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TERPENOIDS OF THE LIVERWORT *FRULLANOIDES DENSIFOLIA* AND *TROCHOLEJEUNEA SANDVICENSIS*

MOTOO TORI, HERNITA ARBIYANTI, ZENEI TAIRA and YOSHINORI ASAKAWA

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro cho, Tokushima 770, Japan

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Key Word Index—*Frullanoides densifolia*; *Trocholejeunea sandvicensis*; Lejeuneaceae; liverwort; Hepaticae; chemosystematics; spirodensifolin A; spirodensifolin B; furanopinguisanol; pinguisane-type sesquiterpenoids; *ent*-kauranols; kaurane-type diterpenoids; lepidozenol; lepidozane-type sesquiterpenoid.

Abstract—Two new rearranged pinguisane-type sesquiterpenoids, spirodensifolin A and B, a pinguisane-type sesquiterpene, isonaviculol, and two new *ent*-kaurane-type diterpenoids, *ent*-kauran-16β-ol-3-one, *ent*-kaurane-3β,16β-diol, have been isolated from the Bolivian liverwort *Frullanoides densifolia*, and a pinguisane-type sesquiterpene, furanopinguisanol and lepidozane-type sesquiterpene alcohol have been isolated from the Japanese *Trocholejeunea sandvicensis*. Their stereostructures have been elucidated by the extensive analysis of the spectral data as well as the X-ray crystallographic analysis.

INTRODUCTION

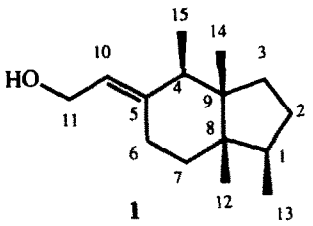
We have previously reported the isolation and structure determination of deoxopinguisone, pinguisanin, dehydropinguisanin, pinguisanolide, dehydropinguisenol (**14**), and pinguisenal, as well as (−)-bicyclogermacrene and aromatic compounds from *Trocholejeunea sandvicensis* [1–3]. Early studies on the flavonoids in Lejeuneaceae, resulted in the isolation of kaempferol-3-methylether from *Frullanoides densifolia* [4, 5]. In a continuation of systematic studies on the Hepaticae and isolation of biologically active substances [6–8], we have reinvestigated the chemical constituents of *F. densifolia* collected in Bolivia, South America, and *T. sandvicensis* collected in Japan. We report the isolation of seven new compounds, isonaviculol (**1**), spirodensifolin A (**2**), spirodensifolin B (**3**), *ent*-kauran-16β-ol-3-one (**4**) and *ent*-kaurane-3β,16β-diol (**5**) from *F. densifolia*, as well as furanopinguisanol (**6**) and (4*S**,5*S**,6*R**,7*R**)-1(10)*E*-lepidozen-5-ol (**7**) from *T. sandvicensis*, respectively. We were also able to isolate compounds **8**, **9** and **10** as artifacts. Their structures have been established by spectral evidence and chemical transformations. In this report, the isolation and structure elucidation, as well as the biogenesis of the new pinguisane-type and rearranged pinguisane-type sesquiterpenoids, and the chemosystematics of the two species are discussed.

RESULTS AND DISCUSSION

The ether extract of *F. densifolia* was chromatographed on a combination of silica gel and Sephadex LH-20 and HPLC, which resulted in the isolation of a new pinguisane-type sesquiterpene **1**, two new rearranged pinguisane-type sesquiterpenes **2** and **3**, which have been preliminarily reported [9], and two new kaurane-type diterpenes, **4** and **5**, together with the previously known naviculol (**11**) [10], ptychanolide (**12**) [11], estafiatin (**17**) [12], dihydroestafiatin (**18**) [13] and *ent*-kauran-16β-ol (**13**) [14–16].

Isonaviculol (**1**)

The IR spectrum of **1**, $C_{15}H_{26}O$ (*m/z* 222 $[M]^+$), exhibited the presence of a hydroxyl group at 3500 cm^{-1}. The ^1H NMR spectrum contained the signals of two tertiary methyl groups at δ 0.62 and 0.66, two secondary methyl groups at δ 0.86 and 0.96, an allylic proton at δ 2.27 (*q*, *J* = 6.8 Hz) which was coupled with a secondary methyl group and a vinyl proton at δ 5.30 (*t*, *J* = 6.9 Hz), as well as an allylic methylene bearing a hydroxyl group at δ 4.19 and 4.22 (each *dd*, *J* = 12.4, 6.8 Hz). The ^{13}C NMR spectrum also suggested the presence of a trisubstituted double bond (δ_C 119.4 *d* and 146.4 *s*) and an allylic hydroxy methyl group (δ_C 59.1 *t*). The INEPT spectra indicated that there were four methyls, four methylenes, two methines and two quaternary carbons (Table 1). This compound gave ^1H NMR (Table 2), ^{13}C NMR and mass spectral data similar to those of naviculol (**11**) [10], which was also isolated from the same liverwort. The structure of **1** was further confirmed by the HMBC experiment (Fig. 1), with the aid of the ^{13}C–^1H COSY spectrum. The protons of the C-12 methyl group showed connectivity to C-7, C-1, C-8 and C-9, the protons of the C-14 methyl group to C-3, C-4, C-8 and C-9, and the C-13 methyl protons to C-2, C-1 and C-8. It also indicated the presence of a correlation between the C-15 methyl protons and the carbons C-4, C-9 and C-5, and the proton at C-10 and the carbons C-6 and C-4, as well as connectivity



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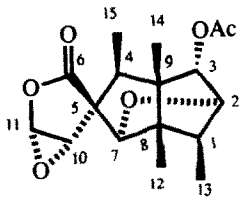

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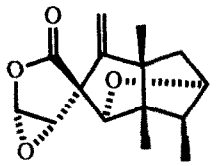

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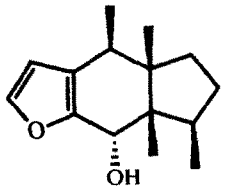

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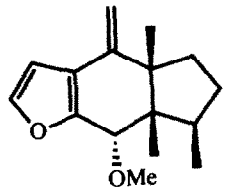

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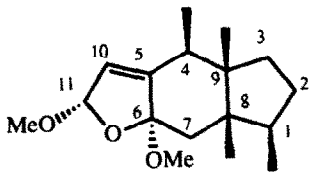

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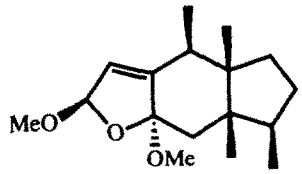

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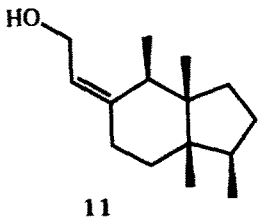

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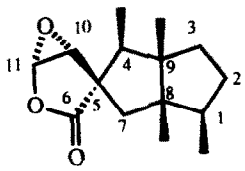

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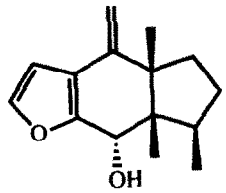

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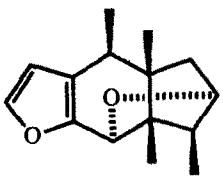

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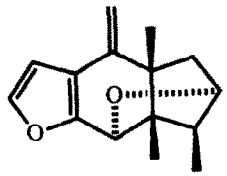

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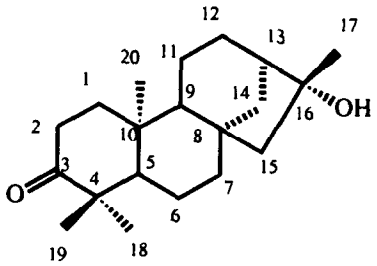
between the protons at C-11 and the carbons C-10 and C-5. In order to clarify the stereostructure of **1**, the difference NOE spectra were measured and the presence of NOE between H-10 and H-15 was observed, indicating that the 5(10)-ene had the *E*-configuration. Hence, compound **1** was the geometrical isomer of naviculol (**11**).

Although the chemical shifts for **1** are significantly different from those for **11** (Table 2), these can be explained by their conformations. Namely, naviculol (**11**) adopts the non-steroid conformatiopn as a preferred one due to severe repulsion between the C-15 methyl group and C-11 methylene, while the predominant conformation for isonaviculol (**1**) is the steroidal one. Each steric energy and conformation calculated by MM2 is shown in Fig. 2, which was supported by the presence of the NOEs (*vide supra*) [10].



