Probing Biotransformation Relationships among Pyridoacridines by Focusing on Oxygenated Analogues

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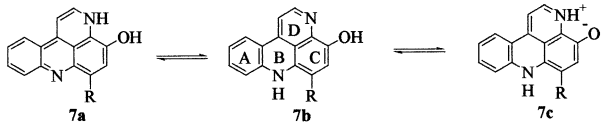
The presence of the MeO-5 in the structure 5-methoxyamphimidine (**5**) is unusual in the light of a recent analysis of the relationships among sponge- and tunicate-derived pyridoacridines. We explore a possible biochemical pathway, from neoamphimidine (**2**) to 5-methoxyamphimidine (**5**). The results of semiempirical and ab initio calculations are presented to help understand the relationships that favor the formation of **5** versus the formation of undiscovered bis-oxygenated pyridoacridines.

Marine-derived pyridoacridines, defined by the fusion of a pyridine D-ring to an acridine A/B/C-ring system, are a fascinating family comprised of more than 55 compounds isolated from sponges and tunicates.[1] The members of two of the five general types of pyridoacridines[2,3] often have a C-8 oxygen substituent as found in the tetracyclic alkaloid styelsamine D (**1**) isolated from tunicates and in the pentacyclic compound neoamphimidine (**2**) obtained from sponges. Curiously, no tetracyclic pyridoacridines possessing the ring system of **1** have been reported from sponges and no pentacyclics having the core of **2** have been isolated from tunicates.[4] Pyridoacridines are thought to arise in nature by a condensation involving tryptophan and dopamine building blocks,[5] which explains the presence of the C-8 oxygen atom in ring C. As an interesting corollary, a recently demonstrated biomimetic synthesis route to styelsamine B (**3**) involves the condensation of kynuramne and *N*-acetyl dopamine.[6] The oxygen functionality at C-8 serves to destabilize the aromatic pyridoacridine chromophore, increasing its reactivity with respect to further biological and chemical transformations. For example, **3** is readily oxidized to cystodytin J (**4**).[6] We recently reported 5-methoxyneoamphimidine (**5**),[3] which possesses both the C-8 and C-5 oxygen substituents, and at the conclusion of this study realized its closely related isomer **6** was unknown, though distantly related to petrosamine.[7] The recent analysis of the "pyridoacridine family tree" by Skyler and Heathcock[8] provides a comprehensive view of many potential biotransformations. On further examination we discovered that the possible steps linking dopamine-derived pyridoacridines to the bis-oxygenated compounds such as **5** or **6** were not discussed. Outlined at this time are the putative steps involved in their transformation from **2**.

It is important to note that styelsamine D (**1**), a substituted 8-hydroxypyrido[2,3,4-*kl*]acridine (**7**), can undergo tautomerization between structures **7a**−**7c** shown in Figure 1. All three structures, **7a**−**7c**, contain a perimeter that is fully conjugated but whose stability is predicted to vary considerably by our gas phase ab initio calculations (Table 1).[9] For structures **7a**−**7c**, the B-ring nitrogen atom is planar. Consistent with previous observations, the calculations predict that the nitrogen of the B-ring should be more basic than the D-ring nitrogen.[5,6] Solvation in water, simulated by the polarizable continuum model (PCM) calculation, yields results shown in Figure 1. These data predict that the energy gap between structures **7b** and **7c** will be substantially reduced in a polar solvent and that the energy difference between **7a** and **7c** is only slightly diminished. The oxidation of **3** to **4** is spontaneous when it is dissolved in DMSO, and this reaction could be facilitated

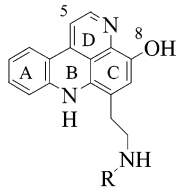


8-hydroxypyrido[2,3,4-*kl*]acridine, R = H
styelsamine A, R = CH_2-CH_2-NH_3

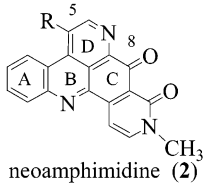
Figure 1. Relationships among 8-hydroxypyrido[2,3,4-*kl*]acridine (**7**) tautomers.

