TABLE V (*Continued*)

No.	Test system[a]	Dose, mg/kg	Survivors	Wt change (T − C),[b] g	Tumor wt[c] (T/C)[b]	% T/C[b]	ED_{50}, μg/ml	Slope
17	AA	10	3/3	22				
		3	3/3	17				
	WA	50	6/6	−80	2.9/8.1	35		

[a] Screening was performed under the auspices of the Cancer Chemotherapy National Service Center according to its protocol.[6] The test systems included: WA, Walker 256 (subcutaneous) in rats, p 11 of protocol; KB, cell culture, p 22; SA, Sarcoma 180 in mice, p 5; FV, solid Friend virus leukemia, p 6; AA, toxicity test. [b] T stands for test animals, C for controls. [c] Tumor weight is in milligrams for SA, FV, and LL, and grams for WA and LE.

30-ml portions of acetonitrile provided an analytical sample, mp 103–104°.

Anal. Calcd for $C_{30}H_{37}Cl_2N_3O_4$: N, 7.31. Found: N, 7.27.

2-Substituted 1,3-Bis(3,4-dimethoxybenzyl)hexahydropyrimidines (Table II) were prepared by the procedure as described in the previous section, from aldehydes and N,N′-bis(3,4-dimethoxybenzyl)-1,3-diaminopropane (VIIIb).

Acknowledgment.—The authors acknowledge Drs. H. B. Wood and J. Leiter of the Cancer Chemotherapy National Service Center for their cooperation in making the screening data available. We also wish to thank Union Carbide Chemical Company for supplying the 1,3-diaminopropane used in this research.

3-Substituted 2-Thiohydrouracils. Synthesis and Antitubercular and Antineoplastic Activities

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Received August 26, 1965

A number of 3-substituted 2-thiohydrouracils were synthesized by cyclization of the appropriate 1-carboxyethyl-3-substituted 2-thioureas using polyphosphoric acid. Infrared spectral characteristics of the –NC=S containing molecules are discussed and compared. The thioureas and resulting thiohydrouracils were tested for their *in vitro* antitubercular activity with minimum inhibitory concentrations ranging from 1.3–2.5 mg % for the thioureas, and from 1.3–5.0 mg % for the thiohydrouracils. Several of the compounds were submitted to the Cancer Chemotherapy National Service Center for screening; the results of this screening are also reported.

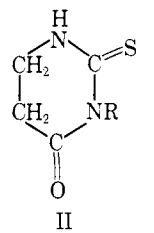
A wide variety of chemical structures have been shown to be effective in the inhibition of the growth of *M. tuberculosis* through *in vitro* testing. These compounds range in complexity of structure from such molecules as the antibiotic streptomycin to the relatively simple substituted thiourea molecule. Numerous investigators have reported upon the synthesis and antitubercular activity of various molecules containing the elements of –NC=S such as thioamides,[1] thioureas,[2] and thiosemicarbazones.[3] Recently we have reported on the activity of some thioureas incorporating heterocyclic N-substitution along with *p*-alkoxyphenyl N-substitution.[4] The present series of compounds was synthesized and examined for the effect of incorporating –NC=S into a cyclic structure such as thiohydrouracil while maintaining certain other features such as the substituents of the previously reported series for comparison.

Chemistry.—The intermediate 1-carboxyethyl-3-substituted 2-thioureas (I) were formed in good yields by treating 3-aminopropionic acid and the appropriately substituted isothiocyanates in aqueous sodium hydroxide at room temperature and subsequent treatment with hydrochloric acid. The substituted carboxyethyl-

$$RNCS + NH_2CH_2CH_2COOH \xrightarrow[2.\ HCl]{1.\ NaOH} RNHCSNHCH_2CH_2COOH$$

I

$$RNHCSNHCH_2CH_2COOH \xrightarrow[PPA]{H_2O}$$



II

thioureas were then cyclized with hot polyphosphoric acid to form the 3-substituted 2-thiohydrouracils (II).

Derzaj-Bizjak and co-workers[5] reported that the condensation of 3-aminopropionic acid with isothiocyanates as reported by Ghosh[6] does not take place except in the case of *o*-tolyl isothiocyanate. These authors used the corresponding ethyl 3-aminopropionate, and, following the condensation with an isothiocyanate, the ester function was hydrolyzed to free the desired 1-carboxyethyl-3-substituted 2-thioureas. We have found that the condensation of the sodium salt of 3-aminopropionic acid with alkyl, cycloalkyl, and aryl isothiocyanates in water pro-

(1) T. S. Gardner, E. Wenis, and J. Lee, *J. Org. Chem.*, **19**, 753 (1954).

