SECONDARY METABOLITES BY CHEMICAL SCREENING. 7†

I. ELAIOPHYLIN DERIVATIVES AND THEIR BIOLOGICAL ACTIVITIES

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The synthesis and the biological activity of 34 acyl derivatives of elaiophylin (**1**) and 6 deglycosidation products were described. Especially the unsymmetric deglycosidation products **33**, **38** and **40** and dimethyloctahydroelaiophylin (**21**) exhibited an activity against nematodes.

Elaiophylin (**1**) was originally isolated from cultures of *Streptomyces melanosporus* and *Streptomyces violaceoniger*[2~5] and it was shown that **1** and azalomycin B were identical[3,5]. Elaiophylin (**1**) is now available in large quantities by fermentation of other *Streptomyces* sp., *e.g.* DSM 3816 and DSM 4137[6]. The macrodiolide antibiotic **1** has a 16-membered unsaturated lactone ring like leucanicidin[7], hygrolidin[8], L-155,175[9], L-681,110[10], the bafilomycins[11] and avermectins[12] and belongs to the small family of macrodiolides with C_2-symmetry like pyrenophorin, vermiculin and conglobatin[13]. The structure of **1** was established by X-ray crystallography[14], chemical degradation[15] and total synthesis[16,17]. Although this compound has stimulated widespread interest due to its C_2-symmetry and biological activity[18,19], little is known about the chemistry of this molecule[15,20~23] and only a few derivatives were reported up to now[1,24,25]. We would now like to report the biological activity of 40 elaiophylin derivatives and their syntheses.

Acylation and Acetalization Reactions

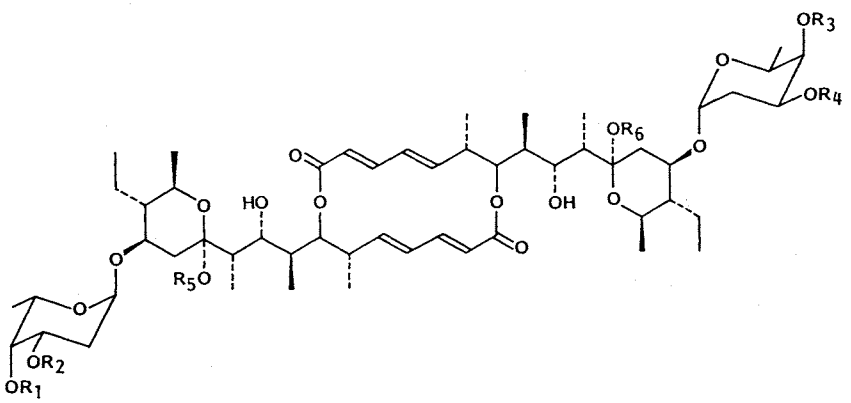
Elaiophylin (**1**), an acid and base sensitive compound, required precautions when reactions were carried out with this macrodiolide. The decomposition can be explained by retro aldol cleavage at C-9/C-10 and elimination of the L-deoxyfucose side chain. This reaction could be avoided by acetalization of **1** by refluxing with methanol of a technical grade[20]. Attempts to reproduce this reaction with crystalline **1** in pure methanol failed, so it could be assumed that impurities in the alcohol catalyzed the reaction. A much more convenient method was the Lewis acid catalyzed acetalization in methanol. Anhydrous $CuCl_2$ or $FeCl_3$ were the best catalysts to perform this reaction within 30 minutes or 1 minute, respectively. Reaction of **1** or **2** in pyridine with the correspondent anhydride led to different acyl derivatives (Table 1) in good yields.

Only the hydroxy groups in the L-deoxyfucose moiety were acylated under these conditions, whereas at 9-OH no reaction took place. This could be explained by steric hindrance of this hydroxy group, because of the hydrogen bond of 11-OH to 9-O and 9-OH hydrogen to the oxygen at C-1'[26].

The acylation could be carried out selectively as it was demonstrated by the bromobenzoylation of **1** to **9**, **10**, **11** and **12**. With only a 1.1-fold excess of *p*-bromobenzoyl chloride compound **9** was prepared, where the equatorial 3''-position of L-deoxyfucose reacted. If a 2.2-fold excess of the reagent was used, the second equatorial position 3''' reacted to **10**. A higher excess of *p*-bromobenzoyl chloride led to a

† See ref 1.

Table 1. Acyl derivatives of elaiophylin.



