(2 - hydroxy - 2 - phenylethyl) - N - ethyl - 2 - methyl - 3 - hydroxy-2-butylamine hydrochloride in 250 ml. of xylene was refluxed overnight. After cooling, the mixture was washed with water. The aqueous layer was separated, treated with excess 50% sodium hydroxide solution, and extracted with ether. The organic layer was dried over magnesium sulfate, filtered, and the solvent removed at reduced pressure. The residue boiled at 90–92°/0.3 mm. The hydrochloride of the distillate was recrystallized from ethyl acetate, m.p. 193–195°.

Anal. Calcd. for $C_{15}H_{24}ClNO$: C, 66.77; H, 8.97. Found: C, 66.96; H, 8.82.

3-(2-Chloroethoxy)-3-methyl-1-butyne (XI).—The 3-chloro-3-methyl-1-butyne, 102.5 g. (1.0 mole), was added dropwise to 500 g. of ethylene glycol containing 60 g. of sodium hydroxide. The mixture was stirred for 2 days after which 500 ml. of water was added, and the mixture extracted with ether. The ether solution was dried over magnesium sulfate, filtered, and the ether was removed from the filtrate at reduced pressure. The residue was distilled, giving 28 g. (22%) of crude 3-(2-hydroxyethxoxy)-3-methyl-1-butyne, b.p. 80–85°/20 mm.

A solution of the crude distillate (0.22 mole) and 71 g. (0.6 mole) of thionyl chloride in 250 ml. of benzene was stirred overnight. The benzene and excess thionyl chloride were removed at reduced pressure and the residue distilled, giving 23 g. (70%) of product, b.p. 56–60°/20 mm.

Anal. Calcd. for $C_7H_{11}ClO$: C, 57.34; H, 7.57. Found: C, 57.18; H, 7.39.

3-(2-Ethylaminoethoxy)-3-methyl-1-butyne (XII).—A mixture of 23 g. (0.157 mole) of 3-(2-chloroethoxy)-3-methyl-1-butyne and 52 g. (0.8 mole) of 70% ethylamine was refluxed for 36 hr. (An additional 25 g. of 10% ethylamine was added after 12 hr.). The cooled mixture was then treated with 15 g. of 10% sodium hydroxide solution and extracted with ether. The ether solution was dried over magnesium sulfate, filtered, and the ether removed. The residue was distilled at reduced pressure giving 10 g. (46%) of product, b.p. 88°/30 mm., $n^{25}D$ 1.4372. The hydrochloride, prepared from a small amount of the distillate, was recrystallized from methyl ethyl ketone, m.p. 76–78°.

Anal. Calcd. for $C_9H_{18}ClNO$: C, 56.38; H, 9.46. Found: C, 56.15; H, 9.43.

2,2,3-Trimethyl-4-ethylmorpholine (XIV).—The 3-(2-ethylaminoethoxy)-3-methyl-1-butyne, 10 g. (0.065 mole), was added dropwise to a mixture of 10 g. of sulfuric acid, 1 g. of red mercuric oxide, 12 ml. of methanol, and 12 ml. of water. (The rate of addition was adjusted to maintain a gentle reflux.) After refluxing for an additional 3 hr., 1 g. of filter aid and 1 g. of powdered charcoal were added, and the warm mixture was filtered with suction. The cooled filtrate was made strongly basic with sodium hydroxide, and extracted with ether. The ether solution was dried over magnesium sulfate and filtered. The filtrate was distilled at reduced pressure and the product collected at 54°/7 mm., giving 3 g. (30%) of a clear oil. The distillate was hydrogenated in 50 ml. of ethanol using 0.1 g. of 5% palladium on carbon as catalyst at approximately 40 p.s.i.g. of hydrogen. The catalyst was removed by filtration, and anhydrous hydrogen chloride was added to the filtrate until the solution was acidic to congo red. The ethanol was removed at reduced pressure and the residue crystallized from ethyl acetate, m.p. 195–197°, giving 3 g. (24%) of product.

Anal. Calcd. for $C_9H_{20}ClNO$: C, 55.50; H, 10.41. Found: C, 55.53; H, 10.58.

