A SYNTHESIS OF HARMALINE[1]

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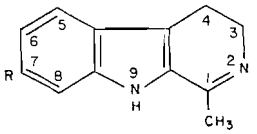
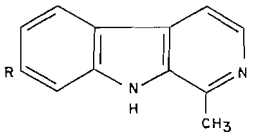
ABSTRACT

The synthesis of 1-methyl-3,4-dihydro-β-carboline (harmalan) and of two of its derivatives by dehydration of the corresponding 1-hydroxymethyl-1,2,3,4-tetrahydro-β-carbolines is described. Harmalan was also obtained by oxidative decarboxylation of 1-methyl-1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid.

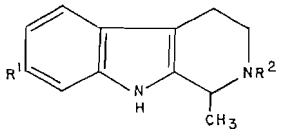
Harmalol (I), isolated from *Peganum harmala* L. (1), and the corresponding methyl ether harmaline (II), obtained from the same plant (1), as well as from *Banisteria caapi* Spruce (2), are the only 3,4-dihydro-β-carboline derivatives hitherto found to occur in nature. All other naturally occurring compounds containing the β-carboline ring system are either fully aromatic (IV) or contain a 1,2,3,4-tetrahydro-structure (VI) (3*a*). Amongst the structurally analogous isoquinoline alkaloids only two, psychotrine and O-methyl-psychotrine (3*b*) have so far been recognized as 3,4-dihydro-derivatives. The 3,4-dihydro-structure thus represents a rare oxidation state in these plant bases.



I R = OH
II R = OCH_3
III R = H



IV R = H
V R = OCH_3



VI $R^1 = R^2 = H$
VII $R^1 = H$, $R^2 = CH_3$
VIII $R^1 = OCH_3$, $R^2 = H$

Although the biosynthesis of the β-carboline alkaloids has not so far been investigated, it has long been assumed (4) that the nucleus arises in the plant by a Mannich-type condensation of tryptamine or oxytryptamine with an aldehyde to yield a tetrahydro-β-carboline derivative. Such condensations have been carried out in vitro under mild conditions of temperature and pH (e.g. (5)), and a number of tetrahydro-β-carbolines have been found in plants (e.g. 1-methyl-1,2,3,4-tetrahydro-β-carboline (eleagnine) (VI) (6, 7, 8), 1,2-dimethyl-1,2,3,4-tetrahydro-β-carboline (leptocladine) (VII) (9), and 1-methyl-7-methoxy-1,2,3,4-tetrahydro-β-carboline (tetrahydroharmine) (VIII) (2, 8)).

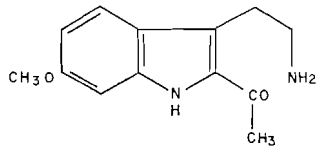
Partial dehydrogenation of these tetrahydro-derivatives has been postulated (4, 7, 10) to account for the origin of the dihydro-derivatives harmaline (II) and harmalol (I), and further loss of hydrogen for that of the β-carbolines (e.g. 1-methyl-β-carboline (harman) (IV) (3*a*) and its 7-methoxy-derivative (harmine) (V) (3*a*)).

If this stepwise oxidation sequence were indeed to represent the biosynthetic origin of dihydro-β-carbolines and β-carbolines, the former might be expected to accompany the latter frequently in the plant. The comparative rarity of the dihydro-derivatives is thus puzzling and led us to consider other possible biogenetic routes.

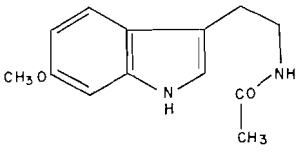
Neither of the reported syntheses of harmaline (II) can be regarded as models for in vivo formation. The synthetic base was first obtained (11) by cyclization of 1-acetyl-2-

[1]*Manuscript received July 2, 1959.*
Contribution from the Department of Chemistry, McMaster University, Hamilton, Ontario.

