MAGNETIC RESONANCE IN CHEMISTRY, VOL. 35, 111–114 (1997)

NMR Spectroscopic Characterization of Isomeric *S*-Oxides Derived from α-Lipoic Acid

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α-Lipoic acid (thioctic acid) was oxidized in saturated $NaHCO_3$ solution by means of hydrogen peroxide. The resulting mixture of four structurally isomeric *S*-oxides (β-lipoic acids, thiosulfinates) was characterized by NMR spectroscopy without chromatographic separation. The regio- and stereochemistry of the *S*-oxides were elucidated using various one- and two-dimensional NMR techniques. The spectroscopic data obtained allow the quantitative determination of the thiosulfinates based on a high-field proton NMR spectrum of the mixture. © **1997 by John Wiley & Sons, Ltd.**

Magn. Reson. Chem. **35**, 111–114 (1997) No. of Figures: 3 No. of Tables: 3 No. of References: 9

Keywords NMR; ^{1}H NMR; ^{13}C NMR; lipoic acid; thioctic acid; thiosulfinate; *S*-oxides

Received 30 May 1996; accepted 12 August 1996

INTRODUCTION

α-Lipoic acid (thioctic acid) shows various pharmacological effects such as enzyme protective and zytoprotective activity, efficacy in hepatic diseases and diabetes mellitus and immunological, anti-inflammatory and anti-arteriosclerotic effects.[1] The function of thioctic acid as a coenzyme in mitochondrial multi-enzyme complexes as a basis of these effects is taken into consideration, in addition to its antioxidative properties and its potency to quench radicals.

Dihydrolipoic acid–lipoic acid is a thiol–disulfide redox system similar to glutathione GSH–GSSG and cysteine–cystine systems. In all cases oxygen radicals are deactivated by the reduced thiol form. The dihydrolipoic acid–lipoic acid system is very interesting because thioctic acid in its oxidized disulfide form is exclusively capable of quenching singlet oxygen in a non-radical chemical reaction.[2]

In the 1970s, Stary and co-workers investigated oxidation products of lipoic acid, which they obtained under various reaction conditions.[3,4] They postulated the presence of four isomeric thiosulfinates and two thiosulfonates based on proton NMR spectroscopic results for mixtures of the *S*-oxides, partly separated by thin-layer chromatography. The number of isomers in the mixtures was elucidated using paramagnetic shift reagents in the NMR experiment. It was found that the relative amount of the different oxides depends on the solvent used in the oxidation reaction. They postulated a zwitterionic intermediate, which is converted into the product through an inter- or intramolecular reaction step. In aprotic solvents and at low temperatures, the intramolecular reaction is preferred, and thus the formation of thiosulfonates. However, to our knowledge, a definitive assignment of the isomeric structures beyond the differentiation of thiosulfinate and thiosulfonate has not been published so far.

In this paper, we give complete ^{1}H and ^{13}C NMR data for the four thiosulfinates of lipoic acid. For the analysis we used a mixture of the *S*-oxides, obtained by oxidation of thioctic acid with H_2O_2 in saturated $NaHCO_3$ solution (Scheme 1). No chromatographic separation steps were performed prior to spectroscopic investigation in $CDCl_3$ solution. Determination of the stereochemistry and the assignment of the spectra were carried out by means of one- and two-dimensional NMR experiments. Especially the ^{13}C chemical shifts of the S-bearing carbon atoms and the stereospecific assignment of diastereotopic CH_2 protons in the five-membered ring were important for the deduction of the structures. With the data presented here, it is possible to identify and quantify the four isomeric forms of β-lipoic acid using a normal high-field proton NMR spectrum.

RESULTS AND DISCUSSION

Thioctic acid was oxidized using 30% H_2O_2 in saturated $NaHCO_3$ solution. For the exact reaction conditions see the Experimental section.

