The tetrachloromethane soln was concd *in vacuo* to give a brown oil which was separated by PLC (Si gel, $CHCl_3$ as eluent). Three fractions contained brominated compounds and they were characterized as follows: R_f = 0.12: 0.04 g of colourless crystals which showed MS and NMR identical to the values found for **4**. The total yield of **4** was 54%.

R_f = 0.26: 0.04 g (9%) of *5-bromo-4-methoxy-2,5-dihydrofuran-2-one* (*5-bromo-4-O-methyltetronic acid*) (**5**) as a brown oil. NMR: Table 1. MS *m/e* (rel. int.): 194 (3), 192 (3), 113 (100); 85 (21), 69 (25). High resolution MS measurement: 191.94407 (calcd for $C_5H_5O_3$ ^{79}Br: 191.94219).

R_f = 0.43: 0.03 g (5%) of *3,5-dibromo-4-methoxy-2,5-dihydrofuran-2-one* (*3,5-dibromo-4-O-methyltetronic acid*) (**6**) as a brown oil. NMR: Table 1. MS *m/e* (rel. int.): 274 (10), 272 (23), 270 (12), 193 (100), 191 (100), 155 (12), 153 (12), 122 (13), 120 (13), 83 (15), 59 (35). High resolution MS measurement: 269.85658 (calcd for $C_5H_4O_3^{79}Br_2$: 269.85270).

5-Hydroxy-4-methoxy-2,5-dihydrofuran-2-one(*5-hydroxy-4-O-methyltetronic acid*) (**2**). The crude compound (**5**) (0.04 g, 0.002 mol) was treated in 2 ml H_2O with Ag_2O (0.025 g, 0.001 mol) for 20 min. The formed AgBr was allowed to deposit, the aq. phase was isolated, and the AgBr was thoroughly washed with H_2O. The combined aq. phases were evapd to dryness to give 0.02 g colourless crystals. Recrystallization from Et_2O afforded crystals 0.01 g (38%) of mp 137–138.5°. NMR: Table 1. MS *m/e* (rel. int.): 130 (31), 85 (18), 84 (40), 69 (100). IR identical to that given for authentic narthogenin.

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STRUCTURE OF LEPIOCHLORIN, AN ANTIBIOTIC METABOLITE OF A FUNGUS CULTIVATED BY ANTS

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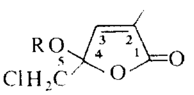
Key Word Index—*Lepiota* species; Basidiomycetes; *Cyphomyrmex costatus*; leaf-cutting ants; Attines; gardening ants; fungal metabolites; lepiochlorin.

A new antibacterial lactol, lepiochlorin, was isolated from liquid cultures of a *Lepiota* species [1], a fungus cultivated by the gardening ants. On the basis of the data presented here, we assign structure **1** for lepiochlorin.

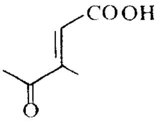
Lepiochlorin, mp 68–70°, did not show a molecular ion in the high resolution EI-MS. It showed intense peaks at *m/e* 113.0228 (M-CH_2Cl; calc. for $C_5H_5O_3$, 113.0238), 85.0303 (113 − CO; calc. for $C_4H_5O_2$, 85.0292). It also showed a weak peak at *m/e* 145.0063 (M − OH; calc. for $C_6H_6ClO_2$, 145.0056). To obtain the correct molecular weight, the chemical ionization spectrum was taken, which showed the highest mass peak at *m/e* 163 (M + H) and the corresponding Cl − 37 isotope peak at *m/e* 165. The high resolution data combined with the chemical ionization results established the molecular formula of lepiochlorin as $C_6H_7ClO_3$. It showed strong end absorption, but no maximum, in the UV spectrum and had ν_{max} at 3250, 1754 and 1670 cm^{-1}. The ^1H NMR spectrum showed a doublet at δ 1.95 (3H, J = 1.5 Hz) for the methyl, a singlet at 3.75 (2H) for the methylene, a broad band at 5.1 (1H) for the hydroxyl, and a quartet at 6.95 (1H, J = 1.5 Hz) for the olefinic proton. The ^{13}C NMR spectrum showed signals at δ 10.13 (CH_3), 46.87 (C-5), 103.69 (C-4), 134.88 (C-2), 145.26 (C-3) and 172.24 (C-1). Thus, the spectral data of lepiochlorin are consistent only with the δ lactol structure **1**.

