primary or secondary amine was heated on an oil bath at 130–140° for 3 hr. with constant stirring. The mixture was then cooled and 20 g. of a 50% aqueous NaOH solution was added. The mixture was extracted with 200 ml. of ether and the extract was dried overnight. The dried extract was then distilled to yield the desired product.

Spasmolytic Activity.—Anticholinergic activity was determined on isolated guinea pig ileum suspended in Locke–Ringer's solution in a water bath at 37°. Acetylcholine (1:50 p.p.m.) was used as the spasmogenic agent. The compounds being tested were added to the medium 30 sec. before the addition of acetylcholine, and the inhibition of the acetylcholine-induced contraction was measured by comparison with control values.

The procedure used to determine the inhibition of $BaCl_2$-induced contractions was the same, except that the final concentration of $BaCl_2$ was 1:10,000 and rabbit ileum was the test tissue. The results are shown in Table II.

Acknowledgment.—The authors wish to express their thanks to Dr. Beiler and his staff at the Research Laboratories of the National Drug Company for the pharmacological evaluation.

Amino Acid Analogs. I. Analogs of the Glutamic Acid–Proline Interconversion. III. Substituted 2-Acetamido-4-benzoylbutyric Acids and 5-Phenylprolines

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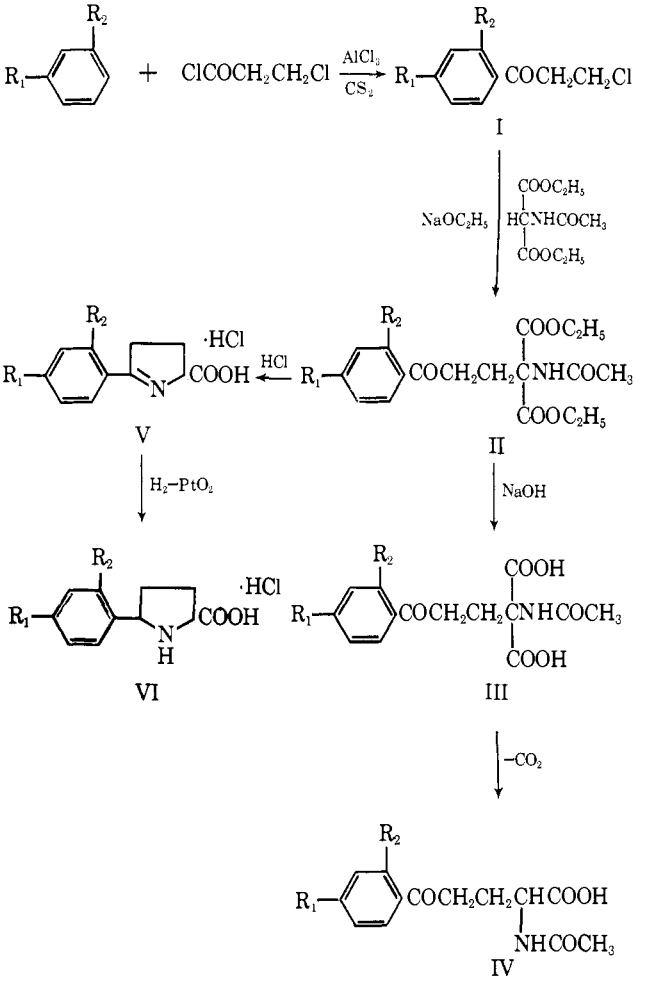
In a previous paper,[2] a rationale for our interest in the preparation of proline and glutamic acid analogs was presented. In that work, the syntheses of 2-acetamido-4-benzoylbutyric acid and 5-phenylproline were reported, and the methods were thought to be suitable for the preparation of aromatic substitution products which were desired for biological study.

In this study, 10 glutamic acid and 10 proline analogs and the necessary intermediates[3] will be described.