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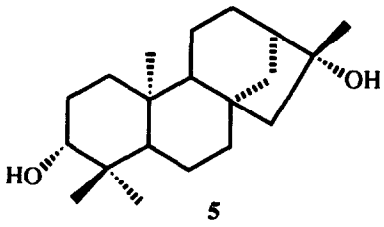

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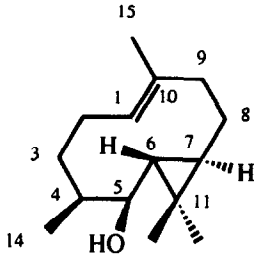

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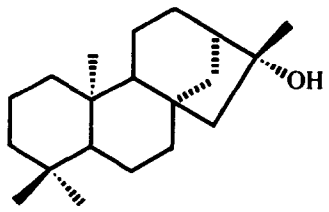

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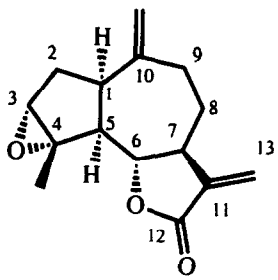

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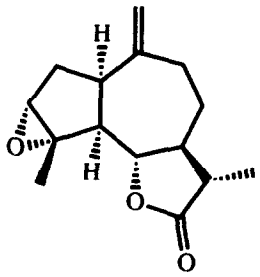

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Spirodensifolin A (2)

Spirodensifolin A (2) was obtained as crystals, mp 193–195°, and its molecular formula, $C_{17}H_{22}O_6$, was determined by the presence of a molecular ion peak at *m/z* 322.1425 in the high resolution mass spectrum (HRMS). The IR spectrum showed the presence of a γ-lactone at 1785 cm^{-1} and an acetoxyl group at 1730 cm^{-1}. The ^1H NMR spectrum of 2 revealed the presence of two secondary methyl groups at δ 1.03 and 1.11, two tertiary methyl groups at δ 1.15 and 1.32, an acetoxyl group at δ 2.17, and five methine protons attached to the carbon bearing oxygen functions at δ 3.93 (*s*), 4.03 (*t*, *J* = 2.0 Hz), 4.38 (*d*, *J* = 2.2 Hz), 5.09 (*d*, *J* = 2.0 Hz), and 5.55 (*d*, *J* = 2.2 Hz). The ^{13}C NMR spectrum also suggested the presence of a γ-lactone group at δ_C 78.8 (*d*) and 175.8 (*s*). The absence of hydroxyl absorption in the IR spectrum indicated that the remaining oxygen atoms were attributed to ether linkages. This assumption was further confirmed by the signals at δ_C 59.2 (*d*), 79.4 (*d*) and 88.8 (*d*) in the ^{13}C NMR spectrum. The planar structure of 2 was determined by the HMBC spectrum (Fig. 3). The protons of the C-12 methyl group showed connectivity to C-1, C-9, C-8 and C-7, and the C-13 methyl protons showed connectivity to C-1, C-8 and C-2. It also showed connectivity between the protons of the C-14 methyl group and the carbons C-4, C-9, C-8 and C-3. The C-15 methyl protons showed connectivity to C-4, C-9 and C-5. Furthermore, it indicated the presence of correlation between the proton at C-4 and the carbons C-15, C-9, C-10, C-8 and C-3, and the proton at C-7 and the carbons C-4, C-9 and C-10. Both protons at C-10 and C-11 showed connectivity to C-6, and connectivity between the proton at C-10 and the carbon C-5 was also observed. These facts indicate the structure of 2 is a rearranged pinguisane-type sesquiterpene having a spirolactone moiety, which is unusual in nature [17]. Its stereochemistry was determined by the presence of NOEs between (i) H-3 and the following protons: H-2, H-13 and H-14, (ii) H-7 and H-12, (iii) H-13 and H-12, (iv) H-13 and H-2 and (v) H-14 and H-15, indicating that all methyl

Table 1. ^{13}C NMR data of compounds **1–3**, **6**, **8–12** and **14** (100 MHz)

C	1*	2*	3†	6†	8†	9†	10†	11* [10]	12†‡	14*
1	36.4	46.5	49.5	37.0	38.8	36.2	36.2	44.0	42.1	38.5
2	29.2	79.4	79.3	29.8	29.5	28.9	28.9	38.3	31.2	29.1
3	34.7	84.6	47.7	34.1	35.1	33.3	33.2	30.4	35.5	34.4
4	40.5	44.7	159.2	33.4	146.4	34.7	34.7	40.6	50.1	145.8
5	146.5	59.6	60.3	120.5	118.5	148.3	148.7	147.3	55.7	118.5
6	25.3	175.8	175.0	150.2	150.3	110.0	112.2	30.0	179.4	149.8
7	32.9	88.8	87.6	65.5	75.8	43.8	43.5	39.5	44.3	66.5
8	45.5	61.7	58.6	47.5[a]	50.8[a]	47.1	47.3	44.3[a]	54.3	50.1[a]
9	50.2	49.6	50.2	51.3[a]	50.3[a]	50.7	50.7	47.8[a]	56.3	50.9[a]
10	119.4	59.2	58.2	109.3	107.2	121.3	122.1	121.7	57.1	106.9
11	59.1	78.8	77.3	142.5	143.0	106.6	108.2	58.6	76.0	143.2
12	19.0	11.8	9.9	13.9[b]	13.6	19.0	19.0	19.2[b]	16.5	12.9
13	15.0	9.7	9.7	15.8[b]	15.1	15.0	15.0	19.4[b]	14.6	15.1
14	15.3	19.7	21.7	14.9[b]	26.0	14.6	14.8	21.9[b]	18.3	25.4
15	12.1	17.1	111.5	14.8[b]	107.8	11.9	11.9	16.6	10.8	107.8
Others		21.1 (OAc)			58.4 (OMe)	49.6 (6-OMe)	48.9 (6-OMe)			
		170.5 (OAc)				55.6 (11-OMe)	54.2 (11-OMe)			

*In $CDCl_3$.
†In C_6D_6.
‡Numbering is used as in other rearranged pinguisane-type compounds, different from the original one [11].
[a,b]Assignments may be interchanged in each vertical column.

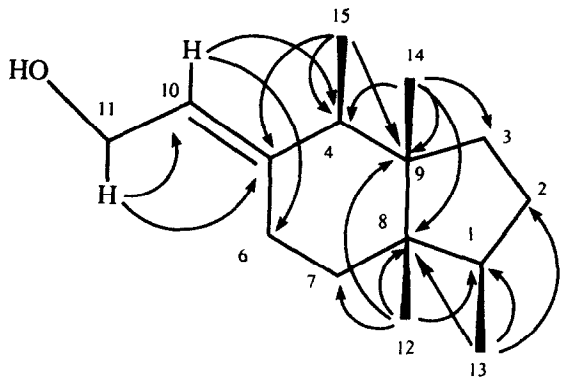


Fig. 1. Long-range correlations for isonaviculol (**1**) detected by the HMBC spectrum.

groups in **2** had the β-orientation and the acetoxyl group was thus α-orientated (Fig. 4). These facts led to the conclusion that the stereostructure of **2** should be expressed as shown, except for the configuration of the epoxide which remains to be established. Although an NOE was observed on H-4 upon irradiation of H-10, both the α- and β-configurations for the epoxide ring can explain these results. Therefore, the X-ray crystallographic analysis of **2** was carried out. The result is shown in the Fig. 5, and the stereochemistry of the epoxide ring is clearly established as depicted in the formula [9].

