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

AXEL GÖHRT and AXEL ZEECK*

Institut für Organische Chemie, Universität Göttingen,
Tammannstraße 2, D-3400 Göttingen, FRG

KLAUS HÜTTER, REINHARD KIRSCH, HEINZ KLUGE and RALF THIERICKE*

Hoechst AG,
Postfach 800 320, D-6230 Frankfurt/Main 80, FRG

(Received for publication April 6, 1991)

The structures of the novel 10-membered lactones, named decarestrictines A_1/A_2 (**1/2**), B (**3**), C_1/C_2 (**5/6**) and D (**7**), are presented. The structures of these secondary metabolites, isolated from different *Penicillium* species, were established by spectroscopic analysis and confirmed by X-ray analysis of **7** and a derivative of **3** leading to the stereochemical information. The decarestrictines vary in the oxygenation pattern between C-3 and C-7 and show structural similarities to known lactones from other fungi.

Decarestrictines are novel 10-membered lactones, which are produced by different strains of *Penicillium*[1]. Some of them have inhibitory effects on the cholesterol biosynthesis. Fermentation conditions of the producing organism, as well as isolation procedures and some properties of the decarestrictines A to D, have already been described in the preceding paper[1]. In this study we present the structural elucidation of these fungal metabolites based mainly on NMR spectroscopy. Additional information concerning the stereochemistry was obtained by X-ray analysis.

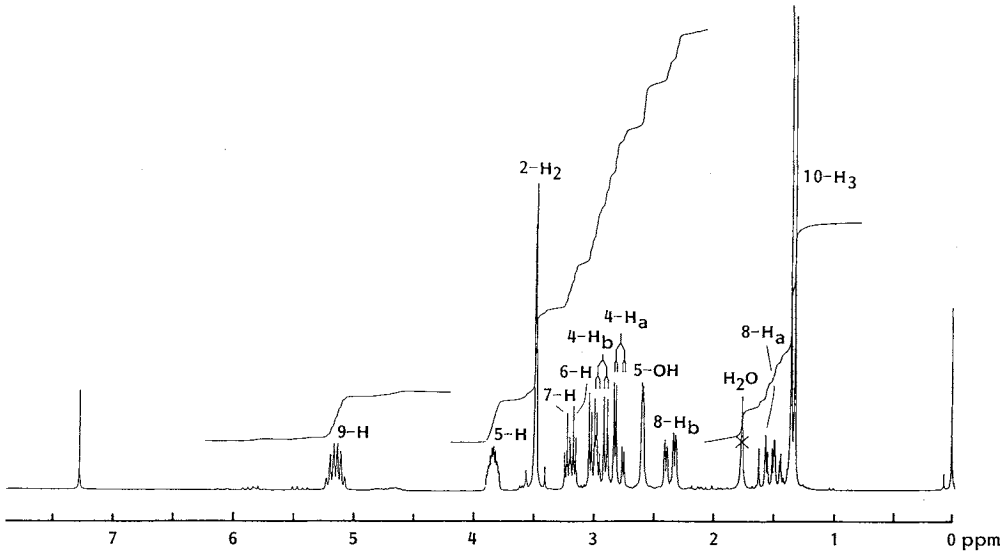
Decarestrictine B

Decarestrictine B ($C_{10}H_{14}O_5$) was purified by repeated silica gel column chromatography to yield 57.4 mg per liter of culture broth of a pure, colorless oil. The IR spectrum[1] revealed carbonyl absorption bands at 1740 and 1700 cm^{-1} due to a lactone/ester group and a non conjugated ketone.

The ^1H NMR spectrum (Fig. 1) showed the presence of a methyl group (δ 1.34, $J=6.4$ Hz) connected with a methine group (δ 5.15, $J=11.6$, 6.4 and 1.4 Hz), a disubstituted oxirane (δ 2.98, $J=9.1$ and 4.0 Hz and δ 3.18, $J=10.4$, 4.3 and 4.0 Hz), a methine group (δ 3.83, $J=9.1$, 3.6 and 2.6 Hz), a hydroxy group at δ 2.22 ($J=2.6$ Hz) and three methylene groups, respectively. One out of these methylene groups appeared as an AB-spin system (δ 3.43, $J=-14.4$ and 0.4 Hz; δ 3.50, $J=-14.4$, 0.8 and 0.7 Hz) supposing its position is located between two carbonyl groups. Signals of these carbonyl groups were observable in the ^{13}C NMR spectrum (Table 1) at δ 200.1 (ketone) and 165.2 (lactone). ^1H-^1H as well as ^1H-^{13}C correlated 2D NMR spectroscopy demonstrated the connectivity of the following sequence: methyl group (δ 1.34, 10-H_3)-methine group (δ 5.15, 9-H)-methylene group (δ 1.52 and 2.35, 8-H_2)-oxirane system (δ 2.98

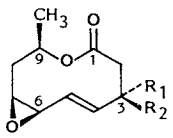
† See ref 1.