(2) L. Doub, L. M. Richardson, D. R. Herbst, M. L. Black, O. L. Stevenson, L. G. Bambas, G. P. Youmans, and A. S. Youmans, *J. Am. Chem. Soc.*, **80**, 2205 (1958).

(3) G. Domagk, *Am. Rev. Tuberc.*, **61**, 8 (1950).

(4) (a) A. C. Glasser and R. M. Doughty, *J. Pharm. Sci.*, **51**, 1031 (1962); (b) *ibid.*, **53**, 40 (1964).

(5) M. Derzaj-Bizjak, S. Oblak, and M. Tisler, *J. Org. Chem.*, **27**, 1343 (1962).

(6) T. N. Ghosh, *J. Indian Chem. Soc.*, **11**, 23 (1934).

TABLE I

1-CARBOXYETHYL-3-SUBSTITUTED 2-THIOUREAS

$CO_2HCH_2CH_2NHCSNHR$

No.	R	Mp, °C[a]	RS[b]	Yield, %	Formula	% N Calcd	% N Found	MIC, mg %
1	n-C_5H_{11}	95–96	B	95	$C_{12}H_{23}N_2O_2S$	10.76	10.68	1.3
2	C_6H_{11}	95–96	A	91	$C_{10}H_{18}N_2O_2S$	12.17	12.34	2.5
3	C_6H_5	118–119[d]	E–W	83	$C_{10}H_{12}N_2O_2S$	12.49	12.51	2.5
4	p-$CH_3OC_6H_4$	159–160[e]	E–W	90	$C_{11}H_{14}N_2O_3S$	11.02	11.07	1.3
5	p-$C_2H_5OC_6H_4$	150–151	E–W	70	$C_{12}H_{16}N_2O_3S$	10.44	10.49	1.3
6	p-(n-C_3H_7O)C_6H_4	125–126	E–W	70	$C_{13}H_{18}N_2O_3S$	9.92	9.99	1.3
7	p-(i-C_3H_7O)C_6H_4	165–166	E–W	96	$C_{13}H_{18}N_2O_3S$	9.92	9.96	2.5
8	p-(n-C_4H_9O)C_6H_4	111–112	E–W	94	$C_{14}H_{20}N_2O_3S$	9.45	9.40	2.5
9	p-(s-C_4H_9O)C_6H_4	133–134	E–W	73	$C_{14}H_{20}N_2O_3S$	9.45	9.45	2.5
10	p-(n-$C_5H_{11}O$)C_6H_4	109–110	B–I	76	$C_{15}H_{22}N_2O_3S$	9.03	8.95	1.3
11	p-(s-$C_5H_{11}O$)C_6H_4	108–110	B–I	75	$C_{15}H_{22}N_2O_3S$	9.03	8.98	2.5

[a] Melting points on Fisher-Johns block are corrected. [b] Recrystallization solvents: A, acetone; B, benzene; C, chloroform; E, ethanol; I, isooctane; W, water. [c] Microanalyses by Dr. Kurt Eder, Geneva, Switzerland. [d] Lit.[5] mp 110°. [e] Lit.[5] mp 127°.

ceeds in satisfactory yields upon stirring at room temperature for periods of from 1 to 4 days.

A variety of dehydrating agents (*p*-toluenesulfonic acid,[7] hydrochloric acid,[8] or acetic anhydride[5,9]) have been used for cyclization of carboxyethylthioureas and related compounds. N,N′-Di(β-carboxyethylthiocarbamyl)ethane has been cyclized with polyphosphoric acid,[10] and we have found this agent satisfactory for the cyclization of the 1-carboxyethyl-3-substituted 2-thioureas we have studied when used for a 2–3-min period of heating at 125–140°.

All members of both series of compounds reacted positively with iodine–sodium azide test solution[11] confirming the presence of a thiocarbonyl group.

Spectral Properties.—Both the substituted thioureas and thiohydrouracils showed characteristic infrared spectra with certain distinguishing bands and shifts appearing during cyclization. In view of the uncertainties concerning the assignment of C=S stretching frequencies in N-containing compounds,[12] we should like to point out some observations as seen in the infrared spectra of 3-*p*-(*n*-propoxy)phenyl-2-thiohydrouracil and 1-carboxyethyl-3-*p*-(*n*-propoxy)phenyl-2-thiourea.[13] The NH stretching of the ArNH- linkage of the arylthioureas produces a sharp, strong band at 3419 cm^{-1} which is lost upon cyclization, exposing the NH stretching in the thiohydrouracils as a broader less intense band at 3425 cm^{-1}. Both compounds show strong NH stretching bands at 3247 cm^{-1} for the thiourea and 3205 cm^{-1} for the thiohydrouracil. A typical carbonyl band of the carboxyl group of the thiourea is seen in its proper position at 1704 cm^{-1} which is shifted to 1725 cm^{-1} after cyclization to the thiohydrouracil. This shift from the normal area of the tertiary amides at 1650 cm^{-1} is brought about by the combined effect of the *para*-substituted phenylamide nitrogen and the thiocarbonyl group.

