Biosynthetic Relationships in the Desertomycin Family

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The minor metabolites oasomycin E, **8**, and F, **9**, isolated from the culture broth of *Streptoverticillium baldacii* subsp. *netropse* (strain FH-S 1625), were structurally characterized as new 42-membered macrolactones of the desertomycin family. A detailed analysis of the fermentation time-course of the producing organism, pH-static fermentations and *in vitro* conversions of the oasomycins result in a complete picture of the biosynthetic relationships of the desertomycin family. Desertomycin A, **1**, the first product detected in the biosynthetic sequence, undergoes an oxidative deamination leading to oasomycin F, 9, which is converted *via* lactonization of the side chain into the main product oasomycin B, **5**. In the late stage of the fermentation, enzymatic demannosylation resulted in the aglyca of the oasomycins **1**, **8** and **7**. In pH-static fermentations these results were successfully used to direct the fermentation process to produce the desired members of the desertomycin family.

The desertomycin family of secondary metabolites from actinomyces consists of desertomycins A,[1-3] **1**, B,[4] **2** and D,[5] **3**, as well as the recently described oasomycins A–D, **4**–**7**.[6] Most of these metabolites bear a 42-membered macrolactone as a characteristic structural element containing five isolated *E*-configurated double bonds, eight methyl branches and twelve hydroxy groups. In contrast, oasomycins C and D, **6** and **7**,[6] were found to be the first representatives of natural products with a 44-membered lactone ring, exhibiting close structural similarities to the parent 42-membered macrolactones. Furthermore, the metabolites of the desertomycin family vary in the constitution of the side-chain located at either C-41 or C-43, as well as in the presence of an α-linked D-mannose moiety attached to the 22-OH. Desertomycin A, **1**, exhibits broad antibacterial and selective antifungal activities,[1] mainly against phytopathogenic fungi, while desertomycin D, **3**, is weakly active. On the other hand, oasomycins A–D, **4**–**7**, were found to be inactive in various antibacterial and antifungal assays. However, in cell-line tests with HEP-G2 liver cells oasomycin A, **4**, showed inhibitory effects on *de novo* cholesterol biosynthesis as well as 50% inhibition at a concentration of 10^{-5} mol dm^{-3} on the muscarine receptor, and weak antiprotozoal activity.[6]

The oasomycins A–D, **4**–**7**,[6] and desertomycin A, **1**, were discovered by chemical screening[7,8] as secondary metabolites of *Streptoverticillium baldacii* subsp. *netropse* (strain FH-S 1625). The individual structural variations in the side chain with close similarities in the 42- and 44-membered macrolactone ring size led us to investigate the biosynthetic relationships of the desertomycin family as well as its biosynthetic origin.[9,10] The presented studies are based on a detailed analysis of the fermentation time-course of the producing organism, pH-static fermentations and examination of the stability of the isolated metabolites at different pH-values. This led to a complete picture of the late biosynthetic sequence involving all the different members of the desertomycin family. Furthermore, two new minor components named oasomycin E, **8**, and F, **9** were discovered, and their structures are reported.

