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CONFORMATIONAL ANALYSIS OF METHYLSUCCINIC ACID BY ^{1}H NMR SPECTROSCOPY‘ AND CIRCULAR DICHROISM

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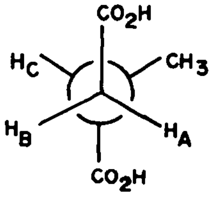
Abstract—The pH-dependence of the ^{1}H NMR and Circular Dichroism (CD) spectra of 2-methylsuccinic acid was investigated. Both spectra undergo dramatic changes between pH 4 and 6, where both carboxylic groups become ionized. From the coupling constants of the tertiary proton with the assigned[1] diastereotopic methylene protons, it is concluded that below pH 4 the *syn*-clinal (2) and above pH 6 the *anti*-periplanar (1) conformation of methylsuccinic acid prevail. The diesters of methylsuccinic acid also assume mainly the *syn*-clinal conformation (2). The pH-dependence of the CD spectra is discussed in terms of conformation and/or ionization effects.

In connection with the use of stereospecifically labelled methylsuccinic acids in enzymic reactions we needed reliable data on the conformation of succinic acid derivatives. Inspection of the literature revealed that in spite of numerous investigations no unambiguous experiments as a basis for secure conclusions are available. We undertook, therefore, an extended study of the pH-dependence of the conformation of methylsuccinic acid which led to some novel conclusions.

^{1}H NMR-SPECTRA OF METHYLSUCCINIC ACID

The 360-NMR spectrum of methylsuccinic acid in deuterium oxide (Fig. 1) shows in the region between 1.8 and 2.8 ppm three groups of signals, corresponding to the methine and the two methylene protons of this acid. The multiplet at lowest field can be assigned to the methine proton which is part of an ABC system and is further coupled with the protons at the methyl group. The two remaining signals can be handled as the AB portion of an ABC system. By means of stereospecifically deuterated methylsuccinic acids the B part at lower field has been assigned[1] to the methylene proton in the *erythro*-position (H_B) with respect to the methyl group (compare 4), whereas the A part at higher field to the methylene proton in the corresponding *threo* position (H_A).

On recording the NMR-spectrum of methylsuccinic acid solutions at different concentrations we noticed that the spectra were very much dependent on pH. Therefore the 360 MHz spectra of methylsuccinic acid between pH 1 and 8 in D_2O were recorded. Some of these spectra are reproduced in Fig. 1 and the coupling constants J_{AC} and J_{BC} are listed in Table 1. While all the signals are continuously shifted to lower fields with decreasing pH, the coupling constants J_{AC} and J_{BC} show a change opposite but complementary to each other. Thus at pH 7 and 8 J_{AC} is greater than J_{BC} and at pH 1–4 the opposite is true. A dramatic change takes place between pH 6 and 4, where one of the carboxylate groups of methylsuccinate is known to be protonated. Of the three staggered conformations of methylsuccinic acid 1, 2 and 3, 3 has two *syn*-clinal relationships and should be the least stable. In conformation 1 the two vicinal protons H_A and H_C are *anti*-periplanar as are protons H_B and H_C in conformation 2. Consequently, the high value of J_{AC} at neutral pH is consistent with the predominance of conformation 1 whereas at acidic pH conformation 2 prevails as indicated by the correspondingly high J_{BC} values. If the empirical method of Snyder[2] is valid also for methylsuccinic acid, the constants for the equilibrium between conformations 1 and 2 (K) can be calculated and



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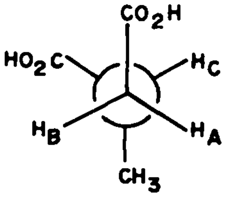

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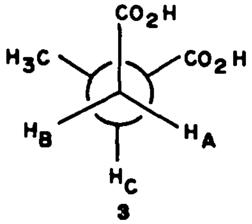

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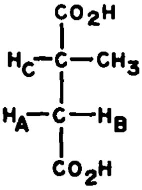

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Fig. 1.

