

# Unimodal Binding of Azulene with $\beta$ -Cyclodextrin: An Intermolecular Nuclear Overhauser Effect Study

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**Abstract.** A nuclear Overhauser effect study of azulene bound to  $\beta$ -cyclodextrin shows that this molecule penetrates into the cavity exclusively from the side of the five-membered ring.

**Key words:** Azulene,  $\beta$ -Cyclodextrin, NOE.

## 1. Introduction

Cyclodextrins (CD), doughnut shaped molecules made of six or more glucose units, have been extensively investigated as enzyme models owing to their ability to form inclusion complexes with a variety of substrates in aqueous solutions [1]. The kinetic as well as the selectivity effects observed [2] depend on the strength of binding of the substrates with the CD and, above all, on the insertion mode whose definition is, accordingly, of paramount importance.

$^1\text{H}$ - and  $^{13}\text{C}$ -NMR studies indicate a substantial orientational preference in the case of CD complexes with polar substrates such as nitrophenols or benzoic acid derivatives [3]. Recently, we observed ( $^1\text{H}$ -NMR and induced c.d.) a more random penetration in the case of substituted cyclohexenones, although preferential binding modes, depending mainly on steric factors, are apparent [4]. Orientational specificity appears to be related to the enhanced polar character of the complexed substrates.

We report here the results of an intermolecular nuclear Overhauser effect (NOE) [5] study of azulene, a weakly polar substrate, bound to  $\beta$ -CD, which shows a clearly unimodal insertion. On the other hand the analogous investigation with  $\alpha$ -CD did not allow any definite conclusion to be made.

## 2. Experimental

The NMR spectra were recorded on a Bruker WP 200 SY instrument.

Owing to the very low solubility of azulene in water, we found it more convenient to isolate the  $\beta$ -CD/azulene complex and to dissolve it in  $\text{D}_2\text{O}$  which was twice distilled under vacuum to remove any trace of  $\text{O}_2$ . For the sake of precision, three experiments were carried out on different samples. The low solubility of the complex compelled us to run the measurements at  $60^\circ\text{C}$ ; at this temperature some azulene sublimates away from the solution onto the tube wall. The effective  $[\text{CD}]/[\text{azulene}]$  ratios, as determined from integrated areas of the NMR spectra,

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Table I. 200 MHz intermolecular nuclear Overhauser effect enhancements from simultaneous irradiation of H-3, H-5, and H-6 of  $\beta$ -CD

	Experiment		
	1	2	3
[CD]/[Azulene]	6.8	4.9	6.2
Azulene proton 2	4.4	5.8	5.3
1,3	6.9	7.7	7.2
4,8	6.4	7.9	7.2
5,7	4.0	4.5	4.1
6	0.0	0.0	0.0

are given in Table I. The usual procedure for NOE difference experiments [6] was modified [7] and the selected resonance region of CD was saturated by a 8 s cyclic perturbation of all lines in the region with a 38–40 dB attenuation of a nominal 0.2 W decoupling power. The % enhancements were obtained from the multiplier of the reference spectrum which brought the observed multiplet to exactly match the corresponding multiplet in the perturbed spectrum. Errors are estimated at about 0.5%.

No  $\alpha$ -CD/azulene complex could be isolated. Thus the solutions were prepared by the following method: a tiny quantity of azulene was added to degassed D<sub>2</sub>O and dissolved by a substantially greater amount of  $\alpha$ -CD.

### 3. Results and Discussion

We report in Table I the % enhancements observed for the different azulene protons in the three experiments. The effects were induced by simultaneous irradiation of the H-3, H-5, and H-6 protons in the  $\beta$ -CD cavity. Selective irradiations were not experimentally feasible because of the near degeneracy of the corresponding signals. On the other hand, irradiation of the H-1, H-2, and H-4 protons, external to the CD cavity, did not give rise to any detectable NOE on the azulene protons.

The most relevant indication of this study is the absence of any enhancement for the H-6 azulene protons, when compared to the relevant effect observed for H-2, the even greater values for H-1,3 and H-4,8, and a lesser effect for H-5,7. These results unambiguously show that azulene is deeply inserted into the cavity, the five-membered ring being totally included and the seven-membered ring partially protruding out of the cavity as illustrated in Figure 1.

