RESULTS AND DISCUSSION

The typical response elicited by dopamine (4 mg) on the isolated *M. mercenaria* heart bathed in 40 mL of artificial sea water is shown in Fig. 1. The heart stopped contracting at once when dopamine was added to the bath, but recovered its contractility within 6 min. When I (8 mg) was added to the bath, 2.5 min before dopamine challenge, it not only prevented cardiac arrest due to dopamine, but also produced a negative inotropic response that was mimicked by the subsequent addition of dopamine (Fig. 2). By contrast, preadministration of the vehicle control for I, in a volume comparable to that of the test drug (0.8 mL), failed to prevent cardiac arrest caused by dopamine; however, the recovery time for the return of contractility was shortened over that induced by dopamine administration alone (Fig. 3).

The average percent of cardiac inhibition attributed to the test drugs and to dopamine challenge is presented in Table I. The number of animals used in each group ranged from 6 to 15. The percent inhibition was calculated by measuring the amplitude of the peak at the moment of test drug challenge and dopamine challenge, and the lowest amplitude within 1 min after administering each agent; *i.e.*, the response recorded in Fig. 1 after the addition to the bath of dopamine alone represents 100% inhibition.

Although each agent, except the solvent control, caused some degree of cardiac inhibition within 1 min of its administration (Table I), propranolol produced the most dramatic and consistent inhibitory responses. Immediately after the addition of the β-blocker to the bath, the heart lost contractility and became spastic, an excitatory response that was identical with that observed after the administration of dopamine alone (Fig. 1). Two and one-half minutes later, the addition of dopamine produced an even greater response.

Initial administrations of phentolamine, tolazoline, and hydralazine yielded consistently positive inotropic responses during the 2.5-min period prior to the addition of dopamine, whereas I caused variable effects (*i.e.*, positive, negative, or no alteration of the inotropic response) prior to the addition of dopamine. Phentolamine mesylate, an α-adrenergic blocker, and I, an experimental hypotensive agent with presumed α-adrenergic blocking activity, appear to act on an adrenergic receptor of an α-configuration within the *M. mercenaria* heart to block the stimulatory effects of dopamine. Of the two compounds, I is the more potent in this respect.

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Identification and Determination of the *S*-Methyl Metabolite of Captopril in Human Plasma by Selected-Ion Monitoring Gas Chromatography–Mass Spectrometry

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Abstract □ The *S*-methyl metabolite of captopril was identified and determined in human plasma by positive chemical ionization selected-ion monitoring gas chromatography–mass spectrometry. After oral administration of 100 mg of captopril to healthy subjects, the maximum plasma level was 60–114 ng/mL. These data for the *S*-methyl metabolite of captopril were correlated to total and unchanged captopril levels.

Keyphrases □ Captopril—identification and determination of the *S*-methyl metabolite in human plasma, gas chromatography-selected-ion monitoring mass spectrometry □ Gas chromatography-selected-ion monitoring mass sepectrometry—determination of the *S*-methyl metabolite of captopril in human plasma after oral administration

Captopril (1-[(2*S*)-3-mercapto-2-methylpropronyl]-L-proline; I) (1, 2) has received considerable attention as an orally active angiotensin-converting inhibitor (3) that is effective in lowering arterial blood pressure (4). The absolute bioavailability and pharmacokinetics of [^{14}C]captopril given orally and intravenously have been reported previously (5). Analysis of the kinetic data suggested that captopril is extensively partitioned into tissue. The interconversion of protein-bound captopril with non-protein-bound mixed disulfides has been suggested as a biotransformation pathway that may extend the pharmacological effects of captopril (5, 6).

Gas chromatography–mass spectrometry (GC–MS) selected-ion monitoring (SIM) methods have been reported for the determination of unchanged captopril in whole blood (7) and total captopril in plasma (8). Total captopril includes unchanged drug, the disulfide dimer of the parent drug, and mixed disulfides with endogenous thiol-containing compounds, *e.g.*, glutathione and cysteine. Recently, the *S*-methyl metabolite of captopril (II) has been identified as a urinary metabolite in humans (9) and rats (6). A GC–MS SIM method was developed to measure the plasma level of the *S*-methyl metabolite (II) after oral administration of captopril. The plasma levels of the metabolite II from samples collected from healthy human subjects after the administration of a single 100-mg tablet of captopril were compared with the free captopril blood levels and total captopril plasma levels determined as IV by GC–MS SIM methods previously reported (7, 8).