Compound No.	R_1	R_2	R_3	R_4	R_5	R_6	Synthesis from	Reagent	Time (hours)	Temperature (°C)	Procedure	Yield (%)
1	H	H	H	H	H	H	—	—	—	—	—	—
2	H	H	H	H	CH_3	CH_3	**1**	$FeCl_3$-CH_3OH	0, 1	25	1	100
3	CH_3CO	CH_3CO	CH_3CO	CH_3CO	H	H	**1**	$(CH_3CO)_2O$	24	25	2	91
4	CH_3CO	CH_3CO	H	CH_3CO	H	H	**1**	$(CH_3CO)_2O$	10	25	2	28
5	CH_3CO	CH_3CO	CH_3CO	CH_3CO	CH_3	CH_3	**2**	$(CH_3CO)_2O$	24	25	2	98
6	$CH_3(CH_2)_3CO$	$CH_3(CH_2)_3CO$	$CH_3(CH_2)_3CO$	$CH_3(CH_2)_3CO$	H	H	**1**	$(CH_3(CH_2)_3CO)_2O$	24	25	2	73
7[a]	C=O		C=O		H	H	**1**	$(C_3H_2N_2CO)_2O$	10	25	2	37
8[a]	C=O		$C_3H_2N_2CO$		H	H	**1**	$(C_3H_2N_2CO)_2O$	10	25	2	35
9[b]	H	*p*-BrC_6H_4CO	H	H	H	H	**1**	*p*-BrC_6H_4COCl	2	25	2	32
10[c]	H	*p*-BrC_6H_4CO	H	*p*-BrC_6H_4CO	H	H	**1**	*p*-BrC_6H_4COCl	6	25	2	54
11[d]	H	*p*-BrC_6H_4CO	*p*-BrC_6H_4CO	*p*-BrC_6H_4CO	H	H	**1**	*p*-BrC_6H_4COCl	12	25	2	8
12	*p*-BrC_6H_4CO	*p*-BrC_6H_4CO	*p*-BrC_6H_4CO	*p*-BrC_6H_4CO	H	H	**1**	*p*-BrC_6H_4COCl	24	25	2	64
13	C_6H_5CO	C_6H_5CO	C_6H_5CO	C_6H_5CO	H	H	**1**	$(C_6H_5CO)_2O$	24	25	2	86
14[e]	$(CH_3)_2$Si-*tert*-Bu	$(CH_3)_2$Si-*tert*-Bu	$(CH_3)_2$Si-*tert*-Bu	$(CH_3)_2$Si-*tert*-Bu	H	H	**1**	$(CH_3)_2$Si-*tert*-Bu-CF_3SO_3	1	25	3	41
15	C_6H_5NHCO	C_6H_5NHCO	C_6H_5NHCO	C_6H_5NHCO	H	H	**1**	C_6H_5NCO	5	25	2	88
16[f]	C_4H_3SCO	C_4H_3SCO	C_4H_3SCO	C_4H_3SCO	H	H	**1**	$(C_4H_3SCO)_2O$	24	25	2	87
17	$CH_3(CH_2)_{10}CO$	$CH_3(CH_2)_{10}CO$	$CH_3(CH_2)_{10}CO$	$CH_3(CH_2)_{10}CO$	H	H	**1**	$(CH_3(CH_2)_{10}CO)_2O$	24	25	2	67
18[g]	C_4H_3OCO	C_4H_3OCO	C_4H_3OCO	C_4H_3OCO	H	H	**1**	$(C_4H_3OCO)_2O$	24	25	2	85
19	$HOOCCH_2CH_2CO$	$HOOCCH_2CH_2CO$	$HOOCCH_2CH_2CO$	$HOOCCH_2CH_2CO$	H	H	**1**	Succinic acid anhydride	24	25	2	56
20	H	CH_3SO_2	H	H	H	H	**1**	CH_3SO_2Cl	1	25	2	34

[a] $C_3H_2N_2$: Imidazole. [b] 1.1-fold excess of the reagent. [c] 2.2-fold excess of the reagent. [d] 3.5-fold excess of the reagent. [e] *tert*-Bu: $C(CH_3)_3$. [f] C_4H_3SCO: thiophene. [g] C_4H_3OCO: furan.

reaction of the less reactive hydroxy groups at position 4″ and 4‴ to **11** and **12**.

Reaction of **1** with carbonyldiimidazole in pyridine yielded the symmetric carbonate (**7**) and unsymmetric carbonate/urethane (**8**). The silylation was carried out with *tert*-butyldimethylsilyl triflate in lutidine/dichloromethane to **14**.

Reaction of **1** with succinic acid anhydride yielded the tetrahemisuccinate (**19**), which was soluble in water.

The hydrogenation of **1** with Pd on charcoal/hydrogen[15] at room temperature in methanol directly gave the dimethyloctahydroelaiophylin (**21**). Obviously, there was a Lewis-acid activity in the Pd-catalyst. Deacetalization was performed with $FeCl_3$ in water-2-propanol to **23**. Another route to **23** was the hydrogenation of **1** in ethyl acetate-2-propanol. The saturated elaiophylins were acylated in the same manner (Table 2) as described for the unsaturated elaiophylins.

If the reaction of **1** was carried out with acetic anhydride or furan carboxylic acid anhydride in pyridine in the presence of catalytic amounts of *N,N*-dimethylaminopyridine, the reaction proceeded under ring opening of the hemiacetal to the corresponding octaacyl derivative (**31**) and the hexaacyl derivative (**32**). In the open form of the hemiacetal ring an acylation of 9-OH was possible, because only one hydrogen bond was left intact. Hydrogenation of **31** with Pd-H_2 gave **33** (Fig. 1).

Another interesting reaction was observed when **2** was stirred with acetic anhydride in pyridine at room temperature for 8 days. Under elimination of the C-11 methoxy group, the unsymmetric enol ether (**34**) and the symmetric enol ether (**35**) were formed.

