(s, 8-Pur). Anal. ($C_{11}H_{15}N_5O_3 \cdot 1^1/_4H_2O$) C, H, N.

(±)-5-Amino-3,6-dihydro-3-[(1α,2β,4α)-2-hydroxy-4-(hydroxymethyl)cyclopentyl]-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (11). A mixture of 150 mg (0.53 mmol) of **9** and 5 mL of 0.25 N aqueous sodium hydroxide was boiled under reflux for 3 h. The mixture was treated with activated charcoal and filtered, and the colorless filtrate was acidified to pH 3 with 1 N HCl. A crystalline precipitate was collected by filtration, washed thoroughly with cold water, and dried in vacuo at 78 °C: yield, 97 mg (69%); mp 278–280 °C dec (inserted at 120 °C, 3 deg/min); UV λ_{max} 270 nm (sh, ε 8200) and 253 (11600) at pH 1; 270 nm (ε 8400) and 253 (11000) at pH 7; 278 nm (ε 10800) and 255 (sh) at pH 13; IR (strong and medium bands) 3360, 3320, 3245, 3200, 1710, 1700, 1645, 1590, 1575, 1535, 1375, 1185, 1040, 1015, 780 cm^{-1}. Anal. ($C_{10}H_{14}N_6O_3$) C, H, N.

(±)-(1α,3α,4β)-3-(2,6-Diamino-9*H*-purin-9-yl)-4-hydroxycyclopentanemethanol (12). A solution of 150 mg (0.53 mmol) of **8** in 15 mL of anhydrous ammonia was heated for 18 h at 90 °C in a stainless steel bomb having a glass liner. The bomb was chilled and opened, ammonia was allowed to evaporate, and the residue was concentrated further with a stream of nitrogen and dissolved in methanol. The solution was filtered and stored at −20 °C, and a white crystalline precipitate was collected by filtration, washed with cold methanol, and dried in vacuo at 78 °C: yield, 74 mg (53%); mp 125–130 °C, resolidified, remelted at 202–204 °C (inserted at 115 °C, 3 deg/min); UV λ_{max} 292 nm (ε 9900), 254 (9700), 219 (22000) at pH 1; 280 nm (ε 10400), 256 (8400), 250 (sh), 216 (28600) at pH 7; 280 nm (ε 10500), 256 (8400), 250 (sh) at pH 13; MS (FAB), *m/e* 265 (M + H), 151 (P + 2H); ^1H NMR (Me_2SO-d_6) δ 1.65 (m, 2 H, 2 and 5), 1.83 (m, 1 H, 5), 2.22 (m, 2 H, 1 and 2), 3.34 (s, H_2O), 3.37 (m, 2 H, CH_2OH), 4.35 (m, 2 H, 3 and 4), 4.61 (m, 1 H, CH_2OH), 5.32 (m, 1 H, sec OH), 5.75 (s, NH_2), 7.76 (s, 8-Pur). A specimen was dried at 100 °C for analysis. Anal. ($C_{11}H_{16}N_6O_2 \cdot {}^1/_2H_2O$) C, H, N.

(±)-(1α,3α,4β)-3-(5,7-Diamino-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)-4-hydroxycyclopentanemethanol (13). A solution of 150 mg (0.527 mmol) of **9** in 20 mL of anhydrous ammonia was heated for 18 h at 60 °C in a stainless steel bomb containing a glass liner. The bomb was chilled and opened, and ammonia was evaporated with a current of nitrogen. The residual solid was dissolved in hot water (6 mL), the hot solution was filtered, and the filtrate was cooled and placed in a refrigerator. The crystalline precipitate was separated by filtration, washed with cold water, and dried in vacuo at 78 °C: yield, 108 mg (77%); mp 220–224 °C dec (inserted at 120 °C, 3 deg/min); UV λ_{max} 285 nm (ε 7500), 254 (9400), 213 (25100) at pH 1; 287 nm (ε 10300), 259 (5700), 223 (25300) at pH 7; 286 nm (ε 10300), 258 (5600), 222 (25100) at pH 13; MS (FAB), *m/e* 266 (M + H), 152 (P + H); ^1H NMR (Me_2SO-d_6) δ 1.70 (m, 1 H, 2), 1.85 (m, 2 H, 5), 2.21 (m, 1 H, 1), 2.32 (m, 1 H, 2), 3.40 (t, CH_2OH), 4.52 (m, 1 H, 4), 4.64 (m, 1 H, 3), 4.62 (m, 1 H, CH_2OH), 5.21 (d, 1 H, sec OH), 6.34 (s, NH_2). Anal. ($C_{10}H_{15}N_7O_2$) C, H, N.

