URDAMYCINS, NEW ANGUCYCLINE ANTIBIOTICS FROM *STREPTOMYCES FRADIAE*

V. DERIVATIVES OF URDAMYCIN A

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(Received for publication September 5, 1988)

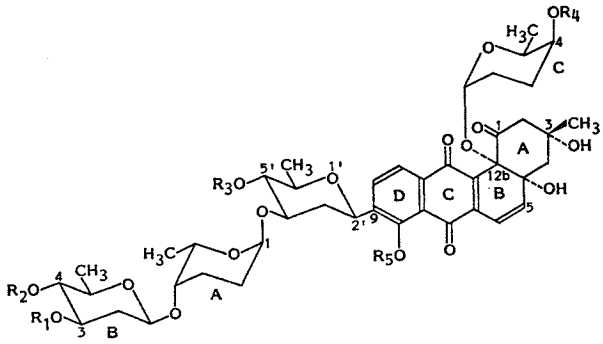
Derivatives of the angucycline urdamycin A (**1**) were prepared in order to study structure-activity relationships in this group of antitumor antibiotics. Derivatives of **1** formed by methanolysis, *O*-acylation, hydrogenation and treatment with diazomethane were isolated and characterized by their spectroscopic data. Urdamycin G (**20**) was isolated from *Streptomyces fradiae* by shortening the fermentation time. The different glycosidation pattern of the aglycone **14** did not lead to significant differences in the biological activity. *O*-Acylation was shown to enhance the *in vitro* activity of **1** against stem cells of murine L1210 leukemia depending on the lipophilicity of the molecules. The importance of the 5,6-double bond of **1** with regard to the antitumor activities is discussed.

The structure of urdamycin A (**1**), the main component among the angucycline antibiotics produced by *Streptomyces fradiae* (strain Tü 2717)[1], was determined from the spectroscopic and chemical data[1,2], and shown to be in accordance with an X-ray analysis[3]. **1** consists of the aglycone aquayamycin (**14**)[4] and three *O*-glycosidically bonded deoxyhexoses, one β-D-olivose (sugar B) and two α-L-rhodinoses (sugars A and C). Similar compounds with the same aglycone have been found in vineomycin A_1[5,6], the saquayamycins[7] and the kerriamycins[8,9]. The structure of urdamycin A (**1**) is identical to that recently published for kerriamycin B[10]. **1** is biologically active against Gram-positive bacteria and stem cells of murine L1210 leukemia. In order to investigate the general chemical behavior of **1** under different reaction conditions and to derive structure-activity relationships, various derivatives of **1** were made and characterized. In addition, deolivosylurdamycin A was isolated from *S. fradiae* as a biosynthetic precursor of **1**.

Methanolysis

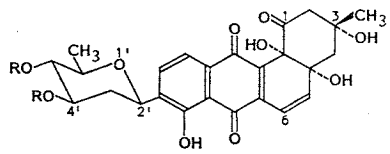
Besides the aglycone **14** derhodinosylurdamycin A (**17**) was obtained by methanolysis of **1** with dichloroacetic acid. The passage of the reaction was observed by HPLC analysis. **17** was the only intermediate detectable during the conversion of **1** to **14**. The lack of resonances of the angular bound rhodinose (sugar C) in the ^1H and ^{13}C NMR spectrum strongly suggested structure **17**, whose molecular formula was supported by the fast atom bombardment mass spectrum (FAB-MS) with the molecular ion at *m/z* 730 (negative ion). The CD spectrum of **17** is identical with that of **14** but differs remarkably from that of **1** in the region of 330 nm by the lack of a positive Cotton effect. We explain this by the influence of the angular sugar C on the conformation of rings A and B. Derhodinosylurdamycin A (**17**) is identical with the previously reported kerriamycin C[8~10]. Obviously the *O*-glycosidic bond of sugar A in **1** is less reactive than that of sugar C but more reactive than that of sugar B.

Scheme 1.

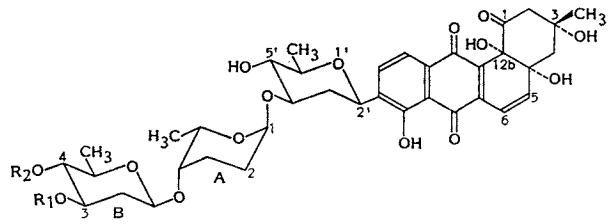


	R_1	R_2	R_3	R_4	R_5
1	H	H	H	H	H
2	Ac	Ac	Ac	Ac	Ac
3	Ac	Ac	Ac	H	Ac
4	Ac	H	H	H	H
5	Ac	Ac	H	H	H
6	Ac	Ac	Ac	Ac	H
7	H	H	Ac	Ac	H
8	H	Oc	H	H	H
9	Oc	H	H	H	H
10	Oc	Oc	H	H	H
11	Oc	Oc	H	Oc	H
12	Oc	Oc	Oc	Oc	H
13	H	H	H	H	CH_3

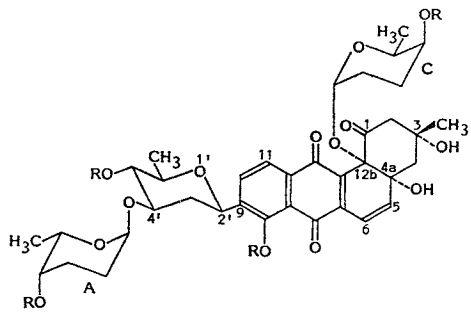
Oc: Octanoyl.



14 R=H
15 R=Ac
16 R=H, 5,6-dihydro



17 $R_1=R_2=H$
18 $R_1=Ac$ $R_2=H$
19 $R_1=R_2=Ac$



20 R=H
21 R=Ac

Urdamycin G

In biosynthetic pathways of oligosaccharide antibiotics the different sugar moieties are connected successively to an aglycone. Thus it might prove possible to obtain urdamycin A variants which may well be important in the biosynthesis by interrupting the fermentation of *S. fradiae* at various stages. We were able to isolate deolivosylurdamycin A as one of the missing links within the urdamycin-series. The new compound was called urdamycin G[11].

The fermentation conditions and the work-up procedure of the urdamycins in general have been described elsewhere[1]. A modified culture medium with soybean meal 2% and glucose 2% was used. The angucycline production was observed by TLC analysis. After a fermentation time of 44 hours

urdamycin G was isolated as the main product (168 mg/liter) together with 100 mg/liter urdamycin A (**1**).

The molecular formula $C_{37}H_{46}O_{14}$ of urdamycin G was confirmed by FAB-MS (*m/z* 714, negative ion). The UV and IR spectra were almost identical with those of **1**. By comparison of its NMR data with those of **1** the lack of the olivose moiety (sugar B in **1**) was evident. A comparison of the δ values and the coupling constants showed that the remaining NMR signals were similar to those of **1**. Thus, urdamycin G was assigned the structure **20** and is identical with the recently described OM-4842 (aggreticin)[12], which is an inhibitor of platelet aggregation. The isolation of **20** and its yield during the course of the fermentation provide evidence to suggest that the last step during the biosynthesis of **1** is the formation of the glycosidic bond between sugars A and B. Because of this fact and in view of the methanolysis studies described above it is arguable whether kerriamycin C[8~10] is a natural product or an artifact caused by the work-up procedure.

O-Acylation

The 3B,4B,4C,5′,8-penta-*O*-acetylurdamycin A (**2**) has already been described[1]. To prepare acyl derivatives of **1** with varying acyl group positions, **1** was treated with acetic and octanoic anhydride, respectively, in the presence of pyridine, sodium acetate and 4-dimethylaminopyridine. **2** was additionally subjected to a basic cleavage.

