Investigations on the Biosynthesis of the Angucycline Group Antibiotics Aquayamycin and the Urdamycins A and B. Results from the Structural Analysis of Novel Blocked Mutant Products

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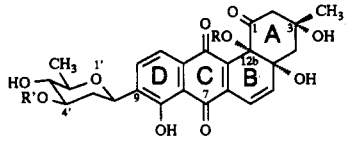
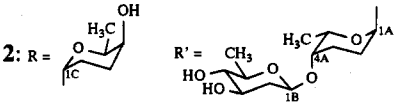
In order to investigate mid and early biosynthetic steps of angucycline group antibiotics, approximately 400 mutants of the urdamycin producer *Streptomyces fradiae* (strain Tü 2717) were prepared, of which ca. 10% were selected for further investigations. The selection criterion, i.e., the consideration of only pale-colored metabolite-producing blocked mutants, yielded several mutants whose block was in close proximity to the known late-stage biosynthetic steps. The product patterns were characterized by TLC and HPLC methods, and the structures of five new and one known (but previously not detectable) metabolites were elucidated (**3**–**8**). Their roles in the biosynthetic pathway leading to aquayamycin (**1**) and on to the urdamycins A (**2**) and B (**9**) are proposed. The glycosylation sequence of the urdamycin group and two additional earlier biosynthetic steps leading to aquayamycin (**1**), the most important angucyclinone, were established in this way.

The angucycline group antibiotics are in ever-growing family of biologically active natural compounds containing more than 100 members.[1] This group of antibiotics has proved to be of broad pharmaceutical interest,[1] and because of their C-glycosidic moiety, some of the members have served as lead structures for synthetic approaches.[1,2] Aquayamycin (**1**) is the most widely occurring aglycon



1: R = R' = H



among the angucycline group of antibiotics. Its importance can be estimated from its occurrence in the most biologically active representatives of the angucycline group.[1] Urdamycin A (**2**), an aquayamycin-containing glycosidic antitumor antibiotic, is the main compound of the urdamycin complex.[3] Classic biosynthetic investigations[3e,4] on the urdamycin complex using radioactive and stable isotope techniques as well as mechanistic studies involving model reactions[5] have elucidated all "late-stage" biosynthetic steps, i.e., interconversion reactions of the different compounds of the antibiotic family. In addition, studies regarding the origin of the oxygen atoms using ^{18}O-labeled precursors have suggested the role of mid-stage biosynthetic steps involved in the formation of aquayamycin (**1**) and thus are of general interest for aquayamycin-containing angucyclines.[6] This article describes experiments following a nonclassical approach (using genetically-altered producing organisms) to investigate further these mid-stage steps of the biosynthetic pathway to angucycline group antibiotics.

Table I. Mutant Strains and Their Product Spectra

mutant strain	products
urd-1	urdamycins A (**2**), B (**9**), G (**10**); 100-1 (**3**); 100-2 (**4**)
urd-2	aquayamycin (**1**); 104-1 (**5**); 104-2 (**6**)
urd-3	urdamycins A (**2**), B (**9**), G (**10**); 124-1 (**7**)
urd-4	rabelomycin (**8**)

Experimental Section

Blocked Mutants. The blocked mutant strains (urd-1, urd-2, and urd-3) were prepared as follows from *Streptomyces fradiae* (Tü 2717). Spores were prepared by the following standard procedure: The spores of five slants of *S. fradiae* were suspended in 5 mL of 0.09 M NaCl solution, homogenized, and filtered through cotton. Three mL of this suspension was preincubated

