A NON-ENZYMATIC REACTION IN THE LATE BIOSYNTHESIS OF THE DECARESTRICTINE FAMILY

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In the late biosynthesis of the decarestrictines, inhibitors of the *de novo* formation of cholesterol isolated from the culture broth of *Penicillium simplicissimum* (FH-A 6090), a common pentaketide precursor undergoes post-polyketide modifications leading to the different members of the decarestrictine family. Besides subsequent enzymatically catalyzed reactions an unexpected non-enzymatic conversion was found to be the key step in the biosynthetic sequence. Under acidic conditions during fermentation the decarestrictines A_1 and A_2 (**3** and **4**) are converted into the main product decarestrictine D (**2**) and the new decarestrictines N (**5**) and O (**6**), whose physico-chemical data are reported. Mechanistic aspects of the non-enzymatic reaction as well as a more detailed picture of the biosynthetic relationships of the decarestrictine family are described. By the application of pH-static fermentations these results were successfully used to manipulate the secondary metabolite pattern of strain FH-A 6090 directing the fermentation process to produce desired members of the decarestrictine family.

The decarestrictines[1~3] were discovered by a chemical screening as products of *Penicillium simplicissimum* (strain FH-A 6090). These secondary metabolites are novel inhibitors of the *de novo* cholesterol biosynthesis, as demonstrated in cell line tests with HEP-G2 liver cells and in *in vivo* studies with normolipidemic rats. Most of the decarestrictines exhibit a 10-membered lactone ring with an exocyclic methyl group as characteristic structural elements[3].

Recently, our biosynthetic studies on the main metabolites decarestrictine B (**1**) and D (**2**) revealed that the carbon skeleton of the 10-membered lactones derive from an acetate polyketide starter unit (C-10/C-9), which is subsequently elongated by four malonate building blocks *via* the polyketide pathway (Fig. 1)[4]. The unusual oxygenation pattern was investigated by feeding experiments with sodium [1-^{13}C, $^{18}O_2$]acetate resulting in [^{18}O]-incorporation into the lactone moiety at C-1 and at C-3 in both, decarestrictine B (**1**) and D (**2**). A fermentation performed under an [$^{18}O_2$]-enriched atmosphere indicated that the oxygen atoms of the oxirane ring in **1** as well as the oxygen at C-7 in **2** are introduced *via* oxygenase catalyzed steps. As a consequence of these earlier studies the decarestrictines obviously arise from a common pentaketide precursor, which is formed by a single polyketide synthetase performancing reduction, dehydration and finally lactonization steps. Subsequently, the proposed intermediate undergoes further post-polyketide modifications to result in the various members of the decarestrictine family[4].

With the aim to manipulate the metabolite pattern we investigated in the late biosynthesis of the decarestrictine family. Reflecting the pH-time course of the fermentation the acid stability of the various decarestrictines was studied. Unexpectedly, a non-enzymatic reaction was found to be a key-step in this

Fig. 1. Biosynthesis of decarestrictine B (**1**) and D (**2**).

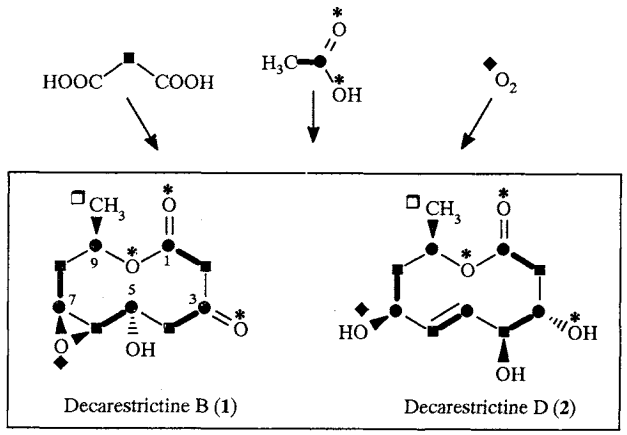


Table 1. Yields of the decarestrictines in non-influenced and pH-static fermentations of *Penicillium simplicissimum* (strain FH-A 6090) after 6 days.

Metabolite	Yield (mg/liter)			
	Non-influenced fermentation	pH 1.0	pH 2.5	pH 5.5
Decarestrictine A_1/A_2 (**3/4**)	5	—	10	20
Decarestrictine B (**1**)	60	—	40	20
Decarestrictine D (**2**)	50	20	25	—
Decarestrictine N/O (**5/6**)	5	—	5	—
Decarestrictine E (**8**)	20	—	10	—
Decarestrictine F (**7**)	5	—	2	—

biosynthetic sequence offering the opportunity to direct the metabolite pattern to desired products by using pH-static fermentation techniques.