Acknowledgment.—The microanalyses were performed by Messrs. William Brown, Howard Hunter, George Maciak, and Alfred Brown. Many of the starting materials were prepared by Dr. Dwight Morrison and Mr. Lawrence White. The infrared spectra were obtained by Mrs. Doris Stephens and Miss Martha Hoffmann. The authors especially wish to thank Dr. Harold Boaz and Messrs. Paul Landis and Donald Woolf, Jr., for their invaluable services in interpreting and compiling the infrared and n.m.r. data. The pressure reactions were carried out by Mr. William Scanlon. The authors also express their sincere appreciation to Dr. George Hennion for his many helpful suggestions and much appreciated encouragement.

Phenylhydrazide as a Protective Group in Peptide Synthesis. The Oxidation of γ-Phenylhydrazides of N-Carbobenzoxy-α-L-glutamylamino Acid Esters with Manganese Dioxide

R. B. KELLY

Research Laboratories of The Upjohn Company, Kalamazoo, Michigan

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The synthesis of some γ-phenylhydrazides of N-carbobenzoxy-α-L-glutamylamino acid esters (III) is described. Treatment of aqueous acetic acid solutions of these compounds with manganese dioxide, at room temperature, results in the rapid oxidation of the phenylhydrazide group to a carboxylic acid leaving the carbobenzoxy group and the ester intact and without racemization. The use of the phenylhydrazide group as a protective group in peptide synthesis is suggested.

An an extension of our synthetic work in the agaritine series,[1] we have synthesized some γ-phenylhydrazides of N-carbobenzoxy-α-L-glutamylamino acid esters (III). These were obtained in good yield by condensing either N-carbobenzoxy-L-glutamic acid γ-phenylhydrazide[1] (Ia) or N-carbobenzoxy-L-glutamic acid γ-(*p*-tolylhydrazide)[1] (Ib) with amino acid esters using N-ethyl-5-phenylisoxazolium-3′-sulfonate[2] as condensing agent. Compounds of type III which have been synthesized to date along with the yields in which they were obtained are listed in Table I.

Aside from their novelty, dipeptides of type III are of interest because of their mode of oxidation with manganese dioxide. When aqueous acetic acid solutions were treated with activated manganese dioxide[3] at room temperature, the phenylhydrazide group was rapidly oxidized to a carboxylic acid,[4] with the evolution of gas, leaving the protecting carbobenzoxy and ester groups intact. A period of thirty to forty minutes is adequate for complete oxidation. The yields of the acid were good, in some cases excellent; a summary of the results obtained is given in Table II.

(1) R. B. Kelly, E. G. Daniels, and J. W. Hinman, *J. Org. Chem.*, **27**, 3229 (1962).

(2) Woodward's reagent, K. R. B. Woodward and R. A. Olofson, *J. Am. Chem. Soc.*, **83**, 1007 (1961); R. B. Woodward, R. A. Olofson, and H. Mayer, *ibid.*, **83**, 1010 (1961).

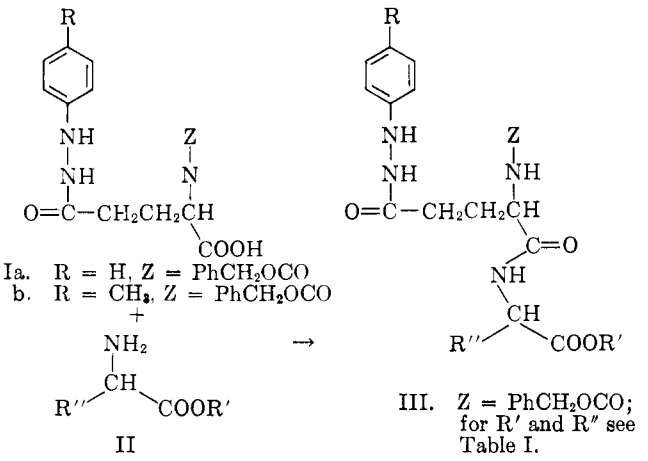
(3) J. Attenburrow, A. F. B. Cameron, J. H. Chapman, R. M. Evans, B. A. Hems, A. B. A. Jansen, and T. Walker, *J. Chem. Soc.*, 1094 (1952).

(4) *Cf.* H. B. Milne, J. E. Halver, D. S. Ho, and M. S. Mason, *J. Am. Chem. Soc.*, **79**, 637 (1957); E. Waldschmidt-Leitz and K. Kühn, *Ber.*, **84**, 381 (1951).