Lepiochlorin formed a monoacetate (**2**) which showed ^1H NMR signals at δ 1.98 (3H, *d*, J = 1.5 Hz), 2.1 (3H, *s*), 3.8 and 4.11 (2H, AB *q*, J = 11 Hz) for the two methylene protons, and 7.16 (1H, *q*, J = 1.5 Hz) for the olefinic proton. The CI-MS gave twin peaks at *m/e* 205 and 207.

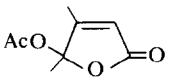
The alternate structure with methyl on C-3 was eliminated by comparison of the ^1H NMR data of **3** and **1** with those of compounds **4** and **5**, respectively. Compound **4** was prepared from *trans*-3-methyl-4-oxo-2-pentenoic acid. In compound **4** the olefinic proton appeared at δ 5.9 and in compound **5** at 7.19 [2]. The chemical



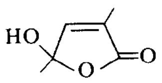
1 R = H
2 R = Ac



3



4



5

shifts of the olefinic protons in **1** (δ 6.95) and **2** (δ 7.16) are comparable to that of compound **5**. In lepiochlorin spectral data does not indicate the presence of any open keto-acid form; however, as has been demonstrated in the case of **5** and similar compounds [2] there may be a tautomeric equilibrium with the cyclic form predominating. This would account for the absence of optical activity in lepiochlorin.

EXPERIMENTAL

CI-MS were determined on a Finnigan MS-3300, High resolution MS were determined at the MS facility at Cornell University. ^{1}H NMR and ^{13}C NMR spectra were determined in CDCl$_3$ soln with TMS as internal standard. The UV spectrum was in EtOH soln.

Cultures. Ant fungus No. W-17 isolated by Weber [3] from ant colony 4471, *Cyphomyrmex costatus* Mann, produced basidiocarps in our laboratory and was identified as a *Lepiota* sp. [1]. The fungus was grown in Fernbach flasks, each containing glass wool and 1 l. of a modified Fries medium in which a vitamin mixture [4] replaced thiamin. Agar discs (5 mm dia) from 6-week-old cultures grown on potato dextrose agar medium, were used as inoculum. Cultures were maintained at 20° in incandescent light (8–10 foot candles) and harvested after 73 days.

Isolation of lepiochlorin. An EtOAc extract of the culture liquids was taken to dryness *in vacuo* and chromatographed on a Si gel column. A fatty material was eluted with petrol and lepiochlorin was eluted with CHCl$_3$. On further purification by PLC (Si gel; CHCl$_3$) lepiochlorin crystallized as needles, mp 68–70° (petrol); yield 8 mg/l.

Acetylation of **1**. Lepiochlorin (30 mg) was mixed with Ac$_2$O (0.5 ml) and Py (0.2 ml) and was kept at room temp. overnight. The mixture was then poured into H$_2$O and was extracted with Et$_2$O. The extract was washed with H$_2$O and the solvent removed to obtain the monoacetate as a colorless oil.

Preparation of the lactone **4**. The commercially available (Aldrich) *trans* 3-methyl-4-oxo-2-pentenoic acid (**3**) (100 mg) was refluxed with Ac$_2$O (5 ml) containing dry NaAc (200 mg) for 2 hr. The mixture was allowed to cool to room temp. and was left overnight, and then was poured into H$_2$O. Extraction with Et$_2$O and removal of the solvents from the extract gave **4** as an oil. It had ν_{max} 1770–1740 (br) and 1665 cm^{-1}; ^{1}H NMR signals at δ 1.7 (3H, *s*) for the C-4 methyl, 2.01 (3H, *d*, J = 1.5 Hz) for the allylic methyl, 2.06 (3H, *s*) for the acetyl and 5.9 (1H, *q*, J = 1.5 Hz) for the olefinic proton. CI-MS showed the highest mass peak at *m/e* 171 (M + H).

Antibiotic activity of **1**. In serial dilution tests, lepiochlorin was active against *Staphylococcus aureus* at a concentration of 60 ppm.

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