Synthesis of Polyglutathione, Polyasparthione, and Related Sequential Polypeptides[1]

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Polypeptides with repeating sequence of glutathione, asparthione, and their α analogs have been synthesized. The protected polypeptides, poly(α-benzyl-L-glutamyl-*S*-benzyl-L-cysteinylglycine) (XIV), poly(α-benzyl-L-aspartyl-*S*-benzyl-L-cysteinylglycine) (XV), poly(γ-benzyl-L-glutamyl-*S*-benzyl-L-cysteinylglycine) (XVI), and poly(β-benzyl-L-aspartyl-*S*-benzyl-L-cysteinylglycine) (XVII), were prepared by self condensation of the corresponding tripeptide pentachlorophenyl esters IV, V, IX, or X and XII or XIII, respectively. The optically pure tripeptide active esters were obtained through the "backing off" method from the C-terminal glycine pentachlorophenyl ester. The *C*- and *S*-benzyl protecting groups from the polymers XIV, XV, XVI, and XVII have been removed with sodium–liquid ammonia to afford polyglutathione (XVIII), polyasparthione (XIX), polyisoglutathione (XX), and polyisoasparthione (XXI), with weight average molecular weights of 9000, 7000, 16,000, and 6000, respectively. Polyglutathione was investigated for radioprotective activity. Polyglutathione shows a growth stimulating effect on *B. subtilis*.

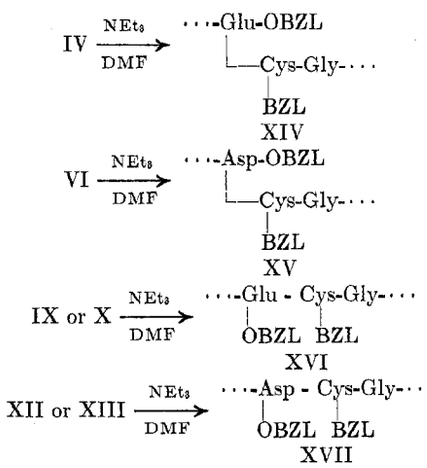
Glutathione[3] has a protective effect against radiation[4] and is considered to act as a detoxicant of hydrogen peroxide generated in cells.[5] It appeared possible that a high-molecular-weight sequential polypeptide containing the repeating unit of glutathione could be stored in the body as an active thiol-containing molecule for prolonged action. Although in recent years a number of sequential polypeptides containing trifunctional amino acids have been synthesized,[6] polypeptides having a repeating sequence of a naturally occurring biologically active peptide are unknown. This paper reports the synthesis of polyglutathione, polyasparthione, and the related α polymers by the pentachlorophenyl ester polymerization method.[6b,e,g-i]

The preparation of the tripeptide active esters IV and VI, which are needed for polycondensation, was achieved in high yields through stepwise lengthening of the peptide chain from the activated C-terminal amino acid, glycine pentachlorophenyl ester, by using the mixed anhydride (M.A.) coupling method, as shown in Scheme I.[7]

Syntheses of the α-tripeptide active esters IX, X and XII, XIII were achieved by using the *N-tert*-butyloxycarbonyl protecting group (Scheme II), since attempted removal of the *N*-benzyloxycarbonyl group from *N*-benzyloxycarbonyl-γ-benzyl-L-glutamyl-*S*-benzyl-L-cysteinylglycine pentachlorophenyl ester (VII), with hydrogen bromide led to a complex mixture.

The tripeptide active ester trifluoroacetate salts IX and XII partly polymerize on standing in solution or drying under vacuum above 50°.

The tripeptide active ester salts were polymerized[6g] in concentrated dimethylformamide solution in the presence of 2 equiv of triethylamine. While the polymerization of the ω-tripeptide active ester salts IV and VI yielded the corresponding polymers XIV and XV, respectively, in 70–80% yield, polymerization of the α-peptide active ester salts IX or X and XII or XIII gave the corresponding polymers XVI and XVII in the range of 55 and 39% yields, respectively.



The *C*- and *S*-benzyl protecting groups of the polymers XIV and XV were removed[8] with sodium–liquid ammonia. The complete removal[9] of the protecting benzyl groups was indicated by the absence of aromatic protons in the pmr spectra of the sodium salts of the deblocked polymers. Polyglutathione (XVIII) and polyasparthione (XIX) were isolated by acidification of the water-soluble sodium salts of the polymers and dialysis, with weight average molecular weights of 9000 and 7000, respectively, in the ultracentrifuge.[10] The iodo-

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(7) Abbreviations follow the rules in "Abbreviated Designation of Amino-Acid Derivatives and Polypeptides" (Information Bulletin No. 25, IUPAC). Other abbreviations follow as used in "Peptides 1968," Proceedings of the 9th European Peptide Symposium, E. Bricas, Ed., Wiley, New York, N. Y., 1968.