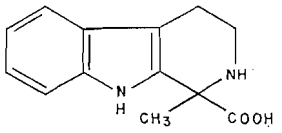
aminoethyl-6-methoxyindole (IX), and later by Bischler–Napieralski ring closure of



(IX)



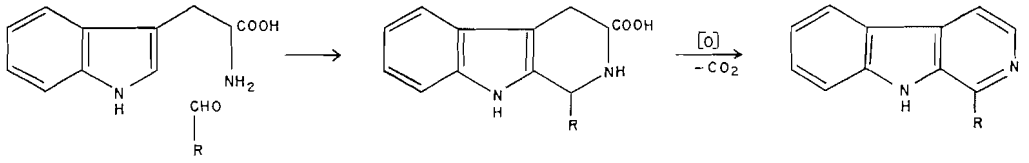
(X)



(XI)

N_β-acetyl-6-methoxytryptamine (X) (12). Much earlier Perkin and Robinson (4) had obtained the compound by oxidation of tetrahydroharmine (VIII) with permanganate in acetone. Under nonacidic conditions dihydro-β-carbolines thus appear to be stable to oxidizing agents. This stability has now been confirmed: 1-methyl-3,4-dihydro-β-carboline (III) (harmalan) was obtained in good yield both by oxidation of 1-methyl-1,2,3,4-tetrahydro-β-carboline (VI) (13) in acetone with permanganate, and also by oxidative decarboxylation of 1-methyl-1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid (XI) (5) with silver oxide in aqueous solution. This latter synthesis of a dihydro-β-carboline was modelled on our studies of oxidative decarboxylation of amino acids (14).

Under acid conditions (nitric acid (15), chromic acid (16), acid permanganate (12, 17)) dihydro-β-carbolines are oxidized to β-carbolines. Oxidation of tetrahydro-β-carbolines under similar conditions does not take place as readily (12) and leads to β-carbolines in poor yield (18). β-Carbolines are obtained in good yield by acid dichromate oxidation of tetrahydro-β-carboline-3-carboxylic acids (XII), decarboxylation accompanying the reaction (19). The biogenetic origin of β-carbolines, without intermediate formation of dihydro-β-carbolines, has been proposed in analogy to this reaction sequence (20).

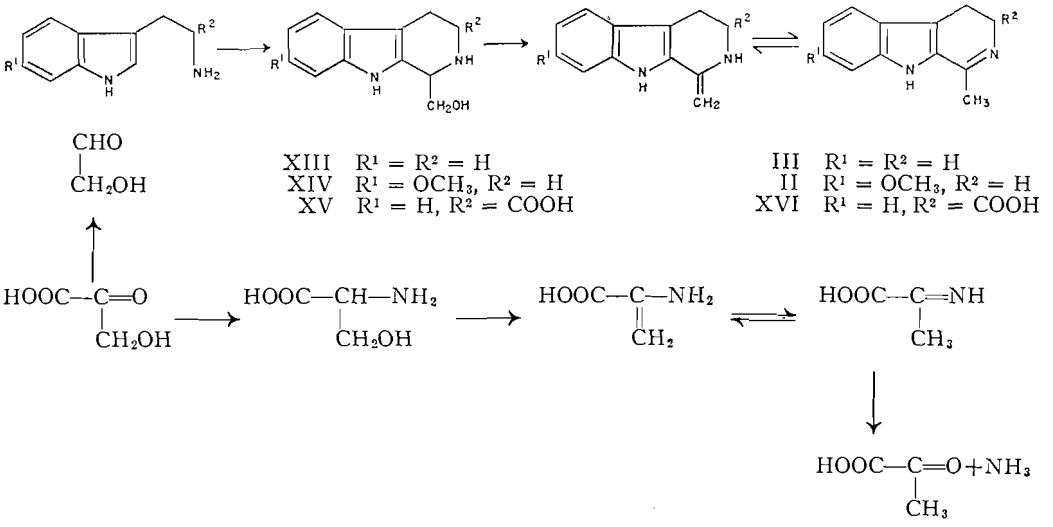


(XII) (IV)

In the present synthesis harmaline was obtained in two physiologically possible steps, the C=N double bond being introduced by dehydration, rather than oxidatively: 6-Methoxytryptamine (21)[2] was condensed with glycolaldehyde to give (±)-1-hydroxymethyl-7-methoxy-1,2,3,4-tetrahydro-β-carboline (XIV) in good yield. This step involves the usual Mannich reaction between a biogenetic amine and an aldehyde. The latter, glycolaldehyde, is known to arise enzymically *inter alia* by decarboxylation of hydroxypyruvic acid (22), a metabolic precursor of serine (23).

