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# Induced Circular Dichroism and UV Spectra of Inclusion Complexes of *N*-alkyl-dihydronicotinamides with Cyclomaltoheptose and Heptakis (2,6-di-*O*-methyl)cyclomaltoheptose

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**Abstract.** The mode of inclusion of *N*-alkyldihydropyridines into native or modified cyclomaltooligosaccharides (cyclodextrins), as inferred from induced CD and UV spectra, was found to depend on the nature of the *N*-alkyl residue of the dihydronicotinamide, on the medium, and on the host–guest molar ratio.

**Key words.** Inclusion complex, cyclodextrins, NADH models.

## 1. Introduction

Cyclodextrins (CDs), toroidally shaped polysaccharides, normally made up of six to eight D-glucose monomers, have been used extensively to model protein–ligand and enzyme–substrate interactions [1].

Crucial to the understanding of the chemical effects of the host–guest interactions is the mode of inclusion (axial, equatorial, lid-type) of the guest molecule in the cavity of the CDs. Induced circular dichroism has been extensively used [2] to define the arrangement of a guest molecule in 1:1 CD complexes from the appearance of the induced band of a given chromophoric portion, as well as from the sign of the induced signals indicating the polarization direction of the included molecule, parallel or perpendicular to the CD cavity axis.

We have undertaken an investigation of the reactivity and selectivity of inclusion complexes of *N*-alkyldihydropyridines into CDs. The substrates have been synthesized and investigated as models of biologically essential coenzymes such as NADH and NADPH, in order to obtain information on the effect of hydrophobic and electrostatic interactions on the redox type reactivity of the complexes and by inference, of their biological counterparts.

As a part of this study, we here report the results of induced CD and UV spectroscopic measurements addressed to the definition of the mode of insertion of dihydronicotinamides **1–3** both in natural $\beta$-CD in 50–50% $H_2O$–MeOH and in heptakis-(2,6-di-*O*-methyl)cyclomaltoheptose (DM$\beta$CD) in methanol.[1]

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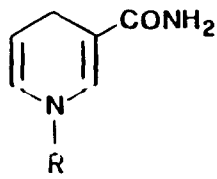



1 : R = $CH_3$

2 : R = $CH_2C_6H_5$

3 : R = $C_{12}H_{25}$

## 2. Experimental

Cyclodextrin was a commercial product and DM$\beta$CD was a gift from Chinoin Pharmaceutical and Chemical Works Ltd., Budapest. They were recrystallized twice from water and stored, as anhydrous materials, over $P_2O_5$ in a vacuum desiccator prior to use. Nicotinamide and the other organic precursors of **1–3** were used without purification. Compounds **1–3** were prepared according to literature procedures [3] and, after purification by column chromatography, their elemental analysis and NMR spectra (see below) were in good agreement with the expected structures.

### 2.1. NMR DATA

$^1$H NMR spectra were recorded on a Bruker WP 200 SY spectrometer operating at 200 MHz, using tetramethylsilane as the internal standard. Intramolecular NOE measurements were carried out to confirm that compounds **1–3** were 1,4-dihydroderivatives and pertinent data are here reported for **2**.

*N-Methyl*-1,4-*dihydronicotinamide*, **1**

$^1$H NMR ($CD_3OD$) $\delta$ 2.90 (*s*, 3H), 3.10 (*m*, 2H), 4.60–4.90 (*m*, 1H), 5.62–5.85 (*m*, 1H), 7.00 (*d*, 1H)

*N-Benzyl*-1,4-*dihydronicotinamide* **2**

$^1$HNMR ($CDCl_3$) $\delta$ 3.10 (*m*, 2H), 4.25 (*s*, 2H), 4.60–4.90 (*m*, 1H), 5.20 (*s*, 2H), 5.60–5.80 (*m*, 1H), 7.10 (*d*, 1H), 7.15–7.40 (*m*, 5H).

*Intramolecular Nuclear Overhauser Effect*: (*proton irradiated*)

Protons enhanced (%) ($H_4$): $H_5$(10.2), $H_7$ (2.8); ($H_8$): $H_6$ (7.3), $H_2$ (14.3); ($H_5$): $H_4$ (2.3), $H_6$ (11.2); ($H_7$): $H_4$ (3.4), $H_2$ (1.3); ($H_6$): $H_5$ (6.8), $H_8$ (1.3); (HAr): $H_8$(2.5).
$H_7 = CONH_2$ $H_8 = CH_2Ar$

*N-Dodecyl*-1,4-*dihydronicotinamide* **3**

$^1$H NMR ($CDCl_3$) $\delta$ 0.79–1.00 (*t*, 3H), 1.20–1.65 (*m*, 2OH), 1.80–2.25 (*m*, 2H), 3.10 (*m*, 2H), 4.60–4.90 (*m*, 1H), 5.25 (*s*, 2H), 5.60–5.80 (*m*, 1H), 7.10 (*d*, 1H).

