# THE STRUCTURE OF MANUMYCIN

## I. CHARACTERIZATION, STRUCTURE ELUCIDATION AND BIOLOGICAL ACTIVITY

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Manumycin (**1**), produced by *Streptomyces parvulus* (strain Tü 64), was isolated from the mycelium by extraction with acetone and could easily be purified chromatographically. Chemical degradation of **1** ($C_{31}H_{38}N_2O_7$) gave 2-acetamino-3-hydroxycyclopent-2-enone (**2**) by acetolysis, 2,4,6-trimethyl-2,4-decadienoic acid (**3**) by alkaline hydrolysis, and 2-(2,4,6-trimethyl-2,4-decadienoylamino)-5,6-epoxy-1,4-benzoquinone (**5**) by mild chromic acid oxidation. In connection with a detailed spectroscopic analysis, the structure of **1** could be elucidated and the (*E*)-configuration of the double bonds in the triene and diene chain was established. Manumycin exhibits biological activity against Gram-positive bacteria and fungi and furthermore, an inhibition of the developmental processes of some insects.

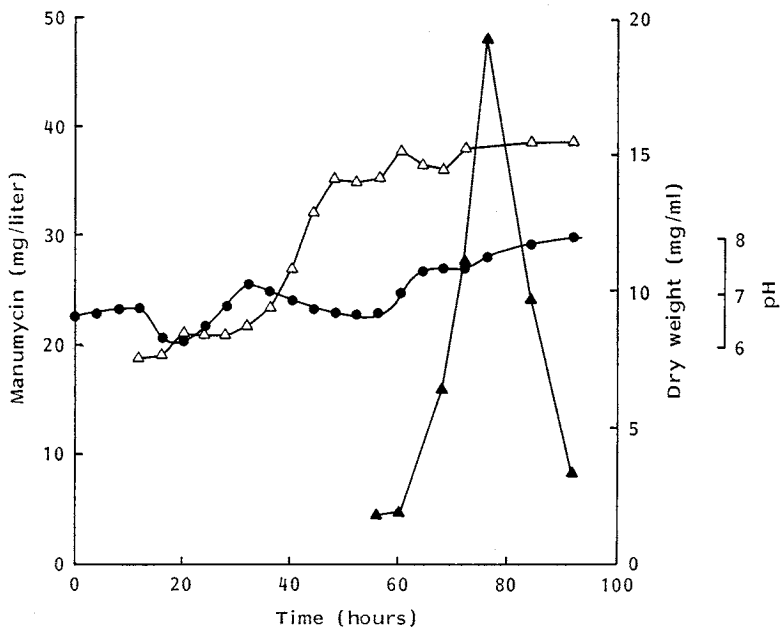
*Streptomyces parvulus* (strain Tü 64) produces the pale yellow antibiotic manumycin[1] in association with more lipophilic red pigments, which were identified as $C_{25}$-prodigiosins[2], and the colorless hydrophilic amino acid antagonist L-2,5-dihydrophenylalanine[3]. Previous reports[1,4] have already described the isolation and structure of manumycin, whose structural elements could not be classified with a known group of antibiotics. In the meantime similar compounds such as asukamycin[5,6], U-62162[7] and U-56,407[8] have been discovered, which could be added to the manumycin group. Structural features incline us to consider these antibiotics as broken chain ansamycins[9]. To verify this hypothesis, biosynthetic studies of manumycin and asukamycin are in progress[10,11].

In our preliminary report[4], chemical degradation reactions have been described to prove the structure of manumycin. Getting more information from subsequent spectra, we are now able to derive the structure of manumycin by a detailed spectroscopic analysis in connection with only a few chemical derivatization reactions. In this full paper we describe the fermentation of strain Tü 64 as well as a simpler isolation method, more biological data and a detailed chemical and spectroscopic characterization of manumycin.

### Fermentation and Isolation

*Streptomyces parvulus* (strain Tü 64) is unstable as its phenotype, and a good logarithmic growth phase is not a reliable indication for producing an identical spectrum of secondary metabolites. The production of the red prodigiosins, starting 36~40 hours after inoculation, does however indicate the formation of manumycin, because these compounds appear simultaneously. The strain Tü 64 was cultivated in 1-liter, 10-liter and 120-liter fermentors, using soybean meal 2% and mannitol 2% as a culture medium. Inoculum was prepared in Erlenmeyer flasks containing the same medium and shaken for 60 hours at 28°C. Fig. 1 shows a typical time course of the fermentation in a 10-liter fermentor. The production of manumycin starts after the log-phase, reaching its maximum 76 hours

Fig. 1. Time course of the fermentation of *Streptomyces parvulus*.
△ Dry weight of the mycelium (mg/ml), ● pH of the culture, ▲ manumycin concentration estimated by TLC scanning at 320 nm.




after inoculation. The yield of manumycin varies and can be up to 100 mg/liter in the culture medium. The antibiotic was obtained by extraction of the mycelium and by partition of the aqueous residue after evaporation between water and chloroform. The organic layer yields a dark red oily crude product, which was triturated by treatment with ice-cold petroleum ether. Crude manumycin could be chromatographically purified on a fast-running silica gel column and on Sephadex LH-20 in chloroform - methanol systems, getting pure manumycin as a pale yellow amorphous powder. This procedure is much easier than the earlier described method[1,4] and leads to a better yield.

## Characterization

Manumycin is soluble in methanol, chloroform, acetone or acetonitrile and insoluble in water or *n*-pentane. It is unstable in the presence of acids and bases and decomposes slowly in protic solvents during chromatographic procedures on silica gel or heating. Surprisingly, it crystallizes from methanol - water systems in pale yellow stars after clearing the solution with charcoal, whereas the antibiotic decomposes to some extent in the mother liquor. The antibiotic is optically active and shows a significant CD spectrum. The molecular formula ($C_{31}H_{33}N_2O_7$) is based on the elemental analysis in connection with the field desorption mass spectrum (FD-MS) (*m/z* 550, $M^+$); the normal electron impact mass spectrum (EI-MS), however, does not show the molecular ion.

