Table I—Cytotoxicity of Fractions from *C. columellaris*[a]

Fractions	ED_{50}, mcg./ml.
A	0.40
B	64.0
C	100.0
D	0.57
E	32.0
F	3×10^{-2}
G	2.4×10^{-2}
Desoxypodophyllotoxin	5.8×10^{-7}

[a] Assays were performed under the auspices of the Cancer Chemotherapy National Service Center. The procedures were those described in *Cancer Chemother. Rep.*, **25**, 1(1962).

Extraction and Preliminary Fractionation of *C. columellaris*— Coarsely ground leaves and stems of *C. columellaris* (5 kg.) were twice extracted continuously with 95% ethanol for 10 hr., and the ethanol extract was concentrated under water pump pressure to a thick dark syrup (A, 1690 g.). A portion of the crude extract (100 g.) was partitioned between water (1.0 l.) and chloroform (2.0 l.), and the two solutions were evaporated under reduced pressure (B, 59.0 g.; and D, 41 g.). The insolubles (C, 6.50 g.) were collected separately.

The chloroform-soluble portions were partitioned between petroleum ether (2.0 l.) and 10% aqueous methanol (1750 ml.), and the two solutions were evaporated under reduced pressure (E, 29.10 g.; and F, 8.0 g.). The 10% aqueous methanol-soluble portions were dissolved in chloroform and chromatographed on a column (60 × 4 cm.) containing 800.0 g. magnesia–silica gel (100–200 mesh, F-101); then they were eluted with 2.0 l. chloroform followed with 1.0 l. 2% methanol in chloroform. The eluents (chloroform and 2% MeOH in chloroform solutions) were combined and evaporated under reduced pressure (G, 4.10 g.).

Isolation of Desoxypodophyllotoxin—A large batch of Fraction G (55.0 g.) was prepared from crude extract (5.0 kg.), dissolved in benzene, added to a column (75 × 6 cm.) of silica gel (1200 g.), and chromatographed, using 20% chloroform in benzene as the solvent. Fractions (30 ml.) were collected and examined by TLC on silica gel G and H, using 1% MeOH in chloroform as eluent and ceric sulfate (3% in 3 *N* sulfuric acid) spray reagent. The fractions richest in desoxypodophyllotoxin were combined, evaporated to dryness (4.20 g.), and crystallized from ethanol. The colorless crystalline product (3.10 g.) was characterized as desoxypodophyllotoxin by mixed melting point, m.p. 166–168°, mixed TLC, and IR spectral comparison with the authentic sample.

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3-Substituted-2-thiohydrothymines as Potential Antitumor Agents

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Abstract □ A series of 3-substituted-2-thiohydrothymines was synthesized through the cyclization of the corresponding 1-(2-methyl)-carboxyethyl-3-substituted-2-thioureas which were formed from the appropriate isothiocyanate and β-aminoisobutyric acid. The cyclized products were screened for antitumor activity against lymphoid leukemia L-1210. Results of the screening indicated the compounds have little significant activity in the test system employed.

Keyphrases □ 2-Thiohydrothymines, 3-substituted—synthesis, antitumor testing □ Antitumor agents, potential—2-thiohydrothymines, synthesized, screened □ IR spectrophotometry—structure, identity

Most chemical agents that prevent cancerous growth do so by affecting nucleotide or nucleic acid metabolism. This is both a conclusion of screening (empirical) and a vindication of the search for such agents on purely theoretical reasons (1). Uncontrolled growth is a hereditary property of the neoplastic cell, and this heritable nature of cancer is one reason for considering interference with nucleic acid metabolism in the search for new agents (2).

An earlier report from these laboratories on the synthesis and antituberculous and antitumor activities of a series of 3-substituted-2-thiohydrouracils showed a degree of antitumor activity slightly above the level of inhibition needed for further experimental testing according to the Cancer Chemotherapy National Service Center (CCNSC) Protocol for tumor weight inhibition (3). Compounds in the thiohydrouracil series, such as 3-*p*-ethoxyphenyl- and 3-*p*-(*n*-butoxyphenyl)-, showed test/control (T/C) % values of 62 and 63, respectively, against Sarcoma 180 as tested by CCNSC.

