Spectrofluorometric Determination of Substituted Tetrahydrocarbazoles by a Methylene Blue Sensitized Photolytic Reaction

J. ARTHUR F. de SILVA [x], NORMAN STROJNY, FELIX RUBIO, JOHN C. MEYER, and BERNARD A. KOECHLIN

Abstract □ A spectrofluorometric method was developed for the determination of 6-chloro-9-[2-(2-methyl-5-pyridyl)ethyl]-1,2,3,4-tetrahydrocarbazole-2-methanol hydrochloride and its carboxylic acid analog in blood and urine. It involves extraction of both compounds at neutral pH, either from blood into ethyl acetate (the residue of which is dissolved in ether) or from urine directly into ether. Both the alcohol and the acid are separated from each other by selective extraction into acid or base, respectively, and then reextracted into ether from the respective aqueous medium by appropriate pH adjustment. The residues of the ether extracts containing the compounds are dissolved separately in 0.25 *N* NH_4OH. Methylene blue is added to all samples, which are then exposed to UV energy for 15 min to produce the fluorophores. The fluorescence of the solutions is read at 370 nm, with excitation at 340 nm. The linear range of quantitation of both compounds is 0.02–10 μg each/ml of final solution. The method was applied to the determination of blood levels and urinary excretion of the alcohol and its acid metabolite in a dog.

Keyphrases □ Tetrahydrocarbazoles, substituted—spectrofluorometric analysis, human blood and urine □ Spectrofluorometry—analysis, various substituted tetrahydrocarbazoles, human blood and urine □ Photolytic reactions—substituted tetrahydrocarbazoles sensitized with methylene blue, spectrofluorometric analysis, human blood and urine □ Antihyperlipidemic agents, potential—various substituted tetrahydrocarbazoles, spectrofluorometric analysis, human blood and urine

Compounds containing the indole moiety have been extensively studied because of their anti-inflammatory (1–4), analgesic, and hallucinogenic (5–7) properties, their inhibition of monoamine oxidase (8), and their cardiovascular effects (5). The compound 6-chloro-9-[2-(2-methyl-5-pyridyl)ethyl]-1,2,3,4-tetrahydrocarbazole-2-methanol (I) hydrochloride and its 2-carboxylic acid analog (II) hydrochloride were synthesized (9) and are of clinical interest, because they have shown marked hypolipidemic activity in animals (10).

Spectrofluorometric analysis of catecholamines (levarterenol and epinephrine) as their trihydroxyindole derivatives has led to the use of condensation reactions to produce fluorescent derivatives. Condensation reactions have been used to determine: (*a*) catecholamines that condense with formaldehyde to form highly fluorescent 3,4-dihydroisoquinolines (11), (*b*) 5-hydroxytryptamine by condensation with *o*-phthalaldehyde as the phthalimidine derivatives (12) and with ninhydrin as the β-carboline derivative (13), (*c*) indole-3-acetic acid by condensation with acetic anhydride (catalyzed with boron trifluoride) as the highly fluorescent indolo-α-pyrone derivative (14), and (*d*) tryptophan by photolytic condensation with acetic acid, using methylene blue as a "sensitizer," to yield highly fluorescent β-carboline derivatives (15, 16).

A spectrofluorometric assay was developed for the quantitation of I and II in blood and urine based on a photolytic reaction of the tetrahydrocarbazole to produce a fluorophore of strong intrinsic fluorescence. The reaction is performed in an alkaline medium catalyzed by methylene blue used as a sensitizer. The overall recovery of both compounds from blood and urine is 90 ± 5.0%. The assay is specific for each compound by virtue of the differential extraction procedure used and has a sensitivity limit of 0.10–0.20 μg/ml of blood or urine using a 2-ml specimen/assay and a sample to blank fluorescence ratio of 2:1 as the limit of detectability.

The method was applied to the determination of the blood levels of I and the urinary excretion of I and its metabolite, II, in a dog following a single intravenous dose of 10 mg/kg.

EXPERIMENTAL

Standard Solutions—Compound I hydrochloride, mol. wt. 391.34, mp 191–193°, and Compound II hydrochloride, mol. wt. 405.33, mp 218.5–220° (pharmaceutical grade purity >98%), are the analytical standards required.

Dissolve 11.02 mg of I hydrochloride and 11.00 mg of II hydrochloride equivalent to 10 mg of the respective free base or acid separately in 100 ml of methanol to give stock solutions containing 100 μg of free base/ml. Dilute 1 ml of each stock solution separately in 10 ml of methanol to give working solutions containing 10 μg/ml, suitable aliquots of which are added to blood or urine as internal standards for the determination of percent recovery. Prepare all solutions in amber actinic volumetric flasks and store in a refrigerator.

