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AROMATIZATION OF 3,4-DIHYDRO-β-CARBOLINE-3-CARBOXYLIC ACID AND ITS DERIVATIVES THROUGH A CARBANION INTERMEDIATE : MECHANISTIC STUDY AND USE IN CHEMICAL SYNTHESIS

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ABSTRACT

3,4-dihydro-β-carboline-3-carboxylic acid, its esters and amide derivatives (AH_2) undergo complete aromatization into corresponding β-carboline derivatives (A) by basic treatment under mild conditions. This, together with the easy obtention of 3,4-dihydro-β-carboline from the parent N^{α}formyltryptophan derivatives constitutes an attractive possibility to obtain complex molecules containing the β-carboline ring.
The mechanism of the reaction was investigated by polarography, U.V.spectrometry and polarimetry.
In alcaline media, (AH_2) undergoes two successive equilibria : the first yielding a C_3 carbanion occurs with suppression of the chirality while the second yields a thermodynamically preferred C_4 carbanion which undergoes aromatization through an oxidative pathway ($K'_a = 2 \times 10^{-14}$ at 45°C).

Compounds containing the β-carboline structure have recently aroused considerable interest in neuropharmacology. Some β-carboline-3-carboxylic acid derivatives are in fact potent inhibitors of the specific binding of ^{3}H-diazepam to its brain receptors (1). It has even been proposed that the β-carboline moiety composes part of the structure of the endogenous ligand of the benzodiazepine receptors, although this has yet to be confirmed.

The rapidly growing interest in the β-carboline derivatives (2,3,4) suffers somewhat from the limitations, imposed by the chemical methods presently available for their preparation. The most common synthesis of β-carboline-3-carboxylic acid derivatives is summarized in Diagram 1.

The reaction of Trp with formaldehyde yields tetrahydro-β-carboline-3-carboxylic acid (AH_4) (5) which is esterified and then oxidized to aromatic β-carboline derivatives (A).

The esterification step is needed to avoid the decarboxylation which would result from oxidative treatment (6). The ester derivative, apart from its own biological interest (1), is the only possible starting point available for the preparation of other 3-carboxylic acid derivatives.

The main limitations of the above synthetic procedure are its low yield after the oxidative step, and the fact that the whole process is rather cumbersome.

Diagram 2 shows an alternative procedure for chemical synthesis.

DIAGRAM 1

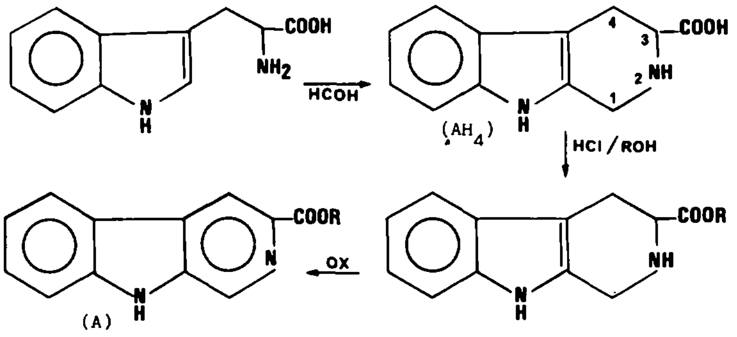
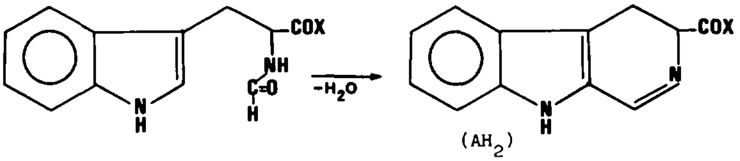


DIAGRAM 2



N^{α} formyl derivatives of tryptophan can in fact be cyclized to (AH_2) derivatives with a high yield, and the reaction can be carried out using dehydrating reagents (7) or anhydrous acidic solvents (8). Under the latter condition, the reaction occurs without loss of steric configuration when optically active Trp derivatives are used as starting material.

This pathway of chemical synthesis can be used with several derivatives, ranging from simple N^{α} formyl Trp to the more complicated peptide molecules with N^{α} formyl Trp as a terminal residue. Last but not least, the dihydro-β-carboline derivatives exibit biological properties similar to those of their fully aromatic analogs (4). In addition, the oxidative treatments used for the aromatization of (AH_4) can also be applied to (AH_2) derivatives.

In the present paper we demonstrate that (AH_2) derivatives are endowed with C-H acidity and generate ambident carbanions when dissolved in alkaline media. These anionic species can be easily oxidized leading to the corresponding fully aromatic derivatives. The use of this reaction for preparative purposes is described for a number of β-carboline derivatives.

RESULTS

Chemical and structural investigations

Table 1 shows the rates of aromatization of methyl-3,4-dihydro-β-carboline-3-carboxylate in basic media.

Table 1. Extent of aromatization of methyl 3,4-dihydro-β-carboline-3-carboxylate , 23°C, 10^{-4}M in :

A = 0.2 M N-ethylmorpholine in methanol
B = 1 M N-ethylmorpholine in methanol
C = 1 M N-ethylmorpholine, 0.2 M acetic acid in methanol

Minutes	Aromatization %		
	A	B	C
10	10	16	23
20	20	27	41
40	32	43	63
80	48	60	88

Aromatization of (AH_2) was recorded by monitoring the decrease of optical density at 319 nm (Fig 1). The aromatization depends on the concentration of the base and, as expected, the acetate anion is more efficient than the tertiary amine. In preparative experiments, the reaction product was isolated and identified as methyl-β-carboline-3-carboxylate (UV spectra, NMR spectra, elemental analysis). The aromatization in particular was clearly demonstrated by NMR spectra as shown in Fig. 2 . NMR spectra of (AH_2) derivative show a triplet at 4.7-5 ppm corresponding to the C-H in position 3 and a doublet at 3.9-4.2 ppm corresponding to the CH_2 in position 4. These signals are not observed in spectra of fully aromatic compounds (A), which show characteristic singlets at 8.82-9 ppm corresponding to the C-H at position 4.

Abnormal peptides containing the 3,4-dihydro-β-carboline-3-carboxylic acid residue can be converted into fully aromatic derivatives by basic treatment (see Experimental). Comparative NMR spectra of peptides containing (AH_2) and (A)moiety are summarized in Tab. 2.

