Hydrolysis of Tetrakis-μ-acetylsalicylato-dicopper(II)

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Abstract □ Hydrolysis rates of acetylsalicylate in the free acid and anion forms and in the dilute solution of the copper complex, tetrakis-μ-acetylsalicylato-dicopper(II), were compared. The hydrolysis rate was unaffected by the presence of copper(II). The pH-dependent rate was 3.64×10^{-4} mmole/liter/min per pH unit at 37° in Ringer's solution. The synthetic procedures commonly used for the preparation of this compound yield a product contaminated with salicylate. This contamination is avoided by synthesis of the compound in methanol, which yields a complex containing no measurable salicylate.

Keyphrases □ Anti-inflammatory agents—aspirin, comparison of hydrolysis rate with that of tetrakis-μ-acetylsalicylato-dicopper(II), pH dependency □ Aspirin—comparison of hydrolysis rate with that of tetrakis-μ-acetylsalicylato-dicopper(II), pH dependency □ Hydrolysis rates—aspirin and tetrakis-μ-acetylsalicylato-dicopper(II), pH dependency

The use of a mixture of copper and salicylate in the treatment of arthritis has been discussed (1–3). Sorenson (4) recently reported that tetrakis-μ-acetylsalicylato-dicopper(II) has greater anti-inflammatory properties than does aspirin (acetylsalicylic acid) alone and suggested that this complex might be the pharmacologically active form of aspirin. This hypothesis generated considerable testing and discussion of the copper complex (5–15), a review of which was given by Sorenson (16, 17).

The evidence presented in the literature concerning Sorenson's hypothesis that the copper complex is the pharmacologically active form of aspirin is limited to observations of the effect of the complex on induced inflammations in laboratory animals (*i.e.*, measurements of paw thickness and volume). It seemed important to assess the relationship between the chemistry of the copper complex and the pharmacological activity of aspirin. The first experiments undertaken involved the hydrolysis of the copper complex to salicylic acid and acetic acid.

The complex was first prepared in 1962 (18), and the X-ray structure was published in 1966 (19). The method used by Manojlovic-Muir (19) yielded a product containing a significant amount of salicylate, so other preparations were sought. This report compares the hydrolysis rates for the complex prepared by three published methods to that for aspirin alone.

EXPERIMENTAL

Materials—The chemicals were reagent grade and were used as obtained with several exceptions. Butyl ether (practical grade) was purified by a literature method (20) and refrigerated in brown glass. Tetrakis-μ-acetylsalicylato-dicopper(II) was prepared by three methods.

Sample B was prepared based on a statement that the complex can be formed in methanol (21). A solution of 25.0 g (0.129 mole) of aspirin in 200 ml of methanol was added to a solution of 13.85 g (0.0694 mole of copper) of copper(II) acetate in 1 liter of methanol. Crystallization of the complex occurred very slowly, and three successive crops of crystals were separated out (53% yield).

Anal.—Calc.: C, 51.25; H, 3.34; Cu, 15.07; O, 30.34. Found[1]: C, 51.04; H, 3.53; Cu, 14.95; O, 30.33.

Sample C was prepared by the method of Sorenson (4), which involves the addition of an aqueous solution of aspirin (pH adjusted to ~11 with 50% NaOH) to an aqueous solution of copper(II) chloride (68% yield).

Found: C, 50.94; H, 3.43; Cu, 14.94; O, 29.96.

Sample D was prepared by the method of Manojlovic-Muir (19) in which stoichiometric amounts of aspirin and copper(II) salicylate are heated in 50% aqueous ethanol to 50° (45% yield).

Found: C, 51.11; H, 3.50; Cu, 15.02; O, 29.90.

Mammalian Ringer's solutions were buffered with acetate to produce solutions of pH 5.8 and with tris(hydroxymethyl)aminomethane to produce solutions of pH 7.5 and 8.8. All solutions were prepared with deionized, glass-distilled water.

Procedures—Hydrolysis was carried out with 2.775 m*M* solutions of aspirin or 0.6953 m*M* solutions of tetrakis-μ-acetylsalicylato-dicopper(II) in the appropriately buffered Ringer's solution at 37° (each mole of complex has 4 moles of acetylsalicylate). Both aspirin and the complex dissolved very slowly. Time zero was taken as the time at which the solid compound was added to the Ringer's solution.

