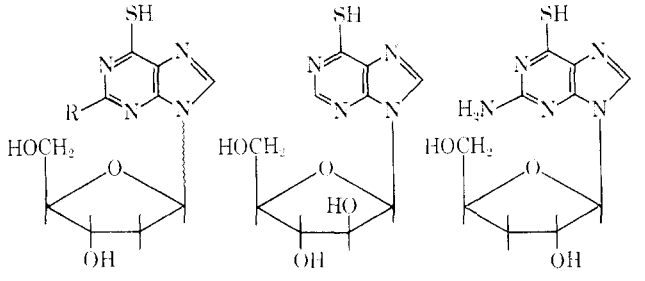
TABLE II
PHYSICAL CONSTANTS FOR NUCLEOSIDE DISULFIDES

Starting thiol	Mp, °C	$[\alpha]_D$, deg	Yield, %	Method	Disulfides λ_{max}, mμ ($\epsilon \times 10^{-3}$)[d]	Formula[c]
α-3	140–150 (eff)	+54.7[a]	32	A	290 (29.6)	$C_{20}H_{22}N_8O_8S_2 \cdot H_2O$
			74	B		
β-3	~100 (eff)	−23.4[b]	80	B	289 (29.7)	$C_{20}H_{22}N_8O_8S_2 \cdot 1.33H_2O$
α-4	208–209	+110[b]	61	A	322 (21.4)	$C_{20}H_{24}N_{10}O_6S_2 \cdot H_2O$
β-4	214–217 (eff)	−33[a]	81	A	322 (21.1)	$C_{20}H_{24}N_{10}O_6S_2 \cdot 1.67H_2O$
β-5	213–216	+13.5[b]	84	A	288 (28.3)	$C_{20}H_{22}N_8O_8S_2$
β-6	219–221 (eff)	−40[a]	39	A	322 (20.9)	$C_{20}H_{24}N_{10}O_6S_2$

[a] H_2O. [b] DMF. [c] All compounds analyzed correctly for C, H, N, O, S. [d] In 1% DMSO–EtOH.

thiols with significant activity in this screen (25% increase in life span) retain activity but show a loss in potency upon conversion to the disulfides.

One of these compounds **5**-S–S has been tested further in *in vitro* systems at the 1.0-m*M* level and has been found to have negligible effects on inhibition of *de novo* purine biosynthesis (−7%) and adenine phosphoribosyl transferase (13%) and no effect on inosine synthesis by intact human erythrocytes. The compound does show significant inhibition of adenosine kinase (65.3%) and has been designated as warranting further study.[11] The disulfide of **5** was administered (intraperitoneally) to BAF1 female mice which were sacrificed after 1 hr and the urine was examined by paper chromatography.[12] The disulfide-treated mice showed a minor spot corresponding to a maximum of 2.7% excretion of **5** as well as several somewhat more intense spots attributable to other metabolites. Mice administered **5** in a companion experiment excreted 37.5% of the unchanged nucleoside during the first hour. Incubation[12] of the disulfide with minces of mouse liver, mouse spleen, and CA755 ascites cells showed a trace conversion to **5** in the spleen mince and no conversion in contact with the other two tissues. It would appear that conversion of the disulfide to the thiol is extremely slow or that **5** is not a major metabolite. The ability of **5** to prolong the life of skin grafts in mice has been recorded previously.[13] A significant increase in the life span of transplanted goldfish scales has also been noted in our laboratories[14] for fish treated with **5**. This activity is retained upon disulfide formation with a lessening in potency (~50%).



3, R = H
4, R = NH_2
5
6

(11) The *in vitro* data were provided by Dr. Florence R. White, Head, Biochemistry Section, Drug Evaluation Branch, CCNSC, National Cancer Institute.

(12) Personal communication, Dr. G. A. LePage, Department of Biochemical Oncology, Stanford Research Institute, Menlo Park, Calif. We are greatly indebted to Dr. LePage for making these results available prior to publication.

