Synthesis and Isolation of Nitro-β-carbolines Obtained by Nitration of Commercial β-Carboline Alkaloids

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Nitration of commercial full aromatic β-carboline alkaloids nor-harmane (**1**), harmane (**2**), harmine (**3**), harmol (**4**), and the 7-acetylated derivative of harmol (**5**) is described. Advantages and disadvantages of different nitration reagents which involve acidic conditions (HNO_3/H^+) and neutral conditions ($Cu(NO_3)_2$; ceric ammonium nitrate) are discussed. A complete ^1H and ^{13}C-nmr characterization including ms and also uv absorption spectra in neutral and acid media is presented. A detailed ei-ms and ld-tof-ms study is enclosed because the nitro-β-carbolines constitute a new family of β-carboline-like chromophores with potential use as matrix in uv-maldi-tof-ms.

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Introduction.

The commercially available β-carboline alkaloids [1] nor-harmane, harmane, harmine, harmol, harmaline and harmalol have been studied mainly because of their biological and pharmacological properties and also because of their photochemical behaviour. They are present in many plant families [2], human urine [3], human lenses [4] and cigarette and tobacco smoke [5]. They are known to be hallucinogens [6], to inhibit the monoamine oxidase (MAO) [7,8] and to have photosensitizing activity towards a variety of systems, including bacteria, fungi, viruses and insects [9,10]. They have been reported to produce singlet oxygen and super oxide radical anion with varying efficiency [11] and to yield under uv irradiation symmetric and asymmetric β-carboline dimers [12]. It is also known that they form charge transfer complexes with flavins [13], the prosthetic group of MAO, and they bind selectively with DNA [14]. Because of their emission properties they have been proposed as fluorescence standards [15,16]. Particular interest has been shown in the study of the acid-base properties of the β-carbolines in aqueous and in organic media, in the ground and in the electronic excited states [17-28]. Electronic excitation results in significant charge-density changes due to the presence of two different types of nitrogen atoms in their structure (pyridinic and pyrrolic); the pK and the excited state pK* values have been determined [16-29] together with some kinetic parameter [29,30]. Besides, fluorescence quantum yields and/or life times have been determined in water at different pH values [28,31]. Recently, they have been described as efficient matrices (photosensitizers) in matrix-assisted ultraviolet laser desorption/ionization time-of-flight mass spectrometry (uv-maldi-tof-ms) for proteins [32-34], synthetic polymers (polyesters [35], polysilesquioxanes [36,37], polyamides [38]) and carbohydrates [32,33]. Specially, the sulfated oligosaccharide uv-maldi-tof-ms analysis has being highly improved [32,39] since commercially available β-carbolines and particularly nor-harmane are used as matrix in polysaccharide analysis, in the negative ion mode [32-34,39].

The simultaneous presence, in the aromatic rings of the molecule, of a group that can be deprotonated, such as NH, and a basic group such as pyridinic nitrogen together with the nature and properties of the low energy electronic excited states cause the special property mentioned above [32-39].

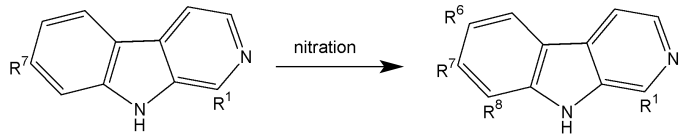
Up to now we have studied for commercially available β-carbolines the photochemical behavior, the effect of the medium on the properties of the ground and the electronic excited states (intermolecular interactions) and their potential use as matrix in uv-maldi-tof-ms [12,22-27,32-39]. In order to study the effect of different substituents present in the β-carboline moiety (intramolecular interactions) on its photochemistry and particularly on its efficiency to work as matrix in uv-maldi-tof-ms analysis of macromolecules, we decided to prepare β-carboline derivatives.

To begin with, preparation of nitro-β-carbolines was selected because the nitro group as a substituent induces strong modification of the acid-base properties and of the nature of the electronically excited states of aromatic compounds.

Although the synthesis of nitro-β-carbolines has been attempted by different authors, the full characterization of these compounds from the usual point of view has not been reported yet. For example, the first preparations of a nitro-harmine were carried out by Fritzsche in 1848 and in 1853 [40,41] who described as product of nitration of harmaline (3,4-dihydro-harmine) with concentrated nitric acid a mononitrated-harmine. This nitration reaction was subsequently carried out by Konovalova *et al*., [42] in 1935 who informed for the mononitro-harmine a mp 204-205°. The position of the nitro group was assumed by these authors to be *para* to indole nitrogen (C-6). Siddiqui *et al*., in 1973, [43] stated that the nitration of harmine proceeds in a very complicated manner and that after considerable experimentation it was possible to obtain mononitro-harmine

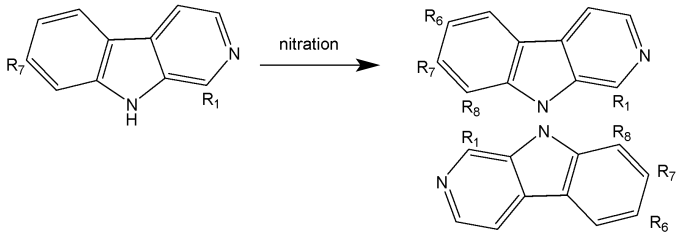
that decomposed at about 260° and whose ^{1}H-nmr spectrum allowed to fix the position of the nitro group at C-6. It is interesting to point that this is the only nitro-β-carboline (Scheme 1, compound **3a**) whose ^{1}H-nmr spectrum has been described in the literature. On the contrary, for the 8-nitro-harmine (Scheme 1, compound **3b**), obtained by oxidation of 8-nitro-harmaline with cromic acid, only the mp was reported in 1935 [42]**,** and for 6,8-dinitro-harmine (**3c**) its preparation has never been reported.

Scheme 1



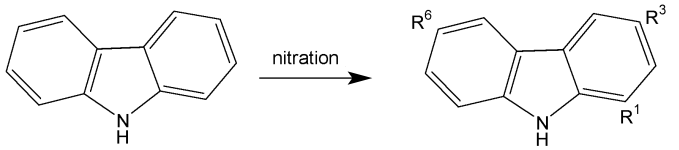
Compound	R^1	R^7
1	H	H
2	Me	H
3	Me	MeO
5	Me	AcO

Compound	R^1	R^6	R^7	R^8
1a	H	NO_2	H	H
1b	H	H	H	NO_2
2a	Me	NO_2	H	H
2b	Me	H	H	NO_2
3a	Me	NO_2	MeO	H
3b	Me	H	MeO	NO_2
3c	Me	NO_2	MeO	NO_2
5a	Me	NO_2	AcO	H
5b	Me	H	AcO	NO_2



	R^1	R^7
4	Me	HO

	R^1	R^6	R^7	R^8
4d	HCO	H	HO	H



6

	R^1	R^3	R^6
6a	NO_2	H	H
6b	H	NO_2	H
6c	NO_2	H	NO_2
6d	H	NO_2	NO_2

About the nitro derivatives of harmane (Scheme 1, compounds **2a** and **2b**), Snyder *et al*., in 1948 [44] described the preparation, by using concentrated nitric acid, of two isomeric nitro-harmanes with the proof of the structure of one of them by using an specific synthetic route. Thus, the higher-melting nitro derivative was shown to be 6-nitroharmane (**2a**) and that with the lower-melting point was supposed presumably to be the 8-nitroharmane (**2b**). This paper includes the description of the ir spectrum of each compound. Besides, there are some papers which describe that by treatment of harmane with nitric acid the 6-nitroharmane was prepared in order to study several properties by using the uv spectra [31,45-47]. Among them, only in references 45 and 46 are the mp's of the prepared nitro-harmane reported as proof of characterization. As part of a study of structure–inhibitory activity relationship of derivatives of β-carbolines, 6-nitroharmane was prepared by treatment of harmane with concentrated nitric acid and was characterized by mp and the m/z value of the molecular ion obtained by ei-ms (M^+, 227) [48].

Finally, in the case of nor-harmane (**1**), after the description of Saxena of a mononitro derivative that he presumed to be the 6-nitronor-harmane (**1a**), obtained by treatment of **1** with concentrated nitric acid [49], several studies have been published. Some of these studies only include the description of the uv of **1a** [50,51] because of its acid-base properties. For others, the only proof of identity given for the nitro derivative presumed to be **1a** is that the method used for its preparation is the treatment of **1** with concentrated nitric acid [52,53]**.** In these cases, the authors do not take into account the possible formation of 8-nitronor-harmane (**1b**).

Here we report full details of various attempts for nitration of the commercial full aromatic β-carboline alkaloids (Scheme 1) nor-harmane (**1**), harmane (**2**), harmine (**3**), harmol (**4**) and *O*-acetyl harmol (**5**) together with a complete characterization of the nitro-β-carbolines obtained. These compounds constitute an interesting family of β-carboline-like chromophores with potential use as uv-maldi matrix. Thus, a detailed ei-ms and ld-tof-ms study (ultraviolet laser desorption/ionization time-of-flight mass spectrometry) is enclosed. In order to compare the ms data obtained for nitro-β-carbolines with those of nitro-carbazoles, the 1-, 3-, 1,6-di- and 3,6-dinitrocarbazoles **6a-d** were also prepared (Scheme 1) and the corresponding ei- and ld-tof- mass spectra were measured.

