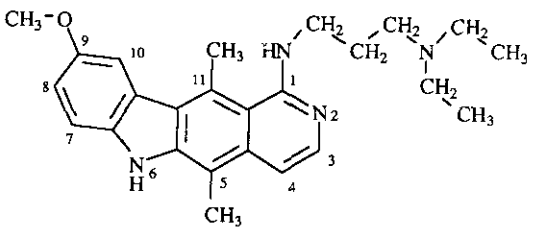
SYNTHESIS OF 9-METHOXYELLIPTICINE *N*-OXIDE AND ITS TRANSFORMATION INTO 1-FUNCTIONALIZED ELLIPTICINE DERIVATIVES

Christian Rivalle* and Emile Bisagni

URA 1387 CNRS, Institut Curie, Section de Biologie, Bât. 110-112, 91405 Orsay, France

Abstract - 9-Methoxy-5,11-dimethyl-(and 5-methyl)-*6H*-pyrido[4,3-*b*]carbazole-1-carbonitriles (**8**) were synthesized through methoxyellipticine *N*-oxides (**4**) and subsequent deoxygenation-functionalization by diethyl cyanophosphonate. Hydration of these nitriles (**8**) into 1-carboxamidoellipticines (**9**) and cytotoxicities of these last compounds are also reported.

9-Methoxyellipticine (9-methoxy-5,11-dimethyl-*6H*-pyrido[4,3-*b*]carbazole) bearing a 3-diethylaminopropylamino chain at the 1-position (BD 84, Retelliptine, (**1**))[1] which display potent antitumor properties,[2-4] was previously described in this laboratory.[1] Its synthesis was performed through a new route including the final D ring closure affording the 9-methoxy-5,11-dimethyl-*6H*-pyrido[4,3-*b*]carbazol-*2H*-1-one (**2a**), precursor of the 1-chloro-9-methoxyellipticine key intermediate (**3a**). Despite the large number of syntheses of ellipticines, repeatedly reported in various reviews,[5-12] the only other method for preparation of this 1-chloro-*6H*-pyrido[4,3-*b*]carbazole (**3a**) is that recently described by Dormoy and Heymes.[13]



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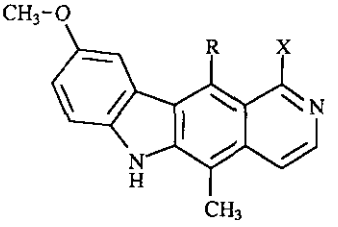


