DNA Intercalating Compounds as Potential Antitumor Agents. 1. Preparation and Properties of 7*H*-Pyridocarbazoles

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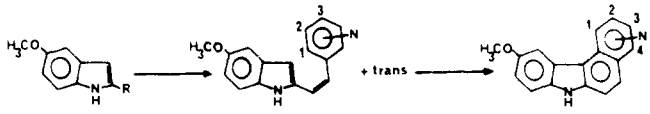
The DNA intercalating compounds derived from 6*H*-pyridocarbazole (ellipticines, olivacines) elicit high antitumor properties. In order to get information about the mechanism of action of these agents it is necessary to study structurally related analogues. For this purpose, various derivatives of the four isomeric 7*H*-pyridocarbazoles were synthesized by a single photochemical process on indolylpyridylethylenes. These derivatives are able to intercalate into DNA. The DNA binding affinities vary in the range of 10^4 to 10^6 M^{-1}, depending mainly on the nature of the substituent, nitrogen quaternization being the most enhancing factor. The position of the pyridinic nitrogen does not markedly affect the DNA binding affinity. Three quaternized compounds elicit a significative but low antileukemic activity on L1210 mice leukemia. The properties of 7*H*-pyridocarbazoles are discussed and compared to those of 6*H*-pyridocarbazoles (ellipticines and olivacines).

Several antitumor drugs, such as actinomycin D,[1] adriamycin,[2] ellipticines,[3] and aminoacridines,[4] interact strongly in vitro with DNA by intercalation.[5,6] Whereas it is easy to understand why DNA-binding compounds exhibit cytotoxic properties, the selectivity of their action toward tumoral cells remains totally unexplained. This situation results from the lack of information on the biochemical differences between normal and malignant cells. Nevertheless, the preparation of new derivatives able to intercalate into DNA remains an attractive way to look for new antitumor drugs. Furthermore, the study of the relation between the DNA binding process and the biological activity of a series of well-chosen compounds can lead to a better understanding of the mechanisms involved.

In the series of 6*H*-pyridocarbazoles, several compounds, mainly derivatives of ellipticine (5,11-dimethyl-6*H*-pyrido[3,4-*b*]carbazole), exhibit antitumor properties.[3,7,8] Activity on human tumors has been reported for two derivatives (9-methoxyellipticine[9] and 2-methyl-9-hydroxyellipticinium acetate).[10] On the other hand, the size and the shape of the 6*H*-pyridocarbazole ring allow an almost perfect positioning in the intercalating site with a maximum overlap between the heterocyclic ring and the DNA base pairs.[5] In addition, the presence of a positive charge on the nitrogen atom in these compounds helps the stabilization of the complex they form with DNA.[3,11] Such characteristic properties are also found in the series of 7*H*- and 11*H*-pyridocarbazoles. It is therefore surprising that so far only a few compounds of these series have been prepared[12-24] and that the antitumor properties have been evaluated only for three[20] of them (in the series of 7*H*-

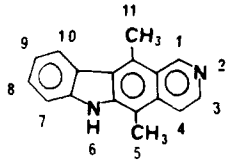
Scheme I



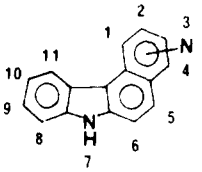
1, R = COOH
2, R = COCl
3, R = $CON(CH_3)_2$
4, R = $CH_2N(CH_3)_2$
5, R = $CH_2N^+(CH_3)_3$, I^-
6, R = $CH_2P^+Ph_3$, I^-

7, 1-N
8, 2-N
9, 3-N

10, 1-N; pyrido-[3,4-*c*]carbazole
11, 3-N; pyrido-[3,2-*c*]carbazole
12, 2-N; pyrido-[4,3-*c*]carbazole
13, 4-N; pyrido-[2,3-*c*]carbazole



5,11-dimethyl-6*H*-pyrido-[4,3-*b*]carbazole (ellipticine)



7*H*-pyridocarbazoles
1-N; pyrido[3,4-*c*]carbazole
2-N; pyrido[4,3-*c*]carbazole
3-N; pyrido[3,2-*c*]carbazole
4-N; pyrido[2,3-*c*]carbazole

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pyrido[2,3-c]carbazoles). It thus seemed of great interest to prepare such derivatives and to study their biological activity.

As a first approach, the 7H-pyridocarbazole series was investigated. For this purpose, a photochemical method able to provide the four isomeric compounds was selected. Synthesis, DNA binding properties, and antitumor activity of several variously substituted 7H-pyridocarbazole derivatives, including quaternary salts, are described.

On the other hand, it has been shown that the dimerization of intercalating agents leads to a very large enhancement of their DNA affinities.[25-27] The 7H-pyridocarbazole derivatives are very appropriate for the preparation and study of such dimeric agents, owing to the easy access to the four isomers. This allows the direct introduction of the chain linking the two rings on the nitrogen atom at different positions. Several such dimers were prepared and studied, some of them exhibiting marked antitumor properties, as reported in the following paper.[28]

Chemistry. Three types of methods have been used in the synthesis of 7H-pyridocarbazoles: the Fischer-type cyclization of indoles,[12-17] the Doebner or Skraup synthesis of quinolines,[18-21] and photochemical synthesis, with indolylpyridylethylenes[22,23] as the starting material. The photochemical synthesis proved to be the most appropriate method for our purpose.

