

Analog of Spermine and Spermidine. I. Synthesis of Polymethylenepolyamines by Reduction of Cyanoethylated α,ω -Alkylenediamines^{1,2}

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A series of linear aliphatic triamines and tetramines has been prepared as homologs of the naturally occurring polyamines, spermine and spermidine, by mono- and symmetrical dicyanoethylation of the appropriate α,ω -alkylenediamines, followed by catalytic reduction of the nitriles. Cyanoethylated derivatives were reduced under unusually mild conditions by means of a commercially available sponge nickel catalyst. Hydrochloride salts of all compounds synthesized have been prepared for biological evaluation. Three triamine trihydrochlorides and one tetramine tetrahydrochloride have shown significant *in vivo* antitumor activity against transplantable mouse tumors. Against KB (human epidermoid carcinoma) cells in a culture system containing calf serum, the synthetic polyamines demonstrate, in general, the same high degree of inhibitory activity shown by spermine and spermidine ($ID_{50} = 1.0\text{--}3.0 \times 10^{-5}$ mmole/ml.).

The naturally occurring polyamines, spermine [N,N'-bis(3-aminopropyl)butane-1,4-diamine (IV, $x = 4$)] and spermidine [N-3-aminopropylbutane-1,4-diamine (III, $x = 4$)], have been known for many years, spermine having been first observed and described by van Leeuwenhoek in 1678 as the crystalline phosphate.³ These bases are found widely distributed in many types of biological material, but the specific role they play is still unknown. Only recently has information become available on their biosynthesis and involvement in various biological systems.⁴

Our interest in the polyamines began with the observation by Dr. R. A. Alarcon in these laboratories of the potent inhibitory activity of spermine and spermidine against neoplastic cells *in vitro* in the presence of calf serum.⁵ These and earlier studies have led to the finding that the inhibitory effect of spermine in bacterial and mammalian cell culture systems is dependent upon the presence of the enzyme spermine oxidase.^{5,6} Previously, it had been shown that spermine exhibits an inhibitory effect on spontaneous mouse tumors *in vivo*⁷ and on the Yoshida sarcoma *in vitro*.⁸ Tokuoka claimed that spermine is present in the serum of cancer patients⁹ and, subsequently, Kosaki, *et al.*, reported that spermine was found to be a component of the phospholipid "malignolipin," allegedly present in human neoplasms.¹⁰

We have undertaken a program of synthesis with the intent of preparing new substances related to spermine and spermidine while retaining or improving the growth-inhibitory properties demonstrated by these bases. Various analogs of these polyamines are being prepared

both to evaluate their biological activity in selected *in vitro* and *in vivo* assay systems and to determine the effect of alteration of chemical structure on the spermine oxidase system. These compounds may be of further interest as complexing agents for deoxyribonucleic acids and as stabilizers for ribosomes, possibilities suggested by recent reports that spermine and spermidine form strong complexes with nucleic acids^{4,11,12} and that ribosomes from *E. coli* are stabilized by spermine¹³ and spermidine.¹⁴ This paper is concerned with the synthesis of spermine and spermidine and homologs of these compounds in which the tetramethylene portion of the molecule shows variation from 2 through 12 methylene units. The products have been prepared by mono- and dicyanoethylation of the appropriate α,ω -alkylenediamines, followed by catalytic reduction of the nitriles under unusually mild conditions.

The monocyanoethylated derivatives (I, Table I) were prepared by warming the appropriate diamine with 1 equiv. of freshly distilled acrylonitrile. For the lower molecular weight diamines, the reaction was run by adding acrylonitrile dropwise into the liquid diamine while the reaction temperature was maintained at 5–10°. After all the acrylonitrile had been added, the reaction was permitted to warm to room temperature and was then heated at 70–80° for 3 hr. In the case of the longer chain diamines, nonane-, decane-, and dodecanediamine, which are solids at room temperature, the general reaction conditions had to be modified. These reactions could be carried out by the gradual addition of acrylonitrile to the liquid diamine, warmed to just above its melting point, or to a solution of the diamine in chloroform. The use of melted diamine was found to be the better of the two methods. In companion runs, N-2-cyanoethyl dodecane-1,12-diamine was obtained in 79% yield from melted diamine, but in only 36% yield when the reaction was conducted in chloroform solution.

The general procedure involving dropwise addition of acrylonitrile into the diamine, followed by gradual

(1) This investigation was supported in part by research grants (CY3335 and C6516) from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

(2) M. Israel, J. S. Rosenfeld, and E. J. Modest, Abstracts of Papers, 144th National Meeting, American Chemical Society, Los Angeles, Calif., April, 1963, p. 43-L.

(3) A. v. Leeuwenhoek, *Phil. Trans. Roy. Soc. London*, **12**, 1040 (1678).

(4) For an excellent biochemical review see H. Tabor, C. W. Tabor, and S. M. Rosenthal, *Ann. Rev. Biochem.*, **30**, 579 (1961).

(5) R. A. Alarcon, G. E. Foley, and E. J. Modest, *Arch. Biochem. Biophys.*, **94**, 540 (1961).

(6) J. G. Hirsch, *J. Exptl. Med.*, **97**, 327 (1953).

(7) E. Boyland, *Biochem. J.*, **35**, 1283 (1941).

(8) K. Miyaki, M. Hayashi, T. Chiba, and K. Nasu, *Chem. Pharm. Bull. (Tokyo)*, **8**, 933 (1960).

(9) T. Tokuoka, *Acta Schol. Med. Univ. Kyoto*, **27**, 241 (1950).

(10) T. Kosaki, T. Ikoda, Y. Kotani, S. Nakagawa, and T. Saka, *Science*, **127**, 1176 (1958), and subsequent publications.

