bearing implants of the same tumor were treated similarly with 0.5% carboxymethylcellulose in 0.85% aqueous NaCl. Beginning 1 day after cessation of treatment, weekly measurements of two perpendicular axes of each tumor in surviving mice were made with vernier calipers. From these values, an average diameter for each experimental group was calculated. Evaluation of inhibitory effect is based on the calculated ratio between the average diameter of tumors in treated mice and that of tumors in control animals. A ratio of 0.75 or less is considered to be indicative of antitumor activity in the Sarcoma 180 system. The mice were considered to have recovered when the tumor was no longer palpable.

The mice were weighed at the times of implantation and of measurement of tumors, and the average change in body weight for each experimental group was calculated. Deaths were recorded daily. Unless otherwise noted deaths may be attributed to the growth of the tumor.

B. Tumor Spectrum.—Tests with other transplanted solid mouse tumor systems were conducted similarly. With the mammary carcinoma E0771, sarcoma T241, and melanoma B16, treatment was begun 1 day after implantation of tumor; with Carcinoma 1025 and the Ridgway osteogenic sarcoma (ROS), treatment was begun 5 days after implanation. In all systems, the mice received 0.5 ml. by the intraperitoneal route, once daily, for 7 days. Inhibition of the growth of the tumors was maximal 1 week after the end of therapy, and evaluations at that time are in Table III. A $T:C$ of 0.7 or less, at a well tolerated dose, for E0771, T241, B16, or ROS, and 0.6 or less for C1025 are considered to be statistically valid indications of significant inhibition of the respective solid tumors.[29] Methods for Ehrlich ascites tumor and Mecca lymphosarcoma were those described.[29]

C. Immuno-Depressant Effect.—The test system used was essentially that of Nathan, *et al.*[7] The tests were carried out as follows: Groups of 10 young adult female Swiss ICR/Ha mice were given a single intraperitoneal injection of tanned sheep erythrocytes, followed several hours later by an intraperitoneal injection of a suspension of the test compound (day 0). Four additional daily injections of the compound were given for a total of five doses. On day 12, all survivors were bled from the retro-orbital venous plexus using heparinized capillary pipettes. Equal amounts of blood from each mouse in the group were pooled and the serum was collected. Twofold serial dilutions of the serum in physiological saline were made, and the heterohemagglutinin antibody titers were measured against sheep erythrocytes. Minimal response (±) readings were confirmed microscopically.

Acknowledgment.—The authors wish to thank Miss Valentina Fetzer, Mr. Raul Pena, Mrs. Miyono Schmid, Miss Barbara Smol, Mrs. Beverly Stern, and Mrs. Marion Eilbert for their technical assistance in conducting the *in vivo* experiments.

(29) F. A. Schmid, J. G. Cappucino, P. C. Merker, and G. S. Tarnowski, *Cancer Res.* (Supplement), in press.

Pyrimidinecarbamates and Thiolcarbamates Derived from Amino- and Oxopyrimidines. II[1,2]

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By reaction of aminopyrimidines with various chlorothiolformates the following new thiolcarbamates were prepared: from 2-aminopyrimidine, the S-methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, *t*-butyl, *n*-octyl, and *p*-chlorophenyl 2-pyrimidinethiolcarbamates; from 5-aminouracil, the S-ethyl, *n*-propyl, isopropyl, *n*-butyl, *t*-butyl, and *n*-octyl 2,4-dioxo-1,2,3,4-tetrahydro-5-pyrimidinethiolcarbamates; from 2,4-diaminopyrimidine, the di-S-ethyl and di-S-*n*-butyl 2,4-pyrimidinebis(thiolcarbamates); from 4,6-diaminopyrimidine, di-S-ethyl 4,6-pyrimidinebis(thiolcarbamate). The reaction of diethyl pyrocarbonate with aminopyrimidines gave new carbamates in good yields: ethyl 6-amino-4-pyrimidinecarbamate, diethyl 4,6-pyrimidinedicarbamate, diethyl 2,4-pyrimidinedicarbamate, and ethyl 2,6-dimethyl-4-pyrimidinecarbamate. The thiolcarbamates were converted to oxygen analogs by treatment with mercuric chloride and triethylamine in the presence of an alcohol. Uracil reacted with ethyl chlorothiolformate at the 1-position to give S-ethyl 3,4-dihydro-2,4-dioxo-1(2H)-pyrimidinecarbothioate. Attack by chloroformates occurred at the same position. None of these substances showed significant activity as an anticancer agent.

The present study is a continuation of an investigation of the preparation and properties of pyrimidinecarbamates and related compounds, which are possible anticancer agents. In the previous work[2] carbamates were obtained by the interaction of alkyl chloroformates with aminopyrimidines. In the current work thiolcarbamates were prepared by the reaction of chlorothiolformates[3] with both mono- and diaminopyrimidines. It was hoped that replacing an oxygen atom by sulfur would increase physiological activity. Another purpose of this work was to determine with certainty the location of the substituting groups.

