Biopharmaceutics of Drugs Administered in Lipid-Containing Dosage Forms I: GI Absorption of Griseofulvin from an Oil-in-Water Emulsion in the Rat

PHILIP J. CARRIGAN and THEODORE R. BATES▲

Abstract □ The GI absorption characteristics of micronized griseofulvin from an oil-in-water emulsion dosage form, an oil suspension, and an aqueous suspension were assessed in the rat. Although there was a slight delay in the time of occurrence of the peak plasma concentration of griseofulvin following its oral administration in the emulsion, this dosage form produced a mean peak plasma antibiotic level 1.5 and 2.3 times higher than the oil and aqueous suspensions, respectively. In addition, the emulsion dosage form significantly increased the time over which plasma concentrations of drug were maintained above 1 mcg./ml. All six rats dosed with the emulsion attained this 1-mcg./ml. plasma level or greater (mean duration of 7.5 hr.), while only two out of six rats dosed with the aqueous suspension (duration of 3.7 and 4.0 hr., respectively) and four out of six rats dosed with the oil suspension (mean duration of four rats of 3.9 hr.) ever achieved this plasma concentration. Based on area under the plasma concentration *versus* time curve measurements, it was established that the bioavailability of micronized griseofulvin can be increased 2.5-fold, as compared to an aqueous suspension, by orally administering this relatively water-insoluble antibiotic in an oil-in-water emulsion dosage form.

Keyphrases □ Griseofulvin (micronized) GI absorption profiles—oil-in-water emulsion compared to oil suspension and aqueous suspension, rats □ Absorption, GI, micronized griseofulvin—plasma concentration *versus* time curves, oil-in-water emulsion compared to oil and aqueous suspensions □ Lipid-containing dosage forms—GI absorption of micronized griseofulvin from oil-in-water emulsion, oil suspension, and aqueous suspension

Griseofulvin is a relatively water-insoluble, systemic, antifungal antibiotic, whose oral absorption has been shown to be dissolution rate limited (1). As a result, its absorption from the GI tract of lower animals (2, 3) and man (4–6) has been characterized as being erratic and incomplete. The rate and extent of absorption of this drug in man have also been shown to be dependent on particle size (7, 8), dosage form (1, 9, 10), and formulation factors (11, 12).

Several investigators demonstrated that the absorption of both regular and micronized griseofulvin in rats (13), guinea pigs (14), and man (5, 7, 15) could be markedly enhanced if coadministered with meals high in fat or triglyceride content. The application of these findings to the clinical treatment of fungal infections offers obvious advantages. However, to date, there are no commercially available dosage forms containing the drug and lipid. Since poor palatability and patient acceptance of an oil suspension dosage form is anticipated, it appears that an oil-in-water emulsion would be the best delivery system for this drug.

The GI absorption of drugs orally administered to intact animals in two- or three-phase, lipid-in-water emulsion dosage forms has received limited attention in the literature (16–21), and no studies have explored the various factors affecting drug release and absorption from such dosage forms. The emulsion dosage form is unique in that it represents the only conventional dosage form containing appreciable quantities of triglycerides or other lipids. The potential influence of the presence of lipid in the GI tract on the absorption of drugs (22) prompted the present preliminary investigation, which was designed to assess the absorption profiles of micronized griseofulvin following its oral administration to rats in the form of an aqueous suspension, a corn oil suspension, and a three-phase corn oil-in-water emulsion dosage form.

EXPERIMENTAL

Materials—The griseofulvin[1] employed in this study was the micronized form (specific surface area 1.32 $m.^2/g.$). Polysorbate[2] 60, mono- and diglycerides of edible fats[3] (I), and corn oil USP[4] were used as received. Anhydrous, reagent grade ether was distilled, immediately prior to use, from an all-glass still having a 47-cm. vigreaux column. Reagent grade anhydrous methanol was treated with activated charcoal[5] to remove fluorescent impurities; water was double distilled.

Dosage Forms—Three dosage forms were subjected to *in vivo* bioavailability testing. The first was an aqueous suspension containing 10 mg. of micronized griseofulvin and 10 mg. of polysorbate 60/ml. The second preparation was a corn oil suspension containing 25 mg. of micronized griseofulvin and 25 mg. of polysorbate 60/ml. The third dosage form was a three-phase oil-in-water emulsion containing 10 mg. of micronized griseofulvin, 10 mg. of polysorbate 60, 10 mg. of I, 0.4 ml. of corn oil, and a sufficient quantity of distilled water to make 1 ml. The emulsion was refined in a blender[6] and finally passed twice through a hand homogenizer[7]. The mean globule size of the internal (oil) phase of the resultant emulsion, as determined by a modification of the photomicrographic method of Singiser (23), was 5.2 μ in diameter (3.8 μ < 90% of globules < 9.7 μ).

