Isolation and Antimicrobial Activity of Dichlororinated Bibenzyl Compound

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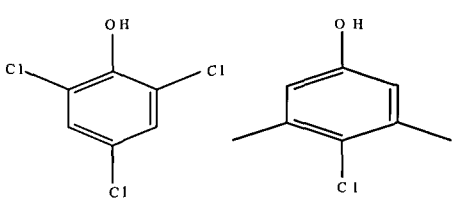
Dichlororinated bibenzyl compound (4) has been isolated from the New Zealand liverwort. This compound was elucidated using 1D/2D-NMR and mass spectral method. The compound (3) inhibited the growth of the Gram positive bacterium *Bacillus subtilis* ATCC 19659, (2 mm inhibition zone and 2 mm inhibition zone at 30 μg/disc), *Candida albicans* ATCC 14053, (2 mm inhibition zone and 2 mm inhibition zone at 30 μg/disc), and the dermatophytic fungi *Trichophyton mentagrophytes* ATCC 28185, (12 mm inhibition zone at 30 μg/disc) and *Cladosporium resinae* ATCC 52833 (2 mm inhibition zone at 30 μg/disc). This bibenzyl compound (4) exhibited antimicrobial activity.

Key words : Bibenzyl compounds (4), 1D/2D-NMR, mass spectral method, *Trichophyton mentagrophytes*, antimicrobial activity

Introduction

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].



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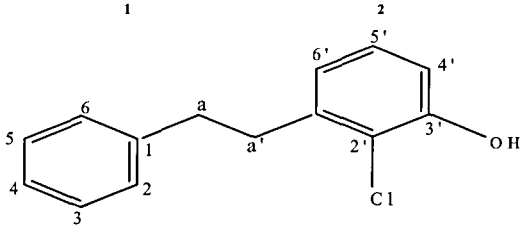

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A crude extract of *R. marginata* showed activity against *Bacillus subtilis, Candida albicans and Trichophyton mentagrophytes* in our antimicrobial screening[3]. 2'-Chloro-3'-hydroxybibenzyl (3) inhibited the growth of the Gram positive bacterium *Bacillus subtilis* ATCC 19659, (2 mm inhibition zone at 30 μg/disc), *Candida albicans* ATCC 14053, (2 mm inhibition zone at 30 μg/disc), and the dermatophytic fungus *Trichophyton mentagrophtes* ATCC 28185, (3 mm inhibition zone at 30 μg/disc)[4]. We now report the dichlororinated bibenzyl compound (4) as the antifungal compound from the New Zealand liverwort.

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Materials and Methods

1. General experimental procedures.

Solvents for extraction and chromatography were distilled prior to use. Preparative silica gel TLC was carried out using Merck DC-plastikfolien Kieselgel 60 F_{254}, visualized with an UV lamp then by dipping in a vanillin solution (1% vanillin, 1% H_2SO_4 in EtOH) and heating. UV spectrum was recorded with a Jasco V-550 UV spectrophotometer. IR spectrum was obtained with a Perkin-Elmer 1600 FTIR as a film on a NaCl disk. NMR spectra were recorded at 25℃ on a Varian INOVA 500 NMR spectrometer operating at 500 MHz for ^1H and 125 MHz for ^{13}C, using solvent signals as references ($CHCl_3$ at 7.25 ppm, $CDCl_3$ at 77.0 ppm). DEPT, HSQC, CIGAR and NOESY experiments were run at 25℃. EIMS was obtained on a VG70-250S double-focusing magnetic sector mass spectrometer. Column chromatography used octadecyl-functionalized sillica gel (Aldrich C_{18}) and 40-63 μm silica gel 60 (Merck). TLC was carried out on silica gel F254 plates (Merck), with the solvent system hexane - ethyl acetate (9 : 1).

2. Plant materials.

Riccardia marginata (*R. marginata*) was collected from New Zealand's sub-Antarctic Auckland Islands in January 1994 (OTA046755), 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℃).

