3.5. *1,7-Heptandiamin* (**2**). – MS.: 130 (3, M^+), 114 (33, $M - NH_2$), 113 (6, $M - NH_3$), 112 (5), 102 (5), 101 (63), 100 (23), 87 (13), 86 (30), 84 (17), 73 (11), 72 (18), 70 (15), 69 (18), 67 (7), 59 (15), 58 (7), 57 (7), 56 (100), 55 (32), 54 (7), 45 (41), 44 (59), 43 (35), 42 (33), 41 (39).

3.6. *1,8-Octandiamin* (**8**). – MS.: 144, (4, M^+), 129 (7), 128 (60, $M - NH_2$), 116 (10), 115 (81), 114 (19), 101 (10), 100 (33), 98 (13), 87 (14), 86 (54), 84 (13), 83 (11), 82 (11), 81 (8), 77 (11), 73 (14), 72 (46), 71 (6), 70 (44), 69 (31), 68 (10), 67 (13), 59 (28), 58 (10), 57 (25), 56 (100), 55 (65), 54 (13), 53 (10), 51 (6), 45 (47), 44 (74), 43 (36), 42 (39), 41 (56), 40 (7).

3.7. *1,9-Nonandiamin* (**5**). – MS.: 158 (6, M^+), 157 (7), 143 (12), 142 (99, $M - NH_2$), 130 (9), 129 (99), 128 (25), 115 (13), 114 (45), 112 (8), 101 (8), 100 (58), 98 (6), 96 (5), 95 (5), 87 (15), 86 (69), 84 (22), 83 (21), 82 (12), 81 (11), 73 (21), 72 (41), 70 (26), 69 (37), 68 (8), 67 (13), 59 (28), 58 (9), 57 (15), 56 (100), 55 (71), 54 (11), 53 (8), 47 (5), 45 (83), 44 (95), 43 (49), 42 (38), 41 (66), 40 (5).

3.8. *1,10-Decandiamin* (**9**). – MS.: 172 (8, M^+), 171 (8), 157 (11), 156 (89, $M - NH_2$), 144 (11), 143 (88), 142 (30), 129 (11), 128 (48), 126 (6), 115 (8), 114 (41), 101 (9), 100 (59), 98 (13), 97 (13), 96 (8), 95 (8), 87 (20), 86 (69), 84 (14), 83 (19), 82 (9), 81 (11), 77 (8), 73 (20), 72 (44), 71 (8), 70 (31), 69 (48), 68 (13), 67 (14), 59 (48), 58 (9), 57 (30), 56 (100), 55 (77), 54 (11), 53 (11), 45 (78), 44 (92), 43 (50), 42 (33), 41 (77), 40 (6).

3.9. *1,12-Dodecandiamin* (**3**). – MS.: 200 (13, M^+), 199 (8), 185 (9), 184 (58, $M - NH_2$), 172 (9), 171 (67), 170 (21), 157 (8), 156 (58), 143 (7), 142 (45), 129 (6), 128 (43), 115 (7), 114 (28), 112 (5), 101 (8), 100 (41), 98 (9), 97 (11), 95 (5), 87 (19), 86 (53), 84 (15), 83 (18), 82 (8), 81 (8), 73 (18), 72 (38), 71 (5), 70 (23), 69 (36), 68 (8), 67 (12), 59 (28), 58 (8), 57 (15), 56 (71), 55 (63), 54 (8), 53 (8), 45 (54), 44 (100), 43 (40), 42 (18), 41 (65).

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13. Pseurotin, a New Metabolite of *Pseudeurotium ovalis* STOLK Having an Unusual Hetero-Spirocyclic System

(Preliminary Communication)

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(17. XI. 75)

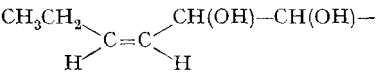
Summary. The structure and absolute configuration of pseurotin (**1**), a new metabolite, isolated from culture filtrates of *Pseudeurotium ovalis* STOLK (*Ascomycetes*), has been shown to be 2-[1′(*S*), 2′ (*S*)-dihydroxyhex-3′-ene-yl]-3-methyl-8(*S*)-methoxy-8-benzoyl-9(*R*)-hydroxy-(5*S*)-1-oxa-7-aza-spiro[4.4]non-2-ene-4,6-dione (**1**), by spectral data and chemical transformations, and by X-ray analysis of its dibromo derivative **2** [1].

