this type of inhibitor is more likely to serve as a selective tool for studying the uptake of neurotransmitters by different tissues and brain regions.

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ACKNOWLEDGMENTS AND ADDRESSES

Received January 20, 1975, from the *Department of Pharmacology, Center for the Health Sciences, University of California at Los Angeles, Los Angeles, CA 90024*

Accepted for publication April 29, 1975.

Supported in part by U.S. Public Health Service Grant MH-23839.

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Synthesis and Biological Evaluation of Tetrakis(acetylsalicylato)-μ-dicopper(II)

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Abstract □ The synthesis of a copper–aspirin chelate, previously reported to be a more active anti-inflammatory agent than aspirin itself, is given. Reaction of potassium acetylsalicylate with cupric sulfate gave a stable copper complex, which analysis and molecular weight determination showed to be a 4:2 chelate structure. Oil–water partition measurements showed the complex to be 10-fold more oil soluble than aspirin. Biological evaluation in rats showed the copper complex of aspirin to be approximately equal to aspirin in reducing carrageenan-induced inflammation, but it was 1.7 times more active than aspirin in reducing the primary lesion of adjuvant arthritis. Whereas aspirin produced a 50% or greater incidence of GI erosions at doses of 100–300 mg/kg in rats, the copper complex caused no erosions in doses up to 1200 mg/kg.

Keyphrases □ Tetrakis(acetylsalicylato)-μ-dicopper(II)— synthesis, biological evaluation, anti-inflammatory activity, physical properties □ Biological evaluation—tetrakis(acetylsalicylato)-μ-dicopper(II), anti-inflammatory activity, rats □ Aspirin–copper complex—synthesis, biological evaluation, anti-inflammatory activity, physical properties □ Copper–aspirin complex—synthesis, biological evaluation, physical properties □ Anti-inflammatory agents, potential—synthesis and biological evaluation of aspirin–copper complex

In 1956, Chenoweth (1) postulated that the salicylates may exert "some if not all" of their biological actions through their ability to bind metal ions, a property that isomers of salicylic acid lack. To test this postulate, a series of substituted salicylic acids and heterocyclic analogs was prepared; their metal-binding avidities were compared with several biological effects to determine possible parallels (2, 3). With hindered (3,5- and 3,6-disubstituted) salicylic acids, for instance, both metal-binding ability and anti-inflammatory activity were lowered (4).

Table I—Oil–Water Partition Coefficients

Compound	pH	K^a
Aspirin–copper complex	4.1	1.3
Salicylic acid	4.1	9.0
Aspirin	4.3	0.11

[a] $K = \frac{C_{\text{oleyl alcohol}}}{C_{\text{water}}}$

Table II—Anti-Inflammatory Activities

Compound	Dose, mg/kg po	Antiedema, Δ, ml (0 *versus* 3 hr)
Aspirin–copper complex	50	+0.40[a]
	100	+0.31[a]
	200	+0.26[a]
Aspirin	50	+0.34[a]
	100	+0.27[a]
	200	+0.20[a]
Controls		+0.55

[a] $p = 0.00$.

Sorenson (5) recently reported that copper chelates of some anti-inflammatory agents, including aspirin, gave marked increases in anti-inflammatory activity compared with the activities of the unchelated agents. Anti-inflammatory activity was also observed for copper chelates of some ligands having no anti-inflammatory properties at all in the nonchelated state. These findings indicate that the copper complexes may be active metabolites of many anti-inflammatory agents. Since the copper(II) complex of

Table III—Effects on Adjuvant-Induced Arthritis

Compound	Dose, mg/kg po	Decrease of Leg Volume[a], %			Body Weight Change[b]	
		Left Leg, Day 3	Left Leg, Day 16	Right Leg, Day 16	Day 3	Day 16
Aspirin	400	27	10 (NS)	25	−4	+2
	200	13	5 (NS)	2 (NS)	NS	NS
	100	8	0 (NS)	2 (NS)	NS	NS
	50	3 (NS)	0 (NS)	0 (NS)	NS	NS
	ED_{25} = 383 (250–748)					
Aspirin–copper complex	400	29	7 (NS)	22	−2	+1
	200	23	8 (NS)	5 (NS)	NS	+2
	100	9	0 (NS)	0 (NS)	NS	NS
	50	12	0 (NS)	13 (NS)	NS	NS
	ED_{25} = 256 (162–502)					
	Relative potency = 1.7 (1.2–2.7)					

[a] NS = nonsignificant. [b] 1, $p < 0.03$; 2, $p < 0.01$; 4, $t > 5.00 < 10.00$; + = increase; − = decrease.

aspirin was prepared in this laboratory previously (6), the synthesis, structure, and some physical and biological properties of this complex are now reported.

