

CHROMATOGRAPHY OF β -CARBOLINES

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Some naturally occurring β -carbolines like harmaline have been known for a considerable time. More recently plant alkaloids such as reserpine have been isolated and found to be of pharmacological importance. This, plus the fact that the β -carbolines are closely related to and can be derived from physiologically occurring tryptamines, *e.g.* serotonin, has resulted in a rapidly increasing interest in the properties of this class of compound.

Other studies in which we have needed to identify the products of synthesis, or metabolites of β -carbolines in biological fluids, have emphasized the utility of chromatographic separation and identification of this class of compound. The chromatographic properties of β -carbolines, however, have not been widely reported, only the characteristics of a limited number of compounds have appeared in the literature¹⁻⁶. Since we had a fairly large and comprehensive series of β -carbolines, many as a result of our synthetic efforts, it appeared well worthwhile to investigate solvents and color reactions which could be used advantageously in the separation and identification of compounds in this class.

EXPERIMENTAL

Materials

β -Carbolines were either obtained from commercial sources (Tables I-III) or prepared in our laboratory.

*Solvent systems**For paper chromatography*

- (A) *n*-Butanol-acetic acid-water (120:30:50).
- (B) *n*-Butanol-isopropanol-ammonium hydroxide-water (3:1:1:1).
- (C) Methanol-butanol-benzene-water (2:1:1:1).

For thin-layer chromatography

- (D) *n*-Butanol-acetic acid-water (4:1:1).
- (E) *n*-Propanol-ammonium hydroxide (8:2).
- (F) Chloroform-methanol (9:1).

Preparation of the thin-layer plates

Thin-layer plates (20 × 20 cm) with a thickness of 0.25 mm were prepared in the usual manner by mixing a slurry of Silica Gel G (Brinkman Instruments, Inc.) and

water in a ratio of 30 g of the adsorbent to 60 ml of water. They were air-dried for 30 min, then activated in an oven for 30 min at 105°.

Detection

U.V. fluorescence

A Mineralight shortwavelength U.V. lamp, Model R51, was used.

Spray reagents

(a) *Xanthydrol*. 1% xanthydrol in 95% ethanol with a few drops of concentrated hydrochloric acid.

(b) *Ehrlich's*. Eight volumes of 2% *p*-dimethylaminobenzaldehyde in 95% ethanol and two volumes of 6 *N* HCl. Chromatograms were heated after the spray.

(c) *Brentamine Fast Blue*. Equal volumes of 0.5% aqueous Brentamine Fast Blue and carbonate-bicarbonate buffer (pH 9.2).

(d) *Diazotized sulfanilic acid*. Spray I: One volume of a mixture of 0.9 g sulfanilic acid, 9 ml of concentrated hydrochloric acid, and 90 ml of water and one volume of 5% aqueous sodium nitrite. Spray II: Two volumes of 10% aqueous sodium carbonate.

(e) *Ninhydrin*. 1% Ninhydrin in *n*-butanol with a few drops of pyridine. Chromatograms were heated after the spray.

(f) *Oxidizing mixture*. 9.8 volumes of 30% aqueous H₂SO₄, 0.2 volumes of 5% aqueous solution of ferric chloride, and 10 volumes of 36% perchloric acid. Chromatograms were heated after the spray.

(g) *Citric acid*. 2% citric acid in acetic anhydride. Chromatograms were heated at 110° for 10–15 min after the spray⁷.

Chromatographic procedure

All solutions were prepared in absolute ethanol (1 mg/ml). Ten μ l of each solution was applied on thin-layer plates, and 25 μ l on Whatman paper No. 1.

The thin-layer chromatograms were developed in air-tight rectangular developing tanks, while the paper chromatograms were developed in a chromatographic chamber using a descending technique. All chromatography was carried out at room temperature. After the chromatograms were dried, the U.V. fluorescence was noted, and the color reaction of these spots with the various spray reagents was determined.

RESULTS AND DISCUSSION

The R_F values shown in Tables I–III are the average of at least four runs on separate chromatograms. Color reactions with the various spray reagents were checked a minimum of three times.

