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MYCORRHIZIN A AND CHLOROMYCORRHIZIN A, TWO ANTIBIOTICS FROM A MYCORRHIZAL FUNGUS OF *MONOTROPA HYPOPITYS* L.

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Abstract—Two new compounds, mycorrhizin A and chloromycorrhizin A, have been isolated from a mycorrhizal fungus of *Monotropa hypopitys* L. and their structures established as 1(*S*),6(*S*),9(*R*) - 6 - hydroxy - 8,8 - dimethyl - 4 - (1 - chloro - prop - 1 - enyl) - tricyclo[4.4.0.0^{1,9}] - 7 - oxa - dec - 3 - ene - 2,5 - dione and its 3-chloro derivative. Several derivatives were prepared and characterized.

A sterile mycelium (D 37) of an ectendomycorrhizal fungus found on the roots of *Monotropa hypopitys* L. has been shown by cross plating to be a strong antagonist of the root rot fungus *Fomes annosus* (Fr.) Cke., which causes large economic losses in Swedish forestry.[1]

An ethyl acetate extract of a filtered culture of D 37 gives three antifungal compounds. Structural investigation on two of these, mycorrhizin A (m.p. 163–165°, $C_{14}H_{15}O_4Cl$) and chloromycorrhizin A (m.p. 122–123°, $C_{14}H_{14}O_4Cl_2$) in combination with an independent X-ray structure determination[2] has now shown that these have the structures **1** and **2** respectively.

Mycorrhizin A **1** and chloromycorrhizin A **2** show (M + 2) peaks in their mass spectra. When deuterium oxide was introduced in the mass spectrometer before introduction of the sample, (M + 3) and (M + 4) peaks appeared. This phenomenon has been observed earlier with certain quinones and is due to reduction of the quinones by moisture in the mass spectrometer.[3,4] A major fragment arises by loss of water from the molecular ion, *m/e* 282 (316).† Other fragments consistent with structures **1** and **2** arise from further loss of one Cl atom and two CO fragments from *m/e* 264 (298).

Compounds **1** and **2** are very labile in basic solution but rather stable in acid media (no change observed in 0.1 M HCl at 60° for 12 hr). On treatment with an alcohol (R–OH, R = Me, Et, n-Pr) and boron trifluoride-etherate, compounds **1** and **2** give the base stable non-hydroxylic products, **3** and **4**, which were shown by ^{13}C NMR to be ketals.[5] The ketals are converted back to **1** and **2**, respectively, on acid hydrolysis; they do not react with sodium periodate.

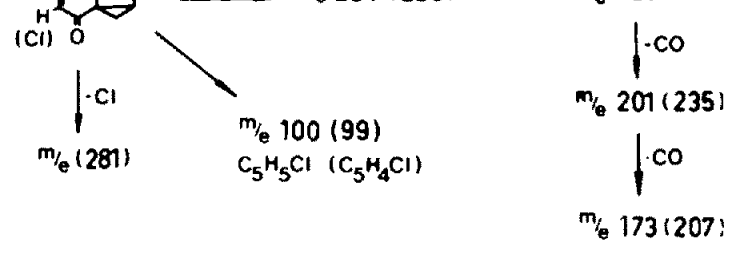
†The data in parentheses are for chloromycorrhizin A.

The proton NMR spectrum of **1** shows a gem-dimethyl group (6H, singlets, δ 1.23 and δ 1.33), a methyl group (3H, doublet of doublets, δ 2.05, $J = 6.8$ Hz and $J = 0.6$ Hz) coupled to two olefinic protons at δ 7.01 ($J = 6.8$ Hz) and δ 7.10 ($J = 0.6$ Hz). The magnitude of the coupling constant $J = 6.8$ Hz is consistent with the presence of an ethylidene group. This assignment is corroborated by ^{13}C NMR data. The olefinic protons show long range coupling ($J = 0.6$ Hz). There is one hydroxyl proton singlet. The signals of the remaining three protons occur as AMX multiplets at δ 1.62–δ 2.25. The NMR spectra (^1H and ^{13}C) of **2** are very similar to the spectra of **1**; they show the ethylidene group signals and are consistent with the substitution of chlorine for a vinylic hydrogen in **2**.

