Synthesis of Potential Antineoplastic Agents. XXXIII. β-Diketone Analogs of the Glutarimide Antibiotics[1]

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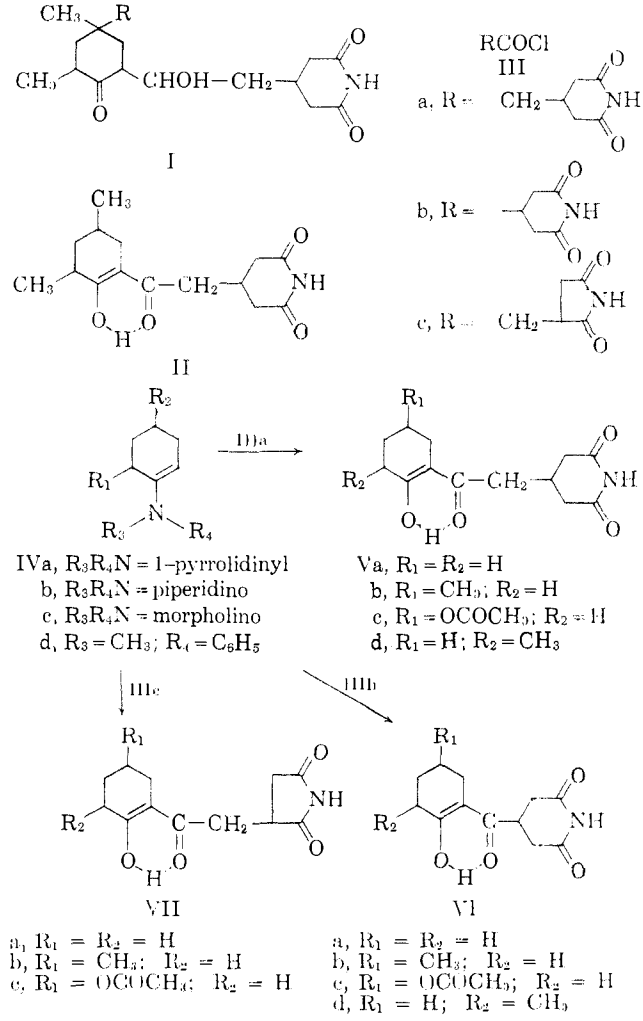
Analogs (V–VII) of the glutarimide antibiotics have been synthesized by the acylation of enamines of cyclohexanone, 2- and 4-methylcyclohexanone, and 4-acetoxycyclohexanone with 3-[(chlorocarbonyl)methyl]-glutarimide (IIIa), 3-(chlorocarbonyl)glutarimide (IIIb), and 2-[(chlorocarbonyl)methyl]succinimide (IIIc) in the presence of triethylamine. The infrared spectra of V–VII indicate that the analogs exist predominantly in the enol form. Three of the analogs showed cytotoxic activity at a concentration of 100 γ/ml. against Eagle's KB cells, but none of the compounds displayed any significant reproducible activity in tests in the Sarcoma 180 and Adenocarcinoma 755 tumor systems.

The structures of cycloheximide[2] (I, R = H), E-73[3] (an antitumor substance from *Streptomyces albulus*, I, R = $OCOCH_3$), streptovitacin A[4] (I, R = OH), streptimidone,[5] inactone,[6] and the other streptovitacins[4] have glutarimide and β-ketol moieties, and the first three of these glutarimide antibiotics are reported to inhibit the growth of experimental neoplasms.[7] Of additional significance to cancer chemotherapy is the inhibitory action of cycloheximide on deoxyribonucleic acid and protein synthesis in certain tumor cell lines.[8] These biological results indicated that analogs of the glutarimide antibiotics may exert antitumor effects. We have synthesized three groups of glutarimide antibiotic analogs in which the β-ketol grouping is replaced by the β-diketone group of dehydro derivatives such as dehydrocycloheximide[2] (II). The first group (V) consists of derivatives in which the degree of substitution of the cyclohexanone ring of cycloheximide and E-73 has been varied. Among the compounds of the second group (VI), these structural variations are combined with a shortening of the side chain by elimination of the methylene group, and in the third group (VII), the structural variations of the cyclohexanone group are combined with the replacement of the glutarimide ring by the succinimide ring.

