Constituents of the Moss *Polytrichum commune*

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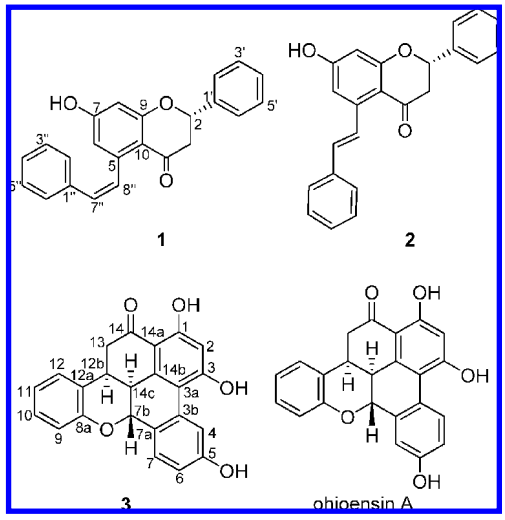
Two unusual flavonones coupled with styryl units, communins A (**1**) and B (**2**), and a new benzonaphthoxanthenone, ohioensin H (**3**), together with 11 known compounds, were isolated from the moss *Polytrichum commune*. The structures of **1**−**3** were assigned by spectroscopic data interpretation. The new compounds were evaluated for cytotoxicity against a small panel of cancer cell lines.

The bryophytes, comprising more than 23 000 species altogether, are classified into three major phyla: mosses, liverworts, and hornworts. Several terpenoids and aromatic compounds with novel skeletons or significant bioactivities have been obtained from mosses and liverworts.[1,2]

Polytrichum commune L. ex Hedw., belonging to the genus *Polytrichum* (Polytrichaceae), is used as a folk remedy to stop bleeding and treat pneumonia, night sweating, and uterine prolapse.[3,4] Previous chemical investigation has resulted in the isolation of 10 benzonaphthoxanthenones with a novel skeleton and two novel cinnamoyl bibenzyls from two mosses, *Polytrichum ohioense* and *P. pallidisetum*. These compounds exhibited cytotoxicity against the 9PS murine leukemia and several other tumor cell lines.[5,6] However, little is known about the chemical constituents of *P. commune* except for a previous report on five lipids and sterols.[4] The current study was carried out to search for novel bioactive metabolites from *P. commune*, leading to the isolation of two unusual flavonone-styryl hybrid molecules, communins A (**1**) and B (**2**), and a new benzonaphthoxanthenone, ohioensin H (**3**), together with 11 known compounds. In this paper, we report the isolation, structure elucidation, and the cytotoxicity testing of the new compounds isolated.



Communin A (**1**) was isolated as colorless needles that analyzed for the molecular formula $C_{23}H_{18}O_3$ by HRESIMS at *m/z* 343.1338 [M + H]$^+$, indicating that **1** possesses 15 degrees of unsaturation. The ^{13}C NMR spectrum of **1** (Table 1) showed 23 signals, consistent with the molecular formula. A carbonyl resonance was observed at δ 191.5 (C-4), which, when combined with a positive reaction in the $NaBH_4$ test and the observation of a sharp IR absorption band at 1741 cm^{-1}, indicated that compound **1** is a flavonone. Also, UV absorptions showed a main peak at 270−295 nm and a shoulder at 316 nm, which are indicative of the presence of a flavonone chromophore.[7]

Table 1. NMR Spectroscopic Data for Compounds **1** and **2**[a]

