29. Isolation and Structure of Pseurotin A, a Microbial Metabolite of *Pseudeurotium ovalis* STOLK with an Unusual Heterospirocyclic System

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Summary

The structure and absolute configuration of pseurotin A, a new metabolite, isolated from culture filtrates of *Pseudeurotium ovalis* STOLK *(Ascomycetes)* has been shown to be (1′*S*,2′*S*,5*S*,8*S*,9*R*,3′*Z*)-8-benzoyl-2(1′,2-dihydroxy-3′-hexenyl)-9-hydroxy-8-methoxy-3-methyl-1-oxa-7-azaspiro[4.4]non-2-ene-4,6-dione (**1**).

1. Introduction. – In 1968 *Sigg et al.* [1] isolated ovalicin from cultures of *Pseudeurotium ovalis* STOLK *(Ascomycetes)* as main metabolite and determined its structure. The biosynthesis [2] and the immuno-suppressive activity [3] of the new compound were studied. As reported in preliminary communications, we have found a second minor metabolite in the cultures of the same organism, named pseurotin A[2]). Its structure **1** was elucidated by spectral data and chemical transformations [4], and by X-ray analysis of its dibromo derivative **2** [5]. In this paper the details of this investigation are presented[3]).

2. Spectral Data and Chemical Transformations of 1. – Pseurotin A (**1**) was isolated from the culture broth of *Pseudeurotium ovalis* (strain S 2269/F) by extraction with ethylene chloride. After final purification of the crude extracts by column chromatography on silica gel, the new metabolite was obtained from dichloromethane/hexane as colourless rhombic crystals of m.p. 162–163.5° and $[\alpha]_D^{20} = -5 \pm 1°$ ($c = 0.5$, methanol). The yield was *ca.* 30–35 mg/liter culture broth.

Pseurotin A (**1**), a neutral substance, is readily soluble in polar solvents but much less soluble in non-polar solvents. Heating of solutions during a longer period of time caused decomposition of the compound. The molecular formula, $C_{22}H_{25}NO_8$, was deduced from elemental analyses. In the mass spectrum the molecular ion is not observed. The highest peak at m/z 399 ($M^+ - 32$) is due to the loss of methanol, as it is frequently observed with methyl ethers (especially with α-methoxy ketones) and methyl esters [6a] [6b]. The difference in mass of 32

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[2]) The compound was called originally pseurotin.

[3]) We thank Drs. *P. Bollinger* and *H.P. Sigg, Sandoz AG*, Basel, for the results of some preliminary experiments and a larger amount of crude pseurotin A.

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is also observed between the peaks at *m/z* 347 ($M^+ - C_5H_8O$) and 315 (base peak). The peaks at *m/z* 326 ($M^+ - 105$), 105 ($C_6H_5CO^+$) and 77 ($C_6H_5^+$), as well as the typical aryl fragments at *m/z* 51 and 39, indicate the presence of an *O*- or *C*-benzoyl group. The frequently occurring mass difference of 18 (H_2O) is probably due to alcoholic hydroxyl groups. One methoxy group is detected by *Zeisel* determination, and one vicinal dihydroxy grouping by periodate titration. Although pseurotin A (**1**) reduces silverdiamine (*Tollens* reaction) to silver, it forms neither an oxime nor a 2,4-dinitrophenylhydrazone under the usual conditions.

The UV. spectrum of pseurotin A (**1**) exhibits absorption maxima at 253 ($\log \varepsilon = 4.22$ in ethanol) compatible with a phenyl ketone [7] and at 2.79 (4.05) nm. The latter band has a slightly bathchromic shift compared with the one of simple 3-(2*H*)-furanone systems [8]. The IR. spectrum (KBr) shows bands at 3570, 3440, 3275 (OH, NH), 1725, 1690–1680 (C=O), 1610 (C=C–C=O), 740 and 705 (monosubstituted benzene ring) cm^{-1}. One of the two C=O stretching vibrations at 1690–1680 cm^{-1} together with the band at 3275 cm^{-1} can be assigned to a lactame. The absence of an amide band II seems to preclude an open chain amide group. The third carbonyl band and the strong C=C stretching vibration at 1610 cm^{-1} indicate the presence of an α,β-unsaturated carbonyl group[4]).

The ^{1}H-NMR. spectrum (DMSO) (s. *Fig.*) of pseurotin A (**1**) indicates the presence of a benzoyl group (multiplets at 8.2 (2 H) and 7.5 ppm (3 H), of one methoxy group (singlet at 3.26 ppm (3 H)) and of an additional methyl group (singlet at 1.65 ppm (3 H)) which is assigned to $H_3C(16)$. On addition of D_2O the singlet of an amide or imide proton at 9.9 ppm[5]) and the doublets of three secondary hydroxyl groups disappear. One of these doublets (6.12 ppm, $J = 7$ Hz, HO–C(9)) forms an *AB*-system with another doublet at 4.41 ppm ($J = 7$ Hz, H–C(9)). By spin-spin decoupling experiments all signals of the side chain from C(10) to C(15) could be assigned. The doublets at 5.68 ppm ($J = 5$ Hz) and 4.78 ppm ($J = 4$ Hz) correspond to HO–C(10) and HO–C(11), and a multiplet at 4.2–4.6 ppm to H–C(10) and H–C(11). A multiplet at 5.1–5.7 ppm is due to the two vinylic protons at C(12) and C(13), and a multiplet at 2.1 ppm and a triplet at 0.90 ppm ($J = 7$ Hz, 3 H) to an ethyl group (C(14) and C(15)) attached to C(13). If the ^{1}H-NMR. spectrum of pseurotin A (**1**) is measured in $CDCl_3$ solution, the signal of H–N is at much higher field (*ca.* 8.5 ppm), but is also very sharp. Its chemical shift depends on the concentration of the solution. Usually these protons appear as a broad signal and only at such a low field if special structural features are encountered [10]. The effect might partially be due to strong H-bonding [11a].

According to these data the following structural elements are present in pseurotin A (**1**): CH(OH), OCH_3, CH_3, NHCO, C_6H_5CO and *cis*-$CH_3CH_2CH{=}CHCH(OH)CH(OH)$.