The β-diketone analogs (V–VII) were synthesized by the modified[9] enamine acylation method of Stork, *et al.*,[10] from an appropriately substituted enamine (IV) and a carboxylic acid chloride (III) in the presence of triethylamine.[11] The enamines were prepared by the method of Herr and Heyl.[12] 3-(Carboxymethyl)glutarimide[13] was prepared by a modification of the method of Lawes[14]; the fusion of methanetriacetic acid with



urea gave a mixture of 3-(carboxymethyl)glutarimide and its amide, and treatment of the mixture with cold nitrous acid gave a 60% yield of 3-(carboxymethyl)-glutarimide. 3-Carboxyglutarimide[15] was prepared in

(1) This investigation was supported by the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. PH-43-64-51, and by the C. F. Kettering Foundation.

(2) E. C. Kornfeld, R. G. Jones, T. V. Parke, *J. Am. Chem. Soc.*, **71**, 150 (1949).

(3) K. V. Rao, *ibid.*, **82**, 1129 (1960).

(4) R. R. Herr, *ibid.*, **81**, 2595 (1959).

(5) R. P. Frohardt, H. W. Dion, Z. L. Zakubowski, A. Ryder, J. C. French, and Q. R. Bartz, *ibid.*, **81**, 5500 (1959); E. E. Van Tamelen and V. Haarstad, *ibid.*, **82**, 2974 (1960).

(6) R. Paul and S. Tchelitcheff, *Bull. soc. chim. France*, 1316 (1955).

(7) F. R. White, *Cancer Chemotherapy Rept.*, **5**, 48 (1959); K. V. Rao and W. P. Cullen, *J. Am. Chem. Soc.*, **82**, 1127 (1960); L. L. Sensenbrenner, *Cancer Chemotherapy Rept.*, **5**, 65 (1959).

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(9) S. Hünig, E. Benzing, and E. Lücke, *Chem. Ber.*, **90**, 2833 (1957).

(10) (a) G. Stork, R. Terrell, and J. Szmuszkovicz, *J. Am. Chem. Soc.*, **76**, 2029 (1954); (b) G. Stork, A. Brizzolara, H. Landesman, J. Szmuszkovicz, and R. Terrell, *ibid.*, **85**, 207 (1963).

(11) In recent communications the synthesis of II by the enamine acylation reaction has been reported: (a) H. J. Schaeffer and V. K. Jain, *J. Pharm. Sci.*, **52**, 509 (1963); (b) F. Johnson, N. A. Starkovsky, A. L. Pagon, and A. A. Carlson, *J. Am. Chem. Soc.*, **86**, 118 (1964).

(12) M. E. Herr and F. W. Heyl, *ibid.*, **74**, 3627 (1952); **75**, 1918 (1953).

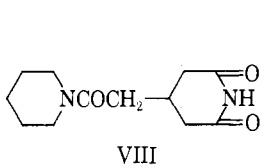
(13) D. D. Phillips, M. A. Acitelli, and J. Meinwald, *ibid.*, **79**, 3517 (1957).

(14) B. C. Lawes, *ibid.*, **82**, 6413 (1960).

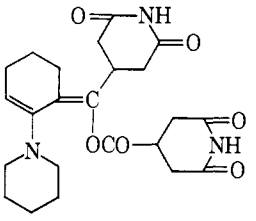
(15) A. L. Langis and R. Gaudry, U. S. Patent 2,871,158 (Feb. 17, 1959); *Chem. Abstr.*, **53**, 12311h (1959).

accordance with the reported procedure from citrazinic acid, and 2-(carboxymethyl)succinimide[16] was produced by the pyrolysis of the crude ammonium salt of 1,2,3-propanetricarboxylic acid. Treatment of the acids with thionyl chloride produced the acid chlorides.