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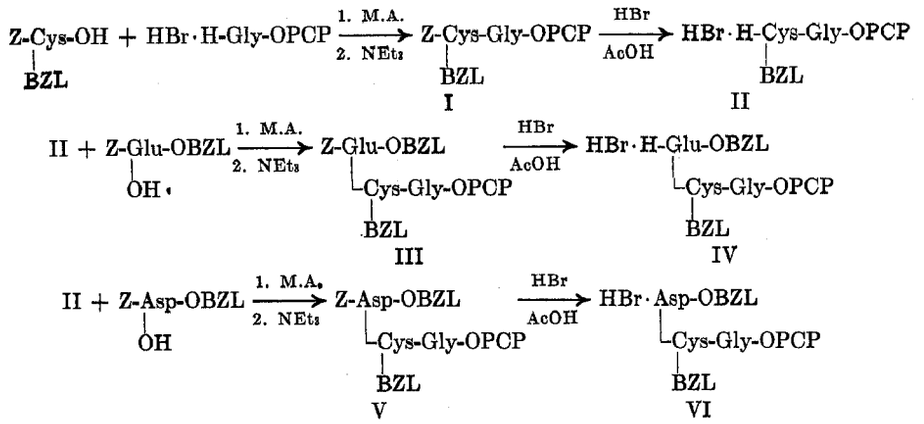
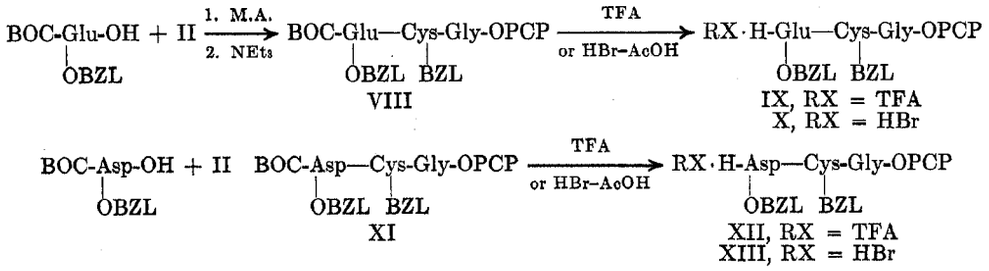
SCHEME I



SCHEME II



metric titration of the thiol groups of dialyzed samples of the polymers XVIII and XIX indicated that only a

negligible amount of disulfide linkage had probably formed under the carefully controlled experimental conditions. Polyisoglutathione (XX) and polyisoasparthione (XXI) were obtained similarly with molec-

ular weights of 16,000 and 6000, respectively. Removal of the *C*- and *S*-benzyl protecting groups of the polymers XIV and XV with anhydrous hydrogen fluoride[11] gave less pure and of lower molecular weight polymers.

During the synthesis of the tripeptide active esters there is no danger of racemization.[12] Racemization of the base-sensitive cysteine residue could occur during polymerization. However, the model dipeptide, *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteinylglycine ethyl ester, in the presence of 7 equiv of triethylamine in the tetrahydrofuran solution showed practically no racemization in 48 hr at room temperature.[13] This result suggests that no racemization of the cysteine residue occurred during polymerization of the tripeptides.

The antiradiation testing of polyglutathione (XVIII) and polyasparthione (XIX) was undertaken by Dr. T. R. Sweeney, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D. C. However, the physical properties of these polymers were unsatisfactory for detailed studies.[14] In addition, Dr. M. Pisano, Department of Biology at St. John's University, investigated the effect of polyglutathione (XVIII) on various microorganisms. Preliminary results disclosed a growth stimulation effect on *Bacillus subtilis* when polyglutathione was present in the medium at a concentration of 25 μg/ml. These results will be published in detail elsewhere in a subsequent paper.

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Experimental Section

Melting points were taken on a Thomas–Hoover apparatus and are uncorrected. The infrared spectra were taken in potassium bromide pellets on a Beckman IR-8 spectrophotometer and only the characteristic strong bands are recorded. The microanalyses were carried out by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y., and Drs. G. Weiler and F. B. Strauss, Oxford, England. Unless otherwise stated, all the analytical samples were dried over phosphorus pentoxide for 20 hr at 56° under high vacuum. The polymers were dried under the same conditions at 78°. Thin layer chromatography was carried out on precoated silica gel analytical plates F254 (Brinkman); unless otherwise stated spots were located by ultraviolet light or exposure to iodine vapor.

***N*-Benzyloxycarbonyl-*S*-benzyl-L-cysteinylglycine Pentachlorophenyl Ester (I).**—A well-stirred solution of 6.90 g (20 mmol) of *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteine in 60 ml of dry tetrahydrofuran containing 2.20 ml (20 mmol) of *N*-methylmorpholine was cooled to −15° (Dry Ice–methanol) and 2.80 ml (21 mmol) of isobutyl chloroformate was added. After 15 min

(14) In a private communication Dr. Sweeney informed us that no satisfactory tests on these polymers could be performed due to their insolubility in biologically suitable vehicles. Both compounds, when placed in aqueous vehicles or suspending agents, form sticky gelatinous clungs which prevent their uniform dispersion in the vehicles. Consequently, more dose cannot be uniformly administered to the test animals through a hypodermic needle. However, one of the samples of polyglutathione (XVIII) was tested under these very unsatisfactory conditions and the following results were obtained in ICR female mice: acute LD_{50} (ip) > 1000 mg/kg; survival after 1000 R ^{60}Co γ radiation, 500 mg/kg, 15 min prerad, 15 mice, 67% survival; 250 mg/kg, 15 min prerad, 15 mice, 7% survival; control, 10 mice, 10% survival. These experiments are suggestive of some radioprotective activity.

