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IDENTIFICATION AND ANALYSIS OF DRUGS IN THE SOLID STATE BY ^{13}C CPMAS NMR: SUXAMETHONIUM CHLORIDE AND HYDROCORTISONUM (CORHYDRON)

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Abstract: Cross-polarization (CP) magic angle spinning (MAS) ^{13}C NMR spectroscopy has become a routine tool in pharmacy, employed to identify and characterize drugs in the solid phase. ^{13}C CPMAS NMR spectra were recorded for solid hydrocortisone 21-hemisuccinate and suxamethonium chloride. White crystalline substances, such as these two drugs, can be easily distinguished; and solid-state ^{13}NMR spectra of remarkably good quality are obtained in less than half an hour. ^{13}C CPMAS chemical shifts for solid suxamethonium chloride and hydrocortisone sodium hemisuccinate are given, as well as cross-polarization kinetic parameters for suxamethonium chloride.

Keywords: suxamethonium chloride, hydrocortisone hemisuccinate, solid-state NMR, ^{13}C CPMAS NMR

In November 2006 Polish newspapers reported news about an outrageous error at Jelfa, the pharmaceutical company based in Jelenia Góra. The company manufactures corhydron, a powerful anti-allergenic drug. Because of the GMP mistake by the manufacturer, some of the vials contained a different active ingredient. Analysts from the National Medicines Institute found that instead of corhydron, the vials contained chlorsuccillin, a muscle relaxant used during surgery. The suspended batches of Corhydron 250 mg were withdrawn from the market by the Main Pharmaceutical Inspectorate (GIF). This manufacturer's mistake was mentioned in the Lancet (1).

Besides the matters connected with logistic and quality system in the pharmaceutical plant, this accident raises the problem of analytical methods suitable for fast identification of unknown solid samples. Techniques typically employed to identify and characterize drugs in the solid phase are: melting point, differential scanning calorimetry, IR spectroscopy and X-ray diffraction. Crystallography produces detailed maps of atomic positions in the unit cell and is an excellent tool of solid-state characterization. However, the problem of obtaining single crystal is difficult to overcome and other methods are often desirable. Nevertheless, the X-ray powder diffraction, especially when using new, fast diffraction techniques, including multilayer mirrors and position-sensitive counters, is a method suitable for pharmaceutical market screening control.

Now that high-resolution ^{13}C NMR spectra of organic solids can be obtained relatively easily, it has become important to popularize solid-state NMR method as a routine tool in pharmacy. Magic angle spinning (MAS) NMR spectroscopy makes significant contribution to the studies on drugs and is applied to confirm their identity, purity and stability (2). It enables nondestructive and fast identification of an active compound since the ^{13}C measurements are performed on pure powdered solid (without dilution with a solvent).

Suxamethonium chloride (succinylcholine or sux; Scheme 1a) is a drug widely used in emergency medicine and anesthesia to induce muscle relaxation. The compound consists of two acetylcholine molecules linked by their acetyl groups. Suxamethonium chloride is a white crystalline substance, odorless and highly soluble in water.

Hydrocortisonum (Cortisol, Corhydron; Scheme 1b) is used to treat asthma and certain allergies. It is a representative of corticosteroids, the

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most frequently used class of anti-inflammatory drugs, important in the therapy of neoplastic, immunological and allergic diseases. Hydrocortisone sodium hemisuccinate is a white crystalline powder, soluble in water.

Therefore, these two drugs cannot be easily distinguished in their crystalline form. In order to determine chemical structure and intermolecular interactions in the solid state, the MAS NMR spectroscopy methods have to be used.

EXPERIMENTAL

Suxamethonium chloride, systematic (IUPAC) name: 2,2'-[(1,4-dioxobutane-1,4-diyl)bis(oxy)]bis--(N,N,N-trimethylethanaminium) and Hydrocortisone sodium 21-hemisuccinate, a water-soluble ester of hydrocortisone (sodium 11,17-dihydroxy-3,20-dioxopregn-4-en-21-yl succinate) were obtained from Pharmaceutical Company Jelfa SA.

Cross polarization (CP) magic angle spinning (MAS) solid-state ^{13}C NMR spectra were recorded at 100.13 MHz on a Bruker DSX-400 instrument. Powder samples were spun at 8-13 kHz in a 4 mm ZrO_2 rotor, contact time of 4-5 ms, repetition time of 6 s and spectral width of 40 kHz were used for accumulation of 400 – 800 scans. Dipolar dephasing pulse sequence (with 50 ms delay time inserted before acquisition) was used to observe selectively the nonprotonated carbon atoms. Chemical shifts were calibrated indirectly through the glycine CO signal recorded at 176.0 ppm relative to TMS.

