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Conformational Studies of Antiradiation Agents by NMR: Cysteamine and Its Derivatives

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Abstract □ The conformations of cysteamine, thiazolidine, and thiazolidine-4-carboxylic acid were determined in aqueous solutions using NMR spectroscopy. At physiological pH, the population ratio of *gauche*- and *trans*-conformers was 3:1. The *gauche*-rotamer is probably responsible for the antiradiation activity and acts through metal chelation involving sulfur and nitrogen atoms. The puckering of the thiazolidine ring was calculated using NMR coupling constants. The observed results were compared with those obtained in the solid state using X-ray diffraction.

Keyphrases □ Cysteamine—and derivatives, NMR conformational study in aqueous solutions □ Thiazolidine—and derivatives, NMR conformational study in aqueous solutions □ NMR—conformational study of cysteamine, thiazolidine, and derivatives in aqueous solutions □ Conformations—cysteamine, thiazolidine, and derivatives, NMR study in aqueous solutions □ Antiradiation agents—cysteamine, thiazolidine, and derivatives, NMR conformational study in aqueous solutions

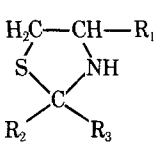
Cysteamine (2-aminoethanethiol, I) is a well-established antiradiation agent (1). The sulfur-substituted derivative of this molecule (2), 2-aminoethanethiosulfuric acid (II), is as active as I. Some 2-substituted thiazolidines (III and IV), prepared by condensing I with carbonyls, are also good antiradiation agents (3). To explain the mechanism of radioprotection offered by these compounds, several postulations (4, 5) have been suggested such as mixed disulfide formation (6) with protein constituents, chelation of vital metal ions (7), and binding to DNA (8).

The conformations in the solid state by X-ray diffraction for these molecules were reported (9–12). Recently, quantum chemical calculations using extended Hückel theory (EHT) and complete neglect of differential overlap (CNDO) methods were made in this laboratory. The results showed that I has both *gauche*- and *trans*-conformations, with a preference toward the *trans*-structure.

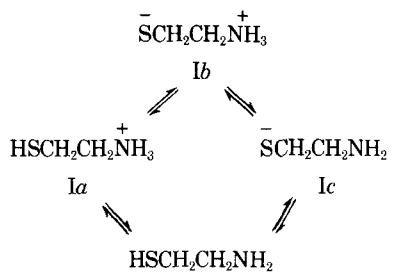
In this paper, the application of NMR spectroscopy for the determination of the preferred conformation in solution of cysteamine and some thiazolidine derivatives is reported. Attempts were made to correlate these findings with their biological action.

$HSCH_2CH_2NH_2$ I

$HO_3SSCH_2CH_2NH_2$ II



III: $R_1 = COOH$, $R_2 = R_3 = H$
IV: $R_1 = H$, $R_2 = R_3 = CH_3$



Scheme I

In physiological solution, cysteamine undergoes multiple ionization (9) (Scheme I). The ionization constants governing these equilibria have been reported, so it is possible to calculate the fraction of each species as a function of pH. The concentration of I is low at all pH values. Form I*a* predominates in acidic media, while I*c* predominates at pH values greater than 12. Form I*b* is present at intermediate pH's and reaches its maximum concentration of 84.6% around pH 9.5. Therefore, NMR studies of cysteamine were made at three different pH values. Other compounds were studied in neutral solution and in deuterochloroform.

EXPERIMENTAL

Materials—Cysteamine hydrochloride[1] was recrystallized from 95% ethyl alcohol until it was free from disulfide traces. 2-Aminoethanethiosulfuric acid and thiazolidine derivatives were prepared using reported procedures (3, 13).

NMR Measurements—Samples were dissolved in deuterium oxide to a known concentration, and spectra[2] were recorded using the sodium salt of 2,2-dimethyl-2-silapentane-5-sulfonic acid as the internal reference. The pH values were adjusted using trifluoroacetic acid.

RESULTS AND DISCUSSION

Spectral Analysis—The studied molecules are of the 1,2-disubstituted ethane type and show the usual rotational isomerism around the carbon–carbon bond. In the case of thiazolidines, this rotation is hindered, but a small degree of conformational freedom is still present due to the puckering modes of the ring. The spectrum of cysteamine recorded in

[1] Fluka grade.
[2] Varian Associates HA-100 and A-60 spectrometers.

