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A REARRANGED ABIETANE-TYPE DITERPENOID FROM THE LIVERWORT *MAKINOA CRISPATA*

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Key Word Index—*Makinoa crispata*; Metzgeriales; liverwort; makinin; 17(15 → 16)abeoabietane-type diterpenoid.

Abstract—Makinin, a new diterpenoid with a 17(15 → 16)abeoabietane skeleton, was isolated from the Taiwanese liverwort *Makinoa crispata*. The structure of makinin was deduced by spectroscopic analysis. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

The Japanese liverwort *Makinoa crispata* had been investigated by Asakawa and co-workers [1–3] and interesting components, such as **1–4**, were reported. However, in our examination of the same species collected at Ali Shan in Taiwan, none of the above mentioned compounds could be detected by GC-mass spectrometry. In the volatile fraction of this Taiwanese species, the major constituents observed were a series of fatty acids as well as their methyl and ethyl esters in addition to a few common sesquiterpenes as minor components. Nevertheless, in a thorough analysis of the non-volatile fraction of this Ali Shan species, a new diterpene aldehyde of rearranged abietane-type, named makinin (**5**), was isolated in a minute amount.

RESULTS AND DISCUSSION

The hexane-extracted oil of the powdered plants was subjected to repeated chromatography on silica gel. The TLC spot with strong UV absorption was isolated and characterized. The ^1H and ^{13}C DEPT NMR spectra, along with the EI-mass spectra of this compound, established a molecular formula of $C_{21}H_{26}O_3$, indicating a diterpene methyl ester with a conjugated aldehyde group and six more sites of unsaturation. Since signals due to two other methyls, five methylenes and three trisubstituted double bonds could also be derived from the ^{13}C DEPT NMR data, the most likely skeleton of this aldehyde appeared to be an abietane-type with a rearranged side-chain on the C-ring. The singlet nature of H-11 and H-12 (both at δ 7.32 *s*, Table 1) was later proved to be a limiting case of an AB spectrum degenerating into an A_2 spectrum in deuterated chloroform, because a pair of doublets at δ 7.01 and 7.03 (J = 8.0 Hz) in deuterated benzene and δ 7.37 and 7.40 (J = 8.8 Hz) in deuterated methanol for these two *ortho*-protons was observed. Structure **5** for this new aldehyde, makinin, was further supported by HMBC (Table 1) and NOESY (Fig. 1) data. The absolute configuration of **5** is tentatively depicted as the antipodal form of abietanes from most vascular plants.

Makinin (**5**) possesses a rearranged 17(15 → 16)abeoabietane skeleton, which has never been reported from liverworts previously [4, 5]. In the present study, five other specimens of the sterile thalli collected from different localities including one from Nara, Japan, were examined by GC-mass spectrometry for components **1–5** and the results are shown in Table 2. Although Asakawa *et al.* [6] have noted that the content of the major components is quite different between the female (**1**) and the male (**2**) gametophytes of *M. crispata*, both thalli do contain the same components. It is interesting to note that components **1–4** were not observed at all in four of the six sterile specimens examined. No matter which part of the gametophytes is studied, the species *M. crispata* may be further chemotaxonomically classified according to the characteristic components **1–4**.

EXPERIMENTAL

Methods. Solvents used for spectral measurements were: $CDCl_3$ (^1H and ^{13}C NMR, 500 MHz), 95% EtOH (UV) and $CHCl_3$ ($[\alpha]_D$). GC-MS: 70 eV; column, DBWAX, 30 m × 0.25 mm, 50–220° (40 min), 5° min^{-1}.

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Table 1. NMR data for makinin (**5**) (in $CDCl_3$, 500 MHz)

Atom	δ ^{1}H (Hz)	δ ^{13}C	HMBC correlated Cs
1 CH_2	2.25* 1.37 (*dt*, *J* = 4.2, 12.4 Hz)	39.2	2, 10, 20
2 CH_2	1.65* 1.98*	19.9	
3 CH_2	2.27* 1.08 (*dt*, *J* = 4.2, 12.4 Hz)	37.5	2, 4, 18, 19
4 4° C	—	44.0	
5 CH	1.55 (*dd*, *J* = 2.5, 12.4 Hz)	52.5	10, 4, 6, 7, 19, 20, 18
6 CH_2	2.15* 1.98*	20.8	5, 7, 8, 10, 4
7 CH_2	2.93 (*ddd*, *J* = 16, 5, 2.7 Hz) 2.80*	31.9	6, 8, 9, 14, 5
8 4° C	—	136.4	
9 4° C	—	152.0	
10 4° C	—	38.9	
11 CH	7.32 (*s*)†	126.5	8, 9, 13
12 CH	7.32 (*s*)†	125.8	8, 9, 13
13 4° C	—	131.2	
14 CH	7.22 (*s*)†	129.7	7, 9, 12, 15
15 CH	7.38 (*d*, *J* = 15.9 Hz)	152.9	12, 14, 17
16 CH	6.6 (*dd*, *J* = 7.7, 15.9 Hz)	127.9	13
17 CHO	9.65 (*d*, *J* = 7.7 Hz)	193.8	16
18 COO	—	177.7	
19 Me	1.25 (*s*)	28.5	3, 4, 5, 19
20 Me	1.0 (*s*)	22.8	1, 5, 10, 9
21 OMe	3.65 (*s*)	51.3	19

*Coupling constants of these undescribed peaks are unclear due to serious overlappings.

