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Orsellides A–E: An Example for 6-Deoxyhexose Derivatives Produced by Fungi

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The antibacterial orsellides A–E (**1**–**5**), novel esters consisting of orsellinic acid (**6**) and a 6-deoxyhexose, were isolated from *Chaetomium sp.* (strain Gö 100/9) together with the known metabolites globosumones A (**7**) and B (**8**). The structures of the new compounds were elucidated by detailed spectroscopic analysis. The biosynthesis of these metabolites was studied by feeding ^{13}C-labelled precursors to growing cultures of strain Gö 100/9. The sugar moieties of the orsellides A–E (**1**–**5**) as well as the side chains of globosumones A (**7**) and B (**8**) originate from carbohydrate intermediates. Thus, the orsellides are rare examples for fungal metabolites containing deoxyhexose building blocks that are normally characteristic for bacterial secondary metabolites.

Introduction

Deoxyhexoses are typical building blocks of bacterial secondary metabolites and are of importance for modulating the biological activity of a large number of antibiotics,[1] e.g. anthracyclines, angucyclines, macrolides and enediynes.[2] The O-glycosidic linkage is formed during the late biosynthesis by glycosyltransferases, which utilize deoxy sugars activated at the anomeric C atom and free aliphatic or phenolic hydroxy groups of an aglycon.[3] Additionally, oligosaccharide chains containing different deoxy sugars exist as part of antibiotics.[4]

In contrast to bacteria, fungi normally are not known to produce deoxyhexoses as part of secondary metabolites. Only a few fungal metabolites are reported, e.g. sordarin[5] and its derivatives [6] Thus it was quite a surprise that *Chaetomium sp.* (strain Gö 100/9), an endophyte from marine algae, produces a couple of secondary metabolites which were identified as deoxy sugar derivatives. We detected these compounds using the well established chemical screening method.[7] In this paper, we describe the isolation and structure elucidation of orsellides A–E (**1**–**5**) as well as feeding experiments with ^{13}C-labelled precursors in order to identify the biogenetic origin of the carbon atoms of the isolated metabolites.

Results and Discussion

Fermentation and Isolation

Chaetomium sp. (strain Gö 100/9) was cultivated in shaking flasks, using a complex medium that consisted of glucose 1%, glycerol 1%, starch 1%, cornsteep powder 0.25%, peptone 0.5%, yeast extract 0.2%, NaCl 0.1%, $CaCO_3$ 0.3%. After four days of fermentation an equal volume of ethyl acetate was added to the culture broth; the mixture was homogenized and centrifuged to remove insoluble material. The liquid phases were separated and the aqueous layer was extracted twice with ethyl acetate. The organic layers were combined and the solvents evaporated. The resulting residue was purified by subsequent chromatography on silica gel, Sephadex LH-20 and RP-18 silica gel to yield 2.5 mg/L of a mixture of orsellide A (**1**) and B (**2**), 1.0 mg/L of orsellide C (**3**), 0.5 mg/L of orsellide D (**4**) and 0.5 mg/L of orsellide E (**5**). As known metabolites orsellinic acid[8] (**6**), globosumones A (**7**) and B[9] (**8**) were identified. All compounds are detectable on silica gel TLC plates with UV-light (254 nm) and when treated with anisaldehyde/H_2SO_4 they show clearly recognizable colour reactions after heating.

Structure Elucidation

The isolated compounds showed similar UV spectra pointing to a similar chromophore, which was identified as orsellinic acid (**6**) by its characteristic NMR spectroscopic data (Table 1 and Table 2). Orsellides A (**1**) and B (**2**) could be separated by preparative HPLC but converted into each other during the removal of the solvent, indicating an equilibrium of two isomers. This assumption was supported by the HRESI-MS spectrum (m/z = 349.08939 $[M + Na]^+$) leading to the same molecular formula ($C_{15}H_{18}O_8$) and by the ^1H NMR spectrum (Table 1), which exhibits signals for two compounds in a ratio of 3:2. Besides the signals for two *meta*-coupled aromatic protons at δ_H = 6.22 und 6.24 ppm and one methyl group at δ_H = 2.51 ppm belonging to an orsellinic acid moiety, signals for one methyl, four methine

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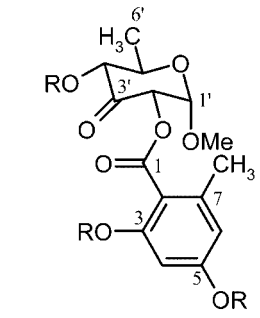

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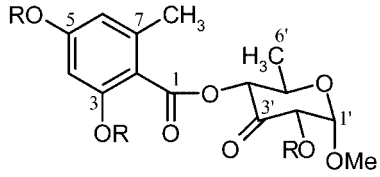

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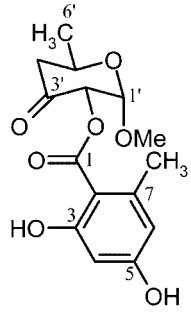

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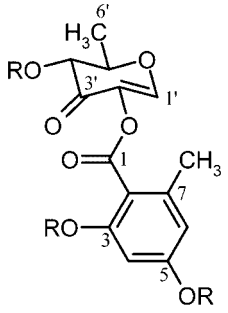

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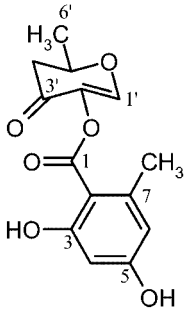

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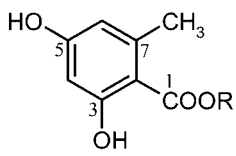
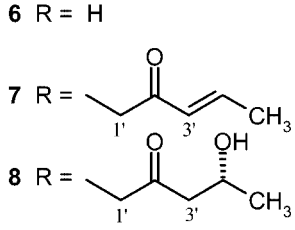




