Pergamon

PII: S0031-9422(97)00902-3

Phytochemistry, Vol. 48, No. 2, pp. 297–299, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

TRITERPENOID CONSTITUENTS OF THE MOSS *FLORIBUNDARIA AUREA* SUBSP. *NIPPONICA*

MASAO TOYOTA, KAZUKO MASUDA and YOSHINORI ASAKAWA*

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

(*Received in revised form* 26 *August* 1997)

Key Word Index—*Floribundaria aurea*; bryophyte; musci; moss; triterpenoids; dammarane; hopane; polypodane.

Abstract—The diethyl ether extract of the moss *Floribundaria aurea* subsp. *nipponica* yielded polypoda-7,13, 17,21-tetraene. This is the first example of its isolation from a moss, although it has been isolated from ferns. Further purification of the extract afforded dammara-17*Z*, 21-diene in addition to diploptene, hop-22 (20)-ene. The structure of dammara-17*Z*, 21-diene was established by extensive 2D NMR techniques. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The bryophytes are classified into liverworts (Hepaticae), hornworts (Anthocerotae) and mosses (Musci), morphologically. The chemical constituents of mosses are clearly different from those of liverworts. Particularly, the mosses have no oil bodies in their leaf cells and contain fatty acids, sterols, triterpenoids and aromatic compounds, whereas most species of liverworts have oil bodies in their cells and produce mono-, sesqui- and di-terpenoids with a variety of carbon skeletons and aromatic compounds as major constituents. Mono- and sesqui-terpenoids are very rare constituents of the mosses [1]. Only three monoterpene hydrocarbons, phellandrene, 3-carene and α-pinene, have been detected in gametophytes of *Splachnum* species by GC-MS [2]. No sesquiterpene has been found in mosses, whereas four diterpenoids have been isolated from different moss species [1].

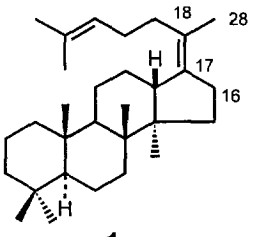
Previous work reported the isolation of triterpenoids such as ursane-, fernane-, friedelane-, hopane-, lupane-, taraxane-, cycloeucalane-, cycloartane-, cyclolaudane-, 24-methylenecycloartane-, norcyclolaudane-, obtusifolane-derivatives from mosses [1]. Here we report the isolation of polypoda-7, 13, 17, 21-tetraene (**3**) and diploptene (**4**) and the structural elucidation of a new dammarane-type triterpene hydrocarbon (**1**) from the moss *Floribundaria aurea* subsp. *nipponica*, a species which has not yet been investigated phytochemically.

RESULTS AND DISCUSSION

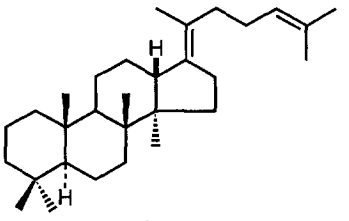
The diethyl ether extract of *F. aurea* subsp. *nipponica* was chromatographed on silica gel and gave a mixture which contained among other things compounds **1**, **3** and **4**. Further purification of the mixture by HPLC on a normal phase column afforded **1**, **3** and **4** in 1.3, 3.6 and 0.2% yield of the total extract, respectively. Compounds **3** and **4** were identified as known compounds by means of their spectral data [3, 4].

The EI-mass spectrum of **1** showed a $[M]^+$ peak at *m/z* 410 and its HR-mass spectrum indicated a molecular formula $C_{30}H_{50}$, confirming six degrees of unsaturation. The ^{13}C NMR spectrum of **1** not only demonstrated the presence of 30 carbon signals including four down field shifted signals at δ 124.7, 126.4, 131.1 and 136.9, but also showed no oxygenated carbon signal. The IR spectrum of **1** showed no absorption band for hydroxyl and carbonyl groups. The above spectral data indicated that **1** was tetracyclic triterpenoid hydrocarbon with two double bonds. Comparison of the ^1H- and ^{13}C-NMR (Table 1) spectral data of **1** and dammara-17*E*, 21-diene (**2**) which has been isolated from the fern *Polypodium fauriei* [5], suggested that **1** was likely to be a dammarane-type triterpene. The chemical shifts of 25 carbon signals of **1** were almost identical to those of **2**, but not those of C-16, 17, 19, 20 and 28. This suggested that **1** was a diastereomer at C-18 of **2**. The gross structure of **1** was proved by extensive 2D NMR experiments involving the determination of its ^1H-^1H COSY, HSQC and HMBC spectra. The geometry of the side chain at C-18 was established by 2D-NOESY spectroscopy. Since a cross-peak between H-28 and

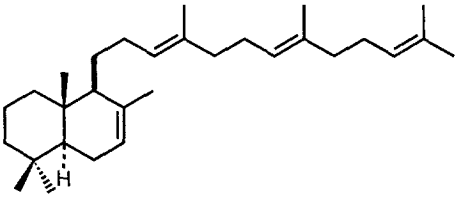
* Author to whom correspondence should be addressed.



