Synthesis and Cytotoxicity of 4-Aminobutyrophenone Hydrochlorides and Their Substituted Pyrimidinylhydrazones[1]

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The hydrochlorides of 4-aminobutyrophenone and its *para*-substituted derivatives were synthesized as amino ketone analogs of γ-aminobutyric acid for biological study. The hydrochlorides exist in the form of open-chain γ-amino ketones rather than the cyclized pyrroline derivatives. These compounds and some of their pyrimidinylhydrazones were tested for growth-inhibitory activity against bacteria, KB and L cell cultures, and the nonparasitic protozoan, *Tetrahymena pyriformis*. The hydrochloride of 4-aminobutyrophenone was also tested against several tumor systems in mice.

Based on biochemical rationale, α- and β-amino ketone analogs of amino acids were synthesized as potential metabolic inhibitors and have been found to be active against microorganisms *in vitro*.[3-5] Although the mechanism of action is not known, the structural similarity between these amino ketone analogs and the parent amino acids seems to provide a chemical basis for the postulated biochemical antagonism. Further, amino ketones are known to antagonize amino acids of related structures.[6] The extension of this concept to other series of amino ketones analogous to natural amino acids might further substantiate this hypothesis.

Among a few naturally occuring γ-amino acids, γ-aminobutyric acid (GABA) has been studied more than other members of this group. Although the presence of γ-aminobutyric acid in bacteria, plants, and mammals has been demonstrated,[7,8] little is known of its physiological significance on microorganisms. GABA is one of the metabolic products in the pathway of arginine metabolism in *Streptomyces griseus*. The deguanidase enzyme systems of this organism have been shown to convert γ-guanidobutyrate to γ-aminobutyrate.[9-11] An adaptive enzyme system in this organism oxidatively decarboxylates arginine to guanidobutyramide, which is hydrolyzed with the formation of guanidobutyrate and is then converted into GABA by deguanidases. GABA can be utilized by some species of bacteria such as *Clostridium aminobutyricum* as a source of carbon, nitrogen, and energy.[12] In mammals, GABA is present exclusively in the spinal cord and brain.[13] The possible role of this amino acid in the transmission of nerve impulses has been expressed by different workers.[14-16]

In view of the wide distribution of γ-aminobutyric acid among biological systems, it was of interest to investigate the biological activity of γ-amino ketones structurally analogous to GABA. This might shed some light on the metabolic significance of this amino acid in bacteria as well as in mammals.

In consideration of the fact that aryl homologs of α- and β-amino ketones possessed equal or greater bacteriostatic activity as compared with alkyl homologs it was decided to prepare 4-aminobutyrophenone and its phenyl-substituted homologs for biological study. A literature survey revealed that an attempted synthesis of 4-aminobutyrophenone hydrochloride resulted in the isolation of 2-phenyl-Δ^2-pyrroline hydrochloride.[17] This further prompted us to attempt the synthesis of 4-aminobutyrophenone and its phenyl substituted homologs for the purpose of exploring the nature of the chemical reaction. Concurrently with the synthetic work, the biological activities of these compounds were studied.

Gabriel and Colman[17] attempted the synthesis of 4-aminobutyrophenone hydrochloride by preparing 4-phthalimidobutyric acid, the acid chloride, and the 4-phthalimidobutyrophenone and subsequently removing the protective phthaloyl group from the ketone with a mixture of concentrated hydrochloric acid and glacial acetic acid in a sealed tube at 150–155° for 1 hr. From the elemental analysis for chlorine, they concluded that dehydration and cyclization had occurred in the course of hydrolysis, and thus they assigned the structure 2-phenyl-Δ^2-pyrroline hydrochloride to the product.

The present report will demonstrate that 4-aminobutyrophenone hydrochloride is obtainable. The reaction sequence of Gabriel and Colman was repeated, the only exception being the conditions of hydrolysis. The removal of the phthaloyl group from 4-phthalimidobutyrophenone was carried out in an open system at about 107° rather than in a sealed tube, because it was felt that the 2-phenyl-Δ^2-pyrroline hydrochloride of Gabriel and Colman must have been derived from the expected 4-aminobutyrophenone hydrochloride under the influence of hydrogen chloride at 150° in the sealed tube. It is reasonable to expect that these

