of dimethyl sulfoxide without compound. Sufficient buffer was used to give a total volume of 5.0 ml in each case.

B. Ascites. The Ehrlich ascites tumor cells were maintained by weekly transfer of 0.1–0.2 ml of the cell-rich ascites fluid each from Swice white mice with 7–10-day tumors. For respiration studies, approximately 4 ml of the ascites fluid was withdrawn from one mouse following cervical dislocation. Normally, at least 500×10^6 cells (2–3 g wet) could be obtained. The cells were washed in isotonic saline before use and were suspended in 10 ml of saline and maintained in ice until used. From 0.2 to 0.4 ml of this suspension was sufficient for individual respiration rate determinations. In measurements of respiratory inhibition the cells were incubated with pure dimethyl sulfoxide (0.1–0.3 ml) as a control or with dimethyl sulfoxide plus chelate (0.1–0.3 ml) as treated in sufficient glucose-free phosphate–Ringer to give a total volume of 3.0 ml. As with the liver slice measurements, the incubation period was 30 min at 37° at which time the respiration rate was recorded using a Yellowsprings Model 53 oxygraph.

Acknowledgment. This work was supported by U.S. Public Health Service Grant No. CA-13481. We are indebted to Mr. Michael A. Pleiss for his skillful technical assistance in the synthetic and biological testing phases of this work.

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Copper Chelates as Possible Active Forms of the Antiarthritic Agents†

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Copper complexes, a unique class of potentially more therapeutically useful antiarthritic agents having both antiinflammatory and antiulcer activities, are presented. Points of interest with regard to their relatively low toxicities and mechanisms of action are discussed.

Cupric acetate was observed to be more active than hydrocortisone in the carrageenan foot edema model of inflammation.[1] To account for this observation, it was suggested that the administration of copper (Cu) in the form of cupric acetate resulted in the formation of Cu chelates in vivo and that these chelates were responsible for the observed antiinflammatory activity as illustrated in Figure 1. Similarly, the observed antiinflammatory activity of the clinically used antiarthritic agents could be attributed to the formation of a Cu chelate or complex in blood since it is well known that serum Cu levels increase markedly in arthritic disease.[2] As a result, it seemed plausible to suggest that a Cu chelate could be formed in vivo and provide the intermediate required for the observation that both Cu and chelating compounds have antiinflammatory or antiarthritic activity.

A search of the literature with regard to the biologic requirements and toxicity of Cu led to the following conclusions. Copper is an essential element and is required for normal metabolism in man.[2-4] Copper, like the essential fats, amino acids, and enzyme cofactors, is required for normal metabolism of all tissues. Since coordinated forms of Cu are always more stable forms, compared to

† This manuscript is dedicated to the memory of a scholar, major professor, and friend, Edward E. Smissman.

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Table I. Antiinflammatory Activities of Cupric Acetate Compared with the Activities of L- and D-Tryptophan, Anthranilic Acid, and 3,5-Diisopropylsalicylic Acid as Well as Their Cu Chelates

Compound	Carrageenan foot edema[a]	Cotton wad granuloma[b]	Polyarthritis[c]	% potency compared with hydrocortisone[d]	% Cu	LD_{50}, mg/kg sc	TI	
							CFE	PA
Cupric acetate	A at 8 sc	I at 100 sc	I at 30 sc	340	31.8	350	70	0
L-Tryptophan	I at 200 sc	NT	NT					
Cu^{II}(L-tryptophan)$_2$	I at 200 sc	I at 100 sc	NT		13.5			
D-Tryptophan	I at 200 sc	NT	NT					
Cu^{II}(D-tryptophan)$_2$	A at 200 sc	I at 100 sc	NT		13.5			
Anthranilic acid	I at 200 sc	NT	I at 30 sc					
Cu^{II}(anthranilate)$_2$	A at 8 sc	A at 25 sc	A at 1.2 sc (+25)	60	18.9	750 ± 106	94	625
3,5-dips acid	I at 200 sc	NT	I at 30 sc					
Cu^{II}(3,5-dips)$_2$	A at 8 sc	A at 5 sc	A at 1.2 sc (+24)	50	12.5	240 ± 33	30	200

[a] The initial screening and subsequent doses studied in this model were 25, 5, 2, 1, 0.5, and 0.2 mg/rat. A weight of compound suspended in 0.2 ml of saline-Tween 80 was rated as active if it caused a significant decrease ($p < 0.05$) in the circumference of the tibiotarsal (ankle) joint compared to controls given a sc injection of 0.2 ml of saline-Tween 80. The lowest active dose was multiplied by 8 to present the tabulated dose in mg/kg. [b] The initial screening and subsequent doses studied in this model were 20, 5, 2, 1, and 0.5 mg/rat given for 2 days. A weight of compound suspended in 0.2 ml of saline-Tween 80 was rated as active if it caused a significant decrease ($p < 0.05$) in the adjusted weight of granuloma tissue encapsulating the cotton pellets compared to controls receiving only 0.2 ml of saline-Tween 80. The lowest active dose was multiplied by 5 to present the tabulated dose in mg/kg. [c] The initial screening and subsequent doses studied in this model were 5, 2, 1, 0.5, 0.2, and 0.1 mg/rat given for 16 days. A weight of compound suspended in 0.2 ml of saline-Tween 80 was rated as active if it caused a significant decrease ($p < 0.05$) in the circumference of the tibiotarsal (ankle) joint compared to controls receiving 0.2 ml of saline-Tween 80. The lowest active dose was multiplied by 6 to present the tabulated dose in mg/kg. [d] Comparison of the dose-response curves obtained with hydrocortisone and the test compound in the carrageenan foot edema model of inflammation. See footnote *a*.

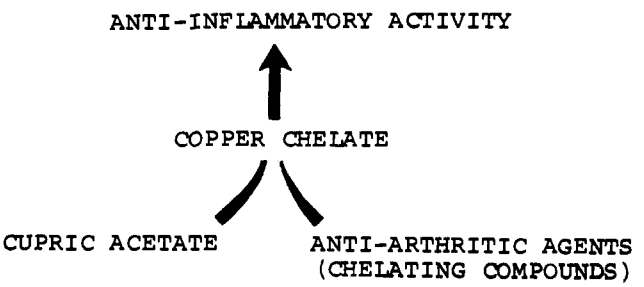


Figure 1. Rationale for the hypothesized active form of antiarthritic compounds.

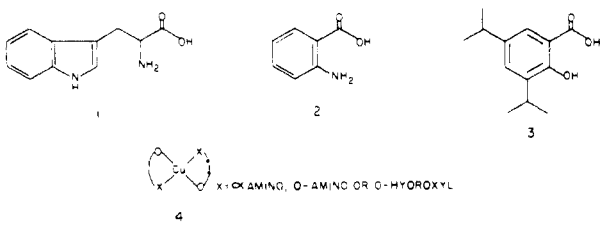
ionized forms, it exists in biological systems as a variety of complexes.[4-8] With the exception of Wilson's disease, which is a genetic disease associated with an accumulation of Cu due to an inability to excrete it, there are no known chronic degenerative diseases in man known to result from nonindustrial exposures to Cu.[2,9,10]

Certainly, not all Cu-containing compounds are nontoxic at all dose levels; but since the body normally handles a tenfold excess of Cu,[4] there must be many nontoxic Cu coordinate compounds present in vivo. One must, therefore, study a particular compound to learn of its relative toxicity.

With this information it was believed to be reasonable to pursue the observed activity of cupric acetate as a potentially useful lead for the development of a better antiarthritic drug which might be more useful in meeting biological requirements in combating a disease process. Fortunately, the hypothesis that Cu chelates were the intermediates responsible for the observed antiinflammatory activity of both cupric acetate and chelating compounds had a simple test. As shown in Figure 1, one only needed to synthesize specific Cu chelates and test them for their antiinflammatory activity. According to pharmacologic theory, if they were the product of chemical transformation in vivo, these chelates would be more active than either cupric acetate or the parent chelating agent. If these Cu chelates were more active than Cu, in the form of cupric acetate, and the parent chelating compounds, they might be correctly considered as the active forms of the antiinflammatory agents. It was felt that if data could be obtained in support of the suggestion that Cu chelates were the active forms of the antiarthritic drugs, then their value in therapy warranted in-depth study. The following are the results obtained in this effort.

Results and Discussion

At the outset, the hypothesis shown in Figure 1 was tested by making and testing Cu chelates prepared from chelating agents that had no known antiinflammatory activity of their own. For this purpose, Cu chelates were synthesized from L- and D-tryptophan (1), anthranilic acid (2), and 3,5-diisopropylsalicylic acid (3,5-dips) (3). All of these 2:1 chelates are believed to be of the square-planar variety[11] with the five bonded atoms of the chelate rings lying in the same plane and possessing general structure 4.



The antiinflammatory activities of these four chelates, their parent compounds, and cupric acetate are presented in Table I. Cupric acetate had earlier been observed to be active (A) in the carrageenan foot edema[12] (CFE) model of inflammation at 8 mg/kg following subcutaneous (sc) administration. However, on follow-up screening it was inactive (I) at the initial screening doses of 100 and 30 mg/kg sc, respectively, in the cotton-wad granuloma[13] (CWG) and therapeutic polyarthritis[14] (PA) models of inflammation, using published statistical methods.[15] The amino acids L- and D-tryptophan were, as expected, inactive in the primary screen and the CFE model, and as customary were not tested (NT) in the two follow-up models of inflammation. Copper D-tryptophan was active only at 125 mg/kg in the CFE model. Both of these were inactive in the CWG model at the initial screening doses of 100 mg/kg.

However, the Cu chelates of both anthranilic acid and 3,5-dips were very much more active than either cupric acetate, anthranilic acid, or 3,5-dips. Copper anthranilate

was active at 8 mg/kg sc in the CFE, 25 mg/kg sc in the CWG, and 1.2 mg/kg sc in the PA models of inflammation. Similarly, Cu 3,5-dips was active at 8 mg/kg sc in the CFE, 5 mg/kg sc in the CWG, and 1.2 mg/kg sc in the PA models of inflammation. At 1.2 mg/kg both copper anthranilate and Cu 3,5-dips gave marked reductions in foot volumes of +25 and +24 in the PA test compared to the standard, butazolidine, which at 6 mg/kg gave a reduction of only +5 when given intragastrically (ig). This +5 reduction in foot volume was a statistically significant ($p < 0.05$) reduction in volume but represented only a minimal reduction in foot volume. The +25 and +24 represent a fivefold decrease in foot volume compared to butazolidine. These large reductions in paw volume suggest that activity might be observed at still lower doses. Both chelates were subsequently shown to be about five times as effective as butazolidine when it was given sc. These two chelates were more active in all of the antiinflammatory tests than either cupric acetate, which was only active in the CFE test, or their parent compounds, which had no measurable activity.

Dose–response plots obtained in progressing from the activity observed at the initial screening dose to the lowest active dose were obtained for cupric acetate, copper anthranilate, and Cu 3,5-dips in the CFE model of inflammation. These dose–response plots were compared with the dose–response plot obtained for hydrocortisone in this model. Based upon these comparisons it was reported that cupric acetate had 340% of the activity of hydrocortisone, while the two chelates had only 60 and 50% of the activity of hydrocortisone. In this acute, 3-hr test, the two chelates appeared to be somewhat less effective than cupric acetate. However, these two chelates were active in all three models of inflammation and did not produce the signs of central nervous system (CNS) toxicity seen with all of the active doses of cupric acetate.