The positive hydroxamic acid test and the fragment *m/z* 43 in the MS. are compatible with both an imide or lactame ($CONH^+$) and an *O*-acetyl group (CH_3CO^+). However, the latter possibility can be ruled out by the absence of

[4]) The spectroscopic data would also be compatible with an α,β-unsaturated imide, α,β-unsaturated lactame or an enamide.

[5]) The appearance of this signal at such a low field is remarkable, even if one assumes a strong association with the solvent [9].

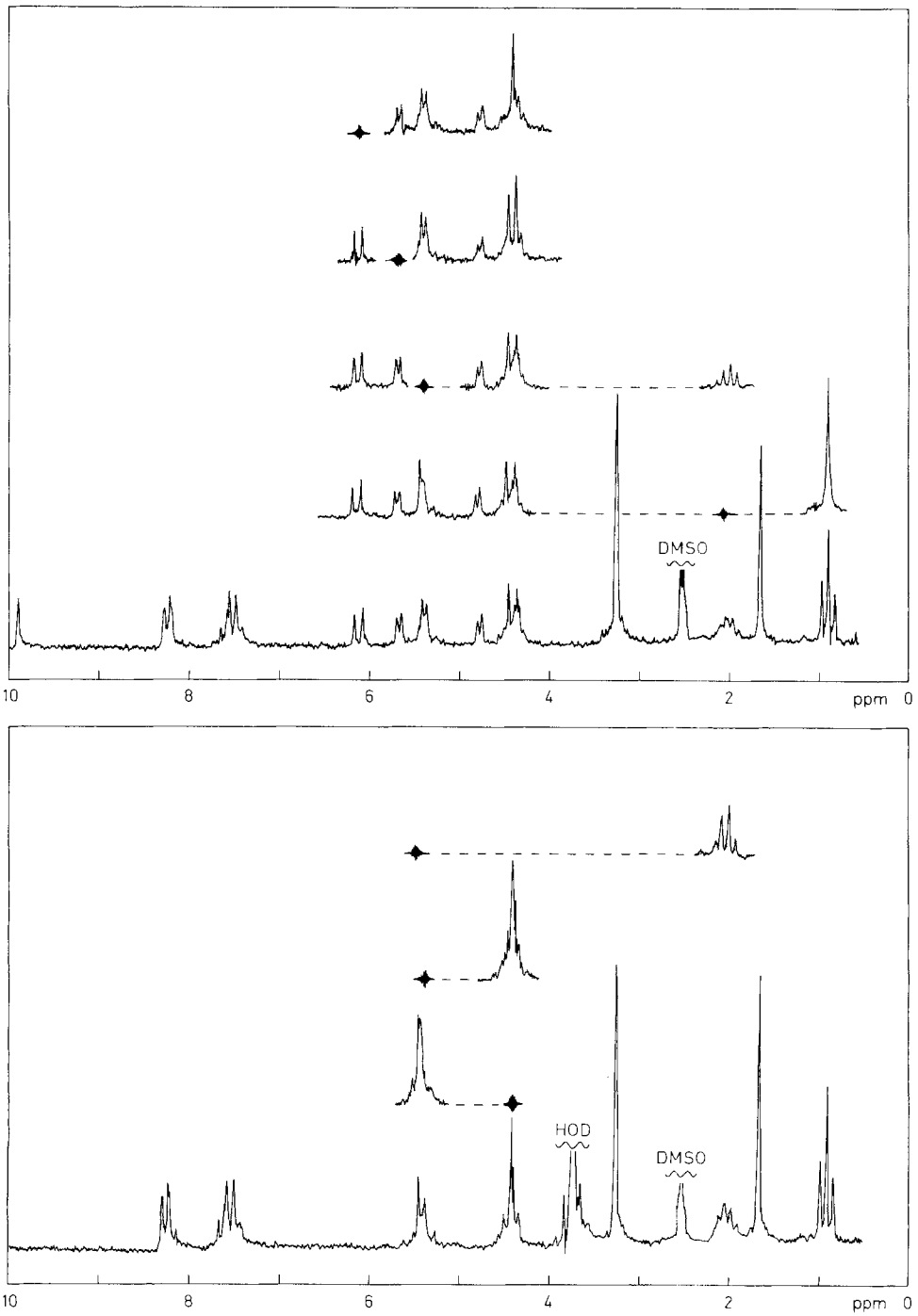


Figure. *^{1}H-NMR. spectrum (100 MHz) of pseurotin A* (**1**) *in $(CD_3)_2SO$ and spin-spin-decoupling experiments after addition of D_2O*

acetate bands in the IR. and of the fragmentation of methyl groups in the MS. The presence of the C_6-side chain, as deduced from the ^{1}H-NMR. spectrum, is also in agreement with the butenyl fragment *m/z* 55 ($C_4H_7^+$) in the MS. An electrocyclic reaction of the radical ion (*McLafferty* rearrangement), as demonstrated by the fragments *m/z* 84 ($C_5H_8O^+$) and *m/z* 347 ($C_{17}H_{17}NO_7$), leads to the cleavage of the vicinal dihydroxy grouping and to the stereoselective hydrogen transfer from the side chain to the larger moiety [6c].

It is interesting to note that the ^{1}H-NMR. data of pyriculol, a metabolite of *Pyricularia oryzae,* which possesses a similar side chain as pseurotin A (**1**) show very similar features [12].

In spite of the reducing properties of **1** the ^{1}H-NMR. spectrum does not reveal an aldehyde proton. This observation points to a masked aldehyde or an α-hydroxy ketone which also give a positive *Benedict* and *Fehling* test [13].

