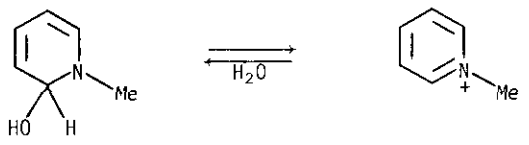
BENZOPHENANTHRIDINIUM SALT EQUILIBRIA[1]

Mary A. Caolo and Frank R. Stermitz*

Department of Chemistry, Colorado State University, Fort Collins, CO 80523 U.S.A.

The iminium ion ⇌ alkanolamine equilibrium position has been determined in various ethanol-buffer mixtures for a number of biologically-active benzophenanthridinium salts. Iminium ion concentration of antitumor active compounds is 90% or higher in 50% EtOH-buffer but much lower (usually 10% or less) for most antitumor inactive compounds.

Nitidine (1) and fagaronine (2) exhibit good antitumor activity in P388 and L1210 mouse leukemia in vivo screens,[2,3] while sanguinarine (9) and chelerythrine (10) are inactive in these screens. 9 and 10 are, however, highly cytotoxic[4] (KB in vitro screen) and exhibit other biological activities as well. The activity dichotomy among these compounds extends to other areas: antifungal activity,[3] NaK-ATPase inhibition[5] and antitubulin effect. A generalized summary is given in Table 1. The biological activity shown by these compounds probably involves the iminium site and hence we have established the position of equilibrium (1) for a number of benzophenanthridinium salts (Table 2).



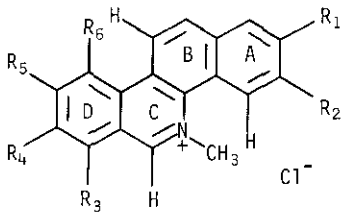
I II (1)

Table 1. Dichotomy of Biological Activities Among Some Benzophenanthridinium Salts.

Structure Type	Antitumor Activity[a]	Cytotoxicity[b]	Antimicrobial Activity[c]	NaK-ATPase Inhibition[d]	Antitubulin Assembly[e]
Nitidine-Fagaronine[f] (1-5)	good	low	low	low	inactive
Sanguinarine-Chelerythrine[f] (9-11)	none	high	high	very high	active

[a]Refs. 2,3. [b]Ref. 4 and private communication (National Cancer Institute). [c]Ref. 3. [d]Ref. 5. [e]This work. [f]Not all of the listed activities have been determined for all compounds of each type.

Table 2. Benzo[c]phenanthridinium Salts and Iminium Ion Concentrations



		% Iminion Ion at pH 7 In		
		50% EtOH	25% EtOH	Buffer Only
1	$R_1 = R_2 = OCH_2O$; $R_4 = R_5 = OCH_3$; $R_3 = R_6 = H$	97	94	89
2	$R_1 = OH$; $R_2 = R_4 = R_5 = OCH_3$; $R_3 = R_6 = H$	98[a]	97[a]	89[a]
3	$R_1 = R_2 = R_4 = R_5 = OCH_3$; $R_3 = R_4 = H$	96	92	89
4	$R_1 = R_2 = R_5 = OCH_3$; $R_3 = R_4 = R_6 = H$	97	97	88
5	$R_1 = R_4 = R_5 = OCH_3$; $R_2 = O\underline{i}Pr$; $R_3 = R_6 = H$	90	89	b
6	$R_1 = R_2 = R_3 = R_6 = H$; $R_4 = R_5 = OCH_3$	91	89	b
7	$R_1 = R_2 = OCH_3$; $R_3 = R_4 = R_5 = R_6 = H$	29	98	89
8	$R_1 = R_2 = R_4 = R_5 = R_6 = OCH_3$; $R_3 = H$	60	81	95
9	$R_1 = R_2 = OCH_2O$; $R_3 = R_4 = OCH_2O$; $R_5 = R_6 = H$	3	14	82
10	$R_1 = R_2 = OCH_2O$; $R_3 = R_4 = OCH_3$; $R_5 = R_6 = H$	10	51	96
11	$R_1 = R_2 = R_3 = R_4 = R_6 = OCH_3$; $R_5 = H$	9	68	81

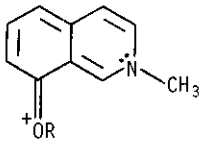
[a]Measurements at pH 6. [b]Not completely soluble.