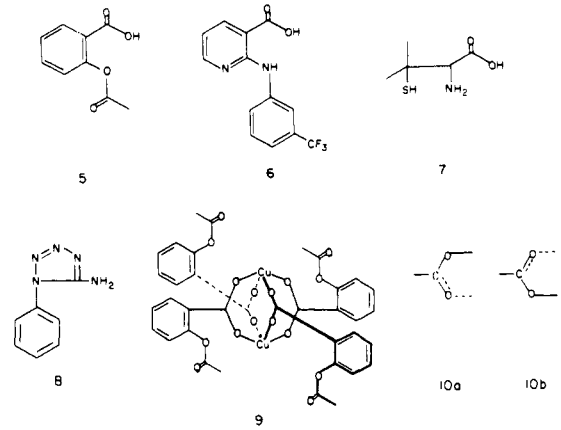
The difference in effectiveness of these compounds does not appear to be related to the amount of Cu in them. Cupric acetate, which was only effective in the CFE model, contained nearly 32% Cu; while copper anthranilate and Cu 3,5-dips, which were active in all three models of inflammation, contained 18.9 and 12.5%, respectively. The two tryptophan chelates contained 13.5% Cu and were inactive by the sc route in all of the test models. Therefore, to account for the observed activity, it is necessary to suppose that the intact chelate mediates the observed antiinflammatory activity, since the observed activity cannot be directly correlated with the activity of the inactive parent compounds or the amount of Cu in the compounds.

To obtain a measure of the acute toxicity of these chelates, the lethal dose in 50% (LD_{50}) of the animals was determined in rats.[16] The LD_{50} for cupric acetate had earlier been shown to be 350 mg/kg sc. The LD_{50} values for copper anthranilate and Cu 3,5-dips were shown to be 750 and 240 mg/kg sc, respectively. Using these data it is possible to evaluate the safety of these compounds by calculating a therapeutic index (TI) for them. The TI, LD_{50}/ED_{50}, for both copper anthranilate and Cu 3,5-dips shows an extremely large separation of lethality and desirable activity.

These two compounds were also evaluated with regard to their production of signs of CNS toxicity. Subcutaneous injections of 20, 40, 80, and 320 mg/kg in mice failed to show signs of CNS stimulation or depression. This was consistent with the lack of observed CNS effects in all of the antiinflammatory testing of these two chelates in rats, although cupric acetate had produced CNS toxicity of unknown pathogenesis at all active doses. It was also found that in mice the LD_{50} values for both of these compounds were greater than 320 mg/kg, the largest dose given.

Toxicity, with regard to liver function,[17,18] was also investigated in rats given these two chelates. Twenty mg/kg, 4–20 times the effective doses of each chelate, was given for 10 days. The usual screen liver function regimen for the evaluation of hepatic toxicity was 10 mg/kg for 4 days, so that giving twice as much for 2.5 times as long was not an experiment weighted in favor of the two chelates. In addition, two doses of cupric acetate, 20 and 10 mg/kg, were tested in the same protocol to distinguish the toxicity of cupric acetate from the two chelates. Serum bromosulfophthalein excretion was not affected following the administration of the two chelates and the two doses of cupric acetate nor was there any evidence of liver parenchymal cell damage or increased permeability with the administration of the two chelates, as evidenced by an elevation of serum glutamic-oxaloacetic transaminase (SGOT) activity. However, rats given cupric acetate had significantly elevated SGOT activities indicating liver cell damage or increased cell membrane permeability at both doses ($p < 0.01$).

Having demonstrated an increase in antiinflammatory activity for the chelates of anthranilic acid and 3,5-dips as well as their safety, as evidenced by their large LD_{50} values, absence of CNS, and liver toxicity, the hypothesis shown in Figure 1 was then tested with clinically used antiarthritic drugs. Copper chelates were prepared from acetylsalicylic acid (aspirin) (**5**), 2-[3-(trifluoromethyl)-phenyl]aminonicotinic acid (niflumic acid) (**6**), D-penicillamine (D-pen) (**7**), and 1-phenyl-5-aminotetrazole (fenamole) (**8**).



5 6 7 8 9 10a 10b

The structure of cupric aspirinate (**9**)[19] is believed to result from intramolecular bonding contributions from the four carboxylate (**10a,b**) groups which facilitate spin coupling of the unpaired electron on each Cu(II) by a super-exchange mechanism.[20–24] The configuration of the carboxylate groups gives rise to a distorted octahedral-bipyrimidal conformation of the binuclear coordination compound. This Cu binuclear,[25] polynuclear,[26] or metal cluster[27] structure is consistent with the structure proposed for the "mono" hydrate of cupric acetate,[28] $Cu_2(OCOCH_3)_4(H_2O)_2$ (**11**). Originally, cupric acetate was suggested to have a weak σ[29] or δ[30] bond formed by the unpaired electrons on the two bivalent Cu [Cu(II)] to account for their closeness in the molecule. However, recent data suggest that closeness of the two Cu(II) is not governed by direct Cu–Cu bonding but by interaction of the unpaired electrons through the carboxylate groups.[28–31] In some cases δ Cu–Cu bonding may contribute more to the stability of the chelate than in others.

It is tempting to suggest that the Cu coordination

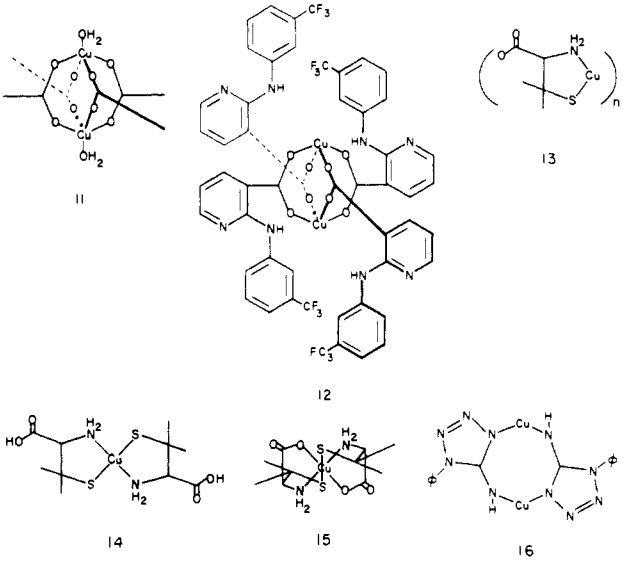
Table II. Comparison of the Antiinflammatory Activities of Some Used Antiarthritic Compounds and Their Copper Chelates

Compound	Carrageenan foot edema[a]	Cotton wad granuloma[b]	Polyarthritis[c]	% potency compared with hydrocortisone[d]	% Cu	LD_{50}, mg/kg	TI CFE	TI PA
Aspirin	A at 64 sc	A at 200 ig	A at 6 sc (+5)	6		1500 ig 790 rt		
Cu^{II}_2(aspirinate)$_4$	A at 8 sc	A at 10 sc	A at 1.2 sc (+27)	130	15.0	760 ± 100 sc	95	633
Niflumic acid	A at 40 ig	A at 25 ig	A at 6 ig (+5)	31		370 ± 25 ig	9	62
Cu^{II}_n(niflumate)$_{2n}$(H_2O)$_n$	A at 8 sc	A at 10 sc	A at 1.2 sc (+18)	180	10.1	650 ± 80 sc	81	542
D-Penicillamine	I at 200 sc	I at 100 sc	I at 30 sc					
Cu^{I}D-pen(H_2O)$_{1.5}$	A at 8 sc	A at 10 sc	NT		26.7			
Cu^{II}_n(D-pen)$_{2n}$(H_2O)$_{2n}$	I at 200 sc	NT	NT		16.1			
Cu^{II}(D-pen disulfide)(H_2O)$_3$	A at 8 sc	A at 25 sc	A at 30 sc (+18)		15.4			
Fenamole	I at 200 sc	A at 100 ig	A at 100 ig (+5)					
Cu^{II}_n(fenamole)$_n$(acetate)$_{2n}$	A at 8 sc	A at 25 sc	A at 30 sc (+5)		18.5			
Cu^{II}_n(fenamole)$_{2n}$(HCl)$_{2n}$	A at 16 sc	A at 10 sc	NT		13.9			

[a] See footnote *a* in Table I. [b] See footnote *b* in Table I. [c] See footnote *c* in Table I. [d] See footnote *d* in Table I.

compounds of the antiarthritic arylcarboxylic and substituted acetic acids have similar structures as illustrated for the Cu chelate of niflumic acid (**12**). The bulk of this acid may provide additional stability as a result of steric hindrance to the approach of a competing, complexing compound.

Three coordination compounds were obtained with D-penicillamine (**7**). The structures of these are at present not known with certainty but from elemental analysis and the available literature[31,32] it seems that the cuprous compound is a polymeric 1:1 coordinate (**13**). Two 2:1 cupric compounds were obtained, one apparently containing Cu–sulfur bonding (**14**) and the other is suggested to contain a disulfide bond (**15**).



Two coordination compounds were obtained when fenamole was allowed to react with $CuCl_2$ and $Cu_2(OCOCH_3)_4(H_2O)_2$. Here it is suggested that two different binuclear chelates were formed, a tetrazolato chelate (**16**) (shown with the two ligands in a single plane and omitting the two projecting above and below this plane) as well as a carboxylato chelate (**17**). A tetrazolato chelate is suggested for **16** since it is known that disubstituted triazines of the type RNHN=NR form binuclear compounds.[20,26] The carboxylato chelate is the result of simple substitution of fenamole for the two molecules of water in $Cu_2(OCOCH_3)_4(H_2O)_2$. Both tetrazolato and carboxylato chelates are of interest, since they offer many possibilities with regard to structural modification and enhanced antiarthritic activity.

Results of tests in the CFE, CWG, and PA models of inflammation are presented in Table II. Comparing the activity observed for aspirin and its Cu chelate demonstrated that cupric aspirinate was more active than aspirin. The chelate was active at one-eighth the lowest active dose of aspirin in the CFE model. No sc data are available for aspirin in the CWG model, so that comparison here is based upon oral data for aspirin. In the PA model, the cupric chelate of aspirin was active at one-fifth the lowest active dose of aspirin and five times as active as aspirin based upon reduction in paw volume (+27). Comparison of the dose–response curves obtained for aspirin and hydrocortisone in the CFE model demonstrated that aspirin had only 6% of the activity of hydrocortisone, while its cupric chelate had 130% of the activity of hydrocortisone. This represents a 20-fold increase in activity for the cupric chelate of aspirin.

In addition, cupric aspirinate was active at 30 mg/kg when given intravenously (iv), while aspirin was inactive. Intravenous testing was done because it was suggested that a compound which was active because it was an irritant, would not be active if given iv.

The Cu chelate of niflumic acid was also found to be active in all three models. Comparison here is somewhat lacking, since one cannot directly compare activities of two compounds given by different routes of administration. It is unfortunate that sc data are not available for niflumic acid in these three models of inflammation. However, the following comparison is viewed as helpful with regard to disclosure of potentially useful information. The parent compound was active at 40 and 25 mg/kg orally in the CFE and CWG models, while the chelate was active at 8 and 10 mg/kg sc in these two models. The lowest active dose in the PA model was 6 mg/kg (+5) orally for niflumic acid and 1.2 mg/kg sc (+18) for Cu niflumate. When both Cu niflumate and niflumic acid were tested iv in the polyarthritic rat, the lowest active dose of niflumic acid was 30 mg/kg, while its Cu chelate was active at 6 mg/kg. The parent compound possessed 31% of the potency of hydrocortisone, while the chelate had 180% of the activity of hydrocortisone in the CFE model.

It is of interest to note that Cu niflumate, having 180% of the potency of hydrocortisone in reducing paw volume, contains 10% Cu, while cupric aspirinate, with 130% of the potency of hydrocortisone, contains 15% Cu. Here again, it is clear that the amount of Cu in the chelate does not directly correlate with the antiinflammatory activity of the chelate.

The clinical use of D-penicillamine (D-pen) as an antiarthritic compound is interesting since it is well known

that it is inactive at the initial screening doses used in these three test models of inflammation. However, two Cu coordination compounds obtained with D-pen, one a cuprous (13) and the other a disulfide cupric complex (15), were found to be active in these three models. The cupric bis(D-pen) compound (14) was found to be inactive at the initial screening dose of 200 mg/kg sc in the CFE model and was not tested in the subsequent follow-up models of inflammation. Both 13 and 15 were active at 8 mg/kg sc in the CFE model. These were also active at 10 and 25 mg/kg sc in the CWG model. In the PA model, only 15 was tested. This compound was found to be active at 30 mg/kg sc with a large reduction in paw swelling. The activities of the D-pen coordination compounds are remarkable, since the parent compound has no activity in these models, even at the large initial screening doses.

