Photochromism of an Anticonvulsant, 1-Diphenylmethyl-4-(6-methyl-2-pyridylmethyleneamino)piperazine, in the Solid State

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Abstract □ The light-induced color change of 1-diphenylmethyl-4-(6-methyl-2-pyridylmethyleneamino)piperazine in the solid state was investigated. Light in the 420–700-nm visible region had no effect. Elevated temperature, dissolution, or prolonged storage in the dark at room temperatures restored the intrinsic color of the compound. IR, UV (solution), NMR, differential scanning calorimetry, and GC methods showed no detectable difference between the long wavelength UV light-exposed (yellow) and unexposed (colorless) samples of the pure compound. Long wavelength UV light exposure studies with several substituted piperazine analogs revealed a structure–activity requirement for the color conversion process. The data indicated that the transformation process from colorless (or faint yellow) to bright yellow is photochromism (phototropy) and is dependent on the intensity of the "action spectrum" in the 300–400-nm region. Studies in the solid state showed that heat-induced fading of the color followed apparent zero-order kinetics. The energy of activation, E_b, for the photochromic conversion process from the metastable (yellow) to the stable (colorless) state was estimated to be about 19 kcal/mole.

Keyphrases □ Photochromism—*N,N*-disubstituted piperazines in solid state, effects of temperature, dissolution, and prolonged storage □ UV—light-induced color changes, *N,N*-disubstituted piperazines in solid state, effects of temperature, dissolution, and prolonged storage □ Piperazines, *N,N*-disubstituted—photochromism in solid state, effects of temperature, dissolution, and prolonged storage □ Structure–activity relationships—photochromism of series of *N,N*-disubstituted piperazines □ Anticonvulsant agents—1-diphenylmethyl-4-(6-methyl-2-pyridylmethyleneamino)piperazine, photochromism studied

1-Diphenylmethyl -4- (6-methyl - 2-pyridylmethyleneamino)piperazine[1] (IV) is a potent anticonvulsant in animals (1). It belongs to a series of hydrazone derivatives of benzhydrylpiperazine that represents a novel chemical structure with such anticonvulsant properties.

During development of a tablet dosage form, IV in its pure state, as well as in the tablet without a color additive, exhibited a gradual change in color when exposed to daylight fluorescent light. This transformation was from colorless (or faint yellow) to bright yellow. The objectives of this investigation were to: (*a*) identify the "action spectrum" of light that induced this change in color, (*b*) study the nature of this reaction and evaluate the physicochemical aspects of the color change with reference to the chemical integrity of the drug, and (*c*) study the kinetics of the color conversion process in the solid state.

EXPERIMENTAL

Materials—Potassium bromide[2], potassium chloride[2], methanol[2], chloroform[2], and deuterated chloroform[3] were analytical reagent grade materials. 1-Diphenylmethyl-4-(6-methyl-2-pyridylmethyleneamino)piperazine[4] (IV), 1-diphenylmethyl-4-(2-pyridylmethyleneamino)piperazine[4] (III), 1-diphenylmethyl-4-(3-pyridylmethyleneamino)piperazine[4] (II), 1-diphenylmethyl-4-(4-pyridylmethyleneamino)piperazine[4] (I), 1-diphenylmethyl-4-(phenylmethyleneamino)piperazine[4] (V), 1-diphenylmethyl-4-(2-pyridyl-*N*-methylammoniummethyleneamino)piperazine bromide[4] (VI), and 1-phenyl-1- (4-chlorobenzyl)methyl-4- (6-methyl-2-pyridylmethyleneamino)piperazine[4] (VII) were supplied as pure compounds[5].

Action Spectrum of IV—Approximately 200 mg of IV[6] was spread on a 43 × 43-mm polyethylene tray[7]. A single light filter or a combination of two filters[8] was placed on the tray top to allow passage of only the desired band of wavelengths in the long wavelength UV or visible light region. The sample assembly was then placed in a light cabinet consisting of six daylight fluorescent lamps[9] mounted in a light fixture 34 cm from the samples.

