elements, analytical results were within ±0.4% of the theoretical values.

***erythro*-9-(2-Hydroxy-3-nonyl)purine (2; Mixture of Two Isomers: 2′*S*,3′*R* and 2′*R*,3′*S*).** Racemic *erythro*-5-amino-4-chloro-6-(2-hydroxy-3-nonyl)pyrimidine (**6**) [mp 121–122 °C; TLC R_f ($CHCl_3$/MeOH/Et_3N, 86:6:8) ~0.5, yield 1.165 g (4.05 mmol)] was synthesized and treated with triethyl orthoroformate according to published procedures.[10] TLC showed the absence of starting material after 1.5 h. After 1 day, the clear solution was evaporated in vacuo to a yellow oil containing crude *erythro*-6-chloro-9-(2-hydroxy-3-nonyl)purine (**5**). An ice-cold ethanolic solution of the crude product was treated with 550 mg of potassium acetate and hydrogenated in the presence of palladium (from 220 mg of 20% palladium oxide on barium carbonate) at atmosphere pressure for 5 h. After filtering and washing the solid with methanol, the solution was concentrated to a black residue. The residue was chromatographed over 15 g of silica gel packed in chloroform. The column was developed with 50 mL of chloroform and then with chloroform containing methanol in increasing concentration: 50 mL of 1%, 50 mL of 2%, 100 mL of 3% (fractions 21–34), and finally 60 mL of 5% (fractions 35–43). Fractions 23–34 were combined to yield 490 mg (46%) of a viscous residue: TLC R_f 0.34 (7% MeOH in $CHCl_3$); UV (MeOH) λ_{max} 264 nm (log ϵ 3.86). Anal. ($C_{14}H_{22}N_4O \cdot 0.15H_2O$) C, H, N.

***erythro*-1,6-Dihydro-6-(hydroxymethyl)-9-(2-hydroxy-3-nonyl)purine (4; Mixture of Four Isomers: 2′*S*,3′*R*,6*R*, 2′*R*,3′*S*,6*R*, 2′*S*,3′*R*,6*S*, and 2′*R*,3′*S*,6*S*).** A solution of 244 mg of **2** (0.92 mmol) in 50 mL of dried methanol in a rotating quartz cylinder (15 cm long × 5 cm o.d.) was irradiated with four 15-W G.E. germicidal lamps as described by Connolly and Linschitz[13] under a nitrogen atmosphere for 230 min. The solution was evaporated in vacuo to 285 mg of solid residue, which was then chromatographed over a column of 2 g of silica gel (8.8 × 1.0 cm o.d.). The column was eluted with 6 mL of chloroform and then with chloroform containing increasing amounts of methanol: 27 mL of 2% (fractions 3–15), 15 mL of 4% (fractions 16–22), 15 mL of 3% (fractions 23–27), and 10 mL of 8% (fractions 28–29). The product was obtained from fractions 18–27 as 157 mg (55%) of solid: TLC R_f 0.19 (15% MeOH in $CHCl_3$); mp ~67–72 °C; UV (H_2O) λ_{max} 292 nm (log ϵ 3.60), 244 (3.37); UV (MeOH) λ_{max} 295 nm (log ϵ 3.65), 245 (3.40); the UV max at 292 nm of a 0.004% aqueous solution decreased by about 5% after 5 days at room temperature; ^1H NMR ($CDCl_3$, after D_2O exchange), 5.11 (dd, 1 H, $HCCH_2OH$, J = 3.5 and 7.0 Hz), 7.0 (s, 1 H, H-2), 7.16 (s, ~0.45 H) and 7.18 (s, ~0.55 H) (H-8 singlets from each of two *dl* pairs); the NMR sample after D_2O exchange showed extensive decomposition after 1 day; mass spectrum, m/e 294.2 (2.85%), 277.2 (7.37%), 264.3 (18.3%), 263.2 (100%), 219.1 (5.62%), 120.9 (19.46%). Anal. ($C_{15}H_{16}N_4O_2 \cdot 0.13CHCl_3$) C, H, N.

Assays of Adenosine Deaminase Inhibitory Activity. A modification of the procedure of Kalckar[14] was used. Calf intestinal mucosal ADA (EC 3.5.4.4; Sigma Chemical Co., type I) suspended in 3.2 M ammonium sulfate (buffer), 50 μL (125 units), was added to 5 mL of a 0.025 M (pH 8) ammonium acetate buffer, and the solution was dialyzed using Spectra/Por 1 cellulose membrane tubing (Fisher) against 500 mL of the same buffer for 48 h at 5 °C, changing the dialyzing bath every 12 h. Such a preparation, useable over a period of a few weeks when stored at 5 °C, was diluted about 20- to 50-fold with 0.050 M (pH 7.5) phosphate buffer, as needed, to a concentration which would give an uninhibited deamination rate of $1–3 \times 10^{-8}$ M/s with ca. 6×10^{-6} M adenosine by the assay procedure below.

The rates of deamination were determined at 25 °C by monitoring for about 3 min the drop in absorbance at 265 nm against six or more varying concentrations of adenosine (8.6×10^{-5} to 6.9×10^{-6} M) and a fixed concentration of the inhibitor (within ±250% of the eventually determined K_i value) in 0.05 M (pH 7.5) phosphate buffer. The reaction was started by adding 20 μL of the diluted ADA to a premixed solution, prepared about 2 min earlier from solutions of the inhibitor (0.02 mL) and of adenosine (3.02 mL). The initial slopes were converted to "molar concentration per second" rates with a factor of 8100 (confirmed experimentally) as $\Delta\epsilon$ between adenosine and inosine. Subsequent Lineweaver–Burk[15] treatment of the data established the type of inhibition (competitive) and gave the K_i values tabulated in Table I.

Acknowledgment. The authors thank Dr. Charles F. Schwender, Warner-Lambert/Parke-Davis, for helpful discussions and Jeff C. Hanvey, University of Alabama, for technical assistance. This work was supported, in part, by Grant CA-26465 (to D.C.B.) from the National Institutes of Health.

(14) Kalckar, H. M.; Shafran, M. *J. Biol. Chem.* **1947**, *167*, 461.
(15) Lineweaver, H.; Burk, D. *J. Am. Chem. Soc.* **1934**, *56*, 658.