Analysis of salicylic acid (due to hydrolysis) was carried out with the following modifications of a published procedure (20). A 300-μl aliquot of the reaction mixture was extracted into 3.00 ml of butyl ether with shaking at 250 rpm for 10 min. The butyl ether contained 4.0 g of malonic acid/liter to facilitate separation and to prevent further hydrolysis. The two-phase mixture was centrifuged at 2500 rpm for 5 min, the ether layer was drawn off, and the UV spectrum was scanned between 330 and 275 nm[2]. The salicylic acid concentration was determined from a standard curve calculated from absorbances at 307 nm by linear regression analysis. The coefficient of determination (22) for standard curves was ≥0.99. Hydrolysis rates were determined by plotting the appearance of salicylic acid with respect to time over at least 4 hr. Slopes were calculated by linear regression analysis.

Samples for thermal analysis were placed in quartz or platinum crucibles and heated from 0 to 1000° over 20 hr in nitrogen or air at a flow rate of ~1 ml/min[3].

RESULTS

The hydrolyses of acetylsalicylate in aspirin and in the three preparations of tetrakis-μ-acetylsalicylato-dicopper(II) in dilute solution at an acidic pH appear in Fig. 1 as a series of nearly parallel lines. The hydrolysis rates were similar at a given pH (Table I), although the *y* intercepts differ. Table I also gives comparable data for the other pH values at which the experiment was performed.

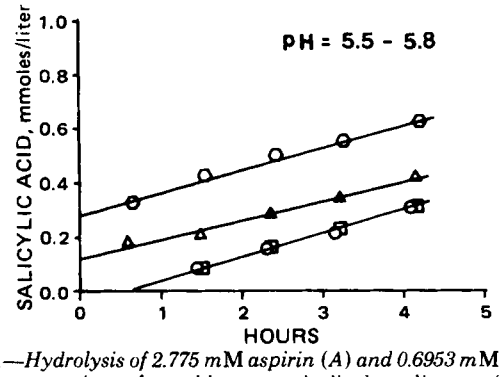

Figure 1—*Hydrolysis of 2.775 m*M *aspirin (A) and 0.6953 m*M *solutions of three preparations of tetrakis-μ-acetylsalicylato-dicopper(II) (B–D) at 37°. Key: ○, Sample A; □, Sample B; △, Sample C; and ○, Sample D.*

[1] All microanalyses for carbon, hydrogen, copper, and oxygen were done by Galbraith Laboratories, Knoxville, TN 37921.

[2] Beckman DB-G spectrophotometer, Beckman Instruments, Fullerton, CA 92634.

[3] Setaram thermoanalyzer G-70 and DTA-80, Marche Instruments, Waco, TX 76710.

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Table I—Hydrolysis Rates of 2.775 m*M* Aspirin (A) and 0.6953 m*M* Solutions of Three Preparations of Tetrakis-μ-acetylsalicylato-dicopper(II) (B–D) at 37° and Three pH Values

Sample	pH Range	pH Mean ± *SD*	Hydrolysis Rate, mmoles/liter/min × 10^3	r^2 [a]	Buffer
A	5.50–5.60	5.56 ± 0.055	1.394	0.9993	Acetate
B	5.75–5.80	5.78 ± 0.027	1.425	0.9956	Acetate
C	5.75–5.85	5.81 ± 0.042	1.155	0.9752	Acetate
D	5.65–5.75	5.71 ± 0.042	1.322	0.9904	Acetate
A	6.95–7.10	7.01 ± 0.065	1.580	0.9967	I[b]
B	6.78–6.90	6.83 ± 0.052	1.963	0.9989	I
C	6.80–6.95	6.83 ± 0.067	1.803	0.9983	I
D	6.75–6.95	6.82 ± 0.076	1.849	0.9906	I
A	8.65–8.80	8.69 ± 0.065	2.528	0.9933	I
B	8.62–8.70	8.65 ± 0.029	2.583	0.9948	I
C	8.55–8.70	8.61 ± 0.055	2.373	0.9868	I
D	8.55–8.70	8.61 ± 0.065	2.111	0.9448	I

[a] Coefficient of determination = $r^2 = \left[\Sigma x_i y_i - \frac{\Sigma x_i \Sigma y_i}{n}\right]^2 \Big/ \left[\Sigma x_i^2 - \frac{(\Sigma x_i)^2}{n}\right]\left[\Sigma y_i^2 - \frac{(\Sigma y_i)^2}{n}\right]$. [b] I = Tris(hydroxymethyl)aminomethane.

If the hydrolysis rates from Table I are plotted *versus* pH (nullifying the effect of any initial salicylate present), they fall on a single line, indicating that aspirin in the free form and in the copper complex hydrolyze at a rate of 3.64×10^{-4} mmole/liter/min per unit of pH increase at 37° (Fig. 2). The coefficient of determination (22) for all of these points was 0.878.