6 R = H
7 R =
8 R =

and one methoxy group could be assigned to the main isomer, named orsellide A (**1**). Its ^{13}C NMR spectrum (Table 2) contained eight signals for the orsellinic acid part and seven signals belonging to one methyl, one methoxy, four methine and one carbonyl group. As the signals for the methine groups are shifted downfield, they should be connected to oxygen, whereas one methine group (δ_C = 100.0) appears to be associated with two oxygen atoms, typical for an anomeric C atom of a sugar. The connectivities between the proton-bearing groups were revealed by a ^1H-^1H COSY experiment, and the elaborated fragments were connected due to an HMBC spectrum leading to a methyl 6-deoxy-3-keto-hexopyranoside derivative. The ^1H NMR and ^{13}C NMR spectra of the minor isomer, named orsellide B (**2**), indicate that the only difference between **1** and **2** is the position of an ester bond between the sugar moiety and the orsellinic acid: orsellide A (**1**) is acylated at position 2′ and orsellide B (**2**) at position 4′, which follows from the ^1H NMR signals of 2′-H and 4′-H, respectively (Table 2). If the mixture of orsellides A (**1**) and B (**2**) was treated with acetic anhydride, the resulting triacetates **1a** and **2a** could be separated by preparative HPLC to obtain pure and stable compounds.

The relative configuration of the sugar part of **1** and **2** was established from coupling constants and by a NOESY experiment. The ^3J coupling constants between 4′-H and 5′-H ($^3J_{H,H}$ = 9.5 and 10.0 Hz, respectively) reveal the *trans* position, while those between 1′-H and 2′-H indicate an axial-equatorial position. From a NOESY experiment, displaying correlations between 2′-H and 4′-H as well as be-

Table 1. ^1H NMR spectroscopic data of the orsellides A–E (**1**–**5**) in $CDCl_3$.

H atom	**1**	**2**	**3**	**4**	**5**
4	6.22 (d, 2.0)	6.20 (d, 2.0)	6.20 (d, 2.0)	6.22 (d, 2.0)	6.13 (d, 2.0)
6	6.24 (d, 2.0)	6.24 (d, 2.0)	6.24 (d, 2.0)	6.25 (d, 2.0)	6.18 (d, 2.0)
8	2.51 (s)	2.45 (s)	2.50 (s)	2.50 (s)	2.44 (s)
3-OH	11.02	11.17	11.02	10.96	10.99
5-OH					6.95
1′	5.22 (d, 4.5)	5.10 (d, 4.5)	5.19 (d, 4.0)	7.55 (s)	7.48 (s)
2′	5.65 (dd, 1.5, 4.5)	4.50 (dd, 0.5, 4.5)	5.56 (dd, 0.5, 4.0)		
4′	3.99 (dd, 1.5, 9.5)	5.25 (dd, 0.5, 10.0)	2.50 (ddd, 0.5, 11.0, 14.0) 2.59 (dd, 3.0, 14.0)	4.19 (d, 13.0)	2.63 (dd, 3.5, 17.0) 2.74 (dd, 13.5, 17.0)
5′	3.81 (dq, 6.5, 9.5)	4.14 (dq, 6.5, 10.0)	4.24 (ddq, 3.0, 6.0, 11.0)	4.36 (dq, 6.5, 13.0)	4.75 (ddq, 3.5, 6.5, 13.5)
6′	1.48 (d, 6.5)	1.44 (d, 6.5)	1.37 (d, 6.0)	1.60 (d, 6.5)	1.52 (d, 6.5)
1′-OCH_3	3.41 (s)	3.44 (s)	3.42 (s)		

Table 2. ^{13}C NMR spectroscopic data (in $CDCl_3$) of the orsellides A–E (**1**–**5**).

C atom	1	2	3	4	5
C-1	169.4	169.7	169.5	169.2	169.4
C-2	104.6	104.6	103.8	104.6	103.9
C-3	165.2	165.5	165.0	165.9	165.7
C-4	101.3	101.4	101.2	101.4	101.3
C-5	161.2	161.2	161.1	161.4	161.8
C-6	111.9	111.8	111.7	111.9	112.0
C-7	145.2	144.2	145.0	144.6	144.4
C-8	24.2	24.4	24.1	24.3	24.3
C-1′	100.0	101.8	100.3	157.8	157.1
C-2′	74.9	75.3	76.0	128.7	131.0
C-3′	200.0	199.6	198.2	188.1	186.3
C-4′	77.9	78.0	48.5	80.8	42.5
C-5′	71.2	68.4	66.0	72.5	66.0
C-6′	18.5	18.7	21.2	17.8	20.1
1′-OCH_3	55.5	56.0	55.4	–	–

tween 5′-H and the methoxy group at C-1′, the two coupling systems could be connected. Additionally, the axial position of the C-1′ methoxy group was confirmed by a coupled ^{13}C NMR spectrum resulting in a C–H coupling constant of $^1J_{C,H}$ = 175 Hz.[10]

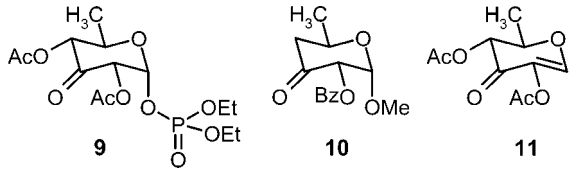
To get more insight into the interconversion of orsellide A (**1**) and B (**2**) the natural mixture (ratio 3:2) was separated by preparative HPLC and the resulting pure **1** was directly analyzed by HPLC at intervals of one hour. At room temperature equilibrium is reached after 40 hours. The interconversion of **1** and **2** occurs by an acyl shift, which proceeds via an orthoester transition state involving a six-membered ring system. The prerequisite for this easily occurring rearrangement should be a flexible conformation of the 3-keto-hexopyranose.