The results of Table 2 are striking and present a chemical property correlation to match the biological activities of Table 1. Compounds 1-5 are all active in in vivo mouse leukemia screens and all exhibit high iminium ion concentration in pure buffer and EtOH/buffer mixtures. Compounds 9-11, *which are all inactive in in vivo screens, have very low iminium ion concentrations in* 50% EtOH solutions and lowered concentrations in 25% EtOH.[6]

A comparison of 6 and 7 with the other compounds is instructive. Compound 7 has no substituents on ring D and is intermediate in iminium ion concentration in 50% EtOH. Placing a substituent at R_5 (1-6) increases iminium ion concentration while placing a substituent at R_3 (9-11) decreases that side of the equilibrium. Compound 6 is anomalous in that it is inactive in the in vivo antitumor screens even though it is 91% in the iminium ion form. Thus, this is a necessary but *not sufficient condition for antitumor activity.*

Why should an alkoxysubstituent at R_3 have such a profound effect on the position of equilibrium (1)? Models indicate that there is strain relief on going from II to I since the N-Me group of II interacts strongly with the H of ring A in the planar II molecule. As polarity of the solvent is decreased (in going from pure buffer to 50% EtOH), the equilibrium should be shifted towards I. Thus, compounds 1-6 must possess some mechanism for stabilizing II relative to I which is not available to 9-11. Resonance interaction of the nonbonded pair on an alkoxy substituent at R_5 with the iminium double bond would certainly assist in stabilizing II:

The same resonance interaction would be available from the R_3 alkoxy, but this would involve a somewhat less important o-quinoid resonance structure:



There is also an important steric interaction to consider. An R_3 substituent would interact with the peri-H of ring C and this would destabilize structure II. If both R_3 and R_4 substituents are present, the steric interaction is enhanced by a buttressing effect.

If steric strain in the planar iminium ion II is an important factor, it was considered that a substituent R_6 might introduce further instability by its interaction with the indicated H on ring B. An R_6 substituent (like one at R_4) would not have a resonance stabilization effect on the iminium ion. We therefore prepared a new benzophenanthridinium salt, 8, which indeed showed a reduced level of II at equilibrium when compared with 1-6. The effect was not as striking as that for 9-11, however. On the basis of comparison with 1-5, we would predict a lowered *in vivo* activity for 8 since it is only 60% in the iminium form in 50% EtOH. (Compound 8 has not yet been found as a natural alkaloid, but could very likely occur naturally. Its synthesis and properties are described in the Experimental.)

Our work correlates with the interesting observation[7] that the pseudoalcoholates of 9 and 10 are more potent antimicrobials than are 9 and 10 themselves. Good antimicrobial activity, in contrast to good antitumor activity, must depend upon high concentration of the I form, which acts as a lipophilic prodrug.[7]

EXPERIMENTAL

Materials

Compounds 2-7 were available from previous syntheses[3] while compounds 9-11 were from isolation procedures.[4,8] Nitidine (1) was obtained from the National Cancer Institute.[9]

Compound 8 was synthesized according to the following outline. Details can be found in a thesis.[10] Condensation of 2-bromo-3,4,5-trimethoxybenzoyl chloride with 6,7-dimethoxyl-1-naphthylamine[3] yielded (89%) 2-bromo-3,4,5-trimethoxy-N-(6,7-dimethoxy-1-naphthyl)benzamide, mp 183-185°; anal. calcd for $C_{22}H_{22}NO_6Br$: C, 55.45; H, 4.55; N, 2.95 and found: C, 55.36; H, 4.85; N, 2.88. The bromoamide was photocyclized in benzene/MeOH to yield (40%) 2,3,8,9,10-pentamethoxybenzo[c]phenanthridine-6-one, mp 265-267°; anal. calcd for $C_{22}H_{21}NO_6$: C, 66.84; H, 5.35; N, 3.54 and found: C, 66.73; H, 5.50; N, 3.29. The amide was treated with $POCl_3$ to yield (44%) 6-chloro-2,3,8,9,10-pentamethoxybenzo[c]phenanthridine, mp 233-236°; anal. calcd $C_{22}H_{20}NO_5Cl$: C, 63.85; H, 4.87; N, 3.38 and found: C, 63.67; H, 4.75; N, 3.39. The 6-chlorobenzophenanthridine was hydrogenated with Raney Ni to yield (70%) 2,3,8,9,10-benzo[c]phenanthridine, mp 230-231°; anal. calcd for $C_{22}H_{21}NO_5$: C, 69.65; H, 5.58; N, 3.69 and found: C, 69.90; H, 5.90; N, 3.51. The benzophenanthridine was methylated with methyl sulfate and converted to the chloride by the usual procedure[3] to yield 2,3,8,9,10-benzo[c]phenanthridinium chloride (8), mp 158-162°; anal. calcd for $C_{23}H_{24}NO_5Cl$: C, 64.26; H, 5.63; N, 3.26 and found: C, 63.98; H, 5.77; N, 3.22