RESULTS AND DISCUSSION

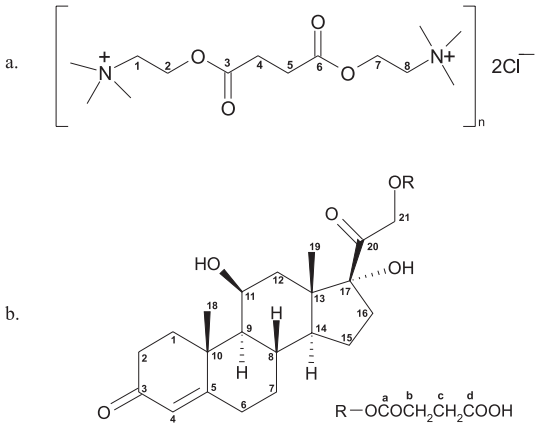
Suxamethonium chloride

Succinylcholine was first applied to clinics in Europe in 1951, and still has clinical utility today, although it exhibits numerous complications. Its role in clinical anesthesia and the mechanism of action as neuromuscular blocking were discussed in 2003 (3). Structurally, succinylcholine consists of two molecules of acetylcholine joined end on end at the acetyl side. The crystal structure of succinylcholine salts, inclucing iodide (4, 5) was established forty years ago. Molecular structures showed extended chains linked by intermolecular NH…O=C hydrogen bonds.

According to our best knowledge, the drug was not studied by means of solid-state NMR. Therefore, ^{13}C CPMAS NMR spectra were recorded, and the spectra recorded at various rotational speeds (4-12 KHz) are illustrated in Figure 1. The signal of C=O group is flanked by rotational side-bands on both sides; it raises technical problem: how fast should the sample rotate to obtain side-bands free spectrum? Large chemical shift anisotropy is typical for carbonyl carbon atoms, and samples with them need to be spun at speeds in excess of 12 kHz. The intensity of central line (isotropic) increases (reaching 95%) at 12 kHz and intensities of side-bands decrease with increasing spinning speed, as illustrated in Figure 2.

The spectrum of suxamethonium chloride exhibited only five resonances; it indicates that two acetylcholine units either have the same geometry and intermolecular contacts or exhibit dynamics i.e. undergo internal rotation. In solution, fast rotation around single bonds takes place, whereas in the solid state frozen conformation usually revealed different chemical sites. Additionally, intermolecular interactions directed to specific sites may produce different chemical shifts for functional groups, such as C=O. The spectra were assigned on the basis of liquid-state chemical shifts and ^{13}C chemical shifts are collected in Table 1. The solid state peaks have chemical shifts almost the same as their liquid state counterparts. The differences $\Delta = \delta_{solution} - \delta_{CPMAS}$ for methylene carbons are less that 1 ppm; deshielding of ca. 2 ppm for solid was observed only for C=O.

It seemed interesting to study molecular dynamics and cross-polarization kinetics in detail. Cross-polarization sequence provides evident advantages, such as signal enhancement and reduction of the measurement time, but the intensity of signals depends on CP parameters. The spectra of suxamethonium chloride recorded with various



Scheme 1. Molecular structures of succinylcholine (a) and hydrocortisone hemisuccinate (b) with carbon numbering.