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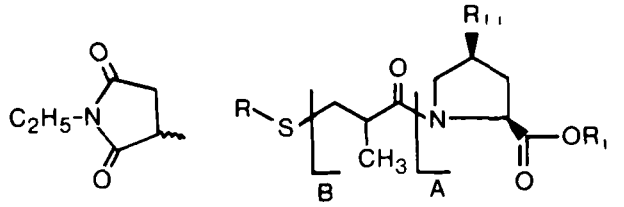

$C_6H_8NO_2$

	R	R_1	R_{11}	MW	MH_+ PCI	A	B
I	H	H	H	217			
II	CH_3	H	H	231			
III	CH_3	H	F	249			
IV	$C_6H_8NO_2$	H	H	342			
V	CH_3	CH_3	H	245	246	128	198
VI	CH_3	CH_3	F	263	264	146	216

EXPERIMENTAL SECTION

Apparatus—A mass spectrometer[1] with a data acquisition system and a GC and interface (7) as well as other equipment and apparatus have been described previously (7, 8).

Reagents—The *S*-methyl metabolite II and its 4-fluoro analogue (III) were pharmaceutical grade materials[2] and were used without additional purification. Redistilled petroleum ether (bp 30–60°C) was first washed with small portions of concentrated sulfuric acid until the discarded sulfuric acid was colorless and then with distilled water prior to distillation. Ammonium hydroxide (3%) was prepared by diluting ammonium hydroxide with distilled water. All other reagents have been previously described; this includes the columns, ethyl acetate, phosphoric acid, methanolic HCl kits, and hydrochloric acid (10).

Extraction of Plasma Samples—The internal reference standard (II) (500 ng) was added to 5 mL of plasma[3] and acidified with 2 mL of 6 M phosphoric acid. The sample was transferred to a 1-g preactivated XAD-2 column (8) by decantation and subsequent rinsing with 0.1 M hydrochloric acid. The washed, vacuum-dried column was eluted with 25 mL of purified ethyl acetate at a rate of 3 mL/min. To the ethyl acetate eluate, collected in a screw-capped tube, (250 × 150 mm), 10 mL of purified petroleum ether and 2 mL of 3% ammonium hydroxide solution were added. The drugs were extracted by shaking into the alkaline aqueous solution. After separation of the layers, the organic phase was removed by aspiration, and 1 mL of 6 M phosphoric acid and 3 g of sodium chloride were added. The solution was washed twice by shaking with 5 mL of ethyl acetate for 5 min and by centrifuging for 5 min. The retained ethyl acetate portions were combined and evaporated to dryness. Compounds II and III were determined as their respective methyl esters, V and VI, by treating the residue with 0.1 mL of 0.8 M methanolic hydrochloric acid solution in a sealed vial at 60°C for 10 min. The solution was evaporated, and the residue was dried in a vacuum desiccator for 1 h. The sample was reconstituted with 20 μL of acetone (10).

Preparation of Standard Curves—Various amounts (0–200 ng of II/mL and 500 ng of III/mL) were added to 5 mL of plasma. A freshly prepared control sample, containing 200 ng of II and 100 ng of III/mL of plasma, was extracted along with each set of samples. These samples were processed by the general scheme described above.

Instrumental Procedure—The combined GC–MS with data system was operated under the SIM mode. In the electron-impact (EI) mode, the unit was tuned from 69 to 502 Da with 1,1,2,2,3,3,4,4,4-nonafluoro-*N*,*N*-bis(nonafluorobutyl)-1-butanamine at an ionization voltage of 70 eV and an emission of 300 μA. The ion source was controlled at a constant temperature of 200°C. Helium was used as the carrier gas at a flow rate of 30 mL/min. For the chromatography in the EI mode, the column was connected through a single-stage jet separator to remove helium from the effluent. Helium, otherwise diverted, was allowed to flow into the ion source from 0.5 min to the end of the acquisition. In positive chemical ionization GC–MS, methane was used as the GC carrier gas and reagent at a flow rate of 15 mL/min. Under these conditions, the source pressure was ~80 Pa. The respective emission and electron energy were 250 μA and 240 V, optimized for the abundance of the *m/z* 219, 414, and 614 ions of 1,1,2,2,3,3,4,4,4-nonafluoro-*N*,*N*-bis(nonafluorobutyl)-1-butanamine.

