METABOLIC PRODUCTS OF MICROORGANISMS. 225[1]
ELLORAMYCIN, A NEW ANTHRACYCLINE-LIKE ANTIBIOTIC FROM *STREPTOMYCES OLIVACEUS*

ISOLATION, CHARACTERIZATION, STRUCTURE AND BIOLOGICAL PROPERTIES

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Elloramycin (**1**), a new antibiotic produced by *Streptomyces olivaceus* strain Tü 2353, was detected by chemical screening. The dark yellow compound, molecular formula $C_{32}H_{36}O_{15}$, is weakly active against a variety of Gram-positive bacteria, especially streptomycetes and against stem cells of L-1210 leukemia. Acidic hydrolysis of the antibiotic liberated elloramycinone (**3**) as aglycone and 2,3,4-tri-*O*-methyl-L-rhamnose, which was identified as methyl glycoside **5b**. The structure of elloramycin was established by comparison of the spectra (UV, ^{1}H NMR, ^{13}C NMR) with those of the known tetracenomycin C (**2**), **3** and the fact that **2** and **3** gave the same tetramethyl ether after permethylation. Elloramycin is an anthracycline-like antibiotic, the aglycone resembles tetracenomycin C, the sugar is connected in a phenolic α-glycosidic linkage.

In the course of our chemical screening[2~5] we look for secondary metabolites of microorganisms, which can be detected on TLC plates by their color or by color reactions using special staining reagents. Elloramycin exhibited a striking appearance in TLC experiments by its dark yellow color, and by fluorescence at 366 nm in UV light. In the following study we describe the fermentation, isolation, physico-chemical characterization, structure elucidation and the biological properties of the new compound. Subject of another publication will be the special chemistry of elloramycin and its derivatives[6].

Fermentation of Elloramycin

To produce elloramycin in larger amounts, 20-liter, 25-liter and 200-liter fermentations were performed. The time course of the fermentation of *Streptomyces olivaceus* in a 25-liter fermentor is shown in Fig. 1. The production of the antibiotic starts early during the beginning of the logarithmical growing phase, and reaches its maximum after about 112 hours, yielding an antibiotic concentration of about 200 mg/liter in the culture medium.

Isolation and Characterization

The crude product, which was obtained by extraction from mycelium (methanol) and the culture filtrate (ethyl acetate) of strain Tü 2353, was chromatographically purified on silica gel and Sephadex

Fig. 1. Time course of fermentation of *Streptomyces olivaceus*.
Symbols: ○ Dry weight of the mycelium (mg/liter), ● pH of the culture, △ elloramycin concentration estimated by HPLC, ▲ elloramycin concentration estimated by disc-diffusion assay.

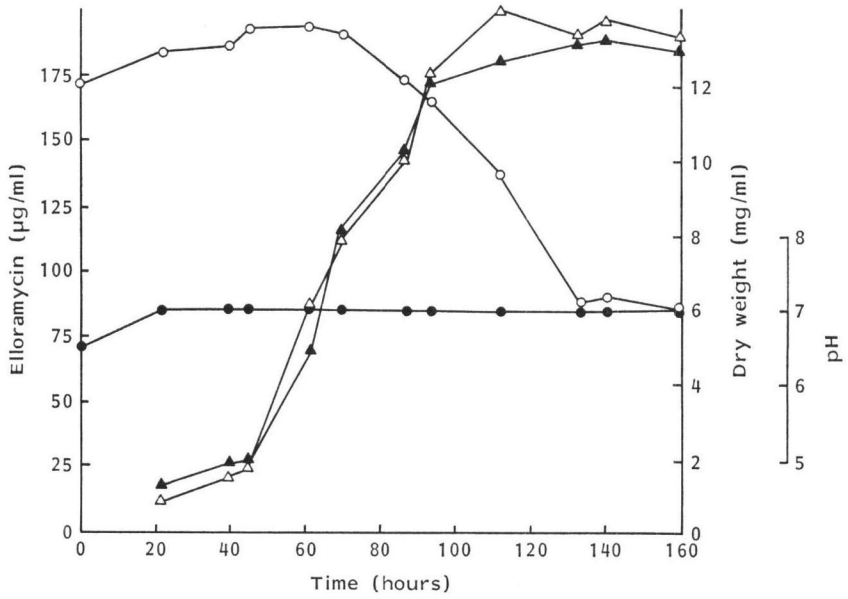
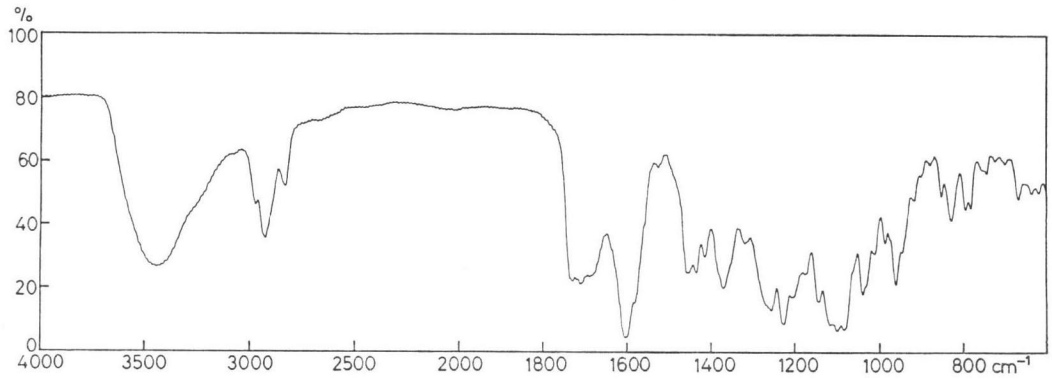


Table 1. Rf values (TLC, silica gel) of elloramycin (**1**), tetracenomycin C (**2**), elloramycinone (**3**) and elloramycinone-tetramethylether (**4**) in different solvent systems.

