

## New Chroman Derivatives from the Japanese Liverworts *Metacalypogeia cordifolia* and *Cephalozia otaruensis*

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The diethyl ether extract of the Japanese liverwort *Metacalypogeia cordifolia* yielded five new chroman type derivatives in addition to known sesquiterpenoids. One of the new chroman derivatives was also isolated from the ether extract of another liverwort, *Cephalozia otaruensis*. Their structures were established by extensive two dimensional (2D) NMR techniques and chemical evidence. They were shown to be 2,2-dimethyl-7-(3-methyl-2-butenyl)-chroman derivatives. This was the first example of the isolation of the chroman-type compounds, although various types of aromatic compounds have been isolated from liverworts.

**Key words** *Metacalypogeia cordifolia*; *Cephalozia otaruensis*; prenylchroman; Hepaticae; Jungermanniales; Calypogeiaceae

The small stem-leafy liverworts, *Metacalypogeia cordifolia* (STEPH.) INOUE and *Cephalozia otaruensis* STEPH. belong to the Calypogeiaceae and Cephalozaceae (Jungermanniales) and grow on moist rocks. Three species of *Metacalypogeia* and nine species of *Cephalozia* are known in Japan. The liverworts, which include Jungermanniales, are rich plant sources of terpenoids with a variety of carbon skeletons.<sup>1)</sup> Classification of the liverworts belonging to the Jungermanniales is morphologically extremely difficult, and thus a study of their chemical constituents is necessary. For this reason we have been investigating the chemosystematics of liverworts. Here we report on the isolation and structure elucidation of five novel chroman derivatives from the Japanese liverworts *Metacalypogeia cordifolia* and *Cephalozia otaruensis* which have not yet been investigated phytochemically.

Compound **1** showed a reddish light brown color when treated with 3% FeCl<sub>3</sub> alcoholic solution on TLC plate. The presence of a phenolic hydroxyl group was apparent from the absorption bands at 3544 (OH), 1456 and 1427 (an aromatic ring) cm<sup>-1</sup> in the Fourier transform (FT)-IR spectrum of **1**. The electron ionization (EI)-MS of **1** showed a molecular ion peak at *m/z* 276. The <sup>1</sup>H-NMR spectrum of **1** (Table 1) showed a methoxyl group at δ 3.80 (3H, s) and four methyl groups at δ 1.31 (6H, s), 1.70 (3H, br s) and 1.73 (3H, br s), and a signal due to the hydroxyl proton at δ 5.23 (1H, s) which disappeared upon addition of D<sub>2</sub>O. Acetylation of **1** with acetic anhydride in pyridine gave a mono acetate **2**, whose IR spectrum exhibited no absorption band for the hydroxyl group, indicating the presence of only one hydroxyl group of **1**. Two mutually coupled equivalent methylene protons were observed at δ 2.73 (2H, t, *J*=6.8 Hz, H-4) and 1.75 (2H, t, *J*=6.8 Hz, H-3), suggesting an isolated C<sub>2</sub> chain as a partial structure of **1**. The <sup>13</sup>C-NMR (Table 1) spectrum showed 17 carbons, including 8 *sp*<sup>2</sup> carbons and an oxygenated carbon as shown in Table 1. Analysis of the extensive two dimensional (2D) NMR spectra supported the structural assignment. In particular, the <sup>1</sup>H-detected heteronuclear multibond connectivity (HMBC) spectrum of **1** indicated the presence of a prenyl group. Correlation of downfield shifted methylene protons at δ 3.29 (2H, d, *J*=7.4 Hz, H-1') which coupled with olefinic proton at δ 5.31 (1H, br t, *J*=7.4 Hz, H-2') to *sp*<sup>2</sup> quaternary carbon at δ 132.9 (s, C-3'), were observed.

The *sp*<sup>2</sup> quaternary carbon further correlated with two vinyl methyl protons at δ 1.70 (H-5') and 1.73 (H-4'). Further correlations are displayed in Fig. 1, and exhibited evidence of the substitution pattern of the prenyl and hydroxyl groups on the chromane aromatic ring. The above features and the structure of **1** were confirmed by the HMQC and HMBC spectral data as shown in Table 1.