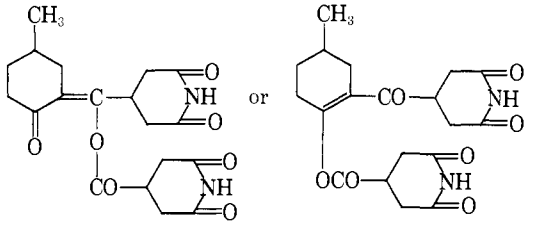
Yields of the β-diketones were generally below 30% except in the case of Va which was obtained in 93% yield. N-Alkylation, N-arylation, and diacylation reactions of enamines are known,[9,10b,17,18] and products of the analogous N-acylation and of diacylation were isolated during the preparation of several of the β-diketones. 3-[(Piperidinocarbonyl)methyl]glutarimide (VIII) was isolated during certain attempts to prepare Va,b,c, and products having elemental analyses in agreement with the diacylated structures IX and X were obtained during the preparation of VIa and VIb, respectively. The infrared spectra of IX and X showed a strong, sharp band at 1750 cm.$^{-1}$ indicative of ester carbonyl absorption. Stork, *et al.*,[10a] noted that alkylation of 2-methylcyclohexanone enamine occurred more sluggishly than alkylation of the unsubstituted cyclohexanone derivative under identical reaction conditions, and Williamson[19] has suggested that this resistance to alkylation was due in part to the steric effects of the 2-alkyl substituent. Schaeffer and Jain[11a] have suggested that low yields result from the acylation of enamines substituted in the 6-position of the cyclohexene moiety because the enamine is a mixture of isomers in which the double bond is at C-1–C-6 or C-1–C-2. The opening of the glutarimide ring during the acylation reaction or during the hydrolysis of the acylated enamine is another possible side reaction which would lower the yields of V–VII.



VIII



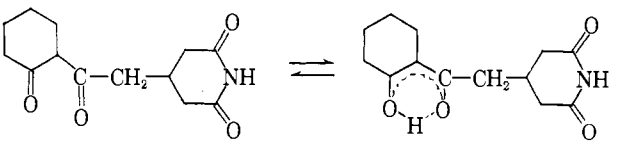
IX



X

The infrared spectra of samples of V–VII that were obtained by crystallization from ethanol–water or less polar solvents suggest that the compounds exist largely in the enol form since all exhibited one or two bands of medium intensity in the range 1560–1630 cm.$^{-1}$.[20] This suggestion is consistent with the observations of Johnson, Gurowitz, and Starkovsky[21] who isolated β-diketones of cycloheximide (I, R = H), isocycloheximide, and neocycloheximide which were readily converted to more stable enolic forms by heating, recrystallization, or solution in a nonpolar solvent. The enol absorption appeared as one broad band in the 1580–1620 cm.$^{-1}$ range in the spectra of Vb,d and VI a,b,d and as two bands in the 1560–1620 cm.$^{-1}$ range in the spectra of Vc, VIc, and the three succinimide derivatives VIIa,b,c. The enol band of Va was present as an incompletely resolved doublet at 1590–1620 cm.$^{-1}$. Hammond, *et al.*,[22] suggested that absorption by β-diketones in the 1120–1230 cm.$^{-1}$ range was perhaps attributable to C–H bending of the hydrogen on the methine carbon in the enol system, and V–VII exhibited a sharp band of medium intensity at 1140–1190 cm.$^{-1}$. In addition to the enol absorption, V displayed an incompletely resolved triplet within the limits of 1675–1730 cm.$^{-1}$, VI absorbed at 1680–1690 cm.$^{-1}$ and at 1730–1740 cm.$^{-1}$, and VII exhibited bands at 1700–1710 cm.$^{-1}$ and at 1765–1770 cm.$^{-1}$. VIc showed a band at 1710 cm.$^{-1}$ which was absent in VIa,b,d, and VIIc displayed a band at 1725 cm.$^{-1}$ which was not present in VIIa,b; these bands probably result from the ester carbonyl absorption of the acetoxy grouping. Such a band could not be distinguished in the spectrum of Vc because of the triplet at 1675–1730 cm.$^{-1}$.

