

NEW STERPURANE AND ISOLACTARANE SESQUITERPENES FROM THE FUNGUS MERULIUS TREMELLOSUS

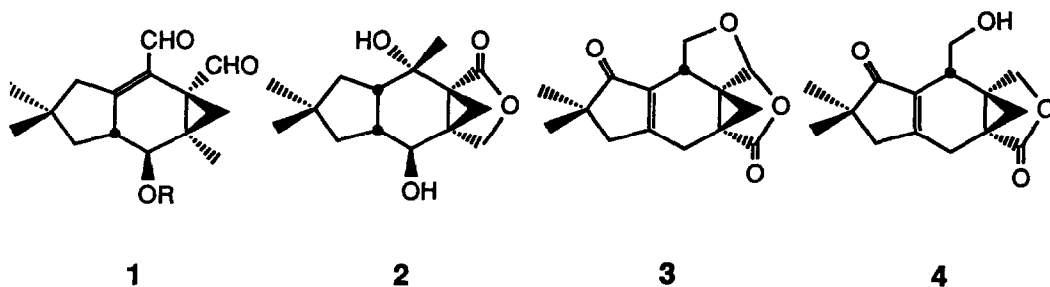
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Abstract : Sterpurane and isolactarane sesquiterpenes have been isolated from a culture of the fungus Merulius tremellosus. The structures of all compounds have been determined by spectroscopic methods, mass and NMR spectroscopy and X-ray structural analyses, and by chemical conversions. Compounds **1a**, **7a**, **8a**, **9a**, **11** and **13** were identified in the cultural fluid, and compounds **7a**, **12a** and **13** in the mycelium. The absolute configuration of the sterpurane **7a** was obtained by X-ray structural analysis of the chloroacetylated derivative **7d**.

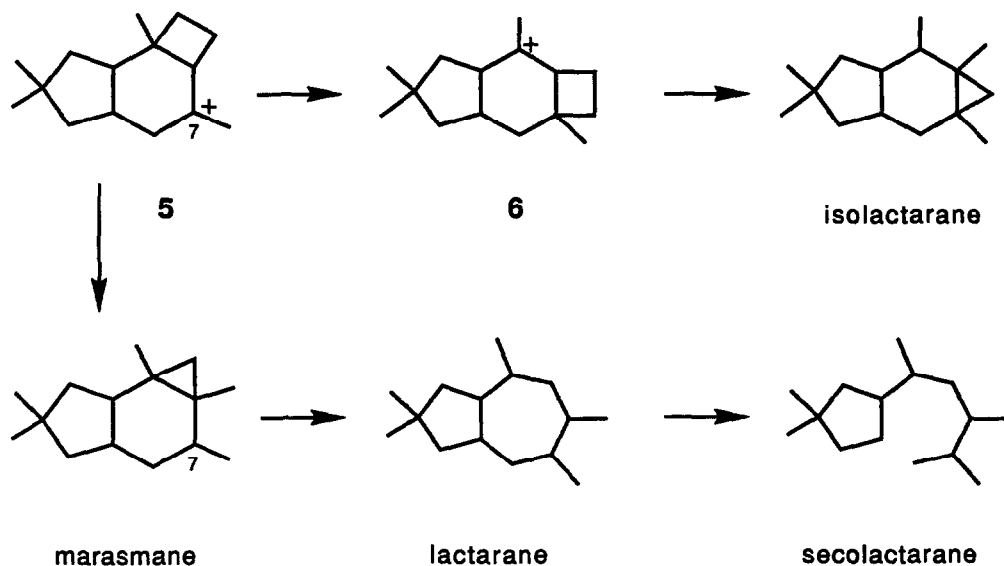
The sesquiterpene merulidial **1a**, isolated from ethyl acetate extracts of submerged cultures of the fungus Merulius tremellosus (Fr.),¹ exhibits antimicrobial, cytotoxic and mutagenic activities.^{2,3} It shares its unsaturated dialdehyde functionality with a number of other biologically active terpenes, isolated from various organisms like fungi, plants, termites and molluscs,^{4,5} which in some cases have been suggested to utilize the unsaturated dialdehydes in natural chemical defense systems. The isolactarane skeleton of merulidial **1a** is less common; the only isolactarane sesquiterpenes reported so far are shown in Scheme 1. Isolactarorufin **2** was isolated from fruit bodies of Lactarius rufus⁶ and L. vellereus,⁷ while sterepolide **3** and dihydrosterepolide **4** were isolated from the fungus Stereum purpureum.⁸