Each test dosage form was assayed in duplicate for drug content by dissolving an aliquot of the preparation in ethanol and subsequently measuring its absorbance at 292 nm. in a recording spectrophotometer[8]. The resultant absorbance values were converted to concentration units with the aid of a Beer's law plot constructed over the 0–10-mcg./ml. range. The constituents of the three dosage forms, in the quantities present in the assay samples, were found not to interfere with the determination of griseofulvin.

***In Vivo* Absorption Studies**—Adult, male, Sprague–Dawley rats[9], weighing 250–300 g., were fasted for 20 hr. prior to, and for a 12-hr. period subsequent to, the initiation of the absorption experiments. However, they were allowed free access to water throughout the experiment. Each freshly prepared dosage form was administered by oral intubation to six rats at a constant griseofulvin dosage level of 50 mg./kg. body weight. The dosing volume employed for

[1] Supplied by Schering Corp., Bloomfield, NJ 07003
[2] Supplied as Tween 60 by ICI America, Inc., Atlas Chemical Division, Wilmington, DE 19899
[3] Supplied as Atmul 84 by ICI America, Inc., Atlas Chemical Division, Wilmington, DE 19899
[4] Amend Drug and Chemical Co., New York, NY 10010
[5] Norit, Fisher Scientific Co., Rochester, NY 14624
[6] Waring blender, model PB-5A, with Polytron Rotor-Stator, VWR Scientific, Rochester, NY 14603
[7] Model 33998-001, VWR Scientific, Rochester, NY 14603
[8] Beckman DB-G, Beckman Instruments Inc., Fullerton, CA 92634
[9] Obtained from Blue Spruce Farms, Altamont, NY 12009

Table I—Plasma Levels of Griseofulvin (Micrograms per Milliliter) following its Oral Administration as a Single 50-mg./kg. Dose in Three Test Dosage Forms

Dosage Form	Rat	Hours 1	2	4	6	8	12	24
Aqueous suspension	1	0.508	1.08	2.04	0.941	0.650	0.450	0.079
	2	0.263	0.527	0.714	0.213	0.276	0.196	0.109
	3	0.162	0.271	0.361	0.199	0.168	0.126	0.027
	4	0.822	1.31	1.08	0.940	0.661	0.469	0.112
	5	0.195	0.389	0.781	0.595	0.307	0.201	0.064
	6	0.237	0.427	0.383	0.245	0.130	0.215	0.058
	Mean (*SE*)[a]	0.364 (0.10)	0.667 (0.17)	0.892 (0.25)	0.522 (0.14)	0.365 (0.10)	0.276 (0.06)	0.075 (0.01)
Oil suspension	7	0.283	0.169	0.645	0.754	0.631	0.178	0.078
	8	0.250	0.359	0.782	0.886	0.681	0.554	0.107
	9	0.416	0.855	2.00	0.491	0.557	0.488	0.043
	10	0.211	0.442	1.08	0.973	0.616	0.246	0.086
	11	0.625	1.39	2.04	2.24	1.70	0.488	0.037
	12	0.363	0.904	1.25	0.779	0.646	0.536	0.031
	Mean (*SE*)[a]	0.358 (0.06)	0.686 (0.18)	1.30 (0.24)	1.02 (0.25)	0.805 (0.18)	0.415 (0.06)	0.063 (0.01)
Oil-in-water emulsion	13	0.313	0.606	1.73	1.84	1.51	0.761	0.055
	14	0.405	0.680	2.96	3.11	2.60	0.494	0.033
	15	0.265	0.737	1.65	1.75	1.78	1.40	0.100
	16	0.438	1.39	2.68	1.99	0.733	0.197	0.035
	17	0.389	1.10	2.15	1.90	0.974	0.315	0.066
	18	0.316	0.619	1.23	1.32	1.19	0.790	0.083
	Mean (*SE*)[a]	0.354 (0.03)	0.855 (0.13)	2.07 (0.27)	1.98 (0.24)	1.46 (0.27)	0.660 (0.18)	0.062 (0.01)

