



# Aromatic compounds from the Ecuadorian liverwort *Marchesinia brachiata*: a revision

F. Nagashima, Y. Murakami, Y. Asakawa

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima, 770-8514, Japan

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## Abstract

3,4-Dimethoxy-1-vinylbenzene and 2,4,5-trimethoxy-1-vinylbenzene, were isolated from the Ecuadorian liverwort, *Marchesinia brachiata*, together with a known flavone, apigenin-7,4'-dimethylether. Their structures were confirmed by extensive NMR spectroscopic analysis. This study corrects our previous reports of eugenol, 3,5-dimethoxy-4-hydroxy- or 3,4-dimethoxy-5-hydroxy-1-allylbenzene in *M. brachiata*, these instead being 3,4-dimethoxy-1-vinylbenzene and 2,4,5-trimethoxy-1-vinylbenzene, respectively. © 1999 Elsevier Science Ltd. All rights reserved.

**Keywords:** *Marchesinia brachiata*; Lejeuneaceae; Liverwort; 3,4-Dimethoxy-1-vinylbenzene; 2,4,5-Trimethoxy-1-vinylbenzene; Apigenin-7,4'-dimethylether; Aromatic compounds; Flavone

## 1. Introduction

As part of the search for new compounds and biologically active substances from bryophytes, we are continuing to study the chemical constituents of liverworts (Asakawa, 1982; 1995; Nagashima et al., 1998; Nagashima et al., in press). Lejeuneaceae, of which there are ca. 3000 species. Most of the species produce mono-, sesqui- and diterpenoids, which are responsible for the oil bodies in each species. The *Marchesinia* species are chemically quite different from other Lejeuneaceae species, because *M. brachiata* and *M. mackaii* elaborate a few simple aromatic compounds, which are then identified as eugenol and its derivatives (Asakawa et al., 1980; Gradstein et al., 1981; Gradstein, 1985). Since chemical differences have been noted in the same species collected in a different locality (Asakawa, 1995; Nagashima et al., in press), we reinvestigated the chemical constituents of the ether extract of *M. brachiata* collected in Ecuador and report the isolation and structure determination of two simple styrene derivatives.

## 2. Results and discussion

The crude extract of *M. brachiata* was analyzed by GC–MS to confirm the presence of two sesquiterpene hydrocarbons,  $\beta$ -caryophyllene and bicyclogermacrene, and two aromatic compounds ( $[M]^+$  164 and  $[M]^+$  194) of which the latter was the major component. The remaining extract was further chromatographed on silica gel, Sephadex LH-20 and MPLC to afford two aromatic compounds, 3,4-dimethoxy-1-vinylbenzene (**1**) and 2,4,5-trimethoxy-1-vinylbenzene (**2**), which corresponded to giving rise to the two peaks ( $[M]^+$  164 and  $[M]^+$  194) by GC–MS and previously known flavone, apigenin-7,4'-dimethylether (**3**) (Biftu & Stevenson, 1978; Asakawa, 1982; Ali et al., 1985; Asakawa, 1985) was also isolated.

The EI mass spectrum of compound **1** gave a  $m/z$  164  $[M]^+$ . In the IR spectrum neither carboxyl nor hydroxyl absorption band was confirmed. The  $^1\text{H}$  NMR spectrum showed the presence of two methoxyl groups, as well as terminal-vinyl protons and aromatic protons. Furthermore, the  $^{13}\text{C}$  NMR spectrum indicated