The molecular formula of orsellide C (**3**) was established as $C_{15}H_{18}O_7$ by HRESI-MS (*m*/*z* = 333 $[M + Na]^+$), differing only in one oxygen from **1**/**2**. The NMR spectra (Table 1 and Table 2) showed the absence of the methine signal of C-4′, which is replaced by a methylene signal (δ_C = 48.5). Thus **3** is the 4′-deoxy derivative of **1**. The relative configuration of **3** was deduced from the coupling constants and a NOESY experiment.

The HRESI-MS of orsellide D (**4**) (*m*/*z* = 317 $[M + Na]^+$) leads to the molecular formula $C_{14}H_{14}O_7$. This suggests an elimination of methanol compared to orsellide A/B (**1**/**2**). In fact, the NMR spectra of **4** don't exhibit signals for a methoxy group any longer and the signal for 2′-H is missing. A signal at δ_H = 7.55 ppm indicates a double bond between C-1′ and C-2′. This assumption is supported by the ^{13}C NMR spectrum displaying signals at δ_C = 128.7 (C-2′) and 157.8 (C-1′). The large difference of the chemical shifts together with the highfield shift of the C-3′ carbonyl group (δ_C = 188.1) reveals the presence of an α,β unsaturated ketone. The enol double bond of the glycal derivative is stabilized by esterification of 2′-OH with orsellinic acid. The *trans* configuration of 4′-H and 5′-H follows from the coupling constant $^3J_{H,H}$ = 13.0 Hz.

The structure elucidation of orsellide E (**5**) was accomplished by comparing the NMR spectroscopic data of **4** and **5** (Table 1), indicating the replacement of the methine group at C-4′ by a methylene group (δ_C = 42.5). Thus orsellide E (**5**) is the 4′-deoxy derivative of **4**.

The absolute configuration of orsellides A–E (**1**–**5**) was determined by comparison of the optical rotation with similar compounds. On the basis of the optical rotation of **1a** ($[\alpha]_D^{20}$ = +79) in comparison to diethyl (2,4-di-*O*-acetyl-6-deoxy-3-keto-α-D-*ribo*-hexopyranosyl)phosphate[11] (**9**) ($[\alpha]_D^{20}$ = +84.5) the absolute configuration of both **1** and **2** can be assigned as *S* for C-1′ and C-2′, while C-4′ and C-5′ are *R*-configured. Thus, orsellides A (**1**) and B (**2**) are esters of orsellinic acid with a methyl 6-deoxy-3-keto-α-D-*ribo*-hexopyranoside moiety.



9 **10** **11**

By comparing the sign of the optical rotation of **3** with that reported for methyl 2-*O*-benzoyl-4,6-dideoxy-3-keto-α-D-*erythro*-hexopyranoside[12] (**10**) the absolute configuration at C-1′ and C-2′ could be identified as *S*, while C-5′ exhibits *R* configuration. The absolute configuration at C-4′ and C-5′ of **4a** was assigned to be *R* by comparison of the optical rotation with 2,4-di-*O*-acetyl-1,5-anhydro-6-deoxy-hex-1-en-3-ulose[11] (**11**).

Globosumones A (**7**) and B (**8**), produced by *Chaetomium globosum*, were identified by comparison of the spectroscopic data with those recently reported.[9] Because our sample of **8** exhibits the same optical rotation as that reported it coincides in the absolute configuration. Comparing the structures of the alcoholic side chain of **8** and the sugar part of **3** the similarity with respect to C-2′ to C-6′ is obvious. Following this notion the reported absolute configuration of C-4′ of **8** should be *R* and not *S*. By analysing the ^1H NMR shifts,[9] which were obtained by applying a modified Mosher's ester method, the reported $\Delta\delta$ values clearly lead to the (*R*)-configuration of C-4′. Thus globusomone B (**8**) was assigned as (4′*R*)-2′-oxo-4′-hydroxypentyl orsellinate.

Biological Activities

The antimicrobial activities of all isolated compounds were determined by agar plate diffusion assays against *Bacillus subtillis*, *Staphylococcus aureus* and *Escherichia coli* (Table 3). The most active compound is orsellide C (**3**). None of the tested metabolites inhibited the growth of *Candida albicans*.

Table 3. Diameter of inhibition zone [mm] caused by 15 µg of the tested compounds in the agar plate diffusion assay (6 mm diameter of the assay discs).

Compound	*E. coli*	*B. subtilis*	*S. aureus*
1 and **2**	11	18	9
3	14	30	12
4	12	21	8
5	–	13	–
1a	8	17	7
2a	10	25	10
4a	8	20	7
6	–	16	–
7	10	15	10
8	8	22	9

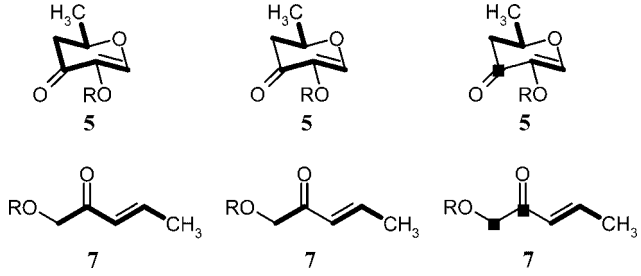
Biosynthetic Studies

While orsellinic acid (**6**) is a widespread fungal tetraketide,[13] the sugar part of orsellides A–E (**1**–**5**) was assumed to be derived from glucose with reference to the well known pathway of deoxy sugars described for bacteria,[1] although there exists no precedent for fungi. Alternatively, the sugar part could originate from the condensation of three acetate units. For that reason we carried out a feeding experiment with [1-^{13}C]acetate, which resulted in an enrichment of C-1, C-3, C-5 and C-7 of orsellinic acid (**6**) as expected. To simplify the isolation of the orsellides a mixture containing the orsellides A, B and D was acetylated with acetic anhydride and then separated by using preparative HPLC. The resulting triacetates **1a**, **2a** and **4a** as well as orsellide C (**3**), isolated from the same feeding experiment, showed the ^{13}C incorporation solely in the orsellinic acid part with specific incorporations between 2.8 and 7.1%.

