrocarbons are summarized in Table 3. For the β-CD complexes, these values were determined in the same high-temperature region as for the γ-CD complexes. As can be seen in Table 3 and Fig. 8, the activation energies for the rotation of molecule **2** in the cavities of β- and γ-CD dimers are low, the ε_a values for β-CD complexes being somewhat higher than for γ-CD

Table 3. Arrhenius parameters of the rotational mobility of spin-labeled indole **2** (ε_a, log*A*) in the ternary complexes of β- and γ-CD in the temperature range of 240—340 K and the environment hydrophobicity parameters (h_s') of **2** in γ-CD complexes at 320—338 K

Complex	ε_a/kcal mol^{-1}	log*A*	h_s'
2β-CD—**2**—C_6H_6	1.82±0.05	8.9±0.04	—
2β-CD—**2**—C_6H_{12}	1.84±0.03	8.8±0.02	—
2γ-CD—**2**—C_6H_6	1.27±0.06	8.55±0.05	0.35
2γ-CD—**2**—C_6H_{12}	1.21±0.07	8.50±0.05	0.38

complexes. It is also worth noting that the rotation frequencies of **2** are rather high for all complexes. Indeed, the values for β- and γ-CD at 295 K are $3 \cdot 10^7$ and $4.5 \cdot 10^7$ s^{-1}, respectively, which corresponds to the rotation correlation times $\tau_R = (1/6)D_R$ of 5.5 and 3.7 ns using the Brownian rotation model.

The mechanism of spin label reorientation and the physical meaning of the activation energies for reorientation in ternary complexes are not entirely clear. In view of rather dense molecular packing in the complexes (see below, quantum chemical calculations), one can suggest that the formation of free volume, which enables spin label reorientation, requires cooperative (self-consistent) motion of both the guest molecules and the host molecule. In this respect, the activation energy cannot be considered as a fixed potential barrier. Apparently, quantitative interpretation of the rotation frequencies and their temperature dependence can be derived from molecular dynamics calculations.

Comparison of the rotation frequencies of **2** in the ternary and binary complexes of β-CD gives a seemingly paradoxical result: despite the fact that averaging of the HFC anisotropy in the ESR spectra is more efficient in ternary complexes (see Fig. 3), the rotation frequencies of **2** in the ternary complexes of β-CD are somewhat lower than in binary complexes (see Ref. 16 and Fig. 8, *a*). This result can be attributed to different characters of guest motion in binary and ternary complexes. In the β-CD dimers, the motion of molecule **2** is rotation in the full solid angle both in binary and ternary complexes. However, as noted above, both the line shapes of the linear spectra and the simulation results are indicative of the different character of motion in binary and ternary complexes: jump diffusion in the former and and nearly Brownian rotation in the latter complexes. It is known[13b] that for the same rotation frequency, averaging of the local magnetic field anisotropy (in this case, the HFC anisotropy) is less efficient for jump than for diffusion type rotation.

The motion of molecules **1** in β-CD is also different for binary and ternary complexes, namely, in binary complexes, it is described satisfactorily by the jump diffusion model,[16] while in ternary complexes, the libration model is more adequate, judging by the ST ESR spectra. Transition to librations is probably due to a decrease in the free volume upon the inclusion of a second guest into the β-CD dimer. However, the fact that **1** is more mobile in the presence of C_6H_{12} rather than C_6H_6 indicates that not only the molecular volume but also the conformational lability of the second guest plays an important role for the dynamics of **1** in the β-CD cavity.

In γ-CD binary complexes, the motion of both spin labels can be represented as librations in a rather narrow angular cone.[16] On going to ternary complexes of **1**, the libration mechanism is retained to at least 295 K, as indi-

cated by their ST ESR spectra; however, libration amplitudes markedly increase and depend substantially on the second guest structure. Unlike **1**, the overall motion of the radical fragment of molecule **2** can be approximated by the rotation in the full solid angle. For both labels, the changes in rotational mobility observed on going from binary to ternary complexes are mainly due to the change in the stoichiometry, *i.e.*, transition from γ-CD monomers to dimers.

