lactone CH_2), 5.22 (doublet, J = 5.0 cps, H-6), 6.00 (quartet, J = 5.0 and 9.0 cps, H-7), and 7.00, 7.40 (aromatic protons).

Methyl 3-Formyl-7-(thiophene-2-acetamido)-3-cephem-4-carboxylate (V).—A mixture of 0.300 g of impure III, 0.900 g of activated MnO_2,[11] and $CHCl_3$ (30 ml) was stirred at room temperature for 20 hr. An additional 0.450 g of MnO_2 was added and the stirring was continued for another 20 hr. Aliquot samples were taken after 18, 24, and 40 hr. The samples exhibited uv absorption (in $CHCl_3$) at 275, 280, and 280 mμ, respectively, due to the cephalosporin nucleus, and at 241 mμ due to the thiophene side chain. The mixture was filtered and the filtrate was evaporated to dryness. The residue was dissolved in $CHCl_3$ and chromatographed on SiO_2 (Mallinckrodt, 15 g). Elution with EtOAc–$CHCl_3$ (1:3) afforded 0.050 g of V, mp 165–170° dec. Recrystallization from 2-propanol gave material with mp 167–170° dec; λ_{max} 233 mμ (ε 10,550), 296 (10,750), and 340 (shoulder);[14] ν_{max} 3.06 (NH), 5.59 (β-lactam C=O), 5.81 (ester C=O), 6.00 (amide I), 6.23 (double bond), and 6.53 μ (amide II); nmr (DMF-d_7) peaks at δ 3.95, 4.03 (C-2 CH_2, ester CH_3, side-chain CH_2), 5.44 (doublet, J = 6.0 cps, H-6), 6.15 (quartet, J = 6.0 and 9.0 cps, H-7), 7.07, 7.47 (aromatic protons), 9.20 (doublet, J = 9.0 cps, NH), and 9.88 (singlet, C11O proton). A satisfactory mass spectrum was not obtained for this compound due to thermal decomposition of the sample in the mass spectrometer.

Anal. Calcd for $C_{15}H_{14}N_2O_5S_2$: C, 49.16; H, 3.85; N, 7.65; S, 17.50. Found: C, 49.15; H, 4.03; N, 7.18; S, 18.03.

Ethylene Acetal of V (VI).—A mixture of 0.090 g of V, 0.20 g of ethylene carbonate, 0.5 ml of ethylene glycol, 0.002 g of *p*-toluenesulfonic acid monohydrate, and 2.5 ml of tetrahydrofuran was allowed to stand at room temperature for 19.5 hr.[15] The mixture was then poured into water and extracted twice (Et_2O–EtOAc). The combined extracts were washed (1 *M* $NaHCO_3$, twice with H_2O, once with saturated NaCl), and then dried (Na_2SO_4). Evaporation of the solvent gave 0.071 g of solid residue which was crystallized from 2-propanol to give 0.036 g of the ethylene acetal (VI): mp 200–205° dec; λ_{max} 236 mμ (ε 12,600) and 260 mμ (ε 7370); ν_{max} 2.97 (NH), 5.60 (β-lactam C=O), 5.77 (ester C=O), 6.04 (amide I), 6.20 (double bond), and 6.50 μ (amide II); nmr ($CDCl_3$) peaks at δ 3.45 (C-2 CH_2), 3.85, 3.97 (acetal CH_2, ester CH_3 and side-chain CH_2), 4.95 (doublet, J = 5.0 cps, H-6), 5.74 (singlet, H-3′), 5.80 (quartet, J = 5.0 and 8.0 cps, H-7), 6.41 (doublet, J = 8.0 cps, NH), and 6.95, 7.22 (aromatic protons). The molecular weight and formula of VI were determined by high-resolution mass spectrometry.