Fig. 1. ^{1}H NMR spectrum of decarestrictine B (**3**) in $CDCl_3$ at 200 MHz.

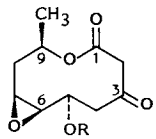


Scheme 1. Structure formulae of decarestrictines.

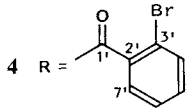
The stereochemistry of **1**, **2**, **5**, and **6** were deduced from the biosynthetic viewpoint.



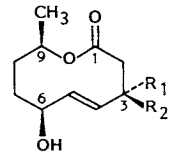
1 R_1=H R_2=OH
2 R_1=OH R_2=H



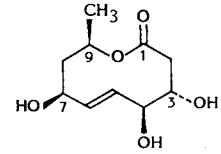
3 R=H



4 R =



5 R_1=OH R_2=H
6 R_1=H R_2=OH



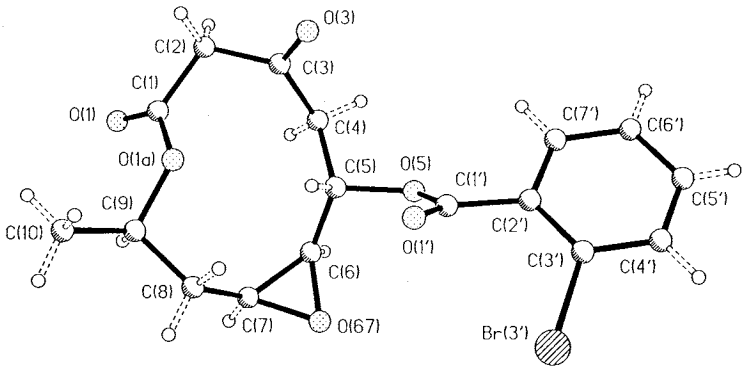
7

Table 1. ^{13}C NMR spectral data of decarestrictines in $CDCl_3$ at 90.5 MHz (δ values in ppm).

Carbon	A_1 (**1**)[e]	A_2 (**2**)[e]	B (**3**)	**4**	C_1 (**5**)[e,f]	C_2 (**6**)[e,f]	D (**7**)[f]
1	170.7	171.1	165.2	165.1	172.0	172.4	174.7
2	45.7	44.4[d]	52.0	51.8	45.4	45.8	35.6
3	71.6[a]	69.3[b]	200.1	198.0	68.7	70.3	75.4
4	129.7	120.1	48.4	47.0	131.9	130.7	73.1
5	133.9	136.7	67.8	69.3	131.9	138.2	129.4
6	55.2[c]	54.1[d]	60.5	57.2	69.0	74.1	135.9
7	55.0[c]	53.5[d]	56.3	55.5	36.2	32.6	73.6
8	34.6	29.0	36.7	36.4	29.2	28.6	44.2
9	71.7[a]	68.2[b]	69.0	69.4	73.9	72.3	69.4
10	21.5	19.0	20.6	20.6	22.1	18.6	21.6

[a~d] The assignments may be exchangeable.
[e] 50.3 MHz.
[f] Recorded in CD_3OD.

Fig. 2. Perspective view of 5-*O*-(3'-bromobenzoyl)-decarestrictine B (**4**) with atom numbering.



and 3.13, 6-H/7-H)-secondary alcohol (δ 3.83, 5-H; 2.22, OH)-methylene group (δ 2.80 and 2.90, 4-H_2)-quaternary carbon atom, *i.e.* a carbonyl group (C-3). Based on the molecular formula, which by both, elemental analysis, and FAB-MS spectroscopy was shown to be $C_{10}H_{14}O_5$ ($(M+H)^+$, *m/z* 215)[1]. Therefore, the molecule has to be cyclized. The ring closure leads to the formation of a 10-membered lactone as shown in formula **3**. This is in accordance to the low-field position of the methine proton attached to C-9 and the absence of a free carboxyl group.

Decarestrictine B (**3**) contains four centers of chirality: C-9, the coupled centers of the oxirane ring at C-6/C-7, and C-5. Force field calculations estimate the four possible diastereomers in its *in vacuo* conformations. Among them, only the 5*S*, 6*R*, 7*S*, 9*R* diastereomer and its enantiomer exhibit a conformation that allows a perfect prediction of the ^3J (^{1}H, ^{1}H) coupling constants *via* KARPLUS' rule. For the three other diastereomers, the predicted values are in severe contradiction to the observed ones. In order to prove the structure elucidation done by NMR spectra analysis and the stereochemistry of **3**, the *o*-bromobenzoate **4** of decarestrictine B (**3**) has been prepared. This compound can easily be crystallized by liquid-liquid diffusion of 2-propanol into a saturated chloroform solution at 8°C. The structure including the absolute configuration of **4** was determined by making use of the anomalous scattering of the bromine atom. As depicted in Fig. 2 the configuration of **4** is 5*S*, 6*S*, 7*S*, and 9*R*. Therefore, decarestrictine B (**3**) is 6,7-epoxy-5-hydroxy-3-oxodecan-9-olide. It is worth mentioning that the *R*/*S*-nomenclature at C-6 has to be changed from the *o*-bromobenzoate **4** to the natural product (**3**: 5*S*, 6*R*, 7*S*, 9*R*).