Activities of both coordination compounds prepared from fenamole and cupric chloride or cupric acetate were just as remarkable. The parent compound was inactive at the initial screening dose of 200 mg/kg sc in the CFE model, active only at the initial screening dose of 100 mg/kg orally in the CWG model, and only active at a dose four times the usual initial screening dose, 120 mg/kg, when given orally. However, the two fenamole compounds were found to be active at 8 and 16 mg/kg in the CFE and 10 and 25 mg/kg, respectively, in the CWG models. In the PA model, only the acetate complex was tested. In this model, it was shown to have only borderline activity (+5) at 30 mg/kg. However, this may be viewed as a substantial increase in activity in comparison to cupric acetate brought about by the substitution of two fenamole molecules for the two H_2O molecules in $Cu^{II}_2(acetate)_4(H_2O)_2$.

The results of the studies reported in Table II support the suggestion that Cu coordination compounds prepared from the clinically used antiarthritic agents are more active than the parent compounds. The suggestion that this activity is due to the coordinate compound in question is supported by the observed enhancement of the antiinflammatory activity and the lack of a direct correlation between antiinflammatory activity and the amount of Cu in the compound, as shown in Table II.

A comparison of the acute toxicity[23] data, in Table II, available for the parent compounds and their chelates, suggests that the Cu chelates are less toxic. The oral and rectal (RT) LD_{50} values[33] for aspirin are given as 1500 and 790 mg/kg, respectively. The LD_{50} for cupric aspirinate was found to be 760 mg/kg when given sc. Since it is quite likely that the LD_{50} for aspirin is less than 760 mg/kg when given sc, it seems safe to suggest that the chelate has a lesser acute toxicity. The case concerning niflumic acid is more certain. The LD_{50} for this chelate, 650 mg/kg sc, was much higher than the value obtained for the parent compound by the oral route, 370 mg/kg. The LD_{50} for niflumic acid, given intraperitoneally (ip), was found to be 155 mg/kg, which would indicate that the LD_{50} value for the sc route would be something between 155 and 370 mg/kg. Based upon these data, it seems reasonable to suggest that in addition to being more potent as antiinflammatory agents, the Cu chelates are less toxic than the parent compounds.

A consequence of this is that the TI values which are a measure of safety and potency, shown in Table II, are larger for the chelates than for the parent compounds. It is to be pointed out and stressed that these values are remarkably large when compared to the values obtained with most other clinically used drugs.

In addition to the marked increase in antiinflammatory activity and reduced toxicity, of even greater interest was the observation that these chelates were potent antiulcer compounds. Since it is well known that clinically used antiarthritic drugs cause ulcers and gastrointestinal distress, the observed antiulcer activity further distinguishes these coordination compounds from their parent compounds. Patients who take these chronically are known to develop peptic ulcers.[34] However, it has been pointed out that part of the arthritic disease syndrome includes peptic ulceration.[34] In the pathological sense, these ulcers can be viewed as extraarticular inflammatory diseases of the stomach. With this understanding, the antiinflammatory Cu coordination compounds were tested for antiulcer activity in the Shay ulcer model.[35] In these studies *intragastric* (*ig*) or *oral* dosing was done, since the site of the lesion is the stomach. Antiulcer activities of the Cu chelates are presented in Table III.

Table III. Antiulcer Activity of the Cu Chelates

Compound	Shay antiulcer act.[a] ig	Corticoid-induced antiulcer act.[b] ig
$Cu^{II}_2(acetate)_4(H_2O)_2$	A at 225	
$Cu^{II}(L\text{-tryptophan})_2$	A at 0.45	A at 113
$Cu^{II}(D\text{-tryptophan})_2$	A at 4.5	
$Cu^{II}(anthranilate)_2$	A at 4.5	A at 113
$Cu^{II}(3,5\text{-dips})_2$	A at 2.3	A at 113
$Cu^{II}_2(aspirinate)_4$	A at 11.3	
$Cu^{II}_n(niflumate)_{2n}$	A at 4.5	
$Cu^{I}D\text{-pen}(H_2O)_{1.5}$	NT	
$Cu^{II}_n(D\text{-pen})_{2n}$	A at 4.5	
$Cu^{II}(D\text{-pen disulfide})(H_2O)_3$	A at 4.5	
$Cu^{II}_n(fenamole)_n(acetate)_{2n}$	A at 4.5	
$Cu^{II}_n(fenamole)_{2n}(HCl)_{2n}$	A at 4.5	

[a] The initial screening and subsequent doses studied in this model were 50, 10, 5, 2, 1, 0.5, 0.2, 0.1, and 0.05 mg/rat. A weight of compound suspended in 1 ml of saline–Tween 80 was rated as active if it significantly inhibited ulceration ($p < 0.05$) compared to controls given only saline–Tween 80. The lowest active dose was multiplied by 4.5 to present the tabulated dose in mg/kg. [b] The only dose tested in this model was 25 mg/rat. A weight of compound suspended in 1 ml of saline–Tween 80 was rated as active if the ulcer index for the treated group was below 13.2 and the ulcer index for the controls was between 13.2 and 18.8. The ulcer index was calculated by adding the average number of ulcers per group of five rats and the average severity per group of five rats and then dividing this sum by ten. The active dose was then multiplied by 4.5 to present the tabulated dose in mg/kg.

Cupric acetate monohydrate was found to be active as an antiulcer compound only at 225 mg/kg when given orally. Activity at 225 mg/kg is viewed as a very low order of potency and may have been a false positive result or irritant-induced activity. Conversely, cupric L-tryptophan was found to be active at 0.45 mg/kg. By any measure, this compound is an extremely potent antiulcer compound. Similarly, Cu 3,5-dips was found to be active at 2.3 mg/kg. The Cu coordination compounds of D-tryptophan, anthranilic acid, niflumic acid, D-penicillamine, and fenamole were all active at 4.5 mg/kg. Copper aspirinate was active at 11.3 mg/kg. These results were most interesting, since the most common reason for withdrawal from aspirin and D-penicillamine therapy of arthritic diseases is the gastrointestinal distress they cause.

For comparison D-tryptophan, anthranilic acid, niflumic acid, and aspirin were tested for antiulcer activity in this model. Both D-tryptophan and niflumic acid were found to be inactive at the initial screening dose of 225 mg/kg ig. Aspirin and anthranilic acid were active at 225 mg/kg ig. Again, if this is real activity in this model then these two compounds lack potency. The question as to whether or not these are irritant-induced or false positive results remains to be answered.

Table IV. Antiinflammatory and Antiulcer Activities of Some Additional Copper Coordination Compounds

Compound	Carrageenan foot edema[a]	Cotton wad granuloma[b]	Polyarthritis[c]	% Cu	Shay antiulcer act.[d] ig
Amino Acids					
Cu^{II}_n(D-aspartate)$_n(H_2O)_{3.5n}$	A at 8 sc	A at 10 sc	NT	32.6	A at 2.3
Cu^{II}_n(L-aspartate)$_n(H_2O)_{3.5n}$	A at 8 sc	NT	NT	32.6	A at 2.3
Cu^{II}_n(L-lysinate)$_n(Cl)_{2n}(H_2O)_{0.5n}$	A at 4 sc	A at 10 sc	NT	21.9	A at 45
Cu^{II}_n(L-lysinate)$_{2n}(Cl)_{2n}(H_2O)_n$	A at 8 sc	NT	NT	14.3	NT
Cu^{II}(DL-tryptophan)$_2$	I at 200 sc	NT	NT	13.5	A at 4.5
Cu^{II}_n(ε-aminocaproate)$_n(Cl)_{1.5n}(CH_3OH)_{0.5n}$	NT	NT	NT	24.0	A at 11.3
Cu^{II}_n(ε-aminocaproate)$_n(Cl)_{2n}(H_2O)_{0.5n}$	NT	NT	NT	23.2	A at 4.5[e]
Amines					
Cu^{II}_2(pyridine)$_2$(acetate)$_4$	A at 16 sc	NT	NT	24.4	A at 2.3
Cu^{II}(pyridine)$_2(Cl)_2$	A at 8 sc	NT	NT	21.6	A at 45
Cu^{II}(morpholine)$_2(Cl)_2(HCl)_2$	A at 4 sc	A at 25 sc	NT	16.6	NT
Cu^{II}_n(histamine)$_n(Cl)_{2n}(HCl)_{2n}$	NT	NT	NT	20.0	A at 45[e]
Heterocyclic Carboxylic Acids					
Cu^{II}_{3n}(nicotinate)$_{4n}(H_2O)_{3n}$	NT	NT	NT	19.0	A at 4.5
Cu^{II}_n(1-carboxyisoquinoline)$_{2n}$	A at 40 sc	A at 25 sc	NT	15.6	A at 225
Cu^{II}_n(2-phenyl-4-carboxyisoquinoline)$_{2n}$-$(H_2O)_{2n}$	NT	NT	NT	10.6	A at 4.5[e]
Cu^{II}_{2n}(2-carboxyindole)$_{3n}$(acetate)$_n$-$(H_2O)_{0.5n}$	A at 8 sc	A at 10 sc	NT	16.5	A at 2.3
Cu^{II}_{2n}(2-carboxyindole)$_{3n}$(acetate)$_n$-$(H_2O)_{3.5n}$	NT	NT	NT	15.4	A at 4.5[e]
Arylacetic Acid					
Cu^{II}_{2n}[1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetate]$_{4n}(H_2O)_{4n}$	NT	NT	NT	7.8	A at 45[e]
Cu^{II}_{2n}[1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetate]$_{4n}(CH_3COCH_3)_{2n}$	NT	NT	NT	7.7	A at 4.5
Pyrazolidinedione					
Cu^{II}_n(4-*n*-butyl-1,2-diphenyl-3,5-pyrazolidinedione)$_{2n}$	NT	NT	NT	11.8	A at 45
Corticoids					
Hydrocortisone 21-phosphate(Na)$_2$	NT	NT	I at 6 sc[f]	0	I at 45[g]
Cu^{II}_{3n}(HC 21-phosphate)$_{2n}(H_2O)_{9n}$	A at 16 sc	A at 25 sc	A at 6 sc (+7)[f]	15.4	A at 22.5
Cu^{II}_{3n}(HC 21-phosphate)$_{2n}(H_2O)_{7n}$	NT	NT	I at 6 sc[f]	15.9	A at 11.3
Hydrocortisone 21-hemisuccinate	NT	NT	A at 6 sc (+7)[f]	0	I at 45[f]
Cu^{II}_{2n}(HC 21-hemisuccinate)$_{4n}(H_2O)_{6n}$ (green)	A at 16 sc	A at 25 sc	A at 6 sc (+7)[f]	6.1	A at 22.5
$Cu^{II}_{2.5n}$(HC 21-hemisuccinate)$_{2n}(H_2O)_{7n}$ (blue)	A at 16sc	A at 5 sc	A at 6 sc (+11)[f]	13.5	A at 45[e]
Cu^{II}_{3n}(dexamethasone 21-phosphate)$_{2n}$-$(H_2O)_{7n}$	NT	NT	NT	15.0	A at 22.5
Cu^{II}_{3n}(dexamethasone 21-phosphate)$_{2n}$-$(H_2O)_{1.5n}$	NT	NT	NT	16.5	A at 22.5
Steroidal Acid					
Cu^{II}_n(17-hydroxy-3-oxo-17α-pregna-4,6-diene-21-carboxylate)$_{2n}(H_2O)_{2n}$	I at 40 sc	NT	I at 30 sc[f]	7.8	A at 22.5

[a] See footnote *a* in Table I. [b] See footnote *b* in Table I. [c] See footnote *c* in Table I. [d] See footnote *a* in Table III. [e] Not tested at lower dose. [f] Only dose tested. [g] Hydrocortisone was inactive at 5 mg ig.