The IR spectra of **1** and **2** show OH absorption, two carbonyl and two olefinic bands and the two bands at about 1380 cm^{-1} characteristic of a gem-dimethyl group. As ^{13}C NMR data (*vide infra*) show the presence of two carbonyl and four olefinic carbons, the compounds must have tricyclic structures containing an ether oxygen.

The UV spectra (λ_{max} 229 and 298 nm for **1** and λ_{max} 246 and 305 nm for **2**) are indicative of a cross-conjugated enone system.

Reduction of the carbonyl groups in **3** with diisobutylaluminium hydride (DIBAL) yields a diol with a vicinal coupling ($J = 6.4$ Hz) between the olefinic proton at δ 6.80 and a carbinolic methine proton. The couplings of the carbinolic protons to the respective hydroxylic protons have been positively identified by exchanging the labile protons with D_2O. The data given so far strongly indicate the presence of the fragment $CH_3CH{=}C(-)-C(=)CH-CO-$ in **1** and **3**. The corresponding part of **2** and **4** should then be $CH_3CH{=}C(-)-C(=)CCl-CO-$. The proton at δ 6.80 of

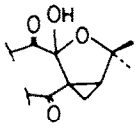


compound **1** shows additional long range coupling to the protons in the ethylidene group. With the structure of **1** at hand an allylic coupling of $J = 1.0$ Hz to the second carbinolic proton could be established (see Experimental).

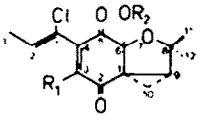
The ^{13}C NMR spectra (see Table 1) of **1** and **2** give evidence for the presence in both compounds of a $-CH_2-$ group and a $-CH-$ group, which give rise to the AMX multiplets in ^1H NMR. This part of the spectrum was clarified by tickling experiments[4] and the protons were found to belong to a 3-membered ring system with $J_{cis} = +8.2$ Hz and $J_{trans} = +6.0$ Hz for the vicinal couplings and $J_{gem} = -4.7$ Hz for the geminal coupling in **2**. Alkyl-substituted cyclopropanes, which occur frequently in natural products, usually have a geminal coupling constant of $J = -4$ to -5.5 Hz.[5]

Chloromycorrhizin A **2** reacts cleanly with one equivalent of sodium periodate to give a compound $C_{14}H_{14}O_5Cl_2$ **5** (the numbering of **1** and **2** is retained for **5**) showing that the hemiketalic hydroxyl group is vicinal to one of the carbonyl groups. The IR spectrum of **5** shows no ketonic bands but has a broad high-frequency carbonyl adsorption consistent with a 5-membered lactone system. From the spectra of **5** and its mode of formation, it must have a lactone–lactol structure. The carboxyl group formed by oxidation of the carbonyl group cyclises immediately with the second carbonyl group.

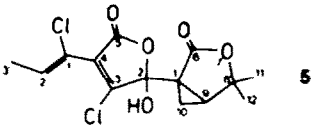
The ^{13}C NMR data (see Table 1) and ^1H NMR data for **3a** require the isopropylidene group to be connected to the fourth oxygen atom and to the $-CH-$ group of the 3-membered ring. A further fragment can then be deduced as:



Combination of all available chemical and spectroscopic evidence however still allowed a number of alternative structures, none of which could be entirely eliminated. Moreover, the absolute configuration remained unspecified. An independent X-ray analysis of chloromycorrhizin A **2** was therefore undertaken.[3] This gave the final stereostructure as **2**. The chemical and spectroscopic evidence presented above then establishes structure **1** for mycorrhizin A.



1 $R_1 = R_2 = H$
2 $R_1 = Cl$, $R_2 = H$
3 $R_1 = H$, $R_2 = CH_3$
4 $R_1 = Cl$, $R_2 = CH_3$



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From the co-occurrence of **1** and **2** it is to be expected that they will have the same absolute configuration. This is supported by the similarity of the CD curves below 260 nm. The difference between **1** and **2** at 370 nm is presumably due to electronic and conformational effects. Similar effects have been observed in the ORD of testosterone and its 4-halo derivatives.[6]

Because of the potential pharmacological interest and the biological activities of these compounds some derivatives were prepared for investigation of structure-

Table 1. ^{13}C NMR data for mycorrhizin A **1**, chloromycorrhizin A **2** and the periodate oxidation product **5** (chemical shifts in δ (ppm) and coupling constants in Hz; TMS as internal standard; $CDCl_3$ for **1** and **2** and CH_3OD for **5**). The numbering system is shown in the text.