By treating 2-aminopyrimidine, 2,4-diaminopyrimidine, and 4,6-diaminopyrimidine with various chlorothiolformates in the presence of a base, thiolcarbamates of the following structures were obtained. The

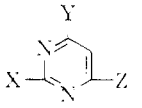
(1) (a) Supported by Public Health Service Research Grant No. CA-03477 from the National Cancer Institute; (b) from the Ph.D. Thesis of Henry Richmond, University of Delaware, 1964.

(2) Part I: E. Dyer, M. L. Gluntz, and E. J. Tanck, *J. Org. Chem.*, **27**, 982 (1962).

(3) The alkyl chlorothiolformates were kindly supplied by the Stauffer Chemical Co.

TABLE I

THIOLCARBAMATES DERIVED FROM AMINOPYRIMIDINES

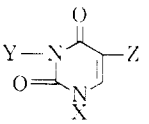


No.	X	Y	Z	Solvent[a]	Base[a]	Temp., °C.	Time, hr.	Products Yield, %	Products Recrystn. solvent[a]	Products M.p., °C.
1	CH_3SCONH	H	H	E	Ap	0	12	22	EA	210–211
2	C_2H_5SCONH[b]	H	H	E	Ap	15	0.5	55	W	147–148
3	n-C_3H_7SCONH	H	H	Py	Py	0	4	29	EA	152–154
4	i-C_3H_7SCONH	H	H	Py	BaO	0	8	11	PE	152–154
5	n-C_4H_9SCONH	H	H	E	NaOH	0	8	69	E–EA	142–144
6	t-C_4H_9SCONH	H	H	Py	Py	0	4	17	PE	166–168
7	p-ClC_6H_4SCONH	H	H	E	BaO	0	5	1	B	182–184
8	n-$C_8H_{17}SCONH$	H	H	E	BaO	0	8	39	E–EA	128–129
9	C_2H_5SCONH	C_2H_5SCONH	H	Py	Py	25	1.5	26	E–EA	201
10	n-C_3H_9SCONH	n-C_4H_9SCONH	H	Py, B	Py	100	0.5	16	E–EA	118–119
11	H	C_2H_5SCONH	C_2H_5SCONH	Py	Py	0	1.0	38	E–EA	242–243

[a] Ap, aminopyrimidine; B, benzene; E, ethanol; EA, ethyl acetate; PE, petroleum ether (b.p. 75–90°); Py, pyridine; W, water. [b] Prepared by T. W. Donahue (M.S. Thesis, University of Delaware, 1962).

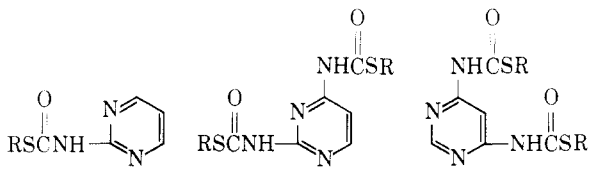
TABLE II

THIOLCARBAMATES DERIVED FROM 5-AMINOURACIL AND FROM URACIL



No.	X	Y	Z	Solvent[a]	Base[a]	Temp., °C.	Time, hr.	Products Yield, %	Products Recrystn. solvent[a]	Products M.p., °C.
12	H	H	C_2H_5SCONH	W	NaOH	0	10	84	E–EA	250–251
13	H	H	n-C_3H_7SCONH	A	Ap	57	1	29	EA	241–243
14	H	H	i-C_3H_7SCONH	A	Ap	0	8	45	E	254–255
15	H	H	n-C_4H_9SCONH	E	NaOH	0	8	61	E–EA	236–238
16	H	H	t-C_4H_9SCONH	A	Ap	57	1	8	E–EA	279–280
17	H	H	n-$C_8H_{17}SCONH$	A	Ap	57	1	65	EA	238–240
18	C_2H_5SCO	H	H	W	NaOH	0	8	64	B–PE	194
19[b]	C_2H_5SCO	CH_3	H					86	EA–PE	103

[a] A, acetone; Ap, aminopyrimidine; B, benzene; E, ethanol; EA, ethyl acetate; PE, petroleum ether (b.p. 75–90°); W, water. [b] Prepared from compound **18** with diazomethane in ether.

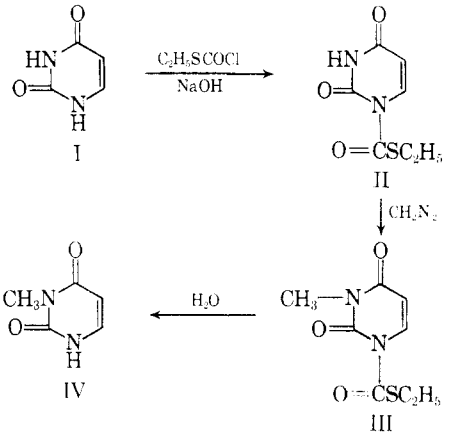


R = alkyl or aryl group

preparation and properties of individual thiolcarbamates are shown in Table I. Nonaqueous systems were used, with ethanol or pyridine as solvent and an excess of the aminopyrimidine, pyridine, or inorganic base as acid acceptor. The reactions proceeded with some difficulty; optimum conditions were found by trial and error.