Instrument—The spectrofluorometer[1] used was equipped with a 150-w xenon arc energy source[2] and photomultiplier[3]. It was used with the 10-nm slit arrangement in all four positions, with the instrument energy adjusted for maximum sensitivity using a Pyrex reference rod and a standard solution of the compound(s) to be analyzed.

Reagents—All reagents were analytical reagent grade (ACS) and were used without further purification; all aqueous solutions were made in distilled deionized water. These solutions included 1.0 *M* (pH 7.0) phosphate buffer, 2 *N* HCl, 0.5 and 10.0 *N* NaOH, 0.25 *N* NH_4OH, and 0.025% methylene blue[4] (USP grade dissolved in water). Ethyl acetate[5] (spectrograde), ether[5] (absolute) from a freshly opened can, chloroform[6], and formaldehyde[7] (37%) were the other reagents used.

Assay in Blood—To 50-ml glass-stoppered centrifuge tubes, add 2 ml of whole blood, 2.0 ml of 1.0 *M* pH 7 buffer, 1.5 g of potassium chloride, and 10 ml of ethyl acetate. Stopper[8] the tubes and extract by shaking for 10 min on a reciprocating shaker. Centrifuge the samples at 0–5° in a refrigerated centrifuge at 2000 rpm for 10 min and transfer the supernate to another 50-ml centrifuge tube. Along with the samples, process a 2-ml specimen of control blood and three 2-ml specimens of control blood to which 0.5, 2.5, and 5.0 μg of I and II (0.05, 0.25, and 0.5 ml of the respective working solutions evaporated to dryness at 40–50° under nitrogen) are added as internal standards.

Repeat the extraction with another 10 ml of ethyl acetate, combine the extracts, and evaporate to dryness under nitrogen in a 60° water bath. Dissolve the residues in 15 ml of ether. Add 5 ml of 0.5 *N* NaOH to the

[1] Model Mark I, Farrand Optical Co., Mount Vernon, N.Y. The spectrofluorometer was equipped with a specially selected high UV energy source and photomultiplier.
[2] Hanovia lamp, Engelhardt Industries, Newark, N.J.
[3] RCA 1-P-28 (lumen output >10).
[4] Eastman Chemical Co.
[5] Mallinckrodt Chemical Co.
[6] Fisher Scientific Co.
[7] Allied Chemical Co.
[8] Teflon stoppers (du Pont).

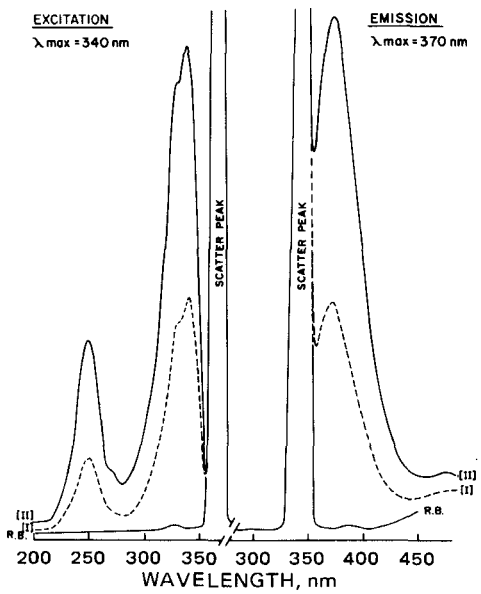


Figure 1—*Excitation and emission spectra of the photochemically produced fluorophores of I and II determined at room temperature for equivalent concentrations of 5 μg/ml.*

ether phase, extract by shaking, centrifuge, and transfer the supernatant ether quantitatively into another 50-ml tube. Wash the sodium hydroxide solution with 10 ml of ether by shaking, centrifuge, and combine the ether wash with the original ether extract. The sodium hydroxide phase contains the carboxylic acid (II) while the ether supernate contains the alcohol (I).

Add 5 ml of 2 *N* HCl to the ether extract containing I and shake for 10 min. Then centrifuge, aspirate, and discard the ether layer. Wash the acid phase containing I with another 10-ml portion of ether, shake, centrifuge, and aspirate off the ether as before. Neutralize the acid with 1.0 ml of 10 *N* NaOH, add 5 ml of 1.0 *M* pH 7.0 phosphate buffer, and extract with 2 × 10-ml aliquots of ether by shaking for 10 min per extraction. Centrifuge and then combine the ether extracts in another 50-ml centrifuge tube.