### 2.2. CIRCULAR DICHROISM AND ULTRAVIOLET MEASUREMENTS

Circular dichroic (CD) measurements were made on a JASCO J-600 spectropolarimeter equipped with a DP-501 N data processor and cylindrical 1-cm fused-quartz cells, for solutions prepared by mixing 0.2 mL of methanolic $10^{-2}$M substrate solution and 0.2, 1 or 1.8 mL of $10^{-2}$ M solutions of $\beta$-CD (in such a way that the resulting medium was in each case a 1 : 1 v/v mixture of the two solvents) or DM$\beta$CD in methanol.

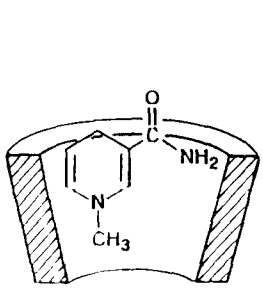
Ultraviolet (UV) spectroscopy was performed on a Perkin-Elmer Lambda 5 instrument.

## 3. Results and Discussion

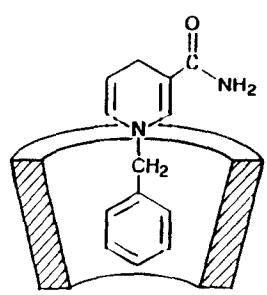
Although $\beta$CD and particularly DM$\beta$CD are largely soluble in a water–methanol mixture, the inclusion complexes of **1–3** with DM$\beta$CD were only sparingly soluble in 1 : 1 water–methanol and could only be investigated in pure methanol. In each case, derivatives **1–3**, in the presence of $\beta$-CD or DM$\beta$CD, exhibit induced optical activity which is diagnostic of the insertion of chromophoric subunits of the guest molecules in the chiral cavity of the receptors [4]. The CD and the UV spectra of 1 mM solutions of both hosts and guests are shown in Figure 1 ($\beta$-CD) and in Figure 2 (DM$\beta$CD). Each complex displays two bands in the regions 350–456 nm and 260–290 nm both characteristic of the conjugated enone systems of the dihydronicotino moiety ($n \rightarrow \pi^\star$ transition) [5].

As shown in Figure 1 the CD spectra of complexes with natural $\beta$-CD show a sharp increase of the 350–356 nm band (of the carbonyl chromophore) on going from CD complexes with **2** to those with **1** and with **3**. Correspondingly, the band at 260–290 nm decreases on going from **2** to **3** and to **1**.

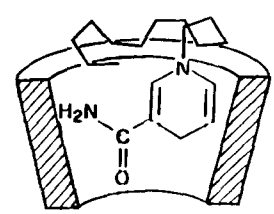
Such a trend clearly indicates that the conjugated carbonyl group of the dihydronicotino moiety is inserted in the cavity to a different extent depending on the structure of the substrate and suggests a change in the insertion mode of the substrates investigated into the CD cavity. Thus, the main mode of inclusion of **2** apparently does not involve insertion of the carbonyl chromophore but rather of the aromatic ring, as shown in the Scheme (mode b). At the other extreme, in the case of **3**, mode c appears as the likely geometry of the complex. The dihydronicotino moiety penetrates inside the cavity and the long paraffinic chain may be assumed to