After crystallization of Va from water, its spectrum showed no band in the 1560–1630 cm.$^{-1}$ range, indicating its conversion to the keto form.[22,23] However, the sharp band at 1140 cm.$^{-1}$ remained. The predominantly enol form, obtained by crystallization from cyclohexane–ethanol, gave an immediate ferric chloride test, whereas the keto form gave a positive test only after a delay.



keto form enol form

Compounds Vd, VId, and VIIb displayed cytotoxic activity at a concentration of 100 γ/ml. against Eagle's KB cells; cell growth in these tests was less than 50% of the growth of the controls. Except VIIa, the dehydrocycloheximide analogs have been screened in the Sarcoma 180 system and, except Vc and VIIa, in the Adenocarcinoma 755 system. Most of the analogs (Va,b, VIb,c,d, and VIIb,c) have also been tested in the Leukemia L1210 system. None of the compounds displayed any significant reproducible activity in these tests. The results are listed in Table I[24] along with results for cycloheximide (I, R = H) and dehydrocycloheximide (II).

(16) F. B. Thole and J. F. Thorpe, *J. Chem. Soc.*, **99**, 1688 (1911).

(17) (a) S. Hünig and E. Lücke, *Chem. Ber.*, **92**, 652 (1959); (b) S. Hünig and W. Lendle, *ibid.*, **93**, 909 (1960).

(18) M. E. Kuehne, *J. Am. Chem. Soc.*, **84**, 840 (1962).

(19) W. R. N. Williamson, *Tetrahedron*, **3**, 314 (1958).

(20) (a) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1958, pp. 142–143; (b) R. D. Campbell and H. M. Gilow, *J. Am. Chem. Soc.*, **82**, 5426 (1960); **84**, 1440 (1962).

(21) F. Johnson, W. D. Gurowitz, and N. A. Starkovsky, *Tetrahedron Letters*, 1167 (1962).

(22) G. S. Hammond, W. G. Borduin, and G. A. Guter, *J. Am. Chem. Soc.*, **81**, 4682 (1959).

(23) The effect of solvent polarity on keto–enol equilibria is well known. See, for example, O. Dimroth, *Ann.*, **399**, 91 (1913); E. M. Kosower, *J. Am. Chem. Soc.*, **80**, 3267 (1958).

(24) Tumor and tissue culture test data were obtained by The Chemotherapy Division of Southern Research Institute under the direction of Drs. F. M. Schabel, Jr., W. R. Laster, and G. J. Dixon.