Rao and Venkataraghavan[14] have examined a number of compounds containing the –NC=S structure, and have indicated that bands analogous to the "amide I, II, and III" could be established based on the "mixed vibrations" of this grouping. These bands are within 1395–1570, 1260–1420, and 940–1140 cm^{-1}, and have been designated as –NC=S bands I, II, and III. Suzuki[15] has established good correlation with these bands through normal, coordinate analyses of thioformamide, thioacetamide, N-methylthioformamide, and N-methylthioacetamide.

Both series of compounds of this study conform to the designation established by Rao and Venkataraghavan with the thiourea under discussion having strong bands at 1543, 1242, and 975 cm^{-1}. Bands at 1543, 1258, and 978 cm^{-1} were seen for the 3-*p*-(*n*-propoxy)phenyl-2-thiohydrouracil. The spectra of the remaining members of the two series studied substantiate the above observations in that they have strong bands in close proximity to those listed above. The most significant and characteristic feature of the cyclization is seen as the loss of the ArNH stretching of the aromatic thioureas at 3419 cm^{-1}, and the appearance of characteristic peaks at 3425 and 3205 cm^{-1} for the cycloalkyl and alkyl members as well as the aromatic members of the series.

Biological Evaluation.—The *in vitro* determination of the tuberculostatic activity was carried out by a serial-dilution technique as previously reported[4a] utilizing a virulent H37Rv strain of *M. tuberculosis var. hominis*[16] grown on Dubos medium with added beef serum. The results of this testing along with the synthetic results are summarized in Tables I and II.

Previous work with some of the same substituents[4] has shown that the most favorable minimum inhibitory concentration which was shown by the thioureas containing a 4-antipyryl along with a *p*-isopropoxyphenyl substituent was 0.16 mg %, and with 4-antipyryl and 2-pyridyl it was 0.63 mg %. The most favorable of the present series was 1.3 mg % seen in a number of the thioureas and thiohydrouracils, but with none

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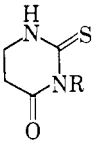
(8) T. B. Johnson and J. E. Livak, *J. Am. Chem. Soc.*, **58**, 299 (1936).
(9) D. L. Gramaise, R. Schwartz, and A. F. McKay, *ibid.*, **80**, 3332 (1958).
(10) A. F. McKay, S. Gelblum, E. J. Tarlton, P. R. Steyermark, and M. A. Mosley, *ibid.*, **80**, 3335 (1958).
(11) F. Feigl, "Spot Tests," Vol. 2 (Organic), Elsevier Publishing Co., Amsterdam, 1954, p 164.
(12) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1958, pp 355–357.
(13) KBr disks on Beckman IR-8 instrument.
(14) C. N. R. Rao and R. Venkataraghavan, *Spectrochim. Acta*, **17**, 541 (1962).
(15) I. Suzuki, *Bull. Chem. Soc. Japan*, **35**, 1456 (1962).
(16) National Tuberculosis Association, Standard Culture Department, Trudeau Laboratory, Saranac Lake, N. Y.

