PERGAMON

Phytochemistry 51 (1999) 743–750

PHYTOCHEMISTRY

Seco-clerodane diterpenoids jamesoniellides H, I and J in axenic cultures of the liverwort *Jamesoniella autumnalis*

Hiroyuki Tazaki[a,*], Hans Becker[b], Kensuke Nabeta[a]

[a]*Department of Bioresource Science, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, 080, Japan*
[b]*fr.12.3 Pharmakognosie und Analytische Phytochemie, Universitat des Saarlandes, D-66041, Saarbrucken, Germany*

Received 23 September 1998; received in revised form 4 January 1999; accepted 4 January 1999

Abstract

Three new seco-clerodane diterpenoids, jamesoniellides H, I and J have been isolated from axenic cultures of the liverwort *Jamesoniella autumnalis*. Their structures were determined by spectroscopic methods. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Jamesoniella autumnalis*; Hepaticae; Axenic culture; Furanoditerpenoids; Jamesoniellides

1. Introduction

Many terpenoids, including irregular skeletal-types, have been isolated from liverworts (Zinsmeister, Becker, & Eicher, 1991; Asakawa, 1982; Huneck, 1983). From the interest of their biosynthesis, we have investigated these compounds by using in vitro cultures of liverworts (Tazaki, Nabeta, Okuyama, & Becker, 1995; Tazaki, Soutome, Iwasaki, Nabeta, & Arigoni, 1997; Nabeta, Komuro, Utoh, Tazaki, & Koshino, 1998). The use of in vitro cultures of liverworts is a promising way around the difficulty of acquiring large amounts of liverworts such as *Jamesoniella autumnalis* (DC) Steph. (Jungermanniaceae) (Schuster, 1983). We have succeeded in the isolation and structure determination of many lignans and diterpenoids, including jamesoniellides A–G from in vitro cultured *Jamesoniella autumnalis* (Tazaki, Adam, & Becker, 1995; Tazaki, Zapp, & Becker, 1995; Tazaki, Nabeta, & Becker, 1998). In our continuing search for terpenoids from the cultured gametophytes of *J. autumnalis*, we report the isolation and characterization of new seco-clerodane type diterpenoids, jamesoniellides H (**1**), I (**3**) and J (**5**).

2. Results and discussion

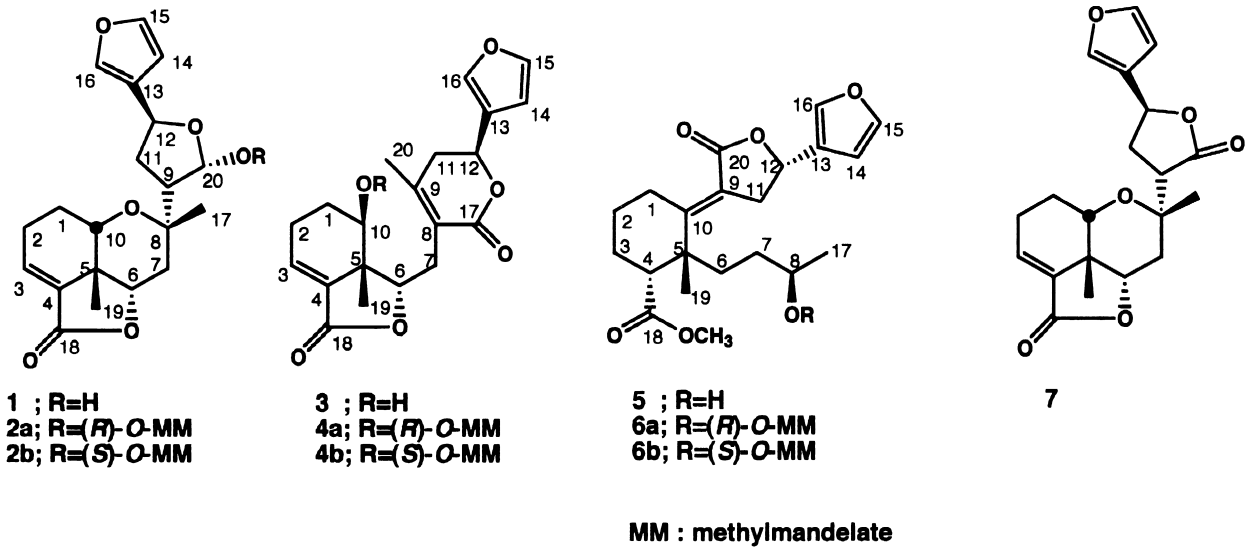
Three diterpenoids **1**, **3**, and **5** were isolated from the Et_2O extract of in vitro cultured *J. autumnalis* by combined CC on silica gel and Sephadex LH-20 and HPLC on Diol and silica gel columns.

