Pergamon

0731-7085(94)E0023-U

Journal of Pharmaceutical & Biomedical Analysis, Vol. 12, No. 7, pp. 855–861, 1994

0731-7085/94 $7.00 + 0.00

Captopril and its probable contaminants: NMR and MS features of analytical value

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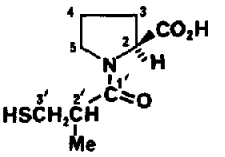
Abstract: The 400 MHz ^{1}H NMR spectrum of captopril in a variety of solvents is analysed and compared with those of epicaptopril and its disulphide analogue. A method for detecting isomeric and oxidative impurities by examination of a ^{1}H NMR spectrum of captopril in DMSO-d_6 is proposed. ^{13}C NMR and MS data enable differentiation of captopril from its disulphide analogue but not from its diastereoisomer epicaptopril.

Keywords: *Captopril; epicaptopril; ^{1}H and ^{13}C NMR spectroscopy; mass spectrometry.*

Introduction

Use of NMR spectroscopy in characterizing captopril **1**, the classical example of an antihypertensive agent which acts by inhibiting angiotensin-converting enzyme [1, 2], is complicated in two respects. Firstly captopril (SS-**1**) exists in the solute state as a binary mixture of amido conformers, and secondly its NMR features are similar to those of its two most likely impurities, namely the RS diastereoisomer (epicaptopril) and the disulphide analogue (two molecules of **1** linked *via* S–S after removal of H_2). The isomeric impurity arises as a result of incomplete removal of the RS-diastereoisomer from reaction mixtures; most syntheses produce significant amounts of the RS-form in addition to the desired SS-form [3] which is the more potent antihypertensive [4] (a stereoselective synthetic procedure has been reported [5]). Captopril is also prone to oxidative linkage of its thiol functions, a liability well-known to drugs of the thiol class.

This paper presents an analysis of the ^{1}H NMR spectra of captopril and its close relatives and shows how the technique has analytical utility in spite of these complications. ^{13}C NMR and MS features of the group are also reported. Literature data on the NMR features of captopril are sparse although Kadin has published data in a monograph on captopril [6]. Nam *et al.* [7] refer to an unanalysed 90 MHz ^{1}H spectrum in $CDCl_3$ [8] while



1 2'S,2S

differentiation of the SS- and RS-diastereoisomers is reported by comparison of the 300 MHz ^{1}H NMR spectra of their S-Bu^t,Bu^t esters [5] but not directly.

Materials and Methods

Samples of captopril, epicaptopril and the disulphide analogue of captopril were kindly supplied by the Bristol-Myers Squibb Company of Princeton, New Jersey. ^{1}H NMR spectra were obtained on a JEOL GX400 spectrometer operating at 399.05 MHz, run mostly at ambient temperature (22 ± 1°C). Samples of 1–5 mg (dependent on their solubility) were dissolved in the appropriate solvent (D_2O, D_2O-NaOD, DMSO-d_6) and examined without degassing, employing the standard conditions of 32K data points with digital resolution of 0.18 Hz per point [9]. By homonuclear ^{1}H–^{1}H correlation spectroscopy (COSY) a 2D spectrum for captopril was obtained using the standard COSY-45 pulse sequence [10]. Solvent resonances (HDO 4.8 ppm, DMSO-d_6 2.5 ppm) were employed as reference signals. The ^{13}C NMR spectra were recorded on a JEOL GX 270 spectrometer at

