Pungent Aromatic Compound from New Zealand Liverwort *Hymenophyton flabellatum*

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Ether extract of the New Zealand liverwort *Hymenophyton flabellatum* produced a pungent principle. The structure has been identified as known compound, 1-(2,4,6-trimethoxy-phenyl)-but-2(*E*)-en-1-one by means of its spectral data, previously isolated from the fern *Arachinoides standishii*. Further isolation of the extract of this species afforded eight aromatic compounds whose structures were determined by spectral analysis. Those compounds were shown to be biogenetically and structurally related to the pungent compound of this species.

Key words Hepaticae; *Hymenophyton flabellatum*; pungent principle; liverwort

Bryophytes are phylogenetically placed between algae and the pteridophytes and are divided into three classes: Musci (mosses), Hepaticae (liverworts) and Anthocerotae (hornworts). We previously reported that chemical constituents among the three classes of bryophytes are totally different, with terpenoids and aromatic components found in liverworts closely related to those found in Phaeophytes (brown algae) in terms of chiroptical properties.[1)]

In the course of our investigation of bioactive substances in bryophytes, we found that some liverworts release pungent, hot-tasting substances. Five pungent compounds have been isolated from liverworts, such as polygodial from *Porella vernicosa*,[2)] sacculatal from *Trichocoleopsis sacculata*,[3)] plagiochilin A from *Plagiochila yokogurensis*,[4)] diplophyllolide from *Chiloscyphus polyanthus* and *Diplophyllum albicans*,[5)] and tulipinolide from *Wiesnerella denudata*[6)] (Fig. 1). Sacculatal and plagiochiline A are the characteristic pungent compounds of liverworts, although polygodial is known as a hot-tasting constituent of higher plant *Polygonum hydropiper*.[7)] Recently, we briefly reported the isolation and identification of hot-tasting compound **1** from the liverwort *Hymenophyton flabellatum* (Labill.).[8)] We now report in detail the isolation and structural elucidation of the pungent principle and four new aromatic compounds, together with four known compounds from New Zealand liverwort *H. flabellatum*.

Endemic to New Zealand and Australia,[9)] the liverwort *H. flabellatum*, belonging to Hymenophytaceae, grows on shaded wet soil, humus and old logs in forest, and beside streams. Previous phytochemical work has resulted in the isolation of flavonoids from this species with division into two chemo types of the genus; however, the hot-tasting constituent of *Hymenophyton* species has not yet been reported.[10)] In reality, *H. flabellatum* shows strong pungency when leaf fragments are chewed. We had an opportunity for collection of this species in New Zealand, and further investigation of this species afforded a hot-tasting compound together with four new aromatic compounds.

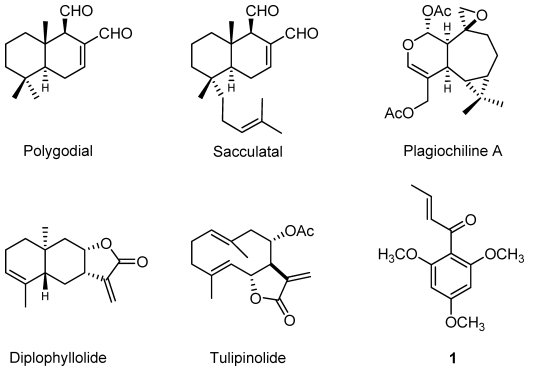

Fig. 1. Hot-Tasting Substances Found in the Liverworts

In order to identify the pungent principle, *Hymenophyton flabellatum* was extracted with ether. The ether extract possessed an incredibly strong pungent taste and was chromatographed on silica gel followed by preparative HPLC to give pure pungent-tasting compound 1-(2,4,6-trimethoxyphenyl)-but-2(*E*)-en-1-one (**1**) (6.6% of the total extract). Isolation of **1** from the fern *Arachinoides standishii*[11,12)] and from *Dysophylla verticillata* (Labiatae)[13)] has been previously reported. Furthermore, antifeeding activity of **1** in the larvae of yellow butterfly, *Eurema hecabe*, has also been reported.[14)]

Further purification of the ether extract gave new aromatic compounds **2** (1.5%), **3** (0.5%), **6** (12.4%), and **7** (0.2%), together with known aromatic compounds **4** (3.0%),[13)] **5** (0.8%),[15)] **8** (1.4%)[15,16)] and **9** (1.8%).[17)]

