Synthesis of 1-((*S*)-3-Acetylthio-2-methylpropanoyl)-L-propyl-L-phenylalanine (Alacepril) and One of Its Active Metabolites, the Desacetyl Derivative (DU-1227)

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The synthesis of 1-((*S*)-3-acetylthio-2-methylpropanoyl)-L-prolyl-L-phenylalanine 1 (alacepril, code name: DU-1219), a clinically used antihypertensive agent, and one of its active metabolites, 1-((*S*)-3-mercapto-2-methylpropanoyl)-L-prolyl-L-phenylalanine 2 (code name: DU-1227), is described. The application of chloral to the *N*-formylation of L-proline is also described. The presence of an intramolecular hydrogen bond in the *trans* conformer of 1 in solution is suggested on the basis of spectroscopic examinations of 1 and its ethyl ester 4.

Keywords alacepril; DU-1219; DU-1227; captopril; antihypertensive agent; active metabolite; 1-formyl-L-proline; chloral; conformation; hydrogen bond

1-((*S*)-3-Acetylthio-2-methylpropanoyl)-L-prolyl-L-phenylalanine **1** (alacepril) is a clinically used antihypertensive agent, and belongs to the category of angiotensin-converting enzyme (ACE) inhibitors. We recently revealed[1] that **1** has characteristic pharmacological properties: when given orally, it exhibited sympatho-inhibitory and *in vivo* ACE-inhibitory actions, which proved to be ascribable to two active metabolites, 1-((*S*)-3-mercapto-2-methylpropanoyl)-L-prolyl-L-phenylalanine **2** (DU-1227) and its further metabolite captopril **3**,[2] respectively.

In the present paper, we describe the synthesis of **1** and its characteristic active metabolite **2**. The structural features of **1** in solution are also described on the basis of infrared (IR) and nuclear magnetic resonance (NMR) examinations of **1** and its ethyl ester **4**.

A practical synthetic route to **1** and **2** is shown in Chart 1. *N*-Formylation of proline in peptide chemistry has been carried out by the formic acid/acetic anhydride procedure,[3] whereas we employed chloral as a formylating reagent. The reaction of L-proline with chloral in the presence of sodium methoxide provided 1-formyl-L-proline **5** in an excellent yield without racemization. This procedure has the advantage that the chloroform formed during the reaction can be more easily removed than the troublesome formic acid and acetic anhydride used in the conventional procedure, and this method can therefore be applied to the large-scale synthesis of **5**. This procedure was also applicable to the formylation of L-phenylalanine and L-methionine.[4] Condensation of **5** with L-phenylalanine methyl ester employing *N*,*N*′-dicyclohexylcarbodiimide (DCC) gave the diprotected dipeptide, 1-CHO–L-Pro–L-Phe–OMe **6**, as an oil. Removal of the two protecting groups was then achieved simultaneously by treatment of **6** with dilute sulfuric acid at 80—85 °C for 2 h to afford the dipeptide, L-Pro–L-Phe **7**, in a good yield. Another key chiral precursor, (*S*)-3-acetylthio-2-methylpropanoic acid, was effectively obtained by means of the same optical resolution as we reported previously.[5] Thus, compound **1** was prepared by acylation of **7** with (*R*)-3-acetylthio-2-methylpropanoyl chloride derived from the corresponding (*S*)-acid. Treatment of **1** with ammonium hydroxide led to **2**, the purification of which was achieved by reverse-phase chromatography.

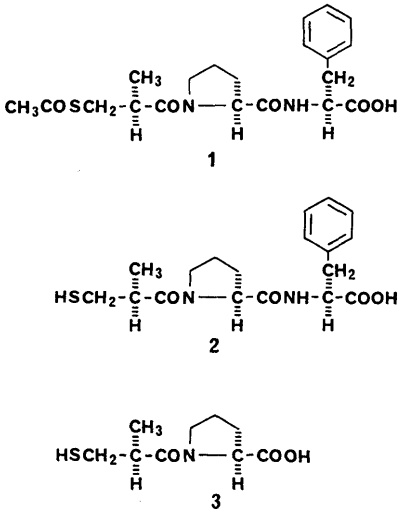
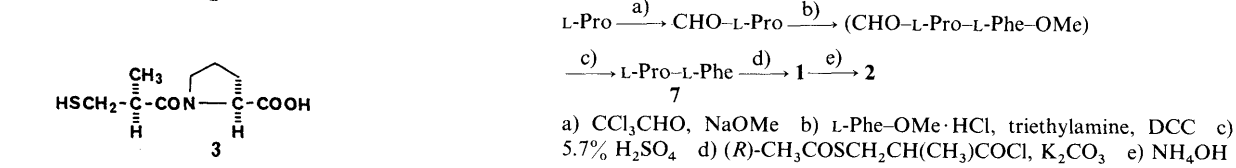