8.58 g (22 mmol) of glycine pentachlorophenyl ester hydrobromide was added followed by 3.0 ml (21 mmol) of triethylamine in 60 ml of precooled tetrahydrofuran. Stirring at −10 to 0° was continued for 30 min followed by another 1.5 hr at 0°. The reaction mixture was diluted with 300 ml of cold water and the white solid peptide was filtered. The precipitate was thoroughly washed with 5% sodium bicarbonate solution, water, 1 *N* hydrochloric acid, water, two 100-ml portions of cold methanol, and finally with ether. White crystalline dipeptide was dried *in vacuo*; yield 12.20 g (93%), mp 189–191°. Recrystallization from tetrahydrofuran–ether afforded 10.15 g of the dipeptide I as fluffy needles, mp 190–191°, $[\alpha]^{22}D$ −31.8° (*c* 2.0, dimethylformamide). It showed a single spot in tlc in benzene–methanol–acetic acid (10:2:1); λ_{max} 5.63 (COOPCP), 5.92 (urethane), 6.08 (amide I), 6.52 (amide II), and doublet at 7.21 and 7.33 μ (pentachlorophenyl).

Anal. Calcd for $C_{26}H_{21}N_2O_5SCl_5$: C, 48.00; H, 3.25; N, 4.30; S, 4.93. Found: C, 48.03; H, 3.34; N, 4.20; S, 5.15.

S-Benzyl-L-cysteinylglycine Pentachlorophenyl Ester Hydrobromide (II).—*N*-Benzyloxycarbonyl-*S*-benzyl-L-cysteinylglycine pentachlorophenyl ester (I), 8 g (12.3 mmol), was pulverized and thoroughly mixed with 25 ml of dry acetic acid followed by the addition of 40 ml of 40% hydrogen bromide in acetic acid. The reaction mixture was shaken from time to time over a period of 20 min. After this time, 25 ml of acetic acid was added and the solid mass was dispersed by vigorous shaking. After 15 min the mixture was diluted with a large excess of dry ether, cooled overnight, and filtered. The light yellow hydrobromide salt was washed with dry ether and dried *in vacuo* over potassium hydroxide; yield 6.19 g (84%), mp 192–193° dec. Two recrystallizations from methanol–ether yielded 5.21 g of colorless needles; mp 193–194° dec; $[\alpha]^{22}D$ 11.3° (*c* 2, dimethylformamide); λ_{max} 3.44 (broad, $-NH_3^+$), 5.61 (COOPCP), 5.99 (amide I), 6.45 (amide II), and doublet at 7.22 and 7.35 μ (pentachlorophenyl); tlc in 1-butanol–pyridine–acetic acid–water (30:20:6:24) gave a single ninhydrin-positive spot.

Anal. Calcd for $C_{18}H_{16}N_2O_3SCl_5Br$: C, 36.18; H, 2.70; N, 4.69; S, 5.37. Found: C, 36.44; H, 2.61; N, 4.32; S, 5.79.

N-Benzyloxycarbonyl-α-benzyl-L-glutamyl-S-benzyl-L-cysteinylglycine Pentachlorophenyl Ester (III).—*N*-Benzyloxycarbonyl-*L*-glutamic acid α-benzyl ester was coupled with the dipeptide hydrobromide II as described previously. The crude product (82.5%), mp 189–190°, was crystallized from tetrahydrofuran–ether to yield pure tripeptide III: mp 191–193°; tlc, single spot in methylene chloride–methanol (9:1); $[\alpha]^{22}D$ −26.15° (*c* 2, dimethylformamide).

Anal. Calcd for $C_{38}H_{34}N_3O_8SCl_5$: C, 52.46; H, 3.94; N, 4.83. Found: C, 52.11; H, 4.07; N, 4.78.

α-Benzyl-L-glutamyl-S-benzyl-L-cysteinylglycine Pentachlorophenyl Ester Hydrobromide (IV).—This compound was prepared using the procedure described for II in 92% yield, mp 148–150° dec. Crystallization from methanol–ether raised the melting point to 152–154° dec. It was recrystallized twice from the same solvent as colorless needles, mp 153–155° dec, $[\alpha]^{22}D$ −18.4° (*c* 2, dimethylformamide).

Anal. Calcd for $C_{30}H_{29}N_3O_8SCl_5Br$: C, 44.11; H, 3.58; N, 5.36. Found: C, 44.00; H, 3.40; N, 5.14.