The IR spectrum (Fig. 2) exhibits characteristic absorption bands in the region of carbonyls and olefins (1600~1700 $cm^{-1}$). The absorption bands at 3400 and 3260 $cm^{-1}$ are characteristic of OH- and NH-groups. The complex $^1H$ NMR spectrum (Fig. 3) shows four NH and OH signals ($\delta$ 13.68, 8.00, 7.82 and 3.14), and furthermore 9 olefinic protons ($\delta$ 7.43~5.34), 23 aliphatic protons ($\delta$ 2.58~0.86) and two oxirane protons ($\delta$ 3.68 and 3.74; $J_{AB}$=5 Hz), one of the latter is coupled with the olefinic proton at 7.43 ($J$=2.5 Hz). $^{13}C$ NMR data are given in Table 1.

Fig. 2. IR spectrum of manumycin (1) in KBr.

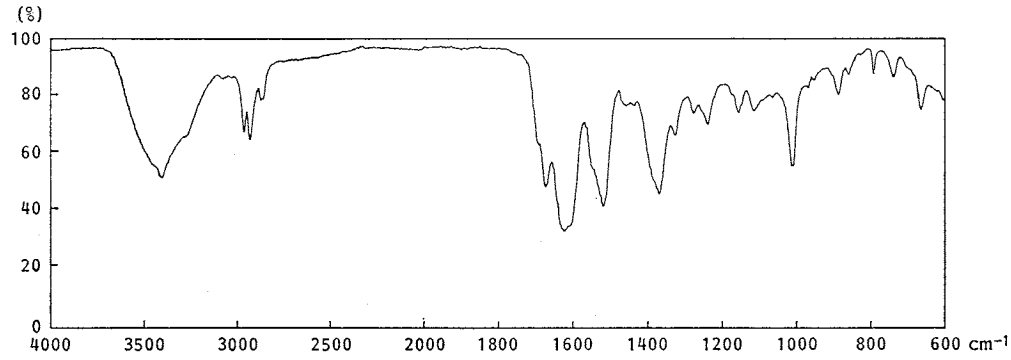
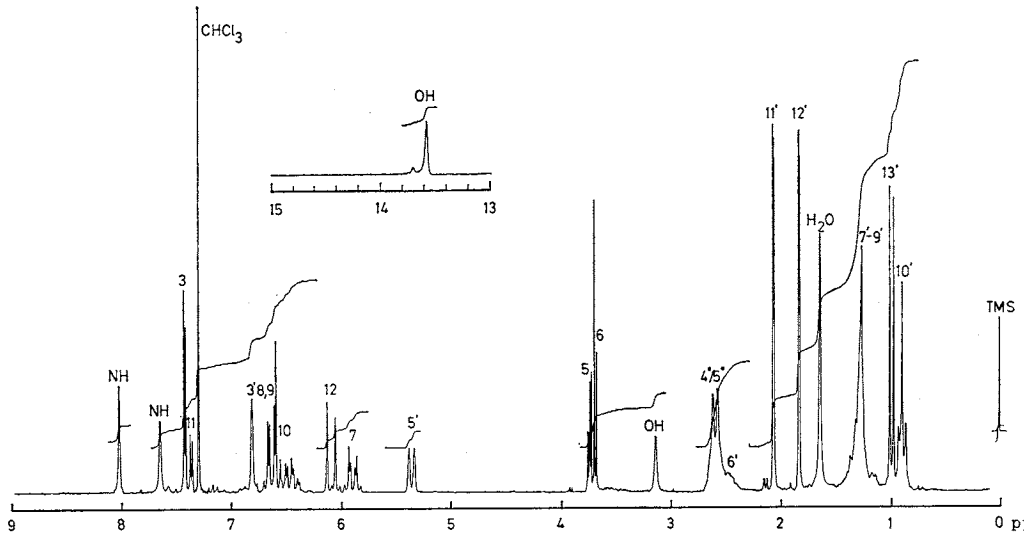



Fig. 3. $^1H$ NMR spectrum of manumycin (1) in $CDCl_3$ at 200 MHz.




## Structural Elucidation

Acetolysis of manumycin yielded a small molecule, 2-acetamino-3-hydroxycyclopent-2-enone (**2**); all data for **2** are in accordance with an authentic sample isolated from limocrocin[12] or moenomycin[13]. Compound **2** shows typical NMR signals corresponding to signals in the spectra of manumycin, confirming the $C_5N$-moiety bond as an amide within the antibiotic. This structural feature is typical of several other secondary metabolites[14,15].

Mild alkaline hydrolysis of manumycin yields the optically active carboxylic acid **3**, containing a conjugated diene chromophore (UV absorption bands see Table 2). The molecular formula ($C_{13}H_{22}O_2$) was established by the high resolution mass spectrum of the methyl ester **4** (*m/z* 224, $C_{14}H_{24}O_2$), which could easily be prepared by methylation of **3** with diazomethane. The $^1H$ NMR spectrum of the acid displayed the following resonances: $\delta$ 0.88, 0.97, 1.86 and 2.02, (4 methyl groups); 1.26 (3 methylene groups); 2.48 (aliphatic methine proton); 5.48 and 7.28 (2 olefinic protons); 10.05 (COOH). The methyl group at $\delta$ 0.88 (t) is connected to the aliphatic chain, consisting of three methylene carbon atoms and a $CH_3$-group at $\delta$ 0.97 (d), which is linked to the carbon atom in the $\alpha$-posi-