It can be seen from Fig. 1, *b* that at high temperatures (320—338 K), the ESR line shape for the 2γ-CD—**2**—C_6H_{12} complex no longer corresponds to the slow rotation region but approaches the fast rotation region. Similar ESR spectra are observed for other complexes of γ-CD not only with **2** but also with **1** (the data are not reported). The outer extrema disappear in this intermediate region and, hence, the $2A_{zz}'$ parameter (the distance between the outer extrema) has no meaning. Therefore, in this temperature region, the relative mobility of **1** and **2** in ternary γ-CD complexes was characterized by the ratio of the hyperfine component amplitudes, the parameter $\rho = \sqrt{I_{+1}/I_{-1}} - 1$, which is known[31] to be proportional, in the fast rotation region, to the rotational correlation time of the nitroxide spin label. As for the parameter S, the $\rho(D_R)$ dependences were calculated and used to determine D_R values for various γ-CD complexes at temperatures of 336—339 K. These data are presented in Fig. 8. It can be seen that, despite a different way of determining the D_R values, they fit satisfactorily into the temperature dependences $D_R(T)$ determined in the slow rotation region.

At the above-indicated temperatures, it was also possible to estimate the isotropic HFC constants (a_{iso}) and then, using the a_{iso} value instead of $2A_{zz}$, and a relation similar to that presented above,[14] to determine the hydrophobicity parameters h_s', which characterize the environment polarity for the spin-labeled guests in the fast rotation region. The a_{iso} values were determined by two methods, in particular, as the distance between the central and the $m = +1$ components and as half the distance between the $m = \pm 1$ components. In the case of complexes with γ-CD, the strongly immobilized ESR signal also contributes to the central component; therefore, the estimation of a_{iso} for these complexes by the former method is less accurate. Nevertheless, the character of variation of h_s' relative to h_s is the same for all the considered complexes (see the data for γ-CD complexes in Table 3). It is remarkable that the h_s' values are significantly smaller than h_s for the same complexes at 77 K in the absence of molecular motion (see Table 1). This difference can be attributed to the fact that at 77 K, the NO group is located in the hydrophobic microenvironment and in a fixed orientation. An increase in the polarity of the environment of the NO group at high temperatures is due to motional averaging of the microheterogeneous environment inside the CD cavities.

Quantum chemical calculations. We calculated the energies and structures of the ternary complexes involving β- and γ-CD by the semiempirical PM3 method. These calculations do not take into account the contribution of the hydrophobic interactions (*i.e.*, the change in the structure of water) to the free energy of binding but they allow one to compare the stabilities of various possible structures of the complexes if the exposures of guest molecules to aqueous phase for these structures are low or nearly equal.

Previously, we noted that the increase in the rotational mobility of spin-labeled indoles observed upon the introduction of an additional guest molecule into the binary γ-CD complexes is rather difficult to interpret *a priori* within the 1 : 1 : 1 stoichiometry. Nevertheless, we calculated the possible structures of complexes in which both guests (the spin label and the hydrocarbon) are located in one γ-CD cavity in order to estimate their formation energies and to compare them with the energies of 2 : 1 : 1 structures. For β-CD, no calculations for 1 : 1 : 1 stoichiometry were carried out, as it has been found previously[15,16] that more stable complexes with the same spin-labeled guests are formed by β-CD dimers even in the absence of hydrocarbons.

The guest molecules were introduced into the cavity of the pre-optimized γ-CD. The complexation energy values were used to characterize the effects of different guests on complex stability. For the 1 : 1 : 1 stoichiometry, these are given by

$$\Delta E_A = \Delta E_{comp} - \Delta E_{CD} - \Delta E_{SL} - \Delta E_{HC},$$

$$\Delta E_C = \Delta E_{comp} - \Delta E_{CD-SL} - \Delta E_{HC};$$

for 2 : 1 : 1 stoichiometry, the expression is similar

$$\Delta E_A = \Delta E_{comp} - \Delta E_{2CD} - \Delta E_{SL} - \Delta E_{HC},$$

$$\Delta E_B = \Delta E_{comp} - 2\Delta E_{CD} - \Delta E_{SL} - \Delta E_{HC},$$

$$\Delta E_C = \Delta E_{comp} - \Delta E_{2CD-SL} - \Delta E_{HC}.$$

Here ΔE_{comp}, ΔE_{CD}, ΔE_{2CD}, ΔE_{SL}, ΔE_{HC}, ΔE_{CD-SL}, and ΔE_{2CD-SL} are the heats of formation of the complex, CD monomer, CD dimer, spin label (SL), hydrocarbon molecule (HC), and CD—SL and 2CD—SL complexes.

