Analyst

PAPER

Vibrational circular dichroism (VCD) methodology for the measurement of enantiomeric excess in chiral compounds in solid phase and for the complementary use of NMR and VCD techniques in solution: the camphor case.†

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† Electronic supplementary information (ESI) available: Linear regressions of the intensity of several VCD bands (ΔAbs.) *vs*. % *ee*; SI1) IR-VCD spectra in CCl_4 solution, SI2) IR-VCD spectra in nujol mull.

Dedicated to Professor Jack D. Dunitz extraordinary contribution to structural chemistry

For the first time, the success of a methodology for the determination of enantiomeric excesses (% *ee*) in chiral solid samples by vibrational circular dichroism (VCD) spectroscopy is reported. We have used camphor to determine the % *ee* in a blind sample constituted by a mixture of its two enantiomers as a test of the validity of our approach. IR and VCD spectra of different enantiomeric mixtures of *R*/*S*-camphor in nujol mulls were recorded and linear regressions of VCD intensities (ΔAbs.) *vs*. % *ee* for selected bands were found. Finally, the VCD intensities of a blind sample were interpolated in these linear regressions, obtaining its % *ee* with a rms of 2.4. These results in the solid phase were complemented with the determination of % *ee* in the liquid phase by VCD and NMR techniques, which are proved to be complementary techniques to carry out this kind of analysis. In the same way as in the VCD solid phase, linear regressions of ΔAbs. *vs*. % *ee* for selected bands were established, obtaining a rms of 1.1 in the % *ee* determination of a blind sample. ^{1}H NMR experiments at 600 MHz using the chiral solvating agent, (*S*,*S*)-ABTE, allow to determine in CD_2Cl_2 solution the proportions of enantiomers with great accuracy. ^{13}C CPMAS NMR spectra prove that this technique cannot be used for conglomerates and/or solid solutions.

1. Introduction

Why is the detection of chirality and enantiopurity in solid samples important? There are two reasons. The trivial one is that there are compounds insoluble in most organic solvents (for instance, most inorganic compounds). The most interesting one is that there are enantiomers that interconvert (racemization) so fast in solution that they can only be isolated and studied in the solid state. This is a rather common situation,[1-5] that we have also observed several times, even with compounds that lacked any stereogenic center like 1*H*-indazoles; these compounds often crystallize in

conglomerates1 formed by chiral helices[6-9] and they cannot be manipulated to prepare mixtures of known enantiomeric excess (*ee*).

A series of papers based on solid-state NMR (SSNMR) and on powder diffraction were used to determine the *ee* of different compounds. We will discuss this topic at the end of the present paper showing that certain compounds cannot be studied by these methods, amongst them camphor.

Racemic camphor crystallizes forming **solid solutions,**[1,10,11] that is, single crystals containing both enantiomers, the D and the L, in any proportion (some authors called this situation a scalemate [12]), all the single crystals being identical, a phenomenon also known as pseudoracemates.[13-15] Note that Dreiling and Gay[16] have used the 3-bromocamphor to justify the Vester-Ulbricht hypothesis of the origin of chirality of biomolecules (Fig. 1).[17,18] In the present paper, we will use indistinctly *R* or *S* and D or L.

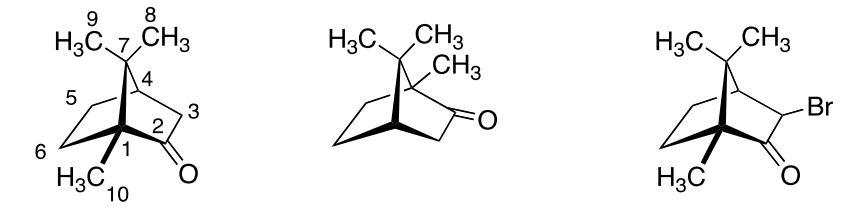


Fig. 1. The structures of camphor enantiomers and a camphor derivative are shown.

Vibrational Circular Dichroism (VCD) is a spectroscopic technique sensitive to chirality that is defined as the difference in the absorbance of left minus right circularly polarized light for a molecule undergoing a vibrational transition.[19] One of the VCD applications is to determine the enantiomeric excess (% *ee*) of mixtures of enantiomers of a chiral molecule. The interest of using this technique arises from the quantity of spectral bands that can be recorded simultaneously in the spectrum. Apart from giving structural information about the molecule, it has been proved that this technique can be used to determine simultaneously the percent *ee* (% *ee*) of one or two chiral species in a given sample in liquid solution.[20,21] The % *ee* of a sample can be defined as the amount of one enantiomer, minus that of the other enantiomer, divided by the sum of their amounts. The expression for the percentage of enantiomeric excess of enantiomer A relative to that of enantiomer B is given by equation 1:[19]

$$\%\ ee\ (A) = \frac{N_A - N_B}{N_A + N_B} \times 100 \qquad (1)$$

where N_A is the number of moles of enantiomer A and N_B that corresponding to enantiomer B. The intensity of the bands of the VCD spectrum of enantiomer A is directly and linearly related with the % *ee* of enantiomer A. For example, the % *ee* (A) for an optically pure sample of enantiomer A is 100 % and the VCD intensity shows its maximum value for it. The value of the racemic mixture is 0 % and –100 % for the optically pure sample of enantiomer B.[20]

Several studies dealing with the analysis of the enantiomeric excess of samples in solution have been carried out. In particular, Freedman *et al.*,[22] Cianciosi *et al.*[23,24] and Spencer *et al.*[25] studied the kinetics of the stereo-specific thermal degradation products of *trans*-dideuterio-cyclopropane from 1989 to 1991. A decade later, Guo *et al.*[20,21,26] reported the use of VCD technique to follow changes in the % *ee* of chiral molecules with time using a flow cell sampling device. They used partial least squares (PLS) chemometric analysis to predict the % *ee*, obtaining accuracies of 1 % in the case of a solution of α-pinene in CCl_4 and 2 % for a two-sample solution to simulate the transformation of *S*-(–)-camphor to *S*-endo-(–)-borneol in the MIR region using VCD spectroscopy. They also carried out this analysis in the Near IR region using VCD, obtaining accuracies of 2 % for species alone (α-pinene, camphor, and borneol) and 3 % for two species [*S*-(–)-camphor and *S*-endo-(–)-borneol].

Additionally, it can be pointed out several works about the study of IR and VCD spectra of camphor,[27,28] and camphor derivatives[27-33] in $CDCl_3$ and CCl_4 solutions[27-33] and in the solid state (KBr pellets[29] or nujol and fluorolube mulls[32]), complemented with DFT calculations. In the references 27, 29 and 31 , UV and CD spectra were also analyzed, with the additional study in the former of the fluorescence and circularly polarized luminescence (CPL) spectra. Note that in the reference 28 the IR and ROA spectra were also recorded and compared with calculations, in order to assess performance of several harmonic and anharmonic computational approaches to model the CH stretching region. In the literature, studies about the IR and VCD analysis in the solid phase (KBr pellets[29,34,35] or nujol and fluorolube mulls[32]) and the structural behaviour of hydrogen-bonded systems in solution and solid phases can be also found.[ref] In the reference ---, where the two enantiomers of a chiral thiophene sulfonamide were analyzed by IR, VCD, Raman and ROA spectroscopies in solution and by X-ray crystallography, IR and VCD spectroscopies also in the solid state (KBr pellets in the case of IR and VCD) with the help of quantum chemical calculations.

For example, T. Wu *et al.* asymmetrically synthesized a novel couple of enantiomers of chiral macrocyclic imines derived from *R/S*-camphor. Using these ligands, they obtained two enantiomeric nickel(II) coordination compounds with predetermined configuration. The chiral ligands and corresponding nickel coordination compounds were characterized by: i) single crystal X-ray crystallography, ii) UV and CD spectroscopies in methanol solution, and iii) IR and VCD spectroscopies in $CDCl_3$ solution and solid state (KBr pellets). The chiroptical properties were further studied using DFT calculations.