Scheme 1

a : R=H, b : R=COCH₃

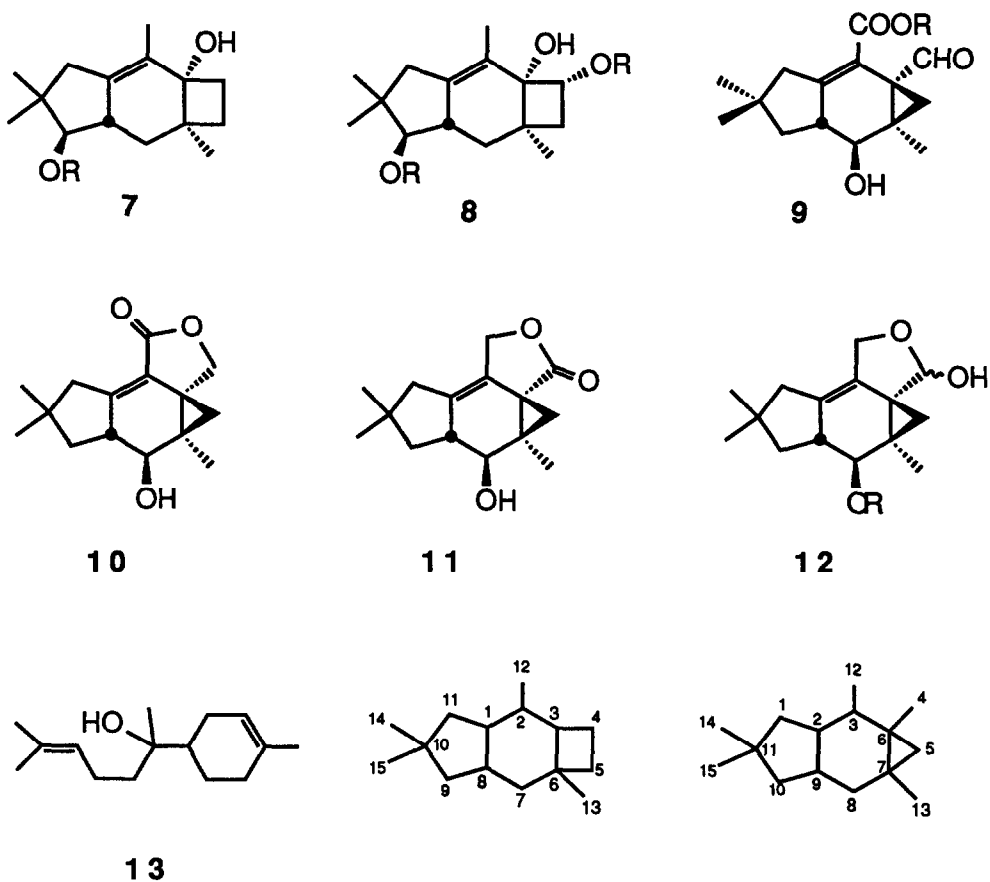
The biosynthesis of the isolactaranes has not been investigated in detail, but they have been suggested to be humulene derived via the protoilludane cation **5** and the sterpurane cation^{6,8} **6** (the latter formed by sliding of the cyclobutane ring^{9,10} of **5**). The isolation of sterepolide **3** and dihydrosterepolide **4** together with several sterpuranes from *Stereum purpureum*^{8,11,12} supports this suggestion. However, isolactarorufin **2** was isolated from fruit bodies of *Lactarius rufus* and *L. vellereus*, species that normally produce marasmane, lactarane and secolactarane sesquiterpenes.¹³ No sterpurane sesquiterpenes have so far been isolated from any *Lactarius* species, and the possibility that an alternative route to the isolactaranes exists must be considered. A marasmane sesquiterpene with a positive charge on C-7 appears to be an intermediate in the biosynthesis of the *Lactarius* sesquiterpenes,¹⁴ and one possibility is that the isolactarane skeleton is formed via this cation by sliding of the cyclopropane ring (similar to the conversion of **5** to **6** mentioned above).



Scheme 2

In order to investigate the nature of the sesquiterpenoids formed together with merulidial **1a** in cultures of *M. tremellosus*, we have in detail analyzed EtOAc extracts of both the culture medium and the mycelium. The fungus was grown in a 100 l fermentor as described previously,² and the culture medium and the mycelium were separated by filtration. The culture medium was extracted immediately with EtOAc while the mycelium was lyophilized prior to extraction with EtOAc. Approximately 50 % of the EtOAc extract of the culture medium consisted of fats, and the majority (3 g) of the remainings was merulidial **1a**. Besides merulidial **1a**, 600 mg tremediol¹⁵ **7a**, 60 mg α -l-bisabolol **13** (identified by spectral comparison with a commercial sample obtained from the company Colimex, Köln, and by its optical rotation¹⁶ $[\alpha]_D^{25} -52^\circ$, c 3.8 in chloroform), 30 mg merulanic acid¹⁵ **9a** (isolated as the methyl ester **9c**), 20 mg merulactone¹⁵ **11**, and 15 mg tremetriol¹⁵ **8a** were isolated by

column chromatography on silica gel. Of these compounds, only bisabolol **13** (50 mg) and tremediol **7a** (10 mg) were found to be present in the mycelium as well as in the culture medium. No traces of merulidial **1a** could be detected by TLC analysis of the mycelium extract, which is somewhat unexpected as the concentration of **1a** in the culture fluid is about 30 mg per liter. However, considering the biological activity of merulidial **1a**, it would be advantageous to the fungus if the concentration of this compound inside the cells is kept as low as possible. The mycelium instead yielded small amounts (30 mg) of the reduced derivative merulialol¹⁵ **12a**, which, in analogy with reduced derivatives of unsaturated dialdehydes in other fungi,^{5, 17} may be formed by the enzymatic reduction of any merulidial **1a** present in the mycelium, possibly in order to avoid exposure to its own toxin.