Solvent system	**1**	**2**	**3**	**4**
$CHCl_3$ - MeOH (9: 1)	0.69	0.55	0.45	0.77
EtOAc - *n*-pentane - AcOH (11: 8: 1)	0.23	0.21	0.21	0.42

Fig. 2. IR spectrum of elloramycin (**1**) in KBr.



LH-20 in chloroform - methanol systems. Elloramycin was obtained as a dark yellow amorphous powder, which was insoluble in water or *n*-pentane and soluble in methanol, chloroform or acetone (Rf values see Table 1). Elloramycin is optically active.

The UV absorption bands at different pH values and the IR spectrum (Fig. 2) in the region between 1800~1200 cm^{-1} demonstrate similarity to tetracenomycin C (**2**)[7~9], which is produced by *S.*

Fig. 3. EI mass spectrum (70 eV) of elloramycin (**1**).

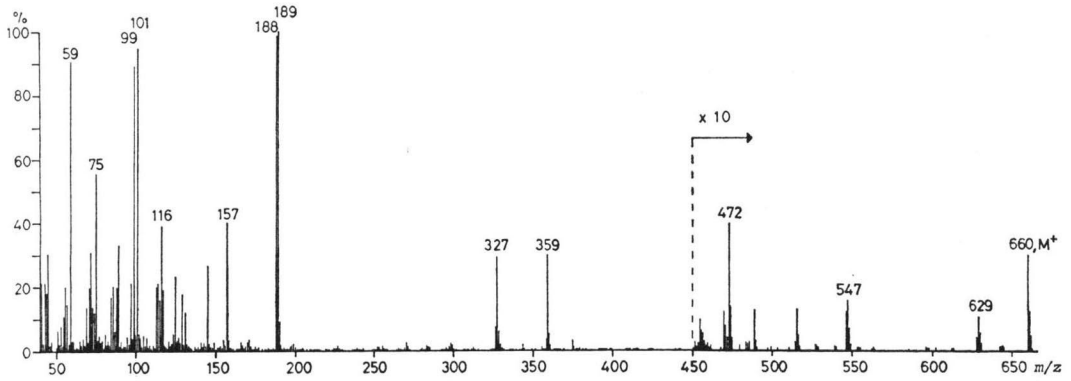
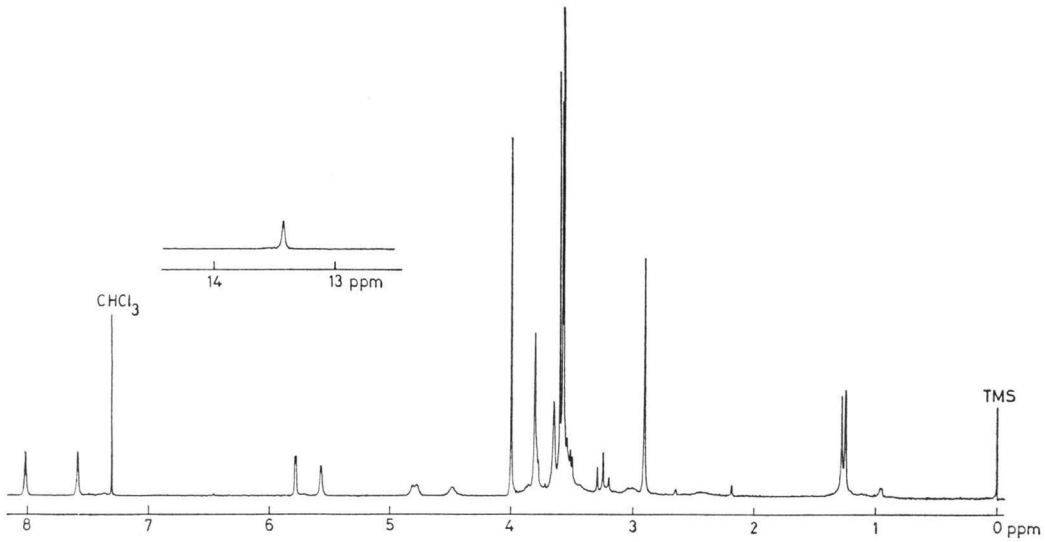


Fig. 4. ^{1}H NMR spectrum of elloramycin (**1**) in $CDCl_3$ at 200 MHz.



glaucescens. The high resolution EI mass spectrum of elloramycin (Fig. 3) and the elemental analysis are in agreement with a molecular formula of $C_{32}H_{36}O_{15}$. Thus, elloramycin differs from tetracenomycin C (**2**) in a $C_8H_{16}O_4$ unit, which occurs in the mass spectrum as base peak at *m/z* 188/189. The loss of this unit from the molecular ion (*m/z* 660) leads to the peak at *m/z* 472 corresponding to the molecular weight of tetracenomycin C.

The ^{1}H NMR spectrum of elloramycin in $CDCl_3$ (Fig. 4, Table 2), in comparison with the data of **2** (Table 2), shows an extensive similarity of the tetracyclic skeleton. Merely the 7-H singlet exhibits a significant shift, and there is a more aliphatic methoxyl singlet (δ 3.65) instead of an aromatic one (δ 4.00) as seen in tetracenomycin C. Additional signals in the elloramycin spectrum occur in the region of a methylated 6-deoxyhexopyranose (Table 4). These results are confirmed by the ^{13}C NMR data (Tables 3 and 4).

