Pergamon

Tetrahedron Letters, Vol. 35, No. 10, pp. 1593-1596, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $6.00+0.00

0040-4039(94)E0092-C

New Compounds Isolated from the Culture Filtrate of the Fungus *Merulius tremellosus*

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Abstract: Extracts of the culture filtrate of the fungus *Merulius tremellosus*, from which isolactarane and sterpurane sesquiterpenes previously have been isolated, has yielded a sesquiterpene lactone with a novel skeleton and a pentacyclic aldehyde. The stuctures of the compounds were determined by mass and NMR spectroscopy and by X-ray structural analysis.

Extracts of the culture filtrate of the fungus *Merulius tremellosus* have previously yielded several isolactarane (e.g. merulidial **1a**) and sterpurane (e.g. tremetriol **2**) sesquiterpenes.[1,2] Merulidial (**1a**) exhibits antibiotic and mutagenic activities,[1,3] which are believed to be linked to its unsaturated dialdehyde functionality.[3] As more merulidial (**1a**) recently was isolated to be used in an ongoing study of quantitative structure-activity relationships for unsaturated dialdehydes, two additional compounds (**3** and **4** in Figure 1) that have previously not been detected were obtained in reasonable amounts (1.1 and 3.7 % of an ethyl acetate extract, respectively). The structures of meruliolactone[4] (**3**) and compound **4** were determined by spectroscopic methods.

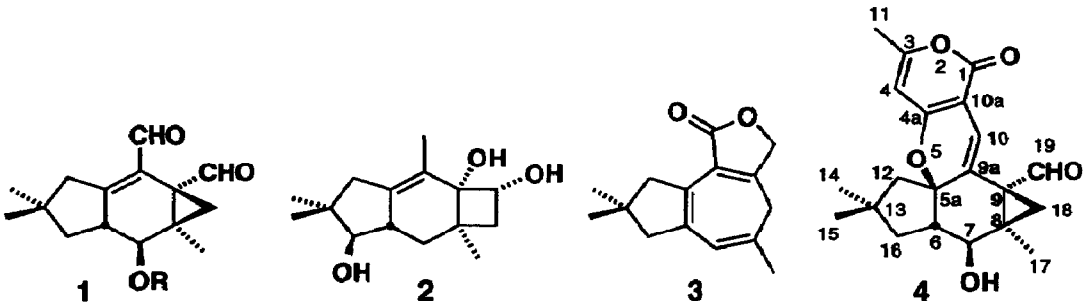
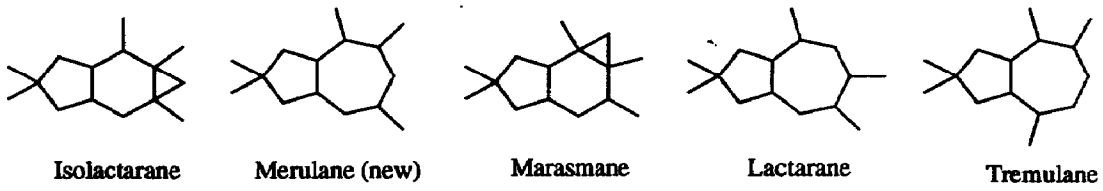
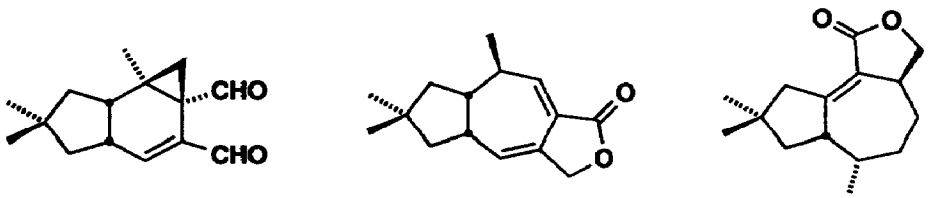


Figure 1. a: R=H, b: R=Ac

Interestingly, the lactone 3 has previously been shown to be one of several products formed during thermal isomerisation of acetylmerulidial (**1b**) at 170 °C in toluene/triethylamine for several hours.[5] This fact

indicates that meruliolactone (3) may be formed from merulidial (1a) during the fermentation. However, the transformation of merulidial (1a) itself to the lactone 3 was never observed *in vitro*. In addition, none of the other products of the thermal isomerisation has been detected in the extracts of the culture filtrate, and the transformation of acetylmerulidial (1b) is completely dependent on the presence of triethylamine. In view of the enzymatic conversion of marasmane sesquiterpenoids to marasmane and lactarane dialdehydes and lactones [e.g. isovelleral (5) and vellerolactone (6)] in fruit bodies of species belonging to *Russulaceae*,[6] the co-formation of meruliolactone (3) and merulidial (1a) is reasonable. Meruliolactone (3) is the first sesquiterpene with this skeleton (for which we propose the name merulane) that has been isolated from a natural source. Recently several sesquiterpenes [e.g. tremulenolide A (7)] with the very similar tremulane skeleton were isolated from the fungus *Phellinus tremulae* (= *Fomes igniarius* var. *populus*).[7]





