Biotransformation of Hinesol Isolated from the Crude Drug *Atractylodes lancea* by *Aspergillus niger* and *Aspergillus cellulosae*

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Biotransformation of the sesquiterpene alcohol hinesol (1) with spasmolytic activity, which was prepared from the rhizome of *Atractylodes lancea*, was carried out by *Aspergillus niger* and *Aspergillus cellulosae* IFO 4040. Compound 1 was easily converted to compounds 2—9 by *A. niger*, and compounds 10 and 11 by *A. cellulosae*, respectively. Their stereostructures were established by a combination of high-resolution NMR spectral analysis, X-ray crystallographic analysis, and chemical reactions such as epoxydation.

Key words *Atractylodes lancea*; hinesol; biotransformation; *Aspergillus niger*; *Aspergillus cellulosae* IFO 4040

We are continuing to study the biotransformation of secondary plant metabolites by microorganisms[1] and mammals[2] from the pharmacological point of view. Previously, we reported the biotransformation of three germacrane-type sesquiterpenoids[3] from the crude drug *Curcuma aromatica*, and 6-gingerol and 6-shogaol[4] from the crude drug *Zingiber officinale* belonging to the Zingiberaceae by the fungus *Aspergillus* niger. In continuation of the biotransformation of the chemical constituents isolated from crude drugs into biologically active compounds, the biotransformation of hinesol (**1**), which has spasmolytic activity,[5] from *Atractylodes lancea* was examined by *A. niger* and *A. cellulosae*. This paper deals with the structure elucidation of 10 metabolites (**2**—**11**) of **1** biotransformed by two fungi.

A. niger was inoculated and cultivated under rotation (100 rpm) in Czapek-peptone medium[6] at 30 °C, pH 7.0, for 2 days. (−)-Hinesol (**1**) (150 mg/200 ml) was added to the medium and further cultivated for 10 days. The crude metabolites obtained from the culture broth by ether extraction were chromatographed on silica gel (*n*-hexane–EtOAc gradient) and a Sephadex LH-20 column ($CHCl_3$-MeOH= 1 : 1) to give eight metabolites, **2** (15%), **3** (11%), **4** (12%), **5** (8%), **6** (7%), **7** (9%), **8** (6%), and **9** (12%). (−)-Hinesol (**1**) (150 mg/200 ml) was cultivated for 8 days by *A. cellulosae* in the same medium to afford **10** (26%) and **11** (14%).

The IR, UV, and ^{1}H-NMR spectra of compound **2**, $C_{15}H_{24}O_2$ (HRMS; $[M]^+$ *m/z* 236.1857), showed the presence of an α, β-conjugated ketone (IR; 1664 cm^{-1}; UV λ_{max} 240 nm [log ε=3.99]; δ_H 5.76 [1H, br s, 1-H]). From careful analysis of its 2D NMR spectrum, the structure of the metabolite **2** was formulated as 2-oxo-hinesol.[5]

Compounds **3** and **4** showed the same molecular formula, $C_{15}H_{26}O_2$, and similar spectral data. Acetylation (Ac_2O, Py) of **3** and **4** afforded the monoacetates **12** and **13**, respectively. The $NaBH_4$ reduction of **2** afforded **3** (2%) as a minor product and **4** (97%) as a major product, whereas the biotrasformation of **1** by *A. niger* afforded **3** and **4** in almost the same yield. Compounds **3** and **12** showed the NOE between H-2 and H-15 in the NOESY spectra (Fig. 1). On the other hand, compounds **4** and **13** showed the NOE between H-2 and H-4 (Fig. 1). Thus the stereostructures of **3** and **4** were formulated as 2α-hydroxyhinesol and 2β-hydroxyhinesol.

Compounds **5** and **6** showed the same molecular formula, $C_{15}H_{26}O_3$, and similar spectral data. The ^{1}H-NMR spectra of **5** and **6** showed the presence of an exomethylene (**5**: δ_H 4.93, 5.16 [br s, H-14]; **6**: δ_H 4.58, 4.98 [br s, H-14]). The relative structure of **5** was established by X-ray crystallographic analysis[7] of **5**, as shown in Fig. 2. The structure of **6** was determined to be the 1β, 2α-dihydroxyl isomer of **5** by the NOESY spectum (Fig. 1) of **6**.

