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Studies with α-Methyl Amino Acids. Resolution and Amino Protection[1a]

John Turk,[1b] G. T. Panse,[1c] and Garland R. Marshall*[1d]

Departments of Biological Chemistry and Physiology and Biophysics, Washington University School of Medicine, St. Louis, Missouri 63110

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We have prepared derivatives of α-methyl amino acids that are suitable for use in peptide synthesis because it appears that peptide hormone analogs containing them might be of special interest. Marshall and Bosshard[2a] and Marshall et al.[2b] have shown by theoretical studies on the allowed dihedral angles of model peptides that the replacement of the α proton of an amino acid residue with a methyl group results in a dramatic reduction of the conformational space available to the backbone of the peptide chain at the position where that residue occurs. These calculations have subsequently been confirmed by others.[3,4] Peptide hormone analogs containing α-methyl amino acid residues should therefore have a sterically rigid backbone conformation at those positions and would correspond closely to "conformational analogs" of the hormone, i.e., analogs which have a primary structure essentially identical with that of the native hormone but which are capable of adopting conformations that would comprise only a small subset of the total set available to the parent molecule.

Should such an analog be biologically active, important constraints might thereby be placed on evolving models of the conformation assumed by the hormone as it interacts with its receptor. In addition, if side-chain interactions were essential for binding, such analogs, which retain all of the native hormone's side chains, might offer a route to inhibitors which bind to the receptor but which are not capable of inducing subsequent events necessary to activity owing to their conformational inflexibility. This sort of inhibitor might be missed by schemes of analog generation which involve varying only the character or position of side chains.

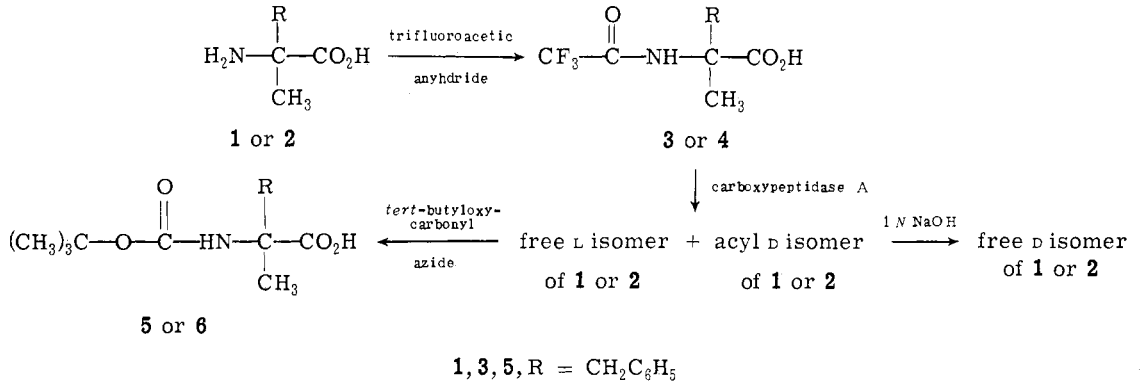
Analogs bearing α-methyl groups would also be expected to be metabolized slowly, since model compounds containing α-methyl amino acids are known to be resistant to chemical hydrolysis[5,6] and to enzymatic attack by both endopeptidases[7] and exopeptidases.[8,9] The synthesis of such analogs might therefore result in the generation of either long-acting agonists or antagonists or of peptides which lack either property but which nonetheless potentiate the effect of endogenous hormone by interfering with its degradation.

We chose to resolve and protect α-methylphenylalanine (2-amino-2-methyl-3-phenylpropionic acid) and α-methylvaline (2-amino-2,3-dimethylbutyric acid) because both phenylalanine and valine occur in angiotensin II, a molecule of current interest. In achieving optical resolution of amino acids, enzymatic digestion of their acylated derivatives is often the method of choice, since it is less tedious and more rapid than many chemical resolution procedures. Since it is known that one enzyme commonly used for such purposes, hog renal acylase I, is unable to catalyze the hydrolysis of *N*-acetyl-α-methylphenylalanine,[7] we decided to use commercially available bovine carboxypeptidase A (CPA). We prepared the *N*-trifluoroacetyl derivatives of the amino acids because they are generally far superior substrates for CPA when compared to the *N*-acetyl derivatives[10] and because such a procedure had proven convenient for the resolution of other amino acids in our laboratory.[11]