An alternative way of synthesizing molecules containing β-carboline is by using β-carboline-3-carboxylic acid as the starting product. The choice of this method depends on whether sufficient quantities of pure β-carboline-3-carboxylic acid are easily available.

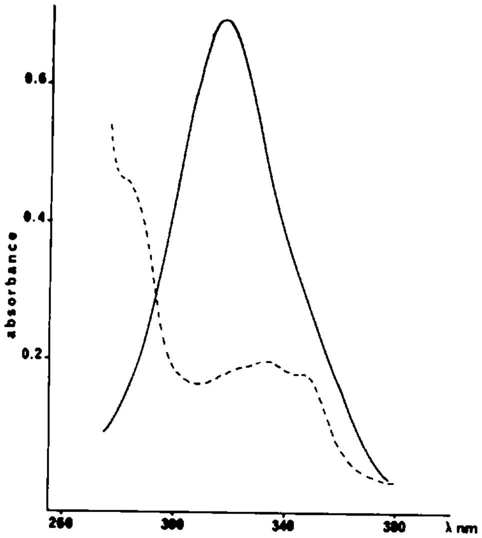


Fig. 1 Comparative spectra of 3,4-dihydro-β-carboline-3-carboxylic acid (———) and its fully aromatic derivative (- - - -). 0.5 x 10^{-4} M in 0.5 M K_2CO_3

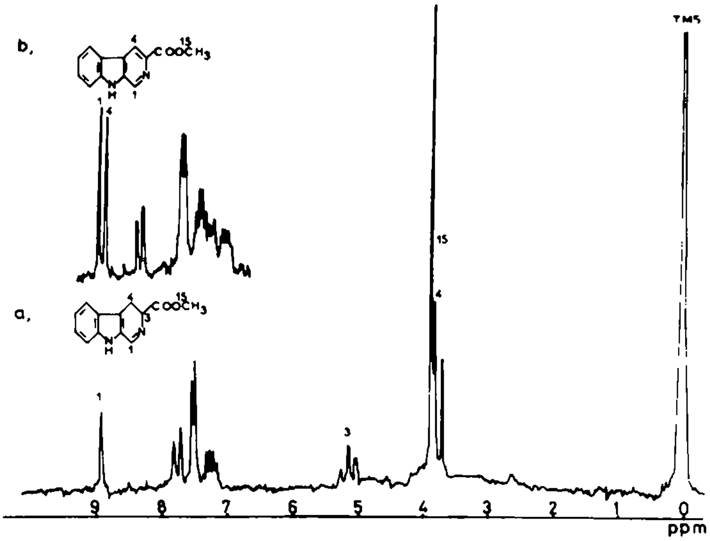
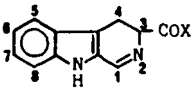
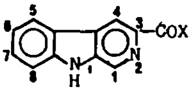


Fig. 2 ^1H NMR spectra in CD_3OD (reference TMS) of :

a) Methyl-3,4-dihydro-β-carboline-3-carboxylate,
b) Methyl-β-carboline-3-carboxylate

Table 2. Chemical shifts (in ppm) with respect to TMS of the characteristic protons of β-carboline derivatives (all measurements were made at room temperature in CD_3OD solutions)

X =					
	C-H (1)	C-H (3)	CH_2(4)	C-H (1)	C-H (4)
-OH	8.9	5	3.8 - 3.68	8.9	9
$-OCH_3$	9	5	3.8 - 3.7	8.82	8.8
-L-Phe·OEt	8.4	4.7	4.14 - 4.23	8.8	8.82
-L-Tyr·OEt	8.4	4.7	4.2 - 4.25	8.8	8.83
-Gly·OEt	8.41	4.7	4.15 - 4.20	8.79	8.82

We have shown in a preceding study (8) that the consecutive conversion Trp ⟶ N$^{\alpha}$formyl-Trp ⟶ 3,4-dihydro-β-carboline-3-carboxylic acid can take place under mild reaction conditions with high yields. 3,4-dihydro-β-carboline-3-carboxylic acid can be fully aromatized without decarboxylation in an alcoholic or aqueous basic solution. Aqueous alcali are more advantageous for preparative purposes because of the greater solubility of 3,4-dihydro-β-carboline-3-carboxylic acid and the physical and chemical properties of its aromatic derivative which facilitate its isolation (see Experimental).

When optically active (AH_2) are used, the aromatization is followed by a suppression of the optical activity of their solutions (Tab.3).

Table 3. Racemization and aromatization of 3,4-dihydro-β-carboline-3-carboxylic acid

Time (min)	% Retention of optical activity (% Aromatization)	
	23°C	45°C
2	94 (2)	82 (12)
5	86 (8)	55 (38)
10	70 (15)	35 (62)
20	47 (28)	2 (80)
45	16 (46)	
90	0 (64)	

This suggests that hydrogen exchange occurs at the chiral center of the molecule yielding the racemic compound according to Diagram 3.

(AH_2) can be considered structurally as an internal Schiff base of the hypothetical 2-formyl tryptophan.

This special structure makes acidic the proton at position 3 which therefore can be removed by a base yielding a carbanion (AH^-).

The ability to produce a carbanion in basic aqueous media accounts for the yield of fully aromatic derivative. However, the loss of the optical activity occurs faster than the formation of the corresponding aromatic derivatives . So, (Tab. 3), part of this work has been devoted to investigating the mechanism of aromatization.

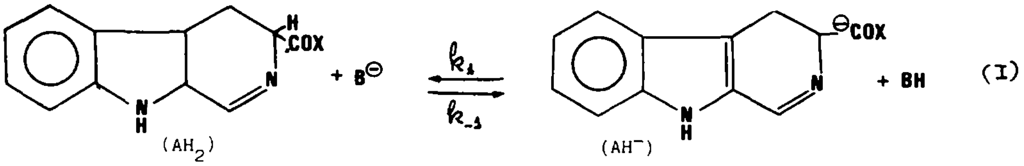
Kinetic investigation of the formation of an ambident carbanion

3,4-dihydro-β-carboline-3-carboxylate undergoes two successive equilibria when dissolved in water-methanol (9 : 1) alkaline media. The first is accompanied by suppression of the optical activity according to the reaction scheme

$$(AH_2) + OH^- \rightleftharpoons (AH^-) + H_2O \qquad (I)$$

Simultaneously the UV spectrum exhibits an evolution. The band situated at 328 nm decreases with time (Tab. 4) while the polarogram shows a slower evolution. The cathodic current recorded within a few minutes after addition of the stock methanolic solution in an aqueous

DIAGRAM 3



buffered solution, (1 : 9) reaches 2e according to the gross electrochemical reaction :

$$>C = N - \ + 2e + 2H^+ \longrightarrow >CH - NH -$$

but it decreases with time in a second step. On the other hand the decrease of the cathodic wave is accompanied at pH > 13.0 by an increase in the anodic wave (Tab. 5). The height of the anodic wave increases with time until it reaches an equilibrium value noted i_{an}^{eq} .