The intensity of the light transmitted through the light filters was measured with a pocket-sized light meter[9]. The total fluorescent light intensity was estimated to be 900 foot-candles. Samples were checked periodically for color change and left under fluorescent light for 96 hr.

Progress of Color Change of IV—Approximately 2.5 g of IV was packed tightly in 13 × 45-mm thin glass cylindrical containers[10] with polyethylene caps. Except for the bottom surfaces, the containers were wrapped with aluminum foil to prevent light exposure. They were then placed inverted in a light cabinet, thus exposing only the unwrapped surface of the container vertically to the daylight fluorescent light for 96 hr. The reaction was monitored every 24 hr by unwrapping and rewrapping the aluminum foil around the containers and noting the color changes.

Structure–Color Activity Relationships in Light—Approximately 200-mg samples of pure crystals of IV and its six analogs were spread on polyethylene trays. These samples were then exposed vertically to a beam of 366 ± 40-nm long wavelength UV light[11]. The light intensity, as monitored by a light meter[12], was approximately 4000 $\mu w/cm^2$. The samples were exposed to light for 1 hr, after which they were compared to unexposed samples of the same lot for color change.

Physicochemical Analysis of Long Wavelength UV Light-Exposed and Unexposed Samples—*UV (Solution) Spectra*—A 10-mg % stock solution of the compound was prepared in spectral grade methanol. All UV spectra were obtained with a 1- or 3-mg % solution of the compound on a ratio recording spectrophotometer[13].

Differential Scanning Calorimetry Profiles—Approximately 4 mg of the sample was placed in the sample container of the instrument[14] under nitrogen atmosphere. The temperature program mode was set at 10°/min and the recording mode was set at 0.5°/2.54 cm for obtaining the endotherms.

IR Spectra—IR spectra on long wavelength UV-exposed and unexposed disks of IV with potassium bromide were obtained as follows. Approximately 0.8 mg of the pure compound powder was weighed on a microanalytical balance[15] and was then transferred along with 250 mg of dry potassium bromide powder into a hard plastic

[1] SC-13504.
[2] Matheson, Coleman & Bell, Norwood, Ohio.
[3] Merck & Co., Rahway, N.J.
[4] Searle Laboratories, Division of G. D. Searle & Co., Chicago, Ill.
[5] From the Chemical Research Department, Searle.
[6] Lot II-128B was used for all experiments.
[7] Sybron Corp., Rochester, N.Y.
[8] G. K. Turner Associates, Palo Alto, Calif.
[9] General Electric Co., Neela Park, Cleveland, Ohio.
[10] Corning Glass Works, Corning, N.Y.
[11] Model B-100A, Ultra-Violet Products, San Gabriel, Calif.
[12] Model J-221, Ultra-Violet Products, San Gabriel, Calif.
[13] Beckman Instruments, Fullerton, Calif.
[14] Dupont Instruments, Wilmington, Del.
[15] Metler Instruments Co., Zurich, Switzerland.

Table I—Effect of Action Spectrum of Light on the Color Change in IV (Powder) Exposed to Daylight Fluorescent Light over 0–96 hr

Light Filter Number	Light Intensity, f.c.[a]	Action Spectrum, nm	Transmitted Light	Color Change Compared with Control
58 and 2A-12	280	500–600 (λ_{max} 525)	Green	None
3 and 48	25	450–500 (λ_{max} 460)	Sky blue	None
7-60	5	300–400 (λ_{max} 360)	Violet	Bright yellow
8 and 5A	25	400–460 (λ_{max} 440)	Greenish blue	None
2A-15 and 23A	300	525–700 (λ_{max} 625)	Reddish orange	None
7-38 (silica)	900	340–700	Colorless	Bright yellow
405	15	380–430 (λ_{max} 410)	Violet blue	Pale yellow
>400 nm, UV protection Plexiglas[b]	900	>400 nm	White	None

[a] f.c. = foot-candles of light. [b] Ultra-violet Products, San Gabriel, Calif.

capsule. The mixture was shaken vigorously on a mechanical shaker[16] for 15 sec, and the powder mixture was transferred into a rectangular-shaped die mounted in a pellet press[17].

