The Epimerization of Sesamin and Asarinin

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Sesamin (**1**) and asarinin (**2**) are two C-7′ epimeric lignans. Molecular modeling by semiempirical methods indicated that **1** is more stable than **2** by about 2.5 kcal/mol. However, epimerization under acidic conditions led to a 44.8/55.2 equilibrium ratio of **1** and **2**. Single-crystal X-ray diffraction analyses indicated that **1** was monoclinic with $a = 10.0435(19)$ Å, $b = 6.9151(8)$ Å, $c = 11.8460(13)$ Å, and **2** was triclinic with $a = 5.595(5)$ Å, $b = 9.5910(18)$ Å, $c = 15.620(4)$ Å. The unexpected equilibrium ratio of **1** and **2** indicated that structural changes are dependent on the conditions of the extraction processes.

Furofuran lignans comprise a large group of natural products characterized by coupling of two phenylpropane (C_6-C_3) units. They are among the largest subclass of lignans with a wide range of biological activities such as antitumor, antimitotic, antimicrobial, and antioxidative.[1] The majority of furofuran lignans have 2,6-diaryl substituents on the *exo* face of the bicyclic skeleton (e.g., sesamin **1**), although those with *endo*, *exo* aryl substitution (e.g., asarinin **2**) and others with *endo*, *endo* substitution are also known.[2] Sesamin (**1**), which can be extracted exclusively from sesame in large quantities, is intriguing due to its highly efficient antioxidant activity.[3] It was also reported that sesamin is a specific inhibitor of Δ^5-desaturase, which catalyzes the conversion of dihomo-γ-linolenic acid to arachidonic acid, in both microorganisms and animals,[4] and sesamin also exerts hypocholesterolemic activity through the inhibition of cholesterol absorption and synthesis.[5] The other report indicated that sesamin prevented liver damage caused by alcohol or carbon tetrachloride and showed a suppressive effect against 7,12-dimethylbenz[*a*]anthracene-induced rat mammary carcinogenesis and antihypertensive effects.[6] Asarinin has also been shown to have several significant biological activities, including antitumor promotion, antiallergic activity, and enhancement of the toxicity of certain insecticides.[7,8]

The radix of *Asarum heterotropoides* Fr. var. *mandshuricum* Kitag. (Xixin) is a traditional herbal medicine listed officially in the Chinese Pharmacopoeia and used as an analgesic, antitussive agent, and expectorant for treatment of influenza, headache, rheumatic pain, and asthma. It is also used as a drug for invigorating blood circulation and eliminating blood stasis.[9] Furofuran lignans have been reported to be widely contained in this plant.[10] In our processes of the isolation of sesamin and asarinin previously, different amounts of **1** and **2** were obtained due to different extraction methods, e.g., soaking at room temperature or vacuum distillation. Several reports also indicated that sesamin epimerized to asarinin during acid-catalyzed conditions. Given the varied biological activities displayed by these two furofuran lignans and their interesting conformational behavior, a more in-depth study on the epimerization process was undertaken.

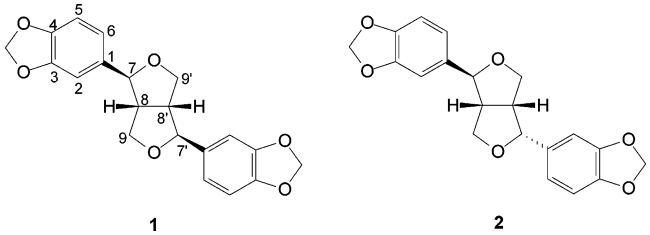

Figure 1. Structures of sesamin (**1**) and asarinin (**2**).

