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Report

Ultraviolet (UV) Absorption and Circular Dichroism (CD) Spectra of Captopril

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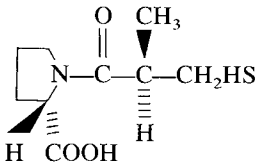
The ultraviolet and circular dichroism spectra of authentic captopril have been obtained, since the reported literature data are inconsistent with those obtained on highly purified material. The UV absorption spectrum consists of a single band maximum at 200 nm, while the CD spectrum consists of a single negative peak located at 210 nm. The CD spectrum of captopril and its other three diastereomers can be explained largely in terms of a summation of the chirality of its individual components, (*S*)-proline and (2*S*)-3-mercapto-2-methylpropionic acid.

KEY WORDS: captopril; ultraviolet absorption; circular dichroism; spectra; diastereomers; chirality.

INTRODUCTION

Captopril, 1-[(2*S*)-3-mercapto-2-methylpropionyl]-(*S*)-proline,



is a potent inhibitor of the angiotensin converting enzyme and, therefore, is used in the treatment of hypertension and congestive heart failure (1). The compound contains two centers of dissymmetry, one associated with the (*S*)-proline portion and the other associated with the 3-mercapto-2-methylpropionic acid side chain. The compound is normally administered as enantiomerically pure, with both centers of dissymmetry being completely resolved.

The physicochemical and analytical characteristics of captopril have been described by Kadin (2) and by Caplar *et al.* (3). Many of the details in these profiles are similar, but the ultraviolet absorption curves are substantially different. Kadin reports a UV spectrum having a single maximum around 200 nm and no other absorption bands at any wavelength. This observation stands in contrast to the profile of Caplar *et al.*, who report the UV absorption of captopril as consisting of two absorptions at 207 and 327 nm. In addition, these workers also reported circular dichroism (CD) band maxima at 225 and 242 nm.

As part of our program to update the analytical profile on captopril, we have investigated the UV spectroscopy of highly purified compound in detail. In the present report, the true UV and CD spectra of captopril are presented. Further investigation into the chirality of captopril was obtained through study of the CD spectra of (*S*)-proline and (2*R*)-3-mercapto-2-methylpropionic acid, each of which separately contains one of the dissymmetric centers of captopril. Finally, the additivity of the component chirality was evaluated through study of the other three captopril diastereomers.

MATERIALS AND METHODS

The highly purified house standard of captopril (lot 34053) was used for all work. This material has been certified by HPLC analysis to be 99.3% pure and free from disulfide contamination (4). All spectroscopic work was carried out in deionized water, at captopril concentrations of 14.3 μg/ml (0.0657 m*M*), 35.7 μg/ml (0.164 m*M*), 89.3 μg/ml (0.411 m*M*), and 446 μg/ml (2.05 m*M*). The pH dependence associated with the spectra was evaluated through characterization of a 13.6 μg/ml (0.0627 m*M*) solution adjusted to pH 4.0, 7.4, and 9.9.

Further spectroscopic characterization was obtained through the study of (*S*)-proline and (2*S*)-3-mercapto-2-methylpropionic acid. The other three diastereomers of captopril, 1-[(2*S*)-3-mercapto-2-methylpropionyl]-(*R*)-proline, 1-[(2*R*)-3-mercapto-2-methylpropionyl]-(*S*)-proline, and 1-[(2*R*)-3-mercapto-2-methylpropionyl]-(*R*)-proline, were also characterized. These compounds were all obtained as highly purified Squibb research standards.

UV absorption spectra were obtained on either a Perkin–Elmer Lambda 3B or a Hewlett–Packard 8450A spectrophotometer. Circular dichroism spectra were obtained on a Spex Jobin–Yvon Model CD6 dichograph.

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RESULTS AND DISCUSSION

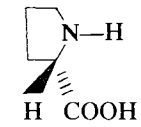
The authentic UV and CD spectra of captopril (14.3 μg/ml, or 0.0657 m*M*) are shown in Fig. 1. The UV absorption consists of a single band, exhibiting a maximum at 200 nm. It was found that the molar absorptivity of this band was 10,500 liter/mol · cm. The CD spectrum consisted of a strong negative band at 210 nm, with a very weak negative band evident around 230 nm. Values calculated for the circular dichroism (Δε) and molar ellipticity [Θ] are found in Table I.

CD spectra were obtained at higher concentrations to evaluate whether the bands reported by Caplar *et al.* (3) could be attributed to some concentration-dependent phenomenon. However, all data measured in spectral regions where the detector was not saturated due to total absorbance by the solution did not reveal the existence of any other CD or absorption bands. Increasing the solution pH from 4 to 10 yielded a slight red shift in the CD minima (209 to 212 nm), with essentially no change in the molar ellipticity values. It was therefore concluded that the bands reported by Caplar *et al.* (3) were not assignable to any pH-induced phenomenon.

