analogous to that used in the preparation of VIa. Crude product was obtained only from the ether extract residue upon trituration in ethanol; yield, 130 mg. (3%); m.p. 134–135°. The analytical sample was obtained by two crystallizations from ethanol, the second time with charcoal decolorization; yield, 55 mg.; m.p. 136–137°; λ_{max}^{EtOH} 292 mμ.

Anal. Calcd. for $C_{14}H_{17}NO_6$: C, 56.95; H, 5.81; N, 4.73. Found: C, 56.81; H, 5.78; N, 4.70.

3-(3-Methyl-2-oxocyclohexylcarbonyl)glutarimide (VId) was obtained from 6-methyl-1-(1-pyrrolidinyl)cyclohexene (2.5 g., 15.1 mmoles), 3-(chlorocarbonyl)glutarimide (2.5 g., 14.2 mmoles), and triethylamine (5 ml.) in dimethylformamide by a procedure analogous to that used in the preparation of VIa. Crude product (310 mg., 8%) was obtained from the ether extract residue. Trituration of the ethyl acetate extract residue failed to yield a precipitate. The analytical sample was obtained by crystallization first from ethanol, and then from ethanol–hexane (4:1); yield, 60 mg.; m.p. 157°; λ_{max}^{EtOH} 297 mμ.

Anal. Calcd. for $C_{13}H_{17}NO_4$: C, 62.13; H, 6.82; N, 5.58. Found: C, 62.16; H, 6.73; N, 5.45.

2-[(2-Oxocyclohexylcarbonyl)methyl]succinimide (VIIa) was obtained from 1-piperidinocyclohexene (2.87 g., 17.4 mmoles), triethylamine (2.75 ml., 19.8 mmoles), and 2-[(chlorocarbonyl)-methyl]succinimide (2.77 g., 15.8 mmoles) by a procedure analogous to that used in the preparation of Va except that chloroform was used as solvent in place of dioxane. The hydrolysis was effected by treatment of the reaction residue with 3 *N* hydrochloric acid. Extraction of the aqueous solution with chloroform gave a brown oil which was redissolved in chloroform. The chloroform solution was extracted with four 10-ml. portions of 5% aqueous sodium hydroxide at 0°, and the combined extracts were acidified at 0° and extracted with four 10-ml. portions of chloroform. Evaporation of the chloroform left a sirup which crystallized on long standing. Crystallization from ethanol–hexane gave 103 mg. (3%) of solid, m.p. 119–121°. Recrystallization from ethanol–hexane raised the melting point to 123–125°. An analytical sample was obtained by another crystallization from ethanol–hexane; yield, 39 mg. (1%); m.p. 129–130°.

Anal. Calcd. for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37; N, 5.90. Found: C, 60.48; H, 6.31; N, 5.75.

2-[(5-Methyl-2-oxocyclohexylcarbonyl)methyl]succinimide (VIIb).—2-[(Chlorocarbonyl)methyl]succinimide (45.2 g., 0.258 mole) in dry tetrahydrofuran (150 ml.) was added rapidly with stirring to 4-methyl-1-(*N*-methylanilino)cyclohexene (56 g., 0.280 mole) in dry tetrahydrofuran (250 ml.) cooled in an ice bath. The mixture was refluxed for 10 min., and triethylamine (19.2 ml., 0.28 mole) in tetrahydrofuran (100 ml.) was added dropwise at reflux in 10 min. Refluxing was continued for 30 min., and the mixture was stirred overnight at room temperature in a nitrogen atmosphere. Evaporation of the reaction mixture *in vacuo* produced a sirup which was stirred for 2 hr. with 10% acetic acid (1100 ml.). The solid phase was collected by filtration and dried *in vacuo*; yield, 11.1 g.; m.p. 138–140°. Refrigerating and scraping the gum adhering to the sides of the container gave more crude product which was crystallized from ethanol (5.9 g., m.p. 140–142°). The two crops were combined and crystallized twice from ethanol (charcoal decolorization); yield, 11.7 g. (18%); m.p. 140–141°; λ_{max} (mμ): in ethanol, 288; at pH 1, 290; at pH 7, 290; and at pH 13, 313.

