Two New Benzophenones From the Endohydric Moss *Polytrichastrum formosum*

Xu-Hong Duan\(^1,2\) , Xue-Wen Zhang\(^1\), Meng Qin\(^3\), Pei He\(^3\), Lin Pei\(^3\), Jian-Cheng Zhao\(^1\), and Yu-Ling Chen\(^1\)

**Abstract**

Two new benzophenone derivatives (1 and 2), were obtained from the endohydric moss *Polytrichastrum formosum*. Their structures were established by 1D and 2D NMR spectroscopic and HRESIMS methods. The cytotoxicity of 1 and 2 against HCT-116, A-549, HepG2 and HeLa cell lines was evaluated, but no obvious cytotoxic activities were observed (IC\(_{50}\) >100 µM). Benzophenones may be characteristic components of Polytrichaceae.

**Keywords**

Polytrichaceae, *Polytrichastrum formosum*, benzophenones, cytotoxicity

Received: January 31st, 2021; Accepted: February 3rd, 2021.

**Graphical Abstract**

Mosses (Musci, Bryophyta) have relatively simple anatomical structures\(^1\) and are currently represented by approximately 14000 species that colonize diverse habitats.\(^2\) The Polytrichaceae family are comprised of 17 genera and approximately 200 species,\(^3\) of which the genera *Polytrichum* and *Polytrichastrum* are among the most familiar of all mosses, especially in boreal and temperate regions, due to their abundance, distinctiveness, and large size.\(^4\) Previous phytochemical investigation of Polytrichaceae mosses has resulted in the isolation of benzonaphthoxanthenones,\(^5-11\) benzophenones,\(^12\) cinnamoyl,\(^1\) coumarin glucosides,\(^13\) and flavonoids\(^10,11\) with broad biological activities such as cytotoxicity,\(^5-7,10,12\) antioxidant,\(^9\) anti-inflammatory,\(^14\) tyrosine phosphatase 1B (PTP1B) inhibition,\(^8\) and anti-neuroinflammatory activities.\(^11\) During continuing chemical research of products from mosses, 2 new benzophenone derivatives (1 and 2) (Figure 1) were obtained from the endohydric moss species *Polytrichastrum formosum*. Herein, we report their chromatographic separation, structure elucidation, and bioactivity.

**Results and Discussion**

Compound 1, obtained as a white powder, had a molecular formula of C\(_{23}\)H\(_{21}\)NO\(_4\) as deduced from its HRESIMS ion at m/z 398.1363 (calcld for C\(_{23}\)H\(_{21}\)NNaO\(_4\), 398.1363). The \(^1\)H NMR spectrum displayed signals for a hydroxy group (\(\delta_H\) 12.58), 2 monosubstituted aromatic rings (\(\delta_H\) 7.63, 2 hours, d, \(J = 7.5\) Hz, 7.51, 2 hours, t, \(J = 7.5\) Hz, 7.59, 1 hour, t, \(J = 7.5\) Hz, 7.18, 1.83, 1 hour, s, 7.61, 2 hours, d, \(J = 7.5\) Hz, 1.83), 2 methylene groups (\(\delta_H\) 3.82, 2 hours, s, 3.92, 2 hours, s), 1 methyl group (\(\delta_H\) 2.16, 3 hours, d, \(J = 6.5\) Hz), and 1 methoxy group (\(\delta_H\) 3.78, 3 hours, d, \(J = 6.5\) Hz).