On further consideration of the general class of compounds, it was felt that modifying the structures to correspond to that of thymine, which has a methyl

Table I—1-(2-Methyl)carboxyethyl-3-substituted-2-thioureas $CO_2HCHCH_3CH_2NHCSNHR$

Number	R	M.p.[a]	RS[b]	Yield, %	Formula	Analysis, %[c] Calcd.	Found
1	C_6H_5—	137–138°	E–H	74	$C_{11}H_{14}N_2O_2S$	C, 55.19 H, 5.99 N, 11.71	C, 54.91 H, 6.02 N, 11.74
2	*p*-$CH_3OC_6H_4$	142–143°	E–H	80	$C_{12}H_{16}N_2O_3S$	C, 53.71 H, 6.01 N, 10.44	C, 53.90 H, 5.89 N, 10.65
3	*p*-$C_2H_5OC_6H_4$—	157–158°	E–H	92	$C_{13}H_{18}N_2O_3S$	C, 55.49 H, 6.09 N, 9.96	C, 55.28 H, 6.37 N, 9.90
4	*p*-(*n*-C_3H_7O)C_6H_4—	136–137°	E–H	44	$C_{14}H_{20}N_2O_3S$	C, 56.73 H, 6.80 N, 9.45	C, 56.74 H, 6.73 N, 9.40
5	*p*-(*iso*-C_3H_7O)C_6H_4—	116–117°	E–H	74	$C_{14}H_{20}N_2O_3S$	C, 56.73 H, 6.80 N, 9.45	C, 56.88 H, 6.97 N, 9.63
6	*p*-(*n*-C_4H_9O)C_6H_4—	99–101°	E–H	77	$C_{15}H_{22}N_2O_3S$	C, 58.04 H, 7.14 N, 9.03	C, 58.14 H, 7.19 N, 9.03
7	*p*-(*n*-$C_5H_{11}O$)C_6H_4—	105–106°	E–H	67	$C_{16}H_{24}N_2O_3S$	C, 59.23 H, 7.45 N, 8.64	C, 59.76 H, 7.33 N, 9.00
8	*p*-FC_6H_4—	157–158°	E–H	63	$C_{11}H_{13}FN_2O_2S$	C, 51.55 H, 5.11 N, 10.93	C, 51.39 H, 4.80 N, 10.68
9	1-$C_{10}H_7$—	141–142°	E–H	54	$C_{15}H_{16}N_2O_2S$	C, 62.16 H, 5.59 N, 9.37	C, 62.24 H, 6.14 N, 9.63
10	*tert*-C_4H_9—	100–101°	B	75	$C_9H_{18}N_2O_2S$	C, 49.51 H, 8.26 N, 12.85	C, 49.39 H, 8.45 N, 12.89

[a] Melting points on Fisher-Johns block—corrected. [b] Recrystallization solvents: E, ethanol; B, benzene; and H, water. [c] Microanalyses by Dr. Kurt Eder, Geneva, Switzerland.

group in the 5-position of the pyrimidine ring, would be worthy of study. Support for this reasoning can be found in consideration of the pathway by which thymine is catabolized, where it has been shown that normal and leukemic leucocytes catabolize thymine to dihydrothymine in the presence of $TPNH_2^+$ (4). Furthermore, thiopyrimidines have been shown to be normal minor constituents of sRNA (5), and the anticytogenic activity of 2-thiothymine on *N. crassa* was determined to meet the criteria of CCNSC in horizontal proliferation tests (6).

DISCUSSION

The compounds of the series being reported were prepared in order to examine the effect of altering the structures in such a manner as to make them coincide with the thiohydrothymine structure and thus to allow some degree of comparison with the antitumor activities of an analogous series of thiohydrouracils reported previously (3). The thiohydrothymines summarized in Tables II and III were prepared through the dehydration and cyclization of the corresponding 1,3-disubstituted thioureas of Table I.

The thioureas of Table I were prepared from the required isothiocyanates and an aqueous solution of the sodium salt of β-aminoisobutyric acid in a general reaction through stirring at room temperature for 1–4 days. Completion of the reaction was indicated by the disappearance of the immiscible isothiocyanate and the loss of the marked odor of the isothiocyanate. *tert*-Butyl isothiocyanate, the only alkyl isothiocyanate used, was reacted with the amino acid in a solution of sodium ethoxide at reflux. Treatment of the reaction mixtures with hydrochloric acid was the procedure used to release the intermediate 1-(2-methyl)carboxyethyl-3-substituted-2-thioureas.