5 6 7

Figure 2

MS and ^{13}C NMR data suggested that the composition of the aldehyde 4 is $C_{21}H_{24}O_5$. On the basis of this assumption, the structure of compound 4 was determined by a single crystal X-ray analysis (see the experimental section). The structure, shown in Figure 3, was solved by direct methods and refined by full matrix least-squares techniques to final discrepancy index of 0.033 for the observed data.[8] Compound 4 appears to be a derivative of merulidial (1a), and Figure 3 displays arbitrarily the (*S*) C-7 configuration in accord with the absolute configuration of merulidial.[2,9] An investigation to determine whether aldehyde 4 is formed from merulidial (1a) is in progress.

The relative stereochemistry of the isolactarane portion of compound 4 was confirmed by NOESY NMR experiments, and except for C-1, C-3 and C-4a (δ 162.0 ppm, δ 162.76 ppm and δ 162.79 ppm) all ^1H and ^{13}C signals were assigned by data from HETCOR experiments. Long-range correlations were observed between 10-H and C-5a, C-9, C-9a, C-19 and δ 162.0 ppm, and between 11-H_3 and C-4 and δ 162.76/162.79 ppm, suggesting that δ 162.0 is either C-1 or C-4a, but not C-3.

Meruliolactone (3) is weakly antibiotic against *Bacillus subtilis*, *Mucor miehei* and *Paecilomyces varioti*, weakly phytotoxic against *Setaria italica*, but not cytotoxic against L1210 cells at 50 μg/ml (the biological assays were performed according to reference 10). None of these activities were detected for compound 4.

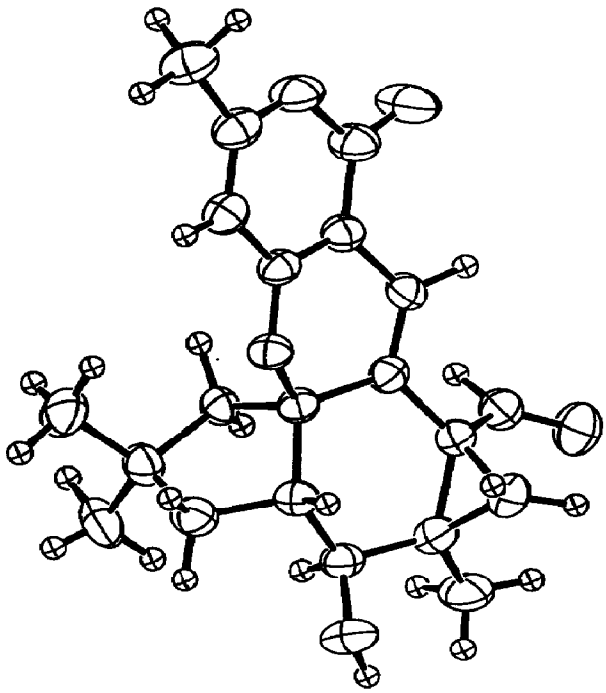


Figure 3. ORTEP view of compound **4**. No absolute configuration is implied.

EXPERIMENTAL

General Procedures. TLC analyses were made on "Merck DC-Alufolien Kieselgel 60 F_{254}" SiO_2 plates, visualised by spraying with anisaldehyde/sulphuric acid and warming to 120°C. The EIMS spectrum (direct inlet, 70 eV) was recorded with a JEOL SX102 spectrometer, and the NMR spectra (in $CDCl_3$) with a Bruker ARX 500 spectrometer at 500.14 MHz for ^1H, and at 125.77 MHz for ^{13}C. The chemical shifts are reported in ppm with the solvent signals (δ_H=7.26 and δ_C=77.0) as reference; the coupling constants are given in Hz. The IR spectrum was recorded with a Perkin-Elmer 298 spectrometer, and the UV spectrum with a Cary 219 spectrometer. The melting point (uncorrected) was determined with a Reichert microscope, and the optical rotation was measured with a Perkin-Elmer 141 polarimeter at 22°C.