---

\(^1\)College of Life Science, Hebei Normal University, Shijiazhuang, People’s Republic of China

\(^2\)College of Pharmacy, Hebei University of Chinese Medicine, Shijiazhuang, People’s Republic of China

\(^3\)Hebei Province Academy of Chinese Medicine Sciences, Shijiazhuang, People’s Republic of China

**Corresponding Author:**
Jian-Cheng Zhao, College of Life Science, Hebei Normal University, Shijiazhuang 050011, People’s Republic of China.
Email: zhaojiancheng@mail.hebtu.edu.cn

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (https://creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).
Natural Product Communications

2 hours, \( d, J = 7.4 \text{ Hz}, 7.30 \), 2 hours, \( t, J = 7.4 \text{ Hz} \), a 1, 2, 4-trisubstituted benzene ring (\( \delta_H 7.54 \), 1 hour, \( d, J = 8.9 \text{ Hz} \); 6.49, 1 hour, \( d, J = 2.1 \text{ Hz} \); 6.38, 1 hour, dd, \( J = 8.9, 2.1 \text{ Hz} \), and an oxygenated methylene group (\( \delta_H 4.51 \), 2 hours, s). The \( ^{13} \text{C} \) NMR spectrum with the assistance of DEPT spectroscopic data revealed the presence of 23 carbon resonances (Table 1), attributed to 7 quaternary (one keto carbonyl), 3 methylene (one oxygenated), and 13 aromatic methine carbons. The above-mentioned information suggested that 1 might be a benzophenone derivative with 3 aromatic rings.

Compound 2 showed a molecular ion peak at \( m/z 336.1207 \) (calcd. for \( \text{C}_{18}\text{H}_{19}\text{NNaO}_{4} \), 336.1206) in its HREIMS, suggesting a molecular formula of \( \text{C}_{18}\text{H}_{19}\text{NO}_{4} \). Comparison of the NMR data of 2 (Table 1) with those of 1 revealed that the main differences were the absence of one monosubstituted aromatic ring and the presence of a methyl group. The COSY correlations of \( H-2' \) and \( H-6' \) (\( \delta_H 7.63 \), 2 hours, d, \( J = 7.4 \text{ Hz} \)) indicated that \( H-2' \) and \( H-6' \) were connected to the same aromatic ring. The HMBC correlations of \( H-2' \) to \( C-1' \) and \( C-2' \) indicated that a methyl group was linked to \( C-2' \). Accordingly, the structure of 2 was elucidated as shown and named 2-(4-benzoyl-3-hydroxyphenoxy)-N-propylacetamide.

The cytotoxicity of the 2 new compounds against HCT-116, A-549, HepG2 and HeLa human cancer cell lines were assessed, but no obvious cytotoxic activity was observed (IC\textsubscript{50} >100 \text{ µM}).

Table 1. \( ^1 \text{H} \) NMR (500 MHz) and \( ^{13} \text{C} \) NMR (125 MHz) Spectroscopic Data for Compounds 1 and 2 in CDCl\textsubscript{3} (\( \delta \) in ppm, \( J \) in Hz).

| Position | \( ^1 \text{H} \) | \( ^{13} \text{C} \) | \( ^1 \text{H} \) | \( ^{13} \text{C} \) |
|----------|----------------|---------|----------------|---------|
| 1        | 114.4          | 114.4   | 114.4          | 114.4   |
| 2        | 166.1          | 166.1   | 166.1          | 166.1   |
| 3        | 6.49 (1 H, d, 2.1) | 102.8   | 6.55 (1 H, d, 1.5) | 102.9   |
| 4        | 163.3          | 163.3   | 163.3          | 163.3   |
| 5        | 6.38 (1 H, dd, 8.9, 2.1) | 106.7   | 6.45 (1 H, dd, 8.8, 1.5) | 106.7   |
| 6        | 7.54 (1 H, d, 8.9) | 135.8   | 7.56 (1 H, d, 8.8) | 135.8   |
| 7        | 200.3          | 200.3   | 200.3          | 200.3   |
| 1'       | 138.1          | 138.1   | 138.1          | 138.1   |
| 2', 6'   | 7.63 (2 H, d, 7.5) | 128.8   | 7.63 (2 H, d, 7.5) | 129.0   |
| 3', 5'   | 7.51 (2 H, t, 7.5) | 128.5   | 7.50 (2 H, t, 7.5) | 128.5   |
| 4'       | 7.59 (1 H, t, 7.5) | 131.9   | 7.58 (1 H, t, 7.5) | 131.9   |
| 1''      | 167.1          | 167.1   | 167.1          | 167.1   |
| 2''      | 4.51 (2 H, s)  | 67.3    | 4.54 (2 H, s)  | 67.3    |
| 1'''     | 3.62 (2 H, q)  | 40.3    | 3.32 (2 H, q)  | 41.0    |
| 2'''     | 2.86 (2 H, q)  | 35.7    | 1.58 (2 H, m)  | 22.9    |
| 3'''     | 138.5          | 0.94 (3 H, t, 7.4) | 11.4 |
| 4'', 8'''| 7.18 (2 H, d, 7.4) | 128.9   | 12.57 (1 H, br s) | 6.52 (1 H, br s) |
| 5'', 7'''| 7.30 (2 H, t, 7.4) | 126.8   |                  |         |
| 6'''     | 7.23 (1 H, t, 7.4) | 6.53 (1 H, br s) |                  |         |
| 12-OH    | 12.58 (1 H, s)  | 12.57 (1 H, br s) |                  |         |
| 1''-NH   | 6.53 (1 H, br s) | 6.52 (1 H, br s) |                  |         |
Experimental