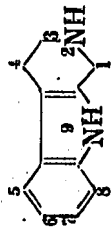
Color reagents such as xanthydrol and Ehrlich's react only with the tetrahydro β -carbolines and not with the dihydro and aromatic compounds. Table IV shows that in the three series of 6-methoxylated β -carbolines, the sensitivity of the tetrahydro compound is in the range of 1–5 μ g, whereas the dihydro and aromatic compounds are not detectable even at 25 μ g. Reaction of the tetrahydro- β -carbolines with Ehrlich's reagent is slow, however, reaction with xanthydrol is fast. In addition, xanthydrol gives a characteristic magenta color with most of the tetrahydro compounds except the 6-substituted derivatives which gives a blue to purple color.

TABLE I

CHROMATOGRAPHY OF SUBSTITUTED 1,2,3,4-TETRAHYDRO- β -CARBOLINES

Colors: B = blue; Br = brown; Gr = green; Gre = grey; Lt = light; Mg = magenta; Or = orange; P = pink; Pu = purple; Q = quench; Redd = reddish; V = violet; Y = yellow.

| | R _F | | Detection | | | | | | a | b | c | d | e | f | g | | | | | | |
|--|---------------------------------|------|--------------------------------------|------|------|------|-------------------|-------------------|-----------------|-------|----------|---|--------|--------|-----------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| | Paper chromatography in solvent | | Thin-layer chromatography in solvent | | | | | | | | | | | | | U.V. fluor. color | | | | | |
| | A | B | C | D | E | F | U.V. fluor. color | U.V. fluor. color | | | | | | | | U.V. fluor. color | U.V. fluor. color | U.V. fluor. color | U.V. fluor. color | U.V. fluor. color | U.V. fluor. color |
| Tetrahydro- β -carbolines ^{a,b} (Noreleagine) | 0.75 | 0.88 | 0.77 | 0.50 | 0.68 | 0.05 | B | Mg | Mg | — | — | — | Or | Gre | Or | | | | | | |
| 1-Methyl- ^b (Tetrahydroharman) | 0.81 | 0.91 | 0.84 | 0.51 | 0.88 | 0.06 | B | Mg | Mg | — | — | — | Pu | Gre | — | | | | | | |
| 1-Ethyl- ^a | 0.83 | 0.91 | 0.89 | 0.54 | 0.84 | 0.07 | B | Mg | Mg | — | — | — | P | P-Gre | — | | | | | | |
| 1,2-Dimethyl- ^{a,b} (Laptocladine) | 0.81 | 0.94 | 0.93 | 0.37 | 0.92 | 0.19 | B | Mg | Mg | — | — | — | — | B | — | | | | | | |
| 1-Methyl-2-(<i>n</i> -propyl)- ^{a,c} (Propyltetrahydroharman) | 0.88 | 0.97 | 0.93 | 0.50 | 0.89 | 0.36 | B | Mg | Mg | — | — | — | P | B-Gre | — | | | | | | |
| 9-Methyl- ^a | 0.77 | 0.89 | 0.84 | 0.47 | 0.78 | 0.21 | B | Mg | Pu ^d | — | — | — | Or | Gre | Or ^d | | | | | | |