Table 1. Relative Energies of the Tautomers in Figure 1

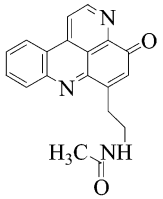
	relative energies (kcal/mol)		
	7a	**7b**	**7c**
AM-1	+11.3	0.0	+23.0
HF/3-21G*	+15.3	0.0	+25.7
B3LYP/6-31+G(d)	+13.3	0.0	+14.5
B3LYP/6-31+G(d)/pcm(water)	+ 9.8	0.0	+ 4.9



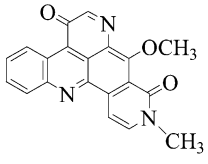
styelsamine D (**1**)
R = H
styelsamine B (**3**)
R = Ac



neoamphimidine (**2**)
R = H
5-methoxyneoamphimidine (**5**)
R = OCH_3



cystodytin J (**4**)



(**6**)

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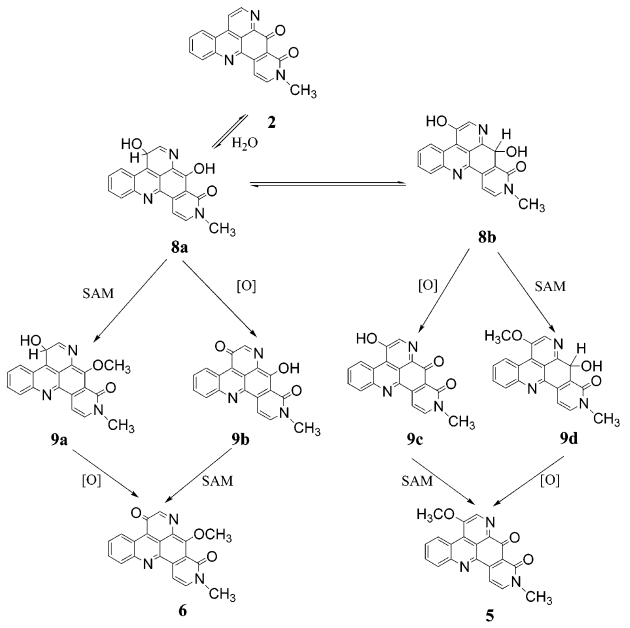

Figure 2. Putative biogenetic pathways between 5-oxygenated and 5,8 bis-oxygenated pyridoacridines.

by having tautomer **7a** (Figure 1) as a relay. Furthermore, it would appear that the stability from π-delocalization is greater for **4** versus **3**. Thus a neoamphimidine (**2**) type structure constitutes an initial low-energy point and provides a biosynthetic end point for dopamine-derived pyridoacridines obtained from sponges.

The multiple pathways shown in Figure 2 rationalize the addition of a second oxygen to the pyridoacridine core and are based on the enhanced reactivity expected at specific atoms in the B/C/D-rings of **2**. For example, a 1,6 Michael-type hydration on **2** could generate intermediate **8a**, which could then undergo three other forward reactions. Compound **8a** could equilibrate by a 1,5 hydride shift to generate **8b**. Our gas phase calculations shown in Figure 3 indicate that both **8′a** and **8′b** could contribute to the equilibrium population, and in solution structure **8′b** predominates. The trapping of the intermediate **8b** via methylation of the oxygen at C-5, leading to **9d**, is the key to our proposed pathway. While similar to reactions discussed by Skyler and Heathcock in their treatise,[6] our focus is on the intermediates of the reactions they propose. The oxidation products, **9b** or **9c** and the two OCH_3 derivatives, **9a** or **9d**, each result from different trapping alternatives for **8a** and **8b**. Both **9c** and **9d** are precursors to known compound **5**, whereas **9a** or **9b** would yield the unknown compound **6**.