mode a




mode b




mode c

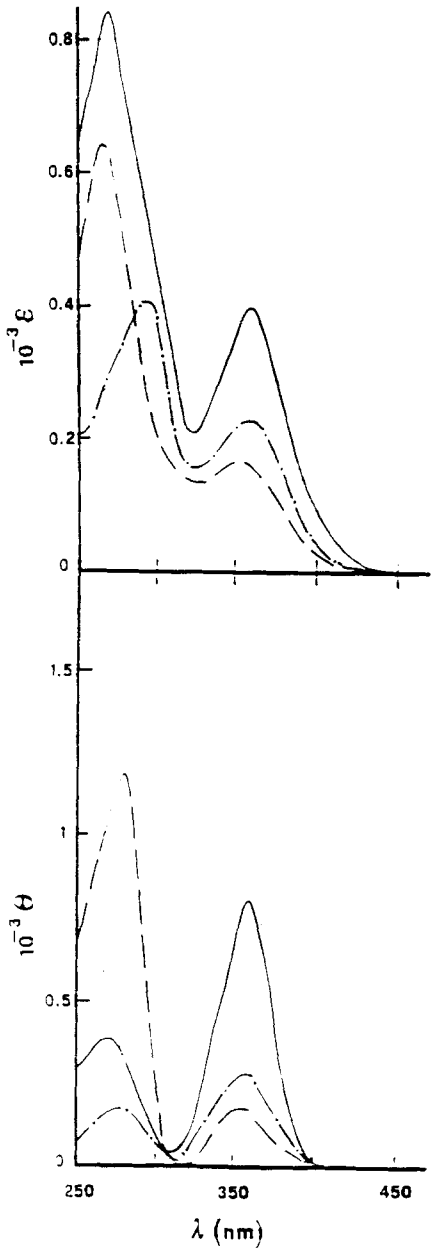



Fig. 1. Induced circular dichroism (bottom) and absorption (top) spectra of dihydronicotinamides 1 (- . -), 2 (- -), 3 (—) $1 \times 10^{-3}$ M in the presence of β-cyclodextrin $10^{-3}$ M in water/methanol 1 : 1 v/v.

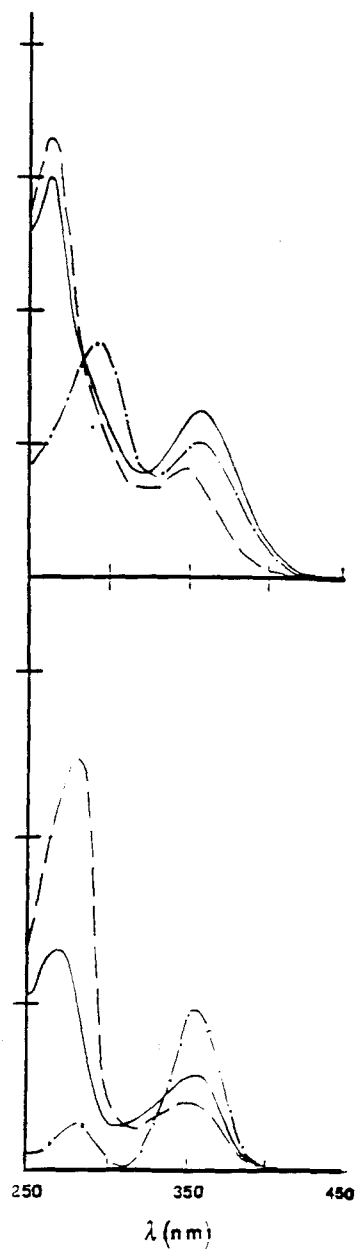



Fig. 2. Induced circular dichroism (bottom) and absorption (top) spectra of dihydronicotinamides 1 (- . -), 2 (- -), 3 (—) $1 \times 10^{-3}$ M in the presence of 2,6-OMe-β-cyclodextrin $10^{-3}$ M in methanol.

be as a lid, on the top of the supramolecular complex. In the case of the complex with **1**, the carbonyl is inserted, although to a lesser extent than with **3**, and a possible mode (a) of insertion where the $CONH_2$ subunit is facing the upper rim of the cavity (possibly hydrogen bonded to the secondary hydroxyls of the CD) may be suggested [6]. It may be noticed that in the case of *p*-nitrophenyl esters of long chain carboxylic acids, there are indications that, in water, the long paraffinic chain is preferentially inside the cavity and the aromatic ring is outside [7], at variance with the mode suggested here for **3**. In the medium used here ($H_2O$/MeOH 1 : 1 v/v), the driving force for penetration of the hydrophobic chain is certainly lower than that in water and this could be the reason for the different behaviour of the two systems. Indeed, the solvent may strongly influence the mode of complexation: quite recently, it has been reported [8] that the inclusion mode of antigenic determinants in CDs may change from an axial to an equatorial type on going from water to *N*,*N*-dimethylformamide.

In the presence of DM$\beta$CD, from the comparison of the induced CD spectra, the mode of inclusion of the benzyl derivative **2** appears similar to that observed with $\beta$-CD. In the case of **1** and **3**, the interaction with the CDs apparently depends on the difference in the geometry of the host and on the nature of the medium. The DM$\beta$CD extends the host cavity relative to that of the native CD and this may make the inside more hydrophobic. As Figure 2 shows for the *N*-methyl derivative **1**, the induced CD band at 360 nm increases, probably due to a deeper insertion. On the other hand, the *N*-dodecyldihydronicotinamide **3** displays a large decrease of the induced CD band at 360 nm; in this case the overall effect is likely to be the result of a diminished binding to the methylated $\beta$-CD and of a stronger influence of the decreased polarity of the medium.