| 9-Ethyl- ^a | 0.81 | 0.91 | 0.88 | 0.53 | 0.77 | 0.26 | P | V | V | Y | — | — | Or | Gre | — | | | | | | |
| 1,9-Dimethyl- ^e | 0.81 | 0.92 | 0.87 | 0.43 | 0.76 | 0.25 | B | Mg | Mg | — | — | — | Pu | Gre | — | | | | | | |
| 1,2,9-Trimethyl- ^a | 0.82 | 0.93 | 0.92 | 0.33 | 0.89 | 0.40 | B | Mg | Mg | — | — | — | Lt-P | Gre | — | | | | | | |
| 2-Acetyl-1-methyl- ^a | 0.91 | 0.93 | 0.91 | 0.81 | 0.81 | 0.93 | Q | Pu | Pu | — | — | — | — | Y | — | | | | | | |
| 1-Benzyl- ^a | 0.85 | 0.94 | 0.91 | 0.74 | 0.91 | 0.73 | B | Mg | P | — | — | — | P | Gre | — | | | | | | |
| 1-Phenyl- ^a | 0.87 | 0.93 | 0.93 | 0.70 | 0.87 | 0.41 | B | Mg | Mg | — | — | — | Pu | Gre | — | | | | | | |
| 1-(1-Naphthyl)- ^a | 0.92 | 0.95 | 0.92 | 0.84 | 0.91 | 0.94 | P | Mg | P | — | — | — | Lt-Gre | Gre | — | | | | | | |
| 1-(2-Naphthyl)- ^a | 0.91 | 0.94 | 0.94 | 0.82 | 0.93 | 0.73 | P | Mg | P | — | — | — | Lt-Gre | Gre | — | | | | | | |
| 1-(4-Pyridyl)- ^a | 0.51 | 0.90 | 0.90 | 0.44 | 0.87 | 0.04 | P | Mg | — | — | — | — | Gr-Y | — | — | | | | | | |
| 1-(3-Hydroxy-5-hydroxy- methyl-2-methyl-4-pyridyl)- 1-Carboxy- ^a | 0.76 | 0.86 | 0.92 | 0.30 | 0.80 | 0.25 | Q | Mg | Mg | Pu | — | — | Pu | — | — | | | | | | |
| 1-Carboxy-9-methyl- 3-Carboxy- ^c (Tetrahydro- norharman-carboxylic acid) | 0.63 | 0.57 | 0.65 | 0.46 | 0.45 | 0.01 | P | Mg | Mg | Y | Re-dd-Br | — | Or | Y | Or | | | | | | |
| 1-Carboxy-3-methyl- 1,3-Dicarboxy-1-methyl- ^b (Eleagine-1,3-dicarboxylic acid) | 0.74 | 0.65 | 0.84 | 0.51 | 0.48 | 0.10 | P | Mg | P | — | — | — | Lt-Or | Y | — | | | | | | |
| 1-Carboxy-1-benzyl- 3-Carboxy-1-benzyl- ^a | 0.62 | 0.45 | 0.57 | 0.50 | 0.30 | 0.03 | B | Mg | Mg | — | — | — | Re-dd | Pu | — | | | | | | |
| 6-Methoxy-1-carboxy- 6-Methoxy- ^a | 0.75 | 0.63 | 0.64 | 0.57 | 0.49 | 0.03 | B | Mg | Mg | Y | — | — | Or | Y | Or | | | | | | |
| | 0.55 | 0.34 | 0.52 | 0.34 | 0.07 | 0.03 | B | Mg | Mg | Br | — | — | Pu | — | — | | | | | | |
| | 0.88 | 0.82 | 0.86 | 0.69 | 0.63 | 0.06 | P | Mg | Mg | Lt-Br | — | — | — | — | Pu | | | | | | |
| | 0.87 | 0.77 | 0.83 | 0.74 | 0.57 | 0.09 | B | Mg | Mg | — | — | — | Pu | — | — | | | | | | |
| | 0.56 | 0.51 | 0.59 | 0.51 | 0.45 | 0.01 | P | Pu | Pu | Y | — | — | Or | Y | Or | | | | | | |
| | 0.70 | 0.82 | 0.75 | 0.46 | 0.67 | 0.03 | P | B | Pu | — | — | — | Or | Pu-Gre | Or | | | | | | |