For the NMR spectroscopic analyses, 0.1–0.6 M solutions of the mixture of *S*-oxides in $CDCl_3$ were used. The one-dimensional proton NMR spectrum shows severe overlap of resonances even at 500 MHz, which

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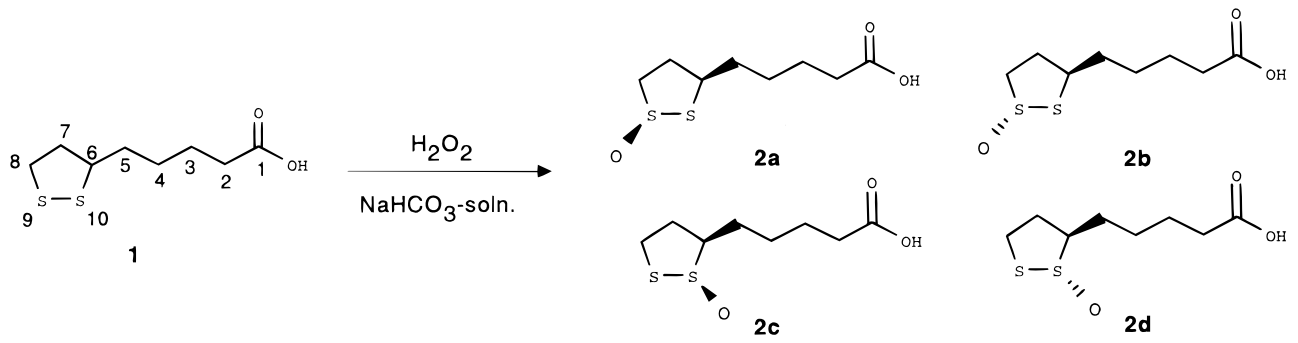
 CCC 0749–1581/97/020111–04 $17.50



Scheme 1.

prohibits a direct assignment of the signals to the corresponding isomers (see Fig. 1). The analysis and assignment of the proton NMR spectrum were carried out by means of an H,H-COSY spectrum and a homonuclear TOCSY spectrum.[5,6] With the TOCSY spectrum a complete separation of the spin systems of the four thiosulfinates **2a–d** is possible (Fig. 1). The assignment of the proton resonances is given in Table 1. The data for thioctic acid (**1**) are also given for comparison.

The assignment of the carbon NMR spectrum was carried out simply by transduction of the proton assignment via inverse detected H,C-correlation experiments (HMQC and HMBC).[7] Figure 2 shows a part of the HMQC spectrum. Table 2 gives the ^{13}C NMR data for

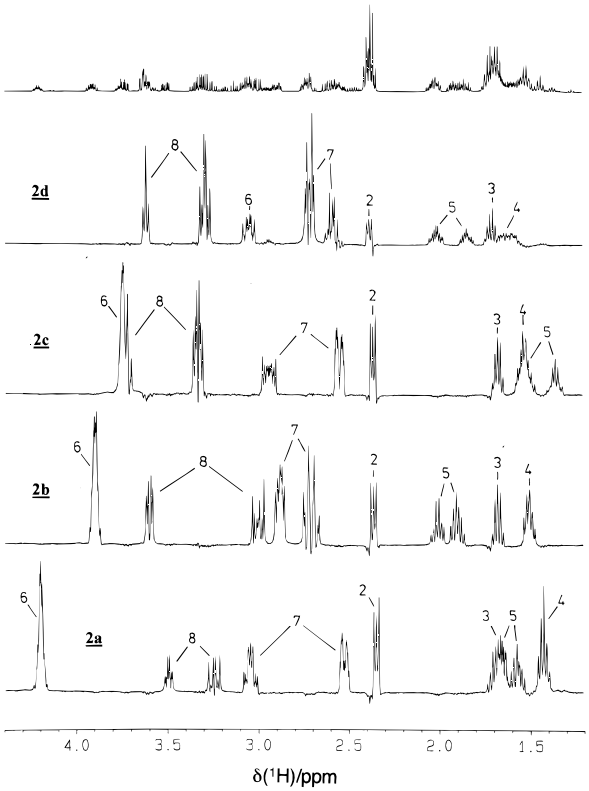


Figure 1. Proton NMR spectrum of the mixture (top) and slices from the TOCSY spectrum for the four thiosulfinate isomers **2a–d** with assignments. The slices were taken at 4.22 ppm (**2a**, H6), 3.92 ppm (**2b**, H6), 3.78 ppm (**2c**, H6) and 2.60 ppm (**2d**, H7′).

