992 J. Med. Chem. 1987, 30, 992-998

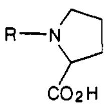
Synthesis and Structure-Activity Relationships of Potent New Angiotensin Converting Enzyme Inhibitors Containing Saturated Bicyclic Amino Acids[1]

C. J. Blankley,* J. S. Kaltenbronn,* D. E. DeJohn, A. Werner, L. R. Bennett, G. Bobowski, U. Krolls, D. R. Johnson, W. M. Pearlman, M. L. Hoefle, A. D. Essenburg, D. M. Cohen, and H. R. Kaplan

Departments of Chemistry and Pharmacology, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, Michigan 48105. *Received November 14, 1986*

The synthesis of a series of novel, potent angiotensin converting enzyme (ACE) inhibitors containing saturated bicyclic amino acids in place of proline is described. Octahydroindole-2-carboxylic acid, octahydroisoindole-1-carboxylic acid, and octahydro-3-oxoisoindole-1-carboxylic acid can replace proline in both sulfhydryl and non-sulfhydryl ACE inhibitors to give compounds equipotent to captopril and enalapril both in vitro and in vivo. Structure-activity relationships are discussed. Compound **11a** (CI-907, indolapril) has advanced to clinical evaluation.

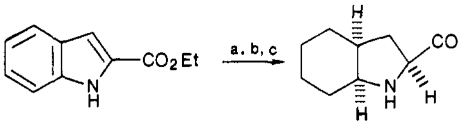
The development of inhibitors of angiotensin converting enzyme [EC 3.4.15.1] (ACE) has resulted in major new agents for the treatment of hypertension. The story of captopril (**1a**) has already become a classic in the field of rational drug design,[2a,b] and this has inspired others to seek additional agents with the same mechanism of action. Several other structurally novel, potent ACE inhibitors have been reported, among them "keto-ACE"[3] (**1e**) and enalapril[4] (**1c**).



1a, R = $HSCH_2CH(CH_3)CO$
b, R = $HSCH_2CH_2CO$
c, R = $PhCH_2CH_2CH(CO_2Et)NHCH(CH_3)CO$
d, R = $PhCH_2CH_2CH(CO_2H)NHCH(CH_3)CO$
e, R = $PhCH_2CH(NHCOPh)COCH_2CH_2CO$

Compounds **1a–e** contain L-proline as the C-terminal portion. Several structure–activity relationship (SAR) studies[2a,b] have indicated that, although this amino acid may not be optimal for in vitro ACE inhibition, it, or other cyclic secondary amino acids, such as thiazolidine-2-carboxylic acid,[5] is most effective for providing useful levels of in vivo activity. Studies on both captopril analogues[2] and simple dipeptide ACE inhibitors[6] showed the enhanced in vitro activity of compounds containing certain lipophilic C-terminal amino acids such as phenylalanine and tryptophan, but these analogues proved to be inferior to the proline derivative in in vivo studies.[2] The susceptibility of the former analogues to nonspecific amidases, in contrast to the proline derivatives, has been cited as the likely reason for diminished oral effectiveness.[2]

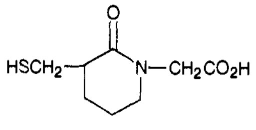
Scheme I[a]



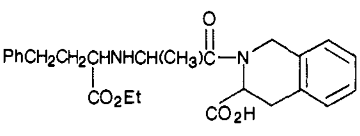
4a, R = H
d, R = Et
e, R = *t*-Bu

[a] (a) H_2, Rh/C, HOAc. (b) Aqueous HCl. (c) *t*-BuOH, H_2SO_4, dioxane.

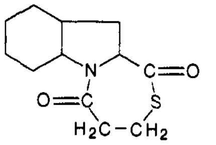
We earlier reported[7] the result of a strategy of "cyclization" of the captopril molecule to provide a hydrolytically stable analogue with the essential functional group requirements intact. This resulted in **2**, a compound in which both in vitro and in vivo ACE inhibition was retained, albeit weakly. Encouraged by this result, we sought to apply a similar reasoning to the proline terminus itself with the idea of converting potent in vitro inhibitors into compounds with equivalent in vivo potency. The success of one such attempt, the transformation of phenylalanine to 1,2,3,4-tetrahydroisoquinoline-3-carboxylic



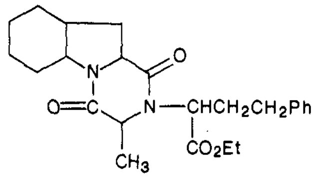
2



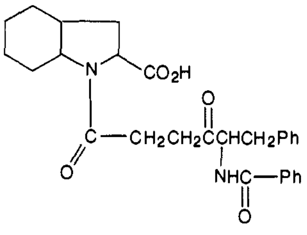
3



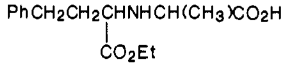
10



12



13



$PhCH_2CH_2CH(CO_2Et)NHCH(CH_3)CO_2H$

14

---

[1] This paper has been presented in part; see *Abstracts of Papers*, 185th National Meeting of the American Chemical Society, Seattle, WA; Americal Chemical Society: Washington, DC, 1983; MEDI 66.

[2] (a) Cushman, D. W.; Ondetti, M. A. *Prog. Med. Chem.* **1980**, *17*, 42–104. (b) Petrillo, E. W., Jr.; Ondetti, M. A. *Med. Res. Rev.* **1982**, *2*, 1–41.

[3] (a) Almquist, R. G.; Chao, W. R.; Wllis, M. E.; Johnson, H. R. *J. Med. Chem.* **1980**, *23*, 1392. (b) Meyer, R. F.; Nicolaides, E. D.; Tinney, F. J.; Lunney, E. A.; Holmes, A.; Hoefle, M. L.; Smith, R. D.; Essenburg, A. D.; Kaplan, H. R.; Almquist, R. G. *J. Med. Chem.* **1981**, *24*, 964. (c) Meyer, R. F.; Essenburg, A. D.; Smith, R. D.; Kaplan, H. R. *J. Med. Chem.* **1982**, *25*, 996.

[4] Patchett, A. A.; Harris, E.; Tristram, E. W.; Wyvratt, M. J.; Wu, M. T.; Taub, R.; Peterson, E. R.; Ikeler, T. J.; Broeke, J. T.; Payne, L. G.; Ondeyka, D. L.; Thursett, E. D.; Greenlee, W. J.; Lohr, N. S.; Hoffsommer, R. D.; Joshua, H.; Ruyle, W. V.; Rothrock, W.; Aster, S. D.; Maycock, A. L.; Robinson, F. M.; Hirschman, R.; Sweet, C. S.; Ulm, E. H.; Gross, D. M.; Vassil, T. C.; Stone, C. A. *Nature (London)* **1980**, *288*, 280.

[5] (a) Funae, Y.; Komori, T.; Sasaki, D.; Yomamoto, K. *Jpn. J. Pharmacol.* **1978**, *28*, 925. (b) Mita, L.; Iwao, J.; Masayuka, O.; Takehisa, C.; Tadashi, I. *Chem. Pharm. Bull.* **1978**, *26*, 1333.

[6] Cheung, H. S.; Wang, F. L.; Ondetti, M. A.; Sabo, E. F.; Cushman, D. W. *J. Biol. Chem.* **1980**, *255*, 401.

[7] Klutchko, S. L.; Hoefle, M. L.; Smith, R. D.; Essenburg, A. D.; Parker, R. B.; Nemeth, V. L.; Ryan, M. J.; Dugan, D. H.; Kaplan, H. R. *J. Med. Chem.* **1981**, *24*, 104.