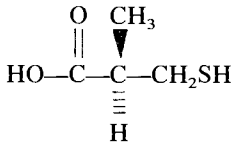
The spectroscopic data obtained on authentic captopril are not reconcilable with the values quoted by Caplar *et al.* (3). Their absorption maximum of 207 nm cannot be reproduced in highly purified samples, and no captopril-related absorption could be located at 327 nm. Caplar *et al.* detected CD bands at 225 and 242 nm in their studies, but the absence of these bands in authentic captopril samples indicates that these cannot be associated with the spectroscopy of captopril. Their spectral data cannot be reproduced either by large increases in captopril concentration or through alterations in solution pH.

Since their data do not correlate with results obtained on authentic captopril, it is concluded that the spectroscopic results reported by Caplar *et al.* must be due to an unknown impurity generated during their method of synthesis. Since these workers did not recrystallize their material, the existence of significant impurity levels is quite likely.

Further characterization of the CD spectrum of captopril was obtained through the separate study of its two components, (*S*)-proline,



and (2*S*)-3-mercapto-2-methylpropionic acid,



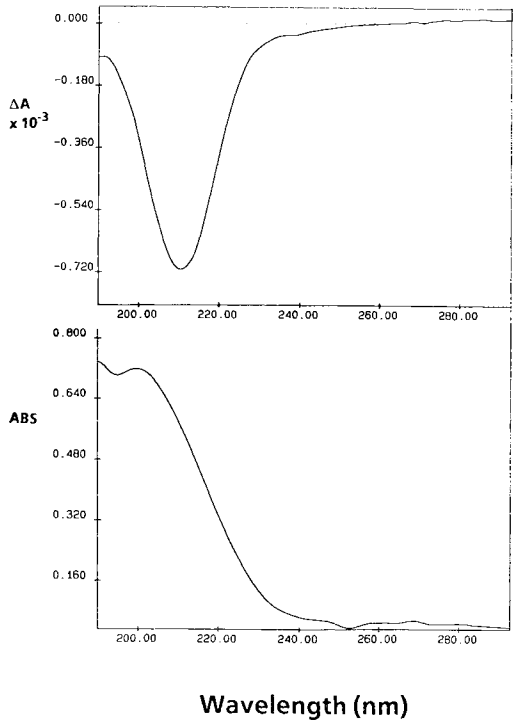


Fig. 1. Ultraviolet absorption (lower trace) and circular dichroism (upper trace) spectra obtained on a 14.3 μg/ml (0.0657 m*M*) solution of captopril, 1-[(2*S*)-3-mercapto-2-methylpropionyl]-(*S*)-proline.

Table I. Chiroptical Properties of Captopril and Related Compounds

Wavelength maximum (nm)	Circular dichroism (liter/mol · cm)	Molar ellipticity (degree · cm²/dmol)
(a) 1-[(2*S*)-3-Mercapto-2-methylpropionyl]-(*S*)-proline, captoril		
210	−9.94	−32,700
(b) (*S*)-Proline		
193	−0.927	−3,050
213	+0.334	+1,100
(c) (2*S*)-3-mercapto-2-methylpropionic acid		
222	−0.638	−2,100
(d) 1-[(2*R*)-3-Mercapto-2-methylpropionyl]-(*R*)-proline		
210	+9.91	+32,700
(e) 1-[(2*S*)-3-Mercapto-2-methylpropionyl]-(*R*)-proline		
195	+4.71	+15,500
218	−7.90	−26,000
(f) 1-[(2*R*)-3-Mercapto-2-methylpropionyl]-(*S*)-proline		
195	+4.77	+15,700
218	−8.02	−26,400

CD and absorption spectra for these two compounds are shown in Figs. 2 and 3, respectively, and the corresponding values for circular dichroism and molar ellipticity are shown

