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Comparison between Roesy and ^{13}C NMR Complexation Shifts in Deriving the Geometry of Inclusion Compounds: A Study on the Interaction between Hyodeoxycholic Acid and 2-Hydroxypropyl-β-Cyclodextrin

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The formation and geometry of the hyodeoxycholic acid (HDCA)/2-hydroxypropyl-β-cyclodextrin (HPβCD) complex in methanol-d_4 solution was determined through a rotating frame nuclear Overhauser (ROESY) experiment. The reported results confirmed those independently and previously obtained though the use of ^{13}C complexation shifts in the same solvent. The ^{13}C approach, which needs shorter experimental times and is currently used in the study of HPβCD/bile acid systems, was then substantiated.

Keywords: hyodeoxycholic acid, 2-hydroxypropyl-β-cyclodextrin, inclusion compound, ROESY, NMR

As it has been recently pointed out in a review by Schneider *et al.*[1] that nuclear magnetic resonance is one of the most important method for structural elucidation in the solution state when supramolecular complexes, such as those formed by cyclodextrins and drugs, are considered. The complexation ability of 2-hydroxypropyl-β-cyclodextrin (HPβCD) towards several bile acids has already been reported.[2–4] The NMR study of these systems through the analysis of ^{1}H and ^{13}C NMR complexation shifts presents some problems. In fact, due to the poor solubility of bile acids in water (this is the reason why they are included in cyclodextrins), the observation of reliable ^{1}H and ^{13}C NMR guest complexation shifts in D_2O solutions is prevented. Moreover, the complexity of both ^{1}H and ^{13}C NMR spectra of HPβCD employed (average MW 1380, average molar substitution 0.6) obscures the detection of host complexation shifts. These problems have been overcome utilizing ^{13}C NMR guest complexation shifts in methanol-d_4 solutions as indicative for the inclusion process.[2–4] In the case of water soluble aromatic compounds, the carbon atoms deeply inserted in the cavity of cyclodextrins are shielded, whereas those closer to the wider rim of the torus are deshielded by complexation with α-cyclodextrin.[5] This behavior has been interpreted as a consequence of the difference in dielectric environment on the basis of reaction field theory by Inoue.[1] Although in

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the case of bile acids and HPβCD the solvent employed, methanol, is less polar than water, the cyclodextrin is structurally different from α-cyclodextrin and the guest is not aromatic, the behavior of ^{13}C NMR guest complexation shifts was found to be qualitative similar.[2-4]

Some perplexities on the use of ^{13}C complexation shifts, especially when quantitative evaluation is considered, have been expressed,[1] owing to the pronounced sensitivity of carbon shielding toward conformational changes and to the usual narrower complexation shift range of ^{13}C with respect to ^{1}H. Nevertheless, bile acids present generally lower ^{1}H than ^{13}C complexation shifts, together with highly overlapped ^{1}H NMR spectra and the analysis of ^{13}C complexation induced shifts is to be preferred, at least at a qualitative level, with respect to that of ^{1}H. A comparison between ^{1}H and ^{13}C complexation shifts for the HDCA/HPβCD complex is reported in Table I; it represents a typical case among HPβCD/bile acid complexes. The carbon complexation induced shifts[9] have been interpreted as proving the insertion of HDCA, from its alkyl chain side, into the HPβCD cavity from the wider rim side. The high shifts experienced by C-21, C-16 and C-12 (for numbering see Scheme 1) should indicate that these carbons are close to the secondary hydroxy rim of HPβCD and the interaction involves the two HDCA rings adjacent the alkyl chain.

