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(+)-OSMUNDALACTONE, γ-LACTONES AND SPIROMENTINS FROM THE FUNGUS *PAXILLUS ATROTOMENTOSUS*

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Key Word Index *Paxillus atrotomentosus*; fungus; Basidiomycetes; Paxillaceae; (+)-osmundalactone; γ-lactones; terphenylquinones; spiromentins.

Abstract—(+)-Osmundalactone, a new natural product, and three γ-lactones, one of which is new, together with eight spiromentins, six of which are new, have been isolated from the fungus *Paxillus atrotomentosus*. Their structures were established by spectroscopic methods and X-ray analysis.

INTRODUCTION

Paxillus atrotomentosus belonging to the Paxillaceae family is a lignicolous mushroom with a large cap and frequently appears on decaying tree trunks. Previous studies on this species have reported on the presence of terpenylquinones [1–5] which include leucomentin [3], flavomentin [4] and spiromentin [4] derivatives. In this paper, we report on the presence of (+)-osmundalactone (**1**), three γ-lactones (**2**–**4**) and eight spiromentins (**5**–**12**). Compound **1**, γ-lactone **4** and spiromentins E–J (**7**–**12**) are new natural products. Compound **1** has previously been obtained from both leucomentin 3 [3] and flavomentin D [4] by chemical reaction.

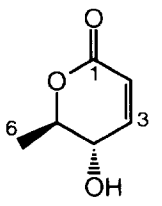
RESULTS AND DISCUSSION

From an ethyl acetate extract of *P. atrotomentosus*, **1**, which was present in very high yield (about 23% of the crude extract), **2**–**4** and **5**–**12** were isolated.

The molecular formula, $C_6H_8O_3$, of **1** was determined by elemental analysis (Found: C, 55.76; H, 6.37. $C_6H_8O_3$ requires: C, 56.23; H, 6.30) and a $[M + H]^+$ peak at *m/z* 129.0558 in its HRCI mass spectrum. The ^{1}H NMR (Table 1), IR, UV and melting point of **1** revealed that this compound has the same structure as osmundalactone, a compound which has been obtained both free [6] and as the hydrolysis product of osmundalin (a glucoside of osmundalactone) [7] from *Osmunda japonica*. An X-ray analysis was carried out on **1**, as suitable single crystals were obtained when crystallized from ethyl acetate. This confirmed the relative stereochemistry to be the same as that of osmundalactone, and an ORTEP drawing of **1** is shown in Fig. 1. In 1974, Hollenbeak and Kuehne established the absolute stereochemistry of osmundalactone, obtained by enzymic hydrolysis of osmundalin, as the (−)-4*R*,5*S* enantiomer [7]. Therefore, the absolute stereochemistry of **1** could be derived from its positive optical rotation ($[\alpha]_D$ + 70.9°) and its negative cotton effect curve at 263 nm ($\Delta\varepsilon$ − 3.27) in its CD spectrum by comparison with (−)-(4*R*,5*S*)-omundalactone {$[\alpha]_D$ − 70.6°; CD: $\Delta\varepsilon_{263}$ (+ 3.86)} obtained as the hydrolysis product of osmundalin. The absolute stereochemistry of **1** from *P. atrotomentosus* is thus established as (+)-(4*S*,5*R*)-osmundalactone.

Compounds **2** and **3** were identified as (4*R*,5*S*)-5-hydroxy-2-hexen-4-olide (**2**) [6–8] and (4*R**,5*S**)-5-hydroxyhexan-4-olide (**3**) [7, 9] by comparing their spectroscopic data with those for authentic samples. We confirmed these structures and fully assigned their ^{1}H (Table 1) and ^{13}C (Table 2) NMR spectra by means of the HMQC and HMBC spectra. Compounds **2** and **3** have also been isolated from *O. japonica* [6]. It is interesting from a biosynthetic point of view that the two chiral centres in **1** (4*S*,5*R*) have the opposite stereochemistry from the two chiral centres in its isomer **2** (4*R*,5*S*).

The HRCI mass spectrometry (MS) of **4** gave the molecular formula $C_8H_{12}O_5$ ($[M + H]^+$ at *m/z* 189.0765). The IR spectrum displayed absorption bands attributable to a hydroxyl group (ν_{max} 3470 cm^{-1}), an acetoxyl group (ν_{max} 1742 cm^{-1}) and a γ-lactone (ν_{max} 1780 cm^{-1}). The ^{1}H NMR spectrum (Table 1) confirmed the presence of an acetoxyl [δ_H2.10 (*s*, 3H), 5.44 (*dt*, *J* = 7.3, 1.6 Hz)] and a secondary alcohol [δ_H2.28 (*brd*, *J* = 4.5 Hz)]. The spectrum also contained signals for a secondary methyl [δ_H 1.33 (*d*, *J* = 6.6 Hz)], a methylene [δ_H3.05 (*dd*, *J* = 18.6, 7.3 Hz), 2.52 (*dd*, 18.6, 1.6 Hz)] and as well as the acetate bearing methine at δ_H5.44 it contained two further methine protons which were attached to oxygenated carbons [δ_H4.35 (*dd*, *J* = 3.2, 1.6 Hz), 4.11 (*m*)]. Spin–spin decoupling of the hydroxyl signal (δ_H2.28) changed the proton signal at δ_H4.11 (*m*) into a clear quartet of doublets (*J* = 6.6, 3.2 Hz). The ^{13}C NMR spectrum (Table 2) indicated the presence of eight carbons: two carbonyl carbons [δ_C174.9 (lactone), 170.3 (acetoxyl)], three oxygenated carbons (δ_C88.2, 69.8,



