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# Incidence of the Peptidic Lactone Opening on the Electrochemical Reduction of Pristinamycin $I_A$.

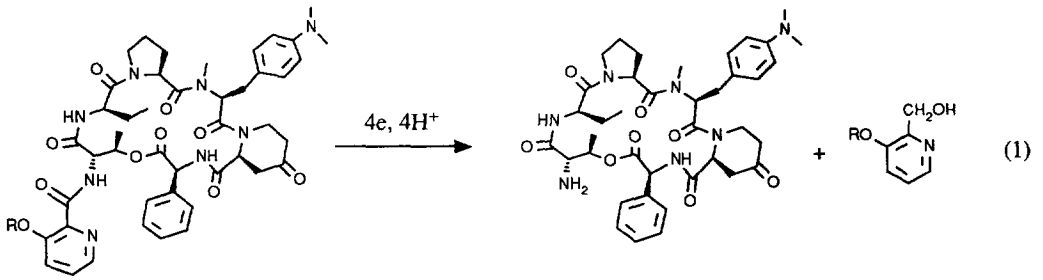
**Martine Largeron[#], Nicolas Auzeil[#], Bouria Dakova[#], Eric Bacqué[‡], Jean-Marc Paris[‡] and Maurice-Bernard Fleury[#*].**

[#]*Laboratoire de Chimie Analytique et Electrochimie associé au CNRS, Faculté de Pharmacie, 4 Avenue de l'Observatoire, 75270 Paris Cedex 06, France.*

[‡]*Rhone-Poulenc Rorer, Centre de Recherches de Vitry Alfortville, 13 quai Jules Guesde, 94403 Vitry sur Seine Cedex, France.*

***Abstract:*** *Comparison of the cathodic behaviour of pristinamycin $I_A$ with an open ring derivative corroborates the role of the steric crowding exerted by the peptidic lactone.* Copyright © 1996 Elsevier Science Ltd

We recently reported that pristinamycin $I_A$ ($PI_A$) could be reduced either electrochemically or chemically, in aqueous acidic solutions, to afford des-3-hydroxy-picolinoyl-pristinamycin $I_A$ (**M**-$NH_2$) in yields ranging from 40 to 50%, along with numerous by-products[1,2].




(1)

R = H ($PI_A$) **M** $NH_2$

R = $CH_3$ , ($PI_A$-$OCH_3$)

In a more detailed study of the electrochemical reduction of $PI_A$ and related streptogramins, we demonstrated the involvement of an original reduction mechanism, proceeding through an electron transfer to the pyridyl nucleus, which initiated three competing reactions[3]:

1 - splitting of the C-N bond, to yield an hydroxymethylpyridine and the free amine **M**-$NH_2$ [eqn. (1)];

2 - splitting of the C-O bond (dehydration), to give the corresponding aminomethylpyridine derivative;

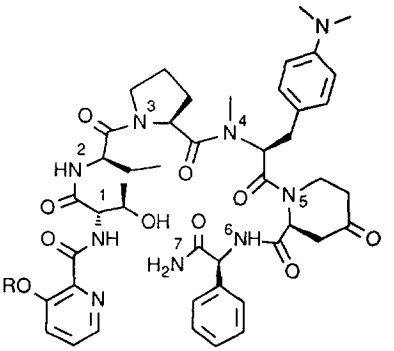
3 - reduction of the heterocyclic nucleus into a tetrahydropyridine derivative.

When compared to previous results concerning the electrochemical reduction of pyridyl carboxamides[4,5], this work highlighted the role of the peptidic lactone residue **M** attached to the amide

nitrogen. This influence was twofold: it favoured the production of the aminomethylpyridine on the one hand, and that of tetrahydropyridine derivatives on the other hand. To the best of our knowledge, the formation of the latter by electrochemical reduction had not been reported previously in aqueous acidic medium.

Moreover, thanks to a series of pyridyl carboxanilide models, we demonstrated that increasing steric hindrance at the amide nitrogen position led to enhanced reduction of the pyridyl ring. Accordingly, 2,6-diisopropylphenyl-3-methoxy-picolinamide was shown to display the same cathodic behaviour as $PI_A$-$OCH_3$ (50% of free amine and 30% of tetrahydropyridine). Likewise, the corresponding hydroxylated derivatives (2,6-diisopropyl-3-hydroxy-picolinamide and $PI_A$) were found identical in their reduction process (50% of free amine, no isolated tetrahydropyridine). Then, we thought that the steric crowding generated by the peptidic macrolactone **M** at the amide nitrogen best explained the specific cathodic behaviour of $PI_A$.