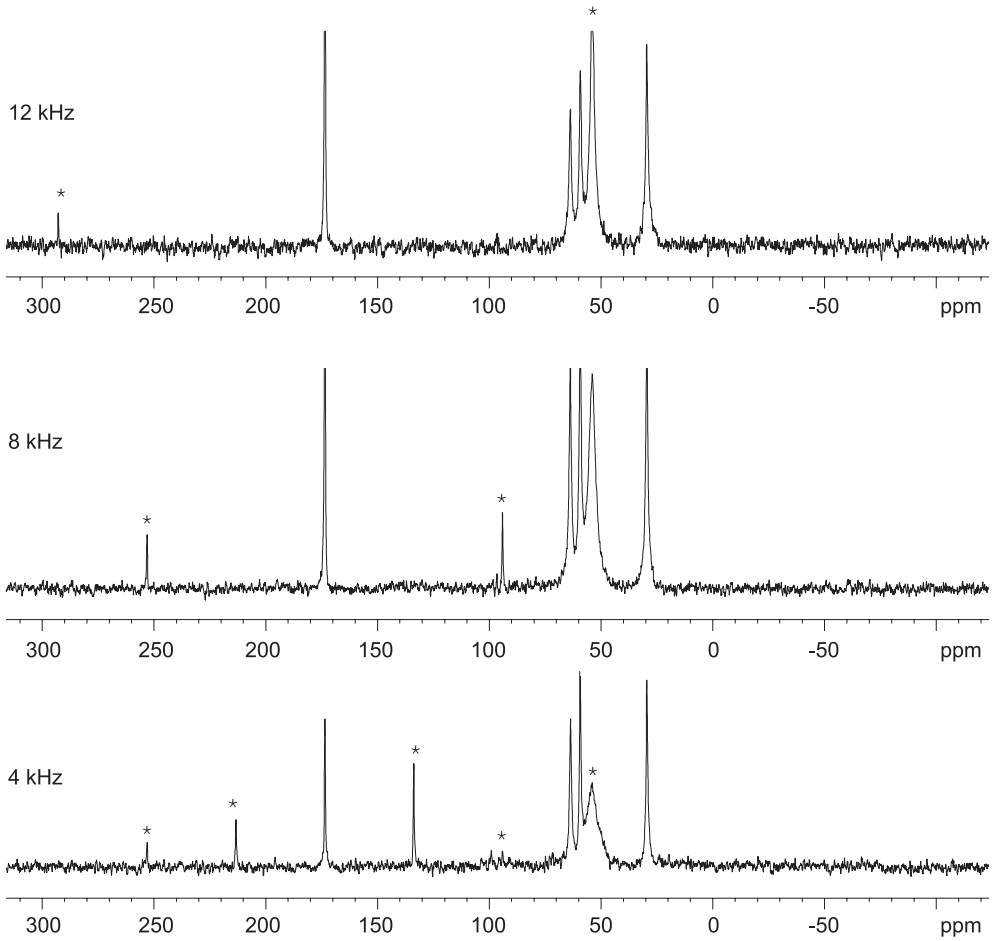


Figure 1. ^{13}C solid state spectra of suxamethonium chloride recorded with different magic angle spinning speeds.

contact times t_{cp} are illustrated in Figure 3. Protonated carbon atoms have fast polarization rate and the signals of methylene groups are intense for short t_{cp}. As expected, quaternary carbon atoms need longer time, especially C=O reaching the maximum with t_{cp} = 3.5 ms. This effect should be taken into account in quantitative measurements, and may be helpful by assignment of the resonances, e.g. distinguishing quaternary carbon atoms from CH or CH_2 ones.

In order to obtain the values of cross-polarization time constant T_{CP} and proton spin-lattice relaxation time in the rotation frame $T_{1\rho}^{H}$ for the I-S model, an unrestricted fit of the experimental data to the equation (1) was performed:

(1) $I(t) = A(1-T_{CP}/T_{1\rho}^{H})^{-1} [\exp(-t/T_{1\rho}^{H}) - \exp(-t/T_{CP})]$, where A is the intensity amplitude.

Cross-polarization in the C-H bonds (CH_2 and CH_3 groups) proceeds according to the I-I*-S model.

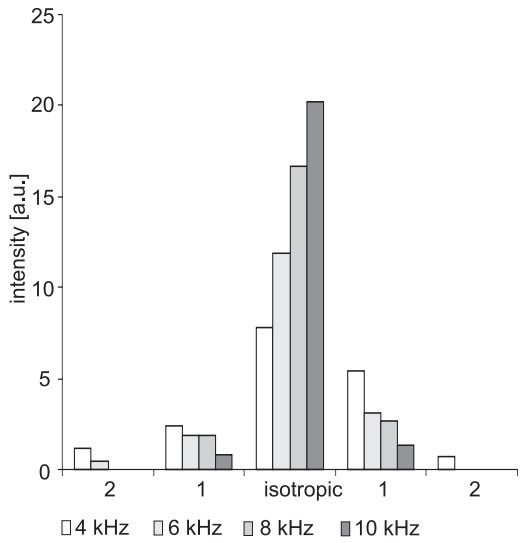


Figure 2. Intensity of isotropic line and two sets of spinning side bands in the spectra recorded at various spinning speeds.

The kinetics within this model is described by equation (2):

(2) $I(t) = A \exp(-t/T_{1\rho}^{I})[1- \lambda \exp(-t/T_{df}) - (1-\lambda) \exp(-1.5t/T_{df}) \exp(-0.5t^2/T_2^2)]$, where T_2 is cross-polarization time constant for this model and T_{df} is the proton spin diffusion.