The structure of elloramycin (**1**) was established by spectral analysis of the antibiotic itself, its hydrolysis products and some derivatives.

Table 2. ^{1}H NMR data of elloramycin (**1**, aglycone part) in comparison with tetracenomycin C (**2**) and elloramycinone (**3**) in $CDCl_3$.

H-Atom	**1** (100 MHz)	**2** (200 MHz)	**3** (80 MHz)
2-H	5.76 d[a]	5.70 d[a]	5.52 d[a]
3-OCH_3	3.80 s	3.86 s	3.77 s
4-H	4.79 m[b]	4.90 m[b]	4.84 m[b]
4-OH	3.04 d[c,d]	2.92 d[c,d]	2.05 br[c]
4a-OH	4.39 s[c]	4.30 s[c]	4.66 s[c]
6-H	8.00 s	7.98 s	7.71 s
7-H	7.56 s	7.17 s	7.16 s
8-OCH_3	—	4.00 s	—
9-$COOCH_3$	3.99 s	4.00 s	4.00 s
10-CH_3	2.90 s	2.85 s	2.96 s
11-OH	13.44 s[c]	13.88 s[c]	14.11 s[c]
12a-OH	—	4.86 s[c]	—
12a-OCH_3	3.65 s	—	3.54 s

[a] J=1.5 Hz.
[b] After exchange with D_2O: d, J=1.5 Hz.
[c] Exchangeable with D_2O.
[d] J=9.5 Hz.
br: Broad.

Table 3. ^{13}C NMR data of elloramycin (**1**, aglycone part), tetracenomycin C (**2**) and elloramycinone (**3**) in DMSO-d_6 at 25.2 MHz.

Region	Assignment	**1**	**2**	**3**
Carbonyl-C	C-12	196.1	197.4	195.0
	C-5	192.7	193.6	193.0
	C-1	190.7	190.8	190.0
	Ester	172.8	174.4	172.3
Aromatic/olefinic-C	C-3	166.8	167.2	170.5
	C-11	165.6	165.7	168.2
	C-8	152.7	157.0	157.2
	C-5a	139.5	140.1	139.6
	C-10	137.3	136.6	137.5
	C-6a	128.9	128.4	128.6
	C-10a	127.7	128.3	127.6
	C-11a	120.6	118.8	120.7
	C-6	119.6	118.8	115.4
	C-7	111.2	108.2	111.3
	C-9	109.3	109.0	108.8
	C-2	100.5	99.6	100.6
Aliphatic-C	C-12a	87.2	84.9	87.8
	C-4a	85.0	83.4	84.5
	C-4	69.3	69.3	69.3
	(O)-CH_3	56.6	56.9	56.3
		55.5	56.6	54.9
		52.5	52.7	51.9
	10-CH_3	20.5	20.7	20.5

Structure Elucidation

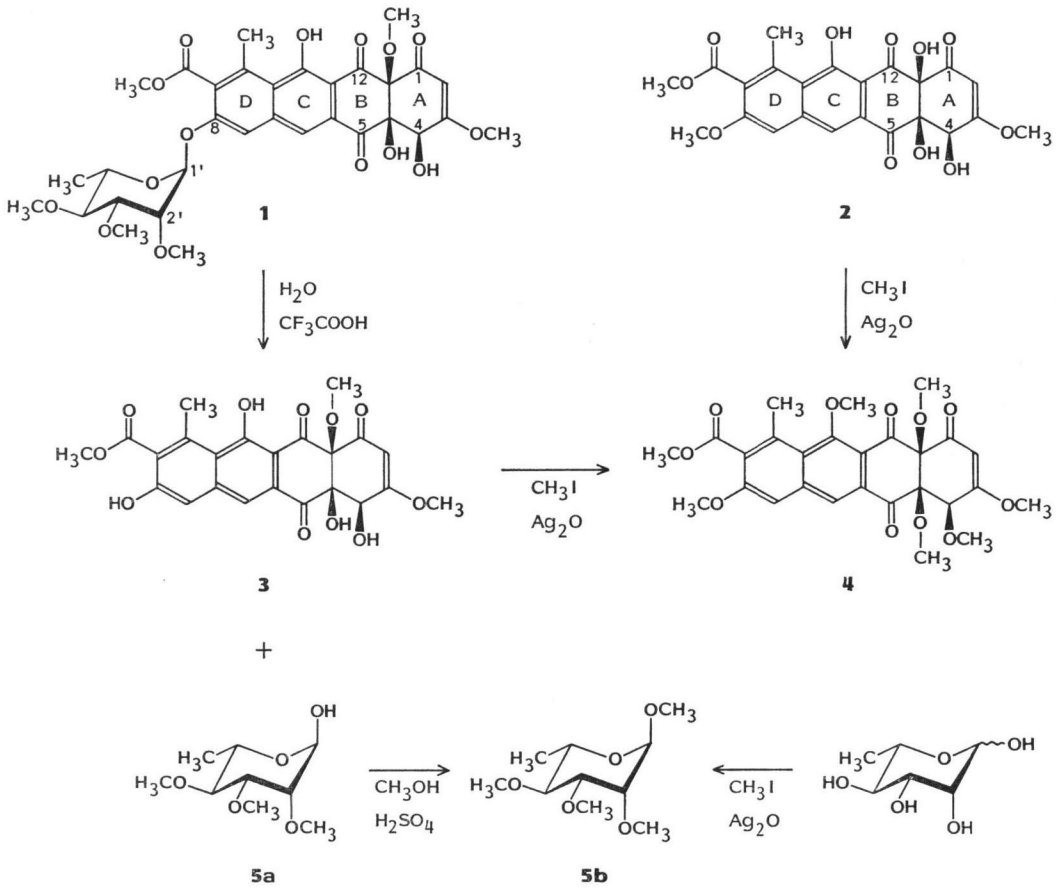
Hydrolysis of elloramycin (**1**) in trifluoroacetic acid containing 1 equivalent water gave the aglycone, called elloramycinone and the sugar (Scheme 1). Especially from the NMR data of the sugar

