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SESQUITERPENE/QUINOL FROM A NEW ZEALAND LIVERWORT, *RICCARDIA CRASSA*

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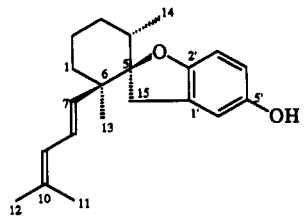
ABSTRACT.—A new sesquiterpene/quinol, with mild cytotoxic and antibacterial activity, has been isolated from a New Zealand collection of the liverwort *Riccardia crassa*. The structure of this compound, riccardiphenol C [**3**], was established by nmr spectroscopy. Closely related compounds previously isolated from a Japanese collection of *R. crassa* were not detected in this collection.

In a continuing search for bioactive natural products with potential as new pharmaceuticals or agrochemicals, we have begun to examine the rich liverwort flora of New Zealand. Two New Zealand *Plagiochila* species have yielded different classes of antifungal compounds (1,2). In this paper, we report the isolation and structure determination of a new sesquiterpene/quinol with weak biological activities from another New Zealand liverwort.

A large collection of liverworts from the high-rainfall area on the west coast of the South Island gave us extracts with a range of biological activities. One bioactive extract was from a liverwort identified as *Riccardia crassa* (Schwaegr.) Carring. & Pears. (Aneuraceae or Riccardiaceae), growing partly submerged in a swamp. This species grows throughout New Zealand in a very wide range of habitats from sea level to above the tree line (3). It has also been recorded in southeastern Australia, Tasmania, southern South America, Java, and the southern tip of Japan (4).

Several classes of compounds have been reported from various *Riccardia* species, including a few compounds with biological activities. Two cytotoxic bisbibenzyls were isolated from *R. multifida*, together with 6-(3-methyl-2-butenyl)indole (5). A piscicidal diterpene dialdehyde was isolated from *R. lobata* var. *yakushimensis* (6). Toyota and Asakawa isolated riccardiphenols A [**1**] and B [**2**],



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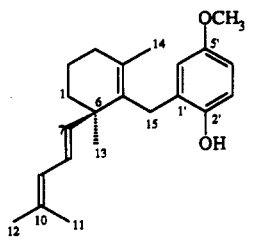

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which contain a sesquiterpene portion attached to a quinol ring, from a Japanese collection of *R. crassa*, but did not mention any biological activities (7). Sesquiterpene/quinones and sesquiterpene/quinols are rare in terrestrial plants, but quite common from marine sources (8). Many of these compounds have shown bioactivity, including antimicrobial, cytotoxic, and antiviral activities (8).

Extracts of our New Zealand collections of *R. crassa* showed mild cytotoxicity (BSC-1 cells) and antimicrobial activity (*Bacillus subtilis*). Reversed-phase flash chromatography of a crude extract gave most of the mass in the non-polar fractions, eluted with MeOH, MeOH-$CHCl_3$ (3:1), and $CHCl_3$. Si gel tlc suggested

that the fraction eluted with MeOH contained mainly one uv-active compound. The ^{1}H-nmr spectrum of this fraction showed aromatic signals, plus conjugated diene signals. However, the chemical shifts of the diene signals differed from those reported for riccardiphenols A [**1**] and B [**2**] (7). This diene was purified by Si gel flash chromatography. Mass spectrometry showed a strong molecular ion at 312.2089 daltons, consistent with the elemental formula of riccardiphenol A [**1**], but the ^{1}H-nmr spectrum did not show the methyl doublet required by that structure (7).

The ^{1}H-nmr spectrum of the diene in C_6D_6 showed better separation of signals than did the spectrum in $CDCl_3$, so the former solvent was used for nmr experiments to establish its structure. The nmr data, including correlations from ^{1}H-^{1}H COSY, 2/3 bond ^{1}H-^{13}C heteronuclear correlation (HETCOR), and nOe interaction experiments, are given in Table 1.

The presumed diene sub-structure was confirmed by a HETCOR nmr experiment, which also showed that this sub-structure was attached to a quaternary carbon (39.68 ppm) bearing a Me group (^{1}H-nmr shift 1.16 ppm). This Me also correlated to a CH_2 unit (^{13}C shift 39.44 ppm). COSY correlations showed this CH_2 to be linked to two further CH_2 groups. The second of these (^{13}C-nmr shift 41.21 ppm) showed a correlation to a Me singlet (^{1}H-nmr shift 1.38 ppm). This Me had to be attached to a quaternary sp^3 carbon, and the only possibility had a chemical shift of 77.45 ppm, showing the attachment of an oxygen. The deductions are summarized in sub-structure A (Figure 1).

