product formed a ppt it was filtered off and recrystd from either C_6H_6 or $CHCl_3$–hexane. (b) When the product remained in soln the EtOH was removed *in vacuo* and the resulting foam was dissolved in $CHCl_3$ and added dropwise, with stirring to 10 vol of hexane. The ppt was filtered off and further purified by a second pptn. Yields varied between 50 and 80%.

Tetrabenzylgentamicin C_2 (III).—$NaBH_4$ (0.43 g) was added to a refluxing soln of pentabenzylidine gentamicin C_2 (0.66 g) in abs EtOH (25 ml). Refluxing was contd for 3.5 hr. The soln was cooled and acidified to pH 1.5 with 1 *N* H_2SO_4–MeOH. After 1 hr at room temp the soln was poured into dil NH_4OH (2 *N*, 200 ml) and extd with $CHCl_3$ (2 × 100 ml). The ext was washed with 5% $NaHSO_3$ and H_2O, dried ($MgSO_4$), filtered, and evapd to yield a colorless foam. Tlc (silica gel, $CHCl_3$–MeOH, 9:1) showed 1 major component, R_f ~0.7, and several minor impurities. Column chromatography on silica gel (45 g) in the same system afforded the pure tetra-*N*-benzylgentamicin C_2 as a colorless foam (0.35 g): mp 63–65°, $[\alpha]^{26}D$ + 80.3° (0.3, $CHCl_3$). *Anal.* ($C_{48}H_{63}N_5O_7$) calcd, C, 69.9; H, 7.97; N, 8.50%. Found, C, 70.6; H, 7.63; N, 7.48. It did not prove possible to obtain a better microanal. and this we attribute to occluded solvent, a problem we have encountered with several other free base aminoglycosides.[9]

(9) Part 2: D. J. Cooper, M. D. Yudis, H. M. Marigliano, and T. Traubel, *J. Chem. Soc.*, submitted for publication.

Catalytic Hydrogenation of Viomycin and Capreomycin[1]

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Viomycin, isolated in 1950 from cultures of *Streptomyces puniceus* and *Streptomyces floridae*,[2] and capreomycin, isolated in 1960 from cultures of *Streptomyces capreolus*,[3] are well-known antibiotics used against streptomycin-resistant strains of *Mycobacterium tuberculosis*. Both cause serious side effects, including kidney damage, vestibular dysfunction, electrolyte imbalance, and hypersensitivity. New derivatives of viomycin and capreomycin that demonstrate biological activity are of interest since any derivative that has fewer or less serious side effects would be important in the treatment of disease.

The perhydrogenated derivatives of both viomycin and capreomycin were suggested by a brief but undetailed report of the catalytic reduction of viomycin.[4] Both viomycin and capreomycin were catalytically hydrogenated, and the purity and properties of the resulting products were studied. Tlc in several solvent systems revealed that the hydrogenated materials were not identical with the starting materials. Both perhydrogenated materials showed only one ninhydrin-positive spot in every solvent system tried. Urea was found in the crude perhydrocapreomycin mixture, but not in the perhydroviomycin preparation.

Although viomycin shows strong absorption in the uv region (λ_{max} 268 (ϵ 23,000) in 0.1 *N* HCl and λ_{max} 282.5 nm (ϵ 14,600) in 0.1 *N* NaOH), perhydroviomycin showed only end absorption in both solvents. Perhydrocapreomycin showed absorption at λ_{max} 256 ($E^{1\%}_{1cm}$ 78) and λ_{max} 263 nm ($E^{1\%}_{1cm}$ 74) in H_2O, in contrast to the absorption of capreomycin complex itself, λ_{max} 266 ($E^{1\%}_{1cm}$ 260) in H_2O.

The nmr spectra of both perhydroviomycin and perhydrocapreomycin were similar to the spectra of the parent compounds except that in each case, the absorption at τ 1.9 in both viomycin and capreomycin had disappeared.

The biological activities of perhydroviomycin and perhydrocapreomycin were measured by the zone inhibition method using *Bacillus subtilis*. Perhydroviomycin was found to be approximately 30% as active as viomycin, and perhydrocapreomycin 43% as active as capreomycin.

In order to demonstrate homogeneity, the perhydroviomycin was chromatographed using a 550 cm × 1.9 cm Sephadex G-15 column. A total recovery of 92% was achieved from the column. A wt curve of individual fractions was plotted, which revealed that 93% of the material recovered was homogeneous, giving a Gaussian peak in the curve. Bioassay of each fraction in the peak revealed an average activity of 30% of the activity of commercial viomycin.

Experimental Section

Perhydroviomycin.—A 6.8-g sample of dried commercial viomycin sulfate was dissolved in 50 ml of 50% aq AcOH. A 10% Pt–C catalyst (7.5 g) was slurried with 250 ml of 50% aq AcOH and equilibrated with H_2. The viomycin sample was introduced with careful exclusion of air, and the soln was stirred under H_2 at room temp and atm pressure for 410 hr. A total of 520 ml of H_2 (STP) was absorbed. The catalyst was filtered off through a bed of Celite, and the filtrate was lyophilized to give 7.3 g of a fluffy, white, amorphous powder. This material was stirred with 90 ml of IR-45(OH^-) ion-exchange resin until the pH was 5.5, the resin was removed by filtration, and the filtrate was applied to a column contg 60 ml of IR-45(SO_4^{2-}) resin and eluted with 250 ml of H_2O. The eluate was lyophilized to give 6.2 g of perhydroviomycin as the stoichiometric sulfate salt. The uv spectrum of this material showed no absorption except end absorption. Tlc in H_2O, 1-BAW,[5] and 1-BAWAA[5] revealed only one ninhydrin-positive spot, which was different from viomycin in R_f value. Bioassay by the zone inhibition method using *B. subtilis* revealed the perhydroviomycin to be 30% as active as viomycin itself.

A 2.4-g sample of the perhydroviomycin was chromatographed on a 550 cm × 1.9 cm Sephadex G-15 column that had been equilibrated with 0.01 *N* HCO_2H. A total of 2.2 g of this material (92.4%) was recovered. A wt curve of the individual fractions was plotted, which showed that 93% of the material recovered was homogeneous and gave a Gaussian peak in the curve. Bioassay of each fraction in the peak revealed an average activity of 30% of the activity of commercial viomycin.

Perhydrocapreomycin.—A 4.5-g sample of dried commercial capreomycin was treated in the same manner as the viomycin except that the soln was stirred under H_2 for 500 hr. A total of 368 ml of H_2 (STP) was absorbed. The hydrogenated material was worked up in the same manner as perhydroviomycin. The uv spectrum of the perhydrocapreomycin showed absorptions at λ_{max} 256 ($E_{1cm}^{1\%}$ 78) and λ_{max} 263 nm ($E_{1cm}^{1\%}$ 74) in H_2O.

(1) This investigation was supported in part by a National Institute of Health Grant.

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(5) 1-BAW is 1-BuOH–AcOH–H_2O (4:5:1); 1-BAWAA is 1-BuOH–AcOH–H_2O–Me_2CO–3 *N* NH_4OH (9:2:4:3:2).

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.

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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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