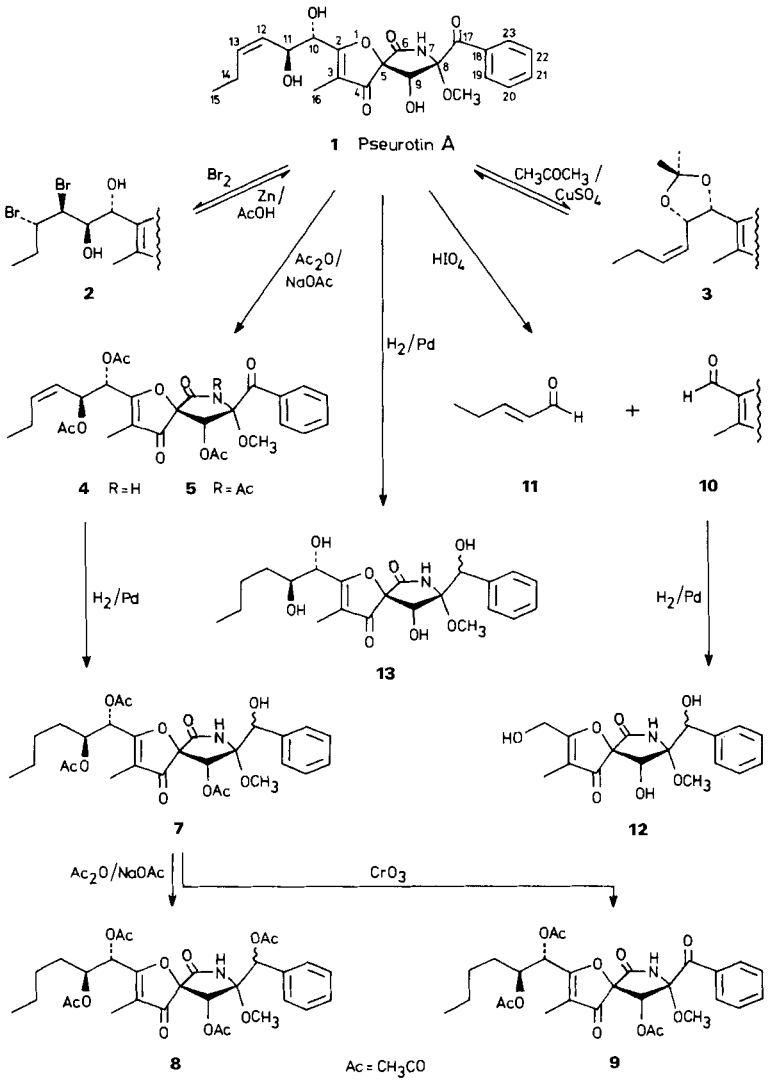
So far a C_5O_2 residue with three degrees of unsaturation has still to be accomodated. The following chemical transformations confirmed these conclusions and provided some indications concerning the nature of the unknown structural elements. The final proof of structure and the determination of the absolute configuration of pseurotin A (**1**) was adduced by the X-ray analysis of the 12,13-dibromo derivative **2** [5].

Pseurotin A (**1**) is relatively stable to cold dilute acids; hot mineral acids provoke decarboxylation. On treatment with NaOH, KOH or $Ba(OH)_2$ an intense red colour appeared and ammonia was released, which was isolated as ammonium picrate. Heating of pseurotin A (**1**) with 0.5 N NaOH or with a saturated methanolic hydrogen carbonate solution led to the formation of benzoic acid and a neutral compound which could not be identified because of its instability.

Oxidation of **1** with HNO_3 followed by esterification with CH_2N_2 gave methyl benzoate. After treatment of **1** with ozone in methanol propionic acid was obtained. It was identified as its benzhydryl ester. Stable products were not obtained, neither with CrO_3/H_2SO_4 in acetone [14] nor with MnO_2 in methylene chloride or boiling benzene [15]. With Ag_2CO_3 on *Celite* in benzene [16] **1** remained unchanged. The reaction of **1** with HIO_4 will be discussed further below.

Treatment of pseurotin A (**1**) with Br_2 in chloroform at 0° yielded a 7:3 mixture of two colourless crystalline isomeric products of similar polarity which were separated by preparative TLC. They had the molecular formula $C_{22}H_{25}Br_2NO_8$. The more polar component was not obtained in pure form because it decomposed quite readily. The less polar compound possesses the structure of (12*S*,13*S*)-12,13-dibromo-12,13-dihydro pseurotin A (**2**) according to X-ray diffraction and the following spectral data.

In the MS. of **2** the peak of M^+ at 591 is again absent, the base peak appearing at *m/z* 105. The fragments *m/z* 488, 486 and 484 show the isotope distribution of a dibromo derivative; they correspond to the fragment $M^+ - 105$. In the ^{1}H-NMR. spectrum of **2** two new signals at 4–5 ppm (multiplet) with $J(12,13) = 6$ Hz appear. This value is in agreement with the (*Z*)-configuration of the original double bond, whose bromination leads to the (12*S*,13*S*)- and/or to the (12*R*,13*R*)-isomer having *threo*-configuration. The torsion angle of both is *ca.* 60° for the most favorable conformation with antiperiplanar arrangement of the Br-atoms. The calculated value of the coupling constant is 2–5 Hz. Bromination of an (*E*)-double bond would yield an *erythro*-isomer having a torsion angle of *ca.* 180° and a coupling constant of 10–13 Hz [11b]. The value of the coupling constant $J(11,12)$



is only 2 Hz in the 12,13-dibromo derivative **2** as compared to 7 Hz in pseurotin A (**1**)[6]). Thus the Br-atoms cause an important change of conformation.

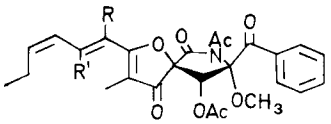
Removal of bromine from **2** with Zn in acetic acid/ether/dimethylformamide [17] led back to **1** in 30–40% yield, thus excluding the possibility of undesired secondary reactions during bromination of **1**. The bromination must occur as *trans*-addition to the double bond, *cis*-addition to an (*E*)-double bond being very unlikely. Since the debromination is a *trans*-elimination the original double bond of pseurotin A (**1**) must possess (*Z*)-geometry.

[6]) This value was determined from a 360-MHz-^{1}H-NMR. spectrum in $(CD_3)_2CO$ by irradiation at the H–C(10) frequency at 4.57 ppm. It caused the simplification of the H–C(11)-signal to a doublet of doublet (allylic coupling with H–C(13)).