2 a, b X = OH

3 a, b X = Cl

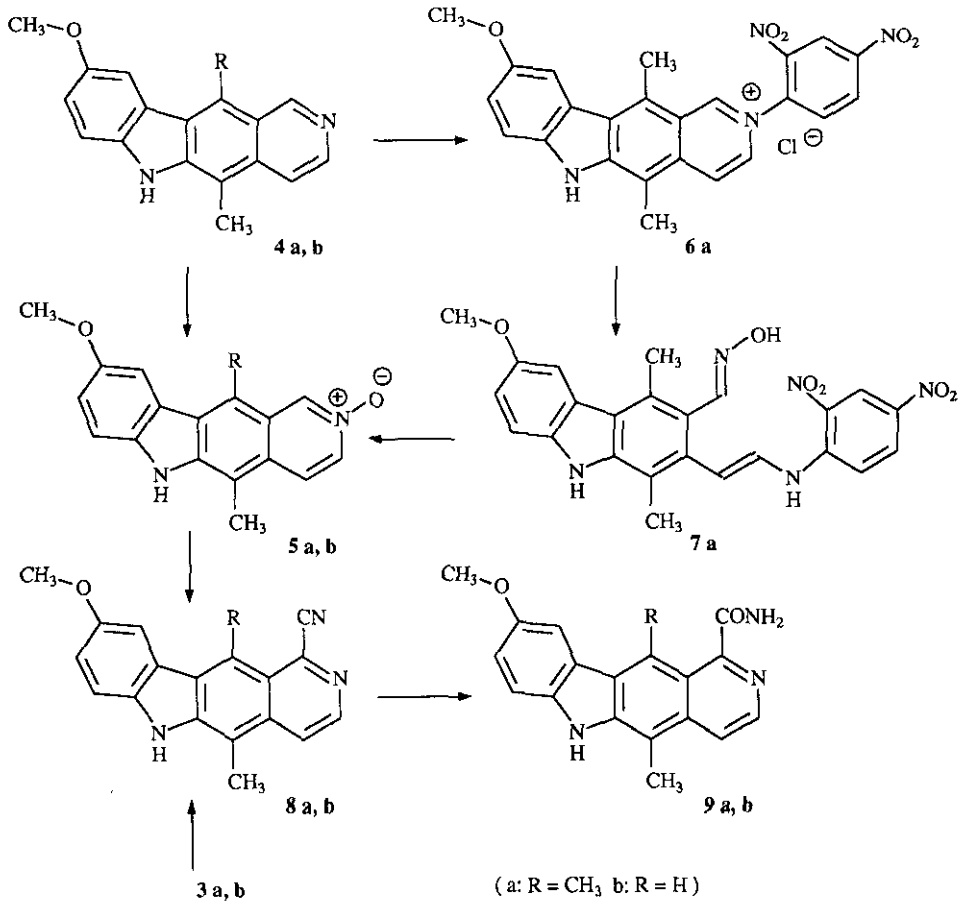
4 a, b X = H

(a: R = CH_3 b: R = H)

In view of what is known on the antitumor properties of *N*-dialkylaminoalkylcarboxamido acridines,[14] new 1-functionalized ellipticine derivatives could be of biological interest. This prompted us to study possible new methods to obtain 1-functionnalized *6H*-pyrido[4,3-*b*]carbazoles. In this paper, we report our results in this area.

A common way to obtain 2-chloropyridines and 1-chloroisoquinolines is the phosphorus oxychloride deoxygenation-chlorination of the corresponding *N*-oxides.[15-16] *A priori*, 1-chloro-9-methoxyellipticine (**3a**) could thus arise from 9-methoxyellipticine *N*-oxide (**5a**). However, this compound was yet unknown and only ellipticine *N*-oxide itself, which was isolated from *Ochrosia vieillardi* and identified by ms, uv and ir spectroscopic data, was reported by Bruneton *et al.*[17] Moreover, these authors failed to prepare this compound from ellipticine by hydrogen peroxide in chloroform or by *p*-nitroperbenzoic acid in various solvents.[17]

In order to obtain *N*-oxide (**5a**) from (**4a**), we then decided to apply the Tamura's method[18] which includes the use of 2,4-dinitrochlorobenzene as quaternarizing agent, opening of the pyridine ring into the aldehyde oxime



Scheme I

and final cyclization in the presence of hydrochloric acid. Applying this method (Scheme I), 2-*N*-(2,4-dinitrophenyl)ellipticinium chloride (**6a**) was obtained (81 %) from **4a**, and hydroxylamine in methanol solution opened the pyridine nucleus of **6a** giving corresponding oxime (**7a**) (57 %). Unfortunately, the conditions, described by Tamura *et al.* [18] for the cyclization of this kind of oxime into corresponding *N*-oxide, did not work in the case of **7a**. To perform this transformation, refluxing ethoxyethanol in the presence of *p*-toluenesulfonic acid was required but the yield did not exceed 24 % with an overall yield of only 11 %. Therefore we tried to prepare this compound by a more direct and efficient method. To our surprise, treatment of 9-methoxyellipticine (**4a**) with *m*-chloroperbenzoic acid (MCPBA) in the mixture chloroform/ethanol 1/1 at reflux and subsequent usual treatment, provided 9-methoxyellipticine *N*-oxide (**5a**) in good yield (64 %). Contrary to the successful transformation of pyridine *N*-oxide and isoquinoline *N*-oxide into 2-chloropyridine and 1-chloroisoquinoline[15] by phosphorus oxychloride alone or in the presence of phosphorus pentachloride, or by sulfuryl chloride, these chlorinating agents did not transform *N*-oxide (**5a**) into the corresponding 1-chloroellipticine (**3a**). Likewise, unsuccessful results were obtained by trying to transform *N*-oxide (**5a**) by acetic anhydride which however transforms the isoquinoline *N*-oxides into isoquinolin-1-ones.[16] The weak nucleophilicity of the chloride ion and acetoxy group probably explain these failures. Indeed, diethyl cyanophosphonate (DEPC), a donor of the more nucleophilic cyano group which transforms isoquinoline *N*-oxide into isoquinoline-1-carbonitrile,[19] reacts with 9-methoxyellipticine *N*-oxide (**5a**) giving corresponding 9-methoxyellipticine-1-carbonitrile (**8a**) as expected. The same compound was also obtained from 1-chloroellipticine (**3a**) by reaction with copper (I) cyanide (49 %).