Anal. Calcd. for $C_{13}H_{17}NO_4$: C, 62.14; H, 6.82; N, 5.58. Found: C, 61.92; H, 6.62; N, 5.57.

2-[(5-Acetoxy-2-oxocyclohexylcarbonyl)methyl]succinimide (VIIc).—2-[(Chlorocarbonyl)methyl]succinimide (8.75 g., 0.05 mole) in dry dimethylformamide (100 ml.) was treated dropwise with stirring in a nitrogen atmosphere at room temperature with 4-acetoxy-1-morpholinocyclohexene (10.7 g., 0.045 mole) in dry dimethylformamide (50 ml.) in 30 min., and the solution was stirred 4 days at room temperature in a nitrogen atmosphere. Evaporation of the dimethylformamide *in vacuo* gave a sirup which was treated with water (50 ml.) and then with *N* sodium hydroxide to pH 5. The solution was extracted continuously with ether for 8 hr., and the extract was evaporated to dryness. Crystallization of the residue from ethanol gave a solid (410 mg.), m.p. 180–182°. A second crop (710 mg.) melted at 146–149°, and a third crop (500 mg.) at 138–143°. The second and third crops were combined and crystallized twice from ethanol; yield, 420 mg. (3%); m.p. 155–157°.

Anal. Calcd. for $C_{14}H_{17}NO_6$: N, 4.73. Found: N, 4.71.

Crystallization of a small amount of the low-melting form from ethanol by seeding with the high-melting form gave the high-melting form. Recrystallization of the first crop (410 mg.) from ethanol produced an analytical sample; yield, 270 mg. (2%); m.p. 182–183°; λ_{max}^{EtOH} 287 mμ.

Anal. Calcd. for $C_{14}H_{17}NO_6$: C, 56.95; H, 5.81; N, 4.73. Found: C, 56.85; H, 5.66; N, 4.73.

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Polypeptides from *p*-Phenylalanine Mustard[1]

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The N-carboxyanhydride (III) of DL-*p*-phenylalanine mustard (I) was obtained, *via* III hydrochloride, from the amino acid I and phosgene, and was converted to two homopolymers (V) of differing molecular weights. The N-carboxyanhydride (IV) of γ-benzyl-L-glutamate and III reacted to form a copolymer VI, which was debenzylated with hydrogen bromide to form VII.

Intense interest in *p*-phenylalanine mustard[2] (*p*-sarcolysin,[2] I) in recent years as an anticancer drug has led to the preparation and study of various analogs and derivatives, in a search for enhanced anticancer properties. Among these derivatives were various oligopeptides,[3-5] of up to five amino acid units. This

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(2) For bibliography and screening data, see *Cancer Chemotherapy Rept.*, **6**, 61 (1960).

(3) J. M. Johnson and J. A. Stock, *J. Chem. Soc.*, 3806 (1962); F. Bergel and J. A. Stock, *ibid.*, 3658 (1960).

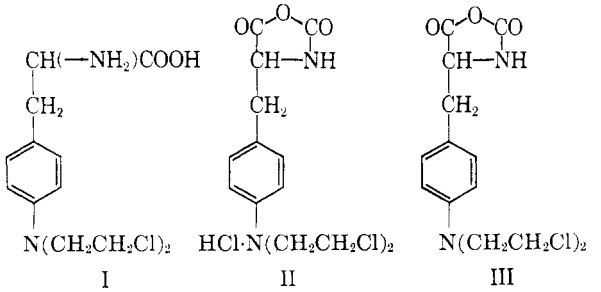
(4) I. L. Knunyants, N. E. Golubeva, K. I. Karpavicius, and O. V. Kil'disheva, *Zh. Vses. Khim. Obshchestva im. D. I. Mendeleeva*, **7**, 238 (1962); *cf. Chem. Abstr.*, **58**, 3505*g* (1963); I. L. Knunyants, N. E. Golubeva, O. V. Kil'disheva, and K. I. Karpavicius, *Puti Sinteza i Izyskania Protivoopukholevykh Preparatov, Tr. Simpoziuma po Khim. Protivoopukholevykh Veshchestv, Moscow*, 5 (1960); *cf. Chem. Abstr.*, **58**, 4647*a* (1963).