2.1. Jamesoniellide H (*1*)

Jamesoniellide H (**1**) was an oil with a molecular formula $C_{20}H_{24}O_6$ from HR-EIMS. The UV spectrum showed an absorption at 235 nm for a conjugated ester. The IR spectrum exhibited absorption bands at 1760 cm^{-1} and 1670 cm^{-1} (α, β-unsaturated 5-ring lactone). The ^1H and ^{13}C NMR spectra of **1** were similar to jamesoniellide E (**7**) (Tazaki et al., 1995), however, compound **1** differed from **7** by the presence of an additional hydroxy function (δ_C 98.9) and the absence of one carbonyl carbon. Additionally, a new signal at δ_H 5.52 in the ^1H NMR spectrum of **1** as compared to **7** showed vicinal couplings with H-9 in the ^1H-^1H COSY. As a consequence of all data, we deduced that C-20 is hydroxylated and the structure of jamesoniel-

* Corresponding author. Tel.: +81-155-49-5541; fax: +81-155-49-5577.
E-mail address: tazaki@obihiro.ac.jp (H. Tazaki)

0031-9422/99/$ - see front matter
PII: S0031-9422(99)00143-0



lide H was formulated as the derivative of **7** represented by **1**. The complete structure of **1** was achieved by following through ^{1}H (Table 1) and ^{13}C NMR assignments (Table 2) with ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, ^{1}H–^{1}H homodecoupling, NOESY, difference NOE and COLOC experiments. The hemiacetal furan ring of **1** was confirmed by H-H couplings (H-12 with H-11α and H-11β; H-9 with H-11α, H-11β, and H-20) observed by ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY experiments. NOEs between 17-Me and H-20, between H-11β and H-12, and between H-11β and H-9 and between H-9 and 17-Me, were in agreement for 17-Me, H-9, H-12 and H-20 in the β-position (Fig. 1). The absolute configuration of jamesoniellide H (**1**) was determined by esterification of the secondary hydroxyl group at C-20. **1** was esterified with (*R*)- and (*S*)-methyl mandelic acid (MM) by the method previously reported by Trost, Belletire, Godleski, McDougal, & Balkovec, 1986. Fig. 2 shows that the configuration of the hydroxyl group at C-20 was *R*, from chemical shift

Table 1
^{1}H NMR data of compounds **1**, **3**, and **5** (270 MHz)

H	**1**	**3**	**5**
1α	1.91 *m*	1.84 *m*	1.96 *m*
1β	1.91 *m*	2.05 *m*	4.31 *ddd* (3.0, 3.1, 15.1)
2α	2.28 *m*	2.43 *m*	1.70 *m*
2β	2.28 *m*	2.43 *m*	1.60 *m*
3α	6.90 *dd* (3.3, 3.6)	6.96 *t* (3.6)	2.05 *dd* (6.1, 12.4)
3β	–	–	1.76 *m*
4	–	–	2.71 *dd* (5.4, 5.6)
6	4.54 *dd* (6.9, 10.0)	4.56 *dd* (4.3, 9.7)	1.83 *m*, 1.55 *m*
7α	1.19 *dd* (10.0, 13.3)	2.63 *m*	1.29 *m*
7β	1.97 *dd* (6.9, 13.3)	2.63 *m*	–
8	–	–	3.69 *m*
9	2.24 *m*	–	–
10	3.96 *dd* (2.8, 3.3)	4.21 *brs*	–
11α	2.11 *m* (5.0, 11.2)	2.75 *ddd* (2.0, 7.7, 14.3)	3.00 *ddd* (3.1, 6.8, 15.7)
11β	1.75 *dd* (10.9,11.2)	3.24 *ddd* (2.0, 6.6, 14.3)	3.56 *ddd* (2.5, 7.6, 15.7)
12	5.13 *dd* (5.0, 10.9)	5.43 *dd* (6.6, 7.7)	5.34 *dd* (6.8, 7.6)
14	6.35 *dd* (1.2, 1.5)	6.36 *d* (0.8)	6.40 *dd* (0.8, 1.8)
15	7.39 *brs*	7.39 *brs*	7.42 *dd* (1.5, 1.8)
16	7.39 *brs*	7.43 *d* (0.8)	7.47 *dd* (0.8, 1.5)
17	1.27 *s*	–	1.16 *d* (6.3)
19	1.23 *s*	1.28 *s*	1.30 *s*
20	5.52 *d* (3.0)	2.34 *t* (2.0)	–
Others	20-OH 2.67 *brs*	–	18-OMe 3.66 *s*

Table 2
^{13}C NMR spectral data of compounds **1**, **3**, and **5** (67.5 MHz, $CDCl_3$)*

C	1	3	5
1	23.3	26.9	25.1
2	21.7	20.7	21.7
3	136.6	136.3	22.3
4	130.4	122.2	51.8
5	38.7	44.7	44.2
6	80.3	86.8	35.8
7	36.9	42.9	33.0
8	77.2	130.7	68.2
9	58.8	149.0	119.5
10	68.3	69.2	160.9
11	34.1	35.1	36.8
12	72.5	70.4	70.2
13	125.2	125.5	125.2
14	108.6	108.2	108.3
15	139.5	143.9	143.9
16	143.5	139.7	139.8
17	20.0	169.0	23.9
18	169.8	169.5	174.5
19	24.3	28.8	24.0
20	98.9	17.6	171.0
Others			51.4 (OMe)

differences defined as $\Delta\delta$ (ppm) $= \delta S - \delta R$ of MM esters (**2a,b**) Trost et al., 1986; Latypov, Seco, Quinoa, & Riguera, 1995). The negative $\Delta\delta$ values of H-11α, H-12, H-14, H-15 and H-16 indicate they are on the same side of the benzene ring in the case of the *S*-MM ester (**2a**), demonstrating the *R*-configuration at C-20 in **1**. Thus, the absolute configuration of **1** is concluded to be as indicated.