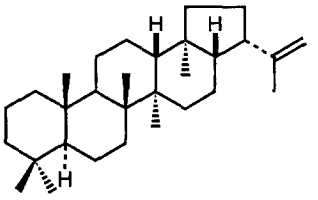
1



2



3



4

H_2-16 was clearly observed, the geometry of the double bond at C-17 of **1** had to be *Z*. It was obvious that the configuration of the side chain of **1** was opposite to that of **2**. Accordingly, the structure of **1** was elucidated as dammara-17*Z*, 21-diene. This is the first isolation of dammarane-type triterpenoid from mosses.

In this investigation, an unstable compound was purified as a major constituent of this species by silica gel chromatography. The presence of a 1,2-disubstituted triple bond (δ_c 78.4 and 79.2) and three double bonds (δ_c 125.4, 126.9, 127.0, 127.2, 128.8 and 131.6) was apparent from its ^{13}C NMR spectrum. While its structural elucidation was being progressed, almost all the compound decomposed. In order to approach its structure, the remaining product was hydrogenated over 5% Pd-C in EtOH to give a saturated glyceride, whose fatty acid moiety was identified by hydrolysis followed by detection of stearic acid as its TMS derivatives by GC-mass analysis. The structure of the unstable compound was likely to be an unsaturated triglyceride, with the additional presence of a 1,2-disubstituted triple bond and three double bonds in the C-18 fatty acid moiety.

Mosses produce various types of triterpenes. This is the first example of the isolation of polypoda-7,13,17,21-tetraene from a moss, although it has been isolated from the ferns *Polystichum ovatopaleaceum*, *P. polyblephalum* and *Cheiropleuria bicuspis* [3, 6].

The chemical constituents of mosses are completely different from those of liverworts. In particular, terpenoid constituents of mosses are very rare, except for triterpenoids, although liverworts contain many classes of terpenoids with a variety of carbon skeletons. On the other hand, the triterpenoid constituents of the mosses are very similar to those of the ferns (Pteridophytes). This is of considerable interest for the discussion of the evolutionary connection between the mosses and the ferns.

EXPERIMENTAL

General

TLC: silica gel precoated glass plates with *n*-hexane–EtOAc (1:1 and 4:1). Detection was with Godin reagent [7]. CC: silica gel 60 (40–63 μm) and Sephadex LH-20, with CH_2Cl_2–MeOH (1:1) as eluant for the latter.

Spectral data

NMR: 150 or 50 MHz for ^{13}C and 600 or 200 MHz for ^1H; EIMS: 70 eV; the temperature programming of GC-mass analysis: isothermal at 50° for 3 min, then 50–250° at 5° min^{-1}, and finally isothermal at 250° for 15 min. Injection temp was 250°. A fused silica column coated with DB-17 (30 m × 0.25 mm i.d., film thickness 0.25μm) was used.

Plant material

Floribundaria aurea subsp. *nipponica* (dry wt. 26.6 g) was collected in March 1997 at Kitagawa-village, Kochi, in Japan. A voucher specimen is deposited at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

Table 1. ^{13}C NMR data for Compounds **1** and **2**

C	1*†	2**‡
1	40.6	40.7
2	18.7	18.8
3	42.2	42.2
4	33.4	33.4
5	57.0	57.1
6	18.6	18.6
7	35.5	35.5
8	40.1	40.1
9	50.7	50.7
10	37.4	37.2
11	21.5	21.5
12	27.1	27.2
13	46.9	47.1
14	50.0	49.7
15	30.1	30.3
16	29.7	28.8
17	126.4	125.8
18	136.9	136.6
19	33.7	37.4
20	28.1	26.1
21	124.7	124.7
22	131.1	131.0
23	33.4	33.4
24	21.5	21.5
25	16.4	15.7§
26	15.7	16.4§
27	16.6	16.5
28	20.8	17.6
29	17.7	17.6
30	25.8	25.7

Measured at 150† and 68† MHz in $CDCl_3$.
* Assignments were confirmed by 2D-NMR techniques.
** Reported literature values [5].
§ Assignment may be reverse.

Extraction and isolation

F. aurea subsp. *nipponica* was extracted with Et_2O for one week. The Et_2O extract (1.06 g) was chromatographed on silica gel divided into 3 frs (fr. I–III) and the pure unstable compound (212 mg). Fr. 1 (250 mg) was rechromatographed on Sephadex LH-20 and purified by prep.-HPLC on a silica gel column using *n*-hexane to give **1** (10.4 mg; 1.3% yield of extract), **3** [3] (38.4 mg; 3.6%) and **4** [4] (2.4 mg; 0.2%), respectively.

