38. Pseurotin B, C, D, and E. Further New Metabolites of *Pseudeurotium ovalis* STOLK

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Summary

The structures **2**–**5** are assigned to the pseurotin B, C, D and E, which are new metabolites of *Pseudeutorium ovalis* STOLK, based on spectroscopic data and chemical transformations.

1. Introduction. – The isolation, spectral data, and chemical properties of pseurotin A (**1**), a minor metabolite of *Pseudeurotium ovalis* STOLK (*Ascomycetes*) have been discussed in preceding papers [1] [2]. It possesses a highly substituted 1-oxa-7-azaspiro[4.4]nonane skeleton, a rare feature in natural product chemistry. We now wish to report on the structural determination of four additional metabolites which we have isolated from culture filtrates of the same microorganism and which we name pseurotin B, C, D, and E. Their structures **2**–**5** are assigned on the basis of their spectral and chemical characteristics.

2. Isolation and Structure. – The ethyl acetate extract of a large scale fermentation (cf. [2]) of *Pseudeurotium ovalis* (strain S 2269/F), which was carried out some years ago[2]), was purified by column chromatography on silica gel. In the more polar fractions the pseurotins B and C (**2** and **3**, respectively) were enriched. They were separated and obtained in pure form by preparative TLC. Pseurotin B (**2**) is crystalline, m.p. 204–206°, with the molecular formula $C_{22}H_{25}NO_9$, which was deduced from the elemental analysis. Pseurotin C (**3**), $C_{22}H_{25}NO_9$, was an oily product. It is not clear whether both substances are genuine metabolites or artefacts which are formed from pseurotin A (**1**) by *in vitro* hydroxylation and isomerization of the double bond. We were not able to detect the compounds in freshly prepared cultures of the *Pseudeurotium ovalis* strains S 2269/F and S 3484/F[3]).

Pseurotin D (**4**), $C_{22}H_{25}NO_8$, was obtained by variation of the culture conditions. If the microorganism was grown on a sucrose instead of glucose medium, *ca.* 40 mg/l of pseurotin A (**1**) and 20 mg/l of pseurotin D (**4**) were isolated. Pseurotin D

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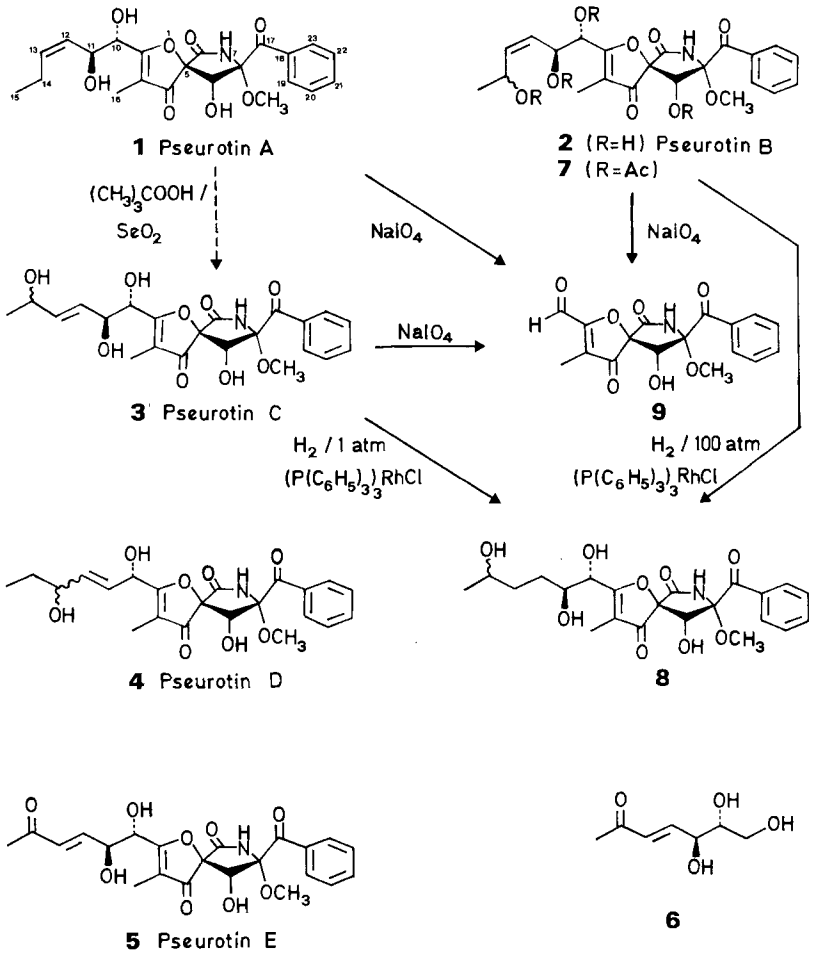

decomposed very readily; it was also obtained in traces when *Pseudeurotium ovalis* was allowed to grow for longer time or at 31° on glucose media.

