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NOTE

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Methoxylaricinolic Acid, a New Sesquiterpene from the Fruiting Bodies of *Stereum ostrea*

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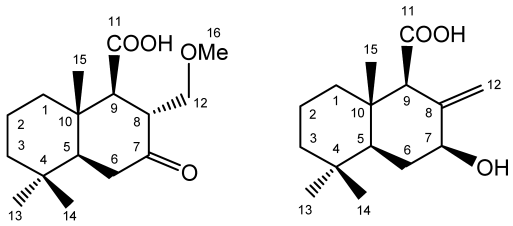
Abstract Methoxylaricinolic acid (**1**), a new sesquiterpene with drimane skeleton was isolated from the fruiting bodies of *Stereum ostrea*, together with the known compound laricinolic acid (**2**). The structure of **1** was determined as 12-methoxy-7-oxo-11-drimanoic acid on the basis of spectroscopic analysis.

Keywords methoxylaricinolic acid, *Stereum ostrea*, chemical structure

Stereum species produce many unique secondary metabolites including sesquiterpenes such as hirsutane [1], sterepolide [2] and sterpurene [3], benzaldehydes [4] and benzofurans [5]. In our previous studies on *Stereum* spp., several antioxidants, hirsutenols A~C [6] and sterins A, B [7] and C [8] were isolated from the culture broth of *Stereum hirsutum* (Willd.: Fr) S. F. Gray (stereaceae). As part of our continuing search for naturally occurring bioactive substances from *Stereum* spp., a new sesquiterpene, methoxylaricinolic acid (**1**), along with the known compound, laricinolic acid (**2**), has been isolated from the methanolic extract of the fruiting bodies of *Stereum ostrea*. In this paper, we describe the isolation, physico-chemical properties, structure determination and antioxidant activity of these compounds.

The air-dried fruiting bodies of *Stereum ostrea* (450 g) were crushed and extracted three times with methanol at room temperature. The combined extract was concentrated *in vacuo* to give a syrup, which was partitioned between chloroform and water. The chloroform-soluble part (7.7 g) was subjected to silica gel column chromatography and eluted by a gradient with increasing amount of methanol in chloroform (from 100 : 1 to 1 : 1, v/v) to give an active fraction. The active fraction was chromatographed on a column of Sephadex LH-20 eluting with chloroform/methanol (1 : 1, v/v), followed by HPLC using a YMC pack ODS-A column (4.6 mm i.d.×150 mm) eluting with acetonitrile/water (70 : 30, v/v) to afford compounds **1** and **2** having retention times of 10.4 and 13.5 minutes, respectively.

The physico-chemical properties of methoxylaricinolic acid (**1**) are as follows; yellow oil, $[\alpha]_D$ −80.0° (*c* 0.01, MeOH), high resolution EI-mass *m/z* 282.1542 M^+ ($C_{16}H_{26}O_4$ requires 282.1533), UV λ_{max} nm (MeOH) 208



Methoxylaricinolic acid (**1**) Laricinolic acid (**2**)

Fig. 1 Structures of methoxylaricinolic acid (**1**) and laricinolic acid (**2**).

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(3.70), 281 (2.90), 314 (2.61) nm, IR (KBr) 3366, 1717, 1606, 1512, 1272, 1058 cm^{-1}. Its molecular formula was determined as $C_{16}H_{26}O_4$ by the high-resolution EI-mass measurement. A close inspection of the ^1H and ^{13}C NMR spectra (Table 1) of **1** by DEPT and HMQC experiments revealed the presence of three tertiary methyl (C-13, C-14 and C-15), five methylenes (C-1, C-2, C-3, C-6 and C-12) including one oxygen-bearing carbon (C-12), three methines (C-5, C-8 and C-9), one methoxyl methyl carbon (C-16) and four quaternary carbons (C-4, C-7, C-10 and C-11). The ^{13}C chemical shifts of two downfield signals corresponding to C-7 (δ_C 213.8) and C-11 (δ_C 178.4) suggested the presence of a ketone and a carboxyl group, respectively. The ^1H-^1H COSY data revealed three partial structures; $–CH_2–CH_2–CH_2–$, $–CH–CH_2–$ and $–CH–CH–CH_2–$. The partial structures were connected by the aid of HMBC spectrum, which displayed ^1H-^{13}C long-range couplings from two singlet methyl protons at δ 0.85 and 0.90 to C-3 (δ_C 42.7), C-4 (δ_C 34.4) and C-5 (δ_C 54.6), from a methine proton at δ 1.36 to C-4 (δ_C 34.4), C-9 (δ_C 62.4), C-10 (δ_C 37.7) and C-13 (δ_C 33.4), from a methylene protons at δ 2.33 and 2.40 to C-7 (δ_C 213.8) and C-10 (δ_C 37.7), from a methine proton at δ 2.83 to C-7 (δ_C 213.8) and C-9 (δ_C 62.4), from a methine proton at δ 2.36 to C-1 (δ_C 40.7), C-10 (δ_C 37.7), C-11 (δ_C 178.4) and C-15 (δ_C 14.7), from a methyl protons at δ 1.23 to C-1 (δ_C 40.7), C-5 (δ_C 54.6), C-9 (δ_C 62.4) and C-10 (δ_C 37.7) and from a methoxyl methyl protons at δ 3.25 to C-12 (δ_C 70.9). These correlations unambiguously led the structure of **1** as 12-methoxy-7-oxo-11-drimanoic acid (Fig. 2). The relative stereochemistry of **1** was established by the NOE correlations from 13-CH_3 to 5-H (δ 1.36) and 6-H (δ 2.33), from 14-CH_3 to 2-H (δ 1.65), 6-H (δ 2.40) and from