In a similar manner tryptamine gave (±)-1-hydroxymethyl-1,2,3,4-tetrahydro-β-carboline (XIII), and *dl*-tryptophan yielded the corresponding 3-carboxylic acid (XV) (24).

[2]*I am grateful to Dr. R. A. Abramovitch, University of Saskatchewan, for a specimen of this material.*



Dehydration of these tetrahydro-derivatives gave the corresponding 3,4-dihydro-β-carbolines. Harmaline (II) was obtained in 70% yield with 90% w/w phosphoric acid, harmalan (III) in 75% yield with phosphoric acid and in 25% yield with 50% w/v sulphuric acid. In each instance the product was indistinguishable from an authentic specimen.[3] 1-Methyl-3,4-dihydro-β-carboline-3-carboxylic acid (XVI) was obtained in moderate yield with 50% w/v sulphuric acid. The course of the dehydration reaction was followed in each case by observing the change in ultraviolet absorption (Figs. 1 and 2).

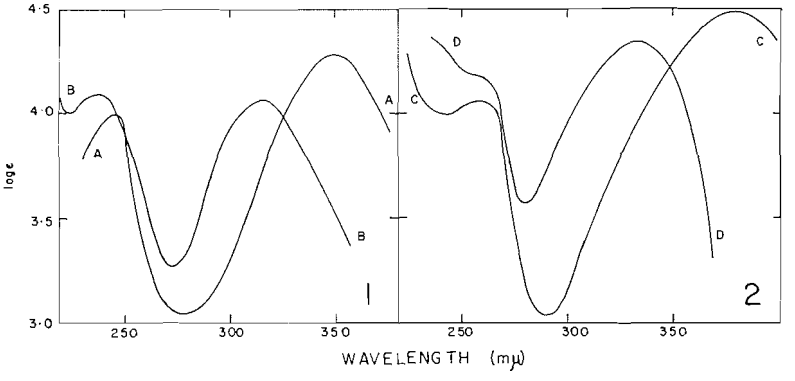
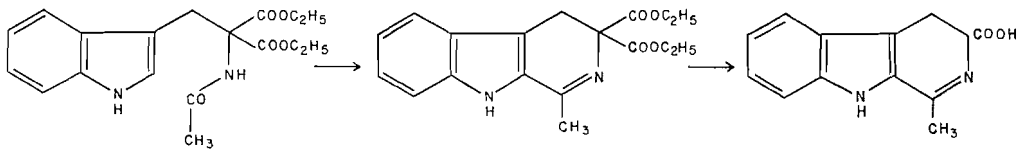


FIG. 1. 1-Methyl-3,4-dihydro-β-carboline (harmalan). Curve A, in 0.1 *N* HCl in aqueous ethanol; curve B, in 0.1 *N* NaOH in aqueous ethanol.
FIG. 2. 1-Methyl-7-methoxy-3,4-dihydro-β-carboline (harmaline). Curve C, in 0.1 *N* HCl in aqueous ethanol; curve D, in 0.1 *N* NaOH in aqueous ethanol.

The dihydro-β-carboline (XVI) derived from tryptophan had not hitherto been described. Another synthetic route was therefore sought to confirm its identity. Bischler–Napieralski ring closure of N_β-acetyltryptophan was not suitable since it has proved unsuccessful with classical dehydrating agents (24, 25) while with polyphosphoric acid

[3]*I am greatly indebted to Dr. T. M. Sharp, Wellcome Laboratories, London, England, for a sample of natural harmaline.*

it was accompanied by decarboxylation and aromatization (26) to yield 1-methyl-β-carboline (IV) instead of the desired product. However, cyclization of ethyl α-acetamido-α-carbethoxy-β-(3-indolyl)-propionate (XVII) (27) gave 1-methyl-3,3-dicarbethoxy-3,4-dihydro-β-carboline (XVIII) (28) and the latter was saponified and decarboxylated, to give a 65% yield of the desired compound (XVI), identical with a specimen obtained by dehydration.