Table 4. Values shown by the main bands recorded at 25°C in buffered water-methanol (9:1) solutions of. 3,4-dihydro-β-carboline-3-carboxylate, biionized species; c = 5 x 10^{-4} M. **(AH$^-$)**

λ nm	235(sh)	280	328	380
ε $M^{-1}cm^{-1}$	15000	11600	9400	2000

When equilibrium is reached, the plot of i_{an}^{eq} as a function of pH increases in the shape of a dissociation curve with the inflexion point at pH 13.7 (K_a' = 2 x 10^{-4}). Simultaneously, the electronic spectrum displays a limiting value ε = 7000 $M^{-1}cm^{-1}$ at 360 nm, in NaOH 1 M medium. The polarographic behaviour corresponds to the occurrence of the tautomeric equilibrium (eqn II).

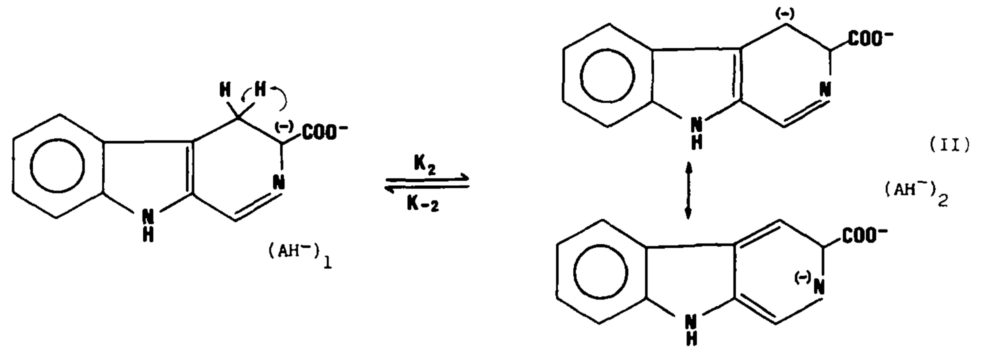
The $(AH^-)_2$ species is no longer reducable but can be oxidized at the mercury electrode according to the gross electrochemical reaction (eqn III).

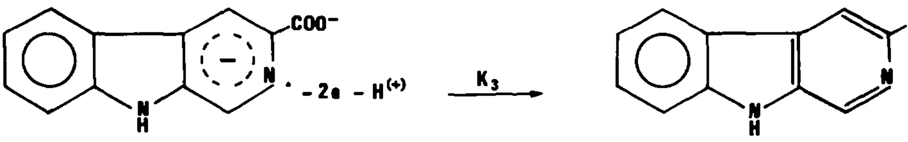
In LiOH 1 M, kinetic data obtained for reaction (eqn I) concerning the decrease of the chirality of the UV absorption at 320 nm yield the experimental rate constant

$k_{obs} = k_1 = 3 \times 10^{-3}\ s^{-1}$ at 45°C.

Table 5. Evolution v.s. time of the cathodic and anodic diffusion controlled current shown by 3,4-dihydro-β-carboline-3-carboxylate in NaOH 1 M, at 45°C methanol 10 %. sensitivity : 250 nm = 2.5 μA ; c = 5 x 10^{-4} M.

t mn	5	15	22	31	45	60	73	88	310
k_{cat}	75	50	36	29	21	17	15	13	15
k_{an}	6	25	43	56	66	68	67	64	22





$(AH^-)_2$ A (III)

Table 6. Experimental rate constants of equilibrium (II) obtained in LiOH media (I > 1) at 45°C by measuring $v = -d\, i_{cat}/dt$.
$c = 5 \times 10^{-4}$ M ; X = OH (carboxylate).

[LiOH] M	0.2	0.5	0.75	1	2
$10^4\ k_{obs}\ s^{-1}$	0.65	2.2	3.2	5.3	8.2
log k	-4.19	-3.66	-3.49	-3.27	-3.09

Table 7. Experimental rate constants of equilibrium (II) obtained in NaOH media (I > 1) at 45°C by measuring $v = -d\, i_{cat}/dt$.
$c = 5 \times 10^{-4}$ M ; X = OH (carboxylate).

[NaOH] M	0.1	0.2	0.5	1	2
$10^4\ k_{obs}\ s^{-1}$	0.81	1.7	4.0	6.7	5.8
log k	-4.09	-3.77	-3.40	-3.17	-3.24

Tables 6 and 7 summarize kinetic data obtained in alkali solutions for the second step which is a first order reaction with respect to dihydro-ß-carboline. The increase of absorbance at 360 nm with time yields a rate constant identical with that shown by the change of the polarographic cathodic current :

$$v = \frac{dA\ 360\ nm}{dt} = -\frac{di_{cat}}{dt} = k_{obs} \cdot [AH_2]$$

The plot of log k_{obs} versus pH (Fig 3) agrees with a kinetic law of the type :

$$v = k \cdot [AH_2] \cdot [OH^-]$$

Assuming that step (I) is fast, and applying the stationary state approximation to the intermediate $(AH^-)_1$, we obtain :

$$[AH^-]_1 = \frac{k_1 \cdot [AH_2] \cdot [OH^-]}{k_{-1} + k_2}$$

$$v = k_2 \cdot [AH^-]_1 = \frac{k_1 \cdot k_2 \cdot [AH_2] \cdot [OH^-]}{k_{-1} + k_2}$$

Since reaction (II) is the rate-determining step, we may assume that k_2 is negligible compared with k_{-1} and thus :

$$v = \frac{k_1}{k_{-1}} \cdot k_2 \cdot [AH_2] \cdot [OH^-]$$

an expression which agrees with the experimental kinetic law.

