NMR Differentiation of Enantiomeric (+)- and (−)-α-Pinene *via* Complexation with Cyclodextrins in Water

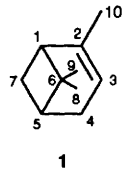
Antigone Botsi,[a] Konstantina Yannakopoulou,*[a] Eugene Hadjoudis[a] and Bruno Perly[b]

[a] *Institute of Physical Chemistry, NCSR 'Demokritos', Aghia Paraskevi 15310, Athens, Greece*
[b] *CEA, Service de Chimie Moleculaire, CEN de Saclay, 91191 Gif sur Yvette Cedex, France*

(+)- and (−)-α-Pinene form diastereoisomeric complexes with α-, 2,3,6-tri-*O*-methyl-α-, 2,3,6-tri-*O*-methyl-β- and 2,3,6-tri-*O*-acetyl-β-cyclodextrin which are distinguishable in ^{1}H and ^{13}C NMR spectra, the α-pinene protons obeying the slow exchange condition during the complexation process; utilization of cyclodextrins as chiral shift reagents for molecules lacking polar groups is consequently proposed.

Cyclodextrins[1] (CDs) are chiral host molecules which form inclusion complexes with a vast number of organic molecules. Owing to the chirality of the CD cavity, complexation with enantiomeric pairs results in the formation of diastereoisomeric complexes, which should show separate guest signals in the NMR spectra for each enantiomer. ^{1}H NMR studies of cyclodextrins as chiral complexing agents for optical purity determinations of pharmaceutically important polar molecules have been reported.[2–4] Application of CDs for analogous NMR investigations of molecules lacking polar groups, for example simple alkenes, has not been explored so far. α-Pinene **1** is such a molecule, a natural terpenoid found as a constituent in many insect pheromones[5] which is used as a starting material for a number of enantioselective chemical transformations. We have found that in D_2O solutions, CDs form diastereoisomeric complexes with racemic α-pinene, which show separate signals for each enantiomer in ^{1}H and ^{13}C NMR spectra. These signals appear at standard frequencies, independent of concentration. We used α-, β- and γ-CD (**2a**, **b**, **c**, respectively), 2,3,6-tri-*O*-methyl-α-, -β- and -γ-CD (**3a**, **b**, **c**, respectively), 2,3,6-tri-*O*-acetyl-β-CD **4** (DS = 14–16) (CYCLOLAB, Hungary) which provided a range of cavity diameters and heights as well as solubilities for selection.[1]

Examination of the ^{1}H NMR spectra of α-pinene in D_2O [Fig. 1(*a*)] in the presence of **2a**, **3a**, **3b** and **4**, revealed a duplication of its signals due to the formation of diastereoisomers [Fig. 1(*b*)(*c*)]. At the same time, shift displacements of the CD protons were also observed,† which typically indicate host–guest association.[5,6] Complexes were formed with **2b** and **c** but their solubilities were very low as indicated by immediate precipitation. On the other hand, there was no splitting of the signals of **1** with **3c** due to the absence of complexation, as evidenced by the lack of shift changes in the CD part of the spectrum. Owing to the complex nature[7] of the ^{1}H NMR spectrum of **1**,‡ the only signals useful to monitor the enantiomeric separation were 8-Me, 9-Me, 10-Me, 3-H and 7-H_{endo}. Table 1 shows the chemical shifts of each diastereoisomer. The chemical shifts of the pure enantiomers in the presence of each CD correspond exactly to those in Table 1, as shown from individual spectra. The largest separation of pinene chemical shifts between diastereoisomeric pairs (Δδ) were induced by **2a** and **4**, the smallest by **3a**. Reliable



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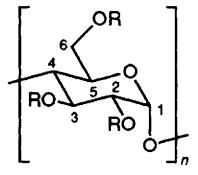

n = 6, 7, 8 : α-, β-, γ-CD
2; R = H
3; R = Me
4; R = Ac

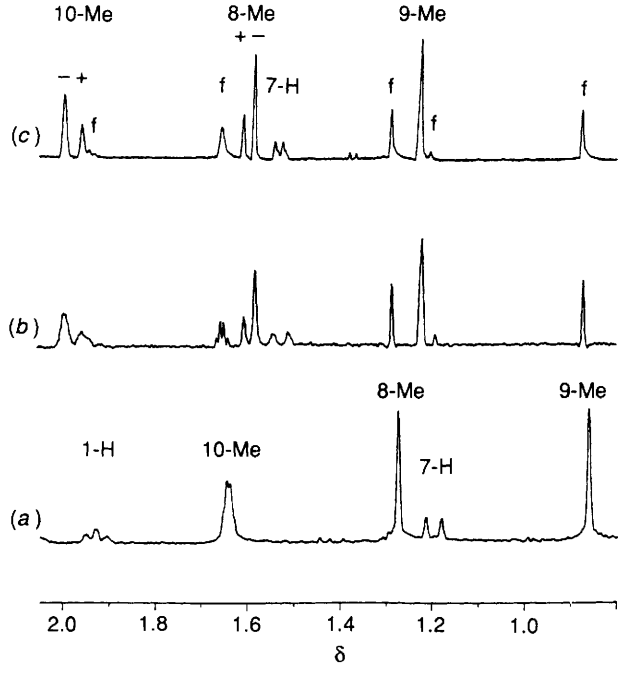

Fig. 1 Partial ^{1}H NMR spectra in D_2O of (*a*) (±)-α-pinene (250 MHz) and (±)-α-pinene/α-CD at (*b*) 250 MHz and (*c*) 500 MHz; f = free α-pinene