In addition to the Shay antiulcer activity, a number of these chelates have been shown to have antiulcer activity in the corticoid induced ulcer model.[36] Copper coordination compounds of L-tryptophan, anthranilic acid, and 3,5-dips were found to be active at the initial screening dose of 115 mg/kg ig, the only dose tested. The remaining compounds were not tested in this model of ulcers.

Although the copper D- and L-tryptophan had no antiinflammatory activity in the CFE model when given sc, they were very potent antiulcer compounds when given ig. The lack of solubility of both of these compounds seems to account for the lack of antiinflammatory activity in the CFE model which is an acute 6-hr test. Presumably, the test compound must be absorbed and distributed to the site of inflammation in a relatively short time interval. Compounds which are insoluble or slowly absorbed are known to be inactive in this model. On the other hand, giving these two compounds by the oral route placed them in a more acidic medium, which is known to increase their solubility, and allows the observation of antiulcer activity. Consistent with this line of reasoning was the observation that both of these compounds had antiinflammatory activity at the initial screening dose of 100 mg/kg in the CWG model when they were mistakenly given ig. They were both inactive in this model when given sc. At the time, this observation was not pursued because of the concern regarding stability of the coordination compounds in acidic media and the need for absorption and peripheral distribution. It was believed that dissociation in the stomach would not allow peripheral distribution to the site of inflammation.

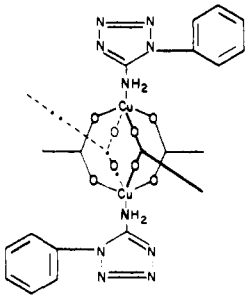
In addition to the Cu coordination compounds already mentioned, a variety of others were made to investigate possible ligand structure–activity relationships. Unfortunately, the data for these compounds are incomplete but the observation that they have both antiinflammatory and antiulcer activity justifies their inclusion and are presented in Table IV.

The first group is composed of amino acid coordination compounds. These were prepared because it is believed

that at least one of the biologically useful forms of Cu in blood and other tissues is an amino acid complex.[37] The structures of these compounds are unknown, with the exception of the square-planar DL-tryptophan chelate. Complexes of D- and L-aspartic acid and L-lysine were found to be active at the 4–10 mg/kg level in the CFE and CWG models of inflammation. Consistent with the earlier observation that copper D-tryptophan and copper L-tryptophan had no antiinflammatory activity, the copper DL-tryptophan derivative was also inactive when given sc. The Cu in these compounds ranges from 13 to 32%, and again there is no direct correlation with the amount of Cu in them and their biological activity. When these amino acid complexes were tested in the Shay model of ulcers, they were shown to have potent antiulcer activity, including the DL-tryptophan derivative which was active at 4.5 mg/kg.

The second group shown in Table IV contains the amines that have been studied. The Cu^{II}_2(pyridine)$_2$(acetate)$_4$[20] compound, prepared by substitution of the two water molecules in Cu^{II}_2(acetate)$_4$(H_2O)$_2$ with pyridine, is of interest since it has the same binuclear structure[20,38,39] as cupric acetate, but it is 100 times more active as an antiulcer compound. The antiinflammatory activity was similar to that observed for cupric acetate hydrate in the CFE model, but no CNS side effects were reported for the lower doses studied. Copper(II)(pyridine)$_2$(Cl)$_2$ is a *trans*-dichloro coordination compound (18)[25] containing only monodentate ligands. It had about the same amount of CFE antiinflammatory activity as the pyridine acetate complex, but only $^1/_{20}$th the antiulcer activity. Here the compound which is suspected to have lesser stability has much less antiulcer activity. The Cu^{II}(morpholine)$_2$(Cl)$_2$(HCl)$_2$ complex (19),[40] which may be viewed as a complex ion, had about the same antiinflammatory activity as the pyridine chloride complex. Unfortunately it has not been evaluated as an antiulcer compound.

The histamine coordination compound, which may be similar to the material described by Walker,[41,42] was of interest since histamine is a potent ulcerogen in this model. However, the histamine coordination compound had antiulcer activity at 45 mg/kg. It has not been tested at a lower dose to evaluate the suggestion that this compound may have antiulcer activity at still lower doses.



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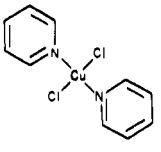

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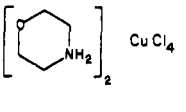

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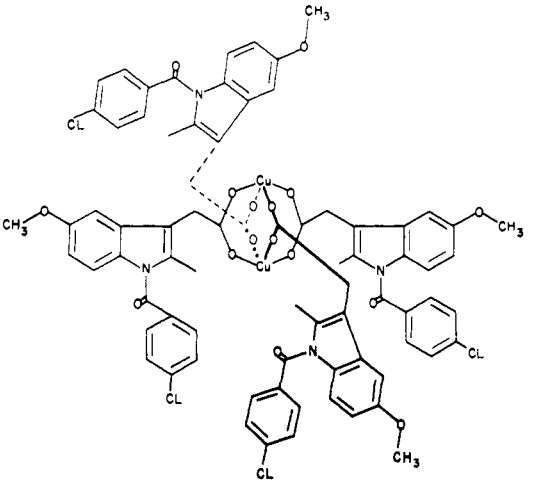
The group of heterocyclic carboxylic acid chelates shown in Table IV has not been adequately evaluated to distinguish members of this class of coordination compounds based upon their antiinflammatory activity. With regard to their antiulcer activity, Cu^{II}_n(1-carboxyisoquinoline)$_{2n}$, which may be square planar, was unique because it was only active at 225 mg/kg. The rest, which are most likely binuclear, were active in the 2.3–4.5 mg/kg dose range.

In the class of arylacetic acids, the antiulcer activities of two solvates of the Cu coordination compound prepared from 1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid (indomethacin) are reported. A binuclear structure (20) is also suggested for both of these compounds. Interestingly, both of these have been shown to have potent antiulcer activity. Unfortunately, they have not been evaluated with regard to their antiinflammatory activity.



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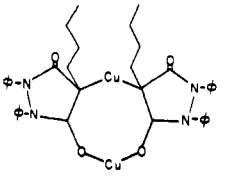
Similarly, a Cu coordination compound prepared from 4-*n*-butyl-1,2-diphenyl-3,5-pyrazolidinedione (phenylbutazone) has been shown to have antiulcer activity. Speculation for the purpose that this serves in scientific discussions, with regard to the structure of this material, derived from the enolate, leads to the square-planar type 4 or the binuclear structure (21). The binuclear structure has been illustrated using only two of the four ligands. Those above and below the plane have been omitted for clarity.



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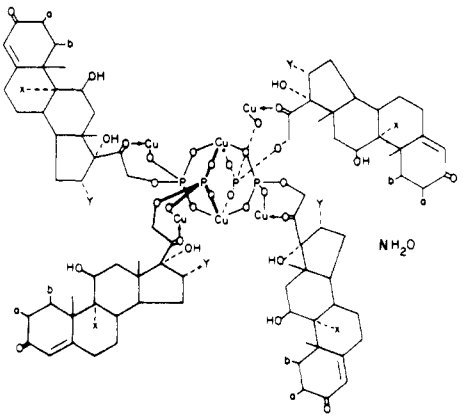
Having concluded that the Cu coordination compounds prepared from the nonsteroidal, antiinflammatory agents were more active than their parent compounds, it was decided to evaluate the possibility that Cu coordinated corticoids might also be more active than their parent compounds. Copper complexes of hydrocortisone 21-phosphate, hydrocortisone 21-hemisuccinate, and dexamethasone 21-phosphate were prepared to test this possibility.

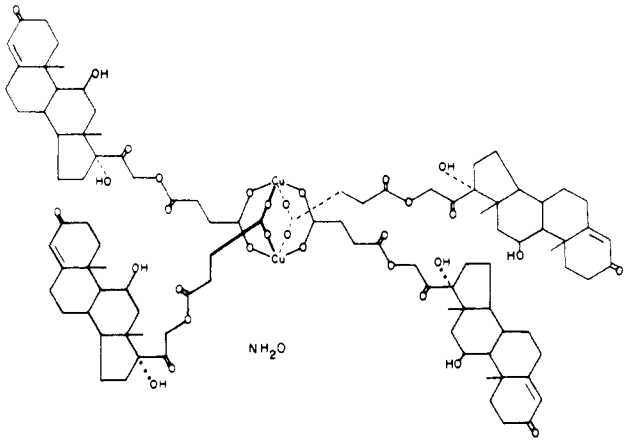
Since the unsubstituted corticoids, commonly used as antiarthritic drugs, are known[43] to only weakly coordinate with Cu, these were not suitable for the preparation of stable isolatable Cu coordination compounds. It was felt that 21-half-acid-esters of sulfuric acid might coordinate well with Cu. Because 21-sulfate substituted corticoids are well-known metabolites of these steroidal alcohols, they were considered ideal for the purposes of preparing isolatable Cu coordination compounds. However, hydrocortisone and dexamethasone 21-sulfates were not as readily available as their 21-phosphates. In addition to hydrocortisone 21-phosphate and dexamethasone 21-phosphate, hydrocortisone 21-hemisuccinate was also available. The hemisuccinate was also believed to be useful in obtaining an isolatable Cu corticoid coordination compound. Structures of these coordination compounds

are also not known with certainty. However, structures are illustrated to indicate what may be interesting features of these coordination compounds.

Both hydrocortisone and dexamethasone phosphate coordination compounds (22) and (23), respectively, were consistently obtained with 3:2 Cu–ligand ratios and were markedly hydrated. The two hydrocortisone 21-hemisuccinate coordination compounds were also highly hydrated but varied in their Cu–ligand ratios in what would seem to be a reasonable suggestion for their basic structure (24).



22 a, b, x and y = H
23 a, b, dehydro, x = F and Y = CH_3



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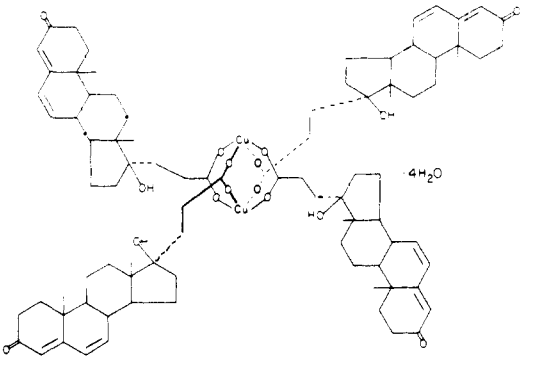
The results of antiinflammatory and antiulcer tests of these compounds are presented in Table IV. Results in the polyarthritis test are those obtained in a single test using the therapeutic model of polyarthritis. For comparison purposes the disodium salt of hydrocortisone 21-phosphate was tested along with the two Cu coordination compounds prepared from it. The disodium salt of hydrocortisone 21-phosphate was tested in the PA model at 6 mg/kg sc, which is a dose which should have been active but, unfortunately, was not. The nonahydrate of $Cu(II)_{3n}$(HC 21-phosphate) was tested in all three models and found to be active. The heptahydrate was also only tested in the PA model and found to be inactive. However, both of these compounds were found to be active antiulcer compounds on testing down to 22.5 and 11.3 mg/kg, respectively. The parent disodium salt was only tested for antiulcer activity at 45 mg/kg, because only limited quantities were available and found to be inactive as expected. Hydrocortisone had also been shown to be inactive, again as expected, as an antiulcer compound at 225 mg/kg.

Phenomenologically, it is of interest to point out that preliminary studies with $Cu(II)_{3n}$(HC 21-phosphate)$_{2n}$-$(H_2O)_{9n}$ demonstrated that it inhibited ulcers in the corticoid-induced ulcer model. This inhibition was observed at 36 and 18 mg/kg and would seem to merit further study.

Hydrocortisone 21-hemisuccinate was found to be active at 6 mg/kg in PA and, as anticipated, inactive as an antiulcer compound at 45 mg/kg. Two coordination compounds prepared from it were also found to be active in the PA model as well as active in the CFE and CWG models of inflammation. Both of these were also shown to have antiulcer activity.

At this time there are no antiinflammatory test results to report for the disodium salt of dexamethasone or the two Cu coordination compounds prepared with it. However, antiulcer tests have demonstrated that both of the Cu coordination compounds were active at 22.5 mg/kg.

