Studies on the Formation of Dyes Derived from Diindolylpyridylmethanes

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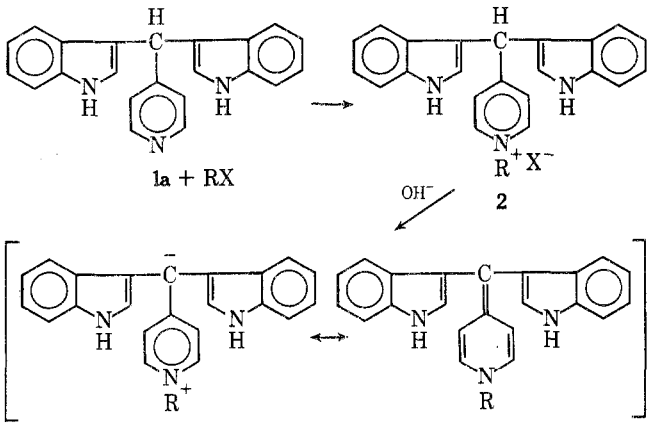
Diindolylpyridylmethane derivatives **1a–l** (except **1k**) upon N-alkylation of the pyridine moiety and treatment with base produce dyes analogous to **3**. Unexpectedly, dye production from **2** was accompanied by rapid autoxidation to yield dye **4** followed by slow dealkylation to dye **5**. Dye **5** was also obtained by Pd/C dehydrogenation of **1a** and alternatively by nitric acid oxidation of **1a**. The corresponding 1-methyldiindolyl **1e** and 1,1′-dimethyldiindolyl **1c** derivatives gave dyes analogous to **3** but not to **4**. The 3-pyridyl derivative **1m** did not form dyes. The kinetics of methylation of seven diindolylpyridylmethane derivatives with methyl iodide in 1:1 (v/v) 2-methoxyethanol–acetonitrile solvent mixture were determined at 30 °C spectrophotometrically and it was found that substituents in the indolyl moiety had little effect on the rate. The second-order rate constants varied between 5.2 and 6.0 $\times 10^{-4}$ l. mol^{-1} s^{-1}. Second-order rate constants for methylation of eight 3- and 4-substituted pyridines with methyl iodide in methanol-d_4 were determined at 30 °C by NMR and were found to range from 0.2 to 5.6 $\times 10^{-5}$ l. mol^{-1} s^{-1}.

While 4-(4-nitrobenzyl)pyridine has been extensively employed for the assay and detection of alkylating agents in the microgram range[1–3], under certain conditions the reagent for unknown reasons displays an unacceptable blank in alkali. Also the rate of alkylation of 4-(4-nitrobenzyl)pyridine is relatively slow at ambient temperatures. Therefore a search was made for reagents that would obviate these disadvantages.

Diindolylpyridylmethane (**1a**) (Scheme I) was expected to yield a colored alkylation product **3** in basic solution. Unexpectedly, **3** has been found to undergo a rapid oxidation followed by a slower dealkylation.

Scheme I

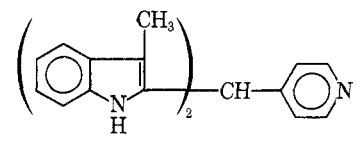


1a + RX → **2** (OH⁻)

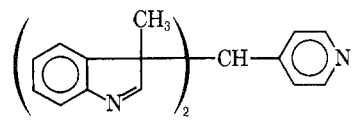
λ_{max}(R = CH_3) 367 nm(ϵ 1.5 × 10^4)

3

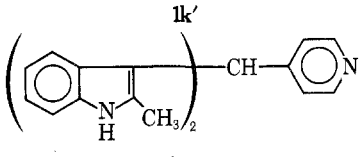
The diindolylpyridylmethanes (Table I) were prepared by condensation of an indole (2 equiv) and 4-pyridinecarboxaldehyde (1 equiv) in ethanolic hydrochloric acid, a method found superior to published procedures.[4,5] All compounds were 3,3′-diindolylpyridylmethanes except for **1k** which resulted from electrophilic attack at the 2 position of skatole. NMR data excluded **1k′** (indole N–H resonances, broad singlet δ 10.45). Compound **1k** differed from **1c** obtained by condensation of 2-methylindole with 4-pyridinecarboxaldehyde. Condensation of formaldehyde and benzaldehyde with skatole reportedly occurred at the 2 position.[6] Unsymmetrical **1e** (accompanied by **1a** and **1b**) was prepared in low yield from equimolar quantities of indole, 1-methylindole, and 4-pyridinecarboxaldehyde. NMR and



1k

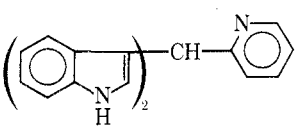


1k′

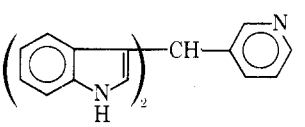


1c

mass spectral data were consistent with the proposed structure. An attempt to isolate the expected carbinol intermediate[5] failed. Use of 2- and 3-pyridinecarboxaldehyde afforded **1l** and **1m.**



