Tetrahedron Vol. 42, No. 1, pp. 409 to 415, 1986
Printed in Great Britain.

0040-4020/86 $3.00 + .00

STUDY OF THE REDUCTIVE METABOLISM PATHWAY OF 4-METHYL-5-(2-PYRAZINYL)-1,2-DITHIOLE-3-THIONE. AN ELECTROCHEMICAL APPROACH.

M. LARGERON, D. FLEURY and M.B. FLEURY*
Laboratoire de Chimie Analytique, U.A. 484 au C.N.R.S., Faculté de pharmacie,
4 avenue de l'Observatoire, 75270 PARIS CEDEX 06.

(*Received in France* 12 *July* 1985)

Abstract - In slightly basic aqueous-ethanol medium or in acetonitrile, electrochemical reduction of 4-methyl-5-(2-pyrazinyl)-1,2-dithiole-3-thione (OLTIPRAZ 35972 R.P., antischistosomal drug) affords a convenient route to pyrrolo[1,2-a]pyrazine derivatives, which are found as metabolites of the drug in host urine. A transient species in the reduction process which is endowed with schistosomicidal activity is isolated.

In a previous paper[1], several mechanistic interpretations of the reaction of various substituted 1,2-dithiole-3-thiones with nucleophiles (alkoxides, thiolates) were proposed. With 4-methyl-5-(2-pyrazinyl)-1,2-dithiole-3-thione 1 (35972 R.P., OLTIPRAZ) as starting material, it was shown that reduction of a transient species by sodium sulphide yields, after methylation, pyrrolo[1,2-a]pyrazine derivatives which are found as metabolites of OLTIPRAZ in mouse urine[2-4]. OLTIPRAZ exhibits schistosomicidal properties but its metabolites are inactive. The analysis of the reduction pathway may be of pharmacological interest since the major metabolite of OLTIPRAZ is a product resulting from a 4e-reduction of the drug.

In addition, electrochemical studies of 1,2-dithiole-3-thiones in dimethylformamide (DMF)[5,6] and in aqueous-ethanol buffered solutions[7] were carried out in our laboratory in order to establish a possible relationship between electrochemical and pharmacological properties. These studies led us to the following conclusions:

1) the life-time of the radical anion produced in DMF by the addition of one electron to a molecule of OLTIPRAZ is considerably longer than that of various other substituted 1,2-dithiole-3-thiones radical anions. This result may be of biological importance if the antischistosomal activity occurs via a radical mechanism.

2) the redox couple 1,2-dithiole-3-thione/2e-reduction product exhibits generally reversible behaviour at the mercury electrode, the 2e-reduction product existing either in cyclic or in opened forms[7]. It is noteworthy that the electrochemical behaviour is noticeably modified when a pyrazinyl group is introduced at the C-5 position: the reduction process becomes irreversible.

The present paper deals with the electrochemical reduction of OLTIPRAZ in slightly basic aqueous ethanol medium or in acetonitrile. This reduction process provides a convenient route to the pyrrolo[1,2-a]pyrazine derivatives isolated as metabolites in mouse urine.

RESULTS AND DISCUSSION

Controlled potential electrolysis in slightly basic aqueous-ethanol medium.

At the dropping mercury electrode, a solution of compound 1 (0.5 mM) exhibits very complex and poorly defined signals owing to the occurrence of catalytic discharges related to surface reactions which are strongly potential dependent, and resulting from the adsorption phenomena occurring at

the solution/electrode interface. When the controlled-potential of the mercury pool working electrode is fixed at -900 mV s.c.e., at a potential immediately after the first catalytic peak and preceding the occurrence of the second cathodic polarographic wave, a coulometric value of 4.0 ± 0.2 is found for n, and the polarogram of the exhaustively electrolyzed solution exhibits one 2e-anodic wave at -780 mV s.c.e.