Carbon atom[a]	Mycorrhizin A	Chloromycorrhizin A	5
1	43.0	42.4	33.9
2	192.6*	189.5*	101.9
3	137.1*	143.0*	151.5
4	144.9	142.8*	127.5
5	192.0*	185.0*	166.3
6	101.2	99.6	173.3
8	82.8	82.5	83.0*
9	44.5	46.0 (170)	31.3
10	16.3	15.6 (168)	14.5
11	24.8*	24.7*	23.3*
12	28.9*	29.2*	28.7*
1'	127.1	122.0	120.5
2'	135.4*	130.8 (155)	133.4
3'	14.9	14.4 (125)	14.1

a) Signals marked (*,*,*) may need to be interchanged within the respective column where they appear.

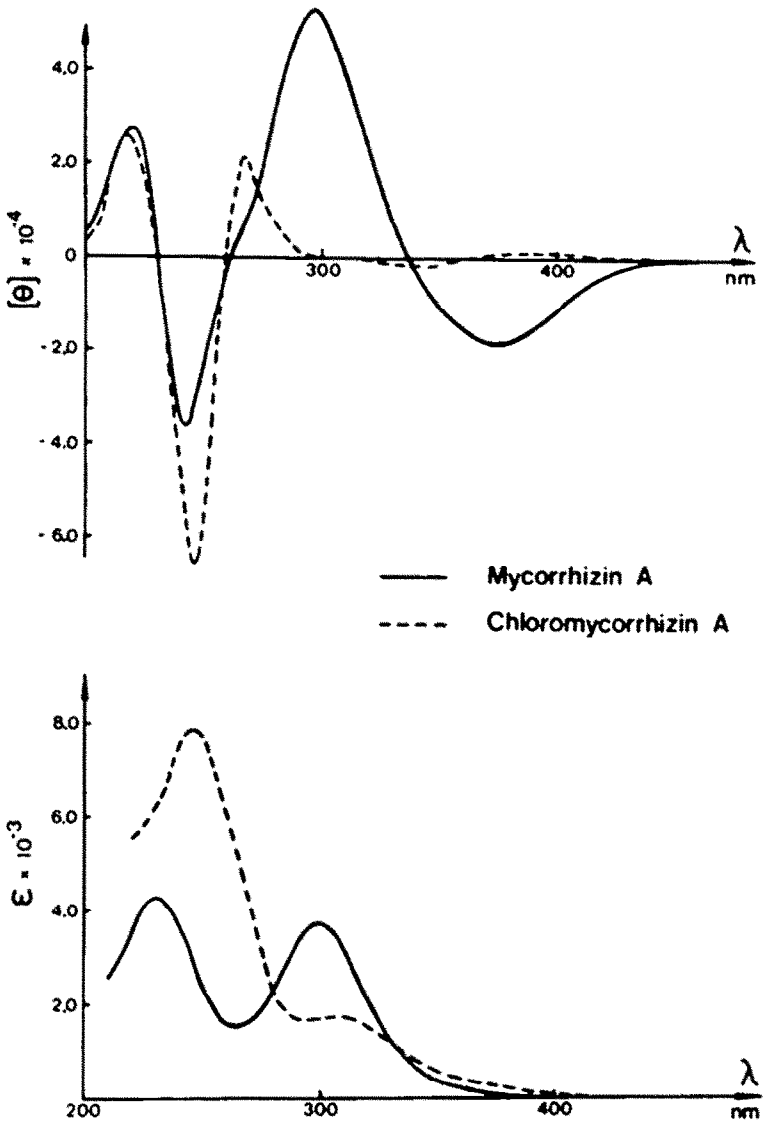
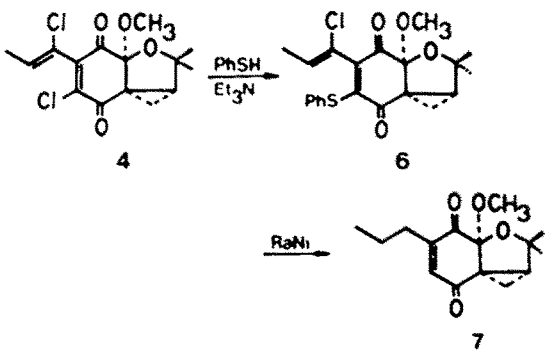