3. Extraction and isolation.

The remaining plant material (46.05 g) from the two *R. marginata* collections was combined, extracted with $CHCl_3$ (4 x 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_2O 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 - ethy acetate mixtures. The first two fractions (14 mg), eluted between hexane and 10% ethyl acetate, were further purified by silica gel TLC (plate thickness, 0.2 mm) using 10% ethy acetate in hexane (developed twice, drying the plates between) to give 2′4′-dichloro-3′-hydroxybibenzyl (4, 3 mg, R_f 0.24): yellow gum; UV (MeaOH) 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-NMR ($CDCl_3$) δ (1H, 7.17, m, 2-H), (1H, 7.27, m, 3-H), (1H, 7.20, m, 4-H), (1H, 7.27, m, 5-H), (1H, 7.17, m, 6-H), (2H, 2.90, m, α), (2H, 2.99, m, α′), (1H, 7.13, d, J=8.5 Hz, 5′-H), (1H, 6.68, d, J=8.5 Hz, 6′-H), (OH, 5.89, s,); 13C-NMR (CDCl3) δ 139.2 (C-1), 128.4 (C-2), 128.4 (C-3), 126.2 (C-4), 128.4 (C-5), 128.4 (C-6), 35.7 (C-α), 35.7 (C-α′), 141.0 (C-1′), 121.0 (C-2′), 147.8 (C-3′), 118.5 (C-4′), 127.3 (C-5′), 121.9 (C-6′); 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 $C_{13}H_{12}Cl_2O$, 266.0265).

4. Screening for antibacterial and antiyeast activities

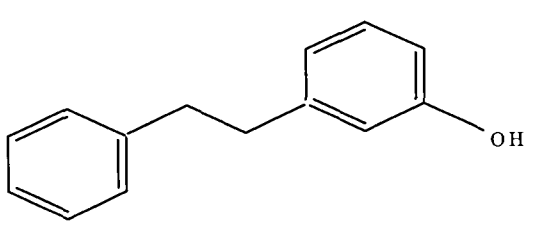
Activity against the following bacterial strains and yeast was tested: multiresistant *Bacillus subtilis* (ATCC 19659), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida albicans* (ATCC 14053) and *Cladosporium resinae* (ATCC 52833). Compounds were dissolved and diluted in an appropriate solvent (usually ethanol : water) to a concentration of 5 mg/mL. Test plates are prepared from Mueller Hinton agar containing extract to give a final concentration of 100 μg extract/mL agar. Activity growing cultures of the test strains were diluted in saline so as to deliver 10^4 colony forming units onto the test, control (solvent), and blank (agar only) plates with a multipoint inoculator. Inoculated plates were incubated overnight at 37℃. Growth on the blank and control plates was checked and, if satisfactory, growth on the test plates was scored for each test strain as follows: (-) inhibition, no reduction in growth compared with the control, (+) inhibition, no growth. Solutions of compound for assay were dried onto 6 mm filter paper disks, which were then placed onto seeded agar Petri dishes and incubated. Activity was observed as a zone of inhibition around the disk, with its width recorded from the edge of the disk in mm. HM and SM refer to the observed margin surrounding the zone of inhibition(H= hazy, S= sharp).

5. Screening for antifungal activity

Activity against the following fungal strain was tested: *Trichophyton mentagraphytes* (ATCC 28185) local strain]. Fungal spore suspensions of the test oraganisms were applied to dextrose agar plates. Aliquots of the compound solutions were applied to filter paper discs, at 30 μg extract/disc, and dired at 37℃ for two hours. These discs were applied to the agar plates, two per plate, and incubated at 28℃.

Results and Discussion

The dichloro compound showed a two-proton ortho-coupled system, with one CH showing NOE interactions with the $-CH_2-CH_2-$ bridge protons (C-α′H, C-αH). This suggested the structure 4, which was supported by 2D-NMR correlations. This compound (4) allowed us to interpret the ESIMS results. The dichloro compound (4) gave both $[M - H]^-$ and $[M_2 - H]^-$. This compound (4) seems to represent successive chlorinaition of bibenzyl (5) by a haloperoxidase, similar to that recently found in the liverworts *Caldariomyces fumigo* and *Bazzania trilobata*.[6] 3-Hydroxybibenzyl (5) has been reported once as a natural product, in another liverwort, Radula frondescens[7], but we did not see NMR signals appropriate for 5 in any chromatographic fractions or in the crude extracts of *R. marginata*.