After the chosen potential is applied, a decrease of the absorption bands shown by the starting material at 300 and 438 nm (spectrum a, Fig. 1) and an increase at 315 and 392 nm are observed. Change in spectra shows four isosbestics points at 310, 330, 405 and 480 nm. The U.V-visible absorption spectrum of the exhaustively electrolyzed solution is given in Fig. 1 and noted b. When the 4e-reduction solution is left in contact with air, the regeneration of 1 is found equal to zero.

Acidification at pH 3.8 of the exhaustively electrolyzed solution (see experimental section) provides species 2a, a compound which was not previously described in the literature. 2a was isolated in 60% yield. U.V-visible absorption spectrum of 2a (spectrum c in Fig. 1) exhibits one band at 500 nm which can be attributed to a thione group. The ^{1}H N.M.R. and mass spectroscopic data are in agreement with structure 2a which possesses a pyrrolo [1,2-a] pyrazine skeleton.

Methylation of the exhaustively electrolyzed solution (see experimental section) provides two major compounds 2b (40% yield) and $(2b)_2$ (30% yield) which possess a pyrrolo [1,2-a] pyrazine skeleton as confirmed by U.V-visible absorption data, ^{1}H N.M.R. and mass spectroscopy. The major one 2b corresponds to the primary metabolite of OLTIPRAZ and the secondary compound is the corresponding disulphide $(2b)_2$.

Reaction pathway:

From these experimental results it can be deduced that the first step implies the transfer of two electrons causing the cleavage of the disulphide bond {eqn. (1)}. This electrochemical reaction is followed by an intramolecular ring-closure reaction yielding a transient species more reducible than the starting material 1, so that the overall reduction reaction involves a 4e-transfer according to scheme 1.

It is worth mentioning with regard to this pathway that:

a) U.V-visible absorption spectra exhibited by similar 2e-reduction products of 4,5-dimethyl-1,2-dithiole-3-thione and 4-methyl-5-phenyl-1,2-dithiole-3-thione have been previously described[7]. However, we were unable to record the U.V-visible absorption spectrum of the 2e-reduction transient species owing to its rapid intramolecular ring-closure yielding the pyrrolo [1,2-a] pyrazine skeleton.

b) pyrrolo [1,2-a] pyrazine ring-closure involves sulphide anion as leaving group {eqn. (2)}. A second 2e-reduction reaction of the transient cationic thione {eqn. (3)} affords, after acidification the pyrrolo [1,2-a] pyrazine species 2a. This second 2e-reduction step can occur either via an electrochemical-reduction process or via the sulphide anion generated in step (2) and exerting reducing properties. It is difficult to choose between the two possibilities. However, experimental results are more in favour of an electrochemical 2e-reduction step:

1) the analysis of the coulometric data gives a total faradaic n value of 4.0 ± 0.2;

2) one mole of sulphide anion per mole of 1 can be characterized by the anodic polarogram of the exhaustively electrolyzed solution according to the electrochemical reaction:

$$Hg + HS^- \longrightarrow HgS + H^+ + 2e;$$

Controlled potential electrolysis in acetonitrile (ACN).

When the electrolysis is carried out at -1050 mV s.c.e., i.e. at a potential corresponding to the plateau of the cathodic polarographic wave, a coulometric value of 2.0 ± 0.1 is found for n. The absorption spectrum of 1 (spectrum a) is replaced by the absorption spectrum noted b in Fig. 2. When the exhaustively reduced solution is left in contact with air, the regeneration of 1 is found equal to zero.

After addition of concentrated perchloric acid, the concentration of added acid being c, where c is the initial concentration of 1, the spectrum becomes analogous to c in Fig. 2. With a 5 mM initial concentration of 1, a red compound partially precipitates in the acidified solution and is isolated by filtration in 25% yield (see experimental section). U.V-visible absorption spectrum,