0022-2623/87/1830-0992$01.50/0

acid and conversion of the latter to highly active in vitro and in vivo ACE inhibitors such as quinapril (3) is being described separately.[8] In this paper we report further results along these lines where we sought to retain the favorable five-membered ring moiety of proline in an enhanced lipophilic environment. To this end, we examined the suitability of octahydroindole-2-carboxylic acid (**4a**) and octahydroisoindole-1-carboxylic acids **5a** and **6a** as alternatives to proline in active ACE inhibitors.

Chemistry

At the time this work was initiated, neither **4a**, **5a**, or **6a** had been described in the literature.[9] We prepared racemic **4a** by the method of Scheme I. Octahydro ethyl ester **4d** showed no chromatographic evidence for the presence of more than one diasteriosomer, and in subsequent reactions, other derivatives of **4a** behaved as single compounds. The indentity of what is at least the highly predominant isomer was ascertained by conversion of **4a** to its 3-bromobenzoyl derivative **7a**, under Schotten–Baumann conditions. X-ray analysis of **7a** established the all-cis disposition of hydrogen atoms at stereocenters around the five-membered ring.[10]

Racemic **4a** was converted to sulfhydryl ACE inhibitor analogues **8a,b** and **9a–d** (Table I) by methods described in the literature[11a] and outlined in Scheme IV. The two diastereomers of the α-methyl compounds **9a,b** could be separated by fractional crystallization.

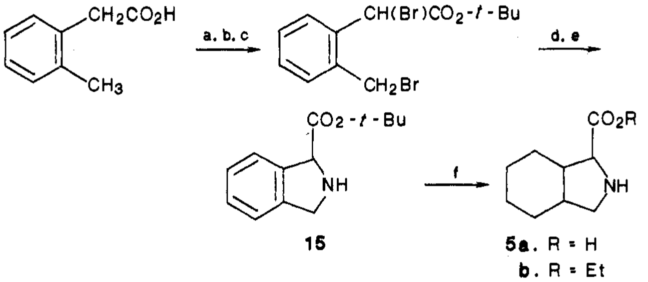
Resolution of **4a** was accomplished initially via separation of α-methylbenzylamine salts of benzoyl derivative **7b**, but subsequently the tartrate salts of the *tert*-butyl ester **4e** also proved effective for this purpose. Since high activity was found to reside in the levorotary isomer, resolved **4b** was used to prepare **8c,d** and **9e,f**.

In most experiments, racemic 3-(acetylthio)-2-methylpropanoyl chloride was used to install the required side chain. Resolution of 3-(benzoylthio)-2-methylpropanoic acid by a literature method[12] provided the preferred *S* enantiomer of the side chain for the preparation of **9e** and **9f**.

The various stereoisomers of non-sulfhydryl compounds **11a–h** (Table II) were prepared by coupling resolved *tert*-butyl esters **4f** and **4g** with the *S,S* and *R,S* diastereomers of **14** (Scheme V). The preparation of the optically pure isomers of **14** has been described.[13] Noteworthy was the fact that steric hindrance of the amine group of **14** obviated the need to protect it during activation and coupling.

Compound **13** was obtained as a mixture of isomers by methods previously described.[3b] Cyclization of **11a** gave **12**, a crystalline material suitable for X-ray analysis.[10] This established the absolute configuration of all chiral centers of **12**, and hence of active isomer **11a** as *S*, in analogy with enalapril.[4] Also confirmed was the relative stereochemistry at the ring junction and carboxyl centers of **11a**.

Scheme II[a]

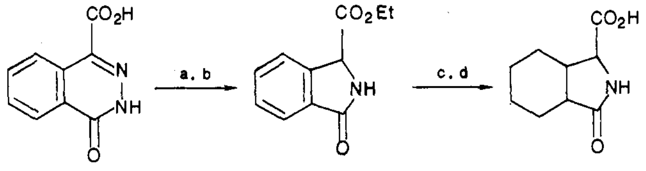


15

5a, R = H
b, R = Et
c, R = *t*-Bu

[a] (a) $SOCl_2$. (b) Br_2, Δ. (c) *t*-BuOH, Py. (d) $PhCH_2NH_2$. (e) H_2, Pd/C. (f) H_2, Rh/C, HOAc.

Scheme III[a]

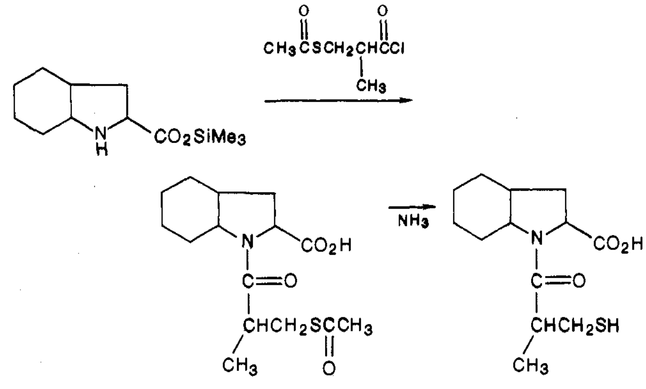


22a, R = H
b, R = Et

6a

[a] (a) Zn/HCl. (b) EtOH/HCl. (c) H_2, Rh/C. (d) NaOH.

Scheme IV



8

The octahydroisoindole-1-carboxylic acid derivatives **5a–c** served as precursors for ACE inhibitors **16** and **17**, which were prepared as outlined in Scheme II. Compounds **5a–c** again behaved as single diastereomers, and it was tentatively presumed that the hydrogens present at the chiral centers have a cis relationship in analogy with **4a,d,e**. These compounds were employed only in racemic form. Non-sulfhydryl analogues **17a,b** were obtained as predominantly single optical isomers by coupling (*S,S*)-**14** with **5c** and separating the resulting diastereomers. Assignments of configuration were made from the activity results and established SAR in analogous systems.[4]

Finally, it was of interest to prepare octahydro-3-oxoisoindole-1-carboxylic acid derivatives **18a–d** and **19** in view of the high activity reported for analogues where pyroglutamic acid replaces proline.[11a,b] The requisite precursor **6** was prepared as shown in Scheme III and again behaved as a single isomer. Is was used in racemic form. The pyroglutamic acid analogues **20a,b**[11a] and **21** were prepared for comparison.[14]

---

[8] Klutchko, S.; Blankley, C. J.; Fleming, R. W.; Hinkley, J. M.; Werner, A. E.; Nordin, I.; Holmes, A.; Hoefle, M. L.; Cohen, D. M.; Essenburg, A. D.; Kaplan, H. D. *J. Med. Chem.* **1986**, *29*, 1953.

[9] A recent paper describes the synthesis of (−)-**4b** and (−)-**4c** by a different procedure. Vincent, M.; Remond, G.; Portevin, B.; Serkiz, B.; Lambie, M. *Tetrahedron Lett.* **1982**, *23*, 1677.

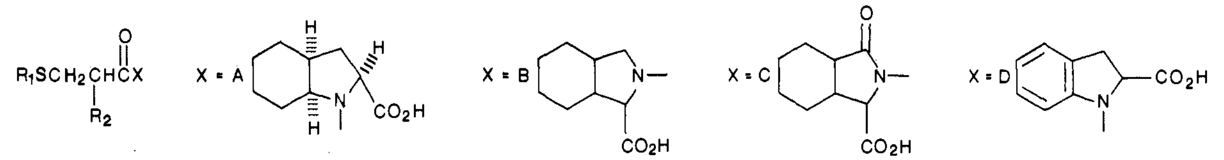
[10] Determined by Dr. A. MacPhail, Duke University. X-ray crystal results on **7a** and **12** are included in the supplementary material for this paper.