5-Aminouracil also gave thiolcarbamates on reaction with alkyl chlorothiolformates (Table II). Schotten–Baumann conditions, which failed with the simple aminopyrimidines, were effective in this case. Another method consisted in heating a suspension of 5-aminouracil in refluxing acetone with the chlorothiolformate; the carbamate dissolved as it formed, and 5-aminouracil hydrochloride precipitated.

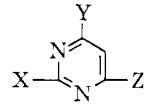
Uracil reacted with alkyl chloroformates to give products assumed[2] to be alkyl uracil-1-carboxylates. The reaction of uracil (I) with ethyl chlorothiolformate has now been shown to give a similar product, S-ethyl 3,-4-dihydro-2,4-dioxo-1(2H)-pyrimidinecarbothioate (II).



The location of the carbothioate group was determined by methylation to form the 3-methyl derivative (III), which was hydrolyzed by boiling water to give 3-

TABLE III

CARBAMATES DERIVED FROM AMINOPYRIMIDINES

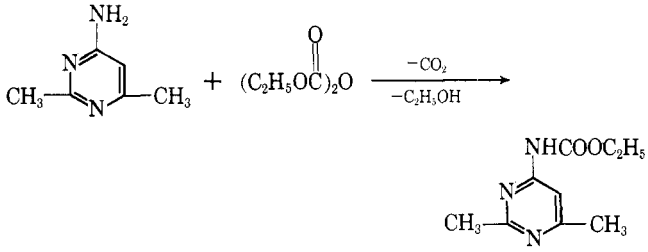


Compd.	X	Y	Z	Yield,[a] % Cf	Yield,[a] % P	Recrystn. solvent[b]	M.p., °C.
20[c,d]	C_2H_5OCONH	NH_2	H	0	73	EA	158
21[e]	C_2H_5OCONH	C_2H_5OCONH	H	0	90	W	170–171
22	H	C_2H_5OCONH	NH_2	20	86	E–EA	196–197
23	H	C_2H_5OCONH	C_2H_5OCONH	25	95	E–EA	266–267
24[d,e]	CH_3	C_2H_5OCONH	CH_3	0	41	PE	79
25[d,f]	C_2H_5OCONH	OH	H	0	52	Et	155–156

[a] Cf, using ethyl chloroformate under Schotten–Baumann conditions; P, using ethyl pyrocarbonate. [b] E, ethanol; EA, ethyl acetate; Et, ether; PE, petroleum ether (b.p. 75–90°); W, water. [c] Prepared by J. V. Miller (M.S. Thesis, University of Delaware, 1962) who separated **20** and **21** by Soxhlet extraction with ether in which **20** was less soluble. [d] Structure assumed by analogy to known compounds, not confirmed by decomposition. [e] Prepared by Colburn.[9] [f] Prepared by R. E. Farris, Jr. (Ph.D. Thesis, University of Delaware, 1964), by heating a mixture of the reactants, without solvent, at 90–95° for 14 hr.

methyluracil (IV), identical in properties with a synthetic specimen prepared by the method of Brown, *et al.*[4] This method is a shorter form of the procedure used by Spector and Keller[5] for proving the structure of acylated uracil. By the same sequence of reactions the structure postulated[2] for the ethyl uracil-1-carboxylate was confirmed.

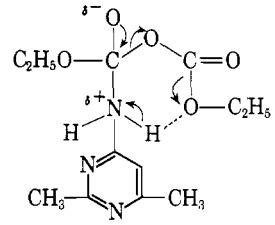
For the preparation of ethyl pyrimidinecarbamates, ethyl pyrocarbonate[6,7] proved to be a more active reagent than ethyl chloroformate, as shown in Table III. Ethyl chloroformate did not react with 2,4-diaminopyrimidine,[8] 4-amino-2,6-dimethylpyrimidine,[2] or 2-amino-4-hydroxypyrimidine[2]; 4,6-diaminopyrimidine gave low yields of products. All of these compounds, however, reacted readily with ethyl pyrocarbonate in ethanol solution. With excess pyrocarbonate, 2,4-



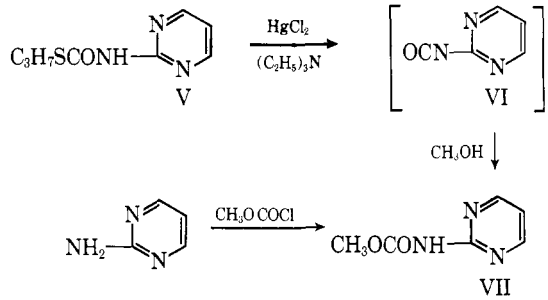
diaminopyrimidine and 4,6-diaminopyrimidine gave good yields of diethyl 2,4-pyrimidinedicarbamate and diethyl 4,6-pyrimidinedicarbamate, respectively; with limited amounts of pyrocarbonate, the monocarbamates were obtained.