	1		**2**	
position	δ_C, mult.[b]	δ_H (*J* in Hz)	δ_C, mult.[b]	δ_H (*J* in Hz)
2	78.5, CH	5.40, dd (3.0, 12.6)	78.5, CH	5.36, dd (2.4, 13.2)
3	44.5, CH_2	α, 3.00, dd (12.6, 16.8) β, 2.73, dd (3.0, 16.8)	45.2, CH_2	α, 2.77, dd, (13.2, 16.8) β, 2.73, dd, (2.4, 16.8,)
4	191.5, qC		192.5, qC	
5	141.8, qC		142.8, qC	
6	112.2, CH	6.18, d (1.2)	108.8, CH	6.34, d (2.4)
7	164.2, qC		164.5, qC	
8	101.9, CH	6.28, d (1.2)	102.5, CH	6.70, d (2.4)
9	163.0, qC		163.2, qC	
10	111.7, qC		111.2, qC	
1′	138.4, qC		138.5, qC	
2′	125.4, CH	7.41,m	125.7, CH	7.42, m
3′	128.0, CH	7.33, m	128.2, CH	7.34, m
4′	126.0, CH	7.29, m	125.9, CH	7.28, m
5′	128.0, CH	7.33,m	128.2, CH	7.34, m
6′	125.4, CH	7.41, m	125.7, CH	7.42, m
1″	136.2, qC		137.1, qC	
2″	128.4[c], CH	7.01, m	126.4[e], CH	7.46, m
3″	127.2[d], CH	7.05, m	128.1, CH	7.28, m
4″	127.8, CH	7.01, m	127.3, CH	7.34, m
5″	127.1[d], CH	7.05, m	128.1, CH	7.28, m
6″	128.2[c], CH	7.01, m	126.3[e], CH	7.46, m
7″	128.1, CH	6.49, d (12.0)	131.1, CH	6.88, d (16.2)
8″	130.8, CH	6.90, d (12.0)	128.4, CH	8.12, d (16.2)

[a] Spectra were recorded in $CDCl_3$ and CD_3OD at 600 and 150 MHz for ^{1}H and ^{13}C NMR, respectively. [b] Chemical shifts and multiplicities based on HSQC and HMBC correlation peaks. [c−e] Assignments for these carbons may be interchangeable.

Analysis the ^{1}H and 2D NMR spectroscopic data allowed the planar structure of **1** to be determined. The ^{1}H NMR spectrum of **1** showed seven aromatic protons due to a monosubstituted phenyl unit at δ 7.41 (2H, m, H-2′, 6′), 7.33 (2H, m, H-3′, 5′), and 7.29 (1H, m, H-4′), a 1,2,3,5-tetrasubstituted phenyl unit at δ 6.28 (1H, d, J = 1.2 Hz, H-8) and 6.18 (1H, d, J = 1.2 Hz, H-6), and three aliphatic protons in an ABX spin system at δ 2.73 (1H, dd, J = 16.8 and 3.0 Hz, H-3β), 3.00 (1H, dd, J = 16.8 and 12.6 Hz, H-3α), and 5.40 (1H, dd, J = 3.0 and 12.6 Hz, H-2). HMBC correlations from H-2 to C-4, from H_2-3 to C-10 and C-1′, from H-6 to C-8 and C-10, from H-8 to C-6 and C-10, and from H-2′ and H-6′ to

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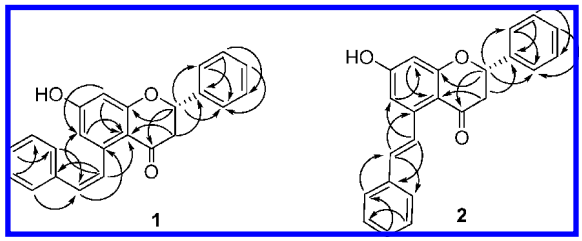

Figure 1. Selected HMBC correlations (H→C) for **1** and **2**.

C-2 (Figure 1) clearly established the presence of a 5,7-disubstituted flavonone unit in communin A (**1**). In addition, the remaining proton signals in the ^{1}H NMR spectrum were readily assigned as another monosubstituted phenyl unit at δ 7.05 (2H, m, H-3″, 5″) and 7.01 (3H, m, H-2″, 4″, 6″) and as a disubstituted double bond at δ 6.49 (1H, d, J = 12.0 Hz, H-7″) and 6.90 (1H, d, J = 12.0 Hz, H-8″). These data, in combination with the observed HMBC correlations from H-2″ and H-6″ to C-7″ and from H-8″ to C-1″, were consistent with the presence of a styryl moiety in **1**. Finally, key HMBC correlations from H-7″ to C-5 and from H-8″ to C-6 and C-10 indicated that this unit is attached to C-5 of the flavonone moiety via a C−C linkage, leaving a hydroxy group to be located at C-7.

