Estimated Formation Constants for the Complexation of Methylmercury(II) by Captopril {1-[(2*S*)-3-Mercapto-2-methyl-1-oxopropyl]-L-proline}: Evidence of Stronger Binding to the *cis* Isomer of the Drug

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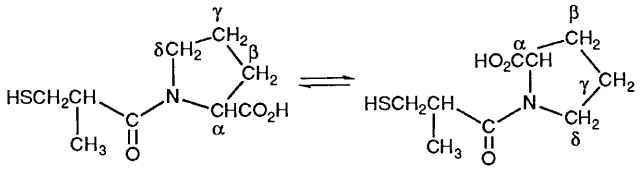
Complexation of CH_3Hg^{II} by captopril {1-[(2*S*)-3-mercapto-2-methyl-1-oxopropyl]-L-proline} has been studied by ^1H and ^{13}C NMR spectroscopy. Very little changes were observed in the equilibrium constants of the *cis* and *trans* isomers of CH_3Hg^{II}–captopril at a 1:1 ratio and free captopril as a function of pH*. However, in the ^{13}C NMR spectrum the chemical shift of the $HSCH_2$ resonance was greater for the *cis* than for the *trans* form at a 1:1 ratio. The formation constants (log K_f) constants for the *cis* and *trans* isomers are estimated to be 16.85 and 16.57 based on the equation log K_f = pK_{SH} + 6.86.

Captopril, 1-[(2*S*)-3-mercapto-2-methyl-1-oxopropyl]-L-proline, is a recently developed drug for the treatment of high blood pressure.[1–3] High blood pressure can result from the production of angiotensin II from inactive angiotensin I, the conversion being catalysed by angiotensin-converting enzyme,[4,5] which is a zinc metalloenzyme.[6] The mechanism by which captopril is thought to be active involves inhibition of angiotensin-converting enzyme, presumably through interaction with the enzyme at its active site.[1–3] More recently captopril has been used with some success to treat rheumatoid arthritis[7] and migraine.[8]

It is well known that proline-containing peptides normally exist as an equilibrium mixture of *cis* and *trans* isomers with respect to the peptide bond involving the proline amino group.[9] Thus, captopril is expected to be present in aqueous solution as the *trans* and *cis* forms, **I** and **II**, respectively, with their relative population dependent on the protonation state of the molecule.[10–13]



I **II**

It has been shown that conformationally restricted angiotensin-converting enzyme inhibitors require a *trans* amide bond of captopril when binding to the enzyme.[14] In view of the above restriction, it is of interest to study the metal-ion binding to captopril using ^1H and ^{13}C NMR spectroscopy by which the two isomers can be studied easily.[13]

In the present study CH_3Hg^{II} has been selected as a metal ion for the following reasons: (*i*) the co-ordination chemistry is very simple, normally binding with thiols, with a co-ordination number of two;[15,16] (*ii*) it usually binds to a thiol group between pH <1 and >13;[17] and (*iii*) the aqueous solution chemistry of CH_3Hg^{II} has been studied in detail so that the comparison of CH_3Hg^{II}–captopril with other thiol systems is easier.[18–23]

Experimental

Chemicals.—The captopril was a gift from the Squibb Institute for Medical Research, Princeton, NJ. The 99.7% D_2O, 40% NaOD in D_2O and 65% DNO_3 in D_2O were obtained from Fluka Chemical Company. Methylmercury(II) iodide (Alfa Division, Ventron Corp.) was converted into a solution of methylmercury(II) hydroxide and standardized as described in the literature.[16,17]

pH *Measurements.*—All pH measurements were made at 25 ± 1 °C with a Fisher model 520 Accumet pH meter, equipped with a Fisher microprobe combination electrode. Fisher-certified buffers of nominal pH 4.00, 7.00 and 10.00 were used for three-point calibration of the pH meter. All pH* measurements were made in D_2O solutions and have not been corrected for deuterium-isotope effects (pH* = pH + 0.40).[24]

NMR Measurements.—Proton NMR spectra were measured at 400 MHz on a Bruker WH-400 spectrometer operating in the pulsed Fourier-transform mode. The probe temperature was 25 °C and ^1H chemical shifts were measured relative to internal *tert*-butyl alcohol and are reported relative to the methyl proton resonance of sodium 4,4-dimethyl-4-silapentane-1-sulphonate (dss). The methyl resonance of t-butyl alcohol is +1.23 ppm relative to dss.