(XVII) (XVIII) (XVI)

A second, as yet unidentified material, differing in infrared and ultraviolet absorption from the main product was also isolated in this reaction.

The dehydration step in the present harmaline synthesis parallels the catabolic pathway of serine (29), enzymatic dehydration to α-aminoacrylic acid, and rearrangement to α-iminopropionic acid. Whereas this compound spontaneously hydrolyzes to pyruvic acid and ammonia, the imino-linkage in harmaline is stabilized by conjugation with the indole system. In vitro this dehydration of serine requires strong acid (30).

The synthesis of harmaline here described is put forward as a possible biogenetic route and an alternative to the oxidative pathway postulated by earlier authors (4, 7, 10). The recent confirmation (31) of the structure of calycotomine as 1-hydroxymethyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline, analogous to the intermediate in the present synthesis, lends additional interest to this hypothesis.

EXPERIMENTAL

1-Methyl-3,4-dihydro-β-carboline (harmalan) (III)

(1) By Dehydration of 1-Hydroxymethyl-1,2,3,4-tetrahydro-β-carboline (XIII)

(±)-1-Hydroxymethyl-1,2,3,4-tetrahydro-β-carboline

Tryptamine hydrochloride (5.9 g, 0.03 moles) was dissolved in 250 ml warm water containing 15 ml 2 *N* HCl (1.095 g, 0.03 moles) and the solution was filtered. To the cold solution was added an aqueous solution of 1.90 g (0.032 moles) glycolaldehyde (32) and the mixture was kept on the steam bath (2 hours) until a sample no longer gave a precipitate with dinitrophenylhydrazine. The cooled solution was then treated with charcoal, filtered, extracted with ether to remove nonbasic impurities, concentrated, and treated with excess of 20% NaOH, when the product precipitated as an oil which crystallized on standing. It was taken up in ether, and the solution was dried with KOH and concentrated to yield an oil which crystallized on digestion with warm water to give the trihydrate of 1-hydroxymethyl-1,2,3,4-tetrahydro-β-carboline (6.25 g, 81%) melting at 138–139° after sintering below 100°. Recrystallization from aqueous ethanol did not raise the melting point. Distillation of a small sample at 2.10^{-2} mm and 140–150° gave an oil, crystallizing into a white solid, melting at 144–145°, which still retained water of crystallization. (Found: H_2O, 19.18. $C_{12}H_{14}ON_2,3H_2O$ requires H_2O, 21.10%. After drying at 120° found: C, 71.5; H, 6.8. $C_{12}H_{14}ON_2$ requires C, 71.5; H, 7.0%.)

The hydrochloride, recrystallized from ethanol, melted at 215–216° after darkening from 205°. (Found: C, 56.3; H, 6.8; N, 10.5. $C_{12}H_{15}ON_2Cl,H_2O$ requires C, 56.1; H, 6.7; N, 10.9%.)

Harmalan

(*i*) *90% w/w* H_3PO_4.—The above tetrahydro-β-carboline trihydrate (3.37 g, 0.013 moles) was wetted with 10 ml water, 100 ml 90% H_3PO_4 was added, and the suspension was kept on the steam bath until the absorption band at 280 mμ, characteristic of the indole chromophore of the starting material, had disappeared. The reaction was complete after 2 hours. The dark brown solution was diluted with water, extracted with ether, treated with charcoal, and made alkaline with 20% NaOH. The precipitate was collected, dried in air, and extracted in the Soxhlet extractor with 70–100° petroleum ether, from which harmalan crystallized in fluffy needles, melting at 178–179° in a total yield of 1.80 g (75%). For analysis a sample was sublimed at 1.10^{-3} mm and 90° C. (Found: C, 77.9; H, 6.4; N, 15.1. Calc. for $C_{12}H_{12}N_2$: C, 78.2; H, 6.6; N, 15.2%.) Infrared absorption (Nujol) (cm^{-1}): 1622 (m), 1605 (m), 1570 (m), 1550 (s). Ultraviolet absorption (Fig. 1) (λ_{max}, mμ (log ε)); in 0.1 *N* HCl: 245 (4.04); 350 (4.28); in 0.1 *N* NaOH: 240 (4.09), 315 (4.06).