The electron ionization-mass spectrum (EI-MS) of **1** showed a molecular ion peak at *m*/*z* 236. IR spectral analysis indicated the presence of an unsaturated carbonyl and an aromatic ring. The ^{1}H-NMR spectrum of **1** showed three methoxyl groups and a di-substituted olefinic proton at δ 6.50 and 6.66, which displayed *trans* configuration by mutual coupling constant (J=15.5 Hz). Analysis of heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC) spectra supported the structural assignment determined as 1-(2,4,6-trimethoxyphenyl)-but-2(*E*)-en-1-one. Whereas the ^{13}C-NMR data of **1** was identical to that of compound **1** found in the fern *Arachinoides standishii*,[11,12)] it was not identical to that of vertinone (**1**)[13)] isolated from *D. verticillata* (Labiatae). For this reason, we attempted to synthesize **1** and directly compare the ^{13}C-NMR data between natural and synthetic materials. The reaction of 1,3,5-trimethoxybenzene with crotonyl chloride using aluminum chloride as catalyst gave 1-(2,4,6-trimethoxy-

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phenyl)-but-2(*E*)-en-1-one, whose ^{13}C-NMR data was completely identical to that of natural product **1** as well as literature values.[12] It was apparent from the above evidence that the assignment of ^{13}C-NMR data in the literature[13] was incorrect.

IR spectrum of **2** showed absorption bands for hydroxyl, carbonyl groups and an aromatic ring at 3585, 1643, 1624 and 1595 cm^{-1}. UV absorption data at 318 nm (log ε, 4.25) provided further evidence for the presence of an aromatic ring. The EI-MS of **2** exhibited an ion peak at *m*/*z* 208 and base peak at *m*/*z* 193. ^{1}H-NMR spectral analysis showed a prominent signal at δ 14.11, indicating the presence of hydrogen bonding in the molecule. The mutually spin–spin coupled proton signals at δ 6.00 (1H, d, J=2.2 Hz) and 5.92 (1H, d, J=2.2 Hz) exhibited the presence of 1,2,4,6-tetrasubstituted aromatic ring in **2**. ^{13}C-NMR spectral analysis demonstrated the resonances of 11 carbons, including a carbonyl carbon at δ 193.1. The presence of *trans*-2-buten-1-one as a partial structure was apparent by further resonance at δ_H 1.97 (3H, dd, J=6.9, 1.5 Hz), 7.07 (1H, dq, J=15, 6.9 Hz) and 7.21 (1H, dq, J=15, 1.5 Hz) in its ^{1}H-NMR, and δ_C 18.7 (q), 142.7 (d), and 131.9 (d) in the ^{13}C-NMR spectra. Furthermore, there is only one possible position, C-6′, for the attachment of the methoxyl group, since the Nuclear Overhauser Enhancement Spectroscopy (NOESY) of 2 showed a correlation between the methoxyl proton and H-2 proton. Consideration of the spectral data established the structure of **2** as 1-(2,4-dihydroxy-6-methoxy-phenyl)-but-2(*E*)-en-1-one.

The structure of **3** was deduced by comparing its spectral data with that of **1**. Since coupling constant 11.5 Hz was observed at δ 6.34 and 6.21 in the ^{1}H-NMR spectrum of **3**, it is clear that the double bond in the α,β-unsaturated ketone was *cis* configuration. The structure of **3** was determined to be 1-(2,4,6-trimethoxy-phenyl)-but-2(*Z*)-en-1-one.