Using the pK'_a value deduced from the plot of i^{eq}_{en} versus pH, we obtain $K'_a = 2 \times 10^{-14}$ for the overall reaction :

$$(AH_2) + OH^- \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} (AH^-)_2 + H_2O$$

In NaOH 1 M, at 45°C :

$$k_{obs} = \frac{K'_a}{K_e} \cdot k_2 \cdot [OH^-] = 6.67 \times 10^{-4} s^{-1}$$

$$k_{obs} = \frac{2 \times 10^{-14}}{10^{-14}} \cdot k_2 \cdot 1 = 6.67 \times 10^{-4} s^{-1}$$

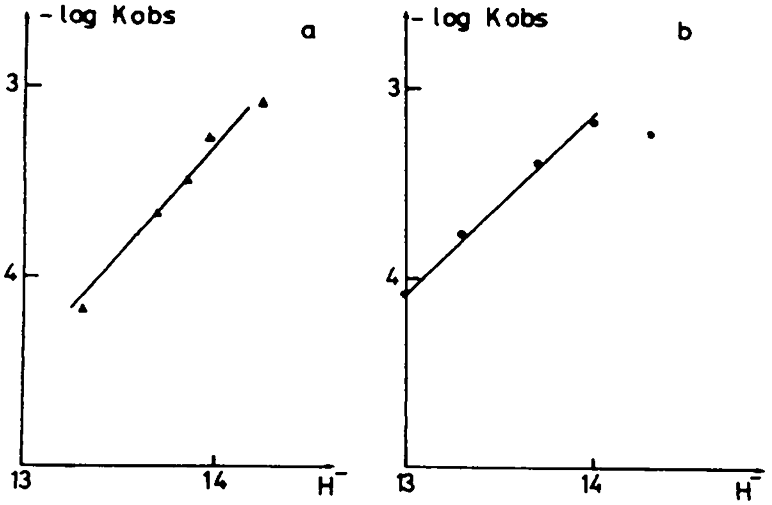


Fig. 3 Plot of log K_{obs} versus pH obtained in LiOH media (Fig. 3a) and in NaOH media (Fig. 3b); X = OH

hence : $k_2 = 3.33 \times 10^{-4} s^{-1}$

and $k_{-2} = \frac{k_2}{K'_a} = \frac{3.33 \times 10^{-4}}{2 \times 10^{-14}} = 1.67 \times 10^{10} M^{-1} s^{-1}$

These values k_2 and k_{-2} are in close agreement with the rate constants determined for other C-H acids (9).

In NaOH 1 M, rate constants k_{obs} were measured at three different temperatures (Tab. 8) The plot of log k_{obs} versus 1/T is a straight line whose slope yields ΔE = 18.4 $k_{cal} \cdot mol^{-1}$.

Table 8. Dependence of the rate constant k_{obs} on the temperature of the supporting electrolyte NaOH 1 M

T°K	303	318	333
$\frac{10^3}{T}$	3.30	3.14	3.00
k_{obs} mn^{-1}	0.011	0.040	0.175
$\log_{10} k$	-1.96	-1.40	-0.76

Aromatization involving carbanion $(AH^-)_2$

The values of half-wave potential listed in table 9 explain the highly oxidizable character of the corresponding carbanion. Hence, in spite of the continuous bubbling of inert gas through the alkaline solution, it was not possible to avoid oxidation yielding ß-carboline (A) according to reaction (eqn III).

The fact that it was not possible to avoid oxidation completely may account for the proportion of carbanion $(AH^-)_2$ determined by anodic polarography, roughly 78 per cent (Fig.4). Thus we may consider the occurrence of two successive first order reactions according to the scheme :

$$(AH_2) + OH^- \xrightarrow{k_2} (AH^-)_2 + H_2O \qquad (II)$$

$$a - (y + z) \qquad\qquad y$$

$$(AH^-)_2 + \frac{1}{2} O_2 \xrightarrow{k_3} (A) + \frac{1}{2} H_2O_2 \qquad (III)$$

$$z$$

where a designates the initial concentration of the reactant (AH_2), y and z the respective concentrations of the carbanion intermediate and the fully aromatized product at t time . (We may assume that step (eqn III) degenerates to order 1 in so far as the concentration of oxygen stays roughly constant in the overall reaction).

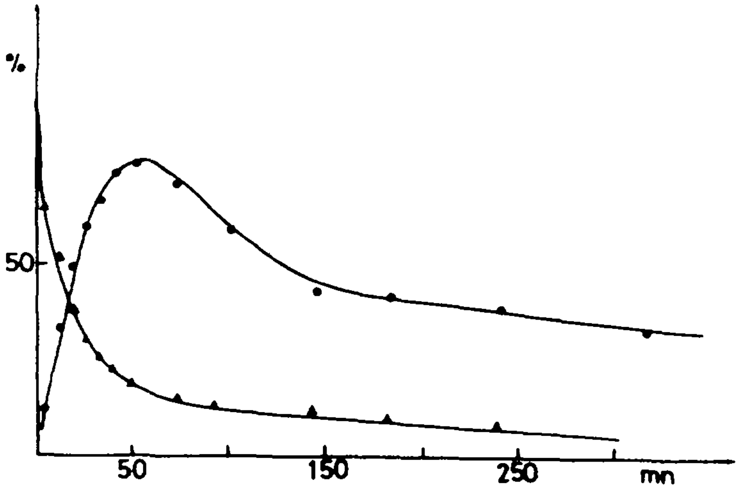


Fig. 4 Percentage of carbanion $(AH^-)_2$ versus time ● Y
Percentage of (AH_2) versus time ▲ a - (Y + z)

Table 9. Oxidation wave of the carbanion $(AH^-)_2$: dependence of half-wave potential on pH

[NaOH] M	0.2	0.5	1	2
$E_{1/2}$mV e.c.s.	-650	-680	-730	-750

Table 10. Determination of the rate constant k_3 in alkaline media ($I > 1$) at 45°C
a. by using the relation $-(r-1)\log(y_m/a) = \log r$ in a continual approach
b. by measuring $v = dA_{260nm}/dt$

[NaOH] M		0.5	1	2
y_m/a		0.536	0.567	0.508
$r = k_2/k_3$		2.4	2.8	2.1
$10^4\, k_3 s^{-1}$	a	1.67	2.33	4.67
	b	1.67	2.33	

From the measurement of

$$v = -d\, i_{cat}/dt = d\, A360\, nm/dt$$

values of k_2 and of the ratio $r = k_2/k_3$ may be deduced using the usual mathematical procedure dealing with the experimental value y_m shown by the plot of y against time (Tab.10):

$$y_m = a \left(\frac{k_2}{k_3} \right)^{\frac{k_2-k_3}{k_3}}$$

$$-(r-1)\log\left(\frac{y_m}{a}\right) = \log r$$

N.B. : when exhaustively formed, the fully aromatic β-carboline (A) exhibits a large absorption band at 260 nm ($\varepsilon = 28000\ M^{-1} cm^{-1}$) (Tab. 11). The increase of (A) 260 nm follows a first order kinetic law and yields a rate constant k_3 agreeing with that obtained by the mathematical treatment applicable to successiye first order reactions (Tab. 10).

