to give 13 g (86%) of a colorless liquid, which solidifed upon cooling, mp 31–33 °C. Its hydrobromide salt (**10**) was prepared in ether and recrystallized from methanol–acetone–ether. Properties of **10** are included in Table I.

5-(4-Methoxyphenyl)-2-*n*-propyl-2-azabicyclo[3.2.1]octane Hydrobromide (11). Method A. A mixture of **10** (1.5 g of the free base, 6.9 mmol), 1.4 g of *n*-propyl iodide (15% excess), and 1.2 g of sodium bicarbonate in 20 mL of DMF was stirred at 80 °C for 16 h. Workup according to the previously described procedure[1] afforded a clear oil, which was converted to 1.8 g of **11** with ethereal HBr. Properties of **11**, and **13** prepared in a similar manner, are included in Table I.

5-(4-Hydroxyphenyl)-2-azabicyclo[3.2.1]octane Hydrobromide (15). Method B. A mixture of **10** (2.7 g, 9.2 mmol) in 20 mL of 48% hydrobromic acid was stirred at 120 °C for 30 min. The excess acid was removed in vacuo, and the solid residue was recrystallized from ethanol–ether to give 2.3 g of **15** as rhombic crystals. Properties of **15**, and of **16–19** prepared in a similar manner, are included in Table I.

5-(4-Methoxyphenyl)-2-phenethyl-2-azabicyclo[3.2.1]octane Hydrobromide (12). Method C. A mixture of 1.9 g (8.7 mmol) of the free base from **10** and 1.7 g of sodium bicarbonate in 40 mL of $CHCl_3$ was treated dropwise with 2.1 g of phenylacetyl chloride during 10 min. After stirring at room temperature for 4 h, the mixture was poured onto 100 g of ice–water, and the $CHCl_3$ solution was washed consecutively with dilute HCl, aqueous $NaHCO_3$, and water. After drying over $MgSO_4$ and removal of solvent, the crude amide (homogeneous by TLC) was reduced with 1.1 g of lithium aluminum hydride in 70 mL of THF for 3 h. Workup in the usual manner led to a pale yellowish oil, which was converted to a crystalline hydrobromide with ethereal HBr. Recrystallization of the crude salt from methanol–acetone–ether gave 1.8 g of **12**. Properties of **12** are included in Table I.

2-(Cyclopropylcarbonyl)-5-(4-hydroxyphenyl)-2-azabicyclo[3.2.1]octane Cyclopropanecarboxylate (14). Method D. To a stirred suspension of 2.0 g (6.9 mmol) of **15** and 5.0 g of triethylamine in 30 mL of $CHCl_3$ was added dropwise 2.35 g of cyclopropylcarbonyl chloride (Aldrich Chemical Co.) in 2 mL of $CHCl_3$. The mixture was stirred at room temperature overnight and concentrated in vacuo to a semicrystalline residue. Trituration with $CHCl_3$, followed by washing and drying ($MgSO_4$), afforded 2.0 g of **14**. Properties of **14** are included in Table I.

2-(Cyclopropylmethyl)-5-(4-hydroxyphenyl)-2-azabicyclo[3.2.1]octane Hydrobromide (20). Method E. A solution of 1.9 g (5.6 mmol) of **14** in 20 mL of THF was added dropwise to a refluxing mixture of 0.6 g of lithium aluminum hydride in 30 mL of THF. The mixture was heated under reflux for 3 h and decomposed with water. Workup in the usual manner led to a phenolic amine, which was converted to 1.6 g of a crystalline hydrobromide (**20**) in ether. Properties of **20** are included in Table I.

[^{3}H]Naloxone Binding. Binding assays were conducted employing modifications of the method described by Pert and Snyder.[6] Male Wistar rats weighing 100–150 g were sacrificed by cervical dislocation and their brains rapidly removed. Brain minus cerebellum was homogenized in 20 volumes of cold 50 mM Tris-HCl, pH 7.4, using a Tekmar homogenizer (30 s at a medium setting of 40–50) and then centrifuged for 10 min at 40000*g*. The pellet was washed twice and the supernatant discarded. The final pellet was then dispersed (Tekmar homogenizer, 5 s at medium speed) in 50 volumes of the 50 mM Tris-HCl buffer. For binding assays, 500 μL of tissue suspension was added to all tubes. For the determination of total binding, each tube contained in final concentration dextrophan (0.1 μM), Tris-HCl, pH 7.4 at 37 °C (50 mM), and [^{3}H]naloxone (5 nM). Nonspecific binding tubes contained levorphanol (0.1 nM), Tris-HCl, pH 7.4 at 37 °C (50 mM), and [^{3}H]naloxone (5 nM). Test drugs were run as follows: Tris-HCl, pH 7.4 at 37 °C (50 mM), [^{3}H]naloxone (5 nM), and varying concentrations of test drug. All incubation mixtures were in a final volume of 1.0 mL. All tubes were incubated for 30 min at 37 °C and the reaction was terminated by filtration through Whatman GF/B filters under vacuum. The filters were washed three times with 5 mL of chilled 50 mM Tris-HCl buffer, pH 7.4, and then transferred to scintillation vials. Ten milliliters of scintillation fluid (3a70B counting cocktail, Research Products, Inc.) was added and radioactivity determined with a Beckman LS-355 liquid scintillation system.

