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NEW 7-ANILINOBENZO[b][1,8]PHENANTHROLINES

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Abstract- The synthesis and ^{1}H nmr spectroscopy of a series of 7-anilinobenzo[b][1,8]-phenanthrolines are reported.

It is well established that direct interaction with DNA is the basis for structural interpretation of many anticancer drugs. Such agents include actinomycins, acridines, anthracyclines, ellipticines and epipodophyllotoxins.[1] Recently, these drugs have been shown to induce protein-associated DNA stand breaks, by stabilizing a "cleavable complex" between topoisomerase II and DNA.[2]
Among 9-anilinoacridine antitumor agents, the derivative 4'-(9-acridinylamino)methanesulfonyl-m-anisidine (m-AMSA) has been specifically relevant and has become a useful clinical agent for the treatment of leukemia and lymphoma, but it is not effective against solid tumors.[3] This limited spectrum of activity has been attributed to its poor distribution in vivo because of the relatively high pKa of the acridine nitrogen, meaning that it is much ionized at the physiological pH. Design of less-basic analogues, with high levels of DNA binding, by shielding of proton approach to such nitrogen atom with convenient 4-and 5-substituents, has been successful.[4,5] However, few works have been carried out concerning extended aromatic chromophores including the benzo[b][1,n]phenanthroline system (n= 7, 8, 10).[6] In this context, the chemistry and antitumor activity of 7-anilino derivatives of the above mentioned system, with n= 7 or 10, have been reported,[7-9] but only the hydrochloride of the AMSA-analogue derived from the benzo[b][1,8]phenanthroline (4k), has been described.[6]
We have focused our interest on the synthesis and ^{1}H nmr spectroscopic study of 7-anilinobenzo[b][1,8]-phenanthrolines (4a-4k)(Scheme I). Ullmann N-arylation of 5-aminoisoquinoline (1) afforded 5-isoquinolylanthranilic acid (2). This reaction has been very much improved with respect to previous results.[10] Thus, using o-iodobenzoic acid and nitrobenzene as solvent instead of other protic polar solvents such as butanol, 3-methyl-1-butanol, 2-ethoxyethanol or pentanol, compound 2 was obtained up to 40% yield (Table 1). The importance of the solvent in the base-catalyzed Ullmann condensations is well known[11,12] but, as far as we know, nitrobenzene is not an usual solvent. Even the more satisfactory synthesis of anthranilic acids, using diphenyliodonium-2-carboxylate as arylating agent[13,14] and 5-aminoisoquinoline, gave very poor yields of 2 (10-15%).[15]
Ring-closure of 2 with phosphorus oxychloride to 7-chlorobenzo[b][1,8]phenanthroline (3) following Elslager method,[10] and substitution by the requisite aniline under rigorous pH control,[16] afforded compounds(4a-4k)in moderate yields. Partial hydrolysis of 3 to the fluorescent compound benzo[b][1,8]-

Table 1: Experimental yields of Ullmann N-arylation of 5-aminoisoquinoline with o-iodobenzoic acid, potassium carbonate and copper powder.

Solvent	bp(°C)	a	b	c
Butanol	117.2	12	20	20
3-Methyl-1-butanol	128.5	15	22	22
2-Ethoxyethanol	135	15	25	30
Pentanol	137.5	15	25	25
Nitrobenzene	210	40	40	40

a) % yield after 24 h of reflux

b) % yield after 48 h of reflux

c) % yield after 96 h of reflux

phenanthrolone (5) was observed in the ring-closure reactions. The amounts of 5 could be reduced by working in anhydrous conditions and using methanesulfonic acid instead of hydrochloric acid. Anyway, the recovered phenanthrolone could be recycled to the 7-chloro derivative (3) by treatment with phosphoryl chloride and phosphorus pentachloride using the procedure reported in the benzo[b]-[1,10]phenanthroline series.[17] Compounds (4) were obtained following the alternative strategy through the 5-isoquinolylanthranilic acid chloride (6) to the unisolated anilides (7), which afforded 4 by treatment with phosphorus oxychloride.[18]

^{1}H Nmr study.