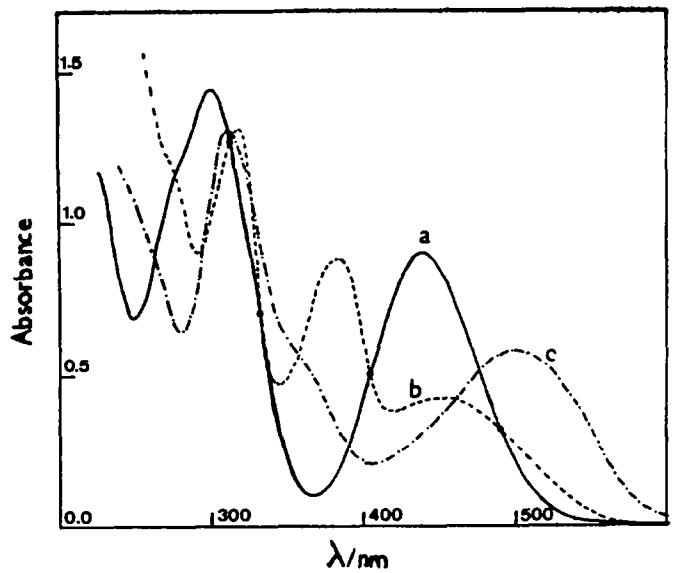
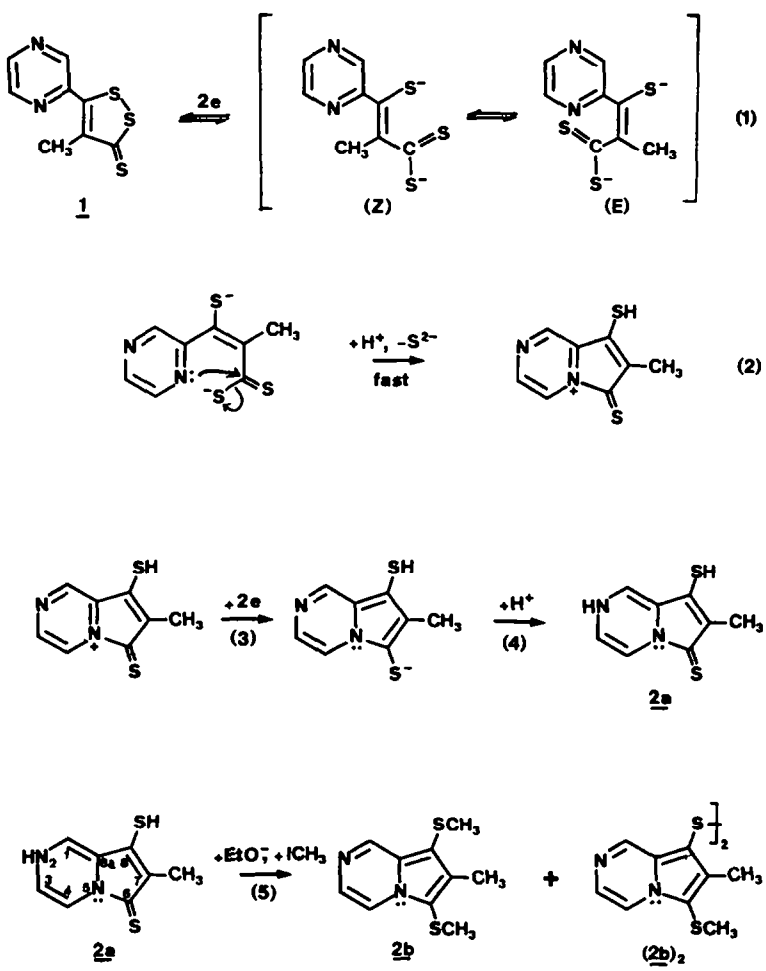


Fig. 1. Spectrophotometric behaviour. Spectrum a, 0.5 mM solution of 1 in slightly basic aqueous-ethanol medium before electrolysis (continuous line); spectrum b, after exhaustive electrolysis (dotted line); spectrum c, 0.5 mM solution of 2a in chloroform-methanol 98:2 (dotted and dashed line); * isosbestic points appearing during the electrochemical reduction.



Scheme 1.

^{1}H N.M.R. and mass spectroscopic data are in agreement with structure 2a.

Methylation of the exhaustively electrolyzed solution (see experimental section) provides the metabolites of OLTIPRAZ above described in this paper and denoted 2b (in 20% yield) and $(2b)_2$ (in 65% yield).