2.2. Jamesoniellide I (3)

The molecular formula of jamesoniellide I (**3**) ($C_{20}H_{22}O_6$) required ten double bond equivalents. The IR spectrum exhibited the presence of a hydroxy group (3450 cm^{-1}), and carbonyl groups (1740, 1730 cm^{-1}). The ^1H NMR spectrum displayed one tertiary methyl group (δ_H 1.28) and a β-substituted furan moiety (δ_H 6.40, 7.42 and 7.47), together with an allylic methyl group (δ_H 2.34, *t*, $J = 2.0$ Hz). The ^{13}C NMR spectrum showed the signals of two methyls, four methylenes, seven methines and seven quaternary carbons, indicating four double bonds (δ_C 108.2, 122.2, 125.5, 130.7, 136.3, 139.7, 143.9, and 149.0) and two carbonyl groups (δ_C 169.5 and 169.0) in the molecule. These observations suggested **3** is a tetracyclic diterpenoid. The complete structure of jamesoniellide I (**3**) was established by the following ^1H–^1H COSY, ^1H–^{13}C COSY, ^1H–^1H homodecoupling, NOESY, difference NOE, and COLOC experiments. ^1H–^1H COSY experiment revealed the sequences C (10) H–C (1) H_2–C (2) H_2–C (3) H, and a set of ABX spin systems, C (6) H–C (7) H_2 and C (11) H_2–C (12) H (Table 1). The complete assignment of those was achieved by ^1H–^{13}C COSY and COLOC experiments (Fig. 3). The quaternary carbon C-5 (δ_C 44.7) showed correlations with 19-Me (δ_H1.28), H-10 (δ_H 4.21) and H-6 (δ_H 4.56). C-8 (δ_C 130.7) showed correlations with H-7 (δ_H 2.63), and 20-Me (δ_H 2.34). C-9 (δ_C 149.0) showed correlations with H-7, 20-Me, and H-11 (δ_H 2.75 and 3.24). The coupling constants ($J = 3.6$ Hz)

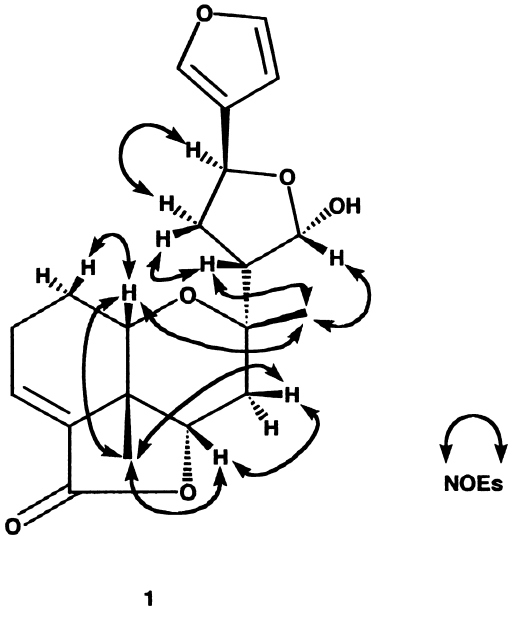


Fig. 1. NOESY correlations used to establish the structure of **1**.

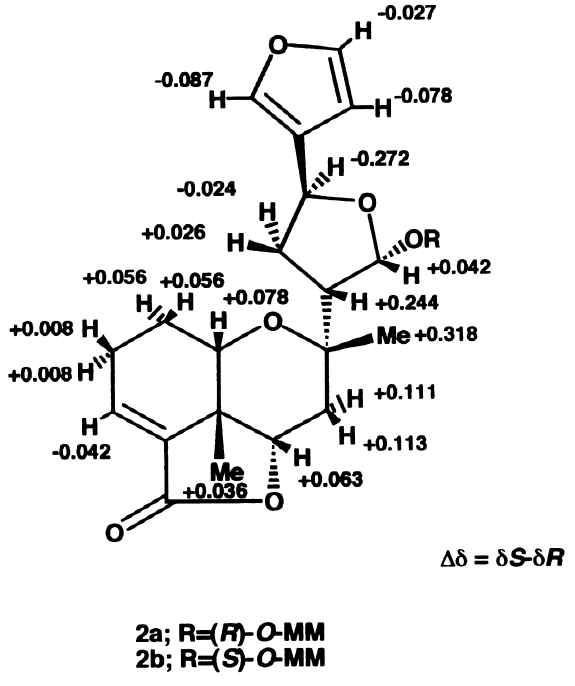


Fig. 2. $\Delta\delta$ values obtained for the methylmandelate of **1**.

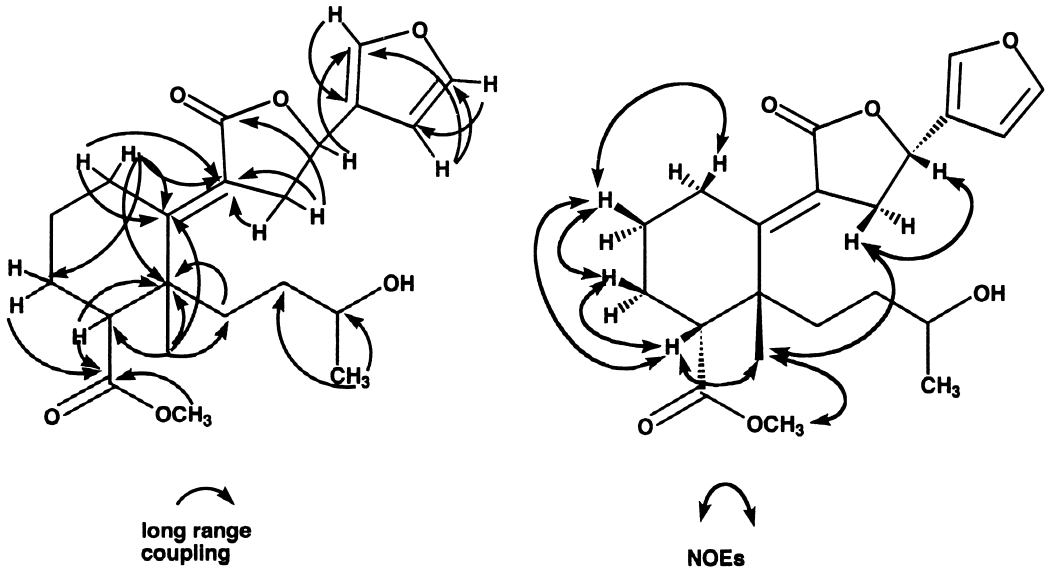


Fig. 3. COLOC and NOESY correlations used to establish the structure of **3**.