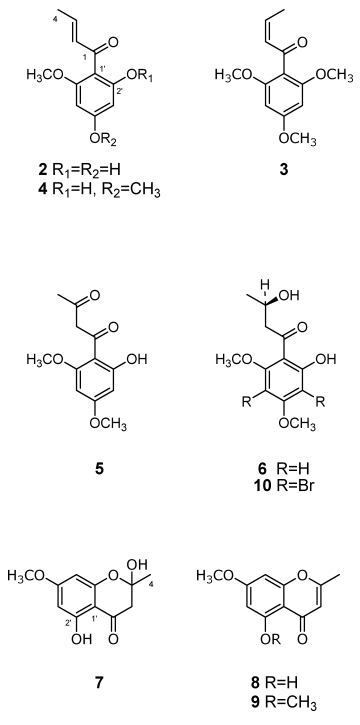

Fig. 2. The Structures of Compounds **2**—**10**

Compound **6**, $[\alpha]_D^{20}$ −64.8 (c=2.82, $CHCl_3$), showed optically active and absorption bands at 3565 (OH), 1620 (C=O) and 1592 (aromatic ring) cm^{-1} in its IR spectrum. The absorption band at 3565 cm^{-1} in the IR spectrum and a proton signal at δ 13.79 (1H, s) in the ^{1}H-NMR spectrum suggested the presence of intramolecular hydrogen bonding. The UV absorption maximum at 288 nm (log ε, 4.16) and mutually spin coupled proton signals at δ 5.92 (1H, d, J=2.5 Hz) and 6.07 (1H, d, J=2.5 Hz) exhibited the presence of 1,2,4,6-tetrasubstituted aromatic ring. Further HMBC spectral analysis of **6** confirmed the structure as 1-(2-hydroxy-4,6-dimethoxyphenyl)-3-hydroxy-1-butanone.

Racemic compound **6** has been reported as a synthetic intermediate of 1-(2,4,6-trimethoxy-phenyl)-but-2(*E*)-en-1-one (**1**) and **4**.[18] Since compound **6** from *H. flabellatum* showed optical activity as described earlier, we attempted to determine the absolute configuration of the hydroxyl group in **6** by X-ray crystallographic analysis of a halogenated derivative. Bromination with *N*-bromosuccinimide afforded **10**, whose EI-mass spectrum showed ion peaks at *m*/*z* 396 [M$^+$] (intensity 3.6%), 398 [M$^+$+2] (7.2%) and 400 [M$^+$+4] (3.7%). This provided evidence for the presence of two bromine atoms in **10**. Since two aromatic proton signals at δ 5.92 (d, J=2.5 Hz) and 6.07 (d, J=2.5 Hz) of **6** were missing in the ^{1}H-NMR spectrum of **10**, the structure was clearly determined. The absolute configuration of the hydroxyl group of **6** was clarified by X-ray crystallographic analysis of crystals of **10** obtained from ether solution. Finally, the absolute structure of **6** was established as 1-(2-hydroxy-4,6-dimethoxyphenyl)-3(*R*)-hydroxy-1-butanone by ORTEP drawing (Fig. 3).

A glycoside of **6** has not only been isolated from ferns *Arachinoides standishii*[12] and *Diplazium nipponicum*,[19] but **6** has also been obtained as an intermediate product in the synthesis of **1** and **4**.[18] This is the first example of the isolation of **6** as a natural product. Assignment of NMR data for **6** is illustrated in Table 1. Furthermore, the absolute configuration at C-3 of an aglycone **6** ($[\alpha]_D$ +7.4)[19] derived from onioside, 1-(2-hydroxy-4,6-dimethoxyphenyl)-3(*S*)-hydroxy-1-butanone 3-*O*-β-D-glucopyranoside isolated from the fern

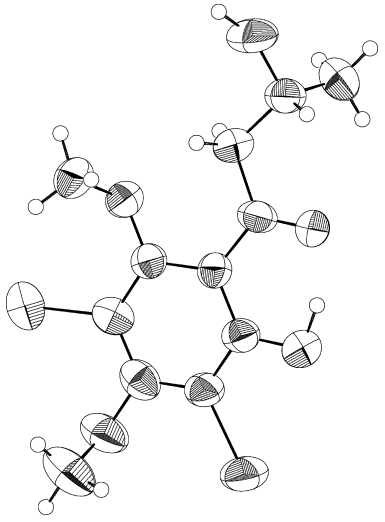

Fig. 3. A Computer Generated Drawing of the Final X-Ray Model of Compound **10**

Table 1. NMR Data for Compounds **2**, **3**, **6**, and **7** in $CDCl_3$

No.	**2**		**3**		**6**		**7**	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1	—	193.1	—	193.7	—	205.0	—	194.6
2	7.21 (dq, 15, 1.5)	131.9	6.34 (dq, 11.5, 1.6)	130.8	3.05 (dd, 18, 9) 3.22 (dd, 18, 2.5)	52.2	2.85 (d, 17) 2.93 (dd, 17, 2)	46.4
3	7.07 (dq, 15, 6.9)	142.7	6.21 (dq, 11.5, 7)	141.5	4.35 (ddddd, 6.5, 6.5, 6.5, 9, 2.5)	64.1	—	100.7
4 (CH_3)	1.97 (dd, 6.9, 1.5)	18.7	2.10 (dd, 7, 1.6)	15.7	1.27 (d, 6.5)	22.4	1.73 (s)	28.6
1′	—	105.8	—	113.9	—	105.9	—	102.4
2′	—	167.8	—	158.7	—	167.7	—	163.9
3′	6.00 (d, 2.2)	96.6	6.11[a] (s)	90.7[a]	6.07 (d, 2.5)	93.7	6.07 (d, 2.5)	94.9
4′	—	162.5	—	162.8	—	166.3	—	167.9
5′	5.92 (d, 2.2)	90.9	6.11[a] (s)	90.7[a]	5.92 (d, 2.5)	91.0	5.98 (d, 2.5)	95.0
6′	—	163.3	—	158.7	—	162.9	—	159.5
2-OCH_3	—	—	3.78[a] (s)	55.9[a]	—	—	—	—
2′-OH	14.11 (s)	—	—	—	13.79 (s)	—	11.95 (s)	—
4′-OCH_3	—	—	3.83 (s)	55.4	3.83 (s)	55.6	3.83 (s)	55.7
4′-OH	5.70 (bs)	—	—	—	—	—	—	
6′-OCH_3	3.88 (s)	55.7	3.78 [a] (s)	55.9[a]	3.86 (s)	55.6	—	—

