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Sesquiterpenoids and diterpenoids from the Chilean liverwort *Lepicolea ochroleuca*

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Abstract

The ether extract of the Chilean liverwort *Lepicolea ochroleuca* yielded three sesquiterpenoids, *ent*-4β-Hydroxy-10α-methoxyaromadendrane, *ent*-3β-Hydroxyspathulenol, and 1,10-Dioxotayloriane, as minor components.The major components were ledol and 13-*epi*-neoverrucosan-5β-ol, four other minor fusicoccanoids were identified.

Keywords: *Lepicolea ochroleuca*; Lepicoleaceae; Liverwort; Isolation; Aromadendrane; Neoverrucosane; Fusicoccane; *ent*-4β-hydroxy-10α-methoxyaromadendrane; *ent*-3β-hydroxyspathulenol; 1,10-dioxotayloriane

1. Introduction

Lepicoleaceae has been considered a rather primitive family in the Hepaticae, and is associated closely with the families Ptilidiaceae and Mastigophoraceae (Schuster, 1980, 1972). In the past, very few chemical studies have been reported for *Lepicolea* species; the only report of chemical investigation was on a Colombian species, *Lepicolea prinosa* (Gradstein, Matsuda & Asakawa, 1981), in which two eudesmane lactones, frullanolide (**1**) and dihydrofrullanolide (**2**) were identified as major constituents, in addition to several other known sesquiterpenoids. On this basis, Asakawa (1982) suggested eudesmanolides as chemical markers for the family Lepicoleaceae.

The GC-MS of an ether extract of the Chilean species *L. ochroleuca* (Cullmann & Becker, 1999) showed a very simple profile with the known sesquiterpenoid ledol (**3**) (Wu, Huang & Chen, 1996) as almost the sole major constituent (64%). The second significant peak was later identified as the diterpenoid alcohol, 13-*epi*-neoverrucosan-5β-ol (**8**) (12%). The areas of all the remaining peaks were smaller than 5%. However, five sesquiterpenoids of the aromadendrane- and seco-aromadendrane-type (**3**–**7**) and five diterpenoids of either the neoverrucosane- or the fusicoccane-type were isolated and identified (**8**–**12**). Three of them are new compounds, their structures, as deduced by spectroscopic analysis, are reported here.

2. Results and discussion

The ether extract (7.7 g) of the crushed plant was subjected to chromatograph on Sephadex (LH-20) and silica gel columns, respectively. Further purification was achieved by HPLC on a silica or a diol column. Among the five sesquiterpenoids isolated, two of them were known compounds. The most abundant component ledol (**3**) had been previously identified in the liverwort species *Cephaloziella recurvifolia* (Wu et al., 1996) and several specimens of *Bazzania tridens* (Wu & Chen, 1996; Wu, 1997; Chang & Wu, 1999). The absolute stereochemistry of the isolated ledol (**3**) was assumed to be the same as in other liverwort species.

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Table 1
^{1}H and ^{13}C-NMR spectral data of sesquiterpenoids **5**–**7**

Atom	**5**		**6**		**7**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	52.2	2.00[a]	46.5	2.12 *m*	221 2	–
2	23.6	1 60[a]	34.4	1.84 *ddd* (12.2, 11 0, 9.1) 1.91 *dd* (12.2, 5.9)	34.7	2.19 *m*
3	41.1	1.59[a]	78.2	3.65 *dd* (9.1, 5.9)	28.0	1.72 *m* 2.00 *m*
4	80.3	–	79.0	–	33.7	2.45 *m*
5	48.1	1.25 *t* (10.7)	51.7	1.44 *dd* (11.2, 10.4)	50.9	1.94 *dd* (10.9, 7.6)
6	28.1	0.40 *dd* (10.7, 9.6)	29.5	0.47 *dd* (11.2, 9.6)	23.8	0.41 *dd* (10.9, 8.5)
7	26.7	0.61 *ddd* (11.1, 9.6, 6.2)	27.6	0.71 *ddd* (11.3, 9.6, 6.2)	26.3	0.63 *q* (8.5)
8	19.8	0.84 *m* 1.82 *m*	24.8	1.00[a] 1.97[a]	19.4	1.56 *m*
9	37.5	1.59[a] 1.68 *td* (12.9, 6.9)	38.9	2.01 *t* (13.1) 2.42 *dd* (13.1, 5.9)	43.6	2.51 *ddd* (16.2, 9.8, 6.5) 2.65 *ddd* (16.2, 9.5, 5.7)
10	79.0	–	152.7	–	209.5	–
11	19.7	–	20.4	–	17.0	–
12	28.7	1.02 *s*	28.6	1.04 *s*	28.9	1.04 *s*
13	16.5	1.02 *s*	16.2	1.02 *s*	15.8	0.94 *s*
14	17.7	1.10 *s*	106.7	4.65 *d* (2) 4.66 *d* (2)	30.0	2.13 *s*
15	24.4	1.23 *s*	23.1	1.22 *s*	16.0	0.94 *d* (7.1)
OCH_3	48.3	3.17 *s*				