between H-3 and H-2α and between H-3 and H-2β indicated that these hydrogens have a gauche relationship. The observed NOEs between H-6 and H-10, between 19-Me and H-1β, between 19-Me and H-10, and between 19-Me and H-6 showed that H-1β, H-10, and 19-Me are all β, establishing the relative stereochemistry of **3** as illustrated in Fig. 3. The absolute configuration of jamesoniellide I (**3**) was determined by esterification of the secondary hydroxyl group at C-10 with (*R*)- and (*S*)-MM. Fig. 5 shows that the configuration of the hydroxyl group at C-10 was *S*, from chemical shift differences defined as $\Delta\delta$ (ppm) $= \delta S$-δR of MM esters (**4a,b**) (Trost et al., 1986; Latypov et al., 1995). The positive $\Delta\delta$ values of H-1, H-2 and H-3 indicate they were on the same side of the benzene ring in the case of the *R*-MM ester (**4b**). Thus, the absolute

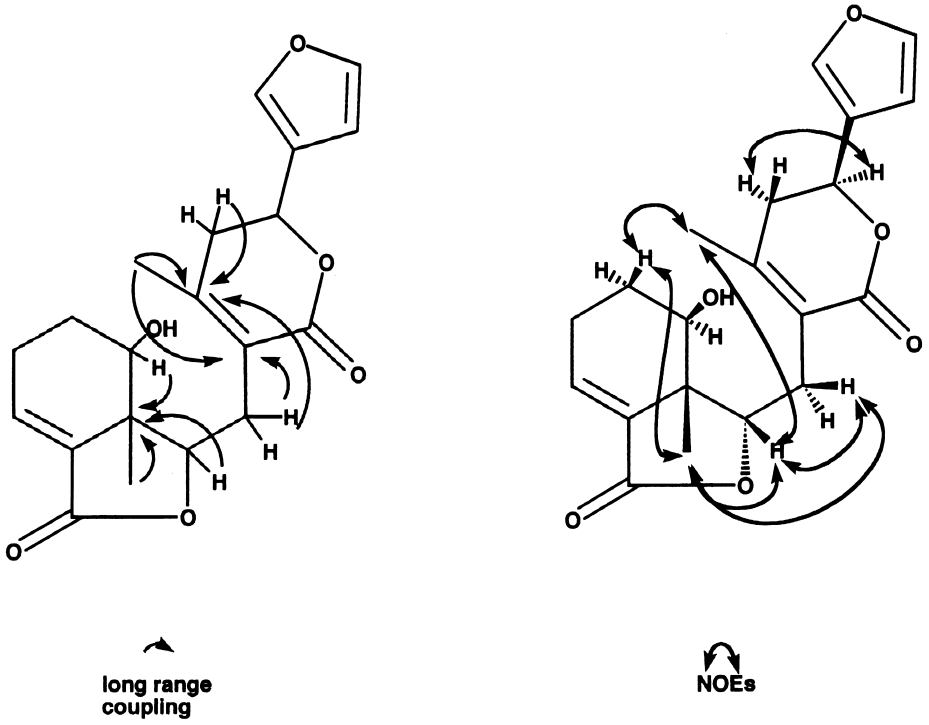


Fig. 4. COLOC and NOESY correlations used to establish the structure of **5**.

configuration of **3** is concluded to be as illustrated in Fig. 5.

2.3. Jamesoniellide J (5)

Jamesoniellide J (**5**) was obtained as a colorless oil with a molecular formula $C_{21}H_{28}O_6$ from HR-EIMS. Further peaks in the mass spectrum revealed the presence of a hydroxy group, m/z 358.1758 ([M-18]+, calcd 358.1780), confirmed by IR spectra (3450 cm^{-1}). The ^{13}CNMR spectrum showed signals of three methyls, six methylenes, six methines and six quaternary carbons, suggesting the presence of three double bonds. The ^{1}H NMR (δ 6.40, 7.42 and 7.47) spectrum suggested the presence of a β-substituted furan ring. The ester signal at 1740 cm^{-1} was overlapped by a carbonyl absorption at 1760 cm^{-1} which, in combination with the carbons at δ 171.0 and 174.5 in ^{13}C NMR, indicated the presence of two carboxyl groups. Low field shift of the olefinic carbon at δ 160.9 and IR (1660 cm^{-1}) suggested one of the double bonds conjugated with a carbonyl function. Therefore, the oxygens in the molecule corresponded to those in one furan ring, one hydroxy group and two carboxyl groups. This suggests that jamesoniellide J (**5**) is a tricyclic diterpenoid. The complete structure of **5** was achieved by following through ^{1}H and ^{13}C NMR assignment with ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY, ^{1}H–^{1}H homodecoupling, DEPT, NOESY and COLOC experiments (Tables 1 and 2). A –CH_2–CH_2–CH_2–CH- unit, of which the proton-line assignment was confirmed by ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY, belonged to a cyclohexane ring. COLOC experiments revealed the connections of the partial structures (Fig. 4). The quaternary carbon C-5 (δ_C 44.2) showed correlations with H-1β (δ_H 1.96), H-4 (δ_H 2.71), H-6 (δ_H 1.55 and 1.83), and 19-Me (δ_H 1.30). C-10 (δ_C 160.9) showed correlations with H-1 (δ_H 1.96 and 4.31), and 19-Me. C-9