Fig. 1. CD spectra and UV absorption of mycorrhizin A and chloromycorrhizin A in ethanol.

activity relationships. Compound **4** on treatment with one equivalent of thiophenol and triethylamine in toluene at −60° gave a deep-yellow compound **6** in good yield.[9] There was no reaction when only triethylamine was present. MS data showed that a phenylmercapto group has been substituted for chlorine on the 6-membered ring. Desulfurisation of **6** with deactivated Raney nickel gave the dehalogenated product **7** in moderate yield.[10,11]



4 6 7

The results of biological and pharmacological tests will be published elsewhere. Mycorrhizin A and chloromycorrhizin A are both highly active against *Fomes annosus*.

EXPERIMENTAL

M.ps were taken on a micro hot-stage and are uncorrected. Unless otherwise stated, proton NMR spectra were determined on a Jeol MH-100 or a Varian XL-100, with a TMS internal standard. The instrument used for ^{13}C NMR data was a Varian XL-100. Mass spectral analyses were determined on a Varian Mat 311 high resolution spectrometer at the Department of Clinical Chemistry, University Hospital, Lund. IR data were obtained on a Perkin Elmer model 257 grating infrared spectrophotometer. UV spectra were obtained on a Bausch & Lomb Spectronic 505 recording spectrophotometer. Specific rotations were measured on a Perkin Elmer Polarimeter 141 and the CD data was recorded on a Jasco J 41-A spectropolarimeter. Merck silica gel 60 (F 254) aluminium sheets, were used for TLC analyses.

Isolation of the metabolites. The sterile mycelium D 37 was cultivated in stirred and aerated, modified Norkrans solution (50 g glucose, 1 g asparagine, 0.6 g KH_2PO_4, 0.4 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 6.6 mg $ZnSO_4 \cdot 7H_2O$, 5.0 mg $MnSO_4 \cdot 4H_2O$, 1.0 mg $CoCl_2 \cdot 6H_2O$, 70 mg $CaCl_2 \cdot 2H_2O$, 0.1 mg thiamine·HCl,

1.2 mg ferric citrate and 1.2 mg citric acid were dissolved in 1 l distilled water and adjusted to pH 5.8 before autoclaving). After 11 days of growth, the medium (25 l) was filtered and extracted with ethyl acetate. The residue (2.4 g) obtained on evaporation of the solvent was fractionated by column chromatography (180 g, SiO_2, 0.063–0.200 mm, deactivated with 20% water) using ethyl acetate:hexane (3:7) as eluent. The fractions were tested for inhibition of the growth of *Fomes annosus* on agar plates. The active fraction (TLC, ethyl acetate:hexane (3:7), R_f = 0.38 for **1** and R_f = 0.43 for **2**) was subjected to partition chromatography using celite (Hyflo) containing 25% of a mixture of dimethylsulfoxide–water (4:1) as the stationary phase; 15% ethyl acetate in hexane was used as eluent. The separation was excellent but highly dependent on the water content of the stationary phase. Three active compounds were isolated. The two main compounds, **1** (25 mg) and **2** (100 mg),† were recrystallised from a mixture of $CHCl_3$ and cyclohexane.