a : R = H
 b : R = COCH₃
 c : R = CH₃
 d : R = COCH₂Cl
 e : R = CO-Ø-Br

Scheme 3

In order to secure the structures proposed by the spectroscopic methods, as well as to assign the absolute configuration in the latter case, X-ray analyses of acetylmerulidial **1b** and chloroacetylremediol **7d** were carried out. The molecular structures obtained are shown in Figure 1. The confidence levels for the determinations of the absolute configurations are on the basis of a Hamilton R-test significantly greater than 90% for acetylmerulidial **1b**, and 99.5% for chloroacetylremediol **7d**. Therefore the assignment of **1b** should be regarded as tentative. The absolute configurations obtained are the same as those proposed previously for other sterpurane¹⁸ and isolactarane^{1,19} sesquiterpenes.

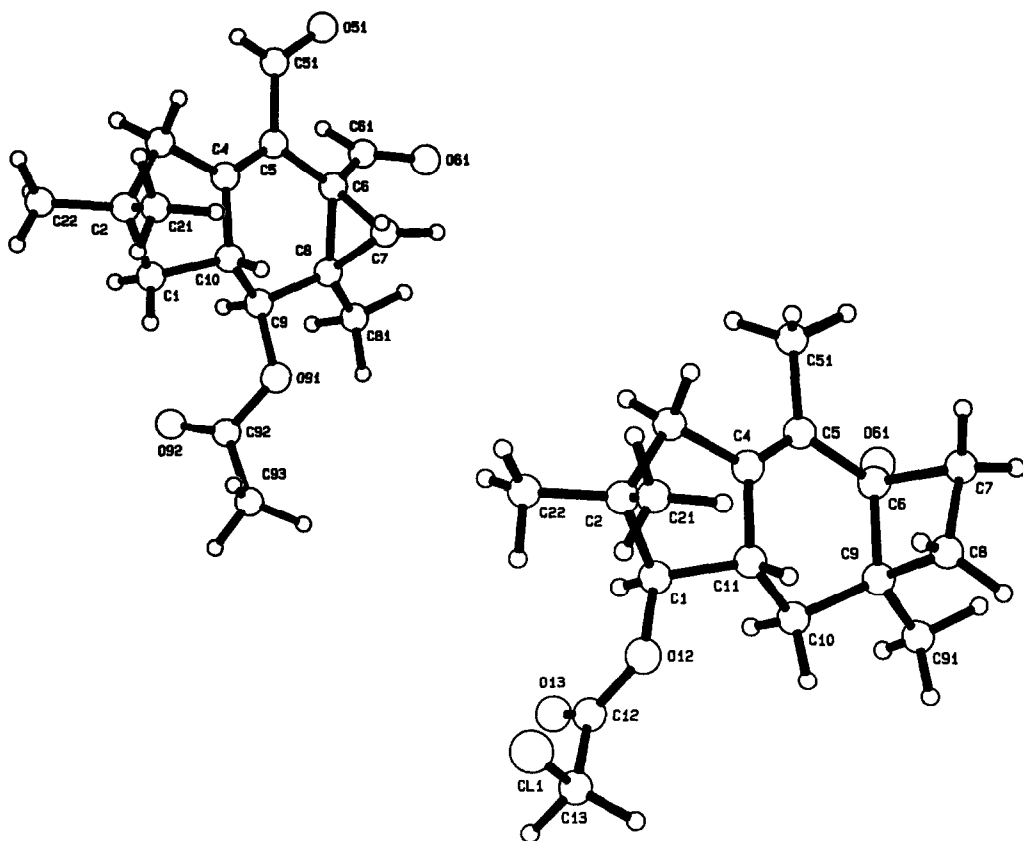


Figure 1. Molecular structures of compounds **1b** and **7d**.

Table 1. Atom coordinates with equivalent isotropic temperature factors for compounds 1b (top) and 7d (bottom).