The facile reaction of ethyl pyrocarbonate with aminopyrimidines might be explained by a cyclic intermediate, formed by the nucleophilic attack of the amino group at the carbonyl site.[9] Electronic shifts would give the resulting pyrimidinecarbamate, ethanol, and carbon dioxide.

The pyrimidine thiolcarbamates could be converted to carbamates by the metal ion assisted removal of mercaptan[10] under mild conditions in the presence of an

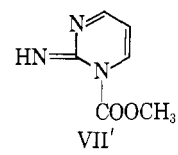


alcohol. For example, the thiolcarbamate V was rapidly decomposed at 40° by mercuric chloride and triethylamine in methanol to give the carbamate VII. The isocyanate VI was the probable intermediate.[10]



The carbamate VII was identical with the substance obtained[2] from the reaction of methyl chloroformate with 2-aminopyrimidine.

Evidence from infrared absorption that the reaction product from 2-aminopyrimidine and methyl chloroformate has the structure VII and not VII′ has been reported.[2] In the current work additional evidence



has been secured from the n.m.r. spectrum in deuteriochloroform.[11] Proton absorption was observed at τ-values of 6.14 (singlet, weight 3), 3.00 (triplet, weight 1), 1.31 (symmetrical doublet, weight 1.9), and 0.24 p.p.m. (broad band, weight 1). These data are in accord with the symmetrical structure VII.

In Table IV are shown the thiolcarbamates which have been converted to carbamates by removal of mercaptan with an alcohol present. In all cases the prod-

(4) D. J. Brown, E. Hoerger, and S. F. Mason, *J. Chem. Soc.*, 211 (1955).
(5) L. B. Spector and E. B. Keller, *J. Biol. Chem.*, **232**, 185 (1958).
(6) E. F. Degering, G. L. Jenkins, and B. E. Sanders, *J. Am. Pharm. Assoc.*, **39**, 624 (1950).
(7) E. Dyer, J. M. Reitz, and R. E. Farris, Jr., *J. Med. Chem.*, **6**, 289 (1963).
(8) L. L. Loney, M.S. Thesis, University of Delaware, 1961.
(9) R. M. Colburn, M.S. Thesis, University of Delaware, 1963.
(10) A. F. Ferris and B. A. Schutz, *J. Org. Chem.*, **28**, 71 (1963).
(11) Kindly done by Dr. G. L. Baker, Department of Chemistry, Montana State College.

TABLE IV

CONVERSION OF PYRIMIDINETHIOLCARBAMATES TO PYRIMIDINECARBAMATES

Compd.	Pyrimidinethiolcarbamate	ROH	Pyrimidinecarbamate
3	S-*n*-Propyl 2-pyrimidinethiolcarbamate	C_2H_5OH	Ethyl 2-pyrimidinecarbamate[a]
		CH_3OH	Methyl 2-pyrimidinecarbamate[b]
9	Di-S-ethyl 2,4-pyrimidinebis(thiolcarbamate)	C_2H_5OH	Diethyl 2,4-pyrimidinedicarbamate
11	Di-S-ethyl 4,6-pyrimidinebis(thiolcarbamate)	C_2H_5OH	Diethyl 4,6-pyrimidinedicarbamate
12	S-Ethyl 2,4-dioxo-1,2,3,4-tetrahydro-5-pyrimidinethiolcarbamate	CH_3OH	Methyl 2,4-dioxo-1,2,3,4-tetrahydro-5-pyrimidinecarbamate[b]

[a] Compound previously described in ref. 2, and by N. P. Buu-Hoi, R. Rips, and C. Derappe, *J. Med. Chem.*, **7**, 364 (1964). [b] Ref 2.

TABLE V

SCREENING DATA ON PYRIMIDINECARBAMATES AND -THIOLCARBAMATES

Compd.	Test system	Dose, mg./kg.	Survivors	Animal wt. change, g.	Tumor wt. or survival days, *T/C*	*T/C*, %
2	S180	500	6/6	−3.9	583/1346	43
		500	5/6	−2.1	441/767	57
2	LE-L1210	350	6/6	−2.7	8.2/8.7	94
2	FV[a]	350	8/10	−2.1	411/954	43
		350	9/10	−1.5	713/877	81
8	S180	500	6/6	−0.8	987/1302	75
8	LE-L1210	400	6/6	−1.8	7.8/9.0	86
8	S91[b]	400	0/10			
12	S180	500	0/6			
		125	6/6	−0.6	1111/871	127
12	LL[c]	100	6/6	0.0	593/857	69
12	LE-L1210	100	6/6	0.7	9.0/9.5	94
18	S180	500	0/6			
		125	0/6			
		31	6/6	−0.6	922/801	115
18	LE-L1210	25	6/6	−0.4	10.8/9.8	110
22	S180	500	0/6			
		100	2/6	−5.0	410/1010	
		25	6/6	−2.1	568/1158	49
		25	6/6	−4.5	1003/850	118
22	LL[c]	20	7/7	−2.2	554/800	69
22	LE-L1210	20	6/6	−1.8	9.8/8.8	102
23	S180	500	2/6	−3.2	480/805	
		250	5/6	−1.5	804/1010	79
23	Ca755	175	10/10	−1.8	1006/1461	68
23	LE-L1210	175	6/6	−1.4	8.7/9.0	96