To demonstrate the origin of the sugar part we next fed [1-^{13}C]glucose, which resulted in a single enrichment of C-1′ of the sugar part of the isolated orsellides. The observed specific incorporations were 4.4% for **1a** and **2a**, 5.4% for **3** and 5.1% for **4a**. An additional feeding experiment with [U-$^{13}C_6$]glucose was carried out leading to orsellide E (**5**) in amounts just enough for ^{13}C NMR analysis. This samples exhibited informative incorporation patterns in its sugar part: (i) [U-$^{13}C_6$]glucose is incorporated directly, (ii) Additional coupling patterns could be observed typical for an incorporation of two C_3 units assigned to C-1′/C-2′/C-3′ and C-4′/C-5′/C-6′, (iii) An additional doublet within the C-2′ coupling pattern typical for an intact C_2 unit (C-1′/C-2′).

The labelling pattern is caused by the known pathways filling the hexose phosphate pool. Both C_3 units orginate from the triose phosphate pool, while the existence of a C_2 unit indicates that the pentose phosphate pathway is involved, too. Identical results have been obtained by feeding [U-$^{13}C_6$]glucose in the case of acyl α-L-rhamnopyranosides from *Streptomyces griseovirides*.[14]

Besides **5**, globosumone A (**7**) could be isolated from the feeding experiment with [U-$^{13}C_6$]glucose and showed an enrichment of all signals belonging to the 2′-oxo-3-pentenyl side chain as well as an incorporation of a C_3 unit at C-3′/C-4′/C-5′ and a C_2 unit at C-1′/C-2′. A third isotopomer is characterised by two single enrichments for C-1′ and C-2′ in connection with a C_3 unit (Scheme 1, Table 4).



Scheme 1.Labelling pattern of orsellide E (**5**) and of globosumone A (**7**) derived from feeding of [U-$^{13}C_6$]glucose [R = orsellinic acid (**6**)].

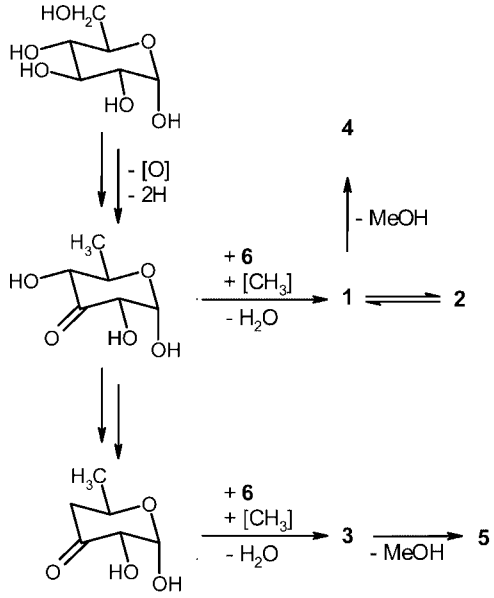
Table 4.Coupling constants of ^{13}C NMR resonances of orsellide E (**5**) and globosumone A (**7**) derived from feeding of [U-$^{13}C_6$]-glucose.

Carbon	**5**	J_{C-C} [Hz]	**7**	J_{C-C} [Hz]
C-1	169.4	78	170.4	78
C-2	103.9	78	105.2	78
C-3	165.7	71	165.2	72
C-4	101.3	71	101.4	72
C-5	161.8	69	160.8	69
C-6	112.0	69	111.5	69
C-7	144.4	44	144.4	44
C-8	24.3	44	24.2	44
C-1′	157.1	88, 5	67.0	44, 19
C-2′	131.0	88, 62, 15	191.9	57, 44
C-3′	186.3	62, 41, 5	127.5	69, 57, 19
C-4′	42.5	41, 35, 15	145.4	69, 42
C-5′	66.0	40, 35	18.7	42
C-6′	20.1	40	–	–

Discussion

On the base of the feeding experiments the origin of the sugar part of the orsellides A–E (**1**–**5**) as well as of the side chains of globosumones A (**7**) and B (**8**) from carbohydrate precursors is undoubted. Orsellides A (**1**) and B (**2**) are derivatives of a 6-deoxy-3-ketohexose, while orsellide C (**3**) is an example for a 4,6-dideoxy-3-ketohexose. Orsellides D (**4**) and E (**5**) are 2,3-dihydro-4-pyrone derivatives. The putative biosynthetic pathway (Scheme 2) starts with the deoxygenation of D-glucose to 6-deoxy-D-glucose. This process has been the subject of intensive research during the last four decades, whereby 6-desoxy-4-keto-D-glucose was identified as a central intermediate.[15,16] The latter is in equilibrium with the 3-keto-6-deoxy-D-glucose, which should be the precursor of orsellides A (**1**) and B (**2**). If a further deoxygenation at C-4′ takes place the so formed 4,6-dideoxy-3-ketohexose, which was found to be an intermediate in the biosynthesis of D-desosamine,[17] gives rise to orsellide C (**3**). To form the orsellides A–C (**1**–**3**) an acylation with orsellinic acid and the formation of a methyl glycoside are necessary. However, the question concerning the sequence of these steps remains open. For the formation of orsellides D (**4**) and E (**5**) we assume an elimination of methanol from

1 or **3**, respectively. To proof this assumption and to exclude the alternative formation of orsellides A–C (**1**–**3**) by addition of methanol to the double bond present in **4** and **5**, a fermentation of *Chaetomium sp.* (strain Gö 100/9) was worked up under exclusion of methanol. No change in the metabolite pattern and yields was observed.