(13) A. P. Kimball, G. A. LePage, B. Bowman, and S. J. Herriot, *Proc. Soc. Exp. Biol. Med.*, **119**, 248 (1965); A. P. Kimball, S. J. Herriot, and P. S. Allenson, *ibid.*, **126**, 181 (1967).

(14) Experiments were carried out under the direction of Dr. L. Levy, Head, Inflammation Section, Biological Sciences Department.

Experimental Section

The thionucleosides used as starting materials were all prepared by literature methods.[2,6–8] The new compounds described all proved homogeneous upon tlc (Eastman Kodak silica gel plates–DMF–$CHCl_3$) or upon paper chromatography (*n*-BuOH–H_2O). Spots were visualized under uv light. Melting points are capillary melting points in open tubes on the Büchi apparatus and are uncorrected. The disulfides tenaciously retained recrystallization solvents which were evident in their nmr spectra (DMSO-d_6).

6,6′-Dithiobis(9-β-D-arabinofuranosyl)-9H-purine (5-S–S) (Method A).—Compound **5** (2.84 g, 0.010 mole) was dissolved in warm (45°) pH 7.6 PO_4^{3-} buffer[4] (700 ml) and the solution was cooled to 30°. An "N I_2" solution[5] (10 ml, 0.005 mole) was added dropwise with stirring at such a rate that the color was dispersed between each addition. A white precipitate separated which was recovered by filtration, washed twice with H_2O (10 ml) and then EtOH (10 ml), then dried *in vacuo* (2.4 g, 84%), mp 228–231°. A portion of the material (1.9 g) was recrystallized by dissolving in DMF (12 ml) and adding H_2O (10 ml) to give colorless crystals (1.89 g), mp 213–216° dec. (See Table II.)

6,6′-Dithiobis[9-(2-deoxy-β-D-*erythro*-pentofuranosyl)]-9H-purine Hydrate (β-3-S–S) (Method B).—β-**3** (0.588 g, 0.0022 mole) was dissolved in warm pH 7.6 PO_4^{3-} buffer[4] (90 ml) and treated dropwise at 38° with a solution of "N I_2"[5] (2.2 ml) as in method A. At the conclusion of the addition the faintly yellow solution was shell frozen and lyophilized. The residue was triturated twice with ice–water (2–3 ml), filtered, and vacuum dried (650 mg). The white solid was recrystallized from warm H_2O (5 ml), with chilling. The crystals were recovered by filtration, washed with cold H_2O (1 ml), and vacuum dried (25°) (0.528 g, 80%), mp ~70° softens, ~110° eff (see Table II).

Acknowledgment.—We are pleased to acknowledge many stimulating and informative discussions with Dr. L. Goodman and his colleagues at Stanford Research Institute, Menlo Park, Calif. Where not otherwise noted results of bioassays were provided by CCNSC.

Synthesis and Evaluation of Salts of Ethylene–Maleic Acid Copolymers as Antitumor Agents[1]

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Copolymers of ethylene and maleic acid inhibit significantly the growth of Sarcoma 180 in mice.[3]

(1) Presented at the Southwest Regional Meeting of the American Chemical Society, Little Rock, Ark., Dec 1967. Abstracted from the M.S. thesis of J. S. H., Oklahoma State University, May 1966.

(2) To whom inquiries should be addressed.

(3) W. Regelson, S. Kukar, M. Tunis, J. Johnson, J. Fields, and E. Ghoseukamp, *Nature*, **186**, 778 (1960).