Results and Discussion.

Nitration Procedures.

Nitration of nor-harmane (**1**) and harmane (**2**) as clean reactions were conducted with HNO_3/H^+ [54,55] with high yields (**1a** (64%), **1b** (30%), **2a** (66%) and **2b** (32%)), recovering in low percentage the corresponding β-carboline. However, this acidic oxidative condition results in lower yields for nitro-harmine derivatives (**3a** (10%), **3b** (5%)) because the following side reactions take place: oxidation of harmine (**3**), a total oxidation/dimerization of harmol (**4**), and the removal of the acetyl group from acetyl-harmol (**5**). Thus, a variety of oxidized products are generated that are unstable and difficult to purify.

Acetyl nitrate [54,55] (*in situ* generation from nitric aid and acetic anhydride) in tetrahydrofuran at 0° also gave the dimmer of harmol, dimeric 1-formyl-harmol (**4d**), from both harmol (**4**) and its acetyl derivative (**5**).

On the contrary, efficient and clean nitration of harmol, as acetyl harmol (**5**), was obtained by treatment with copper(II) nitrate/acetic anhydride [56,57] at room temperature for 48 hours (yields: **5a** 9%; **5b** 33%) although harmol (**4**) itself was completely oxidized even at low temperature. Analogous treatment of nor-harmane (**1**) and harmane (**2**) gave the same reaction pattern as treatment with HNO_3/H^+, but in lower yield (yields: **1a** 32%; **1b** 18%; **2a** 55%; **2b** 5%). For harmine, the reaction was cleaner than that with HNO_3/H^+. After this treatment, harmine was recovered unchanged in high percentage and the yield of the nitro derivatives **3a**, **3b** and **3c** was low (yields: 12%, 4% and 2% respectively).

Cerium ammonium nitrate (CAN) in acetonitrile has been recently described as an efficient reagent for nitration, which does not require acid catalysis [58]. Thus, mononitro carbazoles have been successfully prepared with this reagent. This shows that CAN is a mild nitration reagent because polynitro carbazoles were not obtained [58]. In our experiments with CAN and β-carbolines we improved the work-up of the reaction in order to eliminate excess cerium in the reaction mixture avoiding the formation of β-carboline-Ce complexes. Thus, from harmane and harmine the mononitro derivatives **2a**, **2b**, **3a** and **3b** were obtained in very low yield (17%, 3%, 12% and 4% respectively), together with dimeric products. From nor-harmane, which is not specially activated for electrophilic substitution, the conversion to mononitro derivatives is very low (< 5%) and nor-harmane remains almost unchanged. The fact that in nor-harmane only one ring is activated for the attack by NO_2^+ while in carbazole

Table 1

Static Charge Distribution for β-Carbolines

Compound	Method [a]	Atom number							
		1-C	2-N	3-C	4-C	5-C	6-C	7-C	8-C
1	AM1	-0.088	-0.120	-0.103	-0.127	-0.069	-0.161	-0.095	-0.157
	PM3	-0.061	-0.065	-0.089	-0.096	-0.041	-0.127	-0.074	-0.108
	ZINDO/S	0.112	-0.380	0.116	-0.036	-0.010	-0.037	-0.013	-0.040
1H	AM1	-0.045	-0.071	-0.041	-0.100	-0.041	-0.140	-0.045	-0.151
	PM3	-0.232	0.583	-0.267	0.010	-0.017	-0.094	-0.022	-0.097
1a	AM1	-0.081	-0.113	-0.096	-0.122	0.004	-0.178	-0.032	-0.178
1aH	AM1	-0.035	-0.068	-0.031	-0.096	-0.020	-0.154	-0.001	-0.156
1b	AM1	-0.075	-0.114	-0.096	-0.124	-0.018	-0.185	-0.025	-0.181
1bH	AM1	-0.024	-0.068	-0.037	-0.094	-0.001	-0.155	0.014	-0.165
2	AM1	-0.025	-0.123	-0.096	-0.133	-0.069	-0.162	-0.096	-0.157
	PM3	-0.034	-0.064	-0.083	-0.105	-0.041	-0.127	-0.074	-0.109
	ZINDO/S	0.153	-0.397	0.117	-0.046	-0.011	-0.037	-0.014	-0.041
2H	AM1	0.034	-0.079	-0.041	-0.101	-0.043	-0.140	-0.047	-0.152
	PM3	-0.175	0.556	-0.259	0.004	-0.017	-0.095	-0.023	-0.099
2a	AM1	-0.017	-0.117	-0.089	-0.128	0.004	-0.179	-0.032	-0.178
2aH	AM1	-0.001	-0.069	-0.030	-0.099	-0.020	-0.154	-0.001	-0.156
2b	AM1	-0.011	-0.118	-0.089	-0.131	-0.018	-0.186	-0.025	-0.183
2bH	AM1	-0.001	-0.072	-0.034	-0.099	-0.001	-0.155	0.014	-0.162
3	AM1	-0.032	-0.067	-0.080	-0.111	-0.002	-0.208	0.119	-0.156
	PM3	-0.031	-0.068	-0.079	-0.111	-0.006	-0.166	-0.120	-0.198
	ZINDO/S	0.160	-0.408	0.117	-0.046	-0.010	-0.051	0.159	-0.046
3H	AM1	0.033	-0.089	-0.031	-0.116	0.001	-0.222	0.175	-0.204
	PM3	-0.158	0.528	-0.239	-0.017	0.019	-0.140	0.184	-0.217
3a	AM1	-0.017	-0.117	-0.087	-0.130	0.006	-0.187	0.152	-0.201
3b	AM1	-0.017	-0.063	-0.072	-0.110	0.056	-0.241	0.218	-0.465
3c	AM1	0.015	-0.134	-0.067	-0.134	0.015	-0.163	0.191	-0.161
4	AM1	-0.023	-0.125	-0.093	-0.138	-0.029	-0.245	0.115	-0.193
	PM3	-0.030	-0.068	-0.078	-0.112	-0.002	-0.169	0.131	-0.213
	ZINDO/S	0.155	-0.410	0.110	-0.044	-0.009	-0.055	0.154	-0.033
4H	AM1	0.036	-0.089	-0.031	-0.115	0.003	-0.228	0.173	-0.202
	PM3	-0.159	0.531	-0.239	-0.016	0.024	-0.142	0.194	-0.229
5	AM1	0.026	-0.121	-0.096	-0.131	-0.038	-0.179	0.128	-0.239
	PM3	0.027	-0.122	-0.094	-0.130	-0.040	-0.173	-0.12-	-0.243
	ZINDO/S	0.160	-0.405	0.116	-0.044	-0.011	-0.050	0.158	-0.045
5H	AM1	0.043	-0.090	0.032	-0.110	-0.017	-0.160	0.197	-0.254
	PM3	-0.158	0.527	-0.238	-0.019	-0.015	-0.146	0.183	-0.199
5a	AM1	-0.015	-0.118	-0.084	-0.131	0.028	-0.202	0.202	-0.270
5b	AM1	-0.006	-0.117	-0.085	-0.132	-0.009	-0.202	0.160	-0.199

[a] Single Point calculations were performed using semiempirical methods (AM1; PM3; ZINDO/S) after geometry optimization at HF/6-31G level

both rings are equally activated explains why CAN, a mild nitration reagent, is more effective for mononitration of carbazole than for mononitration of nor-harmane.

Formation of dimeric products from harmane and harmine, which resemble the *N*,*N*'-*bis*-β-carboline symmetric dimers which we obtained by uv-irradiation of these β-carbolines [12], can be explained taking into account the formation of *N*-β-carbolinyl radicals which then yield symmetric dimers. Formation of alkoxy radicals by CAN induced oxidation of alcohols and benzylic alcohols has recently been demonstrated by epr [59] suggesting that CAN works as one electron oxidation reagent. A similar CAN induced radical reaction would account for the dimerization mentioned above and also for the oxidation of the methyl group at C-1 of harmane (**2**) and harmine (**3**) affording **4d**.

Theoretical Calculations.

The substitution patterns for the nitration of β-carbolines **1**-**5** were studied using the net atomic charge values calculated by semiempirical methods (AM1, PM3 and ZINDO/S) after geometry optimization with the HF level of theory and 6-31G basis set.

The nitration patterns observed by using acidic nitration reagents (HNO_3/H^+) and neutral nitration reagents ($Cu(NO_3)_2$; CAN) were predicted using the values calculated by AM1. As can be seen in Table 1, β-carbolines (**1**, **2**, **3**, **4**, and **5**) and cationic β-carbolines (β-carboline + H^+; **1**H, **2**H, **3**H, **4**H and **5**H) have higher charge density values localized at 6-C and 8-C, thus predicting that the incoming NO_2^+ would preferentially attack these positions. In agreement with this fact 6-nitro- and 8-nitro-β-carbolines were obtained from **1**, **2**, **3**, **5**, **1**H, **2**H and **3**H. Net atomic charge values calculated by AM1 also predicted that the mononitrated-β-carbolines might react with NO_2^+ to yield the 6,8-dinitro derivative as we observed for harmine (Scheme 1, compound **3c**).

