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Regioselective Addition of Aniline to 8*H*-Pyrido[2,3,4-*mn*]acridinone: Structure Determination of The Reaction Product

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A combination of NMR techniques, including ^{13}C and ^{1}H DEPT, HMQC and HMBC experiments, was used to assign the structure of the compound formed by addition of aniline to 8*H*-pyrido[2,3,4-*mn*]acridine. From this NMR study, two structures were possible. This ambiguity was removed by regioselective introduction of one deuterium on the starting compound. © **1997 John Wiley & Sons, Ltd.**

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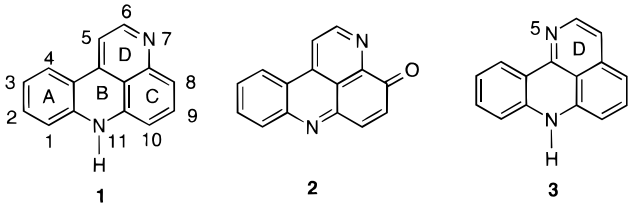
INTRODUCTION

The search for new drugs from marine sources led to the discovery in 1983 of the first compound of a new series of heterocyclic alkaloids known as pyridoacridines.[1] More than 60 compounds have been isolated and identified. The general structure is the 11*H*-pyrido[4,3,2-*mn*]acridine **1** (Scheme 1). A large number of derivatives also bear a methoxy group at position 8, but the most interesting compounds derive from the iminoquinone form, 8*H*-pyrido[4,3,2-*mn*]acridinone **2**. The major interest in these marine pyridoacridines is due to their significant biological properties. Almost all compounds have been reported as being cytotoxic, but other interesting properties have also been mentioned: intercalation in DNA, inhibition of topoisomerase II, anti-HIV activity and Ca^{2+} release activity.[1,2] The identification of the highly condensed heterocyclic structure of the natural pyridoacridines has required extensive NMR studies and especially multi-pulse NMR methods (HMQC, HMBC, INADEQUATE, INAPT).[3] $^{1-3}J_{CH}$ coupling constant analysis has also been used in the resolution of ambiguous structural assignments.[4] In the course of programs devoted to the synthesis and study of DNA-intercalating agents,[5] we have synthesized a structural isomer of the natural pyridoacridine, the 11*H*-pyrido[2,3,4-*mn*]acridine **3**, which differs only in the position of the nitrogen in D ring.[6] Except for the natural product necatorone,[7] this heterocyclic skeleton has not been synthesized and its chemical and biological properties are not known.



1 **2** **3**

Scheme 1

We have started to study in more detail this interesting nucleus and observed the following reactivity. As shown in Scheme 2, the 2-chloro-8-methoxy-11*H*-pyrido[2,3,4-*mn*]acridine[6] **4** was easily oxidized by cerium ammonium nitrate (CAN reagent) to give the 8*H*-8-oxo derivative **5**. This compound reacted with nucleophiles such as aniline to give one major compound. From the chemical point of view, two structures could be considered, **6a** or **6b**. The determination of the structure by a variety of NMR techniques is described, along with the description of an unambiguous synthesis involving the regioselective deuteriation of the starting molecule **4**.

RESULTS AND DISCUSSION

Oxidation of **4** with CAN led to the oxo derivative **5** in 61% yield. The ^{1}H NMR spectrum of **5** showed seven signals in the aromatic region. The reaction of compound **5** with aniline in ethanol in the presence of cerium chloride gave the condensation product **6** in 56% yield after column purification. The ^{1}H NMR spectrum indicated the presence of the aniline nucleus in the new molecule with two possible structures, **6a** or **6b**, with the anilino residue in position 10 or 9, respectively. The mass spectrum was in accordance with this general structure.

