Antimicrobial Chlorinated Bibenzyls from the Liverwort *Riccardia marginata*

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Three new chlorinated bibenzyls **3**, **4**, and **5**, have been isolated from the New Zealand liverwort *Riccardia marginata*. This is the first report of simple chlorinated bibenzyls from any natural source. Compounds **3**–**5** showed antimicrobial activity against *Bacillus subtilis*, *Candida albicans*, and *Trichophyton mentagrophytes*.

The smells of the chlorinated phenol antiseptics TCP (**1**) and Dettol (**2**)[1] are evocative reminders of childhood scrapes for many. Liverworts do not get cut knees, but they do need protection from bacterial and fungal infections in the damp places where they mostly grow.[2] We now report chlorophenol derivatives **3**, **4**, and **5** as antimicrobial compounds from the liverwort *Riccardia marginata* (Colenso) Pearson (family Aneuraceae).

R. marginata is endemic to New Zealand,[3] where it is found growing on old logs in wet forests.[4] No previous chemistry of *R. marginata* has been reported, but the genus *Riccardia* has yielded a range of compounds,[5] including sesquiterpene-linked phenol derivatives,[6,7] indole alkaloids,[8,9] and bis-bibenzyls.[10–12] Brominated bibenzyls have previously been found in the red alga *Polysiphonia urceolata*,[13] but our report is the first of simple chlorinated bibenzyls from any natural source.[14]

A crude extract of *R. marginata* showed activity against *B. subtilis*, *C. albicans*, and *T. mentagrophytes* in antimicrobial screening.[15] The major low-polarity secondary metabolites **3**–**5** were purified by chromatography on reversed-phase C_{18} followed by normal-phase silica. The ^{1}H NMR spectra of these compounds showed $-CH_2-CH_2-$ signals (4H multiplets, δ_H 2.8–3.2) and phenolic OH signals (D_2O-exchangeable broad singlets, δ_H 5.6–6.0). The other signals were of aromatic protons, which were overlapping, especially for the major compound **3**.

Negative electrospray ionization (−ESI) MS gave rather confusing results (see below) for **3**–**5**, but did suggest the presence of chlorine atoms. Electron-impact MS gave molecular ions corresponding to $C_{14}H_{11}Cl_3O$ for **3**, $C_{14}H_{12}Cl_2O$ for **4**, and $C_{14}H_{13}ClO$ for **5**. All these spectra showed strong m/z 91 ions, due to $C_6H_5CH_2^+$. These results, along with the NMR data above, showed that these compounds were three bibenzyls, each with one unsubstituted ring and one ring substituted with −OH and −Cl(s).

The ^{1}H NMR spectrum of the monochloro compound **5** was best dispersed and showed a three-proton spin system due to a 1,2,3-trisubstituted aromatic ring (Table 1). The shielded position of one of the CH signals (δ_H 6.88, δ_C 113.8) suggested that this was *ortho* to the OH, with another CH signal (δ_H 6.74, δ_C 122.0) showing NOE correlations to the $-CH_2-CH_2-$ bridge protons, giving structure **5**. This was confirmed by 2D NMR correlations (Figure 1) and by ^{1}H

3 R₁, R₂, R₃ = Cl
4 R₁, R₂ = Cl R₃ = H
5 R₁ = Cl R₂, R₃ = H
7 R₁, R₂, R₃ = H

6
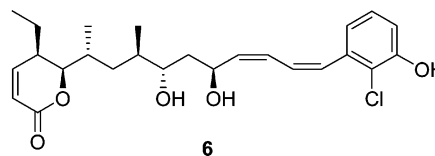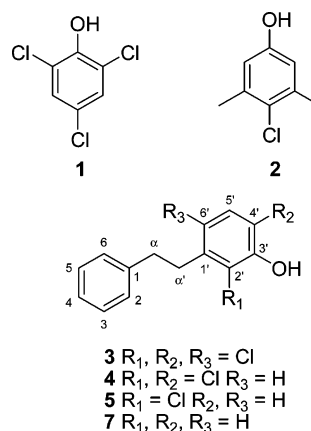
and ^{13}C shifts very similar to the known compound bitungolide A **6**.[16]

The dichloro compound showed a two-proton *ortho*-coupled system, with one CH showing NOE interactions with the $-CH_2-CH_2-$ bridge protons (Table 1). This suggested the structure **4**, which was supported by 2D NMR correlations.

