Methylene Blue Photosensitized Conversion of 3-Substituted Indoles to β-Carboline Derivatives

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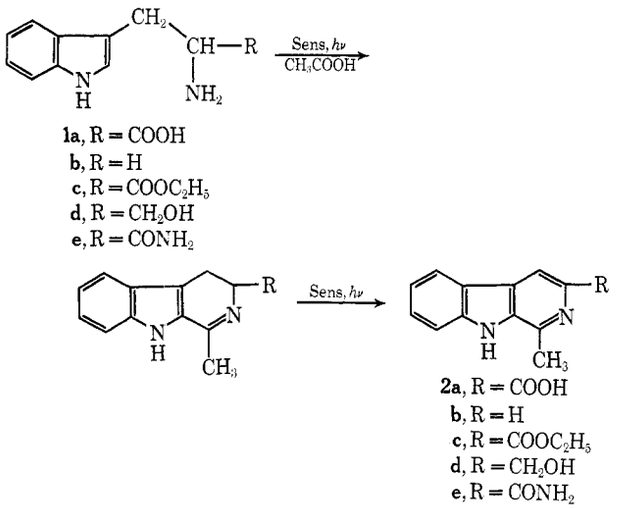
Indole derivatives substituted in the 3 position with a side chain bearing a free β-amino group undergo facile conversion to β-carbolines when a solution of the indole in a carboxylic acid is irradiated with visible light in the presence of methylene blue as the sensitizer. β-Carbolines variously substituted in the 1 and 3 position can be obtained by an appropriate choice of the solvent and indole side chain. The photoreaction appears to proceed through ·OH abstraction from the solvent molecule by photoexcited methylene blue, with formation of an acyl RĊ=O radical, which attacks the 2 position of the indole substrate. The 2-acyl derivative thus obtained undergoes a dark cyclization to β-carboline by a Schiff base formation between the carbonyl function and the side chain amino group. Finally, the dihydro-β-carboline is aromatized by methylene blue photosensitized dehydrogenation.

A previous note from this laboratory[1] described a novel photoreaction of tryptophan (**1a**), leading to the formation of β-carboline derivatives by irradiation of the amino acid with visible light in acetic acid solution and in the presence of methylene blue as the sensitizer. The reaction was found to proceed through a dihydro intermediate according to the following scheme.



By this photoprocess, high yields of 1-methyl-3-carboxy-β-carboline (**2a**) could be easily obtained, whereas the chemical synthesis of this and analogous compounds usually involves complex procedures, yielding relatively low amounts of the desired products.[2-4]

Owing to the importance of β-carbolines in the field of indole alkaloids, it appeared of interest to investigate the preparative and mechanistic features of the aforesaid photoreaction in greater detail. Moreover, on the basis of the reaction scheme outlined above, we explored the possibility of synthesizing differently substituted β-carbolines by changing the type of the solvent, as well as the nature of the side chain in the 3 position of the indole ring.

(1) G. Jori, G. Galiazzo, and G. Gennari, *Photochem. Photobiol.*, **9**, 179 (1969).
(2) R. Tschesche and H. Jenssen, *Chem. Ber.*, **93**, 271 (1960).
(3) H. R. Snyder, C. H. Hansch, L. Katz, S. M. Parmeter, and E. C. Spaeth, *J. Amer. Chem. Soc.*, **70**, 219 (1948).
(4) I. S. Spenser, *Can. J. Chem.*, **37**, 1851 (1959).

Results and Discussion

Irradiation of 3-Substituted Indoles in Acetic Acid Solutions.—The irradiation of the 3-substituted indoles listed in Table I, in either oxygen-saturated or deaerated acetic solutions, caused a gradual disappearance of the starting material, as determined by paper or thin layer chromatography. Concomitantly, new spots were detected which displayed a deep blue fluorescence under 254-mμ light and gave negative color tests with ninhydrin and with Ehrlich's reagent.[5] This strongly suggested that both the side chain amino group and the 2 position of the indole ring were masked as a consequence of the photoreaction. By analogy with our previous findings about the photocyclode hydrogenation process, which **1a** underwent when irradiated under the same conditions,[1] we inferred that the fluorescent products were β-carboline derivatives. This assignment was fully supported by elemental analysis and by spectroscopic characterization of the purified reaction products (see Experimental Section).

TABLE I
FORMATION OF β-CARBOLINES FROM IRRADIATED 3-SUBSTITUTED INDOLES[a]

Substrate	Product(s) obtained	Yield in the aerated solution, %	Yield in the deaerated solution, %
1a	**2a, 2b**	63, 16[b]	71, 24
1b	**2b**	75	93
1c	**2c**	75	95
1d	**2d**	69	87
1e	**2e**	65	88

[a] The irradiations were carried out at 25°, in acetic acid solution and in the presence of methylene blue as the sensitizer, using four 300-W tungsten lamps as the light source. The yields were calculated for the crystallized products. [b] Taken from ref 1.