Measured at 600 MHz for ^1H and 150 MHz for ^{13}C. *a*) Overlapped signals.

Diplazium nipponicum has been established as *S*-configuration by modified Horeau's method. The present work established the configuration as *R* at C-3 of the naturally occurring compound **6** from the liverwort. This is the first example in which compounds from both the liverwort and fern have an enantiomeric relationship.

Compound **7** (EI-MS $[M]^+$ *m*/*z* 224) showed absorption at 3586 cm^{-1} for a hydroxyl group and 1640 cm^{-1} for an unsaturated carbonyl group in the IR spectrum. UV spectral analysis of **7** exhibited a maximum absorption band at 287 nm (log ε, 4.59) indicating the presence of an aromatic ring. NMR spectral data revealed a singlet signal at δ_H 1.73 (s, 3H) which correlated to a hemiketal carbon at δ_C 100.7 and a methylene carbon at δ_C 46.4 in the HMBC of **7**. The correlation of the methylene protons at δ_H 2.85 and 2.93 between the carbonyl carbon at δ_C 194.6 and a quaternary aromatic carbon at δ_C102.4 apparently exhibited the presence of C_4 chain unit attached to aromatic ring, similar to previous compounds **1**—**6**. Further observation of aromatic proton signals at δ 5.98 and 6.07 (each, d, J=2.5 Hz) indicated that 1,2,4,6-tetrasubstituted aromatic ring partially existed in **7**. Extensive ^1H- and ^{13}C-NMR analysis (Table 1) allowed us to define the structure of **7** as 2,5-dihydroxy-7-methoxy-2-methylchroman-4-one. Measurement of the optical rotation was not feasible due to the small sample size.

We have focused on the bioactive constituents and the chemosystematics of bryophytes and pteridophytes as well as the evolutionary relationship between terrestrial spore-forming green plants and algae using their characteristic chemical indicators. The bryophytes are phylogenetically placed between the algae and pteridophytes. Compound **1** and bisbibenzyls[7,20] were elaborated from both liverworts and ferns as chemical indicators that could provide an evolutionary link between liverworts and ferns. On the basis of this result, it is clear that some ferns and liverworts produce similar compounds and appear to be closely related chemically.

Experimental

Plant Material *Hymenophyton flabellatum* (Labill.) Dum. ex Trev. (Herbarium specimen No. NZ-64; dry weight 6.25 g) was collected in December 2000 near the Haast river, on the south island of New Zealand. A voucher specimen has been deposited at Institute of Pharmacognosy, Tokushima Bunri University.

Specimen was extracted with Et_2O for 2 weeks. The ether extract (340.3 mg; yield 5.4%) was chromatographed on silica gel to divide into 11 frs. (fr. 1—11). Fraction 9 (74.6 mg) was rechromatographed on Sephadex LH-20 and further purified by prep HPLC on silica gel column (10 mm i.d. ×250 mm) using *n*-hexane–ethyl acetate (4 : 1, v/v) to give hot-tasting compound, 1-(2,4,6-trimethoxy-phenyl)-but-2(*E*)-en-1-one (**1**) (22.4 mg; 6.6% of total extract), compounds **2** (5.2 mg; 1.5%), **5** (2.7 mg; 0.8%), and **7** (0.8 mg; 0.2%). Fraction 8 (8.2 mg) was purified by prep HPLC on silica gel column (10 mm i.d. ×250 mm) using *n*-hexane–ethyl acetate (7 : 3, v/v) to give compound **3** (1.8 mg; 0.5%). Fraction 10 (63.1 mg) was purified by prep HPLC to give **6** (42.2 mg; 12.4%). Fractions 3 (25.3 mg), 6 (17.1 mg) and 11 (12.8 mg) were further purified by prep HPLC to give **4** (10.2 mg; 3.0%), **8** (4.6 mg; 1.4%), and **9** (6.0 mg; 1.8%), respectively.