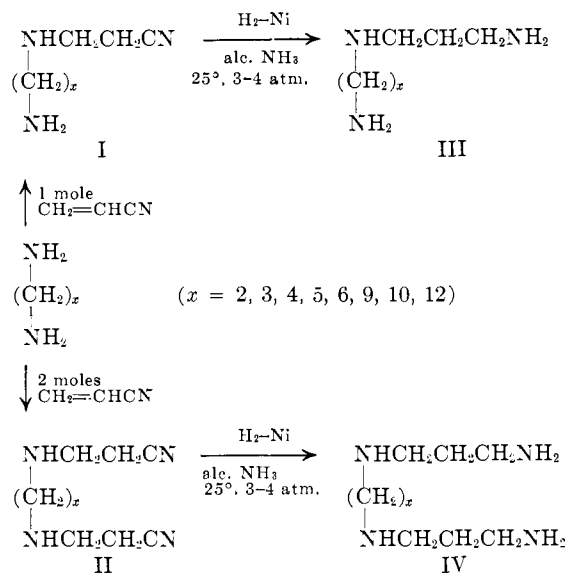
(11) S. Razin and R. Rozansky, *Arch. Biochem. Biophys.*, **81**, 36 (1959).

(12) D. Kaiser, H. Tabor, and C. W. Tabor, *J. Mol. Biol.*, **6**, 141 (1963).

(13) J. L. Collman, B. H. Witherspoon, and E. J. Herbst, *Biochem. Biophys. Acta*, **49**, 422 (1961).

(14) S. S. Cohen and J. Lichtenstein, *J. Biol. Chem.*, **235**, 2112 (1960).

warming to complete the reaction, was intended to decrease the possibility of dicyanoethylation. However, with the lower molecular weight diamines, dicyanoethylation could not be avoided entirely, the yield of the dicyanoethylated product decreasing with increasing methylene chain length. The cyanoethylated products were purified, wherever possible, by vacuum distillation of the reaction mixture in a nitrogen atmosphere. Under high vacuum, a temperature of 160–170° was found to be critical for distillation of cyanoethylated derivatives. Above this temperature, distillation was accompanied by the elimination of acrylonitrile and sometimes by extensive decomposition.



Treatment of the diamine with 2 equiv. of acrylonitrile following the same reaction conditions as before afforded the symmetrically dicyanoethylated compounds II listed in Table II. In one case, where $x = 9$, the dicyanoethylated compound was also prepared by the addition of 1 equiv. of acrylonitrile to purified monocanoethylated compound. No advantage in quality or quantity of product could be observed by this alternate route. All the dicyanoethylated compounds undergo extensive decomposition on distillation. However, where $x = 2$ through 6, it is still possible to isolate and purify the products by vacuum distillation in a stream of nitrogen despite the attendant decrease in yield. Where $x = 9, 10$, or 12, the dicyanoethylated compounds, which are solids at room temperature, cannot be distilled. These products readily eliminate 1 equiv. of acrylonitrile on being heated, and only the monocanoethylated product can be found in the distillate. Thus, N,N' -bis(2-cyanoethyl)nonane-1,9-diamine and N,N' -bis(2-cyanoethyl)dodecane-1,12-diamine (II, $x = 9$ and 12, respectively) were characterized only as their dihydrochloride salts. An analytical sample of N,N' -bis(2-cyanoethyl)decane-1,10-diamine (II, $x = 10$) was obtained by recrystallization of the crude reaction product from a benzene-petroleum ether (b.p. 60–90°) mixture.

Dihydrochloride salts of the mono- and dicyanoethylated diamines were prepared by introduction of anhydrous HCl into an alcoholic solution of the free base, followed by recrystallization of the precipitate from absolute ethanol or water-alcohol mixtures.

Of the sixteen triamines (III) and tetramines (IV) which we have prepared, only five have been previously described in the chemical literature. Spermine has been prepared from N,N' -bis(2-cyanoethyl)butane-1,4-diamine,^{15,16} and C^{14} -labeled spermine and spermidine were obtained by Jackson and Rosenthal¹⁷ from a crude putrescine-acrylonitrile reaction mixture; in each instance Raney nickel was employed at elevated temperatures and high pressures for the reduction. Poppelsdorf and Myerly¹⁸ obtained N -2-aminoethylpropane-1,3-diamine (IV, $x = 2$) as a by-product from the cyclization of N -2-cyanoethylethylenediamine to homopiperazine by means of Girdler G-49A nickel catalyst at high temperature and pressure. Reduction of 6-(N -2-cyanoethyl)aminohexanenitrile to N -3-aminopropylhexane-1,6-diamine (III, $x = 6$) by means of Raney cobalt has been described.¹⁹ Terent'ev, Kost, and Churshina have reported the preparation of bis(3-aminopropyl)amine by sodium-potassium alloy reduction of 3,3'-iminodipropionitrile in 22% yield.²⁰ However, these authors claim that reduction of N -2-cyanoethylpropane-1,3-diamine (I, $x = 3$), for which they report a boiling point 100° greater than for our own sample, afforded only 1,5-diazacyclooctane instead of the expected straight-chain triamine.²¹

The synthesis of spermine, spermidine, and other polyamines prepared during this investigation has been accomplished by catalytic hydrogenation of the corresponding cyanoethylated compound. The sodium-potassium alloy method of Terent'ev, Kost, and Churshina was found to give only dark, viscous, non-distillable polymers. For the catalytic method utilized here, purified cyanoethylated compound was dissolved in ethanol saturated with ammonia, and the resulting solution was shaken on a Parr low-pressure hydrogenator in the presence of sponge nickel catalyst²² at room temperature at an initial hydrogen pressure of 2.81–3.87 kg./cm.² (40–55 p.s.i.). As previously noted for dethiation reactions,²² this catalyst required an apparent bulk density²³ of 0.8–1.0 in order to be effective as a hydrogenation catalyst under these conditions. The presence of ammonia was intended to suppress the formation of secondary amines during reduction of the nitriles,²⁴ and no secondary amine by-product resulting from reduction of the nitrile was ever detected.

