Configurational and Conformational Analysis of Macrocarpals H, I, and J from *Eucalyptus globulus*

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Configurational and conformational analysis of unique phloroglucinol-sesquiterpene coupled compounds, macrocarpals H, I, and J (1—3), showing potent antibacterial activity and inhibitory effect of glucosyltransferase, was made by nuclear Overhauser enhancement (NOE) experiments and computational chemical methods. The configuration at C-9′ of the side chains of 1—3 was elucidated as 1: *S*, 2: *R*, and 3: *S*, by the structures obtained by the Monte Carlo (MC) calculation using MM2* force field implemented in MacroModel/Batchmin, followed by semiempirical molecular orbital calculations, whose stable conformation of macrocarpals H, I, and J was well satisfied by the NOEs observed around H-9′.

Key words macrocarpal; configuration; conformation; Monte Carlo simulation; nuclear Overhauser effect; *Eucalyptus globulus*

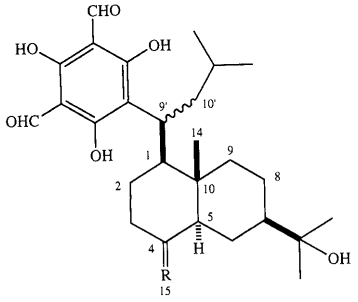
Three new phloroglucinol-sesquiterpene coupled compounds, namely macrocarpals H, I, and J (**1**—**3**) were isolated from the leaves of *Eucalyptus globulus* (Myritaceae) in a previous study,[1] and their chemical structures were determined using the two dimensional (2D)-NMR technique (Fig. 1). These compounds have potent antibacterial activity against cariogenic and periodontopathic bacteria, and have an inhibitory effect on glucosyltransferase. The remaining problem on the chemical structures for us is elucidation of the unknown configuration at C-9′ in isopentyl moiety of **1**—**3**. In the present study, configurational and conformational studies of macrocarpals H, I, and J were made using nuclear Overhauser effect (NOE) experiments and computational calculations including the Monte Carlo (MC),[2] followed by semiempirical molecular orbital calculation method, and the C-9′ configurations and solution form of these compounds was determined.

Results and Discussion

MC Simulation Macrocarpals I and J (**2** and **3**) were configurational isomers with each other at C-9′ in isopentyl moiety. Initially, we investigated this alternative configuration at C-9′ of **2** and **3** by a computational chemical method. Exhaustive conformational search of **2** and **3** was conducted using systematic pseudo MC search for two possible isomers (*R* and *S* isomers at C-9′) of these compounds, followed by molecular mechanics calculations with MM2* force field implemented in MacroModel/Batchmin.[3] A total of 10000 MC steps were performed using different starting geometries given by manual modeling to confirm the reproducibility of calculation results. After the MC conformational search, each of the resulting conformations was subjected to energy-minimization calculation using the MM2* force field, and ranked in order of increasing energy of these conformations. For the compound possessing C-9′*R* configuration, 664 conformers were obtained and the 4 and 12 conformers were found within a range of 1 and 3 kcal/mol of the lowest energy conformation, respectively. On the other hand, 652 conformers were obtained for the C-9′*S* configurational compound and the 4 and 14 conformers were found within a range of 1 and 3 kcal/mol of the lowest one, respectively. For the purpose of precise comparison with energetic stability between these conformers, the lowest 4 energy conformations involved in C-9′*R* and C-9′*S* forms of macrocarpal I and J were further performed by the semiempirical molecular orbital calculation using the PM3 method,[4] respectively. The heat of formation in each 4 conformer with C-9′*R* and C-9′*S* (C-9′*R* derivative −345.686[1.295] kcal/mol; C-9′*S* derivative −344.189[0.355] kcal/mol) and their resulting 4 conformations with each configuration were almost the same [Fig. 2, root mean square deviations (RMSDs)] for carbon atoms: C-9′*R*, 0.98 Å; C-9′*S*, 0.91 Å).

In the side chains of the two conformers, both H-1 and H-9′ take a *trans* conformation with each other, whereas for C-9′–C-10′ rotamer, the conformer with C-9′*R* configuration takes *gauche*$^+$, and that with C-9′*S* *gauche*$^-$.

NOE Relationship of Macrocarpals H, I, and J As shown in a previous paper, ^{13}C chemical shifts of the side chain at C-1 indicated that the configurations at C-9′ of **1** (*δ* 113.8 [6′], 30.3 [9′], 39.4 [10′], 26.9 [11′], 22.3 [12′], 24.9 [13′]) and **3** (*δ* 113.7 [6′], 29.8 [9′], 39.6 [10′], 27.0 [11′], 22.3 [12′], 25.0 [13′]) are the same and different from that of **2** (*δ* 108.9 [6′], 31.6 [9′], 44.5 [10′], 27.4



1: R = CH_2, 9′*S*
2: R = α-OH, β-Me, 9′*R*
3: R = α-OH, β-Me, 9′*S*

Fig. 1. Structures of Macrocarpals H (**1**), I (**2**), and J (**3**)