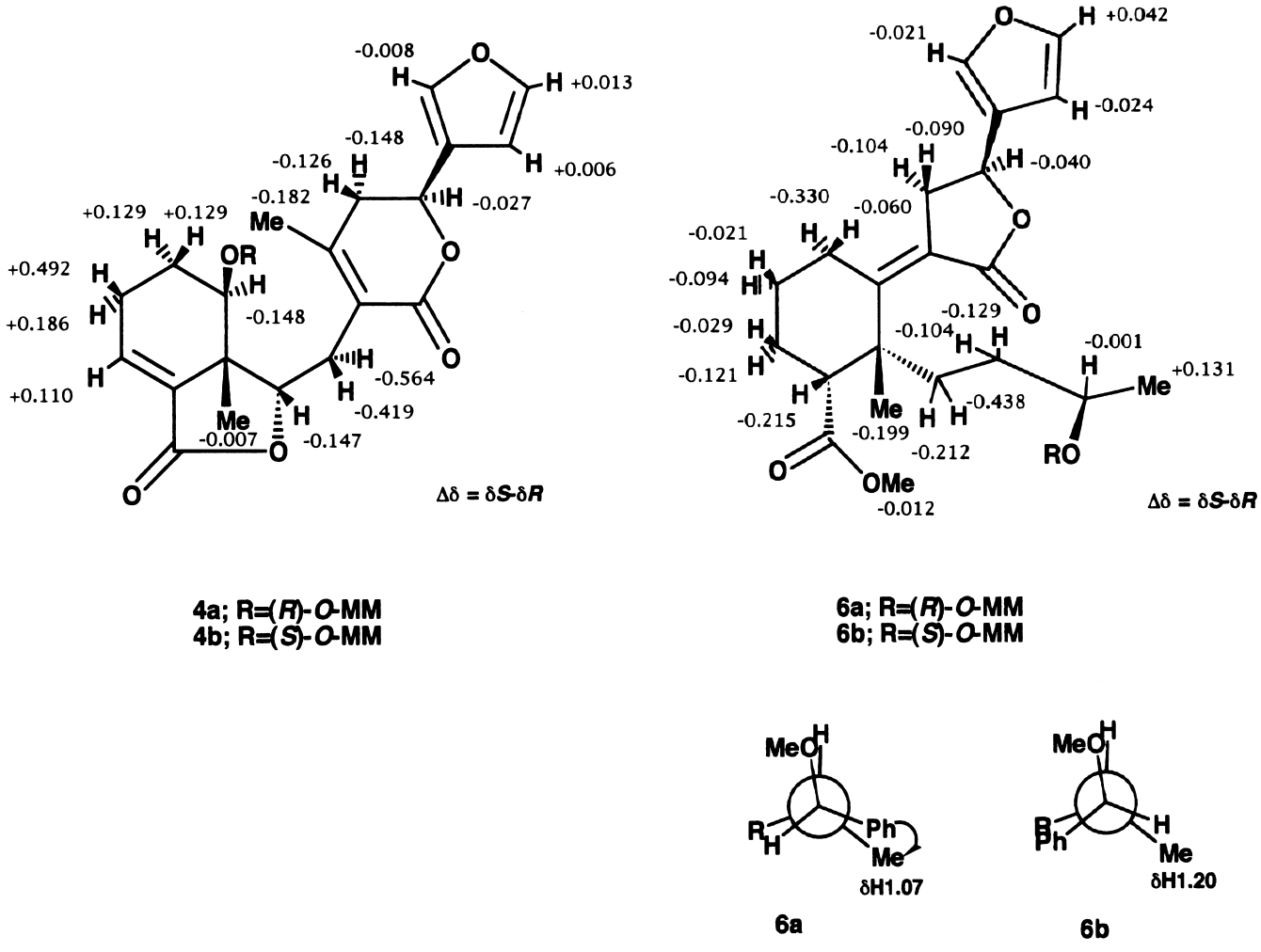


Fig. 5. $\Delta\delta$ values obtained for the methylmandelate of **3** and **5**.

(δ_C 119.5) showed correlations with H-1 and H-11 (δ_H 3.00 and 3.56). The carbonyl carbon C-20 (δ_C 171.0) showed a correlation with H-11. The smaller coupling constants between H-1β and H-2α and between H-1β and H-2β (J=3.0 Hz, each) were resolved by ^{1}H homodecoupling, showing that these hydrogens have a gauche relationship. Thus, the larger coupling constant between H-1α and H-1β (J=15.1 Hz) indicated H-1β should be equatorial. Low field shift of H-1β (δ 4.31), caused by a deshielding effect of an α,β-unsaturated carbonyl group, indicated a geometric isomerism at the C-9/C-10 double bond was *E* configuration. The NOE between 19-Me and H-11 confirmed this. Furthermore, additional NOEs between H-4 and 19-Me indicated that the side chain at C-5 was equatorial (Fig. 4). The configuration of C-8 was established by NMR analyses of (*R*)- and (*S*)-MM esters derived from **5** (Trost et al., 1986). Structures **6a** and **6b** illustrated as Newman projection in which the intervening ester linkage was omitted. 17-Me in **6a** showed its proton shift upfield of the corresponding signal in **6b**. Thus, the configuration of the hydroxyl group at C-8 was *R*. The above results established the absolute stereochemistry of jamesoniellide J (**5**) as illustrated in Fig. 5.