Renin Inhibitors. Substitution of the Leucyl Residues of Leu-Leu-Val-Phe-OCH_3 with 3-Amino-2-hydroxy-5-methylhexanoic Acid

Rodney L. Johnson

Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455.
Received September 14, 1981

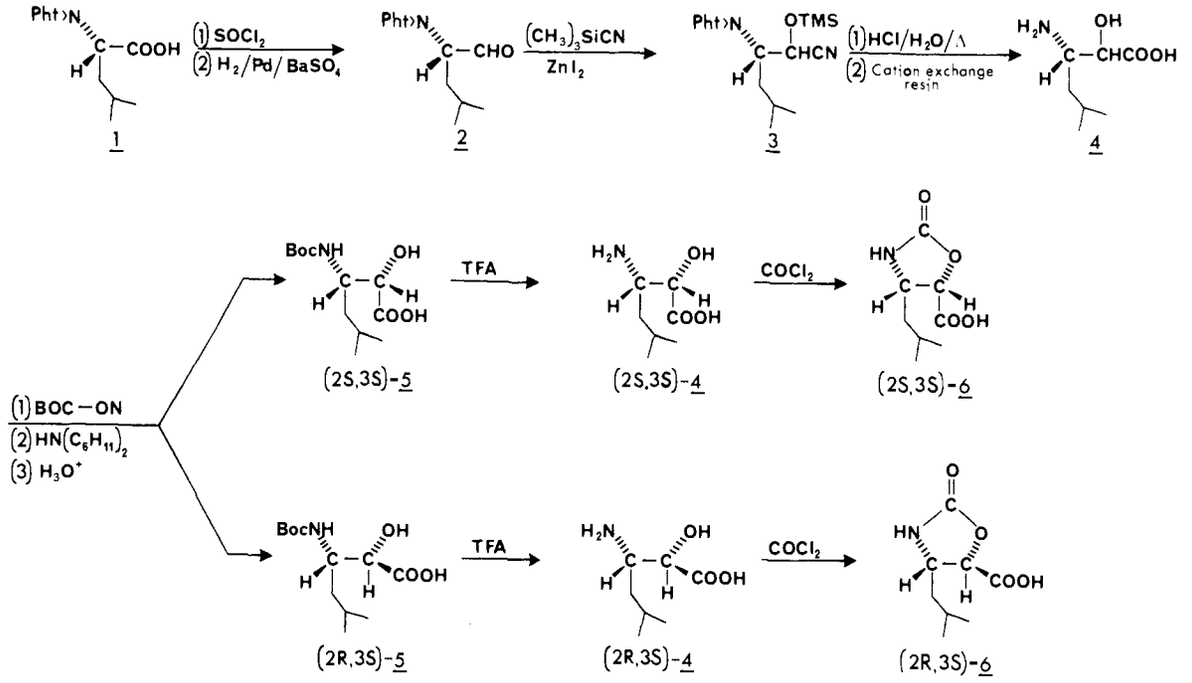
The 2*S*,3*S* and 2*R*,3*S* diastereoisomers of the hydroxy amino acid 3-amino-2-hydroxy-5-methylhexanoic acid (AHMHA) were synthesized and substituted for the leucyl residues of Leu-Leu-Val-Phe-OCH_3 to yield the following analogues: AHMHA-Leu-Val-Phe-OCH_3, AHMHA-Val-Phe-OCH_3, and Leu-AHMHA-Val-Phe-OCH_3. These analogues were tested in vitro for their ability to inhibit human amniotic renin. All of the analogues were found to inhibit renin to some extent with inhibitory constants in the range of 10^{-3} to 10^{-4} M. The analogues AHMHA-Leu-Val-Phe-OCH_3 and AHMHA-Val-Phe-OCH_3 exhibited competitive inhibition when the 2*S*,3*S* isomer of AHMHA was employed and noncompetitive kinetics when the 2*R*,3*S* isomer of AHMHA was used. For the Leu-AHMHA-Val-Phe-OCH_3 analogues, competitive kinetics were observed regardless of the isomer of AHMHA employed. These latter analogues also proved to be the most active in the above series.

In a previous report[1] I described the synthesis and renin inhibitory activity of several *N*-(α-hydroxyalkanoyl) derivatives of Leu-Val-Phe-OCH_3. These compounds were synthesized in an attempt to mimic the postulated transition state of the renin–angiotensinogen reaction. It was felt that the α-hydroxy moiety of the α-hydroxyalkanoyl residue might simulate the hydroxyl moiety that is thought to be formed when the Leu[10] carbonyl group is converted into a tetrahedral intermediate during the enzymatic reaction. The results of this previous study[1] showed that the replacement of the N-terminal leucyl residue of the known substrate analogue inhibitor Leu-Leu-Val-Phe-OCH_3[2] with various α-hydroxyalkanoyl residues led to

(1) R. L. Johnson, *J. Med. Chem.*, **23**, 666 (1980).

0022-2623/82/1825-0605$01.25/0

Scheme I



inhibitors of renin which were up to five times more active than Leu-Leu-Val-Phe-OCH_3.

In an attempt to improve the renin inhibitory activity of the α-hydroxyalkanoyl derivatives, we have in the present study replaced the leucyl residues of the substrate analogue inhibitor Leu-Leu-Val-Phe-OCH_3 with the hydroxy amino acid residue 3-amino-2-hydroxy-5-methylhexanoic acid (**4**, AHMHA). This residue was chosen because it resembled the α-hydroxyalkanoyl residues used previously in that it possessed a hydroxyl group on the α-carbon atom. In addition, it was felt that the presence of the 3-amino group would enhance the aqueous solubility of this type of inhibitor, since the inhibitors could be prepared as hydrochloride salts. Furthermore, the amino moiety could serve as a handle whereby additional amino acid residues could be added to the polypeptide if so desired. The synthesis of hydroxy amino acid **4** and the renin inhibitory activity of the polypeptides in which **4** has been incorporated are described below.