The stereospecific binding of [^{3}H]naloxone was determined by the difference between the total radioactivity bound in the presence of 0.1 μM dextrorphan minus the radioactivity bound in the presence of 0.1 μM levorphanol. The data were converted to percent [^{3}H]naloxone displaced by test drug, and IC_{50} values were obtained using computer-derived Litchfield–Wilcoxin log-probit analyses.

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(7) Compound **20** evoked a 10% antagonistic response when administered sc at 50 mg/kg; see ref 1 for experimental details.

Antiinflammatory Effects of Some Copper Complexes

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Copper complexes of a range of ligands have been prepared and evaluated for antiinflammatory activity and irritancy after oral, subcutaneous, and local administration in rats and guinea pigs. The antiinflammatory activities were found to depend on the species used and the route of administration. When nonantiinflammatory ligands were used, the response was generally dose dependent. With D-penicillamine and when the ligands were themselves antiinflammatory in animal models of inflammation—as was the case with flufenamic acid, levamisole, aspirin, L-histidine, and 2-amino-2-thiazoline—differences in antiinflammatory activity were observed between the copper complexes and the free ligands. In some cases, the copper complexes were the more effective. There was a weak correlation between local (subplantar) irritation and the dose of copper but, for four compounds studied in more detail, the response in the local subplantar test and degree of antiinflammatory activity were not related, suggesting that the action of copper is not solely by a counterirritant mechanism. No obvious differences between the activities of copper(I) and copper(II) compounds were observed, suggesting that a common metabolite may be involved in the antiinflammatory action of copper.

Sorenson[2] has observed that copper complexes of nonantiinflammatory ligands administered subcutaneously to the rat produce an antiinflammatory effect in several animal models of inflammation and that copper complexes

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of antiinflammatory compounds possess greater activity than the ligands themselves. The complexes have also been shown to possess antiulcer activity[1] and to produce less gastric irritation than the ligands.[3a,b] Sorenson attributed the antiinflammatory activity to the action of the complexes in vivo, but Rainsford[4] et al. have suggested that the activity may be due to a counterirritant mechanism caused by free copper ions. The rat has been most widely used for the studies carried out to date, but species differences in antiinflammatory activity and gastric irritancy have been reported.[5-7] In this investigation, we have examined the effects of copper compounds in a rat and guinea pig model of inflammation following oral or subcutaneous routes of administration. Further, in an attempt to evaluate the counterirritant hypothesis,[4,8] the results obtained after local administration have been compared with those obtained after systemic administration. The complexes used covered a range of stabilities and solubilities for both copper(I) and copper(II). Of the ligands used, those classified as potentially active were aspirin (acetylsalicylic acid, asp), *d*-penicillamine (β-mercaptovaline, pen), levamisole (DL-6-phenyl-2,3,5,6-tetrahydro[2,1-*b*]thiazole, LM), flufenamic acid (2-[[3-(trifluoromethyl)phenyl]amino]benzoic acid, fluf), L-histidine, and 2-amino-2-thiazoline (2A2T), an active compound in animal tests. Those classified as inactive were pyridine (py), ethylenediaminetetraacetic acid (EDTA), diaminoethane (en), diethyl dithiocarbamate (Et_2 dtc), anthranilic acid (anth), thiourea (tu), triphenylphosphine (Ph_3P) and the three sulfonamides *N*-[[(1-phenyl-2-hydroxyethyl)amino]ethyl]-*p*-toluenesulfonamide (HS1), *N*-[[(2-phenyl-2-hydroxyethyl)amino]ethyl]-*p*-toluenesulfonamide (HS2), and *N*-[1-phenyl-2-(2-iminothiazolidin-3-yl)ethyl]-*p*-toleuensulfonamide (HS3). This classification is, at best, approximate. In particular, *d*-penicillamine is classified as an antiinflammatory ligand, although no significant antiinflammatory activity has been demonstrated in any animal model of inflammation. It has, however, become established as an effective antiarthritic drug in man. To try to exclude the possibility of any ligand activity caused by the use of a metal complex being attributed to the direct action of copper, penicillamine and anthranilic acid are classified as potentially active ligands.