[a] Friend leukemia (solid form). [b] Cloudman melanoma S91. [c] Lewis lung carcinoma.

ucts were identical with those obtained by reaction of the aminopyrimidine with a chloroformate or diethyl pyrocarbonate. The infrared spectra were superimposable and mixture melting points were unchanged. By analogy to compound VII, it may be postulated that the carbamates and thiolcarbamates obtained from 2-aminopyrimidine, 2,4-diaminopyrimidine, 4,6-diaminopyrimidine, and 5-aminouracil by reaction with chloroformates, chlorothiolformates, and ethyl pyrocarbonate have the substituent groups on the exocyclic nitrogens. Carbamates derived from 4-aminopyrimidine were previously shown,[2] by an independent synthesis, to be of similar structure.

In the reaction of the diaminopyrimidines with chloroformates and ethyl pyrocarbonate, no stable trisubstituted products were isolated. However, benzoyl chloride reacted with 2,4-diaminopyrimidine under Schotten–Baumann conditions to give a tribenzoyl derivative.[8] It is not known whether two benzoyl groups were on an amino nitrogen, as postulated for certain acetyl pyrimidines,[12,13] or whether benzoylation occurred at one or more ring nitrogens, as postulated for tribenzoyl cytosine.[14] Disubstitution on an amino nitrogen was easily produced by reaction of 2-aminopyrimidine with phthalimide to give 2-pyrimidylphthalimide.

Pharmacological Tests.—Testing of a number of the pyrimidine carbamates and thiolcarbamates by the Cancer Chemotherapy National Service Center[15] (Tables V and VI) has shown no significant activity toward Sarcoma 180, Adenocarcinoma 755, Leukemia 1210, Friend virus leukemia (solid form), and the KB cell culture.

Experimental

Melting Points.—Melting points were taken in sealed tubes with a calibrated thermometer in a stirred oil bath.

Pyrimidinecarbamates and Thiolcarbamates.—Certain details of preparation are given in Tables I–III, and one example of each method is described here. Absorption spectra and analytical data are in Table VII.

S-Ethyl 2-Pyrimidinethiolcarbamate.—To a solution of ethyl chlorothiolformate (2.49 g., 0.02 mole) in 100 ml. of ethanol at 12° was added 2-aminopyrimidine (3.8 g., 0.04 mole). The mix-

(12) V. I. Khemelevskii and O. I. Durnitsyna, *Zh. Obshch. Khim.*, **26**, 755 (1956); *Chem. Abstr.*, **50**, 14777 (1956).

(13) W. Pfleiderer and E. Liedek, *Ann.*, **612**, 163 (1958).

(14) D. M. Brown, A. Todd, and S. Varadarajan, *J. Chem. Soc.*, 2384 (1956).

(15) Procedures as described by J. Leiter, A. R. Bourke, S. A. Schepartz, and I. Wodinsky, *Cancer Res.* **20**, 734 (1960).

TABLE VI

SCREENING OF PYRIMIDINECARBAMATES AND -THIOLCARBAMATES WITH KB CELL CULTURE

Compd.	Slope	ED_{50}, γ/ml.	Status
2	−1.4	33	Inactive
8	−0.84	41	Inactive
12	−1.1	36	Inactive
18	−1.03	25	Inactive
22		100	Inactive
23	−0.62	58	Inactive

B. Using Ethyl Chloroformate.—To ethyl chloroformate (0.9 ml., 0.009 mole) was added dropwise 10 ml. of an aqueous solution of 4,6-diaminopyrimidine (0.5 g., 0.0045 mole) and NaOH (0.01 mole). The reaction mixture was stirred for 1 hr. A white precipitate appeared which was recrystallized from an ethanol–ethyl acetate mixture to give 0.26 g. (25%) of a product, m.p. 267–268°.

C. Using the Metal Ion Assisted Elimination of Mercaptan.—To a solution at 40° of di-S-ethyl 4,6-pyrimidinebis(thiolcarbamate) (0.11 g., 0.0038 mole), 0.5 ml. (0.004 mole) of triethylamine, and 50 ml. of absolute ethanol was added 15 ml. of absolute ethanol containing mercuric chloride (0.10 g., 0.00038 mole). A white precipitate immediately appeared which remained after 1 hr. of refluxing. The mixture was cooled and the precipitate filtered. It contained the mercuric mercaptide and diethyl 4,6-pyrimidinedicarbamate. The mercuric mercaptide was extracted with petroleum ether (b.p. 75–90°), and the residue recrystallized as above to give the dicarbamate (0.080 g., 83%) melting at 266–267°. A mixture melting point with the compound prepared by method A showed no depression. The infrared spectra were identical. Yields were frequently higher, for example, 90% in the conversion of **3** to methyl 2-pyrimidinecarbamate.[17]