The number and the position of *O*-acyl groups within the isolated acetates and octanoates could be assigned by ^{1}H NMR spectroscopy, because of the significant downfield shift of the adjacent methine protons caused by the anisotropic effect of the acyloxy groups. The characteristic methine coupling patterns enabled us to initially assign these methine protons to the various individual sugars with the exception of 4B-H and 5′-H, because they have identical coupling constants (J=9.9 Hz). This uncertainty was clarified by ^{1}H-^{1}H correlated two-dimensional NMR spectroscopy (COSY). The results for the sugar moieties of different acyl derivatives are given in Table 1. The acylation of the phenolic hydroxy group could be seen by the lack of the downfield signal of **1** (δ 12.38) and by a change of the chemical shift of 10-H/11-H.

The reaction of **1** with a mixture of acetic anhydride - pyridine (2 : 1) for 8 hours at room tempera-

Table 1. Selected ^{1}H NMR signals of different acetates and octanoates of urdamycin A at 200 MHz in $CDCl_3$ (δ in ppm relative to internal TMS).

Compound	Methine protons							
	4′-H[a]	5′-H[b]	6′-H[c]	3B-H[d]	4B-H[e]	5B-H[f]	4C-H[g]	5A-H[h]
2	3.98	4.82	3.61	4.95[i]	4.74	3.44	4.67	3.87
3	3.98	4.81	3.61	4.95	4.73	3.44	3.49	3.88
4	3.70	3.15	3.56	4.79	3.44	3.29	3.31	4.12
5	3.70[i]	3.17	3.50[i]	4.94	4.74	3.44[i]	3.40[i]	4.12
6	4.00	4.82	3.60[i]	4.95[i]	4.79	3.30	4.67	3.91
7	4.01	4.83	3.61	3.40[i]	3.10	3.27	4.70	3.89
8	3.70	3.17	3.47	3.68	4.48	3.41	3.46	4.11
9	3.70	3.18	3.47	4.80	3.27	3.30	3.46	4.13
10	3.70	3.18	3.47	4.94	4.75	3.41	3.43	4.11
11	3.70	3.17	3.51	4.95	4.77	3.44	4.68	4.12
12	4.02	4.82	3.62	4.93	4.75	3.42	4.68	3.87

Coupling pattern (J in Hz): [a] m (obscured), [b] dd (9, 9), [c] dq (9, 6), [d] ddd (12, 8, 5), [e] dd (9, 9), [f] dq (9, 6), [g] dq (6.5, 9), [h] br s, [i] not assigned exactly.

ture yielded **2** (98%). By shortening the treatment to 7 hours, the less lipophilic 3B,4B,5′,8-tetra-*O*-acetylurdamycin A (**3**, 6%) was isolated, in addition to **2** (92%). Application of sodium acetate as catalyst to a solution of **1** in acetic anhydride for 2 hours yielded 3B-*O*-acetylurdamycin A (**4**, 53%), and, after 10 hours, a second product, characterized as 3B,4B-di-*O*-acetylurdamycin A (**5**, 18%).

The basic cleavage of **2** was carried out in methanol - water (10 : 7), saturated with sodium carbonate, at room temperature. After 10 minutes 3B,4B,5′,4C-tetra-*O*-acetylurdamycin A (**6**, 35%) was found to be the main product besides 5′,4C-di-*O*-acetylurdamycin A (**7**, 12%). As expected, the basic cleavage enabled us to synthesize regio-isomeric acetates. The sequence of acetylation and deacetylation is disturbed at the phenolic position. 8-OH has only a weak reactivity, but the acetylated phenolic ester is easily cleaved.

By treating a solution of **1** in $CHCl_3$ with a mixture containing octanoic anhydride - pyridine (2 : 1) for 6 hours, a number of products could be detected by TLC. Arranged in order of their increasing yield and lipophilic character, three products, 4B-*O*-octanoyl- (**8**, 14%), 3B-*O*-octanoyl- (**9**, 21%) and 3B,4B-di-*O*-octanoylurdamycin A (**10**, 26%) were isolated. 3B,4B,5′,4C-Tetra-*O*-octanoylurdamycin A (**12**) was obtained as the main product (47%) by stirring a chloroform solution of **1**, octanoic anhydride and 4-dimethylaminopyridine (DMAP) for 24 hours, besides 3B,4B,4C-tri-*O*-octanoylurdamycin A (**11**, 14%) as one of several products visible by TLC. Comparing the ^1H NMR data of **11** and **12** with those of **1**, the striking chemical shift of 4′-H and 5A-H is quite interesting. The downfield shift of 4′-H about 0.3 ppm is caused by the anisotropic effect of the ester carbonyl group. This effect is not as strong as that detected for the directly adjacent 5′-H (1.7 ppm). The same effect was observed on 6′-H which may tentatively explained by a *cis* orientation of the acyloxy group with respect to the vicinal hydrogen atom. A similar phenomenon could also be observed for 3B-H, 4B-H and 5B-H in **8**, **9** and **10** as well as in the acetates, and provides proof of the assignment of the acylated positions. The upfield shift of 5A-H of about 0.2 ppm, which could be detected in the acetates too, is obviously induced by the aliphatic residue of the acyloxy group at C-5′. The orientation of this residue and sugar A should be responsible for this remarkable through space induced shift. In the case of **12** all assignments of NMR signals were confirmed by $^1J_{CH}$-heteronuclear shift correlation (HETCOR).

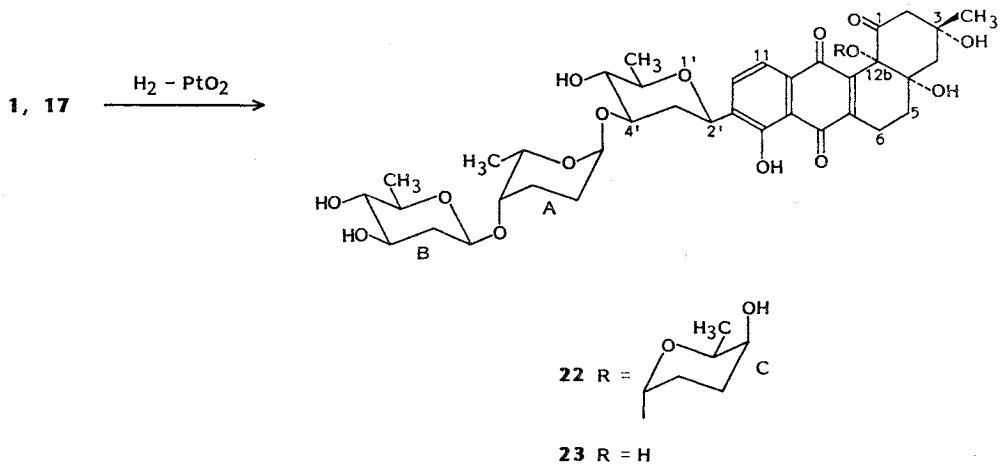
Assuming acylation under kinetic control, the nucleophilicity of the hydroxy groups in **1** could be assigned directly from the yields of the various acyl derivatives formed under the specified conditions. Two different nucleophilicity sequences depending on the acylating agent used were found. For acetic anhydride: 3B-OH>4B-OH>8-OH>5′-OH>4C-OH; for octanoic anhydride: 3B-OH>4B-OH>4C-OH>5′-OH>>(8-OH). As reasons for the differences one should mention a blend of intrinsic reactivity and steric accessibility.