Evaporate the ether extracts containing I to dryness and dissolve the residues in 5 ml of 0.25 *N* NH_4OH. Add 0.1 ml of 0.025% methylene blue solution to each series containing I and II, and mix well on a Vortex action supermixer. Expose all samples to UV energy[9] for exactly 15 min, placing the tubes in a single row approximately 30.5 cm (12 in.) in front of the lamp. After irradiation, to the sample series containing I *only*, immediately add 0.25 ml of formaldehyde to reduce the excess methylene blue reagent to stop any further autocatalyzed photochemical reaction. The final solution is turquoise blue in color, and its fluorescence is read directly. The blue color does not apparently affect the fluorescence reading of I, since the fluorescence due to the reagent blank is quite low. Wash the sample series containing II, which are reddish purple in color, with 5 ml of chloroform to remove the colored material from the sodium hydroxide phase, resulting in a colorless solution containing the fluorophore of II.

The fluorescence of all solutions is read in a 1-cm path quartz cell at 370 nm, with excitation at 340 nm (Fig. 1).

Calculations—Correct all sample readings for their respective control (blank) reading and calculate the concentrations of I and II in the unknowns by interpolation of the fluorescence readings from their respective internal standard curves. The percent recovery from blood or urine is determined by direct comparison of the fluorescence of known amounts of the internal standards against that of similar concentrations of the external standards processed along with the samples to standardize the process of fluorophore formation. The percent recovery of the internal standards should be determined routinely as a check on the reproducibility of the method.

Assay in Urine—To 5 ml of urine in a 50-ml erlenmeyer flask (adjusted approximately to pH 5.5 with 1 *N* HCl), add 5 ml of 0.2 *M* (pH 5.5) acetate buffer and 200 μl of the glucuronidase–sulfatase preparation[10] (2% by volume). Along with the unknowns, process 3 × 5-ml specimens of control urine containing internal standards of I and II added in the same concentrations as in the blood assay.

Incubate all samples at 37° for 2 hr in an incubation shaker[11]. Cool to room temperature and transfer the specimens to 50-ml centrifuge tubes. Titrate the specimen to pH 7.0 with 2 *N* NaOH, add 5 ml of 1.0 *M* (pH 7.0) phosphate buffer and 5.0 g of potassium chloride, and extract with 2 × 10-ml aliquots of ether by shaking for 10 min on a reciprocating shaker. Centrifuge the samples for 10 min (at 0–5° in a refrigerated centrifuge) at 2000 rpm and combine the ether extracts in another 50-ml centrifuge tube. Process the samples from this point using the differential extraction procedure described for blood.

RESULTS AND DISCUSSION

The intense fluorescence of carbazole and polynuclear carbazoles was used in their quantitation in the nanogram range (17). Therefore, the fluorometric analysis of the tetrahydrocarbazoles (I and II) by chemical oxidation to their aromatized carbazole derivatives was investigated. Several oxidation reactions were attempted but did not yield a suitable fluorophore.

A photochemical reaction using methylene blue as a catalytic sensitizer, reported for the formation of highly fluorescent β-carbolines (15, 16), was investigated and gave the most reproducible and optimal yields of a fluorophore for both I and II.

Methylene blue is highly soluble in water, yielding a deep-blue solution (λ_{max} 609 and 668 nm). The free base can be quantitatively extracted into organic solvents (chloroform) at alkaline pH. This fact was used to advantage in the analysis of II in that the reagent was extracted, resulting in a colorless solution of 0.5 *N* NaOH containing the fluorophore of II. This "cleanup" step is not possible in the analysis of I, since both the reagent and the fluorophore are coextracted into organic solvents.

The presence of the methylene blue reagent in the analysis of I appears to catalyze the further reaction of I or its fluorophore, since the fluorescence readings increased on standing, resulting in poor reproducibility, especially in 0.5 *N* NaOH. Formaldehyde was used to oxidize the excess methylene blue to a turquoise blue-colored product, which stabilized the fluorophore of I. The absorption maximum of this colored product (350–400 nm) apparently absorbs sufficient UV energy during fluorometric analysis (excitation of 340 and emission of 370 nm) to quench the fluorescence of I compared to that of II where the methylene blue reagent has been removed.

This fact is seen in the spectra of the photochemical products of the two compounds (Fig. 1) for equivalent concentrations of 5 μg/ml of each. The peak heights of the excitation–emission spectra of I were about half those of II, both determined at the same instrument settings. This difference was probably due to a quenching effect and/or to the fact that the fluorophore of I is chemically different from that of II (Schemes I and II). The fluorescence of the two reaction products, however, was linear with concentration over 0.10–5.0 μg/ml of final solution, with excitation at 340 nm and emission at 370 nm for each compound (Fig. 1).

Characterization of Fluorophores of I and II—Characterization of the reaction products of I and II was attempted following their separation by TLC. Compounds I and II (100 μg of each) were reacted separately as previously described. The reaction products of I were extracted into ether from the reaction medium (0.25 *N* NH_4OH) following neutralization with 1 *N* HCl and pH adjustment with 1 *M* pH 7.0 buffer. The reaction products of II were extracted into ethyl acetate–1-butanol (80:20) following acidification of the reaction medium (0.5 *N* NaOH) with 2 *N* HCl and pH adjustment to 5.5 with 1 *M* phosphate buffer.