The potassium salt of a steroidal diuretic, 17-hydroxy-3-oxo-17-pregna-4,6-diene-21-carboxylate (potassium canrenoate) gave a Cu complex (25), suggested to be binuclear in structure, which had antiulcer activity but did not have any antiinflammatory activity in the CFE and PA models at 40 and 30 mg/kg, respectively, the only doses tested. This compound was originally made to provide



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some indication of stability for these Cu coordination compounds in vivo. The potassium salt of the parent compound is a diuretic, and it was suggested that observed diuresis following the administration of its Cu coordinate could be used as a measure of the compound's stability in vivo. Enough compound was injected sc so that if only 25% dissociated, an ED_{95} ($p < 0.05$) diuretic dose of the free acid would be released. However, no diuresis was observed in the usual assay for diuretic activity[44] and it was concluded that dissociation of the complex failed to result in a blood level of the free acid required to produce diuresis.

A similar study of two of the hydrocortisone coordination compounds 22 and 24 was done to evaluate the possibility that these compounds did not have the corticoid-like diuretic activity in the same model of diuresis.[44] An examination of hydrocortisone 21-phosphate and its 21-hemisuccinate demonstrated that both of these parent compounds had corticoid-like diuretic activity. Both of these increased sodium output and urine volume. Similarly, Cu^{II}_{2n}(HC 21-hemisuccinate)$_{4n}(H_2O)_{6n}$ had corticoid-like diuretic activity. In retrospect, this result is suggested to be due to hydrolysis of the C-21 ester linkage to give free hydrocortisone. Conversely, Cu^{II}_{3n}(HC 21-phosphate)$_{2n}(H_2O)_{9n}$ had no diuretic activity and the observed antiulcer activity of this compound does distinguish it from the parent corticoid with regard to biological activity.

In general, with regard to the antiulcer activity, all of the Cu coordination compounds that were active and decreased the number and size of ulcers were also shown

Table V. Anticholinergic Activity, Qualitative Effects on Mouse Behavior, and Estimations of LD_{50} Values in Mice of a Variety of Coordination Compounds

Compound	Anticholinergic act. % TEA[a]	Anticholinergic act. % atropine[b]	Qualitative behavioral change, mg/kg[c]	LD_{50}, mg/kg[c]
Cu^{II}(L-tryptophan)$_2$	<23	<0.1	I at 320 ig	>320 ig
Cu^{II}(D-tryptophan)$_2$	<23	<1	I at 320 ig	>320 ig
Cu^{II}(anthranilate)$_2$	<11	<1	I at 320 sc	>320 sc
Cu^{II}(3,5-dips)$_2$	<20	<1	I at 320 sc	>320 sc
Cu^{II}_2(aspirinate)$_4$	<20	<1	I at 320 sc	>320 sc
Cu^{II}_n(niflumate)$_{2n}$(H_2O)$_n$	NT	NT	I at 320 sc	>80 < 320 sc
Cu^{II}_nD-pen(H_2O)$_{1.5n}$	NT	NT	I at 320 sc	>80 < 320 sc
Cu^{II}_n(D-pen)$_{2n}$(H_2O)$_{2n}$	NT	NT	Depressant at 320 sc	>80 < 320 sc
Cu^{II}_n(D-pen disulfide)$_n$(H_2O)$_{3n}$	NT	NT	I at 320 sc	>80 < 320 sc
Cu^{II}_n(fenamole)$_n$(acetate)$_{2n}$	<7	NT	I at 320 sc	>320 sc
Cu^{II}_n(fenamole)$_{2n}$(HCl)$_{2n}$	<7	NT	I at 320 sc	>320 sc
Cu^{II}_n(D-aspartate)$_n$	NT	NT	I at 320 sc	>320 sc
Cu^{II}_n(L-aspartate)$_n$	NT	NT	Depressant at 20 ip	>20 < 40 ip
Cu^{II}(DL-tryptophan)$_2$	<20	NT	NT	NT
Cu^{II}_2(pyridine)$_2$(acetate)$_4$	<7	NT	I at 320 sc	>320 sc
Cu^{II}(pyridine)$_2$(Cl)$_2$	NT	NT	I at 320 ip	>80 < 320 ip
Cu^{II}(morpholine)$_2$(Cl)$_2$(HCl)$_2$	NT	NT	I at 320 sc	>320 sc
Cu^{II}_2(salicylate)$_4$(Na)$_4$	NT	NT	I at 320 sc	>320 sc
Cu^{II}_n(salicylate)$_{2n}$(H_2O)$_{4n}$	<20	<1	I at 320 sc	>320 sc
Cu^{II}_n(4-*n*-butyl-1,2-diphenyl-3,5-pyrazolidinedione)$_{2n}$	NT	NT	I at 320 sc	>320 sc

[a] Active anticholinergic compounds produce a 50% decrease in the amplitude of the sustained cat nictitating membrane contraction caused by preganglionic electrical stimulation. The percent potency is estimated on the basis of the ED_{50} determined for TEA. Values which do not differ from control are indicated as "less than" values. Compounds possessing a potency ≥10% TEA were rated as active. [b] A compound is rated as active if it possesses a potency of ≥1% atropine sulfate. In a four-point assay procedure, a comparison is made between the dose of test compound and of atropine sulfate required to cause a 50% decrease in an acetylcholine-induced contraction of rabbit ileum. Values which do not differ from control are indicated as "less than" values. [c] Groups of four mice are injected with doses of test compound varying between 5 and 320 mg/kg. This range will usually include doses which produce minimal to lethal effects. Animals are tested individually at periodic intervals and given scores for psychomotor excitement, depression, and ataxia. Any additional somatic or antonomic effects are recorded. A compound's activity is evaluated by comparing its dose–response curve of excitement–depression scores with curves similarly obtained with a variety of standard compounds. In addition, this procedure permits an estimation of an acute LD_{50}.

to decrease gastrointestinal secretions. In the Shay rat, both acid and pepsin in these secretions were decreased 5 hr after ligation. The possibility that these Cu coordinates decreased pepsin activity by inhibiting pepsin was studied using in vitro techniques.[45] None of the compounds with antiulcer activity inhibited the hydrolysis of denatured hemoglobin by crystalline pepsin in aqueous medium at pH 2.0. The observed antisecretory activity might have been explained as anticholinergic activity but none of the Cu coordination compounds studied had any significant tetraethylammonium bromide[46] (ganglionic) or atropine-like[47] (postganglionic) cholinergic blocking (anticholinergic) activity as shown in Table V.

This lack of anticholinergic activity or blockade of the autonomic nervous system is consistent with the observations that these compounds failed to affect the CNS and cause psychomotor behavioral changes. Qualitative mouse behavioral changes such as psychomotor stimulation, depression, and ataxia were evaluated using published methods,[48] and an estimated LD_{50} for the test compounds was obtained, if lethality occurred in the dose range studied. The data in Table V show that of the compounds studied only two produced behavioral changes.

Copper(II)$_n$(L-aspartate)$_n$, when mistakenly given intraperitoneally (ip), caused depression at 20 mg/kg. The compound Cu^{II}_n(D-pen)$_{2n}$(H_2O)$_{2n}$ was also found to cause depression at 320 mg/kg. However, since the LD_{50} for this compound was found to be between 80 and 320 mg/kg, it is not surprising that at 320 mg/kg these mice were somewhat depressed. The remainder of the compounds tested were inactive with regard to causing behavioral changes at the doses studied and, in general, had LD_{50} values which were between 80 and 320 mg/kg or, as with the majority of the compounds studied, had LD_{50} values greater than 320 mg/kg. These high values in mice are consistent with those reported earlier as being obtained following the administration of these compounds to rats.

The mechanisms of action of these Cu coordination compounds as antiinflammatory and antiulcer agents are unknown. However, it is well known that repair at sites of inflammation, including ulcers, requires the extracellular maturation or cross-linking of the extracellular components, collagen and elastin. Since the enzyme responsible for this, lysyl oxidase, is a Cu-dependent enzyme,[49] this aspect of wound or tissue repair assumes central significance with regard to a plausible role for Cu coordination compounds having both antiinflammatory and antiulcer activity. The notion that these compounds promote tissue repair is consistent with the observation that Cu^{II}(L-tryptophan)$_2$ treated rats, with surgically induced ulcers,[50] healed at a markedly increased rate compared to nontreated controls.[51] On day 5 postulcer induction the treated animals, which were given 5 mg/day or about 50 mg/kg beginning on day 1 postsurgery, were a full 5 days ahead of the nontreated control animals with regard to the healing of these ulcers, and they remained ahead of the nontreated controls throughout the course of the 20-day experiment.

Studies of the inhibition of cathepsin D, a lysosomal proteinase, demonstrated that nearly all of the Cu compounds presented here failed to inhibit this enzyme.[52] The only exception was Cu^{II}_n(niflumate)$_{2n}$(H_2O)$_n$. This would suggest that these coordination compounds as a group were not acting as antiinflammatory agents by this mechanism.

Results of studies with cupric aspirinate demonstrated that it depressed the synthesis of the proinflammatory (vasodilator) prostaglandin, PGE_2, in the carrageenan pouch model of inflammation.[53] This is consistent with the work of Lee and Lands,[54] and recently confirmed by Maddox,[55] who found a depression in PGE_2 synthesis and a concomitant increase in the antiinflammatory (vasoconstrictor) prostaglandin, $PGF_{2\alpha}$, following the addition of copper sulfate or chloride to seminal vesicle homogenates. These results suggest that the mechanism of

action of the Cu coordination compounds may be, at least in part, at the level of the prostaglandin mediation of inflammation. This is to say, these Cu coordination compounds may play a role in decreasing the synthesis of the proinflammatory PGE_2 and, concomitantly, increasing the synthesis of the antiinflammatory $PGF_{2\alpha}$.

A tenable conclusion to be reached from the data presented in this publication is that in all cases Cu coordination compounds were found to be more active as antiinflammatory agents than either Cu, in the form of cupric acetate, or their parent compounds. These results provide evidence for a unique metabolite, a metal coordination compound, which may be responsible for the desired antiinflammatory activity of those agents which have clinical usefulness. That is to say, Cu coordination compounds which have not been generally recognized as possible active metabolites may be responsible for the antiinflammatory activity of the clinically used antiinflammatory agents.

Intuitively, coordination compound stability is in part responsible for the observed antiinflammatory and antiulcer activity. If these coordination compounds were not stable in vivo, then there would be no difference in biological activity between the coordination and parent compounds. The amount of Cu in these compounds does not provide a direct correlation with biological activity, so that it is again necessary to suggest that the coordination compound is an important specie. However, stability is no doubt not the only factor required for activity, since it would seem that utilization of the Cu in the coordination compound would require the appropriate ligand exchange. To fully account for the biological activity of these coordination compounds the remaining parameters employed in drug design must be invoked.

In addition to greater potency, these Cu compounds appear to be less toxic than the parent compounds. The LD_{50}/ED_{50} ratios, which are a measure of safety in therapy, are larger for these compounds than those observed for the parent compounds. Even more remarkable than this, these coordination compounds have potent antiulcer activity which suggests a role in Cu-dependent tissue repair processes associated with connective tissue metabolism of inflammed tissues.

Implicit in all of this is the suggestion that the arthritic diseases may, at least in part, result from an insufficient supply of Cu or the lack of a form of Cu suitable for utilization in normal Cu-dependent metabolic processes required for tissue maintenance. Similarly, a chemical mechanism of coordination is suggested for the etiology of the toxicologic and other biologic manifestations of the various classes of antiarthritic drugs.

Calculations, based upon the use of existing antiarthritic drugs, can be done to show that the amount of Cu ingested while on a therapeutic regimen using a Cu compound is no more than the amount ingested by every individual who eats three well-balanced meals a day. However, it may very well be that individuals with degenerative diseases require an intake which exceeds the "normal" intake.

Experimental Section

All of the antiinflammatory testing was done via the sc route of administration to avoid or minimize the risk of their loss by dissociation. Saline–Tween 80 suspensions were used to avoid the use of suspending agents with carboxyl and other functional groups that might have successfully competed for the Cu in these coordination compounds.

