J-Modulation in 1D NMR ^{1}H Spectrum of Taurine and Aspartate Using Spin-Echo Technique

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This study reports on a theoretical calculation of Hahn's spin-echo experiment in case of a model A_2B_2 spin system with a strongly coupling character and gives the experimental results of one-dimension ^{1}H high-resolution NMR spectra of taurine and aspartate. The calculated amplitudes of the spin-echoes for two different proton groups of taurine are given. Using results of our calculations for taurine, the computer simulations of *J*-modulation are implemented. It is shown that the agreement between the experimental and simulated spectra is good.

Key words: NMR; Amino Acids; A_2B_2 System; *J*-modulation.

Introduction

^{1}H high-resolution NMR spectroscopy is a powerful technique which has been widely using in the investigations of amino acides [1]. By this kind of investigations it is possible to identify and assign the resonances of the ^{1}H spectrum of substances. Furthermore, in recent years it has been recognized that the effect of spin-spin coupling modulates the echoes of a homonuclear spin system where the coupling is first order [2]. For a heteronuclear spin system, *J*-modulation is observed in experiments in which the 180° pulse is deliberately applied to desired spins [3]. In high-resolution spectroscopy, *J*-modulation is usually observed for the first-order spectrum, that is, the chemical shift differences between the coupled nuclei is much larger than the spin-spin coupling constant ($\delta \gg J$). The aim of this work is to show that the effect of spin-spin coupling on echoes might appear in the second-order spectrum (that is, the chemical shift value is not large compared with the spin coupling constant J, e.g. $\delta \simeq J$ or $\delta < J$) [4]. For this purpose, two amino acides, taurine and aspartate, have been chosen as samples which are often found in a high-order or complex spectrum. In our paper we used only taurine as a sample for the A_2B_2 system in the theoretical calculation, but we gave the experimental ^{1}H spectra of aspartate.

Experimental

The samples were obtained from Sigma Chem. Comp. and were prepared by dissolving the powder in a solution of phosphate buffered in D_2O. The concentration of the solution of taurine and aspartate was 0.1 M and 0.006 M, respectively, at pH 7.6.

^{1}H spectra of taurine and aspartate were obtained with an 11.7 T., AMX-500 Bruker Spectrometer operating at a proton resonance frequency of 500.133 MHz. Each spectrum was acquired by summing 32 scans. The total accumulation time was about 30 minutes for each sample. The chemical shifts of the resonances in the spectra of taurine and aspartate residues were measured relative to added TSP (0.000 ppm).

Theory

In a second-order spectrum, the total Hamiltonian of two nuclei can be written as

$$\mathcal{H} = \mathcal{H}_Z + \mathcal{H}_{rf} + \mathcal{H}_{CS} + \mathcal{H}_{SS}\,, \qquad (1)$$

where $\mathcal{H}_Z = \omega_0 (I_{AZ} + I_{BZ})$ is the Zeeman Hamiltonian, $\mathcal{H}_{rf} = -\omega_{rf}\,(I_x \cos \omega t + I_y \sin \omega t)$ the radiofrequency field interaction Hamiltonian, $\mathcal{H}_{CS} = \omega_0\, \delta_{AB}\, (I_{AZ} + I_{BZ})$ the chemical shift Hamiltonian and

$$\mathcal{H}_{SS} = J\,[I_{AZ} + I_{BZ} + 1/2\,(I_{A+} + I_{B+} + I_{A-} I_{B-})] \qquad (2)$$

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the spin-spin coupling Hamiltonian. The Larmor frequency and the strength of the radiofrequency magnetic field are $\omega_0 = -\gamma B_0$ and $\omega_{rf} = -\gamma B_{rf}$, respectively.

As established by Hahn, the free induction decay after a 90° rf pulse is the Fourier transform of a steady state signal, and the 90° τ 180° pulse sequences can provide the measurement of J [5].

In the rotating frame, the Hamiltonian in (1) can be written as

$$\mathcal{H}^r = (\omega_0 - \omega)\, I_z + \mathcal{H}_1 - \omega_{rf}\, I_x , \tag{3}$$

where the superscript r denotes the rotating frame and $\mathcal{H}_1$ is

$$\mathcal{H}_1 = 1/2\,(\delta_A\, I_{AZ} - \delta_B\, I_{BZ}) + [I_{AZ} + I_{BZ} + 1/2\,(I_{A+} I_{B+} + I_{A-} I_{B-})] \tag{4}$$

In (3), the strength of the radiofrequency field is assumed to be much larger than the inhomogenity of the magnetic field, δ and J, and the lengths of pulses can be neglected.