The only systematic ^{1}H nmr study of simple phenanthrolines was reported by Perkampus in 1966.[19] By comparing ^{1}H nmr data of benzo[b][1,8]phenanthroline with those of the 7-chloro derivative (3)(Figure 1) and those of compounds (4a-4k)(Table 3), several interesting features have to be commented.

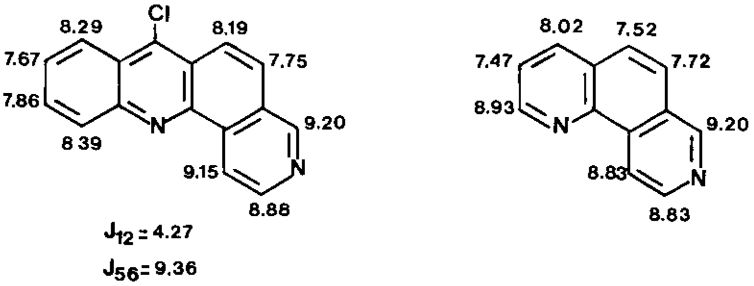
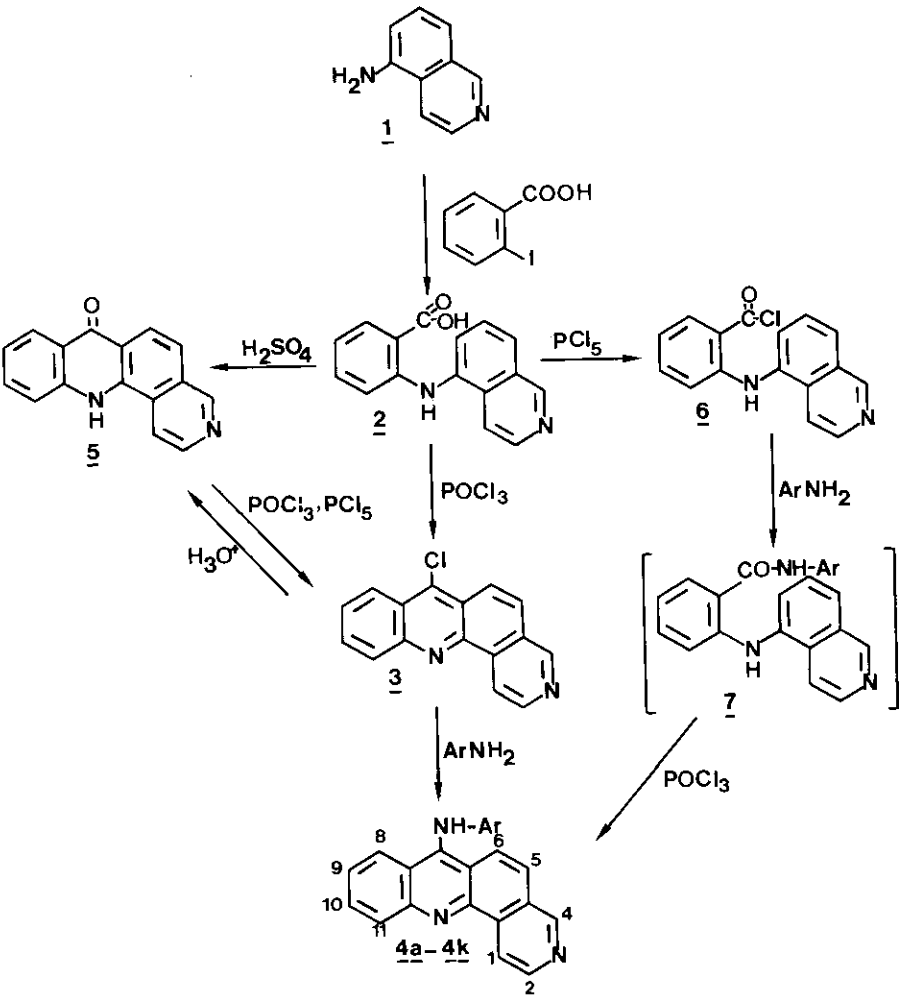
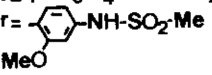


Figure 1. ^{1}H Nmr δ values of 1,8-phenanthroline[19] and 7-chlorobenzo[b][1,8]phenanthroline (3).