TABLE I

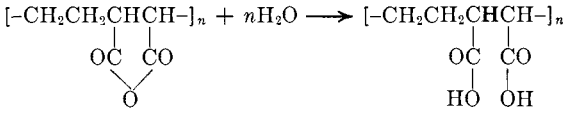
ANTITUMOR SCREENING DATA FOR ETHYLENE–MALEIC ACID COPOLYMERS AND THEIR SALTS[a]

Polymer	Metal	Metal content: Calcd for 1:1 complex, %	Metal content: Found, %	L1210 leukemia of mice: Dose, mg/kg	L1210 leukemia of mice: T/C	Intramuscular Walker sarcoma of rats: Dose, mg/kg	Intramuscular Walker sarcoma of rats: T/C
A	None			400	1.00	400	0.83
	Ni(II)	29.06	8.78	25	1.01		
	Cu(II)	30.60	14.57	20	0.89		
	Co(II)	29.03	6.25	100	1.24	400	0.25
B	None			7	1.14	15	0.28
	Ni(II)	29.06	8.60	10	0.95	10	1.25
	Cu(II)	30.60	8.86	10	0.91	20	0.55
	Co(II)	29.03	5.48	10	0.89	20	0.48
C	None			5	1.02	10	0.41
	Ni(II)	29.06	8.99	20	0.88	10	0.80
	Cu(II)	30.60	10.01	5	0.90	10	0.80
	Co(II)	29.03	6.87	5	0.90	10	0.45

[a] The screening data were supplied through the kindness of Dr. Harry B. Wood, Jr., of the Cancer Chemotherapy National Service Center, National Institutes of Health, Bethesda, Md. Assays were performed according to CCNSC specifications as reported in the reference in ref 6.

Polymers with higher molecular weight averages have greater antitumor activities, but their toxicities per gram increase also. Other functional derivatives such as the amide ammonium salt and the amide acid of the ethylene–maleic acid copolymers are less toxic and somewhat less effective therapeutically when equal weights are used. The purpose of this work was to study several copolymers of ethylene and maleic acid of different molecular weights in order to discover some physical or chemical property which could be related to their antitumor activities.

These polymers are produced by the reaction of ethylene and maleic anhydride under conditions designed to give different average molecular weights. When the anhydrides are stirred in warm water for a few minutes they are converted to water-soluble acids without destruction of the polymer chain as follows.



Since these polymeric acids are good chelating agents we first attempted to determine the abilities of three polymers with different molecular weight averages to complex with copper(II), cobalt(II), and nickel(II) ions using the potentiometric method described by Albert and Serjeant.[4] Since complexation of the metals with the acids releases H^+, the lowering of the pH of the solution caused by addition of the metal ions is a qualitative measure of the extent of complexing between the metal and the acid. Although our results did not allow us to calculate absolute values for the stability constants of the complexes of the polymeric acids and these metal ions, it was clearly demonstrated that the three polymers do not differ significantly from one another in their abilities to complex these metals. Stability constants for the complexes of one ethylene–maleic acid copolymer (polymer B) with several metals have been determined;[5] the mean values of K_{MA} (where

$$K_{MA} = [MA]/[M^{2+}][A^{2-}]f_2^2$$

f_2 is the mean activity coefficient of the divalent ions) for Co(II), Ni(II), and Cu(II) are 4.29×10^8, 3.76×10^8, and 4.45×10^{10}, respectively. In an aqueous solution at pH 7.00 with metal and polymer (based on monomer) concentrations of 1×10^{-5} M, the percentage of Co(II), Ni(II), and Cu(II) complexed by the polymer would be approximately 91, 90, and 99%, respectively.

Cu(II), Co(II), and Ni(II) salts of the three polymers were prepared in order to test their antitumor activities in experimental animals. Since these are polymeric acids the salts do not contain a stoichiometric equivalent of metal; they also contain about 10% of the solvent from which they precipitated. The metal contents of the salts are listed in Table I with the theoretical values for the 1:1 complexes.