Table 1. Chemical shifts and coupling constants (a) in the ^{1}H-NMR-spectrum of methylsuccinic acid at different pH values

	A	B	C	CH_3	J_{AC}	J_{BC}	$K^{(b)}_{calculated}$ 2
pH 8	1.885	2.292	2.395	0.847	10.1	5.0	2.89
pH 7	1.896	2.298	2.402	0.857	9.9	5.1	2.70
pH 6	1.960	2.305	2.430	0.878	9.0	5.8	1.90
pH 5	2.143	2.345	2.520	0.918	6.45	7.7	0.775
pH 4	2.283	2.415	2.613	0.963	5.6	8.7	0.525
pH 3	2.356	2.473	2.675	0.990	5.3	9.0	0.457
pH 2	2.370	2.485	2.687	0.997	5.3	9.1	0.450
pH 1	2.367	2.483	2.687	0.994	5.3	9.1	0.450

a) chemical shifts in ppm (± 0.002) to dioxane = 3.535, coupling constants in Hz (± 0.05).

b) ratio rotamer 1 to 2

are given in the last column of Table 1. However, the assumption of Snyder[2] that the equilibrium concentration of 3 is negligible may not hold at lower pH-values where the *syn*-clinal arrangement of the carboxylic groups is more stable. Interestingly, the same *syn*-clinal conformation prevails for several diesters of methylsuccinic acid in $CDCl_3$ solution, as estimated from the observed coupling constants (Table 2).

These results together with the configurational assignment of the NMR-signals to the diastereotopic methylene protons of methylsuccinic acid[1] shed new light on the conformation of succinic acid derivatives in solution. The *anti*-periplanar arrangement of the negatively charged carboxylate groups (see 1) at higher pH-values is readily explainable on electrostatic grounds. Our conclusion that the *syn*-clinal conformation 2 is predominant at acidic pH and also in several diesters of methylsuccinic acid is at variance with the interpretation of previously published NMR-data.[3] The origin of this erroneous interpretation is the arbitrary assignment of the ^{1}H-NMR-signals from the diastereotopic methylene protons of methylsuccinic acid. Physical measurements other than NMR also led to conflicting conclusions concerning the conformational equilibrium of succinic acid derivatives. Bardet *et al.*[4] interpreted the Raman spectrum of succinic acid in aqueous solution in favour of a *syn*-clinal arrangement of the carboxylic groups and postulated an intramolecular hydrogen bond for its stabilization. However, according to Thompson *et al.*[5] the low value (2.08 D) of the dipole moment of succinic acid in dioxane solutions is in favour of a prevailing *anti*-periplanar arrangement of the carboxylic groups. The available chiroptical measurements (CD and ORD) were interpreted in terms of the rotational equilibria around the bond between the carboxyl- and the α-C-atom (see below), whereas the conformation concerning the two middle C-atoms was taken for granted on the basis of the work of Zetta and Gatti.[3]

Whereas our data unambiguously define a prevailing *syn*-clinal conformation of methylsuccinic acid at acidic pH-values, the physical reasons for such a conformation remain a matter of debate. Because of the small difference between the first and second ionization constants of succinic acid ($\log k_1/k_2 = 1.29$ in water) a strong stabilization of the *syn*-clinal conformation by an intramolecular hydrogen bond is unlikely. Moreover, in the case of the methylsuccinic acid diesters no hydrogen bonds are possible, but the *syn*-clinal conformation is nevertheless more stable. It seems, that a currently undefined interaction of the carboxyl groups (either protonated or alkylated) is responsible for the stabilization of their *syn*-clinal arrangement.

Table 2. Coupling constants (a) in the ^{1}H-NMR-spectrum of some diesters of methylsuccinic acid in $CDCl_3$

	A	B	C	CH_3	J_{AC}	J_{BC}
Dimethyl	2.36	2.69	2.87	1.17	6	8.4
Dibenzyl	2.47	2.80	2.98	1.23	6	8.4
Di-(ß-naphthyl-methyl	2.54	2.88	3.06	1.27	5.4	8.1

a) chemical shifts relative to tetramethylsilane in ppm (± 0.01), coupling constants in Hz (± 0.5).

CIRCULAR DICHROISM OF (S)-METHYLSUCCINIC ACID

The CD of (*S*)-(−)-methylsuccinic acid is heavily dependent on the pH of the solution (Fig. 3, Table 3). In strongly acidic medium in which only the protonated form is present the Cotton effect around 203–207 nm is negative, as it is for (unbuffered) water or ethanol solutions. Approximately at pH 4 this band starts to become smaller and is then blue-shifted; at pH 5 its rotational strength is already less than half of that at pH 2; furthermore, a second, positive Cotton effect appears at 224 nm (+0.04). At pH 6 the band at short wavelengths has already completely disappeared, the positive CD-band (+0.74) has shifted back to the usual position (212 nm), and a very small negative Cotton effect around 252 nm (−0.004) becomes discernible. At still higher pH-