The β-chloropropiophenones (I) were prepared by acylation of the appropriate benzene derivative with β-chloropropionyl chloride by means of aluminum chloride in carbon disulfide, according to Allen, Cressman, and Bell.[4] The chloro ketones were used without further purification in the condensation with ethyl acetamidomalonate using sodium ethoxide in anhydrous ethyl alcohol as the condensing medium. Upon hydrolysis of the acetamidomalonates (II) with alkali, malonic acids (III) were obtained which were decarboxylated to the acetylated glutamic acid analogs (IV). Acid hydrolysis of II yielded 2-(substituted phenyl)-1-pyrroline-5-carboxylic acids (V). Subsequent hydrogenation of the pyrrolines (V) over Adams' catalyst at 3–4 atm. of hydrogen yielded the corresponding proline analogs (VI). These reactions are summarized in Scheme I, and the physical and analytical data on compounds of types II–VI are listed in Tables I–V, respectively.

SCHEME I



a, R_1 = F; R_2 = H
b, R_1 = Cl; R_2 = H
c, R_1 = Br; R_2 = H
d, R_1 = CH_3; R_2 = H
e, R_1 = C_6H_5; R_2 = H
f, R_1 = CH_3O; R_2 = H
g, R_1 = C_6H_5O; R_2 = H
IIh–IVh, R_1 = CH_3CONH; R_2 = H
Vh, VIh, R_1 = R_1 = $NH_2 \cdot HCl$; R_2 = H
i, R_1 = F; R_2 = F
j, R_1 = Cl; R_2 = Cl

The genus *Leuconostoc* is composed of streptococcus-like bacteria that secrete large quantities of gum. These organisms which are prevalent in sugar refineries interfere with the processing of cane sugar.[5] *Leuconostoc mesenteroides* P-60 is a species that has been used as an assay organism for 18 amino acids.[6] Among these are glutamic acid and proline.

To learn whether these glutamic acid and proline analogs possess differences in biological activity from the unsubstituted analogs and to learn whether such compounds could be useful in the cane sugar industry,

(1) To whom requests for reprints should be made: Boyce Thompson Institute for Plant Research, Yonkers, N. Y. 10701.

(2) H. Gershon and A. Scala, *J. Org. Chem.*, **26**, 2347 (1961).

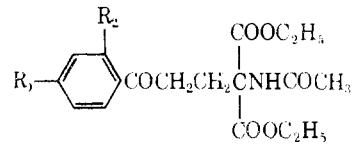
(3) Three of the intermediates prepared in this study, 2-(4-chlorophenyl)-, 2-(4-methoxyphenyl)-, and 2-(4-aminophenyl)-1-pyrroline-5-carboxylic acid, were claimed in a recent patent [F. Leonard, British Patent 977,097 (1964)] but were not characterized. The method of synthesis described in the patent is analogous to that reported by Gershon and Scala[2] for the preparation of 2-phenyl-1-pyrroline-5-carboxylic acid.

(4) C. F. H. Allen, H. W. J. Cressman, and A. C. Bell, *Can. J. Res.*, **8**, 440 (1933).

(5) A. T. Henrici and E. J. Ordal, "The Biology of Bacteria," D. C. Heath and Co., Boston, Mass., 1948, p. 416.

(6) B. F. Steele, H. E. Sauberlich, M. S. Reynolds, and C. A. Baumann, *J. Biol. Chem.*, **177**, 533 (1949).