Results and Discussion

Syntheses. The hydroxy amino acid 3-amino-2-hydroxy-5-methylhexanoic acid (**4**) is a novel amino acid which is found in the small polypeptide amastatin. This polypeptide is produced by streptomyces and has been found to inhibit leucine aminopeptidase and aminopeptidase A.[3] The isomer of **4** which is found in amastatin is the 2*S*,3*R* isomer.[4,5]

In the present study, the 2*S*,3*S* and 2*R*,3*S* isomers of **4** were synthesized as shown in Scheme I. *N*-Phthalyl-L-leucine (**1**) served as the starting material for this synthesis. This material was converted into *N*-phthalyl-L-leucinal through a Rosemund reduction of the acid chloride of **1**. The method used was similar to that previously reported by Balenovic et al.[6,7] The aldehyde was obtained in an overall yield of 64% without racemization. The optical purity of **2** was verified by converting the aldehyde to L-leucinol hydrochloride. This was accomplished by reducing **2** with $NaBH_4$ and then hydrolyzing the resulting product with aqueous hydrochloric acid. The L-leucinol hydrochloride obtained from **2** was found to possess an optical rotation which was very similar to that reported in the literature[8] and identical with that obtained when commercially available L-leucinol (Aldrich Chemical Co.) was converted into its hydrochloride salt. Aldehyde **2** was treated with trimethylsilyl cyanide to give the trimethylsilyl cyanohydrin **3** as a mixture of diastereomers (2*S*,3*S*)-**3** and (2*R*,3*S*)-**3**. The addition of trimethylsilyl cyanide to **2** showed some degree of stereoselectivity in that the ratio of (2*S*,3*S*)-**4** to (2*R*,3*S*)-**4** which was obtained from **3** was found to be consistently in the range of 3:1. The trimethylsilyl cyanohydrin **3** was treated with aqueous acid to give the corresponding cyanohydrin, which in turn was hydrolyzed to the hydroxy amino acid **4** with a mixture of 6 N HCl and dioxane. Passage of the hydrolysis product through a cation-exchange resin provided a 75% yield of **4** as a mixture of diastereomers.

The separation of the 2*S*,3*S* isomer of **4** from the 2*R*,3*S* isomer was accomplished by first converting **4** into its *tert*-butoxycarbonyl derivative using 2-[[(*tert*-butoxycarbonyl)oxy]imino]-2-phenylacetonitrile and then forming the dicyclohexylammonium salt in acetone. On standing, the dicyclohexylammonium salt of 3(*S*)-[(*tert*-butoxycarbonyl)amino]-2(*S*)-hydroxy-5-methylhexanoic acid precipitated from the solution, while the dicyclohexylammonium salt of the other diastereoisomer, 3(*S*)-[(*tert*-butoxycarbonyl)amino]-2(*R*)-hydroxy-5-methylhexanoic acid, remained in solution. The dicyclohexylammonium

(2) T. Kokubu, K. Hiwada, T. Ito, E. Ueda, Y. Yamamura, T. Mizoguchi, and K. Shigezane, *Biochem. Pharmacol.*, **22**, 3217 (1973).
(3) T. Aoyagi, H. Tobe, F. Kojima, M. Hamada, T. Takeuchi, and H. Umezawa, *J. Antiobiot.*, **31**, 636 (1978).
(4) H. Tobe, H. Morishima, H. Naganawa, T. Takita, T. Aoyagi, and H. Umezawa, *Agric. Biol. Chem.*, **43**, 591 (1979).
(5) D. H. Rich, B. J. Moon, and A. S. Boparai, *J. Org. Chem.*, **45**, 2288 (1980).
(6) K. Balenovic, N. Bregant, D. Cerar, D. Fles, and I. Jambresic, *J. Org. Chem.*, 18, 297 (1973).
(7) K. Balenovic, N. Bregant, T. Galijan, Z. Stefanac, and V. Skaric, *J. Org. Chem.*, **21**, 115 (1956).
(8) G. S. Poindexter and A. I. Meyers, *Tetrahedron Lett.*, 3527 (1977).

Table I. Physical Properties of the Protected Polypeptides Containing (2*S*,3*S*)-AHMHA and (2*R*,3*S*)-AHMHA

no.	compd [a]	chirality [b]	yield, %	mp, °C	$[\alpha]_D^{25}$, deg (*c*, CH_3OH)	formula [c]
7	Boc-AHMHA-Leu-Val-Phe-OCH_3	2*S*,3*S*	64	189-191	−60 (0.55)	$C_{33}H_{54}N_4O_8$
8	Boc-AHMHA-Leu-Val-Phe-OCH_3	2*R*,3*S*	81	153-155	−37.2 (0.5)	$C_{33}H_{54}N_4O_8$
9	Boc-AHMHA-Val-Phe-OCH_3	2*S*,3*S*	53	171-174	−57.5 (0.63)	$C_{27}H_{43}N_3O_7$
10	Boc-AHMHA-Val-Phe-OCH_3	2*R*,3*S*	84	122-125	−18 (0.5)	$C_{27}H_{43}N_3O_7$
11	Boc-Leu-AHMHA-Val-Phe-OCH_3	2*S*,3*S*	77	208-209	−67.8 (1.04)	$C_{33}H_{54}N_4O_8$
12	Boc-Leu-AHMHA-Val-Phe-OCH_3	2*R*,3*S*	73	110-112	−37.7 (1.0)	$C_{33}H_{54}N_4O_8$

[a] AHMHA refers to 3-amino-2-hydroxy-5-methylhexanoic acid (4). [b] Chirality of AHMHA. [c] All compounds were analyzed for C, H, and N and were within ± 0.4% of the calculated values.

Table II. Physical Properties of the Deprotected Polypeptides Containing (2*S*,3*S*)-AHMHA and (2*R*,3*S*)-AHMHA

no.	compd [a]	chirality	yield, %	TLC, R_f [c]	mp, °C	$[\alpha]_D^{25}$, deg (*c*, CH_3OH)	formula [d]
13	AHMHA-Leu-Val-Phe-OCH_3·HCl	2*S*,3*S*	100	0.63	219-222	−53.6 (0.5)	$C_{28}H_{47}N_4O_6Cl$
14	AHMHA-Leu-Val-Phe-OCH_3·HCl	2*R*,3*S*	87	0.71	138-140	−23.5 (0.53)	$C_{28}H_{47}N_4O_6Cl$
15	AHMHA-Val-Phe-OCH_3·HCl	2*S*,3*S*	63	0.69	227-229	−45.2 (0.53)	$C_{22}H_{36}N_3O_5Cl$
16	AHMHA-Val-Phe-OCH_3·HCl	2*R*,3*S*	79	0.67	112-115	+12.3 (0.57)	$C_{22}H_{36}N_3O_5Cl$
17	Leu-AHMHA-Val-Phe-OCH_3·HCl	2*S*,3*S*	76	0.72	203-206	−41.9 (0.52)	$C_{28}N_{47}N_4O_6Cl$
18	Leu-AHMHA-Val-Phe-OCH_3·HCl	2*R*,3*S*	84	0.79	128-130	−10 (0.55)	$C_{28}N_{47}N_4O_6Cl$

[a] AHMHA refers to 3-amino-2-hydroxy-5-methylhexanoic acid (4). [b] Chirality of AHMHA. [c] TLC was conducted on silica gel using a solvent system consisting of 1-propanol-NH_4OH (4:1). Compounds were visualized with UV and ninhydrin. [d] All compounds were analyzed for C, H, and N and were within ± 0.4% of the calculated values.