Occurrence of a condensation involving carbanion $(AH^-)_2$

In the pH range 8.0 to 11.0 , the carbanion $(AH^-)_2$ no longer exists in appreciable amounts as indicated by the plot of i^{eq}_{an} v s. pH, whereas the fully aromatic β-carboline (A) is produced in smaller amounts than in alkaline media where it is quantitatively formed. Kinetic data ($v = -dA$ 320 nm/dt) display kinetics of the type :

$$v = k\ .\ [AH_2]^n\ .\ [OH^-]$$

with a half-life depending on the initial concentration of (AH_2) ($1 < n < 2$).

Hence it can be deduced that carbanion $(AH^-)_2$ formed through the gross reaction (eqn II) is later converted into a dimer which disproportionates according to the scheme :

$$(AH_2) + OH^- \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} (AH^-)_2 + H_2O \qquad (II)$$

$$(AH^-)_2 + (AH_2) \underset{k'_{-3}}{\overset{k'_3}{\rightleftharpoons}} (D) \qquad (III')$$

$$(D) + OH^- \xrightarrow{k_4} \underbrace{(AH_4) + (A)}_{P} + H_2O \qquad (IV)$$

Applying the stationary state approximation to the intermediate $(AH^-)_2$ we obtain :

$$K_2.[AH_2].[OH^-] - k_{-2}[AH^-]_2 - k'_3.[AH_2].[AH^-]_2 = 0$$

$$[AH^-]_2 = \frac{k_2.[AH_2].[OH^-]}{k_{-2} + k'_3.[AH_2]}$$

Applying the steady state approximation to the intermediate (D), we obtain :

$$k'_3.[AH_2].[AH^-]_2 - k'_{-3}.[D] - k_4.[D].[OH^-] = 0$$

$$[D] = \frac{k'_3.[AH_2].[AH^-]_2}{k'_{-3} + k_4.[OH^-]}$$

$$v = \frac{d[P]}{dt} = k_4.[D].[OH^-] = \frac{k'_3.k_4.[AH_2].[AH^-]_2.[OH^-]}{k'_{-3} + k_4.[OH^-]}$$

Hence, replacing $[AH^-]_2$ by its value :

$$v = \frac{d[P]}{dt} : \frac{k'_3.k_4.[AH_2].[OH^-]}{k'_{-3} + k_4.[OH^-]} \times \frac{k_2.[AH_2].[OH^-]}{k_{-2} + k'_3.[AH_2]}$$

due to the fact that (*eqn* III') constitutes the rate-determining step :

- in the first term of the right hand side of the equation, k'_{-3} is negligible compared with $k_4.[OH^-]$.
- in the second term of the right hand side of the equation we can disregard the term $k'_3.[AH_2]$ over k_{-2}.

Hence :

$$v = \frac{d[P]}{dt} = \frac{k'_3.k_4.[AH_2].[OH^-]}{k_4.[OH^-]} \times \frac{k_2.[AH_2].[OH^-]}{k_{-2}}$$

$$v = \frac{d[P]}{dt} = \frac{k_2}{k_{-2}}.k'_3.[AH_2]^2.[OH^-]$$

an expression which agrees with the experimental data listed in Tab. 12. The value of the individual rate constant k'_3 can be deduced ;

Table 11. Values shown by the main bands recorded at 25°C in buffered water-methanol (9 : 1) solutions of β-carboline-3-carboxylate ; c = 5 x 10^{-4} M, monoionized species.

λ nm	235	262	280(sh)	333	345	370
$\varepsilon M^{-1} cm^{-1}$	25000	28000	11000	4000	4000	800

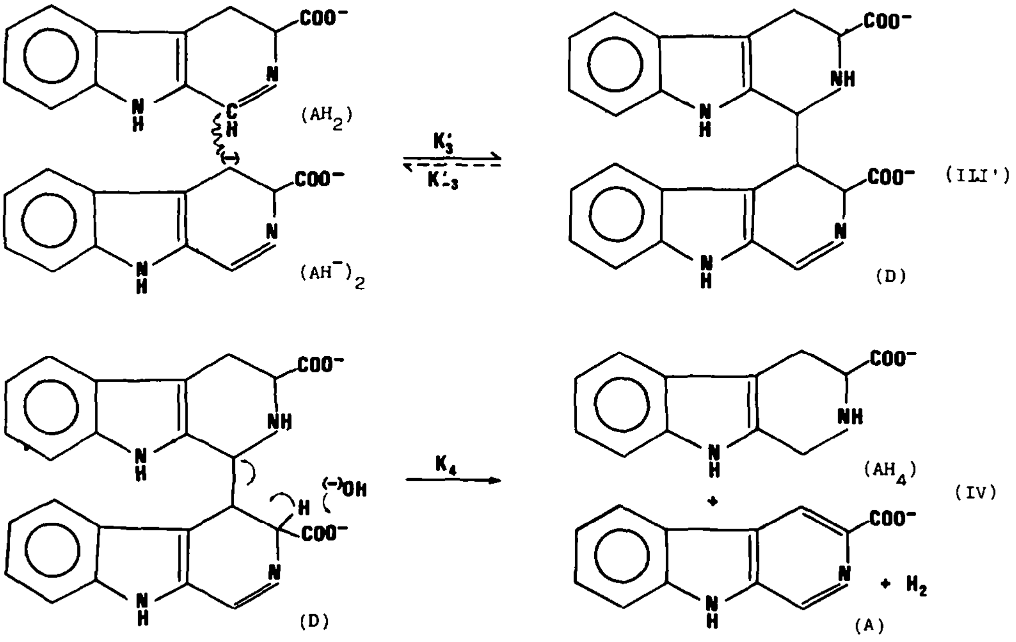


Table 12. Values of the experimental second-order rate constant in buffered water-methanol (9 : 1) solutions determined, at : (*) 60°C or (**) 45°C , by measuring : a, $v = -dA_{320\ nm}/dt$; b, $v = dA_{260\ nm}/dt$; $c = 5 \times 10^{-4}$ M ; X = OH (carboxylate)