It is noteworthy that, as shown in Table I, the yield of β-carbolines was enhanced by performing the irradiation in deaerated solutions. Since the primary step in the formation of β-carbolines involves the interaction between the solvent molecules and the triplet sensitizer (see later), the lower yields obtained upon irradiation in O_2-saturated solutions are probably due to the competition of molecular oxygen with acetic

(5) E. Stahl in "Dünnschicht Chromatographie," Springer-Verlag, West Berlin, 1962, p 503.

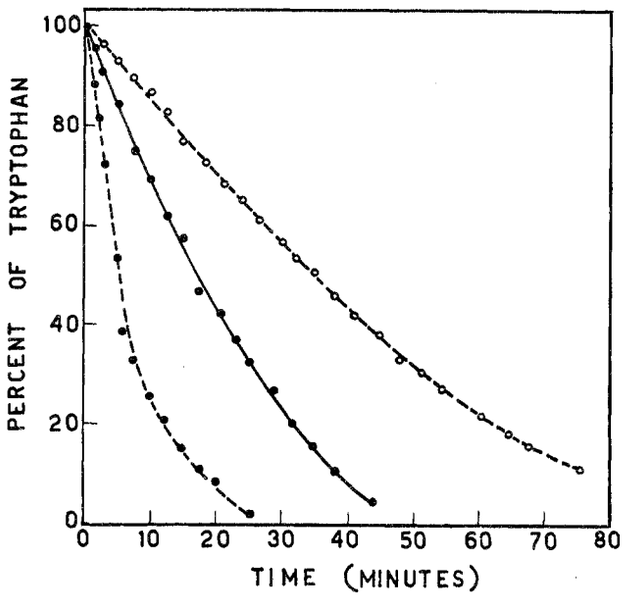


Figure 1.—The time course of tryptophan disappearance upon irradiation at 20° of a 1 m*M* deaerated solution of the amino acid in acetic acid (O---O), isobutyric acid (●—●), and pivalic acid (●---●), and in the presence of equimolar methylene blue as the photosensitizer. The plot obtained upon irradiation in propionic acid solution was closely similar to that shown for acetic acid.

acid for the photoexcited methylene blue. Actually, all the indole derivatives examined, when irradiated in the presence of O_2, gave appreciable amounts of insoluble material, which appeared by chromatographic and colorimetric analysis to be composed mainly of melanines of indole structure.[6] These compounds are known to be produced by photosensitized oxygenation of **1a**.[7]

The lack of side products when the photoreaction is carried out under nitrogen simplifies the experimental procedure for the isolation and purification of β-carbolines and renders this novel preparative approach even more valuable. It is apparent that the requirements to be fulfilled by the side chain for the cyclization to occur are the presence of two carbon atoms and of a free terminal amino group.[8] On the other hand, the yield of β-carbolines is practically independent of the type of the second substituent eventually present on the carbon bearing the NH_2 group (see Table I). This fact opens large possibilities of preparing new β-carbolines; for example, as far as we know, compounds **2d** and **2e** have not been isolated up to now. Moreover, the partial photolytic decarboxylation, which occurs when tryptophan is the starting material,[1] can be avoided by irradiation of tryptophan ethyl ester; the free 3-carboxy-β-carboline can be easily obtained by saponification of **2c**.

The decarboxylated product (**2b**) is quantitatively formed by irradiation of tryptamine.

Irradiation of Tryptophan in Other Acid Organic Solvents.—Changing the length and the branching of the hydrocarbon chain of the organic acid used as the solvent had no marked effect on the yield of the corresponding 1-alkyl-3-carboxy-β-carbolines (Table II).

(6) R. A. Nicolaus, *Gazz. Chim. Ital.*, **85**, 659 (1955).

(7) C. A. Benassi, E. Scoffone, G. Galiazzo, and G. Jori, *Photochem. Photobiol.*, **6**, 857 (1967).

(8) Prolonged irradiation of *N*-acetyltryptophan or of *N*-benzyloxycarbonyltryptophan under the same conditions gave no trace of β-carboline derivatives.

TABLE II

FORMATION OF β-CARBOLINES UPON IRRADIATION OF TRYPTOPHAN IN DIFFERENT SOLVENTS[a]

Solvent	Products formed	Yield, %
Acetic acid	1-Methyl-3-carboxy-β-carboline	71
	1-Methyl-β-carboline	24
Propionic acid	1-Ethyl-3-carboxy-β-carboline	70
	1-Ethyl-β-carboline	22
Isobutyric acid	1-Isopropyl-3-carboxy-β-carboline	75
	1-Isopropyl-β-carboline	23
Pivalic acid	1-*tert*-Butyl-3-carboxy-β-carboline	78
	1-*tert*-Butyl-β-carboline	12

[a] The irradiations were carried out in deaerated solution. All other conditions were the same as described in Table I.