Carbon-13 NMR spectra were measured at 50.3 MHz on a Bruker WH-200 spectrometer operating in the pulsed Fourier-transform mode, with broad-band ^1H decoupling. The ^{13}C chemical shifts were measured relative to internal dioxane but are reported relative to $Si(CH_3)_4$ [the dioxane resonance is +67.4 ppm to higher frequency from $Si(CH_3)_4$]. Positive shifts correspond to less shielding than in $Si(CH_3)_4$.

Sample Preparation.—A 0.15 mol dm^{-3} stock solution of 1:1 ratio of CH_3HgOH–captopril was prepared containing 0.30 mol dm^{-3} $NaNO_3$ in D_2O.[16,17] The pH* of the solution was first decreased to ≈1 by adding DNO_3. The samples were withdrawn under an argon atmosphere into NMR tubes at appropriate intervals as the pH* was increased by titration with NaOD.

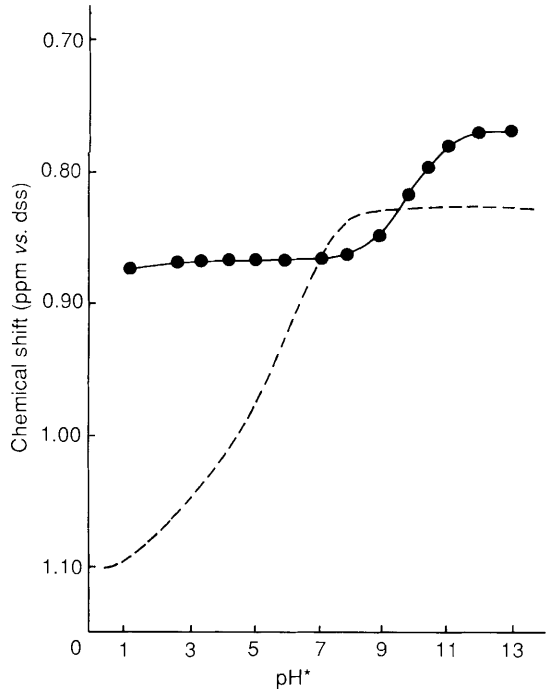


Fig. 1 Proton NMR chemical shift of the resonance for CH_3Hg^{II} in aqueous solution containing (dashed line) 0.19 mol dm^{-3} methylmercury and (solid line) 0.15 mol dm^{-3} CH_3Hg^{II}–captopril (1 : 1) in 0.30 mol dm^{-3} $NaNO_3$ in D_2O. The points for CH_3Hg^{II} itself (dashed line) are taken from refs. 21 and 26

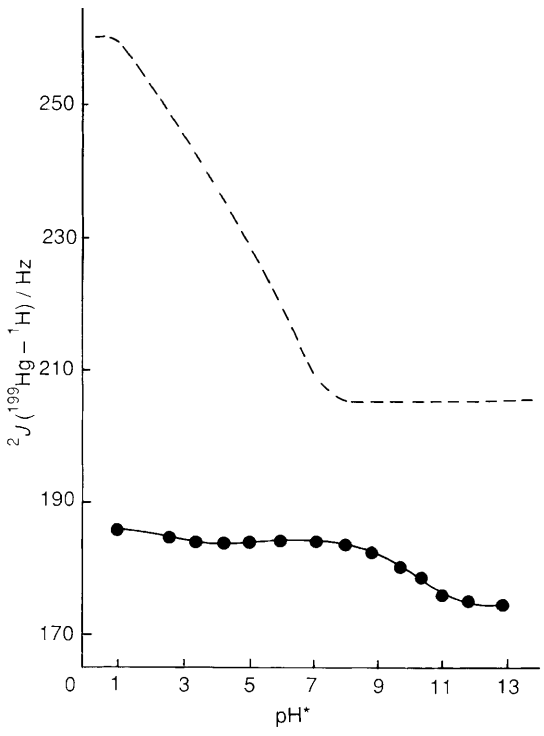


Fig. 2 pH* Dependence of $^2J(^{199}Hg–^1H)$ for CH_3Hg^{II} in aqueous solutions of the compositions given in the legend to Fig. 1

Results

The ^1H NMR chemical shift of the methyl resonance of CH_3Hg^{II} as a function of pH* (refs. 21 and 25–27) is shown in Fig. 1. The chemical shifts of free CH_3Hg^{II} are in the range from δ 1.10 to 0.83 from pH* < 1 to > 13. However, at the 1 : 1 ratio of CH_3Hg^{II}–captopril, it shifted from δ 0.87 to 0.77. Note the methyl resonance remains unshifted to pH* 9 and when pH* was increased further it started to follow a titration curve.