The amplitude of the signal is given by

$$S(t) = Tr\,[\rho(t)\; I_x] , \tag{5}$$

where $\rho(t)$ is the density matrix operator of the spin system. In the rotating frame, considering the evolution of the density matrix operator, the intensity of the spin-echo at 2 τ time can be written as

$$S(2t) = Tr\,[e^{-i\mathcal{H}_1}\,\tau\, e^{-\mathcal{H}_1}\,\tau\, I_x\, e^{i\mathcal{H}_1}\,\tau\, e^{i\mathcal{H}_1}\,\tau] , \tag{6}$$

where τ is the time between the pulses and $\mathcal{H}_1$ has the eigenvalues obtained by reversing all spins in those of $\mathcal{H}_1$ [6].

Results and Discussion

1. Taurine

For our calculations we referred to the results of an experiment which has already been done. The spectrum recorded in the aliphatic region of taurine is shown in Figure 1 (see p. 308). The results of chemical shift assignments, multiplicities and the spin-spin coupling constants are given in Table 1.

Table 1. Proton chemical shift assignments, multiplicities, and coupling constants for taurine and aspartate.

Compound names and assignments		Chemical shift (ppm)	Multiplicity	J (Hz)
Taurine	SCH_2	3.42	*t*	6.35; 6.8
	NCH_2	3.266	*t*	6.65
Asp	αCH	3.90	*dd*	8.75; 3.7
	βCH	2.68	*dd*	17.45; 3.75
	β'CH	2.82	*dd*	17.47; 8.8

Table 2. The wavefunctions for taurine as an A_2B_2 system.

Function notation	Function	State	Wavefunctions
s_2	$\alpha\alpha\alpha\alpha$	s_2	s_2
$1s_1$	$(1/2)^{1/2}(\alpha\beta+\beta\alpha)\alpha\alpha$	$1s_1'$	$1s_1\cos\phi - 2s_1\sin\phi$
$2s_1$	$(1/2)^{1/2}\alpha\alpha(\alpha\beta+\beta\alpha)$	$2s_1'$	$1s_1\sin\phi + 2s_1\cos\phi$
$1s_0$	$\beta\beta\alpha\alpha$	$1s_{-1}'$	$1s_{-1}\cos\phi + 2s_{-1}\sin\phi$
$2s_0$	$\alpha\alpha\beta\beta$	$2s_{-1}'$	$-(1s_{-1})\sin\phi + (2s_{-1})\cos\phi$
$3s_0$	$(1/2)(\alpha\beta-\beta\alpha)(\alpha\beta-\beta\alpha)$	s_{-2}	s_{-2}
$4s_0$	$(1/2)(\alpha\beta+\beta\alpha)(\alpha\beta+\beta\alpha)$	$1a_1'$	$(1a_1)\cos\psi_+ + (2a_1)\sin\psi_+$
$1s_{-1}$	$(1/2)^{1/2}(\alpha\beta+\beta\alpha)(\alpha\beta+\beta\alpha)$	$2a_1'$	$(1a_1)\sin\psi_+ + (2a_1)\cos\psi_+$
$2s_{-1}$	$(1/2)^{1/2}\beta\beta(\alpha\beta+\beta\alpha)$	$1a_{-1}'$	$(1a_{-1})\cos\phi_- - (2a_{-1})\sin\psi_-$
s_{-2}	$\beta\beta\beta\beta$	$2a_{-1}'$	$(1a_{-1})\sin\psi_- + (2a_{-1})\cos\psi_-$
$1a_1$	$(1/2)^{1/2}(\alpha\beta-\beta\alpha)\alpha\alpha$	$3s_0'$	$-3s_0\cos\theta_S + 4s_0\sin\theta_S$
$2a_1$	$(1/2)^{1/2}\alpha\alpha(\alpha\beta-\beta\alpha)$	$4s_0'$	$-3s_0\sin\theta_S + 4s_0\cos\theta_S$
$1a_0$	$1/2(\alpha\beta+\beta\alpha)(\alpha\beta-\beta\alpha)$	$1a_0'$	$1a_0\cos\theta_a - 2a_0\sin\theta_a$
$2a_0$	$1/2(\alpha\beta-\beta\alpha)(\alpha\beta+\beta\alpha)$	$2a_0'$	$1a_0\sin\theta_a + 2a_0\cos\theta_a$
$1a_{-1}$	$(1/2)^{1/2}(\alpha\beta-\beta\alpha)\beta\beta$		
$2a_{-1}$	$(1/2)^{1/2}\beta\beta(\alpha\beta-\beta\alpha)$		