Antiviral Evaluations in Vitro. Compounds **8–13** were tested for their ability to inhibit the cytopathogenic effects produced by strain 377 (TK^+) of HSV-1 or strain MS (TK^+) of HSV-2 replicating in monolayers of Vero cells, strain HF (TK^-) of HSV-1 in monolayers of H.Ep.-2 cells, or strain A_0/PR/8/34 of influenza virus in monolayers of Madin-Darby canine kidney cells. These tests were performed by methods and procedures described previously for the evaluation of compounds for antiviral activity in vitro.[10] The general assay method was described by Ehrlich et al.,[16] but some modifications were incorporated.

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Registry No. **3**, 56-05-3; **4**, 68715-64-0; **5**, 106762-83-8; **6**, 106762-84-9; **7**, 106762-85-0; **8**, 106762-86-1; **9**, 106762-87-2; **10**, 106762-88-3; **11**, 106762-89-4; **12**, 106762-90-7; **13**, 106762-91-8; 4-chloroaniline, 106-47-8.

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Resolution of (±)-2-Tetradecyloxiranecarboxylic Acid. Absolute Configuration and Chiral Synthesis of the Hypoglycemic *R* Enantiomer and Biological Activity of Enantiomers

Winston Ho,* Okan Tarhan,† Timothy C. Kiorpes, Gene F. Tutwiler, and Richard J. Mohrbacher

Departments of Chemical Research and Biological Research, McNeil Pharmaceutical, Spring House, Pennsylvania 19477.
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The resolution of the hypoglycemic agent (±)-2-tetradecyloxiranecarboxylic acid (**3**) as its *d*- and *l*-ephedrine salts is presented. The active enantiomer (*R*)-(+)-**3** was also synthesized by the Sharpless chiral epoxidation procedure and its methyl ester (*R*)-(+)-**4** was shown to be identical with the corresponding ester from the resolved acid. Single-crystal X-ray structure analysis of the diastereomeric salt of (+)-**3** and (−)-ephedrine allowed assignment of (+)-**3** as the *R* configuration. The effects on fatty acid oxidation and glucose tolerance of the racemic and enantiomeric forms of **3**, **4**, and the CoA ester of **3** are presented. A postulated mechanism of action for the active enantiomer as an enantioselective, active-site-directed, irreversible inhibitor of carnitine palmitoyl transferase is suggested.

Racemic methyl 2-tetradecyloxiranecarboxylate (**4**) (methyl palmoxirate) is a potent inhibitor of fatty acid oxidation in vitro and an orally active hypoglycemic agent in rats, dogs, monkeys,[1-3] and humans.[4] In order to study the biological activity of **4** and its precursor, **3**, more thoroughly, we have obtained the two enantiomers of 2-tetradecyloxiranecarboxylic acid (**3**) by classical resolution. We have determined the absolute configuration of the bioactive enantiomer (+)-**3** by X-ray crystallography and

† Visiting Professor from Middle East Technical University, Ankara, Turkey.

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have completed a chiral synthesis of (+)-3 and its methyl ester (+)-4.

Chemistry

Resolution of racemic carboxylic acid **3** was accomplished by fractional recrystallization of the diastereomeric salts generated from (+)- and (−)-ephedrine. The enantiomeric purity of the acids **3** was determined by conversion to their methyl esters **4** with diazomethane followed by 90-MHz ^{1}H NMR analysis using the chiral shift reagent Eu(tfc)$_3$. The methyl ester group resonance from racemic **4** was split into two peaks by addition of 0.3 molar equiv of Eu(tfc)$_3$; the methyl ester of (+)-**4** exhibited only one methyl ester signal under the same conditions. The optical purity was further confirmed by HPLC analysis[5] and shown to be greater than 98%. The enantiomeric purity determinations as observed by HPLC, ^{1}H NMR, and optical rotation were in good agreement.[5]