For all structures with 1 : 1 : 1 stoichiometry, the input position of a spin-labeled guest was specified according to the experimental hydrophobicity parameter in such a way that NO was located in the γ-CD cavity. Calculations for four complexes predict stable states (negative complexation energies) only for two of them, γ-CD—**2**—C_6H_6 and γ-CD—**1**—C_6H_{12} (Table 4). However, even for these complexes, too, the obtained structures seem unlikely. For example, in the γ-CD—**2**—C_6H_6 structure, the indole

Table 4. Energies of formation (ΔE) of 1 : 1 : 1 complexes obtained from the quantum chemical calculations by PM3 method

Complex	$-\Delta E_A$	$-\Delta E_C$
	kcal mol^{-1}	
γ-CD—**1**—C_6H_6*	—	—
γ-CD—**1**—C_6H_{12}	24.3	9.7
γ-CD—**2**—C_6H_6	18.9	5.6
γ-CD—**2**—C_6H_{12}*	—	—

* Calculations for these complexes did not give stable states.

ring is outside and the C_6H_6 molecule barely enters the γ-CD cavity. In the γ-CD—**1**—C_6H_{12} structure, the Me group of molecule **1** and half of the C_6H_{12} molecule occur outside the cavity, and γ-CD is highly distorted. These features should destabilize both structures, which is actually manifested in the ΔE_A and ΔE_C values for both complexes (see Table 4). A comparison of these energies and the corresponding energies for 2 : 1 : 1 complexes (Table 5) indicates that the complexes with γ-CD dimers are much more favorable.

Calculations for the 2 : 1 : 1 structures using different ways of introduction of guest molecules demonstrated

Table 5. Energies of formation (ΔE) of 2 : 1 : 1 complexes

Complex	Structure	$-\Delta E_A$	$-\Delta E_B$	$-\Delta E_C$
		kcal mol^{-1}		
2β-CD—**1**—C_6H_6	**A**	33.0	45.3	13.35
	B	16.4	28.7	3.5
	C	20.2	32.5	0.2
2β-CD—**1**—C_6H_{12}	**A**	35.2	47.5	15.6
	B*	—	—	—
	C	21.1	33.4	1.5
2β-CD—**2**—C_6H_6	**A**	23.0	34.8	6.0
	B	18.5	30.8	1.6
	C	24.2	36.2	7.2
2β-CD—**2**—C_6H_{12}	**A**	25.9	38.2	8.9
	B*	—	—	—
	C	26.9	39.2	9.9
2γ-CD—**1**—C_6H_6	**A**	19.2	39.0	7.2
	B	21.2	41.0	9.15
	C	16.2	36.0	4.1
2γ-CD—**1**—C_6H_{12}	**A**	20.5	40.3	8.5
	B	28.0	47.8	16.0
	C	22.2	42.0	10.15
2γ-CD—**2**—C_6H_6	**A**	26.1	45.9	10.7
	B	21.2	41.0	5.8
	C	20.0	39.8	4.6
2γ-CD—**2**—C_6H_{12}	**A**	25.4	45.5	10.0
	B	25.2	45.0	9.8
	C	21.8	41.6	6.5

* For these structures, no negative formation energies were obtained.