This use of sponge nickel catalyst is apparently the first instance in which a commercially available nickel catalyst has been used for the reduction of nitriles under such moderate conditions. Most previous

(15) H. P. Schultz, *J. Am. Chem. Soc.*, **70**, 2666 (1948).

(16) Sterling Drug Co., British Patent 903,200 (1962).

(17) E. L. Jackson and S. M. Rosenthal, *J. Org. Chem.*, **25**, 1055 (1960).

(18) F. Poppelsdorf and R. C. Myerly, *ibid.*, **26**, 131 (1961).

(19) Badische Anilin- & Soda-Fabrik, German Patent 896,650 (1953); *Chem. Abstr.*, **52**, 12894d (1958).

(20) A. P. Terent'ev, K. I. Churshina, and A. N. Kost, *Zh. Obshch. Khim.*, **20**, 1073 (1950).

(21) A. P. Terent'ev, A. N. Kost, and K. I. Churshina, *ibid.*, **21**, 268 (1951).

(22) Available under water in 32-kg. pails, 50% solids, as sponge nickel hydrogenation catalyst, grade 986, from the Davison Chemical Division, W. R. Grace and Co., Baltimore, Md. We have recently reported on the nature and use of this catalyst for the dethiation of sulfur-containing pyrimidines [H. N. Schlein, M. Israel, S. Chatterjee, and E. J. Modest, *Chem. Ind. (London)*, 418 (1964)].

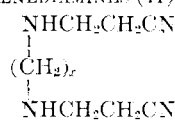
(23) The apparent bulk density is calculated by measuring the dry (110°) weight per unit volume of a settled aqueous suspension of the catalyst.²²

(24) J. C. Robinson, Jr., and H. R. Snyder, "Organic Syntheses," Coll. Vol. 111, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 720.

TABLE I
 MONOCYANOETHYLATED α,ω -ALKYLENEDIAMINES (I) AND THEIR DIHYDROCHLORIDES
 $H_2N(CH_2)_xNHCH_2CH_2CN$

x	B.p., °C. ^a	mm.	% yield ^b	Formula	Base		Hydrogen, %		Nitrogen, %	
					Calcd.	Found	Calcd.	Found	Calcd.	Found
2	107 ^d	1.3	42 ^c	C ₅ H ₁₁ N ₃						
3	86-87 ^o	0.7	53 ^e	C ₆ H ₁₃ N ₃	56.65	56.71	10.31	10.49	33.04	32.97
4	88	0.02	50 ^b	C ₇ H ₁₅ N ₃	59.53	59.42	10.71	10.94	29.76	29.84
5	110	0.12	68 ^b	C ₈ H ₁₇ N ₃	61.89	62.04	11.04	11.18	27.07	27.14
6	146	2.0	46 ⁱ	C ₉ H ₁₉ N ₃	63.86	63.74	11.32	11.30	24.83	25.16
9	152 ^{i,k}	0.3	34	C ₁₂ H ₂₅ N ₃	68.19	68.24	11.92	12.00	19.88	19.84
10	169-170 ^{i,l}	0.4	55	C ₁₃ H ₂₇ N ₃	69.27	69.32	12.08	12.09	18.64	18.38
12	171-176 ^{k,m}	0.3	n	C ₁₅ H ₃₁ N ₃						

^a The boiling points listed are those of the analytical samples. ^b Per cent yield figures represent yields based on distillation fractions collected over a 5° boiling range including the boiling point of the analytical samples. ^c A = absolute ethanol, B = 95% ethanol, C = refluxing 95% ethanol plus sufficient water to dissolve sample. ^d A. P. Terent'ev and A. N. Kost [*Zh. Obshch. Khim.*, **20**, 2069 (1950)] reported b.p. 101° (1.5 mm.); lit.¹⁸ b.p. 106-108° (1.0 mm.). ^e Dicyanoethylated product also present in 25% yield. ^f S. C. Dieker-

 TABLE II
 DICYANOETHYLATED α,ω -ALKYLENEDIAMINES (II) AND THEIR DIHYDROCHLORIDES


x	B.p., °C. ^a	mm.	% yield ^b	Formula	Base		Hydrogen, %		Nitrogen, %	
					Calcd.	Found	Calcd.	Found	Calcd.	Found
2	208-210 ^d	2.5	e	C ₈ H ₁₄ N ₄						
3	176 ^f	0.5	57	C ₉ H ₁₆ N ₄	59.97	60.12	8.95	9.08	31.09	31.82
4	180 ^g	0.7	50	C ₁₀ H ₁₈ N ₄	61.82	61.88	9.34	9.44	28.84	28.59
5	194-196	0.4	93	C ₁₁ H ₂₀ N ₄	63.42	63.44	9.68	9.73	26.90	26.96
6	200-201 ⁱ	1.0	j	C ₁₂ H ₂₂ N ₄	64.82	64.80	9.97	10.14	25.20	24.95
9	k		98	C ₁₅ H ₂₈ N ₄						
10	k,l		99	C ₁₆ H ₃₀ N ₄	69.02	68.80	10.86	11.39	20.13	19.89
12	k		97	C ₁₈ H ₃₄ N ₄						

^a The boiling points listed are those of the analytical samples. ^b The dicyanoethylated compounds undergo extensive decomposition on heating. The yield figures represent, for x = 3, 4, and 5, crude yields of dicyanoethylated compounds after removal of unreacted starting material and monocycanoethylated products by vacuum distillation at bath temperatures no greater than 10° above the boiling points listed in Table I; for x = 9, 10, and 12, the yields are based on crude reaction mixtures. ^c A = absolute ethanol, B = 95% ethanol, C = refluxing 95% ethanol plus sufficient water to dissolve sample, D = aqueous acetone. ^d J. Lincoln, B. Ellis, and G. C.