Deglycosidation Reactions

The acid catalyzed deglycosidation of **1** to **36** and dimethylelaiophylidene (**37**) in a small scale was first described by SEEBACH *et al.*[16] starting from elaiophylin (**1**). In this reaction the acetal **2** was formed as an intermediate. By using **2** as starting material for the deglycosidation, a scale-up to 10 g was made possible. The base sensitivity of elaiophylin (**1**) can be used for a controlled degradation. Heating of **1** with $KHCO_3$ in water-ethyl acetate-ethanol for 4 hours led to a base catalyzed deglycosidation under β-elimination of the deoxyfucose side chain to **38** and **39**[24]. The *trans* configuration of the double bond indicated that the hemiacetal was opened prior to elimination of L-deoxyfucose. Hydrogenation with Pd-H_2 yielded the unsymmetric product **40** and the symmetric 13,13′-dideoxyoctahydroelaiophylidene (**41**) (Fig. 2).

Biological Activities

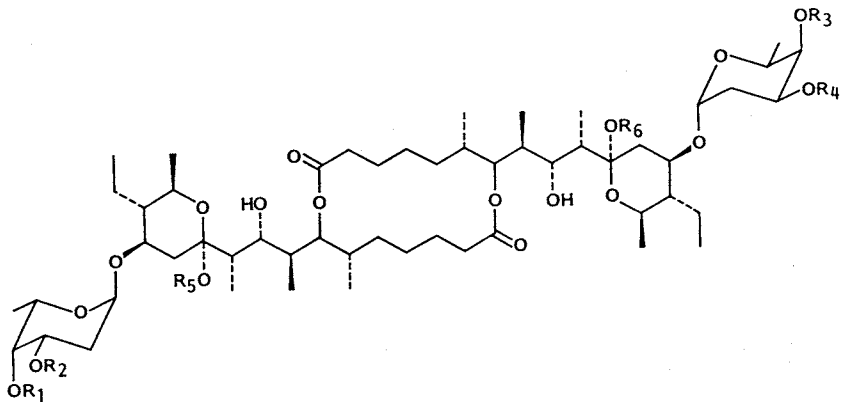
Elaiophylin (**1**) possessed an antibacterial activity against Gram-positive bacteria. The acetalization to **2** led to a 50% reduction of this activity (Table 3). All tetraacyl derivatives were mainly inactive. Only in tetrabutanoate (**6**) some activity remained. The bromobenzoylation of **1** showed that introduction of one acyl group in **9** resulted in some activity against bacteria. In the higher acylated products **10**, **11** and **12** the activity was totally lost.

The dimethyloctahydroelaiophylin (**21**) exhibited antibacterial activity, but surprisingly the octahydroelaiophylin (**23**) was inactive.

The products with the open hemiacetal ring **31**, **32** and **33** exhibited no activity.

While the unsymmetric deglycosidation products **36** and **38** had a small antibacterial activity, the symmetric compounds **37** and **39** were inactive. Maybe this fact as well as the activity of **9** could be explained through the fact that one part of the molecule still had an intact elaiophylin moiety.

Table 2. Acyl derivatives of octahydroelaiophylin.



Compound No.	R_1	R_2	R_3	R_4	R_5	R_6	Synthesis from	Reagent	Time (hours)	Temperature (°C)	Procedure	Yield (%)
21	H	H	H	H	CH_3	CH_3	**1**	Pd-H_2	2	25	4	87
22	CH_3CO	CH_3CO	CH_3CO	CH_3CO	CH_3	CH_3	**21**	$(CH_3CO)_2O$	24	25	2	85
23	H	H	H	H	H	H	**21**	$FeCl_3$-H_2O	0, 2	25	5	94
24	CH_3CO	CH_3CO	CH_3CO	CH_3CO	H	H	**23**	$(CH_3CO)_2O$	24	25	2	93
25	C_6H_5CO	C_6H_5CO	C_6H_5CO	C_6H_5CO	H	H	**23**	$(C_6H_5CO)_2O$	24	25	2	86
26[a]	C_4H_3SCO	C_4H_3SCO	C_4H_3SCO	C_4H_3SCO	H	H	**23**	$(C_4H_3SCO)_2O$	24	25	2	76
27[b]	C_4H_3OCO	C_4H_3OCO	C_4H_3OCO	C_4H_3OCO	H	H	**23**	$(C_4H_3OCO)_2O$	24	25	2	79
28	$CH_3(CH_2)_{10}CO$	$CH_3(CH_2)_{10}CO$	$CH_3(CH_2)_{10}CO$	$CH_3(CH_2)_{10}CO$	H	H	**23**	$(CH_3(CH_2)_{10}CO)_2O$	24	25	2	74
29	C_6H_5CO	C_6H_5CO	C_6H_5CO	C_6H_5CO	CH_3	CH_3	**21**	$(C_6H_5CO)_2O$	24	25	2	62
30	C_6H_5CO	C_6H_5CO	C_6H_5CO	C_6H_5CO	CH_3	H	**21**	$(C_6H_5CO)_2O$	24	25	2	6

[a] C_4H_3SCO: thiophene. [b] C_4H_3OCO: furan.

Fig. 1. Acylation reactions of elaiophylin (**1**) and derivatives.