(*ii*) *50% w/v* H_2SO_4.—The tetrahydro-β-carboline (0.11 g, 0.00043 moles) was treated with an ice-cold mixture of 2 ml 95% H_2SO_4 and 4 ml water. After 2 hours on the steam bath and standing overnight, the solution showed a strong dihydro-β-carboline peak at 350 mμ, but the indole peak at 280 mμ was still apparent. The latter had disappeared after a further 2 hours on the steam bath with an additional 1 ml of 95% H_2SO_4. The solution was diluted with water, treated with charcoal, and the product precipitated by addition of a little 20% NaOH, when harmalan, melting at 176–177°, was obtained in poor yield (25%). When the experiment was carried out at room temperature, even after 1 week unchanged starting material was recovered.

(*2*) *By Oxidation of 1-Methyl-tetrahydro-β-carboline* (VI)

1-Methyl-1,2,3,4-tetrahydro-β-carboline (13) (0.186 g, 0.001 moles) was dissolved in acetone. To the ice-cold solution was added 0.11 g (0.0007 moles) powdered $KMnO_4$. When reaction was complete, MnO_2 was filtered off, the solution evaporated, the residue taken up in ether, dried over NaOH, the solvent removed, and the residual solid extracted with boiling ligroin, from which harmalan (0.11 g, 65%), melting at 176–178°, crystallized.

(*3*) *By Oxidative Decarboxylation of 1-Methyl-1,2,3,4-tetrahydro-β-carboline-1-carboxylic Acid* (XI)

1-Methyl-1,2,3,4-tetrahydro-β-carboline-1-carboxylic Acid

Cooled aqueous solutions of tryptamine hydrochloride (0.98 g, 0.005 moles) and pyruvic acid (0.48 g, 0.0055 moles) were mixed and left at 37° overnight when the product had separated in 85% yield (0.96 g) as colorless crystals, melting with decarboxylation at 217–219°. Recrystallization from dilute aqueous ammonia did not raise the melting point, which is reported as 220° (5).

Harmalan

The above amino acid (0.115 g, 0.0005 moles) in 20 ml water was refluxed 24 hours with freshly precipitated Ag_2O (0.23 g, 0.001 moles). Metallic silver deposited, and the solution gave an ultraviolet absorption curve characteristic of a dihydro-β-carboline. The mixture was filtered hot. On cooling a solid precipitated, which on recrystallization from boiling petroleum ether gave 0.079 g (85%) of harmalan, melting at 178–180°.

The products obtained from each of the above experiments were compared with a sample of harmalan prepared from N_β-acetyltryptamine (12) and were found to have identical ultraviolet (Fig. 1) and infrared (Nujol) spectra, melting points, and mixed melting points.

1-Methyl-7-methoxy-3,4-dihydro-β-carboline (harmaline) (II)

