Tlc of perhydrocapreomycin using 1-BAWAA revealed only one ninhydrin-positive spot, which has the same R_f value of capreomycin. Bioassay revealed perhydrocapreomycin to be 43% as active as capreomycin itself.

Acknowledgment.—The authors are indebted to Dr. Paul D. Shaw and Mr. René deBus, University of Illinois, Urbana, Ill., for the bioassays, and to Parke, Davis and Company and Eli Lilly and Company for their generous gifts of viomycin and capreomycin, resp.

Cobalt Chelates of Schiff Bases of Aromatic Amines as Antitumor Agents

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Some metal chelates have shown activity against tumors of experimental animals.[2-4] We have therefore prepared some Co derivatives of the Schiff bases of salicylaldehyde and various aromatic amines and have determined their activities against L1210 leukemia, ascitic sarcoma 180, and Lewis lung carcinoma in mice.

Similar metal derivatives have been prepared by Bailes and Calvin[5] as O carriers, using Co salts and various Schiff bases. We have found that good yields of pure compounds are obtained from the amine, salicylaldehyde, and $Co(OAc)_2$. Analysis of each compound for Co indicates that 2 molecules of ligand have combined with each Co^{+2} except for III, which has only 1 molecule of ligand for each Co^{2+}. The properties of 12 of these compounds are shown in Table I. The metal chelates are dark-colored solids with limited solubility in organic solvents; 4 dissolve to a small extent in dioxane and in benzene. Their stabilities in aq soln were unknown as were their mode of action in biological systems.

The toxicities of these compounds to mice were determined for 2 routes of administration: ip injection and gavage. Postmortem examination of the animals receiving ip injections showed no drug deposit at the site of injection although the chelates have practically no solubility in H_2O. The antitumor activities of these chelates were evaluated against L1210 leukemia, sarcoma 180 ascites, and the Lewis lung carcinoma using both ip and gavage routes of administration. The results of these tests are shown in Table II. While none of these compounds meet or exceed the CCNSC criteria of significant activity, several of the reported activities are sufficiently close to the borderline to be of possible extrapolative value for further studies. This might be done through a multiple parametric approach.

TABLE I

PREPARATION AND PROPERTIES OF COBALT DERIVATIVES OF SCHIFF BASES

Name	Formula[a]	Mp, °C	Color	Yield, %
Cobalt, bis[*o*-(*N*-phenylformimidoyl)phenolato]-	$C_{26}H_{20}CoN_2O_2$	188–192[b,c]	Reddish yellow	52
Cobalt(II), bis[*o*-(*N*-*o*-tolylformimidoyl)-phenolato]-	$C_{28}H_{24}CoN_2O_2$	195.5–197[d]	Red	95
Cobalt(II), bis[*o*-(*N*-*p*-tolylformimidoyl)-phenolato]-	$C_{28}H_{24}CoN_2O_2$	183–184	Reddish purple	82
Cobalt(II), bis[*o*-[*N*-(*o*-mercaptophenyl)-formimidoyl]phenolato]-	$C_{26}H_{20}CoN_2O_2S_2$	>300	Brown-black	51
Cobalt(II), [*N*-salicylidenenanthranilato(2-)]-	$C_{14}H_9CoNO_3$	196	Brownish orange	68
Cobalt(II), bis[*p*-(salicylideneamino)benzoato]-	$C_{28}H_{20}CoN_2O_6$	>300	Mustard yellow	95
Cobalt(II), bis(salicylaldehydato), dihydrate	$C_{14}H_{10}CoO_4 \cdot 2H_2O$[e]	>300	Yellow	87
Cobalt(II), bis[*o*-[*N*-(*p*-hydroxyphenyl)-formimidoyl]phenolato]-	$C_{26}H_{20}CoN_2O_4$	277–280	Red	98
Cobalt(II), bis[*o*-[*N*-(*o*-methoxyphenyl)-formimidoyl]phenolato]-	$C_{28}H_{24}CoN_2O_4$[c]	288–291	Reddish purple	96
Cobalt(II), bis[*o*-(*N*-(*p*-methoxyphenyl)-formimidoyl]phenolato]-	$C_{28}H_{24}CoN_2O_4$	185.5–186.5	Red-purple	84
Cobalt(II), bis[*o*-[*N*-[*p*-(dimethylamino)phenyl]-formimidoyl]phenolato]-	$C_{30}H_{30}CoN_4O_2$	265–267	Purple	95
Cobalt(II), bis[*o*-[*N*-(*o*-nitro)formimidoyl]-phenolato]-	$C_{26}H_{18}CoN_4O_6$	>300	Light brown	70

[a] All compds were analyzed for Co. The Co content agreed with the theoretical value within acceptable limits. [b] E. M. Hodnett and W. Willie, *Proc. Okla. Acad. Sci.*, **46**, 107 (1966). [c] B. West, *J. Chem. Soc.*, 3115 (1952); no mp given. [d] H. Nishikawa and S. Yamada, *Bull. Chem. Soc. Jap.*, **38**, 1506 (1965). [e] R. H. Bailes and M. Calvin, *J. Amer. Chem. Soc.*, **69**, 1886 (1947).