The antitumor activities of the polymeric acids and their Cu(II), Co(II), and Ni(II) salts have been determined in two tumor systems with the results shown in the table. Significant activity against L1210 mouse leukemia was shown by none of the metal salts.[6] Significant activity against intramuscular Walker sarcoma of the rat was evidenced by some salts of each polymer and by some salts of Co(II) and Cu(II).[7] The polymeric acids appear to have as much antitumor activity as the salts. The Co(II) salts appear to be more active against the Walker sarcoma than the Cu(II) and Ni(II) salts.

Experimental Section

Ethylene–Maleic Anhydride Copolymers.—The inhibition of Sarcoma 180 of mice by the three polymers used in this work was known from an earlier study;[3] the polymers were supplied to us for this work by Dr. John Johnson of Monsanto Company, St. Louis, Mo., who had furnished the polymers for the earlier work.[3] These samples were dried under vacuum at 144° for several hours. The average molecular weights of polymers A and B, determined in anhydrous AcMe by means of a Coleman 115 molecular weight apparatus, were 4100 and 17,000, respectively.

(4) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," John Wiley and Sons, Inc., New York, N. Y., 1962, pp 154–167.

(5) B. J. Felber, E. M. Hodnett, and N. Purdie, *J. Phys. Chem.*, **72**, 2496 (1968).

(6) The effectiveness of a compound against mouse leukemia L1210 is determined by the ability of the compound to lengthen the lives of treated (T) mice bearing this leukemia compared to those not treated (C); the value of T/C must be at least 1.25 for the antileukemia activity to be significant: *Cancer Chemotherapy Rept.*, **25**, 1 (1962).

(7) Effectiveness against intramuscular Walker sarcoma of the rat is measured by weights of tumors of treated rats (T) compared to the tumors of control rats (C); the value of T/C must be no more than 0.63 for significant activity: see reference in ref 6.

The specific viscosities in 1% solutions in DMF for the three polymers are 0.1, 0.6, and 1.0; the average molecular weight of polymer C was calculated to be 43,000.

Determination of Chelating Abilities.—Solutions of 0.02 *N* acid were prepared of each polymer by refluxing an aqueous solution of 1.26 g of the polymer and diluting this to 1 l. at room temperature. This solution was titrated potentiometrically with 0.1 *N* base in order to determine the dissociation constants of the acids. The titration was repeated with Cu(II), Co(II), or Ni(II) ions as perchlorates. The difference in acidity of the solutions for the same amount of base added is an indication of the amount of chelation between the acid and the metal ions.

Synthesis of Metal Complexes.—Samples of each ethylene maleic anhydride copolymer were weighed carefully (6.3 g) and hydrolyzed in aqueous DMF by stirring and warming. A theoretical amount of $Cu(NO_3)_2$ (6.04 g), $CoCl_2$ (5.95 g), or $NiCl_2$ (5.95 g) was added to each solution of polymer and the pH was adjusted to 6 with KOH. The precipitated complex was separated by filtration and centrifugation, and dried to constant weight under vacuum. The salts were analyzed for metal by standard EDTA titrations.

Acknowledgments.—We are grateful to Michael Longmire who performed the metal analyses. Financial support for this work came from the Department of Chemistry, the Lew Wentz Foundation of Oklahoma State University, and the U. S. Public Health Service through a Biomedical Sciences Support Grant.

New Compounds

Synthesis of 3-Hydroxy- and 3-Methoxyindole-2-carboxamides and Esters

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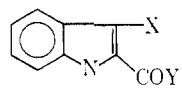
3-Alkoxyindole-2-carboxylates and -2-carboxamides have been shown to possess antiemetic properties.[1] Most of the compounds studied have alkyl or dialkylaminoalkyl side chains. In this communication the preparation of some 3-hydroxyindole-2-carboxamides with aromatic side chains is reported (Table I).