Results and Discussion

Both sesamin (**1**) and asarinin (**2**) have a furofuran backbone, which is similar to those of norbornane and bicyclo[3.3.0]octane. It is well-known that the *exo* isomer is more stable than the *endo* isomer. Sesamin has two substituents in *exo* positions, and asarinin has one substituent in *exo* and the other in *endo* position (Figure 1). Therefore, the conformation of sesamin is supposed to be more stable than that of asarinin. However, in normal isolation processes from *A. heterotropoides* Fr. var. *mandshuricum* Kitag., the relative ratio of the two depends on the methods of extraction. The quantity of **2** was close to that of **1** when soaked in acetone at room temperature. However, when they were isolated by vacuum distillation (10 mmHg, 140 °C, 15 min) or by steam distillation (110 °C, 3 h), the quantity of **2** was 2-fold that of **1**. These results were quite unexpected, and it was suggested that the variations occurred due to acidic conditions, when the plant was extracted at high temperature. A controlled experiment was therefore executed starting from pure samples of **1** and **2**. An amount of pure **1** or **2** was refluxed in acidic MeOH for several hours, while the reaction was monitored periodically to determine the ratio of **1** and **2**. High-performance liquid chromatography (HPLC) was utilized for structure identification using a Cosmosil $5C_{18}$-Ms (5 μm, 4.6 mm × 250 mm) column with MeOH/H_2O (65:35) as the mobile phase. The signals were detected by UV at a wavelength of 240 nm. The results are shown in Figure 2. In these experiments, both pure sesamin and asarinin gave the same ratio of 44.8/55.2 of **1** versus **2**. It appeared that asarinin was a slightly more favored product in these acid-catalyzed epimerization reactions. It was therefore of our interest to examine the crystal structures in order to confirm their molecular geometry.

Crystal structures of the two isomers are shown in Figures 3 and 4. The furofuran structure of sesamin was

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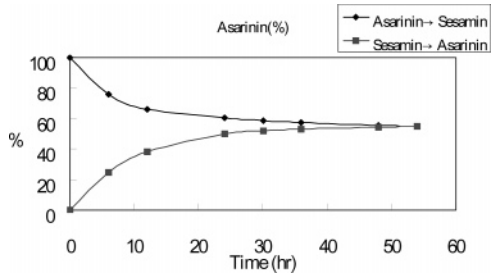

Figure 2. Acid-catalyzed epimerization of sesamin/asarinin.

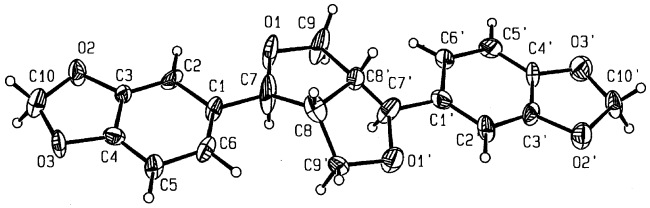

Figure 3. Diagram of sesamin (**1**) by X-ray. Principal ellipses are shown at the 50% probability level; H atoms, as small spheres of arbitrary size.

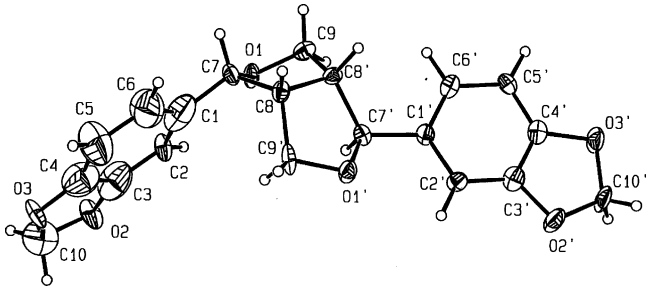

Figure 4. Diagram of asarinin (**2**) by X-ray. Principal ellipses are shown at the 50% probability level; H atoms, as small spheres of arbitrary size.

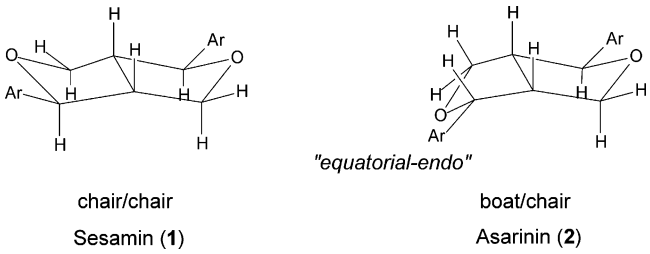

Figure 5. Likely conformations of **1** and **2**.