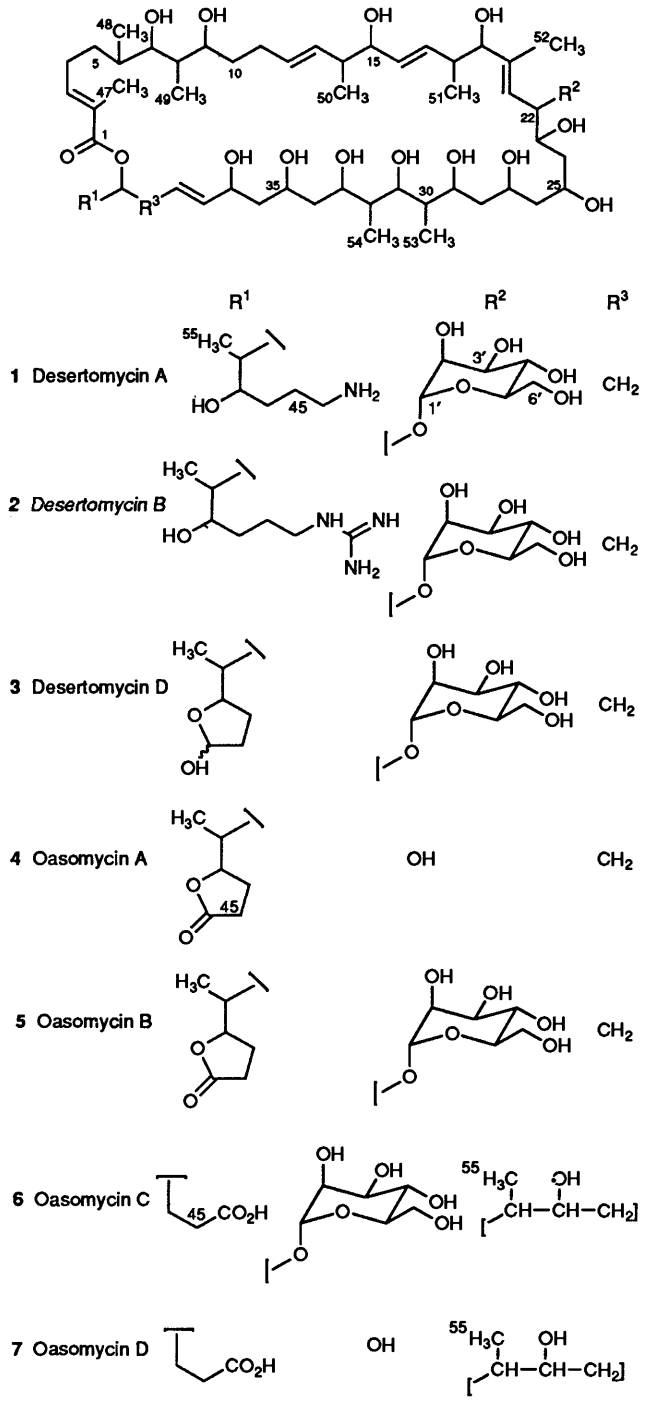
Results and Discussion

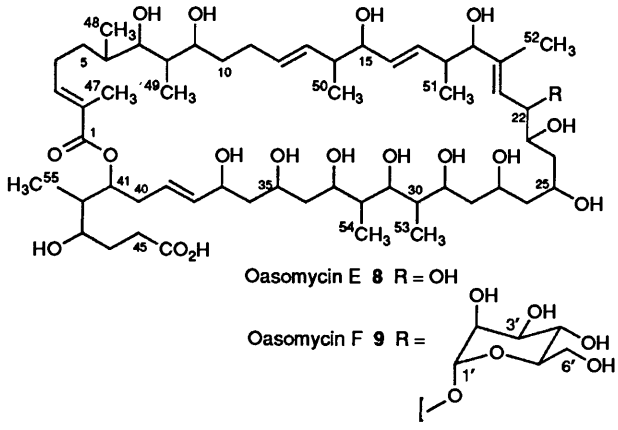
During a detailed analysis of the metabolite pattern produced by *Streptoverticillium baldacii* subsp. *netropse* (strain FH-S 1625) two minor components of the desertomycin family, oasomycin E (240-hour fermentation, yield 30 mg dm^{-3}) and oasomycin F (48-hour fermentation, yield 2.5 mg dm^{-3}), were detected. The isolation and purification of these metabolites mainly followed the procedure which has already been described for oasomycins A–D.[6] The sample of oasomycin F obtained exhibited impurities of desertomycin D, **3** (*ca.* 15%) and oasomycin B, **5** (10%). Oasomycins E and F appeared as amorphous white powders.

Oasomycins E and F were characterized spectroscopically and their molecular weights were determined by FAB-mass spectrometry. Their structures were elucidated by ^1H, ^{13}C, ^1H–^1H-correlation and ^1H–^{13}C-correlation data in comparison with those of the known members of the desertomycin family.[2,6]

The molecular formula $C_{55}H_{96}O_{18}$ of oasomycin E resulting from a FAB-mass spectrum {*m/z* 1067.6 [$(M + Na)^+$], MW 1045.4} was found to be identical with that of oasomycin D, **7**, and differs from that of oasomycin A, **4**, by an additional water molecule. Thus, we assume that the γ-lactone ring of **4** is opened in oasomycin E leading to the presence of a carboxy group at C-46 and a hydroxy group at C-43.[6] In the IR spectrum of oasomycin E an absorption band at ν/cm^{-1} 1685 indicated the existence of the expected carboxy group, while the absorption bands for the OH-groups, the carbonyl group of the macrolactone moiety, the double bonds and the fingerprint region were nearly identical with those of compound **4**. The results from the IR spectrum are in accordance with the chemical shift of C-46 (δ_C 174.9). Deduced from a ^1H–^{13}C NMR spectrum the chemical shifts of the signals, especially those of the critically important atoms $\delta_{C\text{-}41}$ 73.4 and $\delta_{41\text{-}H}$ 5.00, reflected the 42-membered macrolactone of the desertomycin type as the fundamental structural element.[6] In addition, a comparison of the signal shifts at position 43 ($\delta_{C\text{-}43}$ 70.0; $\delta_{43\text{-}H}$ 3.28) of oasomycin E with those of desertomycin A, **1** ($\delta_{C\text{-}43}$ 70.1; $\delta_{43\text{-}H}$ 3.35)[2] proved this result. Thus, oasomycin E exhibits the structure depicted in **8**.

Analogously, oasomycin F, a minor component of a 48-hour fermentation, gave nearly identical IR, UV and NMR spectroscopic data to those obtained for compound **8**. The presence of an additional carbohydrate moiety ($C_6H_{10}O_5$) was deduced from the molecular formula $C_{61}H_{106}O_{23}$ {HR-FAB-MS *m/z* 1205.7226 [$(M - H)^+$], MW 1205.5}. In the ^{13}C NMR spectrum of oasomycin F the expected signals of the 42-membered macrolactone moiety of the desertomycin family as well as the mannose unit attached to 22-OH, which is typical for compounds **1**, **5** and **6**, could be observed. The stereochemistry of the α-glycosidic bond was proven by enzymatic cleavage. The





use of α-D-mannoside mannohydrolase resulted in the deglycosylated compound oasomycin E, **8**, while the corresponding β-D-mannoside mannohydrolase caused no reaction. A comparison of the spectroscopic data with those of the known oasomycins resulted in the structure **9** for oasomycin F with a 42-membered macrolactone ring and a carboxy group at C-46 in the side chain. Thus, compound **9** is the α-D-mannosyl derivative of compound **8**.

In order to study the late biosynthetic sequence of the desertomycin family in *Streptoverticillium baldacii* subsp. *netropse* (strain FH-S 1625) the fermentation time-course was analysed. For this a series of 300 cm^3 Erlenmeyer flasks containing medium B (100 cm^3) were used to determine mycelium dry weight, pH-value and the metabolite pattern produced during a total cultivation of 240 h (Fig. 1). The secondary metabolites were analysed by TLC with 50-fold concentrated extracts of the culture broth [solvent systems: butan-1-ol–acetic acid–water, 4:1:5 (upper phase) and methanol–water, 4:1 (RP_{18}-plates); staining reagent: anisaldehyde–sulfuric acid].