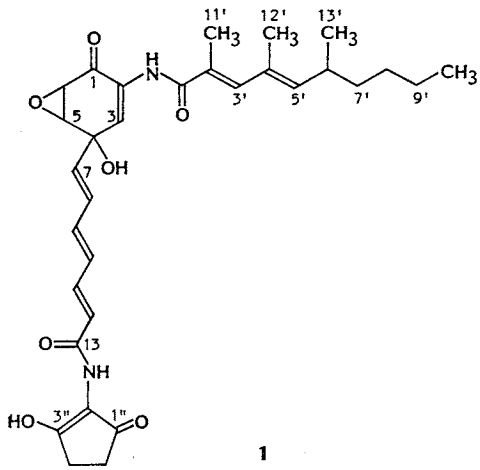
Table 1. $^{13}C$ NMR data of manumycin (**1**), 2,4,6-trimethyl-2,4-decadienoic acid (**3**), methyl 2,4,6-trimethyl-2,4-decadienoate (**4**) and 2-(2,4,6-trimethyl-2,4-decadienoylamino)-5,6-epoxy-1,4-benzoquinone (**5**) in $CDCl_3$.

| C-atom | **1** (100.6 MHz)[a] | **3** (50.3 MHz) | **4** (50.3 MHz)[b] | **5** (50.3 MHz) |
|---|---|---|---|---|
| 1 | 188.9 s | — | — | 188.5 s |
| 2 | 128.1 s | — | — | 139.1 s |
| 3 | 126.3 d | — | — | 114.9 d |
| 4 | 71.3 s | — | — | 191.1 s |
| 5 | 57.5 d | — | — | 52.6 d |
| 6 | 52.9 d | — | — | 53.9 d |
| 7 | 136.5 d | — | — | — |
| 8 | 131.5 d | — | — | — |
| 9 | 139.6 d | — | — | — |
| 10 | 131.7 d | — | — | — |
| 11 | 143.4 d | — | — | — |
| 12 | 121.6 d | — | — | — |
| 13 | 165.5 s | — | — | — |
| 1′ | 168.8 s | 174.6 s | 169.7 s | 168.4 s |
| 2′ | 128.4 s | 123.9 s | 124.7 s | 127.9 s |
| 3′ | 140.2 d | 144.8 d | 143.4 d | 144.0 d |
| 4′ | 129.9 s | 130.6 s | 130.5 s | 129.9 s |
| 5′ | 142.7 d | 145.7 d | 143.6 d | 144.0 d |
| 6′ | 32.9 d | 32.9 d | 32.9 d | 32.9 d |
| 7′ | 37.1 t | 37.0 t | 37.1 t | 37.0 t |
| 8′ | 29.8 t | 29.8 t | 29.8 t | 29.8 t |
| 9′ | 22.8 t | 22.8 t | 22.9 t | 22.8 t |
| 10′ | 14.1 q | 14.1 q | 14.1 q | 13.9 q |
| 11′ | 14.0 q | 13.6 q | 14.1 q | 13.9 q |
| 12′ | 16.5 q | 16.3 q | 16.4 q | 16.3 q |
| 13′ | 20.7 q | 20.7 q | 20.7 q | 20.4 q |

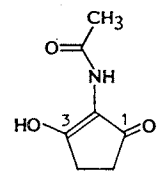
[a] Additional signals for the $C_5N$-moiety: $\delta$ 197.3 (s, C-1″), 115.0 (s, C-2″), 174.0 (s, C-3″), 25.7 (t, C-4″), 32.2 (t, C-5″).

[b] $\delta_{OCH_3}$ 51.8 q.

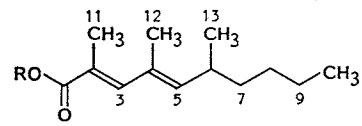
Scheme 1.



**1**



**2**



**3** R = H

**4** R = $CH_3$

Table 2. UV absorption bands in different solvents and $^1H$ NMR data of the $C_6N$-moiety in $CDCl_3$ at 200 MHz of manumycin (**1**), two benzoquinone epoxides (**5** and **6**) and dihydromanumycin (**7**).

| | **1** | **5** | **6** | **7** |
|---|---|---|---|---|
| UV $\lambda_{max}$ nm ($\varepsilon$) | | | | |
| MeOH | 314 (34,600), 278 (36,400) | 318 (15,100), 257 (10,400) | 311 (16,950), 219 (7,950) | 301 (51,700), 263 (48,900) |
| MeOH - HCl | 328 (32,300), 270 (31,700) | 316 (9,800), 257 (10,100) | | |
| MeOH - NaOH | 261 (40,200) | 302 (16,800) | 295 (11,300) | |
| $^1H$ NMR (ppm) (coupling pattern, $J$ in Hz) | | | | |
| 1-H | — | — | — | 4.51 (br) |
| 3-H | 7.51 (d, $J$=2.5) | 7.62 (d, $J$=2.5) | 7.52 (d, $J$=2.5) | 6.17 (d, $J$=2.5) |
| 5-H | 3.72 (dd, $J$=3.5, 2.5) | 3.84 (dd, $J$=3.5, 2.5) | 3.81 (dd, $J$=3.5, 2.5) | 3.36 (dd, $J$=4.4, 2.5) |
| 6-H | 3.65 (d, $J$=3.5) | 3.94 (d, $J$=3.5) | 3.90 (d, $J$=3.5) | 3.61 (dd, $J$=4.4, 2.5) |
| NH | 7.82 (s) | 8.31 (s) | 7.85 (s) | 7.84 (br s) |

tion of the double bond at C-4. The remaining two methyl groups ($\delta$ 1.86, $J$=1 Hz; 2.02, $J$=1 Hz) are allylicly coupled to the two olefinic protons, which indicates a methyl-substituted diene moiety. The positions of these two methyl groups were elucidated by the MS-fragmentation pattern of the methyl ester **4** and the chromic acid oxidation product **5** (see Fig. 5) described below. More detailed spectroscopic investigation confirmed the structure as 2,4,6-trimethyl-2,4-decadienoic acid (**3**), which is amide-bound in the parent compound, manumycin.