3. Experimental

Optical rotations were measured in $CHCl_3$. UV spectra were measured in EtOH. NMR spectra were recorded in $CDCl_3$ soln. using a 400 MHz instrument (H: 400 MHz; C: 100.5 MHz) relative to $CHCl_3$ at δ_H 7.25 and $CDCl_3$ at δ_C 77.0, respectively. ^{13}C multiplicities were determined using the DEPT pulse sequence.

The origin and details of the axenic culture of *J. autumnalis* have been previously reported (Tazaki et al., 1995).

3.1. Extraction and isolation

Powdered dry plant material (815 g) was extracted with Et_2O. The Et_2O extracts (37.03 g) were subjected to vacuum liquid chromatography (VLC) on silica gel and eluted with *n*-hexane containing various concns of EtOAc to give nine fractions of A to I (MeOH) as previously reported (Tazaki et al., 1995). Fr. I was subjected to VLC on silica gel eluted with $CHCl_3$ containing various conc. of MeOH. Elution of the column with $CHCl_3$ gave a yellow oil which was further separated by VLC on silica gel eluted with *n*-hexane containing various conc. of EtOAc. Elution of the column with *n*-hexane-EtOAc (1:1) gave frs. containing compounds **1**, **3** and **5** (1.23 g). Frs. containing **1**, **3** and **5** were further separated by HPLC on a DIOL column (30 × 2.0 cm i.d.) eluted with *n*-hexane-EtOAc (35:65) to give frs. containing **1** and **3** (199.1 mg) and frs. containing **5** (43.9 mg). Compounds **1** (19.1 mg), **3** (15.1 mg) and **5** (12.1 mg) were purified by HPLC on Kieselgel Si60 column (30 × 2.0 cm i.d.) eluted with *n*-hexane-EtOAc (35:65).

3.2. Jamesoniellide H (*1*)

$[\alpha]_D$= − 54.3° (*c* 1.75); HR-MS found $[M]^+$ 360.1578; $C_{20}H_{24}O_6$ requires 360.1573. UV^{λ}_{max} nm (log ϵ): 235 (3.28); IR $\nu^{KBr}_{cm^{-1}}$ 3430, 1760, 1670, 1260, 1165, 1020, 970, 875, 760. ^{1}H and ^{13}C NMR: see Tables 1 and 2, respectively. EIMS *m*/*z* (*rel. int.*): 360 (3), 342 (8), 327 (4), 299 (4), 251 (3), 233 (3), 220 (4), 206 (100), 189 (23), 179 (7), 165 (47), 149 (63), 147 (54), 137 (21), 135 (31), 119 (45), 105 (47), 95 (45), 77 (48), 43 (96).

3.3. (*S*)-*O*-Methylmandelate *2a*

To a stirred solution of compound **1** (1.2 mg) in CH_2Cl_2 (0.5 ml) were added (*S*)-*O*-methylmandelic acid (2.5 mg), 1,3-dicyclohexylcarbodiimide (DCC) (3 mg) and dimethylaminopyridine (DMAP) (1.5 mg). After 15 h, the reaction mixture was filtered. The filtrate was chromatographed on a silica gel column (3 mm × 3 cm) with *n*-hexane-EtOAc (60: 40) to afford purified compound **2a** (1.1 mg). $[\alpha]_D$= − 20.6° (*c* 0.06); ^{1}H NMR ($CDCl_3$): 7.343 (1H, *brs*, H-15), 7.283 (1H, *brs*, H-16), 6.834 (1H, *dd*, J=3.3, 3.6 Hz, H-3), 6.398 (1H, *d*, J=3.0 Hz, H-20), 6.257 (1H, *dd*, J=1.2, 1.5 Hz, H-14), 4.770 (1H, *dd*, J=5.0, 10.9 Hz, H-12), 4.468 (1H, *dd*, J=6.9, 10.0 Hz, H-6), 3.893 (1H, *dd*, J=2.8, 3.3, Hz H-10), 2.497 (1H, *m*, H-9), 2.217 (2H, *m*, H-2), 2.075 (1H, *dd*, J=5.0, 11.2 Hz, H-11α), 1.902 (1H, *dd*, J=6.9, 13.3 Hz, H-7β), 1.817 (2H, *m*, H-1), 1.685 (1H, *dd*, J=10.9, 11.2 Hz, H-11β), 1.205 (3H, *s*, H-19), 1.153 (1H, *dd*, J=10.0, 13.3 Hz, H-7α), 1.114 (3H, *s*, H-17).

3.4. (*R*)-*O*-Methylmandelate *2b*

This compound was obtained from compound **1** (1.3 mg) with (*R*)-*O*-methylmandelic acid by essentially the same procedure as for the preparation of diastereomer **2a**. $[\alpha]_D$= − 30.7° (*c* 0.04); ^{1}HNMR ($CDCl_3$): 7.370 (2H, *brs*, H-15, H-16), 6.876 (1H, *dd*, J=3.3, 3.6 Hz, H-3), 6.357 (1H, *d*, J=3.0 Hz, H-20), 6.334 (1H, *dd*, J=1.2, 1.5 Hz, H-14), 5.042 (1H, *dd*, J=5.0, 10.9 Hz, H-12), 4.405 (1H, *dd*, J=6.9, 10.0 Hz, H-6), 3.816 (1H, *dd*, J=2.8, 3.3, Hz H-10), 2.253 (1H, *m*, H-9), 2.209 (2H, *m*, H-2), 2.048 (1H, *dd*, J=5.0, 11.2 Hz, H-11α), 1.789 (1H, *dd*, J=6.9, 13.3 Hz, H-7β), 1.761 (2H, *m*, H-1), 1.709 (1H, *dd*, J=10.9, 11.2 Hz, H-11β), 1.169 (3H, *s*, H-19), 1.041 (1H, *dd*, J=10.0, 13.3 Hz, H-7α), 0.796 (3H, *s*, H-17).