N-Benzyloxycarbonyl-α-benzyl-L-aspartyl-S-benzyl-L-cysteinylglycine Pentachlorooheny Ester (V).—To the mixed anhydride prepared from 2.072 g (6 mmol) of *N*-benzyloxycarbonyl-L-aspartic acid α-benzyl ester in 30 ml of tetrahydrofuran, 0.67 ml (6 mmol) of *N*-methylmorpholine, and 0.84 ml (6.3 mmol) of isobutyl chloroformate at −15°, 3.588 g (6 mmol) of the dipeptide hydrobromide II was added, followed by 0.84 ml (6 mmol) of triethylamine in 30 ml of cold tetrahydrofuran. After 30 min at −10 to 0°, stirring at 0° was continued for 1 hr. The reaction mixture was worked up as described for I, yield 4.62 g (95%), mp 191–193°. Two recrystallizations from tetrahydrofuran–ether afforded the tripeptide V, mp 193–195°, $[\alpha]^{25}D$ −25.7° (*c* 2, dimethylformamide); tlc in methylene chloride–methanol (9:1) showed a single spot.

Anal. Calcd for $C_{37}H_{32}N_3O_8SCl_5$: C, 51.92; H, 3.77; N, 4.91; S, 3.74; Cl, 20.71. Found: C, 51.90; H, 3.95; N, 4.78; S, 3.94; Cl, 20.41.

α-Benzyl-L-aspartyl-S-benzyl-L-cysteinylglycine Pentachlorophenyl Ester Hydrobromide (VI).—This compound was prepared the usual way. It was recrystallized from methanol–ether in silky, colorless needles: yield 1.25 g (60%); mp 195–196° dec; $[\alpha]^{24}D$ −15.1° (*c* 2, dimethylformamide).

Anal. Calcd for $C_{29}H_{27}O_6N_3SCl_5Br$: C, 43.39; H, 3.39; N, 5.24; S, 3.99. Found: C, 43.19; H, 3.45; N, 5.34; S, 4.11.

N-Benzyloxycarbonyl-γ-benzyl-L-glutamyl-S-benzyl-L-cysteinylglycine Pentachlorophenyl Ester (VII).—The mixed anhydride, prepared from 2.226 g (6 mmol) of *N*-benzyloxycarbonyl-L-glutamic acid γ-benzyl ester, was coupled with 3.588 g (6 mmol) of the dipeptide hydrobromide II, and the reaction mixture was worked up as described for the dipeptide I. The crude product was crystallized from tetrahydrofuran–ether to afford 4.30 g (82%) of VII: mp 203–205°; $[\alpha]^{23}D$ −21.8° (*c* 2, dimethylformamide); tlc showed a single spot in methylene chloride–methanol (9:1).

Anal. Calcd for $C_{36}H_{34}N_3O_8SCl_5$: C, 52.46; H, 3.94; N, 4.83. Found: C, 52.28; H, 3.80; N, 4.71.

***N-tert*-Butyloxycarbonyl-γ-benzyl-L-glutamyl-S-benzyl-L-cysteinylglycine Pentachlorophenyl Ester (VIII).**—Mixed anhydride coupling of *N-tert*-butyloxycarbonyl-L-glutamic acid γ-benzyl ester[15] and dipeptide hydrobromide II gave VIII in 73% yield, mp 150–151° with shrinking above 147°. Recrystallization from tetrahydrofuran–methanol gave gel, which was filtered, washed with ether, and dried to yield the product as a white powder: mp 151–153°, shrinking above 147°; $[\alpha]^{24}D$ −23.1° (*c* 2, dimethylformamide).

Anal. Calcd for $C_{35}H_{36}N_3O_8SCl_5$: C, 50.28; H, 4.34; N, 5.03; S, 3.83; Cl, 21.20. Found: C, 50.13; H, 3.94; N, 5.06; S, 4.18; Cl, 20.77.

γ-Benzyl-L-glutamyl-S-benzyl-L-cysteinylglycine Pentachlorophenyl Ester Trifluoroacetate (IX).—Pentachlorophenyl ester VIII, 1.52 g (1.8 mmol), was treated with 5 ml of cold anhydrous trifluoroacetic acid at room temperature for 40 min. Evaporation of the solvent *in vacuo* below 30° left colorless solid, 1.453 g (95%), mp 151–153° dec, which reprecipitated from methanol solution with ether: mp 151–153° dec; $[\alpha]^{24}D$ −12.45° (*c* 2, dimethylformamide).

Anal. Calcd for $C_{32}H_{29}N_3O_8SCl_5F_3$: C, 45.22; H, 3.44; N, 4.94; S, 3.77. Found: C, 45.64; H, 3.63; N, 5.09; S, 4.42.

γ-Benzyl-L-glutamyl-S-benzyl-L-cysteinylglycine Pentachlorophenyl Ester Hydrobromide (X).—The tripeptide VIII and HBr gave, after recrystallization from methanol–ether, hydrobromide X as fluffy white solid in 86% yield: mp 162–163° dec; $[\alpha]^{23}D$ −9.95° (*c* 2.01, dimethylformamide).