Control of the Metabolite Pattern by pH-Static Fermentations

Regarding the typical pH time course during cultivation it seemed reasonable to suppose a correlation between the pH-value in the fermentation broth and the appearance of the 'decarestrictine D' or 'decarestrictine B'-related compounds[4]. Therefore, pH-static fermentations of the producing organism *Penicillium simplicissimum* (strain FH-A 6090) were considered to gain further information about biosynthetic relationships. In addition, we expected to manipulate the metabolite pattern *via* the pH-value in the fermentation broth. After a 48-hour period of non-influenced cultivation (10-liter scale), constant pH-values (pH 1.0, 2.5, 5.5, and 7.0) were obtained in the fermentation vessel by the addition of 1 M NaOH and/or 0.667 M citric acid. In 12-hour intervals the metabolite pattern was analyzed by TLC chromatography with chloroform - methanol (9 : 1) as solvent system and anisaldehyde-sulfuric acid as staining reagent[1]. The results are summarized in Table 1.

At a constant pH of 2.5 the typical metabolite pattern of a non-influenced fermentation with both major products, decarestrictine B (**1**, 40 mg/liter) and D (**2**, 25 mg/liter) was observed. Exclusively, decarestrictine D (**2**, 20 mg/liter), N and O (**5** and **6**, 5 mg/liter each) were formed at pH 1.0 in an early

stage of the cultivation. However, due to the instability of **5** and **6**, decarestrictine D (**2**) was the only metabolite detectable after 6 days. To intercept acidic catalyzed conversions the decarestrictine producing organism was cultivated at pH 7.0, which resulted in poor growth and suppression of secondary metabolite formation. At pH 5.5, however, a satisfying cell growth and the production of decarestrictine A_1/A_2 (**3/4**, 20 mg/liter) as well as of decarestrictine B (**1**, 20 mg/liter) as the only metabolites was observed.

The pH-static cultivations of *Penicillium simplicissimum* (strain FH-A 6090) allow to manipulate the metabolite pattern to direct the fermentation process to produce desired compounds. At pH 1.0 only the biologically most interesting[1] decarestrictine D (**2**) is present in the culture broth, which simplifies isolation and purification procedures especially in large scale fermentations. On the other hand, decarestrictine B (**1**) as well as the biosynthetic intermediates decarestrictine A_1/A_2 (**3/4**) can be obtained in sufficient yields in pH-static fermentations at pH 5.5, while non-influenced fermentations reveal a mixture of various decarestrictines[1~4].

pH-Stability of the Decarestrictines

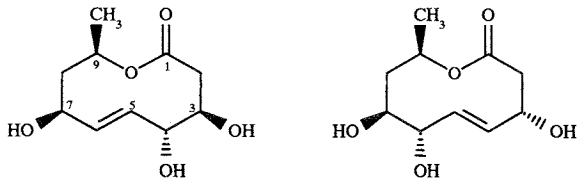
As a consequence of the pH-static fermentation studies the production of the different decarestrictines seems to be mainly dependant on the actual pH-value of the culture broth, which led us to investigate their pH-stabilities. In distilled water, adjusted to pH 2.5 or to pH 7.2 with 0.1 M HCl or 0.1 M NaOH the decarestrictines A to M[2,3] were stirred at 25°C for 7 days. This procedure roughly resembles the conditions in the culture broth of the producing organism strain FH-A 6090 at both, an early and late stage of a typical non-influenced fermentation. In an analytical scale (about 1 mg/ml solution of each metabolite) the stability of the samples were analyzed by TLC chromatography with chloroform-methanol (9:1) and anisaldehyde-sulfuric acid as staining reagent[1]. After 7 days at pH 7.2 decarestrictine B (**1**) showed a slight decomposition (*ca.* 20%) into a number of more polar components, which were not identified. The remaining decarestrictines tested were stable under these conditions. Furthermore, with the exception of decarestrictine A_1/A_2 (**3/4**) and F (**7**) all metabolites appeared to be stable at pH 2.5 in accordance with the results observed during fermentation. After 36 hours a complete conversion of the decarestrictines A_1 and A_2 (**3** and **4**) into decarestrictine D (**2**) as well as into the new decarestrictines N (**5**) and O (**6**) was found. The 3:1 ratio of the diastereoisomeric educts decarestrictine A_1/A_2 (**3/4**) resulted in nearly the same ratio for decarestrictine D (**2**) and the mixture of N and O (**5** and **6**). Applying the pH-static fermentation technique (pH 5.5, 10-liter scale) we obtained satisfying yields (20 mg/liter) of decarestrictine A_1/A_2 (**3/4**) sufficient to study the mechanism of the acidic conversion as well as to elucidate the structures of the new decarestrictines N and O.