1l



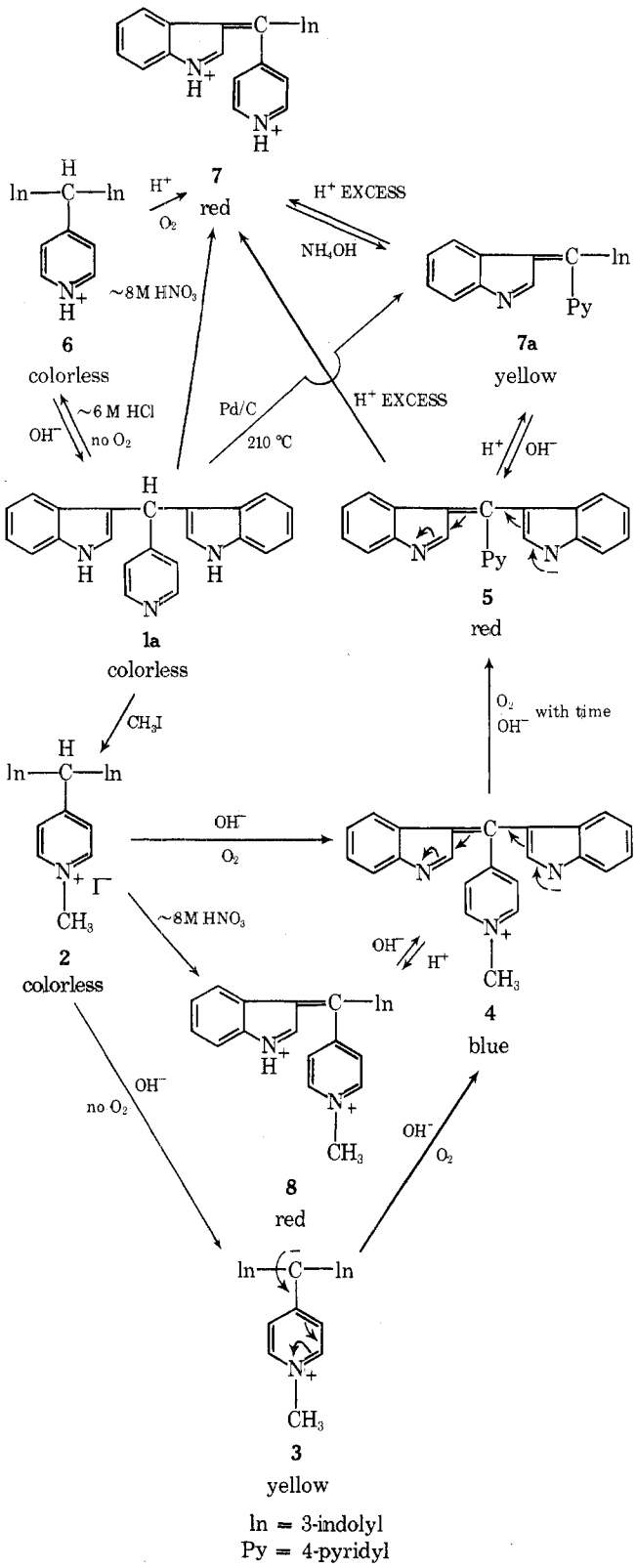
1m

Alkylation of **1a** with methyl iodide gave principally the *N*-methyl pyridinium salt showing poor analytical data. NMR spectra showed an impurity(δ 3.2, Me_2SO-d_6). Analytically pure *N*-methyl salt was prepared from 4-pyridinecarboxaldehyde methiodide and indole in ethanolic hydrogen iodide. The δ 3.2 resonance was greatly reduced. A number of experiments were carried out to probe the structures of the various dyes. Scheme II summarizes these experiments and the structures presented appear to be the' only reasonable ones consistent with the following data.

Treatment of **1a** with excess methyl iodide produced colorless **2.** Compound **1a** with excess methyl iodide and base in the presence of oxygen produced a blue dye, **4** (λ_{max} 575 nm, ϵ 1.4 × 10^4). Compound **2** with base under argon gave a yellow dye, **3,** which immediately turned blue upon exposure to oxygen.

In support of the proposed structures of **3** and **4,** alkylation of di(1-methylindolyl)-4-pyridylmethane (**1b**) followed

Scheme II



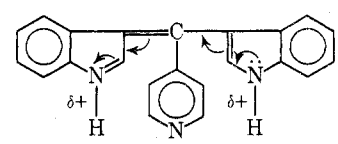
by treatment with base in the presence of oxygen did not form a blue dye. This carbanion is incapable of oxidation to the rosindole chromophore because it lacks the indole NH.

The unsymmetrical **1e** with one indole nitrogen substituted by a methyl group underwent alkylation with excess methyl iodide to produce a colorless product that became red when treated with alkali. This compound can give a carbanion that is oxidized to a rosindole (λ_{max} 543 nm, ϵ 1.6 $\times$ 10^3), but does not have the second indole NH needed to produce blue dye **4**. These experiments showed that one indole NH is necessary for oxidation and two are necessary for the blue dye **4**.[7a]

Compound **2** with 8 M HNO_3 under argon produced a red dye, **8** (λ_{max} 547 nm, ϵ 2.1 $\times$ 10^4), which gave **4** on treatment with deoxygenated base. Compound **4** was converted to **8** on treatment with 8 M HNO_3. Treatment of **1a** with 8 M HNO_3 also produced a red dye, **7** (λ_{max} 543 nm, ϵ 2.0 $\times$ 10^4) whose spectral properties were nearly identical with those of **8** (λ_{max} 547 nm, ϵ 2.1 $\times$ 10^4).

In support of structure **7**, **1a** in 6 M HCl gave a colorless solution, which became red (**7**) upon exposure to oxygen for several days. Compound **7** was not converted to **1a** by base. This established that **7** was an oxidation product of **1a**. Compound **7** (λ_{max} 540 nm, ϵ 2.6 $\times$ 10^4) was likewise produced by refluxing **1a** in nitrobenzene in the presence of 5% Pd/C catalyst,[7b] isolation of the free base **7a**, and dissolution in hydrochloric acid. Compound **7** obtained from **1a** by treatment with 8 M HNO_3 was converted to **7a** with ammonium hydroxide. While a satisfactory elemental analysis was not obtained, the mass spectrum of **7a** showed a parent peak *m/e* 321 (rel intensity 1.0), P − 1 (rel intensity 0.6). The loss of a hydrogen atom from the parent cation would lead to a resonance-stabilized radical cation.