As can be seen in Table 1, the values obtained for β-carbolines **1**, **2**, **3**, **4** and **5**, calculated by the PM3 method, predicted higher charge density values at 6-C and 8-C and for cationic β-carbolines (β-carboline + H^+; **1**H, **2**H, **3**H, **4**H and **5**H) at 1-C and 3-C. The absence of the predicted 1-nitro- and 3-nitro-β-carbolines in the reaction mixture when nitration was performed in acidic conditions (HNO_3/H^+) from **1**H, **2**H, **3**H and **4**H suggests that PM3 does not explain the substitution pattern experimentally obtained.

Finally, as can be seen in Table 1, ZINDO/S calculations for all the β-carbolines studied predicted similar charge values for 4-C, 6-C and 8-C and do not explain the experimental results obtained. 4-Nitro-β-carbolines were not detected in any of the reactions studied.

Table 2

Uv Absorption Sspectra of β-Carbolines in Solution at 298 K [a]

Compound	λ max (nm) (log ε)				Solvent
1	346 (3.61)	332 (3.60)	286 (4.15)		MeCN
1H	374 (3.65)	302 (4.22)			MeCN/H^+ [b]
1a	366 (3.68)	346 (3.69)	332 (3.96)	288 (4.35)	MeCN
1aH	366 (3.67)	336 (3.68)	286 (4.38)		MeCN/H^+
1b	396 (3.68)	302 (3.60)	270 (4.37)		MeCN
1bH	392 (3.67)	296 (3.61)	268 (4.35)		MeCN/H^+
2	344 (3.690	330 (3.68)	286 (4.17)		MeCN
2H	366 (3.73)	300 (4.27)			MeCN/H^+
2a	395 (3.69)	355 (s) (3.68)	310 (3.93)	276 (4.10)	MeCN
2aH	385 (3.72)	345 (s) (3.70)	297 (4.86)		MeCN/H^+
2b	394 (3.67)	348(s) (3.15)	286 (3.85)	278 (s) (3.88)	MeCN
2bH	384 (3.98)	298 (4.25)	268 (4.55)		MeCN/H^+
3	332 (3.68)	324 (3.72)	298 (4.21)		MeCN
3H	360 (3.92)	324 (4.30)			MeCN/H^+
3a	360 (3.75)	332 (3.90)	300 (3.94)	278 (4.15)	MeCN
3aH	356 (3.91)	314 (s) (3.90)	298 (4.10)		MeCN/H^+
3b	372 (3.65)	292 (s) (3.73)	280 (3.98)		MeCN
3bH	362 (3.65)	301 (4.20)			MeCN/H^+
3c	432 (3.57)	380 (3.60)	324 (3.59)	308 (3.60)	MeCN
3cH	476 (3.58)	288 (4.01)	245 (4.10)		MeCN/H^+
4	334 (3.65)	322 (3.77)	300 (4.18)		MeCN
4H	326 (4.30)				MeCN/H^+
5	332 (3.68)	323 (3.71)	298 (4.20)		MeCN
5H	358 (3.93)	324 (4.31)			MeCN/H^+
5a	358 (3.90)	332 (3.89)	300 (3.94)	279 (4.18)	MeCN
5aH	356 (3.93)	319 (3.20)	298 (4.12)		MeCN/H^+
5b	371 (3.66)	292 (s) (3.74)	280 (3.99)		MeCN
5bH	360 (3.65)	300 (4.10)			MeCN/H^+

[a] Concentration: 5.0 x 10^{-5} *M*; λ in nm; ε in M^{-1} cm^{-1}. [b] MeCN with 1% sulfuric acid 0.1 *M*.

The theoretical results predicted by AM1 calculations and the reaction patterns obtained experimentally agree with previous results reported for quinoline [55]. The products arising from nitration clearly showed that one ring reacts faster than the other, and the authors stated that the pyridine ring reactivity was lowered by the factor 2.319 for electrophilic substitution as compared to the other aromatic ring.

Absorption Spectra.

The absorption spectra of β-carbolines **1**, **2**, **3**, **4** and **5** and the nitro-β-carbolines **1a**, **1b**, **2a**, **2b**, **3a**, **3b**, **3c**, **5a** and **5b** in acetonitrile were recorded (Table 2). The absorption spectra of the cationic species were obtained by addition of the appropriate quantity of sulfuric acid.

As we previously described, in non-polar as well as in polar protic and polar aprotic organic solvents β-carbolines **1**, **2**, **3** and **4** exist in the neutral form in the S_0 state, thus, the bands of the neutral species are observed and there is enough evidence that the low energy band is due to a $\pi\rightarrow\pi^*$ transition with typical λ_{max} at 346, 344, 332 and 334 nm, respectively (Table 2) [24-27,60]. These λ_{max} values show a dramatic and clear bathochromic shift (red shift) when the spectra are recorded in acidic medium (Table 2, λ_{max} values for **1**H, **2**H, **3**H and **4**H) owing to the yield of the protonated molecule. In the cationic species the stronger interaction of the pyridine n electron pair involved in the proton bridge with the aromatic ring π system (mesomeric effect; extension of conjugation) would account for the λ_{max} shifts observed. For the 7-acetyl-harmol **5** the same spectroscopical behavior was observed (Table 2, see λ_{max} for **5** and **5H**).

As shown in Table 2, the absorption data of the nitro-β-carboline derivatives in both neutral and protonated forms (**1a** and **1aH**, **1b** and **1bH, 2a** and **2aH**, **2b** and **2bH**, **3a** and **3aH**, **3b** and **3bH**, **3c** and **3cH**, **5a** and **5aH**, **5b** and **5bH**) are quite similar. Protonation of nitro-β-carbolines does not lead to an important bathochromic shift of λ_{max}. On the contrary, in all cases minor to middle clear hypsochromic shifts (blue shifts) were observed (2 to 10 nm). The protonation effect observed on the lowest energy band of the absorption spectra of nitro-β-carbolines suggests that these transitions are in nature different from those of β-carbolines (compounds **1**, **2**, **3**, **4** and **5**) and that some of them clearly are $n\rightarrow\pi^*$ (higher blue shift). However, it is to be noted that in some cases the protonation effect was low or there was no effect at all (Table 2, compounds **1a, 1b** and **3a**) and that extinction coefficient values (ε) are always around 10^4. Probably the close-lying $n\rightarrow\pi^*$ transitions having much lower ε values are likely hidden by the more intense $\pi\rightarrow\pi^*$ ones, and their positions could not be clearly detected even by the protonation effect.

Mass Spectrometry.

Electron Impact Mass Spectrometry (ei-ms).

As it is known, the electron impact induced fragmentation of nitroarene radical-cations proceeds by two basic routes involving expulsion of the NO_2 and NO species as the primary processes. The extent and ease of valence isomerization reflected by the relative abundances of $[M\text{-}NO]^+$ (quinonoid structure) and $[M\text{-}NO_2]^+$ peaks is strongly influenced by the structure of aromatic system, the character of the other substituents and the position of the nitro group [61]. Hydrogen transfer and oxygen migration are the main rearrangements that complicate the spectra of substituted nitroarenes [61].

In the spectra of **1a** and **1b** the molecular ions (M) are quite important (Table 3, ei-ms, 84% and 30%). Comparison of the relative intensities of M peaks with that of $[M\text{-}NO]^+$ and $[M\text{-}NO_2]^+$ (Table 3, $[M\text{-}NO]^+$: m/z 183, 80% and 7%; $[M\text{-}NO_2]^+$: m/z 167, 85% and 23%) provides an explanation of the greater stability of the 6-quinonoid structure obtained from **1a**. This clear difference between the relative intensity of $[M\text{-}NO]^+$ species is also observed in the spectra of the pairs **2a**/**2b** and **3a**/**3b** although the abundances of the $[M\text{-}NO]^+$ fragments are lower (Table 3, $[M\text{-}NO]^+$: m/z 197, **2a**, 10%; **2b**, 5%; m/z 227, **3a**, 9%, **3b**, 3%). For each pair the main fragmentation route involving the NO_2 substituent is the expulsion of this group (Table 3, $[M\text{-}NO_2]^+$: m/z 181, **2a** and **2b**). For compounds **3a** and **3b** the susceptibility of the nitro-nitrite rearrangement is caused by the *ortho* effect of the methoxy group at 7-C. From the methoxy-group, the hydrogen transfer to the nitro group followed by secondary decomposition ($[M\text{-}HNO_2]$), produces more abundant peaks in both spectra than the isomerization to yield $[M\text{-}NO]^+$ and the simple expulsion of NO_2 to yield $[M\text{-}NO_2]^+$ (Table 3, $[M\text{-}HNO_2]$: m/z 210, **3a**, 63%; **3b**, 93%; $[M\text{-}NO]^+$, m/z 227, see above).