Considering Fig. 1 and Table 1, we have a second-order spectrum for taurine ($H_2N{-}CH_2{-}CH_2{-}SO_3H$). For simplicity, we can denote the SCH_2 and NCH_2 of taurine as A and B, respectively Thus we can regard this sample as an A_2B_2 system [7]. For the SCH_2 group of taurine we have the well-known and commonly used quantities $K=0$, $L=0.45$, $M=0$ and $N=13.15$ Hz, and for the NCH_2 group of taurine, $K=0$, $L=0$, $M=0$ and $N=13.3$ Hz. Then the distinction between the quantities of the SCH_2 and NCH_2 groups of taurine arises from the L values. Table 2 shows the wavefunctions for taurine. In Table 2, the wavefunctions include the angles which can be defined as tg $\phi = J/\delta (\pi/2 > \phi > -\pi/2)$, tg $2\,\theta = L/K$ and tg $2\,\psi_\pm = L/M$. For the B group, since $L=0$, the mixed states ($3s_0$, $4s_0$, $1a_0$ and $2a_0$) can be excluded. Furthermore, considering the values of the quantities K, L, M for this group, the angles θ and $\psi_\pm$ can take the values 0° and 45°. On the other hand, for the A group, since $L \neq 0$, the above angles take a value of 180° and the mixed states $3s_0'$, $4s_0'$, $1a_0'$ and $2a_0'$ should be considered. For both cases, the angle ϕ becomes a variable. By considering the wavefunctions in Table 2 and using (6), the calculated spin-echo amplitudes are given in Table 3. In Table 3, the obtained results from the state $3s_0'$

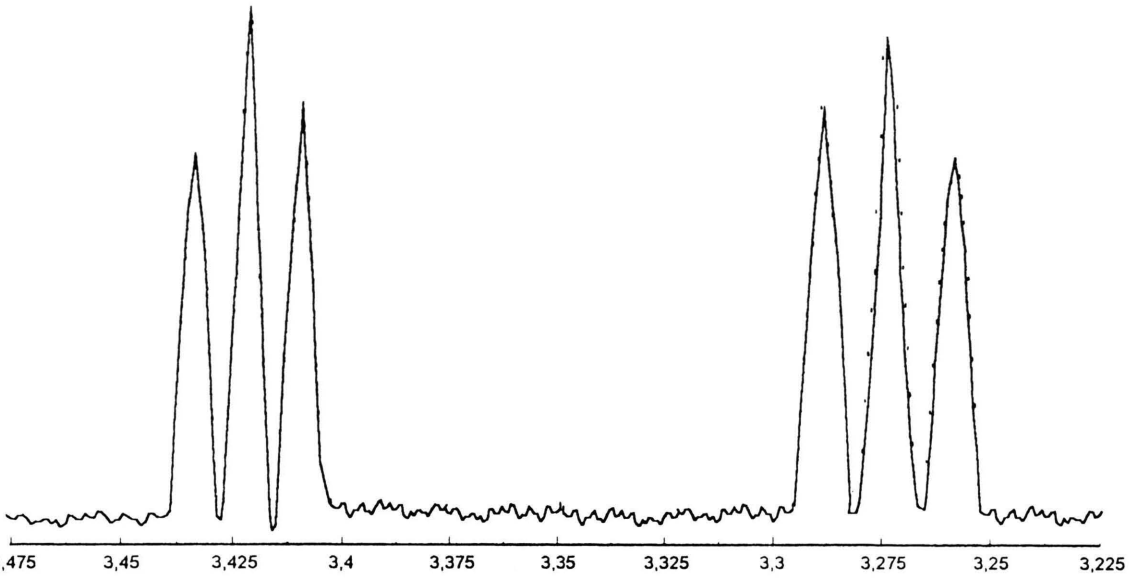

Fig. 2. Simulated spectrum obtained by considering the effect of spin-spin coupling on spin echoes.

