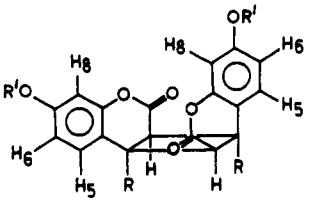
gel column and eluted with a benzene–hexane mixture. The yields of the alkoxycoumarins were 40–60%. The physical and spectral characteristics of a few coumarins as representatives are given in Table III.

**Solubilization of Reactants in Micellar Solutions.** Weighed amounts of powdered alkoxycoumarins were stirred for 12–24 h with 60–80 mL of the surfactant solutions (SDS, CTAB) of concentrations well above critical micelle concentrations (cmc's of SDS and CTAB are $8 \times 10^{-3}$ and $9.5 \times 10^{-4}$ M, respectively). The micellar solutions were filtered through Whatman No. 1 filter paper to remove suspended particles, if any. By a similar procedure micellar solutions containing both coumarin and benzophenone were also prepared.

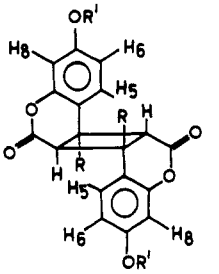
**Irradiation and Workup Procedure.** The clear transparent micellar solutions were irradiated in Pyrex tubes with a 450-W medium-pressure mercury arc lamp (Applied Photophysics Ltd.) for 5–72 h. Bulk irradiations in organic solvents and in aqueous media were also carried out in Pyrex vessels under similar conditions. Irradiations in deuterated organic solvents were carried out in Pyrex NMR tubes. During the course of irradiation the dimer formed in the micellar and aqueous phases precipitate out, on account of its lower solubility in the medium. Due to this, some of the irradiation mixtures become turbid after few hours of exposure. However, prolonged irradiation resulted in neat separation of crystalline products.

These precipitated products from the micellar solutions were collected by filtering the solutions through either a G-4 sintered crucible or Whatman No. 1 filter paper, washed thoroughly with water, and dried slowly in air/air oven. The filtrates were diluted to a concentration that was approximately one-third of the respective cmc's and extracted with ether or chloroform. The contents of the aqueous irradiation mixture were collected by extracting with chloroform. The products in the organic residue were separated from the reactants by preparative TLC (silica gel/benzene–chloroform mixture), purified by recrystallization (chloroform–carbon tetrachloride mixture), and analyzed spectroscopically.

**Structure of the Dimers.** All dimers had elemental analyses (C, H) within calculated values (0.35%). Spectral properties of a few selected dimers are presented in Table IV. The dimers formed upon direct irradiation of **1–12** in organic, aqueous, and micellar media show a closely similar pattern for the aromatic protons ($H_5$, $H_6$, and $H_8$) in the NMR spectrum. All these dimers have been assigned the syn head–tail configuration on the basis of the structure of 7-methoxycoumarin (**1**) dimer which has been unequivocally solved by X-ray analysis.[10] The protons $H_5$, $H_6$, and $H_8$ of the dimers of **1–12** can be described as part of an AMX system. In comparison with the same protons of the monomer, $H_5$ and $H_6$ are slightly shifted upfield while $H_8$ is shifted to a higher field by over 0.6 ppm. This strong shielding effect on $H_8$ caused by the diamagnetic anisotropy of a phenyl nucleus situated in front of this proton is possible only in the syn head–tail configuration[8] shown below.



Syn head-tail dimer



Anti head-tail dimer

The benzophenone-sensitized irradiation products of 4-methyl-7-alkoxycoumarins **7–12** were assigned the anti head–tail configuration by the $^1H$ NMR spectrum of the dimer obtained from **7**. At $\delta$ 3.39 and 1.25 two peaks were observed due to cyclobutane protons and cyclobutane methyl protons, respectively. These protons absorb considerably higher than the corresponding protons in the syn configuration. This shielding effect due to diamagnetic anisotropy effect of both the carbonyl group and the phenyl nucleus is possible in the anti head–tail configuration.