150 mg of the isolated mixture of decarestrictine A_1 and A_2 were treated under the same reaction conditions described above. After 36 hours the reaction mixture was adsorbed on a XAD-16 column, eluted with methanol-water (4:1) and chromatographed on a silica gel column (chloroform-methanol, 9:1). The obtained sample of purified decarestrictine D (**2**, yield 65 mg) corresponds completely with the authentic metabolite[1,2]. The second reaction product (yield 23 mg) appeared to be chromatographically homogenous, but NMR spectroscopic investigations revealed a mixture of two components in a ratio of 4:3. The new compounds were named decarestrictine N (major product) and O.

Decarestrictine N (**5**) and O (**6**)

A HR-FAB mass spectrum of the mixture showed only one molecular ion at $m/z = 217.1076$ [$(M+H)^+$] pointing to an identical composition of decarestrictine N and O ($C_{10}H_{16}O_5$). The structure elucidation of

Fig. 2. Structures of decarestrictine N (**5**) and O (**6**).



Decarestrictine N (**5**) Decarestrictine O (**6**)

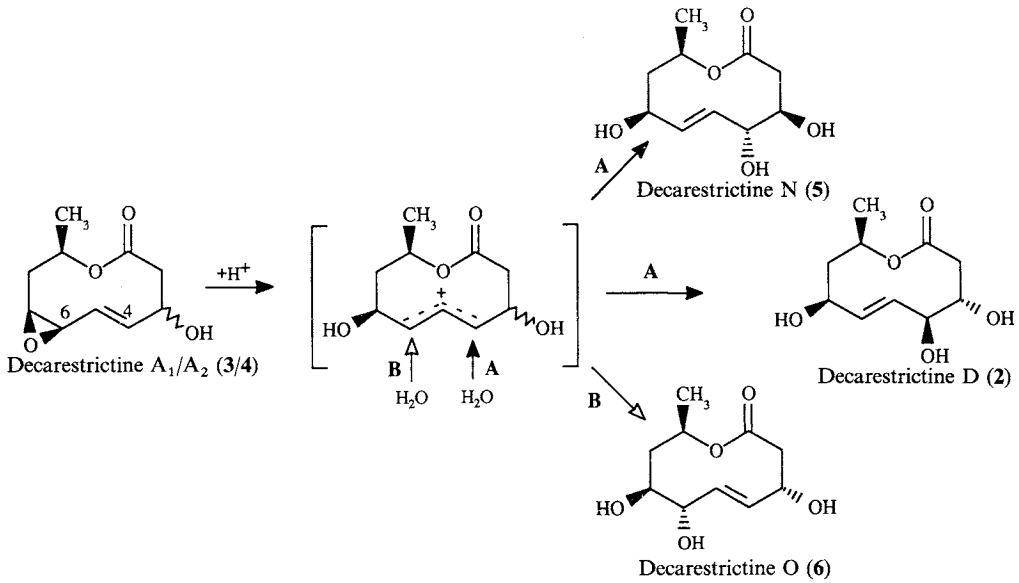
both, decarestrictine N and O from the mixture was possible because of well separated NMR signal patterns in acetone-d_6. *A priory* the ^{13}C NMR spectrum revealed the presence of 19 signals, but a ^{1}H-^{13}C NMR spectrum proved the signal at δ 74.0 to originate from two carbons [C-4 of decarestrictine N (**5**) and C-6 of decarestrictine O (**6**)]. Decarestrictine N exhibits close structural similarities to the 10-membered lactone decarestrictine D (**2**), which was expressed by comparison of the chemical shifts of the ^{13}C NMR spectra (125.7 MHz, acetone-d_6). An identical constitution of decarestrictine N (**5**) in respect to D (**2**) resulted from ^{1}H-^{1}H as well as ^{1}H-^{13}C correlated 2D NMR spectra. However, some differences were found by comparison of the ^{1}H NMR signals. Most strikingly, the coupling constants of 3-H (N (**5**): $J_{3,2\text{-Ha}}=4.0$ and $J_{3,2\text{-Hb}}=10.5$ Hz; D (**2**): $J_{3,2\text{-Ha}}=6.9$, and $J_{3,2\text{-Hb}}=2.4$ Hz) point to an opposite stereochemistry at this center of chirality. Although the signal at δ 4.45 (4-H) appeared as a multiplet, we discuss the 4*R*-configuration because of both, the multiplet signal pattern and mechanistic aspects in the formation of **5** (see below). Inspection of the coupling constants of 7-H and 9-H in **5** gave evidence for analogous configurations at these centers of chirality in comparison to **2**. The *E*-configuration of the double bond at C-5 was deduced from the coupling constant of 5-H/6-H ($J_{5,6}=15.5$ Hz). Compared to **2** decarestrictine N (**5**) obviously exhibit opposite stereochemistry at C-3 and C-4, although all stereochemical aspects have not been proven.