Table III. Renin Inhibitory Activity of Analogues of Leu-Leu-Val-Phe-OCH_3 Substituted with 3-Amino-2-hydroxy-5-methylhexanoic Acid (AHMHA)

no.	analogue [a]	chirality [b]	type of inhibn [c]	K_i, mM
13	AHMHA-Leu-Val-Phe-OCH_3·HCl	2*S*,3*S*	C	0.96
14	AHMHA-Leu-Val-Phe-OCH_3·HCl	2*R*,3*S*	NC	1.52
15	AHMHA-Val-Phe-OCH_3·HCl	2*S*,3*S*	C	2.44
16	AHMHA-Val-Phe-OCH_3·HCl	2*R*,3*S*	NC	1.01
17	Leu-AHMHA-Val-Phe-OCH_3·HCl	2*S*,3*S*	C	0.52
18	Leu-AHMHA-Val-Phe-OCH_3·HCl	2*R*,3*S*	C	0.44
19	Leu-Leu-Val-Phe-OCH_3·HCl [d]		C	1.14
20	L-α-hydroxyisocaproyl-Leu-Val-Phe-OCH_3 [d]		C	0.23

[a] AHMHA = 3-amino-2-hydroxy-5-methylhexanoic acid (4). [b] Chirality of AHMHA. [c] C = competitive; NC = noncompetitive. [d] Renin inhibitory activity reported in ref 1.

salts were converted to (2*S*,3*S*)-**5** and (2*R*,3*S*)-**5**, respectively, with citric acid. These *tert*-butoxycarbonyl derivatives were used in the subsequent coupling reactions. Portions of the *tert*-butoxycarbonyl derivatives were converted into the respective hydroxy amino acid **4** with trifluoroacetic acid, followed by passage through an ion-exchange column. The 2*S*,3*S* isomer of **4** yielded an NMR spectrum in which the α-methine proton appeared as a doublet at δ 4.03 with a coupling constant of 3.5 Hz, while the 2*R*,3*S* isomer yielded an NMR spectrum in which the α-methine proton appeared as a doublet at δ 3.97 with a coupling constant of 4.0 Hz. The optical rotations of (2*S*,3*S*)-**4** and (2*R*,3*S*)-**4** were of the same magnitude but of opposite sign to those reported for (2*R*,3*R*)-**4** and (2*S*,3*R*)-**4** in the literature.[4] The stereochemical assignments were verified by converting (2*S*,3*S*)-**4** and (2*R*,3*S*)-**4** into their respective oxazolidinones, (2*S*,3*S*)-**6** and (2*R*,3*S*)-**6**, with phosgene and then measuring the vicinal coupling, $J_{\alpha,\beta}$, between the protons on the α and β carbons. Such an approach has been used before to determine the absolute stereochemistry of α-amino-β-hydroxy acids[9] and β-hydroxy-γ-amino acids.[10] In these studies it has been shown that the vicinal coupling constant for the *cis*-oxazolidinones is around 9 Hz, while that for the *trans*-oxazolidinones is around 5 Hz. The use of this approach in the present study confirmed the stereochemical assignments made on the isolated diastereoisomers of **4** in that (2*S*,3*S*)-**4** yielded an oxazolidinone in which the vicinal coupling constant was 8.5 Hz, while the (2*R*,3*S*)-**4** isomer yielded an oxazolidinone with a vicinal coupling constant of 4.5 Hz. These results were consistent with the results that Tobe et al.[4] obtained with their oxazolidinone derivatives of (2*R*,3*R*)-**4** and (2*S*,3*R*)-**4**.

The *tert*-butoxycarbonyl derivatives of **4**, (2*S*,3*S*)-**5** and (2*R*,3*S*)-**5**, were coupled to Leu-Val-Phe-OCH_3 and Val-Phe-OCH_3 using the dicyclohexylcarbodiimide/1-hydroxybenzotriazole method of coupling. The various protected polypeptides that were synthesized with the two isomers of **4** are shown in Table I. Table II lists the unprotected forms of the polypeptides shown in Table I. These were obtained by removal of the *tert*-butoxycarbonyl protecting group using 4 N HCl in dioxane.

Biology. Compounds **13**–**18** (Table II) were all tested for their ability to inhibit human amniotic renin. The method of preparation of the enzyme used in this study has been described by us previously,[11] as have the details of the renin assay used.[1] The nature of each compound's inhibitory activity and its inhibitory constant (K_i) were determined with Dixon plots.[12] These plots are shown in Figure 1, and the results are summarized in Table III. Although all of the polypeptides which had hydroxy amino

(9) S. Futagawa, T. Inui, and T. Shiba, *Bull. Chem. Soc. Jpn.*, **46**, 3308 (1973).
(10) D. H. Rich, E. T. O Sun, and E. Ulm, *J. Med. Chem.*, **23**, 27 (1980).
(11) R. L. Johnson, A. M. Poisner, and R. D. Crist, *Biochem. Pharmacol.*, 28, 1791 (1979).
(12) M. Dixon, *Biochem. J.*, **55**, 1970 (1953).