General Experimental Procedures

Optical rotations were measured on a JASCO P-1020 polarimeter in MeOH at room temperature. IR spectra were recorded on a Bruker AVIII 500 MHz spectrometer using KBr pellets, 1D and 2D NMR spectra on a Bruker AVIII 500 MHz spectrometer (Bruker, Karlsruhe, Germany) in CDCl₃ with tetramethylsilane (TMS) as an internal standard, and high-resolution electrospray mass spectrometry (HRESIMS) on an Agilent 6529B Q-TOF instrument (Agilent Technologies, Santa Clara, CA, USA). Preparative HPLC was performed on a Prep-HTGF ODS column (21.2 mm × 250 mm i.d., 7 µm, Agilent Technologies, Santa Clara, CA, USA) with a flow rate of 20.0 mL/min. Silica gel (200, 300 mesh, Qingdao Haiyang Chemical Co., Ltd, Qingdao China), Sephadex LH-20 gel (Amersham Biosciences), MCI (Mitsubishi, Japan) and RP-C₁₈ silica (40, 63 mm, Fuji, Japan) were used for column chromatography.

Plant Material

The endohydric moss, Polytrichastrum formosum (Hedw.) G. L. Sm, was collected from Cape Mount, Dujun city, Guizhou Province (latitude 26°35′42.8″N, longitude 107°38′32.1″E, altitude 1250 m), People’s Republic of China in November, 2019. A specimen (HBSD2019110401) was deposited in the College of Life Sciences, Hebei Normal University.

Extraction and Isolation

The air-dried whole plant (1.3 kg) of P. formosum was powdered and extracted with 95% EtOH by infusion (5 × 5 L, 3 days each). The concentrated extract (39 g) was suspended in H₂O and partitioned with light petroleum, CH₂Cl₂, EtOAc, and n-BuOH, successively. The CH₂Cl₂ extract (7.5 g) was subjected to MCI gel column chromatography eluting with MeOH/H₂O mixtures (30%, 60%-90%), and the 90% MeOH/H₂O fraction (2.6 g) was chromatographed on a silica gel column eluting with light petroleum and acetone mixtures (10:1–5:1–3:1–2:1–1:1) to give 5 fractions, Fr.1–Fr.5. Fraction Fr.3 (650 mg) was separated on an ODS gel column (eluited with MeOH/H₂O, 60%-100% in a step gradient manner) to afford 5 fractions, Fr.3.1–Fr.3.5. Fraction Fr.3.2 (0.92 g) was separated by RP-C₁₈ preparative HPLC with 60% ACN/H₂O and the chromatographic peak at t_R = 19 minutes was collected and purified by Sephadex LH-20 CC (eluited with MeOH) to afford compound 1 (9.3 mg). Compound 2 (6.1 mg) was obtained from the chromatographic peak at t_R = 31 minutes by recrystallization from CDCl₃.