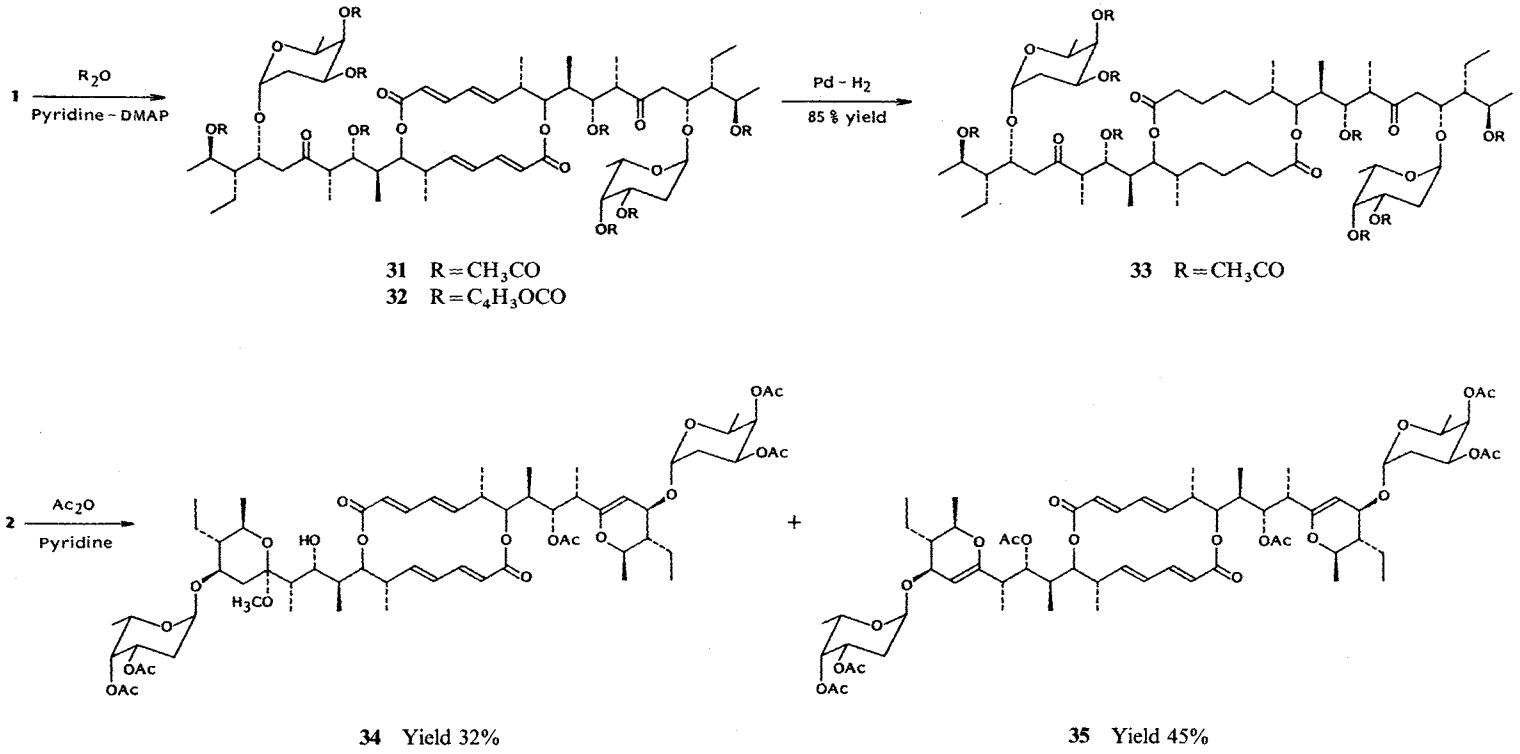


Fig. 2. Deglycosidation reactions of elaiophylin (**1**) and derivatives.