We followed described procedures in synthesizing α-methylphenylalanine (1) and α-methylvaline (2) (Scheme I) from the corresponding ketones, phenylacetone and 2-methyl-3-butanone, respectively.[12,13] These amino acids can be trifluoroacetylated readily with trifluoroacetic anhydride in trifluoroacetic acid by the method of Weygand and Geiger,[14] though not by the milder method of Schallenberg and Calvin[15] which employs *S*-ethylthiol trifluoroacetate in aqueous solution and is an excellent procedure for many amino acids.

Both *N*-trifluoroacetyl-α-methylphenylalanine (3) and *N*-trifluoroacetyl-α-methylvaline (4) are digested stereospecifically by CPA, releasing the L isomers of 1 and 2 and leaving the D isomers of 3 and 4 intact. The protected derivatives can easily be separated from the amino acids by a simple extraction procedure and the D isomers of 1 and 2 can be generated from D isomers of 3 and 4 by mild saponification. The absolute configuration of α-methylphenylalanine has been determined previously,[16,17] and the direction and magnitude of the optical rotation of the free amino

Scheme



1, 3, 5, R = $CH_2C_6H_5$

2, 4, 6, R = $CH(CH_3)_2$

acid appearing in solution after the treatment of racemic **3** or **4** with CPA confirm the expectation that the enzyme selectively digests the L isomers and not the D isomers of these compounds. In this regard, we are grateful to Dr. F. W. Bollinger of Merck Laboratories for supplying us with a sample of chemically resolved L-α-Me-Phe · HCl with which to compare our material. To our knowledge, α-Me-Phe has not been resolved enzymatically before, and neither chemical nor enzymatic resolution of α-Me-Val has been achieved previously.

The susceptibility of these derivatives to degradation by CPA is consistent with the side-chain specificity of the enzyme and with its ability to slowly degrade acyl derivatives of D-alanine. This suggests that this resolution procedure is generally applicable to α-methyl amino acids whose side chains are compatible with the specificity of CPA. In this regard, preliminary studies on the resolution of α-methylleucine by polymer-bound CPA indicate the utility of the enzyme in that form and the general applicability of the procedure. To date, however, the procedure has been applied only to the three α-methyl amino acids mentioned.

Protecting the amino groups of **1** and **2** with a function suitable for use in solid-phase peptide synthesis, such as the *tert*-butyloxycarbonyl function (*t*-Boc),[18] is a more difficult problem. Other investigators have encountered difficulty in preparing *t*-Boc-α-methylalanine (*t*-Boc-2-amino-2-methylpropionic acid), citing optimum yields of from 8.7[19] to 12%[20] under conditions which result in over 80% yields for amino acids routinely used in peptide synthesis. Apparently the problem of steric hindrance due to the extra α-alkyl substituent which complicates the synthesis of *t*-Boc-α-methylalanine is magnified when the amino acid side chain is bulkier than a methyl group. Our yields of *t*-Boc-L-α-methylphenylalanine (**5**) and *t*-Boc-L-α-methylvaline (**6**) were less than 2% and essentially 0%, respectively, under a variety of standard conditions. Procedures which proved to be ineffective in our hands in generating **5** and **6** include that of Schnabel,[21] which employs *t*-Boc azide in aqueous solution, that of Ragnarsson et al.,[22] which employs *t*-Boc phenylcarbonate in dimethyl sulfoxide (DMSO), and a variation on the latter procedure which employs *t*-Boc *p*-nitrophenylcarbonate and hydroxybenzotriazole in dimethylformamide.[23]

Compounds **5** and **6** can be synthesized from **1** and **2** in DMSO at an elevated temperature with an excess of organic base and periodic additions of *t*-Boc azide (to counteract the decomposition of that reagent) over a prolonged period. The yield of **5** so obtained was excellent (70%), and although the yield of **6** was five times lower owing to the added steric problem of the β-branched structure of **2**, this procedure is our current method of choice for preparing *t*-Boc-α-methyl amino acids. The use of compounds **5** and **6** in the solid-phase synthesis of peptide hormone analogs is currently in progress, and their chemical and biological properties will be described in a subsequent report.