Scheme I



4a Ar= C_6H_5
4b Ar= p-C_6H_4-F
4c Ar= p-C_6H_4-OH
4d Ar= p-C_6H_4-OMe
4e Ar= p-C_6H_4-NMe_2
4f Ar= p-C_6H_4-NH-COMe
4g Ar= p-C_6H_4-SO_2-NH_2
4h Ar= p-C_6H_4-SO_2-Me
4i Ar= p-C_6H_4-NH-SO_2-C_6H_5
4j Ar= p-C_6H_4-NH-SO_2-p-C_6H_4-Me
4k Ar=



In spite of the complexity of the aromatic region, most signals could be assigned. The doublets and singlets corresponding to the 1,2, and 4-protons respectively, could be clearly identified since they are the most deshielded signals. Those doublets showed J_{12} values among 4.27 and 5.75 Hz (Table 3). The H-1 protons(δ 9.06-9.41 ppm) were significantly deshielded respect to H-2 protons (δ 8.82-8.93 ppm) while the same chemical shift has been reported for H-10 and H-9 protons in 1,8-phenanthroline (δ =8.83).[19]

Table 2: Analytical data and % yields for benzo[b][1,8]phenanthrolines.

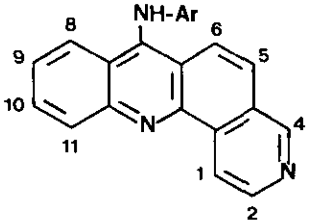
Compound	mp(°C)*	yield(%)	formula	Anal. Calcd (Found) C	H	N
4a	288-289[a]	54	$C_{22}H_{15}N_3$	82.16(82.20)	4.78(4.70)	13.06(13.08)
4b	283[a]	46	$C_{22}H_{14}N_3F$	77.80(77.86)	4.20(4.15)	12.38(12.38)
4c	311-313[b]	59	$C_{22}H_{15}N_3O$	78.19(78.32)	4.56(4.48)	12.48(12.45)
4d	226-227[c]	48	$C_{22}H_{17}N_3O$	78.50(78.61)	4.96(4.87)	11.91(11.95)
4e	193-195[c]	42	$C_{24}H_{20}N_4$	79.06(79.11)	5.56(5.59)	15.36(15.39)
4f	199-200[b]	48	$C_{24}H_{18}N_4O$	76.14(76.20)	4.80(4.75)	14.76(14.80)
4g	308-310[d]	59	$C_{22}H_{16}N_4O_2S$	65.86(65.98)	3.98(4.02)	14.03(13.99)
4h	310-312[a]	62	$C_{23}H_{18}N_4O_2S$	66.57(66.65)	4.46(4.37)	13.55(13.51)
4i	248-250[a]	60	$C_{28}H_{20}N_4O_2S$	70.54(70.60)	4.22(4.19)	11.68(11.75)
4j	260-262[e]	55	$C_{29}H_{22}N_4O_2S$	69.89(71.00)	4.58(4.51)	11.40(11.42)
4k	284-285[a]	58	$C_{24}H_{20}N_4O_3S$	64.81(64.85)	4.56(4.53)	12.62(12.60)

* Solvent of recrystallization: a) Ethyl acetate. b) Ethanol/water. c) Chloroform. d) Acetone. e) Ethanol.

The $\Delta\delta$ values for H-1 can be explained by both, the "peri" effect and a probable intramolecular hydrogen bond $N_{12}\cdots H\text{-}C_1$.[20]

The chemical shifts of the H-4 protons (δ 9.10-9.42 ppm) did not show significant variations in all derivatives, but in many of them the NH signals were present and even overlapped with them (see "others" in Table 3). The other doublet was thus assigned to H-5 and H-6 proton; distinction between them was based on the corresponding values for similar protons in acridines.[20] Protons H-9 and H-10 (which appeared as t,d) and H-8 and H-11 (d,d) were also assigned on the basis of acridine series. The H-2'(6') and H-3'(5') protons showed the lower δ values among the aromatic protons (δ 6.59-7.10 and δ 6.66-7.63 ppm respectively). By comparing ^1H nmr data of benzo[b][1,8]phenanthrol-7-one (5) in

Table 3. ^{1}H Nmr δ and J values (300 MHz, DMSO-d_6) of 7-anilinobenzo[b][1,8]phenanthrolines (4).