The logarithmic growing phase of strain FH-S 1625 had already started 2 h after inoculation and reached the stationary phase about 80 h later. The pH-value increased from 5.9 to 7.8 between 14 and 18 h and remained steady at about 6.4–6.8 until the end of the logarithmic phase. From 80 to 240 h (stationary growing phase) the pH continuously increased to pH 9.0. The production of desertomycin A, **1**, and oasomycin B, **5**, began about 8 h after inoculation. The maximum amount of desertomycin A, **1** (about 15 mg dm^{-3}), was achieved after 22–24 h, while the highest yield of oasomycin B, **5**, (about 100 mg dm^{-3}) was reached after 48–70 h. In the same early fermentation period oasomycins C, **6** and F, **9**, were found to be minor components (maximum yields about 2–5 mg dm^{-3} each). In contrast to the decrease of oasomycin B, **5**, as well as the other early metabolites the amount of the second major product, oasomycin A, **4**, increased and the highest yield (about 100 mg dm^{-3}) was reached after 100 h. Accompanied by the formation of oasomycin A, **4**, the minor metabolites oasomycin D, **7**, and E, **8**, could be detected. The production of compounds **7** and **8** continuously increased during further cultivation, while the amount of **4** decreased.

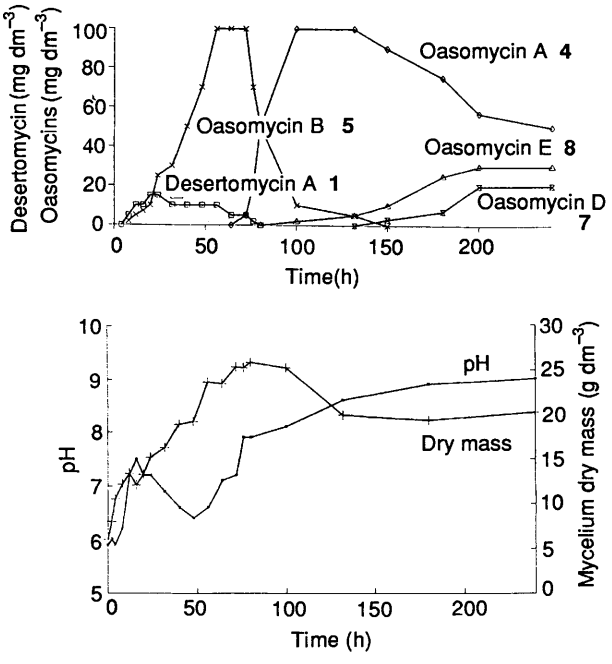


Fig. 1 Typical fermentation time course of *Streptoverticillium baldacii* subsp. *netropse* (strain FH-S 1625) and production of the desertomycins and oasomycins (concentrations estimated by TLC)

Table 1 Maximum yields of the metabolites of the desertomycin family in non-influenced and pH-static fermentations of *Streptoverticillium baldacii* subsp. *netropse* (strain FH-S 1625)

Metabolite	Non-influenced fermentation		pH-Static fermentation (pH 5.0)		pH-Static fermentation (pH 8.0)	
	Time (h)	Yield (mg dm^{-3})	Time (h)	Yield (mg dm^{-3})	Time (h)	Yield (mg dm^{-3})
Desertomycin A **1**	22–24	15	32–240	50	22–24	5
Oasomycin A **4**	80–100	100	32–240	—	80–100	—
Oasomycin B **5**	50–70	100	32–240	< 5	50–70	< 5
Oasomycin C **6**	50–70	5	32–240	—	50–70	20
Oasomycin D **7**	180–240	20	32–240	—	80–100	20
Oasomycin E **8**	150–240	30	32–240	—	80–100	35
Oasomycin F **9**	50–70	< 2	32–240	—	50–70	35

Consequently, the fermentation process of strain FH-S 1625 can be intersected into three parts. During the logarithmic growing phase (part I, 2–80 h) the mannosylated products desertomycin A, **1**, and oasomycin B, **5**, were biosynthesized, whereas parallel to the beginning of the stationary phase a demannosylation leads to the aglycon oasomycin A, **4**, as the typical main product in part II. In the later fermentation stage up to 240 h the appearance of oasomycin D, **7**, and E, **8**, correlates with an increase of the pH-value in the culture broth pointing to alkaline-catalysed pH-dependant conversions (part III).

Considering the typical pH time-course in correlation with the metabolite pattern during cultivation, pH-static fermentations promised to provide further information of biosynthetic relationships in the desertomycin family. The strain FH-S 1625 was grown in 10 dm^3 scales at pH 5.0 and 8.0 nearly reflecting the pH-values of an early and late stage of fermentation. Every 12 h the metabolite pattern produced was analysed by the TLC method described above. The results of the pH-static cultivations in comparison with a typical non-influenced fermentation are summarized in Table 1.

Unexpectedly, at a constant pH of 5.0 desertomycin A, **1**, was the only metabolite detectable during the whole fermentation procedure with a maximum yield of about 50 mg dm^{-3} besides small traces of **5**. Compared with the yield of a non-influenced fermentation the 3-fold increase in the amount of **1** reflected the suppression of the typical metabolic conversions of **1** into oasomycins A, **4**, and B, **5**. Thus, desertomycin A, **1** seems to be an early intermediate, which could be enriched under these cultivation conditions.

The pH-static fermentation at pH 8.0 resulted in a nearly analogous metabolite sequence starting with **1** *via* the glycosylated oasomycins **5**, **6** and **9**, to the corresponding aglyca **4**, **7** and **8** as observed in the non-influenced case. Remarkably, in correlation with non-influenced fermentations the ratio of the amounts of the various metabolites in the early (0–80 h), and late stage of cultivation (80–240 h) differ significantly. After 24 h oasomycin F, **9** (35 mg dm^{-3}), and after 32 h oasomycin C, **6** (20 mg dm^{-3}), appeared to be the major metabolites, while **5** was found to be a minor compound with a yield of < 5 mg dm^{-3}. It seems likely that oasomycin F, **9**, being the main metabolite after 50–70 h of fermentation at pH 8.0, arises directly from the very early metabolite desertomycin A, **1**. In the later fermentation process **9** is converted into the 44-membered macrolactone oasomycin C, **6**. Because demannosylation also takes place in the pH-static fermentation at pH 8.0 the aglyca oasomycins D, **7** and E, **8** could be detected even after 80–100 h of cultivation.