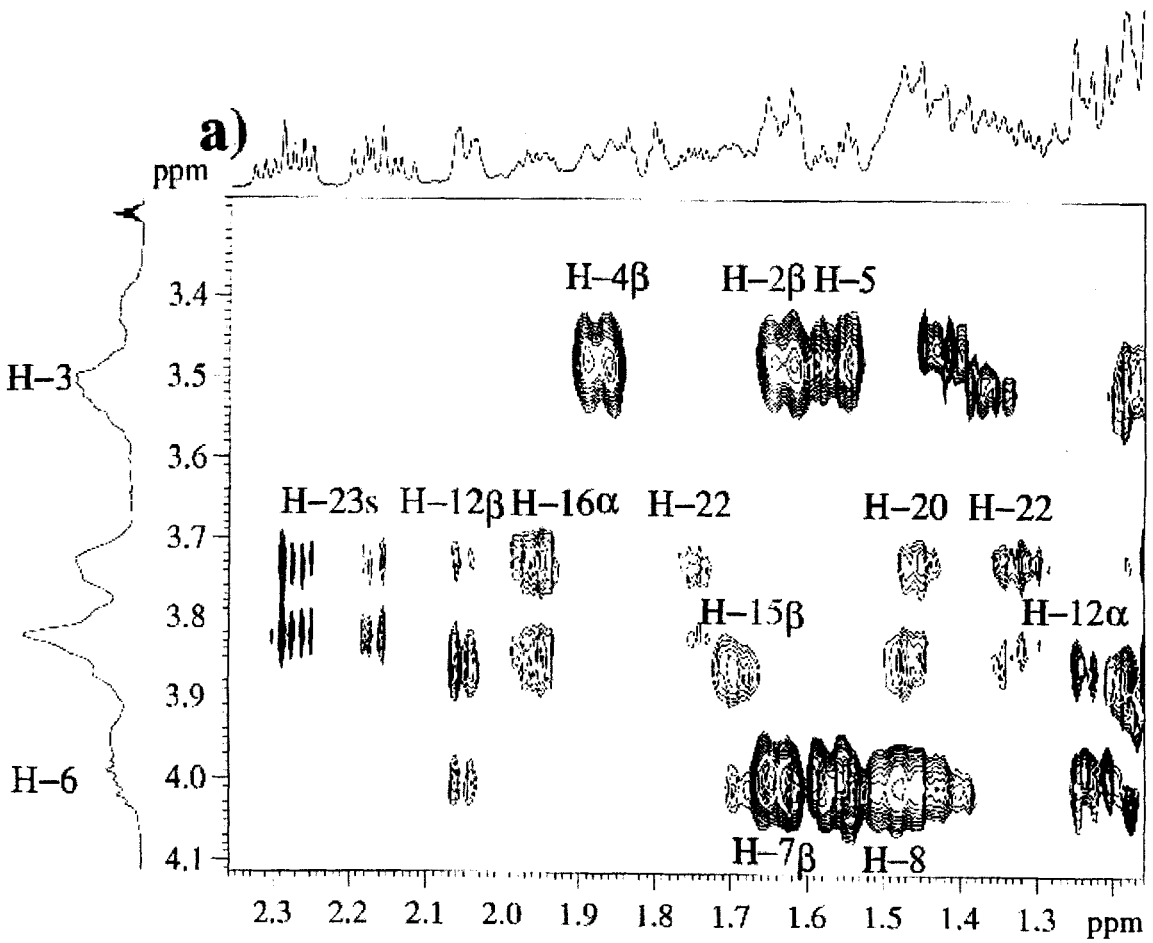
An alternative approach to the characterization of inclusion compounds between drug and 2-hydroxypropyl-β-cyclodextrin in D_2O solution[6,7] is the use of nuclear Overhauser effect spectroscopy in its rotating frame version (ROESY).[8] This experiment allows us to detect when two protons, one from the host and the other from the guest, are spatially close. Also in this case problems arise when cross-peaks are to be assigned because of the complexity of both HDCA and HPβCD ^{1}H NMR spectra. Moreover, these experiments are usually time consuming because intermolecular ROE cross peaks have low intensities and a high number of scans is needed.