To get an almost complete sequence of acylated urdamycin A derivatives, **14**, **17** and **20** were acetylated too. Treating a solution of **14** in acetic anhydride with DMAP led to 4′,5′-di-*O*-acetylurdamycinone A (**15**, 25%), while acetylation of **17** was achieved using acetic anhydride and sodium acetate, giving 3B-*O*-acetyl- (**18**, 21%) and 3B,4B-di-*O*-acetyl-derhodinosylurdamycin A (**19**, 47%). Acetylation of **20** with a mixture of acetic anhydride and pyridine (2 : 1) gave 4A,4C,5′,8-tetra-*O*-acetylurdamycin G (**21**, 65%). Their structures followed unambiguously from the ^1H NMR spectra.

O-Methylation

Using methyl iodide in the presence of potassium carbonate at 40°C, **1** was transformed into the

Scheme 2.



8-*O*-methylurdamycin A (**13**). Comparing the ^{1}H NMR spectrum with that of **1**, **13** exhibited a phenolic methoxy singlet at δ 3.90, and the aromatic 10-H and 11-H signals shifted downfield from δ 7.91/7.67 to δ 7.96/7.92.

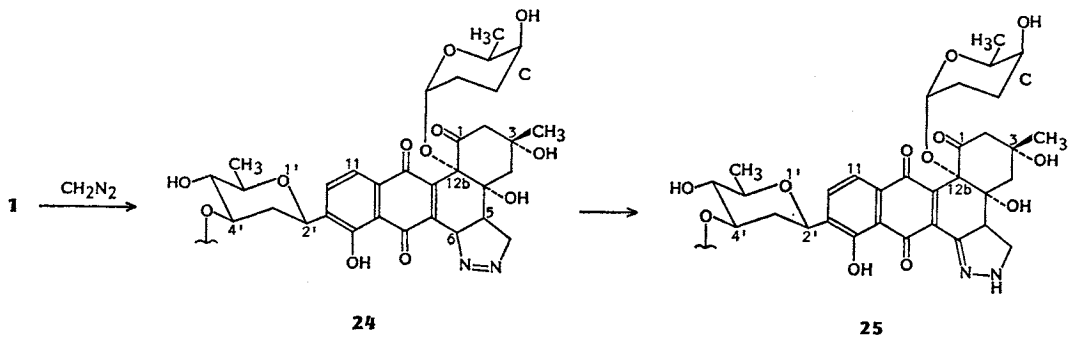
Hydrogenation

Hydrogenation of **1** with H_2 and PtO_2 as catalyst led to the yellow 5,6-dihydrourdamycin A (**22**). The comparison of the ^{1}H NMR spectra of **1** and **22** confirmed the expected structure. Multiplets for 6-H (δ 3.0~3.15) and 5-H (δ 2.25~2.44) were detected instead of the olefinic 5-H/6-H doublets. By hydrogenation of **17** and methanolysis of **22** it was possible to synthesize 5,6-dihydroderhodinosylurdamycin A (**23**) and 5,6-dihydrourdamycinone A (**16**)[4]. The direct hydrogenation of **14** failed under the conditions used.

Reaction with Diazomethane

After treatment of **1** with diazomethane at 0°C in tetrahydrofuran, two new products, one yellow and one red, could be detected by TLC. Purification of the former failed, because it was easily transformed into the red one during further chromatography on silica gel. The elemental analysis of the red product was in agreement with the molecular formula $C_{44}H_{58}N_2O_{17}$, which was confirmed by FAB-MS (*m/z* 886, negative ion). Thus, one equivalent diazomethane had added to urdamycin A (**1**). Comparing the ^{1}H NMR spectra of **1** and of the red product, the lack of the olefinic 6-H/5-H resonances suggested that a cycloaddition of diazomethane at the 5,6-double bond resulted in the adduct **24**. The observed NH-doublet at δ 6.58 pointed to a tautomerism between the yellow intermediate **24** and its red, more stable tautomer **25**. This assumed regioselectivity in the addition of diazomethane was also suggested by the UV data. There is a bathochromic shift of the long-wavelength absorption band (MeOH: 426 nm in **1**, 451 nm in **25**, MeOH - NaOH: 580 nm in **1**, 589 nm in **25**) whose explanation seems to require an extended conjugated system including one nitrogen. This 1,3-dipolar cycloaddition, leading to the compounds **24** and **25**, is well understood with the help of the valence orbital theory[13,14]. The 5,6-double bond is electron deficient and conjugated with the quinone system. The cycloaddition proved to be diastereoselective because there is no doubling of signals in the ^{1}H NMR spectrum of **24** and **25**. The absolute configuration of C-5 in **25** remains unsettled.

Scheme 3.



24 **25**

(Sugar moieties A and B as in **1**)

Table 2. Anticancer assays against L1210, HT 29 and A 549 leukemia cells (IC_{50} values in μg/ml)[15,16].

Compound	MTT-assay			Stem cell assay against L1210 (1 hour expt)
	L1210	HT 29	A 549	
1	7.5	5.0	>10	NT
8	0.38	0.3	0.5	0.09
9	0.36	0.4	1.4	0.26
10	1.4	2.6	5.1	NT
11	>10	>10	>10	NT
12	>10	>10	>10	NT
15	<0.039	0.059	0.09	0.026
18	0.087	0.18	0.086	0.08
19	0.25	0.34	0.24	0.18

NT: Not tested.

Compound	Stem cell assay against L1210	
	Continuous expt	1 hour expt
1	0.55	NT
2	0.055	0.1
3	0.075	NT
4	0.28	2.2
5	0.11	NT
6	0.078	0.25
7	0.13	0.85
13	>10	NT
14	0.32	1.6
17	0.75	1.8
20	0.85	0.35
21	0.036	0.065
22	>10	NT
25	>10	NT

Biological Activities and Discussion

The biological activity of **1** and **14** have already been described[1]. A number of derivatives described above, were tested against L1210, A 549 and HT 29 tumor cells with the stem cell or the proliferation assay (MTT-reduction)[15,16]. In the case of interest, the activity against L1210 tumor cells using the stem cell assay (1 hour experiment) were also tested (Table 2). The comparison of activity between **1**, **14**, **17** and **20** indicated the relative insignificance of the number of sugar moieties attached to the aglycone. The acyl derivatives showed an increasing biological activity in relation to their increasing lipophilic character; **2**, **8** and **21** were the most active derivatives. A further acylation led to the break down of activity. This could be demonstrated in the case of **11** and **12**. Despite the large number of acyl derivatives synthesized, no structure-activity relationships with respect to the hydroxy groups could be found. The data for **8** and **9** provide evidence that the acylation of 4B-OH is slightly more effective than acylation of 3B-OH. Methylation of 8-OH led to a decrease, whereas its acetylation led to an increase of antitumor activity, pointing out the necessity of the phenolic hydroxy group, which may be liberated from esters by esterases in the cells. The diacetate **15** of the aglycone aquaya-

mycin is similarly active like the pentaacetate **2** of urdamycin A, indicating again that the *O*-glycosidically bound sugar moieties are not necessary to induce a basic activity within the angucycline group.

Loss of the 5,6-double bond led to a breakdown of the biological activity (**22** and **25**) and might be explained by the different conformation of rings A and B in **22** and **25** in comparison with **1**. It also may be that the vinylogous 5,6-double bond with its reactivity towards nucleophiles is the most important structural element for preservation of cytotoxicity.

Experimental

General

MP's were determined on a Reichelt hot-stage microscope and are not corrected. IR spectra in pressed KBr discs were recorded on a Perkin-Elmer Model 298 spectrometer, the UV spectra on a Kontron Uvicon 860. If UV data are not represented, the absorption bands are as described in ref 1.

The NMR spectra were determined at 200 MHz with a Varian XL-200 or VXR-200 instrument. Chemical shifts are expressed in δ values (ppm) with TMS as an internal standard. The mass spectra were taken with a Finnigan MAT-8230 (FAB) mass spectrometer. CD spectra were recorded using a Jasco J-500 A spectrometer in combination with a BMC if 800 personal computer.