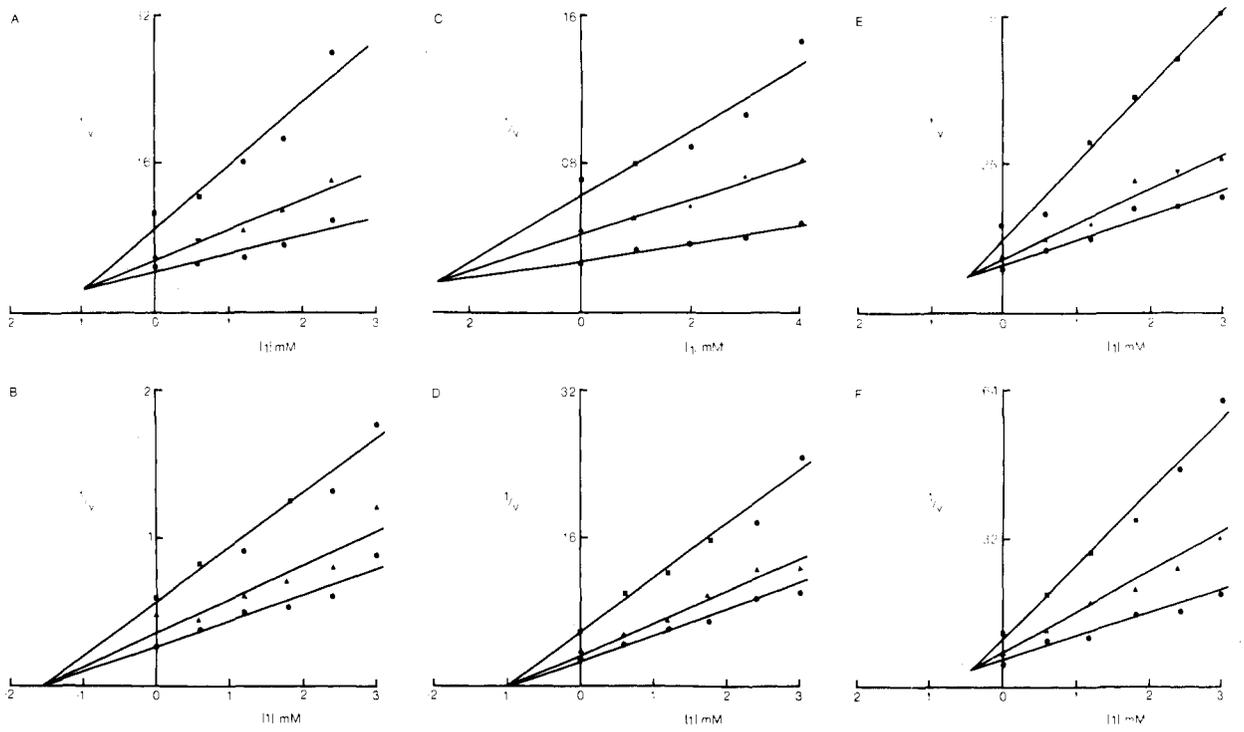


Figure 1. Dixon plots of the inhibition of human amniotic renin by compounds 13–18. For each graph is the reaction velocity (nmol of angiotensin I mL^{-1} h^{-1}) determined at three concentrations of porcine angiotensinogen [0.3 (●), 0.2 (▲), and 0.1 μM (■)] in the presence of varying millimolar concentrations of the given inhibitor: A, compound 13; B, compound 14; C, compound 15; D, compound 16; E, compound 17; F, compound 18.

acid 4 incorporated within their structure inhibited renin to some extent, no dramatic increase was seen when the compounds were compared to the previously reported inhibitors Leu-Leu-Val-Phe-OCH_3 (**19**) and L-α-hydroxyisocaproyl-Leu-Val-Phe-OCH_3 (**20**). Only compounds **17** and **18** were found to be more active than **19**. These two polypeptides were, however, only half as active as **20**. Although incorporation of 3-amino-2-hydroxy-5-methylhexanoic acid (**4**) into the tetrapeptide sequence Leu-Leu-Val-PheOCH_3 did not bring about a dramatic increase in renin inhibitory activity, it did bring about interesting changes in the type of inhibition that the polypeptides containing AHMHA exhibited. In the previous study, all of the *N*-(α-hydroxyalkanoyl) derivatives of Leu-Val-Phe-OCH_3 exhibited competitive inhibition of renin. In the present study, both competitive and noncompetitive inhibition was observed. The type of inhibition which was observed with the polypeptides synthesized in this study seemed to be dependent on two factors: (1) the position of the AHMHA residue in the polypeptide chain and (2) the chirality of the α-carbon atom of the AHMHA residue. When the AHMHA residue was the N-terminal residue and when the chirality of the α-carbon of AHMHA was *S* (compounds **13** and **15**), competitive kinetics were observed. Noncompetitive kinetics were observed when the chirality of the α-carbon of the AHMHA residue was *R* (compounds **14** and **16**). When the AHMHA residue was one of the internal residues (compounds **17** and **18**) of the polypeptide, the analogues exhibited competitive kinetics regardless of the chirality of the α-carbon of the AHMHA residue.

Unlike the previous studies[1] which demonstrated that replacement of the *N*-leucyl residue of **19** with *N*-α-hydroxyalkanoyl residues led to compounds with better renin inhibitory properties, the results of the present study have shown that an analogous substitution using the hydroxy amino acid AHMHA (**4**) does not produce a similar increase in renin inhibitory activity but appears instead to affect the type of inhibition (competitive vs. noncompetitive) that one sees. Studies are currently underway to determine if other hydroxy amino acids can be employed that will not only affect the type of inhibition observed but also increase the renin inhibitory activity.

Experimental Section

Melting points were determined on a Thomas-Hoover Unimelt. Specific rotations were measured with a Perkin-Elmer 141 polarimeter. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. All analytical results were with ±0.4% of the theoreticl values. NMR spectra were recorded on a Varian A-60D spectrometer.