Atom	x/a	y/b	z/c	U _{eq} (Å ² Σ10 ³)
O4	0.3827 (3)	0.1398 (3)	-0.3974 (4)	98 (3)
O8	0.6490 (2)	0.1086 (2)	0.1137 (3)	58 (2)
O12	0.2523 (3)	-0.0491 (2)	-0.3058 (5)	104 (3)
O1'	0.8168 (3)	0.0392 (3)	0.0978 (5)	98 (3)
C1'	0.7596 (4)	0.0945 (3)	0.1520 (6)	70 (3)
C2'	0.7942 (6)	0.1544 (4)	0.2755 (8)	103 (4)
C1	0.4296 (4)	-0.1506 (3)	0.0706 (6)	60 (3)
C2	0.4438 (3)	-0.0631 (2)	0.0022 (4)	46 (2)
C3	0.3999 (3)	-0.0311 (2)	-0.1263 (4)	49 (2)
C4	0.4115 (5)	0.0730 (4)	-0.3438 (6)	74 (3)
C5	0.4087 (3)	0.1298 (3)	-0.0717 (6)	54 (2)
C6	0.4260 (3)	0.0565 (3)	-0.1778 (5)	48 (2)
C7	0.5280 (3)	0.1031 (3)	-0.1035 (5)	50 (2)
C8	0.5950 (3)	0.0502 (2)	0.0080 (5)	49 (2)
C9	0.5237 (3)	-0.0121 (2)	0.0982 (5)	46 (2)
C10	0.5889 (4)	-0.0792 (3)	0.1891 (6)	54 (2)
C11	0.5012 (4)	-0.1505 (3)	0.2175 (5)	55 (2)
C12	0.3096 (4)	-0.0776 (4)	-0.2045 (7)	73 (3)
C13	0.6029 (4)	0.1620 (3)	-0.1977 (6)	73 (3)
C14	0.5611 (5)	-0.2361 (3)	0.2429 (8)	81 (4)
C15	0.4258 (5)	-0.1290 (4)	0.3529 (7)	82 (4)
Cl(a)	1.0417 (1)	0.8724 (1)	0.6681 (2)	86 (1)
Cl'	1.0243 (16)	0.9192 (11)	0.5779 (20)	151 (6)
O3	0.2512 (3)	0.5771 (2)	0.7405 (4)	68 (2)
O9	0.7922 (3)	0.7735 (2)	0.6945 (3)	62 (2)
O1'	0.7431 (4)	0.8303 (2)	0.5012 (4)	93 (3)
C1'	0.8007 (5)	0.8323 (3)	0.6043 (5)	61 (3)
C2'	0.8819 (5)	0.9035 (3)	0.6434 (7)	81 (3)
C1	0.5946 (4)	0.5884 (2)	0.7402 (5)	51 (2)
C2	0.4802 (4)	0.5519 (2)	0.7565 (5)	51 (2)
C3	0.3652 (4)	0.5957 (3)	0.8138 (4)	54 (2)
C4	0.3489 (6)	0.5794 (3)	0.9617 (5)	74 (3)
C5	0.4210 (5)	0.6618 (3)	0.9895 (5)	77 (3)
C6	0.3918 (4)	0.6896 (3)	0.8460 (5)	60 (2)
C7	0.4989 (4)	0.7296 (3)	0.7671 (5)	60 (3)
C8	0.6213 (4)	0.6782 (2)	0.7680 (5)	52 (2)
C9	0.7221 (4)	0.6976 (2)	0.6642 (5)	55 (2)
C10	0.8124 (4)	0.6218 (3)	0.6635 (6)	67 (3)
C11	0.7139 (4)	0.5509 (3)	0.6769 (6)	70 (3)
C12	0.4615 (5)	0.4613 (3)	0.7224 (6)	71 (3)
C13	0.2692 (5)	0.7417 (4)	0.8386 (7)	86 (4)
C14	0.8909 (6)	0.6169 (4)	0.5360 (7)	102 (5)
C15	0.9047 (5)	0.6219 (4)	0.7801 (8)	94 (4)

a) Cl and Cl' are disordered and were assigned site occupation factors of respectively 0.85 and 0.15.

Table 2. ^1H NMR data for tremediol 7a, tremetriol 8a, methyl merulanate 9c, merulactone 11 and merulialol 12a (epimeric mixture 1:1).

Compound	7a	8a	9c	11	12a
H	δ ; multiplicity; I	δ ; multiplicity; I	δ ; multiplicity; I	δ ; multiplicity; I	δ ; multiplicity; I
1a			2.65; dd; 2, 20		1.94; m
1b			2.48; d; 20	1.99; m	1.94; m
4a	2.17; ddd; 10, 10, 10	3.87; dd; 1.6, 6.6	9.41; s (-CHO)		5.29, 5.25; s
4b	1.90; ddd; 2, 8, 10				
5a	1.52; ddd; 8, 9, 10	1.91; dd; 6.6, 13.4		1.82; d; 4.5	1.29, 1.25; d; 4.7
5b	1.22; ddd; 2, 9, 10	1.32; dd; 1.6, 13.4	1.74; d; 5.0	1.38; dd; 1.0, 4.5	0.87, 0.84; d; 4.7
7a	1.67; dd; 6.2, 12.8	1.68; dd; 5.9, 12.6	1.42; d; 5.0		
7b	0.88; dd; 9.0, 12.8	0.87; dd; 11.3, 12.6			
8	2.42; m	2.24; m	3.59; d; 9.3	3.45; d; 10.6	3.53, 3.38; d; 9.0
9	3.15; d; 10.1	3.31; d; 9.9	2.45; m	2.4; m	2.32; m
10a			1.98; ddd; 2, 7, 12	1.96; dd; 4, 12	1.90; m
10b			1.32; t; 12.0	1.26; dd; 9, 12	1.22; m
11a	2.23; dm; 17	2.25; dm; 18			
11b	2.05; dm; 17	2.12; dm; 18			
12a	1.59; m (-CH ₃)	1.58; m (-CH ₃)		4.84; ddt; 2.6, 4.1, 13.1	4.56, 4.54; dm; 13
12b				4.75; ddt; 2.0, 2.8, 13.1	4.47, 4.27; dm; 13
13	1.20; s	1.26; s	1.23; s	1.56; s	1.36, 1.28; s
14	1.06; s	1.09; s	1.12; s	1.06; s	1.04, 1.02; s
15	0.97; s	0.98; s	0.97; s	1.02; s	0.98; s
			-OCH ₃ ; 3.73; s		