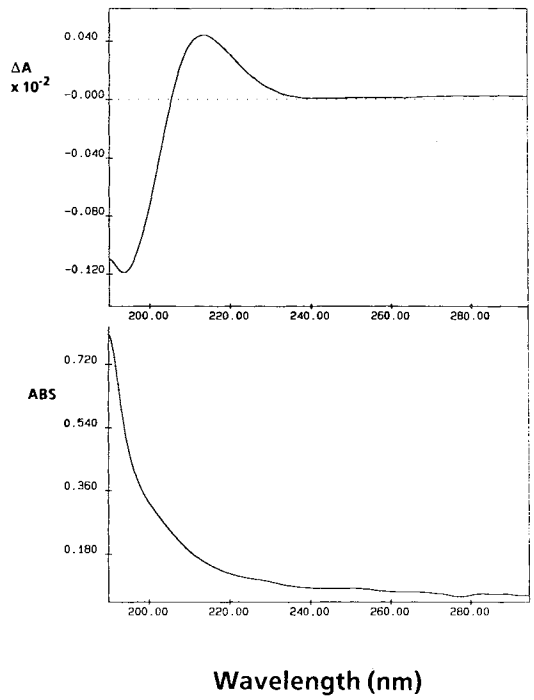


Fig. 2. Ultraviolet absorption (lower trace) and circular dichroism (upper trace) spectra obtained on a 150 μg/ml (1.30 m*M*) solution of (*S*)-proline.

in Table I. The low absorptivity of the two compounds required that the spectra be obtained at significantly higher concentrations than for captopril.

Comparison of all the chiroptical data indicates that the spectroscopic properties of the individual components are significantly altered when they combine to form captopril. The 193-nm negative CD band of (*S*)-proline appears to undergo a red shift to 210 nm in captopril, with the molar ellipticity being greatly increased in the process (going from −3050 to −32,700 degree · cm^2/dmol). At the same time, the positive 210-mm CD band of proline is essentially canceled by the negative CD band of the (2*S*)-3-mercapto-2-methylpropionic acid side chain. The weak negative CD observed for captopril at 230 nm undoubtably represents the residual CD of the chiral side chain which was not canceled by the proline.

The CD spectrum of the enantiomer of captopril, 1-[(2*R*)-3-mercapto-2-methylpropionyl]-(*R*)-proline is shown in Fig. 4, with the associated spectroscopic data given in Table I. As would be anticipated for a true enantiomer, the CD spectra of Figs. 1 and 4 are found to be true mirror images. Comparison of the data in Table I reveals that values calculated for circular dichroism and molar ellipticity are equal but opposite in sign.

CD and absorption spectra of the two mixed diastereomers of captopril, 1-[(2*S*)-3-mercapto-2-methylpropionyl]-(*R*)-proline and 1-[(2*R*)-3-mercapto-2-methylpropionyl]-(*S*)-proline, are found in Figs. 5 and 6, respectively. Values computed for circular dichroism and molar ellipticity are given in Table I. While the CD spectra in Figs. 5 and 6 are mirror images of each other (as would be expected for an enantiomeric pair), they are distinctly different from captopril itself. The low-wavelength proline CD band is evident as a CD peak at 195 nm, although greatly intensified. In the mixed diastereomers, the second proline band now has the same sign as that of the chiral side chain.

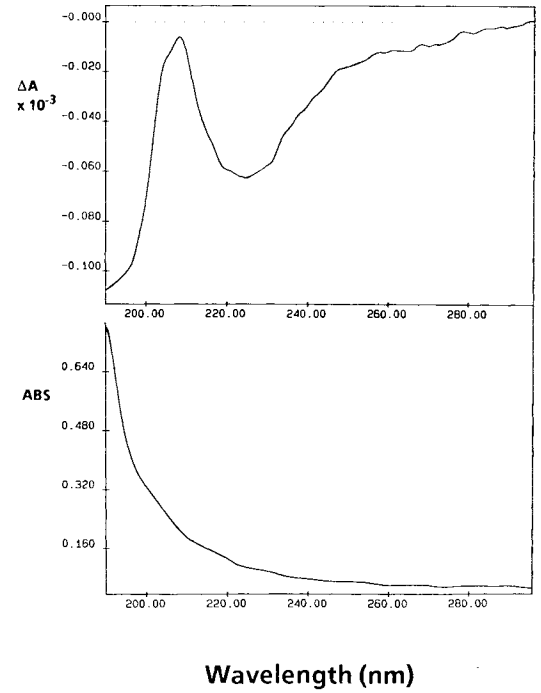


Fig. 3. Ultraviolet absorption (lower trace) and circular dichroism (upper trace) spectra obtained on a 11.4 μg/ml (0.0951 m*M*) solution of (2*S*)-3-mercapto-2-methylpropionic acid.

