Pergamon

0031-9422(95)00760-1

Phytochemistry, Vol. 41, No. 5, pp. 1373–1376, 1996

0031-9422/96 $15.00 + 0.00

PHYTYL ESTERS AND PHAEOPHYTINS FROM THE HORNWORT *MEGACEROS FLAGELLARIS*

MALCOLM S. BUCHANAN, TOSHIHIRO HASHIMOTO and YOSHINORI ASAKAWA

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

(*Received in revised form* 22 *August* 1995)

Key Word Index—*Megaceros flagellaris*; Bryophyte; Anthocerotae; hornwort; phaeophytins; phytyl esters.

Abstract—Two phytyl esters, (2*E*,2′*E*)-phyt-2-enyl phyt-2′-enoate and (2*E*)-phyt-2-enyl phytanoate, which have not been isolated before, seven phaeophytins, β-sitosterol and stigmast-5-en-3-yl hexadecanoate have been isolated from the hornwort *Megaceros flagellaris*. Their structures were established by spectroscopic methods.

INTRODUCTION

The bryophytes are taxonomically placed between the algae and the pteridophytes in the plant kingdom, there being approximately 20000 species in the world [1]. They are morphologically divided into three classes, Musci (mosses, 14000 species), Hepaticae (liverworts, 6000 species) and Anthocerotae (hornworts, 300 species). Of these the most interesting chemically are the liverworts, due to the presence of oil bodies in their cells. Hornworts and mosses do not contain oil bodies and are regarded as chemically simple [2]. Previous studies on hornworts have reported the presence of sterols and sesquiterpenoids detected by GCMS [3–5], lignans [6, 7] and cinnamic acid derivatives [6, 8–10]. Most recently, an alkaloid, anthocerodiazonin, and six glutamic acid amides were isolated from *in vitro* cultures of the hornwort *Anthoceros agrestis* [10]. From the one previous investigation of *Megaceros flagellaris* [6, 7] the lignan megacerotonic acid was isolated. In this paper, we report on the isolation and characterization of the chemical constituents from the hornwort *M. flagellaris* and also discuss its chemosystematics, which is important as only a few species of hornwort have been studied previously.

RESULTS AND DISCUSSION

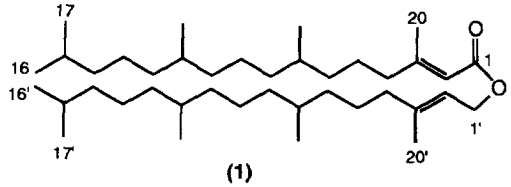
The fresh hornwort, *M. flagellaris*, was extracted with ether and then chromatographed on silica gel and prep. HPLC to give two phytyl esters (**1**, **2**), seven phaeophytins (**3**–**9**), β-sitosterol (**10**) and its palmitate ester, stigmast-5-en-3-yl hexadecanoate (**11**).

Compound **1** revealed a molecular ion peak at *m/z* 588.5847 in its HRMS indicating a molecular formula of $C_{40}H_{76}O_2$. The IR spectrum showed the presence of an α,β-unsaturated ester (ν_{max} 1717, 1649 cm^{-1}). The ^1H NMR spectrum indicated the presence of two olefinic protons [δ_H5.68 (*brq*, J = 1.2 Hz), 5.36 (*brt*, J = 7.1 Hz)] the latter of which couples to a $-CH_2-O-$ group [δ_H4.61 (*d*, J = 7.1 Hz)]. Also present were two olefinic methyls [δ_H2.15 (*d*, J = 1.2 Hz), 1.70 (*br s*)] and eight secondary methyls. The ^{13}C NMR spectrum contained resonances for an α,β-unsaturated ester carbonyl (δ_C166.90), two trisubstituted double bonds [δ_C160.41 (*s*), 142.25 (*s*), 118.42 (*d*), 115.35 (*d*)], an oxygenated methylene (δ_C60.50) in addition to 10 methyls, 18 methylenes and six methines. The above information together with the analysis of 2D NMR spectra (HMQC, phase-sensitive DQF-COSY and HMBC) enabled the partial structures shown in Fig. 1 to be derived. These partial structures together with the remaining eight methylenes, four methines, and four methyls led to structure **1**. It is clear from the ^1H and ^{13}C chemical shifts of the olefinic methyls [3H-20′: δ_C18.74, δ_H2.15 (*d*, J = 1.2 Hz); 3H-20: δ_C16.37, δ_H1.70 (*br s*)] that the two trisubstituted double bonds are *trans* in compound **1**. Thus the structure proposed by Sporle *et al.* [11] should be revised to the 2′*Z* isomer of (2*E*,2′*E*)-phyt-2-enyl phyt-2′-enoate (**1**).