The configuration of the double bond in communin A (**1**) was defined as *Z* from the coupling constant between H-8″ and H-7″ (12.0 Hz) and also the observed NOESY correlations between H-8″ and H-7″ and between H-6 and H-2. The pseudoaxial orientation of H-2 was clearly established by the typically large (12.6 Hz) axial−axial and smaller (3.0 Hz) axial−equatorial coupling *J* values to the adjacent methylene protons at C-3. Like other naturally occurring flavonones,[7,8] the negative optical rotation of **1**, $[\alpha]^{25}_{D}$ −52 (*c* 0.1, MeOH), was consistent with the absolute configuration at C-2 being *S*. Furthermore, the CD spectrum showed a positive Cotton effect at 350 nm and a negative one at 305 nm, in full support of the *S*-configuration at C-2.[9] Consequently, the structure of communin A (**1**) was elucidated as (2*S*,7″*Z*)-7-hydroxy-2-phenyl-5-styrylchroman-4-one.

Communin B (**2**) was isolated as a yellow powder and analyzed for the same molecular formula, $C_{23}H_{18}O_3$, as **1** by HRESIMS at *m*/*z* 343.1340 $[M + H]^+$. The ^{1}H NMR spectrum of **2** was similar to that of **1** except that H-8″ (δ 8.12) was distinctly deshielded. This difference suggested that the *Z* double bond in **1** was replaced in compound **2** by an *E* double bond (16.2 Hz). Similarly, the ^{13}C NMR spectrum showed an *E* double bond at δ 128.4 and 131.1. Comprehensive analysis of 2D NMR data allowed the planar structure of **2** to be assigned. As in **1**, the assignment of a 2*S*-configuration was based on the negative optical rotation of **2**, $[\alpha]^{25}_{D}$ −47 (*c* 0.1, MeOH), and the CD spectrum, which showed a positive Cotton effect at 351 nm and a negative one at 301 nm.[9] Thus, the structure of communin B (**2**) was defined as (2*S*,7″*E*)-7-hydroxy-2-phenyl-5-styrylchroman-4-one.

Compound **3** was obtained as an orange powder, and its HRESIMS showed a molecular ion at *m*/*z* 373.1048 $[M + H]^+$, corresponding to the molecular formula $C_{23}H_{16}O_5$. This molecular formula indicated that compound **3** contains 16 unsaturations, as required for the basic skeleton of a benzonaphthoxanthenone.[5,6] The IR spectrum indicated the presence of intramolecular hydrogen-bonded hydroxy (3500−2500 cm^{-1}) and conjugated carbonyl (1627 cm^{-1}) functionalities. In comparison with the known compound, ohioensin A,[5] **3** showed similar absorptions in the UV spectrum, suggesting the presence of an identical conjugated system.

The ^{1}H NMR coupling patterns observed for compound **3** were identical to those of ohioensin A,[5,6] but the chemical shifts of the protons attributed to the 1,2,4-trisubstituted benzene moiety were slightly different. Comparison of the ^{13}C NMR data of **3** and ohioensin F indicated that C-3b, C-5, and C-7 were shifted

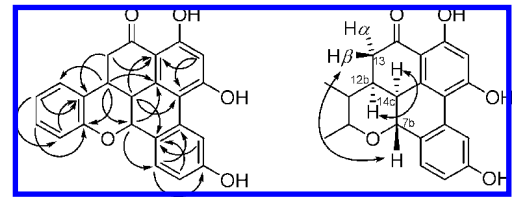

Figure 2. Selected HMBC and NOESY correlations for ohioensin H (**3**).

downfield by 9.0, 44.6, and 17.4 ppm, respectively, while C-4, C-6, and C-7a were distinctly shifted upfield by 13.6, 38.5, and 19.5 ppm, respectively. These spectroscopic differences revealed that compound **3** contains a C-5 hydroxy group rather than the C-6 hydroxy found in ohioensin A. HMBC correlations also positioned the hydroxy at C-5 in compound **3** (Figure 2).

The relative configuration of **3** was assigned by a combination analysis of the coupling constants in the ^{1}H NMR spectrum and NOESY NMR experiments. The splitting pattern and coupling constant of H-7b (d, $J_{7b,14c}$ = 13.8 Hz) indicated that H-7b has a pseudoaxial orientation. NOESY correlations between H-13β and H-7b suggested the proximity of the two protons. Further, correlations from H-14c to 12b allowed the six-membered ketone ring and the dihydropyran ring to be determined as *cis* fused. This was confirmed by the small coupling constant between H-14c and H-12b (J = 7.2 Hz). On the basis of these data, the structure of **3** was established as (7bβ,12bα,14cα)-1,3,5-trihydroxy-7b,12b,13,14c-tetrahydro-14*H*-benzo[*c*]naphtho[2,1,8-*mna*]xanthen-14-one and has been named ohioensin H.