The ^{1}H-nmr spectrum showed a 1′,2′,5′-trisubstituted aromatic ring, and the strong 3-bond correlations to the aromatic protons showed that the 2′- and 5′-positions were oxygenated (^{13}C-nmr shifts 148.18 and 150.26 ppm). The 1′ position bore a CH_2 group, which was also linked to a CH group (^{13}C-nmr shift 49.79). This gave sub-structure B (Figure 1).

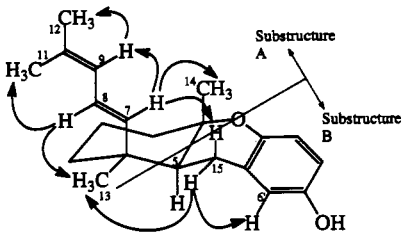

FIGURE 1. Proposed preferred conformation of riccardiphenol C [**3**], plus selected nOe interactions.

Sub-structures A and B accounted for all the atoms of the molecular formula except for one hydrogen (not observed in the ^{1}H-nmr spectrum). Therefore A and B had to be linked to give a cyclohexane ring. This was confirmed by the CH at 49.79 ppm showing a correlation to the Me at 1.16 ppm. The only ambiguity involved the ether linkage. However, the alternative to that shown in structure **3**, i.e., a 4–5′ ether linkage, would give a highly strained 1,3-bridged aromatic structure. The ^{13}C-nmr data for our compound in $CDCl_3$ (see Experimental) showed appropriate shifts when compared with the data for riccardiphenol A [**1**] (7). For example, the C-3 signal is deshielded in **3** compared to **1**, due to β-oxygenation.

The relative stereochemistry of structure **3** (the numbering system is that used for riccardiphenols A and B) was established by nOe experiments (Table 1). Interactions between H-7 and both Me-14 and one H-15 showed that all of these

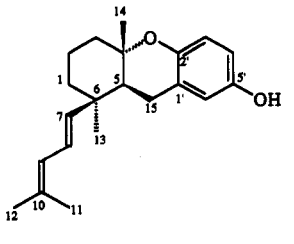

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TABLE 1. Nmr Data for Riccardiphenol C [3].[a]

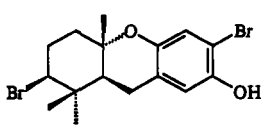
Position	^{13}C[b]	^{1}H[c]	^{1}H-^{1}H correlations[d]	^{13}C-^{1}H correlations[e]	nOe interactions[f]
1	39.44	2.00 (eq, dm, 13)	1.77, 1.63, 1.35	1.16, 2.2	6.54, 2.20[h], 1.63, 1.35
		1.35 (ax, ddd, 13,13,3)[g]	2.00, 1.63		5.88[h], 2.81[h], 2.20[h], 2.00, 1.9, 1.77[h]
2	20.44	1.79 (ax, ddddd, 13,13,13,3,3)	Not resolved	1.95	Not done
		1.63 (eq, ddddd, 13,3,3,3,3)	1.95, 1.35		2.20, 2.00, 1.77
3	41.21	2.20 (eq, dm, 13)	1.95, 1.77	1.38, 2.0	1.95, 1.77, 1.38
		1.95 (ax, ddd, 13,13,3)[g]	Not resolved		Not done
4	77.45	—	—	None seen	—
5	49.79	1.9 (dd, 13,5)[g]	Not resolved	1.16, 2.0, 2.2, 2.8	Not done
6	39.68	—	—	1.16	—
7	135.58	5.88 (d, 15)	6.54, 1.91	6.12, 1.16	6.12, 2.81, 1.38, 1.16
8	125.44	6.54 (dd, 11,15)	6.12, 5.88, 1.91, 1.87	6.12	2.00, 1.87, 1.16
9	127.15	6.12 (br d, 11)	6.54, 1.91	1.91	5.88, 1.91
10	133.43	—	—	1.87	—
11	18.93	1.87 (s)	—	6.12	Not done
12	26.65	1.91 (s)	5.88, 6.12	6.12, 1.87	Not done
13	30.73	1.16 (s)	—	5.88, 1.9	6.71[h], 6.64[h], 6.54, 5.88, 2.72, 1.9
14	19.98	1.38 (s)	—	2.2, 1.9	5.88, 2.81, 2.20, 2.00[h], 1.9[h], 1.77
15	24.11	2.81 (ax, br dd, 13,16)	2.72, 1.9, 6.71	6.71	6.71, 5.88, 1.38
		2.72 (eq, dd, 5,16)	2.81, 1.9		6.71, 5.88[h], 1.16
1′	123.97	—	—	7.11, 2.81/2.72	—
2′	148.18	—	—	6.71	—
3′	118.61	7.11 (d, 8)	6.64	7.11	6.64
4′	115.54	6.64 (dd, 3,8)	7.11, 6.71	None seen	7.11
5′	150.26	—	—	7.11	—
6′	116.83	6.71 (d, 3)	6.64, 2.81/2.72	None seen	2.81, 2.72