TABLE VII

SPECTRAL DATA AND ANALYSES

Compd.	Ultraviolet,[a] mμ	Log ε	Infrared, cm.$^{-1}$ (C=O)	Formula	Calcd., % C	H	N	S	Found, % C	H	N	S
1	229, 269	4.18, 3.59	1639	$C_6H_7N_3OS$	42.59	4.17	24.84	18.97	42.67	4.25	24.46	18.59
2			1650	$C_7H_9N_3OS$	45.88	4.95	22.93		45.82	5.06	23.02	
3	246	4.19	1638	$C_8H_{11}N_3OS$	48.71	5.62	21.30	16.25	48.60	5.52	20.93	16.11
4	247	4.43	1639	$C_8H_{11}N_3OS$	48.71	5.62	21.30	16.25	48.75	5.51	21.52	16.14
5	248	4.35	1645	$C_9H_{13}N_3OS$	51.16	6.20	19.89		51.26	6.42	19.64	
6	248	4.31	1639	$C_9H_{13}N_3OS$	51.16	6.20	19.89	15.18	51.33	6.23	20.09	15.00
7	228, 265	4.39, 3.91	1664	$C_{11}H_8ClN_3OS$	49.72	3.44	15.81	12.07	49.91	3.17	15.61	12.11
8	246	4.19	1653	$C_{13}H_{21}N_3OS$	58.39	7.92	15.72	11.99	58.48	7.55	15.60	11.84
9	242, 244, 282	4.57, 4.57, 4.18	1689, 1639	$C_{10}H_{14}N_4O_2S_2$	41.94	4.93	19.57	22.39	41.97	4.93	19.46	22.26
10	242	4.36	1681, 1639	$C_{14}H_{22}N_4O_2S_2$	49.10	6.48	16.36	18.73	49.22	6.46	15.98	18.41
11	260	3.81	1681	$C_{10}H_{14}N_4O_2S_2$	41.94	4.93	19.57	22.39	42.26	5.08	19.79	22.22
12	244, 287	4.00, 3.88	1706	$C_7H_9N_3O_3S$	39.05	4.21	19.52	14.90	38.92	4.51	19.33	14.98
13	245, 286	4.02, 3.92	1750[c]	$C_8H_{11}N_3O_3S$	41.91	4.84	18.33	13.99	42.03	5.05	18.18	13.90
14	245, 288	4.29, 4.17	1724	$C_8H_{11}N_3O_3S$	41.91	4.84	18.33	13.99	41.73	5.15	18.02	13.59
15	245, 287	4.10, 3.99	1710	$C_9H_{13}N_3O_3S$	44.43	5.39	17.27	13.18	44.49	5.61	16.98	12.91
16	244, 288	4.06, 3.94	1704	$C_9H_{13}N_3O_3S$	44.43	5.39	17.27	13.18	44.44	5.86	17.10	13.16
17	245, 287	3.95, 3.84	1751	$C_{13}H_{21}N_3O_3S$	52.15	7.07	14.04	10.71	52.25	7.26	13.81	10.70
18			1740,[c] 1675	$C_7H_8N_2O_3S$	41.99	4.03	13.99	16.01	42.09	4.22	13.75	15.95
19			1680[c]	$C_8H_{10}N_2O_3S$	44.85	4.70	13.08	14.97	45.04	4.85	13.28	14.86
20	280	4.95	1740	$C_7H_{10}N_4O_2$	46.17	5.54			45.88	5.67		
21	276	5.14	1750	$C_{10}H_{14}N_4O_4$	47.24	5.51	22.04		47.10	5.49	21.80	
22	222, 266	4.62, 3.73	1709	$C_7H_{10}N_4O_2$	46.17	5.54	30.77		46.07	5.83	30.51	
23	222, 264, 269	4.63, 3.83, 3.83	1709	$C_{10}H_{14}N_4O_4$	47.24	5.55	22.04		47.47	6.02	21.84	
24			1740	$C_9H_{13}N_3O_2$	55.38	6.67	21.53		55.86	7.09	21.52	
25			1700,[c] 1650	$C_7H_9N_3O_3$	45.90	4.95	22.94		46.20	5.02	22.59	

[a] All ultraviolet spectra were taken on a Perkin-Elmer Model 202 ultraviolet spectrophotometer. [b] Infrared spectra were taken on the Baird Model B double-beam infrared recording spectrophotometer with potassium bromide pellets. [c] Nujol mull with Perkin-Elmer 137.

ture was stirred for 1 hr. while the temperature slowly rose to 20° and then was poured into 100 ml. of ice–water. The precipitate was recrystallized from ethanol, which gave 2.0 g. (55%) of product, m.p. 147–148°.