TABLE I ^{1}H chemical shifts (ppm) of protons of hyodeoxycholic acid (HDCA) in methanol-d_4 solution and ^{1}H and ^{13}C complexation shifts (ppm) in the presence of 2-hydroxypropyl-β-cyclodextrin (HPβCD)

	δ^1H HDCA		$\Delta\delta^1H^a$		$\Delta\delta\ ^{13}C^b$
	α	β	α	β	
1	1.80	1.05	+0.02	0	+0.06
2	1.33	1.62	+0.06	+0.01	+0.08
3		3.50		0	+0.09
4	1.37	1.87	+0.04	+0.01	+0.03
5		1.57		0	−0.01
6		4.00		+0.01	+0.04
7	1.15	1.61	+0.04	+0.03	−0.03
8		1.49		+0.01	+0.02
9	1.40		+0.02		+0.04
10					−0.03
11	1.44	1.23	+0.05	+0.01	−0.03
12	1.20	2.01	+0.05	+0.04	+0.26
13					+0.10
14	1.16		+0.02		+0.16
15	1.12	1.62	+0.05	+0.08	+0.07
16	1.90	1.32	+0.06	+0.05	+0.36
17	1.15		0		−0.12
18	0.69		+0.04		+0.39
19	0.92		+0.01		−0.06
20	1.45		+0.02		0
21	0.95		+0.01		+0.57
22	1.80,1.28		−0.03,+0.03		+0.15
23	2.32,2.19		−0.03,-0.03		−0.07
24					−0.02

a. $\Delta\delta^1H = \delta^1H$ HDCA/HPβCD − δ^1H HDCA.
b. ref. 9.

Despite the problems above summarized a ROESY study[10] on hyodeoxycholic acid (HDCA)/HPβCD inclusion complex was undertaken with the aim of comparing the information derived from ^{13}C NMR guest complexation