As a consequence of the fermentation studies, the production of the different oasomycins seems to be mainly dependent on the actual pH-value of the fermentation. In order to analyse appropriate instabilities and interconversion reactions, the various oasomycins were stirred in a mixture of water–methanol (1 : 1) at pH-values of 1–12 (addition of 0.1 mol dm^{-3} HCl or NaOH) for 24 h at 30 °C. The results with the aglyca of the oasomycins are summarized in Fig. 2. Oasomycin A, **4**, was found to be stable under acidic conditions, while at a pH-value of 8 about 10–20% of **4** underwent γ-lactone opening to give oasomycin E, **8**. Stronger alkaline conditions favour this reaction. Additionally, at pH > 8.0 oasomycin A, **4**, is also converted into oasomycin D, **7**, which exhibits the already opened γ-lactone moiety as well as the ring-enlarged 44-membered macrolactone. For example, at pH 12, oasomycin A, **4**, yielded a 2:3 mixture of oasomycins D, **7** and E, **8**. The formation of the γ-lactone of oasomycin E, **8** (no re-lactonization of the macrolactone moiety as in the case of oasomycin D, **7**) is already observable at pH values of 5–6. Oasomycin D, **7**, itself appeared to be stable under slightly acidic conditions up to pH 3.0. At a pH < 3.0 ring contraction to the 42-membered macrolactone as well as γ-lactone formation in the side chain is favoured giving rise to oasomycin A, **4**, at pH 1.0 in nearly 100% yield. Under alkaline conditions (pH 8–9) both compounds **7** and **8** resulted in an equilibrium (2:3) of both educts with traces of **4**. In parallel studies, the corresponding glycosylated metabolites of the above mentioned oasomycins showed analogous interconversion reactions.

In combination these results led to the conclusion that, under acidic fermentation conditions, oasomycins A, **4**, and B, **5**, which bear a γ-lactone side chain and a 42-membered macrolactone ring, are the thermodynamically favoured compounds. At pH-values of 6.4–7.0 in the early fermentation stage the main metabolite **5** is formed *via* the biosynthetic intermediate oasomycin F, **9**. Obviously, the formation of the γ-lactone in the side chain is not necessarily catalysed by an enzyme. It is remarkable that oasomycin B, **5**, undergoes γ-lactone opening reactions leading to oasomycin F, **9**, in a pH-static fermentation at pH 8.0. This reaction should also occur in the later stage of a non-influenced cultivation. Under alkaline (fermentation) conditions oasomycin E, **8**, and the corresponding mannosyl derivative **9** undergo ring extension reactions to give the 44-membered macrolactones oasomycins D, **7**, and C, **6**, respectively. At this pH-value a 44-membered ring size may be thermodynamically favoured.

In the biosynthetic sequence desertomycin A, **1**, seems to be the first biosynthetic product which is detectable in the fermentation of strain FH-S 1625. In order to prove the presence of the hypothetical aglycon of **1**, this compound was prepared *in vitro* by a mannoside mannohydrolase catalysed reaction. As expected, β-D-mannoside mannohydrolase caused no reaction, whereas α-D-mannoside mannohydrolase gave *ca.* 50% conversion. The isolated aglycon of **1** was chromatographically characterized [R_f 0.3 in butan-1-ol–acetic acid–water, 4:1:5 (upper phase), and R_f 0.15 in ethyl acetate–methanol–water, 6:2:1] and exhibited the typical colourization reactions of the metabolites from the desertomycin family (*e.g.*

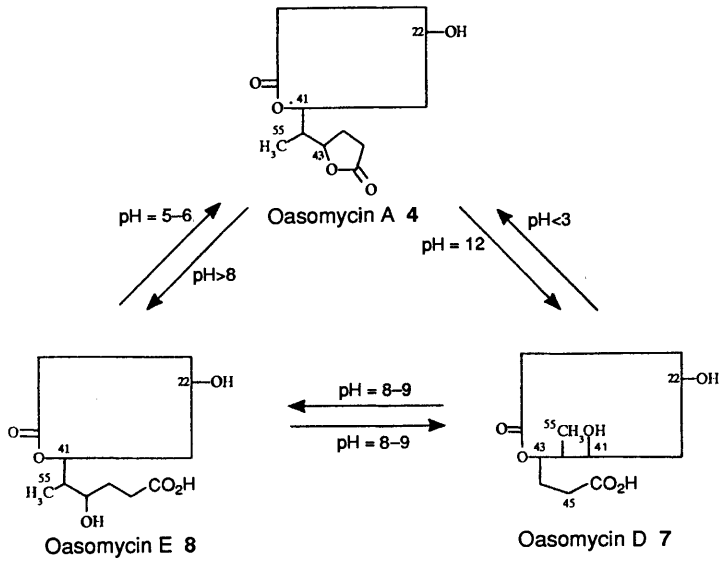


Fig. 2 Acid- and alkaline-catalysed conversions of the oasomycin aglycones

violet to black with anisaldehyde–sulfuric acid and brown with orcinol reagent).[6] A FAB-mass spectrum (*m/z* 1030.4, $C_{55}H_{90}NO_{16}$) established the successful hydrolysis of the mannose unit of **1**. With this sample we tried to detect the desertomycin A aglycon in the culture broth of strain FH-S 1625. However, this particular compound was not detectable at any time during a typical non-influenced fermentation pointing to a strong correlation of mannosylation process and macrolactone biosynthesis.

