Notes

SECONDARY METABOLITES BY CHEMICAL SCREENING. 20†

DECARESTRICTINES, A NEW FAMILY OF INHIBITORS OF CHOLESTEROL BIOSYNTHESIS FROM *PENICILLIUM*: III. DECARESTRICTINES E TO M

SUSANNE GRABLEY, PETER HAMMANN, KLAUS HÜTTER, REINHARD KIRSCH, HEINZ KLUGE and RALF THIERICKE*

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

MARION MAYER and AXEL ZEECK

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

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The decarestrictines[2,3], novel inhibitors of the cholesterol biosynthesis, were discovered by the application of chemical screening[4,5] to different *Penicillium* strains. The previously described decarestrictines A to D[3], 10-membered lactones, vary in their oxygenation pattern ranging from C-3 to C-7 and the location of a double bond. Single crystal diffraction analysis of decarestrictine D (relative configuration) and a bromo-derivative of decarestrictine B (absolute configuration) gave rise to stereochemical information. A more detailed examination of the culture broth of *Penicillium simplicissimum* (strain FH-A 6090) resulted in the detection of minor components of the decarestrictine family. This paper describes the purification, the structures as well as the antihyperlipidemic activities of these novel secondary metabolites.

The decarestrictines could be visualized by TLC-chromatography (Merck, HPTLC ready-to-use plates, Silica gel $60F_{254}$ on glass) using anisaldehyde-sulfuric acid as staining reagent[2] (Table 1). In order to obtain the minor components in sufficient amounts a large scale fermentation (400 liters) of strain FH-A 6090 in a medium containing malt extract 2%, yeast extract 0.2%, glucose 1%, and $(NH_4)_2HPO_4$ 0.05% (pH 6.0 prior to sterilization) was performed[2]. The cultivation was carried out for 8 days at 25°C (aeration: 5.0 liters/minute). The isolation of the decarestrictines mainly followed the procedure previously described[2]. The filtered culture broth (about 380 liters) was adsorbed on Amberlite XAD-16. The resin was washed with 100 liters of deionized water and eluted with 70 liters methanol-water (4:1). The eluate was concentrated *in vacuo* and the remaining aqueous residue was lyophylized to result in 1.5 kg dark brown crude material, which was extracted three times with ethyl acetate (each 20 liters). The evaporated residue of the organic layer was chromatographed on silica gel (column 150 × 10 cm; Silica gel 60 (0.04~0.063 mm, Merck); gradient: 100% ethyl acetate to 60% methanol). Each of the minor components (decarestrictines E to M, **1** to **9**) was further purified by column chromatography on silica gel in various systems containing dichloromethane, *n*-hexane, acetone and methanol and chromatography on Sephadex LH-20 (Pharmacia) using methanol as eluent. The properties of the pure decarestrictines E to M (**1** to **9**) are summarized in Table 1.

The new metabolites were characterized spectroscopically, their molecular formulae were determined by high resolution mass spectra (HREI-MS), and their structures were elucidated by comparing the ^1H, ^{13}C, ^1H-^1H-correlation and ^1H-^{13}C-shift-correlation data to those of the known decarestrictines[2,3] (Tables 2 and 3). The decarestrictines E to K (**1** to **7**) exhibit the typical structural elements of these fungal metabolites: i) The 10-membered lactone ring, ii) an exocyclic methyl group, and iii) additional variations in the oxygenation pattern ranging from C-3 to C-7. Two components (**2** and **4**) comprise *E*- and two (**5** and **7**) *Z*-configurated double bonds (Scheme 1). It is remarkable that decarestrictine I (**5**) possesses a bicyclic ring system in which the 10-membered lactone is bridged forming an ether linkage between C-3 and C-6. With the exception of **4**, **5**, and **7**, the relative configuration of the centers of chirality were determined from the NMR data.

Lack of the 10-membered lactone ring was observed for the remaining decarestrictines L (**8**) and

† Part 19: See ref 1.

Table 1. Properties of decarestrictines E to M (**1** to **9**).