Analyticals

See ref 1.

Derhodinosylurdamycin A (17)

260 μl of dichloroacetic acid were added to a solution of 133 mg **1** in 133 ml MeOH at 4°C. After 72 hours the mixture was neutralized with 2 M NaOH and evaporated to dryness. The crude residue was desalted by dissolving in $CHCl_3$ and filtered. The residue was chromatographed on a Sephadex LH-20 column (2.5×100 cm, MeOH) to yield 95 mg (83%) derhodinosylurdamycin A (**17**) and 14 mg (17%) urdamycinone A (**14**). **14** was shown to be identical with the previous described aquayamycin[4] in all physico-chemical properties.

17: MP 176~177°C; Rf 0.25 ($CHCl_3$ - MeOH, 9:1); IR (KBr) cm^{-1} 3410, 1720, 1658 (sh), 1650 (sh), 1640, 1620 (sh), 1560; UV λ_{max} nm (ε) (MeOH and MeOH - HCl) 427 (4,600), 316 (3,800), 218 (23,600); (MeOH - NaOH) 600 (3,700), 316 (6,900), 282 (7,700), 229 (20,200); ^1H NMR (200 MHz, acetone-d_6) δ 1.16 (d, J=6.5 Hz, 5A-CH_3), 1.23 (d, J=6 Hz, 5B-CH_3), 1.24 (s, 3-CH_3), 1.28 (ddd, J=12, 12 and 12 Hz, 3'-H_{ax}), 1.34 (d, J=6 Hz, 6'-CH_3), 1.49 (dd, J=12 and 12 Hz, 3A-H_{ax}), 1.52 (ddd, J=13, 9 and 5 Hz, 2B-H_{ax}), 1.53 (dd, J=12 and 12 Hz, 2A-H_{ax}), 1.7~2.15 (complex, 4-H_2, 3A-H_{eq}, 2A-H_{eq}), 2.17 (ddd, J=13, 5 and 2 Hz, 3'-H_{eq}), 2.27 (d, J=13 Hz, 2-H_{ax}), 2.71 (ddd, J=13, 5 and 2 Hz, 2B-H_{eq}), 2.94 (ddd, J=10, 10 and 3 Hz, 4B-H), 2.98 (d, J=13 Hz, 2-H_{eq}), 3.19 (ddd, J=10, 10 and 3 Hz, 5'-H), 3.22 (dq, J=6 and 9 Hz, 5B-H), 3.4~3.65 (complex, 4A-H, 3B-H), 3.82 (ddd, J=12, 9 and 5 Hz, 4'-H), 4.23 (dq, J=2 and 6.5 Hz, 5A-H), 4.61 (dd, J=2 and 9.5 Hz, 1B-H), 4.90 (dd, J=2 and 12 Hz, 2'-H), 5.0 (d, J=2 Hz, 1A-H), 6.50 (d, J=10 Hz, 5-H), 6.89 (d, J=10 Hz, 6-H), 7.60 (d, J=8 Hz, 11-H), 7.94 (d, J=8 Hz, 10-H); negative FAB-MS m/z (abundant, %) 731 (100, M+H), 730 (84, M), 713 (31, M−H_2O); CD $\lambda_{extreme}^{MeOH}$ nm ($[\theta]^{20}$) 501 (−3,200), 452 (+400), 402 (−4,400), 290 (+10,500), 273 (+1,080), 254 (−700), 221 (−27,400).

Anal Calcd for $C_{37}H_{46}O_{15}$: C 60.81, H 6.45.
Found: C 60.74, H 6.29.

Urdamycin G (20)

The urdamycin G producing organism *S. fradiae* (strain Tü 2717) was cultured for 44 hours at 28°C in a medium consisting of soybean meal 2%, glucose 2%, 150 ml in 1-liter Erlenmeyer flasks. The pH was adjusted to 7.2 before autoclaving.

The ethyl acetate extract of the culture filtrate was combined with the acetone extract of the mycelium. The organic solvents were removed under vacuum and the aqueous residue dissolved in methanol and extracted twice with *n*-hexane. The methanol layer was evaporated to dryness and chromatogra-

phed on silica gel ($CHCl_3$ - MeOH, 85:15), to yield urdamycin G (**20**; Rf 0.3, $CHCl_3$ - MeOH, 9:1) followed by urdamycin A (**1**; Rf 0.2) and traces of other urdamycins. A typical fermentation yielded 168 mg/liter urdamycin G (**20**) besides 100 mg/liter urdamycin A (**1**).

A final purification using gel filtration on Sephadex LH-20 (MeOH) gave pure **20** as a light orange amorphous powder: MP 141°C; Rf 0.30 ($CHCl_3$ - MeOH, 9:1); IR (KBr) cm^{-1} 3420, 2958 (sh), 2910, 2840, 1718, 1630, 1615, 1555; FAB-MS (negative ions) *m/z* 714 (16%); CD $\lambda^{MeOH}_{extreme}$ nm ($[\theta]^{22}$) 400 (−9,000), 329 (+21,000), 289 (sh, +10,000), 263 (−5,000), 235 (+20,000); ^1H NMR (200 MHz, $CDCl_3$) δ 0.58 (d, J=7 Hz, 5C-CH_3), 0.86 and 1.4~2.2 (complex, 3A-H_2, 2A-H_2, 3C-H_2, 2C-H_2), 1.24 (s, 3-CH_3), 1.24 (d, J=6.5 Hz, 5A-CH_3), 1.30 (obscured, 3′-H_{ax}), 1.43 (d, J=6.0 Hz, 6′-CH_3), 1.84 (d, J=15 Hz, 4-H_{ax}), 2.16 (dd, J=15 and 2 Hz, 4-H_{eq}), 2.46 (ddd, J=13, 5 and 2 Hz, 3′-H_{eq}), 2.52 (d, J=12.5 Hz, 2-H_{ax}), 2.79 (dd, J=12.5 and 2.5 Hz, 2-H_{eq}), 3.19 (dd, J=9 and 9 Hz, 5′-H), 3.4~3.6 (complex, 4C-H, 6′-H), 3.70 (br s, 4A-H), 3.72 (obscured, 5C-H), 3.73 (m, 4′-H), 4.18 (dq, J=6.5 and 2 Hz, 5A-H), 4.88 (dd, J=11.5 and 2 Hz, 2′-H), 5.01 (br s, 1A-H), 5.40 (br s, 1C-H), 6.41 (d, J=10 Hz, 5-H), 6.89 (d, J=10 Hz, 6-H), 7.67 (d, J=8 Hz, 11-H), 7.93 (d, J=8 Hz, 10-H); ^{13}C NMR (50.3 MHz, CD_3OD) δ 16.8 (q, C-6C), 17.4 (q, C-6A), 18.8 (q, C-7′), 24.1 (t, C-3A), 24.3 (t, C-3C), 26.3 (t, C-2A), 26.4 (t, C-2C), 29.9 (q, 3-CH_3), 37.7 (t, C-3′), 44.4 (t, C-4), 54.7 (t, C-2), 67.8 (d, C-4C), 67.9 (d, C-5A), 68.0 (d, C-5C), 72.2 (d, C-2′, C-6′), 76.7 (d, C-4A), 77.1 (s, C-3), 77.7 (d, C-4′), 77.8 (d, C-5′), 82.5 (s, C-4a), 82.7 (s, C-12b), 95.1 (d, C-1A), 95.5 (d, C-1C), 115.3 (s, C-7a), 117.8 (d, C-6), 120.3 (d, C-11), 132.1 (s, C-6a), 134.3 (d, C-10), 138.4 (s, C-11a), 138.9 (s, C-12a), 141.4 (s, C-9), 146.3 (d, C-5), 158.5 (s, C-8), 183.9 (s, C-12), 189.5 (s, C-7), 204.7 (s, C-1).