An IR spectrum on the sample disk referenced against a blank potassium bromide disk was obtained on a double-beam recording IR spectrophotometer[18]. The sample disk was then exposed to long wavelength UV light of 366 ± 40 nm for 1 hr, and another IR spectrum on the sample was obtained to note the spectral change after light exposure.

NMR Spectra—NMR spectra on samples of IV were recorded on a high-resolution NMR spectrometer[19]. The 10% solutions of IV were prepared in deuterated chloroform. The operating temperature probe was held at 37–40°, and 1% tetramethylsilane[18] solution was used as an internal standard.

GC Determination of IV—An in-house method[20] was adopted to identify the compound and its concentration before and after long wavelength UV light irradiation for 2 hr. Light-exposed and unexposed samples of IV were processed as follows.

Each sample (10 mg) was dissolved in 100 ml of spectrograde chloroform, and this stock solution was diluted to obtain a 10-μg/ml concentration of IV. Two microliters of this solution was injected into the GC column[21], which was maintained at 275°. Nitrogen, used as the carrier gas, was passed through the column at a rate of 48 ml/min; detection of the compound was achieved with a flame-ionization detector set at 250°[22].

Kinetic Studies on IV in Solid State—The objectives of these investigations were to: (*a*) identify the absorption spectrum in the visible light region attributable to the metastable (bright-yellow) state of IV, and (*b*) study the kinetics of the color conversion process as a function of temperature.

Approximately 0.5-mm thin transluscent disks from 125 mg of dry unexposed powder of IV were compressed with a 13-mm wide die and punch assembly[23]. The die cavity was filled with the powder, and the punch was placed in position. After 10 min of air evacuation from the die, an initial pressure of 25 tons was applied to the sample using a ring press[24]. The sample was then maintained under 10 tons of pressure for 5 min to obtain disks of good quality.

Two such disks were placed securely in the disk mounts[25]; one was used as a reference to obtain the visible absorption spectrum on the sample disk in the 700–370-nm range. All visible spectra were obtained on a double-beam recording spectrophotometer[26]. After initial scanning for the baseline, the sample disk was exposed on one side to long wavelength UV light of 366 ± 40 nm at 4000 μw/cm^2 intensity for 30 min and the absorption spectrum in the visible region was obtained. Care was exercised to maintain a constant geometry of both the sample and reference disks in their holders.

The sample disk was then placed in a constant-temperature oven at 50 or 70°. At predetermined time intervals, the sample assembly was withdrawn from the oven and cooled for 15 min and the absorption spectrum was recorded. Kinetic data were obtained in triplicate to derive the necessary kinetic parameters.

RESULTS AND DISCUSSION

Identification of Action Spectrum and Nature of Reaction—Experiments were designed to determine: (*a*) the band of wavelengths that induced a color change in the pure compound and (*b*) the progress of the reaction in the solid state. Table I illustrates the results of one study. It demonstrates that long wavelength UV light in the range of 300–400 nm (defined as the action spectrum) was predominantly responsible for inducing the color change. Light radiations above 400 nm were virtually ineffective.

Since transparent materials, such as Plexiglas, prevent the penetration of long wavelength UV light and protect the drug, it was suggested that their use to filter daylight fluorescent light can prevent a color change during large-scale manufacturing of any long wavelength UV light-sensitive compound. The progress of the reaction, as evidenced by color appearance in the light-exposed surface of the glass containers, was restricted to the surface area of light exposure. The possibility of nonradiative interparticulate energy transfer in the solid state was unlikely.