Mycorrhizin A **1**. Bright yellow crystals m.p. 163–165°; $[\alpha]_D^{25}$ + 33.3° (c 1.89, EtOH). Found: C, 59.3; H, 5.4; O, 22.4; Calc. for $C_{14}H_{14}O_4Cl$: C, 59.5; H, 5.3; O, 22.6%). UV: λ_{max}^{EtOH} 229 (4300) and 298 nm (3800); IR (KBr): ν_{max} 3460 (OH), 1720 (C=O), 1672 (C=O), 1612 (C=C), 1574 (C=C), 1384 and 1376 cm^{-1} (gem-dimethyl); ^{1}H NMR ($CDCl_3$): δ 7.10 (1H, m, J = 0.6 and J = 0.6), δ 7.01 (1 H, doublet of quartets, J = 6.8 and J = 0.6), δ 4.36 (1H, s), δ 2.05 (3H, doublet of doublets, J = 6.8 and J = 0.6), δ 2.25 (1H, doublet of doublets, J = 8.0 and J = 5.9), δ 1.91 (1H, doublet of doublets, J = 4.7 and J = 8.0), δ 1.62 (1H, doublet of doublets, J = 4.7 and J = 5.9), δ 1.33 (3H, s) and δ 1.23 (3H, s). See Table 1 for ^{13}C NMR data; CD (4.2 mg/10 ml, EtOH): $[\theta]_{300}$ = + 5721; $[\theta]_{290}$ = + 27597; $[\theta]_{282}$ = − 36347; $[\theta]_{270}$ = + 53175; $[\theta]_{247}$ = − 18308.

Chloromycorrhizin A **2**. Yellow crystals m.p. 122–123°; $[\alpha]_D^{25}$ − 21.6 (c 11.1, EtOH). Found: C, 53.2; H, 4.6; O, 20.1; Calc. for $C_{14}H_{14}O_4Cl_2$: C, 53.0; H, 4.4; O, 20.2%. UV: λ_{max}^{EtOH} 246 (7900) and 305 nm (1730); IR (KBr): ν_{max} 3400 (OH), 1725 (C=O), 1705 (C=O), 1640 (C=C), 1565 (C=C), 1380 and 1371 cm^{-1} (gem-dimethyl); ^{1}H NMR ($CDCl_3$): δ 5.96 (1H, q, J = 6.7), δ 3.81 (1H, s), δ 1.96 (3H, d, J = 6.7), δ 2.30 (1H, doublet of doublets, J = 6.0 and J = 8.2), δ 2.04 (1H, doublet of doublets, J = −4.7 and J = 8.2), δ 1.75 (1H, doublet of doublets, J = −4.7 and J = 6.0), δ 1.33 (3H, s) and δ 1.23 (3H, s). See Table 1 for ^{13}C NMR data; CD (9.4 mg/10 ml, EtOH): $[\theta]_{300}$ = + 3374; $[\theta]_{277}$ = + 26317; $[\theta]_{247}$ = − 65793; $[\theta]_{236}$ = + 21931; $[\theta]_{224}$ = − 1586, $[\theta]_{193}$ = + 1366.

Ketals **3** *and* **4**. Mycorrhizin A **1** (250 mg, 0.89 mmole) was dissolved in 15 ml dry MeOH containing 15 drops $BF_3{\cdot}Et_2O$. The solution was refluxed for 4 h and the solvent was then evaporated. The residue was chromatographed on a SiO_2 column (deactivated with 20% water) with Et_2O:hexane (1:4) as eluent giving 225 mg (86%) of the yellow compound **3**, m.p. 156.5–157°. Found: *m/e* 264.0552 (M$^+$ −CH_3OH). Calc. for $C_{14}H_{13}O_3Cl$: *m/e* 264.0553. UV: λ_{max}^{EtOH} 227 (5100) and 298 nm (3700); IR (KBr): ν_{max} 1718 (C=O), 1671 (C=O), 1615 (C=C) and 1577 cm^{-1} (C=C): ^{1}H NMR ($CDCl_3$): ^{1}H NMR ($CDCl_3$): δ 6.96 (1H, m, J = 0.6 and J = 0.6), δ 6.87 (1H, doublet of quartets, J = 6.8 and J = 0.6), δ 3.27 (3H, s), δ 2.01 (3H, doublet of doublets, J = 0.6 and J = 6.8), δ 2.22 (1H, doublet of doublets, J = 8.2 and J = 6.2), δ 1.83 (1H, doublet of doublets, J = 8.2 and J = 4.4), δ 1.56 (1H, doublet of doublets, J = 4.4 and J = 6.2), δ 1.34 (3H, s) and δ 1.27 (3H, s).

