THEORETICAL CALCULATION-BASED REPRODUCTION OF THERMODYNAMIC, KINETIC BEHAVIORS AND CYTOTOXICITY OF AZASTEGANES

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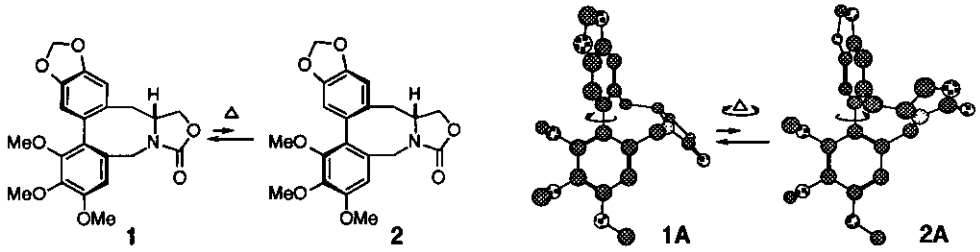
Abstract-Theoretical calculation of azasteganes indicates that the thermodynamic control is operative in the isomerization of the pivotal biphenyl bond of **1** and **2** and kinetic control in epimerization at the sp^3 center of **3** and **4**. The calculated structures qualitatively reproduce the structure-cytotoxicity relationships of azasteganes.

It is well recognized that a computer aided chemistry provides a good guidance for understanding and design of chemical behavior of real and imaginative organic compounds in a variety aspect of organic chemistry. The most useful point by such calculations as molecular mechanics (MM), empirical and *ab initio* methods is the stereostructure of the definite energy. We describe a theoretical calculation-based qualitative reproduction of intriguing thermodynamic and kinetic behaviors-cytotoxicity relationships of azasteganes.



Heating **1** or **2** over the melting point 180-205 °C for 3 h under argon establishes the thermodynamic equilibrium between **1** and **2** in the constant ratio of 40:1 in favor of **1**.[1] Since the equilibrium arises from rotation of the pivotal biphenyl bond, the relative energy difference between **1** and **2** determines the equilibrium ratio. Computation of the total energy of these compounds was carried out by MM and MOPAC of Cache Version 3.6 on Macintosh 840AV served by IBM RS6000 workstation. Some MM parameters were modified to be consistent with the normal pivotal bond length 1.47Å that was obtained from MOPAC PM3 precise mode calculation. Other parameters are used from Cache augmented MM parameters.

The MM calculation of the total energy leads to the energy difference between **1A** and **2A**[2] by 2.9 kcal in

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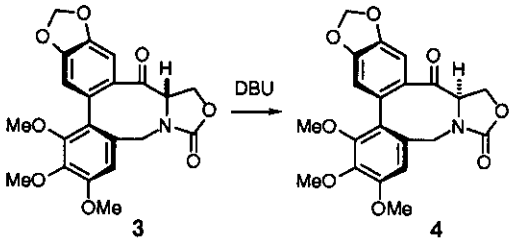
We dedicate this paper to the Memory of the late Professor Yoshio Ban.

favor of **1**, qualitatively reproducing the experimentally observed thermodynamic population. The MOPAC PM3 calculation provided also the same tendency in favor of **1** by 4.1 kcal. The ^{1}H-nmr coupling constants at the benzylic protons of **1** and **2** were reproduced from the calculated structures (**1A**) and (**2A**).[1] Since **1** and **2** have the boat-chair and boat-boat eight-membered ring conformation, respectively, the difference in the stability is reasonably understandable.

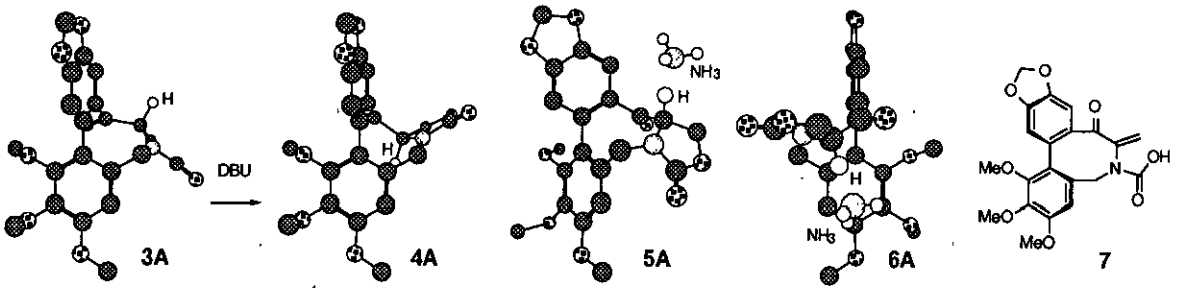
The computation reproduced an unanticipated isomerization of the oxo-analogue (**3**) into **4**. The oxo-compound (**3**) having the same relative configuration as **1** was isomerized in 89% isolated yield upon treatment with DBU at room temperature for 10 min into **4** having the relatively less stable framework **2**.[1] The MM energy of **4** is larger than that of **3** by 1.2 kcal. The MOPAC PM3 calculation also provided the stability of **3** by 1.8 kcal more than **4**. However, the structures (**3A**) and (**4A**) obtained by both computational methods agree well with spectral data of **3** and **4**. The dihedral angles between C=O and the methylenedioxyphenyl ring were 89° and 58°, respectively, for **3A** and **4A**. In fact, the ir absorption maximum of C=O for **3** was 1722 cm^{-1}, indicating almost no conjugation, and 1676 cm^{-1} for **4** indicating existence of conjugation.

