DERIVATIVES OF SAQUAYAMYCINS A AND B

REGIO- AND DIASTEREOSELECTIVE ADDITION OF ALCOHOLS TO THE L-ACULOSE MOIETY

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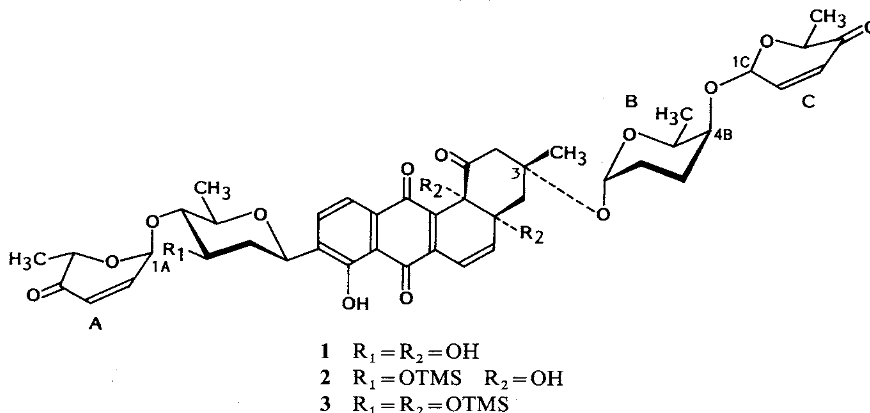
In continuation of our structure-activity investigations on angucycline antibiotics we prepared derivatives of saquayamycins A (1) and B (4) by regio- and diastereoselective nucleophilic addition of different alcohols to the L-aculose moiety. Reversible protection of the 4'-hydroxy group in 1 by silylation allowed a derivatization at both L-aculose moieties without cyclization towards cinerulose B. The *in vitro* cytotoxic activity remained almost unchanged after variation at the L-aculose moieties whereas a change in the aglycone structure led to a total loss of the biological activity.

The saquayamycins A (1) and B (4), produced by *Streptomyces nodusus*, were first described by UMEZAWA *et al.*[1] and recently also isolated from a yet unidentified strain (*Streptomyces* sp. S 6)[†]. **1** consists of the well known aglycone aquayamycin[2] and three *O*-glycosidically bonded deoxyhexoses, one α-L-rhodinose (Sugar B) and two α-L-aculoses (Sugars A and C). In the case of **4** the aculose at C-5' is converted into α-L-cinerulose B[3] which is connected twice, at C-5' and C-4' with the *C*-glycosidic part of the aglycone. The saquayamycins are members of the growing group of angucycline antibiotics which include, for example the urdamycins[4~7] and vineomycin A_1[8]. The recently published platelet aggregation inhibitor PI-083[9] is closely related to saquayamycin B (**4**), except that the 4A-carbonyl group has been reduced to a hydroxy group.

Saquayamycin A (**1**) reveals considerable instability to acids, a limitation in view of the preparation of derivatives. Even contact with silica gel during chromatography is sufficient for the conversion of **1** into **4**[1]. This reaction comprises the addition of the 4'-hydroxy group to the enone system of Sugar A, and gives rise to the assumption that **4** only represents an artifact caused by the work-up procedure.

Scheme 1.



1 $R_1=R_2=OH$
2 $R_1=OTMS$ $R_2=OH$
3 $R_1=R_2=OTMS$

† SEDLACEK, H., *et al.*: Screening of antitumor components using Streptomycetes from new soil isolates. personal communication.

The saquayamycins A and B exhibit a remarkable biological activity against L1210, A549 and HT29 tumor cells using the proliferation assay (MTT-reduction[10]; IC_{50}: 0.004/0.003, 0.2/0.2, 0.06/0.06 μg/ml), but do unfortunately not show an *in vivo* activity due to their considerable toxicity[†]. During our previous investigations of urdamycin A[11] we identified the aquayamycin aglycone to be a very important substructure element with respect to the cytotoxic activity. Regarding this, we intended to vary the L-aculose moiety of **1** and **4** in order to extend our knowledge about structure-activity relationships. Furthermore the L-aculoses are likely to be the best sites to increase the hydrophilic properties of the derivatives. Finally systematic derivatization should lead to a product with reduced toxicity with preservation of the cytotoxic activity. In this paper we describe the nucleophilic addition of different alcohols to **1** and **4**. A particular aim was the reversible protection of 4'-OH in **1** which could help to avoid cyclization to saquayamycin B-type derivatives.