Compound	Yield (mg/liter)	Rf-values (TLC, silica gel)	Color reaction[c]	Molecular formula	MW	HREI-MS (m/z)[d]	$[\alpha]_D^{20}$ (MeOH)
E (**1**)	3	0.95[a] 0.72[b]	blue-brown	$C_{11}H_{16}O_5$	228.25	228.0998 (M^+)	−13.9 (*c* 0.6)
F (**2**)	5	0.55 0.77	red-brown	$C_{10}H_{12}O_4$	196.20	196.0736 (M^+)	nd
G (**3**)	10	0.40 0.70	olivegreen	$C_{10}H_{16}O_5$	216.24	198.0892 ($(M-H_2O)^+$, $C_{10}H_{14}O_4$)	−2.2 (*c* 1.5)
H (**4**)	0.5	0.54 0.77	violet	$C_{10}H_{14}O_4$	198.22	180.0786 ($(M-H_2O)^+$, $C_{10}H_{12}O_3$)	nd
I (**5**)	0.6	0.37 0.77	olivegreen	$C_{10}H_{14}O_4$	198.22	180.0786 ($(M-H_2O)^+$, $C_{10}H_{12}O_3$)	−132.2 (*c* 0.45)
J (**6**)	5	0.61 0.77	red-brown	$C_{10}H_{16}O_4$	200.24	200.1049 (M^+)	nd
K (**7**)	0.4	0.63 0.76	red-brown	$C_{10}H_{14}O_4$	198.22	180.0786 ($(M-H_2O)^+$, $C_{10}H_{12}O_3$)	nd
L (**8**)	0.3	0.48 0.73	ochre	$C_9H_{16}O_3$	172.23	172.1099 (M^+)	+21.8 (*c* 0.5)
M (**9**)	12	0.24 0.65	brown	$C_{10}H_{16}O_5$	216.24	198.0892 ($(M-H_2O)^+$, $C_{10}H_{14}O_4$)	−14.0 (*c* 0.1)

[a] $CHCl_3$-MeOH (9:1).
[b] *n*-BuOH-acetic acid-H_2O (4:1:5, upper phase).
[c] Staining reagent: Anisaldehyde-H_2SO_4.
[d] Found as calcd.
nd: Not determined.

M (**9**). ^1H-^1H as well as ^1H-^{13}C correlated 2D NMR spectroscopy of decarestrictine L (**8**) demonstrated the connectivity of the protons. Based on NMR spectroscopy in combination with information about the molecular formula from the HREI-MS (m/z 172, M^+, $C_9H_{16}O_3$; m/z 154, $(M-H_2O)^+$, $C_9H_{14}O_2$) the structure was proved to be (2α,3β, 6β)-3-hydroxy-6-methyl-2-(propan-2-one-1)-tetrahydropyran (**8**). The relative stereochemistry was proposed from the coupling constants in the ^1H NMR spectrum. Both protons at C-5 exhibited comparable dihedral angles due to its coupling constants ($J_{5eq,6eq}=4.3$ Hz and $J_{5ax,6eq}=4.3$ Hz). Although it seemed that no ideal chair conformation of the 6-membered ring exists, this fact could only be explained by the axial position of the methyl group at C-6. The coupling constant $J_{2ax,3ax}=6.6$ Hz gives rise to the assumption that both, the side chain at C-2 and the hydroxy group at C-3 are in the equatorial position.

The IR spectrum of decarestrictine M (**9**) exhibited characteristic adsorption bands of hydroxy groups at 3370 and 3240 cm^{-1} as well as a lactone moiety at 1755 cm^{-1}. The HREI-MS displayed a fragment at m/z 198 ($(M-H_2O)^+$, $C_{10}H_{14}O_4$). The molecular formula ($C_{10}H_{16}O_5$) was proved by elemental analysis (*Anal* Calcd for $C_{10}H_{16}O_5$: C 55.55, H 7.46, O 37.00; Found: C 55.46, H 7.38, O 37.40). The ^1H NMR spectrum proved the presence of a methyl, three methylene, and five methine groups, respectively. The connectivity of the protons resulted from the analysis of the coupling constants as well as from the ^1H-^1H-COSY-NMR spectrum (Table 2). In combination with the information provided by ^{13}C NMR, and ^1H-^{13}C-NMR spectroscopy decarestrictine M exhibited a bicyclic ring system containing a 7-membered lactone as depicted in **9** (8-hydroxy-2-(2-hydroxyprop-1-yl)-4-oxo-3,9-dioxabicyclo[4.2.1]nonane). In contrast to the bicyclic compound **5**, the lactone moiety in decarestrictine M is formed *via* the 7-OH group. Because of steric reasons, the lactone ring system has to be connected in *cis*-configuration at the tetrahydrofuran ring. The *trans*-positioned proton 4-H_b could be determined by its coupling constant ($J_{3a,4b}=8.3$ Hz), which showed a *cis*-coupling to 5-H ($J_{4b,5}=2.2$ Hz). In combination with the coupling constant of 4-H/5-H_b ($J_{4a,5b}=7.1$ Hz) the *trans* position of these protons was proved. Further stereochemical investigations are currently underway.