In both detection modes, the multiplier and electron voltages were only applied during the data collection period. The separator and transfer lines were kept at 275°C, the ion source was maintained at 250°C, and the injector was kept at 290°C. The silanized glass column (80 cm × 2 mm), packed with 3% OV-101[4], was kept at an isothermal temperature of 190°C for 2.5 min until the analytical components were eluted (~1.5 min) and then temperature programmed to 240°C at a rate of 30°C/min to drive off other components.

About 1–2 μL of solution was evaporated on a GC solids injector. Depending on the mode of ionization, the appropriate ion profiles were obtained by SIM. The procedure for measuring the maximum peak heights and processing the data have been reported previously (7).

[1] HP 5985B; Hewlett Packard, Palo Alto, Calif.
[2] E. R. Squibb and Sons, Princeton, N.J.
[3] Serum may be substituted.

Table I—Recovery of the *S*-Methyl Metabolite of Captopril (II) [a]

Storage Temperature, °C	Recovery, ng/mL Initial	1 Week	4 Weeks
5	1630	1580	1590
5	1600	1560	1600
−20	—	1560	1590
−20	—	1610	1590

[a] Plasma (250 mL) was spiked with [^{14}C]II, subdivided, and stored as 5-mL samples; measured in duplicate at each time-temperature condition.

Table II—Recovery of *S*-Methyl Metabolite of Captopril (II)

Set No.[a]	n[b]	Mean (σ), ng/mL	Δ (Taken − Found)
1	4	98.5 (2.8)	
2	3	99.9 (1.5)	
3	4	100.1 (0.9)	
4	3	98.8 (3.2)	
5	5	99.0 (2.0)	
6	3	99.9 (3.1)	
7	4	99.9 (0.1)	
Mean		99.4 (2.25)	0.6
8	4	12.9 (0.7)	−0.4

[a] Sets 1–7 were spiked with 100 ng/mL of plasma. Set 8 was spiked with 12.5 ng/mL of plasma. [b] Replicate measurements of the same sample.

RESULTS AND DISCUSSION

Although the extraction procedure for the metabolite II was patterned after the method developed for the extraction of IV (7), there were several significant differences. The isolation was accomplished by direct adsorption of II from acidified plasma samples onto XAD-2. The back-extraction was performed with 3% ammonium hydroxide. The solubility of II in ethyl acetate was suppressed by the addition of petroleum ether, which resulted in better phase separation. The compounds were then quantitatively extracted into ethyl acetate from aqueous solutions saturated with sodium chloride to remove additional potentially interfering components. Because significantly lower *S*-methyl metabolite II levels were expected, 5 mL of plasma was processed, with a total of 500 ng of III added as the internal reference standard. It was not practical to isolate and measure total captopril and II concurrently because of the great disparity in their levels. It was decided to process individual samples for the metabolite II in plasma, unchanged captopril in whole blood treated with *N*-ethylmaleimide at the time of sampling, and total captopril in plasma (7, 8). The *S*-methyl metabolite II may be stored for at least 4 weeks at either 5°C or −20°C in plasma without decomposition (Table I).

Methylation of the proline carboxylic acid group was chosen because it forms a low-molecular-weight derivative suitable for GC–MS. Both EI and chemical ionization (CI) MS were explored as potential methods, but the greatest sensitivity and specificity was obtained by CI with the MH^+ of esters V and VI. Under these conditions, a linear calibration curve was obtained with a slope of 1.5 and an intercept of 0.001, as determined by linear regression analysis. A correlation coefficient of 0.9981 was obtained from the calibration data. With each set of extracts, a freshly extracted control was measured as a validation of the method. The 26 replicate measurements of the 100-ng/mL extracted control samples showed a mean *SD* of 2.25 ng/mL and an absolute difference of 0.6 ng/mL from the added amount (Table II). A difference of 0.4 ng/mL was found for the four 12.5-ng/mL spiked samples. The *m/z* 128–*m/z* 146 ions, although producing intense EI ions, also had significant interferences from the coeluting background components.