DISCUSSION

Chemistry—Reaction of the potassium salt of aspirin with cupric sulfate gave an immediate precipitate of an aquamarine complex. Elemental analysis showed it to be a 2:1 aspirin–copper complex without hydration or anions, indicating that four coordination bonds of the copper ion were filled by the organic ligand. Stability constants for the formation of the complex could not be determined because of the rapid hydrolysis of aspirin, which occurred during the attempted determination (by potentiometric titration). However, strong shifts in the IR spectra of the coordinating carbonyl groups indicated appreciable stability of the complex. The complex also has demonstrated remarkable stability in the dry state, remaining stable for years in a glass-stoppered bottle without precautions.

Oil–water partition coefficients, using oleyl alcohol, were determined for both aspirin and the copper(II) complex (Table I). It has been proposed that transport of organic ligands into the cells can be facilitated by the formation of metal complexes (7), and this concept recently was invoked to explain the transport and storage of catecholamines (8). The copper(II) complex of aspirin was found to be 10-fold more lipophilic than aspirin at a comparable pH (~4.0). This finding supports the idea that metal-ion complexation may be of importance for the transport of aspirin into cells.

Examination of the IR spectra of aspirin and its copper(II) complex revealed definite shifts in absorption for both the carboxyl and acetoxy groups (Figs. 1 and 2). The shifts occurred in the direction of longer wavelengths for both carbonyl groups, as expected for metal chelate formation involving covalent bonds. Donation of electrons to metal produces lower excited states and, therefore, shifts to longer wavelengths. Where ionic bonding is involved, these shifts also occur, but to a lesser degree (9). It may be concluded that both the carboxyl and acetoxy groups are involved in binding to the copper ion, which accounts for the stability of the product to the loss of acetic acid.

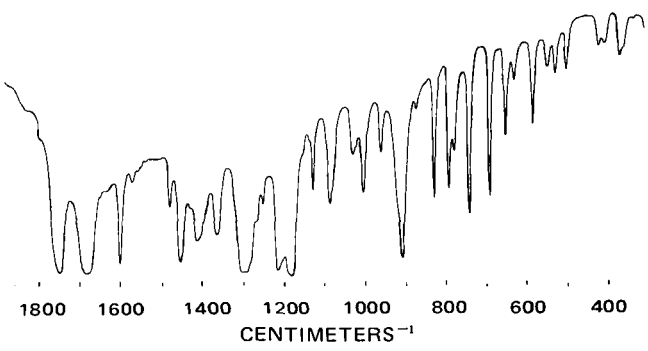

Figure 1—*IR absorption spectrum of aspirin (4.8 mg/g of potassium bromide).*

Previously, a structure was proposed for a copper(II) aspirinate (10) prepared at a higher temperature (50°) and described as "dark blue prisms insoluble in water and many common organic solvents." This structure included four units of acetylsalicylate and two copper ions; each copper ion resided in a distorted octahedral arrangement with five Cu–O bonds and one Cu–Cu linkage. The product reported here most likely has this structure, and a molecular weight determination confirmed the 4:2 ratio of ligand to copper.

Similar attempts to prepare aluminum and ferrous complexes of aspirin gave less satisfactory results. The isolated aluminum complex appeared to be quite stable but did not give suitable analytical results for either a 2:1 or 3:1 complex. The ferrous complex underwent immediate oxidation on exposure to air.

Biological Properties—The anti-inflammatory effect, antiarthritic effect, and ulcerogenic liability of the copper complex of aspirin were determined in rats and compared with the same effects from aspirin. The two agents were approximately equipotent in reducing carrageenan-induced inflammation (Table II). The copper complex, however, was 1.7 times more active than aspirin on the primary lesion (Day 3) of adjuvant arthritis (Table III). The compounds were administered orally.

Oral administration of aspirin to rats resulted in a 50% or greater incidence of GI erosions at doses of 100–300 mg/kg. The copper complex of aspirin caused no erosions in doses up to and including 1200 mg/kg on oral administration (Table IV). There was, however, evidence of hemorrhage caused by the copper complex at doses of 300, 600, and 1200 mg/kg.

EXPERIMENTAL[1]

Tetrakis(acetylsalicylato)-μ-dicopper(II)—Acetylsalicylic acid (3.60 g, 0.02 mole) was allowed to dissolve in a solution of potassium bicarbonate (2.02 g, 0.02 mole) in 30 ml of water. To this solution was added a solution of copper sulfate pentahydrate (2.50 g, 0.01 mole) in 20 ml of water, slowly and with constant stirring. An aquamarine precipitate formed, and the pH of the solution fell from 5.3 to 4.3. The precipitate was collected, washed with water, and dried (calcium chloride), giving a 79% yield; UV (water): 296 nm: IR (potassium bromide): 1755 (ester C=O), 1725 (carboxyl C=O), 1405 (C—O), 1240 (C—O), 1200 (C—O), and 500 (Cu—O) cm^{-1}; IR (potassium bromide) (aspirin): 1750 (ester C=O), 1680 (carboxyl C=O), 1300 (C—O), 1220 (C—O), and 1180 (C—O) cm^{-1}; mol. wt.: theoretical, 843.7, found (Rast, camphor), 854.5.