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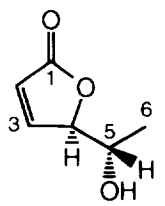

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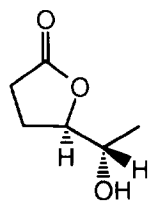

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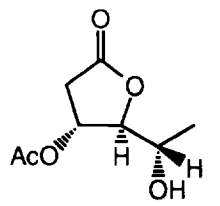

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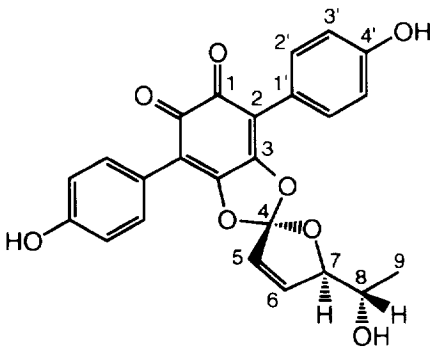

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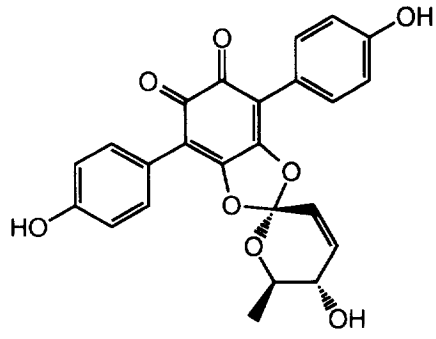

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67.5), a methylene (δ_C 35.7) and two methyls (δ_C 20.9, 18.6). It is evident from these spectral data that **4** is a γ-lactone with an acetoxyl moiety and a 1-hydroxyethyl moiety attached to C-3 and C-4, respectively, and this was confirmed by two-dimensional experiments (phase-sensitive DQF-COSY, HMQC and HMBC).

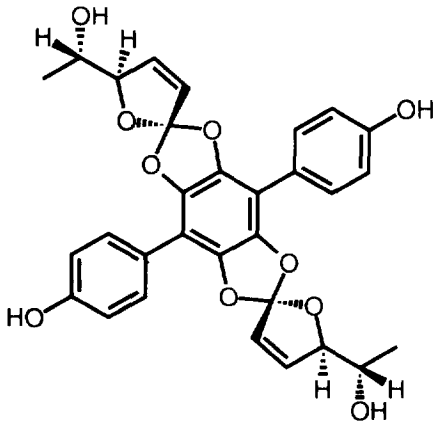
The relative stereochemistry of **4** was determined as follows. The α-orientation of the acetoxyl was established from the J (H-3, H-4) vicinal coupling constant (1.6 Hz). This indicates an H-3, H-4 dihedral angle close to 120° and thus a *trans* relationship between the acetoxyl and 1-hydroxyethyl on the γ-lactone ring. NOE difference experiments confirmed this *trans* relationship: Thus, NOEs were observed between H-5 and H-3 and also between H_3-6 and H-3. The stereochemistry at C-5 is tentatively assigned as *S* based on the co-occurrence of **2**. Compound **4** is, therefore, (3*R**,4*S**,5*S**)-3-acetoxy-5-hydroxyhexan-4-olide.

The absolute stereochemistry of γ-lactones **3** and **4** isolated here are assumed to be the same as that of **2**.

Spiromentins B (**5**) and C (**6**) have previously been isolated from a European species of *P. atrotomentosus* [4] and these same compounds isolated here were identified by extensive one- and two-dimensional NMR spectroscopy (Tables 3 and 4) and their structures were confirmed by comparison of their ^{1}H NMR, UV and IR data with published data [4].

The (+)-FAB-MS of spiromentin E (**7**) gave a peak at m/z 547 $[M + H]^+$, corresponding to a molecular formula of $C_{30}H_{26}O_{10}$. The FAB-MS also showed quasi-molecular ions at m/z 585 $[M + K]^+$ in the (+)-FAB-MS and at m/z 545 $[M - H]^-$ in the (−)-FAB-MS. Its IR spectrum showed the presence of hydroxyl groups (ν_{max} 3375 cm^{-1}) and the UV spectrum showed a band at λ_{max} 301 nm (4.31). The ^{1}H (Table 3) and ^{13}C (Table 4) NMR spectra of **7** resembled those of **5**. Its ^{1}H NMR data (Table 3) contained signals for a *para*-disubstituted benzene ring [AA'BB' spin system: δ_H 7.78 (*m*, 2H); 6.91 (*m*, 2H)], an aromatic hydroxyl [δ_H 8.59 (*brs*)], a disubstituted double bond [δ_H 6.78 (*dd*, J = 5.9, 1.5 Hz); 6.13 (*dd*, J = 5.9, 2.2 Hz)], a secondary alcohol [δ_H 3.78 (*qdd*, J = 6.3, 5.9, 5.6 Hz)], another proton attached to an oxygenated carbon [δ_H 4.81 (*ddd*, J = 5.9, 2.2, 1.5 Hz), a secondary methyl [δ_H 1.22 (*d*, J = 6.3 Hz, 3H)] and another hydroxyl [δ_H 3.95 (*d*, J = 5.6 Hz)]. The ^{13}C NMR spectrum (Table 4) showed 15 signals: six carbons from a *para*-disubstituted benzene ring [δ_C 157.17 (*s*), 130.40 (*d*, 2C), 122.77 (*s*), 115.17 (*d*, 2C)], two olefinic methine carbons (δ_C 137.90, 123.41), two oxygenated carbons [δ_C 89.74 (*d*), 68.77 (*d*)], one methyl (δ_C 18.73) and four other low field quaternary carbons (δ_C 136.99, 136.93, 135.95, 105.94). The FAB-mass spectra indicated a molecular formula of $C_{30}H_{26}O_{10}$; thus, there had to be two identical sets of proton and carbon signals. The most significant difference in the NMR spectra between **5** and **7** was the absence of two carbonyl carbons (δ_C 176.88, 176.83) in the ^{13}C NMR spectrum of **7**. An HMBC experiment was performed on **7**. The quaternary carbon C-4 (δ_C 135.95) was found to have correlations from H-5, H-6 and H-7. The H-2' methine protons [δ_H 7.78 (*m*, 4H)] in the *para*-disubstituted benzene ring correlated with the quaternary carbon C-2 (δ_C 105.94). The above results clearly indicate structure **7** for spiromentin E. The relative stereochemistry at the spirocarbons was deduced from the equivalent chemical shifts of the protons at C-2' and C-3' in both *para*-disubstituted benzene rings.