to $2\,a'_0$ are only given for the SCH_2 group of taurine. From Table 3, the amplitude of the spin-echo can be generalized as

$$S(2\,\tau) \cong S_0\, f(\phi)\ [\cos A'\tau \cos B']\ \tau + \sin A'\tau \sin B'J\tau], \quad (7)$$

where A' and B' are constants and A' includes the chemical shifts δ of the spins. Equation (7) can be expressed as a product of alternating factors in the exponential terms for the nth echo which occurs at $\tau' = 2\,n\,\tau$.

We have implemented numerical simulations of taurine using the above results of our calculation (Figure 2). In the simulated spectrum, the angle ϕ takes values from 0 to $-45°$, while δ_A and δ_B are taken as a parameter for the A and B groups, respectively, and the value of τ is 1 µs. Although Figures 1 and 2 are shown on a different scale, it is clear that the agreement between the simulated and experimental spectra supports the existence of *J*-modulation in the second-order spectrum. However we should mentioned that the "roof" effect must be considered in the simulated spectrum.

2. *Aspartate*

The 1D ^{1}H high-resolution NMR spectrum recorded in the alphatic region of aspartate is shown in Figure 3.

Table 3. The calculated the spin-echo amplitudes given in (6).

State	Amplitude of spin-echo
s_2	$64 \exp[-i\,(15\,\delta_A - 22\,\delta_B + 998\,J)\,\tau]$
$1\,s'_1$	$228 \exp[i\,(-80\,\delta_A + 16\,\delta_B - 5058\,J)\,\tau]$
$2\,s_1$	$(76 \sin^2\phi + 74 \sin 2\phi + 74 \cos^2\phi)$ $\cdot \exp[i\,(100\,\delta_A - 150\,\delta_B - 220\,J)\,\tau]$
$1\,s'_{-1}$	$304 \exp[i\,(46\,\delta_A - 50\,\delta_B - 5656\,J)\,\tau]\,(1 - \sin 2\phi)$
$2\,s'_{-1}$	$(298 \sin^2\phi - 300 \sin 2\phi + 304 \cos^2\phi)$ $\cdot \exp[-i\,(20\,\delta_A - \delta_B + 2760\,J)\,\tau]$
s_{-2}	$64 \exp[-i\,(61\,\delta_A + 59\,\delta_B - 395\,J)\,\tau]$
$1\,a'_1$	$4 \exp[-i\,168\,J\,\tau]$
$2\,a'_1$	$4 \exp[-i\,168\,J\,\tau]$
$1\,a'_{-1}$	$(\sin^2\psi_- - 2 \sin 2\psi_- + 4 \cos^2\psi_-) \exp[-i\,168\,J\,\tau]$
$2\,a'_{-1}$	$(6 \sin^2\psi_- + 2 \sin 2\psi_- + 4 \cos^2\psi_-) \exp[-i\,168\,J\,\tau]$
$3\,s'_0$	$(83 \cos^2\theta_s + 309 \sin 2\theta_s + 443 \sin 2\theta_s)$ $\cdot \exp[i\,(102\,\delta_A - 138\,\delta_B - 11223\,J)]$
$4\,s'_0$	$(174 \sin^2\theta_s + 336 \sin 2\theta_s + 675 \cos 2\theta_s)$ $\cdot \exp[-i\,(164\,\delta_A - 282\,\delta_B + 9873\,J)]$
$1\,a'_0$	$16 \exp[-i\,336\,J\,\tau]$
$2\,a'_0$	$16 \exp[-i\,336\,J\,\tau]$