By comparing physical and spectroscopic data with those reported in the literature, known compounds were identified as β-sitosterol,[4] 7α hydroxysitosterol,[10] ergosterol,[11] 4-hydroxybenzoic acid,[12] 3-methoxy-4-hydroxybenzoic acid,[13] methyl indoline-6-carboxylate,[14] 5-hydroxy-7-methoxy-4*H*-chromen-4-one,[15] 5-hydroxy-6-methoxy-7-*O*-β-D-glucosyl coumarin,[16] β-D-uranallulose,[17] α-D-furanallulose,[17] and sucrose.[17] Except for β-sitosterol, the remaining known compounds were isolated from *P. commune* for the first time.

The unusual flavonone and benzonaphthoxanthenone might be derived from cinnamic acid and 3,5-dioxohexanoic acid via different pathways.[5] Communins A (**1**) and B (**2**) apparently arose from the coupling of 3,5-dioxohexanoic acids with *Z*- or *E*-cinnamic acids, respectively (Figure S1, Supporting Information). When compounds **1**−**3** were evaluated against a small panel of cancer cell lines, none of them were found to be active (IC_{50} < 5 μg/mL).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 341 digital polarimeter. UV and IR spectra were recorded on Shimadzu UV-2550 and Bruker Vector-22 spectrophotometers, respectively. NMR data were acquired on a Bruker 600 NMR spectrometer using TMS as the internal standard, and chemical shifts were recorded as δ values. HRESIMS were obtained on a Q-TOF micro mass spectrometer. Column chromatography was performed using silica gel (200−300 mesh and 10−40 μm; Huiyou Silica Gel Development Co. Ltd., Yantai, People's Republic of China) and Sephadex LH-20 (40−70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden). Spots were visualized under UV light and by spraying with 10% H_2SO_4 followed by heating.

Plant Material. The moss *Polytrichum commune* was collected in Longquan, Zhejiang Province, People's Republic of China, in July 2006. The plant material was authenticated by R.-L.Z. A voucher specimen (no. PC060730) has been deposited at the Department of Biology, School of Life Sciences, East China Normal University, Shanghai.

Extraction and Isolation. The dried plant material (380 g) was ground and percolated with acetone and methanol for one month each. The acetone extract (15 g) was chromatographed over silica gel (250 g), eluting with increasing amounts of EtOAc (0−100%) in petroleum ether, and separated into 10 fractions (F_1−F_{10}) based on TLC analysis.

Fraction F_5 (4.6 g) was fractionated by column chromatography on silica gel, eluting with a gradient system ($CHCl_3$−MeOH, 30:1, 15:1, 5:1), to afford three subfractions (F_{5A}−F_{5C}). Subfraction F_{5B} (126 mg) was further purified by preparative TLC developed with CH_2Cl_2−EtOAc−MeOH (10:1:1) to give **3** (18 mg). Fraction F_7 (1.7 g) was applied to silica gel column chromatography, eluting with a gradient system ($CHCl_3$−MeOH, 10:1, 5:1, 2:1), to provide three subfractions (F_{7A}−F_{7C}). Purification of subfraction F_{7B} (167 mg) by repeated chromatography over Sephadex LH-20 eluting with $CHCl_3$−MeOH (1:1) led to the isolation of compounds **1** and **2** as a mixture. Using preparative TLC (CH_2Cl_2−MeOH−H_2O, 95:10:1), compounds **1** (12 mg) and **2** (6 mg) were obtained in pure form.

Communin A (1): colorless needles ($CHCl_3$−MeOH), mp 212−213 °C; $[\alpha]^{25}_D$ −52 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.83), 267 (3.68), 295 (2.65), 316 (2.59) nm; CD (MeOH) $\Delta\varepsilon = 350$ (+5.0), 305 (−8.1); IR (KBr) ν_{max} 3424, 2925, 2853, 1741, 1648, 1601, 1464, 1383, 1266, 1153, 695 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$−CD_3OD) and ^{13}C NMR (150 MHz, $CDCl_3$−CD_3OD), Table 1; HRESIMS *m/z* 343.1338 ($[M + H]^+$ calcd for $C_{23}H_{19}O_3$, 343.1329, Δ 2.7 ppm).