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(2) See ref. 1. See also: (a) Bolitt, V.; Mioskowski, C. *J. Am. Chem. Soc.* **1991**, *113*, 6320–6321. (b) Matsumotu, T.; Katsuki, M.; Jona, H.; Suzuki, K. *Ibid.* **1991**, *113*, 6982–6992. (c) Tius, M. A.; Gu, X.-q.; Gomez-Galeno, J. *Ibid.* **1990**, *112*, 8188–8189. (d) Yamaguchi, M.; Okuma, T.; Horiguchi, A.; Ikeura, C.; Minami, T. *J. Org. Chem.* **1992**, *57*, 1647–1649. (e) Kim, K.; Reibenspies, J.; Sulikowski, G. *J. Org. Chem.* **1992**, *57*, 5557–5559.
(3) (a) Drautz, H.; Zähner, H.; Rohr, J.; Zeeck, A. *J. Antibiot.* **1986**, *39*, 1657–1669. (b) Rohr, J.; Zeeck, A. *Ibid.* **1987**, *40*, 459–467. (c) Rohr, J.; Zeeck, A., Floss, H. G. *Ibid.* **1988**, *41*, 126–129. (d) Henkel, T.; Ciesiolka, T.; Rohr, J.; Zeeck, A. *Ibid.* **1989**, *42*, 299–311. (e) Rohr, J. *Ibid.* **1989**, *42*, 1482–1488.
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(5) (a) Rohr, J. *J. Chem. Soc., Chem. Commun.* **1989**, 492–493. (b) Rohr, J. *Ibid.* **1990**, 113–114. (c) Rohr, J. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1051–1053.
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Table II. ^{1}H-NMR Data and δ Values (J/Hz) in ppm Relative to Internal TMS

	100-1 (3)[a]	100-2 (4)[a]	104-1 (5)[b]	104-2 (6)[c]	124-1 (7)[c]	rabelomycin (8)[d]
2-H_a	3.34 d (17)	2.88 d (13)	2.91 d (13)	3.00 d (13)	3.00 d (13)	3.01 s
2-H_e	3.20 dd (17, 2)	2.53 dd (13, 2)	2.65 dd (13, 2)	2.67 dd (13, 2)	2.67 dd (13, 2)	3.01 s
3-CH_3	1.48 s	1.15 s	1.28 s	1.35 s	1.35 s	1.47 s
4-H_a	3.05 d (15)	2.32 d (15)	2.77 d (17)	2.78 d (17)	2.80 d (17)	3.08 s
4-H_e	2.88 dd (15, 2)	1.98 dd (15, 2)	2.98 dd (17, 2)	3.03 dd (17, 2)	3.03 dd (17, 2)	3.08 s
5-H	7.63 d (8)	6.48 d (10)	7.28 s			7.00 s
6-H	8.32 d (8)	6.88 d (10)		7.60 s	7.60 s	
9-H						7.25 d (8)
10-H	7.66 d (8)	7.61 d (8)	7.75 d (8)	7.79 d (8)	7.80 d (8)	7.67 dd (8, 8)
11-H	7.64 d (8)	7.90 d (8)	7.45 d (8)	7.48 d (8)	7.48 d (8)	7.65 d (8)
2′-H	4.91 dd (11, 1)	4.90 dd (11, 1)	4.78 dd (11, 1)	4.76 dd (11, 1)	4.78 dd (11, 1)	
3′-H_a	1.2–1.3*	ca. 1.35*	1.2–1.3*	1.30 m*	1.35 m*	
3′-H_e	2.55 td (13, 5, 2)	2.41 td (13, 5, 2)	2.22 dd (12, 5)	2.24 dd (12, 5)	2.00 dd (12, 5)	
4′-H	3.81 td (12, 9, 5)	3.72 td (12, 9, 5)	3.50 td (12, 9, 5)	3.53 td (12, 9, 5)	3.67 td (12, 9, 5)	
5′-H	3.10 dd (9, 9)	3.06 dd (9, 9)	2.88 dd (9, 9)	2.88 dd (9, 9)	2.71 dd (9, 9)	
6′-H	3.54 dq (9, 6)	3.47 (9, 6)	3.35 dq (9, 6)	3.35 dq (9, 6)	3.31 dq (9, 6)	
6′-CH_3	1.37 d (6)	1.35 d (6)	1.25 d (6)	1.28 d (6)	1.28 d (6)	
			Sugar Moieties			
1A-H	5.01 s				4.87 s	
2A-H_2	1.4–2.0**				1.24 m* and 1.71–2.04**	
3A-H_2	1.3 m and 1.9 m				1.20–2.04*	
4A-H	3.57 d (1)				3.43 s	
5A-H	4.22 dq (6, 2)				4.14 dq (6, 2)	
5A-CH_3	1.16 d (6)				1.03 d (6)	
1B-H					4.47 dd (10, 2)	
2B-H_a					1.22 m*	
2B-H_e					2.42 td (12, 5, 2)	
3B-H					3.43 m*	
4B-H					3.06 dd (9, 9)	
5B-H					3.09 dd (9, 6)	
5B-CH_3					1.14 d (6)	
1C-H		5.30 d (1)				
2C-H_2		1.6–2.1*				
3C-H_2		1.6–2.1*				
4C-H		3.36 d (1)				
5C-H		3.66 dq (6, 2)				
5C-CH_3		0.52 d (6)				