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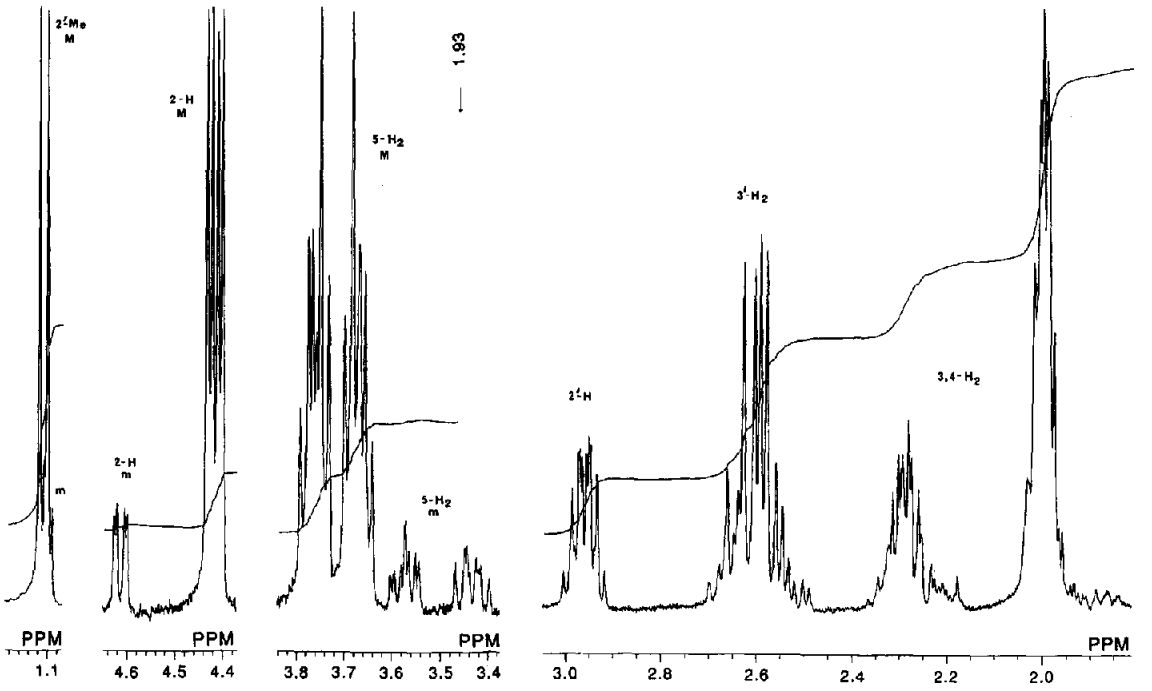


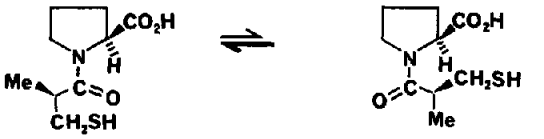
Figure 1
Expansion of the 400 MHz ^{1}H NMR spectrum of captopril in D_2O. Assignments of signals are shown. Where amido conformer signals are resolved, M denotes the major and m the minor resonance.

67.8 MHz and spectral assignments aided by decoupled proton test (DEPT) experiments.

Electron impact (EI) mass spectra at 70 eV were obtained using a 7070E VG Analytical instrument. The chemical ionization spectrum of the disulphide was obtained with isobutane as the reactant gas.

Results and Discussion

The 400 MHz ^{1}H NMR spectrum of captopril in D_2O is resolved into distinct proton groups with coupling interactions clearly apparent for H-2 and methyl protons — other groups give rise to multiplet resonances (Fig. 1). Spectral assignments were facilitated by a COSY experiment and comparisons with a spectrum of proline (Table 1). A feature of the spectrum is the clear duplication of H-2 and H_2-5 resonances, while the major Me doublet signal overlaps a minor doublet near its base. These signal duplications are due to the existence of major and minor amido conformers of captopril (**2** and **3**) whose interconversion rate is slow on the NMR time scale. The major conformer is deduced to be **2** on steric grounds and the lower field chemical shifts of its H_2-5 protons which result from their proximity to the deshielding thiol function (in the minor form **3**, H-2 is proximate to SH and its resonance takes the lower field position). This interpretation is supported by the fact



2 **3**

2 major (close approach of 5-CH_2 protons and SH function possible) ⇌ 3 minor (close approach of CO_2H and SH function possible)

that ^{1}H NMR features of impurities of related structure such as the RS-diastereoisomer and the disulphide analogue do not coincide with the minor resonances of captopril (see below and Table 1). Furthermore, observation of a decrease in the ratio of major to minor signals after a pH rise (from 6:1 in D_2O to 3:1 in D_2O–NaOD, Table 1) is understandable on the basis of the equilibrium **2** ⇆ **3** (ionization of 2-CO_2H will enhance hydrogen bonding interactions between this function and the proximate thiol group in **3** and hence stabilize the minor conformer). A conformer ratio of 5:1 was also

