Aqueous Mixture of Pyrrolidone-5-hydroxamic Acid–Iron (III) Complexes: Isolation and Characterization of Tris(pyrrolidone-5-hydroxamato)iron (III)

M. PEĆAR ˣ, N. KUJUNDŽIĆ, B. MLINAREVIĆ, D. ČERINA, B. HORVAT, and M. VERIČ

Abstract □ The published procedure for the synthesis of pyrrolidone-5-hydroxamic acid was improved. The acidity constant of the pyrrolidone-5-hydroxamic acid was determined as pKa = 8.65. In an aqueous solution of iron (III) ions, pyrrolidone-5-hydroxamic acid binds ferric ion, forming a mixture of mono-, bis-, and tris(pyrrolidone-5-hydroxamato)iron (III) complexes. These complexes were studied by potentiometric and spectrophotometric methods. The tris compound was isolated as dark orange-red crystals and identified according to elemental analysis and IR spectral data as $C_{15}H_{21}FeN_6O_9 \cdot 6H_2O$, having the magnetic moment of 5.67 B.M.

Keyphrases □ Pyrrolidone-5-hydroxamic acid—synthesis, acidity, chelating properties □ (Pyrrolidone-5-hydroxamato)iron (III) complexes—synthesis, characterization, visible and IR spectral data, magnetic moment □ Complexes—iron (III) with pyrrolidone-5-hydroxamic acid, synthesis, characterization, visible and IR spectral data, magnetic moment

In spite of the great interest in iron (III)–hydroxamate complexes and their role in living systems (1–3), the data on heterocyclic aminohydroxamic acid–iron (III) complexes are rather scarce. Iron-containing hydroxamic acids play a functional role in the metabolism of microorganisms (4), and some of them have been patented as metallotherapeutics (5). Free hydroxamic acids are also of biological importance (1, 2, 6) and serve as antibiotics and bacteriostatics. Polyvinylpyrrolidone is used as a substitute for blood plasma (7). Since hydroxamic acids and their iron (III) chelates are of great interest for pharmaceutical sciences (6, 8–11), pyrrolidone-5-hydroxamic acid and its iron (III) complexes have been chosen from the nitrogen heteropentacyclic hydroxamic acids as a model system.

EXPERIMENTAL

Chemicals—Pyrrolidone-5-hydroxamic acid and iron (III) complexes were prepared as subsequently described. All other chemicals were of analytical reagent grade and were used without further purification. The water used in all experiments was distilled from aqueous acidified potassium permanganate solutions and stored in glass[1] containers. The methanol used contained about 0.04% water (determined by the Karl Fischer method).

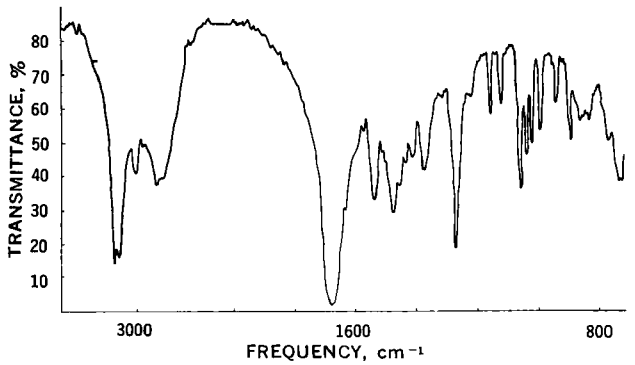
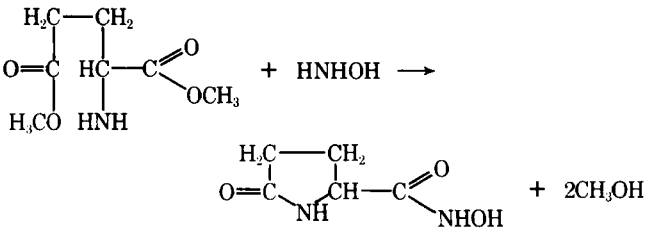


Figure 1—*IR spectrum of I.*



Scheme I

Preparation of Pyrrolidone-5-hydroxamic Acid (I)—The literature method (12) for the synthesis of I was modified as follows. Glutaminic acid, 100 g (0.68 mole), was suspended in 750 ml of anhydrous methanol; dry hydrogen chloride gas was introduced through a glass pipe[2], equipped with a sintered-glass filter, until white fumes appeared above the solution. Then 750 ml of methanol was added and refluxed for 3 hr along with bubbling of dry hydrogen chloride gas. Special care was taken to ensure nonaqueous conditions during the entire procedure since water interferes with the main reaction.