Spiromentin F (**8**), $C_{30}H_{26}O_{10}$ {m/z 547 $[M + H]^+$, (+)-FAB-MS}, was found to have almost identical ^{1}H (Table 3), ^{13}C (Table 4) NMR and other spectroscopic



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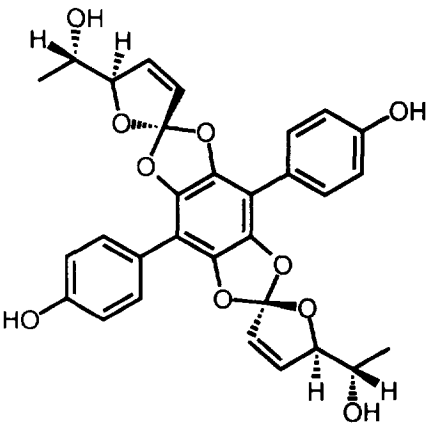

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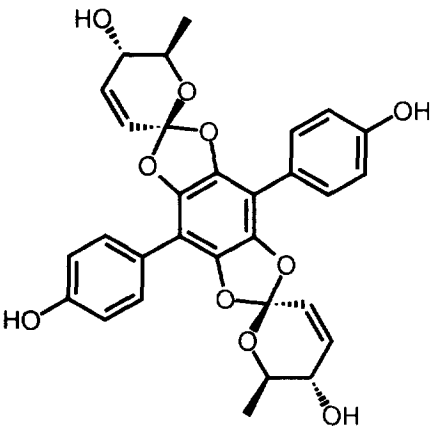

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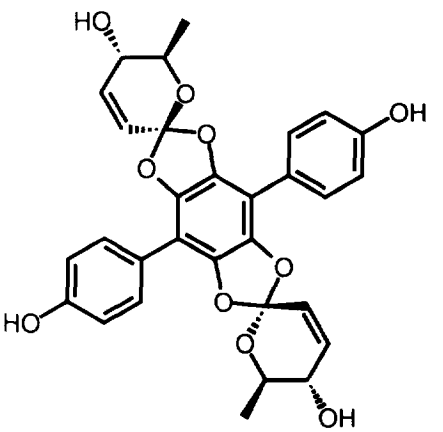

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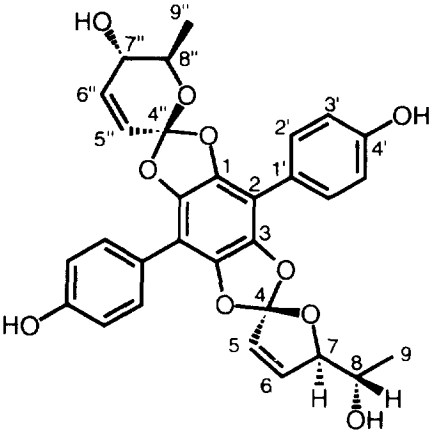

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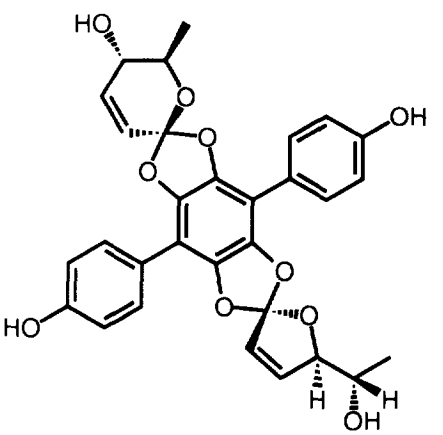

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data as **7**. The only significant difference was that in **7** the chemical shifts of the protons at C-2′ [δ_H7.80 (*m*, 2H); 7.75 (*m*, 2H)] were now different on each *para*-disubstituted benzene ring as were the protons at C-3′ [δ_H6.92 (*m*, 2H); 6.90 (*m*, 2H)]. This evidence revealed that spiromentin F had structure **8**. Thus, spiromentin F (**8**) is a stereoisomer of spiromentin E (**7**) with the opposite stereochemistry at one of the spiro-carbons.