Pseurotin E (**5**), $C_{22}H_{23}NO_9$, can be considered with certainty as a genuine metabolite of the strains S 2269 and S 3484. It was obtained in a yield of *ca.* 6 mg/l of culture broth as colourless oil, if the organism was grown under the usual conditions (glucose medium, s. exper. part). The compound is possibly derived from pseurotin A (**1**). It has been demonstrated by incorporation experiments with [2-$^{13}C^2H_2$]propionate that **5** is not a biogenetic precursor of **1** [3].

The molecular formulae of all new pseurotins, with the exception of the crystalline and relatively stable pseurotin B (**2**), could not be determined by elemental analyses or by mass spectra. It was not possible to observe a peak for the molecular ions, even if the fragmentations were suppressed by field desorption. The thermic lability and the low vapour pressure due to the presence of an additional hydroxyl group in **3**, which required a higher temperature for the

Table 1. *^{1}H-NMR. data (DMSO-d_6) of the hexenyl side chain of the pseurotins A–E (**1**–**5**) and of compound **6***

H-atom[a])	Pseurotins					
	A (**1**)	B (**2**)	C (**3**)	D (**4**)	E (**5**)	Compound **6**
3 H–C(15)	0.90, *t*	1.10, *d*	1.06, *d*	0.80, *t*	2.16, *s*	2.21, *s*
(3 H–C(1))	$J=7$	$J=6$	$J=6.5$	$J=7$		
1 or 2 H–C(14)	~2.1, *m*	~4.4, *m*	4.0–4.4, *m*	~1.35, *m*	–	–
H–C(13)				~3.9, *m*	6.20, *d*×*d*,	6.15, *d*×*d*,
(H–C(3))					$^3J=16$, $^4J=1.5$	$^3J=16$, $^4J=1.5$
	5.2–5.6, *m*	5.3–5.5, *m*	5.5–5.7, *m*			
H–C(12)					6.92, *d*×*d*,	6.98, *d*×*d*,
(H–C(4))					$^3J=16$, $^3J=4.5$	$^3J=16$, $^3J=4.5$
				5.7–5.8, *m*		
H–C(11)						4.0–4.2, *m*
(H–C(5))	4.2–4.6, *m*	~4.4, *m*	4.0–4.4, *m*		4.3–4.6, *m*	
H–C(10)				~5.1, *m*		3.5–3.8, *m*
(H–C(6))						
HO–C(14)	–	4.97[b]), *d*	4.96[b]), *d*	–	–	–
		$J=5$	$J=5.5$			
HO–C(13)	–	–	–	4.72, *d*×*d*,		
				$^3J=4.5$, $^4J=1$		
HO–C(11)	4.78, *d*	4.71[b]), *d*	4.62[b]), *d*	–	5.99, *d*	5.14, *d*
(HO–C(5))	$J=4.5$	$J=4.5$	$J=4.5$		$J=5.5$	$J=5.5$
HO–C(10)	5.68, *d*	5.81, *d*	5.7, *d*	5.91, *d*×*d*,	5.53, *d*	4.77, *d*
(HO–C(6))	$J=5$	$J=5.5$	$J=?$	$^3J=5.5$, $^4J=1$	$J=5.5$	$J=5.5$

[a]) In parenthesis the corresponding H-atoms of **6**.
[b]) Assignment of HO–C(14) and HO–C(11) may be interchanged.

measurement, led to fragmentation patterns which could not be interpreted. However, in pseurotin B (**2**) the peak of highest mass appeared at *m/z* 446 (M^+-1); further significant signals were observed at *m/z* 347 (M^+-100) due to the loss of hydroxypentenal, at *m/z* 342 (M^+-105) due to the loss of the benzoyl group, at *m/z* 315 (M^+-132) due to the loss of hydroxypentenal and methanol and at *m/z* 297 (elimination of water from *m/z* 315). Also the UV. and IR. spectra provided little information on the structures of the new pseurotins. Like pseurotin A (**1**) the pseurotins B (**2**), C (**3**) and E (**5**) show in the UV. spectrum in ethanol a first absorption maximum at 279 nm ($3.7 \leqslant \log\varepsilon \leqslant 4.1$). The benzoyl chromophore absorbs selectively at the same wavelength (253 nm; $4.2 \leqslant \log\varepsilon \leqslant 4.7$) in all four new compounds.

Most useful for the structural determination of the metabolites proved to be the ^{1}H- and ^{13}C-NMR. spectroscopy combined with spin-spin decoupling by irradiation. In *Table 1* the assignments of the H-atoms in the side chains of all five pseurotins and of (5*S*,6*R*,*E*)-5,6,7-trihydroxy-3-heptene-2-one (**6**) which was synthesized as model compound from natural D-erythrose, are summarized. The signals of the benzoyl group and the spirocyclic system were practically superimposable. They are discussed in detail in the preceding communication [2].