Compounds **7** and **8** showed the same molecular formula, $C_{15}H_{24}O_3$, and similar spectral data. Acetylation of **7** and **8** afforded the diacetates **14** and **15**, respectively. The relative structure of **7** was deduced by the NOESY spectrum (Fig. 3) of **14** and finally established by X-ray crystallographic analysis[8] of **14**, as shown in Fig. 4, indicating that **7** contained an intramolecular ether linkage between C-10 and C-11. The structure of **8** was determined to be the 12-hydroxyl isomer of **7** by the NOESY spectum (Fig. 3) of **15**.

The ^{1}H-NMR spectrum of **9**, $C_{15}H_{24}O_4$ showed the pres-

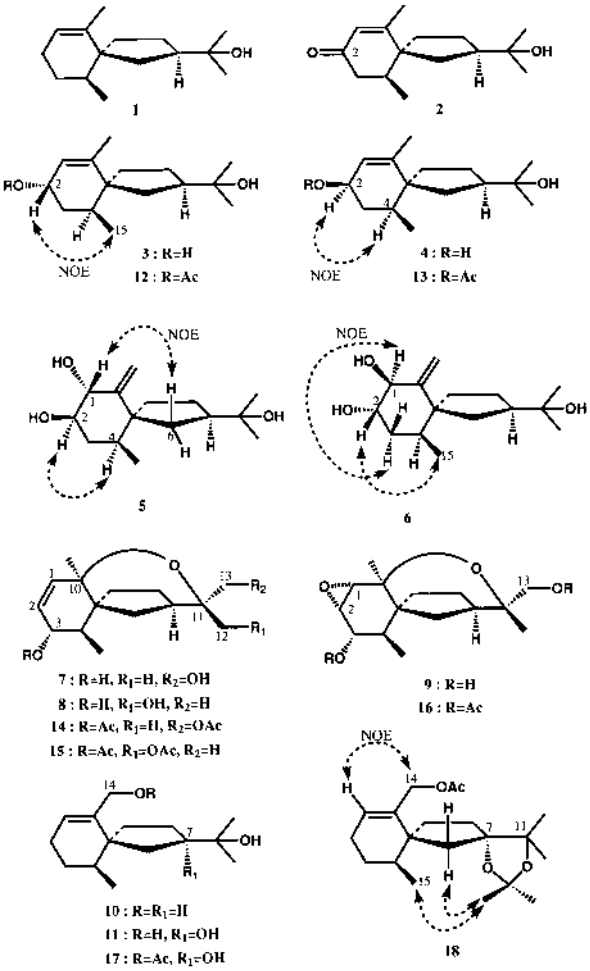

Fig. 1. The Metabolites of (−)-Hinesol (**1**) by *A. niger* and *A. cellulosae*

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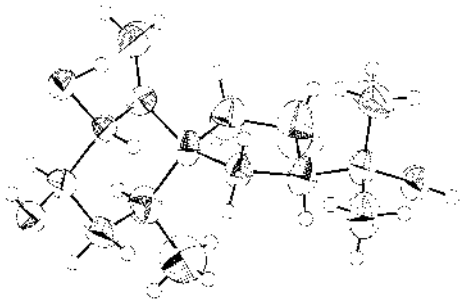

Fig. 2. ORTEP Drawing of Compound **5**

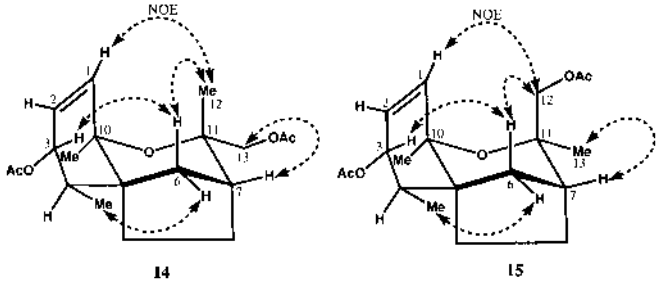

Fig. 3. NOESY Spectra of Compounds **14** and **15**

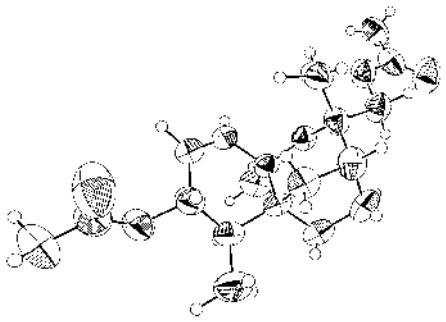

Fig. 4. ORTEP Drawing of Compound **14**

ence of an epoxy ring (δ_H 3.37 [d, J=3.6 Hz, H-1]; 3.46 [dd, J=2.7, 3.6 Hz, H-2]). Acetylation of **9** afforded the diacetate **16**. Epoxydation with MCPBA of **14** gave **16** in 68% yield, indicating that the structure of **9** was the epoxide of **7**.