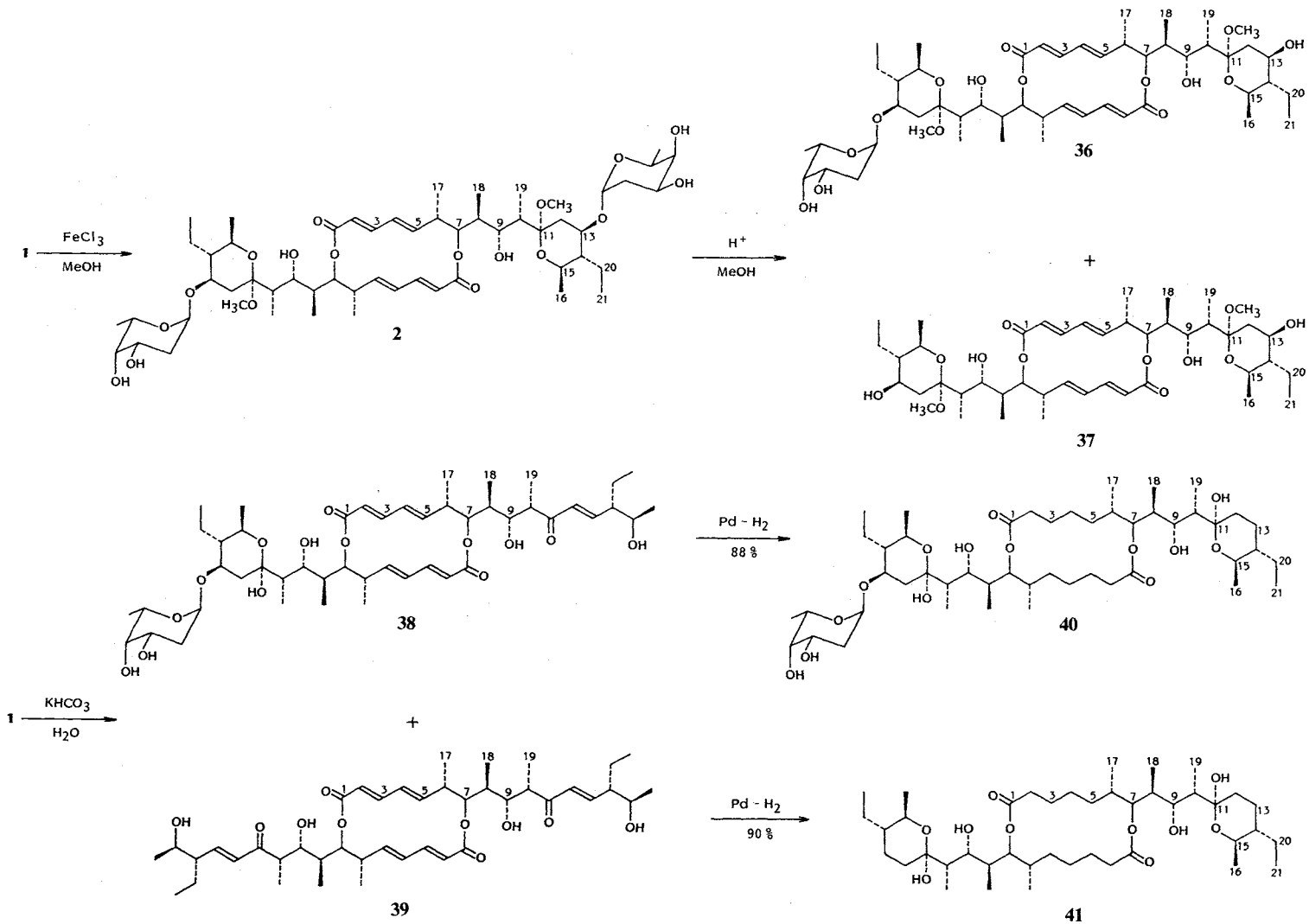


Table 3. Antimicrobial activities of elaiophylin derivatives (MIC in μg/ml).

Compound No.	*Staphylococcus aureus*			*Streptococcus pyogenes*		*S. faecium* A
	SG 511	285	503	308 A	77 A	
1	1.56	1.56	1.56	0.78	1.56	3.13
2	3.13	3.13	3.13	1.56	3.13	3.13
3	>100	>100	>100	100	100	>100
4	>100	>100	>100	6.25	25.0	>100
6	50.0	>100	>100	50.0	>100	>100
7	>100	>100	>100	100	100	>100
9	>100	>100	50.0	3.13	25.0	>100
21	25.0	100	50.0	6.25	12.5	>100
36	>100	>100	>100	25.0	25.0	>100
38	>100	>100	100	25.0	25.0	>100

No antibacterial activities were observed for all other derivatives discussed in this article.

Furthermore, compound **21** and the unsymmetric deglycosidation products **33**, **38** and **40** expressed a strong activity against the nematode *Caenorhabditis elegans* at a concentration of 100 ppm.

In contrast to the hygrolidins where an antifungal activity was observed[8] no antifungal activity of described derivatives against *Candida albicans*, *Trichophyton rubrum*, *Aspergillus niger* and *Microsporum canis* was detected.

Experimental

Analyticals

FAB-MS were recorded on a MS 50 Kratos Analytical with 3-nitrobenzyl alcohol as matrix. MP's were determined with a Reichert hotstage microscope. Optical rotation was measured with a Perkin-Elmer spectrometer 241.

Sensitivity Testing

The sensitivity of bacteria was tested by means of the agar dilution test in Mueller-Hinton agar (Difco). When testing Streptococci, the agar was supplemented with 10% horse blood. Plates were inoculated with a Denley Multipoint inoculator which delivered 5×10^4 cfu of an overnight culture of the strain concerned. The MIC was taken as the lowest concentration at which no visible growth could be detected after 24 hours at 37°C.

General

Compounds **31** and **32** were synthesized as it was described in Procedure 2 for **3** and **18**, but in the presence of 1.0 mmol 4-dimethylaminopyridine. The synthesis of **33** was carried out with the Procedure 4 starting from **31**. Compounds **34** and **35** were prepared with Procedure 2; the reaction time was 8 days. Hydrogenation of **38** to **40** and **39** to **41** was carried out with Procedure 4. Instead of the solvent methanol, ethyl acetate - 2-propanol - water in the ratio 4 : 3 : 2 was used.

The physio-chemical properties of the derivatives are described in Table 4.

Procedure 1

11,11'-Di-*O*-methylelaiophylin (**2**): 10 g (9.8 mmol) elaiophylin (**1**) in 100 ml methanol was stirred with 100 mg $FeCl_3$ for 5 minutes. The reaction mixture was slightly concentrated *in vacuo* until the first crystals precipitated. Cooling the liquid to 4°C yielded 9.3 g (92%) **2**, mp 171 °C.

Procedure 2

1 mmol elaiophylin (**1**) or elaiophylin-derivative was stirred in 20 ml pyridine with an 8-fold excess of

Table 4. Physico-chemical properties of elaiophylin derivatives.