Spiromentins G (**9**) and H (**10**) were obtained as an inseparable 1 : 1 mixture. The ^{1}H (Table 3) and ^{13}C (Table 4) NMR spectra clearly showed this and furthermore the NMR data were very similar to those of spiromentin C (**6**). Again the main difference in the NMR spectra between **6** and the **9**/**10** mixture was the absence of two carbonyl carbons (δ_C176.69, 176.63) in the ^{13}C NMR spectrum of the **9**/**10** mixture. The (+)- and (−)-FAB-

Table 1. ^{1}H NMR data for lactones **1**–**4**

H	1	2	3	4
2	5.88 (*dd*, 10.0, 2.0)	6.19 (*dd*, 5.9, 2.0)	2.60 (*ddd*, 17.8, 10.0, 5.1) 2.55 (*ddd*, 17.8, 10.0, 8.8)	3.05 (*dd*, 18.6, 7.3)β 2.52 (*dd*, 18.6, 1.6)α
3	6.85 (*dd*, 10.0, 2.3)	7.62 (*dd*, 5.9, 1.5)	2.26 (*dddd*, 18.8, 10.0, 8.8, 7.4) 2.17 (*dddd*, 18.8, 10.0, 7.4, 5.1)	5.44 (*dt*, 7.3, 1.6)
4	4.19 (*m*)	4.97 (*ddd*, 4.6, 2.0, 1.5)	4.41 (*td*, 7.4, 3.4)	4.35 (*dd*, 3.2, 1.6)
5	4.33 (*dq*, 8.8, 6.6)	4.05 (*qd*, 6.6, 4.6)	4.13 (*m*)	4.11 (*m*)
6	1.42 (*d*, 6.6)	1.31 (*d*, 6.6)	1.20 (*d*, 6.6)	1.33 (*d*, 6.6)
OH	4.23 (*brd*, 6.2)	3.42 (*brs*)	1.85 (*brd*, 4.2)	2.28 (*brd*, 4.5)
OAc				2.10 (*s*)

Numbers in parentheses are coupling constants (*J*) in Hz.
Assignments confirmed by 2D experiments.

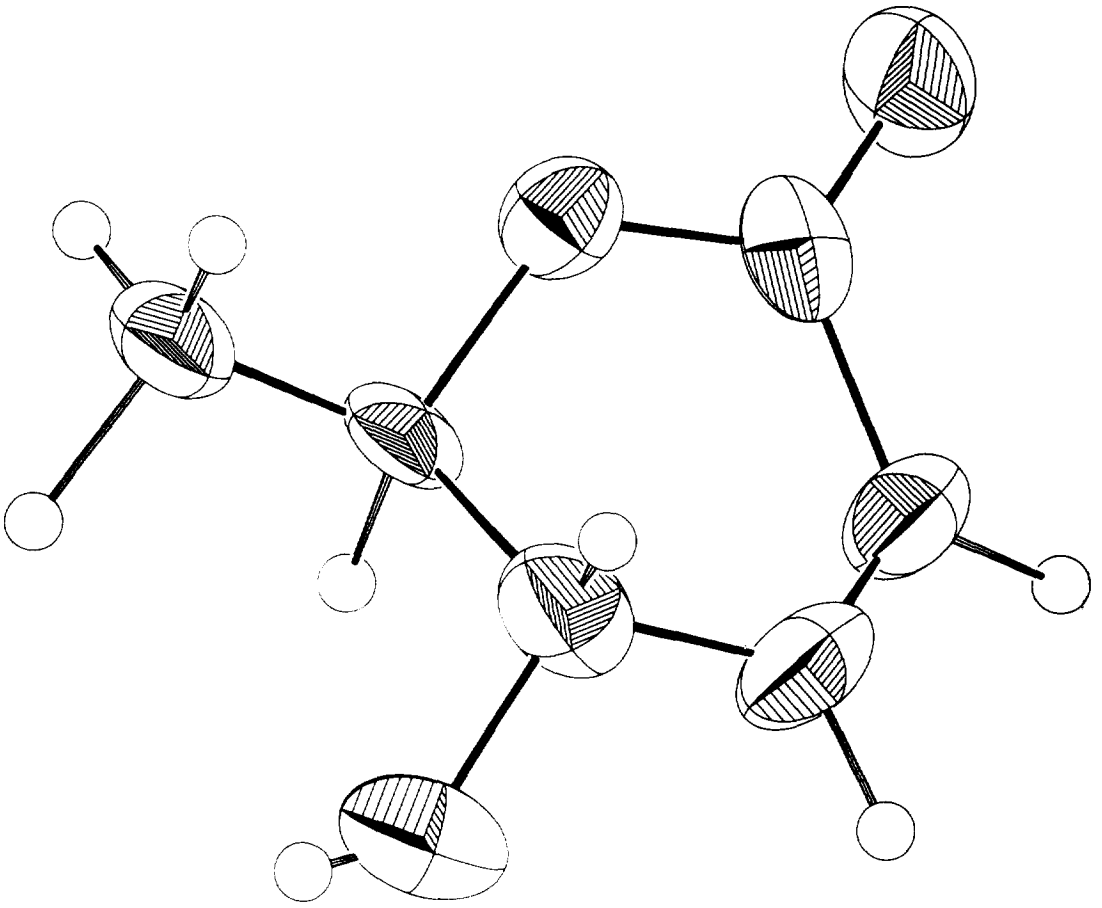

Fig. 1. ORTEP drawing of (+)-fosmundalacton (**1**).