Finally, the trichloro compound showed a singlet for a CH with no NOE interactions with the $-CH_2-CH_2-$ bridge protons or the OH. 2D NMR correlations supported structure **3**. Structures **3**–**5** allowed us to interpret the ESIMS results as showing different proportions of $[M - H]^-$ and $[M_2 - H]^-$ ions. The trichloro compound **3**, with bulky chlorines flanking the OH, showed mainly $[M - H]^-$ with almost no association to give $[M_2 - H]^-$. The dichloro compound **4** gave both $[M - H]^-$ and $[M_2 - H]^-$, and the monochloro compound **5** gave mainly $[M_2 - H]^-$.

Compounds **3**, **4**, and **5** are new, and we could not find any previous reports of natural chlorinated bibenzyls.[14]

These compounds seem to represent successive chlorinations of bibenzyl **7** by a haloperoxidase, similar to that recently found in the liverworts *Caldariomyces fumigo* and *Bazzania trilobata*.[17] 3-Hydroxybibenzyl **7** has been reported once as a natural product, in another liverwort, *Radula frondescens*,[5] but we did not see NMR signals appropriate for **7** in any chromatographic fractions or in

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Table 1. ^{1}H and ^{13}C NMR Spectral Data for Bibenzyls **3–5**[a]

	3		**4**		**5**	
	δ_H mult., (J in Hz)	δ_C	δ_H mult., (J in Hz)	δ_C	δ_H mult., (J in Hz)	δ_C
1		137.1		139.2		140.0
2	7.30 m	128.5	7.17 m	128.4	7.20 m	128.4
3	7.25 m	128.4	7.27 m	128.4	7.28 m	128.4
4	7.22 m	126.3	7.20 m	126.2	7.21 m	126.1
5	7.25 m	128.4	7.27 m	128.4	7.28 m	128.4
6	7.30 m	128.5	7.17 m	128.4	7.20 m	128.4
α	2.82 m	34.1	2.90 m	35.7	2.89 m	36.0
α′	3.15 m	34.0	2.99 m	35.7	3.00 m	36.0
1′		140.9		141.0		141.3
2′		122.0		121.0		119.8[b]
3′		147.0		147.8		151.6[b]
4′		118.8		118.5	6.88 dd (1.5, 8)	113.8
5′	7.33 s	128.2	7.13 d (8.5)	127.3	7.07 t (7.5)	127.5
6′		125.8	6.68 d (8.5)	121.9	6.74 dd (1.5, 7.5)	122.0
OH	5.86 s		5.89 s		5.61 s	

[a] Measured in CDCl$_3$ at 500 MHz for ^{1}H and 125 MHz for ^{13}C, respectively. [b] ^{13}C NMR signals are based on CIGAR correlations.
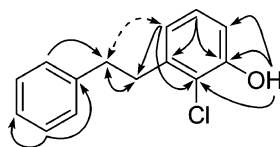
Figure 1. Selected CIGAR (solid line) and NOESY (dotted line) correlations for **5**.

Table 2. Antimicrobial Activities of Bibenzyls **3–5**[a]

compound	*B. subtilis*	*C. albicans*	*T. mentagrophytes*	*C. resinae*
3	2[b]	2	7	1
4	2	2	12	2
5	2	2	3	0

[a] Compounds assayed at 30 μg per disk. [b] Zone of inhibition (extra radius) in mm for 5 mm disks.

the crude extracts of *R. marginata*. However, ^{1}H NMR spectra of the crude screening extracts (after a rapid silica gel cleanup to remove photosynthetic pigments) did show that **3–5** were only present in our West Coast collection of *R. marginata*. The Auckland Islands collection just showed ^{1}H NMR signals for triglycerides. We have previously noted infraspecific variation of secondary metabolites in *R. crassa* with a New Zealand collection yielding riccardiphenol C,[6] and a Japanese collection yielding riccardiphenols A and B.[7]

The *R. marginata* collection containing **3–5** gave an extract with antimicrobial activity against the Gram-positive bacterium *Bacillus subtilis* and the dermatophytic fungus *Trichophyton mentagrophytes*, whereas the Auckland Islands extract was inactive. Assays of **3–5** showed activity against *B. subtilis*, *T. mentagrophytes*, the yeast *Candida albicans*, and the plant pathogenic fungus *Cladosporium resinae* (Table 2), but not against the Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. Dichloro compound **4** gave the largest inhibition zones against the fungi. We presume that these chlorinated bibenzyls protect the liverwort against pathogenic bacteria and fungi, but it is not clear why they would be produced in one location but not another.