The reaction of pseurotin A (**1**) with acetone and $CuSO_4$ at room temperature yielded the isopropylidene derivative **3** which proved to be unstable.

Mild acetylation of the metabolite **1** with acetic anhydride and sodium acetate at room temperature gave the crystalline tri-*O*-acetyl derivative **4** as main product besides the oily tetraacetyl derivative **5**. The ratio **4**/**5** depends on the reaction time, **5** becoming the main product after 40 hours. In the presence of pyridine instead of sodium acetate as base **5** was formed almost exclusively. The spectral data were in agreement with the structures **4** and **5**.

Acetylation of **1** at higher temperature gave instead of **4** and **5** compound **6** as a viscous, unstable, yellow oil. The trienone structure is based on the spectral data.



6a R=H; R'=OAc
6b R=OAc; R'=H

In the UV. spectrum of **6** the longest wavelength band is shifted to 333 nm ($\log\varepsilon = 4.38$). The MS. exhibits a peak at *m/z* 539, 60 mass units lower than the calculated value for **5** ($C_{30}H_{33}NO_{12}$, 599). In the ^{1}H-NMR. spectrum which accounts for a total of 29 protons, signals for three acetyl groups appear at 2.5, 2.3 and 2.1 ppm. Whereas the protons of the *N*-acetyl and phenyl group and H–C(9) have the same chemical shifts as in **5**, the signals at 6.9 ppm (*m*, 1 H) and 6.2 ppm (*m*, 2 H) indicate that only three protons are present for the side chain from C(10) to C(13)).

It is concluded that compound **6** has been formed from the tetraacetyl derivative **5** by elimination of acetic acid in the side chain. A decision between structure **6a** and **6b** is not possible on the basis of the evidence available at present.

Catalytic hydrogenation of the tri-*O*-acetylpseurotin A **4** with 10% Pd/C in ethanol produced the tetrahydro derivative **7**. One of the two possible epimeric alcohols was predominant, the 9-acetoxy group increasing the stereoselectivity of the reduction.

In the IR. spectrum of **7** a new band at 3600 cm^{-1} replaced an absorption at 1690 cm^{-1} in **4**. The UV. maximum at 250 nm had disappeared. In the ^{1}H-NMR. spectrum of **7** all aromatic protons appear as a multiplet at 7.4 ppm. A new singlet at 4.9 ppm is assigned to a benzylic proton adjacent to fully substituted C-atoms[7]). In the range of 1 to 1.8 ppm a multiplet of three methylene groups is visible.

Acetylation of **7** with acetic anhydride and sodium acetate led to the oily tetra-*O*-acetyl derivative **8**, for which the IR. spectrum shows no band at 3600 cm^{-1}. In the ^{1}H-NMR. spectrum the singlet of the benzylic proton is shifted to 6.03 ppm. Oxidation of **7** with CrO_3 gave the ketone **9** as an oil, which exhibited all spectral data characteristic of the benzoyl group.

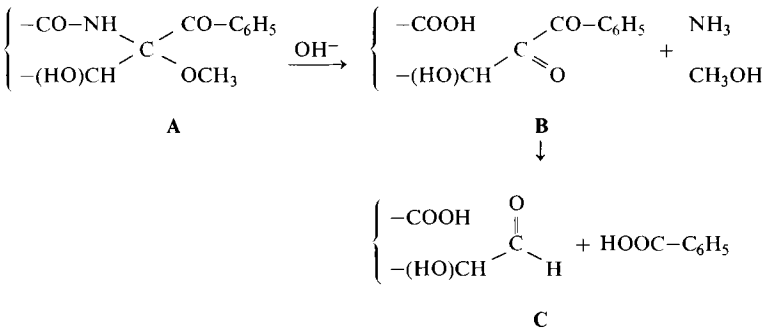
On treatment of pseurotin A (**1**) with 1 equivalent of $NaIO_4$ in aqueous methanol at pH 2 under N_2 in the dark compounds **10** and (*E*)-2-pentenal (**11**) were obtained. The latter was isolated as 2,4-dinitrophenylhydrazone. An acid-catalyzed isomerization of the genuine, thermodynamically less stable (*Z*)-double bond of pseurotin A (**1**) had taken place[8]). The aldehyde **10**, which was isolated

[7]) In $CDCl_3$ as solvent no coupling with the hydroxylic protons can be detected.

[8]) The 2,4-dinitrophenylhydrazone of (*Z*)-2-pentenal [18] is not known. However, the ^{1}H-NMR. analysis of the crude reaction mixture showed that the isomerization had taken place before the aldehyde was trapped by the acidic solution of the hydrazine reagent.

as a yellowish oil in nearly pure form from a second experiment decomposed readily. The splitting of all ^{1}H-NMR. signals of **10** in DMSO led us to assume that an epimerization at C(9) took place besides other reactions. However, in $CDCl_3$ and $(CD_3)_2CO$ **10** was stable enough for recording ^{1}H- and ^{13}C-NMR. spectra, which confirm its structure. The IR. spectrum shows a broadened carbonyl band at 1710–1680 cm^{-1}. The aldehyde band appears at *ca.* 1690 cm^{-1} between the absorption of the benzoyl and α,β-unsaturated keto group.

Whereas oxidation of **10** under various conditions did not yield a stable derivative[9]), catalytic hydrogenation with 10% Pd/C in ethanol gave the somewhat more stable triol **12** as a viscous oil in 84% yield. In the IR. spectrum the carbonyl bands at 1690 and 1680 cm^{-1} were absent indicating the reduction of the aldehyde and 17-keto group. Catalytic hydrogenation of pseurotin A (**1**) with 10% Pd/C in ethanol led to the stable reduction product **13**, which was a viscous oil. In the UV. spectrum of **13** the absorption at 253 nm of **1**, and in the IR. spectrum one carbonyl band of **1** had disappeared. Both **13** and **12** were inseparable mixtures of substituted epimeric benzyl alcohols. The splitting of their signals in their ^{1}H-NMR. spectra indicated close proximity of the C_6H_5CO, CH_3O, CH(OH), and NHCO groups in pseurotin A (**1**) as shown by the partial structure **A**. Such a structural arrangement readily explains the loss of methanol in the mass spectrum of **1**, the chemical shift of its amide proton at 9.9 ppm [19], and its reducing properties. The appearance of an intense red colour and release of ammonia on treatment with $Ba(OH)_2$ is consistent with **A**. It would lead to the 1,2-diketone **B** which eliminates readily benzoic acid under basic conditions (*cf.* [20]).