TABLE I



No.	X	Y	Mp, °C	Recrystn[a] solvent	Yield, %	Formula[b]
1	OH[c]	NHPh	220	A	58	$C_{15}H_{12}N_2O_2$
2	OH[c]	$NHC_6H_4OCH_3$-*p*	238	B	58	$C_{16}H_{14}N_2O_3$
3	OH[c]	$NHC_6H_4OC_2H_5$-*p*	206	B	60	$C_{17}H_{16}N_2O_3$
4	OH[c]		169–170	A	60	$C_{13}H_{14}N_2O_3$
5	OH	OCH_2Ph	152–153	C	51	$C_{16}H_{13}NO_3$
6	OMe	NHPh	176	A	52	$C_{16}H_{14}N_2O_2$
7	OMe	$NHC_6H_4OC_2H_5$-*p*	211	A	54	$C_{18}H_{18}N_2O_3$
8	OMe		150	A	55	$C_{14}H_{16}N_2O_3$
9	H	NHPh	200	A	76	$C_{15}H_{12}N_2O$
10	H	$NHC_6H_4OC_2H_5$-*p*	219	A	71	$C_{19}H_{16}N_2O_2$
11	H		179	A	73	$C_{13}H_{14}N_2O_2$

[a] A = EtOH, B = MeOH, C = C_6H_6–petroleum ether (bp 60–80°). [b] All the analyses were performed for C, H, and N and results were in the range ±0.4%. [c] These compounds first darken and then melt.

Experimental Section[2]

3-Methoxyindole-2-carboxamides (Table I).—Indoxylic ester was prepared by cyclization of *o*-carbomethoxyphenylglycine methyl ester in the presence of NaOMe.[3] The ester was methylated and hydrolyzed with ethanolic KOH.[4] 3-Methoxyindole-2-carboxylic acid (1 g, 0.005 mole) was converted to its acid chloride with $SOCl_2$ (1.42 g, 0.012 mole) in dry Et_2O. After standing for 1 hr at room temperature, Et_2O was removed under reduced pressure and the residual acid chloride was flushed with dry Et_2O to remove the last traces of $SOCl_2$. The acid chloride was then taken up in Et_2O and treated with ethereal $PhNH_2$ (0.96 g, 0.01 mole). The reaction mixture was left for 1.5 hr at 30°, Et_2O was removed, and the solid residue was washed (5% HCl, 5% $NaHCO_3$, H_2O), dried, and crystallized (EtOH), mp 176°, 0.66 g (52%). *Anal.* ($C_{18}H_{14}N_2O_2$) C, H, N.

3-Hydroxyindole-2-carboxamides (Table I).—Chloroacetyl derivatives of amines and benzyl alcohol were prepared by the usual methods.[5]

(a) *o*-Carbomethoxyphenylglycine-*p*-methoxyanilide (Table II).—Chloroacetyl-*p*-anisidine (6.0 g, 0.03 mole) and methyl anthranilate (18.12 g, 0.12 mole) were heated on a steam bath for 8 hr under anhydrous conditions. Dry C_6H_6 (100 ml) was added, and methyl anthranilate hydrochloride was filtered off. C_6H_6 was then removed under reduced pressure and the residue was heated again for another 6 hr and then the hydrochloride was removed as before. The hydrochloride which separated weighed

(1) Société d'Etudes scientifiques et industrielles de l'Ile-de-France, French Patent 1527 (Nov 12, 1962); *Chem. Abstr.*, **58**, 7911*a* (1963).

(2) Melting points are uncorrected.

(3) A. Robertson, *J. Chem. Soc.*, 1937 (1927).

(4) N. T. Modi, Ph.D. Thesis, Aligarh Muslim University, 1966.

(5) (a) W. A. Jacobs and M. Heidelberger, *J. Biol. Chem.*, **21**, 103 (1915); (b) P. Malatesta and G. Migliaceso, *Farmaco, Ed. Sci.*, **11**, 113 (1956); (c) A. L. Remizov and N. V. Khromov Borizov, *Zh. Obshch. Khim.*, **26**, 1451 (1956).