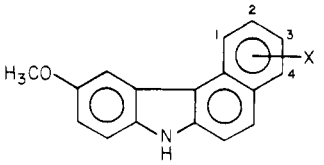
7H-Pyridocarbazoles were prepared according to the Scheme I.[22,24,29] 5-Methoxyindole-2-carboxylic acid (1) was reacted with $SOCl_2$ to give the acid chloride 2; 2 gave the amide 3 by reaction with dimethylamine. The amide 3 was reduced by $LiAlH_4$ to the amine 4, which was quaternized by methyl iodide. The ammonium salt 5 was transformed into phosphonium salt 6. Reaction of 6 with a pyridinealdehyde gave a mixture of the corresponding *cis*- and *trans*-indolylpyridylethylene 7–9. The indolylpyridylethylenes were transformed into 7H-pyridocarbazoles, 10–13, by an oxidative photocyclization in ethanol in the presence of iodine.

We modified the procedure described for the extraction of cis and trans isomers obtained in the Wittig reaction, in order to eliminate $OPPh_3$. The cis and trans isomers were extracted from the reaction mixture by 1 N HCl. The main quantity of $OPPh_3$ remained in the organic phase. The isomers were recovered by simple neutralization of the acidic phase, in the presence of CH_2Cl_2 or $CHCl_3$. Chromatography was then much easier to carry out and gave better yields. Furthermore, the successive steps from 1 to the phosphonium iodide 6 gave nearly quantitative yields.

The photocyclizations led to good yields (Table I), except in the case of *cis*- and *trans*-7, which gave only 25% of 13 (7H-pyrido[2,3-c]carbazole). In this case, secondary reactions were also taking place.

The pK_a of compounds 10 to 19 have been measured by UV spectroscopy and are reported in Table II. The pK_a values are influenced by the position of the pyridinic nitrogen in the heterocycle: the greater the steric hindrance around the nitrogen, the lower the pK. Besides, in the 7H-pyrido[3,2-c]carbazoles favorable mesomeric forms

Table I. Conditions of the Photocyclization of Indolylpyridylethylene Derivatives to 7H-Pyridocarbazoles



no.	X	time of irradiation, h	lamp[a]	yield of photocyclization, %
10	1-N			
11	3-N	24	1	92[b]
12	2-N	90	2, 3	25
13	4-N	320	3	75

[a] 1 = 100-W medium-pressure Hanovia photoreactor; 2 = Rayonet photoreactor (3500 Å); 3 = Hanau TQ 150 high-pressure photoreactor. [b] Total yield for compounds 10 and 11.

resulting from the delocalization of the lone electronic pair of the pyrrolic nitrogen increase the pK values. This feature is also present in compound 10, but the steric hindrance prevents the quaternization even with a methyl group. In relation with the basicity of these compounds, a UV study showed the occurrence of an anhydronium base form at basic pH (14) in the case of compound 23. We checked that no change in the spectrum occurred for compound 24, which has a methylated pyrrolic nitrogen.

As demonstrated by their low pK_a values ($pK_a < 7$), 7H-pyridocarbazoles are not completely protonated at physiological pH. The quaternization of the pyridinic nitrogen is thus necessary to obtain fully charged drugs at this pH, allowing strong interactions with DNA.

Quaternization of pyridinic nitrogens was achieved with the corresponding alkyl halides in DMF.[23] Demethylation of the methoxy group was performed in 48% HBr. Methylation of the pyrrolic nitrogen was obtained by reaction of the pyridocarbazole with NaH and ICH_3 in DMF.[30] For this series, the various compounds synthesized are collected in Tables II and III.

Interaction with DNA. In the first step, the DNA binding constants of 7H-pyridocarbazoles were measured by competition with ethidium bromide.[11] The required presence of a positive charge in the molecule for a strong DNA binding is clearly demonstrated by the increase in the DNA affinity constants (K_{ap}) as a function of the pH (Table II). Consequently, due to their low pK, the 7H-pyridocarbazoles were quaternized in order to obtain permanently, positively charged derivatives at physiological pH. As expected, such quaternization markedly increased the K_{ap} at pH 7.4 (Tables II and III).

The DNA affinities vary only slightly with the position of the nitrogen into the ring of the 7H-pyridocarbazoles, the largest values being obtained for the series of 7H-pyrido[4,3-c]carbazole (compounds 20–22, 25, 27, and 28). This indicates that the subsequent change in the conjugation does not play a crucial role in the formation of the intercalating complex. The influence of the nature of the quaternizing substituent is clearly shown by the values of K_{ap} obtained for compounds 20 (X = 2-N^+CH_3), 21 (X = 2-$N^+(CH_2)_2OH$), and 22 (X = 2-$N^+(CH_2)_2$-1-piperidyl); in this series, the introduction of a more hydrophylic chain lowers the DNA affinity, at pH 7.4. In addition, we demonstrated that the presence of a positively charged nitrogen