In order to establish the absolute configuration for structure–activity relationship (SAR) purposes, a single-crystal X-ray structure analysis was performed on the diastereomeric salt from (+)-**3** and (−)-ephedrine. On the basis of the known absolute configuration of (−)-ephedrine, in which the carbon α to the phenyl ring has the *R* and the carbon β to the ring has the *S* configuraiton, it was determined that (+)-2-tetradecyloxiranecarboxylic acid, (+)-**3**, has the *R* configuration. An ORTEP representation of (*R*)-(+)-**3** giving the crystallographic numbering system (space group *P*2-1, *Z* = 2, *R* = 0.107) is depicted in Figure 1 (supplementary material). Thus, the biologically active enantiomers of acid **3** and methyl ester **4** are (*R*)-(+)-**3** and (*R*)-(+)-**4**, respectively.

We examined the feasibility of synthesizing (*R*)-(+)-**3** and (*R*)-(+)-**4** via the Sharpless chiral epoxidation.[6] Asymmetric synthesis of oxiranecarbinol **2** was accomplished with Ti(O-*i*-Pr)$_4$, (+)-DIPT, and TBHP[6] and allylic alcohol **1** as the starting material to form the enantiomerically pure (−)-**2** in 26% yield[7] and a mixture containing ring-opened products such as **2a**.[8] Oxidation of the oxi-

$CH_3(CH_2)_{13}C(=CH_2)CH_2OH$ (**1**) → $CH_3(CH_2)_{13}$-oxirane-CH_2OH (**2**) + $CH_3(CH_2)_{13}C(OH)(CH_2OH)CH_2O$-*i*-Pr (**2a**)

2 → $CH_3(CH_2)_{13}$-oxirane-CO_2H (**3**) → $CH_3(CH_2)_{13}$-oxirane-CO_2CH_3 (**4**)

$CH_3(CH_2)_{13}$-oxirane-COSCoA ((*R*)-3-CoA ester)

ranecarbinol (−)-**2** gave oxiranecarboxylic acid (+)-**3** in 70% yield (NaIO$_4$, RuCl$_3$). Diazomethane esterification gave (+)-**4** in 61% yield after column chromatographic purification. The ee of the chirally synthesized methyl (+)-2-tetradecyloxiranecarboxylate (**4**) was >94% based on optical rotation as compared to an enantiomerically pure sample. Based on the enantioselectivity of the Sharpless oxidation utilizing (+)-diisopropyl tartrate, the absolute configuration of carbinol **2** is *S* and, thus, ester **4** is expected to have the *R* configuration. This is in agreement with the absolute configuration from our X-ray crystallographic studies.

Biological Results and Discussion

In previous publications, we have presented evidence that inhibition of long-chain fatty acid oxidation by inhibition of carnitine palmitoyl transferase (CPT) results in lowering of blood glucose. Table I lists the biological activity of the enantiomers of **3**, **4**, and **3**-CoA ester at the enzyme level (CPT), tissue level (rat hemidiaphragm palmitic acid oxidation), and the in vivo pharmacological level (glucose tolerance).

Extensive work from our laboratory[9,11,17] has shown that acid **3** (both enantiomers) and racemic ester **4** are converted to their CoA esters, which is the active form in vivo, and that essentially all of the activity resides in the *R* enantiomer.[5] Thus, the active drug, formed in vivo after administration of racemic **4**, is (*R*)-2-tetradecyloxiranecarboxylic acid CoA ester [(*R*)-**3**-CoA]. This CoA ester is closely related in structure to palmitic acid CoA ester, a major, natural substrate for the enzyme carnitine palmitoyl transferase (CPT) located in the mitochondrial membrane of a variety of tissues.[1,3] While the *R* enantiomer of **3** is converted to its CoA ester at a rate more than 10 times faster than (*S*)-**3**[11] to (*S*)-**3**-CoA, this difference is insufficient to explain the virtual inactivity of (*S*)-**3** or (*S*)-**3**-CoA as inhibitors of CPT under the conditions employed.[13] Thus, only (*R*)-**3**-CoA is a potent, enantioselective inhibitor of CPT even though both enantiomers of **3**-CoA ester are expected to be available to the enzyme.