TABLE II

3-SUBSTITUTED 2-THIOHYDROURACILS



No.	R	Mp, °C[a]	RS[b]	Yield, %	Formula	% N[c] Calcd	% N[c] Found	MIC, mg %	ED_{50},[d] μg/ml	% T/C[e,f]
1	n-C_8H_{17}	78–79	E–W	86	$C_{12}H_{22}N_2OS$	11.56	11.49	5.0	1.8	89[g]
2	C_6H_{11}	206–207	E	76	$C_{10}H_{16}N_2OS$	13.20	13.09	2.5	...	...
3	C_6H_5	233–234[h]	A	65	$C_{10}H_{10}N_2OS$	13.58	13.70	1.3	1.0^+	101[g]
4	p-$CH_3OC_6H_4$	234–235[i]	A	76	$C_{11}H_{12}N_2OS$	11.86	11.89	1.3	1.0^+	104[g]
5	p-$C_2H_5OC_6H_4$	229–230	A	86	$C_{12}H_{14}N_2OS$	11.09	11.19	5.0	1.0^+	101[j]
6	p-(n-C_3H_7O)C_6H_4	208–209	A	75	$C_{13}H_{16}N_2OS$	10.60	10.67	5.0	1.0^+	95[j]
7	p-(i-C_3H_7O)C_6H_4	233–234	A	64	$C_{13}H_{16}N_2OS$	10.60	10.70	2.5	1.0^+	97[g]
8	p-(n-C_4H_9O)C_6H_4	213–214	A	86	$C_{14}H_{18}N_2OS$	10.07	10.17	2.5	1.0^+	101[j]
9	p-(s-C_4H_9O)C_6H_4	236–237	A	45	$C_{14}H_{18}N_2OS$	10.07	10.20	5.0	...	...
10	p-(n-$C_5H_{11}O$)C_6H_4	209–210	A	85	$C_{15}H_{20}N_2OS$	9.58	9.57	5.0	1.0^+	101[g]
11	p-(s-$C_5H_{11}O$)C_6H_4	171–172	E	38	$C_{15}H_{20}N_2OS$	9.58	9.86	5.0	...	...

[a] See footnote *a*, Table I. [b] See footnote *b*, Table I. [c] See footnote *c*, Table I. [d] Results of standard KB *in vitro*, tumor-cell inhibition test as carried out by the Cancer Chemotherapy National Service Center representing that dose inhibiting the growth of the KB cell culture to 50% of control growth. [e] Survival time in days in treated and controls reported as % T/C using L1210 lymphoid leukemia as tested by the Cancer Chemotherapy National Service Center. [f] Compounds **5** and **8** showed % T/C values against Sarcoma 180 as tested by CCNSC of 62 and 63, respectively. [g] Dose level 400 mg/ml. [h] Lit.[9] mp 228–229°. [i] Lit.[5] mp 236°. [j] Dose level 500 mg/ml.

involving those substituents seen to be most active from previous work.

In addition to the tuberculostatic testing, compounds from Table II were submitted to the Cancer Chemotherapy National Service Center for screening under the program established for this purpose. The results of their screening are reported in Table II along with the other data.

Experimental Section

Isothiocyanates.—The phenyl isothiocyanate used was a commercial product from Eastman Kodak. The following *p*-alkoxyphenyl isothiocyanates were prepared by the action of sodium chloroacetate upon the appropriate dithiocarbamate with the decomposition of the resulting salt by zinc chloride:[17] *p*-methoxy, *p*-ethoxy, *p*-propoxy, *p*-isopropoxy, *p*-butoxy, *p*-(*sec*-butoxy), *p*-amoxy, and *p*-(*sec*-amoxy). The alkyl and cycloalkyl isothiocyanates were prepared by the method of Moore.[18]

(17) G. J. Vander Kirk, C. W. Pluggers, and G. deVries, *Rec. Trav. Chim.*, **74**, 1262 (1955).

(18) M. L. Moore and F. S. Crossley, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p 599.

1-Carboxyethyl-3-*p*-(*n*-propoxy)phenyl-2-thiourea.—A solution of 4.83 g (0.025 mole) of *p*-propoxyphenyl isothiocyanate and 2.23 g (0.025 mole) of β-alanine in 50 ml of 1 *N* NaOH in a 125-ml extraction flask was stirred at room temperature with a magnetic stirrer until the reactants were in solution. This required 4 days. The solution was filtered and made acid with 10% HCl, and the resultant oil was scratched with chilling in an ice bath to induce crystallization. After standing overnight the solid was filtered, washed twice with cold water, and recrystallized from alcohol and water to yield, after drying, 4.9 g (70%) of white crystalline solid, mp 125–126°.

Anal. Calcd for $C_{13}H_{18}N_2O_2S$: N, 9.92. Found: N, 9.99.

3-*p*-(*n*-Propoxy)phenyl-2-thiohydrouracil.—One gram (0.0035 mole) of 1-carboxyethyl-3-*p*-(*n*-propoxy)phenyl-2-thiourea was added to 0.150 g of polyphosphoric acid which was previously heated to 125–140° in a 50-ml beaker on a hot plate. While the resulting melt was stirred, the temperature was maintained for about 2 min until a yellow-brown color had developed. The beaker was removed from the hot plate, allowed to cool slightly, and 10 ml of water was added with stirring. The resulting solid was filtered, washed twice with cold water, and dried in a desiccator overnight. A buff-colored solid resulted; mp 185–190°. This was recrystallized twice from acetone to yield 7.1 g (75%) of white needles melting at 208–209°.

Anal. Calcd for $C_{13}H_{16}N_2OS$: N, 10.60. Found: N, 10.67.