[11] (a) Imaki, K.; Sakuyama, S.; Okada, T.; Toda, M.; Hayashi, M.; Miyamoto, T.; Kawasaki, A.; Okegawa, T. *Chem. Pharm. Bull.* 1981, *29*, 2210. (b) Condon, M. E.; Petrillo, E. W., Jr.; Ryono, D. E.; Reid, J. A.; Neubeck, R.; Puar, M.; Heikes, J. E.; Sabo, E. F.; Losee, K. A.; Cushman, D. W.; Ondetti, M. A. *J. Med. Chem.* **1982**, *25*, 250.

[12] Iwao, J.; Ooys, M.; Kato, E.; Watanabe, T. Jpn. Kokai Tokkyo Koho 79, 151, 912; *Chem. Abstr.* **1980**, *92*, 215076*q*.

[13] Kaltenbronn, J. S.; DeJohn, D.; Krolls, U. *Org. Prep. Proc. Int.* **1983**, *15*, 35.

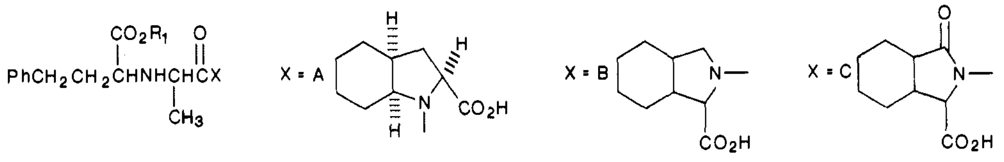
Table I. Physical Properties and in Vitro ACE Inhibitory Activity of Sulfhydryl Analogues



compd	R_1	R_2	X	synth[a] method	recryst solvent	mp, °C	config[b]	$[\alpha]^{23}_D$,[c] deg	formula[d]	IC_{50},[j] μM
8a	CH_3CO	H	A	A	EtOAc	131–133	racemic		$C_{14}H_{21}NO_4S$	0.56
8b	H	H	A	C	MeCN	145–146	racemic		$C_{12}H_{19}NO_3S$	0.013
8c	CH_3CO	H	A	A	EtOAc	110–112	*S*	–51.0	$C_{14}H_{21}NO_4S$	0.48
8d	H	H	A	C	EtOAc	168.5–170	*S*	–68.5	$C_{12}H_{19}NO_3S$	0.0064
9a	CH_3CO	CH_3	A	A	EtOAc	172–173	(*R*,*R* + *S*,*S*)		$C_{15}H_{23}NO_4S$	0.16
9b	CH_3CO	CH_3	A	A	EtOAc	151.5–153.5	(*R*,*S* + *S*,*R*)		$C_{15}H_{23}NO_4S$	35.0
9c	H	CH_3	A	C	EtOAc	155–156	(*R*,*R* + *S*,*S*)		$C_{13}H_{21}NO_3S$	0.0077
9d	H	CH_3	A	C	EtOAc	141–142	(*R*,*S* + *S*,*R*)		$C_{13}H_{21}NO_3S$	1.10
9e	PhCO	CH_3	A	A	EtOAc	184.5–185.5	(*S*,*S*)	–135.6	$C_{20}H_{25}NO_4S$	0.54
9f	H	CH_3	A	C	MeCN	145–148	(*S*,*S*)	–53.5	$C_{25}H_{44}N_2O_3S$	0.0052
10	*m*				EtOAc	148–152	racemic		$C_{12}H_{17}NO_2S$	0.007
16a	CH_3CO	H	B	B	EtOAc	140–145	racemic		$C_{14}H_{21}NO_4S$	1.2
16b	H	H	B	C		170–175 dec	racemic		$C_{12}H_{18}NO_3S\cdot C_{12}H_{23}N^{e,i}$	0.024
16c	CH_3CO	CH_3	B	B		oil	mixture		$C_{15}H_{23}NO_4S\cdot 0.3CHCl_3{}^{f}$	0.42
16d	H	CH_3	B	C		119–129	mixture		$C_{13}H_{21}NO_3S^{g}$	0.028
18a	CH_3CO	H	C	B	MeCN	198–203	racemic		$C_{14}H_{19}NO_5S\cdot C_{12}H_{23}N^{i}$	0.66
18b	H	H	C	C	MeCN	188–208	racemic		$C_{12}H_{17}NO_4S\cdot C_{12}H_{23}N^{i}$	0.013
18c	CH_3CO	CH_3	C	B	EtOAc	185–188	mixture		$C_{15}H_{21}NO_5S\cdot C_{12}H_{23}Ni$	0.3
18d	H	CH_3	C	C	MeCN	191–200 dec	mixture		$C_{13}H_{19}NO_4S\cdot C_{12}H_{23}N^{h,i}$	0.014
20a	H	H	*n*	B	MeCN	185–191 then 225–227	*S*	–31.7	$C_{20}H_{34}N_2O_4S$	0.96[l]
20b	H	CH_3	*n*	B	MeCN	188–191.5	(*S*,*S*)	–58.6	$C_{21}H_{36}N_2O_4S$	0.0093[l]
23a	H	H	D				racemic			0.14[k]
23b	H	CH_3	D				(*S*,*S*)			0.0037[k]
captopril (**1a**)	H	CH_3	proline				(*S*,*S*)		$C_{12}H_{17}NO_2S$	0.012
1b	H	H	proline				*S*			0.82

[a] See Experimental Section. [b] "Mixture" refers to an unseparated mixture of diastereomers. [c] c 1.0, 1:1 MeOH/0.1 N HCl. [d] Analyses for C, H, N were within ±0.4% except as noted. [e] C: calcd, 65.71; found, 65.24. [f] C: calcd, 52.62; found, 51.95. [g] C: calcd, 57.53; found, 56.17. [h] N: calcd, 6.50; found, 5.89. [i] Isolated as the dicyclohexylamine salt. [j] Molar concentration required for 50% inhibition. [k] Reference 15. [l] See reference 11a. The authors found IC_{50} values for **20a** of 0.06 μM and for (*S*,*S*)-**20b** 0.0036 μM. [m] See the structure for **10**. [n] Pyroglutamic acid.

Table II. Physical Properties and in Vitro ACE Inhibitory Activity of Non-Sulfhydryl Analogues