more twisted than that of asarinin, as both angles C7−C8−C9′ (125.3°) and C9−C8′−C7′ (128.2°) in sesamin are larger than those (114.9°, 113.6°) in asarinin (see Supporting Information) to reduce proton interactions at C7 and C7′. Two phenyl substituents at C7 and C7′ of sesamin are almost in the same plane as its central furofuran structure with torsional angles C6−C1−C7−C8 = 18.7° and C6′−C1′−C7′−C8′ = 43.9°. However, torsional angles C6−C1−C7−C8 and C6′−C1′−C7′−C8′ in asarinin are 72.8° and 30.1°, respectively. In asarinin one of the phenyl substituents is almost perpendicular to its central furofuran structure and the substituent is located at the "*endo*" position. The stability of asarinin can be explained by the boat/chair conformation of its furofuran rings (Figure 5). In this form the phenyl group occupies the *equatorial-endo* position and steric hindrance is substantially reduced.

The heats of formation (HF) of the two isomers were calculated by semiempirical methods. The HFs of **1** and **2** were estimated by AM1 with full geometry optimization to be −151.96 and −149.64 kcal/mol, respectively. The potential energy of **1** is lower than that of **2** by 2.32 kcal/mol. A similar result can be obtained by the PM3 method, where **1** is more stable than **2** by 2.74 kcal/mol. For epimerization to occur, a process of protonation followed by ring-opening must be involved. These processes depend significantly on the polarity of solvents. The thermodynamic equilibrium between **1** and **2** also depends on the hydrogen-bonding nature of the solvents, as both structures are highly oxygenated. These factors, which were not considered thoroughly by the simple empirical modeling, may override the small HF difference.

In summary, we have presented evidence that either asarinin or sesamin can epimerize under acidic conditions. The crystal structures of both isomers were confirmed by X-ray crystal diffraction analyses. Their heats of formation were estimated by semiempirical methods, which showed that **1** is more stable than **2** by about 2.5 kcal/mol. The experimental equilibrium ratio of 44.8/55.2 between sesamin and asarinin was unexpected. This indicated that these compounds can epimerize in an unexpected way during the extraction processes. Indeed, previous studies revealed that in the commercial preparation of sesamin epimerization occurred during acid-clay bleaching in an oil refining process and a mixture of sesamin and asarinin in almost equal amounts was formed.[11] As a result, hepatic fatty acid oxidation, mainly ascribed to asarinin, increased. Thus, the decoction process of herbal medicines should be carried out more carefully, especially for the complex traditional Chinese medicines.

Experimental Section

Materials. *Asarum heterotropoides* var. *mamdshuricum* was obtained from the market in Taiwan, and its origin was verified by Prof. C. S. Kuoh. A voucher specimen was deposited in the herbarium of Cheng Kung University, Tainan, Taiwan. The standard sesamin (**1**) and asarinin (**2**) were obtained from *A. heterotropoides*. var. *mamdshuricum*, and the purities were determined by HLPC analysis. All reagents were analytical grade. MeOH and HCl were obtained from E. Merck (Germany).

Epimerization Study. A sample (20 mg of sesamin **1** or asarinin **2**) was dissolved in 50 mL of 10% (w/w) HCl/EtOH. The solution was stirred under reflux (about 85 °C). The reaction was monitored by HPLC until equilibrium was reached. After every hour 1 mL of the reaction solution was diluted to 10 mL with MeOH and injected into HPLC for quantitative analysis. The HPLC was equipped with a Hitachi L-6200 pump, a Hitachi L-4000 UV detector, and a Cosmosil $5C_{18}$-MS (5 μm, 4.6 mm × 250 mm) column. The mobile phase comprised a mixture of MeOH/H_2O (65:35). The flow rate was 0.8 mL/min, and the detecting wavelength was 240 nm. The reactions reached equilibrium at 1.5 h, where the mixture contained 44.8% of **1** and 55.2% of **2**.

Computation. Molecular modeling was performed by semiempirical methods AM1 and PM3 with full geometry optimization. These methods were implanted in the Spartan '04 1.0.1 software (Wavefunction Inc.) and were executed on a personal computer.

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Supporting Information Available: X-ray crystallographic data of compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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