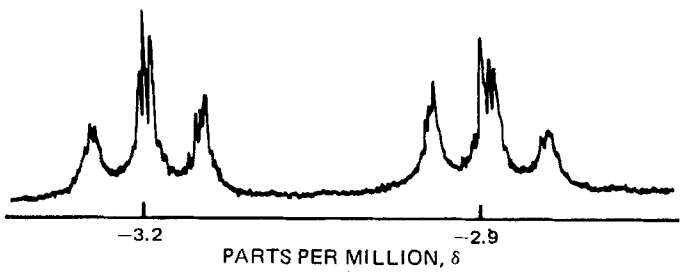


Figure 1—*The 100-MHz spectrum of cysteamine in deuterium oxide (pH 7).*

a neutral medium (pH 7.0) is shown in Fig. 1. Figures 2 and 3 show the spectra of thiazolidine-4-carboxylic acid hydrochloride and 2,2′-dimethylthiazolidine hydrochloride, respectively.

The chemical shift and coupling constant data for all these molecules are given in Table I. The spectra of I and IV show two methylene proton absorptions due to the CH_2CH_2 fragment. The chemical shift difference in acidic solution is greater than in neutral and basic solutions, indicating the effect of pH on the acid–base equilibrium. The spectrum is of the *AA′BB′* type and was analyzed by an iterative LAOCOON program (14). As usual with such spectra, only the sum of the coupling constants ($J_{AB} + J_{AB'}$) was estimated accurately. The spectrum of thiazolidinecarboxylic acid, on the other hand, is of the *ABC* type and all coupling constants were evaluated accurately. 2-Aminoethanethiosulfuric acid shows a single methylene proton at −3.00 ppm (δ), indicating a very small chemical shift difference (spectrum not shown).

Conformational Analysis of Cysteamine—Due to rotation about the carbon–carbon bond, the spectrum is an average of the individual rotamers, each of which belongs to the *AA′BB′* class (15). In the system of three equilibrating staggered rotamers, G, T, and G′ (Fig. 4), the weighted mean of the couplings between protons *A* and *B* are observed.

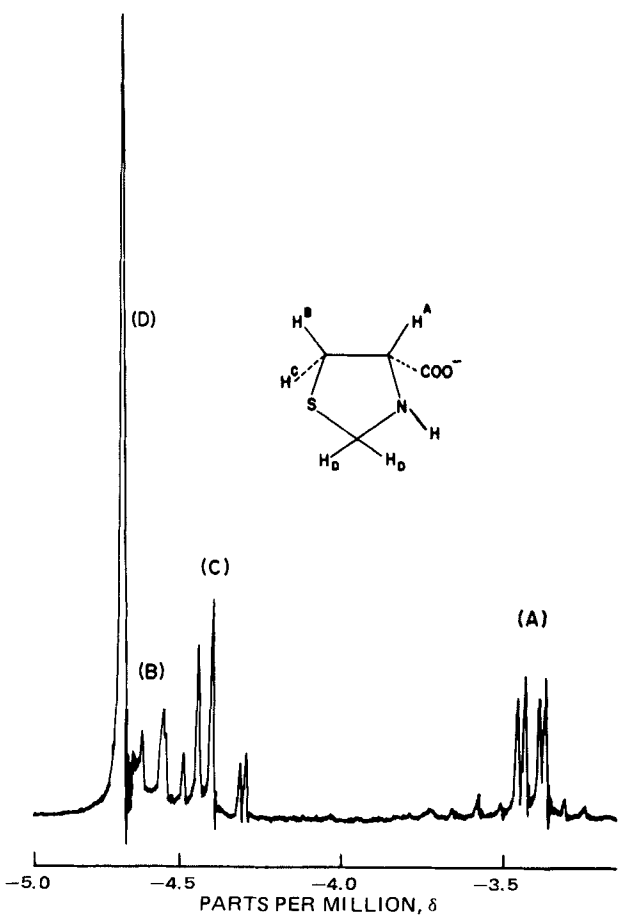


Figure 2—*The 100-MHz spectrum of thiazolidine-4-carboxylic acid in deuterium oxide.*