[a]Recorded in C_6D_6 solution.
[b]Shift in ppm.
[c]Shift in ppm; orientation (ax=axial, eq=equatorial); multiplicity (d=doublet, br=broad, s=singlet, m=multiplet); couplings in Hz.
[d]From COSY experiment.
[e]From HETCOR experiment, optimized for 2/3 bond couplings.
[f]From NOGGLE experiment.
[g]Couplings from NOGGLE experiment.
[h]NOe due to irradiation of overlapping signal.

substituents were on the same face of the cyclohexane ring. Conformational searching of the proposed structure **3** was carried out using MacroModel software (9) and the MM2 force field (10). The most stable conformation found is illustrated in Figure 1, but other rotamers about the 6–7 and 8–9 bonds were predicted to have significant populations. The observed coupling constants and nOe interactions (Table 1) are consistent with the conformation shown in Figure 1.

We have named this new compound riccardiphenol C [**3**] because of its close structural similarity to riccardiphenols A [**1**] and B [**2**]. The absolute stereochemistry of **3** is arbitrarily shown to be the same at C-6 as in **1** and **2**, but absolute stereochemistries have not been proven for any of these compounds (7). Riccardiphenols A [**1**] and C [**3**] represent cyclizations to opposite faces of the 4,5 double bond of a demethylated derivative of riccardiphenol B [**2**]. A monoterpene/quinol, 4-isocymobarbatol [**4**], shares many of the structural features of riccardiphenol C [**3**] (11). Compound **4**, which has antimutagenic properties, was isolated from a marine green alga.

Riccardiphenol C [**3**] was one of the major components in our crude EtOH extract of *R.* crassa. It could be clearly seen in the ^{1}H-nmr spectrum ($CDCl_3$) of the crude extract, and the purified yield was about 4 mg per 1 g of dry liverwort. We did not detect either riccardiphenols A or B, which were found in the Japanese collection of *R. crassa* (7). This is an interesting example of intra-specific variation in liverwort metabolites. Riccardiphenol C [**3**] was also a major component of a collection of *R. crassa* from the sub-Antarctic Auckland Islands.



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However, we could not detect **3** in any other *Riccardia* species that we have collected in New Zealand, so sesquiterpene/quinols may prove to be a taxonomic marker for this species.

Riccardiphenol C [**3**] showed slight cytotoxicity against BSC-1 cells, at 60 μg/disk, but not against P-388 cells (IC_{50}>25 μg/ml). It was slightly active against *B. subtilis*, at 60 μg/disk, but not against the fungi *Candida albicans* or *Trichophyton mentagrophytes*. There have been some reports of a marine sesquiterpene/quinol, avarol, having activity against the human immunodeficiency virus (HIV) (8). However, the U.S. National Cancer Institute did not detect any anti-HIV activity in our crude extract of *R. crassa* (12).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All solvents were distilled before use and were removed by rotary evaporation at temperatures up to 45°. Octadecyl-functionalized Si gel (Aldrich) was used for reversed-phase flash chromatography, and Davisil, 35–70 μm, 150 Å was used for Si gel flash chromatography. Tlc was carried out using Merck DC-Plastikfolien Kieselgel 60 F_{254}, visualized with a uv lamp then by dipping in a vanillin solution (1% vanillin, 1% H_2SO_4 in EtOH), and heating. Mass, uv, and ir spectra were recorded on Kratos MS-80 (electron impact, 70 eV), Shimadzu UV 240, and Perkin-Elmer 1600 spectrometers, respectively. Nmr spectra, with C_6D_6 or $CDCl_3$ solutions at 25°, were recorded at 300 MHz for ^{1}H and 75 MHz for ^{13}C on a Varian VXR-300 spectrometer (C_6D_6), and at 200 MHz for ^{1}H and 50 MHz for ^{13}C on a Varian Gemini spectrometer ($CDCl_3$). Chemical shifts are given in ppm on the δ scale referenced to the solvent peaks C_6HD_5 at 7.40 and C_6D_6 at 128.70, or $CHCl_3$ at 7.25 and $CDCl_3$ at 77.00. Details of antimicrobial, antiviral, and P-388 assays have been given previously (1). Our method of conformational searching has been described elsewhere (13).