pH		7.0(*)	8.0(*)	11.0(*)	11.35(**)	11.80(**)
$k_{obs} M^{-1} s^{-1}$	a	1.00	1.33	0.87	1.33	1.75
	b	0.92	1.50	0.87		

for instance at pH 11.0 :

$$k_{obs} = \frac{K'_a}{K_e} . k'_3 . [OH^-] = 0.866\ M^{-1}s^{-1} \text{ at } 60°C$$

$$k_{obs} = \frac{2 . 10^{-14}}{10^{-14}} . k'_3 . 10^{-3}$$

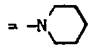
$$k'_3 = \frac{0.866}{2 \times 10^{-3}} = 4.33 \times 10^2\ M^{-1}s^{-1} \text{ at } 60°C$$

N.B. : in less basic medium (pH 8.0) the rate of the condensation increases, corresponding to a faster reaction (eqn III') of the carbanion with the conjugated immonium cation (AH_3^+).

Effect of replacement of the carboxylate group by an ester or an amide group

The replacement of the carboxylate group by an ester or an amide group enhances the yield in dimeric product.

In slightly basic media, when X = OEt , the determination of $v = -d\ i_{cat}/dt$ follows a second order kinetic law in ester according to the experimental data listed in Tab. 13. Thus, it can be conclude that the ester group favors the carbanion and its subsequent dimerization.

The behavior of the X = -N substituted derivative depends, in alkaline media , on the nature of the background electrolyte.

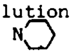
In LiOH media (Tab. 14) (Fig. 5a) the behaviour resembles that of the carboxylate species (except for the increase of the rate constant k_2), while in NaOH media a kinetic law of the type :

$$v = -\frac{d\ i_{cat}}{dt} = k . [AH_2]^2 . [OH^-]^{-1}$$

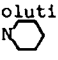
is established (Tab. 15) (Fig. 5b).

Table 13. Effect of the concentration in 3,4-dihydro-β-carboline-3-ester on the half life of the dimerization, in buffered solution pH 8.2, at 45°C

$10^4 . C_{ester}$ M	k_{obs} $M^{-1}s^{-1}$	$t_{1/2}$ mn
1.25	1.11	120
2.50	1.00	67
5.00	1.03	32

Table 14. Values of the experimental first-order rate constant in LiOH water-methanol solutions (9 : 1) determined at 45°C by measuring $v = -d\ i_{cat}/dt$; $c = 5 \times 10^{-4}$ M ; X = N

[LiOH] M	0.2	0.5	1	2
$10^4\ k_{obs} s^{-1}$	5.2	4.8	9.7	12.5
log k	-3.28	-3.32	-3.01	-2.90

Table 15. Values of the experimental second-order rate constant in NaOH water-methanol solutions (9 : 1) determined at 45°C by measuring $v = -d\ i_{cat}/dt$; $c = 5 \times 10^{-4}$ M ; X = N

[NaOH] M	0.2	0.5	1	2
k_{obs} $M^{-1}s^{-1}$	2.17	2.00	0.55	0.19
log k	0.34	0.30	-0.26	-0.72

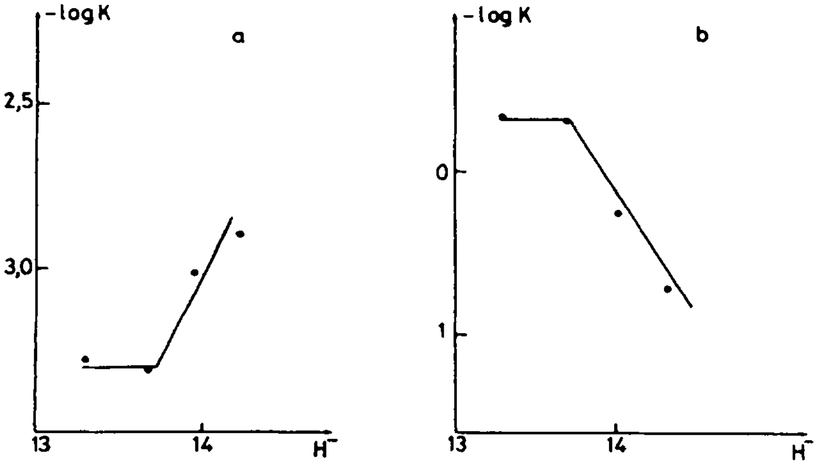


Fig. 5 Plot of log K_{obs} versus pH obtained in LiOH media (Fig. 5a) and in NaOH media (Fig. 5b) ; X = 

This expression agrees with the following mechanism :

$$(AH^-)_2 + H_2O \underset{k_2}{\overset{k_{-2}}{\rightleftharpoons}} (AH_2) + OH^-$$

$$(AH^-)_2 + (AH_2) \xrightarrow{k_3} (D)$$

Applying the steady state approximation to the intermediate (AH_2), we obtain :

$$[AH_2] = \frac{k_{-2} . [AH^-]_2}{k_2 . [OH^-] + k_3 . [AH^-]_2}$$

$$v = \frac{d[D]}{dt} = k_3 . [AH_2] . [AH^-]_2$$

$$v = \frac{k_{-2} . k_3 . [AH^-]_2^2}{k_2 . [OH^-] + k_3 . [AH^-]_2}$$

We can assume that the step (eqn III) is limiting and disregard the term $k_3 . [AH^-]_2$ over $k_2 . [OH^-]$; hence :

$$v = \frac{k_{-2}}{k_2} . [AH^-]_2^2 . \frac{1}{[OH^-]}$$

This expression agrees with the experimental data listed in Tab . 15 . In NaOH 0.5 M , $k_{obs} = 2\ M^{-1}s^{-1}$ at 45°C.

$$k_{obs} = \frac{k_{-2}}{k_2} . \frac{1}{[OH^-]} = 2\ M^{-1}s^{-1}$$

$$\frac{k_{-2}}{k_2} = \frac{K_e}{K'_a} = 2 \times 0.5 = 1$$

Hence $K_a = 10^{-14}$ at 45°C.