Obviously, the biosynthetic pathways leading to the decarestrictine family will start from a common

Table 2. ^{1}H NMR data (360 MHz, $CDCl_3$, internal standard TMS, δ (ppm), J (Hz)) of the decarestrictines E to M (**1** to **9**). Mutual interproton couplings are given only once, at their first occurrence in the table.

Proton	E (**1**)	F (**2**)	G (**3**)[a]	H (**4**)[b]	I (**5**)	J (**6**)[c]	K (**7**)[d]	L (**8**)	M (**9**)[e,f,g]
2-H_a	3.47, d, $J_{2a,2b}=-15.0$	2.88, d, $J_{2a,2b}=-14.6$	3.40, dd, $J_{2a,2b}=-15.6$, $J_{2a,4a}=0.8$	3.23, d, $J_{2a,2b}=-12.9$	2.64, dd, $J_{2a,2b}=-14.1$, $J_{2a,3}=0.7$	3.41, dd, $J_{2a,2b}=-15.0$, $J_{2a,4b}=0.5$	3.47, d, $J_{2a,2b}=-12.9$	4.01, ddd, $J_{2,3}=6.6$, $J_{2,7a}=8.2$, $J_{2,7b}=4.9$	2.92, dd, $J_{2a,2b}=-16.1$, $J_{2a,3}=5.1$
2-H_b	3.44, dd, $J_{2b,4a}=0.5$	2.88, d	3.40, d	3.46, d	2.75, dd, $J_{2b,3}=8.1$	3.37, d	3.48, d	—	2.65, dd, $J_{2b,3}=4.6$
3-H	—	—	—	—	5.11, dddd, $J_{3,4}=2.0$, $J_{3,5}=0.7$	—	—	3.39, m	4.46, dddd, $J_{3,4a}=1.7$, $J_{3,4b}=8.3$
4-H_a	2.90, ddd, $J_{4a,4b}=-14.1$, $J_{4a,5}=4.5$	5.70, d, $J_{4,5}=8.9$	3.00, ddd, $J_{4a,4b}=-15.9$, $J_{4a,5}=2.7$	3.14, m, $J_{4a,5}=7.2$	5.97, ddd, $J_{4,5}=6.5$, $J_{4,6}=0.7$	2.71, ddd, $J_{4a,4b}=-14.8$, $J_{4a,5a}=11.0$, $J_{4a,5b}=3.9$	2.91, dd, $J_{4a,4b}=-12.5$, $J_{4a,5}=5.0$	1.58, m	2.04, ddd, $J_{4a,4b}=-13.8$, $J_{4a,5}=7.1$
4-H_b	2.63, dd, $J_{4b,5}=3.1$	—	2.67, dd, $J_{4b,5}=5.0$	3.14, m, $J_{4b,5}=7.2$	—	2.31, dddd, $J_{4b,5a}=4.9$, $J_{4b,5b}=4.9$	2.83, dd, $J_{4b,5}=9.0$	1.72, m	1.89, ddd, $J_{4b,5}=2.2$
5-H_a	3.29, ddd, $J_{5,6}=9.1$	5.13, dd, $J_{5,6}=6.5$	3.50, dddd, $J_{5,6}=9.7$, $J_{5,5\text{-OH}}=10.1$	5.45, m, $J_{5,6}=15.6$	5.89, ddd, $J_{5,6}=2.0$	2.04, m	4.78, ddd, $J_{5,6}=8.0$	1.73, m, $J_{5a,6}=4.3$	4.29, dddd, $J_{5,5\text{-OH}}=4.2$
5-H_b	—	—	—	—	—	1.63, m	—	1.87, m, $J_{5b,6}=4.3$	—
6-H_a	2.98, dd, $J_{6,7}=4.1$	3.48, dd, $J_{6,7}=2.3$	3.58, ddd, $J_{6,7a}=1.9$, $J_{6,7b}=5.6$	5.69, dd, $J_{6,7}=9.3$	4.97, ddd, $J_{6,7}=2.4$	1.70, m	5.60~5.75, dd, $J_{6,7}=11.0$	3.95, qdd, $J_{6,10}=6.6$	3.85, dd, $J_{6,7}=5.2$