In addition, small amounts of the decarboxylated compounds were constantly obtained. In all cases, the formation of decarboxylated products was avoided when tryptophan ethyl ester was the starting material. This allowed us to isolate the corresponding 1-alkyl-β-carbolines in almost quantitative yields.

The rate of conversion of **1a** to β-carbolines increased as the carbon atom adjacent to the carboxyl function of the organic acid changed from primary to secondary and to tertiary (Figure 1). Conversely, no photoreaction of **1a** occurred when the irradiations were run in formic acid or in trichloroacetic acid solution. It thus appears that the presence of electron-donating substituents near the COOH group in the solvent molecule favors the photocyclization process, whereas the reaction is inhibited by the presence of electron-withdrawing groups.

The Reaction Mechanism. A. The Attacking Species.—The stoichiometry of β-carboline formation, as well as the accelerating power of electron-donating groups in the solvent molecule, can be reasonably interpreted by hypothesizing that the organic acid attacks the indole derivatives either as an acylium cation, $R-\overset{+}{C}=O$, or as an acyl radical, $R-\dot{C}=O$. The ability of acetyl radicals to promote the conversion of **1a** to β-carbolines is demonstrated by the following experiment.

Paper chromatographic analysis of 2 m*M* solutions of **1a** in 25% aqueous acetone, which had been irradiated with 254-mμ light (Mineralight lamp), in a N_2 atmosphere, showed that, besides other unidentified products, appreciable amounts of **2a** were produced. Since, under our conditions, almost all of the incident light was absorbed by acetone, it appears reasonable to infer that **2a** is formed by attack from the acetyl radicals deriving from the photolysis of acetone.[9] Furthermore, the presence of radical intermediates in the methylene blue sensitized process can be deduced by the inhibitory effect displayed by radical scavengers, such as hydroquinone.[10]

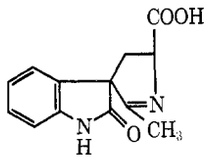
The photoreaction was also inhibited by skatole (3-methylindole). After 1 hr irradiation of an equimolar mixture of skatole and **1a** in deaerated acetic acid, the yield of β-carboline dropped from about 70%

(9) M. I. Christie, J. M. Collins, and M. A. Voisey, *Trans. Faraday Soc.*, **61**, 462 (1965).

(10) In the presence of 10 m*M* hydroquinone, prolonged irradiation caused no detectable modification of tryptophan.

to 22%. Irradiation of skatole alone under the same conditions gave an over 70% yield of 2-acetyl skatole. These findings strongly support the hypothesis that the attacking species is the R–Ċ=O radical, which should originate by an interaction between the photoexcited sensitizer[11] and acetic acid. The detailed features of such interaction are not yet fully understood. A possible reaction pathway involves the photolytic detachment of one $\cdot N(CH_3)_2$ radical from methylene blue and the subsequent attack of the dye radical on acetic acid, leading to the R–Ċ=O species and 3(or 6)-dimethylamino-6(or 3)-hydroxyphenthiazine. Indeed, prolonged irradiation of methylene blue alone in deaerated acetic acid solution led to a major product, whose elemental analysis corresponded to a derivative of methylene blue in which one $N(CH_3)_2$ group has been replaced by an OH group. Moreover, the infrared spectrum of the compound clearly showed the presence of the OH function. The presence of deaminated species among the products of the photobleaching of methylene blue was postulated by Obata.[12]

B. The Position of Attack on Tryptophan.—Charge density calculations[13] indicate that the 2 and 3 positions of the indole ring are the most suitable for an electrophilic attack. The experimental evidence supports this conclusion.[14–16] In our case, it is likely that the radical attack occurs in the 2 position. Actually, 2-hydroxytryptophan failed to undergo any reaction when irradiated under the same conditions as used for tryptophan. If the 3 position had been the site of attack by R–Ċ=O, the spirocycloindolenine **3** should



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be formed, analogously to what has been observed by other authors[17] for tryptophan derivatives masked in the 2 position. Further evidence supporting our hypothesis is provided by the unique formation of 2-acetylskatole upon methylene blue sensitized irradiation of skatole in deaerated acetic acid solution. On the other hand, the possibility of formation of *N*-acetyltryptophan as an intermediate is ruled out by the failure of this compound to undergo photocyclization to β-carbolines.[8]

A definite demonstration of the intermediate formation of 2-acetyltryptophan was achieved by irradiation of 1 m*M* *N*-*tert*-butoxycarbonyl-L-tryptophan ethyl ester in 80% acetic acid solution, where this compound is quite stable in the dark. After 3 hr of irradiation a photoproduct was isolated which gave a negative response to a colorimetric test with the Ehrlich reagent, showing that the 2 position was masked; its elemental analysis and ir spectrum were consistent with the presence of a $COCH_3$ group. After removal of the *tert*-butoxycarbonyl group by treatment with HCl, the expected **2c** was isolated.