6-Methoxytryptamine (21) (0.033 g, 0.00017 moles) was dissolved in 3.5 ml 0.1 *N* HCl (0.00035 moles) and heated on the steam bath with 0.011 g (0.00019 moles) glycolaldehyde, until a sample no longer gave a precipitate with dinitrophenylhydrazine (1½ hours). The solution was filtered, concentrated, washed into a continuous extractor, overlayered with ether, and made alkaline with 10 ml 0.5 *N* NaOH. Eighteen hours' extraction, drying over KOH and removal of solvent, gave 50 mg of a white crystalline solid, melting at 170–175°, after sintering at 85°, presumably a hydrate of 1-hydroxymethyl-7-methoxy-1,2,3,4-tetrahydro-β-carboline (XIV). (Ultraviolet absorption (λ_{max}, mμ (log ε)): 225 (4.37), 275 (3.53), 295 (3.65) in ethanol.) Without further purification, this base was treated with 2.5 ml 90% H_3PO_4 and kept on the steam bath for 2 hours, when the solution gave an ultraviolet curve identical with that of natural harmaline (footnote 2). The solution was diluted with water, placed in a continuous extractor, made alkaline with 20% NaOH, and the product was extracted into ether. The ether extract was dried, the solvent removed, and the product distilled at 10^{-3} mm and 120–140° to yield 27 mg (72%) harmaline, melting at 235–237°, identical with an authentic specimen in infrared and ultraviolet absorption (Fig. 2), R_f value (0.89) in 95% ethanol/.880 ammonia (99:1), melting point and mixed melting point. (Found: C, 72.6; H, 6.6; N, 13.1. Calc. for $C_{13}H_{14}ON_2$: C, 72.8; H, 6.6; N, 13.1%.) Infrared absorption in (Nujol) (cm^{-1}): 1620 (s), 1600 (m), 1570 (m), 1535 (s). Ultraviolet absorption (Fig. 2) (λ_{max}, mμ (log ε)): in 0.1 *N* HCl: 260 (4.07), 380 (4.49); in 0.1 *N* NaOH: 260 (4.18) (shoulder), 335 (4.34).

A portion was converted to the picrate, orange needles, melting at 228–229° after sintering at 215°, after recrystallization from aqueous ethanol, identical with the picrate derived from the authentic sample.

1-Methyl-3,4-dihydro-β-carboline-3-carboxylic acid (XVI)

(*1*) *By Dehydration of 1-Hydroxymethyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic Acid* (XV)

1-Hydroxymethyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic Acid

dl-Tryptophan (1.02 g, 0.005 moles) was dissolved in *N* HCl (5 ml, 0.005 moles), 0.33 g glycolaldehyde (0.0055 moles) was added, and the mixture was kept on the steam bath for 2 hours when a sample no longer gave a precipitate with dinitrophenylhydrazine. The dark solution was treated with charcoal, the pH of the filtrate was adjusted to pH 7, and a small amount of precipitate was removed by filtration. On concentration the product crystallized, and on recrystallization from boiling water was obtained in 51% yield (0.63 g) as long needles, decomposition 256–257° after darkening from 235°. No attempt was made to separate the diastereoisomers. A melting point of 234° was reported (24) for the product derived from *l*-tryptophan. (Found: C, 63.1; H, 5.7; N, 11.6. Calc. for $C_{13}H_{14}O_3N_2$: C, 63.4; H, 5.7; N, 11.4%.)

1-Methyl-3,4-dihydro-β-carboline-3-carboxylic Acid

The above amino acid (0.37 g, 0.0015 moles) was treated with an ice-cold solution of 2 ml 95% H_2SO_4 in 5 ml H_2O. After keeping this solution at room temperature for 48 hours no absorption at 355 mμ was observed. The solution was then kept on the steam bath for

2 hours, when absorption at 355 mμ appeared, but since the ultraviolet absorption band at 280 mμ was still strong, a further 1 ml 95% H_2SO_4 was added. After 2 hours the reaction was complete. The solution was diluted with water, absorbed on a Zeocarb cation exchange resin in the acid form, and eluted with ammonia. The eluate was concentrated and the residue taken up in aqueous ethanol, treated with charcoal and allowed to stand at 0°, giving 88 mg (26%) of yellow crystals, melting at 189–190° after darkening at 185°, of the dihydrate of 1-methyl-3,4-dihydro-β-carboline-3-carboxylic acid. (Found: H_2O, 14.0. $C_{13}H_{12}O_2N_2,2H_2O$ requires H_2O, 13.6%. After drying at 100° found: C, 68.2; H, 5.4; N, 12.0. $C_{13}H_{12}O_2N_2$ requires C, 68.4; H, 5.3; N, 12.3%.) Infrared absorption (Nujol) (cm^{-1}): 1655 (m), 1632 (m), 1615 (m), 1592 (s), 1570 (s), 1523 (m), 1445 (s). Ultraviolet absorption (λ_{max}, mμ (log ε)): in 0.1 *N* HCl: 245 (3.97), 355 (4.29); in 0.1 *N* NaOH: 235 (4.10), 315 (4.07).