The nitrile (**8a**) was subjected to hydration conditions by hydrogen peroxide in alkali media. 9-Methoxyellipticine-1-carboxamide (**9a**) was thus obtained in 64 % yield.

The same reactions have been performed by starting from 11-desmethylellipticine (**4b**). *N*-Oxide (**5b**), nitrile (**8b**) and carboxamide (**9b**) have been successively obtained in the same conditions and with comparable yields. The only difference which must be pointed out is the rate of nitrile to amide transformation which is slow for **8a** and normal for **8b** (7 days/4 hours). Steric hindrance is probably concerned in this case.

From the biological point of view, the reported antitumor properties of acridines bearing a *N*-substituted carboxamidogroup[14] whose unsubstituted nucleus is inactive, justify the testing of ellipticine-1-carboxamides (**9a, b**). They were studied *in vitro* on the growth of L1210 Leukaemia cells in culture in the conditions given in a preceding paper from this laboratory. [20] Toxic at 5.10^{-6}M and inactive at 10^{-6}M concentrations, these compounds were thus almost as active as 9-methoxyellipticines (**4a, b**).

In conclusion, the deoxygenation-functionalization of ellipticine *N*-oxides did not work for the introduction of a weakly nucleophilic chloride ion. However the successful transformation of ellipticine *N*-oxides into ellipticine-1-carbonitriles provides a new way to 1-functionalized ellipticine derivatives.

EXPERIMENTAL

All melting points were determined with a Reichert hot-stage microscope and are uncorrected. ^{1}H-Nmr spectra were recorded on a Bruker AC200 spectrometer. Mass spectra were obtained with a (AEI) MS-9 spectrometer, ICSN, CNRS, 91190 Gif-sur-Yvette. Elemental analyses were performed at the ICSN, CNRS.

9-Methoxy-5,11-dimethyl-*6H*-pyrido[4,3-*b*]carbazole *N*-oxide (5a).

Method A (from 9-metoxyellipticine (**4a**)). To the mixture of 9-methoxyellipticine (**4a**) (553 mg, 2 mmol) in boiling $CHCl_3$/EtOH mixture 1/1 (40 ml), MCPBA (720 mg, 4.4 mmol) was progressively added. The mixture turned homogenous after addition of 400 mg (2.4 mmol) of MCPBA and the reflux was maintained 2 h. The solvents were removed by evaporation and the residue was stirred in 1 N ammonium hydroxide. The solid was filtered, washed with water, and recrystallized from EtOH to yield **5a** as beige needles, 452 mg, 64 %, mp > 260°C. *Anal.* Calcd for $C_{18}H_{16}N_2O_2$, 3.5 H_2O : C, 61.24 ; H, 6.47 ; N, 7.88. Found : C, 60.84 ; H, 6.47 ; N, 7.88. ^{1}H-Nmr ($(CD_3)_2SO$) δ : 2.81 (3H, s, 5-CH_3), 3.14 (3H, s, 11-CH_3), 3.94 (s, 3H, OCH_3), 7.35 (1H, dd, J = 8.7 Hz, J = 2.1 Hz, 8-H), 7.52 (1H, d, J = 8.7 Hz, 7-H), 7.89 (1H, d, J = 2.1 Hz, 10-H), 8.09 (2H, br s, 3-H + 4-H), 9.20 (1H, s, 1-H), 11.32 (1H, s, NH).