Spirodensifolin B (**3**)

Spirodensifolin B (**3**) was obtained as a minor component, and its molecular formula, $C_{15}H_{18}O_4$, was determined by HR mass spectrometry (*m/z* 262.1221 $[M]^+$). The IR spectrum showed the band due to a γ-lactone (1790 cm^{-1}) group. The ^1H NMR spectrum indicated the signals due to a secondary methyl group (δ 1.04), two tertiary methyl groups (δ 1.18, 1.23), four methine protons attached to the carbon bearing oxygen functions (δ 3.90, 4.08, 4.24, 5.58), and an exomethylene group (δ 5.12, 5.28). The above spectral data resembled those of **2**, except for the presence of two protons of an exomethylene group and the absence of a secondary methyl group and an acetoxyl group. The spectral data indicated that the secondary methyl group at C-4 in **2** was replaced by the exomethylene group in **3** and the acetoxyl group at C-3 by the hydrogen. Thus, the above results, together with the co-occurence of **2** in the same liverwort, established the structure of spirodensifolin B as **3**.

ent-*Kauran*-16β-*ol*-3-*one* (**4**)

The major component, *ent*-kauran-16β-ol-3-one (**4**), was obtained as crystals, mp 162–163°. The HR mass spectrum suggested the molecular formula $C_{20}H_{32}O_2$ (*m/z* 304.2420 $[M]^+$), thus indicating five degrees of unsaturation in the molecule. Since the ^{13}C NMR spectrum contained only one carbonyl resonance (δ_C 218.1), the molecule should be tetracyclic. An absorption at 1700 cm^{-1} in the IR spectrum confirmed the presence of a ketone in the molecule. The IR spectrum also showed the band due to a hydroxyl group at 3300 cm^{-1}, which was confirmed by the resonance at δ_C 79.2 (*s*) in the ^{13}C NMR spectrum. The ^1H NMR spectrum indicated the presence of four tertiary methyl groups at δ 1.03, 1.07, 1.08 and 1.38. In order to clarify the partial structures of **4**, the

Table 2. ^{1}H NMR data of compounds **1**, **6**, **8–11** and **14** (400 MHz)

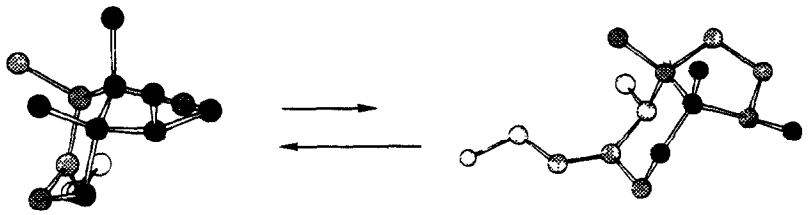
H	1*	11*[10]	6*	6†	8†	9†	10†	14*
1	‡	1.68 *m*	‡	1.69 *m*	1.73 *m*	2.99 *m*	2.79 *m*	‡
2	‡	1.81 *m*	‡	‡	1.03 *m*	1.75 *m* (α)	1.77 *m* (α)	‡
		1.89 *m*	‡	‡	1.73 *m*	1.19 *m* (β)	1.18 *m* (β)	‡
3	‡	1.14 *m*	‡	‡	1.60 *m*	1.60 *td* (9.8, 3.7) (α)	1.59 *td* (9.8, 3.7) (α)	‡
		1.26 *m*	‡	‡	2.08 *br t*	1.28 *td* (12.0, 5.4) (β)	1.28 *td* (12.0, 5.4) (β)	‡
4	2.27 *q* (6.8)§	2.46 *q* (7.3)	2.58 *q* (7.3)	2.40 *q* (7.1)	—	2.34 *qd* (6.8, 1.6)	2.34 *m*	—
5	—	—	—	—	—	—	—	—
6	‡	2.34 *ddd* (α) (13.7, 12.5, 4.4) 1.98 *ddd* (β) (13.7, 5.4, 5.4)	—	—	—	—	—	—
7	‡	1.34 *m* (α) 1.45 *ddd* (β) (13.7, 10.4, 5.8)	4.34 *s*	4.28 *s*	3.92 *s*	2.39 *d* (14.2) (α) 1.43 *d* (14.2) (β)	1.73 *d* (14.2) (α) 2.34 *d* (14.2) (β)	4.45 *s*
8	—	—	—	—	—	—	—	—
9	—	—	—	—	—	—	—	—
10	5.30 *t* (6.9)	5.40 *dd* (7.3, 6.4)	6.23 *br s*	6.08 *d* (2)	6.31 *br s*	5.36 *br s*	5.36 *br s*	6.47 *d* (1.9)
11	4.19 *dd* (12.4, 6.8) 4.22 *dd* (12.4, 6.8)	4.10 *dd* (6.6, 6.4) 4.17 *dd* (7.3, 6.6)	7.34 *br s*	7.17 *d* (2)	7.01 *s*	5.27 *s*	5.72 *br s*	7.34 *d* (1.9)
12	0.62 *s*	0.94 *s*	1.00 *s*	0.93 *s*	1.03 *s*	0.53 *s*	0.52 *s*	1.22 *s*[a]
13	0.86 *d* (6.8)	0.92 *d* (7.3)	0.86 *d* (6)	0.67 *d* (6.4)	0.66 *d* (6.2)	0.94 *d* (6.8)	0.94 *d* (6.8)	0.81 *d* (6.6)
14	0.66 *s*	0.94 *s*	0.89 *s*	0.88 *s*	1.43 *s*	0.58 *s*	0.69 *s*	1.04 *s*[a]
15	0.96 *d* (6.8)	1.09 *d* (7.3)	1.16 *d* (7.3)	1.03 *d* (7.1)	5.10 *s* 5.30 *d*	0.86 *d* (6.8)	0.87 *d* (6.8)	5.14 *s* 5.29 *s*
Others					3.39 *s* (OMe)	3.21 *s* (OMe) 3.33 *s* (OMe)	2.95 *s* (OMe) 3.31 *s* (OMe)	

*In $CDCl_3$.
†In C_6D_6.
‡Not assigned due to severe overlap of the signals.
§Coupling constants (*J* in Hz) are given in parentheses.
[a]Assignments may be interchanged in each vertical column.

Table 3. ^{1}H NMR data of **2**, **3** and **12** (400 MHz)

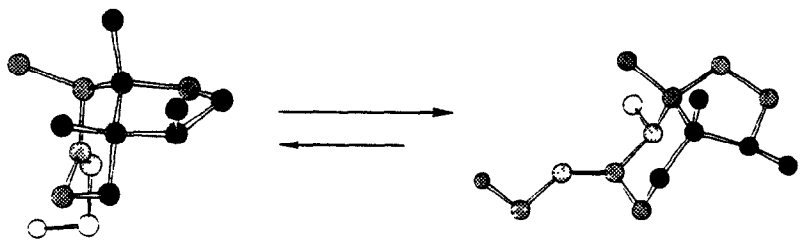
H	2†	3†	3‡	12† [11]§	12‡
1	1.98 *qd* (7.6, 2.0)‖	*	1.67 *brq* (7.3)	2.38 *m*	2.44 *m*
2	4.03 *t* (1.7)	4.08 *brs*	3.67 *brs*	1.3–1.4 *m*	1.18 *m*
				1.71 *m*	1.70 *m*
3	5.09 *d* (1.7)	*	1.55 *dd* (13.8, 1.7)	1.3–1.4 *m*	1.09 *m*
			1.64 *brd* (13.8)	1.87 *m*	1.43 *m*
4	2.76 *q* (7.8)	—	—	2.61 *q* (7.7)	2.63 *q* (7.3)
5	—	—	—	—	—
6	—	—	—	—	—
7	3.93 *s*	3.90 *s*	3.62 *s*	1.39 *d* (13.6)	0.79 *d* (14.2)
				1.99 *d* (13.6)	1.69 *d* (14.2)
8	—	—	—	—	—
9	—	—	—	—	—
10	4.38 *d* (2.2)	4.24 *d* (2.2)	4.00 *d* (2.2)	3.55 *d* (2.4)	2.69 *d* (2.4)
11	5.55 *d* (2.2)	5.58 *d* (2.2)	4.71 *d* (2.2)	5.52 *d* (2.4)	4.72 *d* (2.4)
12	1.32 *s*	1.18 *s*	0.91 *s*	10.49 *s*	0.79 *s*
13	1.11 *d* (7.6)	1.04 *d* (7.3)	0.56 *d* (7.3)	0.84 *d* (7.7)	0.73 *d* (6.8)
14	1.15 *s*	1.23 *s*	1.11 *s*	0.93 *s*	0.60 *s*
15	1.03 *d* (7.8)	5.12 *s*	4.96 *s*	0.98 *d* (7.7)	0.91 *d* (7.3)
		5.28 *s*	5.18 *s*	5.40 *s*	
Others	2.17 *s* (OAc)				

*Not assigned.
†In $CDCl_3$.
‡In C_6D_6.
§Numbering is used as in other rearranged-pinguisane type compounds, different from the original one [11].
‖Coupling constants (*J* in Hz) are given in parentheses.



31.66 kcal **34.03 kcal**

Naviculol 11



32.03 kcal **31.06 kcal**

Isonaviculol 1

Fig. 2. Conformations and steric energies for naviculol (**11**) and isonaviculol (**1**) calculated by MM2.