The ^{13}C NMR spectrum of decarestrictine O (**6**) exhibits the typical signals of a 10-membered lactone of the decarestrictine type, namely an exocyclic methyl group (δ 21.4, q, C-10), a lactone carbonyl group (δ 170.7, s, C-1), as well as two CH_2-groups (δ 45.1, t, C-2 and δ 39.8, t, C-8). A ^{1}H-^{1}H COSY NMR spectrum showed a connected coupling pattern from 2-H_2 to 10-H_3: methyl group (δ 1.28, 10-H_3), methine group (δ 5.04, 9-H), methylene group (δ 1.31, 8-H_b; δ 2.31, 8-H_a), two methine groups (δ 3.40, 7-H and δ 4.25, 6-H), the methine protons of a double bond (δ 5.55, 5-H and δ 5.40, 4-H), a further methine group (δ 4.45, 3-H), and a methylene group (δ 1.36, 2-H_b; δ 2.90, 2-H_a). The signal assignments are in accordance with the data obtained from a ^{1}H-^{13}C 2D NMR spectrum. Based on proton coupling constants **6** exhibits an identical stereochemistry at C-3 (*R*) and C-7 (*S*) than decarestrictine D (**2**). Mechanistic aspects of the oxirane ring opening let us discuss a *trans*-position of the hydroxy groups at C-6/C-7. A close examination of the culture broth of strain FH-A 6090 had already shown that a TLC spot corresponding to decarestrictine N (**5**) and O (**6**) is detectable in the early acidic fermentation stage[4].

Mechanistic Aspects of the Non-Enzymatic Reaction

In contrast to the epoxide moiety containing decarestrictines B (**1**), E (**8**) and F (**7**), the oxirane ring in decarestrictine A_1/A_2 (**3**/**4**) is accessible for acid catalyzed opening (pH 2.5 in the fermentation broth). Obviously, a regio- and stereoselective ring opening resulted in allylic carbocation intermediates being stereoisomers at C-3. The oxygen of the former epoxide remains at C-7 in *S*-configuration, which had

Fig. 3. Proposed mechanism of the non-enzymatic conversion of decarestrictine A_1/A_2 (**3**/**4**) into decarestrictine D (**2**), N (**5**), and O (**6**).