Penta-*O*-acetylurdamycin A (**2**)

90 mg of **1** were dissolved in a mixture of 15 ml acetic anhydride and 7.5 ml pyridine. After stirring for 8 hours at room temperature the solution was poured into ice-water. After 5 hours it was extracted 3 times with $CHCl_3$ and evaporated to dryness. Pyridine residues were removed by dissolving several times in toluene and evaporating. The residue was chromatographed on a silica gel column (30×2 cm, $CHCl_3$ - MeOH, 100:2), the main fraction was rechromatographed on Sephadex LH-20 column (100×2.5 cm, MeOH) to yield 110 mg (98%) **2** as a yellow powder, which was shown to be identical with the previously described **2**[1] in all physico-chemical properties.

3B,4B,5′,8-Tetra-*O*-acetylurdamycin A (**3**)

123 mg of **1** was treated as described in the last section, but it was poured into ice-water after 7 hours. The same work up procedure yielded 148 mg (92%) of **2** and 11 mg (6%) of **3**: MP 160°C; Rf 0.49 ($CHCl_3$ - MeOH, 9:1); IR (KBr) cm^{-1} 3450, 2980, 2938, 2870, 1781, 1745, 1664, 1632, 1598, 1561; UV λ_{max} nm (ε) (MeOH and MeOH - HCl) 360 (4,770), 313 (5,500), 258 (15,300); (MeOH - NaOH) 401 (3,100), 316 (7,700), 255 (12,600), 224 (17,000); ^1H NMR (200 MHz, $CDCl_3$) see Table 1, δ 2.04, 2.06, 2.08, 2.38 (s, CH_3CO), all other resonances are very similar to those found for **1**.

3B-Mono-*O*-acetylurdamycin A (**4**)

To a solution of 36 mg of **1** in 10 ml acetic anhydride was added 20 mg of sodium acetate and stirred for 2 hours. The mixture was poured into ice-water, extracted 4 times with $CHCl_3$ and evaporated to dryness. The residue was chromatographed on silica gel column (50×2 cm, CH_2Cl_2 - EtOH, 9:1). The main fraction was evaporated and purified on a Sephadex LH-20 column (100×2.5 cm, MeOH) to yield 20 mg (53%) of **4**: MP 178°C; Rf 0.31 ($CHCl_3$ - MeOH, 9:1); IR (KBr) cm^{-1} 3430, 2980, 2935, 1727, 1660 (sh), 1640, 1621, 1565; ^1H NMR (200 MHz, $CDCl_3$) see Table 1, δ 2.11 (s, CH_3CO), all other resonances are very similar to those found for **1**.

Anal Calcd for $C_{45}H_{58}O_{18}$: C 60.82, H 6.53.
Found: C 60.83, H 6.70.

3B,4B-Di-*O*-acetylurdamycin A (**5**)

A mixture of 50 mg of **1**, 20 mg sodium acetate and 15 ml acetic anhydride was stirred for 16 hours. It was poured into ice-water, extracted with 3 times with $CHCl_3$ and evaporated to dryness. The residue was chromatographed on a silica gel column (30×2 cm, CH_2Cl_2 - EtOH, 95:5). The two main products were purified on a Sephadex LH-20 column (100×2.5 cm, MeOH) to yield 10 mg (18%)

of **5** and 10 mg (19%) of **4**.

5: MP 169°C; Rf 0.52 ($CHCl_3$ - MeOH, 9:1); IR (KBr) cm^{-1} 3440, 2980, 2935, 2870, 1745, 1735, 1658, 1640, 1621, 1563; ^1H NMR (200 MHz, $CDCl_3$, assigned by ^1H-^1H COSY) see Table 1, δ 2.01, 2.04 (s, CH_3CO), all other resonances are very similar to those found for **1**.

Basic Cleavage

3.5 ml of a saturated sodium carbonate solution was added to a solution of 73 mg **2** in 10 ml MeOH and 7 ml water. After stirring for 10 minutes the reaction was stopped by adding acetic anhydride. It was poured into ice-water, extracted 5 times with $CHCl_3$ and evaporated to dryness. The residue was chromatographed on a silica gel column (50×2.5 cm, CH_2Cl_2 - EtOH, 9:1). The two main fractions were evaporated and rechromatographed on Sephadex LH-20 column (100× 2.5 cm, MeOH) to yield 31 mg (35%) of 3B,4B,5′,4C-tetra-*O*-acetylurdamycin A (**6**) and 10 mg (12%) of 5′,4C-di-*O*-acetylurdamycin A (**7**).

6: MP 184°C; Rf 0.49 ($CHCl_3$ - MeOH, 9:1); IR (KBr) cm^{-1} 3440, 2940, 1740, 1660 (sh), 1640, 1625, 1565; ^1H NMR (200 MHz, $CDCl_3$) see Table 1, δ 2.02, 2.12, 2.14, 2.15 (s, CH_3CO), all other resonances are very similar to those found for **2**.

Anal Calcd for $C_{51}H_{64}O_{21}$: C 60.41, H 6.32.
Found: C 60.42, H 6.50.

7: MP 175~181°C; Rf 0.26 ($CHCl_3$ - MeOH, 9:1); IR (KBr) cm^{-1} 3450, 1740, 1660, 1640, 1622, 1565; ^1H NMR (200 MHz, $CDCl_3$) see Table 1, δ 2.01, 2.10 (s, CH_3CO), all other resonances are very similar to those found for **1**.

Anal Calcd for $C_{47}H_{60}O_{19}$: C 60.71, H 6.46.
Found: C 60.80, H 6.45.

Octanoylation of **1**

A): 69 mg of **1** was dissolved in a mixture consist of 2 ml $CHCl_3$, 1.5 g octanoic anhydride and 0.76 g pyridine and stirred for 6 hours at room temperature. The solution was poured into ice-water and, after 3 hours, was extracted 3 times with $CHCl_3$ and evaporated. Pyridine residues were removed by dissolving several times in toluene and evaporating. The remaining orange oil was chromatographed on a silica gel column (40×3 cm, CH_2Cl_2 - MeOH - triethylamine, 9:1:0.01). Three products were isolated and purified on Sephadex LH-20 column (100×2.5 cm, MeOH) to yield 10.7 mg (13.5%) of 4B-mono-*O*-octanoylurdamycin A (**8**), 16.5 mg (20.8%) of 3B-mono-*O*-octanoylurdamycin A (**9**) and 23.1 mg (25.8%) of 3B,4B-di-*O*-octanoylurdamycin A (**10**).

8: MP 138°C; Rf 0.41 ($CHCl_3$ - MeOH, 9:1); IR (KBr) cm^{-1} 3440, 2940, 2870, 1730, 1640, 1620, 1560; ^1H NMR (200 MHz, $CDCl_3$) see Table 1, δ 0.88 (octanoyl-CH_3), all other resonances are very similar to those found for **10**.

9: MP 123°C; Rf 0.42 ($CHCl_3$ - MeOH, 9:1); IR (KBr) cm^{-1} 3440, 2940, 2870, 1730, 1640, 1620, 1560; ^1H NMR (200 MHz, $CDCl_3$) see Table 1, δ 0.88 (octanoyl-CH_3), all other resonances are very similar to those found for **10**.