3.5. Jamesoniellide I (3)

$[\alpha]_D = -1.1°$ (*c* 0.91); HR-MS found $[M]^+$ 376.1853; $C_{21}H_{28}O_6$ requires 376.1886, $[M-H_2O]^+$ 358.1758; $C_{21}H_{26}O_5$ requires 358.1780. UV^{λ}_{max} nm (log ϵ): 243 (3.59); IR $\nu^{KBr}_{cm^{-1}}$ 3450, 1760, 1740, 1670, 1660, 1320, 1260, 1180, 1020, 750. ^1H and ^{13}C NMR: see Tables 1 and 2, respectively. EIMS *m/z* (*rel. int.*): 358 (3), 313 (5), 297 (6), 285 (10), 267 (11), 237 (16), 225 (20), 197 (25), 167 (15), 147 (17), 131 (22), 109 (34), 91 (45), 81 (86), 67 (38), 55 (83), 41 (100).

3.6. (S)-O-Methylmandelate 4a

To a stirred solution of compound **3** (1.2 mg) in CH_2Cl_2 (0.5 ml) were added (*S*)-*O*-methylmandelic acid (2.5 mg), DCC (3 mg) and DMAP (1.5 mg). After 5 h, the reaction mixture was filtered. The filtrate was chromatographed on a silica gel column (3 mm × 3 cm) with *n*-hexane-EtOAc (60:40) to afford purified compound **4a** (0.9 mg). $[\alpha]_D = +5.0°$ (*c* 0.05); ^1H NMR ($CDCl_3$): 7.482 (1H, *brs*, H-16), 7.435 (1H, *brs*, H-15), 6.822 (1H, *t, J* = 3.6 Hz, H-3), 6.406 (1H, *brs*, H-14), 5.449 (1H, *dd, J* = 6.6, 7.7 Hz, H-12), 5.449 (1H, *brs*, H-10), 4.437 (1H, *dd, J* = 4.3, 9.7 Hz, H-6), 3.198 (1H, *ddd, J* = 2.0, 6.6, 14.3 Hz, H-11β), 2.688 (1H, *ddd, J* = 2.0, 7.7, 14.3 Hz, H-11α), 2.333 (1H, *m*, H-7β), 2.250 (3H, *t, J* = 2.0 Hz, H-20), 2.140 (1H, *m*, H-2α), 1.989 (1H, *m*, H-7α), 1.783 (2H, *m*, H-1), 1.633 (1H, *m*, H-2β), 1.295 (3H, *s*, H-19).

3.7. (R)-O-Methylmandelate 4b

This compound was obtained from compound **3** (1.3 mg) with (*R*)-*O*-methylmandelic acid by essentially the same procedure as for the preparation of diastereomer **4a**. $[\alpha]_D = -20.7°$ (*c* 0.05); ^1H NMR ($CDCl_3$): 7.473 (1H, *brs*, H-16), 7.449 (1H, *brs*, H-15), 6.932 (1H, *t, J* = 3.6 Hz, H-3), 6.412 (1H, *brs*, H-14), 5.421 (1H, *dd, J* = 6.6, 7.7 Hz, H-12), 5.300 (1H, *brs*, H-10), 4.290 (1H, *dd, J* = 4.3, 9.7 Hz, H-6), 3.072 (1H, *ddd, J* = 2.0, 6.6, 14.3 Hz, H-11β), 2.540 (1H, *ddd, J* = 2.0, 7.7, 14.3 Hz, H-11α), 2.326 (1H, *m*, H-2α), 2.125 (1H, *m*, H-2β), 2.069 (3H, *t, J* = 2.0 Hz, H-20), 2.002 (2H, *m*, H-1), 1.913 (1H, *m*, H-7β), 1.425 (1H, *m*, H-7α), 1.289 (3H, *s*, H-19).

3.8. Jamesoniellide J (5)

$[\alpha]_D = -5.9°$ (*c* 0.44); HR-MS found $[M]^+$ 358.1446; $C_{20}H_{22}O_6$ requires 358.1417, $[M - H_2O]^+$ 340.1303; $C_{20}H_{20}O_5$ requires 340.1311. UV^{λ}_{max} nm (log ϵ): 236 (4.19); IR $\nu^{KBr}_{cm^{-1}}$ 3450, 1740, 1730, 1620, 1450, 1190, 1160, 870, 750, 600. ^1H and ^{13}C NMR: see Tables 1 and 2, respectively. EIMS *m/z* (*rel. int.*): 358 (0.3), 340 (2), 325 (4), 304 (3), 276 (4), 261 (4), 233 (4), 215 (5), 203 (15), 185 (16), 165 (13), 149 (22), 131 (15), 119 (25), 105 (30), 91 (49), 77 (43), 67 (26), 51 (21), 44 (100).

