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Scapaundulins A and B, Two Novel Dimeric Labdane Diterpenoids, and Related Compounds from the Japanese Liverwort *Scapania undulata* (L.) Dum.

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Abstract : Two novel dimeric labdane diterpenoids, scapaundulins A (**4**) and B (**5**), have been isolated from the ether extract of the Japanese liverwort *Scapania undulata* (L.) Dum., together with three new labdane diterpenoids (**1-3**) and their structures characterized by spectroscopic techniques, especially 2D-NMR and mass spectrometry. The structures of scapaundulins possess a C_2 axis of symmetry.

The liverworts (Hepaticae) contain a wide variety of terpenoids and aromatic compounds which constitute the characteristic oil bodies, and occasionally produce their own peculiar dimeric compounds (*e.g.*, bisbibenzyls, dimeric isocuparanes) possessing interesting biological activities.[1, 2]

The European liverwort, *Scapania undulata* (L.) Dum. (Family; Scapaniaceae) has been phytochemically investigated by a few groups and the isolation of many *ent*-sesquiterpenoids, labdane diterpenoids and phenolic compounds has been reported.[3] However, there is only one report of the isolation of *ent*-sesquiterpenoids from the Japanese *S. undulata*.[3b]

It is well-known that there are many geographical races of European *S. undulata*.[4] In order to compare the chemical constituents of European and Japanese *S. undulata*, we re-examined the chemical constituents of the Japanese species and isolated two novel dimeric labdanes (**4**, **5**) and related compounds (**1-3**). In this paper we describe the isolation and the structure elucidation of these new diterpenoids.

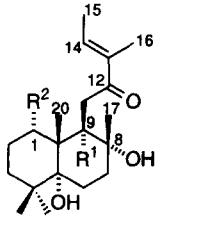
The liverwort *S. undulata* (no. 96169) was collected in 1996 at Omogo-kei, Ehime, Japan. The plant material (258.7 g) was dried for one day, mechanically ground and extracted with ether for one week. The ether extract (3.62 g) was chromatographed on silica gel, followed by Sephadex LH-20 and further purified by HPLC to give three new labdane diterpenoids, 5α, 8α, 9α-trihydroxy-13*E*-labden-12-one (**1**) (145.6 mg), 1α, 5α, 8α-trihydroxy-13*E*-labden-12-one (**2**) (8.4 mg) and 5α, 8α-dihydroxy-13*E*-labden-12-one (**3**) (4.9 mg), two novel dimeric labdanes, scapaundulins A (**4**) (16.7 mg) and B (**5**) (8.7 mg), together with the known sesquiterpenoids, (-)-longiborneol[3a, b, d, i] (122.3 mg) and (-)-longipinanol[3d, i] (26.3 mg).

Compound **1**[5] was obtained as a single crystal from an *n*-hexane - EtOAc (9:1) and its X-ray crystallographic analysis was carried out.[5b] The ORTEP diagram was drawn in Fig. 1. The absolute configuration of **1** was determined by the differential CD curve before and after addition of $Eu(fod)_3$[6] to a CCl_4 solution.[7] The 8, 9-diol of **1** showed a positive peak at 301 nm ($\Delta\varepsilon$ +2.85), corresponding to a positive chirality between the glycol hydroxyl groups. Thus, the absolute structure of **1** was depicted as Fig. 1.

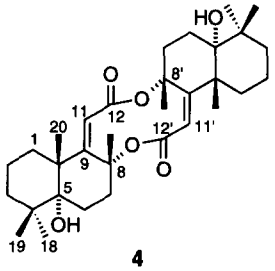
The structures of two new labdanes (**2**, **3**)[8, 9] were established by the comparison of the ^1H and ^{13}C NMR spectra with those of **1** and the analyses of their 2D-NMR spectra.