***N*-Phthalyl-L-leucinal (2).** Phthalyl-L-leucine (1; 25 g, 0.096 mol) in anhydrous benzene (200 mL) was treated with thionyl chloride (11.9 g, 0.1 mol). The solution was refluxed for 6 h and then stripped of solvent in vacuo to give 26.8 g (100%) of crude phthalyl-L-leucyl chloride as a light orange oil. This material was dissolved in 100 mL of *m*-xylene, and 5% $Pd/BaSO_4$ (3 g) was added to the solution. The mixture was heated to 110 °C. Hydrogen was bubbled into the mixture until there was no evidence of HCl being emitted from the reaction (approximately 6 h). The mixture was cooled and then filtered to remove the catalyst. The filtrate was stripped of solvent in vacuo to give a clear oil, which was dissolved in Et_2O. This solution was washed with 1 M $NaHCO_3$ and then dried over $MgSO_4$. The ether was removed in vacuo to afford 20 g of an oil. Petroleum ether (60–70 °C) was added to the oil, and the resulting mixture was triturated at −70 °C. The petroleum ether was decanted off, and fresh petroleum ether was added. The mixture was warmed to room temperature, and the white precipitate present was collected to give 15 g (64%) of the desired aldehyde **2**: mp 44–46 °C; $[\alpha]^{25}_D$ −30.1° (*c* 1.45, benzene); NMR ($CDCl_3$) δ 9.90 (s, 1 H, CHO), 7.77 (m, 4 H, aromatic H's), 4.76 (dd, *J* = 8.5 and 9.5 Hz, 1 H, α-CH), 1.9–2.3 (m, 2 H, β-CH_2), 1.2–1.8 (m, 1 H, γ-CH), 0.97 [dd, 6 H, $(CH_3)_2$]. TLC analysis on silica gel using a solvent system consisting of $CHCl_3$–CH_3OH (9:1) gave a single spot (R_f 0.77) after both UV and iodine visualization.

In order to verify the optical purity of phthalyl-L-leucinal, a portion of the above material was converted to the known compound L-leucinol hydrochloride in the following manner. To a

cooled (4 °C) solution of **2** (1 g, 4 mmol) in EtOH (10 mL) was added in a dropwise manner a solution of $NaBH_4$ (0.15 g, 4 mmol) in EtOH (10 mL). After the addition of the $NaBH_4$ was complete, the solution was stirred for an additional 30 min at 4 °C. Acetic acid (0.3 mL) was then added to decompose the excess $NaBH_4$. The EtOH was removed in vacuo, and the resulting residue was partitioned between H_2O and EtOAc. The EtOAc layer was dried (Na_2SO_4) and then stripped of solvent to give a colorless oil. TLC and NMR indicated that no aldehyde was present. The oil was dissolved in dioxane (20 mL). Concentrated HCl (20 mL) was added, and the resulting solution was heated at reflux for 24 h. The solution was cooled and then washed with EtOAc (50 mL), followed by Et_2O (2 × 30 mL). The aqueous layer was evaporated to dryness, and the residue remaining was crystallized from EtOH–Et_2O to give 0.27 g (43%) of L-leucinol hydrochloride: mp 105 °C; $[\alpha]^{22}_D$ +12.0° (*c* 1.0, EtOH) [lit.[8] $[\alpha]^{20}_D$ +11.1° (EtOH)].

3-Amino-2-hydroxy-5-methylhexanoic Acid (4). Phthalyl-L-leucinal (**2**; 7.0 g, 28.5 mmol) was dissolved in CH_2Cl_2 (20 mL). This solution was added to a three-neck flask containing ZnI_2 (10 mg). The system was placed under a nitrogen atmosphere and then treated with trimethylsilyl cyanide (4.5 mL, 33.8 mmol). The solution was stirred at room temperature until no aldehyde peak could be observed in the NMR spectrum of the reaction mixture (approximately 12 h). The solution was stripped of solvent in vacuo to yield quantitatively the trimethylsilylcyanohydrin **3** as an oil. This material was immediately treated with 60 mL of 10% HCl. The mixture was stirred for 1.5 h, after which time it was extracted with CH_2Cl_2 (2 × 20 mL). The combined methylene chloride extracts were dried ($MgSO_4$) and then evaporated to dryness under reduced pressure to give an oil, which solidified on standing. The solid was triturated with petroleum ether (30–60 °C) and then collected by suction filtration to give 6.8 g (86%) of the cyanohydrin of phthalyl-L-leucinal as a brown solid, mp 113–117 °C. The crude diastereoisomeric mixture of cyanohydrin was dissolved in 120 mL of a 1:1 mixture of 6 N HCl and dioxane. The solution was refluxed for 48 h and then stripped of dioxane in vacuo. The remaining aqueous solution was washed with Et_2O (2 × 50 mL) and then evaporated to dryness in vacuo. The residue was dissolved in a small amount of water and then placed on a Bio-Rad AG-50W-X8 cation-exchange column containing 16 g of resin. The column was washed with distilled water until the pH of the eluant was neutral. At this time the hydroxy amino acid was eluted from the column with 2 N NH_4OH. The fractions containing **4** were evaporated to dryness, and the residues were triturated with acetone to afford 3 g (75%) of the mixture of diastereomers.

3(*S*)-Amino-2(*S*)-hydroxy-5-methylhexanoic Acid [(2*S*,3*S*)-4]. 3(*S*)-Amino-2(*RS*)-hydroxy-5-methylhexanoic acid (**4**; 3.5 g, 21.7 mmol) was dissolved in H_2O (30 mL) along with NEt_3 (2.4 g, 24 mmol). To this solution was added a solution of Boc-ON (5.9 g, 24 mmol) in dioxane (30 mL). The solution was stirred at room temperature overnight and then diluted with H_2O (60 mL) and EtOAc (60 mL). The aqueous layer was isolated and acidified with 10% citric acid. The acidified solution was extracted with EtOAc (2 × 50 mL). The combined EtOAc extracts were dried ($MgSO_4$) and then stripped of solvent in vacuo to give 5.7 g (100%) of the *tert*-butoxycarbonyl derivative of **4** as a viscous oil.

The *tert*-butoxycarbonyl derivative was dissolved in acteone (50 mL) and then treated with an equivalent amount of dicyclohexylamine (3.93 g, 21.7 mmol). On standing at room temperature, 5.63 g of fine white crystals was deposited. These were collected and recrystallized from CH_3OH–acetone to give 4 g of the dicyclohexylammonium salt of 3(*S*)-[(*tert*-butoxycarbonyl)-amino]-2(*S*)-hydroxy-5-methylhexanoic acid: mp 168–170 °C; $[\alpha]^{25}_D$ −16.2° (*c* 1.11, MeOH). Anal. ($C_{24}H_{46}N_2O_5$) C, H, N.