Addition of Methanol

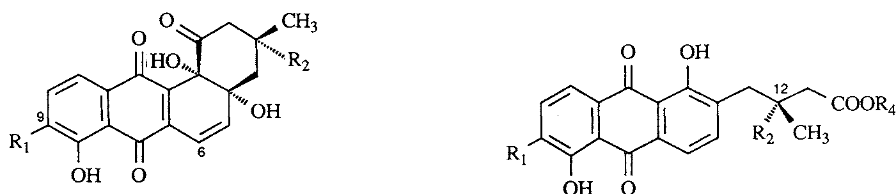
The nucleophile methanol reacts with **4** at $-20°C$ in the presence of diisopropyl amine (2%) as a catalyst. Two new yellow products were detected by TLC and chromatographically purified. The ^1H NMR spectrum of the less lipophilic compound showed a large similarity to **4**. Differences were evident in the downfield region ($\delta > 6$ ppm), *e.g.*, the 2C-H and 3C-H signals were missing, and in the upfield region, where a new AB system (δ 2.84 and 2.65) and a multiplet (δ 3.78) as well as a methoxy singlet at δ 3.41 appeared (Table 1). In view of the unquestionable up-field shift of 5C-H and 1C-H, the alteration of the molecule should be located at Sugar C and was in accordance with structure **5**. The molecular ion of the FAB-MS showed the molecular formula to be $C_{44}H_{52}O_{17}$, which confirmed the addition of one molecule of methanol to **4**. The reaction was obviously under regioselective control, despite the presence of a second vinylogous system, the C-5/C-6 double bond of the aglycone. Considering the ^1H and ^{13}C NMR spectra, the L-aculose was converted into a new α-L-cinerulose A derivative diastereoselectively.

The rules for the nucleophilic addition of lithium carbanions to L-aculose, worked out by PAULSEN and KOEBERNIK[12], give an excellent clue to determine the absolute configuration at C-2C in the newly formed sugars. We found only the *trans* (*threo*) product, which was deduced from NMR by observing the coupling constants between 1C-H and 2C-H and by comparison of these data with those described by PAULSEN *et al.* This stereospecifity is probably due to a strong steric and electrostatic interaction between the attacking nucleophile and the axial oxygen at C-1C. This kind of selectivity could be observed in all further derivatives as described below.

The ^1H NMR spectrum of the second, more lipophilic product pointed to a principle change of the aglycone structure in comparison with **4**. Signals of two chelated phenolic hydroxy groups at δ 13.11 and 13.16 as well as two aromatic AB systems at δ 7.92/7.86 and 7.78/7.69 were detected. Furthermore, two aliphatic methylene groups were observed at δ 3.27/3.14 and δ 2.78/2.60 as well as a methoxy singlet (δ 3.70, Table 1), leading to structure **11**, which is closely related to the aglycone of vineomycin B_2 (**12**)[13] and its methyl ester, respectively. The sugar reasonances of **11** remained almost constant in comparison with those of **5**. The FAB-MS unequivocally offered evidence for the expected molecular formula $C_{45}H_{54}O_{17}$. The transformation of the aquayamycin aglycone into the vineomycin B_2 aglycone (**13**), which has the same anthraquinone system as assumed for **11**, has already been described by UMEZAWA *et*

† See footnote on p. 830.