Anal. Calcd for $C_{30}H_{29}N_3O_8SCl_5Br$: C, 44.11; H, 3.58; N, 5.36; S, 3.92. Found: C, 43.41; H, 3.57; N, 5.20; S, 3.89.

***N-tert*-Butyloxycarbonyl-β-benzyl-L-aspartyl-S-benzyl-L-cysteinylglycine Pentachlorophenyl Ester (XI).**—The mixed anhydride prepared from 3.557 g (11 mmol) of *N-tert*-butyloxycarbonyl-L-aspartic acid β-benzyl ester[16] was coupled to 6.578 g (11 mmol) of dipeptide hydrobromide II. The tripeptide was worked up as described previously, to afford 8.75 g (90%) of XI, mp 164–166°. This was precipitated as gel from tetrahydrofuran–methanol. After drying 6.53 g of the pure tripeptide XI was obtained as a white powder: mp 168–169°; $[\alpha]^{23}D$ −26.3° (*c* 2.03, dimethylformamide); tlc in methylene chloride–methanol (9:1) gave a single spot.

Anal. Calcd for $C_{34}H_{34}N_3O_8SCl_5$: C, 49.68; H, 4.16; N, 5.11; S, 3.90; Cl, 21.56. Found: C, 50.05; H, 4.32; N, 5.30; S, 4.04; Cl, 21.90.

β-Benzyl-L-aspartyl-S-benzyl-L-cysteinylglycine Pentachlorophenyl Ester Trifluoroacetate (XII).—Pentachlorophenyl ester XI, 3 g (3.65 mmol), was treated with 10 ml of anhydrous trifluoroacetic acid; after 10 min crystalline white solid separated. The reaction mixture was diluted with ether and the precipitated solid was washed with ether and dried *in vacuo* over potassium hydroxide: yield 3.01 g (98%); mp 170° dec with charring; $[\alpha]^{24}D$ −17.3° (*c* 2, dimethylformamide).

Anal. Calcd for $C_{31}H_{27}N_3O_8Cl_5SF_3$: C, 44.54; H, 3.26; N, 5.03; S, 3.84. Found: C, 45.72; H, 3.15; N, 5.25; S, 4.31.

β-Benzyl-L-aspartyl-S-benzyl-L-cysteinylglycine Pentachlorophenyl Ester Hydrobromide (XIII).—This compound was obtained from pentachlorophenyl ester (XI), the usual way in 92% yield: mp 196–198° dec; $[\alpha]^{23}D$ −12 .6° (*c* 2, dimethylformamide).

Anal. Calcd for $C_{29}H_{27}N_3O_8SCl_5Br$: C, 43.39; H, 3.39;

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N, 5.24; S, 3.99. Found: C, 43.39; H, 3.45; N, 5.37; S, 3.98.

Poly(α-benzyl-L-glutamyl-S-benzyl-L-cysteinylglycine)(XIV).—To a solution of 1.635 g (2 mmol) of α-benzyl-L-glutamyl-S-benzyl-L-cysteinylglycine pentachlorophenyl ester hydrobromide (IV) in 2 ml of purified dimethylformamide,[6g] 0.56 ml (4 mmol) of purified triethylamine[6g] was added and left on a shaker for 48 hr. The solid reaction mass was triturated with 150 ml ether and centrifuged. The residue was triturated with ether (three 100-ml portions), with methanol (two 100-ml portions), finally with ether and dried: yield 787 mg (84%); λ_{max} 5.78 (COOBZL), 6.09 (amide I), 6.55 μ (amide II), the pentachlorophenyl ester carbonyl band and the doublet completely disappeared.

Anal. Calcd for $(C_{24}H_{27}N_3O_5S)_\infty$: C, 61.38; H, 5.80; N, 8.95; S, 6.83. Found: C, 60.87; H, 5.66; N, 8.87; S, 6.97.

Poly(α-benzyl-L-aspartyl-S-benzyl-L-cysteinylglycine) (XV).—To a suspension of 803 mg (1 mmol) of α-benzyl-L-aspartyl-S-benzyl-L-cysteinylglycine pentachlorophenyl ester hydrobromide (VI) in 1.5 ml of dimethylformamide, 0.28 ml (2 mmol) of triethylamine was added and the mixture was left in a mechanical shaker for 48 hr. The polymer was worked up following the procedure described above to afford 330 mg (73%) of white powder.

Anal. Calcd for $(C_{23}H_{25}N_3O_5S)_\infty$: C, 60.65; H, 5.53; N, 9.22; S, 7.04. Found: C, 60.95; H, 5.73; N, 8.90; S, 7.22.

Poly(γ-benzyl-L-glutamyl-S-benzyl-L-cysteinylglycine) (XVI).