1-(2,4,6-Trimethoxy-phenyl)-but-2(*E*)-en-1-one (1) ^1H-NMR (200 MHz, $CDCl_3$) δ: 6.34 (1H, dq, J=15, 1.5 Hz), 6.67 (1H, dq, J=15, 6.9 Hz), 1.90 (3H, dd, J=6.9, 1.5 Hz), 6.12 (2H, s), 3.75 (6H, s), 3.83 (3H, s). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 194.7 (C-1), 134.2 (C-2), 145.2 (C-3), 18.3 (C-4), 111.8 (C-1′), 158.5 (C-2′, 6′), 90.7 (C-3′, 5′), 162.2 (C-4′), 55.9 (2′, 6′-OCH_3), 55.4 (4′-OCH_3). EI-MS *m*/*z* (rel. int.): 236 $[M^+]$ (83), 221 (38), 195 (100), 181 (25). IR ($CHCl_3$) cm^{-1}: 1649, 1607, 1590. UV λ_{max} (EtOH) nm (log ε): 291 (3.52), 223 (4.22), 211 (4.33).

Synthesis of 1-(2,4,6-Trimethoxy-phenyl)-but-2(*E*)-en-1-one (1) To a solution of crotonyl chloride (0.76 g) in CS_2 (15 ml), aluminum chloride (0.77 g) and 1,3,5-trimethoxybenzene (0.4 g) was added and the mixture was heated under reflux for 4 h. Excess water and $CHCl_3$ were added to the reaction mixture. The organic layer was washed with 10% Na_2CO_3 and then with water. The organic layer was dried over Na_2SO_4, and the solvent was evaporated under reduced pressure to give a residue (0.61 g). The residue was purified by Sephadex LH-20 using CH_2Cl_2–MeOH (1 : 1, v/v) and prep HPLC, affording pure **1** (17.3 mg).

1-(2,4-Dihydroxy-6-methoxy-phenyl)-but-2(*E*)-en-1-one (2) NMR data were described in Table 1. EI-MS *m*/*z* (rel. int.): 208 $[M^+]$ (11), 193 (100), 178 (16), 167 (24), 69 (11). IR ($CHCl_3$) cm^{-1}: 3585, 1643, 1624, 1595, 1574, 1429, 1339. UV λ_{max} (EtOH) nm (log ε): 318 (4.25), 250 (sh) (3.95), 209 (4.31).

1-(2,4,6-Trimethoxy-phenyl)-but-2(*Z*)-en-1-one (3) NMR data were described in Table 1. EI-MS *m*/*z* (rel. int.): 236 $[M^+]$ (87), 205 (38), 195 (100), 168 (44), 137 (17). IR ($CHCl_3$) cm^{-1}: 1607, 1466, 1414, 1157, 1132. UV λ_{max} (EtOH) nm (log ε): 297 (3.64), 227 (4.15), 204 (4.58).

1-(2-Hydroxy-4,6-dimethoxyphenyl)-3(*R*)-hydroxy-1-butanone (6) NMR data were described in Table 1. mp 67—68 °C. $[\alpha]_D^{20}$ −64.8 (c=2.82, $CHCl_3$). EI-MS *m*/*z* (rel. int.): 240 $[M^+]$ (8.5), 207 (23.5), 181 (100), 154 (13.6). IR ($CHCl_3$) cm^{-1}: 3565, 1620, 1592, 1418, 1160, 1116. UV λ_{max} (EtOH) nm (log ε): 325 (3.88), 288 (4.16), 211 (4.65).

Bromination of 6 with *N*-Bromosuccinimide *N*-Bromosuccinimide (10 mg) was added to a solution of **6** (5.9 mg) in CCl_4 (0.4 ml), and the reac-

tion mixture was refluxed for 1 h. After cooling, the reaction mixture was passed through a short column on celite, then purified by prep HPLC to give **10** (3.8 mg). **10**: $[\alpha]_D^{20}$ −23.4 (c=0.38, $CHCl_3$). EI-MS m/z (rel. int.): 400 (3.7), 398 (7.2), 396 [M^+] (3.6), 367 (47.9), 365 (100), 363 (50.1), 341 (41.1), 339 (86.1), 337 (46.2). ^{1}H-NMR ($CDCl_3$; 300 MHz): 1.29 (d, 3H, J=6.3 Hz), 3.22 (dd, J=18.4, 8.5 Hz), 3.34 (dd, J=18.4, 3 Hz), 3.92 (s), 3.96 (3H, s), 4.41 (1H, m).