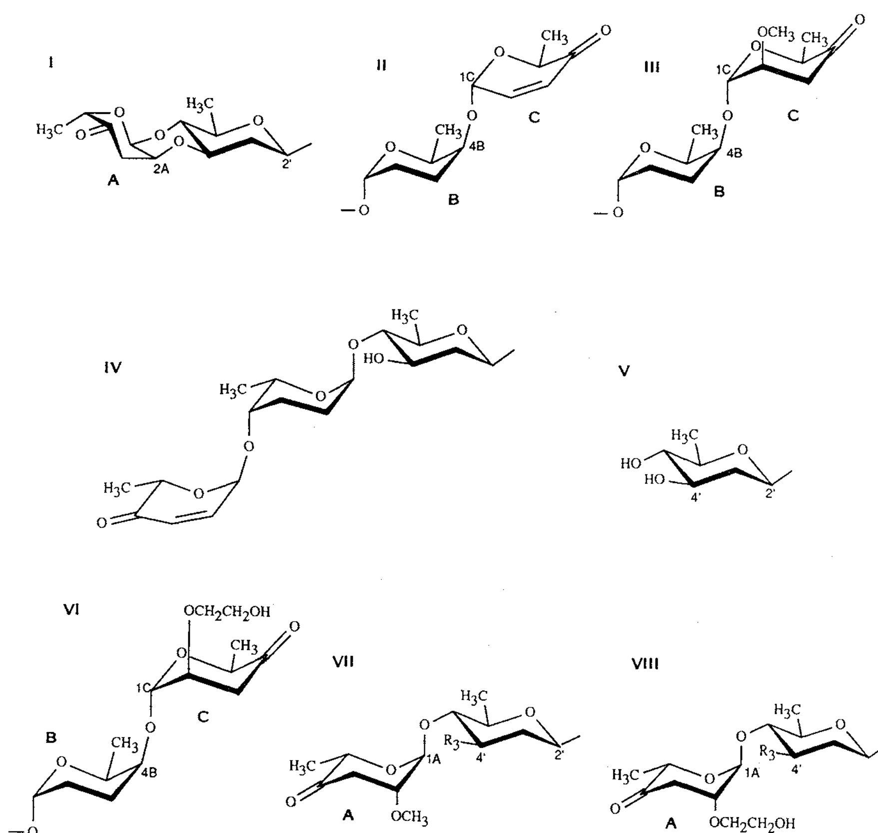
Scheme 2.



4	R_1=I		R_2=II
5	R_1=I		R_2=III
6	R_1=I		R_2=VI
7	R_1=VII	(R_3=-OTMS)	R_2=III
8	R_1=VII	(R_3=-OH)	R_2=III
9	R_1=VIII	(R_3=-OTMS)	R_2=VI.
10	R_1=VIII	(R_3=-OH)	R_2=VI

11	R_1=I		R_2=III	R_4=-CH_3
12	R_1=IV		R_2=II	R_4=-H
13	R_1=V		R_2=-OH	R_4=-H
14	R_1=VII	(R_3=-OTMS)	R_2=III	R_4=-CH_3

Sugars:



al.[3], using hydrochloric acid as a catalyst. But in contrast to an acidic catalysed ring opening reaction, the nucleophilic addition of methanol to the C-1 carbonyl group maintained all *O*-glycosydic bonds. Thus our reaction represents a new derivatization method for all angucycline antibiotics possessing the aquayamycin aglycone.

Addition of 1,2-Ethanediol

The reaction with 1,2-ethanediol as nucleophile should expand the knowledge about the addition of

Table 1. Selected ^{1}H NMR signals of different derivatives accessible by nucleophilic addition of alcohols at 200 MHz in $CDCl_3$ (δ in ppm relative to internal TMS).

Proton	1	4	5	11	6	14	8	10	Coupling (*J* in Hz)
2′	4.88	4.96	4.97	5.01	4.96	4.91	4.87	4.85	dd (2; 10)
3′-eq	2.56	2.45	2.45	2.49	2.45	2.58	2.53	2.51	ddd (2; 5; 12)
4′	3.91	3.81	3.82	3.83	3.81	3.89	3.86	*	ddd (2; 8; 12)
5′	3.21	3.48	3.49	3.50	*	3.22	3.20	3.20	dd (9; 9)
6′	3.58	3.55	3.57	3.57	*	3.56	3.57	*	dd (6; 9)
7′	1.39	1.40	1.40	1.41	1.40	1.40	1.38	*	d (6)
1A	5.37	5.17	5.19	5.19	5.19	5.03	5.01	5.05	d (3)
2A	6.86[c]	4.34	4.34	4.34	4.34	*	*	*	ddd (1.5; 3; 3)
3A-ax	6.15[b]	2.65[a]	2.66[a]	2.64[a]	2.66[a]	2.60	2.66	2.67	dd (6; 16)
3A-eq	—	—	—	—	—	2.83	2.83	2.86	dd (4; 16)
5A	4.76	4.71	4.71	4.74	4.72	4.38	4.37	4.38	q (7)
6A	1.45	1.38	1.38	1.38	1.38	1.34	1.32	1.35	d (7)
1C	5.26	5.26	4.95	4.91	4.99	4.88	4.93	4.96	d (3)
2C	6.90[c]	6.88[c]	3.78	3.78	3.96	*	*	*	ddd (3; 4; 6)
3C-ax	6.10[b]	6.09[b]	2.65	2.64	2.65	2.60	2.63	2.63	dd (6; 16)
3C-eq	—	—	2.84	2.83	2.87	2.81	2.81	2.84	dd (4; 16)
5C	4.54	4.55	4.20	4.21	4.20	4.19	4.17	4.17	q (7)
6C	1.37	1.37	1.31	1.29	1.32	1.25	1.28	1.30	d (7)
$O-CH_3$	—	—	3.41	3.38	—	3.41	3.41	—	s 3H
$O-CH_3$	—	—	—	—	—	3.36	3.39	—	s 3H
$O-(CH_2)_2$	—	—	—	—	3.6~3.8	—	—	3.5~3.8	m 4H
$O-(CH_2)_2$	—	—	—	—	—	—	—	3.8~4.0	m 4H