The spectra were recorded in CDCl_3 at 299.9 MHz with TMS as internal standard, δ is given in ppm and J in Hz.

The spectral data for tremetriol **8a** are very similar to those of tremediol **7a**. The most characteristic spectroscopic feature of both compounds is that the base peaks of their high resolution mass spectra are formed by loss of C_2H_4 from the molecular ion of tremediol **7a**, and C_2H_4O from the molecular ion of tremetriol **8a**. Exactly the same phenomena have previously been observed for other sterpuranes,^{11,12} and this appears to be typical for the sterpurane sesquiterpenes. Except for the different molecular ions, the mass spectra of the two compounds are almost identical, suggesting that a hydrogen in the cyclobutane ring of tremediol **7a** has been replaced by a hydroxyl group in tremetriol **8a**. The NMR data (1H and ^{13}C) of the two compounds are quite similar, except for differences supporting the above suggested substitution of a hydrogen for a hydroxyl (for NMR data of both compounds, see Tables 2 and 3). The position of the additional hydroxyl group in tremetriol **8a** was determined by NOE experiments with tremetriol **8a** as well as its p-bromobenzoyl ester **8e** (for which the 1H NMR signals were better resolved). When 12- H_3 was irradiated, a NOE could be observed on 4-H (6% in compound **8a** and 7% in compound **8e**). Irradiation of 5- H_a in the ester **8e** resulted in NOE:s on 4-H (17%) and 8-H (13%), while irradiation of 8-H gave a 9% NOE on 5- H_a .

Table 3. ^{13}C NMR data for tremediol **7a**, tremetriol **8a**, methyl merulanate **9c**, merulactone **11** and merulialol **12a** (epimeric mixture 1:1).

Compound	7a	8a	9c	11	12a
C					
1	132.2 ^a ; s	131.2 ^a ; s	45.3 ^a ; t	43.2 ^a ; t	43.7, 43.8 ^a ; t
2	135.8 ^a ; s	137.3 ^a ; s	156.1; s	124.5 ^b ; s	126.9, 127.1 ^b ; s
3	76.1; s	76.7; s	120.4; s	129.8 ^b ; s	129.1, 130.3 ^b ; s
4	24.7 ^b ; t	75.5; d	197.9; s	176.9; s	98.5, 102.0; d
5	35.8 ^b ; t	36.2 ^b ; t	20.4; t	25.4; t	17.5, 21.3; t
6	46.3 ^c ; s	44.9 ^c ; s	34.8 ^b ; s	31.6 ^c ; s	27.4, 27.8 ^c ; s
7	36.3 ^b ; t	37.3 ^b ; t	37.4 ^b ; s	34.6 ^c ; s	38.4, 38.5 ^c ; s
8	45.4; d	45.4; d	74.6; d	78.1; d	78.0, 78.1; d
9	87.8; d	87.8; d	46.4; d	44.6; d	44.7, 45.4; d
10	41.8 ^c ; s	41.8 ^c ; s	48.0 ^a ; t	45.7 ^a ; t	45.8, 46.3 ^a ; t
11	45.3; t	45.1; t	40.2 ^b ; s	38.9 ^c ; s	38.8, 39.8 ^c ; s
12	30.3; q	29.7; q	167.2; s	68.1; t	67.4, 68.0; t
13	14.6; q	14.3; q	16.4; q	14.7; q	17.2, 17.5; q
14	25.0 ^d ; q	24.4 ^d ; q	28.1 ^c ; q	28.4 ^d ; q	28.5, 28.6 ^d ; q
15	26.1 ^d ; q	27.1 ^d ; q	29.5 ^c ; q	29.1 ^d ; q	29.2, 29.5 ^d ; q
OCH ₃			51.6; q		

The spectra were recorded in CD_3OD (compounds **7a** and **8a**) or $CDCl_3$ (compounds **9c**, **11** and **12a**), at 75.4 MHz with TMS as internal standard, and δ is given in ppm. a, b, c and d are interchangeable.