TABLE I

TUMOR AND CYTOTOXICITY TEST DATA

Compound	Tumor	Dosage, mg./kg./day	Mortality	Wt. change, g., test/control	T/C[a]	Ratio, %	Cytotoxicity data, Eagle's KB cell line T − C_0/C − C_0[b]
Va	S180	500	0/6	−0.4/+1.5	1014/870	117	0.79
	Ad755	450	1/9	+1.9/+2.5	950/808	118	
	L1210	450	0/6	+0.0/−0.1	8.7/9.7	90	
Vb	S180	500	0/6	−0.6/+1.5	1124/870	129	0.56
	Ad755	450	1/10	+1.0/+2.5	395/808	49	
	Ad755	450	2/10	+1.0/+2.4	1297/1086	119	
	L1210	450	1/6	+0.5/−0.1	9.4/9.7	97	
Vc	S180	500	0/6	−0.3/+1.6	638/649	98	0.69
Vd	S180	500	2/6	−1.1/−1.1	598/1115	53	−0.02
	S180	500	1/6	−1.9/+0.0	625/952	65	
	Ad755	450	0/10	+1.9/+2.2	1061/1192	89	
VIa	S180	500	1/6	−0.0/+0.3	439/583	75	0.54
	Ad755	450	1/9	+2.7/+2.4	1093/1086	100	
	Ad755	350	2/10	+1.8/+3.5	869/1400	62	
VIb	S180	500	0/6	+1.8/+0.3	1646/1139	144	0.69
	S180	250	0/6	−0.4/−1.3	888/869	102	
	Ad755	450	0/10	+1.4/+2.0	1294/983	131	
	L1210	450	0/6	+0.1/−0.1	8.5/8.8	96	
VIc	S180	500	0/6	−1.5/−1.0	1576/1474	106	0.70
	Ad755	400	0/10	+1.7/+2.2	1501/1181	127	
	L1210	400	0/5	−0.4/−1.1	8.9/9.4	89	
VId	S180	500	0/6	−1.2/−0.1	609/1076	56	0.19
	Ad755	400	1/10	+0.3/+2.0	1093/983	111	
	L1210	400	0/6	+0.4/+0.7	8.0/8.5	94	
VIIa							0.61
VIIb	S180	500	0/6	−0.7/+0.8	1572/1389	113	0.41
	Ad755	500	1/10	+1.2/+1.6	944/875	107	
	L1210	500	0/6	−0.7/+0.2	9.0/9.0	100	
VIIc	S180	500	1/6	−0.5/+0.8	1610/1389	115	0.71
	Ad755	500	3/10	+2.1/+2.6	676/1162	58	
	L1210	500	0/6	−0.9/−0.6	7.7/8.6	89	
II	S180	500	5/6				
	S180	500	1/6	−2.0/+0.1	465/1168	39	
	S180	500	2/6	−1.0/+1.0	705/1930	37	
	S180	250	0/6	−1.0/+1.0	1170/1930	61	
	Ad755	125	4/10				
	Ad755	125	0/10	−2.3/−0.3	542/802	67	
	Ad755	62	0/10	+0.6/+0.6	1075/921	116	
I (R = H)	S180	24	1/10	+2.0/+2.9	449/1179	38	
	Ad755	50	2/20	+1.2/+2.4[c]	103/884[c]	10[c]	
	Ad755	34.5	5/30	−1.0/+1.9[d]	161/1267[d]	12[d]	
	Ad755	17.25	0/30	+1.8/+1.4[d]	728/1267[d]	58[d]	
	L1210[e]	57	0/36		11.3/8.4[f]	31[f]	

[a] T/C = treated/controls. T and C represent tumor weights in mg. for S180 and Ad755 and survival time in days for L1210. [b] T − C_0 and C − C_0 represent γ of protein in treated and control systems in tests at a dose level of 100 γ/ml. [c] Average of two tests. [d] Average of three tests. [e] Administered every other day instead of daily. [f] Average of six tests.

Experimental

Melting points were determined with a Kofler Heizbank melting point apparatus and are corrected. Infrared spectra were determined with samples in pressed potassium bromide disks and with a Perkin-Elmer Model 21 or 221G spectrophotometer. Ultraviolet spectra were determined with a Cary Model 14 recording spectrophotometer; extinction coefficients at absorption maxima are not reported for V-VII because the maxima decreased with time and appeared to approach a constant value.

Enamines.—The enamines were prepared by the method of Herr and Heyl[12] from cyclohexanone, 2- and 4-methylcyclohexanones, and 4-acetoxycyclohexanone and a secondary amine (pyrrolidine, piperidine, morpholine, or N-methylaniline).

3-(Carboxymethyl)glutarimide.—Methanetriacetic acid[25] (19 g., 0.1 mole) and urea (6.7 g., 0.11 mole) were heated to 200° in an oil bath. The melt was heated with stirring until ammonia evolution ceased, cooled to 125°, and dissolved in water (100 ml.). Cold, concentrated sulfuric acid (100 ml.) was added with stirring to the cold solution (0°) which was then treated with aqueous sodium nitrite (8.3 g., 0.12 mole, in 100 ml. of water) over a period of 1 hr. The solution was stirred overnight at room temperature, neutralized with sodium carbonate, and extracted continuously with ethyl acetate for 6 hr. Acidification of the aqueous phase (pH 3) and continuous extraction with ethyl acetate for 24 hr. produced the acid: yield, 10 g. (60%); m.p. 171–174°. One recrystallization from absolute ethanol or dioxane–chloroform afforded the pure acid, m.p. 175°; lit.[13] m.p. 172–173°.