| | | | | | | | | | | | | | | |
|--|------|------|------|------|------|------|------|-----------------|------|---------|-------|----|-------|---|
| 6-Methoxy-1-methyl- ^b (6-Methoxytetrahydroharman) | 0.79 | 0.87 | 0.83 | 0.49 | 0.78 | 0.06 | P | B-Pu | Pu | Lt-Br | — | P | Gre | — |
| 6-Methoxy-1,9-dimethyl- ^f | 0.78 | 0.82 | 0.91 | 0.25 | 0.88 | 0.49 | P | B | Pu | Lt-Br | — | — | — | — |
| 6-Methoxy-1-benzyl- ^a | 0.86 | 0.93 | 0.92 | 0.68 | 0.90 | 0.66 | P | B | Pu | — | — | — | Gre | — |
| 6-Methoxy-2-acetyl-1-methyl- | 0.90 | 0.91 | 0.90 | 0.79 | 0.84 | 0.89 | P | B | Pu | — | — | — | Gr | — |
| 7-Methoxy-1-methyl- | 0.74 | 0.86 | 0.82 | 0.50 | 0.80 | 0.06 | B-Gr | Mg | B | Y-Br | — | — | P-Gre | — |
| 7-Methoxy-1,2-dimethyl- ^b (2-Methylheptaforine) | 0.77 | 0.93 | 0.92 | 0.35 | 0.84 | 0.12 | B | Mg | Pu | Lt-Br | — | — | P-Gre | — |
| 7-Methoxy-1-methyl-2-(<i>n</i> -propyl)- ^c (Propyltetrahydroharmanine) | 0.86 | 0.93 | 0.92 | 0.48 | 0.88 | 0.34 | B | Mg | Pu | Lt-Br | — | — | Gre | — |
| 7-Methoxy-2-acetyl-1-methyl- | 0.89 | 0.90 | 0.92 | 0.73 | 0.79 | 0.89 | B | Mg | B | — | — | — | Gr | — |
| 6-Hydroxy-1-methyl- ^b (6-Hydroxytetrahydroharman) | 0.64 | 0.77 | 0.73 | 0.46 | 0.75 | 0.00 | P | B-Pu | B-Pu | Pu | Br | P | Gre | — |
| 6-Hydroxy-2-acetyl-1-methyl- | 0.84 | 0.83 | 0.88 | 0.76 | 0.75 | 0.50 | P | B-Gr | B | Pu | Br | — | Gre | — |
| 7-Hydroxy-1-methyl- ^b (Tetrahydroharmanol) | 0.67 | 0.78 | 0.74 | 0.49 | 0.71 | 0.00 | B | Mg | Pu | Redd-Br | Br | — | P-Gre | — |
| 7-Hydroxy-1,2-dimethyl- ^{a,b} (2-Methyltetrahydroharmanol) | 0.73 | 0.20 | 0.77 | 0.32 | 0.21 | 0.00 | B | Mg | Mg | Redd-Br | Or-Br | — | — | — |
| 7-Hydroxy-2-acetyl-1-methyl- | 0.86 | 0.87 | 0.92 | 0.76 | 0.74 | 0.60 | B | Mg | B | Redd-Br | Or-Br | — | Gre | — |
| 6-Benzoyloxy-1-methyl- | 0.86 | 0.92 | 0.90 | 0.56 | 0.78 | 0.08 | P | Pu | Pu | Y-Br | Br | Pu | Lt-Br | — |
| 6-Benzoyloxy-2-acetyl- | 0.93 | 0.94 | 0.93 | 0.85 | 0.91 | 0.94 | P | B | Pu | — | — | — | Gre | — |
| 6-Fluoro- | 0.80 | 0.91 | 0.91 | 0.49 | 0.74 | 0.05 | B | Pu | Mg | — | — | Or | Or | — |
| 6-Methyl- | 0.79 | 0.91 | 0.82 | 0.50 | 0.71 | 0.05 | B | B | Mg | — | — | Or | Gre | — |
| 1-Oxo- ^b | 0.86 | 0.85 | 0.84 | 0.72 | 0.83 | 0.60 | B | Mg ^d | Mg | — | — | — | P | — |
| 1-Oxo-6-chloro- ^e (6-Chloro-1-tetrahydronorharmanone) | 0.87 | 0.88 | 0.90 | 0.72 | 0.76 | 0.67 | B | — | — | — | — | — | Gre | — |
| 1-Oxo-6-fluoro- ^e (6-Fluoro-1-tetrahydronorharmanone) | 0.86 | 0.88 | 0.86 | 0.83 | 0.73 | 0.57 | B | — | — | — | — | — | Gre | — |
| 1-Oxo-6-methyl- ^c | 0.89 | 0.89 | 0.85 | 0.82 | 0.86 | 0.67 | B | — | — | — | — | — | Gre | — |
| 1-Oxo-6-nitro- ^c | 0.76 | 0.93 | 0.87 | 0.61 | 0.53 | g | P | — | Y | — | — | Pu | — | — |
| 1-Oxo-6-methoxy- ^c | 0.87 | 0.78 | 0.81 | 0.77 | 0.81 | 0.74 | B | Pu ^d | Pu | — | — | — | Gre | — |
| 1-Oxo-8-chloro- ^c | 0.87 | 0.91 | 0.91 | 0.72 | 0.79 | 0.74 | B | — | — | — | — | — | — | — |
| 1-Oxo-8-methyl- ^c | 0.89 | 0.88 | 0.89 | 0.70 | 0.76 | 0.76 | B | Mg ^d | Mg | — | — | — | — | — |
| Corynanthine ^h | 0.86 | 0.97 | 0.93 | 0.50 | 0.89 | 0.42 | B | Mg | Mg | — | — | — | Gre | — |
| Yohimbine ^{a,c} | 0.81 | 0.94 | 0.91 | 0.44 | 0.87 | 0.54 | B | Mg | Mg | — | — | Pu | Gre | — |
| 9-Methyl-yohimbine | 0.84 | 0.86 | 0.93 | 0.42 | 0.85 | 0.54 | B | Mg | Mg | Lt-Br | — | Pu | Gre | — |
| Rauniltidine ^e | 0.82 | 0.92 | 0.93 | 0.44 | 0.89 | 0.86 | Gr | Mg | Mg | Lt-Br | — | — | Y | — |