Having established the initial formation of 2-acetyltryptophan, the subsequent step must be the formation of a dihydro-β-carboline by a dark condensation between the side chain NH_2 group and the acetyl group.

C. The Dehydrogenation of the Dihydro-β-carbolines.—Methylene blue can photosensitize the dehydrogenation of the intermediate dihydro-β-carbolines. Actually, we found that, upon irradiation of the dihydro derivative either in acetic acid or in aqueous solution, **2a** is formed with a concomitant enhancement of the photobleaching of methylene blue, which is probably converted to the leuco dye;[18] in particular, after 3 hr of irradiation in deaerated aqueous solution, the presence of 1 m*M* dihydro-β-carboline increased the amount of photobleached dye from 5.7 to about 23%, as deduced from absorbance measurements at 665 mμ. On the other hand, even after prolonged irradiation in the presence of O_2, practically no decoloration of methylene blue was observed. This is in agreement with the well known reversibility of the conversion of this dye to the leuco form.[18]

It is noteworthy that the photodehydrogenation of the dihydro intermediates was efficiently sensitized also by the aforesaid hydroxy derivative of methylene blue. This process may become important in the later stages of the photoreaction.

Experimental Section

All melting points were determined with Kofler micro hot stage and are uncorrected. Spectra were measured with a Cary Model 15 spectrophotometer and with a Perkin-Elmer 317 grating infrared spectrophotometer.

Thin layer chromatography was performed on silica gel coated plates (ascending technique) using the mixture 1-butanol–water–acetic acid (80:20:20, v/v/v) as eluent; paper chromatograms were run on Whatman No. 1 paper (descending technique), using 5% ammonia (solvent 1) and the Partridge mixture (solvent 2) as eluents.

The time course of tryptophan photodegradation was followed by the spectrophotometric procedure detailed elsewhere.[19] The indole derivatives were obtained from Fluka and appeared to be homogeneous by tlc in different solvents. Methylene blue was purchased from Merck; the organic acids were products of Carlo Erba and were distilled under reduced pressure before use. 2-Hydroxytryptophan was kindly supplied by Dr. A. Fontana of this Institute.

Analytical Scale Irradiations.—In all cases, 4 ml of a 1 m*M* substrate solution, added in the dark with an equimolar amount of sensitizer, was introduced into Pyrex test tubes (1.2 × 12 cm) and exposed to the light of four 300-W tungsten lamps, using the same experimental conditions as previously described.[19] The temperature was maintained at 20 ± 1° by circulating water. The solutions were deaerated by bubbling ultrapure N_2 for at least 15 min prior to and during illumination. In some experiments, the irradiations were performed in the presence of hydroquinone or ferrous sulfate in tenfold molar excess over the substrate.

Photocyclodehydrogenation of 3-Substituted Indoles in Acetic Acid.—In a 300-ml Pyrex cylinder was placed 0.5 mmol of substrate, plus an equimolar amount of methylene blue and 250 ml of acetic acid. The vessel was placed in a water bath at 20 ± 1° and irradiated by means of the lamp system described above, at a

(11) The conversion of tryptophan to β-carbolines is completely inhibited by running the irradiation in the presence of Fe^{2+} ions. Since paramagnetic species are known to quench the excited triplet states by enhancing the spin–orbit coupling, this fact suggests that the triplet state of the dye is the reactive intermediate.

(12) H. Obata, *Bull. Chem. Soc. Jap.*, **34**, 1057 (1961).

(13) W. E. Kurtin and P. S. Song, *Photochem. Photobiol.*, **7**, 263 (1968).

(14) A. H. Jackson and P. Smith, *Tetrahedron*, **24**, 2227 (1968).

(15) A. H. Jackson, B. Naidoo, and P. Smith, *Tetrahedron*, **24**, 6119 (1968).

(16) E. Scoffone, A. Fontana, and R. Rocchi, *Biochemistry*, **7**, 971 (1968).

(17) A. H. Jackson and P. Smith, *Tetrahedron*, **24**, 403 (1968).

(18) Y. Usui, H. Obata, and M. Koizumi, *Bull. Chem. Soc. Jap.*, **34**, 1049 (1961).