Purity assessment of synthesized compounds usually includes melting-point data. All samples of tetrakis-μ-acetylsalicylato-dicopper(II) decomposed without melting. Samples B and C turned from the original blue to green to brown between 220 and 235°. Sample D began to turn green at 180°. Thermal analysis in either air or nitrogen indicated an endothermic reaction at ~240° for all of the samples, which did not correspond to any weight loss. When heated in air, all samples decomposed in three exothermic steps between 250 and 370° to a weight corresponding to copper(II) oxide. Sample D slowly lost ~5% of its weight prior to the onset of the described reactions.

DISCUSSION

Hydrolysis rates of free and bound acetylsalicylate are nearly identical at a given pH, but the curves in Fig. 1 are not superimposable. Extrapolation of hydrolysis curves to zero time in Fig. 1 indicates the initial presence of salicylic acid in Samples C and D. Melting-point and thermal analysis data indicate an impurity in Sample D but not in Sample C. The *y* intercepts in Fig. 1 indicate approximately twice as much salicylate in Sample D as in Sample C. The contamination of Samples C and D with salicylic acid undoubtedly is due to the preparation methods. At the high pH used in the preparation of Sample C (4), hydrolysis occurs rapidly, causing the product to be contaminated with salicylate. Copper(II) salicylate was used in the reaction mixture for the preparation of Sample D (19) and probably contaminated the product. Recrystallization was not mentioned in the original literature for either procedure and was not used in the preparations made in this study.

The solubility of the complex is very slight in any common solvents (23). Thus, contamination by salicylate in the methods used for Samples C and D would be difficult if not impossible to avoid. Furthermore, contamination was not apparent from IR spectra or elemental analysis (nor was such a problem mentioned in the original literature). The *y* intercept for Sample B in Fig. 1 coincides with that of aspirin, indicating that Sample B does not contain salicylate. The data presented here appear to indicate that tetrakis-μ-acetylsalicylato-dicopper(II) should not be prepared in a solution of basic pH and perhaps should be prepared in a nonaqueous solvent such as methanol to prevent contamination with salicylate. This precaution is especially important in experiments concerning the pharmacological activity of the copper complex since salicylate also is pharmacologically active.

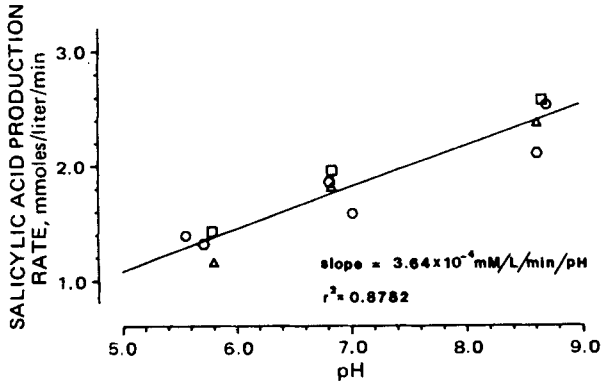

Figure 2—*Hydrolysis rate of aspirin in the free form (A) and in three preparations of tetrakis-μ-acetylsalicylato-dicopper(II) (B–D) as a function of pH. Key: ○, Sample A; □, Sample B; △, Sample C; and ○, Sample D.*

For aspirin alone as well as for Sample B of tetrakis-μ-acetylsalicylato-dicopper(II), Fig. 1 shows an induction period before the appearance of the hydrolysis product, salicylic acid. This induction period would not be present if zero time had been set when all compounds had dissolved (*i.e.*, the vertical axis would be moved to the right by ~30 min). However, the resulting plots would show some salicylic acid in all of the complex preparations and in the aspirin itself (as was the case in preliminary experiments), because hydrolysis begins as soon as any compound is present in solution. To show unambiguously that some salicylate contaminates two of the complex preparations, zero time was taken as described. Use of a sonicator to speed dissolution decreased the length of this period, so it was assumed that the time lag before the appearance of salicylate represents the time required to dissolve the compound.

As observed from Fig. 2, the hydrolysis rate of aspirin, measured by the appearance of salicylic acid, increases with increasing pH and is the same in the complex solutions as in the free form under the experimental conditions. Some inferences concerning the structure in solution may be drawn from this and other information. The crystal structure of the complex has binuclear units of tetrakis-μ-acetylsalicylato-dicopper(II) in which each of the four carboxyl groups is attached to both copper ions. The binuclear units are interconnected into a polymeric structure by a fifth copper–oxygen bond from the acetyl group of a neighboring residue. This bond is nearly 0.3 Å longer than the carboxyl copper–oxygen bonds (19).