In pseurotin B (**2**) the signal of the $H_3C(15)$-group is split into a doublet ($J=6$ Hz) and shifted to lower field by *ca.* 0.2 ppm compared to the corresponding signal of **1**. The multiplet at 2.1 ppm for the 2 H–C(14) of **1** is not observed for **2**, but an additional doublet at 5.0 ppm ($J=5$ Hz) appears

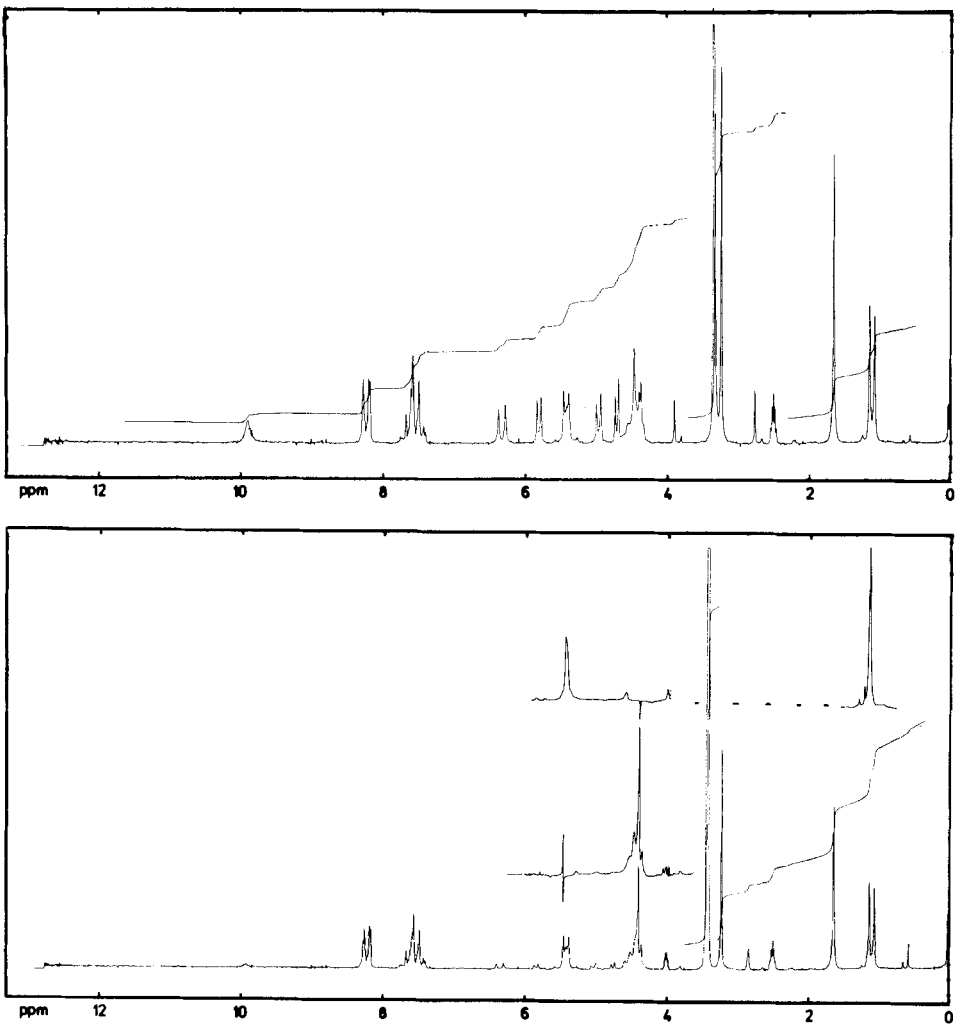

Fig. 1. *^{1}H-NMR. spectrum of pseurotin B* (**2**) *in $(CD_3)_2SO$ (90 MHz) and spin-spin decoupling experiments after addition of D_2O*

which is assigned to HO–C(14). Five protons were exchanged by D-atoms after addition of D_2O: the proton of the amide group at 9.9 ppm, *H*O–C(14), *H*O–C(11) at 4.7 ppm, *H*O–C(10) at 5.8 ppm, and *H*O–C(9) at 6.3 ppm.

Treatment of pseurotin B (**2**) with acetic anhydride/sodium acetate at room temperature led to the 9,10,11,14-tetra-*O*-acetyl derivative **7** as expected, whose ^{1}H-NMR. spectrum exhibited a 12-proton signal at 2.1 ppm.

The ^{1}H-NMR. spectrum of pseurotin B (**2**) with decoupling experiments after deuterium exchange, which confirmed the assignments, is shown in *Figure 1*. Unfortunately it was not possible to produce spectra of first order by single frequency decoupling using 90 MHz, because many resonance frequencies are too close to each other and the double bond causes allylic and probably homoallylic couplings [4a]. In spite of these disadvantages the method furnished useful indications. Irradiation of

