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CHLOROMYCORRHIZINOL A, A FUROCHROMAN FROM AN ISOLATE OF THE ROOTS OF *MONOTROPA HYPOPITYS*

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Key Word Index—*Monotropa hypopitys*; Ericaceae; *Fomes annosus*; Polyporaceae; furochroman; chloromycorrhizinol A; anodic oxidation; structural determination.

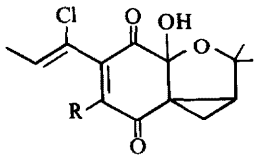
Abstract—A new furochroman, chloromycorrhizinol A, has been isolated together with mycorrhizin A and chloromycorrhizin A from an isolate (D 37) from the roots of *Monotropa hypopitys*. Its structure, determined by spectroscopic and chemical methods, is 3,4-dichloro-5,7-dihydroxy-2,8,8-trimethyl-furo-[3,2-h]-chroman.

INTRODUCTION

The structures of two new antifungal compounds, mycorrhizin A (**1**) and chloromycorrhizin A (**2**), obtained from an isolate (D 37) from the roots of *Monotropa hypopitys* L. were reported recently [1]. Further investigations on the ethyl acetate extract of the culture medium of D 37 have now led to the isolation of a phenolic isomer of chloromycorrhizin A which lacks antifungal activity.

RESULTS AND DISCUSSION

The new substance, named chloromycorrhizinol A (**3**) was isolated in small quantities as a white crystalline compound by column chromatography. The pure compound had mp 201–203°. High resolution MS determination gave the molecular formula $C_{14}H_{14}O_4Cl_2$. Chloromycorrhizinol A is sensitive to air oxidation.



(**1**) R = H
(**2**) R = Cl

Scheme 1

The presence in **3** of a substituted benzofuran ring system was indicated by the appearance of UV maxima at 302 (2600), 293 (2900), 266 (13900), 257 (13600), 248 (9600) and 219 nm (29900) [2]. The ^{13}C NMR spectrum exhibited fourteen carbon signals, two of which overlapped at δ 107.86 ppm. The IR and PMR spectra indicated the presence of: three tertiary methyl groups, two in geminal positions (δ 1.30 and 1.38) and one on an aromatic ring (δ 2.36); two benzylic methylene protons (δ 2.68 and 3.02) and one methine proton (δ 3.80) giving ABX type signals; and two hydroxyl protons. There was no indication of the presence of carbonyl groups. Consequently, it was concluded that the remaining two oxygen atoms were present in ether linkages, and that the compound was tricyclic.

On catalytic hydrogenation **3** gave a chlorine-free derivative $C_{14}H_{18}O_4$ (**5**), the spectral data for which suggested a 2,3-dihydrobenzofuran substructure. In the PMR spectrum signals at δ 2.68 and 3.19 were assigned to benzylic methylene protons on the dihydrofuran ring. These protons had a geminal coupling constant of 15.4 Hz and furthermore showed a long-range coupling (J = 0.9) [3] to an aromatic proton and identical vicinal couplings (J = 8.6) to an adjacent methine proton at δ 4.75. This data allowed the assignment of the partial structures shown below to chloromycorrhizinol A.

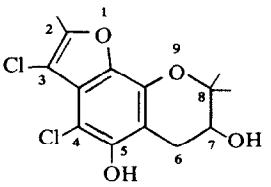
Scheme 2

Reduction of **3** with magnesium in isopropanol gave a compound, **4**, still containing one chlorine atom and retaining the benzofuran chromophore. The new aromatic proton was uncoupled, confirming the allocation of the reduced chlorine atom to the benzene ring.

In order to establish the tricyclic ring system, the chemical shift of the methine proton at δ 3.80 in **3** was compared with the corresponding proton in decurcinol (**8**) (δ 3.85) [4], in lomatin (**9**) (δ 3.87) [4] and in stenocarpoquinone-A (**10**) (δ 3.97) [6]. These shifts are quite different from those in columbianetin (**11**) (δ 4.77) [4] and in marinesin (**12**) (δ 4.73) [4]. This strongly suggested that the second heterocyclic ring in chloromycorrhizinol A (**3**) was six- rather than five-membered.

The MS of compounds **3**–**6** could be interpreted as is shown in Scheme 5 for the title compound. The retro-Diels–Alder reaction thus not only established the dihydropyran ring but also confirmed the location of the hydroxyl group. Analogous fragmentations of decurcinol (**8**) [4], lomatin (**9**) [5] and stenocarpoquinone-A (**10**) [6] have been reported.