(19) G. Jori, G. Galiazzo, A. Marzotto, and E. Scoffone, *Biochim. Biophys. Acta*, **154**, 1 (1968).

distance of 40 cm. Deaeration of the solutions was achieved by thoroughly flushing ultrapure N_2 through three porous disks at the bottom of the vessel. When tlc showed total disappearance of the original compound, the irradiation was stopped, the solvent was removed by lyophilization, and the residue was taken up with water and loaded on a column (1.3 × 50 cm) of a carboxylic resin (Amberlite CG-50, 200–400 mesh). The column was eluted with 2% ammonia; 2-ml fractions were collected, which were analyzed by tlc or paper chromatography. In the case of the solutions irradiated in the presence of oxygen, prior to loading on the column, a trace of insoluble material was removed by centrifugation; the residual solution was further purified by preparative paper chromatography,[20] using 5% ammonia as eluent. The blue fluorescent strip was eluted with absolute methanol and taken to dryness.

In the case of tryptamine, one fraction was obtained from the column (R_{f_1} 0.11, R_{f_2} 0.32); crystallization from 80% methanol gave white needles of 1-methyl-β-carboline (**2b**), 64.5 mg (75%) for solutions irradiated in O_2, 80 mg (93%) after irradiations in N_2. The identification of the product was achieved by mixture melting point and by uv and ir comparison with a sample of **2b** prepared independently.[1]

Anal. Calcd for $C_{12}H_{10}N_2$: C, 79.09; H, 5.48; N, 15.68. Found: C, 79.12; H, 5.50; N, 15.62.

In the case of tryptophan ethyl ester, one fraction was again isolated (R_{f_1} 0.18, R_{f_2} 0.42), which was found, after crystallization from absolute methanol, to be 1-methyl-3-ethoxycarbonyl-β-carboline (**2c**) (95.2 mg, 75% after irradiation in O_2; 110.7 mg, 95% after irradiations under N_2): mp 248–249°; uv max (95% methanol) 234 mμ (log ε 4.581), 268.5 (4.202), 334.5 (3.598), 347 (3.605); ir (KBr) 1720 (aryl ester C=O), 1250 and 1120 cm^{-1} (aryl ester CO).

Saponification of **2c** by the method of Tschesche, *et al.*,[21] yielded the free acid (**2a**, yield 85%), identified by mixture melting point and by uv and ir comparison with a sample of authentic **2a**.

Anal. Calcd for $C_{15}H_{14}N_2O_2$: C, 70.81; H, 5.83; N, 5.52. Found: C, 71.02; H, 5.97; N, 5.42.

In the case of tryptophanol, the isolated product (R_{f_1} 0.14, R_{f_2} 0.59) was identified as 1-methyl-3-hydroxymethyl-β-carboline (**2d**): 73.2 mg, 69% after irradiation in O_2; 88.3 mg, 87% after irradiation under N_2; mp 263–265°; uv max (95% methanol) 212 mμ (log ε 4.537), 236 (4.477), 277.5 (4.316), 329 (3.617), 345 (3.588); ir (KBr) 3360 (broad, OH) 1050 cm^{-1} (broad, primary OH).

Anal. Calcd for $C_{13}H_{12}N_2O$: C, 73.49; H, 5.66; N, 13.42. Found: C, 73.22; H, 5.78; N, 13.36.

In the case of tryptophanamide, the isolated product (R_{f_1} 0.36, R_{f_2} 0.44) was found to be 1-methyl-3-carbamoyl-β-carboline (**2e**) after crystallization from 80% methanol: 73.1 mg, 65% after irradiation in O_2; 99 mg, 88% after irradiation under N_2; mp 235–237°; uv max (95% methanol) 228 mμ (log ε 4.762), 260.5 (4.573), 288 (4.105), 321 (3.276), 353 (3.421); ir (KBr) 3330 and 3190 (medium, NH), 1645 (amide I), 1630 cm^{-1} (amide II).

Anal. Calcd for $C_{13}H_{11}N_3O$: C, 69.35; H, 4.88; N, 18.67. Found: C, 69.48; H, 4.65; N, 18.72.

Photochemical Conversion of Tryptophan to 1-Alkyl-3-carboxy-β-carbolines.—In a typical experiment, 51 mg (0.25 mmol) of tryptophan was dissolved in 250 ml of the appropriate solvent and mixed with an equimolar amount of methylene blue. The solutions were deaerated and irradiated by the experimental arrangement previously described. When all the tryptophan had reacted, as shown by tlc, the irradiation was stopped and the solvent was removed by rotary evaporation and by repeated lyophilization. The residue was taken up with the minimal amount of water and loaded on a column of Amberlite CG-50 (0.9 × 55 cm).