Anal. Calcd for $C_{17}H_{18}N_2O_6S_2$: mol wt, 410.06220. Found: *m/e*, 410.06064.[16]

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(14) The Δ²-aldehyde (VII) was reported to have λ_{max}^{EtOH} 292 mμ (ε 13,600): R. B. Woodward, K. Heusler, J. Gosteli, P. Naegeli, W. Oppolzer, R. Ramage, S. Ranganathan, and H. Vorbrüggen, *J. Am. Chem. Soc.*, **88**, 852 (1966).

(15) The authors wish to thank Dr. E. Fornefeld for this procedure in which ethylene carbonate acts as a water scavenger.

(16) The mass spectrum was taken on a CEC Model 21-110A-1 spectrometer; ionizing potential 70 ev, temperature of the ion source 180°.

Synthesis of α-Methyl-DL$_g$-glutathione

DANIEL F. VEBER, KARL PFISTER, III, AND RALPH HIRSCHMANN

Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey

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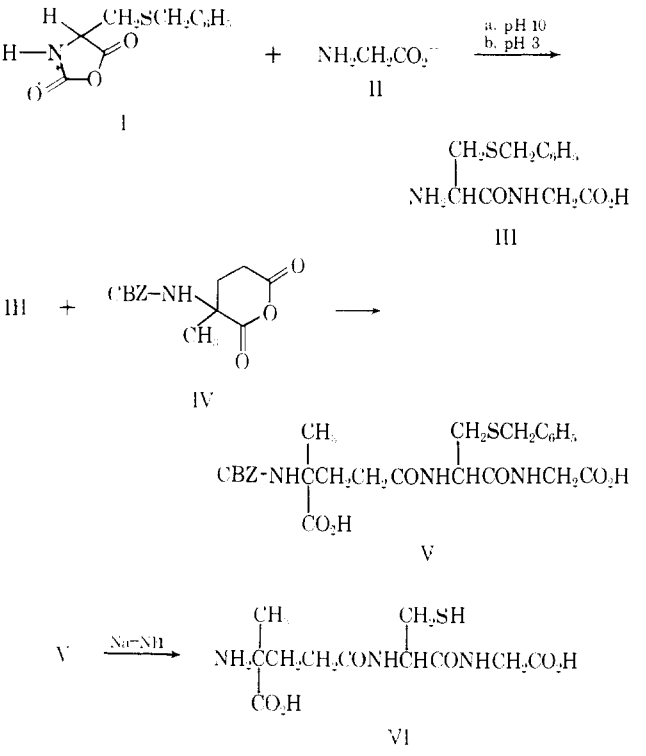
In recent years α-methylamino acids have proven to be of considerable interest in the study of enzyme mechanisms[1] and in medicinal chemistry.[2,3] However, to our knowledge these derivatives have not been introduced into the sequence of a biologically active peptide. We have carried out such an alteration at the glutamic acid residue of glutathione.

α-Methyl-DL$_g$-glutathione (VI) was prepared by the route outlined in Scheme I. S-Benzyl-L-cysteinyl-

SCHEME I



glycine (III) was prepared by the N-carboxyanhydride procedure.[4] In our initial preparations, III was isolated and purified. It was then allowed to react with CBZ-DL-α-methylglutamic anhydride[5] in aqueous dioxane at pH 8. However, the purification was later found to be unnecessary. On adjusting the pH to 2 and extracting with ethyl acetate, CBZ-α-methyl-DL-glutamyl-S-benzyl-L-cysteinylglycine (V) was obtained as a noncrystalline solid having a trace of CBZ-α-methylglutamic acid as impurity as shown by thin layer chromatography. The blocking groups of V were removed by sodium in liquid ammonia and the resulting thiol VI was purified through the copper(I) salt. The gross structure of VI was established by its elemental analysis and iodometric and base titration. The N-ethylmaleimide derivative moved as a single component by electrophoresis (pH 7.5 and 4.2) and by paper chromatography in several solvent systems.