The dicyclohexylammonium salt was partitioned between EtOAc and 10% citric acid solution. The EtOAc layer was dried ($MgSO_4$) and then evaporated to dryness in vacuo to give quantitatively (2*S*,3*S*)-**5** as a glassy solid. This material was used in all subsequent coupling reactions. A portion of (2*S*,3*S*)-**5** (0.5 g, 1.9 mmol) was dissolved in 10 mL of CH_2Cl_2. To this solution was added 10 mL of trifluoroacetic acid. The solution was stirred at room temperature for 45 min and then stripped of solvent in vacuo. The residue obtained was dissolved in H_2O and then placed on a column containing 16 g of AG-50W-X8 cation-exchange resin. The hydroxy amino acid was eluted from the column using 2 N NH_4OH. Fractions containing (2*S*,3*S*)-**4** were evaporated to dryness to give a white solid, which after trituration with acetone was collected to give 290 mg (94%) of product: mp 237–239.5 °C dec; $[\alpha]^{25}_D$ −30.6° (*c* 0.53, AcOH). [lit.[4] reports mp 195–197 °C and $[\alpha]^{24}_D$ +34° (*c* 0.5, AcOH) for the enantiomer (2*R*,3*R*)-**4**]; NMR (D_2O) δ 4.03 (d, 1 H, *J* = 3.5 Hz, α-CH), 3.33–3.77 (m, 1 H, β-CH), 1.1–1.8 (m, 3 H, CH_2CH), 0.87 (dd, 6 H, CH_3). Anal. ($C_7H_{15}NO_3$) C, H, N.

3(*S*)-Amino-2(*R*)-hydroxy-5-methylhexanoic Acid [(2*R*,3*S*)-4]. The filtrate remaining from the crystallization of the dicyclohexylammonium salt of (2*S*,3*S*)-**5** was stripped of solvent in vacuo, and the residue was dissolved in EtOAc. This solution was washed with 10% citric acid (2 × 30 mL), dried ($MgSO_4$), and then evaporated to dryness to give 1.18 g of (2*R*,3*S*)-**5** as a glassy solid.

A portion of (2*R*,3*S*)-**5** (0.39 g, 1.5 mmol) was treated in the same manner as described above for (2*S*,3*S*)-**5**. A yield of 176 mg (74%) was obtained after recrystallization from a mixture of 1 propanol and H_2O: mp 228–229 °C, $[\alpha]^{25}_D$ +26.9° (*c* 0.32, AcOH) [lit.[4] reports mp 188–189 °C and $[\alpha]^{22}_D$ −28° (*c* 0.5, AcOH) for the enantiomer (2*S*,3*R*)-**4**]; NMR (D_2O) δ 3.90 (d, 1 H, *J* = 4 Hz, α-CH), 3.27–3.67 (m, 1 H, β-CH), 1.27–1.93 (m, 3 H, CH_2CH), 0.88 (d, 6 H, CH_3). Anal. ($C_7H_{15}NO_3$) C, H, N.

Oxazolidinone Derivative of (2*S*,3*S*)-4 [(2*S*,3*S*)-6]. Hydroxy amino acid (2*S*,3*S*)-**4** (100 mg, 0.62 mmol) was dissolved in 35 mL of 1 M KOH. The solution was cooled in an ice bath and then treated with 40 mL of 12.5% phosgene in toluene. The mixture was stirred vigorously for 1.5 h, after which time the layers were separated. The basic aqueous layer was acidified with 10% HCl and then extracted with EtOAc. The EtOAc layer was dried ($MgSO_4$) and then stripped of solvent in vacuo to afford 42 mg (36%) of crystalline oxazolidinone: mp 139–140.5 °C, $[\alpha]^{25}_D$ −36.2° (*c* 0.53, MeOH); NMR ($CDCl_3$) δ 9.01 (s, 1 H, CO_2H), 6.93 (s, 1 H, NH), 5.06 (d, 1 H, *J* = 8.5 Hz, C*H*OH), 4.0–4.46 (m, 1 H, CHN), 1.2–1.8 (m, 3 H, CH_2CH), 0.97 (dd, 6 H, CH_3).

Oxazolidinone Derivative of (2*R*,3*S*)-4 [(2*R*,3*S*)-6]. Hydroxy amino acid (2*R*,3*S*)-**4** (100 mg, 0.62 mmol) was treated in a manner analogous to that described above for the oxazolidinone derivative of (2*S*,3*S*)-**4**. Oxazolidinone (2*R*,3*S*)-**6** was obtained as an oil: yield 70 mg (60%); NMR ($CDCl_3$) δ 10.9 (br s, 1 H, COOH), 7.18 (br s, 1 H, NH), 4.68 (d, *J* = 4.5 Hz, 1 H, C*H*OH), 3.78–4.12 (m, 1 H, CHN), 1.5–1.83 (m, 3 H, CH_2CH), 0.98 (d, 6 H, CH_3).

General Procedure for the Coupling of the Protected Hydroxy Amino Acids to the Polypeptide Segments Leu-Val-Phe-OCH_3 and Val-Phe-OCH_3. The Boc-protected hydroxy amino acid (1.5 mmol), 1-hydroxybenzotriazole (2 mmol), and the polypeptide segment (Val-Phe-OCH_3·HCl or Leu-Val-Phe-OCH_3·HCl, 1.5 mmol) were dissolved in 5 mL of a mixture of THF and DMF (1:1). After *N*-methylmorpholine (1.5 mmol) was added to the solution, the solution was cooled in an ice bath. A solution of dicyclohexylcarbodiimide (1.5 mmol) in THF (2 mL) was added. The solution was stirred for 18 h, during which time the solution was slowly allowed to come to room temperature. After this time, the mixture was cooled and the precipitate of dicyclohexylurea was removed by filtration. The filtrate was stripped of THF in vacuo, leaving a DMF solution behind. This solution was treated with 1 M $NaHCO_3$. The precipitate that formed was collected, washed with H_2O, 10% citric acid, and H_2O, and then recrystallized from a mixture of methanol and H_2O to give the protected polypeptides shown in Table I.

General Procedure for the Removal of the *tert*-Butoxycarbonyl Protecting Group. The *tert*-butoxycarbonyl-protected polypeptide (0.6 mmol) was treated with 5 mL of 4 N HCl in dioxane for 45 min at room temperature. The solution was stripped of dioxane and HCl in vacuo to give a solid residue. This material was triturated with ether, collected, and dried in vacuo over KOH to give the unprotected polypeptides shown in Table II as hydrochloride salts.

Inhibition Studies. The ability of compounds 13–18 to inhibit renin was measured by determining the inhibitory constant (K_i) of each compound. Activated human amniotic prorenin served as the source of renin for these studies.[11] The K_i and the type of inhibition of each compound was determined through the use

of Dixon plots.[12] Data for these plots were obtained by measuring the reaction velocities of renin at three concentrations of porcine angiotensinogen (0.1, 0.2, and 0.3 μM) in the presence of varying concentrations of each inhibitor.