The <sup>1</sup>H-, <sup>13</sup>C-NMR (Tables 2, 3), IR and EI-MS spectra of

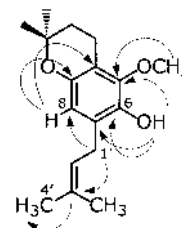


Fig. 1. The Arrows Display Selected Long Range H-C Correlations of Compound **1** (H→C)

Table 1. NMR Spectral Data for Chroman Derivative **1**

No.	<sup>1</sup> H <sup>a)</sup>		<sup>13</sup> C <sup>b)</sup>	
		Long-range H/C correlations <sup>c)</sup>		Long-range H/C correlations <sup>c)</sup>
2	—		73.3	H-4, 6', 7', 3
3	1.75 (2H, t, <i>J</i> =6.8 Hz)	C-4a, 2, 6', 4	32.2	
4	2.73 (2H, t, <i>J</i> =6.8 Hz)	C-8a, 5, 4a, 2, 3	17.5	
4a	—		111.9	H-3, 4, 8
5	—		144.2	H-4, OCH <sub>3</sub> , OH
6	—		139.8	
7	—		127.0	H-1', 2', OH
8	6.40 (s)	C-8a, 6, 4a, 7	112.8	
8a	—		147.0	H-4, 8
1'	3.29 (2H, d, <i>J</i> =7.4 Hz)	C-2', 3', 6, 7, 8	28.1	
2'	5.31 (br t, <i>J</i> =7.4 Hz)	C-7, 1', 4', 5'	122.0	
3'	—		132.9	H-1', 4', 5'
4'	1.73 (br s)	C-2', 3', 5'	25.8	
5'	1.70 (br s)	C-2', 3', 4'	17.7	
6', 7'	1.31 (6H, s)	C-2, 3	26.7 <sup>d)</sup>	
OCH <sub>3</sub>	3.80 (s)	C-5	60.4	
OH	5.23 (s)	C-5, 6, 7		

a) Measurement in chloroform-*d*<sub>1</sub>, 600 MHz for <sup>1</sup>H and 150 MHz for <sup>13</sup>C. b) Assignments were confirmed by HMQC spectrum. c) Confirmed by HMBC spectrum. d) Two signals were overlapped.

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Table 2.  $^1\text{H-NMR}$  Data for Compounds **3**–**6**

No.	<b>3</b> <sup>a)</sup>	<b>4</b> <sup>a)</sup>	<b>5</b> <sup>a)</sup>	<b>6</b> <sup>b)</sup>
3	1.77 (2H, t, $J=6.8$ Hz)	1.74 (2H, t, $J=6.5$ Hz)	1.76 (2H, t, $J=6.8$ Hz)	1.74 (2H, t, $J=6.8$ Hz)
4	2.62 (2H, t, $J=6.8$ Hz)	2.43 (2H, t, $J=6.5$ Hz)	2.68 (2H, br t, $J=6.8$ Hz)	2.70 (2H, br t, $J=6.8$ Hz)
6	6.17 (s)	6.40 (t, $J=2$ Hz)	—	—
8	—	—	6.10 (s)	6.30 (s)
1'	3.32 (2H, br d, $J=7.4$ Hz)	3.09 (2H, d, $J=7.4$ Hz)	2.68 (2H, br t, $J=6.8$ Hz)	2.70 (2H, br t, $J=6.8$ Hz)
2'	5.33 (br t, $J=7.4$ Hz)	5.13 (br t, $J=7.4$ Hz)	1.78 (2H, t, $J=6.8$ Hz)	1.76 (2H, t, $J=6.8$ Hz)
4'	1.73 (3H, br s)	1.75 (3H, br s)	1.32 (3H, s)	1.34 (3H, s)
5'	1.74 (3H, br s)	1.63 (3H, br s)	1.32 (3H, s)	1.34 (3H, s)
6', 7'	1.34 (6H, s)	1.38 (6H, s)	1.30 (6H, s)	1.29 (6H, s)
OCH <sub>3</sub>	3.76 (3H, s)	—	—	3.81 (s)
OH	5.26 (s)	—	5.61 (s)	—

a) Assignments were confirmed by  $^1\text{H-}^1\text{H}$  COSY, HMQC and HMBC spectra in chloroform- $d_1$ . b) Tentative assignment.