compd	R_1	X	synth[a] method	mp, °C	config[b]	$[\alpha]^{23}_D$,[c] deg	formula[d]	IC_{50},[k] μM
11**a**	C_2H_5	A	D	amorph	*SSS*	–28.8	$C_{24}H_{34}N_2O_5\cdot HCl\cdot 0.5H_2O$	0.080
11**b**	H	A	E	165–166 dec	*SSS*	–34.0	$C_{22}H_{30}N_2O_5$	0.0024
11**c**	C_2H_5	A	D	amorph	*RSS*	–66.8	$C_{24}H_{34}N_2O_5\cdot 0.5H_2O^{e}$	1.30
11**d**	H	A	E	amorph	*RSS*		$C_{22}H_{30}N_2O_5$	0.011
11**e**	C_2H_5	A	D	amorph	*SSR*	+28.8	$C_{24}H_{34}N_2O_5\cdot 0.25H_2O^{f}$	57.0
11**f**	H	A	E	147–149 dec	*SSR*	+41.9	$C_{22}H_{30}N_2O_5\cdot H_2O^{g}$	2.4
11**g**	C_2H_5	A	D	amorph	*RSR*	–1.9	$C_{24}H_{34}N_2O_5\cdot HCl\text{-}H_2O^{h}$	>100
11**h**	H	A	E	124–126 dec	*RSR*	+8.9	$C_{22}H_{30}N_2O_5\cdot 0.5H_2O$	6.4
12	*m*			138–141	*SSS*	–53.5	$C_{24}H_{32}N_2O_4$	>100
13	*m*			amorph	mixture		$C_{28}H_{32}N_2O_5\cdot 0.5H_2O^{i}$	0.011
17a	C_2H_5	B	D	198–216 dec	*SSS*	–33.6	$C_{24}H_{34}N_2O_5\cdot HCl$	0.17
17b	C_2H_5	B	D	204–214 dec	*SSR*	+24.8	$C_{24}H_{34}N_2O_5\cdot HCl\cdot 0.8H_2O$	7.50
17c	H	B	E	186–190	*SSS*	–36.5	$C_{22}H_{30}N_2O_5\cdot 1.2H_2O$	0.0026
17d	H	B	E	145–163	*SSR*	+33.2	$C_{22}H_{30}N_2O_5\cdot 1.5H_2O$	0.018
19	C_2H_5	C	D	amorph	(*SSS* + *SSR*)	+9.5	$C_{24}H_{32}N_2O_8\cdot HCl\cdot 1.2H_2O^{j}$	0.34
21	C_2H_5	*n*	D	amorph	*SSS*	–17.1[l]	$C_{20}H_{26}N_2O_6\cdot HCl\cdot 1.1H_2O$	0.063
enalapril (1**c**)	C_2H_5	*o*			*SSS*			0.14
enalaprilat (1**d**)	H	*o*			*SSS*			0.0023
1**e**	*m*							0.0032

[a] See Experimental Section. [b] "Mixture" refers to an unseparated mixture of diastereomers. The *S* and *R* configurations refer to the optical centers present as one proceeds from left to right in the structure as indicated. The bicyclic amino acid portion is considered as a unit and is designated as either *S* or *R*. [c] c 1.0, 1:1 MeOH/0.1 N HCl. [d] Analyses for C, H, N were within ±0.4% except as noted. [e] H: calcd, 7.62; found, 7.13. [f] H: calcd, 7.59; found, 6.80. [g] H: calcd, 7.67; found, 7.15. [h] H: calcd, 7.69; found, 6.97. [i] N: calcd, 5.77; found, 5.28. [j] C: calcd, 57.35; found, 56.85. [k] Molar concentration required for 50% inhibition. [l] Reference 14 reports $[\alpha]^{23}_D$ –31.8° (c 0.96, EtOH). [m] See text. [n] Pyroglutamic acid. [o] Proline.

Table III. Summary of Effects of Certain ACE Inhibitors on Blood Pressure in the Conscious Renal (2 Kidney/1 Clip) Hypertensive Rat

			mean aortic blood pressure	
compd	dose,[a] mg/kg po	no. tested	base line,[b] mmHg	max change,[c] mmHg
8b	3	5	198 ± 5	−73 (at 8 h)
	10	5	198 ± 5	−89 (at 3 h)
	30	5	198 ± 5	−91 (at 5 h)
9c	0.1	4	184 ± 7	−16 (at 6 h)
	0.3	4	185 ± 14	−19 (at 8 h)
	1.0	4	178 ± 9	−72 (at 5 h)
	3.0	4	190 ± 5	−81 (at 5 h)
	30	4	197 ± 6	−115 (at 10 h)
9f	30	3	193 ± 6	−72 (at 6 h)
16b	30	3	184 ± 3	−76 (at 6 h)
16d	30	4	152 ± 3	−50 (at 6 h)
18b	30	5	205 ± 6	−57 (at 8 h)
18d	30	3	192 ± 19	−100 (at 8 h)
20b	30	3	185 ± 13	−48 (at 8 h)
11**a**	0.1	4	185 ± 5	−52 (at 10 h)
	0.3	4	191 ± 10	−73 (at 5 h)
	1.0	4	190 ± 5	−72 (at 3 h)
	10.0	4	197 ± 7	−86 (at 6 h)
	30.0	4	185 ± 5	−102 (at 6 h)
11**b**	30	4	198 ± 5	−104 (at 5 h)
13	30	4	169 ± 2	−27 (at 8 h)
17a	3	4	199 ± 12	−87 (at 6 h)
1**a** (captopril)	0.3	4	189 ± 6	−25 (at 2 h)
	3.0	4	192 ± 7	−93 (at 6 h)
	30.0	4	186 ± 4	−101 (at 6 h)
1**c** (enalapril)	0.3	4	201 ± 9	−39 (at 5 h)
	1.0	4	175 ± 2	−24 (at 8 h)
	3.0	4	200 ± 5	−56 (at 5 h)
	10.0	4	199 ± 5	−56 (at 4 h)

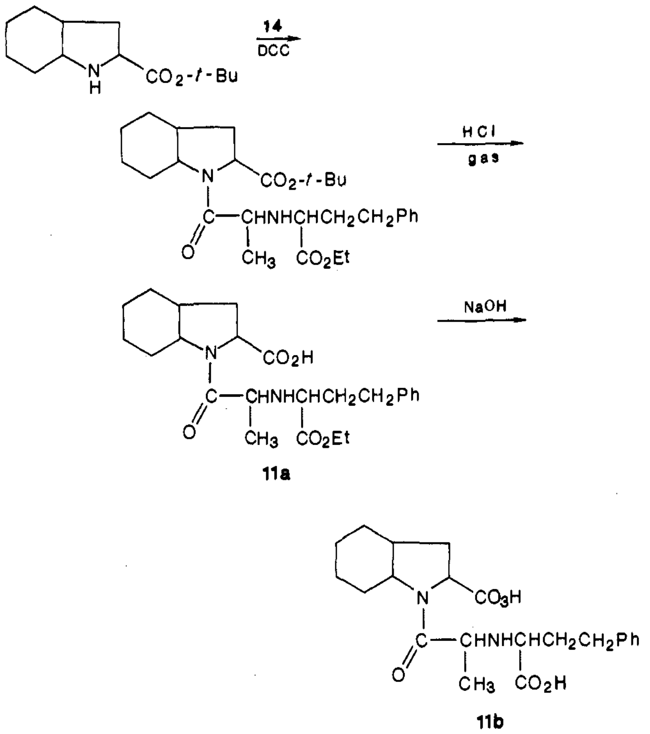
[a] All compounds were dissolved/suspended in 4% gum acacia with the exception of 11**b**, which was dissolved in a 0.1% aqueous solution of methocel, and **13**, which was dissolved in 5% EtOH. [b] All values are the mean ± 1 SEM. [c] Average result for three to five animals. Vehicle controls average 183 ± 5 mmHg with maximum decreases about 16–20 mmHg, or 10%. Hence, blood pressure decreases in excess of 20 mmHg are taken as significant for the purposes of this comparison.

Table IV lists the physical constants of the new amino acids and derivatives used in this study.