Compound	H_4	H_1	H_2	H_6	H_5	H_8	H_{11}	H_9	H_{10}	$H_{3'(5')}$	$H_{2'(6')}$	Others
3	9.20	9.15	8.88	8.19	7.75	8.29	8.39	7.67	7.86			
	s	d	d	d	d	m	m	m	m			
		$J_{1,2}$= 4.3		$J_{5,6}$= 9.4								
4a	9.36	9.13	8.91	8.05	7.85	8.32		7.65	7.93	7.23	6.94-6.87	6.87($H_{4'}$)
	s[a]	d	d	d	d	m		t,d	t,d	t	m	
		$J_{1,2}$= 5.2		$J_{5,6}$= 9.5				$J_{9,10}$= 7.1		$J_{2',3'}$= 7.5		
4b	9.34	9.12	8.90	8.04	7.88	8.30		7.65	7.93	7.07	6.92	
	s[a]	d	d	d	d	m		t,d	t,d	m	m	
		$J_{1,2}$= 5.2		$J_{5,6}$= 9.5				$J_{9,10}$= 7.9				
4c	9.12	9.06	8.82	8.03	7.70	8.22	8.28	7.52	7.83	6.84	6.75	9.20 ; 9.27
	s	d	d	d	d	d,d	d,d	t,d	t,d	d	d	s(NH) s(OH)
		$J_{1,2}$= 5.3		$J_{5,6}$= 8.5		$J_{8,9}$= 8.1		$J_{9,10}$= 7.4		$J_{2',3'}$= 8.7		
						$J_{10,11}$= 8.5						
4d	9.31	9.10	8.87	8.05	7.76	8.27	8.30	7.58	7.88	6.93	6.87	3.74 ; 9.24
	s	d	d	d	d	d,d	d,d	t,d	t,d	d	d	s(OCH_3) s(NH)
		$J_{1,2}$= 5.5		$J_{5,6}$= 9.1		$J_{8,9}$=		$J_{9,10}$= 7.9		$J_{2',3'}$= 8.7		
						$J_{10,11}$= 8.3						
4e	9.12	9.06	8.82	8.05	7.68	8.20	8.30	7.50	7.82	6.90	6.68	9.12 ; 2.85
	s	d	d	d	d	d,d	d,d	t,d	t,d	d	d	s(NH) s(NMe_2)
		$J_{1,2}$= 5.3		$J_{5,6}$= 8.7		$J_{8,9}$=		$J_{9,10}$= 7.7		$J_{2',3'}$= 8.6		
						$J_{10,11}$= 8.5						
4f	9.41	9.27	8.93	8.10	7.84	8.35	8.47	7.62	7.97	7.62	7.10	10.09; 2.15
	s	d	d	d	d	d,d	d,d	t,d	t,d	d	d	s(NH) s($COCH_3$)
		$J_{1,2}$= 5.7		$J_{5,6}$= 8.6		$J_{8,9}$= 8.1		$J_{9,10}$= 7.3		$J_{2',3'}$= 8.7		
						$J_{10,11}$= 8.5						
4g	9.35	9.13	8.90	8.37	8.24	8.25	8.38	7.71	7.96	7.63	6.85	9.70 ; 7.14
	s	d	d	d	d	d,d	d,d	t,d	t,d	d	d	s(NH) s(SO_2NH_2)
		$J_{1,2}$= 5.7		$J_{5,6}$= 8.6		$J_{8,9}$= 8.3		$J_{9,10}$= 7.7		$J_{2',3'}$= 8.5		
						$J_{10,11}$= 8.5						
4h	9.30	9.08	8.85	8.01	7.82	8.27		7.61	7.88	7.08	6.84	9.39 ; 9.29
	s	d	d	d	d	d,d		t,d	t,d	d	d	s(NH) s(NH)
		$J_{1,2}$= 5.6		$J_{5,6}$= 9.3		$J_{8,9}$=		$J_{9,10}$= 7.9		$J_{2',3'}$= 8.9		2.92
						$J_{10,11}$= 8.5						s(SO_2CH_3)
4i	9.38	9.16	8.91	8.33	8.22	7.94		7.80-7.35		6.98	6.85	10.06 ; 7.80-7.53
	s	d	d	d	d	m		m, overlap.		d	d	s(NH) (overlap. m, SO_2Ph)
		$J_{1,2}$= 5.5		$J_{5,6}$= 8.3						$J_{2',3'}$= 8.7		