^a Hydrochloride salt.

^b Regis Chemical Company, Inc.

^c Aldrich Chemical Company, Inc.

^d Color appeared after heating.

^e Sulfate salt.

^f Orion Laboratories.

^g Streak.

^h International Chemical & Nuclear Corporation.

TABLE II

CHROMATOGRAPHY OF SUBSTITUTED DIHYDRO- β -CARBOLINES

Colors: B = blue; Br = brown; Bt = bright; Gr = green; Gre = grey; Lt = light; Or = orange; Pu = purple; Redd = reddish; Y = yellow.

| | <i>R_F</i> | | | | | | <i>Detection</i> | | | | | | | | | | | |
|--|--|----------|----------|---|----------|----------|--------------------------|----------------------------------|----------|----------|----------|----------|----------|----------|---|---|---|-------|
| | <i>Paper chromatography in solvent</i> | | | <i>Thin-layer chromatography in solvent</i> | | | <i>U.V. fluor. color</i> | <i>Color with spray reagents</i> | | | | | | | | | | |
| | <i>A</i> | <i>B</i> | <i>C</i> | <i>D</i> | <i>E</i> | <i>F</i> | | <i>a</i> | <i>b</i> | <i>c</i> | <i>d</i> | <i>e</i> | <i>f</i> | <i>g</i> | | | | |
| (a) <i>3,4-Dihydro-β-carbolines</i> | | | | | | | | | | | | | | | | | | |
| 6-Methoxy-1-methyl ^a (6-Methoxyharmalan) | 0.77 | 0.90 | 0.87 | 0.42 | 0.87 | 0.08 | Bt-Y | — | — | Br-Pu | — | — | — | — | — | — | — | Lt-Br |
| 7-Methoxy-1-methyl ^b (Harmaline) | 0.79 | 0.92 | 0.86 | 0.42 | 0.86 | 0.04 | Bt-Gr | — | — | Br-Pu | — | — | — | — | — | — | — | Lt-Br |
| 7-Hydroxy-1-methyl ^{c,d} | 0.71 | 0.75 | 0.80 | 0.45 | 0.75 | 0.00 | Bt-B-Gr | — | — | Redd-Br | Or-Br | — | — | — | — | — | — | Lt-Br |
| (b) <i>1,2-Dihydro-β-carboline</i> | | | | | | | | | | | | | | | | | | |
| 6-Methoxy-2-methyl-1-oxo- | 0.83 | 0.80 | 0.83 | 0.74 | 0.77 | 0.93 | B | — | — | Lt-Pu | Lt-Br | — | — | — | — | — | — | Gre |

^a Regis Chemical Company.^b Mann Research Laboratories, Inc.^c Aldrich Chemical Company, Inc.^d Hydrochloride salt.