HMBC experiment was examined with the aid of the ^{13}C–^{1}H COSY spectrum. It showed connectivities as shown in Fig. 6. The carbonyl group was assigned to C-3 based upon the observation of connectivity between both the C-18 and C-19 methyl protons and C-3. On the bases of the above data, compound **4** was thus suggested to be a kaurane-type diterpene possessing a carbonyl group on C-3 as shown. This assumption was further confirmed by Wolff–Kishner reduction of **4**, which gave a corresponding deoxo compound whose spectral data including the optical rotation were identical with those of *ent*-kauran-16β-ol (**13**) [14–16], isolated from the same liverwort. Thus, the structure of **4** was established as *ent*-kauran-16β-ol-3-one.

ent-*Kaurane*-3β,16β-*diol* (**5**)

ent-Kaurane-3β,16β-diol (**5**) was also obtained as crystals, mp 202–203°, and its molecular formula was determined to be $C_{20}H_{34}O_2$ by the appearance of a molecular ion peak at *m/z* 306.2553 in the HR mass spectrum. The ^{1}H NMR spectrum of **5**, similar to that of **4**, contained the signals due to four tertiary methyl groups at δ0.77, 0.97, 1.02 and 1.36 and a proton attached to the carbon bearing a hydroxyl group at δ3.19 (*dd*, $J = 5.5$ and 10.9 Hz). The ^{13}C NMR spectrum also indicated the presence of hydroxyl group at δ_C 79.1 (*d*) and 79.3 (*s*). The above data along with the co-occurence of **4** in the same liverwort are consistent with the presence of a hydroxyl group at C-3 in place of the carbonyl group in **4**, and hence the compound **5** was suggested to be kaurane-3,16-diol. The double doublets resonance at δ3.19 (axial) indicated the α-position of the hydroxyl group at C-3. Treatment of **4** with $NaBH_4$ in methanol gave a diol which was found to be completely identical with **5** in all respects. This fact confirmed the absolute configuration of *ent*-kaurane-3β,16β-diol as **5**.

From the *n*-hexane extract of *T. sandvicensis* was isolated a new pinguisane-type sesquiterpenoid **6**, and a new lepidozane-type sesquiterpenoid **7**, in addition to the known pinguisanin (**15**) [18, 19], dehydropinguisanin (**16**) [1], dehydropinguisenol (**14**) [1] and ptychanolide (**12**) [11]. The pinguisanes containing methoxyl groups, compounds **8**, **9** and **10** were also identified as artifacts. Each of them was obtained as a pure compound by a combination of column chromatography on silica gel and Sephadex LH-20 and HPLC.

Furanopinguisanol (**6**)

The molecular formula of furanopinguisanol (**6**) was determined to be $C_{15}H_{22}O_2$ from the HR mass spectrum (*m/z* 234.1613 $[M]^+$). The IR spectrum showed the presence of a hydroxyl group at 3425 cm^{-1}. The ^{1}H NMR spectrum of **6** contained the signals of two protons of a typical α,β-disubstituted furan ring at δ6.23 and 7.34 (each 1H, *br s*), two tertiary methyl groups at δ0.89 and 1.00, two secondary methyl groups at δ0.86 and 1.16, and a proton on a carbon bearing a secondary hydroxyl group at δ4.34 (*s*). The above spectral data were quite similar to those of dehydropinguisenol (**14**), suggesting that the exomethylene group at C-4 in **14** was replaced by the methyl group in **6**. The information on the relative stereochemistry was deduced from NOE difference experiments (Fig. 7). NOEs were observed between (i) H-12 and the following protons: H-7, H-13 and H-14, (ii) H-13 and H-7, and (iii) H-15 and H-14, indicating that all methyl groups in **6** had the β-orientation. Hence, the hydroxyl group had the α-orientation. Hydrogenation of **14** with 10% Pd-C as the catalyst afforded a corresponding dihydro derivative (yield 19%) whose spectral data

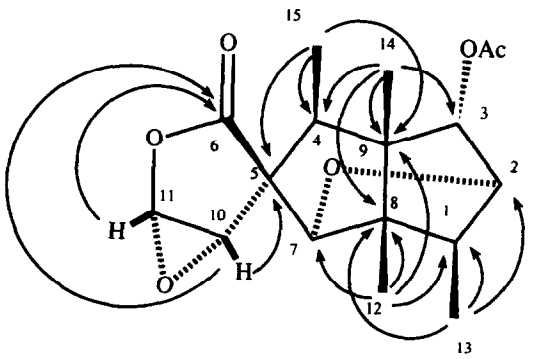


Fig. 3. Long-range correlations for spirodensifolin A (**2**) detected by the HMBC spectrum.

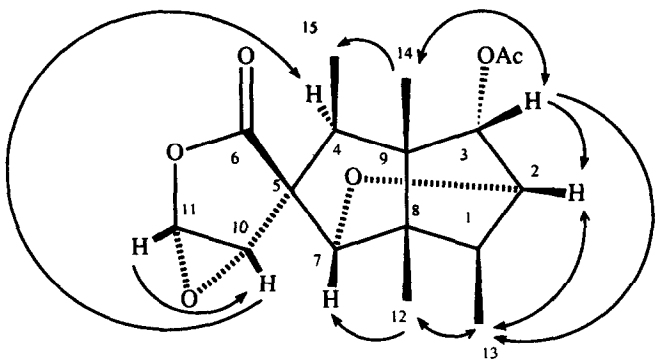


Fig. 4. NOEs detected by the NOE difference spectra of spirodensifolin A (**2**).

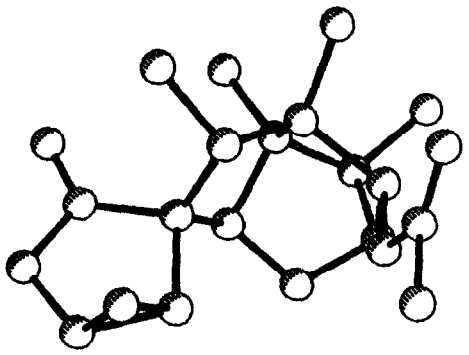


Fig. 5. Perspective view of spirodensifolin A (2).

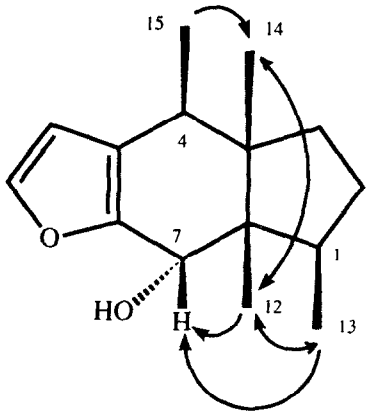


Fig. 7. NOEs detected for furanopinguisanol (6).

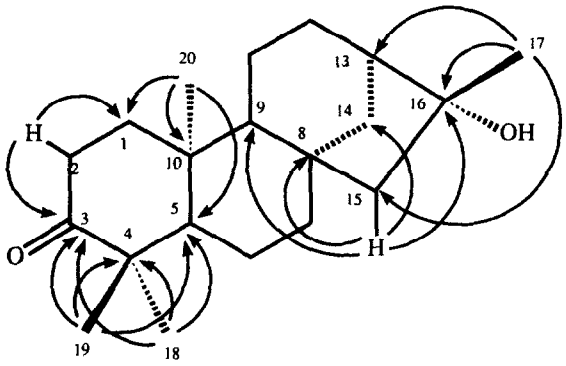


Fig. 6. Long-range correlations for 4 detected by the HMBC spectrum.

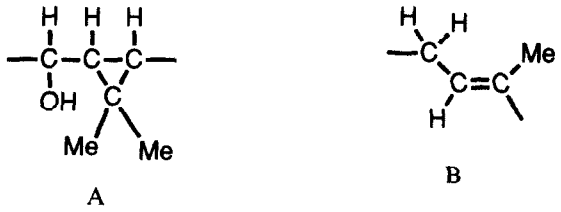


Fig. 8. Partial structures for compound 7.

including the optical rotation were identical with those of **6**. From the above data as well as the co-existence of dehydropinguisenol (**14**) in the same liverwort, it was surmised that the structure of furanopinguisanol was as shown in **6**.