MS gave quasi-molecular ions at *m/z* 547 $[M + H]^+$ and 545 $[M - H]^-$, respectively, indicating a mixture of $C_{30}H_{26}O_{10}$ isomers. From an HMBC experiment recorded on the **9**/**10** mixture, correlations were observed between the quaternary carbon C-4 (δ_C 121.76, 121.70) and H-5, H-6 and H-8. Also, the H-2′ methine protons in the *para*-disubstituted benzene ring correlated with the quaternary carbon C-2 (δ_C 106.90). This spectroscopic evidence, together with the co-occurrence of other spiromentins, indicates structures **9** and **10** for spiromentins G and H, respectively.

Spiromentins I (**11**) and J (**12**) were obtained as a mixture, but further purification by preparative TLC yielded a pure sample of one of these compounds while the other still formed part of a mixture. The HR (+)-FAB-MS of the pure sample gave a peak at *m/z* 547.1619 $[M + H]^+$,

Table 2. ^{13}C NMR data for lactones **1**–**4**

C	1	2	3	4
1	164.1 *s*	173.4 *s*	177.2 *s*	174.9 *s*
2	119.6 *d*	122.4 *d*	28.6 *d*	35.7 *t*
3	150.2 *d*	153.9 *d*	21.0 *d*	69.8 *d*
4	67.0 *d*	87.0 *d*	83.3 *d*	88.2 *d*
5	79.2 *d*	67.4 *d*	67.4 *d*	67.5 *d*
6	17.9 *q*	18.7 *q*	17.7 *q*	18.6 *q*
OAc				170.3 *s*
				20.9 *q*

Assignments confirmed by 2D experiments.

corresponding to a molecular formula of $C_{30}H_{26}O_{10}$. The (+)-FAB-MS of the mixture gave quasi-molecular ions at *m/z* 547 $[M + H]^+$, 569 $[M + Na]^+$ and 585 $[M + K]^+$, indicating a mixture of $C_{30}H_{26}O_{10}$ isomers. It was evident from the ^1H (Table 5) and ^{13}C (Table 4) NMR spectra that spiromentins I and J have one spiro-moiety (lactone acetal unit) the same as in **7** and **8** and the other spiro-moiety (lactone acetal unit) the same as in **9** and **10**. Long-range correlations in the HMBC spectrum of the **11**/**12** mixture were observed between (i) C-2 and H-2′, (ii) C-4 and H-5, H-6 and H-7, and (iii) C-4″ and H-5″, H-6″ and H-8″. On the basis of the above findings spiromentins I and J were assigned structures **11** and **12**, respectively. The ^1H (Table 5) and ^{13}C (Table 4) NMR data assigned to the other compound in the mixture were obtained by careful analysis of the NMR spectra for both the mixture and the pure compound. It was not possible to assign the spectroscopic data to a particular structure.

It is noteworthy that no leucomentins or flavomentins, which were isolated from European *P. atrotomentosus*, were detected in the Japanese species studied here.

EXPERIMENTAL

General. TLC and PLC: Merck precoated Silica gel 60 F_{254} with visualization under UV light (254 nm) and by spraying with 30% H_2SO_4 and heating. R_f values are for EtOAc as eluent. Flash CC: Silica gel 60 (40–63 μm) and Sephadex LH-20 (MeOH–CH_2Cl_2, 1:1). HPLC: Chemcosorb 5Si-U10 × 250 mm (B). Mps: uncorr.

Spectral data. NMR (^1H, 600 MHz; ^{13}C, 150 MHz): $CDCl_3$ and Me_2CO-d_6 solns relative to TMS at $\delta_H 0$, $CDCl_3$ at $\delta_c 77.0$ and CD_3COCD_3 at $\delta_c 29.0$. Multiplicities were determined by DEPT experiments and/or by an HMBC experiment. IR: $CHCl_3$ and MeOH solns. UV: EtOH and H_2O: CD: dioxane. $[\alpha]_D$: $CHCl_3$, EtOH and H_2O. CIMS: 70 eV with CH_4 as reagent gas. (+)-FAB-MS: *m*-nitrobenzyl alcohol matrix. (−)-FAB-MS: triethylglycol matrix.

Fungus *P. atrotomentosus* was collected from a dead and decaying pine tree in Tokushima Prefecture, Sanagouchi-son (Tokuenji temple) in October 1992. A voucher specimen is deposited at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

Extraction and isolation. The fresh material (638 g) was cut into small pieces and extracted with EtOAc. After filtration and solvent evapn, 12.8 g of extract was obtained. The extract (12.8 g) was then subjected to CC on Sephadex LH-20 (MeOH–CH_2Cl_2, 1:1) and 13 frs were collected. Frs 5 (6.56 g), 6 (1.98 g) and 7 (593 mg) were each further fractionated by flash CC over silica gel [*n*-hexane–EtOAc (fr. 5) and MeOH–CH_2Cl_2 (frs 6 and 7) gradients]. Final purification of these frs was by PLC [*n*-hexane–EtOAc (1:4), MeOH–CH_2Cl_2 (1:9)] and HPLC [MeOH–CH_2Cl_2 (1:19), CH_2Cl_2–Me_2CO–*n*-hexane (16:13:11)] to give **1** (2.92 g, R_f 0.44), **2** (195 mg, R_f 0.39), **3** (2 mg, R_f 0.33) and **4** (10 mg, R_f 0.54), **5** (36 mg, R_f 0.44), **6** (48 mg, R_f 0.50), **7** (17 mg, R_f 0.45), **8** (11 mg, R_f 0.45) a mixt. of **9** and **10** (76 mg, R_f 0.54) and