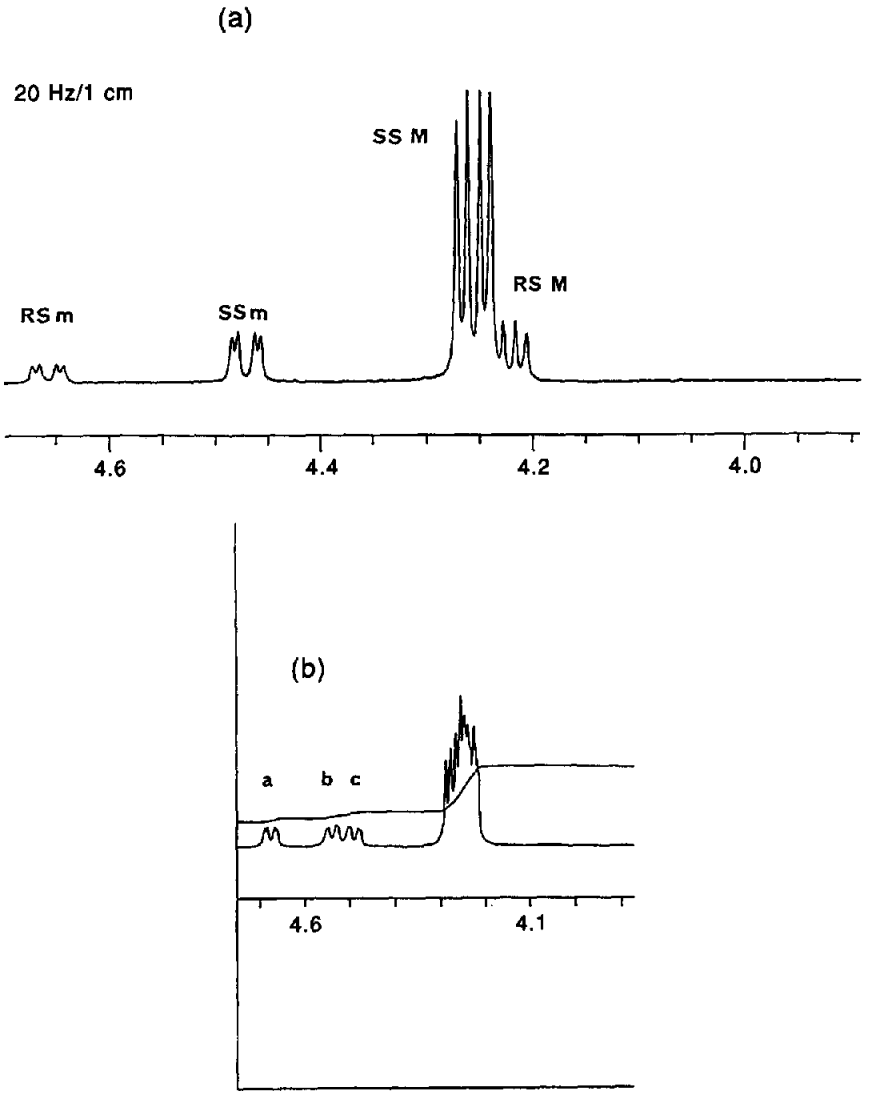
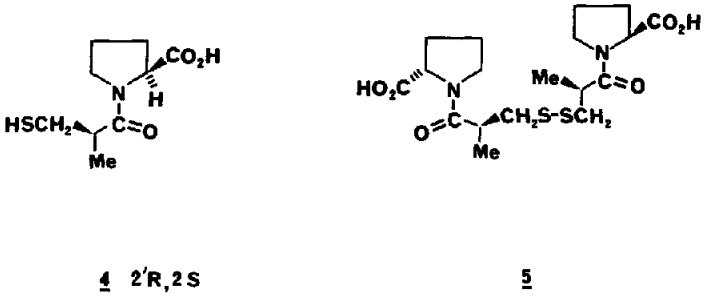


Figure 2
Part of the 400 MHz ^{1}H NMR spectrum of (a) a binary mixture of captopril (SS-**1**) (16 mg) and epicaptopril (RS-**4**) (4 mg) in DMSO-d_6 showing the major (M) and minor (m) H-2 signals, and (b) a tertiary mixture of captopril (16 mg), epicaptopril (4 mg) and the disulphide **5** (2 mg) in DMSO-d_6 showing clear resolution of the minor H-2 signals: a. RS (**4**); b. disulphide (**5**); c. SS (**1**).