Experimental Section

Preparation of Complexes. $Cu^{II}(anth)_2$,[1] $Cu^{II}(H_2EDTA)\cdot H_2O$,[9] $K_2Cu^{II}(EDTA)\cdot 2H_2O$,[9] $Cu^{II}(en)_2Cl_2\cdot 5H_2O$,[10] $Cu^{II}(py)_2Cl_2$,[1] $Cu_2^{II}(py)_2(acetate)_4$,[1] $Cu^{II}(Et_2\ dtc)_2$,[11] CuCl,[12] $[Cu^{II}(tu)_3]_2SO_4\cdot 2H_2O$,[12] $Cu^{I}(PPh)_3Cl$,[3b] $Cu^{I}(PPh_3)_2(Et_2\ dtc)$,[10] $Cu^{II}(His)_2(NO_3)_2\cdot H_2O$[15] and $Cu^{I}(pen)$[1] were prepared, using methods described in the literature. $Cu^{II}(en)_2(NO_3)_2\cdot H_2O$ and $Cu^{II}(en)_2SO_4\cdot H_2O$ were prepared from copper nitrate and copper sulfate, respectively, by the method used to prepare $Cu^{II}(en)_2Cl_2\cdot 0.5H_2O$. $Cu^{II}_2(OAc)_4(H_2O)_2$, Cu_2O, and $CuCl_2\cdot 2H_2O$ were obtained directly from BDH and used without further purification. The other copper complexes of ligands HS 1, HS 2, and HS 3 were prepared as described by Bailey et al.[16] Elemental analysis of carbon, hydrogen, and nitrogen was determined and found to be within ±0.3% of the theoretical result except where specified below.

Electronic spectra were recorded on a Unicam SP1800 spectrophotometer.

$Cu^{II}(S1)_2$. A solution of $CuCl_2\cdot 2H_2O$ (0.11 g) in methanol (5 mL) was added to a solution of HS1 (0.875 g) in methanol (50 mL). The blue solution obtained was made alkaline by adding 5 mL of a methanolic solution of sodium hydroxide dropwise. The mixture was cooled to 0 °C and left at this temperature for 24 h. The purple crystals obtained were filtered, washed with 5 mL of methanol, and air-dried. Anal. ($C_{34}H_{44}N_4O_6S_2Cu$) C, H, N.

$Cu^{II}(S2)Cl$. This compound was prepared by allowing HS2 (0.55 g) in methanol (25 mL) to react with $CuCl_2$ (0.22 g) in methanol (20 mL). A 1 M methanolic solution of sodium hydroxide was added to this green solution until the mixture just started to become cloudy. Blue needles precipitated on standing overnight at 0 °C. These were filtered, washed with 5 mL of methanol, and air-dried. Anal. ($C_{17}H_{22}N_2O_3SCu$) H; N: calcd, 47.2; found, 46.6.

$Cu^{II}(S3)_2$. A warm solution (35 °C) of copper acetate (0.4 g) in ethanol (40 mL) was added to a warm solution of the ligand (1.51 g) in ethanol (40 mL). A gray-green solid precipitated on cooling the dark-green solution to room temperature. The product was filtered, washed with ethanol (2 × 25 mL) and ether (2 × 25 mL), and air-dried. Anal. ($C_{36}H_{42}N_6O_4S_4Cu$) C, H, N.

$Cu^{II}_2(asp)_4$ was prepared by dissolving acetylsalicylic acid (30 g) in water (200 mL) at 0 °C with 50% (w/v) sodium hydroxide solution so that the pH remained below 11. On completion, the final pH was about 8. A solution of $CuCl_2\cdot 2H_2O$ (56 g) in water (500 mL) was added over a 10-min period. The blue precipitate obtained was washed with water, acetone, and diethyl ether and left to air-dry. Anal. ($C_{36}H_{28}Cu_2$) C, H, N.

$Cu^{II}(py)_2(asp)_2$ was prepared by dissolving copper(II) aspirinate (1 g) in 15 mL of pyridine preheated to 60 °C. The mixture was refluxed for 15 min. Diethyl ether was added to the cooled solution, until precipitation was complete. The lilac-colored product was filtered, washed with diethyl ether, and air-dried. Anal. ($C_{28}H_{24}N_2O_8Cu$) C, H, N.

$Cu^{I}(PPh_3)asp$ was prepared by refluxing a mixture of copper(II) aspirinate (1 g) and triphenylphosphine (2.18 g) in methanol (40 mL) until the solution was colorless. The solution was allowed to stand at 0 °C for 12 h. The white crystals obtained were filtered, washed with methanol, and air-dried. Anal. ($C_{27}H_{22}O_4PCu$) C, H, N.

$Cu^{II}(LM)_2Cl_2$ was prepared by dissolving 0.5 g of levamisole in ethanol (20 mL) and adding it dropwise to a stirred solution of $CuCl_2\cdot 2H_2O$ (0.11 g) in ethanol. The brown solution was left at 0 °C overnight. The green complex precipitated and was washed with ethanol and diethyl ether before air-drying. Anal. ($C_{22}H_{22}N_4S_2Cl_2Cu$) C, H, N.