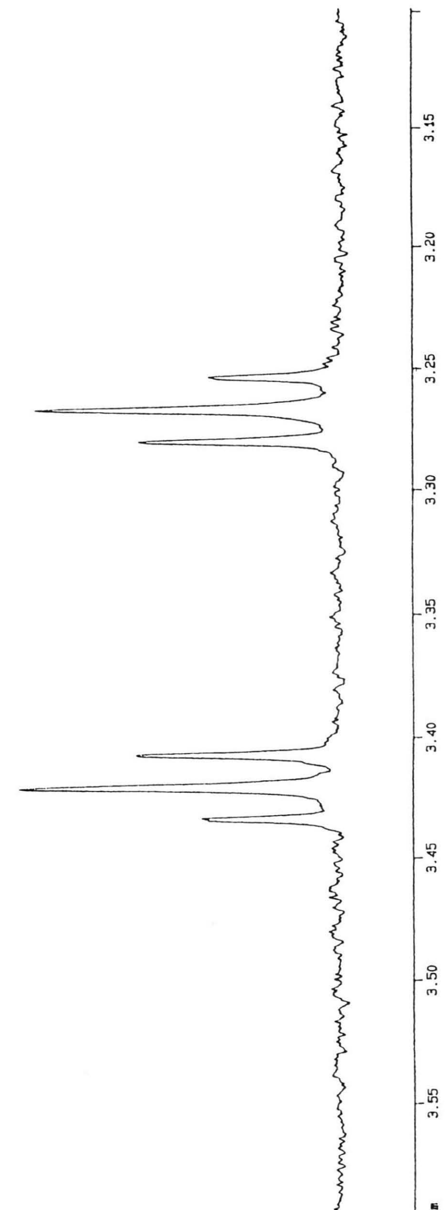

Fig. 1. The experimental ^{1}H- n.m.r. spectrum of taurine.