Biological Results and Discussion

The assay for in vitro ACE inhibition was performed as previously described[7] with Hip-Gly-Gly as the synthetic substrate. In vitro results are reported in Tables I and II. It is clear that, allowing for racemic mixtures in some cases, all of the bicyclic analogues are at least as potent in vitro as their monocyclic counterparts. In the sulfhydryl series, the derivative with the octahydroindole-2-carboxylic acid in place of proline (compound **9f**) actually improves the IC_{50} by 2.3-fold. A recent study has reported a similar activity enhancement of a related proline replacement, indoline-2-carboxylic acid[15] (IC_{50} = 0.0037 μM for **23b**). It is quite clear though that the additional geometrical features present in **4b** can impart enhanced in vitro activity to analogues with less than optimal side chains, at least when compared with the proline series. For example, compare **8d** (IC_{50} = 0.006 μM), **1b** (IC_{50} = 0.82 μM),[16] and **23a** (IC_{50} = 0.14 μM). This represents an activity enhancement of 136-fold for **8d** relative to **1b** and 23-fold for **8d** over **23a**. Data given for the isomeric octahydroisoindole analogues suggest that similar activity enhancements are obtained in these series as well. Quantitation is not possible, however, due to the unavailability of pure chiral diastereoisomers. Cyclic analogue **10** was prepared as a possible prodrug form of **8b**. Surprisingly this compound showed in vitro activity comparable to that of **8b**. It was not determined, however, whether **8b** was actually formed under the assay conditions.

Scheme V



With regard to the non-sulfhydryl analogues, a similar pattern is observed. Optimal isomers **11b** and **1d** have nearly identical in vitro activities, while suboptimal **11d** shows a 75-fold activity enhancement over the corresponding diastereoisomer of **1d** (IC_{50} = 0.82 μM).[4] Again, the octahydroisoindole derivatives give comparable activity to their monocyclic analogues, reflecting further a parallelism in the SAR for the captopril and enalapril types. Although the data of Table II does not allow a definitive judgment on the effect of octahydroindole vs. proline in the "keto-ACE" type of inhibitor, we have observed the substantial activity enhancing effect of the octahydroindole-2-carboxylic acid in other series of non-sulfhydryl ACE inhibitors.[17]

Oral activity of selected analogues in 2-kidney-1-clip Goldblatt hypertensive rats is reported in Table III. All of the optimum diastereoisomers show potent activity in this model. Additional pharmacology of **11a** (CI-907, SCH 31846, indolapril) has been reported elsewhere,[18a–c,19] as well

---

(14) Compound **21** was recently reported. See: Johnson, A. L.; Price, W. A.; Wong, P. C.; Vavala, R. F.; Stump, J. M. *J. Med. Chem.* **1985**, *28*, 1596.

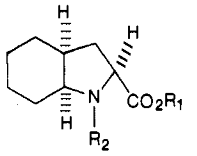
(15) Kim, D. H.; Guinosso, C. J.; Buzby, G. C., Jr.; Herbst, D. R.; McCaully, R. J.; Wicks, T. C.; Wendt, R. L. *J. Med. Chem.* **1983**, *26*, 394.

(16) Reference 2 gives IC_{50} values of **1d** as 0.20 μM and **1a** as 0.023 μM; Hip-His-Leu as substrate.

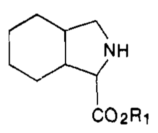
(17) Roark, W. H.; Tinney, F. J.; Cohen, D.; Essenburg, A. D.; Kaplan, H. R. *J. Med. Chem.* **1985**, *28*, 1291.

(18) (a) Kaplan, H. R.; Burmeister, W.; Essenburg, A.; Major, T. C.; Mertz, T.; Randolph, A. *Pharmacologist* **1982**, *24*, 176 (Abstr 446). (b) Ryan, M. J.; Boucher, D. M.; Cohen, D. M.; Olszewski, B. J.; Singer, R. M.; Smith, R. D.; Kaplan, H. R. *Pharmacologist* **1982**, *24*, 176 (Abstr 447). (c) Ryan, M. J.; Boucher, D. M.; Cohen, D. M.; Essenburg, A. D.; Major, T. C.; Mertz, T. E.; Olszewski, B. J.; Randolph, A. E.; Singer, R. M.; Kaplan, H. R. *J. Pharmacol. Exp. Ther.* **1984**, *228*, 312. (d) Borondy, P. E.; Michniewicz, B. M.; Yakatan, G. J. *Pharmacologist* **1982**, *24*, 95 (Abstr 14).

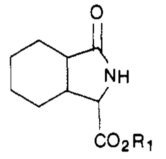
Table IV. New Amino Acids and Derivatives



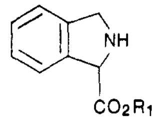
4, 7



5a-c



6



15

compd	R_1	R_2	salt	mp, °C	$[\alpha]^{23}{}_D$,[a] deg	formula[b]
4a	H	H		238–240	racemic	$C_9H_{15}NO_2$
4b	H	H		268–270 dec	−47.9	$C_9H_{15}NO_2$
4c	H	H		267–268 dec	+48.4	$C_9H_{15}NO_2$[c]
4d	C_2H_5	H		oil	racemic	$C_{11}H_{19}NO_2$[d]
4e	t-C_4H_9	H		oil	racemic	$C_{13}H_{23}NO_2$[c]
4f	t-C_4H_9	H		oil	−29.4	$C_{13}H_{23}NO_2$[c]
4g	t-C_4H_9	H		oil	+29.9	$C_{13}H_{23}NO_2$[c]
5a	H	H	HCl	257–262 dec	racemic	$C_9H_{15}NO_2{\cdot}HCl{\cdot}0.2H_2O$
5b	C_2H_5	H	HCl	174–176	racemic	$C_{11}H_{19}NO_2{\cdot}HCl$
5c	t-C_4H_9	H	HCl	170–176 dec	racemic	$C_{13}H_{23}NO_2{\cdot}HCl{\cdot}0.15H_2O$
6	H	H		205–213 dec	racemic	$C_8H_{13}NO_3$
7a	H	3-BrPhCO		171–174	racemic	$C_{16}H_{18}BrNO_3$
7b	H	PhCO		191–193	racemic	$C_{16}H_{19}NO_3$
7c	H	PhCO		169–171	−48.9	$C_{16}H_{19}NO_3$
7d	H	PhCO		169–171	+50.5	$C_{16}H_{19}NO_3$[c]
15	t-C_4H_9	H	HCl	148–154 dec	racemic	$C_{13}H_{17}NO_2{\cdot}HCl{\cdot}0.2H_2O$

[a] c 1, MeOH. [b] Analyses for C, H, N were within ±0.4% except as noted. [c] Not analyzed. [d] C: calcd, 66.97; found, 66.54.

as pharmacokinetic studies preparatory to clinical development.[18d]

In summary, we have described the potent ACE inhibitory and blood pressure lowering effects of a number of saturated bicyclic amino acid derivatives. These not only confirm the salutory effect on ACE activity of additional lipophilic character at the proline site in the model proposed by Ondetti et al.[2a,b] but also add information about bulk and shape tolerance in this region. The question of real interest now is to what extent these altered geometrical and physicochemical properties will manifest themselves in a physiological setting.

Experimental Section

Melting points were determined in a Thomas-Hoover capillary melting point apparatus or a Mel-Temp apparatus. Infrared (IR) data were recorded on a Beckman IR-9 or IR-7 prism grating instrument on a Digilab FTS-14 interferometer. Nuclear magnetic resonance measurements (NMR) were made on a Bruker WH-90 pulsed Fourier transform instrument. IR and NMR were compatible with the assigned structures. Homogeneity of the products was determined by ascending thin-layer chromatography (TLC) on precoated TLC sheets (silica gel 60 F 254, Merck), using principally the solvent system HOAc–MeCN–toluene (1:9:10). HPLC analyses were carried out on a C18 reverse phase Alltech column using 40% acetonitrile/60% 0.005 M Pic A as the solvent. Peaks were detected by UV at 210 mm.