[a] Standard error of the mean in parentheses.

the aqueous suspension and emulsion dosage forms was 5.0 ml./kg. body weight. To maintain the volume of oil administered in the form of the corn oil suspension consistent with that of the emulsion dosage form, a dosing volume of 2.0 ml./kg. body weight was used. The animals were subsequently placed in restraining cages, and approximately 0.5-ml. blood samples were collected from the tail vein into heparinized tubes at 1, 2, 4, 6, 8, and 12 hr. postadministration. Twenty-four-hour samples were obtained *via* decapitation. The blood samples were then centrifuged, and a 0.2–0.3-ml. aliquot of the plasma obtained was accurately measured directly into glass-stoppered extraction vessels. A 2.0-ml. plasma sample was obtained at the 24-hr. time period. All plasma samples were immediately frozen solid and assayed for intact drug content within 48 hr. of collection.

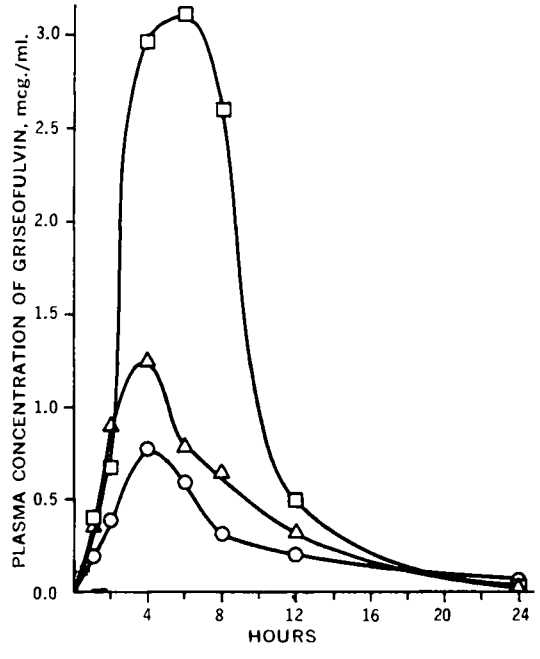


Figure 1—*Representative plasma concentrations of griseofulvin as a function of time following the oral administration of a 50-mg./kg. dose of micronized griseofulvin to rats in the form of an aqueous suspension* (○), *a corn oil suspension* (△), *and a corn oil-in-water emulsion* (□) *dosage form.*

Assay Procedure for Griseofulvin in Plasma—The specific spectrofluorometric procedure of Shah *et al.* (24) for plasma griseofulvin was appropriately modified for handling small plasma samples. The method essentially involves extracting griseofulvin from the plasma samples under neutral pH conditions into 7 ml. of freshly distilled ether and subsequently evaporating to dryness an aliquot (3–5 ml.) of the organic phase. The residue is then reconstituted with 2 ml. of a 1:1 (v/v) mixture of water and methanol and washed with 2 ml. of reagent grade hexane; the fluorescence of the hydroalcoholic phase was measured on a spectrofluorometer[10] using activation and emission wavelengths of 303 and 428 nm. (uncorrected), respectively. Subsequently, 4 drops of concentrated sulfuric acid was added to the sample and the fluorescence was redetermined.

On each assay day, a linear calibration curve of net fluorescence intensity (sample minus quench reading) *versus* concentration, over the range of 0.0071–0.143 mcg./ml. of hydroalcoholic solution, was constructed by carrying standard solutions of griseofulvin through the entire extraction procedure. The net fluorescence values of the extracted plasma samples were first corrected for the small positive intercept value of the calibration plot and then converted to concentration units by dividing the corrected net values by the least-squares slope value of the calibration plot.

Recovery studies performed with plasma samples containing 0.040–0.800 mcg. of griseofulvin indicated that the extraction procedure employed was capable of quantitatively removing the drug from plasma [mean recovery $\pm SD$ ($n = 6$) was $105 \pm 2.4\%$].

RESULTS AND DISCUSSION

To provide a basis for valid comparisons, control dosage forms employed in this *in vivo* absorption study (*i.e.*, aqueous and oil suspensions) were designed to incorporate certain components present in the emulsion dosage form. For example, both control dosage forms contained the same amount of nonionic surfactant (polysorbate 60) and the same particle-size fraction of drug. In addition, animals dosed with either the oil suspension or emulsion dosage form received an equivalent amount of triglyceride (corn oil).