Method B (from oxime (**7a**)). A mixture of oxime (**7a**) (95 mg, 0.2 mmol) and *p*-toluenesulfonic acid (300 mg, 1.6 mmol) in ethoxyethanol (30 ml) was refluxed for a 20 h period. Solvent was evaporated *in vacuo* and water was added to the residue. After neutralization to pH 7 with 1N NaOH, this solution was extracted with $CHCl_3$ (10 x 20 ml). The organic layer was washed with water and dried ($MgSO_4$). After evaporation *in vacuo*, the residue was triturated with Me_2CO to give *N*-oxide (**5a**) (14 mg, 24 %) in all respects identical to the compound obtained by *N*-oxidation, method A.

9-Methoxy-5-methyl-*6H*-pyrido[4,3-*b*]carbazole *N*-oxide (5b). Using the same technique as for **5a** from **4a**, **5b** (65 %) was obtained as beige crystals, mp > 260°C (from EtOH). Anal. Calcd for $C_{17}H_{14}N_2O_2$, 2.5 H_2O : C, 63.14 ; H, 5.92 ; N, 8.66. Found : C, 62.94 ; H, 6.03 ; N, 8.50. ^{1}H-Nmr ($(CD_3)_2SO$) δ : 2.85 (3H, s, 5-CH_3), 3.92 (3H, s, OCH_3), 7.18 (1H, dd, J = 6.8 Hz, J = 2.5 Hz ; 8-H), 7.50 (1H, d, J = 6.8 Hz, 7-H), 7.85 (1H, d, J = 2.5 Hz), 8.10 (2H, br s, 3-H + 4-H), 8.58 (1H, s, 11-H), 9.02 (1H, s, 1-H), 11.33 (1H, br s, NH).

6-Methoxy-1,4-dimethyl-2-[2-(2,4-dinitrophenylamino)vinyl]-*9H*-carbazol-3-carbaldehyde oxime (7a). A mixture of ellipticine (**4a**) (1 g, 3.6 mmol) and 2,4-dinitrochlorobenzene (730 mg, 3.6 mmol) was heated at 50°C for 15 h and the resulting mixture was taken up in boiling acetone. After cooling, the solid product was filtered and washed with Et_2O giving the quaternarized ellipticine (**6a**) as violet needles (1.4 g, 81 %) used without further purification for the next step. ^{1}H-Nmr ($(CD_3)_2SO$) δ : 2.97 (3H, s, 5-CH_3), 3.32 (3H, s, 11-CH_3), 3.99 (3H, s, OCH_3), 7.41 (1H, dd, J = 8.8 Hz, J = 2.15 Hz, 8-H), 7.73 (1H, d, J = 8.8 Hz, 7-H), 8.02 (1H, d, J = 2.15 Hz, 10-H), 8.49 (1H, d, J = 8.65 Hz, 6'-H), 8.70 (2H, m, 3-H + 4-H), 9.03 (1H, dd, J = 8.65 Hz, J = 2.4 Hz, 5'-H), 9.22 (1H, d, J = 2.4 Hz, 3'-H), 10.38 (1H, s, 1-H), 12.52 (1H, s, NH).