**Registry No.** **1**, 531-59-9; **2**, 71783-00-1; **3**, 71783-02-3; **3** (syn-HT dimer), 85389-91-9; **4**, 85389-84-0; **5**, 85405-69-2; **6**, 85389-85-1; **6** (syn-HT dimer), 85389-92-0; **7**, 2555-28-4; **7** (anti-HT dimer), 85389-95-3; **8**, 85389-86-2; **8** (syn-HT dimer), 85389-93-1; **9**, 85389-87-3; **10**, 85389-88-4; **11**, 85389-89-5; **11** (syn-HT dimer), 85389-94-2; **12**, 85389-90-8.

# Synthesis of Novel 3-Methylstatine Analogues. Assignment of Absolute Configuration[1a]

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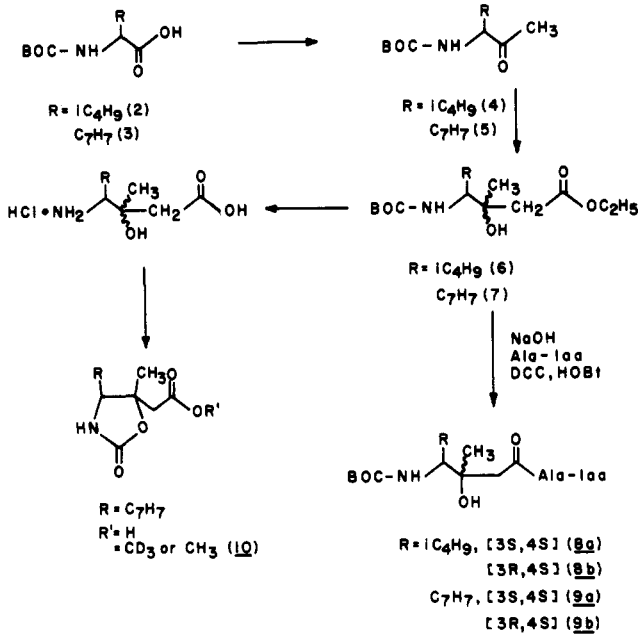
*Received September 20, 1982*

The synthesis and stereochemical assignments of two new analogues of statine are reported. Boc-L-leucine was reacted with methyllithium in dimethoxyethane to produce the corresponding methyl ketone in 45–75% yield. Addition of ethyl lithioacetate at −78 °C to the methyl ketones gave Boc-(*S*)-4-amino-3-hydroxy-3,6-dimethylheptanoic acid ethyl ester [Boc-Me$^3$Sta-OEt] in 75–78% yield as a mixture of 3-position diastereomers. The corresponding Me$^3$AHPPA derivatives [(*S*)-4-amino-3-hydroxy-3-methyl-5-phenylpentanoic acid] were synthesized as a pair of diastereomers starting from Boc-L-Phe-OH. The absolute configurations of both Me$^3$Sta and Me$^3$AHPPA diastereomers were established by converting the free amino acids to the corresponding trideuteriomethyl ester oxazolidones by reaction with phosgene followed by esterification with $^2H_3CI$ and $Cs_2CO_3$. $^{13}C$ NMR and $^1H$ nuclear Overhauser effect difference spectra established the following chiralities for the oxazolidone obtained from Me$^3$AHPPA: major diastereomer (70%) 3*S*,4*S*; minor diastereomer (30%) 3*R*,4*S*. Similarly the major Me$^3$Sta diastereomer (>90% of mixture) was shown to have the 3*S*,4*S* configuration and the minor diastereomer to have the 3*R*,4*S* configuration. Pepstatin analogues containing the 3*R*,4*S* diastereomer of Me$^3$Sta or Me$^3$AHPPA are better inhibitors of pepsin and cathepsin D than the corresponding 3*S*,4*S* diastereomers.