Based on the results of equilibrium solubility studies performed at 25°, it was established that at the time of dosing a maximum of only 0.5, 2.4, and 2.7% of the orally administered dose of drug was

[10] Aminco-Bowman spectrophotofluorometer, American Instrument Co., Silver Spring, MD 20910

Table II—Peak Plasma Levels (C_{max}) and Its Time of Occurrence (T_{max}) following Oral Administration of Micronized Griseofulvin (50 mg./kg.) in Three Test Dosage Forms

	Aqueous Suspension		Oil Suspension		Oil-in-Water Emulsion	
	C_{max}, mcg./ml.	T_{max}, hr.	C_{max}, mcg./ml.	T_{max}, hr.	C_{max}, mcg./ml.	T_{max}, hr.
	1.31	2.0	2.24	6.0	3.11	6.0
	0.36	4.0	0.75	6.0	1.78	8.0
	0.71	4.0	1.25	4.0	2.15	4.0
	2.04	4.0	2.00	4.0	2.68	4.0
	0.78	4.0	1.08	4.0	1.84	6.0
	0.43	2.0	0.89	6.0	1.32	6.0
Mean (SE)[a]	0.94 (0.26)	3.3	1.37 (0.25)	5.0	2.15 (0.27)	5.7
Statistical significance[b]	N.S.		$p < 0.05$			
	$p < 0.02$					
Coefficient of variation, %	67.8	—	44.7	—	30.4	—

[a] Standard error of the mean in parentheses. [b] Determined by Student's t test.

in solution in the aqueous suspension, oil suspension, and emulsion dosage forms, respectively. Hence, differences encountered among the absorption characteristics of the drug from the three dosage forms cannot be attributed to this potential variable.

The results of *in vivo* absorption studies are summarized in Table I. Figure 1 depicts representative plasma griseofulvin concentration *versus* time plots obtained following the oral administration of a single 50-mg./kg. dose of micronized griseofulvin to individual rats in the aqueous suspension, oil suspension, and oil-in-water emulsion dosage forms. Examination of this figure, together with the mean data for the six animals dosed with each test preparation (Table II), reveals that a delay existed in the attainment of peak plasma drug levels following oral administration of both the oil suspension and emulsion as compared to the aqueous suspension (*i.e.*, 5–6 hr. *versus* 3 hr.). However, the magnitude of the mean peak plasma level of griseofulvin from the emulsion was approximately 1.5 and 2.3 times higher than from the oil and aqueous suspensions, respectively (Table II). These differences are highly significant as determined by Student's t tests performed on the mean data.

Griseofulvin blood levels greater than 1 mcg./ml. are required to effect a cure in human *Tinea capitis* infections (25). The same minimum effective blood level was also reported for dogs (26). If it is assumed that a similar value exists for the rat, an indication of differences among the dosage forms as to the duration of therapeutic levels may be realized. An interesting finding was that all six rats receiving the emulsion maintained a plasma level of 1 mcg./ml. or greater for approximately 6–11 hr. (mean duration of 7.5 hr.), while only two out of six rats dosed with the aqueous suspension (duration of 3.7 and 4.0 hr., respectively) and four out of six rats dosed with the oil suspension (mean duration of four rats of 3.9 hr.) ever achieved this proposed plasma level.

By using the trapezoidal rule, the area under the plasma griseofulvin level *versus* time curve from time zero to 24 hr. was determined for each animal and test preparation. The results of these area calculations, which reflect the amount of griseofulvin absorbed in 24 hr., are summarized in Table III. A one-way analysis of variance performed on the area under the plasma level–time curve for the three dosage forms yielded an F value of 10.4 (df 2,15) with $p < 0.005$. Individual differences between two treatments were tested statistically using Student's t test (Table III). As can be seen from an examination of the mean area under plasma level-time curve values, there is no statistical difference between the fraction of the oral dose of griseofulvin absorbed from the aqueous and oil suspensions. However, there is a highly significant 1.6- and 2.5-fold increase in the amount of drug absorbed from the emulsion dosage form as compared to the oil and aqueous suspensions, respectively. As reflected by the magnitudes of the coefficients of variation listed in Table II, the uniformity of absorption of micronized griseofulvin decreased in the following order: emulsion > oil suspension > aqueous suspension.