$Cu^{II}(LM)_2I$ was prepared by stirring together levamisole (0.5 g) and copper(I) iodide (0.23 g) in absolute ethanol for 24 h. The white solid was filtered, washed with ethanol, and air-dried. Anal. ($C_{22}H_{22}N_4S_2ICu$) C, H, N.

$Cu^{II}(2A2T)_4Cl_2$ was prepared by adding dropwise a solution of $CuCl_2$ (0.46 g) in ethanol (10 mL) to a stirred solution of the ligand (2 g) in ethanol (30 mL). The mixture was cooled in ice and the dark-blue complex precipitated. Anal. ($C_{12}H_{24}N_8S_4Cl_2Cu$) C, H; N: calcd, 26.5; found, 27.0.

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Table II. Biological Activities of Compounds in the Rat[a]

test:	rat paw kaolin edema	rat gastric irritancy	rat paw kaolin edema	kaolin and compd edema[b]
rte of admin:	oral	oral	sc	local (subplantar)
	A. inactive ligands[c]			
Cu_2O	I at 50	0 at 50	A at 30	NE
CuCl	I at 300[e]	+40 at 300	A at 30	+88
$[Cu^I(tu)_3]_2SO_4 \cdot 2H_2O$	A at 300	+10 at 300	A at 30	+146
Cu^IPPh_3Cl	I at 300	0 at 300	A at 100	−8
$Cu^I(PPh_3)_2(Et_2\ dtc)$	I at 300	0 at 300	I at 300	NE
$Cu^{II}(H_2EDTA) \cdot H_2O$	I at 300	0 at 300	A at 30[d]	+48
$K_2Cu^{II}EDTA \cdot 2H_2O$	I at 300	0 at 100	A at 100	+104
$Cu^{II}(S1)_2$	I at 300	+30 at 300	I at 300	NE
$Cu^{II}(S2)Cl$	I at 300	0 at 300	A at 100	NE
$Cu^{II}(S3)_2$	I at 300	0 at 300	NE	NE
$Cu^{II}(en)_2Cl_2 \cdot 0.5H_2O$	A at 100	0 at 300	A at 30[e]	NE
$Cu^{II}(en)_2(NO_3)_2 \cdot H_2O$	A at 300[f]	+93 at 300	NE	+94
$Cu^{II}(en)_2SO_4 \cdot H_2O$	I at 300[f]	+37 at 300	NE	+97
$Cu^{II}(py)_2Cl_2$	I at 300	+20 at 300	A at 30	+317
$Cu^{II}(py)_2(OAc)_4$	I at 300	0 at 300	A at 30	+429
$Cu^{II}(OAc)_4(H_2O)_2$	I at 50	0 at 50	NE	NE
$CuCl_2 \cdot 2H_2O$	I at 50	0 at 50	NE	NE
$Cu^{II}(Et_2\ dtc)_2$	NE	NE	I at 300	NE
	B. active ligands[c]			
aspirin	A at 300	+23 at 300	A at 300	+31
$Cu^{II}(py)_2(asp)_2$	A at 300	+3 at 300	A at 100	NE
$Cu^{II}_2(asp)_4$	A at 300	+27 at 300	A at 10	+182
$Cu^I(PPh_3)_3asp$	I at 300	0 at 300	I at 100	−15
flufenamic acid	A at 10	0 at 100	NE	NE
$Cu^{II}(fluf)_2 \cdot 0.5H_2O$	A at 10	0 at 100	NE	+20
$Cu^{II}(arth)_2$	I at 300	0 at 300	NE	+30
levamisole	A at 100	0 at 100	NE	NE
$Cu^{II}(LM)_2Cl_2$	A at 30	0 at 300	A at 100	+465
$Cu^I(LM)_2I$	I at 300	0 at 300	A at 300	NE
d-penicillamine	I at 300	+20 at 300	NE	NE
$Cu^I(pen) \cdot 5H_2O$	I at 300	+17 at 300	A at 100	NE
2-amino-2-thiazoline	A at 300	+80 at 300	NE	NE
$Cu^{II}(2A2T)_4Cl_2$	A at 300	0 at 300	A at 30	+167
$Cu^{II}(His)_2(NO_3)_2 \cdot 2H_2O$	*g*	+30 at 30	A at 30	+78[h]

[a] $p < 0.05$; five animals per group. [b] 3 mg/paw administered; % increase or decrease. [c] Abbreviations used: I = inactive at the dose stated (mg/kg); A = active ($p < 0.05$) at the dose stated (mg/kg); NE = not examined. [d] 3/5 sacrificed at 100 mg/kg because of severe scratching of injection site by animals. [e] 3/5 rats died at 30 mg/kg. [f] Animals showed tense body tone, hypothermia, and reduced spontaneous activity. [g] Caused an increase in paw edema at 100 and 30 mg/kg. [h] 5/5 died at 300 mg/kg.