The purification of the limited amounts of merulanic acid **9a** from acidic impurities was difficult, the crude fraction was therefore treated with diazomethane and merulanic acid **9a** was isolated as its methyl ester **9c** after silica gel chromatography. The suggested structure is supported by its spectral data, and was confirmed by the spectral comparison of the product obtained by reduction of methyl merulanate **9c** with NaBH₄, with the previously reported lactone **10**.¹ The spectral data of merulactone **11** are very similar to those of lactone **10**, although the carbonyl absorption in the IR spectrum (1775 cm⁻¹) suggests that the lactone functionality of merulactone **11** is no longer α,β -unsaturated. In addition, long-range couplings in the ¹H NMR spectrum of merulactone **11** between 12-H₂ and both 1-H₂ and 9-H, confirm that the lactone is positioned as suggested. The NMR spectra of merulialol **12a**, which in organic solvents exists as an epimeric mixture (1:1), show large similarities with those of merulactone **11**. The major difference is the loss of the signals for a lactone functionality, and the gain of signals for a hemiacetal functionality, and this is supported by the high resolution mass spectra and IR spectra. The long-range couplings between 12-H₂ and 1-H₂, as well as between 12-H₂ and 9-H, are approximately the same as in merulactone **11**. Furthermore, careful reduction of merulidial **1a** with NaBH₄ gave, among other reduction products, a product identical to the isolated merulialol **12a**. Acetylation of merulialol **12a** yielded, after chromatography on silica gel, the monoacetate **12b**, which spectral data support the suggested structure.

The different oxidation patterns in the sterpurane and isolactarane sesquiterpenes isolated in this investigation excludes tremediol **7a** and tremetriol **8a** as precursors to merulidial **1a**. However, if merulidial **1a** has a sterpurane precursor, the hydroxylation of the cyclobutane ring in tremetriol **8a** indicates how an isolactarane C-4 aldehyde (like merulidial **1a**) could be formed from a hydroxylated sterpurane precursor via a presumably enzyme-catalyzed pinacol-type of rearrangement, as depicted in Figure 2.

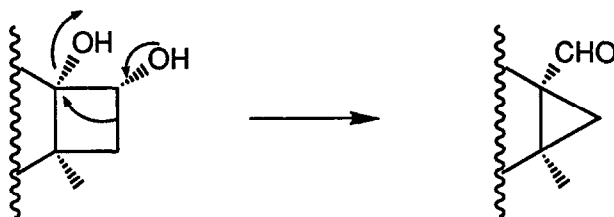


Figure 2

All isolated sesquiterpenes were assayed for antimicrobial and cytotoxic activities.²⁰ Besides merulidial **1a**,² only α -l-bisabolol **13** showed any activity at all (α -l-bisabolol **13** is a known antibiotic previously isolated from various plants¹⁶). In view of the mutagenic activity of merulidial **1a** in the Ames Salmonella assay,³ methyl merulanate **9c**, merulactone **11** and merulialol **12a** were assayed for direct-acting mutagenic activity in the same assay (tester strains TA98, TA100 and TA2637). However, all three compounds were found to be inactive.

This is the second time sterpurane and isolactarane sesquiterpenes have been isolated together from the same species, supporting the suggested biogenetic relationship between the two groups. No obvious sterpurane precursor to the isolactaranes was found in this investigation, and it could be worthwhile to investigate whether *M. tremellosus* incorporates labelled acetate into merulidial **1a** according to the suggested biosynthetic pathway.^{8,10}

EXPERIMENTAL

X-ray structural analyses of **1b** and **7d**:

1b: Crystal data - $C_{17}H_{22}O_4$, $M = 290.4$.

Orthorhombic, $a = 11.644(4)$, $b = 15.676(4)$, $c = 8.826(3)$ Å, $V = 1611(1)$ Å³ (by least squares refinement of diffractometer angles for 25 automatically centered reflections, $\lambda = 1.54184$ Å), space group $P2_12_12_1$, $Z = 4$, $D_x = 1.20$ g cm⁻³, $F(000) = 624$, $\mu(\text{Cu-K}\alpha) = 6.5$ cm⁻¹. Data collection - crystal size 0.48 x 0.48 x 0.48 mm Enraf-Nonius CAD4 diffractometer, ω scan mode with scan width 0.75 + 0.14 tan θ° , $2\theta \leq 140^\circ$ (+ h , + k , + l), graphite-monochromated Cu-K α radiation. 1759 independent reflections, giving 1559 with $I \geq 3\sigma(I)$; absorption corrections with ψ scan. 3 standard reflections monitored regularly showed variations $< \pm 1\%$. Structure Solution and Refinement - Direct methods followed by full-matrix least squares refinement for all atoms (H isotropic). $\sum_w (F_o - F_c)^2$ was minimized with weights $w = 1/[\sigma^2(E_o) + p^2 E_o^2]$, $p = 0.006$. Final R and R_w values for **1b** are 0.0614 and 0.0614, for the enantiomorph 0.0616 and 0.0615. The deterioration was significant at the 90% level.

7d: Crystal data - $C_{17}H_{25}O_3Cl$, $M = 312.8$.