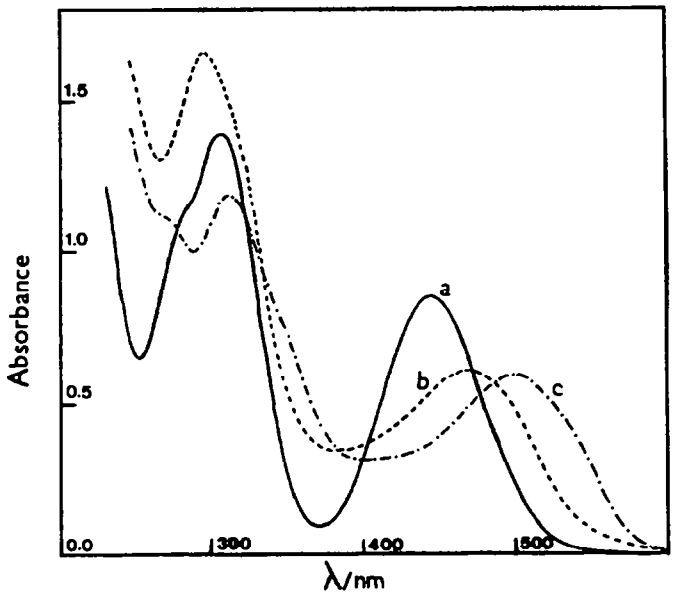


Fig. 2. Spectrophotometric behaviour. Spectrum a, 0.5 mM solution of 1 in ACN before electrolysis (continuous line); spectrum b, after exhaustive electrolysis (dotted line); spectrum c, after acidification of the exhaustively reduced solution (dotted and dashed line). Background, 0.1 M T.B.A.P.

Reaction pathway:

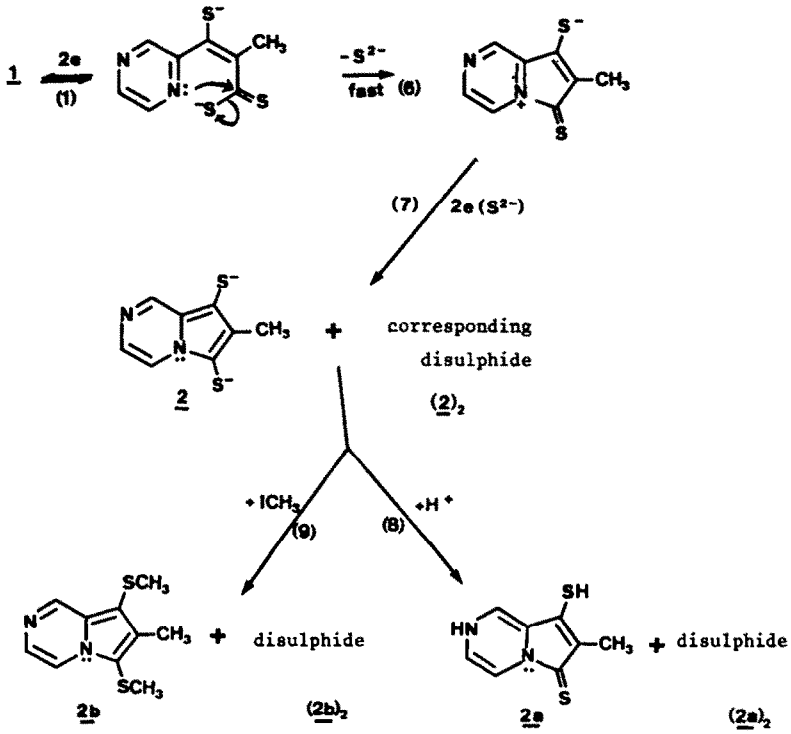
Previous experimental results suggest the transfer of two electrons causing the cleavage of the disulphide bond according to step (1) proposed in scheme 1. This electrochemical reaction is followed by the intramolecular ring-closure reaction affording the pyrrolo[1,2-a]pyrazine skeleton {eqn. (6)}. As pyrrolo[1,2-a]pyrazine species are compounds resulting from a 4e-reduction process while only two electrons are involved in the electrochemical reduction, it is obvious that the two other electrons are produced by the sulphide anion generated in equation (6).