After removal of excess hydrochloric acid by evaporation under reduced pressure, the resulting oil-like liquid was diluted with 300 ml of methanol. The addition of the chloroform solution of ammonia liberated the dimethyl ester of glutaminic acid and precipitated ammonium chloride, which was filtered off. Methanol and chloroform were removed from the filtrate by distillation, and 95 g (1.36 mole) of hydroxylamine dissolved in methanol was added to the rest of the filtrate.

The mixture was allowed to stand in a refrigerator for 4 days. During that period, condensation as well as cyclization took place (Scheme I). The crystallized product was filtered, washed with cool methanol, and recrystallized from hot water; the total yield before recrystallization was 67 g (68%), mp 165.5–167.5°.

Anal.—Calc. for $C_5H_8N_2O_3$ (mol. wt. 144.14): C, 41.66; H, 5.59; N, 19.44. Found: C, 41.69; H, 5.35; N, 19.47.

Preparation of Tris(pyrrolidone-5-hydroxamato)iron (III) Complex—Ferric chloride hexahydrate, 1.35 g (0.005 mole), was

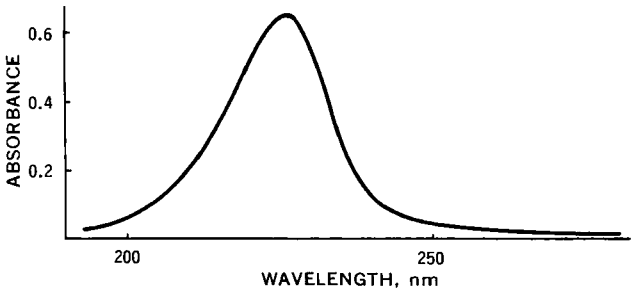


Figure 2—*UV spectrum of 0.003 M I at pH 6.7, using 1-cm quartz cells.*

[1] Pyrex.
[2] Jena.