Pepstatin, isovaleryl-L-valyl-L-valyl-[(3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoyl]-L-alanyl-(3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid (**1**), abbreviated Iva-Val-Val-Sta-Ala-Sta, a pentapeptide first isolated by

0022-3263/83/1948-1876$01.50/0 

Scheme I




Umezawa and co-workers[1b] from bacterial culture broths, is a low-molecular-weight inhibitor of aspartylproteases, e.g., pepsin, renin, and cathepsin D,[2] that can lower blood pressure in animal models of hypertension.[3,4] Numerous pepstatin analogues have been synthesized in our laboratory, and their binding to pepsin has been demonstrated kinetically.[7-11] The (3*S*)-hydroxyl group of statine in the third residue has been shown to be necessary for tight-binding inhibition of pepsin[5,6] because changing the stereochemistry at C(3) in this essential Sta residue from *S* to *R* led to a 1000-fold-weaker inhibitor of pepsin.[9] One statine analogue (4-amino-3-hydroxyl-5-phenylpentanoic acid, abbreviated AHPPA), derived by replacing the isobutyl side chain with a benzyl side chain, has been reported and used to prepare the pepstatin analogue, isovaleryl-Val-(3*S*,4*S*)-AHPPA-Ala-Iaa (Iaa, isoamylamide), an effective inhibitor of pepsin ($K_i = 0.9 \times 10^{-9}$ M).[7]

We have synthesized analogues of Sta and AHPPA in which the single proton on C(3) is replaced by a methyl group (Me$^3$Sta, Me$^3$AHPPA). During this work it became evident that one diastereomer of both Me$^3$Sta and Me$^3$AHPPA was especially effective as an inhibitor of either pepsin or cathepsin D when incorporated into pepstatin derivatives. An accurate determination of the chirality of this hydroxyl group therefore was needed. We report here a convenient, high-yield synthesis of the 3-methyl Sta and AHPPA derivatives (Me$^3$Sta, Me$^3$AHPPA) suitable for use in peptide synthesis. The determination of the absolute configuration of the C(3) carbon by $^{13}$C NMR and difference nuclear Overhauser effect techniques is also reported

## Results and Discussion

Preparation of the 3-methyl Sta and AHPPA derivatives is shown in Scheme I. The anhydrous Boc-amino acids **2**, **3** were allowed to react with methyl lithium in dimethoxyethane (DME). The methyl ketones were isolated in 45–75% yield. Addition of ethyl lithioacetate at −78 °C to ketones **4**, and **5** in THF gave 75–78% yields of the esters **6**, and **7**, which were isolated as mixtures of diastereomers. One diastereomer of **6** is formed with a high degree of stereoselectivity (i.e., major isomer:minor isomer = ≥90:10, based on NMR). On the other hand, the diastereomers of **7** were obtained as a 70:30 mixture. Several attempts to separate the respective diastereomers by standard column chromatography over silica gel or by high-pressure liquid chromatography (HPLC) were unsuccessful. However, after coupling the amino acids to L-Ala-Iaa to form the dipeptides **8** and **9**, the diastereomers were successfully separated by standard silica gel chromatography.

Peptides derived from diastereomers of **8a**, **8b** and **9a**, **9b** were tested as inhibitors of pepsin. We found that peptides containing the minor isomers were consistently better inhibitors of pepsin and cathepsin D than those incorporating the corresponding major diastereomers.[12]

To assign the stereochemistry at C(3), we prepared the rigid oxazolidone derivative **10** (Scheme I). The Me$^3$AHPPA derivative **10** was used because the different intensity of the peaks for the major and minor isomers facilitated their assignment. $^{13}$C NMR data established that the peak due to methyl at C(3) in **10** derived from (3*S*,4*S*)-Me$^3$AHPPA resonated 4.50 ppm upfield relative to that of the derivative of **10** derived from (3*R*,4*S*)-Me$^3$AHPPA (20.58 vs. 25.08 ppm). The same upfield shift was observed for the C(2) carbon (39.61 ppm for 3*R*,4*S*; 44.69 ppm for 3*S*,4*S*) in the other diastereomer. These chemical shift differences are due to steric compression[17] between C(5) and an adjacent carbon and are consistent with the assigned stereochemistry. The chemical shifts of carbons at C(3), C(4), and C(5) are identical in both diastereomers.