When the enantiomers of a sample are only stable in the solid state, the most used method to determine the absolute configuration has been -until a few years ago- X-ray crystallography based on anomalous scattering, but apart from some cases, the presence of a heavy atom is required.[6] It has been reported a method using VCD spectroscopy together with quantum chemical calculations to determine the absolute configuration of solid chiral materials,6-78 but not a methodology to determine the % *ee* of these solid samples. Thus, to our knowledge there are no previous studies in the literature on the determination of % *ee* in the solid phase. Nujol or fluorolube mulls are less used than solution phase to study chiral molecules due to the possible presence of undesired artifacts in the measured VCD spectrum with sample preparation.[19] Several methodologies have been proposed in order to eliminate contributions of linear birefringence and linear dichroism from VCD spectra of mulls or films.[36,37]

For the first time, we have recorded the VCD spectra of nujol mulls of mixtures with different *R*/*S* proportions of solid camphor to calculate linear regressions with several VCD bands and interpolate a blind sample with it, obtaining accurate values of the % *ee* in a quantitative way. The experimental procedure followed to record the VCD spectra of solid samples has been reported by Merten *et al.*[36] It is important to remark the importance of determining % *ee* in the solid phase with the use of the VCD technique, since it could open a door for its application in the chirality field of solid materials. Besides, reference compounds in VCD, such as camphor, could be used as internal references together with the target solid sample studied in nujol mull to determine the % *ee* of the target solid sample.

We have complemented this analysis in the solid phase with the determination of the *R*/*S* proportions of camphor mixtures using both NMR technique in the presence of a chiral solvating agent (CSA)[38-41] in CD_2Cl_2 solution and VCD spectroscopy in CCl_4 solution.7-89,[20,32] Likewise, trying to test the reliability of the methodology proposed, a blind experiment was carried out.

Two previous publications reported the enantiodifferentiation of camphor enantiomers by ^1H NMR spectroscopy. Dodziuk *et al*. used α-cyclodextrin complexes with *R*- and *S*-camphor (1*R*,4*R* and 1*S*,4*S*) in D_2O finding that the interaction energies of both complexes are different.[42] This difference was used by Hill *et al*. to differentiate both enantiomers using a symmetrical achiral molecule, a prochiral solvating agent, pro-CSA, a complex structure derived from a porphyrin [N_{21},N_{23}-*bis*(4-bromobenzyl)-5,10,15,20-tetrakis(3,5-di-*t*-butyl-4-oxocyclohexadien-2,5-ylidene)

porphyrinogen].[43]

2. Experimental section

We have used commercial samples of D-(+), L-(–) and racemic camphor, all of them purchased from Sigma-Aldrich (now Merck): there named (1*R*)-(+)-camphor, (1*S*)-(–)-camphor and (±)-camphor, respectively.

2.1. Preparation of the NMR samples

The amount necessary for ^{1}H NMR experiments was about 10 mg, the mixtures range from 9:1 to 1:9 of D- and L-enantiomers (the racemic was used for the 5:5 mixtures). A Metler AE260 Delta Range Analytical Balance was used. The reproducibility (standard deviation) is 0.1 mg and the linearity: ± 0.2 mg. Remember that the error in the ratio is larger in the extremities than in the center, for instance, for a 9:1 mixture is ±1 and for 5:5 is 0.2 (dimensionless).

2.2. NMR in solution

All enantiodistinction spectra were obtained and analyzed at several temperatures between 260 and 273 K using CD_2Cl_2 as solvent. Previous experiments using $CDCl_3$ show instability, a precipitate appears and the ^{1}H-NMR spectra change with time. All experiments were acquired on a Bruker AVANCE spectrometer (Bruker BioSpin, Rheinstetten, Germany) operating at 600.13 MHz proton frequency, equipped with a 5 mm inverse probe and a z-axis pulsed field gradient accessory.

The enantiodiscrimination analysis was carried out adding additive quantities of (*S*,*S*)-ABTE [α,α'-bis(trifluoromethyl)-9,10-anthracenedimethanol][38,44] to 10-11 mg of camphor in 0.6 ml of CD_2Cl_2 to obtain increasing separation of signals corresponding to *R* and *S* enantiomers. The corresponding ratios between both compounds were calculated through the values of the integration of the resolved NMR signals.

2.3. IR and VCD spectra

We specify below the amount necessary for solution and solid phases. A Sartorius Entris Analytical Balance was used. Its reproducibility (standard deviation) is 0.1 mg and the linearity: ± 0.2 mg.

The IR and VCD spectra of the different D/L proportions of camphor mixtures in CCl_4 solution (liquid phase) and nujol mulls (solid phase) were recorded at room temperature using a JASCO FVS-4000 FTIR spectrometer, equipped with MCT (2000-900 cm^{-1}) detector.

2.3.1. VCD in solution

These experiments were carried out in carbon tetrachloride using a liquid standard cell equipped with BaF_2 windows, a resolution of 4 $cm^{-1,}$ 2000 scans and path lengths of 50 μm and 100 μm according to the % *ee* of the different mixtures. The following mixtures of 20 mg of camphor (*R*, *S* or *R*+*S*) were dissolved in 100 μl of CCl_4 (1.314 M): 20 mg of *R*-(+)-camphor (corresponding to 100% *ee*), 16 mg *R* + 4 mg *S* (60% *ee*), 12 mg *R* + 8 mg *S* (20% *ee*), 20 mg *S*-(–)-camphor (corresponding to –100% *ee*), 16 mg *S* + 4 mg *R* (–60% *ee*), 12 mg *S* + 8 mg *R* (–20% *ee*) and 10 mg *R* + 10 mg *S* (0% *ee*). The enantiomeric excesses were calculated using eq. (1). The baseline was corrected by subtracting the CCl_4 signals from the raw VCD spectra of the different enantiomeric mixtures. We averaged the CCl_4 corrected VCD spectra of the two pure enantiomers obtaining the VCD baseline, which was subtracted from all the CCl_4 corrected VCD spectra of the different enantiomeric mixtures giving the baseline corrected VCD spectra.

2.3.2. VCD in the solid state

We have prepared different suspensions of 30 mg of camphor (*R*, *S* or *R*+*S*) in 50 μl of nujol corresponding to the following mixtures: 30 mg of *R*-(+)-camphor (corresponding to 100% *ee*), 24 mg *R* + 6 mg *S* (60% *ee*), 18 mg *R* + 12 mg *S* (20% *ee*), 30 mg *S*-(–)-camphor (corresponding to –100% *ee*), 24 mg *S* + 6 mg *R* (–60% *ee*), 18 mg *S* + 12 mg *R* (–20% *ee*), 15 mg *R* + 15 mg *S* (0% *ee*). Special attention is needed when working with solid samples in circular dichroism spectroscopy.[36,37] In fact, we have measured the mulls at several positions by rotating the sample around both the beam propagation axis (90º and 180º, face A) and that perpendicular to it (180º, face B) to obtain the "true" VCD peaks and ascertain the absence of artifacts in the VCD spectra.[36,37] The spectra were recorded using a liquid standard cell equipped with BaF_2 windows where the mulls were placed (resolution: 4 cm^{-1}, scans: 2000-8000, in blocks of 2000 scans). For the baseline correction, we subtracted the nujol signals from the raw VCD spectra of the different enantiomeric mixtures. Later, we averaged the (nujol corrected) VCD spectra of the pure enantiomers obtaining the VCD baseline, which was subtracted from VCD spectrum of each enantiomeric mixture giving the corresponding baseline corrected VCD spectrum. Note that nujol is a refined liquid paraffin (nonvolatile and unreactive oil) formed by a hydrocarbon mixture of high molecular weight.[45,46]

3. Results and discussion

This paper comprises three parts all of them concerning the determination of the enantiomeric excess (% *ee*) of mixtures of D- and L-camphor: i) in solution by ^{1}H NMR in the presence of a chiral solvating agent [*(S,S)*-ABTE]; ii) in solution by VCD, and iii) in the solid state by VCD.

3.1. Solution experiments by NMR

The spectra showed in Fig. 2 correspond to a ratio of (*rac*)-camphor *vs*. *(S,S)*-ABTE of 0.00, 0.40, 0.85, 1.00 and 1.25. The optimized quantity of *(S,S)*-ABTE for a good enantiodifferentiation was determined empirically [(*rac*)-camphor/*(S,S)*-ABTE ≈ 0.8].