$Cu^{II}(fluf)_2 \cdot 0.5H_2O$. The sodium salt of flufenamic acid was prepared in situ by adding a 50% (w/v) aqueous solution of sodium hydroxide to a stirred suspension of the acid (0.93 g) in water (25 mL) so that the final pH was below 9 when all of the acid was in solution. Addition of a stoichiometric amount of copper chloride in aqueous solution (10 mL) gave immediate precipitation of the copper complex, which was then washed with water and air-dried. Anal. ($C_{28}H_{20}N_2O_{4.5}F_6Cu$) C, H, N.

Pharmacology. The methodology used for induction of kaolin paw edema has been described in detail elsewhere.[7] Male Wistar rats (CE/CFHB, 80–100 g) or female Dunkin-Hartley guinea pigs (150–180 g) were used in groups of five for all experiments. Edema in both species was routinely evaluated 4 h after the subplantar administration of a 10% kaolin (BDH Ltd.) suspension in 0.9% (w/v) sodium chloride solution. Paw edema (measured as paw diameter) in animals treated with copper complexes was expressed as a percentage increase (+) or decrease (−) of the edema elicited in vehicle-treated animals.

Gastric irritancy in rats was assessed 6 h after oral administration of drug by visual examination of stomach lesions.[17] Stomachs from guinea pigs were assessed for gastric irritation 24 h after drug administration, because the gastric lesions were of a different nature in guinea pigs, with far greater hemorrhaging being apparent.

All compounds were administered in 5% mulgofen, a polyoxyethylated vegetable oil (EL-719, GAF Co., Manchester), in distilled water (0.2 mL/100 g of body weight) 1 h before kaolin administration. Animals were dosed with drugs on a weight of drug (mg) per weight of animal (kg) basis.

Local irritation was evaluated by subplantar injection of 7, 3, 1, or 0.3 mg of copper complex with kaolin into a hind paw of the rat.[7] The increase (+) or decrease (−) in the paw diameter compared to controls treated with kaolin alone was expressed in percentage terms.

Table III. Biological Activity of Copper Complexes Administered Orally in the Guinea Pig[a] ($p < 0.05$, $n = 5$)

compd	biological activity[b]: paw edema	biological activity[b]: gastric irritancy
$Cu^{II}(py)_2Cl_2$	A at 100	+40 at 300
$Cu^{II}_2(py)_2(OAc)_4$	A at 300	+60 at 300
$Cu^{II}_2(OAc)_4(H_2O)_2$	I at 50	+30 at 50
$CuCl_2 \cdot 2H_2O$	I at 50	+60 at 50
$Cu^{II}(Et_2\ dtc)_2$	I at 300	+13 at 300
Cu_2O	A at 30	+45 at 50
CuCl	I at 50[c]	+60 at 50
$Cu^I(PPh_3)_2(Et_2\ dtc)$	I at 300	+10 at 300

[a] $p < 0.05$; $n = 5$. [b] Abbreviations used: A = active ($p < 0.05$) at the dose stated (mg/kg); B = inactive at the dose stated (mg/kg). [c] At 300 mg/kg, 5/5 animals died. At 50 mg/kg, 2/5 dead at 24 h.

Results

Electronic spectra (Table I, see paragraph at the end of this paper concerning supplementary material) show that,

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Table IV. Statistical Correlations for Copper Complexes in the Kaolin Edema and Paw Irritancy Models

parameters correlated	species	rte of admin	n	r	p
ln dose of Cu and AI act. for all act. compds	rat	sc	24	-0.581	0.01
ln dose of Cu and AI act. excluding insoluble $Cu^{II}PPh_3Cl$ and $Cu^{II}(S1)Cl$	rat	sc	18	-0.769	0.001
ln dose of Cu irritancy on subplantar (local) admin	rat	local	27	0.443	0.05
ln dose of Cu and AI act.	rat	oral	27	-0.470	0.02
AI response and gastric irritancy	rat	oral	33	-0.333	NS[b]
ln dose of Cu and AI act. (all compds tested)	GP[a]	oral	17	-0.433	NS
ln dose of Cu and AI act. (for soluble compds)	GP	oral	11	-0.673	0.05
gastric irritancy and AI act. (all compds)	GP	oral	14	-0.560	0.05
gastric irritancy and AI act. (soluble compds)	GP	oral	10	-0.395	NS

[a] GP = guinea pig. [b] NS = not significant.