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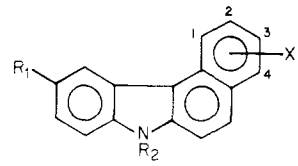
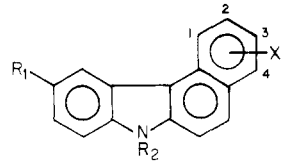
Table II. Physical Properties and DNA Affinity Constants of Nonquaternized Pyridocarbazoles



no.	R_1	R_2	X	mp, °C	formula[a]	anal.	pK	K_{ap},[b] × 10^5 M^{-1} pH 7.4	pH 6	pH 5
10	OCH_3	H	1-N	169-170	$C_{16}H_{12}N_2O$	C, H, N	5.25	0.18	0.32	1
11	OCH_3	H	3-N	254	$C_{16}H_{12}N_2O$	C, H, N	6.4	0.77	1.1	1.5
12	OCH_3	H	2-N	288	$C_{16}H_{12}N_2O$	C, H, N	5.75	0.35	0.44	1.7
13	OCH_3	H	4-N	230	$C_{16}H_{12}N_2O$	C, H, N	5.6	0.16	0.40	1.2
14	OH	H	1-N	183	$C_{15}H_{10}N_2O$	C, H, N	5.35	0.16	0.3	1.15
15	OH	H	2-N	>290	$C_{15}H_{10}N_2O$	C, H, N	6.4	0.42	1.15	3
16	OH	H	3-N	>290	$C_{15}H_{10}N_2O$	C, H, N	6.75	0.19	0.82	2.4
17	OCH_3	CH_3	1-N	110	$C_{17}H_{14}N_2O$	C, H, N	5.35	0.23	0.37	1.57
18	OCH_3	CH_3	2-N	127	$C_{17}H_{14}N_2O$	C, H, N	5.9	1.46	2.5	5.3
19	OCH_3	CH_3	3-N	156	$C_{17}H_{14}N_2O$	C, H, N	6.6	1.23	4.67	11

[a] Analyses for the indicated elements were within 0.4% of the theoretical values for the formulas given. [b] The affinity constants (K_{ap}) of the drugs for calf thymus DNA were measured on a Zeiss PMQ II apparatus by competition with ethidium bromide, in 0.2 M buffer, Tris-HCl, pH 7.4, cacodylate, pH 6.0, or acetate, pH 5.0. Scatchard plots were calculated with a 9810 A Hewlett Packard calculator.

Table III. DNA Binding Constants and Anti-L1210-Activity of Quaternary Salts



no.	R_1	R_2	X	mp, °C	formula	anal.[a]	K_{ap} × 10^5,[b] M^{-1}	MTD, mg/kg	OD fraction MTD	% T/C[c]	p[d]
20	OCH_3	H	2-N^+CH_3, I^-	>290	$C_{17}H_{15}N_2OI$	C, H, N, I	2.9	50	0.5		ns
21	OCH_3	H	2-$N^+(CH_2)_2OH$, I^-	>290	$C_{18}H_{19}N_2O_2I \cdot 1.5H_2O$	C, H, N, I	1.1	100	0.5	116	<0.05
22[e]	OCH_3	H	2-$N^+(CH_2)_2$N-1-piperidyl, Cl^-	>290	$C_{23}H_{26}N_3OCl \cdot 1.5H_2O \cdot 1HCl$	C, H, N, O, Cl	1.2	10	1[e]		ns
23	OCH_3	H	3-N^+CH_3, I^-	>290	$C_{17}H_{15}N_2OI$	C, H, N, I	2	50	0.5		ns
24	OCH_3	CH_3	3-N^+CH_3, I^-	>290	$C_{18}H_{17}N_2OI$	C, H, N, I	3.1	150	0.5	115	<0.05
25	OH	H	2-N^+CH_3, I^-	>290	$C_{16}H_{13}N_2OI \cdot 0.3H_2O$	C, H, N, I	1.8	25	1		ns
26	OH	H	3-N^+CH_3, I^-	290	$C_{16}H_{13}N_2OI \cdot 1H_2O$	C, H, N, I	0.67	50	0.5		ns
27	OCH_3	CH_3	2-N^+CH_3, I^-	>290	$C_{18}H_{17}N_2OI$	C, H, N, I	6.95	25	0.7	122	<0.001
28	OCH_3	CH_3	2-$N^+(CH_2)_2$N-1-piperidyl, Cl^-	>290	$C_{24}H_{28}N_3OCl \cdot 1H_2O \cdot 1HCl$	C, H, N, O, Cl	3	10	0.5		ns
29[f]	H	H	4-N^+CH_3, I^-	280	$C_{16}H_{13}N_2I$	C, H, N, I	0.6	50	0.5		ns