To a cooled (0°C) solution of compound (**6a**) (240 mg, 0.5 mmol) in MeOH (250 ml), the hydroxylamine hydrochloride (70 mg, 1 mmol) and triethylamine (101 mg, 1 mmol) dissolved in MeOH (20 ml) were added. After 2 h stirring, addition of hydroxylamine hydrochloride (70 mg, 1mmol) was repeated and the mixture was stirred 4 h further. The mixture was evaporated under reduced pressure and the residue was taken up in boiling MeOH (30 ml), and filtered. To the filtrate, water (60 ml) was added and the precipitate was collected, washed with water and Et_2O to provide the oxime (**7a**) (140 mg, 57 %) as brick-red powder, mp 160°C. *Anal.* Calcd for $C_{24}H_{21}N_5O_6$: C, 60.80 ; H, 4.73 ; N, 14.34. Found : C, 60.62 ; H, 4.45 ; N, 14.73. ^{1}H-Nmr ($(CD_3)_2SO$) δ :

2.54 (5-CH_3 under DMSO), 2.86 (3H, s, 4-CH_3), 3.90 (3H, s, OCH_3), 5.93 (2H, 2d degenerated, 2H vinyl), 7.13 (1H, dd, J = 8.8 Hz, J = 2.2 Hz, 7-H), 7.52 (1H, d, J = 8.8 Hz, 8-H), 7.71 (1H, d, J = 2.2 Hz, 5-H) ; 7.98 (1H, d, J = 9.6 Hz, 6'-H), 8.28 (1H, s, CH oxime), 8.41 (1H, dd, J = 2.6 Hz, J = 9.6 Hz, 5'-H), 8.48 (1H, s, OH oxime), 8.87 (1H, d, J = 2.6 Hz, 3'-H), 11.40 (1H, s, 9-NH).

9-Methoxy-5,11-dimethyl-*6H*-pyrido[4,3-*b*]carbazole-1-carbonitrile (8a).

Method A (from *N*-oxide (**5a**)). A mixture of *N*-oxide (**5a**) (585 mg, 2 mmol), triethylamine (210 mg, 2.1 mmol), and diethyl cyanophosphonate (DEPC) (1300 mg, 8 mmol) was refluxed during 24 h. After evaporation in vacuo, water was added and the mixture was adjusted to pH 12 with 1N NH_4OH. The resulting precipitate was collected by filtration, dried and recrystallized from EtOH to afford the expected nitrile (**8a**) (380 mg, 63 %) as red-brown crystals, mp > 260°C. Anal. Calcd for $C_{19}H_{15}N_3O$, 1.5 H_2O : C, 68.40 ; H, 5.68 ; N, 13.29. Found : C, 68.41 ; H, 5.98 ; N, 13.03. ^{1}H-Nmr ($(CD_3)_2SO$) δ : 2.85 (3H, s, 5-CH_3), 3.58 (3H, s, 11-CH_3), 3.91 (3H, s, OCH_3), 7.31 (1H, dd, J = 8.7 Hz, J = 1.9 Hz, 8-H), 7.57 (1H, d, J = 8.7 Hz, 7-H), 7.95 (1H, d, J = 1.9 Hz, 10-H), 8.31 (1H, d, J = 6.8 Hz, 4-H), 8.58 (1H, d, J = 6.8 Hz, 3-H), 11.51 (1H, s, NH).

Method B (from chloroellipticine (**3a**)). A solution of 1-chloroellipticine (**3a**) (155 mg, 0.5 mmol) and copper (I) cyanide (50 mg, 0.55 mmol) in dimethylformamide (2 ml) was refluxed during 2 h under nitrogen. After cooling, the mixture was poured into a solution of 10 % aqueous sodium cyanide (20 ml) and stirred 1 h. The solid was collected, washed with water and dried. Crystallization from toluene afforded the nitrile (**8a**) (72 mg, 49 %) identical to the compound obtained from *N*-oxide (**5a**).