Determination of the structure of compound 6

The structure of **6** was initially characterized by ^{1}H and ^{13}C and DEPT edited.[8] These spectra were recorded in

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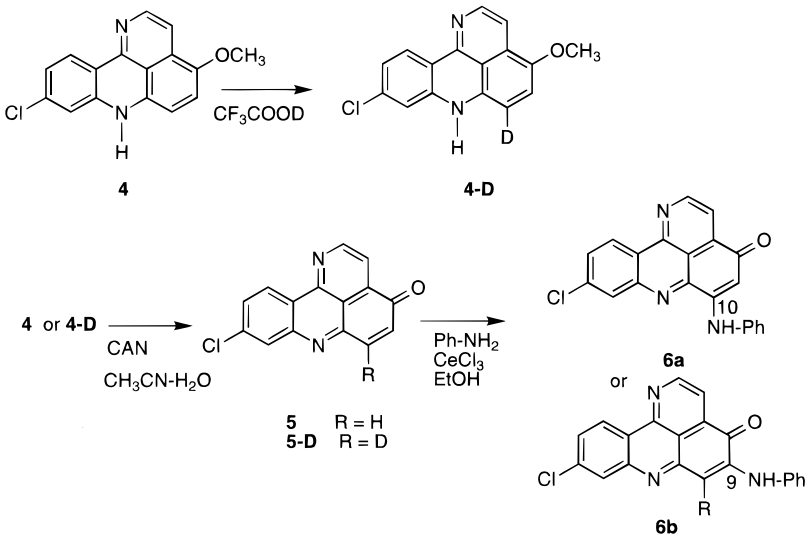


Scheme 2

$CDCl_3$ and, owing to the low solubility of the compound, a 0.17 mM concentration was used. The ^1H spectrum showed five doublets and a singlet characteristic of a substituted pyridoacridine along with the AA′BB′C coupling pattern corresponding to the aniline residue. The NH appeared as an exchangeable singlet at 8.87 ppm. Comparison of the coupling constants in **5** and **6** indicated that the addition had occurred on ring C. In **5**, the C ring was characterized by two doublets (H-9 and H-10) with a coupling constant $J = 10.3$ Hz that was missing in compound **6**. However, discrimination between positions 9 and 10 was not possible at this stage and, despite the low concentration of **6** in $CDCl_3$, we started heteronuclear 2D experiments. An HMQC spectrum[9] allowed the identification of all the protonated carbons. The remainder of the skeleton was deduced from HMBC[10] experiment optimized for $J = 5$ Hz. Two-, three-, four- and five-bond ^{13}C–^1H peaks were observed (see Table 1). We found a large number of four- and five-bond correlations as highlighted in Fig. 1. The observed correlations could well correspond to structure **6a** or **6b** depending on the assignment of a weak long-range coupling between H-6 and either C-9 or C-10 or *vice versa* (^5J or ^6J). The W geometry between H-6 and C-9 would be more in agreement with a ^5J coupling and therefore with structure **6a**.

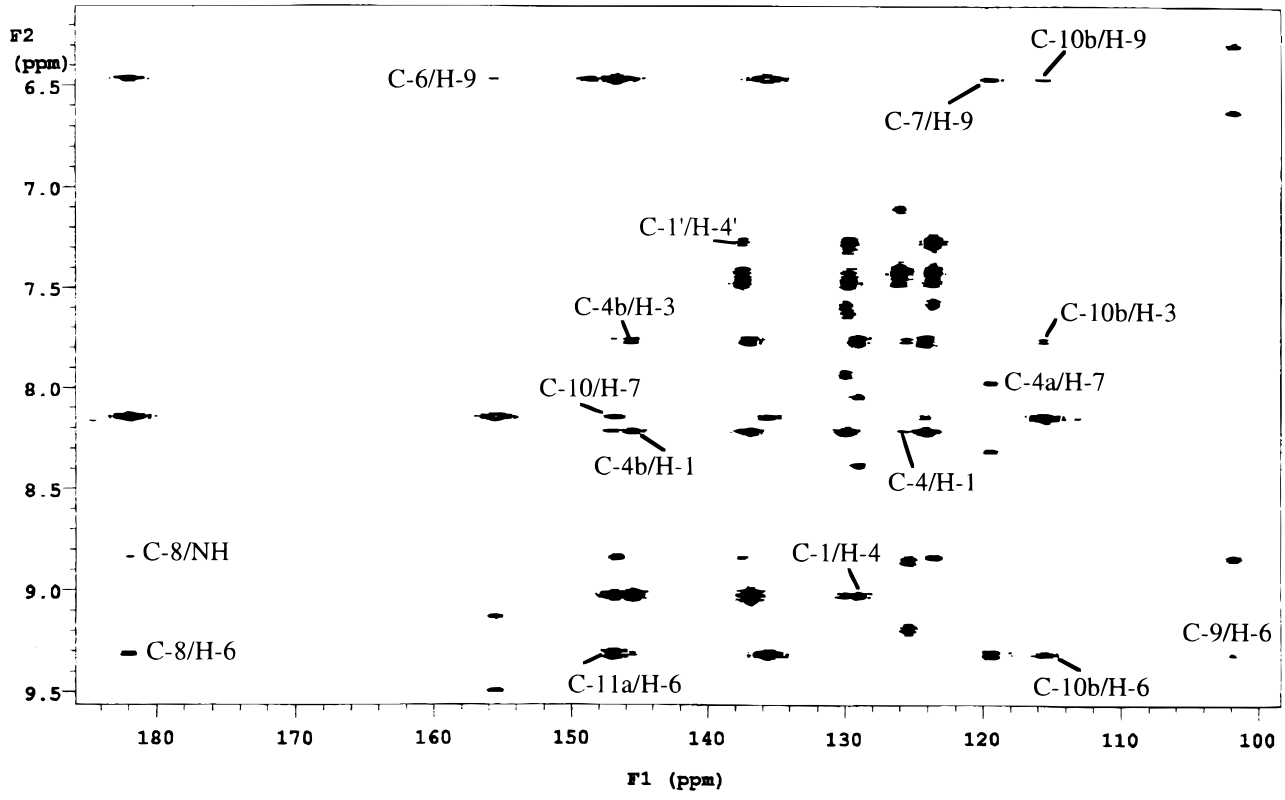
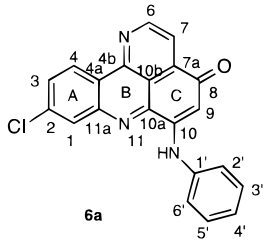