4 2'R,2S **5**

Structures **4** and **5**.

Table 1
400 MHz ^{1}H NMR characteristics of captopril, epicaptopril and the disulphide analogue of captopril[a]

Compound and solvent	H-2[d]	H_2-5[d]	(Me)CH-2′
Captopril (SS **1**) in D_2O[b]	major dd 4.42 (8.7, 4.1) minor dd 4.61 (7.5, 2.5) ratio ~6:1[c]	major 3 3.72–3.80 m 3.62–3.72 minor m 3.54–3.62 m 3.38–3.48 ratio ~6:1	m 2.85–3.0
Epicaptopril (RS **4**) in D_2O	major dd 4.41 (9.3, 4.5) minor masked by HDO band	major m 3.6–3.7 minor m 3.35–3.50 ratio ~4:1	m 2.8–2.9[e]
Captopril (SS **1**) in D_2O–NaOD	major dd 4.09 (8.6, 4.4) minor dd 4.23 (8.85, 2.7) ratio ~3:1	manor m 3.6–3.7 m 3.5–3.6 minor m 3.2–3.35 m 3.35–3.45 ratio ~3:1	m 2.05–2.2[e]
Epicaptopril (RS **4**) in D_2O–NaOD	major[m] dd 4.55 (8.7, 4.5) minor dd 4.11 (9.2, 4.9) ratio ~1.5:1	m 3.4–3.8 major and minor signals overlap	
$HSCH_2$-3′	H_2-3,4[d]	(Me)CH-2′	
m 2.45–2.7	m 2.25–2.35 m 2.15–2.25 m 1.95–2.02	major d 1.14 (6.7) minor d 1.1 (6.7) (integrals overlap)	
m 2.55–2.65 m 2.35–2.55	m 2.1–2.25 m 2.0–2.1 m 1.8–1.95	major d 0.98 (6.8) minor d 0.93 (6.4) ratio ~4:1	
m 2.05–2.2 m 2.2–2.6	m 1.85–1.95 m 1.7–1.85	major d 0.99 (6.4) minor d 1.02 (6.1) (integrals overlap)	
[f]	[f]	manor d 0.96 (6.7) minor d 1.05 (6.7) ratio ~4:3	
Compound and solvent	H-2[d]	H_2-5	(Me)CH-2′
Captopril (SS **1**) in DMSO-d_6-D_2O[g,j]	major dd 4.27 (8.7, 4.2) minor dd 4.55 (8.5, 2.1) ratio ~5.1	major m 3.6–3.7 m 3.5–3.6 minor m 3.3–3.5 ratio ~5:1	[h]
Epicaptopril (RS **4**) in DMSO-d_6-D_2O[j]	major dd 4.24 (8.6, 4.2) minor brd[i] 4.68 (~9) ratio ~3.5:1	major m 3.65–3.75 m 3.55–3.65 minor m 3.35–3.45 ratio ~3.5:1	[h]

(Table 1 continued from previous page)

Compound and solvent	H-2[d]	H_2-5	(Me)CH-2′
Disulphide **5** in DMSO-d_6	major[l] dd 4.22 (8.8, 3.9) minor brd 4.54 (8.3) ratio ~5:1	major m 3.5–3.7[k] minor m 3.3–5.45	[h]
$HSCH_2$-3′	H_2-3,4[d]	(Me)CH-2′	
[h]	[h]	major d 1.07 (6.7) minor d 1.1 (~8) (integrals overlap)	
[h]	[h]	major d 1.06 (6.4) minor d 1.01 (5.9) ratio ~3:1	
[h]	[h]	major d 1.1 (6.8) unresolved signal near base	