2-(4-Benzoyl-2-Hydroxyphenoxy)-N-Phenethylacetamide (1)

White amorphous powder; [α]D_{25}^{25} −1.9 (c 0.1, MeOH); UV (MeOH) λ_{max} (log e) 205 (4.38), 243 (3.86), 284 (4.05), 324 (3.81) nm; IR (KBr) ν_{max} 3351, 1651, 1627, 1543, 1346, 1261, 1223, 1192, 1112, 1053, 700 cm⁻¹; ¹H and ¹³C NMR see Table 1; HRESIMS m/z 398.1363 [M + Na]^+ (calcd. for C₂₃H₂₁NNaO₄, 398.1363).

2-(4-Benzoyl-3-Hydroxyphenoxy)-N-Propylacetamide (2)

Colorless crystals (CDCl₃); [α]D_{25}^{25} −5.7 (c 0.1, MeOH); UV (MeOH) λ_{max} (log e) 204 (4.26), 243 (3.86), 284 (4.05), 327 (3.81) nm; IR (KBr) ν_{max} 3390, 1679, 1627, 1546, 1505, 1283, 1171, 1131, 1045, 702 cm⁻¹; ¹H and ¹³C NMR see Table 1; HRESIMS m/z 336.1207 [M + Na]^+ (calcd. for C₁₈H₁₉NNaO₄, 336.1206).

Cytotoxicity

Cytotoxicity was measured using the sulforhodamine B method with human colon cancer (HCT-116), human lung carcinoma (A-549), liver hepatocellular carcinoma (HepG2), and human cervical carcinoma (HeLa)cell lines. The half-maximal inhibitory concentration value was analyzed using GraphPad Prism 5 software. Adriamycin was used as a positive control.¹⁷

Conclusion and Discussion

Two new benzophenones, 2-(4-benzoyl-2-hydroxyphenoxy)-N-phenethylacetamide (1) and 2-(4-benzoyl-3-hydroxyphenoxy)-N-propylacetamide (2), were isolated from the endohydric moss P. formosum. The 2 compounds showed no activity against 4 hours suman cancer cell lines. Our findings enrich the knowledge of the phytochemical content of Polytrichaceae. In addition, our previous research found that benzophenones were isolated from the genera Pogonatum and Polytrichum.¹²,¹³ From
the perspective of phytochemical taxonomy, it shows that these 3 genera are closely related. Benzophenone compounds may be characteristic chemical constituents of the Polytrichaceae.

Declaration of Conflicting Interests

Polytrichaceae. may be characteristic chemical constituents of the these 3 genera are closely related. Benzophenone compounds for the perspective of phytochemical taxonomy, it shows that these.