Communin B (2): yellow powder ($CHCl_3$−MeOH), mp 247−250 °C (dec); $[\alpha]^{25}_D$ −47 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.31), 267 (2.20), 291 (2.02), 322 (1.99) nm; CD (MeOH) $\Delta\varepsilon = 351$ (+0.8), 301 (−0.5); IR (KBr) ν_{max} 3425, 2931, 2856, 1740, 1648, 1604, 1464, 1396, 1268, 1163, 720 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$−CD_3OD) and ^{13}C NMR (150 MHz, $CDCl_3$−CD_3OD), Table 1; HRESIMS *m/z* 343.1340 ($[M + H]^+$ calcd for $C_{23}H_{19}O_3$, 343.1329, Δ 3.3 ppm).

Ohioensin H (3): brilliant orange powder ($CHCl_3$−MeOH), mp 270−272 °C (dec); $[\alpha]^{25}_D$ −22 (*c* 0.15, MeOH); UV (EtOH) λ_{max} (log ε) 359 (2.73), 272 (4.67), 216 (4.74) nm; IR (KBr) ν_{max} 3500−2500, 1627, 1384, 1235, 575 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 12.07 (s, OH-1), 11.21 (s, OH-3), 9.62 (s, OH-5), 8.22 (1H, d, $J = 9.0$ Hz, H-7), 7.40 (1H, dd, $J = 1.2$, 7.2 Hz, H-12), 7.28 (1H, d, $J = 2.4$ Hz, H-4), 7.21 (1H, ddd, $J = 1.2$, 7.2, 7.8 Hz, H-10), 7.04 (1H, dd, $J = 1.2$, 7.2 Hz, H-9), 6.98 (1H, ddd, $J = 1.2$, 7.2, 7.8 Hz, H-11), 6.76 (1H, dd, $J = 2.4$, 9.0 Hz, H-6), 6.41 (1H, s, H-2), 5.08 (1H, d, $J = 13.8$ Hz, H-7b), 3.58 (1H, ddd, $J = 4.8$, 7.2, 15.0 Hz, H-12b), 3.21 (1H, dd, $J = 7.2$, 13.8 Hz, H-14c), 2.96 (1H, dd, $J = 15.0$, 15.0 Hz, H-13β), 2.79 (1H, dd, $J = 4.8$, 15.0 Hz, H-13α); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 201.0 (C, C-14), 162.2 (C, C-3), 160.9 (C, C-1), 156.2 (C, C-5), 152.3 (C, C-8a), 139.4 (C, C-3b), 139.3 (C, C-14b), 129.7 (CH, C-12), 129.3 (CH, C-7), 127.9 (CH, C-10), 123.8 (C, C-12a), 121.2 (CH, C-11), 121.1 (CH, C-7a), 116.9 (CH, C-9), 114.3 (C, C-3a), 113.7 (CH, C-6), 110.5 (C, C-14a), 111.0 (CH, C-4), 101.8 (CH, C-2), 69.5 (CH, C-7b), 41.9 (CH_2, C-13), 37.5 (CH, C-14c), 27.5 (CH, C-12b); HRESIMS *m/z* 373.1084 ($[M + H]^+$ calcd for $C_{23}H_{15}O_5$, 373.1071, Δ 3.6 ppm).

Cytotoxicity Assay. The cytotoxic activity was determined against five human cancer cell lines, human lung carcinoma (A549), human hepatoma carcinoma (HepG2), huamn bowel carcinoma (LOVO), human breast adenocarcinoma (MDA-MB-435), and human T cell leukemia (6T-CEM), obtained from the American Type Culture Collection (ATCC, Rockville, MD). Cells were seeded in 96-well plates at a cell density of 3000 per well and were treated 24 h later with various concentrations of compounds **1**−**3**. After 24 h of incubation, MTT was added to all wells. Plates were incubated for another 24 h, and cell viability was measured by observing absorbance at 570 nm on an enzyme-labeled detector (Denley MK-2). Doxorubicin was used as the positive control, and compounds **1**−**3** gave IC_{50} values of 0.10 (A549), 1.7 (HepG2), 0.13 (LOVO), 0.11 (MDA-MB-435), and 0.0022 (6T-CEM).

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Supporting Information Available: HRESIMS, NMR, and CD spectra of compounds **1**−**3** and biogenetic scheme of compounds **1** and **2** are available free of charge via the Internet at http://pubs.acs.org.

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