* Obscured by solvent or other signal(s). ** Complex. [a] 200 MHz, acetone-d_6. [b] 200 MHz, DMSO-d_6. [c] 500 MHz, DMSO-d_6. [d] 500 MHz, $CDCl_3$.

at 28 °C for 90 min with an additional 7 mL of a clear medium (1% glucose, 1% bacto-peptone, 0.1% casamino acids, 0.2% yeast extract, 0.6% NaCl, pH 7.0) to start DNA replication. After centrifugation (20 min, 5000 rpm), the spores were resuspended in 2 mL of 0.05 M Tris–maleic acid (pH 9) buffer and incubated for 2 h at 37 °C with 4 mg of NTG (*N*-methyl-*N*′-nitro-*N*′-nitrosoguanidine).[7] Mutant strain urd-4 was prepared analogously from strain urd-2. The mutant strains (urd-1, urd-2, urd-3, and urd-4) were selected (vide infra) and isolated from single clones.

Selection Criteria and Detection of New Metabolites. Only those mutants were selected which showed a light discoloration of the agar (DIFCO Bi-Tek agar 21%, with a growth medium containing 10% malt extract, 4% yeast extract, 4% glucose, and 0.2% $CaCO_3$ in 1 L of water, pH adjusted on 7.2 before autoclaving; growth period: 1 week at 28 °C), i.e., which produced only "pale-colored" metabolites. Since the urdamycin complex antibiotics are intensively colored, this selection method led naturally to potential mutant strains. The detection of novel metabolites was achieved by TLC (silica gel, $CHCl_3/CH_3OH$ = 85/15) and HPLC (RP-18 silica gel, $CH_3CN/H_2O/CH_3OH$ = 5/9/1 for 16 min; gradient: 1 min, after 17 min: $CH_3CN/H_2O/CH_3OH$ = 15/2/3).

Fermentation. *Streptomyces fradiae* (Tü 2717) and the mutant strains urd-1, urd-2, and urd-3 were cultured in 2–20-L volumes (20–200 250-mL Erlenmeyer flasks with three baffles, each containing 100 mL of medium) in the standard medium (2% soybean meal, 2% glucose, pH adjusted at 7.2 before autoclaving). The fermentation of mutant urd-4 was performed in a medium containing 2% glucose, 1% soy peptone, 0.2% $CaCO_3$, 0.0001% $CoCl_2$. The pH was adjusted at 7.2 before autoclaving. The product spectrum of the mutant strains is summarized in Table I.

Isolation and Characterization of the New Metabolites. General purification: silica gel (first column 3.5 × 30 cm, $CHCl_3$/MeOH = 4/1, second column 3.5 × 30 cm, $CHCl_3$/MeOH = 85/15) and Sephadex LH 20 (column 100 × 2.5 cm, MeOH). In some cases, the purification with silica gel and/or Sephadex had to be repeated. $[\alpha]_D$ values and $[\theta]_D$ values are always given at room temperature (20 °C).