Figures 1 and 2 show the UV absorption spectra of 1 : 1 complexes formed at the same concentration of the host-guest complex reported for the circular dichroism study. The absorption spectra are not usually very diagnostic for inclusion complex formation but the spectra of complexes of **1**–**3** are significantly different, although they are related to substrates having the same chromophore. When included in the native CD, they show a small difference in the absorbance for methyl- and benzyldihydronicotinamide **1** and **2**, and a relatively large increase of the absorption maximum at 356 nm for the dodecyldihydronicotinamide **3**. In the last case, according to the indication obtained from the circular dichroism measurements the substrate chromophore is forced to move (see Scheme) from a polar environment ($H_2O$/MeOH) into a rather hydrophobic one ($\beta$-CD cavity), and this accounts for the most important changes in intensity, band shape, or wavelength of the absorption observed among the complexes investigated. For the DM$\beta$CD inclusion complexes, on the other hand, the UV effects are minimized and this is probably due to the reduced difference between the polarity of the solvent and the cavity of the host.

Figures 3 and 4 show the dependence of the induced CD intensity of **1**–**3** at 356 nm as the host–guest molar ratio changes from 1 to 10. At least in the case of *N*-methyl (**1**) and *N*-benzyl (**2**) derivatives, a slight increase of the induced CD intensity is observed up to a host–guest molar ratio of 5, and this is probably related only to an increase of the complex concentration. Interestingly, at the highest molar ratio (host/guest = 10) the intensity of the induced CD bands

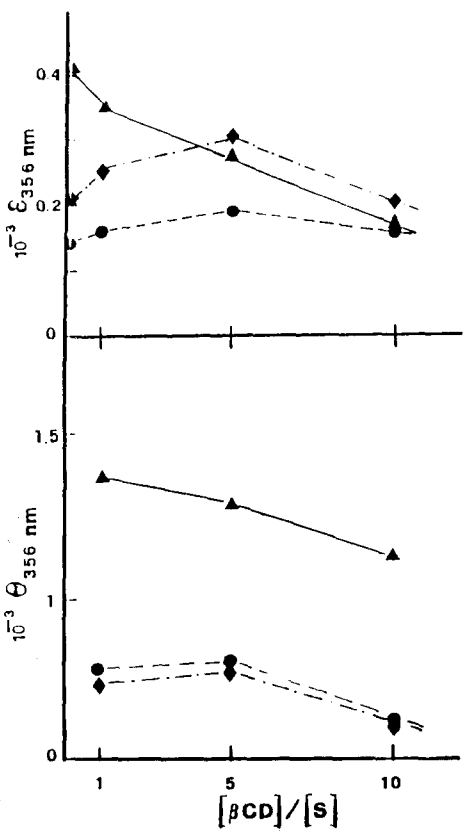



Fig. 3. Induced ellipticity (bottom) and absorption maximum (top) at 356 nm as a function of the molar ratio $\beta$-cyclodextrin/dihydronicotinamides (*S*): 1 (◆), 2 (●), 3 (▲). [*S*] = $1 \times 10^{-3}$ M.

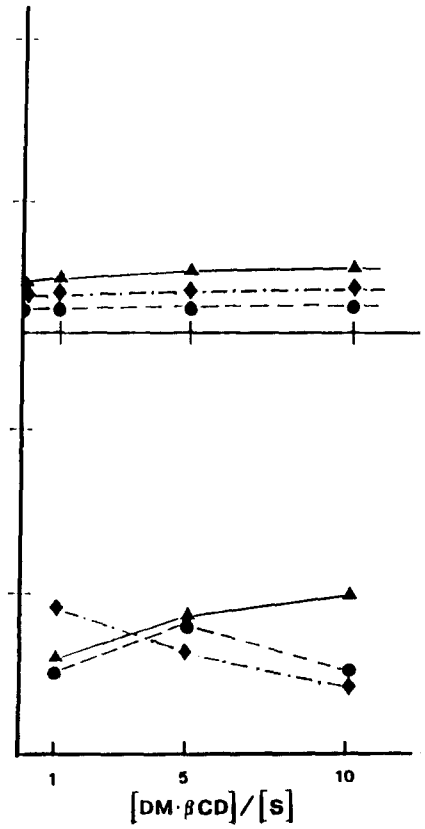



Fig. 4. Induced ellipticity (bottom) and absorption maximum (top) at 356 nm as a function of the molar ratio 2,6-OMe-$\beta$-cyclodextrin/dihydronicotinamides (*S*): 1 (◆), 2 (●), 3 (▲). [S] = $1 \times 10^{-3}$ M.

decreases to the lowest level. This loss of induced chirality, nearly total for **1** and **2**, may be explained by the ejection of the guest from the host cavity probably due to the formation of large $\beta$-CD aggregates, as observed, at least in aqueous solution, and reported by Nicolis [9]. Moreover, a $\beta$-cyclodextrin polymeric aggregate may be formed in concentrated solution, or it could generate a trimeric complex (2 : 1) with the guest inserted in the groove, thus decreasing the intensity of the induced CD bands [10]. As a matter of fact, in crystalline complexes [11] it has been observed that $\beta$-cyclodextrin molecules are linked through hydrogen bonds involving the secondary hydroxyl groups to form a polymeric structure with a head-to-head arrangement.