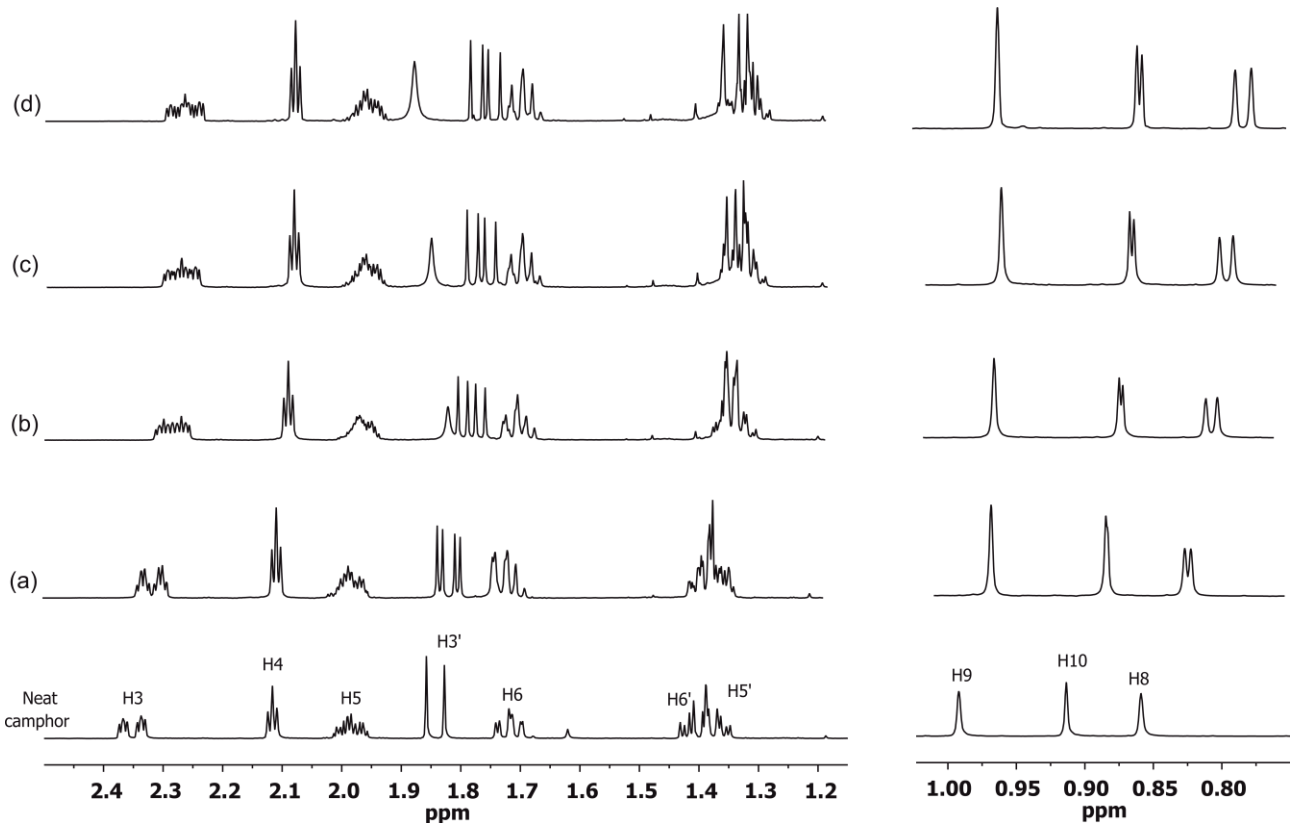


Fig. 2. [1]H-NMR 600.13 MHz at 270 K of 10 mg of (*rac*)-camphor in 0.6 ml of CD_2Cl_2 plus four increasing quantities of *(S,S)*-ABTE (from a to d). The spectra correspond to ratios of (*rac*)-camphor of 0.00 (neat camphor) *vs*. *(S,S,)*-ABTE, (a) 0.40; (b) 0.85; (c) 1.00, and (d) 1.25.

Our independent assignment of camphor signals (Fig. 3) is consistent with previous literature results.[47]

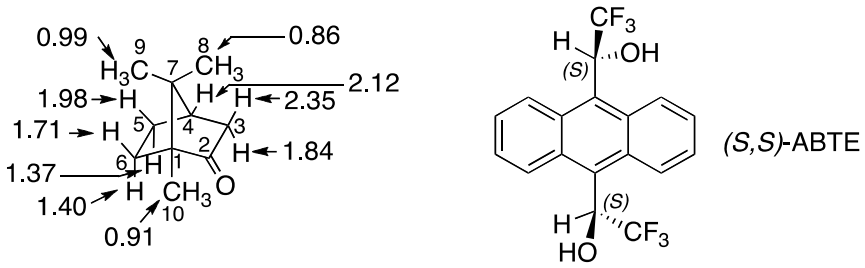


Fig. 3. The chemical shifts (ppm) assignment of all protons of camphor (left) and the structure of the employed CSA (right) are shown.

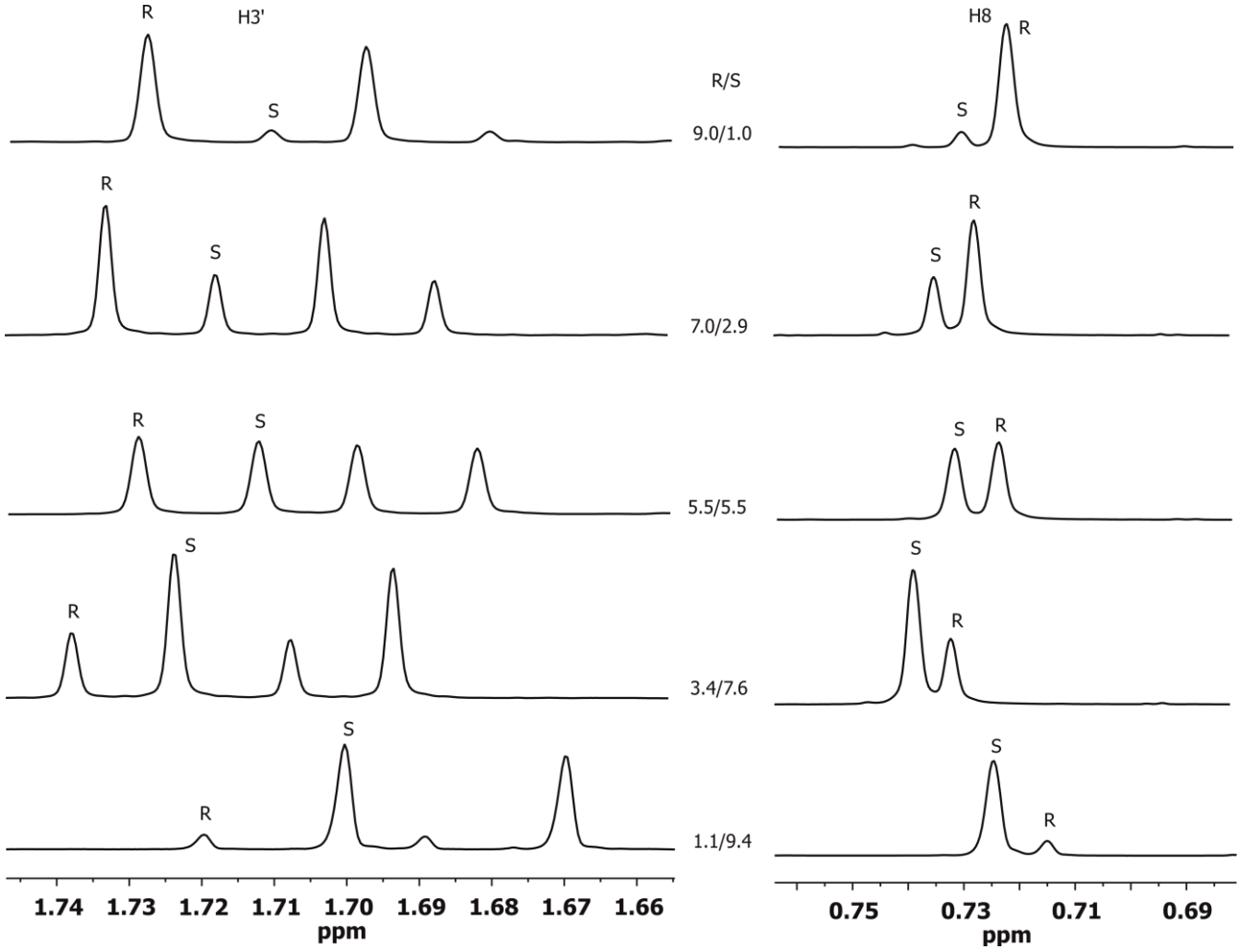


Fig. 4. Five examples of enantiodiscrimination of camphor using *(S,S)*-ABTE. [1]H NMR at 600.13 MHz for proton H3' and methyl H8 and camphor *R/S* ratios are shown. *Q* = (from top to bottom): 9.00, 2.41, 1.00, 0.45 and 0.12.

The standardization experiments were tested at 303, 270 and 260 K, being temperatures near 270 K the best for the desired analysis. The samples were prepared dissolving the following mixtures of *R*- and *S*-camphor in 0.6 ml of CD_2Cl_2 (Fig. 4 and Table 1).

Table 1. Results corresponding to Fig. 2.