[a] Overlapped or obscured peaks ( ) J, in Hz.

Compound **4** displayed very similar ^{1}H and ^{13}C-NMR spectra to those of ledol (**3**) except that two secondary methyl groups were observed instead of one. Subsequently, HMBC correlations indicated that the hydroxyl function should be placed at C-1, leading to structure **4**. The ^{13}C-NMR spectral data of **4** were identical with those published for palustrol (Cheer, Smith, Djerassi, Tursch, Braekman & Daloze, 1976). Compound **4** was, however unstable, and readily decomposed; the stereochemistry depicted in **4** thus assumes the same absolute configuration as ledol (**3**), with an α-cyclopropyl group.

The ^{1}H-NMR spectrum of **5** again indicated an aromadendrane skeleton, with two cyclopropyl protons resonating at α 0.40 (*dd*, 10.7, 9.6) and 0.61 (*ddd*, 11.1, 9.6, 6.2) (Table 1). Other resonances included four singlet methyls (δ 1.02, 1.02, 1.10 and 1.23), two quaternary carbons (*δ* 80.3, *s*; 79.0, *s*) bearing a hydroxyl group and a methoxyl group (δ 3.17, *s*), respectively. Correlations in the ^{1}H–^{1}H COSY and HMBC spectra led to the identification of two oxygenated carbons as shown in **5**. The relative stereochemistry was determined from the NOESY spectrum as shown in Fig. 1; again an α-orientation of the cyclopropyl group was assumed. Compound **5** is thus *ent*-4β-hydroxy-10α-methoxyaromadendrane.

The ^{1}H-NMR spectral data of **6** (Table 1) appeared very similar to that of spathulenol, which is an artefact observed in many liverworts (Toyota et al., 1996). The obvious difference was a secondary hydroxyl group at δ 3.65 (*dd*, 9.1, 5.9), which was placed at C-3 on the basis of COSY and HMBC correlations. The relative stereochemistry of the chiral carbons was apparent from the NOESY correlation shown in Fig. 1. Compound **6** is thus *ent*-3β-hydroxyspathulenol.

The ^{1}H-NMR spectrum of compund **7** showed two cyclopropyl protons at δ 0.41 (*dd*, 10.9, 8.5) and 0.63 (*q*, 8.5) (Table 1), as well as four methyls at δ 1.04 (*s*), 0.94 (*s*), 0.94 (*d*, 7.1), and 2.13 (*s*). The singlet at *δ* 2.13 (*s*, 3H) suggested a methyl ketone functional