Fig. 4. Long range coupling pattern of the ester carbonyl in **4** ($J$ in Hz), determined by selective decoupling experiments in $CDCl_3$ at 200 MHz.

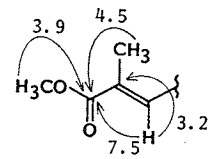
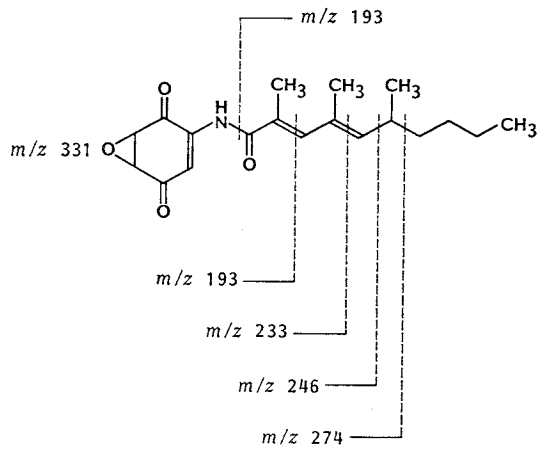


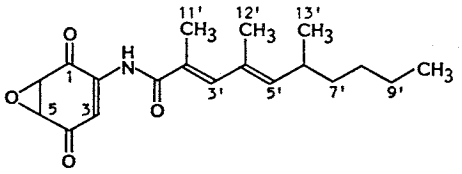

Fig. 5. EI-MS fragmentation pattern of **5** at 70 eV.



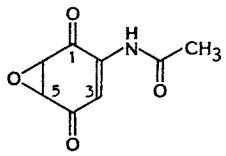

The configuration of the two double bonds of **3** was derived by partially decoupled $^{13}C$ NMR spectra and nuclear Overhauser effect (NOE) difference spectra of the methyl ester **4**. C-1 ($\delta$ 169.7) in the coupled spectrum was split by the protons of 2-$CH_3$, 3-H, and the methoxy group. Selective hetero-decoupling experiments resulted in the elucidation of the corresponding coupling constants (see partial formula **4**), the value of 7.5 Hz for $^3J_{C\text{-}1/3\text{-}H}$ indicated the (*E*)-configuration for the double bond at C-2, while the coupling constant of the (*Z*)-isomer should be larger than 12 Hz[16]. This is in accordance with the down-field shift of 3-H ($\delta$ 7.14) under the influence of the carbonyl group. The NOE between 3-H and 5-H with an enhancement of 8.5% (irrad. 5-H; 10%: irrad. 3-H) pointed out the (*E*)-configuration of this double bond.

Mild chromic acid oxidation of manumycin leads to the optically active compound **5**, which

Scheme 2.



5



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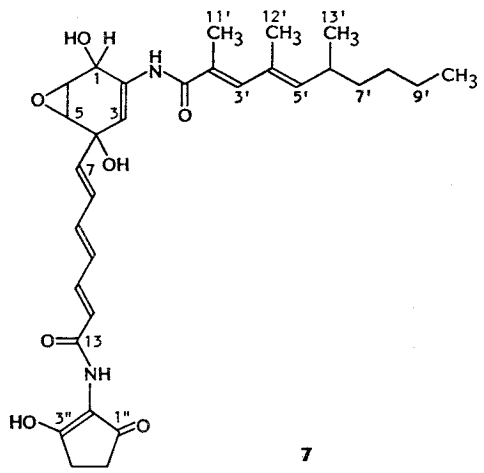

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shows carbonyl groups and olefinic bonds (1684 (sh), 1675 and 1608 $cm^{-1}$) and a NH group (3356 $cm^{-1}$) in its IR spectrum. The UV absorption bands (see Table 2) suggest a quinone-like chromophore. High resolution EI-MS yields a molecular ion at *m/z* 331 ($C_{19}H_{25}NO_4$) and typical key fragments (see Fig. 5), which established the presence of the amide-bound 2,4,6-trimethyl-2,4-decadienoic acid within **5**. The mass spectrum of **5** provides a supplementary proof of the diene side chain's constitution. The $^1H$ NMR spectrum confirms this result. The ABX-protons (Table 2), also detectable in manumycin, belong to the remaining $C_6H_3O_3$ moiety. $^{13}C$ NMR of **5** (see Table 1) and selective decoupling experiments of the ABX-system in comparison with the model compound **6** (see Table 2) established the structure of the oxidation product as a 2-amino-5,6-epoxy-1,4-benzoquinone with the amide-bound acid **3**.

The NMR data of manumycin suggest that all carbon atoms of the degradation products **2** and **5** are part of the antibiotic molecule. Based on the molecular formula of manumycin, seven carbon atoms are still missing. One of these carbon atoms is an amide carbonyl group ($\delta$ 165.4) and the remaining atoms belong to six olefinic CH-groups, forming a triene chain (NMR signals see Tables 1 and 3). Both the isolation of **5** from chromic acid oxidation and the absence of a C-4 carbonyl group (no signal at $\delta$ 191.1) in manumycin give rise to the extension of **5** at this C-atom. Thus, the triene chain is the missing linkage between the cyclohexane epoxide and the amide-bound $C_5N$-moiety.