Acidification of 2 gives 2a {eqn. (8)} while methylation of 2 and $(2)_2$ yields 2b and $(2b)_2$ {eqn. (9), scheme 2}.

It is noteworthy, with regard to this pathway, that:

1) controlled potential electrolysis carried out in slightly basic aqueous-ethanol medium or in ACN medium yield similar results. However, 2a is obtained in low yield in ACN medium (25%). So, it seems reasonable to assume that this low yield is due to an oxidation of the thiolate group at the C-8 position of the pyrrolo[1,2-a]pyrazine ring {eqn. (7)} yielding the corresponding disulphide. This oxidation should occur more slowly in slightly basic aqueous-ethanol buffered media as the pyrrolo[1,2-a]pyrazine ring possesses a thiol group in place of the thiolate group {eqn. (2)}. The protonation of the thiolate group should not occur in ACN medium.

2) when the electrolysis is carried out at -1050 mV s.c.e. in the presence of an excess of methyl iodide (see experimental section), compound 2b is obtained in 75% yield at the exclusion of compound $(2b)_2$. In these well defined conditions, due to the instantaneous methylation of 2 yielding 2b, oxidation to disulphide species no longer occurs. In this case, the electrochemical reduction agrees with the sequence proposed in scheme 2 but excluding formation of disulphides.



Scheme 2.

In comparison with the electrochemical behaviour of various substituted 1,2-dithiole-3-thiones[5,7] it is obvious that the pyrazinyl substituent exerts a determining influence: the 2e-reduction transient species yielded in step (1) undergoes rapid intramolecular ring-closure reaction which cannot occur when the pyrazinyl ring is not present. Intramolecular pyrrolo[1,2-a]pyrazine ring-closure implies sulphide anion as leaving group {step (2) or (6)}: the second 2e-reduction reaction occurs via an electrochemical process in slightly basic aqueous-ethanol medium {eqn. (3)} or via the sulphide anion generated in equation (6) in ACN medium.

It is particulary interesting to note that the major metabolites of OLTIPRAZ i.e. pyrrolo[1,2-a] pyrazine derivatives 2b and $(2b)_2$ can be alternatively obtained in vitro by an electrochemical-reduction process or by the use of nucleophiles (alkoxides, thiolates). Also, it is worth mentioning that the transient species 2a, which was not previously described in the literature, is endowed with schistosomicidal activity[8], a result which is of interest from a pharmacological point of view since the major metabolites 2b and $(2b)_2$ are devoid of such an activity.

Acknowledgements: The authors thank Dr. M. VUILHORGNE for ^{1}H N.M.R., mass spectra and fruitful discussion.

EXPERIMENTAL

General: 4-methyl-5-(2-pyrazinyl)-1,2-dithiole-3-thione 1 was supplied by RHONE-POULENC-SANTE. The solvents used for extractions and chromatography were obtained from S.D.S (puran purity grade). ACN, tetrabutylammonium perchlorate (T.B.A.P.) and methyl iodide were MERCK products.

Apparatus, as well as procedures have been described elsewhere[9]. Only apparatus, cells and electrodes used for electrochemical studies will be described.

Apparatus: Polarographic measurements were made with a three-electrode TACUSSEL polarograph, mark PRG-5. Current-potential measurements were recorded on a TACUSSEL EPL-2B recorder.

Controlled potential electrolysis was carried out using a three-compartment water-jacketed cell whose counter and reference electrode compartments were filled with the background solution; a TACUSSEL PRT 2000 potentiostat, a TACUSSEL IG5-N electronic integrator and a general purpose milliammeter were included in the circuit.