Difference nuclear Overhauser effect spectra confirm the $^{13}$C NMR stereochemical assignment. The deuterated methyl ester **10**, prepared by reaction of $Cs_2CO_3$ and $CD_3I$ with the oxazolidone, was used in order to eliminate overlap of the $OCH_3$ resonance and the C(4) proton resonance of the minor 3*R*,4*S* isomer. When the C(3) methyl protons at 1.65 ppm were irradiated, the C(4) proton resonance (3.89 ppm) from the minor isomer was enhanced (8%), establishing that the C(4) H and the C(3) $CH_3$ groups are close to each other (Figure 1 available in supplementary material). No enhancement of the C(4) proton signal (4.09 ppm) in the major isomer was observed when

(1) (a) Abbreviations used for amino acids follow IUPAC-IUP tentative rules as described in: *J. Biol. Chem.* **1972**, *247*, 977. All amino acids are of the L configuration. Sta, statine or 4-amino-3-hydroxyl-6-methylheptanoic acid; AHPPA, 4-amino-3-hydroxyl-5-phenylpentanoic acid; Iva, isovaleryl; Iaa, isoamylamide; Boc, *N-tert*-butyloxycarbonyl; NMR, nuclear magnetic resonance; DME, dimethoxyethane; THF, tetrahydrofuran; TLC, thin-layer chromatography; IR, infrared; DIPA, diisopropylamine; NMM, *N*-methylmorpholine; HOBT, 1-hydroxybenzotriazole; DCC, dicyclohexylcarbodiimide; DMF, dimethylformamide; $Me_4Si$, tetramethylsilane. (b) Umezawa, H.; Aoyagi, T.; Morishima, H.; Matsusaki, M.; Hamada, H.; Takeuchi, T. *J. Antibiot.* **1970**, *23*, 259.

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the C(3) $CH_3$ (1.59 ppm) was irradiated. Thus the $^{13}C$ NMR and $^1H$ NOE data establish that the absolute configuration of the minor isomer is 3*R*,4*S* and the configuration of the major isomer is 3*S*,4*S*.

The fact that the (3*R*,4*S*)-Me$^3$AHPPA and -Me$^3$Sta containing peptides are good inhibitors of pepsin and cathepsin D and better than the 3*S*,4*S* derivatives (in some cases by more than 1000-fold) is surprising because in the corresponding Sta or AHPPA peptides the (3*S*)-hydroxyl derivatives are 1000-fold better inhibitors.[7-11] Thus, assignment of the stereochemistry of these compounds only by comparing their respective kinetic inhibition constants would have given incorrect assignments of chirality. The mechanism of the inhibition of pepsin and cathepsin D by peptides containing these new amino acid derivatives will be reported separately.

## Experimental Section

Elemental analyses were performed by Galbraith Labs., Inc., TN. Melting points were determined on a Fischer-Johns melting point apparatus and are uncorrected.

$^{13}C$ NMR spectra were recorded on a JEOL FX 90 Q (22.5 KHz) spectrometer. $^1H$ NMR and nuclear Overhauser effect (NOE) data were recorded on a Bruker WH-270 instrument. Procedural details have been reported previously.[14] Chemical shifts are reported as $\delta$ units (ppm) relative to $Me_4Si$ (tetramethylsilane) as an internal reference.

Thin-layer chromatography (TLC) was performed on silica gel G plates by using the following solvent systems: (A) 20% ethyl acetate in toluene, (B) 4% methanol in chloroform, (C 6% methanol in chloroform, and (D) 7% methanol in chloroform.

***N*-(*tert*-Butyloxycarbonyl)-3-amino-5-methyl-2-hexanone (4).** Anhydrous Boc-leucine (2.9 g, 12.5 mmol, **2**) was dissolved in dimethoxyethane (60 mL) and cooled at 0 °C under a nitrogen atmosphere. An ethereal solution of methyl lithium (28 mL of a 1.6 M solution, 37.6 mmol, 3 equiv) then was added dropwise (1 h) to the vigorously (mechanically) stirred solution, and the slightly turbid solution was stirred at 0–5 °C for an additional 5 h. The reaction mixture then was added dropwise to a vigorously stirred ice–water mixture. The ketone was extracted with ether (4 × 60 mL). The combined organic phase was washed with brine, dried ($MgSO_4$), and concentrated in vacuo to afford a pale yellow oil (1.22 g, 43%), which solidified to a waxy solid when stored at −20 ° for 18 h. TLC analysis showed one major spot: $R_f$(A) 0.42 (≥95%), 0.27 (very minor), 0.14 (very minor). The ketone was used without further purification: IR (neat, thin film) 1810 $cm^{-1}$ (sh) (ketone carbonyl); $^1H$ NMR (270 MHz, $CDCl_3$) $\delta$ 0.93 (3 H, $J$ = 6.0 Hz, Leu $\delta$-$CH_3$), 0.97 (d, 3 H, $J$ = 6.0 Hz, Leu $\delta'$-$CH_3$), 1.37–1.76 (m, 12 H, includes singlet at 1.43), 2.20 (3 H, $COCH_3$), 4.32 (br m, 1 H, $\alpha$-CH), 4.97 (br, 1 H, NH).