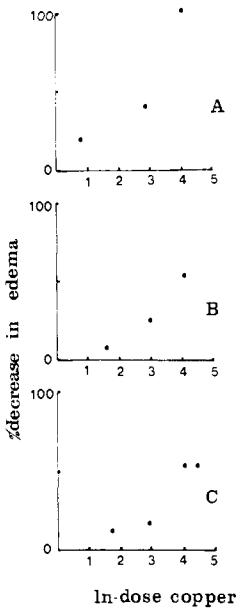

Figure 1. Dose–response of three orally active copper compounds in the rat paw kaolin model of inflammation: (A) $Cu^{II}(en)_2Cl_2 \cdot 5H_2O$; (B) $Cu^{II}(en)_2(NO_3)_2 \cdot H_2O$; (C) $[Cu^{II}(tu)_3(SO_4)_2] \cdot 2H_2O$.

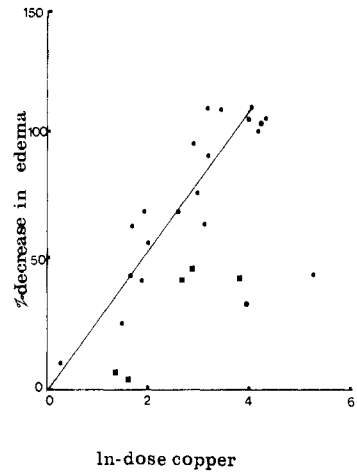

Figure 2. Relationship between dose of copper and response in the rat paw kaolin model of inflammation for all copper compounds administered by subcutaneous injection. The insoluble complexes $Cu^I(PPh_3)Cl$ and $Cu^{II}S2Cl$, which appear to produce a somewhat lower edema than the soluble complexes, are marked.

with the exceptions of $Cu^I(PPh_3)$asp, Cu^I(pen)·1.5H_2O, and $Cu^I(LM)_2I$, the new complexes contain copper(II), since all show the typical d–d absorption band between 600 and 800 nm.

Biological Tests. The results of the biological tests in rats are given in Table II. Section A includes the ligands which are not thought to have antiinflammatory (AI) activity and section B includes penicillamine and the ligands which are active. Table IV gives the results of a limited number of tests in guinea pigs.

Effect of Oral Administration of Copper Compounds of Nonactive Ligands against Kaolin-Induced Paw Edema in Rats. Of the compounds examined orally in the kaolin model, $Cu^{II}(en)_2(NO_3)_2 \cdot H_2O$ and $[Cu^I(tu)_3]_2SO_4 \cdot 2H_2O$ significantly inhibited the edema ($p < 0.05$) at a dose of 300 mg/kg and $Cu^{II}(en)_2Cl_2 \cdot 0.5H_2O$ effectively inhibited the response at 100 mg/kg (Table II). As shown in Figure 1, oral activity of these three compounds was dose related. $Cu^{II}(en)_2SO_4 \cdot 0.5H_2O$ possessed significant activity at a dose of 300 mg/kg. There was a correlation ($p < 0.02$; $r = -0.470$) between the percent change in edema and the ln dose of copper when all orally administered compounds tested were included in the sample.

Effect of Subcutaneous Administration of Copper Compounds of Nonactive Ligands against Kaolin-Induced Paw Edema in Rats. Twelve copper compounds were tested sc in rats and of these, nine significantly inhibited ($p < 0.05$) the edema. Six of the compounds were active at the lowest dose administered (30 mg/kg). Both $[Cu^I(tu)_3]_2SO_4 \cdot 2H_2O$ and $Cu^{II}(en)_2Cl_2 \cdot 0.5H_2O$ were more active when administered sc compared with oral administration. The AI activity produced on sc administration of copper compounds was generally dose related. A plot of AI activity vs. the ln of the dose of copper is shown for copper complexes administered sc to rats (Figure 1). There was a statistically significant linear correlation ($n = 24$; $r = -0.581$; $p < 0.01$) between the two parameters and a more obvious correlation when the points arising from treatment by the two insoluble complexes $Cu^I(PPh_3)Cl$ and $Cu^{II}(S2)Cl$ were excluded ($n = 18$; $r = -0.769$; $p < 0.001$).

Irritancy of Copper Compounds Following Subplantar Injection along with Kaolin. The effect of injecting 3 mg of each of the copper compounds into a hind paw of rats is shown in Table II. Apart from the insoluble $Cu^I(PPh_2)Cl$, all the complexes examined enhanced the edema formation produced by kaolin. A correlation ($n = 27$; $r = 0.443$; $p < 0.05$) was found between the percent increase in paw diameter and ln dose of copper for the compounds examined in this test. Figure 3 illustrates the comparison between the percent change in the kaolin edema when the compounds were administered sc and the