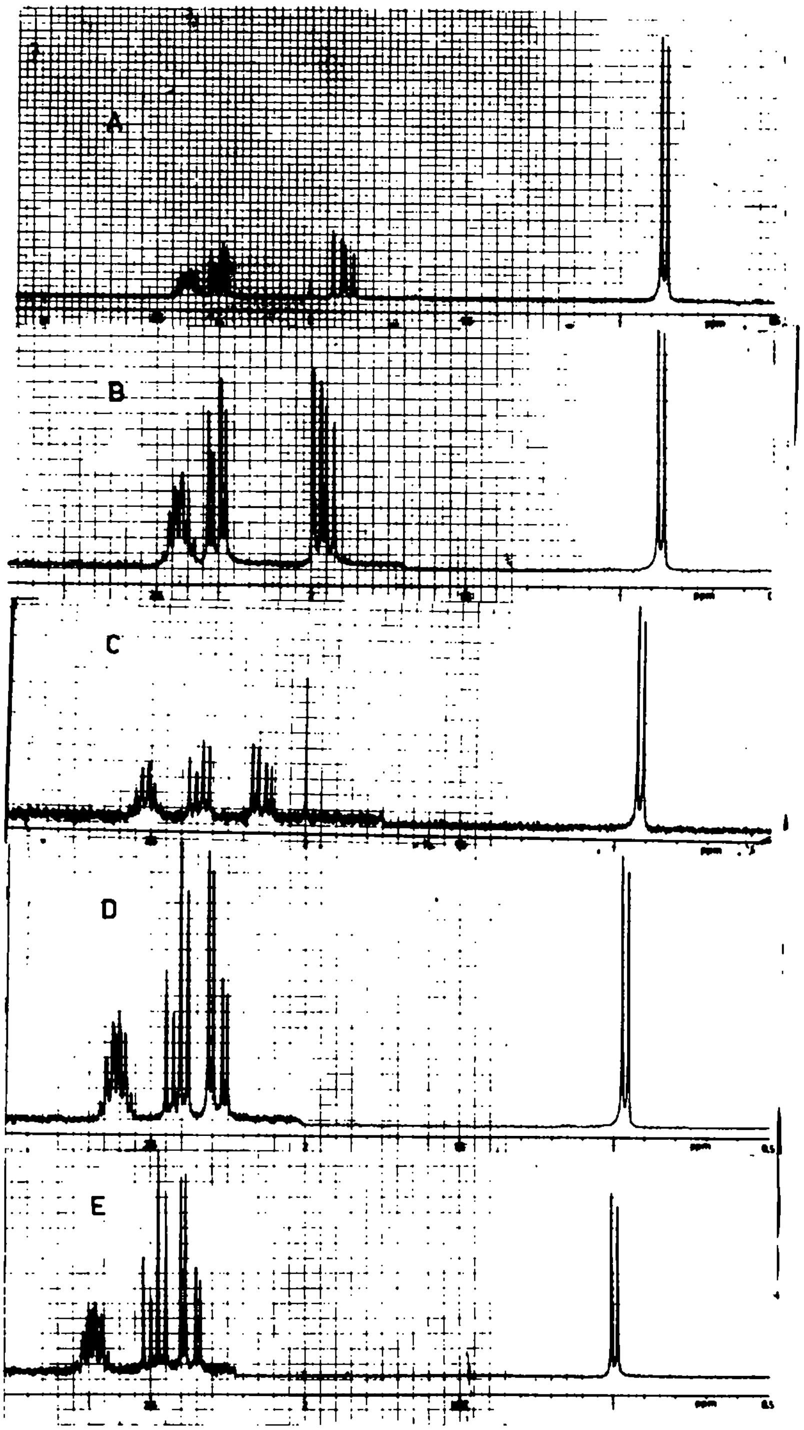

Fig. 2. ^{1}H NMR-spectra of methylsuccinic acid in ^2H_2O buffered at p^{2}H = 7 (A), p^{2}H = 6 (B), p^{2}H = 5 (C), p^{2}H = 4 (D) and p^{2}H = 2 (E).

values (7; 8) these CD-bands become only slightly stronger. This behaviour of pronounced band shifting (between pH 4 and 6) is typical for the overlap of two bands of opposite signs,[6,7] the real positions of the maxima may differ only by a few nm.

This bisignated behaviour of the CD of methyl succinic acid and its dimethyl ester (in organic solvents) has already been observed earlier,[8,9] and was ascribed to the presence of (at least) two conformers which differ in the torsion angle around the bond between the tertiary carbon atom of the chain and its adjacent COOH-group. It was furthermore assumed that (i) no discernible interaction exists between the two carboxylic chromophores, and (ii) that the CD is mainly determined by that carboxyl group which is adjacent to the chiral centre. Only the *anti*-periplanar conformation 1 of the (HOO)C–C–C–C(OOH) chain was taken into consideration.