***N*-(*tert*-Butyloxycarbonyl)-4-amino-3-hydroxy-3,6-dimethylheptanoic Acid Ethyl Ester (Boc-Me$^3$Sta Ethyl Ester, 6).** Following the procedure for the synthesis of Boc-statine ethyl ester,[13] a hexane solution of *n*-butyllithium (5 mL of a 1.6 M solution, 8 mmol), diisopropyl amine (DIPA) (0.81 g, 8 mmol) in THF (10 mL), ethyl acetate (0.70 g, 8 mmol), and ketone **4** (1.22 g, 5.3 mmol) gave the desired product **6** in 78% yield after purification by silica gel column chromatography (eluting with 6% ethyl acetate in Skellysolve B): mp 61–62 °C.; TLC and HPLC (reversed phase C18 column, 63–70% acetonitrile gradient in 0.1% $H_3PO_4$ in 20 min) gave only a single peak; $[\alpha]_D$ −23° (*c* 1.6, methanol); $^1H$ NMR (270 MHz, $CDCl_3$) $\delta$ 0.92 (t, 6 H, $J$ = 6 Hz), 1.17 (s, C(3) $CH_3$ minor isomer), 1.23 (s, C(3) $CH_3$ major isomer), 1.28 (t, 3 H, $J$ = 7 Hz $CH_3$ of ethyl) 1.37–1.67 (m, 12 H, includes 1.44, s, Boc), 2.53 (2 H, AB quartet, $J$ = 14.0 Hz, C(2) $H_2$), 3.55 (m, 1 H, C(4) H), 4.00 (s, 1 H, OH), 4.19 (m, 2 H, $CH_2$ of ethyl), 4.62 (d, 1 H, $J$ = 9.5 Hz, NH).

Anal. Calcd for $C_{16}H_{31}NO_5$: C, 60.54; H, 9.84; N, 4.41. Found: 60.51; H, 9.84; N, 4.40.

***N*-(*tert*-Butyloxycarbonyl)-3-amino-4-phenyl-2-butanone (5).** The title compound was prepared from Boc-Phe-OH (**3**) (25.00 g, 0.094 mol) and methyl lithium (3 equiv) in DME by the same procedure as for preparation of **4**; 18.52 g (74.7%) of **5** was obtained as a semisolid. TLC showed ≥95% **5**: $R_f$(C) 0.59 and two very minor spots; $^1H$ NMR (270 MHz, $CDCl_3$) $\delta$ 1.41 (s, 9 H, Boc), 2.13 (s, 3 H, $COCH_3$), 3.04 (m, 2 H, C(4) $H_2$), 4.54 (m, 1 H, C(3) H), 5.13 (br d, 1 H, NH), 7.15–7.32 (m, 5 H).

***N*-(*tert*-Butyloxycarbonyl)-4-amino-3-hydroxyl-3-methyl-5-phenylpentanoic Acid Ethyl Ester (Boc-Me$^3$AHPPA Ethyl Ester, 7).** Following the same procedure used to prepare **6**, the title compound was prepared from the ketone **5** (18.36 g, 0.069 mol), DIPA (14.66 mL, 0.10 mol), *n*-butyllithium (43.75 mL, 2.4 M *n*-hexane solution], and ethyl acetate (10.2 mL, 0.105 mol). The yield after column chromatography over silica gel (solvent, 15% ethyl acetate in Skellysolve B) was 19.00 g (77.6%): mp 73–74 °C; $R_f$(A) 0.21; $R_f$(C) 0.64; $^1H$ NMR (270 MHz, $CDCl_3$) $\delta$ 1.15–1.32 (m, 12 H, Boc, $CH_3$ of ethyl), 1.36 (s, 3 H, C(3) $CH_3$), 2.43–2.76 (m, 3 H, C(5) H, C(2) H), 3.08–3.26 (m, 1 H, C(5) H), 3.76 (m, $\alpha$-CH of minor isomer), 4.14–4.23 (m, 3 H, $CH_2$ of ethyl, $\alpha$-CH of major isomer), 4.32 (s, 1 H, OH), 4.47 (d, $J$ = 9.6 Hz, NH of minor isomer), 4.66 (d, $J$ = 9.6 Hz, NH of major isomer), 7.18–7.30 (m, 5 H, aromatic).