3-Carboxyglutarimide was prepared by the procedure of Langis and Gaudry.[15]

2-(Carboxymethyl)succinimide was prepared by a method different from that reported.[16] 1,2,3-Propanetricarboxylic acid (75 g., 0.43 mole) was added slowly with stirring to concentrated ammonium hydroxide (150 ml.) cooled in an ice–salt bath. Evaporation of the solution produced a residue which was distilled *in vacuo*. The brown distillate (53.5 g.) was dissolved in glacial acetic acid (100 ml.) and the solution decolorized with charcoal. Crystallization was accomplished by the cautious addition of chloroform; yield, 28.1 g. (42%); m.p. 148° (lit.[16] m.p. 127–128°); $\bar{\nu}$ (cm.$^{-1}$): 3430 (OH); 3160, 3080 (NH); 1780 (imide

(25) E. P. Kohler and G. H. Reid, *J. Am. Chem. Soc.*, **47**, 2803 (1925).

C=O); and 1715 sh, 1700, 1680 sh (COOH and imide C=O).

Acid Chlorides.—3-[(Chlorocarbonyl)methyl]glutarimide[13] (IIIa) and 3-(chlorocarbonyl)glutarimide (IIIb) were prepared by refluxing the acid with excess thionyl chloride, adding benzene, cooling, collecting the crystalline product, and recrystallizing from benzene. 2-[(Chlorocarbonyl)methyl]succinimide (IIIc) was prepared by refluxing the acid with excess thionyl chloride, evaporating the mixture to dryness *in vacuo*, allowing the dark residue to crystallize, and recrystallizing from benzene.

3-[(Chlorocarbonyl)methyl]glutarimide had m.p. 140° (lit.[13] m.p. 129–130°); $\bar{\nu}$ (cm.$^{-1}$): 3270, 3155 (NH); 1830 (COCl); and 1730 sh, 1695 (imide C=O).

3-(Chlorocarbonyl)glutarimide had m.p. 118–119°.

Anal. Calcd. for $C_6H_6ClNO_3$: C, 41.10; H, 3.44; Cl, 20.2; N, 7.98. Found: C, 41.25; H, 3.46; Cl, 20.1; N, 7.86.

2-[(Chlorocarbonyl)methyl]succinimide had m.p. 75–78°.

Anal. Calcd. for $C_6H_6ClNO_3$: C, 41.10; H, 3.44; Cl, 20.2; N, 7.98. Found: C, 41.41; H, 3.63; Cl, 19.8; N, 8.12.

3-[(2-Oxocyclohexylcarbonyl)methyl]glutarimide (Va).—To a cold (12°) solution of 1-piperidinocyclohexene (4.8 g., 29 mmoles) and dry triethylamine (5 ml., 36 mmoles) in dry dioxane (50 ml.) was added dropwise with stirring a solution of 3-[(chlorocarbonyl)-methyl]glutarimide (5 g., 26.5 mmoles) in dry dioxane (75 ml.). After the addition, the bath temperature was raised to 40° and kept at that temperature for 1 hr. The mixture was stirred overnight at room temperature and evaporated (*in vacuo*) to a sirup. Hydrolysis of the sirup was effected by codistillation *in vacuo* with three 75-ml. portions of water. The residue was triturated in four 10-ml. portions of water, collected by filtration, and dried *in vacuo;* yield, 5.2 g. (78%); m.p. 133°. Evaporation of the trituration filtrate produced a residue from which an additional 15% of the β-diketone was extracted with benzene. The two crops were combined and crystallized from ethanol–benzene (4:1); yield, 4.34 g. (66%); m.p. 147°. Recrystallization from water gave a product with a melting point of 152° that appears to be the keto form according to its infrared spectrum.

Anal. Calcd. for $C_{13}H_{17}NO_3$: C, 62.14; H, 6.82; N, 5.57. Found: C, 62.34; H, 6.56; N, 5.33.

Recrystallization from cyclohexane–ethanol produced a solid melting at 139° that appears to be predominantly the enol form according to its infrared spectrum; λ_{max} (mμ): in ethanol, 291; at pH 1, 295; at pH 7, 295; and at pH 13, 316.5.

Anal. Found: C, 61.89; H, 6.77; N, 5.57.