Orthorhombic, $a = 10.321(2)$, $b = 16.092(4)$, $c = 10.171(4)$ Å, $V = 1689(1)$ Å³ (by least squares refinement of diffractometer angles for 25 automatically centered reflections, $\lambda = 1.54184$ Å), space group $P2_12_12_1$, $Z = 4$, $D_x = 1.23$ g cm⁻³, $F(000) = 672$, $\mu(\text{Cu-K}\alpha) = 20.7$ cm⁻¹. Data collection - crystal size 0.52 x 0.36 x 0.34 mm Enraf-Nonius CAD4 diffractometer, ω scan mode with scan width 0.85 + 0.14 tan θ° , $2\theta \leq 140^\circ$ (+ h , + k , + l), graphite-monochromated Cu-K α radiation. 1840 independent reflections, giving 1666 with $I \geq 3\sigma(I)$; absorption corrections with ψ scan. 3 standard reflections monitored regularly showed variations $< \pm 1\%$. Structure Solution and Refinement - Direct methods followed by full-matrix least squares refinement with H atoms riding on C atoms. $\sum_w (F_o - F_c)^2$ was minimized with weights $w = 1/[\sigma^2(E_o) + p^2 E_o^2]$, $p = 0.006$. Final R and R_w values for **7d** are 0.065 and 0.066, for the enantiomorph 0.074 and 0.077. The deterioration was significant at the 99.5% level. The chlorine atom is disordered; site occupation factors of 0.85 and 0.15 were assigned to the two positions.

Column chromatography was performed on "Merck Lobar pre-packed" silica gel columns eluted with ethyl acetate : petroleum ether mixtures. Preparative TLC was performed on "Merck DC Fertigplatten, Kieselgel 60" (layer thickness 0.25 mm) with diethyl ether, or ethyl acetate : petroleum ether mixtures. Analytical TLC was performed on "Merck DC Alufolien, Kieselgel 60" with ethyl ether. ¹H and ¹³C NMR spectra were obtained on

a Varian XL-300 spectrometer in CDCl_3 or CD_3OD solutions with tetramethylsilane as the internal standard. The coupling constants J are given in Hz. IR spectra were recorded on a Perkin Elmer 1420 instrument. UV spectra were taken with a Varian Cary 17 spectrometer. High resolution mass spectra were obtained on an AEI MS 50 instrument. Optical rotations were recorded at 22 °C.

Tremediol **7a** (600 mg) was obtained as a crystalline white solid, mp 134-135°C. R_F 0.30 (ethyl acetate : petroleum ether). $[\alpha]_D^{22} -71^\circ$ (c 1.1 in diethyl ether). UV (ethanol) : no maximum above 210 nm. IR (KBr) : 3370, 2950, 1450, 1370, 1050, and 985 cm^{-1} . ^1H NMR see Table 2. ^{13}C NMR see Table 3. MS, m/z : 236.1769 (M^+ , 30%, calculated for $\text{C}_{15}\text{H}_{24}\text{O}_2$ 236.1776), 208, (100%), 190 (25%), 137 (50%), 136 (58%), 135 (65%), 121 (33%).

Chloroacetyltremediol **7d** was obtained as a crystalline white solid, mp 99-100 °C (EtOAc:heptane), after chloroacetylation of tremediol with chloroacetyl chloride in pyridine, and chromatography on silica gel. ^1H NMR (CDCl_3): 4.66, d, C(9)H, $J_{8-9}=10.0$; 4.09, s, OCOCH_2Cl ; 2.62, m, C(8)H ; 2.30, d, C(11)Ha, $J_{11a-11b}=17$; 2.16, d, C(11)Hb, $J_{11a-11b}=17$; C(4)Ha, 2.13, ddd, $J_{4a-4b}=10$, $J_{4a-5a}=10$, $J_{4a-5b}=10$; 2.00, ddd, C(4)Hb, $J_{4a-4b}=10$, $J_{4b-5a}=8$, $J_{4b-5b}=2$; 1.63, m, C(12)H₃ ; 1.53, dd, C(7)Ha, $J_{7a-8}=6.0$, $J_{7a-7b}=13.2$; 1.46, ddd, C(5)Ha, $J_{4a-5a}=10$, $J_{4b-5a}=8$, $J_{5a-5b}=9$; 1.23, ddd, C(5)Hb, $J_{4a-5b}=10$, $J_{4b-5b}=2$, $J_{5a-5b}=9$; 1.20, 1.06, and 1.06, s, C(13)H₃, C(14)H₃, and C(15)H₃ ; 1.00, dd, C(7)Hb, $J_{7b-8}=11.4$, $J_{7a-7b}=13.2$.

