layer) and (2) cyclohexane–EtOAc (3:7). Radioactively labelled 19-deoxylimonoate 3-methyl-^{14}C ester was obtained from R. D. Bennett of our laboratory. Compound **4** was synthesized by the method of Poling *et al.* [7], and **5** was synthesized from 2-diethylaminoethylchloride and 3,4-dimethylphenol according to Schuetz and Baldwin [13]. X-77 Spreader, Ortho, was purchased from Chevron Chemical Company, San Francisco, California. A Meyer tree and an unidentified lemon tree were used. The latter was grown from a seedling.

Quantitative analyses of limonoids. Compound **2** was extracted from leaves in the form of **1**, and its quantity was estimated by the procedure described previously [5].

Spray treatments. The aq. solns of **4** and **5** containing 0.1% of the X-77 spreader were sprayed on branches from the top to about 50 cm down, where phloem tissues were cut to prevent translocation of the chemicals to other branches.

Analyses of labelled metabolites. Labelled metabolites were extracted from leaves as described previously [5]. A portion of the extract, which contained about 2500 cpm, was spotted on a thin layer plate and developed with solvent (2). The chromatogram was analyzed with a radiochromatogram scanner.

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ANTIMICROBIAL COMPOUNDS OF THE MARINE RED ALGA *MARGINISPORUM ABERRANS*

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Key Word Index—*Marginisporum aberrans*; Corallinaceae; red alga; *p*-hydroxybenzaldehyde; dichloroacetamide; 3,5-dinitroguaiacol; antimicrobial activity.

The fresh alga (15 kg) was washed with water, air-dried and extracted with MeOH. The extract showed marked antimicrobial activity against *Bacillus subtilis*. After removing the solvent, the residue was separated into *n*-hexane- and Et_2O-soluble neutral, EtOAc-soluble acidic and Et_2O-soluble basic fractions. Si gel chromatography of the Et_2O-soluble neutral fraction (*n*-hexane–EtOAc, 3:1) gave an active compound (8 mg), mp 113–114°, which was found to be identical with *p*-hydroxybenzaldehyde by comparison (mmp, IR, NMR, MS) with authentic sample. Recently, Fenical and McConnell [1] also isolated *p*-hydroxybenzaldehyde as an antimicrobial component of the red alga *Dasya pedicellata* var. *stanfordiana*.

Continued elution gave dichloroacetamide (15 mg), mp 98–99°, which was identical in all respects (IR, NMR, MS) to an authentic sample. In 1967, Khaskin and coworkers [2] synthesized a variety of amides and measured their antimicrobial activities, and they found dichloroacetamide showing moderate activity against *Botorytis cinerea* and *Alternaria radicina*.

Si gel chromatography of the EtOAc-soluble acidic fraction using $CHCl_3$–EtOAc (5:1) and crystallization from *n*-hexane–Et_2O afforded 3,5-dinitroguaiacol (10 mg), mp 124–125°; $\nu_{max}^{CCl_4}$ cm^{-1}: 3500, 1625, 1570, 1550 and 1355; λ_{max}^{MeOH} nm (log ε): 213(4.33), 266(3.98), 332(3.81) and 410(3.04); $\delta_{TMS}^{CDCl_3}$ (100 MHz): 4.06(3H, s), 8.02(1H, d J = 3 Hz), 8.74(1H, d, J = 3 Hz) and 11.22(1H, s).

Direct comparison (mmp, IR, NMR) with an authentic sample confirmed the identity. 3,5-Dinitroguaiacol showed marked antimicrobial activity against *B. subtilis*.

This is the first report of the isolation of dichloroacetamide and 3,5-dinitroguaiacol as natural products.

EXPERIMENTAL

Plant. Marginisporum aberrans (Yendo) Johansen et Chihara (Corallinaceae). The identification was carried out by Dr. T. Masaki, Faculty of Fisheries, Hokkaido University, Hakodate, Japan, *Source*. Cape Omaezaki, Shizuoka Prefecture, Japan.

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QUINONES FROM *PEREZIA RUNCINATA*

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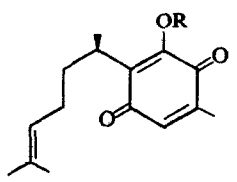
Key Word Index—*Perezia runcinata*; Compositae; roots; hydroxyperezone monoisovaleryl esters; isovalerylperezone; benzoquinones.

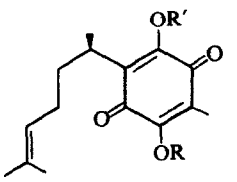
The hexane extract of 24 g of the roots of *Perezia runcinata* yielded 600 mg of a red oil which by PMR analysis showed the presence of perezone type signals, in addition to other peaks. The oil was separated by chromatography into perezone isovalerate (or *O*-isovalerylperezone) and the two hydroxyperezone monoisovalerates which were identified spectroscopically and by hydrolysis which gave the respective parent quinones and isovaleric acid.

Definitive proof of the structure of isovalerylperezone (**1b**) was obtained by alkaline hydrolysis which afforded perezone (**1a**) identified by standard procedures with an authentic sample [1] and isovaleric acid, characterized by its PMR spectrum and comparison of the corresponding anilide derivative with a sample prepared specifically [2].

Chemical confirmation of the hydroxyperezone esters was also obtained by alkaline treatment, which yielded hydroxyperezone and isovaleric acid, characterized as the anilide [2]. Thus only the position of the ester has to be defined in order to establish the complete structures.



1a: R = H
b: R = i-Val



2a: R = H, R' = i-Val
2b: R = Me, R' = i-Val
3a: R = i-Val, R' = H
3b: R = i-Val, R' = Me

In earlier work [3] we were able to define the esterifying position of a monoangeloyl hydroxyperezone by methylation of the free alcohol group, removal of the ester group and comparison of the product with a sample synthesized from perezone (**1a**). Therefore a portion of the red oil was esterified with Me_2SO_4, yielding a mixture of the two isomers **2b** and **3b** in almost equimolecular proportion, since in the PMR spectrum the hydroxyl signal originally at 7.04 ppm disappeared, two methoxyl singlets at 3.98 and at 4.02 ppm appeared and two quinonoid methyl signals at 1.88 and 1.93 ppm are now seen, instead of one signal at 1.91 ppm present before methylation.

The structural assignment of **2b** as the less polar constituent and of **3b** as the more polar one was done after detailed PMR considerations. Comparison of the chemical shifts of the quinonoid methyl group in 3-hydroxythymoquinone [4] with its corresponding methyl ether and in 6-hydroxythymoquinone also with the derived ether, reveals that the chemical shift difference for the *C*-methyl group associated with the methoxylation process is greater in the 3-substituted compounds than in the 6-substituted series, since in the first case the *O*-methyl group is introduced adjacent to the existing quinonoid methyl group. Similar differences are observed up on comparison of the spectrum of the mixture **2a** and **3a** with the spectra of the *O*-methylated derivatives **2b** and **3b**. Furthermore, treatment of this derivative with $Eu(DPM)_3$ shift reagent, revealed that greater shifts are induced to both the *C*-methyl and *O*-methyl groups of **3b** than to the same groups in **2b**. This is in agreement with the structural assignment of the isomers, since on one hand there appears to be no association at the ester carbonyls, as deduced from the chemical shifts of the