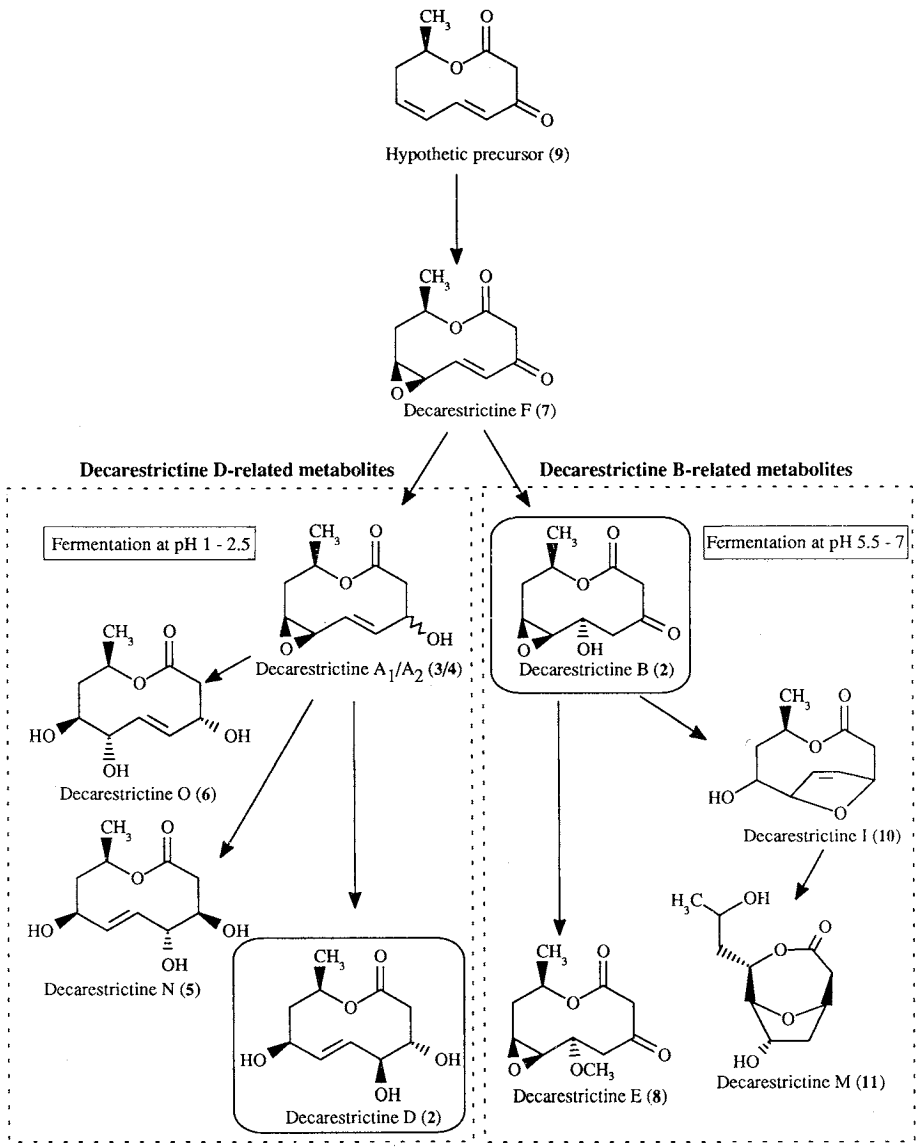
been proven *via* fermentations in [$^{18}O_2$]-enriched atmosphere causing label-incorporation into 7-OH in decarestrictine D (**2**, see Fig. 1)[4]. In an S_N1-type reaction the hypothetic carbocation intermediate reacts with a molecule of water either at C-4 or at C-6. Considering the stereochemistry at C-3 as well as the ratio of the educts (3 : 1), decarestrictine A_1 (**3**; major product) resulted in both, decarestrictine D (**2**; attack at C-4) and decarestrictine O (**6**; attack at C-6). In analogy, decarestrictine A_2 (**4**) is converted into decarestrictine N (**5**; attack at C-4), while the expected minor product from the attack at C-6 could not be isolated. Since decarestrictine D (**2**) and N (**5**) were found to be the major products of this non-enzymatic step, the nucleophilic addition is favoured at position C-4 in the hypothetic intermediate. Stereochemically, the spatial position of the α-hydroxy groups should direct the nucleophile to result in *trans*-diols either at C-3/C-4 in **2** and **5** or at C-6/C-7 in **6**.

Discussion

In combination, the results from the analysis of the fermentation time course[1,4], the experiments with stable isotope-labeled precursors[4], the pH-static cultivations as well as non-enzymatic conversions of the different decarestrictines illustrate the biosynthetic relationships in the decarestrictine family, depicted in Fig. 4.

Obviously, the fungal polyketide synthase forms the hypothetic intermediate **9**, leading to decarestrictine F (**7**) by oxygenation of the *Z*-configurated double bond at C-6/C-7 *via* a monooxygenase catalyzed reaction[4]. At this point, the biosynthetic pathway branches off into two parts, namely to the 'decarestrictine B'-, and to the 'decarestrictine D'-related compounds. A non-stereospecific reduction of the keto group at C-3 in decarestrictine F (**7**) results in the diastereoisomeric mixture decarestrictine A_1/A_2 (**3**/**4**, ratio 3 : 1). Being the key step in the following course of conversions the pH-value in the fermentation broth triggers the metabolite pattern produced. Under acidic conditions (pH < 3.0) the intermediates A_1/A_2 were converted in a non-enzymatic reaction into decarestrictine D (**2**), the biologically most interesting compound, as well as the new minor components decarestrictine N (**5**) and O (**6**).

Fig. 4. Biosynthetic relationships of the decarestrictine family.