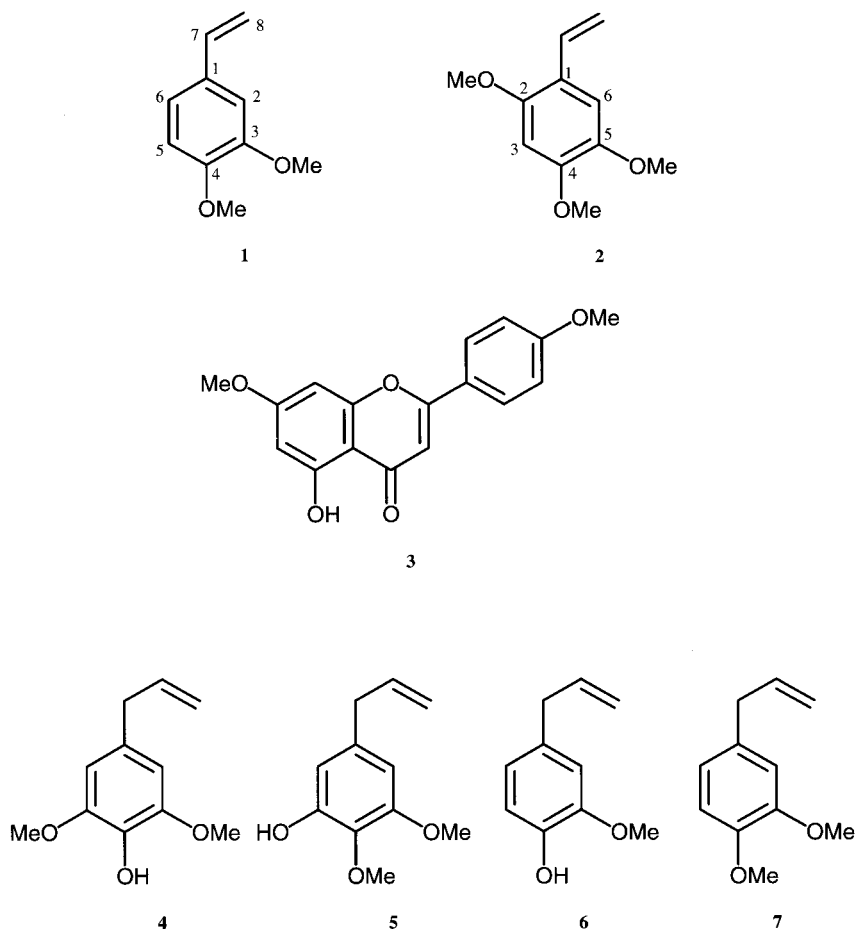


Fig. 1

the presence of ten carbons. The splitting pattern of each  $^1\text{H}$  NMR signal suggested that compound **1** is a styrene-type compound with two methoxyl groups. The substitution pattern of the two methoxyl groups was confirmed by analysis of its NOESY spectrum. Namely, NOEs were observed between (i)  $\text{C}_3\text{-OMe}$  and H-2, (ii)  $\text{C}_4\text{-OMe}$  and H-5, (iii) H-8 and H-2, and (iv) H-7 and H-6, respectively. Thus, the structure of **1** was established to be 3,4-dimethoxy-1-vinylbenzene.

The EIMS spectrum of **2** gave a  $m/z$  194  $[\text{M}]^+$  and its molecular formula was confirmed to be  $\text{C}_{11}\text{H}_{14}\text{O}_3$  by HR mass spectrum. This compound was very unstable in weak acid even in  $\text{CDCl}_3$  or on the TLC to give unidentified material. Its  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra resembled those of **1**, except for the presence of an additional methoxyl group, suggesting that **2** might be a trimethoxystyrene. To clarify the substitution pattern, a NOESY experiment was carried out, and NOEs were observed between (i)  $\text{C}_2\text{-OMe}$  and H-3, (ii)  $\text{C}_4\text{-OMe}$  and H-3, (iii)  $\text{C}_5\text{-OMe}$  and H-6, and (iv) H-6

and H-8, respectively. Accordingly, **2** was established to be 2,4,5-trimethoxy-1-vinylbenzene.

3,4-Dimethoxy-1-vinylbenzene (**1**) has been detected in the thalloid liverworts, *Asterella*-like species (Hashimoto et al., 1993) and *Conocephalum conicum* (Toyota et al., 1997), which are morphologically quite distinct from the *Marchesinia* species, and also has been found in coffee aroma (Stoll et al., 1967) and *Coreopsis fasciculata* (Compositae) (Bohlmann et al., 1983). 2,4,5-Trimethoxy-1-vinylbenzene (**2**) has been obtained not only from an unidentified Jamaican liverwort (Connolly, 1985), but also from *Pachypodanthium staudtii* (Annonaceae) (Waterman, 1976; Ngadjui et al., 1989). However, the  $^{13}\text{C}$  NMR data of these compounds have not been reported. The *Marchesinia* species are chemically very different from the other subfamilies of the Lejeuneaceae, because of the presence of styrene derivatives as the major components. Occurrence of styrene derivatives are very rare in the Hepaticae, only two species belonging to the

Marchantiales (Hashimoto et al., 1993; Toyoto et al., 1997) and an unidentified species as mentioned above (Connolly, 1985) have been identified. *Leptlejeunea elliptica*, belonging to the subfamily of the Lejeuneaceae, is chemically similar to the *Marchesinia* species, because the former species elaborates *p*-hydroxy-, *p*-methoxy- and *p*-acetoxy ethylbenzenes as major components.