Some years ago the main metabolite from culture filtrates of the fungus *Pseudeurotium ovalis* STOLK (*Ascomycetes*), ovalicin, was isolated, its structure was elucidated [2], and its immunosuppressive activity [3] and its biosynthesis [4] were studied. We now report on the isolation[1]) and structural determination of a second, minor, metabolite of this microorganism, which we name pseurotin (**1**).

Pseurotin (**1**) is characterized by a novel, highly substituted and functionalized spirocyclic system containing oxygen and nitrogen atoms. It is a neutral, optically active substance ($[\alpha]_D^{20} = -5° \pm 2°$, methanol), m.p. 162–163.5° (colourless rhombic crystals from dichloromethane/hexane); it showed no antibacterial or antifungal activity[2]). The molecular formula, $C_{22}H_{25}NO_8$, was deduced from elemental microanalyses. In the mass spectrum[3]) the molecular ion was not observed, the first peak being at *m/e* 399 ($M^+ - 32$), due to the loss of methanol (*cf.* [5]). The peaks at *m/e* 326 ($M^+ - 105$), 105 and 77 indicated the presence of a benzoyl group. One methoxyl group was revealed by a *Zeisel* determination, and one glycol group was detected by periodate titration. In the UV., pseurotin exhibited absorption maxima at 253 (4.22) and 280 (4.05) nm (log ε) (ethanol). The IR. spectrum (KBr) showed bands at 3275 (OH, NH), 1725 (C=O), 1680–1690, 740 and 705 (benzene ring) cm^{-1}. The 100 MHz ^{1}H-NMR. spectrum[4]) ($(CD_3)_2SO$) of **1** indicated the presence of the benzoyl group (multiplets at 8.24 and 7.5 ppm (2 H and 3 H respectively)), of one methoxy group (singlet (3 H) at 3.26 ppm) and of an additional methyl group (singlet (3 H) at 1.65 ppm). On addition of D_2O the singlet of an amide proton (at 9.9 ppm) and the doublets of three secondary hydroxyl groups disappeared. One of these doublets (6.12 ppm, $J = 7$ Hz, C(9)–OH) forms an *AB*-system with another doublet at 4.41 ppm ($J = 7$ Hz, C(9)–H). By spin-spin decoupling experiments all remaining signals were assigned to the protons of the side chain from C(10) to C(15) and the two remaining hydroxyl groups; the doublets at 5.68 ppm ($J = 5$ Hz) and 4.78 ppm ($J = 4$ Hz) correspond to C(10)–OH and C(11)–OH, a multiplet at 4.2–4.6 ppm to C(10)–H and C(11)–H respectively. A multiplet at 5.1–5.7 ppm ($J = 7$ and 11 Hz) is due to two vinylic protons at C(12) and C(13), and a pseudoquintet at 2.07 ppm ($J \approx J' \approx 7$ Hz, 2 H) 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).

These data reveal the presence of the following structural elements in pseurotin (**1**):

–CH(OH)–; $-OCH_3$; $-CH_3$; –NHCO–; C_6H_5CO–;



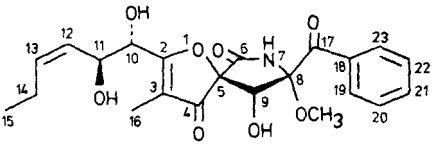
A C_5O_2 residue with four degrees of unsaturation has still to be accomodated. The following chemical transformations confirmed these conclusions. Ozonolysis of **1** yielded propionic acid which was identified as its benzhydryl ester. Oxidation of **1**

[1]) We thank Mr. *F. Geissmann & A. Tóth, Sandoz A.G.*, for the isolation and purification of larger amounts of this substance.

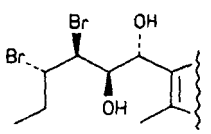
[2]) We thank Dr. *E. Härri, Sandoz A.G.*, for microbiological experiments.