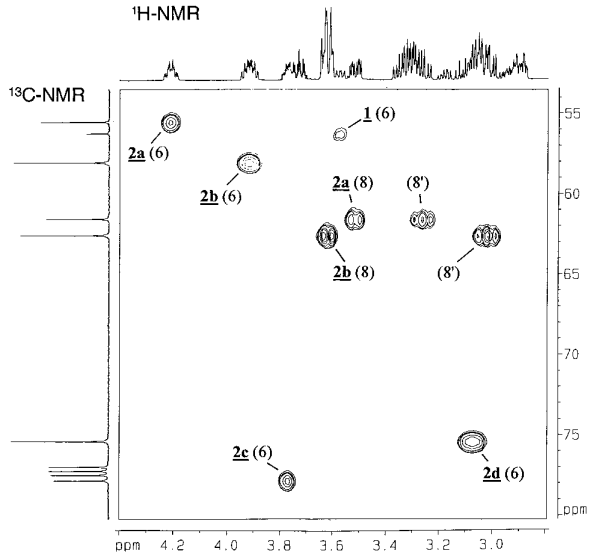


Figure 2. Part of the heteronuclear HMQC spectrum, showing the direct proton–carbon correlations of CH(6) for the four thiosulfinate isomers **2a–d** and of CH2(8) for **2a** and **2b**. The sample contains a small amount of thioctic acid (**1**); the respective CH(6) correlation is also labelled.

Table 1. Proton NMR data (ppm) for thioctic acid (1) and thiosulfinates 2a–d in $CDCl_3$ at 303 K

Position	1	2a	2b	2c	2d
1 (COOH)	9.3 (br)	9.3 (br)	9.3 (br)	9.3 (br)	9.3 (br)
2, 2′	2.38	2.36	2.38	2.39	2.41
3, 3′	1.68	1.71	1.69	1.70	1.72
4, 4′	1.50	1.44	1.52	1.56	1.65
5	1.70	1.70	2.02	1.50	2.04
5′	1.70	1.57	1.91	1.37	1.86
6	3.57	4.22	3.92	3.78	3.08
7	2.46	3.06	2.90	2.96	2.73
7′	1.92	2.54	2.72	2.56	2.60
8	3.18	3.51	3.62	3.75	3.64
8′	3.12	3.26	3.02	3.35	3.31

Table 2. Carbon NMR data (ppm) for thioctic acid (1) and thiosulfinates 2a–d in $CDCl_3$ at 303 K

Position	1[8]	2a	2b	2c	2d
1	179.87	178.19	178.46	178.36	178.38
2	33.83	33.54	33.67	33.66	33.52
3	24.39	24.32	24.35	24.23	24.46
4	28.66	27.94	28.77	27.16	28.04
5	34.59	35.45	36.12	26.90	27.09
6	56.29	55.60	58.11	77.78	75.34
7	40.23	35.14	35.29	33.98	33.90
8	38.51	61.60	62.61	37.19	36.73

the four thiosulfinates **2a–d** in comparison with **1**. For the assignment of the carbon spectrum of **1**, see Ref. 8.

Important for the differentiation of the regio- and stereochemistry are the chemical shifts of the sulfur-bearing groups CH(6) and CH_2(8) in the dithiolane ring. The assignment of the constitutional isomers is possible due to a 20–25 ppm low-field shift of the carbon atoms next to the oxidized sulfur. Thus, the inspection of the ^{13}C NMR data shows immediately that isomers **2a** and **2b** are oxidized at S(9) and **2c** and **2d** are oxidized at S(10). This interpretation is corroborated additionally by the shift of the C(5) carbons: due to the oxidation of the neighbouring group S(10) the signal is shifted to high field.[9]

The question of whether the position of the *S*-oxygen is *cis* or *trans* relative to the carboxypentyl side chain can be answered for isomers **2c** and **2d** in a simple way: because of the anisotropy of the SO group the neighbouring proton CH(6) is shifted to high field when the oxygen and the proton are positioned on the same side of the five-membered ring.[9] Thus, the configuration of thiosulfinate **2c** is *cis* and **2d** is *trans*. As expected, the shifts of the carbon atoms C(5) show a small opposite effect.

In contrast, the similar classification of isomers **2a** and **2b** is not straightforward. For this attempt the stereospecific assignment of the diastereotopic protons H(8) and H(8′) must be known. This assignment was elucidated by an H,H-NOESY spectrum at 238 K. An NOE effect of proton H(6) at 3.92 ppm to the high-field shifted H(8′) at 3.02 ppm showed that in isomer **2b** both protons are positioned on the same side of the ring. Therefore, thiosulfinate **2b** has a *trans* and **2a** a *cis* configuration. This is supported also by the relative high-field shift of proton H(6) in isomer **2b** compared with **2a**. No effects were found between H(6) and H(8,8′) of isomer **2a** in the same NOESY spectrum.