Decarestrictines A, C, and D

The decarestrictines A, C, and D were purified by repeated silica gel and gel permeation chromatography[1] to yield 4.8 mg/liter of decarestrictine A, 5.4 mg/liter of C, and 47 mg/liter of D, respectively. These compounds seemed to be homogeneous on TLC. The ^{1}H NMR spectrum of decarestrictine A revealed a mixture of diastereomers (A_1/A_2) in a ratio of 3:1. The structural elucidation of both compounds from the mixture was possible because of the well separated signal patterns. Both, decarestrictines A_1 and A_2 are 10-membered lactones showing similarities to decarestrictine B (**3**). One of the differences is a double bond between C-4/C-5 (two olefinic protons in A_1: δ 5.84/5.42, J=16.8 Hz; in A_2: δ 6.07/5.77, J=15.5 Hz), whose coupling constants confirmed (*E*)-configurations of the double bonds in both metabolites. In addition, the carbonyl group at C-3 in **3** was found to be reduced in decarestrictines A_1/A_2. Thus, the ^{1}H, ^{1}H-COSY NMR spectrum showed a connected coupling pattern from 2-H_2 to 10-H_3. A hypothetical dehydration reaction followed by hydrogenation of the molecule explains the molecular formula $C_{10}H_{14}O_4$ for the

decarestrictines A_1/A_2. This is in accordance with the data obtained by FAB-MS spectroscopy, which showed only one molecular ion for both compounds at *m/z* 199 $(M+H)^+$. Therefore, decarestrictines A_1 (**1**) and A_2 (**2**) are diastereomers of 6,7-epoxy-3-hydroxy-4-decen-9-olide, most likely with different stereochemistry at C-3. The relative and absolute configuration (in relation to **3**) is depicted in Scheme 1.

Shown by NMR spectra, decarestrictine C consists of two components (C_1/C_2) in the ratio of nearly 1:1. GC-MS analysis of trimethylsilylated derivatives excluded the presence of a dimer, because each of the separated di-*O*-trimethylsilyl ethers (M^+: *m/z* 344) indicated the molecular formulae of both natural products to be $C_{10}H_{16}O_4$. The ^1H NMR spectrum of the C_1/C_2 mixture exhibits olefinic protons, methine protons of secondary alcohols, methyl and methylene groups. As demonstrated by ^{13}C NMR spectroscopy, neither oxirane nor ketone was present in these compounds. It was possible to attribute the ^1H NMR signals to both components in a ^1H, ^1H-2D-COSY NMR spectrum at 200 MHz, in which two independent coupling patterns gave rise to the proton connectivity from 10-H_3 to 2-H_2. The assignments, based on the methyl group of each component, were proven by selective ^1H-[^1H] decoupling experiments and checked by ^1H,^{13}C-2D-COSY NMR at 360 and 90 MHz, respectively. The first signal (δ 1.15) was attributed to decarestrictine C_1, the latter (δ 1.20) to C_2. We propose the structure of both components

Fig. 3. ^1H,^1H-COSY NMR spectrum of decarestrictine D (**7**) in $CDCl_3$ at 200 MHz.