10: MP 122°C; Rf 0.50 ($CHCl_3$ - MeOH, 9:1); IR (KBr) cm^{-1} 3440, 2930 (sh), 2860, 1730, 1635 (sh), 1560; ^1H NMR (200 MHz, $CDCl_3$, assigned by ^1H-^1H COSY) see Table 1, δ 0.58 (d, J= 7 Hz, 5C-CH_3), 0.87, 0.88 (t, J=6 Hz, octanoyl-CH_3), 1.17 (d, J=6 Hz, 5B-CH_3), 1.20 (d, J=6 Hz, 5A-CH_3), 1.25 (s, 3-CH_3), 1.40 (obscured, 3′-H_{ax}), 1.4~1.7 (complex, 3A-H_{ax}), 1.43 (d, J=6 Hz, 6′-CH_3), 1.51 (obscured, 2A-H_{ax}), 1.53 (obscured, 3C-H_{eq}), 1.76 (obscured, 2B-H_{ax}), 1.80 (obscured, 4-H_{ax}), 1~2 (complex, 3C-H_{ax}, 2C-H_2), 1.95 (obscured, 3A-H_{eq}), 2.14 (obscured, 2A-H_{eq}), 2.15 (obscured, 4-H_{eq}), 2.39 (obscured, 2B-H_{eq}), 2.43 (obscured, 3′-H_{eq}), 2.48 (d, J=13 Hz, 2-H_{ax}), 2.80 (dd, J=13 and 2 Hz, 2-H_{eq}), 3.55 (s, 4A-H), 3.68 (dq, J=7 and 2 Hz, 5C-H), 4.59 (dd, J=10 and 2 Hz, 1B-H), 4.86 (dd, J=12 and 2 Hz, 2′-H), 5.04 (s, 1A-H), 5.42 (s, 1C-H), 6.44 (d, J=10 Hz, 5-H), 6.92 (d, J=10 Hz, 6-H), 7.70 (d, J=8 Hz, 11-H), 7.95 (d, J=8 Hz, 10-H).

B): To a solution of 113.7 mg of **1** in 5 ml $CHCl_3$ and 1.85 g octanoic anhydride were added 102 mg of DMAP. After 24 hours it was evaporated and the oily residue was treated with ice-water. It was extracted twice with CH_2Cl_2, dried and evaporated to yield a yellow oil. Octanoic acid residues were removed by column chromatography on silica gel column (30×3 cm, CH_2Cl_2 - MeOH - tri-

ethylamine, 9:1:0.01). The combined evaporated fractions were rechromatographed on a silica gel column (30×3 cm, EtOAc - petroleum ether, 1:1). The two main fractions were purified on Sephadex LH-20 column (100×2.5 cm, MeOH) to yield 22.7 mg (14.1%) of 3B,4B,4C-tri-*O*-octanoylurdamycin A (**11**) and 86.4 mg (47.4%) of 3B,4B,5′,4C-tetra-*O*-octanoylurdamycin A (**12**).

11: MP 95°C; Rf 0.79 ($CHCl_3$ - MeOH, 9:1); IR (KBr) cm^{-1} 3400, 2930 (sh), 2860, 1730, 1655, 1640, 1620, 1560; ^1H NMR (200 MHz, $CDCl_3$) see Table 1, δ 0.84, 0.87, 0.88 (octanoyl-CH_3), all other resonances are very similar to those found for **10**.

12: MP 78~80°C; Rf 0.79 ($CHCl_3$ - MeOH, 9:1); IR (KBr) cm^{-1} 3460, 2925 (sh), 2860, 1730, 1655, 1640, 1620, 1560; ^1H NMR (200 MHz, $CDCl_3$, assigned by $^1J_{C,H}$-heteronuclear shift correlation) see Table 1, δ 0.50 (d, J=7 Hz, 5C-CH_3), 0.83, 0.87, 0.88, 0.90 (t, J=6 Hz, octanoyl-CH_3), 1.17 (d, J=6 Hz, 5B-CH_3), 1.18 (obscured, 5A-CH_3), 1.24 (s, 3-CH_3), 1.25 (obscured, 6′-CH_3), 1~2 (complex, 2C-H_2, 3C-H_2), 1.2~1.5 (complex, 2A/3A-H_{ax}), 1.4~1.6 (complex, 2A-H_{eq}), 1.7~1.8 (complex, 4-H_{ax}), 1.7~2.0 (complex, 3A-H_{eq}), 1.76 (obscured, 2B-H_{ax}), 1.8~2.1 (complex, 2A-H_{eq}), 2.17 (dd, J=16 and 3 Hz, 4-H_{eq}), 2.38 (ddd, J=12, 5 and 2 Hz, 2B-H_{eq}), 2.51 (d, J=13 Hz, 2-H_{ax}), 2.63 (ddd, J=12, 5 and 2 Hz, 3′-H_{eq}), 2.79 (dd, J=13 and 3 Hz, 2-H_{eq}), 3.43 (s, 4A-H), 3.71 (dq, J=2 and 7 Hz, 5C-H), 4.54 (dd, J=10 and 2 Hz, 1B-H), 4.89 (dd, J=12 and 2 Hz, 2′-H), 4.95 (s, 1A-H), 5.4 (s, 1C-H), 6.42 (d, J=10 Hz, 5-H), 6.90 (d, J=10 Hz, 6-H), 7.67 (d, J=8 Hz, 11-H), 7.90 (d, J=8 Hz, 10-H); ^{13}C NMR (50.3 MHz, $CDCl_3$, assigned by $^1J_{C,H}$-heteronuclear shift correlation) δ 14.1 (q, octanoyl-CH_3), 16.5 (q, C-6C), 17.2 (q, C-6B), 17.7 (q, C-6A), 18.1 (q, C-7′), 22.5, 22.6 (t, octanoyl-CH_2), 23.8 (t, C-3C), 24.2 (t, C-3A), 24.5 (t, C-2A), 24.9 (t, octanoyl-CH_2), 25.1 (t, C-2C), 28.8, 28.9, 29.0, 29.1, 29.2 (t, octanoyl-CH_2), 29.9 (q, 3-CH_3), 31.6, 34.2, 34.3, 34.5 (t, octanoyl-CH_2), 36.2 (t, C-2B), 36.6 (t, C-3′), 43.2 (t, C-4), 53.9 (t, C-2), 65.7 (d, C-5C), 66.0 (d, C-5A), 68.5 (d, C-4C), 70.0 (d, C-5B), 70.4 (d, C-3B), 71.0 (d, C-2′), 72.2 (d, C-4′), 73.6 (d, C-4B), 74.6 (d, C-6′), 75.1 (d, C-5′), 75.2 (s, C-3), 76.6 (d, C-4A), 80.2 (s, C-4a), 81.8 (s, C-12b), 92.7 (d, C-1A), 94.5 (d, C-1C), 100.9 (d, C-1B), 113.7 (s, C-6a), 116.7 (d, C-6), 120.2 (d, C-11), 130.1 (s, C-7a), 133.7 (d, C-10), 137.2 (s, C-11a), 138.0 (s, C-12a), 138.7 (s, C-9), 144.8 (d, C-5), 157.7 (s, C-8), 172.6, 173.1, 173.3 (s, octanoyl-CO), 182.0 (s, C-12), 187.6 (s, C-7), 201.4 (s, C-1); negative FAB-MS *m/z* (abundance, %) 1,350 (26, M+H), 1,349 (48, M).

Anal Calcd for $C_{75}H_{112}O_{21}$: C 66.74, H 8.38.
Found: C 66.61, H 8.23.