(4*S**,5*S**,6*R**,7*R**)-1(10)*E*-*Lepidozen*-5-*ol* (7)

Compound **7** had molecular formula $C_{15}H_{26}O$, which was determined by HR mass spectrometry (*m/z* 222.1981 $[M]^+$). The IR spectrum of **7** exhibited the presence of a hydroxyl group at 3400 cm^{-1}. From the ^1H NMR spectrum, it was shown that there were signals due to a cyclopropane at δ −0.2 (*ddd*, J = 8.1, 5.5, 2.4 Hz) and 0.24 (*dd*, J = 9.2, 5.5 Hz), two tertiary methyl groups at5 δ0.99 and 1.00, one secondary methyl group at δ1.10 (*d*, J = 7.3 Hz), a vinyl methyl group at δ1.51, a vinylic proton at δ5.23 (*t*, J = 7.9 Hz), and a proton attached to the carbon bearing a hydroxyl group at δ3.05 (*dd*, J = 9.2, 2.2 Hz). The ^{13}C NMR spectrum also suggested the presence of a secondary hydroxyl group at δ_C 77.0 (*d*) and a trisubstituted double bond at δ_C 127.4 (*d*) and 133.2 (*s*). Furthermore, the signals of four methyls, four methylenes, three methines and a quaternary carbon appeared in the ^{13}C NMR spectrum (see Experimental). The above spectral data coupled with the molecular formula indicated that **7** was a bicyclic sesquiterpenoid with one hydroxyl and one trisubstituted double bond. In order to clarify the partial structure of **7**, the ^{13}C–^1H and ^1H–^1H COSY spectra were measured and thereby the partial structures A and B were confirmed (Fig. 8). Furthermore, the HMBC experiment was examined and correlation between each methyl group and the other atoms was obtained as shown in Fig. 9. The difference NOE spectra showed the NOEs between (i) H-7 and H-4 and H-5, and (ii) H-5 and H-4. Thus, compound **7** has a lepidozane skeleton [20] with the most probable configurations as shown in the formula.

Dehydropinguisenol methyl ether (8)

The molecular formula of **8** was determined as $C_{16}H_{22}O_2$ by the presence of a molecular ion peak at *m/z* 246.1627 $[M]^+$. The IR and ^1H NMR spectra revealed the presence of an exomethylene group (1640, 870 cm^{-1}; δ5.10, 5.30) and a furan ring (1585, 1500 cm^{-1}; δ6.31, 7.01). The ^1H NMR spectrum also showed the presence of a secondary methyl group (δ0.66), two tertiary methyl groups (δ1.03, 1.43), a methoxyl group (δ3.39) and a proton on the carbon bearing a secondary methoxyl group (δ3.92). The above spectral data were similar to those of **14**, except for the presence of a methoxyl group. The absence of hydroxyl absorption in the IR spectrum indicated that the hydroxyl group at C-7 in **14** was replaced by the methoxyl group in **8**. Thus, the above

observations and the co-occurence of **14** in the same liverwort led to the structure of **8** to be a methoxy derivative of **14**. This might be formed by dehydration of **14** and attack of methanol during column chromatography of Sephadex LH-20 using chloroform–methanol as an eluent.

6α,11α-*Dimethoxypinguis*-5(10)-*ene* (**9**)

The molecular formula for **9**, $C_{17}H_{28}O_3$, was determined by its HR mass spectrum [*m/z* 280.2016 $[M]^+$), indicating four degrees of unsaturation. The ^1H NMR spectrum contained the signals attributable to two secondary methyl groups at δ0.86 and 0.94, two tertiary methyl groups at δ0.53 and 0.58, two methoxyl groups at δ3.21 and 3.33, vinyl proton at δ5.36, and a methine proton attached to the carbon bearing an oxygen function at 5.27. The ^{13}C NMR spectrum also suggested the presence of a trisubstituted double bond and two methoxyl groups as well as a methine and a quaternary carbon bearing oxygen functions. The complete decoupled ^{13}C NMR and INEPT spectra indicated that there were four methyls, three methylenes, two methines and two quaternary carbons (Table 1). The long-range ^{13}C–^1H COSY coupled with ^{13}C–^1H and ^1H–^1H COSY spectra allowed complete assignment of all proton and carbon resonances to the pinguisane-type skeleton. The relative stereochemistry was deduced from NOE difference experiments (Fig. 10). NOEs were observed between (i) H-12 and H-13, and H-7β, (ii) H-14 and H-7β, (iii) OMe-6 and H-4, and (iv) H-4 and H-1, indicating that all methyl groups in **9** had the β-orientation and the C-6 methoxyl group was α-orientated. The α-configuration of the C-11 methoxyl group was suggested by the relatively upfield chemical shift of H-11 (δ5.27) (*vide infra*) [21]. These facts led to the conclusion that the stereostructure of **9** should be expressed as shown.

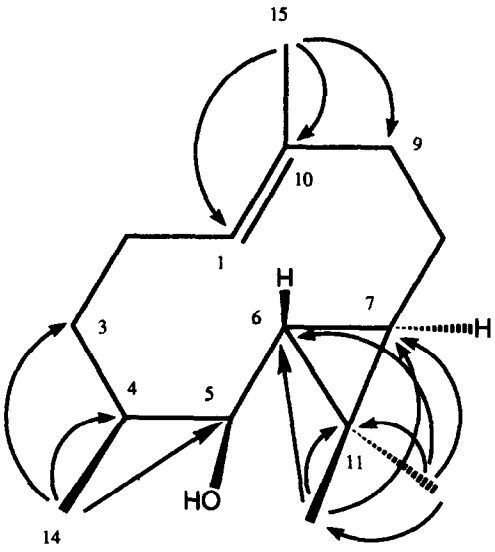


Fig. 9. Long-range correlations detected for **7** by the HMBC spectrum.

6α,11β-*Dimethoxypinguis*-5(10)-*ene* (**10**)

Compound **10** has the same molecular formula as compound **9**, $C_{17}H_{28}O_3$ (*m/z* 280.2039 $[M]^+$), and the IR spectrum showed the presence of a double bond (1665, 1465 cm^{-1}). The ^1H NMR spectrum of **10** was very similar to that of **9**, which contained the signals due to two secondary methyl groups (δ0.87, 0.94), two tertiary methyl groups (δ0.52, 0.69), two methoxyl groups (δ2.95, 3.31), an isolated methylene group (δ1.73, 2.34), a vinyl proton (δ5.36) and a proton on a carbon bearing oxygen functions (δ5.72). This compound also had a ^{13}C NMR spectrum similar to that of **9**, indicating that it had the same planar structure as **9**. In order to clarify the stereostructure of **10**, the difference NOE spectra were measured and the NOEs between (i) H-12 and H-2β, and H-3β, (ii) H-13 and H-12, (iii) H-14 and H-2β, and (iv) OMe-6 and H-4, and H-11 were observed (Fig. 11). These results indicated that all methyl groups in **10**, as well as

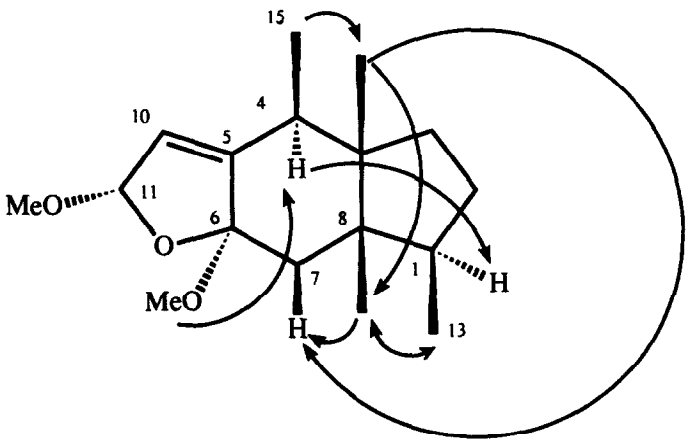


Fig. 10. NOEs detected for compound **9**.

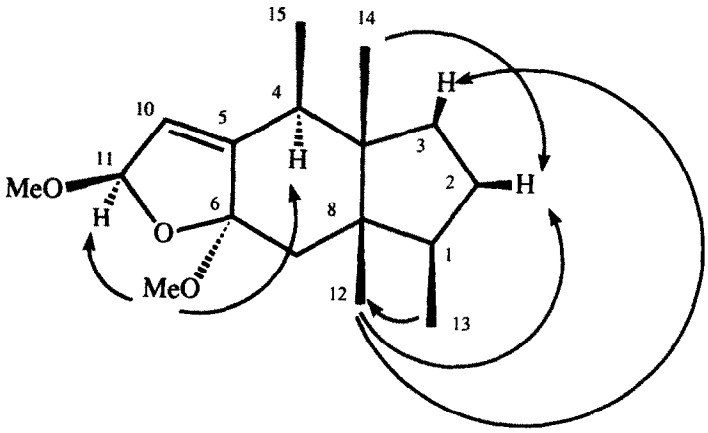


Fig. 11. NOEs detected for compound **10**.

the C-11 methoxyl group, were β-orientated, and that the C-6 methoxyl group had the α-orientation. The relatively downfield chemical shift of the H-11 (δ5.72) also suggested the configuration of the C-11 methoxyl group to be β-orientated (*vide supra*) [21]. Hence, compound **10** was identified as shown.