Compound **7a** was converted to a dihydrochloride, **7**, whose NMR showed a sharp two-proton resonance for the α-indole and indolinenyl protons (δ 8.6) and no triarylmethinyl proton. This established the structure of **7**. The monoprotonated form of **7a** showed λ_{max} 512 nm. The



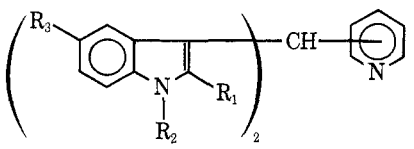
monoprotonated form of **7a**

NMR of **7a** (Me_2SO-d_6–D_2O) showed no triarylmethinyl proton and two equivalent α-indolyl protons (δ 8.0) shifted upfield from **7**, as expected. The absence of α-indolyl and indolinenyl proton resonances was due to rapid deuteron exchange with solvent. These observations were in accord with **7** and **7a**. Treatment of **7a** (λ_{max} 450 nm, ϵ 2.6 $\times$ 10^4) with excess methanolic sodium hydroxide yielded **5** (λ_{max} 521 nm, ϵ 3.6 $\times$ 10^4). Thus, **7a** contained an acidic proton (a conjugated indole NH), two basic sites (pyridyl N and indolinenyl N), and no triarylmethinyl proton and had molecular weight 321 (mass spectrum). Coupled with the fact that **7a** was obtained by oxidation or catalytic dehydrogenation, these data firmly established the structures of **7a** and **5**.

TLC analysis of a preparative scale solution of **5** on silica gel gave a compound whose R_f value was identical with that of **7a**. These data indicate the loss of an *N*-methyl group in the conversion of **4** to **5**. Compound **2** was treated preparatively with potassium hydroxide in methanol to produce **5**. The product was isolated by evaporation and extraction with benzene. TLC analysis of the solid recovered from an aliquot of the benzene solution indicated one component (R_f 0.5, **7a**) and a minor contaminant (R_f 0.7). A portion of the benzene solution was evaporated on the probe of the mass spectrometer. The mass spectrum showed a major *m/e* 321 and an unexplained weaker peak *m/e* 323. The latter *m/e* cannot be ascribed to **1a** because the characteristic blue color was not obtained upon treatment with dimethyl sulfate and base.[8]

A portion of the benzene solution of **7a** obtained from **4** was extracted with aqueous hydrochloric acid and the extract was evaporated to dryness. The NMR in methanol-d_4 showed the characteristic A_2B_2 pattern of a 4-substituted

Table I. Diindolylpyridylmethanes[a]



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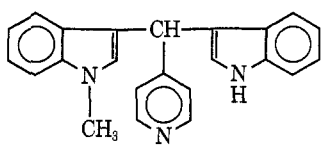
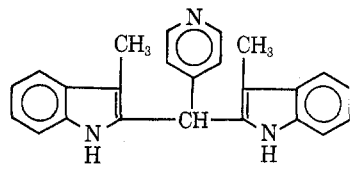


1e



1k

Compd	Ar	R_1	R_2	R_3	Mp, °C	Elemental anal., % Calcd C	Calcd H	Calcd N	Found C	Found H	Found N
1a	4-Pyridyl[c]	H	H	H	156–158 dec	81.7	5.3	13.0	81.9	5.5	13.0
1b	4-Pyridyl[d]	H	CH_3	H	184–185 dec	82.0	6.0	12.0	81.7	6.3	11.7
1c	4-Pyridyl[e]	CH_3	H	H	241–244 dec	78.0	6.3	11.4	78.0	6.0	11.5
1d	4-Pyridyl	H	H	CH_3	256–260 dec	82.0	6.0	12.0	81.7	5.9	12.0
1e[b]					146–148 dec	81.9	5.7	12.4	80.1	5.7	12.0
1f	4-Pyridyl	H	H	Cl	261–263 dec	67.4	3.9	10.7	67.1	4.0	10.5
1g[b]	4-Pyridyl	H	H	Br	275–277 dec	54.9	3.1	8.7	55.2	3.3	9.4
1h	4-Pyridyl	H	H	CN	274–276 dec	77.2	4.0	18.8	77.0	4.2	18.8
1i	4-Pyridyl	H	H	COOH	185–188 dec	70.1	4.2	10.2	69.0	4.3	10.3
1j[b]	4-Pyridyl	H	H	CH_3O	181–185 dec	75.2	5.5	11.0	74.6	5.6	10.9
1k					264–266 dec	82.0	6.0	12.0	81.9	6.2	12.2
1l	2-Pyridyl	H	H	H	212 dec	81.7	5.3	13.0	81.9	5.3	13.1
1m	3-Pyridyl	H	H	H	102–107 dec	81.7	5.3	13.0	83.1	5.7	10.7

[a] Substantial parent peaks were shown by these compounds. [b] Analyses unsatisfactory; homogeneous by TLC under conditions that separate 1a and 1b. [c] Lit. mp 152–155°C dec (ref 4) and 155–156°C dec (ref 5). [d] Lit. mp 186–188°C dec (ref 4). [e] Lit. mp 249–250°C uncorrected (ref 13).