From Table I, it is evident that the *R*-(+) enantiomers of 2-tetradecyloxiranecarboxylic acid, **3**, and its methyl ester, **4**, possess the biological activity and that the *S* enantiomers are essentially inactive. Furthermore, in numerous in vitro and in vivo experiments,[3,5,9] we have demonstrated that racemic acid **3** and its methyl ester **4** have equivalent biological activity. The racemic methyl ester **4** was chosen for clinical trial on the basis of greater

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(7) Prof. Sharpless (*J. Org. Chem.* **1984**, *49*, 731) reported a 15% yield of **2** using Ti(O-*i*-Pr)$_4$ and a 51% yield (ee 95%) using Ti(O-*t*-Bu)$_4$.

(8) Prof. Sharpless (see ref 7) observed that 2-allylic alcohols (such as **1**) undergo asymmetric epoxidation readily but that the oxiranemethanols formed (**2**) are sensitive to ring-opening reaction by 2-propanol, TBHP, or the starting allylic alcoholic.

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(13) Inhibition of CPT at 10, 20, 50, and 100 nM over 5 min.[5]

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(15) To date we have not studied the binding affinity of (*R*)- and (*S*)-**3**-CoA esters for CPT. There is evidence that the binding site on CPT for fatty acyl CoA esters is not highly selective at the acyl-CoA region of the substrate molecule. Wieland et al. (Ciardelli, T.; Stewart, C. J.; Seeliger, A.; Wieland, T. *Liebigs Ann. Chem.* **1981**, 828) demonstrated that both $CH_3(CH_2)_{15}CH_2SCH_2NHCoA$-residue, a desoxy analogue of acyl-CoA, and $CH_3(CH_2)_{14}C(O)CH_2CH_2NHCoA$-residue were competitive inhibitors of CPT. Considering the observations that (*R*)-**3**-CoA must bind to CPT prior to alkylation of the enzyme and the apparent lack of structural selectivity of CPT toward binding acyl-CoA structures, we feel that it is most likely that (*S*)-**3**-CoA will be found to bind to CPT.

(16) The X-ray crystallographic determination was performed by the Molecular Structure Corp., College Station, TX.

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Table I. 2-Tetradecyloxiranecarboxylic Acid and Derivatives: Effects on Fatty Acid Oxidation and Glucose Tolerance

2-tetradecyloxirane-carboxylic acid derivatives	inhibn of CPT:[a] IC_{50}, μM	inhibn palmitate oxidation in vitro:[b] IC_{50}, μM	rat glucose tolerance test[c]: max % lowering at 100 mg/kg po	rat glucose tolerance test[c]: min effective dose, mg/kg po
(±) acid **3**	0.04	2.2[e]	53[e]	5[e]
(*R*)-(+)-**3**	0.03	1.2	25 (10 mg/kg)	5
(*S*)-(−)-**3**	inact	inact	inact	inact
(±) ester **4**	0.09	2.0[e]	45 (75 mg/kg)[e]	2–4[e]
(*R*)-(+)-**4**	0.04			
(*S*)-(−)-**4**	inact			
±-(**3**)-CoA ester	0.03			
(*R*)-**3**-CoA ester[d]	0.02			
(*S*)-**3**-CoA ester[d]	inact			

[a] Inhibition of carnitine palmitoyl transferase was assessed with isolated rat liver mitochondria as described in ref 5; IC_{50} values determined by using these assay conditions are lower than those reported in ref 3. [b] Inhibition of [1-^{14}C]palmitate oxidation was determined with rat hemidiaphragms as described in ref 1. [c] The rat glucose tolerance test was performed as described in ref 2. The minimum effective dose is the lowest dose giving a statistically significant ($p < 0.05$) lowering at any time point. [d] The nomenclature (*R*)- or (*S*)-**3**-CoA is meant to denote only the absolute configuration of acid **3**. [e] Values from ref 3.

stability than the racemic acid **3**.