The cross-polarization kinetic parameters obtained using I-S and I-I*-S models are collected in Table 1. Signal intensity for C=O carbon atom versus contact time and fitting to the I-S model (T_{CP} = 1 ms and $T_{1\rho}$ = 4.6 ms) is illustrated in Figure 4a. Cross-polarization kinetics for methylene carbon atom (δ 59 ppm) and fitting to I-I*-S model gives T_2 = 2.5 ms and T_{df} = 20 ms (Figure 4b). Fast decay of signals intensities at longer contact times suggests the absence of intramolecular dynamics. Flexible

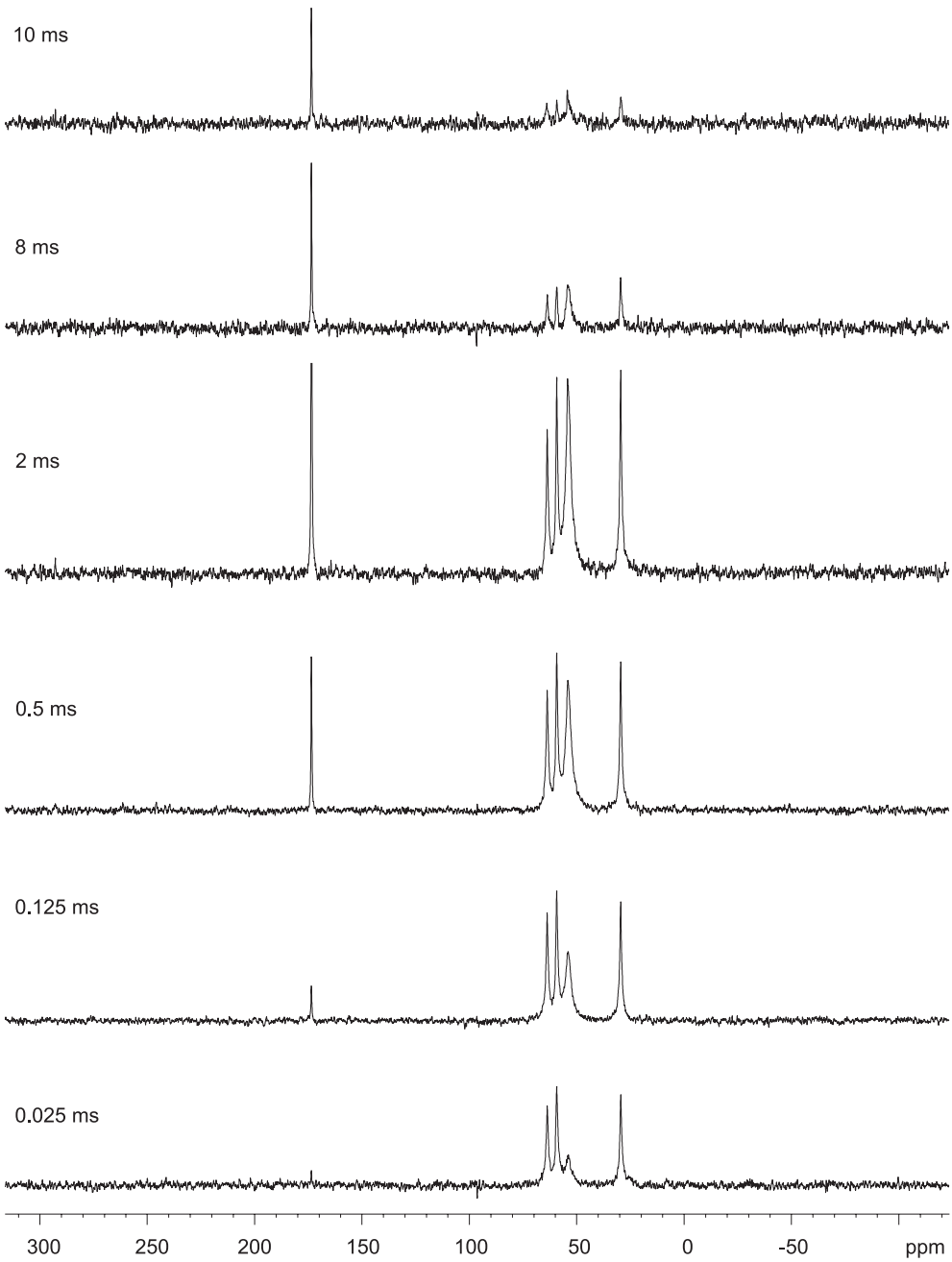


Figure 3. The ^{13}C CP MAS spectra of suxamethonium chloride recorded with various contact times.