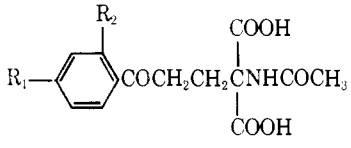
TABLE I
SUBSTITUTED ACETAMIDO(2-BENZOYLETHYL)MALONIC ESTERS (II)



II	R_1	R_2	Yield, %	M.p.,[a] °C.	Formula	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N
a	F	H	46.9	88–90	$C_{18}H_{22}FNO_6$	58.85	6.04	3.81	59.07	5.94	3.70
b	Cl	H	56.0	114–115	$C_{18}H_{22}ClNO_6$	56.32	5.78	3.65	56.49	5.74	3.51
c	Br	H	67.8	126–127	$C_{18}H_{22}BrNO_6$	50.48	5.18	3.27	50.63	5.12	3.24
d	CH_3	H	39.2	101–103	$C_{19}H_{25}NO_6$	62.80	6.93	3.86	62.54	6.90	4.08
e	C_6H_5	H	51.0	102–104	$C_{24}H_{27}NO_6$	67.75	6.40	3.29	67.69	6.33	3.20
f	CH_3O	H	42.5	87–90	$C_{19}H_{25}NO_7$	60.15	6.64	3.69	60.12	6.58	3.64
g	C_6H_5O	H	55.8	102–104	$C_{24}H_{27}NO_7$	65.29	6.17	3.17	65.08	5.93	3.11
h	CH_3CONH	H	39.8	150–152	$C_{20}H_{26}N_2O_7$	59.10	6.45	6.89	59.01	6.52	6.66
i	F	F	55.0	95–97	$C_{18}H_{21}F_2NO_6$	56.10	5.49	3.64	56.58	5.41	3.56
j	Cl	Cl	33.3	96–97	$C_{18}H_{21}Cl_2NO_6$	51.69	5.06	3.35	51.42	5.22	3.05

[a] Analytical samples; crystallized from isopropyl alcohol.

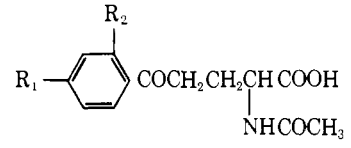
TABLE II
SUBSTITUTED ACETAMIDO(2-BENZOYLETHYL)MALONIC ACIDS (III)



III	R_1	R_2	Yield, %	M.p.,[a] °C. dec.	Formula	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N
a	F	H	56.3	145	$C_{14}H_{14}FNO_6$	54.02	4.53	4.50	54.23	4.82	4.33
b	Cl	H	95.0	125	$C_{14}H_{14}ClNO_6$	51.31	4.31	4.27	51.54	4.58	4.63
c	Br	H	97.5	209	$C_{14}H_{14}BrNO_6$	45.18	3.79	3.76	45.27	3.99	3.57
d	CH_3	H	61.0	160–164	$C_{15}H_{17}NO_6$	58.63	5.58	4.56	58.40	5.21	4.87
e	C_6H_5	H	95.6	240–243	$C_{20}H_{19}NO_6$	65.03	5.18	3.79	65.42	5.31	3.48
f	CH_3O	H	99.0	130–133	$C_{15}H_{17}NO_7$	55.73	5.30	4.33	55.91	5.62	4.52
g	C_6H_5O	H	64.1	156–158	$C_{20}H_{19}NO_7$	62.33	4.97	3.64	62.61	5.33	3.84
h	CH_3CONH	H	77.1	137–140	$C_{16}H_{18}N_2O_7$	54.86	5.18	8.00	54.50	5.02	8.40
i	F	F	62.8	114	$C_{14}H_{13}F_2NO_6$	51.07	3.98	4.25	50.62	4.59	3.52
j	Cl	Cl	66.0	100–102	$C_{14}H_{13}Cl_2NO_6$	46.43	3.62	3.87	46.52	3.99	3.90

[a] Analytical samples; crystallized from methyl alcohol.