Fig. 1

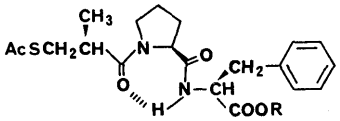
Acyl prolines[6] and peptides[7] containing proline are well known to have both stable *trans* and *cis* conformers about the amide bond of an X-Pro moiety. The ^{13}C-NMR spectrum of **1** in deuterium chloroform ($CDCl_3$) revealed the presence of the *trans* and *cis* conformers, which were assigned on the basis of paired chemical shifts (δ (*trans*): 24.75, δ (*cis*): 21.92) of the γ-carbon of the proline ring, according to the empirical rule reported by Dorman and Bovay.[6] The ratio of *trans* to *cis* was estimated to be approximately 9 : 1 on the basis of the relative intensities of the paired peaks of the γ-carbon. The ratio in deuterium dimethyl sulfoxide (DMSO-d_6), however, was changed to approximately 2 : 1. On the other hand, *N*-acetyl-L-proline *N*-methylamide[8] and *tert*-butoxycarbonyl-L-prolyl-D-alanine[9] in nonpolar solvents have been reported to exist mainly in the *trans* conformation with an intramolecular seven-atom (C_7) hydrogen bond. We have therefore paid particular attention to the assessment of the *trans* conformer of **1**.

We examined the amide NH absorption band of the IR



a) CCl_3CHO, NaOMe b) L-Phe–OMe·HCl, triethylamine, DCC c) 5.7% H_2SO_4 d) (*R*)-$CH_3COSCH_2CH(CH_3)COCl$, K_2CO_3 e) NH_4OH

Chart 1



1: R=H
4: R=Et

Fig. 2. C_7 Hydrogen-Bonded Structures of the *trans* Conformers of **1** and **4**

spectra of the ethyl ester **4** as a model compound of **1**, since the amide NH absorption band of **1** was masked by the carboxylic acid absorption band. The IR spectrum of **4** in $CHCl_3$ showed a free NH absorption at 3420 cm^{-1} and a broader associated NH absorption at 3290 cm^{-1}. No significant changes in the relative intensities of the two NH absorptions were observed over the concentration range of 2×10^{-2} to 1×10^{-3} M. This suggests that the broader NH absorption is due to the intramolecularly hydrogen-bonded NH. In addition, ^{1}H-NMR examinations of **4** revealed that the values of the temperature coefficients, $\Delta\delta/\Delta T$, for NH chemical shifts of the *trans* and *cis* conformers in dilute $CDCl_3$ solution (2×10^{-2} M) were 1.6×10^{-3} and 3.4×10^{-3} ppm·deg^{-1}, respectively. The smaller value for the NH proton of the *trans* conformer is close to that reported for an intramolecularly hydrogen-bonded NH of other peptides.[10] We have also measured the carbonyl absorptions of **4** in $CHCl_3$: three carbonyl absorptions were observed at 1735 (ester), 1675 (thioester and secondary amide) and 1630 (tertiary amide) cm^{-1}. The lower shifted frequency and broadening of the tertiary amide carbonyl absorption indicate that the carbonyl group is hydrogen-bonded. In addition, the NH frequency (3290 cm^{-1}) corresponds to that reported for a C_7 hydrogen bond of other peptides.[10] These findings suggest that, of the two possible C_7 and C_5 hydrogen-bonds, the C_7 hydrogen bond is in fact formed in the *trans* conformer of **4** (Fig. 2).