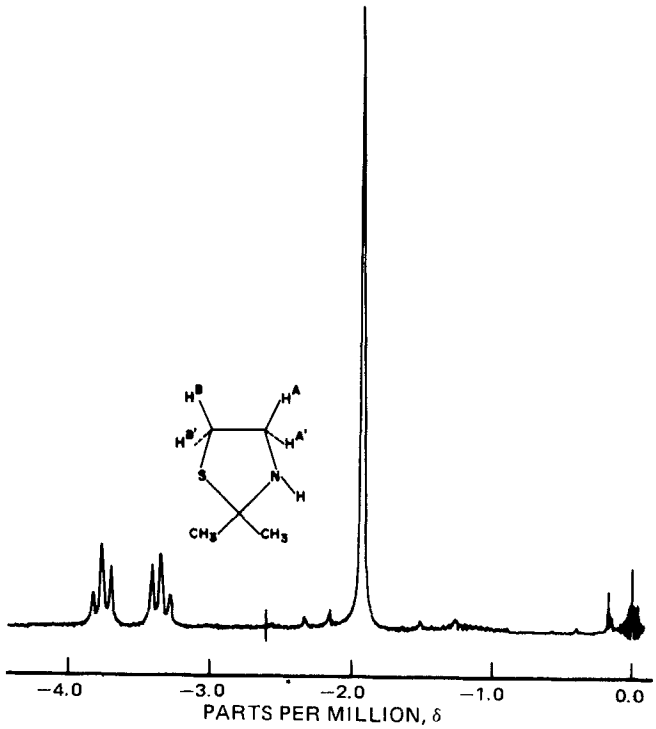


Figure 3—*The 100-MHz spectrum of 2,2-dimethylthiazolidine in deuterochloroform.*

In the present case, rotamers G and G′ are identical. The observed coupling constants (J_{AB} and $J_{AB'}$) contain information about the relative populations of the three rotamers.

In an $X\text{-}CH_2CH_2\text{-}Y$ fragment, the *vicinal* coupling constants depend on the electronegativity of the substituents and the dihedral angles. Abraham and Gatti (16) made a detailed analysis of the two effects. For staggered conformations of 1,2-disubstituted ethanes, the individual *gauche-* and *trans-vicinal* proton–proton couplings (J_g and J_t) are related to the electronegativities of the substituent atoms X and Y as follows.

For *trans*-rotamer T:

$$J_g{}^T = 1.35 + 0.63(E_X + E_Y) \quad \text{(Eq. 1)}$$

$$J_t{}^T = 18.07 - 0.88(E_X + E_Y) \quad \text{(Eq. 2)}$$

For *gauche*-rotamers G and G′:

$$J_g{}^G = 8.94 - 0.94(E_X + E_Y) \quad \text{(Eq. 3)}$$

$$J_{g'}{}^G + J_t{}^G = 26.92 - 2.03(E_X + E_Y) \quad \text{(Eq. 4)}$$

where E_X and E_Y are Huggins electronegativities (17) for atoms S and N, which are 2.60 and 3.05, respectively. From Eqs. 1–4, $J_g{}^T = 4.91$, $J_t{}^T = 13.04$, $J_g{}^G = 3.63$, and $J_t{}^G = 11.83$. From these values, one estimates $(J_{AB} + J_{AB'})^T = J_g{}^T + J_t{}^T = 17.95$ and $(J_{AB} + J_{AB'})^{G,G'} = \frac{1}{2}[(J_{AB} + J_{AB'})^G + (J_{AB} + J_{AB'})^{G'}] = \frac{1}{2}[3J_g{}^G + J_t{}^G] = 11.36$. The population of the *trans*-isomer, p_T, can now be evaluated using the observed values of $(J_{AB} + J_{AB'})$:

$$(J_{AB} + J_{AB'})_{obs} = 17.95p_T + 11.36(1 - p_T) \quad \text{(Eq. 5)}$$

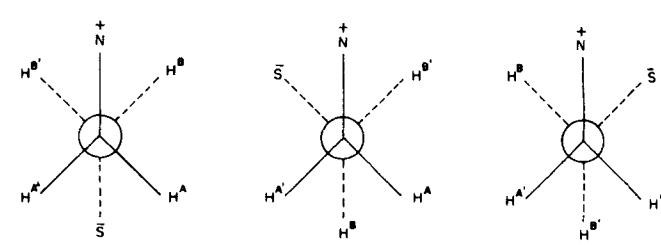


Figure 4—*Conformational rotamers of cysteamine.*

Table I—Proton Chemical Shifts and Coupling Constants in Cysteamine and Thiazolidines