Scheme 2. Putative biosynthetic pathway for the orsellides A–E (**1**–**5**) starting from D-glucose.

6-Deoxyhexoses are widespread as part of bacterial secondary metabolites whereas 4,6-dideoxy sugars are quite rare. 6-Deoxy-3-ketohexoses are also no typical building blocks produced by bacteria. There exist only a few examples for secondary metabolites containing a 6-desoxy-3-keto-*ribo*-hexopyranoside: Aurantinin B[18] produced by *Bacillus aurantinus* and datiscoside[19] isolated from dried twigs of *Datisca glomerata*. Admittedly, aurantinin B is β-linked and datiscoside contains the α-L-form while the sugar part of orsellides A (**1**) and B (**2**) is a methyl 6-deoxy-3-keto-α-D-*ribo*-hexopyranoside. The 3-keto-4,6-dideoxy sugar of orsellide C (**3**) is part of spectinomycin[20] and of five natural derivatives of erythromycin[21] The glycal-type sugar moieties of orsellides E (**4**) and F (**5**) are not reported as parts of secondary metabolites so far.

Remarkably is the preservation of D-series of the hexose during the deoxygenation and the generation of methyl α-D-glycosides. This observation is in contrast to the rule established e.g. for macrolides: Deoxyhexoses as part of glycosides exist predominantly in the α-L or β-D configuration.[22]

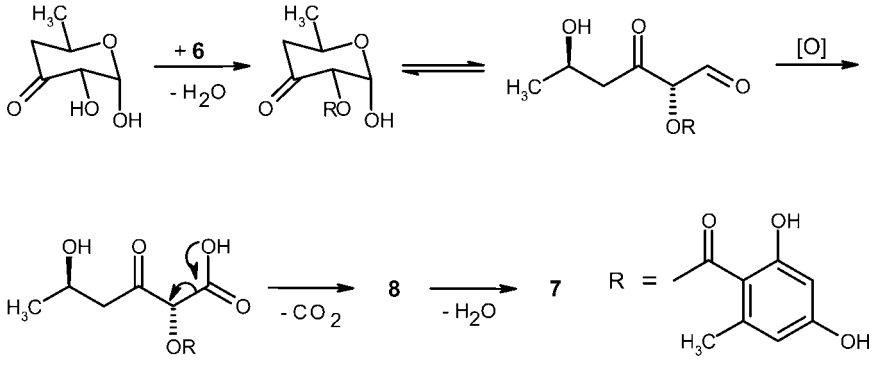
Since feeding experiments with [U-$^{13}C_6$]glucose resulted in a typical labelling of the 2-oxopent-3-enyl side chain of globosumone A (**7**) it is in all likelihood that the sugar part of **7** and **8** is derived from a 5-deoxypentose, which is generated by decarboxylation of 4,6-dideoxy-3-oxogluconic acid (Scheme 3). It seems to be most likely, that acylation with orsellinic acid (**6**) takes place before decarboxylation since the fungal strain has the ability to esterify 6-deoxyhexoses at a defined position.

The orsellides A–E (**1**–**5**) are rare examples for deoxy sugar containing secondary metabolites produced by a fungal strain. Since deoxy sugars are well known metabolites of bacteria we assume that *Chaetomium sp.* strain Gö 100/9 gained the ability to produce deoxy sugars through horizontal gene transfer from bacteria. It would be exciting to study this question in detail.

Experimental Section

General Remarks: ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ with Varian Inova 600 (600 MHz). Chemical shifts are expressed in δ values (ppm) with solvents as internal standards. The mass spectra were taken with a Finnigan LC-Q. IR spectra were recorded with a Perkin–Elmer FT IR-1600 spectrometer as KBr pellets. UV spectra were recorded with a Kontron Uvikon 860 spectrophotometer. TLC was carried out on silica gel 60 F_{254} plates (Merck, 0.25 mm) and column chromatography on silica gel (< 0.08 mm, Macherey–Nagel,) or Sephadex LH-20 (Pharmacia). R_f values were determined on 20 × 20 cm plates, the evaluation length was 10 cm. Compounds were detected under a UV lamp at 254 nm and sprayed with anisaldehyde/H_2SO_4 followed by heating.

Fermentation and Isolation: *Chaetomium sp.* (strain Gö 100/9) was maintained as a stock culture on agar slants consisting of malt extract (1%), yeast extract (0.4%), glucose (0.4%), $CaCO_3$ (0.03%), agar (2%), pH = 7.0 prior to sterilization. Fermentations were carried out in 250-mL Erlenmeyer flasks with three indentations. Each flask was filled with 100 mL of a complex medium (glucose 1%, glycerol 1%, starch 1%, cornsteep powder 0.25%, peptone 0.5%,



Scheme 3. Putative pathway for the formation of globosumone A (**7**) and B (**8**).

yeast extract 0.2%, NaCl 0.1%, $CaCO_3$ 0.3%) and sterilized at 121 °C for 30 minutes. The cultures were inoculated with a 1-cm^2 piece of agar from 7-days-old cultures and incubated for 96 hours at 28 °C with a rotary shaker (180 rpm).