In the case of irradiation in acetic acid solution, elution of the column with water gave one product (40.7 mg, yield 71%), which was identified as 1-methyl-3-carboxy-β-carboline on the basis of elemental analysis and chromatographic R_f values[1] as well as by mixture melting point and uv and ir comparison with a sample of the same product previously prepared.[1] Subsequent elution from the column with 3% ammonia gave a second fraction (10.9 mg, yield 24%), which appeared by elemental, chromatographic, and spectroscopic analysis to be identical with a sample of authentic 1-methyl-β-carboline.[1]

After irradiation in propionic acid solution, elution from the column with water gave one fraction which, after crystallization from 80% methanol, was identified as 1-ethyl-3-carboxy-β-carboline: 47 mg, 70%; mp 295–297°. The melting point, as well as the uv and ir spectra, were coincident with those of a sample of the same product prepared from tryptophan according to Tschesche, *et al.*[2,21]

Anal. Calcd for $C_{14}H_{12}N_2O_2$: C, 70.20; H, 5.03; N, 11.59. Found: C, 69.70; H, 5.10; N, 11.55.

Subsequent elution with 3% ammonia gave a second fraction, which was crystallized from methanol and identified as 1-ethyl-β-carboline: 10.8 mg, 22%; mp 244–246°; the uv and ir spectrum were coincident with a sample of the same product prepared from tryptamine by a chemical procedure.[2]

Anal. Calcd for $C_{13}H_{12}N_2$: C, 78.6; H, 6.18; N, 13.98. Found: C, 78.9; H, 6.15; N, 14.15.

After irradiation of tryptophan in the presence of isobutyrric acid as the solvent, elution from Amberlite with water gave one product (R_{f_1} 0.73, R_{f_2} 0.50) which, after crystallization from 80% methanol, was identified as 1-isopropyl-3-carboxy-β-carboline: 47.5 mg, 75%; mp 277–278°; uv max (95% methanol) 232 mμ (log ε 4.603), 279 (4.326), 331 (3.602), 345 (3.613); ir (KBr) 1685 (aryl C=O), 1385 and 1370 cm^{-1} (strong, isopropyl C–H bending).

Anal. Calcd for $C_{15}H_{14}N_2O_2$: C, 70.82; H, 5.55; N, 11.02. Found: C, 70.23; H, 5.62; N, 11.20.

A second fraction (R_{f_1} 0.33, R_{f_2} 0.38) was isolated by elution with 3% ammonia. Crystallization with 80% methanol gave pale yellow needles of 1-isopropyl-β-carboline: 12.6 mg, 23%; mp 225–228°; uv max (95% methanol) 212 mμ (log ε 4.803), 268 (3.992), 307 (2.654), 360 (2.812); ir (KBr) 3345 (indole NH), 1380 and 1370 cm^{-1} (isopropyl C–H bending).

Anal. Calcd for $C_{14}H_{14}N_2$: C, 79.95; H, 6.71; N, 13.33. Found: C, 79.73; H, 6.70; N, 13.42.

Finally, after irradiation in pivalic acid solution, one fraction (R_{f_1} 0.78, R_{f_2} 0.57) was isolated by elution with water; it was crystallized from 80% methanol and identified as 1-*tert*-butyl-carboxy-β-carboline: 52.3 mg, 78%; mp 293–295°; uv max (methanol) 227 mμ (log ε 4.715), 282 (4.22), 332 (3.487), 348 (3.608); ir (KBr) 1685 (aryl C=O), 1390 and 1365 cm^{-1} (weak, *tert*-butyl C–H bending).

Anal. Calcd for $C_{16}H_{16}N_2O_2$: C, 71.68; H, 6.34; N, 10.45. Found: C, 71.35; H, 6.35; N, 10.62.

Elution with 3% ammonia yielded a second product (R_{f_1} 0.18, R_{f_2} 0.25), which after crystallization from 80% methanol was shown to be 1-*tert*-butyl-β-carboline: 6.7 mg, 12%; mp 208–211°; uv max (methanol) 208 mμ (log ε 4.77), 271.5 (4.082), 310 (2.835), 353 (2.874); ir (KBr) 3350 (indole NH), 1385 and 1360 cm^{-1} (*tert*-butyl C–H bending).

Anal. Calcd for $C_{15}H_{16}N_2$: C, 80.04; H, 7.14; N, 12.5. Found: C, 79.95; H, 7.08; N, 12.35.

Irradiation of *tert*-Butoxycarbonyl-L-tryptophan Ethyl Ester in 80% Acetic Acid Solution.—The apparatus described above was used to irradiate, under N_2, 20 mg of *tert*-butoxycarbonyl-L-tryptophan ethyl ester and 25 mg of methylene blue dissolved in 40 ml of 80% acetic acid. After 5 hr of illumination, the solvent was removed by lyophilization. Chromatography of the residue on a column of Amberlite CG-50 (see above) yielded 18 mg of one product, which gave a negative color test with the Ehrlich reagent,[5] showing that the 2 position of the indole ring was masked. The ir spectrum (Nujol) showed, besides the peak at 1730 cm^{-1} (aliphatic ester C=O), the presence of a band at 1680 cm^{-1}, which can be assigned to a ketonic –C=O conjugated with an aromatic system. The elemental analysis of the product was in agreement with the expected one for 2-acetyl-*tert*-butoxycarbonyl-L-tryptophan ethyl ester.