Table 3. Quantitative determination of thioctic acid (1) and thiosulfinates 2a–d in $CDCl_3$, 303 K

Isomer	Concentration (mol%)
1	10.5
2a	16.2
2b	23.7
2c	16.5
2d	33.1

A quantitative analysis of the four thiosulfinate isomers in the mixture is possible using the non-overlapping signals in the one-dimensional proton NMR spectrum. In the mixture a small amount of thioctic acid (**1**) was present. The quantitative data are given in Table 3.

CONCLUSION

Thioctic acid (**1**) was oxidized by H_2O_2 to the thiosulfinate isomers **2a–d**. The mixture of the products was characterized by NMR spectroscopy without chromatographic separation. A complete assignment of the ^1H and ^{13}C NMR spectra of the four isomers were made. Interpretation of the spectroscopic data revealed **2a** and **2b** to be S(9)-sulfoxides and **2c** and **2d** S(10)-sulfoxides. The configuration of **2b** and **2d** is *trans* whereas that of **2a** and **2c** is *cis*. The one-dimensional high-field proton spectrum allows a rapid quantitative analysis of the thiosulfinate mixture.

This example shows that NMR techniques are a very powerful tool even for the analysis of mixtures containing compounds with very similar molecular structures.

EXPERIMENTAL

NMR

All NMR spectra were recorded on a Bruker AMX-500 NMR spectrometer (500.13 MHz for protons, 125.77 MHz for carbon). For all experiments the mixture of thiosulfinates was dissolved in $CDCl_3$. The concentrations used were 0.3 mol l^{-1} for the one-dimensional spectra, H,H-COSY, H,H-TOCSY and HMQC, 0.1 mol l^{-1} for the NOESY experiment and 0.6 mol l^{-1} for the HMBC spectrum. All spectra were recorded at 303 K except the NOESY (238 K). For all experiments standard pulse programs of the Bruker library were used (UXNMR-Package 920801). One-dimensional ^1H spectrum, pulse program zg30, spectral width 10 204 Hz (20 ppm), 64 K data points; one-dimensional ^{13}C spectrum, pulse program zgpg, spectral width 33 333 Hz (265 ppm), 128 K data points; H,H-COSY, pulse program cosy90, spectral width 6024 Hz (12 ppm), 2 K data points in F_2, 256 experiments in F_1; TOCSY, pulse program mlevtp, mixing time 70 ms, spectral width 2262 Hz (0.5–5 ppm = 4.5 ppm), 4 K data points in F_2, 1 K experiments in F_1, exponential multiplication (1.5 Hz) and zero-filling in both dimensions; NOESY, pulse program noesytp, mixing time 250 ms, temperature 238 K, spectral width 5050 Hz (-0.5 to 9.5 ppm = 10 ppm), 4 K data points in F_2, 512 experiments in F_1, SINE multiplication (SSB = 2) in both dimensions; HMQC, pulse program inv4tp, spectral width 5050 Hz (10 ppm) in F_2 and 25 000 Hz (199 ppm) in F_1, 2 K data points in F_2, 512 experiments in F_1, processing with QSINE (SSB = 2) in both dimensions, zero-filling in F_1; HMBC, pulse program inv4nd, spectral width 5050 Hz

(10 ppm) in F_2 and 25 000 Hz (199 ppm) in F_1, 2 K data points in F_2, 256 experiments in F_1, processing with QSINE (SSB = 2) in both dimensions, zero-filling in F_1.

Synthesis of 2a–d

A 20.8 g (0.1 mol) amount of α-lipoic acid was dissolved in 300 ml of saturated $NaHCO_3$ solution and 41 ml (0.4 mol) of 30% H_2O_2 were added within 1.5 h, during which the temperature rose to a maximum of 45 °C and the yellow colour disappeared. After standing overnight, 300 ml of dichloromethane were added and the pH was adjusted to 1 by addition of 37% hydrochloric acid. The organic solution was collected, dried over anhydrous $MgSO_4$ and evaporated to dryness, yielding 9.1 g (41%) of β-lipoic acid as an oil.

Acknowledgements

We thank S. Hübner, M. Schneider and I. Schmidt for recording the NMR spectra and Professor C. Griesinger, University of Frankfurt, for helpful discussions.

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