Experimental Section

All melting points were determined in open capillaries and are uncorrected. Thin layer chromatography on silica gel G plates (Brinkman) was performed on each compound in two systems, 1-butanol–pyridine–acetic acid–H_2O (15:10:3:12) and 1-butanol–acetic acid–H_2O (4:1:1). The R_f values will be designated BP R_f and BAW R_f. Elemental analyses were performed by PCR Inc., Gainesville, Fla., or by Galbraith Laboratories, Knoxville, Tenn.

Preparations of *N*-Trifluoroacetyl-α-methylphenylalanine (3) and *N*-Trifluoracetyl-α-methylvaline (4). Either 17.1 g (0.1 mol) of α-methylphenylalanine (**1**) or 13.1 g (0.1 mol) of α-methylvaline (**2**) was dissolved in 60 ml of trifluoroacetic acid and chilled to 0°. A total of 17.6 ml (0.12 mol) of trifluoroacetic anhydride was then added over a period of 5 min and the resulting solution was stirred at 0° for 1 hr. The solvent and excess anhydride were then evaporated under reduced pressure and the resulting oil was diluted with 100 ml of H_2O. Aliquots of 2 *N* NaOH were added to the aqueous slurry with vigorous stirring until a pH of 7 was achieved, at which point a clear solution was obtained that was treated with charcoal, filtered, and adjusted to pH 3. The precipitated **3** or **4** was collected by filtration, and the mother liquor was extracted three times with ethyl acetate. The extracts were combined, dried with Na_2SO_4, concentrated under reduced pressure, and seeded with crystals of **3** or **4**. The resulting solid was washed with hexane, combined with the first crop, and recrystallized from benzene–hexane (for **3**) or from ethyl acetate–toluene (for **4**). The yield of **3** was 75% (20.6 g) and its physical characteristics follow: mp 162–163°; BAW R_f 0.68, BP R_f 0.65. Anal. Calcd for $C_{12}H_{12}NO_3F_3$: C, 52.37; H, 4.36; N, 5.09. Found: C, 52.08; H, 4.61; N, 4.80. The yield of **4** was 72% (16.4 g) and its physical characteristics follow: mp 112–113°; BAW R_f 0.70, BP R_f 0.60. Anal. Calcd for $C_8H_{12}NO_3F_3$: C, 42.29; H, 5.28; N, 6.15. Found: C, 42.11; H, 5.33; N, 6.12.

Enzymatic Digestion. Either 13.8 g (0.05 mol) of **3** or 10.4 g (0.05 mol) of **4** was added to 400 ml of H_2O and stirred vigorously while 2 *N* NaOH was added until a clear solution of pH 7.2 was obtained. A total of 20 mg of DFP-treated carboxypeptidase A (Sigma Chemical Co., St. Louis, Mo.) was then added, and the solution was maintained at 37° by a thermostated water bath and at pH 7.2 by a Radiometer pH-Stat. After 16 hr of gentle stirring, the solution was adjusted to pH 5, treated with charcoal, and filtered to remove the enzyme. It was then adjusted to pH 3 with 1 *N* HCl and extracted three times with ethyl acetate. The treatment from this point on depended on whether **3** or **4** had been the starting material.

For **3** the aqueous solution was neutralized and concentrated by boiling until the L isomer of **1** began to crystallize, and then it was allowed to cool to room temperature. The crystals were collected by filtration, and a second crop was harvested from the mother liquor. The two crops were combined and dried overnight at 70° under reduced pressure. The yield was 78% (3.32 g). The material is pure enough for most synthetic purposes at this point but does contain some NaCl, which can be removed by recrystallization from H_2O. The physical characteristics follow: mp 279–280° dec (compare 275–276° for racemate); BAW R_f 0.45, BP R_f 0.62; $[\alpha]^{25}_{300}$ −47.8° (H_2O, *c* 0.716). Anal. Calcd for $C_{10}H_{13}NO_2$: C, 67.04; H, 7.32; N, 7.82. Found: C, 67.32; H, 7.40; N, 7.69.