100-1 (3). Production: 10 mg/L. $C_{31}H_{34}O_{10}$ (566.61); yellow solid, mp 165 °C, soluble in $CHCl_3$ or methanol. R_f 0.53, R_{rel} 16.66 min. $[\alpha]_D$: 154 (*c* = 0.02, MeOH). IR (KBr): 3420, 2970 sh, 2930, 1705, 1685, 1632, 1590 cm^{-1}. UV (MeOH) and (MeOH/HCl) λ_{max} (log ε): 268 (4.418), 408 (3.647) nm; (MeOH/NaOH) λ_{max} (log ε) 247 (4.477), 266 (4.482), 502 (3.934) nm. ^{1}H-NMR: see Table II. ^{13}C-NMR: see Table III. EI-MS (70 eV) *m/z* (abundance): 548 (M − H_2O, 4, calcd for $C_{31}H_{32}O_9$ and found 548.2046), 434 (548 − rhodinose, 36), 114 (rhodinose, 28), 69 (30), 58 (50), 57 (76), 44 (100).

100-2 (4). Production: 10 mg/L. $C_{31}H_{36}O_{12}$ (600.62); light orange solid, mp 152 °C, soluble in $CHCl_3$, methanol, or acetone. R_f 0.35, R_{rel} 6.12 min. $[\alpha]_D$: 113 (*c* = 0.05, MeOH). IR (KBr): 3430, 1728, 1657 sh, 1638, 1620 cm^{-1}. UV (MeOH) λ_{max} (log ε): 219 (4.468), 318 (3.625), 426 (3.709) nm; (MeOH/HCl) λ_{max} (log ε) 218 (4.517), 315 (3.780), 422 (3.748) nm; (MeOH/NaOH) λ_{max} (log ε) 226 (4.491), 324 (3.907), 584 (3.661) nm. ^{1}H-NMR: see Table II. ^{13}C-NMR: see Table III. DCI-MS (NH_3) *m/z* (abundance): 618 (0.4, $[M + NH_4^+]^+$), 583 (0.2, $[M - H_2O + H^+]^+$), 504 (9), 469 (10), 451 (16), 435 (10), 150 (13), 133 (38), 132 (100, olivose + H^+, 100), 115 (100, rhodinose). Anal. Calcd (found): C, 61.99 (61.85); H, 6.04 (6.28).

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Table III. ^{13}C NMR Data (δ in ppm, Multiplicity from ATP[a] or DEPT[b] Spectrum, the Assignments Are Confirmed by C/H-COSY Experiments, for 6 and 7 also by HMBC[c] Spectra)

	100-1 (3)[d]	100-2 (4)[d]	104-2 (6)[e]	124-1 (7)[e]
C-1	196.9 s	202.6 s	197.2 s	197.0 s
C-2	54.2 t	54.7 t	52.9 t	52.8 t
C-3	72.6 s	76.2 s	71.4 s	71.3 s
3-CH_3	30.1 q	29–30 q*	30.2 q	30.0 q
C-4	44.8 t	44.1 t	37.6 t	37.5 t
C-4a	150.1 s	81.6 s	125.7 s	126.0 s
C-5	134.9 d	146.4 d	160.7 s	160.1 s
C-6	129.5 d	117.3 d	112.1 d	112.0 d
C-6a	134.1 s	137.9 s	133.9 s	133.9 s
C-7	189.1 s	189.7 s	188.0 s	187.9 s
C-7a	115.9 s	115.0 s	114.7 s	114.7 s
C-8	158.9 s	158.2 s	157.1 s	157.1 s
C-9	137.1 s	141.2 s	136.0 s	135.7 s
C-10	134.4 d	134.1 d	133.2 d	133.4 d
C-11	119.5 d	119.9 d	118.3 d	118.3 d
C-11a	137.8 s	131.9 s	134.2 s	134.1 s
C-12	183.4 s	183.4 s	180.8 s	180.8 s
C-12a	135.2 s	139.0 s	138.0 s[f]	138.0 s[g]
C-12b	134.1 s	81.6 s	137.1 s[f]	136.9 s[g]
C-2′	71.8 d	71.9 d	70.5 d	70.2 d
C-3′	37.9 t	40.8 t	40.1 t	38–40 t*
C-4′	79.7 d	77.1 d	71.7 d	74.7 d
C-5′	76.7 d	78.6 d	77.0 d	76.8 d
C-6′	77.2 d	73.3 d	76.1 d	70.4 d
6-CH_3	18.8 q	18.6 q	18.4 q	18.4 q
		Sugar Moieties		
C-1A				92.0 d
C-2A				24.0 t[h]
C-3A				23.9 t[h]
C-4A				75.3 d[i]
C-5A				65.3 d
C-6A				16.9 q
C-1B				100.9 d
C-2B				36.0 t
C-3B				76.1 d[i]
C-4B				74.5 d
C-5B				71.5 d
C-6B				18.0 q
C-1C		95.0 d		
C-2C		26.2 t		
C-3C		23.8 t		
C-4C		67.1 d		
C-5C		67.8 d		
C-6C		16.9 q		