The respective organic solvent extracts were evaporated to about 100 μl, transferred quantitatively onto silica gel chromatoplates[12], and developed to 15 cm in acetone–ammonium hydroxide (90:10). In the respective extracts, I migrated with R_f Ω 0.9; its reaction product had R_f Ω 0.7, while II had R_f Ω 0.2 and its reaction product had R_f Ω 0.3. The respective reaction products were eluted from the silica gel into ethanol and then examined by UV absorption, fluorescence, and phosphorescence emission spectrophotometry.

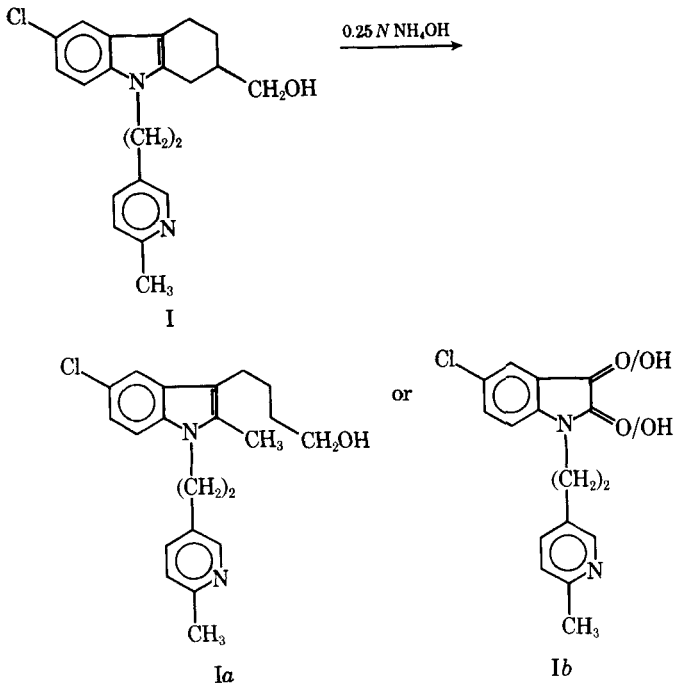
The UV spectra of the authentic compounds in methanol showed a major band at 235–237 nm with minor bands at 265 and 290 nm. Fol-

[9] Pyro-Lux R-57 lamp (Luxor Corp., New York, N.Y.), contained in an aluminum foil-lined reflector box of sufficient size [61 × 61 × 61 cm (2 × 2 × 2 ft)] for adequate ventilation.

[10] Glusulase enzyme preparation containing 100,000 units of glucuronidase and 50,000 units of sulfatase/ml, Endo Laboratories, Garden City, N.Y.

[11] Dubnoff, GCA/Precision Scientific, Chicago, Ill.

[12] E. Merck F_{254} silica gel G, 60-μm particle size, 250-μm bed thickness, Brinkmann Instruments, Westbury, N.Y.



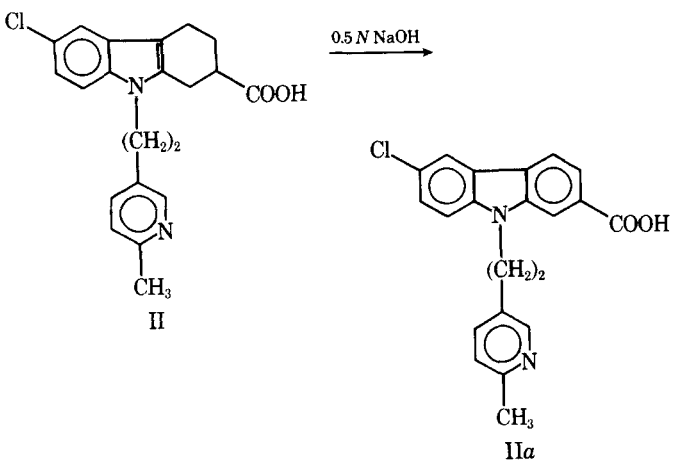
Scheme I

lowing photochemical reaction, the spectrum of I showed a bathochromic shift to 258 nm with secondary peaks at 330 and 343 nm, respectively. The spectrum of II also showed a similar bathochromic shift to longer wavelengths, with the major band at 250 nm and minor bands at 308, 330, and 345 nm. These UV spectral changes indicated major structural changes.