TABLE III
SUBSTITUTED 2-ACETAMIDO-4-BENZOYLBUTYRIC ACIDS (IV)

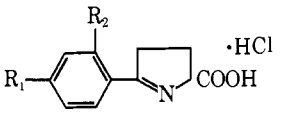


IV	R_1	R_2	Yield, %	M.p.,[a] °C.	Formula	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N
a	F	H	47.0	150	$C_{13}H_{14}FNO_4$	58.42	5.28	5.24	58.32	5.29	5.08
b	Cl	H	46.2	200–202	$C_{13}H_{14}ClNO_4$	55.03	4.97	4.94	54.70	4.94	4.83
c	Br	H	65.0	213–215	$C_{13}H_{14}BrNO_4$	47.58	4.30	4.27	47.19	4.29	4.16
d	CH_3	H	35.5	155	$C_{14}H_{17}NO_4$	63.86	6.51	5.32	63.79	6.40	5.07
e	C_6H_5	H	75.3	220–222	$C_{19}H_{19}NO_4$	70.14	5.89	4.31	70.29	6.04	4.05
f	CH_3O	H	23.3	169–170	$C_{14}H_{17}NO_5$	60.21	6.14	5.02	60.43	5.78	5.02
g	C_6H_5O	H	20.0	208–211	$C_{19}H_{19}NO_5$	66.85	5.61	4.10	66.77	5.65	3.97
h	CH_3CONH	H	52.2	165–168	$C_{15}H_{18}N_2O_5$	58.81	5.92	9.15	59.25	6.25	9.54
i	F	F	55.5	168–170	$C_{13}H_{13}F_2NO_4$	54.74	4.59	4.91	54.51	4.68	4.87
j	Cl	Cl	58.2	162–164	$C_{13}H_{13}Cl_2NO_4$	49.07	4.12	4.40	49.15	4.23	4.21

[a] Analytical samples; crystallized from aqueous methyl alcohol.

a random sampling of five glutamic acid and five proline analogs was made. The test organism (*L. mesenteroides* P-60) was exposed to the compounds in a medium prepared to simulate cane sugar juice. The results are summarized in Table VI, and it can be seen that in some cases the substituents on the benzene ring of the 2-acetamido-4-benzoylbutyric acid and proline derivatives increased the antibacterial effect of the

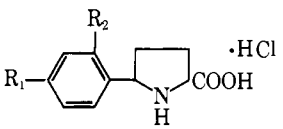
TABLE IV
SUBSTITUTED 2-PHENYL-1-PYRROLINE-5-CARBOXYLIC ACID HYDROCHLORIDES (V)



V	R_1	R_2	Yield, %	M.p.,[a] °C. dec.	Formula	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N
a	F	H	83.0	108–110	$C_{11}H_{11}ClFNO_2$	54.22	4.55	5.75	54.54	4.93	5.30
b	Cl	H	73.8	107–109	$C_{11}H_{11}Cl_2NO_2$	50.79	4.26	5.39	50.75	4.32	5.42
c	Br	H	52.5	192–194	$C_{11}H_{11}BrClNO_2$	43.37	3.64	4.60	43.40	3.65	4.64
d	CH_3	H	91.5	70	$C_{12}H_{14}ClNO_2$	60.13	5.89	5.84	60.02	5.68	5.52
e	C_6H_5	H	89.8	90	$C_{17}H_{16}ClNO_2$	67.66	5.34	4.64	67.98	5.30	4.23
f	CH_3O	H	97.7	110	$C_{12}H_{14}ClNO_3$	56.36	5.52	5.48	56.62	5.81	5.28
g	C_6H_5O	H	86.8	123	$C_{17}H_{16}ClNO_3$	64.25	5.08	4.41	64.42	5.35	4.16
h	$NH_2 \cdot HCl$	H	80.0	252	$C_{11}H_{14}Cl_2N_2O_2$	47.67	5.09	10.11	47.65	5.12	10.20
i	F	F	92.5	112–114	$C_{11}H_{10}ClF_2NO_2$	50.49	3.85	5.35	50.71	3.97	4.95
j	Cl	Cl	85.6	172–174	$C_{11}H_{10}Cl_3NO_2$	44.85	3.42	4.76	45.22	3.76	4.50

[a] Analytical samples; crystallized from methyl alcohol–ether mixtures.