Figure 1. 500 MHz ^1H–^{13}C HMBC map optimized for $J = 5$ Hz. Specifically, four-bond and five-bond correlations are labelled. Some residual ^1J couplings are still present.

Table 1. $^{13}C^a$ and $^1H^a$ chemical shifts for compound 6a and *n*-bond connectivity peaks from HMBC[b]



6a

Carbon	δ_C	δ_H	*n*-Bond connectivities
1	129.1	8.26	H-3 (^3J), H-4 (^4J)
2	136.9		H-4 (^3J), H-1 (^2J), H-3 (^2J)
3	129.9	7.79	H-1 (^3J), H-4 (^2J)
4	125.4	9.07	H-1 (^4J), H-3 (^2J)
4a	124.2		H-1 (^3J), H-3 (^3J), H-7 (^5J)
4b	145.5		H-4 (^3J), H-1 (^4J), H-3 (^4J)
6	155.5	9.34	H-7 (^2J), H-9 (^5J)
7	119.4	8.17	H-6 (^2J), H-9 (^4J)
7a	135.6		H-6 (^3J), H-9 (^3J), H-7 (^2J)
8	181.9		H-7 (^3J), H-9 (^2J), H-6 (^4J), NH (^4J)
9	102.9	6.48	NH (^3J), H-6 (^5J)
10	146.7		NH (^2J), H-9 (^2J), H-7 (^5J)
10a	148.6		H-9 (^3J)
10b	115.5		H-7 (^3J), H-6 (^4J), H-9 (^4J), H-3 (^5J)
11a	146.9		H-4 (^3J), H-1 (^2J), H-6 (^5J)
1′	137.4		H-2′/H-6′ (^2J), H-3′/H-5′ (^3J), H-4′ (^4J) NH (^2J)
2′/6′	123.5	7.42	H-3′/H-5′ (^2J), H-6′/H-2′ (^3J), H-4′ (^3J) NH (^3J)
3′/5′	129.6	7.46	H-5′/H-3′ (^3J), H-2′/H-6′ (^{2}J), H-4′ (^2J)
4′	126.0	7.26	H-3′/H-5′ (^2J), H-2′/H-6′ (^3J)
NH		8.87	

[a] Chemical shifts on δ scale (ppm) in $CDCl_3$ at 500 MHz for ^1H and 125 MHz for ^{13}C.
[b] HMBC spectrum optimized for $J = 5$ Hz.