Crystal Data for 1-(3,5-dibromo-2-hydroxy-4,6-dimethoxyphenyl)-3(*R*)-hydroxy-1-butanone (10) All diagrams and calculations were performed using maXus (Bruker Nonius, Delft & MacScience, Japan). Mo$K\alpha$ (λ=0.71073). Orthorhombic $P2_12_12_1$, a=4.484 (2) Å, b=14.4210 (8) Å, c=22.102 (5) Å, V=1429.2 (8) Å^3, Z=4, D_x=1.850 mg m^{-3}, μ=5.684 mm^{-1}, final residuals R(all)=0.0750, R(gt)=0.0580, and S(ref)=1.084.

2,5-Dihydroxy-7-methoxy-2-methyl-chroman-4-one (7) NMR data were described in Table 1. EI-MS m/z (rel. int.): 224 [M^+] (91), 209 (66), 207 (79), 182 (21), 167 (100) 140 (28). IR ($CHCl_3$) cm^{-1}: 3586, 1640, 1578, 1315, 1157, 1073. UV λ_{max} (EtOH) nm (log ε): 325 (sh) (3.88), 287 (4.59), 212 (4.65).

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References

1) Asakawa Y., *J. Bryol.*, **14**, 59—70 (1986).
2) Asakawa Y., Toyota M., Takemoto T., *Phytochemistry*, **17**, 457—460 (1978).
3) Asakawa Y., Takemoto T., Toyota M., Aratani T., *Tetrahedron Lett.*, **16**, 1407—1410 (1977).
4) Asakawa Y., Toyota M., Takemoto T., *Tetrahedron Lett.*, **18**, 1553—1556 (1978).
5) Asakawa Y., Toyota M., Takemoto T., Suire C., *Phytochemistry*, **18**, 1007—1009 (1979).
6) Asakawa Y., Matsuda R., Takemoto T., *Phytochemistry*, **19**, 567—569 (1980).
7) Asakawa Y., "Progress in the Chemistry of Organic Natural Products," Vol. 42, ed. by Herz W., Kirby G. W., Moore R. E., Steglich W., Tamm Ch., Springer, Vienna, 1982.
8) Asakawa Y., Toyota M., Oiso Y., Braggins J. E., *Chem. Pharm. Bull.*, **49**, 1380—1381 (2001).
9) Allison K. W., Child J., "The Liverworts of New Zealand," University of Otago Press, Dunedin, 1975, p. 245.
10) Markham K. R., Porter L. J., Campbell E. O., Chopin J., Bouillant M.-L., *Phytochemistry*, **15**, 1517—1521 (1976).
11) Tanaka N., Maehashi H., Saito S., Murakami T., Saiki Y., Chen C.-M., Iitaka Y., *Chem. Pharm. Bull.*, **27**, 2874—2876 (1979).
12) Tanaka N., Maehashi H., Saito S., Murakami T., Saiki Y., Chen C.-M., Iitaka Y., *Chem. Pharm. Bull.*, **28**, 3070—3077 (1980).
13) Chakrabarti A., Chakraborty D. P., *Phytochemistry*, **27**, 3683—3684 (1988).
14) Numata A., Katsuno T., Yamamoto K., Nishida T., Takemura T., Seto K., *Chem. Pharm. Bull.*, **32**, 325—331 (1984).
15) Ahluwalia, V. K., Kumar D., *Indian J. Chem.*, **15B**, 514—518 (1977).
16) Tsui W.-Y., Brown Geoffrey D., *Phytochemistry*, **43**, 871—876 (1996).
17) Morimoto M., Tanimoto K., Nakano S., Ozaki T., Nakano A., Komai K., *J. Agric. Food Chem.*, **57**, 389—393 (2003).
18) Banerji A., Goomer N. C., Kalena G. P., *Synthetic Commun.*, **10**, 851—856 (1980).
19) Hori K., Satake T., Saiki Y., Murakami T., *Yakugaku Zasshi*, **110**, 315—320 (1990).
20) Oiso Y., Toyota M., Asakawa Y., *Chem. Pharm. Bull.*, **47**, 297—298 (1999).