Melting and decomposition ranges were determined in a Thomas-Hoover capillary melting point apparatus and are corrected. In general, these compounds did not melt but decomposed on heating over the reported decomposition range or at the decomposition point, giving a red or black mass. Frequently subtle and clear color changes were observed on heating up to the initial temperature of the decomposition range or point. Microanalyses were performed by the Analytical Department of Searle Laboratories. Where the analyses are indicated only by the symbols of the elements, the analytical values were within ±0.4% of the theoretical values. Reference uv, visible, and ir spectra were obtained for each compound. Air drying of samples was accomplished by drawing air through the sample contained in a sintered glass filter funnel which was attached to a laboratory vacuum line or water aspirator. Drying at reduced pressure was accomplished in a GCA/Precision Scientific heated vacuum desiccator attached to a laboratory vacuum line.

Bis[L-tryptophanato(*O*,*N*)]copper(II) [Cu^{II}(L-tryptophan)$_2$] (4) (Method A). The L-tryptophan (5.0 g, 0.025 mol) was dissolved in 100 ml of H_2O with a solution of NaOH (50%), filtered, and back-titrated if necessary with a solution of HCl (10%) until indicator paper showed the solution to be weakly basic. This solution was then dropped into 100 ml of H_2O containing $CuCl_2$ dihydrate (3.3 g, 0.021 mol). After stirring for about 1 hr a precipitate formed and was collected by filtration. This blue precipitate was washed with H_2O and diethyl ether, dried at 100°C and 15 mmHg overnight, and weighed (4.7 g, 82% yield). A sample of this material on heating turned brown at 240°C and finally decomposed at 260°C. Anal. ($C_{22}H_{22}N_4O_4Cu$) C, H, N.

Bis[D-tryptophanato(*O*,*N*)]copper(II) [Cu^{II}(D-tryptophan)$_2$] (4). This coordination compound was prepared as described for the L isomer (method A) using 5.0 g (0.021 mol) of D-tryptophan. After collecting the precipitate by filtration and washing with H_2O, diethyl ether, and acetone (250 ml), the precipitate was dried overnight at 100°C and 15 mmHg and weighed (4.3 g, 75% yield). A sample of this material decomposed slowly on heating to 269°C. Anal. ($C_{22}H_{22}N_4O_4Cu$) C, H, N.

Bis[anthranilato(*O*,*N*)]copper(II) [Cu^{II}(anthranilate)$_2$] (4). The sodium salt of anthranilic acid (5 g, 0.04 mol) was prepared as described in method A in 150 ml of H_2O with 50% NaOH. This solution was dropped into 300 ml of a stirred aqueous solution of $CuCl_2$ dihydrate (2.5 g, 0.016 mol). The precipitate which formed was removed by filtration and washed with H_2O and diethyl ether (5 × 50 ml). After drying overnight at 120°C and 15 mmHg the material weighed 6.1 g (99% yield). A sample of this greenish blue material began to decompose on heating to 240°C and continued to do so on heating to 290°C. Anal. ($C_{14}H_6N_2O_4Cu$) C, H, N.

Bis[(3,5-diisopropylsalicylato)(*O*,*O*)]copper(II) [Cu^{II}(3,5-dips)$_2$] (4). A solution of the sodium salt of 3,5-diisopropylsalicylic acid (5 g, 0.023 mol) was prepared as described in method A and added to 300 ml of a stirred aqueous solution of $CuCl_2$ dihydrate (0.59 g, 0.0336 mol). A brown precipitate formed which when recrystallized from ether gave green crystals. These crystals were filtered and dried at 125°C and 15 mmHg for 3 hr. The resulting brown crystalline material melted with decomposition over the range of 142–144°C. Anal. ($C_{26}H_{34}O_3Cu$) C, H.

Tetrakis-μ-acetylsalicylato-dicopper(II) [Cu^{II}_2(aspirinate)$_4$] (9). The sodium salt of acetylsalicylic acid was prepared by dissolving acetylsalicylic acid (30 g, 0.165 mol) in 200 ml of H_2O at 0°C with 50% NaOH so that the pH did not go above 11.0 and rarely reached 11.0. This was done over a period of 45–60 min. The final pH of the solution was about 8.7. The $CuCl_2$ solution, prepared by adding 56.5 g (0.330 mol) of $CuCl_2$ dihydrate to 500 ml of water, was added to a stirred solution of sodium acetylsalicylate during a period of 10–15 min. Following the completion of this addition the blue precipitate was collected by filtration, washed with H_2O (500 ml × 2), acetone (400 ml × 2), and diethyl ether (300 ml), and left to dry on a filter funnel attached to a water aspirator. After 2 days the powder was dried at 50°C for 6–7 hr and weighed (31.3 g, 90.6% yield). A sample of this material did not melt but turned brown on heating to 300°C. Anal. ($C_{36}H_{28}O_{16}Cu_2$) C, H.

2-[3-(Trifluoromethyl)phenyl]aminonicotinato$_{2n}$(aqua)$_n$copper(II)$_n$ [Cu^{II}_n(niflumate)$_{2n}$(H_2O)$_n$] (12). The sodium salt of 2-[3-(trifluoromethyl)phenyl]aminonicotinic acid (20 g, 0.0708 mol) was prepared as described in method A. The solution of this salt was then added to about 300 ml of a saturated,

stirred solution of cupric acetate monohydrate. The resultant greenish precipitate was collected by filtration and dissolved in 200 ml of diethyl ether. The ether solution was then dropped into about 4 l. of boiling Skellysolve A. The resultant precipitate was collected from the hot solution by filtration, dried at 125°C for 3 hr at 15 mmHg, and weighed (16 g, 70% yield). A sample of this material melted with decomposition over the range of 201–208°C. Anal. ($C_{52}H_{36}O_{10}N_8F_{12}Cu$) C, H, N.

(D-Penicillaminato)$_n$(aqua)$_{1.5n}$copper(I)$_n$ [Cu^I_n(D-pen)$_n$(H$_2$O)$_{1.5n}$] (13). D-Penicillamine (5 g, 0.0335 mol) was dissolved in 50 ml of water. The solid cupric acetate monohydrate (6.68 g, 0.017 mol) was then added to the solution at such a rate so as to not exceed its rate of solution. Upon the completion of this addition the solution was dark gray. About 50–100 ml of H_2O was then added and the mixture left to stir for about 30 min. The resultant gray precipitate was collected by filtration leaving a blue filtrate (125 ml). This blue filtrate was subsequently diluted with about 375 ml of acetone and set aside. The air-dried gray powder weighed 4.3 g (54.0% yield). A sample of this material decomposed over the range of 155–157°C. Anal. ($C_5H_9SNO_{3.5}Cu$) C, H, N.

(D-Penicillaminato)$_{2n}$(aqua)$_{2n}$copper(II)$_n$ [Cu^{II}_n(D-pen)$_{2n}$(H$_2$O)$_{2n}$] (14). On standing, the acetone diluted blue filtrate described in the preparation of Cu^I_n(D-pen)$_n$(H$_2$O)$_{1.5n}$ gave a gray precipitate which was collected by filtration and this filtrate also set aside. The gray solid was washed with 60 ml of water and the remaining light tan solid washed with 60 ml of acetone, air-dried, and weighed (1.15 g, 17.4% yield). A sample of this solid melted with decomposition over the range of 155–157°C. Anal. ($C_{10}H_{24}O_6S_2N_2Cu$) C, H, N.

(D-Pencillamine disulfide)$_n$(aqua)$_{3n}$copper(II)$_n$ [Cu^{II}_n(D-pen disulfide)$_n$(H$_2$O)$_{3n}$] (15). The acetone–water filtrate obtained after removing the Cu^{II}_n(D-pen)$_{2n}$(H$_2$O)$_{2n}$ from the blue acetone filtrate, described above, was concentrated to about 100 ml and diluted with 400 ml of acetone. A blue precipitate (1.3 g, 9.4% yield) was obtained following filtration, washing with acetone, and air drying. A sample of this material decomposed over the range of 157–158°C. After drying twice at 73° and 15 mmHg overnight, a sample of this material decomposed over the range of 173–175°C. Anal. ($C_{10}H_{24}N_2S_2O_7Cu$) C, H, N.

(Acetato)$_{2n}$(1-phenyl-5-aminotetrazolato)$_n$copper(II)$_n$ [Cu^{II}_n(fenamole)$_n$(acetate)$_{2n}$] (17). Cupric acetate monohydrate (5 g, 0.012 mol) was dissolved in 20 ml of H_2O. This solution was diluted with 100 ml of methanol. When 5 g (0.031 mol) of 1-phenyl-5-aminotetrazole was added, a blue gel was obtained. This gel was filtered and the resulting blue flakes were washed with about 400 ml of methanol until the washings were no longer blue. The filtrate was then concentrated to about 150 ml and stored for about 1 week in the refrigerator. A precipitate formed and was removed by filtration. This green crystalline solid was air-dried and weighed (3.8 g, 17.9% yield). A sample of this solid decomposed over the range of 186–189°C. Anal. ($C_{22}H_{26}N_{10}O_8Cu_2$) C, H, N.

(1-Phenyl-5-aminotetrazolato)$_{2n}$(chloride)$_{2n}$copper(II)$_n$ [Cu^{II}_n(fenamole)$_{2n}$(HCl)$_{2n}$] (16). 1-Phenyl-5-aminotetrazole (5 g, 0.012 mol) was dissolved in 30 ml of methanol; then 5 g (0.029 mol) of $CuCl_2$ dihydrate dissolved in 25 ml of methanol was added to the stirred solution of tetrazole. The resulting solution was filtered and set aside. Three subsequent crops of a green solid were obtained following filtration and concentration of the filtrate. The combination of these were air-dried and weighed (5 g, 17.7% yield). A sample of this material decomposed on heating over the range of 184–185°C. Anal. ($C_{14}H_{14}N_{10}CuCl_2$) C, H, N.

(D-Aspartato)$_n$(aqua)$_{3.5n}$copper(II)$_n$ [Cu^{II}_n(D-aspartate)$_n$(H$_2$O)$_{3.5n}$]. This material was made in a manner similar to method A using D-aspartic acid in place of L-tryptophan. The yield of this substance was of the order of 75%. A sample of this material decomposed on heating up to and over the range of 210–212°C. Anal. ($C_4H_{12}NO_{7.5}Cu$) C, H.

(L-Aspartato)$_n$(aqua)$_{3.5n}$copper(II)$_n$ [Cu^{II}_n(L-aspartate)$_n$(H$_2$O)$_{3.5n}$]. This material was made in a manner similar to method A using L-aspartic acid in place of L-tryptophan. The yield of this substance was of the order of 75%. A sample of this material decomposed on heating up to and over the range of 210–212°C. Anal. ($C_4H_{12}NO_{7.5}Cu$) C, H.

(L-Lysinato)$_n$(chloro)$_{2n}$(aqua)$_n$copper(II)$_n$ [Cu^{II}_n(L-lysinate)$_n$(Cl)$_{2n}$(H$_2$O)$_n$]. This material was made in a manner similar to method A using L-lysine in place of L-tryptophan. The yield of this substance was of the order of 50%. A sample of this material decomposed on heating over the range of 169–170°C. Anal. ($C_6H_{16}N_2O_3CuCl_2$) C, H, Cl, N.

(L-Lysinato)$_{2n}$(chloro)$_{2n}$(aqua)$_n$copper(II)$_n$ [Cu^{II}_n(L-lysinate)$_{2n}$(Cl)$_{2n}$(H$_2$O)$_n$]. This material was made in a manner similar to method A using L-lysine in place of L-tryptophan. The yield of this material was of the order of 50%. A sample of this compound decomposed on heating up to and over the range 210–214°C. Anal. ($C_{12}H_{32}N_4O_5CuCl_2$) C, H, Cl.