Table II. Second-Order Rate Constants for the Reaction of Diindolylpyridylmethanes and 4-(4-Nitrobenzyl)pyridine with Methyl Iodide at 30°C[a]

Compd	Substituent	$k_2 \times 10^4$, l. mol^{-1} s^{-1}	λ_{max}, nm	$\epsilon \times 10^{-4}$, M^{-1} cm^{-1} Calcd	Corrected
1a	H	5.9	575[b]	1.4	1.4
1c	2-CH_3	5.9	644[b]	1.0	1.1
1d	5-CH_3	5.9	578[b]	1.2	1.3
1f	5-Cl	5.2	556[b]	1.3	1.3
1g	5-Br	5.7	559[b]	1.1	1.3
1h	5-CN	6.0	532[c]	1.4	1.4
1j	5-CH_3O	5.9	562[b]	1.1	1.3
4-(4-Nitrobenzyl)pyridine[e]		4.5	559[d]	2.5	2.5

[a] Second-order rate constants were obtained by dividing the pseudo-first-order rate constants by the methyl iodide concentration. [b] For compound 4. [c] For compound 5. [d] For the dye.[3] [e] Registry no., 1083-40-3.

pyridine, the equivalent α hydrogens of indole, the eight benzenoid protons, and no triarylmethinyl proton. Significantly, the *N*-methyl resonance was present as only a minor feature (<10 mol %) of the spectrum. This spectrum had the significant features of the rosindole **7** prepared via the catalytic route except for an unexplained resonance at δ 7.5, assigned to an impurity. All the evidence points to a loss of an *N*-methyl group in the conversion of **4** to **5**. While nucleophilic displacement on the pyridinium *N*-methyl group was not anticipated, there is ample evidence for carbanion formation at this site;[9,10] and thus, a likely pathway is the oxidative cleavage of the methyl–nitrogen bond via a carbanion as suggested by Corwin et al. for viologen dealkylation.[11] When compound **9**, the *N*-benzyl chloride analogue of **2**, was treated with base, benzaldehyde was detected by GLC analysis. Alkylation of **7a** was attempted with excess dimethyl sulfate followed by addition of base. This procedure, however, did not produce the characteristic blue color of **4**. The most likely side reaction to account for this failure was the alkylation of the indolenine nitrogen, the most basic site in **5**.[12]

The presence of the pyridinium moiety in the rosindole has a marked effect on the rosindole chromophore. The rosindole **7a** in alkali has λ_{max} 521 nm (ϵ 3.6 × 10^4) while **4** has λ_{max} 575 nm (ϵ 1.4 × 10^4). This bathochromic shift seems unusually large for a substituent in a cross conjugated position.

In the 2-pyridyl series, the independently prepared *N*-methylpyridinium compound on treatment with alkali and oxygen gave a dye with λ_{max} 550 nm (ϵ 2.8 × 10^4). Qualitatively, the 2-pyridyl compound **1l** underwent alkylation much more slowly than **1a**. The corresponding 3-pyridyl compound gave a colorless solution in base suggesting that neither a carbanion nor a rosindole was formed. Evidently conjugation of the triarylmethyl carbanion center with the pyridinium nitrogen is necessary for ready carbanion formation and carbanion formation is a prerequisite for facile oxidation to rosindole. The λ_{max} of the dye resulting from treatment of the 2-pyridinium compound with base was 25 nm hypsochromically shifted compared to the 4-pyridinium compound. The structure of the 2-pyridinium dye would be expected to deviate from coplanarity more than that of the 4-pyridinium dye.

All of the di(5-substituted indolyl)-4-pyridylmethane compounds **1d–g** (Table I) gave dyes upon alkylation and subsequent treatment with base. The spectral characteristics are given in Table II.

The N-methylated 5-cyano derivative (**1h**) formed a blue dye in base that was rapidly converted to the red dye (λ_{max} 532 nm, ϵ 2.8 × 10^4) presumably corresponding to the deal-

Table III. Second-Order Rate Constants for the Reaction of 3- and 4-Substituted Pyridines with Methyl Iodide at 30°C[a]

Substituent	H	4-CH_3	4-CN	3-CN	4-CH_2OH	3-CH_2OH	4-C_6H_5	3-$CONH_2$
$k_2 \times 10^5$, l. mol^{-1} s^{-1}	4.0	5.5	0.4	0.2	2.8	5.6	4.7	1.7
Registry no.	110-86-1	108-89-4	100-48-1	100-54-9	586-95-8	100-55-0	939-23-1	98-92-0

[a] Obtained by NMR.

kylated product **5**. The conversion of the blue dye from the methiodide of **1h** to the red dye was complete in 5 min while the conversion of **4** to **5** required ca. 8 h under the same conditions.

Kinetics of Alkylation of Substituted Pyridines. Kinetic studies were carried out on the rates of methylation with excess methyl iodide of the substituted diindolylpyridylmethanes. The reactions were carried out at 30 °C in a 1:1 (v/v) 2-methoxyethanol–acetonitrile solvent mixture with a 1000-fold excess of methyl iodide. An aliquot of the reaction mixture was withdrawn periodically, diluted with aqueous base, and the absorbance read at the appropriate λ_{max} (Table II). The reaction was followed for 2–3 half-lives. After approximately 15 h, the absorbance of an aliquot was read and the molar absorptivity was calculated assuming complete reaction. The molar absorptivities are recorded in Table II. The use of the 15-h absorbance as the infinity value for the kinetic calculations resulted in poor pseudo-first-order plots. By trial and error a molar absorptivity value was chosen to give a straight-line first-order plot. The corrected molar absorptivities are shown in the last column of Table II. The second-order rate constants were calculated using the corrected molar absorptivities. The rate constants were found to be insensitive to substituents in the indole ring (Table II).