Cells and electrodes: For polarographic experiments, a water-jacketed TACUSSEL CPRA cell was used. In slightly basic aqueous-ethanol medium, the reference electrode was an aqueous KCl-saturated calomel electrode (S.C.E.) TACUSSEL C 10 to which all potentials are referred. In ACN medium, the reference electrode was an aqueous KCl-S.C.E. METROHM EA 441-1. The aqueous KCl solution was enclosed in a first compartment which was put inside a second compartment containing a 0.1 M solution of T.B.A.P. in ACN. The first and the second compartments on one hand, and the second compartment and the bulk solution on the other, were separated with sintered porous glass disks. The counter-electrode was a platinum TACUSSEL Pt 11. The working dropping mercury electrode was obtained with a TACUSSEL CMT 10/24 capillary; the drop-time was controlled by a TACUSSEL MPO 3 drop-knocker, for experiments with solid working electrode, we used a TACUSSEL EDI electrode with a platinum ferrule.

For controlled potential electrolysis, the working electrode was either a platinum grid (6 cm diam.) in ACN medium or a mercury pool (60 cm^2 area) in slightly basic aqueous-ethanol solution. The counter electrode was a platinum foil. The reference electrode was above described.

Compound 2a:

method A:

200 ml of an aqueous-ethanol (1:1) buffered solution (0.1 M carbonate, pH 10.0, measured before adding ethanol) of 1 (0.1 mmol) was exhaustively reduced under nitrogen at the mercury pool working electrode (U = -900 mV s.c.e.). When the electrolysis was complete, the alkaline solution was acidified by sulphuric acid to pH 6.5 and evaporated in vacuo at 35°C. The resulting solution (20 ml) was acidified by sulphuric acid to pH 3.8 and extracted with ethyl acetate. The organic phase, dried over anhydrous sodium sulphate and evaporated to dryness gave 2a as a red solid (13 mg, 60% yield, decomposes above 205°C).

^{1}H N.M.R., 250 MHz, DMSO d^6, δ: 2.15 (s,3H,CH_3); 7.10 (d,1H,H-3,$J_{\{(H-3)-(H-4)\}}$ = 5.5 Hz); 7.70 (bs, 1H,H-1); 7.95 (d,1H,H-4,$J_{\{(H-3)-(H-4)\}}$ = 5.5 Hz); 11.40 (bs,1H,NH).

mass spectrum (E.I.): m/z = 196 ($M^{+\cdot}$); m/z = 163 (M^+ - SH); m/z = 123; m/z = 79.

U.V-visible (chloroform-methanol 98:2), λ_{nm} (ε_M $l\,mol^{-1}\,cm^{-1}$): 305 (13000); 350 (5000,sh); 500 (6000).

As 2a undergoes a slow transformation when left in contact with air, elemental analysis was not performed.

Method B:

1 (1 mmol) was dissolved in an ACN + T.B.A.P. 0.1 M solution (200 ml) and the resulting solution exhaustively reduced, under nitrogen at a platinum electrode (E = -1050 mV s.c.e.). When the electrolysis was complete, the solution was acidified by adding concentrated perchloric acid (1 mmol). A precipitate immediately appeared in the solution which was concentrated in vacuo at 30°C (40 ml). The red precipitate thus obtained was filtered off, washed with ACN and then dried over P_2O_5 at reduced pressure for 18 hr. Preparative T.L.C. (chloroform-methanol 95:5) enabled the separation of compound 2a (55 mg, 25% yield).

7-methyl-6,8-dimethylthio-pyrrolo [1,2-a]pyrazine 2b and 7-methyl-6 (or 8-)-methylthio-pyrrolo [1,2-a]pyrazine disulphide $(2b)_2$

The methylated derivatives 2b and $(2b)_2$ can be isolated as follows:

a) the organic phase obtained using the above mentioned method A was made alkaline under nitrogen by adding sodium ethoxide (0.3 mmol), methylated with an excess of methyl iodide (10 mmol) and evaporated to dryness. Preparative T.L.C. (toluene-acetone 80:20) provided two major compounds, 2b (8 mg, 40% yield, Rf = 0.45) and $(2b)_2$ (6 mg, 30% yield, Rf = 0.20).

U.V-visible absorption, ^{1}H, ^{13}C N.M.R., mass spectroscopic data concerning 2b had been described in a previous paper[1].