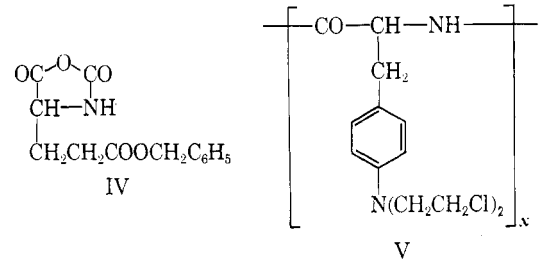
(5) (a) F. Bergel, J. A. Stock, and R. Wade in "Biological Approaches to Cancer Chemotherapy," R. J. C. Harris, Ed., Academic Press, New York, N. Y., 1961, p. 125; (b) L. F. Larionov, *ibid.*, p. 139.

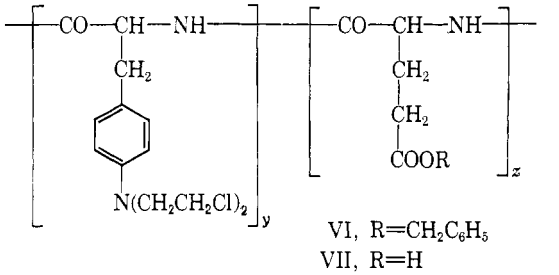
paper concerns the preparation of a homopolymer of DL-*p*-phenylalanine mustard and of a copolymer with L-glutamic acid. Modification of a polyserine[6] and, briefly, of albumin[5a] with substituents containing latent nitrogen mustard groups have been reported; beyond this, V, VI, and VII are apparently the first polymers which contain nitrogen mustard moieties.

Amino acid N-carboxyanhydrides are commonly prepared and used as intermediates in the polymerization of amino acids.[7,8] The N-carboxyanhydride (III) of *p*-phenylalanine mustard was obtained by the familiar method of treating the amino acid in dioxane suspension with phosgene.[7-10] The anhydride was precipitated as hydrochloride salt II of the weakly basic mustard nitrogen, and was converted to free base III with silver carbonate in a dichloromethane suspension. Either of two isomorphous forms of III could be obtained, depending on the method of isolation. The anhydride III was surprisingly stable, considering the lability of many N-carboxyanhydrides. The substance could be stored without special precautions, and upon fusion it did not evolve carbon dioxide with the formation of a polymer.

The polymerization of III could be followed qualitatively by the loss of characteristic anhydride bands and the appearance of peptide (*i.e.*, amide) bands in the infrared spectrum. A polymer V (x = *ca.* 10) of low molecular weight (2000–3000) was obtained from III in 10% dioxane solution with sodium methoxide as initiator (anhydride:initiator, A:I = 200:1); presence of enough methanol to solubilize the methoxide was essential. Polymers V (x = 50–100) of estimated molecular weights in the range 10,000–30 000 were obtained from III in dichloromethane solution with triethylamine as initiator (A:I = 200:1), and also in chloroform solution without added initiator. The polymers were isolated by precipitation from solution with petroleum ether and were obtained as amorphous solids.







A copolymer VI was prepared by treating equimolar quantities of III and γ-benzyl-L-glutamate N-carboxyanhydride (IV) in dioxane solution with methanolic sodium methoxide (total A:I = 200:1). There was infrared spectral evidence for the absence of the anhydrides III and IV and for the presence of both the phenylalanine mustard and benzyl glutamate components in VI; elemental analysis indicated about twice as much of the mustard component was present, *i.e.*, $y:z$ = 2:1. The molecular weight of VI was not determined because of chloroform insolubility.[11]

The copolymer VI was debenzylated to form the copolymer VII containing free carboxyl groups, by treatment in dichloroacetic acid solution with anhydrous hydrogen bromide[12] at room temperature for 1 hr. Conversion of ester functions to free acid functions could be observed qualitatively in the infrared spectrum. The copolymer VII was insoluble when treated with 0.1 *M* sodium hydroxide, but partial conversion to sodium carboxylate functional groups was observed in the infrared. Elemental analysis suggested the ratio $y:z$ in VII was about 6:5. The copolymers VI and VII are no doubt random block copolymers. Variations in the $y:z$ ratio from VI to VII and in different experiments were attributed to loss of atypical, lower molecular weight species upon precipitation of VI and VII at the various steps involved.