(1) This investigation was supported by Public Health Service Grants CA-06364-03 and CA-06364-04 from the National Cancer Institute.
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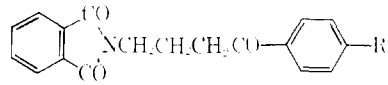
(3) S. S. Cheng and S. Jonsson, *J. Am. Pharm. Assoc., Sci. Ed.* **49**, 611 (1960).
(4) S. S. Cheng, S. Jonsson, and F. T. Semeniuk, *J. Pharm. Sci.*, **51**, 108 (1962).
(5) S. S. Cheng, S. Jonsson, and F. T. Semeniuk, *ibid.*, **51**, 976 (1962).
(6) H. P. v. Hahn and F. E. Lehman, *Helv. Physiol. Pharmacol. Acta*, **16**, 107 (1958).
(7) M. N. Camien, L. E. McClure, A. Lepp, and M. S. Dunn, *Arch. Biochem. Biophys.*, **43**, 378 (1953).
(8) V. A. Najjar, and J. Fisher, *J. Biol. Chem.*, **206**, 215 (1954).
(9) N. V. Thoai, J. L. Hatt, T. T. An, and J. Roche, *Biochim. Biophys. Acta*, **22**, 337 (1956).
(10) N. V. Thoai, J. L. Hatt, and T. T. An, *ibid.*, **22**, 116 (1956).
(11) I. Roche, N. V. Thoai, and J. L. Hatt, *Compt. Rend. Soc. Biol.*, **150**, 1686 (1956).
(12) J. K. Hardman and E. C. Stadtman, *J. Bacteriol.*, **85**, 1326 (1963).
(13) J. Awapara, A. J. Landua, R. Fuerst, and B. Seale, *J. Biol. Chem.*, **187**, 35 (1950).
(14) K. A. C. Elliott and H. H. Jasper, *Physiol. Rev.*, **39**, 383 (1959).
(15) B. Sisken, K. Sano, and E. Roberts, *J. Biol. Chem.*, **236**, 503 (1961).
(16) J. H. Gaddum, *Nature*, **197**, 741 (1963).
(17) S. Gabriel and J. Colman, *Ber.*, **41**, 513 (1908).

TABLE I

4-PHTHALIMIDOBUTYROPHENONES

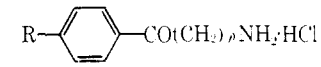


No.	R	Mp, °C	Reaction Temp, °C	Yield, %	Formula	Calcd, % C	H	N	X[a]	Found, % C	H	N	X[a]
1	H	130–131	75	65	$C_{18}H_{15}NO_3$	74.0	5.41	4.77	...	73.7	5.35	4.95	...
2	CH_3	115–116	90	67	$C_{19}H_{17}NO_3$	74.23	5.58	4.56	...	74.41	5.33	4.31	...
3	CH_3O	110–111	75	35	$C_{19}H_{17}NO_4$	70.56	5.30	4.33	...	71.0	5.50	4.50	...
4	Br	135–136	90	48	$C_{18}H_{14}BrNO_3$	58.06	3.79	3.76	21.48	58.64	3.81	3.74	22.0
5	Cl	120–121	90	69	$C_{18}H_{14}ClNO_3$	65.94	4.31	4.27	10.82	66.14	4.12	4.31	11.0
6	F	105–107	75	40	$C_{18}H_{14}FNO_3$	69.43	4.50	4.53	6.10	69.66	4.41	4.52	5.88

[a] X = halogen.

TABLE II

4-AMINOBUTYROPHENONE HYDROCHLORIDES AND 2-AMINOACETOPHENONE HYDROCHLORIDES



No.	n	R	Mp, °C[a]	Yield, %	Formula	Calcd, % C	H	N	Cl	Br	F	Found, % C	H	N	Cl	Br	F
7	3	H	215–216 dec	62	$C_{10}H_{14}ClNO$	60.13	7.07	7.02	17.77	...	...	60.43	7.27	7.22	18.10	...	...
8	3	CH_3	142–143	60	$C_{11}H_{16}ClNO$	61.82	7.55	6.56	16.60	...	...	62.20	8.00	6.40	17.07	...	...
9	3	CH_3O	195–197	59	$C_{11}H_{16}ClNO_2$	57.50	7.01	6.09	15.44	...	...	57.48	7.02	5.90	15.64	...	...
10	3	Br	246–250 dec	73	$C_{10}H_{13}BrClNO$	43.09	4.70	5.02	12.73	28.69	...	43.37	4.40	5.13	12.91	29.09	...
11	3	Cl	204–206	72	$C_{10}H_{13}Cl_2NO$	51.28	5.60	5.98	30.32	...	...	51.11	5.61	6.10	30.21	...	...
12	3	F	202–203	50	$C_{10}H_{13}ClFNO$	55.16	6.02	6.44	16.30	...	8.73	55.56	5.90	6.60	17.0	...	9.10
16	1	Br	285 dec	73	$C_8H_9BrClNO$	38.34	3.63	5.59	14.16	31.91	...	38.09	3.80	5.60	[b]	[b]	...
17	1	Cl	279 dec	68	$C_8H_9Cl_2NO$	46.6	4.40	6.80	34.42	...	...	47.1	4.42	6.80	35.0	...	...