Compound No.	Formula	Calcd		Found		MW	Observed mass (m/z)[a]	Optical rotation $[\alpha]_D^{20}$	MP (°C)
		C	H	C	H				
1	$C_{54}H_{88}O_{18}$	63.3	8.7	63.0	8.7	1,025.3	1,047		
2	$C_{56}H_{92}O_{18}$	63.8	8.8	63.4	8.1	1,053.3	1,075		171
3	$C_{62}H_{96}O_{22}$	62.4	8.1	62.0	8.3	1,193.4	1,215	−66° (*c* 1, CH_2Cl_2)	—
4	$C_{60}H_{94}O_{21}$	62.6	8.2	62.5	8.1	1,151.4	1,173	−60° (*c* 1, CH_2Cl_2)	—
5	$C_{64}H_{100}O_{22}$	62.9	8.3	62.9	8.4	1,221.5	1,243	−60° (*c* 1, CH_2Cl_2)	—
6	$C_{74}H_{120}O_{22}$	65.3	8.9	65.7	9.0	1,361.7	1,383	−81° (*c* 1, CH_2Cl_2)	172~173
7	$C_{56}H_{84}O_{20}$	62.4	7.9	62.2	8.1	1,077.3	1,098	+3° (*c* 1, CH_2Cl_2)	206~207
8	$C_{63}H_{90}N_4O_{21}$	61.1	7.3	61.3	7.6	1,239.4	1,261	−42° (*c* 1, CH_2Cl_2)	158~160
9	$C_{61}H_{91}BrO_{19}$	60.6	7.6	60.8	7.8	1,208.3		−56° (*c* 1, CH_2Cl_2)	158~160
10	$C_{68}H_{94}Br_2O_{20}$	58.7	6.8	58.8	7.1	1,391.3		−93° (*c* 1, CH_2Cl_2)	205
11	$C_{75}H_{97}Br_3O_{21}$	57.2	6.2	57.2	6.4	1,574.3		−125° (*c* 1, CH_2Cl_2)	180~182
12	$C_{82}H_{100}Br_4O_{22}$	56.0	5.7	56.2	5.8	1,757.3		−132° (*c* 1, CH_2Cl_2)	193~194
13	$C_{82}H_{104}O_{22}$	63.3	7.3	63.2	7.1	1,441.7	1,463	−96° (*c* 1, CH_3OH)	—
14	$C_{78}H_{144}O_{18}Si_4$	63.2	9.8	63.4	9.7	1,482.3		−37° (*c* 1, CH_3OH)	196~199
15	$C_{82}H_{108}N_4O_{22}$	65.6	7.2	65.3	7.5	1,501.8	1,523		—
16	$C_{74}H_{96}O_{22}S_4$	60.6	6.6	60.3	6.4	1,465.8	1,487		207~209
17	$C_{102}H_{176}O_{22}$	69.8	10.1	69.3	10.2	1,754.5	1,776		152~153
18	$C_{74}H_{96}O_{26}$	63.4	6.9	63.2	7.0	1,401.6	1,423		186~187
19	$C_{70}H_{104}O_{30}$	59.0	7.4	59.4	7.5	1,425.6		−58° (*c* 1, CH_2Cl_2)	—
20	$C_{55}H_{90}O_{20}S$	59.9	8.2	60.0	8.1	1,103.4	1,125		—
21	$C_{56}H_{100}O_{18}$	63.4	9.5	63.0	9.1	1,061.4	1,083	−50° (*c* 1, CH_3OH)	112~114
22	$C_{64}H_{108}O_{22}$	65.5	8.8	65.6	8.3	1,229.5	1,251	−53° (*c* 1, $CHCl_3$)	188
23	$C_{54}H_{96}O_{18}$	62.7	9.4	62.4	9.2	1,033.3	1,055	−77° (*c* 1, CH_2Cl_2)	196~198
24	$C_{62}H_{104}O_{22}$	62.0	8.7	62.4	8.3	1,201.5	1,223	−86° (*c* 1, CH_2Cl_2)	124~125
25	$C_{82}H_{112}O_{22}$	67.9	7.8	67.4	8.0	1,449.7		−106° (*c* 1, CH_3OH)	190
26	$C_{74}H_{104}O_{22}S_4$	60.3	7.1	60.4	7.4	1,473.9	1,495		202~204
27	$C_{74}H_{104}O_{26}$	63.1	7.4	63.4	7.5	1,409.6	1,431		195~197
28	$C_{102}H_{184}O_{22}$	69.5	10.5	69.3	10.2	1,762.6	1,784		102~103
29	$C_{84}H_{116}O_{22}$	68.3	7.9	68.0	7.1	1,477.8	1,477		—
30	$C_{84}H_{108}O_{22}$	68.1	7.8	68.0	7.1	1,463.8	1,485		143~145
31	$C_{70}H_{104}O_{26}$	61.7	7.7	61.8	7.8	1,361.6		+10° (*c* 1, CH_3OH)	190
32	$C_{94}H_{104}O_{34}$	63.5	5.9	63.6	6.0	1,777.8	1,799		—
33	$C_{70}H_{112}O_{26}$	61.4	8.2	61.2	8.3	1,369.6	1,391	−24° (*c* 1, CH_3OH)	—
34	$C_{65}H_{98}O_{22}$	63.4	8.0	63.2	8.1	1,231.5	1,253		—
35	$C_{66}H_{96}O_{22}$	62.9	8.3	62.4	8.2	1,241.5	1,263	−60° (*c* 1, CH_2Cl_2)	—
36	$C_{50}H_{82}O_{15}$	65.1	8.9	65.5	8.7	923.2		+46° (*c* 1, CH_2Cl_2)	156~158
37	$C_{44}H_{72}O_{12}$	66.6	9.2	66.9	9.4	793.0	815	+108° (*c* 1, CCl_4)	—
38	$C_{48}H_{76}O_{14}$	65.7	8.7	65.4	8.9	877.1	899	+13° (*c* 1, CH_3OH)	—
39	$C_{42}H_{64}O_{10}$	69.2	8.8	69.7	8.8	729.0	751	+107° (*c* 1, CH_3OH)	—
40	$C_{48}H_{86}O_{14}$	65.1	9.7	64.8	9.4	887.2	909		—
41	$C_{42}H_{76}O_{10}$	68.1	10.3	68.5	10.1	741.1	763		—