The presence of eugenol (**6**), methyleugenol (**7**) and 3,5-dimethoxy-4-hydroxyallylbenzene (**4**) or 3,4-dimethoxy-5-hydroxyallylbenzene (**5**) was previously suggested by GC–MS analysis of *M. brachiata* collected in Saba (Gradstein et al., 1981). Methyleugenol has also apparently been found in *M. mackaii* collected in the UK [7]. However, the molecular weights ( $m/z$  164  $[M]^+$  and  $m/z$  194  $[M]^+$ ) and fragmentation patterns of the aromatic compounds assigned to compounds **6** and **4** or **5** were in fact identical to those of **1** and **2**. It is then concluded that the previously identified compounds (**6**, and **4** or **5**) by GC–MS in *M. brachiata* (Gradstein et al., 1981; Gradstein, 1985) were actually **1** and **2**, respectively. There are ostensibly no metabolic differences between *M. brachiata* collected in the Netherlands Antilles, Saba and that in Ecuador Province, Los Rios, since the same sesquiterpene hydrocarbons,  $\beta$ -caryophyllene and bicyclogermacrene and styrene derivatives (**1** and **2**), were present as major components in both species.

### 3. Experimental

Melting points were uncorr. Chemical shift values were expressed in  $\delta$  (ppm) downfield from tetramethylsilane as an internal standard ( $^1\text{H}$  NMR) and  $\delta$  77.03 (ppm) from  $\text{CHCl}_3$  and/or  $\delta$  128.00 (ppm) from  $\text{C}_6\text{D}_6$  as a standard ( $^{13}\text{C}$  NMR). TLC spots were visualized under UV (254 nm) light and by spraying with 10%  $\text{H}_2\text{SO}_4$  or Godin reagent (Godin, 1954) followed by heating at  $110^\circ$ . Capillary GC–MS: 70 eV; GC condition; column DB-1, 0.25 mm  $\times$  30 m; column temperature  $50^\circ\text{C}$  (5 min hold)– $250^\circ\text{C}$  temperature program  $5^\circ\text{C}/\text{min}$ ; carrier gas: He; detector temperature  $280^\circ\text{C}$ ; injection temperature  $250^\circ\text{C}$ .

**Plant material.** *Marchesinia brachiata* (Sw.) Schiffn. was collected in Ecuador in September, 1997, and identified by Professor S.R. Gradstein. The voucher specimen was deposited at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

**Extraction and isolation.** The dried *M. brachiata* (20 g) was mechanically ground and twice extracted with  $\text{Et}_2\text{O}$  for 1 week. A small amount of the extract was analyzed by GC–MS to detected limonene (0.11%), 3,4-dimethoxy-1-vinylbenzene (5.3%),  $\beta$ -caryophyllene (5.1%), bicyclogermacrene (15.6%) and 2,4,5-trimethoxy-1-vinylbenzene (62%). The remaining

extract was divided into 10 fractions by column chromatography on Silica gel (*n*-hexane–EtOAc gradient). Fraction 4 was rechromatographed on Sephadex LH-20,  $\text{SiO}_2$  and finally purified by MPLC (Lobar<sup>®</sup> column; Si 60, *n*-hexane–EtOAc 95:5) to give 3,4-dimethoxy-1-vinylbenzene (**1**) (21 mg) (Stoll et al., 1967; Bohlmann et al., 1983; Hashimoto et al., 1993; Toyoto et al., 1997). 2,4,5-Trimethoxy-1-vinylbenzene (**2**) (60 mg) (Waterman, 1976; Connolly, 1985; Ngadjui et al., 1989) was isolated from fraction 6 by rechromatography on Sephadex LH-20 and  $\text{SiO}_2$  (*n*-hexane–EtOAc 9:1). Fraction 8 was rechromatographed on Sephadex LH-20 and  $\text{SiO}_2$  (*n*-hexane–EtOAc 1:1) to give apigenin-7,4'-dimethylether (**3**) (15 mg) (Biftu & Stevenson, 1978; Asakawa, 1982; Ali et al., 1985; Asakawa, 1995).

**3,4-Dimethoxy-1-vinylbenzene (1).** FTIR  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 1510, 1460, 1265, 1140, 1025; HREIMS;  $m/z$  164.0832. Calculated for  $\text{C}_{10}\text{H}_{12}\text{O}_2$ : 164.0837;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  6.97 (1H, *d*,  $J=2.0$  Hz, H-2), 6.82 (1H, *d*,  $J=8.0$  Hz, H-5), 6.94 (1H, *dd*,  $J=8.0, 2.2$  Hz, H-6), 6.65 (1H, *dd*,  $J=17.6, 11.0$  Hz, H-7), 5.15 (1H, *dd*,  $J=11.0, 0.8$  Hz, H-8), 5.61 (1H, *dd*,  $J=17.6, 0.8$  Hz, H-8'), 3.91 (3H, *s*, 3-OMe), 3.88 (3H, *s*, 4-OMe);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  130.7 (C-1), 108.5 (C-2), 148.9, 149.0 (C-3 or C-4), 111.0 (C-5), 119.4 (C-6), 136.4 (C-7), 111.8 (C-8), 55.8, 55.9 (3-OCH<sub>3</sub> or 4-OCH<sub>3</sub>); EIMS  $m/z$  (rel. int): 164 $[M]^+$  (100), 149(44), 121(16), 103(17), 91(20), 77(15), 65(5), 51(5), 39(3); UV  $\lambda_{\text{max}}$  nm(log  $\epsilon$ ): 298(2.81), 260(3.26), 213(3.45) ( $c$   $4.63 \times 10^{-4}$ , EtOH).