Table 4. ^{1}H and ^{13}C NMR signals of elloramycin (**1**, sugar part) in comparison with methyl 2,3,4-tri-*O*-methyl-α-L-rhamnopyranoside (**5b**), δ in ppm relative to internal TMS (J in Hz).

Position	^{1}H NMR		^{13}C NMR	
	1 (Sugar part)[a]	**5b**[b]	**1** (Sugar part)[c]	**5b**[d]
1	5.57 d (2)	4.66 d (2)	94.3	99.1
2	3.78[e]	3.54 dd (2, 3)	75.4	77.8
3	3.49 dd (3, 9.5)	3.33 dd (3, 9.5)	80.3	82.1
4	3.24 dd (9.5, 9.5)	2.97 dd (9.5, 9.5)	80.8	82.8
5	3.59[e]	3.45[e]	68.7	68.3
6	1.27 d (6)	1.18 d (6.5)	17.7	18.3
1-OCH_3	—	3.29 s	—	55.1
OCH_3	3.57 s	3.36 s	56.8	57.4
	3.58 s	3.38 s	58.4	59.1
	3.60 s	3.45 s	59.9	60.7

[a] $CDCl_3$: 200 MHz, [b] CD_3CN: 200 MHz, [c] DMSO-d_6: 25.2 MHz, [d] CD_3CN: 50.3 MHz, [e] overlapped.

Scheme 1. Structure elucidation of elloramycin (**1**).



moiety of elloramycin (Table 4) in comparison with those of methyl glycosides given in literature[10], a permethylated rhamnose was supposed to be the sugar moiety of elloramycin. The deduction of the relative stereochemistry of this sugar by the ^{1}H NMR data was difficult, because the methoxy

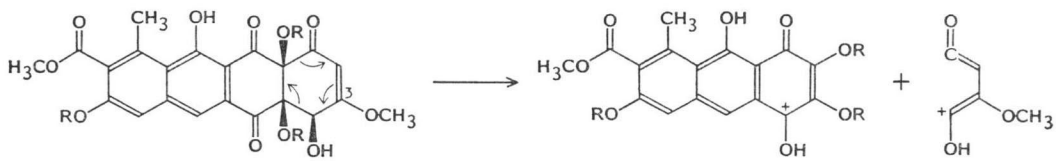
signals overlap with some CH signals. To prove the rhamnose-hypothesis and to get the absolute configuration of the sugar, L-(+)-rhamnose was permethylated yielding the methyl 2,3,4-tri-*O*-methyl-α-L-rhamnopyranoside (**5b**) as main product. This derivative was in all respects identical with the methyl glycoside obtained by hydrolysis from elloramycin after methylation of the anomeric hydroxyl group with acidic methanol. The same optical rotation of both compounds ($[\alpha]_D^{24}$ −62°, *c* 1, chloroform) established the L-configuration of the elloramycin sugar.

Elloramycinone (**3**) and tetracenomycin C (**2**) gave the same molecular ion at *m/z* 472 ($C_{23}H_{20}O_{11}$). They are isomers but not identical, which can be seen by the Rf values (Table 1), the different chemical shifts of the methoxy groups in the NMR spectra (Tables 2 and 3) and a second phenolic hydroxyl group for elloramycinone (**3**), recognizable in its alkaline UV spectrum (λ_{max} 315 nm). If the only difference between elloramycinone and tetracenomycin C is the methylation pattern, identical methyl ethers should be received by permethylation of both compounds. Indeed the methylation with methyl iodide-siver(I) oxide yielded permethylethers (**4**), which were identical in all respects (Scheme 1) including their CD spectra. Thus, elloramycinone and tetracenomycin C have the same skeleton, similar substituents and a corresponding stereochemistry. The structure of elloramycinone (**3**) follows from the known absolute configuration of tetracenomycin C (**2**)[8].

The glycosidic linkage of the sugar to elloramycinone (**3**) cannot be placed at C-3 or C-4, because from the retro-dien cleavage of elloramycin (**1**) in the EI mass spectrum (Fig. 3, Scheme 2) derives, that the sugar remains at the bigger fragment. C-3 is occupied by a methoxy group (δ 3.80), C-4 by a hydroxyl group (δ 3.04), which follows from the fragmentation pattern and the ^{1}H NMR data, especially the coupling constant (*J*=9.5 Hz) between 4-H/4-OH (Table 2). Thus, the additional absorption band in the alkaline UV spectrum of elloramycinone (**3**) compared with elloramycin (**1**) itself could only originate from a new phenolic hydroxyl group at C-8, which is liberated during hydrolysis. Because of the chemical shift (δ 94.3) and the $^1J_{C-H}$ coupling constant of the anomeric C of the sugar moiety in elloramycin (*J*=170 Hz), the mode of linkage is regarded as α[11].