TABLE I

DIPEPTIDES OF TYPE III

γ-Phenylhydrazide of N-carbobenzoxy-α-L-glutamyl-	R	R′	R″	Yield, %
Glycine ethyl ester (IIIa)	H	C_2H_5	H	77
Glycine methyl ester (IIIb)	H	CH_3	H	86
L-Valine methyl ester (IIIc)	H	CH_3	$(CH_3)_2CH$	80
L-Leucine ethyl ester (IIId)	H	C_2H_5	$(CH_3)_2CHCH_2$	74
L-Phenylalanine ethyl ester (IIIe)	H	C_2H_5	$PhCH_2$	71
L-Methionine methyl ester (IIIf)	H	CH_3	CH_3—S—$(CH_2)_2$	73
L-Methionine methyl ester (IIIg)	CH_3	CH_3	CH_3—S—$(CH_2)_2$	85



The oxidation products of IIIa and IIIc were each characterized by saponification to the dicarboxylic acid and decarbobenzoxylation to the free dipeptide. A comparison of the optical rotations of the unsubstituted dipeptides with values reported in the literature indicated that no racemization had occurred.

The above results suggest the use of the phenylhydrazide group for protection of carboxylic acids in peptide chemistry, especially in situations where alkaline saponification of esters is undesirable.

The presence of methionine and, no doubt, other sulfur-containing amino acids, introduces complications due to the possibility of oxidation of the sulfide to a sulfoxide. Under the conditions of the reaction, methionine itself is converted to a mixture of 67% methionine sulfoxide and 33% methionine. There is the possibility, as yet not fully explored, of rectifying this situation by mild reduction of the oxidation product with mercaptans[5] or, alternatively, by substituting methionine sulfoxide[5] for methionine and reducing when convenient.

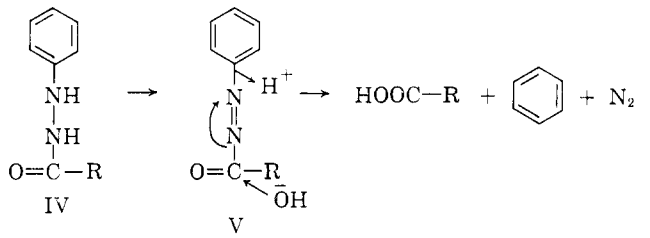
The oxidation of phenylhydrazides to carboxylic acids with manganese dioxide may be explained by assuming a preliminary oxidation to the azo compound (IV → V), followed by the heterolytic elimination of nitrogen with the addition of water or acetic acid (as shown in V) to give a carboxylic acid, benzene, and nitrogen. If acetate ion, rather than hydroxyl ion, is involved, a mixed anhydride would result which, on hydrolysis, would yield an acid. Since there are other possibilities, for example the involvement of phenyldiimide as an intermediate, the mechanism of this reaction is being investigated.

(5) B. Iselin, *Helv. Chim. Acta*, **44**, 61 (1961).

TABLE II

OXIDATION OF γ-PHENYLHYDRAZIDES WITH MANGANESE DIOXIDE

Substrate	Product	Yield, %
IIIa	N-Carbobenzoxy-α-L-glutamylglycine ethyl ester	82
IIIb	N-Carbobenzoxy-α-L-glutamylglycine methyl ester	92
IIIc	N-Carbobenzoxy-α-L-glutamyl-L-valine methyl ester	62
IIId	N-Carbobenzoxy-α-L-glutamyl-L-leucine ethyl ester	78
Ia	N-Carbobenzoxy-L-glutamic acid	76



The high yield of carboxylic acid (Table II) apparently precludes a homolytic elimination of nitrogen to produce acyl and phenyl radicals. However, given suitable conditions, one might expect the homolytic elimination to occur. In either event, the utilization of this type of reaction as an intramolecular bonding reaction may be feasible. This aspect is now under investigation.

Experimental

Melting points were determined on a Kofler block and are corrected.