In methanol, the DM$\beta$CD complexes show a random trend which is difficult to rationalize, while the UV spectra, in the same range of relative concentration, show (Figures 3 and 4) the same trend observed in the case of the $\beta$-CD complexes (in water/methanol) and only a slight dependence on the host/guest molar ratio.

## 4. Conclusion

Our results indicate that the inclusion complexes of dihydronicotinamide models, in both $\beta$-CD or DM$\beta$CD, are widely influenced by the type of substituent on the ring. In particular, at least in the water/methanol mixture used here, a long alkyl chain may force the chromophore deeply into the cavity, at variance with what has been observed in water [7]. Changing the solvent from a 1:1 water–methanol mixture to pure methanol did not result in important changes in the mode of inclusion. Literature reports [2b, 8] indicate that remarkable changes in the inclusion geometry, e.g. from axial to equatorial or lid-type, may occur on going from pure water to less polar solvents. Unfortunately, solubility problems did not allow us to explore the system in water as planned.

## Acknowledgments

Financial support from CNR (Rome) and the technical collaboration of Mr. E. Castiglione is gratefully acknowledged.

## Note

[1]Intermolecular Nuclear Overhauser Effect (NOE) experiments could have provided further important indications. In the present case, however, at least using our current instrumentation, it was not possible to obtain any significant integration of the NOE signals due to the complexity of the NMR spectra of the inclusion systems.

## References

1. R. Breslow: *Science* (Washington, D.C.) **218**, 532 (1982); I. Tabushi: *Acc. Chem. Res.* **15**, 66 (1982); J. Szejtli: *Cyclodextrin Technology*, Kluwer Academic Publisher, Dordrecht, (1988), pp. 365–403.
2. (a) N. Kobayashi: *J. Chem. Soc., Chem. Commun.* 918 (1988); (b) N. Kobayashi: *ibid.* 1126 (1989); (c) N. Kobayashi and M. Opallo: *ibid.* 477 (1990); (d) K. Harata and H. Vedaira: *Bull. Chem. Soc. Jpn.* **48**, 375 (1975); (e) K. Harata: *ibid.* **52**, 1807, (1979); (f) Y. Ishizuka, Y. Nagawa, H. Nakanishi and A. Kuboyama: *J. Incl. Phenom.* **9**, 219 (1990).
3. M. E. Brewster, A. Sumay, K. Czako, D., Winwood, H. Farag, and N. Bodor: *J. Org. Chem.* **154**, 3271 (1984) and references cited therein.
4. G. M. Bonora, R. Fornasier, P. Scrimin, and U. Tonellato; *Carbohydr. Res.*, **147**, 205 (1986).
5. J. Palecek, L. Ptackova, and J. Kuthan: *Coll. Czech. Chem. Commun.* **34**, 427 (1969).
6. M. Sakurai, M. Kitagawa, H. Goshi, Y. Inoue and R. Chujo: *Carbohydr. Res.* **198**, 181 (1990) and references cited therein.
7. G. M. Bonora, R. Fornasier, P. Scrimin and U. Tonellato: *J. Chem. Soc. Perkin Trans.* 2, 367 (1985).
8. A. F. Danil de Namor, R. Traboulssi, and D. F. V. Levis: *J. Chem. Soc. Chem. Commun.* 751, (1990).
9. A. W. Coleman, N. Keller, J. P. Dalbiez, J. Nicolis, M. Drifford, and M. Miocque: *Workshop on Supramolecular Organic Chemistry and Photochemistry*, Universität des Saarlandes (1989).
10. J. S. Manka and D. S. Lawrence; *Tetrahedron Lett.* 7341 (1989); T. V. S. Rao and D. S. Lawrence, *J. Am. Chem. Soc.* **112**, 3614 (1990).
11. C. Betzel, W. Saenger, B. E. Hingerty and G. M. Brown: *J. Am. Chem. Soc.* **106**, 7545 (1984); V. Zabel, W. Saenger and S. A. Mason: *ibid.* **108**, 3664 (1986).