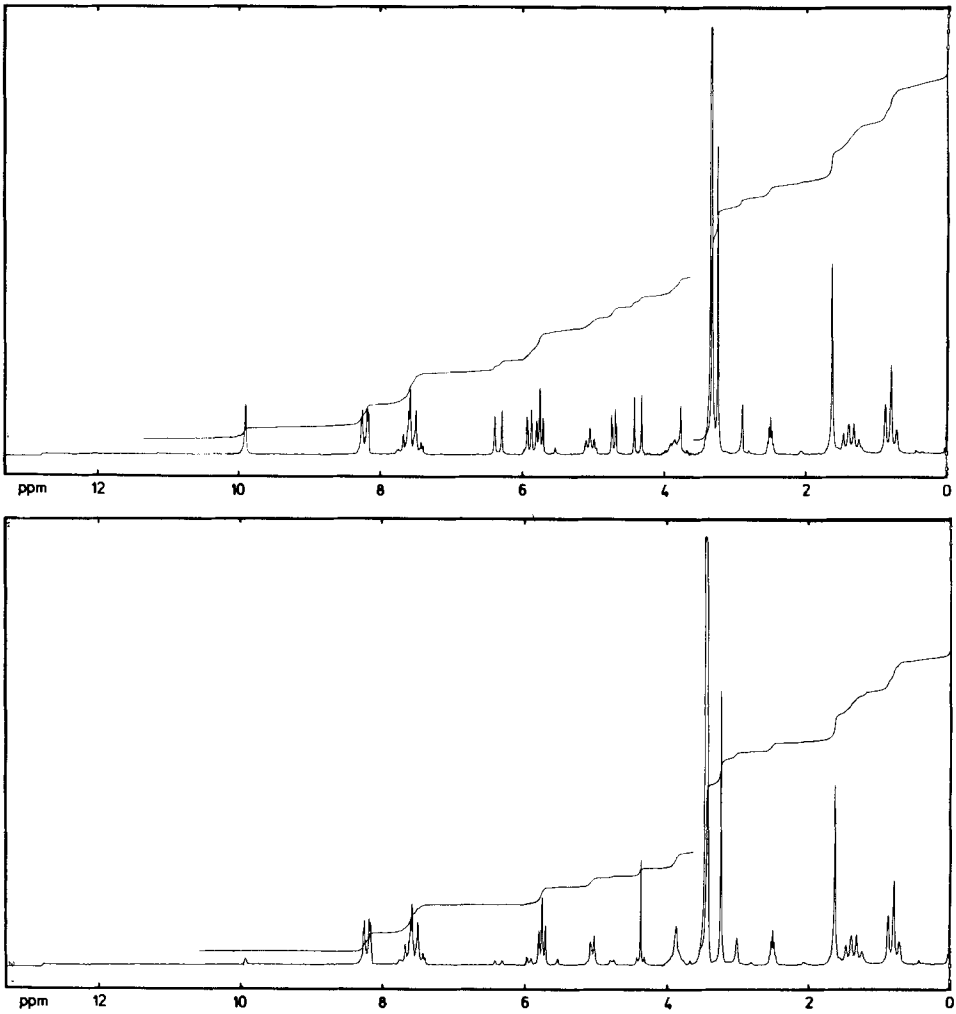

Fig. 2. *^{1}H-NMR. spectrum of pseurotin D (**4**) in $(CD_3)_2SO$ (90 MHz) before and after addition of D_2O*

the $H_3C(15)$-signal of **2** changed only little the splitting pattern of the signals at 4.4 ppm, because next to the singlet of H–C(9) the protons H–C(10), H–C(11) and H–C(14) show also almost the same chemical shift with mutual coupling. On the other hand the $H_3C(15)$-signal collapses to a singlet upon irradiation at 4.4 ppm. This irradiation influences also both olefinic H-atoms (H–C(12) and H–C(13)); they appear as a broadened singlet, because they are decoupled simultaneously from H–C(10), H–C(11) and H–C(14), and because their chemical environment is very similar. As expected irradiation of H–C(12) and H–C(13) changed the pattern of the multiplet at 4.4 ppm (methine protons).

In an analogous manner the structures of the pseurotins C (**3**) and D (**4**) can be deduced. In both cases it was possible to assign all ^{1}H-NMR. signals to the 25 protons by double resonance experiments. The ^{1}H-NMR. spectra of **2** and **3** are very similar, but they show a characteristic difference of the splitting pattern of the olefinic protons. The least certain assignments are those of the signals of pseurotin D (**4**), the most labile metabolite. *Figure 2* shows the ^{1}H-NMR. spectrum of **4** in DMSO-d_6