Table 3. ^1H NMR data for spiromentins B, C and E–H (**5**–**10**)

H	5	6	7	8	9 + 10
5	6.31 (*dd*, 5.9, 2.2)	6.10 (*brd*, 10.0)	6.13 (*dd*, 5.9, 2.2)	6.15 (*dd*, 5.9, 2.2)	5.990 (*dd*, 10.0, 2.2, 2H) 5.973 (*dd*, 10.0, 2.2, 2H)
6	6.95 (*dd*, 5.9, 1.5)	6.50 (*brd*, 10.0)	6.78 (*dd*, 5.9, 1.5)	6.78 (*dd*, 5.9, 1.5)	6.325 (*dd*, 10.0, 1.7, 2H) 6.319 (*dd*, 10.0, 1.7, 2H)
7	5.04 (*ddd*, 5.4, 2.2, 1.5)	4.11 (*m*)	4.81 (*ddd*, 5.9, 2.2, 1.5)	4.80 (*ddd*, 5.9, 2.2, 1.5)	4.022 (*dm*, 9.0, 4H)
8	3.88 (*qd*, 6.3, 5.4)	4.11 (*m*)	3.78 (*qdd*, 6.3, 5.9, 5.6)	3.77 (*dq*, 6.3, 5.9)	4.092 (*dq*, 9.0, 6.3, 4H)
9	1.24 (*d*, 6.3)	1.40 (*d*, 6.3)	1.22 (*d*, 6.3)	1.22 (*d*, 6.3)	1.305 (*d*, 6.3, 2H) 1.304 (*d*, 6.3, 2H)
2′	7.50 (*m*, 2H) 7.53 (*m*, 2H)	7.52 (*m*, 2H) 7.49 (*m*, 2H)	7.78 (*m*, 4H)	7.80 (*m*, 2H) 7.75 (*m*, 2H)	7.788 (*m*, 2H) 7.761 (*m*, 4H) 7.730 (*m*, 2H)
3′	6.892 (*m*, 2H) 6.886 (*m*, 2H)	6.904 (*m*, 2H) 6.903 (*m*, 2H)	6.91 (*m*, 4H)	6.92 (*m*, 2H) 6.90 (*m*, 2H)	6.946 (*m*, 2H) 6.941 (*m*, 4H) 6.934 (*m*, 2H)
8-OH	4.21 (*brs*)	4.81 (*brs*)	3.95 (*d*, 5.6, 2H)	3.95 (*brs*, 2H)	4.619 (*brs*, 4H)
Ar-OH	8.64 (*brs*, 2H)	8.65 (*brs*, 2H)	8.59 (*brs*, 2H)	8.63 (*brs*, 2H)	8.651 (*brs*, 4H)

Numbers in parentheses are coupling constants (*J*) in Hz.
Assignments confirmed by 2D experiments.

Table 4. ^{13}C NMR data for spiromentins **5–12**

C	5	6	7	8	9, 10	11/12†	12/11
1 *s*	176.88	176.69	136.99(2C)*	136.93(2C)*	137.80(2C)*	137.51*	137.53*
	176.83	176.63			137.62(2C)*	137.15*	137.21*
2 *s*	112.35	113.12	105.94(2C)	105.97	106.90(4C)	106.52	106.37
	112.27	112.85		105.83		106.38	106.34
3 *s*	154.84	155.38	136.93(2C)*	136.93(2C)*	137.29(2C)*	137.02*	137.04*
	154.75	154.87			137.13(2C)*	137.01*	136.99*
4 *s*	137.44	123.95	135.95(2C)	135.98(2C)	121.76(2C)	135.95, 4	136.10, 4
					121.70(2C)	121.59, 4″	121.63, 4″
5 *d*	122.47	120.21	123.41(2C)	123.48(2C)	122.41(2C)	123.51, 5	123.52, 5
					122.32(2C)	122.36, 5″	122.40, 5″
6 *d*	140.11	141.99	137.90(2C)	137.83(2C)	139.72(2C)	137.97, 6	137.70, 6
					139.52(2C)	139.62, 6″	139.58, 6″
7 *d*	92.02	66.96	89.74(2C)	89.74(2C)	67.76(2C)	89.69, 7	89.74, 7
					67.74(2C)	67.65, 7″	67.69, 7″
8 *d*	68.09	77.13	68.77(2C)	68.80(2C)	74.74(4C)	68.66, 8	68.73, 8
						74.69, 8″	74.72, 8″
9 *q*	18.51	17.12	18.73(2C)	18.74(2C)	17.27(4C)	18.64, 9	18.70, 9
						17.26, 9″	17.26, 9″
1′ *s*	120.95	120.94	122.77(2C)	122.80	122.77	122.46	122.75
	120.92	120.72		122.73	122.60(2C)	122.41	122.57
					122.46		
2′ *d*	131.23(4C)	131.25(2C)	130.40(4C)	130.39(4C)	130.40(2C)	130.37(2C)	130.40(2C)
		131.22(2C)			130.37(4C)	130.34(2C)	130.37(2C)
					130.34(2C)		
3′ *d*	114.87(4C)	114.93(2C)	115.17(4C)	115.15(4C)	115.27(2C)	115.23(2C)	115.18(2C)
		114.90(2C)			115.23(4C)	115.18(2C)	115.12(2C)
					115.17(2C)		
4′ *s*	157.49	157.60	157.17(2C)	157.18(2C)	157.26(2C)	157.42(2C)	157.21
	157.47	157.52			157.22(2C)		157.17

*Values may be interchanged.
†Data for pure sample.
Assignments confirmed by 2D experiments

a mixt. of **11** and **12** (88 mg, R_f 0.51). PLC [MeOH–CH_2Cl_2 (1:9)] of the **11/12** mixt. ((88 mg) resulted in a small amount (8 mg) of one of these compounds being isolated pure.