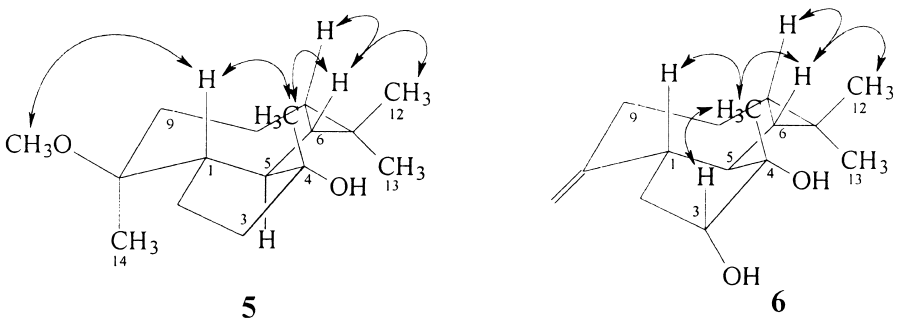
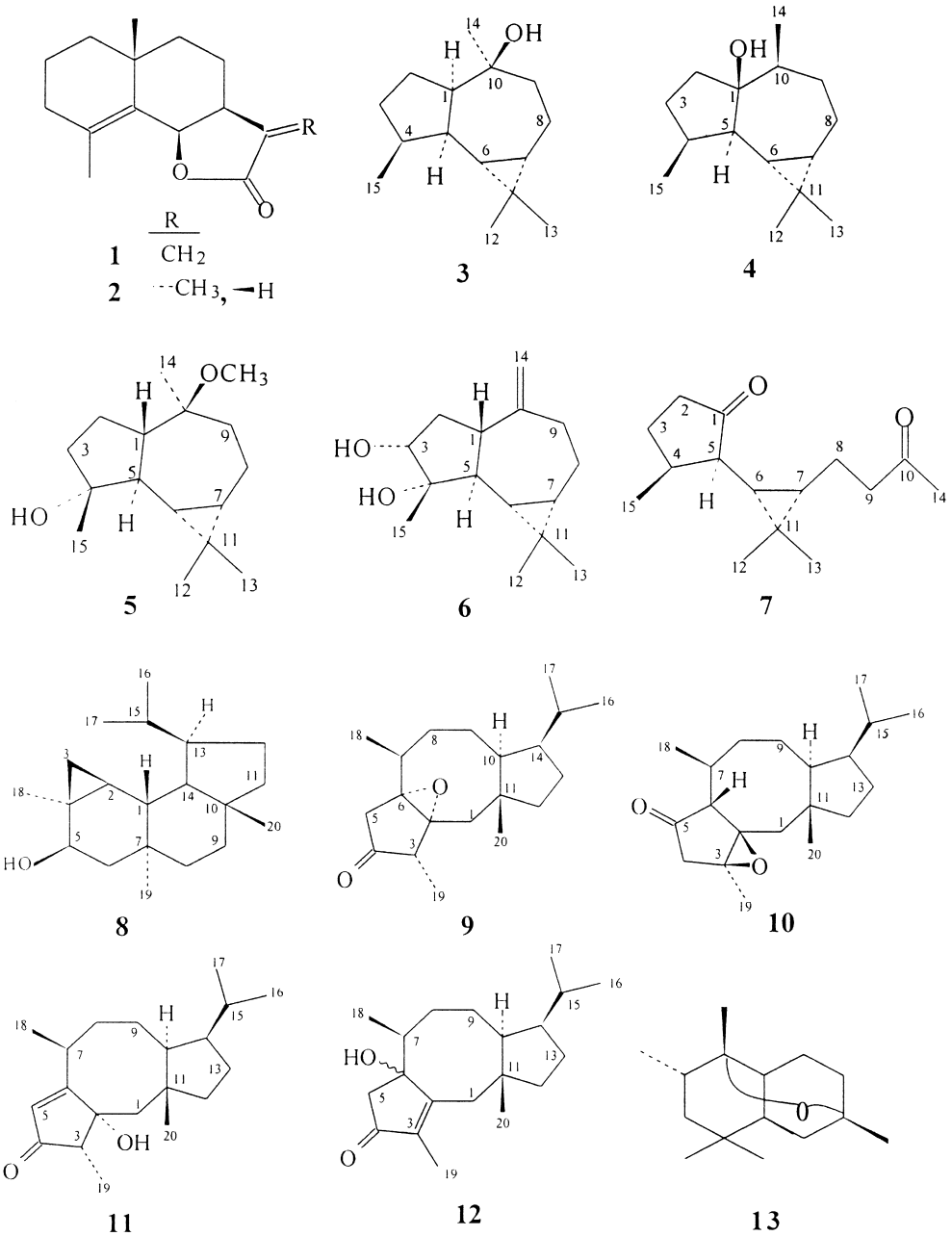


Fig. 1. NOE's observed for compounds **5** and **6**.

group. The presence of two ketonic carbonyl carbons at δ 221.2 and 209.5 led to the conclusion that **7** had a cleaved aromadendrane skeleton. To accommodate the formation of a methyl ketone from the aromadendrane skeleton, either C_1–C_{10} or C_4–C_5 must be cleaved. Correlations from $^1H-{}^1H$ COSY, $^{13}C-{}^1H$ COSY and HMBC spectra agreed well with a cleavage at C_1–C_{10} and an oxo group at C_1. Previously, a number of 1,10-secoaromadendrane-type of sesquiterpenoids have been isolated from the liverwort *Mylia taylorii* (Matsuo & Takaoka, 1990; Asakawa, 1995), but none of them was identical to compound **7**. However, structure **7** was once obtained as a synthetic compound (Birch, Grimshaw, Speake, Gascoigne & Hellyer, 1959), yet without any spectral data reported. Thus, **7** was 1,10-dioxotayloriane. The stereochemistry of **7** was assumed to be as shown on the basis of co-occurrence with other aromadendranes. Neither NOESY nor NOE difference spectra provided any information about the relative stereochemistry of compound **7**.