[a] All 4-aminobutyrophenone hydrochlorides (n = 3) showed the phenomenon of dehydration before melting and gave an aromatic odor resembling that of pyrrolines. All 2-aminoacetophenone hydrochlorides (n = 1) decomposed gradually above 220°. [b] Analysis was not performed for this element.

conditions might favor the cyclization of the γ-amino ketone to form a pyrroline derivative. The product isolated from our experiment proved to be the expected 4-aminobutyrophenone hydrochloride rather than the pyrroline derivative. Verification was provided by elemental analyses and by spectral evidence. The same reaction condition was applied with equal success to the hydrolysis of five other 4-phthalimidobutyrophenones with a *para* substituent on the benzene nucleus. The elemental analyses and spectroscopic evidence proved that all the hydrolytic products are the expected 4-aminobutyrophenone hydrochlorides rather than the pyrroline derivatives.

The hydrochlorides of 4-aminobutyrophenone and its *para*-substituted homologs were tested for their cytotoxicities against protozoa, mammalian cells, and several species of bacteria. The pyrimidinylhydrazone derivatives of some 4-aminobutyrophenones were also prepared and their cytotoxicities were tested in the same screening systems in view of the biological significance of pyrimidines as important components of nucleic acids. As can be seen from the data presented below, the pyrimidinylhydrazones of 4-aminobutyrophenones in general possess cytotoxicities about twice as great as that of the parent 4-aminobutyrophenones.

Experimental Section[18]

4-Chloro-4-phthalimidobutyrophenone.—γ-Phthalimidobutyryl chloride (40 g, 0.158 mole) was dissolved in 120 ml of dry hot chlorobenzene. Anhydrous $AlCl_3$ (practical grade) (72 g, *ca.* 0.54 mole) was added in six portions during a 1-hr period while the solution was kept vigorously stirred and maintained at 90°. The reaction mixture was stirred for an additional 2 hr and then was poured into a mixture of 200 ml of 1 *N* HCl and 200 g of crushed ice. The aqueous layer was separated and extracted with four portions of 60 ml of benzene and the extracts were combined with the chlorobenzene solution. The organic solution was first washed with water then with 5% Na_2CO_3 solution and dried (Na_2SO_4). The solid obtained from vacuum distillation of the solution was recrystallized from 95% alcohol to give 36 g (69%) of white crystals, mp 120–121°. Other 4-phthalimidobutyrophenones prepared by a similar procedure are listed in Table I.

4′-Chloro-4-aminobutyrophenone Hydrochloride.—4′-Chloro-4-phthalimidobutyrophenone (32.96 g, 0.1006 mole) was dissolved in 350 ml of hot glacial acetic acid. Concentrated HCl (100 ml) was gradually added to the well-stirred solution. The mixture was then heated to reflux for 14 hr while a constant stream of HCl gas was passed through it. The temperature of the boiling mixture was about 107°. After heating for 24 hr, the mixture was cooled in an ice bath and filtered to remove phthalic acid. Phthalic acid (6.5 g) was obtained from the crude product after purification through its sodium salt. A second batch of 2.5 g of phthalic acid was obtained after the filtrate was concentrated; total yield 9 g.

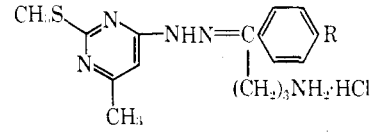
The filtrate from the removal of phthalic acid was concentrated to dryness and the syrupy liquid was dissolved in 30 ml of absolute alcohol. The crystals which separated out on standing were collected by filtration to give 12.8 g of the hydrochloride, mp 204–206°. A second batch (5.7 g) was obtained by the addition of anhydrous ether to the mother liquid. The two crops gave a total of 18.5 g (78%) of the hydrochloride. Other 4-aminobutyrophenone hydrochlorides prepared from the hydrolyses of the corresponding 4-phthalimidobutyrophenones by a similar procedure are listed in Table II along with 2-aminoacetophenone hydrochlorides which are included as model compounds for comparing their biological activities.

Attempted Preparation of Picrates.—4′-Chloro-4-aminobutyrophenone hydrochloride (0.1040 g, 0.00044 mole) was dissolved in 2 ml of boiling 95% alcohol. To this solution 4 ml of a 4% solution of picric acid in 95% alcohol was added dropwise and a yellow precipitate formed immediately. The reaction mixture was allowed to stand at room temperature for 10 min and then

(18) Microanalyses were performed by Alfred Bernhardt Microanalytical Laboratories, Ruhr, Germany. All melting points were taken in a Fisher-Johns melting point apparatus and are corrected.