$(2b)_2$, yellow solid, m.p. 160°C, had:

^{1}H N.M.R., 250 MHz, $CDCl_3$, δ: 2.25 (s,3H,CH_3 or SCH_3); 2.35 (s,3H,CH_3 or SCH_3); 7.55 (d,1H,H-3, $J_{\{(H-3)-(H-4)\}}$ = 5 Hz); 7.90 (s,1H,H-1); 8.10 (dd,1H,H-4,$J_{\{(H-3)-(H-4)\}}$ = 5 Hz, $J_{\{(H-1)-(H-4)\}}$ = 2 Hz).

mass spectrum (E.I.) and (D.C.I.): m/z = 418 ($M^{+\cdot}$); m/z = 209; m/z = 194; m/z = 123; m/z = 79.

U.V-visible (100% EtOH), λ_{nm} (ε_M $l\,mol^{-1}\,cm^{-1}$): 242 (16000); 302 (4000); 312 (4000); 350 (3600); 372 (3200).

Elemental analysis: found (C, 51.44; H, 4.43; N, 13.29; S, 30.68%) $C_{18}H_{18}N_4S_4$ requires (C, 51.67 H, 4.30; N, 13.39; S, 30.62%).

b) 1 (1 mmol) was dissolved in an ACN + T.B.A.P. 0.1 M solution (200 ml) and the resulting solution exhaustively reduced, under nitrogen, at a platinum working electrode (E = -1050 mV s.c.e.). When the electrolysis was complete, the solution was methylated instantaneously with an excess of methyl iodide (10 mmol) and evaporated to dryness in vacuo at 30°C. The residue was poured into ethyl ether-chloroform (80:20) (100 ml), in which the solubility of T.B.A.P. was very low. T.B.A.P. was filtered off and the solution containing the methylated derivatives evaporated to dryness. Chromatography of the residue (toluene-acetone 90:10) enabled the separation of 2b (45 mg, 20% yield) and $(2b)_2$ (150 mg, 65% yield).

c) 1 (0.4 mmol) was dissolved in an ACN + T.B.A.P. 0.1 M solution (200 ml). After addition of an excess of methyl iodide (20 mmol), the resulting solution was exhaustively reduced under nitrogen at a platinum working electrode (E = -1050 mV s.c.e.). When the electrolysis was complete, the solution was evaporated to dryness in vacuo at 30°C. Chromatography of the residue (dichloromethane-acetone 12:0.5), after elimination of T.B.A.P. (see §b), provided 2b (65 mg, 75% yield).

REFERENCES

1. M.B. FLEURY, M. LARGERON, M. BARREAU and M. VUILHORGNE, Tetrahedron, in press.
2. RHONE-POULENC-INDUSTRIE, Brevets français N° 760 3604, 10 February 1972; N° 7636 901, 23 December 1976; brevet belge N° 851 262, 9 August 1977.
3. J.P. LEROY, M. BARREAU, C. COTREL, J. JEANMART, M. MESSER and F. BENAZET, in W. Siegenthaler and R. Lüthy (eds) Current Chemotherapy. Proceedings of the 10 th international congress of chemotherapy vol. 1 American Society of Microbiology, Washington, 1978 p. 148, Cur. Chemother. 1, (1978) 148.
4. A. BIEDER, B. DECOUVELEARE, C. CAILLARD, H. DEPAIRE, D. HEUSSE, C. LEDOUX, M. LEMAR, J.P. LEROY L. RAYNAUD, C. SNOZZI and J. GREGOIRE, Arzneim. Forsch. (Drug. Res.) 33 (II), (1983), 1289.
5. J. MOIROUX, S. DEYCARD and M.B. FLEURY, J. Electroanal. Chem. 146, (1983), 313.
6. J. MOIROUX and S. DEYCARD, J. Electrochem. Soc. 131, (1984), 2840.
7. M. LARGERON, D. FLEURY and M.B. FLEURY, J. Electroanal. Chem. 167, (1984), 183.
8. J.P. LEROY, Private Communication.