**2,4,5-Trimethoxy-1-vinylbenzene (2).** mp.  $54\text{--}57^\circ\text{C}$ ; FTIR  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 1515, 1466, 1217, 1120, 1035; HREIMS;  $m/z$  194.0935. Calculated for  $\text{C}_{11}\text{H}_{14}\text{O}_3$ : 194.0943;  $^1\text{H}$  NMR ( $\text{C}_6\text{D}_6$ ):  $\delta$  6.24 (1H, *s*, H-3), 7.10 (1H, *s*, H-6), 7.36 (1H, *dd*,  $J=17.9, 11.3$  Hz H-7), 5.20 (1H, *dd*,  $J=11.3, 1.6$  Hz, H-8), 5.68 (1H, *dd*,  $J=17.9, 1.4$  Hz, H-8'), 3.32 (3H, *s*, 2-OMe), 3.39 (3H, *s*, 4-OMe), 3.45 (3H, *s*, 5-OMe);  $^{13}\text{C}$  NMR ( $\text{C}_6\text{D}_6$ ):  $\delta$  119.3 (C-1), 152.1 (C-2), 98.9 (C-3), 151.0 (C-4), 144.7 (C-5), 111.4 (C-6), 131.8 (C-7), 111.8 (C-8), 56.0 (2-OMe), 55.8 (4-OCH<sub>3</sub>), 56.5 (4-OCH<sub>3</sub>); EIMS  $m/z$  (rel. int): 194 $[M]^+$  (100), 179(61), 151(46), 136(13), 121(7), 91(11), 69(11), 51(8), 39(4); UV  $\lambda_{\text{max}}$  nm(log  $\epsilon$ ): 316(2.52), 259(2.79), 210(2.97) ( $c$   $6.31 \times 10^{-4}$ , EtOH).

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## References

- Ali, M. S., Iqbal, J., & Llyas, M. (1985). *Chemistry and Industry*, 15, 276.
- Asakawa, Y., Tokunaga, N., Takemoto, T., Hattori, S., & Mizutani, M. (1980). *Journal of the Hattori Botanical Laboratory*, 47, 153.
- Asakawa Y. (1982). Herz W., Grisebach H., Kirby G.W. (Ed.), *Progress in the chemistry of organic natural products* (vol. 42, p. 1). Vienna: Springer.
- Asakawa Y. (1995). Herz W., Kirby G.W., Moore R.E., Steglich W., Tamm Ch (Ed.), *Progress in the chemistry of organic natural products* (vol. 65, p. 1). Vienna: Springer.
- Biftu T., Stevenson R. (1978). *Journal of the Chemical Society, Perkin Transactions I* 360.
- Bohlmann, F., Ahmed, M., Grenz, M., King, R. M., & Robinson, H. (1983). *Phytochemistry*, 22, 2858.
- Connolly, J. D. (1985). In R. I. Zalewski, & J. J. Skolik, *Natural Products Chemistry* (p. 3). Amsterdam: Elsevier.
- Godin, P. (1954). *Nature (London)*, 174, 134.
- Gradstein, S. R., Matsuda, R., & Asakawa, Y. (1981). *Journal of the Hattori Botanical Laboratory*, 50, 231.
- Gradstein, S. R. (1985). In *Contributions to a monograph of the Lejeuneaceae subfamily Ptychanthoideae* (p. 63). Vaduz: J. Cramer.
- Hashimoto, T., Asakawa, Y., Nakashima, K., & Tori, M. (1993). *Journal of the Hattori Botanical Laboratory*, 74, 121.
- Nagashima F., Takaoka S., Asakawa Y. (1998). *Phytochemistry* (in press).
- Nagashima, F., Suzuki, M., Takaoka, S., & Asakawa, Y. (1998). *Chemical & Pharmaceutical Bulletin*, 46, 1184.
- Ngadjui, B. T., Lontsi, D., Ayafor, J. F., & Sondengam, B. L. (1989). *Phytochemistry*, 28, 231.
- Stoll, M., Winter, M., Gautschi, F., Flament, I., & Willhalm, B. (1967). *Helvetica Chimica Acta*, 50, 628.
- Toyota, M., Saito, T., Matsunami, J., & Asakawa, Y. (1997). *Phytochemistry*, 44, 1265.
- Waterman, P. G. (1976). *Phytochemistry*, 15, 347.