Regioselective deuteriation

To assign unambiguously the structure of **6**, we chose to replace one hydrogen of ring C of the starting compound **5** by deuterium. This was done at an earlier stage of the synthetic pathway, on **4**, by regioselective H–D exchange in acidic medium. Compound **4** was dissolved in CF_3COOD and allowed to stand at 25 °C. The reaction was followed by ^1H NMR by diluting samples of the reaction mixture in CD_3OD at different time intervals. With time, the two doublets at 7.25 and 7.60 ppm attributed to H-10 and H-9, respectively, disappeared, being replaced by a singlet at 7.60 ppm. The H–D exchange followed pseudo-first-order kinetics, a 0.017 h^{-1} rate constant was measured (half-life 40 h). Compound **4-D** was isolated in 62% yield after treatment in basic medium. The ^1H NMR spectrum of the free base of **4-D** contained six aromatic protons with a singlet at 7.15 ppm for H-9 or H-10, a singlet for the methoxy group at 3.87 ppm and a broad signal for the NH function at 10.49 ppm. The position of deuterium was established by NOE experiments. Irradiation of the methoxy group in position 8 ($\delta = 3.87$ ppm) resulted in an enhancement of the singlet at 7.15 ppm that was therefore attributed to H-9 and also has a small effect on the doublet at 7.28 that corresponded to H-7. Compound **4-D** was then oxidized by CAN to give the corresponding **5-D** compound that was characterized in ^1H NMR by the presence of signals of six aromatic protons. The reaction between **5-D** and aniline in ethanol in the presence of cerium chloride followed by HPLC gave the same condensation product **6**. The ^1H NMR spectrum of the compound formed from the deuteriated **5-D** was identical with that observed for the compound formed from the non-labelled **5**. Especially the singlet at 6.48 ppm attributed to the single proton of ring C was observed in both compounds, indicating that the deuterium atom of **5-D** was substituted in the reaction.

CONCLUSION

The structure assignment of highly condensed heterocyclic aromatic compounds may be complicated and require the use of multi-pulse NMR methods (HMBC, HMQC). For **6**, a surprisingly large number of long-range ^{13}C–^1H couplings were observed. This observation can be related to the fully conjugated planar structure of **6**. Introduction of a deuterium on the starting molecule **4** by regioselective H–D exchange at position 10 in acidic medium allowed the differentiation of

the two possible reaction centers (C-9 or C-10) in **5-D**. ^{1}H NMR spectra of the products **6** obtained from the reaction of **5** and **5-D** with aniline were identical, indicating that the reaction center was C-10, i.e. the structure is **6a**. The use of selectively deuteriated **5-D** will be helpful in the study of other chemical properties of this compound.

EXPERIMENTAL

Two-dimensional NMR measurements were carried out on a Varian UNITY plus 500 spectrometer. Saturated solutions of **6** (*ca.* 2–3 mg in 0.7 ml of $CDCl_3$) were used. H–D exchange was followed on a Bruker WP250 spectrometer. Residual peaks of the solvent were used as internal references ($CDCl_3$ at 7.24 ppm and CD_3OD at 3.30 ppm).

Syntheses

Deuteration of 4. Compound **4**[6] (0.2 g, 0.71 mmol) dissolved in TFA-*d* (5 ml), was stirred at room temperature for 5 days. The monodeuteriated compound **4-D** was precipitated by pouring the mixture into 1 M sodium hydroxide. The solid was collected by filtration, washed twice with water and dried. Compound **4-D** was thus obtained in 62% yield (0.12 g, 0.44 mmol). ^{1}H NMR (200 MHz, DMSO-d_6), δ (ppm) 10.49 (s, 1H, NH), 8.23 (d, 1H, J = 8.5 Hz, H-4), 8.16 (d, 1H, J = 6 Hz, H-6), 7.28 (d, 1H, J = 6 Hz, H-7), 7.15 (s, 1H, H-9), 7.01 (s, 1H, H-1), 6.96 (d, 1H, J = 8.5 Hz, H-3), 3.87 (s, 3H, OCH_3); MS (DCI, NH_3, isobutane), m/z 283 (M^+).

H–D exchange was followed by ^{1}H NMR: a stock solution of **4** (20 mg) in 5 ml of deuteriated trifluoroacetic acid was kept at 25 °C. At different time intervals, 0.1 ml of sample was collected, diluted with 0.5 ml of tetradeuteromethanol and analyzed by ^{1}H NMR (250 MHz).