The nature of the glutamyl peptide link (α *vs.* γ) is an important one. The use of glutamic anhydride generally leads to the formation of an α-peptide as the

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(5) CBZ = carbobenzoxy.

major product, the amount of γ-peptide depending *inter alia* on the reaction solvent and nature of the amino blocking group.[6] We hoped that the α-methyl group would present a steric hindrance to reaction at the α-carbonyl thus leading to a preferential reaction at the γ-carbonyl.[7] This was indeed found to be true since only a single tripeptide could be found in the product by thin layer chromatography and electrophoresis. That this peptide contains a γ-glutamyl linkage was established by the Van Slyke nitrogen analysis and the p*K*'s determined by base titration.

The Van Slyke analysis for amino nitrogen is known to give approximately 2 equiv of nitrogen for a γ-glutamyl peptide while giving only 1 equiv of nitrogen for an α-glutamyl peptide as well as most other peptides.[8] VI like glutathione gave 2 equiv of nitrogen, while a control, α-glutamylleucine, gave only 1 equiv of nitrogen.

The $pH_{1/2}$'s in a base titration were found to be 3.5 and 8.5, the same as those obtained for a sample of glutathione. The α-peptide would be expected to be a weaker acid than glutathione.[9]

Biology.[10]—α-Methyl-DL$_g$-glutathione was compared to glutathione in a number of standard cell cultures at levels of 0.5–256 μg/ml. Their cytotoxicities as determined by total cell protein[11,12] were found not to differ significantly. Other data indicated inhibition by VI (10^{-3} *M*) of yeast glutathione reductase[13] in the presence of glutathione.

Experimental Section[14]

α-Methyl-DL-glutamic Anhydride (IV). **A.**—α-Methyl-DL-glutamic acid (5 g, 0.017 mole) was suspended in 20 ml of acetic anhydride and stirred until a homogeneous solution was obtained (5 hr). The Ac_2O was removed *in vacuo* at 50–60° and the residual oil was flushed several times with $CHCl_3$. The resulting oil was triturated with ether to give 3.6 g of IV (76% yield). An analytical sample, mp 113–113.5°, was obtained by recrystallization from CH_2Cl_2–ether. *Anal.* Calcd for $C_{14}H_{15}NO_5$: C, 60.66; H, 5.45; N, 5.05. Found: C, 60.58; H, 5.27; N, 5.01.

B.—α-Methyl-DL-glutamic acid (8.31 g, 0.03 mole) was dissolved in 250 ml of dioxane. Dicyclohexylcarbodiimide (6.18 g, 0.03 mole) was added with stirring and the solution was allowed to stand at 25° for 18 hr. The precipitate of dicyclohexylurea was removed by filtration and washed with 20 ml of dioxane. The resulting solution was then used in subsequent reactions assuming a 100% conversion to IV.

S-Benzyl-L-cysteinylglycine (III).—Glycine (7.5 g 0.1 mole) was dissolved in 1 l. of a solution of potassium borate buffer (1 *M*) at pH 11.0 and the solution was cooled to 0°. This solution was placed in a Waring blender containing 400 g of ice. N-Carboxy-S-benzyl-L-cysteine anhydride (24.9 g, 0.105 mole) was added with high-speed stirring and the stirring was continued for 90 sec. The reaction mixture was filtered, the pH was adjusted to pH 4.5, and the resulting insolubles were removed by filtration. The filtrate containing S-benzyl-L-cysteinylglycine was used to prepare V without isolation as described below or the dipeptide was isolated in the following way. III was adsorbed on 500 ml of Pittsburg 01 granular carbon. The column was washed with 3 l. of water to remove salts and residual glycine, and III was eluted with 5% AcOH in 50% aqueous acetone. After evaporating the eluate to dryness, III was crystallized from water to give 15.4 g (57% yield), $[\alpha]^{25}D$ +28.8° (*c* 2, 1 *N* NaOH) [lit.[15] $[\alpha]D$ +27° (*c* 2, 1 *N* NaOH)], equiv wt (base titration) 263 (calcd 286).