[a] Analyses for the indicated elements were within ±0.4% of the theoretical figures for the formulas given. [b] Binding constants (K_{ap}) of the drugs for calf thymus DNA were measured on a Zeiss PMQ II apparatus in 0.2 M Tris-HCl buffer, pH 7.4. [c] T/C = (treated survival/control) × 100. Figures are quoted for L1210 assays employing an initial tumor burden of 10^5 cells implanted ip, and the drugs were injected ip 24 h later in one single injection. These assays were carried out with the acetate derivatives for their better solubility in water. [d] The statistical significance of the results was determined using Student's t test. ns = not significant. [e] Compound 22 was administered by iv route. Its ip toxicity is very high: MTD < 5 mg/kg. [f] Compound 29 was synthesized by quaternization of 7*H*-pyrido[2,3-*c*]carbazole.[22]

on the quaternizing substituent strongly enhances the DNA affinity. The K_{ap} value of compound **22**, with an ethylpiperidine quaternizing chain, is increased at acidic pH: at pH 5, K_{ap} of **22** is equal to 5.2×10^5 M^{-1}, a value four times greater than that obtained at pH 7.4. Such a feature occurs in the case of **28**, with the same quaternizing chain. Besides, in this latter case, as already reported for the ellipticine series,[3] the methylation of the pyrrolic nitrogen equally enhances the DNA affinity. As expected, the combination of these two modifications leads to the highest K_{ap} value in our series at pH 5: $K_{ap} = 1.8 \times 10^6$ M^{-1}, a value six times greater than that obtained at pH 7.4.

At physiological pH, however, the highest DNA affinity is that of compound **27** ($R_1 = OCH_3$; $R_2 = CH_3$; X = 2-N^+CH_3, I^-). The replacement of a 10-methoxy group by a 10-hydroxy substituent does not produce a great change in the DNA affinity, as opposed to the results obtained in the ellipticine series.[3]

Interestingly the DNA-binding constant of 6*H*-pyrido[4,3-*b*]carbazole (5,11-didemethylellipticine,[3] $K_{ap} = 1.0 \times 10^4$ M^{-1}) is of the same order as those of the 7*H*-pyridocarbazoles (Table II). Furthermore, the addition of methyl groups on the 6*H*-pyridocarbazole ring leads to a 15-fold DNA affinity increase [for example, 5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole (ellipticine) $K_{ap} = 1.5 \times 10^5$ M^{-1}]. These results emphasize the importance of the introduction of methyl groups for the stability of the intercalating complex. Such a feature also appears in the case of compounds **18** and **19**, where methylation of the pyrrolic nitrogen substantially increases the K_{ap} values (comparison with **12** and **11**, respectively).

Table IV. Intercalation Parameters of 7H-Pyridocarbazoles

compd	slope[a]	r_e[b]	ϕ,[b] deg
24	2.9	0.1	13
25	2.5	0.09	14.5
27	2.3	0.09	14.5
29	3	0.11	12
9-OCH_3-Ell[c]	2.9	0.08	14.7
EthBr	2.3	0.05	26[d]

[a] The slope is that of the curve log $([\eta]/[\eta]_0) = f[\log(1 + 2r)]$, measuring the length increase of sonicated calf thymus DNA in the presence of increasing amounts of drugs. See Experimental Section and text. [b] r_e = number of dye molecules bound per nucleotide, providing the maximum of viscosity of covalently closed circular DNA of phage PM2. ϕ = unwinding angle of the DNA helix caused by the added dye, calculated on the basis of an unwinding angle of 26° for ethidium bromide. See Experimental Section and text. [c] 9-Methoxyellipticine, ref 33, recalculated for an unwinding angle of 26° for ethidium bromide. [d] Ethidium bromide, ref 37.

As mentioned above, the shape and the size of 7H-pyridocarbazoles should allow their relatively easy intercalation between the DNA base pairs. The intercalating ability of these compounds was investigated in order to check this assumption. Table IV shows the results of such a study for four typical compounds corresponding to different positions of the pyridinic nitrogen in the 7H-pyridocarbazole ring.

Among the different tests available to demonstrate and study DNA intercalation, two methods have been selected, which have already been used in the ellipticine series:[3] the measurement of the unwinding of the DNA helix using covalently closed circular DNA and that of the lengthening of DNA resulting from the DNA binding of these compounds.[31-34]

Actually, when a compound intercalates into DNA, the DNA length is necessarily increased by 3.4 Å and the DNA helix must unwind.[35] This provides a change in the viscosity of the DNA solution. Theoretical treatment[36] shows that if log $([\eta]/[\eta]_0)$ is plotted vs. log $(1 + 2r)$, where $[\eta]$ and $[\eta]_0$ are the intrinsic viscosities of sonicated DNA in the presence and in the absence of the compound, respectively, and r is the number of compound molecules bound per nucleotide of DNA; a slope value of 3 is expected. However, experimental slope values are between 2.3 and 3 for different classical monointercalators.[33] This feature is discussed in more detail in the following paper.[28] Futhermore, the intercalation process leads to different DNA unwinding angles, varying from 12 to 26°.[32,33,37]

The DNA length increase is most easily measured by viscosimetry[33] using sonicated DNA. The DNA unwinding was also measured by viscosimetry following the relaxation