Compound **8** was a diterpenoid alcohol and the second major component of the oil as shown by its GC-MS ($[M]^+$ 290). Its ^{1}H-NMR spectrum displayed signals for three cyclopropyl protons, three tertiary methyls, one isopropyl group, and one secondary hydroxyl group. These characteristics indicated that **8**



had a verrucosane skeleton. Both the ^{1}H and ^{13}C-NMR data of **8** were identical with those reported for 13-*epi*-neoverrucosan-5β-ol (Fukuyama, Masuya, Tori, Kido, Wakamatsu & Asakawa, 1988; Asakawa, Masuya, Tori & Fukuyama, 1988), a component of the liverworts *Plagiochila stephensoniana* (Fukuyama et al., 1988), *Schistochila nobilis* (Asakawa et al., 1988), *Heteroschyphus planus* (Hashimoto, Nakamura, Tori, Takaoka & Asakawa, 1995) and *Fossombronia alaskana* (Grammes, Burkhardt, Veith, Huch & Becker, 1997).

Four other minor diterpenoids with a fusicoccane skeleton were also isolated. Two were identified as fusicogigantone A (**9**) and fusicogigantone B (**10**), both of which were first isolated from the liverwort *Pleurozia gigantea* (Asakawa, Lin, Tori & Kondo, 1990). The other two were anadensin (**11**) and fusicoauritone (**12**). Anadensin was the first fusicoccane compound to be isolated from a liverwort (Huneck, Baxter, Cameron, Connolly & Rycroft, 1983). Fusicoauritone was also a component of the liverwort *Anastrophyllum auritum* (Zapp, Burkhardt & Becker, 1994).

The species *Lepicolea ochroleuca* elaborates sesquiterpenoids with an aromadendrane skeleton and diterpenoids with neoverrucosane and fusicoccane skeletons. No eudesmane sesquiterpenoids were observed. Therefore, *L. ochroleuca* elaborates different type of sesquiterpenoids from that of *L. pruinosa*. From a chemical point of view, the three primitive families Ptilidiaceae. Mastigophoraceae and Lepicoleaceae do not appear to be as close as they were once thought to be on a morphological basis.

3. Experimental

3.1. General

Solvents used for spectral measurements were: $CDCl_3$ (^{1}H and ^{13}C-NMR, 500 and 125 MHz), and CH_2Cl_2 ($[\alpha]_D$). HPLC analyses were carried out either on a LiChrospher Si60 (5 μm, 4.6 × 250 mm) or a LiChrospher Diol column (5 μm, 4 × 250 mm), eluted by an *n*-hexane–EtOAc gradient. GC-MS: 70 eV, column, DBWAX, 30 × 0.25, 50–220°C (40 min), 5°C/min.

3.2. Plant material

Plants of *Lepicolea ochroleuca* were collected and identified by Professor Mues (Saarland University) in Chile, South America. Specimens were deposited in the herbarium of Saarland University, Germany.

3.3. Extraction and isolation

Air-dried and powdered whole plants (300 g) were soxhlet-extracted with ethyl ether. The crude extract (7.7 g) was first subjected to chromatography on Sephadex LH-20 and then on silica gel (230–400 mesh) using an *n*-hexane–EtOAc gradient. Fr. 6 (10–15% EtOAc–hexane) afforded ledol (**3**) (464 mg) and fr. 8 (20% EtOAc–hexane) furnished 13-*epi*-neoverrucosan-5β-ol (**8**) (99 mg). Further purification by HPLC, compounds **4** (2.6 mg), **9** (15.4 mg), and **10** (4.7 mg) were obtained from fr. 4 (5–10% EtOAc–hexane), compound **7** (1.2 mg) from fr. 7 (15% EtOAc–hexane), compound **11** (1 mg) from fr 9 (20–25% EtOAc–hexane), compounds **5** (1.7 mg), **6** (1.6 mg), and **12** (0.5 mg) from fr. 11 (30–35% EtOAc–hexane). In addition to the isolated components, peculiaroxide (**13**) that was previously isolated from a Taiwanese liverwort *Plagiochila peculiaris* (Wu, Huang & Shih, 1993) was identified by GC-MS (4% of the volatile oil) as well.