Table III

The Pyrimidinylhydrazones of 4′-Halogeno-4-aminobutyrophenone Hydrochlorides



No.	R	Mp, °C	Yield, %	Formula	Calcd, % C	H	Br	Cl	F	N	S	Found, % C	H	Br	Cl	F	N	S
13	Br	221–223 dec	76	$C_{16}H_{21}BrClN_5S$	44.60	4.92	18.56	8.23	...	16.26	7.43	44.51	5.11	18.51	8.21	...	16.16	*a*
14	Cl	218–222 dec	65	$C_{16}H_{21}Cl_2N_5S$	49.74	5.48	...	18.37	...	18.13	8.29	49.55	5.47	...	18.43	...	18.16	8.47
15	F	206–210 dec	73	$C_{16}H_{21}ClFN_5S$	51.96	5.73	...	9.60	5.14	18.94	8.66	52.01	5.74	...	9.80	5.12	18.69	8.58

[a] Analysis was not performed for this element.

was filtered. The precipitate was washed with 95% alcohol and recrystallized from 8 ml of boiling 95% alcohol, yielding 0.1438 g (76%) of shining yellow crystals, mp 215–218° dec. The elemental analyses of the product agree with the structure for the corresponding 2-(4-chlorophenyl)-Δ^1-pyrroline picrate.

6-Methyl-2-methylthio-4-pyrimidinylhydrazone of 4′-Chloro-4-aminobutyrophenone.—4′-Chloro-4-aminobutyrophenone hydrochloride (1.760 g, 0.0075 mole) was dissolved in 10 ml of water. To this solution was added a solution of 1.275 g (0.0075 mole) of 6-methyl-2-methyl-thio-4-pyrimidinylhydrazine[19] in 10 ml of hot water. A white precipitate formed immediately. The reaction mixture was heated to 80° for 5 min, allowed to cool to room temperature, and filtered. The white powder obtained was recrystallized from 12 ml of boiling water; the yield was 1.5 g (52%). The compound decomposed between 218–222°. The elemental analysis and the following chemical reactions indicated that the hydrazone existed in the form of an amine hydrochloride. The aqueous solution of this product gave a white precipitate of AgCl upon addition of $AgNO_3$, and a yellow precipitate upon addition of an aqueous solution of picrolonic acid. The alcoholic solution of this product yielded a yellow precipitate upon the addition of picric acid. The hydrazones are listed in Table III.

4′-Chloro-4-(dichloroacetamido)butyrophenone.—To a solution of 0.79 g (0.0033 mole) of 4′-chloro-4-aminobutyrophenone hydrochloride in 7 ml of water was gradually added 4 ml of 16% NaOH and 1.60 g (0.0109 mole) of dichloroacetyl chloride while the mixture was kept vigorously stirred and cooled in an ice bath. The pH of this mixture was then adjusted to 10 by the dropwise addition of 16% NaOH solution. An oil formed in the course of the reaction, which solidified gradually into a white powder. The solid was collected by filtration, washed with water, and then treated with 4 ml of 0.3 *N* HCl and again washed with 3 ml of water. The crude product was recrystallized from absolute alcohol by adding water dropwise to the solution. The scale-like crystals (0.35 g, 36.5%) melted at 102–104°.

Anal. Calcd for $C_{12}H_{12}Cl_3NO_2$: C, 46.68; H, 3.92; N, 4.54; Cl, 34.48. Found: C, 46.69; H, 4.13; N, 4.37; Cl, 34.23.

The 2,4-dinitrophenylhydrazone melted at 222–224°.

Anal. Calcd for $C_{18}H_{16}Cl_3N_5O_5$: C, 44.23; H, 3.29; Cl, 21.77; N, 14.33. Found: C, 44.09; H, 3.23; Cl, 21.72; N, 14.26.

Structure Elucidation.—The elemental analyses of all the hydrochlorides are consistent with data expected for 4-aminobutyrophenones, but do not agree with those for pyrroline derivatives. Since pyrroline derivatives possess less hydrogen and oxygen (equivalent to 1 mole of H_2O), the calculated percentage elemental contents of carbon, nitrogen, and chlorine should be significantly greater than those for the corresponding 4-aminobutyrophenones. Thus the percentage contents calculated for phenylpyrroline hydrochloride, $C_{10}H_{12}ClN$, are: C, 66.10; H, 6.66; N, 7.77; Cl, 19.53; for 4-aminobutyrophenone hydrochloride, $C_{10}H_{14}ClNO$: C, 60.13; H, 7.07; N, 7.02; Cl, 17.77; O, 8.01. The percentages found by analysis are C, 60.43; H, 7.27; N, 7.52; Cl, 18.40, thus excluding the structure for phenylpyrroline hydrochloride.