* Obscured by solvent. [a] APT = attached proton test. [b] DEPT = distortionless enhancement by polarization transfer. [c] HMBC = hetero multiple-bond connectivity. [d] 50.3 MHz, acetone-d_6. [e] 125.7 MHz, DMSO-d_6. [f–i] Assignments are interchangeable.

104-1 (5). Production 2 mg/L.[8] $C_{25}H_{24}O_9$ (468.46); yellow solid, soluble in DMSO or MeOH. R_f 0.21, R_{rel} 5.8 min. ^1H-NMR: see Table II.

104-2 (6). Production 1.5 mg/L. $C_{25}H_{24}O_9$ (468.46); yellow solid, mp 177 °C dec, soluble in DMSO or MeOH. R_f 0.26, R_{rel} 5.8 min. $[\alpha]_D$: 78 (c = 0.0026, MeOH). CD (MeOH) $\lambda_{extr.}$ ($[\theta]_D \times 10^{-5}$): 330 sh (−0.41), 298 (−1.17), 268 (−0.1), 253 (−0.49), 216 (+1.05) nm. IR (KBr): 3400 br, 1710, 1635, 1568, 1440, 1365, 1280 cm^{-1}. UV (MeOH) λ_{max} (log ϵ): 257 (4.095), 285 (4.178), 385 (3.703) nm; (MeOH/HCl) λ_{max} (log ϵ) 260 (3.985), 285 (4.220), 403 (3.664) nm; (MeOH/NaOH) λ_{max} (log ϵ): 252 (4.191), 295 (3.740), 328 (3.719), 371 (3.700), 478 (3.271) nm. ^1H-NMR: see Table II. ^{13}C-NMR: see Table III. EI-MS (70 eV) m/z (abundance): 450 (12, M − H_2O, HR calcd for $C_{25}H_{22}O_8$ and found 450.1314), 346 (10), 254 (5), 210 (8), 97 (10), 83 (15), 69 (20), 57 (33), 44 (100).

124-1 (7). Production 1 mg/L. $C_{37}H_{44}O_{14}$ (712.75); dark yellow solid, mp 178 °C dec, soluble in DMSO. R_f 0.26, R_{rel} 6.06 min. $[\alpha]_D$ not observable. CD (MeOH) $\lambda_{extr.}$ ($[\theta]_D \times 10^{-5}$): 327 sh (−0.35), 298 (−1.33), 262 (−0.05), 253 (−0.16), 216 (+1.27) nm. IR (KBr): 3300, 2970, 2930, 2880 sh, 1700, 1630, 1554, 1440, 1365, 1280, 1080, 1060, 1013 cm^{-1}. UV (MeOH) λ_{max} (log ϵ): 258 (4.065), 285 (4.161), 383 (3.620) nm; (MeOH/HCl) λ_{max} (log ϵ): 260 (3.561), 285 (4.007), 403 (1.880) nm; (MeOH/NaOH) λ_{max} (log ϵ): 244 (4.483), 399 (4.000), 467 (3.997) nm. ^1H-NMR: see Table II. ^{13}C-NMR: see Table III. DCI-MS (NH_3) m/z (abundance): 730 ($[M + NH_4^+]^+$, 19), 712 (M^+, 40), 610 (17), 608 (17), 506 (10), 392 (10), 262 ([Oliv-Rho + NH_3], 100), 148 ([Oliv + NH_3], 10).