Compound **5** ($M^{+\cdot}$, m/z 240), the 7-acetyl derivative of harmol, mainly loses CH_2CO (ketene) (Table 3, m/z 198, 100%). The nitro derivatives of compound **5**, **5a** and **5b**, containing the NO_2 substituent next to the 7-acetyloxy group do not show significant $[M\text{-}HNO_2]^+$ peaks (m/z 238). By comparison of the spectra of **3a**, **3b**, **5a** and **5b** in connection with HNO_2 expulsion it seems that hydrogen transfer is a specific rearrangement only possible if the intramolecular hydrogen bridge involves the formation of stable 5 to 7 membered rings as an intermediate [61]. From the molecular ions (Table 3, m/z 285, **5a**, 4%; **5b**, 6%) the main fragmentation path is the subsequent expulsion of CH_2CO (Table 3, m/z 243, **5a**, 100%; **5b**, 37%) and NO_2 (Table 3, m/z 197, **5a**, 56%; **5b**, 39%). The differences in the spectra of **5a** and **5b** are determined by the subsequent expulsion of NO (Table 3, m/z 213, **5a**, 10%; **5b**, < 1%) and CO (Table 3, m/z 185, **5a**, 3%; **5b**, < 1%) from the fragment of m/z 243, which is favored when the stable 6-quinonid structure can be formed (compound **5a**).

The intensities of $[M\text{-}O]^{+\cdot}$ ions are low or just do not appear in the spectra of the nitro-β-carbolines studied. After the expulsion of the nitro subtituent the main fragmentation path of **1a**, **1b**, **2a**, **2b**, **3a**, **3b**, **5a** and **5b** are

Table 3

Ld-tof-ms and Ei-ms Signals of β-Carbolines, Nitro-β-carbolines, Carbazole and Nitrocarbazoles [a]

		ld-tof-ms			ei-ms
Comp	MW	Meth. [a]	Positive ion mode	Negative ion mode	Positive ion mode
1	168	A	334 (5), 169 (100)	225 (5), 167 (100), 112 (90)	
		B	335 (36), 169 (100), 132 (19)	225 (10), 167 (100), 112 (90)	
1a	213	A	214 (20), 198 (10), 184 (18), 168 (100)	496 (4), 422 (6), 212 (100), 196 (18), 182 (14), 166 (28), 112 (6)	213 ($M^{+\cdot}$, 84), 183 (80), 167 (85), 155 (44), 140 (100), 129 (8), 113 (82), 99 (11), 83 (16), 69 (35), 46 (36)
		B	425 (8), 214 (22), 198 (10), 184 (18), 168 (100)	496 (14), 422 (10), 212 (100), 196 (20), 182 (10), 166 (20), 112 (16)	
1b	213	A	492 (5), 214 (100), 198 (18), 184 (8), 168 (70), 129 (30)	212 (100), 196 (25), 112(8)	213 ($M^{+\cdot}$, 30), 183 (7), 167 (23), 155 (5), 140 (23), 129 (6), 113 (11), 97 (14), 83 (18), 69 (32), 46 (100)
		B	492 (15), 214 (100), 198 (28), 184 (9), 168 (75), 129 (23)	223 (7), 212 (100), 196 (28), 112(10)	
2	182	A	362 (4), 183 (100)	181 (100), 112 (35)	
		B	363 (14), 183 (100)	246 (21), 224 (35), 181 (88), 112 (100)	
2a	228	A	362 (5), 229 (10), 199 (8) 183 (100)	227 (100), 211 (24), 197 (20), 181 (18), 112 (78), 96 (64)	227 ($M^{+\cdot}$, 100), 210 (2), 197 (10), 181 (63), 169 (21), 154 (22), 140 (8), 127 (34), 113 (15), 97 (17), 84 (10), 78 (19), 73 (11), 69(4), 63 (20), 57 (7), 45 (10)
		B	362 (20), 229 (8), 199 (8) 183 (100)	324 (10), 248 (6), 227 (78), 211 (20), 197 (12), 181 (10), 134 (48), 113 (100), 96 (34)	
2b	228	A	444 (10), 408 (13), 229 (100), 213 (20)	453 (4), 405 (3), 227 (100), 211 (18), 112 (8)	227 ($M^{+\cdot}$, 80), 210 (2), 197 (5), 181 (41), 169 (5), 154 (11), 140 (3), 127 (12), 113 (6), 99 (4), 88 (3), 77 (9), 71 (16), 69 (100), 57 (33), 44 (58)
		B	444 (15), 408 (10), 229 (100), 213 (20)	453 (10), 405 (8), 227 (100), 211 (10), 112 (16)	
3	212	A	423 (3), 213 (100)	211 (100), 112 (35)	
		B	423 (6), 213 (100)	246 (20), 224 (80), 211 (75), 112 (100)	
3a	257	A	258 (100), 242 (20), 228 (60), 212 (76), 182 (6)	256 (100), 240 (14), 226 (16)	257 ($M^{+\cdot}$, 100), 227 (9), 212 (32), 210 (63), 199 (16), 197 (18), 181 (79), 169 (42), 154 (28), 140 (23), 127 (36), 113 (21), 99 (13), 88 (14), 77 (25), 71 (35), 69 (22), 57 (42), 45 (54)
		B	513 (3), 258 (100), 242 (15), 228 (56), 212 (68), 182 (4)	511 (20), 497 (8), 483 (12), 256 (100), 240 (24), 226 (10)	
3b	257	A	258 (100), 242 (70), 228 (12), 212 (3)	256 (100), 24 (24), 226 (24), 150 (14)	257 ($M^{+\cdot}$, 63), 227 (3), 212 (12), 210 (93), 199 (11), 197 (11), 181 (93), 169 (37), 154 (55), 140 (41), 127 (56), 113 (40), 99 (30), 88 (37), 77 (48), 71 (43), 69 (30), 63 (100), 57 (17), 45 (75)
		B	512 (5), 280 (12), 271 (34), 258 (100), 242 (64), 228 (14), 212 (6)	300 (15), 271 (10), 256 (100), 240 (24), 226 (24), 150 (14)	
3c	302	A	346 (2), 324 (1), 303 (100), 287 (42), 273 (20), 257 (58), 227 28), 211 (10), 197 (16), 181 (3)	301 (44), 285 (100), 271 (56), 255 (42), 197 (30), 179 (32)	302 ($M^{+\cdot}$, 4), 285 (1), 270 (1), 257 (2), 255 (3), 243 (2), 225 (1), 213 (2), 147 (5), 136 (4), 121 (4), 97 (11), 83 (23), 77 (4), 71 (10), 69 (50), 63 (1), 59 (3), 58 (2), 57 (13), 56 (56), 46 (30), 45 (100)
		B	346 (14), 324 (10), 303 (100), 287 (22), 273 (12), 257 (38), 227 (18), 211 (8), 197 (6), 181 (8)	601 (8), 301 (100), 285 (100), 271 (10), 255 (10)	
4	198	A	212 (10), 199 (100), 39 (12), 23 (26)	393 (20), 233 (80), 197 (100), 113 (48), 97 (49), 62 (20), 34 (28)	
		B	212 (5), 199 (100), 39 (10), 23 (26)	393 (8), 233 (12) , 197 (24), 113 (100), 97 (7), 69 (58), 34 (26)	
5	240	A	479 (10), 241 (100), 212 (18), 198 (28), 39 (44)	275 (100), 239 (72), 197 (12), 132 (70), 113 (28), 62 (58), 35 (36)	240 ($M^{+\cdot}$, 17), 198 (100), 182 (2), 170 (13), 154 (2),140 (4), 128 (2), 115 (6), 99 (3), 97 (3), 85 (3), 75 (5), 63 (5), 43 (46)
		B	479 (10),241 (100), 212 (18), 198 (28), 39 (8)	275 (100), 239 (52), 197 (6), 132 (17), 62 (2)	

Table 3 (continued)

		ld-tof-ms			ei-ms
Comp	MW	Meth. [a]	Positive ion mode	Negative ion mode	Positive ion mode
5a	285	A	286 (100), 270 (14), 256 (28), 241 (26), 213 (6)	284 (100), 268 (14), 254 (22), 238 (28), 223 (22)	285 ($M^{+\cdot}$, 4), 243 (100), 225 (5), 213 (10), 197 (56), 185 (3), 182 (9), 169 (21), 167 (24), 155 (7), 140 (18), 128 (8), 114 (13), 101 (9), 99 (9), 88 (8), 77 (9), 71 (18), 69 (12), 57 (22), 43 (68)
		B	286 (100), 270 (14), 256 (28), 241 (26), 213 (6)	284 (100), 268 (14), 254 (22), 238 (28), 223 (22)	
5b	285	A			285 ($M^{+\cdot}$, 6), 243 (37), 225 (5), 197 (39), 184 (3), 182 (1), 169 (4), 167 (11), 155 (2), 140 (6), 128 (3), 114 (5), 101 (3), 99 (2), 88 (3), 77 (3), 71 (4), 69 (5), 57 (7), 43 (100)
		B			
6	167	A [b]	334 (18), 167 (100)	166 (100), 79 (18), 63 (28)	
6a	212	A [b]	213 (100), 197 (32), 183 (30), 167 (16)	211 (100), 195 (28), 181 (4)	213 (1.7), 212 ($M^{+\cdot}$, 5), 181 (4), 166 (4), 164 (5), 154 (3), 140 (4), 139 (4), 128 (5), 113 (3), 98 (15), 82 (22), 69 (31), 63 (19), 45 (100)
6b	212	A	213 (34), 197 (86), 183 (100), 167 (10)	211 (100), 195 (18), 181 (22)	213 (15), 212 ($M^{+\cdot}$, 100), 182 (26), 166 (63), 164 (14), 154 (13), 140 (18), 139 (53), 128 (4), 113 (6), 98 (2), 82 (7), 69 (24), 63 (11), 45 (14)
6c	257	A [b]	Clusters (551, 494, 466, 422, 279, 250), 258 (14), 242 (14), 212 (68), 196 (6), 182 (100), 166 (10)	Clusters (510, 300), 256 (100)	258 (9), 257 ($M^{+\cdot}$, 57), 241 (4), 236 (3), 227 (17), 211 (21), 194 (3), 181 (12), 165 (20), 164 (27), 153 (9), 138 (12), 137 (10), 129 (3), 111 (12), 97 (22), 83 (28), 69 (45), 58 (61), 56 (60), 47 (54), 46 (87), 45 (100)
6d	257	A [b]	Clusters (494, 302, 280, 250), 258 (20), 242 (76), 228 (100), 212 (4), 196 (6), 182 (6), 166 (6)	Clusters (574, 510, 300, 270) 256 (100), 240 (14), 226 (26)	258 (8), 257 ($M^{+\cdot}$, 56), 241 (1), 227 (15), 211 (15),197 (2), 181 (17), 165 (13), 164 (19), 152 (5), 138 (9), 137 (7), 129 (3), 111 (5), 97 (9), 83 (11), 71 (12), 69 (17), 58 (28), 56 (26), 47 (51), 46 (100), 45 (62)