In pH-static fermentations (pH > 3.0) the decarestrictines A_1/A_2 (**3**/**4**) appeared to be stable. Besides **3** and **4** a second major metabolite, decarestrictine B (**1**), was formed. Starting from decarestrictine F (**7**) a regio- and stereoselective addition of water at the double bond at C-4/C-5 seems to result in decarestrictine B (**1**), because in experiments with both, [^{18}O]-labeled acetate and oxygen gas no label-incorporation into 5-OH was detectable[4]. This supports the assumption that 5-OH originates *via* a hydratase catalyzed reaction from water. Further enzymatic derivatization of decarestrictine B (**1**) seem to yield in additional 'decarestrictine B'-related compounds like decarestrictine E (**8**), I (**10**), and M (**11**). Remarkably, in static (non-shaken) cultivations using roux-flasks (pH 4.5 to 6.0) decarestrictine D (**2**) embodies the most stabile and main metabolite during 40 days, whereas *e.g.* decarestrictine B (**1**), underlies catabolic processes. The observed stability of **2** correlates with the isolation of decarestrictine D (identical with tuckolide) from the sclerotium of *Polyphorus tuberaster*, called 'the stone that grows'[13].

Up to our present knowledge, non-enzymatic steps in the biosynthesis of secondary metabolites have

been reported only in rare cases. ROHR[5~8] described that in the biosynthetic formation of the urdamycin family the S-methyl group of urdamycin E is introduced by a non-enzymatic nucleophilic Michael addition of methylmercaptane at the 5-position of urdamycin A (=aquayamycin). Furthermore, urdamycin C and D are formed by condensations of urdamycin A with 4-hydroxypyruvic acid and indole-3-pyruvic acid, respectively, while urdamycin H is build up *via* an unusual non-enzymatic ring contraction reaction. The spontaneous reactions without participation of enzymes were found to be late steps in the biosynthetic sequence of the urdamycin family. A second example has been observed for the late biosynthesis of the macrolactones of the desertomycin family, in which γ-lactone formation in the side chain as well as ring enlargement reaction from a 42- to a 44-membered macrolactone moiety not necessarily need enzymatic catalysis[9]. These results are in accordance with the above mentioned findings in the decarestrictine family, in which non-enzymatic steps are also involved in the late biosynthetic sequence. We presume a more frequent occurrence of non-enzymatic reactions in the secondary metabolism of organisms, especially of those, which result in a wide spectrum of related compounds[6]. These aspects reveal further arguments in the discussion of meaning and evolution of secondary metabolism[10,11]. Regarding the 'playing-ground hypothesis' of ZÄHNER[12] a further facette has to be considered with the coincidentally occurring transformations of secondary metabolites by non-enzymatic reactions.

Experimental

General

FAB mass spectra were taken with a Finnigan MAT 8230. ^1H and ^{13}C NMR spectra were measured with Varian VXR-500S. Chemical shifts are expressed in δ values (ppm) with tetramethylsilane (TMS) as internal standard. The multiplicities of the ^{13}C NMR values were assigned by attached proton test (APT). Fermentation was carried out in a 10-liter fermenter (Biostat E) from Braun Dissel (Melsungen, Germany). TLC was performed on silica gel plates (Merck, HPTLC-ready-to-use-plates, silica gel $60F_{254}$ on aluminium foil or glass) and column chromatography on silica gel 60 (0.040~0.063 mm, Merck) and Sephadex LH-20 (Pharmacia).

Fermentation

Penicillium simplicissimum (strain FH-A 6090, deposited in the German Culture Collection: DSM 4209) was grown on agar slates containing malt extract 2%, yeast extract 1%, $(NH_4)_2HPO_4$ 0.05%, agar 1.5%, pH 6.0 prior to sterilization (medium A). A piece of agar (1 cm^2) from this storage culture was used to inoculate a 300-ml Erlenmeyer flask containing 100 ml of medium A omitting agar (medium B). After cultivation on a rotary shaker (140 rpm) at 25°C for 5 days 300 ml of these cultures were used to inoculate a 10-liter fermenter with pH-measuring and regulation equipment containing medium B (200 rpm, aeration 5 liters/minute). The fermentation process was analyzed for a total of 7 days. 48 hours after inoculation the pH-value was adjusted to pH 1.0, 2.5, 5.5, or 7.0 with 0.667 M citric acid and/or 1 M NaOH. Every 12 hours a 10 ml sample of the culture broth was extracted twice with 5 ml of ethyl acetate. The combined organic layers were evaporated to dryness and the oily crude extract was dissolved in 200 μl of chloroform - methanol (9 : 1). The production of secondary metabolites was analyzed by TLC chromatography (solvent system: $CHCl_3$ - MeOH, 9 : 1; staining reagent: anisaldehyde-sulfuric acid). In order to isolate the decarestrictines A_1/A_2 (**3**/**4**) the culture of the pH-static fermentation at pH 5.5 was harvested after 72 hours. The isolation and purification protocol followed the procedure as previously described[1].