 TABLE III
 N-3-AMINOPROPYL- α,ω -ALKYLENEDIAMINES (III) AND THEIR TRIHYDROCHLORIDES
 $H_2N(CH_2)_xNHCH_2CH_2CH_2NH_2$

x	B.p., °C. ^a	mm.	% yield ^b	Formula	Base		Hydrogen, %		Nitrogen, %	
					Calcd.	Found	Calcd.	Found	Calcd.	Found
2	63-64 ^d	0.2	54	C ₅ H ₁₃ N ₃	51.24	51.20	12.90	13.06	35.86	36.04
3	80-81 ^e	1.0	61	C ₆ H ₁₅ N ₃	54.92	55.02	13.06	13.06	32.03	31.89
4	82-84 ^f	0.1	66	C ₇ H ₁₇ N ₃						
5	94-95	0.3	64	C ₈ H ₁₉ N ₃	60.32	60.24	13.20	13.30	26.89	26.73
6	104-105 ^{h,i}	0.2	51	C ₉ H ₂₁ N ₃						
9	135 ^j	0.2	39	C ₁₂ H ₂₉ N ₃	66.91	67.13	13.57	13.52	19.51	19.55
10	144-145 ^k	0.2	84	C ₁₃ H ₃₁ N ₃	68.06	68.24	13.63	14.02	18.32	17.88
12	153-154 ^l	0.5	47	C ₁₅ H ₃₅ N ₃	69.99	69.90	13.71	13.59	16.33	16.32

^a The boiling points listed are those for the analytical samples. ^b Per cent yield figures represent yields based on distillation fractions collected over a 5° range including the boiling point of the analytical samples. ^c A = 95% ethanol, B = refluxing 95% ethanol plus sufficient water to dissolve sample, C = 95% ethanol plus ether to precipitate. ^d Lit.¹⁸ b.p. 72° (1.0 mm.). ^e Lit.²¹ b.p. 105-106° (5-6 mm.) for free base and decomposition at 259° for the trihydrochloride. ^f The infrared spectrum of this material was identical

investigators²⁵ have reduced cyanoethylated amines, and nitriles in general, with W-2 Raney nickel at elevated temperatures (100-150°) and pressures [105.5-351.6 kg./cm.² (1500-5000 p.s.i.)]. There have been some sporadic reports in which nitriles have been reduced in the presence of Raney nickel at 4 atm. but at temperatures of 70-100°. ²⁶ Adkins and Billica²⁷

reported the preparation of W-6 Raney nickel and its use for the reduction of nitriles at room temperatures and low pressures; this catalyst is, however, quite hazardous to prepare and to handle and loses its activity in 2 weeks when stored under ethanol in a refrigerator. Sponge nickel catalyst has other advantages: it re-

(25) See, for example, F. C. Whitmore, *et al.*, *J. Am. Chem. Soc.*, **66**, 725 (1944), and ref. 15-17.

(26) See, for example, W. F. Haddock and C. S. Hamilton, *J. Am. Chem. Soc.*, **64**, 1309 (1942); and C. F. H. Allen and C. V. Wilson, *ibid.*, **80**, 105-106 (1958).
 (27) H. Adkins and H. R. Billica, *J. Am. Chem. Soc.*, **70**, 695 (1948).

M.p., °C. (solvent ^c)	Dihydrochloride							
	Carbon, %		Hydrogen, %		Nitrogen, %		Chlorine, %	
	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found
129-131 ^f (C)	32.26	32.07	7.05	7.17	22.58	22.21	38.10	37.89
216-218 ^g (C)	36.01	36.17	7.55	7.62	21.00	20.98	35.44	35.43
207.5-211 (B)	39.26	39.39	8.00	8.16	19.62	19.36	33.11	32.95
189-190 (A)	42.11	42.11	8.39	8.52	18.42	18.27	31.08	31.01
204-207 (A)	44.63	44.64	8.74	8.75	17.35	17.05	29.28	29.55
206-208 (A)	50.70	50.71	9.59	9.64	14.78	14.73	24.95	24.99
219-222 (A)	52.34	52.44	9.80	9.67	14.08	13.89	23.78	23.69
228-232 (A)	55.21	55.01	10.19	10.23	12.88	12.92	21.73	21.67

man and J. Simon [*J. Org. Chem.*, **22**, 259 (1957)] reported hygroscopic needles, m.p. 129-131°; our product showed no evidence of hygroscopicity. ^g Lit.²¹ b.p. 203-206° (3.0 mm.) for free base and decomposition above 180° for dihydrochloride. ^h Some dicyanoethylated product also formed. ⁱ Dicyanoethylated compound also present in 15% yield. ^j Some decomposition on distillation. ^k Low-melting solid. ^l White solid, m.p. 34-38°. ^m Extensive decomposition on heating. ⁿ 79% crude yield.

M.p., °C. (solvent ^c)	Dihydrochloride							
	Carbon, %		Hydrogen, %		Nitrogen, %		Chlorine, %	
	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found
192-203 (C)	40.18	40.23	6.74	6.91	23.43	23.30	29.65	29.59
240 dec. (D)	42.69	42.78	7.16	7.16	22.13	22.01	28.01	27.98
233-239 ^h dec. (C)	44.95	44.77	7.54	7.66	20.97	20.97	26.54	26.52
217-220 dec. (B)	46.98	46.66	7.88	7.89	19.92	20.14	25.22	25.26
228-232 (C)	48.81	48.72	8.19	8.36	18.98	18.96	24.02	23.88
222-235 dec. (A)	53.45	53.39	8.96	8.99	16.61	16.80	21.02	21.25
222-225 (C)	54.70	54.95	9.18	8.98	15.95	15.81	20.18	20.14
230-233 (C)	56.98	56.96	9.56	9.68	14.77	14.66	18.69	18.65

Richardson [British Patent 613,807 (1948)] reported b.p. 186-192° (0.2 mm.). ^e Obtained in 25% yield as by-product of the monocyanooethylation reaction. ^f Lit.²¹ b.p. 263-265° (3.0 mm.). ^g Previously reported to be nondistillable even at pressures of 0.01 mm. ^h Lit. m.p. 232-233° dec.,¹⁵ 243-244°.¹⁶ ⁱ Lit.² b.p. 230-238° (2.0 mm.). ^j Obtained in 15% yield as by-product of the monocyanooethylation reaction. ^k Low-melting solid; complete decomposition on distillation. ^l Analytical sample reprecipitated from benzene-petroleum ether mixture, m.p. 47-49°.