(2) By Malonic Ester Synthesis

Ethyl α-Acetamido-α-carbethoxy-β-(3-indolyl)-propionate (XVII)

This was prepared by the pyridine method of Howe, Zambito, Snyder, and Tishler (27) which was found more satisfactory than their other methods. It was obtained as colorless crystals, melting at 142–144°.

1-Methyl-3,3-dicarbethoxy-3,4-dihydro-β-carboline (XVIII) (*cf.* (*28*))

Ethyl α-acetamido-α-carbethoxy-β-(3-indolyl)-propionate (1.04 g, 0.003 moles) was gently warmed with 5 ml $POCl_3$ for 45 minutes, when the band at 280 mμ in the ultraviolet spectrum of the original solution had been completely replaced by the band at 355 mμ. The reaction mixture had become dark brown. Excess $POCl_3$ was distilled off at room temperature at 10^{-3} mm, and the brown tarry residue triturated with excess NH_3. On standing overnight the tarry salt had been converted into the solid, crude base, which was filtered off and taken up in ether. The ether solution was washed with dilute NH_3 and H_2O, and the product then extracted into 0.02 *N* HCl. The acid solution was immediately treated with dilute ammonia, and the product re-extracted into ether and dried over Na_2SO_4. Removal of solvent gave a yellow solid which was dissolved in acetone. Addition of 70–100° petroleum ether and cooling gave the product as long yellow needles, melting at 154–156°, which was identical in infrared and ultraviolet absorption with the product described by Hardegger and Corrodi (28), melting at 147–149°[4] (yield 0.45 g, 44%). (Found: C, 65.6; H, 6.3; N, 8.7. Calc. for $C_{18}H_{20}O_4N_2$: C, 65. 8; H, 6.1; N, 8.5%.) Infrared absorption (cm^{-1}) 1725 (s), 1612 (w), 1595 (m), 1570 (w), 1540 (m), 1500 (w). Ultraviolet absorption (λ_{max}, mμ (log ε)): in 0.1 *N* HCl: 245 (4.11), 355 (4.40); in 0.1 *N* NaOH: 240 (4.18), 318 (4.22).

1-Methyl-3,4-dihydro-β-carboline-3-carboxylic Acid

1-Methyl-3,3-dicarbethoxy-3,4-dihydro-β-carboline (0.2 g, 0.00061 moles) was wetted with a little ethanol, suspended in 1.22 ml *N* NaOH (0.00122 moles), and warmed for 2 hours. A small quantity of tarry material was filtered off and the filtrate acidified by addition of 19 ml 0.982 *N* HCl (0.00183 moles) and decarboxylation accelerated by keeping the mixture on a steam bath for 30 minutes. A small amount of solid was filtered off. The solution was applied to a column of Dowex 50, was washed free of chloride ion, and the product eluted with *M* NH_3. Concentration gave a small amount of a substance, crystallizing as long needles and melting at 188–190°, which gave an infrared spectrum ((Nujol) (cm^{-1}): 1620 (m), 1545 (m), 1510 (w)) different from that of the expected product (*vide supra*) and an ultraviolet absorption which even after repeated recrystallization of

[4]*I thank Dr. E. Hardegger for a reference sample of this material.*

the substance showed, in addition to the dihydro-β-carboline bands at 245 mμ and 355 mμ in acid solution and 240 mμ and 318 mμ in alkaline solution, another band at 278 mμ in acid solution and at 256 mμ (shoulder) in alkaline solution.

Further concentration of the solution gave the desired product (0.09 g, 64%) as yellow crystals, melting at 186–188°, identical in infrared (Nujol) and ultraviolet absorption (*vide supra*), melting point, and mixed melting point with that obtained by dehydration.

ACKNOWLEDGMENTS

Financial assistance by the National Research Council of Canada and by the Ontario Research Foundation is gratefully acknowledged.

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