Biogenesis of pinguisane-type and rearranged pinguisane-type sesquiterpenoids

The existence of pinguisane-type sesquiterpenoids is very interesting since their structures are difficult to rationalize in terms of the isoprene rule. We propose the possible biogenetic pathway for the formation of the pinguisane-type sesquiterpenoids as shown in Scheme 1.

The mode of formation of the rearranged pinguisane-type sesquiterpenoids in the plant was suggested by Connolly [22]. Rearrangement of the 5α,6α-epoxide (**19**) and 5β,6β-epoxide (**20**) of pinguisane provides a possible route, leading via **21** and **22**, respectively, to the spiro system with the correct stereochemistry (Scheme 2). On the other hand, Takeda *et al.* [11] suggested that the pinguisane-type sesquiterpenoid, i.e. deoxopinguisone (**23**), is formed from the rearranged pinguisane-type sesquiterpenoid by the mechanism as shown in Scheme 3.

Pinguisane-type sesquiterpenoids, usually rare in nature, have been frequently encountered only in liverworts. From the results discussed above, it is very interesting from the taxonomic view-point that *Frullanoides densifolia* and *Trocholejeunea sandvicensis* as well as *Ptychantus striatus*) [11] (Lejeuneaceae) produce the same pinguisane-type sesquiterpenoids as *Porella navicularis* [10], *P. platyphylla* [18, 19], *P. densifolia* [23] and *P. japonica* [24] (Porellaceae), although the species belonging to both families are morphologically quite different. Pinguisane-type sesquiterpenoids have also been found in *Ptilidium ciliare* [25] and *P. pulcherrimum* [26] (Ptilidiaceae) belonging to the Jungermanniales and *Aneura pinguis* [27, 28] (Aneuraceae) belonging to the Metzgeriales. Schuster [29] proposed that the Jungermanniales and Metzgeriales originated in a common ancestor. On the other hand, Asakawa [30] independently reported the same conclusion as described above by the chemosystematics results. These experimental results further support the above taxonomically and evolutionally important relationship between the two orders of the Jungermanniales and the Metzgeriales.

Dehydropinguisenol (**14**) showed cytotoxic activity (ED_{50} 12.5 $\mu g\,ml^{-1}$) against KB cells.

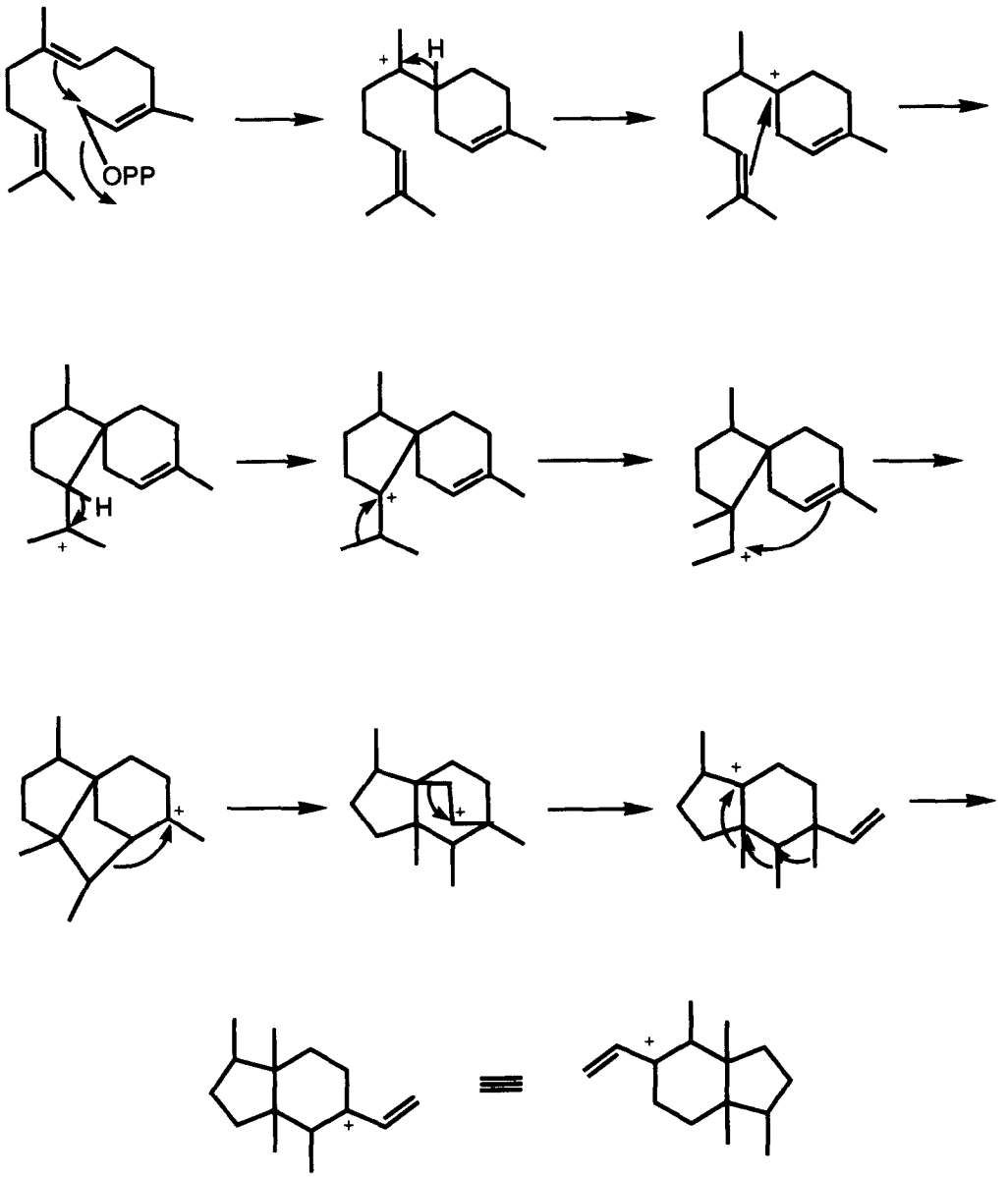
EXPERIMENTAL

Mps: uncorr. ^1H NMR: 90 or 400 MHz, ^{13}C NMR: 100 MHz (in $CDCl_3$ or C_6D_6 soln, TMS as int. standard). CC: silica gel 60 (70–230 mesh, Merck) and Sephadex LH-20. TLC: silica gel $60F_{254}$ 0.25 mm (Merck). Spots were visualized by UV (254 nm) and 30% H_2SO_4.

Plant material. The liverwort *Frullanoides densifolia* Radii was collected in Bolivia in November 1989 and was identified by R. Grandstein, University of Utrecht. The voucher specimen is deposited in the Herbarium at the University of Utrecht. *Trocholejeunea sandvicensis* (Gott.) Mizut. was collected at Tokushima city in June 1990 and was identified by Y. Asakawa. The voucher specimen is deposited at the Herbarium of Institute of Pharmacognosy, Tokushima Bunri University.

Extraction and isolation. (i) The air-dried powdered *F. densifolia* (150 g) was extracted with Et_2O for a month at room temp. The crude extract (3.65 g) was fractionated by CC on silica gel with a step gradient (C_6H_6–EtOAc) to give 6 fractions of A (C_6H_6, 490 mg), B (C_6H_6–EtOAc, 19:1) (397 mg), C (C_6H_6–EtOAc, 9:1) (2.36 g), D (C_6H_6–EtOAc, 4:1) (50.6 mg), E (C_6H_6–EtOAc, 1:1) (55.2 mg) and F (EtOAc) (88.7 mg). Further sepn of fr. B by Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) and silica gel CC gave **12**.

Fr. C, which was divided into 4 parts (C1, C2, C3 and C4), was further sepd. Purification of fr. C1 with HPLC (*n*-hexane–EtOAc, 9:1) yielded **3** (5.0 mg) and sepn of fr. C2 with HPLC (CH_2Cl_2–MeOH, 99:1) gave **2** (90 mg), **18**



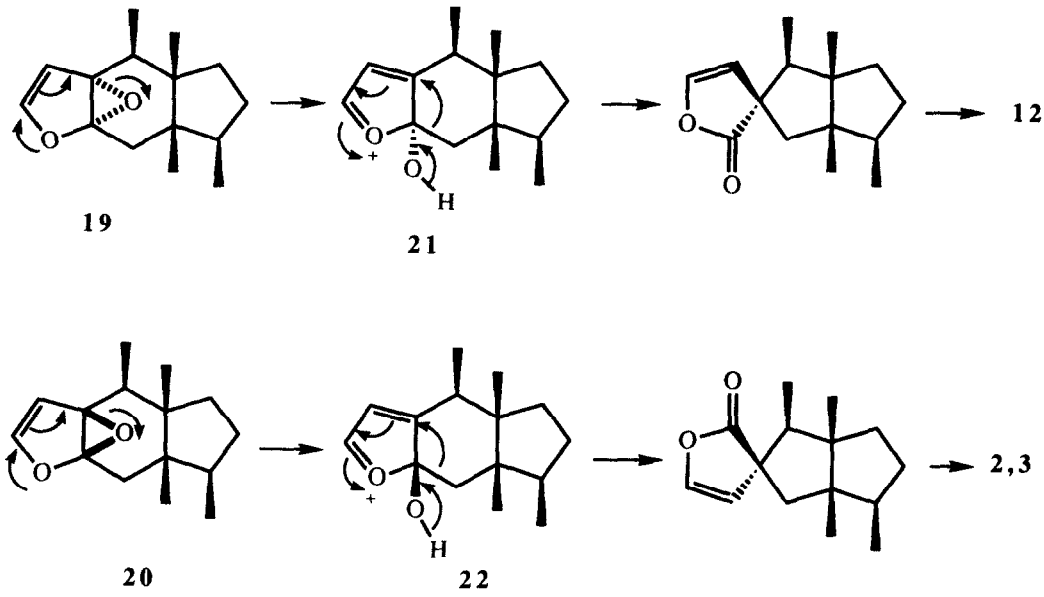
Pinguisanes

Scheme 1. Plausible biosynthetic pathway for the pinguisane-type sesquiterpenoids.

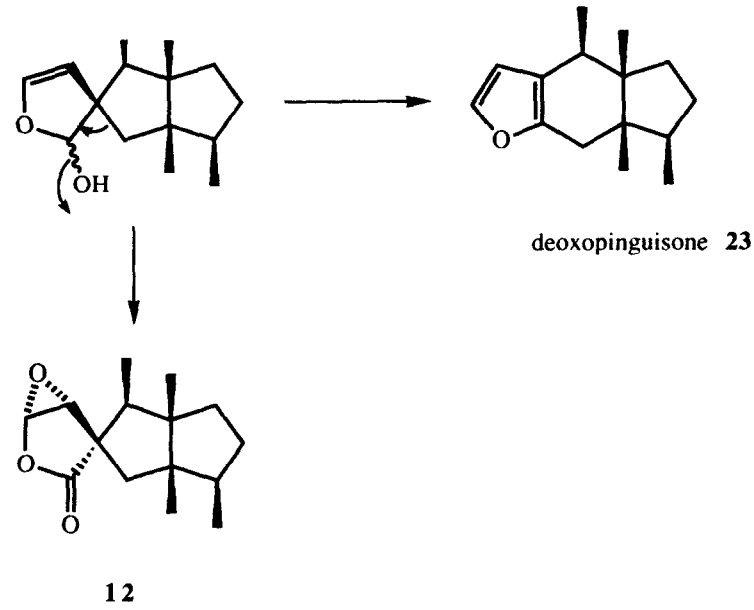
(21.4 mg) and **17** (13.6 mg). Sepn of fr. C3 with HPLC (*n*-hexane–EtOAc, 17:3) yielded **1** (3.8 mg), **11** (1.8 mg) and **13** (3.4 mg) as the minor components. Purification of fr. C4 by Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) yielded the major product **4** (170 mg). The diol **5** (26.5 mg) was isolated from fr. D by Sephadex LH-20 ($CHCl_3$–MeOH, 1:1). (ii) Ground *T. sandvicensis* (1.3 kg) was extracted with *n*-hexane for one week at room temp. The crude extract (22.8 g) was sepd directly by CC on silica gel using a *n*-hexane–EtOAc gradient to yield 7 fractions: fr. A (*n*-hexane) (0.97 g), fr. B (*n*-hexane–EtOAc, 19:1) (4.77 g), fr. C (*n*-hexane–EtOAc, 9:1) (8.67 g), fr. D (*n*-hexane–EtOAc, 17:3) (1.66 g), fr. E (*n*-hexane–EtOAc, 4:1) (2.43 g), fr. F (*n*-hexane–EtOAc, 1:1) (1.00 g) and fr. G (EtOAc). Fr. C was further sepd by HPLC (*n*-hexane–EtOAc, 9:1) to give **16** (12.6 mg), **15** (7.8 mg), **6** (79.8 mg), **14** (285.3 mg), **7** (25 mg) and **12** (6.9 mg). Sepn of fr. D by Sephadex LH-20 ($CHCl_3$–MeOH, 1:1), silica gel CC and HPLC (*n*-hexane–EtOAc, 19:1) gave **8** (36.5 mg), **9** (32.4 mg) and **10** (24.4 mg).

Isonaviculol (**1**). Oil; $[\alpha]_D^{25}$ −17.9° ($CHCl_3$; *c* 0.84); EI-MS: *m/z* 222 $[M]^+$ (10), 204 (17), 189 (37), 123 (90), 109 (100), 95 (50), 81 (49), 67 (35), 55 (40), 41 (60); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 3300.

Spirodensifolin A (**2**). Crystals, mp 193–195°; $[\alpha]_D^{22}$ −40.6° ($CHCl_3$; *c* 1.33); CD $[\theta]_{296nm}$ −260 ($CHCl_3$); HRMS: *m/z* 322.1425 $[M]^+$, calcd for $C_{17}H_{22}O_6$: 322.1416; EI-MS: *m/z* 322 $[M]^+$ (0.5), 306 (0.2), 262 (19),



Scheme 2. Plausible biosynthetic pathway into compounds **2**, **3** and **12** [22].



Scheme 3. Possible biosynthetic pathway into **12** and **23** [11].

205 (19), 165 (32), 149 (39), 137 (56), 97 (37), 55 (47), 43 (100); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1785, 1730, 1060, 850.

X-Ray analysis of spirodensifolin A (**2**). The compound was obtained from Et_2O–CH_2Cl_2 as thin leaf-like crystals, orthorhombic, space group $P2_12_12_1$, $a = 13.625$ (6), $b = 31.051$ (4), $c = 7.580$ (3) A, $F(000) = 1367$, $M_r = 322.357$, $Dx = 1.335$, $\mu(MoK\alpha) = 0.9426$. Of 2454 total unique reflections, 850 were considered to be observed at the level of $F_0 > 3.0\sigma(F_0)$. The final R value was 0.0999. The supplementary materials have been deposited at the Cambridge Crystallographic Data Centre [9].

Spirodensifolin B (**3**). Oil; $[\alpha]_D^{18} -36.8°$ ($CHCl_3$; c 0.31); HRMS: m/z 262.1221 $[M]^+$, calcd for $C_{15}H_{18}O_4$: 262.1205; EI-MS: m/z 262 $[M]^+$ (4), 234 (3), 218 (3), 205 (49), 163 (18), 135 (66), 119 (29), 109 (38), 97 (100) and 43 (28); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1790, 1640, 1385, 1310, 1075, 970, 850.

ent-*Kauran*-16*β*-*ol*-3-*one* (**4**). Crystals, mp 162–163°; $[\alpha]_D^{22} -71.7°$ ($CHCl_3$; c 0.46); CD: $[\theta]_{290nm} -3500$ (MeOH); HRMS: m/z 304.2420 $[M]^+$, calcd for $C_{20}H_{32}O_2$: 304.2402; EI-MS: m/z 304 $[M]^+$ (38), 286 (68), 246 (47), 231 (43), 200 (60), 147 (55), 119 (55), 105 (60), 94

(66), 81 (55), 55 (52), 43 (100); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300 (br), 1700. ^1H NMR (400 MHz, $CDCl_3$): δ 1.03 (3H, *s*, H-18), 1.07 (3H, *s*, H-19), 1.08 (3H, *s*, H-20), 1.38 (3H, *s*, H-17), 1.58 (2H, *s*, H-15), 1.89 (1H, *s*, H-13), 2.47 (2H, *dd*, $J=8.8$, 6.3 Hz, H-2); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 17.7 (*q*, C-20), 18.5 (*t*), 20.9 (*q*), 21.6 (*t*), 24.4 (*q*, C-17), 26.6 (*t*), 27.2 (*q*), 34.0 (*t*, C-2), 37.2 (*t*), 38.5 (*s*, C-10), 39.3 (*t*, C-1), 40.9 (*t*, C-14), 45.0 (*s*, C-8), 47.2 (*s*, C-4), 48.8 (*d*, C-13), 54.3 (*d*, C-5), 55.5 (*d*, C-9), 57.4 (*t*, C-15), 79.2 (*s*, C-16), 218.1 (*s*, C-3).