Scapaundulin A (**4**) was obtained as colorless needles, $[\alpha]_D^{23}$ -60.2° (*c* 0.96, $CHCl_3$). The CIMS of **4** exhibited a $[M+H]^+$ peak at *m/z* 529 (rel. int. 8) and fragment peaks at 511 (2), 293 (2), 275 (14), 265 (100), 247 (63), 201 (7), 137(4). HRCIMS measurements gave the exact mass of the $[M+H]^+$ ion at 529.3534 corresponding to the molecular formula $C_{32}H_{49}O_6$ (calcd. $[M+H]^+$, 529.3529). The IR (neat) and UV spectra (MeOH) showed the presence of a hydroxyl group (3518 cm^{-1}) and an α, β-unsaturated lactone group [1740 cm^{-1}; λ 218 nm (log ε=4.03)].

Scapaundulin B (**5**), was isolated as a pale yellowish oil, $[\alpha]_D^{21}$ +48.3° (*c* 0.58, $CHCl_3$). In the EI and CIMS of **5**, $[M]^+$, $[M/2]^+$, $[M+H]^+$ or $[M/2+H]^+$ peaks were not observed; however, the negative-FABMS showed a $[M-H]^-$ peak at *m/z* 639 while positive-FABMS also gave a $[M+K]^+$ peak at *m/z* 679 (HRFABMS $C_{40}H_{64}O_6K$, calcd. 679.4330). The IR spectrum (neat) exhibited absorption bands corresponding to a hydroxyl group (3355 cm^{-1}). Since neither ketone nor ester absorption bands were observed in the IR spectrum, the remaining oxygen function might be either an ether or a hydroxyl group. The UV spectrum (MeOH) showed a maximum at λ 220 nm (log ε=3.89) ascribable to a conjugated double bond.



1 : R^1=OH, R^2=H
2 : R^1=H, R^2=OH
3 : R^1=H, R^2=H



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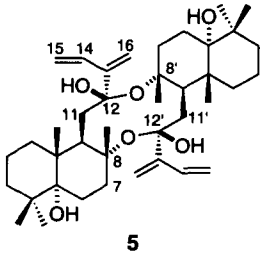

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Table 1. ^{1}H- (600 MHz) and ^{13}C- NMR (150 MHz) Data for Scapaundulins in $CDCl_3$ *

Position No.	Scapaundulin A (**4**)		Scapaundulin B (**5**)	
	^{1}H	^{13}C	^{1}H	^{13}C
1 / 1'	1.99 (dddd, 13.7, 13.7, 4.9, 0.8, ax.) 1.45 (brd, 13.7, eq.)	30.4	1.49 (ddd, 12.9, 12.9, 4.4, ax.) 0.99 (brd, 12.9, eq.)	31.6
2 / 2'	1.82 (qt, 13.7, 3.8, ax.) 1.61[a] (m, eq.)	17.5	1.66-1.77[e] (ax.) 1.42 (m, eq.)	17.4
3 / 3'	1.73[b] (ddd, 13.7, 13.7, 4.1, ax.) 1.22 (brd, 13.7, eq.)	35.8	1.61-1.70[e] (ax.) 1.17 (brd, 13.2, eq.)	36.0
4 / 4'	------	38.4	------	38.1
5 / 5'	------	78.2	------	77.1
6 / 6'	1.91 (ddd, 13.2, 13.2, 4.7, ax.) 1.76[b] (m, eq.)	24.4	1.66-1.77[e] (ax.) 1.66-1.77[e] (eq.)	25.7
7 / 7'	2.02 (dddq, 13.2, 13.2, 4.7, 0.8, ax.) 2.08 (ddd, 13.2, 4.7, 4.7, eq.)	34.9	2.02 (ddd, 12.9, 12.9, 4.6, ax.) 1.80[c] (m, eq.)	34.3
8 / 8'	------	86.5	------	83.0
9 / 9'	------	182.7	2.59 (t, 10.4)	49.5
10 / 10'	------	44.1	------	40.0
11 / 11'	5.58 (s)	112.4	1.82[c] (2H, d, 10.4)	33.1
12 / 12'	------	172.3	------	113.3
13 / 13'	------	-----	------	145.8
14 / 14'	------	-----	6.39 (ddd, 17.9, 11.5, 0.8)	134.9
15 / 15'	------	-----	5.16 (ddd, 11.5, 1.6, 0.5) 5.60 (dd, 17.9, 1.6)	115.9
16 / 16'	------	-----	5.37 (d, 1.9) 5.53 (ddd, 1.9, 0.8, 0.5)	117.9
17 / 17'	1.60[d] (3H, d, 0.8)	26.4	1.38 (3H, d, 0.8)	23.3
18 / 18'	0.94 (3H, s)	27.8	0.93 (3H, s)	28.0
19 / 19'	1.09 (3H, s)	23.5	1.04[d] (3H, s)	23.7
20 / 20'	1.37 (3H, d, 0.8)	21.8	1.04[d] (3H, s)	18.7
-OH	1.29 (brs, 5 / 5'-OH)		1.42 (brs, 5 / 5'-OH) 8.00 (s, 12 / 12'-OH)	