Physicochemical Aspects of Color Conversion—Samples of IV exposed to long wavelength UV light for 2 hr were analyzed by differential scanning calorimetry, IR (solid state), NMR, and UV (solution) spectroscopy methods. No detectable change was observed in the spectra or thermograms of either long wavelength UV-exposed or unexposed samples. Light-exposed samples that had turned yellow were subjected to elevated temperature storage at 70° for 2 hr, and the original color was restored. The UV spectrum (solution) and differential scanning calorimetry profile of this light- and heat-exposed sample were identical to those of the unexposed sample stored at room temperature.

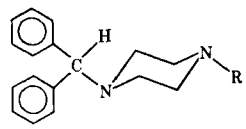
Furthermore, repetition of the light exposure/heat (70°)/light exposure cycle for five consecutive times did not produce any observable "fatigue" in the color change phenomenon of IV. GC analysis of these exposed and unexposed controls showed the same retention time (6.0 min). The potency of the irradiated material was also retained, as evidenced by comparison of the peak heights of control and irradiated samples at equivalent concentrations in the chromatographic solvent (chloroform). Also, the TLC profiles[27] of light-exposed and unexposed samples of IV were identical and showed no impurity spots on the developed plates.

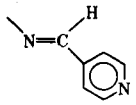
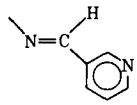
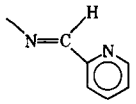
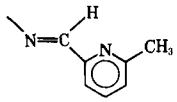
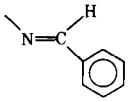
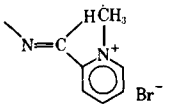
Effect of Long Wavelength UV Light on IV and Related Compounds: Possible Structure–Photochromic Activity Relationship—Photochromism refers to the ability of solid organic or inorganic compounds to undergo a transient color change on exposure to an action spectrum of light (2, 3). This color change is dependent on temperature and the duration and intensity of light exposure. However, dissolution, heat treatment, or prolonged storage in the dark

[16] Crescent Dental Manufacturing Co., Chicago, Ill.
[17] Maintenance Department, Searle Laboratories, Chicago, Ill.
[18] Model IR-12, Beckman Instruments, Fullerton, Calif.
[19] Varian Associates, Palo Alto, Calif.
[20] Mr. L. Kosobud, Bioanalytical Department, Searle Laboratories, Chicago, IL 60648, personal communication.
[21] OV-17 (1.5%) coated on Chromosorb-WH, Ohio Valley Speciality Chemical Co., Marietta, Ohio.
[22] Packard Instruments, Downers Grove, Ill.
[23] Beckman Instruments, Fullerton, Calif.
[24] Model RHC—D-01, Research & Industrial Instruments Co., London, England.
[25] Model INS-HV, Beckman Instruments, Fullerton, Calif.
[26] Acta CV double-beam recording spectrophotometer, Beckman Instruments, Fullerton, Calif.

[27] TLC analysis was performed with activated silica gel-coated glass plates. A compound sample (50 and 100 μg) dissolved in methanol was spotted and the plates were developed by the ascending technique, using benzene–ethanol–ammonium hydroxide (92.5:7:0.5) as the solvent system. Detection of the compound was done under long and short wavelength UV light.

Table II—Relationship between Some Physicochemical Parameters and the Effect of Long Wavelength UV Light Exposure on the Color of Substituted Benzhydrylpiperazines



Number	R	λ_{max} (Solution), nm	Melting Point (Differential Scanning Calorimetry)	Color[a]	
				Initial	Long Wavelength UV Light Exposed
I[b]		313	129°	Faint yellow	Faint yellow
II[c]		304	120°	Pale yellow	Pale yellow
III[d]		308	165°	Pale yellow	Bright yellow
IV[e]		310	137°	Faint yellow	Bright yellow
V[f]		297	135°	Colorless (white)	Colorless (white)
VI[g]		372	235°	Orange	Bright orange

[a] The color was compared to an unexposed control by visual inspection after 1 hr of irradiation of samples from the same lot. [b] SC-12757. [c] SC-13249. [d] SC-13300. [e] SC-13504. [f] SC-12123. [g] SC-21503.

at ambient temperature reverses this color change. Several organic compounds, including anils, semicarbazones, and hydrazones, exhibit photochromism in the solid state (2). Among semicarbazones, thiacetazone, an antitubercular drug, darkens in color when exposed to light (4).