Anal. Calcd for $C_{19}H_{29}NO_5$; C, 64.93; H, 8.32; N, 3.99. Found C, 65.03; H, 8.49; N, 3.88.

**[*N*-(*tert*-Butyloxycarbonyl)-4-amino-3-hydroxy-3,6-dimethylheptanoyl]alanine Isoamylamide (8).** Saponification of compound **6** (3.17 g, 0.01 mol) was carried out by using 5 mL of 2 N NaOH in 30 mL of dioxane/$H_2O$ (2:1) for 5 h; 2.8 g (96.9%) of pure Boc-Me$^3$Sta-OH was obtained as a syrup. This free acid (2.89 g, 0.01 mol) was coupled with HCl·H-Ala-Iaa derived from Boc-Ala-Iaa[7] (2.58 g, 0.01 mol) in the presence of NMM (1.1 mL, 0.01 mol), HOBt (1.68 g, 0.011 mol), and DCC (2.06 g, 0.01 mol) in 20 mL of DMF.

The separation of these diastereomers was carried out by silica gel chromatography, eluting with 2% methanol in chloroform: **8a** (pure major isomer, 3**S**,4**S**); 3.400 g (79.1%); mp 129–130 °C; $R_f$(C) 0.37; $^1H$ NMR (270 MHz, $CDCl_3$) $\delta$ 0.89–0.96 (m, 12 H, Leu $\delta,\delta'$-$CH_3$, 2 H $C_3$ Iaa), 1.20 (s, 3 H, C(3) $CH_3$), 1.37–1.68 (m, 18 H, includes 1.45, s, Boc, 1.38, d, $J$ = 6.5 Hz, Ala $\beta$-$CH_3$), 2.42 (AB quartet, $J$ = 14.0 Hz, C(2) H), 3.27 (q, 2 H, $J$ = 7.5 Hz, $NHCH_2$-Iaa), 3.52 (m, 1 H $\alpha$-CH), 4.45 (quintet, 1 H, $J$ = 6.5 Hz, $\alpha$-CH), 4.57 (s, 1 H, OH), 4.72 (d, 1 H, $J$ = 9.5 Hz, NH), 6.33 (br t, 1 H, Iaa), 6.87 (d, 1 H, $J$ = 6.8 Hz NH).

Anal. Calcd for $C_{22}H_{44}N_3O_5$: C, 61.36; H, 10.30; N, 9.76. Found: C, 61.40; H, 10.07; N 9.68.

**8b** (pure minor isomer, 3*R*,4*S*): 411 mg (9.6%); mp 151–152 °C; $R_f$(C), 0.31. $^1H$ NMR (270 MHz, $CDCl_3$) $\delta$ 0.87–0.96 (m, 12 H, Leu $\delta,\delta'$-$CH_3$ 2 $CH_3$ Iaa), 1.12 (s, 3 H, C(3) $CH_3$), 1.38–1.76 (m, 18 H, includes 1.46, s, Boc), 2.35 (AB quartet, 2 H, $J$ = 14.0 Hz, C(2) H), 3.25 (m, 2 H, $NHCH_2$ Iaa), 3.62 (m, 1 H, $\alpha$-CH), 4.38–4.48 (m, 2 H, 2 NH), 5.26 (s, 1 H, OH), 6.15 (d, 1 H, $J$ = 7.0 Hz, NH), 7.24 (br t, 1 H, NH). An additional 4.3% (183 mg) of a mixture of diastereomers was obtained.

