CONFORMATION OF TROPOLONE RING IN ANTILEUKEMIC TROPOLOISOQUINOLINE ALKALOIDS

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Conformational analysis of antileukemic tropoloisoquinoline alkaloids isolated from *Cissampelos pareira* was conducted by thermodynamic proton nuclear magnetic resonance (^{1}H NMR) studies. The line-broadening of one of methoxy methyl signals can be explained by the tropolone ring-puckering process. Analysis by dynamical simulated annealing and modified neglect of differential overlap (MNDO) calculations also supported puckering of tropolone ring system.

KEYWORDS conformation; puckering; tropolone; tropoloisoquinoline alkaloid; NMR; simulated annealing

Novel antileukemic tropoloisoquinoline[1] and azafluoranthene[2] alkaloids have been isolated from *Cissampelos pareira* (Menispermaceae), and their structures and antileukemic activities have already been reported. In previous paper,[1] solid state conformations of novel tropoloisoquinoline alkaloids, pareirubrines A and B, were analyzed by X-ray crystallographic method, and it was shown that the molecules including both isoquinoline and tropolone rings were almost completely planar. This solid state conformation was identical with those possessing a tropolone ring system, such as imerubrine.[3]

In ^{1}H NMR spectrum, the signal of one methoxyl group substituted at C-11 of isoimerubrine was broadened more than the other methoxyl group substituted at the isoquinoline skeleton. However, the signal of one methoxyl group substituted at C-12 of pareirubrine A was sharp, similar to the other methoxyl groups. The results obtained by thermodynamic ^{1}H-NMR study and simulated annealing are used to determine the conformational behavior of the tropoloisoquinoline alkaloids in solution. In this paper, conformational analysis of tropolone ring in tropoloisoquinoline alkaloids by use of ^{1}H NMR studies and energy calculations including simulated annealing using molecular dynamics (MD) and MNDO calculations are described.

As can be seen from Figs. 1 and 2, which show the structures of pareirubrine A (**1**) and isoimerubrine (**2**), and their ^{1}H NMR spectra, four methoxy methyl signals in **1** were observed as a sharp singlet at 300K. However, in **2** one of them was shown to be a slightly broad singlet. When temperature was decreased to 260K at intervals of 10 degrees, the broad methoxy methyl signal of **2**, which was attached at C-11 of the tropolone ring, was further broadened. However, the shape of the corresponding methoxy methyl signal attached at C-12 of **1** did not change at even 260K. Pareirubrine A (**1**) exists as tautomeric mixture (Fig. **1a** and **b**) in solution. The cause of this broadening signal cannot be regarded as only the attached position of methoxyl group and/or the above tautomerism.

Conformational analysis of the tropolone ring of **1** and **2** was conducted by the computational chemical method.[4] The possibility of using molecular dynamics techniques as a tool for simulated annealing is tested in the case of the molecule of tropoloisoquinoline alkaloids. At the beginning of the 1980s, Kirkpatrick et al. suggested a simulated annealing

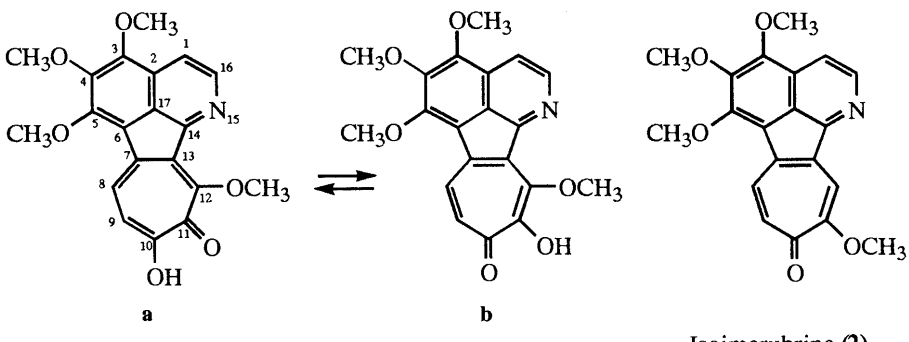
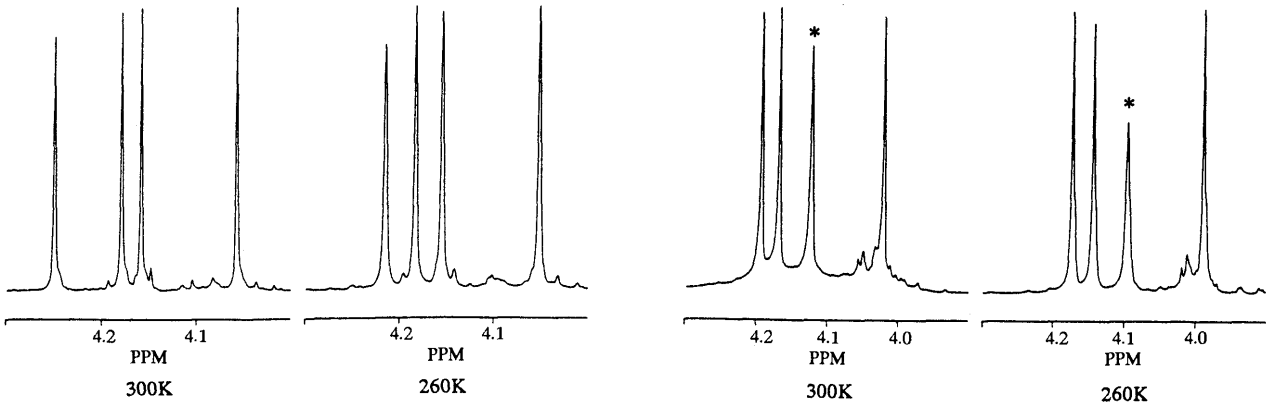