To assess whether the heteroaromatic substitution (R) adjoining the hydrazone [>N—N=C(—H)R] had any effect on the light-induced color transformation process, several analogs of IV were examined for color change after exposure to long wavelength UV light for 1 hr (Table II). From these data, it is evident that *meta*- and *para*-substitution of the pyridyl moiety on the hydrazone inhibited the color change, suggesting that an electron resonance requirement was necessary for the change to occur. The time required for discoloration of III was prolonged over that necessary (about 1 min) for the discoloration of IV under similar conditions. Thus, a $+I$ (inductive) and, possibly, a hydrophobic effect of the methyl group appear to contribute to the stabilization of the light-activated intermediate.

Quaternization of the pyridyl nitrogen (VI) did not inhibit the color change significantly. However, the original color was restored in a relatively short time (about 24 hr). Except for V, which showed a shift of 7 nm toward shorter wavelengths, none of these compounds showed any significant alterations in their UV spectra after exposure to long wavelength UV light for 1 hr.

Possible Mechanisms for Photochromic Process—Longwave UV light-excited molecules of IV in the crystalline lattice are subjected to an electron delocalization process. The involvement of an *ortho*-substituted pyridyl nitrogen is likely since *meta*- and *para*-substituted analogs of IV (I and II) are relatively stable to long wavelength UV light irradiation. The melting points of the compounds studied (Table II) range from 120 to 235° and appear to show no correlation with the photochromic process. This finding suggests that crystal lattice differences between these six compounds are of minor importance.

A possible mechanism of this light-induced phenomenon is that the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ electronic transitions in the excited (singlet and/or triplet) state of the molecule may lead to a stabilized resonating structure between the pyridyl and the piperazyl nitrogen atoms (Scheme I). The proposed mechanism considers the steric and resonance requirements essential for a photochromic process. A molecular model, constructed to envision the feasibility of the proposed mechanism, indicated that an *anti–syn*-transformation around the hydrazone moiety in the light-excited state may allow close proximity of the piperazine and pyridyl nitrogen. Thus, either inter- or intramolecular electrostatic forces of the molecules on the light-penetrable surface of a crystal lattice can stabilize the resonating photochromic system to produce the metastable state. Therefore, the mechanism appears to be realistically attractive and can be considered proven only if and when a compound with a carbon in place of the nitrogen atom on the piperazyl ring remains the same in color after exposure to long wavelength UV light. No such compound was available to confirm this possibility.