Experimental Section

General Experimental Procedures. UV spectra were recorded with a Jasco V-550 UV spectrophotometer. IR spectra were obtained with a Perkin-Elmer 1600 FTIR as a film on a NaCl disk. NMR spectra were recorded at 25 °C on a Varian INOVA 500 NMR spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C, using solvent signals as references (C*H*Cl$_3$ at 7.25 ppm, *C*DCl$_3$ at 77.00 ppm). ESI mass spectra

were acquired using a Shimadzu LC-MS QP8000α mass spectrometer, and EIMS were obtained on a VG70-250S double-focusing magnetic sector mass spectrometer. Column chromatography used octadecyl-functionalized silica gel (Aldrich C$_{18}$) and 40–63 μm silica gel 60 (Merck). TLC was carried out on silica gel F$_{254}$ plates (Merck), with the solvent system hexane–EtOAc (9:1). Solvents for extraction and chromatography were distilled prior to use.

Plant Material. *Riccardia marginata* was collected from New Zealand's sub-Antarctic Auckland Islands in January 1994 (OTA 046755) and near the Kiahoka Lakes, Cape Farewell, West Coast, New Zealand (OTA 050850), in March 1999. Collections were identified by R. Tangney, and voucher specimens are deposited in the University of Otago Herbarium. The samples were air-dried (30 °C), and subsamples (5 g) were shaken overnight in EtOH (50 mL) to give extracts for biological screening, as reported previously.[15]

Extraction and Isolation. The remaining plant material (46 g) from the two *R. marginata* collections were combined, extracted with CHCl$_3$ (4 × 460 mL), and concentrated under vacuum to give a deep green solid residue (1.67 g). The residue was chromatographed on C$_{18}$ using a steep, stepped solvent gradient from H$_2$O to MeOH to CHCl$_3$ to hexane. The fraction that eluted at 100% MeOH (221 mg) was chromatographed further on silica gel, eluting with hexane–EtOAc mixtures. The first two fractions (14 mg each), eluted between hexane and 10% EtOAc, were further purified by silica gel TLC (plate thickness, 0.2 mm) using 10% EtOAc in hexane (developed twice, drying the plates between) to give 2,4-dichloro-3-hydroxybibenzyl (**4**) (3 mg, R_f 0.24), 2-chloro-3-hydroxybibenzyl (**5**) (1 mg, R_f 0.25), and 2,4,6-trichloro-3-hydroxybibenzyl (**3**) (6 mg, R_f 0.34).

2,4,6-Trichloro-3-hydroxybibenzyl (3): yellow gum; UV (MeOH) λ_{max} (log ϵ) 217 (4.3), 289 (3.5), 296 (3.5) nm; IR (film) ν_{max} 3380, 2940, 2869, 1559, 1500, 1452, 1422, 1315, 1291, 1273, 1226, 1148, 805, 770, 758, 741, 729, 688 cm^{-1}; ^{1}H and ^{13}C NMR in Table 1; EIMS m/z 304 (2), 303 (1), 302 (8), 300 [M$^+$] (9), 211 (5), 209 (5), 91 (100); HREIMS m/z 299.9889 (calcd for C$_{14}$H$_{11}$Cl$_3$O, 299.9875).

2,4-Dichloro-3-hydroxybibenzyl (4): yellow gum; UV (MeOH) λ_{max} (log ϵ) 209 (4.3), 279 (3.1), 286 (3.2) nm; IR (film) ν_{max} 3404, 2940, 2857, 1450, 1419, 1340, 1249, 1192, 1147, 803, 753, 710, 698 cm^{-1}; ^{1}H and ^{13}C NMR in Table 1; EIMS m/z 270 (1), 268 (8), 267 (2), 266 [M$^+$] (13), 177 (10), 175 (15), 91 (100); HREIMS m/z 266.0254 (calcd for C$_{14}$H$_{12}$Cl$_2$O, 266.0265).

2-Chloro-3-hydroxybibenzyl (5): yellow gum; UV (MeOH) λ_{max} (log ϵ) 212 (4.0), 276 (3.2), 282 (3.1) nm; IR (film) ν_{max} 3523, 2917, 2845, 1580, 1450, 1292, 1190, 788, 757, 699 cm^{-1}; ^{1}H and ^{13}C NMR in Table 1; EIMS m/z 235 (1), 234 (7), 233 (3), 232 [M$^+$] (18), 141 (15), 91 (100); HREIMS m/z 232.0651 (calcd for C$_{14}$H$_{13}$ClO, 232.0655).

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