It appears that we have uncovered one of the relatively rare cases of a drug acting as an enantioselective, active-site-directed, irreversible inhibitor of an enzyme by covalent-bond formation.[5,9] Our proposed mechanism is similar to that proposed by Shaw[10] with the affinity alkylation reagent L-*N*-tosylphenylalanine chloromethyl ketone (L-TPCK) as an irreversible inhibitor in which the L isomer, but not the D isomer, was shown to alkylate the enzyme chymotrypsin. To date, biochemical studies have shown that (*R*)-**3**-CoA ester meets most of the criteria for this mechanism of action, as outlined by Walsh,[14] i.e., enzyme specificity, inactivation by covalent bond formation, and pseudo-first-order, time-dependent kinetics of inactivation with loss of activity observed following addition of the natural substrate.

In summary, we postulate that racemic mixtures of either **3** or **4** are converted to their **3**-CoA esters and that both (*R*)- and (*S*)-**3**-CoA esters bind[15] reversibly to the fatty acyl CoA specific site on CPT. We suggest that only the *R* enantiomer is sufficiently proximal to a nucleophilic site on CPT to allow rapid reaction with the oxirane ring and, thus, irreversible inhibition of CPT. Further investigation will reveal which amino acid of CPT becomes alkylated and which carbon of the fatty acid oxidation inhibitor (*R*)-2-tetradecyloxiranecarboxylic acid has formed a covalent bond with the enzyme.

Experimental Section

Melting points are uncorrected and were taken on Thomas-Hoover Uni-Melt or Laboratory Devices Mel-Temp melting point apparatuses in capillary melting point tubes. UV spectra were determined on a Cary 14 spectrophotometer and IR spectra on a Perkin-Elmer 552 infrared spectrophotometer. The 90-MHz ^{1}H NMR spectra were obtained on a Perkin-Elmer R-32 NMR spectrometer using Me_4Si as the internal standard. Spectral data for each compound supported the assigned structure, and all elemental and Karl-Fischer analyses were within 0.4% of calculated values.

(*S*)-2-Tetradecyloxiranemethanol (2). To a solution of Ti(O-*i*-Pr)$_4$ (4.83 g, 0.017 mol) and (+)-diisopropyl tartrate (3.98 g, 0.017 mol) in 150 mL of CH_2Cl_2 cooled to −23 °C was added a solution of 2-methylenehexadecan-1-ol (**1**)[3] (4.33 g, 0.017 mol) in 50 mL of CH_2Cl_2. To this mixture was added a solution of *tert*-butyl hydroperoxide (TBHP) (10 mL) in CH_2Cl_2, and the reaction mixture was maintained at −27 °C for 18 h. A solution of water (7 mL) and acetone (400 mL) was added gradually, and the reaction mixture was allowed to warm to room temperature. After addition of ether and water, the organic phase was separated, filtered (to remove titanium hydroxide), and dried (Na_2SO_4) and solvent evaporated to give 2.8 g of crude product. GLC–mass spectroscopy showed the product to contain an impurity that had a molecular weight of 316, which is presumed to be **2a**.[8] The mixture was purified by flash column chromatography (silica gel, hexane–ethyl acetate, 85:15), giving 1.2 g (26%) of pure **2**: mp 25 °C; $[\alpha]^{25}_D$ −9.45° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.6–3.8 (m, 2 H, CH_2OH), 2.85 (d, 1 H, oxirane proton), 2.65 (d, 1 H, oxirane proton), 2.15 (m, 1 H, OH), 1.25 (m, 26 H, CH_2), 0.90 (t, 3 H, CH_3). Anal. Calcd for $C_{17}H_{34}O_2$: C, 75.50; H, 12.67. Found: C, 75.53; H, 12.71.

Methyl (*R*)-2-Tetradecyloxiranecarboxylate (4). To a stirred mixture of **2** (0.27 g, 0.001 mol) in CCl_4 (2 mL)–CH_3CN (3 mL) was added a solution of $NaIO_4$ (0.64 g, 0.003 mol) in 3 mL of H_2O followed by 0.098 g (0.0004 mol) of $RuCl_3$. The reaction mixture was stirred at 25 °C for 5 h. Methylene chloride (10 mL) was added and the organic layer was separated. The aqueous phase was extracted three times with methylene chloride and the combined organic extract was concentrated to dryness. The resulting residue was diluted with 20 mL of ether, filtered, and dried (Na_2SO_4). The solvent was evaporated to give 0.20 g (70%) of crude (*R*)-2-tetradecyloxiranecarboxylic acid (**3**).