The structures of the 4-aminobutyrophenone hydrochlorides are further proved by the formation of derivatives of the carbonyl and amino functions. The preparation of 4′-chloro-4-(dichloroacetamido)butyrophenone and its 2,4-dinitrophenylhydrazone provides evidence for the presence of amino and carbonyl groups in the product.

In addition, the infrared absorption data indicate the presence of a conjugated carbonyl function (1640–1680 cm^{-1}, 6.10–5.95 μ strong absorption) and an ammonium structure (2200–3000 cm^{-1}, 4.54–3.33 μ broad band) in all the hydrochlorides examined in KBr pellets or Nujol mulls. The unusually low frequency of infrared absorption for these phenyl-conjugated carbonyls compared with the usual range of 1665–1700 cm^{-1} (6.00–5.88 μ) should not be mistaken as the absorption frequency of $-N{=}C<$ (1610–1660 cm^{-1}, 6.21–6.01 μ). In fact, the infrared absorption maximum for $-N{=}C<$ of 2-phenyl-Δ^1-pyrroline occurs at 1620 cm^{-1} (6.17 μ) according to a French patent.[20] This latter evidence further indicates that the product we obtained from the hydrolysis of 4-phthalimidobutyrophenone is not a hydrochloride of 2-phenyl-Δ^1-pyrroline but 4-aminobutyrophenone, since the product has an absorption maximum at the frequency of 1680 cm^{-1} (5.95 μ). Further evidence that the hydrolytic product from 4′-methoxy-4-phthalimidobutyrophenone is not a hydrochloride of the corresponding pyrroline derivative comes from the fact that 2-(4-methoxyphenyl)-Δ^1-pyrroline displays an absorption maximum at 6.20 μ[21] (1613 cm^{-1}). Thus this compound should be 4′-methoxyl-4-aminobutyrophenone hydrochloride, since its absorption maximum occurs at 6.10 μ (1640 cm^{-1}).

Growth Inhibition Tests against Bacteria.—The antibacterial activity of all the hydrochlorides was tested *in vitro* by the broth dilution method as reported previously.[5] The organisms used in the tests were obtained from American Type Culture Collection: *Escherichia coli* 11775, *Klebsiella pneumoniae* 13883, *Proteus vulgaris* 13315, *Pseudomonas aeruginosa* 10145, and *Staphylococcus aureus* 6538P. The growth was measured in terms of turbidity and absorbance at 490 mμ, with a Spectronic 20 colorimeter after 36 hr of incubation at 37°. The logarithms of the per cent inhibition were plotted *vs.* the drug concentrations. The 50% inhibition dose was then read from the logarithmic plots. The minimum total inhibitory concentration (MIC) observed and the ID_{50} are recorded in Table IV.

Growth Inhibition Tests against *Tetrahymena pyriformis*.—The significance of using the nonparasitic protozoa, *Tetrahymena pyriformis*, as an *in vitro* screening system for potential antibacterial agents has been rationalized.[22] It has also been demonstrated that this system is able to detect the greatest number (73/89) of tumor-active agents among twelve microorganisms used as screening tools.[23]

In our test, the ciliate *T. pyriformis* ATCC 10542 was used. The stock culture was maintained at room temperature in broth containing 2% proteose–peptone (Difco) and 0.2% liver fraction 2 (Wilson Laboratories), and was transferred to fresh broth every 3 days. For each compound, the test was carried out in a duplicate series of 50-ml erlenmeyer flasks containing 6 ml of broth and the proper amount of drug to give final drug concentrations of 1000, 750, 500, 250, 125, 50, 20, and 0 μg/ml. Each flask was inoculated with 0.3 ml of a 3-day broth culture and incubated at room temperature for 72 hr. The ID_{50} and MIC were determined in the same manner as described for antibacterial screening. The data are presented in Table IV.

Cytotoxicity Test against the L Strain of Mouse Fibroblasts and the KB Strain of Human Epidermoid Carcinoma Cells.—

(19) K. Takagi and T. Ueda, *Chem. Pharm. Bull.* (Tokyo), **11**, 1382 (1963).

(20) French Patent 1,330,761 (1963); *Chem. Abstr.*, **60**, 2906*a* (1964).

(21) A. I. Meyers, *J. Org. Chem.*, **24**, 1233 (1959).

(22) G. W. Kidder and V. C. Dewey, *J. Biol. Chem.*, **178**, 383 (1949).

(23) G. E. Foley, R. E. McCarthy, V. M. Binns, E. E. Snell, B. M. Gurard, G. W. Kidder, V. C. Dewey, and P. S. Thayer, *Ann. N. Y. Acad. Sci.*, **76**, 413 (1958).