**[*N*-(*tert*-Butyloxycarbonyl)-4-amino-3-hydroxyl-3-methyl-5-phenylpentanoyl]alanine Isoamylamide (9).** Compound **7** (3.00 g, 8.54 mmol) was saponified (2 N NaOH, 4.27 mL) to give 2.65 g (96.3% of the free acid; mp 111–113 °C. The free acid (2.76 g, 8.54 mmol) was coupled with HCl·H-Ala-Iaa derived from Boc-Ala-Iaa (2.20 g, 8.5 mmol] using NMM (0.936 ml, 8.5 mmol], HOBt [1.439 g, 9.39 mmol] and DCC [1.76 g, 8.54 mmol). The obtained crude product was dissolved in 35 mL of ethyl acetate and filtered. The filtrate was allowed to stand at room temperature for 3 h, during which time the pure major isomer (3*S*,4*S*) precipitated. The solid was collected, washed with 5 mL of cold ethyl acetate, and dried to give 2.23 g (56.5%). The filtrate was evaporated to dryness. Separation of diastereomers from the residue of the filtrate was carried out by silica gel chromatography, eluting with 2% methanol in chloroform. The combined yield of **9a** (pure major isomer, 3*S*,4*S*) was 2.51 g (63.4%): $R_f$(D) 0.49; mp 155–156 °C; $^1H$ NMR (270 MHz, $CDCl_3$) $\delta$ 0.91 (d, 6 H, $J$ = 6.5 Hz, 2 $CH_3$ Iaa), 1.13–1.66 (m, 18 H, includes 1.38, d, $J$ = 6.0 Hz, Ala $\beta$-$CH_3$, 1.34, s, 3 H, C(3) $CH_3$, 1.27, s, 9 H, Boc), 2.49 (AB quartet, 2 H, $J$ = 14.0 Hz, C(2) H), 2.68 (d, 1 H, $J$ = 14.0 Hz, C(5) H), 3.27 (q, 2 H, $J$ = 7.0 Hz, $NHCH_2$ Iaa), 3.72 (br m, 1 H, C(4) H), 4.45 (quintet, 1 H, $J$ = 7.0 Hz, $\alpha$-CH), 4.76 (d, 1 H, $J$ = 9.5 Hz, NH), 4.93 s, 1 H, OH), 6.28 (br t, 1 H, NH), 6.79 (d, 1 H, $J$ = 7.0 Hz, NH), 7.17–7.28 (m, 5 H, aromatic).

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Anal. Calcd for $C_{25}H_{42}N_3O_5$: C, 64.63; H, 9.11; N, 9.04. Found: C, 64.75; H, 8.91; N 9.04.

**9b** (pure minor isomer, 3*R*,4*S*): 540 mg (13.6%); mp 156–157 °C, $R_f$(D) 0.44; $^1$H NMR (270 MHz, $CDCl_3$) δ 0.86 (d, 6 H, *J* = 6.0 Hz, 2 $CH_3$ Iaa), 1.23–1.74 (m, 18 H, includes 1.23, s, C(3) $CH_3$, 1.28, s, Boc, 1.40, d, *J* = 6.5 Hz, Ala β-$CH_3$), 2.31–2.55 (m, 3 H, C(5) H, C(2) H), 3.17 (m, 2 H, $NHCH_2$ Iaa), 3.42 (br dd, 1 H, C(5) H), 3.78 (m, 1 H, C(4) H), 4.37–4.48 (m, 2 H, α-CH, NH), 5.58 (s, 1 H, OH), 6.16 (d, 1 H, *J* = 6.5 Hz, NH), 7.15–7.28 (m, 6 H, NH, aromatic). An additional 665 mg (16.8%) mixture of diastereomers was obtained.

**[$^2H_3$]Methyl (4*S*)-Benzyl-5-methyloxazolidone-5-acetate (10).** The oxazolidone ring system was prepared by the method reported for the synthesis of a homologue.[7] Starting with compound **7** (1.408 g, 4 mmol), 536 mg (54.0%) ≥95% pure oxazolidone carboxylic acid was obtained as a semisolid.