The ethyl acetate extracts containing the D isomer of **3** ($[\alpha]^{25}D$ −30.0 (ethyl acetate, *c* 10.3)) were combined, dried with Na_2SO_4, and concentrated under reduced pressure. The residual syrup was dissolved in 60 ml of ethanol to which 60 ml of 1 *N* NaOH was added, and the solution was allowed to stand for 72 hr. It was then neutralized with HCl and evaporated to dryness. The salty residue was dissolved in H_2O and the free D isomer of **1** was obtained in the manner described above for the L isomer. The yield was 74% (2.96 g), and the physical characteristics follow: mp 279–280° dec; BAW R_f 0.46, BP R_f 0.62; $[\alpha]^{25}_{300}$ +47.8° (H_2O, *c* 0.716).

For **4** the aqueous solution was neutralized and evaporated to dryness. The salty residue was purified by 100 transfers in a 1-butanol–H_2O–trifluoroacetic acid (50:50:1) countercurrent distribution system. The amino acid was located by performing thin layer chromatography on the contents of each tube, and the contents of tubes 40–60 were pooled and evaporated to dryness. The white residue was dissolved in H_2O, and the solution was neutralized with NH_4OH and boiled until crystallization began. It was then allowed to cool to room temperature and the crystals were collected by filtration. A second crop was collected, combined with the first, lyophilized twice from H_2O, and dried overnight at 70° under reduced pressure. The yield was 70% (2.30 g) and the physical characteristics follow: mp 281–282° (sublimes without melting, compare 273–274° for the racemate); BAW R_f 0.29, BP R_f 0.54; $[\alpha]^{25}D$ −3.92° (H_2O, *c* 1.31). Anal. Calcd for $C_6H_{13}NO_2$: C, 54.96; H, 9.93; N, 10.69. Found: C, 55.18; H, 9.89; N, 10.68.

The ethyl acetate extracts containing the D isomer of **4** ($[\alpha]^{25}_{400}$ −18.6 (ethanol, *c* 1.5)) were treated in the same manner as for compound **3** except that after saponification and neutralization, the solution was evaporated to dryness, and the salty residue containing the D isomer of **2** was purified in the manner described above for the L isomer. The yield was 69% (2.28 g) and the physical characteristics follow: mp 281–282° (sublimes without melting); BAW R_f 0.28, BP R_f 0.54; $[\alpha]^{25}D$ = +3.90° (H_2O, *c* 1.31).

Preparation of the Dicyclohexylamine Salts of *N*-*tert*-Butyloxycarbonyl-L-α-methylphenylalanine (5) and *N*-*tert*-Butyloxycarbonyl-L-α-methylvaline (6). Either 1.71 g (0.01 mol) of **1** or 1.31 g (0.01 mol) of **2** was dissolved in 50 ml of dimeth-

yl sulfoxide with 3.5 g (0.03 mol) of tetramethylguanidine. A total of 1.5 ml (0.01 mol) of *t*-Boc azide was added immediately, and the solution was stirred at 40° for 3 weeks with the addition of 0.4 ml of *t*-Boc azide every 3 days. The solution was then diluted with 150 ml of H_2O, brought to pH 2.5 with the addition of solid $NaHSO_4$, and extracted five times with ethyl acetate. The extracts were combined, dried with Na_2SO_4, and evaporated under reduced pressure. The viscosity of the residual oil was reduced by the addition of a small amount of ethyl acetate, and an excess of dicyclohexylamine was added. The resultant crystalline salts were collected by filtration and recrystallized from ethyl acetate. The yield of **5** was 70% (3.22 g), and its physical characteristics follow: mp 230–231°; BAW R_f 0.81, BP R_f 0.70; $[\alpha]^{25}D$ +16.8° (2% acetic acid in ethanol, *c* 0.46). Anal. Calcd for $C_{27}H_4N_2O_4$: C, 70.43; H, 9.57; N, 6.09. Found: C, 70.41; H, 9.68; N, 6.04. The yield of **6** was 13% (0.533 g), and its physical characteristics follow: mp 181–182°; BAW R_f 0.74, BP R_f 0.72; $[\alpha]^{25}_{300}$ +32.6° (ethanol, *c* 2.06). Anal. Calcd for $C_{23}H_{44}N_2O_4$: C, 66.97; H, 10.79; N, 6.74. Found: C, 66.91; H, 10.68; N, 6.78.