Fermentation, extraction and chromatographic separation. The fungus was grown in a 20 l fermentor as described previously,[1] and the culture medium was after filtration (to remove the mycelium) passed through a DIAION HP 21® polymer column. The absorbed materials were eluated with acetone that was evaporated, and the residue (containing some water) was extracted several times with EtOAc. The extracts were combined and dried, and the solvent was removed under reduced pressure yielding 3.82 g. The pure compounds were isolated by preparative chromatography on silica gel with heptane-EtOAc mixtures, or toluene-MTBE 1:1, or CH_2Cl_2-EtOAc 6:1; 42 mg of meruliolactone (**3**) and 140 mg of compound **4** were obtained.

X-ray structure analysis. 4 ($C_{21}H_{24}O_5$) crystallises in the monoclinic space group $P2_1$ (No. 4) with a = 10.009(3), b = 9.225(2), c =10,381(2) Å, β = 107.98(4)°, V = 911.7(3) Å^3, Z = 2, D_{calc} = 1.30 g·cm^{-3}, $F(000)$ = 380, μ = 0.86 cm^{-1}. The structure was refined to R= 0.033, wR= 0.047 for 1580 observed reflections with I > 3σ(I); 330 parameters were refined.[8] Colourless crystals of **4** were grown from an heptane-EtOAc solution. A crystal was mounted on a Huber diffractometer with graphite-monochromated Mo $K\alpha$ radiation ($\lambda_{\alpha 1}$ = 0.70932 Å), $2\theta_{max.}$ = 50°, 1726 independent reflections measured, T = 295 K. Three standard reflections measured every hour did not show any significant variation. Intensities were corrected for absorption using the ψ-scan method. The correction factors varied between 0.93 and 1.00. Anisotropic temperature factors were introduced for all non-hydrogen atoms. Isotropic temperature factors were refined for the hydrogen atoms.

***rel*-(5aR,6S,7S,8R,9R)-9-Formyl-7-hydroxy-3,8-dimethyl-1-oxo-5a,6-(2,2-dimethyl-propano)-8,9-methano-5a,6,7,8,9-pentahydro-2,5-dioxaanthracene (4)** was obtained as colourless rods from heptane-EtOAc, mp 210-212 °C (decomposed). R_F 0.36 (heptane-EtOAc 1:2). $[\alpha]_D$ -58° (*c* 1.00, $CHCl_3$). MS [*m/z* (% rel. int.)]: 356.1619 (M^+, 82 %, calculated for $C_{21}H_{24}O_5$ 356.1624), 327 (48), 286 (55), 285 (46), 173 (42), 91 (58), 86 (64), 84 (100), 43 (64). UV (EtOH) $\lambda_{max.}$ (ε): 347 nm (7550), 251 nm (9470). IR ($CHCl_3$): 3420, 2920, 2860, 1720, 1705, 1640, 1620, 1560, 1445, 1220, 1150, 1035, and 995 cm^{-1}.

^{1}H NMR: 9.70, s, 19-H; 6.33, s, 10-H; 5.83, m, 4-H; 3.79, d, $J_{7a\text{-}6b}$=8.3, 7-H_a; 2.23, dd, $J_{12b\text{-}6b}$=1.5, $J_{12a\text{-}12b}$=14.9, 12-H_b; 2.22, d, $J_{4\text{-}11}$=0.7, 11-H_3; 2.18, m, 6-H_b; 2.02, dd, $J_{6b\text{-}16b}$=6.9, $J_{16a\text{-}16b}$=13.2, 16-H_b; 1.85, dd, $J_{6b\text{-}16a}$=3.6, $J_{16a\text{-}16b}$=13.3, 16-H_a; 1.74, d, $J_{18a\text{-}18b}$=4.6, 18-H_a; 1.39, d, $J_{12a\text{-}12b}$=14.9, 12-H_a; 1.30, d, $J_{18a\text{-}18b}$=4.6, 18-H_b; 1.27, s, 17-H_3; 1.14, s, 14-H_3; 1.13, s, 15-H_3; ^{13}C NMR: 197.8, C-19; 162.8, 162.8 and 162.0, C-1, C-3 and C-4a; 127.4, C-9a; 117.5, C-10; 101.6, C-10a; 100.0, C-4; 90.0, C-5a; 73.4, C-7; 50.8, C-6; 48.5, C-12; 42.6, C-16; 40.4, C-9; 36.6, C-13; 36.4, C-8; 32.5, C-15; 31.7, C-14; 24.6, C-18; 20.2, C-11; 17.8, C-17.

ACKNOWLEDGEMENT

Financial support from the Swedish Natural Science Research Council is gratefully acknowledged.

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(*Received in UK* 29 *November* 1993; *accepted* 7 *January* 1994)