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However, ^{1}H-NMR spectrum of the crude screening extracts (after a rapid silica gel cleanup to remove photosynthetic pigments) did show that 4 was only represent in our West Coast

collection of *R. marginata*. The Auckland Islands collection just showed ^{1}H-NMR signals for triglycerides. The *R. marginata* collection containing 4 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. Assay of 4 showed activity against *B. subtilis*, *T. mentagrophytes*, the yeast *Candida albicans*, and the plant pathogenic fungus *Cladosporium resinae* (Table 1), but not against the Gram-negative bacteria Escherichia coli and *Pseudomonas aeruginosa*. Dichloro compound (4) gave the strongest inhibition zones against the fungus. We presume that this chlorinate bibenzyl protect the liverwort against pathogenic bacteria and fungi, but it is not clear why this would be produced in one location but not another.

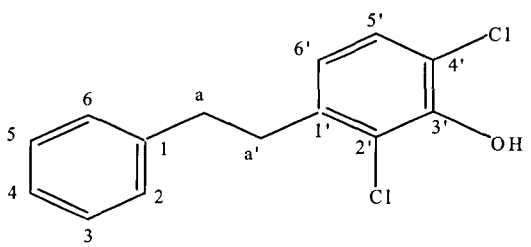

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Fig. 1. The structure of 2',4'-dichloro-3'-hydroxybibenzyl (4).

Table 1. Antimicrobial activity of 2 ' 4'-dichloro-3'-hydroxybibenzyl (4) from *R. marginata*.

Tested material	Antimicrobial activitya					
	B.subtilis	*C.albicans*	*T.ment.*	*E.coli*	*C.resinae*	*P.aeruginosa*
4	HM 2	HM 2	HM 12	-	HM 2	-
Chloramphenicol	SM 13	0	0	0	0	0
Nystatin	0	SM 12	SM 6	0	SM 10	0
Gentamycin	0	0	0	SM 9	0	SM 10

[a]Width of zone of inhibition in mm: 30 μg/disc: -: not detected, 0: not determined. Chloramphenicol: 30 μg/disc, Gentamycin: 30 μg/disc, Nystatin: 100 unit/disc. HM: Hazy margin, SM: Sharp margin, numbers refer to zone of inhibition (mm)

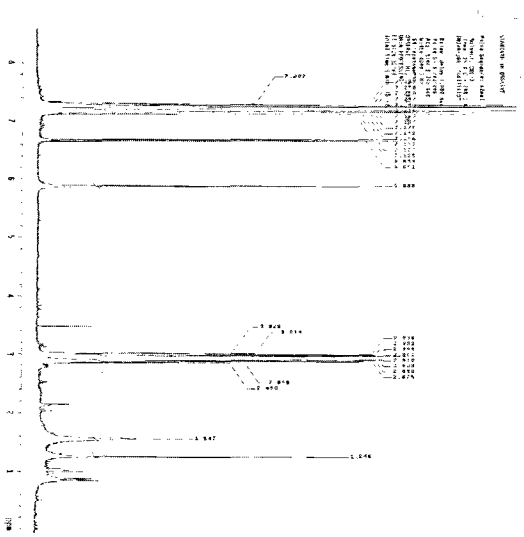

Fig. 2. ^{1}H-NMR Spectrum of 2',4'-dichloro-3'-hydroxybibenzyl (4).

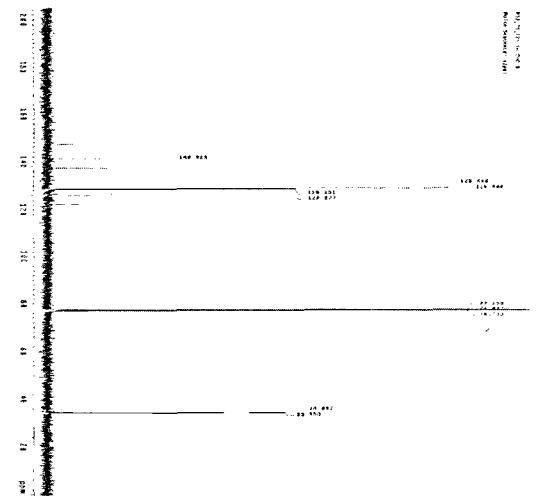

Fig. 3. ^{13}C-NMR Spectrum of 2',4'-dichloro-3'-hydroxybibenzyl (4).