Table 3. Cont.

Compound	H_4	H_1	H_2	H_6	H_5	H_8	H_{11}	H_9	H_{10}	$H_{3'(5')}$	$H_{2'(6')}$	Others
<u>4j</u>	9.27 s	9.08 d $J_{1,2}$= 5.3	8.86 d	7.92 d $J_{5,6}$= 8.7	7.76 d	8.20 d,d $J_{8,9}$= $J_{10,11}$= 8.7	8.27 d,d	7.59 t,d $J_{9,10}$= 7.9	7.88 t,d	6.93 d $J_{2',3'}$= 8.4	6.77 d	9.88 ;9.30 s(NH) s(NH) 7.60 and 7.37 d d $J_{2',3'}$= 8.1 (SO_2Ph) 2.36 s(CH_3)
<u>4k</u>	9.10 s	9.30 d $J_{1,2}$= 4.3	8.87 d	8.02 d $J_{5,6}$= 9.5	7.77 d	8.26 m		7.57 t,d $J_{9,10}$= 7.5	7.88 t,d	6.66 d $J_{2',3'}$= 8.7	6.59 d	9.53 ; 8.65 s(NH) s(NH) 6.70 ; 3.77 s(H-3') s(OCH_3) 2.99 s(SO_2CH_3)

[a] Overlapped with NH

DMSO-d_6, with those of acridone in DMSO-d_6(Figure 2), we can see similar chemical shifts for H-6 (8.38 ppm, d) and H-8 (8.31 ppm, d,d) protons of <u>5</u> and H-1 proton of acridone. It is interesting to comment *the assignement given to H-1 (8.84 ppm) and H-2 (8.90 ppm) protons in* <u>5</u>. *While* δ values of H-2 protons in compounds (<u>3</u>, <u>4</u> and <u>5</u>) are very similar, significant Δδ values(at about 0.30 ppm) were found between H-1 protons of compounds (<u>3</u> and <u>4</u>)(at about δ 9.15 ppm) and the same proton in <u>5</u> (at δ 8.84 ppm) which can be related to the lack of the above mentioned hydrogen bond $N_{12}\ldots HC_1$ in compound <u>5</u>.

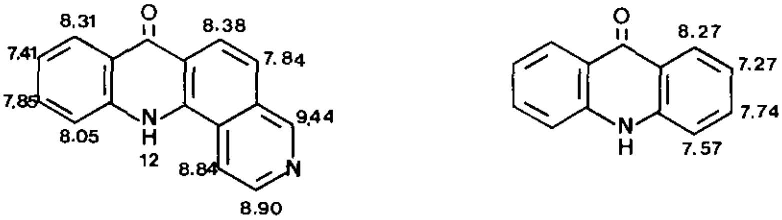


Figure 2. ^{1}H Nmr δ values of acridone and 12-<u>H</u>-benzo[b][1,8]phenanthrol-7-one (<u>5</u>) in DMSO-d_6