[a] br s (2H; 3A-H) [b] d (J=11 Hz; 3A-H) [c] dd (J=3 and 11 Hz).
* Obscured.

alcohols to the aculose and, with respect to the biological activity, reduce the lipophilicity of **4**. The use of 1,2-ethanediol - methanol (1 : 1) as reagent and solvent, respectively, and diisopropyl amine as a catalyst led to the expected product **6**, besides **4** and **5**. The ^{1}H NMR spectra of **5** and **6** were almost identical. In comparison, only the small downfield shift of 2C-H and the new complex multiplet between δ 3.6 and 3.76, deriving from the ethanediol protons represent remarkable differences and confirmed the proposed structure. The FAB-MS in conjunction with the elemental analysis suggested the molecular formula of **6** to be $C_{45}H_{54}O_{18}$.

In conclusion the described experiments give support to the assumption that the nucleophilic addition of alcohols to **4** as well as to all other natural products containing an aculose sugar moiety is applicable to a large number of alcohols. As a consequence, the polarity of the whole molecule can be varied by different functional groups at the L-aculose moiety.

Nucleophilic Addition after Silylation of 1

Direct addition of methanol to saquayamycin A (**1**) remained unsuccessful, because the alcohol competes poorly with the 4′-OH leading predominantly to **5**. The unwanted intramolecular addition could be prevented by silylation of the 4′-OH before addition of the nucleophile. Silylation of **1** was carried out with trimethylsilyl chloride (TMSCl) and ethyldiisopropyl amine as a catalyst. It led to the desired 4′-*O*-trimethylsilylsaquayamycin A (**2**, 90%, 8 hours) besides further silylated 4′,4a,12b-tri-*O*-trimethylsilyl-saquayamycin A (**3**) which was obtained as the main product after 15 hours. The structure of **2** was established from the ^{1}H NMR spectrum, showing the singlet of the trimethylsilyl group at δ 0.08 and the resonance of the 5′-H significantly shifted downfield at δ 3.44. **2** reacted with the nucleophiles described

Table 2. Anticancer assay against L1210, HT29 and A549 leukemia cells (IC_{50} values in μg/ml)[†,10,14].

Compound	L1210	MTT-assay HT29	A549	Stem cell assay against L1210 (1 hour expt)
1	0.004	0.2	0.06	0.042
4	0.003	0.2	0.06	0.024
5	0.007	0.3	0.15	0.022
6	0.03	0.7	0.6	0.04
8	0.009	0.02	0.1	0.02
10	0.029	0.1	0.47	0.088
11	>1	>1	>1	Not tested

above. Treatment with methanol resulted in three products whose ^{1}H NMR spectra exhibited two methoxy singlets at δ 3.40 and 3.35 as well as all expected sugar resonances, indicating the addition of methanol to both L-aculoses. The first product was identified as **14** (17%) and represents an analogue of **11**. The two other products were the desired derivatives of saquayamycin A (**1**), **7** (24%) and **8** (27%), the latter has already been desilylated at 4'-OH. Using a mixture of 1,2-ethanediol - ethanol - diisopropyl amine (4:3:0.1), **2** could be transformed into the new product **9** (29%), which still contained the trimethylsilyl group, but also into **6** (25%). The last step of this derivatization was planned to be the liberation of 4'-OH in **9**. Although different chemical techniques were tested, the easiest method of cleavage was found to be the hydrolysis at atmospheric humidity for one week, giving **10** in almost 100% yield. The ^{1}H NMR spectrum of **10** and the FAB-MS showed the addition of ethanediol to both L-aculoses.