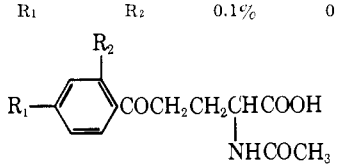
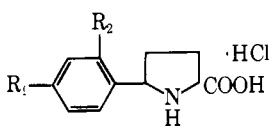
TABLE V
SUBSTITUTED 5-PHENYLPROLINE HYDROCHLORIDES (VI)



VI	R_1	R_2	Yield, %	M.p.,[a] °C.	Formula	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N
a	F	H	92.5	140	$C_{11}H_{13}ClFNO_2$	53.78	4.92	5.70	53.55	4.90	5.30
b	Cl	H	80.0	150–152	$C_{11}H_{13}Cl_2NO_2$	50.40	5.00	5.34	50.22	5.07	5.21
c	Br	H	92.0	93	$C_{11}H_{13}BrClNO_2$	43.09	4.27	4.57	43.01	4.12	4.36
d	CH_3	H	93.0	109–111	$C_{12}H_{16}ClNO_2$	59.63	6.67	5.80	59.71	6.85	5.51
e	C_6H_5	H	96.5	190	$C_{17}H_{18}ClNO_2$	67.21	5.97	4.61	66.94	5.63	4.25
f	CH_3O	H	84.8	177–179	$C_{12}H_{16}ClNO_3$	55.93	6.26	5.44	55.68	6.24	5.21
g	C_6H_5O	H	96.8	188–190	$C_{17}H_{18}ClNO_3$	63.85	5.67	4.38	63.61	5.56	4.11
h	$NH_2 \cdot HCl$	H	88.8	137	$C_{11}H_{16}Cl_2N_2O_2$	47.32	5.78	10.04	47.28	5.75	9.95
i	F	F	84.0	175–177	$C_{11}H_{12}ClF_2NO_2$	50.11	4.59	5.31	50.20	4.41	4.91
j	Cl	Cl	70.0	203–205	$C_{11}H_{12}Cl_3NO_2$	44.55	4.08	4.72	44.50	3.97	4.22

[a] Analytical samples; crystallized from methyl alcohol–ether mixtures.

TABLE VI
ANTIBACTERIAL ACTIVITY OF SELECTED 2-ACETAMIDO-4-BENZOYLBUTYRIC ACIDS AND 5-PHENYLPROLINES AGAINST *L. mesenteroides* P-60 IN SIMULATED CANE SUGAR JUICE

Compd.	R_1	R_2	Growth at levels tested[a] 0.1%	0.5%	1.0%
					
Ref. 2	H	H	+	+	−
IVa	F	H	+	+	−
IVe	C_6H_5	H	+	−	−
IVf	CH_3O	H	+	−	−
IVi	F	F	+	−	−
					
Ref. 2	H	H	+	+	−
VId	CH_3	H	+	−	−
VIe	C_6H_5	H	+	−	−
VIf	CH_3O	H	+	−	−
VIj	Cl	Cl	+	−	−

[a] + = growth, − = complete inhibition.

compound, but the low level of activity of the test compounds would make them useless for the purpose of the study undertaken.

All of these compounds were screened by the Cancer Chemotherapy National Service Center (NIH) against at least three mouse tumors, Sarcoma 180, Carcinoma 755 or Ehrlich ascites, and leukemia L1210. These data are contained in Table VII.