Preparation of the N-carboxyanhydride of *m*-phenylalanine mustard[2] was investigated with the same procedures as with the *para* isomer. Although infrared spectra showed that both the *meta* hydrochloride corresponding to II and the free base corresponding to III were formed, these *meta* isomers were more sensitive to atmospheric moisture and could not be obtained in a state of analytical purity. Further reactions were not attempted with these isomers.

Biological Data.[13]—The two polymers V (x = 10 and x = 50–100) and the copolymer VII were tested in rats bearing Walker Ca 256 (subcutaneous) implanted tumors. Toxicities (LD_{10}, in mg./kg./day) were *ca.* 47, *ca.* 41, and >50, respectively. The polymer V (x = 10) had a therapeutic index ($LD_{10}:ED_{90}$) of <1; V (x = 50–100) and VII were inactive.

The carboxy anhydride III was inactive when tested against Sarcoma 180 and Lymphoid Leukemia L-1210

(6) M. Szekerke, *Nature*, **199**, 280 (1963).

(7) C. H. Bamford, A. Elliott, and W. E. Hanby, "Synthetic Polypeptides," Academic Press, New York, N. Y., 1956, Chapters 2 and 3.

(8) E. Katchalski and M. Sela, *Advan. Protein Chem.*, **13**, 243 (1958).

(9) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 2, John Wiley and Sons, Inc., New York, N. Y., 1961, pp. 867–868.

(10) K. Heyns, W. Walter, and H. F. Grützmacher, *Ann.*, **609**, 209 (1957).

(11) A soluble copolymer could be obtained by reaction of III and IV in reagent chloroform solution without added initiator; the molecular weight then was in excess of 10,000. As measured by changes in viscosity, the fairly rapid polymerization in chloroform solution was preceded by a long induction period.

(12) C. J. Fox, U. S. Patent 3,004,004 (March 26, 1958); *Chem. Abstr.*, **56**, 3639*g* (1962); *cf.* M. Sela and R. Arnon, *J. Am. Chem. Soc.*, **82**, 2625 (1960); E. R. Blout and M. Idelson, *ibid.*, **78**, 497 (1956); D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952).

(13) The compounds were screened under the auspices of the Cancer Chemotherapy National Service Center according to its protocols, outlined in *Cancer Chemotherapy Rept.*, **25**, 1 (1962).

in mice, and was toxic (0/6 survivors) at a dose of 12.5 mg./kg./day. Preliminary results with III on mice implanted with Lewis Lung Carcinoma indicate some activity against this tumor at a dose of 1.25 mg./kg./day.

Experimental[14]

DL-4-{*p*-[Bis(2-chloroethyl)amino]benzyl}-2,5-oxazolidinedione Hydrochloride (II).—With the procedure and precautions described in ref. 9, a suspension of 6.12 g. (20 mmoles) of *p*-sarcolysin[15] in 120 ml. of dioxane was treated with a stream of phosgene. After a few minutes, a clear solution formed but then precipitation commenced almost immediately. After 1 hr., when phosgene was no longer absorbed, the reaction mixture was cooled, and precipitated II was collected on a filter and washed with ether. The product weighed 6.21 g. (84%), m.p. 109–114°. New bands at 4.3 μ (R_3NH^+) and 5.38, 5.43, and 5.62 μ (C=O) appeared in the infrared spectrum.

Anal. Calcd. for $C_{14}H_{17}Cl_3N_2O_3$: C, 45.7; H, 4.66; Cl, 28.9; N, 7.62. Found: C, 46.0; H, 4.49; Cl, 28.7; N, 7.46.