Biological Activity and Discussion

All important derivatives described above were tested against L1210, A549 and HT29 tumor cells with the proliferation assay (MTT-reduction)[10] and against L1210 tumor cells using the stem cell assay (1 hour experiment, Table 2). As expected, the change of the aglycone structure in **11** resulted in a loss of activity and offered further evidence for the necessity of an unmodified aquayamycin aglycone to provide a resonable cytotoxic activity. With regard to the biological activity of the other derivatives, variation of the L-aculoses did not have a plainly recognizable effect compared to the educts except for the selective 10-fold increase in the activity of **8** against HT29 tumor cells. The first *in vivo* tests with **8** and **10** resulted in a disappearance of toxicity as well as biological activity compared to **1** and **4** presumably because penetration through the cell walls was inhibited. Further investigations are currently under way.

Experimental

General

See ref 12. If UV data are not represented, the absorption bands are as described in ref 1.

Assay Methods for Antitumor Activities[†,14]

Proliferation Assay (MTT-Reduction): Exponentially growing L1210, A549 or HT29 tumor cells at a density of 5×10^3/ml in Roswell Park Memorial Institute (RPMI) medium was incubated in a 96 well microtiter plate for 72 hours (37°C, 5% CO_2, 95% relative humidity) with various concentrations of each test substance. Control consisted of cells exposed to fresh medium only. Quadruplicate wells were prepared for each drug concentration and for control. After 65 hours 50 μl 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT, 2.5 mg/ml phosphate buffered sodium chloride solution, pH 7.2) were added. The MTT will be reduced by viable cells to a red insoluble formazan dye. After additional 7 to 24 hours

† See footnote on p. 830.

incubation (depending on the cell used) the supernatant medium was carefully removed. The formazan dye was solubilized by adding 100 μl DMSO to each well followed by gentle shaking. The extinction is measured for each well using a Multiscan photometer 340 CC, Fa. Flow, at 492 nm.

Results were expressed as the ratio of the extinction after incubation with test substances over that of control. The coefficient of variation for replicate experiments was less than 15%.

Effect on Stem Cells of L1210 (1 hour Experiment): The assay was performed according to the procedure of HAMBURGER and SALMON with some modifications described below. Conditioned medium was replaced by McCoy 5A. Number of cells plated was reduced to 5×10^2 cells/plate due to the high plating efficiency of the tumor cell lines. Cells were incubated with various concentrations of the test substance for 1 hour at 37°C. Thereafter the cells were washed twice with McCoy 5A and finally plated in an agar upper layer according to the method of HAMBURGER and SALMON. Plates were stored in an incubator with 5% CO_2, 20% O_2 and 95% relative humidity for 5~7 days at 37°C. After this time colonies with a diameter >60 μm were counted using an inverted microscope. Results were expressed as percentage of the number of colonies formed from treated cells over an untreated control. The coefficient of variation of repeated experiments was less than 15%.

Addition of Methanol to **4**

A solution of 50 mg **4** in 50 ml MeOH was treated with 1 ml diisopropyl amine at −20°C and stirred for 20 hours. After neutralization with acetic acid and evaporation to a volume of 5 ml, 100 ml water were added and the mixture was extracted 3 times with $CHCl_3$. The residue of the concentrated extract was chromatographed on a silica gel column (30×2.5 cm, $CHCl_3$-MeOH, 95:5). The two main fractions were further purified on a Sephadex LH-20 column (100×2.5 cm, MeOH) to yield 24 mg (46.2%) of **5** and 18.2 mg (34.5%) of **11**.