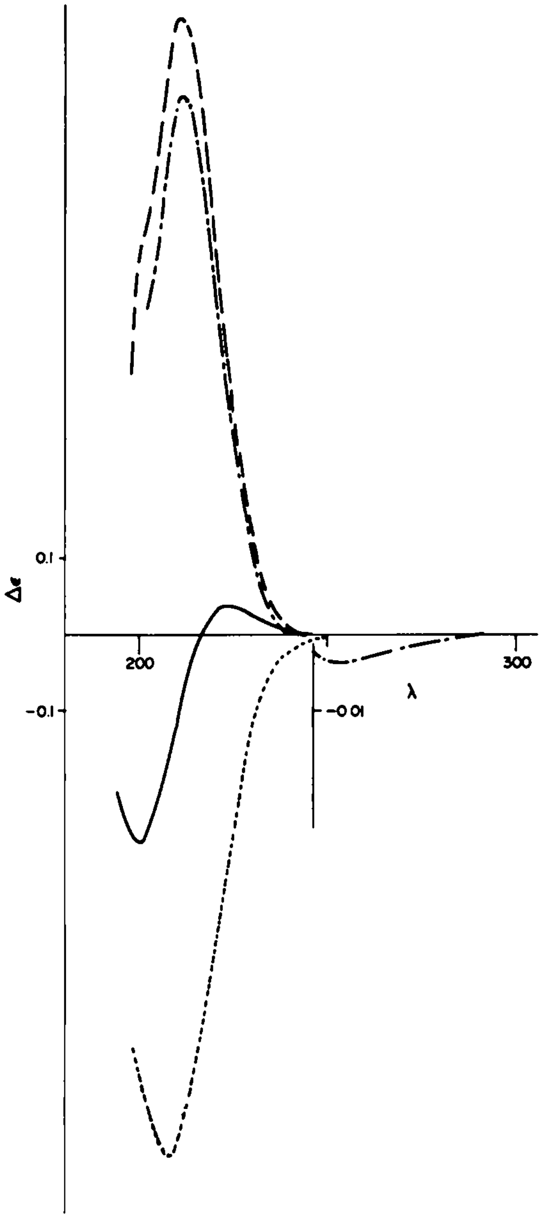

Fig. 3. CD-spectra of (*S*)-(–)-methylsuccinic acid in buffered aqueous solution: pH 2 (——), pH 5 (——), pH 6 (–·–·–), and pH 8 (---).

The ^{1}H NMR results presented here prove that in acidic medium actually the *syn*-clinal conformation 2 is predominant. However, this has only negligible influence upon the CD. Furthermore the ^{1}H NMR spectra give no information about the preferred conformation of the COOH groups. If the *syn*-periplanar conformation of the C_α–X with the C=O of a carboxylic group is indeed the energetically favoured one, then that bond is achirally disposed with respect to the chromophore (torsion angle 0°), whereas the remaining two bonds connected to C_α (torsion angles $+120°$ and $-120°$) are chirally arranged.

We can thus treat this situation as if we had a chiral second sphere,[10,11] and the well established rule for lactones[12,13] can then be applied. This states that a negative torsion angle ($|\omega| > 90°$) (O=)C–C(–C) gives rise to a negative Cotton effect around 210–220 nm. The situation of the chiral second sphere is not influenced by the conformation within the backbone chain. Furthermore, the second sphere for the $-CH_2-COOH$ chromophore is either achiral (torsion angle 0°) or racemic (approximately equal population of the conformations with torsion angle $+120°$ and $-120°$). Thus, the main influence on the CD should come from the other carbonyl chromophore.

If the C_α–C_β-bond is *syn*-periplanar to the C=O, the torsion angle (O=)C–C(–CH_2) is $-120°$ for (*S*)-(–)-methylsuccinic acid and a negative CD is predicted (the CD-contribution of the C–H bond is for such chromophores in general assumed to be negligible compared to that of a C–C bond). With a *syn*-periplanar arrangement of the methyl group a positive CD is expected. For the conformation with the C–H bond *syn*-periplanar to the C=O second-sphere contributions will cancel approximately. The observed negative CD for (*S*)-(–)-methyl succinic acid is thus in agreement with the preponderance of that conformation in which the CH_2 (and not the methyl) is *syn*-periplanar to the C=O, as was also assumed by other authors.[8,9] No second Cotton effect could be observed at longer wavelengths.