3.9. (S)-O-Methylmandelate 6a

To a stirred solution of compound **5** (1.2 mg) in CH_2Cl_2 (0.5 ml) were added (*S*)-*O*-methylmandelic acid (2.5 mg), DCC (3 mg) and DMAP (1.5 mg). After 8 h, the reaction mixture was filtered. The filtrate was chromatographed on a silica gel column (3 mm × 3 cm) with *n*-hexane-EtOAc (60:40) to afford purified compound **6a** (1.2 mg). $[\alpha]_D = +20.0°$ (*c* 0.12); ^1H NMR ($CDCl_3$): 7.453 (1H, *brs*, H-16), 7.427 (1H, *brs*, H-15), 6.378 (1H, *brs*, H-14), 5.294 (1H, *dd, J* = 6.8, 7.6 Hz, H-12), 4.853 (1H, *m*, H-8), 4.218 (1H, *ddd, J* = 3.0, 3.1, 15.1 Hz, H-1β), 3.645 (3H, *s,* 18-OMe), 3.422 (1H, *ddd, J* = 2.5, 7.6, 15.7 Hz, H-11β), 2.789 (1H, *ddd, J* = 3.1, 6.8, 15.7 Hz, H-11α), 2.346 (1H, *dd, J* = 5.4, 5.6 Hz, H-4), 1.840 (1H, *dd, J* = 6.1, 12.4 Hz, H-3α), 1.709 (1H, *m*, H-2α), 1.680 (1H, *m*, H-3β), 1.580 (1H, *m*, H-2β), 1.569 (1H, *m*, H-1α), 1.541 (1H, *m*, H-6), 1.300 (1H, *m*, H-7), 1.205 (1H, *m*, H-7′), 1.204 (3H, *d, J* = 6.3 Hz, H-17), 1.027 (3H, *s*, H-19), 0.719 (1H, *m*, H-6′).

3.10. (R)-O-Methylmandelate 6b

This compound was obtained from compound **5** (1.3 mg) with (*R*)-*O*-methylmandelic acid by essentially the same procedure as for the preparation of diastereomer **6a**. $[\alpha]_D = -18.3°$ (*c* 0.1); ^1HNMR ($CDCl_3$): 7.474 (1H, *brs*, H-16), 7.385 (1H, *brs*, H-15), 6.402 (1H, *brs*, H-14), 5.334 (1H, *dd, J* = 6.8, 7.6 Hz, H-12), 4.854 (1H, *m*, H-8), 4.278 (1H, *ddd, J* = 3.0, 3.1, 15.1 Hz, H-1β), 3.657 (3H, *s*, 18-OMe), 3.512 (1H, *ddd, J* = 2.5, 7.6, 15.7 Hz, H-11β), 2.893 (1H, *ddd, J* = 3.1, 6.8, 15.7 Hz, H-11α), 2.561 (1H, *dd, J* = 5.4, 5.6 Hz, H-4), 1.961 (1H, *dd, J* = 6.1, 12.4 Hz, H-3α), 1.899 (1H, *m*, H-1α), 1.803 (1H, *m*, H-2α), 1.754 (1H, *m*, H-6), 1.709 (1H, *m*, H-3β), 1.601 (1H, *m*, H-2β), 1.429 (1H, *m*, H-7), 1.309 (1H, *m*, H-7′), 1.226 (3H, *s*, H-19), 1.157 (1H, *m*, H-6′), 1.073 (3H, *d, J* = 6.3 Hz, H-17).

Appendix

Seco-clerodane diterpenoids jamesoniellides H, I and J in axenic cultures of the liverwort *Jamesoniella autumnalis*

Hiroyuki Tazaki, Hans Becker, Kensuke Nabeta
Department of Bioresource Science, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro 080, Japan

Three new seco-clerodane diterpenoids, jamesoniellides H, I and J have been isolated from axenic cul-

tures of the liverwort *Jamesoniella autumnalis*. Their structures were elucidated on the basis of spectroscopic evidence.

References

Asakawa, Y. (1982). *Progress in the Chemistry of Organic Natural Products*, *vol. 42* (p. 1). Springer.

Huneck, S. (1983). *New Manual of Bryology (Schuster RM)*, *vol. 1* (p. 1). Nichinan, The Hattori Botanical Laboratory, Japan: .

Latypov, S. K., Seco, J. M., Quinoa, E., & Riguera, R. (1995). *J. Org. Chem.*, *60*, 504.

Nabeta K., Komuro K., Utoh T., Tazaki H., Koshino H. 1998 Chem. Commun., p. 169.

Schuster, R. M. (1983). *New Manual of Bryology (Schuster RM)*, *vol. 1* (p. 463). The Hattori Botanical Laboratory, Nichinan, Japan: .

Tazaki, H., Nabeta, K., Okuyama, H., & Becker, H. (1995). *Biosci. Biotech. Biochem.*, *59*, 158.

Tazaki, H., Adam, K-P., & Becker, H. (1995). *Phytochemistry*, *40*, 1671.

Tazaki, H., Zapp, Y., & Becker, H. (1995). *Phytochemistry*, *39*, 859.

Tazaki H., Soutome H., Iwasaki T., Nabeta K., Arigoni D. 1997 Chem. Commun., p. 1101.

Tazaki, H., Nabeta, K., & Becker, H. (1998). *Phytochemistry*, *48*, 681.

Trost, B. M., Belletire, J. L., Godleski, S., McDougal, P. G., & Balkovec, M. (1986). *J. Org. Chem.*, *51*, 2370.

Zinsmeister, H. D., Becker, H., & Eicher, T. (1991). *Angew. Chem., Int. Ed. Engl.*, *30*, 130.