Rabelomycin (8). Production 0.1 mg/L. $C_{19}H_{14}O_6$ (338.32); orange solid, soluble in $CHCl_3$, DMSO. R_f 0.64, R_{rel} 16.83 min. $[\alpha]_D$: −130 (c = 0.01, MeOH). IR (KBr): 3420, 2955 sh, 2925, 2855, 1720 sh, 1630, 1600 sh, 1500, 1460, 1380 cm^{-1}. UV (MeOH) λ_{max} (log ϵ): 223 (4.224), 263 (4.251), 434 (3.555) nm; (MeOH/HCl) λ_{max} (log ϵ): 228 (4.309), 266 (4.274), 426 (3.731) nm; (MeOH/NaOH) λ_{max} (log ϵ): 256 (4.228), 317 (3.844), 502 (3.662) nm. ^1H-NMR: see Table II. EI-MS (70 eV) m/z (abundance): 338 (M^+, 5), 320 (M − H_2O, 100), 292 (18), 280 (22), 130 (68), 77 (74), 57 (100). Negative FAB-MS: 338 ([M + H − H]$^-$, 100).

NMR Experiments. ^1H- and ^{13}C-NMR spectra were recorded at 200 or 500 MHz and 50.3 or 125.7 MHz, respectively. The ^1H- and the ^{13}C-NMR signals of the blocked mutant products **3**, **4**, **5**, and **8** were assigned on chemical shift theory and in comparison with **2**, **9**, and **10** (assigned unequivocally in context with previous biosynthetic studies).[3c–e,4] The signals of compounds **6** and **7** were assigned also on 2D heteronuclear and homonuclear correlations, such as 2D-H,H-COSY (correlation spectroscopy) experiments for ^1H assignments. APT (attached proton test) or phase sensitive DEPT (distortionless enhancement by polarization transfer) and C,H-COSY or HMQC (heteronuclear multiple-quantum connectivity) were used for the determination of the carbon multiplicities and the proton-bearing carbons, respectively. The quarternary carbons were assigned on 2D long-range coupling experiments with the HMBC (= heteronuclear multiple-bond connectivity) pulse sequence showing sufficient $^2J_{C-H}$ and $^3J_{C-H}$ long-range couplings for unequivocal assignments (if not marked otherwise in Table III).