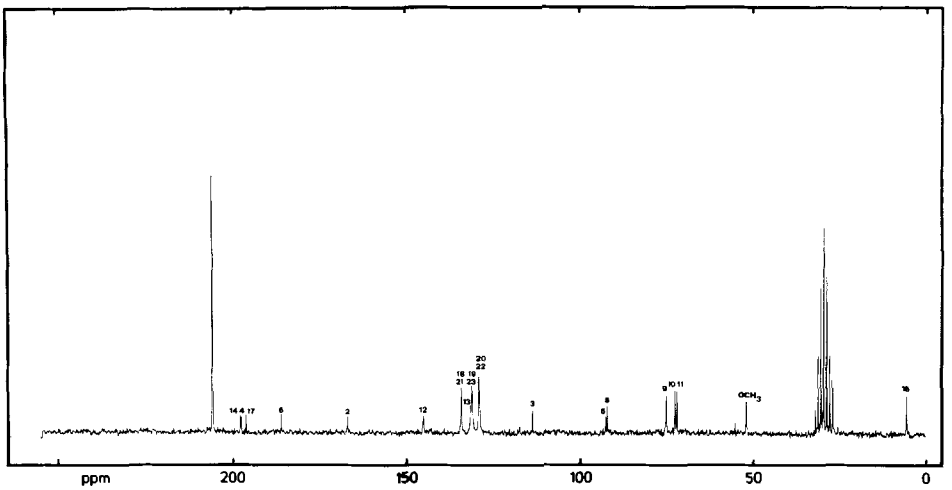

Fig. 3. *^{13}C-NMR. spectrum of pseurotin E* (**5**) *in $(CD_3)CO$ (22.63 MHz);* 40 mg dissolved in 0.4 ml of solvent, puls angle 45°, distance of pulses 2 s, 35,000 pulses

before and after the exchange with D_2O. A characteristic feature is the paramagnetic shift of H–C(10) of **4** compared to **2**, which could be expected in view of the additional deshielding effect of the rearranged double bond [4b]. The configuration of the olefinic 11,12-double bond could not be determined, even not after the measurement of the ^{13}C-NMR. spectrum (s. below).

In the case of pseurotin E (**5**) the signals of the olefinic protons are well separated (α,β-unsaturated ketone); based on their coupling constant ($J = 16$ Hz) the (*E*)-configuration could be assigned unambigously. The (5*S*,6*R*,*E*)-5,6,7-trihydroxy-3-heptene-2-one (**6**) showed practically identical ^{1}H-NMR. data as **5** for the methyl group and the olefinic H-atoms (see *Table 1*).

Further valuable indications for the structural elucidation of the new metabolites were provided by the ^{13}C-NMR. spectra. The chemical shifts of the C-atoms of the spirocyclic systems and the benzoyl groups appear in the usual region with nearly identical frequencies. They are discussed elsewhere [2] [3]. *Figure 3* shows the ^{13}C-NMR. spectrum of pseurotin E (**5**) in $(CD_3)_2CO$ with the complete assignment of the signals. The chemical shifts of the hexenyl side chain C-atoms are listed and compared in *Table 2* for **1**–**5**. The correlation of the data with the proposed structures is excellent.

The additional hydroxyl group in **2** and **3** shows up by the strong shift of the resonance of C(14) compared to **1**. In the spectrum of **5** an additional carbonyl signal is observed. Furthermore the chemical shift of 9.9 ppm of the 15-methyl group in **4** is typical. It corresponds almost exactly to the value calculated for the structural element $CH_3CH_2CH(OH)$ [5].

The structure elucidation of pseurotin A (**1**) which was ascertained by additional measurements of derivatives and the incorporation of doubly labeled biogenetic precursors [3], facilitated the structure assignment of **2**–**5** to a large extent. The signal of C(15) appears in **2** and **3** at a field which is 10 ppm lower than in **1**, a position which is characteristic for an additional hydroxyl group in α-position. In **5** the usual frequency of a methyl ketone is observed. Whereas the strong downfield shift of the β-atom (C(12)) in α,β-unsaturated carbonyl system like **5** is characteristic as a consequence of the deshielding, the adjacent C-atom undergoes a slight shift to lower frequencies [6].

As far as configuration is concerned, we assume without strict proof, that the pseurotins B (**2**) and C (**3**) which are probably secondary transformation products,

Table 2. *^{13}C-NMR. data of the hexenyl side chains of the pseurotins A–E* (**1–5**) *and the dihydro derivative* **8**

C-atom	Pseurotins					
	A (**1**)	B (**2**)	C (**3**)	D (**4**)	E (**5**)	Compound **8**
C(15)	14.1[a])	23.8[a])	23.9[a])	9.9[b])		23.6[a])
	14.5[b])		23.7[b])	9.6[c])	27.6[c])	
	14.0[c])					
C(14)	20.6[a])	62.7[a])	66.8[a])		198.0[b])	66.0[a])
	21.8[b])		68.0[b])	29.7[c])	198.8[c])	
	21.4[c])					
C(13)	134.0[a])	137.3[d])	137.2[a])	69.8[b])[e])	131.6[b])	35.1[a])
	135.9[b])		138.1[b])		131.2[c])	
	137.0[c])					
C(12)	128.4[a])	128.3[a])	127.8[a])	137.7[b])	145.4[b])	28.4[a])
	129.0[b])		128.4[b])	137.1[c])	143.5[c])	
	126.6[c])					
C(11)	68.3[a])	68.3[a])	72.6[a])[d])	127.4[b])	72.3[b])	71.8[a])
	69.7[b])		73.1[b])	126.6[c])	70.9[c])	
	70.2[c])					
C(10)	72.0[a])	71.5[a])	73.2[a])[d])	73.2[b])[e])	72.9[b])	72.2[a])
	72.8[b])		74.0[b])		72.7[c])	
	71.0[c])					

[a]) In $(CD_3)_2SO$.
[b]) In $(CD_3)_2CO$.
[c]) In $CDCl_3$.
[d]) Assignments of C(11) and C(10) may be interchanged.
[e]) Assignments of C(13) and C(10) may be interchanged.

and pseurotin E (**5**) which we believe to be biogenetically linked to pseurotin A (**1**) possess the same absolute configuration at C(10) and C(11) as **1**. For pseurotin D (**4**) which might have been formed from **1** by an allylic rearrangement only speculations are possible.