5: MP 162~164°C; Rf 0.78 ($CHCl_3$-MeOH, 95:5); IR (KBr) cm^{-1} 3440, 2970, 2930, 2880, 1725, 1655, 1637, 1620, 1560; negative FAB-MS *m/z* (abundance, %) 853 (18%, M+2H−H), 852 (30%); UV $\lambda_{max}^{MeOH\ and\ MeOH-HCl}$ nm (ε) 202 (12,500), 218 (15,900), 314 (2,850), 424 (3,450); $\lambda_{max}^{MeOH-NaOH}$ nm (ε) 229 (14,500), 275 (12,400), 386 (2,950), 560 (3,900); ^{1}H NMR (200 MHz, $CDCl_3$) see Table 1, all other resonances are very similar to those found for **4**; ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 14.9 q (C-6C); 40.0 t (C-3C); 57.1 q ($O-CH_3$); 71.1 d (C-5C); 76.9 d (C-2C); 100.5 d (C-1C); 207.7 s (C-4C), all other resonances are very similar to those found for **4**.

Anal Calcd for $C_{44}H_{52}O_{17}$: C 61.96, H 6.15.
Found: C 61.36, H 6.07.

11: MP 111~112°C; Rf 0.86 ($CHCl_3$-MeOH, 95:5); IR (KBr) cm^{-1} 3450, 2980, 2930, 2880, 1730, 1625, 1580; negative FAB-MS *m/z* (abundance, %) 867 (25%, M+2H−H); 866 (50%); positive FAB-MS *m/z* 866 (0.8%, M^+); UV λ_{max}^{MeOH} nm (ε) 202 (23,950), 230 (46,200), 258 (27,300), 294 (9,300), 426 (12,600), 440 (12,500); $\lambda_{max}^{MeOH-NaOH}$ nm (ε) 273 (32,100), 291 (8,900), 392 (4,500), 478 (5,800), 519 (7,900); ^{1}H NMR (200 MHz, $CDCl_3$, see Table 1) δ 1.14 (3H, d, J=6 Hz, 5B-CH_3), 1.43 (3H, s, 12-CH_3), 1.23~1.51 (2H, m, 2B/3'-H_{ax}) 1.82~2.02 (2H, m, 3B-H_2), 2.04 (m, 2B-H_{eq}), 2.60 (1H, d, J=14 Hz, 11/13-H), 2.78 (1H, d, J=14 Hz, 11/13-H), 3.14 (1H, d, J=14 Hz, 11/13-H), 3.27 (1H, d, J=14 Hz, 11/13-H), 3.63 (br s, 4B-H), 3.70 (s, 14-OCH_3), 4.03 (dq, J=2 and 7 Hz, 5B-H), 5.20 (br s, 1B-H), 7.69 (d, J=8 Hz, 8-H), 7.78 (d, J=8 Hz, 7-H), 7.86 (d, J=8 Hz, 4-H) 7.92 (d, J=8 Hz, 3-H), 13.11 (s, OH), 13.16 (s, OH).

Addition of 1,2-Ethanediol to **4**

A solution of 14 mg **4** in 1 ml 1,2-ethanediol, 1 ml MeOH and 0.05 ml diisopropyl amine was stirred for 2 hours at −20°C. After neutralization with acetic acid and concentration, the oily residue was chromatographed on a silica gel column (30×3 cm; $CHCl_3$-MeOH, 9:1). The orange colored fraction was rechromatographed on a silica gel column (30×2.5 cm, $CHCl_3$-MeOH, 9:1). The main product was purified on a Sephadex LH-20 column (100×2.5 cm, MeOH) to yield 7 mg (46.5%) **6**.

6: MP 143~144°C; Rf 0.43 ($CHCl_3$-MeOH, 95:5); IR (KBr) cm^{-1} 3450, 2980, 2940, 2880, 1730, 1660, 1640, 1620, 1560; negative FAB-MS *m/z* (abundance, %) 883 (6%, M+2H−H); 882 (2%); UV $\lambda_{max}^{MeOH\ and\ MeOH-HCl}$ nm (ε) 205 (38,000), 217 (38,000), 319 (12,300), 425 (5,550); $\lambda_{max}^{MeOH-NaOH}$ nm (ε) 212 (48,600), 274 (20,900), 538 (5,400); ^{1}H NMR (200 MHz, $CDCl_3$) see Table 1, all other resonances are very similar to those found for **4**; ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 14.9 q (C-6C); 40.5 t (C-3C); 61.9 t; 70.8 t

($2\times OCH_2$); 71.1 d (C-5C); 76.7 d (C-2C); 100.3 d (C-1C), all other resonances are very similar to those found for **4**.