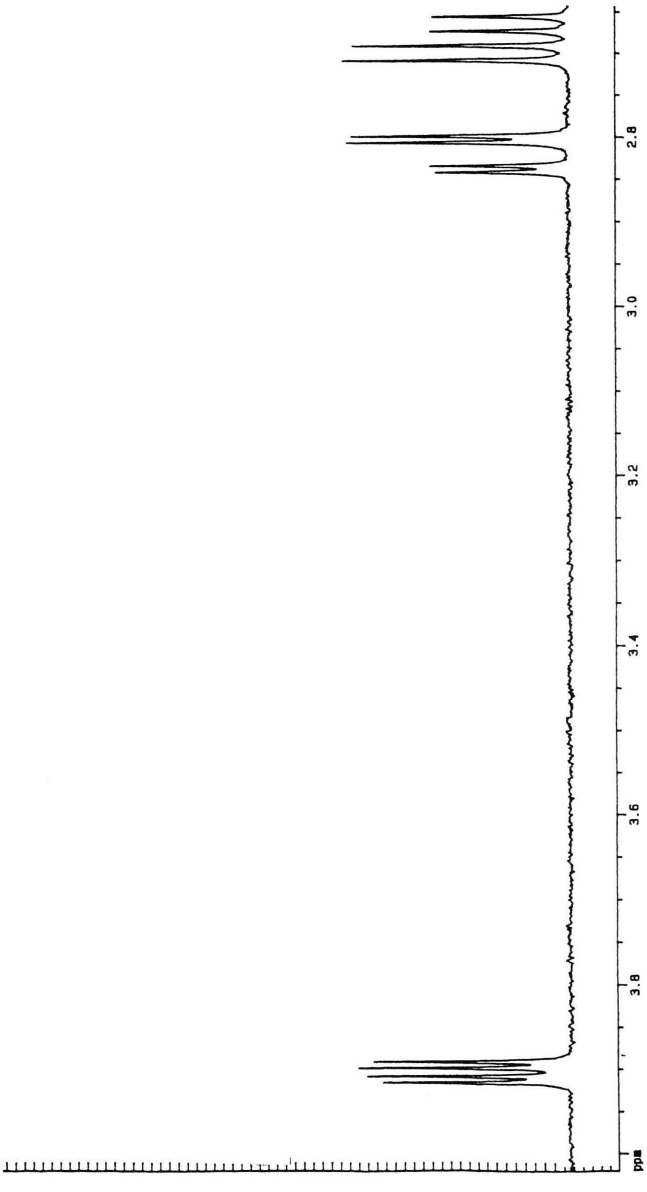

Fig. 3

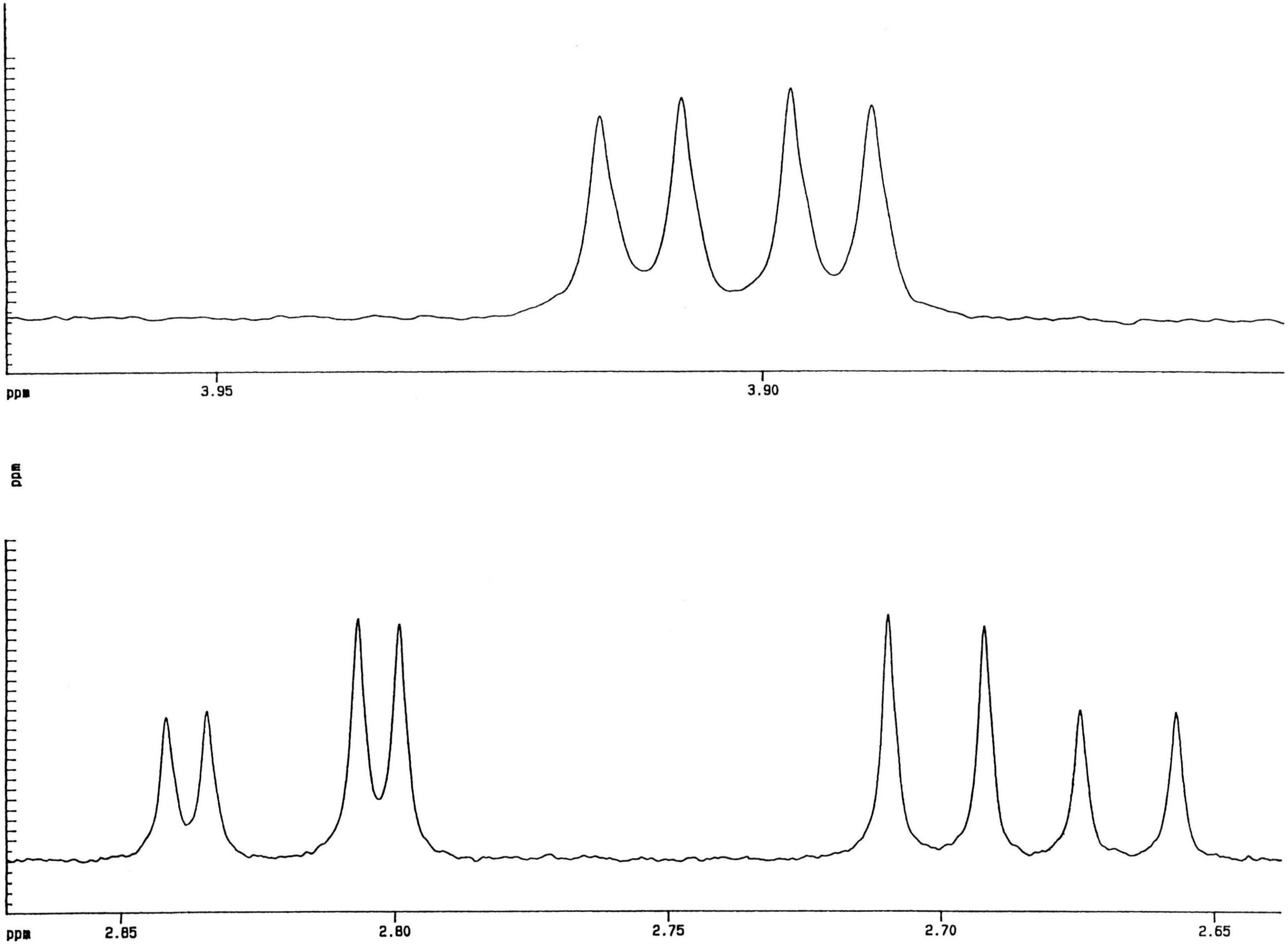

Fig. 3. The experimental ^{1}H- n.m.r. spectrum of aspartate. (a) The resonances of αCH proton and (b) the resonances of βCH_2 protons of aspartate.

The results of the chemical shift assignments, multiplicities and the spin-spin coupling constants are given in Table 1. The resonances of the αCH and βCH_2 protons of aspartate are given separately in Figs. 3a and b. The αCH proton and non-equivalent β-methylene protons of aspartate are split into a double doublet at 3.90, 2.82, and 2.68 ppm, respectively.

It can be easily seen that the second-order effects play an important role in the ^{1}H spectrum of aspartate. We did no theoretical calculation of the spin-echo amplitudes in the case of aspartate because it is very tedious and time consuming.

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