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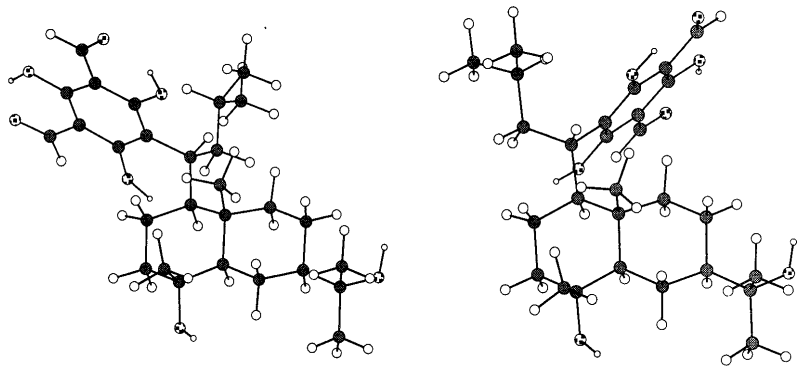
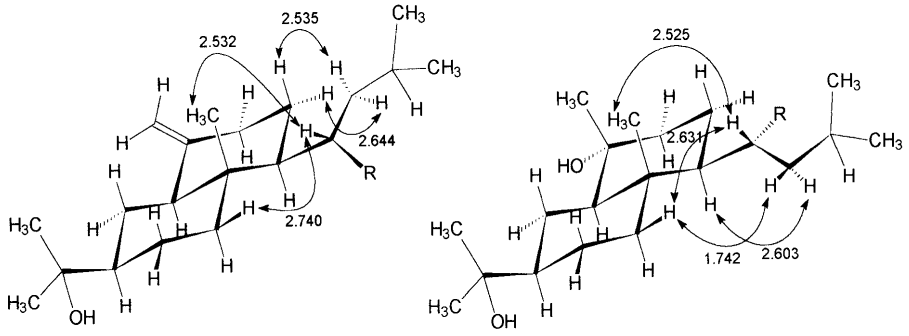


2 3

Fig. 2. Perspective Views of the Lowest Energy Conformer of Each of **2** and **3** by MC/MO Calculations



1 2

Fig. 3. Some Characteristic NOE Correlations around H-9′ of **1** and **2**

Numbers show the distances (Å) of the lowest energy conformer of each by MO calculation. NOE correlations of **3** were also observed like those of **1** (R = phloroglucinol moiety).

[11′], 21.9 [12′], 24.5 [13′]).

NMR spectra were measured in pyridine-d_5 and the solution conformations of **1**—**3** were deduced on the basis of data from NOE correlations in phase sensitive NOE spectroscopy (NOESY) spectra.[5] The NOE correlations around the side chains at C-1 of **1**—**3** and the corresponding distances obtained from the lowest energy conformations of **2** and **3** are depicted in Fig. 2. The NOE correlations of macrocarpal I (**2**) are well satisfied by the conformation possessing C-9′*R* configuration obtained by MC/MO calculations as follows. The NOE enhancements were observed among H-9′ on the side chain, Me-14, and H-9β in **2**. In addition, NOE enhancements were seen between H-10′a (Pro-*R*) and H-9β, and between H-10′b (Pro-*S*) and H-1, suggesting that C-9′ in **2** adopts *R*-configuration. No NOE between H-1 and H-9′ indicates that both protons take *trans* conformation, being identical with that obtained by calculation.

Different NOE correlations were observed in macrocarpals H and J (**1** and **3**) as follows: NOE correlations between H-10′a (Pro-*S*) and an axial proton of H-2, and between H-10′b (Pro-*R*) and an equatorial proton of H-2 were observed. As in **1**, NOE correlations among H-9′, H-9β, and Me-14 at C-10 were also observed. These correlations were identical with the conformation with C-9′*S* configuration obtained by calculation.

The NOE experiments of macrocarpals H (**1**), I (**2**), and J (**3**) showed the correlations which can be explained for **2** by C-9′*R*, and **1** and **3** by C-9′*S* configurations.

Experimental

Materials Macrocarpals H, I, and J were isolated from the leaves of *Eucalyptus globulus* according to the method described previously.[1]

NMR ^{1}H and ^{13}C-NMR spectra were recorded on Varian Unity 400 spectrometers. Each 10 mg of macrocarpals H, I, and J in a 5 mm tube (0.5 ml pyridine-d_5, degassed) was used for the homonuclear measurements. The spectra were recorded at 300 K. A phase sensitive NOESY experiment was made with a mixing time of 600 ms.

Computational Methods Computer modeling experiments were carried out using the MacroModel program (version 4.5) on an IRIS 4D computer (Indigo^2 R4400). Molecular mechanics and MC calculations were performed with the MM2* force field with a distance-dependent dielectric, $\varepsilon = R_{ij}$. The extended cut off distances employed were 8 Å for van der Waals, 20 Å for charge/electrostatics and 10 Å for charge/multiple electrostatics. The obtained structures were energy minimized by the use of the derivative convergence criteria at a value of 0.001 kJ/Å-mol.

Each MC search was carried out using the pseudo MC routine in MacroModel. The closure bond was chosen at two bonds in rings A and B with a closure limit of 1—4 Å. A total of 664 structures for C-9′*R* isomer and 652 structures for C-9′*S* isomer were obtained after minimization using molecular mechanics calculation of MM2* force field. In each isomer, 4 conformers were found within a range of 1 kcal/mol of global minima, followed by the semiempirical molecular orbital calculation using the PM3 method.

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