M.p., °C. (solvent ^c)	Trihydrochloride							
	Carbon, %		Hydrogen, %		Nitrogen, %		Chlorine, %	
	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found
223-228 (B)	26.50	26.44	8.01	8.00	18.55	18.63	46.94	46.72
270 dec. ^e (C)	29.95	29.89	8.38	8.47	17.47	17.36	44.21	44.37
255-257 ^g (B)								
250-252 (A)	35.76	35.78	9.00	9.13	15.64	15.64	39.57	39.61
254-256 (B)	38.24	38.15	9.27	9.30	14.86	14.74	37.63	37.59
279-282 dec. (B)	44.37	44.28	9.93	9.90	12.94	12.92	32.75	32.97
283-286 dec. (B)	46.09	46.06	10.12	10.17	12.41	11.93	31.40	30.90
290-295 dec. (B)	49.12	49.23	10.45	10.43	11.46	11.38	29.00	28.94

with that obtained from an authentic sample of spermidine (from L. Light and Co., Ltd., Colnbrook, Bucks, England) and purified by vacuum distillation [b.p. 84-86° (0.2 mm.)]. ^g Lit.¹⁷ m.p. 257-258°. ^h M.p. 37-39°. ⁱ Lit.¹⁹ b.p. 104° (0.4 mm.) and m.p. 38.4°. ^j Low-melting solid. ^k M.p. 56-59°. ^l M.p. 57-66°.

tains its activity on storage under water for long periods of time (over 2 years), possesses a very low degree of pyrophoricity, and can be handled safely in large quantities.

All of the triamines and tetramines thus obtained, except IV ($x = 10$ and 12), were purified by vacuum distillation; the two exceptions are high-boiling solids which can be distilled only under forcing conditions. The free bases and their hydrochloride salts, prepared

by the addition of anhydrous HCl to an alcoholic solution of the purified polyamine, are listed in Tables III and IV.

Biological Activity.—Of the 32 compounds reported in this paper, all but two (IV, $x = 10$ and 12) have been examined in various *in vitro* and *in vivo* bioassay systems at the Children's Cancer Research Foundation. Antitumor activity *in vivo* has been evaluated against four transplantable mouse tumors by the standard

TABLE IV
 N,N'-Bis(3-aminopropyl)- α,ω -alkylenediamines (IV) AND THEIR TETRAHYDROCHLORIDES

x	B.p., °C. ^a	mm.	yield ^b	Formula	Carbon, %		Hydrogen, %		Nitrogen, %	
					Calcd.	Found	Calcd.	Found	Calcd.	Found
2	118	0.2	63	C ₈ H ₂₂ N ₄	55.13	55.13	12.72	12.79	32.15	32.27
3	97-100	0.07	52	C ₉ H ₂₄ N ₄	57.40	57.53	12.85	12.65	29.75	29.52
4	141-142 ^c	0.5	25	C ₁₀ H ₂₆ N ₄						
5	150 ^d	0.2	63	C ₁₁ H ₂₈ N ₄	61.05	60.66	13.05	12.90	25.89	26.19
6	164-166 ^e	0.5	35	C ₁₂ H ₃₀ N ₄						
9	168-170 ^{f,i}	0.2	47	C ₁₅ H ₃₈ N ₄	66.11	66.14	13.31	13.40	20.56	20.02
10	<i>j,k</i>		95	C ₁₆ H ₃₈ N ₄						
12	190-210 ^{l,m}	0.3	45	C ₁₈ H ₄₂ N ₄	68.72	68.60	13.46	13.31	17.81	17.56

^a The boiling points listed are those of the analytical samples. ^b Per cent yield figures represent yields based on distillation fractions collected over a 5° range including the boiling point of the analytical samples except for $x = 10$ and 12, in which case the compounds are high-boiling solids and not easily distillable. ^c The tetrahydrochloride salts were all recrystallized from aqueous ethanol. ^d Previously reported²⁰ but without adequate characterization; the authors did not isolate the free base and gave only an unacceptable N analysis and a decomposition point of 150° for the tetrahydrochloride. ^e Identical with samples of spermine obtained from various sources. ^f Lit. m.p. 310-311° dec.,¹³ 309-311°.¹⁵ This sample of spermine tetrahydrochloride proved to be identical with that isolated from beef heart muscle⁶ and with samples obtained from various other sources. ^g Low-melting solid. ^h M.p. 46-50°. ⁱ A dihydrate salt consisting

assay procedures employed here.²⁸ Four hydrochloride salts (III, $x = 3, 5, 9$, and IV, $x = 9$) and the dinapionate salt of IV ($x = 9$) show significant antitumor activity; the data are summarized in Table V. The tetrahydrochloride salt of IV, $x = 9$, also produced a 51% increase in survival at a dosage of 20 mg./kg. in the WR-3 rat leukemia tumor system.²⁸

Against KB cells (human epidermoid carcinoma) in a culture system containing calf serum,²⁹ the triamines

and tetramines demonstrate, in general, the same high degree of inhibitory activity shown by spermine and spermidine, *i.e.*, 50% inhibiting doses (ID₅₀ values) in the range of 1-7 γ /ml. on a weight basis, equivalent to a molar concentration range of 1.0-3.0 $\times 10^{-5}$ mmole/ml.