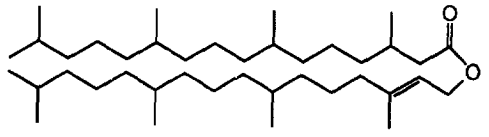
The CIMS of compound **2** gave the molecular formula $C_{40}H_{78}O_2$ ($[M + H]^+$ at *m/z* 591.6066). It was clear from the spectroscopic data of **2** that it was the 2′,3′-dihydro derivative of **1**. Thus compound **2** is (2*E*)-phyt-2-enyl phytanoate.

This is the first report of the isolation of (2*E*,2′*E*)-phytenyl phytenoate (**1**) and its 2′,3′-dihydro derivative (**2**) from a natural source. However, (2*E*,2′*Z*)-phyt-2-enyl phyt-2′-enoate has been isolated from the liverwort *Monoclea gottschei* ssp. *neotropica* [11] and there have been reports of phytyl esters which were detected by GC-mass spectrometry [12].

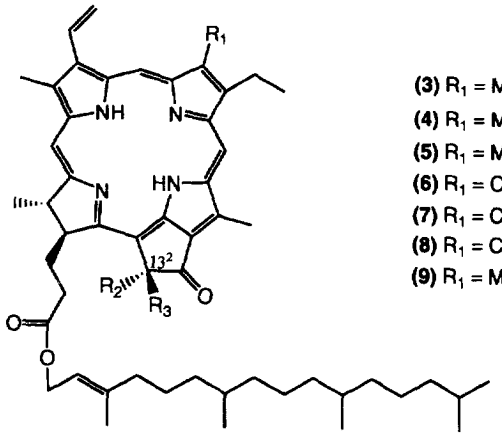
Phaeophytin a (**3**), 13^2-hydroxy-(13^2-*R*)-phaeophytin a (**4**), 13^2-hydroxy-(13^2-*S*)-phaeophytin a (**5**), phaeophytin b (**6**), 13^2-hydroxy-(13^2-*R*)-phaeophytin b (**7**), 13^2-hydroxy-(13^2-*S*)-phaeophytin b (**8**) and pyrophaeophytin a (**9**) were isolated from this extract. The compounds isolated here (**3**–**9**) are all known natural



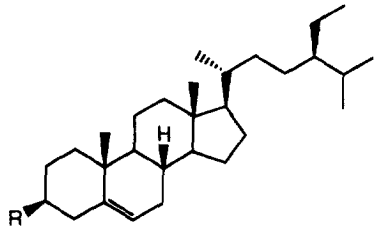
(1)



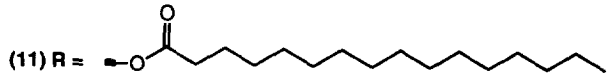
(2)



(3) R_1 = Me, R_2 = CO_2Me, R_3 = H
(4) R_1 = Me, R_2 = OH, R_3 = CO_2Me
(5) R_1 = Me, R_2 = CO_2Me, R_3 = OH
(6) R_1 = CHO, R_2 = CO_2Me, R_3 = H
(7) R_1 = CHO, R_2 = OH, R_3 = CO_2Me
(8) R_1 = CHO, R_2 = CO_2Me, R_3 = OH
(9) R_1 = Me, R_2 = H, R_3 = H



(10) R = OH



(11) R =

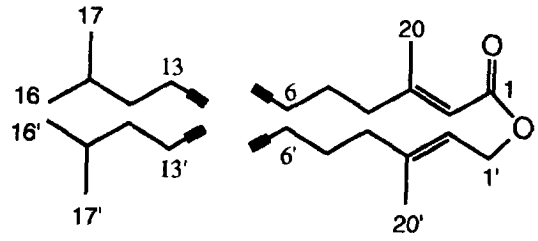


Fig. 1. Partial structures of compound **1**.

products and were identified by comparison of their NMR data with published data [13–16]. Neither terpenoids, alkaloids nor lignans were detected in the present hornwort species. The identification of phaeophytins in this species is reported for the first time in the Anthocerotae. Compounds **4**, **5**, **7** and **8** have been isolated from silkworm excreta [13] and the moss, *Entodon rubicundus* [H. Nozaki, personal communication].