[a] MNa^+.

the anhydride, carboxylic acid halide or isocyanate at room temperature for the appropriate time. After hydrolysis with water the aqueous phase was extracted three times with 50 ml ethyl acetate. The organic layer was washed with 20 ml 0.1 N hydrochloric acid, 20 ml saturated sodium carbonate solution and 20 ml water. After evaporation the residue was separated on silica gel with a linear gradient of ethyl acetate - hexane (1 : 5) to ethyl acetate.

Procedure 3

3″,3‴,4″,4‴-Tetra-*O*-(*tert*-butyldimethylsilyl)elaiophylin (**14**): 800 mg (0.78 mmol) elaiophylin (**1**), 1.29 g (12 mmol) 2,6-lutidine and 1.08 ml (4.7 mmol) *tert*-butyldimethylsilyl triflate were dissolved at 0 °C in 10 ml dry methylene chloride. After 20 minutes the cooling bath was removed and the solution was

warmed up to 25°C. Dilution with 200 ml diethyl ether, extraction three times with saturated $NaHCO_3$-solution, drying with Na_2SO_4 and evaporation gave a syrup. Chromatography on silica gel with methylene chloride with an increasing gradient of diisopropyl ether gave 474 mg (41%) **14** as a white foam.

Procedure 4

11,11'-Di-*O*-methyloctahydroelaiophylin (**21**): 1 g (1 mmol) elaiophylin (**1**) was dissolved in 50 ml methanol and 400 mg Palladium on charcoal (Merck) was added. The hydrogenation was carried out at room temperature for 2 hours. The reaction mixture was filtrated and concentrated until the first crystals of **21** were observed; yield 920 mg (87%).

Procedure 5

Octahydroelaiophylin (**23**): 1g (1 mmol) **23** was dissolved in 20 ml water and 40 ml 2-propanol and stirred with 100 mg $FeCl_3$ for 10 minutes at room temperature. The reaction mixture was concentrated until the first crystals were obtained; yield 980 mg (95%).

Procedure 6

Deglycosidation of Elaiophylin to **36** and 11,11'-Di-*O*-methylelaiophylidene (**37**): 10 g (9.5 mmol) **2** in 200 ml methanol was stirred with 500 mg 4-toluenesulfonic acid for 3 hours at 20°C. After dilution with water and neutralization with $NaHCO_3$ the methanol was evaporated. The remaining water phase was extracted with ethyl acetate three times. After drying with Na_2SO_4 and evaporation of the solvent the residue was chromatographed on silica gel with diethyl ether - hexane (1 : 4); yield 3.6 g (41%) **36** and 2.8 g (37%) **37**.

Procedure 7

Deglycosidation to **38** and **39**: 10 g (9.8 mmol) elaiophylin (**1**) was refluxed in 150 ml water, 50 ml ethanol and 70 ml ethyl acetate with 25 g $KHCO_3$ for 6 hours. The organic solvents were evaporated and the water phase was extracted four times with ethyl acetate. The organic phase was evaporated and the residue was chromatographed on silica gel with the solvent chloroform - methanol (40 : 1); yield 2.9 g (34%) **38** and 3.7 g (52%) **39**.

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References

1) HAMMANN, P. & G. KRETZSCHMAR: Secondary metabolites by chemical screening. 6. Cleavage of elaiophylin and transformation into a spiroketal building block. Tetrahedron 46: 5609~5616, 1990
2) ARCAMONE, F. M.; C. BERTAZOLLI, M. GHIONE & T. SCOTTI: Melanosporin and elaiophylin, new antibiotics from *Streptomyces melanosporus* (sive mela nosporofaciens) n. sp. Gion. Microbiol. 7: 207~216, 1959
3) ARAI, M.: Azalomycins B and F, two new antibiotics. I. Production and isolation. J. Antibiotics, Ser. A 13: 46~50, 1960
4) ARAI, M.: Azalomycins B and F, two new antibiotics. II. Properties of azalomycins B and F. J. Antibiotics, Ser. A 13: 51~56, 1960
5) FIEDLER, H.-P.; W. WÖRNER, H. ZÄHNER, H. P. KAISER, W. KELLER-SCHIERLEIN & A. MÜLLER: Metabolic products of microorganisms. 200. Isolation and characterization of niphithricins A, B, and elaiophylin, antibiotics produced by *Streptomyces violaceoniger*. J. Antibiotics 34: 1107~1118, 1981
6) GRABLEY, S.; P. HAMMANN, W. RAETHER, J. WINK & A. ZEECK: Sencondary metabolites by chemical screening. II. Amycins A and B, two novel niphimycin analogs isolated from a high producer strain of elaiophylin and nigericin. J. Antibiotics 43: 639~647, 1990
7) ISOGAI, A.; S. SAKUDA, S, MATSUMOTO, M. OGURA, K. FURIHATA, H. SETO & A. SUZUKI: The structure of leucanicidin, a novel insecticidal macrolide produced by *Streptomyces halstedii*. Agric. Biol. Chem. 48: 1379~1381,