The stereochemistry of the triene chain was confirmed by selective decoupling experiments. In benzene-$d_6$, the coupling constants of **1** are $^3J_{7,8}$=15 Hz, $^3J_{8,9}$=11 Hz, $^3J_{10,11}$=11 Hz and $^3J_{11,12}$= 15 Hz. Because of the signal complexety in benzene-$d_6$, $^3J_{9,10}$ could not be determined. Using different solvents the triene protons in manumycin exhibit significant differences in the chemical shifts (see Table 3), and the coupling constant $^3J_{9,10}$ (14.5 Hz) was determined in pyridine-$d_5$. Thus, manumycin shows the all-(*E*)-configuration in the triene moiety and differs remarkably from asukamycin[6] and U-56,407[8].

To confirm the proposed linkage of the $C_{13}$-acylamino side chain at C-2 of the cyclohexenone-epoxide, manumycin was reduced with sodium borohydride to the dihydro derivative **7**, which could be chromatographically purified on a Sephadex LH-20 column. The hydrogenation took place at

Table 3. Chemical shifts ($\delta$ in ppm) of the triene protons of manumycin (**1**) in different solvents at 200 MHz.

| Proton | $CDCl_3$ | Acetone-$d_6$ | Benzene-$d_6$ | Pyridine-$d_5$ | DMSO-$d_6$ |
|---|---|---|---|---|---|
| 7-H d | 5.89 | 6.11 | 5.49 | 6.28 | 5.99 |
| 8-H dd | 6.5~6.7[a] | 6.5~6.7[a] | 6.91 | 7.09 | 6.4~6.9[a] |
| 9-H dd | 6.5~6.7[a] | 6.5~6.9[a] | 6.2~6.4[a] | 6.80 | 6.4~6.9[a] |
| 10-H dd | 6.47 | 6.5~6.9[a] | 6.2~6.4[a] | 6.52 | 6.4~6.9[a] |
| 11-H dd | 7.36 | 7.40 | 7.55 | 7.71 | 7.25 |
| 12-H d | 6.18 | 6.5~6.9[a] | 6.13 | 6.77 | 6.4~6.9[a] |

[a] Not definitely assigned.

the C-1 carbonyl group of manumycin, indicated by a new 1-H signal at $\delta$ 4.51 and a significant upfield shift of 3-H. Selective decoupling experiments confirmed the assignment of all protons of the six-membered ring (Table 2). The coupling constants between 1-H and 6-H ($J$=2.5 Hz) and the missing of a coupling between 1-H and 3-H proved the position of the $C_{18}$-acylamido side chain at C-2.

$^1H$-$^1H$ and $^1H$-$^{13}C$ two-dimensional (2D) NMR spectra of **1** confirmed the assignments of the proton and carbon atom signals. The three olefinic C-atoms C-2 (q), C-2′ (q) and C-4′ (q) were assigned by their signal coupling patterns derived from full-coupled $^{13}C$ NMR spectra followed by selective irrad. of 10′-$H_3$ and 11′-$H_3$ (Table 1).

## Biological Activity

In the disc-diffusion assay, manumycin inhibited the growth of Gram-positive bacteria and fungi and showed no activity against Gram-negative bacteria and yeasts[1]. A low but significant cytotoxic effect on stem cells of L1210 leukemia ($IC_{50}$ 0.93 $\mu$g/ml) was observable. In laboratory and outdoor tests, using *Lepidoptera* and *Coleoptera* as test organisms, manumycin proved to be a development restrictor in addition to its repellent effect on larvae who want to feed on it. Good effects on the eggs and larvae of *Pieris brassicae* and *Epilachna varivestis* were achieved by means of a 0.05%-solution. Concentrated solutions did not lead to an effective increase. Compared to chitin synthetase-inhibitors, its effect sets in too slowly, so that damages to leaves cannot be avoided. Manumycin was inactive against *Tetranychus urticae* and *Myzus persicae*.

## Experimental

### General

Melting points were determined on a Reichelt hot-stage microscope and are not corrected. IR spectra in pressed KBr discs were recorded on Perkin-Elmer Model 137 and Perkin-Elmer Model 297 spectrometers, the UV spectra on a Zeiss DMR 21 spectrophotometer. Optical rotations were recorded with a Perkin-Elmer Model 241 polarimeter. All CD spectra were obtained in various solvents using of a Jasco J-500A spectropolarimeter. The intensities of the TLC spots were measured with a Desaga Chromatogramm Densitometer CD 50. $^1H$ NMR spectra were determined at 100 MHz with a Varian HA-100 and Varian XL-100, at 200 MHz with a Varian XL-200 or at 400 MHz with a Bruker WM-400. $^{13}C$ NMR spectra were obtained on a Varian XL-100 (25.2 MHz), Varian XL-200 (50.4 MHz) or Bruker WM-400 (100.6 MHz). Chemical shifts are expressed in $\delta$ values (ppm) with TMS as an internal standard. The multiplicities of the $^{13}C$ NMR values were assigned by attached proton test (APT) or distortionless enhancement of polarization transfer (DEPT) techniques. The mass spectra were taken by a Varian MAT-311a (EI) and a Finnigan MAT-8230 (FD) mass spectrometer.

### Analyticals

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), silica gel 0.04~0.063 mm (Macherey & Nagel) and Sephadex LH-20 (Pharmacia).