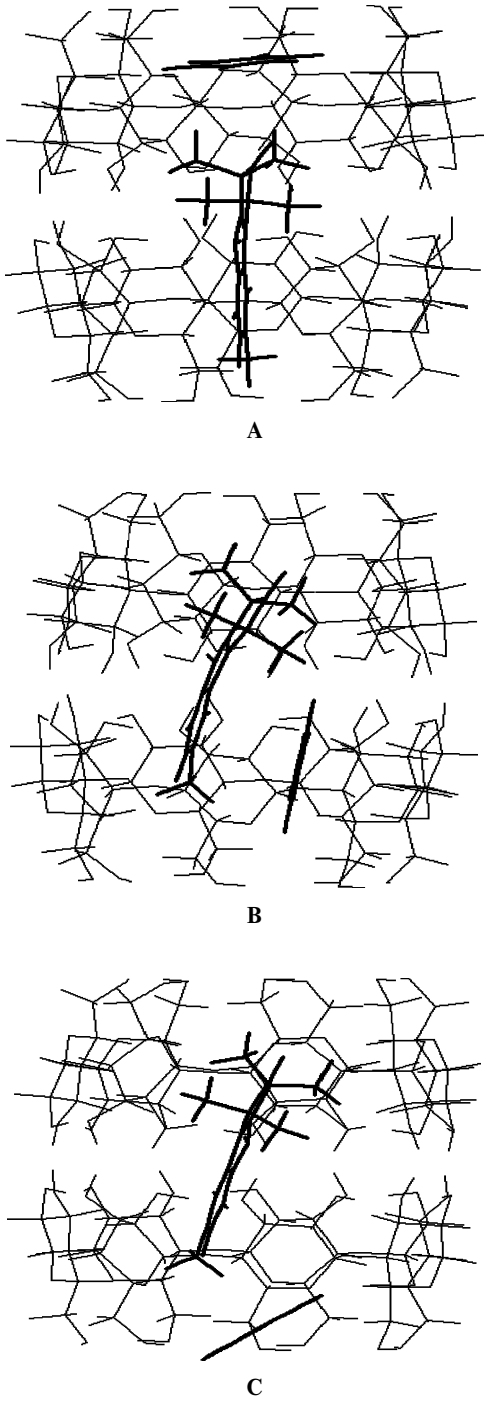

Fig. 9. Three variants (**A—C**) of arrangements of guests in the β-CD dimer cavity of the 2β-CD—**1**—C_6H_6 complex. The structures were determined by PM3 calculations.

that stable states (*i.e.*, having negative ΔE_A, ΔE_B, ΔE_C) are formed in all complexes with three main arrangements of the guests (Fig. 9): the hydrocarbon molecule is located in the vicinity of the NO group in the same CD molecule (**A**) or in another CD molecule parallel (**B**) or perpendicular (**C**) to the indole ring. The ΔE_A, ΔE_B, and ΔE_C

values for these states for different complexes are given in Table 5.

Complexes with β-CD. It is seen in Table 5 that for complexes of **2** with C_6H_6 or C_6H_{12}, structures **A** and **C** are more stable and their energies are similar. Unlike the complexes of **2**, for the complexes formed by **1** with C_6H_6, or C_6H_{12}, structure **A** is much more stable than **C**. In structures of type **A** of the complexes with C_6H_6, the indole fragments of molecules **1** or **2** are oriented parallel to the dimer axis; in the complexes with C_6H_{12}, molecule **2** deflects from this axis by ~10°. The bulky tetramethylpyrrolidine (**1**) or tetramethylpiperidine (**2**) parts of the labels occupy the widest region at the dimer center, while the indole group completely resides in the second CD molecule. The C_6H_6 or C_6H_{12} molecules occur near the NO group parallel (**1**) or at a ~20° angle (**2**) to the dimer bases. The distances from the O atom of the NO group to the nearest H atom of the C_6H_6 or C_6H_{12} molecule in the β-CD—**2**—C_6H_6 and β-CD—**2**—C_6H_{12} complexes are equal to 3.14 and 1.89 Å, respectively, while in β-CD—**1**—C_6H_6 and β-CD—**1**—C_6H_{12}, these values are— 3.55 and 2.5 Å.

As noted above, the experimental hydrophobicity parameter (h_s) for complexes with **2** substantially increases on going from binary to ternary β-CD complexes (see Table 1 and 2). These data are in better agreement with type **A** structures in which the NO groups of the spin labels are located at a smaller distance from the hydrocarbon molecule than in structures **B** and **C**. The same type (**A**) exists for the complexes of **1** with β-CD and both hydrocarbons, which is in good agreement with hydrophobicity data. The h_s parameter for the complexes of **1** and **2** with C_6H_{12} is much greater than the h_s values for the same complexes with C_6H_6 (see Table 1). As follows from the calculated structures of type **A**, these differences may be attributed to the fact that the distances from the NO group to the C_6H_{12} molecule are markedly shorter than to the C_6H_6 molecule. In addition, as noted above, the differences in h_s are partly due to lower electronic polarizability of the aliphatic cyclohexane compared to the aromatic benzene.