TABLE III

CHROMATOGRAPHY OF SUBSTITUTED AROMATIC β -CARBOLINES

Colors: B = blue; Br = brown; Bt = bright; Gr = green; Gre = grey; Lt = light; Or = orange; Pu = purple; Redd = reddish; P = pink; V = violet; Y = yellow.

| | R_F | | | | | | Detection | | | | | | | | | |
|---|---------------------------------|------|------|--------------------------------------|------|------|---------------------------|---|---|---------|-------|----|-------|-------|--|--|
| | Paper chromatography in solvent | | | Thin-layer chromatography in solvent | | | Color with spray reagents | | | | | | | | | |
| | A | B | C | D | E | F | U.V. fluor. color | a | b | c | d | e | f | g | | |
| β -Carbolines (Norharman) | 0.77 | 0.90 | 0.92 | 0.57 | 0.87 | 0.57 | Bt-B | — | — | — | — | Or | P | V | | |
| 1-Methyl ^a (Harman) | 0.83 | 0.90 | 0.93 | 0.52 | 0.85 | 0.54 | Bt-B | — | — | — | — | — | P | V | | |
| 1-Ethyl ^b | 0.87 | 0.93 | 0.95 | 0.56 | 0.89 | 0.59 | Bt-B | — | — | — | — | — | P | V | | |
| 9-Methyl- | 0.80 | 0.91 | 0.92 | 0.40 | 0.88 | 0.86 | Bt-B | — | — | — | — | Or | P | Lt-Br | | |
| 1-Benzyl- | 0.91 | 0.94 | 0.96 | 0.63 | 0.88 | 0.98 | Bt-B | — | — | — | — | — | P | V | | |
| 1-Phenyl- | 0.89 | 0.93 | 0.96 | 0.63 | 0.89 | 0.99 | Bt-B | — | — | — | — | — | P | V | | |
| 1-(4-Pyridyl)- | 0.83 | 0.91 | 0.93 | 0.54 | 0.86 | 0.51 | Y | Y | Y | — | — | — | Y | — | | |
| 1-(2-Naphthyl)- | 0.90 | 0.94 | 0.97 | 0.71 | 0.88 | 0.96 | B-Gr | Y | Y | — | — | — | Gre | — | | |
| 6-Methoxy-1-methyl ^c (6-Methoxyharman) | 0.82 | 0.87 | 0.90 | 0.52 | 0.86 | 0.47 | Bt-B-Gr | — | — | — | — | — | Y | Lt-B | | |
| 6-Methoxy-1-benzyl ^b | 0.90 | 0.94 | 0.95 | 0.65 | 0.90 | 0.98 | Bt-Gr | — | — | — | — | — | — | — | | |
| 7-Methoxy-1-methyl ^e (Harmine) | 0.83 | 0.86 | 0.91 | 0.54 | 0.88 | 0.31 | Bt-B | — | — | Lt-Br | — | — | P-Gre | V | | |
| 7-Hydroxy-1-methyl ^{b,f} (Harmol) | 0.77 | 0.76 | 0.87 | 0.58 | 0.80 | 0.10 | Bt-B | — | — | Redd-Br | Or-Br | — | P-Br | V | | |
| 7-Hydroxy-1,2-dimethyl ^e (2-Methylharmine) | 0.81 | 0.63 | 0.87 | 0.29 | 0.31 | 0.00 | Bt-B | — | — | Lt-Br | — | — | Gre | V | | |
| 2-Methyl ^e (2-Methylnorharman) | 0.80 | 0.68 | 0.85 | 0.29 | 0.30 | 0.00 | Bt-B | — | — | — | — | Or | P | V | | |
| Sempervirine nitrated ^d | 0.88 | 0.84 | 0.89 | 0.51 | 0.65 | 0.06 | B | — | — | — | — | — | — | — | | |

^a Aldrich Chemical Company, Inc.