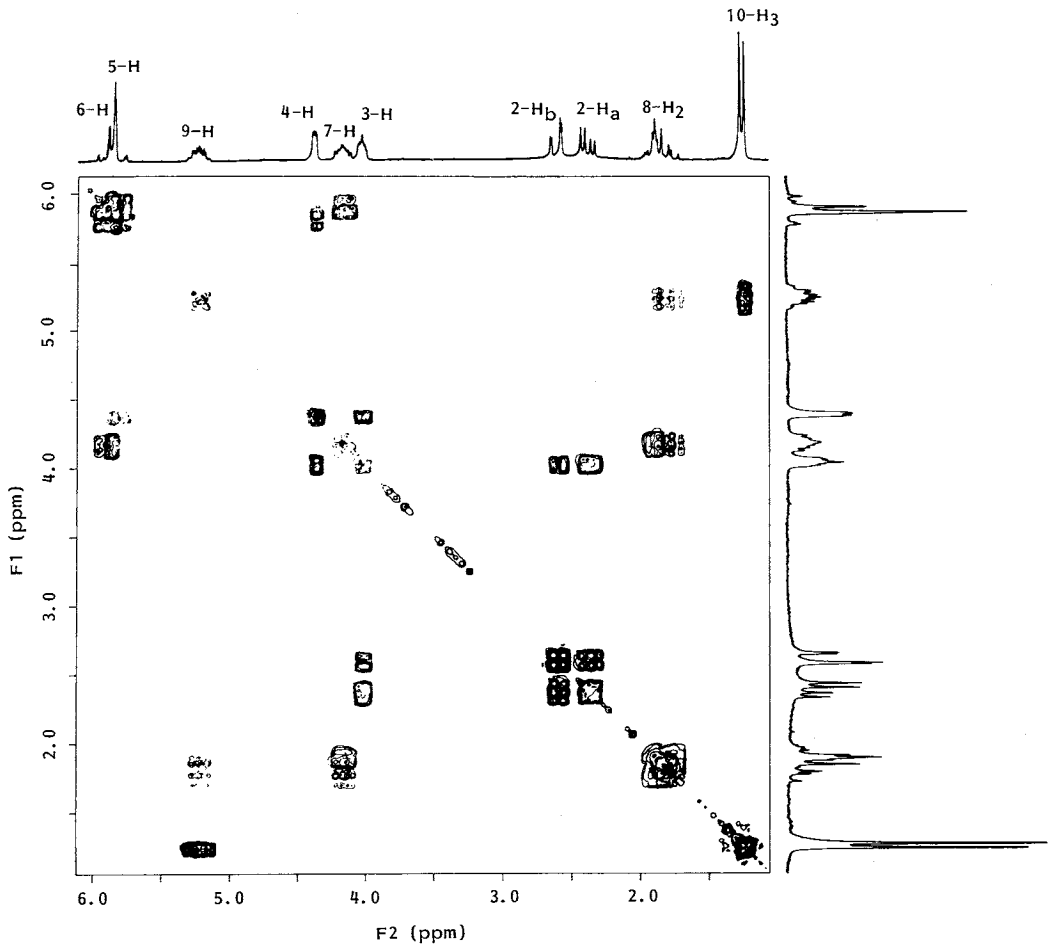
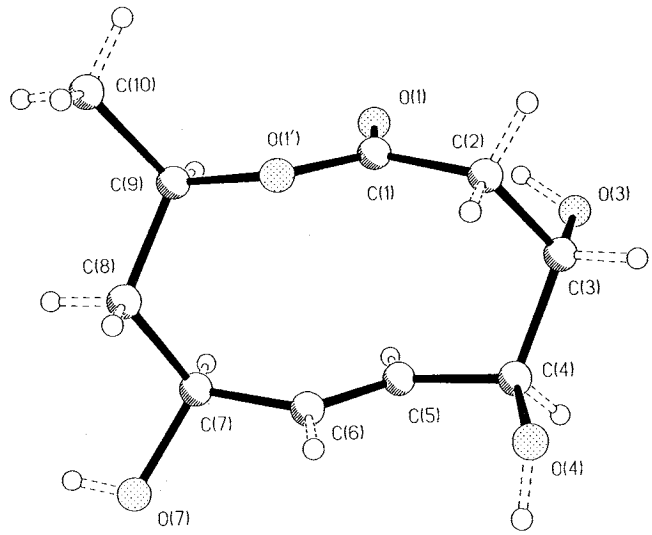


Fig. 4. Perspective view of decarestrictine D (**7**) with atom numbering.
Only the relative configuration resulted from the X-ray analysis.



being diastereomers of 3,6-dihydroxy-4-decen-9-olide (**5** and **6**). In addition, we assume a different stereochemistry at C-3. This is because of the changes in ^1H, ^1H coupling constants along with the remarkable shift of some ^1H NMR signals, due to conformational changes of the 10-membered lactone rings. As indicated by different line shapes, isomer C_1 is conformationally flexible, while C_2 behaves like a rather rigid 10-membered lactone ring like decarestrictine D (**7**). In relation to decarestrictines A and B, we expected the relative and absolute configuration as being shown in Scheme 1, although this has not been proven.

In contrast to A and C, decarestrictine D is homogeneous. Spectroscopical characterization pointed out the similarity to the above described 10-membered lactones. One *E*-configurated double bond is present in this metabolite (δ 5.73, J=15.8, 3.0 and −0.5 Hz, 5-H; δ 5.82, J=15.8, 9.5 and 1.2 Hz, 6-H). A ^1H,^1H-COSY NMR spectrum revealed a connected coupling pattern from 2-H to 10-H (Fig. 3). Decarestrictine D is the most polar component among the decarestrictines due to three hydroxy groups. It is easily crystallized by liquid-liquid diffusion of *n*-hexane into a saturated isoamyl acetate solution at 8°C. The structure of decarestrictine D (**7**), as well as the relative configuration of the centers of chirality, was ascertained by X-ray analysis (Fig. 4). Therefore, decarestrictine D was identified as 3,4,7-trihydroxy-5-decen-9-olide given in formula **7**, or less likely its enantiomer.