A. Polymerization of the Trifluoroacetate Salt IX.—γ-Benzyl-L-glutamyl-S-benzyl-L-cysteinylglycine pentachlorophenyl ester trifluoroacetate (IX) (850 mg) was polymerized and the product was worked up following the method described above to yield 254 mg (54%) of XVI.

Anal. Calcd for $(C_{24}H_{27}N_3O_5S\cdot H_2O)_\infty$: C, 59.12; H, 5.58; N, 8.62; S, 6.58. Found: C, 59.33; H, 5.68; N, 8.82; S, 7.40.

B. Polymerization of the Hydrobromide Salt X.—γ-Benzyl-L-glutamyl-S-benzyl-L-cysteinylglycine pentachlorophenyl ester hydrobromide (X) (2.45 g) was polymerized and worked up according to the method described previously. The polypeptide XVI was obtained as a white solid, yield 0.809 g (57%).

Anal. Found: C, 60.35; H, 5.37; N, 9.13; S, 7.52.

Poly(β-benzyl-L-aspartyl-S-benzyl-L-cysteinylglycine) (XVII).

A. Polymerization of the Trifluoroacetate Salt XII.—β-Benzyl-L-aspartyl-S-benzyl-L-cysteinylglycine pentachlorophenyl ester trifluoroacetate (XII) (1.67 g) gave polymer XVII following the usual procedure, yield 248 mg (27%).

Anal. Calcd for $(C_{23}H_{25}N_3O_5S\cdot H_2O)_\infty$: C, 58.33; H, 5.71; N, 8.87; S, 6.77. Found: C, 58.54; H, 5.61; N, 9.31; S, 7.29 (0.07% residue).

B. Polymerization of the Hydrobromide Salt XIII.—β-Benzyl-L-aspartyl-S-benzyl-L-cysteinylglycine pentachlorophenyl ester hydrobromide (XIII) (2.40 g) was polymerized to afford 537 mg (39%) of polymer XVII.

Anal. Calcd for $(C_{23}H_{25}O_5N_3S\cdot{}^1/_2H_2O)_\infty$: C, 59.47; H, 5.64; N, 9.05; S, 6.90. Found: C, 59.73; H, 5.32; N, 9.40; S, 7.46.

Removal of the *C*- and *S*-Benzyl Protecting Groups of the Polymers with Sodium–Liquid Ammonia. Polyglutathione (XVIII).—To a stirred suspension of 1.7 g of the polymer XIV in 200 ml of freshly distilled (from sodium) liquid ammonia, 800 mg of finely cut sodium was added. Each portion of sodium was added when the blue color had faded. At the end, the blue color was allowed to persist for 30 min, after which the excess of sodium was destroyed by the addition of a few crystals of ammonium chloride. The ammonia was allowed to evaporate under a stream of oxygen-free nitrogen (purified by passing through Fieser's solution) and the white residue was completely freed from ammonia in a vacuum desiccator over sulfuric acid. The residue was dissolved in 50 ml of freshly distilled water containing 1 ml of 1% ethylenediaminetetracetic acid sodium salt, adjusted to about pH 6 with acetic acid, and lyophilized. The residual white solid was taken in 25 ml of freshly distilled water, acidified with 2 *N* hydrochloric acid to about pH 3, and dialyzed against six 1000-ml portions of freshly distilled water under a nitrogen atmosphere, until free of chloride ion. After lyophilization the polymer XVIII was obtained as a fluffy white solid: yield 516 mg (59%); $\bar{M}_w$ = 9000 by sedimentation equilibrium. The sodium salt of the polymer XVIII in D_2O (10%) showed the complete absence of aromatic protons in the nmr (60 Mcps). The analytical sample was prepared by washing the polymer with water, absolute ethanol, and peroxide-free ether under an oxygen-free nitrogen atmosphere and dried.

Anal. Calcd for $(C_{10}H_{15}N_3O_5S)_\infty$: C, 41.52; H, 5.23; N, 14.52; S, 11.08; SH, 11.14. Found: C, 41.58; H, 5.85; N, 14.87; S, 10.65; SH, 9.48 by iodometric titration.

Polyasparthione (XIX).—The protecting benzyl groups from polymer XV (1.0 g) were removed in 200 ml of liquid ammonia with 350 mg of sodium. The reaction mixture was worked up following the conditions described for the preparation of XVIII. After dialysis and lyophilization, the polymer XIX was obtained as a fluffy white solid: yield 265 mg (60%); $\bar{M}_w$ = 7000 by sedimentation equilibrium.

Anal. Calcd for $(C_9H_{13}N_3O_5S)_\infty$: C, 39.27; H, 4.76; N, 15.26; S, 11.65; SH, 11.20. Found: C, 39.27; H, 5.16; N, 15.14; S, 11.54; SH, 10.29.

Polyisoglutathione (XX).—The polymer XVI (500 mg) was treated as above to obtain the free polymer XX in 58% yield; $\bar{M}_w$ = 16,000 by sedimentation equilibrium.

