Chem. Pharm. Bull. **46**(3) 542—544 (1998) Vol. 46, No. 3

EUDESMANE- TYPE SESQUITERPENOIDS FROM JAPANESE LIVERWORT *FRULLANIA TAMARISCI* SUBSP. *OBSCURA*

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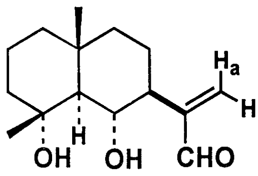
The diethyl ether extract of the Japanese liverwort *Frullania tamarisci* subsp. *obscura* yielded a new sesquiterpene aldehyde in addition to a new dimeric sesquiterpenoid with a eudesmane-type carbon skeleton. Their structures were established by extensive 2D NMR techniques and chemical evidence. They were shown to be 4α,6α-dihydroxy-11(13)-eudesmen-12-al and its dimer with 4-*epi*-arbusculin A at the C-4 ether linkage.

KEY WORDS liverwort; *Frullania tamarisci* subsp. *obscura*; sesquiterpenoid; dimeric eudesmane-type carbon skeleton; chemosystematics

Frullania species are rich sources of terpenoids, especially eudesmane-type sesquiterpenoids.[1,2) Phytochemical investigation of some of these epiphytic liverworts has led to the isolation of eudesmane- and eremophilane-type sesquiterpene lactones which cause intense allergenic contact dermatitis[3), a pacifigorginane-type sesquiterpene alcohol, tamariscol[4), and bibenzyl derivatives.[5) Further investigation of the chemical constituents of *Frullania tamarisci* subsp. *obscura* resulted in the isolation of two new eudesmane-type sesquiterpenoids.

F. tamarisci subsp. *obscura* (#97092; 29.6 g) was collected in August 1997 at Kainan-cho (altitude 100 m), Tokushima, Japan. The ether extract (0.67 g) of *F. tamarisci* subsp. *obscura* was chromatographed repeatedly and subjected to preparative HPLC to give a new sesquiterpene aldehyde **1** (4.5 mg; 0.68% in yield of the total extract) and a new dimeric sesquiterpene lactone **2** (81.1 mg; 12.1%), together with two known related compounds **3**[6) (78.1 mg; 11.7%) and **4**[6) (16.1 mg; 2.4%).

Compound **1**, $[\alpha]_D$-15.9° ($CHCl_3$, *c* 0.23) showed a yellow coloration when treated with 2,4-dinitrophenylhydrazine on a TLC plate. The EIMS spectrum of **1** showed a molecular ion peak at *m/z* 252. The presence of a formyl and hydroxyl group was apparent from the absorption bands at 1694 and 3318 cm^{-1} in the FT-IR spectrum. Further evidence for the presence of the formyl group was provided by a proton signal at δ 9.60 (*s*) in the ^{1}H NMR spectrum of **1**. On the other hand, compound **4**, which has been isolated from Japanese *F. tamarisci* subsp. *obscura* collected in Hiroshima, was also isolated from the present species. The ^{1}H and ^{13}C NMR spectra of **1** were similar to those of **4**, except for the appearance of a methyl at δ_H1.34 and δ_C23.6 in place of the presence of an exocyclic methylene proton signal of **4**. The stereochemistry of **1** was established by 2D NOESY spectrum as follows. The important cross peaks between H-14 and H-6, and H-15 and H-6 indicated that two methyl groups at C-4 and 10, and a carbinyl proton at C-6 were in an axial



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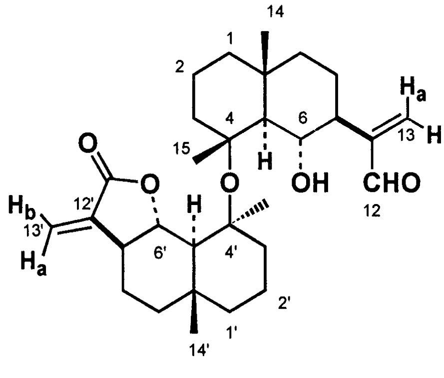

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configuration. Consideration of these spectral data led to the conclusion that the structure of **1** was 4α,6α-dihydroxy-11(13)-eudesmen-12-al. The absolute configuration of known compound **3**$^{6)}$ has been established and that of **1** was tentatively assigned as shown, since compounds **1** and **3** coexist within the present species.