Compound	Chemical Shift (Downfield) A	B	C	Coupling Constant, Hz	p_T
Cysteamine[a]					
pH 3	−3.11	−3.54	—	$J_{A,B} \sim 6.5$, $J_{A,B'} \sim 7.8$ $\lvert J_{A,B} + J_{A,B'} \rvert = 14.3$	0.44
pH 7	−3.04	−3.35	—	$J_{A,B} \sim 7.2$, $J_{A,B'} \sim 5.8$ $\lvert J_{A,B} + J_{A,B'} \rvert = 13.0$	0.25
pH 11	−3.05	−3.37	—	$J_{A,B} \sim 6.8$, $J_{A,B'} \sim 6.8$ $\lvert J_{A,B} + J_{A,B'} \rvert = 13.6$	0.34
Thiazolidinecarboxylic acid[a]	−3.43	−4.58	−4.46	$J_{A,B} \sim 6.6$, $J_{A,C} \sim 2.1$, $J_{B,C} \sim -11.7$	—
Dimethylthiazolidine[b]	−3.35	−3.78	—	$J_{A,B} \sim 6.6$, $J_{A,B'} \sim 6.6$ $\lvert J_{A,B} + J_{A,B'} \rvert = 13.2$	—

[a] In deuterium oxide relative to sodium 2,2-dimethyl-2-silapentane-5-sulfonate. [b] In deuterochloroform relative to tetramethylsilane.

or:

$$p_T = \frac{(J_{AB} + J_{AB'})_{obs} - 11.36}{6.59} \quad \text{(Eq. 6)}$$

The population analysis of the rotamer isomers is given in Table I.

Two factors can contribute to errors in the estimates of the population distributions reported in Table I. Basic values of component coupling constants, estimated using empirical generalizations made on the basis of experimental measurements on a large number of compounds (16), may be in error. Recently, Feeney (18) made an accurate determination of component coupling constants for carbon–carbon bonds in peptides using data from methyl-substituted piperidine and cyclohexylamine. The electronegativities of the substituents in this case are similar to those in cysteamine, as are the component coupling constants for the G and T isomers. Nevertheless, errors of ~0.5 Hz in the estimation of $J_{AB} + J_{AB'}$ for either the G or T isomer are possible and will be reflected as a maximum error of 6 in the estimated population of the *trans*-conformer.

Errors also may occur in the observed values of $J_{AB} + J_{AB'}$. In neutral and acidic media, the NMR lines are fairly sharp and the sum of the coupling constants can be estimated to an accuracy of ±0.1 Hz. This value corresponds to an accuracy of ±2 in estimated percentage values of p_T. However, in basic solutions, the linewidths are about 1 Hz and the sum of the estimated coupling constants can be in error by ±0.5 Hz, leading to an error of ±10 in the percentage values of p_T.

Clearly, a significant population of both *gauche*- and *trans*-conformers is present at all three pH values. The population distribution of p_G to p_T is close to the value of 2:1 expected from purely statistical considerations. If it is assumed that the component coupling constants are not sensitive to the ionization state of the molecule, then the population distribution does show a detectable change with pH. At physiological pH, the ratio of p_G to p_T is around 3:1. A slightly higher population of the *gauche*-conformer around pH 7 may be associated with a preferential stabilization of such a conformer by electrostatic charge–charge interaction or the nitrogen–hydrogen–sulfur–hydrogen bond.

Puckering Angle in Thiazolidine and Thiazolidinecarboxylic Acid—Thiazolidine and its derivatives cannot acquire fully staggered conformations about their various single bonds because of the restrictions imposed by ring closure. In general, a saturated five-member ring system may lie in a twisted conformation, with two of its atoms lying out of the plane defined by the other three.

It is possible to estimate the dihedral angle (δ) along the nitrogen–carbon–carbon–sulfur bond (Fig. 5) using *vicinal* NMR coupling constants between protons on the two carbon atoms[3]. In general, a Karplus-type relationship between the *vicinal* hydrogen–carbon–carbon–hydrogen coupling constant and the relevant dihedral angle (ϕ) is used in such studies:

$$J(\phi) = A\cos^2\phi + B\cos\phi + C \quad \text{(Eq. 7)}$$

The first term in the Karplus relationship generally dominates, since the value of A is almost 10 times larger than that of B or C.