The location of the phenolic hydroxyl group was



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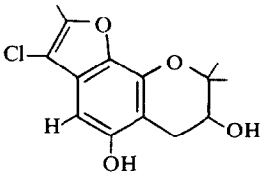

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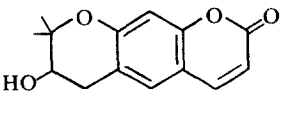

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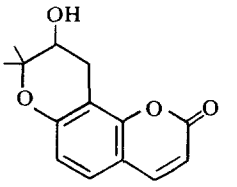

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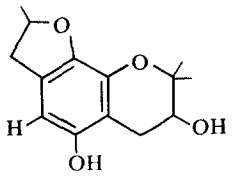

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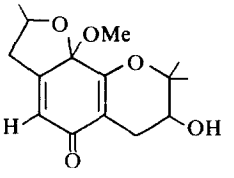

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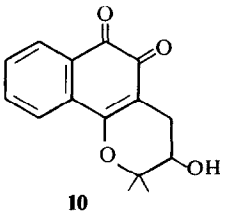

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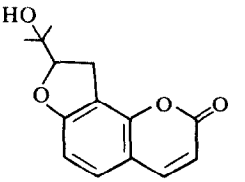

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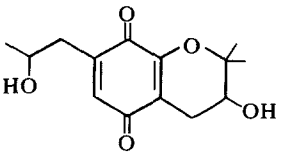

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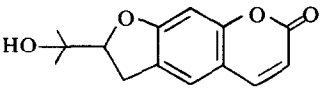

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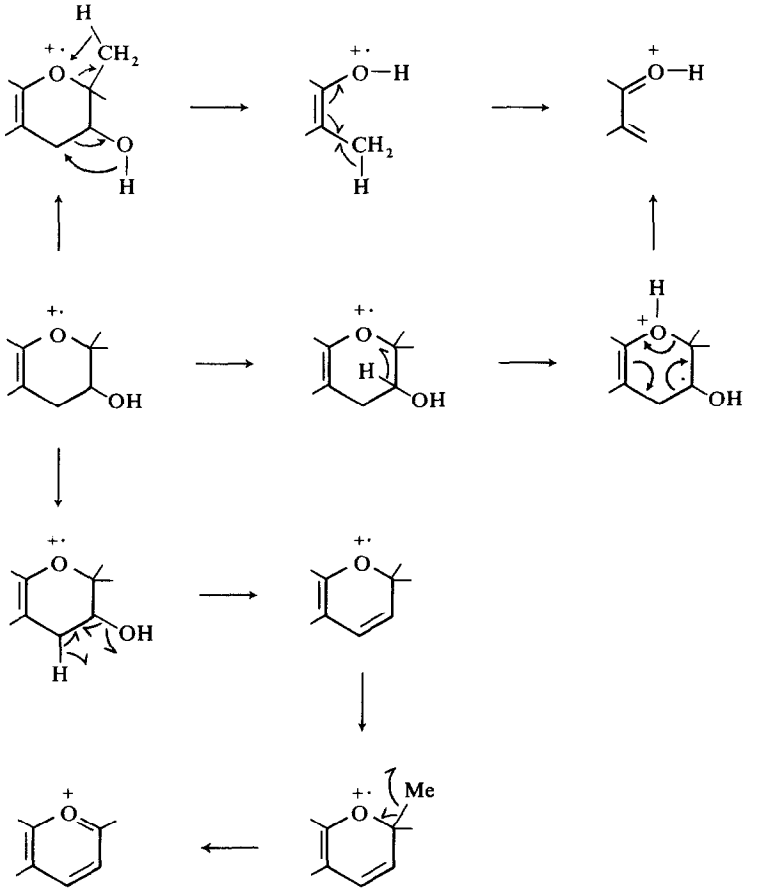
Scheme 3

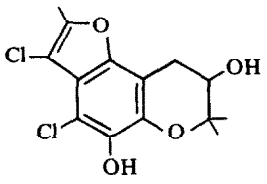
Scheme 4



Scheme 5. MS fragmentation of chloromycorrhizinol A (**3**) and related compounds (**4–6**).

corroborated by an electrochemical oxidation experiment. At a platinum anode in acetonitrile–methanol (1:1) the hydrogenation product **5** was converted to the corresponding dialkoxycyclohexadienone **6** (UV and MS). Hydrolysis yielded the quinone **7**, which did not react with sodium periodate. The dihydropyran ring was thus retained intact during the oxidation. The quinone **7** showed the spectroscopic properties of a 2-alkoxy-3,6-dialkyl-1,4-benzoquinone [7].