Compound. **1**. Oil; $[\alpha]_D + 50.0$ ($CHCl_3$, *c* 0.52); EIMS *m/z* (rel. int.): 410 $[M]^+$ (96), 341 (96), 299 (31), 205 (42), 192 (30), 191 (100), 149 (54), 147 (30), 137 (33), 135 (36), 109 (35), 95 (34), 69 (33); HR-MS: found 410.3917 $C_{30}H_{50}$, requires 410.3913; ^1H NMR; δ 0.81 (6H, *s*, H-24 and H-27), 0.848 (3H, *s*, H-25), 0.853 (3H, *s*, H-23), 0.98 (3H, *s*, H-26), 1.58 (3H, *ddd*, *J* = 2.5, 1.4, 1.4 Hz, H-28), 1.60 (3H, *br s*, H-29), 1.68 (3H, *br s*, H-30),0.78 (1H, *dd*, *J* = 12, 2 Hz, H-5), 0.80 (1H, *m*, H-lax.), 1.12 (1H, *m*, H-15), 1.14 (1H, *ddd*, *J* = 13, 13, 3.8, H-3ax.), 1.22 (1H, *dddd*, *J* = 13, 13, 13, 3.3 Hz, H-11ax.), 1.30 (1H, *ddd*, *J* = 12, 2.5, 2.5 Hz, H-7eq.), 1.61 (1H, *m*, H-2eq.), 1.66 (1H, *br d*, *J* = 13.7 Hz, H-1eq.), 1.98 (1H, *m*, H-20), 2.04–2.06 (3H, *m*, H_2-19 and H-20′), 2.10 (1H, *m*, H-16), 2.17 (1H, *br q*, *J* = 9 Hz, H-16′), 2.21 (1H, *ddd*, *J* = 12.6, 7, 4 Hz, H-12eq.), 2.32 (1H, *br d*, *J* = 11.5 Hz, H-13), 5.11 (1H, *br m*).

Unstable compound. Oil; ^1H NMR(200 MHz in C_6D_6); δ 0.90 (*t*, *J* = 7.5 Hz), 1.24, 1.34, 2.03, 2.74, 2.90, 4.00, 4.23 and 5.36 (each, *br*); ^{13}C NMR (50 MHz in C_6D_6); δ 14.0, 17.0, 18.2, 20.3, 23.8, 25.25, 25.31, 28.1, 33.0, 33.2, 61.8, 68.9, 78.4, 79.2, 125.4, 126.9, 127.0, 127.2, 128.8, 131.6, 171.7 and 172.0.

Derivatives of the unstable compound. A soln of the unstable compound (100 mg) in EtOH (2 ml) was stirred for 1 h on 5% Pd–C under H_2. The catalyst was filtered off and washed with Et_2O. The soln was evapd to dryness. The hydrogenated product (14.5 mg) was dissolved in 5% KOH–MeOH (2 ml), then heated at 80° for 30 min. H_2O was added to the mixt and extracted with Et_2O. A soln of the Et_2O extract (ca 1 mg) in MeCN (200 μl) was treated with N, O-Bis (trimethylsilyl)-acetamide (BSA) (50 μl) for 30 min. GC-mass analysis of the reaction mixt detected stearic acid as its TMS ester.

Acknowledgements—We thank Dr Z. Iwatsuki (Hattori Botanical Laboratory, Okazaki Branch Office, Aichi, Japan) for kindly identifying the moss.

REFERENCES

1. Asakawa, Y. In *Progress in the Chemistry of Organic Natural Products*. Vol 65, eds. W. Herz, G. W. Kirby, R. E. Moore, W. Steglich and Ch. Tamm, Springer, Vienna, 1995, pp. 1–562.
2. Koponen, A., Koponen, T., Pyysalo, H., Himberg, K. and Mansikkamäki, P. in *Bryophytes: Their Chemistry and Chemical Taxonomy*, ed. H. D. Zinsmeister and R. Mues, Oxford University Press, Oxford, 1990, p. 449.
3. Shiojima, K., Arai, Y., Masuda, K., Kamada, T. and Ageta, H. *Tetrahedron Letters*, 1983, **24**, 5733.
4. Ageta, H., Shiojima, K., Suzuki, H. and Nakamura, S. *Chemical & Pharmaceutical Bulletin*, 1993, **41**, 1939.
5. Arai, Y., Yamaide, M., Yamazaki, S. and Ageta, H. *Phytochemistry*, 1991, **30**, 3369.
6. Kamaya, R., Tanaka, Y., Hiyama, R. and Ageta, H. *Chemical & Pharmaceutical Bulletin*, 1990, **38**, 2130.
7. Godin, P. *Nature*, 1954, **174**, 134.