γ-(CBZ-α-methyl)-DL-glutamyl-S-benzyl-L-cysteinylglycine (V). **A. From Isolated III.**—III (8.04 g, 0.03 mole) and 5.04 g, (0.06 mole) of $NaHCO_3$ was dissolved in 500 ml of H_2O and the pH was adjusted to 8.0 at 25°. A solution of 0.03 mole of IV in 270 ml of dioxane (prepared from CBZ-α-methyl-DL-glutamic acid and dicyclohexylcarbodiimide as described above) was added over a period of 20 min and the pH was held at 8.0 by the addition of a solution of 3 *N* NaOH. The reaction mixture was stirred 1 additional hr and the pH was adjusted to 1.5. Water (500 ml) was added and the mixture was extracted twice with 500-ml portions of ethyl acetate. The combined EtOAc extracts were dried (Na_2SO_4) and evaporated to yield 7.6 g (47%) of V as an amorphous solid contaminated with a trace of CBZ-α-methyl-DL-glutamic acid as shown by thin layer chromatography. This material was not purified further but carried to the next step where the impurity was more easily removed.

B. Without Isolation of III.—A solution of III [prepared from 4.03 g, 0.017 mole, of N-carboxy-S-benzyl-L-cysteine anhydride and 1.12 g, 0.015 mole, of glycine (as described above)] in 150 ml of borate buffer was acidified to pH 2.5 to decarboxylate the carbamate of III. After the solution had stood for 1 hr, the pH was taken to 8.0 and a solution of IV in dioxane (prepared by allowing 2.81 g, 0.010 mole, of α-methyl-DL-glutamic acid to react with dicyclohexylcarbodiimide in 80 ml of dioxane) was added with stirring over a period of 20 min while the pH was held at 8.0. The reaction mixture was stirred for 1 additional hr and V was isolated as described in A (yield 4.0 g, 73% based on the limiting reagent IV). Removal of the blocking groups from this material as described below gave analytically pure α-methyl-DL$_g$-glutathione.

α-Methyl-DL$_g$-glutathione.—Sodium was added to a solution of V (4.0 g, 0.0073 mole) (prepared in B above) in 250 ml of liquid NH_3 until a light blue color remained for 5 min. The excess sodium was removed by the addition of NH_4Cl and the NH_3 was removed under a stream of N_2. The residual solids were dissolved in 125 ml of a 0.5 *N* solution of H_2SO_4 and the solution was extracted with ethyl acetate to remove dibenzyl. VI was then precipitated as the copper salt (as in the isolation of glutathione[16]), which was isolated by centrifugation and dialyzed to remove the equivalent of H_2SO_4 associated with it. This product was treated with H_2S and the CuS was removed by filtration. The resulting solution was lyophilized to give VI as a fluffy powder. The yield was 0.630 g (72% yield based on V).

Anal. Calcd for $C_{10}H_{19}N_3O_6S$: C, 41.12; H, 5.96; N, 13.08; S, 9.98; 2 equiv of N_2, 8.72; mol wt, 321. Found: C, 41.25; H, 5.70; N, 13.28; S, 10.14; Van Slyke amino N analysis, 8.3; mol wt (NaOH), 311 ($pH_{1/2}$ 3.5, 8.5) (I_2), 315.

Paper chromatography and electrophoresis were carried out on the N-ethylmaleimide (NEM) derivative.[17] Circular paper chromatography gave R_2 0.32 (solvent a) and 0.53 (b) compared with R_2 0.26 (a) and 0.43 (b) for the N-ethylmaleimide derivative of glutathione. VI-NEM moves 9.6 cm on electrophoresis on S and S No. 598 paper at pH 7.5 and a voltage gradient of 11.3 v/cm for 3.5 hr and 10.4 cm at pH 4.15 and a voltage gradient of 13.7 v/cm for 3.75 hr. It was found to be a single component in all systems. Glutathione-NEM moved 9.6 and 11.5 cm, respectively, under the above conditions.

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