N-Carbobenzoxy-α-L-glutamylglycine Ethyl Ester γ-Phenylhydrazide (IIIa).—A suspension of 2.026 g. (0.008 mole) of N-ethyl-5-phenylisoxazolium-3′-sulfonate[2] in 50 ml. of acetonitrile was treated with a solution of 2.968 g. (0.008 mole) of N-carbobenzoxy-L-glutamic acid γ-phenylhydrazide (Ia) in 25 ml. of acetonitrile containing 1.11 ml. (0.008 mole) of triethylamine. The mixture was stirred at room temperature for 30 min. by which time all material was in solution. The solution was treated with 1.17 g. (0.008 mole) of glycine ethyl ester hydrochloride in 25 ml. of acetonitrile containing 1.11 ml. of triethylamine. The resulting solution was left at room temperature overnight then evaporated *in vacuo* at room temperature. The residue was crystallized from 0.5% sodium bicarbonate solution and the crystalline product was washed well with 0.5% sodium bicarbonate solution and water. The dried product (2.895 g., 77%) melted at 162–165°, had λ_{max}^{EtOH} 233 mμ (ε 11,500) and 282 mμ (ε 1690), ν_{max}^{Nujol} 3209, 3090, 3050, 3030, 1735, 1695, 1665, 1650, 1605, 1550, 1500, 1255, 1235, 1065, 755, 700, and 690 $cm.^{-1}$.

Anal. Calcd. for $C_{23}H_{25}O_6N_4$: C, 60.51; H, 6.18; N, 12.28. Found: C, 60.57; H, 5.94; N, 12.33.

N-Carbobenzoxy-α-L-glutamylglycine Methyl Ester γ-Phenylhydrazide (IIIb).—This was prepared by the procedure detailed in the preceding experiment from 0.002 mole each of Ia and glycine methyl ester hydrochloride. Crystallization of the crude product from ethyl acetate–petroleum ether (b.p. 30–60°) gave 706 mg. (86%) of the desired product melting at 163–165°. For analysis the product was recrystallized from ethyl acetate–petroleum ether, m.p. 164–166°, λ_{max}^{EtOH} 233 mμ (ε 11,200) and 282 mμ (ε 1530), infrared spectrum similar to IIIa.

Anal. Calcd. for $C_{22}H_{26}O_6N_4$: C, 59.72; H, 5.92; N, 12.67; O; 21.69. Found: C, 59.42; H, 5.86; N, 12.61; O, 21.52.

N-Carbobenzoxy-α-L-glutamyl-L-valine Methyl Ester γ-Phenylhydrazide (IIIc).—This was prepared by the procedure detailed in the first experiment from 0.030 mole each of Ia and L-valine methyl ester hydrochloride. The crystalline product was obtained in 80% yield (11.59 g.). Recrystallization from 80% methanol gave a sample which melted at 125–130°, resolidified and remelted at 166–169°, λ_{max}^{EtOH} 233 mμ (ε 11,400) and 282 mμ (ε 1450), infrared spectrum similar to IIIa.

Anal. Calcd. for $C_{25}H_{32}O_6N_4$: C, 61.96; H, 6.66; N, 11.57. Found: C, 61.56; H, 6.14; N, 11.65.

N-Carbobenzoxy-α-L-glutamyl-L-leucine Ethyl Ester γ-Phenylhydrazide (IIId).—This was prepared by the procedure detailed in the first experiment from 0.002 mole each of Ia and L-leucine ethyl ester hydrochloride. The crystalline product (740 mg., 74%) had m.p. 146–153°. Recrystallization from methanol–water afforded a sample with m.p. 154–157°, λ_{max}^{EtOH} 234 mμ (ε 12,200) and 282 mμ (ε 2000), infrared spectrum similar to IIIa.

Anal. Calcd. for $C_{27}H_{36}O_6N_4$: C, 63.26; H, 7.08; N, 10.93. Found: C, 63.08; H, 6.68; N, 11.09.

N-Carbobenzoxy-α-L-glutamyl-L-phenylalanine Ethyl Ester γ-Phenylhydrazide (IIIe).—This was prepared by the procedure detailed in the first experiment from 0.002 mole each of Ia and L-phenylalanine ethyl ester hydrochloride. The product (775 mg., 71%) had m.p. 177–179°, λ_{max}^{EtOH} 233 mμ (ε 11,500) and 282 mμ (ε 1460), infrared spectrum similar to IIIa.

Anal. Calcd. for $C_{30}H_{34}O_6N_4$: C, 65.85; H, 6.27; N, 10.27. Found: C, 65.43; H, 6.61; N, 10.22.

N-Carbobenzoxy-α-L-glutamyl-L-methionine Methyl Ester γ-Phenylhydrazide (IIIf).—This was prepared by the procedure detailed in the first experiment from 0.005 mole each of Ia and L-methionine methyl ester hydrochloride. The crystalline product (1.88 g., 73%) had m.p. 163–167°. Crystallization from ethanol–water gave a sample which had m.p. 164–168°, λ_{max}^{EtOH} 233 mμ (ε 11,000) and 282 mμ (ε 1290), infrared spectrum similar to IIIa.