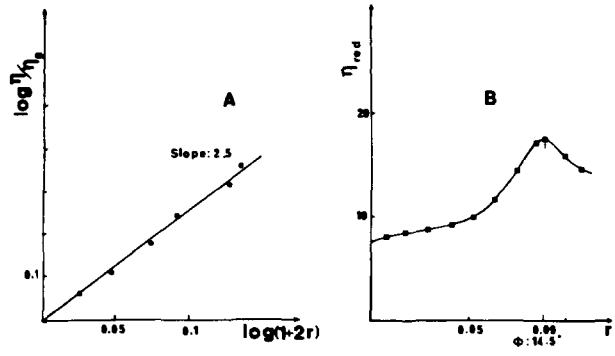


Figure 1. Lengthening and unwinding of DNA by compound **25**. (A) Length increase of sonicated calf thymus DNA. The lengthening of the DNA helix is proportional to the slope of the plot log $([\eta]/[\eta]_0)$ vs. log $(1 + 2r)$, where $[\eta]$ and $[\eta]_0$ are the intrinsic viscosities of sonicated DNA in the presence and in the absence of the compound, respectively, and r is the number of compound molecules bound per nucleotide of DNA; this slope is expected to be between 2.3 and 3 for a monointercalating agent[33] (see discussion in the text). DNA concentration is 100 μg/mL in 0.2 M acetate buffer, pH 5.0. (B) Unwinding of covalently closed DNA from PM_2 phage. The reduced viscosity, η red, is measured as a function of r. The value of r which provides a maximum of viscosity (r_e) is equal to 0.09. Under the same conditions, the r_e value of ethidium bromide is 0.05, corresponding to an unwinding angle of 26°. The unwinding angle ϕ caused by compound **25** is therefore 14.5°. Experiments were performed in 0.2 M acetate buffer, pH 5.0. DNA concentration is 100 μg/mL.

of supercoiled PM2 DNA:[33,34] Figure 1 shows typical values measured for these two parameters. Table IV reports the results obtained for several 7H-pyridocarbazoles in comparison with 9-OCH_3 ellipticine and ethidium bromide.

The magnitudes of the unwinding of the DNA helix caused by the different derivatives are very close to each other whatever the nature of the substituents and the position of the intracyclic nitrogen. This is quite different from the results found in the ellipticine series where the nature of the 9-substituent strongly affects the unwinding angle,[3] as shown by its value in the 9-hydroxy- (25°), 9-methoxy- (19.5°), unsubstituted- (14.7°), and 9-aminoellipticine (8.6°). The value of the unwinding angle found in the crystalline ellipticine–ICpG minihelical complex is 26°.[5]

Hence, the values obtained from crystallographic data on intercalated RNA minihelices are not necessarily identical with those found for DNA structure in solution. This was illustrated by the difference in the unwinding angles for proflavine–CpG and proflavine–dCpG complexes, which are 4 and 19°, respectively.[38] The different behavior in the 7H-pyridocarbazole series and the 6H-pyridocarbazole series (ellipticines) could be related to the difference in the overall dimension of the two kinds of intercalating rings. The more curved shape of 7H-pyridocarbazole could allow DNA intercalation with fewer constraints.

All these experiments clearly demonstrate that 7H-pyridocarbazoles intercalate into DNA. However, the results obtained with the series of 7H-pyridocarbazole and comparison with those obtained in the series of 6H-pyridocarbazole[3] (ellipticine derivatives) show that neither the changes in the position of the nitrogen atom into 7H-pyridocarbazoles nor the changes in the localization of the pyridinic ring into the tetracycle affect the DNA intercalating ability.

Antitumor Properties. The antitumor activities of the 7H-pyridocarbazoles were determined using L1210 murine leukemia and the results are reported in Table III. The

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activities of compounds **21**, **24**, and **27** are low but statistically significant.

It must be noted that the only three active drugs are quaternized, whereas the parent compounds are completely inactive (data not reported). Two of the active drugs belong to the 7*H*-pyrido[4,3-*c*]carbazole series with the pyridinic nitrogen in position 2. Although a simple correlation between antitumor activity and DNA binding does not appear, it can be observed that the most potent derivative, **27**, elicits the highest DNA affinity.

It is interesting to compare the properties of 6*H*- and 7*H*-pyridocarbazoles. The derivatives of 6*H*-pyridocarbazoles which can be compared with the compounds studied in this paper are the derivatives of 5- and/or 11-demethylellipticine. None of these latter compounds have ever been found to be active.[3,24,39] It appears therefore, as already noted,[3] that the presence of methyl substituents on the intercalating ring is a critical factor for the DNA binding affinity, as well as for biological efficacy. Such a critical role of methyl groups is also found in the series of actinomycin. For instance, the 4,6-didemethylactinomycin C_1 has a DNA binding affinity 30 times smaller than actinomycin C_1 and is devoid of biological activity.[1]

In this work it has been shown that 7*H*-pyridocarbazoles can lead to derivatives able to intercalate into DNA with properties similar to those of 6*H*-pyridocarbazoles (ellipticines). The simplest compounds already prepared have elicited slight antitumor properties. These parent compounds can be modified along two lines to try to increase their pharmacological activity: (a) the preparation of adequately substituted monomeric derivatives in order to mimic the features of ellipticines and actinomycins (such derivatives are presently under investigation in our laboratory); (b) the increase of their DNA binding affinity (this can readily be done through the preparation of dimeric molecules). In the following paper,[28] we show that such dimeric molecules possess high antitumor activity.