Reaction of pseurotin A (**1**) with $NaBH_4$ in ethanol as well as with $LiAlH_4$, led – not unexpectedly in view of the different reducible functional groups – to complex mixtures of unseparable and unstable products[10]).

The final determination of the structure and the absolute configuration of pseurotin A (**1**) as (1′*S*,2′*S*,5*S*,8*S*,9*R*,3′*Z*)-8-benzoyl-2-(1′,2′-dihydroxy-3′-hexenyl)-9-hydroxy-8-methoxy-3-methyl-1-oxa-7-azaspiro[4.4]non-2-ene-4,6-dione was achieved by an X-ray diffraction analysis of the dibromo derivative **2** [5].

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[9]) The desired carboxylic acid is probably decarboxylated. In addition, the resulting furanone is probably sensitive to further oxidation.

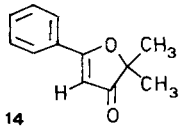
[10]) Selective reductions were achieved with $NaBH_3CN$ and $H_2/((C_6H_5)_3P)_3RhCl$ [21].

Table. *Chemical shifts (in ppm) of the C-atoms in the ^{13}C-NMR. spectra of pseurotin A* (**1**)

C-atom	in $(CD_3)_2CO$	in $(CD_3)_2SO$	C-atom	in $(CD_3)_2CO$	in $(CD_3)_2SO$
C(2)	167.3	166.6	C(13)	135.9	134.0
C(3)	113.6	111.6	C(14)	21.8	20.6
C(4)	197.9	196.7	C(15)	14.5	14.1
C(5)	92.7	92.4	C(16)	5.8	5.6
C(6)	187.7	186.8	C(17)	196.2	196.4
C(8)	92.4	91.1	C(18)	134.7	133.6
C(9)	75.3	75.0	C(19) and C(23)	131.3	130.2
C(10)	72.8	72.0	C(20) and C(22)	129.2	128.4
C(11)	69.7	68.3	C(21)	134.7	133.6
C(12)	129.0	128.4	CH_3O	52.2	51.7

3. ^{13}C-NMR. spectra. – The ^{13}C-NMR. spectra of pseurotin A (**1**) were measured especially in view of the biogenetic incorporation experiments with ^{13}C-labeled precursors. The proton-noise-decoupled spectrum exhibits only 19 signals for the 22 C-atoms. The aromatic part gave only three lines. The assignments which are summarized in the *Table* are based on the comparison with the off-resonance-decoupled spectrum and with the spectra obtained from the derivatives such as (12*S*,13*S*)-12,13-dibromo-12,13-dihydropseurotin A (**2**), 12,13,17-(17-*O*)-tetrahydropseurotin A (**13**), 12,13-dihydropseurotin A and 17,(17-*O*)-dihydropseurotin A. The synthesis of the latter two compounds and the spectra of all derivatives and of the four other pseurotins, which agree well with the here presented assignments, are discussed elsewhere [21] [22]. The unambiguous distinction of C(5) and C(8) whose chemical shifts are separated only by 0.3 ppm in acetone was achieved by the incorporation of doubly labeled [2,3-$^{13}C_2$]phenylalanine [21].

4. Final Remarks. – The 1-oxa-7-azaspiro[4.4]nonane skeleton is a very rare system and only a few derivatives are known. Thus, comparison of spectral data with those of pseurotin A (**1**) are limited to partial structures such as dihydro-



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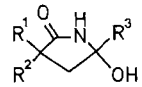

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furanone derivatives. Useful examples are bullatenone (**14**) and some other substituted dihydrofuranones, whose spectral characteristics agree well with those of the corresponding structural elements of **1** [23]. Also the ^{1}H-NMR. data of 5-hydroxy-2-pyrrolidones **15** revealed some interesting parallels to the ones of pseurotin A (**1**) [19].

Having established the structure of the new microbial metabolite the question of the biogenetic formation of the unusual spirocyclic system and its substitution pattern arises. Experimental studies which lead to a proposal of a hypothetic biogenetic pathway are reported in [21].

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Experimental Part

General Methods. S. [24]. Moreover, the 100-MHz-^{1}H-NMR. spectra (CW) were determined on a *Varian* HA 100 D spectrometer in the Institute of Physical Chemistry, Basel, or in the spectral laboratories of *Sandoz AG*, Basel. The mass spectra (*m/z*) were measured by Mr. *A. Raas* at the Institute of Physical Chemistry, Basel, on a *Hitachi Perkin-Elmer* model RMU 7 instrument. The optical rotation was determined on a *Perkin-Elmer* model 141 polarimeter. Preparative thin-layer chromatography (TLC.) was carried out on silica gel 60 PF_{254} from *E. Merck AG*, Darmstadt, and column chromatography on silica gel 0.063–0.2 mm *(Merck)*.