Another experiment, analogous to the preparation of Va except that addition of IIIa was made at 35–40° and hydrolysis was conducted in 4.8 *N* hydrochloric acid at 0°, gave a 51% yield of the N-acylation product, **3-[(piperidinocarbonyl)methyl]glutarimide (VIII),** and no β-diketone. It was purified by crystallization from ethanol–hexane followed by crystallization from benzene–hexane, m.p. 138–140°.

Anal. Calcd. for $C_{12}H_{18}N_2O_3$: C, 60.48; H, 7.61; N, 11.76. Found: C, 60.59; H, 7.56; N, 12.07.

Compound VIII was isolated similarly during the attempted preparation of Vb and Vc in experiments in which the addition of IIIa was made at 35–45°.

3-[(5-Methyl-2-oxocyclohexylcarbonyl)methyl]glutarimide (Vb).—4-Methyl-1-piperidinocyclohexene (10.7 g., 60 mmoles), triethylamine (10 ml., 71.5 mmoles), and 3-[(chlorocarbonyl)-methyl]glutarimide (10.5 g., 55 mmoles) in dioxane gave Vb by a procedure analogous to that used in the preparation of Va. Extraction of the residue from the hydrolysis step with ether and evaporation of the ether gave 9.5 g. of crude product which was crystallized from ethanol–benzene (4:1); yield, 4.5 g. (31%); m.p. 160°; λ_{max} (mμ): at pH 1, 294; at pH 7, 294; and at pH 13, 313.

Anal. Calcd. for $C_{14}H_{19}NO_4$: C, 63.38; H, 7.22; N, 5.28. Found: C, 63.18; H, 7.31; N, 5.52.

3-[(5-Acetoxy-2-oxocyclohexylcarbonyl)methyl]glutarimide (Vc).—Compound Vc was obtained from 4-acetoxy-1-piperidinocyclohexene (2.68 g., 12 mmoles), triethylamine (1.8 ml., 13 mmoles), and 3-[(chlorocarbonyl)methyl]glutarimide (1.89 g., 10 mmoles) in dioxane by a procedure analogous to that used in the preparation of Va except that hydrolysis was accomplished by stirring the sirupy reaction residue with 10% hydrochloric acid (28 ml.) at 0° for 30 min. and at room temperature for 3 hr. The crystalline product was collected by filtration and recrystallized from benzene–hexane and again from benzene; yield, 0.65 g. (22%); m.p. 147–150°. An analytical sample was obtained by another recrystallization from benzene; m.p. 147–148°; λ_{max} (mμ): in ethanol, 290; at pH 1, 295; at pH 7, 295; and at pH 13, 314.

Anal. Calcd. for $C_{15}H_{19}NO_6$: C, 58.24; H, 6.19; N, 4.53. Found: C, 58.08; H, 6.07; N, 4.74.

3-[(3-Methyl-2-oxocyclohexylcarbonyl)methyl]glutarimide[11a] (Vd).—Compound Vd was obtained from 6-methyl-1-(1-pyrrolidinyl)cyclohexene (3.6 g., 22 mmoles), triethylamine (3.5 ml., 25 mmoles), and 3-[(chlorocarbonyl)methyl]glutarimide (3.8 g., 20 mmoles) in dioxane by a procedure analogous to that used in the preparation of Va. The product crystallized from aqueous solution during the hydrolysis step; yield, 1.3 g. (25%). Extraction of the aqueous filtrate with ether afforded an additional 0.4 g. of crude product. The two portions were combined and crystallized from ethanol–water; yield, 1.1 g.; m.p. 131°; λ_{max} (mμ): pH 1, 296; pH 7, 296; pH 13, 316.5.

Anal. Calcd. for $C_{14}H_{19}NO_4$: C, 63.38; H, 7.22; N, 5.28. Found: C, 63.17; H, 7.32; N, 5.38.