The ketal **4** was prepared in an analogous procedure giving a yield of 85% of **4**, m.p. 86–87°. Found: *m/e* 298.0143 (M$^+$ −CH_3OH). Calc. for $C_{14}H_{12}O_3Cl_2$: *m/e* 298.0164. UV: λ_{max}^{EtOH} 236 (7100) and 302 nm (2300); IR (KBr): ν_{max} 1720 (C=O), 1708 (C=O), 1678 (C=C) and 1584 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$): ^{1}H NMR ($CDCl_3$): δ 5.98 (1H, q, J = 6.8), δ 3.30 (3H, s), δ 1.96 (3H, d, J = 6.8), δ 2.32 (1H, doublet of doublets, J = 8.0 and J = 6.2), δ 1.96 (1H, doublet of doublets, J = 8.0 and J = 4.0), δ 1.70 (1H, doublet of doublets, J = 4.0 and J = 6.2), δ 1.33 (3H, s) and δ 1.24 (3H, s). ^{13}C NMR ($CDCl_3$): 188.1, 185.4, 144.5 (br, 2 C), 131.1, 122.3, 102.4, 82.8, 52.4, 45.0, 42.2, 28.8, 24.6, 16.6 and 14.1 ppm.

On hydrolysis of the ketals **3** and **4** in aqueous dioxane containing HBF$_4$ the hemiketals **1** and **2**, respectively, were recovered.

Reduction of **3** *with di-isobutylaluminum hydride (DIBAL)*: **3a**. To 180 mg (0.6 mmole) of **3** was added 10 ml dry benzene and the solution was cooled to +5° before adding 1.8 mmole DIBAL in benzene over 15 min. After stirring for 90 min Et_2O (30 ml) was added and the solution was shaken as quickly as possible with cold 0.5 M HCl; the organic phase washed twice with cold water, dried with Na_2SO_4 and evaporated. The residue was chromatographed on a SiO_2 column (deactivated with 20% water) and eluted with diethyl ether:hexane (1:3) giving 23.3 mg (13%) of a pure oily product **3a**. Found: *m/e* 268.0850 (M$^+$ −CH_3OH). Calc. for $C_{14}H_{13}O_3Cl$: *m/e* 268.0866. *UV*: λ_{max}^{EtOH} 238 nm (14400); IR: ν_{max} 3410 (OH, broad), 1660 (C=C) and 1635 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$): δ 6.80 (1H, m, J = 6.4, J = 0.6, J = 0.6 and J = 1.0), δ 6.15 (1H, doublet of quartets, J = 6.6 and J = 0.6), δ 4.52 (1H, doublet of doublets, J = 2.6 and J = 1.0), δ 3.82 (1H, doublet of doublets, J = 10.0 and J = 6.4), δ 3.23 (3H, s) δ 3.02 (1H, d, J = 10.0), δ 2.95 (1H, d, J = 2.6), δ 1.90 (3H, doublet of doublets, J = 6.6 and J = 0.6), δ 1.49 (3H, s), δ 1.32 (3H, s), δ 1.52–2.04 (3H, m).

Oxidation product **5** *(oxidation of* **2** *with sodium periodate)*. Chloromycorrhizin A **2** (940 mg, 3.0 mmole) and $NaIO_4$ (1.60 g) were dissolved in 115 ml MeOH:water (1:1). After 55 h at room temperature the MeOH was evaporated and the aqueous solution was extracted (three times) with Et_2O. After drying over Na_2SO_4 the solvent was evaporated. The residue was chromatographed on a SiO_2 system (gradient elution from 10% to 50% ether in hexane) giving white crystals of **5** (322 mg). With a conversion of 41% the yield of **5** was 80%, m.p. 207–208°. Found: *m/e* 332.0201. Calc. for $C_{14}H_{14}O_4Cl_2$: *m/e* 332.0219. UV: λ_{max}^{EtOH} 217 (8200) and 269 nm (5800); IR (KBr): ν_{max} 3270 (OH, broad), 1760 (C=O), 1755 (C=O), 1680 (C=C) and 1635 cm^{-1} (C=C); ^{1}H NMR (CD_3OD): δ 6.60 (1H, q, J = 6.8), δ 4.64 (1H, s), δ 1.92 (3H, d, J = 6.8), δ 1.52 (3H, s), δ 1.37 (3H, s), δ 2.49 (1H, doublet of doublets, J = 5.0 and J = 8.0), δ 1.74 (1H, doublet of doublets, J = 5.0 and J = 8.0) and δ 1.20 (1H, t, J = 5.0 and J = 5.0).