* Chemical shifts from TMS (multiplicity, *J* in Hz).
[a-d] Overlapped signals, [e] Complex multiplet.

The ^{13}C NMR spectra of scapaundulins A (**4**) and B (**5**) exhibited only 16 and 20 signals, respectively. Thus, it is concluded that **4** and **5** are the symmetrical dimeric compounds. The NMR spectra (Table 1) showed simplification due to homotropic behavior of the two halves of the molecule. The homotropic half-structure of scapaundulins was deduced from careful analysis of the 2D-NMR employing ^1H-^1H-COSY, NOESY, HSQC, and HMBC spectra.

In the HMBC spectrum of **4**, the observed long-range ^1H-^{13}C couplings for H-11(11') / C-8(8'), C-9(9'), C-10(10'), C-12(12') suggested the presence of an α, β- unsaturated lactone function and led to the gross structure of a 13, 14, 15, 16- tetranorlabdane skeleton.

The relative stereochemistry of **5** at C-12 was determined by the presence of an NOESY cross-peak at H-9 / H-16. Furthermore, in the ^1H NMR spectrum of **5**, the signal of 12-OH was indicated at δ 8.00 (s, interchangeable with D_2O) which was deshielded by the effect of the hydrogen bond between 12β-OH and 12' β-OH. This result suggested the relative stereochemistry at C-12 and the dimeric structure of **2**.

Comparison of the ^{13}C chemical shift of C-8 in the symmetrical dimers (**4** and **5**, each δ 86.5, 83.0) with those of compounds **1-3** (δ 72.4-74.0), is the most important key point in deduction of the dimeric structures. The two identical monomeric units confirmed by 2D-NMR are connected *via* carboxyl linkages and/or hemiacetal linkages from C-8 of one half to C-11 of the other half. Thus, the ^{13}C chemical shifts of C-8 in both scapundulins appeared at lower field than in **1-3**. Thus, the structures of **4** and **5** result in the presence of C_2 axis of symmetry which gives rise to homotropic behavior of the NMR spectra.

These structures are also in good agreement with the unsaturation degree from the molecular formulae, which possess the central 10-membered ring connecting the two labdane moieties.

The dimeric scapaundulins A (**4**) and B (**5**) might be biosynthesized from the monomeric labdanes such as **1-3**. The present new labdanes are significant chemical markers of the Scapaniaceae.

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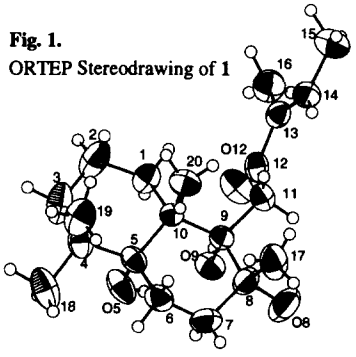
5. (**1**): (a) Colorless crystals; mp 126 °C; $[\alpha]_D^{24}$ +41.7° (*c* 0.24, $CHCl_3$); UV (MeOH) λ max (log ε) 235 (4.15) nm; FTIR (neat) ν max 3414 (OH), 2955 (CH), 1640 (C=O), 1451, 1391, 1244, 990 cm^{-1}; ^1H-NMR ($CDCl_3$, 600 MHz)

δ 2.06 (ddd, 12.4, 12.4, 4.7, H-1ax.), 0.67 (ddd, 12.4, 3.8, 3.8, H-1eq.), 1.65 (qt, 14.3, 4.7, H-2ax.), 1.50 (brd, 14.3, H-2eq.), 1.93 (ddd, 14.0, 14.0, 5.2, H-3ax.), 0.99 (m, H-3eq.), 1.71 (ddd, 14.0, 14.0, 3.8, H-6ax.), 1.62 a (m, H-6 eq.), 2.13 (dddd, 14.0, 14.0, 3.3, 0.8, H-7ax.), 1.56 (ddd, 14.0, 3.8, 3.8, H-7eq.), 2.75, 3.28 (each 1H, d, 16.2, H-11), 6.84 (qq, 6.9, 1.1, H-14), 1.93 (3H, dq, 6.9, 1.1, H-15), 1.79 (3H, quintet, 1.1, H-16), 1.31 (3H, t, 1.1, H-17), 0.91 (3H, s, H-18), 0.99 (3H, s, H-19), 1.10 (3H, d, 0.8, H-20), 6.31 (s, 5-OH), 3.24 (q, 1.1, 8-OH), 7.77 (s, 9-OH); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ 29.8(C-1), 18.4(C-2), 35.5(C-3), 38.9(C-4), 77.7(C-5), 25.6(C-6), 33.7(C-7), 74.0(C-8), 86.8(C-9), 45.6(C-10), 32.0(C-11), 206.8(C-12), 139.3(C-13), 140.1(C-14), 15.3 (C-15), 11.3(C-16), 24.0(C-17), 27.8(C-18), 24.7(C-19), 20.5(C-20); EIMS (+) *m/z* (rel. int.) 338 (10; $[M]^+$), 320 (28), 302 (78), 287 (6), 263 (17), 240 (36), 205 (12), 194 (27), 176 (49), 161 (35), 136 (49), 126 (14), 98 (28), 83 (100), 55 (49); HREIMS $C_{20}H_{34}O_4$ 338.2458, requires 338.2457; CD [CCl_4+Eu(fod)$_3$ (1 x 10^{-4} *M*)], (*c* 7.5 x 10^{-5} *M*) $\Delta\Delta\varepsilon_{301nm}$ +2.85.

(b) The crystal data for **1** are as follows : Crystal dimensions = 0.50 x 0.30 x 0.20 mm, Orthorhombic, Space group $P2_12_12_1$ (no.19), a = 12.000 (2)Å, b = 24.203 (5)Å, c = 6.582 (2)Å, V = 1911.7 (8)Å^3, Z = 4, F(000) = 720, D_{calc} = 1.15 g cm^{-3}, μ (Cu Kα) = 6.01 cm^{-1}, Final R and R_w were 0.046 and 0.057 for 1673 reflections with $I > 3\sigma(I)$. The structure was solved by direct method (SIR 92) and refined by full-matrix least-squares techniques. Diffraction data were obtained using a Mac Science MXC18 diffractometer at room temperature.

Fig. 1.
ORTEP Stereodrawing of **1**



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8. (**2**): Oil; $[\alpha]_D^{22}$ -9.3° (*c* 0.28, $CHCl_3$); UV (MeOH) λ max (log ε) 232 (4.03) nm; FTIR (neat) ν max 3345 (OH), 2953 (CH), 1644 (C=O), 1453, 1391, 1292, 1088, 1061, 1026, 907, 733 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ 3.39 (ddd, 3.3, 3.3, 3.3, H-1), 2.04 (2H, m, H-2ax. and H-3ax.), 1.69 (m, H-2eq.), 1.05 (m, H-3eq.), 1.56 (dddd, 14.0, 12.6, 4.4, 2.5, H-6ax.), 1.64 (2H, m, H-6eq. and H-7eq.), 2.09 (dddq, 14.3, 12.6, 4.4, 1.1, H-7ax.), 3.04 (dd, 5.8, 3.3, H-9), 2.38 (dd, 17.9, 5.8, H-11), 3.21 (dd, 17.9, 3.3, H-11'), 7.05 (qq, 7.1, 1.1, H-14), 1.89 (3H, dq, 7.1, 1.1, H-15), 1.81 (3H, quintet, 1.1, H-16), 1.19 (3H, d, 1.1, H-17), 1.00 (3H, s, H-18), 0.99 (3H, s, H-19), 0.89 (3H, s, H-20), 5.85 (dd, 3.3, 1.4, 1-OH), 5.30 (d, 2.5, 5-OH), 1.16 (brs, 8-OH); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ 72.7 (C-1), 19.1(C-2), 32.5(C-3), 38.7(C-4), 77.7(C-5), 25.4(C-6), 38.2(C-7), 73.7(C-8), 44.7(C-9), 44.4(C-10), 32.5(C-11), 206.1(C-12), 138.1(C-13), 139.3(C-14), 15.0(C-15), 11.5(C-16), 24.0(C-17), 28.0(C-18), 24.5(C-19), 24.5(C-20); EIMS (+) *m/z* (rel. int.) 338 (3; $[M]^+$), 320 (20), 302 (82), 287 (23), 284 (41), 269 (27), 221 (49), 187 (19), 142 (62), 123 (24), 99 (15), 83 (100), 55 (34); HREIMS $C_{20}H_{34}O_4$ 338.2459, requires 338.2457.
9. (**3**): Oil; $[\alpha]_D^{22}$ +30.3° (*c* 0.33, $CHCl_3$); UV (MeOH) λ max (log ε) 230 (3.94) nm; FTIR (neat) ν max 3482 (OH), 2936 (CH), 1659 (C=O), 1454, 1368, 1303, 1082, 752 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ 1.55 (ddd, 13.2, 13.2, 3.9, H-1ax.), 0.94 (brd, 13.2 H-1eq.), 1.65-1.72 (5H, complex multipulet, H-2ax., H-3ax., H-6, H-7eq.), 1.42 (m, H-2eq.), 1.10 (brd, 15.1, H-3eq.), 2.13 (ddd, 14.0, 14.0, 4.9, H-7ax.), 3.10 (dd, 5.5, 4.4, H-9), 2.72 (dd, 17.3, 4.4, H-11), 2.76 (dd, 17.3, 5.5, H-11'), 6.81 (qq, 6.9, 1.1, H-14), 1.86 (3H, dq, 6.9, 1.1, H-15), 1.77 (3H, t, 1.1, H-16), 1.13 (3H, s, H-17), 0.89 (3H, s, H-18), 0.99 (3H, s, H-19), 1.01 (3H, s, H-20), 2.58 (brs, 5-OH), 2.56 (brs, 8-OH); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ 31.7(C-1), 17.8(C-2), 35.5(C-3), 38.2(C-4), 76.8(C-5), 24.9(C-6), 38.3(C-7), 72.4(C-8), 47.9(C-9), 42.4(C-10), 32.7(C-11), 203.1(C-12), 138.5(C-13), 136.3(C-14), 14.8(C-15), 11.6(C-16), 23.6(C-17), 28.0(C-18), 24.0(C-19), 19.0(C-20); EIMS (+) *m/z* (rel. int.) 322 (9; $[M]^+$), 304 (18), 286 (32), 271 (32), 251 (26), 231 (9), 203 (16), 147 (14), 119 (20), 95 (11), 83 (100), 69 (12), 55 (33), 43 (13); HREIMS $C_{20}H_{34}O_3$ 322.2513, requires 322.2508.

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