[a] Chemical shifts in ppm (solvent reference: D_2O 4.8 ppm; DMSO-d_6 2.5 ppm); coupling constants (line separations) in parentheses (Hz); abbreviations: d doublet, dd doublet of doublets, m multiplet, br broad.
[b] Assignments supported by a COSY-45 ^1H, ^1H 2D plot which showed the required cross peaks e.g. CH-2′ m (2.85–3.0 ppm) with 2.45–2.7 m (CH_2-3′) and 1.14 d(Me).
[c] From integral trace of spectrum.
[d] cf. S-Proline in D_2O: H-2 4.05 ppm; H_2-5 near 3.3; H_2-3,4 near 2.3 and 1.95 (all m).
[e] A degree of overlap amongst CH-2′, CH_2-3′ and H_2-3,4 multiplets is evident from the integral ratios.
[f] Series of m 1.7–2.7 ppm showing extensive overlap.
[g] One drop of D_2O added to provide common band for exchangeable protons.
[h] Series of m 1.8–3.0 ppm showing extensive overlap.
[i] Few Hz splitting just resolved.
[j] Evidence for presence of epimer from low intensity H-2 signals.
[k] Includes HDO signal.
[l] Evidence of presence of **1** and/or **4** from low intensity H-2 signals (4.25, 4.26 ppm).
[m] Proximity to HDO band may distort integral value.

Table 2
67.8 MHz ^{13}C NMR characteristics of captopril, epicaptopril and the disulphide analogue of captopril[a]

Compound and solvent	C-2	C-3	C-4	C-5	C = O[b]	C-2′	C-3′	Me-2′
Captopril **1** in D_2O[c]	58.8 (59.5)	28.6 (30.45)	24.0 (21.7)	47.4 (47.3)	175.8 176.1	41.4 (42.0)	26.2[d]	15.6
Captopril **1** in DMSO-d_6	58.3 (58.6)	28.8 (30.8)	24.4 (22.1)	46.6 (46.1)	172.6 173.1 (173.4)	41.1 (41.3)	27.2[e]	16.6
Epicaptopril **4** in DMSO-d_6	59.5 (60.1)	29.6 (31.6)	25.1 (23.1)	47.7 (47.1)	174.7 174.3 (174.9, 175)	42.0 (42.3)	28.7[e] (28.4)	17.1 (17.5)
Disulphide **5** in DMSO-d_6	58.3 (58.5)	28.6 (30.8)	24.3 (21.9)	46.4 (45.9)	172.1 173.2 (172.6, 173.6)	36.8[e]	41.2[d] (40.6)	16.4

[a] Chemical shifts in ppm (references: external TMS for D_2O and 39.6 ppm solvent signal for DMSO-d_6); minor resonances in parentheses. Assignments were supported by DEPT experiments.
[b] 2-CO_2H and amido C = O (C-1′).
[c] cf. S-Proline in D_2O: C-2 61.6, C-3 29.7, C-4 24.4, C-5 46.5 (all ppm) [ref 14]; C-3, C-4 and C-3′ assignments may be interchanged.
[d] cf. C-3 of L-cysteine HCl in D_2O: 27.4 ppm; C-3 of L-cystine 2HCl in D_2O, 39.0 ppm [ref 11].
[e] Minor signal obscured by solvent resonance.

found for captopril as solute in DMSO-d_6 (Table 1).

^{1}H NMR spectral characteristics of the RS-diastereoisomer (**4**, epicaptopril) and disulphide analogue (**5**) were similar to those of captopril in all three solvents employed (Table 1); a pH rise likewise favoured and minor conformer of **4**.

Spectra of mixtures of captopril and epicaptopril displayed well resolved 4-line signals (dd) due to H-2 of the minor conformer of each component (obscured by the HDO band in D_2O but resolved when the band was moved upfield by a temperature rise to 80°C). Corresponding major H-2 signals overlapped but provided clear evidence of both isomers (Fig. 2a). The two components of a mixture of captopril and its disulphide in DMSO-d_6 could be identified in the same manner as could a tertiary mixture of captopril, **4** and **5** (remarkably, the three minor H-2 dd signals were all resolved at 400 MHz, Fig. 2b).

Examination of the ^{1}H NMR spectrum of a captopril sample in DMSO-d_6 therefore, allows detection of its two more probable impurities; the sensitivity of the procedure will depend on the operating frequency of the spectrometer (the higher the better) and may be established through spiking experiments.