Experimental Section

Melting points were determined on a Kofler apparatus and are not corrected. The structures of the products were established by NMR spectra obtained on a Varian T-60 or a Brüker 270 MHz spectrometer. UV spectra were recorded using a SP-820 UV spectrometer; p*K* values were determined by the method of Albert and Sergeant using UV spectrophotometry. Analysis indicated only by symbols of the elements means that analytical results obtained for those elements were within ±0.4% of the theoretical values.

***N*,*N*-Dimethyl-5-methoxyindole-2-carboxamide (3).** To a suspension of 5-methoxyindole-2-carboxylic acid (**1**; 17.1 g; 85.5 mmol) in 235 mL of benzene was added, at 20 °C, 23.8 mL (335 mmol) of freshly distilled thionyl chloride; the mixture was stirred for 10 min and warmed up to 55 °C over a 15-min period. Stirring was continued for 150 min. The resulting suspension was kept for 3 h at room temperature and then filtered. The yellow solid was rapidly dissolved in 260 mL of anhydrous ether. The mother liquor was concentrated in vacuo and the yellow solid which precipitated was also dissolved in ether (50 mL). The ether phases were then collected and added to 60 mL of dimethylamine (33%, w/v, benzenic solution) over a period of 45 min at room temperature. A white crystalline solid immediately appeared. The mixture was stirred for 15 min and 160 mL of water was added. The white solid was thoroughly washed with water (final pH 6–7): yield 15.4 g (79%); mp 207 °C. Anal. ($C_{12}H_{14}N_2O_2$) C, H, N.

5-Methoxy-2-[(dimethylamino)methyl]indole (4). Lithium aluminum hydride (5.65 g, 145 mmol) was added in 5 min at 10–15 °C to 113 mL of anhydrous THF. The cooling bath was removed and a suspension of 14.3 g (65.5 mmol) of amide **3** in 385 mL of THF was then added over a period of 45 min. The temperature rose to 35 °C. The mixture was stirred during 3 h at 45–50 °C, then cooled down to −10 °C, and 85 mL of water was added cautiously.

The resulting solid was filtered and washed with THF; the filtrate was evaporated in vacuo. The residue was treated with 23 mL of 2 N sodium hydroxide and extracted with ether (2 × 100 mL). The extracts were washed with water (100 mL) and then with a saturated solution of NaCl (100 mL), dried over sodium sulfate, and evaporated in vacuo to give 13.35 g (~100%) of the crude amine **4** as a brown oil, which was not further purified.

5-Methoxy-2-[(dimethylamino)methyl]indole Methiodide (5). A solution of methyl iodide (4.3 mL; 1.1 × 52.8 mmol) in 27.5 mL of ethyl acetate was added to a solution of amine **4** (12.8 g; 62.8 mmol) in 55 mL of ethyl acetate. The temperature rose to 35 °C and a solid appeared immediately. The mixture was stirred for 1 h at 45–55 °C and kept for 2 h at 25 °C. The solid was filtered and 19.5 g of **5** was obtained: 88% yield; mp 184 °C. Concentration of the mother liquor gave 850 mg of **5**: total yield 92%; mp 184 °C. Anal. ($C_{13}H_{19}IN_2O$) C, H, N, I.

5-Methoxy-2-[(triphenylphosphonio)methyl]indole Iodide (6). A solution of 20.1 g (58 mmol) of **5** and 20 g (1.31 × 58 mmol) of triphenylphosphine in 190 mL of DMF was gradually heated up to 120–130 °C over 1 h and stirred for 24 h at 120–130 °C, under a nitrogen atmosphere. The mixture was then kept for one night at room temperature, without stirring, after which the solvent was evaporated. The residual brown oil was diluted with benzene, and the triphenylphosphonium salt **6** crystallized: yield 24.6 g (77%); mp 260 °C. Anal. ($C_{28}H_{25}INOP$) C, H, N, I, P.

***cis*- and *trans*-1-(5-Methoxy-2-indolyl)-2-(4-pyridyl)-ethylene (*cis*- and *trans*-9).** Sodium methoxide (64 mL; 260 mg of sodium in 64 mL of methanol) and 4-formylpyridine (1.7 mL; 18 mmol) in 30 mL of methanol were simultaneously added at 60–65 °C to a solution of **6** (8.1 g, 14.75 mmol) in 250 mL of methanol. The mixture was stirred for 24 h under nitrogen gas and concentrated. The trans isomer of **9** crystallized: yield 1.73 g; mp 252 °C.

The mother liquor was evaporated, diluted with 100 mL of methylene chloride, washed four times with 100 mL of water, and extracted four times with 250 mL of 1 N HCl. The acid phases were basified with a saturated aqueous solution of Na_2CO_3 (final pH 9) and extracted with 300 mL of methylene chloride; the extract was dried over sodium sulfate and evaporated in vacuo. Chromatography over a silica gel column (Et_2O–$CHCl_3$, 1:2) gave 177 mg of *trans*-**9**, mp 250 °C, and 630 mg of *cis*-**9**, mp 143 °C: total yield 70% (cis + trans). Anal. ($C_{16}H_{14}N_2O$) C, H, N.