(+)-(4S,5R)-*Osmundalactone* (**1**). Crystals from EtOAc, mp 81.5–82.5°. $[\alpha]_D$ + 70.9 (H_2O, *c* 1.27). HRCIMS: *m/z* 129.0558 $[M + H]^+$ calc. for $C_6H_9O_3$: 129.0552; CIMS *m/z* (rel. int.): 129 $[M + H]^+$ (100), 111 (32), 103 (8), 87 (79), 73 (40), 45 (30). Elemental analysis (Found: C, 55.76; H, 6.37. $C_6H_8O_3$ requires: C, 56.23; H, 6.30). UV λ_{max} (H_2O) nm (log ε): 203 (3.8); IR ν_{max} cm^{-1}: 3405 (OH), 1726 (C = O), 1260 (C–O); ^1H NMR: Table 1; ^{13}C NMR: Table 2; CD: $\Delta\varepsilon_{310}$ (0), max. $\Delta\varepsilon_{263}$ (− 3.27), $\Delta\varepsilon_{240}$ (0), max. $\Delta\varepsilon_{227}$ (+ 1.51), $\Delta\varepsilon_{215}$ (0).

Crystal data. $C_6H_8O_3$, M_r 128, orthorhombic, space group $P2_12_12_1$ with a = 10.613(6) Å, b = 13.445(9) Å, c = 4.413(3) Å, V = 629.7(7) Å^3, Z = 4, D_{obs} = 1.30 g cm^{-3}, D_{calc} = 0.99 g cm^{-3}, Cu K_α radiation (λ = 1.54178, μ = 8.28 cm^{-1}). Diffraction measurements were made on a Mac Science MXC 18 diffractometer using Cu K_α radiation. Of 608 reflections 574 were unique. The structure was solved by SHELXS-86 and refined by full matrix least squares. The function $\Sigma[w(|F_o|^2 - |F_c|^2)^2]$ was minimized, in which $w = 1.0/[\sigma^2 F_o|^2 + 0.0000|F_o|^2]$. The reflections used were 566 and the number of variables was 116. Final R = 0.060, R_w = 0.073, S = 3.11. The maximum negative and positive peaks in the final difference map were − 0.31 and + 0.30 eÅ^{-3}, respectively.

(4R,5S)-5-*Hydroxy*-2-*hexen*-4-*olide* (**2**). Oil, $[\alpha]_D$ + 112.6° ($CHCl_3$, *c* 0.64). HRCIMS: *m/z* 129.0556 $[M + H]^+$ calc. for $C_6H_9O_3$: 129.0552; CIMS *m/z* (rel. int.): 129 $[M + H]^+$ (100), 111 (39), 85 (49), 45 (6), 41 (6); UV λ_{max} (H_2O) nm (log ε): 204 (4.04); IR ν_{max} cm^{-1}: 3433 (OH), 1740 (C=O); ^1H NMR: Table 1. ^{13}C NMR: Table 2.

(4R*,5S*)-5-*Hydroxyhexan*-4-*olide* (**3**). HRCIMS: *m/z* 131.0707 $[M + H]^+$ calc. for $C_6H_{11}O_3$: 131.0708; CIMS *m/z* (rel. int.): 131 $[M + H]^+$ (45), 113 (100), 85 (45), 41 (10); IR ν_{max} cm^{-1}: 3440 (OH), 1744 (C=O); ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Hydrogenation of (4R,5S)-5-*hydroxy*-2-*hexen*-4-*olide* (**2**). A soln of **2** (18.5 mg) in EtOH (5 ml) containing 5% Pd on C (1 mg) was stirred with H_2 at room temp and 1 atm for 14 hr. The mixt. was then filtered through celite and concd. The product (17.9 mg) contained **3** and *ca* 8% of one of its diastereomers. These were identified from the ^1H NMR spectrum of the product [7, 9]. The signals for H-5 [δ_H 3.79 (*m*)] and H_3-6 [δ_H 1.26 (*d*, *J* = 6.5 Hz)] in the

Table 5. ^{1}H NMR data for spiromentins I and J (**11** and **12**)