In **1**, a broad tertiary amide carbonyl absorption was observed at 1620 cm^{-1} in dilute $CHCl_3$ solution (1×10^{-3} M), and the $\Delta\delta/\Delta T$ values for the NH chemical shifts (in $CDCl_3$) of the *trans* and *cis* conformers were 1.3×10^{-3} and 5.0×10^{-3} ppm·deg^{-1}, respectively. This close similarity of **1** with **4** implies that the *trans* conformer of **1** in $CHCl_3$ forms the C_7 hydrogen bond analogously to that of **4**. On the other hand, **1** and **4** in DMSO-d_6, a strong proton-acceptor, appeared to form no intramolecular hydrogen bond, because no significant differences between the $\Delta\delta/\Delta T$ values ($5.4-6.3\times10^{-3}$ ppm·deg^{-1}) of the NH protons for the *trans* and *cis* conformers were observed.

Experimental

Melting points were determined on a Yanaco micro melting point apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian XL-300 with tetramethylsilane as an internal standard, respectively, and chemical shifts are presented only for the *trans* conformer as the major component. IR spectra of solutions were recorded on a Jasco A-102 spectrophotometer with a KRS-5 cell (1.0 mm). Rotations were observed with a Jasco DIP-4 digital polarimeter. Solutions were dried over anhydrous sodium sulfate.

1-Formyl-L-proline (5) A 24% solution (22.7 g, 0.1 mol) of sodium methoxide in methanol was added to a mixture of L-proline (11.5 g, 0.1 mol) in 1,2-dichloroethane (60 ml). Chloral (16.2 g, 0.11 mol) was added to the resultant solution over a period of 30 min with stirring at room temperature. The solution was stirred for an additional hour and then evaporated. The residue was dissolved in H_2O (50 ml). The solution was neutralized with dilute sulfuric acid and concentrated to dryness *in vacuo*. Isopropyl alcohol (50 ml) was added to the residue, and the insoluble material was removed by filtration. Diethyl ether (500 ml) was added to the filtrate and the mixture was allowed to stand in a refrigerator overnight to afford 13.6 g (95.1%) of **5**; mp 93—94 °C; $[\alpha]_D^{20}$ −123° (c=1.0, EtOH) (lit.[11] mp 88—91 °C, $[\alpha]_D^{20}$ −125° (c=1, EtOH)). *Anal.* Calcd for $C_6H_9NO_3$: C, 50.34; H, 6.34; N, 9.79. Found: C, 50.16; H, 6.35; N, 9.88.

L-Prolyl-L-phenylalanine (7) A solution of DCC (28.8 g, 139 mmol) in dichloromethane (CH_2Cl_2, 50 ml) was added to a stirred mixture of **5** (20 g, 139 mmol), L-phenylalanine methyl ester hydrochloride (30 g, 139 mmol), and triethylamine (14.1 g, 139 mmol) in CH_2Cl_2 (300 ml). The mixture was stirred overnight at room temperature. The precipitate was removed by filtration, and the filtrate was evaporated. The residue was dissolved in ethyl acetate and the solution was allowed to stand in a refrigerator for 2 h. The precipitate was removed by filtration, and the filtrate was washed successively with aqueous sodium bicarbonate, water, and 10% citric acid. The organic layer was dried and evaporated to afford 40.6 g of the protected dipeptide **6** as a viscous oil. A solution of sulfuric acid (13.6 g, 139 mmol) in H_2O (225 ml) was added to the oil, and the mixture was heated at 80—85 °C for 2 h with stirring. After being cooled, the resultant solution was adjusted to pH 5.5 and then allowed to stand overnight in a refrigerator to afford 31.3 g (86%) of **7**. Recrystallization from dilute ethanol yielded a sample for microanalysis; mp 255—258 °C; $[\alpha]_D^{26}$ −42.2° (c=1.0, 6 N HCl) (lit.[12] mp 264 °C, $[\alpha]_D^{25}$ −41.7° (c=1.0, 6 N HCl)). *Anal.* Calcd for $C_{14}H_{18}N_2O_3$: C, 64.11; H, 6.92; N, 10.68. Found: C, 64.28; H, 7.08; N, 10.75.