pH-Stability of the Decarestrictines

In 1 ml of distilled water, which had been adjusted at pH=2.5 with 0.1 N HCl or at pH=7.2 with 0.1 N NaOH, about 1 mg of the different decarestrictines were stirred at 25°C for a total of 7 days. After 1, 6, 14, 22, 36, and 144 hours the stability of each sample was analyzed by TLC chromatography (solvent system: $CHCl_3$ - MeOH, 9 : 1; staining reagent: anisaldehyde-sulfuric acid).

Decarestrictine N (**5**) and O (**6**)

150 mg of a mixture of decarestrictine A_1/A_2 (**3**/**4**, 3 : 1) yielded from a pH-static fermentation at pH 5.5 were dissolved in 50 ml of distilled water, adjusted to pH=2.5 with 0.1 N HCl, and stirred at 25°C for

36 hours. TLC analysis revealed about 100% conversion (after 6 hours *ca.* 40%) into decarestrictine D (**2**) and N/O (**5/6**) in a ratio of 3:1. The mixture was neutralized by the addition of 0.1 N NaOH and adsorbed on an Amberlite XAD-16 column (10 × 3 cm). After washing the column with 50 ml of distilled water, the products were eluted with 50 ml of methanol. The dried eluate (135 mg) was chromatographed on silica gel (column: 30 × 2, chloroform - methanol, 9:1) to yield 65 mg (43.3%) of pure decarestrictine D (**2**) and 23 mg (15.3%) of a mixture of decarestrictine N (**5**), and O (**6**) in a ratio of 4:3. The mass spectra and NMR data were obtained from the mixture. NMR signal assignments arose from partial subtraction of the signals.

Decarestrictine N (**5**): Rf 0.26 (chloroform - methanol, 9:1), 0.90 (1-butanol - acetic acid - water, 4:1:5, upper phase), violet colorization after staining with anisaldehyde-sulfuric acid on silica gel TLC plates; HRFAB-MS *m/z* 217.1076 [$(M+H)^+$], MW 216.24 ($C_{10}H_{16}O_5$); ^{1}H NMR (500 MHz, acetone-d_6) δ 1.17 (d, $J_{10,9}=6.6$ Hz, 10-H_3), 1.36 (dd, $J_{2a,2b}=-15.0$, and $J_{2a,3}=4.0$ Hz, 2-H_a), 2.36 (dd, $J_{2b,2a}=-15.0$, and $J_{2b,3}=10.5$ Hz, 2-H_b), 2.40 (m, 8-H_2), 3.67 (ddd, $J_{3,2a}=4.0$, and $J_{3,2b}=10.5$ Hz, 3-H), 4.45 (m, 4-H), 4.67 ~ 4.74 (m, 7-H and 9-H), 5.70 (ddd, $J_{5,6}=15.5$, $J_{5,4}=3.0$, and $J_{5,7}=1.0$ Hz, 5-H), 5.99 (ddd, $J_{6,5}=15.5$, $J_{6,4}=1.5$ Hz, and $J_{6,7}=5.5$ Hz, 6-H); ^{13}C NMR (125.7 MHz, acetone-d_6) δ 23.2 (q, C-10), 39.4 (t, C-2), 44.8 (t, C-8), 67.9 (d, C-9), 70.0 (d, C-7), 74.0 (d, C-4), 76.1 (d, C-3), 126.7 (d, C-5), 131.7 (d, C-6), 170.3 (s, C-1).