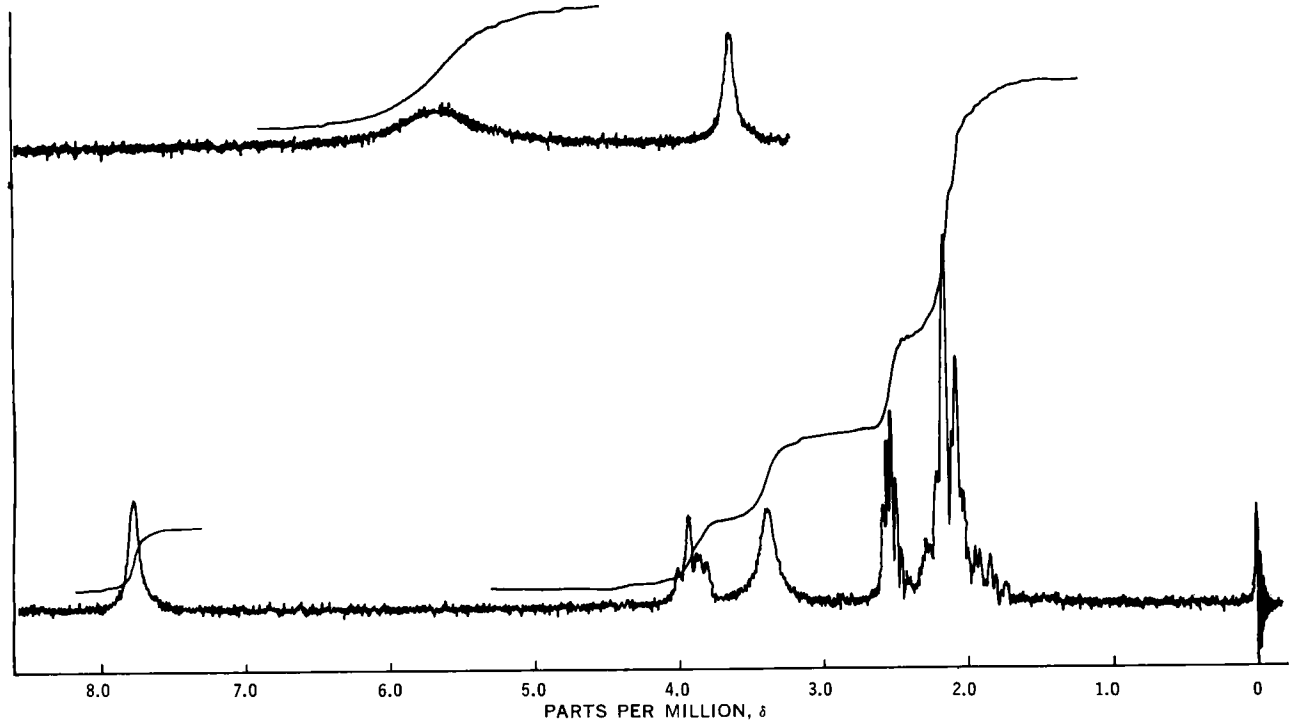


Figure 3—*NMR (60-MHz) spectrum of I in deuterated dimethyl sulfoxide.*

dissolved in 5 ml of water and mixed with 5 ml of solution containing 2.16 g (0.015 mole) of I and 0.60 g (0.015 mole) of sodium hydroxide. Acetone was then added slowly, with rigorous stirring, until the liquid over the resinous black mass turned colorless or pale yellow. The supernatant liquid was decanted from the solid, which was dried in air and finally vacuum desiccated at room temperature over phosphorus pentoxide, yielding 2.0 g (67%).

Anal.—Calc. for $C_{15}H_{21}FeN_6O_9 \cdot 6H_2O$ (mol. wt. 593.33): C, 30.35; H, 5.60; Fe, 9.42; N, 14.17. Found: C, 30.47; H, 4.37; Fe, 9.43; N, 14.08.

Physical Measurements[3]—To determine the acid strength of I, a potentiometric titration at 25° was performed and the pKa value of this acid was calculated from the plot of milliliters of base *versus* pH. By assuming the activity coefficient of unity for the neutral hydroxamic acid species and calculating the activity coefficient (f_{A^-}) for the pyrrolidone-5-hydroxamate anion from the Debye–Huckel equation, $f_{A^-} = -0.51\sqrt{\mu}$, where μ is ionic strength, the pKa value was found to be 8.65.

RESULTS AND DISCUSSION

Preparation and Characterization of I—The literature method (12) for the synthesis was modified since it started with a 10-fold increase in the amount of the reactants and the reaction mixture was allowed to stand much longer for cyclization and condensation; more importantly, anhydrous conditions were ensured all of the time. Finally, it may be noticed that dry hydrogen chloride gas was introduced through a sintered-glass filter, thus forming a large amount of bubbles. These modifications resulted in a higher yield (68 *versus* 52%) and a pure product.

The IR spectrum (Fig. 1) shows the characteristic functional groups in the I molecule. The UV spectrum of this acid (Fig. 2) shows an absorption peak at 225 nm, which can be assigned to the electronic transitions at the keto groups in the I molecule. This absorption peak is shifted to a longer wavelength in comparison with the analogous absorption maximum in the acetamide molecule at 208 nm (13), which suggests that the electron transfer in I takes place between closer π-energy levels than in the acetamide molecule.

The NMR spectrum of I (Fig. 3) shows two peaks of multiplet structure at 2.03 and 3.90 ppm, the first of which is exhibited by the four hydrogens of the two methylene groups and the second by a single proton attached to the ring carbon. The two other signals at 7.76 and 9.80 ppm are assigned to the single hydrogen attached to the ring nitrogen and to the two hydroxylamine protons, respectively. The NMR spectrum of I is in agreement with its proposed structure.

The acidity constant of I, pKa = 8.65, can be compared with the analogous values of benzhydroxamic acid, pKa = 8.79, and acethydroxamic acid, pKa = 9.37 (14). The increase of acidity in the sequence acethydroxamic acid < benzhydroxamic acid < I is in agreement with the fact that the pyrrolidone ring has a lower electron-donating ability than the phenyl and/or the methyl groups.

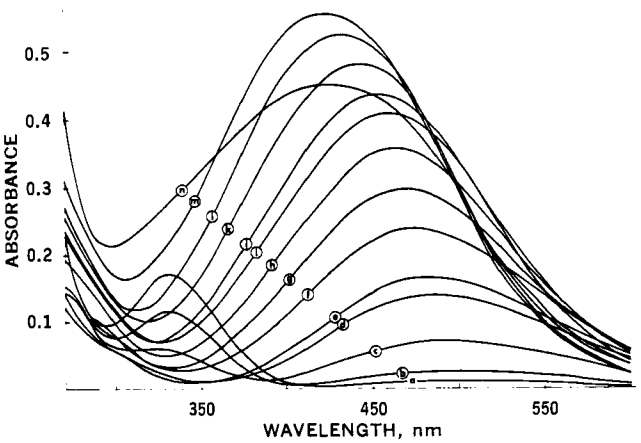


Figure 4—*Absorption spectra of 0.0002 M $FeCl_3$ and 0.002 M I using 1-cm quartz cells at the following pH values: a, 0.70; b, 1.25; c, 1.50; d, 1.72; e, 1.87; f, 2.20; g, 2.80; h, 3.13; i, 3.58; j, 3.96; k, 4.42; l, 5.00; m, 6.50; and n, 9.00.*