3.4. Compound characterization

3.4.1. ent-4β-Hydroxy-10α-methoxyaromadendrane (*5*)

Oil; $[\alpha]_D + 9.2$ (*c* 0.085, CH_2Cl_2), IR (film) ν_{max} cm^{-1}: 3120–3680; GC-MS (EI) *m/z* (rel. int.): 252 ($[M]^+$, 2), 162 (36), 107 (28), 93 (33), 85 (50), 55 (30), 43 (100), 41 (35); NMR spectral data: Table 1.

3.4.2. ent-3β-Hydroxyspathulenol (*6*)

Oil; $[\alpha]_D - 9.9$ (*c* 0.08, CH_2Cl_2): IR (film) ν_{max} cm^{-1}: 3150–3650; NMR spectral data Table 1.

3.4.3. 1,10-Dioxotayloriane (*7*)

Oil; $[\alpha]_D + 30.2$ (*c* 0.06, CH_2Cl_2); IR (film) ν_{max} cm^{-1}: 1736, 1710; GC-MS(El) *m/z* (rel. int.): 236 ($[M^+$, 2), 178 (40), 139 (55), 121 (32), 107 (30), 95 (35), 81 (35), 43 (100), NMR spectral data Table 1.

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References

Asakawa, Y. (1995). In W. Hertz, G. W. Kirby, R. E. Moore, W. Steglich, & Ch. Tamm, *Progress in the chemistry of organic natural products, vol. 65* (pp. 36–39). Wien: Springer.

Asakawa, Y. (1982). *J. Hattori. Bot. Lab*, *53*, 283.
Asakawa, Y., Lin, X., Tori, M., & Kondo, K. (1990). *Phytochemistry*, *29*, 2597.
Asakawa, Y., Masuya, T., Tori, M., & Fukuyama, Y. (1988). *Phytochemistry*, *27*, 3509.
Birch, A. J., Grimshaw, J., Speake, R. N., Gascoigne, R. M., & Hellyer, R. O. (1959). *Tetrahedron Lett*, *3*, 15.
Chang, R.-C., & Wu, C.-L. (1999). *J. Chin. Chem. Soc*, *46*, 191.
Cheer, C. J., Smith, D. H., Djerassi, C., Tursch, B., Braekman, J. C., & Daloze, D. (1976). *Tetrahedron*, *32*, 1807.
Cullmann, F., Becker, H. (1999). *Phytochemistry*, *52*, 1651.
Fukuyama, T., Masuya, T., Tori, M., Kido, M., Wakamatsu, M., & Asakawa, Y. (1988). *Phytochemistry*, *27*, 1797.
Gradstein, S. R., Matsuda, R., & Asakawa, Y. (1981). *J. Hattori Bot. Lab*, *50*, 231.
Grammes, C., Burkhardt, G., Veith, M., & Huch & Becker, H. (1997). *Phytochemistry*, *44*, 1495.
Hashimoto, T., Nakamura, I., Tori, M., Takaoka, S., & Asakawa, Y. (1995). *Phytochemistry*, *38*, 119.
Huneck, S., Baxter, G., Cameron, A. F., Connolly, J. D., & Rycroft, D. S. (1983). *Tetrahedron Lett*, *24*, 3787.
Matsuo, A., & Takaoka, D. (1990). In H. D. Zinsmeister, & R. Mues, *Bryophytes: Their chemistry and chemical taxonomy* (p. 59). Oxford: Clarendon Press.
Schuster, R. M. (1980). *The hepaticae and anthocerotae of North America*, *vol. 1* (pp. 382–758). New York: Columbia University Press.
Schuster, R. M. (1972). *J. Hattori. Bot. Lab*, *36*, 321.
Toyota, M., Koyama, H., Mizutani, M., & Asakawa, Y. (1996). *Phytochemistry*, *41*, 1347.
Wu, C.-L. (1997). *Youji Huaxue*, *17*, 28.
Wu, C.-L., & Chen, J.-R. (1996). *J. Chin. Chem. Soc*, *42*, 597.
Wu Jr, C.-L., Huang, C.-D., & Shih, T.-L. (1993). *Tetrahedron Lett*, *34*, 4855.
Wu, C.-L., Huang, Y.-M., & Chen, J.-R. (1996). *Phytochemistry*, *42*, 677.
Zapp, J., Burkhardt, G., & Becker, H. (1994). *Phytochemistry*, *37*, 787.