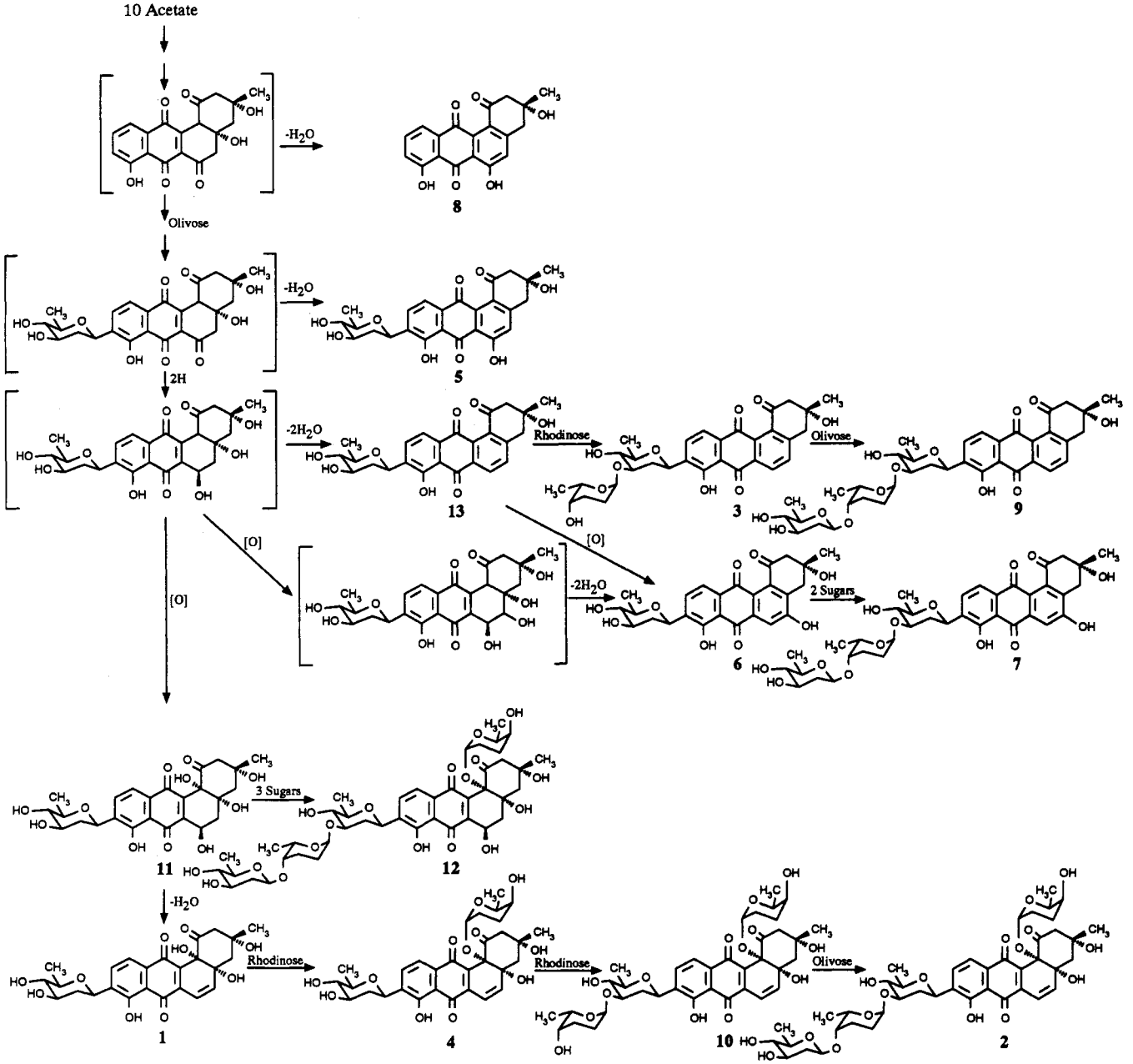
Results and Discussion

The preselection of mutants blocked in the urdamycin pathway was made possible by the lack of significant discoloration of the agar plates. The wild-type strain produces a palette of intensively colored products, causing a remarkable darkening of the agar. The selection criterion, in this instance the consideration of only weakly colored colonies, naturally resulted in mutants blocked near the known late-stage biosynthetic steps, which normally lead to intensively colored compounds. Furthermore, the structure elucidation of the blocked mutant products was facilitated by this approach, since the chemical structures were somewhat close to the known metabolites.

Besides known products of the wild-type strain, e.g., the urdamycins A (**2**), B (**9**), and G (**10**) as well as aquayamycin (**1**), five novel products (**3**–**7**) plus rabelomycin (**8**) were isolated and their structures elucidated. The roles of these compounds in the biosynthetic scheme (Scheme I) can be suggested as follows: 100-1 (**3**), intermediate of the shunt pathway leading to urdamycin B (**9**); 100-2 (**4**), intermediate of the main biosynthetic pathway immediately after aquayamycin (**1**); with this metabolite the glycosylation sequence of the urdamycin family could be determined unambigously; 104-1 (**5**), shunt product deriving from an intermediate two steps in front of urdamycinone F (**11**); 104-2 (**6**), shunt product (due to an unspecific oxygenase?), representing a previously undiscovered shunt pathway via urdamycinone B (**13**) or via a hypothetical ring B triol structure. The latter can dehydrate either to **5** or to **6**; 124-1 (**7**), glycosylated 104-2

(8) Compound 104-1 (**5**) is irregularly produced by the mutant strain urd-2. **5** could be isolated only once and decomposed after characterization by ^1H-NMR. Several attempts for its further isolation failed (including variation of the fermentation conditions). The detection was complicated, since the R_f and the R_{rel} values of **5** are identical with those of **6**.