Isolation of pseurotin A (**1**)[11]. The culture broth from a 450 l fermentation of *Pseudeurotium ovalis* STOLK (S 2269/F) (120 h, 27°) was extracted with ethylene chloride. After evaporation of the solvent the residue was dissolved in 5 liter of methanol (90%) and washed 3 times with 3 liter of petroleum ether. The methanolic phase was concentrated to half the volume, diluted with 500 ml of water, and the organic solvent evaporated i.V. The aqueous residue was carefully extracted with $CHCl_3$ yielding 37.3 g of an oil, from which after addition of ether 5.1 g of crystalline **1** could be isolated. After column chromatography on silica gel additional 7.5 g of pure **1** could be obtained, m.p. after several recrystallizations from CH_2Cl_2/hexane 162–163.5°, $[\alpha]_D^{20} = -5 \pm 1°$ ($c = 0.5$, methanol). – IR. (KBr): 3570, 3440, 3275, 2965, 1725, 1690–1680, 1610, 740, 705. – UV. (ethanol): 279 (4.05), 253 (4.22). – ^{1}H-NMR. (100 MHz, DMSO-d_6): 0.90 (*t*, $J = 7$, 3 H); 1.65 (*s*, 3 H); 2.1 (*m*, 2 H); 3.26 (*s*, 3 H); 4.41 (*d*, $J = 7$, 1 H; *s* after exchange with D_2O); 4.2–4.6 (*m*, 2 H); 4.78 (*d*, $J = 4$, 1 H, exchangeable with D_2O); 5.1–5.7 (*m*, 2 H); 5.68 (*d*, $J = 5$, 1 H, exchangeable with D_2O); 6.12 (*d*, $J = 7$, 1 H, exchangeable with D_2O); 7.4–7.8 (*m*, 3 H); 8.2–8.4 (*m*, 2 H); 9.9 (*s*, 1 H; exchangeable with D_2O). – ^{13}C-NMR.: *cf. Table.* – MS.: no M^+ (calc. for $C_{22}H_{25}NO_8$: 431.4), 399, 347, 326, 315.

Periodate-cleavage of pseurotin A (**1**). A solution of 150 mg (0.35 mmol) of **1** in 5 ml of methanol was added to a solution of 250 mg of $NaIO_4$ in 10 ml of water whose pH-value was adjusted with 0.5N H_2SO_4 to 2. The mixture was stirred under N_2 in the dark for 3 h. After evaporation of the solvent at 20° i.V. the aqueous residue was extracted in a continuous liquid-liquid extractor with ethyl acetate yielding 102 mg of nearly pure *8-benzoyl-9-hydroxy-8-methoxy-3-methyl-4,6-dioxo-1-oxa-7-azaspiro[4.4]non-2-en-2-carbaldehyde* (**10**) 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-C-atom can not be detected).

For identifying *(E)-2-pentenal* (**11**) 1 g (2.3 mmol) of **1** was treated with HIO_4 as above. The crude product was extracted with ether instead of ethyl acetate. From the distillate **11** could be trapped as 2,4-dinitrophenylhydrazone yielding, after recrystallization from ethanol, 24 mg of cinnabar red needles of m.p. 159–160° (lit. [25]: 160°).

$C_{11}H_{12}N_4O_4$ (264) Calc. C 50.00 H 4.58 N 21.20% Found C 50.22 H 4,75 N 21.02%

Preparation of (12S,13S)-12,13-dibromo-12,13-dihydropseurotin A (**2**). A solution of 57 mg (0.36 mmol) of Br_2 in 2.1 ml of $CHCl_3$ was added slowly in the dark to a stirred solution of 154 mg (0.36 mmol) of **1** in 5 ml of $CHCl_3$. The temp. was kept at 0° by cooling with ice. After stirring the mixture for additional 5 min the solvent was evaporated i.V. (20°). The yellow residue was diluted with ether yielding a fine precipitate from which by TLC. (benzene/isopropyl alcohol 97.5:2.5) 70 mg of **2** could be isolated as yellow crystals, m.p. 154–156° (decomp.) after recrystallization from ethanol/ether. Besides, 25 mg of a more polar component were obtained thought to be the (12*R*,13*R*)-isomer. Purification of the mother liquor by column chromatography on silica gel (benzene/isopropyl alcohol) afforded additional 30 mg of **2**. Crystals for X-ray analysis were obtained by diluting slowly a solution of **2** in ethyl acetate with cyclohexane at 70°. $[\alpha]_D^{20} = -28°$ ($c = 0.4$, methanol), $d_4^{20} = 1.645 \pm 0.015$ g/cm^3. – IR. (KBr): 2970, 1720–1670, 1615, 740, 690. – ^{1}H-NMR. (100 MHz, DMSO-d_6): 1.01 (*t*, $J = 7$, 3 H); 1.70 (*s*, 3 H); 1.8–2.2 (*m*, 2 H); 3.30 (*s*, 3 H); 4.0–4.2 (*m*, 1 H; *d*×*d* after exchange

[11]) The isolation of pseurotin A (**1**) was done by Mr. *F. Geissmann* and *A. Toth* at *Sandoz AG*, Basel.

with D_2O, $J=2.8$); 4.3–4.6 (*m*, 1H); 4.48 (*d*, $J=8$, 1H; *s* after exchange with D_2O); 4.58 (*m*, 1H; *d* after exchange with D_2O, $J=8$); 4.77 (*d*×*d*, $J=2$ and 6, 1H); 5.32 (*d*, $J=7$, 1H; exchangeable with D_2O); 6.2 (*d*, 1H; exchangeable with D_2O); 6.28 (*d*, $J=8$, 1H; exchangeable with D_2O); 7.4–7.8 (*m*, 3H); 8.2–8.4 (*m*, 2H); 9.98 (*s*, 1H; exchangeable with D_2O). – MS.: no M^+ (calc. for $C_{22}H_{25}Br_2NO_8$: 591±2), 486±2, 105.

$C_{22}H_{25}Br_2NO_8$	Calc.	C 44.69	H 4.26	Br 27.03	N 2.37%
(591)	Found	„ 45.16	„ 4.31	„ 26.68	„ 2.39%

Pseurotin A (**1**) *from* **2**. To a solution of 83 mg (0.14 mmol) **2** in 10 ml of ether containing 3 ml of DMF and 4 drops of acetic acid were added 100 mg of activated zinc dust (2N HCl). The reaction was followed by TLC. After 3 h the mixture was diluted with ether to 150 ml and washed with 0.5N HCl and water. Evaporation of the solvent yielded a brown foam which was further purified by repeated TLC. (benzene/ethyl acetate/isopropyl alcohol 40:58:2; ethyl acetate/methanol 98.5:1.5) giving 18 mg of pure **1** identical with an authentic sample (Rf in different solvents, optical rotation, IR. and ^{1}H-NMR.).