Anal Calcd for $C_{45}H_{54}O_{18}$: C 61.12, H 6.16.
Found: C 61.12, H 6.21.

4'-*O*-Trimethylsilylsaquayamycin A (**2**)

A solution of 30.2 mg dried saquayamycin A (**1**) in 2 ml TMSCl was treated with 0.1 ml ethyldiisopropyl amine for 8 hours at room temperature. Immediately after concentration the residue was dissolved in 2 ml of a mixture of $CHCl_3$ - ethyldiisopropyl amine (9 : 1) and chromatographed on silica gel (column 40 × 2.5 cm, $CHCl_3$ - MeOH, 9 : 1) and Sephadex LH-20 (100 × 2.5 cm, MeOH) to yield 29.7 mg (90.4%) **2** besides small amounts of **1** and 4',4a,12b-tri-*O*-trimethylsilylsaquayamycin A (**3**).

2: ^1H NMR (200 MHz, $CHCl_3$) δ 0.10 (9H, s, TMS), 2.40 (ddd; J=2, 5 and 13 Hz, 3'-H_{eq}), 3.46 (dd, J=9 and 9 Hz, 5'-H), 3.52 (dq, J=6 and 9 Hz, 6'-H), 3.90 (ddd, J=5, 9 and 11 Hz, 4'-H), 4.84 (dd, J=2 and 10 Hz, 2'-H), 4.86 (q, J=6 Hz, 5A-H), 5.43 (d, J=4 Hz, 1A-H), 6.08 (d, J=11 Hz, 3A-H), 6.87 (dd, J=4 and 11 Hz, 2A-H), all other resonances are very similar to those found for **1**.

4',4a,12b-Tri-*O*-trimethylsilylsaquayamycin A (**3**)

An analogous experiment as described above for **2** gave 24 mg (64.4%) **3** by extending the reaction time from 8 to 15 hours. The purification was achieved by chromatography on silica gel (column 30 × 2.5 cm, ethyl acetate - petroleum ether, 1 : 1) and precipitating **3** by adding a concentrated $CHCl_3$ solution to *n*-hexane.

3: ^1H NMR (200 MHz, $CDCl_3$) δ −0.1 (6H, s, TMS), 0.06 (3H, s, TMS), 0.10 (9H, s, TMS), 0.14 (9H, s, TMS), 1.1～1.6 (m, 4-H_{ax}), 1.1～1.6 (m, 3'-H_{ax}), 2.54 (dd, J=2 and 14 Hz, 4-H_{eq}), 2.39 (m, 3'-H_{eq}), 2.37 (d, J=13 Hz, 2-H_{ax}), 3.00 (dd, J=2 and 13 Hz, 2-H_{eq}), 3.46 (dd, J=9 and 9 Hz, 5'-H), 3.51 (m, 6'-H), 3.90 (m, 4'-H), 4.85 (dd, J=2 and 10 Hz, 2'-H), 4.87 (q, J=7 Hz, 5A-H), 5.43 (d, J=3 Hz, 1A-H), 6.07 (d, J=11 Hz, 3A-H), 6.35 (d, J=10 Hz, 5-H), 6.84 (d, J=10 Hz, 6-H), 6.87 (dd, J=4 and 11 Hz, 2A-H), all other resonances are very similar to those found for **1**.

Addition of Methanol to **2**

A solution of 22 mg **2** in 20 ml MeOH was treated with 0.2 ml diisopropyl amine at −20°C. After 21 hours one neutralized with acetic acid and added water. Extraction of the products with $CHCl_3$ and chromatography on silica gel (column 40 × 2.5 cm, $CHCl_3$ - MeOH, 93 : 7) gave 4 mg (16.7%) **14**, 5.7 mg (24%) **7** and 5.9 mg (27%) **8**.