A previous study of thiohydrouracils (3) indicated that polyphosphoric acid was a useful cyclization agent. Indeed, the substituted thioureas of Table I, with the exception of No. 10 which is quite hindered, were cyclized in good yields using polyphosphoric acid at 140–150°. The required isothiocyanates were prepared using thiophosgene and the appropriate amine in a water–chloroform media (7) and were isolated by vacuum distillation of the isothiocyanate.

Spectral correlations of the structures were good in that the IR spectra[1] of both the thioureas and thiohydrothymines showed good agreement in a comparison of spectra of related thioureas and thiohydrouracils (3). The compounds of Tables I and II show IR absorption in general agreement with the —NC=S bands I, II, and III of Rao and Venkataraghaven (8). The spectra of the aromatic substituted, cyclized thiohydrothymines showed the same characteristic loss of the sharp 2.95-μ aromatic NH—stretching band after cyclization of the thioureas as was seen for the thiohydrouracils. The thiohydrothymines could be further identified following cyclization through the appearance of strong absorption around 8.5 μ, which was usually resolved into sharp bands at 8.4, 8.5, and 8.7 μ. Absorption in this area of the spectrum can be assigned to the C=S stretching of the thioamide bond, which was displaced from the normal area of 8.9 μ by the substituted aromatic ring attached to the No. 3 nitrogen of the heterocyclic ring.

The thiohydrothymines were tested under the direction of CCNSC for antitumor activity in mice at dosage levels of 400 mg./kg., with the exception of Compound 5 which proved toxic at this level. The compounds were tested against L-1210 lymphoid leukemia and displayed no significant activity. In this respect, the T/C % values compared quite closely to the analogous thiohydrouracils.

EXPERIMENTAL

Aryl Isothiocyanates—Phenyl, *p*-fluorophenyl, naphthyl, and *tert*-butyl isothiocyanates were used.[2] The remaining isothiocyanates were prepared from thiophosgene and the appropriate amine according to the typical procedure used for *p*-*n*-amoxyphenylisothiocyanate. A solution of 17.9 g. (0.1 mole) of *p*-*n*-amoxyaniline (9) in 50 ml. of chloroform was added with stirring to 11.5 g. (7.3 ml.) of thiophosgene in 80 ml. of water in a 300-ml. flask under a reflux condenser while held at 0° by an ice–salt bath in an efficient hood. The ice was allowed to melt, and the temperature was allowed

[1] Beckman model IR-8 using KBr disks.
[2] Commercial products, Eastman Kodak.

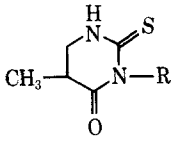

Table II—3-Substituted-2-thiohydrothymines

Number	R	m.p.[a]	RS[b]	Yield, %	Formula	Analysis, %[c] Calcd.	Found
1	C_6H_5—	197–198°	A–B	78	$C_{11}H_{12}N_2OS$	C, 60.00 H, 65.45 N, 12.73	C, 60.13 H, 5.57 N, 12.77
2	*p*-$CH_3OC_6H_4$—	186–187°	A	69	$C_{12}H_{15}N_2O_2S$	C, 57.61 H, 5.62 N, 11.21	C, 57.64 H, 5.69 N, 11.23
3	*p*-$C_2H_5OC_6H_4$—	192–194°	A	70	$C_{13}H_{17}N_2O_2S$	C, 59.10 H, 6.06 N, 10.60	C, 58.91 H, 6.27 N, 10.60
4	*p*-(*n*-C_3H_7O)C_6H_4—	184–185°	A–B	54	$C_{14}H_{19}N_2O_2S$	C, 60.41 H, 6.52 N, 10.14	C, 60.40 H, 6.57 N, 10.17
5	*p*-(*iso*-C_3H_7O)C_6H_4—	208–209°	A–B	84	$C_{14}H_{19}N_2O_2S$	C, 60.41 H, 6.52 N, 10.14	C, 60.48 H, 6.49 N, 10.21
6	*p*-(*n*-C_4H_9O)C_6H_4—	179–180°	A–B	71	$C_{15}H_{21}N_2O_2S$	C, 61.64 H, 6.85 N, 9.59	C, 61.79 H, 6.99 N, 9.56
7	*p*-(*n*-$C_5H_{11}O$)C_6H_4—	211–213°	A–B	63	$C_{16}H_{23}N_2O_2S$	C, 62.73 H, 7.19 N, 9.15	C, 62.96 H, 7.21 N, 9.16
8	*p*-FC_6H_4—	214–215°	A–B	83	$C_{11}H_{11}FN_2OS$	C, 55.51 H, 4.62 N, 11.76	C, 55.58 H, 4.90 N, 11.73
9	1-$C_{10}H_7$—	208–210°	A–B	74	$C_{15}H_{14}N_2OS$	C, 66.73 H, 5.42 N, 10.32	C, 66.72 H, 5.35 N, 10.37