1-((*S*)-3-Acetylthio-2-methylpropanoyl)-L-prolyl-L-phenylalanine (1) A solution of (*R*)-3-acetylthio-2-methylpropanoyl chloride (6.9 g, 38.2 mmol) in tetrahydrofuran (THF, 10 ml) was added to a vigorously stirred solution of **7** (10 g, 38.2 mmol) and potassium carbonate (7.9 g, 57 mmol) in THF (10 ml)/H_2O (40 ml) under cooling. The mixture was stirred for 2 h at room temperature and then washed with ethyl acetate. The aqueous layer was acidified with dilute HCl and then extracted with CH_2Cl_2. The organic layer was dried and evaporated. The residue was recrystallized from acetonitrile to give 10.1 g (65%) of **1**; mp 155—157 °C, $[\alpha]_D^{26}$ −81.6° (c=1.0, EtOH). IR (KBr): 3250 (NH), 1745 (COOH), 1688 (COS), 1650 (CONH), 1625 (CON) cm^{-1}. IR (1×10^{-3} M solution in $CHCl_3$): 1750 (COOH), 1680 (COS and CONH), 1620 (CON) cm^{-1}. ^{1}H-NMR (in $CDCl_3$) δ: 1.07 (3H, d, J=6.8 Hz, CH_3), 1.66—2.36 (4H, m, Pro-β, γ), 2.32 (3H, s, CH_3CO), 2.75 (1H, m, CH), 2.93 (1H, dd, J=13.5, 6.4 Hz, 1H of CH_2), 3.03 (1H, dd, J=14.2, 7.2 Hz, 1H of Phe-β), 3.07 (1H, dd, J=13.5, 7.6 Hz, 1H of CH_2), 3.23 (1H, dd, J=14.2, 5.6 Hz, 1H of Phe-β), 3.35—3.54 (2H, m, Pro-δ), 4.61 (1H, m, Pro-α), 4.77 (1H, ddd, J=7.7, 7.5, 7.2 Hz, Phe-α), 7.10—7.31 (5H, m, arom.), 7.26 (1H, d, J=7.5 Hz, NH), 7.52 (1H, brs, COOH). ^{13}C-NMR (in $CDCl_3$) δ: 16.81, 24.75 (Pro-γ), 27.52, 30.63, 32.08, 37.41, 38.41, 47.40, 53.35, 59.92, 126.87, 128.36, 129.37, 136.29, 171.15, 173.67, 175.21, 196.11. *Anal.* Calcd for $C_{20}H_{26}N_2O_5S$: C, 59.09; H, 6.45; N, 6.89; S, 7.89. Found: C, 59.03; H, 6.68; N, 7.04; S, 7.90.

1((*S*)-3-Mercapto-2-methylpropanoyl)-L-prolyl-L-phenylalanine (2) A solution of **1** (15.0 g, 36.9 mmol) in 14% ammonium hydroxide (200 ml) was stirred for 2 h at room temperature. The solution was acidified with concentrated HCl and then extracted with ethyl acetate. The organic layer was dried and evaporated. The residue was chromatographed on reverse-phase ODS-Q3 (purchased from Fuji Gel Hanbai Co., Ltd., Japan.) with 40% dioxane[13] containing 1% acetic acid as an eluant under medium pressure. The desired fraction (210 ml) was diluted with water and then lyophilized to afford an amorphous powder, which was dried over phosphorus pentoxide for 5 d *in vacuo* to give 8.1 g (59%) of **2**; $[\alpha]_D^{26}$ −63.8° (c=1.0, EtOH). IR (4×10^{-3} M solution in $CHCl_3$): 1750 (COOH), 1670 (CONH), 1630 (CON) cm^{-1}. ^{1}H-NMR (in CD_3CN) δ: 1.06 (3H, d, J=6.7 Hz, CH_3), 1.68 (1H, dd, J=9.0, 8.0 Hz, SH), 1.71—2.00 (4H, m, Pro-β, γ), 2.45 (1H, m, 1H of CH_2), 2.72 (1H, m, 1H of CH_2), 2.78 (1H, m, CH), 2.97 (1H, dd, J=14.0, 7.8 Hz, 1H of Phe-β), 3.15 (1H, dd, J=14.0, 5.3 Hz, 1H of Phe-β), 3.56 (2H, m, Pro-δ), 4.39 (1H, dd, J=7.5, 4.1 Hz, Pro-α), 4.60 (1H, ddd, J=8.0, 7.6, 5.3 Hz, Phe-α), 7.03 (1H, d, J=7.6 Hz, NH), 7.18—7.35 (5H, m, arom.), 9.45 (1H, brs, COOH). ^{13}C-NMR (in CD_3CN) δ: 17.43, 25.42 (Pro-γ), 28.19, 29.12, 37.98, 42.86, 48.35, 54.31, 60.87, 127.76, 129.34, 130.38, 137.94, 172.76, 173.01, 175.93. *Anal.* Calcd for $C_{18}H_{24}N_2O_4S\cdot0.5H_2O$: C, 58.60; H, 6.69; N, 7.59; S, 8.69. Found: C, 58.45; H, 6.68; N, 7.35; S, 8.44.