Discussion

Decarestrictines represent a family of novel 10-membered lactones produced by different strains of *Penicillium*. So far, six components of the family of decarestrictines have been identified. The identical carbon skeleton, which forms a 10-membered lactone ring, varies in the oxygenation patterns ranging from carbon 3 to 7 and creation of one *E*-configurated double bond located either at C-4 or at C-5. This seems to be a result of dehydration during biosynthesis. We assume that the producing strains possess interesting oxidase and reductase activities with a defined regioselectivity, because the methylene groups at C-2 and C-8 remain unchanged. X-Ray studies of the crystalline bromobenzoate **4** proved the (*R*)-configuration

of C-9. From the biosynthetic point of view, we suppose that all isolated decarestrictines belong to the 9*R*-series.

However, the decarestrictines are not the first representatives of oxygenated decan-9-olides. Diplodialides from *Diplodia pinea*[2,3], pyronolides from *Pyrenophora teres*[4,5], and achaetolide[6] from *Achaetomium crystalliferum* exhibit related 10-membered lactone structures differing in the substitution pattern. Some of these compounds act as steroid hydroxylase inhibitors and show morphogenic activity on fungi. Further studies on the other representatives of the decarestrictine family are in progress.

Experimental

General

MP's were determined on a Reichert hot-stage microscope and are not corrected. ^1H and ^{13}C NMR spectra were measured with Bruker AM 360 and Varian VXR-200. Chemical shifts are expressed in δ values (ppm) with TMS, $CDCl_3$ or CHD_2OD as internal standards. The multiplicities of the ^{13}C NMR values were assigned by attached proton test (APT) or DEPT techniques. Chemical shifts and coupling constants (360 MHz) were determined by iterative spectrum simulation with PANIC (Bruker software). The mass spectra were taken by a Varian MAT 311a (EI, DCJ with NH_3 (200 eV)), a Finnigan MAT 8230 (FAB, matrix: glycerol or 3-nitrobenzylalcohol), and GC mass spectroscopy with Hewlett Packard model HP 5840/5940 (SE-54 fused silica, 25 m, He, 70 eV).

Decarestrictines A_1/A_2 (1/2)

The isolated sample[1] named decarestrictine A was a mixture of diastereomers in a ratio of 3:1. The NMR data were obtained from the mixture and the assignments arose from partial subtraction of the signals.

Decarestrictine A_1 (**1**, major component): ^1H NMR (200 MHz, $CDCl_3$) δ 1.45 (d, $J_{10,9}=7$ Hz, 10-H_3), 1.80 (ddd, $J_{8a,8b}=15$, $J_{8a,9}=11$ and $J_{8a,7}=10$ Hz, 8-H_a), 2.05 (ddd, $J_{8b,8a}=15$, $J_{8b,7}=5$ and $J_{8b,9}=1.5$ Hz, 8-H_b), 2.33 (dd, $J_{2a,2b}=11.9$ and $J_{2a,3}=9.5$ Hz, 2-H_a), 2.83 (dd, $J_{2b,2a}=11.9$ and $J_{2b,3}=6.6$ Hz, 2-H_b), 3.05 (ddd, $J_{7,8a}=10$, $J_{7,8b}=5$ and $J_{7,6}=4.2$ Hz, 7-H), 3.57 (dd, $J_{6,5}=8$ and $J_{6,7}=4.2$ Hz, 6-H), 4.65 (ddq, $J_{9,8a}=11$, $J_{9,10}=7$ and $J_{9,8b}=1.5$ Hz, 9-H), 4.65 (ddd, $J_{3,2a}=9.5$, $J_{3,4}=8.6$ and $J_{3,2b}=6.6$ Hz, 3-H), 5.42 (dd, $J_{5,4}=16.8$ and $J_{5,6}=8$ Hz, 5-H), 5.84 (dd, $J_{4,5}=16.8$ and $J_{4,3}=8.6$ Hz, 4-H); ^{13}C NMR (50.3 MHz, $CDCl_3$) see Table 1.

Decarestrictine A_2 (**2**, minor component): ^1H NMR (200 MHz, $CDCl_3$) δ 1.39 (d, $J_{10,9}=6.8$ Hz, 10-H_3), 1.85 (ddd, $J_{8a,8b}=16$, $J_{8a,7}=6.5$ and $J_{8a,9}=4.8$ Hz, 8-H_a), 2.18 (m, 8-H_b), 2.46 (dd, $J_{2a,2b}=12$ and $J_{2a,3}=3$ Hz, 2-H_a), 2.63 (dd, $J_{2b,2a}=12$ and $J_{2b,3}=4.2$ Hz, 2-H_b), 3.19 (ddd, $J_{7,8a}=6.5$, 5 and 4 Hz, 7-H), 3.50 (m, 6-H), 4.8 (m, 3-H and 9-H), 5.77 (ddd, $J_{5,4}=15.5$, 2 and 1 Hz, 5-H), 6.07 (ddd, $J_{4,5}=15.5$, 1.8 and 1.5 Hz, 4-H); ^{13}C NMR (100.6 MHz, $CDCl_3$) see Table 1.