[a] See Table I. [b] Recrystallization solvents: A, acetone; and B, benzene. [c] See Table I.

to rise to that of the room. Two layers developed upon standing overnight. The upper layer consisted of a white, milky emulsion which was broken by the addition of a saturated sodium chloride solution. Following the cracking of the emulsion, the layer was separated and dried over sodium sulfate, and the chloroform evaporated. The residue was subjected to vacuum distillation at 1 mm. to yield 14 g. (63%) of *p*-*n*-amoxyphenylisothiocyanate, b.p. 142–144°. Reported 147–150° (0.8 mm.) (10). IR (film) 4.8 μ.

1-(2-Methyl)carboxyethyl-3-*p*-(*n*-amoxy)phenyl-2-thiourea—A 0.03 *M* solution of *p*-*n*-amoxyphenylisothiocyanate (6.8 g.) and β-aminoisobutyric acid (3.2 g.) in 35 ml. of 1 *N* aqueous sodium hydroxide solution was stirred on a magnetic stirrer in a stoppered 125-ml. extraction flask for 4 days at room temperature. The reaction mixture was worked up in an analogous manner to that used by Glasser and Doughty (3) to yield 7.3 g. of product, m.p. 94–103°, which, after recrystallization from alcohol–water, gave 6.5 g. of 1-(2-methyl)carboxyethyl-3-(*p*-*n*-amoxy)phenyl-2-thiourea as a white crystalline solid, m.p. 105–106°. IR (KBr) 2.95, 3.15, 3.40, 5.96, 6.52, 6.68, 8.08, 9.81, 12.02 μ. (See Table I, No. 7.)

Table III—Antitumor Screening Results for 3-Substituted-2-thiohydrothymines[a]

Number[b]	Dose[c]	Survivors	Animal Wt. Diff., T/C	Average Evaluation[d] Test	Control	T/C, %
1	400	6/6	−1.8	8.8	9.1	96
2	400	5/6	−0.2	8.4	9.1	92
3	400	6/6	−1.7	9.0	9.1	98
4	400	6/6	−1.2	9.3	9.1	102
5	25	6/6	−1.5	9.0	9.3	96
6	400	6/6	−1.3	8.5	9.1	93
7	400	6/6	−2.1	8.5	9.1	93
8	400	6/6	−2.1	8.5	9.1	93
9	400	6/6	−1.0	9.3	9.1	102

[a] CCNSC according to protocol using lymphoid leukemia L-1210. [b] Table II. [c] Milligrams/kilogram in BDF mice. [d] Tumor weight in grams.

3-*p*-(*n*-Amoxy)phenyl-2-thiohydrothymine—A sample of 3.8 g. of 1-(2-methyl)carboxyethyl-3-(*p*-*n*-amoxy)phenyl-2-thiourea was cyclized using 1 g. of polyphosphoric acid as previously described (3) to yield 3.6 g. of product, m.p. 199–208°. Recrystallization from acetone–benzene gave 2.3 g. (64%) of 3-*p*-(*n*-amoxy)phenyl-2-thiohydrothymine as a white crystalline solid, m.p. 212–213°. IR(KBr) 3.14, 3.38, 5.82, 6.40, 6.62, 7.26, 8.05, 8.40, 8.51, 8.68 μ. (See Table II, No. 7.)

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