Equilibria Measurements

Equilibrium constants for equilibrium I were calculated by observing the long wave uv band (350-450 nm) for each of the benzophenanthridinium salts. There is no absorbance at that wavelength for any of the alkanolamines I and a maximum absorbance in each case for 100% iminium ion II in pH 2 buffer. Intermediate values are observed for pH 7 buffer and buffer/alcohol mixtures. Details of the calculations and actual K values (from which the iminium per cents of Table 2 were obtained) are in a thesis.[10] Anomalous uv spectra were obtained for fagaronine (2) at pH 7, probably due to partial ionization of the phenolic hydrogen. Measurements of the equilibrium constants for fagaronine were therefore made at pH 6, where the uv-visible spectrum was not affected by such ionization.

Bioassays

Antitubulin tests were performed by Prof. J. Bamburg, Department of Biochemistry, as follows. Chick embryo brain microtubules, cycled two times by disassembly in cold (1-2°C) and reassembly at 37°C, were disassembled, pelletable material removed by centrifugation, and 4 aliquots (0.5 mg/ml) placed in glass cuvettes at 4°C. Appropriate amounts of drug were added and after 0-20 minutes, the cuvettes were placed in a multichannel Beckman recording spectrophotometer equipped with a constant temperature cuvette chamber (37°C) and changes in OD (340 nm) recorded over a 20 minute

period. The assembly and disassembly buffer contained 0.1 M MES, 0.5 mM $MgCl_2$, 1 mM EGTA and 0.1 mM GTP. Glycerol (4 M) is present during all assembly steps except the final monitored assembly.

1, 2, and 3 were all ineffective in inhibiting tubulin assembly at concentrations below 1.25×10^{-4} M (highest concentration tested). At this same concentration, 9 completely inhibited the assembly of microtubules, while a concentration of the drug equal to 1×10^{-5} M had no inhibition of assembly at all. The ID_{50} was calculated to be about 3×10^{-5} M. The tubulin concentration used in this assay was 0.5 mg/ml which corresponds to a protein concentration of about 4.5×10^{-6} M.

REFERENCES

1. *This work was supported by grant CA 19243 from the National Cancer Institute.*
2. a. K. Y. Zee Cheng and C. C. Cheng, J. Heterocycl. Chem., 1973, 10, 85;
   b. W. M. Messmer, M. Tin-Wa, H. H. S. Fong, C. Bellvelle, N. R. Farnsworth, D. J. Abraham and J. Trojanek, J. Pharm. Sci., 1972, 61, 1858.
3. F. R. Stermitz, J. P. Gillespie, L. S. Amoros, R. Romero, T. A. Stermitz, K. A. Larson, S. Earl and J. E. Ogg, J. Med. Chem., 1975, 18, 708.
4. F. R. Stermitz, K. A. Larson and D. K. Kim, J. Med. Chem., 1973, 16, 939.
5. H. G. Cohen, K. D. Straub, C. Tiefenback, E. E. Seifen and F. R. Stermitz, Biochem. Pharmacol., 1978, in press.
6. For an alternate study on equilibrium (1), which included 1, 9 and 10, see: V. Simanek, V. Preininger, S. Hegerova and F. Santavy, Coll. Czech. Chem. Commun., 1972, 37, 2746. For a recent comprehensive review see: V. Simanek and V. Preininger, Heterocycles, 1977, 6, 475.
7. L. A. Mitscher, Y. H. Park, D. Clark, G. W. Clark, P. D. Hammesfahr, W.-N. Wu and J. L. Beal, Lloydia, 1978, 41, 145.
8. D. K. Kim and F. R. Stermitz, Phytochemistry, 1975, 14, 834.
9. We are indebted to Dr. J. Dorous for arranging for this material.
10. M. A. Caolo, *Ph.D. thesis, Colorado State University*, 1978.

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