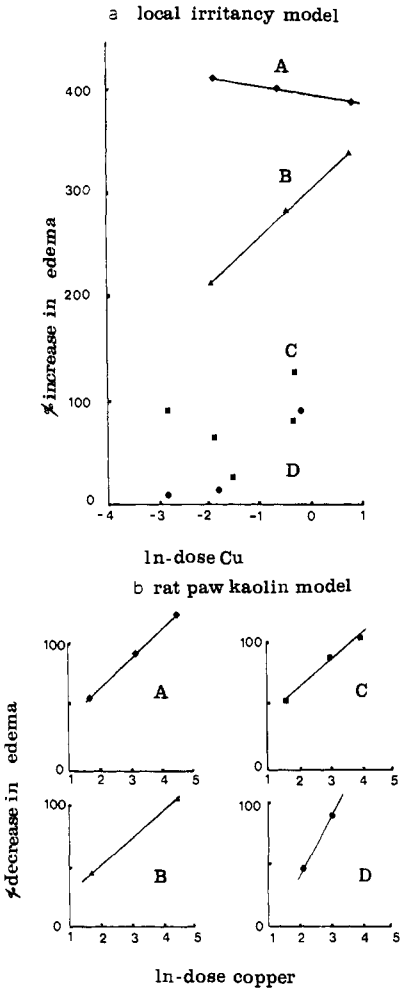

Figure 3. A comparison of the response of the compounds $Cu^{II}(py)_2(OAc)_2$ (A), $Cu^{II}(py)_2$ (B), $[Cu^{II}(tu)_3(SO_4)_2]\cdot H_2O$ (C), and $[Cu^{II}(en)_2]^{2+}$ as nitrate and sulfate (D) in the local irritancy test (Figure 3a), with the response of the same compounds injected subcutaneously in the rat paw kaolin model (Figure 3b).

local irritancy of the same compounds at different doses. The strongly irritant compounds show dose-related responses in both tests, but they were no more effective than the weakly irritant compounds in the kaolin edema antiinflammatory test.

Effect of Oral Administration of Copper Compounds of Nonactive Ligands against Kaolin-Induced Paw Edema in the Guinea Pig. In this model of inflammation, two out of eight copper complexes examined significantly inhibited the edema (Table III). These were $Cu^{II}(py_2)_2Cl_2$ and $Cu^{II}_2py_2(OAC)_4$, both of which were inactive when administered orally in the rat paw edema model. Copper(I) chloride was only evaluated at 50 mg/kg because of toxicity at higher doses.

Gastric Irritancy of Copper Compounds after Oral Administration to Rats and Guinea Pigs. A shown in Table II, 13 of the 32 compounds tested orally in rats caused stomach irritation, although in most cases swelling and large quantities of mucus-like secretions were observed. When administered to guinea pigs, all eight compounds tested were irritating to the stomach (Table III). In this species, a great deal of hemorrhaging was produced by the complexes, as is known to be the case for many existing antiinflammatory drugs.

Effect of Oral and Subcutaneous Administration of Copper Compounds of Active Ligands against Kaolin-Induced Paw Edema in Rats. The purpose of these experiments was to compare the effects of the ligands with their copper complexes. The results suggest that at the dose levels used, and compared to the ligand alone, there is an improvement in the antiinflammatory activity with some copper complexes. The gastric irritancy results indicate that the copper complexes were less irritating than the free ligands.

Statistical Correlations. Groups of either three or five animals were used throughout this work, and the results were evaluated using the Student's t test. In evaluating the activity of compounds in the kaolin edema model, a probability of <0.05 was chosen. Compounds were designated as active where the lower limit of probability indicated antiinflammatory activity compared to the control. In the figures, the mean value computed is plotted. Correlations between the various parameters measured are summarised in Table IV. It should be noted that antiinflammatory activity is measured as a reduction in paw size from the control level.

Discussion

The results given above for copper aspirinate administered orally and subcutaneously are in general agreement with those of other authors.[4,7,16,17] A few of the other compounds mentioned above have been reported on by others but not in a similar context and so a direct comparison is not possible.

The general conclusions from our results may be summarized as follows: (i) More copper compounds produced an antiinflammatory response after sc administration than after oral administration. In general, these compounds are also effective at lower doses after sc administration. (ii) Significant oral activity can be obtained at fairly high dose levels with a limited number of compounds. Some of these do not contain ligands, which are expected to produce any antiinflammatory effect. Thus, it would seem that copper compounds have an appreciable antiinflammatory action by either route of administration. (iii) The antiinflammatory activity appeared to be related to the dose of copper, particularly for soluble compounds administered sc to the rat. (iv) The correlation between gastric irritancy and antiinflammatory response in the rat and guinea pig was not significant at $p < 0.05$. Thus, the possibility of the oral antiinflammatory response arising from a counterirritation effect of gastric irritation seems unlikely, particularly since some compounds produced gastric irritation and no antiinflammatory response. (v) Local irritancy was weakly related to the dose of copper (Table III), perhaps suggesting that the effect of a counterirritation mechanism was appreciable on sc administration. However, the slopes of the dose–response curves for individual compounds varied considerably (Figure 3). Also, the local irritancy test produced a different order when the compounds were ranked for effectiveness than that observed after sc injection in the rat paw edema model, suggesting that the counterirritant mechanism was not the only antiinflammatory action produced by sc administration of copper compounds. (vi) When copper complexes of ligands which themselves were active antiinflammatory agents were tested for antiinflammatory effects, differences were observed between complex and free ligand which in some cases would suggest that, at equal doses, the copper complexes were more effective antiinflammatories than the corresponding ligands. The effect is very dependent on the complex chosen. The gastric irritancy results suggested that the copper complexes were less irritant than the free ligands. (vii) The toxicity of the copper complex of L-histidine was unexpected in that, of the compounds tested, only copper histidinate is possibly present in vivo although in much lower concentrations than those used here. The