Fermentation Studies

*Streptomyces parvulus* (strain Tü 64) was cultured at 28°C on agar slants composed of malt extract (Difco) 1%, glucose 0.4%, yeast extract (Difco) 0.4% and agar-agar (Difco) 2%, pH 7.3 adjusted with 2 M NaOH. 4~5 days after inoculation, sporulation is accompanied with an appearance of red pigments ($C_{25}$-prodigiosins) to follow 24~48 hours later. After a 10-day incubation period the red mycelia were suspended in a 1,000-ml Erlenmeyer flask containing 100 ml of a sterile medium consisting of degreased soybean meal 2% and mannitol 2%, adjusted at pH 7.5 with 2 M NaOH. Shaken for 60 hours at 28°C this culture was used to inoculate further Erlenmeyer flasks, 1 liter as well as 10-liter and 120-liter fermentors (inoculation volume: 25 ml/liter) containing the same medium. In order to produce manumycin the cultures were shaken at 28°C or incubated using air supply at *ca.* 45 liters/hour. If necessary the anti-foaming compound Niax Polyol PPG 2025 (1:9 in EtOH) was added. The fermentation time-course is depicted in Fig. 1. After 76 hours of cultivation the culture broth was adjusted to pH 4.5 with 2 M HCl, stirred with Hyflo-Celite (50 g/liter), and filtered. The culture filtrate was discarded, and the precipitate was extracted three times with acetone (250 ml/liter culture broth). In vacuum, the acetone was evaporated and the remaining aqueous residue was extracted four times with chloroform (30 ml per liter of the culture broth). The combined organic layers were evaporated to dryness in order to obtain a dark red oily crude product, which, by trituration with ice-cold petroleum ether, yielded a dark red amorphous powder.

Isolation of Manumycin

In a fast-running column chromatography (silica gel, 20×8.5 cm; $CHCl_3$ - MeOH, 9:1), 10 g of the crude powder were roughly purified, leading to 5.3 g of an enriched product (*ca.* 60% manumycin). 200 mg of this product were additionally purified by repeated chromatography on a Sephadex LH-20 column (100×2.5 cm, eluting with $CHCl_3$), yielding 120 mg of pure manumycin (**1**) as a pale yellow amorphous powder. The overall isolated yield amounted to 90 mg per liter of the culture broth.

MP 139~141°C (dec); $[\alpha]_D^{20}$ −185° (*c* 0.4, $CHCl_3$); Rf 0.51 ($CHCl_3$ - MeOH, 9:1); IR (KBr, see Fig. 2) $cm^{-1}$ 3400, 3260 (sh), 1690 (sh), 1668, 1620, 1603 (sh), 1005; UV see Table 2; $^1H$ NMR (200 MHz, $CDCl_3$, see Fig. 3, values completing Tables 2 and 3) $\delta$ 0.86 (t, $J$=6.4 Hz, 10′-$H_3$), 0.93 (d, $J$=6.4 Hz, 13′-$H_3$), 1.12~1.44 (br m, 7′-$H_2$, 8′-$H_2$ and 9′-$H_2$), 1.81 (d, $J$=1.6 Hz, 12′-$H_3$), 2.04 (d, $J$=1.6 Hz, 11′-$H_3$), 2.35~2.60 (br m, 6′-H), 2.58 (s, 4″-$H_2$ and 5″-$H_2$), 5.34 (d, $J$=10 Hz, 5′-H), 7.32~7.43 (m, including signals of 3′-H and 3-H), 8.00 (s, NH), 13.68 (br s, OH); $^1H$ NMR (200 MHz, pyridine-$d_5$, values completing Table 3) $\delta$ 0.86 (t, $J$=6.4 Hz, 10′-$H_3$), 0.93 (d, $J$=6.4 Hz, 13′-$H_3$), 1.08~1.36 (br m, 7′-$H_2$, 8′-$H_2$ and 9′-$H_2$), 1.73 (d, $J$=1.6 Hz, 12′-$H_3$), 2.12 (d, $J$=1.6 Hz, 11′-$H_3$), 2.30~2.60 (br m, 6′-H), 2.44 (s, 4″-$H_2$ and 5″-$H_2$), 4.00 (d, $J$=4 Hz, 6-H), 4.12 (dd, $J$=4 and 2 Hz, 5-H), 5.31 (br d, $J$=9.5 Hz, 5′-H), 7.05 (br s, 3′-H), 8.02 (d, $J$=2.5 Hz, 3-H), 8.76 (s, NH), 9.06 (s, NH); $^{13}C$ NMR (100.6 MHz, $CDCl_3$) see Table 1; CD $\lambda_{extreme}^{CHCl_3}$ nm ($[\theta]\times10^4$) 317 (−4.2), 284 (+4.1), 261 (+1.6); FD-MS *m/z* 550 ($M^+$), 532 ($M-H_2O$).

*Anal* Calcd for $C_{31}H_{38}N_2O_7$: C 67.62, H 6.96, N 5.09.
Found: C 67.69, H 7.06, N 4.95.

2-Acetamino-3-hydroxycyclopent-2-enone (**2**)

A solution of 493 mg **1** in 8 ml acetic anhydride was heated for 5 hours at 150°C in a sealed tube. The red solution was evaporated to dryness, extracted by means of boiling MeOH and filtered. The filtrate was poured into twice the quantity of water, filtered, concentrated to a third of the volume and filtered again. The precipitates were discarded and the filtrate was dried and purified by column chromatography on silica gel (20×1.8 cm, $CHCl_3$ - MeOH, 9:1). **2** was crystallized from cyclohexane to yield 44.5 mg (32%) colorless needles: MP 166~167°C (literature[17] 166~168°C); Rf 0.13 ($CHCl_3$ - MeOH, 98:2); IR (KBr) $cm^{-1}$ 3247, 1689, 1602, 1544; UV $\lambda_{max}^{MeOH}$ nm ($\varepsilon$) 257 (14,200); $\lambda_{max}^{MeOH-HCl}$ 257 (23,800); $\lambda_{max}^{MeOH-NaOH}$ 259 (23,800); $^1H$ NMR (100 MHz, $CDCl_3$) $\delta$ 2.20 (s, $NCOCH_3$), 2.53 (br s, 4-$H_2$

and 5-$H_2$), 8.15 (br s, NH), 13.20 (s, 3-OH); MS (70 eV) *m/z* (relative intensity) 155 (38%, $M^+$), 113 (100%), 96 (5%), 85 (9%).