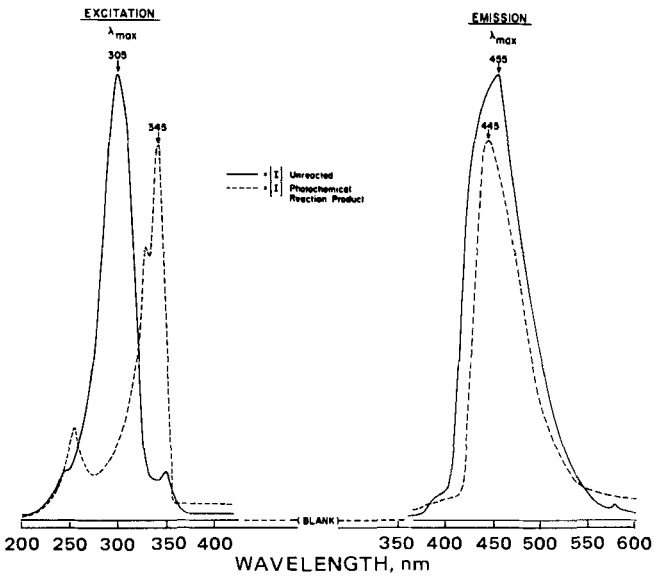
The fluorescence spectra of the reaction products of I and II (5 μg/ml) at room temperature (Fig. 1) were practically identical. Both exhibited excitation maxima at 250 and 340 nm and a broad emission maximum at 370 nm; the excitation bands corresponded fairly well with the UV absorption maxima of the reaction products.

Further insight into the character of these products was sought from a comparison of their phosphorescence spectra (77° K) in ethanol (18) with those of I and II. The spectra showed characteristic differences, permitting certain structural deductions by reference to spectra of known analogous compounds (19). The phosphorescence spectra of unreacted authentic I (Fig. 2A) and II (Fig. 3A) were almost identical, with excitation–emission maxima at 305 and 455 nm, respectively. The excitation spectra of both reaction products possessed considerable fine structure. The spectrum of I (Fig. 2A) had excitation maxima at 250, 330, and 345 nm (major bands); that of II (Fig. 3A) had maxima at 250, 315 (major bands), 340, 350, 365, and 380 nm, indicating a higher degree of aromaticity.

The emission spectrum of the reaction product of I showed a hypsochromic shift to a single maximum at 445 nm; that of II showed a similar



Scheme II



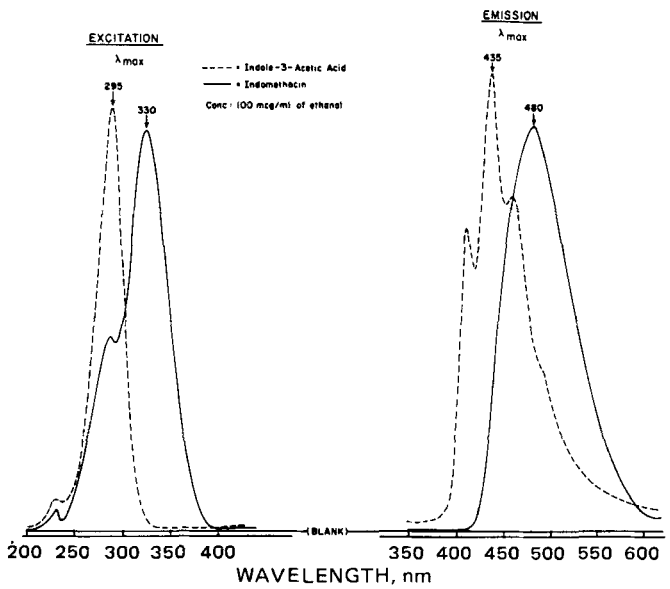


Figure 2—*Phosphorescence spectra in ethanol (77° K) of (top) authentic I and its photochemical reaction product and (bottom) indole acetic acid and indomethacin.*

shift, with a characteristic doublet at 440 and 450 nm of approximately equal intensity. The striking differences in the phosphorescence spectra suggested marked structural differences in the respective reaction products.

The phosphorescence excitation–emission spectrum of the reaction product of I (Fig. 2A) exhibited maxima at 345 and 445 nm, respectively. The absence of a high degree of fine structure suggests that it is not a carbazole derivative. The phosphorescence spectral characteristics were fairly similar to that of indomethacin, whose excitation–emission maxima were at 330 and 480 nm, respectively (also with very little fine structure). Indole-3-acetic acid (III), whose excitation–emission maxima were at 295 and 435 nm, respectively, showed a more complex emission spectrum (Fig. 2B) (20) than either indomethacin (IV) or the reaction product of I. This result was probably due to a reduction of the aromaticity of the indole ring in indomethacin and in the reaction product of I because of the electron-withdrawing effects of the *p*-chlorobenzoyl group in the former and the *p*-methyl-3-pyridylethyl group in the latter, which are attached to the indole nitrogen.

These similarities suggest that the tetrahydrocarbazole ring has either been partially cleaved, resulting in a product structurally similar to indomethacin (Scheme I, Ia), or totally cleaved with possible concomitant oxidation of the indole ring to an indoxyl derivative (Scheme I, Ib). The formation of such an indole or indoxyl derivative could also lead to further

Table I—Luminescence Properties of I and II, Their Photochemical Reaction Products, and the Analogous Carbazole, V, as a Reference Compound

Compound	Fluorescence (25°) Excitation/ Emission Maxima, nm	Intensity, $TM^a/\mu g/ml$	Sensitivity Limit, $\mu g/ml$	Phosphorescence (77° K) Excitation/ Emission Maxima, nm	Intensity, $TM/\mu g/ml$	Sensitivity Limit, $\mu g/ml$
I	—	Nil 100 $\mu g/ml$	>100 $\mu g/ml$	302/455	10	10.0
I Reaction product	340/370	250	0.2	345/445	Ω 300	0.5[b]
II	—	Nil	>100 $\mu g/ml$	305/450	10	10.0
II Reaction product	340/370	550	0.1	305/445	Ω 450	0.5[b]
V	310/400	1250	0.02	312/445, 475	4100	0.001

[a] Fluorescence (TM) units = transmittance (T) × meter multiplier factor (M). [b] Phosphorescence of the reaction products of I and II was approximately corrected for reaction efficiency, extraction efficiency, and efficiency of TLC elution.

reaction under the photolytic conditions to form other condensation products which would be difficult to isolate (21).

The phosphorescence excitation–emission spectrum of the reaction product of II (Fig. 3A) was similar in its fine structure to that of 6-chloro-2-carbazolecarboxylic acid (V) (Fig. 3B). The excitation spectrum of the reaction product of II and V exhibited similar fine structure with multiple peaks at 250, 300, 315 (major bands), 340, 350, and 365 nm. The emission spectra of the reaction product of II and V exhibited a characteristic doublet with maxima at 430 and 445 nm for II and 445 and 475 nm for V. The similarities in the spectral characteristics of the photochemical reaction product of II to those of V and of other known carbazoles (17, 18, 22) strongly suggest that it is a carbazole derivative (Scheme II, II*a*). Attempts to characterize these products further by electron-impact mass spectrometry were unsuccessful due to the poor fragmentation patterns obtained.