Coupling of Bicyclic Amino Acids with Sulfur-Containing Side Chains Leading to Analogues of the Sulfhydryl Type. A General Procedure Illustrated with the Preparation of (2α,3aβ,7aβ)-1-[3-(Acetylthio)-2-methyl-1-oxopropyl]octahydro-1*H*-indole-2-carboxylic Acid (9a and 9b). Method A. A mixture of 3.0 g (0.02 mol) of the amino acid **4a** and 3.5 g (0.02 mol) of hexamethyldisilazane in 15 mL of CH_3CN was treated with a few drops of chlorotrimethylsilane and heated at reflux for 3 h. The resulting solution was then cooled in an ice bath and treated dropwise with a solution of 3.2 g (0.02 mol) of 3-(acetylthio)-2-methylpropionyl chloride in 5 mL of CH_3CN. After 1 h at 0 °C, the solution was allowed to warm to room temperature and then warmed to reflux, allowing the major portion of the chlorotrimethylsilane and CH_3CN to escape. The solution was then recooled to 0 °C and 0.5 mL of H_2O added, and the solution was warmed briefly to reflux and filtered through Celite. Removal of the solvent under reduced pressure left an oil as a mixture of diastereomers.

Trituration of the oil with Et_2O caused separation of the less soluble diastereomer **9a**. Several recrystallizations from EtOAc gave pure **9a**. HPLC analysis showed only one diastereomer.

Extensive fractional recrystallization of the material obtained from the mother liquors and soluble in cyclohexane gave a sample of the more soluble isomer **9b**. HPLC analysis showed less than 8% of **9a** present.

Alternate Coupling Leading to Analogues of the Sulfhydryl Type. A General Procedure Illustrated with the Preparation of 2-[3-(Acetylthio)-1-oxopropyl]octahydro-1*H*-isoindole-1-carboxylic Acid (16a). Method B. A mixture of 2.0 g (9.7 mmol) of **5a** and 2.3 g (29 mmol) of pyridine in 25 mL of THF was cooled in ice and treated dropwise with 1.8 g (10.8 mmol) of 3-(acetylthio)propionyl chloride. After 2 h in the cold, the mixture was allowed to warm to room temperature over 1 h and then concentrated under reduced pressure. The residue was taken up in EtOAc and washed twice with dilute HCl. Drying of the EtOAc over $MgSO_4$ and removal of the solvent under reduced pressure left an oil which solidified. Two recrystallizations from EtOAc gave 1.3 g (45%) of **16a**.

Removal of the Acetyl Group, a General Procedure Illustrated with the Preparation of (2α,3aβ,7aβ)-Octahydro-1-(3-mercapto-2-methyl-1-oxopropyl)-1*H*-indole-2-carboxylic Acid (9c). Method C. Under N_2, a solution of 1.0 g (3 mmol) of **9a** in 10 mL of a 5 N solution of NH_3/MeOH was stirred at room temperature for 2.5 h. The mixture was concentrated under reduced pressure and taken up in H_2O. The pH was brought to 2.0 with 10% $NaHSO_4$ and extracted twice with EtOAc. The EtOAc was washed with saturated NaCl and dried over $MgSO_4$. Removal of the solvent under reduced pressure left 0.8 g of a white solid. Recrystallization from EtOAc gave pure **9c**.

Coupling of Bicyclic Amino Acids Leading to Analogues of the Non-Sulfhydryl Type (Table II). A General Procedure Illustrated with the Preparation of [2*S*-[1-[*R-(*R**)],2α,3aβ,7aβ]]-1-[2-[[1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]octahydro-1*H*-indole-2-carboxylic Acid Hydrochloride (11a). Method D.** A solution of 8.0 g (28.6 mmol) of (*S*,*S*)-14,[13] 6.62 g (28.6 mmol) of 4f, and 3.87 g (28.6 mmol) of 1-hydroxybenzotriazole in 120 mL of THF was cooled in ice and treated dropwise with a solution of 5.91 g (28.6 mmol) of *N*,*N*′-dicyclohexylcarbodiimide in 15 mL of THF. The solution was stirred at 0 °C for 1 h and then at room tem-

---

(19) Baum, T.; Sybertz, E. J.; Ahn, H. S.; Watkins, R. W.; Powell, M. L.; LaRocca, P. T. In *New Drugs Annual: Cardiovascular Drugs*; Scriabine, A., Ed.; Raven: New York, 1985; Vol. 3, p 43.

perature overnight. The dicyclohexylurea was filtered off and the solvent removed under reduced pressure. The residue was taken up in EtOAc and washed with saturated $NaHCO_3$ and then saturated NaCl. After drying over $MgSO_4$, the solvent was removed under reduced pressure. The residue was taken up in hexane, charcoaled, and filtered through Celite, and the solvent was removed under reduced pressure to give 13.9 g of the crude diester as an oil; $[\alpha]^{23}{}_D$ −69.3° (*c*, 1, MeOH). HPLC analysis showed the diester to be 97% pure.

A solution of 13.9 g (28.6 mmol) of the diester in 135 mL of CH_2Cl_2 was cooled to 0 °C and saturated with HCl gas. After the solution was allowed to stand at 0 °C overnight, the solvent was removed under reduced pressure. Additional CH_2Cl_2 was added and the solvent removed again. The residue was then taken up in 100 mL of CH_2Cl_2, charcoaled, filtered through Celite, and concentrated under reduced pressure to a foam. After trituration with Et_2O, the solid was dissolved in H_2O, filtered, and lyophilized to give 12.1 g (93% yield) of **11a** as an amorphous solid. HPLC analysis showed this to be 99% pure.

Hydrolysis to the Diacid. A General Procedure Illustrated with the Preparation of [2*S*-[1-[*R-(*R**)],2α,3aβ,7aβ]]-1-[2-[(1-Carboxy-3-phenylpropyl)amino]-1-oxopropyl]octahydro-1*H*-indole-2-carboxylic Acid (11b). Method E.** A solution of 10.0 g (21.4 mmol) of **11a** in 50 mL of H_2O was treated with 50 mL (70.7 mmol) of 1.4 N NaOH and stirred at room temperature overnight. The pH was brought to 4.5 with dilute HCl, a small amount of insoluble material filtered off, and the filtrate lyophilized. The residue was treated with warm EtOH to remove NaCl and the EtOH then removed under reduced pressure. The residue was purified by ion-exchange chromatography on Amberlite IR-120, eluting with 2 N NH_4OH. Removal of the solvent under reduced pressure and trituration of the residue with H_2O gave 6.61 g (76.7%) of **11b** as a white solid.

(6aα,10aα,11aα)-Decahydro-1*H*,5*H*-[1,4]thiazepino[4,3-*a*]indole-1,5-dione (10). A solution of 0.4 g (1.9 mmol) of *N*,*N'*-dicyclohexylcarbodiimide and 0.2 mL of pyridine in 35 mL of CH_2Cl_2 was cooled in ice and treated dropwise with a solution of 0.5 g (1.9 mmol) of **8b** in 5 mL of CH_2Cl_2 over 20 min. The mixture was then stirred for 2 days at room temperature, filtered, and concentrated under reduced pressure. The product was isolated by chromatography on silica gel, eluting with $CHCl_3$/MeOH/HOAc (196/3/1). Combination of the appropriate fractions and recrystallization from EtOAc gave pure **10**.