Details of the ^{13}C NMR spectra of captopril, epicaptopril and the disulphide **5** are given in Table 2. Assignments of resonances were aided by DEPT experiments and by comparisons with spectra of proline, cysteine and cystine. For reasons of solubility, spectral comparisons are best made using the common solvent DMSO-d_t. With few exceptions, all signals of spectra recorded in DMSO-d_6 were in duplicate form, a further demonstration of conformer equilibria. The small differences in chemical shift observed between equivalent carbon signals made differentiation of the compounds by ^{13}C NMR difficult. However, the spectrum of the disulphide **5** is readily distinguished from those of **1** and **4** by the low field position of the methylene carbon adjacent to sulphur (cf. β-carbon resonances of cysteine HCl 27.4 ppm, and cystine HCl 39.0 ppm in D_2O [11].

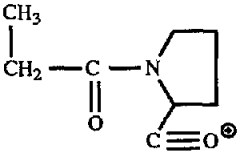
Apart from an entry in a pharmaceutical MS collection [12], most MS studies on captopril relate to derivatives employed in quantitative procedures [13]. Details of the present 70 eV electron impact spectrum of captopril are given in Table 3. Most of the ions, including $[M]^+$.

Table 3
Mass spectral features of captopril and its corresponding disulphide

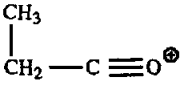
Captopril (S,S 1) ion (*m/z*)	Percentage abundance[a] 70eV EI	low ev EI
217 $[M]^+$	10	90
199[b]	7.5	100
184[b]	7.5	80
173[b]	7.5	—[f]
140	10	80
126	10	—
75[c]	12.5	—
70[d]	100	—
41[e]	25	—

Disulphide **5** ion (*m/z*)	Percentage abundance[a,g] 70eV EI	CI(isobutane)
365	<2.5	12.5
337	40	<5
239	20	<5
205	20	<5
193	<5	20
186	20	12.5
160	50	<5
154	<10	100[i]
149	20	<10
146	20	<5
130	30	10
123	5	35
114	25	<10
106	7.5	30
105	20	<10
91	60	15
85	20	52.5
81	30	30
69	50	72.5
67	20	60
57	100[i]	[h]
41	65	[h]

[a] To nearest multiple of 2.5.
[b] Possible losses from $[M]^+$: 199, $-H_2O$ (18); 184, $-SH$ (33); 173, $-CO_2$ (44).
[c] Possible structure $HSCH_2C^+H(Me)$.
[d] Possible loss of SH from $HSCH_2CH(Me)CO^+$(*m/z* 103).
[e] Possible loss of H_2S (34) from *m/z* 75 ion.
[f] 60% in low eV EI MS of epicaptopril.
[g] Mostly ions >20%; many lines above 20% were recorded below *m/z* 100 and not all are listed.
[h] Not scanned below *m/z* 60.
[i] Probable Structure:



[i] Probable Structure:



are of low intensities (<20%) relative to the base peak (*m/z* 70) and may be accounted for by loss of small neutral molecules or radicals from the molecular ion. The base peak (absent

in a low eV EI spectrum) may arise from loss of SH from $HSCH_2CHMeCO^+$. Although captopril and epicaptopril could not be differentiated by mass spectrometry (their EI MS showed no significant difference, spectra (70 eV and CI) of the disulphide **5** differed radically from those of **1** and **4**. MS details for **5** are included in Table 3; many lines were recorded but no $[M]^+$ or $[M + 1]^+$. (CI) ion observed. Structures for the base peaks, *m/z* 57 (EI) and 154 (CI), are suggested.

Conclusions

Analytical data presently reported demonstrate the value of routine examination of samples of captopril by high-field NMR spectroscopy (preferably as solutes in DMSO-d_6) for purposes of identity and detection of isomeric and oxidative (disulphide) contaminants. The EI mass spectrum of captopril is characteristic of overall structures but fails to differentiate the RS-diastereoisomer. However, samples of captopril and its disulphide analogue may readily be distinguished by MS.

A flow injection analysis of captopril and discussion of other quantitative methods are presented in a recent paper.

Acknowledgements — We thank Mr S.J. Lucania and Dr E. Petrillo (both of Bristol-Myers Squibb) for supply of materials and valuable advice, and Messrs R. Hartell and D. Wood (NMR data) and C. Cryer (MS data) for recording the spectra.

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[Received for review 4 January 1994]