TABLE IV

THE *in Vitro* ANTIMICROBIAL ACTIVITY

Compd	*E. coli*	*K. pneumoniae*	*P. vulgaris*	*P. aeruginosa*	*S. aureus*	*T. pyriformis*
	MIC × 10^{-4} mole/l.[a]					
7	55.2	..	55.2	37.6	..	55.0
8	46.8	46.8	46.8	35.0	46.8	23.4
9	..	..	43.6	32.7	..	43.6
10	17.9	32	10.8	26.9	17.9	3.59
11	21.3	32	21.1	32.1	10.7	5.34
12	46.0	46	34.5	33	.	23
13	11.6	17.3	5.81	11.6	5.81	2.32
14	12.9	12.9	6.48	25.9	12.9	3.24
15	27.0	27.1	20.6	20.3	27.1	6.77
16	20.9	4.99	4.99	1.96	3.19	4.99
17	24.3	6.07	6.07	24.3	4.86	6.07
	ID_{50}, μg/ml[b]					
7	27.6	>55.2	20.3	15.0	>55.2	9.27
8	14	37.5	12.9	11.9	32.8	2.58
9	>43.6	>43.6	10	15.3	>43.6	3.70
10	4.13	3.77	2.15	14.4	6.45	1.44
11	4.49	5.56	6.63	18.2	8.5	1.28
12	22.5	21.8	11.3	13.1	>46	6.2
13	2.34	3.02	1.05	9.06	4.18	0.81
14	5.18	3.89	3.24	14.3	11.9	1.94
15	8.12	5.68	16.5	6.5	14.9	3.65
16	23.8	3.59	1.59	12.4	1.99	3.19
17	16.0	2.43	2.19	14.1	2.19	3.89

[a] MIC = the minimum total inhibitory concentration. [b] ID_{50} = the 50% inhibitory concentration.

Since Eagle and Foley[24] pointed out the possibility of using tissue culture cytotoxicity tests for the detection of carcinolytic agents, a considerable amount of work[25-27] has been conducted to improve the reliability and simplicity of the method. The cell culture method is now used as a primary screen in the Cancer Chemotherapy National Service Center (CCNSC) cell culture program for selecting possible antitumor agents.[28]

The cytotoxicity test procedure of our laboratory is similar to that used by CCNSC screening laboratories. CCNSC used the procedure of Smith, *et al.*,[27] which was a simplification of Eagle and Foley's original method[24] for the *in vitro* screening. A modification of these procedures was adopted for our experiments described herein. The main reason for the modification was based on the fact that most of the compounds that we tested at moderate concentrations caused an early detachment of the monolayer after 5–7 hr of incubation. Thus, in processing the culture for protein analysis, the growth medium containing the detached cells, the washings, and the Lowry's solution C used for dissolving the cells were all passed through a sintered-glass funnel in order to avoid losing the protein.

The cell lines used for the cytotoxicity test were the L strain of mouse fibroblasts and the KB strain of human epidermoid carcinoma cells obtained from Microbiological Associates, Bethesda, Md. Since the monolayer cultures of HeLa, KB, human leukemia (J-111), human embryonic intestine (Henle), human liver (Chang), human conjunctiva (Chang), human thymus (Foley), human amnion (Foley), monkey kidney (Eagle & Foley), and mouse sarcoma (S180) tested by Eagle and Foley[24,26] have been found to respond similarly to drugs, the KB strain of human carcinoma was selected as the only cell line for routine screening of cytotoxic compound in the CCNSC program because of its rapid and reproducible growth rate as a monolayer culture.[28] In our experiments, the L strain of mouse fibroblast was also included because it has the same advantages possessed by the KB cell.

The L-cell monolayers were cultured in medium 199 containing 10% horse serum, whereas the KB cultures were grown in Eagle medium containing 10% calf serum. The test cultures were grown as monolayers on one glass wall (approximately 14 cm^2 surface) of 28-g screwcap prescription bottles in 6 ml of medium. Twelve hours after the plantation, monolayers which had attached well to the glass were selected. Several cultures were sampled for the determination of the initial protein content. At the same time, 0.1 ml of drug solution containing different amounts of the test compound was added to the growth media of the test cultures, whereas only growth medium was used for the control cultures. The test and control cultures were incubated at 37° for 72 hr and then chilled at 5° until the time for processing. Samples from the control group were analyzed for final protein content.