The mycelium was extracted twice with acetone and the culture filtrate was extracted at pH 4 with ethyl acetate (3×). The crude residue was purified by column chromatography on silica gel (CH_2Cl_2/methanol, 9:1), Sephadex LH-20 (methanol) and preparative HPLC (column: Jasco Nucleodur 100 C18, 5 µm, 20 mm × 250 mm; mobile phase 40% AcCN; flow rate: 15.0 mL/min. UV detection: 220 nm). Orsellides A–E (**1**–**5**) were eluted at 11.2, 13.4, 23.7, 12.4 and 19.7 minutes, respectively. To simplify the isolation for the feeding experiments a mixture containing orsellides A, B and D was acetylated with acetic anhydride, and the triacetates were separated by preparative HPLC (column: Jasco Nucleodur 100 C18, 5 µm, 20 mm × 250 mm; mobile phase 50% AcCN; flow rate: 15.0 mL/min. UV detection: 220 nm).

Labelled Compounds: ^{13}C-Labelled compounds were of 99% ^{13}C atom purity. 1.0 g/L (12.0 mmol/L) [1-^{13}C]acetate (Campro Scientific), 0.5 g/L (2.8 mmol/L) [1-^{13}C]glucose (CIL Inc.) and [U-$^{13}C_6$]-glucose (CIL Inc.).

Feeding Experiments: Feeding experiments were carried out in 250-mL Erlenmeyer flasks under conditions as described before. Aqueous solutions of the labelled precursors were adjusted to pH 7 and added in four equal aliquots following the pulse-feeding method 78, 84, 90 and 96 hours after incubation. The cultures were harvested after 108 hours and worked up as described before. Addition of [1-^{13}C]acetate doubled the isolated amount of orsellinic acid (**6**) to 40 mg/L. The yields of orsellides A–D (**1**–**4**) were also increased to 5.0 mg/L for the mixture of orsellides A (**1**) and B (**2**), 2.5 mg/L for orsellide C (**3**) and 2.5 mg/L for orsellide D (**4**), while orsellide E (**5**) could not be isolated. Therefore, feeding experiments with [1-^{13}C]glucose and [U-$^{13}C_6$]glucose were carried out under addition of unlabeled acetate (1.0 g/L, 12.0 mmol/L). Addition of [1-^{13}C]glucose lead to the isolation of orsellides A–D (**1**–**4**) in the same amounts as reported for the feeding of [1-^{13}C]acetate, whereas the experiment with [U-$^{13}C_6$]glucose solely led to the isolation of 1.5 mg/L of orsellide C (**3**), 1.0 mg/L of orsellide E (**5**) and 1.0 mg/L of globosumone A (**7**). Specific incorporation rates were calculated according to Scott et al. [23]

Orsellide A [Methyl 2-*O*-(2,4-Dihydroxy-6-methylbenzoyl)-6-deoxy-3-keto-*α*-D-*ribo*-hexopyranoside] (1) and Orsellide B [Methyl 4-*O*-(2,4-Dihydroxy-6-methylbenzoyl)-6-deoxy-3-keto-*α*-D-*ribo*-hexopyranoside] (2): (characterisation as a 3:2 mixture). $C_{15}H_{18}O_8$ (326.30). R_f value: 0.64 ($CHCl_3$/MeOH, 9:1). Colour reaction with anisaldehyde/H_2SO_4: auburn. IR (KBr): $\tilde{\nu}$ = 3429, 2928, 2864 (sh), 1719, 1631, 1457, 1341, 1241, 1169, 1097 cm^{-1}. UV (MeOH): λ_{max} (lg ε) = 216 (4.48), 261 (4.17), 299 (3.83) nm; MeOH/HCl: λ_{max} (lg ε) = 216 (4.52), 262 (4.20), 299 (3.85) nm; MeOH/NaOH: λ_{max} (lg ε) = 210 (4.44), 232 (sh, 4.19), 304 (4.29) nm. $[\alpha]_D^{20}$ = +58 (c = 0.1 in MeOH). CD (MeOH): λ_{max} ([Θ]) = 268 (2089). ^1H NMR (600 MHz, $CDCl_3$): see Table 1. ^{13}C NMR (150.8 MHz, $CDCl_3$): see Table 2. ESI-MS: *m*/*z* (%): positive mode = 349 (30) $[M + Na]^+$, 675 (100) $[2M + Na]^+$; negative mode = 325 (95) $[M - H]^-$, 651 (100) $[2M - H]^-$. HRESI-MS: *m*/*z* 349.08939 $[M + Na]^+$, calculated for $C_{15}H_{18}O_8Na$ and found.

Orsellide C [Methyl 2-*O*-(2,4-Dihydroxy-6-methylbenzoyl)-4,6-dideoxy-3-keto-*α*-D-*erythro*-hexopyranoside] (3): $C_{15}H_{18}O_7$ (310.30). R_f value: 0.81 ($CHCl_3$/MeOH, 9:1). Colour reaction with anisaldehyde/H_2SO_4: auburn. IR (KBr): $\tilde{\nu}$ = 3415, 2977, 2934, 2840 (sh), 1739, 1650, 1621, 1451, 1326, 1258, 1167, 1114, 1049 cm^{-1}. UV (MeOH): λ_{max} (lg ε) = 216 (4.39), 265 (4.19), 302 (3.76) nm; MeOH/HCl: λ_{max} (lg ε) = 216 (4.37), 265 (4.19), 302 (3.76) nm; MeOH/NaOH: λ_{max} (lg ε) = 210 (4.30), 242 (3.98), 306 (4.37) nm. $[\alpha]_D^{20}$ = +45 (c = 0.1 in MeOH). CD (MeOH): λ_{max} ([Θ]) = 259 (–5176), 278 (5666). ^1H NMR (600 MHz, $CDCl_3$): see Table 1. ^{13}C NMR (150.8 MHz, $CDCl_3$): see Table 2. ESI-MS: *m*/*z* (%): positive mode = 333 (100) $[M + Na]^+$, 643 (50) $[2M + Na]^+$; negative mode = 309 (100) $[M - H]^-$. HRESI-MS: *m*/*z* 333.09447 $[M + Na]^+$, calculated for $C_{15}H_{18}O_7Na$ and found.