In combination, the results from the analysis of the fermentation time course, pH-static cultivations and *in vitro* conversions of the different oasomycins illuminate the biosynthetic relationships of the desertomycin family, depicted in Fig. 3. The desertomycin family arise from a common precursor, which is obviously built up *via* the polyketide pathway and the carbohydrate pool[9,10] resulting in desertomycin A, **1**, the first metabolite isolated from strain FH-S 1625.[2] The discovery of desertomycin B, **2**,[4] which bears a guanidino moiety in its side chain, can be explained by hydrolysis of the guanidino group to the primary amine of **1** *via* a guanidinobutyrase.[11] Thus, **2** may be the ultimate biosynthetic precursor of the desertomycin family, even though this compound is not detectable in the fermentation broth of strain FH-S 1625. On the other hand, we assume different amino acids could also serve as a polyketide starter unit for the formation of the macrolactone moiety.[9,10] Desertomycin A, **1**, may undergo oxidative deamination to the terminal aldehyde, which results in the diastereoisomeric mixture of hemiacetals named desertomycin D, **3**.[5] A further oxygenation step of **3** led to the carboxylic acid derivative oasomycin F, **9**, which acts as a parent compound for further conversions leading to the various oasomycins.

Oasomycin F, **9**, seems to be converted into oasomycin B, **5**, by lactonization of its γ-hydroxycarboxylic acid side chain and into oasomycin C, **6**, by a ring extension reaction forming the 44-membered macrolactone. The latter reaction is favoured under more alkaline fermentation conditions. In the late fermentation stage, oasomycins B, **5**, C, **6** and F, **9**, are converted into their corresponding aglyca oasomycins A, **4**, D, **7** and E, **8**, respectively, by α-D-mannosidase catalysed reactions.[6,10] However, analogous interconversions, as described for the glycosylated compounds, were found for the corresponding aglyca in the later fermentation stage. Thus, in between the formation of the various oasomycins starting from oasomycin F, **9**, only the demannosylation is enzymatically catalysed, while γ-lactone formation and opening reactions as well as the macrolactone enlargement and contraction processes seem to be non-enzymatic steps. As depicted in Fig. 3 the main route of the biosynthesis proceeds from the early minor metabolite desertomycin A, **1**, *via* the intermediate oasomycin F, **9**, to the first major component oasomycin B, **5**. During further cultivation of the producing organism, **5** is demannosylated to yield in the second major product oasomycin A, **4**, which is subsequently converted into oasomycin E, **8**, and oasomycin D, **7**. Studies with labelled precursors, which confirm the biosynthetic conclusions of the desertomycin family outlined above, will be the subject of an additional paper.[9]

The knowledge of the late biosynthesis in the desertomycin family has been used successfully to manipulate the metabolite pattern of *Streptoverticillium baldacii* subsp. *netropse* (strain FH-S 1625) directing the fermentation process to produce desired metabolites. Variations of the fermentation time led either to glycosylated compounds such as oasomycin B, **5** (50–70 h), or to the corresponding aglycons such as oasomycin A, **4** (80–100 h). On the other hand the enzymatic deamination step can be completely blocked by pH-static fermentations at pH 5.0 resulting in desertomycin A, **1**, as the major metabolite produced in yields of about 50 mg dm^{-3}. The carboxylic acid derivatives of the oasomycins can be obtained *via* pH-static fermentations at pH 8.0, however, a convenient chemical derivatization starting from oasomycins A, **4**, or B, **5**, respectively, seems to be the more elegant route.

It is obvious from our studies that the mannose moiety is linked to a macrolactone intermediate in an early stage of the biosynthesis. We presume the mannosylation to be necessary for transport processes through the cell membrane (the oasomycins as well as desertomycin A are present in the culture filtrate). On the other hand, the organism seems to acquire a new carbohydrate pool with the demannosylation in a late stage of the fermentation (stationary growing phase). Analogously, nitrogen storage followed by re-use may also argue for the deamination process.

Non-enzymatic reactions occurring in biosynthetic sequences are rarely found. However, examples in which late steps in the biosynthesis were caused by spontaneous reactions obviously involving no enzymes have been described for the streptomycetes metabolites of the urdamycin family[12–15] and for the fungal 10-membered lactones of the decarestrictine family.[16]