6-H_b	—	—	—	—	—	1.56, m	—	—	—
7-H_a	3.05, ddd, $J_{7,8a}=4.3$, $J_{7,8b}=10.4$	2.91, ddd, $J_{7,8a}=10.0$, $J_{7,8b}=5.0$	1.30, dddd, $J_{7a,7b}=-15.6$, $J_{7a,8a}=10.3$, $J_{7a,8b}=1.3$	4.12, ddd, $J_{7,8a}=4.3$, $J_{7,8b}=10.7$	3.99, dddd, $J_{7,8a}=11.0$, $J_{7,8b}=2.4$, $J_{7,7\text{-OH}}=4.6$	3.68, m, $J_{7,8a}=1.9$, $J_{7,8b}=9.4$	5.60~5.75, ddd, $J_{7,8a}=4.5$, $J_{7,8b}=6.5$	2.69, dd, $J_{7a,7b}=-15.5$	4.20, ddd, $J_{7,8a}=10.1$, $J_{7,8b}=3.0$
7-H_b	—	—	1.70, dddd, $J_{7b,8a}=1.3$, $J_{7b,8b}=8.3$	—	—	—	—	2.76, dd	—
8-H_a	2.35, ddd, $J_{8a,8b}=-14.8$, $J_{8a,9}=1.2$	2.00, ddd, $J_{8a,8b}=-14.7$, $J_{8a,9}=2.0$	1.77, dddd, $J_{8a,8b}=-14.9$, $J_{8a,9}=11.0$	1.88, ddd, $J_{8a,8b}=-14.3$, $J_{8a,9}=1.7$	2.05, ddd, $J_{8a,8b}=-14.4$, $J_{8a,9}=11.1$	1.87, ddd, $J_{8a,8b}=-14.2$, $J_{8a,9}=3.1$	2.97, ddd, $J_{8a,8b}=-14.2$, $J_{8a,9}=5.0$	—	1.81, ddd, $J_{8a,8b}=-14.6$, $J_{8a,9}=3.0$
8-H_b	1.54, ddd, $J_{8b,9}=11.5$	2.04, ddd, $J_{8b,9}=11.0$	1.92, dddd, $J_{8b,9}=4.0$	1.71, ddd, $J_{8b,9}=10.8$	1.69, ddd, $J_{8b,9}=1.6$	1.90, ddd, $J_{8b,9}=11.0$	2.03, ddd, $J_{8b,9}=2.0$	—	1.53, ddd, $J_{8b,9}=9.7$
9-H	5.11, qdd, $J_{9,10}=6.3$	4.78, qdd, $J_{9,10}=6.6$	5.05, qdd, $J_{9,10}=6.2$	5.08, qdd, $J_{9,10}=6.4$	5.08, qdd, $J_{9,10}=6.4$	5.18, qdd, $J_{9,10}=6.1$	5.13, qdd, $J_{9,10}=6.8$	2.21, 3H, s	3.69, qddd, $J_{9,10}=6.2$, $J_{9,9\text{-OH}}=4.6$
10-H_3	1.33, d	1.23, d	1.31, d	1.29, d	1.29, d	1.30, d	1.23, d	1.22, d	1.08, d
11-H_3	3.50, s	—	—	—	—	—	—	—	—

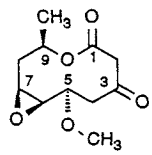
[a] $\delta_{5\text{-OH}}$ 3.85; $\delta_{6\text{-OH}}$ 2.94 (both signals: s, broad, exchangeable with D_2O).
[b] $\delta_{7\text{-OH}}$ 2.88 (s, broad, exchangeable with D_2O).
[c] $\delta_{7\text{-OH}}$ 2.01 (s, broad, exchangeable with D_2O).
[d] $\delta_{5\text{-OH}}$ 1.94 (s, broad, exchangeable with D_2O).
[e] DMSO-d_6.
[f] $\delta_{5\text{-OH}}$ 5.02 ($J_{5\text{-OH},5}=4.2$); $\delta_{9\text{-OH}}$ 4.61 ($J_{9\text{-OH},9}=5.0$).
[g] For comparison reasons the numbering system is used in analogy to the 10-membered lactones.

Table 3. ^{13}C NMR data of the decarestrictines E to M (**1** to **9**) in $CDCl_3$ (90.5 MHz, δ values in ppm, TMS as internal standard), multiplicity assignments by attached proton test (APT).