EXPERIMENTAL

^{1}H Nmr spectra were recorded on a Varian VXR-300 (300 MHz) and a Brucker WM-200-SY (200 MHz), DMSO-d_6 was used as solvent, and TMS was added in all cases as an internal standard. Elemental analyses were determined on a Perkin-Elmer 2400 CHN microanalyzer. Melting points are uncorrected and were measured using a Büchi capillary melting point apparatus. Reactions were monitored by thin layer chromatography on aluminium plates coated with silica gel with fluorescent indicator (Scharlau f 530). Separations by flash chromatography were performed on silica gel columns (SDS 60 A CC). 5-Aminoisoquinoline was obtained from isoquinoline following literature references.[21]

N-(5-Isoquinolyl)anthranilic Acid Hydrochloride,(2)

A mixture of 5-aminoisoquinoline(17.5 g, 120 mmol), o-iodobenzoic acid (29.8 g, 120 mmol), anhydrous potassium carbonate (18 g, 130 mmol), copper powder (0.44 g, 6.8 mmol),and dry nitrobenzene (250 ml) was stirred and boiled at reflux for 24 h. The reaction mixture was cooled and the solid was collected by filtration and washed with acetone/ethyl ether (1:1)(400 ml). The remaining solid was then extracted in a Soxhlet apparatus with ether (700 ml) for 8 h. After evaporating the solvent, the residue was treated with 3N hydrochloric acid (70 ml) in vacuo. Crystallization from methanol yielded 14.5 g (40%) of pure 2 as yellow crystals, mp 266-268°C(dec.)(lit.[10] mp 262-264°C). Ir: 3240, 3020, 1680, 1600 cm^{-1}. ^{1}H-Nmr(200 MHz, $CDCl_3$) δ: 6.93 (dd, $J_{7,8}$= 7.7 Hz, $J_{6,8}$= 1.3,1H, H-8); 7.56-7.80 (m, 2H, H-6 and H-7); 7.93 (d, $J_{3,4}$= 6.0 Hz, 1H, H-4); 8.78 (d, $J_{3,4}$= 6.0 Hz, 1H, H-3); 8.20-8.60 (m, 4H, H-3'-H-6'); 9.30 (s, 1H, H-1); 12.05 (s, 1H, CO_2H).

12H-Benzo[b][1,8]phenanthrol-7-one, (5)

A solution of 2(3.9 g, 15 mmol) in conc. sulfuric acid(50 ml) was warmed at 100°C for 4 h. The cooled solution was poured on ice-water(250 ml) and the precipitate salt was filtered, and dissolved in water. The solution was neutralized with 30% NaOH and the precipitate was washed with water, and dried in vacuo. Recrystallization from ethanol gave 2.20 g(60% yield) of pure 5, mp 352-353°C. Ir:3100-3000, 1620 cm^{-1}. Anal. Calcd for $C_{16}H_{10}N_2O$: C, 78.03; H, 4.09; N, 11.37. Found: C, 77.99; H, 4.05; N, 11.36.

7-Chlorobenzo[b][1,8]phenanthroline, (3)

It was prepared from 2 following reference 10 and from 5 following reference 17.

N-5-Isoquinolylanthranilic Acid Chloride, (6)

It was prepared from 2 following reference 18.

General procedures for the synthesis of 7-anilinobenzo[b][1,8]phenanthrolines, (4)

Method A.- To a solution of 3 (1g, 3.8 mmol) and the adecuate aniline (3.8 mmol) in the minimum amount of anhydrous ethanol to be solved at reflux, methanesulfonic acid (0.4 g, 4 mmol) was added and the mixture was refluxed for 30 min. After cooling, the precipitate salt was filtered, and dissolved in water. The solution was neutralized with 30% NaOH and the precipitate was washed with water, and dried in vacuo. The product thus obtained was a mixture of 5 and the corresponding 4 which was separated by flash chromatography on silica gel columns and ethyl acetate as solvent.

Method B.- To a solution of 6 (11.26 g, 40 mmol) in dry benzene (100 ml, 40 mmol) was added a solution of the corresponding aniline (40 mmol) in the same solvent (50 ml) and the mixture was refluxed for 40 min. Phosphorus oxychloride (20 ml, 219 mmol) was added afterwards, and the reflux was continued for 24 h. Addition of water (100 ml) and cooling at 0°C caused the precipitation of a solid which was chromatographied as it was described in method A.

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