Anal. Calcd. for $C_{25}H_{32}O_6N_4S$: C, 58.12; H, 6.24; N, 10.85; S, 6.21. Found: C, 58.00; H, 6.05; N, 10.93; S, 5.90.

N-Carbobenzoxy-α-L-glutamyl-L-methionine Methyl Ester γ-(*p*-Tolylhydrazide) (IIIg).—This was prepared by the procedure detailed in the first experiment from 0.010 mole each of Ib and L-methionine methyl ester hydrochloride. The product (4.51 g., 85%) had m.p. 180°. Crystallization from methanol–water afforded a sample with m.p. 190–193°, λ_{max}^{EtOH} 236 mμ (ε 12,000) and 285 mμ (ε 1580), infrared spectrum similar to IIIa.

Anal. Calcd. for $C_{26}H_{34}O_6N_4S$: C, 58.84; H, 6.46; N, 10.56; S, 6.04. Found: C, 58.88; H, 6.20; N, 10.62; S, 5.71.

Oxidation of N-Carbobenzoxy-α-L-glutamylglycine Ethyl Ester γ-Phenylhydrazide (IIIa) with Manganese Dioxide.—A solution of 3.42 g. (0.0075 mole) of IIIa in 100 ml. of 60% acetic acid was treated with 3.92 g. of activated manganese dioxide.[3] The mixture was stirred at room temperature for 30 min., more (0.50 g.) manganese dioxide was added, and stirring was continued for 15 min. Solid material was removed by filtration and washed with four 20-ml. portions of glacial acetic acid then with 25 ml. of ethyl acetate. The combined filtrate and washings were saturated with hydrogen sulfide gas, with cooling, and evaporated *in vacuo*[6] at 25–30°. The residue was dissolved in 100 ml. of saturated sodium bicarbonate solution and the aqueous solution was extracted with three 70-ml. portions of ethyl acetate. The ethyl acetate extracts were extracted with two 100-ml. portions of saturated sodium bicarbonate solution. The ethyl acetate extracts were discarded and the combined aqueous extracts were acidified to congo red with 6 *N* hydrochloric acid and extracted with three 500-ml. portions of ethyl acetate. The ethyl acetate extracts were washed with 100 ml. of 1 *N* hydrochloric acid then with two 100-ml. portions of water. The combined ethyl acetate extracts, dried over sodium sulfate, yielded on evaporation to dryness *in vacuo*, 2.25 g. (82%) of N-carbobenzoxy-α-L-glutamylglycine ethyl ester with m.p 102–106° (*on some occasions it resolidified and melted at 125–126°*). Recrystallization from ethyl acetate–petroleum ether gave a sample with m.p. 108.5–109.5° (lit.,[7] 120–122°, 126°, 122°); characterized by saponification to N-carbobenzoxy-α-L-glutamylglycine (see col. 2).

(6) For the best results the last traces of acetic acid should be removed in high vacuum.

(7) (a) W. J. Le Quesne and J. T. Young, *J. Chem. Soc.*, 1959 (1950); (b) M. Bergman, L. Zervas, and J. S. Fruton, *J. Biol. Chem.*, **111**, 225 (1935); (c) W. Grassman and F. Schneider, *Biochem. Z.*, **273**, 452 (1934).

Anal. Calcd. for $C_{17}H_{22}O_7N_2$: C, 55.73; H, 6.05; N, 7.65. Found: C, 55.68; H, 6.41; N, 7.57.

N-Carbobenzoxy-α-L-glutamylglycine.—N-Carbobenzoxy-α-L-glutamylglycine ethyl ester (1.09 g., 0.003 mole) from the preceding experiment was dissolved in 6 ml. of 1 *N* sodium hydroxide and left at room temperature for 1 hr. The alkaline solution was diluted with 14 ml. of water and extracted with three 15-ml. portions of ethyl acetate and the ethyl acetate extracts were extracted with two 10-ml. portions of saturated sodium bicarbonate solution. The ethyl acetate extracts were discarded and the aqueous layers combined, acidified to congo red with 6 *N* hydrochloric acid, and extracted with three 60-ml. portions of ethyl acetate. The ethyl acetate extracts were washed with two 15-ml. portions of 0.1 *N* hydrochloric acid and 15 ml. of water. The combined ethyl acetate extracts, dried over sodium sulfate, yielded, on evaporation *in vacuo* 0.560 g. (56%) of crystalline N-carbenzoxy-α-L-glutamylglycine with m.p. 155°. Recrystallization from ethyl acetate yielded a sample with m.p. 156–158° (lit.,[7c] 143°); characterized by decarbobenzoxylation to α-L-glutamylglycine (see below).