For protein analysis of the cultures, the scheme of Oyama and Eagle[29] was used. This is based on the procedure of Lowry, *et al.*,[30] using Folin–Ciocalteau phenol reagent[31] for the colorimetric determination of protein in terms of tyrosine, by the reaction between tyrosine and phosphotungstic–phosphomolybic compounds.[32]

The cytotoxicity of different concentrations of drugs on the test cultures was determined in terms of the inhibition of protein synthesis of the cells and was expressed in per cent inhibition of protein synthesis, $\{1 - [(T - C_0)/(C - C_0)]\}100$. In this formula, T is the protein content of the test culture, C_0 is the initial protein content for each culture, and C is the final protein content of the control. To find the 50% inhibition dose (ID_{50}) of the drug being tested, the percentages of inhibition were plotted against drug concentrations, and the ID_{50} was read from the plot. The data on cytotoxicity tests of the compounds are presented in Table V.

TABLE V

THE *in Vitro* INHIBITORY EFFECT ON THE GROWTH OF MAMMALIAN CELL CULTURES

Cell culture	ID_{50}, 10^{-5} *M* (μg/ml)				
	9[a]	10[a]	11[a]	16	17
KB[b]	17.4 (40)	10.8 (30)	3.42 (8)	17.9 (45)	3.89 (8)
L[c]	95.8 (220)	51.7 (144)	63.3 (148)	13.2 (33)	2.91 (6)

[a] Eagle medium, calf serum 10%. [b] Medium 199, horse serum 10%. [c] In the alkaline culture medium the compound exists in the form of 2-substituted Δ^1-pyrroline.

***In Vivo* Antitumor Screening of 4-Aminobutyrophenone Hydrochloride.**—4-Aminobutyrophenone hydrochloride was selected for *in vivo* screening against four transplantable mouse tumors, namely, Adenocarcinoma 755 (Ca755), lymphoid leukemia L1210, Ehrlich asicites carcinoma, and Sarcoma 180 (S180). The tests were performed by CCNSC screening laboratories. The screening against Ehrlich ascites carcinoma was carried out in our laboratory as described previously.[33,34] The data are recorded in Table VI. The 6-methyl-2-methylthio-4-pyrimidinylhydrazone of 4'-bromo-4-aminobutyrophenone hydrochloride was also tested against Ehrlich ascites tumor in mice. A daily dose of

TABLE VI

THE CARCINOSTATIC ACTIVITY OF 4-AMINOBUTYROPHENONE HYDROCHLORIDE[a] ON TRANSPLANTED MOUSE TUMORS[b]

Tumor	Dose, mg/kg/day	Survivors	Tumor growth, T/C, mg or ml	% inhib
Ca775	90	10/10	486/988	50
	110	10/10	685/1457	53
Ehrlich ascites	30	7/8	2.3/2.78	17
	120	3/8	2.07/2.65	22
L1210, ascites form	90	6/6	8.8/9.0	3
	110	6/6	8.3/8.5	33
S180	125	4/6	528/906	42
	125	6/6	522/799	35

[a] In physiological pH the compound exists in the form of 2-phenyl-Δ^1-pyrroline. [b] Screenings were performed by CCNSC laboratories, with the exception of the test *vs.* Ehrlich ascites which was carried out in our laboratory.

(24) H. Eagle and G. E. Foley, *Am. J. Med.*, **21**, 739 (1956).
(25) G. E. Foley and H. Eagle, *Cancer Res.*, **18**, 1012 (1958).
(26) H. Eagle and G. E. Foley, *ibid.*, **18**, 1017 (1958).
(27) C. G. Smith, W. L. Lummis, and J. E. Grady, *ibid.*, **19**, 843, 847 (1959).
(28) J. Leiter, B. J. Abbott, and S. A. Schepartz, *ibid.*, **25** (Suppl. I), 1 (1965).
(29) V. I. Oyama and H. Eagle, *Proc. Soc. Exptl. Biol. Med.*, **91**, 305 (1956).
(30) O. H. Lowry, N. J. Rosebrough, A. L. Fair, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
(31) O. Folin and V. Ciocalteau, *ibid.*, **73**, 627 (1927).
(32) O. Folin and W. Denis, *ibid.*, **12**, 245 (1912).
(33) J. E. Wilson, J. L. Irvin, J. E. Suggs, and K. Liu, *Cancer Res.*, **19**, 272 (1959).
(34) J. E. Wilson, J. E. Suggs, and J. L. Irvin, *ibid.*, **21** (Suppl. 10), 632 (1961).

30 mg/kg of body weight caused only 3% inhibition, but the tumor growth could be inhibited to 85% by increasing the dose to 120 mg/kg.