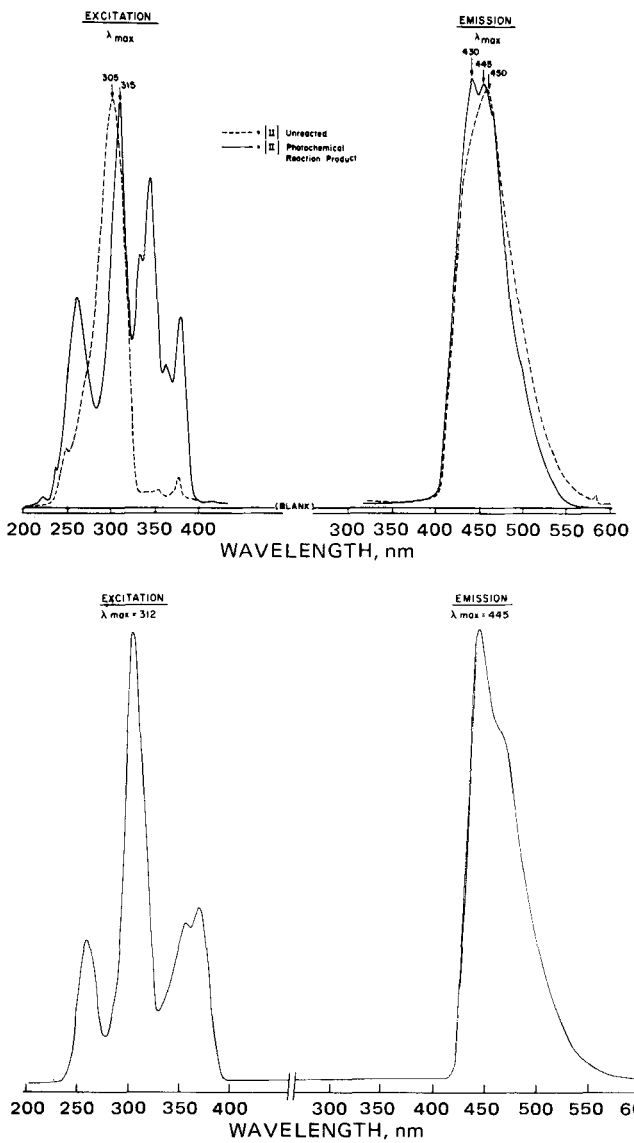


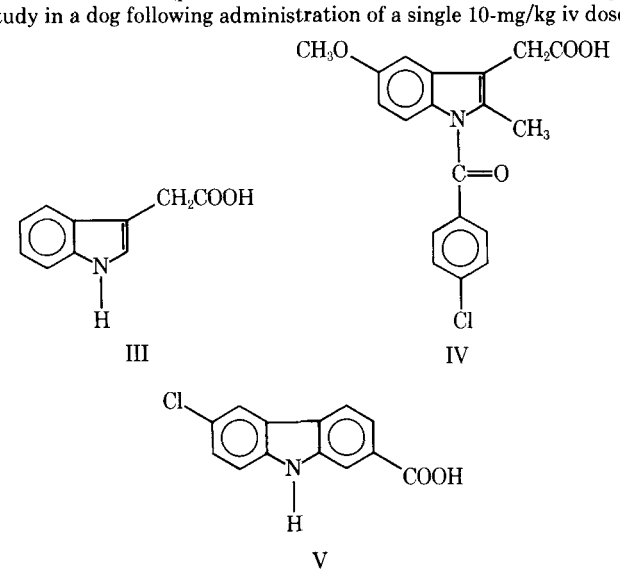
Figure 3—*Phosphorescence spectra in ethanol (77° K) of (top) authentic II and its photochemical reaction product compared with that of (bottom) V, a 9-unsubstituted analog. (Attenuation = 3×.)*

The relative sensitivities of the luminescence properties of I, II, their photochemical reaction products, and the analogous carbazole (V) are given in Table I. Compounds I and II had no measurable fluorescence at room temperature, whereas their intrinsic phosphorescence provided detection limits of 10 μg for I and 0.5–1.0 μg for II and can also be used to analytical advantage where high sensitivity is not a requirement.

Assay Specificity—Preliminary studies in the dog indicated that the carboxylic acid analog, II, is not a major metabolite. Compound I is excreted as a glucuronide conjugate in the urine and feces[13]. However, the presence of small amounts of II in blood or urine would impair the accuracy of the assay for I if it is not resolved from it, because of the relatively high fluorescence yield of its photochemical reaction product.

The spectrofluorometric assay is specific for each compound due to the differential extraction procedure used to separate the two compounds prior to photochemical irradiation and quantitation. The overall recovery of I hydrochloride or II hydrochloride added to blood or urine in the concentration range of 0.10–10.0 $\mu g/ml$ is of the order of 90 ± 5.0% (SD). The sensitivity limits of the assay for I and II are 0.15–0.20 and 0.10–0.15 $\mu g/ml$, respectively, of blood or urine using a 2-ml specimen per assay and a sample–blank fluorescence ratio of 2:1 as the limit of quantitation.

Applications to Biological Specimens—The blood levels and urinary excretion of I and II (produced as a metabolite) were determined in a pilot study in a dog following administration of a single 10-mg/kg iv dose of



[13] S. A. Kaplan, D. E. Maynard, A. Maggio, V. Mattaliano, C. Abruzzo, and S. Cotler, to be published.

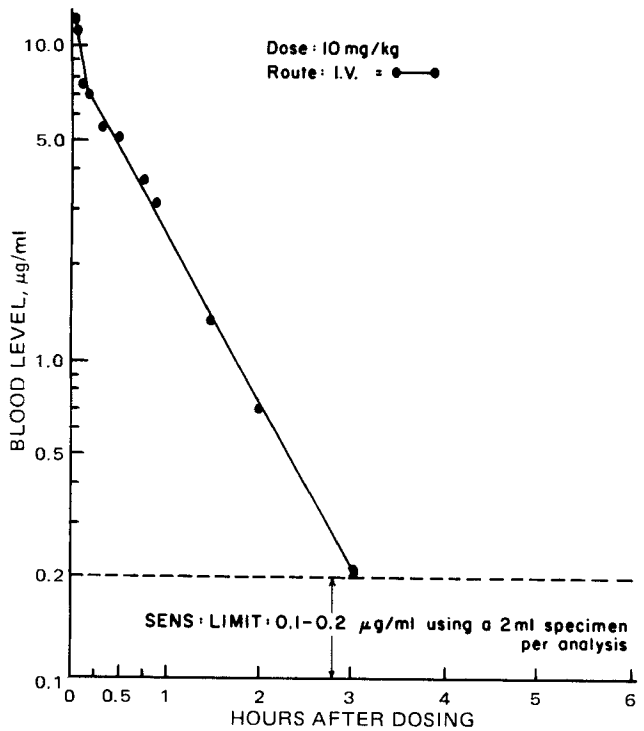


Figure 4—*Blood level curves of I in a dog following the administration of a single 10-mg/kg iv dose of I hydrochloride.*

I hydrochloride. The blood levels of the intact drug I (Fig. 4) showed a biphasic fall-off pattern with an apparent half-life of elimination of about 30 min. Urinary excretion of the parent drug accounted for only 0.1% of the administered dose, present mainly as a glucuronide–sulfate conjugate. No measurable levels of II were seen in either the blood or urine. The utility of the assay was demonstrated in the evaluation of the biopharmaceutic and pharmacokinetic profile of the drug in the dog[13].