If this rearrangement occurred the configuration at C(10) would also be (*S*). The configuration at C(13) and C(10) of **4** could certainly be derived from an X-ray analysis, but so far no suitable crystalline specimens neither of the metabolite nor of derivatives thereof are available.

Although pseurotin B (**2**) and C (**3**) are probably not genuine metabolites the hydroxylation which leads to them seems nevertheless to be a stereoselective process giving predominantly one of the two possible epimers. But from the spectral data it is impossible to deduce the configuration at C(14).

The determination of the configuration at the double bond was mainly based on the γ-effect [7] observed in the ^{13}C-NMR. spectrum, since the ^{1}H-NMR. coupling constants of the olefinic protons cannot be detected directly (except in the case of **5** as consequence of the higher order of the spectrum.

Thus in pseurotin B (**2**) C(11) and C(14) are shifted to higher field by *ca.* 4 ppm compared to pseurotin C (**3**). The γ-effect confirms also the (*E*)-configuration in pseurotin E (**5**), whereas no unequivocal statement can be made for pseurotin D (**4**) because the assignment of the signals of C(10) and C(13), respectively, is not clear. This assignment depends on the configuration of the C(11),C(12)-double bond of **4**; for (*Z*)-configuration a signal at *ca.* 69 ppm is expected for C(13), for (*E*)-configuration a signal at *ca.* 73 ppm. Since the chemical shifts of H–C(10), H–C(13) differ by more than 1 ppm, an unequivocal assignment could probably be achieved by a single-frequency

decoupled ^{13}C-NMR. spectrum. In this manner a determination of the configuration of the double bond should be possible too.

3. Chemical Transformations. – The very small amounts of new metabolites available limited the chemical transformations needed for the structural elucidation. However, it was possible to correlate the pseurotins B (**2**) and C (**3**) by selective hydrogenation of both under homogenous catalysis using tris(triphenylphosphine)-rhodium(I) chloride in ethanol/benzene. The products were identical possessing structure **8** with a saturated side chain as demonstrated by the ^{13}C-NMR. spectrum (s. *Table 2*). The spectrum exhibits two additional sp^3-signals at 35.1 ppm for C(13) and 28.4 ppm for C(12); the signals of the olefinic C-atoms have disappeared. The same chemical shifts within the experimental error for all C-atoms of the two samples of **8** and an identical coupling constant $J_{14,15}$ of 6.2 Hz allowed the conclusion that the unknown configuration at C(14) is the same in both pseurotin B (**2**) and C (**3**). It is interesting to note that **2** having a (*Z*)-double bond could be hydrogenated only under more energetic conditions.

All pseurotins possessing a vicinal diol group could be cleaved by sodium periodate in aqueous methanol. They yielded the very unstable aldehyde **9**. It was purified by column chromatography and characterized by the ^{1}H- and ^{13}C-NMR. spectra. The latter were in full agreement with structure **5**.

Attempts to carry out an allylic oxidation on pseurotin A (**1**) were rather disappointing. The system proved to be quite stable to selenium dioxide. However, treatment of **1** with SeO_2/*t*-butylhydroperoxide [8] was accompanied by a slightly enhanced decomposition leading to pseurotin C (**3**).

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Experimental Part

General Methods. S. [2].

Media and isolation of products. The microorganism was fermented or grown in shake cultures. The sterilized medium was innoculated with a spore suspension of *Pseudeurotium ovalis* STOLK (S 2269/F and S 3484/F) under sterile conditions. The 500-ml-*Erlenmeyer* flasks, each containing 200 ml of medium, were shaken on a rotatory shaker at 200 revolutions/min at 28° for 8 days. Both the mycelium and the medium were then twice extracted with ethyl acetate. The organic layer was washed with a small amount of water, dried (Na_2SO_4) and evaporated i.V. (40°). The dark-brown residue was chromatographed on silica gel (100:1) using increasing amounts of methanol in CH_2Cl_2. The isolated yields are given in *Table 3* and refer to a 1 liter scale. In *Figure 4* a TLC. in CH_2Cl_2/methanol 93:7 of all five pseurotins is reproduced showing increasing polarity from left to right.

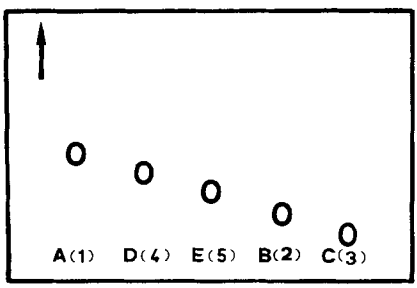

Figure 4

Table 3

	Pseurotins				
	A (**1**)	B (**2**)	C (**3**)	D (**4**)	E (**5**)
Medium A[a])	65–100 mg	–	–	[c])	≤ 8 mg
Medium B[b])	30– 50 mg	–	–	15–20 mg	–

[a]) Medium A: 20 g of glucose, 2 g of peptone, 2 g of malt extract, 2 g of yeast extract, 2 g of KH_2PO_4, 2 g of $MgSO_4 \cdot 7\,H_2O$ per liter of demineralized water; pH (non adjusted) 4.8–5.3.