One remaining ambiguity, namely the position of the angular methoxy and hydroxyl group at C-4a and C-12a, respectively, should be solved by an oxidative degradation and/or by an acetonide derivative of elloramycin. While the oxidative degradation with sodium periodate yielded several unstable products[8], the acetonide derivative could be received in acceptable yields. Molecular model studies confirm that the energetically favored 5-membered 4,4a-acetonide ring of elloramycin (**1**) is the most probable derivative and the remaining methoxy group is attached at C-12a. When we have finished our studies a X-ray analysis of elloramycin supplied the final proof of this conclusion and the whole structure[12].

Scheme 2. Retro-dien cleavage of elloramycin (**1**) in the EI mass spectrum.



R=H, CH_3, Sugar

Biological Activity

In the disc-diffusion assay the sensibility of various bacteria and fungi were tested against elloramycin (**1**) in comparison with tetracenomycin C (**2**, Table 5). The results show that the biological activity spectra of both antibiotics are very similar against Gram-positive bacteria such as streptomycetes, and against related organisms. Among the streptomycetes *S. prasinus* is striking by its high sensibility against elloramycin. The *in vitro* test against L-1210 leukemia cells showed a factor 100 weaker activity than doxorubicin[13]. An *in vivo* test against leukemia P 388 in mice showed no effect.

Table 5. The biological activity of elloramycin and tetracenomycin C against various microbial strains.

Tested organisms	Inhibitory diameter (mm)	
	Elloramycin	Tetracenomycin C
Achromobacter geminiani	—	—
Escherichia coli	—	—
Bacillus brevis	—	13
B. subtilis	—	—
Micrococcus luteus	0/14	0/15
M. roseus	—	—
Arthrobacter aurescens	11	18
A. crystallopoietes	15	18
Brevibacterium flavum	15	24
Corynebacterium rathayi	—	—
Streptomyces diastatochromogenes	21	30
S. glaucescens	10	Sp
S. lavendulae	16	18/31
S. phaeochromogenes	22	10/22
S. prasinus	28	22/30
S. violaceus-niger	18	31
S. violaceoruber	22	14
Botrytis cinerea	—	—
Mucor miehei	—	—

Sp: Trace, —: no inhibition.
Concentration: 1 mg/ml.

Discussion

Elloramycin (**1**) is chemically related to tetracenomycin C (**2**) and belongs to the group of anthracycline related antibiotics. The antibacterial activity of both compounds is comparable and a similar mechanism of action by intercalation of the molecule into the DNA[7] is assumed. However, a weaker *in vitro* antitumor activity in comparison with tetracenomycin C and no *in vivo* antitumor activity in contrast to tetracenomycin C could be detected. Elloramycin is a phenolic glycoside, this glycoside-type is rather seldom among the secondary metabolites of streptomycetes. Substances of similar appearance are the antitumor antibiotics chartreusin[14~16] and chromocyclomycin[17]. L-Rhamnose and its partially methylated derivatives are frequently occurring as sugar parts of antibiotics. Elloramycin (**1**) is the first secondary metabolite of microorganisms with this sugar permethylated. This high grade of methylation explains the lipophilic character of the molecule and may be one reason for its weaker cytotoxic activity[6].

Experimental

General

UV spectra were recorded using a Zeiss DMR 21 spectrometer, IR spectra in pressed KBr disks using a Perkin-Elmer Model 298 spectrometer. The NMR spectra were determined with a Varian XL-100 or XL-200, respectively. Chemical shifts (δ in ppm) are reported relative to internal tetramethylsilane. The mass spectra were obtained on a Varian MAT 731 using direct probe insert, high resolutions with perfluorokerosine as a standard. Thin-layer chromatography (TLC) was performed on silica gel plates (Macherey & Nagel Sil G/UV 254+366, 0.25 mm silica gel on glass), column chromatography on silica gel 60 (<0.08 mm, Macherey & Nagel).

For quantitative determination all samples were analyzed by using a Spectra Physics Model SP 8000 B Liquid Chromatograph, a Spectra Physics Model 770 Spectrometer Detector and a Perkin-Elmer Model ISS 100 Autosampler. The reversed phase column (120×4.6 mm) and the precolumn (40×4.6 mm) were packed with Shandon ODS Hypersil 5 μm. The mobil phase of the isocratic elution system consisted of doubly distilled water - acetonitrile (6: 4) and 0.1% phosphoric acid. The

flow rate was 2 ml/minute with a pressure of approximately 110 bar.

The detector wavelength was set at 288 nm (sensitivity: 80 mV, full scale). The samples were prepared as following: the culture filtrate was adjusted to a final AcOH concentration of 0.1%, centrifugated, and 10 μl were injected into reversed phase columns.

Bacterial Strains

The standard strains for the activity spectrum of elloramycin were obtained from the stock culture collection in our laboratories or from ATCC. The antibiotic-producing microorganism (Tü 2353) was a new soil isolate (soli sample from Ellora, India), classified according HÜTTER[18] and BERGEY[19] as *Streptomyces olivaceus*.

Fermentation Studies

S. olivaceus was cultured for 50 hours at 27°C in a medium consisting of starch 2% and soybean meal 2% (100 ml medium in 500-ml Erlenmeyer flask with one intrusion, the pH was adjusted to 7.0 before autoclaving). These cultures were used as inoculum for the 25-liter fermentor (Giovanola Frères Sa, Monthey, Schweiz). Elloramycin was produced in this tank under following conditions: 5% inoculum was transferred to a 25-liter fermentor containing 25 liters medium (s.a.) and run at 27°C and 1,000 rpm at 0.5 vvm (volume air per volume culture fluid per minute). To prevent an increasing of pH over 7.0, 3 N H_2SO_4 was added automatically.