Anal. Calcd for $(C_{10}H_{15}N_3O_5S)_\infty$: C, 41.52; H, 5.23; N, 14.52; S, 11.08; SH, 11.14. Found: C, 41.72; H, 5.45; N, 13.92; S, 10.82; SH, 11.52.

Polyisoasparthione (XXI).—Polymer XVII (500 mg) gave, after similar treatment, 133 mg (60%) of XXI as a fluffy white solid, $\bar{M}_w$ = 6000 by sedimentation equilibrium.

Anal. Calcd for $(C_9H_{13}N_3O_5S)_\infty$: C, 39.27; H, 4.76; N, 15.26; S, 11.65; SH, 11.20. Found: C, 39.97; H, 5.37; N, 14.92; S, 11.26; SH, 10.82.

Removal of the *C*- and *S*-Benzyl Protecting Groups of the Polymers by Anhydrous Hydrogen Fluoride. Polyglutathione (XVIII).—To a stirred solution of 2 g of the polymer XIV in 15 ml of trifluoroacetic acid containing 2.5 ml of anisole, *ca.* 20 ml of anhydrous hydrogen fluoride was collected at Dry Ice–methanol bath temperature. The solution turned light yellow to red color. After stirring at −15 to 0° for 1 hr, the reaction mixture was stirred at room temperature for about 18 hr. The hydrogen fluoride was removed with a stream of oxygen-free nitrogen and the polymer was precipitated by addition of peroxide-free ether, centrifuged, washed with four 200-ml portions of ether to afford white amorphous polymer, and dried overnight at 78° (0.1 mm), yield 1.20 g, $\bar{M}_w$ = 5000 by sedimentation equilibrium.

Anal. Calcd for $(C_{10}H_{15}N_3O_5S)_\infty$: C, 41.52; H, 5.23; N, 14.52; S, 11.08. Found: C, 43.97; H, 4.88; N, 11.42; S, 9.01.

Polyasparthione (XIX).—Polymer XV was similarly treated with anhydrous hydrogen fluoride. The reaction mixture was worked up following the conditions as described for the preparation of XVIII. The polymer was obtained as white amorphous powder and dried at 78° (0.1 mm) overnight, yield 2.87 g, $\bar{M}_w$ = 4600 by sedimentation equilibrium.

Anal. Calcd for $(C_9H_{13}N_3O_5S)_\infty$: C, 39.27; H, 4.76; N, 15.26; S, 11.65. Found: C, 41.71; H, 5.06; N, 12.22; S, 9.44.

Weight-Average Molecular Weights.—Weight-average molecular weights were determined in the Spinco Model E analytical ultracentrifuge by the sedimentation equilibrium method in Tris buffer (pH 7.8). The calculations used were those given by Schachman.[10] Measurements were made at concentrations in the range of 0.7–1% at 22–24°, at a rotor speed of 16,000 rpm and a schlieren angle of 65°, assuming partial specific volume of 0.72.

Registry No.—I, 32296-65-4; II, 32296-66-5; III, 32296-79-0; IV, 32380-99-7; V, 32296-80-3; VI, 32296-81-4; VII, 32296-82-5; VIII, 32296-67-6; IX, 32296-68-7; X, 32296-69-8; XI, 32296-70-1; XII, 32296-71-2; XIII, 32296-72-3; XIV (polymer), 32270-57-8; XIV (repeating unit), 32355-52-5; XV (polymer), 32270-58-9; XV (repeating unit), 32355-53-6; XVI (polymer), 32270-59-0; XVI (repeating unit), 32355-54-7; XVII (polymer), 32270-60-3; XVII (repeating unit), 32355-55-8; XVIII (polymer), 32270-61-4; XVIII (repeating unit), 32355-57-0; XIX (poly-

mer), 32270-62-5; XIX (repeating unit), 32355-56-9; XX (polymer), 32270-63-6; XX (repeating unit), 32355-58-1; XXI (polymer), 32270-64-7; XXI (repeating unit), 32355-59-2.

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Synthesis of *dl*-Hedycaryol[1]

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The synthesis of *dl*-hedycaryol (**5**) according to Scheme I is described. Dimethyl 4-hydroxyisophthalate (**1**) was hydrogenated over ruthenium dioxide and the resulting dimethyl hydroxycyclohexanedicarboxylate mixture **11** was acetylated. The acetate **12** was pyrolyzed at 260° in the presence of potassium acetate and the major product, dimethyl cyclohexene-2,4-dicarboxylate (**13**), was separated by distillation. Addition of methyllithium to **13** yielded the bis tertiary diol **15** which was selectively dehydrated to 2-(2-propenyl)-4-(2-hydroxy-2-propyl)-1-cyclohexene (**2**) by heating in dimethyl sulfoxide. Conversion of **2** to octalone **3** was effected by a previously established sequence: 1,4 cycloaddition of ethyl α-acetoxyacrylate, lithium aluminum hydride reduction, and sodium periodate oxidation. Direct angular methylation of **3** was effected by treatment with a mixture of sodium hydride and methyl iodide in dimethoxyethane (conditions corresponding to the reaction of kinetically generated enolates). The resulting octalone mixture **4** was correlated with γ-eudesmol (**41**) and epi-γ-eudesmol (**42**) by Wolff–Kishner reduction. From the reduction of the octalone mixture with lithium aluminum hydride a crystalline diol **43** was obtained which was converted to monotosylate **44** which yielded hedycaryol (**5**) upon treatment first with diborane and then with aqueous sodium hydroxide. The properties of synthetic *dl*-**5** match those reported for the natural *d* enantiomer.