The available coupling constants, $J_g^G(\phi = 60°)$ and $J_t^G(\phi = 180°)$, for cysteamine can be used to calibrate the Karplus relationship for thiazolidine. However, these data are insufficient for estimating all three parameters. Fortunately, as will be seen later, results are not sensitive to values of B and C. Accordingly, attempts are made on the following combinations of A, B, and C values to estimate δ in thiazolidine and thiazolidinecarboxylic acid: (*a*) $A = 10.93$, $B = 0$, and $C = 0.90$ (analogous to the relationship suggested by Feeney (18) for amino acids); (*b*) $A = 12.7$, $B = 0.9$, and $C = 0$; (*c*) $A = 11.8$, $B = 0$, and $C = 0$; and (*d*) $A = 14.5$, $B = 0$, and $C = 0$. The estimated value of δ does not differ by more than ±5° from the mean values reported in the subsequent discussion.

For thiazolidine itself, two identical conformations correspond to positive or negative values of δ. The NMR spectrum of the NCH_2CH_2S fragment is a weighted average of the two identical conformers. As is evident from Fig. 5*b*, the sum of coupling constants $J_{AB'} + J_{A'B}$ is related to δ by the following equation:

$$(J_{AB'} + J_{A'B})_{obs} = \tfrac{1}{2}[2J(\delta) + J(120 - \delta) + J(120 + \delta)] \quad \text{(Eq. 8)}$$

Using a value of 13.2 Hz for $J_{AB'} + J_{A'B}$, one obtains $\delta = 42 \pm 3°$.

With thiazolidinecarboxylic acid, the two conformers become nonequivalent, but the NMR spectrum (*ABC* type) enables one to determine which of the two conformers is preferred because both J_{AB} and J_{AC} are measurable. The observed values of 2.1 Hz for J_{AC} and 6.6 Hz for J_{AB} indicate that the preferred conformer in solution is as shown in Fig. 5c with $\delta = 45 \pm 5°$. The result is not significantly affected when the parameters of the Karplus relationship are calibrated using $J_g^G = 3.2$ and $J_t^G = 11.4$, which are the values obtained when allowing for the electronegativity of the substituent on thiazolidine.

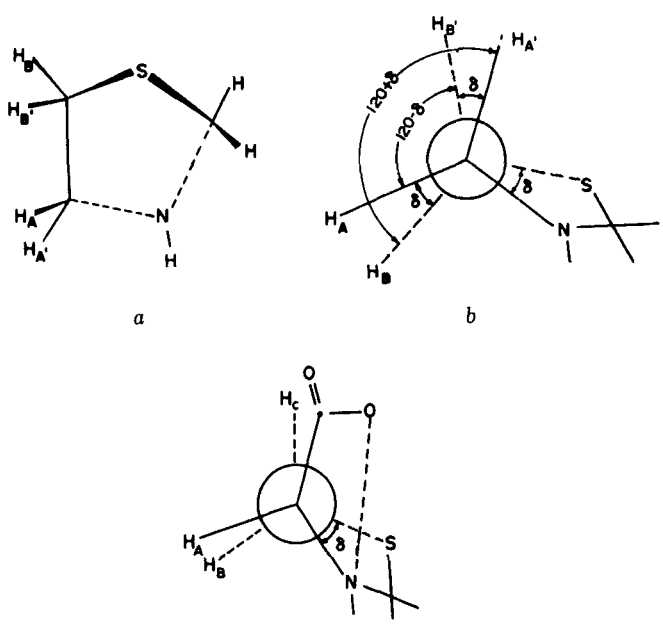
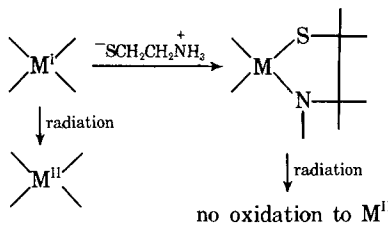


Figure 5—*Conformations of thiazolidine. Key:* a, *ring puckering of thiazolidine showing both nitrogen and sulfur atoms puckered with respect to the plane passing through the three carbon atoms (twisted conformation);* b, *dihedral angles around the carbon–carbon bond in thiazolidine; and* c, *ring puckering in thiazolidinecarboxylic acid.*

[3] In principle, it is also possible to estimate dihedral angles along the carbon–carbon–nitrogen–carbon and carbon–nitrogen–carbon–sulfur bonds through hydrogen–carbon–nitrogen–hydrogen coupling constants. Unfortunately, such coupling constants were not detected in the molecules under study.