The major constituent of this hornwort was the sterol, β-sitosterol (**10**). This was isolated along with a small amount of stigmast-5-en-3-yl hexadecanoate (**11**). These common plant constituents were identified by comparison of their spectroscopic data with published data [17–20].

Although the chemical constituents of hornworts have not been studied thoroughly, *M. flagellaris* is chemically quite different from the five hornwort genera, *Anthoceros*, *Dendroceros*, *Folioceros*, *Notothylas* and *Phaeoceros*, which have been investigated so far [3–10]. Furthermore, the compounds isolated from the species studied here support the hypothesis that hornworts are chemically

simple [2]. Their structures are simple when compared with the liverworts which are a rich source of terpenoids and aromatic compounds possessing a variety of novel and interesting structures [1]. This indicates that the biosynthetic pathways in hornworts are not as sophisticated and thus they are a more primitive class of plants than the liverworts.

EXPERIMENTAL

General. TLC and PLC: Merck precoated silica gel 60 F_{254}; visualised under UV light (254 nm) and by spraying with 30% H_2SO_4 and heating. Flash CC: Silica gel 60 (40–63 μm). HPLC: Chemcosorb 5Si-U 10 × 250 mm (B).

Spectral data. NMR spectra (^1H, 600 MHz; ^{13}C, 150 MHz) were recorded for $CDCl_3$ solutions relative to TMS at $\delta_H 0$ and $CDCl_3$ at $\delta_C 77.0$. Multiplicities were determined by DEPT experiments. IR spectra and $[\alpha]_D$ were measured for $CHCl_3$ solutions. EIMS were measured at 70 eV. CIMS were obtained at 70 eV with CH_4 as the carrier gas.

Plant material. Megaceros flagellaris was collected in Kagoshima Prefecture (Japan) on 20 November 1993. A voucher specimen is deposited at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

Extraction and isolation. The fresh material (176 g) was extracted with Et_2O to yield 1.96 g of crude extract. The crude extract (1.96 g) was chromatographed by flash CC over silica gel using an *n*-hexane–EtOAc gradient to yield ten fractions. Frs 2 (215 mg) and 3 (280 mg) were combined and further fractionated by PLC (*n*-hexane–EtOAc 24:1). Purification of the subsequent frs by HPLC yielded the following compounds: (2*E*,2′*E*)-phyt-2-enyl phyt-2′-enoate (**1**) (160 mg) [HPLC: *n*-hexane–EtOAc (99.7:0.3), *n*-hexane–CH_2Cl_2 (3:2)]; (2*E*)-phyt-2-enyl phytanoate (**2**) (37 mg) [HPLC: *n*-hexane–EtOAc (99.7:0.3), *n*-hexane–CH_2Cl_2 (13:7)] and stigmast-5-en-3-yl hexadecanoate (**11**) (12 mg) [HPLC: *n*-hexane–EtOAc (99.7:0.3), *n*-hexane–CH_2Cl_2 (3:2)]. Fr. 5 (187 mg) was purified by HPLC using *n*-hexane–EtOAc (3:1) and then CH_2Cl_2–MeOH (49:1) to afford β-sitosterol (**10**) (110 mg). Fr. 6 (336 mg) was separated further by PLC (CH_2Cl_2–EtOAc 17:3) to yield β-sitosterol (**10**) (120 mg) and three other frs. The first of these frs yielded phaeophytin a (**3**) (94 mg), pyrophaeophytin a (**9**) (6 mg) both purified by HPLC (CH_2Cl_2–EtOAc 19:1) and 13^2-hydroxy-(13^2-*R*)-phaeophytin a (**4**) (6 mg), 13^2-hydroxy-(13^2-*S*)-phaeophytin a (**5**) (6 mg) both also purified by HPLC [CH_2Cl_2–EtOAc (19:1), *n*-hexane–EtOAc (3:1), *n*-hexane–CH_2Cl_2–EtOAc (47:47:6)]. Frs 7 and 8 were combined (202 mg). Purification of these two frs by HPLC yielded phaeophytin b (**6**) (33 mg) [CH_2Cl_2–EtOAc (19:1), *n*-hexane–CH_2Cl_2–EtOAc (9:9:2)], 13^2-hydroxy-(13^2-*R*)-phaeophytin b (**7**) (5 mg) and 13^2-hydroxy-(13^2-*S*)-phaeophytin b (**8**) (5 mg) [CH_2Cl_2–EtOAc (19:1), *n*-hexane–CH_2Cl_2–EtOAc (47:47:6)]. Phaeophytins a (**3**) and b (**6**) consist of 16% and 19% (NMR peak areas), respectively, of the 13^2-*S* isomer.