Mixture	*R*-Camphor/mg	*S*-Camphor/mg	Total camphor amount/mg	*R/S* quotient (*Q*)	*(S,S)*-ABTE/mg	Camphor/ *(S,S)*-ABTE quotient
1	9.0	1.0	10	9.00	13.7	0.73
2	7.0	2.9	9.9	2.41	13.1	0.76
3[a]	5.5	5.5	11	1.00	13.5	0.81
4	3.4	7.6	11	0.45	12.9	0.85
5	1.1	9.4	10.5	0.12	14.0	0.75

[a] From 11 mg of (*rac*)-camphor.

The ^{1}H NMR experiments were obtained in standard conditions with a pulse of 30º and a delay time between pulses (32) of 1 sec. The FIDs were directly Fourier transformed without any apodization.

In ^{13}C NMR, the addition of ABTE also split several signals (Fig. 5) but for quantitative analysis they are less useful than those obtained by ^{1}H NMR.

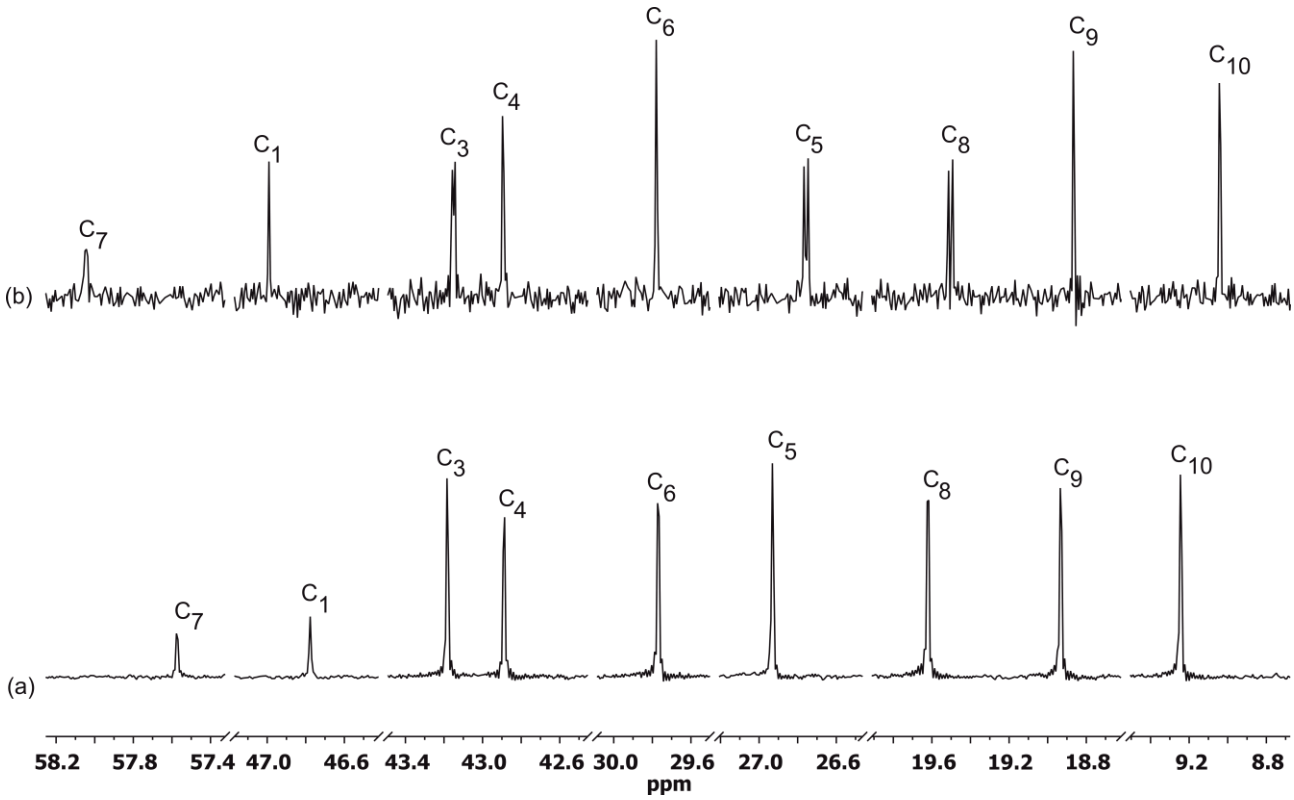


Fig. 5. ^{13}C NMR spectra (150.9 MHz) of (*rac*)-camphor (a) and after addition of 1.25 eq. of *(S,S)*-ABTE in CD_2Cl_2 at 250 K (b).

The results of the NMR experiments are reported in Table 2. Ratios are defined as $Q = R/S$. For statistical reasons we have considered reciprocal experiments (*e.g.* 7.0/2.9 and 2.9/7.0) as independents.

Table 2. Ratios obtained by simple integration and by deconvolution.

Simple integration

Exp	Weight/mg	Ratio (*Q*)	H3' *R*	H3' *S*	*R/S* Ratio	H8 *S*	H8 *R*	*S/R* Ratio
1	9.0/1.0	9.00	100	10.3	9.70			
	1.0/9.0	0.11				50.3	572.5	0.09
2	9.4/1.1	8.55				589.5	80.7	7.30
	1.1/9.4	0.12	13.7	100	0.14			
3	5.5/5.5	1.00	100	89.4	1.12	450.1	508.0	0.89
4	7.0/2.9	2.41	100	40.8	2.45			
5	2.9/7.0	0.41				211.0	497.8	0.42
6	7.6/3.4	2.24				464.8	155.9	2.98
7	3.4/7.6	0.45	41.8	100	0.42			

Deconvolution

Exp	Weight/mg	Ratio (*Q*)	H3' *R*	H3' *S*	*R/S* Ratio	H8 *S*	H8 *R*	*S/R* Ratio
1	9.0/1.0	9.00	517113	53919	9.59			
	1.0/9.0	0.11				132429	1630718	0.08
2	9.4/1.1	8.55				1727328	212050	8.15
	1.1/9.4	0.12	75201	573473	0.13			
3	5.5/5.5	1.00	271492	260091	1.04	737864	910995	0.81
4	7.0/2.9	2.41	373383	176052	2.12			
5	2.9/7.0	0.41				478052	1188589	0.40
6	7.6/3.4	2.24				1222104	640570	1.91
7	3.4/7.6	0.45	181554	417723	0.43			

We have statistically analyzed these results. *Q* exp. corresponds to the values obtained by weighting. Note that % *ee* (*S*) = (1 – *Q*)/(1 + *Q*) x 100.

Q exp. = (1.000±0.042) *Q* integration, n = 10, R^2 = 0.984, rms = 0.54 (2)

Q exp. = (0.994±0.022) *Q* deconvolution, n = 10, R^2 = 0.996, rms = 0.29 (3)

Q integration = (0.984±0.041) *Q* exp., n = 10, R^2 = 0.984, rms = 0.54, (worse point, H8 7.30, fitted 8.42) (4)

Q deconvolution = (1.002±0.022) *Q* exp., n = 10, R^2 = 0.996, rms = 0.29 (worse point, H3' 9.59, fitted 9.02) (5)

Q deconvolution = (1.053±0.023) *Q* exp., H3', n = 5, R^2 = 0.998, rms = 0.22 (6)

Q deconvolution = (1.002±0.022) *Q* exp., H8, n = 5, R^2 = 0.999, rms = 0.13 (7)

3.2. Solution experiments by VCD

The IR and VCD spectra of the different enantiomeric mixtures above mentioned are reported in Fig. 6. As expected the IR spectra of both enantiomers are identical while the VCD spectra are practically "mirror-images" (Fig. 6, top). On the bottom side, the variation in intensity of several VCD bands with the *ee* is apparent. Linear regressions of ΔAbs. *vs*. % *ee* values were then carried out for several bands appearing at 1296.9, 1244.8, 1046.2, 947.8, 933.4 and 924.7 cm^{-1} (see Table 3 and Supporting Information). We have multiplied the ΔAbs. values by 10^6 to have larger

coefficients. The correlation coefficients are > 0.99 for all the bands, except for that at 933.4 cm^{-1}. Our IR and VCD spectra of *R* and *S* camphor in CCl_4 solution are very similar to those appearing in previous works.[20,48-50]