Biological Assay

The disc-diffusion assay was used for measuring the antibiotic content of the cultures, and to determine the antibacterial and antifungal spectrum of elloramycin.

Quantitative determination of elloramycin with *S. prasinus* as test organism: To produce spores of *S. prasinus* this organism was cultured on a sporulation medium consisting of yeast extract 0.4%, malt extract 1%, glucose 0.4% and agar 2% (100 ml medium in 500-ml Erlenmeyer flask, the pH was adjusted to 7.2 before autoclaving). As inoculum one used a 50-hour preculture. After an incubation time of 14 days, the spores were harvested and the density of the spore solution was adjusted to an OD 1.3 (578 nm). 0.5 ml of this suspension were mixed with 200 ml of sporulation medium, and test solutions were applied to the filter discs (ϕ 6 mm). The plates were incubated for 24 hours at 37°C.

Isolation of Elloramycin (1)

A crude extract of elloramycin (5 g) was dissolved in $CHCl_3$ - acetone (1: 1) and precipitated in petroleum ether. The precipitate was chromatographed three times on a column (30×10.5 cm) of silica gel with $CHCl_3$ - MeOH (9: 1) as eluant. The blue fluorescent fractions (UV light, 366 nm) contained 590 mg slightly contaminated elloramycin, which was purified on Sephadex LH-20 (column: 200×2.5 cm) with MeOH as solvent. The main zone gave 510 mg chromatographically pure substance, which was dissolved in 5 ml $CHCl_3$ and precipitated by pouring into *n*-pentane. Dark yellow amorphous powder: mp 156°C; $[\alpha]_D^{24}$ +67° (*c* 1.0, $CHCl_3$); Rf values see Table 1; IR (KBr, Fig. 2) 1735, 1710, 1690, 1603, 1585 (sh) cm^{-1}; UV λ_{max}^{MeOH} (ε) 436 (6,500), 411 (11,900), 390 (9,750), 287 (32,600) nm; $\lambda_{max}^{MeOH-HCl}$ 436 (sh), 410 (10,900), 390 (10,700), 287 (39,100) nm; $\lambda_{max}^{MeOH-NaOH}$ 444 (12,500), 425 (sh), 252 (34,500) nm; ^1H NMR (200 MHz, $CDCl_3$) see Fig. 4 and Tables 2 and 4; ^{13}C NMR (25.2 MHz, DMSO-d_6) see Tables 3 and 4; MS (70 eV, Fig. 3) *m/z* (abundant) 660 (3%, M^+, high resolution found: 660.2056, calcd for $C_{32}H_{36}O_{15}$: 660.2054), 547 (2%, M−113, cleavage of ring A), 472 (4%, $C_{23}H_{20}O_{11}$), 359 (31%), 327 (30%), 189 (100%, $C_9H_{17}O_4$), 188 (99%), 157 (39%), 145 (26%), 116 (38%), 101 (95%), 99 (88%), 75 (57%), 59 (90%).

Anal Calcd for $C_{32}H_{36}O_{15}$: C 58.18, H 5.49, 6 CH_3O 28.19.
Found: C 57.76, H 5.66, CH_3O 25.64.

Elloramycinone (3)

50 mg of elloramycin (**1**) were treated with 20 ml of TFA and a drop of H_2O. The solution was stirred for 30 minutes at 50°C and then diluted with 100 ml $CHCl_3$. After extraction of the acid with water, the organic layer was evaporated to dryness. The yellow residue was purified by chromatography on silica gel (column: 20×2.5 cm, $CHCl_3$ - MeOH, 9: 1) and Sephadex LH-20 (column: 50×

2.5 cm, MeOH). The eluate of the main yellow zone was evaporated to dryness, the residue was resolved in 3 ml of acetone and precipitated as yellow, amorphous powder in *n*-pentane, to yield 29 mg of **3** (81%): mp 195°C (dec); $[\alpha]_D^{24}$ +74° (*c* 0.1, MeOH); Rf see Table 1; IR (KBr) 1735 (sh), 1712, 1680 (sh), 1604 cm^{-1}; UV (MeOH and MeOH - HCl) λ_{max} (ε) 412 (11,400), 394 (10,800), 289.5 (35,600) nm; $\lambda_{max}^{MeOH-NaOH}$ 440 (10,000), 357 (7,400), 315 (17,700), 280 (11,400), 245 (22,100) nm; ^1H NMR (80 MHz, $CDCl_3$) see Table 2; ^{13}C NMR (25.2 MHz, DMSO-d_6) see Table 3; MS (70 eV) *m/z* (abundant) 472 (1%, M^+, high resolution calcd for $C_{23}H_{20}O_{11}$ and found: 472.1006), 442 (3%), 358 (5%, M−114, cleavage of ring A), 326 (10%), 114 (16%, M−358), 85 (11%), 69 (25%), 44 (100%).

Anal Calcd for $C_{23}H_{20}O_{11}$: C 58.48, H 4.27.
Found: C 58.04, H 4.60.

Methyl 2,3,4-Tri-*O*-methyl-α-L-rhamnopyranoside (**5b**)

From Elloramycin: The sugar, obtained from a prezone of the silica gel chromatography after hydrolysis of **1** with TFA (see above) as a colorless syrup (*ca.* 10 mg, detectable with 10% molybdatophosphoric acid in EtOH solution as blue spot on silica gel TLC plates), was dissolved in 50 ml MeOH with 10 drops of conc H_2SO_4 and stirred at room temp for 36 hours. The H_2SO_4 was neutralized with a saturated solution of $Ba(OH)_2$ in H_2O, the precipitated $BaSO_4$ was removed by centrifugation. After removal of the solvents the residual syrup was purified by chromatography on silica gel (column: 20× 2.5 cm) with CH_2Cl_2 - MeOH (95: 5) as solvent, to yield 10 mg **5b** as colorless syrup.