ent-*Kaurane-3β,16β-diol* (**5**). Crystals, mp 202–203°; $[\alpha]_D^{20}$ −36.8° ($CHCl_3$; *c* 0.19); HRMS: *m/z* 306.2553 $[M]^+$, calcd for $C_{20}H_{34}O_2$: 306.2559; EI-MS: *m/z* 306 $[M]^+$ (1), 288 (28), 270 (18), 255 (30), 227 (41), 187 (13), 135 (37), 119 (42), 105 (48), 94 (89), 43 (65); ^1H NMR (400 MHz, $CDCl_3$): δ 0.77 (3H, *s*), 0.97 (3H, *s*), 1.02 (3H, *s*), 1.36 (3H, *s*), 1.55 (2H, *s*), 3.19 (1H, *dd*, $J=10.6$, 5.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 15.4 (*q*), 17.8 (*q*), 18.1 (*t*), 20.2 (*t*), 24.5 (*q*), 26.9 (*t*), 27.3 (*t*), 28.3 (*q*), 37.6 (*t*), 38.7 (*t*), 38.8 (*s*), 39.1 (*s*), 42.0 (*t*), 45.1 (*s*), 49.0 (*d*), 55.1 (*d*), 56.7 (*d*), 57.9 (*t*), 79.1 (*d*), 79.3 (*s*).

Wolff–Kishner reduction of ent-*kauran-16β-ol-3-one* (**4**). A mixt. of ketone **4** (50 mg), hydrazine hydrate (0.6 ml), diethylene glycol (3.0 ml) and KOH (50 mg) was heated at 180° for 3 hr and the excess solvent was distilled off. The mixt. was heated again at 240° for 7 hr. 1 N HCl soln was added to the reaction mixt. which was extracted with Et_2O. The extract was dried over $MgSO_4$ and concd. Purification by CC using C_6H_6–EtOAc as the eluent, leaving pure crystals of **13** (37 mg); mp 218–220° (lit. [15] 216–217°); $[\alpha]_D^{22}$ −37.7° ($CHCl_3$; *c* 1.22) (lit. [15] −41°); EI-MS: *m/z* 290 $[M]^+$ (3), 272 (34), 257 (55), 229 (30), 123 (61), 106 (68), 94 (100), 81 (57), 69 (69), 55 (56), 41 (69); ^1H NMR (400 MHz, $CDCl_3$): δ 0.80 (3H, *s*), 0.84 (3H, *s*), 1.02 (3H, *s*), 1.36 (3H, *s*); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 17.8 (*q*), 18.0 (*t*), 18.6 (*t*), 20.5 (*t*), 21.6 (*q*), 24.5 (*q*), 27.0 (*t*), 33.3 (*s*), 33.6 (*q*), 37.7 (*t*), 39.4 (*s*), 40.4 (*t*), 42.1 (*t* × 2), 45.4 (*s*), 49.1 (*d*), 56.3 (*d*), 56.9 (*d*), 58.1 (*t*), 79.3 (*s*).

$NaBH_4$ *reduction of* ent-*kauran-16β-ol-3-one* (**4**). A mixt of **4** (11 mg) and $NaBH_4$ (20 mg) in MeOH was stirred at room temp. for 3 hr. The reaction mixt. was diluted with H_2O and extracted with $CHCl_3$. The extract was washed with satd NaCl, dried over $MgSO_4$ and concd. Recrystallization from *n*-hexane and $CHCl_3$ gave pure crystals of **5**; mp 202–203°; $[\alpha]_D^{20}$ −31.0° ($CHCl_3$; *c* 0.29).

Furanopinguisanol (**6**). Oil; $[\alpha]_D^{16}$ −1.96° (MeOH; *c* 0.51); HRMS: *m/z* 234.1613 $[M]^+$, calcd for $C_{15}H_{22}O_2$: 234.1620; EI-MS: *m/z* 234 $[M]^+$ (11), 216 (68), 201 (32), 173 (21), 160 (77), 145 (58), 125 (100), 109 (92), 95 (38), 70 (48), 61 (49); IR ν_{max}^{film} cm^{-1}: 3425, 1000.

Hydrogenation of dehydropinguisenol (**14**). A mixt. of **14** (37.2 mg) and 10% Pd-C (5 mg) in EtOAc (2 ml) was stirred under H_2 atoms. at room temp. for one day. The catalyst was removed by filtration and the solvent was concd to give a residue, which was sepd by CC on silica gel. Elution with C_6H_6–EtOAc yielded **6** (7.3 mg).

(4S*,5S*,6R*,7R*)-1(10)E-*Lepidozen-5-ol* (**7**). Oil; $[\alpha]_D^{20}$ −29.9° ($CHCl_3$; *c* 1.22); CD: $[\theta]_{285nm}$ +40 ($CHCl_3$); HRMS: *m/z* 222.1981 $[M]^+$, calcd for $C_{15}H_{26}O$: 222.1983; EI-MS: *m/z* 222 $[M]^+$ (2), 189 (4), 161 (11), 122 (18), 109 (26), 95 (32), 82 (100), 67 (40), 41 (49); IR ν_{max}^{film} cm^{-1}: 3400, 1450, 1375; ^1H NMR (400 MHz, C_6D_6): δ −0.2 (1H, *ddd*, $J=8.1$, 5.5, and 2.4 Hz, H-7), 0.24 (1H, *dd*, $J=9.2$, 5.5 Hz, H-6), 0.99 (3H, *s*), 1.00 (3H, *s*), 1.10 (3H, *d*, $J=7.0$ Hz, H-14), 1.51 (3H, *s*, H-15), 1.57 (1H, *m*, H-4), 1.93 (1H, *m*, H-2), 2.14 (1H, *m*, H-2), 3.05 (1H, *dd*, $J=9.2$, 2.2 Hz, H-5), 5.23 (1H, *t*, $J=7.9$ Hz, H-1); ^{13}C NMR (100 MHz, C_6D_6): δ_C 17.3 (*q*, C-15), 18.0 (*s*, C-11), 19.9 (*q*, C-14), 22.0 (*q*), 22.3 (*q*), 25.8 (*t*, C-2), 27.3 (*t*, C-8), 28.2 (*d*, C-7), 30.1 (*t*, C-3), 35.5 (*d*, C-4), 37.8 (*d*, C-6), 38.9 (*t*, C-9), 77.0 (*d*, C-5), 127.4 (*d*, C-1), 133.2 (*s*, C-10).

Dehydropinguisenol methyl ether (**8**). Oil; $[\alpha]_D^{22}$ +82.3° ($CHCl_3$; *c* 0.13); HRMS: *m/z* 246.1627 $[M]^+$, calcd for: $C_{16}H_{22}O_2$: 246.1620; EI-MS: *m/z* 246 $[M]^+$ (11), 231 (4), 215 (6), 199 (7), 171 (2), 149 (13), 121 (7), 109 (16), 70 (13), 43 (100); IR ν_{max}^{film} cm^{-1}: 1640, 1585, 1500, 1445, 1375, 1340, 1140, 940, 870.

6α,11α-*Dimethoxypinguis-5(10)-ene* (**9**). Oil; HRMS: *m/z* 280.2016 $[M]^+$, calcd for $C_{17}H_{28}O_3$: 280.2039; EI-MS: *m/z* 280 $[M]^+$ (3), 248 (42), 216 (71), 201 (38), 160 (42), 138 (82), 125 (62), 109 (100), 70 (19), 43 (83); IR ν_{max}^{film} cm^{-1}: 1665, 1445, 1370, 1335, 1295.

6α,11β-*Dimethoxypinguis-5(10)-ene* (**10**). Oil; HRMS: *m/z* 280.2059 $[M]^+$, calcd for $C_{17}H_{28}O_3$: 280.2039; EI-MS: *m/z* 280 $[M]^+$ (1), 248 (9), 216 (99), 201 (47), 159 (36), 138 (70), 119 (14), 109 (100), 69 (29), 43 (61); IR ν_{max}^{film} cm^{-1}: 1665, 1465, 1445, 1375, 1300, 935.

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