*Anal* Calcd for $C_7H_9NO_3$: C 54.19, H 5.85, N 9.03.
Found: C 54.36, H 5.88, N 8.94.

2,4,6-Trimethyl-2,4-decadienoic Acid (3)

675 mg **1** were dissolved in 400 ml 0.1 M NaOH and stirred for 30 minutes at 50°C. The dark red solution was acidified with 0.5 M oxalic acid (pH 2) and extracted with $CHCl_3$. The dried organic layer was evaporated to a brown syrupy oil, which was chromatographed on silica gel (column 60×2.5 cm) eluting with $CHCl_3$ - MeOH (99 : 1). Further purification was carried out by chromatography on Sephadex LH-20 (60×2.5 cm) eluting with $CHCl_3$ to yield 226 mg (88%) of **3** as a pale yellow oil: $[\alpha]_D^{22}$ −75.8° (*c* 1.6, $CHCl_3$); Rf 0.43 ($CHCl_3$ - MeOH, 9 : 1); IR (KBr) $cm^{-1}$ 2920, 1680, 1652, 1620; UV $\lambda_{max}^{EtOH}$ nm (ε) 264 (15,100); $\lambda_{max}^{EtOH-HCl}$ 265 (16,500); $\lambda_{max}^{EtOH-NaOH}$ 255 (13,000); $^1H$ NMR (200 MHz, $CDCl_3$) δ 0.88 (t, *J*=7 Hz, 10-$H_3$), 0.97 (d, *J*=6 Hz, 13-$H_3$), 1.26 (br m, 7-$H_2$, 8-$H_2$ and 9-$H_2$), 1.86 (d, *J*=1 Hz, 12-$H_3$), 2.02 (d, *J*=1 Hz, 11-$H_3$), 2.48 (m, 6-H), 5.48 (d, *J*=10 Hz, 5-H), 7.28 (3-H, overlapped by $CDCl_3$), 10.05 (br s, COOH); $^{13}C$ NMR (50.3 MHz, $CDCl_3$) see Table 1; MS (70 eV) *m/z* (relative intensity) 210 (8%, $M^+$, high resolution calcd for $C_{13}H_{22}O_2$ and found: 210.1620), 165 (4%), 153 (5%), 125 (100%), 112 (33%), 107 (12%).

Methyl 2,4,6-Trimethyl-2,4-decadienoate (4)

A 0.4 M solution of etheral diazomethane was added dropwise to a stirred solution of 29.1 mg **3** in 15 ml $CHCl_3$ until there was no more compound **3** detectable by TLC. Evaporation to dryness yielded 30 mg (97%) of **4** as a pale yellow oil: $[\alpha]_D^{20}$ −55.8° (*c* 1.2, $CHCl_3$); Rf 0.78 ($CHCl_3$ - MeOH, 9 : 1); IR (film) $cm^{-1}$ 1713, 1625; UV $\lambda_{max}^{EtOH}$ nm (ε) 265 (16,300); $^1H$ NMR (200 MHz, $CDCl_3$) δ 0.88 (t, *J*=6.8 Hz, 10-$H_3$), 0.97 (d, *J*=7.5 Hz, 13-$H_3$), 1.26 (br m, 7-$H_2$, 8-$H_2$ and 9-$H_2$), 1.83 (d, *J*=1.27 Hz, 12-$H_3$), 2.01 (d, *J*=1.34 Hz, 11-$H_3$), 2.46 (m, 6-H), 3.76 (s, $OCH_3$), 5.40 (dt, *J*=9.7 and 1.3 Hz, 5-H), 7.14 (t, *J*=1.3 Hz, 3-H); $^{13}C$ NMR (50.3 MHz, $CDCl_3$) see Table 1; MS (70 eV) *m/z* (relative intensity) 224 (7%, $M^+$, high resolution calcd for $C_{14}H_{24}O_2$ and found: 224.1776), 193 (3%, M−$OCH_3$), 165 (6%, M−$COOCH_3$), 139 (100%), 126 (40%), 107 (59%).

2-(2,4,6-Trimethyl-2,4-decadienoylamino)-5,6-epoxy-1,4-benzoquinone (5)

775 mg **1** were dissolved in 30 ml acetic acid, distributed into 10 flasks, and then stirred at room temp. After 0, 1, 2, 4 and 5 hours 0.6 ml of a chromic acid solution (530 mg $CrO_3$ in 30 ml 60% acetic acid) were added to each flask. Six hours later the reaction mixtures were poured together, diluted with 250 ml of 2 M HCl, and extracted five times with 200 ml ether. The organic layer was dried with $Na_2SO_4$, filtered and evaporated to dryness. The brown oily residue was purified by silica gel column chromatography (100×2 cm, $CHCl_3$ - MeOH, 9 : 1) to yield 105 mg (37%) of **5** as a yellow oil: $[\alpha]_D^{20}$ −8.1° (*c* 1.0, $CHCl_3$); Rf 0.66 ($CHCl_3$ - MeOH, 95 : 5); IR (KBr) $cm^{-1}$ 3380, 1684 (sh), 1675, 1608, 1009; UV see Table 2; $^1H$ NMR (200 MHz, $CDCl_3$) δ 0.88 (t, *J*=7 Hz, 10′-$H_3$), 0.98 (d, *J*=6 Hz, 13′-$H_3$), 1.16~1.48 (br m, 7′-$H_2$, 8′-$H_2$ and 9′-$H_2$), 1.84 (d, *J*=1 Hz, 12′-$H_3$), 2.08 (d, *J*=1 Hz, 11′-$H_3$), 2.38~2.58 (br m, 6′-H), 3.84 (dd, *J*=3.5 and 2.5 Hz, 5-H), 3.94 (d, *J*=3.5 Hz, 6-H), 5.42 (d, *J*=10 Hz, 5′-H), 6.86 (br s, 3′-H), 7.62 (d, *J*=2.5 Hz, 3-H), 8.32 (br s, NH); $^{13}C$ NMR (22.63 MHz, $CDCl_3$) see Table 1; CD $\lambda_{extreme}^{CHCl_3}$ nm([θ]×$10^{-4}$) 365 (+2.499), 316 (−4.126), 242 (+1.507), 219 (−2.698); MS (70 eV) *m/z* (relative intensity) 331 (9.5%, $M^+$, high resolution calcd for $C_{19}H_{25}NO_4$ and found 331.1783), 274 (3%, $C_{15}H_{18}NO_4$, 274.1079), 246 (100%, $C_{13}H_{12}NO_4$, 246.0766), 233 (10.8%, $C_{12}H_{11}NO_4$, 233.0684), 193 (30.2%, $C_{13}H_{21}O$, 193.1591), 153 (63.3%).