Anal. Calcd for $C_{20}H_{26}N_2O_5$: C, 64.17; H, 6.95; N, 7.48. Found: C, 63.98; H, 6.80; N, 7.51.

Removal of the *tert*-butoxycarbonyl group by treating the product (10 mg) with 2 *N* HCl for 3 hr at 50° yielded a new product, whose chromatographic and spectral features were coincident with those found for 1-methyl-3-ethoxycarbonyl-β-carboline (**2c**)

Irradiation of Skatole in Acetic Acid Solution.—The apparatus described above was used to irradiate, under N_2, 20.8 mg of skatole and 32 mg of methylene blue dissolved in 40 ml of acetic acid. After 3 hr of illumination, tlc showed that skatole (R_{f_2} 0.95) was almost completely converted to one product (R_{f_2} 0.83), which

(20) G. Jori, G. Gennari, G. Toniolo, and E. Scoffone, *J. Mol. Biol.*, in press.

(21) R. Tschesche, H. Jenssen, and P. M. Rangachari, *Chem. Ber.*, **91**, 1732 (1958).

gave negative color tests with the Ehrlich reagent,[5] suggesting that the 2 position of the indole moiety was masked. The product was isolated by chromatographing the lyophilized residue on a column (1.0 × 42 cm) of the sulfonic resin Dowex-50, using 1% ammonia as eluent: 21.2 mg, 72.7%; the ir spectrum (KBr) gave a peak at 1690 cm^{-1}, clearly showing the presence of a carbonyl group conjugated with an aromatic system; the elemental analysis was in agreement with the expected one for 2-acetylskatole.

Anal. Calcd for $C_{11}H_{11}NO$: C, 74.68; H, 6.79; N, 8.64. Found: C, 74.19; H, 6.85; N, 8.65.

Irradiation of Methylene Blue in Acetic Acid Solution.—In one experiment, 100 mg of methylene blue in 250 ml of acetic acid were irradiated for 40 hr under N_2 by the apparatus previously described. Tlc analysis showed that methylene blue was slowly converted to three products. The major product (R_{f_2} 0.65) was isolated by chromatographing the lyophilized irradiated mixture on an alumina column (2 × 50 cm), using a 1:1 (v/v) CH_3OH–$CHCl_3$ mixture as eluent. The visible absorption maximum of the product in chloroform was located at 625 mμ; such a blue shift with respect to methylene blue (absorption maximum 637 mμ) was observed by Obata[12] for hydroxyphenthiazines. The ir spectrum (KBr) differed from that of methylene blue for the presence of a broadened band at 3340 cm^{-1} and of a doublet at 1200 cm^{-1}, as it is typical of phenolic OH. On the basis of these evidences, we tentatively identify the product as 3(or 6)-hydroxy-6(or 3)-dimethylaminophenothiazine.

Anal. Calcd for $C_{14}H_{14}N_2SO$: C, 61.84; H, 5.58; N, 12.02; S, 13.75. Found: C, 61.73; H, 5.55; N, 12.10; S, 13.69.

Registry No.—**2a**, 22329-38-0; **2b**, 486-84-0; **2c**, 33821-71-5; **2d**, 33821-72-6; **2e**, 23256-12-4; tryptophan, 6159-33-7; 1-ethyl-3-carboxy-β-carboline, 33821-74-8; 1-ethyl-β-carboline, 20127-61-1; 1-isopropyl-3-carboxy-β-carboline, 33821-76-0; 1-isopropyl-β-carboline, 22314-95-0; 1-*tert*-butyl-3-carboxy-β-carboline, 33821-78-2; 1-*tert*-butyl-β-carboline, 33821-79-3; 2-acetyl-*tert*-butoxycarbonyl-L-tryptophan ethyl ester, 33821-80-6; 2-acetylskatole, 16244-23-8; methylene blue, 61-73-4; 3-hydroxy-6-dimethylaminophenthiazine, 33821-82-8; 6-hydroxy-3-dimethylaminophenthiazine, 33821-83-9.