Anal. Calcd. for $C_{15}H_{18}O_7N_2$: C, 53.25; H, 5.36; N, 8.28. Found: C, 53.44; H, 5.26; N, 8.21.

α-L-Glutamylglycine.—N-Carbobenzoxy-α-L-glutamylglycine (100 mg., 0.0003 mole) in 25 ml. of 40% methanol was hydrogenated over 15 mg. of palladium–charcoal (10%) catalyst for 90 min. at 2 atm. The catalyst was removed by filtration and washed with 50 ml. of warm water. The combined filtrate and washings were evaporated *in vacuo* and the residue, on crystallization from water–ethanol yielded 57 mg. (95%) of α-L-glutamylglycine, m.p. 175–177°, $[\alpha]^{25}D$ +78° (*c* 2.56, water) (lit.,[7c,8] m.p. 180°, 220°, 175–176°; $[\alpha]D$ (in water) +80.3°, +79.5 ± 1°, +80 ± 1°).

Anal. Calcd. for $C_7H_{12}O_5N_2$: C, 41.17; H, 5.92; N, 13.72. Found: C, 41.01, H, 5.63; N, 14.07.

Oxidation of N-Carbobenzoxy-α-L-glutamylglycine Methyl Ester γ-Phenylhydrazide (IIIb) with Manganese Dioxide.—A solution of 221 mg. (0.0005 mole) of IIIb in 8 ml. of 50% acetic acid was stirred with 218 mg. of activated manganese dioxide[3] for 30 min. The reaction mixture was worked up by the procedure detailed in the preceding oxidation experiment. The crude product, on crystallization from ethyl acetate–petroleum ether, yielded 162 mg. (92%) of N-carbobenzoxy-α-L-glutamylglycine methyl ester with m.p. 93–95°.

Anal. Calcd. for $C_{16}H_{20}O_7N_2$: C, 54.54; H, 5.72; N, 7.92. Found: C, 54.55; H, 5.38; N, 7.71.

Oxidation of N-Carbobenzoxy-α-L-glutamyl-L-valine Methyl Ester γ-Phenylhydrazide (IIIc) with Manganese Dioxide.—A solution of 2.43 g. (0.005 mole) of IIIc in 40 ml. of 60% acetic acid was stirred with 2.61 g. of activated manganese dioxide[3] for 30 min., then more (0.20 g.) manganese dioxide was added and stirring was continued for 15 min. The reaction mixture was worked up as in the preceding oxidation experiments. The crude product, on crystallization from ethyl acetate–petroleum ether with charcoal treatment, yielded 1.22 g. (62%) of N-carbobenzoxy-α-L-glutamyl-L-valine methyl ester with m.p. 117–120°. Recrystallization from ethyl acetate–petroleum ether yielded a sample with m.p. 120.5–121°; characterized by saponification to N-carbobenzoxy-α-L-glutamyl-L-valine (see below).

Anal. Calcd. for $C_{19}H_{26}O_7N_2$: C, 57.85; H, 6.65; N, 7.10. Found: C, 57.66; H, 6.85; N, 7.33.

N-Carbobenzoxy-α-L-glutamyl-L-valine.—N-Carbobenzoxy-α-L-glutamyl-L-valine methyl ester (5.92 mg., 0.0015 mole) from the preceding experiment was dissolved in 3.3 ml. of 1 *N* sodium hydroxide and 2 ml. of water and left at room temperature for 1 hr. The alkaline solution was diluted with 15 ml. of water and extracted with 5 ml. of ethyl acetate. The ethyl acetate extract was extracted with two 2.5-ml. portions of 1 *N* sodium hydroxide and discarded. The combined aqueous layers were acidified to congo red with 6 *N* hydrochloric acid, diluted to 25 ml. with water, and extracted with four 25-ml. portions of ethyl acetate. The ethyl acetate extracts were washed with two 20-ml. portions of 1 *N* hydrochloric acid then with two 10-ml. portions of water. The combined ethyl acetate extracts, dried over sodium sulfate, yielded, on evaporation *in vacuo* and crystallization of the residue from ethyl acetate–petroleum ether, 545 mg. (96%) of N-carbobenzoxy-α-L-glutamyl-L-valine with m.p. 133–134°. Recrystallization from ethyl acetate–petroleum

(8) (a) G. Amiard, R. Heymes, and L. Velluz, *Bull. soc. chim. France*, 97 (1956); (b) J. Rudinger, *Chem. Listy*, **48**, 244 (1954).

ether gave a sample with m.p. 134–135° (lit.,[9] 131–133°); characterized by decarbobenzoxylation to α-L-glutamyl-L-valine (see below).