DL-4-{*p*-[Bis(2-chloroethyl)amino]benzyl}-2,5-oxazolidinedione (III).—A suspension of 5.98 g. (16.2 mmoles) of the hydrochloride II and 6.35 g. (23.0 mmoles) of silver carbonate in 800 ml. of dichloromethane was stirred and heated under reflux for 1 hr. The silver salts were collected and washed on a filter, and the filtrate was concentrated *in vacuo*. The residual sirup (5.13 g., 95%) crystallized upon trituration with a few drops of dichloromethane, and was recrystallized from ethyl acetate (60 ml.) upon addition of petroleum ether (b.p. 30–60°). When *ca.* 60 ml. had been added, a yellow oil separated and was discarded: the supernatant solution was decanted and diluted further with 340 ml. of petroleum ether. The first crop weighed 3.49 g. (65%), m.p. 118–127°; additional crops obtained from the mother liquors raised the yield to 85%. The infrared spectrum was free of the band in II at 4.3 μ and exhibited bands at 3.09 μ (NH), 5.40 and 5.63 μ (C=O, anhydride), and at 6.19, 6.53, 10.68, and 11.22 μ. This material was used in subsequent experiments.

Anal. Calcd. for $C_{14}H_{16}Cl_2N_2O_3$: C, 50.8; H, 4.87; Cl, 21.4; N, 8.46. Found: C, 50.8; H, 4.98; Cl, 21.3; N, 8.48.

When in one experiment the initial sirup was reprecipitated directly, without the dichloromethane treatment, II was obtained in an isomorphous form, also analytically pure, m.p. 97–103°. The infrared spectrum exhibited the same bands at 5.40, 5.63, 6.19, and 6.53 μ, but the NH band was at 2.91 and the band at 10.68 μ was replaced by one at 10.75 μ with characteristic side peaks at 10.97 and 11.13 μ.

Poly-DL-*p*-sarcolysin (V, x = *ca.* 10).—A solution of 496 mg. (1.5 mmoles) of anhydride III in 4.95 ml. of dry dioxane treated with 0.0075 mmole of methanolic sodium methoxide (0.405 mg. in 0.41 ml.) was protected from moisture and allowed to stand for 117 hr. The bright yellow solution was poured into 50 ml. of petroleum ether (b.p. 30–60°). A yellow oil separated, which partly solidified, and, after decantation of the supernatant liquid, was dissolved in 8 ml. of ethyl acetate. This solution was poured into 30 ml. of petroleum ether and the resultant light yellow precipitate was collected, 300 mg., m.p. 87–98°, mol. wt. 3000. The inherent viscosity of a 0.1% chloroform solution was too low to be measured; infrared data: 3.03 (NH), 6.0 (amide C=O), 6.55 (amide II), 8.49 (Ar–N mustard; intensity relative to weak bands at 8.28, 8.61, and 8.72 μ was same as in *p*-sarcolysin), and 12.35 μ (*p*-C_6H_4); a weak band at 5.80 μ was tentatively assigned to $-COOCH_3$, possibly arising from chain termination with the methanolic methoxide initiator. Anhydride bands at 5.40 and 5.63 μ were absent.

Anal. Calcd. for $H(C_{13}H_{16}Cl_2N_2O)_{10}OCH_3$: C, 54.2; H, 5.69; Cl, 24.4; N, 9.64. Found: C, 54.1; H, 5.80; Cl, 24.5; N, 9.48.

Poly-DL-*p*-sarcolysin (V, x = 50–100).—The same procedure and amounts as above were used, but with dichloromethane as solvent and triethylamine (0.75 mg. in 0.15 ml. of dioxane) as initiator. After 140 hr., the product was obtained as a fine white precipitate and was reprecipitated from ethyl acetate with petroleum ether to yield 350 mg., which softened and became transparent at 120–150° but did not melt; mol. wt. *ca.* 12,500 and >20,000 for two preparations. Inherent viscosities were 0.08–0.12 in 0.1% chloroform solution. The infrared spectrum was the same as above, except for absence of the weak band at 5.80 μ.

Anal. Calcd. for $(-C_{13}H_{16}Cl_2N_2O-)_x$: C, 54.4; H, 5.62; Cl, 24.7; N, 9.75. Found: C, 54.3; H, 5.68; Cl, 24.5; N, 9.50.