The h_s parameters in the ternary complexes are greater for **1** than for **2**, although the distance from the O atom of the NO group to the H atom of the hydrocarbon molecule is somewhat greater for the former complexes. These differences seem to be due to the fact that the NO group in planar molecule **1** is oriented nearly along the dimer axis, in the region with high hydrophobicity, whereas in **2**, it deviates from this axis due to the nonplanar structure of the piperidine ring.

Importantly, for complexes with **2**, calculations predict considerable deformations of the β-CD dimer. These deformations are manifested, first, as an increase in the distances between the O atoms of secondary OH groups of neighboring CD molecules and, second, as an elliptical distortion of the broad bases (heads) of CD. Indeed, the distances between the corresponding O atoms of neighboring CD molecules vary from 2.65 to 3.38 Å (according to our calculations, this distance in the initial dimer containing no guests is the same for all O...O pairs being equal to 2.65 Å). Both β-CD "heads" are extended in mutually perpendicular directions, the ratio of the ellipse semiaxes being increased up to 1.24. For the complex with C_6H_6, the deformation is more pronounced than for the complex with C_6H_{12}. For the complexes with **1**, the deformation of the dimer is unsignificant, which can be attributed to more compact packing of molecule **1** and the hydrocarbon in the cavity of the 2β-CD dimer causing no deformation of the hydrogen bonds between the β-CD monomers. The strained complexes with **2** are expected to be less stable than the complexes with **1**, which is actually observed for the calculated complexation energies (see Table 5).

Complexes with γ-CD. It can be seen from Table 5 that for the complexes of **2** with both hydrocarbons, structure **A** is more favorable. In this structure, molecule **2** in the 2γ-CD—**2**—C_6H_6 complex fits almost completely into the cavity of one γ-CD with a bend of 40° to the dimer axis, the NO group being located in the transient region between the "heads" of the γ-CD molecules. This arrangement may be responsible for the relatively low hydrophobicity parameter (h_s = 0.39, see Table 1) of the NO group in this complex, because the NO group is located in the region of the secondary OH groups and the hydrogen bonds between the CD monomers. In the 2γ-CD—**2**—C_6H_{12} complex, molecule **2** is bent at a smaller angle (20°) to the γ-CD dimer axis. The parameter h_s for this complex is higher than for 2γ-CD—**2**—C_6H_6 (see Table 1). This is apparently due to the fact that the hydrophobicity is higher near the γ-CD dimer axis than along the perimeter of the wide opening (head) of γ-CD containing hydrogen bonds between the two γ-CD molecules.

In the 2γ-CD—**1**—C_6H_6 complex, structure **A** in which the spin-labeled guest is arranged nearly along the dimer axis is also more favorable. The C_6H_6 molecule is located near the narrow base of CD almost parallel to it, which creates the hydrophobic environment of the NO group. For the 2γ-CD—**1**—C_6H_{12} complex, structure **B** in which the C_6H_{12} molecule is located in the vicinity of the indole group (see Fig. 9) proved to be energetically favorable (see Table 5). Nevertheless, since the NO group is arranged virtually along the dimer axis, the hydrophobicity parameter is high. It is even higher than that for the 2γ-CD—**1**—C_6H_6 complex, because the electronic polarizability of the aliphatic cyclohexane is lower than that of aromatic benzene. Unlike the β-CD complexes, no deformation of the dimer takes place for γ-CD upon the formation of ternary complexes, evidently, because of greater size of γ-CD cavity.

* * *

Thus, solid-phase ternary complexes of β- and γ-CD with two types of guest molecules, namely, spin-labeled indoles (pyrrolidinemethylindole (**1**) and piperidineindole (**2**)) and hydrocarbons (benzene and cyclohexane), were prepared for the first time. A study of all eight complexes revealed the influence of the CD structure, a nature of hydrocarbon, and spin label structure on the stoichiometry of the complexes, guest orientation in the cavity, the hydrophobicity of its environment, and molecular dynamics. The hydrophobicity of the environment of the reporter NO group and the dynamics of spin-labeled guests in most of these complexes differ appreciably from these characteristics of the corresponding binary complexes.