***cis*- and *trans*-1-(5-Methoxy-2-indolyl)-2-(2-pyridyl)-ethylene (*cis*- and *trans*-7) and *cis*- and *trans*-1-(5-methoxy-2-indolyl)-2-(3-pyridyl)ethylene (*cis*- and *trans*-8)** were similarly prepared: *cis*-**7**, mp 124 °C; *trans*-**7**, mp 147 °C (75%, cis + trans). Anal. ($C_{16}H_{14}N_2O$) C, H, N. *cis*-**8**, mp 124 °C; *trans*-**8**, mp 179–180 °C (70%, cis + trans). Anal. ($C_{16}H_{14}N_2O$), C, H, N.

10-Methoxy-7*H*-pyrido[4,3-*c*]carbazole (12). A solution of 1.2 g (4.8 mmol) of a mixture of *cis*- and *trans*-**9** and 330 mg of iodine in 1000 mL of ethanol was irradiated for 90 h in a Rayonet photoreactor at 3500 Å. Ethanol was then concentrated to 100 mL and **12** crystallized: yield 735 mg; mp 280 °C. The mother liquor was treated with 230 mL of water, 1.8 g of potassium carbonate and 50 mg of sodium carbonate; final pH 9. A solution of 1.8 g of sodium thiosulfate in 16 mL of water was then added, and the ethanol evaporated in vacuo. The aqueous residue was extracted with 100 mL of chloroform and 268 mg of **12** precipitated: mp 285 °C. Recrystallization from DMF gave 897 mg (75%) of **12**, mp 288 °C. Anal. ($C_{16}H_{12}N_2O$) C, H, N.

10-Methoxy-7*H*-pyrido[3,2-*c*]carbazole (11) and 10-methoxy-7*H*-pyrido[3,4-*c*]carbazole (10) were similarly prepared (the conditions are given in Table I) from a mixture of *cis*- and *trans*-**8**. In this case, a final chromatography over a silica gel column (C_6H_6–Et_2O, 1:4) has been used to separate the two pyridocarbazoles **11** and **10**. **11**: 63% yield; mp 254 °C. Anal. ($C_{16}H_{12}N_2O$) C, H, N. **10**: 29% yield; mp 169–170 °C. Anal. ($C_{16}H_{12}N_2O$) C, H, N.

10-Methoxy-7*H*-pyrido[2,3-*c*]carbazole (13) was similarly prepared (conditions in Table I) from a mixture of *cis*- and *trans*-**7**. A final chromatography over a silica gel column (C_6H_6–Et_2O, 1:4) has been used to purify the pyridocarbazole **13**: 25% yield; mp 230 °C. Anal. ($C_{16}H_{12}N_2O$) C, H, N.

10-Hydroxy-7*H*-pyridocarbazoles 14–16. 10-Methoxy-7*H*-pyridocarbazole (400 mg, 1.6 mmol) in 3.5 mL of 48% aqueous

HBr was stirred for 5 h at 100 °C under nitrogen gas. The solution was then cooled down to 40 °C and poured into 120 mL of water and treated with Na_2CO_3 until no more precipitation occurred; final pH 9. Recrystallization of the precipitate in methanol gave about 50% of the hydroxy compound. **14**: 55% yield; mp 283 °C. Anal. ($C_{15}H_{10}N_2O$) C, H, N. **15**: 50% yield; mp >290 °C. Anal. ($C_{15}H_{10}N_2O$) C, H, N. **16**: 46% yield; mp >290 °C. Anal. ($C_{15}H_{10}N_2O$) C, H, N.

7-Methyl-10-methoxy-7*H*-pyridocarbazoles 17–19. Sodium hydride (150 mg, 3.18 mmol) was added to a solution of 250 mg (1.4 mmol) of pyridocarbazole in 3 mL of dry DMF. The mixture was stirred for 1.5 h under nitrogen gas. A solution (0.11 mL) of methyl iodide in 3 mL of dry DMF was then added dropwise. The reaction was kept stirring for one night at 25 °C, and ether (1 mL) was added to facilitate crystallization of the 7-methyl-10-methoxy-7*H*-pyridocarbazoles. **17**: 60% yield; mp 170 °C. Anal. ($C_{17}H_{14}N_2O$) C, H, N. **18**: 62% yield; mp 127 °C. Anal. ($C_{17}H_{14}N_2O$) C, H, N. **19**: 60% yield; mp 156 °C. Anal. ($C_{17}H_{14}N_2O$) C, H, N.

Quaternization of 7*H*-Pyridocarbazoles by Methyl Iodide (20, 23–27 and 29). The corresponding 7*H*-pyridocarbazole (0.8 mmol) was dissolved into 3 mL of DMF at 50 °C and 2.5 mL (4.2 mmol) of ICH_3 was added. The mixture was stirred for 3 h at 50–55 °C and concentrated in vacuo. Recrystallization of the precipitate in methanol gave about 70% of the desired 7*H*-pyridocarbazolium salts. **20**: 73% yield; mp >290 °C. Anal. ($C_{17}H_{15}N_2OI$) C, H, N, I. **23**: 68% yield; mp >290 °C. Anal. ($C_{17}H_{15}N_2OI$) C, H, N, I. **24**: 68 yield; mp >290 °C. Anal. ($C_{18}H_{17}N_2OI$) C, H, N, I. **25**: 73% yield; mp >290 °C. Anal. ($C_{16}H_{13}N_2OI \cdot 0.3H_2O$) C, H, N, I. **26**: 60% yield; mp 290 °C. Anal. ($C_{16}H_{13}N_2OI$) C, H, N, I. **27**: 78% yield; mp >290 °C. Anal. ($C_{18}H_{17}N_2OI$) C, H, N, I. **29**: 66% yield; mp 280 °C. Anal. ($C_{16}H_{13}N_2I$) C, H, N, I.