9-Methoxy-5-methyl-*6H*-pyrido[4,3-*b*]carbazole-1-carbonitrile (8b). Using method A, *N*-oxide (**5b**) provided **8b** in 55 % yield as beige crystals, mp 245°C (from toluene). Anal. Calcd for $C_{18}H_{13}N_3O$, 0.5 C_7H_8, H_2O : C, 73.59 ; H, 5.45 ; N, 11.86. Found : C, 73.89 ; H, 5.15 ; N, 11.78. ^{1}H-Nmr ($(CD_3)_2SO$) δ : 2.90 (3H, s, 5-CH_3), 3.96 (3H, s, OCH_3), 7.26 (1H, dd, J = 8.7 Hz, J = 2.4 Hz, 8-H), 7.53 (1H, d, J = 8.7 Hz, 7-H), 8.19 (1H, d, J = 2.4 Hz, 10-H), 8.35 (1H, d, J = 6 Hz, 4-H), 9.58 (1H, d, 3-H), 9.01 (1H, s, 11-H), 11.55 (1H, s, NH), and 2.34 (1/2 3H, s, CH_3 tol.), 7.26 (1/2 5H, m, H Ar tol.).

Using method B but starting from **3b**, **8b** was obtained in 52 % yield. It was identical to the compound obtained from *N*-oxide (**5b**).

9-Methoxy-5,11-dimethyl-*6H*-pyrido[4,3-*b*]carbazole-1-carboxamide (9a). To a solution of nitrile (**8a**) (118 mg, 0.39 mmol) in MeOH (12 ml) refluxed under nitrogen, 4N NaOH (0.05 ml, 0.2 mmol) and 30 % H_2O_2 (0.16 ml, 1.56 mmol) were added. The resulting heterogeneous mixture was refluxed for a 7 days period while additions of the same reagents were repeated nine times. The solution thus became homogeneous and the nitrile (**8a**) disappeared (tlc monitoring). After evaporation *in vacuo*, water was added and the solid was filtered, washed with water and with EtOH to afford the expected carboxamide (**9a**) (80 mg, 64 %) as yellow ochre powder, mp > 260°C. Anal. Calcd for $C_{19}H_{17}N_3O_2$, 2H_2O : C, 64.21 ; H, 5.95 ; N, 11.82. Found : C, 63.88 ; H, 5.96 ; N, 11.6. ^{1}H-Nmr ($(CD_3)_2SO$) δ : 2.83 (3H, s, 5-CH_3), 3.26 (3H, s, 11-CH_3), 3.93 (3H, s, OCH_3), 7.24

(1H, dd, J = 8.7 Hz, J = 2.3 Hz, 8-H), 7.53 (1H, d, J = 8.7 Hz), 7.78 (1H, br s, NH amide), 7.88 (1H, d, J = 2.3 Hz, 10-H), 8.01 (1H, d, J = 6 Hz, 4-H), 8.17 (1H, br s, NH amide), 8.35 (1H, d, J = 6 Hz, 3-H), 11.28 (1H, s, 6-NH). Ms (CI) *m/z* : 320 (M + 1).

9-Methoxy-5-methyl-6*H*-pyrido[4,3-*b*]carbazole-1-carboxamide (9b). Using the above mentioned technique, but starting from nitrile (**8b**), this carboxamide (**9b**) was obtained after 4 h at reflux (67 %), mp > 260°C (from AcOEt). Anal. Calcd for $C_{18}H_{15}N_3O_2$, 0.5 $C_4H_8O_2$: C, 68.75 ; H, 5.48 ; N, 12.02. Found : C, 68.78 ; H, 5.31 ; N, 11.66. ^{1}H-Nmr ($(CD_3)_2SO$) δ : 2.88 (3H, s, 5-CH_3), 3.95 (3H, s, OCH_3), 7.21 (1H, dd, J = 8.7 Hz, J = 2.4 Hz, 8-H), 7.54 (1H, d, J = 8.7 Hz, 7-H), 7.76 (1H, br s, NH amide), 7.87 (1H, d, J = 2.4 Hz, 10-H), 8.13 (1H, d, J = 6 Hz, 4-H), 8.26 (1H, br s, NH amide), 8.45 (1H, d, J = 6 Hz, 3-H), 9.51 (1H, s, 11-H), 11.32 (1H, s, 6-NH). Ms (CI), m/z : 306 (M + 1).

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