Bis[DL-tryptophanato(*O*,*N*)]copper(II) [Cu^{II}(DL-tryptophan)$_2$] (4). This coordination compound was prepared and isolated as described in method A. Anal. ($C_{22}H_{22}N_4O_4Cu$) H; C: calcd, 56.22; found, 55.58.

(ε-Aminocaproato)$_n$(chloro)$_{1.5n}$(methanol)$_{0.5n}$copper(II)$_n$ [Cu^{II}_n(ε-aminocaproate)$_n$(Cl)$_{1.5n}$(CH$_3$OH)$_{0.5n}$]. This coordination compound was prepared by stirring a suspension of 10 g (0.08 mol) of ε-aminocaproic acid in 200 ml of methanol and slowly adding 10 g (0.065 mol) of solid cupric chloride dihydrate. The resultant green precipitate was collected by filtration, washed with methanol, dried at 25°C and 15 mmHg overnight, and weighed (10.5 g, 51% yield). A sample of this material decomposed over the range of 157–158°C. Anal. ($C_7H_{15.5}O_{2.5}CuCl_{1.5}$) C, H, Cl.

(ε-Aminocaproato)$_n$(chloro)$_{2n}$(aqua)$_{0.5n}$copper(II)$_n$ [Cu^{II}_n(ε-aminocaproate)$_n$(Cl)$_{2n}$(H$_2$O)$_{0.5n}$]. This coordination compound was obtained from the filtrate described in the preparation of Cu^{II}_n(ε-aminocaproate)$_n$(Cl)$_{1.5n}$(CH$_3$OH)$_{0.5n}$. Following concentration of the filtrate and methanol washings to about 100 ml a bluish green precipitate formed. This precipitate was collected by filtration, dried at 25°C at 15 mmHg, and weighed (4.6 g, 21% yield). A sample of this material decomposed on heating over the range of 193–194°C. Anal. ($C_6H_{14}NO_{2.5}Cl_2Cu$) C, H, Cl.

Tetrakis-μ-acetato-bis(monopyridino)dicopper(II) [Cu^{II}_2(pyridine)$_2$(acetate)$_4$]. This coordination compound was prepared by adding 10 g (0.025 mol) of cupric acetate monohydrate to 70 ml of pyridine and the mixture heated while stirring at 100°C. The hot suspension was filtered and the resulting precipitate collected by filtration and washed with 200–300 ml of diethyl ether. A sample of this green solid decomposed on heating over the range of 214–216°C. When the ether–pyridine filtrate cooled a second precipitate, which was bluish, was obtained. Removal by filtration and washing with ether gave a second crop of the green material in the filtrate. This green solid had a decomposition range of 216–218°C. A mixture decomposition range of 216–218°C was observed for a sample of the combination of the two green solids. The total yield was 12 g (92%). Anal. ($C_{18}H_{22}N_2O_8Cu_2$) C, H, N.

Dipyridinodichlorocopper(II) [Cu^{II}(pyridine)$_2$(Cl)$_2$] (18). This compound was prepared by dissolving 9.42 g (0.062 mol) of $CuCl_2$ dihydrate in 95% ethanol and adding 15 g (0.19 mol) of pyridine slowly to the stirred solution. The resultant blue precipitate was removed by filtration, washed with 95% ethanol (200 ml), dried at about 50°C for 24 hr, and weighed (19.8 g, 35.6% yield). A sample of this material decomposed over the range 225–275°C. Anal. ($C_{10}H_{10}N_2CuCl_2$) C, H.

Dimorpholiniumtetrachlorocopper(II) [Cu^{II}(morpholine)$_2$(Cl)$_2$(HCl)$_2$] (19). This compound was prepared according to the published procedure of Ruggeberg et al.[40] Starting with 14.5 g (0.167 mol) of morpholine the coordination compound was obtained in 41% yield. A sample of this green crystalline material melted with decomposition over the range of 167–170°C. Anal. ($C_8H_{20}N_2O_2CuCl_4$) C, H, N.

(Histamino)$_n$(chloro)$_{2n}$(hydrochloro)$_{2n}$copper(II)$_n$ [Cu^{II}_n(histamine)$_n$(Cl)$_{2n}$(HCl)$_{2n}$]. This coordination compound was prepared by mixing 5 g (0.055 mol) of histamine hydrochloride and 5 g (0.048 mol) of cupric chloride dihydrate in 200 ml of methanol and concentrating to 135 ml. On standing a tan solid precipitated. This was removed by filtration and the filtrate concentrated to 80 ml. Upon addition of 40 ml of diethyl ether to this concentrate a light green solid precipitated. After removal by filtration and air drying this material was weighed (4.0 g, 23% yield). A sample decomposed over the range of 185–189°C with softening at 182°C. Anal. ($C_5H_{10}N_3Cl_4Cu$) C, H, N.

(Pyridine-3-carboxylato)$_{2n}$(aqua)$_{1.5n}$copper(II) [Cu^{II}_{2n}(nicotinate)$_{4n}$(H$_2$O)$_{3n}$]. This coordination compound was

prepared by dissolving 10 g (0.08 mol) of nicotinic acid in 100 ml of water with concentrated NH_4OH so that the final pH was 7.0. A cupric chloride solution, prepared by dissolving 21.6 g (0.14 mol) of cupric chloride dihydrate in 200 ml of water, was stirred while the ammonium salt of nicotinic acid was added dropwise. The blue precipitate was collected by filtration, washed with 500 ml of water, and air-dried. The resulting material was dried at 80°C and weighed (10.7 g, 80% yield). A sample of this material decomposed on heating up to and through the range of 265–266°C. Anal. ($C_{24}H_{22}O_{11}N_4Cu_2$) C, H, N.

(Isoquinoline-1-carboxylato)$_{2n}$copper(II)$_n$ [Cu$^{II}_n$(1-carboxyisoquinoline)$_{2n}$]. The copper coordination compound of 1-carboxyisoquinoline (5 g, 0.029 mol) was prepared by adding to its solution of the sodium salt, prepared as in method A in 200 ml of water, 60 ml of a saturated aqueous solution of cupric acetate monohydrate. The resultant purple precipitate was collected by filtration, washed with 500 ml of water, and dried overnight at 100°C and 15 mmHg. A sample of this material (4.0 g, 70.2% yield) decomposed over the range of 295–296°C. Anal. ($C_{20}H_{12}N_2O_4Cu$) C, H, N.

(2-Phenylisoquinoline-4-carboxylato)$_{2n}$(aqua)$_{2n}$copper(II)$_n$ [Cu$^{II}_n$(2-phenyl-4-carboxyisoquinoline)$_{2n}$(H$_2$O)$_{2n}$]. This coordination compound was synthesized from the sodium salt of phenylcinchoninic acid (25 g, 0.15 mol), which was prepared as described in method A, in 550 ml of water. The solution of the sodium salt was dropped into a stirred solution of cupric chloride dihydrate (14.2 g, 0.09 mol). The resulting green precipitate was collected by filtration, washed with methanol and water, and then air-dried and weighed (29.5 g, 67% yield). A sample of this material decomposed on heating over the range of 228–229°C. Anal. ($C_{64}H_{48}N_4O_{12}Cu_2$) C, H, N.

(Indole-2-carboxylato)$_{3n}$(acetato)$_n$(aqua)$_{0.5n}$copper(II)$_n$ [Cu$^{II}_n$(2-carboxyindole)$_{3n}$(acetate)$_n$(H$_2$O)$_{0.5n}$]. This copper coordination compound was prepared from the parent acid (4.5 g, 0.028 mol) as in method A, using cupric acetate. The green precipitate was collected by filtration, air-dried for several days, suspended in boiling methanol, and again collected by filtration. It was then dried at 100°C and 15 mmHg overnight and at 125°C and 15 mmHg for 3 hr. A sample of this material (3.0 g, 23.3% yield) decomposed over the range of 249–255°C. Anal. ($C_{29}H_{22}N_3O_9Cu$) C, H, N.

(Indole-2-carboxylato)$_{3n}$(acetato)$_n$(aqua)$_{3.5n}$copper(II)$_n$ [Cu$^{II}_n$(2-carboxyindole)$_{3n}$(acetate)$_n$(H$_2$O)$_{0.5n}$]. This material was prepared as described in method A and dried at 100°C and 15 mmHg over the weekend. A sample of this material did not melt but did turn brown, as did Cu$^{II}_n$(2-carboxyindole)$_{3n}$(acetate)$_n$(H$_2$O)$_{0.5n}$, on heating to 260°C. Anal. ($C_{29}H_{28}N_3O_{12}Cu$) C, H, N.

[3-(*p*-Chlorophenyl)-3,4,5,6-tetrahydro-β-carboline-5-carboxylato]$_{2n}$(aqua)$_{2n}$copper(II)$_n$ [Cu$^{II}_n$(cp-tcca)$_{2n}$(H$_2$O)$_{2n}$]. The copper coordination compound of the parent acid (5 g, 0.015 mol) was prepared as described in method A. An olive drab precipitate was collected by filtration and washed with 500 ml of H_2O, 300 ml of diethyl ether, and then with acetone until the washings were colorless. This material was dried at 100°C overnight and 110°C at 15 mmHg for 3 hr before dissolving in acetone and precipitated with Skellysolve B. This material (2 g, 40% yield) was then dried overnight at 60°C and 15 mmHg and again at 125°C and 15 mmHg. A sample of this material decomposed over the range of 205–210°C. Anal. ($C_{36}H_{32}Cl_2N_4O_6Cu$) C, H; N: calcd, 7.46; found, 6.96.

(3,4,5,6-Tetrahydro-β-carboline-5-carboxylato)$_{2n}$(aqua)$_{2.5n}$copper(II)$_n$ [Cu$^{II}_n$(tcca)$_{2n}$(H$_2$O)$_{2.5n}$]. The copper coordination compound of the parent acid (5 g, 0.023 mol) was prepared as described in method A. This dark green solid was washed with 500 ml of water, then suspended in 500 ml of boiling acetone, and collected by filtration. Drying was done at 100°C at atmospheric pressure for 24 hr and then at 110°C and 15 mmHg for 3 hr. Subsequent leaching with hot propylene glycol gave an insoluble material (3.3 g, 52.8% yield) which rapidly decomposed on heating to 294°C. Anal. ($C_{24}H_{27}N_4O_{6.5}Cu$) C, H, N.

(Hydrocortisone 21-phosphato)$_{2n}$(aqua)$_{9n}$copper(II)$_{3n}$ [Cu$^{II}_{3n}$(HC 21-phosphate)$_{2n}$(H$_2$O)$_{9n}$] (22). This coordination compound was prepared by dissolving 1 g (0.002 mol) of the disodium salt of hydrocortisone 21-phosphate in 25 ml of water and adding this solution dropwise to a stirred solution of cupric acetate monohydrate to 25 ml of water. After the addition was complete, stirring was continued for 0.5 hr before the light blue precipitate was collected by filtration and washed with 500 ml of water before air drying. The yield was 0.85 g (34% yield). On heating a sample of this material to 209°C it decomposed. Anal. ($C_{42}H_{78}O_{25}P_2Cu_3$) C, H.

(Hydrocortisone 21-phosphato)$_{2n}$(aqua)$_{7n}$copper(II)$_{3n}$ [Cu$^{II}_{3n}$(HC 21-phosphate)$_{2n}$(H$_2$O)$_{7n}$] (22). This coordination compound was prepared by dissolving 1 g (0.002 mol) of the disodium salt of hydrocortisone 21-phosphate in 100 ml of water, adding 1 drop of concentrated hydrochloric acid to give a pH of 6.6, and adding this solution dropwise to a stirred solution of cupric chloride dihydrate (1 g, 0.006 mol) in 50 ml of water. After the addition was complete the mixture was allowed to stir for 1 hr and the light blue precipitate collected by filtration, washed with 200 ml of water, air-dried, and weighed (400 mg, 33% yield). A sample of this material gradually decomposed on heating to 210°C. Anal. ($C_{42}H_{74}O_{23}P_2Cu_3$) C, H.