PLANT MATERIAL.—*R. crassa* was first collected from south of Haast, on the west coast of the South Island, New Zealand, in October 1992 (University of Otago Herbarium specimen OTA 046607), and again from the same location in June 1993 (OTA 046627). *R. crassa* was also collected from Tagua Bay, Carnley Harbour, Auckland Islands, in January 1994 (OTA 046732).

EXTRACTION AND ISOLATION.—Initial screening was carried out on extracts produced by shaking air-dried (30°) material (5.0 g) overnight in EtOH (50 ml). These extracts, at 30 μl/disk, all gave slight inhibition of *B. subtilis* and marginal cytotoxicity against BSC-1. Dried, ground *R. crassa* (27 g) was extracted with EtOH (1×300 ml and 3×100 ml) and $CHCl_3$ (1×100 ml) by homogenizing and filtering to give a dark-green gum (1.115 g, 50% BSC-1 cytotoxicity at 300 μg/disk). This extract was subjected to reversed-phase flash chromatography over C_{18} Si gel (1.065 g, precoated on 2.13 g C_{18}, loaded on 11 g C_{18} column) eluted in steps from H_2O to $CHCl_3$. One of the largest fractions, eluted with MeOH, contained a uv-active component by tlc (0.25 g, 50% BSC-1 cytotoxicity at 300 μg/disk). Chromatography of this fraction over Si gel (12 g column) developed with hexane-EtOAc (5:1) gave **3** (0.065 g).

Riccardiphenol C [**3**].—Pale yellow oil; Si gel tlc R_f 0.3 (hexane-EtOAc, 5:1); $[\alpha]_{589}$ +26°, $[\alpha]_{577}$ +35°, $[\alpha]_{546}$ +37°, $[\alpha]_{435}$ +50°, $[\alpha]_{405}$ +61° (c=0.5, $CHCl_3$); ir ν max (film) 3368, 2921, 2867, 1703, 1649, 1616, 1491, 1447, 1376, 1338, 1278, 1218, 1142, 1120, 1093, 984, 957, 880, 804, 782, 755 cm^{-1}; uv λ max (MeOH) (log ε) 295 (3.19), 239 (4.07), 234 (4.08) nm, (MeOH/NaOH) 309 (3.23), 240 (4.16), 234 (4.15) nm; eims *m/z* 312.20892 ($[M]^+$, 1%, $C_{21}H_{28}O_2$ requires 312.20893), 230 (13), 215 (28), 189 (67), 161 (15), 147 (13), 135 (43), 123 (23), 107 (28), 93 (30), 77 (11); ^1H nmr ($CDCl_3$) δ 6.52–6.66 (3H, m), 6.24 (1H, dd, J=11 and 15 Hz), 5.82 (1H, br d, J=11 Hz), 5.69 (1H, br d, J=15 Hz), 2.58–2.72 (2H, m), 1.774 (3H, br s), 1.767 (3H, br s), 1.110 (3H, s), 1.095 (3H, s); ^{13}C nmr ($CDCl_3$) 148.57 (C-5′), 146.98 (C-2′), 134.36 (C-7), 133.61 (C-10), 125.61 (C-9), 124.60 (C-8), 123.27 (C-1′), 117.50 (C-3′), 115.66 (C-6′), 114.35 (C-4′), 76.92 (C-4), 48.91 (C-5), 40.04 (C-3), 38.91 (C-6), 38.73 (C-1), 29.89 (C-13), 25.95 (C-12), 23.19 (C-15), 19.50 (C-2), 19.15 (C-14), 18.39 (C-11). Nmr data in C_6D_6 are listed in Table 1. Compound **3** gave a 50% cytotoxic effect against BSC-1 cells at 60 μg/disk; a 2-mm zone of inhibition of *B. subtilis* at 60 μg/disk; and no effects against P-388 leukemia cells at 25 μg/ml.

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