[3] The pH and potentiometric measurements were performed with a type MA 5701 ISKRA pH meter and a Radiometer type SRB2c/ABUlb/TTA3 titrigraph. IR spectra from 4000 to 400 cm^{-1} were obtained using a Perkin-Elmer model 457 grating spectrophotometer and KBr disks. The polystyrene spectrum was used for calibration. Proton magnetic resonance spectra were obtained with a Varian model T-60 NMR spectrometer. UV and visible spectra were obtained with a Opton PMQ II single-beam spectrophotometer and a Cary model 16K recording spectrophotometer equipped with quartz cells. Determinations of the magnetic susceptibility were made by means of the Gouy method. Elemental analyses were performed by the Research Institute of the Pharmaceutical and Chemical Works "Pliva," Zagreb.

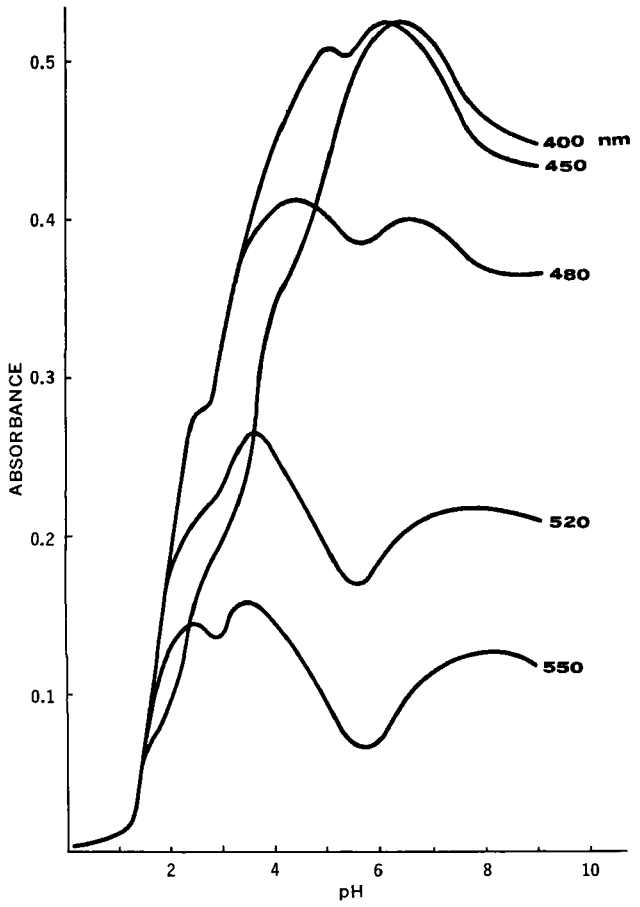


Figure 5—*Absorbance of 0.0002* M *$FeCl_3$ and 0.002* M *I* versus *pH at different wavelengths, using 1-cm quartz cells.*

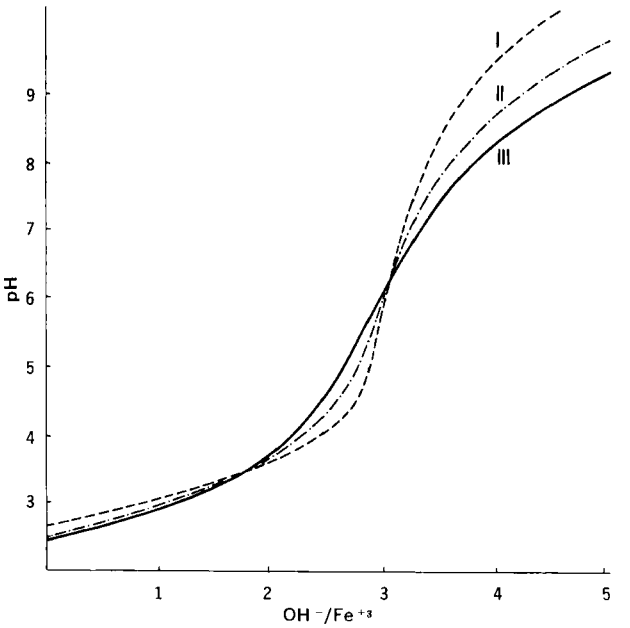


Figure 6—*Potentiometric titration of 0.002* M *$FeCl_3$ containing 0.002* M *I (curve I), 0.004* M *I (curve II), and 0.006* M *I (curve III) with 0.006* M *NaOH.*