These findings leave the two alternative structures **3** and **13** for chloromycorrhizinol A. The co-occurrence of mycorrhizin A (**1**) and chloromycorrhizin A (**2**) in the medium of D 37 gives strong support to **3** rather than structure **13** for chloromycorrhizinol A.



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Scheme 6

EXPERIMENTAL

The reported mps were determined on a Kofler micro hot stage and are uncorr. PMR spectra were recorded at 100 MHz in CD_3OD with TMS as int. stand. if not otherwise stated. The ^{13}C-NMR data were determined with a Jeol FX 60 instrument. Merck Si gel 60 (F 254) aluminium sheets were used for TLC analysis.

Isolation of chloromycorrhizinol A (**3**). The sterile mycelium D 37 was cultivated with stirring and aeration in a modified Norkrans soln [1]. After 11 days, the medium (25 l.) was filtered and extracted with EtOAc. The residue (2.4 g) obtained on evapn of the solvent was fractionated by CC (180 g Si gel, 0.063–0.200 mm, deactivated with 20% H_2O) using EtOAc–hexane (3:7) as eluent. A chromatographically homogenous fraction with R_f 0.27 (TLC, EtOAc–hexane, 3:7), which lacked activity against *Fomes annosus* (Fr.) Cke., afforded a phenol, chloromycorrhizinol A, as white crystals from $CHCl_3$–cyclohexane.

Chloromycorrhizinol A (**3**). White crystals mp 201–203°; $[\alpha]_D^{25}$ +30.9 (*c* 1.7, EtOH). Found: *m/e* 316.0284. Calc. for $C_{14}H_{14}O_4Cl_2$: *m/e* 316.0270. UV λ_{max}^{EtOH} nm: 302 (2600, inflexion), 293 (2900), 266 (13900), 257 (13600), 248 (9600, inflexion) and 219 (29900); λ_{min}^{EtOH} nm: 278 (2300), 261 (12400) and 242 (8000); IR ν_{max}^{KBr} cm^{-1}: 3590, 3340, 1635, 1619, 1600 and 1495; ^1H NMR: δ 1.30 (3H, *s*), 1.38 (3H, *s*), 2.36 (3H, *s*), 3.80 (1H, *dd*, *J* = 5.6 and 7.3), 2.68 (1H, *dd*, *J* = 7.3 and 17.3), 3.02 (1H, *dd*, *J* = 5.6 and 17.3) and 4.82 (2H, *s*); ^{13}C-NMR: 11.36 (Me), 20.94 (Me), 25.49 (Me), 28.08 (CH_2), 69.96 (CH), 78.73 (C) and aromatic carbons at 101.3, 107.86 (2C), 122.88, 138.22, 138.71, 148.53 and 152.02; MS (probe) 70 eV *m/e* (rel. int.): 318 [M^+ + 2] (25), 316 [M^+] (38), 298 (2), 283 (9), 246 (38), 245 (100) and 71 (8). (Found: C, 53.1; H, 4.4; O, 20.3. Calc. for $C_{14}H_{14}O_4Cl_2$: C, 53.0; H, 4.4; O, 20.2%).

Reduction of **3** *with Mg/i-PrOH.* Chloromycorrhizinol A (**3**) (39.7 mg) in *iso*-PrOH (1 ml) was added dropwise to Mg (25 mg) and a crystal of I_2 in decalin (5 ml). The soln was kept at 150°. After 8 hr the soln was filtered and acidified with 0.1 M HCl. The aq. layer was extracted with Et_2O. The organic layer was washed with H_2O and then dried ($MgSO_4$). Mycorrhizinol A (**4**) (35.4 mg, 96%) was isolated by Si gel chromatography using Et_2O–hexane as eluent. R_f = 0.32 (Et_2O). Found: *m/e* 282.0659. Calc. for $C_{14}H_{15}O_4Cl$: *m/e* 282.0659. $[\alpha]_D^{22}$ +20.5 (*c* 1.48, EtOH). UV λ_{max}^{EtOH} nm: 298 (1735, inflexion), 289 (1925), 262 (7200, inflexion), 254 (8100), 245 (6250, inflexion) and 216 (21800), λ_{min}^{EtOH} nm: 275 (1400) and 237 (5000); IR ν_{max}^{KBr} cm^{-1}: 3400, 1641, 1620, 1600 and 1495; ^1H NMR: δ 1.30 (3H, *s*) 1.40 (3H, *s*), 2.36 (3H, *s*), 3.76 (1H, *dd* *J* = 5.6 and 7.3), 2.65 (1H, *dd*, *J* = 7.3 and 17.3), 3.00 (1H, *dd*, *J* = 5.6 and 17.3), 6.4 (1H, *s*) and 4.79 (2H, *s*); MS (probe) 70 eV *m/e* (rel. int.): 284 [M^+ + 2] (10), 282 [M^+] (28), 264 (1), 249 (6), 212 (23), 211 (100) and 71 (3).