H	**11**/**12**	**12**/**11**
5	6.13 (*dd*, 5.9, 2.2)	6.16 (*dd*, 5.9, 2.2)
5″	5.96 (*dd*, 10.0, 2.2)	5.99 (*dd*, 10.0, 2.2)
6	6.77 (*dd*, 5.9, 1.5)	6.77 (*dd*, 5.9, 1.5)
6″	6.32 (*dd*, 10.0, 1.7)	6.32 (*dd*, 10.0, 1.7)
7	4.80 (*ddd*, 5.9, 2.2, 1.5)	4.81 (*ddd*, 5.9, 2.2, 1.5)
7″	4.01 (*ddd*, 9.0, 2.2, 1.7)	4.02 (*ddd*, 9.0, 2.2, 1.7)
8	3.76 (*qd*, 6.3, 5.9)	3.77 (*qd*, 6.3, 5.9)
8″	4.09 (*dq*, 9.0, 6.1)	4.09 (*dq*, 9.0, 6.1)
9	1.22 (*d*, 6.3)	1.22 (*d*, 6.3)
9″	1.30 (*d*, 6.1)	1.30 (*d*, 6.1)
2′	7.77 (*m*, 2H)	7.79 (*m*, 2H)
	7.76 (*m*, 2H)	7.74 (*m*, 2H)
3′	6.94 (*m*, 2H)	6.928 (*m*, 2H)
	6.92 (*m*, 2H)	6.927 (*m*, 2H)
8-OH	*	2.86 (*brs*, 2H)
Ar-OH	*	*

*Signals not identified in spectrum at this concentration. Numbers in parentheses are coupling constants (J) in Hz. Assignments confirmed by 2D experiments.

diastereomer could clearly be seen. Therefore, **2** must also consist of a small amount of one of its diastereomers and this explains the difference in specific rotation between **2** ($[\alpha]_D$ + 112.6°) and the value reported for this compound ($[\alpha]_D$ + 88.8°) [8]. It was not possible to identify the presence of the diastereomer from the spectroscopic data for **2**.

(3R*,4S*,5S*)-3-*Acetoxy*-5-*hydroxyhexan*-4-*olide* (**4**). Oil, $[\alpha]_D$ − 26.4° ($CHCl_3$, c 0.34). HRCIMS: m/z 189.0765 $[M + H]^+$ calc. for $C_8H_{13}O_5$: 189.0763; CIMS m/z (rel. int.): 189 $[M + H]^+$ (100), 129 (84), 111 (74), 85 (20), 41 (51); IR ν_{max} cm^{-1}: 3470 (OH), 1780 (γ-lactone C=O), 1742 (acetate C=O); 1235, 1170 (C–O); ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

Spiromentin E (**7**). Amorphous solid, $[\alpha]_D$ + 42.3 (EtOH, c 0.52). (+)-FAB-MS m/z: 546 $[M]^+$, 547 $[M + H]^+$, 585 $[M + K]^+$; (−)-FAB-MS m/z: 545 $[M - H]^-$, 546 $[M]^-$; UV λ_{max} (EtOH) nm (log ε): 301 (4.31); IR ν_{max} cm^{-1}: 3375 (OH), 1613, 1537, 1493, 1364, 1208, 1144, 1069, 837, 777; ^{1}H NMR: Table 3; ^{13}C NMR: Table 4.

Spiromentin F (**8**). Amorphous solid, $[\alpha]_D$ + 29.9 (EtOH, c 0.33). (+)-FAB-MS m/z: 546 $[M]^+$, 547 $[M + H]^+$, 569 $[M + K]^+$, 585 $[M + K]^+$; UV λ_{max} (EtOH) nm (log ε): 301 (4.22); IR ν_{max} cm^{-1}: 3365 (OH), 1613, 1537, 1493, 1364, 1206, 837, 777; ^{1}H NMR: Table 3; ^{13}C NMR: Table 4.

Spiromentins G and H (**9** *and* **10**) (1:1 *mixt.*). Amorphous solid, $[\alpha]_D$ + 74.7° (EtOH, c 1.41). (+)-FAB-MS m/z: 546 $[M]^+$, 547 $[M + H]^+$, 585 $[M + K]^+$; (−)-FAB-MS m/z: 545 $[M - H]^-$, 546 $[M]^-$; UV λ_{max} (EtOH) nm (log ε): 300 (4.32); IR ν_{max} cm^{-1}: 3440 (OH), 1615, 1540, 1495, 1460, 1399, 1236, 1185, 1145, 1073, 988, 837, 777; ^{1}H NMR: Table 3; ^{13}C NMR: Table 4.

Spiromentins I/J (**11**/**12**) (*pure sample*). Amorphous solid, $[\alpha]_D$ + 42.6° (EtOH, c 0.31). HR-(+)-FAB-MS: m/z 547.1619 $[M + H]^+$ calc. for $C_{30}H_{27}O_{10}$: 547.1604; (+)-FAB-MS m/z: 546 $[M]^+$, 547 $[M + H]^+$, 569 $[M + Na]^+$, 585 $[M + K]^+$, UV λ_{max} (EtOH) nm (log ε): 300 (4.41); IR ν_{max} cm^{-1}: 3290 (OH), 1615, 1539, 1454, 1397, 1186, 1063, 837; ^{1}H NMR: Table 5; ^{13}C NMR: Table 4.

Spiromentins I/J (**11**/**12**) (3:4 *mixt.*). Amorphous solid, $[\alpha]_D$ + 57.4° (EtOH, c 1.10). (+)-FAB-MS m/z: 546 $[M]^+$, 547 $[M + H]^+$, 569 $[M + Na]^+$, 585 $[M + K]^+$; UV λ_{max} (EtOH) nm (log ε): 300 (4.50); IR ν_{max} cm^{-1}: 3380 (OH), 1613, 1539, 1493, 1454, 1397, 1144, 1073, 837, 779; ^{1}H NMR: Table 5; ^{13}C NMR: Table 4.

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