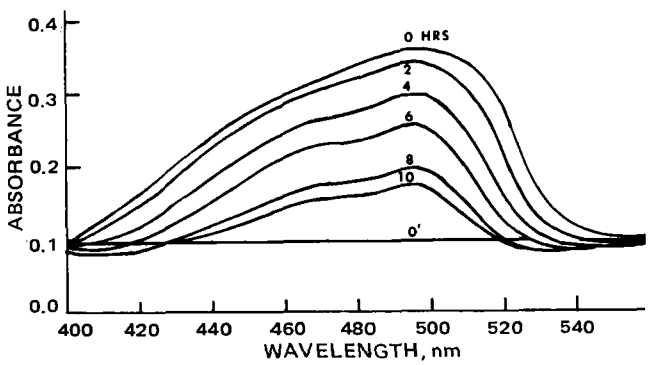
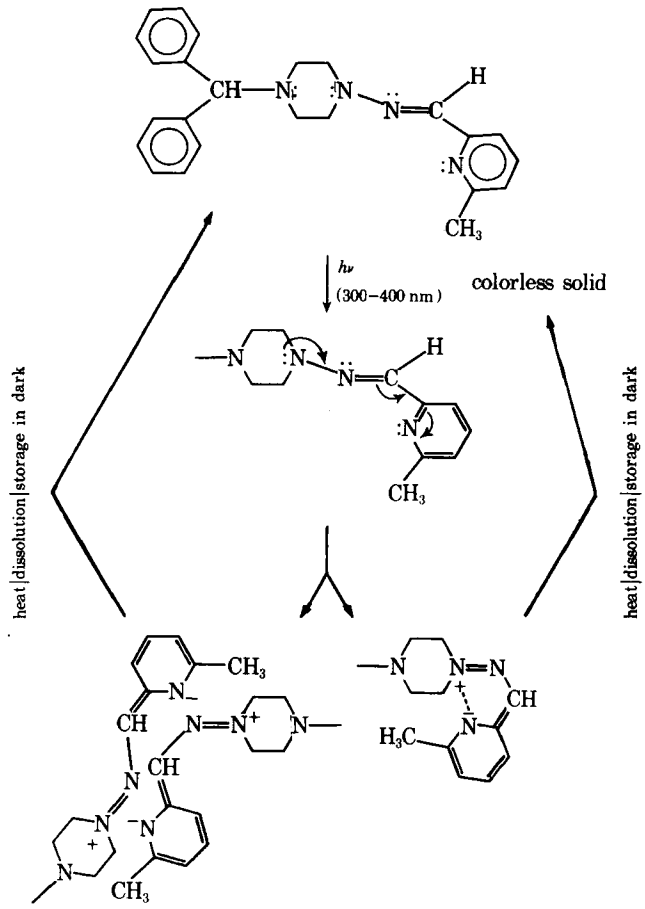


Figure 1—*Profile of visible absorption spectra obtained on a sample disk of IV prior to (0') and after (0) irradiation and successive periods of 2, 4, 6, 8, and 10 hr of heating at 50°.*



Scheme I—Mechanism for photochromism of IV

Compound VII did not produce any color change when exposed to light. The UV (solution) and IR spectra and the differential scanning calorimetry profiles of the light-exposed and unexposed samples of this compound were identical. The presence of a heavy atom, such as chlorine, in the vicinity of the resonating hydrazone moiety probably enhances the intercrossing of the excited-state electronic transitions, thus inhibiting the transformation of IV from the stable (colorless) state to a metastable (yellow) solid state. This conclusion appears consistent with the observed absence of photochromic activity of some chloro-substituted hydrazone derivatives (2).

Thus, it can be concluded that photochromism of IV in the solid state is an interesting, but known, property inherent in some hydrazone derivatives. Since the compound retains its physicochemical integrity in fluid solutions, its biological effects as a potent anticonvulsant compound in animals are most likely independent of its photochromic property in the solid state.

Kinetics of Photochromism of IV in Solid State—Figure 1 is a profile of visible absorption spectra obtained on a long wavelength UV-exposed disk of IV subjected to intermittent heat treatment at 50°. The absorption band at 500 nm (visible region) is attributed to the metastable photochromic (yellow) state. Such an assignment of the absorption spectrum is reasonable, since most yellow-colored compounds, such as riboflavin, bilirubin, and tartrazine yellow, absorb this bluish-green band of light. Furthermore, intermediates with an absorption maximum at 500 nm have been detected for a number of bioorganic reactions of pyridoxal and its derivatives with biogenic amines (5). Extension of the conjugation with the pyridyl moiety participating in such resonating structures has been proposed for the appearance of the colored intermediate (5, 6). Since the absorption maximum of the photochromic state of IV is at 494 nm (~500 nm), the possibility of an extended resonating structure lends credence to the proposed mechanism for the photochromism of IV (Scheme I).