6 - Methoxy - 8,8 - dimethyl - 3 - phenylmercapto - 4 - (1 - chloro - prop - 1 - enyl) - tricyclo[4.4.0.0^{1,6}] *- 7 - oxa - dec - 3 - ene - 2,5 - dione* **6**. To the ketal **4** (100 mg, 0.30 mmole) in toluene (3 ml), thiophenol (0.30 mmole) and triethylamine (0.30 mmole) were added with cooling (−60°). After 30 min cooling was interrupted and the mixture allowed to reach room temperature. The resulting suspension was filtered ($Et_3NH^+Cl^-$ precipitate) and the filtrate concentrated. The residue was chromatographed on SiO_2 (diethyl ether:hexane (1:9)). The deep yellow oily product **6** (106 mg, 87%) analyzed for m/e 404.0861. Calc. for $C_{21}H_{17}O_4SCl$: *m/e* 404.0880; UV: λ_{max}^{EtOH} 240 (13400, inflection), 256 (10500, inflection) and 360 nm (7100); IR (film); ν_{max} 1702 (C=O), 1690 (C=O), 1652 (C=C) and 1637 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$): δ 7.4 (5H, s), δ 3.40 (3H, s), δ 5.83 (1H, q, J = 6.9), δ 1.32 (3H, s) δ 1.24 (3H, s), δ 1.75 (3H, d, J = 6.9), δ 1.73 (1H, doublet of doublets, J = 7.3 and J = 4.4), δ 1.65 (1H, doublet of doublets, J = 7.3 and J = 4.4), δ 2.29 (1H, t, J = 7.3 and J = 7.3).

6 - Methoxy - 8,8 - dimethyl - 4 - propyl - tricyclo[4.4.0.0^{1,6}] *- 7 - oxa - dec - 3 - ene - 2,5 - dione* **7**. Compound **6** (87 mg, 0.22 mmole) was dissolved in 15 ml acetone and an excess of a suspension of Raney nickel W-2 in acetone was added. (The Raney nickel was deactivated by keeping in acetone for 1 h before use.) After stirring for 30 min. the solution was filtered through celite, the solvent was evaporated and the residue chromatographed on a SiO_2 column ($CHCl_3$:hexane = 1:1) giving 17.5 mg (31%) of the oily product **7**. Found: *m/e* 232.1129 (M$^+$ −CH_3OH). Calc. for $C_{14}H_{16}O_3$: *m/e* 232.1099. UV λ_{max}^{EtOH} 231 nm (11300); IR (film): ν_{max} 1703 (C=O), 1676 (C=O) and 1612 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$): δ 6.70 (1H, t, J = 1.4), δ 3.30 (3H, s), δ 2.59 (2H, doublet of triplets, J = 9.0 and J = 1.4), δ 1.52 (2H, m, J = 8.2 and J = 9.0), δ 1.33 (3H, s), δ 1.23 (3H, s), δ 2.27 (1H, doublet of doublets, J = 6.7 and J = 9.2), δ 1.95 (1H, doublet of doublets, J = 4.5 and J = 9.2), δ 1.59 (1 H, doublet of doublets, J = 4.5 and J = 6.7) and δ 0.98 (3H, t, J = 8.2).

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†Recent results indicate that the yields of compounds **1** and **2** may be raised about ten-fold by adding $CuSO_4{\cdot}5H_2O$ (1 mg/l), $Na_2MoO_4{\cdot}2H_2O$ (2 mg/l) and H_3BO_3 (0.25 mg/l) to the culture medium.

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