(2E,2′E)-*Phyt-2-enyl phyt-2′-enoate* (**1**). Oil, $[\alpha]_D$ 0. HRMS: *m/z* 588.5847 $[M]^+$ calculated for $C_{40}H_{76}O_2$: 588.5845; EIMS *m/z* (rel. int.): 588 $[M]^+$ (73), 293 (100), 278 (62), 123 (46), 111 (33), 95 (47), 83 (43), 69 (49) 57 (56); IR ν_{max} cm^{-1}: 2928, 1717 (C=O), 1649 (C=C), 1462, 1219, 1146; ^1H NMR: $\delta_H 5.68$ (*br q*, J = 1.2 Hz, H-2′), 5.36 (*brt*, J = 7.1 Hz, H-2), 4.61 (*d*, J = 7.1 Hz, 2H-1), 2.15 (*d*, J = 1.2 Hz, 3H-20′), 2.10 (*m*, 2H-4′), 2.00 (*m*, 2H-4), 1.70 (*br s*, 3H-20), 1.52 (*sept.*, J = 6.6 Hz, 3H-15/3H-15′), 0.867, 0.866 (both *d*, J = 6.6 Hz, 3H-16/3H-16′/3H-17/3H-17′), 0.850, 0.849, 0.844, 0.844 (all *d*, J = 6.6 Hz); ^{13}C NMR: $\delta_C 166.90$ (*s*, C-1′), 160.41 (*s*, C-3′), 142.25 (*s*, C-3), 118.42 (*d*, C-2), 115.35 (*d*, C-2′), 60.50 (*t*, C-1), 41.26 (*t*, C-4′), 39.88 (*t*, C-4), 39.36 (*t*, C-14), 39.36 (*t*, C-14′), 37.42, 37.40, 37.34, 37.28, 37.28, 37.28 (all *t*, C-8/8′/10/10′/12/12′), 36.64 (*t*, C-6), 36.54 (*t*, C-6′), 32.78, 32.78, 32.67, 32.64 (all *d*, C-7/7′/11/11′), 27.96 (C-15), 27.96 (C-15′), 25.04 (*t*, C-5), 24.86 (*t*, C-5′), 24.79 (*t*, C-13), 24.79 (*t*, C-13′), 24.46 (*t*, C-9), 24.46 (*t*, C-9′), 22.71, 22.71, 22.63, 22.63 (all *q*, C-16/16′/17/17′), 19.74, 19.74, 19.71, 19.64 (all *q*, C-18/18′/19/19′/20′), 18.74 (*q*, C-20′), 16.37 (*q*, C-20).

(2E)-*Phyt-2-enyl phytanoate* (**2**). Oil, $[\alpha]_D$ 0. HRCIMS: *m/z* 591.6066 $[M + H]^+$ calculated for $C_{40}H_{79}O_2$: 591.6080, CIMS *m/z* (rel. int.): 591 $[M + H]^+$ (17), 341 (27), 311 (61), 278 (100), 197 (46), 153 (46), 125 (57), 111 (64), 97 (45); IR ν_{max} cm^{-1}: 2926, 1738 (C=O), 1462; ^1H NMR: $\delta_H 5.34$ (*brt*, J = 7.1 Hz, H-2), 4.59 (*d*, J = 7.1 Hz, 2H-1), 2.30 (*dd*, J = 14.4, 6.1 Hz, H-2′), 2.10 (*dd*, J = 14.4, 8.2 Hz, H-2′), 2.00 (*m*, 2H-4), 1.69 (*brs*, 3H-20), 1.52 (*sept.*, J = 6.6 Hz, 3H-15/3H-15′), 0.93 (*d*, J = 6.6 Hz, 3H-17′), 0.868, 0.866 (both *d*, J = 6.6 Hz, 3H-16/3H-16′/3H-17/3H-17′), 0.851, 0.842, 0.845, 0.845 (all *d*, J = 6.6 Hz); ^{13}C NMR: δ_C 173.39 (*s*, C-1′), 142.59 (*s*, C-3), 118.19 (*d*, C-2), 61.08 (*t*, C-1), 42.00 (*t*, C-2′), 39.86 (*t*, C-4), 39.37 (*t*, C-14), 39.37 (*t*, C-14′), 37.45, 37.45, 37.29, 37.29, 37.12, 37.06 (all *t*, C-8/8′/10/10′/12/12′), 37.36 (*t*, C-4′), 36.63 (*t*, C-6), 32.79, 32.79, 32.75, 32.67 (all *d*, C-7/7′/11/11′), 30.44 (*d*, H-3′), 29.70 (*t*, C-6′), 27.97 (C-15), 27.97 (C-15′), 25.04 (*t*, C-5), 24.79 (*t*, C-13), 24.79 (*t*, C-13′), 24.47 (*t*, C-9), 24.47 (*t*, C-9′), 24.33 (*t*, C-5′), 22.71, 22.71, 22.63, 22.63 (all *q*, C-16/16′/17/17′), 19.75, 19.75, 19.71, 19.70, 19.66 (all *q*, C-18/18′/19/19′/20′), 16.36 (*q*, C-20).