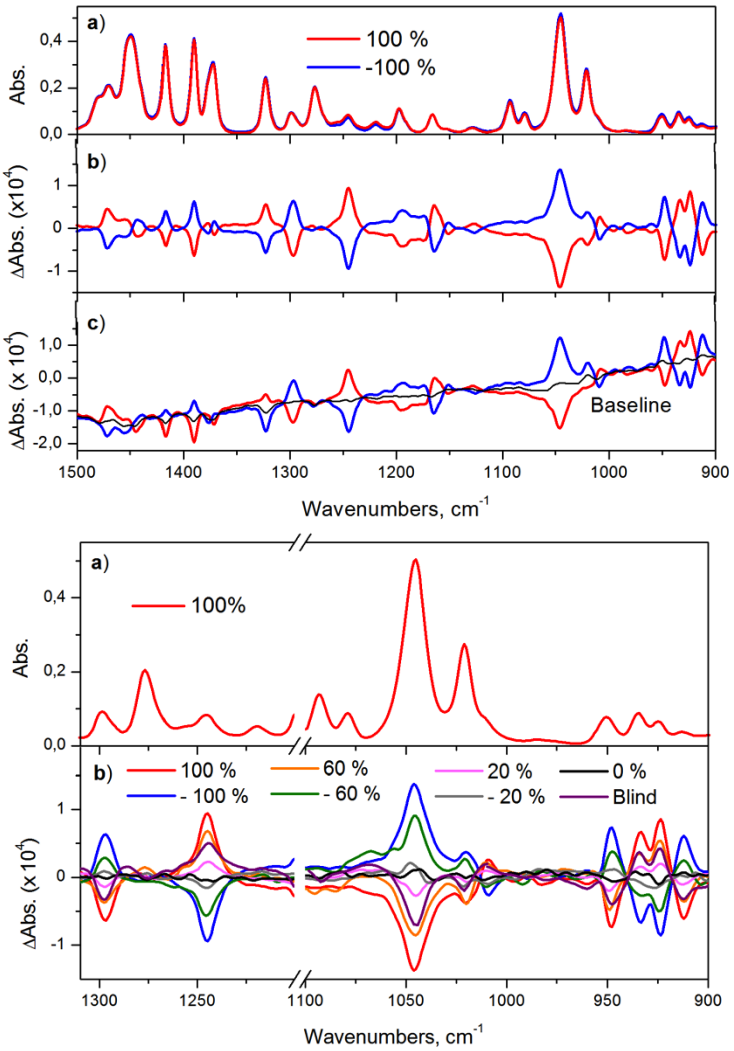


Fig. 6. At the top, experimental IR (a) and VCD (b and c) spectra of the *R*-(+)-camphor (100% *ee*, in red) and *S*-(-)-camphor (–100% *ee*, in blue) in CCl_4 solution. Panel b) shows the baseline corrected VCD spectra and panel c) the VCD spectra with the subtraction of the CCl_4 signals. At the bottom, experimental IR spectrum (a) of *R*-(+)-camphor (in red) and experimental baseline corrected VCD spectra (b) of the two optically pure enantiomers (blue and red), two mixtures of enantiomers, *i.e.*, 60% *ee* (in orange) and –60 % *ee* (in green), two 20% *ee* (rose and grey), the racemic mixture (black) and a blind sample (purple) in CCl_4 solution.

Table 3. Regression lines obtained by VCD in CCl_4 solution corresponding to 10^6 ΔAbs. = a + b *ee* %

Band (cm^{-1})	Intercept (a)	Slope (b)	R^2
1296.9	–2.1	–60.62	0.994
1244.8	2.2	96.28	0.995
1046.2	1.2	–138.65	0.996
947.8	–2.5	–71.72	0.997
933.4	2.6	62.21	0.986
924.7	–0.3	82.50	0.993

The intercept should be close to 0; the non-zero values obtained for them are very small and not significant. The slopes are positive or negative depending on the band; the most sensitive (larger slope) corresponds to the 1046.2 cm^{-1} band. The quality of the regressions, as measured by the values of the correlation coefficients, is comparable to those obtained by ^{1}H NMR.

3.3. Solid state results by VCD

Fig. 7 shows the IR and VCD spectra recorded for the different enantiomeric mixtures aforementioned in nujol mull. Our IR and VCD spectra of the two pure enantiomers of camphor are in agreement with those recorded in nujol mulls in the literature.[50,51] Additionally, Table 4 contains the results of the linear regressions carried out for three selected bands, *i.e.* 1244.8, 933.4 and 924.7 cm^{-1}.

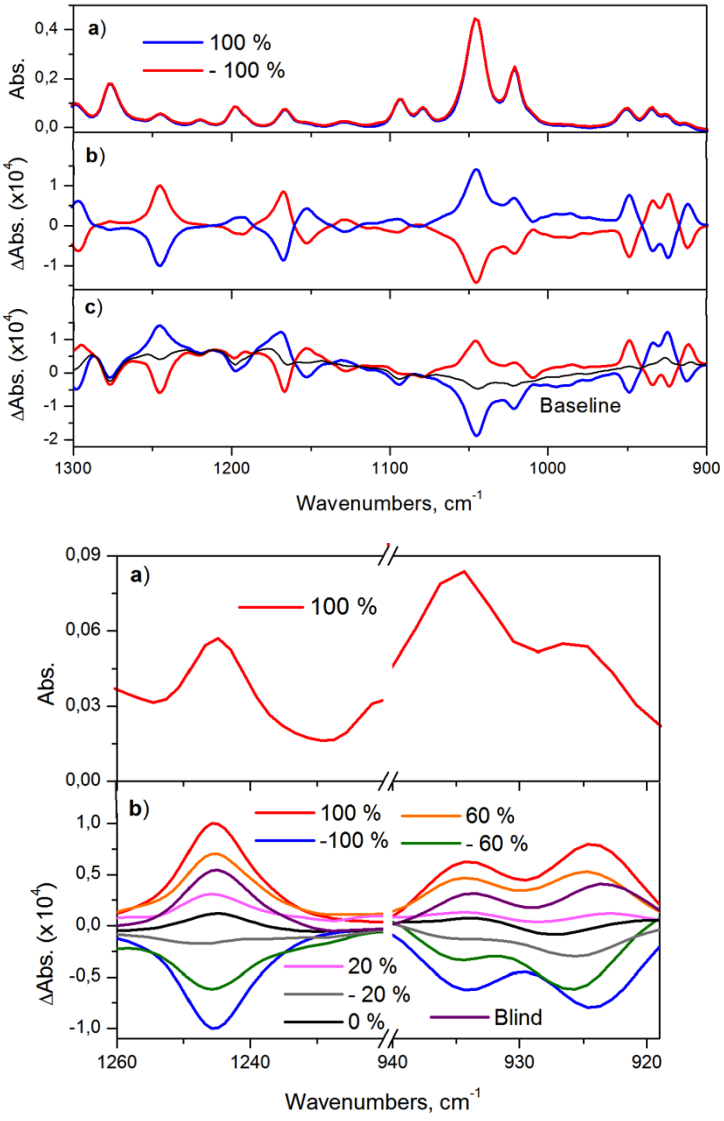


Fig. 7. At the top, experimental IR (a) and VCD (b and c) spectra of the *R*-(+)-camphor (100% *ee*, in red) and *S*-(–)-camphor (–100% *ee*, in blue) in nujol mull. Panel b) shows the baseline corrected VCD spectra and panel c) the VCD spectra with the subtraction of the nujol signals. At the bottom, experimental IR spectrum (a) of *R*-(+)-camphor (in red) and experimental baseline corrected VCD spectra (b) of the two optically pure enantiomers (blue and red), two mixtures of enantiomers, *i.e.*, 60% *ee* (in orange) and –60 % *ee* (in green), two 20% *ee* (rose and grey), the racemic mixture (black) and a blind sample (purple) in nujol mull.

Table 4. Regression lines obtained by VCD in nujol suspension using 10^6 ΔAbs.

Band (cm^{-1})	Intercept (a)	Slope (b)	R^2
1244.8	5.1	102.58	0.994
933.4	2.9	62.45	0.992
924.7	–3.9	83.23	0.990

The value and sign of the slopes between solution and solid state should be the same for a given band. This is the case of the three bands that have been analyzed in solution and in the solid phase, *i.e.*, 1244.8 cm^{-1} (+96.28 *vs*. +102.58), 933.4 cm^{-1} (62.21 *vs*. 62.45) and 924.7 cm^{-1} (82.50 *vs*. 83.23). In the three cases, the slopes are somewhat larger in the solid state than in solution.