REFERENCES

(1) H. J. Sanders, *Chem. Eng. News*, **Aug. 12, 1968,** 46.

(2) R. E. Harman, M. A. P. Meisinger, G. E. Davis, and F. A. Kuehl, *J. Pharmacol. Exp. Ther.*, **143,** 215 (1964).

(3) H. B. Hucker, A. G. Zacchei, S. V. Cox, D. A. Brodie, and N. H. R. Cantwell, *ibid.*, **153,** 237 (1966).

(4) D. E. Duggan, A. F. Hogans, K. C. Kwan, and F. G. McMahon, *ibid.*, **181,** 563 (1972).

(5) T. A. Slotkin, V. di Stefano, and W. Y. W. Au, *ibid.*, **173,** 26 (1970).

(6) T. A. Slotkin and V. di Stefano, *Biochem. Pharmacol.*, **19,** 125 (1970).

(7) T. A. Slotkin and V. di Stefano, *J. Pharmacol. Exp. Ther.*, **174,** 456 (1970).

(8) B. T. Ho, W. M. McIssac, and K. E. Walker, *J. Pharm. Sci.*, **57,** 1364 (1968).

(9) L. Berger and A. J. Corraz, U.S. pat. 3,862,953 (Jan. 28, 1975) and U.S. pat. 3,896,145 (July 22, 1975).

(10) C. Dalton and W. R. Pool, *J. Pharm. Sci.*, **66,** 348 (1977).

(11) H. Corradi and N. A. Hillarp, *Helv. Chim. Acta*, **46,** 2425 (1963).

(12) R. P. Maickel and F. P. Miller, *Anal. Chem.*, **38,** 1937 (1966).

(13) W. B. Quay, *J. Pharm. Sci.*, **57,** 1568 (1968).

(14) A. Stoessl and M. A. Venis, *Anal. Biochem.*, **34,** 344 (1970).

(15) G. Jori, G. Galiazzo, and G. Gannari, *Photochem. Photobiol.*, **9,** 179 (1969).

(16) G. Cauzzo and G. Jori, *J. Org. Chem.*, **37,** 1429 (1972).

(17) M. Zander "Phosphorimetry—The Application of Phosphorescence to the Analysis of Organic Compounds," Academic, New York, N.Y., 1968, pp. 95–106, 158–194.

(18) J. A. F. de Silva, N. Strojny, and K. Stika, *Anal. Chem.*, **48,** 144 (1976).

(19) J. N. Bridges and R. T. Williams, *Biochem. J.*, **107,** 225 (1968).

(20) W. A. Remers, in "Indoles," part I, W. J. Houlihan, Ed., in "The Chemistry of Heterocyclic Compounds," A. Weissberger and E. C. Taylor, Eds., Wiley, New York, N.Y., 1972, pp. 19–33.

(21) R. J. Sundberg, "The Chemistry of Indoles," in "Organic Chemistry," A. T. Blomquist, Ed., Academic, New York, N.Y., 1970, pp. 282–315.

(22) N. Strojny and J. A. F. de Silva, *J. Chromatogr. Sci.*, **13,** 583 (1975).

ACKNOWLEDGMENTS AND ADDRESSES

Received March 1, 1976, from the *Department of Biochemistry and Drug Metabolism, Hoffmann-La Roche Inc., Nutley, NJ 07110.*

Accepted for publication May 18, 1976.

The authors thank Mr. L. Berger of the Chemical Research Division for the authentic standards and chemical analogs and Mr. R. McGlynn for the drawings.

[x] To whom inquiries should be directed.

Oxidative Reactions of Hydroxylated Chlorpromazine Metabolites

RICHARD L. McCREERY

Abstract □ The oxidation pathways of two hydroxylated chlorpromazine metabolites were investigated using modern electrochemical techniques. Upon oxidation, the 7-hydroxy derivative of chlorpromazine rapidly reacts to form the 7,8-dihydroxy derivative and a substituted quinone. The oxidation potentials for both compounds were determined in the pH 3–8 range. The importance of these redox reactions and potentials to the pharmacology of the materials is discussed.

Keyphrases □ Chlorpromazine metabolites—oxidation pathways, electrochemical study, pH 3–8 □ Oxidation pathways—hydroxylated chlorpromazine metabolites, electrochemical study, pH 3–8 □ Electrochemistry—study of oxidation pathways of hydroxylated chlorpromazine metabolites □ Tranquilizers—chlorpromazine metabolites, electrochemical study of oxidation pathways

The widespread use of phenothiazine derivatives in the treatment of mental illness has inspired a large amount of research on their chemical properties and reactions. Of relevance to the present discussion are studies of the importance of redox reactions to the metabolism and mode of action of chlorpromazine and related neuroleptic drugs.