Anal.—Calc. for $C_{18}H_{14}CuO_8$: C, 51.21; H, 3.34; Cu, 15.07. Found: C, 51.19; H, 3.46; Cu, 15.27.

Oil–Water Partition Coefficients—Oil–water partition coeffi-

[1] The melting point was taken on a Mel-Temp apparatus and is uncorrected. Microanalyses for carbon and hydrogen were done by F. B. Strauss, Oxford, England. UV absorption spectra were determined with a Beckman model DU spectrometer, and IR absorption spectra were obtained with a Perkin-Elmer model 457 spectrometer in potassium bromide pellets. A copper analysis was performed by ashing the dried complex at 1000° and weighing the copper oxide formed.

Table IV—Ulcerogenic Liability

Compound	Dose, mg/kg po	Erosions	Average Number of Erosions
Aspirin	300	5/8	0.9
	200	7/8	4.1
	100	4/8	0.9
	50	2/8	0.4
Aspirin–copper complex	1200	0/8	0
	600	0/8	0
	300	0/8	0
	100	0/8	0

cients were determined by shaking 100 ml of a 0.002 *M* solution of acetylsalicylic acid or its copper complex with an equal volume of oleyl alcohol. After 4 hr of shaking, the aqueous layer was separated and heated at 70° for several hours to hydrolyze the complex. The hydrolyzed mixture was filtered, and the liberated salicylic acid was measured spectrophotometrically at 296 nm.

Anti-Inflammatory Activity—Groups of eight male Charles River Wistar rats were dosed orally with the respective drug or vehicle (tragacanth) and hydrated. One hour later, 0-hr (normal) paw measurements were made, and the animals were immediately injected with 0.05 ml of a 1% carrageenan (in 0.9% sodium chloride) suspension into the plantar surface of the right hindpaw. Paw measurements were made 3 hr after carrageenan injection, according to the procedure of Walz *et al.* (11). A statistically significant effect was determined by using the Student *t* test.

Adjuvant-Induced Arthritis Test—The procedure of Walz *et al.* (12) was followed. Eight groups of eight Charles River Wistar rats, 160–190 g, were given the following doses of drug orally in tragacanth (0.5%): aspirin—50, 100, 200, and 400 mg/kg; and copper complex of aspirin—50, 100, 200, and 400 mg/kg (aspirin as base). Sixteen rats were given 0.5% tragacanth as a vehicle control. On the afternoon of the same day, adjuvant arthritis was induced by the injection of 0.75 mg of *Mycobacterium butyricum*[2], suspended in white paraffin oil, into the left hind footpad of each rat.

Drug dosing was continued daily for 4 days (Day 0 through Day 3). On Day 3, the animal's left hindleg volume was determined plethysmographically. Leg volume was also determined on Day 16. Body weight changes with respect to Day 0 were calculated for Day 3 and Day 16. The ED_{25} (the dose giving a 25% decrease in leg volume) was calculated from a dose-response curve fitted by computer. All data were statistically analyzed for significant differences between drug-treated and control animals by the Student *t* test.

Ulcerogenic Liability Test—Groups of eight Charles River or Carworth Wistar rats, 210–240 g, were dosed orally with aspirin (50, 100, 200, or 300 mg/kg) and with the copper complex of aspirin (100, 300, 600, or 1200 mg/kg) (aspirin as base). After dosing, the animals were fasted for 18 hr. Then the animals were sacrificed, and the stomachs were removed, washed, cut along the greater curvature, and pinned to a cork sheet. The mucosal surface was examined under low magnification. Incidence of erosions and average number of erosions were recorded.

[2] Difco.

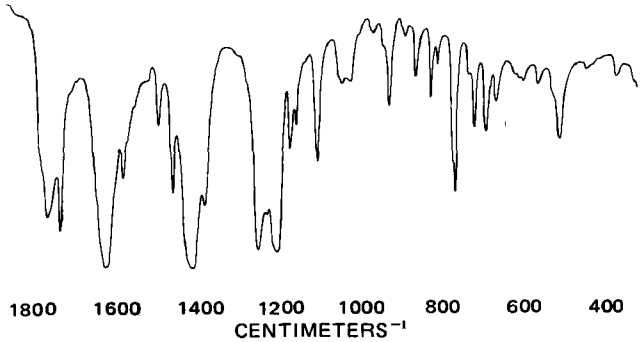

Figure 2—*IR absorption spectrum of the copper complex of aspirin (5.1 mg/g of potassium bromide).*

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ACKNOWLEDGMENTS AND ADDRESSES

Received June 3, 1974, from the **Department of Chemistry, Massachusetts College of Pharmacy, Boston, MA 02115,* and [‡]*Smith Kline and French Laboratories, Philadelphia, PA 19101*

Accepted for publication April 28, 1975.

D. A. Williams and W. O. Foye express their appreciation to the Dow Chemical Co. for financial assistance.

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