The ^{1}H NMR spectrum of **2**, $[\alpha]_D +2.8^\circ$ ($CHCl_3$, *c* 4.05) seemingly exhibited the signals due to a 1:1 mixture of **1** and **3**. However, the comparative TLC analysis of **2** with **1** and **3**, which were isolated from the present species, showed different *Rf* values. Furthermore, since the EIMS spectrum showed a molecular ion peak at *m/z* 484, it was clear that **2** was a dimeric eudesmane-type sesquiterpenoid. While the signals for a methyl group on C-4 of **1** and **3** appeared at δ 1.34 and 1.44, the resonance for the methyl groups at δ 1.56 and 1.57 was confirmed in the ^{1}H NMR spectrum of **2**. The 2D-NMR experiments involving the determination of ^{1}H-^{1}H COSY and HMQC spectra were effective for complete assignments (Table 1 in reference site) of all carbons and protons, and the carbon-carbon connectivities of the molecule of **2** were established by HMBC spectrum. Further confirmation of the structure of **2** was provided by chemical evidence. Treatment of **2** with *p*-TsOH gave **3** (in 46.5% yield, $[\alpha]_D +45.4^\circ$) and **4** (34.8%), for which the spectral data, including optical rotation, were in agreement with those of **3** ($[\alpha]_D +47^{\circ\,6)}$) and **4**. Thus the absolute configuration of **1** might be as shown in Fig. 1. The relative conformation was shown to be **2** in Fig. 1, since a cross peak between the C-6 hydroxyl proton at δ 5.20 (d, *J*=2 Hz), which disappeared upon addition of D_2O, and two protons of H-6' and 14' was observed in its NOESY spectrum.