^b Hydrochloride salt.

^c Regis Chemical Company.

^d Mann Research Laboratories, Inc.

^e Calbiochem.

^f K & K Laboratories, Inc.

TABLE IV

SENSITIVITY OF COLOR REAGENTS TO 6-METHOXY- β -CARBOLINES ON TLC

| Compound | Xanthydrol | Ehrlich's | Ninhydrin |
|---|-----------------------|-----------------------|--|
| 6-Methoxy-1-methyl-tetrahydro- β -carboline | 1 μ g (positive) | 1 μ g (positive) | 1 μ g (negative) 5 μ g (positive) |
| 6-Methoxy-1-methyl-dihydro- β -carboline | 25 μ g (negative) | 25 μ g (negative) | 25 μ g (negative) |
| 6-Methoxy-1-methyl- β -carboline | 25 μ g (negative) | 25 μ g (negative) | 25 μ g (negative) |

Two color reagents, the acidic diazo reagent and α -nitroso- β -naphthol, are commonly used for detecting 6-hydroxylated and 5-hydroxylated indoles respectively⁸. However, they are found not to be specific for the 7-hydroxylated and 6-hydroxylated β -carbolines (Table V).

TABLE V

COLOR REACTIONS OF SOME HYDROXYLATED β -CARBOLINES AND INDOLES WITH ACIDIC DIAZO REAGENT AND α -NITROSO- β -NAPHTHOL ON TLCColors shown after the arrow (\rightarrow) were recorded 30 min after the appearance of the first colors.

| Compound ^a | Acidic diazo reagent ^b | α -Nitroso- β -naphthol ^{b,c} |
|------------------------------------|-----------------------------------|---|
| (a) 6-Hydroxy- β -carbolines | | |
| 1-methyl-tetrahydro- | Light purple ^d | Grey \rightarrow grey violet |
| 1-methyl-N-acetyl-tetrahydro- | Purple | Pink \rightarrow grey violet |
| (b) 7-Hydroxy- β -carbolines | | |
| 1-methyl-tetrahydro- | Violet \rightarrow redd. violet | Grey \rightarrow grey violet |
| 1,2-dimethyl-tetrahydro- | No color | Grey \rightarrow grey |
| 1-methyl-N-acetyl-tetrahydro- | Cherry red | Grey \rightarrow grey violet |
| 1-methyl-3,4-dihydro- | No color | Grey \rightarrow grey |
| 1-methyl- | No color | Grey \rightarrow grey |
| (c) 5-Hydroxy-indoles | | |
| Serotonin | No color | Grey \rightarrow grey violet |
| N-Acetylserotonin | No color | Grey \rightarrow grey violet |
| (d) 6-Hydroxy-indoles | | |
| 6-Hydroxy-N-acetyltryptamine | Brownish red | Grey pink \rightarrow grey violet |
| Melatonin | Brownish red | Purple \rightarrow deep purple |
| (e) 5,6-Dihydroxy-indole | | |
| 5,6-Dihydroxytryptamine | Purple | Grey \rightarrow grey purple |

^a For the acidic diazo reagent 10 μ g of each compound were used, and 25 μ g for the α -nitroso- β -naphthol.

^b Reference 8.

^c The same reagent gave same colors on paper.

^d Color appeared after one hour.

Both the aromatic and tetrahydro β -carbolines with no substituents on the C-1 position, the *nor* compounds, give an orange color reaction with 1% ninhydrin in *n*-butanol after heating. The 2-deaza compound carbazole, however, does not react.

With the various color reagents described it is possible to detect almost all the β -carbolines with the exception of 1-oxo compounds, which are difficult to dissolve and require large amounts of material for visible detection.

The fluorescent characteristics of U.V. light are useful in differentiating the aromatic from the tetrahydro compounds. The aromatic β -carbolines are more fluorescent than the tetrahydro compounds. The 6-hydroxylated and methoxylated compounds usually have a pink fluorescence, whereas the 7-hydroxylated and methoxylated compounds tend to have a blue fluorescence.