Experimental Section

General Synthesis.—$Co(OAc)_2$ (25 mmoles) was placed in a 500-ml flask equipped with an N_2 inlet. Solvent (generally 40 ml of EtOH) was added to the metal acetate and stirred magnetically until the acetate had dissolved. A primary amine (50 mmoles) and 100 mmoles of anhyd Na_2CO_3 were added to the flask with continuous stirring. Salicylaldehyde (50 mmoles), dissolved in 40 ml of the solvent, was placed in the addition funnel. The system was flushed with N_2, and the soln of salicylaldehyde was added with stirring to the mixt. The reaction mixt was warmed until CO_2 evoln ceased and then held somewhat below the bp of the solvent for 15–20 min. The ppt which formed was filtered from the reaction mixt, washed with distd H_2O, and with EtOH, and dried under vacuum at room temp.

(1) The biological phase of this work was conducted under Grant CA-03287 from the National Cancer Institute.

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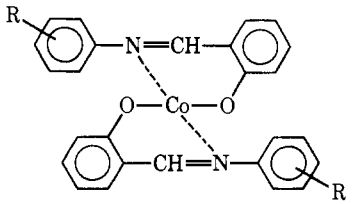
TABLE II: ANTITUMOR ACTIVITIES OF COBALT CHELATES



Compound	R	Formula	Tumor system[a]	Route	Dose, mg/kg	No. of treatments	Survivors	Weight change T/C, g	Results T/C,%
I	*o*-CH_3	$C_{28}H_{24}CoN_2O_2$	L1210	Ip	100	6	6/6	−2.0/+2.6	121
				Gavage	400	5	5/5	−0.9/+2.6	109
			S180A	Ip	100	5	6/6	−2.5/+4.8	84
				Gavage	400	4	6/6	−1.1/+4.8	59
			LLCa	Ip	100	9	6/6	−1.1/+1.1	49
				Gavage	300	9	6/6	−1.1/+1.1	55
II	*p*-CH_3	$C_{28}H_{24}CoN_2O_2$	L1210	Ip	75	6	6/6	−1.0/+2.6	124
				Gavage	400	5	6/6	−1.5/+2.6	110
			S180A	Ip	75	5	6/6	+0.2/+4.8	85
				Gavage	400–200	3	4/6	−1.3/+4.8	70
			LLCa	Ip	75	9	5/6	+0.4/+1.1	74
				Gavage	300	9	6/6	−0.9/+1.1	66
III	*o*-CO_2H	$C_{14}H_9CoNO_3$[b]	L1210	Ip	50	6	6/6	−0.5/+2.0	114
				Gavage	400	5	6/6	+0.2/+2.0	110
			S180A	Ip	50	6	6/6	−2.5/+3.1	101
				Gavage	400	5	4/6	−3.7/+3.1	115
			LLCa	Ip	50	9	6/6	−0.6/−0.1	75
				Gavage	400	9	5/6	−1.8/−0.1	66
IV	*p*-CO_2H	$C_{28}H_{20}CoN_2O_6$	L1210	Ip	50	6	6/6	−2.1/+2.0	113
				Gavage	400	5	6/6	−0.2/+2.0	101
			S180A	Ip	50	6	6/6	+2.7/+6.0	111
				Gavage	400	6	6/6	+1.6/+6.0	100
			LLCa	Ip	50	9	6/6	−0.2/0	100
				Gavage	400	9	6/6	0/0	92
V	*p*-OH	$C_{26}H_{20}CoN_2O_4$	L1210	Ip	25	5	6/6	+1.1/+2.0	111
				Gavage	400	5	6/6	−0.8/+2.0	104
			S180A	Ip	25	6	6/6	+3.1/+3.1	97
				Gavage	400	6	6/6	−2.3/−3.1	114
			LLCa	Ip	25	9	6/6	+0.3/+0.1	78
				Gavage	400	9	5/6	+0.3/+0.1	62
VI	*o*-OCH_3	$C_{28}H_{24}CoN_2O_4$	L1210	Ip	150	5	6/6	−1.5/+2.6	110
				Gavage	400	5	6/6	+0.2/+2.6	107
			S180A	Ip	150	5	6/6	+0.8/+4.8	63
				Gavage	400	5	6/6	+4.0/+4.8	82
			LLCa	Ip	150	9	6/6	−0.8/+1.1	68
				Gavage	400	9	6/6	+1.0/+1.1	70
VII	*p*-OCH_3	$C_{28}H_{24}CoN_2O_4$	L1210	Ip	100	6	5/6	−2.1/+2.6	114
				Gavage	400	5	6/6	−0.7/+2.6	104
			S180A	Ip	100	5	6/6	−1.8/+4.8	90
				Gavage	400	3	6/6	−3.0/+4.8	56
			LLCa	Ip	100	9	6/6	−0.8/+1.1	65
				Gavage	300	9	6/6	+0.6/+1.1	96
VIII	*p*-$N(CH_3)_2$	$C_{30}H_{30}CoN_4O_2$	L1210	Ip	50	5	6/6	+0.7/+2.0	103
				Gavage	50	5	6/6	+2.5/+2.0	100
			S180A	Ip	50	6	6/6	+0.8/+3.1	62
				Gavage	50	6	6/6	+2.8/+3.1	103
			LLCa	Ip	50	9	6/6	−0.6/−0.1	93
				Gavage	50	9	6/6	−0.6/−0.1	81
IX	*o*-NO_2	$C_{26}H_{18}CoN_4O_6$	L1210	Ip	50	6	6/6	−1.2/+2.0	115
				Gavage	400	5	6/6	−1.3/+2.0	106
			S180A	Ip	50	6	6/6	+3.3/+6.0	87
				Gavage	400	6	6/6	+2.8/+6.0	100
			LLCa	Ip	50	9	6/6	−0.9/0	103
				Gavage	400	9	5/6	0/0	108
X	*c*	$C_{14}H_{10}CoO_4 \cdot 2H_2O$	L1210	Ip	50	6	6/6	−3.8/+2.0	123
				Gavage	200	5	6/6	+1.7/+2.0	103
			S180A	Ip	50	6	6/6	+2.6/+6.0	92
				Gavage	400	6	6/6	+4.8/+6.0	99
			LLCa	Ip	50	9	5/6	−0.2/0	74
				Gavage	200	9	6/6	−1.0/0 —	71