Crude **3** from above was dissolved in ether and treated with excess ethereal CH_2N_2. The solvent was removed and the resulting oil was purified by column chromatography on silica gel (2% ether in petroleum–ether) to afford 0.13 g of pure **4** (61% yield): mp 43 °C; $[\alpha]^{25}_D$ +10.27° (*c* 0.5, $CHCl_3$). This sample was shown to be identical (TLC, GLC, ^{1}H NMR) to the *R* isomer of **4** obtained by resolution.

A sample of (*R*)-2-tetradecyloxiranecarboxylic acid (**3**) obtained by resolution (see below) was converted to its methyl ester **4** by CH_2N_2 treatment: mp 45 °C; $[\alpha]^{25}_D$ +10.89° (*c* 0.5, $CHCl_3$).

Resolution of Racemic 2-Tetradecyloxiranecarboxylic Acid. The resolution of (±)-**3** and the details of the HPLC analysis of the phenacyl derivative of **3** are described in detail in the supplementary notes in ref 5. The (−)-ephedrine salt of (±)-**3** was recrystallized 11 times from acetone to give a 10% yield of **3**-(−)-ephedrine salt: mp 64–65 °C; $[\alpha]^{25}_D$ −14.5 °C (*c* 1.0, $CHCl_3$). Acidification of an aqueous solution of the salt gave (*R*)-(+)-2-tetradecyloxiranecarboxylic acid [mp 64–62 °C, $[\alpha]^{25}_D$ +15.96° (*c* 0.99, $CHCl_3$)]. Similarly, (*S*)-(−)-**3** [mp 60–62 °C, $[\alpha]^{25}_D$ −15.44° (*c* 0.68, $CHCl_3$)] was obtained from its (+)-ephedrine salt (mp 86–88 °C).

X-ray Crystallographic Analysis[16] of (−)-Ephedrine (+)-2-Tetradecyloxiranecarboxylate. Data were collected on an Enraf-Nonius CAD4 diffractometer (Cu Kα radiation, λ = 1.541 84 Å) equipped with a graphite crystal, incident beam monochromator. The software programs employed were part of the Enraf-Nonius Structure Determination Package and private programs of Molecular Structure Corporation and implemented on a linked PDP-11/45-11/60 computer. The diasteremeric salt of (+)-2-tetradecyloxiranecarboxylic acid and (1*R*,2*S*)-(−)-ephedrine was recrystallized from acetone to provide a colorless, needlelike cyrstal (0.05 × 0.1 × 0.3 mm): $C_{27}H_{47}NO_4$; M_r 449.68; monoclinic, a = 9.801 (1) Å, b = 5.854 (2) Å, c = 25.254 (6) Å, d_{obsd} = 1.05 g/cm^3, β = 101.17 (1)°, V = 1421.4 Å^3, for Z = 2 mole-

cules/unit cell, d_{calcd} = 1.05 g/cm^3, space group P2-1. Data were collected to a maximum 2θ of 120.0°. Of a total of 1915 reflections, 1844 were unique and not systematically absent (the data was corrected for Lorentz and polarization factors, but not for absorption); 982 reflections with $F_o^2 > 3.06\ (F_o^2)$ were used for structural analysis. Calculation and refinement was carried out by the full-matrix least-squares method. Final anisotropic refinement of non-hydrogen atoms (hydrogen atoms not calculated) gave $R = 0.107$ and $Rw = 0.141$ where $R = (\sum||F_o|-|F_c||/\sum|F_o|$, $Rw = [\sum(|F_o| - |F_c|)^2/\sum F_o^2]_{1/2}$, and the function minimized was $(\sum|F_o| - |F_c|)^2$. The final difference map showed a highest peak of 0.14 (3) e/A^3. Tables of atomic positional parameters, bond distances and angles, useful least-square planes, and thermal parameters are available as supplementary material. The absolute configuration of (+)-3 is R.