Fig. 1. Molecular Structures of Pareirubrine A (**1**) and Isoimerubrine (**2**)
Compound **1** exists in two states (**a** and **b**) in solution.



Pareirubrine A (**1**) Isoimerubrine (**2**)

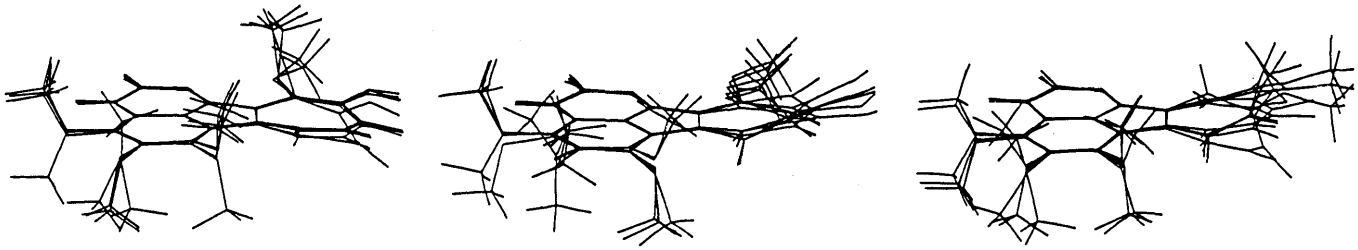
Fig. 2. Expansion of Four Methoxyl Groups in ^{1}H-NMR Spectra of Pareirubrine A (**1**) and Isoimerubrine (**2**) at 300 and 260K Asterisks show the broad signals.

algorithm using a Monte Carlo technique.[5] The method, applied to a broad class of problems, has also shown its practical utility in the case of conformational problems.[6] Simulated annealing is a method by which molecular dynamics is used to sample many low-energy conformations. The basic premise of the technique is to hold a molecule at a specified "plateau" temperature for some time, then to anneal the sample decreasing a ramped temperature to a specified minimum. Finally, the number of cycles is provided with a configuration from the minimum temperature.

The starting geometry of pareirubrine A (**1a**) for the simulation was chosen as the crystal structure reported in the previous paper, and that of **1b** and isoimerubrine (**2**) was modeled by modification of the structure of **1a**. A simulation was performed using a time step of 1 fs, and the structures were sampled every 90 fs. Each system was equilibrated for 5400 fs with a thermal bath at 500K and thereafter successively for 900 fs with a thermal bath 10 K lower in temperature until a final temperature of 10 K was obtained. Ten cycles are performed, giving a total simulation time of 63 ps, and each frozen conformation as sampled from the minimum temperature at 10K. The resulting geometry was minimized by use of semiempirical MNDO calculation which neglects diatomic-differential overlap approximation until the maximum derivative was <0.001 kcal/Å. The minimized structures were ranked in order of increasing energy and each five low energy conformation that match each isoquinoline skeleton was depicted in Fig. 3.

Simulated annealing supports the conclusion that the molecule is interconverting between two rapidly nonplanar molecular conformations of near envelope type with C-10 or C-11 carbons out of the mean plane of the remaining isoquinoline atoms. As can be seen from Fig. 3, the puckering amplitude of **2** is larger than those of **1a** and **b**.

It is well known that tropolone undergoes a tautomeric hydrogen shift reaction, as shown in Fig. 1.[7] In solution, ^{1}H and ^{13}C NMR spectra always exhibit averaged signals due to the two tautomeric forms. On the other hand, the structural change due to ring puckering is not well studied. The interpretation of the peak broadening shows that the tropolone ring skeleton of tropoloisoquinoline is not rigidly planar due to ring puckering vibrations. The nonequivalence that might be caused by anisotropic effect of tropolone ring and ketone at C-10 of **2** makes the methoxy methyl signal at C-11 broaden slightly. The methoxyl signals at the tropolone ring of colchicine,[9] which has been isolated from *Colchicum* genus and shows specific strong binding to tubulin, was also shown to broaden in $CDCl_3$. This can also be explained by the tropolone ring-puckering process.



a b

Pareirubrine A (1) Isoimerubrine (2)

Fig. 3. Five Low-Energy Conformer of Pareirubrine A (**1a** and **b**) and Isoimerubrine (**2**) Superimposed for Best Fit on Isoquinoline Heavy Atoms

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