Table III—Bioavailability of Micronized Griseofulvin following Its Oral Administration as a Single 50-mg./kg. Dose in Three Test Dosage Forms

	Area under Plasma Level–Time Curve from Time Zero to Twenty-Four Hours (mcg.-hr./ml.)		
	Aqueous Suspension	Oil Suspension	Oil-in-Water Emulsion
	14.1	20.5	25.5
	3.36	9.32	16.0
	5.96	12.2	19.3
	13.2	12.5	25.3
	6.44	11.2	16.8
	4.59	7.12	15.9
Mean (SE)[a]	7.95 (1.87)	12.1 (1.86)	19.8 (1.85)
Statistical significance[b]	N.S.	$p < 0.02$	
	$p < 0.005$		
Coefficient of variation, %	57.4	37.6	22.8

[a] Standard error of the mean in parentheses. [b] Determined by Student's t test.

The physicochemical and/or physiological mechanism(s) by which the presence of triglyceride in the emulsion dosage form markedly enhances the absorption characteristics of griseofulvin, and possibly other poorly absorbed drugs, is currently under intensive investigation in these laboratories. Several possible physiological mechanisms being explored involve the influence of unemulsified and emulsified triglyceride (corn oil) on the rate of gastric emptying, GI motility, and bile flow. The results of these studies will be subsequently reported.

REFERENCES

(1) R. M. Atkinson, C. Bedford, K. J. Child, and E. G. Tomich, *Antibiot. Chemother.*, **12**, 232(1962).

(2) C. Bedford, D. Busfield, K. J. Child, I. MacGregor, P. Sutherland, and E. G. Tomich, *Arch. Dermatol.*, **81**, 735(1960).

(3) H. M. Sharpe and E. G. Tomich, *Toxicol. Appl. Pharmacol.*, **2**, 44(1960).

(4) E. G. McNall, *Antibiot. Ann.*, **7**, 674(1959–1960).

(5) R. G. Crounse, *J. Invest. Dermatol.*, **37**, 529(1961).

(6) A. Gonzalez-Ochoa and M. Ahumada-Padilla, *Arch. Dermatol.*, **81**, 833(1960).

(7) R. G. Crounse, *ibid.*, **87**, 176(1963).

(8) H. Blank, *Amer. J. Med.*, **39**, 831(1965).

(9) S. Riegelman, *Drug Inform. Bull.*, **3**, 59(1969).

(10) W. L. Chiou and S. Riegelman, *J. Pharm. Sci.*, **60**, 1376 (1971).

(11) B. Katchen and S. Symchowicz, *ibid.*, **56**, 1108(1967).

(12) S. Symchowicz and B. Katchen, *ibid.*, **57**, 1383(1968).

(13) M. Kraml, J. Dubuc, and D. Beall, *Can. J. Biochem. Physiol.*, **40**, 1449(1962).

(14) G. A. Greco, E. L. Moss, Jr., and E. J. Foley, *Antibiot. Ann.*, **7**, 663(1959 1960).

(15) P. Kabasakalian, M. Katz, B. Rosenkrantz, and E. Townley, *J. Pharm. Sci.*, **59**, 595(1970).

(16) S. E. Svenson, W. F. Delorenzo, R. Engelberg, M. Spooner, and L. O. Randall, *Antibiot. Med.*, **2**, 148(1956).

(17) C. W. Daeschner, W. R. Bell, P. C. Stivrins, E. M. Yow, and E. Townsend, *J. Dis. Child.*, **93**, 370(1957).

(18) L. A. Elson, B. C. V. Mitchley, A. J. Collings, and R. Schneider, *Eur. J. Clin. Biol. Res.*, **15**, 87(1970).
(19) L. Diamond, *Arch. Int. Pharmacodyn. Ther.*, **185**, 246 (1970).
(20) R. H. Engle and M. J. Fahrenbach, *Proc. Soc. Exp. Biol. Med.*, **129**, 772(1968).
(21) J. G. Wagner, E. S. Gerard, and D. G. Kaiser, *Clin. Pharmacol. Ther.*, **7**, 610(1966).
(22) T. R. Bates and M. Gibaldi, in "Current Concepts in the Pharmaceutical Sciences: Biopharmaceutics," J. Swarbrick, Ed., Lea & Febiger, Philadelphia, Pa., 1970, p. 95.
(23) R. E. Singiser, Ph.D. dissertation, University of Connecticut, Storrs, Conn., 1959.
(24) V. P. Shah, S. Riegelman, and W. L. Epstein., *J. Pharm. Sci.*, **61**, 635(1972).
(25) E. J. Grin and M. Denic, *Acta Med. Iugoslav.*, **19**, 53(1965).
(26) W. L. Chiou and S. Riegelman, *J. Pharm. Sci.*, **59**, 937(1970).