Experimental³⁰

Freshly distilled acrylonitrile (b.p. 76-77°) was used exclusively throughout this work. The alkylenediamines containing from 2-6 carbon atoms were redistilled prior to use. All distillations of cyanoethylated and polyamine products were carried out in a nitrogen atmosphere. Infrared spectra were obtained by means of a Perkin-Elmer Model 137B spectrophotometer. Spectra of the free bases were determined in chloroform solution or as thin films, and those of the hydrochloride salts as potassium bromide disks. Melting points were taken by the capillary method in a modified Wagner-Meyer melting point apparatus³¹ at a heating rate of 1°/min. within 10⁹ of the melting point. Analytical samples of the hydrochloride salts were dried at 70° *in vacuo* for 17 hr. over phosphorus pentoxide.

The following experimental procedures illustrate the general methods used to prepare the compounds described in the tables. For each type of compound (I, II, III, or IV), the infrared spectra vary only slightly for the members of the homologous series. The infrared data given for specific compounds are therefore illustrative of the other members of that series.

Monocyanoethylation. N-2-Cyanoethylpropane-1,3-diamine (I, $x = 3$).—Acrylonitrile (10.6 g., 0.2 mole) was added dropwise over a 15-min. period to 1,3-propanediamine (14.8 g., 0.2 mole) with stirring and ice bath cooling. The reaction mixture was stirred at 5° for 20 min., allowed to warm gradually to 45°, and held at that temperature for 0.5 hr., after which time it was heated in a boiling water bath for 2 hr. On vacuum distillation the mixture yielded two fractions, a lower boiling fraction (16 g.), 90-103° (1.0 mm.), and a higher fraction (dicyanoethylation product, 3.5 g.), 166-170° (1.0 mm.). The lower boiling fraction was distilled twice to give 14.5 g. of material boiling at 84-87° (0.7 mm.); a sample boiling at 86-87° (0.7 mm.) was obtained for analysis; infrared spectrum (film): 2.98 (sh), 3.02, 3.40, 3.59, 4.45, 6.26, 6.83, 7.05, 7.35, 8.85, 9.30 μ .

The dihydrochloride salt was prepared by dissolving 3 g. of the redistilled product in 40 ml. of absolute ethanol and saturating

 TABLE V
 INHIBITION OF MOUSE TUMOR SYSTEMS BY SELECTED
 POLYMETHYLENEPOLYAMINES

Compd.	Tumor system ^a	Dose, ^b mg./kg.	Survival increase, ^c %	Tumor inhibition ^d , %	
				day	%
III-3HCl ($x = 3$)	B	80	+23	11	+75
III-3HCl ($x = 5$)	C	100	+36	14	+76
III-3HCl ($x = 9$)	C	80	+24	12	+72
IV-4HCl ($x = 9$)	A	25	+45	<i>d</i>	
	B	25	+62	9	+100
	C	25	+58	12	+100
	D	25	+5	15	+47
IV-dinapionate ($x = 9$)	B	80	-11	16	+66
	C	50	+44	14	+100
	D	80	+1	14	+64

^a Transplantable mouse tumors employed²⁸: A, L1210 ascitic lymphatic leukemia in the BDF/1 hybrid; B, P1534 lymphatic leukemia in the DBA/2 inbred strain; C, C1498 myelogenous leukemia in the C57BL/6 inbred strain; D, DBRB mammary carcinoma in the DBA/1 inbred strain. ^b Intraperitoneal administration once daily starting with first post-tumor inoculation day and continuing until death of last animal. Hydrochloride salts were administered in aqueous solution and the napionate salt as a suspension in 10% aqueous Tween 80. ^c Mean survival increase above 20% and tumor inhibition (reduction in mean tumor size) above 50% denote significant antitumor activity. ^d Evaluation based only on survival.

(28) The four tumor screen includes the following transplantable mouse tumors: L1210 ascitic lymphatic leukemia in the BDF/1 hybrid, P1534 lymphatic leukemia in the DBA/2 inbred, C1498 myelogenous leukemia in the C57BL/6 inbred, and DBRB mammary carcinoma in the DBA/1 inbred strain. The standard assay procedures have been described [C. L. Maddock, G. J. D'Angio, S. Farber, and A. H. Handler, *Ann. N. Y. Acad. Sci.*, **89**, 380 (1960)].

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M.p., °C. dec. ^a	Tetrahydrochloride							
	Carbon, %		Hydrogen, %		Nitrogen, %		Chlorine, %	
	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found
290-300 ^d	30.01	30.01	8.19	8.20	17.50	17.42	44.30	44.22
295-300	32.34	32.34	8.45	8.58	16.77	16.60	42.44	42.46
312-314.5 ^f								
298-305	36.36	36.19	8.90	8.98	15.47	15.48	39.15	39.11
310-315	38.30	38.41	9.11	9.11	14.89	14.74	37.70	37.65
306-308	43.06	43.10	9.64	10.08	13.39	13.18	33.90	33.72
300	44.44	44.15	9.81	9.78	12.96	12.80	32.79	32.35
300-305	46.95	46.73	10.07	10.16	12.17	11.96	30.80	30.70

of 1 molecule of *N,N'*-bis(3-aminopropyl)nonane-1,9-diamine and 2 molecules of naphthalene-1,5-disulfonic acid (naphonic acid) was prepared by mixing warm aqueous solutions of the tetrahydrochloride salt (1 part) and naphonic acid disodium salt (2 parts). The product was submitted for analysis after recrystallization from water, no m.p. below 310°. *Anal.* Calcd. for $C_{33}H_{50}N_4O_{12}S_4 \cdot 2H_2O$: C, 47.59; H, 6.14; N, 6.35; S, 14.53. Found: C, 47.69; H, 6.44; N, 6.31; S, 14.78. ^f M.p. 80-82°. ^k A tetrapicric mono-hydrate has been prepared (m.p. 185-190°, alcohol). *Anal.* Calcd. for $C_{30}H_{50}N_{16}O_{28} \cdot H_2O$: C, 39.35; H, 4.29; N, 18.33. Found: C, 39.54; H, 4.65; N, 18.11. ⁱ Boiling point obtained under forcing conditions. ^m Recrystallized from water, m.p. 52.6°.

the resulting solution with anhydrous HCl. Crystallization of the white precipitate (4.6 g.) from aqueous ethanol gave 3.8 g. (82% recovery) of white platelets, m.p. 216-219°; infrared spectrum (KBr): 3.18 (sh), 3.40, 3.64, 3.82 (sh), 4.00 (sh), 4.04, 4.12, 4.44, 5.00, 5.35, 6.22, 6.61, 6.72, 6.82, 6.98, 7.08, 7.18, 7.30, 7.48, 7.64, 7.83, 8.11, 8.64, 9.50, 9.95 μ .