mixed complex copper histidinate cysteinate has been suggested as having antiinflammatory activity.[18] (viii) Although only a few copper(I) complexes were examined, the results in this study suggest that copper(I) and copper(II) compounds have similar properties when absorbed, both could produce oral and sc activity, and both could produce gastric and local irritation. Since these effects were, in general, dose related for both oxidation states and since Cu(I) and Cu(II) undergo quite different reactions in vitro, a catalytic mechanism of action caused by the exogenous form of the copper is unknown. Copper(I) is a soft Lewis acid and copper(II) is intermediate between hard and soft. Thus, copper(I) would be expected to react with the softer thiols in vivo and copper(II) with the harder N and O donors. It might be expected, therefore, that, initially at least, they would each produce different metabolites. The similarity of their responses in the edema model suggests that both copper(I) and copper(II) are acting by the same mechanism. Thus, probably a series of reactions for both sc and orally administered complexes is involved, leading to the formation of a common metabolite. This could be a different species from that produced following normal absorption of copper through the gut. (ix) The activity of the few active oral compounds could be due to their bypassing of the normal oral copper absorption route. In one orally active compound, Cu_2O, most of the additional copper absorbed is carried in serum on albumin, giving an abnormal copper serum distribution.[19] Another method of absorption would be by topical application. The use of copper bangles in this field has been investigated,[20] and it is claimed that the bangles are effective.

Thus, it is clear that some copper-containing compounds do possess antiinflammatory activity in animal models and the copper would appear to be the active moiety. Species differences, methods of application, and the nature of the copper complex are important variables. Counterirritation caused by injection of potentially irritant compounds may affect the degree of antiinflammatory response, but it would not appear to be the sole mechanism of action of copper. Further work is required, in particular to elucidate the nature of the different copper species present in vivo after sc and oral administration.

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Supplementary Material Available: Table I, containing electronic spectra of some compounds prepared (1 page). Ordering information is given on any current masthead page.

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Synthesis and Antiinflammatory Activity of Some 2-(Substituted-pyridinyl)benzimidazoles[1]

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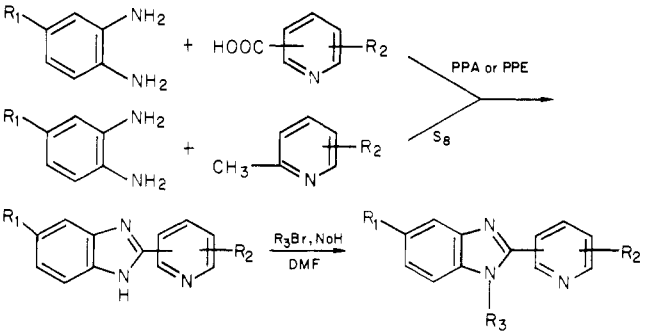
A series of 2-(2-pyridinyl)benzimidazoles was synthesized and evaluated for antiinflammatory activity by the carrageenan-induced rat paw edema assay. Among several active derivatives, 2-(5-ethylpyridin-2-yl)benzimidazole (6) was selected for further study. A comparison of compound 6 with phenylbutazone and tiaramide revealed that 6 possesses stronger activity in acute inflammatory models possibly with slightly less gastrointestinal irritation than both phenylbutazone and tiaramide.

Aryl- and heteroarylalkanoic acids have been explored widely as nonsteroidal antiinflammatory agents.[2] Among them, 2-aryl- and 2-heteroaryl-5-benzoxazolealkanoic acids[3] and 2-aryl-5-benzothiazolealkanoic acids[4] were reported to exhibit potential antiinflammatory activities. Acidic antiinflammatory agents tend to accumulate in acidic sites of animal organs, such as inflamed tissue, stomach, and kidney;[5] therefore, gastrointestinal irritation is likely to be produced. Some nonacidic 2-arylbenzazoles, dichlorophenylbenzoxazole[6] and fluorophenylbenzimidazole,[7] have been shown to exhibit antiinflammatory activity and so we thought it pertinent to prepare a group of nonacidic 2-heteroarylbenzazoles in order to derive a new class of antiinflammatory agents with less gastrointestinal irritation.

Scheme I



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