[a] Spectra were obtained by loading two drop of the β-carboline solution (Method A: 1 mg per ml of DMSO; Method B: 0.5 mg per ml of acetonitrile – water trifluoroacetic acid 0.1 % (3:1, v/v)) on the stainless steel probe. λ_{exc} = 337 nm; [b] Similar spectra were obtained by using Method B.

similar to those described for the corresponding β-carboline [12,62].

Finally, although very clear ei-ms spectra have been reported for dinitroaromatic compounds, *e.g.*, dinitrocarbazoles [61], (see in Table 3 ei-ms data for 1,6-dinitro and 3,6-dinitrocarbazole, compounds **6c** and **6d**, respectivley), peaks of very low intensity were obtained from the dinitro derivatives of harmine (Table 3, compound **3c**) by ei-ms even at very soft experimental conditions (low temperature at the injection chamber; low voltage for the ionizing electron source).

Laser Desorption/Ionization Time-of-flight Mass Spectrometry (ld-tof-ms).

The laser desorption/ionization (λ_{exc} = 337 nm) time-of-flight mass spectra (ld-tof-ms) of nitro-β-carbolines have been recorded in positive and negative ion modes. Samples were prepared in two different solvents: dimethylsulfoxide (DMSO) (Method A) and acetonitrile – water trifluoroacetic acid 0.1% (3:1, v/v) (MeCN:H_2O TFA 0.1%) (Method B) because as we have reported the quality of the ld-ms depends on the sample preparation method [32]. Spectra obtained from both solutions are quite similar. As expected, those obtained from DMSO showed a variable amount of clusters with m/z two or three times the molecular mass whose intensity depended simultaneously on the laser power and on the effectiveness of the solvent evaporation process. In positive and negative ion modes the peaks corresponding to $[M+H]^+$ and $[M-H]^-$ were detected in general as one of the most abundant signal (Table 3, ld-tof-ms data; see for each compound values for $[M+H]^+$ and $[M-H]^-$ in the corresponding mode). In both modes, the molecular ion generated suffers the expulsion of O and NO yielding abundant $[M+H-O]^+$ and $[M+H-NO]^+$ signals in positive ion mode, and $[M-H-O]^-$ and $[M-H-NO]^-$ signals in negative ion mode, respectively. These are the main fragmentation paths of 6-nitro and 8-nitro-β-carbolines **1a, 1b**, **2a, 2b**, **3a, 3b, 5a** and **5b** (see as example in Table 3, ld-tof-ms; positive ion mode, Method A, $[M+H-O]^+$: **1a,** 198 (10%), **1b**, 198 (18%); **2b**, 213 (20%); **3b**, 242 (70%); **5a**, 270 (14%); $[M+H-NO]^+$: **1a,** 184 (18%), **1b**, 184 (8%); **2a,** 199 (8%); **3a**, 228 (60%); **3b**, 228 (12%); **5a**, 256 (28%)). Besides $[M+H-NO_2]^+$ signals in positive ion mode and

$[M\text{-}H\text{-}NO_2]^-$ signals in the negative ion mode together with the above mentioned signals were observed.

The fragmentation processes of the dinitro derivative of harmine (6,8-dinitro-harmine; **3c**) is not very complicated although two nitro groups are present. In this case, although the signal corresponding to the molecular ion is quite important (Table 3, ld-tof-ms; **3c**, positve ion mode, m/z 303 (100%); negative ion mode, m/z 301 (44 and 100%), the other most abundant ion in negative mode is the $[M\text{-}H\text{-}O]^-$ (Table 3, ld-tof-ms; **3c,** m/z 285, 100%) being the peaks corresponding to $[M\text{-}H\text{-}NO]^-$ (m/z 271 (56%)) and $[M\text{-}H\text{-}NO_2]^-$ (m/z 255 (50%) also important. The presence of the second NO_2 group in the molecule, placed at the same benzenic ring, would be the cause of the higher stability shown by the $[M\text{-}H\text{-}O]^-$ in the negative ion mode. Comparison of the spectra obtained from the 6,8-dinitro-harmine **3c** by ei-ms and by ld-tof-ms techniques shows that the latter is a very useful technique because of its soft desorption/ionization conditions compound **3c** can be effectively ionized to intact monocharged molecules in gas phase. Besides, the soft character of the latter technique together with the different stability of ions and radical-ions in the gas state would explain the noticeable high abundance of the $[M+H\text{-}O]^+$ and $[M\text{-}H\text{-}O]^-$ species obtained in the respective ion mode, by ld-tof-ms analysis.

Similar results were obtained when we compared the ld-tof-ms and the ei-ms obtained from carbazole (**6**), 1-nitrocarbazole (**6a**), 3-nitrocarbazole (**6b**), 1,6-dinitrocarbazole (**6c**) and 3,6-dinitrocarbazole (**6d**). As is shown in Table 3, $[M+H\text{-}O]^+$ and $[M\text{-}H\text{-}O]^-$ species are formed efficiently by the former technique (Table 3, ld-tof-ms; positive ion mode: **6a,** m/z 197 (32%), **6b**, m/z 197 (86%), **6c,** m/z 242 (14%), **6d,** m/z 242 (76%); negative ion mode: **6a,** m/z 195 (28%), **6b**, m/z 195 (18%), **6d,** m/z 240 (14%)), while they are not detected by using the latter (Table 3, ei-ms data for compounds **6a**, **6b**, **6c** and **6d**). The species $[M+H\text{-}NO]^+$, $[M+H\text{-}NO_2]^+$, $[M\text{-}H\text{-}NO]^-$ and $[M\text{-}H\text{-}NO_2]^-$ were also observed, especially in the negative ion mode. It is noteworthy to emphasize that from the dinitrocarbazoles **6c** and **6d** both NO_2 substituents are expelled efficiently in the positive ion mode, while in negative ion mode the species are more stable keeping both (Table 3, ld-tof-ms, negative ion mode: compound **6c**) or at least one NO_2 group (Table 3, ld-tof-ms, negative ion mode: compound **6d**).

Finally, it is noteworthy to point the fact that after the expulsion of the nitro substituent the β-carboline like structure remaining does not suffer the typical cleavage that β-carbolines show by ei-ms technique (expulsion of HCN and C_2H_2 fragments *inter alia*). As expected they showed at that m/z region, m/z ≤ β-carboline molecular mass, signals quite similar to those observed in the ld-tof-ms of the corresponding β-carboline (see in Table 3, ld-tof-ms for **1**, **2**, **3**, **4** and **5**).

Studies in connection with the potential use of this family of nitro-β-carbolines as matrices in the matrix-assisted ultraviolet-laser desorption/ionization time-of-flight mass spectrometry (uv-maldi-tof-ms) analysis of macromolecules such as synthetic polymers, biopolymers and proteins, are in progress in our laboratory and the preliminary results are quite good.

EXPERIMENTAL

Dichloromethane, chloroform, acetic acid, ethanol, hexane, ethyl acetate and other reagents used were of analytical grade. Solvents were freshly distilled and dried before using. β-Carbolines (9*H*-pyrido[3,4-*b*]indoles), *i.e.*, nor-harmane (9*H*-pyrido[3,4-*b*]indole, **1**), harmane (1-methyl-9*H*-pyrido[3,4-*b*]indole, **2**), harmine (7-methoxy-1-methyl-9*H*-pyrido[3,4-*b*]-indole, **3**), harmol (1-methyl-9*H*-pyrido[3,4-*b*]indol-7-ol, **4**), harmaline (7-methoxy-1-methyl-3,4-dihydro-9*H*-pyrido[3,4-*b*]-indole), carbazole (**6**), 2,5-dihydroxybenzoic acid (gentisic acid, DHB) and 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid, SA) were purchased from Aldrich, Sigma and Wako Pure Chemical Ind. Japan. Ceric ammonium nitrate ($Ce(NH_4)_2(NO_3)_6$; CAN), ammonium ferric (II) sulfate hexahydrate ($(NH_4)_2Fe(SO_4)_2.6H_2O$, Mohr salt), EDTA, copper (II) nitrate trihydrate ($Cu(NO_3)_2.3H_2O$), sodium nitrite, acetic anhydride, nitric acid, glacial acetic acid were purchased from Aldrich.