Anal. Calcd. for $C_{18}H_{24}O_7N_2$: C, 56.83; H, 6.36; N, 7.37; O, 29.44. Found: C, 56.84; H, 6.40; N, 7.39; O, 29.54.

α-L-Glutamyl-L-valine.—N-Carbobenzoxy-α-L-glutamyl-L-valine (300 mg., 0.00079 mole) from the preceding experiment, in 40 ml. of 60% methanol, was hydrogenated over 25 mg. of palladium–charcoal (10%) catalyst at 2 atm. for 1 hr. The catalyst was removed by filtration and washed with 25 ml. of warm water. The combined filtrate and washings were evaporated *in vacuo* and the residue, on crystallization from water–ethanol, yielded 145 mg. (75%) of α-L-glutamyl-L-valine, m.p. 188°, $[\alpha]^{25}D$ +24.4 (*c* 2.46 in 0.1 *N* hydrochloric acid) [lit.,[9] m.p. 189–190°; $[\alpha]^{15}D$ +24.5 (in dilute hydrochloric acid)].

Anal. Calcd. for $C_{10}H_{18}O_5N_2$: C, 48.77; H, 7.37; N, 11.38. Found: C, 48.78; H, 7.06; N, 10.93.

Oxidation of N-Carbobenzoxy-α-L-glutamyl-L-leucine Ethyl Ester γ-Phenylhydrazide (IIId) with Manganese Dioxide.—A solution of 1.025 g. (0.002 mole) of IIId in 50 ml. of 50% acetic acid was stirred with 1.044 g. of activated manganese dioxide[3] for 30 min., more (0.250 g.) manganese dioxide was added and stirring was continued for 15 min. The reaction mixture was worked up as in the preceding oxidation experiments to give 0.663 g. (78%) of N-carbobenzoxy-α-L-glutamyl-L-leucine ethyl ester with m.p. 87–90°. Crystallization from ethyl acetate–hexane afforded a sample with m.p. 92–95° (lit.,[9] 88–94°).

Anal. Calcd. for $C_{21}H_{30}O_7N_2$: C, 59.70; H, 7.16; N, 6.63. Found: C, 60.02; H, 6.86; N, 6.70.

Oxidation of N-Carbobenzoxy-L-glutamic Acid γ-Phenylhydrazide (Ia) with Manganese Dioxide.—A solution of 1.86 g. (0.005 mole) of Ia in 50 ml. of 50% acetic acid was stirred with 2.18 g. of activated manganese dioxide[3] for 30 min. The solid material was removed by filtration and washed with 25 ml. of 50% acetic acid, 10 ml. of methanol, and finally with 100 ml. of warm ethyl acetate. The combined filtrate and washings were worked up as in the preceding oxidation experiments. The crude product yielded, on crystallization from ethyl acetate–petroleum ether, 1.071 g. (76%) of N-carbobenzoxy-L-glutamic acid with m.p. 118–120° (lit.,[10] 120°), identical to a sample prepared by carbobenzoxylation of L-glutamic acid (m.p., mixed m.p., and infrared spectrum).

Anal. Calcd. for $C_{13}H_{15}O_6N$: C, 55.51; H, 5.38; N, 4.98. Found: C, 55.58; H, 5.20; N, 4.94.

Oxidation of Methionine with Manganese Dioxide.—A solution of 149 mg. of L-methionine in 5 ml. of 50% acetic acid was stirred with 435 mg. of activated manganese dioxide[3] for 45 min. The mixture was filtered and the solid material washed with 10 ml. of glacial acetic acid and 10 ml. of water. The combined filtrate and washings were saturated with hydrogen sulfide gas then evaporated *in vacuo* at 25–28°. Analysis of the residue on a Beckman/Spinco amino acid analyzer (Model 120) showed that the amino acid content consisted of 67% methionine sulfoxide and 33% methionine.