Fig. 2 shows the mercury–proton coupling constant of CH_3Hg^{II} as a function of pH*. The value of $^2J(^{199}Hg–^1H)$ was 260 Hz at pH* ≈ 1 for free CH_3Hg^{II}. This decreased to 205 Hz at about pH* 8. However, for CH_3Hg^{II}–captopril, the value was 185 Hz in the range pH* 1–9 which decreased to 172 Hz from pH* 9 to 13.

These results indicate that captopril binds to CH_3Hg^{II} forming a CH_3Hg^{II}–captopril complex up to pH* 9. At higher pH* the OH^- competes with captopril as shown in equation (1).[16–21,25–27]

$$CH_3Hg–SR + OH^- \Leftrightarrow CH_3HgOH + RS^- \quad (1)$$

The ^{13}C NMR chemical shifts of the $HSCH_2CH$ (C^2) and $HSCH_2$ (C^3) resonances of captopril in the presence and absence of CH_3Hg^{II} are shown in Fig. 3. The resonances of the *cis* isomer were shifted more than those of the *trans* isomer. As noted in Table 1, very little changes are observed in the chemical shifts of other captopril resonances. Note that the chemical shifts of the C^2 and C^3 resonances at a 1 : 1 ratio CH_3Hg^{II}–captopril remained constant to about pH* 9; at higher pH* the chemical shifts of these two resonances were shifted toward the free positions.

The fractional concentrations of the two isomers were determined as a function of pH* by using the relative intensities of the two multiplet patterns in the δ 3.4–3.9 region of the ^1H NMR spectrum.[13] The fractional concentrations of free captopril and CH_3Hg^{II}–captopril (1 : 1) as a function of pH* are shown in Fig. 4 The equilibrium constants for the **I** ⇔ **II**

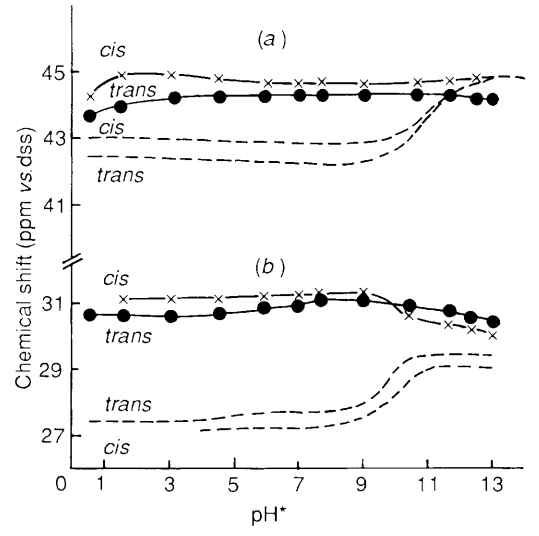


Fig. 3 pH* Dependence of the ^{13}C NMR chemical shift of the C^2 (*a*) and C^3 resonances (*b*) of captopril in solutions containing (solid lines) 0.15 mol dm^{-3} CH_3Hg^{II}–captopril (1 : 1) and (dashed lines) 0.15 mol dm^{-3} captopril itself in 0.30 mol dm^{-3} $NaNO_3$ in D_2O

equilibrium, K_{eq} = [**II**]/[**I**], are given in Table 2 for free captopril (H_2A, HA^- and A^{2-} ions where H_2A = fully protonated molecule at low pH, HA^- = CO_2^- deprotonated molecule at neutral pH, and A^{2-} = fully deprotonated molecule) and at a 1 : 1 ratio of CH_3Hg^{II}–captopril.

Discussion

The binding of captopril with zinc(II), cadmium(II), lead(II) and copper(II) has been reported.[28,29] Since these studies were carried out by potentiometric titrations the *cis* and the *trans* isomers were not resolved. The interaction of captopril with gold(I) has also been reported recently.[30] The chemical shift differences of the *cis* and the *trans* isomers bonded to Au^I were not resolved because of the overlapping of some resonances.