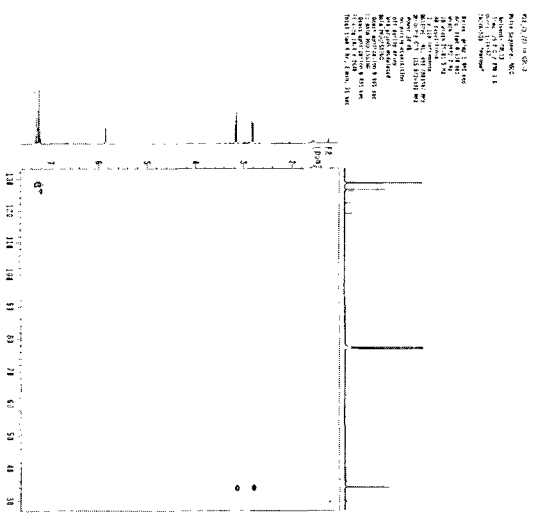

Fig. 4. SHQC Spectrum of of 2',4'-dichloro-3'-hydroxybibenzyl (4).

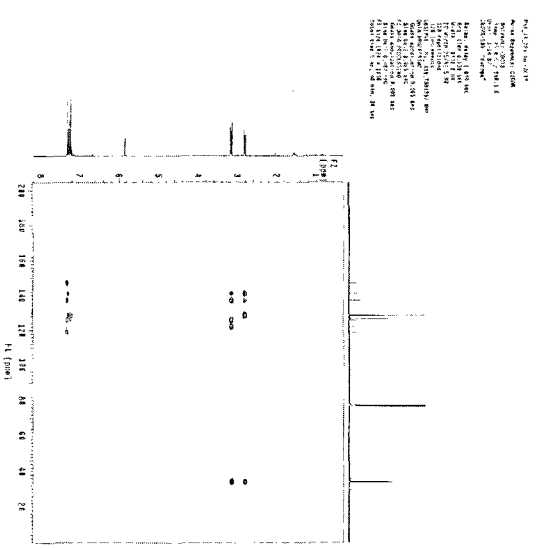

Fig. 5. CIGAR Spectrum of of 2',4'-dichloro-3'-hydroxybibenzyl (4).

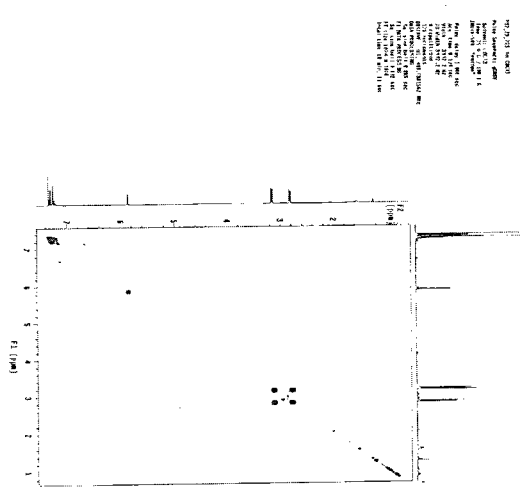

Fig. 6. COSY Spectrum of of 2',4'-dichloro-3'-hydroxybibenzyl (4).

In conclusion, dichlororinated bibenzyl compound (4) inhibited the growth of the Gram positive bacterium Bacillus subtilis ATCC 19659, (2 mm inhibition zone at 30 μg/disc), Candida albicans ATCC 14053, (2 mm inhibition zone at 30 μg/disc), and the dermatophytic fungi Trichophyton mentagrophytes ATCC 28185, (12 mm inhibition zone at 30 μg/disc) and Cladosporium resinae ATCC 52833 (2 mm inhibition zone at 30 μg/disc).

Acknowledgements

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