[3*S*-[2(*R),3α,5aβ,9aβ,10aβ]]-Decahydro-3-methyl-1,4-dioxo-α-(2-phenylethyl)pyrazino[1,2-*a*]indole-2(1*H*)-acetic Acid** Ethyl Ester **(12).** A solution of 9.4 g (20 mmol) of **11a** and 3.0 g (20 mmol) of 1-hydroxybenzotriazole in 200 mL of THF was cooled to 0 °C and treated with 21 mL (21 mmol) of a 1 M solution of *N*,*N'*-dicyclohexylcarbodiimide in THF. After the mixture was stirred at room temperature for 16 h, the dicyclohexylurea was filtered off and the filtrate concentrated under reduced pressure. The residue was taken up in EtOAc and washed with saturated $NaHCO_3$ and then saturated NaCl. After drying over $MgSO_4$ and removal of the solvent under reduced pressure, the crude product was obtained. Recrystallization from MeOH gave 4.2 g of **12**, mp 143–146 °C.

1-[5-(Benzoylamino)-1,4-dioxo-6-phenylhexyl]octahydro-1*H*-indole-2-carboxylic Acid (13). To a slurry of 16.2 g (0.05 mol) of 6-phenyl-5-(benzoylamino)-4-oxohexanoic acid in 100 mL of CH_3CN was added in portions 12.0 g (0.074 mol) of carbonyldiimidazole. On warming to 30 °C, solution soon occurred and the solution was kept at this temperature for 0.5 h. The solution was cooled in ice and treated with a solution of 8.46 g (0.05 mol) of **4a** in 100 mL of CH_3CN. The solution was then allowed to stir at room temperature overnight. The solvent was removed under reduced pressure, and the residue taken up in CH_2Cl_2 and washed with dilute HCl and then with water. After drying over $MgSO_4$, the solvent was removed under reduced pressure, leaving 20.8 g of the crude product as a foam. A portion of this was chromatographed on silica gel, eluting with EtOAc. Combining the appropriate fractions gave **13** as a mixture of diastereomers.

(2α,3aβ,7aβ)-Octahydro-1*H*-indole-2-carboxylic Acid Ethyl **Ester (4d).** A solution of 100 g (0.53 mol) of indole-2-carboxylic acid ethyl ester[20] in 1 L of absolute EtOH and 32 mL of concentrated H_2SO_4 was hydrogenated in a Parr sapparatus with 4.0 g of a 10% Rh/C catalyst. After the required amount of H_2 had been taken up, the catalyst was removed by filtration, and the filtrate was evaporated in vacuo. The syrupy residue was dissolved in ice water and first neutralized with K_2CO_3 and then made basic with $KHCO_3$. The mixture was extracted twice with Et_2O and the Et_2O washed with saturated NaCl. Drying over Na_2SO_4 and removal of the solvent under reduced pressure gave 78.5 g of the ester as a colorless oil of high purity.

(2α,3aβ,7aβ)-Octahydro-1*H*-indole-2-carboxylic Acid (4a). A solution of 2.0 g (0.01 mol) of **4d** in 25 mL of 15% HCl was heated at reflux for 4 h and then evaporated to dryness in vacuo. Recrystallization from CH_3CN/EtOAc gave 1.7 g of hydrochloride salt, mp 186–187 °C dec.

The free acid was obtained by dissolving 1.2 g of the hydrochloride in 10 mL of H_2O and adding 2 N NaOH to pH 6.5. The resulting solution was evaporated to dryness under reduced pressure and the residue then refluxed with 50 mL of CH_3CN and filtered hot. Concentration of the filtrate to 10 mL and cooling gave 0.5 g of pure **4a**.

(2α,3aβ,7aβ)-1-Benzoyloctahydro-1*H*-indole-2-carboxylic Acid (7b). A solution of 58.32 g (0.344 mol) of **4a** in 290 mL of H_2O was cooled in ice and treated dropwise simultaneously over a 1-h period with 2 N NaOH and 42.0 mL (0.36 mol, a 5% XS) of benzoyl chloride, while the pH was maintained between 10 and 11 and the temperature between 5 and 10 °C. When the addition was complete, the mixture was allowed to stir at 5 °C for 3 h while base was added to maintain the pH at 10–11. Approximately 350 mL of 2 N NaOH was used. The pH was brought to 1.8 with dilute HCl and the solid collected and washed with water. Recrystallization from EtOH/H_2O gave 91.0 g (96.6%) of pure acid, mp 191–193 °C.

(2α,3aβ,7aβ)-1-(3-Bromobenzoyl)octahydro-1*H*-indole-2-carboxylic Acid (7a). By a procedure similar to the above, but using 3-bromobenzoyl chloride, there was obtained **7a** as a crystalline solid.

Resolution of 7b To Give 7c. A solution of 92.93 g (0.7668 mol) of (*S*)-(−)-α-methylbenzylamine in 1670 mL of MeOH was treated with 209.6 g (0.7668 mol) of **7b** and the mixture swirled. Almost all dissolved. The solution was warmed slightly on the steam bath and filtered. The filtrate was diluted with 3.0 L of EtOAc and seeded. Crystallization started at once. After swirling occasionally at room temperature for 1 h, the mixture was placed in the cold room overnight. The solid was collected and washed with cold EtOAc. There was obtained 114.4 g (75.6%) of the salt; mp 210–213 °C; $[\alpha]^{23}{}_D$ −48.3° (*c* 1.04, MeOH).

Recrystallization of 109.4 g of this salt from 1.1 L of MeOH and 2.2 L of EtOAc gave 87.87 g of pure salt; mp 210–214 °C; $[\alpha]^{23}{}_D$ −50.0° (*c* 1.02, MeOH).

A suspension of 87.87 g of the salt in 1617 mL of H_2O and 650 mL of MeOH was broughtr to pH 1.5 with dilute HCl. Almost all dissolved and another solid formed. After being stirred at room temperature for 1 h, the mixture was diluted with 878 mL of H_2O and cooled. The solid was collected and washed with H_2O. There was obtained 52.63 g of pure **7c**.

Resolution of 7b To Give 7d. By a procedure similar to the above but using (*R*)-(+)-α-methylbenzylamine as the resolving agent there was obtained **7d** as a crystalline solid.

Hydrolysis of 7c To Give 4b. A suspension of 66.28 g (0.242 mol) of **7c** in 660 mL of concentrated HCl and 660 mL of H_2O was heated at reflux for 4 h. All material was in solution after approximately 15 min. At the end of the heating period the solution was diluted with 660 mL of H_2O and cooled. The precipitated benzoic acid was filtered off and the filtrate extracted twice with $CHCl_3$. The pH was brought to 6.4 with 50% NaOH and the material concentrated to a slush on a rotary evaporator. This material was transferred to a tray and dried in a vacuum oven, leaving 516 g of a white solid. This material was digested with two 3-L portions of boiling anhydrous EtOH. Removal of the EtOH under reduced pressure left 52.5 g of the crude product. This material was desalted by passing over an Amberlite IR-120 column, eluting with 2 N NH_4OH. Removal of the solvent under

---

(20) Noland, W. E.; Baude, F. J. *Organic Syntheses*; Wiley: New York, 1973; Collect. Vol. V, p 567.

reduced pressure left 41 g of a white solid. Two recrystallizations from MeOH/anhydrous EtOH gave 36.01 g (87.8%) of resolved product, **4b**.

Hydrolysis of 7d To Give 4c. Hydrolysis of **7d** followed by a workup similar to the above gave **4c**.