These calculations suggest us that the easy isomerization of **3** into **4** is governed by kinetic factors rather than the thermodynamic stability. Inspection of the calculated structure of **3A** reveals much more easier access to the proton that will be deprotonated by the base. On the contrary, the corresponding proton of **4A** suffers severe steric shielding by the trimethoxyphenyl ring, not allowing approach of bulky DBU.



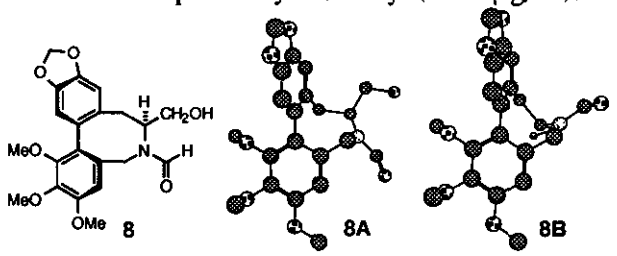
Indeed, calculation of **5A** and **6A**, representing the imaginary structures approaching ammonia, instead of DBU, to the proton of **3** and **4**, provided the energy difference by 3.4 kcal (1.6 kcal by PM3) in favor of **5A**. The enol formed from **5A** tautomerizes back to a mixture of **3** and **4**, and is reproduced from **3**.

It is also noteworthy that the another possible intermediate **7** in the isomerization may be omitted, since **7** would result in the corresponding decarboxylated amine.

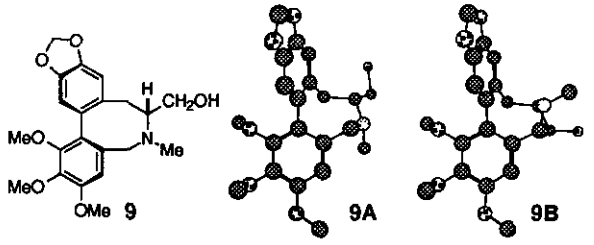


The structures calculated by the MM reproduce the structure-cytotoxicity relationships. Previously we have proposed and confirmed the cytotoxic stereostructures of (-)-isopicrostegane and (-)-azastegane (**1**)[1] that are characterized by a boat-chair eight-membered ring conformation, *R* configuration around the pivotal bond, and the carbonyl group placing near the trimethoxyphenyl group.[3] According to the guidelines, **1** and **2** are

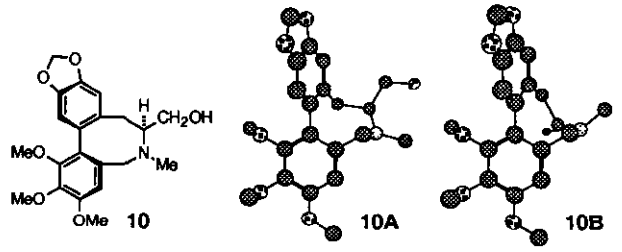
active and inactive, respectively.[1] However, the inactive structure (2) (45 μg/ml growth inhibition of KB cell) was reduced to a *N*-formyl derivative (8) that recovered potent cytotoxicity (<0.3 μg/ml). The calculation-based structures of 8 are **8A** and **8B**. *The less stable structure (8B) has the* boat-boat conformation similar to the parent (2) that does not exert cytotoxicity. The more stable structure (**8A**) by a factor of 3.2 kcal than **8B** has a boat-chair conformation and carbonyl orientation rather similar to the potent cytotoxic compound (**1**) (<0.3 μg/ml). It is apparent from the predominant structure (**8A**) that the reduced **8** exerts potent cytotoxicity.



Reduced *N*-methyl analogues, **9** from active **1** and **10** from inactive **2**, exert the same level cytotoxicity, 1.5 and 1.1 μg/ml, respectively. These are also reasonably reproduced by calculation. The preferred conformation of **9** is **9A** rather than **9B** by a factor of 2.6 kcal.



The boat-boat conformation of **10B** is less stable than the boat-chair conformation (**10A**) by 1.6 kcal, clearly indicating the predominant conformation of **10** to be **10A**. Both preferred conformations of **9** and **10** are quite similar and easy to understand the same level cytotoxicity by contrast to the parent compounds active **1** and inactive **2**.



In conclusion, the thermodynamic as well as kinetic behaviors of azastegane analogues are reasonably reproduced by theoretical calculations. The stereostructures calculated by MM and MOPAC also well provide the structure-cytotoxicity relationships of azasteganes. Further studies toward logical design of antitumor molecular structures are in progress in our laboratories.[4]

REFERENCES AND NOTES

1. Y. Kubota, H. Kawasaki, K. Tomioka, and K. Koga, *Tetrahedron*, **1993**, *49*, 3081, and references cited therein.
2. Stereostructure was presented by Chem3D drawing transferred from the Cache structure. Protons were omitted for clarification except for **3A-6A**.
3. K. Tomioka, T. Ishiguro, H. Mizuguchi, N. Komeshima, K. Koga, S. Tsukagoshi, T. Tsuruo, T. Tashiro, S. Tanida, and T. Kishi, *J. Med. Chem.*, **1991**, *34*, 54.
4. We are grateful to The Osaka University, ISIR/WRI, Joint Research Program "Advanced Materials Creation and Their Limit State Prediction for Environment Preservation" for partial support of the work.

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