A molecular compound composed of 1 mole of product and 1 mole of CO_2 was prepared by saturating a solution of 1.65 g. of free base in 20 ml. of ethanol with dry CO_2 . The white precipitate (2.07 g., 93%) was separated and purified by dissolving the product in a minimal volume of water, filtering off any insoluble residue, and reprecipitating the carbonate salt by the addition of acetone. A sample thus purified was dried at 40° *in vacuo* over phosphorus pentoxide for analysis; m.p. 118-131° dec.

Anal. Calcd. for $C_6H_{18}N_3 \cdot CO_2$: C, 49.11; H, 7.65; N, 24.54; N/H, 3.21. Found: C, 49.08; H, 7.71; N, 24.31; N/H, 3.15.

Agreement of the found N/H ratio with the calculated value for this molecular compound and with that of the free base (I, $x = 3$) (calcd. N/H, 3.21) supports the formula assignment of the molecular compound.

***N,N'*-2-Cyanoethyl-dodecane-1,12-diamine (I, $x = 12$).**—1,12-Dodecane diamine (16.4 g., 0.08 mole) was melted in a water bath at 70° and to the melt was added dropwise with stirring 5.45 ml. of acrylonitrile (4.36 g., 0.08 mole). After total addition, the bath temperature was raised to 100° and maintained there for 3 hr. On cooling, the reaction product hardened into a white solid. Vacuum distillation first removed a small amount of unreacted diamine, b.p. 102-140° (0.3-1.0 mm.); above a temperature of 155° (0.5 mm.), decomposition became excessive and the distillation was discontinued. The remaining material weighed 16.4 g. (79%); infrared spectrum ($CHCl_3$): 2.81 (sh), 2.94 (sh), 3.10, 3.40, 3.50, 4.44, 6.32, 6.85, 7.05, 7.30-7.40, 8.90, 9.15, 9.50 μ .

The dihydrochloride salt (m.p. 228-232°) was obtained in the usual manner.

Dicyanoethylation. *N,N'*-Bis(2-cyanoethyl)pentane-1,5-diamine (II, $x = 5$).—Acrylonitrile (45.2 g., 0.854 mole) was added dropwise with stirring and ice bath cooling into 43.6 g. of 1,5-pentanediamine (0.427 mole). The cooling bath was allowed to warm gradually and then heated to boiling, and the reaction mixture was maintained at 100° for 3 hr. The flask was set for vacuum distillation and the wax bath temperature was slowly increased to no higher than 130° with an internal pressure of 0.6 mm. A fraction containing unreacted diamine and a small amount of monocyanoethylated material was collected and the distillation was discontinued. The contents of the flask weighed 83.1 g., equivalent to a 93% yield of crude product. Vacuum distillation of 18.4 g. of crude product gave, with excessive decomposition, 7.9 g. (43% recovery) of material boiling at 196-204° (0.7 mm.); infrared spectrum ($CHCl_3$):

3.00, 3.10, 3.40, 3.50, 4.45, 6.10 (sh), 6.32, 6.85, 7.08, 7.30-7.40, 7.58, 8.30, 8.88, 9.15, 9.54 μ .

The dihydrochloride salt was prepared in the usual way, m.p. 217-220° dec.; infrared spectrum (KBr): 3.40, 3.50, 3.58 (sh), 3.82 (sh), 4.00 (sh), 4.08, 4.15, 4.43, 6.18, 6.43 (broad), 6.75 (sh), 6.82, 6.95 (sh), 7.10, 7.28, 7.49, 7.73, 8.08, 8.91, 9.45 (sh), 9.55 μ .

***N,N'*-Bis(2-cyanoethyl)decane-1,10-diamine (II, $x = 10$).**—A flask containing 20.94 g. (0.122 mole) of 1,10-diaminodecane was warmed at 80° in a water bath to melt the diamine. To the melt was added dropwise with stirring 16.2 ml. of acrylonitrile (12.93 g., 0.244 mole). The temperature was maintained at 80° for 1 hr. and then raised to 100° for 2 hr. On cooling, the reaction mixture solidified (33.6 g., 99%, m.p. 40-45°). The product was purified by repeated precipitation from benzene-petroleum ether (b.p. 60-90°) to give an analytical sample, m.p. 47-49°.

The dihydrochloride was obtained in 87% yield, m.p. 222-225°.

Catalytic Reduction of Cyanoethylated Compounds. *N,N'*-2-Aminoethylpropane-1,3-diamine (III, $x = 2$).—*N,N'*-2-Cyanoethyl-ethylenediamine (21.9 g., 0.194 mole) was dissolved in 175 ml. of absolute ethanol. The solution was cooled in an ice bath and saturated with ammonia gas at 0°. A teaspoonful (approximately 5 ml.) of sponge nickel catalyst²² was added, and the mixture was shaken overnight under hydrogen on a Parr low-pressure hydrogenator at an initial pressure of 3.94 kg./cm.² (56.0 p.s.i.) (theoretical hydrogen pressure drop, 2.21 kg./cm.²; actual, 2.22 kg./cm.²). The catalyst was removed by suction filtration and washed twice with ethanol. The filtrate and washings were combined and the ethanol was evaporated on a rotary evaporator at the water pump. The crude product showed the absence of nitrile absorption in the infrared. A fraction boiling at 64-72° (0.3 mm.) was collected by fractional vacuum distillation (15.7 g., 69%). Redistillation afforded a fraction (12.3 g., 54%) boiling at 63-64° (0.2 mm.), a sample of which was used for analysis; infrared spectrum ($CHCl_3$): 2.80 (sh), 2.93, 3.11, 3.40, 3.50, 6.33, 6.88, 7.29, 7.41, 7.95, 8.41, 8.90, 9.18, 9.53 μ .