Catalytic hydrogenation of **3**. Chloromycorrhizinol A (**3**) (83.9 mg) in absolute EtOH (5 ml) and 5% Pd/C (30 mg) was shaken for 10 hr over H_2 (3 atm). After filtration and removal of the solvent in vacuo at room temp., the crude product was subjected to PLC using Et_2O–hexane (1:1) giving **5** (60.0 mg, 91%). R_f = 0.23 (Et_2O). Mp 178–181°, $[\alpha]_D^{22}$ +30.0 (*c* 0.9, EtOH). Found: *m/e* 250.1249. Calc. for $C_{14}H_{18}O_4$: *m/e* 250.1205. UV λ_{max}^{EtOH} nm: 294 (3600), 227 (8100, inflexion) and <220, λ_{max}^{EtOH} nm: 260 (750); IR: ν_{max}^{Film} cm^{-1}: 3400, 1635, 1609 and 1595; ^1H NMR (CD_3COCD_3): δ 1.23 (3H, *s*), 1.34 (3H, *s*), 1.39 (3H, *d*, *J* = 6.4), 4.75 (1H, *m*, *J* = 6.4, 8.6 and 8.6), 2.68 (1H, *m*, *J* = 8.6, 15.4 and 0.9), 3.19 (1H, *m*, *J* = 8.6, 15.4 and 0.9), 6.25 (1H, *t*, *J* = 0.9), 3.73 (1H, *dd*, *J* = 5.6 and 7.3), 2.53 (1H, *dd*, *J* = 7.3 and 17.3), δ 2.88 (1H, *dd*, *J* = 5.6 and 17.3) and 3.2 (2H, *s*); MS (probe) 70 eV *m/e* (rel. int.): 250 [M^+] (82), 232 (4), 217 (47), 179 (100) and 71 (8).

Anodic oxidation of **5**. Phenol **5** (10.5 mg), $LiClO_4$ (300 mg) and $NaHCO_3$ (100 mg) were dissolved in a mixture of dry MeOH (20 ml) and dry CH_3CN (20 ml). The electrode arrangement was a cylindrical platinum foil anode with a tungsten wire cathode positioned on the centre line of the anode together with a SCE (LiCl) reference (Radiometer model K901). The electrolysis was carried out at constant potential (0.75 V) with stirring and at room temp. in a 100 ml beaker. After electrolysis the electrolyte was poured into cold 0.1 M HCl and shaken vigorously for a few min. EtOAc was added and the organic phase was washed twice with a phosphate buffer (pH 6–7), twice with H_2O and then dried ($MgSO_4$). Evapn of the solvent gave a crude yellow oily product which on purification by PLC using Et_2O as eluent gave the quinone **7** (6.6 mg, 59%); R_f = 0.34 in Et_2O–EtOAc (8:2). Found: *m/e* 266.1168. Calc. for $C_{14}H_{18}O_5$: *m/e* 266.1154. UV λ_{max}^{EtOH} nm: 393 (940), 265 (11800) and <220, λ_{min}^{EtOH} nm: 226 (4300). IR ν_{max}^{Film} cm^{-1}: 3400, 1675, 1646 and 1602.

A separate analytical expt gave the intermediate dialkoxycyclohexadiene **6** UV λ_{max}^{EtOH} nm: 307 (3800) and 235 (10100), λ_{min}^{EtOH} nm: (1500); MS (probe) 70 eV *m/e* 280 [M^+], 249 [$M^+ - 31$], 231 [$M^+ - 31 - 18$] and 179 [$M^+ - 31 - 70$].

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