Thin layer chromatography (tlc) analysis was performed with aluminium silica gel sheets (0.2 layer thickness, silica-gel 60 F254). Products were isolated by preparative thick layer chromatography and column chromatography which was carried out using silica gel 200-400 mesh 60Å and ethyl acetate and ethyl acetate - ethanol as eluent. Melting points are uncorrected. ^1H- and ^{13}C-nmr spectra were run in deuterochloroform, deuterochloroform/methanol-d_4 and dimethylsulfoxide-d_6 at 200 and 500 MHz. Chemical shifts are reported in ppm values, using tetramethylsilane as internal standard. The uv spectra were run in acetonitrile and acetonitrile – sulfuric acid solutions of the β-carbolines (10^{-4} *M*). Electron impact mass spectra (ei-ms) and high-resolution mass spectra (hrms) were obtained under electron impact (70 eV). The ratios m/z and the relative intensities are reported.

Laser desorption/ionization time-of-flight mass spectrometry (ld-tof-ms): measurements were performed using: i) Shimadzu Kratos, Kompact MALDI III UV-laser desorption time-of-flight mass spectrometer and ii) Shimadzu Kratos, Kompact MALDI 4 (Pulsed Extraction). Both spectrometers are equipped with a pulsed nitrogen laser (λ = 337 nm; pulse width = 3 ns), the latter only with tuneable time delay capability. The analyzer was used at accelerating voltage of 20kV. The laser can be fired at any spot or fired continuously along a selected length of the sample holder. The sample was irradiated just above the threshold for obtaining ions. Thus, the irradiance used for producing a mass spectrum was analyte dependent. Usually 50 spectra were accumulated. All mass spectra were taken (a) in the positive ion mode and in the negative ion mode and (b) in the linear and in the reflectron mode.

Stock solution of β-carbolines (10^{-2} *M*) were prepared by dissolving 10^{-5} moles of the compound in 1 ml of the selected solvent (Method A: dimethylsulfoxide (DMSO); Method B: acetonitrile - water-trifluoroacetic acid 0.1% (3:1) (v:v)). The solutions were kept in the dark at low temperature to prevent photochemical decomposition.

Sample preparation method: ld-tof-ms experiments using β-carbolines as analytes, a sample deposit, typically 0.5 µl of the solution, was placed on the sample probe tip (2 mm diameter; stainless steel). Thus the solvent was removed by room temperature evaporation by forced air (film). Then, additional 0.5 µl of the same solution was placed on the same probe tip covering the first deposited film, and again the solvent removed by forced air. Spectra were calibrated by use of (a) internal calibrant reagent; *e.g.*: gramicidin S, Cs, Na and (b) standard chemicals as external calibrant reagent (gentisic acid and sinapinic acid) using Kratos Kompact calibration program.

7-Acetyloxy-1-methyl-9*H*-pyrido[3,4-*b*]indole (**5**).

A mixture of harmol (204.7 mg, 1.03 mmoles) (commercial 1-methyl-9*H*-pyrido[3,4-*b*]indol-7-ol (harmol, **4**)) and acetic anhydride (1.0 ml) in pyridine (0.5 ml) was stirred at room temperature during 20 hours. In order to prevent the hydrolysis of the acetyloxy group, addition of methanol and/or sodium bicarbonate aqueous solution was avoided. Instead of these chemicals, toluene was added in order to yield the pyridine – toluene azeotrope and then the system was evaporated *in vacuo*. The solid residue obtained was purified by silica-gel column chromatography eluted with ethyl acetate and ethyl acetate - ethanol mixtures. Compound **4** (205 mg, 83% yield) was obtained as white needles (ethanol), mp 155°; ^{1}H-nmr (deuteriochloroform/methanol-d_4): δ 8.46 (broad singlet (bs), 1H, NH), 8.32 (d, 1 H, 3-H, J = 5.4 Hz), 7.91 (d, 1H, 5-H, J = 8.7 Hz), 7.63 (d, 1 H, 4-H, J = 5.4 Hz), 7.20 (d, 1 H, 8-H, J = 2.0 Hz), 6.96 (dd, 1 H, 6-H, J = 8.7, 2.0 Hz), 2.77 (s, 3 H, CH_3), 2.39 ppm (s, 3 H, CH_3CO); ^{13}C-nmr (deuteriochloroform/methanol-d_4): δ 170.3 (CO), 150.8 (7-C), 141.4 (1-C), 140.2 (9a-C), 136.6 (3-C), 135.2 (8a-C), 127.9 (4b-C), 122.1 (5-C), 119.1 (4a-C), 113.8 (6-C), 112.6 (4-C), 104.7 (8-C), 20.9 (CH_3), 18.9 ppm (CH_3CO); hrms: Calcd. for $C_{14}H_{12}N_2O_2$ 240.089878, found: 240.089947.

Anal. Calcd. for $C_{14}H_{12}N_2O_2$: C, 69.98; H, 5.03; N, 11.66. Found: C, 69.96; H, 5.04; N, 11.65.

Nitration of β-Carbolines.

-$Ce(NH_4)_2(NO_3)_6(H_20)_6$ (CAN) Method 1.

The β-carboline (0.62 mmole) and CAN (1.1 mmoles) were separately dissolved in acetonitrile (100 ml) and then mixed together. The resulting mixture was stirred in the dark for 6 hours at 90º until tlc indicated that the reaction was complete. All these reactions were carried out under normal (air) atmosphere. Acetonitrile was evaporated *in vacuo* to give a light brown-greenish oily residue. The residue was separated by column flash chromatography (silica gel-ethyl acetate-ethanol mixtures) to give **1a**, **1b**, **1c** and polynitrated-β-carboline dimeric compounds and cerium-polynitrated-β-carboline complexes.

$Ce(NH_4)_2(NO_3)_6(H_20)_6$ (CAN) Method 2.

In order to remove more efficiently the remaining cerium to obtain compounds of higher purity we proceeded as follows: 30 ml of water solution containing Mohr salt (1.1 mmoles, $(NH_4)_2Fe(SO_4)_2$•$6H_2O$)) was added to the solid residue obtained after acetonitrile evaporation. The mixture was stirred at room temperature for one hour. Then ethylene diamine tetraacetic acid EDTA (2.5 mmol) was added to complex both Fe^{3+} and Ce^{3+}. Stirring was continued for another hour. After that, potassium carbonate was added until the solution became neutral (pH = 7). The aqueous solution was washed with dichloromethane (3 x 15 ml). The combined organic extracts were washed with water (100 ml), and the organic layer was dried over sodium sulfate, filtered and evaporated *in vacuo* to give an almost colorless residue. The residue was treated by flash column chromatography following the procedure described.

Olah's Method.

A mixture of copper (II) nitrate trihydrate (200 mg, 0.83 mmole) and acetic anhydride (1.5 ml) was stirred at room temperature for 1.5 hours. After that the β-carboline compound (0.72 mmole) was added to the mixture, and stirring was continued for 24 hours. Additional copper (II) nitrate trihydrate (100 mg, 0.83 mmole) was added, and stirring was continued for another 24 hours. Then methanol (10 ml) was added. After 30 minutes of stirring volatiles were evaporated *in vacuo*, 20 ml of water were added to the remaining solution, and the mixture was stirred for 30 minutes the water solution was adjusted to pH 14 by potassium carbonate. The aqueous solution was washed with dichloromethane (3 x 15 ml). The combined extracts were washed with water (100 ml), and the organic layer was dried over sodium sulfate, filtered and evaporated *in vacuo* to give a solid residue. The residue was separated by flash column chromatography as described above.

Nitric Acid - Acetic Acid Method.

A slurry of the β-carboline (0.5 mmole) and 0.5 mmole of sodium nitrite in glacial acetic acid (6 ml) was stirred at 30-40° for 1.5 hours. Then, 1.4 ml of the mixture concentrated nitric acid – glacial acetic acid (1:1, v:v) was slowly added. The resultant mixture was stirred at 40-45° for additional 3.5 hours and left overnight at room temperature. Water was added (100 ml), the solution was neutralized with potassium carbonate and the workup was conducted as described above.

6-Nitro-9*H*-pyrido[3,4-*b*]indole (6-Nitro-nor-harmane) (**1a**).

Compound **1a** was obtained as yellow needles (ethanol), mp 255° [lit 325-326° [49]; 340-342° [52]); ^{1}H-nmr (deuteriochloroform/methanol-d_4): δ 10.17 (bs, 1H, NH), 9.14 (d, 1 H, 5-H, J = 2.2 Hz), 8.91 (s, 1H, 1-H), 8.47 (dd, 1 H, 7-H, J = 2.2, 9.1 Hz), 8.44 (d, 1 H, 3-H, J = 5.2 Hz), 8.15 (d, 1 H, 4-H, J = 5.2 Hz), 7.65 ppm (d, 1 H, 8-H, J = 9.1 Hz); ^{13}C-nmr (deuteriochloroform): δ 143.7 (6-C), 140.2 (9a-C), 139.6 (3-C), 138.0 (8a-C), 134.9 (1-C), 127.9 (4b-C), 123.2 (5-C), 121.6 (4a-C), 119.2 (7-C), 115.4 (4-C), 112.2 (8-C) ppm; hrms: Calcd. for $C_{11}H_7N_3O_2$ 213.053827, found 213.053932.