4′,5′-Di-*O*-acetylurdamycinone A (**15**)

A solution containing 76 mg of **14** and 102 mg of DMAP in 12 ml acetic anhydride was stirred for 5 hours. The mixture was poured into ice-water, extracted 3 times with $CHCl_3$ and evaporated to dryness. The residue was chromatographed on silica gel column (30×3 cm, CH_2Cl_2 - MeOH, 9:1). The main fraction was rechromatographed on a silica gel column (30×2 cm, CH_2Cl_2 - MeOH, 9:1) and purified on Sephadex LH-20 (100×2.5 cm, MeOH) to yield 22 mg (24.7%) of **15**: MP 169°C; Rf 0.57 ($CHCl_3$ - MeOH, 9:1); IR (KBr) cm^{-1} 3410, 2970, 2930, 2850, 1745, 1655, 1640, 1620, 1585, 1565; ^1H NMR (200 MHz, $CDCl_3$) δ 1.31 (d, J=6.5 Hz, 6′-CH_3), 1.52 (ddd, J=12, 12 and 12 Hz, 3′-H_{ax}), 2.03, 2.09 (s, CH_3CO), 2.61 (ddd, J=12, 5 and 2 Hz, 3′-H_{eq}), 3.70 (dq, J=6.5 and 9 Hz, 6′-H), 4.84 (dd, J=9 and 9 Hz, 5′-H), 5.19 (ddd, J=12, 9 and 5 Hz, 4′-H), all other resonances are very similar to those found for **14**.

Anal Calcd for $C_{29}H_{30}O_{12}$: C 61.05, H 5.30.
Found: C 60.45, H 5.71.

Acetylation of **17**

20 mg of sodium acetate were added to 52.3 mg of **17** dissolved in 12 ml acetic anhydride. After stirring for 72 hours the mixture was poured into ice-water, extracted with $CHCl_3$ and evaporated to dryness. The residue was chromatographed on a silica gel column (30×3 cm, $CHCl_3$ - MeOH, 85:15). The two eluated fractions were purified on Sephadex LH-20 (100×2.5 cm, MeOH) to yield 11.7 mg (21.2%) of 3B-mono-*O*-acetylderhodinosylurdamycin A (**18**) and 27.7 mg (47.5%) of 3B,4B-di-*O*-acetylderhodinosylurdamycin A (**19**).

18: MP 154~159°C; Rf 0.41 ($CHCl_3$ - MeOH, 9:1); IR (KBr) cm^{-1} 3430, 2980, 2940, 2880, 1735, 1660, 1640, 1625, 1585, 1565; negative FAB-MS *m/z* (abundance, %) 774 (8, M+2H−H), 773

(17, M+2H−2H); ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.12 (s, CH_3CO), 3.2~3.4 (complex, 4B-H, 5B-H), 4.77 (m, 3B-H), all other resonances are very similar to those found for **17**.

19: MP 140~143°C; Rf 0.55 ($CHCl_3$ - MeOH, 9:1); IR (KBr) cm^{-1} 3430, 2940, 2880, 1750, 1660, 1640, 1620, 1565; negative FAB-MS *m/z* (abundance, %) 815 (29, M+2H−2H), 814 (10, M−H); ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.04, 2.06 (s, CH_3CO), 3.2~3.4 (complex, 5B-H), 4.76 (dd, J= 9 and 9 Hz, 4B-H), 4.99 (obscured, 3B-H), all other resonances are very similar to those found for **17**.

4A,4C,5',8-Tetra-*O*-acetylurdamycin G (**21**)

A mixture of 12 ml acetic anhydride - pyridine (2:1) and 20 mg **20** was stirred for 5.5 hours at room temperature. After hydrolysis on ice it was extracted with $CHCl_3$ and evaporated to dryness. Pyridine residues were removed by dissolving several times in toluene and evaporating. The residue was chromatographed on a silica gel column (30×2.5 cm, CH_2Cl_2 - EtOH, 95:5). The main fraction was purified on Sephadex LH-20 column (100×2.5 cm, MeOH) to yield 16 mg (65%) **21**: MP 161°C; Rf 0.60 ($CHCl_3$ - MeOH, 9:1); IR (KBr) cm^{-1} 3450, 2990, 2950, 1788, 1742, 1670, 1657 (sh), 1603, 1566; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.51 (d, J=7 Hz, 5C-CH_3), 1.16 (d, J=6 Hz, 5A-CH_3), 1.26 (s, 3-CH_3), 1.29 (d, J=6 Hz, 6'-CH_3), 1.2~2.2 (complex, 2A-H_2, 3A-H_2, 3'-H_{ax}, 4-H_2, 2C-H_2, 3C-H_2), 2.01, 2.11, 2.14 (s, 5'-, 4A-, 4C-$COCH_3$), 2.49 (s, $COCH_3$), 2.55 (dd, J=13 and 2 Hz, 2-H_{ax}), 2.61 (ddd, J=12, 9 and 2 Hz, 3'-H_{eq}), 2.83 (dd, J=13 and 2 Hz, 2-H_{eq}), 3.6~3.7 (complex, 6'-H, 5C-H), 3.96 (dq, J=9 and 6 Hz, 5A-H), 3.98 (m, 4'-H), 4.70 (br s, 4C-H), 4.84 (br s, 4A-H), 4.86 (dd, J=9 and 9 Hz, 5'-H), 4.90 (dd, J=12 and 2 Hz, 2'-H), 5.00 (br s, 1A-H), 5.49 (s, 1C-H), 6.42 (d, J=10 Hz, 5-H), 6.42 (d, J=10 Hz, 6-H), 8.06 (d, J=8 Hz, 11-H), 8.15 (d, J=8 Hz, 10-H); CD $\lambda_{extreme}^{MeOH}$ nm ($[\theta]^{20}$) 501 (−4,600), 450 (+1,400), 394 (−12,000), 308 (+20,300), 295 (sh, +16,500), 266 (−11,200), 233 (+25,600).

5,6-Dihydrourdamycin A (**22**)

25 mg of **1** dissolved in 2 ml MeOH were hydrogenated with H_2 at room temperature for 2 hours in the presence of 12 mg of platinum (IV)-oxide. The solution was filtered and evaporated to dryness. The residue was chromatographically purified on Sephadex LH-20 column (100×2.5 cm, MeOH) to yield 24.4 mg (97%) of **22** as a yellow powder: MP 215°C; Rf 0.38 ($CHCl_3$ - MeOH, 9:1); IR (KBr) cm^{-1} 3440, 1735 (sh), 1725, 1720 (sh), 1660, 1633, 1609; UV λ_{max} nm (ε) (MeOH and MeOH - HCl) 430 (1,700), 410 (sh, 1,600), 280 (200), 246 (3,110), 218 (13,600); (MeOH - NaOH) 541 (1,800), 323 (sh, 2,300), 283 (4,400), 229 (sh, 10,000); ^{1}H NMR (200 MHz, acetone-d_6) δ 2.25~2.44 (complex, 5-H_2), 3.00~3.15 (complex, 6-H_2), all other resonances are very similar to those found for **1**; CD $\lambda_{extreme}^{MeOH}$ nm ($[\theta]^{20}$) 500 (+1,200), 440 (+4,700), 355 (−1,900), 308 (sh, +3,500), 274 (+10,600), 245 (+2,100), 221 (+9,100).

Anal Calcd for $C_{43}H_{58}O_{17}$: C 60.92, H 6.85.
Found: C 60.87, H 6.80.