7: ^1H NMR (200 MHz, $CDCl_3$) δ 0.06 (9H, s, TMS); 1.25 (m, 6A-, 6B-CH_3), 1.99 (m, 2B-H_{eq}), 2.40 (ddd, J=2, 5 and 12 Hz, 3'-H_{eq}), 2.47 (d, J=13 Hz, 2-H_{ax}), 2.62 (dd, J=6 and 16 Hz, 3A-, 3C-H_{ax}), 2.77 (dd, J=4 and 16 Hz, 3C-H_{eq}), 3.48 (dd, J=9 and 9 Hz, 5'-H), 3.53 (m, 6'-H), 3.68 (ddd, J=3, 4 and 6 Hz, 2C-H), 3.70 (br s, 4B-H), 3.76 (ddd, J=3, 4 and 6 Hz, 2A-H), 4.60 (q, J=7 Hz, 5A-H), 5.13 (d, J=3 Hz, 1A-H), 5.24 (br s, 1C-H), all other resonances are very similar to those found for **8**.

8: MP 70～73°C; IR (KBr) cm^{-1} 3450, 2930 (sh), 2860, 1730, 1660, 1640, 1620, 1565; negative FAB-MS *m/z* (abundance, %) 886 (3%), 885 (5%; M+2H−H); UV $\lambda_{max}^{MeOH\ and\ MeOH\text{-}HCl}$ nm (ε) 203 (18,050), 218 (20,100), 317 (3,400), 425 (3,700); $\lambda_{max}^{MeOH\text{-}NaOH}$ nm (ε) 211 (26,800), 230 (24,050), 278 (12,200), 536 (6,000); ^1H NMR (200 MHz, $CDCl_3$) see Table 1, all other resonances are very similar to those found for **1**.

14: ^1H NMR (200 MHz, $CDCl_3$) see Table 1, δ 0.02 (9H, s, TMS), all other resonances are very similar to those found for **11**.

Addition of 1,2-Ethanediol to **2**

Diisopropyl amine (0.1 ml) was added to a solution of 37.5 mg **2** in a mixture of 4 ml 1,2-ethanediol and 3 ml EtOH at −20°C. After 4.5 hours one neutralized with acetic acid, added water and extracted 5 times with $CHCl_3$. The extracted products were chromatographed on silica gel (column 40 × 2.5 cm, $CHCl_3$ - MeOH, 9 : 1). Both main fractions were purified on Sephadex LH-20 (100 × 2.5 cm, MeOH) to yield 12.2 mg (28.6%) **9** and 9.3 mg (25.1%) of **6**.

9: ^1H NMR (200 MHz, $CDCl_3$, δ 0.07 (9H, s, TMS), 1.22 (d, J=7 Hz, 6C-CH_3), 1.24 (d, J=7 Hz,

6A-CH_3), 1.24 (d, J=6 Hz, 6B-CH_3), 1.36 (s, 3-CH_3), 1.40 (d, J=6 Hz, 7'-CH_3), 1.78 (d, J=15 Hz, 4-H_{ax}), 1.97 (m, 2B-H_{eq}), 2.40 (ddd, J=2, 5 and 12 Hz, 3'-H_{ax}), 2.62 (dd, J=7 and 16 Hz, 3A-, 3C-H_{ax}), 2.80 (dd, J=4 and 16 Hz, 3A-H_{eq}), 4.60 (q, J=7 Hz, 5A-H), 5.15 (d, J=3 Hz, 1A-H), all other resonances are very similar to those found for **10**.

Hydrolysis of the TMS-group of **9**

Compound **9** (12.2 mg) were subjected to atmospheric humidity at room temperature for 7 days and subsequently chromatographed on Sephadex LH-20 (100 × 2.5 cm, MeOH) to yield 10.9 mg (96%) **10**.

10: MP 95 ~ 100°C; Rf 0.38 ($CHCl_3$ - MeOH, 9 : 1); IR (KBr) cm^{-1} 3450, 2960, 2930, 2860, 1730, 1660, 1640, 1625, 1565; negative FAB-MS m/z (abundance, %) 946 (2.5%); 945 (1%; M+2H−H); UV $\lambda_{max}^{MeOH\ and\ MeOH-HCl}$ nm (ε) 203 (16,900), 218 (19,100), 316 (3,250), 426 (3,800); $\lambda_{max}^{MeOH-NaOH}$ nm (ε) 211 (31,000), 228 (23,250), 524 (7,850); ^{1}H NMR (200 MHz, $CDCl_3$) see Table 1, all other resonances are very similar to those found for **1**.

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