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Registry No. 1, 88393-66-2; (S)-2, 88424-62-8; (±)-3, 81556-15-2; (S)-(–)-3, 106974-46-3; (R)-(+)-3, 106974-51-0; (–)-3((+)-ephedrine salt), 106974-49-6; (–)-3((–)-ephedrine salt), 106974-50-9; (R)-3-CoA ester, 92142-38-6; (S)-3-CoA ester, 106974-48-5; (±)-4, 92982-25-7; (S)-(–)-4, 106974-47-4; (R)-(+)-4, 106974-52-1; CPT, 9068-41-1.

Supplementary Material Available: Illustrations showing the atom-numbering system of 3 and (–)-ephedrine and tables of positional and thermal parameters, bond distances, bond angles, and torsional angels (4 pages). Ordering information is given on any current masthead.

Long-Acting Angiotensin II Inhibitors Containing Hexafluorovaline in Position 8

Kun-hwa Hsieh,*† Philip Needleman,‡ and Garland R. Marshall*‡

Department of Biochemistry, University of Colorado Health Sciences Center, Denver, Colorado 80262, and Department of Pharmacology, Washington University School of Medicine, St. Louis, Missouri 63110. *Received July 21, 1986*

An improved synthesis of hexafluorovaline (Hfv) derivatives, i.e., DL-Hfv-OBzl and Boc-DL-Hfv, is described. Incorporation of hexafluorovaline into angiotensin resulted in [Sar[1],Hfv[8]]AII and [Sar[1],D-Hfv[8]]AII. At the nanogram/milliliter dose range, the L congener was 20–100 times more active as either angiotensin agonist or angiotensin antagonist than its D diastereomer on isolated tissue preparations. At the microgram dose range, both [Sar[1],Hfv[8]]AII and [Sar[1],D-Hfv[8]]AII were significantly more effective than [Sar[1],Leu[8]]AII as angiotensin II inhibitors, producing prolonged blockade of the pressor response toward angiotensin II for over 1 h.

Recent advances in recombinant biotechnology have enabled the elucidation of a variety of protein factors that may have an important role in cancer or other diseases, i.e., angiogenesis factor,[1] platelet-derived growth factor,[2,3] and atrial natriuretic hormone.[4,5] It is anticipated that further identification of their active sequences,[6] followed by the development of orally effective drugs from these peptide structures, will lead to many new therapeutic possibilities. Toward this goal, both drug design and peptide formulation have been experimented with, and metabolically stable luteinizing hormone-releasing hormone analogues[7,8] and liposome-entrapped insulin[9] have been found orally active. Nevertheless, a systematic assessment of the parameters involved in gastrointestinal absorption of peptides is needed, so that strategies generally useful for promoting their oral effectiveness can be developed. For this purpose, angiotensin II (Asp-Arg-Val-Tyr-Val-His-Pro-Phe) represents an especially stringent model, in that its many aromatic and polar side chains provide multiple foci for digestive degradations. In addition, its highly hydrophilic–ionic nature at physiological pH is contraindicative to membrane transport, such that even liposomal encapsulation did not afford orally active angiotensin II.[10]

In initial studies,[11] we incorporated the highly fluorinated $\gamma,\gamma,\gamma,\gamma',\gamma',\gamma'$-hexafluorovaline (Hfv) into angiotensin II in order to develop a probe for ^{19}F NMR conformational analysis of the hormone–receptor interaction and, possibly, for receptor localization.[12] We observed that while hexafluorovaline can substitute for valine to give the fully active [Hfv[5]]angiotensin II (133% activity), its derivatives are resistant to proteolytic digestion by the enzymes carboxypeptidase A, hog renal acylase, and α-chymotrypsin. The metabolic stability of hexafluorovaline coupled with its highly lipophilic–electronegative nature makes this amino acid an especially promising candidate for further development of orally active peptide drugs. In this study, we address the need for suitably protected derivatives of hexafluorovaline that can be used in standard procedures of peptide synthesis, so that its ability to promote the oral effectiveness of peptides can be assessed in the future. In addition, the in vitro and in vivo activities of [Sar[1],Hfv[8]]AII

† University of Colorado Health Sciences Center.
‡ Washington University School of Medicine.

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