Registry No.—DL-**1**, 1132-26-9; D-**1**, 17350-84-4; L-**1**, 23239-35-2; DL-**2**, 26287-62-7; D-**2**, 53940-82-2; L-**2**, 53940-83-3; DL-**3**, 53940-84-4; D-**3**, 53940-85-5; DL-**4**, 53940-86-6; D-**4**, 53940-87-7; **5**, 53940-89-9; **6**, 53940-91-3; *t*-Boc azide, 1070-19-5.

References and Notes

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Ring Opening of 3-Chloro-4-nitroisothiazole with Amines

Martin Winn

Department of Medicinal Chemistry, Abbott Laboratories, North Chicago, Illinois 60064

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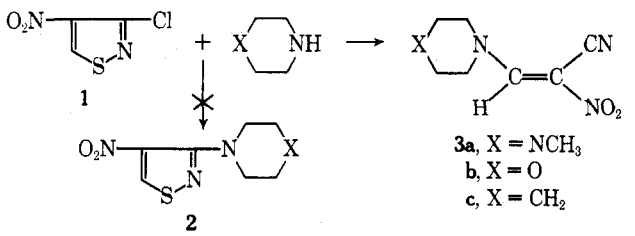
In an attempt to prepare 3-(*N*-methylpiperazino)-4-nitroisothiazole (**2a**), 3-chloro-4-nitroisothiazole (**1**)[1] was treated with *N*-methylpiperazine. Unexpectedly, a ring opening occurred leading to the enamine **3a** rather than **2** (Scheme I). The evidence for this is as follows.

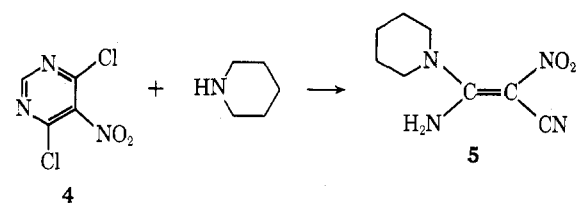
Scheme I



The product did not contain sulfur and analyzed as $C_8H_{12}N_4O_2$. The ir spectrum showed a C≡N band (2200 cm^{-1}), a strong C=C band (1620 cm^{-1}), and an NO_2 band (1280 and 1490 cm^{-1}) which had been shifted to longer wavelength owing to conjugation with an amino group. A normal C=C—NO_2 should have peaks at 1524 ± 4 and 1353 ± 6 cm^{-1}.[2] The NMR chemical shift of the olefinic proton (δ 8.50) enabled us to assign the configuration of **3a** as having a trans relationship between the amine and NO_2 nitrogens. Matter et al.[3] have shown that the equation $\delta = 5.25 + Z_{gem} + Z_{trans} + Z_{cis}$ is useful in determining the chemical shift of protons on substituted ethylenes where *Z*'s are parameters for various substituents listed in his paper. Together with the parameter for NO_2 determined by Descotes et al.,[4] one can calculate the chemical shift for the olefinic proton of **3a** as follows. δ = 5.25 + 1.17 (conjd NR_2) + 0.55 (*trans*-CN) + 1.67 (*cis*-NO_2) = 8.64. The calculated chemical shift for the other isomer is δ = 5.25 + 1.17 + 0.75 (*cis*-CN) + 0.46 (*trans*-NO_2) = 7.63. Clearly the observed value (8.50) is closer to that calculated for **3a** than its isomer.

There are no other 3-amino-2-nitroacrylonitriles reported in the literature, the nearest analog being **5**.[5] This compound, prepared by ring opening of the dichloronitropyrimidine **4** (Scheme II), shows ir maxima at 1643 cm^{-1} (C=C).

Scheme II



The monobasic amines, morpholine and piperidine, react in a like manner if triethylamine is present.

Two possible mechanisms for the formation of **3** are given in Scheme III. The first involves a nitrile sulfide in-

Scheme III