Figure 2 is a zero-order plot of absorbance at 494 nm *versus* time of sample exposure to 50°. It illustrates the apparent zero-order kinetics of the photochromic transformation of IV from a metastable

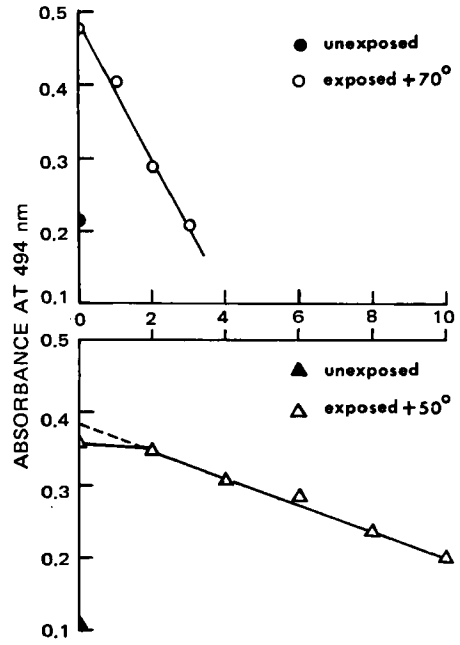


Figure 2—*Plots illustrating kinetics of photochromic conversion of IV from metastable (colored) to stable (colorless) solid state.*

(yellow) state to a stable (colorless) solid state at two defined temperatures. The rate constant for the photochromic conversion process is the slope of this plot at a given temperature. Equation 1 can be used to estimate the energy of activation, E_b, required for a reaction to proceed (7):

$$\log \frac{k_2}{k_1} = \frac{E_b}{2.303R} \frac{T_2 - T_1}{T_1 T_2} \qquad \text{(Eq. 1)}$$

where k_1 and k_2 are rate constants, T_1 and T_2 are temperatures, and R is the gas constant for the kinetic process occurring during the study of a reaction. With these parameters, the energy of activation, E_b, for the conversion of IV from a metastable (yellow) to a stable (colorless) solid state was estimated to be 19.0 kcal/mole.

Figure 3 is a proposed potential energy diagram illustrating the reversibility of the photochromic transformation of IV from a stable (colorless) state, *A*, to a metastable (yellow) state, *B*. For a reactant to go to a product *via* an intermediate requires energy and involves bond breaking processes (7). Also, in most cases, the product is at a lower energy level than the reactant. Since no bond breaking processes are involved in this case, as evidenced by the spectroscopy data, it is reasonable to expect that the metastable (yellow) state, *B*, of IV is at a higher energy level than its stable (colorless) state, *A*. Consequently, the estimated energy of activation (E_b = 19 kcal/mole) is assignable for the reversal of the metastable state, *B*, to the stable state, *A*, *via* an intermediate, X_3 (Fig. 3). This activation energy is within the range reported for photochromic systems (3). Light in the vicinity of 366 ± 40 nm provided efficient excitation energy transfer equivalent to about 80 kcal/mole (8). This was evident from the observation that

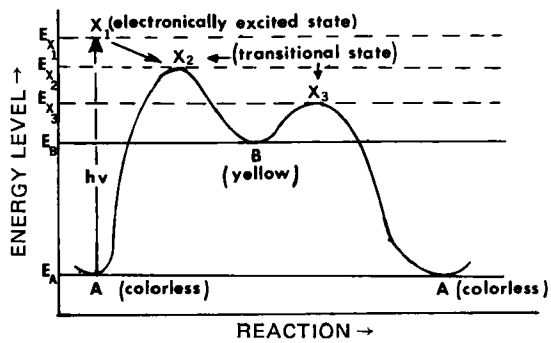


Figure 3—*Potential energy diagram illustrating the energy difference between the stable (A, colorless) and metastable (B, colored) solid state of IV; X is the transitional intermediate.*

an absorption band in the 400–550-nm region appeared within minutes after irradiation of the sample disk with long wavelength UV light of 366 ± 40 nm.

SUMMARY

Compound IV exhibited photochromism in the solid state. This light-induced color conversion process was typical of many hydrazone derivatives and did not affect the chemical nature or purity of the compound in solution. Conditions of elevated temperature, dissolution, and prolonged storage in the dark at ambient temperatures restored the original color of the compound. An action spectrum in the 300–400-nm range induced the change in color. A structure–photochromic activity relationship among several analogs of IV was observed, from which a possible mechanism for the photochromic conversion of IV from a stable to a metastable solid state was proposed.