Esterification of this acid (536 mg, 2.17 mmol) was carried out by reaction with $Cs_2CO_3$ (1.70 mL of 20% aqueous solution) and $CD_3I$ (0.149 mL, 2.39 mmol) as reported.[15] The ester was then purified by silica gel chromatography, eluting with 1% methanol in chloroform to give 463 mg (80.8%) of pure **10**.

**Acknowledgment.** This work was supported by a grant from the National Institutes of Health (No. AM 20100).

**Registry No. 2**, 13139-15-6; **3**, 13139-15-6; **4**, 85613-63-4; **5**, 85613-64-5; (3*S*,4*S*)-**6**, 85613-65-6; (3*S*,4*S*)-**7**, 85613-66-7; (3*R*,4*S*)-**7**, 85613-61-2; (3*S*,4*S*)-**7** free acid, 85613-67-8; (3*S*,4*S*)-**8a**, 85613-68-9; (3*R*,4*S*)-**8b**, 85613-69-0; (3*S*,4*S*)-**9a**, 85613-70-3; (3*R*,4*S*)-**9b**, 85613-71-4; **10**, 85613-72-5; Boc-Ala-Iaa, 72155-58-9; H-Ala-Iaa·HCl, 72155-60-3; Boc-Me$^3$Sta-OH, 85613-73-6; ethyl acetate, 141-78-6.

**Supplementary Material Available:** Full NMR NOE data and spectra for compound **10** (1 page). Ordering information is given on any current masthead page.

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# General-Base Catalysis in the Reaction of Water with Activated Aromatic Substrates. The Hydrolysis of 3-Methyl-1-picrylimidazolium Ion

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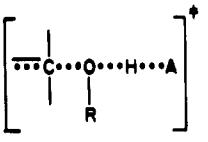
*Received November 17, 1982*

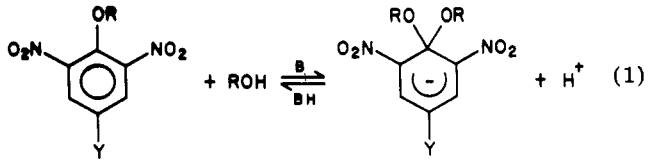
The rate of hydrolysis of 3-methyl-1-picrylimidazolium ion was investigated between pH 1.7 and 9.3 in the presence of several buffer bases at various concentrations. The reaction is strongly catalyzed by oxygen bases. The catalytic constants including water and $OH^-$ are spread over a range of ten powers of ten and show a good Brønsted correlation with $\beta = 0.62$. The kinetic solvent isotopic effect for the water-, acetate-, and $OH^-$-catalyzed reactions are 2, 0.86, and 0.84, respectively. The mechanism of catalysis is discussed, and it is concluded that it represents concerted addition of water to the aromatic ring.

The reaction of water and alcohols with the carbonyl carbon occurs by a concerted mechanism with an important component of proton transfer in a mobile transition state.[1]



This mechanism is supported by a large number of structure–reactivity correlations which provide evidence for a fully concerted mechanism.[2]

Similar results have been found for the addition–elimination of alcohols to activated aromatic compounds (eq 1)[3] where the general base–acid catalysis was interpreted in terms of a concerted mechanism.



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On the other hand, the reaction of water with activated aromatic substrates is known in less detail mainly because most aromatic nucleophilic substitution reactions have been studied at high pH where the hydroxide ion catalyzed reaction is predominant. It has been suggested that water addition to 4,6-dinitrobenzofuroxan occurs through a concerted mechanism.[4] Also related are the studies on pseudobase formation from water addition to quaternary nitrogen heterocycles.[5]

We recently reported a study of the hydrolysis of *N*-picrylimidazole (**1**) and interpreted the general buffer catalysis observed along the same lines.[6] At low pH, the reaction folows the rate law of eq 2, where the term $k_{SH}$

$$v = k_0[S] + k_s[S][B] + k_{SH}[S][BH] \quad (2)$$

was attributed to the reaction of water with *N*-picrylimidazolium cation (**2**), catalyzed by general bases, but this mechanism is kineticaly indistinguishable from others that involve **1** and a general-acid catalyst in the transition state. We now report a study of the hydrolysis of 3-methyl-1-picrylimidazolium cation (**3**) which is expected to be a good model for **2**.

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