Decarestrictine B (3)

^1H NMR (200 MHz, $CDCl_3$; 360 MHz, $CDCl_3$, 303 K, 0.04 M, see Fig. 1) δ 1.34 (d, $J_{10,9}=6.4$ Hz, 10-H_3), 1.52 (ddd, $J_{8ax,8eq}=-14.7$, $J_{8ax,9}=11.6$ and $J_{8ax,7}=10.4$ Hz, 8-H_{ax}), 2.22 (d, $J=2.6$ Hz, 5-OH, exchangeable with D_2O), 2.35 (ddd, $J_{8ax,8eq}=-14.7$, $J_{8eq,7}=4.3$ and $J_{8eq,9}=1.4$ Hz, 8-H_{eq}), 2.80 (ddd, $J_{4a,4b}=13.4$ and $J_{4a,5}=3.6$ and 0.7 Hz, 4-H_a), 2.90 (dddd, $J_{4a,4b}=-13.4$, $J_{4b,5}=6.2$, 0.8 and 0.4 Hz, 4-H_b), 2.98 (dd, $J_{5,6}=9.0$ and $J_{6,7}=4.0$ Hz, 6-H), 3.18 (ddd, $J_{7,8a}=10.4$, $J_{7,8b}=4.3$ and $J_{6,7}=4.0$ Hz, 7-H), 3.43 (dd, $J_{2a,2b}=-14.4$ and 0.4 Hz, 2-H_a), 3.50 (ddd, $J_{2a,2b}=-14.4$, 0.8 and 0.7 Hz, 2-H_b), 3.83 (dddd, $J_{6,5}=9.1$, $J_{5,4b}=6.2$, $J_{5,4a}=3.6$ and $J_{5,OH}=2.6$ Hz, 5-H), 5.15 (ddq, $J_{9,8ax}=11.6$, $J_{9,10}=6.4$ and $J_{9,8eq}=1.4$ Hz, 9-H); ^{13}C NMR (90.5 MHz, $CDCl_3$, 303 K, 0.04 M) see Table 1.

5-*O*-(3'-Bromobenzoyl)-decarestrictine B (4)

A solution of 267 mg (1.25 mmol) **3** in CH_2Cl_2, 230 mg dicyclohexylcarbodiimide (1.12 mmol), 300 mg 2-bromobenzoic acid (1.50 mmol) and a small crystal of 4-*N*,*N*-dimethylaminopyridine were stirred for 2 hours at room temperature. The mixture was extracted three times with H_2O (25 ml), the organic layer was dried with Na_2SO_4 and evaporated to dryness. Column chromatography on silica gel (CH_2Cl_2 - ethyl acetate, 10:1) yielded 400 mg (81%) colorless crystalline **4**.

MP 124°C; $[\alpha]_D^{20}$ +16° (*c* 1.0, $CHCl_3$); Rf 0.40 ($CHCl_3$ - *n*-hexane - MeOH, 20:20:1); IR (KBr) cm^{-1}

3060, 1720, 1590; UV $\lambda_{max}^{CHCl_3}$ nm (ε) 240 (6,142), 285 (980); ^{1}H NMR (360 MHz, $CDCl_3$, 308 K, 0.08 M) δ 1.41 (d, $J_{10,9}=6.3$ Hz, 10-H_3), 1.79 (ddd, $J_{8ax,8eq}=-14.8$, $J_{7,8ax}=11.7$ and 10.2 Hz, 8-H_{ax}), 2.40 (ddd, $J_{8eq,8ax}=-14.8$, $J_{8eq,7}=4.1$, $J_{8eq,9}=1.5$ Hz, 8-H_{eq}), 3.00 (dd"d" (d" means pseudo doublet), $J_{4a,4b}=-13.3$, $J_{4a,5}=6.5$ and $J=0.4$ Hz, 4-H_a), 3.16 (dd, $J_{4a,4b}=-13.3$ and $J_{4b,5}=4.7$ Hz, 4-H_b), 3.17 (ddd, $J_{7,8ax}=10.2$, $J_{7,8eq}=4.1$ and $J_{6,7}=3.8$ Hz, 7-H), 3.19 (dd, $J_{5,6}=11.2$ and $J_{6,7}=3.8$ Hz, 6-H), 3.40 (dd, $J_{2a,2b}=-13.5$ and 0.4 Hz, 2-H_a), 3.69 (d, $J_{2b,2a}=-13.5$ Hz, 2-H_b), 5.20 (ddq, $J_{9,8ax}=11.7$, $J_{9,10}=6.3$ and $J_{9,8eq}=1.5$ Hz, 9-H), 5.48 (ddd, $J_{5,6}=11.2$, $J_{4b,5}=4.7$, and $J_{5,4a}=6.5$ Hz, 5-H), 7.34 (m, $J_{5',6'}=8.0$, $J_{5',4'}=7.4$ and 1.7 Hz, 5'-H), 7.38 (m, $J_{4',3'}=7.8$, $J_{4',5'}=7.4$ and 1.1 Hz, 4'-H), 7.67 (m, $J_{5',6'}=8.0$, 1.1 and 0.2 Hz, 6'-H), 7.86 (m, $J_{4',3'}=7.8$, 1.7 and 0.2 Hz, 3'-H); ^{13}C NMR (90.5 MHz, $CDCl_3$, 303 K, 0.08 M) values completing Table 1: δ 121.9 (s, C-3'), 127.2 (d, C-6'), 131.6 (d, C-4'), 131.6 (d, C-7'), 132.8 (s, C-2'), 134.4 (d, C-5'), 165.0 (s, C-1', assignment is interchangeable with δ 165.0, C-1).