S-*n*-Octyl 2,4-Dioxo-1,2,3,4-tetrahydro-5-pyrimidinethiolcarbamate.—To a suspension of 5-aminouracil (0.64 g., 0.0051 mole) in 500 ml. of refluxing acetone was added 3 ml. (0.015 mole) of *n*-octyl chlorothiolformate. At the end of 2 hr. the reaction mixture was cooled. A yellow precipitate of undissolved 5-aminouracil and 5-aminouracil hydrochloride was filtered. The filtrate was evaporated and the wool-like compound (0.49 g., 65% based on 0.0025 mole of 5-aminouracil) was recrystallized from ethyl acetate. The final melting point was 238–240°.

Diethyl 4,6-Pyrimidinedicarbamate. A. Using Ethyl Pyrocarbonate.—To a solution of 4,6-diaminopyrimidine[16] (0.5 g., 0.0045 mole) in 20 ml. of ethanol was added dropwise with stirring 1.2 ml. (0.0084 mole) of ethyl pyrocarbonate. Bubbles of CO_2 were observed. The ethanol was evaporated to dryness. A drop more of ethyl pyrocarbonate caused no further reaction. Recrystallization from ethanol and ethyl acetate gave a white powder (1.0 g., 95%) melting at 266–268°. Ethyl 4-amino-6-pyrimidinecarbamate was prepared in the same manner by using a 1:1 *M* ratio of pyrocarbonate to pyrimidine.

S-Ethyl 3,4-Dihydro-2,4-dioxo-3-methyl-1(2H)-pyrimidinecarbothioate and Its Conversion to 3-Methyluracil.—An ether solution of diazomethane[18] (25 ml., 0.09 mole) was added to 50 ml. of ether containing S-ethyl 3,4-dihydro-2,4-dioxo-1(2H)-pyrimidinecarbothioate (1 g., 0.005 mole) prepared from uracil and ethyl chlorothiolformate using Schotten–Baumann conditions. At the end of 20 hr. of standing at room temperature, the ether was evaporated. The white residue was recrystallized from ether and then from a 1:1 mixture of ethyl acetate and petroleum ether (b.p. 75–90°) to give the product (0.92 g., 86%), melting at 103°.

(16) D. J. Brown, *J. Chem. Soc. Japan, Ind. Chem. Sect.*, **69**, 353 (1950).

(17) Done by Mary Ann Findeisen.

(18) J. A. Moore and D. E. Reed, *Org. Syn.*, **41**, 16 (1961).

A solution of S-ethyl 3,4-dihydro-2,4-dioxo-3-methyl-1(2H)-pyrimidinecarbothioate (0.10 g., 0.00047 mole) in 20 ml. of distilled water was heated on a steam bath for 5 hr. Evaporation of the water left a white residue which was first treated with ether to extract any starting material. Extraction with ethyl acetate gave 0.05 g. (86%) of 3-methyluracil, m.p. 179°. A mixture melting point with analyzed material prepared from 2-thiouracil by the method of Brown, *et al.*,[1] showed no depression. The infrared spectra were superimposable.

Tribenzoyl-2,4-diaminopyrimidine.[8]—To 10 ml. of water solution containing 0.8 g. (0.02 mole) of NaOH and 1.0 g. (0.0091 mole) of 2,4-diaminopyrimidine was added dropwise 2.5 ml. (0.025 mole) of benzoyl chloride. After stirring at room temperature overnight, the aqueous solution was decanted from a yellow gum which was made granular by stirring with methanol. Recrystallization from methanol gave 2 g. (53%) of white product, m.p. 230–231°.

Anal. Calcd. for $C_{25}H_{18}N_2O_3$: C, 71.08; H, 4.30; N, 13.27. Found: C, 70.84; H, 4.21; N, 13.52.

2-Pyrimidylphthalimide.—A test tube containing a well-ground mixture of 2-aminopyrimidine (0.95 g., 0.01 mole) and phthalic anhydride (1.48 g., 0.01 mole) was heated at 140° for 90 min. After cooling, the solid was extracted with ethanol, ethyl acetate, and acetone. Recrystallization from ethyl acetate gave white crystals of product (0.54 g.), m.p. 120°. The yield was 65% based on the 2-aminopyrimidine used (0.6 g. was recovered).

Anal. Calcd. for $C_{12}H_7N_3O_2$: C, 63.99; H, 3.13; N, 18.61. Found: C, 64.12; H, 3.05; N, 18.57.

Ascending Paper Chromatography.—Several pyrimidinecarbamates and thiolcarbamates were chromatographed to test for homogeneity, using a 5:3 mixture of 1-butanol and 5 *N* acetic acid at room temperature. Each gave only a single dark spot, observed under ultraviolet light. Values of the ratio R_f pyrimidine-R_f adenine, using adenine as internal standard, were as follows: **1**, 1.29; **3**, 1.58; **4**, 1.67; **5**, 1.54; **6**, 1.54; **7**, 1.66; **8**, 1.62; **10**, 1.74; **11**, 1.58; **12**, 1.35; **13**, 1.60; **14**, 1.54; **15**, 1.54; **16**, 1.10; **17**, 1.54.