2-Acetamino-5,6-epoxy-1,4-benzoquinone (6)

2.25 mg sodium perborate in 240 ml water were added to a stirred solution of 780 mg 2-acetamino-1,4-benzoquinone[18], dissolved in 360 ml EtOH. Two minutes later the solution was acidified to pH 5 with 2 M HCl and extracted four times with 100 ml $CHCl_3$. The organic layer was dried with $Na_2SO_4$, filtered and evaporated to dryness. The residue was chromatographed twice on a silica gel column (30×2.2 cm) in $CHCl_3$. Further purification was carried out by sublimation at 100°C in a high vacuum apparatus to yield 81 mg (9.5%) of **6** as a yellow amorphous powder: MP 166~167°C (dec);

Rf 0.51 ($CHCl_3$ - MeOH, 9:1); UV see Table 2; $^1H$ NMR (100 MHz, $CDCl_3$) $\delta$ 2.22 (s, $NCOCH_3$), further signals see Table 2.

*Anal* Calcd for $C_8H_7NO_4$: C 53.04, H 3.90, N 7.73.
Found: C 53.10, H 3.97, N 7.60.

Dihydromanumycin (7)

100 mg manumycin were dissolved in 20 ml MeOH and 5 ml water. In small portions, 7 mg sodium borohydride were added and stirred for 2 minutes. The reaction mixture was diluted with 100 ml water, adjusted to pH 3 with 0.5 M oxalic acid and extracted with $CHCl_3$. The organic layer was washed with water, dried with $Na_2SO_4$, and filtered by adding a small amount of MeOH. After evaporation, the remaining residue was chromatographed on Sephadex LH-20 (100×2.5 cm, $CHCl_3$) and twice on Sephadex LH-20 (100×2.5 cm, MeOH). Seven zones could be detected and the main product (Rf 0.20, $CHCl_3$ - MeOH, 19:1; 0.66, $CHCl_3$ - MeOH, 9:1) consisted of pure **7** (57 mg, 57%) being a yellow amorphous powder: MP 160°C (dec); IR (KBr) $cm^{-1}$ 3367, 3279, 1678 (sh), 1660 (sh), 1641 (sh), 1616, 1600, 1005; UV see Table 2; $^1H$ NMR (200 MHz, $CDCl_3$ - $CD_3OD$) $\delta$ 0.88 (t, $J$= 6.5 Hz, 10′-$H_3$), 0.96 (d, $J$=6.5 Hz, 13′-$H_3$), 1.26 (br s, 7′-$H_2$, 8′-$H_2$ and 9′-$H_2$), 1.80 (s, 12′-$H_3$), 2.02 (s, 11′-$H_3$), 2.46 (br s, 6′-H), 2.57 (br s, 4″-$H_2$ and 5″-$H_2$), 3.36 (dd, $J$=4.4 and 2.5 Hz, 5-H), 3.61 (dd, $J$=4.4 and 2.5 Hz, 6-H), 4.51 (br, 1-H), 5.33 (br d, $J$=10 Hz, 5′-H), 5.88 (br d, $J$=14 Hz, 7-H), 6.12 (d, $J$=16 Hz, 12-H), 6.17 (br s, 3-H), 6.30~6.66 (m, 3 olefinic protons), 6.78 (s, 3′-H), 7.22~7.44 (m, 1 olefinic proton, overlapped by $CDCl_3$), 7.84 (br s, NH), 8.06 (br s, NH); $^{13}C$ NMR (50.3 MHz, $CDCl_3$) $\delta$ 167.7 (s, C-1′), 165.5 (s, C-13), 130.0 (s), 129.9 (s), 129.4 (s), 127.9 (d), 122.6 (d, C-12), 113.9 (s, C-2″), 111.8 (d, C-3), 71.3 (s, C-4), 63.5 (d, C-1), 56.7 (d, C-5), 53.7 (d, C-6), 36.4 (t, C-7′), 32.1 (d, C-6′), 29.1 (t, C-8′), 22.1 (t, C-9′), 20.6 (q, C-13′), 16.3 (q, C-12′), 13.9 (q, C-10′), 13.8 (q, C-11′); CD $\lambda_{extreme}$ ($CHCl_3$ - MeOH, 9:1) nm ($[\theta]\times10^4$) 322 (+3.0), 285 (−2.6), 249 (−1.7).

*Anal* Calcd for $C_{31}H_{40}N_2O_7$: C 67.37, H 7.30, N 5.07.
Found: C 67.49, H 7.28, N 5.00.

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