In order to confirm the role of **M**, we chose to study the electrochemical behaviour of compounds **1** and **2**, i.e. linear hexapeptides whose sequence was identical to that of macrolide **M**, but characterized by increased conformational freedom around the picolinamide nitrogen position. The lactone ring was opened by ammonolysis[6], giving **1** (or **2**), a C-terminal amide, in roughly 70% isolated yield[7].



**1**: R = H

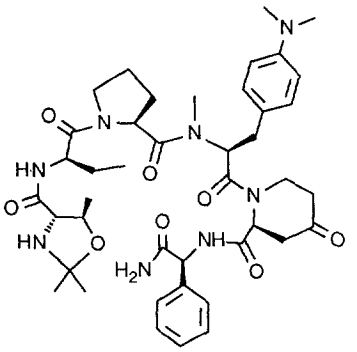
**2**: R = $CH_3$

The cyclic voltammogram of **1** (or **2**) in aqueous 0.5 mol.$L^{-1}$ sulfuric acid solution, at a stationary mercury electrode, showed two distinct reduction peaks $Pc_1$ and $Pc_2$, around -800 and -1050 mV vs. saturated calomel electrode (s.c.e.) respectively, the sweep rate being 0.2 V.$s^{-1}$. When the potential scan was reversed after $Pc_1$ or after $Pc_2$, no anodic peak was recorded in the reverse sweep of the cyclic voltammogram of **1** (or **2**), showing the irreversibility of the cathodic process.

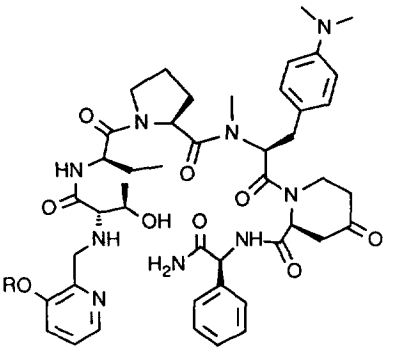
When the controlled potential (E) of the mercury pool working electrode was fixed at -850 mV s.c.e., i.e. at a potential immediately following the first cathodic peak $Pc_1$ and preceding the second peak $Pc_2$, a coulometric value of 4.0 ± 0.2 was found for the number of electrons (n) involved in the reduction of one molecule of **1** (or **2**). As the electrolysis proceeded, a decrease in the first cathodic peak $Pc_1$ was observed and the voltammogram of the exhaustively reduced solution exhibited a sole cathodic peak at -1050 mV s.c.e. Finally, preparative scale electrolysis[8] allowed the isolation of two major compounds **3** and **4** (or **5**), in respectively 45% and 30% yields. Note that the expected amine was isolated as an oxazolidine **3**[9], as a consequence of the reaction of the aminoalcohol with acetone upon chromatography.

When E was fixed at -1100 mV s.c.e., i.e. at a potential immediately following the second cathodic peak $Pc_2$, a coulometric value of 5.0 ± 0.2 was found for n; no cathodic peak was observed in the cyclic voltammogram of the exhaustively reduced solution of **1** (or **2**). Then, compound **3** was isolated as the sole

product in 75% yield, owing to the reduction of aminomethylpyridine **4** (or **5**) which produced the corresponding picoline and oxazolidine **3**. The fact that picoline and oxazolidine **3** were reliably formed from aminomethylpyridine was confirmed by a direct electrolysis of **4** (or **5**) performed at -1100 mV s.c.e. which yielded, after consumption of 2 Faraday. $mol^{-1}$, oxazolidine **3** in quantitative yield.



**3**



**4**: R = H

**5**: R = $CH_3$

On the basis of these findings, it appeared that replacement of the peptidic lactone residue **M** by the corresponding linear hexapeptide induced noticeable changes in the electrochemical behaviour of pyridyl carboxamide.

When working at -850 mV s.c.e., comparison of the results obtained from **1** or **2** with that exhibited by $PI_A$ or $PI_A$-$OCH_3$ showed that the yield of aminomethylpyridine remained unchanged (30%), while the yield of amine derived from the C-N bond splitting reaction was found three fold higher (45% against 15%).