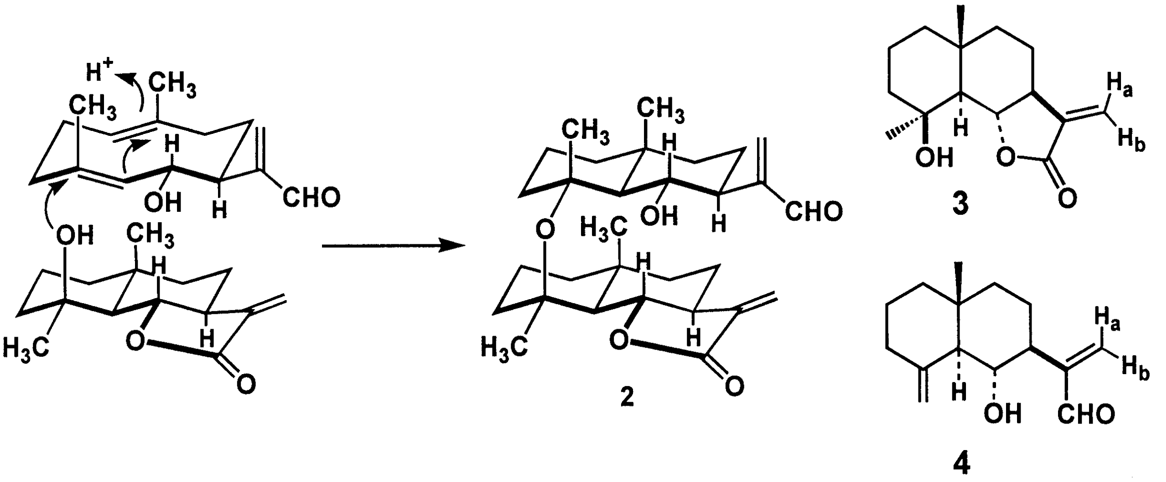

Fig. 1. Possible Biogenetic Pathway and Conformation of **2**

The intermediate cationic species formed in the cyclization procedure might be attacked by a molecule of 4-*epi*-arbusculin A (**3**) in the possible biogenetic pathway shown in Fig. 1. Kraut$^{7)}$ has reported the isolation of dimeric eudesmane-type sesquiterpene lactone, muscicolides A and B from *F. muscicola*. Furthermore, Connolly$^{8)}$ has reported the isolation of related dimeric lactones with a C-C bond at C-3 and 4' of costunolide from *F. tamarisci* subsp. *tamarisci*, a species which was stored in the laboratory for at least one year. In the present work, the major constituent **2** of this species was isolated from fresh materials. We found at least two different chemical races in this species, one of which contained **2** (14.2%; the average yield from the ether extracts); on the other hand, the other did not give the dimeric sesquiterpenoid **2** in examinations to date.

ACKNOWLEDGMENTS We thank Dr. M. Mizutani (Hattori Botanical Laboratory, Nichinan, Miyazaki, Japan) for his identification of the species. We are indebted to Dr. M. Tanaka (TBU) for measurements of the NMR spectra.

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Table 1. 600 MHz NMR Data for Compounds **1** and **2** in $CDCl_3$

	^1H		^{13}C	
No.	**1**	**2**	**1**	**2**
1	1.20 (m, ax) 1.38 (m, eq)	1.20 (m, ax), 1.35 (brd, *J*=12 Hz, eq)	41.3	41.2
2	1.56 (m, 2H)	1.52 (m, 2H)	19.6	19.6
3	1.47 (m, ax) 1.78 (dddd, J=12, 5, 3, 3 Hz eq)	1.83 (ddd, *J*=12, 12, 6 Hz, ax) 2.20 (brd, *J*=12 Hz, eq)	43.0	42.4
4	-----	-----	73.8	85.1
5	1.45 (d, *J*=10 Hz)	1.58 (d, *J*=10 Hz)	58.0	58.4
6	4.08 (t, *J*=10 Hz)	3.93 (ddd, *J*=10, 10, 2 Hz)	72.4	70.9
7	2.64 (ddd, *J*=14, 10, 4 Hz)	2.61 (ddd, *J*=12, 10, 5 Hz)	46.8	46.5
8	1.54 (m, eq), 1.72 (qd, *J*=12, 4 Hz)	1.53 (m, 2H)	26.3	26.6
9	1.31 (td, *J*=12, 4 Hz), 1.38 (m, eq)	1.30 (m, 2H)	43.5	44.6
10	-----	-----	36.3	37.0
11	-----	-----	151.7	152.3
12	9.60 (s)	9.54 (s)	195.5	195.0
13	6.15 (s, H_b), 6.43 (s, H_a)	6.06 (s, H_b), 6.32 (*s*, H_a)	135.6	133.9
14	0.98 (s)	1.02 (s)	19.7	20.4
15	1.34 (s)	1.57 (s)	23.6	22.7
6-O<u>H</u>		5.20 (d, *J*=2 Hz)		
1'		1.19 (m, ax), 1.48(*m*, eq)		42.6
2'		1.45 (m, eq), 1.91 (ddddd, *J*=14, 14, 14, 3, 3 Hz, ax)		18.5
3'		1.26 (ddd, *J*=12, 12, 5 Hz, ax) 2.26 (brd, *J*=12 Hz, eq)		40.3
4'		-----		80.0
5'		1.30 (d, *J*=11 Hz)		60.8
6'		4.00 (t, *J*=11 Hz)		80.3
7'		2.43 (ddddd, *J*=12, 11, 3, 3, 3 Hz)		51.6
8'		1.66 (dddd, *J*=12, 12, 12, 4 Hz, ax.) 1.89 (m, eq)		21.6
9'		1.22 (m, ax), 1.45 (m, eq)		43.3
10'		-----		37.3
11'		-----		139.3
12'		-----		170.6
13'		5.34 (d, *J*=3 Hz H_a), 6.03 (d, *J*=3 Hz H_b)		116.6
14'		1.19 (s)		21.1
15'		1.56 (s)		31.7

(Received December 19, 1997; accepted February 6, 1998)