For comparison, rates of alkylation of various pyridine compounds were determined by NMR (Table III) by measurement of the rates of appearance of the *N*-methyl resonance and disappearance of the methyl iodide resonance. The reactions were carried out using equimolar concentrations of reactants.

The data in Table III show that substituents introduced directly into the pyridine ring show less than a 30-fold variation in the rate of alkylation, so it is not surprising that rates of alkylation of compounds **1a–j** are relatively insensitive to substitution in the indole nucleus.

Quantitative comparison of the data in Table II and III is not possible owing to solvent difference.

Experimental Section

Melting points are corrected. Elemental analyses were performed by the Chemical Laboratory, Edgewood Arsenal. Infrared spectra were determined on a Perkin-Elmer 257 spectrophotometer. Electronic spectra were obtained on a Cary 14 spectrophotometer. The NMR spectra were measured with a Varian A-60D instrument using Me_2SO-d_6, acetone-d_6, or methanol-d_4 and Me_4Si as the internal standard. Mass spectra were obtained on a Perkin-Elmer Hitachi Model RMU-6E at 70 eV. For gas chromatography a Hewlett-Packard 5750 instrument was used. Unless otherwise noted thin layer chromatography (TLC) was performed on Eastman silica gel chromatogram sheets containing a fluorescent indicator. Compounds were visualized with 254-nm light. Molar absorptivities are given in M^{-1} cm^{-1} units.

Melting points and analytical data for the diindolylpyridylmethanes are given in Table I.

3,3′-Diindolyl-4-pyridylmethane (1a) was prepared by dissolving indole (2.34 g, 0.02 mol) and 4-pyridinecarboxaldehyde (1.07 g, 0.01 mol) in ethanol (100 ml). Acid (20 ml of concentrated HCl) was added and the solvent was allowed to stand at 25 °C for 2 h. Water (300 ml) was added giving a fine white precipitate. The milky suspension was neutralized with concentrated NH_4OH, yielding a yellow precipitate. After filtration and drying, 2.87 g (89% yield) of **1a** was obtained. The solid was recrystallized from methanol to obtain white prisms. After a tenfold dilution of a methanolic solution of **1a** with 1:1 (v/v) concentrated HCl–H_2O no color was visible. However, the solution slowly turned red. After 198 min the spectrum was obtained: λ_{max} 545 nm (ϵ 8×10^1) (**1a** → **6** → **7** incomplete reaction). After diluting a methanolic solution of **1a** tenfold with 1:1 (v/v) concentrated HNO_3–H_2O a red solution was obtained, λ_{max} 543 nm (ϵ 2.0×10^4) (**1a** → **7**).

3,3′-Di(5-cyanoindolyl)-4-pyridylmethane (1h) was prepared from 5-cyanoindole (2.84 g, 0.02 mol) and 4-pyridinecarboxaldehyde (1.07 g, 0.01 mol) in ethanol (150 ml) with 30 ml of concentrated HCl. After 1 week at 25 °C, water (300 ml) was added and the solution was neutralized with NH_4OH. The mixture was cooled and the white crystals of **1h** were filtered and dried (2.29 g, 61.4% yield). The solid recrystallized from methanol.

The other symmetrical compounds in Table I, **1b, 1c, 1d, 1f, 1g, 1i, 1j, 1k, 1l,** and **1m,** were prepared similarly with reaction times varying from 2 h to 1 week with yields varying from 40 to 60%. Satisfactory analyses were generally obtained by slow recrystallization overnight from methanol at 25 °C.[13]

3,3′-Di(5-carboxyindolyl)-4-pyridylmethane (1i) was prepared on one-tenth the scale used for **1a.** A mixture of five products resulted (TLC, hexane–ethyl ether–ethanol, 5:5:2). The crude product was chromatographed on neutral alumina (Woelm activity I) eluting with acetonitrile until all the impurities were removed (TLC). The product **1i** was eluted with methanol.

3-Indolyl-3′-(1-methylindolyl)-4-pyridylmethane (1e) was prepared by allowing indole (1.17 g, 0.01 mol), 1-methylindole (1.31 g, 0.01 mol), and 4-pyridinecarboxaldehyde (1.07 g, 0.01 mol) to react in ethanol (100 ml) containing concentrated HCl (20 ml) at 25 °C for 2 h. Water (300 ml) was added and the mixture was neutralized with NH_4OH. The precipitate was filtered, dried, and chromatographed on basic alumina (Woelm activity I), eluting with dichloromethane.

3,3′-Diindolyl-4-pyridylmethane Methiodide (2). Method A. Compound **1a** (1 g) was dissolved in acetone (20 ml) containing methyl iodide (20 ml). After 1 h the yellow crystals were filtered and dried, giving 0.35 g (24% yield) of **2.** The solid recrystallized from methanol gave an unsatisfactory elemental analysis. The purity of **2** was monitored by measuring the molar absorptivity of **4** at 575 nm by dissolving **2** in a 1:1 (v/v) mixture of 2-methoxyethanol and acetonitrile, diluting to 10^{-4} M, and adding an equal volume of 2 N NaOH. The molar absorptivities that were obtained using **2** after successive recrystallizations were 1.43×10^4, 1.43×10^4, and 1.41×10^4, respectively.