(Hydrocortisone 21-hemisuccinato)$_{4n}$(aqua)$_{6n}$copper(II)$_{2n}$ [Cu$^{II}_{2n}$(HC 21-hemisuccinate)$_{4n}$(H$_2$O)$_{6n}$] (24). This coordination compound was prepared by dissolving 1 g (0.002 mol) of hydrocortisone-21-hemisuccinic acid in 250 ml of water with concentrated ammonium hydroxide. The resulting pH was 9.0 and was adjusted to pH 7.0 with a 10% solution of hydrochloric acid. This solution was then added dropwise to a stirred solution of cupric chloride dihydrate 1 g (0.006 mol) dissolved in 250 ml of water. The resulting light blue-green precipitate was collected, air-dried, and weighed (1 g, 96% yield). A sample of this material decomposed on heating over the range of 191–195°C. Anal. ($C_{100}H_{144}O_{38}Cu_2$) C, H.

(Hydrocortisone 21-hemisuccinato)$_{4n}$(aqua)$_{7n}$copper(II)$_{2.5n}$ [Cu$^{II}_{2.5n}$(HC 21-hemisuccinate)$_{2n}$(H$_2$O)$_{7n}$] (24). This coordination compound was prepared by dissolving 1 g (0.002 mol) of hydrocortisone-21-hemisuccinic acid in 20 ml of water with concentrated ammonium hydroxide. The resulting pH was 9.5. This solution was then added dropwise to a stirred solution of cupric chloride dihydrate (1 g, 0.006 mol) dissolved in 15 ml of water. The light blue precipitate which formed was collected by filtration, air-dried, and weighed (1.2 g, 99% yield). A sample of this material decomposed on heating over the range of 196–197°C. Anal. ($C_{100}H_{160}O_{46}Cu_5$) C, H.

(9α-Fluoro-11β,17α,21-trihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione-21-phosphato)$_{2n}$(aqua)$_{7n}$copper(II)$_{3n}$ [Cu$^{II}_{3n}$(dexamethasone 21-phosphate)$_{2n}$(H$_2$O)$_{7n}$] (23). This coordination compound was prepared by dropping a solution of the disodium salt of dexamethasone 21-phosphate (9 g, 0.017 mol) dissolved in 100 ml of water into a stirred solution of 100 ml of water containing 4.6 g (0.003 mol) of cupric chloride dihydrate. After the addition was completed an additional 300 ml of water was added. The resulting light blue precipitate was collected by filtration, washed with water, air-dried, and weighed (8.1 g, 75% yield). A sample of this material gradually decomposed on heating to 300°C. Anal. ($C_{88}H_{140}O_{46}P_4F_4Cu_6$) C, H.

(9α-Fluoro-11β,17α,21-trihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione-21-phosphato)$_{2n}$(aqua)$_{1.5n}$copper(II)$_{3n}$ [Cu$^{II}_{3n}$(dexamethasone 21-phosphate)$_{2n}$(H$_2$O)$_{1.5n}$] (23). This coordination compound was prepared by taking 2 g (0.008 mol) of Cu$^{II}_{3n}$(dexamethasone 21-phosphate)$_{2n}$(H$_2$O)$_{7n}$ and suspending it in stirred methanol for 2 hr to remove some of the water of hydration. After air drying this material was dried at 45°C and 15 mmHg overnight. A sample of this material also decomposed on heating to 300°C. Anal. ($C_{88}H_{118}O_{35}P_4F_4Cu_6$) C, H.

[1-(*p*-Chlorobenzoyl)-5-methoxy-2-methylindole-3-acetato]$_{4n}$(aqua)$_{4n}$copper(II)$_{2n}$ [Cu$^{II}_{2n}$[1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetate]$_{4n}$(H$_2$O)$_{4n}$] (20). This coordination compound was synthesized from the sodium salt of the parent acid (5 g, 0.014 mol), prepared as in method A, in 200 ml of water. The solution of the sodium salt was dropped into a stirred 300-ml water solution of cupric chloride dihydrate (1.95 g, 0.013 mol). The resultant green precipitate was collected by filtration, washed with water, air-dried, and weighed (5.6 g, 98% yield). A sample of this material decomposed on heating to 190°C. Anal. ($C_{76}H_{68}O_{20}N_4Cl_4Cu_2$) C, H, N.

[1-(*p*-Chlorobenzoyl)-5-methoxy-2-methylindole-3-acetato]$_{4n}$(acetone)$_{2n}$copper(II)$_{2n}$ [Cu$^{II}_{2n}$[1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetate]$_{4n}$-

$(CH_3COCH_3)_{2n}$] (20). This compound was prepared in a manner similar to that described for Cu^{II}_{2n}[1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetate]$_{4n}(H_2O)_{4n}$, using twice the amount of parent acid and cupric chloride dihydrate. However, after the green precipitate was collected by filtration it was leached with 1 l. of acetone and the leachate concentrated to 500 ml. On standing, additional green crystals formed in the acetone solution. These were collected by filtration, air-dried, and weighed (6.9 g, 62% yield). A sample of this material decomposed on heating up to and over the range of 190–193°C. Anal. ($C_{82}H_{72}O_{18}N_4Cl_4Cu_2$) C, H, N.

(4-*n*-Butyl-1,2-diphenyl-3,5-pyrazolidinedione)$_{2n}$copper(II)$_n$ [Cu^{II}_n(4-*n*-butyl-1,2-diphenyl-3,5-pyrazolidinedione)$_{2n}$] (21). A solution of the sodium salt of the parent compound (5 g, 0.015 mol) dissolved in 50 ml of 95% ethanol was diluted with 150 ml of H_2O. To this stirred solution was added 2.73 g (0.007 mol) of cupric acetate monohydrate in small aliquots. The greenish precipitate which formed was collected by filtration, dried at 95°C and 15 mmHg overnight, and weighed (4.5 g, 94.5% yield). A sample of this material softened and melted over the range of 65–75°C. Anal. ($C_{38}H_{38}N_4O_4Cu$) C, H, N.

(17-Hydroxy-3-oxo-17α-pregna-4,6-diene-21-carboxylato)$_{2n}$(aqua)$_{2n}$copper(II)$_n$ [Cu^{II}_n(17-hydroxy-3-oxo-17α-pregna-4,6-diene-21-carboxylato)$_{2n}(H_2O)_{2n}$] (25). The potassium salt of the parent acid (5 g, 0.013 mol) was dissolved in 50 ml of water. This solution was dropped into a stirred solution of cupric acetate monohydrate, prepared by dissolving 5 g (0.012 mol) in 50 ml of water. After the addition was completed the mixture was left to stir for an additional 0.5 hr before removing the precipitate by filtration. This precipitate was washed with 500 ml of water before air drying, followed by drying at 30°C and 15 mmHg over the weekend. A 5-g (24%) yield was obtained. A sample of this material decomposed on heating over the range of 168–169°C. This material was redried at 40°C and 15 mmHg before obtaining elemental analysis. Anal. ($C_{88}H_{128}O_{22}Cu_2$) C, H.

Acknowledgment. I am particularly grateful to my colleagues at Searle Laboratories for having done nearly all of the biological testing of these compounds. These include Dr. R. Aspinal and Mr. Bergstrom for the anti-inflammatory work, Mr. B. Bianchi and Mr. M. Hurley for the antiulcer and anticholinergic studies, Mr. T. Martinez for the LD_{50} determinations, Dr. H. Lennon for the rat liver toxicologic studies, Dr. C. Craig for the qualitative mouse behavior and LD_{50} estimations, Dr. L. Hofmann for the diuretic studies, and to Mr. M. Wegner, Mr. R. Niinemae, and Mr. D. Bozian for chemical technical assistance. I also wish to thank the G. D. Searle Co. for allowing me to have the compounds and data obtained with them. I also wish to express my gratitude to Professor A. M. Dannenberg, Jr., of the Johns Hopkins University, School of Hygiene and Public Health, for his cathepsin D inhibition work, to Professor S. F. Townsend of the University of Cincinnati, College of Medicine, for his rate of ulcer healing studies, and to Dr. A. L. Willis of the Hoffmann-La Roche Research Institute for his studies of prostaglandin synthesis.

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Podophyllotoxin Analogs. 1. Synthesis and Biological Evaluation of Certain *trans*-2-Aryl-*trans*-6-hydroxymethyl-3-cyclohexenecarboxylic Acid γ-Lactones as Antimitotic Agents†,1

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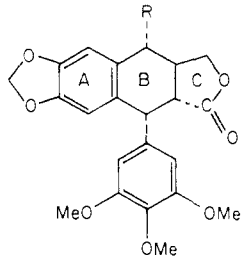
A series of cis and trans bicyclic lactones was prepared as congeners of podophyllotoxin (1) and evaluated as antimitotic agents both in cell cultures grown in vitro and in an in vitro protein binding assay. All compounds displayed insignificant activity—a result which may reflect insufficient structural similarity to podophyllotoxin or which may be interpreted as in agreement with previous observations of the stereochemical requirements for antimitotic activity defined for 1.

Podophyllotoxin (1) is a naturally occurring lignan lactone which is found in several species of *Podophyllum*, more commonly known as American mandrake or May apple. In the late 1940's and early 1950's the anticancer action of 1 was demonstrated.[2-4]



1, R = OH
2, R = H

Clinical testing, however, has revealed the inadequacies of 1 in the treatment of most cancers.[5] Moreover, the severe damage to rapidly proliferating tissues, consistent with a mechanism of cellular destruction based upon arrest of dividing cells at mitosis,[6] as well as its toxic effect on the CNS has limited its usefulness in cancer chemotherapy. Consequently a variety of semisynthetic and synthetic analogs has been prepared[7-19] in attempts to exploit the antitumor activity of podophyllotoxin in a molecule presenting more defined and narrow pharmacological properties. The most therapeutically useful substances arising from those studies to date appear to be several of the semisynthetic derivatives, notably podophyllic acid ethyl hydrazide[20-22] and 4'-demethylepipodophyllotoxin 9-(4,6-*O*-2-thenylidene)-β-D-glucopyranoside,[23-26] both of which have been used clinically with some success.

† Dedicated to Edward E. Smissman who was Chairman of this Department from 1960 until his death, July 1974.

The present study was initiated with an aim toward determining the minimum structural (and stereochemical) requirements necessary for the antimitotic activity of podophyllotoxin. In this regard our efforts have initially been directed toward the synthesis of B–C ring analogs of 1. In this first report we wish to relate the synthesis and biological evaluation of certain *trans*-2-aryl-*trans*-6-hydroxymethyl-3-cyclohexenecarboxylic acid γ-lactones (**12**), compounds which perhaps more appropriately could be termed analogs of deoxypodophyllotoxin (**2**), a known cytotoxic derivative present in several plants.[27-30]

Chemistry. The synthetic route employed for the preparation of the all-cis bicyclic lactones **7** is outlined in Scheme I. Treatment of the arylallylcarbinol **3**, prepared from the benzaldehyde and allyl chloride,[31] with TsOH in benzene at reflux for 2 hr generated the arylbutadiene, which was not isolated. Subsequent reaction of the preformed diene with maleic anhydride in benzene as solvent at reflux for 3 (**3b**) or 24 hr (**3a**) or alternatively in a steel autoclave at 120° for 24 hr (**3c**) afforded the Diels–Alder adducts **4a–c** in good yield. Reduction of anhydrides **4** with either $NaBH_4$–THF, Red-Al–C_6H_6, $LiAlH_4$–THF at −55°, or Li(*O*-*t*-Bu)$_3$AlH–C_6H_6 gave, in all cases, two products **5** and **6**, in roughly equal proportions, easily separable by column chromatography or fractional crystallization. One of the products (**5**) was found to be lactonic in nature (ir, C=O, 1770 cm^{-1}) while the other was determined to be a hydroxy acid (ir, broad OH, C=O, 1700 cm^{-1}) (Table I). Treatment of **6** with DCC yielded a different lactone **7**. These results differ from the metal hydride reduction of anhydrides reported by others[32,33] wherein only the lactone resulting from reduction of the most hindered carbonyl was formed.

That lactones **5** and **7** were indeed positional isomers as depicted and not geometrical isomers was shown by dehydrogenation experiments. When **5** and **7** were heated