Table 1. ^{13}C chemical shifts (δ, ppm) for carbons of suxamethonium chloride in solution (DMSO-d_6) and solid state, and cross polarization parameters (ms) as obtained using two CP models.

C			Carbon type	Model I-S		Model I*I-S	
	$\delta_{solution}$	δ_{CPMAS}		T_{CP}	$T_{1\rho}$	T_2	T_{df}
1,8	63,47	63,5	CH_2	–	4.1 ± 0.2	2.6 ± 0.1	22.7 ± 1.6
2,7	58,06	59,3	CH_2	–	3.9 ± 0.1	2.4 ± 0.2	19.4 ± 1.2
3,6	171,43	173,5	C=O	1.09 ± 0.07	4.6 ± 0.2	–	–
4,5	28,54	29,4	CH_2				
CH_3	52,79	53,8	NCH_3	0.22 ± 0.01	4.1 ± 0.2	–	–

Table 2. ^{13}C NMR chemical shifts (δ, ppm) of cortisone hemisuccinate in solution (acetone-d_6) and solid state; the differences $\Delta = \delta_{solution} - \delta_{CPMAS} > 1$ ppm.

C	$\delta_{solution}$	δ_{CPMAS}	Δ
1	32.26	32.8	
2	33.46	32.8	
3	204.69	201.7	3.0
4	121.39	121.9	
5	175.34	175.0	
6	32.43	32.8	
7	31.48	32.8	-1.3
8	30.52	32.8	-2.3
9	56.09	56.4	
10	39.05	39.5	
11	69.06	68.0	
12	39.57	39.5	
13	47.62	47.4	
14	52.48	52.3	
15	23.56	23.5	
16	32.99	32.8	
17	90.13	89.5	
18	16.78	17.2	
19	20.78	20.6	
20	209.28	209.0	
21	68.12	68.0	
succinate anion			
a	176,08	179.6	-3.5
b	28,96	30	
c	28,67	30	
d	170,94	172.2	-1.2

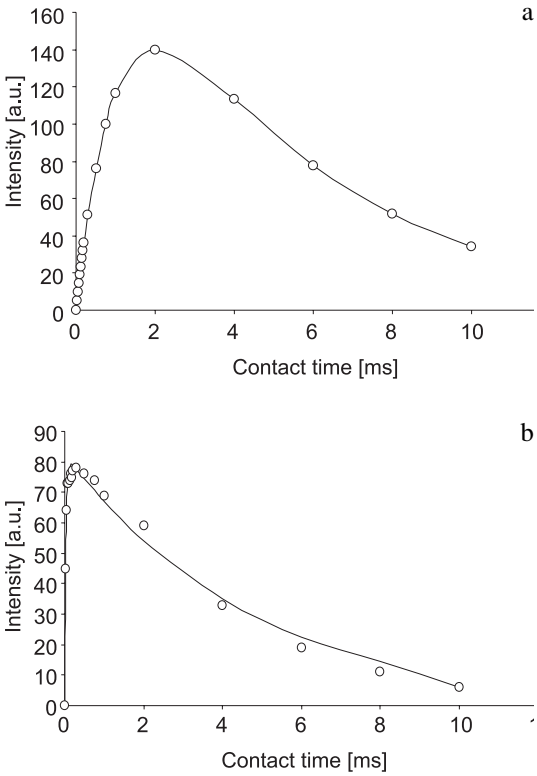


Figure 4. Cross-polarization kinetics for: (a) C=O carbon (I-S model) and (b) CH_2 (I-I*S model) of suxamethonium chloride. Signal intensity in arbitrary units (a.u.), contact time in ms.

molecular fragments are usually characterized by very long relaxation times $T_{1\rho}{}^{H}$. The CP curves for carbon atoms of beta-carotene chain ended with plateau (6).