(1) The environment hydrophobicity determined in the absence of molecular motion at 77 K in most ternary complexes is substantially higher than in the binary CD complex. However, the hydrophobicity of the environment of the NO group in the γ-CD complexes decreases at high temperatures, apparently, due to its averaging upon the rotational motion of spin labels in the microheterogeneous γ-CD cavity.

(2) The rotational mobility of the spin-labeled guest characterized by the spectral parameter $S = 1 - A_{zz}'/A_{zz}$ is also higher for most ternary complexes, in particular, for the γ-CD complexes than for the corresponding binary complexes. This is, most probably, a result of transition to the 2γ-CD—**2**(**1**)—C_6H_6(C_6H_{12}) stoichiometry in the ternary complexes from 1 : 1 stoichiometry in the binary γ-CD complexes. Analysis of the ST ESR spectra of the ternary complexes led to the conclusion that the motions of label **1** are fast librations in an angular cone, while those of **2** are, most probably, rotations in a relatively wide or full solid angle. This result indicates that the character of rotation in the ternary and binary complexes may also be considerably different: previously,[16] it was shown that motion of both spin-labeled guests in γ-CD can be described as fast librations, whereas for β-CD, the jump rotation model is more adequate. Finally, analysis of the linear ESR spectra for label **2** demonstrated that the rotation of this label in ternary complexes (unlike that in binary complexes) is better described by the Brownian rather than jump rotation model.

The amplitudes of the libration motion of the anisotropy axis (z) of spin label **1** in the ternary complexes ($\Delta\theta$) determined from the parameters A_{zz}' increase, in all complexes, with temperature. The $\Delta\theta$ values for the complexes of **1** with γ-CD are greater than with β-CD, which may be a consequence of the larger cavity diameter in the γ-CD dimer. Note that the mobility of **1** is much higher in the complexes with C_6H_{12} than in the complexes with C_6H_6, despite the greater molecular volume of C_6H_{12}. This difference may be attributed to intramolecular chair-to-boat transitions, which take place in C_6H_{12}, thus producing free volume fluctuations in the CD cavity, which induce the librations of molecule **1**. Unlike **1**, the influence of the structure of the second guest on the rotational mobility of **2** is insignificant for both the complexes with β-CD and with γ-CD. The reason is that the conformational transitions of the piperidine ring make a substantial contribution to the rotational mobility of **2** and, hence, the intramolecular dynamics of the second guest (C_6H_{12}) has a slight influence on the rotational mobility of molecule **2**.

The results of calculation of the energies and geometries of ternary complexes are generally well correlated with the experimental data on the effects of components: CD, spin-labeled indoles, and hydrocarbon, on the hydrophobicity of the environment of the NO group. Indeed, the noticeably lower hydrophobicity of the environment of the NO group of molecule **2** in the complexes with γ-CD compared to that in the β-CD complexes is due to the fact that the spin label **2** in the γ-CD complexes is tilted at an angle of about ~40° to the dimer axis, with a result that the NO group is located near the perimeter of the wide opening of γ-CD near the secondary OH groups and hydrogen bonds between CD monomers, *i.e.*, in rather hydrophilic environment. In the β-CD complexes, molecule **2** is arranged virtually along the dimer axis (with slight deviations in some cases) where the environment is more hydrophobic, which is reflected in a higher h_s value. Molecule **1** both in β-CD and γ-CD complexes is oriented along the dimer axis (see Fig. 9). As in the case of **2**, this gives rise to the hydrophobic environment of the NO group of this label in the complexes. The second factor is the distance from the NO group to the hydrocarbon molecule. It follows from calculations that C_6H_{12} is less remote from NO than C_6H_6 in all complexes. This correlates with the higher hydrophobicity of the NO environment in all C_6H_{12}-containing complexes compared to C_6H_6-containing complexes. In addition, as we have noted previously, the higher environment hydrophobicity in the presence of C_6H_{12} than in the presence of C_6H_6 can result from lower polarizability of aliphatic cyclohexane compared to aromatic benzene.

The results obtained demonstrate the possibility of appreciable modification of the physical properties (polarity and molecular mobility) of the model functional molecules (spin labels) upon introduction of a second guest molecule into cyclodextrin cavities.

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