Method B. Methyl iodide (15.6 g, 0.11 mol) was added to 4-pyridinecarboxaldehyde (10.7 g, 0.1 mol) in 10 ml of acetonitrile. After refluxing for 15 h, the solution was cooled and poured into acetone (300 ml). The precipitate was collected and dried. A portion of the crude 4-pyridinecarboxaldehyde methiodide (0.01 mol) was dissolved in ethanol (100 ml) containing indole (0.02 mol and concentrated HI (10 ml). After 2 h at 25 °C, 300 ml of water was added. The precipitate was filtered, dried, and recrystallized from methanol, mp 200–208 °C dec (darkens above 116 °C). Anal. Calcd for $C_{23}H_{20}N_3I$: C, 59.4; H, 4.3; N, 9.0; I, 27.3. Found: C, 59.3; H, 4.5; N, 9.1; I, 27.3. The NMR spectral data were in accord with the assigned structure except for an unexplained resonance at δ 3.2. The molar absorptivity obtained for this product **2** after treatment with alkali at 575 nm was 1.43×10^4.

Preparation of the Benzyl Chloride Salt of 1a (9). Compound **9** was prepared by dissolving **1a** (3.23 g, 0.01 mol) and benzyl chloride (1.27 g, 0.01 mol) in benzene (50 ml). After refluxing for 18 h the precipitate was collected and dried to obtain 1.16 g (26% yield) of **9.** The product was recrystallized from water containing sodium chloride, mp 175 °C dec (darkens above 150 °C). Anal. Calcd for $C_{29}H_{24}N_3Cl \cdot H_2O$: C, 74.4; H, 5.6; N, 9.0; Cl, 7.6. Found: C, 74.9; H, 5.5; N, 9.4; Cl, 7.3.

Reaction of 9 with Potassium Hydroxide in Methanol and Gas Chromatography of Products. Compound **9** (1 g) in 1 N methanolic KOH (20 ml) was stirred for 2 h at 25 °C and acidified with 10% HCl. The mixture was evaporated in a stream of air. The semisolid mass was extracted with benzene (20 ml) and the solution was analyzed by GLC (6-ft column containing UCW 98 on 80–100 mesh Chromosorb W at 110 °C). The single peak had the retention time of benzaldehyde.

Preparation of Rosindoles. 3-Indolinylidene-3′-indolyl-4-pyridylmethane (7a). Method A. Compound **1a** (0.5 g) in nitroben-

Table IV. Chemical Shifts of the Pyridinium Methiodides[a]

Pyridine substituent[b]	H	4-CH_3	3-$CONH_2$	3-CN	4-CN	3-CH_2OH	4-CH_2OH	4-C_6H_5
$\overset{+}{N}$–Me resonance, ppm	4.51	4.39	4.55	4.58	4.60	4.48	4.43	4.47
Registry no.	930-73-4	2301-80-6	6456-44-6	1004-16-6	1194-04-3	42330-63-2	43330-64-3	36913-39-0

[a] CH_3I resonance varied between δ 2.16 amd 2.19 depending on the substituted pyridine that was used.

zene (60 ml) was treated with 5% Pd/C catalyst (Engelhard) (0.3 g). The mixture was refluxed under argon for 4 h. TLC (ethyl acetate) of a 1-μl aliquot of the solution showed no starting material. The solution was extracted with 200 ml of 1:4 (v/v) concentrated HCl–H_2O. The acid solution was extracted with one 500-ml and two 300-ml portions of benzene. The aqueous phase was neutralized with NH_4OH. The air-sensitive product was filtered, washed with water, and dried in vacuo for 16 h. The dried solid (**7a**) weighed 0.37 g, λ_{max} (methanol) 450 nm (ϵ 1.6 × 10^4). A solution prepared by dissolving **7a** in a stoichiometric amount of HCl (aqueous) showed λ_{max} 512 nm (ϵ 1.3 × 10^4). A methanolic solution of **7a** diluted tenfold with 1:1 (v/v) concentrated HNO_3–H_2O showed λ_{max} 540 nm (ϵ 2.6 × 10^4) (**7a** → **7**). The methanolic solution of **7a** diluted tenfold with 2 N NaOH (aqueous) gave λ_{max} 521 nm (ϵ 3.6 × 10^4) (**7a** → **5**). TLC of **7a** on silica gel (acetone) indicated a major spot, R_f 0.5, with a minor spot, R_f 0.7. The mass spectrum showed a major peak, *m/e* 321 (**7a**), and a weaker peak, *m/e* 323. The compound corresponding to *m/e* 323 was not identical with the starting **1a** (mol wt 323) by TLC or colorimetric analysis. The R_f 0.7 spot on a TLC strip turned purple on exposure to acid vapors and on subsequent treatment with base it became deep red. Similar behavior was observed with the R_f 0.5 spot. Preparative chromatography (2.0 mm silica gel plates) using acetone gave two bands. The lower band was removed and extracted with methanol. Both the R_f 0.5 and the R_f 0.7 spots were evident in a TLC of the extract. Furthermore, the extract contained a relatively larger proportion of the R_f 0.7 material as compared with the sample chromatographed. This result suggests that the two materials were interconverted in the elution process.

Method B. Compound **1a** (1 g) was stirred in 1:1 (v/v) concentrated HNO_3–H_2O. The deep purple solution was neutralized with NH_4OH. The precipitate was filtered and washed with water. TLC on silica gel (acetone) showed spots at R_f 0.5 and 0.7 which showed the same color reactions and mass spectral data as the product from method A.

3-Indolinylidene-3′-indolyl-4-pyridylmethane Dihydrochloride (Dihydrochloride of 7a). The synthesis was the same as that for **7a** (method A) except that the aqueous acid extract was evaporated without neutralization. The recovery of the purple dihydrochloride **7** was 96%. The NMR spectrum in methanol-d_4 showed no triarylmethinyl proton (δ 6.0), but showed a 4-substituted pyridine A_2B_2 quartet: δ 9.25 (2 H, α-pyridine, *J* = 6.0 Hz), 8.42 (2 H, β-pyridine, *J* = 6.0 Hz), 8.60 (s, 2 H, α-indole), 6.8–8.1 (m, 10 H, benzenoid).