Bose and Patel (21) modified this complex, substituting dimethylformamide or dimethyl sulfoxide in the terminal positions for the bond from the acetyl group. They reported that the copper–ligand (dimethylformamide or dimethyl sulfoxide ligand) bond was unusually weak for copper(II) complexes of these ligands. Kato *et al.* (24) reported evidence indicating that the copper(II) complexes of several carboxylic acids that are dimeric in crystalline form are monomeric in aqueous solution. If, in dilute solution, a monomer exists rather than a polymerized dimer, then solvent molecules will occupy the terminal positions and the acetyl group will not be bonded to copper. Thus, hydrolysis would occur at a rate similar to that in free aspirin.

There also is the possibility that the complex is completely dissociated. Sorenson was critical of several experiments on the pharmacological effectiveness of tetrakis-μ-acetylsalicylato-dicopper(II), in which the complex was administered orally in water solution, and suggested that in water the complex probably was dissociated (6, 17), especially at the stomach pH. It has not been possible to determine the extent of complexation under the conditions of this experiment due to the complexity of the solutions.

The stability constant of a 1:1 complex of copper and acetylsalicylate determined polarographically in phosphate buffer at pH 7.4 was 1000

(25). Gaur and Palrecha (26) also determined the stability constants polarographically with a sodium perchlorate supporting electrolyte (no buffer given) and found that 1:1, 1:2, and 1:3 species were formed, having stability constants of 80, 540, and 1610, respectively. Williams *et al.* (10) attempted to determine a stability constant potentiometrically but were unable to do so due to the rapid hydrolysis of acetylsalicylate. It is possible that at the concentrations employed and in the absence of excess acetylsalicylate ion, little complexed species exists. In this case, hydrolysis rates should not be different for solutions made from the copper complex than for those of aspirin.

In summary, the pH-dependent hydrolysis of aspirin was found to occur at the same rate in the free form as in a dilute solution of the tetrakis-μ-acetylsalicylato-dicopper(II) complex. Preparation of this complex in aqueous solution, especially a basic solution, yielded a compound contaminated with significant amounts of salicylic acid.

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NOTES

Quantitation of the Antineoplastic Agent Indicine-*N*-oxide in Human Plasma by Differential Pulse Polarography

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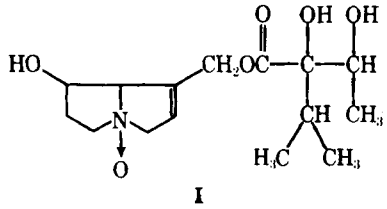
Abstract □ A sensitive and reproducible differential pulse polarographic method of analysis was developed for indicine-*N*-oxide (NSC 132319) in human plasma. Lyophilized plasma is extracted with methanol, and the extract is chromatographed over partially deactivated aluminum oxide and reversed-phase silica gel columns. Indicine-*N*-oxide is eluted from the silica gel column with 25% aqueous methanol and quantitated by differential pulse polarography by measurement of the peak current at -0.72 ± 0.03 v (*versus* the saturated calomel electrode). Recovery of indicine-*N*-oxide from plasma was $88 \pm 7\%$ (*SD*) in the 1–20-μg/ml range. The method was linear over the range of 0.5–10 μg/ml of pH 4.6 buffer.

Keyphrases □ Indicine-*N*-oxide—quantitation by differential pulse polarography, human plasma □ Antineoplastics—indicine-*N*-oxide, quantitation by differential pulse polarography, human plasma □ Polarography, differential pulse—indicine-*N*-oxide, quantitation in human plasma

Indicine-*N*-oxide (I) (NSC 132319) is an antineoplastic agent (1) that recently entered clinical trials. The purpose of this investigation was to develop a polarographic method of analysis for use in clinical studies on the physiological disposition of the drug. Assays based on GLC (2) and GLC–mass spectrometry (3–5) have been reported. These assays required derivatization of either indicine-*N*-oxide or its reduced form to a volatile species prior to analysis. The method reported here for the analysis of the drug in biological samples does not require derivatization and is not subject to interference from the reduced form.

With recent advances in instrumentation (6), differential pulse polarography has become a rapid and inexpensive technique. A quantitative polarographic assay was developed in these laboratories for indicine-*N*-oxide in human plasma that combines sufficient sensitivity, reproducibility, and ease of operation for the potential routine analysis of suitable clinical samples (7).



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