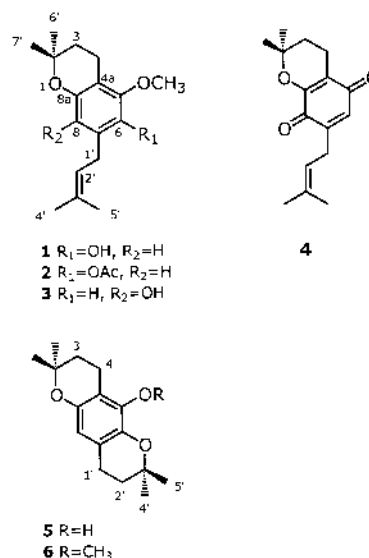
Table 3.  $^{13}\text{C-NMR}$  Data for Compounds **3**–**6**

No.	<b>3</b> <sup>a)</sup>	<b>4</b> <sup>a)</sup>	<b>5</b> <sup>a)</sup>	<b>6</b> <sup>b)</sup>
2	75.0	78.0	73.4	73.3
3	32.3	31.4	32.3	32.9
4	17.0	16.0	17.0	17.4
4a	107.9	117.0	106.5	113.5
5	150.2	187.4	142.4	145.9
6	101.6	132.3	133.8	140.0
7	123.6	145.8	119.2	120.7
8	136.8	182.3	106.5	111.2
8a	141.1	152.8	147.1	146.6
1'	28.4	27.2	22.0	22.6
2'	122.7	118.2	33.2	32.5
3'	132.3	136.1	74.7	73.7
4'	25.8	25.8	26.8 <sup>c)</sup>	26.8 <sup>c)</sup>
5'	17.8	17.7	26.8 <sup>c)</sup>	26.8 <sup>c)</sup>
6', 7'	26.7 <sup>d)</sup>	26.4 <sup>d)</sup>	26.6 <sup>c,d)</sup>	26.7 <sup>c,d)</sup>
OCH <sub>3</sub>	55.6	—	—	59.8

a) Assignments were confirmed by HMQC and HMBC spectra in chloroform- $d_1$ . b) Tentative assignment. c) Signals may be interchangeable in each vertical column. d) Two signals were overlapped.

**3** were similar to those of **1**. The partial structure of a prenylated chroman moiety was confirmed by the HMBC spectrum of **3**. In particular, correlations between methoxyl protons at  $\delta$  3.76 (s) and equivalent methylene protons at  $\delta$  2.62 (2H, t,  $J=6.8$  Hz, H-4) and an aromatic proton at  $\delta$  6.17 (s, H-6), which further correlated with H-1' at  $\delta$  3.32 (2H, d,  $J=7.4$  Hz) and H-2' at  $\delta$  5.33 (br t,  $J=7.4$  Hz) in the nuclear Overhauser effect spectroscopy (NOESY) spectrum of **3**, supported the positioning of the hydroxyl group at C-8. The above evidence allowed the conclusion that compound **3** only differed from **1** by the replacement of a proton by the hydroxyl group at C-8.

The structure of **4** was deduced by comparing its spectral data with those of **1** and **3**. The EI-MS spectrum of **4** showed a molecular ion peak at  $m/z$  260. Whereas the hydroxyl group absorption band was missing in the IR spectrum of **4**, the presence of a carbonyl group ( $1650\text{ cm}^{-1}$ ) was confirmed. Analysis of the HMBC and HMQC spectra of **4** supported the presence of a prenyl group. Correlation between an olefinic proton at  $\delta$  6.40 (t,  $J=2$  Hz, H-6) and allyl methylene protons at  $\delta$  3.09 (2H, br d,  $J=7.4$  Hz, H-1') in the  $^1\text{H-}^1\text{H}$  correlation spectroscopy (COSY) spectrum of **4**, indicated the presence of a long-range proton spin coupling between H-1' and the olefinic proton which correlated with car-



bonyl carbon at  $\delta$  182.3 (s, C-8) and 117.0 (s, C-4a) in the HMBC spectrum of **4**. The above features and the structure of **4** were confirmed by extensive 2D NMR experiment. The structure of **4** was established as a 2,2-dimethyl-7-(3'-methyl-2'-butenyl)-chroman-5,8-quinone.