5,6-Dihydroderhodinosylurdamycin A (**23**)

26 mg of **17**, dissolved in 1.5 ml MeOH were hydrogenated with H_2 at room temperature for 2 hours in the presence of 8 mg of platinum(IV)-oxide. By using the same work up procedures as described immediately above, 17 mg (65%) of **23** were obtained: MP 170°C; Rf 0.47 ($CHCl_3$ - MeOH, 9:1); UV λ_{max} nm (ε) (MeOH and MeOH - HCl) 430 (4,300), 321 (sh, 2,300), 270 (sh, 7,500), 241 (sh, 10,700); (MeOH - NaOH) 548 (4,300), 326 (sh, 3,800), 281 (8,200), 226 (22,500); IR (KBr) cm^{-1} 3430, 1725, 1660 (sh), 1640, 1615; ^{1}H NMR (200 MHz, acetone-d_6) δ 2.25~2.55 (complex, 5-H_2), 3.04~3.15 (complex, 6-H_2), all other resonances are very similar to those found for **17**.

Anal Calcd for $C_{37}H_{48}O_{15}$: C 60.59, H 6.55.
Found: C 60.55, H 6.38.

5,6-Dihydrourdamycinone A (**16**)

56 mg of **1** were hydrogenated as described in the previous sector. After evaporation, the residue was treated with a mixture H_2O, MeOH and trifluoroacetic acid for 10 minutes. It was extracted 3 times with EtOAc and evaporated. The crude residue was chromatographed on a silica gel column (50×1.5 cm, CH_2Cl_2 - EtOH, 85:15). The main fraction was purified on Sephadex LH-20

(100×2.5 cm, MeOH) to yield 22 mg (69%) of **16**: MP 181°C; Rf 0.50 ($CHCl_3$ - MeOH, 9:1); IR (KBr) cm^{-1} 3430, 2935, 1733, 1661, 1640, 1615; UV λ_{max} nm (ε) (MeOH) 429 (4,800), 274 (7,820), 250 (8,650); (MeOH - NaOH) 545 (4,130), 287 (8,100); ^1H NMR (200 MHz, $CDCl_3$ - acetone-d_6, 1:1) δ 1.26 (s, 3-CH_3), 1.38 (d, J=6 Hz, 6′-CH_3), 1.8~2.3 (complex, 4-H_2, 5-H_2), 2.46 (ddd, J=12, 5 and 2 Hz, 3′-H_{eq}), 2.6~3.05 (complex, 2-H_2, 6-H_2), 3.15 (dd, J=9 and 9 Hz, 5′-H), 3.50 (dq, J=9 and 6 Hz, 6′-H), 3.80 (ddd, J=12, 9 and 5 Hz, 4′-H), 4.91 (dd, J=10 and 2 Hz, 2′-H), 7.58 (d, J=8 Hz, 11-H), 7.88 (d, J=8 Hz, 10-H).

Anal Calcd for $C_{25}H_{28}O_{10}$: C 61.47, H 5.74.
Found: C 61.36, H 5.87.

8-*O*-Methylurdamycin A (**13**)

To a solution of 55 mg **1** in 25 ml acetone were added 50 mg of potassium carbonate and 50 μl of methyl iodide. After stirring for 100 hours at 40°C the mixture was filtered and evaporated to dryness. The residue was chromatographed on a silica gel column (30×2.5 cm, $CHCl_3$ - MeOH, 85:15). Further purification was carried out by chromatography on Sephadex LH-20 (100×2.5 cm, MeOH) to yield 22 mg (40%) of **13**: MP 176°C; Rf 0.41 ($CHCl_3$ - MeOH, 9:1); IR (KBr) cm^{-1} 3440, 1730, 1665, 1635, 1585, 1575; UV λ_{max}^{MeOH} nm (ε) 377 (3,470), 308 (3,510), 250 (sh, 11,500); ^1H NMR (200 MHz, acetone-d_6) δ 3.90 (s, 8-OCH_3), 5.04 (dd, J=10 and 2 Hz, 2′-H), 7.92 (d, J=8 Hz, 11-H), 7.96 (d, J=8 Hz, 10-H), all other resonances are very similar to those found for **1**; CD $\lambda_{extreme}^{MeOH}$ nm ($[\theta]^{24}$) 500 (−4,100), 400 (−7,500), 318 (+21,900), 292 (sh, 10,900), 266 (−6,000), 233 (23,800).

Addition of Diazomethane to **1**

6 ml of a diazomethane solution (0.4 molar in diethyl ether) were added dropwise to a solution of 96 mg **1** in 15 ml THF at 0°C. After stirring for 25 minutes the solution was evaporated to dryness. The residue was chromatographed on silica gel (Merck lobar column B LiChroprep Si 60, $CHCl_3$ - MeOH, 85:15) yielding from the slower running zone a red product and from a faster zone a mixture of a yellow and a red product. The latter fraction was rechromatographed under the same condition. The combined fractions with the red product were purified on Sephadex LH-20 (100×2.5 cm, MeOH) to yield 70 mg (70%) **25** as a red powder: MP 190°C; Rf 0.22 ($CHCl_3$ - MeOH, 9:1); IR (KBr) cm^{-1} 3430, 1728, 1660, 1640 (sh), 1550; UV λ_{max} nm (ε) (MeOH) 451 (5,100), 278 (7,600); (MeOH - HCl) 443 (4,800), 316 (4,200), 247 (12,200); (MeOH - NaOH) 589 (4,000), 444 (2,800), 312 (6,400); negative FAB-MS *m/z* (abundance, %) 886 (0.9, M^+), 765 (0.6, M−rodinose); ^1H NMR (200 MHz, $CDCl_3$) δ 0.66 (d, J=6.5 Hz, 5C-CH_3), 1.22 (s, 3-CH_3), 1.27 (d, J=6 Hz, 5A-CH_3), 1.32 (d, J=6 Hz, 5′-CH_3), 1.43 (d, J=6 Hz, 5B-CH_3), 1.3~2.1 (complex, 4-H_2, 3′-H_{ax}, 2A-H_2, 3A-H_2, 2B-H_{ax}, 2C-H_2, 3C-H_2), 2.29 (ddd, J=12, 5 and 2 Hz, 2B-H_{eq}), 2.47 (ddd, J=12, 5 and 2 Hz, 3′-H_{eq}), 2.54 (d, J=13 Hz, 2-H_{ax}), 2.80 (dd, J=13 and 3 Hz, 2-H_{eq}), 2.8~3.2 (obscured, 4B-H), 3.20 (dq, J=9 and 6 Hz, 5B-H), 3.22 (dd, J=9 and 9 Hz, 5′-H), 3.30 (br s, 4C-H), 3.4~3.8 (complex, 5-H, CH_2 at C-5, 6′-H, 4A-H, 3B-H, 5C-H), 3.92 (ddd, J=12, 9 and 5 Hz, 4′-H), 4.18 (dq, J=6.5 and 2 Hz, 5A-H), 4.55 (dd, J=10 and 2 Hz, 1B-H), 4.92 (dd, J=12 and 2 Hz, 2′-H), 5.05 (br s, 1A-H), 5.38 (br s, 1C-H), 6.58 (d, J=2 Hz, NH), 7.72 (d, J=8 Hz, 11-H), 7.89 (d, J=8 Hz, 10-H); CD $\lambda_{extreme}^{MeOH}$ nm ($[\theta]^{24}$) 490 (8,200), 400 (sh, −2,600), 347 (−9,700), 286 (33,700).

Anal Calcd for $C_{44}H_{58}N_2O_{17}$: C 59.59, H 6.55, N 3.16.
Found: C 59.60, H 6.56, N 3.08.

Acknowledgment

We would like to thank Prof. H. ZÄHNER, Institut für Biologie der Universität Tübingen (FRG) for providing us with the strain *S. fradiae* (Tü 2717). We are grateful to Dr. H. P. KRAEMER and Dr. H. H. SEDLACEK (Behring-Werke A.G., Marburg, FRG) for the *in vitro* cytotoxic activity test. This work was supported by the Fond der Chemischen Industrie.

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