Table 1 Carbon-13 NMR chemical shifts (ppm) of free captopril and CH_3Hg^{II}–captopril (1:1) at various pH*

Assignment	Free captopril at pH* 0.59	CH_3Hg^{II}–captopril pH* 0.40	Δ[a]	Free captopril at pH* 7.45	CH_3Hg^{II}–captopril pH* 7.20	Δ[a]	Free captopril at pH* 12.30	CH_3Hg^{II}–captopril pH* 12.85	Δ[a]
C^3 *trans*	27.630	30.655	+3.025	27.730	30.898	+3.168	29.689	30.466	+0.777
cis	*b*	31.625	—	27.560	31.161	+3.610	29.101	30.089	+0.988
C^2 *trans*	42.553	43.757	+1.204	42.333	44.161	+1.828	44.832	44.242	−0.59
cis	43.141	44.566	+1.425	42.850	44.674	+1.824	*b*	44.971	—
CH_3 *trans*	16.898	17.095	+0.197	16.901	17.338	+0.437	16.751	17.310	+0.559
cis	*b*	17.634	+0.736	16.750	17.175	+0.425	16.089	16.933	+0.844
CON *trans*	177.153	177.069	−0.084	176.505	176.823	+0.318	178.476	177.000	−1.476
cis	*b*	*b*	—	177.373	*b*	*b*	179.726	*b*	—
C_α *trans*	60.049	60.067	+0.018	62.550	62.682	+0.132	62.475	62.655	+0.180
cis	*b*	60.525	+0.476	63.210	63.329	+0.119	63.211	63.329	+0.118
C_β *trans*	29.836	*b*	—	30.433	30.493	+0.060	30.497	30.688	+0.191
cis	31.673	*b*	—	32.190	32.190	0.000	32.261	32.165	−0.096
C_γ *trans*	25.204	25.155	−0.049	25.130	25.130	0.000	25.131	25.101	−0.030
cis	*b*	*b*	—	23.290	23.214	−0.076	23.293	23.214	−0.079
C_δ *trans*	48.655	48.637	−0.018	48.800	48.906	+0.106	48.802	48.879	+0.077
cis	47.722	47.854	+0.132	47.921	48.070	+0.149	47.772	47.936	+0.164
CO_2^- *trans*	177.006	176.638	+0.632	180.534	180.827	+0.293	178.402	—	—
cis	*b*	*b*	—	*b*	*b*	—	179.729	—	—

[a] Δ = Chemical shift difference between free and bound captopril: +shift = low-field shift, −shift = high-field shift. [b] Resonance is either overlapped or not detected.

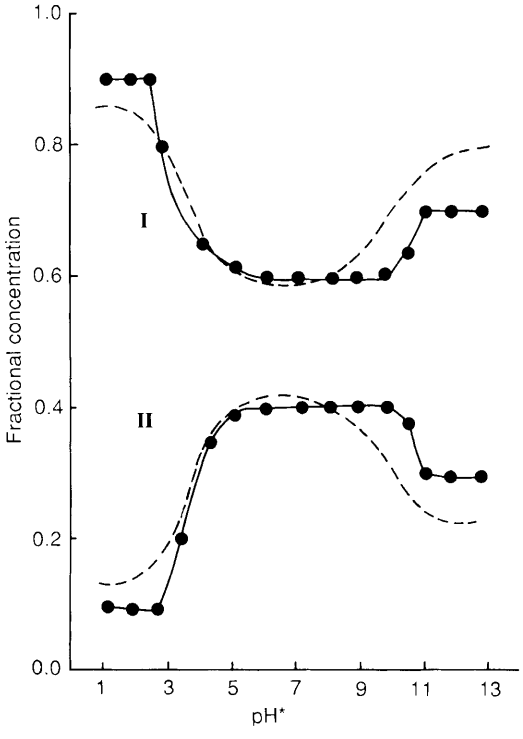


Fig. 4 Fractional concentrations of the *trans* (**I**) and *cis* (**II**) isomers as a function of pH* determined by using the relative intensities of the two multiplet patterns in the δ 3.4–3.9 region of the ^1H NMR spectra. The points for the dashed lines are taken from ref. 13 (0.5 mol dm^{-3} captopril itself), the points for the solid lines from the 0.15 mol dm^{-3} CH_3Hg^{II}–captopril (1:1) in 0.30 mol dm^{-3} $NaNO_3$ in D_2O

Nonetheless, captopril replaced thiomalate as a free ligand forming $[Au(HA)_2]^-$ complex at a 1:2 ratio of Au^I–captopril at the physiological pH*.