X-Ray Analysis of **4**

4 (molecular formula: $C_{17}H_{17}BrO_6$, $M_r=397.2$) was crystallized by liquid-liquid diffusion of 2-propanol into a saturated $CHCl_3$ solution at 8°C. Crystal size $1.2\times0.8\times0.2$ mm^3, monoclinic, space group $P2_1$, $a=7.773(2)$, $b=9.208(2)$, $c=11.997(3)$ Å, $\beta=92.09(4)$, $V=858.1(4)$ Å^3, $Z=2$, $D_{calc}=1.537$ Mg/m^3, $\mu(MoK_\alpha)=2.397$ mm^{-1}, Siemens-Stoe AED2 diffractometer, data collection with profile-fitting method[7] at −120°C, 2θ range = 8 to 55°, 2,974 reflections measured, 2,761 unique reflections, 2,609 with $|F|>4\sigma F$ treated as observed, semi empirical absorption correction (272 azimuthal scans), structure solved by PATSEE[8,9] with C_7O_2Br as search fragment. Although the positions of all H-atoms were located by difference electron-density synthesis, a riding model with idealized hydrogen geometry was employed for refining them. The H-atom displacement parameters were refined isotopically. They were constrained to be equal for all H-atoms except those on C-10. The anisotropic refinement converged at $R=0.0480$ ($wR=0.0661$ with weights $W^{-1}=\sigma_F^2+0.0005F^2$). η-refinement[10] gave $\eta=1.01(3)$. Further details of the crystal structure investigations are available on request (from the Fachinformationszentrum Energie, Physik, Mathematik GmbH, D-7514 Eggenstein-Leopoldshafen 2 (FRG), on quoting the depository number CSD-55307 (**4** and **7**), the names of the authors and the journal citation).

Decarestrictines C_1/C_2 (**5**/**6**)

The isolated compound[1] was a mixture of diastereomers in a ratio of 1:1. The NMR data were taken of the mixture and the assignments resulted from a ^{1}H-^{1}H-COSY NMR spectrum.

5: ^{1}H NMR (200 MHz, CD_3OD) δ 1.15 (d, $J_{9,10}=6.3$ Hz, 10-H_3), 1.43 (dd, $J=16$ and 7.5 Hz, 1 proton), 1.80~2.00 (m, 3 protons), 2.48/2.52 (m, 2-H_2), 4.38 (m, 6-H), 4.66~4.78 (m, 3-H, 9-H), 5.78 and 5.88 (AB-system, each ddd, $J=16$, 2.3 and 1 Hz and $J=16$, 2.8 and 1.4 Hz, 5-H, 4-H); **6**: ^{1}H NMR (200 MHz, CD_3OD) δ 1.21 (d, $J_{9,10}=6.3$ Hz, 10-H_3), 1.60~1.80 (m, 4 protons), 2.30 (dd, $J_{2a,2b}=13.4$ and $J_{2a,3}=5.6$ Hz, 2-H_a), 2.92 (dd, $J_{2a,2b}=13.4$ and $J_{2b,3}=7.7$ Hz, 2-H_b), 3.94 (m, 6-H), 4.56 (dddd, $J_{2b,3}=7.7$, $J_{3,4}=6.8$, $J_{2a,3}=5.6$ and 1 Hz, 3-H), 4.99 (dq, $J_{9,10}=6.7$ and 3.5 Hz, 9-H), 5.40 and 5.55 (AB = system, each dd, $J_{4,5}=16$ and $J_{3,4}=6.8$ Hz, 4-H and $J_{4,5}=16$ and $J_{5,6}=8.2$ Hz, 5-H); ^{13}C NMR (50.3 MHz, CD_3OD) of **5** and **6** see Table 1.