3.4. Test: blind sample

To test the validity of our approach we will report here, as a separate section, both experimental and discussion, the results obtained with a blind sample (known only to one of us, R.M.C.). Two sample preparations were used:

a) 750 mg of *S*-camphor and 260 mg of *R*-camphor (total 1010 mg) were mixed carefully using a pestle mortar. Aliquots of the mixture were sent to the NMR (A.V. & E.M.) and to the VCD (M.M.Q.M, J.R.A.M. & J.J.L.G.) groups without indicating the composition. The mixture corresponds to $Q = R/S = 0.347$ and to % *ee* (*R*) = 48.5%.

b) 100 mg of the unknown *R*- and *S*-camphor mixture were dissolved in 10 ml of CD_2Cl_2 obtaining a 10 mg/ml concentration. 1 ml of this solution was introduced into a 5 mm NMR tube. The solvent was evaporated until reach a volume of 0.6 ml. Then 13.4 mg of *(S,S)*-ABTE (0.57 eq.) were added. The ^{1}H-NMR 600 MHz at 270 K spectrum was acquired in standard conditions with a pulse of 30 degree waiting 1 sec. between the scans (32). A Gaussian function with lb = –0.9 and gb = 0.8 was applied prior to the Fourier transformation. In Fig. 8 is represented the result obtained which corresponds to R/S = 0.370, *i.e*. 27.0% R, 73.0% S, which corresponds to % *ee* (*R*) = 46.0% while the experimental value is 48.5%. Corrections using the empirical equations (6) and (7) does not improve the result because the slopes are close to 1. Using deconvolution, the same results are obtained.

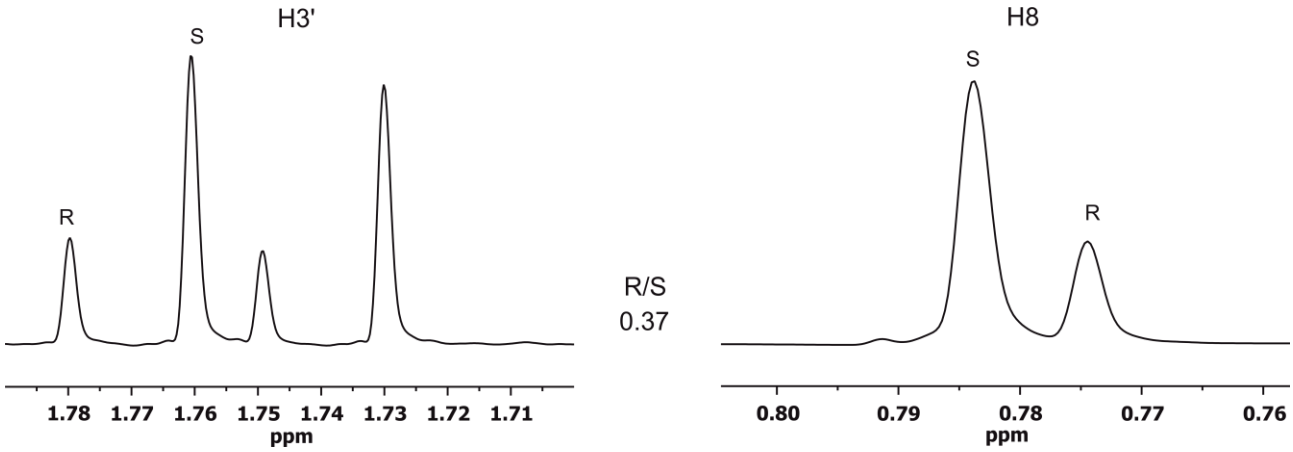


Fig. 8. ^{1}H NMR 600 MHz at 270 K of 0.6 ml of the blind sample solution in CD_2Cl_2 after the addition of 0.57 eq. of *(S,S)*-ABTE.

c) We recorded the IR and VCD spectra of this blind sample in CCl_4 solution and in the solid state (see Figs. 6 and 7). The ΔAbs. of this sample with unknown % *ee* was interpolated using the corresponding equations of the linear regressions carried out for each band, obtaining an average % *ee* value of 50.7 with a rms of 1.1 in the case of the solution and an average % *ee* value of 48.0 with a rms of 2.4 for nujol mulls. These results concerning the interpolation of ΔAbs. of the blind sample are gathered in Tables 5 and 6. The accuracies that have been achieved, that is, 1.1 % (solution) and 2.4 % (mull), are within the range obtained by other authors with the same spectral resolution (4 cm^{-1}) in solution.[20] Surprisingly, the % ee results obtained in nujol mulls by interpolation are closer to actual % *ee* value of the blind sample than those obtained in CCl_4 solution, although the rms value in solution is lower than that in nujol mulls. This can be due to the fact that six bands have been analyzed in solution and only three in nujol mulls.

Thus, it can be observed that the analysis of the % *ee* in the solid state can be carried out using reference compounds used in VCD, as camphor, obtaining reasonable results. Depending on the structure of the compound, there will be a specific number of bands in the useful 2000-1500 cm^{-1} and 1300-900 cm^{-1} spectral regions.

VCD spectroscopy shows several advantages with respect to other analytical techniques, as the presence in the spectra of different vibrational bands allowing to identify individual peaks in a mixture of two or more molecules.[20] Additionally, each point in a single species VCD spectrum represents an independent value of the % *ee*.[20]

Table 5. Results of the interpolated % *ee* of the blind sample for selected VCD bands in CCl_4 solution.

Band (cm^{-1})	Interpolated % *ee*	Actual % *ee*	Variance (%)
1296.9	49.6	48.5	–1.1
1244.8	49.6		–1.1
1046.2	50.8		–2.3
947.8	51.7		–3.2
933.4	52.1		–3.6
924.7	50.3		–1.8
RMSE	1.1		

Table 6. Results of the interpolated % *ee* of the blind sample for selected VCD bands in nujol mull.

Band (cm^{-1})	Interpolated % *ee*	Actual % *ee*	Variance (%)
1244.8	48.0	48.5	0.5
933.4	45.7		2.8
924.7	50.4		–1.9
RMSE	2.4		

3.5. The use of alternative methodologies

In recent years a series of methods mainly based on solid state NMR but, occasionally, on X-ray powder diffraction (XRPD),[52,53] have been published that allow to determine the % *ee*. Three different methods based on NMR are known: use of isotropic chemical shifts, application of ODESSA (one-dimensional exchange spectroscopy by sideband alternation[54-56]) and use of the chemical shifts tensors.[57] Compounds studied with these methods include tartaric acids,[58] organophosphorus compounds,[59] oxazaphosphorinanes,[60] *O*-phosphorilated amino acids,[61] cyclophosphamide,[62] valine,[63] benzodiazacoronands,[64] proline[65,66] and BINOL.[12]

Note that ODESSA which needs the observation of spinning sidebands is particularly well suited for molecules containing a unique atom with a specific spin, for instance, only one atom of

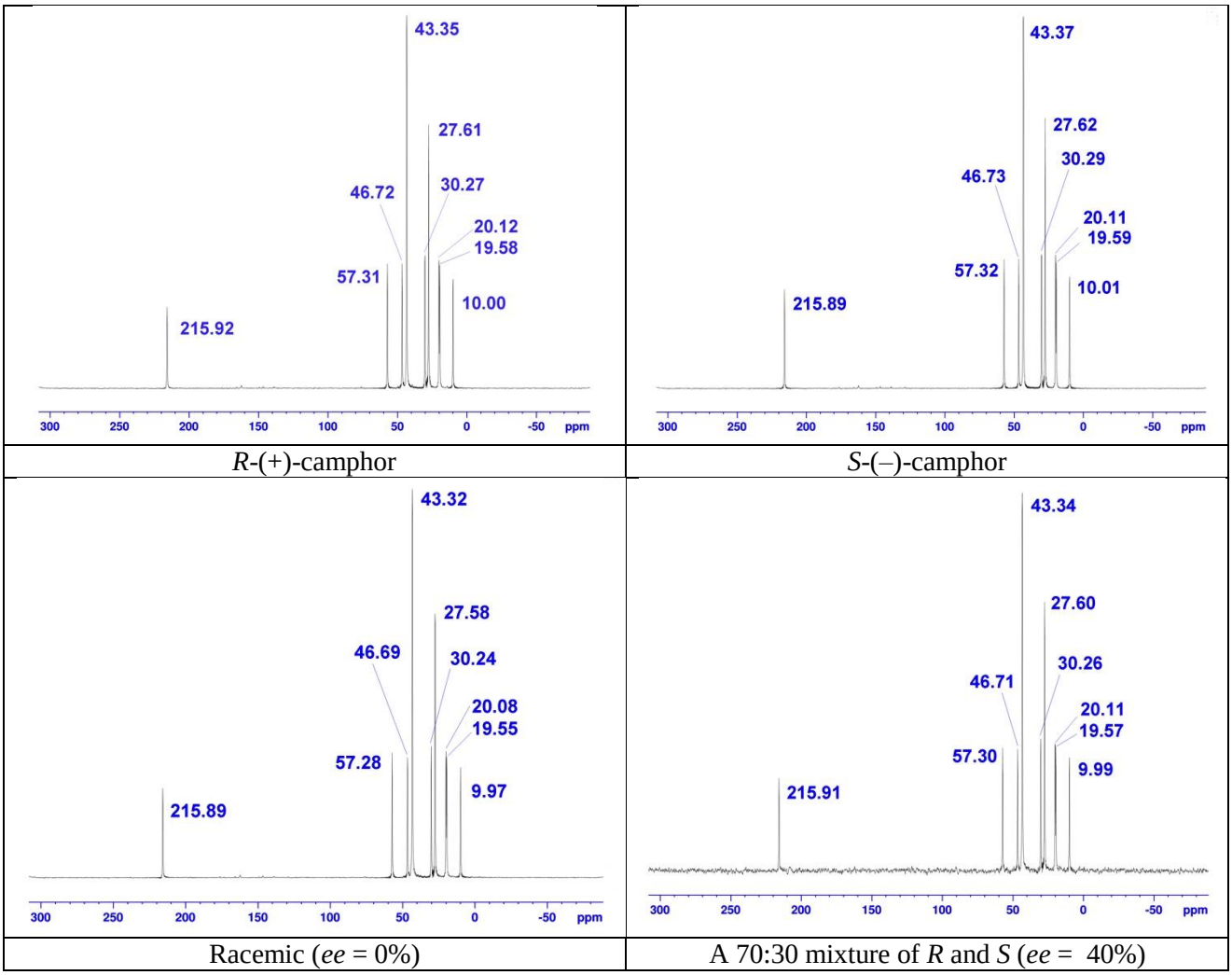
^{31}P;[55,59,60-62] otherwise, the superposition of spinning sidebands of several carbon atoms will result in a very complex experiment in ^{13}C SSNMR.