From L-(+)-Rhamnose: A solution of 1 g L-rhamnose monohydrate in 50 ml MeOH was treated with 1 ml conc H_2SO_4 and stirred at room temp for 36 hours. The H_2SO_4 was neutralized and the barium sulfate separated as above. After removal of the solvents, the remaining syrup was dissolved in 20 ml of DMF and treated with 30 ml methyl iodide and 2 g silver(I) oxide. This black suspension was stirred until it became white. The silver salts were removed by centrifugation after dilution with 50 ml DMF and 50 ml $CHCl_3$. The supernatant was extracted with 100 ml 5% aq potassium cyanide solution and with 100 ml H_2O afterwards. The organic layer was concentrated *in vacuo*, the residual syrup was purified on silica gel (column: 50×2.5 cm) with petroleum ether - ether (6: 4) as solvent. Yielding 300 mg **5b** from the main zone (TLC control): $[\alpha]_D^{24}$ −62.5° (*c* 1.0, $CHCl_3$); ^1H NMR (200 MHz) and ^{13}C NMR (50.3 MHz) in CD_3CN see Table 4; MS (70 eV) *m/z* (abundant) 189 (1%, M−OCH_3, high resolution calcd for $C_9H_{17}O_4$ and found: 189.1127), 176 (1%), 155 (1%), 129 (2%), 119 (1%), 101 (18%), 97 (2%), 88 (100%), 75 (23%), 73 (19%), 40 (17%).

Anal Calcd for $C_{10}H_{20}O_5$: C 54.53, H 9.15.
Found: C 53.80, H 8.29.

Methylation of Elloramycinone and Tetracenomycin C

A stirred solution of 50 mg elloramycinone (**3**) in 20 ml methyl iodide was treated with 10 mg silver(I) oxide at room temp for 60 hours. The silver salts were separated by centrifugation, the supernatant was evaporated to dryness. The residue was chromatographed several times on silica gel (plates, 20×40 cm, $CHCl_3$ - MeOH, 95: 5) and Sephadex LH-20 (column: 100×2.5 cm) with $CHCl_3$ and MeOH as eluants yielding 8 mg pure elloramycinone tetramethylether (**4**, the middle zone of the lipophilic products on silica gel TLC, $CHCl_3$ - MeOH, 98: 2) and two different elloramycinone trimethylethers[6].

277 mg **2** were methylated under same conditions and separated as described. The tetramethylether (**4**, 28 mg) was found in the eluate between two different (also identical with those of **3**) trimethylethers. A saturated solution of **4** in $CHCl_3$ was poured drop by drop in *n*-pentane giving a pale yellow, amorphous powder.

MP 144°C; Rf see Table 1; IR (KBr) 1738, 1715, 1692, 1662, 1610 cm^{-1}; UV λ_{max}^{MeOH} (ε) 390 (3,400), 340 (sh, 4,300), 286.5 (35,600) nm; ^1H NMR (100 MHz, $CDCl_3$) δ 2.77 (s, 10-CH_3), 3.27 (s, 4a-OCH_3), 3.64 (s, 4-OCH_3), 3.73 (s, 12a-OCH_3), 3.79 (s, 3-OCH_3), 3.90 (s, 11-OCH_3), 3.95 (s, 8-OCH_3), 3.98 (s, 9-$COOCH_3$), 4.86 (s, 4-H), 5.31 (s, 2-H), 7.09 (s, 7-H), 8.14 (s, 6-H); CD ($CHCl_3$) $\lambda_{extreme}$ ($[\theta]^{22} \times 10^{-4}$) 400 (−0.8), 350 (+0.6), 325 (0), 306 (+0.3), 281 (−5.9), 261 (−1.8), 250 (−2.9) nm; MS (70 eV) *m/z* (abundant) 528 (24%, M^+, high resolution calcd for $C_{27}H_{28}O_{11}$ and found: 528.1632), 497 (41%, M−OCH_3), 465 (43%, 497−CH_3OH), 128 (100%), 113 (28%), 45 (28%).