Aqueous Mixture of Mono-, Bis-, and Tris(pyrrolidone-5-hydroxamato)iron (III) Complexes—The behavior of the solution containing 2×10^{-4} *M* ferric ions and 2×10^{-3} *M* I (mole ratio = 10) is represented in Fig. 4 over pH 0.70–9.00. Curve a, corresponding to pH 0.70, is similar to that of ferric ion with the absorption peak at 335 nm. However, a new peak at 500 nm appears, which probably indicates the formation of new absorbing species between the ferric ion and I. The increase in pH up to 6.50 results in the disappearance of the 335-nm peak and a shift of the absorption peak from 500 to 430 nm. This observation may suggest the formation of at least two absorbing species between the ferric ion and I. A further increase in pH causes a change of curve m (pH 6.50) to curve n (pH 9.00), which is accompanied by the precipitation of ferric hydroxide.

The pH dependence of the absorbance at several wavelengths of the solution with mole ratio = 10 is shown in Fig. 5. The appearance of the three peaks can be clearly recognized on each curve and may be tentatively assigned to the formation of the three different iron (III)–I species. The existence of these three different species is consistent with the visible color changes of the iron (III)–I solution; a pH decrease or ferric-ion concentration increase turns the solution violet, a pH increase to approximately 4 changes the violet color to red, and further pH increases to the neutral and slightly basic regions or an increase in the concentration of I turns the original red color to a golden yellow.

By following Job's (15) method of continuous variation, the plots of the absorbance *versus* iron fractions x, where x = [Fe]/[Fe] + [I], show the maxima at x = 0.5, 0.33, and 0.25. The assumption may be made that iron (III) forms complexes with I in the stoichiometric ratios of iron (III)–I of 1:1, 1:2, and 1:3. The acidic behavior of all three complexes is shown in Fig. 6, which shows potentiometric titration of the solution containing different I–iron (III) ratios of 1 (curve I), 2 (curve II), and 3 (curve III). All three potentiometric curves intersect at the joint inflection point, showing the existence of the three neutral species of the possible formulation $Fe(C_5H_7O_3N_2)(OH)_2$, $Fe(C_5H_7O_3N_2)_2(OH)$, and $Fe(C_5H_7O_3N_2)_3$.

On the basis of these observations, the behavior of the iron (III)–I solution may be tentatively represented by Scheme II.

$$Fe^{+3} + HC_5H_7O_3N_2 \rightleftharpoons Fe(C_5H_7O_3N_2)^{+2} + H^+$$

$$Fe(C_5H_7O_3N_2)^{+2} + HC_5H_7O_3N_2 \rightleftharpoons Fe(C_5H_7O_3N_2)_2^{+} + H^+$$

$$Fe(C_5H_7O_3N_2)_2^{+} + HC_5H_7O_3N_2 \rightleftharpoons Fe(C_5H_7O_3N_2)_3 + H^+$$

Scheme II

Tris(pyrrolidone-5-hydroxamato)iron (III)—The IR spectrum of the recrystallized product shows characteristic bands of I, including a rather broad band at 3000 cm^{-1} due to the crystal water.

The magnetic moment of tris(pyrrolidone-5-hydroxamato)iron (III) complex was 5.67 B.M.[4], which is very close to the full high-spin value of 5.92 B.M. for the five unpaired electrons per iron atom. The measured value of 5.67 B.M. is in agreement with the figures for the other high-spin iron (III)–hydroxamato complexes, such as ferrichrome (5.68 B.M.), ferrioxamine A (5.73 B.M.), and ferrioxamine B (5.86 B.M.)(2).

The spectral data and the elemental analyses given in the *Experimental* section confirm the formulation as $Fe(C_5H_7O_3N_2)_3 \cdot 6H_2O$.