Hydrocortisone hemisuccinate

In the medical literature, reports on an anaphylactoid reaction to hydrocortisone sodium succinate given intravenously are rare. However, the corticosteroid-induced adverse events observed in adults represent a broad clinical spectrum, as lastly reviewed (7, 8). Hydrocortisone sodium succinate, a water-soluble ester which is used for intravenous administration, is hydrolyzed to the hydrocortisone, the presumed active compound. The hydrolysis of hydrocortisone 21-hemisuccinate has been studied (9) in alkaline aqueous solution. Several methods have been described for determining hydrocortisone and hydrocortisone succinate in plasma; however, the widely used liquid chromatography (HPLC) and

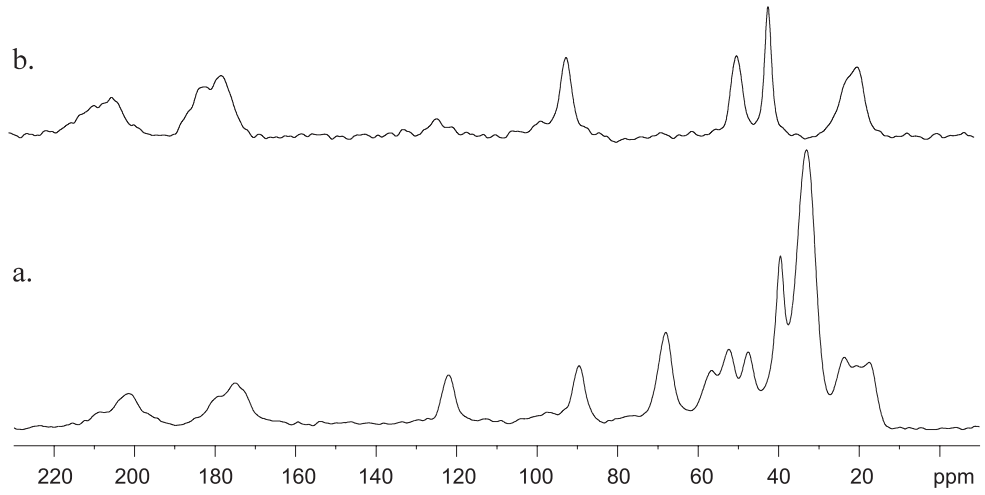


Figure 5. ^{13}C CPMAS NMR spectra of cortisone hemisuccinate: a) standard and b) with depolar dephasing.

isotope dilution-mass spectrometry are time-consuming and expensive (10). For the future NMR study of the metabolism in humans isotopically labeled compounds are necessary. The synthesis of multi-labeled cortisol and cortisone with ^{13}C and ^{2}H was described (11), and their ^{13}C and ^{1}H NMR spectra were fully assigned.

Cortisone in the form of the acetate ester exists in solid phase in several polymorphic forms, also as solvated crystals (pseudopolymorphs). Six crystalline forms were distinctly found by the analysis of solid state ^{13}C NMR spectra of nine various samples (12). Crystal structures are known for three forms and, therefore some correlation between NMR and XRD results could be made. Chemical shift differences between solid forms appear in the side chain and the conformations of ring A. Chemical shift of C3=O is larger for solid sample than for solution spectra, the effect is related with hydrogen bonding. Separate CH and CH_2 resonances in ^{13}C MAS spectra can be obtained by polarization inversion following cross polarization (a CPPI procedure). This procedure has been applied to polymorphism situations, as illustrated by Harris (13) for form I of cortisone acetate. However, there is no solid-state NMR data on hydrocortisone hemisuccinate.

^{13}C CPMAS NMR spectra were recorded for solid hydrocortisone 21-hemisuccinate, and standard spectrum is shown in Figure 5a. Chemical shifts are collected in Table 2. The spectra were assigned on the basis of liquid-state chemical shifts and dipolar dephasing (DD) experiment The solid state peaks have chemical shifts almost the same as their liquid state counterparts. The differences of 3.0-3.5 ppm were observed for carbonyl carbons. Deshielding of 3.5 ppm occurs for carbonyl carbon atom (a) of ester linkage. These results indicate that steroid structure is relatively rigid in solution, without much conformational freedom and resulting average of chemical shifts. The signals of methylene carbon atoms: 1, 2, 6, 7, 8 and 15, as well as those of succinate (b, c) give rise to a broad peak at 30-33 ppm. The resonances of quaternary carbon atoms C10 and C13 can be easily distinguished in the spectrum recorded with DD pulse sequence (Figure 5b). All resonances appear to be broader then usually observed for crystalline steroid-type compounds, indicating that the studied sample is amorphous.

CONCLUSIONS

^{13}C CPMAS NMR allows for fast identification of solid organic material, without any preparation procedures. The advantage of solid-state technique is also its non-destructive character; the sample removed from rotor can be further used for analysis by solution NMR or chromatography. It is evident at first sight that the ^{13}C MAS spectra of hydrocortisone and succinylcholine are quite different, although both compounds have carbonyl and methylene groups.

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