The main and obvious conclusion of all these experiments is that they are restricted to differentiate enantiomers and racemates, because they crystallize in different point groups (different crystallites)[52,55,56,59-63,67-69] (one of the clearer illustration is reported in reference 66).

Therefore, these methodologies do not apply to conglomerates nor to solid solutions because the crystal contains either only one enantiomer or both enantiomers with the same point group, thus being identical irrespective of the enantiomeric composition. In the case of fast interconverting enantiomers, one crystallization batch will contain a mixture of crystals each one of one enantiomer that cannot be studied by NMR. A possibility explored with little success is the addition of a chiral auxiliary;[12] possibly a chemical interaction (co-crystal) is necessary and a physical one (mixture) is not sufficient enough.

According to these considerations, camphor chirality cannot be determined by ^{13}C SSNMR. Camphor spectrum has been reported several times. It was pointed out that line broadening is particularly small if the molecule crystallizes to give so-called "plastic crystals", a well known example being camphor.[70] Prout reported temperature effects of the deoxycholic acid clathrates with camphor with special interest in phase changes.[71]

We recorded at 100.74 MHz (see experimental details in our previous publications9,[72]) four camphor spectra (Fig. 9). They are all identical and similar to those reported previously.[70]



R-(+)-camphor | *S*-(–)-camphor

Racemic (*ee* = 0%) | A 70:30 mixture of *R* and *S* (*ee* = 40%)

Fig. 9. ^{13}C CPMAS NMR spectra of the four camphor samples.

4. Conclusions

The main conclusions of the work here reported are:

1. Concerning the NMR use in solution we have found: i) the largest errors correspond to large ratios where the weighting error is maximum; (ii) deconvolution improves the results, compare eq. (2) and (3); (iii) practically, since what is required is to estimate the "true" values from the NMR experiments, the best equation is (4) [compare (4) and (5)]; (iv) it is better to use H8 than H3' or H8+H3', compare eqs. (7) (RMS residual = 0.13), (6) (RMS residual = 0.22) and (5) (RMS residual = 0.29). Note also that the intercept of eq. (7) is closer to 1.000 than that of eq. (6). The use of chiral reagents in SSNMR has not yet been reported.

2. Concerning the use of VCD we have shown, for the first time, how to determine % *ee* values in the solid phase (nujol mulls) with the use of the VCD spectroscopy using camphor as an example. Additionally, % *ee* values in solution phase have been measured by VCD and NMR techniques. The rms values of these determinations were approximately 1-2 % at 4 cm^{-1} resolution in the case of VCD, which is a reasonable level of accuracy. This methodology to measure % *ee* in nujol mulls could be applied to other chiral solid compounds in addition to camphor, opening thus a door of application in the chirality field. Additionally, camphor could be used as an internal reference to determine enantiomeric excesses in unknown % *ee* samples, by comparing the VCD intensities of the two samples.

3. The method we advocate, the use of VCD in the solid state, can be used for those cases, like camphor, that are not amenable to NMR experiments.

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References

1 J. Jacques, A. Collet and S. H. Wilen, *Enantiomers, Racemates, and Resolutions*, Krieger Publishing Co, Malabar, Florida, 1994.

2 C. P. Brock, W. B. Schweizer and J. D. Dunitz, *J. Am. Chem. Soc.*, 1991, **113**, 9811–9820.

3 C. Viedma, B. J. V. Verkuijl, J. E. Ortiz, T. de Torres, R. M. Kellogg and D. G. Blackmond, *Chem. Eur. J.*, 2010, **16**, 4932–4937.

4 S. Gonella, G. Levilain and G. Coquerel, *J. Therm. Anal. Calorim.*, 2011, **103**, 125–129.

5 *Advances in Organic Crystal Chemistry. Comprehensive reviews*, eds. R. Tamura and M. Miyata, Springer, Tokyo, 2015.

6 S. Graus, R. M. Tejedor, S. Uriel, J. L. Serrano, I. Alkorta and J. Elguero, *J. Am. Chem. Soc.*, 2010, **132**, 7862–7863.

7 J. J. López-González, F. P. Ureña, J. R. A. Moreno, I. Mata, E. Molins, R. M. Claramunt, C. López. I. Alkorta and J. Elguero, *New J. Chem.*, 2012, **36**, 749–758.

8 J. R. Avilés Moreno, M. M. Quesada Moreno, J. J. López González, R. M. Claramunt, C. López, I. Alkorta and J. Elguero, *ChemPhysChem*, 2013, **14**, 3355–3360.