Experimental Section

Chemical.[7] ***p*-Acetamido-β-chloropropiophenone (Ih).**—A mixture of 137 g. (1.01 moles) of acetanilide, 210 g. (1.58 moles) of anhydrous $AlCl_3$, and 400 ml. of dry carbon disulfide was prepared. β-Chloropropionyl chloride (100 g., 0.79 mole) was added dropwise with vigorous agitation, during 2 hr., and the temperature of the reaction was maintained at about 20° by means of a water bath. Upon completion of addition of the β-chloropropionyl chloride, agitation was continued overnight. The product was drowned in a slurry of 500 g. of ice in 200 ml. of concentrated HCl, and the solid material that formed was removed by filtration, washed thoroughly with water and dried at 35° under vacuum. The yield of product was 173 g. (97.5%), m.p. 128–140° dec. An analytical sample was crystallized from a mixture of acetone and ethyl alcohol, m.p. 154–157° dec.

(7) This work was completed several years ago, and melting points were then taken in a Hershberg melting point apparatus and are uncorrected. The synthetic procedures are general.

TABLE VII

SUMMARY OF ANTICANCER SCREENING DATA AGAINST SARCOMA 180, CARCINOMA 755 OR EHRLICH ASCITES, AND LEUKEMIA L1210[a]

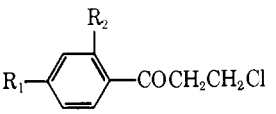
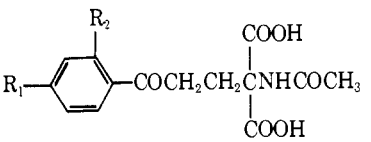
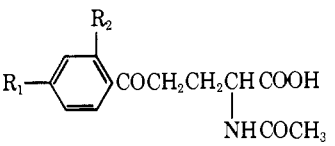
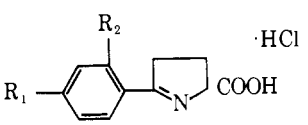
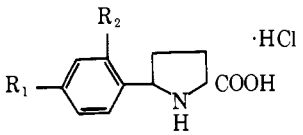
			S180		Ca755 or E.A.			L1210	
Compd.	R_1	R_2	NTL,[b] mg./kg.	T/C,[c] %	NTL,[b] mg./kg.		T/C,[c] %	NTL,[b] mg./kg.	T/C,[c] %
									
Ref. 2	H	H	65	93	65	E[d]	84	50	93
Ia	F	H	125	50	6	C[d]	87	87	71
			125	76					
Ib	Cl	H	25	68	5	C	87	20	70
Ic	Br	H	50	90	2.5	C	103	20	78
Id	CH_3	H	50	58	11	C	93	45	90
Ie	C_6H_5	H	125	65	56	C	68	76	98
If	CH_3O	H	125	63	200	C	70	400	86
Ig	C_6H_5O	H	25	108	11	C	61	22	72
Ih	CH_3CONH	H	500	99	400	C	72	200	105
Ii	F	F	500	62	400	C	74	200	107
									
Ref. 2	H	H	125	189	125	E	82	90	89
			125	106					
IIIa	F	H	500	96	450	E	84	450	77
IIIb	Cl	H	125	84	112	C	64	125	102
IIIc	Br	H	500	79	350	E	131	350	91
IIId	CH_3	H	500	84	450	E	105	450	97
IIIe	C_6H_5	H	125	124	100	E	109	100	125
IIIg	C_6H_5O	H	250	166	225	C	64	225	87
IIIh	CH_3CONH	H	500	121	450	E	93	450	97
IIIi	F	F						450	86
IIIj	Cl	Cl	500	87	450	C	62	450	93
									
Ref. 2	H	H	500	90	450	C	78	500	88
IVa	F	H	500	79	450	C	66	450	81
IVb	Cl	H	500	95	400	C	44	200	87
					400	C	108		
IVc	Br	H	500	72	400	C	66	400	69
IVd	CH_3	H	500	117	450	C	92	450	86
IVe	C_6H_5	H	500	102	450	C	64	125	82
IVf	CH_3O	H	500	78	400	C	52	400	92
					400	C	60		
IVg	C_6H_5O	H	500	71	90	C	94	90	86
IVh	CH_3CONH	H	500	99	400	C	77	400	85
IVi	F	F	500	72	350	C	84	350	92
IVj	Cl	Cl	500	85	450	C	88	450	98
									