Table 1 ^{1}H NMR frequencies[a] (δ) of free α-pinene **1** and complexed with **2**, **3** and **4**, where $\Delta\delta = \delta_{CD/(+)\text{-}1} - \delta_{CD/(-)\text{-}1}$

Compound	8-Me	9-Me	10-Me	7-H_{endo}	3-H
1 (rac)	1.275	0.862	1.642	1.198	5.177
2a/(+)-**1**	1.608	1.233	1.959	1.530	5.666
2a/(−)-**1**	1.585	1.224	1.996	1.532	5.608
Δδ	0.023	0.009	−0.037	−0.002	0.058
3a/(+)-**1**	1.482	1.081	1.850	1.287	5.446
3a/(−)-**1**	1.481	1.073	1.840	1.311	5.457
Δδ	0.001	0.008	0.010	−0.024	−0.011
3b/(+)-**1**	1.453	1.033	1.802	1.335	5.400
3b/(−)-**1**	1.440	1.027	1.790	1.352	5.378
Δδ	0.013	0.006	0.012	−0.017	0.022
4/(+)-**1**	1.451	1.082	1.798	—[b]	—[b]
4/(−)-**1**	1.440	1.059	1.839	—[b]	—[b]
Δδ	0.011	0.023	−0.041	—	—

[a] Experimental conditions: spectra were acquired on Bruker spectrometers AC-250, AMX-500 or AMX-600 at 298 K using a maximum of 256 scans. [b] Obscured by other peaks.

† The region δ 5–3 is characteristic[6] for each CD; interaction of a guest molecule with the cavity is evidenced by chemical shift changes, corresponding mainly to the inner CD protons, 3-H and 5-H.[6]

‡ Spectral assignment in D_2O was aided by 2D H–H and H–C correlation and double quantum spectroscopy, using the standard Bruker software.

integration at 250 MHz can thus be attained, which is even better if the spectra are obtained at higher fields. The optical purity of a mixture can thus be readily assessed. Integration of the ^{1}H NMR signals of racemic **1** in the presence of a CD gives the following (−)/(+) ratios: **2a/1** : 2.00; **3a/1** : 1.10; **3b/1** : 1.05; **4/1** : 1.00. These ratios reflect the relative size of the diastereoisomeric binding constants (K_R and K_S) associated with the complexation process, and express the relative preference of a given CD toward each of the enantiomers. They also show that different chemical shifts of (+)- and (−)-pinene protons are observed even when there is no selective binding, as for example with peracetylated β-CD **4**.

The exchange between free and complexed enantiomeric **1** is slow, since the uncomplexed species is present (Fig. 1) and no chemical shift variations of the guest signals are observed upon varying the concentrations. In the slow exchange condition the chemical shifts of each diastereoisomer are real and not time-averaged, thereby permitting the observation of two distinct signals even when the differences (Δδ) between δ_+ and δ_- are very small. On the contrary, in the fast exchange reduced signal separation (Δδ) is observed as the result of average shifts between complexed and uncomplexed enantiomers. In addition, the observation of separate signals for each enantiomer could require a study of varying concentrations, if the diastereoisomeric K values are similar. Fast exchange is most commonly observed in CD complexes.

It is important to point out that the use of CDs as chiral shift reagents does not seem to require enantioselective binding but only an interaction of a chiral guest with the CD cavity. If two enantiomers can be discriminated by NMR using a particular CD, it is likely that they can also be separated chromatographically with the same CD. Indeed, (+)- and (−)- α-pinene have been separated using α-CD[8] and permethylated β-CD[9] as stationary phases in gas chromatography. Direct correlation, however, between solution NMR and gas chromatography is not safe,[9] as apart from inclusion, other mechanisms may contribute to enantioselection in each of the methods.

The above results were confirmed by the ^{13}C NMR spectra, in which splitting of the pinene signals due to the formation of diastereoisomers could also be observed. The solubilities of the so-formed complexes were 7 and 10 mmol dm^{-3} for **2a/1** and **3a/1**, respectively, inadequate to achieve a good signal-to-noise ratio for the guest signals within a reasonable amount of time. With **3b**, however, a very good spectrum was easily obtained. The pinene carbon atoms C-1, C-2, C-3, C-5 and C-6 were split by δ 0.06, 0.14, 0.11, 0.15 and 0.08, respectively. The ^{13}C NMR spectra can therefore provide a means of detecting the presence of enantiomers,[10] especially if the ^{1}H NMR spectra are complicated. Investigation of the structures of the complexes in aqueous solution, involving detailed NMR studies will be presented shortly.[11]

The known chiral lanthanide shift reagents are expensive and not very effective in separating NMR signals of enantiomeric molecules which lack polar groups.[12] Such studies are scarce in the literature,[12,13] and thus cyclodextrins can provide convenient alternative chiral shift reagents for optical purity examination of alkenes and related molecules by NMR.

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