Experimental

General.—IR spectra in pressed KBr discs were recorded on

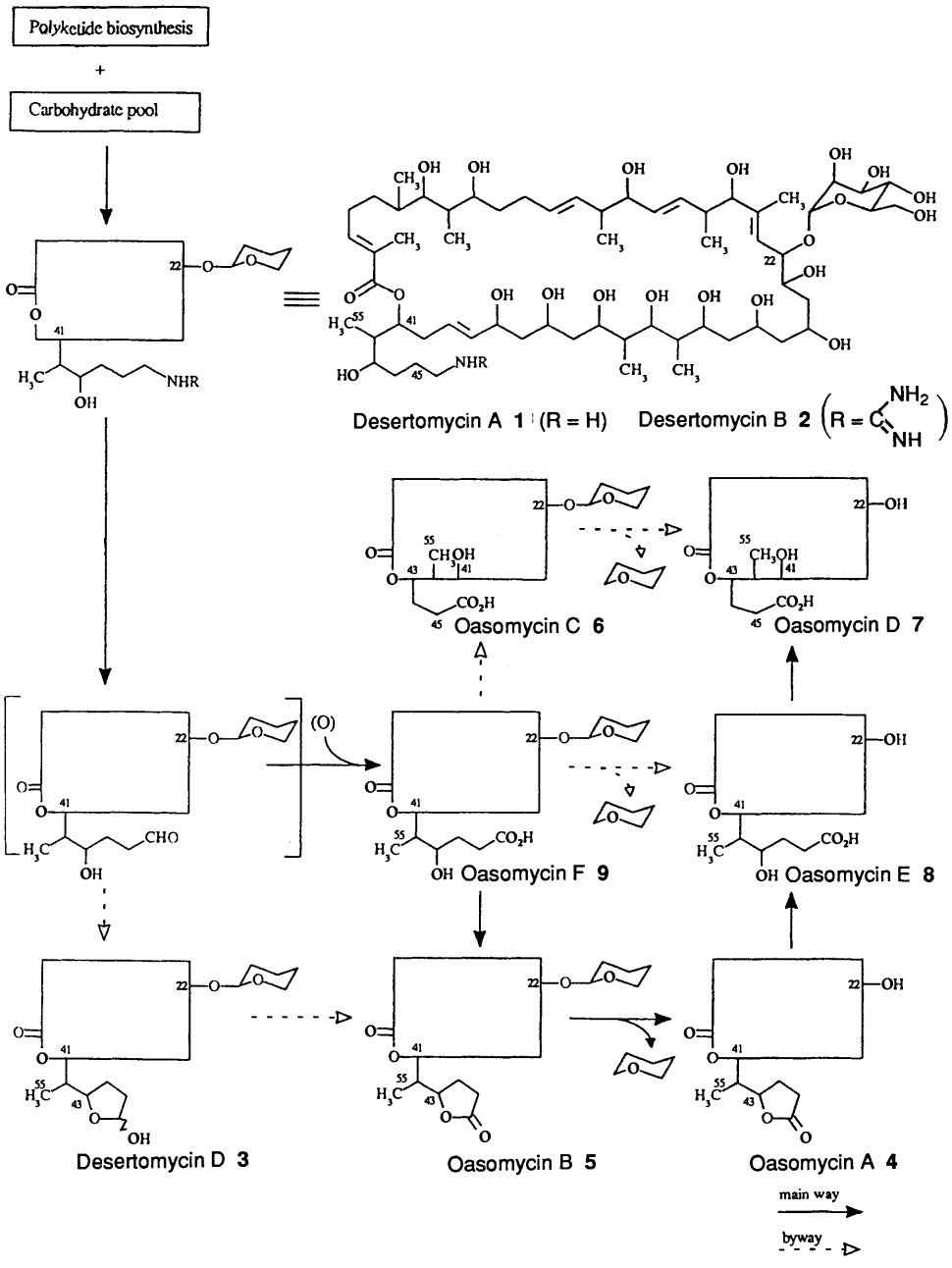


Fig. 3 Biosynthetic relationships in the desertomycin family

a Perkin-Elmer Model 297 spectrometer. FAB mass spectra were taken with a Finnigan MAT 8230 (matrix: α-nitrobenzyl-alcohol, glycerol). NMR spectra were measured with a Varian VXR-500S spectrometer. Chemical shifts are expressed in δ values with tetramethylsilane as internal standard. TLC was performed on silica gel plates (Merck, HPTLC ready-to-use plates, silica gel $60F_{254}$ on glass, RP_{18}-plates on glass), and column chromatography on silica gel 60 (Merck, 0.040 × 0.063 mm) or Sephadex LH-20 (Pharmacia). HPLC was performed on Kontron equipment (pump: 414, sampler 360, detector 420, mixing chamber M800, data system 450, detection at 228 nm, column: Beckmann Ultrasphere ODS, 10 × 0.40 cm, 10 μm, RP-18, methanol–water (78:22), 1 cm^3 min^{-1}). Fermentation was carried out in 1 and 10 dm^3 fermenters (Biostat M and E) as well as in a 200 dm^3 fermenter from Braun Dissel (Melsungen, Germany). Malt and yeast extracts were purchased from Difco Ltd., and all chemicals from Riedel de Haen. α-D-Mannoside mannohydrolase [EC 3.2.1.24, jack beans, suspension in 3.0 mol dm^{-3} $(NH_4)_2SO_4$, pH 7.5, activity 20 units mg^{-1} protein] and β-D-mannoside mannohydrolase [EC 3.2.1.25, snail acetone powder, suspension in 3.0 mol dm^{-3} $(NH_4)_2SO_4$ and 10 mmol dm^{-3} sodium acetate, pH 4.0, activity 5–30 units cm^{-3}] were purchased from Sigma.

Culture.—*Streptoverticillium baldacii* subsp. *netropse* (strain FH-S 1625, deposited in the German Culture Collection: DSM 5990) was grown on agar slants containing malt extract 1%, yeast extract 0.4%, glucose 0.4%, $CaCO_3$ 0.06%, agar 2%, pH 7.0 prior to sterilization (medium A), and soybean meal (degreased) 2%, mannitol 2%, pH 7.2 prior to sterilization (medium B). The strain was stored in glycerol (50%) at −20 °C.

Fermentation.—Cultivation of strain FH-S 1625 was performed in a two-step fermentation. The glycerol-containing storage mixture (3 cm^3) was used to inoculate 300 cm^3 Erlenmeyer flasks containing medium B (100 cm^3). The flasks were cultivated on a rotary shaker (180 rpm) for 3–5 days at 30 °C. These cultures were used to inoculate fermenters (1, 10

and 200 dm^3 working volume) containing medium B (inoculation volume 1.5%, 200 rpm, 30 °C, aeration 5 dm^3 min^{-1}). The cultivation time varied from 48 to 240 h. Foaming could be decreased using ethanolic polyol solutions (*e.g.* Niax Polyol). In the same way the precultures were used to inoculate further 300 cm^3 Erlenmayer flasks containing medium B (100 cm^3), which were cultivated on a rotary shaker (180 rpm) at 30 °C.