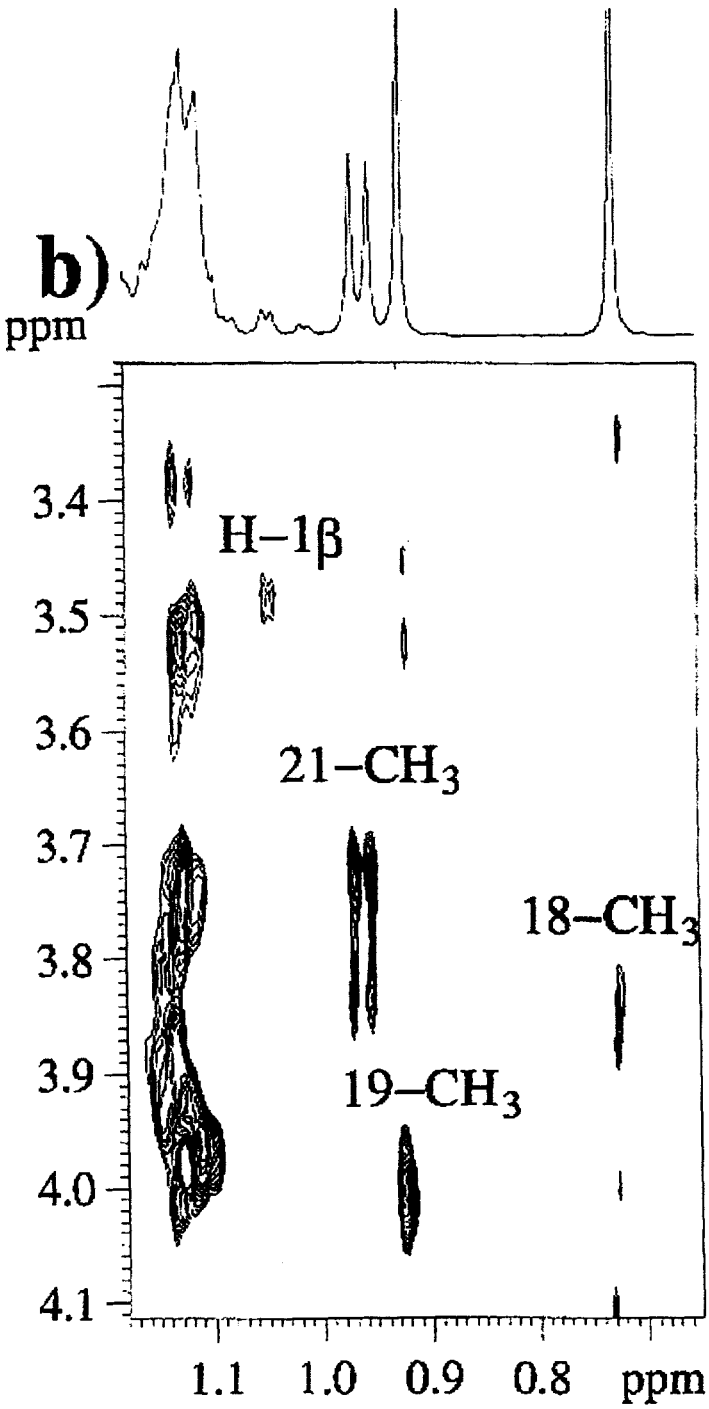


FIGURE 1 Partial ROESY spectra of HDCA/HPβCD complex in methanol-d_4 solution. The labels refer to HDCA protons, for HPβCD protons chemical shifts see Table II. The two *f*2 regions a) 2.35–1.16 ppm and b) 1.18–0.65 ppm were plotted with different intensities. The strongest ROE cross peaks are intramolecular

induced shifts[9] with that derived from nuclear Overhauser effect spectroscopy in methanol-d_4 solution. DQS,[11] HSQC[12] and HMBC[13] experiments were also carried out to locate the major signal of the HPβCD employed, both in the free and in the complexed state. The results are collected in Table II and show that two major groups of signals can be distinguished: those deriving from unsubstituted units and those coming from 2-substituted rings. A third minor fraction was not completely characterized and is not reported. Only minor changes (within 0.01 ppm for ^1H and 0.05 ppm for ^{13}C) were observed in ^1H and ^{13}C NMR chemical shifts when passing from free to complexed HPβCD in methanol-d_4 solution.

The assignments of proton signals of HPβCD enabled the interpretation of intermolecular cross peaks of the ROESY spectrum (Fig. 1) to be made. Fig. 1 shows that, apart from strong intermolecular ROEs between H-6 and H-3 of HDCA (at 4.01 and 3.50 ppm, respectively) and their neighboring protons, a number of intermolecular peaks between HPβCD and HDCA protons appears, the pattern of which resembles that found for the complex between HPβCD and ursodeoxycholic acid in D_2O solution.[6] In particular, we can note that H-23s and 21-CH_3 of HDCA are spatially close to H-5 and H-6s of HPβCD, H-22s are close to H-5 of HPβCD, H-16α and H-20 are near to both H-5 and H-3 of HPβCD, whereas 18-CH_3, H-12s, H-14 and H-15β are closer to H-3 than to H-5 of HPβCD. The only intermolecular ROEs confidently assignable to H-3 proton of HPβCD 2-substituted ring (the signal of which is very close to that of H-6 of HDCA) are with H-12β and H-15β. These last cross-peaks can hardly be seen as intramolecular in origin. The overall picture derived from ROE data confirm that the relative orientation between HDCA and HPβCD is that shown in Fig. 2: the alkyl side chain of the former is close to the primary hydroxy rim of the latter, whereas protons from the two rings of HDCA adjacent to the alkyl chain are close to the wider rim of HPβCD.

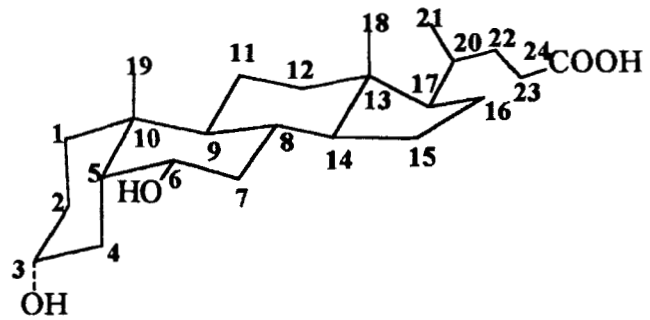
In conclusion, we have definitely demonstrated the geometry of the supramolecular complex as derived from ROE data, Fig. 2, agrees with that previously deduced from ^{13}C complexation shifts of HDCA in the same solvent,[9] and for other HPβCD/bile acid complexes previously studied both in methanol and in water solution.[2–4,6] These results reinforce the use of ^{13}C complexation shifts in methanol-d_4 solution, at least when HPβCD/bile acid complexes are concerned, as a simple and time saving (experimental times are reduced to about 1/10) tool to obtain information on the geometry of the supramolecular complex.