Scheme I. Middle Biosynthetic Steps to Aquayamycin (1) and Forward to Urdamycin A (2)

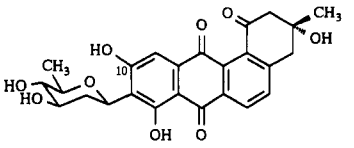


(6); this example shows again (see also urdamycinone F 11 → urdamycin F 12, and urdamycinone B 13 → urdamycin B 9) that the glycosyl-transferases of *S. fradiae* serve regiospecifically 12b-O and the OH groups of the glycosidic moieties, respectively, but accept structurally different aglycone moieties; and rabelomycin 8, its meaning here is a shunt product (probably immediately) in front of the C-glycosylation step. Rabelomycin was already discovered as a minor metabolite of another producer of aquayamycin-containing angucyclines[9] but could never be detected in the urdamycin-producing wild strain *S. fradiae*. These interpretations are summarized in Scheme I.

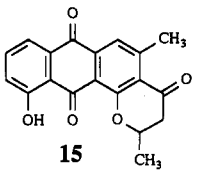
The above criteria for the selection of blocked mutants turned out to be sufficient to gain important new biosynthetic information, even though most of the blocked mutant strains produce only known metabolites. Some mutants were recognized as incompletely blocked ("leaky mutants", e.g., urd-1 and urd-3); most of them were not investigated further. Since we looked for mutants which produce only pale yellow or orange metabolites, we expected to find intermediates or shunt products of midstage biosynthetic reactions.

In combination with the recently described ^{18}O-experiments,[6] the studies with blocked mutants extend the biosynthetic scheme of the urdamycin family toward earlier steps. Our use of blocked mutants in biosynthetic studies is yet another example of the large potential of this method for biosynthetic investigations, namely the elucidation of earlier biosynthetic steps, which cannot be obtained from feeding experiments with labeled precursors. Two previously unknown biosynthetic steps leading to aquayamycin (1) could be postulated and are the earliest yet elucidated on the aquayamycin pathway. The structure shown for the mutant product 104-1 (5), which is produced irregularly in detectable quantities, is based on our experience in NMR interpretation of this group of antibiotics. Structure 14 as the best alternative to 5 might be considered also. In the latter case, 104-1 would reflect

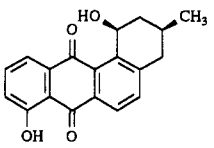
(9) Imamura, N.; Kakinuma, K.; Ikekawa, N.; Tanaka, H.; Ōmura, S. *J. Antibiot.* 1982, *35*, 602–608.



14



15



16

on an intermediate still carrying the 10-OH group, which is normally lost through dehydration for the formation of the aromatic ring D. We are still working on the isolation of sufficient amounts of 104-1 to determine its structure unambigously.

The gap between the elucidated biosynthetic steps and the biogenesis of the tetracyclic ring frame from 10 acetate units is nevertheless still large. In our continuing studies with *S. fradiae* (Tü 2717) we will employ only those mutants without colored products. This is a more difficult approach than the current one, since additional experiments, such as cosynthesis assays, will be necessary.

Our working hypothesis for angucyclines, anthracyclines, and similar multicyclic aromatic polyketide systems includes a successive closure of the four rings of the tetracyclic system.[10] Thus, in future studies, we hope to discover intermediates (or shunt products derived therefrom) in which one or more of the ring-closing cyclases are blocked, causing the formation of tricyclic or even bicyclic intermediates. Other angucycline-producing strains can also be considered, especially the emycin-producer *Streptomyces* sp. strain FHS-1114. It produces emycin B (**15**) besides the main product emycin A[11] (**16**, a typical angucyclinone), which can be postulated as resulting from a bicyclic intermediate.[10]

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(10) Rohr, J. *J. Org. Chem.* **1992**, *57*, 5217–5223.
(11) Dobreff, S. Ph.D. thesis, University of Göttingen, Germany, 1989.