Decarestrictine D (**7**)

MP 116°C; ^{1}H NMR (200 MHz, $CDCl_3$; 360 MHz, CD_3OD, 303 K, 0.23 M, see Fig. 3) δ 1.20 (d, $J_{10,9}=6.4$ Hz, 10-H_3), 1.71 (ddd, $J_{8ax,8eq}=-14.0$, $J_{8ax,7}=11.1$ and $J_{8ax,9}=11.0$ Hz, 8-H_{ax}), 1.85 (ddd, $J_{8eq,8ax}=-14.0$, $J_{8eq,7}=3.6$ and $J_{8eq,9}=1.6$ Hz, 8-H_{eq}), 2.30 (dd, $J_{2a,2b}=-14.0$ and $J_{2a,3}=6.9$ Hz, 2-H_a), 2.58 (dd, $J_{2b,2a}=-14.0$ and $J_{2b,3}=2.4$ Hz, 2-H_b), 3.92 (ddd, $J_{3,2a}=6.9$, $J_{3,4}=4.7$ and $J_{3,2b}=2.4$ Hz, 3-H), 4.07 (ddd"d", $J_{7,8ax}=11.0$, $J_{7,8eq}=3.6$, $J_{6,7}=9.5$ and −0.5 Hz, 7-H), 4.18 (ddd, $J_{4,3}=4.7$, $J_{4,5}=3.0$ and 1.2 Hz, 4-H), 5.15 (ddq, $J_{9,8ax}=11.1$, $J_{9,10}=6.4$ and $J_{9,8eq}=1.6$ Hz, 9-H), 5.73 (dd"d", $J_{5,6}=15.8$, $J_{5,4}=3.0$ and −0.5 Hz, 5-H), 5.82 (ddd, $J_{5,6}=15.8$, $J_{6,7}=9.5$ and 1.2 Hz, 6-H); ^{13}C NMR (90.5 MHz, $CDCl_3$, 303 K, 0.23 M) see Table 1.

X-Ray Analysis of **7**

7 (molecular formula: $C_{10}H_{16}O_5$, $M_r=216.2$) was crystallized by liquid-liquid diffusion of *n*-hexane into a saturated isoamyl acetate solution at 8°C. Crystal size $0.23\times0.23\times0.23$ mm^3, monoclinic, space

group C2, $a=24.605(6)$, $b=5.528(1)$, $c=7.899(2)$ Å, $\beta=93.83(3)$, $V=1{,}072.0(4)$ Å^3, $z=4$, $D_{calc}=1.340$ Mg/m^3, $\mu(MoK_\alpha)=0.100\,mm^{-1}$, Siemens-Stoe AED2 diffractometer, data collection with profile-fitting method[7] at -120°C, 2θ range = 8 to 55°, 2,206 reflections measured, 2,206 unique reflections, 2,025 with $|F|>4\sigma F$ treated as observed, structure solved by direct methods using SHELXS-86[8,11]. An empirical isotropic extinction correction $\chi=0.0041$ (4) was applied where $F^*=F[1+0.002\chi F^2/\sin(2\theta)]^{-0.25}$, and anisotropic refinement converged at $R=0.0324$ ($wR=0.0441$ with weights $W^{-1}=\sigma_F^2+0.0003F^2$). For further details see X-ray analysis of **4**.

Acknowledgment

We thank Dr. R. KLEIN for force field calculations and Mrs. U. BODE for recording NMR spectra. This work was supported by the Bundesministerium für Forschung und Technologie (BMFT, grant 0319311B). The authors are responsible for the contents within this publication.

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) TATSUYOSHI, I. & K. WADA: A steroid hydroxylase inhibitor, diplodialide A from *Diplodia pinea*. J. Chem. Soc. Chem. Commun. 1975: 209~210, 1975
3) WADA, K. & T. ISHIDA: A steroid hydroxylase inhibitor, diplodialide A, and related metabolites from *Diplodia pinea*. J. Chem. Soc. Perkin Trans. I 1979: 1154~1158, 1979
4) NUKINA, M.; T. SASSA & M. IKEDA: A new fungal morphogenic substance, pyrenolide A from *Pyrenophora teres*. Tetrahedron Lett. 21: 301~302, 1980
5) SUZUKI, S.; A. TANAKA & K. YAMASHITA: Synthesis and biological activity of (±)-pyrenolide B. Agric. Biol. Chem. 51: 3095~3098, 1987
6) BODO, B.; L. MOLHO, D. DAVOUST & D. MOLHO: Fungal macrolides: Structure determination and biosynthesis of achaetolide, a lactone from *Achaetomium crystalliferum*. Phytochemistry 22: 447~451, 1983
7) CLEGG, W.: Faster data collection without loss of precision. An extension of the learnt profile method. Acta Crystallogr. (A) 37: 22~28, 1981
8) SHELDRICK, G. M.: SHELXS-86. University of Göttingen, 1986
9) ROGERS, D.: On the application of Hamilton's ratio test to the assignment of absolute configuration and an alternative test. Acta Crystallogr. (A) 37: 734~741, 1981
10) EGERT, E.: Patterson and Pattersons. *Ed.*, J. P. GLUSKER, Oxford University Press, 1987
11) EGERT, E. & G. M. SHELDRICK: Search for a fragment of known geometry by integrated patterson and direct methods. Acta Crystallogr. (A) 41: 262~268, 1985