Note

A Novel Kauranoid Isolated
from *Gibberella fujikuroi*

Noboru MUROFUSHI

Department of Agricultural Chemistry,
The University of Tokyo,
Bunkyo-ku, Tokyo 113, Japan

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In the process of surveying the metabolites produced by *Gibberella fujikuroi*, a new kauranoid was isolated. I report here its structural determination.

An ethyl acetate-soluble neutral fraction was obtained from the culture filtrate of *G. fujikuroi* and several known kauranoids were isolated by the application of several purification procedures. In the fraction containing 7 β -hydroxykauranolide, an unknown kauranoid-like compound was detected, its *R_f* value being a little higher than that of 7 β -hydroxykauranolide on thin layer chromatography (TLC). Separation by preparative TLC, followed by recrystallization from dichloromethane-*n*-hexane gave a crystalline compound (**1**), mp 197~198°C.

The high resolution mass spectrum of **1** indicated its molecular formula to be C₂₀H₃₂O₂ (M⁺: calcd. 304.2400, found 304.2429). The IR spectrum of **1** revealed a hydroxyl absorption at 3420 cm⁻¹ but not any carbonyl band. The mass spectrum of trimethylsilylated **1** gave a molecular ion peak at *m/z* 376, indicating the presence of only one hydroxyl group in **1**. The ¹H NMR spectrum* showed the presence of four tertiary methyl groups (δ 0.85, 0.88, 1.10, 1.36, 3H, s) and the absence of an unsaturated bond. The ¹³C NMR spectrum of **1** showed the presence

of four methyls, seven methylenes, five methines and four carbons carrying no hydrogen atom. These observations indicate that **1** is a diterpene with a five-ring system where an ether ring is included. Assuming that **1** belongs to the kaurane derivatives, which are major metabolites of *G. fujikuroi*, the structure of **1**, based on its spectral features was determined as follows.

Four methyls were allocated to 4-C (two methyls), 10-C and 16-C, and an ether linkage was placed on 16-C and another carbon, the possible position of which is 11-C or 12-C. 11-C is more likely, based on the fact that the signal at δ 4.45 (broad singlet with triplet-like shoulders, *J* = 3.4 Hz, *W*_{1/2} = 8.5 Hz) is very close to the 11-H signal of Pterokaurane L₅ (**2**, δ 4.42, broad t, *J* = 2.5 Hz)¹⁾ in the ¹H NMR spectra measured in deuteriopyridine.

1 was treated with mesyl chloride in pyridine to give a mesylate (**3**, NMR: δ 3.03, 3H s; δ 4.61, 1H dd, *J* = 6 Hz, 12 Hz), which was refluxed in collidine to give an olefin (**4**, NMR: 5.33, 1H dd, *J* = 10 Hz, 3 Hz; δ 5.65, 1H, dd, *J* = 10 Hz, 2 Hz). Catalytic reduction of **4** gave **5**, which showed no change either under the concentrated acidic condition or by treatment with oxidation reagents. **4** was treated with osmic acid to give a diol (**6**, NMR: δ 3.40, 1H d, *J* = 3 Hz; δ 3.90, 1H dd, *J* = 12 Hz, 3 Hz) in which the vicinal hydroxyls are in β -configuration by the attack of the reagent from the less hindered β -side. **6** was treated with sodium periodate to give a dialdehyde (**7**) in which the one aldehyde group links to a tertiary carbon and the other to a quaternary carbon (NMR: δ 9.66, 1H s; δ 9.83, 1H d, *J* = 5 Hz). These results indicate that a hydroxyl group in **1** exists in 6-C or 7-C. In this alternative, the 7-hydroxyl with α -configuration (equatorial) is more valid, based on the coupling pattern of the carbinol proton in the NMR spectrum (δ 3.51, 1H dd, *J* = 10 Hz, 4.8 Hz). This conclusion was substantiated by a comparison of the 7-H signal in the NMR spectrum of **1** measured in deuteriopyridine (δ 3.77, 1H dd, *J* = 11.2 Hz, 4.4 Hz) with that of **8**

* NMR data in this paper are those measured in deuteriochloroform unless otherwise stated.

TABLE I. ASSIGNMENTS OF SIGNALS IN
NMR SPECTRUM OF **1** MEASURED
IN CDCl₃

Long range coupling was observed between 11-H and 13-H, and between 14 α -H and 15 β -H.

Assigned proton	Chemical shift (ppm)	Shape of signal	Coupling const. (Hz)
6 α -H	1.33	q	10.7
6 β -H	1.72	dd	4.8, 10.7
7-H	3.53	dd	4.8, 10.7
9-H	1.49	br. s	
11-H	4.35	br. s	$W_{1/2}=9.3$
12 α -H	2.11	d	11.5
12 β -H	1.93	m	
13-H	2.26	t	6.3
14 α -H	1.65	dd	3.7, 12.3
14 β -H	1.75	dd	6.3, 12.3
15 α -H	1.83	d	11.5
15 β -H	1.48	dd	3.7, 11.5

(δ 3.67, 1H dd, $J=11.7$ Hz, 3.9 Hz), which was obtained from 7-oxokaurenoic acid²⁾ (**9**) by a lithium aluminum hydride reduction. Further, **9** and the oxidation product (**10**) of **1** revealed a similar Cotton effect (in methanol, **10**, 294 nm, $[\theta]+8600$; **9**, 294 nm, $[\theta]=+9500$) in their CD spectra. Thus, the structure of **1**, *ent*-11 α ,16 α -epoxy-7 β -hydroxykaurane, was established. In addition the intensive ¹H NMR studies, including spin-decoupling experiments, further confirmed the structure, as shown in the accompanying Table I.

Although **1** was isolated from the extract of a culture filtrate of *G. fujikuroi*, the possibility that **1** is an artifact from **11** remains. This is because some 11 β -hydroxykaurenoids isolated from liverwort,³⁾ fern¹⁾ and seed

plant,⁴⁾ and gibberellin A₃₅⁵⁾ which has the same moiety, were found to be converted into compounds having the 11~16 ether linkage by treatment with acid.^{1,3~5)} However, the occurrence of **11** in the culture filtrate of *G. fujikuroi* has not yet been confirmed.

Some kaurenoids obtained as metabolites of *G. fujikuroi* have an *ent*-7 α -hydroxyl and/or an *ent*-11 β -hydroxyl group and their configurations opposite to those in **1** should be noted. Furthermore, the stage of hydroxylation to the ring carbons in *ent*-kaurene is of interest. Generally it occurs after the oxidation of 19-C but the occurrence of **1** (or **11**) suggests another possible metabolic route of *ent*-kaurene in the fungus.

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