[a] L1210 = lymphoid leukemia L1210; S180A = ascitic sarcoma 180; LLCa = Lewis lung carcinoma. [b] 1:1 chelate. [c] Bis(salicylaldehydato)cobalt.

All samples were analyzed for their metal content by standard methods. The org matter was first destroyed by oxidn with a mixt of HNO_3–H_2SO_4. Complexometric titrations with standard EDTA soln were performed with murexide indicator in some cases and in others the Hg–EDTA electrode[6] was used as the indicator.

Antitumor Tests.—The methods used in this investigation for the evaluation of antitumor activity in mice have been described elsewhere.[7] The finely ground drugs were suspended in sterile distd H_2O with the addn of a small drop of Tween 80. Doses were chosen on the basis of preliminary toxicity tests. The drugs were given fresh daily ip or by gavage at approximately the max tolerated dose starting 24 hr after tumor inoculation. The index of evaluation for the ascitic tumors is T/C [(mean survival time of treated mice)/(mean survival time of control mice)] × 100. For L1210 a $T/C \geq 125$ is considered positive; for S-180 ascites a % $T/C \geq 150$ is required. In the solid tumor Lewis lung carcinoma % T/C is [(mean tumor weight of treated mice)/(mean tumor weight of control mice)] × 100. A %T/C value ≤ 30 is considered pos.

Spectroscopic Studies.—Schiff bases of salicylaldehyde and arom amines in EtOH have characteristic uv absorptions at 300–360 and at 250–290 nm.[8] The Schiff bases corresponding to the 4 sol Co derivatives show absorbances in anhyd PhH in these regions also. The Co derivatives of these compds in anhyd PhH absorb in the 380- to 420-nm region in addn to the 300- to 360-nm and 250- to 290-nm regions. However, when the Co chelates of these Schiff bases were placed in anhyd dioxane, the spectra changed within a few min. In the case of II an absorption peak at 390 nm decreased and in the case of I a peak at 340 nm increased in intensity. These changes, which were not observed with anhyd PhH solns, could have been caused by reaction with a small amt of H_2O left in the dioxane or by complexation with the dioxane itself.

Solubilities.—The solubilities of I, II, VII, and VIII were detd in anhyd PhH. The other compds did not dissolve to any measurable extent in this solvent. The solvent was dried for 5 days by passing it repeatedly through a Soxhlet extractor filled with 4-A Molecular Sieves;[9] care was then taken to avoid absorption of H_2O from the atm.