Hedycaryol (**5**) is a biogenetically important sesquiterpene, isomeric with and derived from *trans,trans*-farnesol and the progenitor of a further set of sesquiterpene skeletal families, exemplified most directly by the eudesmols (hydronaphthalenes) and bulnesol and guaiol (hydroazulenes), related by acid-catalyzed cyclizations, and by elemol, related *via* the Cope rearrangement.[3]

Hedycaryol has a relatively recent history; although its biogenetic involvement as a 1,5-cyclodecadiene was appreciated in print in 1953,[4] the structure of the first sesquiterpene 1,5-cyclodecadiene was not established until 1959,[5] and hedycaryol itself was not isolated until 1968.[6] This article records the synthesis of *dl*-hedycaryol.

Synthetic Scheme.—The synthesis was planned and carried out according to the accompanying flow chart, in four main sections, A, B, C, and D (see Scheme I). This approach concedes to nature, at least temporarily, the exclusive ability to transform acyclic precursors directly to *trans,trans*-1,5-cyclodecadienes.[7] The present synthesis involves fragmentation of the appropriately functionalized and substituted hydronaphthalene precursor to the ten-membered ring of hedycaryol which is specifically a 1,5-dimethyl-*trans,trans*-1,5-cyclodecadiene bearing a 2-hydroxy-2-propyl chain at C-8. This was accomplished by Marshall's method:[8] hydroboration of octalyl tosylate **44** and subsequent generation of *dl*-hedycaryol at a relatively low temperature (65°) by fragmentation in the presence of base. Some care is necessary in planning and carrying out the synthesis because hedycaryol is relatively unstable thermally, rearranging to elemol (**6**) with a half-life of 3 hr at 100°,[6] and it is very susceptible to cyclization in the presence of acids.[9]

Sections B and C together exemplify the preparation of 9-methyl-$\Delta^{4(10)}$-1-octalones *via* 1,4 cycloaddition to a 1-isopropenylcyclohexene (B)[10] and subsequent angular methylation (C).[11] Section B involves the regiospecific but indirect 1,4-cycloaddition of ketene (which itself favors 1,2 cycloaddition).[12] This can be accomplished by the use of α-acetoxyacrylates and related substances.[10] Although the involvement of angular methylation as an essential part of the planned synthesis might be considered to be poor strategy, it may be noted that introduction of the angular methyl group by direct alkylation of $\Delta^{4(10)}$-1-octalones (β,γ-unsaturated) is a viable method, whereas direct angular methylation of 1-decalones is not. Moreover, prior incorporation of the methyl, using the same overall approach, can be summarily dismissed because of the failure of 1-methyl-2-isopropenylcyclohexenes to un-

(1) (a) The investigation was supported by Public Health Service Research Grants GM 09759, GM 14133, and GM 16338 from the Division of General Medical Sciences, U. S. Public Health Service. (b) The article is abstracted from the Ph.D. theses of C. E. S., University of Wisconsin, 1968, and H. C. K., Wesleyan University, 1971. The synthesis was first presented at the 155th National Meeting of the American Chemical Society, San Francisco, Calif., April 1968, Abstract P-2.

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(3) Sesquiterpene biogenetic relations are reviewed by W. Parker, J. S. Roberts, and R. Ramage, *Quart. Rev., Chem. Soc.*, **21**, 321 (1967).

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(5) J. B. Hendrickson, *Tetrahedron*, **7**, 82 (1959); V. Herout, M. Horak, B. Schneider, and F. Sorm, *Chem. Ind. (London)*, 1089 (1959).

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(7) For an interesting, although unsuccessful, synthetic approach of this type see E. J. Corey and E. A. Broger, *Tetrahedron Lett.*, 1779 (1969).

(8) J. A. Marshall and G. L. Bundy, *Chem. Commun.*, 854 (1967).

(9) The conversion of *d*-**5** to a mixture of eudesmols has been reported to occur upon refluxing a solution in ether containing 1% *p*-toluenesulfonic acid.[6] Our results provide an even more striking illustration of the sensitivity of **5** to acid, synthetic material suffering cyclization to the eudesmols in buffered acetic acid with a half-life of 15 min at 60°. The product of this reaction also consisted of a mixture of α-, β-, and γ-eudesmols; moreover, specific examination by glpc for the presence of bulnesol, which is biogenetically formed from **5** as are the eudesmols, by a simple acid-catalyzed cyclization (albeit anti-Markovnikov in the case of bulnesol), failed to detect any (<2%), and an intriguing problem of biogenetic simulation remains.

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