References

1. Zajączkowski U, Grabowska K, Kokor G, Kruk M. On the benefits of living in clumps: a case study on Polytrichastrum formosum. *Plant Biol*. 2017;19(2):156-164. doi:10.1111/plb.12532
2. Asakawa Y. Recent advances in phytochemistry of bryophytes-acetogenins, terpenoids and his(bibenzyl)s from selected Japanese, Taiwanese, New Zealand, Argentinean and European liverworts. *Phytochemistry*. 2001;56(3):297-312. doi:10.1016/S0031-9422(00)00454-4
3. Flora Yunnanica. *Institutum Botanicum Kunmingense Academiae Sinicae*. Science Press; 1998:19. 589.
4. Bell NE, Hyvönen J. A phylogenetic circumscription of Polytrichastrum (Polytrichaceae): reassessment of sporophyte morphology supports molecular phylogeny. *Am J Bot*. 2010;97(4):566-578. doi:10.1093/aib/abo.0900161
5. Zheng GQ, Chang CJ, Stout TJ, Clardy J, Cassady JM, et al. Ohioensin-A: a novel benzonaphthoxanthenone from *Polytrichum obioense*. *J Am Chem Soc*. 1989;111(14):5500-5501. doi:10.1021/ja00196a085
6. Zheng GQ, Chang CJ, Stout TJ, Clardy J, Ho DK, Cassady JM. Ohioensins: novel benzonaphthoxanthenones from *Polytrichum obioense*. *J Org Chem*. 1993;58(2):366-372. doi:10.1021/jo00054a019
7. Zheng GQ, Ho DK, Elder PJ, Stephens RE, Cottrell CE, Cassady JM. Ohioensins and pallidisetins: novel cytotoxic agents from the moss *Polytrichum pallidisetum*. *J Nat Prod*. 1994;57(1):32-41. doi:10.1021/np50103a005
8. Seo C, Choi Y-H, Sohn JH, et al. Ohioensins F and G: protein tyrosine phosphatase 1B inhibitory benzonaphthoxanthenones from the Antarctic moss *Polytrichastrum alpinum*. *Bioorg Med Chem Lett*. 2008;18(2):772-775. doi:10.1016/j.bmcl.2007.11.036
9. Bhattacharj HD, Paudel B, Lee HK, Oh H, Yim JH. *In vitro* antioxidant capacities of two benzonaphthoxanthenones: ohioensins F and G, isolated from the Antarctic moss *Polytrichastrum alpinum*. *Z Naturforsch C J Biosci*. 2009;64(3-4):197-200. doi:10.1515/znc-2009-3-408
10. Fu P, Lin S, Shan L, et al. Constituents of the moss *Polytrichum commune*. *J Nat Prod*. 2009;72(7):1335-1337. doi:10.1021/np800830v
11. Guo Z-F, Bi G-M, Zhang Y-H, Li J-H, Meng D-L. Rare benzonaphthoxanthenones from Chinese folk herbal medicine *Polytrichum commune* and their anti-neuroinflammatory activities in *vitro*. *Bioorg Chem*. 2020;102:104087. doi:10.1016/j.bioorg.2020.104087
12. Duan X-H, Zhan J-C, Li P, Pei L, He P, Wang R. Two new benzophenones from endohydric moss *Polytrichum Commune*. *Nat Prod Res*. 2019;33(19):2750-2754. doi:10.1080/14786419.2018.1499638
13. Jung M, Zinsmeister HD, Geiger H. New three- and Tetraox-ygenated coumarin glucosides from the mosses *Atrichum undulatum* and *Polytrichum formosum*. *Zeitschrift fur Naturforsch C*. 1994;49(11-12):697-702. doi:10.1515/znc-1994-11-1201
14. Byeon H-E, Um SH, Yim JH, Lee HK, Pyo S. Ohioensin F suppresses TNF-α-induced adhesion molecule expression by inactivation of the MAPK, Akt and NF-κB pathways in vascular smooth muscle cells. *Life Sci*. 2012;90(11-12):396-406. doi:10.1016/j.lfs.2011.12.017
15. Tantakul C, Mancewar W, Sripisut T, et al. New benzophenones and xanthenes from *Cratoxylum sumatranum* ssp. *serifolium* and their antibacterial and antioxidant activities. *J Agric Food Chem*. 2016;64(46):8755-8762. doi:10.1021/acs.jafc.6b03643
16. Rouis Z, Abid N, Aouni M, et al. Benzophenone glycosides from *Amphanthoxanthenones from Chinese folk herbal medicine Polytrichum commune* and their anti-neuroinflammatory activities in *vitro*. *Bioorg Chem*. 2020;102:104087. doi:10.1016/j.bioorg.2020.104087
17. Duan X-H, Zhan J-C, Li P, Pei L, He P, Wang R. Two new benzophenones from endohydric moss *Polytrichum Commune*. *Nat Prod Res*. 2019;33(19):2750-2754. doi:10.1080/14786419.2018.1499638
18. Duan X-H, Zhao J-C, Li L, Pei L, He P, Wang R. Two new benzophenones from endohydric moss *Polytrichum Commune*. *Nat Prod Res*. 2019;33(19):2750-2754. doi:10.1080/14786419.2018.1499638