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(9) W. J. Le Quesne and J. T. Young, *J. Chem. Soc.*, 1954 (1950).

(10) M. Bergman and L. Zervas, *Ber.*, **65**, 1192 (1932).

Synthesis of Sulfur Analogs of Inositol (Dimercaptocyclohexanetetrols). Nuclear Magnetic Resonance Configurational Proofs[1,2]

G. E. McCasland,[3] Stanley Furuta, Arthur Furst, L. F. Johnson,[4] and J. N. Shoolery[4]

Institute of Chemical Biology, University of San Francisco, San Francisco 17, California, and Varian Associates, Palo Alto, California

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Two stereoisomers of 5,6-dimercapto-1,2,3,4-cyclohexanetetrol have been synthesized and their configurations determined by means of nuclear magnetic resonance spectra and optical rotation calculations. Reaction of dextrorotatory 3,4:5,6-di-*O*-isopropylidene-1,2-anhydro-*allo*-inositol with carbon disulfide gave a mixture of two diastereomeric trithiocarbonate diketals (m.p. 204°, 191°). The 204° diastereomer on reduction gave a tetroldithiol diketal, m.p. 91°, shown by n.m.r. to have the (126/345) configuration. This product was converted to the tetroldithiol, its hexaacetate, and a mercaptole diketal, all of the same configuration. The 191° trithiocarbonate diketal on similar treatment gave a tetroldithiol diketal, tetroldithiol, tetroldithiol hexaacetate, mercaptodithiolane diketal, and mercaptodithiolane pentaacetate, all necessarily having the (125/346) configuration. The trithiocarbonate derivatives give optical rotatory dispersion spectra with well defined Cotton effects and have ultraviolet specific rotations exceeding plus or minus 22,000 degrees. An attempt to predict the monochromatic optical rotations of the two tetroldithiols gave results which were in the right order, but showed large deviations in magnitude from the experimentally found values.

Continuing our studies[2,5] on the stereochemistry and nuclear magnetic resonance spectra of alicyclic type carbohydrates (cyclitols), we wish to report the synthesis and characterization of what appear to be the first known sulfur analogs of inositol.[5a] Our present products include two optically active diastereomers of 5,6-dimercapto-5,6-dideoxyinositol (XIV, XVII) and corresponding hexaacetate (XV, XVIII), diisopropylidene (X, XII), and trithiocarbonate (VI, VIII) derivatives, and one active diastereomer of a mercaptolediketal (XI) and of a mercaptodithiolane diketal (XIII) and pentaacetate (XIIIa).

This program of studies on sulfur-containing cyclitols has been initiated, partly in the hope of discovering substances of biological value,[6] and partly in order to expand knowledge in certain relatively unexplored areas of

(1) Presented at Brussels, Belgium, in June, 1962, to the I.U.P.A.C. Symposium International de Chimie Organique consacré à l'Étude des Produits Naturels. Certain aspects of this research were reported to the Division of Carbohydrate Chemistry at the 141st National Meeting of the American Chemical Society, Washington, D. C., March, 1962.

(2) Paper XIII on cyclitol stereochemistry by G. E. McCasland and coworkers; for preceding paper see J. N. Shoolery, L. F. Johnson, Stanley Furuta, and G. E. McCasland, *J. Am. Chem. Soc.*, **83**, 4243 (1961).

(3) To whom any requests for reprints should be sent: Institute of Chemical Biology, University of San Francisco, San Francisco 17, Calif.

(4) Varian Associates.

(5) G. E. McCasland, Stanley Furuta, L. F. Johnson, and J. N. Shoolery, *J. Am. Chem. Soc.*, **83**, 2335 (1961).

(5a) Note Added in September, 1962: We have recently prepared a monomercaptocyclohexanepentol (hexaacetate m.p. 182°) by reaction of 1,2-anhydro-*cis*-inositol diketal with benzyl mercaptan, reduction and hydrolysis. Details will be given in a subsequent publication.

(6) Biological interest in inositol sulfur analogs arises in part from the fact that *myo*-inositol is essential for growth of isolated normal and malignant human cells in tissue culture [see H. Eagle, *J. Biol. Chem.*, **226**, 191 (1957)] and also for certain microörganisms. There is also some reason to believe that inositol sulfur analogs may show activity as antitoxic agents for metals or as drugs for radiation protection.