[b]) Medium B: 85 g of sucrose, 10 g of L-asparagine, 3.5 g of $(NH_4)_2SO_4$, 10 g of KH_2PO_4, 2 g of $MgSO_4 \cdot 7\,H_2O$, 0.075 g of $CaCl_2 \cdot 2\,H_2O$, 0.005 g of $ZnSO_4 \cdot 7\,H_2O$, 0.005 g of $Na_2B_4O_7 \cdot 10\,H_2O$, 0.002 g of $FeSO_4 \cdot 7\,H_2O$ per liter of demineralized water; pH was adjusted to 4.5 with HCl.

[c]) In traces (≤ 1 mg) **4** was also produced if the organism was allowed to grow for a longer period of time or at elevated temp. (31°).

Pseurotin D (**4**) and E (**5**) were further purified by TLC. (CH_2Cl_2/methanol 93:7), pseurotin A (**1**) could be crystallized from THF/hexane. *Pseurotin D* (**4**)[4]): yellowish lac. – ^{1}H-NMR. (DMSO-d_6): *cf. Table 1;* moreover, 1.64 (*s*, 3 H); 3.25 (*s*, 3 H); 4.38 (*d*, *J* = 9, 1 H; *s* after exchange with D_2O); 6.34 (*d*, *J* = 9, 1 H; exchangeable with D_2O); 7.4–7.8 (*m*, 3 H); 8.2–8.3 (*m*, 2 H); 9.92 (*s*, 1 H; exchangeable with D_2O). – ^{13}C-NMR.: *cf. Table 2.*

Pseurotin E (**5**): colourless lac. – IR. (KBr): 3500–3200, 2960, 2920, 1720–1680, 1620, 1100. – UV. (ethanol): 279, 253. – ^{1}H-NMR. (DMSO-d_6): *cf. Table 1;* moreover, 1.64 (*s*, 3 H); 3.26 (*s*, 3 H); 4.40 (*d*, *J* = 9, 1 H; *s* after exchange with D_2O); 6.34 (*d*, *J* = 9, 1 H; exchangeable with D_2O); 7.4–7.8 (*m*, 3 H); 8.2–8.3 (*m*, 2 H); 9.96 (*s*, 1 H; exchangeable with D_2O). – ^{13}C-NMR.: *cf. Table 2.*

The probably not genuine pseurotin B (**2**) and C (**3**) were isolated by column chromatography from the crude extract of a 500-l fermentation carried out a few years ago and purified by TLC. (CH_2Cl_2/methanol 93:7). *Pseurotin B* (**2**): colourless crystals, m.p. 204–206° (THF/ether). – IR. (KBr): 3440, 3340, 3240, 2980, 2920, 1720, 1690, 1605. – UV. (ethanol): 279, 253. – ^{1}H-NMR. (DMSO-d_6): *cf. Table 1;* moreover, 1.65 (*s*, 3 H); 3.24 (*s*, 3 H); 4.41 (*d*, *J* = 9, 1 H; *s* after exchange with D_2O); 6.33 (*d*, *J* = 9, 1 H; exchangeable with D_2O); 7.4–7.8 (*m*, 3 H); 8.2–8.3 (*m*, 2 H); 9.94 (*s*, 1 H; exchangeable with D_2O). – ^{13}C-NMR.: *cf. Table 2.* – MS.: 446, 347, 342, 315, 297

$C_{22}H_{25}NO_9$	Calc.	C 59.05	H 5.63	N 3.13 O 32.18%
(447.4)	Found	„ 59.16	„ 5.74	„ 3.15 „ 32.26%

Pseurotin C (**3**): colourless foam. – IR. (KBr): 3500–3200, 2970, 2930, 1720, 1700–1680, 1620. – UV. (ethanol): 279, 253. – ^{1}H-NMR. (DMSO-d_6): *cf. Table 1;* moreover, 1.63 (*s*, 3 H); 3.24 (*s*, 3 H); 4.40 (*d*, *J* = 9, 1 H; exchangeable with D_2O); 6.27 (*d*, *J* = 9, 1 H; exchangeable with D_2O); 7.4–7.8 (*m*, 3 H); 8.2–8.3 (*m*, 2 H); 9.92 (*s*, 1 H; exchangeable with D_2O). – ^{13}C-NMR.: *cf. Table 2.*