Orsellide D [(2*R*,3*R*)-5-*O*-(2,4-Dihydroxy-6-methylbenzoyl)-3-hydroxy-2-methyl-2,3-dihydro-4-pyrone] (4): $C_{14}H_{14}O_7$ (294.26). R_f value: 0.63 ($CHCl_3$/MeOH, 9:1). Colour reaction with anisaldehyde-H_2SO_4: auburn. M.p. 81 °C. IR (KBr): $\tilde{\nu}$ = 3431, 2930, 2858 (sh), 1743 (sh), 1691 (sh), 1655 (sh), 1625, 1505, 1453, 1391, 1319, 1256, 1178 cm^{-1}. UV (MeOH): λ_{max} (lg ε) = 215 (4.11), 269 (3.97), 299 (sh, 3.49) nm; MeOH/HCl: λ_{max} (lg ε) = 215 (4.08), 267 (3.97), 299 (sh, 3.48) nm; MeOH/NaOH: λ_{max} (lg ε) = 216 (4.00), 234 (sh, 3.70), 276 (sh, 3.71), 302 (3.85) nm. $[\alpha]_D^{20}$ = +58 (c = 0.1 in MeOH). CD (MeOH): λ_{max} ([Θ]) = 205 (–8254), 264 (6777). ^1H NMR (600 MHz, $CDCl_3$): see Table 1. ^{13}C NMR (150.8 MHz, $CDCl_3$): see Table 2. ESI-MS: *m*/*z* (%): positive mode = 317 (100) $[M + Na]^+$; negative mode = 293 (100) $[M - H]^-$, 587 (20) $[2M - H]^-$. HRESI-MS: *m*/*z* 317.06317 $[M + Na]^+$, calculated for $C_{14}H_{14}O_7Na$ and found.

Orsellide E [(2*R*)-5-*O*-(2,4-Dihydroxy-6-methylbenzoyl)-2-methyl-2,3-dihydro-4-pyrone] (5): $C_{14}H_{14}O_6$ (278.26). R_f value: 0.73 ($CHCl_3$/MeOH, 9:1). Colour reaction with anisaldehyde/H_2SO_4: auburn. M.p. 81 °C. IR (KBr): $\tilde{\nu}$ = 3386, 2980, 2936, 1661, 1622, 1503, 1448, 1385, 1315, 1258, 1181 cm^{-1}. UV (MeOH): λ_{max} (lg ε) = 215 (4.11), 269 (3.97), 299 (sh, 3.49) nm; MeOH/HCl: λ_{max} (lg ε) = 215 (4.35), 269 (4.32), 301 (sh, 3.79) nm; MeOH/NaOH: λ_{max} (lg ε) = 210 (4.30), 242 (3.99), 306 (4.38) nm. $[\alpha]_D^{20}$ = +88 (c = 0.1 in MeOH). CD (MeOH): λ_{max} ([Θ]) = 273 (12497). ^1H NMR (600 MHz, $CDCl_3$): see Table 1. ^{13}C NMR (150.8 MHz, $CDCl_3$): see Table 2. ESI-MS: *m*/*z* (%): positive mode = 301 (100) $[M + Na]^+$; negative mode = 277 (100) $[M - H]^-$. HRESI-MS: *m*/*z* 301.06856 $[M + Na]^+$, calculated for $C_{14}H_{14}O_6Na$ and found.

Acetylation of Orsellides A, B and D (1, 2 and 4): To a mixture containing **1**, **2** and **4** (5–10 mg) in pyridine (1 mL) acetic anhydride (2 mL) was added. The solution was stirred for 4 to 6 hours at room temperature, hydrolyzed and the aqueous layer was extracted three times with CH_2Cl_2. The crude mixture obtained after evaporation of the organic layer was separated by preparative HPLC to yield **1a**, **2a** and **4a**.

3,5,4′-Tri-*O*-acetylorsellide A (1a): $C_{21}H_{24}O_{11}$ (452.41). R_f value: 0.81 ($CHCl_3$/MeOH, 9:1). Colour reaction with anisaldehyde/H_2SO_4: auburn. M.p. 48–52 °C. IR (KBr): $\tilde{\nu}$ = 3432, 2936, 1771, 1745, 1616, 1448, 1371, 1260, 1195, 1135, 1075 cm^{-1}. $[\alpha]_D^{20}$ = +79 (c = 0.1 in MeOH). CD (MeOH): λ_{max} ([Θ]) = 205 (19616), 249 (–6958). ^1H NMR (600 MHz, $CDCl_3$): δ = 1.38 (d, J = 6.0 Hz, 3 H, 6′-H_3), 2.16 (s, 3 H, Ac-CH_3), 2.22 (s, 3 H, Ac-CH_3), 2.26 (s, 3 H, Ac-CH_3), 2.48 (s, 3 H, 8-H_3), 3.40 (s, 3 H, 1′-OCH_3), 4.11 (dq, J = 6.0, 10.0 Hz, 1 H, 5′-H), 5.05 (dd, J = 1.0, 10.0 Hz, 1 H, 4′-H), 5.15 (d, J = 4.0 Hz, 1 H, 1′-H), 5.53 (dd, J = 1.0, 4.0 Hz 1 H, 2′-H), 6.79 (d, J = 2.0 Hz, 1 H, 6 H), 6.88 (d, J = 2.0 Hz, 1 H, 4-H). ^{13}C NMR (150.8 MHz, $CDCl_3$): δ = 18.4 (q, C-6′), 20.4 (q, Ac-CH_3 or C-8), 20.8 (q, Ac-CH_3 or C-8), 20.9 (q, Ac-CH_3 or C-8), 21.1 (q, Ac-CH_3 or C-8), 55.6 (q, 1′-OCH_3), 67.9 (d, C-5′), 75.7 (d, C-2′), 77.6 (d, C-4′), 99.7 (d, C-1′), 114.4 (d, C-4), 121.4 (d, C-6), 122.1 (s, C-2), 140.9 (s, C-7), 150.0 (d, C-3), 152.2 (s, C-5), 164.1 (s, C-1), 168.5 (s, Ac-CO), 169.0 (s, Ac-CO), 169.2 (s, Ac-CO), 192.5 (s, C-3′).