DISCUSSION

In basic water-methanol (9 : 1) media (AH_2) undergoes two successive equilibria. The first is accompanied by suppression of the chirality. This result suggests the cleavage of the C_3-H bond according to a kinetic law of the type :

$$v = k_1 . [AH_2] . [OH^-]$$

found : $k_1 = 3 \times 10^{-3} s^{-1}$, in NaOH 1 M at 45°C.

Concerning the second equilibrium, there is spectrometric (shown by $v = d\,A\,360\ nm/dt$) and electrochemical evidence (shown by $v = -d\,i_{cat}/dt$) of the occurrence of a slow tautomerism :

$$(AH^-)_1 \rightleftharpoons (AH^-)_2 .$$

The appearance of an anodic current may account for the formation of a highly oxidizable species, i.e. resonant carbanion $(AH^-)_2$. Plotting the value of the anodic current i_{an}^{eq} as a function of pH gives the value $K'_a = 2 \times 10^{-14}$, at 45°C, for the gross reaction :

$$(AH_2) + OH^- \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} (AH^-)_2 + H_2O$$

assuming that $K_a' = 2 \times 10^{-14}$, values of $k_2 = 3.33 \times 10^{-4} s^{-1}$ and $k_{-2} = 1.67 \times 10^{10} M^{-1}s^{-1}$ are obtained in NaOH 1 M .

The resonant carbanion $(AH^-)_2$ may undergo two competing reactions in water–methanol basic media.

a) Oxidation to fully aromatic ß-carboline (A):

This reaction occurs predominantly when the background electrolyte consists of LiOH rather than NaOH, or when the NaOH concentration is high enough to avoid any antecedent protonation [NaOH] > 0.5 M . Note that oxidation does not take place through the indole ring nitrogen as previously assumed (10).

The oxidation rate constant was determined according to the usual mathematical treatment of successive first order reactions :
in NaOH 0.5 M (I = 1) found at 45°C :
$k_3 = 1.67 \times 10^{-4} s^{-1}$.

b) Dimerization

In slightly basic media , in the pH range 8.0-12.0, the fully aromatic ß-carboline (A) is produced in smaller amounts (50-70 per cent) than in alkaline media. The increase of the band situated at 260 nm with time follows a second order reaction according to the equation :

$$v = k_{obs} \cdot [AH_2]^2 \cdot [OH^-]$$

measured :

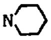
$$k_{obs} = 0.87\ M^{-1}s^{-1} \quad \text{at pH 11.0 and 45°C}$$

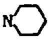
hence :

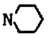
$$k_{dim} = 4.33 \times 10^2\, M^{-1}s^{-1} \quad \text{at pH 11.0 and 45°C.}$$

The dimer disproportionates, yielding roughly 50 % fully aromatic ß-carboline (A) and 50 % tetrahydrocarboline (AH_4), in agreement with previous results (11).

Finally, the preference for one of the two competitive pathways –a) oxidation or b) dimerization–disproportionation – depends on :

α) the nature of the X group. With X = OH (carboxyl) reaction b) does not occur in NaOH media, in the concentration range 0.1 < c < 0.5 M, but predominates when X = N

ß) the acidity of the solution. With X = N or OEt, b) constitutes the major reaction in slightly basic media, in pH range 8.0-11.8.

γ) the nature of the background electrolyte in alkaline media, with X = N , b) occurs :

- as a minor reaction in LiOH solution
- predominantly in NaOH solution , in the concentration range 0.1 < c < 1 M .

The corresponding kinetic data may be summarized vs. the pH value as indicated in Tab. 16.

The data of Tab. 16 indicates that aqueous LiOH constitutes the most specific medium for the aromatization of (AH_2) derivatives. However the choice of the reaction conditions also depends on the presence of other functional groups. Actually some (AH_2) derivatives aromatize in aqueous LiOH as well as in NaOH while other derivatives (i.e. esters) need the use of anhydrous alcohols as reaction solvent.

The two successive conversions :
N^{α}Formyl Tryptophan → 3,4-Dihydro-ß-carboline → ß-carboline which occur respectively through dehydration in acidic media and aromatization under basic conditions, constitute a alternative pathway to the Pictet-Spengler reaction (5) for the synthesis of ß-carboline derivatives and perhaps a model of their biosynthesis.

EXPERIMENTAL

Apparatus

Optical activities were measured with a type 71 ROUSSEL–JOUAN polarimeter.

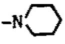
U V spectra were recorded on a VARIAN electroscan spectrophotometer.

^{1}H NMR spectra were obtained with a model CFT-20 VARIAN spectrometer.

pH values were determined with a TACUSSEL TS 70 N pH meter.

The polarograms were recorded on a three-electrode TACUSSEL polarograph, mark PRG. 5. Data were recorded on a TACUSSEL EPL-2 recorder.

Table 16. 3-substituted 3,4-dihydro-ß-carbolines
Rate constants obtained in various water-methanol (9 : 1) media for the two competitive pathways : a = oxidation and b = dimerization

3-substituent	COO^-	COOEt	CO-N 
pH 8.0 60°C		b $K = 1\ M^{-1}s^{-1}$	
pH 11.0 60°C	b $k_{obs} = 0.866\ M^{-1}s^{-1}$ $k_{dim} = 433\ M^{-1}s^{-1}$		
LiOH 0.5 M 45°C	a $k_{obs} = 2.2 \times 10^{-4} s^{-1}$	a $k_{obs} = 5 \times 10^{-4} s^{-1}$	a $k_{obs} = 6 \times 10^{-4} s^{-1}$
NaOH 0.5 M 45°C	a $k_{obs} = 4.0 \times 10^{-4} s^{-1}$		b $k_{obs} = 2\ M^{-1}s^{-1}$ $K'_a = 10^{-14}$
NaOH 1 M 45°C	a $k_{obs} = 6.67 \times 10^{-4} s^{-1}$ $k_2 = 3.33 \times 10^{-4} s^{-1}$ $k_{-2} = 1.67 \times 10^{10} M^{-1} s^{-1}$ $K'_a = 2 \times 10^{-14}$		b $k_{obs} = 0.5\ M^{-1}s^{-1}$

Cell and electrodes

For polarographic experiments, a water-jacketed TACUSSEL CPRA cell was used, with an aqueous saturated TACUSSEL C 10 electrode which is the reference for all potentials cited. The counter electrode was a platinum TACUSSEL PT 11 electrode.