The spectral data of **10**, $C_{15}H_{26}O_2$, resembled those of **1**, except for the presence of a primary hydroxyl group (δ_H 4.04, 4.16 [d, J=12.6 Hz, H-14]) in place of the vinyl methyl group observed in **1**, establishing that the structure of **10** was the C-14-hydroxylated derivative of **1**.

The ^{13}C-NMR spectrum of **11**, $C_{15}H_{26}O_3$, showed the presence of two tertiary alcohols (δ_C 74.5, 87.6 [each s]). Acetylation of **11** gave the monoacetate **17**, followed by reaction with 2, 2-dimethoxypropane and *p*-TsOH to give the acetonide **18**. The structure of **11** was determined to be the 7α, 14-dihydroxyl derivative of **1** by the NOESY spectrum (Fig. 1) of **18**.

In the time course of biotrasformation of (−)-hinesol (**1**), it was converted into 2-hydroxyhinesols (**3** and **4**) after 24 h, and both compounds were successively converted into 2-oxohinesol (**2**) and compounds **5**—**9** by rearrangement and intramolecular etherification. 1-Aminobezotriazole, an inhibitor of cytochrome P-450, inhibited the oxidation process of **1** into **3** and **4**. Possible metabolic pathways of **1** by *A. niger* might be as shown in Fig. 5. It is noteworthy that the

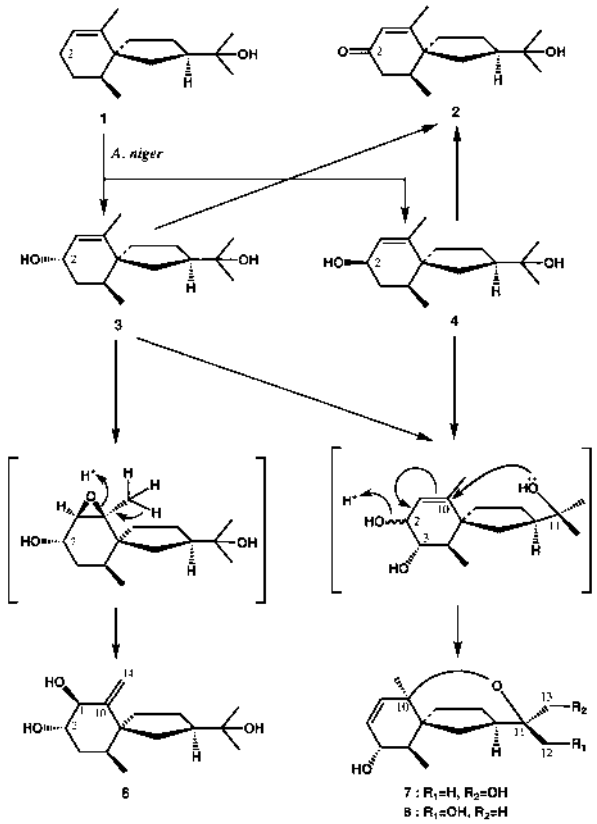

Fig. 5. Possible Metabolic Pathways of Hinesol (**1**) by *A. niger*

metabolic pathways of **1** are strikingly different between *A. niger* and *A. cellulosae*. Intramolecular etherification and rearrangement occured in *A. niger*, and hydroxydations of the five-membered ring at C-7 and the vinyl methyl group at C-14 of **1** occured in *A. cellulosae*. The biotrasformation of **1** by *A. niger* is very similar to that of oral administration to mammals since **1** was mainly converted into **2**—**4** by rabbits.[9]

References and Notes

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7) The crystal data for **5** are as follows: monoclinic; space group $P2_1$ with a=7.642 (1), b=18.353 (8), c=10.688 (5) Å, β=92.50 (5)°, V=1497.65 (1) Å^3, Z=4, and μ (Cu K-α)=5.765 mm^{-1} by Mac Science MXC 18 instrument. Final R value was 0.067 for 1741 reflections.
8) The crystal data for **14** are as follows: monoclinic; space group $P2_1$ with a=11.349 (2), b=7.857 (2), c=10.601 (2) Å, β=99.53 (1)°, V=932.2 (2) Å^3, Z=2, and μ (Cu K-α)=7.244 mm^{-1} by Mac Science MXC 18 instrument. Final R value was 0.065 for 1604 reflections.
9) Lie K., Toyota M., Asakawa Y., unpublished data.