1-((*S*)-3-Acetylthio-2-methylpropanoyl)-L-prolyl-L-phenylalanine Ethyl Ester (4) 1-(3-Dimelthylaminopropyl)-3-ethylcarbodiimide hydrochloride (3.0 g, 15.6 mmol) was added to a stirred solution of 1-((*S*)-3-acetylthio-2-methylpropanoyl)-L-proline[2] (4.0 g, 15.4 mmol), L-phenyl-

alanine ethyl ester hydrochloride (3.54 g, 15.4 mmol), and *N*-methylmorpholine (3.12 g, 30.8 mmol) in CH_2Cl_2 (100 ml). The mixture was stirred for 2 h at room temperature and then washed successively with aqueous sodium bicarbonate, 10% HCl, and brine. The organic layer was dried and evaporated to afford 6.0 g of an oil. The oil was chromatographed on silica gel. The product was recrystallized from ether/hexane to give 3.8 g (57%) of **4**; mp 68—69 °C, $[\alpha]_D^{26}$ −104° (c = 1.0, EtOH). IR (KBr): 3290 (NH), 1735 (CO), 1690 (COS), 1640 (CO) cm^{-1}. IR (2×10^{-2} M solution in $CHCl_3$): 3420 (NH), 3290 (NH), 1735 (CO), 1680 (COS and CONH), 1630 (CON) cm^{-1}. ^{1}H-NMR (in $CDCl_3$) δ: 1.09 (3H, d, J = 6.8 Hz, CH_3), 1.22 (3H, t, J = 7.1 Hz, CH_3 of Et), 1.80—2.39 (4H, m, Pro-β, γ), 2.32 (3H, s, CH_3CO), 2.75 (1H, m, CH), 2.94 (1H, dd, J = 13.4, 6.4 Hz, 1H of CH_2), 3.02 (1H, dd, J = 14.0, 6.8 Hz, 1H of Phe-β), 3.08 (1H, dd, J = 13.4, 7.8 Hz, 1H of CH_2), 3.15 (1H, dd, J = 14.0, 6.0 Hz, 1H of Phe-β), 3.37—3.53 (2H, m, Pro-δ), 4.15 (2H, q, J = 6.8 Hz, CH_2Me), 4.60 (1H, m, Pro-α), 4.75 (1H, ddd, J = 8.2, 6.6, 5.9 Hz, Phe-α), 7.10—7.30 (5H, m, arom.), 7.21 (1H, d, J = 8.2 Hz, NH). ^{13}C-NMR (in $CDCl_3$) δ: 14.11, 17.06, 24.86 (Pro-γ), 27.28, 30.62, 32.17, 37.90, 38.37, 47.24, 53.46, 59.68, 61.36, 126.89, 128.35, 129.30, 136.22, 170.88, 171.34, 174.76, 196.03. *Anal.* Calcd for $C_{22}H_{30}N_2O_5S$: C, 60.81; H, 6.96; N, 6.45; S, 7.38. Found: C, 60.57; H, 7.10; N, 6.30; S, 7.34.

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