Discussion

The Structure of Picrates.—The elemental analyses of the picrates prepared from 4′-aminobutyrophenone hydrochlorides indicate that the products have been dehydrated. Perhaps dehydration occurred during the process of converting the hydrochlorides into the picrates. Since all the picrates were subjected to prolonged heating in absolute alcohol during recrystallization, it seemed reasonable to suspect that the heating caused dehydration. In order to test this premise, the attempted picrate of 4′-chloro-4-aminobutyrophenone was also prepared by adding an aqueous solution of picric acid to the aqueous solution of 4′-chloro-4-aminobutyrophenone hydrochloride. The precipitate was thoroughly washed with water and dried at 40°. The infrared spectrum and the melting point of this product proved that the compound was identical with a sample of 2-(4-chlorophenyl)-Δ^1-pyrroline picrate. This experiment indicated that dehydration and cyclization took place at room temperature and was not due to the influence of heat during recrystallization. The detailed structures of these pyrroline picrates will be discussed in a subsequent paper.

Structure–Activity Relationships.—The *in vivo* antitumor screening of 4-aminobutyrophenone hydrochloride indicated that this compound possessed a weak carcinostatic activity, but the effect was not significant from a practical view point. The *in vitro* cytotoxicity tests against protozoa, bacteria, and mammalian cells disclosed three interesting generalities for structure–activity relationships. In general the growth-inhibitory activity of the pyrimidinylhydrazones of 4-aminobutyrophenone hydrochlorides are twice as great as that of the parent 4-aminobutyrophenone hydrochlorides. The second generality points out that the growth-inhibitory activities are greater for the hydrochlorides with an electronegative halogen atom at the *para* position of the benzene nucleus than for those carrying an electropositive moiety such as hydrogen, methyl, or methoxyl at the same position of the phenyl ring. The third generality indicates that the growth-inhibitory activities of the 4′-halogeno-4-aminobutyrophenone hydrochlorides and their pyrimidinylhydrazones vary with the sizes of the halogen atoms. Thus the activity of the bromo derivative is greater than that of the chloro derivative, which, in turn, is greater than that of the fluoro derivative (Tables IV and VII). Consequently, it seems that both inductive and mesomeric effects play a role in determining the magnitudes of the growth-inhibitory activities of the compounds being tested.

TABLE VII
RELATIVE GROWTH-INHIBITORY ACTIVITIES OF 4-AMINOBUTYROPHENONE HYDROCHLORIDES AND 2-AMINOACETOPHENONE HYDROCHLORIDES

Organism	Order of activity[a]
E. coli	13 > 10 > 11 > 14 > 15 > 8 > 17 > 12 > 16 > 7 > 9
K. pneumoniae	16 > 13 > 17 > 10 > 14 > 11 > 15 > 12 > 8 > 9 > 7
P. vulgaris	13 > 16 > 17 > 10 > 14 > 11 > 9 > 12 > 8 > 15 > 7
P. aeruginosa	15 > 13 > 8 > 16 > 17 > 12 > 14 > 10 > 7 > 9 > 11
S. aureus	16 > 17 > 13 > 10 > 11 > 14 > 15 > 8 > 9 > 12 > 7
T. pyriformis	13 > 10 > 11 > 14 > 8 > 16 > 15 > 9 > 17 > 12 > 7
KB cell culture	17 > 10 > 16 > 9 > 11
L cell culture	17 > 16 > 10 > 11 > 9

[a] The numbers correspond to the compounds listed in Tables II–III.

Among five species of bacteria and one species of protozoa tested, the *P. aeruginosa* test system seemed to show more irregularities in adhering to the above generalities than other test systems.

Some Herbicidal Silicon Compounds

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A large number of organofunctional silicon compounds were examined for biological activity. A small, closely related group of (haloalkyl)silicon compounds was found to have strong herbicidal activity in both preemergence and postemergence screening tests, and to act in some cases as defoliants. The active compounds are leachable in moist soil but seem to become fixed upon drying. These compounds seem to be the first organosilicon compounds that have been found to have herbicidal activity.

A program of screening silicon compounds for biological activity revealed a small group of closely related structures that have strong herbicidal activity. Although there exists extensive literature concerned with the herbicidal properties of thousands of chemical compounds, no reference could be found for such activity in an organosilicon compound. Therefore, these results were especially interesting as the first of their kind.

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Active compounds had $RCHXSiMeY_2$ for their structure with R = H or CH_3; X = Cl, Br, or I; and Y being Cl, F, or an alkoxy group. Variations of this structure such as RCX_2SiMeY_2 or RCHXSiMeY or $RCHXSiY_3$ showed no activity. (Trichloromethyl)-methyldimethoxysilane [$Cl_3CMeSi(OMe)_2$] was active and an exception to the above generalizations.

Hydrolysis of Y and the formation of siloxane polymers would be expected during the testing of all of the active compounds. Therefore the corresponding polymer $[ClCH_2MeSiO]_x$ was prepared. It had very little