Elloramycin-4,4a-acetonide

79 mg elloramycin (**1**) were solved in 10 ml of 2,2-dimethoxypropane and treated with 5 mg of *p*-toluolsulfonic acid. After stirring 3 hours at 70°C the reaction was allowed to continue at 50°C overnight. Chromatography on silica gel (column: 50×2.5 cm) with $CHCl_3$ - MeOH (85: 15) and Sephadex LH-20 (column: 100×2.5 cm, MeOH) yielded elloramycin (**1**, 65 mg), elloramycinone (**3**, 5 mg) and elloramycin-4,4a-acetonide (25 mg), which was eluted first: IR (KBr) 3440, 1740 (sh), 1728, 1706, 1620, 1605 cm^{-1}; UV λ_{max}^{MeOH} (ε) 439 (sh, 3,000), 411 (11,400), 392 (10,600), 287.5 (37,400), 239 (22,600); $\lambda_{max}^{MeOH-HCl}$ as MeOH, without the shoulder at 439 nm; $\lambda_{max}^{MeOH-NaOH}$ 440 (13,000), 420 (sh, 12,200), 281 (sh, 17,500), 251 (31,100) nm; ^1H NMR (80 MHz, $CDCl_3$) δ 1.26 (d, J=6 Hz, 5′-CH_3), 1.46, 1.77 (s, acetonid-CH_3), 2.89 (s, 10-CH_3), 3.31 (dd, J=9/9 Hz, 4′-H), 3.45 (dd, J=3/9 Hz, 3′-H), 3.55, 3.56, 3.59 (s, 2′-, 3′-, 4′-OCH_3), 3.66 (s, 12a-OCH_3), 3.75 (s, 3-OCH_3), 3.75 (2′-H, overlapped), 3.98 (s, 9-$COOCH_3$), 4.80 (s, 4-H), 5.55 (s, 2-H), 5.70 (d, J=2 Hz, 1′-H), 7.47 (s, 7-H), 7.88 (s, 6-H), 14.10 (s, 11-OH, exchangeable with CD_3OD); CD (MeOH) $\lambda_{extreme}$ ($[\theta]^{22}\times10^{-4}$) 340 (−1.6), 295 (+0.8), 282 (−1.2), 257 (+6.3), 239 (−2.4) nm; MS (70 eV) m/z (abundant) 700 (6%, M^+, high resolution calcd for $C_{35}H_{40}O_{15}$ and found: 700.2367), 454 (4%), 399 (5%), 189 (100%), 188 (97%), 157 (24%), 145 (15%), 131 (6%), 129 (11%), 125 (14%), 116 (15%), 113 (11%), 101 (54%), 99 (53%), 97 (8%), 89 (22%), 85 (10%), 75 (28%), 59 (58%).

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References

1) Werner, G.; H. Hagenmaier, H. Drautz, A. Baumgartner & H. Zähner: Metabolic products of microorganisms. 224. Bafilomycins, a new group of macrolide antibiotics. J. Antibiotics 37: 110~117, 1984
2) Zähner, H.; H. Drautz & W. Weber: Novel approaches to metabolite screening. *In* Bioactive Microbial Products. Search and Discovery. *Eds.*, J. D. Bu'Lock *et al.*, pp. 51~70, Academic Press, New York, 1982
3) Drautz, H.; H. Zähner, E. Kupfer & W. Keller-Schierlein: Isolierung und Struktur von Streptazolin. Helv. Chim. Acta 64: 1752~1765, 1981
4) Noltemeyer, M.; G. M. Sheldrick, H.-U. Hoppe & A. Zeeck: 2-Ethyl-5-(3-indolyl)oxazole from *Streptomyces cinnamomeus* discovered by chemical screening. Characterization and structure elucidation by X-ray analysis. J. Antibiotics 35: 549~555, 1982
5) Keller-Schierlein, W.; D. Wuthier & H. Drautz: (3*S*,8*E*)-5-Oxo-8-decen-1,3-diol, ein Stoffwechselprodukt von *Streptomyces fimbriatus*. Helv. Chim. Acta 66: 1253~1261, 1983
6) Rohr, J. & A. Zeeck: Structure-activity relationships of elloramycin and tetracenomycin C. Ph. D. Thesis, Univ. Göttingen, 1984
7) Weber, W.; H. Zähner, J. Siebers, K. Schröder & A. Zeeck: Tetracenomycin C. Arch. Microbiol. 121: 111~116, 1979
8) Lazar, G.; H. Zähner, S. Breiding, M. Damberg & A. Zeeck: 3-Demethoxy-3-ethoxy-tetracenomycin C. J. Antibiotics 34: 1067~1068, 1981
9) Weber, W.; H. Zähner, J. Siebers, K. Schröder & A. Zeeck: Tetracenomycins — new antibiotics from *Streptomyces glaucescens*. Actinomycetes Zbl. Bakteriol. (Suppl. 11): S465~S468, 1981
10) Haverkamp, J.; M. J. A. De Bie & J. E. G. Vliegenthart: ^{13}C-and ^1H-NMR spectroscopy of permethylated gluco-, galacto-, and manno-pyranoses and their 6-deoxy analogues. Carbohydr. Res. 39: 201~211, 1975
11) Bock, K.; I. Lundt & C. Pedersen: Assignment of anomeric structure to carbohydrates through geminal ^{13}C-H coupling constants. Tetrahedron Lett. 13: 1037~1040, 1973
12) Jones, P. G.; G. M. Sheldrick, J. Rohr & A. Zeeck: Elloramycin, $C_{32}H_{36}O_{15}$. Acta Cryst. C41: 255~257, 1985

13) KRAEMER, H. P. & H. H. SEDLACEK: A modified screening system to select new cytostatic drugs. Behring Inst. Mitt. 74: 301~328, 1984
14) SIMONITSCH, E.; W. EISENHUTH, O. A. STAMM & H. SCHMID: Über die Struktur des Chartreusins. I. Helv. Chim. Acta 47: 1459~1475, 1964
15) EISENHUTH, W.; O. A. STAMM & H. SCHMID: Über die Struktur des Chartreusins. II. Helv. Chim. Acta 47: 1475~1484, 1964
16) YAGI, M.; T. NISHIMURA, H. SUZUKI & N. TANAKA: Chartreusin, an antitumor glycoside antibiotic, induces DNA strand scission. Biochem. Biophys. Res. Commun. 98: 642~647, 1981
17) BERLIN, Y. U.; M. N. KOLOSOV, I. V. VASINA & I. V. YARTSEVA: The structure of chromocyclomycin. J. Chem. Soc. Chem. Commun. 1968: 762~763, 1968
18) HÜTTER, R.: Systematik der Streptomyceten. Karger AG, Basel, 1967
19) BUCHANAN, R. & N. GIBBONS: BERGEY's Manual of Determinative Bacteriology. Williams & Wilkins Co., Baltimore, 1974