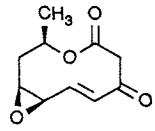
Carbon	E (**1**)[a]	F (**2**)[b]	G (**3**)	H (**4**)[b]	I (**5**)	J (**6**)	K (**7**)[b]	L (**8**)	M (**9**)[d,e]
1	165.4 s	165.6 s	166.1 s	166.7 s	172.9 s	166.7 s	165.6 s	—	171.9 s
2	52.1 t	51.4 t	52.3 t	51.0 t	43.2 t	51.7 t	53.5 t	72.0 d	45.1 t
3	200.3 s	198.4 s	205.3 s	199.1 s	82.7 d	203.0 s	198.6 s	69.1 d	71.1 d
4	46.2 t	136.4 d	41.5 t	41.1 t	133.0 d	39.4 t	51.4 t	28.2 t	40.8 t
5	77.3 d	124.7 d	72.8 d	121.5 d	128.1 d	21.3 t	69.7 d	26.9 t	72.9 d
6	59.6 d	53.5 d	75.2 d	139.8 d	93.5 d	36.8 t	136.4 d	67.4 d	86.9 d
7	53.2 d	63.7 d	30.3 t	72.7 d	72.2 d	69.2 d	124.6 d	46.2 t	76.4 d
8	36.7 t	30.5 t	32.8 t	48.5 t	40.4 t	44.2 t	30.5 t	207.9 s	45.1 t
9	69.0 d	69.7 d	75.1 d	69.3 d	74.2 d	71.7 d	63.6 d	30.5 q	62.1 d
10	20.6 q	17.8 q	20.4 q	21.3 q	21.9 q	20.8 q	17.7 q	18.1 q	24.0 q

[a] δ_{OCH_3} 57.4 (q).
[b] 50.3 MHz.
[c] 50.3 MHz, CD_3OD.
[d] DMSO-d_6.
[e] See [g] in Table 2.

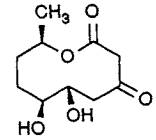
Scheme 1.



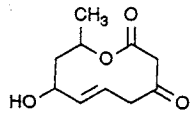
Decarestrictine E (**1**)



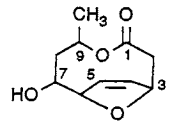
Decarestrictine F (**2**)



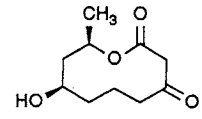
Decarestrictine G (**3**)



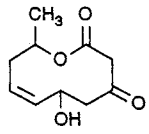
Decarestrictine H (**4**)



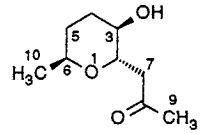
Decarestrictine I (**5**)



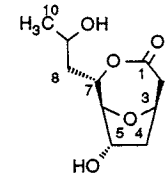
Decarestrictine J (**6**)



Decarestrictine K (**7**)



Decarestrictine L (**8**)



Decarestrictine M (**9**)

Table 4. Biological activities of the decarestrictines E to M (**1** to **9**). Incorporation of [^{14}C]acetate into the sterol fraction of HEP-G2 liver cell cultures[2] (concentration: 1.0×10^{-7}).

Compound	Sodium [^{14}C]acetate incorporation (%) into the cholesterol fraction
E (**1**)	105
F (**2**)	63
G (**3**)	125
H (**4**)	67
I (**5**)	82
J (**6**)	74
K (**7**)	93
L (**8**)	129
M (**9**)	34
Control	100

decaketide precursor. However, decarestrictines L (**8**) and M (**9**) may be shunt products on the way to the 10-membered lactones.

In cell line tests with HEP-G2 liver cells[6,7] the decarestrictines showed more or less potent inhibitory effects on cholesterol biosynthesis[2]. This was tested *via* sodium [^{14}C]acetate incorporation into the cholesterol fraction of the HEP-G2 cell cultures. The data of decarestrictines E to M (**1** to **9**) are presented in Table 4. A detailed investigation of structure-activity relationships including a number of derivatives is still under progress. It is worth mentioning that besides the most potent decarestrictine D[2], which exhibits a 10-membered lactone moiety, decarestrictine M (**9**) reveals a good activity in the HEP-G2 cell assay. In contrast, evaluation of the activity in normolipemic rats (oral application 15 mg/kg and day for a total of 7 days) showed that decarestrictine M (**9**) is less active than decarestrictine D[2] (antiatherogenic index: HDL-cholesterol/LDL-cholesterol = 4.63 (93% of the control)).

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