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Specific TLC Tissue Residue Determination of Sulfadiazine following Fluorescamine Derivatization

CARL W. SIGEL [x], JOSEPH L. WOOLLEY, Jr., and CHARLES A. NICHOL

Abstract □ A spectrodensitometric method for the direct determination of sulfadiazine at the tissue residue level (0.1 ppm) is based upon the measurement of the fluorescence of a sulfadiazine–fluorescamine derivative formed directly on a TLC plate by dipping it into a fluorescamine solution. The linear dynamic range for the assay is about 150 from 200 to 0.2 ng, the lower limit of sensitivity. Recoveries from various spiked tissues including milk, eggs, liver, kidneys, muscle, skin, and fat varied with the tissue type but were reproducible. This assay technique has also been used for the assay of sulfamethoxazole and has been explored for use in specifically assaying sulfonamide mixtures.

Keyphrases □ Sulfadiazine—specific TLC tissue residue determination following fluorescamine derivatization □ Fluorescamine—used to prepare sulfadiazine derivative, specific TLC tissue residue determination of sulfadiazine □ TLC—tissue residue determination, sulfadiazine, fluorescamine derivatization

The antibacterial activity of trimethoprim is potentiated by administering it with a sulfonamide (1). Investigation of the use of the combination[1] of trimethoprim with sulfadiazine for treatment of infections in food-producing animals required the development of a method capable of determining sulfadiazine tissue concentrations down to 0.1 ppm. Previous work in this laboratory showed that quantitative TLC could be applied to the direct and specific determination of sulfamethoxazole extracted from plasma by measuring its absorbance on a silica gel plate (2). Although quite satisfactory for kinetic measurement of drug levels in body fluids, this procedure lacked the sensitivity needed for the determination of tissue residue levels of sulfadiazine. Therefore, derivatization on the TLC plate to form a fluorescing compound was considered as an alternative. Fluorescamine, which was first reported as a means of generating a fluorescing derivative of primary amino acids (3), was tested. The reagent was used for detecting amino acids after TLC development, but the fluorescing zones were not quantitated (4).

Sulfonamides and fluorescamine can form fluorescing derivatives directly on silica gel TLC plates, and the resulting zones can be quantitated using a scanning spectrodensitometer. These observations led to a sensitive and specific assay for sulfadiazine, which was applied to the determination of the drug at tissue residue levels. Determination of other sulfonamides, singly and in combination, was explored and will be discussed.

EXPERIMENTAL

Thin-Layer Plates—Silica gel 60 plates without fluorescent indicator (20 × 20 cm, 0.25 mm) were used[2].

Solvents and Solutions—All solvents were reagent grade[3] and all were distilled before use. The tissue homogenization buffer was 0.56 *M* sodium chloride, 0.01 *M* sodium phosphate dibasic, and 0.0013 *M* sodium phosphate monobasic (pH 7.4). Solutions of sulfadiazine (1.0 mg) in 100 ml of ethyl acetate and solutions of triethanolamine (1.25 ml) in 250 ml of chloroform were prepared each week. Fluorescamine[4] (25 mg) was dissolved in 250 ml of acetone. The solution was used for derivatization of not more than 15 plates and was not kept longer than 2 weeks.

Instrumentation—Plates were spotted with an automatic spotter[5] or by hand. Fluorescence was determined by scanning TLC plates with a spectrodensitometer[6], using the reflectance mode and only the sample beam with a secondary cutoff filter at 400 nm. Excitation was at 290 nm for the sulfadiazine–fluorescamine derivative, and total emission (above 400 nm) was determined using a density computer[7]. Peak areas were electronically integrated[8], and standard curves were calculated using the method of least squares.

Sulfadiazine Determination—*Procedure for Milk and Muscle*—In a stainless steel or glass cup kept at 4°, tissue (10 g) was homogenized in saline phosphate buffer (15 ml) using a tissue homogenizer[9]. After adjusting the pH to 6.0 with 1 *M* phosphoric acid, an aliquot equivalent to 1 g of tissue was pipetted into a 15-ml glass-stoppered centrifuge tube. The homogenate was extracted with three 3-ml portions of ethyl acetate. The combined

[1] Tribrissen, Cooper, Inc.

[2] E. M. Laboratories, Inc.
[3] Mallinckrodt.
[4] Fluram, Roche Diagnostics.
[5] Analytical Instrument Specialties Multi-spotter.
[6] Schoeffel model SD 3000 using a reflectance mode assembly.
[7] Schoeffel SDC 30.
[8] Autolab System IV.
[9] Virtis "45."