A last comment is to be made on the competition that can be expected between HDCA and methanol for the cavity of HPβCD. A study on effects of ethanol on complexation of HPβCD with testosterone[14] (in aqueous solution) has shown that a competition occurs in this case. On the other hand, a lower affinity of methanol, with respect to ethanol, for βCD has also been reported.[15] The present results unambiguously show that the inclusion complex between HDCA and HPβCD in methanol-d_4 solution forms, hence ^{13}C shifts of HDCA, and of bile acids in general, in the presence of HPβCD in methanol-d_4 are correctly attributed to complexation.

TABLE II ^1H and ^{13}C NMR chemical shifts (δ, ppm) of 2-hydroxypropyl-β-cyclodextrin (HPβCD) in methanol-d_4.[a]

ring	*H-1/C-1*	*H-2/C-2*	*H-3/C-3*	*H-4/C-4*	*H-5/C-5*	*H-6/C-6*	*H-a/H-a'/C-a*	*H-b/C-b*	*CH_3/CH_3*
unsubstituted	4.97/104.0	3.49/74.5	3.87/75.2	3.50/83.5	3.72/74.0	3.83/62.2	3.53/3.72/79.2		1.13/20.1[b]
2-substituted	5.10/102.2	3.41/83.2–84.0	3.99/74.7	3.50/83.5	3.70/73.8	[c]/62.2	3.60/3.76/79.8		1.11/19.6/19.7[b]

[a] only minor changes (within 0.01 ppm for ^1H and 0.05 ppm for ^{13}C) were observed in ^1H and ^{13}C NMR spectra and in 2D spectra when passing from free to complexed HPβCD in methanol-d_4 solution.[b] assignment may be reversed
[c]protons signal are in the range 3.95–3.75 ppm