[3]) We thank Dr. *H. Lichti, Sandoz A.G.*, for determination of high resolution mass spectra on a CEC 21-110 B instrument. Other mass spectral measurements on a AEI-MS 30 spectrometer by Drs. *C. Pascual* and *J. Vogt*, Physikalisch-chemisches Institut der Universität Basel, are gratefully acknowledged.

[4]) Chemical shifts are given in δ-values, relative to tetramethylsilane (TMS) as internal standard, with $\delta_{TMS} = 0$ ppm.



1 Pseurotin



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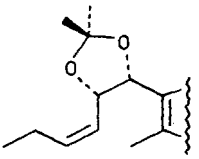

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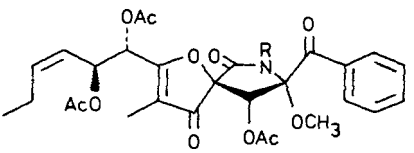
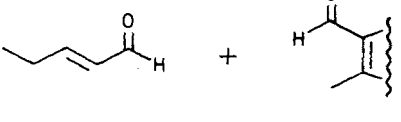
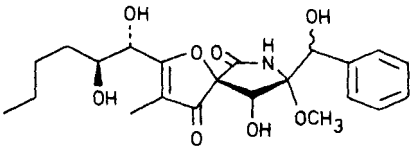


4 R=H **5** R=Ac



6 **7**



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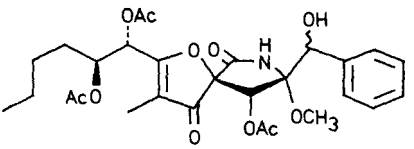

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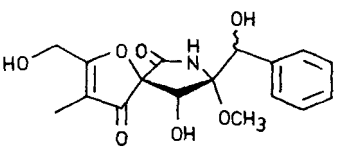

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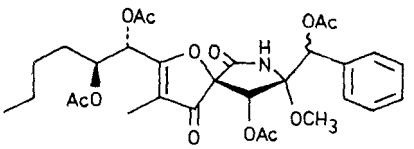

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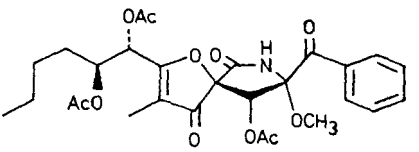
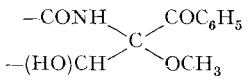


12

$Ac = CH_3CO$

with HNO_3 followed by esterification with CH_2N_2 gave methyl benzoate. Treatment of **1** with Br_2 in chloroform at 0° led to crystalline 12,13-dibromopseurotin (**2**), $C_{22}H_{25}Br_2NO_8$, m.p. 154–156°, $[\alpha]_D^{20} = -28° \pm 2°$ (methanol). In the ^{1}H-NMR. spectrum of **2** two new signals at 4–5 ppm (multiplet; $J = 6$ Hz) replaced those of the two vinylic protons. Removal of bromine with Zn in acetic acid/ether led to the original antibiotic **1** (satisfactory overall yield), thus excluding the possibility of undesired secondary reactions during bromination. The isopropylidene derivative **3** proved to be unstable. Mild acetylation of pseurotin (**1**) gave a mixture of crystalline tri-O-acetyl derivative **4**, $C_{28}H_{31}NO_{11}$; m.p. 126–128°, $[\alpha]_D^{20} = -23° \pm 2°$ (methanol), and tetraacetyl derivative **5** as a viscous oil, $[\alpha]_D^{20} = -66° \pm 2°$ (methanol), which contained in addition an N-acetyl group. Catalytic hydrogenation of **4** with Pd/C (10%) in ethanol produced the tetrahydro derivative **9**, $C_{28}H_{35}NO_{11}$; which was characterized by its spectral data. In the IR. spectrum of **9**, 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 **9** all aromatic protons appeared as a multiplet at 7.4 ppm; a new singlet at 4.9 ppm was assigned to a benzyl proton; in the range of 1 to 1.8 ppm a multiplet of three methylene groups could be seen. Acetylation of **9** with acetic anhydride/sodium acetate led to the oily tetra-O-acetyl derivative **11**, $C_{30}H_{37}NO_{12}$, for which the IR. spectrum showed no band at 3600 cm^{-1} and in the ^{1}H-NMR. spectrum the singlet of the benzylic proton was shifted to 6.01 ppm. Oxidation of **9** with CrO_3 gave the ketone **12**, $C_{28}H_{33}NO_{11}$, as an oil which exhibited all spectral data characteristic for the benzoyl group.