(2α,3aβ,7aβ)-Octahydro-1*H*-indole-2-carboxylic Acid 1,1-Dimethylethyl Ester (4e). A solution of 14.23 g (0.084 mol) of **4a** in 150 mL of dioxane contained in a pressure vessel was treated with 15 mL of concentrated H_2SO_4 and 84 g of isobutylene and kept at 20 °C for 20 h with stirring. The mixture was then poured into ice water containing 45 mL (0.86 mol) of 50% NaOH solution and the mixture extracted three times with ether. The ether was washed with H_2O and then saturated NaCl solution. Drying over $MgSO_4$ and removal of the ether under reduced pressure gave 14.4 g (76% yield) of the ester as an oil. The ester is sufficiently pure for use in subsequent reactions.

Resolution of 4e To Give 4f. A solution of 3.3 g (0.022 mol) of *l*-tartaric acid (unnatural tartaric acid) in 20 mL of hot THF was treated with 5.0 g (0.022 mol) of **4e** in 10 mL of THF and the resulting solution was allowed to stand at room temperature overnight. The precipitated solid was collected and dried, giving 3.9 g of salt; mp 147–148 °C; $[\alpha]^{23}{}_D$ −26.9° (*c* 1.09, MeOH). Recrystallization from 80 mL of EtOAc/MeOH (1:1) while the temperature was maintained at 0 °C overnight gave 3.11 g of pure salt; mp 152–153 °C; $[\alpha]^{23}{}_D$ −31.7° (*c* 1.1, MeOH).

A suspension of 3.06 g of this salt in 30 mL of Et_2O was shaken with 30 mL of 5% Na_2CO_3 solution. The layers were separated, and the aqueous phase was extracted two times with Et_2O. The combined Et_2O layers were washed with H_2O and dried over $MgSO_4$. Removal of the Et_2O under reduced pressure gave 1.82 g of **4f** as an oil. The product was sufficiently pure for use in subsequent reactions.

Resolution of 4e To Give 4g. A solution of 17.17 g (0.114 mol) of *d*-tartaric acid (natural tartaric acid) in 130 mL of hot THF was treated with a solution of 25.78 g (0.114 mol) of **4e** (recovered from previous resolutions and enriched in the (+)-rotating isomer) in 85 mL of THF and allowed to stand at room temperature overnight. The precipitated solid was collected and dried, giving 36.6 g of salt. Recrystallization from 750 mL of EtOAc/MeOH (1:1) gave 31.55 g of pure salt; mp 149–150 °C dec; $[\alpha]^{23}{}_D$ +31.5° (*c* 1.02, MeOH).

A suspension of this salt in 320 mL of Et_2O was shaken with 320 mL of 5% Na_2CO_3 solution. The layers were separated, and the aqueous phase was extracted two times with Et_2O. The combined Et_2O layers was washed with H_2O and dried over $MgSO_4$. Removal of the solvent under reduced pressure gave 18.57 g of **4g** as an oil. The product was sufficiently pure for use in subsequent reactions.

Octahydro-1*H*-isoindole-1-carboxylic Acid Hydrochloride (5a) and Esters 5b and 5c. A solution of 2.14 g of methyl 2,3-dihydro-1*H*-isoindole-1-carboxylate hydrochloride[21] in 100 mL of MeOH and 5 mL of HOAc was hydrogenated in a Parr apparatus using 0.5 g of a 10% Rh/C catalyst. When the required amount of hydrogen had been taken up, the catalyst was filtered off and the filtrate concentrated under reduced pressure. Trituration of the residue with Et_2O gave a solid. Recrystallization from $MeOH/Et_2O$ gave pure methyl octahydro-1*H*-isoindole-1-carboxylate hydrochloride, mp 181–183 °C.

A solution of 6.25 g of this compound was dissolved in 55 mL of 10% HCl and heated at reflux for 4 h. The solvent was removed under reduced pressure and the residue recrystallized from H_2O to give **5a**.

The esters **5b** and **5c** were prepared by hydrogenating the corresponding esters of 2,3-dihydro-1*H*-isoindole-1-carboxylic acid hydrochloride. These in turn were prepared according to ref 21 (see Scheme II) by substituting EtOH or *t*-BuOH for the MeOH used in the esterification of α,α′-dibromo-2-methylbenzeneacetyl chloride. Cyclization with benzylamine gave the 2,3-dihydro-2-(phenylmethyl)-1*H*-isoindole-1-carboxylic acid esters in 35–40% yield. Debenzylation was accomplished by hydrogenation with 20% Pd/C in MeOH.

Octahydro-3-oxo-1*H*-isoindole-1-carboxylic Acid (6). A solution of 15 g of ethyl 2,3-dihydro-3-oxo-1*H*-isoindole-1-carboxylic acid ethyl ester,[22] **22b** in 110 mL of THF, and 110 mL of absolute EtOH was hydrogenated in a Parr apparatus using 1.0 g of 10% Rh/C as a catalyst. After the required amount of hydrogen had been absorbed, the catalyst was filtered off and the filtrate concentrated under reduced pressure. Recrystallization of the residue from EtOH gave octahydro-3-oxo-1*H*-isoindole-1-carboxylic acid ethyl ester, mp 149–157 °C.

A solution of 5 g of this ester in 10 mL of EtOH and 48 mL of 1 N NaOH was stirred at room temperature overnight. After removal of the EtOH under reduced pressure and adjustment of the pH to 2.0, the crude acid precipitated. Recrystallization from H_2O gave **6**.

Biological Methods. A. In Vitro ACE Inhibition. The in vitro ACE inhibitory activity was determined by a radioassay procedure reported previously.[7] Activity is reported as the IC_{50}, which is the approximate molar concentration of test compound causing a 50% inhibition of the control converting-enzyme activity. The test solutions were prepared by dissolving 2–5 mg of test compound in 1 mL of Me_2SO and diluting to the desired concentration with a pH 8 buffer of 0.05 M Hepes (Calbiochem), 0.1 M NaCl, and 0.6 M Na_2SO_4 in H_2O.

B. Blood Pressure and Heart Rate Test in the Conscious Rat. Hypertension of renal origin was produced in 4-week-old Sprague–Dawley male albino rats (Charles River; Wilmington, MA) by placing a silver clip (0.2-mm gap) around the left renal artery near the aorta and leaving the contralateral kidney intact. Hypertension was allowed to develop for 4–8 weeks. The rats were then cannulated for blood pressure monitoring as described previously.[23] Only rats with mean aortic blood pressure of > 160 mmHg were used. At the time of cannulation the rats reighed 280–320 g. The rats were given free access to a standard lab chow (5012 Purina; Richmond, IN) and tap water and were maintained on a 12 h dark/12 h light cycle. One minute running average values of heart rate and aortic blood pressure (mean, systolic, and diastolic) for each rat were recorded every 30th min by means of a computer-assisted data capture system.

Acknowledgment. We thank Dr. F. A. MacKellar and associates for the analytical and spectral data, D. Schweiss for the preparation of compound **12**, and Dr. R. Meyer for the preparation of compound **13**. We also thank Prof. A. MacPhail of Duke University for the X-ray crystallographic determinations.

Supplementary Material Available: X-ray data, fractional coordinates and thermal parameters, and interatomic distances and angles for compounds **12** and **10** (19 pages). Ordering information is given on any current masthead page.

---

(21) Cignarella, G.; Cerri, R.; Grella, G.; Sanna, P. *Gazz. Chim. Ital.* **1976**, *106*, 65.

(22) Darapsky, A.; Heinrichs, P. *J. Prakt. Chem.* **1936**, *146*, 307.

(23) Smith, R. D.; Wood, T. J.; Tessman, D. K.; Olszewski, B. J.; Currier, G.; Kaplan, H. R. *DHEW Publ.* (*NIH*) (*U.S.*) **1980**, *41*, 1473.