The pH dependence of the methyl resonance in the ^1H NMR spectrum of CH_3Hg^{II} is due to the titration of $[Hg(CH_3)(OH_2)]^+$ with OH^-.[25–27] When captopril was added to the CH_3Hg^{II} solution at a 1:1 ratio, the CH_3 resonance of CH_3Hg^{II} remained unshifted until pH* 9 and then titrated at higher pH*.[21] The coupling constant $^2J(^{199}Hg–^1H)$ also shows similar results which indicate that captopril forms a stable complex

Table 2 Equilibrium constants K_{eq} = [II]/[I] of the *trans* (**I**) and *cis* (**II**) isomers of captopril itself[a] and CH_3Hg^{II}–captopril at a 1:1 ratio[b]

pH	Species	Captopril	CH_3Hg^{II}–captopril
0.59	H_2A[c]	0.17	0.11
7.43	HA^-	0.69	0.64
12.30	A^{2-}	0.30	0.43

[a] Values are taken from ref. 13. [b] This work. [c] For the definition of H_2A, HA^- and A^{2-} see the text.

Table 3 Formation constants for methylmercury(II)–thiol complexes

Ligand	pK_{SH}	log K_f	Ref.
Mercaptoacetic acid	10.08	16.92	17
Mercaptoethanol	9.62	16.12	17
N-Acetylpenicillamine[a]	10.19	16.76	17
Mercaptosuccinic acid	10.26	17.31	17
Captopril *cis*	9.99	16.85	*b*
trans	9.71	16.57	*b*

[a] Penicillamine = 3-mercaptovaline. [b] This work; the log K_f values are estimated by using log K_f = pK + 6.86 as given in ref. 17.

which is in fast exchange. Beyond pH* 9 the OH^- competes with captopril as shown in equation (1).[16–21,25–27]

Reid and Rabenstein[17] measured the formation constants for various thiols with CH_3Hg^{II} and proposed a general equation log K_f = pK_{SH} + 6.86. Based on this, as noted in Table 3, we found the log K_f for the *cis* and *trans* isomers for captopril to be 16.85 and 16.57 respectively. The formation constants for other thiols are also compared. Fig. 5 shows the two different isomers bonded to CH_3Hg^{II}.

As noted in Table 1, the C^3 and C^2 resonances for the *cis* isomer were shifted by 3.610 and 1.824 ppm and for the *trans* isomer 3.168 and 1.828 ppm respectively at the physiological pH*. The other resonances were shifted little compared to those of C^3 and C^2. This indicates that CH_3Hg^{II} binds *via* the SH, consistent with other CH_3Hg^{II}–thiol systems.[16–21] It binds to the *cis* isomer strongly compared to the *trans* isomer as observed by the chemical shift differences.

The ^1H coupling constant $^2J(^{199}Hg–^1H)$ observed for CH_3Hg^{II}–captopril at neutral pH* is 184.0 Hz compared to the values for hydroselenoacetic acid and mercaptoacetic acid which are reported to be 167[31] and 172.0 Hz.[17] The log K_f for

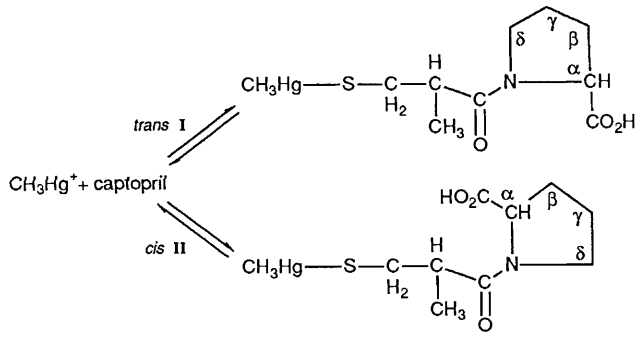


Fig. 5 The *cis* and *trans* isomers of captopril bonded to CH_3Hg^{II} *via* the SH binding site

these two ligands were reported to be 17.36[31] and 16.92,[17] respectively. This suggests that the smaller the coupling constants the stronger is the complex. In the present system, ^2J decreased from 260 to 185 Hz at a 1:1 ratio of CH_3Hg^{II}–captopril at the physiological pH* which strongly indicates that CH_3Hg^{II} forms a stronger complex with captopril. The methyl resonance of CH_3Hg^{II} is in fast exchange with both isomers and therefore ^2J was not resolved for these two isomers.

The pK values for the CO_2^- group did not change significantly in the presence of CH_3Hg^{II} for either isomer. The K_{eq} show very little difference at pH* 0.4, 7.20 and 12.85. This indicates that the conformations of the *cis* and *trans* isomers along the peptide bond remain the same at a 1:1 ratio of CH_3Hg^{II}–captopril compared to captopril itself.

The data presented here show that the *cis* isomer binds to CH_3Hg^{II} more strongly than does the *trans* isomer. This conclusion is based on the formation constants and chemical shift difference between the free and bound *cis* and *trans* isomers of captopril.

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