For polarographic measurements, the dropping mercury electrode consisted of a TACUSSEL CMT 10/24 capillary.
Capillary constants in deoxygenated 1 M KCl with open circuit and a mercury height of 60 cm (uncorrected for back pressure) were $m = 0.65\ g.s^{-1}$ and $t = 6$ s . The drop time t was controlled by a TACUSSEL MPO3 drop-knocker.

Stock solutions and buffers

5 mM stock solutions of (A) and (AH_2) derivatives were prepared in methanol.

For pH < 2, solutions of sulfuric acid were used ; for pH 2-10, phosphate buffers were prepared from 0.5 M Na_2HPO_4 ; for pH > 12, solutions of sodium hydroxide were used and the ionic strength I was kept constant by addition of sodium chloride : I = 1 .

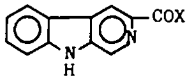
Materials

The present study was performed using : D- and L-3,4-dihydro-ß-carboline-3-carboxylic acid (I'); methyl-3,4-dihydro-ß-carboline-3-carboxylate (II'); D,L-3,4-dihydro-ß-carboline-3-carbonyl-L-glycine ethyl ester (III'); L-3,4-dihydro-ß-carboline-3-carbonyl-L-leucine ethyl ester (IV'); L-3,4-dihydro-ß-carboline-3-carbonyl-L-phenylalanine ethyl ester (V'); L-3,4-dihydro-ß-carboline-3-carbonyl-L-tyrosine ethyl ester (VI'). The synthesis of compounds I', II', III', IV', V' and VI' was described previously (8).

Methyl-ß-carboline-3-carboxylate : L-I'·HCl (1g) was added to a chilled solution of thionyl chloride (1.5 ml) in methanol (30 ml) and the mixture was shaken vigorously at room temperature. At the end of this time, the solid had completely dissolved. The solution was concentrated under reduced pressure and an excess of anhydrous ethyl ether was added. The precipitated L-II'·HCl was filtered, washed with ether, and dried in a vacuum over NaOH pellets; yield 85 % of theory; m.p. 188 (m.p. 185 for D,L form). II'·HCl (0.265 mg) was dissolved in methanol (10 ml) containing triethylamine (10 %) and acetic acid (2 %). The reaction mixture was refluxed for 60 minutes, concentrated under reduced pressure and diluted with an excess of chloroform. After washing twice with $NaHCO_3$ (5 %) and NaCl (10 %) in water, the organic layer was dried over Na_2SO_4, and evaporated under reduced pressure. The methyl-ß-carboline-3-carboxylate separate as crystals from chloroform/petroleum ether (see Tab. 2).

ß-carboline-3-carboxylic acid : I'·HCl (2.5g) was dissolved in NaOH (10 ml) and the reaction mixture was heated at 50°C for 40 minutes. The ß-carboline-3-carboxylic acid monohydrate separate in crystalline form as the result of a drop-by-drop addition of acetic acid (10 %) in water (50 ml) under vigorous stirring. The product was collected by filtration, washed with cold aqueous acetic acid (5 %) and dried under vacuum over KOH pellets (Tab. 17).

Table 17. Properties of β-carboline-3-carboxylic acid derivatives



X-substituent	Yield %	m.p. °C	Calculated % C	Calculated % H	Calculated % N	Found % C	Found % H	Found % N
$-OH^a$	70	250	62.60	4.38	12.17	62.45	4.45	12.14
$-OCH_3$	83	185	69.01	4.46	12.38	68.49	4.71	12.29
-L-Phe•OEt	75	86	71.30	5.46	10.85	71.23	5.60	10.82
-L-Tyr•OEt	68	104	68.45	5.25	10.42	68.25	5.30	10.42
-L-Leu•OEt	68	91	67.97	6.56	11.89	67.78	6.62	11.84
-Gly•OEt	64	186	64.63	5.09	14.14	64.46	5.10	14.16

[a] monohydrate

β-carboline-3-carboxy amino acid ester : In a typical experiment, V' ($0.5\ 10^{-2}$M) was dissolved in ethanol (100 ml) containing triethylamine (10 %) and acetic acid(2 %). The reaction mixture was refluxed 1 h, concentrated under reduced pressure, diluted with an excess of ethyl acetate and successively washed with $NaHCO_3$ (5 %) and NaOH (10 %). The organic layer was concentrated under reduced pressure and diluted with petroleum ether until cloudy. On cooling, the product separated as pale yellow crystals. The same procedure was used to obtain β-carboline-3-carbonyl-glycine ethyl ester, β-carboline-3-carbonyl-L-leucine ethyl ester, β-carboline-3-carbonyl-L-tyrosine ethyl ester (Tab. 17).

Kinetic measurements

Polarimetry. All kinetics were measured at 518 nm in capped 0.7 dm tubes of 7 ml capacity provided with a water jacket for temperature control. In a typical experiment, 10 mg of L-I'•HCl was weighed in a 50 ml flask which was thermostated at 23°C, together with a solution of 1N NaOH. To start the reaction, 10 ml of NaOH were pipetted on to L-I'•HCl which dissolved instantly. The timer was started and the reaction solution was quickly transferred to the polarimeter tube thermostated at 23°C and readings began. The first reading could be obtained in 1.5 /2 minutes : the optical activity at zero time was determined by extrapolating initial readings to zero time and the results were considered satisfactory if they were reproducible in three different runs. In a parallel experiment an identical solution of L-I'•HCl was allowed to stand at 23°C. At suitable intervals, 0.1 ml were withdrawn and diluted with 5 ml of 0.5 M of K_2CO_3 to check the extent of aromatization. This was done by U.V. measurements at 319 nm (Fig. 2).

U.V. spectrometry . Ionization of (AH_2) derivatives was monitored by recording the decrease of optical density at 328 nm with time of their basic solutions.

The subsequent tautomeric equilibrium was accompanied by an increase in the absorbance at 360 nm.

The final aromatization of (AH_2) was monitored by recording the increase of optical density at 260 nm.

Polarography. For polarography, the 0.5 mM stock solutions of (AH_2) in methanol was diluted tenfold with the buffer or supporting electrolyte used so that the final solution contained 10 % methanol.

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