Acknowledgements—We thank Miss Y. Kan (TBU) for measurement of 600 MHz NMR spectra and Miss Y. Okamoto (TBU) for measurement of mass spectra. Thanks are also due to Y. Tada, S. Kanayama and T. Yoshida for helping to collect the plant material and to Dr M. Toyota (TBU) for his assistance in carrying out this work. To Prof. H. Nozaki (Okayama University of Science, Japan) we express our appreciation for helpful discussions. We would also like to acknowledge financial support by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare, Japan, and to the Japan Society for the Promotion of Science for the award of a postdoctoral fellowship (to M.S.B.).

REFERENCES

1. Asakawa, Y. (1982) in *Progress in the Chemistry of Organic Natural Products*, Vol. 42 (Herz, W., Grisebach, H. and Kirby, G. W., eds), p. 1. Springer, Wien.
2. Asakawa, Y. (1986) *J. Bryol.* **14**, 59.
3. Asakawa, Y., Tokunaga, N., Toyota, M., Takemoto, T., Hattori, S., Mizutani, M. and Suire, C. (1979) *J. Hattori Bot. Lab.* **46**, 67.
4. Asakawa, Y., Tokunaga, N., Takemoto, T., Hattori, S., Mizutani, M. and Suire, C. (1980) *J. Hattori Bot. Lab.* **47**, 153.
5. Asakawa, Y. (1982) *J. Hattori Bot. Lab.* **53**, 283.
6. Takeda, R., Hasegawa, J. and Shinozaki, M. (1990) *Tetrahedron Letters* **31**, 4159.
7. Takeda, R., Hasegawa, J. and Shinozaki, M. (1990) in *Bryophytes; Their Chemistry and Chemical Taxonomy* (Zinsmeister, H. D. and Mues, R., eds.), p. 201. Oxford University Press, Oxford.
8. Mendez, J. and Sanz-Cabanilles, F. (1979) *Phytochemistry* **18**, 1409.
9. Mues, R. (1985) *Abstr. Botan.* **9**, Suppl. 2, 171.
10. Trennheuser, F., Burkhard, G. and Becker, H. (1994) *Phytochemistry* **37**, 899.
11. Sporle, J., Becker, H., Allen, N. S. and Gupta, M. P. (1991) *J. Hattori Bot. Lab.* **70**, 151.
12. Cranwell, P. A. (1986) *Org. Geochem.* **10**, 891.
13. Nakatani, Y., Ourisson, G. and Beck, J.-P. (1981) *Chem. Pharm. Bull.* **29**, 2261.
14. Boxer, S. G., Closs, G. L. and Katz, J. J. (1974) *J. Am. Chem. Soc.* **96**, 7058.
15. Zass, E., Isenring, H. P., Etter, R. and Eschenmoser, A. (1980) *Helv. Chim. Acta* **63**, 1048.
16. Lotjonen, S. and Hynninen, P. H. (1983) *Org. Magn. Reson.* **21**, 757.
17. Steele, J. A. and Mosettig, E. (1963) *J. Org. Chem.* **28**, 571.
18. Matsumoto, T., Shigemoto, T. and Itoh, T. (1983) *Phytochemistry* **22**, 2622.
19. Seo, S., Sankawa, U., Seto, H., Uomori, A., Yoshimura, Y., Ebizuka, Y., Noguchi, H. and Takeda, K. (1986) *J. Chem. Soc. Chem. Commun.* 1139.
20. Arai, Y., Koide, N., Ohki, F., Ageta, H., Yang, L.-L. and Yen, K.-Y. (1994) *Chem. Pharm. Bull.* **42**, 228.