The kinetics of photochromism of IV were apparently zero order for the return of the metastable (yellow) state to the stable (colorless) state. The energy of activation for this process was about 19 kcal/mole. The appearance and disappearance of an absorption band in the visible region, with a concomitant change in the color of the compound after exposure to long wavelength UV light, were evidence for the presence of a metastable state, which reverts back to the stable state of IV with heat treatment. These studies allowed novel and direct application of absorption spectrophotometry for determining the kinetics of photochromism in the solid state.

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ACKNOWLEDGMENTS AND ADDRESSES

Received October 14, 1975, from *Searle Laboratories, Division of G. D. Searle & Co., Chicago, IL 60680*

Accepted for publication December 8, 1975.

The author thanks Mrs. Iris A. Brener and Mr. Dale Chappelow for technical assistance. He also thanks Dr. E. LeVon, Dr. H. Dryden, Dr. G. Lenz, Dr. R. Bible, and Mr. A. Damascus for helpful discussions. The encouragement of Dr. H. J. Lambert and Dr. K. Rorig to study this phenomenon in depth is very much appreciated.

Absorption, Distribution, Metabolism, and Excretion of Furosemide in Dogs and Monkeys I: Analytical Methodology, Metabolism, and Urinary Excretion

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Abstract □ ^{35}S-Furosemide was administered to beagle dogs and rhesus monkeys in an oral solution on a single and a 20 repeated 5-mg/kg/day dosing regimen. Following the single dose, 25.0% (dogs) and 24.0% (monkeys) of the dose were excreted in the urine in 24 hr. TLC analysis demonstrated that both species had similar excretory patterns; *i.e.*, over 80% of the amount excreted in the urine was present as unchanged furosemide and the remainder was composed of a known metabolite, saluamine, and an as yet unidentified metabolite(s). The repetitive dosing regimen did not appear to alter significantly either the total amount recovered in the 24-hr urine or the excretion pattern. Studies in dogs showed that only 50–60% of furosemide was absorbed from oral solution. A significant biliary secretion elimination pathway for furosemide also was observed.

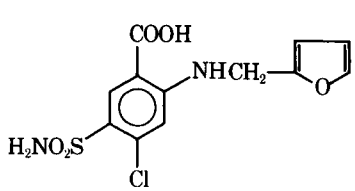
Keyphrases □ Furosemide and metabolite—TLC analysis, urine, dogs and monkeys □ TLC—analysis, furosemide and metabolite, urine, dogs and monkeys □ Excretion—furosemide, TLC analysis, urine, dogs and monkeys □ Pharmacokinetics—furosemide, dogs and monkeys compared □ Diuretics—furosemide, TLC analysis, urine, dogs and monkeys

Despite the widespread clinical use of diuretics in the treatment of edematous states and essential hypertension, there exist little published data on the comparative pharmacokinetics of these agents. This laboratory is involved in a major effort to elucidate the pharmacokinetic profiles of various diuretics and to compare the absorption, distribution, metabolism, and excretion patterns of these agents in several animal species including humans. The present work reports some findings with one diuretic, furosemide, in beagle dogs and rhesus monkeys.

Furosemide, 4-chloro-*N*-furfuryl-5-sulfamoylanthranilic acid (I), is a potent, orally effective diuretic. It exerts its major effect by inhibiting sodium reabsorption in the proximal convoluted tubule and the loop of Henle (1).

4-Chloro-5-sulfamoylanthranilic acid (saluamine) (II) was reported to be the major metabolite of furosemide in humans and dogs (2). Saluamine also was identified as a metabolite in a study on the distribution and urinary excretion of furosemide in the rat (3).

The purpose of the present studies was to determine



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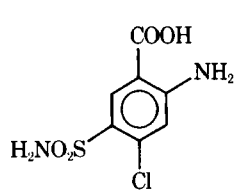

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