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Mechanism and Catalysis for Furfural Phenylhydrazone Formation[1]

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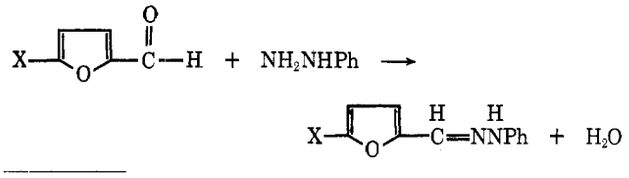
As is typical for the addition of amines to carbonyl compounds, the reaction of 5-substituted furfurals with phenylhydrazine exhibits rate-determining attack of the nucleophile under acidic conditions and rate-determining decomposition of the carbinolamine intermediate under neutral and basic conditions. The attack of phenylhydrazine on these substrates is subject to general acid catalysis by carboxylic acids, the Brønsted exponent $\alpha = 0.35$. Dehydration of the carbinolamine intermediates occurs *via* acid-catalyzed, pH-independent, and, in the case of the nitro derivative, base-catalyzed reaction pathways.

The principal features of the mechanisms for addition of weakly basic amines to carbonyl compounds[2,3] have been derived from a series of studies employing simple aliphatic and aromatic aldehydes and ketones.[4-12] Both to broaden the basis upon which our conclusions are founded and to explore substituent effects in heterocyclic aromatic systems, the kinetics of 5-substituted furfural phenylhydrazone formation have been investigated. While this study has, for the most part, reinforced previous conclusions, some interesting differences in detail do appear. The results are presented below.



Experimental Section

Materials.—5-Methylfurfural was prepared according to the method of Rinkes,[13] bp 84–86° (3 mm) [lit.[13] bp 83–85° (15 mm)]. The product is stable for several months at −10° in the absence of light and oxygen. Dilute solutions in 20% aqueous ethanol were found to be stable for several days at 0° in the dark. 5-Bromofurfural, mp 84° (lit.[14] mp 82°), and 5-nitrofurfural, mp 35–36° (lit.[15] mp 35–36°), were also prepared according to published procedures.[14-16] All other reagents employed were obtained commercially and, with the exception of reagent grade inorganic salts, were either redistilled or recrystallized prior to use. Solutions of phenylhydrazine were prepared just prior to use, as were those of carboxylic acids in 20% ethanol, to avoid formation of the ethyl esters.

Kinetic measurements[4,6,9] were carried out spectrophotometrically at 25° with the aid of a Zeiss PMQ II spectrophotometer equipped with a cell holder through which water from a thermostated bath was continuously circulated. Reaction kinetics were monitored by observing the appearance of the furfural phenylhydrazones at appropriate wavelengths in solutions containing initial concentrations of the aldehydes of 5×10^{-5} *M*: 5-methyl, 343 nm; unsubstituted, 340 nm; 5-bromo, 348 nm; 5-nitro, 464 nm. In all cases a sufficient excess of phenylhydrazine was employed to ensure that pseudo-first-order kinetic behavior would be obtained. First-order rate constants were evaluated from plots of log ($OD_\infty - OD_t$) against time in the usual manner. Second-order rate constants were obtained by dividing first-order constants by the concentration of phenylhydrazine free base. In the pH region in which phenylhydrazine attack is principally rate-determining, rate constants have been

(1) Supported in part by the Conselho Nacional de Pesquisas and by the Fundacão de Amparo à Pesquisa do Estado de São Paulo.
(2) W. P. Jencks, *Progr. Phys. Org. Chem.*, **2**, 63 (1964).
(3) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969.
(4) W. P. Jencks, *J. Amer. Chem. Soc.*, **81**, 475 (1959).
(5) E. H. Cordes and W. P. Jencks, *ibid.*, **84**, 826, 832 (1962).
(6) E. H. Cordes and W. P. Jencks, *ibid.*, **84**, 4319 (1962).
(7) L. do Amaral, W. A. Sandstrom, and E. H. Cordes, *ibid.*, **88**, 2225 (1966).
(8) R. B. Martin, *J. Phys. Chem.*, **68**, 1369 (1964).
(9) M. P. Bastos and L. do Amaral, *J. Org. Chem.*, **36**, 3412 (1971).
(10) J. M. Sayer and W. P. Jencks, *J. Amer. Chem. Soc.*, **91**, 6353 (1969).
(11) J. Hine, F. A. Via, J. K. Gotkis, and J. C. Craig, Jr., *ibid.*, **92**, 5186 (1970).
(12) J. Hine, J. C. Craig, Jr., J. G. Underwood, II, and F. A. Via, *ibid.*, **92**, 5194 (1970).
(13) I. J. Rinkes, "Organic Syntheses," Collect. Vol. II, Wiley, New York, N. Y., 1943, p 393.
(14) Z. N. Nazarova, *Dokl. Akad. Nauk Uzbek SSR*, 40 (1953).
(15) H. Gilman and G. F. Wright, *J. Amer. Chem. Soc.*, **52**, 2550 (1930).
(16) H. Gilman and G. F. Wright, *Iowa State College J. Sci.*, **4**, 53 (1929).