In Erlenmeyer flasks the fermentation process was analysed using a total cultivation time of 10 d. Samples were collected between 0 and 64 h in 2 h intervals between 68 and 156 h in 4 h intervals and between 162 and 240 h in 6 h intervals. The pH-value was measured using a pH-electrode. Mycelium dry weight was determined after centrifugation (4000 rpm, 10 min) of culture broth (10 cm^3) and drying the pellet at 105 °C. The remaining fermentation broth (80 cm^3) was separated into the culture supernatant and the mycelium by centrifugation (4000 rpm, 10 min). Supernatant (50 cm^3) was adsorbed on Amberlite XAD-16 (column: 4 × 2 cm). The column was washed with distilled water (25 cm^3) and eluted with methanol–water (4:1) (50 cm^3). The eluate was evaporated to dryness and redissolved in methanol–water (1:1) (1 cm^3). The mycelium obtained from the centrifugation step was extracted with acetone (100 cm^3) in an ultrasonic bath (15 min). The suspension was filtered, the filtrate was evaporated to dryness and redissolved in methanol–water (1:1) (1 cm^3). The production of secondary metabolites was visualized by TLC analysis on silica gel plates [solvent system: butan-1-ol–acetic acid–water, 4:1:5 (upper phase), staining reagent: anisaldehyde–H_2SO_4].

pH-*Static Fermentation*.—Cultivation and analysis of the obtained samples was performed exactly as described above. 10 dm^3 Fermenters (culture medium B, 500 rpm, aeration 0.5 Vvm, 30 °C) with pH-measuring and regulating equipment were used. Constant pH-values (pH 5.0 and 8.0) were obtained by addition of 2 mol dm^{-3} NaOH and 0.667 mol dm^{-3} citric acid.

Isolation.—For isolation and purification of oasomycin E, **8**, and desertomycin A, **1**, from 200 dm^3 fermentations (cultivation time for the production of **8**: 240 h; for **1**: 24 h) and oasomycin F, **9**, from 10 dm^3 fermentations (48 h) an analogous procedure was applied as previously described.[6] HPLC-purification [RP-18; column: 10 × 0.40 cm; 10 μm; 1 cm^3 min^{-1}; methanol–water (78:22)] yielded 10 mg dm^{-3} of pure amorphous oasomycin E, **8**, 2.5 mg dm^{-3} of oasomycin F, **9** (impurities **3**: 15%, **5**: 10%) and 15 mg dm^{-3} of pure desertomycin A, **1**. On both, silica gel- and RP_{18}-TLC plates these compounds showed violet to black colourization with anisaldehyde–H_2SO_4 reagent, brown colourization with orcinol, grey to brown colourization with Ehrlich's reagent, and a weak pink colourization with blue tetrazolium reagent.

Enzymatic Reactions.—To desertomycin A, **1** (1 mg) and oasomycin F, **9** (1 mg) each dissolved in 0.01 mol dm^{-3} phosphate buffer (1 cm^3, pH 6.5), α- or β-D-mannoside mannohydrolase solution (0.1 cm^3) was added and the mixture was stirred at 30 °C. The reaction was monitored by TLC as described in the fermentation section using oasomycin E, **8**, as a control. After 3 days reaction with α-D-mannoside hydrolase as catalyst about 30% of both desertomycin A, **1**, and oasomycin F, **9**, were deglycosylated to desertomycin A-aglycon and oasomycin E, **8**, respectively. A 5 day reaction period yielded about 50% demannosylation of the educts. The use of β-D-mannoside hydrolase caused no cleavage of the glycosidic bond.

Oasomycin E **8**.—R_f 0.46 (ethyl acetate–methanol–water, 6:2:1), 0.55 (butan-1-ol–acetic acid–water, 4:1:5, upper phase), 0.91 [ethanol–ammonia (15%)–water, 8:1:1], 0.46 (RP_{18}-plates, methanol–water, 4:1); $[\alpha]_D^{25}$ 0 (*c* 0.65 in methanol); λ_{max}(MeOH)/nm 220 (ε/dm^3 mol^{-1} cm^{-1} 13 200) and 202 (20 400); ν_{max}/cm^{-1} (KBr) 3390br, 2960, 2930, 1685, 1640 and 1565; δ_H(500 MHz; [$^2H_6$]-DMSO) 0.56 (54-Me), 0.60 (49-Me), 0.68 (48-Me), 0.72 (53-Me), 0.76 (55-Me), 0.76 (50-Me), 0.94 (51-Me), 1.03 (34-H_2), 1.12 and 1.38 (10-H_2), 1.17 (24-H_2), 1.20 (36-H_2), 1.26 and 1.46 (5-H_2), 1.28 (26-H_2), 1.32 (32-H), 1.32 and 1.62 (44-H_2), 1.35 (30-H), 1.42 (28-H_2), 1.44 (52-Me), 1.46 (6-H), 1.49 (8-H), 1.70 (47-Me), 1.74 (42-H), 1.82 and 2.10 (11-H_2), 2.05 (14-H), 2.07 (4-H_2), 2.10 (18-H), 2.14 (40-H_2), 2.18 (45-H_2), 3.18 (7-H), 3.28 (43-H), 3.42 (19-H), 3.55 (29-H), 3.60 (9-H), 3.62 (31-H), 3.68 (15-H), 3.78 (23-H), 3.80 (25-H), 3.82 (27-H), 3.85 (35-H), 4.00 (22-H), 4.05 (33-H), 4.10 (37-H), 5.00 (41-H), 5.20 (21-H), 5.28 (16-H), 5.32 (13-H), 5.32 (17-H), 5.33 (12-H), 5.40 (38-H), 5.45 (39-H) and 6.65 (3-H); δ_C(125.7 (MHz; [$^2H_6$]-DMSO) 9.6 (C-53), 10.4 (C-54), 10.4 (C-55), 11.2 (C-49), 11.5 (C-52), 12.1 (C-47), 12.2 (C-48), 15.3 (C-50), 16.8 (C-51), 26.0 (C-4), 28.4 (C-44), 28.9 (C-11), 30.7 (C-45), 32.1 (C-10), 32.3 (C-40), 32.9 (C-5), 34.3 (C-6), 39.2 (C-30), 39.3 (C-18), 40.0 (C-32), 40.4 (C-24), 41.3 (C-8), 41.5 (C-42), 42.2 (C-34), 42.2 (C-14), 42.2 (C-28), 45.6 (C-26), 45.7 (C-36), 63.7 (C-35), 63.8 (C-25), 66.7 (C-33), 66.8 (C-37), 66.8 (C-27), 70.0 (C-43), 70.8 (C-22), 70.8 (C-23), 70.8 (C-31), 72.0 (C-9), 72.8 (C-29), 73.4 (C-41), 74.3 (C-15), 74.6 (C-7), 81.0 (C-19), 122.9 (C-39), 126.8 (C-21), 127.1 (C-2), 129.5 (C-12), 130.9 (C-16), 132.5 (C-13), 132.6 (C-17), 137.7 (C-38), 138.1 (C-20), 141.9 (C-3), 166.4 (C-1) and 174.9 (C-46); *m/z* (HR-FAB-MS) 1067.6494 [$(M + Na)^+$], 1083.6 [$(M + K)^+$]; MW 1045.4 ($C_{55}H_{96}O_{18}$).