Copoly-γ-benzyl-L-glutamate-DL-*p*-Sarcolysin (VI, $y:z$ *ca.* 2:1).—A dioxane solution (119 ml.) of 20.0 mmoles each of *p*-sarcolysin carboxyanhydride (III, 6.62 g.) and γ-benzyl L-glutamate N-carboxyanhydride[16] (IV, 5.26 g.) was treated with 0.2 mmole of methanolic sodium methoxide (10.8 mg. in 1.50 ml.). After 3 weeks, the product was precipitated with 1100 ml. of petroleum ether as a sticky yellow solid, and then reprecipitated (from 100 ml. of ethyl acetate with 1200 ml. of petroleum ether) as a light amorphous solid weighing 7.35 g., which did not melt but became transparent at 85–125°; infrared data (sample cast from chloroform): 3.01 (NH), 5.73 (C=O, OBz), 6.0 (C=O, amide), 6.55 (amide II), 8.45 (Ar–N mustard), 8.6 shoulder (OBz), 12.35 (*p*-C_6H_4), 13.4 (C_6H_5 plus C–Cl), and 14.3 μ weak (C_6H_5); anhydride bands at 5.3–5.63 μ in III and IV were completely absent.

Anal. Calcd. for $[(-C_{13}H_{16}Cl_2N_2O-)_2(-C_{12}H_{13}NO_3-)]_x$: C, 57.5; H, 5.72; Cl, 17.9; N, 8.84. Found: C, 56.8; H, 5.86; Cl, 17.9; N, 8.86.

Copoly-L-glutamic Acid-DL-*p*-Sarcolysin (VII, $y:z$ *ca.* 6:5).—A stirred solution of 7.35 g. of the copolymer VI in 100 ml. of dichloroacetic acid was treated with a gentle stream of anhydrous hydrogen bromide for 1 hr., while an orange oil separated. The mixture was stirred 1 hr. longer, and poured with stirring into 1 l. of anhydrous ethyl ether. The pale orange precipitate (6.60 g.) was collected (infrared absorption at 3.8–4.2 μ indicated it was partly the hydrobromide) and washed with water until the washings were free of bromide ion. Reprecipitation from dimethylformamide solution (40 ml.) with 500 ml. of water removed traces of hydrogen bromide and afforded 4.8 g. of fluffy, light brown solid, which did not melt below 280° and was insoluble in chloroform, benzene, ethanol, and acetone. The infrared spectrum, compared to that of VI, was free of benzoate absorption (5.73, 8.6, and 14.3 μ), and exhibited new COOH bands at 3.66 and 3.73 (weak), and at 5.80 μ (medium).

Anal. Calcd. for $[(-C_{13}H_{16}Cl_2N_2O-)_5(-C_5H_7NO_3-)_5]_x$: C, 51.7; H, 5.09; Cl, 18.1; N, 10.1. Found: C, 51.6; H, 5.58; Cl, 18.8; N, 9.90.

Acknowledgment.—The authors are grateful to Dr. Boris Weinstein, now at Stanford University, for some of the exploratory experiments in this field. The authors are indebted to Dr. D. J. Lyman and staff for studying the inherent viscosities of V, to Dr. Peter Lim for infrared interpretations, and to Mr. O. P. Crews and staff for preparing certain intermediates.

(14) Melting points were observed on a Fisher-Johns hot stage and corrected. Infrared spectra were determined in Nujol mulls, except as noted for VI; the significant bands recorded were medium to strong in intensity, unless labeled as weak. Molecular weights were determined with a Mechrolab vapor pressure osmometer; values above 10,000 could only be estimated, and no great accuracy is attributed to them. Inherent viscosity equals [ln (flow time solution/flow time solvent)]/concentration in g./100 ml.; values were determined at 30° in chloroform at a concentration of 0.1. The dioxane used was distilled from calcium hydride; I was dried at 60° (2 mm.).

(15) V. N. Konyukhov and Z. V. Pushkareva, *Puti Sinteza i Izyskania Protivoopukholevykh Preparatov. Tr. Simpoziuma po Khim. Protivoopukholevykh Veshchestv, Moscow*, 40 (1960); *cf. Chem. Abstr.*, **58**, 2501g (1963).

(16) Cyclo Chemical Corporation, Los Angeles, Calif.