SCHEME 1 3α,6α-dihydroxy-5β-cholan-24-oic acid, HDCA

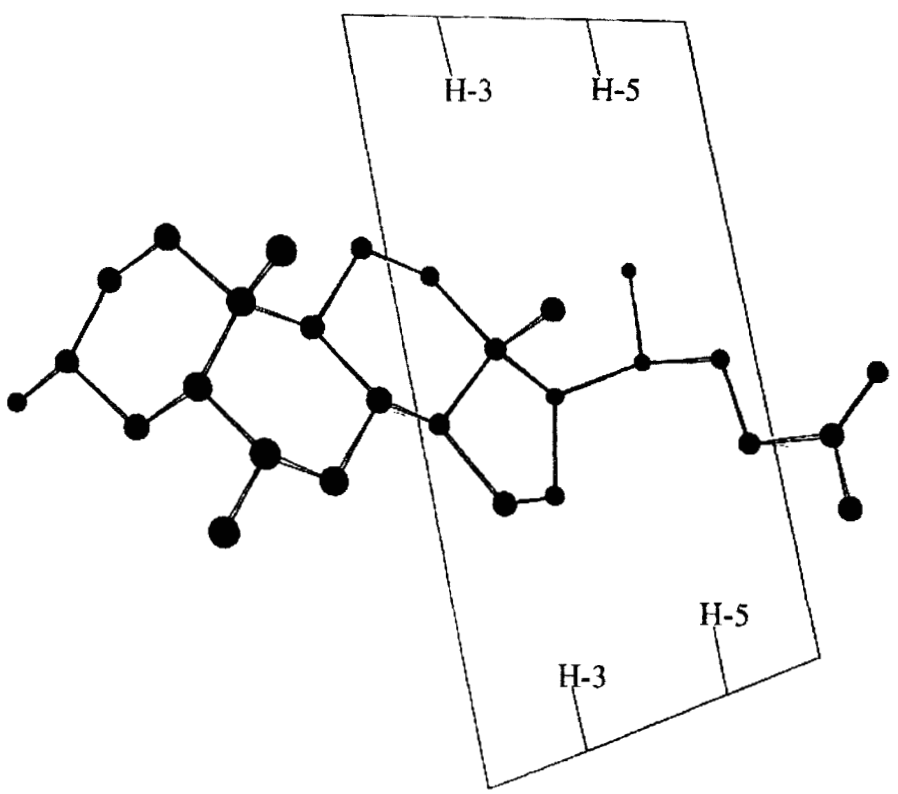


FIGURE 2 Geometry of the HDCA / HPβCD inclusion complex

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References

[1] Schneider, H.-J.; Hacket, F.; Rüdiger, V.; *Chem. Rev.* 1998, 98, 1755.
[2] Vandelli, M.A.; Salvioli, G.; Mucci, A.; Panini, R.; Malmusi, L.; Forni, F.; *Int. J. Pharm.* 1995, 118, 77.
[3] Mucci, A.; Vandelli, M. A.; Salvioli, G.; Malmusi, L.; Forni, F.; Schenetti, L.; *Supramolecular Chem.* 1996, 7, 125.
[4] Mucci, A.; Schenetti, L.; Salvioli, G.; Vandelli, M. A.; Forni, F.; *J. Incl. Phenom.* 1996, 26; 233.
[5] Inoue, Y.; *Annu. Rep. NMR Spectrosc.* 1993, 27, 59.
[6] Mucci, A.; Schenetti, L.; Vandelli, M. A.; Forni, F.; Ventura, P.; Salvioli, G.; *J. Chem. Soc., Perkin Trans. 2* 1996, 2347.
[7] Mucci, A.; Schenetti, L.; Vandelli, M.A.; Ruozi, B.; Forni, F.; *J. Chem. Res. (S)* 1999, 414.
[8] Bothner-By, A. A.; Stephens, R. L.; Lee, J.-M.; Warren, C. D.; Jeanlos, R. W.; *J. Am. Chem. Soc.* 1984, 106, 811.
[9] Vandelli, M.A.; Ruozi, B.; Forni, F.; Mucci, A.; Salvioli, G.; Galli, E.; *J. Incl. Phenom.*, 2000, 37, 237.
[10] HPβCD (Aldrich Chemical Co., Milwaukee, WI, USA) and HDCA (Sigma Chemical Co., St. Louis, MO, USA) were used as received from the manufacturers. The inclusion complex was prepared at a 1:1 molar ratio of HDCA to HPβCD. Typically, 1.38 g (1 mM) of HPβCD was dissolved at room temperature in 50 mL of water to which 30 mL of ethyl alcohol containing 392 mg (1 mM) of HDCA was added. The solution was evaporated immediately after its preparation under vacuum in a rotary evaporator (model R-114; Büchi, Buchs, Switzerland) at a temperature of 45°C. ^{1}H and ^{13}C NMR spectra were obtained at 303 K using a Bruker AMX-400 WB spectrometer operating at 400.13 and 100.61 MHz, respectively, on 3 10^{-2} M CD_3OD solutions. δ values refer to internal CHD_2OD and $^{13}CD_3OD$ set to 3.30 and 49.3 ppm, respectively. Typical digital resolution was ± 0.1 and ± 0.3 Hz for ^{1}H and ^{13}C NMR spectra, respectively. Conditions for ROESY[8] phase-sensitive spectrum *via* time-proportional phase incrementation (TPPI) were: presaturation of residual HDO signal during relaxation delay 0.5 s; mixing time 200 ms; spin-lock field 4000 Hz; spectral width 9 ppm; 4096 complex points in $f2$; 256 $t1$ values and 192 scans per $t1$ value, total acquisition time: 18 hours. Lorentz-Gauss enahancement (LB=-0.5; GB=0.01) in $f1$ and square-sine function (SSB=2) in $f2$ were applied before Fourier transformation.
[11] Mareci, T.; Freeman, R.; *J. Magn. Reson.* 1983, 51, 531.
[12] Bodenhausen, G.; Ruben, D. J.; *Chem. Phys. Lett.* 1980, 69, 185.
[13] Bax, A.; Summers, M.F.; *J. Am. Chem. Soc.* 1986, 108, 2093.
[14] Pitha, J.; Hoshino, T.; *Int. J. Pharm.* 1992, 80, 243.
[15] Matsui, Y.; Mochida, K.; *Bull. Chem. Soc. Jap.* 1979, 52, 2808.