The EI-MS of **5** gave a molecular ion peak at  $m/z$  262. The IR spectrum of **5** showed the presence of a hydroxyl group ( $3535\text{ cm}^{-1}$ ). The  $^1\text{H-NMR}$  spectrum showed the signals of four methyl groups at  $\delta$  1.32 (6H, s) and 1.30 (6H, s) and an aromatic ring proton at  $\delta$  6.10 (s). Two mutually coupled methylene groups showed doubling of the signals in the  $^1\text{H-NMR}$  spectrum of **5** at  $\delta$  1.76/1.78 (each 2H, t,  $J=6.8$  Hz) and  $\delta$  2.68 (4H, br t,  $J=6.8$  Hz). Thus, two isolated C<sub>2</sub> chains were present in the molecule of **5**. The structures of **5** and **6** were established by extensive 2D NMR experiments. Methylation of **5** afforded a mono methyl ether, whose  $^1\text{H-NMR}$  spectrum was identical to that of **6**, providing further evidence for the structure.

Isolation of chroman-type compound was the first achieved, although many aromatic compounds with a variety of types have been isolated from liverworts. *Calypogeia* species belongs to the Calypogeiaceae and are very characteristic, since they produce 1,4-dimethyl azulene derivatives.<sup>1)</sup> The present *Metacalypogeia cordifolia* (Calypogeiaceae) does not contain any azulene compounds. This

work is still in progress.

### Experimental

**General Experimental Procedures** TLC was carried out on silica gel precoated glass plates with *n*-hexane–EtOAc (1 : 1, 4 : 1). Detection was with Godin reagent.<sup>2)</sup> For normal phase column chromatography (CC), Silica gel 60 (40–63  $\mu\text{m}$ ) was used. The mixture of  $\text{CH}_2\text{Cl}_2$ –MeOH (1 : 1) was used for CC on Sephadex LH-20 as solvent.

Melting point was determined by a Yanagimoto micro melting point apparatus and is uncorrected. NMR spectra were recorded at 150 or 50 MHz for  $^{13}\text{C}$  and 600 or 200 MHz for  $^1\text{H}$  on a Varian UNITY 600 or 200, and chemical shifts are given in  $\delta$  (ppm) with tetramethylsilane as an internal standard. UV spectra were recorded in spectroscopic grade EtOH on a Hitachi U-3000. IR spectra were measured on a JASCO FT/IR-410 in  $\text{CHCl}_3$ . EI-MS spectra were recorded on a Hewlett-Packard HP-6890 gas chromatograph with 5972A mass selective detector. The temperature programming of GC-mass analysis was performed from 50  $^\circ\text{C}$  isothermal for 3 min, then 50–250  $^\circ\text{C}$  at 5  $^\circ\text{C min}^{-1}$ , and finally isothermal at 250  $^\circ\text{C}$  for 15 min. Injection temperature was 250  $^\circ\text{C}$ . A fused silica column coated with DB-17 (30 m  $\times$  0.25 mm i.d., film thickness 0.25  $\mu\text{m}$ ) was used.

**Plant Materials** *Metacalypogeia cordifolia* (STEPH.) INOUE (dry weight; 7.30 g) and *Cephalozia otaruensis* STEPH. (4.52 g) were collected in March 2000 at Otoyō in Kochi. The voucher specimens (*M. cordifolia*; #20112, *C. otaruensis*; #20026) are deposited at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

Each species of liverwort was gently washed with water, impurities removed and extracted with  $\text{Et}_2\text{O}$  for 7 d, then filtered through a short pad column (ca. 20 mm).

**Extraction and Isolation** The ether extracts (0.42 g) of *M. cordifolia* and *C. otaruensis* (0.1 g) were chromatographed on silica gel and Sephadex LH-20 to give a new chroman derivative **1** (28.9 mg; 6.9% of the total extract of the former species, 1.53 mg; 1.5% from the latter species) as major constituents of the two species. Further purification with preparative HPLC afforded four new chroman derivatives **3**–**6** (**3**: 12.8 mg; 3.0%, **4**: 3.5 mg; 0.8%, **5**: 10.0 mg; 2.4%, **6**: 6.2 mg; 1.5%) from the former species. The GC mass analysis of the extract of *M. cordifolia* detected  $\beta$ -barbatene,  $\beta$ -bisabolene and cuparene as the major constituent of sesquiterpene hydrocarbons.