In the pH-range between approximately 4 and 7 several species must be present, viz. the undissociated diacid, the two monoanions, and the dianion, and all may adopt several conformations. At pH 8 only the dianion should remain, and the ratio of backbone conformations 1–2 is approximately 3 : 1 as inferred from the ^{1}H NMR spectra. The interpretation of the UV-absorption spectrum of the carboxylate chromophore is still a matter of debate. Calculations showed[14] that the two n-orbitals of a_1- and b_2-symmetry are of lower energy than that of the π^0-orbital. Extensive CI should render, however, the A_2 ($n^- \rightarrow \pi^-$) and B_1 ($n^+ \rightarrow \pi^-$)-state more stable than the B_2-state ($\pi^0 \rightarrow \pi^-$). The origin of the band between 220 and 240 nm in the crystal spectrum is, however, not yet clear.

The $A_1 \rightarrow B_1$-transition is formally electrically allowed. Its electric transition moment is, however, quite small because of very weak overlap; on the other hand this transition is magnetically allowed with $m_y \neq 0$. The obvious transition from which the missing μ_y can be stolen is the $A_1 \rightarrow A_2$ ($\pi^0 \rightarrow \pi^-$)-transition. Applying qualitative MO-theory[15] for the determination of the sign of the CD to that problem leads to the result that an intrachromophoralic contribution to the CD has the opposite sign to that obtained interchromophoralically from, e.g. a $\sigma \rightarrow \sigma^*$-transition in the chirally arranged

Table 3. CD-data of (*S*)-(−)-methylsuccinic acid (maxima)

pH of buffered aqueous solution or solvent	Concentration (mmol/l)	Wavelength (nm)	$\Delta\epsilon$
2	1.917	207	−0.69
4	1.431	204	−0.54
5	1.468	224	+0.04
		200	−0.28
6	1.758	252	−0.04
		212	+0.71
7	1.754	251	−0.09
		212	+0.88
8	1.552	212	+0.82
H_2O (unbuffered)	1.828	205	−0.64
ethanol	2.025	240	+0.12
		207	−0.46

C_α–C_β-bond. Without more model compounds at hand and with the uncertainty about the favoured conformation of the COO^--group in solution we prefer to present our experimental result only and not attempt to assign the two observed bands to any specific transition. Whether these two belong to two different conformations or to two different transitions (e.g. the $n^- \rightarrow \pi^-$ and the $n^+ \rightarrow \pi^-$) can not be decided from the present data.

EXPERIMENTAL

Materials. Methylsuccinic acid dibenzylester (b.p. 180°/0.01 Torr) and methylsuccinic acid bis (naphthylmethyl)ester (m.p. 76–77°) were obtained from Dr. M. Fountoulakis.[16] Optically pure (*S*)-(−)-methylsuccinic acid (kindly donated by Prof. H. Simon, Munich[17]) was further purified by chromatography on Dowex 1' and recrystallized from water (m.p. 110–111°).

^1H NMR-*measurements* were carried out at 23° with a Bruker WH-360 instrument. The results are given in Fig. 2 and in Tables 1 and 2.

Preparation of the solutions for the ^1H NMR-measurements: 416.8 mg (2.365 mmol) of methylsuccinic acid (m.p. 110–111°, recrystallized from ethyl acetate) were dissolved in 4.73 ml 1 N sodium hydroxide solution. Evaporation of the solvent and drying *in vacuo* yielded 516.3 mg of the disodium salt.

The buffer solutions were prepared by mixing appropriate amounts of 0.1 M ^2HCl and 0.1 M KCl (p^2H 1–4) or 0.1 M $K^2H_2PO_4$ and 0.1 M $Na_2{}^2HPO_4$ (p^2H 5–8) in deuterium oxide (99.98%) as solvent. In 1 ml of each buffer solution 2 mg of the disodium salt of methylsuccinic acid were dissolved. The p^2H of the eight final solutions were measured with a pH-meter at 17° and the following values were found: 1.05, 2.05, 3.05, 3.95, 5.05, 5.95, 6.95 and 7.95, respectively. An error of ± 0.05 p^2H-values was possible.

CD-measurements. The buffer solutions were prepared in a similar way as for the ^1H NMR-measurements, except that non-deuterated reagents and solvents were used. The concentrations of (*S*)-(−)-methylsuccinic acid are given in Table 3. The CD curves were obtained with the dichrograph MARK III of Jobin-Yvon at room temperature in cells of 0.02–2.00 cm path-lengths.

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