Ref. 2	H	H	500	56	350	C	50	500	90
						C	78		
Va	F	H	100	37	90	C	124	90	131
			100	123					
Bb	Cl	H	500	72	175	C	44	350	102
					175	C	80		
Vc	Br	H	250	103	225	C	49	225	80
					112	C	103		
Vd	CH_3	H	500	102	200	C	75	400	90
Ve	C_6H_5	H	125	96	112	C	87	112	80

TABLE VII (*Continued*)

Compd.	R_1	R_2	S180 NTL,[b] mg./kg.	S180 T/C,[c] %	Ca775 or E.A. NTL,[b] mg./kg.		Ca775 or E.A. T/C,[c] %	L1210 NTL,[b] mg./kg.	L1210 T/C,[c] %
Vf	CH_3O	H	500	68	350	C	90	350	102
Vg	C_6H_5O	H	125	98	112	C	116	112	85
Vh	$NH_2 \cdot HCl$	H	250	50 61	112	C	68	225	87
Vi	F	F	125	99	88	C	108	88	103
Vj	Cl	Cl	125	78	100	C	59	100	103



Ref. 2	H	H	500	100	450	C	58	225	72
VIa	F	H	250	78	200	C	95	200	91
VIb	Cl	H	125	66	112	C	82	112	129
VIc	Br	H	500	99	350	E	82	350	73
VId	CH_3	H	250	75	100	C	100	100	104
VIe	C_6H_5	H	125	75	56	C	93	112	96
VIf	CH_3O	H	500	102	225	C	137	450	88
VIg	C_6H_5O	H	500	72	225	C	108	110	104
VIh	$NH_2 \cdot HCl$	H	375	80	320	C	55	320	106
VIi	F	F	500	62	200	C	70	400	89
VIj	Cl	Cl	100	89	80	C	63	80	104

[a] We are indebted to Dr. Howard W. Bond, Cancer Chemotherapy National Service Center, National Institutes of Health, Bethesda 14, Md., for making these data available to us. The details of the screening procedures can be found in *Cancer Chemotherapy Rept.*, **1**, 42 (1959). [b] NTL = maximum nontoxic level. [c] T/C = treated tumor/control tumor. [d] E = Ehrlich ascites, C = Carcinoma 755.

Anal. Calcd. for $C_{11}H_{12}ClNO_2$: C, 58.54; H, 5.32; N, 6.28. Found: C, 58.63; H, 5.80; N, 6.00.

Ethyl 2-Acetamido-4-(3-*p*-acetamidobenzoyl)-2-carbethoxybutyrate (IIh).—To a mixture of 76 g. (0.35 mole) of ethyl acetamidomalonate, 79 g. (0.35 mole) of Ih, and 150 ml. of anhydrous ethyl alcohol kept at 20° was added a solution of 9 g. (0.39 g.-atom) of sodium in 150 ml. of anhydrous ethyl alcohol, dropwise with agitation. Agitation was continued overnight at room temperature. Alcohol was removed by flash evaporation, and the residue was extracted with 500 ml. of methylene chloride and washed free of salts with two 100-ml. portions of water. Methylene chloride was flash evaporated, and the residue was crystallized from isopropyl alcohol and yielded 58 g. of product, m.p. 149–158°, 39.8% yield. An analytical sample was crystallized from isopropyl alcohol, m.p. 150–152°.

Acetamido[3-(*p*-acetamidophenyl)-3-oxopropyl]malonic Acid (IIIh).—IIh (20.5 g., 0.05 mole) was suspended in a solution prepared from NaOH (10 g., 0.25 mole) dissolved in a mixture of 90 ml. of water and 40 ml. of methyl alcohol. After standing overnight at 40°, the hydrolysate was acidified with concentrated HCl. The precipitate was filtered, washed free of chloride, and dried at 50° under vacuum. The yield of malonic acid was 13.5 g. (77.1%), m.p. 137–140° dec. Recrystallization from methyl alcohol did not change the melting point.