The equilibrium relationships between these compounds need to be stated. Compounds **9b** and **9c** are tautomers. Compounds **9a** and **9d** differ in the placement of the methyl group; equilibration between these requires reversion back to **8a** and **8b**, a process we argue would be very slow. The gas phase and aqueous calculations of Table 1 indicate the relative stability order **9′b** > **9′c**, **9′d** > **9′a** and **5′** > **6′**. On the basis of the isolation of **5** and not **6** we predict that methylation of **8b** occurs prior to oxidation. Were this not the case, that is, if **8a** or **8b** undergoes oxidation, we would predict that **9b** and **9c**, the products of oxidation, would equilibrate, the equilibrium favoring **9b**, leading to the formation of **6** over **5**. Equilibration between **5** and **6** would not be direct but could occur by the very intermediates discussed above.

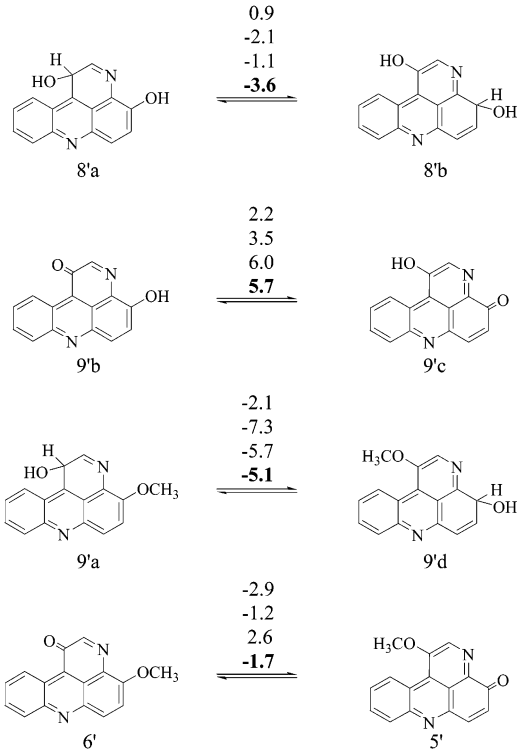

Figure 3. Semiempirical and ab initio calculated energy differences among tetracyclic pyridoacridines. Results in kcal/mol: (top) AM-1; (second) HF/3-21G*; (third) B3LYP/6-31+G(d)//3-21G*; (bottom) B3LYP/6-31+G(d)//3-21G*//PCM, water.

In the future we hope to apply the relationships outlined in Figure 2[10] that imply the existence of **6** alongside **5**, by discovering additional sponge- and/or microorganism-derived oxygenated pyridoacridine analogues. Our continuing goal is to explore the bioactivity properties of such congeners because we found **5** was murine solid tumor selective.[5] We believe that the observations presented above, when placed alongside those recently published,[6] represent a powerful tool for the design of isolation, semisynthesis, and biosynthetic experiments to explore additional dimensions of marine pyridoacridines

Experimental Section

General Experimental Procedures. All calculations were performed in Cartesian coordinates using GAUSSIAN98.[11] Standard basis sets 3-21G* and 6-31+G(d) were used. The polarizable continuum model is that of Tomasi,[12] the default for rev.A.7 of GAUSSIAN98, with the flag solvent = water. Zero-point energies were scaled separately from the vibrational partition function, giving an overall scaled zero-point and thermal energy correction for each calculation.[13]

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Supporting Information Available: Input coordinates, computed energies of each species, in Hartrees, and zero-point corrections are available free of charge via the Internet at http://pubs.acs.org.

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(10) An alternative to the oxidation pathway proposed here is the action of a single enzyme. The "bay region" of polyaromatic hydrocarbons (PAH) such as benzo[*a*]pyrene undergoes epoxidization or hydroxylation by the action of a liver cytochrome p450. By analogy the bay region, position 5, of pyridoacridines may be subject to a similar cytochrome p450. See: (a) Stansbury, K. H., Flesher, J. W.; Gupta, R. C. *Chem. Res. Toxicol.* **1994**, *7*, 254−259. (b) Seidel, A.; Friedberg, T.; Lollmann, B.; Schwierzok, A.; Funk, M.; Frank, H.; Holler, R.; Oesch, F.; Glatt, H. *Carcinogenesis* **1998**, *19*, 1975−1981.
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