When working at -1100 mV s.c.e., no tetrahydropyridine was detected using **2** as starting material, while the yield of amine increased from 45% to 75%. Then, the cleavage of the C-N bond became the sole route, without evidence of the formation of the tetrahydropyridine. This behaviour obviously resulted of conformational changes associated with the opening of the peptidic lactone residue **M**, which induced a decrease of the steric crowding in the neighbourhood of the picolinamide group. Finally, these results provide further indications that the specific behaviour of the picolinamide of $PI_A$ stems from the steric crowding generated by the peptidic macrolactone **M** at the amide nitrogen position.

## References and notes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7. ***Linear hexapeptide 1***: $^1$H NMR (300 MHz, $CDCl_3$): δ 0.25 (dd, 1H, $5\beta_2$, J = 6 Hz, J = 16 Hz), 0.90 (t, 3H, 2γ, J = 6 Hz), 1.25 (d, 3H, 1γ, J = 6 Hz), 1.60 (m, 1H, $2\beta_2$), 1.85 (m, 4H, $2\beta_1$, $3\beta_2$, $3\gamma_1$ and $3\gamma_2$), 2.00 to 2.20 (m, 4H, $3\beta_1$, $5\beta_1$, $5\delta_1$ and $5\delta_2$), 2.50 (dt, 1H, $5\epsilon_2$, J = 5 Hz, J = 14 Hz), 2.85 [s, 6H, $N(CH_3)_2$], 3.10 (dd, 1H, $4\beta_2$, J = 4 Hz, J = 14 Hz), 3.20 (m, 1H, $4\beta_1$), 3.35 (s, 3H, $NCH_3$), 3.50 (m, 2H, $3\delta_1$ and $3\delta_2$), 4.55 (m, 3H, 1α, 1β and $5\epsilon_1$), 4.70 to 4.90 (m, 3H, 2α, 3α and 5α), 5.40 (dd, 1H, 4α, J = 4 Hz, J = 14 Hz), 5.60 (d, 1H, 6α, J = 8 Hz), 6.30 (broad s, 1H, 7NH, $D_2O$ exchanged), 6.60 (d, 2H, 4ϵ, J = 8 Hz), 6.80 (broad s, 1H, 7NH, $D_2O$ exchanged), 7.10 (d, 2H, 4δ, J = 8 Hz), 7.20 [m, 7H, 1'$H_4$, aromatic(6) and 2NH ($D_2O$ exchanged)], 7.40 (m, 1H, 1'$H_5$), 8.15 (dd, 1H, 1'$H_6$, J = 5 Hz, J = 1 Hz), 8.90 (d, 1H, 1NH, J = 8 Hz, $D_2O$ exchanged), 9.10 (d, 1H, 6NH, J = 8 Hz, $D_2O$ exchanged), 11.7 [s, 1H, 1'OH(3), $D_2O$ exchanged]; $^{13}$C NMR (75 MHz, $CDCl_3$): δ 9.7 (2γ), 19.0 (1γ), 24.4 (3γ), 25.3 (2β), 27.8 (3β), 31.4 ($NCH_3$), 35.7 (4β), 37.2 (5β), 38.9 (5δ), 40.1 (5ϵ), 40.5 [$N(CH_3)_2$], 47.0 (3δ), 52.0 (5α), 54.5 (2α), 56.9 (3α), 57.0 (6α), 57.8 (4α), 58.2 (1α), 66.6 (1β), 112.8 (4ϵ), 122.1 (4γ), 125.8 and 128.0 [1'CH(4) and 1'CH(5)], 126.5, 128.7 and 128.9 [CH, aromatic(6)], 130.3 (4δ), 130.8 (1'C-2), 137.8 [Cq, aromatic(6)], 140.0 [1'CH(6)], 150.3 [Cq-$N(CH_3)_2$], 157.5 (1'C-3), 168.0, 169.4, 169.7, 169.9, 171.6, 172.4 and 172.5 (CO, amides), 204.4 (5γ); MS (DCI): m/z = 886 ($MH^+$).