On treatment of pseurotin (**1**) with HIO_4 compound **7** and (*E*)-pent-2-ene-al (**6**) (2,4-dinitrophenylhydrazone, $C_{11}H_{12}N_4O_4$, m.p. 159–160°) were obtained due to acid-catalyzed isomerization of the genuine *Z*-double bond. The aldehyde **7** proved to be unstable, but by catalytic hydrogenation with Pd/C (10%) in ethanol it gave the somewhat more stable triol **10** (viscous oil), characterized by its IR. and ^{1}H-NMR. spectra. Catalytic hydrogenation of pseurotin (**1**) itself with Pd/C (10%) in ethanol led to the stable reduction product **8**, $C_{22}H_{29}NO_8$ (viscous oil). Both **8** and **10** were inseparable mixtures of the substituted epimeric benzyl alcohols, but splitting of signals in their ^{1}H-NMR. spectra indicated close proximity of the C_6H_5CO–, CH_3O–, –CH(OH)– and –NHCO– groups in pseurotin (**1**):



Such a structural arrangement explains readily the loss of methanol in the mass spectrum of pseurotin (**1**) [5], the chemical shift of its amide proton at 9.9 ppm [6], and its reducing properties. The appearance of an intense red colour and release of ammonia on treatment with $Ba(OH)_2$ is consistent with the presence of this structural unit, likewise the elimination of benzoic acid with 5N NaOH (*cf.* [7]). In contrast, pseurotin (**1**) is relatively stable to cold dilute acids; hot mineral acids provoke decarboxylation.

For the final determination of the structure and the absolute configuration of pseurotin (**1**), the dibromo derivative **2** proved to be suitable for X-ray diffraction analysis. Details of the structure analysis are given in the following paper [1].

The investigations at the Institut für Organische Chemie were also supported by the *Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung* (Projects No. 2.675.72 and 2.0550.73), which is gratefully acknowledged.

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14. The Crystal and Molecular Structure of 12,13-Dibromopseurotin

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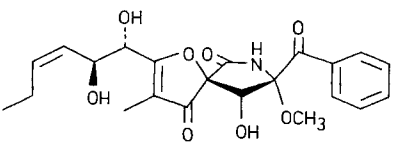
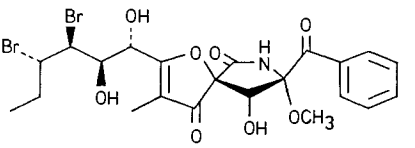
(17. XI. 75)

Summary. The crystal structure of 12,13-dibromopseurotin has been determined by single crystal X-ray analysis. The crystals belong to space group $P2_1$ with $a = 16.75$, $b = 9.63$, $c = 7.42$ Å, $\beta = 95.9°$, $Z = 2$. The structure was solved by the heavy atom technique and refined to $R = 0.062$ with 819 significant reflexions for 299 parameters.

The isolation, chemical and spectral characterisation of pseurotin (**1**), a minor metabolite from the culture filtrate of *Pseudeurotium ovalis* STOLK, is reported in the preceding paper [1]. Although many of the structural fragments of the molecule were identified by spectroscopic data, an X-ray analysis was necessary to reveal the complete structure with the unexpected spiro ring-system. Crystals of the 12,13-dibromo derivative **2** turned out to be suitable for this purpose (*cf.* [1]).



1 Pseurotin



2

Crystal Data. – The 12*S*,13*S*-dibromo derivative of pseurotin, $C_{22}H_{25}Br_2NO_8$, $M = 591.3$, was crystallised from cyclohexane/ethyl acetate 1:1. The thin prismatic