The trihydrochloride salt was obtained by bubbling anhydrous hydrogen chloride into a cold alcohol solution containing 11.4 g. of free base. The white precipitate (21.5 g., 98%, m.p. 219-225°) was recrystallized from aqueous ethanol giving white prismatic plates, m.p. 220-225° (16.54 g.). Prolonged drying (102 hr.) at 75° *in vacuo* over phosphorus pentoxide raised the melting point to 223-228°. A sample melting at this range was used for the analysis; infrared spectrum (KBr): 3.42, 3.50 (sh), 3.70, 3.89, 4.04, 4.15, 6.22, 6.60, 6.73, 6.85, 7.11, 7.21, 7.41, 7.75, 7.90, 8.62, 8.99, 9.42, 9.61, 9.88, 10.11 μ .

***N,N'*-Bis(3-aminopropyl)pentane-1,5-diamine (IV, $x = 5$).**—A solution containing 15.0 g. of *N,N'*-bis(2-cyanoethyl)pentane-1,5-diamine (0.072 mole) and ca. 5 ml. of sponge nickel catalyst in 150 ml. of absolute ethanol saturated with NH_3 was shaken

under hydrogen at an initial pressure of 3.87 kg./cm.² (55.0 p.s.i.) on a Parr hydrogenator. A decrease in hydrogen pressure of 1.62 kg./cm.² was observed (calcd., 1.64). After removal of the catalyst, the ethanol was evaporated on a rotary evaporator. The residue, on vacuum distillation, yielded a fraction which boiled at 144–158° (0.2 mm.) and which hardened into a white solid on cooling (9.78 g., 63%). Redistillation afforded 7.48 g. (76% recovery) of material boiling at 144–150° (0.2 mm.); a sample boiling at 150° (0.2 mm.) was used for analysis: infrared spectrum (CHCl₃): 2.85 (sh), 2.95 (sh), 3.12, 6.32, 6.89, 6.95 (sh), 7.30, 7.41 (sh), 7.95, 8.92, 9.15, 9.52 μ .

The tetrahydrochloride salt was prepared in the usual manner in 74% yield, m.p. 298–305° dec. after recrystallization from aqueous ethanol.

When a warm solution of 320 mg. of free base (1.5 mmoles) in ethanol was added to a warm solution of 1.28 g. (5.6 mmoles) of picric acid in ethanol, a yellow crystalline picrate formed instantly (1.46 g., 92%). Several recrystallizations from water, with better than 90% recovery each time, gave a product de-

composing at 227°. Analysis indicated the sample to be a tetrapicrate monohydrate. The sample was dried at 70° for 72 hr. *in vacuo*.

Anal. Calcd. for C₂₁H₂₈N₄·4C₆H₃N₃O₇·H₂O: C, 36.53; H, 3.68; N, 19.47. Found: C, 36.51; H, 3.78; N, 19.54.

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Compounds Derived from the Mannich Bases of β -Phenylpropiophenone

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Various substituted β -phenylpropiophenones, obtained by hydrogenating the appropriate chalcone, have been allowed to react with secondary bases and formaldehyde to give the Mannich bases II. These have been reduced or treated with Grignard reagents, the resulting alcohols have been acylated and dehydrated, and the olefinic products of dehydration have been hydrogenated to the paraffins. Cyclization of the chalcones provided a series of substituted indanones which were treated in the same manner as the β -phenylpropiophenones. Many of the compounds prepared had interesting pharmacological activities.

Whereas the use of the Mannich bases of alkylphenones¹ and desoxybenzoin² as valuable intermediates for the preparation of physiologically active compounds is of long standing, little work has been reported³ on the Mannich bases of the β -phenylpropiophenones, either as chemical entities or potential pharmaceuticals.

Taking the substituted phenylpropiophenones (I) (Scheme I) we have prepared the Mannich bases (II) which with sodium borohydride gave the secondary alcohols (III, R₅ = H) or with a Grignard reagent gave the tertiary alcohols (III, R₃ = alkyl, cycloalkyl, phenyl, or aminoalkyl). The tertiary alcohols could then be dehydrated by boiling gently with HCl in acetic acid to give the olefins (IV) usually as a mix-

ture of *cis-trans* isomers. With ethylmagnesium bromide as the Grignard reagent the resulting tertiary alcohol (III, R₁ = R₂ = H; R₃ = R₄ = CH₃; R₅ = CH₂CH₃) could theoretically dehydrate in both of two ways to give the olefin of type IV and, by eliminating a proton from the ethyl group, compound VI. In earlier work,⁴ the somewhat simpler tertiary alcohol VII eliminated away from the ethyl group to give VIII analogous to type IV. However, our dehydrated material was shown to be VI by both the isolation of acetaldehyde as its 2,4-dinitrophenylhydrazone from the products of ozonolysis and the n.m.r. spectrum which showed two pairs of doublets (mixture of *cis-trans* isomers) at τ 3.9–4.2, indicative of a vinyl proton.

The catalytic reduction of these dehydrated compounds (IV) proceeded well only when R₅ was an alkyl group; when R₅ was phenyl, the alkane (V) was prepared directly from the substituted benzhydrol (III, R₅ = C₆H₅) by reduction with sodium in liquid ammonia.

The pyridyl chalcone IX obtained by condensing 2-acetylpyridine with benzaldehyde⁵ gave, on reduction, the analogous alkanone⁶ which with phenyllithium gave the tertiary alcohol X; the same alcohol was obtained by reacting 2-pyridyllithium with β -phenylpropiophenone, though in neither reaction was the yield good. With hydrazine and sodium ethoxyethoxide⁷ the pyridyl chalcone IX gave the pyridyl-

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