Acetylation of **1** *with acetic anhydride/sodium acetate.* A solution of 100 mg (0.23 mmol) of **1** in 5 ml of acetic anhydride was shaken 18 h together with 0.5 g of anhydrous sodium acetate. The mixture was then poured onto ice/water and extracted with ether. After evaporation of the solvent the residue was recrystallized from ether to yield 40 mg of *(9-O),(10-O),(11-O)-triacetylpseurotin A* (**4**) as colourless needles, m.p. 126–128°, $[\alpha]_D^{20}=-23°$ ($c=0.23$, methanol). – UV. (methanol): 253 (4.12), 279 (4.01). – IR. (CH_2Cl_2): 3400, 3000, 1760, 1720, 1690, 1650, 1600. – ^{1}H-NMR. (90 MHz, $CDCl_3$): 0.99 (*t*, $J=7$, 3H); 1.79 (*s*, 3H); 2.02 (*s*, 9H); 2.0–2.2 (*m*, 2H); 3.50 (*s*, 3H); 5.27 (br. *d*×*d*, $J=11$ and 9, 1H); 5.57 (*d*, $J=7$, 1H); 5.71 (*s*, 1H); 5.7 (*m*, 1H); 5.9 (br. *d*×*d*, $J=9$ and 7, 1H); 7.6 (*s*, 1H); 7.4–7.8 (*m*, 3H); 8.2–8.4 (*m*, 2H). – MS.: no M^+ (calc. for $C_{28}H_{31}NO_{11}$: 557), 525, 392, 370, 328, 105.

$C_{28}H_{31}NO_{11}$ (557) Calc. C 60.31 H 5.60 N 2.51% Found C 60.19 H 5.67 N 2.57%

Column chromatography of the mother liquor on silica gel (benzene/ethyl acetate 98:2) afforded beside 22 mg of **4** 25 mg of the less polar *7,(9-O),(10-O),(11-O)-tetraacetylpseurotin A* (**5**) as colourless oil, $[\alpha]_D^{20}=-66°$ ($c=0.27$, methanol). – IR. ($CHCl_3$): 3000, 1750, 1720, 1690, 1640, 1600. – ^{1}H-NMR. (90 MHz, $CDCl_3$): 1.03 (*t*, $J=7$, 3H); 1.82 (*s*, 3H); 2.05 (*s*, 3H); 2.07 (*s*, 3H); 2.10 (*s*, 3H); 2.0–2.2 (*m*, 2H); 2.55 (*s*, 3H); 3.58 (*s*, 3H); 5.29 (br. *d*×*d*, $J=11$ and 9, 1H); 5.64 (*s*, 1H); 5.72 (*d*, $J=7$, 1H); 5.8 (*m*, 1H); 5.96 (br. *d*×*d*, $J=9$ and 7, 1H); 7.4–7.8 (*m*, 3H); 8.2–8.4 (*m*, 2H).

Acetylation of **1** *with acetic anhydride/pyridine.* A solution of 100 mg (0.23 mmol) of **1** in 2.5 ml of pyridine/acetic anhydride was stirred for 40 h at 40°. The solvent was evaporated i.V. and the residue dried at 0.005 Torr/RT. Separation of the crude product by TLC. (benzene/isopropyl alcohol 98.5:1.5) yielded 70 mg of **6** thought to be either *7,(9-O),(10-O)-triacetyl-11-desoxy-10,11-didehydro-pseurotin A* (**6b**) or the *7,(9-O),(11-O)-triacetyl-10-desoxy-10,11-didehydro-derivative* (**6a**. – UV. (ethanol): 333 (4.38), 250 (4.23). – IR. ($CHCl_3$): 3000, 1760, 1725, 1705, 1700, 1615. – ^{1}H-NMR. (60 MHz, $CDCl_3$): 1.05 (*t*, $J=7$, 3H); 1.85 (*s*, 3H); 2.1 (*s*, 3H); 2.0–2.3 (*m*, 2H); 2.3 (*s*, 3H); 2.55 (*s*, 3H); 3.6 (*s*, 3H); 5.65 (*s*, 1H); 6.1–6.3 (*m*, 2H); 6.8–7.1 (*m*, 1H); 7.3–7.7 (*m*, 3H); 7.9–8.2 (*m*, 2H). – MS.: 539 (M^+, calc. for $C_{28}H_{29}NO_{10}$: 539), 497, 484, 406, 392, 332, 105.

If the reaction was carried out at RT. and worked up in a similar manner **5** was isolated in about 50% yield.

Preparation of (9-O),(10-O),(11-O)-triacetyl-12,13,17,(17-O)-tetrahydropseurotin A (**7**). A solution of 95 mg (0.17 mmol) of **4** in 15 ml of ethanol was hydrogenated with 10 mg of 10% Pd/C as catalyst. When 2 mol-equiv. of H_2 were consumed the suspension was filtered and the filtrate evaporated to dryness. Purification of the crude product by TLC. (ethyl acetate/methanol 97:3) yielded 70 mg of **7**. – UV. (methanol): 278 (3.94). – IR. (CH_2Cl_2): 3400, 2980, 1750, 1715, 1645, 1380. – ^{1}H-NMR. (60 MHz, $CDCl_3$): 0.9 (br. *t*, $J=7$, 3H); 1.0–1.7 (*m*, 6H); 1.7 (*s*, 3H); 1.8 (*s*, 3H); 2.0 (*s*, 3H); 2.1 (*s*, 3H); 3.65 (*s*, 3H); 4.95 (*s*, 1H); 5.0–5.3 (*m*, 1H); 5.55 (*s*, 1H); 5.65 (*d*, $J=6$, 1H); 7.3–7.6 (*m*, 5H); 7.85 (*s*, 1H).