Solubilities in PhH (given in Table III) were detd spectroscopically by use of Beer's law plots on solns of known concns.

TABLE III

SOLUBILITIES AND RATES OF HYDROLYSIS

Compound	R	Solubilities of chelates in benzene, g/100 ml	Half-times of hydrolysis, min: Schiff base	Half-times of hydrolysis, min: Cobalt deriv
I	*o*-CH_3	0.143	7.1	5.8
II	*p*-CH_3	0.166	6.5	5.4
VII	*p*-CH_3O	0.041	17	22
VIII	*p*-$N(CH_3)_2$	0.031	1.8	3.0

These solubilities differ widely and are related to the melting points; the compds with the higher melting points being less sol.

For the study of the rates of hydrolysis, each of the 4 sol compds was dissolved in 1 ml of anhyd dioxane and at time zero this soln was mixed rapidly with 100 ml of pH 7.0 phosphate buffer. An aliquot of the soln was placed in a quartz cell and the uv spectrum was scanned from 220 to 400 nm in about 4 min with a Cary 14 recording spectrophotometer. The spectrum was recorded at various times until no further changes were seen.

The absorption peak at 380–390 nm, characteristic of the Co chelates, disappeared in the first 5 min and was replaced by a peak at 330–340 nm which is characteristic of the Schiff bases. The latter decreased at a measurable rate and so was used for detn of the rate of hydrolysis. A peak at 255 nm, characteristic of salicylaldehyde,[10] increased slowly and was also used for studies on the rate of hydrolysis.

The half-times of approach to equil, calcd by the method of Reeves,[11] are shown in Table III for the Co chelates and the Schiff bases. The values obtd for the Schiff bases are in agreement with the values found by Reeves.[11]

Acknowledgment.—Grateful acknowledgment is made of the assistance of the staff of the Research Foundation of Oklahoma State University in preparing this report.

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Synthesis and Antifungal Activity of Substituted Carbanilic Acid Esters

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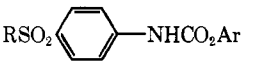
As part of a continuing study of the chemical and pharmacological properties of substituted carbamate esters,[1-4] new compds listed in Table I were prepared by Curtius degradation of appropriate azides. New azides prepared are tabulated in Table II.

TABLE I



Compd	R	Ar	Mp, °C	Yield, %	Formula[a]
1	CH_3	TCP[b]	185	32	$C_{14}H_{10}Cl_3NO_4S$
2	C_2H_5[c]	TCP	208	41	$C_{15}H_{12}Cl_3NO_4S$
3	C_6H_5	TCP	195	44	$C_{19}H_{12}Cl_3NO_4S$
4	H_2N	TCP	235	49	$C_{13}H_9Cl_3N_2O_4S$
5	$(CH_2)_4N$	TCP	190	44	$C_{17}H_{16}Cl_3N_2O_4S$
6	$O(CH_2)_4N$	TCP	260	52	$C_{17}H_{16}Cl_3N_2O_5S$
7	CH_3	PCP[d]	145	45	$C_{14}H_8Cl_6NO_4S$
8	C_2H_5[c]	PCP	127	77	$C_{15}H_{10}Cl_5NO_4S$
9	*p*-FC_6H_4	PCP	170	75	$C_{19}H_9Cl_5FNO_4S$
10	H_2N	PCP	230	31	$C_{13}H_7Cl_5N_2O_4S$
11	$(CH_3)_2N$[c]	PCP	174	83	$C_{15}H_{11}Cl_5N_2O_4S$
12	$(CH_2)_4N$	PCP	135	80	$C_{17}H_{12}Cl_5N_2O_4S$
13	$O(CH_2)_4N$	PCP	172	39	$C_{17}H_{12}Cl_5N_2O_5S$
14	$(CH_2)_5N$[e]	PCP	180	72	$C_{18}H_{14}Cl_5N_2O_4S$
15	Cyclo $C_6H_{11}NH$[e]	PCP	155	65	$C_{19}H_{17}Cl_5N_2O_4S$

[a] All compds were analyzed for C, H, and the results were satisfactory. Ir and nmr spectra were as expected. [b] TCP = 2,4,6-trichlorophenyl. [c] Azide was prepd according to ref 1. [d] PCP = pentachlorophenyl. [e] Azide was prepd according to ref 2.

All compds prepared were tested *in vitro* for antifungal activity against *Candida albicans*, *Aspergilus niger*, and *Penicillum sp.* Concentrations of 10 and 25 μg/ml of each compd in BBL Sabouraud dextrose agar medium were used. Compds were dissolved in acetone (1.5 mg/ml), diluted with hot culture medium to the desired concn, and autoclaved at 120° for 1 hr.

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