Decarestrictine O (**6**): Rf 0.26 (chloroform - methanol, 9:1), 0.90 (1-butanol - acetic acid - water, 4:1:5, upper phase), violet colorization after staining with anisaldehyde-sulfuric acid on silica gel TLC plates; HRFAB-MS *m/z* 217.1076 [$(M+H)^+$], MW 216.24 ($C_{10}H_{16}O_5$); ^{1}H NMR (500 MHz, acetone-d_6) δ 1.28 (d, $J_{10,9}=6.5$ Hz, 10-H_3), 1.31 (8-H_b), 1.36 (dd, $J_{2a,2b}=15.0$, and $J_{2b,3}=2.0$ Hz, 2-H_b), 2.31 (m, $J_{8a,8b}=13.0$, and $J_{8a,7}=3.6$ Hz, 8-H_a), 2.90 (dd, $J_{2b,2a}=14.0$, and $J_{2a,3}=7.0$ Hz, 2-H_a), 3.40 (m, 7-H), 4.25 (m, $J_{6,5}=8.5$ Hz, and $J_{6,4}=1.0$ Hz, 6-H), 4.45 (m, 3-H), 5.04 (qd, $J_{9,10}=6.5$ Hz, and $J_{9,8b}=3.6$ Hz, 9-H), 5.40 (ddd, $J_{4,5}=16.0$, $J_{3,4}=9.0$, and $J_{4,6}=1$ Hz, 4-H), 5.55 (ddd, $J_{5,6}=8.5$, $J_{5,4}=16.0$, and $J_{3,5}=0.5$ Hz, 5-H); ^{13}C NMR (125.7 MHz, acetone-d_6) δ 21.4 (q, C-10), 39.8 (t, C-8), 45.1 (t, C-2), 71.3 (d, C-3), 73.2 (d, C-9), 74.0 (d, C-6), 76.9 (d, C-7), 131.1 (d, C-4), 135.8 (d, C-5), 170.7 (s, C-1).

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References

1) GRABLEY, S.; E. GRANZER, K. HÜTTER, D. LUDWIG, M. MAYER, R. THIERICKE, G. TILL, J. WINK, S. PHILIPPS & A. ZEECK: Secondary metabolites by chemical screening. 8. Decarestrictines, a new family of inhibitors of cholesterol biosynthesis from *Penicillium*. I. Strain description, fermentation, isolation, and properties. J. Antibiotics 45: 56 ~ 65, 1992
2) GÖHRT, A.; A. ZEECK, K. HÜTTER, R. KIRSCH, H. KLUGE & R. THIERICKE: Secondary metabolites by chemical screening. 9. Decarestrictines, a new family of inhibitors of cholesterol biosynthesis from *Penicillium*. II. Structure elucidation of the decarestrictines A to D. J. Antibiotics 45: 66 ~ 73, 1992
3) GRABLEY, S.; P. HAMMANN, K. HÜTTER, R. KIRSCH, H. KLUGE, R. THIERICKE, M. MAYER & A. ZEECK: Secondary metabolites by chemical screening. 20. Decarestrictines, a new family of inhibitors of cholesterol biosynthesis from *Penicillium*: III. Decarestrictines E to M. J. Antibiotics 45: 1176 ~ 1181, 1992
4) MAYER, M. & R. THIERICKE: Biosynthetic studies on the decarestrictine family. J. Chem. Soc. Perkin Trans. I 1993: 495 ~ 500, 1993
5) ROHR, J.: Biosynthetic formation of the *S*-methyl group of the angucycline antibiotic urdamycin E. J. Chem. Soc. Chem. Commun. 1989: 492 ~ 493, 1989
6) ROHR, J.: A novel type of non-enzymatic reaction during the late steps in the biosynthesis of the angucycline antibiotics urdamycin C and D. J. Chem. Soc. Chem. Commun. 1990: 113 ~ 114, 1990
7) ROHR, J.: A novel type of ring contraction as a non-enzymatic step in the later stages of the biosynthesis of urdamycin H. Angew. Chem. Int. Ed. Engl. 102: 1051 ~ 1052, 1990
8) ROHR, J. & R. THIERICKE: Angucycline group antibiotics. Nat. Prod. Rep. 9: 103 ~ 137, 1992

9) MAYER, M. & R. THIERICKE: Biosynthetic relationships in the desertomycin family. J. Chem. Soc. Perkin Trans. I., in press
10) HASLAM, E.: Secondary metabolism—fact and fiction. Nat. Prod. Rep. 3: 217~249, 1986
11) WILLIAMS, D. H.; M. J. STONE, D. R. HOUCK & S. K. RAHMAN: Why are secondary metabolites (natural products) biosynthesized? J. Nat. Prod. 52: 1189~1208, 1989
12) ZÄHNER, H.: What are secondary metabolites? Folia Microbiol. 24: 435~443, 1979
13) AYER, W. A.; M. SUN, L. M. BROWNE, L. S. BRINEN & J. CLARDY: Chemical investigation of the metabolites from the canadian tuckahoe, *Polyporus tuberaster*. J. Nat. Prod. 55: 649~653, 1992