Acyltryptamines. IV.[1] Azepino[5,4,3-*cd*]indoles

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5-Acetyl-8-chloro-1,2,3,4-tetrahydro-1-oxo-β-carboline (IIb) was obtained from the cyclization of 2,3-piperidinedione 3-[(3-acetyl-6-chlorophenyl)hydrazone], prepared by coupling diazotized 3-acetyl-6-chloroaniline with 3-carboxy-2-piperidone. The chlorine substituent was used to block the undesired cyclization at C-6. Acid treatment of 4-acetyl-2-carboxy-7-chlorotryptamine, obtained from the alkaline hydrolysis of IIb, gave 9-chloro-3,4-dihydro-6-methyl-1H-azepino[5,4,3-*cd*]indole (IVa) and the corresponding 2-carboxylic acid (IVb). Catalytic reduction of IV resulted in removal of chlorine followed by saturation of the C=N bond. Acylation of IVa with acetic anhydride gave 5-acetyl-9-chloro-3,4,5,6-tetrahydro-6-methylene-1H-azepino[5,4,3-*cd*]-indole (VII) which was hydrolyzed to 7-chloro-4,N-diacetyltryptamine (VIII). Treatment of IV with KBH_4 or $LiAlH_4$ resulted in reduction of the C=N bond without loss of chlorine. Other reactions included N-1 and N-5 alkylation and conversion of the carboxyl substituent at C-2 to carbethoxy, hydroxymethyl, trimethoxybenzoyloxymethyl, piperidinocarbonyl, and piperidinomethyl groups. A limited pharmacological evaluation of the azepinoindoles failed to uncover any significant effects at nontoxic dose levels.

Previous studies in this laboratory on the synthesis of acyltryptamines[2] showed that 4-acetyl-2-carboxytryptamine is readily cyclized to a derivative of azepino[5,4,3-*cd*]indole. This finding suggested an investigation of some of the chemical and pharmacological properties of this novel nucleus.

According to our previous communication, cyclization of the (*m*-acetylphenyl)hydrazone of 2,3-piperidinedione resulted in a mixture consisting of approximately three parts of 7-acetyl-1,2,3,4-tetrahydro-1-oxo-β-carboline[3] and one part of 5-acetyl-1,2,3,4-tetrahydro-1-oxo-β-carboline (IIa) (Chart I). This unfavorable proportion limited the availability of azepinoindole since only the 5-acyl isomer (IIa) can be utilized for its synthesis. For this reason, it was decided to prevent the formation of the undesirable isomer by the use of a chloro substituent as a removable blocking group. Accordingly, diazotized 3-acetyl-6-chloroaniline was coupled with 3-carboxy-2-piperidone to give 2,3-piperidinedione 3-[(3-acetyl-6-chlorophenyl)hydrazone] (I). Cyclization of I in refluxing formic acid gave the desired 5-acetyl-β-carboline derivative IIb in high yield. Alkaline hydrolysis of IIb resulted in the formation of 5-acetyl-2-carboxy-7-chlorotryptamine (III), which on refluxing for 100 hr. in hydrochloric acid–acetic acid mixture gave 9-chloro-3,4-dihydro-6-methyl-1H-azepino[5,4,3-*cd*]indole (IVa) and the corresponding 2-carboxylic acid (IVb) in a ratio of approximately 1:3. The rate of decarboxylation is apparently decelerated by the negative effect of chlorine upon the electron density at the indole nitrogen.[4] In contrast, the decarboxylation of the corresponding chlorine-free acid was completed within 6 hr.[2]

The effects of chlorine were also noticeable in other phases of this sequence. For example, when the coupling reaction was carried out in normal fashion, complete conversion to an unidentified, amorphous red product took place. This was avoided by lowering the pH of the reaction mixture to 1–2 from the usual 3–4.

After fulfilling its function by directing the cyclization of the hydrazone in the desired manner, the chlorine was removed by catalytic hydrogenation over palladium on carbon. Interrupting the reduction of IVa after the uptake of 1 mole of hydrogen permitted the isolation of 3,4-dihydro-6-methyl-1H-azepino[5,4,3-*cd*]indole (V) described in part II of this series.[2] If the reduction were allowed to proceed to completion, satura-

(1) Paper III in this series: M. von Strandtmann, C. Puchalski, and J. Shavel, Jr., *J. Med. Chem.*, **7**, 141 (1964).

(2) M. von Strandtmann, M. P. Cohen, and J. Shavel, Jr., *ibid.*, **6**, 719 (1963).

(3) The generally accepted β-carboline nomenclature takes precedence over the systematic name, 1H-pyrido[3,4-*b*]indole, according to A. M. Patterson, L. T. Capell, and D. F. Walter, "The Ring Index," 2nd Ed., American Chemical Society, Washington, D. C., 1960.

(4) Protonation of the nitrogen is the first step in the decarboxylation of indole-2-carboxylic acids, according to R. A. Abramovitch, *J. Chem. Soc.*, 881 (1956).