Preparation of (9-O),(10-O),(11-O)-triacetyl-12,13-dihydropseurotin A (**9**). A solution of 10 mg (0.1 mmol) of CrO_3 in 0.1 ml of water was diluted with 0.5 ml of acetic acid. This reagent was added

to a stirred solution of 20 mg (0.036 mmol) of **7** in 0.5 ml of acetic acid. After standing for 1 h the starting material had disappeared in TLC. The solution was diluted with 3 ml of water and extracted with ether. The organic layer was dried (Na_2SO_4) and concentrated to about 0.5 ml. After addition of 2 drops of conc. NH_3 solution to neutralize the acid, the crude product was purified by TLC. (CH_2Cl_2/methanol 98:2) to yield 11 mg of **9** as a colourless solid. – IR. (CH_2Cl_2): 3420, 2980, 1760, 1740, 1720, 1690, 1650, 1600. – ^{1}H-NMR. (90 MHz, $CDCl_3$): 0.9 (br. *t*, $J=6$, 3 H); 1.1–1.8 (*m*, 6 H); 1.83 (*s*, 3 H); 2.05 (*s*, 3 H); 2.09 (2 *s*, 6 H); 3.54 (*s*, 3 H); 5.1–5.4 (*m*, 1 H); 5.70 (*d*, $J=5$, 1 H); 5.76 (*s*, 1 H); 7.35–7.8 (*m*, 3 H); 7.44 (*s*, 1 H; exchangeable with D_2O); 8.2–8.4 (*m*, 2 H).

Preparation of 12,13,17,(17-O)-tetrahydropseurotin A (**13**). A solution of 500 mg (1.16 mmol) of **1** in 25 ml of ethanol was hydrogenated with 50 mg of 10% Pd/C. When 2 mol-equiv. of H_2 were consumed the catalyst was filtered off and the solvent evaporated i.V. Column chromatography of the residue on silica gel (benzene/ethyl acetate 9:1) afforded 440 mg of **13** as colourless oil (epimeric mixture). – UV. (ethanol): 279 (3.97). – IR. (KBr): 3600–3200, 2950, 2920, 1720–1680, 1620, 690. – ^{1}H-NMR. (100 MHz, DMSO-d_6): 0.92 (br. *t*, $J\approx5$, 3 H); 1.1–1.6 (*m*, 6 H); 1.62 (*s*, 3 H); 3.28, 3.48 (2 *s*, 6/3 H and 3/3 H); 3.5–3.7 (*m*, 1 H); 4.19 (*d*, $J=9$, 1 H; exchangeable with D_2O); 4.35 (*m*, 1 H); 4.47 (*d*, $J=6$, 1 H; exchangeable with D_2O); 4.65, 4.68, 4.76, 4.79 (4 *m*, 1/3 H, 2/3 H, 1/3 H, and 2/3 H, resp.; 4 *s* after exchange with D_2O); 5.68 (*d*, $J=5$, 4/3 H; exchangeable with D_2O); 5.90 (*d*, $J=4$, 2/3 H; exchangeable with D_2O); 7.2–7.5 (*m*, 5 H); 8.98, 9.40 (2 *s*, 1/3 H, and 2/3 H, resp.; exchangeable with D_2O).

Preparation of (9-O),(10-O),(11-O),(17-O)-tetraacetyl-12,13,17,(17-O)-tetrahydropseurotin A (**8**). Compound **8** could be obtained either by acetylation of **7** with acetic anhydride/sodium acetate or directly from **13** in the following manner: 124 mg (0.28 mmol) of **13** and 0.5 g of anhydrous sodium acetate were shaken for 18 h in 5 ml of acetic anhydride at RT. The mixture was then poured onto crashed ice and extracted carefully with ether. The combined organic extracts were washed with phosphate-buffer solution (pH 7) and water, dried and evaporated i.V. The crude product was purified by TLC. (benzene/isopropyl alcohol 95:5) to yield 54 mg of **8** as epimeric mixture. – IR. (CH_2Cl_2): 3390, 2950, 1750, 1715, 1640, 1370, 1220. – ^{1}H-NMR.[12]) (60 MHz, $CDCl_3$): 0.9 (br. *t*, 3 H); 1.2–1.8 (*m*, 6 H); 1.75 (*s*, 3 H); 1.8 (*s*, 3 H); 2.05 (*s*, 3 H); 2.1 (*s*, 3 H); 2.2 (*s*, 3 H); 3.6 (*s*, 3 H); 5.1–5.4 (*m*, 1 H); 5.55 (*s*, 1 H); 5.75 (*d*, $J=7$, 1 H); 6.0 (*s*, 1 H); 7.0 (1 H; exchangeable with D_2O); 7.2–7.5 (*m*, 5 H).

Preparation of (5S,8S,9R)-9-hydroxy-8-hydroxybenzyl-2-hydroxymethyl-8-methoxy-3-methyl-1-oxa-7-azaspiro[4.4]non-2-ene-4,6-dione (**12**). Under the same conditions as **1** 135 mg (0.39 mmol) of **10** were hydrogenated (20 mg 10% Pd/C). TLC. of the crude product (ethyl acetate/methanol 98:2) yielded 115 mg of **12** (epimeric mixture with respect to C(α) of the benzyl radical). – IR. (CH_2Cl_2): 3600, 3400, 1730, 1705, 1635. – ^{1}H-NMR. (100 MHz, DMSO-d_6): 1.59 (*s*, 3 H); 3.26, 3.45 (2 *s*, 3/2 H, and 3/2 H, resp.); 4.32 (*d*, $J=9$, 1/2 H; exchangeable with D_2O); 4.43 (*d*, $J=6$, 2 H; br. *s* after exchange with D_2O); 5.65, 5.7 (2 *d*, 1 H; 2 *s* after exchange with D_2O); 5.8 (2 *d*, 1 H; br. *s* after exchange with D_2O); 5.60 (*t*, $J=6$, 1 H; exchangeable with D_2O); 5.9 (*d*, 1/2 H; exchangeable with D_2O); 5.67, 5.87 (2 *d*, $J=4$, twice 1/2 H; exchangeable with D_2O); 7.2–7.5 (*m*, 5 H); 8.96, 9.34 (2 *s*, twice 1/2 H; exchangeable with D_2O).

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[12]) The indirect approach to **8** *via* hydrogenation of **4** ($\rightarrow$**7**) and subsequent acetylation gave predominantly one of the two isomers. The NMR. data correspond to this compound.

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