3,5,2′-Tri-*O*-acetylorsellide B (2a): $C_{21}H_{24}O_{11}$ (452.41). R_f value: 0.81 ($CHCl_3$/MeOH, 9:1). Colour reaction with anisaldehyde/H_2SO_4: auburn. M.p. 49 °C. IR (KBr): $\tilde{\nu}$ = 3432, 2931, 2870 (sh), 1771, 1750, 1619, 1369, 1196, 1136 cm^{-1}. CD (MeOH): λ_{max} ([Θ]) = 210 (–28891), 247 (14369). ^1H NMR (600 MHz, $CDCl_3$): δ = 1.41 (d, J = 6.0 Hz, 3 H, 6′-H_3), 2.20 (s, 3 H, Ac-CH_3), 2.26 (s, 3 H, Ac-CH_3), 2.27 (s, 3 H, Ac-CH_3), 2.47 (s, 3 H, 8-H_3), 3.42 (s, 3 H, 1′-OCH_3), 4.11 (dq, J = 6.0, 10.0 Hz, 1 H, 5′-H), 5.13 (d, J = 4.0 Hz, 1 H, 1′-H), 5.18 (dd, J = 1.0, 10.0 Hz, 1 H, 4′-H), 5.46 (dd, J = 1.0, 4.0 Hz 1 H, 2′-H), 6.82 (d, J = 2.0 Hz, 1 H, 6-H), 6.88 (d, J = 2.0 Hz, 1 H, 4-H). ^{13}C NMR (150.8 MHz, $CDCl_3$): δ = 18.4 (q, C-6′), 20.4 (q, Ac-CH_3 or C-8), 20.5 (q, Ac-CH_3 or C-8), 21.0 (q, Ac-CH_3 or C-8), 21.1 (q, Ac-CH_3 or C-8), 55.6 (q, 1′-OCH_3), 67.8 (d, C-5′), 74.9 (d, C-2′), 78.3 (d, C-4′), 99.7 (d, C-1′), 114.2 (d, C-4), 121.2 (d, C-6), 123.0 (s, C-2), 139.7 (s, C-7), 149.1 (d, C-3), 151.9 (s, C-5), 164.2 (s, C-1), 168.6 (s, Ac-CO), 169.0 (s, Ac-CO), 169.4 (s, Ac-CO), 192.5 (s, C-3′).

3,5,4′-Tri-*O*-acetylorsellide D (4a): $C_{20}H_{20}O_{10}$ (420.37). R_f value: 0.81 ($CHCl_3$/MeOH, 9:1). Colour reaction with anisaldehyde-H_2SO_4: auburn. M.p. 48–52 °C. IR (KBr): $\tilde{\nu}$ = 3448, 2935, 1765, 1702, 1626, 1372, 1188, 1133, 1059, 1017 cm^{-1}. $[\alpha]_D^{20}$ = +91 (c = 0.1 in MeOH). CD (MeOH): λ_{max} ([Θ]) = 270 (38234). ^1H NMR (600 MHz, $CDCl_3$): δ = 1.49 (d, J = 6.0 Hz, 3 H, 6′-H_3), 2.19 (s, 3 H, Ac-CH_3), 2.26 (s, 3 H, Ac-CH_3), 2.27 (s, 3 H, Ac-CH_3), 2.49 (s, 3 H, 8-H_3), 4.64 (dq, J = 6.0, 12.5 Hz, 1 H, 5′-H), 5.41 (d, J = 12.5 Hz, 1 H, 4′-H), 6.82 (d, J = 2.0 Hz, 1 H, 6-H), 6.90 (d, J = 2.0 Hz, 1 H, 4-H), 7.46 (s, 1 H, 1′-H). ^{13}C NMR (150.8 MHz, $CDCl_3$): δ = 17.3 (q, C-6′), 20.5 (q, Ac-CH_3 or C-8), 20.7 (q, Ac-CH_3 or C-8), 20.9 (q, Ac-CH_3 or C-8), 21.1 (q, Ac-CH_3 or C-8), 72.6 (d, C-5′), 78.3 (d, C-4′), 114.2 (d, C-4), 121.3 (d, C-6), 122.2 (s, C-2), 130.3 (s, C-2′), 140.5 (s, C-7), 149.6 (d, C-3), 152.3 (s, C-5), 156.1 (d, C-1′), 163.5 (s, C-1), 168.5 (s, Ac-CO), 169.0 (s, Ac-CO), 169.3 (s, Ac-CO), 181.7 (s, C-3′).

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