1984

8) SETO, H.; H. AKAO, K. FURIHATA & N. ŌTAKE: The structure of a new antibiotic, hygrolidin. Tetrahedron Lett. 23: 2667～2670, 1982
9) GOETZ, M. A.; P. A. MCCORMICK, R. L. MONAGHAN, D. A. OSTLIND, O. D. HENSENS, J. M. LIESCH & G. ALBERS-SCHONBERG: L-155, 175: A new antiparasitic macrolide. Fermentation, isolation and structure. J. Antibiotics 38: 161～168, 1985
10) HENSENS, O. D.; R. L. MONAGHAN, L. HUANG & G. ALBERS-SCHÖNBERG: Structure of the sodium and potassium ion activated adenosinetriphosphatase inhibitor L-681,110. J. Am. Chem. Soc. 105: 3672～3679, 1983
11) WERNER, G.; H. HAGENMAIER, H. DRAUTZ, A. BAUMGARTNER & H. ZÄHNER: Metabolic products of microorganisms. 224. Bafilomycins, a new group of macrolide antibiotics. Production, isolation, chemical structure and biological activity. J. Antibiotics 37: 110～117, 1984
12) DAVIES, M. G. & R. H. GREEN: Avermectins and milbemycins. Nat. Prod. Rep. 3: 87～121, 1986
13) ŌMURA, S. (*Ed.*): Macrodiolide Antibiotics. Chemistry, Biology, and Practice. Academic Press, 1984
14) NEUPERT-LAVES, K. & M. DOBLER: 24. Stoffwechselprodukte von Mikroorganismen. 221.Röntgenstrukturanalyse von Elaiophylin. Helv. Chim. Acta 65: 262～267, 1982
15) KAISER, H. P. & W. KELLER-SCHIERLEIN: 41. Stoffwechselprodukte von Mikroorganismen. 202. Strukturaufklärung von Elaiophylin: Spektroskopische Untersuchung und Abbau. Helv. Chim. Acta 64: 407～424, 1981
16) SEEBACH, D.; H.-F. CHOW, R. F. W. JACKSON, M. A. SUTTER, S. THAISRIVONGS & J. ZIMMERMANN: (+)-11,11'-Di-*O*-methyl-elaiophylidene, preparation from elaiophylin and total synthesis from (*R*)-3-hydroxybutyrate and (*S*)-malate. Liebigs Ann. Chem. 1986: 1281～1308, 1986
17) TOSHIMA, K.; K. TATSUTA & M. KINOSHITA: Total synthesis of elaiophylin (azalomycin B). Tetrahedron Lett. 27: 4741～4744, 1986
18) JACKSON, R. F. W.; M. A. SUTTER & D. SEEBACH: Preparation of (2*E*,4*E*,6*S*,7*S*,10*E*,12*E*,14*S*,15*S*,16*S*)-7,15-bis-(1-hydroxymethylethyl-6,14-dimethyl-8,16-dioxa-2,4,10,12-cyclo-hexadecatetraene-1,9-dione. A building block of the synthesis of elaiophylin. Liebigs Ann. Chem. 1985: 2313～2327, 1985
19) WAKAMATSU, T.; S. YAMADA & H. NAKAMURA: Enantioselective synthesis of 16-membered α,β-unsaturated macrodiolide: A model system in elaiophylin. Heterocycles 25: 43～46, 1987
20) YOKURA, S.; Y. SATO, A. HASHIMATA & S. KIUCHI (Tokyo Tanabe Seiyaku): Azalomycin B Derivate sowie Mittel gegen Geschwüre mit denselben als Wirkstoff. Jpn. Kokai 36295 ('86), Feb. 20, 1986 [CA 106: 67626j, 1987]
21) TAKAHASHI, S.; M. ARAI & E. OHKI: Chemical studies on azalomycins. I. Preliminary study on azalomycin-B. Chem. Pharm. Bull. 15: 1651～1656, 1967
22) TAKAHASHI, S.; M. KURABAYASHI & E. OHKI: Chemical studies on azalomycins. II. Sugar component of azalomycin-B. Chem. Pharm. Bull. 15: 1657～1661, 1967
23) TAKAHASHI, S. & E. OHKI: Chemical studies on azalomycins. III. Alkaline degradation of azalomycin-B. Chem. Pharm. Bull. 15: 1726～1732, 1967
24) HAMMANN, P. & G. KRETZSCHMAR: Secondary metabolites by chemical screening. 5. Cycloaddition reactions of elaiophylin. Tetrahedron 46: 5603～5608, 1990
25) HAMMANN, P. & G. KRETZSCHMAR: Secondary metabolites by chemical screening. 3. Side chain modification of elaiophylin. Z. Naturforsch. 45B: 517～519, 1990
26) LEY, S. V.; D. NEUHAUS & D. J. WILLIAMS: A conformational study of elaiophylin by X-ray crystallography and difference ^{1}H NMR methods; observation of a selective sign reversal of the nuclear Overhauser effect. Tetrahedron Lett. 23: 1207～1210, 1982