Scheme II

Comparison with Conformational Studies by Other Methods—While the solid-state and solution data on the preferred conformations of the compounds are not identical, they do show a high degree of similarity. For cysteamine in deuterium oxide at physiological pH, the relative population of the *gauche*-conformer is greater (75%) than the *trans*-conformer. X-ray diffraction studies (9), on the other hand, showed that in the solid state the molecule crystallizes only in the *gauche*-form. In crystals, the observed structure is stabilized by intramolecular nitrogen–hydrogen–sulfur bonds.

For thiazolidinecarboxylic acid, X-ray crystallography indicates that the ring has a one-atom pucker (envelope) conformation, with the sulfur atom lying about 0.8 Å ($\delta = 27°$) out of the plane described by the other four atoms. The solution data indicate a slightly higher pucker ($\delta = 45°$) for both thiazolidine and thiazolidinecarboxylic acid. If the bond length and bond angles observed in the solid state are preserved in solution, then the NMR data indicate that both sulfur and nitrogen atoms are puckered relative to the plane described by the three ring carbon atoms. The structure of thiazolidinecarboxylic acid is held in the crystal by several intra- and intermolecular NH···O=C hydrogen bonds. When such hydrogen bonds are replaced by solute–solvent hydrogen bonds in aqueous solutions, the molecular conformation apparently switches to a more relaxed two-atom twisted conformation.

The solution results are in better agreement with those predicted by EHT and CNDO theory. Theoretical results correspond more closely to conformations for isolated molecules and indicate a slight preference for the intramolecularly hydrogen-bonded form of I*b* while almost equal energies are predicted for the G, T, and G′ conformers of I*a* and I*c*. For thiazolidine systems, such calculations indicate that a two-atom twisted conformation (with $\delta = 38°$) is most stable.

Biological Action—Based on the conformer population of cysteamine in solution, it can be argued that the radioprotective action of this molecule arises from a structure in which the essential atoms, sulfur and nitrogen, are placed in a *gauche*-arrangement. In this conformation, the sulfur and nitrogen atoms come in close proximity and their steric repulsion may be offset by intramolecular hydrogen bond NH···S and charge interactions. This type of molecular arrangement allows the possibility of chelation of metal ions involving sulfur and nitrogen atoms (Scheme II) and prevents their oxidation by free radicals formed in the biophase by the radiolysis of water (19). With thiazolidines, the antiradiation action may be due to their *in vivo* hydrolysis (20) to a protective compound, cysteamine, which then undergoes molecular arrangement as shown in Scheme II.

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Radioimmunoassay of Indomethacin in Biological Fluids

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Abstract □ A radioimmunoassay was developed for the determination of indomethacin in biological fluids at concentrations as low as 50 ng/ml. Antibodies were produced in rabbits immunized with a conjugate of bovine serum albumin and indomethacin. This conjugate was prepared by an *N*-hydroxysuccinimide active ester procedure. Antiserums exhibited minimal cross-reactivity with the *O*-desmethyl and deschlorobenzoyl metabolites. However, the glucuronide conjugate was about three times as reactive as indomethacin, thus invalidating direct determinations of indomethacin in urine. This difficulty was circumvented by analyzing urine aliquots before and after conjugate hydrolysis. Concentrations of free and conjugated indomethacin were calculated by difference.

Keyphrases □ Indomethacin—radioimmunoassay, human plasma and urine □ Radioimmunoassay—indomethacin, human plasma and urine □ Anti-inflammatory agents—indomethacin, radioimmunoassay, human plasma and urine

Analyses of the widely used anti-inflammatory agent indomethacin[1] have previously been accomplished by spectrofluorometric (1) or radioisotopic (2) methods. These procedures are useful in certain situations but are subject to limitations. The spectrofluorometric method lacks sensitivity, and the presence of other fluorescent materials,

[1] Indocin, Merck Sharp & Dohme.