The addition of alkyl groups to positions 2 and 9 of the tetrahydro- β -carbolines increases the R_F values relative to the parent compounds in chloroform-methanol on thin-layer chromatography. This suggests an attraction, possibly through the formation of hydrogen bonding, between the silica and the N-H linkage of both the piperidine and pyrrole moieties of the tetrahydro- β -carbolines. If the pyrrole hydrogen is involved in hydrogen bonding, the replacement of this hydrogen by alkyl groups will reduce the attraction of β -carbolines for the silica, and, as a result, the 9-alkylated compounds readily migrate in the polar solvent such as chloroform-methanol. A similar increase in R_F values due to the alkylation on position 9 is seen in the aromatic series. For the 2-alkylated tetrahydro- β -carbolines, the high R_F values can be attributed to the decrease in the basicity of the piperidine function, since alkylation of the parent compounds converts a strongly basic secondary amine to a weakly basic tertiary amine and thus weakens the bonding to the silica. For the same reason the aromatic β -carbolines being weaker in basicity than the tetrahydro compounds have higher R_F values in chloroform-methanol. When both the positions 2 and 9 of the tetrahydro compounds are methylated as in the case of 1,2,9-trimethyltetrahydro- β -carboline, further weakening of the attraction to the silica results in a rise of R_F value above the 1,2- and 1,9-dimethyl- β -carbolines.

The 1-aryl derivatives tend to have a high R_F in butanol-acetic acid-water on paper chromatography and in chloroform-methanol on thin-layer chromatography. The exceptions are the 1-(4-pyridyl)tetrahydro- β -carboline and its aromatic analog, whose low R_F values in chloroform-methanol indicate a strong attraction between the pyridyl group and the silica.

The bonding of the hydroxylated β -carbolines to the silica is clearly seen in all three series from their low R_F values. With the methoxylated derivatives, bonding to the silica is relatively less and the resulting R_F values are somewhat higher, however they are comparatively lower than the parent compounds with no substituents on the benzene ring.

Neutral compounds like 2-acetyl- and 1-oxo-tetrahydro- β -carbolines have high R_F values.

The 1-carboxy substituted compounds tend to run slow in all the basic solvents. The addition of another polar carboxyl group to the position 3 of tetrahydro- β -carboline causes a further decrease in R_F value, as shown in 1,3-dicarboxy-1-methyl-tetrahydro- β -carboline.

The quaternary compounds tested remained at the origin in chloroform-methanol on thin-layer chromatography. They also run slower in acidic and basic solvent systems on the same adsorbent.

In conclusion, the data derived from the chromatography of the series of 73 β -carbolines would indicate that the best chromatographic system for the separation

of compounds of this class is a neutral and polar solvent such as chloroform-methanol on thin-layer chromatography with Silica Gel G.

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SUMMARY

Chromatography of a series of seventy-three β -carbolines was carried out on both paper and thin-layer plates in acidic, basic, and neutral solvents. The best solvent for the separation is the neutral and polar solvent such as chloroform-methanol on thin-layer chromatography with Silica Gel G. In general, tetrahydro- β -carbolines are more sensitive to xanthydrol than to Ehrlich's reagent. The former reagent is also more specific for tetrahydro compounds. Both the aromatic and tetrahydro compounds with no substituents on position 1 give an orange color reaction with 1% ninhydrin in *n*-butanol after heating.

The fluorescent characteristics of U.V. light are useful in differentiating the aromatic from the tetrahydro derivatives. The aromatic compounds are more fluorescent than the tetrahydro compounds. The 6-hydroxylated and methoxylated compounds usually have a pink fluorescence whereas the 7-hydroxylated and methoxylated compounds tend to have a blue fluorescence. Acidic diazo reagent and α -nitro- β -naphthol, which are commonly used for detecting 6-hydroxylated and 5-hydroxylated indoles respectively, are found not to be specific for the 7-hydroxylated and 6-hydroxylated β -carbolines.

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