8. A typical procedure was as follows: 200 mL of an aqueous sulfuric acid (0.5 mol. $L^{-1}$) solution of linear hexapeptide (0.4 mmol) were reduced under nitrogen, at 25°C, in a 3-compartment cell (cathode: mercury pool; anode: platinum foil). After exhaustive electrolysis, i.e. when a steady state minimum value of the current was recorded, the resulting solution was neutralized by a sodium carbonate solution (5 mol. $L^{-1}$) and extracted with dichloromethane (200 mL). The organic phase was dried over anhydrous magnesium sulfate and the solvent removed under reduced pressure at 30°C. The residue was chromatographied on silica.

9. ***oxazolidine 3***: $^1$H NMR (300 MHz, $CDCl_3$): δ 0.30 (dd, 1H, $5\beta_2$), 0.95 (t, 3H, 2γ), 1.35 and 1.45 (s, 3H, $CH_3$, oxazolidine), 1.40 (d, 3H, 1γ), 1.50 to 1.80 (m, 5H, $2\beta_1$, $2\beta_2$, $3\beta_2$, $3\gamma_1$ and $3\gamma_2$), 2.00 to 2.20 (m, 4H, $3\beta_1$, $5\beta_1$, $5\delta_1$ and $5\delta_2$), 2.60 (dt, 1H, $5\epsilon_2$), 2.80 [s, 6H, $N(CH_3)_2$], 3.00 (dd, 1H, $4\beta_2$), 3.25 (m, 1H, $4\beta_1$), 3.35 (s, 3H, $NCH_3$), 3.45 (d, 1H, 1α), 3.55 (m, 2H, $3\delta_1$ and $3\delta_2$), 4.05 (q, 1H, 1β), 4.50 to 4.90 (m, 4H, 2α, 3α, $5\epsilon_1$and 5α), 5.45 (dd, 1H, 4α), 5.60 (d, 1H, 6α), 6.55 (broad s, 1H, 7NH, $D_2O$ exchanged), 6.60 (d, 2H, 4ϵ), 6.90 (broad s, 1H, 7NH, $D_2O$ exchanged), 7.10 (d, 2H, 4δ), 7.25 [m, 5H, aromatic(6)], 7.50 (d, 1H, 2NH, $D_2O$ exchanged), 8.75 (d, 1H, 6NH, $D_2O$ exchanged). $^{13}$ C NMR (75 MHz, $CDCl_3$): δ 9.9 (2γ), 20.5 (1γ), 24.5 (3γ), 25.5 (2β), 26.5 and 28.3 ($CH_3$, oxazolidine), 27.9 (3β), 31.4 ($NCH_3$), 35.8 (4β), 37.1 (5β), 39.0 (5δ), 40.0 (5ϵ), 40.4 [$N(CH_3)_2$], 47.1 (3δ), 51.2 (5α), 54.0 (2α), 57.1 (3α), 57.4 (6α), 57.8 (4α), 67.0 (1α), 75.9 (1β), 94.7 (Cq, oxazolidine), 112.8 (4ϵ), 122.1 (4γ), 126.3, 127.8 and 128.5 [CH, aromatic(6)], 130.2 (4δ), 137.8 [Cq, aromatic(6)], 150.3 [Cq-$N(CH_3)_2$], 167.7, 169.7, 171.4, 171.5, 171.6 and 173.0 (CO, amides), 204.5 (5γ); MS (DCI): m/z = 803 ($MH^+$).

***aminomethylpyridine 4:*** see oxazolidine **3**, with the exception of the following signals: $^1$H NMR (300 MHz, $CDCl_3$): δ 3.70 (broad s, 1H, 1NH, $D_2O$ exchanged), 3.90 to 4.15 (dd, 2H, 1'$\underline{CH_2}$-NH), 7.15 to 7.30 [m, 7H, 1'$H_4$, 1'$H_5$ and aromatic(6)], 7.95 (dd, 1H, 1'$H_6$), 11.5 [s, 1H, 1'OH(3), $D_2O$ exchanged]; $^{13}$ C NMR (75 MHz, $CDCl_3$): δ 52.0 (1'$\underline{CH_2}$-NH), 67.9 (1β), 123.4 and 123.6 [1'CH(4) and 1'CH(5)], 139.4 [1'CH(6)], 144.7 (1'C-2), 153.5 (1'C-3); MS (DCI): m/z = 872 ($MH^+$).

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