Chemical and Spectral Characterization of Structures in Scheme II. A. Demonstration of an Oxidizable Intermediate 3 in the Conversion of 2 → 4. Compound **2** in 2-methoxyethanol–acetonitrile–aqueous 2 N NaOH (1:1:2 v/v) was converted to the blue dye **4** in the presence of air, λ_{max} 393 nm (ϵ 1.4 × 10^4), 575 (1.4 × 10^4). When the same solutions of **2** and NaOH were deoxygenated with argon (5 min) prior to mixing, there resulted a green solution with λ_{max} 367 nm (rel intensity 10) and 575 (rel intensity 1) (**2** → **3**). The 575-nm band was attributed to **4** resulting from incomplete deoxygenation. This solution upon exposure to air immediately turned blue, λ_{max} 393 and 575 nm (rel intensities 1:1) (**3** → **4**).

B. Demonstration of Demethylation in the Conversion of 4 → 5. A blue solution of **4** in 1.8 N methanolic KOH became red [after 16 h, λ_{max} 525 nm (ϵ 3.3 × 10^4) (**4** → **5**)]. Treatment of **2** in methanol (1 ml) with 2.5 N NaOH (aqueous) (9 ml) for 1 h gave a red solution of **5**, λ_{max} 525 nm. A sample of **7a** (prepared by catalytic dehydrogenation of **1a**) in 10% methanol–1.8 N NaOH (aqueous) showed the same spectral characteristics as **5** derived from **4**, λ_{max} 521 nm (ϵ 3.6 × 10^4). An acidified solution of **5** derived from **4** showed λ_{max} 546 nm (ϵ 1.5 × 10^4) (**5** → **7**) and an acidic solution of **7a** showed λ_{max} 540 nm (ϵ 2.6 × 10^4) (**7a** → **7**). The lower extinctions of **5** and **7** derived from **4** were attributed to side reactions.[8] On diluting a methanolic solution of **2** tenfold with 1:1 (v/v) concentrated HNO_3–H_2O there was obtained a red solution, λ_{max} 547 nm (ϵ 2.1 × 10^4) (**2** → **8**). A blue solution of **4** obtained by diluting a methanolic solution of **2** tenfold with 2.5 N NaOH (aqueous), upon acidification with 1:1 (v/v) concentrated HNO_3–H_2O gave a red solution, λ_{max} 546 nm (ϵ 1.6 × 10^4) (**4** → **8**). Compound **8** was prepared from **2** in the absence of oxygen using HNO_3 and converted to **4** with 2:N NaOH (aqueous) in the absence of oxygen.

TLC Evidence for Structures in Scheme II. Compound **2** (1 g, 0.02 mol) was dissolved in 60 ml of 0.33 N methanolic KOH. The blue solution was stirred in air for 30 min, giving a red solution. The methanolic solution was evaporated. The solid was triturated with 50 ml of water and extracted with benzene. The remaining violet solid, insoluble in benzene and water, was dissolved in acetone to yield a yellow-orange solution. TLC on silica gel (acetone) of the benzene- and the acetone-soluble materials each showed one major spot (R_f 0.5 for the benzene-soluble product and 0.7 for the acetone-soluble product). A minor spot in each TLC indicated that each of the materials was contaminated with the other. Evaporation of an aliquot of the benzene soluble product on the probe of the mass spectrometer and subsequent analysis showed a major *m/e* 321 (**7a**) with a weaker peak *m/e* 323 assigned to the contaminant. The acetone-soluble product showed a major *m/e* 323. The benzene solution containing **7a** was extracted with 1:4 (v/v) concentrated HCl–H_2O and the purple acidic extract was evaporated in a stream of air to dryness to obtain a dark purple solid. The NMR spectrum of this sample revealed all of the resonances of the dihydrochloride **7**, which was obtained catalytically (method A). The characteristic quaternary methyl resonance of **2** (δ 4.0) was weak, corresponding to <10 mol %. Furthermore, the triarylmethinyl proton (δ 6.0) was not detected.

Kinetics of Alkylation of Diindolylpyridylmethanes. Into a 50-ml volumetric flask was placed 45 ml of 1:1 (v/v) 2-methoxyethanol and acetonitrile solvent mixture and the flask was thermostated at 30 °C. A solution of diindolylpyridylmethane (ca. 10^{-2} M) in the solvent mixture was added followed by methyl iodide (0.5 ml, 1.1 g). The flask was filled to the mark with thermostated solvent mixture. Periodically a 1-ml aliquot was withdrawn, placed in a 3-ml cuvette, and treated with 1 ml of 2 N NaOH (aqueous). After 2 min the absorbance was read at the appropriate λ_{max} (see Table II). The pseudo-first-order rate constants were calculated from $k = t^{-1} \ln (OD_\infty - OD_0)/(OD_\infty - OD_t)$ and second-order rate constants were obtained by dividing by [CH_3I].

When the rate of alkylation of the 5-cyano compound **1h** was determined, the procedure had to be modified owing to the instability of the blue dye analogous to **4**. After a 1-ml aliquot was taken and treated with 1 ml of 2 N NaOH (aqueous), the solution was allowed to stand for 18 h (overnight) prior to reading the absorbance. During this period the solution became red (structure corresponding to **5**) and the absorbance was read at 532 nm.