Oxidation of 4 and 4-D, formation of 5 and 5-D. Compound **4** (0.1 g, 0.36 mmol) was dissolved in hot acetonitrile (200 ml). To this solution was added CAN (0.6 g, 1.1 mmol) dissolved in the minimum amount of water. After 1 h of stirring at room temperature, the solution was concentrated under vacuum, diluted with water and **5** was extracted with dichloromethane. The organic layers were collected, dried over magnesium sulfate and concentrated. The solution was kept at 0 °C and the desired compound **5** was precipitated by adding diethyl ether. It was obtained in 61% yield (0.6 g, 0.22 mmol), m.p. 185 °C (decomp.). ^{1}H NMR (200 MHz, $CDCl_3$), δ (ppm) 9.41 (d, 1H, J = 4.6 Hz, H-6), 9.05 (d, 1H, J = 8.7 Hz, H-4), 8.23 (d, 1H, J = 2 Hz, H-1), 8.19 (d, 1H, J = 4.5 Hz, H-7), 7.87 (d, 1H, J = 10.3 Hz, H-10), 7.76 (dd, 1H, J = 8.7 and 2 Hz, H-3), 6.96 (d, 1H, J = 10.3 Hz, H-9); MS (DCI, NH_3, isobutane), m/z 267 (M^+); IR (KBr), 3060, 1660, 1585, 1430, 1330, 1290, 1270, 1180, 1150, 1080, 920, 900, 830 cm^{-1}; analysis, calculated for $C_{15}H_7N_2OCl + 0.5\ H_2O$, C 65.47, H 2.75, N 10.18; found, C 65.40, H 2.52, N 9.98%.

The same reaction was performed with **4-D** to give **5-D**. ^{1}H NMR (200 MHz, $CDCl_3$), δ (ppm) 9.41 (d, 1H, J = 4.6 Hz, H-6), 9.05 (d, 1H, J = 8.7 Hz, H-4), 8.23 (d, 1H, J = 2 Hz, H-1), 8.19 (d, 1H, J = 4.5 Hz, H-7), 7.76 (dd, 1H, J = 8.7 and 2 Hz, H-3), 6.96 (s, 1H, H-9); MS (DCI, NH_3, isobutane), m/z 269 [$(M + 1)^+$].

Reaction with aniline, formation of 6. Compound **5** (0.04 g, 0.15 mmol) was dissolved in ethanol (50 ml) warmed at 50 °C. To this solution was added cerium chloride heptahydrate (1 g) and aniline (0.1 ml, 1.1 mmol). After 7 h of stirring, a new excess of cerium chloride (0.5 g) and aniline (0.1 ml) was added. Stirring was continued for 1 day and the solution was evaporated to dryness. The residue was diluted with 1 M sodium hydroxide and the solution was extracted with dichloromethane. The organic layers were collected, washed with water, dried on magnesium sulfate and concentrated under vacuum. Compound **6** was precipitated by adding cyclohexane and was obtained in 56% yield (0.03 g, 0.084 mmol), m.p. 243–245 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm), 9.34 (d, 1H, J = 4.5 Hz, H-6), 9.07 (d, 1H, J = 8.7 Hz, H-4), 8.87 (s, 1H, N-H), 8.26 (d, 1H, J = 2 Hz, H-1), 8.17 (d, 1H, J = 4.5 Hz, H-7), 7.79 (dd, 1H, J = 8.7 and 2 Hz, H-3), 7.52–7.24 (m, 5H, Ph-H), 6.48 (d, 1H, J = 10.3 Hz, H-9); MS (EI), 359 ($M^+ + 2$, ^{37}Cl), 357 (M^+, ^{37}Cl); IR (KBr), 3340, 1635, 1585, 1570, 1530, 1450, 1435, 1350, 1320, 1270, 1175, 1080, 935, 840, 810 cm^{-1}; analysis, calculated for $C_{21}H_{12}N_3OCl$, C 70.5, H 3.38, N 11.74; found, C 70.28, H 3.17, N 11.57%.

The same reaction was performed with **5-D**.

NMR experiments

For the HMQC and HMBC experiments the ^{1}H spectral width was 1750 Hz and the ^{13}C width 15 080 Hz. The two experiments were recorded with 2048 points, zero-filled to 4096 in f_2 and 32 scans were accumulated for each of the 256 increments in t_1, zero-filled to 2048. For the HMQC experiment, a BIRD nulling delay of 0.5 s was used. Shifted sine-bell weighting was used in f_1 and Gaussian weighting in f_2.

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