The enzymatic assay was carried out in a manner identical with that described previously.[1] Reaction velocities were expressed as the number of nanomoles of angiotensin I generated per milliliter per hour. Three determinations were made for each inhibitor concentration at each substrate level. A plot of $1/v$ vs. inhibitor concentration was made for each compound tested. All lines were calculated by linear regression analysis. The $-[I]$ value at the intersection of the three substrate line gave the K_i of the compound, while the competitive or noncompetitive nature of each inhibitor was determined by whether the point of intersection was above or on the x axis.

Acknowledgment. The excellent technical assistance of Karen Verschoor is acknowledged. This study was supported in part by a grant from the Graduate School of the University of Minnesota and by Grants HL 24795 and HL 00932 from the National Heart, Lung, and Blood Institute.

Book Reviews

Progress in Macrocyclic Chemistry. Volume 2. Edited by Reed M. Izatt and James J. Christensen. Wiley, New York. 1981. xi + 347 pp. 15 × 23 cm. $50.00.

As the title denotes, this book contains information on synthetic multidentate macrocyclic compounds of which crown ethers were the first. The five chapters are (1) "Complexation of Arenediazonium Ions by Multidentate Ligands", (2) "Stability Constants of Cation–Macrocycle Complexes and Their Effect on Facilitated Membrane Transport Rates", (3) "Solute Binding to Polymers with Macroheterocyclic Ligands", (4) "Synthetic Chiral Receptor Molecules from Natural Products", and (5) "Cyclopeptide Macrocycles".

For the medicinal chemist interested in utilizing multidentate macromolecules, Chapters 1–3 will provide detailed information about the phenomena and help to explain the results of the experimental behavior. For example, Chapter 1 includes discussions of spectral studies of arenediazonium salt–crown ether complexes and crown ethers as phase-transfer catalysts in various arenediazonium salt reactions. Chapter 3 covers interaction of macrocycles with ions and ion pairs in media of low polarity and ion binding to poly(vinyl) crown ethers in nonaqueous media, while Chapter 2 deals with subjects like factors affecting complex stability and an electrostatic model for cation–macrocyclic binding. These latter two topics should interest people concerned with receptor-site binding and fit.

For the medicinal chemist with stereochemical interests, Chapter 4 will be a delight. The design and synthesis of various systems, utilizing chiral natural products, plus a discussion of complexation and catalysis of these multidentate macrocycles are covered. The fifth chapter on cyclopeptide macrocycles encompasses the total topic from conventions and nomenclature to design and synthesis to compounds with specific cation binding potential and compounds which mimic metal-binding sites of metalloproteins.

The book is good technically with very few typographical errors. The literature coverage is thorough through 1979 with selected references from 1980. This reviewer suggests that this book will be of value to medicinal chemists working in advanced aspects of multidentate macrocycles, to those involved in ion binding studies, and to those involved in stereochemical problems.

College of Pharmacy **C. F. Barfknecht**
University of Iowa
Iowa City, Iowa 52242

Advances in Cyclic Nucleotide Research. Volume 14. Edited by J. E. Dumont, P. Greengard, and G. A. Robinson. Raven Press, New York. 1981. 724 pp. 16 × 24 cm. $79.00.

This volume includes 55 of the invited lectures presented at the Fourth International Conference on Cyclic Nucleotides held in Brussels, July, 1980. Also included are 330 abstracts of poster presentations. Interestingly, many lectures cover areas not involving cyclic nucleotides but related through indirectly linked mechanisms. For example, questions are posed regarding the mechanisms of α- and β-adrenergic effects which are not mediated by adenylate cyclase. Consequently, the sections present several considerations of the role of calcium as a second messenger and of the significance of protein phosphorylation regulated by factors other than cyclic nucleotides or calcium. Many of the lectures are illustrated with helpful diagrams of models and hypotheses. Unfortunately, the sections and abstracts are not divided according to any scheme or field; this is a particular drawback in the abstracts part of the book. Several issues are addressed which clearly transcend the early phenomenological approach seen in the cyclic nucleotide field a decade ago. A functional and mechanistic approach is now being utilized to examine questions dealing with the role of GTP, calcium-binding proteins, "guanomodulin", and refractoriness in hormone–receptor activity. The mechanisms underlying the relationship between the phosphodiesterases and the regulation of cyclic nucleotide levels are also presented. Several investigations are now describing the architectural relationships among the various components of the cyclic AMP and cyclic GMP metabolizing systems in intact cells.

Tufts University **Jeffrey B. Blumberg**
Boston, Massachusetts 02111

Alkaloid Chemistry. By Manfred Hesse. Translated by I. Ralph C. Bick. Wiley, New York. 1981. xii + 231 pp. $28.50.

This volume is a translation of *Alkaloidchemie*, which was published in 1978. It was written for the scientist who is just beginning the study of alkaloidal natural products. Thus, it is not encyclopedic in its coverage of the field nor exhaustive in its discussion of individual topics. Leading references are given for each chapter or major section. Structural examples are used liberally throughout the text.

The brief initial chapters include the "Concept and Definition of an Alkaloid", "Nomenclature", and "Artifacts". "Classification of Alkaloids" is the longest chapter, covering all of the major classes and subclasses with examples and species of origin. The examples are well chosen to illustrate the structural variations within each class. The "Dimeric Alkaloids and Bisalkaloids" are covered in a separate chapter. The concepts of alkaloid biogenesis are illustrated with the benzylisoquinoline alkaloids. A discussion of the techniques used to study the biosynthesis of the *Papaver* alkaloids serves to demonstrate the methodology of such studies. Plumerane alkaloids are used to illustrate "Aspects of Chemotaxonomy".

The structural elucidation of villastonine in an excellent example with which to demonstrate the use of modern methods of structural investigation. From these mostly spectral techniques the author moves into a detailed description of the use of degradation reactions (Hofmann, Emde, and von Braun). The final chapter contains "Examples of Alkaloid Synthesis". A synthesis of mesembrine, the biomimetic synthesis of porantherine, the synthesis of oncinotine (from Hesse's own work), and a stereoselective synthesis of vincamine are discussed in detail. These