ACKNOWLEDGMENTS AND ADDRESSES

Received February 16, 1973, from the *Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14214*

Accepted for publication April 19, 1973.

Supported in part by a grant from the Harilian Corporation, Greenwich, CT 06830, and by General Research Support Grant FR5-501RR-05454-10 from the General Research Support Branch, Division of Research Facilities and Resources, National Institutes of Health, Bethesda, MD 20014

▲ To whom inquiries should be directed.

Photolytic Decomposition of *N*-(2,6-Dichloro-*m*-tolyl)anthranilic Acid (Meclofenamic Acid)

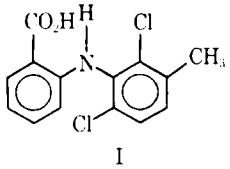
JOSE PHILIP▲ and DALE H. SZULCZEWSKI

Abstract □ Exposure of dilute solutions of *N*-(2,6-dichloro-*m*-tolyl)anthranilic acid to visible or UV light results in fairly rapid decomposition with concurrent formation of approximately equimolar amounts of 8-chloro-7-methylcarbazole-1-carboxylic and 8-chloro-5-methylcarbazole-1-carboxylic acids.

Keyphrases □ *N*-(2,6-Dichloro-*m*-tolyl)anthranilic acid (meclofenamic acid)—isolation, identification of photolytic decomposition products □ Meclofenamic acid [*N*-(2,6-dichloro-*m*-tolyl)anthranilic acid]—isolation, identification of photolytic decomposition products □ Photolysis, *N*-(2,6-dichloro-*m*-tolyl)anthranilic acid (meclofenamic acid)—isolation, identification of decomposition products

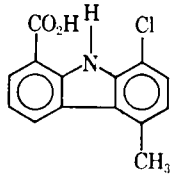
N-(2,6-Dichloro-*m*-tolyl)anthranilic acid (meclofenamic acid)[1], I, is an investigational new drug being evaluated for use as an anti-inflammatory agent. Results from previous studies in these laboratories indicated that one possible route of decomposition involved decarboxylation to the corresponding substituted diphenylamine in accord with the general chemistry of anthranilic acids. This reaction, however, proceeds at an appreciable rate only at elevated temperatures (100° for strongly acidic solutions or above the melting point, 258.5–259.5° in the case of the solid state) and would have little relevancy to dosage form shelflife at near ambient temperatures.

As part of continuing preformulation studies, the

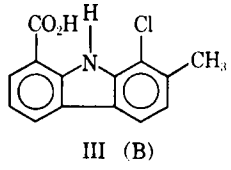


I

[1] Parke, Davis & Co.



II (A)



III (B)

influence of light, as another condition of stress, was studied.

DISCUSSION

Sequential UV spectra obtained during exposure of a dilute solution of I (c 0.00426%, methanol) to visible light[2] are shown in Fig. 1. There was a fairly rapid spectral change during the initial period of exposure, after which the spectra become essentially constant. Spectra obtained from aqueous alkaline solutions exposed in the same manner were qualitatively similar, whereas like solutions stored in the dark gave spectra invarient with time.

A thin-layer chromatogram obtained on a methanolic solution of I (R_f 0.7) exposed to the point of spectral redundancy showed the formation of at least two reaction products (1, R_f 0.4; and 2, R_f 0.31), one of which predominated. Available data indicate that I undergoes a facile reaction in the presence of light, but the nature of this reaction is unknown. Relevant information from the literature is rather scarce; however, the work of Bowen and Eland (1) indicated that diphenylamine is converted to carbazole in the presence of light.

To determine the nature of this reaction, reaction products were isolated for identification. A large volume of a more concentrated methanolic solution of I was exposed to UV light[3] (c 0.1%), and the solvent was removed under vacuum. The residue was recrystallized to remove residual I and the minor decomposition product. During the recrystallization, fractions were obtained whose R_f values were the same as the major decomposition product but whose

[2] Light cabinet (a steel cabinet equipped with several 100-w. incandescent bulbs).
[3] Hanovia Letheray germicidal lamps.