3-(2-Oxocyclohexylcarbonyl)glutarimide (VIa).—3-(Chlorocarbonyl)glutarimide (2.8 g., 16 mmoles) and 1-piperidinocyclohexene (2.8 g., 17 mmoles) were mixed at room temperature and treated immediately with dry dimethylformamide (20 ml.). Triethylamine (5 ml.) was added dropwise with stirring in 30 min. at room temperature in a nitrogen atmosphere. Stirring was continued for 1 hr. after the amine addition, and the mixture was stoppered and allowed to stand overnight at room temperature. Evaporation of the reaction mixture *in vacuo* with mild heating produced a sirup which was hydrolyzed at 0° with 6 *N* hydrochloric acid. The β-diketone was obtained by extraction of the aqueous system with three 50-ml. portions of ether, neutralization of the aqueous layer, extraction with three 50-ml. portions of ether, then with ethyl acetate (50 ml.), evaporation of the ethyl acetate and the combined ether extracts separately, and trituration of the two residues in ethanol. From the ether extract was obtained 375 mg. of crude product, from the ethyl acetate extract, 210 mg. Crystallization of the crude product from ethanol gave 400 mg. (10%) of solid, m.p. 170–173°. The analytical sample was obtained by crystallization (charcoal decolorization) from ethanol, m.p. 175°, λ_{max}^{EtOH} 295 mμ.

Anal. Calcd. for $C_{12}H_{15}NO_4$: C, 60.77; H, 6.38; N, 5.90. Found: C, 60.71; H, 6.25; N, 5.88.

Another experiment, analogous to the preparation of VIa except that the reaction mixture was stirred for 3.5 hr. at room temperature after the triethylamine addition and allowed to stand 5 hr. instead of overnight, gave a different product. After removal of the volatile components *in vacuo*, the mixture was poured into water and stirred for 5 min. A solid (3.1 g.) was collected by filtration and was purified by washing with hot ethanol and crystallizing from ethanol–dimethylformamide, and then from ethanol. Analyses of the product (m.p. 220° with decomposition) were consistent with structure IX.

Anal. Calcd. for IX ($C_{23}H_{29}N_3O_6$): C, 62.30; H, 6.58; N, 9.46. Found: C, 62.04; H, 6.67; N, 9.40.

3-(5-Methyl-2-oxocyclohexylcarbonyl)glutarimide (VIb) was obtained from 4-methyl-1-piperidinocyclohexene (3.1 g., 17 mmoles), 3-(chlorocarbonyl)glutarimide (2.8 g., 16 mmoles), and triethylamine (5 ml.) in dimethylformamide by a procedure analogous to that used in the preparation of VIa. The crude product, obtained by trituration of the ether and the ethyl acetate extract residues in ethanol, was crystallized from ethanol; yield, 520 mg. (13%); m.p. 178°; λ_{max} (mμ): at pH 1, 298; at pH 7, 299; and at pH 13, 319.

Anal. Calcd. for $C_{13}H_{17}NO_4$: C, 62.13; H, 6.82; N, 5.58. Found: C, 62.14; H, 6.89; N, 5.58.

In a related, large-scale experiment, treatment of the acid chloride in dimethylformamide with the enamine in dimethylformamide followed by adding triethylamine, stirring 4 days at room temperature, evaporating the mixture to a sirup, and triturating in 3 *N* hydrochloric acid, gave a solid which was washed with hot ethanol and purified by crystallization from dimethylformamide–ether. Analyses of the product (m.p. 229–232° dec.) were consistent with structure X.

Anal. Calcd. for X ($C_{19}H_{22}N_2O_7$): C, 58.45; H, 5.68; N, 7.18. Found: C, 57.88; H, 5.56; N, 7.26.

The yield, based on structure X, was 24%. In addition, a 12% yield of VIb was isolated from the hydrochloric acid trituration filtrate.

3-(5-Acetoxy-2-oxocyclohexylcarbonyl)glutarimide (VIc) was obtained from 4-acetoxy-1-piperidinocyclohexene (3.8 g., 17 mmoles), 3-(chlorocarbonyl)glutarimide (2.8 g., 16 mmoles), and triethylamine (5 ml.) in dimethylformamide by a procedure

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 $C_{13}H_{19}NO_5$: 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. 138–149°. 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 (1419 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 $C_{15}H_{17}NO_6$: 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 $C_{15}H_{17}NO_6$: 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).

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