Compound **1**: IR ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 3545, 1488, 1456, 1427, 1159, 1054. UV  $\lambda_{\text{max}}$  (EtOH) nm (log  $\epsilon$ ): 212 (4.25), 293 (3.59). EI-MS  $m/z$ : 276 ( $\text{M}^+$ ), 221, 220 (100%), 205, 177, 165, 148. HR-EI-MS  $m/z$  [ $\text{M}]^+$ : 276.1702 (Calcd for  $\text{C}_{17}\text{H}_{24}\text{O}_3$ : 276.1725). Compound **3**: IR ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 3546,

1628, 1601, 1498, 1455, 1441, 1385, 1371, 1159, 1113. UV  $\lambda_{\text{max}}$  (EtOH) nm (log  $\epsilon$ ): 213.5 (4.3), 288.5 (3.48). EI-MS  $m/z$ : 276 ( $\text{M}^+$ ), 221, 220, 189, 177 (100), 165, 136. HR-EI-MS  $m/z$  [ $\text{M}]^+$ : 276.1721 (Calcd for  $\text{C}_{17}\text{H}_{24}\text{O}_3$ : 276.1725). Compound **4**: IR ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 1650, 1631, 1604, 1393, 1373, 1295, 1156, 1115, 908. UV  $\lambda_{\text{max}}$  (EtOH) nm (log  $\epsilon$ ): 203 (4.05), 265.5 (3.82), 395 (2.87). EI-MS  $m/z$ : 260 ( $\text{M}^+$ ), 245 (100), 204, 189, 161. HR-EI-MS  $m/z$  [ $\text{M}]^+$ : 260.1388 (Calcd for  $\text{C}_{16}\text{H}_{20}\text{O}_3$ : 260.1412). Compound **5**: mp 181–185  $^\circ\text{C}$  (recrystallized from hexane–AcOEt). IR ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 3534, 1501, 1456, 1447, 1332, 1159, 1121, 1065, 960, 908. UV  $\lambda_{\text{max}}$  (EtOH) nm (log  $\epsilon$ ): 211 (4.22), 296 (3.24). EI-MS  $m/z$ : 262 ( $\text{M}^+$ ), 206 (100), 177, 163, 151. HR-EI-MS  $m/z$  [ $\text{M}]^+$ : 262.1552 (Calcd for  $\text{C}_{16}\text{H}_{22}\text{O}_3$ : 262.1568). Compound **6**: IR ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 1479, 1457, 1427, 1370, 1159, 1121, 1080, 964. UV  $\lambda_{\text{max}}$  (EtOH) nm (log  $\epsilon$ ): 212 (4.39), 300 (3.63). EI-MS  $m/z$ : 276 ( $\text{M}^+$ ) (100), 221, 220, 205, 177, 165, 148. HR-EI-MS  $m/z$  [ $\text{M}]^+$ : 276.1727 (Calcd for  $\text{C}_{17}\text{H}_{24}\text{O}_3$ : 276.1725).

**Methylation of 5 with Trimethylsilyldiazomethane ( $\text{CH}_3)_3\text{SiCHN}_2$**  To a solution of **5** (4.0 mg) in MeOH (0.5 ml), excess  $(\text{CH}_3)_3\text{SiCHN}_2$  in *n*-hexane (1 ml) was added. The mixture was left overnight and evaporated to yield a reaction mixture. Purification of the mixture by HPLC afforded a mono methyl ether (3.1 mg). The  $^1\text{H-NMR}$  spectrum of the monomethyl ether was identical to that of **6**.

**Acetylation of 1** Compound **1** (10 mg) was acetylated with  $\text{Ac}_2\text{O}$  (1 ml) and dry pyridine (1 ml) overnight at room temperature. The usual work-up afforded an acetate **2** (11.3 mg). Compound **2**:  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 200 MHz):  $\delta$  1.34 (6H, s), 1.68, 1.74 (each 3H, br s), 1.76 (2H, t,  $J=7$  Hz), 2.33 (3H, s), 2.73 (2H, t,  $J=7$  Hz), 3.14 (2H, d,  $J=7$  Hz), 3.78 (3H, s), 5.22 (1H, brt,  $J=7$  Hz), 6.46 (1H, s); IR ( $\text{CHCl}_3$ )  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 1756, 1579, 1457, 1419, 1371, 1344, 1223.

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