The experiment carried out with  $\alpha$ -CD revealed only a small enhancement of the azulene H-1,3 protons. This suggests that although azulene can spend most of its time outside  $\alpha$ -CD, in the complex only the external protons of the five-membered ring can come into sufficiently close proximity with the inner protons of  $\alpha$ -CD.

Inspection of CPK models indicates that the other possible mode of insertion with the seven-membered ring totally inside  $\beta$ -CD is also possible. However this would imply an early steric interaction with the internal cavity wall which would not allow a deep insertion of the molecule. The steric argument is enforced by the finding that whereas the  $\beta$ -CD-azulene complex can be easily isolated following standard procedures [8], the analogous complex with  $\alpha$ -CD could not be obtained even under forcing conditions. The smaller cavity of  $\alpha$ -CD cannot

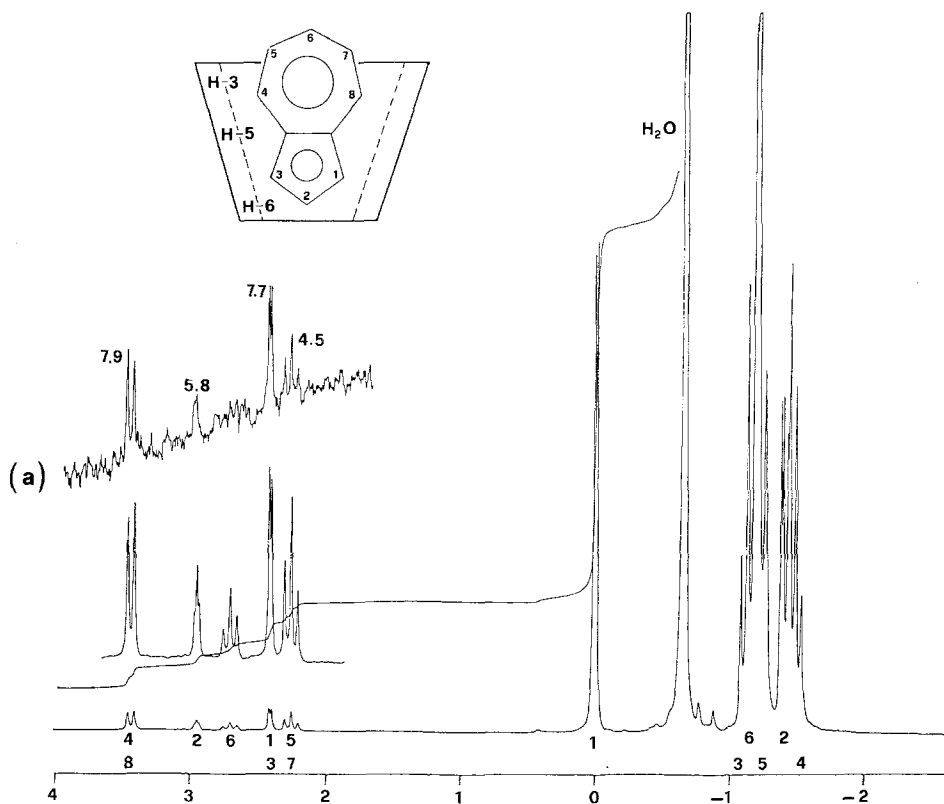


Fig. 1.  $\beta$ -CD/azulene complex in  $D_2O$  at  $60^\circ C$  (experiment 2). Ppm scale referenced to the H-1 resonance of  $\beta$ -CD. Trace (a) gives the NOE difference spectrum; numbers are % enhancements from saturation of the H-3, H-5, and H-6 resonances of  $\beta$ -CD and are obtained as described in the experimental section. The bands in the 0 to  $-2$  ppm region arise from  $\beta$ -CD, while the azulene resonances occur in the 2 to 4 ppm region.

accommodate azulene for sufficient insertion: partial filling and only partial expulsion of water molecules from the cavity do not allow the formation of any stable  $\alpha$ -CD azulene complex.

No definite conclusion can be drawn on the nature of the  $\alpha$ -CD complex. In the case of the  $\beta$ -CD complex, the unambiguous NOE results demonstrate that orientational specificity can be attained also in the case of a weakly polar guest molecule when the relative sizes of substrate and CD allow a complete filling of the cavity [9].

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