The levels of the *S*-methyl metabolite II were low (Table III) relative to both unchanged and total captopril levels for the same subjects (Table IV). For the comparison, the unchanged captopril blood values were multiplied by two to approximate the plasma levels. The parameter *D* is defined as the

[4] On 80/100 Supelcoport; Bellefonte, Pa.

Table III—*S*-Methyl Metabolite of Captopril Plasma Levels After Oral Administration of Captopril [a]

Time, h	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	Mean (σ)[b]
0	0.1	0.2	0.2	0.1	0	0.5	0	0.2 (0.2)
0.5	102	7.3	13	36.7	9	43.3	29.7	34.4 (30.5)
1	114	49.4	42.4	73.0	48.6	94.6	84.9	72.4 (25.0)
2	80.2	65.0	62.5	42.1	60.7	78.2	71.7	65.8 (11.9)
3	39.8	46.6	45.2	27.3	35.0	47.5	47.9	41.3 (7.2)
4	27.3	39.4	32.1	21.8	21.2	40.5	37.8	31.4 (7.6)
6	16.2	21.0	15.9	9.9	12.0	17.1	25.3	16.8 (4.8)
8	9.3	14.4	10.0	8.2	9.5	14.2	13.3	11.3 (2.4)
12	5.4	7.4	6.2	3.6	3.8	4.9	8.1	5.6 (1.6)
24	1.4	1.4	1.0	0.4	0.7	1.2	0.7	1.0 (0.4)

[a] Single 100-mg dose. Values are in nanograms per milliliter. [b] *SD*, seven subjects.

Table IV—Mean Values (ng/mL) of Captopril and Metabolites in Plasma Equivalents [a]

Time, h	Total T	2 × Unchanged[b] ($2F$)	Disulfide Metabolites[c] (D)	*S*-Methyl Metabolites[d] (S)	R[e]
0	—	4	—	0.2	—
0.5	1750	1346	404	34.4	0.020
1	3140	1672	1468	72.4	0.023
2	2400	596	1804	65.8	0.027
3	1570	200	1370	41.3	0.026
4	1150	42.7	1107	31.4	0.027
6	680	29.6	650	16.8	0.025
8	460	13.0	447	11.3	0.025
12	270	—	270	5.6	0.021
24	80	—	80	1.0	0.0125[f]

[a] Seven subjects. [b] Assumes plasma is one-half the volume of whole blood. [c] Difference $D = T - 2F$ = disulfide metabolites derived from captopril. [d] Taken from the mean values shown in Table III. [e] $R = S/T$. The mean from samples taken at 0.5–12 h was 0.024; (σ/mean) × 100 = 11. [f] Excluded from determination of the means.

difference between the total captopril level and twice the free captopril level as an expression of the disulfide levels. Uniformly, the individual values followed exactly the mean values (Table IV). Whereas the unchanged captopril levels predominate during the first hour, the disulfides derived from captopril were the significant components after that. Levels of the metabolite II followed most closely the total drug concentrations. The mean ratio between the metabolite II and total captopril, R, was 0.024 for samples taken at 0.5–12 h (Table IV). No zero values of metabolite II were observed in any of the samples taken during the first 12 h, and more than half of the samples collected over 24 h had values of ≥1 ng/mL.

In recent reports, various discussions have been concerned with the concept of interconversion of protein-bound drug to "free" captopril (5) and of a subsequent conversion of captopril or its disulfides to the *S*-methyl metabolite by *S*-methyltransferase (6). The role of *S*-methylation, other than as a metabolic pathway, is not well understood. The discussion following a recent presentation highlights this point (9). This analytical method, combined with the previously reported methods for determining unchanged (7) and total (8) captopril, allows profiling to study the role of *S*-methylation in renal function. Future steady-state studies, combined with pulsed deuterated analogue administration, might help in establishing the role of protein-bound captopril in deep compartments *versus* circulating protein-bound blood components.

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