Kinetics of Alkylation of Substituted Pyridines. Pyridine or a substituted pyridine (0.16 mmol) was dissolved in methanol-d_4 (0.4 ml), placed in an NMR tube, and thermostated at 30 °C for 10 min. To the solution was added 22.7 mg (0.16 mmol) of methyl iodide using a 10-μl syringe as a weight buret. Measures were taken to ensure thorough mixing. The samples were maintained at 30 °C in a constant-temperature bath and read periodically in the NMR spectrometer for short periods of time at 33 °C. Where the kinetic runs required extended reaction times, the samples were quenched overnight in dry ice and thawed rapidly the following morning. Periodic determination was made of the NMe/MeI resonance ratios as a function of time. Table IV summarizes the chemical shifts measured for the *N*-methylpyridines. The second-order rate constants were determined using the integrated form of the second-order rate law for reactants at equal concentrations (eq 1)

$$kt = x/a(a - x) \quad (1)$$

where $x(a - x)$ is the ratio of the *N*-methyl integral to the methyl iodide integral and *a* is the methyl iodide concentration determined by weight at $t = 0$. Molarity of the methyl iodide was calculated assuming a volume of 0.4 ml with no adjustment for volume changes upon mixing. The run-to-run reproducibility was ca. 10% of the rate constant.

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Registry No.—**1a**, 21182-09-2; **1b**, 21182-15-0; **1c**, 1053-39-0; **1d**, 57637-71-5; **1e**, 57637-72-6; **1f**, 57637-73-7; **1g**, 57637-74-8; **1h**, 57637-75-9; **1i**, 57637-76-0; **1j**, 57637-77-1; **1k**, 57637-78-2; **1l**, 57637-79-3; **1m**, 21182-11-6; **2**, 57637-80-6; **3**, 57637-81-7; **4**, 57637-82-8; **5**, 57637-83-9; **7a**, 1099-94-1; **7a** 2HCl, 57637-84-0; **9**, 57637-85-1; indole, 120-72-9; 4-pyridinecarboxaldehyde, 872-85-5; 5-cyanoindole, 15861-24-2; 1-methylindole, 603-76-9; benzyl chloride, 25168-05-2; methyl iodide, 74-88-4.

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A Synthetic Approach to the Cephalotaxine Skeleton

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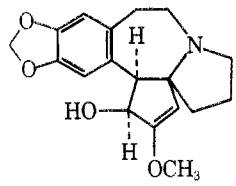
Several possible routes to the synthesis of the alkaloid cephalotaxine have been explored. Friedel–Crafts cyclization of 1-[2-(3,4-methylenedioxyphenyl)ethyl]pyrrole-2-carboxylic acid, followed by reduction with hydrogen over rhodium on charcoal, gave 8,9-methylenedioxy-1,2,3,6,11,11a-hexahydro-5*H*-pyrrolo[2,1-*b*][3]benzazepine.

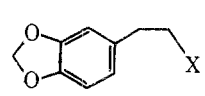
The alkaloid cephalotaxine (I), found in the plum yew, has been assigned an absolute structure based on a combination of chemical, spectral, and x-ray diffraction data.[1–6]



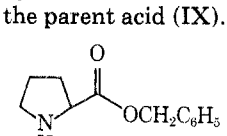
I

Esters of cephalotaxine derived from substituted malic and tartaric acids are known as harringtonines,[7–10] and are of interest because of their antitumor properties.[11] With a view to potential medicinal applications, work was begun in the fall of 1971 on the synthesis of the parent ring system, which, if successful, could be extended to other alkaloids in this series.

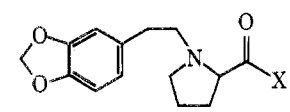
Treatment of 1,2-methylenedioxybenzene (II) with bromine gave 3,4-methylenedioxybromobenzene (III), as well as a small amount of 4,5-methylenedioxy-1,2-dibromobenzene (IV).[12] Generation of the Grignard reagent from the bromide III, followed by the addition of ethylene oxide, formed 3,4-methylenedioxyphenethyl alcohol (V). Refluxing alcohol V with phosphorus tribromide then produced 4-(2-bromoethyl)-1,2-methylenedioxybenzene (VI). Alkylation of benzyl prolinate (VII) by the bromide VI went smoothly; the intermediate benzyl ester (VIII) was not isolated, but was hydrogenated to the parent acid (IX).



V, X = OH
VI, X = Br



VII



VIII, X = $OCH_2C_6H_5$
IX, X = OH
X, X = Cl

The next step, Friedel–Crafts cyclization of compound IX, rather surprisingly failed because decarbonylation of the proline carboxyl group occurred with remarkable ease when attempts were made to prepare the acid chloride X. Interestingly, the treatment of proline (XI) with thionyl chloride has been said to give prolyl chloride hydrochloride (XII); unfortunately, no analytical or spectral data were provided to support the assigned structure.[13] By contrast, two other reports on the preparation of amino acid acyl chloride hydrochlorides are known and appear to be correct.[14,15] Treatment of acid IX with thionyl chloride, phosphorus trichloride, or oxalyl chloride yielded in all cases a new compound (XIII). The structure assigned to XIII was supported by the absence of a carbonyl group in the infrared and a correct proton count in the nuclear magnetic resonance spectrum for both the methylenedioxyphenylethyl and prolyl groups. These results can be explained by postulating the existence of a "reverse-Koch" reaction (Scheme I). Here, we assume the initial conversion of the acid IX to the desired acyl chloride X, which then undergoes decomposition either by nucleophilic attack or by internal rearrangement to yield the iminium chloride XII. Some support for this idea was found when it was observed that a solution of IX in methylene dichloride at −70 °C on treatment with trifluoroacetic acid evolved carbon monoxide. The same result was obtained when other modes of ring cyclization were tried with IX, for example, treatment