9 M. M. Quesada-Moreno, J. R. Avilés-Moreno, J. J. López-González, K. Jacob, L. Vendier, M. Etienne, I. Alkorta, J. Elguero and R. M. Claramunt, *Phys. Chem. Chem. Phys.*, 2017, **19**, 1632–1643.
10 J. E. Anderson and W. P. Slichter, *J. Chem. Phys.*, 1964, **41**, 1922–1928.
11 Y. Zhen, H. Tanaka, K. Harano, S. Okada, Y. Matsuo and E. Nakamura, *J. Am. Chem. Soc.*, 2015, **137**, 2247–2252.
12 K. D. Klika, *J. Spectrosc.*, 2013, 970654 (6 pages).
13 E. L. Eliel and S. H. Wilen, *Stereochemistry of Organic Compounds*, John Wiley & Sons, New York, 1994.
14 A. G. Mitchell, *J. Pharm. Pharmaceut. Sci.*, 1998, **1**, 8–12.
15 Y. Corvis and P. Espeau, *Thermochim. Acta*, 2012, **539**, 39–43.
16 J. M. Dreiling and T. Gay, *J. Phys. Rev. Lett.*, 2014, **113**, 118103.
17 F. Vester, T. L. V. Ulbricht and H. Krauch, *Naturwissenschaften*, 1959, **46**, 68–69.
18 T. L. V. Ulbricht and F. Vester, *Tetrahedron*, 1962, **18**, 629–637.
19 L. A. Nafie, *Vibrational Optical Activity. Principles and Applications*, Wiley, Chichester, 2011.
20 C. Guo, R. D. Shah, R. K. Dukor, X. Cao, T. B. Freedman and L. A. Nafie, *Anal. Chem.*, 2004, **76**, 6956–6966.
21 C. Guo, R. D. Shah, R. K. Dukor, X. Cao, T. B. Freedman and L. A. Nafie, *Appl. Spectrosc.*, 2005, **59**, 1114–1124.
22 T. B. Freedman, S. J. Cianciosi, N. Ragunathan, J. E. Baldwin and L. A. Nafie, *J. Am. Chem. Soc.*, 1991, **113**, 8298–8305.
23 S. J. Cianciosi, K. M. Spencer, T. B. Freedman, L. A. Nafie and J. E. Baldwin, *J. Am. Chem. Soc.*, 1989, **111**, 1913–1915.
24 S. J. Cianciosi, N. Ragunathan, T. B. Freedman, L. A. Nafie, D. K. Lewis, D. A. Glenar and J. E. Baldwin, *J. Am. Chem. Soc.*, 1991, **113**, 1864–1866.
25 K. M. Spencer, S. J. Cianciosi, J. E. Baldwin, T. B. Freedman and L. A. Nafie, *Appl. Spectrosc.*, 1990, **44**, 235–238.
26 C. Guo, R. D. Shah, J. Mills, R. K. Dukor, X. Cao, T. B. Freedman and L. A. Nafie, *Chirality*, 2006, **18**, 775–782.
27 G. Longhi, E. Castiglioni, S. Abbate, F. Lebon and D. A. Lightner, *Chirality*, 2013, **25**, 589–599.
28 J. Hudecová, V. Profant, P. Novotná, V. Baumruk, M. Urbanová and P. Bouř, *J. Chem. Theory Comput.*, 2013, **9**, 3096–3108.
29 T. Wu, C.-H. Li, Y.-Z. Li, Z.-G. Zhang and X.-Z. You, *Dalton Trans.*, 2010, **39**, 3227–3232.
30 S. Abbate, L. F. Burgi, F. Gangemi, R. Gangemi, F. Lebon, G. Longhi, V. M. Pultz and D. A. Lightner, *J. Phys. Chem. A*, 2009, **113**, 11390–11405.
31 H. Sato, Y. Mori, Y. Fukuda and A. Yamagishi, *Inorg. Chem.*, 2009, **48**, 4354–4361.
32 M. M. Quesada-Moreno, J. R. Avilés-Moreno, J. J. López-González, R. M. Claramunt, C. López, I. Alkorta and J. Elguero, *Tetrahedron Asymm.*, 2014, **25**, 507–515.
33 T. Wu and X. You, *J. Phys. Chem. A*, 2012, **116**, 8959−8964.
34 H. Sato, I. Kawamura, A. Yamagishi and F. Sato, *Chem. Lett.*, 2017, **46**, 449–452.
35 J. E. Rode, J. Cz. Dobrowolski, K. Lyczko, A. Wasiewicz, D. Kaczorek, R. Kawęcki, G. Zając and M. Baranska, *J. Phys. Chem. A*, 2017, **121**, 6713−6726.
36 C. Merten, T. Kowalik and A. Hartwig, *Appl. Spectrosc.*, 2008, **62**, 901–905.
37 T. Buffeteau, F. Lagugné-Labarthet and C. Sourisseau, *Appl. Spectrosc.*, 2005, **59**, 732–745.
38 M. Pomares, F. Sánchez-Ferrando, A. Virgili, A. Alvarez-Larena and J. F. Piniella, *J. Org. Chem.*, 2002, **67**, 753–758.
39 J. Comelles, C. Estivill, M. Moreno-Mañas, A. Virgili and A. Vallribera, *Tetrahedron*, 2004, **60**, 11541–11546.

40 C. Estivill, M. Pomares, M. Kotev, P. Ivanov and A. Virgili, *Tetrahedron: Asymm.*, 2005, **16**, 2993–2997.
41 I. Alkorta, J. Elguero, A. Font, J. Galcera, I. Mata, E. Molins and A. Virgili, *Tetrahedron*, 2014, **70**, 2784–2795.
42 H. Dodziuk, A. Ejchart, O. Lukin and M. O. Vysotsky, *J. Org. Chem.*, 1999, **64**, 1503–1507.
43 J. Labuta, S. Ishihara, T. Sikorsky, Z. Futera, A. Shundo, L. Hanyková, J. V. Burda, K. Ariga and J. P. Hill, *Nat. Commun.*, 2013, **4**, 2188, 1–8.
44 A. Virgili, M. M. Quesada-Moreno, J. R. Avilés-Moreno, J. J. López-González, M. A. García, R. M. Claramunt, M. R. Torres, M. L. Jimeno, F. Reviriego, I. Alkorta and J. Elguero, *Helv. Chim. Acta*, 2014, **97**, 471–490.
45 R. W. Hannah, *Simple Sampling* (Chapter 8), and J. D. Russell, *Infrared spectroscopy of Inorganic Compounds* (Chapter 18), in Laboratory Methods in Vibrational Spectroscopy, eds. H. A Willis, J. H. van der Maas and R. G. J. Miller, 3rd Edition, Wiley, Chichester, 1987.
46 N. Sheppard, *The Historical Development of Experimental Techniques in Vibrational Spectroscopy in Introduction to the theory and Practice of Vibrational Spectroscopy* (Chapter 1) in Theory and Instrumentation in Handbook of Vibrational Spectroscopy, eds. J. M. Chalmers and P. R. Griffiths, Wiley, Chichester, 2002, vol. 1.
47 R. J. Abraham and N. J. Ainger, *J. Chem. Soc. Perkin Trans. 2*, 1999, 441–448.
48 P. J. Stephens, *Encyclopedia of Spectroscopy and Spectrometry*, ed. J. Lindon, 2nd Edition, Academic Press, London, 2010, pp. 2910–2915.
49 Y. He, B. Wang, R. K. Dukor and L. A. Nafie, *Appl. Spectrosc.*, 2011, **65**, 699–723.
50 J. R. Avilés Moreno, F. Partal Ureña and J. J. López González, *Asian J. Spectrosc.*, 2010, **14**, 1–21.
51 L. A. Nafie, R. K. Dukor and T. B. Freedman in *Handbook of Vibrational Spectroscopy*, *Vibrational Circular Dichroism*, eds. J. M. Chalmers and P. R. Griffiths, John Wiley & Sons, Chichester, 2006.
52 E. J. Tdeusiak, R. Kruszynski and K. Misiura, *Polish J. Chem.*, 2004, **78**, 431–435.
53 E. Tadeusiak, R. Kruszynski, A. T. Kruszynska, T. Sieranski and B. Lewinski, *Eur. J. Chem.*, 2010, **1**, 319–321.
54 V. Gérardy-Montouillout, C. Malveau, P. Tekely, Z. Olender and Z. Luz, *J. Magn. Reson. Series A*, 1996, **123**, 7–15.
55 M. J. Potrzebowski, *Eur. J. Org. Chem.*, 2003, 1367–1376.
56 M. J. Potrzebowski, A. Jeziorna and S. Kazmierski, *Concepts Magn. Reson. A*, 2008, **32**, 201–218.
57 H. Duddeck and E. Díaz Gómez, *Chirality*, 2009, **21**, 51–68.
58 H. D. W. Hill, A. P. Zens and J. Jacobus, *J. Am. Chem. Soc.*, 1979, **101**, 7090–7091.
59 K. V. Anderson, H. Bildsøe and H. J. Jakobsen, *Magn. Reson. Chem.*, 1990, **28**, S47–S51.
60 M. J. Potrzebowski, E. Tadeusiak, K. Misiura, W. Ciesielski, G. Bujacz and P. Tekely, *Chem. Eur. J.*, 2002, **8**, 5007–5011.
61 M. J. Potrzebowski, X. Assfeld, K. Ganicz, S. Olejniczak, A. Cartier, C. Gardiennet and P. Tekely, *J. Am. Chem. Soc.*, 2003, **125**, 4223–4232.
62 E. J. Tadeusiak, S. Olejniczak and W. Ciesielski, *Heteroatom Chem.*, 2004, **15**, 388–394.
63 E. J. Tadeusiak, W. Ciesielski and S. Olejniczak, *Magn. Reson. Chem.*, 2006, **44**, 905–908.
64 J. Pacholczyk, J. Kalisiak, J. Jurczak and M. J. Potrzebowski, *J. Phys. Chem. B*, 2007, **111**, 2790–2799.
65 R. T. Berendt and E. J. Munson, *J. Pharm. Sci.*, 2011, **100**, 1879–1891.
66 R. T. Berendt and E. J. Munson, *CrystEngComm*, 2012, **14**, 2479–2488.
67 W. L. Bragg, *The Crystalline State 1* (Chapter 5), Bell and Sons, London, 1955.
68 G. H. Stout and L. H. Jensen, *X-ray Structure Determination: a practical guide*, Macmillan, Michigan, 1968, pp. 144-145.

69 *Structure elucidation in organic chemistry. The search for the right tools*, eds. M.-M. Cid and J. Bravo, Wiley-VCH, Weinheim, 2015.
70 T. N. Mitchell and B. Costisella, *NMR - From spectra to structures*, Springer, Berlin, 2007.
71 K. Prout, *Croat. Chem. Acta*, 2002, **75**, 817–833.
72 I. Alkorta, J. Elguero, M. Pérez-Torralba, C. López and R. M. Claramunt, *Magn. Reson. Chem.*, 2015, **53**, 353–362.