†In deuterated benzene, chemical shifts (300 MHz) for these atoms are: H-11 δ 7.03 (*d*, *J* = 8.0 Hz), H-12 δ 7.01 (*dd*, *J* = 8.0, 1.5 Hz), H-14 δ 6.85 (*br s*). In deuterated methanol, chemical shifts (300 MHz) for these atoms are: H-11 δ 7.37 (*d*, *J* = 8.8 Hz), H-12 δ 7.40 (*dd*, *J* = 8.8, 1.5 Hz), H-14 δ 7.34 (*br s*).

Table 2. Distribution of characteristic components in *Makinoa crispata* collected at different localities

Species	**1**	**2**	**3**	**4**	**5**	C_{16} acid	Other C_{15}s	Other C_{20}s
AL	− −	− −	− −	− −	+	+ + +*	+	− −
TP	− −	− −	− −	− −	+	+	+	+ +
YY	− −	− −	− −	− −	+	+	+ + +	+
FC	− −	− −	− −	− −	− −	+	+	− −
FS	+ +	− −	(+)†	+ +	− −	+	+	+ +
JN	− −	+ +	(+)†	+ +	− −	+	+	− −
JT‡	+ +	+ +	+	+	NR	NR	NR	NR

*Series of fatty acids (C14–C22) including their methyl and ethyl esters.

†Not compound **3**, but one of the same confertifoline skeleton by judging from the GC-MS data {$[M]^+$ 248 (43), 233 (70), 205 (35), 91 (100), 83 (85)}.

‡Data taken from refs [1–3].

AL = Ali Shan; TP = Taiping Shan; YY = Yuenyang Lake; FC = Fenchi Lake; FS = Fu Shan; JN = Kasuga Mountain, Nara city, Japan; JT = Tokushima, Japan.

NR = not reported.

Plant material. Collection sites and collection dates of the liverwort specimens are as follows: Ali Shan (2200 m), Chiayi Hsien, 1992; Taiping Shan (2000 m), Ilan Hsien, 1994; Yuenyang Lake (1700 m), Hsinchu Hsien, 1987; Fenchi Lake (1400 m), Chiayi Hsien, 1995; Fu Shan (800 m), Ilan Hsien, 1995; Kasuga Mountain (JN) (300 m), Nara city, Japan, 1990. The JN species was identified by Prof. N. Kitagawa (Nara University of Education, Japan) and the remaining species by Dr K. Yamada (Ise-shi, Japan). All voucher specimens were deposited at the Department of Chemistry, Tamkang University.

Extraction and isolation. Air-dried and powered sterile plants (AL species, 160 g) were extracted with *n*-hexane. The crude extract (1.2 g) was subjected to CC on silica gel (70–230 mesh) using a *n*-hexane–EtOAc gradient. Fr. 5 (15% EtOAc–hexane) was re-chromatographed on silica gel (230–400 mesh, pure

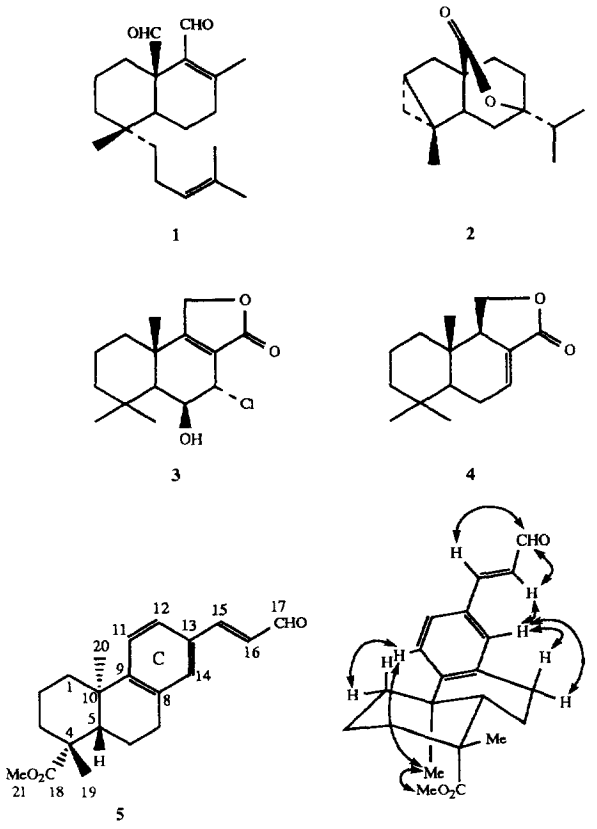

Fig. 1. Partial NOEs of **5**.

C_6H_6) to afford **5** (2 mg). A GC-MS examination of the hexane-extracted oil indicated fatty acids of C_{15}, C_{16} (major), C_{18}, C_{20} and C_{22}, Me esters of C_{16}, C_{18}, C_{20} and C_{22}, Et esters of C_{16} (major), C_{17}, C_{18}, C_{20} and C_{24} and trace sesquiterpenes β-barbatene and spathulenol.

Makinin (**5**). Oil; $[\alpha]_D -71°$ (*c* 0.07, $CHCl_3$); UV λ_{max}^{EtOH} nm (ε): 220.5 (6490), 289.5 (6606); IR ν_{max}^{film} cm^{-1}: 2870, 2855, 1730, 1683; EIMS *m/z* (rel. int.): 326 $[M]^+$(8), 266 $[M-60]^+$ (32), 251 (100), 144 (72); GC-MS $R_t = 67$ min, 200–250°, 5° min^{-1}; ^1H and ^{13}C NMR data: Table 1.

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