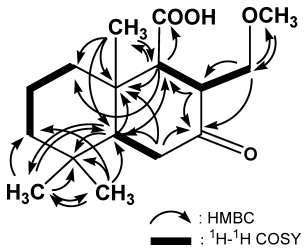

Fig. 2 HMBC correlations of methoxylaricinolic acid (**1**).

Table 1 ^1H and ^{13}C NMR data of methoxylaricinolic acid and laricinolic acid in CD_3OD

Position	Methoxylaricinolic acid		Laricinolic acid	
	δ_C	δ_H	δ_C	δ_H
1	40.7	1.37 (m)	39.8	1.19 (m)
		1.76 (m)		1.65 (m)
2	19.7	1.48 (m)	19.9	1.17 (m)
		1.65 (m)		1.65 (m)
3	42.7	1.22 (m)	42.9	1.23 (m)
		1.46 (m)		1.46 (m)
4	34.4		34.2	
5	54.6	1.36 (dd, 13.7, 3.9)	53.7	1.18 (dd, 12.9, 2.2)
6	39.9	2.33 (dd, 15.6, 3.9)	34.2	1.35 (ddd, 12.9, 12.0, 10.8)
		2.40 (dd, 15.6, 13.7)		2.03 (ddd, 12.0, 5.6, 2.2)
7	213.8		73.3	3.99 (br dd, 10.8, 5.6)
8	51.3	2.83 (ddd, 12.7, 4.4, 2.0)	147.6	
9	62.4	2.36 (d, 12.7)	62.6	2.70 (br s)
10	37.7		39.9	
11	178.4		174.7	
12	70.9	3.36 (dd, 9.3, 4.4)	105.8	4.80 (s)
		3.73 (dd, 9.3, 2.0)		5.20 (s)
13	33.4	0.85 (s)	33.9	0.92 (s)
14	21.5	0.90 (s)	22.2	0.87 (s)
15	14.7	1.23 (s)	14.7	1.05 (s)
16	59.3	3.25 (s)		

Chemical shifts in ppm from TMS as internal standard.
^1H and ^{13}C NMR were measured at 600 MHz and 150 MHz, respectively.

15-CH_3 to 2-H (δ 1.65), 6-H (δ 2.40) and 8-H (δ 2.83) and 15-H (δ 1.23) and from 9-H to 1-H (δ 1.37) and 5-H (δ 1.36), and was identical to that of laricinolic acid (**2**) described below. The co-occurrence in the fruiting bodies of *Stereum ostrea* and the same relative stereochemistry of **1** and **2** suggested that the absolute stereochemistry of **1** might be identical to that of **2**. Based on the evidence described above, methoxylaricinolic acid was assigned as a new sesquiterpene of drimane skeleton.

The molecular formula of laricinolic acid (**2**) was established as $C_{15}H_{24}O_3$ by high-resolution ESI-mass measurement. The ^1H and ^{13}C NMR spectra (Table 1) in the aid of DEPT and HMQC data revealed the presence of one exocyclic double bond (C-8 and C-12), three methyls (C-13, C-14 and C-15), four methylenes (C-1, C-2, C-3 and C-6), three methines (C-5, C-7 and C-9) including one oxygen-bearing carbon (C-7), two quaternary carbon (C-4 and C-10) and one carbonyl carbon (C-11). These data were in good agreement with those of laricinolic acid, a sesquiterpene of the drimane type isolated from the wood-rotting fungus *Laricifomes officinalis* [9]. **2** was confirmed to be identical to laricinolic acid by interpretation of HMBC, which exhibited the long-range correlations from 5-H to C-4, C-9, C-10 and C-13, from 9-H to C-1, C-8, C-10, C-11 and C-15, from 12-H to C-7, C-8 and C-9. Other physico-chemical properties were well matched with those of laricinolic acid [9].

Compounds **1** and **2** were not isolated by following a specific bioassay-guided separation protocol. In a panel for antioxidant effect, however, these compounds exhibited marginal inhibitory activity with an IC_{50} of 50 μg/ml (vitamin E, 1 μg/ml) against lipid peroxidation in rat liver microsomes evaluated by the thiobarbituric acid method [10].

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