Anal. Calcd. for $C_{11}H_7N_3O_2$: C, 63.97; H, 3.31; N, 17.71. Found: C, 63.99; H, 3.30; N, 17.70.

8-Nitro-9*H*-pyrido[3,4-*b*]indole (8-Nitro-nor-harmane) (**1b**).

Compound **1b** was obtained as yellow needles (ethanol), mp 215°; ^{1}H-nmr (deuteriochloroform): δ 12.45 (bs, 1H, NH), 9.11 (s, 1 H, 1-H), 8.78 (d, 1H, 3-H, J = 5.3 Hz), 8.49 (d, 2 H, 5-H and 7-H, J = 7.4), 8.26 (d, 1 H, 4-H, J = 5.3), 7.47 ppm (dd, 1 H, 6-H, J = 7.4 Hz); ^{13}C-nmr (dimethylsulfoxide-d_6): δ 142.8 (9a-C), 139.7 (3-C), 133.4 (1-C), 132.5 (8a-C), 131.4 (8-C), 129.9 (5-C), 127.9 (4b-C), 124.3 (7-C), 123.6 (4a-C), 119.1 (6-C), 112.3 (4-C) ppm; hrms: Calcd. for $C_{11}H_7N_3O_2$ 213.053827, found: 213.054005.

Anal. Calcd. for $C_{11}H_7N_3O_2$: C, 63.97; H, 3.31; N, 17.71. Found: C, 63.96; H, 3.32; N, 17.72.

6-Nitro-1-methyl-9*H*-pyrido[3,4-*b*]indole (6-Nitroharmane) (**2a**).

Compound **2a** was obtained as yellow needles (ethanol), mp 225° (d) [lit 300-301° [44,46]; 296-298° [48]); ^{1}H-nmr (deuteriochloroform): δ 10.17 (bs, 1H, NH), 9.08 (d, 1 H, 5-H, J = 1.8 Hz), 8.50 (d, 1H, 3-H, J = 5.5 Hz), 8.39 (d, 1 H, 4-H, J = 5.5 Hz), 7.99 (dd, 1 H, 7-H, J = 1.8, 5.1 Hz), 7.91 (d, 1 H, 8-H, J = 5.1 Hz), 2.87 ppm (s, 3 H, CH_3); ^{13}C-nmr (dimethylsulfoxide-d_6): δ 143.1 (1-C), 140.9 (6-C), 140.2 (9a-C), 138.9 (3-C), 135.6 (8a-C), 129.7 (5-C), 127.5 (4b-C), 122.9 (7-C), 120.7 (4a-C), 113.3 (8-C), 112.4 (4-C), 20.2 ppm (CH_3); hrms: Cal. for $C_{12}H_9N_3O_2$ 227.069477, found: 227.069000.

Anal. Calcd. for $C_{12}H_9N_3O_2$: C, 63.43; H, 3.99; N, 18.50. Found: C, 63.41; H, 3.98; N, 18.52.

8-Nitro-1-methyl-9*H*-pyrido[3,4-*b*]indole (8-Nitroharmane) (**2b**).

Compound **2b** was obtained as yellow needles (ethanol), mp 205-207° [lit 210° [44]); ^{1}H-nmr (dimethylsulfoxide-d_6): δ 11.72 (bs, 1H, NH), 8.76 (d, 1 H, 7-H, J = 7.3 Hz), 8.49 (d, 1H, 5-H, J = 8.4 Hz), 8.37 (d, 1 H, 3-H, J = 5.1), 8.09 (d, 1 H, 4-H, J = 5.1), 7.48 (dd, 1 H, 6-H, J = 8.4, 7.3 Hz), 2.92 ppm (s, 3 H, CH_3); ^{13}C-nmr (dimethylsulfoxide-d_6): δ 143.8 (1-C), 140.4 (9a-C), 139.5 (3-C), 135.0 (8a-C), 129.7 (8-C), 126.8 (5-C), 125.8 (4b-C), 124.2 (7-C), 121.6 (4a-C), 119.2 (6-C), 112.7 (4-C), 20.9 ppm (CH_3); hrms: Calcd. for $C_{12}H_9N_3O_2$ 227.069477, found: 227.069535.

Anal. Calcd. for $C_{12}H_9N_3O_2$: C, 63.43; H, 3.99; N, 18.50. Found: C, 63.41; H, 3.99; N, 18.52.

6-Nitro-7-methoxy-1-methyl-9*H*-pyrido[3,4-*b*]indole (6-Nitroharmine) (**3a**)**.**

Yellow needles (ethanol), mp 265º (d) ; ^{1}H-nmr (deuteriochloroform): δ 11.98 (bs, 1H, NH), 8.93 (s, 1 H, 5-H), 8.26 (d, 1H, 3-H, J = 5.4 Hz), 7.99 (d, 1 H, 4-H, J = 5.4 Hz), 7.20 (s, 1 H, 8-H), 4.03 (s, 3 H, CH_3O), 2.74 ppm (s, 3 H, CH_3); ^{13}C-nmr (dimethylsulfoxide-d_6): δ 155.1 (7-C), 146.1 (1-C), 140.2 (9a-C), 135.8 (8a-C), 133.2 (3-C), 129.8 (6-C), 126.5 (4b-C), 122.3 (5-C), 115.1 (4-C), 113.0 (4a-C), 96.1 (8-C), 57.5 (CH_3O), 17.5 ppm (CH_3); hrms: Calcd. For $C_{13}H_{11}N_3O_3$ 257.080041, found: 257.080451.

Anal. Calcd. for $C_{13}H_{11}N_3O_3$: C, 60.64; H, 4.31; N, 16.39. Found: C, 60.62; H, 4.30; N, 16.41.

8-Nitro-7-methoxy-1-methyl-9*H*-pyrido[3,4-*b*]indole (8-Nitroharmine) (**3b**).

Compound **3b** was obtained as yellow needles (ethanol), mp 212-213° [lit 204-205° [43]); ^{1}H-nmr (deuteriochloroform): δ 9.90 (bs, 1H, NH), 8.42 (d, 1 H, 3-H, J = 5.4 Hz), 8.3 (d, 1H, 5-H, J = 8.7 Hz), 7.73 (d, 1 H, 4-H, J = 5.4), 7.00 (d, 1 H, 6-H, J = 8.7), 4.13 (s, 3 H, CH_3O), 2.86 ppm (s, 3 H, CH_3); ^{13}C-nmr (dimethylsulfoxide-d_6): δ 153.7 (7-C), 142.9 (1-C, 9a-C), 139.4 (3-C), 134.3 (8a-C), 127.8 (5-C), 127.2 (4b-C), 117.6 (4a-C, 8-C), 112.3 (4-C), 105.8 (6-C), 57.5 (CH_3O), 20.7 ppm (CH_3); hrms: Calcd. for $C_{13}H_{11}N_3O_3$ 257.080041, found: 257.080146.

Anal. Calcd. for $C_{13}H_{11}N_3O_3$: C, 60.64; H, 4.31; N, 16.39. Found: C, 60.65; H, 4.30; N, 16.40.

6,8-Dinitro-7-methoxy-1-methyl-9*H*-pyrido[3,4-*b*]indole (6,8-Dinitroharmine) (**3c**).

Compound **3c** was obtained as yellow needles (ethanol), mp 238° (d); ^{1}H-nmr (deuteriochloroform): δ 9.94 (bs, 1H, NH), 8.93 (s, 1 H, 5-H), 8.56 (d, 1H, 3-H, J = 5.3), 7.84 (d, 1 H, 4-H, J = 5.3), 4.21 (s, 3 H, CH_3O), 2.91 ppm (s, 3 H, CH_3); ^{13}C-nmr (dimethylsulfoxide-d_6): δ 158.6 (7-C), 145.7 (1-C), 140.8 (9a-C), 134.6 (3-C), 133.9 (8a-C), 128.3 (6-C), 127.9 (4b-C), 124.2 (5-C), 119.4 (4a-C and 8-C), 112.8 (4-C), 65.1 (CH_3O), 29.5 ppm (CH_3); hrms: Calcd. for $C_{13}H_{10}N_4O_5$ 302.065120, found: 302.065368.

Anal. Calcd. for $C_{13}H_{10}N_4O_5$: C, 51.66; H, 3.33; N, 18.54. Found: C, 51.68; H, 3.32; N, 18.55.

6-Nitro-7-acetyloxy-1-methyl-9*H*-pyrido[3,4-*b*]indole (6-nitro-7-acetoxy-harmol) **(5a)**.