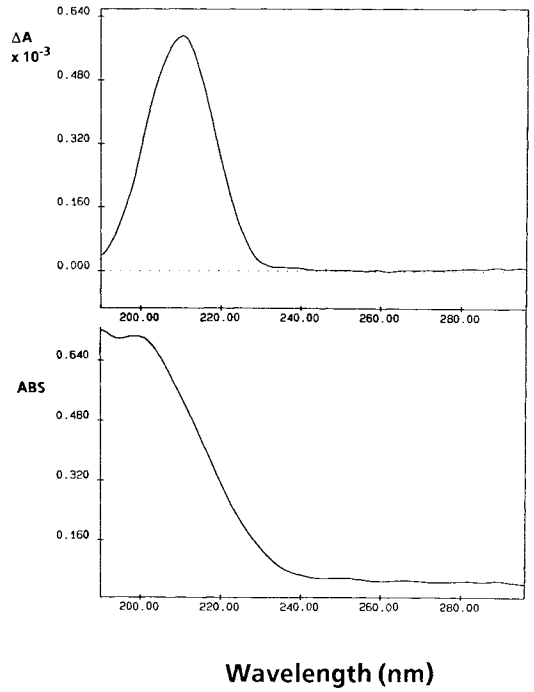


Fig. 4. Ultraviolet absorption (lower trace) and circular dichroism (upper trace) spectra obtained on a 13.4 μg/ml (0.0617 m*M*) solution of 1-[(2*R*)-3-mercapto-2-methylpropionyl]-(*R*)-proline.

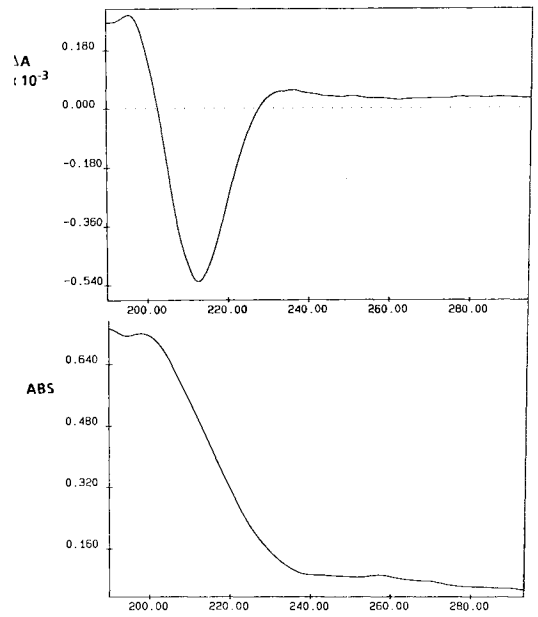


Fig. 5. Ultraviolet absorption (lower trace) and circular dichroism (upper trace) spectra obtained on a 13.4 μg/ml (0.0617 m*M*) solution of 1-[(2*S*)-3-mercapto-2-methylpropionyl]-(*R*)-proline.

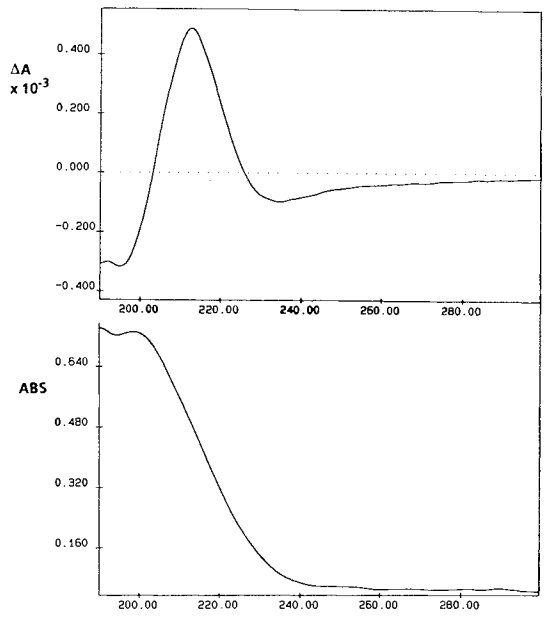


Fig. 6. Ultraviolet absorption (lower trace) and circular dichroism (upper trace) spectra obtained on a 14.1 μg/ml (0.0649 m*M*) solution of 1-[(2*R*)-3-mercapto-2-methylpropionyl]-(*S*)-proline.

As a result, a strong CD peak is now observed at 218 nm (also intensified with respect to the component bands).

SUMMARY

The ultraviolet and circular dichroism spectra of highly purified, authentic captopril, its component fragments, and other three diastereomers have been obtained. The UV absorption spectrum of captopril consists of a single band maximum at 200 nm, while the CD spectrum consists of a single negative peak located at 210 nm. The CD spectrum of captopril and its other three diastereomers can be explained largely in terms of summation of the chirality of its individual components, (*S*)-proline and (2*S*)-3-mercapto-2-methylpropionic acid, although the individual chiralities are increased in the diastereomer compounds.

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