Periodate cleavage of **1**, **2** *and* **3**. A solution of 18 mg (0.04 mmol) of **1**, **2** or **3** in *ca.* 4 ml of methanol was cooled in an ice bath. A solution of 20 mg (0.09 mmol) of $NaIO_4$ in a minimal amount of water was added dropwise and the mixture stirred after removing the cooling. The oxidation was followed by TLC. (CH_2Cl_2/methanol 93:7). After ½ h all starting material had disappeared. After filtration and washing with methanol, the filtrate was concentrated i.V. (30°). The residue was purified by column chromatography on *florisil* (100–200 mesh *(Sigma);* CH_2Cl_2/methanol) yielding about 10 mg of the unstable *8-benzoyl-9-hydroxy-8-methoxy-3-methyl-4,6-dioxo-1-oxa-7-azaspiro[4.4]-non-2-ene-2-carbaldehyde* (**9**) as colourless oil. – IR. (CH_2Cl_2): 3520, 3400, 2940, 1745, 1700, 1680, 1600. – UV. (ethanol): 277, 252. – ^{1}H-NMR. (90 MHz, $CDCl_3$): 2.02 (*s*, 3 H); 3.41 (*s*, 3 H); 3.9–4.2 (br., 1 H; exchangeable with D_2O); 4.72 (br., 1 H; *s* after exchange with D_2O); 7.4–7.8 (*m*, 3 H); 7.8 (br., 1 H; exchangeable with D_2O); 8.2–8.4 (*m*, 2 H); 9.96 (*s*, 1 H). – ^{13}C-NMR. (22.63 MHz, $(CD_3)_2CO$): 5.3, 52.2, 76.1, 92.4, 93.5, 120.8, 129.2 (2 C), 131.3 (2 C), 134.6 (2 C), 165.9, 172.1, 195.9, 199.7 (1 signal for a sp^2-carbon can not be detected).

[4]) The systematic name of **4** is: (5*S*,8*S*,9*R*)-8-benzoyl-2-(1′,4′-dihydroxy-2′-hexenyl)-9-hydroxy-8-methoxy-3-methyl-1-oxa-7-azaspiro[4.4]non-2-ene-4,6-dione.

The other fragment from **2** and **3**, (*E*)- and (*Z*)-4-hydroxy-2-pentenal, respectively, or the corresponding cyclic hemiacetals, could never be observed although many attempts were made to trap them. For the (*Z*)-2-pentenal from **1** *cf.* [2].

Hydrogenation of pseurotin B (**2**). After adding 67 mg of tris(triphenylphosphine)rhodium(I) chloride to a solution of 100 mg (0.22 mmol) of **2** in 15 ml of ethanol/benzene 1:1, the mixture was stirred under a H_2-pressure of 100 atm during 7 h at RT. The solvent was then removed i.V., and the crude extract separated from the catalyst by passing it through a short column. Purification by TLC. (CH_2Cl_2/methanol 93:7) gave 40 mg of *12,13-dihydropseurotin B* (**8**). – ^{1}H-NMR. (DMSO-d_6): 1.01 (*d*, J=6, 3 H); 0.8–1.8 (*m*, 4 H); 1.65 (*s*, 3 H); 3.25 (*s*, 3 H); 3.2–3.8 (*m*, 2 H); 4.39 (*d*, J=9, 1 H; *s* after exchange with D_2O); 4.58 (br., 1 H; exchangeable with D_2O); 4.3–4.6 (*m*, 2 H; 1 H exchangeable with D_2O); 5.69 (*d*, J=6, 1 H; exchangeable with D_2O); 6.28 (*d*, J=9, 1 H; exchangeable with D_2O); 7.3–7.8 (*m*, 3 H); 8.2–8.3 (*m*, 2 H); 9.94 (*s*, 1 H; exchangeable with D_2O). – ^{13}C-NMR.: *cf. Table 2.*

Hydrogenation of pseurotin C (**3**). About 200 mg of crude **3** containing *ca.* 0.2 mmol of **3** were dissolved in 30 ml of ethanol/benzene 1:1, 135 mg tris(triphenylphosphine)rhodium(I) chloride were added, and the mixture was hydrogenated under normal pressure at RT. over night. Work-up as above (**2** → **8**), and purification by column chromatography on silica gel and TLC. (CH_2Cl_2/methanol 93:7) gave 30 mg of *12,13-dihydropseurotin C*, identical by ^{1}H- and ^{13}C-NMR. with 12,13-dihydropseurotin B (**8**).

Synthesis of (5S,6R,E)-5,6,7-trihydroxy-3-heptene-2-one (**6**). Stabilized *Wittig*-reagent (3.82 g; 12 mmol) prepared from triphenylphosphine and chloroacetone according to [9] was added to a solution of 1 g (8.3 mmol) of D-erythrose in 20 ml of DMF. The mixture was stirred at 65° for 4 h. The solvent was then removed i.HV., the dark-brown residue dissolved in hot water and extracted with CH_2Cl_2. The organic layer was discarded, and the aqueous extract yielded after evaporation 1.3 g of a yellow oil which contained according to ^{1}H-NMR. *ca.* 70% of **6**. It was further purified by column chromatography on silica gel to give TLC.-pure (BuOH sat. with water), but unstable **6** as colourless oil. – ^{1}H-NMR.: *cf. Table 1.*

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