2-(2-Hydroxyethyl)-10-methoxy-7*H*-pyrido[4,3-*c*]carbazolium Chloride (21). Chloroethanol (2.5 mL) was added to a solution of 300 mg (1.2 mmol) of **12** in 10 mL of DMF, and the mixture was stirred overnight at 65 °C. DMF was evaporated to half its amount in vacuo, and methanol was added (3 mL); **21** crystallized: 58% yield; mp >290 °C. Anal. ($C_{18}H_{19}N_2OI \cdot 1.5H_2O$) C, H, N, I.

Quaternization of 12 and 18 by Chloroethylpiperidine (22 and 28). A solution of 0.8 mmol of chloroethylpiperidine hydrochloride in 10 mL of water was neutralized by 59 mg of Na_2CO_3, and the base was extracted with 5 mL of chloroform. The organic phase was dried over calcium chloride and poured into a solution of 0.5 mmol of **12** or **18** in 3 mL of DMF. The mixture was kept overnight at 80 °C. The precipitate was washed with ether and dried in vacuo. This precipitate was then dissolved in 15 mL of methanol and 0.1 mL of water, 0.2 mL of 2.2 N HCl in methanol was added, the methnaol was evaporated, and the resulting precipitate was thoroughly washed with ether to remove the excess of HCl. This solid was then dried in vacuo, over calcium chloride, at 80 °C. **22**: 50% yield; mp >290 °C. Anal. ($C_{23}H_{24}N_3OCl \cdot 0.5H_2O \cdot 1HCl$) C, H, N, O, Cl. **28**: 50% yield; mp >290 °C. Anal. ($C_{24}H_{28}N_3OCl \cdot 1H_2O \cdot 1HCl$) C, H, N, O, Cl.

Determination of K_{ap} Values. A Zeiss PMQ-2 spectrophotometer with a ZFM-4 fluorescence attachment was used at the maximum sensitivity. Excitation of the buffer solution (3 mL in 1-cm path-length glass cells) was achieved using a high-pressure mercury lamp and a 546-nm (M-466) filter. The wavelength of emission was 590 nm and a slit of 0.2 mm was used at the entrance of the monochromator. The temperature was 25 °C in all the experiments.

The buffers were 0.2 M Tris-HCl, pH 7.4, sodium cacodylate, pH 6, or sodium acetate, pH 5. Four cells were used simultaneously for each experiment. The first cell contained the buffer, the second one DNA (4 μg/mL), and the third and fourth ones contained the agent plus DNA. The compounds were used at a concentration of 10^{-5} M. Microliter portions of EthBr (ethidium bromide) (100 μg/mL in buffer) were added. Scatchard plots of competition were calculated with a 9810-1 Hewlett Packard calculator.

Viscosimetry. Viscosimetric measurements were performed at 25 °C in a semimicro dilution capillary viscosimeter with a suspended level (Cannon Instrument Co, State College, Pa.) mounted in a highly accurate thermostated water bath. Flow times were measured to ±0.1 ms by the combined use of photoelectric sensors and an electronic timer.[31]

The unwinding angle of the DNA helix caused by the binding of the different derivatives was measured with covalently closed circular DNA from PM2 phage.[31] The reduced viscosities were calculated in units of dL/g, and η red is plotted vs. r, the number of bound compounds per nucleotide of DNA, as described by Saucier et al.[33]

To measure the length increase of short DNA segments, the intrinsic viscosity of sonicated calf thymus DNA in the presence of an increasing concentration of agents was measured. Log $[\eta]/[\eta]_0$ was plotted as a function of log $(1 + 2r)$ where $[\eta]$ and $[\eta]_0$ are the intrinsic viscosities of the DNA measured in the presence and in the absence of bound agent, respectively, and r is the number of bound agent per nucleotide of DNA. These two procedures have been previously described by Saucier et al.[33]

Biological Testing. L1210 cells (10^5) were inoculated intraperitoneally into DBA_2 mice. There were 20 to 30 animals per group. Twenty-four hours later, one group was treated with an intraperitoneal inoculum of the studied drug and another group (control group) received the same volume of the solvent used to dissolve the compound under study. Deaths were recorded every day at the same hour. Animals which survived for more than 45 days were considered cured. The mean survival time of treated animals (T) was compared to that of control animals (C). The increases in life span (ILS) were calculated as $T/C \times 100$.

The acute toxicity was determined by the usual procedure. The highest dose which could be administered without causing animal death (MTD) was taken as unity, and dosages were expressed in fraction of MTD to appreciate the chemotherapeutic indexes. The statistical significance of the results was determined using Student's t test.

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