Oasomycin F **9**.—R_f 0.35 (ethyl acetate–methanol–water, 6:2:1), 0.33 (butan-1-ol–acetic acid–water, 4:1:5, upper phase), 0.72 [ethanol–ammonia (15%)–water, 8:1:1], 0.33 (RP_{18}-plates, methanol–water, 4:1); δ_H(500 MHz; [$^2H_6$]-DMSO) 0.56 (54-Me), 0.58 (49-Me), 0.68 (48-Me), 0.68 (55-Me), 0.75 (53-Me), 0.80 (50-Me), 0.96 (51-Me), 1.14 (34-H_2), 1.14 and 1.36 (10-H_2), 1.18 (24-H_2), 1.18 (36-H_2), 1.20 and 1.41 (5-H_2), 1.20 and 1.58 (44-H_2), 1.21 (26-H_2), 1.32 (32-H), 1.38 (30-H), 1.46 (6-H), 1.46 (28-H_2), 1.50 (52-Me), 1.50 (8-H), 1.80 (47-Me), 1.80 and 2.06 (11-H_2), 2.00 (14-H), 2.00 (42-H), 2.06 (4-H_2), 2.14 (18-H), 2.14 (45-H_2), 2.18 (40-H_2), 3.19 (7-H), 3.36 (4′-H), 2.28 (5′-H), 3.46 (43-H), 3.50 (19-H), 3.50 (2′-H), 3.52 (3′-H), 3.52 (6′-H), 3.56 (29-H), 3.59 (9-H), 3.68 (15-H), 3.74 (31-H), 3.75 (23-H), 3.78 (35-H), 3.78 (27-H), 3.81 (25-H), 4.03 (33-H), 4.07 (37-H), 4.12 (22-H), 4.60 (1′-H), 4.96 (41-H), 5.16 (21-H), 5.28 (16-H), 5.34 (12-H), 5.36 (13-H), 5.36 (17-H), 5.43 (38-H), 5.43 (39-H) and 6.60 (3-H); δ_C(125.7 MHz; [$^2H_6$]-DMSO) 9.6 (C-53), 10.4 (C-54), 10.4 (C-55), 11.2 (C-49), 11.6 (C-52), 12.2 (C-47), 12.2 (C-48), 15.2 (C-50), 16.7 (C-51), 26.0 (C-4), 28.4 (C-44), 28.9 (C-11), 31.5 (C-45), 32.1 (C-10), 32.3 (C-40), 32.9 (C-5), 34.3 (C-6), 38.9 (C-30), 39.2 (C-18), 40.4 (C-32), 40.5 (C-42), 40.8 (C-24), 41.4 (C-8), 42.2 (C-14), 42.2 (C-34), 42.3 (C-28), 45.6 (C-26), 45.7 (C-36), 61.3 (C-6′), 63.5 (C-25), 63.9 (C-35), 66.7 (C-27), 66.8 (C-33), 66.8 (C-37), 67.2 (C-4′), 69.4 (C-23), 70.0 (C-43), 70.6 (C-2′), 70.8 (C-31), 70.9 (C-3′), 72.1 (C-9), 72.9 (C-29), 73.4 (C-41), 73.5 (C-5′), 73.7 (C-22), 74.4 (C-15), 74.7 (C-7), 80.7 (C-19), 95.9 (C-1′), 122.2 (C-21), 122.9 (C-39), 127.1 (C-2), 129.5 (C-12), 131.0 (C-16), 132.7 (C-17), 132.6 (C-13), 137.7 (C-38), 141.9 (C-3), 143.3 (C-20), 166.4 (C-1) and 175.7 (C-46); *m/z* (HR-FAB-MS) 1205.7226 [$(M - H)^+$]; MW 1205.5 ($C_{61}H_{106}O_{23}$).

pH-*Stability of the Oasomycins*.—In a mixture of distilled water and methanol (1:1) (1 cm^3), which had been adjusted to pH values in the range 1–12 with 0.1 mol dm^{-3} HCl or 0.1 mol dm^{-3} NaOH, the different oasomycins (*ca.* 1 mg) were stirred at 30 °C for a total of 24 h. Each sample was analysed by TLC [butan-1-ol–acetic acid–water, 4:1:5 (upper phase), and methanol–water, 4:1, RP_{18}-plates; staining reagents: anisaldehyde-H_2SO_4; orcinol].

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