Yellow needles (ethanol), mp 250° (d); ^{1}H-nmr (deuteriochloroform/methanol-d_4): δ 14.4 (bs, 1H, NH), 9.25 (s, 1 H, 5-H), 8.35 (d, 1H, 3-H, J = 5.5 Hz), 8.16 (d, 1 H, 4-H, J = 5.5 Hz), 7.51 (s, 1 H, 8-H), 2.76 (s, 3 H, CH_3), 2.33 ppm (s, 3 H, CH_3CO; ^{13}C-nmr (dimethylsulfoxide-d_6): δ 168.9 (C=O), 143.5 (7-C), 143.0 (1-C), 142.8 (9a-C), 139.2 (3-C), 135.8 (8a-C), 134.4 (6-C), 127.1 (4b-C), 121.1 (5-C), 118.4 (4a-C), 113.2 (4-C), 107.1 (8-C), 20.6 (CH_3), 20.3 ppm (CH_3CO); hrms: Calcd. for $C_{14}H_{11}N_3O_4$ 285.074956, found: 285.075046.

Anal. Calcd. for $C_{14}H_{11}N_3O_4$: C, 58.94; H, 3.89; N, 14.73. Found: C, 58.92; H, 3.88; N, 14.74.

8-Nitro-7-acetyloxy-1-methyl-9*H*-pyrido[3,4-*b*]indole (8-Nitro-7-acetoxyharmol) (**5b**).

Compound **5b** was obtained as yellow needles (ethanol), mp 190°; ^{1}H-nmr (dimethylsulfoxide-d_6): δ 11.76 (bs, 1H, NH), 8.67 (d, 1 H, 5-H, J = 8.4), 8.36 (d, 1H, 3-H, J = 5.5 Hz), 8.06 (d, 1 H, 4-H, J = 5.5 Hz), 7.27 (s, 1 H, 6-H, J = 8.4 Hz), 2.87 (s, 3 H, CH_3), 2.40 ppm (s, 3 H, CH_3CO); ^{13}C-nmr (deuteriochloroform/methanol-d_4): δ 168.7 (C=O), 144.1 (7-C and 9a-C), 143.6 (1-C), 139.6 (3-C), 135.4 (8a-C), 133.7 (4b-C), 128.5 (5-C), 126.5 (8-C), 123.0 (4a-C), 115.6 (6-C), 112.7 (4-C), 20.8 (CH_3), 20.6 ppm (CH_3CO); hrms: Calcd. for $C_{14}H_{11}N_3O_4$ 285.074956, found: 285.075075.

Anal. Calcd. for $C_{14}H_{11}N_3O_4$: C, 58.94; H, 3.89; N, 14.73. Found: C, 58.96; H, 3.88; N, 14.74.

1-Nitrocarbazole (**6a**).

Compound **6a** was obtained as yellow needles (ethanol), mp 195° (lit 197-198° [61,62]); ^{1}H-nmr (dimethylsulfoxide-d_6): δ 12.15 (bs, 1H, NH), 8.62 (d, 1 H, 4-H, J = 7.3 Hz), 8.41 (d, 1 H, 8-H, J = 8.4 Hz), 8.31 (dd, 1H, 5-H, J = 1.1, 7.8 Hz), 8.24 (d, 1 H, 2-H, J = 8.0 Hz), 7.51 (dd, 1 H, 7-H, J = 7.3, 8.4 Hz), 7.35 (dd, 1 H, 3-H, J = 7.3, 8.0 Hz), 7.29 ppm (dd, 1 H, 6-H, J = 1.1, 7.3, 7.8 Hz); ^{13}C-nmr (dimethylsulfoxide-d_6): δ 140.6 (1-C), 132.8 (4-C), 131.6 (9a-C), 130.9 (4a-C), 127.9 (3-C), 127.1 (7-C, 8a-C), 121.6 (2-C), 121.4 (4b-C), 120.5 (5-C), 118.2 (6-C), 112.6 ppm (8-C).

3-Nitrocarbazole (**6b**).

Compound **6b** was obtained as yellow needles (ethanol), mp 215° (lit 215-216° [61,62]); ^{1}H-nmr (dimethylsulfoxide-d_6): δ 12.02 (bs, 1H, NH), 9.15 (d, 1 H, 4-H, J = 1.8 Hz), 8.36 (d, 1H, 5-H, J = 8.0 Hz), 8.29 (dd, 1 H, 2-H, J = 1.8, 8.8 Hz), 7.65 – 7.58 (m, 2 H, 1-H and 8-H), 8.51 (dd, 1 H, 6-H, J = 6.9, 8.0 Hz), 7.29 ppm (dd, 1 H, 7-H, J = 6.9, 7.7 Hz); ^{13}C-nmr (dimethylsulfoxide-d_6): δ 143.1 (3-C), 140.8 (9a-C), 139.6 (8a-C), 127.3 (7-C), 122.2 (4a-C), 121.9 (4b-C), 121.1 (2-C), 120.9 (5-C), 120.0 (6-C), 116.9 (4-C), 111.7 (1-C), 110.9 ppm (8-C).

1,6-Dinitrocarbazole (**6c**).

Compound **6c** was obtained as yellow needles (ethanol), mp 343° [lit 344-346° [61,62]); ^{1}H-nmr (dimethylsulfoxide-d_6): δ

12.71 (bs, 1H, NH), 9.30 (d, 1 H, 5-H, J = 2.1 Hz), 8.90 (d, 1H, 4-H, J = 7.7 Hz), 8.58 – 8.39 (m, 2 H, 2-H and 7-H), 7.88 (d, 1 H, 8-H, J = 9 Hz), 7.49 ppm (dd, 1 H, 3-H, J = 7.7, 7.9 Hz); ^{13}C-nmr (dimethylsulfoxide-d_6): δ 144.0 (6-C), 132.4 (1-C), 132.1 (9a-C), 129.2 (4-C), 129.1 (8a-C), 124.6 (4a-C), 123.2 (3-C), 122.6 (7-C), 121.4 (4b-C), 119.9 (2-C), 116.5 (5-C), 112.9 ppm (8-C).

3,6-Dinitrocarbazole (**6d**).

Compound **6b** was obtained as yellow needles (ethanol), mp 385° [lit 386-387° [61,62]); ^{1}H-nmr (dimethylsulfoxide-d_6): δ 12.60 (bs, 1H, NH), 9.45 (d, 1 H, 5-H, J = 2.1 Hz), 8.90 (d, 1H, 4-H, J = 7.7 Hz), 8.58 – 8.39 (m, 2 H, 2-H and 7-H), 7.88 (d, 1 H, 8-H, J = 9 Hz), 7.49 ppm (dd, 1 H, 3-H, J = 7.7, 7.9 Hz); ^{13}C-nmr (dimethylsulfoxide-d_6): δ 149.8 (3-C and 6-C), 141.0 (8a- and 9a-C), 122.7 (2-C, 4a-C, 4b-C and 7-C), 118.8 (4-C and 5-C), 112.4 ppm (1-C and 8-C).

Nitration of 7-Hydroxy-1-methyl-9*H*-pyrido[3,4-*b*]indole (**4**, Harmol).

Nitric Acid - Acetic Acid – THF at 0° Method.

Harmol (**4**, 80 mg) was dissolved in a mixture of glacial acetic acid (6 ml) and tetrahydrofuran (2 ml). To this solution, stirred and kept at 0°, a mixture of nitric acid (1 ml) and glacial acetic acid (1 ml) was added dropwise during 3 hours. After that the system was stirred at room temperature overnight. Water was added (100 ml), and the aqueous solution was neutralized with potassium carbonate, then saturated with sodium bicarbonate and finally washed with dichloromethane (3 x 15 ml). The combined extracts were washed with water (100 ml), and the organic layer was dried over sodium sulfate, filtered and evaporated *in vacuo* to give a solid residue. The residue was separated by flash column chromatography as described above.

N,*N*'-Bis(7-hydroxy-1-formyl-9*H*-pyrido[3,4-*b*]indole) (**4d**).

Compound **4d** was obtained as yellow needles (ethanol), mp 385°; H-nmr (dimethylsulfoxide-d_6): δ 10.63 (s, 1H, HCO), 8.52 (s, 1 H, NH), 8.02 (d, 1H, 3-H, J = 6.6 Hz), 7.67 (d, 1 H, 5-H, J = 8.7 Hz), 7.50 (d, 1 H, 4-H, J = 6.6 Hz), 6.20 (d, 1 H, 6-H, J = 8.7 Hz), 5.75 ppm (s, 1 H, 8-H); ms (ei): m/z 422 (2), 380 (4), 305 (3), 233 (< 1), 220 (2), 212 (< 1), 203 (1), 191 (2), 178 (2), 161 (12), 150 (22), 137 (20), 123 (22), 120 (18), 96 (42), 86 (84), 66 (100), 58 (40), 55 (82), 45(100), 43 (100), 41(100); ms (ld-tof-ms; positive mode): m/z 423 (30), 213 (100).

Calculations.

The static charge distribution for predicting chemical reactivity of β-carbolines **1-4**, nitro-β-carbolines and possible reaction intermediates were calculated by using the semiempirical parametrized AM1, PM3 and ZINDO/S methods as implemented in HyperChem 5.1 Suite program [63]. These results were obtained from calculations performed after transferring the geometries optimized by *ab initio* calculations (HF level; 6-31G basis set; Gaussian 98W) [64].

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