DL-2-Acetamido-4-(*p*-acetamidobenzoyl)butyric acid (IVh) was obtained by heating 12 g. (0.034 mole) of IIIh in 250 ml. of water for 2 hr. under reflux. Upon cooling, 5.5 g. (52.2%) of product was obtained, m.p. 155–166°. An analytical sample was prepared by crystallization from aqueous methyl alcohol, m.p. 165–168°.

2-(*p*-Aminophenyl)-1-pyrroline-5-carboxylic Acid Dihydrochloride (Vh).—IIh (22 g., 0.054 mole) was heated under reflux with 150 ml. of concentrated HCl overnight. The solution was evaporated to dryness under vacuum, and the residue was dissolved in water, decolorized with charcoal, and evaporated again. The product was then dissolved in methyl alcohol and allowed to stand in the freezer for several days when 12 g. (80%) of compound was obtained which melted at 240–242° dec. An analytical sample was obtained by crystallization from a mixture of methyl alcohol and ether, m.p. 252° dec.

5-(*p*-Aminophenyl)proline Dihydrochloride (VIh).—Compound Vh (14.7 g., 0.053 mole) was dissolved in 150 ml. of methyl alcohol and was hydrogenated in a Parr hydrogenator in the presence of 50 mg. of platinum oxide under 3–4 atm. When the theoretical uptake of hydrogen was observed, the catalyst was removed by filtration and the solvent was evaporated under a stream of air. The product (13 g., 88.8%) obtained melted at 131–137°. For analysis, a sample was recrystallized from a mixture of methyl alcohol and ether, m.p. 137°.

Microbiological Assay.—Aqueous medium (100 ml.) contained sucrose (10 g.); glucose (1.0 g.); yeast extract, Difco (1.0 g.); peptone, Difco (0.3 g.); beef extract, Difco (0.2 g.); and inorganic salts (0.3 g.). The salt mixture was composed of K_2HPO_4 (150 mg.), $(NH_4)_2SO_4$ (150 mg.), sodium citrate (149 mg.), $CaCl_2$ (25 mg.), $MgSO_4 \cdot 7H_2O$ (25 mg.), $FeNH_4SO_4$ (0.5 mg.), and $ZnSO_4 \cdot 7H_2O$ (0.5 mg.). Solutions of the test compounds were made in water and the pH was adjusted to 7 with NH_4OH. The levels of compound were set so that 1 ml. of solution when diluted to 10 ml. would yield final concentrations of 0.1, 0.5, and 1.0%, respectively. A solution (1 ml.) of test compound, made sterile by filtration through a Seitz filter, was added aseptically to 9 ml. of medium, also made sterile by filtration through a Seitz filter. Inoculation with *L. mesenteroides* P-60 was effected by addition of 2 loopfuls of an 18-hr. culture of the organism in Eugon broth (BBL). After incubation for 24 hr. at 37°, the culture tubes were examined for turbidity in a Klett–Summerson colorimeter using a No. 66 filter. The data recorded indicated 100% inhibition as compared to growth.

Reduction of Steroidal Enamines with Potassium Borohydride

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de Winters, *et al.*, and others[1–4] have reported that the removal of the oxygen function at C-3 of Δ^4-3-keto steroids resulted in compounds with anabolic or progestational activity. Kincl and Dorfman[5] showed

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(2) N. E. Borglin, *Acta Endocrinol., Suppl.*, 58 (1960).

(3) O. Halpern, J. A. Edwards, and J. A. Zderic, *Chem. Ind.* (London), 1571 (1962).

(4) K. Irmscher, H. G. Kraft, and K. Bruckner, *J. Med. Chem.*, **7**, 345 (1964).