Tremetriol **8a** (15 mg) was obtained as a crystalline white solid, mp 160-164°C. R_F 0.45 (diethyl ether). $[\alpha]_D^{22} +43^\circ$ (c 0.8 in diethyl ether). UV (ethanol) : no maximum above 210 nm. IR (KBr) : 3340, 2900, 1260, 1125, 1090, 1060, and 1020 cm^{-1} . ^1H NMR see Table 2. ^{13}C NMR see Table 3. MS, m/z : 252.1745 (M^+ , 0.5%, calculated for $\text{C}_{15}\text{H}_{24}\text{O}_3$ 252.1725), 208, (100%), 190 (29%), 175 (36%), 137 (46%), 136 (49%), 135 (24%), 121 (30%).

p-Bromobenzoyl ester of tremetriol (**8e**) was obtained as a crystalline white solid, mp 139-140.5 °C ($\text{H}_2\text{O}:\text{EtOH}$), after p-bromobenzoylation of tremetriol with p-bromobenzoyl bromide in pyridine. ^1H NMR (CDCl_3): 7.94 and 7.61, m, aromatic-Hg; 4.97, dd, C(4)H, $J_{4-5a}=6.8$, $J_{4-5b}=1.5$; 4.89, d, C(9)H, $J_{8-9}=9.6$; 2.67, m, C(8); 2.42, d, C(11)Ha, $J_{11a-11b}=18$; 2.29, d, C(11)Hb, $J_{11a-11b}=18$; 2.09, dd, C(5)Ha, $J_{4-5a}=6.8$, $J_{5a-5b}=13.8$; 1.78, m, C(12)H₃; 1.63, dd, C(7)Ha, $J_{7a-7b}=13.3$, $J_{7a-8}=5.8$; 1.60, dd, C(5)Hb, $J_{4-5b}=1.5$, $J_{5a-5b}=13.8$; 1.34, 1.19, and 1.12, s, C(13)H₃, C(14)H₃, and C(15)H₃; 1.13, m, C(7)Hb.

Methyl merulanate **9c** (30 mg) was obtained as a crystalline white solid, mp 121-124°C. R_F 0.60 (diethyl ether). $[\alpha]_D^{22} -59^\circ$ (c 2.5 in chloroform). UV (ethanol) λ_{max} (ϵ) : 231 (5.900) and 250 (infl.). IR (KBr) : 3470, 2950, 1705 (broad), 1630, 1430, 1250, 1040, 970 and 860 cm^{-1} . ^1H NMR see Table 2. ^{13}C NMR see Table 3. MS, m/z : 278.1514 (M^+ , 8%, calculated for $\text{C}_{16}\text{H}_{22}\text{O}_4$ 278.1518), 247, (25%), 246 (100%), 228 (20%), 218 (61%), 217 (76%), 203 (57%).

Merulactone **11** (20 mg) was obtained as a colourless oil. R_F 0.28 (ethyl acetate : petroleum ether 1:1). $[\alpha]_D^{22} -38^\circ$ (c 1.5 in diethyl ether). UV (ethanol) λ_{max} (ϵ) : 230 (4.800). IR (neat) : 3420, 2950, 1765, 1460, 1365,

1215, 1040, and 1010 cm^{-1} . ^1H NMR see Table 2. ^{13}C NMR see Table 3. MS, m/z : 248.14 11(M^+ , 94%, calculated for $\text{C}_{15}\text{H}_{20}\text{O}_3$ 248.1412), 233, (43%), 230 (37%), 215 (72%), 175 (100%), 119 (96%), 91 (88%).

Merulialol **12a** (30 mg, epimeric mixture 1:1) was obtained as a crystalline white solid, mp 62-67°C. R_F 0.30 (ethyl acetate : petroleum ether 2:3). $[\alpha]_D^{+30}$ (c 3.0 in chloroform). UV (ethanol) λ_{max} (ϵ): 220 (2.400). IR (KBr): 3380, 2950, 1460, 1020, and 900 cm^{-1} . ^1H NMR see Table 2. ^{13}C NMR see Table 3. MS, m/z : 250.1554 (M^+ , 5%, calculated for $\text{C}_{15}\text{H}_{22}\text{O}_3$ 250.1569), 232, (100%), 217 (53%), 204 (95%), 175 (63%), 119 (66%), 91 (87%).

Acetylmerulialol **12b** (epimeric mixture 1:1) was obtained as a slightly yellow oil after acetylation of merulialol **12a** with acetic anhydride in pyridine, and chromatography of the crude product on silica gel. ^1H NMR (CDCl_3): 5.29 and 5.26, s, C(4)H; 5.07 and 4.95, d, C(8)H, $J_{8-9}=9.7$; 4.55 and 4.30, m, C(12)H₂; 2.49, m, C(9)H; 2.14 and 2.13, s, OCOCH_3 ; 1.97, m, C(1)H₂; 1.66, m, C(10)Ha; 1.40 and 1.37, d, C(5)Ha, $J_{5a-5b}=4.8$; 1.26, m, C(10)Hb; 1.24, 1.16, 1.05, 1.04 and 0.96, s, C(13)H₃, C(14)H₃ and C(15)H₃; 0.89 and 0.88, d, C(5)Hb, $J_{5a-5b}=4.8$. IR (neat): 3420, 2960, 1740, 1260, 1090, 1020 and 800 cm^{-1} . MS, m/z : 292.1668 (M^+ , 9%, calculated for $\text{C}_{17}\text{H}_{24}\text{O}_4$ 292.1674), 232, (29%), 231 (33%), 217 (39%), 214 (45%), 187 (49%), 186 (94%).

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The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication.